Optical properties of DNA-hosted silver clusters

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CHAPTER 1

Introduction

1.1 Metal clusters

Metal clusters have attracted a lot of attention mostly due to the fact that their size and shape determine their optical properties [1–5]. When the dimensions of the metal objects become comparable to the mean free path (MFP) of electrons in the metal, then the dielectric response function becomes wavelength-dependent. This typically happens at scales below 100 nm (the MFP for silver is 52 nm). However, if the dimension of the particle becomes comparable to the Fermi wavelength (for Ag: λ_F=0.55 nm), then the energy levels become discrete.

The small clusters are usually unstable and tend to aggregate which leads to the loss of their individual physical properties. Therefore, numerous capping techniques have been developed to stabilize the clusters. For example, the ligands used to protect the clusters from the environment are dendrimers [6], polymers [7], peptides [8], even single stranded RNA [9] and DNA [10].

1.2 DNA-hosted silver clusters

In this thesis we focus on DNA-hosted silver clusters (Ag:DNAs) (Figure 1.1 a, b). The main purpose is to investigate the optical properties of silver clusters, either at the level of individual emitters or as a collection of emitters positioned on DNA scaffolds.

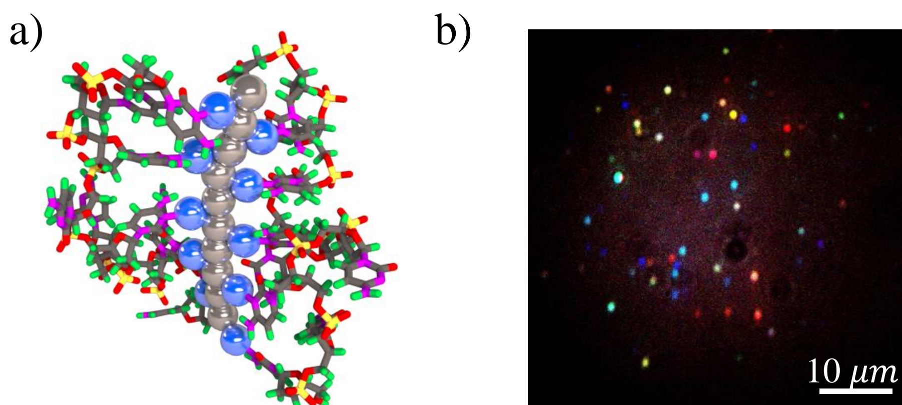

Figure 1.1: Single stranded DNA can stabilize fluorescent silver clusters. a) Neutral core of silver atoms is represented with gray spheres while positive ions are shown as blue spheres. Positive ions connect the neutral core and surrounding DNA bases [19]. b) False-color fluorescent image (Chapter 2) of the Ag:DNAs immobilized on the silica substrate [20].

While most of the cations interact with the negatively charged backbone of DNA, silver ions bind preferentially to the heterocyclic ring nitrogen on DNA bases [11, 12]. The base sequence determines the size and shape of the clusters which directly influences their optical properties [13]. Together with their small size, the tunability of the emission wavelength in the range from 400-900 nm, makes them suitable for various applications which drive the research in this field. These DNA-hosted metal clusters proved to be great sensors for single base mutations [14, 15] and mercury ions [16], and they also show a great potential for biological research [17, 18].

Thorough mass spectrometry studies of purified Ag:DNA material have discovered that the fluorescent clusters contain both positive ions and neutral atoms [19]. From the model proposed by Schultz and coworkers (Figure 1.1 a), it seems that the neutral core of silver atoms is surrounded by silver cations, which mediate between the core and the surrounding DNA bases [19]. In a more detailed study, it was further established that the number of ions and neutral atoms are not random, but rather 'magic' [21], shaping the size and the optical properties of the clusters.

For a better understanding of their optical tunability, one has to consider

the electronic structure of silver atoms. The energy necessary to excite the d-band transitions of silver is much higher than the energy necessary to excite the collective excitation mode of s-electrons. Furthermore, the optical transitions of the chains of silver atoms is length-dependent [22]. The model of the linear chain of atoms somewhat corresponds to the previously proposed nanorod model [19].

1.3 DNA scaffolds

The appealing possibilities of binding between complementary DNA strands paved the way to the construction of elaborate DNA scaffolds. In 2006, Paul Rothemund showed in his pioneering work [23] that short DNA strands with careful sequence design can fold long strands of M13 virus. A new field of DNA origami exploded and since then the creation of 2D [23] and 3D objects [24] with nanometer precision became simplified.

Generally speaking, DNA scaffolds stabilize organic dye molecules [25, 26], plasmonic particles [27], but also change a conformation on demand [28, 29] . Most recent results show that the DNA structures can be utilized for the drug delivery and to initiate an immune response [30, 31].

In our research, we use short DNA oligonucleotides which represent modular components in building quasi 3D objects [32–35]. Namely, the sequence of bases in the oligonucleotides is chosen such that one oligonucleotide is piecewise complementary to at least two other oligonucleotides.

For example, only 5 different types of nucleotides form a tile, which is a 'unit cell' of double-crossover (DX) tubes [33] as will be discussed in Chapter 4. The tiles are designed such that the complementary short sticky ends connect and form a larger 3D structure (Figure 1.2 a, b). On the other hand, 10 strands (Chapter 5) or 6 strands (Chapter 6) can be interwoven such that they form half crossover (HX) tubes [35, 36]. While DX tubes allow the direct synthesis of Ag:DNAs on the pre-defined positions (DNA loops) [33], the HX tubes give a possibility to attach subsequently purified Ag:DNAs [35] (Figure 1.2 c, d).

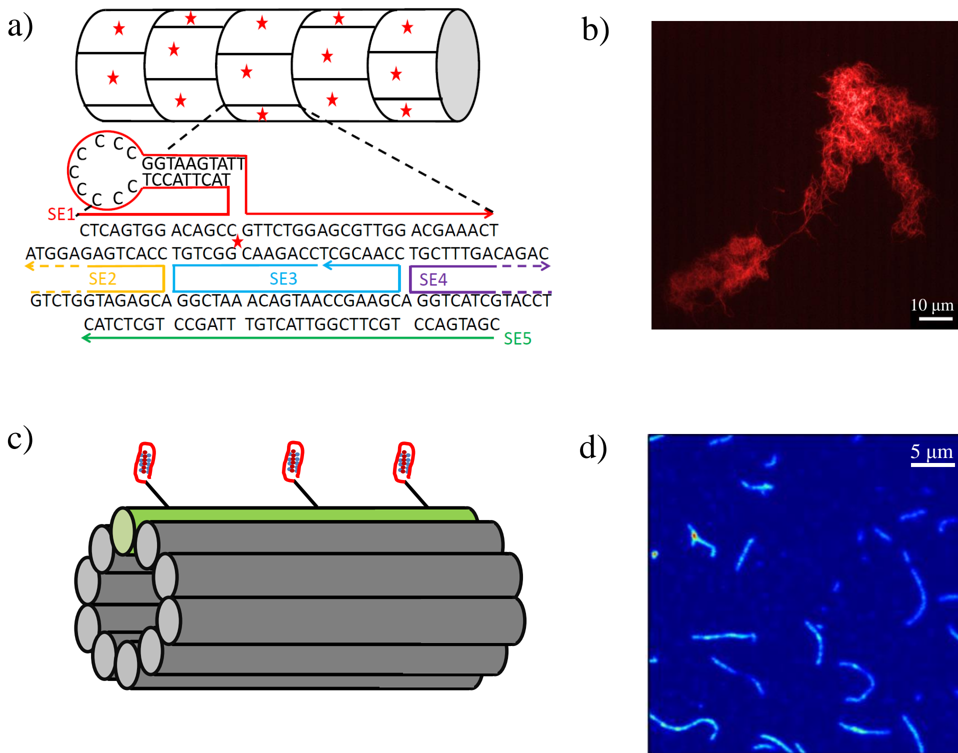

Figure 1.2: DNA tubes for organization of Ag:DNAs. a) Five different strands form a 'unit cell' of the DX tubes. Silver clusters are stabilized in the loop of strand SE1-9C [33]. b) Fluorescent image of the tangles of tubes immobilized in PVA (scale bar: 10 μm). c) Ten oligonucleotides form HX tube to which we attach purified Ag:DNAs [35]. d) Fluorescent image of the tubes immobilized in PVA (imaged area: 40X40 μm).

1.4 Experimental techniques

The synthesis procedure of Ag:DNAs consists of several steps: a water solution of DNA strands which can stabilize the cluster is mixed in a buffer (ammonium acetate/magnesium acetate) with silver nitrate ($AgNO_3$) and subsequently reduced by sodium borohydride ($NaBH_4$). Shortly after reduction, the solution changes color, but in order to reach a maximum fluorescence intensity, we kept our sample overnight at 4°C.

To form DNA tubes, we follow the procedure developed by O'Neill and

coworkers for DX tubes [33], and Yin and coworkers for HX tubes [34] with modifications suggested by Schiffels et al. and Copp et al. [35, 36] . A solution of carefully selected DNA strands was mixed in a buffer (ammonium acetate/magnesium acetate) and heated to 95°C. Then the system is slowly cooled down ($\sim$ 48 hours) in a thermos bottle to room temperature. In the case of DX tubes, where we synthesized the emitters directly on the tubes, the addition of $AgNO_3$ was followed by the addition of the reducing agent $NaBH_4$. On the other hand, the purified Ag:DNA emitters were added to the solution of HX tubes, and made connections through the docker-linker complementary bonds (Chapter 5).

The characterization of the emitters in solution was performed by UV-visible spectrophotometry (Cary 50 Bio) and fluorimetry (Cary Eclipse Varian). These two techniques enabled us to monitor the absorption and emission properties of the emitters in solution.

For single emitter spectroscopic cryogenic measurements we constructed the setup schematically presented in Figure 1.3. In this configuration it was not only possible to detect an ensemble of emitters immobilized in poly(vinyl alcohol) (PVA) by CCD camera as presented in Figure 1.1 b (in this case we used a lens to illuminate a larger area on the sample), but also to align a diffraction limited laser beam to individual emitters. The emission is then detected by a fiber-coupled spectrometer or a single-photon sensitive counting module (APD).

To perform polarization measurements we slightly modified the setup for spectroscopic measurements (Figure 1.4), introducing the computer controlled polarization rotator in the excitation path and the polarizer in the emission path. A wide field illumination lens enabled us to excite many emitters at the same time, improving the efficiency of data collection.

The setup schematically presented in Figure 1.5 was used for time-resolved measurements. A pulsed laser with a repetition rate of 20 MHz excited the emitters immobilized in PVA. For the data acquisition and data processing we used SymPho Time 200 PicoQuant software.

Microscopy was performed on commercially available setups: Fluorescence microscope (Zeiss Axiovert 40 CFL) with 100X oil- immersion objective and Nikon eclipse Ti-E microscope system with a Nikon 100x/1.45 NA Oil immersion DIC H objective.

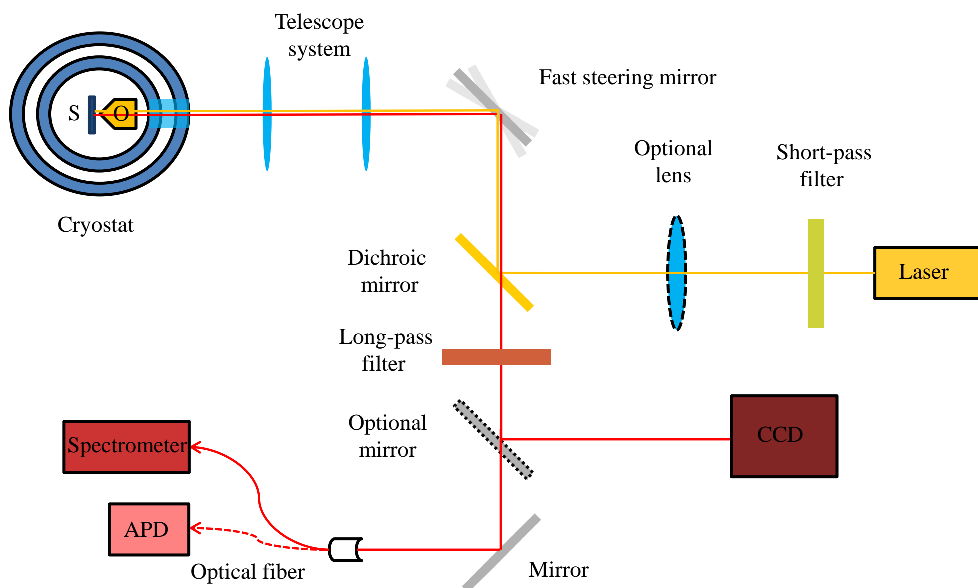

Figure 1.3: Setup for spectroscopic cryogenic measurements. Laser light from a tunable ring dye laser (Coherent 899) is filtered through the short-pass filter (edge at 600 nm). An optional lens is removed when we perform single-molecule type of measurements. Laser light is further reflected by the dichroic mirror and a fast steering mirror through the telescope system and objective (O). The illuminated sample (S) emits light which is directed with the same objective through the dichroic mirror and long-pass filter (edge at 600 nm). To detect the ensemble of emitters by a CCD camera, we use an optional mirror (an optional lens is also in the system). For single molecule measurements, light is directed by the mirror to the optical fiber and further to the single-photon counter (APD) or Spectrometer.

1.5 Thesis outline

In this thesis we are mainly focusing on the properties of the DNA-hosted silver clusters at the single emitter level. Previous research was mostly dedicated to the bulk solution properties at room temperature. We immobilize the emitters in PVA and perform cryogenic spectroscopic measurements on the single emitter level. Furthermore, we examine the formation of Ag:DNA on the DNA scaffolds such as DNA tubes and compare the properties of such emitters to the properties of free individual emitters. Time-resolved properties of individual emitters and densely packed Ag:DNAs have attracted our

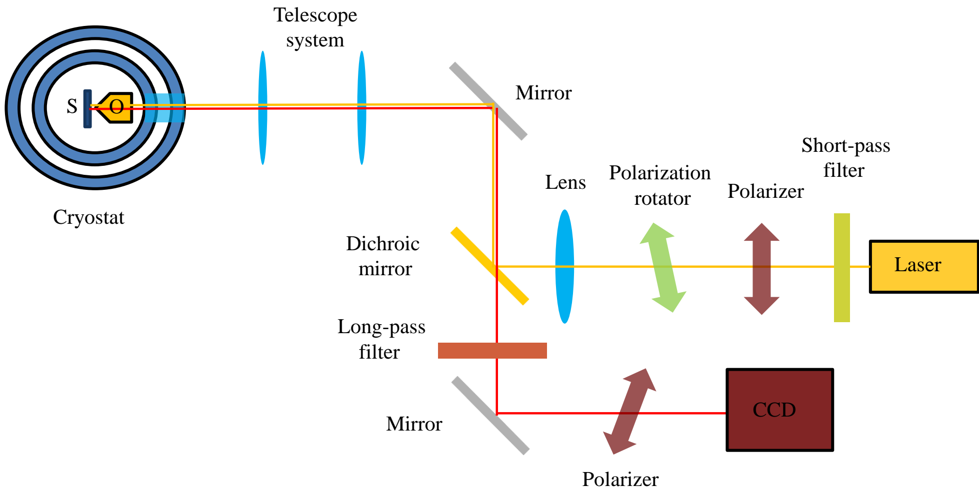

Figure 1.4: Setup for polarization cryogenic measurements. Laser light from a tunable ring dye laser (Coherent 899) is filtered through the short-pass filter (edge at 600 nm) and a polarizer. A polarization rotator is used to rotate the polarization angle of the excitation light. A lens inserted in the excitation beam enables the illumination of the larger field of view (diameter $\sim$ 100 μm). Since the emitters are spatially well separated, we can extract the excitation and emission properties from each of them. Laser light is further reflected by the dichroic mirror and another mirror through the telescope system and objective (O). The illuminated sample (S) emits light which is directed with the same objective through the dichroic mirror and long-pass filter (edge at 600 nm). To detect the ensemble of emitters by the CCD camera, the light is reflected by the mirror and transmitted through the polarizer.

attention as well. Finally, we use the knowledge gained on the organization of the Ag:DNAs to guide the assembly of large colloidal particles (d= 1 μm) into flexible strings.

In Chapter 2, we present low temperature spectroscopic measurements of the Ag:DNAs. We show that by removing the ensemble broadening, we can probe the line widths and brightness of individual Ag:DNA emitters. The brightness of the emitters roughly increases five times from 295 to 1.7 K while the emission line width decreases two times, indicating the plasmonic properties of the Ag:DNAs.

Chapter 3 contains low temperature polarization measurements on the immobilized Ag:DNAs. From the polarization data, we conclude that the

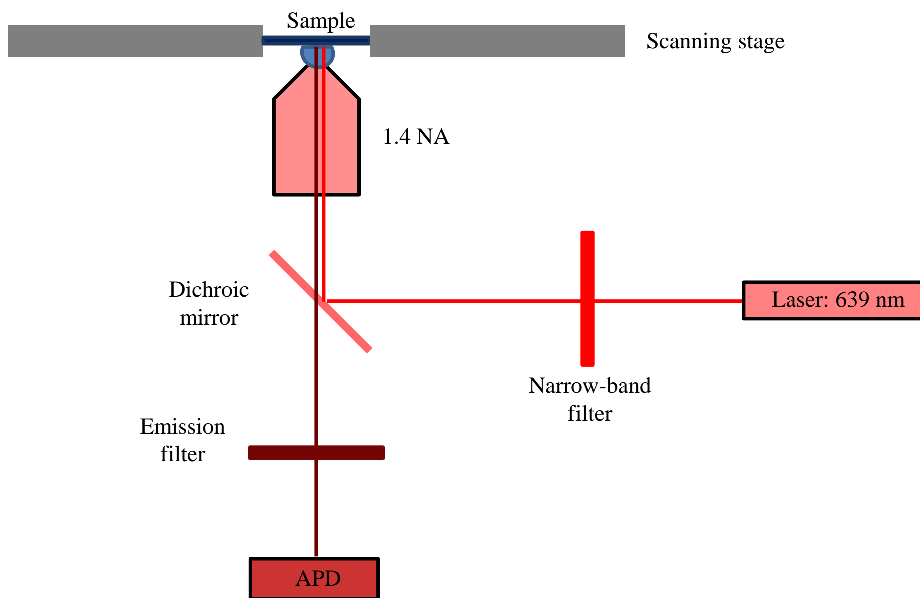

Figure 1.5: Setup for time-resolved measurements. A pulsed laser light (λ= 639 nm) is filtered through the narrow-band filter and reflected by the dichroic mirror through the oil-immersion objective onto the sample mounted on the scanning stage. The emitted light is collected by the objective and transmitted through the dichroic mirror and emission filter to a single-photon counter (APD).

emission is strongly polarized, whereas the excitation does not strongly depend on the polarization of the excitation light. This brings us to the conclusion that the clusters are rod-shaped and that the DNA is involved in the excitation/emission process.

In Chapter 4 we focus on the synthesis of Ag:DNAs on the DNA tubes. We discover that the emitters formed on the tubes show different temperature stability with respect to their free counterparts. The temperature increase leads to the deterioration of free emitters, whereas the emitters formed on much larger structures, tiles and tube, show the increase in fluorescence as a consequence of formation of new fluorescent emitters. This is supported by the increase of absorption.

Lifetime measurements on the individual emitters and emitters organized on the DNA tubes with high densities are presented in Chapter 5. Single emitters show single exponential decay after the excitation with short laser

light pulses, while the emitters immobilized on the DNA tubes show double exponential decay path. This can be explained by the interaction between the emitters.

In Chapter 6, we describe how to use the properties of DNA tubes with sticky ends (similar to those used in Chapter 5) to glue together colloidal particles into stable strings. Generally, the superparamagnetic particles align in magnetic field, but the thermal fluctuations break strings after the magnetic field is switched off. Here we show that the DNA nanotubes can be used as a nano-contact glue between the DNA functionalized colloids.