DEPARTMENT OF PHYSICS UNIVERSITY OF JYVÄSKYLÄ RESEARCH REPORT No. 9/2017

NANODEVICES BY DNA BASED GOLD NANOSTRUCTURES

BY KOSTI TAPIO

Academic Dissertation for the Degree of Doctor of Philosophy

To be presented, by permission of the Faculty of Mathematics and Science of the University of Jyväskylä, for public examination in Auditorium FYS1 of the University of Jyväskylä on December 19, 2017 at 12 o'clock noon



Preface

The work reviewed in this thesis has been carried out during the years 2012-2017 at the Department of Physics in the University of Jyväskylä.

First of all I would like to thank my supervisor Adj. Prof. Jussi Toppari for his valuable guidance during my Master's and Ph.D. studies and allowing me to work in his group for many years. In addition, I would like to thank especially Prof. Vesa Hytönen, Prof. Janne Ihalainen, Dr. Alli-Mari Liukkonen and Dr. Sanna Auer for their advices and guiding related to biology-oriented studies, which played a significant role in this thesis.

It has been pleasure to work in the Nanoscience Center for many years and I would like to thank the following people who have been supporting me during that time: Prof. Markus Ahlskog, Dr. Veikko Linko, Dr. Kimmo Kinnunen, Dr. Siim Pikker, Prof. Gerrit Groenhof, Mr. Andrii Torgovkin, Mr. Shao Dongkai, Mr. Geng Zhouran and Dr. Janne Simonen. Special mention goes also to the former members of my group, Dr. Svitlana Baieva, Dr. Tommi Isoniemi and Dr. Tibebe Lemma, for fruitful discussion and to my students, Mr. Janne Saastamoinen and Ms. Sailee Shroff, for their help. In general, I would express my gratitude to the whole staff of Nanoscience Center.

During my time at the University of Jyväskylä I have met a lot of wonderful people. From the first years in the university, I remember fondly Mr. Sampsa Vihonen and Dr. Armen Julukian, two people who always bring a smile to my face. I had the privilege to share the office with Mr. Boxuan Shen, Mrs. Leena Sovijärvi and Dr. Matti Hokkanen and I warmly remember going to lectures together. Also, I would like to thank Mr. Timo Jetsonen and Mrs. Riikka Huikkonen, living in Jyväskylä would not have been so fun and lively without them.

Lastly, I wish to thank my parents Matti and Pirkko, my brother Joel and my sisters Milka and Maaret for their love, empathy and support during my studies. Financial support from the Finnish Cultural Foundation, Tekes and Academy of Finland are gratefully acknowledged.

Jyväskylä, December 2017

Kosti Tapio

Abstract

Kosti Tapio Nanodevices by DNA based gold nanostructures Jyväskylä: University of Jyväskylä, 2017, 134 p. (Research report/Department of Physics, University of Jyväskylä, ISSN 0075-465X; 9/2017) ISBN 978-951-39-7307-0 (paper copy) ISBN 978-951-39-7308-7 (PDF) diss.

In this thesis DNA based structures were utilized to create gold nanostructures for nanosensing and nanoelectronic applications. In the past, both of these fields have been dominated by the conventional lithography methods, e.g., electron beam lithography and UV-lithography, but more recently scaling down the components by these techniques has become increasingly more complex and costly. Especially in the micro- and nanoelectronics, the increase in the component density and thus computational power would require fabrication of sub-10-nm components, which is challenging for the top-down approaches. Aforementioned developments have led researchers to seek alternative methods to fabricate these components using socalled bottom-up approaches, that could offer less complex, faster and cost-efficient ways to fabricate the desired structures. Two of the most promising candidates for this task have been the deoxyribonucleic acid and metallic nanoparticles due to their unique optical, mechanical and chemical properties, which allow almost seamless interfacing between the two, yet still incorporate their essential optical and electrical properties, that is typically more difficult to achieve using other pairs of organic and inorganic compounds.

Three distinct fabrication methods were investigated to create three different nanodevices. The new DNA assisted lithography method was used to create metasurfaces covered with arbitrary, highly defined metallic shapes, e.g., nanoantenna bowties. The more traditional hybridization based patterning of gold nanoparticles on DNA template was used to create DNA and gold nanoparticle assemblies, which applicability as a single electron transistor was demonstrated. Finally, DNA and gold nanoparticle based assembly was utilized as an electric field controllable probe to investigate the folding and unfolding properties of a hairpin-DNA molecule.

Metallic bowtie antennas have interested researchers due to the high field en-

hancement between the two triangles, which could be used in e.g. surface-enhanced Raman spectroscopy. However, the current fabrication techniques have been mostly limited to infrared region due to the size and shape restrictions. By using dark field microscopy, we have showed that the new fabrication method is able to produce highly defined structures in a wafer scale and having their desired optical properties at visible regions even on high-refractive index substrates, where both of the features have not been feasible to accomplice before.

Single stranded DNA functionalized gold nanoparticles are one of the standard tools to develop nanoscale applications, from nanopatterning to diagnostic detection. Functionalization scheme using DNA and AuNPs was utilized to fabricate two vastly different assemblies: pearl-like, three gold nanoparticle linear chain on DNA template and AuNPs coated with biotinylated DNA strands, which were further immobilized to chimeric avidin coated gold surface via strong biotin-avidin interaction. For the former case, dielectrophoresis trapping was employed to position these pearl-like DNA-AuNP assemblies between a fingertip electrode structure for current-voltage characterization. It was observed that the plain, pearl-like DNA-AuNP assemblies did not conduct a current, which was most probably due to too large air gaps between the AuNPs. Thus the structures were extruded larger by chemical gold growth process. After that the current started to flow when a threshold voltage was reached, i.e, where after the Coulomb blockade was observed for a few samples from 4.2 K up to room temperature. For the latter case, the sandwich assembly of gold surface-avidin-DNA-AuNP was used to study the conformational changes of a hairpin-DNA by electric field induced motion of the AuNP, where the motion of gold nanoparticles either caused the DNA to stretch and unfold or relax and fold back.

Keywords DNA, self-assembly, hairpin-DNA, origami, TX-tile structure, DNA hybridization, gold nanoparticles, functionalization, surface plasmon, chimeric avidin, biotin, immobilization, electrostatic manipulation, nanoactuator, dark field microscopy, single electron transistor, Coulomb blockade, differential conductance.

Author's address	Kosti Tapio Department of Physics University of Jyväskylä Finland
Supervisor	Adj. Professor Jussi Toppari Department of Physics University of Jyväskylä Finland
Reviewers	Professor Ilko Bald Institute of Chemistry University of Potsdam Germany
	Lecturer Dr. Matteo Castronovo Faculty of Mathematics and Physical Sciences School of Food Science and Nutrition University of Leeds United Kingdom
Opponent	Professor Adam Woolley Department of Chemistry and Biochemistry Brigham Young University United States of America

Tiivistelmä (abstract in Finnish)

Kosti Tapio Nanodevices by DNA based gold nanostructures Jyväskylä: University of Jyväskylä, 2017, 134 p. (Research report/Department of Physics, University of Jyväskylä, ISSN 0075-465X; 9/2017) ISBN 978-951-39-7307-0 (paper copy) ISBN 978-951-39-7308-7 (PDF) diss.

Väitöskirjani keskittyy metallisten nanorakenteiden valmistamiseen DNA-pohjaisten rakenteiden avulla, joita voidaan hyödyntää sähköisinä sekä optisina komponentteina tai antureina. Perinteisesti tällaisten komponenttien valmistamiseen käytetään elektronisuihku- ja UV-litografiaa, joissa suuren kokoluokan kappaleessa pyritään eri prosessointimenetelmillä, kuten syövyttämällä, tekemään pieniä komponentteja. Kuitenkin komponenttien koon pienentyessä alle 100 nanometriin niiden valmistus näillä litografiamenetelmillä on entistä vaikeampaa ja monimutkaisempaa. Tämän takia on alettu kehittämään ns. "alhaalta-ylös" menetelmiä, joissa yksittäisistä atomeista, molekyyleistä tai nanohiukkasista pyritään kokoamaan nanokomponentteja liittämällä niitä toisiinsa. "Alhaalta-ylös" menetelmien kohdalla kaksi hyvin yleistä ja tässäkin väitöskirjassa käytettyä materiaalia ovat deoksiribonukleiinihappo eli DNA ja kultananohiukkaset, koska näitä materiaaleja voidaan helposti yhdistää toisiinsa menettämättä kummankaan materiaalin haluttuja optisia, kemiallisia tai sähköisiä ominaisuuksia.

Väitöskirjassani tutkittiin kolmen eri valmistusmenetelmän soveltuvuutta nanorakenteiden tai -komponenttia valmistuksessa. DNA-avusteisessa litografiamenetelmässä tarkoituksena oli tuottaa safiiri- tai piinitridi-pintoja, jotka olivat kauttaaltaan päällystetty halutulla metallisella nanorakenteella kuten rusetin muotoisella kultananorakenteella. Rusetin muotoiset rakenteet valittiin tutkimukseen, koska ne ovat herättäneet kiinnostusta johtuen valon niiden ympärilleen virittämistä vahvoista sähkökentistä, joita voidaan hyödyntää esimerkiksi pintavahvistetussa Raman spektroskopiassa. Kuitenkin aallonpituus, jolla valo vahvimmin virittää sähkökenttiä, riippuu hyvin vahvasti nanorakenteen koosta. Johtuen nykyisten valmistusmenetelmien rajoituksista tämä aallonpituus on yleensä infrapuna-alueella. DNA-avusteisen litografia menetelmän avulla onnistuttiin pienentämään rusetin muotoisten rakenteiden kokoa niin, että muodostuneiden rakenteiden optiset ominaisuudet olivat näkyvällä aallonpituusalueella.

Toisessa käytetyssä menetelmässä tutkittiin itsejärjestäytyvien DNA-nanorakenteiden käyttöä sähköisenä piirilevynä ja erityisesti siihen liitettyjen kultananohiukkasten kykyä muodostaa yhden elektronin transistori. Kultananohiukkaset aseteltiin suoraan ketjuun DNA:sta valmistetun ns. TX-tiili-nanorakenteiden päälle käyttäen kemiallisia menetelmiä ja syntyneet rakenteet vangittiin nanoelektrodien väliin sähköisiä mittauksia varten käyttäen dielektroforeesia. Vangitut rakenteet johtivat sähköä hyvin heikosti johtuen liian isoista ilmaraoista kultananohiukkasten väleillä. Ilmarakoja pienennettiin kasvattamalla kultananohiukkasia suuremmiksi kemiallisella kullan pelkistysreaktiolla. Kasvatuksen jälkeen virta-jännite- sekä differentiaalisen konduktanssin mittauksissa havaittiin Coulombin saarto, joka on tärkein vaatimus sille, että rakenne voisi toimia yhden elektronin transistorina.

Viimeisessä tutkimuksessa kultananohiukkasta ja yksijuosteista, biotiinin sisältävää, itseensä pariutuvaa DNA-molekyyliä käytettiin muodostamaan sähkökentällä ohjattava nanoanturi, jonka avulla tutkittiin tämän DNA-molekyylin hybridisaatiota itsensä kanssa ja kaksoiskierteen avautumista. Kultahiukkaset päällystettiin DNA-molekyyleillä, jolloin hiukkaset saatiin liitettyä kultapinnalle kiinnitettyihin kimeerisiin avidiineihin. Kultapintaan yhdistetyllä jännitteellä pystyttiin säätelemään varautuneen kultananohiukkasen etäisyyttä kultapinnasta samalla, kun sen paikkaa havainnointiin pimeäkenttäspektroskopian avulla. Kultananohiukkasen liike kultapinnasta poispäin aiheutti niiden välissä olevan DNA-molekyylin venymisen ja työssä havaittiin, että tietyillä sähkökentän voimakkuuksilla DNAmolekyylin kaksoiskierre saatiin avattua.

Avainsanat DNA, itsejärjestäytyvyys, origami, TX-tiili rakenne, hybridisaatio, kultananohiukkanen, funktionalisointi, pintaplasmonit, kimeerinen avidiini, biotiini, sähkökenttä ohjaus, nanoanturi, pimeäkenttä spektroskopia, yhden elektronin transistori, Coulombin saarto, differentiaalinen konduktanssi.

List of Publications

The main results of this thesis have been reported in the following articles:

- **Pub.I** K. TAPIO, J. LEPPINIEMI, B. SHEN, V.P. HYTÖNEN, W. FRITZSCHE AND J.J.TOPPARI, *Toward Single Electron Nanoelectronics Using Self-Assembled DNA Structure*. Nano Lett.**16** (11) (2016) 6780–6786.
- **Pub.II** K. TAPIO AND J.J. TOPPARI, *Characterization of emergence of the Coulomb blockade in a pearl-like DNA-AuNP assembly.* SAME Journal.5 (3) (2017) 31–44.
- **Pub.III** B. SHEN, V. LINKO, K. TAPIO, M.A. KOSTIAINEN AND J.J.TOPPARI, *Custom-shaped metal nanostructures based on DNA origami silhouettes*. Nanoscale 7 (26) (2015) 11267-11272.
- **Pub.IV** B. SHEN, V. LINKO, K. TAPIO, S. PIKKER, T. LEMMA, A. GOPINATH, K.V. GOTHELF, M.A. KOSTIAINEN AND J.J.TOPPARI, *Plasmonic nanostructures through DNA-assisted lithography*. Accepted for publication.
- **Pub.V** K. TAPIO, S. DONGKAI, S. AUER, J.-P. TUPPURAINEN, M. AHLSKOG, V.P. HYTÖNEN AND J.J.TOPPARI, DNA-nanoparticle actuator enabling optical monitoring of nanoscale movements induced by electric field. Submitted for publication.

Author's contribution

In the articles Pub.I and Pub.II, the author wrote most of the publication, fabricated all of the samples, carried out most of the measurements and did almost all of the data analysis.

In the articles Pub.III and Pub.IV, the author was mainly involved in the optical measurements and data analysis and had a major contribution in the writing of the publication.

In the article Pub.V, the author was responsible for the designing of the experimental setup, performed almost all of the measurements and the data analysis and wrote most of the publication.

Other works that the author contributed:

- **B.I** V. LINKO, B. SHEN, K. TAPIO, J.J.TOPPARI, M.A. KOSTIAINEN AND S. TUUKKANEN, *One-step large-scale deposition of salt-free DNA origami nanostructures*. Sci.Rep. 5 (2015) 15634.
- B.II V. HYNNINEN, L. VUORI, M. HANNULA, K. TAPIO, K. LAHTONEN, T. ISONIEMI, E. LEHTONEN, M. HIRSIMÄKI, J.J. TOPPARI, M. VALDEN AND V.P. HYTÖNEN, Improved antifouling properties and selective bio-functionalization of stainless steel by employing heterobifunctional silane-polyethylene glycol overlayers and avidin-biotin technology. Sci.Rep. 6 (2016) 29324.
- B.III B. SHEN, ,K. TAPIO, V. LINKO, M.A. KOSTIAINEN AND J.J.TOPPARI, *Metallic Nanostructures Based on DNA Nanoshapes*. Nanomaterials **6** (8) (2016) 146.
- **B.IV** V. LINKO, S. NUMMELIN, L. AARNOS, K. TAPIO, J.J. TOPPARI AND M.A. KOSTIAINEN, *DNA-Based Enzyme Reactors and Systems*. Nanomaterials **6** (2016) 139.
- B.V J. WANG, K. TAPIO, A. HABERT, S. SORGUES, C. COLBEAU-JUSTIN, B. RATIER, M. SCARISOREANU, J.J. TOPPARI, N. HERLIN-BOIME AND J. BOUCLÉ, Influence of Nitrogen Doping on Device Operation for TiO2-Based Solid-State Dye-Sensitized Solar Cells: Photo-Physics from Materials to Devices. Nanomaterials 6 (3) (2016) 35.
- **B.VI** L. NUUTTILA, K. TAPIO, T. LEMMA, J. A. IHALAINEN, N.V. TKACHENKO, J.J. TOPPARI AND H. LEHTIVUORI, *Enhancing fluorescence of monomeric bacteriophytochromes by plasmonic nanoparticles*. Submitted for publication.

Contents

Pr	Preface				
A	ostrac	t	3		
Ti	iviste	lmä (abstract in Finnish)	7		
Li	st of l	Publications	9		
1	Intro 1.1 1.2 1.3 1.4	oduction Deoxyribonucleic acid Surface plasmons: From inception to modern day Colloidal gold particles The content and the aims of the thesis	9 9 10 11		
2	Back 2.1	kground and theory DNA structure 2.1.1 DNA self-assembled nanoconstructs 2.1.2 DNA self-assembled nanostructures in nanopatterning 2.1.3 DNA based assemblies as actuators and sensors Synthesis and applications of colloidal gold nanoparticles	13 13 15 18 21 23		
		 2.2.1 Chemical synthesis and functionalization of colloidal nanoparticles 2.2.2 Chemical growth of metallic nanostructures 	23 26		
	2.3	 Plasmonic properties of metallic nanostructures and nanoparticles 2.3.1 Propagating surface plasmon polaritons 2.3.2 Surface plasmon resonance and Kretschmann Configuration 2.3.3 Non-propagating surface plasmons 2.3.4 Quasistatic approximation and spherical metal nanoparticles 2.3.5 Optical characterization methods 	26 27 31 34 35 45		
	2.42.5	 Single electron effects	49 50 52 55 56		
3	Plas 3.1	monic metasurface and probe studies Gold nanostructures by DNA Assisted Lithography 3.1.1 Fabrication of planar, arbitrary shaped metal nanostructures	59 59		
		using DALI method	60		

	~ ~	3.1.3 Optical characterization of different planar gold structures	65
	3.2	Electric field guided DNA and AuNP based nanoactuator	68
		3.2.1 Assembly and operation of the DNA-AuNP actuator	68
		3.2.2 Electric field manipulation	74
4	DN	A and AuNP based single electron transistor	81
	4.1	Fabrication of a linear, pearl-like three nanoparticle chain on DNA	
		template	81
	4.2	Dielectrophoretic trapping of the DNA-AuNP assembly	85
	4.3	Electrical characterization of the DNA-AuNP chain assembly	86
		4.3.1 Measurements setup	86
		4.3.2 I-V-characterization of the DNA-AuNP assembly	87
		4.3.3 Differential conductance characterization	91
5	Con	clusions and outlook	93
Aŗ	ppen	dixes	117

Chapter 1

Introduction

1.1 Deoxyribonucleic acid

Deoxyribonucleic acid or DNA is and has been an essential part of human evolution. Intricate in design and prone for mutations, the genetic coding of DNA contains the key to life itself, which is the reason why this molecule has prompted a lot of interest over more than two centuries [1, 2]. Although necessary for the evolution of life, its existence remained mystery until 1860s, when the first hints of the DNA were discovered by Friedrich Miescher [1]. The research on the nature and structure of DNA continued until 1953, when Watson and Crick proposed that the DNA forms a three dimensional double helix structure [3]. This realization was due to several related studies conducted in the same period. First, it was suggested by Phoebus Levene [4] that the DNA is composed of phosphate-backbone, sugar and bases, and later Chargaff [5] continued on this by dividing the bases in the two complementary pairs: adenine-thymine and cytosine-guanine. It was also proposed, that these pairs do not crosslink in neutral pH conditions. Finally, the X-ray crystallography studies of DNA by Franklin and Wilkins [6] allowed Watson and Crick to introduce their double helix model, where bases of individual DNA strand would bind to another strand, which had the complementary base sequence. Together these findings laid foundation for what has become the DNA nanotechnology.

For few decades after the work by Watson and Crick, the DNA related research concentrated on, e.g., discovery of the human genome and trying to understand the nature of DNA coding. Also during this period, oligonucleotide synthesis, DNA sequencing and thermal cycling based on polymerase chain reaction (PCR) started to emerge, where the base sequences of arbitrary single DNA strands could be precisely determined and produced [7–9]. This revolutionized many fields of industry by enabling, e.g., tailoring of plants to be resistant against insects or to enhance production of certain proteins in food industry [10], cloning of plasmid pBR322 for cheap mass-production of human insulin in medical industry [11] and the DNA fingerprinting method for forensic science familiar from TV series and movies [12]. At the same time, a smaller research area started to evolve, where synthetic DNA strands could be used to form desired nanostructures. These studies started by Nadrian Seeman [13] have over the year bloomed into a wide research field known as the structural DNA nanotechnology.

1.2 Surface plasmons: From inception to modern day

In contrast to DNA, the research on Surface Plasmons (SP) has been more turbulent and the studies of SP can be roughly divided into two parts: the surface plasmon polariton (SPP) and the localized surface plasmons (LSP). Although fairly similar in concept, distinctions between the two can be drawn from the historical perspectives and the nature of the SP. The LSP based applications such as colloidal nanoparticles have been mainly produced using chemical methods, where the unique and peculiar LSP properties of metallic (gold) nanoparticles has been utilized since the times of the Roman empire [14], while the field of SPPs typically involves modern physics in sample fabrication and utilization and has been mainly developed during the same time as the DNA nanotechnology.

The starting point, when the whole field of study of the SPP started to mature, can be pinpointed to the end of the 19th century and the beginning 20th century. The work done by Zenneck and Sommerfeld [15] introduced key concept of electromagnetic plane waves propagating along an interface of two media in 1900s. Later in 1950s and 1960s Pines and Bohm [16], Ritchie [17] and Stern and Ferrell [18] furthered the understanding of SPPs. It was at this point that the SPP was described as an excited collective electron wave propagating on the interface of the metal and dielectric interface. During 1960s and the following decade, these concepts were studied extensively by, e.g., Kretschmann [19], Otto [20] and Fleischmann *et al.* [21] with the development of techniques such as Surface enhanced Raman Spectroscopy (SERS) and total internal reflection based Surface Plasmon Resonance (SPR).

At the same time on LSP side, the work on the theory of the absorption and the scattering of spherical, metallic nanoparticles by Mie [22], development of synthesis methods to produce colloidal particles by Bredig [23] and later by Turkevich [24] and studies of stability under different ionic conditions by Smoluchowski [23] fueled the interest on the LSP properties of the metallic nanoparticles. These findings have led into applications in the fields of, e.g., fluorescence enhancement [25, 26], catalysis [27, 28] and non-invasive bioimaging [29, 30]. More recently, the combination of nanoparticle probes/actuators and functional surface [31, 32] or metasurfaces [33] have attracted interest of researchers due to possible applications in super-resolution imaging or molecular logic gates [34–37]. Fabrication and utilization of such surfaces is also one of the topics investigated in this thesis.

1.3 Colloidal gold particles

Third subject of this thesis is the incorporation of DNA and colloidal gold nanoparticles (AuNP) into nanoelectronics. Starting from the work on semiconductor transistors by Bardeen, Brattain and Shockley in 1940s [38], one of the aims in 20th and 21st century is the development of evermore sophisticated electronic devices. The fundamental requirement for this is the increasing miniaturization of the electronic components, since the computational power is directly proportional to the number of component or, in other words, the density of the components. From 1940s and continuing until the present day, this miniaturization has required several innovations to shrink the transistors in the integrated circuits (IC) well below 1 µm range, typically using conventional lithography methods. However, when scaling close to sub-10-nanometer scale, these conventional top-down methods have become increasingly complex and costly, although resolution of 7 nm has been demonstrated in literature [39]. For these reasons, there has been growing interest for alternative bottom-up methods to overcome these limitations.

Recently, one of the more interesting electronic components or devices has been a single electron transistor (SET) due to the simple design of the structure and confined size, which could further drive the miniaturization of the transistors in the ICs. The main drawback of the SET is the physical size dependent operation temperature, which has mostly meant that UV-lithography fabricated SETs have been operating only at cryogenic temperatures. Here, the bottom-up techniques can offer simpler and straight forward way to produce SETs with the desired dimensions. In the past, techniques such as phase-shift mask UV-lithography [40], deposition of cadmium selenide nanocrystals [41] and molecular deposition at cryogenic temperatures [42] have been used to fabricate SETs working at room temperature. However, the implementation of these techniques to the semiconductor industry remains challenging due to, e.g., post-purification of unwanted debris or the highly sophisticated (complex) nature of the fabrication process. Meanwhile, gold nanoparticles have also peaked interest in the nanoelectronic application, since their size can be scaled down to few nanometers and they offer fairly simple fabrication tool due to readily accessible functionalization schemes.

1.4 The content and the aims of the thesis

The premise of this thesis, as it is written in the title, is to tackle the aforementioned challenges of the fabrication and utilization of metasurfaces, nanoscale actuators and nanoelectronics components by use of two different materials: gold and DNA. This choice is due to several reasons. First, gold is an unique material for the synthesis, since it does not form native oxide unlike silver and copper, plasmonic properties of gold are in the visible region, which makes many applications easier to real-

ize, and it has easily exploitable chemistry to attach certain chemical groups [43–45]. Secondly, DNA has the robust and well-defined self-assembled properties and can be easily functionalized with desired chemical groups, which allows attachment to gold nanoparticles. The aim of the thesis is to further advance the development of DNA and gold based nanostructures and their applications.

The chapter 2 of the thesis introduces the theoretical background of the DNA nanotechnology, colloidal particles, single electron nanoelectronics and surface plasmons. Also, the SP related imaging techniques are presented in the context of the experimental studies of the thesis. The chapter 3 bundles together the fabrication and the optical characterization of gold nanoparticle-DNA based nanoactuator and the DNA assisted lithography made gold nanostructures. Different fabrication steps of the nanoactuator assembly are presented and analyzed and binding affinity between the immobilized actuator and the surface is optimized. Then the nanoactuator is used to study the folding properties of single stranded DNAs and a looped hairpin-DNA. In addition, the new DNA assisted lithography method is employed to fabricate metasurface covered with arbitrary shaped metallic structures like bowtie antennas and cross structures. It is also demonstrated that the maximum enhancement wavelength of these antennas is in the visible region, which has been difficult realize with other approaches. The chapter 4 involves fabrication of linear, pearl-like assembly of gold nanoparticles using a DNA template. To demonstrate that this assembly functions as a single electron transistor, it was first trapped between fingertip electrodes using dielectrophoresis. Next, the differential conductance and currentvoltage characteristics were measured, where the Coulomb blockade behavior, that is prerequisite for the SET, was observed even at room temperature. Finally, the conclusions and future prospects for the different approaches are discussed briefly.

Chapter 2

Background and theory

This chapter is divided roughly into five parts. First part is devoted to describe the structure of DNA, different fabrication and nanopatterning schemes of DNA nanoassemblies and how DNA and gold nanoparticle based nanoassemblies can be utilized as bioactuators and biosensors. Next, the synthesis, chemical properties and chemical growth of gold nanoparticles are explained in details. After this, the plasmonic properties of gold nanoparticle assemblies and gold nanoshapes are discussed in the context of the Mie theory and the Quasistatic approximation. The final two sections present the theoretical backgrounds for single electron effects and also introduce dielectrophoresis, that is used to isolate nanoobjects for electrical studies.

2.1 DNA structure

In nature, the DNA forms double helix structures as shown in figure 2.1, which consist of two single stranded DNAs (ssDNAs) woven together via hydrogen bonds. The ssDNA itself consists of 2'-deoxyribonucleotide monomer units or nucleotides in short, that are composed of three different subunits [3,46]: aromatic nitrogenous base, phosphate group and 2'-deoxyribose (monosaccharide or sugar). The 5' end of the phosphate group of one nucleotide can be linked to the 3' end of the sugar subunit of another nucleotide via phosphodiester (PO_4^{-3}) bonds thus creating chain of nucleotides or oligonucleotides with negatively charged sugar-phosphate backbone. There are four distinct bases as shown in figure 2.1a: adenine, thymine, cytosine and guanine. These bases form complementary pairs, and in neutral pH conditions this pairing will follow the Watson-Crick base pairing [3]: adenine pairs with thymine and cytosine with guanine.

When two ssDNAs with complementary base sequences are mixed together, they will form the double helix structure or double stranded DNA (dsDNA) (figure 2.1a). Here, the complementary bases of the two strands are linked together via hydrogen bonds and along the strands the nucleobases interact via base-stacking interactions (van der Waals and dipole-dipole), which will stabilize the whole chain.



FIGURE 2.1: (a) Illustration of the DNA double helix. Aromatic bases are connected to the deoxyriboses (sugar) from one side and the phosphate groups from the other side of the sugar. Due to the specific hydrogen bonding in neutral pH conditions, the adenine base will only bind to thymine base and similar pairing happens with cytosine and guanine. The nucleotides are connected together by formation of phosphodiester bonds between the 5' and 3' ends of the phosphate groups.(b)-(d) Structural view of B-DNA, A-DNA and Z-DNA, respectively. Figure b-d created using UCSF Chimera and Nucleic Acid Builder [47,48].

For these reasons, the more the strands have mismatches in pairing the less stable the resulting dsNDA is. It should be noted that any two arbitrary DNA strands will try to form double helix on their longest complementary sequence parts, which is also utilized in formation of DNA nanostructures.

The helix structure itself can have several different forms depending on the conditions of the surrounding medium (see figure 2.1b-d) [3, 6, 49–51]: B-form (B-DNA), A-form (A-DNA) and Z-form (Z-DNA). B-DNA is the most stable form under physiological conditions. Its helix is right-handed with the diameter of 2 nm, 10.5 base pairs (bs) per helical turn and the helical rise is 0.34 nm per base pair. A-DNA has the same handed turning, but the diameter and the helical turn are slightly larger (2.6 nm and 11 bp per turn) and the helical rise is slightly smaller (0.26 nm per bp). In contrast to B- and A-DNA, Z-DNA is left-handed with the helical rise 0.38 nm, the helical turn 12 bp per turn and the diameter of 1.8 nm.

More exotic forms of DNA are G-quadruplex (G4-DNA) and triplex structures (H-DNA) [52, 53]. The G4-DNA structure is formed in guanine rich strands, where one, two or four strands form stacked, square planar arrays of four guanines via Hoogsteen hydrogen bonding [54]. In the presence of metal cation like K⁺ or Mg²⁺, these G4-DNA will self-assemble into stable, long wires called G-wires [55], which can be used as switches or nanowires [56, 57]. In the case of H-DNA, third DNA strand is attached to dsDNA either by T binding to T-A base pairs, or G or N-3 protonated C (C+) to C-G pairs. The N-3 protonation requires acidic pH conditions, and hence the triplex form is not common in nature. Still, H-DNA structures can be stabilized at physiological conditions by, e.g., polyvalent cations like spermine and spermidine or modification with phosphorothioate groups [58].

Overall, the structure of oligonucleotides with complementary pairing scheme is the basis for encoding the genetic information with DNA, since the base sequences contained within the nucleotide chains are unique for different physiological functions in nature. This is also exploited in the synthetically made DNA self-assembled nanostructures, where the base sequences of the used oligonucleotides are designed in such way, that when the mixture of the oligonucleotides is heated to elevated temperature and cooled down to room temperature, the desired structure will selfassemble. The DNA self-assembly into nanostructures or nanoshapes, the folding process and the temperature ramping has been discussed extensively in the literature [59–61], which is outside of the scope of this thesis. Instead, the resulting DNA self-assembled structure will be discussed in the following sections.

2.1.1 DNA self-assembled nanoconstructs

The dawn of synthetic DNA nanostructures can be attributed to fabrication of the branched DNA junctions by Nadrian Seeman [13, 62] as illustrated in figures 2.2a and 2.2b. The revolution was to utilize so-called "sticky-ends", which are unpaired ssDNA overhangs protruding from the structure, and that can hybridize with complementary, targeted "sticky-end"-strands. These "sticky-ends" established building-block approach, where overhangs from one branched junction or block would attach to several other blocks thus forming a 2D network as shown in figures 2.2b and 2.3a. However, the branched junctions are not very stiff, which is crucial in many applications. This led into development of crossover junctions, where large number of short ssDNAs are partially hybridized with each other to form crossover junctions between two or more dsDNAs. The resulting 2D DNA-structures have been formed from, e.g., double crossover tiles (DX-tile) [63], triple crossover tiles (TX-tile, see figure 2.3b) [64] and tensegrity triangles [65]. These crossover based til structures have been used as building blocks to create grids [66,67], ribbons [66], triangles [65], nanotubes [68] and even 3D lattice structures [69].



FIGURE 2.2: (a) Structural view of the Holliday junction. (b) The schematic view of the Holliday junction hybridization scheme.

However, this partial hybridization scheme of short ssDNAs is inherently unstable due to sensitivity on the stoichiometrical ratio between the strands: missing



FIGURE 2.3: Building block approach to fabricate DNA self-assembled structures. (a) Sketch of tensegrity array and an AFM image of the array on a mica surface. The scale bar is 100 nm.(b) Left: Sketch of TX-tiles A, B, C, C'and D and different array configurations. Right: AFM image of ABC'D TX-tile array. Adapted with permission from [65,67]. Copyright 2000 and 2006 American Chemical Society.

even one strand will destabilize the structure, and achieving even decent yields for complex structures requires multiple reaction steps followed by laborious purification. Also, limiting the size of the overall structures in building block approach sets extra self-terminating constrains into the design of the blocks, which is not necessary desirable. These underlying problems led into development of the DNA origami approach [70], where, instead of using multiple short oligonucleotides, one long, 7.429 kb viral scaffold strand (M13mp18) is folded into desired, arbitrary shape using shorter staple strands in an one-pot experiment with relatively high yields (see figure 2.4). To emphasize popularity of DNA origami, several softwares have been developed for designing origamis, even with twist corrections [71, 72]. One major advantage of DNA origami over the building block approach is that all the staple strands can be easily extended to incorporate "sticky-ends" to anchor different nanomaterials, which can be utilized for non-periodic patterning with 4-6 nm resolution [70,73]. The DNA origami design was later extended to 3D structures, e.g., boxes or containers [74,75], bars [72], bridges [72] and curved 3D structures [76] by introduction of the familiar crossovers or using continuous DNA origami sheets (see figures 2.4a and 2.4b).



FIGURE 2.4: DNA origami approach to fabricate DNA self-assembled structures. The scale bars are 100 nm in a and 20 nm in b.(a) On the left is a structural view of DNA origami, where single long scaffold strand is folded into 2D shape using shorter stable strands (colored lines). Different 2D DNA origami based nanoshapes are presented in the right panel, where the upper row shows the schematic view and the mid and the lower rows show atomic force microscope images of the folded structures. (b) On the left is schematic view of gear structure and on the right are transmission electron microscope images of the same gear structure. Figure (a) adapted with the permission from [70]. Copyright Nature Publishing group, 2006. Figure (b) adapted with the permission from [76]. Reprinted with permission from AAAS.

Besides the DNA origami and building block approaches, polygonal DNA meshes can be used to form DNA nanostructures [77]. Similarly as in the origami approach, one long scaffold strand is folded into the desired shape using smaller staple strands. However, the scaffold strand length and sequence are determined by first dividing the structure into triangular meshes, where each side of the triangle consists of one DNA double helix, and then solving the composition of the strands using computational methods with following constrains: the used scaffold strand should not cross itself and the vertex junction should always be planar. Figure 2.5 shows two different 3D shapes folded using the polygonal meshing method: a bottle and a helix with pentagonal cross-sections. Here, the advantage compared to the DNA origami is the higher complexity, thermal and low-salt stability of the folded structures, but functionalization and use as a breadboard can be more challenging due to higher flexibility. In the context of this thesis, we are considering the planar 2D TX-tile and DNA origami based platforms for either nanoparticle patterning or creating metallic shapes.

For any electrical applications, it is important to know the nature of DNA conductivity, which has been a hot debate for more than a decade. Plethora of studies has been published on the matter, results ranging from insulating, to semiconducting and all the way to ohmic or even superconducting behaviour [78–81]. Also, the conductivity has been found to be strongest along the helical axis and heavily dependent on the connections between the DNA and the external contact electrodes.



FIGURE 2.5: DNA meshing approaches to fabricate a DNA self-assembled structures. The scale bars are 50 nm.(a),(b) Left: structural view of helix and bottle shape. Right: SEM images of the corresponding folded structures. Figures adapted with the permission from [77]. Copyright Nature Publishing Group, 2015.

Although the exact nature of the DNA conductivity has not been fully clarified, several theories [82–84] exist to explain the variations in the experiments. These include tunneling through the DNA double helix, charge hopping between discrete bases and charge hopping between base domains. The discrete base hopping model involves charge hopping between guanine bases and tunneling thought any thymineadenine base pairs. The domain model has similar premise but guanine bases are now extended to several bases, where electron distribution throughout the domain is delocalized due to matching π -orbital stacking, and charges tunnel from one domain to another. It is evident that the hopping mechanisms depend on the sequence and the conformation of the DNA and can extend to long distances, but the tunneling can be considered relevant only in short distances. In our case, the TX-tile structure presented and used in this thesis has been proven to be very poor conductors in dry or ambient conditions [85], so we will exclude the intrinsic conductivity of the DNA from any electrostatic descriptions.

2.1.2 DNA self-assembled nanostructures in nanopatterning

As already discussed, plain DNA structures can be considered as poor conductors in dry state due to loss of the stacked alignment of the basepairs [78, 79, 84]. Although dry DNA structures are in a collapsed state, they will conserve the overall shape and retain any existing attachments to the nanocompounds, which makes it ideal for nanoscale patterning. The patterning schemes usually involve protruding ssDNAs as anchoring points, since ssDNA can be readily modified to incorporate



FIGURE 2.6: DNA self-assembled nanostructures as templates. The scale bars are 200 nm in j and l, 100 nm in h-p and 20 nm in c.(a),(b) Left: Sketch of biotin modified DNA grids, where every second or all junctions can used to immobilize streptavidins (STV). Right: AFM images of the grids with immobilized STVs.(c) STVs immobilized on biotin modified origami to display the coat of arms of Ukraine.(d) Sketch of binding of biotin-ssDNA functionalized CNTs to SVT modified origamis.(e),(f) AFM images of CNT aligned on DNA origamis.(g),(h) Illustration of X shape by DNA origami structure and transmission electron microscope image of the same shape after gold cluster coating.(i) SEM and (j) AFM images of letter H patterned on rectangular DNA origamis by biotin-ssDNA coated AuNPs. (k) and (l) shows similar pattern for letter O as in figure i and j. Figures (m)-(p) show how the initial four AuNP pattern can be extruded larger using chemical gold growth methods. Figures (a),(b) and (i)-(p) adapted with the permission from [86,87]. Copyright 2005 and 2011 American Chemical Society. Figure (c) adapted with the permission from [88]. Copyright IOP Publishing, 2009. Figures (d)-(h) adapted with the permission from [89,90]. Copyright John Wiley and Sons, 2011.

desired chemical groups like biotin and thiol (SH) or nanocompounds with complementary ssDNAs can be connected to them. In the latter case, the phosphate backbone of the hybridized double helix between nanocompound and DNA template is partially disconnected, which can be problematic in some cases. However, the backbone can be post-treated using ligase to covalently bond together the 3' end of the nanocompound side and 5' end of the DNA template.

The DNA patterning has included, e.g., biotin modified grid structure or origami to attach streptavidins [86,88] (see figures 2.6a-c), "sticky-end" attachment of DNA coated carbon nanotubes to DNA origami template [91], attachment of biotinylated ssDNA coated CNTs to biotin modified origami template using streptavidins as linkers [89] (see figures 2.6d-f), direct immobilization of amine coated gold clusters to DNA origami using Coulombic attraction [90] (see figures 2.6g and 2.6h) and attachment of ssDNA coated metallic NPs via "sticky-ends" hybridization to origami template [87,92–94] (see figures 2.6i-p). Especially the hybridization scheme involving gold nanoparticles and DNA has been widely used in this field, and it is also utilized in this thesis. Establishing firm binding requires several (over 10) ssDNA anchoring points for one AuNP, although even one anchoring point has been utilized. During the hybridization, the DNA platforms and the ssDNA coated AuNPs are mixed in either 1:1 ratio or using overabundant amount of AuNPs in solution. Then the mixture is either incubated at room temperature for several hours or a temperature ramp is utilized to first dissociate the undesired DNA bindings and then hybridize the complementary DNA bindings. In general, the AuNP-DNA platform attachment scheme has a higher yield (77-98%) [92-94], when there are multiple anchoring points and an overabundant amount of AuNPs. Dropping the ratio to 1:1 or using less anchoring points results in a lower yield (\sim 45%) [95]. Also, the room temperature incubation is sufficient if the AuNPs are anchored using multiple "sticky-ends".

After attachment or immobilization, these DNA-AuNP assemblies can be posttreated by growing the AuNP larger using chemical methods as shown in figures 2.6m-p, which will be described in the later sections. This tactic has also been successfully incorporated to DNA mold casting technique to produce different metallic nanoshapes [96,97]. It should be pointed out here, that in the aforementioned techniques the DNA structure itself does not metallize or cause growth. On the other hand, the direct metallization of ssDNAs, dsDNA and DNA self-assembled structures can be achieved using different chemical reactions and has been studied quite extensively with different metal, e.g., silver [98], palladium [99], nickel [100],copper [101] and platinum [102]. However, the results have mostly been grainy structures, which is not desirable in the fabrication of electronic or plasmonic components.

2.1.3 DNA based assemblies as actuators and sensors

One of the earliest applications of nanotechnology are the biological sensors, which have their roots in the medical and the food industry [103] to detect, e.g., pathogens and antibodies. Traditional sensors such as ELISA immunosensors have been utilized since 1960s, but due to the poor sensitivity and detection limit of these sensors researchers have sought new methods and materials to overcome the limitations. In a related field, micro- and nanoactuators have attracted attention over the years as promising and novel techniques for, e.g., motors [32], computing [104, 105] and nanoreactors [106]. These actuators have also been incorporated into sensing, where a typical sensor consists of a probe immobilized to a sensor substrate. The immobilization is typically via either a targeted molecule or a compound used to recognize the target material. This probe can be also manipulated using, e.g., magnetic [107] and electric fields [108, 109], solution flow [110, 111], optical tweaking [112] and molecular, protein or ionic interactions [113, 114]. Here, DNA and gold nanoparticles have been heavily utilized in both actuator and sensor applications. Since one of the aims of this thesis is to fabricate a new DNA and gold nanoparticle based actuators and sensors, it is suitable to review some of the DNA and gold nanoparticle related advancement in this field.

One of the more extensively studied schemes is the DNA origami based actuators. For example, Ke *et. al* [114] designed four-arm, rhombus shaped DNA origami structure shown in figure 2.7a, where the distance between the two capture strands (green lines protruding from the scaffold) could be adjusted by changing the length of the strut (blue and black lines) via hybridization of ssDNAs with varying lengths. By binding two inactive fragments of enhanced green fluorescent protein (eGFP) to the capture strands, it was demonstrated that this structure could be utilized as a switch, since the eGFP fragments exhibit fluorescence only when they are brought close enough to each other. Individual DNA strands can also be moved, stretched and even cleaved using electric fields. Gao *et al.* [116] demonstrated that surface immobilized dsDNAs can be selectively removed from the surface by Coulombic interactions between electric field and negatively charged dsDNAs. The selectivity was achieved by tuning the length of the dsDNA, where the shorter human genomic DNA was removed before the longer λ -DNA.

Figures 2.7b and 2.7c show two examples of AuNP based actuator or sensor system. Block *et al.* [111] demonstrated that the solution flow can be used to move surface immobilized gold nanoparticles with the diameter up to 105 nm. Here, nanoparticle tracking analysis (NTA) [117] was applied to solve the hydrodynamic radius *R* of the particles. In a more static approach, Pilo-Pais *et al.* [115] used gold nanoparticle and DNA origami assembly to enhance SERS signal of 4-aminobenzene-thiol (4-ABT). Here, a rectangular DNA origami was used as a breadboard to attach four gold nanoparticles (tetramer) or one particle (monomer) into the corners of the origami. These particles were then enlarged using silver metallization kit to pro-



FIGURE 2.7: Different DNA based nanoactuator and sensor systems.(a) The upper row shows schematic images of four-arm, rhombus shaped DNA origami actuator, where the distance between the two capture strands (green lines) can be adjusted by hybridizing the strut with different ssDNAs(blue lines). The lower row shows TEM images of the four-arm actuator corresponding to the different hybridization situations. The scale bars are 20 nm. (b) A schematic view of AuNP based actuator system, where streptavidin functionalized AuNPs are bound to biotin coated glass surface. The particle is moved using fluid flow and the particle position is tracked by surfaceenhanced ellipsometric contrast imaging. The trajectory data of the AuNP can be fitted to the Einstein-Smoluchowski relation to solve the hydrodynamic shear force F_s , which can be then related to the hydrodynamic radius of the particle R. (c) The SERS spectra of 4-ABT molecule detected using four nanoparticle assembly shown in the insets. Figures (a) and (b) adapted with the permission from [111,114]. Copyright Nature Publishing Group, 2015 and 2016. Figure (c) adapted with the permission from [115]. Copyright 2014 American Chemical Society.

duce hot spots between the particles and thiolated 4-ABT was covalently linked to the surfaces of the particles. The SERS signal of the immobilized 4-ABT was measured from ensembles of monomers and tetramers, where the characteristic SERS signal was only fully detected in the case of tetramers and the signal enhancement was at least factor of 100 larger compared to the monomers.

In this thesis, two different approaches are studied for biosensor or bioactuator fabrication. One approach utilizes a sandwich-type sensor system, where the analyzed DNA strand is placed between the probe AuNP and the gold sensor surface, and the AuNP position is controlled or moved reversibly using electric fields to change the conformation of the linker DNA strands. In the other approach, we will use a new DNA Assisted Lithography (DALI) method to fabricate desired gold nanostructures with unique and strong plasmonic properties, that can be used for detecting different molecules in SERS. Certain aspects of both of these approaches have been already realized as shown in figures 2.7b and 2.7c, but here the goal is to broaden the applicability of these methods, e.g., to extend the operation to the visible spectrum by the DALI method and to study *in situ* conformational changes of individual proteins and enzymes.

2.2 Synthesis and applications of colloidal gold nanoparticles

In practice, the fabrication process of the studied nanoparticles and structures is often as important as the intricate characterization methods used to study their properties. Even the most novel features and effects can be easily lost due to insufficient resolution and heterogeneity of the sample, which emphasizes the proper choice of the fabrication methods. The studies in this thesis cover two different routes to produce nanoassemblies: (i) purely chemical synthesis of colloidal metal nanoparticles, their functionalization with single stranded DNAs, hybridization to DNA scaffolds and further chemical growth, and (ii) aforementioned chemical vapor deposition based DNA assisted lithography method. The former method has been utilized over two decades to produce spherical nanoparticles in a liquid medium and utilize them as building block to fabricate more complex structures [118], whereas the latter DALI method has been more recently developed by us to fabricate planar nanostructures on a substrate. In the following sections I will briefly introduce the chemical synthesis route, and the DALI method will be covered in chapter 3.

2.2.1 Chemical synthesis and functionalization of colloidal nanoparticles

The chemical synthesis of nanoparticles, their functionalization with different chemical groups and molecules as well as the already mentioned utilization in nanopatterning, sensing and actuation, has been studied since the 1950s. Common examples of the liquid-phase chemical methods to synthesize spherical gold nanoparticles involve reduction of chloroauric acid (HAuCl₄) by, e.g., heat [24,119], reducing agents [120–123], combination of heat and reducing agents [124] or UV-irradiation [125] to zerovalent gold atom Au^0 and subsequent collisions of the zerovalent atoms with each other to form larger clusters called nuclei seeds. This process will continue as long as there are excess metal ions and seeds, and to control the size of the final gold nanoparticles, it is vital to introduce stabilizing or capping agents into the synthesis process. The capping can be achieved via electrostatic or steric stabilization [126], where the former utilizes ionic double layers to induce ionic repulsion between the formed AuNPs thus preventing the aggregation process. In the latter the AuNPs are coated with organic molecules to prevent the agglomerations. By far the most commonly used stabilizing agent is trisodium citrate (TSC, $Na_3C_6H_5O_7$), which creates anionic surface charge on the AuNPs. In addition, the synthesis can utilize surfactants to produce directional growth [126], where, e.g., cubes [127], rods [128], hexagons [127, 129] and rectangles [127] has been realized.

The aforementioned functionalization of AuNPs with DNA strands protruding from them can be achieved by several methods: after AuNP synthesis, thiolated ssDNAs are chemisorbed to the surface of AuNPs using strong interaction between the thiol and gold atoms [120] via, e.g., salt aging process [130–134], surfactant aided process [135, 136] or using specialized pH-assisted method [137]. The protruding DNA strands can be then used to anchor the particle to other nanostructures or surfaces via either complementary DNA strand hybridization scheme or by receptor ligand interaction as described in the previous sections, where the ligand is chemically introduced to the free end of the DNA strand before functionalization with AuNPs.



FIGURE 2.8: Illustration of spherical gold nanoparticle synthesis and the DNA hybridization. The gold salt (HAuCl₄) is reduced to zerovalent gold atoms, which collide and grown into larger particles, until the process is stabilized using, e.g., surfactant. In the following DNA hybridization step, the thiolated ssDNA strands are chemisorbed slowly on the surface of the AuNP using, e.g., salt aging methods.

Figure 2.8 illustrates a general synthesis process to fabricate spherical gold nanoparticles [123] and the subsequent functionalization. The chloroauric acid is first reduced in the presence of TSC and reducing agent like sodium borohydride (NaBH₄) to Au¹⁺ ions. Due to excess amount of A¹⁺ ions, three A¹⁺ ions react to from one A³⁺ and two Au⁰. When the solution has supersaturated with the zerovalent A⁰, they collide with each other and aggregate creating small seed particles. As the seeds grow, they start developing more defined crystal structures with faces, where the stabilizing agents attach and thus the further growth and aggregation process is slowed down. Here, it is crucial to have a vigorous stirring for uniform size distribution of the resulting particles. Without any further stabilization agents, the size of the particles can be scaled from few nanometers to 20 nm, but, e.g., addition of hydroquinone [122] the size can be increased up to 300 nm. In general,

the concentration and the size of the particles are tuned by adjusting the amount of reducing agents and the chloroauric acids.

The quintessential functionalization process of AuNPs with thiolated DNA strands consist of chemisorption of the DNA molecules to the surface of the citrate capped AuNP followed by the straightening and reorientation of the molecule [130]. The negatively charged DNA is repulsed by the citrate capped AuNP, which can be overcome by adding sodium chloride in stepwise manner in presence of overabundant amount of thiolated ssDNAs [131,132] commonly known as the salt-aging process. The thiol-groups form strong bonds with the gold nanoparticle thus preventing the detachment of the DNA strands. With the salt-aging process, it is crucial to do the addition of sodium in stepwise manner, since too high amount of salt will aggregate AuNPs before the DNA is adsorbed to protect them. It is also crucial to tune the amount of sodium and the rate in which the sodium is added, if the diameter of particle is increased above 50 nm, to compensate the increasing instability [130,134]. For purely citrate capped AuNPs, this means that the functionalization of DNA strands requires from 1-2 days for small particles (<50 nm) to 4-5 days for larger particles (>50 nm) [138,139]. The resulting functionalized particles are more stable under elevated temperatures (80 °C) and salt concentrations [130–134].

Another functionalization process involves use of surfactants, e.g., nonionic fluorosurfactant Zonyl (FSN) [136] and sodium dodecylsulfate (SDS) [135] to stabilize the AuNP during the addition of the salts. For SDS, the thiolated DNA strands are mixed with AuNPs (diameter up to 250 nm) and the SDS concentration is adjusted to 0.01 M at room temperature. The solution is then incubated for 20 min and NaCl and PBS buffer is added to increase concentration of NaCl to 0.05 M. After this solution is sonicated for 10 s and incubated for 20 min at RT. This NaCl addition process, sonication and incubation is repeated until the concentration of NaCl is 1.0 M, where the concentration of NaCl is increased by steps of 0.05 M. The stabilization process is followed overnight and the excess DNA is removed by centrifugation.

For the FSN process, AuNPs are first incubated in FSN solution, then the resulting FSN-capped AuNPs are mixed with the thiolated ssDNAs, NaCl concentration is adjusted to 1.0 M and the solution is incubated for 2 h. The FSN-capping prevents also unspecific binding of the nucleobases of the DNA strands to the surface of the AuNPs during the adsorption process, since only the thiol-gold interaction is strong enough to substitute the FSN. The advantage of both surfactant approaches is that the process can be applied to particles with diameter from 13 nm to 100 nm.

The pH-assisted method was developed as a quick and easy method to functionalize nanoparticles. Whereas other methods can take several days to attach DNA strands to AuNPs, the pH-assisted method takes merely few minutes and can be used to exert better control over the DNA adsorption process [137]. Similarly as before, thiolated DNA strands are mixed with the citrate capped AuNPs and incubated for 1 min. Then the pH of the mixture is adjusted to 3 using citrate-HCl buffer (citrate end concentration 10-20 mM) and incubated for 3 min. The final product is stable even in 1 M NaCl buffer and pH can be adjusted after the functionalization to neutral. The fast reaction rate could be attributed to the change in the charge of adenine and cytosine and partial protonation of citrate and phosphate, which will reduce the repulsion between AuNPs and DNA strands.

Out of the three different functionalization approaches, the salt aging technique was employed in the thesis. Although other techniques are less time consuming and can offer better stability, the aging technique produces samples with only DNA on the surface of the AuNP and the use of surfactants might interfere with the studied folding properties or the conformation of the DNA molecule. The pH-assisted method was tested during the studies, but, contrary to the Zhang *et al.* observations, the author did not found the method stable enough to functionalize 80 nm gold particles used in the nanoactuator fabrication.

2.2.2 Chemical growth of metallic nanostructures

Metallic (gold) nanostructures can be extruded larger using chemical growth of gold, also known as gold-based Autometallography (AMG) [140]. AMG was originally developed for visualizing small colloidal nanoparticles (1-5 nm) in transmission electron microscopy (TEM) in 1980s using silver compounds. It was extended later to gold, where chloroauric acid or gold salt is reduced onto pre-existing gold seed particles or gold structures in presence of catalyst, e.g., hydroxylamine (NH₂OH) to grow them larger [141,142]. The growth continuous until most of the reagents are consumed and the growth rate depends on the initial size of the structures and the number of nucleation centers [141, 143]: diffusion of reagents to particle with larger surface area is more pronounced than for smaller particles. The rate can be thus tuned down by addition of extra nucleation centers or by diluting the concentration of reagents, e.g., gold salt or reducing agents, which both were employed in this thesis to decelerate the growth rate. The gold growth reaction can be stopped using sodium thiosulfate (1% in aqueous solution), but this has a risk of background staining. To avoid this, it is recommended to stop the reaction using less invasive distilled water washing [140].

2.3 Plasmonic properties of metallic nanostructures and nanoparticles

Metals are considered to be one of the quintessential nanomaterials for optical applications due to the unique plasmonic properties, which can be understood either as propagating or non-propagating excitation of electron oscillation on a metaldielectric interface (surface plasmons, SP). This concept was first introduced by Pines and Bohn in 1952 as quantized bulk plasma oscillations of electrons in a metal foils, although it should be noted, that nowadays the Pines and Bohn plasma oscillations are called bulk plasmons to separate them from the actual surface plasmons [16]. Here, we consider plasmonic nanoparticles and nanostructures in respect to classical electrodynamics and Maxwell's equations, since the complex quantum mechanical description can be wrapped up under the dielectric function or permittivity [144] with the assumption that the mean free path of electrons is smaller than any defining feature length in the studied objects [144]. This is a fact that should be kept in mind when considering SP systems and, in practice, the classical electrodynamics and bulk dielectric constants can be utilized to study particles and structures with dimensions ~ 10 nm or above [144]. The next two sections will cover both propagating and non-propagating (localized) surface plasmons and how they relate to the experimental work of the thesis.

2.3.1 Propagating surface plasmon polaritons

The existence and creation of surface plasmons can be worked out via the classical electrodynamics by solving the dispersion relation in figure 2.9a from the time- and space-dependent Maxwell's equations

$$\nabla \times \vec{E} + \frac{\partial \vec{B}}{\partial t} = 0, \qquad (2.1)$$

$$\nabla \times \vec{H} - \frac{\partial \vec{D}}{\partial t} = \vec{j}, \qquad (2.2)$$

$$\nabla \cdot \vec{D} = \rho, \tag{2.3}$$

$$\nabla \cdot \vec{B} = 0, \tag{2.4}$$

where \vec{E} is the electric field vector, \vec{B} is the magnetic induction, \vec{H} is the magnetic field vector, \vec{D} is the electric displacement field, ρ is the charge density, which in SP case is the charge density of the free electrons in a metal, and \vec{j} is the free electric current density in the material. The presence of matter introduces two more equations to describe the relationship between the vector fields as following.

$$\vec{D} = \epsilon_0 \epsilon_r \vec{E},\tag{2.5}$$

$$\vec{B} = \mu_0 \mu_r \vec{H},\tag{2.6}$$

where ϵ_0 and μ_0 are the permittivity and the permeability of the free space and ϵ_r and μ_r are the relative permittivity and permeability of the material.



FIGURE 2.9: (a) The dispersion relation of SPP (red curve). For SPP excitation, the light line (dashed and solid black lines) needs to intersect the SPP curve. (b) Schematic view of charge distribution and electric fields of SPP propagating to z-direction on a metal-dielectric interface.

The starting point of the derivation of the dispersion relation is to consider excited SPs in the absence of the excess charge and current densities ($\vec{j} = 0$, $\rho = 0$ and $\nabla \cdot \vec{D} = 0$). We can solve the differential equation for the electric field \vec{E} of the SPs by combining equations 2.1, 2.2, 2.3, 2.4, 2.5 and 2.6 and assuming that changes in the permittivity ϵ_r and permeability μ_r are negligible.

$$\nabla \times \vec{H} - \frac{\partial \vec{D}}{\partial t} = \vec{j} = 0,$$

$$\Rightarrow \nabla \times \left(\frac{\partial \vec{B}}{\partial t}}{\mu_0 \mu_r}\right) - \epsilon_0 \epsilon_r \frac{\partial^2 \vec{E}}{\partial t^2} = 0,$$

$$\Rightarrow \nabla \times \left(\frac{-\nabla \times \vec{E}}{\mu_0 \mu_r}\right) - \epsilon_0 \epsilon_r \frac{\partial^2 \vec{E}}{\partial t^2} = 0,$$

$$\Rightarrow -\frac{1}{\mu_0 \mu_r} \left(\nabla \left(\sum_{i=0} \hat{E}\right) - \nabla^2 \vec{E}\right) - \epsilon_0 \epsilon_r \frac{\partial^2 \vec{E}}{\partial t^2} = 0,$$

$$\Rightarrow \nabla^2 \vec{E} - \epsilon_0 \mu_0 \epsilon_r \mu_r \frac{\partial^2}{\partial t^2} \vec{E} = 0,$$

(2.7)

The equation 2.7 is the *Helmholtz equation* representing a harmonic field, where the propagating light is then described by the time-harmonic electromagnetic field \vec{E} , \vec{D} , \vec{B} and \vec{H} , that have the general form of \vec{F} as shown in equations 2.8 and 2.9.

$$\vec{F} = \vec{F}_0 e^{i\left(\vec{k}\cdot\vec{r}-\omega t\right)},\tag{2.8}$$

$$\vec{k} = k_x \hat{x} + k_y \hat{y} + k_z \hat{z}, \tag{2.9}$$

TABLE 2.1: Different material categories based on the relative permittivity and the permeability of the material.

Туре	$\epsilon'_r \operatorname{sign}$	μ'_r sign
Double positive (DPS)	+	+
Double negative (DNG)	-	-
ϵ'_r negative (ENG)	-	+
μ'_r negative (MNG)	+	-

where $\vec{F_0}$ is the amplitude of vector field, ω is the angular frequency, \vec{r} is the position vector of the field and \vec{k} is the complex propagation wave vector. How the material reacts to the electromagnetic radiation is mainly set by the material parameters, namely the ϵ_r and μ_r , and the geometrical shape. For this reason, the materials are separated into four distinct categories based on the sign of the real part of the ϵ'_r and μ'_r as shown in table 2.1.

Since we are only considering non-magnetic materials, the relative permeability of the material is assumed to be $\mu_r = 1$ in the following derivations. The figure 2.9b illustrates surface plasmon polariton propagating or oscillating towards positive z-direction in the interface of the dielectric and metal. Basically, SPP could have either transverse electric or magnetic mode as pictured on figure 2.9b. The boundary conditions dictate that the magnetic and electric fields in z-directions must be continuous, which will set conditions for the propagating SPs. For this, we need to derive the electric field E_z and the magnetic field H_z using Maxwell's equations and the harmonic wave solutions *F* in the equations 2.1, 2.2,2.3,2.4, 2.5, 2.6, 2.8 and 2.9.

$$\nabla \times \vec{E} = -\frac{\partial \vec{B}}{\partial t} = -\mu_0 \mu_r \frac{\partial}{\partial t} \left(\vec{H}_0 e^{i(\vec{k} \cdot \vec{r} - \omega t)} \right) = i\mu_0 \mu_r \omega \vec{H},$$

$$\Rightarrow H_z = \frac{1}{i\omega\mu_0\mu_r} \left(\frac{\partial E_y}{\partial x} - \frac{\partial E_x}{\partial y} \right),$$

$$\nabla \times \vec{H} = \frac{\partial \vec{D}}{\partial t} = \epsilon_0 \epsilon_r \frac{\partial}{\partial t} \left(\vec{E}_0 e^{i(\vec{k} \cdot \vec{r} - \omega t)} \right) = -i\omega\epsilon_0 \epsilon_r \vec{E},$$

$$\Rightarrow E_z = \frac{i}{\omega\epsilon_0\epsilon_r} \left(\frac{\partial H_y}{\partial x} - \frac{\partial H_x}{\partial y} \right).$$

(2.10)
(2.11)

The electric and magnetic fields are influenced by material parameters, we can assign wave vector $k_{x,m} = ik_0\gamma$ for the metal substrate and $k_{x,d} = ik_0\delta$ for the dielectric medium and define the propagation constant $\beta = k_z/k_0$, where the subscript *d* refers to dielectric, *m* to metal and γ and δ are the decay constants of the polariton in metal and dielectric, respectively. Because time-dependency is only present in the harmonic part, we can remove it and the time independent H_z and E_z can be solved from equations 2.8, 2.10 and 2.11 in both the metal substrate and the dielectric medium.

$$H_{z,d} \stackrel{E_x=0}{=} \frac{1}{i\omega\mu_0\mu_d} \frac{\partial}{\partial x} \left(E_{y0} \cdot e^{i(ik_0\delta x + k_0\beta z)} \right) = \frac{iE_{y0}}{\omega\mu_0} \frac{k_0\delta}{\mu_d} e^{(-k_0\delta x + ik_0\beta z)}, \tag{2.12}$$

$$H_{z,m} = -\frac{iE_{y0}}{\omega\mu_0} \frac{k_0\gamma}{\mu_m} e^{(k_0\gamma x + ik_0\beta z)},$$
(2.13)

$$E_{z,d} \stackrel{H_x=0}{=} \frac{i}{\omega\epsilon_0\epsilon_d} \frac{\partial}{\partial x} \left(H_{y0} \cdot e^{i(ik_0\delta x + k_0\beta z)} \right) = -\frac{iH_{y0}}{\omega\epsilon_0} \frac{k_0\delta}{\epsilon_d} e^{(-k_0\delta x + ik_0\beta z)}, \tag{2.14}$$

$$E_{z,m} = \frac{iH_{y0}}{\omega\epsilon_0} \frac{k_0\gamma}{\epsilon_m} e^{(k_0\gamma x + ik_0\beta z)},$$
(2.15)

 E_z and H_z in equations 2.12, 2.13, 2.14 and 2.15 depict oscillating fields illustrated in figure 2.9b. Similar electric and magnetic field can be solved for the $E_{x,d/m}$ and $H_{x,d/m}$. The decay constants γ and σ are related to the propagation constant β

$$\gamma^2 = \beta^2 - \epsilon_r \mu_r, \tag{2.16}$$

$$\sigma^2 = \beta^2 - \epsilon_r \mu_r, \tag{2.17}$$

which can be derived from the wave equation 2.7 and equations 2.13 and 2.15 [144]. Since the magnetic field for TE-mode and the electric field for TM-mode in z-direction must be continuous at the interface, we can derive the conditions for the propagating surface plasmon polaritons from equations 2.12, 2.13, 2.14, 2.15, 2.16 and 2.17.

$$H_{z,m} (x = 0) = \frac{iE_{y0}}{\omega\mu_0} \frac{k_0\gamma}{\mu_m} e^{ik_0\beta z} = H_{z,d} (x = 0) = \frac{iE_{y0}}{\omega\mu_0} \frac{k_0\delta}{\mu_d} e^{ik_0\beta z},$$

$$\Rightarrow \frac{\gamma}{\mu_m} = \frac{\delta}{\mu_d},$$

$$\Rightarrow \left(\frac{\gamma}{\mu_m}\right)^2 = \left(\frac{\delta}{\mu_d}\right)^2,$$

$$\Rightarrow \frac{\beta_{TE}^2 - \epsilon_m\mu_m}{\mu_m^2} = \frac{\beta_{TE}^2 - \epsilon_d\mu_d}{\mu_d^2},$$

$$\Rightarrow \beta_{TE}^2 \left(\frac{1}{\mu_m^2} - \frac{1}{\mu_d^2}\right) = \frac{\epsilon_m\mu_m}{\mu_m^2} - \frac{\epsilon_d\mu_d}{\mu_d^2},$$

$$\Rightarrow \beta_{TE} = (\pm) \sqrt{\frac{\epsilon_m\mu_m\mu_d^2 - \epsilon_d\mu_d\mu_m^2}{\mu_d^2 - \mu_m^2}},$$
(2.18)

where β_{TE} is the propagation constant for TE mode. Similarly the propagation constant β_{TM} can be derived for the TM mode.
$$E_{z,m} (x = 0) = \frac{iH_{y0}}{\omega\epsilon_0} \frac{k_0 \gamma}{\epsilon_m} e^{ik_0 \beta z} = E_{z,d} (x = 0) = \frac{iH_{y0}}{\omega\epsilon_0} \frac{k_0 \delta}{\epsilon_d} e^{ik_0 \beta z},$$

$$\Rightarrow \beta_{TM} = \sqrt{\frac{\epsilon_m \mu_m \epsilon_d^2 - \epsilon_d \mu_d \epsilon_m^2}{\epsilon_d^2 - \epsilon_m^2}},$$
(2.19)

Notably, the β_{TE} in equation 2.18 does not have solution in our case, since now $\mu_m = \mu_d = 1$, so SPP on a metal-dielectric interface cannot have TE polarization. For TM-mode, the equation 2.19 has converging solutions at $\mu_m = \mu_d = 1$ and SPPs with TM polarization can exist on a metal-dielectric surface. When inserting $\mu_m = \mu_d = 1$ into equation 2.19, we get well known solution

$$\beta_{TM} = \sqrt{\frac{\epsilon_m \mu_m \epsilon_d^2 - \epsilon_d \mu_d \epsilon_m^2}{\epsilon_d^2 - \epsilon_m^2}} \xrightarrow{\mu_r = 1} \sqrt{\frac{\epsilon_m \epsilon_d (\epsilon_d - \epsilon_m)}{(\epsilon_d - \epsilon_m) (\epsilon_d + \epsilon_m)}} = \sqrt{\frac{\epsilon_m \epsilon_d}{\epsilon_m + \epsilon_d}}.$$
 (2.20)

Equation 2.20 is also known as the dispersion relation, where the permittivities describe how much resistance/attenuation both materials will inflict on the electric field. Figure 2.9a shows the plot of dispersion relation for Drude metal [145], where $\beta_{TM} = k_z/k_0$, $k_z = \omega/c$, $\epsilon_m = 1 - \omega_p^2/\omega^2$, $\omega_p = \omega_{sp}\sqrt{1 + \epsilon_d}$ is the plasma frequency of the free electron gas, ω_{sp} is the characteristic surface plasmon frequency and ω is the angular frequency. It is evident that the free photon momentum (the light line in figure 2.9a) is always smaller than what is required to excite SPPs, and only in the presence of higher refractive index material the SPP can be excited (the tilted light line).

In general, the real part β' for both TM and TE modes is related to the existence of SPPs. Thus, the interface between two material will support SPPs in TM mode if one material has negative ϵ (ENG) and in TE mode if the ϵ and μ are both negative (DNG). Typically the metals are ENG-type materials and dielectrics are PNG type, which is the reason why only TM mode SPPs can be excited in metal-dielectric interface. The imaginary part β'' , on the other hand, is associated to the exponential decay of SPPs. For ideal situation, i.e., no surface contamination and smooth surfaces, where one of the materials at the interface has negative/complex permittivity ϵ_r or permeability μ_r , SP has a finite propagation distance. For purely non-complex permittivities SP would propagate indefinitely. In reality however, the decay process will be heavily damped by the surface roughness and contaminations regardless of the permittivity.

2.3.2 Surface plasmon resonance and Kretschmann Configuration

The excitation of SPP requires that the momentum and energy of the incident light matches with the created SPP. This requirement cannot be matched on flat metaldielectric interface, when excited directly with incident light, since the surface parallel wave vector of the plasmon has always higher momentum than the incident light. This problem can be circumvented by introduction of surface roughness or grating. Another common way to excite propagating SSPs is to employ the Kretschmann configure illustrated in figure 2.10a. Here, a double interface structure is used to excite SPPs, which propagates on the interface of the metal (gold) and dielectric medium (water or air). Excitation is done from the prism side, since, in the context of dispersion relation, the prism can be considered modifying the momentum of the free photon as shown in figure 2.9a (solid line), where intersection of the SPP curve and the tilted light line indicates plasmon excitation.



FIGURE 2.10: (a) Schematic view of the Kretschmann configuration. Figure created using Blender [146]. (b) Reflectance \Re versus the incident angle of excitation laser curves in water (red) and air (black) media, where the laser wavelength is 670 nm. The dip indicates excitation of surface plasmons on the media-metal interface, where the dip position depends on the refractive indexes of the metal and dielectric. The total reflection angle θ_{tir} indicates the point, when the plasmonic coupling start to take effect. Data simulated using Winspall-software [147].

In the following discussion, the metal layer is always assumed to be gold, since it was only metal used in the measurements employing the Kretschmann configuration. For SP excitation, it is assumed that the thickness of the gold layer in figure 2.10a is thin enough, so that the evanescent tail of the excitation light can penetrate through the gold layer. Other requirement is that the surface parallel component of the k-vector of the excitation light needs to match the β_{TM} (momentum) of the created SPP, which is only possible if the refractive index of the dielectric is smaller than that of the prism. To characterize this, we plotted the reflectance \Re of the Kretschmann configuration using Fresnel equations [144] for TM polarized excitation using Winspall-software (see figure 2.10b). The thickness of the gold layer is 42 nm, the prism is made from SF10 glass ($n_p \approx 1.73$) and the dielectric medium is either water or air. Intensity of the reflected light in total reflection has dependency on the incident angle θ of the light, since, in certain θ angles, the energy and momentum of the incident light is transferred to excitation of SPPs, which can be seen as a dip in the intensity as shown in figure 2.10b. The dip angle is also known as the resonance angle, and the position of the resonance angle is heavily influenced by the material parameters of the metal, prism and the dielectric. For example, water and air have completely different resonance angles as shown in figure 2.10b, where the reflectance dips are at 32° and at 66° for pure air and water medium, respectively.

Since the momentum and energy of the light is transferred to the SPP, we can correlate the wave vector $k_0\beta'$ of the SPP to the incident angle of the light as following $\theta = \sin^{-1} (\beta'_{TM}/n_p)$, where the β' is the real part of the propagation constant in equations 2.18 and 2.19, which solely depends on the dielectric constants of the medium and the metal. The changes in the permittivity or the refractive index of the medium close to the interface will have drastic changes in the reflection angle and SPR signal, which can be used to detect attachment of particles, molecules or proteins to the metal layer.

Adsorption of proteins and biomolecules to the gold surface increases the refractive index near the gold-medium interface, since these compounds have higher, non-complex permittivity than the surrounding dielectric medium [148], which leads into higher propagation constant and increase in the resonance angle θ . This effect is known as the mass-contribution effect. However, the situation is not so simple for materials with complex permittivities, e.g., metallic nanoparticles, and we have to consider two extreme cases where particle is far away from or close to gold surface. Essentially, when particle is far away from the surface, the coupling between surface and particle is weak and the particle has just the mass-contribution, i.e., positive shift. When brought close to gold surface, the particle and surface will form a coupled plasmonic state. Depending on the distance between the particle and the surface, the excitation light and the size of the particle, this new coupled state can either decrease, increase or not alter at all the refractive index near the gold surface thus leading into decreased, increased or non-altered angle shift.

This behavior can be interpreted via the localized surface plasmon resonance (LSPR) of AuNP: when the particle is brought closer to gold surface, the LSPR is shifted to higher wavelength. If the wavelength of the excitation laser overlaps with the shifted localized surface plasmon resonance of the particle, then depending on the coupling the resulting state has negative, zero or slightly positive effect on the surface conditions, i.e., on the permittivity and the refractive index and thus on the reflectance. If the two wavelengths do not overlap at any distance between the particle and the surface, then the closer the particle is to the surface the more it will increase the refractive index, resulting in higher positive shift. Uchiho *et al.* [149] reported, that detection of 120 nm particle close to gold surface using 630 nm laser resulted in decrease of the reflection angle θ due to resonance overlapping, but when switched to 835 nm, longer wavelength laser only increase in the θ was observed.

2.3.3 Non-propagating surface plasmons

In contrast to propagating SPPs, the localized (non-propagating) surface plasmons (LSP) are, in essence, oscillation motion of excited electrons on conduction band of the finite sized metallic nanostructure: the incident light sets electrons in oscillation motion, that can lead to dipole or even higher degree charge separation in the structure (see figure 2.11). Although similar in nature, the SPPs and LSPs differ in few key aspects.



FIGURE 2.11: (a) Schematic illustration of localized surface plasmon resonance of a spherical nanoparticle. The electric field of the incident light causes electron to oscillate, which creates dipole within the particle. (b) Scattering cross section at different angles. The incident light coming from left has higher probability of scattering to forward (0°) than back (180°).

First, LSPs can be directly excited on surface of the structure by incident light, which was prohibited in the case of SPP. Secondly, due to spatial confinement, the excitation can relax through either radiative or non-radiative path: in non-radiative relaxation, electron-hole pairs are excited inside the sphere that in turn will excite phonons, which then dissipates as heat. Radiative path involves emission of photon, generally at lower energy and longer wavelength. The radiative and non-radiative processes ae typically labeled as scattering and absorption processes. Due to the dipole excitation, there are characteristic wavelengths, where the mechanical oscillation frequency of the electrons matches the excitation frequency and thus LSPs are excited most efficiently, which are also known as localized surface plasmon resonances (LSPR). Both processes and thus the related LSPRs are heavily influenced by the local permittivity or the refractive index around the metallic nanostructure. Here, we are particularly interested in the scattering process, since it extends to farfield and thus can be directly measured. The scattering and the absorption processes are competing processes with different probabilities (cross sections). In general, for smaller particles the absorption cross section dominates over the scattering cross section and vice versa when the particle size increases.

Third, since electron band structure is material and geometry dependent, various nanostructures and -shapes have different efficiency to either scatter or absorb different wavelengths of electromagnetic radiation. In addition, the forward- and backscattering efficiencies are also material, size and shape dependent, and also the experiment geometry plays an important factor in the scattering processes, where the forward scattering is typically preferable due to higher cross section compared to the backscattering as shown in figure 2.11b.

In the upcoming sections, the analytical solution for localized surface plasmons of a spherical (gold) nanoparticle will be derived based on Quasistatic theory, which is valid if the dimension of the particle is less than 1 % of the wavelength of the incident light. Unfortunately, there does not exist any analytical model for non-spherical nanostructures or nanoparticle, which is why we have to rely on numerical methods to solve the LSP spectra of metallic cross and bowtie structures, which were used in the studies of this thesis. For this, we will utilize Finite Element Methods (FEM) and employ the Mie theory to solve the scattering cross section Q_{sc}

$$Q_{sc} = \lim_{R >>r} \frac{R^2}{\pi r^2} \int_0^{2\pi} \int_0^{\pi} \vec{n} \cdot \vec{S}_{sc} \cdot \sin\left(\theta\right) d\theta d\phi, \qquad (2.21)$$

where *R* is the radius of the observations, *r* is the radius of the particle, \vec{n} is the vector normal to the investigated plane and \vec{S}_{sc} is the pointing vector of the scattered field. Here, the Q_{sc} is the ratio between the power of the scattered (far-field) light and the power of the incident light, where both are normalized per unit area. Both TM and TE polarized light are used in the FEM simulations, since these two form orthogonal basis to represent the other polarization modes.

2.3.4 Quasistatic approximation and spherical metal nanoparticles

One common approach to characterize plasmonic nanostructure is to define the different LSPRs of the structure. The simplest plasmonic nanostructure and only one with adequate theoretical model is the spherical metal nanoparticle due to high symmetry factor. Initially, we are considering the characteristic LSPR of small metallic spherical nanoparticles in terms of Quasistatic approximation, which will be then expanded to larger particles. As it was in the case of SPPs, we need to derive the electric field \vec{E} in and around the particle to solve the conditions for the LSP creation. Following discussion is based on the theoretical description given by Maier, Huffman and Jackson [145, 150, 151]. The electric field \vec{E} is assumed to be uniform inside and around the particle($|\vec{E}_{\infty}| = E_0$, see figure 2.12a), the sphere has dielectric function ϵ_m and it is surrounded by medium with a dielectric constant ϵ_d .

Taking classical electrostatic approach and solving the Laplace equation 2.22,



FIGURE 2.12: (a) Sketch of spherical nanoparticle under influence of an electromagnetic field. (b) The scattering and absorption spectra of 20 nm AuNP in water and air.

we can calculate electric potential inside (Φ_{in}) and outside (Φ_{out}) of the sphere

$$E = -\nabla\Phi, \tag{2.22}$$

$$\Phi_{in} = \sum_{j} A_j r^j P_j(\cos(\theta)), \qquad (2.23)$$

$$\Phi_{out} = \sum_{j} \left(B_j r^j + C_j r^{-(j+1)} \right) P_j(\cos(\theta)),$$
(2.24)

where Φ_{in} and Φ_{out} follow the Legendre Polynomial formula due to the azimuthal symmetry [150, 151], *a* is the radius of the sphere, \vec{r} is the position vector and the coefficients A_i , B_i and C_i are set by the boundary conditions, when $r \to \infty$ and r = a. When far away from the nanoparticle $(r \to \infty)$, the potential should converge into $\Phi_{out} \to -E_0 r \cos(\theta)$. The boundary conditions at the surface of the sphere require that the tangential components of the electric fields are equal and the normal components of the displacement fields are equal. All these requirements lead into solution for equations 2.23 and 2.24 (see appendix for more detailed derivation), where B_j , A_j and C_j are zero for $j \neq 1$ and

$$\Phi_{in} = -\frac{3\epsilon_d}{\epsilon_m + 2\epsilon_d} E_0 r \cdot \cos\left(\theta\right), \qquad (2.25)$$

$$\Phi_{out} = -E_0 r \cdot \cos\left(\theta\right) + \frac{\epsilon_m - \epsilon_d}{\epsilon_m + 2\epsilon_d} E_0 a^3 \frac{\cos\left(\theta\right)}{r^2},$$
(2.26)

Essentially equations 2.25 and 2.26 describe superposition of the external electric field and a dipole moment inside the sphere, where the polarizability α is the

ability of the incident light to induce dipole momentum inside the sphere.

$$\Phi_{out} = -E_0 r \cdot \cos(\theta) + \alpha \frac{E_0 \cos(\theta)}{4\pi r^2},$$

$$\alpha = \frac{(4\pi a^3) \cdot (\epsilon_m - \epsilon_d)}{2\epsilon_d + \epsilon_m}.$$
(2.27)

Notably, polarizability depends on the permittivity of the sphere, which depends on the wavelength of the incident light. The scattering and absorption cross section can be then calculated using the polarizability from equation 2.27 as following [151]

$$Q_{sc} = \frac{k^4 \left|\alpha\right|^2}{6\pi} = \frac{8\pi k^4 a^6}{3} \left|\frac{\epsilon_m - \epsilon_d}{\epsilon_m + 2\epsilon_d}\right|^2,$$
(2.28)

$$Q_{abs} = k \cdot \operatorname{Im}[\alpha] = 4\pi \cdot k \cdot a^3 \cdot \operatorname{Im}\left[\frac{\epsilon_m - \epsilon_d}{\epsilon_m + 2\epsilon_d}\right],$$
(2.29)

where $k = 2\pi/\lambda$. The polarization of a nanoparticle can be perceived as motion, where the electron cloud of the particle is set to oscillate from one side of the particle to the other as shown in figure 2.11a, forming dipole along the polarization axis. This process depends on the wavelength of the incident light, and the peak maximum in the equations 2.27,2.28 and 2.29 is the localized surface plasmon resonance, where the electrons are the most efficiently set into oscillation motion. By plotting the equations 2.28 and 2.29 for different metals and media, one can solve the LSPR position of scattering and absorption of a spherical metal nanoparticle. The figure 2.12b shows the scattering and absorption spectra of 20 nm in diameter gold nanoparticle in both air and water, where, e.g., the scattering LSPR are at 520 nm and 530 nm for air and water, respectively, which is in a close agreement with literature [152].

So far we have considered that the electric field inside the nanoparticle is constant, which has the consequence that the LSPR positions from equations 2.28 and 2.29 are independent of the particle size and thus only valid for small particles. For larger spherical particles, we need to include retardation effects on the excitation field over the particle, where, as the size of the particle increases, the restoration force and the energy of the dipole excitation decrease. For this, the Mie theory provides better approximation, and we will consider first-order corrections of the Quasistatic approximation [22], where the polarizability α_{mie} is

$$\alpha_{mie} = \frac{V \cdot \left(1 - \frac{(\epsilon_m + \epsilon_d) \cdot x^2}{10}\right)}{\frac{1}{3} + \frac{\epsilon_d}{\epsilon_m - \epsilon_d} - \frac{(\epsilon_m + 10 \cdot \epsilon_d) \cdot x^2}{30} - i \cdot \frac{4\pi^2 \epsilon_d^{1.5}}{3} \frac{V}{\lambda^3}},$$
(2.30)

where $x = \pi a / \lambda$, λ is the wavelength of the light and *V* is the volume of the sphere.

Here, we ignore higher than second-order terms. The quadratic terms of *x* in denominator and nominator are the effect of retardation of the depolarization field inside the particle and the retardation of exciting field near particle, which both shift the overall LSPR wavelength. Notably, the volume dependent term in the denominator will redshift the LSPR peak as the particle size increases. This can be seen in figure 2.13, where the absorption and scattering cross sections of a 80 nm spherical gold particle redshifts to longer wavelengths compared to the 20 nm particle, which is again in a fairly good agreement with the theoretical values [152].



FIGURE 2.13: (a)The absorption and (b) spectra of a 80 nm AuNP in both air and water, when considering first order correction to the dipole excitation.

Besides the dipole excitation, nanoparticles can have higher order poles, e.g., quadrupole and octopole, present at higher energies, but typically they have weaker scattering and absorption cross sections. Due to these reasons, the higher order poles are not considered in the framework of this thesis. The Quasistatic theory and the correction terms are quite accurate for relatively small spherical particles in noninteracting regime, when concentration in solution or density on substrate is low enough and there are no matrix effect originating from lattice arrangement of particles.

In the next sections, we will shift focus to discuss about more complex structures and assemblies, such as the LSP of a spherical nanoparticle close to a metal surface, cross structure and bowtie structure, which are beyond the discussed Quasistatic and Mie approximations. The exact analytical derivations are difficult for these constructs, so I am presenting both an intuitive model to explain the electron excitation phenomena in these structures and more accurate FEM simulations of the scattering and absorption spectra using equation 2.21 in Comsol.

Spherical nanoparticle close to metallic surface

The utilization of DNA-AuNP based nanoactuator, where AuNPs are moved on and off a gold surface, is investigated in this thesis. If the particle is far away from the surface, the plasmonic excitation and the LSPR behavior by the incident light follows the framework discussed in the previous section. However, when the distance between the surface and the particle is 20 nm or less, the particle starts to interact with the gold surface and forms a new coupled state with altered plasmonic properties, where the LSPR position is redshifted.



FIGURE 2.14: (a) Sketch of AuNP close to a gold surface and under illumination of incident light coming from angle θ in respect to the surface normal. The angle θ is 70° in all of the simulations. (b),(c) When the dipole-excited particle is close to the gold surface, the conducting surface can be considered as a mirror particle with an opposite polarization \vec{p} . The mutual dipole symmetry axes are marked with dashed lines.(d) Scattering spectra of a 80 nm AuNP in respect to the distance *d* between the nanoparticle and the gold surface.(e) The field enhancement of the 80 nm AuNP, when the distance *d* is 3 nm, the excitation wavelength is 615 nm and the light is TM polarized. The field maximum is located in the gap, which corresponds to the parallel dipole configuration in figure c. (f) The field enhancement of the 80 nm AuNP, when the distance *d* is 3 nm, the excitation wavelength is 550 nm and the light is TM polarized. The field is highest in the gap and along the perimeter of the AuNP, which corresponds to the antiparallel dipole configuration in figure b.

Intuitively, we can explain the behavior using classical electrodynamics. The

particle excitation using incident light coming at an angle θ with momentum k (see figure 2.14a) can be divided into two orthogonal directions, x-y-plane and the z-axis. In both cases, the charged particle interacts with its own reflected dynamic electric field, so that the gold surface can be effectively thought as a dynamic mirror particle with the opposite dipole momentum in respect to mutual symmetry axis and the same distance from the surface [153]. The x-y-plane mode in the figure 2.14b involves the induced dipole momentum parallel to the plane, where the mirror particle has a dipole momentum in the opposite direction. The z-axis excitation in the figure 2.14c will produce surface normal dipole momentum for the real particle and the mirror particle, where the dipole of the real particle is pointing towards the surface and in the opposite direction for the mirror particle. The key difference is that the x-y-plane plasmonic excitation has antiparallel dipole orientation and the z-axis excitation induces parallel orientation, meaning that both result in redshift of the scattering spectrum, but the parallel interaction is more pronounced than the antiparallel interaction [154–156]. This would lead into two peaks, where one shifts faster to longer wavelengths than the other. We will label the stronger peak as the gap mode peak and the weaker peak as in-plane mode peak, corresponding to the associated directions.

More quantitative analysis is performed using FEM simulations, where the distance *d* between a 80 nm AuNP and a Au-surface is varied from 1 nm to 20 nm for both TM and TE polarized light with the incident angle of 70°. The arbitrary limit of 1 nm is due to the quantum mechanical effect, which start to play a major role after that limit and which the FEM simulations cannot handle anymore properly. From the TM simulations, when particle is far away from the surface ($d \sim 20$ nm), there is only one peak at 565 nm, which corresponds to the previously discussed dipole excitation of a spherical particle, but now it is shifted slightly to higher wavelengths due to the z-direction coupling with the surface. When the particle is brought close to sample surface, the scattering spectra initially redshifts and after 4 nm distance a secondary peaks appear around 530-550 nm as shown in figure 2.14d. These values also agree with the literature [154, 156–158]. Similar results were obtained for TE polarization (see figure 3.8 in chapter 3).

Figures 2.14e and 2.14f show the field enhancements for the 615 nm excitation peak and the 550 nm excitation peak with the distance d of 3 nm, where the electric field is located purely in the gap for the 615 nm excitation and mainly along the perimeter of the particle for the 550 nm excitation. This means that the former peak corresponds to the z-direction excitation (gap mode) shown in figure 2.14c, which is redshifting to longer wavelengths as the distance between particle and surface is decreasing. The peak around 550 nm is associated to excitation by in-plane component of the electric field, but the peak is initially blueshifted compared to plain AuNP due to higher, particle dipole enhanced dielectric function near the surface. This peak also slightly redshifts to longer wavelengths as the distance d decreases.

Planar, metallic cross structure on a substrate

The derivations of the LSP behavior thus far have been fairly simple due to the high symmetry of the spherical nanoparticles, where the dipole excitation is essentially independent of the azimuthal angle. Although this is not the case for many other structures, the symmetry features generally dictate the LSPR properties of the examined structures. For the cross structure in figure 2.15a, the symmetry features would be the arms in both vertical (orange line) and horizontal (blue line) directions and we will consider excitation along these directions next.

To qualitatively understand the situation, we will first examine the LSPR modes of ellipsoid and rod nanostructures, since cross can be though as a combination of two intersecting rods. For a rough approximation, the rod structure can be considered as an oblate ellipsoid, since ellipsoid has similar LSPR behavior as the sphere except there are three major axes with different dipole excitations instead of one dipole mode (see figure 2.15b). The polarizability of an ellipsoid can be calculated from equation 2.31 below. The material is gold and the medium is air [144].

$$\alpha_{i} = \frac{4\pi\epsilon_{0}\epsilon_{d}ab^{2}\left(\epsilon_{m} - \epsilon_{d}\right)}{3\left(\epsilon_{d} + A_{i}\left(\epsilon_{m} - \epsilon_{d}\right)\right)},$$

$$A_{long} = \frac{1 - E^{2}}{E^{2}} \left(\frac{1}{2E} \ln\left(\frac{1 + E}{1 - E} - 1\right)\right)$$

$$A_{short} = \frac{1 - A_{long}}{2},$$

$$E^{2} = 1 - \frac{b^{2}}{a^{2}},$$
(2.31)

where *a* is the length of the long axis, *b* is the length of the short axes and A_i is the shape factor, where *i* denotes either the long or short axis. The scattering cross section for both long axis and short axis is shown in figure 2.15b, where the two short axes have the same length (b = 20 nm), the long axis *a* is 72 nm long and medium is air. As expected, the LSPR peak of the long axis is at longer wavelengths compared to the shorter axes. The ellipsoid has typically more rounded peak when compared to the spherical gold nanoparticle due to the continuous shape change from long axis to short axis. Also, it should be noted that the oblate ellipsoid exhibit lightning rod effect, where the sharp, pointy ends will have significantly higher local electric fields due to needle-like shape, which can be used, e.g., to enhance chemical reactions near the tip of the ellipsoid.

Now we can turn the focus on the rod structure. There is not any analytical model for the rod, so we use FEM in Comsol to calculate the scattering cross section for a rod with similar dimension as the previously calculated oblate ellipsoid (see figure 2.15c and appendix figure A.12), but both in air and on a sapphire substrate (refractive index = 1.76), where the medium is air. Similarly as in the case of the ellipsoid, there exist two peaks, which correspond to the longitudinal axis and the



FIGURE 2.15: (a) A sketch of a cross structures, where the vertical (long) and horizontal (short) directions are depicted by the orange dotted line and dashed blue line, respectively.(b) The scattering spectra of oblate ellipsoid, where two main LSPR peaks related to the short and the long axes are presented. The inset shows the simulated ellipsoid structure. (c) The scattering and absorption spectra of a rod structure, in air and on a substrate, where the excitation is along the long axis. The inset above shows the simulated rod structure.(d) Field enhancement of the rod structures in figure c.

two transverse axes, where the longitudinal peak is at higher wavelength compared to the transverse axes.

The longitudinal LSPR scattering peak is at 620 nm and the transverse peak 430 nm in air, and when on substrate the longitudinal LSPR scattering peak is at 688 nm the transverse peak is 522 nm. Notably, the transverse scattering cross section of the rod is much smaller than the absorption cross section (500 nm), which is close

to the absorption cross section of the spherical nanoparticle in figure 2.12b. Also, the longitudinal peak in air is close to the long axis peak of the ellipsoid, and the shift between air and substrate can be attributed to the higher refractive index of the sapphire. Overall, the peaks are sharper and in the longer wavelengths than in the case of the ellipsoid due to the sharper features and the high refractive index substrate. Unsurprisingly, the rod exhibits also the lightning rod effect, which can contribute to the field enhancement at the ends of the rod (see figure 2.15d).



FIGURE 2.16: (a) The scattering spectra and (b) the field enhancement of the cross structure depicted in the figure 2.15a. The different spectra are simulated with different excitation polarizations with colors corresponding to the polarization directions shown in Figure 2.15a. The electric field is highest at the tips of the arms.

By combining the two simpler cases, we can finally analyze the LSPR behavior of the cross structure. Like in the case of the rod, FEM simulations are employed and we are considering the cross structure in the figure 2.15a, where one of the arms has the length of 72 nm and the other on has 65 nm, the thickness of the structure is 20 nm, the width of the arms is 25 nm and the ends are rounded. The cross is simulated on a sapphire substrate and in air. The scattering cross section has two main peaks in figure 2.16a (simulated with different polarizations) corresponding to the two longitudinal modes of the different arms, that are fairly close to that of the rod on substrate. The difference in the LSPR wavelength might be due to the coupling between the two arms. The two dominating longitudinal peaks are to be expected, since the LSPR modes of the arms have higher statistical weight when compared to excitation in any other equivalent angle. For example, the excitation in between the arms is much weaker than along the arms (see figure 2.16a), and the LSPR mode in this directions is mainly a mixture of the LSPR peaks of the two dominating arms. This suggests that the principle LSPR modes are along the arms. Similarly as the ellipsoid and the rod, the field enhancement in figure 2.16b shows

that the fields are highest at the tips of the arms (see appendix figures A.11 and A.12).

So far we have only considered an ideal cross with sharp features. However, due to the limitation in fabrication processes, the shape and especially the edges of the cross can be rounded. The consequence of this is that the overall scattering peaks of the cross have LSP features from both oblate ellipsoid and rod shapes, which can influence the LSPR wavelengths and the roundness of the peaks.

Planar, metallic bowtie structures on substrate

The bowtie structure has interested researchers due to the high field enhancement between the triangles, which is due to combination of three factors: collective lightning rod effect of the triangles, dual-dipole effect and the LSPR of the triangles. The FEM simulations of a symmetric bowtie structure on a sapphire substrate and in air are shown in figure 2.17a. There are two dominant LSPR peaks at 725 nm and 665 nm, which correspond to the excitation polarization along the gap (gap mode) and another one along the edges of the triangles (edge mode). The field enhancements at the LSPR positions in the figures 2.17b and 2.17c show that the highest field enhancement is located within the gap, as expected, which has been reported in the literature also [159–161].

The figure 2.17 depicts a perfect bowtie on a substrate, which is hard to realize in actual fabrication process, and there are several factors which will then influence the final LSPR positions. The angle between the bowties affects the LSPR modes of the bowtie. Chien *et al.* reported [160] that initially when the angle is shifted from the perfect alignment, the gap mode is redshifted due to mixing of the edge modes and the gap mode. If the angle is shifted more than 40°, then two opposite edges from both triangles start to face each other, which blueshifts the LSPR peak as the angle is increased, and the gap mode is slowly lost.

Another important factor for the LSP properties of the bowtie is the size of the gap, since the field enhancement and the LSPR peak position of the gap mode heavily depend on it. Fromm *et al.* [162] have investigated the effect of the gap in a gold bowtie structure and discovered that when the distance between the two symmetric triangles is less than twice the length of one triangle, then shrinking of the gap will results in redshift of the LSPR peak of the gap mode and the peak intensity increases. Similar findings have been reported by other groups [163]. If the triangles separate more than twice the length, then by increasing the distance the LSPR peak will redshift.

Third important parameter is the opening angle of the triangle and sharpness of the tip. Ding *et al.* have demonstrated that increasing the opening angle initially blueshifts the LSPR peak, but when the angle is over 80° then the LSPR peak starts to redshift due to higher order modes appearing at large angles [164]. If the tip is truncated, the LSPR peak blueshifts to shorted wavelengths [165]. Fourth factor is



FIGURE 2.17: (a) The scattering spectra of a bowtie structure, when the polarization of the electric field is along the gap (blue) and perpendicular to the gap (orange).(b) The field enhancement of the bowtie structure for excitation at 725 nm, when the electric field is along the gap.(c) The field enhancement of the bowtie structure for excitation at 665 nm, when the electric field is perpendicular to the gap.

the size of the bowties. As the edge lengths increases, than the LSPR peak redshifts [163], which is in agreement with the general behavior of any plasmonic structure discussed thus far.

2.3.5 Optical characterization methods

The optical characterization of above-mentioned individual metallic structures is fairly challenging due to relatively weak scattering, which is even more pronounced in fast paced dynamic experiments. Also, due to the size and reactivity of the samples, it is vital to avoid unnecessary physical and chemical modification of target species during the measurements. These requirements mean that the characterization methods need to be non-invasive and parallel in nature. I have employed a quite commonly-used resonance angle SPR (RA-SPR), dynamic light scattering (DLS), atomic force microscopy (AFM) and dark field (DF) microscopy to characterize the studied system. Other possible methods, e.g., near-field scanning optical microscopy (SNOM) [166] could be used to characterize the studied systems, but these techniques are limited by either the resolution or operation environment (only air).

In the following sections, the theory of DLS is briefly discussed, whereas the DF microscopy is introduced in the chapter 3. Theory of the SPR was already introduced in previous sections, and here the discussion focuses on the mechanics of experiments.

Resonant angle surface plasmon resonance detection of nanocompounds

The RA-SPR has proven to be powerful method to detect binding of different chemicals, biomolecules, proteins and nanoparticles to different substrates with real-time and label-free detection [149,167–169]. It is typically employed in studies of layerby-layer self-assemblies [170], determination of binding constants [171] and thermodynamic analysis of organic compounds [172]. Also, it doesn't suffer from harsh chemical treatments and does not require sensitive detection of, e.g., fluorescence signal, which makes it more applicable in study of biological samples. The used SPR setup utilizes the previously discussed Kretschmann configuration shown in figure 2.10a, and the detection scheme is illustrated in figure 2.18. Here, in the context of this thesis, we are considering immobilization and dissociation of gold nanoparticle, proteins and biomolecules. The sensor (substrate) surface is typically gold, since it is an inert metal with relatively sharp SPR response.

As already established, the incident laser light excites SPP at the interface of the dielectric and the metal, which can be detected as a dip in the reflectance measurement intensity as shown in figure 2.10b. This dip angle or resonant angle is monitored during the SPR measurements (Point 1. in figure 2.18). The excitation of SPPs and the dip depends on the dielectric functions of the metal and the medium as depicted by equation 2.20, and the absorbed proteins and biomolecules shift the dip angle (point 2. in figure 2.18): typically for DPS material like protein, the shift is positive and saturates after the surface is mostly covered by the material in question, while the metal nanoparticles can have positive or negative shift depending on the coupling between the gold surface and the nanoparticle.

Since the SPP excitation is sensitive to dielectric function changes until 100 nm above the gold substrate, attachment of additional biomolecules and nanoparticles can be further characterized (point 3. in figure 2.18). Finally, the dissociation of nanocompounds can be studied, which is seen as a negative shift in the angle (point 4. in figure 2.18), although decrease in signal does not indicate what species are dissociated from the surface.

There are two important factors that need to be considered during the RA-SPR measurements. First, the dielectric functions depend on temperature, which needs to be constant during the measurements. Secondly, the medium in SPR is typically biological buffer, and changes in the buffer condition affect the dielectric function of the medium, which is typically detected as a (negative) shift in the SPR signal, known as bulk effect.



FIGURE 2.18: The operation principle of SPR. Initial starting point (1.) is a plain (gold) surface, which has the dip in the SPR signal at the angle α . As biomolecules, e.g., antibodies are adsorbed (point 2.) to the surface, the angle α increases, which can be seen as a positive shift in the SPR signal and the signal saturates when the surface is saturated with the biomolecules. This process can be further continued by introduction of one or more compounds, that will attach either to the existing surface-bound molecules or to the surface itself, where the association of the new compound can be seen as positive shift in the SPR signal. The dissociation of biomolecules can be detected as a negative shift (point 4.).

Dynamic light scattering characterization

The development of the dynamic light scattering has a fairly interesting background. The basic concepts of scattering theory of small spherical particles were introduced by Rayleigh and Tyndall in late 19th century and early 20th century and extended later to larger and non-spherical particles by Debye [173]. Other important early contributors to the development of the light scattering techniques were Smoluchowski and Einstein, who related the fluctuation of the scattered light from a medium to the fluctuation of the dielectric constant of the same medium. The final piece in the puzzle to study the nanoparticles using the light scattering techniques was the introduction of lasers in 1960s, which enabled study of weakly scattering systems. When combined together, these advances enabled light scattering, which have been utilized quite extensively to study, e.g., structure of solid, liquid crystal, viruses

and biomolecules as well as studying the dynamics of nanoparticles in dilute solutions [174, 175].



FIGURE 2.19: The operation principle of DLS. Scattering of light from nanoparticle is detected at angle alpha, where the fluctuations are correlated to the size of the particle using autocorrelation function.

The schematic view of DLS setup and operation is shown in figure 2.19. The detection and monitoring of nanoparticles is based on the interaction between the incident laser and randomly fluctuating AuNP. The monochromatic laser beam hits the scattering volume V of the studied medium, which can be divided into subregions. Each subregion can be considered as a scattering point for the incident planewave, and if the dielectric function of the medium is constant (no particle in volume V), then only forward scattering occurs due to constructive and destructive interferences of each subregion. When a particle enters the volume V, the superposition of the scattering processes of the subregions is non-zero in other directions than the forward direction. As the particle moves randomly from subregion to subregion the scattering intensity at the angle φ fluctuates randomly in time. The random fluctuations of the intensity and the particle position can be correlated together using the equations 2.32, 2.33 and 2.34 [173], where the autocorrelation function of the particle's Brownian motion and the scattering intensity fluctuation g_2 can be then related

to the particle size using Stokes-Einstein relation in equation 2.35 [173].

$$g_2(q,t) = \frac{\langle I_s(q,t)I_s(q,t+\tau)\rangle}{\langle |I_s(q,t)|^2\rangle},$$
(2.32)

$$g_2(q,t) = 1 + \beta |g_1(q,t)|^2, \qquad (2.33)$$

$$g_1(q,t) = e^{-q^2 D\tau},$$
(2.34)

$$R = \frac{kT}{6\pi\eta D},\tag{2.35}$$

Here I_s is the intensity of the scattered light, β is the coherence factor, q is the scattering vector, τ is the time lag between the scattering event and the recording, D is diffusion coefficient, R is the hydrodynamic radius of the particle, T is the temperature and η is the viscosity of the medium. It should be noted that the equation 2.32 assumes monodispersed sample, and for polydispersed sample multiple auto-correlation functions should be fitted.

The DLS experiments in this thesis were used to study aggregation processes, which inherently compromise polydispersed samples. However, the used instrumentation was only suitable for monodispersed analysis, so the results of the DLS analysis give only qualitative assessment of the aggregation. This is still enough in our case, since we wanted to determine the point, where aggregation is minimized and the sample is still roughly monodispersed.

2.4 Single electron effects

Single-electron electronics, where individual electrons are tunneling through artificially fabricated tunnel junctions in a controllable manner, were first realized in 1980s by Fulton and Dolan [176]. Later in 1991, Averin and Likharev [177] developed theory for the single electron tunneling and provided several applications based on their work, which was then expanded by Mooij and Geerlings [178], Averin and Nazarov [179] and van Houten, Beenakker and Staring [180]. The reason why these devices have only very recently caught wider attention of researchers is the requirement for the small tunnel junctions to have capacitances at least at range of 10^{-15} F or smaller, which corresponds to operation temperature of 1 K. Controllable fabrication of small enough tunnel junctions has required tremendous advances in the lithography methods, and it is still challenging to fabricate metallic single electron devices that operate at room temperature. This is one of the main objectives of this thesis. Single electron phenomena studies have encompassed several different materials and their combinations, e.g., normal metals [181], superconductors [182], carbon nanotubes [183] and semiconductors [41], but the framework of this thesis involves only the (normal) metal leads. The superconductor and semiconductor offer possible follow up research topics.

In the upcoming sections, the operation conditions, the bias voltage and gate voltage dependency of the single electron transistor is defined based on the perturbation theory, Fermi golden rule and single electron box [184]. This approach is valid as long as the resistances of the tunnel barriers are much higher than the resistance quantum $R_K = h/e^2 \approx 25.813 \text{ k}\Omega$, which, as will be shown in chapter 4, is true for DNA and gold nanoparticle based assemblies.

2.4.1 The Coulomb blockade, single electron box and single electron transistor

Single electron transistor illustrated in figure 2.20a is a device comprising of an island, drain and source electrodes with tunneling barriers to the island, and a gate electrode, which is capacitively coupled to the island. The tunnel junctions are assumed to be identical, which is in the context of the thesis fairly reasonable approximation. The operation of single electron transistor is based on the concept of the discrete electron tunneling into the island and the consecutive tunneling away from the island through the other tunnel junctions. This process is influenced by the temperature, the size of the island, i.e., the capacitance of the island C_i , the voltage between the drain and the source, the gate voltage and the resistances of the system. Here, we are considering an ideal system at low temperature with no external resistances.



FIGURE 2.20: (a)A schematic illustration of a single electron transistor.(b) An equivalent circuit of a single electron transistor. (c) An equivalent circuit of a single electron box.

To understand the operation principle of the single electron transistor in figure 2.20b, we will first examine the simpler model of a single electron box, which consists of one tunnel barrier with the capacitance C_j , a capacitive junction C_G and gate voltage V_g as shown in figure 2.20c. Let's set initial condition as such that the gate voltage $V_g = 0$ V and there are no excess electrons on the island, i.e., the island is neutral. Now, non-zero V_g allows tunneling of discrete number of electrons $n = \pm 1, \pm 2...$, and any electron tunneling to the island will disturb the collective electron distribution due to the electrostatic repulsion, causing the system to reorient to find a new equilibrium position. We can derive the charging energy E_{ch} of the island by using elementary Kirchhoff's laws and assuming that the *n* excess electrons are divided equally between the tunnel junction and the capacitor [185].

$$E_{ch}(n, V_g) = \frac{(e \cdot n - V_g C_g)^2}{2C_i},$$
(2.36)

where the capacitance of the island $C_i = C_j + C_g$. Since the single electron transistor (SET) can be considered as two single electron boxes, the charging energy of the SET island has similar form as the single electron box, except $C_i = 2C_j + G_g$. We can now calculate the energy needed to add one electron to the island, ΔE_{ch} , as

$$\Delta E_{ch}(n, V_g) = E_{ch,i}(n+1, V_g) - E_{ch,i}(n, V_g),$$

$$\Delta E_{ch}(n, V_g) = \frac{\left(e \cdot (n+1) - V_g C_g\right)^2}{2C_i} - \frac{\left(e \cdot n - V_g C_g\right)^2}{2C_i},$$

$$\Delta E_{ch}(n, V_g) = \frac{1}{2C_i} \left(e^2 \left(n^2 + 2n + 1\right) - 2V_g C_g(n+1)e - \left(e^2 n^2 - 2V_g C_g ne\right)\right),$$

$$\Delta E_{ch}(n, V_g) = \left(n + \frac{1}{2} + \frac{V_g C_g}{e}\right) \frac{e^2}{C_i}.$$
(2.37)

From the equation 2.37 it is evident that the energy changes in discrete intervals, which means that the island has Coulombic energy bands as shown in figure 2.21. This energy band structure generates interesting conditions for electron tunneling: at low temperature, if the energy level of the left junction eV_L is lower than the highest unoccupied state, then electrons cannot tunnel to the island due to Coulombic repulsion, and if the energy level of the right junction eV_R is higher than the highest occupied state, then electrons cannot tunnel from the island. This is known as the Coulomb blockade effect and it is the basis for the SET. The tunneling only happens, when the external biasing is set high enough so that electrons can tunnel on and off the islands as shown in figure 2.21b. Also, by changing the gate voltage, one can shift the energy state and allow electron tunneling through the island.

If the temperature is increased, electrons have enough thermal energy to tunnel through the barriers into higher energy levels and the Coulomb blockade is not observable. The operation temperature of the SET, where the Coulomb blockade is still observable, can be evaluated at the point where the required charging energy $\Delta E_{ch,i}$ and the thermal energy k_bT are equal.

$$k_b T = \Delta E_{ch,i} \left(n, V_g \right),$$

$$k_b T \xrightarrow{V_g \approx 0 V} \frac{\left(n + \frac{1}{2} \right) \cdot e^2}{C_i}$$

$$k_b T \propto \frac{1}{C_i} \propto \frac{1}{V},$$



FIGURE 2.21: (a) The energy diagram of the SET in absence of an external biasing. The island exhibits the Coulomb blockade effect, where electron tunneling is blocked due lack of available energy states. (b) The energy diagram of the SET with external biasing. Now the electrons can tunnel through the barrier, since both sides of the island have the correct biasing.

where *V* is the volume of the island. If we approximate tunnel junctions as a plate capacitors with 1 nm air gaps, then for SET to work at room temperature the size of island should be in order of $10 \text{ nm} \times 10 \text{ nm}$, which has been fairly challenging to realize using the conventional top-down lithography methods.

So far we have only conceptually discussed about the different conditions and operation of SET and, for more tangible assessment, the next section is devoted to derive the gate-dependent I-V-characteristics of SET and for discussion about the effect of cotunneling processes on the I-V-characteristics.

2.4.2 I-V-characteristics of SET

We start the derivations of the I-V-characteristics by considering the SET shown in figure 2.20b. The tunneling process is described as an event, where either an electron tunnels from k state of drain lead to the j state of the island or an electron tunnels from j state of the island onto l state of the source lead. In the former case the island has gained an extra electron or an excited state, that is relaxed in the latter case. Importantly, we are only considering single tunneling events and the tunneling to island and from island need not to involve same state of the island nor the processes need to be simultaneous. This formalism also considers that during the tunneling process the junction not involved in the tunneling process can be effectively considered as a plain capacitor, meaning that we will only consider first order term in the perturbation theory. Some of the higher order processes will be discussed later, like the cotunneling processes, which involve the simultaneous tunneling.

The theory can be worked out with the tunneling Hamiltonian functions, which

essentially describe tunneling as a creation and annihilation of electrons in the drain, source and island. Using this formalism, we can define the tunneling rates Γ_{jk} without bias voltage by the Fermi golden-rule argument [181]

$$\Gamma_{jk} = \frac{1}{e^2 R_t} \frac{\Delta E_{ch,i}^{jk}}{\exp\left[\frac{\Delta E_{ch,i}^{jk}}{k_b T}\right] - 1},$$
(2.38)

$$\Delta E_{ch}\left(n, V_g, V_{L/(R)}\right) =^{+}_{(-)} \left(\left(n + \frac{1}{2} + \frac{V_g C_g}{e}\right) \frac{e^2}{C_i} - V_{L/(R)} \right),$$
(2.39)

where *j* and *k* denote the electron tunneling from *j* side to *k* side of the tunnel junction, R_t^{-1} is the tunnel conductance of the junction *jk* and V_L and V_R are the bias voltages at the left and right junctions. Terms e^2/C_i and k_bT from equations 2.37, 2.38 and 2.39 are combined into constant $A = e^2/(C_ik_bT)$, which is essentially the attenuation term of the exponent in the equation 2.38. In the previous section, the Coulomb blockade was explained intuitively by purely considering classical electrodynamics. Here, we have provided justification for this argument, since the Coulomb blockade is only observable if the constant $A = e^2/(C_ik_bT) > 1$, meaning that the current is suppressed due to the low tunneling probability.

As mentioned, it is assumed that both tunneling junctions are identical and they have the same tunneling conductance R_t^{-1} . The charging energy ΔE_{ch} can be calculated from the equation 2.37, but we have to take into account that electron tunneling to island (denoted as i) will increase the charging energy and electron tunneling from island will decrease the charging energy, meaning $\Delta E_{ch}^{ji} \rightarrow + |\Delta E_{ch}^{ji}|$ and $\Delta E_{ch}^{ik} \rightarrow - |\Delta E_{ch}^{ik}|$. Given this definition, the tunneling current I_L through the left junction of the SET can be calculated from the tunneling rates using equation 2.38

$$I_{L} = -e \sum_{n} \left[\Gamma_{Li} \left(n \right) - \Gamma_{iL} \left(n \right) \right] p \left(n, t \right), \qquad (2.40)$$

where *p* is the probability that there is n electrons in the island and *L* denotes the left junction. The framework of this thesis considers only low DC-biased systems, so the current $I = I_L = I_R$. We can simplify the equation 2.40 with two assumptions: tunneling follows two state limit, where the only relevant changes are from state n to n+1 and the reverse process n+1 to n, and we assume symmetric biasing $V_L = -V_R = V/2$. The two state assumption gives fairly accurate description of the I-V characteristics at low temperatures and qualitative reference for other cases. Taking these assumptions into accounts the equation 2.40 simplifies into

$$I = -e \frac{\Gamma_{Li}(n) \cdot \Gamma_{iR}(n+1) - \Gamma_{Ri}(n) \cdot \Gamma_{iL}(n+1)}{\Gamma_{Li}(n) + \Gamma_{iR}(n+1) + \Gamma_{Ri}(n) + \Gamma_{iL}(n+1)}.$$
(2.41)

The current *I* is plotted in figures 2.22a and 2.22b from equations 2.37, 2.38, 2.39 and 2.41. For plotting convenience, we are using terms IR_tC_i/e , C_gV_g/e and VC_i/e

instead of *I*, V_q and *V*.



FIGURE 2.22: (a)The gate dependent I-V characteristics of SET. (b) The I-V-curves corresponding to the same colored lines in a. The threshold voltages for the black curve are indicated by the green dashed lines.

The current dependency on the bias voltage V has plateau regions (Coulomb blockade) and symmetric, parabolic or linear conduction regions as shown in figures 2.22a and 2.22b. Also, one can observe, that the current is oscillating in periodic manner, when gate voltage V_g is increased, which is known as the Coulomb oscillations. The threshold voltage V_{th} in figure 2.22b defines the boundary between the conducting and non-conducting regions.

The theoretical derivations so far have considered SET in such conditions, that the $e^2/(C_i k_b T) > 1$ and the Coulomb blockade is effectively blocking any tunneling at low bias voltages, which is also the case in the experimental part of the thesis. It should be still noted that there exist higher order tunneling processes than the discussed first order term, which can affect the experimental results. If the bias-voltage is relative high, then the two-state assumption does not anymore hold and we allow higher transitions than n to n+1, which allow process like cotunneling. In the cotunneling, the island experiences an electron-hole excitation, where (i) an electron tunnels first from left junction onto the island and simultaneously an electron tunnels from the island to the other side of the right junction or (ii) an electron first tunnels through the right junction and it is simultaneously replaced by an electron tunneling through the left junction. Typically these electrons do not occupy the same state of the island, in which case the process is called "inelastic" cotunneling. This will result in tunneling processes, where, even near zero temperature, tunneling current is non-zero at the Coulomb blockade region and the current $I \propto V^3$. Complementary to "inelastic" tunneling, there also exist "elastic" cotunneling, where one electron tunnels from left junctions to the island and then through the right junction. The tunneling rate for "elastic" cotunneling is lower than the "inelastic" cotunneling,

but it is directly proportional to the bias voltage *V*, which makes it viable even in low voltage region.

2.4.3 The capacitance and the threshold voltage of linear AuNP chain

The crucial part of SET is the precise creation of the isolated island, which can be achieved by many different ways, e.g., buckling carbon nanotubes [183] and fabrication of silicon nanowires between electrodes using UV lithography [40]. Another viable option to form the islands is to arrange spherical gold nanoparticles in a pearl-like chain, where each nanoparticle forms an island. As already discussed, the capacitance of the island defines the operation temperature and the threshold voltage between low conduction and high conduction regions. In order to work out the capacitance of a nanoparticles within the pearl-like chain of nanoparticle C_{Σ} , we have to solve the capacitance *C* of two spherical conducting object from equation 2.42 [186].

$$C = 2\pi\epsilon_0\epsilon_d a \sum_n \frac{\sinh\left(\ln\left(D + \sqrt{D^2 - 1}\right)\right)}{\sinh\left(n \cdot \ln\left(D + \sqrt{D^2 - 1}\right)\right)},$$
(2.42)

where the *a* is the radius of the sphere, ϵ_m is the dielectric constant of the medium in between the spheres, constant D = (2a + d)/2a and *d* is the smallest distance between the surfaces of the objects. The investigated pearl-chain is defined as chain of *n* particles on insulating (silicon oxide) substrate with external contacts at the ends as shown in figure 2.23. Here, we assume that the insulating layer is thick enough that the capacitive coupling to the substrate is neglectable and, for simplicity, particles are equally separated from each other and the external contacts.



FIGURE 2.23: The capacitance calculation of pearl-like spherical gold nanoparticle chain, where particle-particle and particle-contact distances are d. The chain is divided from the middle into k and N-k branches with the capacitances C_{left} and C_{right} , respectively. By summing the capacitances of these two branches one can solve the capacitance of the middle island.

The capacitance C_{Σ} can be solved by grounding both external contacts of the chain and by applying Kirchhoff's laws [187], where the number of junctions or capacitance is N = n + 1. The threshold voltage, when the external biasing exceeds the Coulomb blockade threshold, can be then calculated using equations 2.42 and

2.43, where the capacitance *C* is solved numerically.

$$C_{\Sigma} = C_{left} + C_{right} = \frac{2C}{N},$$

$$E_{ch} = eV_{th} = \frac{e^2}{C_{\Sigma}} = \frac{N \cdot e^2}{2C},$$

$$\Rightarrow V_{th} = \frac{N \cdot e}{2C}.$$
(2.43)

2.5 Dielectrophoretic trapping of nanoparticles

The electrical characterization of any nanomaterial or -compound requires establishing suitable external contacts between the measurement setup and the sample, which can be fairly challenging for a suspended sample like the pearl-like, linear assembly of three nanoparticles investigated in this thesis. There are few general methods to realize this: One way is to randomly deposit investigated materials on, e.g., silicon surface and post-fabricate contact electrodes, which is a process regularly employed to fabricate carbon nanotube transistors [188]. Other possibility is to utilize AFM to make either one or both external contacts and apply voltage to the AFM tip(s) to measure the I-V-curves [189].

However, both of these methods require either specialized instrumentation or the fabrication of contacts involves chemical processing, which can affect the properties of the studied species. Instead, we are using dielectrophoresis (DEP), which is a relatively simple way to trap chemically intact assemblies between fingertip electrodes, although the positioning of the assembly to achieve suitable tunneling junction still remains a challenge.

In this section, I will provide the fundamentals of dielectrophoresis and how it is related to the trapping of the studied assemblies. Dielectrophoresis is a phenomenon [190–192], where a polarizable particle in an external inhomogeneous electric field experiences a non-zero force, which drives the particle to either the maxima or the minima of the electric field (see figure 2.24a). If the same particle is in a homogeneous field, it does not experience any driving force (figure 2.24b).

The derivation of the DEP force and conditions for trapping are analogous to the aforementioned dipole excitation of metallic nanoparticles. Here, we assume that polarizable particles do not influence the imposed electric field or that the particles interact with each other, which is fairly good approximation if the concentration of the investigated species is low enough so that they can be considered noninteracting. In DEP, the electric field \vec{E} causes charge separation into poles ($\pm q$) within the particle, and both poles experience different Coulombic or electrophoretic forces $\vec{F_p}$ and $\vec{F_n}$ (see figure 2.24a). For a polarizable particle in homogeneous field, these



FIGURE 2.24: The fundamentals of dielectrophoresis. (a) Inhomogeneous electric field causes non-zero net force on the particles, that drives them towards either the maxima or minima of the electric field gradient. The propagation direction depends on the permittivities of the materials (ϵ_1 and ϵ_2) and the medium (ϵ_d). (b) In a homogenous electric field, a polarizable particle experience zero net force.

forces cancel each other and the overall force is zero. The net force \vec{F}_{DEP} is

$$\vec{F}_{DEP} = \vec{F}_p - \vec{F}_n = q\vec{E}\left(\vec{r} + \vec{d}\right) - q\vec{E}\left(\vec{r}\right),$$
 (2.44)

where \vec{r} is the position vector of the $\vec{F_n}$ and \vec{d} is the vector between the poles. Since the distance *d* is small, we can expand the term $\vec{E}(\vec{r} + \vec{d})$ in the equation 2.44 as Taylor series

$$\vec{E}\left(\vec{r}+\vec{d}\right) = \vec{E}\left(\vec{r}\right) + \vec{d} \cdot \nabla \vec{E}\left(\vec{r}\right) + O\left(d^{2}\right),$$

$$\vec{F}_{DEP} = q\left(\vec{E}\left(\vec{r}\right) + \vec{d} \cdot \nabla \vec{E}\left(\vec{r}\right) + O\left(d^{2}\right)\right) - q\vec{E}\left(\vec{r}\right),$$

$$\vec{F}_{DEP} \approx q\vec{d} \cdot \nabla \vec{E} = \vec{p} \cdot \nabla \vec{E},$$
(2.45)

where $\vec{p} = q\vec{d}$ is the dipole momentum of the particle. The consequence of the equation 2.45 is that the DEP force is driving the particle toward the extreme of the field gradient. The dipole momentum \vec{p} can be expressed as

$$\vec{p} = \alpha \left(V, \epsilon_d, K_i \right) \vec{E},\tag{2.46}$$

$$\alpha = 3V\epsilon_d \cdot \operatorname{Re}\left[K_i\left(\epsilon_p, \epsilon_d\right)\right],\tag{2.47}$$

where α is the effective polarizability of the particle, which depends on the volume V of the particle, the permittivity of the dielectric medium ϵ_d , the permittivity of the particle ϵ_p and the Clausius-Mossotti factor K_i [193]. Typically the Clausius-Mossotti factor does not have any analytical solution and, for simplicity, we are considering

an ellipsoid, since the Clausius-Mossotti factor for ellipsoid can be expressed analytically as

$$K_i(\epsilon_p, \epsilon_d) = \frac{\epsilon_p - \epsilon_d}{3 \cdot (\epsilon_p + N_i(\epsilon_p - \epsilon_d))},$$
(2.48)

where N_i is the depolarization factor of ellipsoid. Here, the direction of the DEP force depends heavily on the permittivities of the particle ϵ_p and surrounding medium ϵ_d , where two similar particles or ellipsoids with different permittivities can travel to opposite directions as shown in figure 2.24a in accordance to the Clausius-Mossotti factor in equation 2.48.

In liquid medium, the particles are always in random Brownian motion, the DEP trapping requires that the DEP force \vec{F}_{DEP} overcomes the Brownian force \vec{F}_B . To compare the two forces, we have to combine equations 2.45, 2.46 and 2.47

$$\vec{F}_{DEP} \propto V \epsilon_d \operatorname{Re}\left[K_i(\epsilon_p, \epsilon_m)\right] \nabla\left(E^2\right),$$
(2.49)

$$\vec{F}_B \propto V^{-1/3}$$
. (2.50)

The equations 2.49 and 2.50 have several consequences. Since the DEP force is proportional to the volume of the particle V and the Brownian force is inversely proportional to V, the DEP trapping should favor larger particles over smaller ones, which can be exploited to trap only the desired particles by filtering away the larger undesired particles before the trapping. Secondly, the DEP force is frequency dependent, since the factor $\text{Re}[K_i]$ depends on the frequency and, because the factor can be positive or negative, the direction of the DEP force can be changed by tuning the frequency or changing the material. In this context, the DEP is defined as positive, if the DEP force is pointing towards the field maximum, and negative, if the DEP force is pointing towards field minimum.

In this thesis, we are using well-established DEP trapping scheme [85, 194], where a sinusoidal AC voltage is applied to a fingertip electrode structures to attract DNA and AuNP based nanostructures towards electric field maxima located in the fingertips. The two opposite field maxima will align then nanostructures to the gap of the electrode structure, where after the I-V-characteristics of the trapped structures can be measured.

Chapter 3

Plasmonic metasurface and probe studies

3.1 Gold nanostructures by DNA Assisted Lithography

Over the past decades, the fields of nanoelectronics and -optics have greatly benefited from the development of conventional nanolithography methods, e.g., UVlithography and electron beam lithography (EBL). Especially the field of optics has had plethora of applications ranging from fluorescence [161, 195, 196], Raman [21, 197, 198] and IR spectroscopy [199, 200] to metasurface [201] and refractive index based sensing [202, 203]. As discussed earlier, to satisfy the demand for ever diminishing electric and optical components with high resolution and fast production has become recently more difficult, since the traditional lithography methods have been limited by the serial nature of the fabrication process (EBL) or by the increasing complexity of the instrumentation (photo-lithography). These limitations have created a demand for alternative, bottom-up methods to fabricate micro- and nanodevices, which would circumvent the issues of serial fabrication or overly complex instrumentation.

To tackle this, the soft-lithography approaches [204] utilizing self-assembly and molecular-scale structures have been investigated during the past few decades. As mentioned in the earlier chapters, one of the more interesting molecules in this field has been the DNA molecule, since different materials, e.g., metallic nanoparticles, enzymes and carbon nanotubes can be attached to it via chemical methods. Although these chemical methods are parallel in nature and the actual size of the devices can be scaled to 100 nm, typically the resolution is somewhat limited by the random nature of the fabrication process. This can reduce or limit the operation range and conditions of the device, e.g., the resonance wavelength of a fluorescence enhancer, or there are unwanted by-products after synthesis, which has to be removed for the method to be applicable for the industry. To combine the strengths of the conventional- and soft-lithography worlds, we have devised the DNA Assisted Lithography (DALI) method, where the high parallel throughput, high yield and superior resolution of the DNA self-assembly are combined with robustness of the conventional lithography procedures.

In this section, the DALI method is utilized to create arbitrary shaped plasmonic nanostructures on transparent surfaces and we characterized the optical properties of the created structures. In addition, we will demonstrate that the DALI method is a viable option to create large scale, planar and cost efficient metasurfaces with sub-10-nm feature size.

3.1.1 Fabrication of planar, arbitrary shaped metal nanostructures using DALI method

The different steps of the DALI methods are illustrated in figure 3.1, where three distinct shapes were selected for fabrication: a so-called Seeman Tile (ST), a bowtie origami (BO) and a chiral double-L (CDL). The key point of the method is to convert the shape of surface immobilized DNA origamis directly into metallic nanostructures (see figure 3.1a), where the high spatial resolution and the arbitrary design of the DNA origami structures can be exploited. Other methods, e.g., metal-seed based lithography [205] utilize similar idea, but the resolution is limited to the size and the packing density of the AuNPs, and the end result is typically a granular structure that retain their original features as long as the substructure size is larger than 50 nm [90].

The fabrication process consists of several steps as shown in the figure 3.1b. First, the sapphire (Al_2O_3) or silicon nitride (Si_3N_4) substrate is cleaned with acetone and isopropanol (Step 1), then an amorphous silicon layer is grown on top of the substrate (Step 2) using plasma enhanced chemical vapor deposition (PECVD). The surface is turned hydrophilic by oxygen plasma treatment and DNA origamis are deposited on the surface (Step 3). A silicon dioxide layer is then grown on top of the silicon layer using CVD method (Step 4), where the origami shapes inhibit the growth of SiO_2 leaving origami shaped holes into the silicon layer. The silicon layer underneath the SiO_2 is etched through at the origami openings using reactive ion etching (RIE, Step 5). Next, a metal layer is evaporated using physical vapor deposition (PVD) in ultra-high vacuum (Step 6). In the last two final steps, the silicon dioxide and silicon layer are removed using hydrogen fluoride (HF)-based wet etching (Step 7) and oxygen plasma etching in RIE (Step 8), and the sample is ready for optical characterization.

The figure 3.1c shows STs, BOs on a silicon nitride substrate and CDLs on a sapphire substrate, where the whole substrate is covered with the metallic structures. The height of all of the fabricated gold structures was 20 nm. The STs and CDLs had roughly the length of 100 nm and the width of the arms is 20 nm. The bowties had the length of 125 nm (long axis) with sub-60-nm triangles and the gap



FIGURE 3.1: Description of the DNA assisted lithography method and the fabrication results.(a) Left column: illustrations of the different origami structures. Middle column: AFM images of the corresponding origami deposited on silicon. Right column: SEM images of the resulting metal structures. (b) Different fabrication steps of the DALI methods. (c) SEM images of STs and BOs on a silicon nitride surface and CDLs on a sapphire substrate.

size is 8-20 nm. The STs and CDLs had roughly 50 % yields and the bowties had either 50 % yield (0 nm < gap <40 nm) or 76 % yield (gap > 40 nm). Also, the CDL fabrication yielded distribution of 51 % S-shaped and 49 % Z-shaped CDLs. The STs and CDLs were analyzed based on how correctly all the arms were formed, whereas the bowties were evaluated based on the gap size, the angle between the triangles and how correctly the triangles were formed. The extra criteria for bowties are due to the fact that all of them influence the LSPR wavelength of the bowties.

Typical problems for the fabricated STs and CDLs were missing or malformed arms, which are most probably caused by the deposition process: if the arms roll up or touch each other after the deposition, undesired shapes will be present in the final results. The triangles of the bowties were typically quite nicely formed, but the control over the gap size and especially the angle between the triangles was less precise. The angle differences are again due to flexibility of the DNA origami during the deposition.

All these discoveries highlight that the DNA origami deposition process is a key factor in the DALI method, and extra attention needs to be put into design of the DNA origami to avoid the flexibility issues. One advantage of the DALI compared to chemical methods is that essentially structures can be fabricated from any material that can be physically evaporated and is not to be dissolvable in HF, which includes large variety of metals and semiconductors [206]. Besides the gold structures in figure 3.1, we have demonstrated that nanostructures can be fabricated from copper and silver (see appendix Pub.III).

3.1.2 Optical measurement setup

The optical microscopy has been utilized for a long time in research and industry and there exist multiple different imaging method, e.g., bright field, dark field, differential interference contrast (DIC) microscopy. Especially the dark field microscopy have utilized for metal nanoparticle detection due to simple instrumentation and high sensitivity and contrast, where even individual nanoparticles can be probed [156, 160]. Here, we describe the used DF microscope setup and how a typical experiment was carried out to characterize different nanoassemblies and nanostructures.

The microscope system for measurements of individual nanoparticles and nanostructures consisted of uprights microscope system coupled to a spectrograph equipped with EMCCD-camera via optical fiber(see figure 3.2). The fiber was connected from open end to one of the output ports of the microscope and from the collimator end to a custom-made switch box. The idea of the switch box is to allow bidirectional signaling, where the output signal, i.e., the light coming from the sample is focused after the collimator to the slit of the spectrograph using a lens. The input signal is a LED light, which can be used to illuminate the position of the fiber on the sample surface. Samples were monitored and images of the sample surface taken using Canon EO5 6D camera, which was connected to the other output port of the microscope.

We employed Olympus BX51TRF-microscope with several MPLANFL-N brightfield/darkfield objectives (5x / 20x / 50x), where the 50x objective was used to measure spectra of nanoparticles. For excitation, either Olympus 75 W Xenon lamp (L2194-01) or Olympus 100 W halogen lamp inside IR-lamp housing (Olympus U-LH100IR-1-7) was utilized. The Xenon lamp was used in the reflection measurements and the halogen lamp in the transmission measurements. The EMCCD-camera was Andor Ivac DR-324B-FI and the spectrograph was Princeton Instruments (Acton) SP2150. Both systems were controlled using Andor Solis (version 4.18). The optical fiber was Thorlabs UM22-300-custom with the core size of 300 µm. For the polarization measurements, we used Olympus U-AN360-3 analyzer and Thorlabs linear polarizer (LPVISE200-A 2). To illuminate the position of the fiber on the sample surface, we used Thorlabs M530L3 mounted LED ($\lambda_{max} = 530$ nm). The microscope system with the spectrograph was placed inside an electromagnetically shielded room, and the connection between the microscope system and the measurement computer was established via USB fiber extender (Black box IC404A). This was done to maintain the electrical isolation and to reduce background illumination.



FIGURE 3.2: Single particle spectroscopy (SPS) setup. (a) The SPS setup in the polarization dependent transmission dark field measurements during the initial analyzer-polarizer calibrations. (b) The same SPS setup as in (a) but during the DF measurements. (c) The SPS setup in the reflectance DF measurements. (d) Typical DF image of a DALI fabricated sample. The green circle spot indicates the fiber position and the gold/yellow rectangle highlights the area in e. (e) SEM image of a malformed CDL and two bowties on a sapphire surface.

Two different kinds of measurements were performed during the studies of this thesis. Either polarization dependent scattering spectra of nanoshapes on transparent sapphire substrate were recorded or movement of spherical gold nanoparticles on top of non-transparent gold surface was observed by measuring the scattering spectra. For the former case, we used transmitted light in the dark field mode, where the dark field was achieved using Olympus dark field condenser (U-DCW) as shown in figures 3.2a and 3.2b.

The polarization measurements were carried out in several steps. In the first step, the sample on a sapphire chip was mapped by taking images of the same sample area using both dark field imaging and scanning electron microscope (SEM) (see figures 3.2d and 3.2e). From the SEM images, we calculated the angle ϕ between the primary axes of the structures and either horizontal or vertical axis of the grid (red scattered lines in figure 3.2d). Then the sapphire chip was placed to the microscope together with the polarizer and analyzer as shown in figure 3.2a. A narrow single slit mask was placed on top of the polarizer in such a way that the opening of the slit followed the polarization axis of the polarizer and both the mask and the polarizer were brought as close to chip as possible. Now the image of the slit, which is related to the polarization angle, could be seen on the sample surface, and the slit line was turned so that it was parallel to either vertical or horizontal axis. The analyzer was turned, until minimum signal was detected. Now the polarization angle of the analyzer was perpendicular to the polarization angle of the polarizer and followed either the vertical or horizontal axis of the grid. The polarizer was removed and the dark field condenser was put in its place (see figure 3.2b). Immersion oil was placed on the condenser, which was then pushed as close to the microscope slide as possible. The system was left to settle for 1 h, and after this the fiber spot was placed on the target of interest, the analyzer was turned so that we started from one of the primary axis of the target and the polarization dependent scattering spectra were recorded for different polarization angles from 0° (starting position) to 180° (back to the same position). Typically the integration time was 6 s with 10 averaged scans. Integration time can be extended, but the background radiation starts to influence the measurement after 2 min and after 5 min it significantly hinders the measurements.

The measurements of spherical gold nanoparticles were done in a reflection mode as shown in figure 3.2c. The deposition of the DNA coated AuNPs is fully described in the section 3.2, and here we assume that AuNP have been immobilized to the gold surface via linker DNAs and observed using the microscope setup. Similarly as before, the optical fiber was used to select the interesting particles and individual scattering spectra under different biasing voltages were recorded using 3-5 s integration time with 3 scan averaging. One issue here is that we are not using water immersion objective, which results in reflection from the interfaces between ITO cover and both air and water medium. These reflections are more pronounced if the ITO cover is not perfectly perpendicular to the objective and thus a lot of care

should be used when sealing the chamber with ITO cover.

3.1.3 Optical characterization of different planar gold structures

The metallic bowtie shape is widely harnessed as an antenna for different optical applications due to the highly confined plasmonic mode in the gap as discussed in the theory section. Thus, it is important to determine the wavelength dependent field enhancement. Especially in surface-sensing [207] or fluorescence enhancement [161] it is important to tune the LSPR wavelength to the visible spectrum, which mainly requires that the size of the antenna is below 100 nm (on a glass substrate). Other main factors that influence the LSPR peak position are the angle between triangles and the gap size. To assess the applicability of the DALI fabricated bowties, individual bowties and STs were characterized by measuring the polarization dependent LSPR scattering spectra as shown in figure 3.3, where the STs are used as a control due to the aforementioned symmetric optical response.

The experimental and the already introduced simulated LSPR scattering spectra for the BOs are shown in figures 3.3a, 3.3c and 3.3e and for the STs in figures 3.3b, 3.3d and 3.3f. For the BOs, the LSPR peaks were located along the gap (λ_{max} from 704 nm to 814 nm) and perpendicular to the gap (λ_{max} from 650 nm to 700 nm) and, for the STs, the LSPR peaks were mostly located along the arms (λ_{max} from 646 nm to 690 nm). It should be pointed out that most of the LSPR peaks are in the visible spectrum despite the high refractive index of the sapphire substrate (n \approx 1.76). The LSPR peak of the BO gap mode has higher intensity and is at longer wavelengths compared to the perpendicular mode, which has been reported earlier in literature and is in agreement with the theory [161, 163, 207]. In the case of STs, the peaks should overlap if the arms are symmetric, and asymmetric arms induce a slight displacement. These findings are also in good agreement with the FEM simulated data (dashed lines in figures 3.3a and 3.3b). Although the LSPR maxima of both STs and BOs are at similar wavelengths, the key difference is the maximum obtained field enhancement as shown in the insets of the figures 3.3a and 3.3b, where the gap mode of the BO has roughly four times higher field enhancement compared to the other ST or BO modes.

As discussed in the fabrication section, the BOs and STs have some variations in the physical dimension and the characteristic features, which will then influence the optical properties. For BOs, the perpendicular modes are roughly at a constant wavelength, but the gap mode peaks have larger variations. The size and the shape of the BO triangles are approximately the same for all of the samples, but the gap size and the angles are differing. As discussed in the theory sections, if the triangles are close to each other, the LSPR peak will blueshift as the gap between triangles increases, and when the angle between the triangles deviates from symmetric 180° bowtie case, the peak will redshift up to 40° angle. When comparing the different BOs, the BO with the LSPR peak at the longest wavelength (figure 3.3e) has also



FIGURE 3.3: The polarization dependent scattering spectra of the metallic bowties and STs. Arrows in the insets mark the linear polarization directions. (a),(c),(e) LSPR scattering spectra of metallic bowtie structures. The higher redshift of the gap mode peak in e compared to a and c is due to narrower gap. (b),(d),(f) LSPR scattering spectra of metallized cross structures. The dominant peak positions in b and d are along the arms, whereas in figure f they are between the arms.

the smallest angle shift (7 $^{\circ}$ from the perfect bowtie alignment) and the smallest gap (5 nm) compared to the other BO samples in figure 3.3. As a comparison the sample in figure 3.3a has twice the gap and 25 $^{\circ}$ angle shift. This would suggest that the gap size has overall larger impact on the LSPR maxima than the angle shift, hence the redshift in the figure 3.3e.
Most of the STs had the LSPR maxima along the arms, which is expected from the theory sections. However, for some samples the maxima were between the arms as shown in figure 3.3f. The exact reason for this is unknown. However, there are several possible factors that can explain this. The ST in the figure 3.3f is slightly more rounded than the other samples in figures 3.3b and 3.3d, which might cause disk-like qualities, and, as discussed in the theory part, the maxima would then be at the elliptical axes, that are not necessary along the arms. Also, the height profile of STs might not be flat but rather granular, which can influence the optical properties of the sample. Despite these undesired varieties, the BO antenna covered sapphire substrates were successfully used in SERS to detect two common markers Rhodamine 6G and 2,2-bipyridine as shown in figure 3.4, meaning that the DALI method is robust enough to produce functioning metasurfaces.



FIGURE 3.4: The SERS spectra of Rhodamine 6G and 2,2-bipyridine on bowtie metasurface (inset). The flat spectra under the SERS spectra are the Raman signals recorded by the same settings but at a position without bowties.

3.2 Electric field guided DNA and AuNP based nanoactuator

This chapter involves fabrication and characterization of reversibly controllable nanoactuator assembly, where 80 nm AuNPs functionalized with biotinylated single stranded DNAs are immobilized on chimeric avidin coated gold surface. The chimeric avidin and biotin binding scheme was used, since the avidin-biotin interaction is one of the strongest non-covalent bondings (Ka $10^{15}M^{-1}$) [208]. This assembly was used to study the folding properties of a hairpin-DNA strand by moving the charged AuNPs using electric field, which also stretched the hairpin-DNA at the same time. The detection of the AuNP motion is done by tracking the localized surface plasmon resonance of the AuNPs.

One of the main challenges is to induce and detect motion for a nanoparticle via electric field, since e-field manipulation is typically done for large assemblies of nanoparticles, where the collective force induces motion. This technique is feasible, if the charge of the particles is high enough. The technique could be also extended to study other proteins and biomolecules and besides the conformational studies the nanoactuator motion can be utilized in applications, e.g., surface enhanced Raman spectroscopy and fluorescence enhancement, where generally the optical properties of such probes cannot be adjusted after the fabrication or if it is possible, then the process is irreversible.

3.2.1 Assembly and operation of the DNA-AuNP actuator

The nanoactuators have had many different implementations ranging from polymer brushes to enzyme-based biosensors. Typically, these sensors consist of a substrate (sensor surface) and a probe, that is immobilized to the substrate. The actuator may require manipulation of the probe using solution flow, electric or magnetic fields or optical trapping.

One of the key features or challenges in such a system is the attachment scheme between the probe and the substrate. To assess the efficiency of our attachment scheme, we divided the assembly of our actuator in several parts as shown in figure 3.5a-c: The first step was the binding of neutral, cysteine-tagged chimeric avidins on the gold substrate, then surface passivation of the substrate using blocking agents and finally the immobilization of the AuNPs functionalized with biotinylated ss-DNA.

This assembly was used first to characterize folding properties of single stranded DNA with varying lengths (3 nm, 8 nm and 15 nm), and these results were then applied to study folding properties of hairpin-DNA (see appendix table A.1 for DNA sequences). The lengths of 8 nm and 15 nm were chosen, since they correspond to open (15 nm) and folded (8 nm) conformations of the hairpin-DNA. These DNA coated particles were fabricated using the salt-aging method (see appendix Pub.V).



FIGURE 3.5: Sketch of different DNA-AuNP actuator assembly steps.(a) Binding of cysteine-tagged chimeric avidin to the gold surface.(b) Passivating rest of the surface using blocking agent.(c) Immobilization of biotinylated AuNPs.(d)-(f) Depending on amount of biotin-avidin binding, distance between AuNP and gold surface varies (d_1 vs d_2). By applying electric field, AuNP can be moved away from the surface (d_3) or brought close to the surface (d_1).

The nanoactuator operation involves motion of the charged AuNP using electric fields as shown in figures 3.5d, 3.5e and 3.5f and the simultaneous detection of the localized surface plasmon resonance (LSPR) of the AuNP. The electric field is applied to the liquid medium surrounding the actuator by biasing the sensor surface and the counter electrode above it. The distance between the gold surface and the AuNPs heavily influences the LSPR scattering spectra of the AuNPs as discussed in the theory section, which can be therefore used to track the AuNP position.

One vital feature in the operation and immobilization of the DNA-AuNP probe is the amount of bonds between the chimeric avidin coated gold surface and biotinylated-AuNPs. It has been shown [209] that the attachment and detachment of nanoparticles from surfaces depends on the binding geometry and surface density of the receptor (avidin) and its ligand (biotin). This would mean that AuNPs with high amount of biotin-avidin bonds are very tightly bound to the sensor surface and less probable to detach from the surface (figure 3.5d versus figure 3.5e), but, on the other hand, have less degree of motion available, which can be detrimental for the applicability of the actuator. To assess this, we are varying the surface density of the biotinylated DNAs by substituting them with short blocking-DNAs and then characterizing the motion of AuNPs with different biotin densities under influence of electric field.

Attachment of proteins and biotin-AuNPs to sensor surfaces

The surface attachment of chimeric avidin and blocking agent (BSA or SH-PEG) was characterized using RA-SPR, which is sensitive technique to characterize protein, biomolecule and, to some extent, nanoparticle immobilization as discussed in the theory section. The experiment was carried out by first flushing the gold surface with PBS buffer followed with 16 min injection of the chimeric avidin solution (75 μ g/ml) diluted in PBS buffer. After the injection, the gold surface was flushed with PBS buffer and the next compound was injected for 16 min.



FIGURE 3.6: The RA-SPR curves showing the attachment of chimeric avidin and blocking agents to the gold surface and subsequent immobilization of AuNPs functionalized with biotinylated-DNAs.(a) RA-SPR curve for immobilization of AuNPs functionalized with 15 nm linker DNA (upper) or 8 nm linker DNA (lower). (b) Hairpin-DNA-AuNP immobilization with (lower) or without (upper) extra biotin passivation. The avidin attachment follows similar pattern as in a and it is therefore cut from the graph. The green dotted and dashed lines indicate positive SPR shift for the non-biotin-passivated sample and negative shift for the biotin-passivated sample, respectively. (c) RA-SPR sensor surface after AuNP immobilization in figure b (upper curve) without biotin passivation.(d) RA-SPR sensor surface after AuNP immobilization in figure b (lower curve) with biotin passivation.(e) Plain sensor surface before any attachments.

The binding of chimeric avidin and surface passivation of the gold surface can be seen as a positive angle shift of the SPR resonance in figures 3.6a and A.1: the injection of chimeric avidin and blocking agent is followed by a linear rise in the resonant angle, which saturates after 3.5 - 4.5 min for avidin and $\sim 1 \text{ min}$ for the blocking agent. This is expected, since the chimeric avidin and both BSA and SH-PEG are DPG materials that only increase the refractive index and thus also the resonant angle. The saturation of each compound indicates, that the surface is mostly covered by the compound(s) and the equilibrium between association and dissociation has been reached. The blocking agent saturation is also quicker than the avidin saturation. This is reasonable since the surface is already covered by the avidins and the blocking agents are much smaller than the avidin and thus bind more rapidly to the surface.

For AuNP immobilization, we observed that the AuNP functionalized with 15 nm DNA strands had overall higher positive shift compared to the AuNPs functionalized with 8 nm DNA strands (see figure 3.6a). As discussed in the theory section, this difference comes most probably from the coupling effects between AuNP and the gold surface, where longer DNA linkers have higher positive shift due to domination of the mass-contribution over the coupling effects. The small negative shift at the beginning of the AuNP injection and at the end of the injection is due to the bulk effects.

The hairpin-DNA-AuNP had also a small positive shift in the SPR signal as shown in figure 3.6b (the two green dotted lines) indicating binding of the AuNPs. By passivating the avidins bound to the surface with free biotin before AuNP injection, the corresponding shift was negative (the two green dashed lines in figure 3.6b), which means that AuNPs are not binding efficiently and rather compounds are dissociating from the surface. This was further confirmed by the dark field images in figure 3.6c-e, where the non-biotin-passivated surface shows larger amount of bound AuNPs compared to the biotin-passivated surface, which is closer to the plain sensor surface shown in figure 3.6e. In addition, the hairpin-DNA-AuNP shift is more comparable to the shift of the 8 nm DNA strand than to the shift of the 15 nm DNA strand, which means that the hairpin-DNA is mostly in a folded state. The results together indicate, that the attachment scheme between the surface bound avidin and the biotinylated-AuNPs is functioning as intended.

Assessment of interactions between hairpin-DNA-AuNP and chimeric avidins

It was concluded in the last section, that the biotinylated-AuNPs are attaching to the chimeric avidin coated gold surface. However, the SPR measurements do not specify the degree of the bonding between the two, which can affect the motion of the AuNPs as shown in figure 3.5. To characterize this, we minimized the number of biotins on the hairpin-DNA-AuNPs by substituting part of the hairpin-DNA with a shorter blocking-DNA. This was done during the DNA adsorption on the AuNP surface by mixing the hairpin-DNA with blocking-DNA from ratio of 1:0 (pure hairpin-DNA) to 0:1 (pure blocking-DNA). These different AuNP batches were characterized with dynamic light scattering by measuring the initial hydrodynamic diameter D_{ini} (see appendix figure A.3), which was found to overall decrease as the amount of hairpin-DNA was decreasing due length difference between the hairpin-DNA and the blocking-DNA.

Next, we tested the binding efficiency of each batch by aggregating them with chimeric avidins in solution, where the hydrodynamic diameter D_{final} should increase due to aggregation as shown in figure 3.7a. The aggregation process was then characterized by plotting the relative increase of the hydrodynamic radius $D_{rel} = (D_{final} - D_{ini})/D_{ini}$ as show in figure 3.7b, where increase in the hydrodynamic diameter should be more pronounced for samples with higher biotin density.



FIGURE 3.7: Characterization of the binding efficiency of chimeric avidins and the hairpin-DNA-AuNP.(a) Schematic view of the chimeric avidin and biotin-AuNPs aggregation process.(b) The relative increase in diameter D_{rel} versus hairpin-DNA to blocking-DNA ratio, where the size of the aggregated species decrease overall as amount of hairpin-DNA decreases.

It was observed, that the plain AuNPs had 20 % increase in the hydrodynamic diameter when mixed with avidins, which is most probably due to either the non-specific binding of the chimeric avidin to AuNPs or buffer related bulk effects. Also, the pure blocking-DNA-AuNPs had similar increase, which indicates that the DNA strands themselves do not cause aggregation. The lowest mixture of hairpin-DNA and blocking-DNA (1:999 or 0.1 %) showed still significant increase (30 %) compared to the control samples. If assuming that the 20 % increase for control samples is due to bulk effects and there are no dimer forming in the 0.1 % sample, then the increase from 20 % to 30 % would correspond to roughly a size of one avidin layer (see appendix Pub.V for calculations).

As the amount of hairpin-DNA was increased, the relative hydrodynamic diameter also increased until ratio of 1:1. After this, the relative hydrodynamic diameter started to decrease, which indicates that the aggregation process is hindered. This is reasonable, since too high surface density of biotins does not allow correct orientation between the chimeric avidin and biotins and thus the binding is hindered [210]. Since the aggregation is still happening at 0.1 % ratio, this ratio was selected for further studies and we fabricated AuNP functionalized with ssNDA (3 nm, 8 nm and 15 nm) with the same ratio and, as a control sample, purely hairpin-DNA functionalized AuNPs were fabricated as well.

Simulations of the LSPR of AuNP coupled to gold surface

The AuNP close to a conductive surface was introduced in the theory section, where the detection of AuNP position and hence the motion can be tracked by measuring the LSPR scattering spectra of the AuNP. Since we cannot directly correlate position (distance *d* in figure 3.8) of our nanoactuator to the LSPR spectra experimentally, the coupling depended LSPR spectra of AuNP close to a Au-surface was solved using Finite Element Method (FEM) in Comsol as show in figure 3.8. In the experiments, we used unpolarized light, so both TM- and TE-polarized light were used in simulations.



FIGURE 3.8: (a) The simulated TM polarized and (b) the simulated TE polarized LSPR scattering spectra of AuNP close to a gold surface. (c) The simulated LSPR peak versus the distance *d* plot, where the resonance of the AuNP is redshifting as the distance is decreasing due to strong plasmonic coupling with the surface. There are also secondary peaks forming after 4 nm due to in-plane dipole-dipole interactions.

For both polarizations, it was observed that the main LSPR peak shifts from 570 nm to 660 nm, when the distance d between the particle and surface changes from 20 nm to 1 nm. Also, there is a secondary peak appearing around 550 nm, when distance d is less than 4 nm. As explained in the theory section, this is due to interaction between the real particle and a conductive surface induced imaginary mirror particle. The main LSPR peak is related to the parallel dipole excitation of the spherical nanoparticle, which experiences redshift as the distance d is decreased.

The secondary peak corresponds to the in-plane dipole-dipole interaction, which has similar behavior but starts at shorter wavelengths. Both of these interactions create strong electric fields, that are confined between the particle and the gold surface (see figures 2.14e, 2.14f and A.5a-d). The distance *d* versus the LSPR peak maxima is plotted in figure 3.8c, where it can be seen that the sensitivity of the coupled system increases as the distance decreases. We used average values between the TM and TE polarized curves to correlate the distance to the LSPR maxima in the experiments due to the use of unpolarized light.

3.2.2 Electric field manipulation

The results of the preliminary tests were combined to construct the final nanoactuator assembly. The AuNP immobilization and the consecutive measurements were done in a sample holder with a liquid flow cell (see appendix figure A.2), which was designed so that the liquid could be pumped in and out, while a biasing voltage could be applied between the gold surface and the ITO glass counter electrode above it. The immobilized AuNPs could be observed at all times though the ITO glass.

Typical immobilization experiment was done by first treating the hydrophilic Au-surface on silicon chip with chimeric avidins and BSA, connecting the surface to the holder, sealing the surface inside a chamber with the ITO glass, pumping 50 µl of solution containing the biotinylated AuNPs using ISMATEC tubing pump and waiting 15-90 min until enough particles were immobilized. The surface was never allowed to dry during the sample preparation and measurements. Then the chamber was flushed, contacts with the ITO and the gold-surface were established with external circuit and the large scale motion was analyzed for both the ssDNAs and the hairpin-DNA functionalized AuNPs (ratio 0.1%). Also, single particle motion was recorded for the hairpin-DNA-AuNPs (both 0.1% and pure hairpin-DNA).

Large scale analysis of the nanoactuator motion under influence of electric field

The large-scale measurements were carried out to assess the degree of controllability and to characterize the average particle motion, that is influenced by the conformation and structure of the anchoring molecules. The measurements started by sweeping the bias voltage from negative to positive and back in steps, and between each step the system was let to stabilize for 1 min before taking dark field images of the samples (see appendix figure A.6). These images were then processed to extract the RGB data of each particle with respect to the bias voltage. The data was further converted into Hue (H) values (see appendix), because the conversion between the wavelength data and the Hue representation is relatively easy, since the visible spectrum can be presented as a linear scale of Hue from 0° to 360°. However, the exact conversion depends on the measurement system, and we needed to calibrate the conversion by measuring simultaneously the LSPR scattering spectra and the RGB values of several different colored particles and plotting the H values versus the LSPR wavelength curve (see appendix figure A.7). As a result, the LSPR wavelength can be connected to the distance *d* between the particle and the gold surface as shown in figure 3.8c.

It was found out that $H = 0^{\circ} - 20^{\circ}$ (wavelength = 600-620 nm, see figure A.7) roughly corresponds to the situation, where particles are close to surface (\leq 5 nm or less from figure 3.8c), and the H value over 60° (wavelength > 580 nm, see figure A.7) corresponds to case, where particles are far away from the surface (\sim 20 nm from figure 3.8c). The 5 nm limit is of importance, since it corresponds to the thickness of the avidin layer [211]. So if considering the full particle motion, i.e., from close to surface to far away and back, which relates to DNA unfolding and stretching, the minimum shift in the Hue is about 40°. To characterize this motion, we only selected for further analysis the particles that had at least one Hue shift over 40°, when the voltage switched several times from negative to positive side or vice versa.

The motion of particles in respect to the bias voltage and with the above threshold for the Hue changes was evaluated for the different ssDNAs (3 nm, 8 nm and 15 nm) and the hairpin-DNA functionalized AuNPs as shown in figures 3.9 and 3.10. These particles could be roughly categorized into four different groups: the particles in group 1 were pushed with negative voltage away from the surface (\sim 100°) and pulled towards the surface using positive bias voltage (orange histogram in figures 3.9 and 3.10), the particles in group 2 behaved opposite to group 1 in respect to the bias voltage (blue histogram), the particle in group 3 got stuck eventually far away from the surface (violet histogram) and the particles in group 4 got stuck close to the gold surface (green histogram). The black histogram shows overall data of the particles that had at least one 40° or larger shifts.

The reason for different behavior for group 1 and group 2 is not exactly clear, but it can be partially explained by the charge distribution of the used biotinylated-AuNPs (see appendix figure A.4), since there exist both negatively and positively charged particles in the 8 nm ssDNA and hairpin-DNA samples, which both have also significant portion of the particles in group 2 as shown in figures 3.9c and 3.10a. The group 2 is missing for 15 nm and 3 nm ssDNA samples, since both samples do not have a significant amount of positively charged particles (see appendix figure



FIGURE 3.9: The histogram data of the large scale motion of (a) the 15 nm ssDNA, (b) the 3 nm and (c) the 8 nm ssDNA-AuNP samples.



FIGURE 3.10: (a) The histogram data of the large scale motion of the hairpin-DNA-AuNP sample. (b)-(e) Illustration of hairpin-DNA-AuNP position under different bias voltages. The histogram data in figures b, c, d and e depicts the first (V= 0 V), third (V= -0.1 V), fifth (V= -0.2 V) and eighth (V= +0.1 V) histogram starting from the bottom in figure (a) (orange histogram). In the absence of electric field and for small field \vec{E}_2 the hairpin is closed (hybridized) and the particle is close to surface ($H_1 \approx 0^\circ$, indicated by the dotted line). When high enough field \vec{E}_3 is applied, the hairpin unfolds and the particle is moved far away from the surface ($H_2 \approx 100^\circ$, indicated by the dashed line). When opposite field \vec{E}_4 is applied, the particle is brought close to surface and the hairpin can fold back. Compared to the case a of plain ssDNA linker, a clear quantization/discretization to the folded and unfolded hairpin states (shown as the dotted and dashed lines) is visible instead or smooth drift of a Gaussian distribution.

A.4). Other possible explanation is the double-layer formation on the AuNP or the gold surface induced by the free ions in the solutions, which can affect the overall charge of the particles. These speculations still leave much room for interpretation and the AuNP behavior under electric field should be studied further to fully assess the situation.

The voltage dependent behavior of the 15 nm and 8 nm ssDNA-AuNP are shown in figures 3.9a and 3.9c, where the orange and blue histograms for 8 nm ssDNA-AuNP sample and the orange histogram for 15 nm ssDNA-AuNP sample show that the position of the ssDNA-AuNP distribution (Gaussian) is shifting mainly in a continuous manner as the voltage is swept. This is expected for a nanoactuator anchored via a flexible linker under thermal fluctuations. The position of the ssDNA-AuNPs with 3 nm linkers seemed to just disperse implying that the linker is too short to allow free movement of the particle and the observed motion of the particle might be caused by irreversible conformational changes in the chimeric avidin platform.

In contrast, the motion of hairpin-DNA sample seemed to fluctuate between 0° (close to the surface) and 100° (far away from the surface), when the biasing voltage was swept from negative to positive values and vice versa. This behavior is quite opposite compared to the 15 nm and 8 nm ssDNA-AuNP samples. This implicates that the unfolding of the hairpin has a certain voltage threshold, which has to be exceeded in order to push the particle away from the surface. When pulled towards the surface the hairpin refolds back, and thus we observe quantized fluctuation motion of the hairpin-DNA-particles.

Initially, the 15 nm and 8 nm ssDNA and hairpin-DNA functionalized AuNPs can be manipulated using e-fields, but eventually the collective motion was lost as seen in the two upmost histograms in the figures 3.9 and 3.10. The amount of movable particle was, e.g., for the hairpin-DNA-AuNPs 6.5% in group 1 and 5.1% in group 2 (see appendix Pub.V for more details), and most of the particles were unmovable and located near the gold surface (see appendix figure A.8) meaning that there are still a lot of contacts between the chimeric avidins and the biotinylated AuNPs.

Electric field manipulation of individual hairpin-DNA-AuNPs

The LSPR scattering spectra of individual hairpin-DNA-AuNPs (both 0.1% and pure hairpin-DNA) were also analyzed under influence of electric fields. During the large scale analysis, the most promising particles were selected for analysis (see appendix for experimental details). The results of the measurements are shown for both samples in figures 3.11, A.9 and A.10, where the figures 3.11a and 3.11b shows the LSPR scattering spectra of particles A and B with 0.1% hairpin-DNA, which had larger shifts in the LSPR peak compared to the particles C and D in figures 3.11c and 3.11d.



FIGURE 3.11: Manipulation of individual AuNP by electric field. (a),(b) E-field dependent LSPR scattering spectra of 0.1 % hairpin-DNA particles A and B, when voltage is swept from positive to negative side. The bolded lines have the corresponding bias voltages listed next to them. The purple and the blue lines indicate 530 nm and 660 nm peaks, respectively. (c),(d) E-field dependent LSPR scattering spectra of pure hairpin-DNA particles C and D, when voltage is swept from positive to negative side. (e),(f) DF images of the particle A under negative (e) and positive (f) bias voltages. (g),(h) Similar DF images for particle B as in e and f.

Voltage steps between each curve in figure 3.11a are 0.05 V in the first sweep from positive to negative voltage (starting from the bottom of the graph) and the subsequent sweeps have 0.1 V voltage step. Voltage steps are 0.1 V in figure 3.11b

and 0.5 V in figures 3.11c and 3.11d. The shifts could be also observed as color changes in the dark field images as shown in figures 3.11e and 3.11f for the particle A and in figures 3.11g and 3.11h for the particle B.

Notably, particles A and B needed lower biasing voltage of ± 0.5 V to have a significant movement of the LSPR peak compared to the voltage requirement of ± 3 V for particles C and D. This is reasonable, since particles C and D have higher amount of biotin groups on surface of the particle and thus there are more anchoring points between the surface and the particle compared to the particles A and B, which have higher degree of motion.

The main scattering peak of the particles A and B are typically around 570-585 nm, when they are pushed with negative voltage and when pulled with positive voltage, the LSPR peak shifts to 600-640 nm. From the simulations, the 570 nm peak corresponds to distance *d* of 18 nm and the 600-640 nm peaks to 2-5 nm distance above the gold surface. In addition, there are secondary peaks at 535 nm and 660 nm for particles A and B, when they are pushed with negative voltage. Based on FEMsimulations, these peaks should be related to the short distances between the gold surface and the particle, and they should not be present when a particle is pushed away from the surface. The averaging time during the experiments was relatively long (3-5 s), so we observe the average position of the measured particles in respect to the gold surface. This indicates that, when pushing the particles with less hairpin-DNA away from the surface, they are fluctuating on and off the surface and thus we have both the main peaks and the less pronounced secondary peaks.

For particles C and D, the main scattering peaks were located at 590 nm and 623 nm (pushed away) and at 620 nm and 643 nm (pulled toward). In this case, the motion of the particles is well-defined and more rigid compared to the particles A and B and there are no secondary peaks observed during the particle C and D measurements. Again, the different behavior is due to different amount of biotin-avidin bonds. For particle C, the peak shifts would correspond to motion from 7 nm above the surface to distance of 2.5 nm and, for particle D, the shift would be from 3 nm to 1 nm. These results suggest, that range of particle motion is heavily influenced by the biotin and avidin density, and by changing the densities one can tune the motion of the particle and the available wavelength range.

Chapter 4

DNA and AuNP based single electron transistor

This chapter contains the fabrication and the electrical characterization of a pearllike linear assembly consisting of TX-tile based self-assembled DNA structure and gold nanoparticles, where we demonstrate that this assembly could be used as a single electron transistor. The TX-tile structure was chosen, since it has quite small size (55 nm in length), which makes it suitable to build confined structures such as SET, and its conductivity has been proven to be minimal in dry conditions [85]. After the fabrication process, the assembly was trapped between a fingertip-electrode structure using dielectrophoresis, and the I-V- and differential conductance characteristics of the trapped assembly was measured. Since the trapped, plain assemblies showed only non-conducting behavior, we had to further process them by chemically growing the assemblies larger to reduce the air gaps within the trapped assembly-electrode system. After the chemical treatment, few assemblies showed the desired Coulomb blockade behavior. We investigated the conditions to observe the Coulomb blockade by characterizing the tunneling resistance of the trapped and chemically enhanced assemblies.

4.1 Fabrication of a linear, pearl-like three nanoparticle chain on DNA template

The linear TX-tile structure consisting of B-tile, A-tile and B-tile (B-A-B or BAB, appendix Pub.I) was fabricated using previously established protocols [85]. The structure was slightly modified from the original to have single "sticky end" overhangs protruding from each tile. These overhangs were used as anchoring points to attach AuNPs functionalized with complementary single stranded DNA as shown in figure 4.1a. These "sticky ends" were modified to the existing BAB structure by cutting one strand in each tile into two parts and extending these parts to protrude from the

structure. Here, a special care was taken to ensure that the "sticky ends" were facing the same direction. The "sticky end" to "sticky end" distance on the formed BAB structure is 14 nm and by attaching 10-12 nm particle the distance between the particles would be roughly 2-4 nm, which starts to be in the range of optimal tunneling junctions [212–215]. The thiolated ssDNAs at the ends of the BAB are used to anchor the BAB-AuNP assembly to the fingertip electrode structure during trapping.



FIGURE 4.1: (a) Schematic view of the pearl-like BAB-AuNP assembly. Three nanoparticles are attached to the BAB structure (A-tile, B-tile, A-tile) via ssDNA anchors (red and green lines). The air gaps form tunnel junctions with capacitances *C*. (b) An AFM image of fabricated linear BAB self-assembled structures in dry conditions. The inset shows the blue circled BAB structures, where the height profile is shown on the right plot. Reprinted with the permission from [216]. Copyright 2016 American Chemical Society.

The fabrication of BAB structure started with Kinase-Ligase treating the used DNA strands 1-14 (except the strand 7, see appendix table A.3 for the DNA strands sequences). The treatment was carried out by first mixing 0.6 µl of T_4 poly-Kinase, 27.6 µl of T_4 Ligase, 6.9 µl of [tris-Acetate-EDTA] (TAE) buffer and 0.9 µl of TAE with 0.5 M Magnesium Acetate (MgAce) together. We added 1.5 µl of the Kinase-Ligase mixture to 10 µl solutions each containing one of the DNA strand 1-6 and 3 µl to 20 µl solutions each containing one of the DNA strands 8-14, where the end concentration

of MgAce was 12.5 mM. These DNA strand solutions were incubated at 37 °C for 1 h. After this, all the strand solution were mixed together (total volume 200 μ l) and 60 μ l of the solution containing DNA strand 7 in TAE / 12.5 mM MgAce buffer was added.

The mixture was divided into four separate 1.5 ml PCR-tubes and the solutions were heated to 90 °C, incubated for 30 s and then slowly cooled down to 20 °C with ramping rate of 0.01 °C/s. Next, the solutions were combined and the final product was Ligase-treated by mixing 40 µl of T_4 Ligase with similar TAE and MgAce concentration to the 260 µl of the final BAB solution and incubating the solution 2 h at room temperature. The fabricated BAB structure can be seen in figure 4.1b, where the average length of the structure is 50-60 nm and the height is 1-2 nm, which correspond to the theoretical size 55 nm of the BAB structure and the height of the a DNA double helix [85].

The pearl-like BAB-AuNP assemblies were formed using the BAB structure and two different AuNPs, that attached separately to the "sticky end" extension protruding from tile A and B (see figure 4.1a). These AuNPs were labeled as AuNP_A for tile A and AuNP_B for tile B, and the functionalization of the AuNPs were carried out using the salt-aging method [131,217,218]. The average diameter of the particle were 10-15 nm (LSPR at 520 nm, see appendix Pub.I). The fabrication process of the assemblies was done by mixing solution containing BAB with solutions containing two different AuNPs in ratio of BAB:AuNP_A:AuNP_B = 1 : 1 : 2, the MgAce concentration was adjusted to 7-8 mM, the mixture was heated up to 45 °C and slowly cooled to 21 °C with the ramping rate of 0.0025 °C/s. An example of the results is shown in figure 4.2.

The fabrication yield of the BAB-AuNP assemblies (trimers) was 19-45 %, size of the AuNPs was 4-7 nm and the typical length of these assemblies is 50-100 nm, which corresponds quite well to the theoretical size of the BAB-AuNP assembly. For the yield analysis, we included all the structures that were under 110 nm, since any larger structures would most probably correspond to a case, where two BAB are already linked together. For the size variation it should be mentioned, that the tip related convolution in the lateral resolution of the AFM makes the structures to appear larger than they are, so the 100 nm size is quite reasonable. The average size of the AuNPs in the trimer assemblies is smaller than the average diameter of the AuNP_A or AuNP_B (10-15 nm) and the fabrication process frequently resulted also in formation of dimer BAB-AuNP assemblies, which is possible due to Coulombic repulsion between the charged AuNPs, i.e., particles needed to be smaller to attach all three. This is further indicated in figure 4.2 by the bending and curving of the linear chains of AuNPs.

The origin of the issue is the single DNA strand attachment scheme, since particles have quite high range of motion available during the fabrication and they try to minimize the repulsion by maximizing the distance between each other, and



FIGURE 4.2: AFM images of fabricated linear, pearl-like DNA-AuNP assemblies. (a) Overall image of the fabricated assemblies. The scale bar is 200 nm. Green circles highlight some of the three AuNP chain structures. (b),(c) Zoomed images with cross sections of individual DNA-AuNP assemblies. Figure adapted with the permission from [216]. Copyright 2016 American Chemical Society.

hence the bending. This can be also reason for smaller particles, since they would be more separated from each other. This could be solved by adding multiple binding sites to each tile, which has been done quite frequently in other studies [219, 220]. However, this would be quite difficult for the BAB structure, since one would need to cut more DNA strands from each tile, and the cutting process could destabilize the whole structure.

We further highlighted the importance of the annealing process by either doing the fabrication with faster ramping rate (0.01 °C/s) or just incubating at room temperature for 3 h or 14 h. In these cases only few linear chains were found and mostly particles and the BAB structures were located separately (see appendix Pub.I). This can be attributed to the DNA hybridization: after annealing at high temperatures the poorly formed, partially hybridized BAB-AuNP assemblies will dissociate first and the bond with highest dissociation energy (trimers) will form first, when the temperature is lowered. At lower temperature, the partially hybridized BAB-AuNP assemblies are not dissociated fully, which will lead into loosely bound particle that can come off at any point. This temperature dependent hybridization process is also quite sensitive to amount of "sticky end" bindings, and by increasing the amount of binding site one could reduce the temperature and the ramping rate. It should be noted here that I tried to utilize gel electrophoresis to isolate trimers from other products, but this resulted in separation of AuNPs from BAB structure (data not shown) most probably due to the single ssDNA attachment scheme.

4.2 Dielectrophoretic trapping of the DNA-AuNP assembly

In order to study the electrical properties of the pearl-like BAB-AuNP assembly, we needed to connect it to an external circuitry. This was achieved by trapping the assemblies between fingertip-electrodes using dielectrophoresis. A typical trapping experiment was performed as following: 12.5 µl of BAB-AuNP sample was mixed with 37.5 µl of 6 mM Hepes, 2 mM NaOH, 1 mM MgAce buffer (buffer A) and then filtered using 0.45 µm centrifuge filter for 6 min at 2000 rcf. The filtered solution was diluted with the buffer A until the estimated concentration of BAB-AuNP assemblies was under 10 pM based on the theoretical concentration of the BABs. Fingertipelectrode structure on a silicon oxide chip was placed inside a shielded box and the electrodes were connected to a function generator via $100 \,\mathrm{M}\Omega$ front resistor. Then 10 µl of the diluted BAB-AuNP solution was pipetted on the gap region of the electrode structure (see figure 4.3a). For the trapping, we used sinusoidal voltage with frequency of 12.5 MHz, amplitude of $1 V_{pp}$ and trapping time was varied between 5-10 min. After trapping, the electrode was dried using N_2 flow and outcome of the trapping was confirmed using AFM in dry conditions. The figures 4.3b-d show images of typical trapped assemblies.



FIGURE 4.3: (a) AFM image of fingertip-electrode before trapping. (b)-(d) AFM images of trapped DNA-AuNP assemblies. The trapped structures in (c) and (d) are labeled as sample 1 and sample 2.

We mainly managed to trap trimer structures, which is reasonable since the trapping process favors larger structures. This was also the reason for filtering the samples, since we wanted to remove possible larger aggregated structures, which are present in the fabrication as show in figure 4.2. The figure 4.3d shows an image of trapped BAB-AuNP assembly, where the three nanoparticles can be seen as bumps

indicated in the cross section by dashed, orange lines. The size of the trapped trimers is also larger than in figure 4.2 (7-12 nm versus 4-7 nm), which is again reasonable due to DEP favoring of larger structures.

4.3 Electrical characterization of the DNA-AuNP chain assembly

4.3.1 Measurements setup

The current-voltage and the differential conductance characterization was performed using setup illustrated in figure 4.4. Different measurement chambers were used for different temperature ranges. For room temperature and normal humidity measurements, we placed the fingertip electrode with the trapped sample inside a shielded box (see appendix Pub.I for more details), where the contacts were made by pressing steel needles on the contact pads of the electrode. For low temperature measurements (4.2 - 77 K), the sample was glued to a home-made sample stage (dipstick) and aluminum wires were bonded to the contact pads of the electrode structure using F&K Delvotec 5430 bonder. Then the dipstick was immersed first to liquid nitrogen and then to liquid helium in a cryogenic dewar.

In both temperature ranges, the chambers with the samples were placed inside an electromagnetically shielded room prior to the measurements and they were connected to the preamplifiers as shown in figure 4.4. To adjust the temperature from 4.2 K to 10 K, temperature gradient inside the dewar was utilized by raising the holder with the sample above and beyond the liquid helium level and monitoring the temperature using a Cernox CX-1030 thin film sensor (Lake Shore Cryotronics) located next to the sample. The DC-voltage and the AC-sinusoidal voltage were remotely controlled using a home-made, battery-powered voltage box connected to the computer via National instruments PCI interface card (NI PXI-8335 MIX-3) in NI-chassis (NI-8335) and multifunction data acquisition card (National instruments NI-PXI-6251). The data was recorded using the same data acquisition card and Labview program (National Instruments).

For the I-V-measurements, we used Stanford Research System SR560 voltage preamplifier and SR570 current preamplifier. The sensitivity of the current preamplifier was 10^{-9} A/V and 3 Hz low pass filtering with 6 dB attenuation slope was used to remove external noises for both current and voltage preamplifiers. In general, the measurements were carried out by sweeping the voltage from -3 V to +3 V and back with ~ 1.5 mV steps and 1-1.5 s settling time as well as 2000 point averaging after each step.

In differential conductance measurements, the AC-voltage and the AC-current were recorded using Stanford Research System SR830 DSP Lock-in Amplifiers, that were in series connected to the voltage and the current preamplifiers as shown in



FIGURE 4.4: The electric measurement setup used in the single electron transistor characterization.

figure 4.4. In this case, both preamplifiers had 3-100 Hz band pass filtering and both lock-in amplifiers had time constant of 3 s and sensitivity of 100 mV_{max} . The applied sinusoidal AC-voltage had peak-to-peak voltage of 4 mV at 13 Hz frequency. Typically, the differential voltage and differential current were recorded by similarly sweeping the voltage from -3 V to +3 V and back.

4.3.2 I-V-characterization of the DNA-AuNP assembly

The I-V-characterization of sample 1 (figure 4.5a) and 2 (figure 4.5d) after trapping is shown in figure 4.5c and 4.5f (black lines). If the sample was acting as a single electron transistor, one would expect similar behavior as shown in figure 2.22a with non-

conducting and conducting regions. However, we observed only non-conducting, $T\Omega$ -range behavior. This is most probably due to too large air gaps between the particles, since the size of the particle is less than 10 nm after trapping, which would correspond to higher than 2 nm tunnel junctions.



FIGURE 4.5: (a) AFM images of trapped BAB-AuNP assembly (sample 1) before and (b) after gold growth.(c) I-V-characteristics of sample 1 before (black line) and after (green) gold growth. (d) AFM images of trapped BAB-AuNP assembly (sample 2) before and (e) after gold growth. (f) I-V-characteristics of sample 2 before (black line) and after (green) gold growth. Figure reprinted with the permission from [216]. Copyright 2016 American Chemical Society.

To improve the tunneling probability, we used chemical gold enhancement kit (Nanoprobes) to grow the AuNPs and the electrode structure large to shrink the air gaps. This was achieved using stepwise growth scheme, where in the first step we used higher growth rate to increase the height of the sample roughly 10 nm. In the following steps, the growth rate was slowed down so that the height increase was 2-3 nm per step. Both growth processes were accomplished by mixing the four reagents of the kit (enhancer, activator, buffer and initiator) as following: 3 µl of

 $12 \times$ diluted enhancer was mixed with 3 µl of activator and incubated for 5 min. Then 3 µl of initiator and 3 µl of $12 \times$ diluted buffer were added to the mixture and 10μ l of the final solution was added to the electrode sample for 30 s incubation. After incubation, the sample was rinsed with 60 µl of MilliQ water several times and dried using N₂ flow. The difference in the growth rates was achieved by mixing 3 µl of 5-10 nm AuNP (C_{AuNP} =0.46 nM) to the final solution to compete with the reduction reaction.

To characterize the gold growth process and to determine the point for the Coulomb Blockade effect, we calculated the resistance of the sample after each growth step by fitting a current versus voltage curve in equation 4.1 that takes into account charging currents induced by the capacitive elements [221] (see figure 4.6a).

$$I = \frac{V}{R} + I_0 \alpha \frac{1 - \alpha^n}{1 - \alpha} \tag{4.1}$$

where I_0 is the maximum charging current at the bias transients, α is a constant including the ratio of the stabilization time of the measurement and the time constant of the charging current, n refers to the nth measurement point and V_n is the corresponding voltage. The results for sample 1 and sample 2 are shown in figure 4.6b, where the resistance overall decreases with each growth step and under 100 MΩ the Coulomb blockade is observed as shown in figures 4.5c and 4.5f (green lines). It is hard to exactly predict from the resistance curves when gold enhanced BAB-AuNP assemblies start to exhibit Coulomb blockade, but on average, 2-4 growth steps with slower growth rate after the one faster growth step were required to achieve the Coulomb blockade. Typically, the result was grainy structures as shown in figures 4.5b (sample 1) and 4.5e (sample 2). The yield of the structures displaying the Coulomb blockade was 12 %.

The I-V-characteristics of sample 1 and 2 in figure 4.5c and 4.5f shows the Coulomb blockade region even at room temperature. The threshold voltages were -0.5 V and +0.25 V for the sample 1 and -1.9 V and +1 V for sample 2. If assuming that the island of the SET consist of three separate nanoparticle, then by using equations 2.42 and 2.43 the threshold voltage of the three particle island is 0.36 V, which is in a close agreement with the threshold voltages of the sample 1 but not with the sample 2. The higher threshold voltage of sample 2 can be due to the granular structure, since the formed grains could form separate islands themselves. Then the structure would not necessary consist of just three nanoparticles but several more particles that each would increase the threshold voltage as shown in equation 2.43.

To estimate the overall composition of the sample 2, we assumed that the size of the contributing BAB-AuNP structure was 50-100 nm (\sim electrode gap size), which consist of *n* island (AuNPs), the distance *d* between nanoparticles is roughly 1 nm and the contributing BAB-AuNP chains consist of *n* linearly and equally spaced particles. Then by varying the particle diameter *a* and n, we solved the possible



FIGURE 4.6: (a) The I-V-characteristics of sample 1 after 2nd growth step (black circles) and the charging dependent resistance curve (red line, fitted using the equation 4.1). (b) The resistance of the trapped, gold enhanced samples 1 and 2 after each gold growth step. Figure adapted with the permission from [222]. Copyright River Publishers, 2017.

compositions using equation 2.42 and 2.43 that would correspond to the threshold voltage of 1 V. The end results was that 6-8 particles with 8-11 nm diameter was required to meet the criteria, which is close to the observed gold growth of the sample 2 (10-20 nm) in figure 4.5e making this a plausible explanation.

This has implications that the Coulomb blockade might not originate from the original BAB-AuNP assembly but rather from the grainy outcome of the gold enhancement process. To verify this, we repeat the trapping and the gold growth for plain BAB structures, which in most cases resulted in the electrode structure with non-existing gap (resistance = $k\Omega - T\Omega$, see appendix Pub.I). However, for one plain, trapped BAB structure we could observe the Coulomb blockade. It should be noted that the key advantage of the BAB-AuNP trimers is that it acts as a starting point for the gold growth, which has higher probability to form the correct isolated construction, where the Coulomb blockade can be observed even at room temperature.

Both samples showed strong fluctuation and sudden jumps in the current, which can be due to thermal effects, e.g., thermal hopping. To reduce or eliminate these effects, we cooled the sample 1 down to 4.2 K and measured the I-V-characteristics and the differential conductance (see next section) in various temperatures between 4.2 K and the room temperature as shown in figure 4.7. As temperature is lowered, the thermal fluctuations in the current are clearly decreasing, but there are still sudden jumps in the current (red circles in figure 4.7), which now seems to happens between specific conducting states. Also, the curves at 77 K (orange and violet curves in the inset) show the current rapidly switching between two different I-V-characteristics resembling random telegraph-like noise. Most probably both of these effects are due to random fluctuations in the background charge, which



FIGURE 4.7: I-V characteristic of chemically grown sample 1 from 4.2 K to room temperature. The inset shows the current in more details, where rapid switching and jumps between different I-V curves (red circles) can be seen. Figure reprinted with the permission from [216]. Copyright 2016 American Chemical Society.

can be considered as quick manipulation of the gate voltage of the SET [212,213,215].

4.3.3 Differential conductance characterization

The differential conductance of the sample 1 was measured at four different temperatures (4.2, 5.2, 5.9 and 10.2 K) as shown in figure 4.8, where the Coulomb blockade can be seen as a sharp and clear plateau in the conduction near zero voltage. The differential conductance dG curves seems to fluctuate outside the blockade region, which can be due to different resistances at different junctions or due to different electronic states within single nanoparticle of the chemically enhanced assembly. Also, the threshold voltage seems to drastically change as shown in figure 4.8a, even in a constant temperature. This is again most probably due the changes in background charge, since the main factors, that influence the threshold voltage, are the gate voltage and the temperature, and we have two different differential conduction curves for consecutive measurements at same temperature in figure 4.8a (black and red lines). This background charge induced gating effect further indicates that the trapped and gold enhanced BAB-AuNP assemblies are acting as a SET.



FIGURE 4.8: The differential conductance of the BAB-AuNP assembly (sample 1) at (a) 4.2 K, (b) 5.2 K,(c) 5.9 K and (d) 10.2 K. The red and black lines in figure a represent two consecutive measurements at 4.2 K, where the changes in the threshold voltage are most probably due to changes in the background charge. Figure adapted with the permission from [222]. Copyright River Publishers, 2017.

Chapter 5

Conclusions and outlook

The main goal of this thesis concerns producing different plasmonic and electronic structures or assemblies using DNA and gold (nanoparticles), since both materials offer unique approaches to bottom-up fabrication and the interfacing between the two is well-established. Although they have been widely utilized over many decades due to the parallel, mass scale fabrication of millions of produced copies, the applications in electronics and plasmonics still remain elusive and the work in this thesis focuses on bringing forth those innovations that allow the DNA and gold (nanoparticles) to be more widely applied in creation of, e.g., metasurfaces or nanoprobes.

One of the primary constraints in both electronic and plasmonic applications has been the metallization and the nanopatterning schemes of DNA, where the shape or resolution of produced assemblies has been fairly limited. To tackle this, we have developed a new novel DALI method to produce metasurfaces that were successfully demonstrated to function, e.g., as a SERS enhancer. The resolution of the DALI method could be scaled down to sub-10-nm, which rivals the conventional electron beam lithography and has significantly higher variability compared to the chemical methods. However, the method has currently two main limiting factors: the HF wet etching and the flexibility of the DNA platform. The HF etching mainly constrains the choice of metals and substrates, but the DALI methods still covers quite extensively number of metals and semiconductors. The flexibility of the DNA platform could be seen as missing arms in the case of CDLs and angle variety of the bowties, and these defects will influence the optical properties of the metasurfaces. This emphasizes that it is of highest paramount to correctly design the platform to minimize undesired rolling and twisting. Also in some applications, the random orientation of the structures after deposition is not desirable and thus the future development of the DALI method should concentrate on improving the deposition step and redesigning the layer composition to replace the HF etching with more subtle chemical etching.

Second paper in this thesis was devoted to realize electric field guided, AuNP

and DNA based actuator and we used this assembly to analyze the conformation changes of hairpin-DNA. It was discovered that the hairpin-DNA behavior under electric field resembled fluctuation between two binary states when compared to the behavior of ssDNA linkers, which indicates that the opening and folding of the hairpin-loop sets extra constrains to the motion of the hairpin-DNA. Besides DNA, we are confident that this actuator scheme could be expanded to study other biomolecules and proteins in the future, where the control of the actuator could be established using, e.g., pH, magnetic particle and magnetic fields or combination of several different techniques. For example, the probe scheme could be utilized to study the behavior of Influenza hemagglutinin (HA) glytoprotein inside cells, where the conformation of HA depends on the pH of the cell.

Also, we assessed the overall properties and applicability of the actuator from two different perspectives. First, one main advantage of our actuator assembly is the reversibly tunable optical properties at the post-fabrication state, which could be useful diagnostics, e.g., for real-time detection of different fluorophores. Although some methods in the past have been able to modify LSPR wavelength of the AuNP probe after fabrication by, e.g., altering the dielectric function of the substrate, these changes are typically irreversible.

Second, the motion and degree of controllability was investigated by altering the amount of biotin-avidin attachments between the particles and the gold surface. High amount of biotin-avidin bindings forces the particle close to surface, where the motion of the particle under electric field is quite limited but at the same time less prone to fluctuations. When the amount of bindings is reduced, the range of the motion is more extensive but also the fluctuation increase as was seen in the individual LSPR scattering measurements. These findings implicate that the surface immobilization scheme plays a crucial role in the operation of the actuator, and by tuning the amount of biotins or avidins one can tune the system to incorporate the desired optical or mechanical response. Other factor, which could be used to tune the behavior of the actuator but was not taken into account in this thesis, is the size of the AuNPs. This has direct impact on the optical properties, where the LSPR wavelength can be further tuned, and it most probably affects the motion as well, since the size and the mass have huge impact on diffusive motion of the suspended objects.

In the final work, we utilized more traditional DNA hybridization methods to construct DNA and AuNP based assembly that was demonstrated to act as a single electron transistor. Dielectrophoresis trapping was employed to trap these assemblies between electrodes to characterize their electrical properties. The DNA-AuNP assemblies initially only exhibited non-conducting behavior, which could be attributed to the large air gaps at the junctions. This problem was solved by growing the AuNPs larger using chemical enhancement kit, after which few structures showed the Coulomb blockade behavior. We also characterized the growth mechanism to determine the point, where the Coulomb blockade was observable, by measuring the resistance of the trapped sample after each growth step. Here, it was discovered that the Coulomb blockade became observable after the resistance of the trapped assembly was reduced from few T Ω to under 100 M Ω .

Although, we were missing the gate voltage dependent measurement, which would have been the ultimate proof for assemblies to function as SETs, the temperature independent fluctuation and oscillation in the I-V-characteristics and the changing differential conductance curves suggest that the random fluctuations of the background charges are influencing the trapped BAB-AuNP system. This can be considered as a gating effect, which implies that the trapped assemblies are acting as SETs. For any future implementations in fabrication of nanoelectronics, the design of the DNA platform and the "sticky-end" attachment scheme should be improved so that the gold growth is not anymore required. One way to achieve this could be utilization of DNA origami structures with multiple anchoring points, where particle to particle separation of 3 nm or under has been already achieved.

Bibliography

- [1] DAHM, R., Discovering DNA: Friedrich Miescher and the yearly years of nucleic acid research. Hum. Genet. **122** (2008) 565–581.
- [2] TRAVERS, A. AND MUSKHELISHVILI, G., DNA structure and function. FEBS J. 282 (2015) 2279–2295.
- [3] WATSON, J. D. AND CRICK, F. H. C., Molecular structure of Nucleic Acids. Nature 171 (1953) 737–738.
- [4] OLBY, R., *The path to the Double Helix: The Discovery of DNA* (Dover Publications, Mineola, New York, US, 1994).
- [5] CHARGAFF, E., LIPSHITZ, R., GREEN, C., AND HODES, M. E., *The composition of the deoxyribonucleic acid of salmon sperm*. J. Biol. Chem. **192** (1951) 223–230.
- [6] WILKINS, M. H. F., STOKES, A. R., AND WILSON, H. R., *Molecular Structure* of *Deoxypentose Nucleic Acids*. Nature **171** (1953) 738–740.
- [7] SANGER, F. AND COULSON, A. R., A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J. Mol. Biol. **94** (1975) 441–446.
- [8] MAXAM, A. M. AND GILBERT, W., A new method for sequencing DNA. Proc. Natl. Acad. Sci. 74 (1977) 560–564.
- [9] WEIER, H. U. AND GRAY, J. W., *A programmable system to perform the polymerase chain reaction*. DNA. 7 (1988) 441–447.
- [10] ADRIO, J.-L. AND DEMAIN, A. L., Recombinant organisms for production of industrial products. Bioeng Bugs 1 (2010) 161–131.
- [11] GOEDDEL, D. V., KLEID, D. G., BOLIVAR, F., HEYNEKER, H. L., YANSURA, D. G., CREA, R., HIROSE, T., KRASZEWSKI, A., ITAKURA, K., AND RIGGS, A. D., Expression in Escherichia coli of chemically synthesized genes for human insulin. Rec. Meat. Conf. Proc. 35 (1982) 36–41.
- [12] JEFFREYS, A. J., WILSON, V., AND THEIN, S. L., Individual-specific 'fingerprints' of human DNA. Nature **316** (1985) 76–79.

- [13] SEEMAN, N. C., Nucleic acid junctions and lattices. J. Theor. Biol. 99 (1982) 237– 247.
- [14] FREESTONE, I., MEEKS, N., SAX, M., AND HIGGITT, C., The Lycurgus Cup A Roman Nanotechnology. Gold Bull. 40 (2007) 270–277.
- [15] SOMMERFELD, A., Über die Ausbreitung der Wellen in der drahtlosen Telegraphie. Ann. Physik **333** (1909) 665–736.
- [16] PINES, D. AND BOHM, D., A Collective Description of Electron Interactions: II. Collective vs Individual Particle Aspects of the Interactions. Phys. Rev. 85 (1952) 338–353.
- [17] RITCHIE, R. H., Plasma Losses by Fast Electron in Thin Films. Phys. Rev. 106 (1957) 874–881.
- [18] STERN, E. A. AND FERRELL, R. A., Surface Plasma Oscillations of a Degenerate *Electron Gas.* Phys. Rev. **120** (1960) 130–136.
- [19] KRETSCHMANN, E. AND RAETHER, H., Radiative decay of non-radiative surface plasmons excited by light. Z. Naturforsch. 23 (1968) 2135–2136.
- [20] OTTO, A., Excitation of nonradiative surface plasma waves in silver by the method of frustrated total reflection. Z. Phys. A-Hadron Nucl. **216** (1968) 398–410.
- [21] FLEISCHMANN, M., HENDRA, P. J., AND MCQUILLAN, A. J., *Raman-spectra of pyridine adsorbed at a silver electrode*. Chem. Phys. Lett. **26** (1974) 163–166.
- [22] MIE, G., Beiträge zur Optik trüber Medien, speziell kolloidaler Metallösungen. Ann. Physik **330** (1908) 377–445.
- [23] TURKEVICH, J., Colloidal Gold. Part I: Historical and preparative aspects, morphology and structure. Gold Bull. **18** (1985) 86–91.
- [24] TURKEVICH, J., STEVENSON, P. C., AND HILLIER, J., A study of the nucleation and growth processes in the synthesis of colloidal gold. Discuss. Faraday. Soc. 11 (1951) 55–75.
- [25] MOSKOVITS, M., Surface-enhanced spectroscopy. Rev. Mod. Phys. 57 (1985) 783– 826.
- [26] LAKOWICZ, J., Radiative decay engineering 5: metal-enhanced fluorescence and plasmon emission. Anal. Biochem. **337** (2005) 171–194.
- [27] CHA, D. Y. AND PARRAVANO, G., Surface Reactivity of Supported Gold.I. Oxygen Transfer Between Carbon Monoxide and Carbon Dioxide. J. Catal. 18 (1970) 200– 211.

- [28] VALDEN, M., LAI, X., AND GOODMAN, D. W., Onset of Catalytic Activity of Gold Clusters on Titania with the Appearance of Nonmetallic Properties. Science 281 (1998) 1647–1650.
- [29] SHEN, T., WEISSLEDER, R., PAPISOV, M., JR., A. B., AND BRADY, T. J., Monocrystalline iron oxide nanocompounds (MION): Physicochemical properties. Magn. Reson. Med. 29 (1993) 599–604.
- [30] DANIEL, M.-C. AND ASTRUC, D., Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology. Chem. Rev. 104 (2004) 293–346.
- [31] MILTON, J. A., PATOLE, S., YIN, H., XIAO, Q., BROWN, T., AND MELVIN, T., Efficient self-assembly of DNA-functionalized fluorophores and gold nanoparticles with DNA functionalized silicon surfaces: the effect of oligomer spacers. Nucleic Acids Res. 41 (2013) e80.
- [32] CHA, T.-G., PARK, J., CHEN, H., SALGADO, J., LI, X., MAO, C., AND CHOI, J. H., A synthetic DNA motor that transports nanoparticles along carbon nanotubes. Nat. Nanotechnol. 9 (2013) 39–43.
- [33] WANG, Z., CHENG, F., WINSOR, T., AND LIU, Y., Optical chiral metamaterials: a review of the fundamentals, fabrication methods and applications. Nanotechnology 27 (2016) 412001.
- [34] PENDRY, J. B., Negative Refraction Makes a Perfect Lens. Phys. Rev. Lett. 85 (2000) 3966–3969.
- [35] ZHANG, X. AND LIU, Z., *Superlenses to overcome the diffraction limit*. Nat. Mater. 7 (2008) 435–441.
- [36] YANG, J., SONG, Z., LIU, S., ZHANG, Q., AND ZHANG, C., Dynamically Arranged Gold Nanoparticles on DNA Origami for Molecular Logic Gates. Appl. Mater. Interfaces 8 (2016) 22451–22456.
- [37] WANG, D., FU, Y., YAN, J., ZHAO, B., DAI, B., CHAO, J., LIU, H., HE, D., ZHANG, Y., FAN, C., AND SONG, S., *Molecular Logic Gates on DNA Origami Nanostructures for MicroRNA Diagnostics*. Anal. Chem. 86 (2014) 1932–1936.
- [38] RIORDAN, M., HODDESON, L., AND HERRING, C., The invention of transistor. Rev. Mod. Phys. 71 (1999) S336–S345.
- [39] MOJARAD, N., GOBRECHT, J., AND EKINCI, Y., Interference lithography at EUV and soft X-ray wavelengths: Principles, methods, and applications. Microelectron. Eng. 143 (2015) 55–63.

- [40] SUN, Y., RUSLI, AND SINGH, N., Room-Temperature Operation of Silicon Single-Electron Transistor Fabricated Using Optical Lithography. IEEE Trans. Nanotechnol. 143 (2011) 96–98.
- [41] KLEIN, D. L., ROTH, R., LIM, A. K. L., ALIVISATOS, A. P., AND MCEUEN, P. L., A single-electron transistor made from a cadmium selenide nanocrystal. Nature 389 (1997) 699–701.
- [42] KUBATKIN, S., DANILOV, A., HJORT, M., CORNIL, J., BRÉDAS, J.-L., STUHR-HANSEN, N., HEDEGÅRD, P., AND BJØRNHOLM, T., Single-electron transistor of a single organic molecule with access to several redox states. Nature 425 (2003) 698–701.
- [43] S. DUROCHER, A. R., HAMM, C., RANGAN, C., MITTLER, S., AND MUTUS, B., Disulfine-linked, gold nanoparticle based reagent for detecting small molecular weight thiols. J. Am. Chem. Soc. 131 (2009) 2475–2477.
- [44] UVDAL, K., PERSSON, I., AND LIEDBERG, B., *Tricyclohexylphosphine adsorbed* on gold. Langmuir **11** (1995) 1252–1256.
- [45] LEFF, D. V., BRANDT, L., AND HEATH, J. R., Synthesis and characterization of hydrophobic organically-soluble gold nanocrystals functionalized with primary amines. Langmuir **12** (1996) 4723–4730.
- [46] SEEMAN, N. C., Nanomaterials Based on DNA. Annu. Rev. Biochem. 79 (2010) 65–87.
- [47] PETTERSEN, E. F., GODDARD, T. D., HUANG, C. C., COUCH, G. S., GREEN-BLATT, D. M., MENG, E. C., AND FERRIN, T. E., USCF Chimera – a visualization system for exploratory research and analysis. J. Comput. Chem. 25 (2004) 1605–1612.
- [48] MACKE, T. J. AND CASE, D. A., Molecular Modeling of Nucleic Acids (American Chemical Society, Washington DC, USA, 1997).
- [49] LANGRIDGE, R. AND WILSON, H. R., The molecular configuration of deoxyribonucleic acid: I. X-ray diffraction study of a crystalline form of the lithium salt. J. Mol. Biol. 2 (1960) 19–37.
- [50] LANGRIDGE, R., MARVIN, D. A., SEEDS, W. E., AND WILSON, H. R., The molecular configuration of deoxyribonucleic acid: II. Molecular models and their fourier transforms. J. Mol. Biol. 2 (1960) 38–62.
- [51] GESSNER, R. V., FREDERICK, C. A., QUIGLEY, G. J., RICH, A., AND WANG, A. H.-J., The Molecular Structure of the Left-handed Z-DNA Double Helix at 1.0-Å Atomic Resolution. J. Biol. Chem. 264 (1989) 7921–7935.

- [52] BOCHMAN, M. L., PAESCHKE, K., AND ZAKIAN, V. A., DNA secondary structures: stability and function of G-quadruplex structures. Nat. Rev. Genet. 13 (2012) 770–780.
- [53] ZHAO, J., BACOLLA, A., WANG, G., AND VASQUEZ, K. M., Non-B DNA structure-induced genetic instability and evolution. Cell. Mol. Life Sci. 67 (2010) 43–62.
- [54] HOOGSTEEN, K., The Crystal and Molecular Structure of a Hydrogen-Bonded Complex Between 1-Methylthymine and 9-Methyladenine. Acta. Cryst. 16 (1963) 907–916.
- [55] MARSH, T. C., VESENKA, J., AND HENDERSON, E., A new DNA nanostructure, the G-wire, imaged by scanning probe microscopy. Nucleic Acids Res. 23 (1995) 696–700.
- [56] MIYOSHI, D., KARIMATA, H., WANG, Z. M., KOUMOTO, K., AND SUQI-MOTO, N., Artificial G-wire switch with 2,2'-bipyridine units responsive to divalent metal ions. J. Am. Chem. Soc. 129 (2007) 5919–5925.
- [57] KOTLYAR, A. B., BOROVOK, N., MOLOTSKY, T., COHEN, H., SHAPIR, E., AND PORATH, D., Long, Monomolecular Guanine-Based Nanowires. Adv. Mater. 17 (2005) 1901–1905.
- [58] JAIN, A., WANG, G., AND VASQUEZ, K. M., DNA triple helices: biological consequences and therapeutic potential. Biochimie **90** (2008) 1117–1130.
- [59] KOPIELSKI, A., SCHNEIDER, A., CSÁKI, A., AND FRITZSCHE, W., Isothermal DNA origami folding: avoiding denaturing conditions for one-pot,hybrid-component annealing. Nanoscale 7 (2015) 2102–2106.
- [60] WEI, X., NANGREAVE, J., JIANG, S., YAN, H., AND LIU, Y., Mapping the Thermal Behavior of DNA Origami Nanostructures. J. Am. Chem. Soc. 135 (2013) 6165–6176.
- [61] FU, Y., ZENG, D., CHAO, J., JIN, Y., ZHANG, Z., LIU, H., LI, D., MA, H., HUANG, Q., GOTHELF, K. V., AND FAN, C., Single-Step Rapid Assembly of DNA Origami Nanostructures for Addressable Nanoscale Bioreactor. J. Am. Chem. Soc. 135 (2013) 696–702.
- [62] FU, T.-J., TSE-DINH, Y.-C., AND SEEMAN, N. C., Holliday Junction Crossover Topology. J. Mol. Biol. 236 (1994) 91–105.
- [63] WINFREE, E., LIU, F., WENZLER, L. A., AND SEEMAN, N. C., Design and selfassembly of two-dimensional DNA crystals. Nature **394** (1998) 539–544.

- [64] WEI, B. AND MI, Y., A New Triple Crossover Triangle (TXT) Motif for DNA Self-Assembly. Biomacromolecules 6 (2005) 2528–2532.
- [65] LIU, D., WANG, M., DENG, Z., WALULU, R., AND MAO, C., Tensegrity: Construction of Rigid DNA Triangles with Flexible Four-Arm DNA Junctions. J. Am. Chem. Soc. 126 (2004) 2324–2325.
- [66] YAN, H., PARK, S. H., FINKELSTEIN, G., REIF, J. H., AND LABEAN, T. H., DNA-templated self-assembly of protein arrays and highly conductive nanowires. Science 301 (2003) 1882–1884.
- [67] LABEAN, T. H., YAN, H., KOPATSCH, J., LIU, F., WINFREE, E., REIF, J. H., AND SEEMAN, N. C., Construction, Analysis, Ligation, and Self-Assembly of DNA Triple Crossover Complexes. J. Am. Chem. Soc. 122 (2000) 1848–1860.
- [68] LIU, H., CHEN, Y., HE, Y., RIBBE, A. E., AND MAO, C., Approaching the limit: Can one DNA oligonucleotide assemble into large nanostructures. Angew. Chem. In. Ed. 45 (2006) 1942–1945.
- [69] ZHENG, J., BIRKTOFT, J. J., CHEN, Y., WANG, T., SHA, R., CONSTANTINOU, P. E., GINELL, S. L., MAO, C., AND SEEMAN, N. C., From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal. Nature 461 (2009) 74–77.
- [70] ROTHEMUND, P. W. K., Folding DNA to create nanoscale shapes and patterns. Nature **440** (2006) 297–302.
- [71] DOUGLAS, S. M., MARBLESTONE, A. H., TEERAPITTAYANON, S., VAZQUEZ, A., CHURCH, G. M., AND SHIH, W. M., *Rapid prototyping of 3D DNA-origami* shapes with caDNAno. Nucleic Acids Res. 37 (2009) 5001–5006.
- [72] DOUGLAS, S. M., DIETZ, H., LIEDL, H., HÖGBERG, B., GRAF, F., AND SHIH, W. M., Self-assembly of DNA into nanoscale three-dimensional shapes. Nature 459 (2009) 414–418.
- [73] PINHEIRO, A. V., HAN, D., SHIH, W. M., AND YAN, H., *Challenges and opportunities for structural DNA nanotechnology*. Nat. Nanotechnol. **6** (2011) 763–772.
- [74] ANDERSEN, E. S., DONG, M., NIELSEN, M. M., JAHN, K., SUBRAMANI, R., MAMDOUH, W., GOLAS, M. M., SANDER, B., STARK, H., OLIVEIRA, C. L. P., PEDERSEN, J. S., BIRKEDAL, V., BESEBBACHER, F., GOTHELF, K. V., AND KJEMS, J., Self-assembly of a nanoscale DNA box with a controllable lid. Nature 459 (2009) 73–76.
- [75] KE, Y., SHARMA, J., LIU, M., JAHN, K., LIU, Y., AND YAN, H., Scaffolded DNA Origami of a DNA Tetrahedron Molecular Container. Nano Lett. 9 (2009) 2445– 2447.
- [76] DIETZ, H., DOUGLAS, S. M., AND SHIH, W. M., Folding DNA into Twisted and Curved Nanoscale Shapes. Science 324 (2009) 725–730.
- [77] BENSON, E., MOHAMMED, A., GARDELL, J., MASICH, S., CZEIZLER, E., ORPONEN, P., AND HÖGBERG, B., DNA rendering of polyhedral meshes at the nanoscale. Nature **523** (2015) 441–444.
- [78] LINKO, V., PAASONEN, S.-T., KUZYK, A., TÖRMÄ, P., AND TOPPARI, J. J., Characterization of the Conductance Mechanisms of DNA Origami by AC Impedance Spectroscopy. Small 5 (2009) 2382–2386.
- [79] PORATH, D., BEZRYADIN, A., DE VRIES, S., AND DEKKER, C., Direct measurement of electrical transport through DNA molecules. Nature **403** (2000) 635–638.
- [80] FINK, H.-W. AND SCHÖNENBERGER, C., Electrical conduction through DNA molecules. Nature 398 (1999) 407–410.
- [81] KASUMOV, A. Y., KOCIAK, M., GUÉRON, S., REULET, B., VOLKOV, V. T., KLI-NOV, D. V., AND BOUCHIAT, H., *Proximity-Induced Superconductivity in DNA*. Science 291 (2001) 280–282.
- [82] BOON, E. M. AND BARTON, J. K., *Charge transport in DNA*. Curr. Opin. Struct. Biol. **12** (2002) 320–329.
- [83] ELEY, D. D. AND SPIVEY, D. I., Semiconductivity of Organic Substances. Trans. Faraday Soc. 58 (1962) 411–415.
- [84] ENDRES, R. G., COX, D. L., AND SINGH, R. R. P., The quest for high-conductance DNA. Rev. Mod. Phys. 76 (2004) 195–214.
- [85] LINKO, V., LEPPINIEMI, J., PAASONEN, S.-T., HYTÖNEN, V. P., AND TOPPARI, J. J., Defined-size DNA triple crossover construct for molecular electronics: modification, positioning and conductance properties. Nanotechnology 22 (2011) 275610.
- [86] PARK, S. H., YIN, P., LIU, Y., REIF, J. H., LABEAN, T. H., AND YAN, H., Programmable DNA Self-Assemblies for Nanoscale Organization of Ligands and Proteins. Nano Lett. 5 (2005) 729–733.
- [87] PILO-PAIS, M., GOLDBERG, S., SAMANO, E., LABEAN, T. H., AND FINKEL-STEIN, G., Connecting the Nanodots: Programmable Nanofabrication of Fused Metal Shapes on DNA Templates. Nano Lett. 11 (2011) 3489–3492.

- [88] KUZYK, A., LAITINEN, K. T., AND TÖRMÄ, P., DNA origami as a nanoscale template for protein assembly. Nanotechnology **20** (2009) 235305.
- [89] ESKELINEN, A.-P., KUZYK, A., KALTIAISENAHO, T. K., TIMMERMANS, M. Y., NASIBULIN, A. G., KAUPPINEN, E. I., AND TÖRMÄ, P., Assembly of Single-Walled Carbon Nanotubes on DNA-Origami Templates through Streptavidin-Biotin Interaction. Small 7 (2011) 746–750.
- [90] SCHREIBER, R., KEMPTER, S., HOLLER, S., SCHÜLLER, V., SCHIFFELS, D., SIMMEL, S. S., NICKELS, P. C., AND LIEDL, T., DNA origami-templated growth of arbitrarily shaped metal nanoparticles. Small 7 (2011) 1795–1799.
- [91] MAUNE, H. T., HAN, S.-P., BARISH, R. D., BOCKRATH, M., GODDARDIII, W. A., ROTHEMUND, P. W. K., AND WINFREE, E., Self-assembly of carbon nanotubes into two-dimensional geometries using DNA origami templates. Nat. Nanotechnol. 5 (2010) 61–66.
- [92] KUZYK, A., SCHREIBER, R., FAN, Z., PARDATSCHER, G., ROLLER, E.-M., HÖGELE, A., SIMMEL, F., GOVOROV, A. O., AND LIEDL, T., DNA-based selfassembly of chiral plasmonic nanostructures with tailored optical response. Nature 483 (2012) 311–314.
- [93] KUZYK, A., SCHREIBER, R., ZHANG, H., GOVOROV, A. O., LIEDL, T., AND LIU, N., Reconfigurable 3D plasmonic metamolecules. Nat. Mater. 13 (2014) 862– 866.
- [94] PEARSON, A. C., LIU, J., POUND, E., UPRETY, B., WOOLLEY, A. T., DAVIS, R. C., AND HARB, J. N., DNA Origami Metallized Site Specifically to Form Electrically Conductive Nanowires. J. Phys. Chem. B 116 (2012) 10551–10560.
- [95] SHARMA, J., CHHABRA, R., ANDERSEN, C. S., GOTHELF, K. V., YAN, H., AND LIU, Y., Toward Reliable Gold Nanoparticle Patterning On Self-Assembled DNA Nanoscaffold. J. Am. Chem. Soc. 130 (2008) 7820–7821.
- [96] HELMI, S., ZIEGLER, C., KAUERT, D. J., AND SEIDEL, R., Shape-controlled synthesis of gold nanostructures using DNA origami molds. Nano Lett. 14 (2014) 6693– 6698.
- [97] SUN, W., BOULAIS, E., HAKOBYAN, Y., WANG, W. L., GUAN, A., BATHE, M., AND YIN, P., *Casting inorganic structures with DNA molds*. Science 346 (2014) 1258361.
- [98] KEREN, K., KRUEGER, M., GILAD, R., BEN-YOSEPH, G., SIVAN, U., AND BRAUN, E., Sequence-specific molecular lithography on single DNA molecules. Science 297 (2002) 72–75.

- [99] RICHTER, J., SEIDEL, R., KIRSCH, R., MERTIG, M., POMPE, W., PLASCHKE, J., AND SCHACKERT, H. K., Nanoscale Palladium Metallization of DNA. Adv. Mater. 12 (2000) 507–510.
- [100] BECERRIL, H. A., LUDTKE, P., WILLARDSON, B. M., AND WOOLLEY, A. T., DNA-templated nickel nanostructures and protein assemblies. Langmuir 22 (2006) 10140–10144.
- [101] MONSON, C. F. AND WOOLLEY, A. T., DNA-templated construction of copper nanowires. Nano lett. **3** (2003) 359–363.
- [102] MERTIG, M., CIACCHI, L. C., SEIDEL, R., AND POMPE, W., DNA as a Selective *Metallization Template*. Nano lett. **2** (2002) 841–844.
- [103] UPDIKE, S. J. AND HICKS, G. P., *The Enzyme Electrode*. Nature **214** (1967) 986–988.
- [104] CHURCH, G. M., GAO, Y., AND KOSURI, S., Next-Generation Digital Information Storage in DNA. Science **337** (2012) 1628.
- [105] BURNS, J. R., PREUS, S., SINGLETON, D. G., AND STULZ, E., A DNA based fivestate switch with programmable reversibility. Chem. Commun. 48 (2012) 11088– 11090.
- [106] LIU, M., FU, J., HEJESEN, C., YANG, Y., WOODBURY, N. W., GOTHELF, K., LIU, Y., AND YAN, H., A DNA tweezer-actuated enzyme nanoreactor. Nat. Commun. 4 (2013) 2127.
- [107] BANCHELLI, M., NAPPINI, S., MONTIS, C., BONINI, M., CANTON, P., BERTI, D., AND BAGLIONI, P., *Magnetic nanoparticle clusters as actuators of ssDNA release*. Phys. Chem. Chem. Phys. 16 (2014) 10023–10031.
- [108] SCHLICKE, H., BATTISTA, D., KUNZE, S., SCHRÖTER, C. J., EICH, M., AND VOSSMEYER, T., Freestanding Membranes of Cross-Linked Gold Nanoparticles: Novel Functional Materials for Electrostatic Actuators. Appl. Mater. Interfaces 7 (2015) 15123–15128.
- [109] WEIR, M. P., HERIOT, S. Y., MARTIN, S. J., PARNELL, A. J., HOLT, S. A., WEB-STER, J. R. P., AND JONES, R. A. L., Voltage-Induced Swelling and Deswelling of Weak Polybase Brushes. Langmuir 27 (2011) 11000–11007.
- [110] FRIEDRICH, R., BLOCK, S., ALIZADEHHEIDARI, M., HEIDER, S., FRITZSCHE, J., ESBJÖRNER, E. K., WESTERLUND, F., AND BALLY, M., A nano flow cytometer for single lipid vesicle analysis. Lab on a Chip 17 (2017) 830–841.

- [111] BLOCK, S., FAST, B. J., LUNDGREN, A., ZHDANOV, V. P., AND HÖÖK, F., Twodimensional flow nanometry of biological nanoparticles for accurate determination of their size and emission intensity. Nat.Commun. 7 (2016) 12956.
- [112] JIANG, C., MCCONNEY, M. E., SINGAMANENI, S., MERRICK, E., CHEN, Y., ZHAO, J., ZHANG, L., AND TSUKRUK, V. V., Thermo-Optical Arrays of Flexible Nanoscale Nanomembranes Freely Suspended over Microfabricated Cavities as IR Microimagers. Chem. Mater. 18 (2006) 2632–2634.
- [113] GU, H., YANG, W., AND SEEMAN, N. C., A DNA scissors device used to measure MutS binding to DNA mis-pairs. J. Am. Chem. Soc. 132 (2010) 4352–4357.
- [114] KE, Y., MEYER, T., SHIH, W. M., AND BELLOT, G., Regulation at a distance of biomolecular interactions using a DNA origami nanoactuator. Nat. Commun. 7 (2015) 10935.
- [115] PILO-PAIS, M., WATSON, A., DEMERS, S., LABEAN, T. H., AND FINKEL-STEIN, G., Surface-Enhanced Raman Scattering Plasmonic Enhancement Using DNA Origami-Based Complex Metallic Nanostructures. Nano Lett. 14 (2014) 2099–2104.
- [116] GAO, L., WU, J., AND GAO, D., Separation of long DNA molecules through cleavage of hydrogen bonds under a stretching force. Appl. Phys. Lett. **91** (2007) 113902.
- [117] DRAGOVIC, R. A., GARDINER, C., BROOKS, A. S., TANNETTA, D. S., FER-GUSON, D. J. P., HOLE, P., CARR, B., REDMAN, C. W. G., HARRIS, A. L., DOBSON, P. J., HARRISON, P., AND SARGENT, I. L., Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis. Nanomedicine 7 (2011) 780–788.
- [118] ALIVISATOS, A. P., JOHNSSON, K. P., PENG, X., WILSON, T. E., LOWETH, C. J., BRUCHEZ, M. P. J., AND SCHULTZ, P., Organization of "nanocrystal molecules" using DNA. Nature 382 (1996) 609–611.
- [119] FRENS, G., Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. Nature Phys. Sci. **241** (1973) 20–22.
- [120] BRUST, M., WALKER, M., BETHELL, D., SCHRIFFRIN, D. J., AND WHYMAN, R., Synthesis of Thiol-derivatised Gold Nanoparticles in a Two-phase Liquid-Liquid System. Chem. Commun. 7 (1994) 801–802.
- [121] MARTIN, M. N., BASHAM, J. I., CHANDO, P., AND EAH, S.-K., Charged Gold Nanoparticles in Non-Polar Solvents: 10-min Synthesis and 2D Self-Assembly. Langmuir 26 (2010) 7410–7417.

- [122] PERRAULT, S. D. AND CHAN, W. C. W., Synthesis and Surface Modification of Highly Monodispersed, Spherical Gold Nanoparticles of 50-200 nm. J. Am. Chem. Soc. 131 (2009) 17042–17043.
- [123] JANA, N. R., GEARHEART, L., AND MURPHY, C. J., Seeding Growth for Size Control of 5-40 nm Diameter Gold Nanoparticles. Langmuir 17 (2001) 6782–6786.
- [124] ZIEGLER, C. AND EYCHMÜLLER, A., Seeded Growth Synthesis of Uniform Gold Nanoparticles with Diameters of 15-300 nm. J. Phys. Chem. C 115 (2011) 4502– 4506.
- [125] KIMLING, J., MAIER, M., OKENVE, B., KOTAIDIS, V., BALLOT, H., AND PLECH, A., *Turkevich Method for Gold Nanoparticle Synthesis Revisited*. J. Phys. Chem. B 110 (2006) 15700–15707.
- [126] SPERLING, R. A. AND PARAK, W. J., Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. Phil. Trans. R. Soc. A 368 (2010) 1333–1383.
- [127] SAU, T. K. AND MURPHY, C. J., Room Temperature, High-Yield Synthesis of Multiple Shapes of Gold Nanoparticles in Aqueous Solution. J. Am. Chem. Soc. 126 (2004) 8648–8649.
- [128] PÉREZ-JUSTE, J., PASTORIZA-SANTOS, I., LIZ-MARZÁN, L. M., AND MUL-VANEY, P., Synthesis, characterization and applications. Coord. Chem. Rev. 249 (2005) 1870–1901.
- [129] HU, J., WANG, Z., AND LI, J., Gold Nanoparticles With Special Shapes: Controlled Synthesis, Surface-enhanced Raman Scattering, and The Application in Biodetection. Sensors 7 (2007) 3299–3311.
- [130] MIRKIN, C. A., LETSINGER, R. L., MUCIC, R. C., AND STORHOFF, J. J., A DNA-based method for rationally assembling nanoparticles into macroscopic materials. Nature 382 (1996) 607–609.
- [131] STORHOFF, J. J., ELGHANIAN, R., MUCIC, R. C., MIRKIN, C. A., AND LETSINGER, R. L., One-Pot Colorimetric Differentiation of Polynucleotides with Single Base Imperfections Using Gold Nanoparticle Probes. J. Am. Chem. Soc. 120 (1998) 1959–1964.
- [132] ELGHANIAN, R., STORHOFF, J. J., MUCIC, R. C., LETSINGER, R. L., AND MIRKIN, C. A., Selective Colorimetric Detection of Polynucleotides Based on the Distance-Dependent Optical Properties of Gold Nanoparticles. Science 277 (1997) 1078–1081.

- [133] FESTAG, G., STEINBRÜCK, A., WOLFF, A., CSÁKI, A., MÖLLER, R., AND FRITZSCHE, W., Optimization of Gold Nanoparticle-Based DNA Detection for Microarrays. J. Fluoresc. 15 (2004) 161–170.
- [134] CSÁKI, A., MÖLLER, R., STRAUBE, W., KÖHLER, J. M., AND FRITZSCHE, W., DNA monolayer on gold substrates characterized by nanoparticle labeling and scanning force microscopy. Nucleic Acids Res. 29 (2001) e81.
- [135] HURST, S. J., LYTTON-JEAN, A. K. R., AND MIRKIN, C. A., *Maximizing DNA* Loading on a Range of Gold Nanoparticle Sizes. Anal. Chem. **78** (2006) 8313–8318.
- [136] ZU, Y. AND GAO, Z., Facile and Controllable Loading of Single-Stranded DNA on Gold Nanoparticles. Anal. Chem. **81** (2009) 8523–8528.
- [137] ZHANG, X., SERVOS, M. R., AND LIU, J., Instantaneous and Quantitative Functionalization of Gold Nanoparticles with Thiolated DNA Using a pH-Assisted and Surfactant-Free Route. J. Am. Chem. Soc. 134 (2012) 7266–7269.
- [138] LIU, J. AND LU, Y., Accelerated Color Change of Gold Nanoparticles Assembled by DNAzymes for Simple and Fast Colorimetric Pb2+ Detection. J. Am. Chem. Soc. 126 (2004) 12298–12305.
- [139] JIN, R., WU, G., LI, Z., MIRKIN, C. A., AND SCHATZ, G. C., What Controls the Melting Properties of DNA-Linked Gold Nanoparticle Assemblies. J. Am. Chem. Soc. 125 (2003) 1643–1654.
- [140] GU, J. AND HACKER, G. W., Gold and Silver Staining: Techniques in Molecular Morphology (CRC Press, New York, NY, 2002).
- [141] FESTAG, G., STEINBRÜCK, A., CŚAKI, A., MÖLLER, R., AND FRITZSCHE, W., Single particle studies of the autocatalytic metal deposition onto surface-bound gold nanoparticles reveal a linear growth. Nanotechnology **18** (2007) 015502.
- [142] BROWN, K. R., WALTER, D. G., AND NATAN, M. J., Seeding of colloidal Au nanoparticle solutions. 2. Improved control of particle size and shape. Chem. Mater. 12 (2000) 306–313.
- [143] LACKIE, P. M., HENNESSY, R. J., HACKER, G. W., AND POLAK, J. M., Investigation of immunogold-silver staining by electron microscopy. Histochemistry 83 (1985) 545–550.
- [144] SARID, D. AND CHALLENER, W., Modern Introduction to Surface plasmons: Theory, Mathematica Modeling, and Applications (Cambridge University Press, Cambridge, UK, 2010).

- [145] MAIER, S. A., *PLASMONICS: FUNDAMENTALS AND APPLICATIONS* (Springer Science, New York, USA, 2007).
- [146] BLENDER, Open Source 3D Creation Suite, https://www.blender.org/ (2017). [Online; accessed 7-septemper-2017].
- [147] RES-TEC, WinSpall data analysis software, http://www.res-tec.de/ index.html (2017). [Online; accessed 31-septemper-2017].
- [148] PITERA, J. W., FALTA, M., AND VAN GUNSTEREN, W. F., Dielectric Properties of Proteins from Simulation: The Effects of Solvent, Ligands, pH and Temperature. Biophys. J. 80 (2001) 2546–2555.
- [149] UCHIHO, Y., SHIMOJO, M., FURUYA, K., AND KAJIKAWA, K., Optical Response of Gold-Nanoparticle-Amplified Surface Plasmon Resonance Spectroscopy. J. Phys. Chem. C 114 (2010) 4816–4824.
- [150] JACKSON, J. D., *Classical Electrodynamics* (Johnson Wiley & Sons, Inc., New York, NY, US, 1999).
- [151] HUFFMAN, D. R., Absorption and Scattering of Light by Small Particles (WILEY-VCH Verlag GmbH & Co., Weinheim , Germany, 1983).
- [152] NANOCOMPOSIX, Mie Theory Calculator, https://nanocomposix.eu/ pages/tools (2017). [Online; accessed 7-septemper-2017].
- [153] CHANCE, R. R., PROCK, A., AND SILBEY, R., Frequency shifts of an electricdipole transition near a partially reflecting surface. Phys. Rev. A 12 (1975) 1448– 1452.
- [154] MOCK, J. J., HILL, R. T., DEGIRON, A., ZAUSCHER, S., CHILKOTI, A., AND SMITH, D. R., Distance-Dependent Plasmon Resonant Coupling between a Gold Nanoparticle and Gold Film. Nano lett. 8 (2008) 2245–2252.
- [155] DATHE, A., ZIEGLER, M., HÜBNER, U., FRITZSCHE, W., AND STRANIK, O., Electrically Excited Plasmonic Nanoruler for Biomolecule Detection. Nano lett. 16 (2016) 5728–5736.
- [156] LUMDEE, C., YUN, B., AND KIK, P. G., *Gap-Plasmon Enhanced Gold Nanoparticle Photoluminescence*. ACS Photonics **1** (2014) 1224–1230.
- [157] LUMDEE, C., TOROGHI, S., AND KIK, P. G., Post-fabrication voltage controlled resonance tuning of nanoscale plasmonic antennas. ACS Nano 6 (2012) 6301–6307.
- [158] DRISKELL, J. D., LIPERT, R. J., AND PORTER, M. D., Labeled Gold Nanoparticles Immobilized at Smooth Metallic Substrates Systematic Investigation of Surface

Plasmon Resonance and Surface-Enhanced Raman Scattering. J. Phys. Chem. B **110** (2006) 17744–17451.

- [159] WANG, Q., LIU, L., WANG, Y., LIU, P., JIANG, H., XU, Z., MA, Z., OREN, S., CHOW, E. K. C., LU, M., AND DONG, L., *Tunable Optical Nanoantennas Incorporating Bowtie Nanoantenna Arrays with Stimuli-Responsive Polymer*. Sci. Rep. 5 (2015) 18567.
- [160] CHIEN, M.-H., NIEN, L.-W., CHAO, B.-K., LI, J.-H., AND HSUEH, C.-H., Effects of the rotation angle on surface plasmon coupling of nanoprisms. Nanoscale 8 (2016) 3660–3670.
- [161] KINKHABWALA, A., YU, Z. F., FAN, S. H., AVLASEVICH, Y., MULLEN, K., AND MOERNER, W. E., Large single-molecule fluorescence enhancements produced by a bowtie nanoantenna. Nat. Photonics 3 (2009) 654–657.
- [162] FROMM, D. P., SUNDARAMURTHY, A., SCHUCK, P. J., KINO, G., AND MO-ERNER, W. E., Gap-Dependent Optical Coupling of Single Bowtie Nanoantennas Resonant in the Visible. Nano lett. 4 (2004) 957–961.
- [163] KANIBER, M., SCHRAML, K., REGLER, A., BARTL, J., GLASHAGEN, G., FLAS-SIG, F., WIERZBOWSKI, J., AND FINLEY, J. J., Surface plasmon resonance spectroscopy of single bowtie nano-antennas using a differential reflectivity method. Sci. Rep. 6 (2016) 23203.
- [164] DING, W., BACHELOT, R., KOSTCHEEV, S., ROYER, P., AND DE LAMAESTRE, R. E., Surface plasmon resonance in silver Bowtie nanoantennas with varied bow angles. J. Appl. Phys. 108 (2010) 124314.
- [165] FISCHER, H. AND MARTIN, O. J. F., *Engineering the optical response of plasmonic nanoantennas*. Opt. Express **16** (2008) 9144–9154.
- [166] ASH, E. A. AND NICHOLLS, G., Super-Resolution Aperture Scanning Microscope. Nature 237 (1972) 510–512.
- [167] SHANKARAN, D. R., GOBI, K. V., AND MIURA, N., Recent advancements in surface plasmon resonance immunosensors for detection of small molecules of biomedical, food and environmental interest. Sens. Actuator 121 (2007) 158–177.
- [168] POHANKA, M., SKLÁDAL, P., AND KROÈA, M., Biosensors for Biological Warfare Agent Detection. Def. Sci. J. 57 (2007) 185–193.
- [169] AKIMOTO, T., SASAKI, S., IKEBUKURO, K., AND KARUBE, I., Effect of incident angle of light on sensitivity and detection limit for layers of antibody with surface plasmon resonance spectroscopy. Biosens. Bioelectron. **12** (2000) 355–362.

- [170] BABA, A., PARK, M.-K., ADVINCULA, R. C., AND KNOLL, W., Simultaneous Surface Plasmon Optical and Electrochemical Investigation of Layer-by-Layer Self-Assembled Conducting Ultrathin Polymer Films. Langmuir 18 (2002) 4648–4652.
- [171] VUIGNIER, K., SCHAPPLER, J., AND VEUTHEY, J.-L., *Drug-protein binding: a critical review of analytical tools*. Anal. Bioanal. Chem. **398** (2010) 53–66.
- [172] DE MOL, N. J., DEKKER, F. J., BROUTIN, I., FISCHER, M. J. E., AND LISKAMP, R. M. J., Surface Plasmon Resonance Thermodynamic and Kinetic Analysis as a Strategic Tool in Drug Design. Distinct Ways for Phosphopeptides to Plug into Srcand Grb2 SH2 Domains. J. Med. Chem. 48 (2005) 753–763.
- [173] CHU, B., Laser light Scattering: Basic Principles and Practice, Second Edition (Academic Press, Elsevier, Cambridge, US, 1991).
- [174] LI, Y., LUBCHENKO, V., AND VEKILOV, P. G., The use of dynamic light scattering and Brownian microscopy to characterize protein aggregation. Rev. Sci. Instrum. 82 (2011) 053106.
- [175] LIM, J., YEAP, S. P., CHE, H. X., AND LOW, S. C., *Characterization of magnetic nanoparticle by dynamic light scattering*. Nanoscale Res. Lett. **8** (2013) 381.
- [176] FULTON, T. AND DOLON, G., Observation of single-electron charging effects in small tunnel junctions. Phys. Rev. Lett. **59** (1987) 109–112.
- [177] AVERIN, D. V. AND LIKHAREV, K. K., *Mesoscopic Phenomena in Solids* (Elsevier, Amsterdam, 1991).
- [178] GEERLIGS, L. J., ANDEREGG, V. F., HOLWEG, P. A. M., MOOIJ, J. E., POTH-IER, H., ESTEVE, D., URBINA, C., AND DEVORET, M. H., *Frequency-locked turnstile device for single electrons*. Phys. Rev. Lett. **64** (1990) 2691.
- [179] AVERIN, D. V. AND NAZAROV, Y. V., Virtual electron diffusion during quantum tunneling of the electric charge. Phys. Rev. Lett. **65** (1990) 2446.
- [180] VAN HOUTEN, H. AND BEENAKKER, C. W. J., Billiard model of a ballistic multiprobe conductor. Phys. Rev. Lett. 63 (1989) 1893.
- [181] GRABERT, H. AND DEVORET, M. H., Single Charge Tunneling: Coulomb Blockade Phenomena in Nanostructures (Plenum Press, New York, US, 1992).
- [182] KIM, J., OH, S., PARK, J. W., LEE, J.-O., KWON, H. C., PARK, S. I., KIM, K. T., YOO, K. H., AND KIM, J.-J., Effect of cross capacitance in three junction single-electron transistor. Phys. Rev. B 59 (1999) 1629–1632.

- [183] POSTMA, H. W. C., TEEPEN, T., YAO, Z., GRIFONI, M., AND DEKKER, C., Carbon Nanotube Single-Electron Transistor at Room Temperature. Science 293 (2001) 76–70.
- [184] DITTRICH, T., HÄNGGI, P., INGOLD, G., KRAMER, B., SCHÖN, G., AND ZW-ERGER, W., Quantum Transport and Dissipation (Wiley-VCH, Weinheim, Germany, 1997).
- [185] KOUWENHOVEN, L. P., VAN DER VAART, N. C., JOHNSON, A. T., KOOL, W., HARMANS, C. J. P. M., WILLIAMSON, J. G., STARING, A. A. M., AND FOXON, C. T., Single electron charging effects in semiconductor quantum dots. Z. Phys. B.-Condensed Matter 85 (1991) 367–373.
- [186] RAWLINS, A. D., Note on the Capacitance of Two Closely Separated Spheres. IMA J. Appl. Math. 34 (1985) 119–120.
- [187] MELSEN, J. A., HANKE, U., MÜLLER, H.-O., AND CHAO, K.-A., Coulomb blockade threshold in inhomogeneous one-dimensional arrays of tunnel junctions. Phys. Rev. B 55 (1997) 10639–10642.
- [188] TANS, T. J., DEVORET, M. H., DAI, H., THESS, A., SMALLEY, R. E., GEER-LING, L. J., AND DEKKER, C., Individual single-wall carbon nanotubes as quantum wires. Nature 386 (1997) 474–477.
- [189] HEIM, T., DERESMES, D., AND VUILLAUME, D., Conductivity of DNA probed by conducting-atomic force microscopy: Effects of contact electrode, DNA structure, and surface interactions. J.Appl.Phys. 96 (2004) 2927–2936.
- [190] POHL, H. A., The Motion and Precipitation of Suspensoids in Divergent Electric Fields. J. Appl. Phys. 22 (1951) 869.
- [191] POHL, H. A., Dielectrophoresis: The Behavior of Neutral Matter in Nonuniform Electric Fields (Cambridge University Press, Cambridge, UK, 1978).
- [192] JONES, T. B., *Basic theory of dielectrophoresis and electrorotation*. IEEE EMBS Magazine **22** (2003) 33.
- [193] ZHENG, L., BRODY, J. P., AND BURKE, P. J., Electronic manipulation of DNA, proteins, and nanoparticles for potential circuit assembly. Biosens. Bioelectron. 20 (2004) 606–619.
- [194] SHEN, B., LINKO, V., DIETZ, H., AND TOPPARI, J. J., Dielectrophoretic trapping of multilayer DNA origami nanostructures and DNA origami-induced local destruction of silicon dioxide. Electrophoresis 36 (2015) 255–262.

- [195] GERSTEN, J. AND NITZAN, A., Spectroscopic properties of molecules interacting with small dielectric particles. J. Chem. Phys. **75** (1981) 1139–1152.
- [196] LAKOWICZ, J. R., GEDDES, C. D., GRYCZYNSKI, I., MALICKA, J., GRYCZYN-SKI, Z., ASLAN, K., LUKOMSKA, J., MATVEEVA, E., ZHANG, J. A., AND BADUGU, R., Advances in surface-enhanced fluorescence. J. Fluoresc. 14 (2004) 425–441.
- [197] ALBRECHT, M. G. AND CREIGHTON, J. A., Anomalously intense Raman-spectra of pyridine at a silver electrode. J. Am. Chem. Soc. **99** (1977) 5212–5217.
- [198] KLEINMAN, S. L., FRONTIERA, R. R., HENRY, A. I., DIERINGER, J. A., AND DUYNE, R. P. V., Creating, characterizing, and controlling chemistry with SERS hot spots. Phys. Chem. Chem. Phys. 15 (2013) 21–36.
- [199] HARTSTEIN, A., KIRTLEY, J. R., AND TSANG, J. C., Enhancement of the infraredabsorption from molecular monolayers with thin metal overlayers. Phys. Rev. Lett. 45 (1980) 201–204.
- [200] HATTA, A., OHSHIMA, T., AND SUËTAKA, W., Observation of the enhanced infrared-absorption of para-nitrobenzoate on Ag island films with an Atr technique. Appl. Phys. A **29** (1982) 71–75.
- [201] YU, N. AND CAPASSO, F., Flat optics with designer metasurfaces. Nature 13 (2014) 139–150.
- [202] WILLETS, K. A. AND DUYNE, R. P. V., Localized surface plasmon resonance spectroscopy and sensing. Annu. Rev. Phys. Chem. 58 (2007) 267–297.
- [203] HOMOLA, J., Surface Plasmon Resonance Based Sensors (Springer-Verlag, Berlin/Heidelberg, Germany, 2006).
- [204] QIN, D., XIA, Y., AND WHITESIDES, G. M., Soft lithography for micro- and nanoscale patterning. Nat. Protoc. 5 (2010) 491–502.
- [205] SHEN, B., TAPIO, K., LINKO, V., KOSTIAINEN, M. A., AND TOPPARI, J. J., Metallic Nanostructures Based on DNA Nanoshapes. Nanomaterials 6 (2016) 6080146.
- [206] WILLIAMS, K. R., GUPTA, K., AND WASILIK, M., *Etch Rates for Micromachining Processing - Part II.* J. Microelectromech. Syst. **12** (2003) 761–778.
- [207] LIU, N., TANG, M. L., HENTSCHEL, M., GIESSEN, H., AND ALIVISATOS, A. P., Nanoantenna-enhanced gas sensing in a single tailored nanofocus. Nat. Mater. 10 (2011) 631–636.

- [208] WILCHEK, M. AND BAYER, E. A., *The avidin-biotin complex in bioanalytical applications*. Anal. Biochem. **171** (1988) 1–32.
- [209] LUNDGREN, A., AGNARSSON, B., ZIRBS, R., ZHDANOV, V., REIMHULT, E., AND HÖÖK, F., Nonspecific Colloidal-Type Interaction Explains Size-Dependent Specific Binding of Membrane-Targeted Nanoparticles. ACS Nano 10 (2016) 9974– 9982.
- [210] CASWELL, K. K., WILSON, J. N., BUNZ, U. H. F., AND MURPHY, C. J., Preferential End-to-End Assembly of Gold Nanorods by Biotin-Streptavidin Connectors. J. Am. Chem. Soc. 125 (2003) 13914–13915.
- [211] VIKHOLM-LUNDIN, I., AUER, S., PAAKKUNAINEN, M., MÄÄTTÄ, J. A. E., MUNTER, T., J.LEPPINIEMI, HYTÖNEN, V. P., AND TAPPURA, K., Cysteinetagged chimeric avidin forms high binding capacity layers directly on gold. Sensors and Actuators B 171-172 (2012) 440–448.
- [212] LIKHAREV, K. K., Single-electron devices and their applications. Proc. IEEE 87 (1999) 606–632.
- [213] YADAVALLI, K. K., ORLOV, A., SNIDER, G. L., AND KOROTKOV, A. N., Single electron memory devices: Towards background charge insensitive operation. J. Vac. Sci. Technol., B: Microelectron. Process. Phenom. 21 (2003) 2860–2864.
- [214] ALTSHULER, B. L., LEE, P. A., AND WEBB, R. A., *Mesoscopic Phenomena in Solids* (Elsevier, Amsterdam, 1991).
- [215] KOROTKOV, A. N., Single-electron logic and memory devices. Int. J. Electron. 86 (1999) 511–547.
- [216] TAPIO, K., LEPPINIEMI, J., SHEN, B., HYTÖNEN, V. P., FRITZSCHE, W., AND TOPPARI, J. J., Toward Single Electron Nanoelectronics Using Self-Assembled DNA Structure. Nano lett. 16 (2016) 6780–6786.
- [217] ZHANG, X., SERVOS, M. R., AND LIU, J., Surface Science of DNA Adsorption onto Citrate-Capped Gold Nanoparticles. Langmuir 28 (2012) 3896–3902.
- [218] CSÁKI, A., KAPLANEK, P., MÖLLER, R., AND FRITZSCHE, W., The optical detection of individual DNA-conjugated gold nanoparticle labels after metal enhancement. Nanotechnology 14 (2003) 1262–1268.
- [219] THACKER, V. V., HERRMANN, L. O., SIGLE, D. O., ZHANG, T., LIEDL, T., BAUMBERG, J. J., AND KEYSER, U. F., DNA origami based assembly of gold nanoparticle dimers for surface-enhanced Raman scattering. Nat. Commun. 5 (2013) 3448.

- [220] SHEN, X., ASENJO-GARCIA, A., LIU, Q., JIANG, Q., DE ABAJO, F. J. G., LIU, N., AND DING, B., *Three-Dimensional Plasmonic Chiral Tetramers Assembled by DNA Origami*. Nano lett. 13 (2013) 2128–2133.
- [221] KUZYK, A., *Molecular devices for nanoelectronics and plasmonics*, Ph.D. thesis, Department of Physics, University of Jyväkylä (2009).
- [222] TAPIO, K. AND TOPPARI, J. J., Characterization of Emergence of the Coulomb Blockade in a Pearl-Like DNA-AuNP Assembly. SAME 5 (2016) 31–44.
- [223] AGOSTON, M., Computer graphics and Geometric Modelling v.1: Implementation and Algorithms (Springer, London, UK, 2004).
- [224] TO HSV, R., Math behind colorspace conversion, RGB-HSL, http://www.niwa. nu/2013/05/math-behind-colorspace-conversions-rgb-hsl/ (2017). [Online; accessed 30-january-2017].

Appendix A

Solution for electrostatic potential in Quasistatic approximation

The electrostatic potential outside (Φ_{out}) and inside (Φ_{in}) of a spherical gold nanoparticle is solved from Laplace equation 2.22 and they have general form of

$$\Phi_{in} = \sum_{j} A_j r^j P_j(\cos(\theta)), \tag{A.1}$$

$$\Phi_{out} = \sum_{j} \left(B_j r^j + C_j r^{-(j+1)} \right) P_j(\cos(\theta)),$$
(A.2)

where Φ_{in} and Φ_{out} follow the Legendre Polynomial formula due to the azimuthal symmetry [150, 151], \vec{r} is the position vector and the coefficients A_i , B_i and C_i are set by the boundary conditions, when $r \to \infty$ and r = a. We are using the spherical coordinates in all of the derivations. The simplest condition to start with is the limit $r \to \infty$, since in this limit the electric field is assumed to be constant in respect to z-coordinate as shown in figure 2.12a. Since $|\vec{E}| = \text{constant}$ and $\vec{E} = -\nabla \Phi$, then $\Phi_{out} \to -E_0 z = -E_0 r \cdot \cos(\theta)$, when $r \to \infty$ and $z = r \cdot \cos(\theta)$ from figure 2.12a. The constants B_j can be also solved from equation A.2 at the limit $z \sim r \to \infty$

$$\begin{split} \Phi_{out} &= \sum_{j=0}^{\infty} \left(B_j r^j + C_j r^{-(j+1)} \right) P_j(\cos(\theta)) = -E_0 r \cos(\theta), \\ -E_0 r \cdot \cos(\theta) &= B_0 + B_1 \cdot r \cdot \cos(\theta) + \sum_{j=2}^{\infty} \left(B_j r^j + C_j r^{-(j+1)} \right) P_j(\cos(\theta)) \\ B_1 &= -E_0, j = 1. \\ B_j &= 0, \forall j \neq 1. \end{split}$$

In the above derivations, the Legendre Polynomials $P_0 = 1$ and $P_1 = \cos(\theta)$ and only j = 1 term is non-zero, since only the first order terms are present both sides of the equation. The boundary conditions at r = a require that tangential components of the electric field \vec{E} are equal and normal component of the displacement field \vec{D} are equal for Φ_{in} and Φ_{out} . By solving the tangential conditions we can derive relation between A_j and C_j as following:

$$\begin{split} E_{in/out} &= -\nabla \Phi_{in/out} = -\left(\frac{\partial}{\partial r}\hat{r} + \frac{1}{r}\frac{\partial}{\partial \theta} + \frac{1}{r\sin(\theta)}\frac{\partial}{\partial \phi}\hat{\phi}\right)\Phi_{in/out}(r=a) \\ &-\frac{1}{a}\frac{\partial}{\partial \theta}\Phi_{in}(r=a) = -\frac{1}{a}\frac{\partial}{\partial \theta}\Phi_{out}(r=a), \\ &\frac{\partial\Phi_{in}(r)}{\partial \theta} = \frac{\partial}{\partial \theta}\left(\sum_{j}A_{j}r^{j}P_{j}(\cos(\theta))\right) = \sum_{j}A_{j}r^{j}\frac{\partial P_{j}(\cos(\theta))}{\partial \theta}, \\ &\frac{\partial\Phi_{out}(r)}{\partial \theta} = \frac{\partial}{\partial \theta}\left(\sum_{j}\left(B_{j}r^{j} + C_{j}r^{-(j+1)}\right)P_{j}(\cos(\theta))\right)\right), \\ &\frac{\partial\Phi_{out}(r)}{\partial \theta} = \sum_{j}\left(B_{j}r^{j} + C_{j}r^{-(j+1)}\right)\frac{\partial P_{j}(\cos(\theta))}{\partial \theta}, \end{split}$$

Since $P_0(\cos(\theta)) = 1$ and $P_1(\cos(\theta)) = \cos(\theta)$, then above equation simplify into

$$-\frac{1}{a}\frac{\partial}{\partial\theta}\Phi_{in}(r=a) = -\frac{1}{a}\frac{\partial}{\partial\theta}\Phi_{out}(r=a),$$
$$\sum_{j}A_{j}a^{j}\frac{\partial P_{j}(\cos(\theta))}{\partial\theta} = \sum_{j}\left(B_{j}a^{j} + C_{j}a^{-(j+1)}\right)\frac{\partial P_{j}(\cos(\theta))}{\partial\theta},$$
$$A_{1}\cdot a\cdot\frac{\partial P_{1}(\cos(\theta))}{\partial\theta} + \sum_{j=2}^{\infty}A_{j}a^{j}\frac{\partial P_{j}(\cos(\theta))}{\partial\theta} = B_{1}\cdot a\cdot\frac{\partial P_{1}(\cos(\theta))}{\partial\theta} + \sum_{j=1}^{\infty}C_{j}a^{-(j+1)}\frac{\partial P_{j}(\cos(\theta))}{\partial\theta}.$$

Since above equation must hold for all θ [150], then $A_j = C_j = 0$, when $j \neq 1$, and the equation above simplifies into

$$\sum_{j=2}^{\infty} \left(\underbrace{A_j}_{=0} \cdot a^j - \underbrace{C_j}_{=0} \cdot a^{-(j+1)} \right) \cdot \underbrace{\frac{\partial P_j}{\partial \theta}}_{\neq 0} + \left(A_1 \cdot a - B_1 \cdot a - C_1 \cdot a^{-2} \right) \cdot \underbrace{\frac{\partial P_1}{\partial \theta}}_{\neq 0} = 0,$$
$$A_1 \cdot a = B_1 \cdot a + C_1 a^{-2},$$
$$A_1 = -E_0 + C_1 a^{-3}.$$

The condition for the normal component of the displacement field \vec{D} in equation 2.5 can be used to solve the coefficients A_1 and C_1 , where the other coefficients A_j and C_j coefficient are assumed to be zero.

$$\begin{split} \vec{D}_{in}(r=a) &= \vec{D}_{out}(r=a), \\ \Rightarrow \epsilon_0 \epsilon_m \nabla \Phi_{in}(r=a) &= \epsilon_0 \epsilon_d \nabla \Phi_{out}(r=a), \\ \frac{\partial}{\partial r} \left(\epsilon_m \Phi_{in}(r=a) \right) &= \frac{\partial}{\partial r} \left(\epsilon_d \Phi_{out}(r=a) \right), \\ \frac{\partial \Phi_{in}(r)}{\partial r} &= \frac{\partial}{\partial r} \left(A_1 r P_1(\cos(\theta)) \right) = A_1 \cdot \cos(\theta), \\ \frac{\partial \Phi_{out}(r)}{\partial r} &= \frac{\partial}{\partial r} \left(\left(B_1 \cdot r + C_1 r^{-2} \right) P_1(\cos(\theta)) \right) \right) = \left(B_1 - 2 \cdot C_1 \cdot r^{-3} \right) P_1(\cos(\theta)), \\ \Rightarrow \frac{\partial \Phi_{out}(r)}{\partial r} &= -E_0 \cdot \cos(\theta) - 2 \cdot C_1 \cdot r^{-3} \cdot \cos(\theta), \\ \epsilon_m A_1 \cdot \cos(\theta) &= -E_0 \epsilon_d \cdot \cos(\theta) - 2 \epsilon_d \cdot C_1 \cdot r^{-3} \cdot \cos(\theta), \\ \Rightarrow \epsilon_m A_1 &= -E_0 \epsilon_d - 2 \epsilon_d \cdot C_1 \cdot r^{-3}, \\ \Rightarrow \epsilon_m \left(-E_0 + C_1 a^{-3} \right) &= -E_0 \epsilon_d - 2 \epsilon_d \cdot C_1 \cdot r^{-3}, \\ \Rightarrow C_1 &= \frac{E_0}{a^3} \frac{\epsilon_m - \epsilon_d}{\epsilon_m + 2 \epsilon_d}, \\ \Rightarrow A_1 &= -E_0 + C_1 a^{-3} = -\frac{3 \epsilon_d E_0}{\epsilon_m + 2 \epsilon_d}. \end{split}$$
(A.3)

By substituting the coefficients A_1 and C_1 from equations A.3 and A.4 and $B_1 = -E_0$ into equations A.1 and A.2, the final forms of the Φ_{in} and Φ_{out} are

$$\begin{split} \Phi_{in} &= -\frac{3\epsilon_d}{\epsilon_m + 2\epsilon_d} E_0 r \cdot \cos\left(\theta\right), \\ \Phi_{out} &= -E_0 r \cdot \cos\left(\theta\right) + \frac{\epsilon_m - \epsilon_d}{\epsilon_m + 2\epsilon_d} E_0 a^3 \frac{\cos\left(\theta\right)}{r^2}, \end{split}$$

Nanoactuator experiments

DNA strands for nanoactuator experiments

The thiol-modified DNA strands were purchased from Integrated DNA technologies (IDT,Coralville, Iowa, USA) and the strand sequences are shown in table A.1.

Strand	DNA sequence
hairpin-DNA	/5BiosG/AAAAAAAAAAAAAAAAATACCCGGGCGT-
	TTTTCGCCCGGGTAAAAAAAA/3ThioMC3
DNA-s15nm	/5BiosG/ACACACACACACACACACACACACAC-
	ACACACACACACACACACA/3ThioMC3-D/
DNA-s8nm	/5BiosG/ACACACACACACACACACACACAC/3ThioMC3-D/
DNA-s3nm	/5BiosG/ACACACACAC/3ThioMC3-D/

TABLE A.1: The see	quences of the	different DI	NA strands.
--------------------	----------------	--------------	-------------

Additional SPR data



FIGURE A.1: Chimeric avidin, BSA and biotin-AuNP immobilization on gold surface. (a) The binding of chimeric avidin and the blocking agent (BSA) was detected as a positive shift in the resonant angle. (b) The SPR characterization of the assembly steps for chimeric avidin, SH-PEG and 8 nm linker DNA coated AuNP immobilization. The binding of chimeric avidin and blocking agent (SH-PEG) is detected as a positive shift in the resonant angle but, for the 8 nm linker DNA coated AuNPs, the overall shift is negligible due to the plasmonic coupling between the surface and the AuNPs. The dark field images of the arbitrary, plain gold surface (left inset) and the channel after the AuNP deposition (right inset) show still that the AuNPs are binding to the surface.



FIGURE A.2: Different preparation stages of the actuator experiment and an image of the sample holder. (a) A schematic view of the holder before any modifications. The dotted lines indicate the fluid channels. (b) Aluminum contacts are added for the ITO cover glass. The dashed line indicates the cross section in a. (c) The aluminum contact is added for the hydrophilic gold coated silicon chip, which is then glued to the contact. (d) Chimeric avidin and BSA solution are incubated for 30 min, followed by washing step. (e) ITO cover glass is placed over the gold surface to seal the surface into a chamber. The dashed line indicates the cross section in f. (f) A schematic side view of the holder configuration in e. The fluid channels are wetted before the AuNP deposition. (g) During the biotin-AuNP immobilization, the AuNP solution is injected to the chamber and incubated for 15 to 90 min. Then pump is turned on and NaPhos and NaCl buffer is added while pumping the excess liquid away. (h) Image of the sample holder without the ITO cover glass. The length of the holder is 75 mm and the width is 26 mm.

Sample holder in the nanoactuator experiments

The sample holder used in the nanoactuator measurement is illustrated in figure A.2h together with the different preparation stages of the experiment in figures A.2a-g. Initially, new aluminum contacts were established on the holder using aluminum tape (figures A.2a and A.2b). Next, an aluminum contact was added for the hydrophilic, gold coated silicon chip, which was glued to the contact using silver paste and varnish (figure A.2c). The silver paste was only added to the backside gold contacts and it was circled using the varnish. This was followed by addition of the TCEP-treated chimeric avidin solution and the solution was incubated for 30 min (figure A.2d). The incubation was followed by washing the surface with 60 µl

of PBS three times and addition of 0.5 mg/ml BSA solution, which was then incubated for 30 min. The following washing step was done similarly as before except using 0.1 mM NaPhos and 1 mM NaCl buffer (pH 7.6).

After BSA incubation and washing, the surface was not allowed to dry and ITO cover glass was fixed on top of the surface using scotch tape (figure A.2e). To acquire smoother flow through the channels, the both fluid channels were wetted using the same NaPhos and NaCl buffer (figure A.2f). We placed the holder in the microscope and ISMATEC tubing pump (ISM597D) was connected to the output channel. The AuNPs were immobilized on the BSA-chimeric avidin coated surface by injecting 50-100 µl of biotin-AuNP solution (c = 7.7-22.3 pM) to the input channel and waiting 15 to 90 min until enough particles were bound to the surface (figure A.2g). Then excess particles were washed away by pumping \sim 4 ml of the same NaPhos and NaCl buffer through the chamber.

Hydrodynamic diameter of hairpin-DNA and ssDNA coated AuNPs

BECKMAN Coulter N5 submicron Particle size Analyzer was used to measure the the hydrodynamic diameter of the hairpin-DNA functionalized AuNPs. The measurement started by first washing three times the 4 ml cuvettes with ddH_2O . The water was filtered before using 0.1 µm pore size syringe filter. Next, 10 µl of the sample was mixed with 4 ml of ddH_2O , bubbles on the cuvette walls were removed and the cuvette was placed inside the analyzer. Thermally stabilization time was 5 min, and after the stabilization the average hydrodynamic diameter was measured for 5 runs, which each lasted 120 s. The results are shown in figure A.3.



FIGURE A.3: (a) The initial hydrodynamic diameter of different batches of hairpin-DNA functionalized AuNPs in respect to the ratio of hairpin-DNA and blocking-DNA. (b) The polydispersity index for the same samples as in a.

Zeta potential of the hairpin-DNA and ssDNA coated AuNPs

The zeta potentials of different DNA coated AuNPs were measured using Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Typical experiment included diluting the sample $400 \times$ using 10 mM NaPO_4 , 1 mM NaCl buffer in disposable folded capillary cells (count rate 20-107 kcps), placing the cell into Zetasizer and measuring the zeta potential at $20 \,^{\circ}$ C temperature for $60 \,\text{s}$. The results are shown in figure A.4, where the positive and negative peaks are listed in the table A.2.

Sample	Neg. Peak (mV)	Pos. Peak (mV)	Neg. AuNP (%)	Pos. AuNP (%)
hairpin-DNA	-27.3	+20.8	94.2	5.8
DNA-s15nm	-48.6	-	99.1	0.9
DNA-s8nm	-35.1	+24.0	96.5	3.5
DNA-s3nm	-36.9	-	98.7	1.3

TABLE A.2: The charge composition of the biotin-AuNP samples



FIGURE A.4: The charge distribution of (a) 0.1% hairpin-DNA-AuNP sample, (b) 3 nm ssDNA linker sample,(c) 8 nm ssDNA linker sample and (d) 15 nm ssDNA linker sample.

6 (a) F/ 7.5 4.5 7 6.5 4 6 3.5 5.5 3 5 4.5 2.5 4 2 3.5 1.5 3 (d)E/E_o E/E_o (C)30 70 25 60 20 50 40 15 30 10 20 5 10

Additional simulation data for AuNP-Au-surface coupled system

FIGURE A.5: (a) The field enhancement of the AuNP-Au-substrate system for TM polarization, when the excitation wavelength is 565 nm and the distance d is 20 nm. (b) The field enhancement of the AuNP-Au-substrate system for TE polarization, when the excitation wavelength is 580 nm and the distance d is 20 nm. (c) The field enhancement of the AuNP-Au-substrate system for TE polarization, when the excitation wavelength is 545 nm and the distance d is 3 nm. (d) The field enhancement of the AuNP-Au-substrate system for TE polarization, when the excitation wavelength is 545 nm and the distance d is 3 nm. (d) The field enhancement of the AuNP-Au-substrate system for TE polarization, when the excitation wavelength is 615 nm and the distance d is 3 nm.

Analysis immobilized biotin-AuNPs from the dark field images

During the measurements, after each voltage step an optical image of the surfaceimmobilized AuNPs was taken as shown in figure A.6. The images were post-processed by adjusting the brightness and the contrast to remove the background. The RGB data of the particle in the images were next analyzed using ImageJ-software by creating Region-Of-Interest (ROI) maps, where particles were selected by drawing circular ROI areas around them and assigning number for each particle. The surface defects were left out of the ROI maps at this point. We defined the maps for each sample separately and the RGB values of the particles were traced from first image (deposition) to last image. The RGB data was converted then to HSV data using equations A.5 and A.6 [223,224].

$$\begin{cases}
M = \max \left(R, G, B \right) \\
m = \min \left(R, G, B \right) \\
C = M - n
\end{cases}$$
(A.5)

$$H = \begin{cases} 60^{\circ} \cdot \left(\frac{G-B}{C} \cdot \text{mod}6\right) \\ 60^{\circ} \cdot \left(\frac{B-R}{C} + 2\right) \\ 60^{\circ} \cdot \left(\frac{R-G}{C} + 4\right) \end{cases}$$
(A.6)



FIGURE A.6: (a)-(f) DF images of surface immobilized 0.1 % hairpin-DNA-AuNPs before deposition and under different voltage biasing. The movable (changes color) particles are highlight with the orange circles.



Hue versus LSPR wavelength calibration data

FIGURE A.7: The Hue versus the LSPR wavelength calibration curve.

N=240 N=258 (b) (d) -0.5V -0.5V 90 60-0 0-+0.5V +0.5V 60-90 N=1217 (c) 0 0 0V 0V +0.5V 270 90 60-Norana Maria 0 0 0 ٥V -0.5V -0.5V 270-90-60-N=774 (a) 0-0 0 -0.4V -0.4V +0.2V -0.4V 300 270-60-90 -0.3V 0 0 0 0 0V -0.3V -0.3V 300-270-90-60 0 0 0 0--0.2V -0.2V -0.2V -0.2V 270-300-60-90 -0.1V 0 0 0 0--0.1V -0.1V -0.1V 270-300-90 60-M 0-0 0 0-0V 0V 0V 0V 270-300 90-60-0 2001 Conut 0 0 0 0 +0.2V +0.5V +0.5V +0.5V 270-90 60-0 0 0 0-+0.4V +0.4V +0.1V +0.4V 270-300-90-60-M 0 0 0 0 +0.3V +0.3V +0.3V 0V 300-270-90 60-III. 0 0 0-+0.2V 0 -0.2V +0.2V +0.2V 270-300-90-60-0-0 0-0 +0.1V -0.1V +0.1V +0.1V 270-300 60-90 0 0 0 0-0V 0V 0V 0V 300-270-90-60-0 0 0 0 100 H (degree) 100 H (degree) 100 H (degree) 200 200 100 H (degree) 200 Ò 200 0 0 0

Histogram data of all samples without any threshold

FIGURE A.8: The overall histogram data of the AuNPs bound on gold surface. The numbers above the histograms indicate the number of analyzed particles. (a) Hairpin-DNA-AuNP sample. (b) 3 nm, (c) 15 nm and (d) 8 nm ssDNA-AuNP samples.



Additional data for individual AuNP manipulation experiments

FIGURE A.9: Additional spectra of the manipulation of individual AuNP by electric field. (a)The LSPR scattering spectra of pure hairpin-DNA-AuNP. The voltage step between each curve is 1 V. (b) The LSPR scattering spectra of 0.1% hairpin-DNA-AuNP. The voltage step between each curve is 0.5 V.



FIGURE A.10: Additional spectra of the manipulation of individual AuNP by electric field. (a)-(d) The particles in a and c have a double peak, when pulled with positive, and single main peak, when pushed with negative voltage. The particle in d has a single main peak, when pushed with negative and pulled with positive voltage, and a small hump at 535 nm indicated by orange line, when pulled with positive voltage. The particle in b has double peak with negative voltage and single peak with positive voltage. The voltage steps are 0.5 V in figure a and c, 1 V in figure b and 0.5 V to 1 V in figure d.

Single electron transistor experiments

TX-tile DNA strand

The thiol-modified DNA strands were purchased from Integrated DNA technologies (IDT,Coralville, Iowa, USA) and the TX-tile strand sequences are shown in table A.3.

Strand	DNA sequence		
AuNPA	AAGAAGAAGAAGAAG		
$AuNP_B$	TTTTTTTTTTTTTT		
BAB-ssDNA-1a	ATCGAGAGACATAACTGCT-		
	TGACCACGCTGTACGAAG		
BAB-ssDNA-1b	TTCTTCTTCTTCTTCCTTCGTTTC-		
	GGAACCTGACTCCTAATCAGCA		
BAB-ssDNA-2	AGGAGTCACTCTCGATGCCAGACG		
BAB-ssDNA-3	GGTATAGTATGCAACGTGAATGAACA-		
	AGGTGAGGTGTCAATGGAGATGAATGTTC		
BAB-SSDNA-4	ATCTCCATTGACAGGTCAAGCAGTTA-		
DAD-55DINA-4	TGTGGTTCTGCATACTATACCGAATGTTC		
BAB-ssDNA-5	ATAGCACCACTGCAAGGCCAGACG		
BAB-ccDNA-6	CTTGCAGTCCTTGTTCATTCACGTCGA-		
DAD-55DINA-0	TACAGCGTCCTCAGGTGCTATAATCAGCA		
BAB-ssDNA-7	TGGAGCGACATG		
BAB-SSDNA-8	AGATAACATAAGGACACTTAGGAATC-		
DAD-SSDINA-0	CAGTACTGACACAGTTGGAACATTC		
BAB-ssDNA-9	CAACTGTGTGAATGGGACTTTGCTGATT		
BAB-seDNA-10	AGCTGAACCTACAGTCATACGACTCGA-		
DAD-SSDINA-10	ACACGTAGTATCATCTAGCGTCTGGC		
BAB-seDNA-110	CTAGATGATACTACGGCTACTCAGTACT-		
DAD-SSDINA-IIC	GGATTCCTAAGTGAATTGGACTCAAC		
BAB-seDNA-120	AAAAAAAAAAAAAAGTTGATTGATCATGATGTTCGAGT-		
DAD-55DINA-120	CGTATGACTGTACTATCTCCTTATGTTATCTCATGTCGCTCCA		
BAB-ssDNA-13e	AAGTCCCATTGTAGCTCATGATCGTCCAATT-		
	GATAGCCCTGTTGACATGTCGCTCCA		
BAB-ssDNA-14	TCAACAGGGGGTTCAGCTCATGTCGCTCCA		

TABLE A.3: The sequences of the different TX-tile DNA strands.

Fabrication of fingertip electrodes

Fabrication started by cutting a silicon dioxide wafer (oxide thickness = 300 nm) into smaller chips with area~ 0.5 cm^2 . After cutting, the chips were washed in heated acetone path, then sonicated in isopropanol for 30 s and finally dried using N_2 flow.

Next 100 nm PMMA layer was spin coated using 950 PMMA A2 resist and Bictec model-Sp100 spinner, where the speed was 2000 rpm and the spinning time was 60 s, and the chips were baked for 5 min at $160 \,^{\circ}$ C.

Fingertip-electrode pattern (see appendix Pub.I) was exposed to the PMMAcoated chips using Raith E-line ebeam writer, where the acceleration voltage was 20 kV. The exposed areas were developed in isopropanol-MIBK-solution (volume ratio 3:1) for 30 s and the reaction was stopped by placing the chips to isopropanol path for 30 s. The chips were dried using N_2 flow and cleaned from PMMA leftovers in reactive ion etcher (RIE, Oxfords instruments Plasmalab80plus) using oxygen plasma (15 W, 10 s exposure time). After this, the Jyväskylä self-made UHV e-beam evaporator was used to evaporate first a 2 nm titanium adhesion layer and then a 20 nm gold layer. As a final step, the PMMA was removed by keeping the chips in acetone path overnight, gently blowing acetone on the surface of the chips using a syringe while still immersed, sonicating chips in isopropanol for 30 s and then finally drying the chips using N_2 flow. Digital Instruments Dimension 3100 atomic force microscope was used to analyze the results.

DNA assisted lithography experiments



Additional field enhancement images for plasmonic structures

FIGURE A.11: (a) The field enhancement of the cross structure at 685 nm excitation wavelength, where the highest FE is at the tips of the short arm. (b) The field enhancement of the cross structure at 700 nm excitation wavelength, where the highest FE is at the tips of both arms.



FIGURE A.12: Additional field enhancement images of cross structure. (a),(b) Top and side view of field enhancement of rod structure on a sapphire substrate, where the excitation wavelength is at 515 nm.