

## **1 Title of the project**

Single molecule microscopy on energy transfer across a chain of nanoparticles seated on a DNA origami

## **2 Abstract**

Silver nanoparticles are interesting objects in plasmonics, bearing the hope of making nanoscale waveguides and nano-photonics circuits. DNA origami [1] is a good candidate of making photonic nanostructures and bio-inorganic hybrids. We proposed an experiment of making donor-nanoparticle-acceptor energy transfer cascade in nanometer precision by the help of DNA origami nanotechnology, and recording single energy transfer route using single molecule fluorescence microscopy. We are hoping to have more insights of energy transfer efficiencies between silver nanoparticles as well as between nanoparticle and molecules.

## **3 Applicant**

Qi Liu

## **4 Institute**

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## **5 Duration of the project**

4 years, starting September 2012

## **6 Personnel**

### **6.1 Senior scientists**

Tobias Schnitzler	Daily guidance, 20%
Maxim S. Pchenitchnikov	Guidance, 10%
Andreas Herrmann	Guidance, 5%
Paul H. M. van Loosdrecht	Promotor and guidance, 5%

## 6.2 Junior scientists and technical assistance

PhD position	New hire, full time
Ben Hesp	Technical assistance 5%
Foppe de Haan	Technical assistance 5%

## 7 Cost Estimate

### 7.1 Personnel positions

1 ‘onderzoeker in opleiding’ position for 4 years, k€ 204

### 7.2 Running budget

Travel/subsistence,  $4 \times k€ 15$ .

### 7.3 Equipment

Nanoparticles, DNA, fluorophores and other chemicals	k€ 70
Microscope objective and other optics	k€ 60
Misc.	k€ 6

### 7.4 Budget summary

	2012	2013	2014	2015	2016	Total
Personnel PhD	17	51	51	51	34	204
Running budget	5	15	15	15	10	60
Equipment	26	50	20	20	20	136
TOTAL	48	116	86	86	64	400

## 8 Research Programme

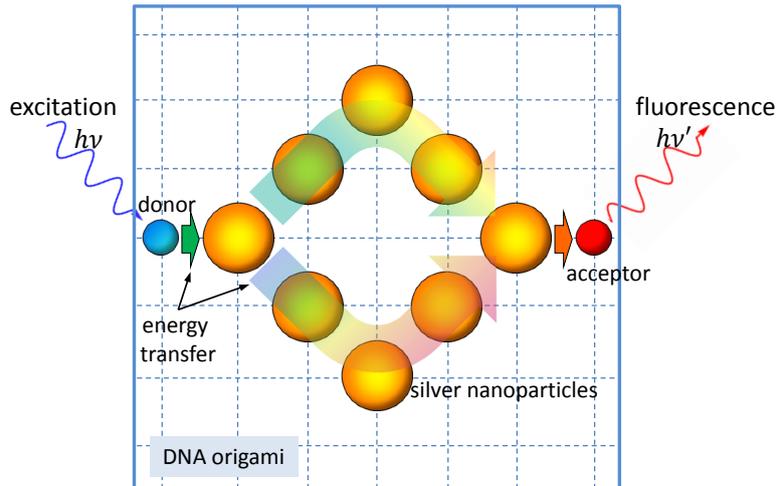
### 8.1 Introduction

Better understanding of energy transfer processes at the nano-scale not only improves our knowledge about the microscopic world, but also helps advances in technical applications, such as for making nano-scale photonic devices. Particularly, nanoparticles made of gold or silver have drawn much attention due to the coupling of local electromagnetic field (light) and free electron resonances (surface plasmons) of the particles. This localized and sometimes enhanced electromagnetic field makes such nanoparticles promising to confining and guiding light at nanometer scale, much below the diffraction limit. For example, a chain of closely spaced silver nanoparticles have been shown the capability as an optical nano-waveguide both theoretically [3] and indirect measured using near-field scanning microscope [4].

Here we propose an experiment to measure the transport efficiency of a chain of silver nanoparticles (AgNPs) using single molecule fluorescence microscopy. Energy input to the AgNP chain is excitation energy of an excited fluorescent molecule (donor) positioned at the front of the chain, and output being the fluorescence from another dye (acceptor) seated near the end of the chain. Similar to fluorescence resonance energy transfer (FRET) microscopy, the fluorescence intensity of these two fluorophores will be detected simultaneously and in a single molecule fashion. Such microscopy technique has been widely used in especially biology [5].

Another novel aspect of our proposed experiment is that we are going to implement a recently developed technique, DNA origami, as a nanoscale breadboard to construct the donor-AgNPs-acceptor complex at 100 nm scale. Via self assembly of complementary DNA single strands attached with fluorophores or AgNPs correctly, the complex structure can be fabricated in nanometer precision. Such biochemical synthesis methods have been developed rapidly after its invention in 2006, now ready to handle gold and silver nanoparticles [6, 7].

A candidate design of such DNA origami-AgNP-fluorophore complex is depicted in figure 1, where energy can be transferred from donor to acceptor via two chains of



**Figure 1:** Schematic show of a design of two pathways of silver nanoparticles (in orange) as intermediate energy transfer channel between a donor dye (blue) and an acceptor dye (red) positioned on a piece of square DNA origami (blue grids) of size  $\sim 100$  nm. In energy transfer process, donor dye is excited by a laser (blue wave arrow), followed by several steps of energy transfer (indicated by wide arrow), from donor dye to nanoparticle, then across the nanoparticle chain and finally transferred to the acceptor. Sizes not to scale.

AgNPs. Apart from helping to show the energy transfer efficiency of AgNP chains, such a 2-pathway construction will also shed light upon whether or not the energy transfer from 2 routes added up coherently.

## 8.2 Methods

In the proposed experiment, two main things need to be addressed: synthesis of the nanostructure using DNA origami technique and single molecule fluorescence microscope imaging.

### **Synthesis of DNA origami complex**

DNA origami can be viewed as the art of folding DNA strands, where a long single strand DNA (ssDNA) (called scaffold) and its complementary part, many short ssDNA (called staples), are mixed together. After all the complementary part bind together in a self-assembled fashion, a structure of crossed linked double strand DNA formed with relatively rigid shape and size [1]. By modifying particular staple strands priorly with fluorophores, gold nanoparticles (AuNPs) or AgNPs etc., one can include these objects into the DNA origami structure, with the distance controlled by DNA double strand framework.

Most of the studies involve gold instead of silver because AuNPs are chemically more stable and easier to be modified using strong Au-thiol interaction. However in terms of plasmonic energy transfer efficiency, AuNPs suffer from large dissipation because of larger ohmic loss compared to silver. Thus we choose AgNPs considering the energy transfer efficiency, despite that properly binding AgNPs to DNA origami is in general more difficult. We are going to use Hao's method [7] to tackle this key problem by coating AgNPs with chimeric phosphorothioated DNA (ps-po DNA) single strands (making use of Ag-S interaction) and attaching to DNA origami by binding to sticky ends extruding from the origami. Fluorophores will be binded to DNA origami by modified corresponding staple strands with such dyes, same as DNA labeling.

As a starting point, we are going to use a 50 nm×50 nm DNA origami, single chain of three 10 nm diameter AgNPs (absorption at ~400 nm) spaced 20 nm from each other, Alexa Fluor 350(346 nm absorption, 442 nm emission) as donor and Texas Red (583 nm absorption, 603 nm emission) as acceptor. Quality control will be done with the help of AFM and TEM.

### **Two color single molecule fluorescence microscopy**

We are going to use it to detect the fluorescence signal from both donor and acceptor at the same time. A dilute solution containing DNA origami-AgNP-fluorophore complex will be imaged in both blue and red channels under the excitation of UV laser light. The signal is collected using a high-resolution objective and an EMCCD camera, resulting a pixel size of 80 nm. The blinking fluorescence from single donor and acceptor will be recorded over time, and the energy efficiency is estimated using the ratio of photon counts in two channels [8].

To fix the nanostructure position, microscope slides will be cleaned and modified with

a layer of biotin, which binds specifically to sticky ends of DNA origami which contains e.g. streptavidin [9]. Anti-oxidation reagent will also be added to reduce photobleaching of the dyes [10].

### 8.3 Preliminary investigation

#### Theoretical consideration

We've done point dipole calculation to investigate plasmonic coupling in a chain structure made of AgNPs. The results showed that for there 20 nm diameter separated by 30 nm, the transfer efficiency for longitudinal electromagnetic field around 400 nm is estimated to be  $\sim 30\%$  ( $100\times$  less for AuNPs with similar parameters).

For energy transfer between nanoparticles and fluorophores, it is shown that short distance (compared to radius of the nanoparticle) mainly leads to quenching of fluorescence, i.e. energy transfer from dye to nanoparticle; while slightly larger distance leads to enhancement of fluorescence, i.e. energy transfer fro nanoparticle to dye. We can thus put the donor fluorophore closer to AgNP and acceptor slightly further to facilitate total energy transfer efficiency.

#### Test experiments: DNA origami attached with AuNPs and microscopy

We've tested the whole synthesis process of attaching AuNPs onto DNA origami. AFM imaging showed a clear and integrate square shape of DNA origami molecule, and TEM imaging showed chains of AuNPs with defined distance. Stable and efficient output DNA origami-AuNPs complex was not yet achieved though.

The fingerprint stepwise blinking fluorescence of a test dye molecule have also been achieved, showing the capability of dual-color single molecule fluorescence imaging.

### 8.4 Scientific goals

1. Achieving stable and efficient synthesis of DNA origami-AgNP-fluorophore complex, initially a simple structure containing a chain of 3 nanoparticles. Difficulty of this part is the efficiency binding of AgNPs to DNA origami which is limited by chemical stability of AgNP and silver-sulfur bond strength for AgNP-phosphorothioated DNA binding. Feasibility of such synthesis has already been addressed above. Final goal is to make 2 pathways of AgNPs in order to see any coherent inference of two energy transfer paths.
2. Two-color fluorescence microscopy of single complexes dispersed on modified surface of a microscope slide. Starting point is a single molecule FRET microscopy where direct energy transfer between donor and acceptor is investigated. Then the efficiency of AgNPs intermediated energy transfer between donor and acceptor will be measured with different AgNP structure. This will ultimately shed some light on the plasmonic

transport properties of AgNP chains and the nature of nanoscale energy transfer between molecules and nanoparticles.

## 8.5 Collaborative expertise

The experiments requires specialized knowledge of DNA biochemistry and single molecule imaging. Andreas Herrmann is an associate professor in Zernike Institute for Advanced Materials, with much experience in organic and polymer chemistry. He and Dr. Tobias Schnitzler will be guiding the synthesis processes. Maxim Pchenitnikov has a lot of experiments experience as well theoretical knowledge of laser spectroscopy and microscopy. He will be guiding the microscopy experiments. Inside Zernike Institute, we also have a group of single molecule biophysics, who will equipment us with many modern single molecule microscopy techniques.

## 8.6 Timeline

Learning synthesis: to 1.5 years after starting. Synthesis of simple test structure with single chain of AgNPs.

Learning microscopy: 1.5-2.5 years after starting. Carrying out single molecule microscopy on donor-acceptor pair on DNA origami.

First 3-step energy transfer experiments: 3 years after starting. Stable and efficient synthesis of DNA origami-AgNPs-fluorophore complex and routinely single molecule fluorescence microscopy. Analyzing and interpretation.

## 9 Infrastructure

Synthesis will be done in the group of polymer chemistry and bioengineering (PCBE), and microscopy carried out in the group optical condensed matter physics (OCMP). Both groups are inside of Zernike Institute for Advanced Materials, which has a very collaborative atmosphere and many experts in chemistry and physics.

## 10 Application perspective in industry, other disciplines or society

The experiments bears importance for realizing nano-photonic computing circuits using surface plamons of noble metals, inspiring next generation high-speed computers and nano-optics. Since its bio-friendly nature, such methods can be also applied in bio-sensing and medicines.

Student hired for this project will receive deep inter-disciplinary research training, building up expertise in both biochemical synthesizing and microscopy physics.

## References

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