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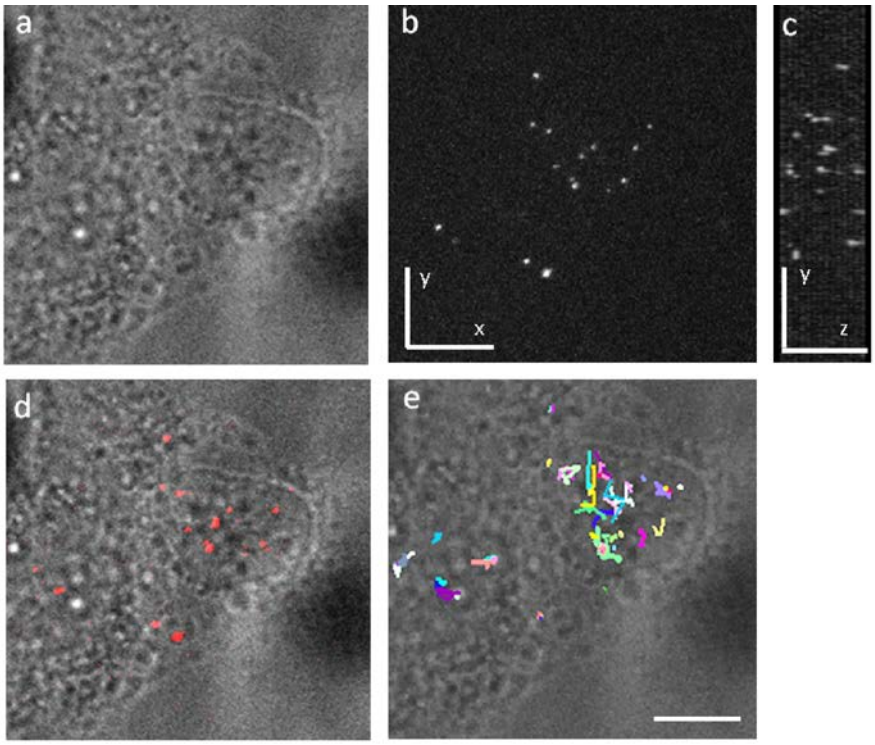
Nederlandse Organisatie voor Wetenschappelijk Onderzoek

Annual report 2016

FOM programme nr. 135
'Single gold nanorods in live cells'



A HeLa cell microinjected with GNRs. a) transmission image. b and c)two-photon luminescence images. d) The overlap of transmission image and two-photon luminescence e) traces of single GNRs in the cytoplasm and nucleus. Scale bars correspond to 10 µm.

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Content

1. Scientific results 2016

In the past year, the joint efforts to use Gold Nanorods (GNRs) as specific labels for long term tracking of single molecules in cells were wrapped up. We produced GNRs in several sizes and chemical modifications, inserted them in three different cell lines by cell squeezing, electroporation incubation and/or microinjection. 3D-tracking using multiple-focus two-photon microscopy was performed on each of these samples followed by detailed analysis of individual tracks in individual cells.
The proof-of-principle results showed the potential of using GNRs for in cellulo single-molecule tracking. However, the experiments proved to be rather complex in practice, which was highly limiting for biologically more relevant experiments involving specifically targeted proteins. Complexity includes the limited shelf time of synthesized GNRs in combination with long cell culturing procedures, balancing small sizes for minimal cellular interference at the cost of increased aggregation with larger GNRs that cannot penetrate the nucleus but yield larger signal, and large stochastic variations in mobility behavior versus surprisingly short luminescence stability in cells. The many challenges in the main theme of the programme leaded to several sideways, including extensive simulations, single-molecule tracking of YFP-labeled glucocorticoid receptors and temperature sensing with GNRs.

Contributions per group

Oio: Sara Carozza, PI: John van Noort
In her final year Oio Sara Carozza completed simulations and experiments on the detection of dynamic changes of diffusion of gold nanorods. The key challenge is the discriminate brief reductions in diffusion from stochastic movements by diffusion. We show that detection of such events is generally not limited by the tracking accuracy. These results are in press and are highly significant for quantifying binding events for example of transcription factors to DNA.
In close collaboration with Oio Keizer and postdoc Boyle, several GNR transfection methods were tested and compared using PEG-modified GNRs. In general, bulk transfection methods were shown to affect cell viability significantly. The best results, though low in throughput, were obtained by microinjection. Next to cell viability, the mobility of single GNRs was quantified in detail. A variation of mobility profiles was found that were split into immobile (typically 10%), confined (about 30%) and free diffusion. These findings, using relatively inert GNRs, set the basis for GNRs that are functionalized to target specific components.
Also in a highly cooperative spirit, GNRs functionalized with nuclear localization signals (NLS) peptides were scrutinized for the mobility in cells. Carozza's contribution consisted of the imaging and track-analysis. Again, a large fraction of GNRs were shown to be restricted in movement. Nevertheless, specific targeting of the nucleus could be demonstrated. Interestingly, the traces in the cells were significantly shorter than those in a better controlled, similarly viscous environment like glycerol. We attributed this behavior to changes in the local environment and thus the refractive index, which would shift the plasmon resonance away from the used laser frequency.
Finally, Carozza investigated and optimized the multi-focus scanning two-photon setup for excitation spectroscopy of multiple GNRs in parallel. It turned out that the obtained spectra featured several peaks that could not be attributed to the plasmon resonance of a single GNR. We correlated optical images with electron microscopy images to rule out GNR clustering and scrutinized the setup for spectral responses of the used optical components. Though Carozza could not finish this part completely, a new PhD student picked up this project and was able to fix the problems in spectroscopy, making two-photon sensing applications feasible. A joint paper is expected from these efforts.

Oio: Veer Keizer, PI: Marcel Schaaf
Oio Keizer set up the equipment for microinjection of GNRs into cultured cells, and this procedure is now routinely used in our laboratory. Currently, we are able to inject GNRs into either the nucleus or the cytoplasm of the cells. Subsequently the injected cell can be transferred to the 2-photon microscope for imaging of the rods. Because the localization of the injected cells is marked, they can be rapidly identified under this microscope. Using this procedure two projects were performed. First, the mobility pattern of non-functionalized GNRs was determined in the cytoplasm and in the nucleus of the cells. To our surprise, no significant difference was observed in the GNR mobility between these two compartments, suggesting that the viscosity of the cyto- and nucleoplasm is the main determinant of GNR mobility. Second, GNRs were functionalized with a NLS in order to

study whether GNRs can be imported from the cytoplasm into the nucleus by the nuclear pore system. Upon optimization of the NLS sequence and the experimental conditions, we found that GNRs can be imported into the nucleus, but that this process is very inefficient. About 10% of functionalized GNRs ends up in the nucleus. Importantly, we also showed that non-functionalized GNRs never translocate from the cytoplasm to the nucleus.
In the remaining time, we aim to inject GNRs functionalized with a Halo-ligand into the cytoplasm of cells expressing the glucocorticoid receptor fused to a Halo tag. Upon activation of the receptor with a hormone, the receptor's natural NLS is exposed and it should translocate it to the nucleus and find its DNA target.

Oio: Aquiles Carattino, PI: Michel Orrit
The main result of the spectroscopy part in 2016 has been the discovery, characterization, and applications of anti-Stokes photoluminescence of gold nanorods. The photoluminescence can be excited by a one-photon process (by contrast with the two-photon excitation used above). The signal is enhanced by the plasmon resonance of the rod, the anti-Stokes part, at shorter waves than the laser excitation wavelength. This can be optimized by placing the laser on the red wing of the plasmon resonance. Because anti-Stokes processes in molecules are weak, gold nanorod anti-Stokes luminescence can be detected on a very low background of autofluorescence in cells, and even on the background of fluorescence of heavily stained cells. One can thus identify gold nanorods even in highly fluorescent media such as stained cells. This work (Carattino et al. Biophys. J. 2016) has been done in collaboration between two groups in the programme's network.
The anti-Stokes luminescence spectrum, being activated by thermal energy, is very sensitive to temperature. It can be used to monitor the local temperature around a rod, or to measure the temperature increase due to light absorption by the rod, a particularly useful thermometer for photothermal therapy applications. Moreover, the temperature determination being based on a spectrum measurement is absolute and requires only measurement of two spectra excited by two different wavelengths (e.g., 532 nm and 633 nm).

There has been no time in the PhD period of A. Carattino to address the interaction of trapped nanorods with cells membranes and cell components. Yet, this very important and promising problem should be pursued in the future, provided adequate funding is obtained.

Postdoc: Aimee Boyle, PI: Alexander Kros
Various sizes GNRs were produced and it was established that smaller GNRs were not suitable for microinjection into cells because of clogging. Moreover, imagingis less sensitive because of the greatly reduced quantum yield. This line of research was not pursued further. Regular sized (40 x 10 nm) GNRs were functionalized with NLS peptides or Halo ligands. The NLS was found to work – GNRs were translocated to the nucleus, although many appeared to be stuck or to surround the nucleus, possibily because the GNRs were too large to pass through the nuclear membrane.

2. Added value of the programme

The research in this programme is critically dependent on the combination of chemistry, biology and physics. Without such a consortium, it would not have been possible to benefit from the unique optical properties of GNRs for cell imaging. This mutual dependence ensures coherence between the projects, though it is also the cause of some delays.
Throughout the programme we kept close contact with Peter Zijlstra in Eindhoven, who attended the programme meetings regularly.
Part of the VICI programmes awarded to Kros and Van Noort build on the works of this programme. The VENI grant of Boyle continues with protein delivery methods. A synergy project in the NWO zwaartekracht programme Nanofront was granted to Orrit for GNR-basedsensing. Many results in the programme, including nuclear targeting and GNR tracking in cells and anti-Stokes photoluminescence in cell microscopy, could not have been done without the preparation and regular contact between the teams involved in the project.

3. Personnel

Postdocs Capoulade and Boyle had left the programme in 2015. Thanks to a Veni grant, Boyle was able to continue to partially contribute to the programme. Aquiles Carattino graduated on 9 March 2017. Sara Carozza

has submitted her concept thesis to the reading committee. Veer Keizer is on track and will finish her research in October (an extension was granted for her COR activities).

4. Publications

a. Scientific (refereed) publications

- A. Carattino, V.I.P. Keizer, M.J.M. Schaaf, M. Orrit, Background Suppression in Imaging Gold Nanorods through Detection of Anti-Stokes Emission, Biophysical Journal, 111, 2492-2499, 2016 (DOI: 10.1016/j.bpj.2016.10.035).
- A. Carattino, S. Khatua, M. Orrit, In situ tuning of gold nanorod plasmon through oxidative cyanide etching, Physical Chemistry Chemical Physics, 18, 15619-15624, 2016 (DOI: 10.1039/c6cp01679k).
- A. Carattino, V. Keizer, M. Orrit, Background-Suppression in the Detection of Gold Nanoparticles in Cells through Anti-Stokes Photoluminescence, Biophysical Journal, 110, 486A-486A, 2016; Meeting Abstract.

In press:

- Accuracy of the detection of binding events using 3D single particle tracking. S. Carozza, J. Culkin, J. van Noort. BMC Biophysics.

Submitted:

- Model-free analysis of step-size distributions to detect and quantify stochastic processes in live cells. S. Coppola, V.I.P. Keizer, M.J.M. Schaaf, T. Schmidt.

In preparation:

- DNA-binding determines dynamics of the glucocorticoid receptor in living cells. V.I.P. Keizer, M. van Royen, S. Coppola, B. Geverts, A.B. Houtsmuller, T. Schmidt, M.J.M. Schaaf.
- Parallel 2 photon spectroscopy of single gold nanorods, R. Vlieg, S. Carozza, J. Capoulade and J. van Noort.

b. Presentations at (inter)national scientific conferences

- Talk by A. Carattino: 'Background-Suppression in the Detection of Gold Nanoparticles in Cells through Anti-Stokes Photoluminescence', 22nd International Workshop on 'Single Molecule Spectroscopy and Super-resolution Microscopy in the Life Sciences', Berlin, September 16, 12:00pm..
- Talk by A. Carattino: 'Anti-Stokes luminescence of gold nanorods', MNO, Lyon, November 4, 11:50am.
- Poster by V. Keizer: 'DNA-binding dynamics of glucocorticoid receptor revealed by particle image correlation spectroscopy' Biophysical Society meeting, February 27 - March 6, 2016 (Los Angeles).
- Poster by V. Keizer: 'DNA-binding dynamics of the glucocorticoid receptor' Nuclear Receptor Research Network meeting, November 30, 2016 (Amsterdam).
- Poster by S. Carozza: 'Tracking Single Gold Nanorods in Live Cells' Quantitative Bioimaging meeting, January 13-15, 2016 (Delft).
- Poster by S. Carozza: 'Quantification of Gold Nanorods Mobility in Live Cells' Dutch Biophysics meeting , October 3-4, 2016 (Veldhoven).

5. PhD defences

Aquiles Carattino, PhD defense 9 March 2017.
Sara Carozza, concept thesis submitted, expected defense in June 2017.
Veer Keizer, due to graduate at the end of 2017.

6. Valorisation, outreach and patents

Our efforts have attracted the attention of environmental toxicologists (Martine Vijver, Leiden University) who are interested in tracking various nanometalic particles in small organisms like zebra fish embryos. The ability to resolve the position of multiple nanoparticles with nanometric accuracy, complemented by spectral and orientation measurements was also noticed by Eike Kiltz and Ulrich Rührmair (Horst Görtz Institut, Bochum, Germany) who have an interest in using nanoparticles as for security applications. Aquiles Carattino has started a one-person company in January 2017 and plans to operate it after his PhD defense. The company will provide software and control solutions for academia and industry.

7. Vacancies

All postions have been filled according to plan; 1 Oio is still working on the programme.

FOM - 12.0254/5
datum: 01-01-2017

APPROVED FOM PROGRAMME

Number	135.
Title (code)	Single gold nanorods in live cells (SGC)
Executive organisational unit	BUW
Programme management	Dr.ir. S.J.T. van Noort
Duration	2012 – 2017
Cost estimate	M€ 1.4

Concise programme description

a. Objectives

The aim of this programme is to develop the methodology to probe the function and mechanics of a wide range of biomolecules in a living cell. We will use a single gold nanorod as a probe for tracking and force/torque transduction on single molecules. Kros will synthesize gold nanorods with a surface chemistry that allows us to incorporate a specific functionality onto the probe and to control conjugation with selected proteins inside the cell, that are genetically tagged by Schaaf. Tagged proteins will be followed in three dimensions for arbitrarily long times using the two-photon microscope and tracking algorithms developed by Van Noort. Orrit will further develop the optical trapping and manipulation of a single gold nanorod *in vitro*.
We will use this new technology to study and develop delivery strategies for nanorods into the cell, to optimize detection and manipulation techniques for chromatin biophysics *in vitro*, to reveal the mechanisms of glucocorticoid receptor (GR) based regulation *in vivo* and to probe chromatin organization *in vivo*.

b. Background, relevance and implementation

Single-molecule fluorescence and force spectroscopy provide novel insight into the dynamics and interactions of biomolecules. Yet, current *in vitro* experiments are not always relevant to complex and variable cell conditions. Tracking and manipulating single molecules *inside* a cell is still not possible because (1) current fluorescent labels exhibit poor optical stability and (2) the bulky microbeads employed in optical or magnetic tweezers easily interfere with cell function.
We will employ gold nanorods as a solution to both these issues. Recently we have developed methods to track and trap single gold nanorods *in vitro,* and in this programme we will advance these methods further to probe specific biomolecules inside a cell. Because gold nanorods don't blink or bleach we can follow a labeled biomolecule for arbitrary long times. Moreover, their small volume (comparable to a big protein complex) warrants a significantly reduced invasiveness compared to microbeads generally used for force spectroscopy. Being small, they are also less prone to undesired non-specific interactions with cell components.
Our consortium will use the physical knowledge on microscopy and force spectroscopy, chemical knowledge on the synthesis and functionalization of gold nanoparticles, as well as specific biological knowledge to address the glucocorticoid signaling pathway. All participants are located in one building, ensuring a close and efficient collaboration. By joining the expertise from such diverse disciplines we will be able to address one of the most pertinent challenges in biophysics and cell biology i.e. tracking and manipulating the journey of individual molecules through the cell.

Funding

salarispeil cao per 01-01-2016

Via BUW

bedragen in k€	≤ 2016	2017	2018	2019	2020	≥ 2021	Totaal
FOM-basisexploitatie	1.325	46	-	-	-	-	1.371
FOM-basisinvesteringen	-	-	-	-	-	-	-
Doelsubsidies NWO	-	-	-	-	-	-	-
Doelsubsidies derden	-	-	-	-	-	-	-
Totaal	1.325	46	-	-	-	-	1.371

Source documents and progress control

a) Original programme proposal: FOM-11.1199
b) Ex ante evaluation: FOM-11.1412
c) Decision Executive Board: FOM-12.0253

Remarks

The final evaluation of this programme will consist of a self-evaluation initiated by the programme leader and is foreseen for 2017.

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Subgebied: 100% FL

Overview of projects and personnel

Workgroup FOM-L-21

Leader	Prof.dr. M.A.G.J. Orrit
Organisation	Leiden University
Project (title + number)	GNRs for single molecule detaction andmanipulation 11SGC02

FOM employees on this project

Name	Position	Start date	End date
A.J. Carattino	PhD	1 October 2012	31 December 2016

Workgroup FOM-L-36

Leader	Dr.ir. S.J.T. van Noort
Organisation	Leiden University
Project (title + number)	Two-photon tracking of functional GNRs in livecells 11SGC01

FOM employees on this project

Name	Position	Start date	End date
S. Carozza	PhD	1 July 2012	31 December 2016

Workgroup FOM-L-37

Leader	Dr. M.J.M. Schaaf
Organisation	Leiden University
Project (title + number)	GR signaling exposed with GNRs 11SGC03

FOM employees on this project

Name	Position	Start date	End date
V.I.P. Keizer	PhD	1 September 2012	31 August 2017