INSD NanoScience Seminar (No.14)

Date and Time: **Monday, July 27th, 2015, 16:15-17:25**

Place: **Room 305 INSD Seminar Room, Interdisciplinary Research Building 3rd floor, Toyonaka Campus**

1) 16:15~16:50

Title: **Mechanisms of membrane transport: a single-molecule view**

Lecturer: Prof. Thorben Cordes

Zernike Institute, University of Groningen, The Netherlands

Membrane transporters are vital to any living system and involved in the translocation of a wide variety of substrates. Despite their importance, all proposed molecular models for transport are based on indirect evidence due to the inability of classical biophysical and biochemical techniques to visualize dynamic structural changes. My group has recently started to use single-molecule fluorescence microscopy to characterize conformational states and changes in ATP-binding cassette (ABC) transporters in vitro to directly observe how different steps in transport are coordinated.[1]

In the first part of my talk I focus on the homodimeric GlnPQ complex, a bacterial ABC-importer, possessing two different substrate-binding proteins (SBDs) per single translocator. To decipher how conformational changes within the different subdomains drive transport, we use a combination of single-molecule methods and classical biochemical techniques (calorimetry and uptake assays). We demonstrate by single-molecule Förster resonance energy transfer (FRET) that the two SBDs intrinsically transit from open to closed ligand-free conformation, and the proteins capture their amino acid ligands via an induced-fit mechanism. High-affinity ligands elicit transitions without changing the closed-state lifetime, whereas low-affinity ligands dramatically shorten it. We show that SBDs in the closed state compete for docking onto the translocator, but remarkably the effect is strongest without ligand. We find that the rate-determining steps for translocation depend on the SBD and the amino acid transported. We conclude that the lifetime of the closed conformation controls both SBD docking to the translocator and substrate release.[1]

In the second part of the talk, I describe our latest developments of “enabling technology” for mechanistic studies: using photophysical tricks, we present a simple two-colour FRET assay that allows either to monitor multiple distances within protein complexes or simultaneously reveals one FRET-based distance and the presence of a second protein. I finally summarize our contributions towards the development of "self-healing" organic fluorophores[2-3] and their applications in single-molecule FRET or super-resolution microscopy.

[1] G. Gouridis et al., ***Nature Structural & Molecular Biology*** *22* **(2015)** 57-64.
[2] J.H.M. van der Velde et al., ***ChemPhysChem*** 14 **(2013)** pages 4084-4093.
[3] J.H.M. van der Velde et al., ***Journal of Physical Chemistry Letters*** 5 **(2014)** pages 3792-3798.

2) 16:50~17:25

Title: **Control of nanoparticle self-assemblies using distorted liquid crystals**

Lecturer: Dr. Emmanuelle Lacaze

Paris Institute of Nano-Sciences, France

Directed assembly of nanoparticles is a promising alternative for original nanoparticle organizations. New kinds of optical properties are expected when semi-conductive or metallic nanoparticles are concerned. Using liquid crystal matrices oriented by their interfaces, it is possible to induce anisotropic nanoparticle organizations. We can then investigate the influence of these matrices on the optical properties of the nanoparticles. We focus here on fluorescent semi-conducting nanorods, which behave like single photon emitters and gold nanoparticles for their plasmon resonance properties.

I will show how to create arrays of straight topological defects (dislocations) in thin smectic films that act as efficient traps for a specific localization and orientation of nanoparticles.
For trapped fluorescent nanorods, a fine control of the polarization of the single photons is then obtained. Similarly the orientation of gold nanorods leads to the control of their luminescence as well as their plasmon resonance by light polarization.

When the nanoparticle concentration is increased, we show that single chains are formed, and can lead to a strong electromagnetic coupling between the particles. We are not only capable of linearly confined the particles, but also of varying the inter-particle interactions and thus modify their optical properties which are sensitive to the inter-particle distance.

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