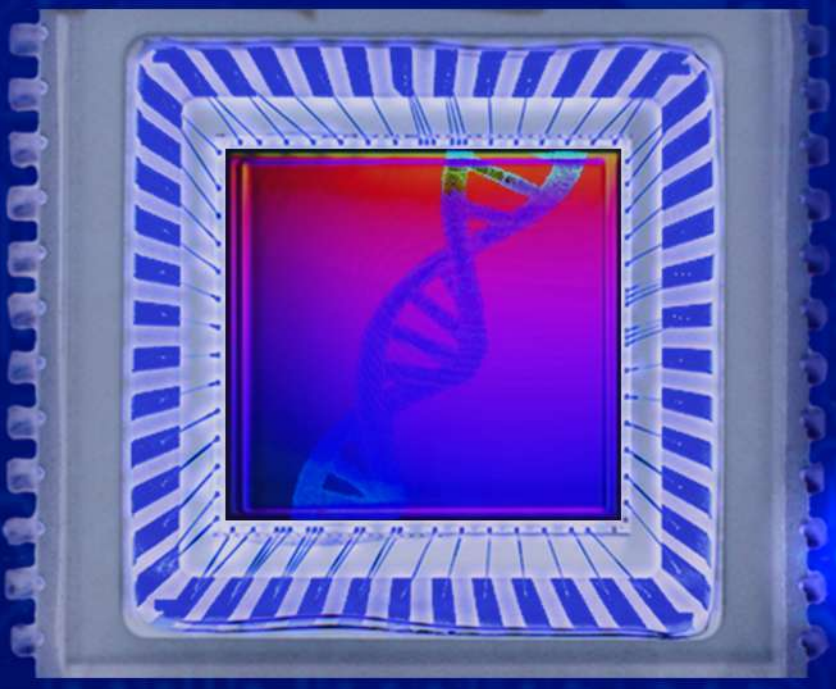
S3IC 2023

Single-Molecule **S**ensors and nano**S**ystems International Conference

November 22-24, 2023 | BARCELONA

Book of Abstracts



PremC
Conferences, Events & Workshops

premc.org/S3IC

ISSN 2678-3002

Table of Contents

Co-translational protein folding, one molecule at a time

Wednesday, 22nd November - 09:00: (Auditorium) - Oral

Prof. Carlos Bustamante [1]

1. *University of California*

TBD

Toward on-chip molecular fingerprinting of heterogeneous cell secretomes

Wednesday, 22nd November - 09:40: (Auditorium) - Oral

Prof. Romain Quidant [1]

1. *ETH Zürich*

Cells release a variety of substances to maintain homeostasis, communicate with other cells, and support essential bodily functions. These secretomes include individual proteins like cytokines and hormones, as well as lipid-bound extracellular vesicles (EVs) that transport proteins and genetic material. However, our understanding of cell secretions has been limited by a lack of methods combining sensitivity, high-throughput, and molecular fingerprinting to accurately reconstruct heterogenous biological nanoparticle populations. In this presentation, we present our recent technological advances toward novel optofluidic platforms addressing some of these existing challenges. The first platform brings together state-of-the-art digital holography with multilayer PDMS microfluidics and is applied to profiling four distinct ovarian cell-derived EV populations over a panel of surface protein biomarkers. The second platform combines hyperspectral imaging with droplet microfluidics enabling high-throughput biomarker detection in picoliter volumes.

“Quantum Biology”: how nature harnesses quantum processes to function optimally, and how might we control such quantum processes to therapeutic and tech advantage

Wednesday, 22nd November - 10:50: (Auditorium) - Oral

Dr. Clarice D. Aiello [1]

1. *Quantum Biology Tech Lab*

Imagine driving cell activities to treat injuries and disease simply by using tailored magnetic fields. Many relevant physiological processes, such as: the regulation of oxidative stress, proliferation, and respiration rates in cells; wound healing; ion channel functioning; and DNA repair were all demonstrated to be controlled by weak magnetic fields (with a strength on the order of that produced by your cell phone). Such macroscopic physiological responses to magnetic fields are consistent with being driven by chemical reactions that depend on the electron quantum property of spin. In the long-term, the electromagnetic fine-tuning of endogenous “quantum knobs” existing in nature could enable the development of drugs and therapeutic devices that could heal the human body — in a way that is non-invasive, remotely actuated, and easily accessible by anyone with a mobile phone. However, whereas spin-dependent chemical reactions have been unambiguously established for test-tube chemistry (bearing uncanny similarities with what physicists call “spin quantum sensing”), current research has not been able to deterministically link spin states to physiological outcomes *in vivo* and in real time. **With novel quantum instrumentation, we are learning to control spin states within cells and tissues, having as a goal to write the “codebook” on how to deterministically alter physiology with weak magnetic fields to therapeutic and technological advantage**.

Nanofluidic Scattering Microscopy: Mass Photometry of Macromolecules In Motion

Wednesday, 22nd November - 11:17: (Auditorium) - Oral

Mr. Bohdan Yeroshenko [1], ***Mr. Henrik Klein Moberg*** [1], ***Dr. David Albinsson*** [2], ***Dr. Daniel Midtvedt*** [3], ***Dr. Joachim Fritzsche*** [1], ***Prof. Giovanni Volpe*** [3], ***Prof. Christoph Langhammer*** [4]

1. *Chalmers University of Technology,* ***2.*** *Envue Technologies,* ***3.*** *University of Gothenburg,* ***4.*** *Chalmers university of technology*

Nanofluidic scattering microscopy is an optical microscopy technique that allows for the characterization of individual, moving macromolecules in liquids within a nanochannel. By employing differential imaging and background subtraction, the interference between light scattered by the nanochannel and the particles is measured, which allows us to estimate the light scattering cross-section of macromolecules, a property closely related to molecular weight. Particles confined within the nanochannel diffuse along it while remaining in focus. This feature enables the reconstruction of molecular trajectories and the calculation of hydrodynamic radii. Image data is processed using neural networks, which enhances data interpretation. The method has been successfully used to measure macromolecules such as Bovine Serum Albumin (BSA, ~66 kDa) [1], and is capable of analyzing macromolecules below 60 kDa.

[1] Špačková, B., Klein Moberg, H., Fritzsche, J. et al. Label-free nanofluidic scattering microscopy of size and mass of single diffusing molecules and nanoparticles. Nat Methods 19, 751–758 (2022). https://doi.org/10.1038/s41592-022-01491-6

The influence of ascorbic acid and iron loading on the dynamic behaviour of single, native ferritin

Wednesday, 22nd November - 11:34: (Auditorium) - Oral

Mr. Arman Yousefi [1], ***Dr. Cuifeng Ying*** [1], ***Dr. Christopher Parmenter*** [2], ***Mrs. Mahya Assadipapari*** [1], ***Mr. Gabriel Sanderson*** [1], ***Mr. Ze Zheng*** [1], ***Dr. Lei Xu*** [1], ***Mr. Saaman Zargarbashi*** [1], ***Dr. Graham Hickman*** [3], ***Prof. Mohsen Rahmani*** [1]

1. *Advanced Optics and Photonics Laboratory, Department of Engineering, School of Science and Technology, Nottingham Trent University, Nottingham NG118NS, United Kingdom,* ***2.*** *Nanoscale and Microscale Research Centre, University of Nottingham, Nottingham NG7 2RD, United Kingdom,* ***3.*** *School of Science and Technology, Nottingham Trent University, Nottingham NG11 8NS, United Kingdom*

Introduction:

The study of protein function and conformational changes is important for understanding their role in biological processes and diseases. Studying proteins at a single-molecule level provides a detailed understanding of molecular mechanisms, chemical processes, and heterogeneity across molecular systems. In this work, plasmonic nanotweezers using a double-nanohole (DNH) nanostructure were used to study individual, unlabelled apo- and holo-ferritins in a liquid environment.[1] Also, the effect of ascorbic acid (AA) concentration on the dynamic behaviour of single ferritin was investigated.

Method:

We employed a focused ion beam (FIB) to create DNH structures (gap size: 15-20 nm) in a gold film.[2] A laser (852 nm) with polarisation perpendicular to the gap direction excites the plasmonic resonance, which generates field enhancement in the gap of the DNH (Fig.1). This enhanced field is capable of trapping proteins within the DNH in a liquid environment. Optical trapping experiments were conducted on ferritin proteins, then Fe^{2+}, Fe^{3+}, and AA solutions were introduced to analyse protein behaviour at the trapping site.

Figure 1. Monitoring of iron loading on single ferritin using DNH structure.[1]

Results and Discussion:

The transmission signal of single apo- and holo-ferritin trapped in the DNH revealed that the RMS of apo-ferritin is higher than its holo counterpart, indicating the stability of holo-ferritin with an iron core. Additionally, trapping holo-ferritin led to a 41% larger change in average transmission signal than trapping apo-ferritin, suggesting a higher polarisability due to its larger size and higher conductivity. For the first time, this study showed real-time tracking of iron mineralisation in unmodified single ferritins, uncovering the structural dynamics of their three-fold channels involved in iron entry and transfer. Furthermore, we demonstrated that ferritin's channels exhibit increased dynamics at concentrations above 5 mM of AA, leading to higher iron release from the protein. Our method shows the potential of plasmonic nanotweezers for studying the dynamics of single proteins.

References:

1. Yousefi, Arman, et al. "Optical Monitoring of In Situ Iron Loading into Single, Native Ferritin Proteins." Nano Letters 23.8 (2023): 3251-3258.

2. Pang, Yuanjie, and Reuven Gordon. "Optical trapping of a single protein." Nano letters 12.1 (2012): 402-406.

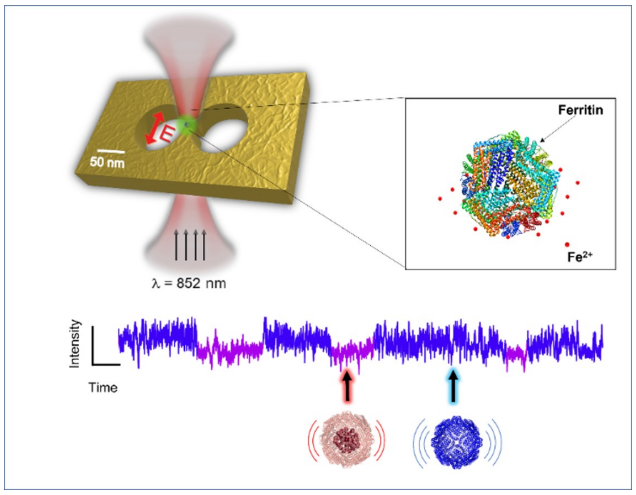


Figure 1.jpg

New methodologies for single molecule imaging using the fluorous effect

Wednesday, 22nd November - 11:51: (Auditorium) - Oral

Dr. Marina Santana Vega [1], ***Dr. Carlos Bueno Alejo*** [2], ***Dr. Andrea Taladriz Sender*** [3], ***Mr. Max Wills*** [4], ***Prof. Ian Eperon*** [4], ***Prof. Glenn Burley*** [3], ***Prof. Andrew Hudson*** [4], ***Prof. Alasdair Clark*** [1]

1. *University of Glasgow,* ***2.*** *School of Chemistry, University of Leicester, University Road, Leicester, United Kingdom, LE1 7RH.,* ***3.*** *University of Strathclyde,* ***4.*** *School of Chemistry, University of Leicester, University Road, Leicester, United Kingdom, LE1 7RH, Leicester Institute of Structural & Chemical Biology, Henry Wellcome Building, University of Leicester, Lancaster Road, LE1 7HB*

Introduction:

Perfluorinated phases favour interactions with other perfluorinated phases, excluding themselves from aqueous and organic ones, a property known as the fluorous effect. Our recent research demonstrates the utilization of fluorous brushes to prevent non-specific binding of single proteins for mass photometry studies (Figure1), proving their anti-fouling properties at the single molecule level.[1] Expanding upon this work, we now look at the potential of these brushes for total internal reflection fluorescence (TIRF) microscopy, by exploiting the high affinity of fluorous-fluorous interactions[2] that allows us to specifically anchor DNA oligonucleotides to the brush using perfluorinated tails (Figure 2).

Methods:

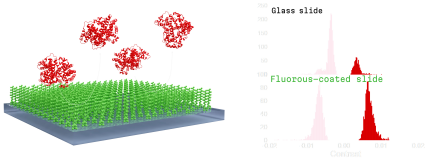
Perfluorinated brushes were created upon self-assembly of perfluorinated silanes on microscope glass slides. Commercially available DNA-oligonucleotides were modified with perfluorinated tags using standard solid-phase methods on a DNA/RNA synthesiser. Picomolar solutions of the oligonucleotides were prepared in T-50 buffer and injected in a chamber for 5 min before rinsing with the same buffer. The samples were characterised using a TIRF microscope setup and the diffusion coefficients calculated using custom-made software.

Results and discussion:

Fluorous-tagged DNA oligonucleotides specifically bind the fluorous brush. The interaction is sufficiently strong that the substrate can be rinsed with buffer inside a microfluidic chamber without removing the tethered oligos. Our observations prove that the fluorous-fluorous interaction is dynamic; tethered DNA oligonucleotides diffuse across the fluorous brush. We have been able to characterise this diffusion using machine learning software developed in house. We believe these findings will enable new experimental possibilities for colocalization microscopy, and even label-free detection of single molecules.

[1]Bueno Alejo, C. J., Santana Vega, M., Chaplin, A. K., Farrow, C., Axer, A., Burley, G. A., Dominguez, C., Kara, H., Paschalis, V., Tubasum, S., Eperon, I. C., Clark, A. W., Hudson, A. J., Surface passivation with a perfluoroalkane brush improves the precision of single molecule measurements. *Submitted.*

[2]Flynn, G., Withers, J., Macias, G., Sperling, J., Henry, S., Cooper, J., Burley, G. and Clark, A., 2017. Reversible DNA micro-patterning using the fluorous effect. *Chemical Communications*, 53(21), pp.3094-3097.



Fluorous brushes prevent non-specific binding of single proteins.jpg

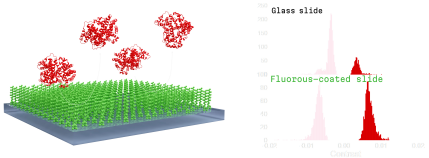


Figure 1 - fluorous brushes prevent non-specific binding of single proteins.jpg

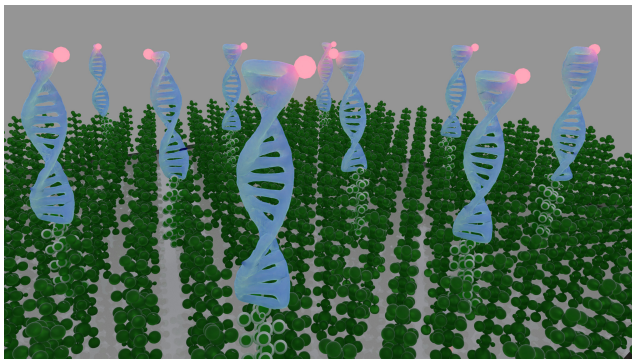


Figure 2 - shcematic representation of fluorous-tethered dna oligonucleotides.jpg

Mechanism and function of Smc5/6-mediated DNA loop extrusion

Wednesday, 22nd November - 10:50: (Room 607) - Oral

Dr. Eugene Kim [1]

1. *Max Planck Institute of Biophysics*

Structural maintenance of chromosome (SMC) complexes are essential for genome organization and functions. Unlike condensin and cohesin, the molecular functions of Smc5/6 have so far much less understood. Recently, we discovered the Smc5/6's loop-extruding motor activity by real-time *in vitro* single-molecule imaging, establishing loop extrusion as a conserved mechanism among all eukaryotic SMC complexes. While the kinetics of the Smc5/6-mediated loop extrusion appeared to be similar to those from condensin and cohesin, we found distinct mechanistic details that are specific to Smc5/6 complex, namely, on Smc5/6 dimerization-mediated loop extrusion and down-regulation of Nse5/6 on Smc5/6-mediated loop extrusion. Furthermore, our recent in vivo analysis found that Smc5/6 associates with transcription-induced positively supercoiled chromosomal DNA and links these regions in cis. Mechanistically, single-molecule imaging reveals that dimers of Smc5/6 specifically recognize the tip of positively supercoiled DNA plectonemes, and efficiently initiates loop extrusion to gather the supercoiled DNA into a large plectonemic loop.

[1]Pradhan, B. *et al.* The Smc5/6 complex is a DNA loop-extruding motor. *Nature* **616**, 843–848 (2023).

[2]Jeppsson, K. et al. Loop-extruding Smc5/6 organizes transcription-induced positive DNA supercoils. *bioRxiv* (2023): 2023-06

Developing a motility-based whole-cell biosensor

Wednesday, 22nd November - 11:17: (Room 607) - Oral

Ms. Diana Coroiu [1], ***Dr. Amritha Janardanan*** [1], ***Dr. James Flewellen*** [1], ***Prof. Teuta Pilizota*** [1]

1. *Institute of Cell Biology, The University of Edinburgh*

Introduction

Many bacteria possess a regulatory network that enables them to navigate their environment. *Escherichia coli's* (chemotactic) network relies on transmembrane receptor proteins that convey environmental information by initiating a signalling cascade that ultimately leads to the change in the rotational direction of their flagellum. The latter is predominantly composed of the bacterial flagellar motor and a stiffer longer filament. The change of the direction of the flagellum is a result of the change of the direction of the motor, which is caused by the interaction with the phosphorylated CheY (CheY-P) protein. Thus, the concentration of CheY-P reflects the environmental concentration of molecules of interest to the cell, and determines the frequency of the motor's rotational direction changes. Here, we wish to exploit the system to sense molecules of interest to humans by coupling different sensing components with the bacterial flagellar motor via a CheY** protein that mimics CheY-P.

Methods

We developed a rhamnose-sensing *E. coli* K-12 strain whose frequency of the motor's rotational direction changes increases with increasing concentrations of rhamnose. We deleted the wild type *cheY* gene and introduced a construct containing the *chey*** gene controlled by a rhamnose-inducible promoter (PrhaBAD) and constitutively expressed PrhaBAD activator *rhaS*. The strain also encodes a more hydrophobic flagellar filament that facilitates quantifying the rotational direction of the motor, as follows: (i) the truncated flagellum adheres to glass slides and the rotating cell bodies (driven by the motor) can be visualised with optical microscopy (tethered cell assay), or (ii) the cell bodies are surface-immobilised and polystyrene beads attached to the truncated filament so that their rotation can be tracked using either back focal plane interferometry or impedance-based measurements.

Results and discussion

Because the flagellar motor is highly sensitive to CheY**, even small concentrations of rhamnose can be detected. Initial results indicate that the sensor is at least 10 times more sensitive than its GFP-based equivalent. We explore both theoretically and experimentally the limit of sensitivity of our new sensor. Our work enables a new class of whole-cell sensors whose output can be read out electrically and on a single-cell level.

Hybrid gold-DNA origami nanostructures for colorimetric sensing

Wednesday, 22nd November - 11:34: (Room 607) - Oral

Ms. Claudia Corti [1], ***Ms. Elise Gayet*** [1], ***Ms. Nesrine Aissaoui*** [2], ***Ms. Sylvie Marguet*** [3], ***Mr. Gaetan Bellot*** [2], ***Mr. Sébastien Bidault*** [1]

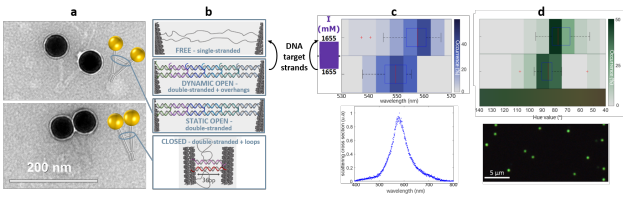
1. *Institut Langevin, ESPCI Paris, Université PSL, CNRS, Paris, France,* ***2.*** *CBS - Centre de Biochimie Structurale, CNRS, INSERM, Montpellier, France,* ***3.*** *CEA Saclay, Univ. Paris-Saclay, IRAMIS, NIMBE, Gif-sur-Yvette, France*

To develop a colorimetric sensing platform compatible with single-molecule detection, we assemble gold-nanosphere dimers on a 3D Y-shaped DNA origami that acts as a nanoscale actuator (Fig.1-a). DNA origamis are a flexible platform to produce nanostructures that shift their morphology when interacting with specific targets, such as DNA/RNA strands, proteins, or specific cations [1]. To translate such conformational changes in colorimetric information, we exploit the nanoscale dependence of plasmon coupling between two gold nanospheres. We demonstrated that dark-field microscopy allows the far-field monitoring of nanoscale distance changes in single gold dimers on a simple color camera [2,3]. The scaffold of our DNA origami features an active site with a conformation that can be tuned by hybridizing specific DNA single strands (Fig. 1-b). The morphology of the hybrid nanostructure is governed by the geometry of the DNA origami but also by steric and electrostatic repulsions between the nanospheres. We observed that the difference in conformation of the active site is only visible in the optical response for high ionic strength, when these steric and electrostatic repulsions are reduced. One-step colorimetric sensing of DNA single strands is achieved at high ionic strength using a strand displacement reaction. These measurements are carried out both by performing single-nanostructure scattering spectroscopy (Fig.1-c) and by monitoring the hue of single dimers in dark-field images (Fig.1-d), obtaining similar statistical responses. These results open exciting perspectives for the colorimetric sensing of individual DNA strands on a color camera.

Figure 1: (a) EM images of 40nm-gold-spheres dimers assembled with DNA origami (b) Different conformations of the active site (c) Distributions of resonance wavelength (top) of single-nanostructure scattering spectra (bottom) (d) Distributions of maximum hue value of single-nanostructures (top), retrieved from dark-field color-camera images (bottom).

References

- [1] Y. Ke et al., Nat. Commun, 7:10935, 2016
- [2] L. Lermusiaux et al., ACS Nano, 9:978, 2015
- L. Lermusiaux et al., Langmuir, 34:14946, 2018



Tem images - active site design - spectral and rgb data.png

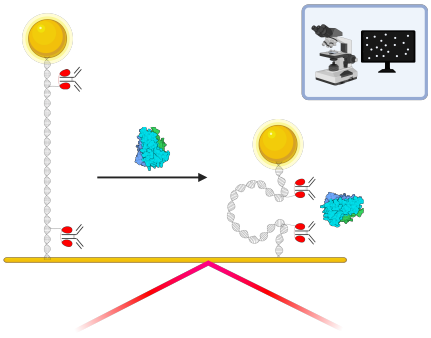
A DNA nanoswitch as an architecture for continuous biosensing with single molecule detection

Wednesday, 22nd November - 11:51: (Room 607) - Oral

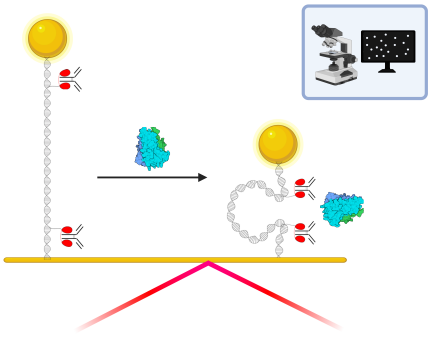
Ms. Anna Swietlikowska [1], ***Mr. Livio Oliveira de Miranda*** [1], ***Prof. Peter Zijlstra*** [1], ***Prof. Maarten Merkx*** [1]

1. *Eindhoven University of Technology*

There is an increasing need for biosensors that can measure biomarkers continuously. An accurate diagnosis of certain diseases, such as sepsis, depends on it. The technology of similar sensor can be based on an enzymatic reaction (like for glucose monitoring) or on conventional binders as aptamers or antibodies. The suitable binders should also be put in the right molecular architecture. Hence, I developed a molecular switch, made of DNA, that undergoes a large conformation change when the target biomarker is bound. The system is based on long quasi double stranded DNA. One structure is functionalized in two regions and upon analyte binding, two distant parts of this nanoswitch are brought together and form a loop. In this research, I took advantage of protein G dimer, a generic antibody binding protein that was conjugated to maleimide-modified DNA. The protein was then successfully photo-crosslinked to antibodies targeting inflammation biomarkers: anti IL-6 and infliximab, anti TNFα. The presence of analytes was detected in various ways, depending on the sensor design. Firstly, the split NanoLuc system was genetically fused to protein G and an analyte was detected by bioluminescence. Upon addition of TNFα, the bioluminescence level increased five times. Although, it is not really suitable for continuous biosensing (constant substrate addition), it is an effective tool to characterize the switching behavior in function of the distance between binders. Interestingly, the higher the effective concentration of antibodies was, the less noticeable hook effect was. Secondly, the use of such a modular material as DNA enables easy immobilization of a sensor on gold surface and attachment of the gold nanoparticle on another side. When light beam was introduced with total internal reflection, only the particles close to the surface were observed. Upon target analyte addition, the particles were brought to the surface and each one could be tracked under the microscope. The DNA nanoswitch is suitable for continuous biosensing with most of the available antibodies. The next step would be to incorporate binders with faster dissociation rates to enable the use of this sensor in a continuous manner.



Swietlikowska s3ic.png



Swietlikowska s3ic1.png

Conduction properties of bacterial nanowires probed by Scanning Dielectric Microscopy

Wednesday, 22nd November - 10:50: (Room 608) - Oral

Prof. Gabriel Gomila [1]

1. *University of Barcelona*

Long-range electron conduction in cable bacteria filaments has attracted considerable attention recently due to its unusual characteristics in the biological world. Electronic conduction in cable bacteria filaments mediate spatially segregated redox reactions in marine sediments between regions rich in, respectively, sulfur and oxygen, located centimeters apart. Electronic conduction takes place through Ni rich protein nanofibers ~50 nm in diameter located in the bacteria periplasm below the outer cell membrane, with conductivity values in the range ~1-10 S/cm, exceeding by more than 6 orders of magnitude the conductivity of the best conducting protein nanowires. Electrical measurements are performed usually with two (and four) microelectrode set-ups, typically separated from few micrometers to centimeters, which generally show poor stability in ambient conditions. Here I present the recent theoretical and experimental developments we made to access the conduction properties of these protein nanofibers by means of scanning dielectric microscopy (SDM) [1]. SDM, being based in the detection of alternating electric fields and forces, provides access to the conductive properties of single protein nanofibers without physically contacting them, enabling stable measurements and systematic electric transport studies. I will report on the results we have obtained with the application of SDM to protein nanowires from cable bacteria. [1] H. T.S. Boschker,(…), G. Gomila and F. Meysman, Efficient long-range conduction in cable bacteria through nickel protein wires, Nature Communications 12, 3996 (2021).

Electron transfer measurement in cytochrome c

Wednesday, 22nd November - 11:17: (Room 608) - Oral

Dr. Alexandre Gomila [1], Dr. Gonzalo Pérez-Mejías [2], Dr. Alba Nin-Hill [3], Dr. Alejandra Guerra-Castellano [2], Ms. Laura Casas-Ferrer [1], Ms. Sthefany Ortiz-Tescari [1], Prof. Antonio Díaz-Quintana [2], Prof. Josep Samitier [1], Prof. Carme Rovira [3], Prof. Miguel A. De la Rosa [2], Prof. Irene Díaz-Moreno [2], Prof. Pau Gorostiza [1], Dr. Marina I. Giannotti [4], Dr. Anna Lagunas [4]

1. *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST),* ***2.*** *Institute for Chemical Research–cicCartuja, Universidad de Sevilla, Consejo Superior de Investigaciones Científicas (CSIC),* ***3.*** *University of Barcelona, Department of Inorganic and Organic Chemistry, Institute of Theoretical Chemistry (IQTCUB),* ***4.*** *CIBER-BBN, ISCIII*

Introduction: Interprotein electron transfer underpins essential biological processes including mitochondrial respiration. Cytochrome *c* (C*c*) is a mitochondrial hemeprotein that exerts multiple functions. C*c* localizes in the intermembrane mitochondrial space, shuttling electrons from cytochrome bc_1 complex (Cbc_1, complex III) to cytochrome *c* oxidase (C*c*O, complex IV). C*c* also takes part in other redox reactions within mitochondria including a preapoptotic function. C*c* activities are tightly regulated by posttranslational modifications such as phosphorylation. Here, we used electrochemical scanning tunneling microscopy (EC-STM), and computational methods to investigate the molecular mechanism of ET between Cc and Cbc_1, and by using a phosphomimetic mutant, we studied ET regulation by C*c* phosphorylation.

Methods: We measured ET between C*c* and Cbc_1 using the cross complex between human C*c* and the soluble domain of plant cytochrome Cc_1. The effects of C*c* phosphorylation were studied using the phosphomimetic mutant Y48*p*CMF. C*c* and Cc_1 were immobilized on the probe and the sample electrodes of EC-STM, respectively, through Cys moieties. Experiments were conducted in an electrochemical cell under bipotentiostatic control and in physiological buffer. Current-distance (I-z) electrochemical spectroscopy was conducted, and faradaic leakage current was maintained below a few pA over the entire recording range through probe insulation.

Results: Iz measurements showed that the current decays along more than 10 nm in the solution. Computational simulations showed cation exclusion and the formation of a charge conduit at the active interface between the two proteins (Gouy-Chapman conduit), facilitating long-range ET. EC-STM measurements also demonstrated that long-range ET is impaired by the phosphomimetic mutant Y48*p*CMF C*c*. MD simulations showed that the negative charge introduced prevents cation exclusion between the proteins' active sites, enabling charge screening and weakening the Gouy-Chapman conduit, which is completely disrupted by phosphate groups.

Discussion: We demonstrated inter-protein long-distance ET through the aqueous solution between C*c* and Cc_1.Those results suggested that ET could take place without the need of establishing a well-defined protein complex, thus balancing high specificity with weak binding to maintain the high turnover rate. Upon phosphorylation, the long-distance charge conduit between the protein partners is disrupted, impairing the flow of electrons, anticipating a change in the biological function.

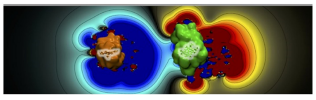


Figure. Side view of the equipotential lines from -1.1 kT/e (red) to 1.1 kT/e (blue) calculated by Poisson-Boltzmann equation (APBS) showing the formation of a charge conduit between C*c* (orange) and Cc_1 (green). Proteins are superimposed for visualization purposes.

Figure.jpg

Designing a single molecule refractive index sensor based on DNA origami and plasmonic nanoparticles

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Mr. Malthe von Tangen Sivertsen [1], ***Mr. Kasper Okholm*** [1], ***Prof. Duncan Sutherland*** [1]

1. *Aarhus University*

Single-molecule biosensing provides statistical distributions, and real-time information, giving insight into events that would be averaged out in ensemble measurements [1]. Most single-molecule techniques rely on a label, commonly a fluorophore, which poses limitations, such as photobleaching, and is invasive on the system, why label-free studies are of high interest [1,2,3]. The localized surface plasmon resonance (LSPR) effect of metal nanoparticles can be used for label-free study of molecules, as the LSPR frequency changes with the local refractive index of the particle leading to an intensity change of the scattered light [1,2,3].

Here, we propose a single molecule refractive index sensor based on DNA origami and plasmonic nanoparticles. Key for the design is the placement of two nanorods, with high aspect ratio, end-to-end with high alignment, and small gap size, to get a very strong and highly localized hot spot needed for sensing [4,5,6,7]. The accurate placement of the rods is achieved using a novel DNA origami design (fig. 1, fig 2a) where DNA functionalized rods are bound to a cavity groove, and the central 3-layer origami rod, ensuring alignment of the rods and a fixed gap size around 10 nm. The binding ligand for the molecule can be placed via the origami in the hot spot. As shown in figure 2b, preliminary data shows proof of concept where two rods attach as designed. To further increase the sensitivity of the sensor it is envisioned to use core-shell gold-silver rods coated in DNA (fig. 3) made following the protocol from Dass et al., [8]. Hereby the plasmonic properties of silver allow to shift the LSPR into the visible range and increase the sensor sensitivity.

[1] Beuwer, Prins, and Zijlstra, *Nano Letters,* 2015, 15, 3507−3511

[2] Celiksoy et al., *J. Phys. Chem. Lett.* 2020, 11, 4554−4558

[3] Ye et al., *Nano Lett.* 2018, 18, 6633−6637

[4] Trofymchuk et al., *ACS Nano*, 2023, 17(2), 1327−1334

[5] Funston et al., *Nano Letters,* 2009 vol. 9, No 4 1651-1658

[6] Becker et al., *Plasmonics,* 2010 5:161–167

[7] Zhao et al., *Nano Res.* 2022 15, 1327–1337

[8] Dass et al., *Mater. Adv.,* 2022, 3, 3438

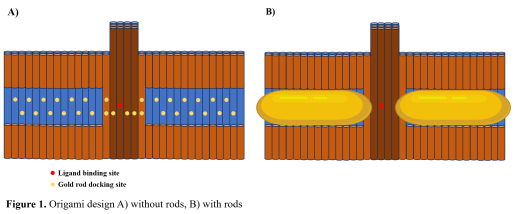


Figure 1. Origami design A) without rods, B) with rods

Figure1 submission.png

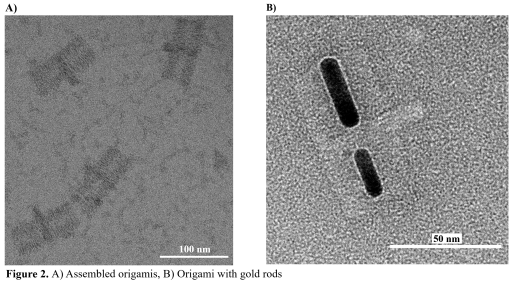


Figure 2. A) Assembled origamis, B) Origami with gold rods

Figure2 submission.png

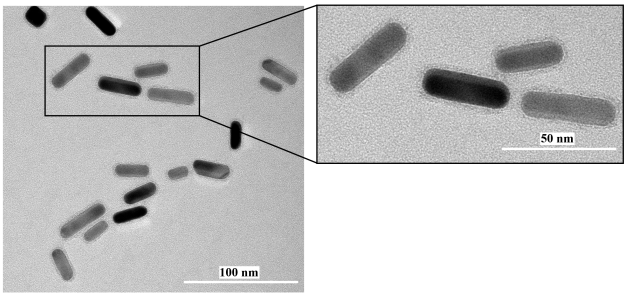


Figure 3. Gold silver core shell rods coated with DNA. Insert is a zoom-in

Figure3 submission.png

Single molecule orientation localization microscopy for supramolecular polymers

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Dr. Hailin Fu [1], ***Dr. Yuyang Wang*** [1], ***Prof. Peter Zijlstra*** [1], ***Prof. E.W. (Bert) Meijer*** [1]

1. *Eindhoven University of Technology*

Introduction

Complex molecular systems composed of supramolecular building blocks have shown great potential in the development of polymer materials.[1] It is essential to understand the structural nature of various supramolecular assemblies which is characterized by highly dynamic bonding. Albertazzi et al[2] applied stochastic optical reconstruction microscopy (STORM) to reveal the structure and dynamics of single supramolecular fibers. In STORM-like super-resolution techniques, single fluorescent molecules conjugated to monomers are inserted or exchanged with the supramolecular structure. However, powerful as super-resolution fluorescence microscopy in spatial and temporal resolution, information on single molecule orientation that stems from the interaction of single fluorescent molecules with supramolecular systems is neglected.

Methods

We report a microscopic technique to reveal the spatial, temporal and orientational information of fluorescently labeled supramolecular assemblies. In addition to traditional STORM, we extract the orientation information encoded in the point spread functions (PSFs) of single fluorescent dyes covalently linked with supramolecular structures using a polarization camera based method for super-resolution instant orientation microscopy (POLCAM)[3]. A polarization camera with embedded wire gird polarizers directly on CMOS sensor distinguishes the emission patterns of differently linearly-polarized single molecules in/out of the image plane, and the images containing spatial, temporal and orientational information of single molecules are used to resolve the structure and orientations of supramolecular assemblies.

Results

We measure single molecule orientations using POLCAM method. We use an open-source algorithm for fast analysis of single molecule localizations and orientations with high precision. With elongated structures, the supramolecular polymers could form into tactoids, a liquid crystal state, through liquid liquid phase separation. The orientation of the supramolecular polymer fibrils is closely related to the type and organization of the supramolecular polymer liquid crystals. Using POLCAM method, we could scan the orientation of the fluorescent labels and therefore the orientation of the supramolecular polymer fibrils. It provides a way to track the internal organization of the supramolecular polymer liquid crystals as a function of time and crowding conditions.

[1] Aida, T.*Israel Journal of Chemistry* **2020**, *60* (1–2), 33–47.

[2] Albertazzi, L.*Science* **2014**, *344* (6183), 491–495.

[3] Bruggeman, E.bioRxiv **2023**.02.07.527479

Multimode optomechanical weighing and elastometry of individual nanoparticles

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Mr. Louis Waquier [1], ***Dr. Samantha Sbarra*** [2], ***Mr. Stephan Suffit*** [2], ***Dr. Aristide Lemaître*** [3], ***Dr. Ivan Favero*** [2]

1. *Matériaux et Phénomènes Quantiques, Université Paris Cité, Paris, France,* ***2.*** *Matériaux et Phénomènes Quantiques, Université Paris Cité, CNRS, Paris, France,* ***3.*** *Centre de Nanosciences et de Nanotechnologies, Université Paris-Saclay, CNRS, Palaiseau, France*

We demonstrate multimode optomechanical sensing of individual nanoparticles with a radius between 75 and 150 nm. A semiconductor optomechanical disk resonator is optically driven and detected under ambient conditions, as nebulized nanoparticles land on it. Multiple mechanical and optical resonant signals of the disk are tracked simultaneously, providing access to several pieces of physical information about the landing analyte in real time. Thanks to a fast camera registering the time and position of landing, these signals can be employed to weigh each nanoparticle with precision. Sources of error and deviation are discussed and modeled with care, eventually providing a procedure to evaluate the elasticity of the nanoparticles on top of their mere mass. The device is optimized for the future investigation of biological particles in the high megadalton range, such as large viruses .

Fig1 : Top: optomechanical disk sensor (left), with a single landed nanoparticle (right)

Fig2 : Mechanical (red) and optical (blue) signals upon time, during the arrival of a single nanoparticle landing from a mist of suspended nanoparticles sprayed onto the sensor.

Fig3 : Nanoparticles landed on optomechanical disk sensors. Ref : S. Sbarra, L. Waquier, S. Suffit, A. Lemaître, I. Favero. Nano Letters 22 (2), 710-715 (2022)

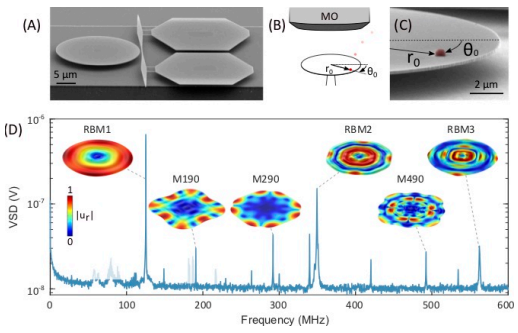


Figure1.jpg

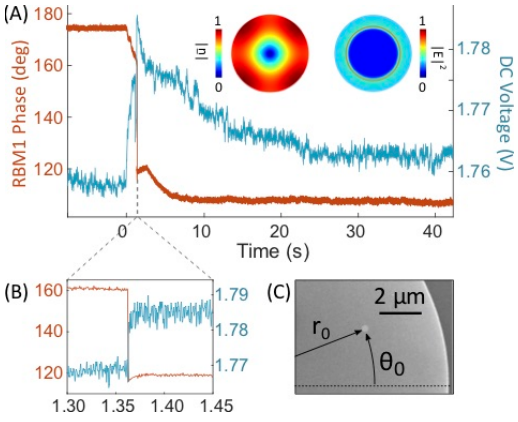


Figure2.jpg

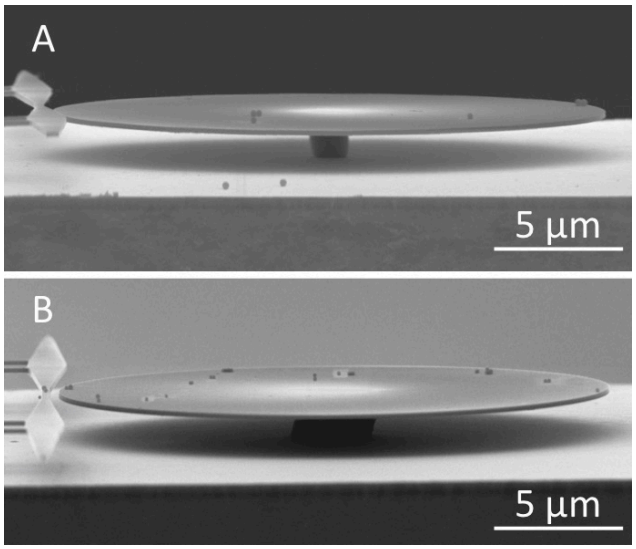


Figure3.jpg

Sensing with single organic molecules at room temperature

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Ms. Anežka Dostálová [1], ***Dr. Robert Stárek*** [1], ***Prof. Miroslav Jezek*** [1]

1. *Palacký University Olomouc*

Introduction

Single emitters such as organic molecules are growing in importance in quantum optics, biomedical imaging, and sensing. The implementations of molecular sensors are predominantly limited to cryogenic temperatures, thereby significantly reducing their applicability. We present the utilization of organic molecules as sensors operated at room temperature. We monitor statistical properties of the light emitted by single molecules and their clusters and study changes in the dynamics under various ambient conditions.

Methods

Our approach is based on a fluorescence microscope scheme consisting of an excitation path and an emission path separated by a dichroic mirror, as indicated in Fig. 1. Both confocal and wide-field imaging are possible, as well as a total internal reflection regime of the excitation. A sample containing single terrylene molecules embedded in a crystal host is prepared by spin coating and placed above a high numerical aperture objective. The individual molecules are single-photon sources with highly non-classical emission exhibiting strong antibunching. Advanced techniques are utilized for emission characterization, such as multiplexed photon-number-resolving detection, photon statistics reconstruction, various criteria of nonclassicality, and novel machine learning modalities.

Results

We experimentally demonstrated the single-photon nature of light emitted by single terrylene molecules and observed saturation behavior of the emission rate with increasing excitation power. Photon statistics and correlation properties of the detected signals from molecular clusters were examined to resolve the number of individual emitters, and dependence on the excitation power was studied. We are working towards enhancement of sensing abilities and the counting of single emitters aided by deep learning.

Discussion

Sensors based on single fluorescent molecules and their clusters hold a promise of a significant advancement of nanosensing and quantum sensing. The combination of the measured statistical and correlation features of the emitted light with the developed deep learning models will ensure less sensitivity to noise, improving the sensors' performance. The room temperature operation and novel statistical readout methods open the path to a broad range of new applications, including minimally-invasive and in-vivo sensing.

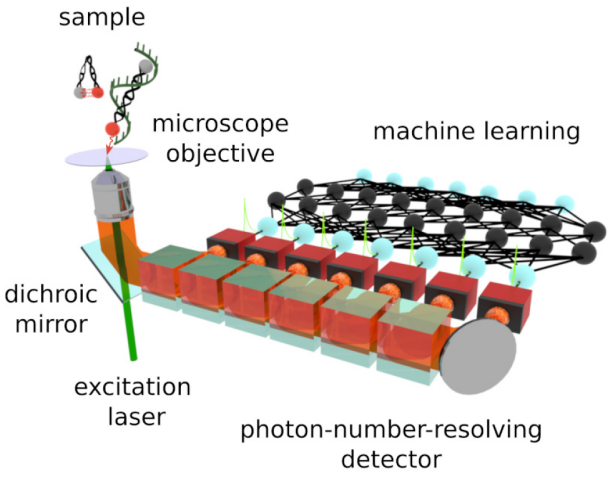


Figure1 fluorescence emission and analysis scheme.png

Reversible Covalent Bonds for Trapping Single Proteins Transiently in Nanopores

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Mr. Yuanjie Li [1], ***Prof. Michael Mayer*** [1], ***Dr. Saurabh Awasthi*** [2]

1. *Adolphe Merkle Institute, University of Fribourg, Chemin des Verdiers 4, CH-1700, Switzerland,* ***2.*** *Department of Biotechnology, National Institute of Pharmaceutical Education and Research, Raebareli (NIPER-R), Lucknow-226002, Uttar Pradesh, India*

Single-molecule level characterization of individual proteins by solid-state nanopores has shown substantial promise. Fast translocation time through the nanopore and bandwidth limitations of the instrument make it challenging to characterize the size and shape of individual proteins accurately. In this work, we coated the walls of solid-state nanopores with a polymer (PAcrAm-g-PEG) that minimizes non-specific interactions with proteins while exposing azide groups. Reaction of these azide groups via a DBCO-activated linker with a phenylboronic acid group (PBA) makes it possible to trap glycated proteins by taking advantage of reversible covalent bond formation between PBA and vicinal diols of glycated amino acid residues in proteins. Dwell time analysis revealed two populations of resistive pulses: Short-lived signals from free translocations (< 0.5 ms[MOU1]) and long-lived signals (0.5 ms – 2 s[MOU2]) from transient covalent bonds between glycated proteins and PBA. Control experiments using nanopores coated with PAcrAm-g-PEG but without PBA groups or with unglycated proteins confirmed that the long dwell time events only occur in the presence of both glycated proteins and PBA groups. Variations in the applied potential difference or the pH value of the recording buffer demonstrate the ability to control the trapping time during protein translocation through nanopore. Using covalent trapping of proteins, we determine the approximate size and shape of proteins with an approximately 15 % improvement in accuracy compared to free translocation. This approach, hence, selectively extends the residence time of natively glycated proteins (or of proteins that are intentionally modified with chemical groups that bear vicinal diols) thereby providing selectivity and improving the accuracy of single-molecule level protein characterization.

Improved Reuse and Storage Performances at Room Temperature of a New Environmental-Friendly Lactate Oxidase Biosensor Made by Ambient Electrospray Deposition (ESD)

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Dr. Antonella Cartoni [1], ***Dr. Mattea Carmen Castrovilli*** [2], ***Dr. Viviana Scognamiglio*** [3], ***Dr. Emanuela Tempesta*** [4], ***Dr. Jacopo Chiarinelli*** [2], ***Dr. Maria Antonietta Parracino*** [2], ***Dr. Valeria Frisulli*** [3], ***Dr. Maria Teresa Giardi*** [5], ***Dr. Lorenzo Avaldi*** [2], ***Dr. Danae Rossi*** [6]

1. *Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Roma, Italy,* ***2.*** *Institute of Structure of Matter-CNR, (ISM-CNR), Area della Ricerca di Roma 1, Via Salaria km 29.300, 00015, Monterotondo, Italy,* ***3.*** *Institute of Crystallography, National Research Council, Area Della Ricerca di Roma 1, Via Salaria Km 29.300, 00015, Monterotondo, Italy,* ***4.*** *CNR-Institute of Environmental Geology and Geoengineering (CNR-IGAG), Area della Ricerca Roma1, Via Salaria km 29.300, 00015, Monterotondo, Italy,* ***5.*** *Biosensor Srl, Via Degli Olmetti 4, 00060, Formello, Rome, Italy,* ***6.*** *University of Rome Sapienza*

A new biosensor for lactate detection has been developed using an environmentally friendly approach [1,2,3,4]. The biosensor is based on lactate oxidase (LOX) and has remarkable capabilities for reuse and storage at room temperature. The manufacturing technique employed is ambient electrospray deposition (ESD), which enables efficient and sustainable immobilization of the LOX enzyme on a cost-effective commercial screen-printed Prussian blue/carbon electrode (PB/C-SPE).

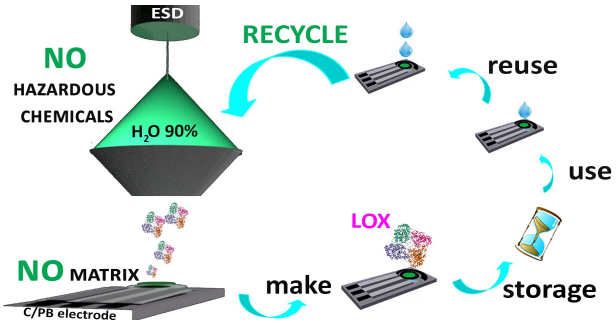
The study demonstrates that the ESD technology allows the biosensor to be stored at ambient pressure and temperature for extended periods without affecting the enzymatic activity. The biosensor can be stored for up to 90 days without requiring specific storage conditions, and it can be reused for up to 24 measurements on both freshly prepared electrodes and electrodes that are three months old.

The LOX-based biosensor exhibits a linear range of lactate detection between 0.1 and 1 mM, with a limit of detection of 0.07±0.02 mM. Additionally, it does not exhibit any memory effects. The immobilization process does not involve the use of entrapment matrices or hazardous chemicals, making it environmentally sustainable and non-toxic compared to current methods.

Furthermore, the application of a new electrospray deposition cycle on previously used biosensors rejuvenates their performance, making them comparable to freshly made biosensors. This highlights the excellent recycling potential of the technique, eliminating the waste associated with disposable devices.

References:

1. M. C. Castrovilli et al. *Green Chem. 2023,* 25, 5257–5266.
2. M. C. Castrovilli et al. *ACS Sustainable Chem. Eng. 2022*, 10, 1888–1898.
3. M.C. Castrovilli et al. *Biosens. Bioelectron. 2020*, 163, 112299.
4. A. Cartoni *Ateneo Sapienza 2021* RP12117A5D2DEE6B


ESD
NO
HAZARDOUS
CHEMICALS
H2O 90%
RECYCLE
reuse
use
LOX
storage
make
NO MATRIX
C/PB electrode

Toc new.jpg

Exploration of single-molecule protein dielectrophoresis by means of trapping and actuation

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Mrs. Janike Bolter [1], ***Mr. Jamal Soltani*** [1], ***Mr. Jacco Ton*** [2], ***Dr. Théo TRAVERS*** [2], ***Mr. Dmytro Shavlovskyi*** [1], ***Mr. Daniel Wijnperle*** [1], ***Prof. Michel Orrit*** [2], ***Dr. Sergii Pud*** [1]

1. *BIOS Lab-on-a-Chip at MESA+ Institute for Nanotechnology, University of Twente, Enschede,* ***2.*** *Huygens-Kamerlingh Onnes Laboratory, Leiden University*

Introduction

Dielectrophoresis (DEP) is an electrokinetic effect often used to trap and manipulate proteins using an AC electric field in lab-on-a-chip applications. It causes the protein to experience a force, which is aligned with the spatial gradient of the electric field and depends on the protein's shape, size, and charge properties. However, due to its complexity this force is hard to quantify and therefore most models oversimplify it, which leads to poor understanding of the interaction between the proteins and a spatially inhomogeneous electric field. We aim to gain a deeper understanding of protein DEP by building a single-molecule DEP tool to study it.

Methods

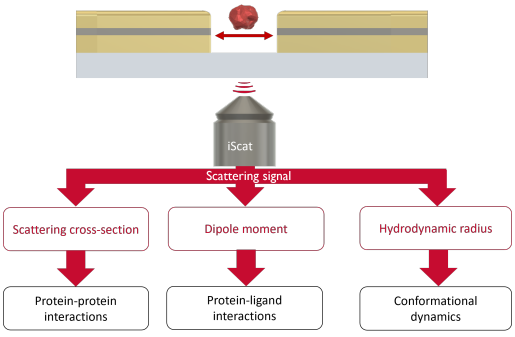
We combine interferometric scattering microscopy (iSCAT) with DEP actuation in a nano-electrode trap to investigate the behaviour of proteins in high (up to 10^7 V/cm) electric fields. By alternating the electric field strength, we want to actuate the trapped protein. This motion is dependent on the protein properties, it's environment and parameters of the AC field.

Results

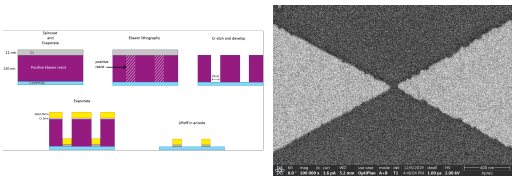
So far, we managed to build a confocal iScat microscope, which can detect trapping and show manipulation of polystyrene nano-particles down to 30 nm with a SNR of 2 at 1 ms integration time. Furthermore, we established a protocol for consistent nano-electrode production with gap-sizes of down to 20 nm.

Discussion

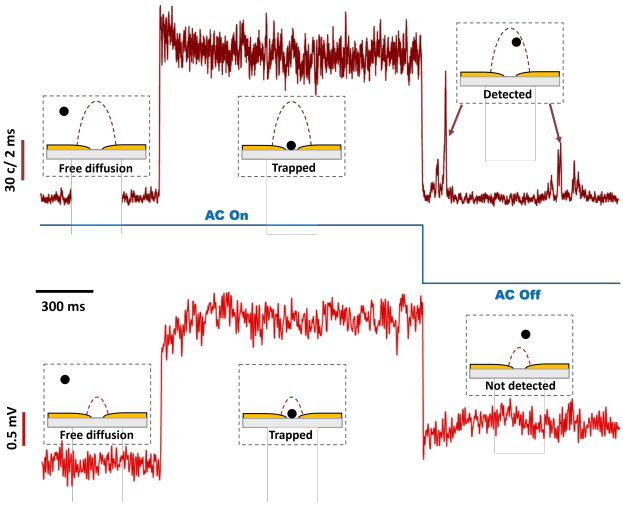
Now that our team has shown the feasibility of this technique with nano-particles, we can move on to proteins. A protein with a defined shape and constant shape is needed, for easy comparison with particles. On top of that, it needs to be labelled to compare iScat and fluorescence in our setup. A group of protein which form hollow, round and consistently sized proteins is the perfect object for this: encapsulines. They can be loaded with fluorophores but also heavier molecules, which enables calibration of our setup. Afterwards we can move onto cellular salt concentrations and a wide range of other proteins.



Proact scematic.png



Nanoelectrodefabrication.png



Timetrace trappednanoparticle fl iscat.png

Single-Molecule Footprinting of Netropsin-DNA Binding

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Dr. Isabel Pastor [1], ***Dr. Marc Rico-Pasto*** [2], ***Dr. Felix Ritort*** [3]

1. *University of Barcelona, Department of Condensed Matter Physics,* ***2.*** *nit of Biophysics and Bioengineering, Department of Biomedicine, School of Medicine and Health Sciences, University of Barcelona,* ***3.*** *Small Biosystems Lab, Departament de Física de la Materia Condensada, Facultat de Física, Universitat de Barcelona, 08028 Barcelona, Spain*

How small drugs interact with DNA molecules is a hot topic in biophysics, with biomedicine and drug delivery implications. In general, ligands tend to bind DNA at specific sequences motifs; however, non-specific binds with reduced affinity to other motifs also occur, making ligand-DNA binding an exciting question to address. While bulk methods offer a generic binding map to different motifs, single-molecule techniques [1], and their analytical tools can provide a far more accurate picture of the binding energy landscape.

Here, we studied netropsin, a small minor groove binder with antiviral and antibiotic properties that binds dsDNA in AT-rich regions, preferably to a 5'-AATT-3' motif. We investigated the interaction of netropsin with different DNA hairpins. We used an optical tweezers setup to investigate the netropsin-DNA binding under mechanical unzipping experiments. By classifying the unfolding trajectories recorded from non-equilibrium experiments, we derived the association and dissociation rates, as well as the Gibbs binding energies, all in agreement with those estimated from bulk assays. Furthermore, we extended the Continous Effective Barrier Approach [2-3] to derive the binding motif configuration. Here, we considered that each base pair included in the binding motif has an extra energy. Then, we fitted the experimental unfolding kinetic rates for those trajectories with netropsin bound to the effective kinetic barrier considering the Kramers solution to evaluate the DNA-single-netropsin binding energy. We found that netropsin only extra-stabilizes two of the four bases in the binding site.

[1] Manosas, M., Camunas-Soler, J., Croquette, V., and Ritort, F. (2017). Nature Communications, 8, 304

[2] Alemany, A., Ritort, F. (2017) J. Phys. Letters, 8(5), 895–900.

[3] Rico-Pasto, M., Alemany, A., Ritort, F. (2022) J. Phys. Letters, 13, 1025–1032

Investigating the velocity and diffusion of helicase gp41 using magnetic tweezers: A theoretical approach

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Mr. Victor Rodríguez Franco [1], ***Dr. Maria Manosas*** [1], ***Dr. Felix Ritort*** [1]

1. *Small Biosystems Lab, Departament de Física de la Materia Condensada, Facultat de Física, Universitat de Barcelona, 08028 Barcelona, Spain*

Helicases are molecular motors that play a crucial role in DNA replication and repair by unwinding double-stranded DNA. They convert the energy of ATP hydrolysis in directed motion and work, promoting the DNA unwinding reaction. Magnetic tweezers allow to manipulate DNA molecules and follow the helicase motion on the DNA in real time and at the single-molecule level by tracking the changes in the DNA extension. With these assays, one can measure the motor's velocity and diffusivity. Changing the force and the ATP concentration is a way to test the mecano-chemical cycle of the motor. While the relationship between helicase velocity and the force or ATP concentration has been extensively studied, the dependence of the diffusion coefficient with these parameters remains unknown.

In this study, we experimentally investigate the behavior of the gp41 helicase using magnetic tweezers under different ATP concentrations and forces. By analyzing the motor's velocity and diffusion coefficient, we aim to gain insights into its mechanisms and develop a theoretical model that can reproduce these experimental results. We propose a theoretical model based on the random walk theory, where a walker can either move forward, backward or remain in the same position with probabilities P_+, P_- and P_0, with characteristic waiting times exponentially distributed with constants and respectively. This model is expected to provide a simple framework to describe the mechanisms of molecular motors from the analysis of the velocity and diffusion coefficient under different force and ATP conditions .

NON-POROUS COORDINATION POLYMERS ACTING AS POROUS AS A VERSATILE PLATFORM FOR REVERSIBLE OPTO-ELECTRONIC READ-OUTS DISPLAYS

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

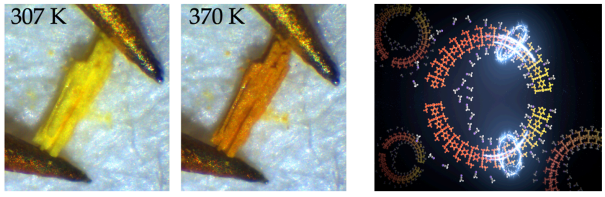
Dr. Jose Sanchez Costa [1]

1. *IMDEA Nanociencia*

The efficient and cost-effective detection of harmful volatile organic compounds (VOCs) is a major health and environmental concern in industrialised societies. To this end, tailor-made porous coordination polymers are emerging as promising molecular sensing materials due to their responsivity to a wide range of external stimuli. Non-porous coordination polymers (npCPs) capable to accommodate molecules through internal lattice reorganization[1,2] are uncommon materials with applications in sensing and selective gas adsorption.[3]
In this presentation, a non-porous crystalline Fe(II) coordination polymer with remarkable sensing activity is presented.[4] The desorption of interstitial host molecules are accompanied by magneto-structural transitions easily detectable in the optical and electronic properties of the material. This structural change, and therefore its (opto)electronic readout, is reversible upon exposure to the source vapors. In addition, the *insitu* exchange of these weakly bound starting hosting molecules by others, results in a different optical response.[5] Therefore, the color and conduction properties are determined by the weakly bonded molecules in the lattice.
These findings open the door to a novel concept of non-porous switchable protonic conductors and capacitive sensors that operate at low humidity levels and with selectivity for different molecules. These materials can therefore provide a versatile platform for the fabrication of tailor-made detectors with a variety of readout options from optical, magnetic to electron transport measurements.

References

[1] E. Coronado, M. Giménez-Marqués, G. M. Espallargas, L. Brammer, *Nat. Commun.* **2012**, 3, 828.
[2] J. Sánchez Costa, S. Rodríguez-Jiménez, G. A. Craig, B. Barth, C. M. Beavers, S. J. Teat, G. Aromí, *J. Am. Chem. Soc.* **2014**, *136*, 3869.
[3] S. Rodríguez-Jiménez, H. L. Feltham, S. Brooker, *Angew. Chemie - Int. Ed.* **2016**, *55*, 15067.
[4] E. Resines-Urien, E. Burzurí, E. Fernandez-Bartolome, M.A. García García-Tuñón, P. de la Presa, R. Poloni, S. J. Teat and J. Sanchez Costa, *Chem. Sci.*, **2019**,10, 6612-6616.
[5] A. Develioglu, E. Resines-Urien, R. Poloni, L. Martín-Pérez, J. Sanchez Costa and E. Burzurí, *Adv. Sci.* **2021**, 2102619.



Captura de pantalla 2023-09-16 a las 20.33.38.png

Electric-field driven microfluidic devices for affinity-based protein separation

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Mr. Alexandre S. Avaro [1], ***Prof. Andrew D. Griffiths*** [1], ***Prof. Juan G. Santiago*** [2]

1. *Laboratoire de Biochimie, ESPCI Paris, Université PSL, CNRS UMR 8231, Paris, France.,* ***2.*** *Department of Mechanical Engineering, Stanford University*

Single-domain antibodies, often called nanobodies, are increasingly popular in diagnostics and therapeutics applications due to their high binding affinity, specificity, and small size. There has been considerable work on the directed evolution of such proteins, which include library preparation, in-vitro expression, and selection. However, selection protocols suffer from low reaction rates, and are not sufficiently selective to the strength of protein-to-protein interactions.

To address this issue, we are developing a rapid on-chip device for selection and separation of nanobodies based on their binding affinity to specific target proteins. We use isotachophoresis (ITP), which is an electrophoretic technique that selectively preconcentrates, separates and purifies ionic samples using a leading (LE) and a trailing electrolyte (TE). Sample species focus if their mobility magnitude is higher than the TE's co-ion and lower than the LE's co-ion. The use of ITP for improving immunoassays remains largely unexplored, partly due to the higher complexity of the manipulation of proteins on chip.

Mutant nanobodies are covalently bound to their corresponding gene and undergo ITP in a custom microfluidic chip. We are developing a method whereby the applied electric field and ITP drive nanobody-DNA complexes to migrate and focus at a sharp peak at in a concentrated ITP zone. This peak of high concentration progresses through a matrix of immobilized target protein, such that high-affinity nanobodies bind to the matrix. The goal is to create the physical separation of high- and low-affinity nanobodies. High-affinity nanobodies can then be collected back from the microfluidic chip, without the need for many manual washing and elution steps. We perform a model selection using the green fluorescent protein (GFP) as a model target, and we show that the ITP-based selection enables to retrieve nanobody-gene constructs for high-affinity binders. ITP-based selection has potential to achieve fast selection of high-affinity binders, and may allow for the tuning of the selection and reduction of the number of manual steps by automating the selection process on-chip.

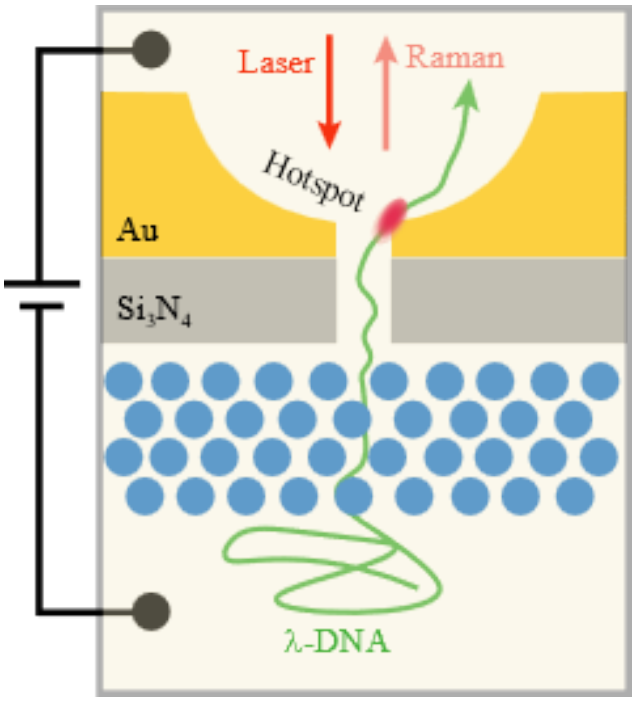
Plasmonic bowl-shaped nanopore using Raman Spectroscopy for sequencing of translocating DNA

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Dr. Sudarson Sinha [1], ***Prof. Francesco DeAngelis*** [2], ***Dr. Yingqi Zhao*** [3], ***Dr. Aliaksandr Hubarevich*** [1], ***Dr. Marzia Iarossi*** [1]

1. *Istituto Italiano di Tecnologia, Via Morego 30, 16163, Genova, Italy,* ***2.*** *Italian Institute of Technology,* ***3.*** *Faculty of Medicine, Faculty of Biochemistry and Molecular Medicine, University of Oulu, Aapistie 5 A, 90220 Oulu, Finland*

Despite having challenges to work with a single molecule, it is now well-recognized to perform an experiment close to a single molecular regime. However, the present techniques are based on fluorophore tagging. Out of various label free detection, Raman spectroscopy is an emerging method due to its selective signals in fingerprint regime. Plasmonic nanopores are on the other hand, useful to control the spatial confinements in single molecule regime. A nice composition of these two methodologies could be useful for the achievements of the detection and sequencing of DNA and proteins in a single molecular regime. The ability of recognizing the sequence of DNA in single nucleotide regime or sequence of proteins in single amino acid level have been demonstrated. In these cases, a single polymeric unit is absorbed on the plasmonic surface and delivered through plasmonic pores. Here, we demonstrated on bowl-shaped gold nanopores capable of direct Raman detection of single λ-DNA molecules in flow through pore. The bowl shape allows two advantages in these experiments. The foremost optical effect enables to focus the incident laser into the nanopore to generate a single hot spot with no cut off in pore size. The second one is the trapping of the particle due to its shape. The curved surface allows the electrical trapping of the molecule efficiently and guides the unidirectional movements. Therefore, we achieved ultrasmall focusing of NIR light in a spot of 3 nm that enables to detect 7 consecutive bases along the DNA chain during the translocation. Furthermore, we found a novel electro-fluidic mechanism to manipulate the molecular trajectory within the pore volume so that the molecule is pushed toward the hot spot thus improving the detection efficiency.



Sudarson abstract.png

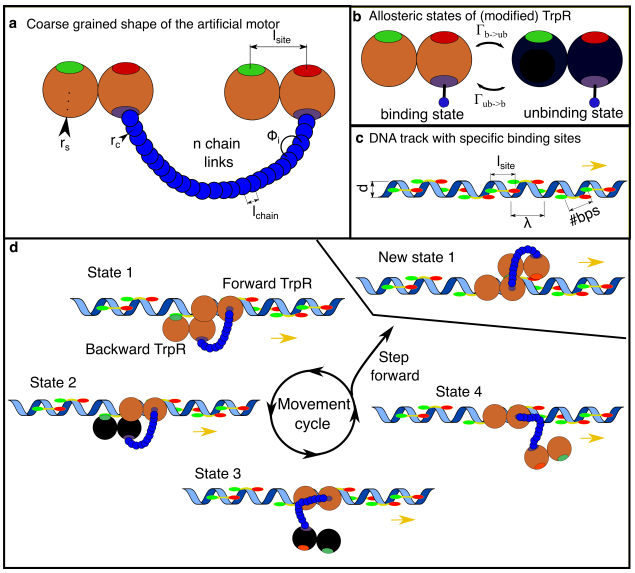
Understanding an artificial motor proteins using Langevin simulations

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Dr. Michael Konopik [1], ***Mr. Olivier Laprévote*** [2], ***Prof. Paul Curmi*** [3], ***Prof. Birte Höcker*** [2], ***Prof. Heiner Linke*** [1], ***Prof. Ralf Eichhorn*** [4]

1. *Lund University,* ***2.*** *Universität Bayreuth,* ***3.*** *UNSW Sydney,* ***4.*** *Nordita*

Motor proteins are essential for the existence of cellular life. They help to overcome the diffusive nature of the nanoscale world and facilitate directed movements.
Famous cases are the myosin or kinesin motor families, responsible for transport processes.
While the results of protein motor stepping can generally be experimentally observed, it is difficult to understand what the actual microscopic behavior patterns are that result in these steps, due to having to resolve the Å-nm structures in time steps of ns or ps. It is expected that diffusion is an integral part of the movement of these motors.
One way to help understand the details of e.g. a step of a myosin V motor protein is to simulate the movement with a coarse grained model taking into account its structure and the effects of the thermal environment [1].
In our study, we analyse a concept for an artificial motor [2] (fig. 1) of two (modified) tryptophan repressor (TrpR) proteins being linked together with a glycine-serine chain, walking along a specific DNA track. This motor concept utilizes its inherent asymmetry by exploiting a chemical cycle akin to ATP-ADP to only allow the 'back' positioned TrpR to unbind, which then can bind in front of the still bound TrpR, reversing their roles and thus facilitating processive, autonomous movement.
We use (overdamped) Langevin dynamics describing translational and rotational degrees of freedom [1,3] of these motors. We further model the motor by employing a coarse grained approach not from first principles but using plausible models for the involved parts.
We analyze what makes the motor perform the autonomous processive movement, which constraints are needed on the motor parameters to allow for moving, and the efficiency with which it can move.
This coarse grained analysis allows us to both understand better the fundamental movement of biological motors, but also help in the direct design process of the actual artificial motors.
Citations:
[1] Craig, Linke: PNAS 106, 43 (2009)
[2] Linke et al., Biophysical Reviews volume 12, pages 1041–1054 (2020)
[3] Naess, Elgsaeter, Macromol. Theory Simul. 2004, 13 (2004)



Cycle visualization.png

The BIODIVERSI project for the discovery of biomarkers

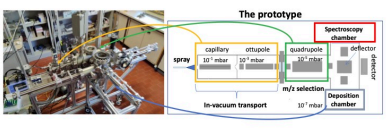
Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Dr. Anna Rita Casavola [1], Dr. Donatella Schiumarini [1], Dr. Jacopo Chiarinelli [1], Dr. Francesco Porcelli [1], Dr. Alessandro Grottesi [2], Dr. Paola Bolognesi [1], Dr. Antonella Cartoni [3], Dr. Lorenzo Avaldi [1]

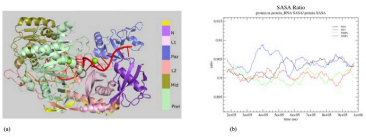
1. *a) CNR-Istituto di Struttura della Materia, Area della Ricerca di Roma 1, CP 10 Monterotondo Scalo, Italy,* ***2.*** *b) Cineca, Via dei Tizii, 6, Rome, Italy,* ***3.*** *University of Rome Sapienza*

The BIODIVERSI project aims to develop a new technology for the discovery and characterization of biomarkers in their native form. The project focuses on miRNAs, non-coding single-stranded RNA sequences, which negatively regulate gene expression. Changes in their expression are monitors of several pathologies.
In the project a set-up based on ElectroSpray Ionization mass spectrometry was implemented (fig.1); the instrument consists of i) an ESI source, ii) a section where the analyte is transferred in vacuum and the solvent evaporation occurs, iii) a quadrupole filter where the m/z selection is performed, iv) a diagnostic chamber and the deflector towards a deposition chamber.
The mass spectrometric measurements [1,2] performed were supported by theoretical calculation (molecular dynamics simulations [3]) on conformational dynamics, protein-ligand interactions, solvent effects of the miRNA strands as isolated structures and as complex in the minimal RISC configuration. The theoretical calculation, performed with Gromacs software, consists in distinct MD trajectories in NPT (P = 1 atm, T = 300 K) ensemble, for a sampling time of 1 μs each, in pure water and water/methanol (70:30 molar ratio). The Force Field used is Amber99sb-ilsdn and TIP3P model is employed to define water interactions; for mixed solvent simulation, GAFF topology for methanol was generated using acpype python tool.
The Ago2 protein consists of 859 amino acids forming 4 globular domains and 2 linkers (fig.2a). The system is connected through a dense network of hydrogen bonds: the strength of the connection between the different units determines the stability of the system, the external exposure of the ligands, influencing the movements of the globular domains and the ligand stress. Based on our analyses, for the time interval considered, the complex retains its cohesion (see fig.2b). The water-methanol system shows greater fluctuations and more widespread minima in the Gibbs map.
The MS spectrum of isolated miRNA strand is shown in fig. 3 (right panel), side by side to the theoretical MD structure of the miRNA that shows the folding of the system. In this case, the peaks with high mass/charge ratio result as the dominant ones.

1Annu.Rev.Biophys.2022.51:63–77
2Chem.Rev.2022,122,7720-7839
3JCellBiochem,2019,120,19915–19924



Casavola fig.1.jpg



Casavola fig.2.jpg

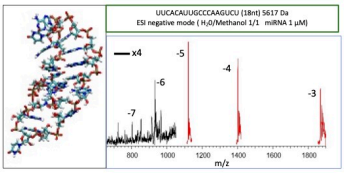


Fig.3 miRNA structure from PDB entry (4w5q) and its ESI mass spectra in negative mode in a large m/z range.

Casavola fig.3.jpg

Multiplexing nanobody kinetics measurements at the single-molecule level

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Ms. Ghada Mansour [1], ***Dr. Sebastian Hutchinson*** [2], ***Ms. Ellyn Redheuil*** [1], ***Mr. Ahmed Rehan*** [1], ***Dr. Adeline Pichard-Kostuch*** [2], ***Dr. Marco Ribezzi-Crivellari*** [2], ***Prof. Andrew D. Griffiths*** [1]

1. *Laboratoire de Biochimie, ESPCI Paris, Université PSL, CNRS UMR 8231, Paris, France.*, ***2.*** *Quantum-Si, France*

Single-molecule methods provide useful insights into the intermolecular variations and functional differences of individual molecules and have revealed much about the complexity of biological processes. Today it is possible to monitor binding kinetics at the single molecule level in a highly parallel fashion using zero-mode waveguide (ZMW) arrays. This allows monitoring the binding/unbinding of fluorescently-labelled molecules on millions of immobilised targets simultaneously, obtaining a full kinetic description of their interactions, offering a complementary picture to classical techniques but also uncovering important details that are missed in bulk studies. In our work, we use ZMW arrays to study the binding kinetics of antibody-antigen interactions at the single-molecule level. Moreover, we demonstrate that it is possible to couple these binding kinetics measurements with single-molecule protein sequencing to multiplex kinetic analysis to panels of nanobodies. This new approach to the study of nanobodies will help us understand the sequence/function relationship in nanobodies and open new directions in nanobody affinity maturation.

Semi-Empirical Haken-Strobl Model for Molecular Spin Qubits

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Ms. Katy Aruachan [1], ***Prof. Yamil Colon*** [2], ***Prof. Daniel Aravena*** [1], ***Prof. Felipe Herrera*** [1]

1. *Universidad de Santiago de Chile,* ***2.*** *University of Notre Dame*

Understanding the mechanisms that determine relaxation times of molecular spin qubits is essential for applications in precision measurements and quantum information processing [1]. Recent spin-echo experiments on the spin relaxation times of molecular spin qubits as a function of magnetic field and temperature have stimulated the development of phenomenological and ab-initio quantum mechanical modeling techniques [1-3]. We propose an alternative semi-empirical approach for building Redfield quantum master equations using a stochastic Haken-Strobl model with fluctuating molecular gyromagnetic tensors and local magnetic fields, parametrizing the corresponding bath spectral densities using only a small number of T_1 relaxation measurements [4]. Taking a vanadium-based spin qubit as a case study, we compute relaxation (T_1) and decoherence (T_2) timescales, extrapolating over a broad range of temperatures and magnetic fields beyond the experimental conditions used to parametrized the model. The theoretical predictions agree quantitatively with experiments [3] and represent a solid foundation for the theoretical characterization of other spin qubits, which could be beneficial for designing novel molecule-based quantum magnetometers.

References:

[1] T. Yamabayashi, M. Atzori, L. Tesi, G. Cosquer, F. Santanni, M.-E. Boulon, E. Morra, S. Benci, R. Torre, M. Chiesa, L. Sorace, R. Sessoli, M. Yamashita, *Scaling Up Electronic Spin Qubits into a Three-Dimensional Metal–Organic Framework*, J. Am. Chem. Soc., 140, 38, 12090–12101, (2018).

[2] A. Lunghi, and S. Sanvito, *How do phonons relax molecular spins?* Sci. Adv.5,eaax7163 (2019).

[3] L. Tesi, A. Lunghi, M. Atzori, E. Lucaccini, L. Sorace, F. Totti, R. Sessoli, *Giant spin-phonon bottleneck effects in evaporable vanadyl-based molecules with long spin coherence*, Dalton Trans., 45, 16635-16643 (2016).

[4] K. Aruachan, Y. Colón, D. Aravena, F. Herrera, *Semi-empirical Haken–Strobl model for molecular spin qubits*, New J. Phys. 25 093031 (2023)

Two methods for studying the dynamics of the bioluminescent enzyme: NanoLuc

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Ms. Alice Attenborough [1], ***Prof. Frank Vollmer*** [1], ***Dr. Daniel Kattnig*** [1]

1. *University of Exeter*

Bioluminescence is the light emission from an enzyme-catalysed reaction in a wide variety of living organisms and is used in many different biological studies. Whispering gallery mode (WGM) sensing involves the resonance of light at a particular wavelength in a cavity, this wavelength changes when a molecule interacts with the evanescent field of the cavity. WGM sensing may gain new insights into the dynamics of the luciferase, NanoLuc. The dynamics of NanoLuc can also be measured by detecting the bioluminescence directly. To compare the methods, NanoLuc was attached to the tip of an optical fibre, and the bioluminescence was detected with a single photon avalanche diode (SPAD). Plasmonic gold nanorods, which enhance the WGM signal, were also attached to the tip of an optical fibre. Nanoluc was attached to the gold nanorods, and the bioluminescence could be detected.

Acousto-optical ultra-low concentration nanoparticle detection

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Dr. Robert Stárek [1], ***Prof. Miroslav Jezek*** [1]

1. *Palacký University Olomouc*

Introduction:
The advances in nanotechnology and life sciences bring a demand for nanoparticle detection. Optical scattering methods provide a convenient way to detect and characterize nanoparticles. Detecting nanoparticles diluted at concentrations lower than a few ng/ml in macroscopic volumes represents an open problem. Inspired by a lock-detection scheme, we propose an acoustically modulated dynamic light scattering modality and demonstrate its performance in nanoparticle detection.

Methods:
We utilize a dynamic light scattering apparatus, depicted in Figure A, operating at 810 nm with constant 0.3 mW optical power. We added an ultrasonic transducer to the cuvette holder to introduce an ultrasonic oscillatory motion to nanoparticles highly diluted in a macroscopic liquid sample. Fourier analysis is used to locate a distinct peak at the frequency of the transducer. Figure B shows such a peak for nanoparticles (orange) and pure solvent (blue). As a sample, we used polystyrene nanoparticles of 400 nm diameter diluted in ultra-pure water and contained in cleaned cuvettes. We measured the height of the spectral peak expressed as multiples of the spectral baseline standard deviation as the function of concentration. We performed this analysis also for a control group of clean solvents and compared the peak heights.

Results:
Figure C compares the peak heights produced by the diluted nanoparticle solution (orange) with those obtained by pure solvent (blue). We see that a difference is still visible down to a concentration of 100 picograms per milliliter. In contrast, the detection based on count rate, calculated from the same time trace depicted in Figure D (same color coding), requires approximately an order of magnitude higher concentration.

Discussion:
The results indicate an order of magnitude increase in sensitivity when compared to the scattered intensity-based detection. Due to the non-specificity of the method, we were limited by the cleanliness of our workspace. We believe that the advantage could be improved. Moreover, the choice of a shorter wavelength, higher optical power, and longer integration times could push the detection limit even more. Advantageously, the method is not limited to magnetic or fluorescent nanoparticles. Another advantage is its robustness to stray light and laser fluctuations.

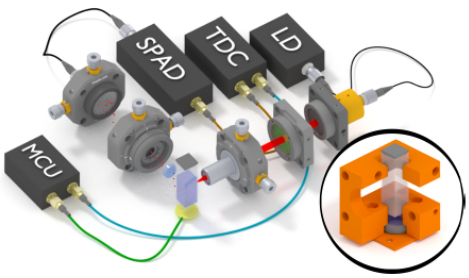


Figure a.png

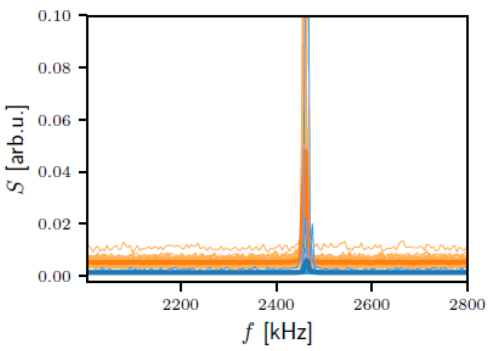


Figure b.png

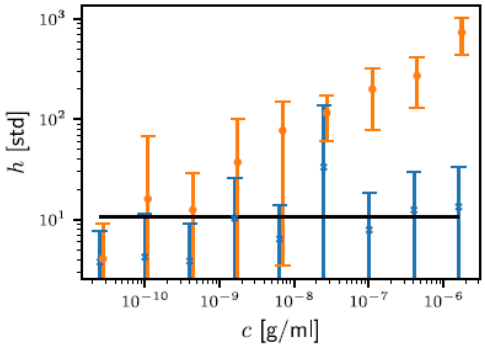


Figure c.png

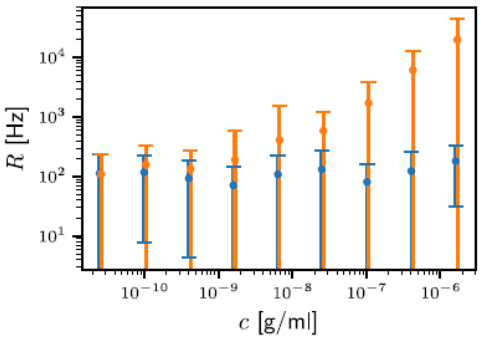


Figure d.png

Sensing of single emitters enabled by silver nanostructures

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Dr. Marcin Szalkowski [1], ***Ms. Karolina Sulowska*** [1], ***Mr. Michał Mac*** [1], ***Prof. Joanna Niedziółka-Jönsson*** [2], ***Prof. Dorota Kowalska*** [1], ***Prof. Dawid Piątkowski*** [1], ***Prof. Sebastian Maćkowski*** [1]

1. *Nicolaus Copernicus University in Toruń,* ***2.*** *Institute of Physical Chemistry, PAS*

Specific sensing of highly diluted analytes, preferably down to the level of single molecules, is of high interest for nowadays medicine, biochemistry and biotechnology. Between the other methods, fluorescence microscopy is recognized as one of the best tools for such purpose due to fast and high sensitivity detection applicable also for *in vivo* real time measurements and possibility to distinguish the analytes based on their spectroscopic properties. However, it still remains a challenge and access to such techniques is limited, as highly sensitive and thus expensive detection systems are required. One of the remedies for such issue is the enhancement of the emission intensity provided by utilization of plasmonically active nanostructures, resulting in improved signal to noise ratio, which can be also further improved by utilization of the functionalized surfaces decorated with the chemical groups of high affinity to the analytes.

We present studies of the two types of silver nanostructures – wet chemistry synthesized silver nanowires and the photochemically printed arbitrary shaped silver nanopatterns. Both of them feature the plasmon activity in the visible range enabling enhancement of the emission intensity of the fluorophores (Fig. 1) [1-3]. This nanoscale effect, combined with microscale size of these structures, enables effortless detection of the luminescent analytes. Furthermore, their surface can be chemically modified to specifically bond desired analytes, providing simultaneously the distance between the analytes and silver surface preventing against the parasitic effect of emission quenching. We demonstrate real time observation of the sensing of the biomolecules by chemically functionalized silver nanostructures [3], including also the detecting of the presence of single molecules [4].

Research was supported by the project 2021/41/N/ST7/03528 funded by the National Science Centre of Poland, as well as by “Excellence Initiative - Research University” of the NCU in Toruń under the “Debuts” program (No. 4101.00000066).

References:

1. Szalkowski M., et al. *Int. J. Mol. Sci.* **2020**, *21*, 2006.
2. Kowalska D., et al. *Sci. World J.* **2013**, *2013*.
3. Szalkowski M.et al. *Sensors* **2018**, *18*.
4. Sulowska K.et al. *Methods Appl. Fluoresc.* **2020**, *8*, 045004.

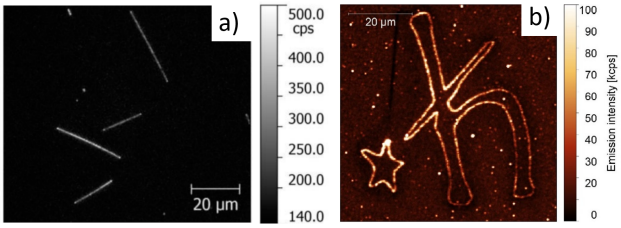


Figure 1. demonstration of plasmon enhanced sensing of a photosynthetic proteins with silver nanowires and b upconverting nanocrystals with arbitrarily shaped laser printed silver nanopatterns.png

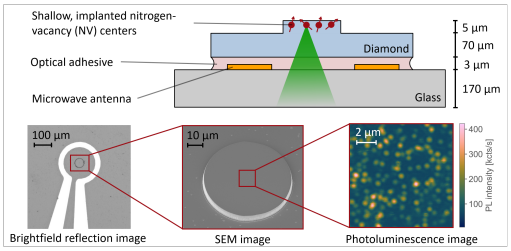
Planar scanning probes – A new platform for nanoscale magnetometry with NV centers and near-field microscopy

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

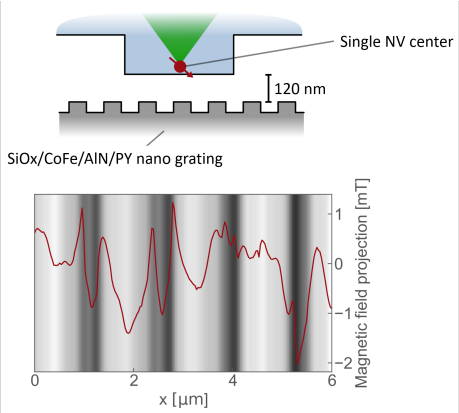
Mr. Paul Weinbrenner [1], ***Ms. Patricia Quellmalz*** [2], ***Dr. Christian Giese*** [2], ***Ms. Monika Scheufele*** [3], ***Mr. Manuel Müller*** [3], ***Dr. Matthias Althammer*** [3], ***Dr. Stephan Geprägs*** [3], ***Prof. Rudolf Gross*** [3], ***Prof. Friedemann Reinhard*** [1]

1. *University of Rostock,* ***2.*** *Fraunhofer Institute for Applied Solid State Physics IAF,* ***3.*** *Walther-Meißner-Institute*

We present the application of a new scanning probe technique to magnetometry with nitrogen-vacancy (NV) centers in diamond. Instead of using sharp tips, we use flat mesas (shallow pillars) with a lateral size of 50 µm and a height of up to 5 µm. Due to their geometry, these so-called planar scanning probes offer a unique advantage for nanoscale magnetometry and novel optical near-field sensors.
Despite their large lateral size, they can still be scanned at a standoff distance of several nanometers if the probe and sample are parallel to each other. To achieve this alignment, we use a tilt measurement technique based on interference reflection microscopy. The distance between the probe and sample can be determined optically with nanometer precision, by using techniques from total internal reflection microscopy.
We fabricate planar diamond probes and use NV center quantum sensors for magnetic field measurements. The lateral size of the planar probes enables highly parallel scanning probe magnetometry. Additionally, the fabrication is less complex compared to conventional diamond tips.
We use such a planar probe, that contains single NV centers with different orientations to image different components of the stray field of magnetic nanostructures.
With this new approach to scanning probe measurements, we propose the emergence of new material systems as sensors for nanoscale imaging. These next-generation sensors include plasmonic nanostructures, localized defects in 2D materials and single dye molecules, encapsulated in hBN.



Diamond planar scanning probe schematic.png



Nanograting magnetic field measurement.png

Hypertension alters the function and expression profile of the peptide cotransporters PEPT1 and PEPT2 in the rodent renal proximal tubule

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Dr. Othman Alghamdi [1]

1. *University of Jeddah, College of Science, Department of Biological Sciences*

Introduction: Hypertension is a major risk factor for kidney and cardiovascular disease. The treatment of hypertensive individuals by selected ACE inhibitors and certain di-and tripeptides halts the progression of renal deterioration and extends life-span. Renal reabsorption of these low molecular weight substrates are mediated by the PEPT1 and PEPT2 cotransporters. **Aim**: This study aims to investigate whether hypertension and ageing affects renal PEPT cotransporters at gene, protein expression and distribution as well as function in the superficial cortex and the outer medulla of the kidney. **Methods**: Membrane vesicles from the brush border (BBMV) and outer medulla (OMMV) were isolated from the kidneys of young Wistar Kyoto (Y-WKY), young spontaneously hypertensive (Y-SHR), and middle aged SHR (M-SHR) rats. Transport activity was measured using the substrate, β-Ala-Lys (AMCA). Gene expression levels of PEPT genes were assessed with qRT-PCR while renal localisation of PEPT cotransporters was examined by immunohistochemistry with Western Blot validation. **Results**: The Km and Vmax of renal PEPT1 were decreased significantly in SHR compared to WKY BBMV, whilst the Vmax of PEPT2 showed differences between SHR and WKY. By contrast to the reported cortical distribution of PEPT1, PEPT1-staining was detected in the outer medulla, whilst PEPT2 was expressed primarily in the cortex of all SHR; PEPT1 was significantly upregulated in the cortex of Y-SHR. **Conclusion**: These outcomes are indicative of a redistribution of PEPT1 and PEPT2 in the kidney proximal tubule under hypertensive conditions that has potential repercussions for nutrient handling and the therapeutic use of ACE inhibitors in hypertensive individuals.

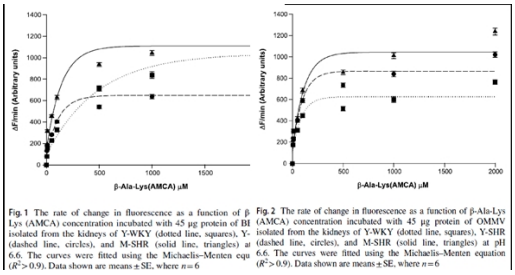


Fig. 1 The rate of change in fluorescence as a function of β Lys (AMCA) concentration incubated with 45 μg protein of BI isolated from the kidneys of Y-WKY (dotted line, squares), Y- (dashed line, circles), and M-SHR (solid line, triangles) at 6.6. The curves were fitted using the Michaelis–Menten equ ($R^2 > 0.9$). Data shown are means ± SE, where $n = 6$

Fig. 2 The rate of change in fluorescence as a function of β-Ala-Lys (AMCA) concentration incubated with 45 μg protein of OMMV isolated from the kidneys of Y-WKY (dotted line, squares), Y-SHR (dashed line, circles), and M-SHR (solid line, triangles) at pH 6.6. The curves were fitted using the Michaelis–Menten equation ($R^2 > 0.9$). Data shown are means ± SE, where $n = 6$

1.jpg

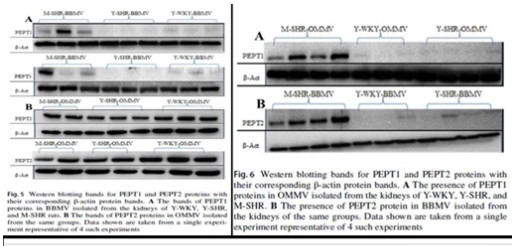


Fig. 5 Western blotting bands for PEPT1 and PEPT2 proteins with their corresponding β-actin protein bands. **A** The bands of PEPT1 proteins in BBMV isolated from the kidneys of Y-WKY, Y-SHR, and M-SHR rats. **B** The bands of PEPT2 proteins in OMMV isolated from the same groups. Data shown are taken from a single experiment representative of 4 such experiments

Fig. 6 Western blotting bands for PEPT1 and PEPT2 proteins with their corresponding β-actin protein bands. **A** The presence of PEPT1 proteins in OMMV isolated from the kidneys of Y-WKY, Y-SHR, and M-SHR. **B** The presence of PEPT2 protein in BBMV isolated from the kidneys of the same groups. Data shown are taken from a single experiment representative of 4 such experiments

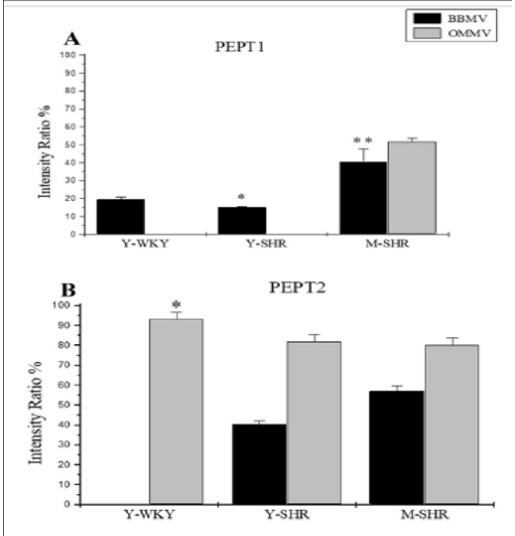
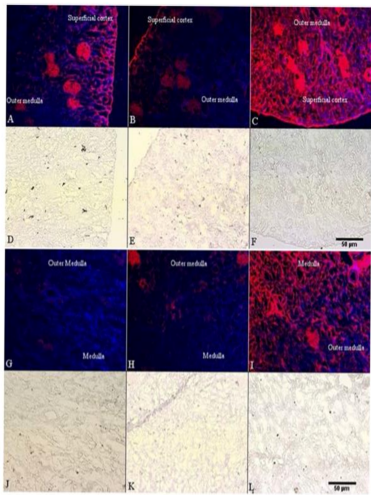


Fig. 7 Bar graphs comparing the relative level of PEPT1 (**A**) and PEPT2 (**B**) protein expression in BBMV and OMMV isolated from Y-WKY, Y-SHR and M-SHR. Results are expressed as the intensity ratio (%) with respect to β-actin. **A** $*p = 0.02$ vs. Y-WKY; $**p < 0.05$ vs. Y-WKY and Y-SHR. **B** $p < 0.05$ vs. Y-SHR and M-SHR. Data shown are means ± SE, where $n = 4–6$

2.jpg

Fig. 3 Fluorescent images of kidney cross sections showing immunolocalisation of PEPT1 in the cortex of Y-WKY (**A**), Y-SHR (**B**) and M-SHR (**C**) kidneys by the red immuno-fluorescent staining TSA-CY 3.5 amplification kit and anti-PEPT1 mouse monoclonal antibody. PEPT1 was also present in the outer medulla and medulla of M-SHR (**C** and **I**) and with low presence in Y-SHR (**H**), but not in Y-WKY kidneys (**G**). DAPI staining DNA (blue) was merged with the specifc-PEPT1 staining. Brightfield images were also acquired for the same sections (**D**–**F** and **J**–**L**). Data shown are taken from a single experiment representative of 4 such experiments



3.png

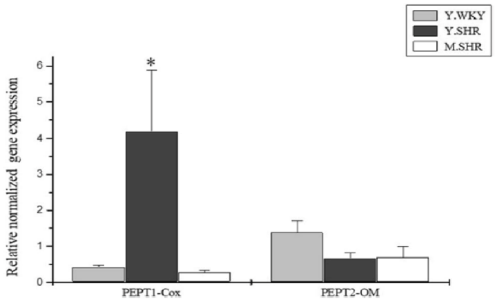


Fig. 8 Relative normalised expression of PEPT1 and PEPT2 genes (against two housekeeping genes SDHA, and β-Actin) was measured by QRT-PCR and ΔΔ Cq method using Bio-Rad CFX manager 3.1. These genes are measured in the Cortex (Cox) and Outer medulla (OM) of young adult WKY, young adult SHR, and middle-aged SHR rat kidneys, as colours shown in the graph. $*p < 0.05$ vs Y-WKY and M-SHR. Data shown are means ± SE, where $n = 5$

4.png

Single-molecule detection, analysis, and manipulation with advanced modes of atomic force microscopy

Wednesday, 22nd November - 13:30: (Poster Aera) - Poster

Mr. Jan Přibyl [1], ***Mrs. Radka Obořilová*** [1], ***Dr. Jarmila Mlčoušková*** [2]

1. *CEITEC MU, Masaryk University,* ***2.*** *Faculty of Medicine, Masaryk University*

Introduction

Sensors as analytical units are often combined with biomolecules as sensitive detection elements; we are talking about so-called biosensors. Signal transducers are electrodes, photometric, fluorescent, optical, or acoustic devices. The atomic force microscope (AFM) was then used to image the structure or directly the processes on the surface of biosensor interfaces.

Over the years, the AFM technique has also improved, moving from a pure microscope to a device that actually contains nanosensors, providing much more information than simple topography, although it still provides valuable information. The sensitive cantilever of this instrument then allows single molecules to be monitored by imaging, where resolution has been significantly improved by new measurement modes (QI, PFQNM, etc.).

Single-Molecule Force Spectroscopy (SMFS) is a special mode that uses a cantilever for a tractable nanosensor to measure the intramolecular forces associated with the unfolding and folding molecular structures. The mentioned cantilever can then be used to destructively study synthetic membranes (phospholipid bilayers, SLBs) when higher forces are applied, and the breakdown occurs, which can be observed on the measured curves, and the thickness and quality of these layers can be determined from the specific patterns.

Force microscope is well combined with other techniques, which are often other types of microscopy. An interesting combination is the combination of microfluidics inside the cantilever. This combined technique is then called FluidFM. It finds its application, for example, in pipetting very small volumes that can be sensitively delivered (or removed) into a single cell.

These examples of the use of AFM in analysing single molecules, molecular forces, or combining this microscopic technique with microfluidics will be demonstrated on selected examples studied in our laboratory.

Results

Single DNA molecule and the structure of DNA origami (complex of tens of DNA chains) are shown in Figure 1. The force-distance curve recorded while unfolding a single collagen fibre is presented in Figure 2. Finally, Figure 3 visualizes the AFM probe penetration through the phospholipidic bilayer – the experiment scheme and typical curves recorded during the SLB membrane can be found here.

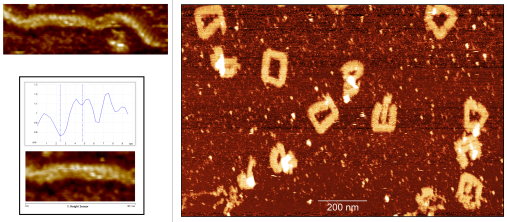


Figure1.png

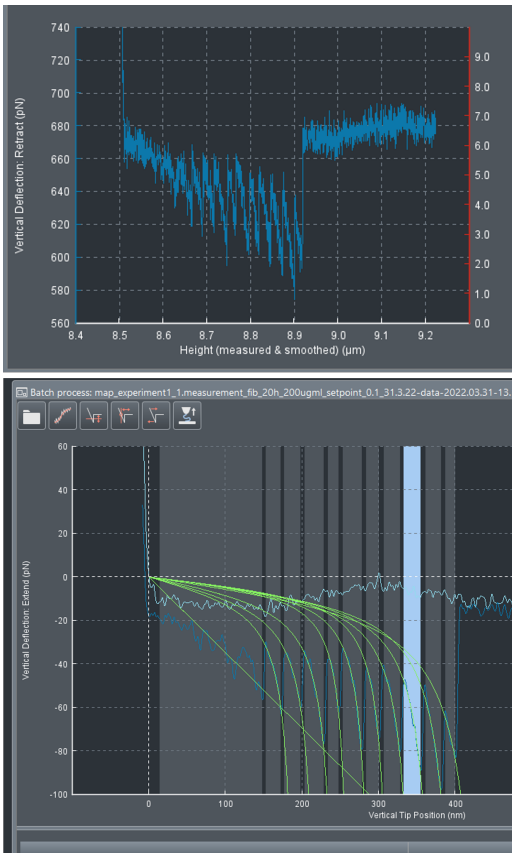


Figure2.png

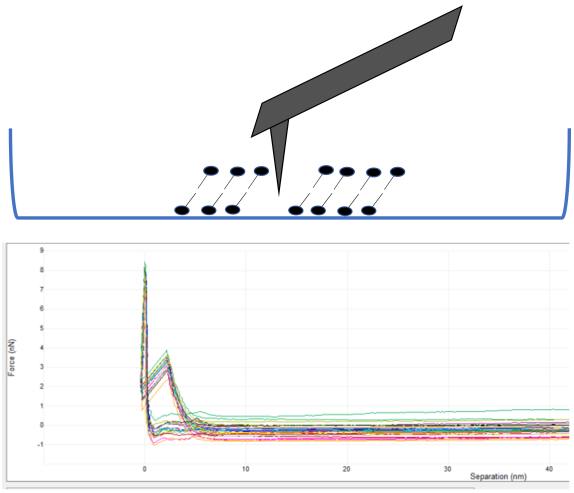


Figure3.png

AAdventures in DNA and chromatin replication using single-molecule biophysics

Wednesday, 22nd November - 14:30: (Auditorium) - Oral

Prof. Nynke Dekker [1]

1. *Technical University of Delft*

Over the past few decades, single-molecule biophysics together with biochemical approaches have made substantial contributions to our understanding of molecular machines. This has led to increased understanding of their mechanochemical cycles, their interplay with other proteins, and their functioning within the cellular context. An ongoing challenge is to probe more complex molecular machines from a biophysical perspective and to do so while maintaining an acceptable degree of experimental yield. I will describe how we have navigated this challenge in the context of the eukaryotic replisome, the multi-protein protein complex that copies the DNA in all of our cells. While the overall outline of replisome assembly in eukaryotes is understood, much remains to be learned about the dynamics of the individual proteins on the DNA and how these contribute to the formation and activity of proper replisomes. Probing at the single-molecule level can help to investigate such dynamics. Using integrated optical trapping and confocal microscopy, as well as TIRF microscopy, I will show how we can now dissect how protein binding, diffusion, sequence recognition, and protein-protein interactions play

important roles in the assembly of the pre-replication complex and the replicative holo-helicase CMG, and how certain of these interactions are altered in the context of chromatin.

Quantum and quantum-limited methods for molecular imaging

Wednesday, 22nd November - 15:10: (Auditorium) - Oral

Prof. Warwick Bowen [1]

1. *University of Queensland,*

Quantum measurement and control techniques provide new ways to image biological structures and observe their dynamics. In this presentation, I will provide an overview of applications in two areas: (1) the use of quantum correlated light sources to supress photon shot noise, enabling faster imaging with contrast beyond what would otherwise be possible, and (2) the use of nanoscale optical cavities to both enhance light-molecule interactions and change the nature of these interaction. For the former, I will focus on coherent Raman microscopy experiments in my laboratory, where we have shown that the shot noise limit can be overcome in chemically specific imaging of biological specimens allowing imaging contrast beyond what is otherwise possible without photodamage to the specimen. For the latter, I will focus on experiments using optical nanocavities to trap single protein dynamics, observe their motions at nanosecond speeds, and ultimately to control their long-range collective motions and understand the effects of these motions on function. Depending on time, I may also discuss quantum-limited interferometric imaging techniques that provide heatmaps of the activity within living cells without labels.

Maxwell Demon that Can Work at Macroscopic Scales

Wednesday, 22nd November - 16:20: (Auditorium) - Oral

Prof. Massimiliano Esposito [1]

1. *University of Luxembourg*

We propose an electronic implementation of an autonomous Maxwell demon which, as expected, stops working in the macroscopic limit as the dynamics becomes deterministic. However, we find that if the power supplied to the demon is scaled up appropriately, the deterministic limit is avoided and the demon continues to work. The price to pay is a decreasing thermodynamic efficiency. A chemical implementation of this Maxwell demon will also be briefly discussed. Our results suggest that novel strategies may be found in nonequilibrium settings to bring to the macroscale nontrivial effects so far only observed at microscopic scales.

N. Freitas and M. Esposito, “A Maxwell demon that can work at macroscopic scales”, Phys. Rev. Lett. 128, 120602 (2022).
N. Freitas and M. Esposito, “Information flows in macroscopic Maxwell’s demons”, Phys. Rev. E 107, 014136 (2023).
M. Bilancioni, M. Esposito and N. Freitas, “A chemical reaction network implementation of a Maxwell demon”, arXiv:2307.14994.

Tracking motion of tethered particles with nanoscale tunnel junctions

Wednesday, 22nd November - 16:47: (Auditorium) - Oral

Mr. Juraj Topolancik [1], ***Dr. Patrick Braganca*** [2], ***Dr. Seoung-Ho Shin*** [1], ***Mr. Flint Mitchell*** [1]

1. *Scientific Advisor,* ***2.*** *Western Digital Corporation*

Real-time monitoring of Brownian motion of nanoparticles confined to solid surfaces with flexible molecular tethers is a powerful technique for studying nanoscale thermodynamics and single-molecule interactions. The motion is traditionally tracked optically using video microscopy or light scattering spectroscopy to detect physically or biochemically induced changes in particle mobility. Here we introduce magnetic detection as a viable alternative to single-particle optical tracking.

Magnetic sensor miniaturization driven by numerous advances in nanofabrication and thin-film magnetism has reduced the device dimensions down to the nanoscale. Magnetic tunnel junctions (MTJs) used in commercial hard disk drives (HDDs) represent the culmination of these research and development efforts. These ultra-sensitive spin-valves have been meticulously engineered to detect magnetization direction of nanoscopic islands written in HDD recording media. Here we repurpose MTJs to detect stochastic motion of tethered superparamagnetic nanoparticles immersed in a solution.

Figure 1 shows schematic representation and scanning electron micrographs of an MTJ with a tethered nanoparticle. We explain the sensor operation and describe its low-frequency current noise spectral characteristics. At low bias voltages the spectra are dominated by 1/f flicker noise represented as a straight line with a slope of ≈ (-1) in the log-log plot of power spectral density versus the frequency, *i.e., P(f) ≈1/f*. Tunneling current magnitude is determined by the relative magnetization orientation of the free and reference layers. A nanoparticle moving in a thermal bath perturbs the direction of the free layer as it collides with solvent molecules. Stochastic particle motion thus modulates magnetic sensor response and gives rise to a Lorentzian bulge with a characteristic corner frequency (f_c) and the high-frequency tail of $P(f) \approx 1/f^2$, as shown schematically (Fig. 2A) and measured experimentally (Fig. 2B). The feature is analogous to the power spectra of light scattered off trapped particles analyzed in optical tweezer single-molecule experiments. We further demonstrate experimentally and explain why at higher biases the system behaves as an active medium and the particle motion becomes super-diffusive with $P(f) \approx 1/f^{\alpha}$, where $\alpha > 2$. This observation makes tethered magnetic nanoparticle tracking a potentially useful tool for studying nonequilibrium dynamics phenomena at the nanoscale.

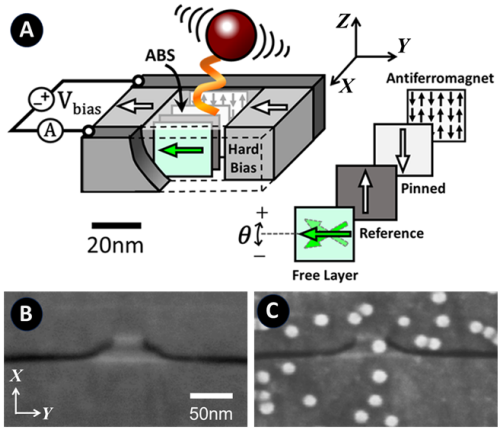


Figure 1 (A) Schematic of a commercial HDD MTJ with a magnetic nanoparticle (MNP) tethered to the air bearing surface (ABS). (B) SEM images of ABS without, and (C) with an MNP.

Figure 1 device schematic.png

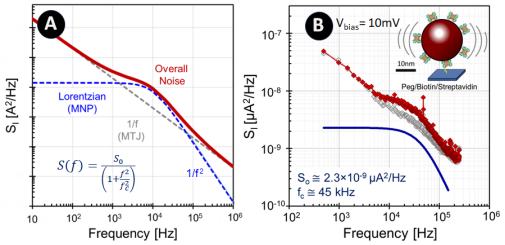


Figure 2 (A) Schematic illustration of a Lorentzian bulge in MTJ PSD created by a tethered MNP, and (B) measured PSD of an MTJ without (gray) and with a Peg-Biotin-Streptavidin tethered nanoparticle (red).

Figure 2 measured noise spectra.png

Intracavity dual-beam optical trap with transverse offset

Wednesday, 22nd November - 17:04: (Auditorium) - Oral

Dr. Antonio Ciarlo [1], ***Dr. Giuseppe Pesce*** [2], ***Dr. Fatemeh Kalantarifard*** [3], ***Prof. Parviz Elahi*** [3], ***Dr. Agnese Callegari*** [1], ***Prof. Giovanni Volpe*** [1], ***Prof. Antonio Sasso*** [4]

1. *Physics Department, University of Gothenburg,* ***2.*** *Physics Department, University of Naples Federico II; Physics Department, University of Gothenburg,* ***3.*** *Physics Department, Boğaziçi University,* ***4.*** *Physics Department, University of Naples Federico II*

Intracavity optical tweezers are a valuable tool for capturing microparticles in water by exploiting the nonlinear feedback effect induced by particle motion when confined in a laser cavity. This feedback effect arises as a consequence of the particle confinement inside a laser cavity, leading to fluctuations in the optical losses of the cavity due to Brownian motion. Our study extends intracavity optical trapping to both single-beam and counter-propagating dual-beam configurations, allowing us to investigate what happens when the two beams are slightly misaligned.

We used a 1030-nm Yb-doped ring fiber laser (pumped at 976 nm) with a hybrid optical path that allows light propagation in both fiber and air (see Fig-ExperimentalSetup.png). To switch between single-beam and dual-beam configurations, a free-space removable isolator is incorporated, resulting in a single-beam configuration when the isolator is installed and a dual-beam configuration when the isolator is removed. We tracked particle positions in 3D using digital holographic microscopy and simultaneously measured the powers of the two counter-propagating beams, providing insight into the feedback effect. A crucial aspect of our experiment is the ability to introduce a transverse offset between the two optical beams in the two-beam configuration, resulting in periodic particle motion (see Fig-TrajectoryAndPower.png and Fig-Trajectory3D.png).

Our study has revealed a periodic orbital rotation of the particle that is closely related to the behavior of the two laser beam powers. We investigated the effect of beam separation and laser pump power on this phenomenon (see Fig-RotationalFrequency.png).

This phenomenon results from the interplay of gradient force, scattering force, and nonlinear feedback. The trapped particle undergoes periodic transitions between the two traps, causing a periodic variation in the laser power of the two beams. As a result, the particle acts as a micro-isolator, attenuating the beam in which it is trapped and amplifying the other beam. It was also observed that the duration of the transition increases as the pump power decreases and the distance between the two traps increases.

Future research will focus on refining the trapping configurations to exploit their potential for precise particle manipulation in the field of nanothermodynamics.

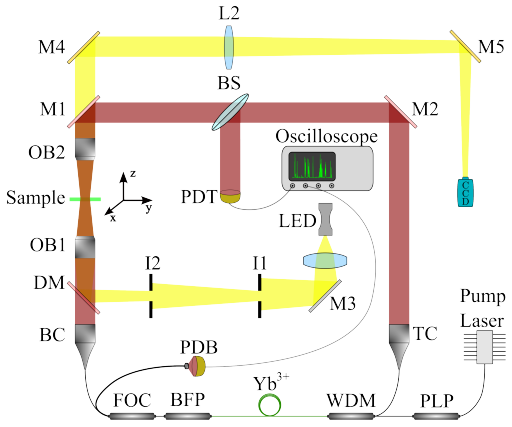


Fig-experimentalsetup.png

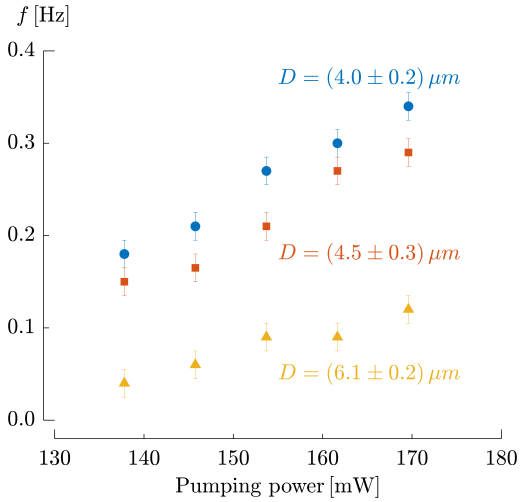


Fig-rotationalfrequency.png

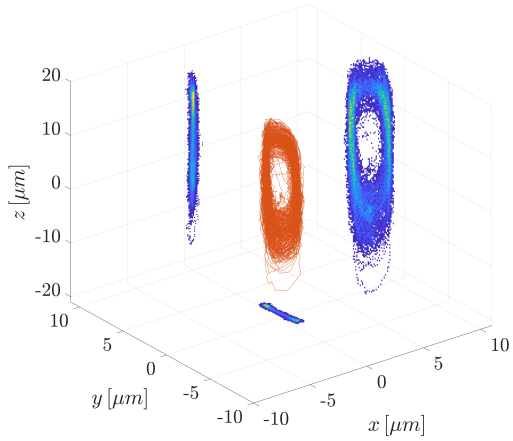


Fig-trajectory3d.png

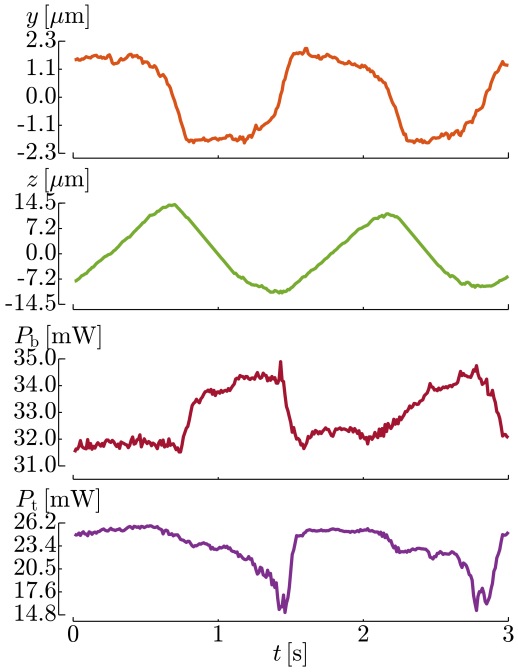


Fig-trajectoryandpower.png

A piston like information engine operating on a colloidal suspension

Wednesday, 22nd November - 17:21: (Auditorium) - Oral

Prof. Yael Roichman [1], ***Mr. Gilad Pollack*** [1], ***Dr. Remi Goerlich*** [2], ***Prof. Saar Rahav*** [3]

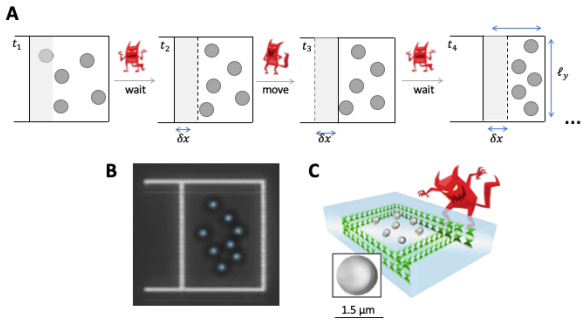
1. *School of Physics and Astronomy, the Raymond and Beverly Sackler Faculty of Exact Sciences, Tel Aviv University,* ***2.*** *School of Chemistry, the Raymond and Beverly Sackler Faculty of Exact Sciences, Tel Aviv University,* ***3.*** *Schulich Faculty of Chemistry, Technion-Israel Institute of Technology*

Thermodynamic principles lie at the heart of the design of every engine and refrigerator around us. As technology progresses toward miniaturization, it becomes essential to develop thermodynamics principles that apply to engines operating in microscopic noisy and active environments. Information engines, the most famous of which is Szilard's engine, use a Maxwell demon to convert information into work, and have been experimentally implemented. These engines are characterized by using measurements of a system's microstate to perform feedback on the system and produce useful work. While some properties of these engines are similar to heat engines, others are unique to them and many recent works have explored such properties extensively. Our work expands on these advancements, by studying the operation of a phase-space contracting engines, such as the piston-based compression engine, on a many-body microscopic system.

Here we use optical trapping to construct an optical piston that compresses a colloidal suspension converting information through feedback to stored energy in the form of increased osmotic pressure of the colloidal suspension. The engine is operated stepwise. In each step the demon measures the occupancy of an area near the piston wall and decides if it can move the wall without pushing any particle (see figure). The measure information is then given by the probability of finding the shaded area empty, p, and its counterpart, q=1-p.

We demonstrate that work can be extracted from this system, experimentally, when the piston is released and the suspension is allowed to push a larger colloid in a deterministic manner.

We characterize the operation of the engine in different configuration numerically, to extend the range of the studied engine parameters and conditions, showing universal features in the operation of the engine. We show that for any such engine, operating on a system in equilibrium, the average work gained per measurement has a universal form depending on a single parameter, p. The maximal average extractable work gained per measurement is always given by the same expression depending only on temperature.



Barcelona nov 23.png

Optomechanical measurement of single nanodroplet evaporation with millisecond time-resolution

Wednesday, 22nd November - 17:38: (Auditorium) - Oral

Dr. Samantha Sbarra [1], ***Mr. Louis Waquier*** [1], ***Mr. Stephan Suffit*** [1], ***Dr. Aristide Lemaître*** [2], ***Dr. Ivan Favero*** [1]

1. *Matériaux et Phénomènes Quantiques, Université Paris Cité, CNRS, Paris, France,* ***2.*** *Centre de Nanosciences et de Nanotechnologies, Université Paris-Saclay, CNRS, Palaiseau, France*

Abstract

Tracking the evolution of an individual nanodroplet of liquid in real-time remains an outstanding challenge. Here a miniature optomechanical resonator detects a single nanodroplet landing on a surface and measures its subsequent evaporation down to a volume of twenty attoliters. The ultra-high mechanical frequency and sensitivity of the device enable a time resolution below the millisecond, sufficient to resolve the fast evaporation dynamics under ambient conditions. Using the device dual optical and mechanical capability, we determine the evaporation in the first ten milliseconds to occur at constant contact radius with a dynamics ruled by the mere Kelvin effect, producing evaporation despite a saturated surrounding gas. Over the following hundred of milliseconds, the droplet further shrinks while being accompanied by the spreading of an underlying puddle. In the final steady-state after evaporation, an extended molecular film is stabilized on the surface. Our optomechanical technique opens the unique possibility of monitoring all these stages in real-time.

Figure 1 : SEM picture of an optomechanical disk resonator

Figure 2 : Optical and mechanical frequency shifts after the droplet landing

Figure 3 : Measurement of the volume of the droplet as it evaporates and progressively evolves into a molecular film

Paper

Sbarra, S., Waquier, L., Suffit, S., Lemaître, A., & Favero, I. (2022). Optomechanical measurement of single nanodroplet evaporation with millisecond time-resolution. *Nature Communications*, *13*(1), 6462.

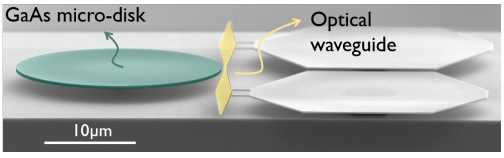


Fig1.png

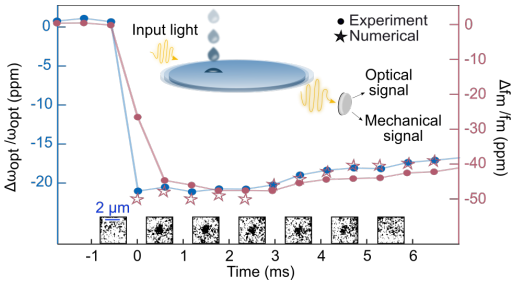


Fig2.png

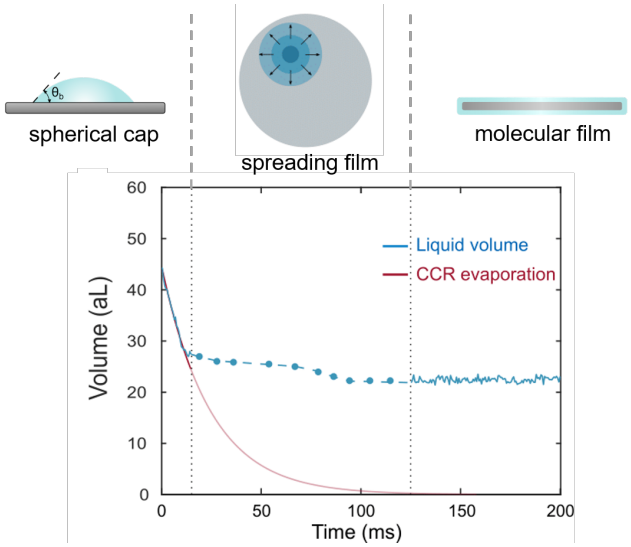

spherical cap
spreading film
molecular film
Liquid volume
CCR evaporation
Volume (aL)
Time (ms)
0
10
20
30
40
50
60
0
50
100
150
200

Fig3.png

Stacking cooperativity in single-stranded DNA

Wednesday, 22nd November - 17:55: (Auditorium) - Oral

Dr. Xavier Viader-Godoy [1], ***Dr. Maria Manosas*** [1], ***Dr. Felix Ritort*** [1]

1. *Small Biosystems Lab, Departament de Física de la Materia Condensada, Facultat de Física, Universitat de Barcelona, 08028 Barcelona, Spain*

Base stacking is a key driving force in nucleic acid stabilization. The measurement of the stacking energies is challenging due to their weakness in single-stranded DNA and the inextricable separation from hydrogen bonding energies in double-stranded DNA. We carry out mechanical unfolding experiments of short poly-dA sequences of 20-40 bases using optical tweezers and derive the base stacking energies with 0.01 kcal/mol accuracy. We introduce a helix-coil model to investigate the stacking-unstacking transition reproducing the force-extension curves. We find a salt-dependent cooperativity parameter, γ_{ST} = 0.86(2) + 0.06(2) log C (kcal/mol), and a stacking energy per base, ΔG_{ST} 0.14(3) kcal/mol. Stacking is predominantly cooperative with a static correlation length at zero force, ξ_{ST} 3 − 4 bases, which reaches a maximum at 15 − 20 pN of ξ_{ST} 5 − 10 bases, consistent with the shoulder observed in the force-extension curves. Finally, both ξ_{ST} and γ_{ST} monotonically increase with salt enhancing the cooperativity of the stacking-unstacking transition. Salt corrections in γST demonstrate that double-helix stability is primarily due to stacking.

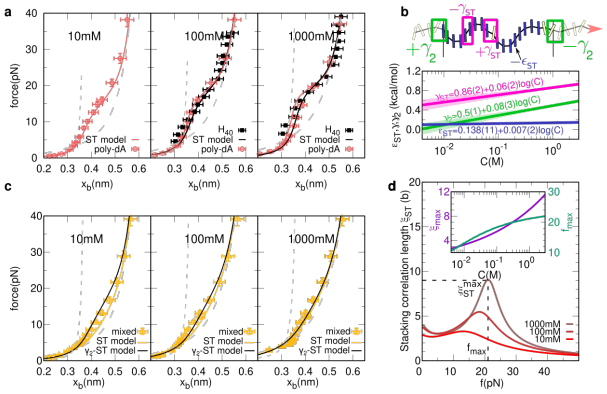


Figure4 model2.png

Temperature sensing at the microscale with optically trapped microgels.

Wednesday, 22nd November - 18:12: (Auditorium) - Oral

Prof. Raúl Rica [1]

1. *Universidad de Granada*

Microgels are soft systems comprised of crosslinked hydrogels that often exhibit thermoresponsiveness and collapse above a volume phase transition temperature (VPTT). The proximity of the VPTT to physiological temperatures (e.g., ≈32 °C for pNIPAM) together with their capability to carry cargo makes them very well suited to develop interesting applications, including their use as drug-delivery carriers, their potential applicability in the exploration of synthetic cell research, the design of microswimmers that move thanks to the responsiveness, and, more recently, to locally sense temperature [1].

In this work, we discuss the dynamics of a composite made of a pNIPAM microgel decorated with magnetite nanocubes that can both locally heat up and identify the surpass of threshold temperature determined by the VPTT of the microgel. Above a certain laser power, a single decorated microgel features a sharp volume phase transition, i.e., occurs at a particular power value. Since the VPTT can be modified by adding amphoteric and other functional groups to the microgel polymer network, the system can be used as a local probe tuned to keep heating under a certain threshold that might be identified as critical. We discuss the dynamics of such a system when trapped in optical tweezers. The self-heating microgels also exhibit an unexpected and intriguing bistability behaviour above the critical power, probably due to partial collapses of the microgel. These results set the stage for further studies and the development of applications based on the hot Brownian motion of soft particles [2].

[1] Muñoz□Ortiz, T., Alayeto, I., Lifante, J., Ortgies, D. H., Marin, R., Rodríguez, E. M., ... & Jaque, D. (2023). Three□dimensional Optical Coherence Thermometry Using Polymeric Nanogels. *Advanced Materials*, 2301819.

[2] Fernandez□Rodriguez, M. A., Orozco□Barrera, S., Sun, W., Gámez, F., Caro, C., García□Martín, M. L., & Rica, R. A. (2023). Hot Brownian Motion of thermoresponsive microgels in optical tweezers shows discontinuous volume phase transition and bistability. *Small*, 2301653.

Plasmonic Solid State Nanopores for single biomolecule identification

Wednesday, 22nd November - 16:20: (Room 607) - Oral

Prof. Francesco DeAngelis [1]

1. *Italian Institute of Technology*

Identification of peptides and proteins is central to proteomics. Protein sequencing is mainly conducted by insensitive mass spectroscopy because proteins cannot be amplified, which hampers applications such as single-cell proteomics and precision medicine. The commercial success of portable nanopore sequencers for single DNA molecules has inspired extensive research on proteins based on electrical or optical readout. In this regard, a large variety of nanopores, both biological and solid state have been developed. However, when moving from DNA to proteins some major challenges remain: (1) DNA bases are just 4 against the amino acids which are 20 hence their discrimination only by using electrical current levels or colorimetric readout is extremely difficult; (2) spatial and temporal resolution (sensitivity) to detect single amino acids within the same molecule; and (3) controlling the motion of proteins into the nanopores. Similar difficulties affect the post-transcriptional modification of RNA. In this context, the emergence of label-free optical analysis based on plasmonic enhancement shows great promises to address the first two challenges [1,2]. In fact, plasmonic nanopores can both confine and enhance the local electromagnetic field into the pore. The confinement improves the spatial resolution while the enhancement helps to increase sensitivity. Notably, Raman spectroscopy provides unique molecular fingerprints to discriminate all the 20 amino acids [2]. We will present our latest results on plasmonic nanopores combined with Raman Spectroscopy for single-amino-acid identification [3,4]. Molecules in solutions are delivered into solid state plasmonic nanopores by means of electrophoresis and detected by plasmonic enhanced Raman scattering. In addition, we discuss the manipulation of molecule translocation and liquid flow in plasmonic nanopores for controlling molecule movement and for enabling high-resolution reading of protein/molecule sequences [3]. We envision that a combination of Raman spectroscopy with plasmonic nanopores can succeed in single-molecule protein sequencing in a label-free way.

References

[1] Jian-An Huang et al., Nature communications, 10 (2019), 1, 1-10.

[2] Yingqi Zhao et al., ACS Photonics, 9 (2022), 3, 730-742.

[3] Yingqi Zhao et al., Nano Lett. 23 (2023), 11, 4830–4836.

[4] M. Iarossi et al., Adv. Funct. Mater. 2023, 2301934

Enhancing the Sensitivity of Silica Microresonator-based Biosensors through Adding a Perovskite Coating

Wednesday, 22nd November - 16:47: (Room 607) - Oral

Dr. Mandana Jalali [1], ***Prof. Daniel Erni*** [2]

1. *General and Theoretical Electrical Engineering (ATE), Faculty of Engineering, University of Duisburg-Essen, D-47048 Duisburg,* ***2.*** *General and Theoretical Electrical Engineering (ATE), Faculty of Engineering, University of Duisburg-Essen,D-47048 Duisburg*

Optical microresonators sustaining whispering gallery modes (WGMs) have been validated as ultra-sensitive biosensing platforms, capable of resolving entities as small as individual viruses. The sensitivity is governed by the resonator's quality factor, mode volume, and the localized electric field intensity. Advancements in the sensor's detection limits could result in specificity along with the ultra-sensitivity, making them appropriate for fundamental studies in natural and life sciences.

A promising approach to enhance the sensitivity consists of depositing a thin dielectric coating with a larger refractive index to the spherical microresonator (cf. Fig.1b)). This yields a significant spatial shift of the WGM profile towards the surface (while still keeping a large Q factor) together with a stronger mode confinement, and thus an increase of the electric field intensity at the sensing location near the surface.

In this study, a 200nm $CsPbI_3$

perovskite coating is added to the 30µm SiO_2 microsphere structure. The proposed structure is modelled and simulated in an aquatic environment, using the FEM-based COMSOL Multiphysics simulation platform, where the quality factor as well as the electric field intensity at the microresonator's surface are determined as figures of merits. It's shown that inclusion of the perovskite coating increases the quality factor 5.8 times, while decreasing the mode volume to about 0.17 times of the initial mode volume. The electric field intensity at the surface of the coating gets 3 times stronger, showing that the microresonator's sensitivity is already prominently enhanced. Afterwards, a 50nm sized exosome with an effective refractive index of 1.384 (based on an effective medium analysis), is positioned in the vicinity of both microsphere versions (i.e. with and without coating). The resulting wavelength shift of the resonant WGM due to presence of the exosome is computed. The results show that the corresponding spectral shift for the uncoated microresonator amounts to 21fm, whereas this shift increases to 8.5pm for the microresonator with the 200nm perovskite coating. This is more than 3 orders of magnitudes implying an increase in sensitivity in a similar range, and thus validating the functionality of the proposed biosensor concept.

Tab. 1 The resonance wavelength, quality factor, mode volume as well as the electric field strength at the microresonator's surface, for a 30µm Silica microsphere in water without any coating, with 200nm PS, PMMA, Si_3N_4, TiO_2, as well as a $CsPbI_3$ perovskite coatings.

	Resonance wavelength (nm)	Quality factor	Mode volume (m^3)
Uncoated	772.42	2.8×10^5	1.9×10^{-19}
PS	797.61	1.1×10^6	8.4×10^{-20}
PMMA	780.54	2.0×10^5	1.1×10^{-19}
Si_3N_4	868.25	7.7×10^5	3.0×10^{-20}
TiO_2	883.67	7.8×10^5	2.8×10^{-20}
$CsPbI_3$	898.58	1.6×10^6	3.3×10^{-20}

Tab1.png

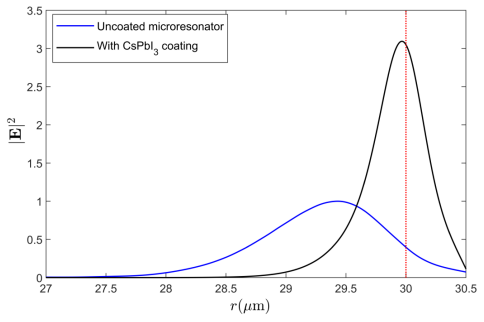


Fig. 1 The normalized electric field strength along a radial line near the 30µm silica microsphere's surface without any coating together with a 200nm $CsPbI_3$ coating. The red vertical dotted line represents the position of the microsphere surface.

Fig1.png

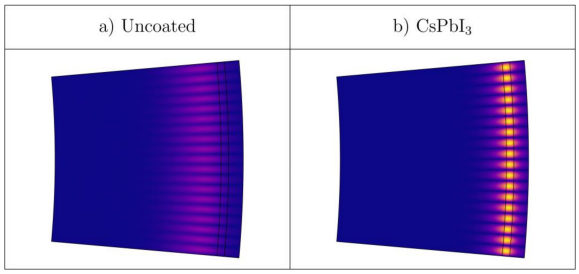


Fig. 2 The electric field profile of the excited whispering gallery modes for a) uncoated, b) 200nm thick $CsPbI_3$ coated 30μm silica microsphere in water.

Fig2.png

Nanosecond transient plasmonic single-protein detection

Wednesday, 22nd November - 17:04: (Room 607) - Oral

Dr. Martin Baaske [1]

1. *Max Planck Institute of Biophysics*

Fluorescence-based detection schemes exhibit detection bandwidths, which are limited by photo-bleaching and photon emission-rates. Scattering-based techniques face no such restrictions and thus promise the observation of faster single-molecule dynamics over longer periods. Recently, we have demonstrated that single nanoparticles and proteins traversing the sub-attoliter detection volumes of plasmonic nanorods in Brownian motion can be observed with nanosecond temporal resolution [1,2]. This is enabled via combination of polarization-sensitive and interferometric nanorod read-out and photothermal calibration [3]. Our fast plasmonic detection method enables the direct interrogation of single-protein motion and dynamics on time scales compatible with molecular dynamics simulations.

[1] M.D. Baaske, P.S. Neu, M.Orrit. *Label-free Plasmonic Detection of Untethered Nanometer-sized Brownian Particles*, ACS Nano (2020)

[2] M.D. Baaske, N. Asgari, D. Punj, M. Orrit. *Nanosecond time scale transient optoplasmonic detection of single proteins*, Science Advances (2022)

[3] M.D. Baaske et al, N.Asgari, P.Spaeth, S. Adhikari, M. Orrit. *Photothermal Spectro-Microscopy as Benchmark for Optoplasmonic Bio-Detection Assays*, Journal of Physical Chemistry C (2021)

Continuous single-molecule sensing based on particle motion: how do single-molecule sensors change over several days?

Wednesday, 22nd November - 17:21: (Room 607) - Oral

Mr. Chris Vu [1], ***Mr. Sebastian Cajigas*** [2], ***Dr. Junhong Yan*** [2], ***Dr. Arthur de Jong*** [3], ***Prof. Menno Prins*** [1]

1. *Department of Biomedical Engineering, Eindhoven University of Technology,* ***2.*** *Helia Biomonitoring,* ***3.*** *Department of Applied Physics, Eindhoven University of Technology*

Introduction

Biosensing by Particle Motion (BPM) is a sensing method with single-molecule resolution designed to enable the continuous monitoring of biomolecules at low concentrations, such as nucleic acids, metabolites, proteins and hormones[1,2,3]. The method relies on optically tracking the motion of individual biofunctionalized particles (1 µm in diameter) that interact with a biofunctionalized sensor surface. The particles switch between bound and unbound states due to reversible single-molecule interactions of analyte molecules and affinity molecules (Figure 1). To enable continuous sensor operation over days and weeks, detailed understandings of the functionality and stability of the molecules and their mechanisms are required.

Methods

We experimentally quantified characteristic parameters of the single-molecule sensors over several days: number of particles, mobility patterns, switching activity, and bound and unbound state lifetimes of specific and non-specific interactions. We additionally studied the binding behavior of individual particles to collect information about loss processes on a single-molecular level. The parameters were studied for different densities of binder molecules and assay conditions.

Results

Particle populations were identified and classified based on their switching frequency and mobility patterns. Particles with high switching frequencies showed a gradual lowering in activity over time and increased unbound state lifetimes, with change rates on the order of 10^{-6} s^{-1}. These active particles were shown to become either more mobile or less mobile over time. Controls without binder molecules showed that non-specific interactions had negligible effects on the switching activity. Thus the reduction of switching activity over long time spans can be attributed to losses of specific binding functionality on particles and/or surface, and to the loss of mobility due to multivalent binder interactions, decreasing the effective association rate between particle and surface.

Discussion

The BPM sensor can function continuously over several days, while showing changes that relate to the molecular constituents of the sensor. The presented experimental framework facilitates a systematic evaluation of functionality changes and gives opportunities to investigate the long-term limits of continuous single-molecule sensors.

References

1. Visser, E. et al., Nature Communications (2018)
2. Buskermolen, A. et al. Nature Communications (2022)
3. Vu, C. et al., Analytical Chemistry (2023)

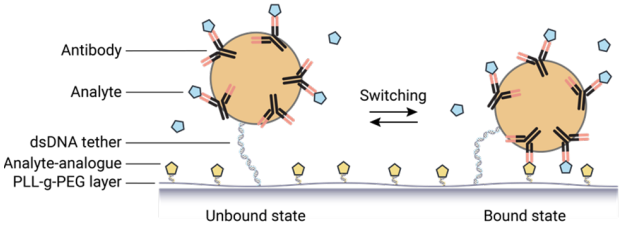


Figure 1. quantification of small molecules using biosensing by particle mobility bpm with a competitive assay format..png

Real-time Tracking of Protein Corona Formation on Single Nanoparticles

Wednesday, 22nd November - 17:38: (Room 607) - Oral

Dr. Mathias Dolci [1], ***Dr. Yuyang Wang*** [1], ***Mr. Sjoerd Nooteboom*** [1], ***Dr. Paul Soto Rodriguez*** [2], ***Prof. Samuel Sanchez*** [3], ***Dr. Lorenzo Albertazzi*** [1], ***Prof. Peter Zijlstra*** [1]

1. *Molecular Biosensing. Department of Applied Physics and Science Education, Eindhoven University of Technology.,* ***2.*** *Departmento de Fisica, Unversidad de la Laguna,* ***3.*** *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain.*

Introduction

For many applications in nanomedicine, nanomaterial injection directly into biological fluids is required. The spontaneous interaction of proteins present in such fluids leads to the formation of a shell, known as a "Protein Corona" (PC).[1] The formation of PCs undeniably results in changes of the physico-chemical properties of nanoparticles, and thus reduces their targeting efficiency. It is therefore essential to fully understand the mechanisms of PC formation in order to predict their future behavior and hence optimize their design.[2]

Methods

Here we introduce a method which combines dark-field microscopy with a microfluidic system to track the formation of PC in-situ, at the single-particle level.[3] The use of a total internal reflection configuration enables the monitoring of protein adsorption on the surface of nanoparticles directly in undiluted blood serum without being affected by background signal (Fig.1). The method, which relies on changes in scattering, provides a way to investigate both plasmonic and non-resonant dielectric particles.[4]

Results and Discussion

Our method provides millisecond temporal resolution without the need for a spectrometer, allowing many particles to be compared in parallel. As a result, statistics can be established for protein adsorption during injection of full serum (Fig.2). Two systems were investigated: silica and gold particles with sizes of ~100 nm. Timetraces, giving access to adsorption kinetics and final shifts, combined with electromagnetic simulations were used to quantify the adsorption kinetics.

We find that the corona is predominantly determined by the surface chemistry rather than the underlying nanoparticle material or size. The extraction of kinetic parameters showed an anti-cooperative behavior during the formation of PC. In addition, the study of particles with porous structures reveal multiphase behavior during the protein adsorption, involving the migration of proteins into the pores (Fig.3). Finally, the versatility of the method opens up the possibility of extending the scope of the study to other types of materials, structures or functionalities to relate the formation of PC's to the performance of particles in biosensing applications.

(1) Ke,P.C.;Lin,S.;Parak,W.J.;Davis,T. P.;Caruso,F. *ACS Nano* **2017**,*11*(12),11773–11776.

(2) Breznica,P.;Koliqi,R.;Daka,A. *Medicine and Pharmacy Reports* **2020,**93(4),342-350.

(3) Dolci,M;Wang,Y;Nooteboom,S;Rodriguez,P;Sanchez,S;Albertazzi,L.;Zijlstra,P. *ACS Nano* **2023**.

(4) Zijlstra,P.;Paulo,P.M.R.;Orrit,M. *Nature Nanotechology* **2012**,7(6),379–382.

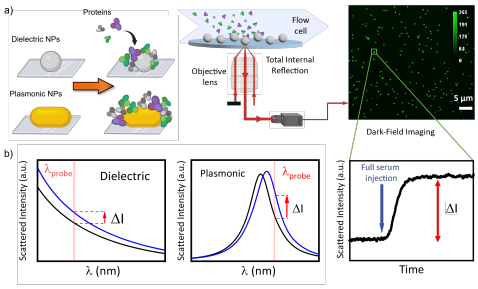


Figure1. a schematic representation of the pc formation and the setup b scattering of dielectric and plasmonic particle with an example of timetrace on a single particle.png

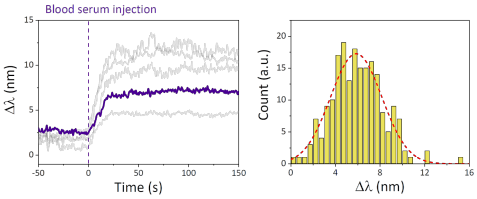


Figure2. timetrace of the plasmon shift of different single nanorods in the field of view after injection of blood serum and the associated histogram of the plasmon shift.png

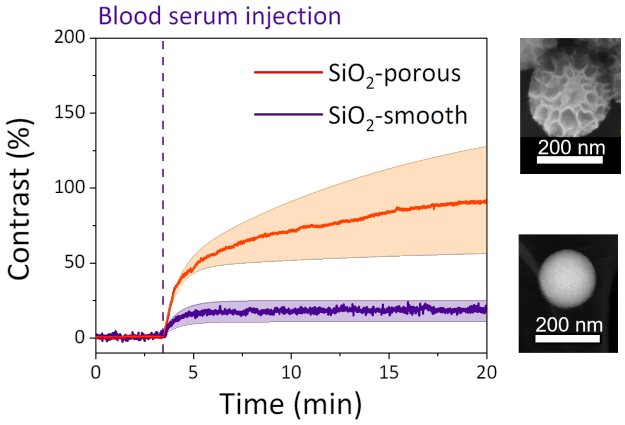


Figure3. averaged timetraces during pc adsorption on sio2 nanoparticles with a smooth surface purple line and a porous structure red line upon exposure to fbs.png

Optical Microcavity Measurement of Aspect Ratio of Gold Nanorods in Water

Wednesday, 22nd November - 17:55: (Room 607) - Oral

Ms. Yumeng Yin [1], ***Dr. Aurelien Trichet*** [1], ***Prof. Jason Smith*** [1]

1. *University of Oxford*

It is significant yet challenging to measure the shape of nanoparticles in fluids. Shape measurement can help characterise nanoparticles and detect rod-shaped bacteria in water. Here, we introduce a new technique for measuring the aspect ratio (μ) of a single gold nanorod.

The microcavity forms highly resonant modes with a highly reflected distributed Bragg reflector coating on the planar and concave surfaces. Fig.1 shows a gold nanorod diffusing in the laser-illuminated microcavity. By modulating the cavity length, we repeatedly sweep a cavity mode through resonance and analyse the transmission. An avalanche photodiode (APD) records the resonant peak's intensity, while the polarimeter measures the degree of linear and circular polarization, S_1 and S_3 (Fig.1).

Fig.2a shows the change in the full width at half maximum (FWHM) of APD resonant peaks with sample A over a 200ms period with the gold nanorod solution. Increases in FWHM indicate increases in intra-cavity losses, implying a nanorod's presence. The anisotropic polarisability of a gold nanorod causes the transmitted light to deviate from its original left-circularly polarised state, and a non-zero S_1 peak appears. By applying an analytic model to fit the APD and S_1 peaks (Fig.2b, c), we could obtain a polarisability-related parameter Φ. We employ two fitting models for the distribution of Φ to obtain μ with the loss mechanism of only absorption and with absorption and scattering considered. Summaries of a single-particle event with sample A and B are illustrated in Fig.3a-d. It gives 2.00±0.02 and 1.66±0.05 without and with scattering for sample A, and 1.87±0.02 and 1.69±0.04 for sample B. Fig.3a, c show a flat distribution of azimuthal angle in the microcavity plane (Fig.3e) as expected. Then we compare the statistical results of 30 particles each for sample A and B with the scanning electron microscopy (SEM) results (Fig.4). The optical microcavity sensing method shows a similar distribution of sample A and B with SEM results despite the absolute value difference. Our technique opens the new possibility of real-time and single-particle shape measurements, which have promising applications in nanoparticle characterisation, water monitoring and beyond.

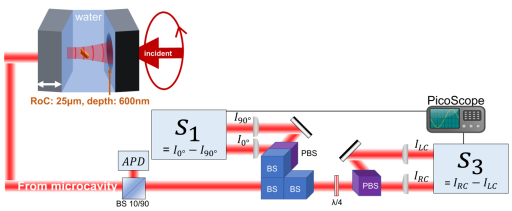


Fig.1 schematic of the microcavity and polarimeter.png

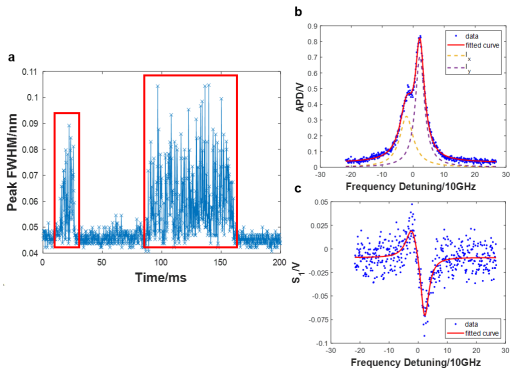


Fig.2 single particle event and individual fittings of apd and s1.png

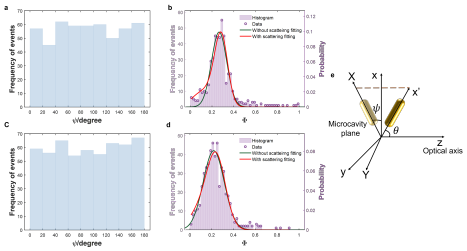


Fig.3 summary of a single-particle event for sample a and b.png

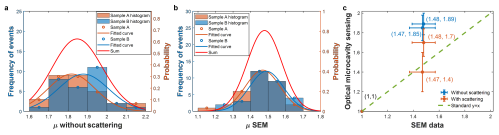


Fig.4 summary of 30 particles using optical microcavity sensing and sem.png

Hexagonal plasmonic nanostructure arrays for high-throughput multicolor single-molecule detection

Wednesday, 22nd November - 18:12: (Room 607) - Oral

Mr. Ediz Kaan Herkert [1], ***Mr. Roger Pons Lanau*** [2], ***Mr. Lukas Lau*** [2], ***Prof. Maria F. Garcia-Parajo*** [2]

1. *ICFO-Institut de Ciencies Fotoniques, The Barcelona Institute of Science and Technology,* ***2.*** *ICFO-Institut de Ciencies Fotoniques, The Barcelona Institute of Science and Technology, 08860 Castelldefels (Barcelona), Spain*

Biosensing applications based on fluorescence detection often require single-molecule sensitivity in the presence of strong background signals. Plasmonic nanoantennas are particularly suitable for these tasks, as they can confine and enhance light in volumes far below the diffraction limit. Antenna-in-box (AiB) platforms achieved high single-molecule detection sensitivity at high fluorophore concentrations by placing gold nanoantennas inside nanoapertures.[1] Further improvements in terms of signal enhancement and background reduction have been recently achieved using hybrid AiBs made of gold and aluminum.[2] However, the full potential of AiB platforms has not yet been exploited as most studies are based on a confocal single-color fluorescence detection scheme restricting their broad applicability and throughput.

We overcome these limitations by introducing aluminum-based hexagonal close-packed AiBs (HCP-AiBs) that enable high-density, multicolor single-molecule detection providing unprecedented high throughput. We demonstrate parallel dual color readout of >1000 HCP-AiBs through an alternating excitation-wavelength scheme and epi-fluorescence detection. We show that this biosensing platform does not only provide three orders of magnitude faster data acquisition than a confocal scheme, but also allows alignment-free correlative multicolor studies by incorporating optical fiducial markers. We illustrate through FDTD simulations how HCP-AiBs can be optimized for fluorescence detection throughout the visible regime and discuss how the fluorescence excitation enhancement of HCP-AiBs is affected by cavity and grating effects originating from the dense hexagonal packing of nanoapertures. We foresee that these types of nanophotonic biosensors will enable the quick acquisition of statistically robust datasets while providing multicolor single-molecule sensitivity beyond the capabilities of current biosensors.

(1) Flauraud, V.; Regmi, R.; Winkler, P. M.; Alexander, D. T. L.; Rigneault, H.; Van Hulst, N. F.; García-Parajo, M. F.; Wenger, J.; Brugger, J. In-Plane Plasmonic Antenna Arrays with Surface Nanogaps for Giant Fluorescence Enhancement. *Nano Lett.* **2017**, *17* (3), 1703–1710. https://doi.org/10.1021/acs.nanolett.6b04978.

(2) Herkert, E. K.; Bermeo Alvaro, D. R.; Recchia, M.; Langbein, W.; Borri, P.; Garcia-Parajo, M. F. Hybrid Plasmonic Nanostructures for Enhanced Single-Molecule Detection Sensitivity. *ACS Nano* **2023**. https://doi.org/10.1021/acsnano.3c00576.

How kinesins walk - it’s not how you thought!

Wednesday, 22nd November - 16:20: (Room 608) - Oral

Prof. Erik Schäffer [1]

1. *University of Tübingen*

TBD

Plasmon-enhanced single-molecule biosensing for continuous monitoring in complex matrices

Wednesday, 22nd November - 16:47: (Room 608) - Oral

Mr. Vincenzo Lamberti [1], ***Prof. Peter Zijlstra*** [2], ***Dr. Mathias Dolci*** [3]

1. *Department of Applied Physics, Eindhoven University of Technology,* ***2.*** *Eindhoven University of Technology,* ***3.*** *Molecular Biosensing. Department of Applied Physics and Science Education, Eindhoven University of Technology.*

Introduction

Continuous and compact sensors capable of tracking pico- to nanomolar concentrations over long time spans while maintaining high specificity in interference-rich media are the next fundamental challenge in biosensing. Single-molecule (SM) resolution results in digital sensor signals that eliminate the effects of mechanical and temperature drift[1], and are thus poised to generate a breakthrough in the field of long-term continuous monitoring. Fluorescence microscopy allows for SM sensors with highly specific sandwich assays for nucleic acids [2] and proteins[3], however, current sensors do not enable continuous sensing because current implementations require high-affinity interactions.

Results and Discussion

Here we propose a novel sensing method based on plasmon-enhanced fluorescence[4] in combination with a low-affinity and thus reversible sandwich assay (Fig 1.a-b). We demonstrate continuous monitoring SM biosensing directly in complex media (blood serum). Plasmon-enhancement (Fig 1.c), generates very strong SM signals enabling the use of low-affinity (reversible) interactions that enable continuous monitoring of analyte concentrations over extended timespans. Employing a widefield optical interrogation, we probe hundreds of nanoparticles simultaneously (Fig 1.d) for high statistical precision.

Analysis of time-traces allows for accurate event counting and harvesting of SM dynamic information (Fig 2.a). We analyze the sensor response in terms of kinetic binding rates (Fig 2.b) that are converted to an analyte concentration. We show sensing in undiluted biological media overcoming the background autofluorescence thanks to plasmon-enhanced SM signals for a relevant cancer marker (Fig 2.c) with 100 pM limit-of-detection. With the use of low-affinity interactions, we are able to reversibly track pico- to nanomolar analyte concentrations over more than 3h (Fig 3) with a sample-to-data response of 10 minutes. Enhanced SM signals enabled us to drastically miniaturize the optical setup into a compact and cheap platform for application in point-of-care diagnostics, monitoring of industrial processes, and safekeeping of the environment.

[1] Dey, S., Dolci, M., & Zijlstra, P. *ACS Phys. Chem Au*, 3(2), 143–156. (2023).

[2] Hayward, S. L. et al. J. Am. Chem. Soc, 140(37), 11755–11762. (2018).

[3] Chatterjee, T., et al. PNAS, 117(37), 22815–22822. (2020).

[4] Horáček, M., Engels, D. J., & Zijlstra, P. Nanoscale, 12(6), 4128–4136. (2020).

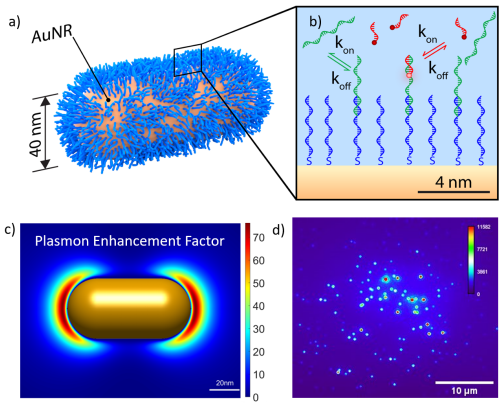


Fig1 assaydesign.pef.fov.png

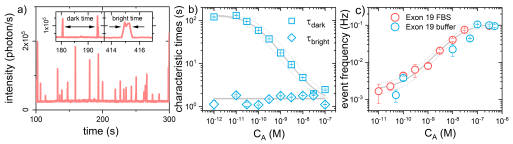


Fig2 examplett bufferresponse exon19complexmedia.png

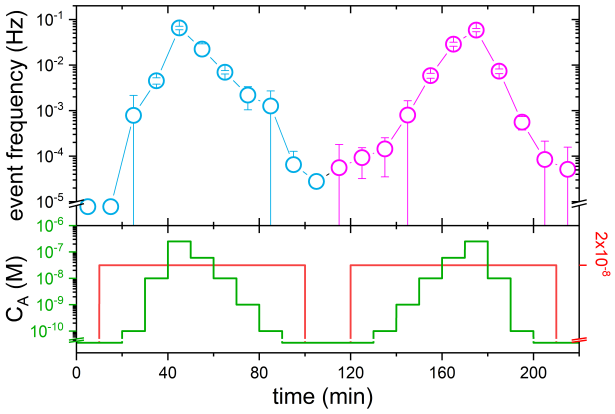


Fig3 continousmonitoring.png

Adsorption and Kondo signature of stable π-radical BDPA on Cu(100)

Wednesday, 22nd November - 17:04: (Room 608) - Oral

Dr. Jacob Teeter [1], ***Dr. Daniel Miller*** [2], ***Dr. Stefan Müllegger*** [1]

1. *Johannes Kepler Universität Linz,* ***2.*** *Hofstra University*

Introduction

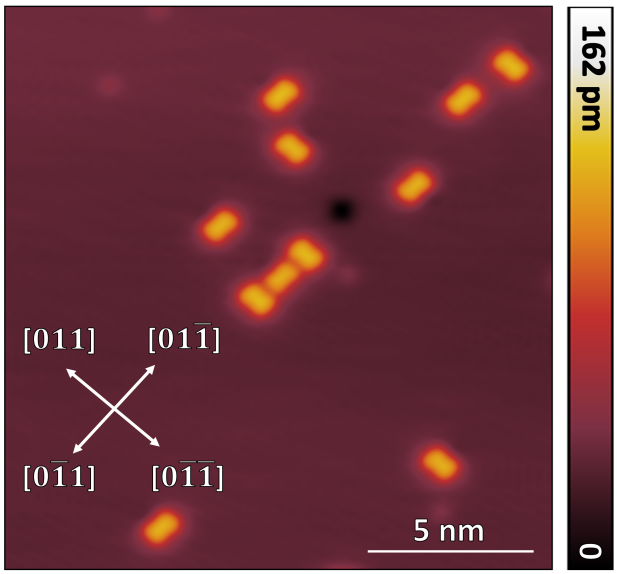
Stable organic radicals can serve as model systems for investigating metal-free magnetic phenomena. We have investigated one such species, α, γ-bisdiphenylene-β-phenylallyl (BDPA), at the single-molecule level on Cu(100).

Methods

Ultra-high vacuum (UHV) scanning tunneling microscopy (STM) and spectroscopy (STS) were performed using a commercial Omicron Polar STM operated at at 6 K. Spectroscopic measurements were acquired with a commercial Pt-Ir tip using an external lock-in amplifier at 773 Hz and a typical modulation voltage of 5 mV (zero-to-peak). The substrate employed was a Cu(100) crystal obtained from Surface Preparation Laboratory. Density functional theory computations which account for London dispersion forces were performed to elucidate upon the adsorption structure and orientation of the BDPA radical on the Cu(100) surface.

Results and Discussion

Our investigations revealed structural and electronic features of BDPA on Cu(100), including a strong preferential adsorption orientation and the presence of a Kondo-like electronic signature that suggests survival of the unpaired electron spin.



Bdpa on cu100 and orientation.png

Fast molecule-spanning dynamics in a multi domain protein by single-molecule fluorescence

Wednesday, 22nd November - 17:21: (Room 608) - Oral

Ms. Veronika Frank [1], ***Dr. Benedikt Sohmen*** [1], ***Dr. Steffen Wolf*** [2], ***Dr. Jean-Benoît Claude*** [3], ***Dr. Jérôme Wenger*** [3], ***Prof. Thorsten Hugel*** [4]

1. *Institute of Physical Chemistry, University of Freiburg, Freiburg, Germany,* ***2.*** *Institute of Physics, University of Freiburg, Freiburg, Germany,* ***3.*** *Fresnel Institute, CNRS, Aix-Marseille University, Marseille, France,* ***4.*** *Institute of Physical Chemistry & BIOSS and CIBSS Signalling Research Centres, University of Freiburg, Freiburg, Germany*

Understanding the functions of proteins requires a complete picture of their dynamics on different length and time scales: Slow protein dynamics (microsecond to minute time scale) are associated with (large) conformational changes, whereas picosecond dynamics have been linked to side chain movements. Up to now, the nanosecond time scale domain has barely been explored.

A combination of single-molecule fluorescence techniques and full-atom MD simulations reveals rapid fluctuating motions within the protein on the hundreds of nanoseconds time scale, encompassing the entire multi-domain heat shock protein Hsp90 – a chaperone associated with cancer and neurodegenerative deseases [1].

These fast molecule-spanning dynamics depend on the state of the protein and are influenced by helper proteins (cochaperones) of Hsp90. Therefore, we anticipate that they are important to promote allosteric communication. To improve the signal-to-noise in these measurements, we are currently establishing the use of zero-mode waveguide nanoapertures. Further experiments will uncover the functional significance of molecule-spanning dynamics.

[1] Sohmen, B., Beck, C., Frank, V., Seydel, T., Hoffmann, I., Hermann, B., Nüesch, M., Grimaldo, M., Schreiber, F., Wolf, S., Roosen-Runge, F., Hugel, T., The onset of molecule-spanning dynamics in heat shock protein Hsp90, accepted

Combination of TIRF and Mass Photometry for single molecule detection of biomolecules

Wednesday, 22nd November - 17:38: (Room 608) - Oral

Dr. Carlos Bueno Alejo [1], ***Prof. Andrew Hudson*** [1], ***Prof. Ian Eperon*** [1]

1. *School of Chemistry, University of Leicester, University Road, Leicester, United Kingdom, LE1 7RH, Leicester Institute of Structural & Chemical Biology, Henry Wellcome Building, University of Leicester, Lancaster Road, LE1 7HB*

Single molecule spectroscopy enables unique insight into the analysis of biomolecular interactions. Most commons techniques use fluorescence proteins or biomolecules with fluorescence tags as reporter of that interactions, so only one type of information is gathered. For other type of information different techniques are required. For instance, a fairly new technique called Mass Photometry, based on scattering microscopy, is used to gather mass information of single biomolecules.

In order to get the full picture of biological processes it would be very useful to collect different type of information from the same sample. There are various examples of multicolor fluorescence microscopy, or methodologies to gather spectral and time resolved fluorescence information from the same sample but not that many involving the use of completely different techniques on the same spot.

With that in mind, we here in report on the development of a new technique combining total internal reflection fluorescence microscopy (TIRF) and Mass photometry (MP) in order to gather fluorescence and mass information from the same biomolecules. In order to do that we combined MP with rarely used variant of TIRF called light guide TIRF (Lg-TIRF). This fluorescence technique allows us to separate the excitation pathway from any of the emission ones which provide us with the opportunity to choose the illumination wavelength that we need, even multiple ones. This development could play a paramount role in the research of biological processes like protein-protein or protein-nucleic acid interactions.

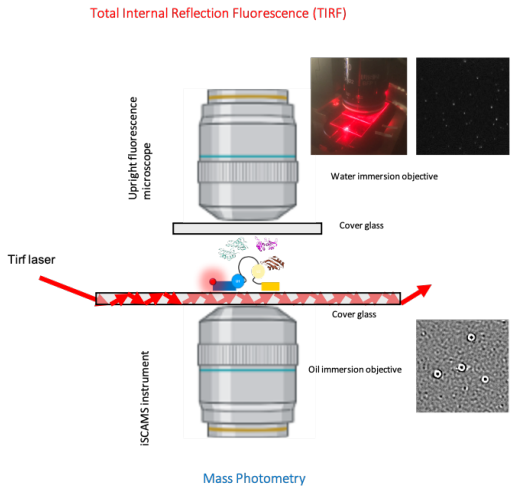


Fig abstract.png

Label-free tracking of proteins through plasmon enhanced interference

Wednesday, 22nd November - 17:55: (Room 608) - Oral

Mr. Matthew Peters [1], ***Mr. Declan McIntosh*** [1], ***Dr. Alexandra Branzan Albu*** [1], ***Dr. Cuifeng Ying*** [2], ***Dr. Reuven Gordon*** [1]

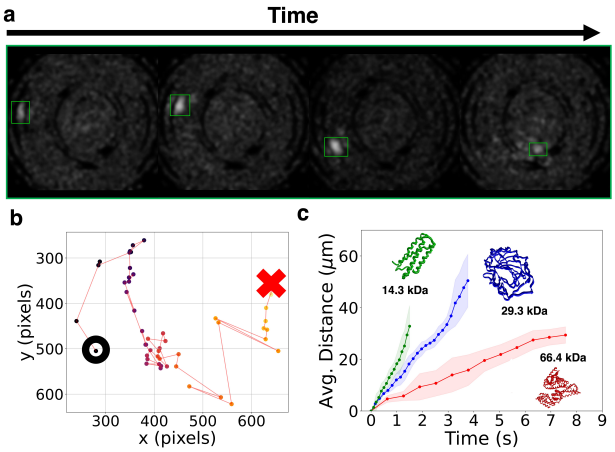
1. *Department of Electrical Engineering, University of Victoria, Victoria, B.C.,* ***2.*** *Advanced Optics and Photonics Laboratory, Department of Engineering, School of Science and Technology, Nottingham Trent University, Nottingham NG118NS, United Kingdom*

The dominant approaches to observing single proteins modify them with fluorescent labels and/or tethers that alter the biophysical properties and limit observation time. It is desirable to have complementary methods that can directly observe single unmodified proteins. This is challenging since particles much smaller than the wavelength of light have scattering that diminishes with the sixth power of the radius and quickly becomes insignificant. Interferometric scattering (iSCAT), plasmonic scattering (PSM), and nanochannel scattering microscopy (NSM) have detected unlabelled, large proteins. By combining surface plasmon interference with reflected laser interference, we track the diffusion of single unmodified proteins as small as 14kDa (r~2nm) in real time in plasmon-enhanced protein tracking with interference (PEPTI).

Gold double nanohole sample preparation was performed as described in past works. Proteins came from Sigma Aldrich MWGF70-1KT. Bovine Serum Albumin (BSA), Carbonic Anhydrase (CA), and Cytochrome C (CTC) were prepared with a concentration of 0.1wt%/vol in 0.01M phosphate buffer solution.

Surface plasmons are launched by focusing an 850nm laser on a gold nanostructure. Single proteins approach the nanostructure, and the surface plasmons interfere with the scattering of the protein. The evanescent nature of the surface plasmon means that smaller proteins get closer to the surface (on the order of nanometers), and the interference is stronger. We achieve a signal-to-noise ratio of 3.5 for a single 14.3kDa protein. Detection of a single CTC over time is shown in Figure 1(a), the tracked position of the protein is seen in Figure 1(b), and the molecular weight dependence on diffusion is seen in Figure 1(c) for three proteins (over 15 different observations). The results follow the expected trend that smaller proteins diffuse faster.

Scattering from the DNH is polarization sensitive, and aligning the laser polarization to excite both polarization components gave the best result for imaging. Significantly larger contrast is seen when compared to NSM, PSM, and iSCAT: neither plasmonic, nor reflection interference play the sole role in PEPTI. A combination of effects are present to provide such an exceptionally strong signal. The benefit of PEPTI is the minimal processing required, single frame removal enables real-time imaging of single proteins.



Pepti protein tracking.jpg

Cold phase transition in RNA

Wednesday, 22nd November - 18:12: (Room 608) - Oral

Dr. Paolo Rissone [1], ***Mr. Aurelien Severino*** [1], ***Dr. Isabel Pastor*** [1], ***Prof. Felix Ritort*** [1]

1. *University of Barcelona, Department of Condensed Matter Physics*

RNA's diversity of behaviors and structures has impacted life since *primordia.* The promiscuity of base pairing and stacking interactions makes RNA a unique biopolymer with many functions, from information carrier to regulatory and enzymatic activity. Single-molecule force spectroscopy [1] has revealed an extraordinary tool to investigate RNA folding [2, 3] in previously unexplored conditions.

Here, we present force spectroscopy measurements on RNA hairpin structures at near-to-freezing temperatures, showing that the most basic secondary structure element, a Watson-Crick hairpin, unexpectedly misfolds into a highly compact tertiary fold. RNA cold misfolding appears as a ubiquitous phenomenon triggered by the large flexibility of the RNA chain and specific heat change at low temperatures, facilitating the formation of non-native contacts, misfolding, and cold denaturation. Our results show a universal RNA phase transition at low temperatures, with maximum RNA stability at 5°C where water density is maximum and cold denaturation at -50°C. RNA cold misfolding opens a paradigm for novel RNA functionalities and catalytic activities at subfreezing temperatures.

1. Rissone, P., Pastor, I. and Ritort, F., 2023. *Unraveling RNA by Mechanical Unzipping*. arXiv preprint arXiv:2303.14065.

2. Rissone, P., Bizarro, C. V., & Ritort, F. (2022). *Stem–loop formation drives RNA folding in mechanical unzipping experiments.* Proceedings of the National Academy of Sciences, 119(3), e2025575119.

3. Rissone, P. and Ritort, F., 2022. *Nucleic Acid Thermodynamics Derived from Mechanical Unzipping Experiments*. Life, 12(7), p.1089.

Virus traps and other molecular machines of the future

Thursday, 23rd November - 09:00: (Auditorium) - Oral

Prof. Hendrik Dietz [1]

1. *Technical University of Munich*

TBD

Plasmonic and dielectric nanostructures for enhanced light harvesting, emission control, and nanometrology

Thursday, 23rd November - 09:40: (Auditorium) - Oral

Prof. Stefan Maier [1]

1. *Imperial College London*

The first part of my talk focuses on disordered arrays of plasmonic colloids that enable broadband optical absorption. This is due to equipartition of energy and convergence of internal mode lifetimes. The optical response can be tuned via coupling to an underlying Fabry-Perot cavity, enabling narrow reflection suitable for color generation. Surprisingly, the far-field properties, i.e. the perceived color in reflection, is independent of the colloidal material, with potential applications in solar light harvesting for energy conversion and photocatalysis. Due to reciprocity, such disordered surfaces can also be used to enhance light extraction from underlying high-index dielectric layers, via simple deposition of the disordered array on for example commercial GaN LEDs. In the second part I will present recent research on dielectric metasurfaces for enhanced light/matter interactions, such as control of non-linear emission of low-dimensional materials, strong coupling with intrinsic excitons, and chiral sensing.

Stochastic microscopy of living cells: towards a thermodynamic theory of biological performance (provisional)

Thursday, 23rd November - 10:50: (Auditorium) - Oral

Prof. Francisco Monroy [1]

1. *Complutense University of Madrid*

TBD

Label-free single-molecule detection of biomolecules in free motion using Nanofluidic Scattering Microscopy

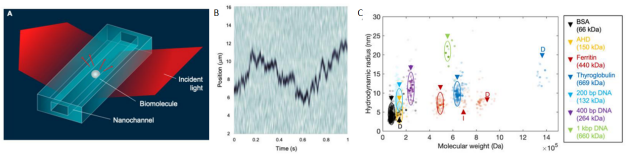
Thursday, 23rd November - 11:17: (Auditorium) - Oral

Dr. Tereza Roesel [1], ***Dr. Barbora Špačková*** [1]

1. *Institute of Physics Czech Academy of Sciences*

Label-free characterization aims to complement single-molecule fluorescence microscopy, in cases where the labelling with a fluorophore may perturb the chemical or structural properties of the studied biomolecule. An alternative approach, interferometric scattering microscopy (iSCAT), has allowed to resolve individual biomolecules in a label-free manner. However, iSCAT requires attaching biomolecules to a surface, possibly influencing the properties and reactivity of such molecules. Recently, the ground-breaking method of Nanofluidic Scattering Microscopy (NSM) has been developed [1]. The NSM enables the label-free imaging of individual biomolecules diffusing inside a nanofluidic channel. It overcomes the limitation of extremely low efficiency of Rayleigh scattering from very small biomolecules. The biomolecules are introduced into nanofluidic channels fabricated onto an oxidized silicon wafer. The nanochannels themselves exhibit strong light-scattering properties and are therefore detectable by dark-field optical microscopy. The light scattered by the biomolecule and the nanochannel interferes, culminating in an optical contrast that is several orders of magnitude greater than the contrast produced solely by a biomolecule positioned outside the nanochannel. Consequently, the individual biomolecule becomes directly observable, enabling precise tracking of its movement within the nanochannel over time. The nanochannels are tens to hundreds of nanometres wide (adjusted to the targeted biomolecule size) and tens of micrometres long. A distinctive characteristic of the NSM technique lies in its capability to not only image individual biomolecules but also to determine two of their attributes: molecular weight (through its effect on optical contrast) and hydrodynamic radius (via statistical analysis of its stochastic motion), from which additional insights into the biomolecule's conformation can be inferred. In its first iteration, NSM has successfully detected biomolecules down to the range of tens of kDa. This presentation will delve into innovative approaches aimed at pushing the boundaries of NSM capabilities. Emphasis will be placed on harnessing alternative optical configurations of the experimental setup, alongside employing cutting-edge data processing techniques. These enhancements pave the way for groundbreaking applications in biomolecular research, enabling the scrutiny of biomolecular interactions at the single-molecule level, all in a label-free and tether-free format.

[1] Špačková, B. et al. Nat. Methods **19**, 751– 758 (2022).



Nsm principle.png

Real-time microsecond dynamics of single biomolecules probed by plasmon-enhanced fluorescence

Thursday, 23rd November - 11:34: (Auditorium) - Oral

Mr. Sjoerd Nooteboom [1], ***Mr. Kasper Okholm*** [2], ***Mr. Vincenzo Lamberti*** [1], ***Mr. Bas Oomen*** [1], ***Prof. Duncan Sutherland*** [2], ***Prof. Peter Zijlstra*** [1]

1. *Eindhoven University of Technology,* ***2.*** *Aarhus University*

Introduction

Biomolecules such as DNA and proteins are at the basis of all processes in living organisms. Living processes involve intra- and intermolecular dynamics on timescales from nanoseconds to many seconds, including (un)folding, conformational dynamics, and transient interactions. Understanding such mechanisms is key to understanding disease and subsequent drug development. Typically, molecular mechanisms are studied using single-molecule fluorescence or Förster resonance energy transfer (smFRET) [1]. However, the brightness of the required fluorophores is limited by saturation and blinking, prohibiting direct observation of sub-ms processes in most cases [2]. Recently, fluorescence enhancement in zero-mode waveguides was shown to enable sub-ms observation of fluorophores [3].

Methods

In this work, we use the strong plasmonic fluorescence enhancement provided by DNA functionalized gold nanorods [4] to probe real-time single-molecule DNA dynamics [5] (Fig. 1). The plasmon increases the saturation photon count rate by enhancing the fluorophore's radiative decay rate, while we use chemical additives to suppress blinking. Real-time microsecond dynamics are then captured on an avalanche photodiode.

Results and Discussion

We demonstrate the possibility to harvest on the order of 10 million fluorescence photons/s from a single biomolecule (Fig. 2), and use the dramatically increased signal-to-noise ratio at short timescales to study microsecond dynamics of ultralow affinity DNA interactions for the first time (Fig. 3). In addition, we also exploit the strong field gradients around the nanoparticle, which lead to spatial variations in fluorescence enhancement factor. This allows us to directly observe conformational dynamics of multivalent DNA constructs on timescales down to 10 us (Fig. 4). Our results pave the way towards real-time microsecond studies of more complex biomolecular systems such as enzymes and intrinsically disordered proteins.

[1] Bandyopadhyay, D.; Mishra, P. P. (2021). Front. Mol. Biosci., 8 (September), 1–18.

[2] Ha, T.; Tinnefeld, P. (2012). Annu. Rev. Phys. Chem., 63 (1), 595–617.

[3] Tiwari, S.; Wenger, J. et al. (2023). Adv. Opt. Mater. 11 (13), 2300168.

[4] Wang, Y.; Zijlstra, P. et al. (2020). J. Phys. Chem. Lett., 11, 1962–1969.

[5] Nooteboom, S. W.; Zijlstra, P. et al. (2022). Small, 18 (31), 2201602.

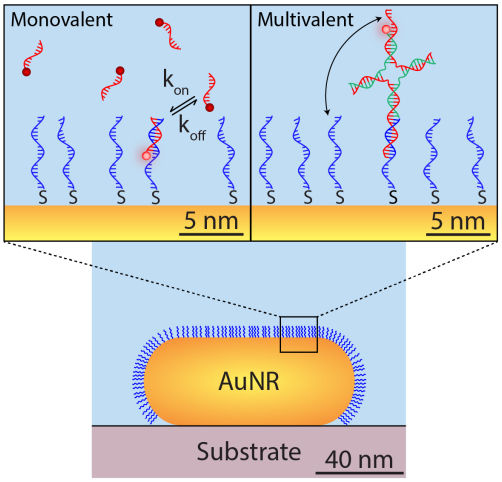


Figure 1 - overview of the method.png

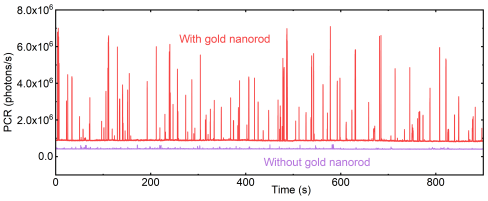


Figure 2 - enhanced dna-paint signal.png

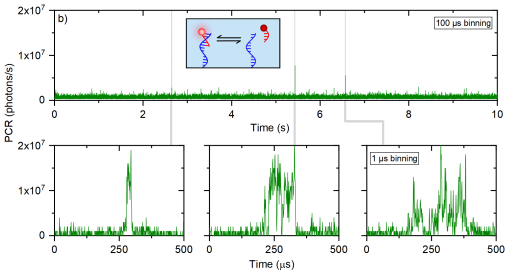


Figure 3 - dna interaction with only 5 base pairs.png

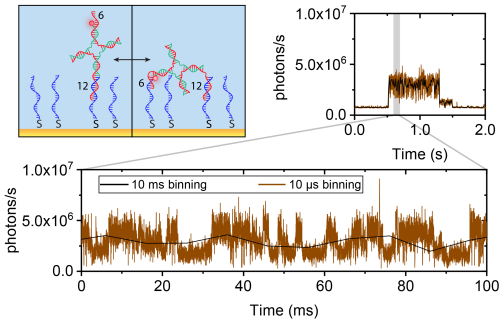


Figure 4 - multivalent interaction.png

Minitweezers 2.0: paving the way for fully autonomous optical tweezers experiments

Thursday, 23rd November - 11:51: (Auditorium) - Oral

Mr. Martin Selin [1], ***Dr. Antonio Ciarlo*** [2], ***Dr. Giuseppe Pesce*** [3], ***Dr. Joan Camuñas-Soler*** [4], ***Dr. Vinoth Sundar Rajan*** [5], ***Prof. Fredrik Westerlund*** [6], ***Prof. Marcus Wilhelmsson*** [7], ***Dr. Isabel Pastor*** [8], ***Dr. Felix Ritort*** [9], ***Dr. Steven Smith*** [10], ***Prof. Giovanni Volpe*** [2]

1. *Physics Department, University of Gothenburg, 412 96 Gothenburg,* ***2.*** *University of Gothenburg,* ***3.*** *University of Naples Federico II; University of Gothenburg,* ***4.*** *Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, University of Gothenburg;Wallenberg Centre for Molecular and Translational Medicine, Sahlgrenska Academy, University of Gothenburg,* ***5.*** *Department of Life Sciences, Chalmers University of Technology, 41296 Gothenburg; Department of Chemistry and Chemical Engineering, Chalmers University of Technology, 41296 Gothenburg,* ***6.*** *Department of Life Sciences, Chalmers University of Technology, 41296 Gothenburg,* ***7.*** *Department of Chemistry and Chemical Engineering, Chalmers University of Technology, 41296 Gothenburg,* ***8.*** *University of Barcelona, Department of Condensed Matter Physics,* ***9.*** *Small Biosystems Lab, Departament de Física de la Materia Condensada, Facultat de Física, Universitat de Barcelona, 08028 Barcelona, Spain,* ***10.*** *Steven B. Smith Engineering, Los Lunas, New Mexico*

Since their invention by Ashkin et al. in the 1980s, optical tweezers have evolved into an indispensable tool in physics, especially in biophysics, with applications spanning from cell sorting to stretching single DNA strands. By the 2000s, commercial systems became available. Nevertheless, owing to their unique requirements, many labs prefer to construct their own, often drawing inspiration from existing designs.

A prominent optical tweezers design is the “miniTweezers” system, pioneered by Bustamante’s group in the late 1990s. This system has been widely adopted globally for force spectroscopy experiments on single molecules, including DNA, proteins, and RNA.

In this presentation, we unveil an advanced iteration of the miniTweezers. By enhancing its control and acquisition capabilities, we’ve augmented its versatility, enabling new experiment types. A significant breakthrough is the integration of real-time image feedback, which paves the way for automated procedures via deep learning-based image analysis, the first of which we demonstrate in this presentation.

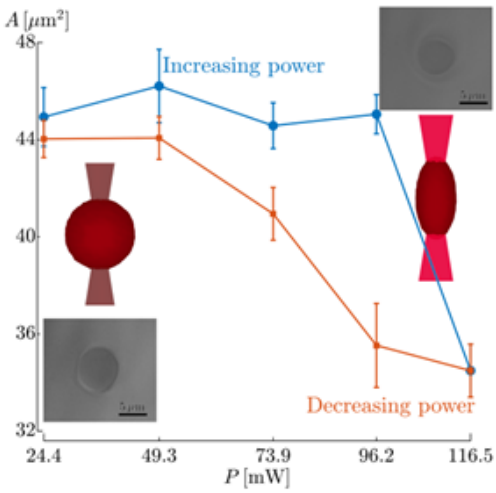
We showcase this system’s capabilities through three distinct experiments:

A pulling experiment on a 1/3 λ DNA strand. By tethering DNA between two polystyrene beads - one anchored in a micropipette and the other manipulated by the tweezer - we illustrate near-complete automation, with the system autonomously handling bead trapping, attachment of the DNA and the pulling procedure.

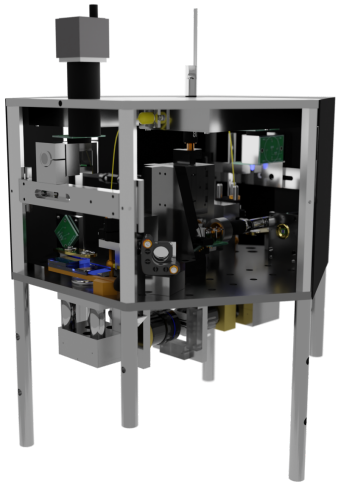
An exploration of Coulomb interactions between charged particles. Here, one particle remains in a micropipette, while the other orbits the stationary bead, providing a 3D map of the interaction.

A non-contact stretching experiment on red blood cells is conducted under low osmotic pressure conditions. Modulating the laser power induces cell elongation along the laser's propagation direction. By correlating this elongation with the optical force exerted by the lasers, we present a simple and non-invasive method to measure membrane rigidity.

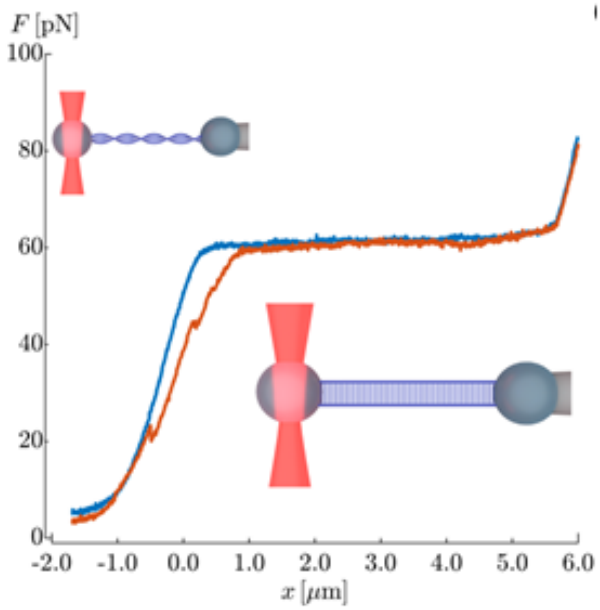
In summary, these advancements mark a significant leap in the capabilities and applications of optical tweezers in biophysics. As we push the boundaries of automation and precision, we envision a future where such instruments can unravel even more intricate molecular interactions and cellular mechanics, setting the stage for groundbreaking discoveries.



Redbloodcellsstretching.png



Instrument.png



Dnastretching.png

Pushing the limits of detection of biomolecules with light and machine learning

Thursday, 23rd November - 10:50: (Room 607) - Oral

Prof. Giovanni Volpe [1]

1. *University of Gothenburg*

The domain of biomolecule analysis is witnessing a paradigm shift with the advent of advanced label-free detection techniques, such as nanofluidic scattering microscopy (NSM). NSM ushers in a novel era of detecting and analyzing single biomolecules in real-time without the need for surface binding—addressing a significant limitation of current methods. At the heart of this advancement is the integration of sophisticated machine learning algorithms with NSM, which markedly amplifies its detection capabilities. This study illustrates how machine learning algorithms are tailored to discern intricate patterns in light scattering data, enabling the quantification of molecular weight and hydrodynamic radius with unprecedented precision. The versatility of machine learning further extends to the rapid classification of biomolecular conformations, monitoring of dynamic conformational changes, and identification of interactions within biofluids. Specifically, the application of this technology in analyzing conditioned cell culture medium reveals its potential in detecting extracellular vesicles and probing into single-cell secretomes. This convergence of machine learning with NSM not only pushes the limits of detection but also opens up new avenues for real-time analysis of biomolecules, potentially revolutionizing our understanding of cellular processes and molecular diagnostics.

Single-layer MoS2 Nanopores for Coarse-Grained Sequencing of Proteins

Thursday, 23rd November - 11:17: (Room 607) - Oral

Dr. Adrien Nicolaï [1], ***Ms. Andreina Urquiola Hernandez*** [1], ***Dr. Patrice Delarue*** [1], ***Dr. Christophe Guyeux*** [2], ***Prof. Patrick Senet*** [1]

1. *Université de Bourgogne,* ***2.*** *Université de Franche Comté*

Solid-State Nanopores (SSN) made of 2-D materials such as molybdenum disulfide (MoS_2) have emerged as one of the most versatile sensors for single-biomolecule detection, which is essential for early disease diagnosis (biomarker detection). One of the most promising applications of SSN is DNA and protein sequencing, at a low cost and faster than the current standard methods. The detection principle relies on measuring the relatively small variations of ionic current as charged biomolecules immersed in an electrolyte traverse the nanopore, in response to an external voltage applied across the membrane. The passage of a biomolecule through the pore yields information about its structure and chemical properties, as demonstrated experimentally particularly for DNA molecules. Indeed, protein sequencing using SSN remains highly challenging since the protein ensemble is far more complex than the DNA ensemble [1].

In this work, we performed extensive unbiased all-atom classical Molecular Dynamics simulations to produce data of translocation of biological peptides through single-layer MoS_2 nanopores. Peptide made of the 20 different amino acids were chemically linked to a short polycationic charge carrier [2]. First, ionic current time series were computed from MD and peptide-induced blockade events were extracted and characterized using structural break detection. Second, clustering of ionic current drops and duration using Gaussian Mixture Model was applied. Using this technique, we demonstrate that each amino acid presents a large diversity of ionic current characteristics, however, charged amino acids were distinguished from the others. Finally, classification was also implemented to identify residue motifs inside the nanopore. We show that the classification performance depends on the position of the amino acid in the sequence, as well as its charge property (neutral, positive or negative). These promising findings may offer a route toward protein sequencing using MoS_2 solid-state nanopores.

References

[1] **A. Nicolaï** and P. Senet. Challenges in Protein Sequencing using MoS_2 Nanopores, **2022**. In Bowen, W., Vollmer, F., Gordon, R. (eds) Single Molecule Sensing Beyond Fluorescence. Nanostructure Science and Technology. Springer, Cham. https://doi.org/10.1007/978-3-030-90339-8_11

[2] M. D. Barrios Pérez et al. Improved model of ionic transport in 2-D MoS_2 membranes with sub-5 nm pores. *Appl. Phys. Lett.*, **2019**, 114(2):023107.

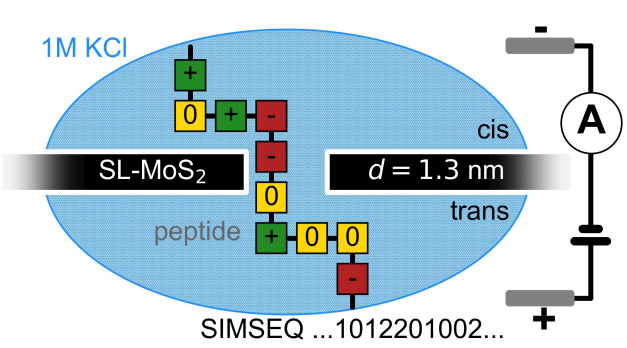


Fig toc.jpg

Machine-learning-based Single-molecule Quantification of Circulating MicroRNA Mixtures

Thursday, 23rd November - 11:34: (Room 607) - Oral

Prof. Yuval Ebenstein [1]

1. *Tel Aviv University*

MicroRNA (miR) are small non-coding RNAs that regulate gene expression and are emerging as powerful indicators of diseases. MiRs are secreted to blood plasma and thus may report on systemic aberrations at an early stage via liquid biopsy analysis. We present a method for multiplexed single-molecule detection and quantification of a selected panel of miRs. The proposed assay does not depend on sequencing, requires less than one ml of blood and provides fast results by direct analysis of native, unamplified miRs. This is enabled by a novel combination of compact spectral imaging together with a machine learning based detection scheme that allows simultaneous multiplexed classification of multiple miR targets per sample. The proposed end-to-end pipeline is extremely time efficient and cost-effective. We benchmark our method with synthetic mixtures of three target miRs, showcasing the ability to quantify and distinguish subtle ratio changes between miR targets.

The Mean Back Relaxation: a new observable to quantify non-equilibrium from simple trajectories

Thursday, 23rd November - 10:50: (Room 608) - Oral

Prof. Timo Betz [1]

1. *Georg August University Göttingen*

Understanding life is arguably among the most complex scientific problems faced in modern research. From a physics perspective, living systems are complex dynamic entities that operate far from thermodynamic equilibrium. This active, non-equilibrium behavior, with its constant hunger for energy, allows life to overcome the ever-dispersing forces of entropy and drives cellular organisation. Unfortunately, most analysis methods provided by the toolbox of statistical mechanics cannot be used in such non-equilibrium situations, forcing researchers to use sophisticated and often invasive approaches to study the mechanistic processes inside living organisms. Here we introduce a new observable coined the mean back relaxation. Based on three-point probabilities, and exploiting Onsager's regression hypothesis, it extracts additional information from passively observed trajectories compared to classical observables such as the mean squared displacement. The mean back relaxation is able to detect broken detailed balance in systems confined in stationary or actively diffusing potentials. We show in experiment and theory that it gives access to the non-equilibrium generating energy and the viscoelastic material properties of a well-controlled artificial system, and we experimentally demonstrate that it does so even for a variety of living systems, revealing an astonishing relation between the mean back relaxation and the active mechanical energy. Based on these findings, we conclude that it acts as a new marker of non-equilibrium dynamics. Combining, in a next step, passive fluctuations with the extracted active energy allows to overcome a fundamental barrier in the study of living systems; it gives access to the viscoelastic material properties from passive measurements.

Dielectrophoresis as a label-free tool to study properties of single sub-30 nm nanoparticles

Thursday, 23rd November - 11:17: (Room 608) - Oral

Dr. Sergii Pud [1], ***Mr. Jacco Ton*** [2], ***Dr. Théo TRAVERS*** [2], ***Mr. Jamal Soltani*** [1], ***Mrs. Janike Bolter*** [1], ***Mr. Daniel Wijnperle*** [1], ***Mr. Dmytro Shavlovskyi*** [1], ***Prof. Michel Orrit*** [2]

1. *BIOS Lab-on-a-Chip at MESA+ Institute for Nanotechnology, University of Twente, Enschede,* ***2.*** *Huygens-Kamerlingh Onnes Laboratory, Leiden University*

Dielectrophoresis (DEP) is a phenomenon where polarizable objects experience a force in an inhomogeneous electric field. This force has been used for manipulating biological entities such as cells, vesicles, and proteins at the microscale. We aim to harness the complexity of DEP to develop a technology that allows for real-time analysis of the physical parameters of trapped nanoparticles and proteins.

Methods

Our experimental setup employs gold nanoelectrodes with gaps as narrow as 20 nm (Figure 1). We conduct imaging through a custom-built confocal iScat microscope, which is capable of combined scattering and fluorescence detection. One of the unique features of this microscope is its real-time monitoring capability. This enables the system to trigger a response from the DEP trap immediately when a particle enters, ensuring precise control over the trapping process

Results

We have successfully trapped sub-30 nm polystyrene particles using DEP at sub-5V peak-to-peak voltages. Once a particle is trapped, our system offers the flexibility to release it on demand(Figure 2). This feature enables us to study the interaction of individual particles with the electric field. To understand the minimum voltage required for particle release, we conducted an experiment where the amplitude of the AC voltage applied to the trap is gradually reduced. This methodology allows us to quantify the release voltages for each particle and to calculate the distribution of these voltages (Figure 3).

Discussion

Figure 2 illustrates the trapping of a 30 nm polystyrene particle. Subfigures 2B and 2C display the fluorescence and scattering signatures of the trapped particle. Occasionally, we observe particles passing by the trap without being captured (Figure 2B), yet showing no corresponding scattering signature, which suggests that the scattering detection volume is smaller than fluorescence one. This could be caused by the plasmon resonance shift of the DEP electrodes contributing to the scattering response. Figure 3B reveals a broad distribution of release voltages, reflecting the inhomogeneity in particle sizes and the stochastic nature of the release process. We plan to refine our experimental setup to quantify the potential of DEP traps more accurately by using autocorrelation of the fluorescent and scattering signals.

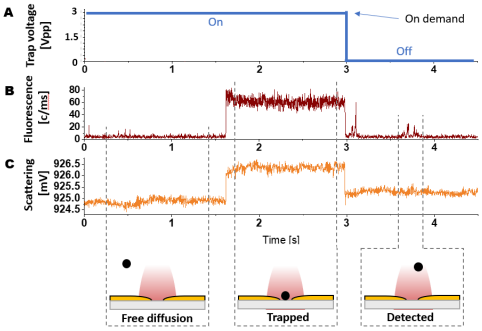


Figure 2.png

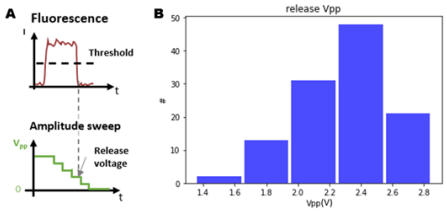


Figure 3.png

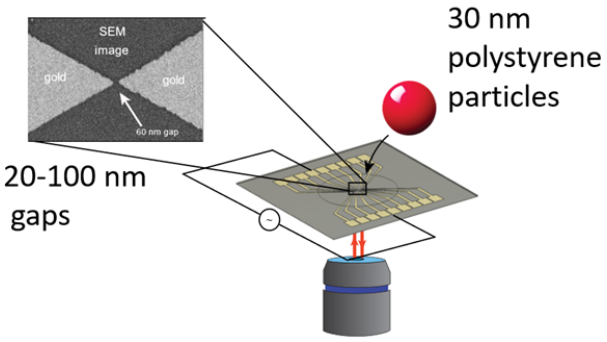


Figure 1.png

Development of Plasmonic-Nanopore Sensing for Thermally Controlled Biopolymer Sequencing Applications

Thursday, 23rd November - 11:34: (Room 608) - Oral

Dr. Tadas Penkauskas [1], ***Mr. Addhyaya Sharma*** [1], ***Mr. Shankar Haridas*** [1], ***Dr. Joseph Robertson*** [1]

1. *National Institute of Standards and Technology*

Resistive-pulse nanopore techniques have enabled rapid, label-free, amplification-free, low cost and high-throughput single-molecule analysis. In a typical nanopore measurement, ionic current is measured as it passes through a nanopore in response to an applied electric field. When individual molecules enter the cavity of the nanopore, a resistive pulse is measured which can be used to characterize the size, shape, structure, dynamics and chemical interactions of the analyte molecule[1]. Combination of a high-resolution nanopore, a molecular motor to control the translocation rate and advanced computer algorithms has led to successful commercialization of nanopore-based DNA sequencing[2]. This progress in genomics offers a roadmap to similar field-revolutionizing tool for proteomics and clinical diagnostics. However, protein sequencing is still in its early stages and faces multiple challenges unique to protein nature, such as structured protein unfolding, translocation control and discrimination of the 20 proteinogenic amino acids (AAs).

To access the single-molecule level manipulation and characterization of biopolymers, we are developing novel protein-DNA origami hybrid structures that allow precise positioning of localized surface plasmon resonance (LSPR) particles (Figure 1). Such setup facilitates generation of precise thermal gradients at and across the nanopore in yoctoliter volumes[3]. In order to analyze the assembly and its kinetics, we implemented multiple surface-sensitive techniques and electrochemical methods. To predict the temperature distribution within the structure, we employ COMSOL simulations to investigate the 3D temperature gradient resulting from laser-induced heating of gold nanoparticles (Figure 2). We also explore the effects of various parameters such as the number, position, and size of the gold nanoparticles on the resulting temperature distribution. In parallel, translocation of short polypeptide is being analyzed in order to investigate conformational dynamics and transport, as well as to establish the unique signals for identification of individual AAs.

Incorporation of such structures with rapid optical temperature control, has the potential to increase speed and efficiency of nanopore measurements, opening the door for applications in a wide array of difficult to control and unstable systems.

1. J. Robertson, et al. BBA-Biomembr. 2021. https://doi.org/10.1016/j.bbamem.2021.183644.
2. M. Jain, et al. Genome Biol. 2016. https://doi.org/10.1186/s13059-016-1103-0.
3. J. Reiner, et al. JACS 2013. https://doi.org/10.1021/ja309892e

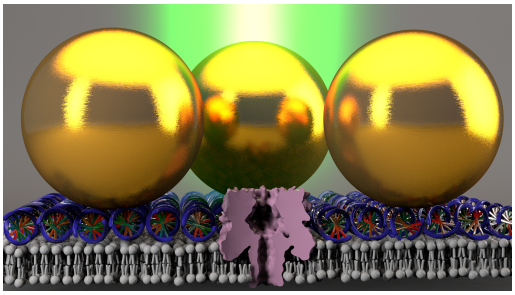


Figure1 plasmonic nanopore.png

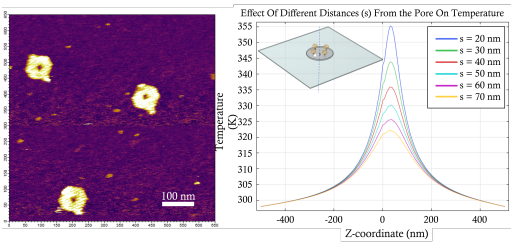


Figure2 afm comsol.png

Single-Molecule Thermodynamic Penalties Applied by Whispering Gallery Mode Biosensors

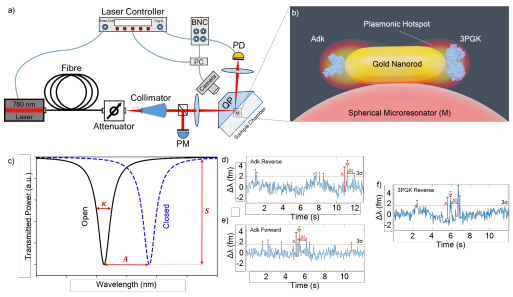
Thursday, 23rd November - 11:51: (Room 608) - Oral

Mr. Matthew Houghton [1], ***Dr. Nikita Toropov*** [2], ***Dr. Stefan Bagby*** [3], ***Prof. Frank Vollmer*** [1]

1. *University of Exeter,* ***2.*** *University of Southampton,* ***3.*** *University of Bath*

Whispering gallery mode (WGM)-based plasmonically enhanced microcavity sensors are proving to be an excellent tool to study biomolecules. Advantages, including single-molecule sensing and including lack of labels, could allow native properties of these biomolecules to be investigated. Recently, plasmonically-enhanced WGM biosensors have been used to measure thermodynamic quantities such as the specific heat capacity of activation of enzyme-mediated catalysis (1). Based on this technology, we introduce a new method for measuring thermodynamic penalties applied to enzyme conformational changes in the form of optical forces that may be used to control turnover. We introduce an equation which links the properties of the sensor (linewidth, coupling efficiency, wavelength, input laser power) with amplitudes of wavelength shifts when an enzyme undergoes turnover in the enhanced near-field of the PE-WGM sensor, calculating the work done by the sensor on the enzyme. The relationship between WGM intensity and work-done appears to be linear when investigating two enzymes: adenylate kinase (Adk), in both the forward and reverse directions, and 3-phosphoglycerate kinase (3PGK). The gradient of this linearity is different, appearing to correlate with the enzyme volume. Jarzinski's estimator was then used to calculate the the free energy change associated with this process: termed here as the free-energy penalty applied to the enzyme undergoing turnover in the near-field. Finally, we control these values for their radius and find the force applied to each enzyme is constant, and differences in work due to this force being applied over a greater distance. This could provide the basis of controlling enzyme turnover by thermodynamics means- we make the transition from open-to-closed (or vice-versa) non-spontaneous and hence prevent turnover from taking place.

(1) S. Subramanian *et al.* Sensing enzyme activation heat capacity at the single-molecule level using gold-nanorod-based optical whispering gallery modes. *ACS applied nano materials* **4**, 4576-4583, doi:10.1021/acsanm.1c00176 (2021).



Summary figure 1 v2.4.png

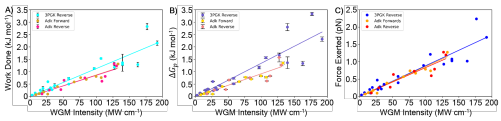


Figure 2 v.4.png

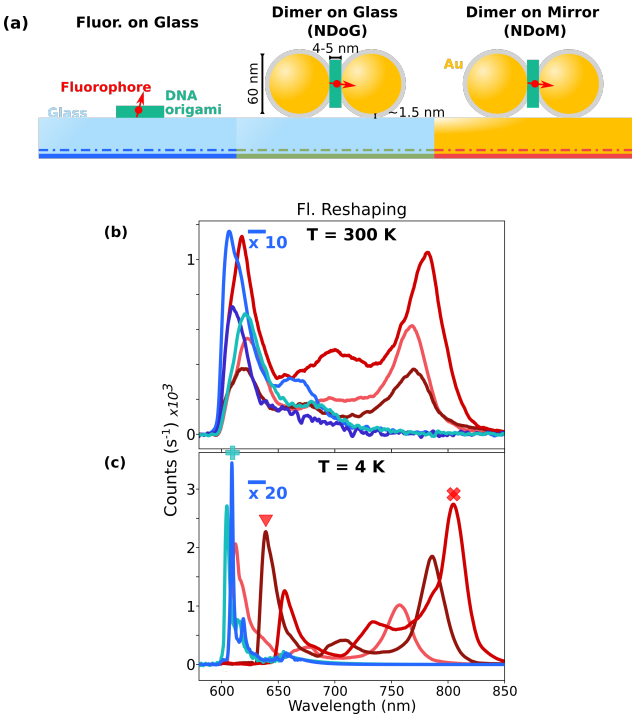
Spectral reshaping of a single fluorophore in a plasmonic cavity

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Mr. Sachin Verlekar [1], ***Ms. Maria Sanz-Paz*** [2], ***Dr. Mario Zapata*** [3], ***Dr. Mauricio Pilo-Pais*** [2], ***Prof. Ruben Esteban*** [3], ***Prof. Javier Aizpura*** [3], ***Prof. Christophe Galland*** [1]

1. *Institute of Physics, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland,* ***2.*** *Department of Physics, University of Fribourg, Fribourg CH-1700, Switzerland,* ***3.*** *Materials Physics Center CSIC-UPV/EHU, 20018 Donostia-San Sebastián, Spain*

Efficiently exciting, detecting and controlling the light emitted by individual molecules is instrumental to a number of novel technologies ranging from bio-imaging and molecular sensing to quantum optics and nanophotonics. A particularly powerful and versatile method to tailor the properties of light emission is to engineer the local photonic environment through the coupling to a nanocavity. In this way, one can modify the decay pathways, boost the outgoing photon rate, activate new emission channels and reshape the fluorescence spectrum. Here, we discover that the coupling of individual molecules to plasmonic nanocavities seems to introduce dephasing channels not predicted by the conventional Purcell effect. The excess dephasing is evidenced by measurements at cryogenic temperatures; when a fluorophore is placed in the gap of a plasmonic dimer on a metallic film, cavity-enhanced emission can be observed far detuned from the zero-phonon-line (ZPL) – at detunings that are up to two orders of magnitude larger than the fluorescence linewidth of the bare dye. This behavior is well reproduced by accounting not only for the simulated local density of optical states but also for a dephasing rate that is at least 4 times larger than predicted by the Purcell effect alone.



S3ic fig.png

Plasmon-enhanced fluorescence probing of fast protein interactions using low-quantum-yield dyes

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Mr. Roy Teeuwen [1], ***Dr. Souvik Ghosh*** [2], ***Dr. Johannes Broichhagen*** [2], ***Prof. Maarten Merkx*** [1], ***Prof. Peter Zijlstra*** [1]

1. *Eindhoven University of Technology,* ***2.*** *Leibniz-Forschungsinstitut für Molekulare Pharmakologie*

Introduction

Sub-populations of abnormal protein-protein interactions (PPIs) can cause severe diseases. Single-molecule-fluorescence microscopy enables characterization of PPIs. However, physiologically, many protein species occur at high nanomolar to micromolar concentrations. Matching such conditions experimentally requires corresponding probe concentrations, causing an overwhelming background signal. Plasmon-enhanced-fluorescence (PEF)[1] has enabled the use of such high concentrations by exploiting the strongly confined near-field around a plasmonic nanoparticle in combination with solution-phase quenchers to reduce the background[2]. However, these quenchers strongly perturb PPIs, thereby prohibiting further application of PEF to proteins. Here, we overcome this challenge by custom conjugation of low-QY dyes to the proteins, enabling the study of protein interactions using PEF.

Methods

As model system we employ PDZ(protein)–peptide interactions. Using cysteine-maleimide chemistry, proteins are labeled with either high or low QY dyes (ATTO643-Mal, QY~62%, and ATTOMB2-Mal, QY~1%). Immobilized gold nanorods are decorated with the docking peptide. Labeled PDZ proteins that bind docking peptides on the nanorods are predicted to emit strongly enhanced fluorescence (Figure 1). Correspondingly, single-particle fluorescence time-traces are used to follow the protein-peptide interactions.

Results

PDZ labeled with the high QY dye, in combination with a common solution-phase quencher (tryptophan), showed strongly perturbed kinetics (Figure 2). Using the low QY dye without quencher efficiently decreased the background signal (Figure 3). As predicted by recent simulations[3], the low QY dyes' emission was strongly enhanced by the particles' plasmons, resulting in strong fluorescence readout signals. Corresponding fluorescence time-traces (Figure 4) show high binding signal intensities, whilst retaining a minimal background at high (100 nM) protein concentrations.

Discussion

The results with the high QY dye-protein conjugates combined with solution-phase quenchers clearly showed the requirement for another approach to minimize the background. To this end, the use of an inherently low QY dye successfully minimized the background, whilst retaining strong fluorescence readout signals due to the PEF approach. Correspondingly, the developed approach paves the way towards studying unperturbed PPIs at physiologically relevant concentrations, with single-molecule resolution.

References

(1)Wang,Y.;Horáček,M.;Zijlstra,P. *J.Phys.Chem.Lett.* **2020**, *11* (5), 1962–1969.

(2)Punj,D.;Regmi,R.;Devilez,A.;Plauchu,R.;Moparthi,S.B.;Stout,B.;Bonod,N.;Rigneault,H.;Wenger,J. *ACS Photonics* **2015**, *2* (8), 1099–1107.
(3)Lu,X.;Ye,G.;Punj,D.;Chiechi,R.C.;Orrit,M. *ACS Photonics* **2020**, *7* (9), 2498–2505.

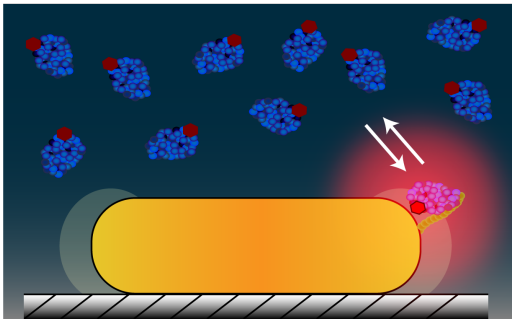


Figure 1 binding of labeled protein to immobilized binding partner results in strongly enhanced fluorescence signal..png

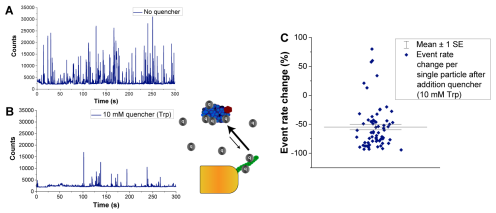


Figure 2 fluorescence time-trace of a particle before a and after b adding quencher. c event rate changes of many particles after addition quencher..png

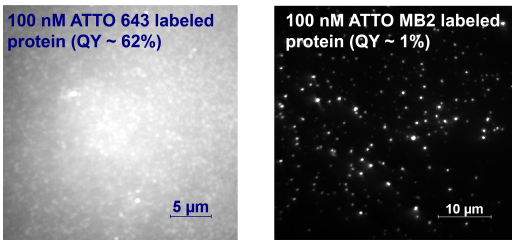


Figure 3 optical field of view of outlined assay using 100 nm of protein labeled with a common high qy dye left and with a low qy dye right ..png

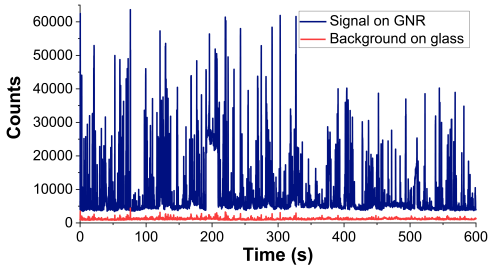


Figure 4 particle time-trace at 100 nm of low-qy-dye-protein. protein-peptide binding yields strong bursts whilst the background remains minimal..png

Measuring Nanobody Kinetics at the Single-Molecule Level

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Ms. Ellyn Redheuil [1], ***Dr. Sebastian Hutchinson*** [2], ***Ms. Ghada Mansour*** [1], ***Dr. Adeline Pichard-Kostuch*** [2], ***Mr. Ahmed Rehan*** [1], ***Dr. Marco Ribezzi-Crivellari*** [2], ***Prof. Andrew D. Griffiths*** [1]

1. *Laboratoire de Biochimie, ESPCI Paris, Université PSL, CNRS UMR 8231, Paris, France.,* ***2.*** *Quantum-Si, France*

Antibodies are a paradigm for high-affinity, protein-based binding reagents and are extremely important in biotechnological, diagnostic, and therapeutic applications. Of special interest are nanobodies, recombinant variable domains from heavy-chain-only antibodies. Nanobodies have several advantages: their small molecular weight, superior solubility and stability and clearance rate. One particular use of nanobodies is their use in imaging, which requires tailoring affinity and specificity for their targets. Despite their interest, nanobodies have only recently been used in single-molecule assays, e.g. PAINT imaging, which require careful tuning of their kinetic properties.

In this work we demonstrate the detection of nanobody-antigen binding at the single-molecule level. We first demonstrate the measurement of interaction kinetics between an immobilised GFP target and a LaG-16 antibody in solution, with similar results to bulk-derived kinetics constant. We then demonstrate the same kind of experiments in a more complex situation with multiple nanobodies.

A unified approach based on dielectrophoresis and SERS for protein transport and sensing

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Dr. Siarhei Zavatski [1], ***Prof. Olivier Martin*** [1]

1. *Nanophotonics and Metrology Laboratory (NAM), Ecole Polytechnique Fédérale de Lausanne (EPFL)*

The development of single-molecule sensing techniques relies heavily on devices innovation. The key component of these sensing devices is their ability to transport objects in and out of the sensing area. Ultimately, this must ensure delicate and fast spatial manipulation, down to the nanoscale. Dielectrophoresis (DEP) has established itself as a particularly well-suited experimental technique that meets this requirement. However, the current DEP theory does not provide a suitable accuracy to model molecular DEP behaviour, especially for proteins. Nevertheless, experimental protein manipulation by DEP has been efficiently conducted since the 1990s.

To support a smooth transition from the mature DEP framework at the microscale towards applications for nanosized molecular particulates, in this presentation, we focus on acquiring the currently missing experimental data on the magnitude of the so-called Clausius-Mossotti (CM) factor, which defines the protein polarizability in a nonuniform electric field and, subsequently, the DEP force. To achieve this, we utilize an innovative dielectrophoretic platform in the form of metallic sawtooth microelectrode arrays with various gap distances between adjacent electrode pairs (Figure 1). The CM factor is determined for three fluorescently labeled proteins (lysozyme, bovine serum albumin (BSA), and lactoferrin) through the measurement of the fluorescence intensity after they have been trapped near electrodes. This way, we can identify the minimum electric field gradient required to overcome dispersive forces (Figure 2). By careful elimination of various sources of experimental errors, we show the significant discrepancy between the obtained CM values and the current DEP model predictions. The results obtained in this work may serve as a quantitative reference to guide further developments in protein DEP theories.

We also combine our DEP platform with Ag nanoparticles fabricated by the solid-state dewetting approach (Figure 3) and acquire surface-enhanced Raman scattering (SERS) spectra of BSA (Figure 4) to reveal the potential of applying DEP for upgrading current sensing protocols. A long-term objective is to construct a composite microfluidic device capable of simultaneous particle separation, transport, trapping, and sensing. Such a device may not only push the limits of the forefront micro- and nanosensing techniques but also become extremely useful for daily life applications.

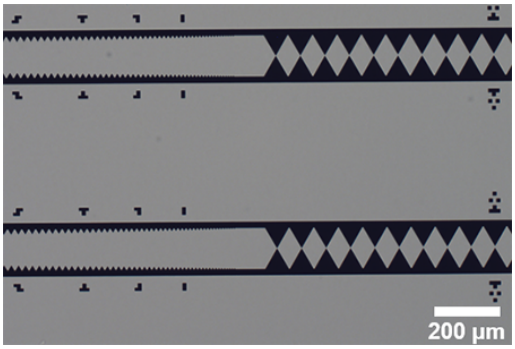


Figure 1 - optical image of metallic sawtooth microelectrode arrays with various gap distances between adjacent electrode pairs.png

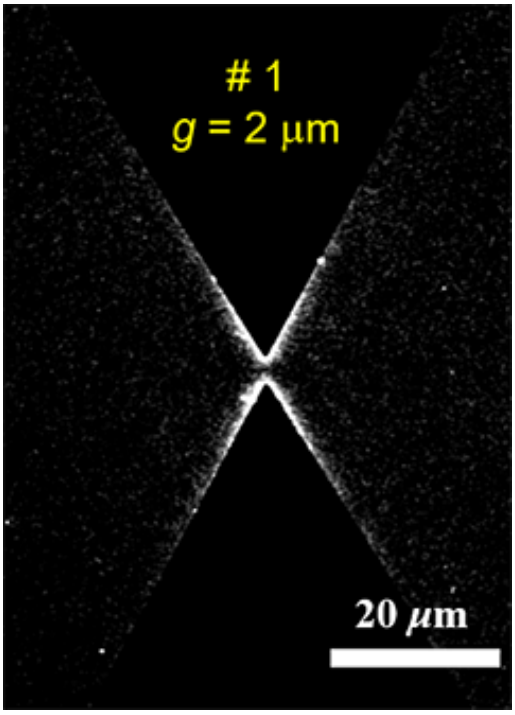


Figure 2 - fluorescence image acquired after protein dep trapping near sawtooth microelectrodes.png

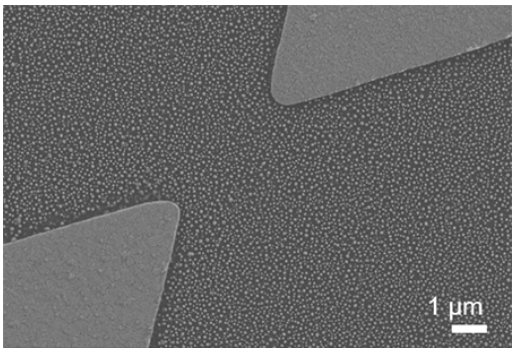


Figure 3 - sem image of dep microelectrodes after fabrication of ag nanoparticles.png

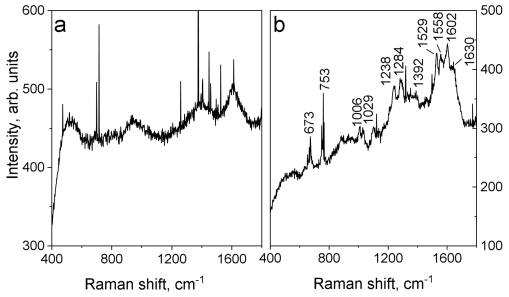


Figure 4 - sers spectra of bsa acquired by dep microelectrodes covered by ag nanoparticles before a and after b conduction of dep trapping experiment.png

Ambient Electrospray Deposition: an Efficient Technique to Immobilize Laccase on cheap electrodes with unprecedented Reuse and Storage Performances

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Dr. Mattea Carmen Castrovilli [1], ***Dr. Emanuela Tempesta*** [2], ***Dr. Antonella Cartoni*** [3], ***Dr. Paolo Plescia*** [2], ***Dr. Paola Bolognesi*** [1], ***Dr. Jacopo Chiarinelli*** [1], ***Dr. Pietro Calandra*** [4], ***Dr. Nunzia Cicco*** [5], ***Dr. Maria Filomena Verrastro*** [6], ***Dr. Diego Centonze*** [7], ***Ms. Ludovica Gullo*** [8], ***Dr. Alessandra Del Giudice*** [8], ***Dr. Luciano Galantini*** [9], ***Dr. Lorenzo Avaldi*** [1]

1. *Institute of Structure of Matter-CNR, (ISM-CNR), Area della Ricerca di Roma 1, Via Salaria km 29.300, 00015, Monterotondo, Italy,* ***2.*** *CNR-Institute of Environmental Geology and Geoengineering (CNR-IGAG), Area della Ricerca Roma1, Via Salaria km 29.300, 00015, Monterotondo, Italy,* ***3.*** *University of Rome Sapienza,* ***4.*** *CNR-Institute for the Study of Nanostructured Materials (CNR-ISMN), Area della Ricerca Roma1, Via Salaria km 29.300, 00015 Monterotondo, Italy,* ***5.*** *CNR-Institute of Methodologies for Environmental Analysis (CNR-IMAA), Contrada Santa Loja, Tito Scalo, 85050 Potenza, Italy,* ***6.*** *Istituto di Struttura della Materia-CNR (ISM-CNR), Contrada Santa Loja, Tito Scalo 85050, Potenza, Italy,* ***7.*** *Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università degli Studi di Foggia, via Napoli, 25, 71122 Foggia, Italy,* ***8.*** *Department of Chemistry, Sapienza University, P.le Aldo Moro 5, 00185 Roma, Italy,* ***9.*** *Department of Chemistry, Sapienza University, P.le Aldo Moro 5, 00185 Roma, Italy More by Luciano Galantini*

The Electrospray Ionisation (ESI), a well established technique widely used to produce ion beams of biomolecules in mass spectrometry (ESI-MS), can be used for ambient soft landing of enzymes on a specific substrate[1]. In this work we show how the ambient electrospray deposition (ESD) technique can be successfully exploited for manufacturing a new promising green friendly electrochemical amperometric Laccase based biosensor with unprecedented reuse and storage performance.

These biosensors have been manufactured by spraying a laccase solution of 2μg/μL at 20% of methanol on a commercial carbon screen printed electrode (C-SPE) using a custom ESD set-up.

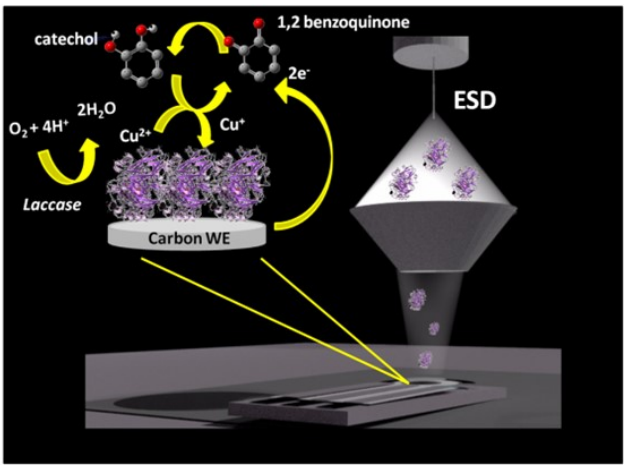
The laccase-based ESD biosensor has been tested against catechol compound in the linear range 2–100 μM, with a limit of detection of 1.7 μM, without interference from cadmium, chrome, arsenic and zinc and without any memory effects, but showing a matrix effect in lake and well water.

The ESD biosensor shows enhanced performances compared to the ones fabricated with other immobilization methods, like dropcasting. Indeed it retains a 100% activity up to two months of storage at ambient conditions without any special care and a working stability up to 63 measurements on the same electrode just prepared and 20 on one year old electrode subjected to redeposition together with a 100% resistance to use of the same electrode in subsequent days[2].

The ESD method is a one-step, environmentally friendly method allowing deposition of the bio-recognition layer without using any additional chemicals. The promising results in terms of storage and working stability obtained also with the more fragile lactate oxidase enzyme[3], suggest these improvements should be attributed to the ESD technique rather than to the bioreceptor, highlighting how the ESD could be useful in reducing pollution from disposable devices.

The understanding at the molecular level of this new promising biosensor by using different spectroscopies, microscopies and analytical techniques is the subject of our new PRIN 2022 project ESILARANTE[4]

1. Castrovilli, M.C. et al. (2020), *Biosens. Bioelectron.*, 163, 112299
2. Castrovilli, M.C. et al. (2022), *ACS Sustainable Chem. Eng.* 10, 5, 1888–1898.
3. Castrovilli M.C. et al. (2023) *Green Chem.* 25, 5257-5266
4. ESILARANTE 202283YHXY Project PRIN 2022



Esd laccase.jpg

Fuel-driven processes in DNA-biosensors investigated by optical tweezers

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Dr. Xavier Viader-Godoy [1], ***Dr. Riccardo Tancredi*** [1], ***Dr. Erica Del Grosso*** [2], ***Dr. Francesco Ricci*** [2], ***Dr. Leonard Prins*** [3], ***Dr. Annamaria Zaltron*** [1]

1. *Via Francesco Marzolo, 8, Dipartimento di Fisica e Astronomia Galileo Galilei, Università degli Studi di Padova, 35131 Padova, Italy.*, ***2.*** *Dipartimento di Scienze e Tecnologie Chimiche, Università degli Studi di Roma Tor Vergata,* ***3.*** *Via Francesco Marzolo, 1, Dipartimento di Scienze Chimiche, Università degli Studi di Padova, 35131 Padova, Italy.*

Over the past decades, synthetic nucleic acids (DNA and RNA) have emerged as ideal components for self-assembly processes. The vast majority of these self-assembled systems exploit thermodynamics as a driving force, which means that the final structure resides at the thermodynamic equilibrium and its assembly is more stable than the separate components. Recently, it has been demonstrated that DNA hairpins are particularly suited for developing chemical-fuel driven systems, thanks to the high predictability of the molecular recognition processes occurring in the fuel-receptor complex. However, a full control over the energy storage and dissipative mechanisms can be achieved only through a deep knowledge of the kinetics and thermodynamics of the DNA-based machine. Most of the current knowledge on the structure and functionality of biomolecules has been derived thanks to biochemical bulk measurements. The aim of this project is to investigate at the single-molecule level an energy-dissipating biochemical system based on a DNA hairpin by means of optical tweezers (OT).

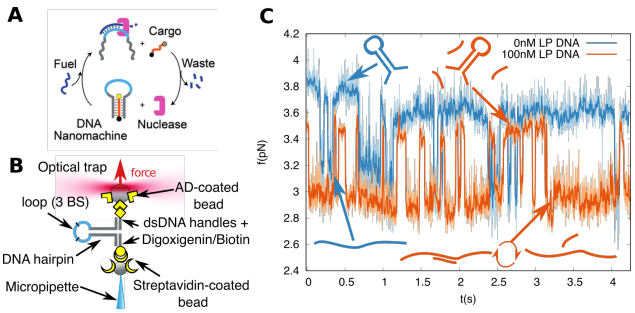


Fig poster bioforphys.png

Molecular probes for the monitoring of neuron membrane potential with two-photon microscopy

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Ms. Zsofia Horvath [1], ***Ms. Reka Lanyi*** [2], ***Dr. Zoltan Mucsi*** [2], ***Dr. Levente Cseri*** [1]

1. *BrainVisionCenter; Budapest University of Technology and Economics,* ***2.*** *BrainVisionCenter*

Introduction

The detailed understanding of neuron activity and network structures holds the promise of new treatments for neurological diseases and the development of the next generation of brain–computer interfaces. To effectively study neurons, the spikes of swift membrane potential change should be detectable and observable over time. Voltage sensitive dyes (VSDs) enable the fast non-invasive simultaneous monitoring of membrane potential in a large number of neurons with fluorescence microscopy, but the currently available sensors are limited by their low fluorescence enhancement and brightness. A better understanding of the voltage sensing mechanism and a facile screening method of candidate molecules could lead to the development of more efficient VSDs.

Methods

VSDs were synthesized from commercially available chemicals in 6–8 synthetic steps. Time-dependent density functional theory (DFT) calculations were carried out in Gaussian 16 with B3LYP method and 6-311+G(d,p) basis set to predict spectroscopic properties. Two-photon microscopy experiments were conducted on a 3D acousto-optic laser-scanning microscope (ATLAS, Femtonics). Femtosecond laser pulses were provided by a Chameleon Discovery laser (Coherent) operated at 840 nm. Voltage signals were induced in HEK cells using whole-cell patch-clamp method.

Results & Discussion

This work focuses on several aspects of VSD development. First, the role of the lipophilic wire part of a prominent VSD were systematically modified synthetically and studied by computational methods to explore its role in the voltage sensing mechanism and to identify a pathway to property improvements. Second, novel VSDs were synthesized based on a tetraoxaazapentacene fluorophore to acheive high photostability and improved two-photon fluorescence properties. Finally, the novel VSDs were tested in single-photon spectroscopic experiments using a liposomal system and two-photon microscopic experiments with HEK cells.

Optical contrast inversion of metal nanoparticles in a nanofluidic channel

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

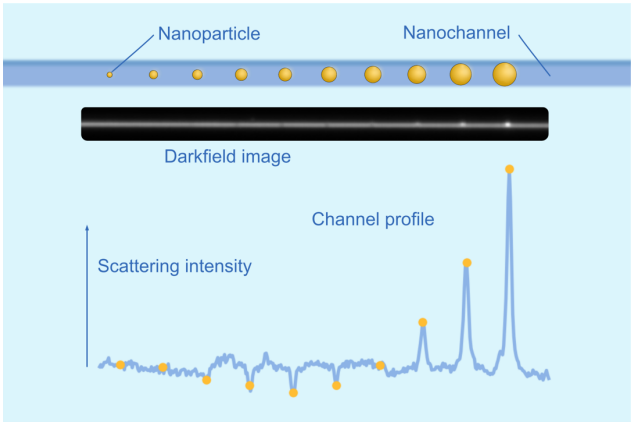
Ms. Lova Wilske [1], ***Prof. Christoph Langhammer*** [1]

1. *Chalmers university of technology*

Nanofluidic systems enable the control of fluid flow at the nanoscale and are traditionally used in biophysics. Recently, we have developed what we call Nanofluidic Reactors (Levin, S.; et al. *ACS Nano* **2022,** *16* (9), 15206-15214.) to study catalysis on single metal nanoparticles and Nanofluidic Scattering Microscopy (NSM) (Špačková, B.; et al. *Nature Methods* **2022,** *19* (6), 751-758.) for label-free mass and size determination of single biomolecules. In both these applications of nanofluidics we either used the plasmonic light scattering properties of the metal catalyst nanoparticles or the light scattering properties of a nanofluidic channel *individually* as the signal transducing mechanism.

Here, we investigate the interesting regime of the interplay of the plasmonic and light scattering signatures of single metal nanoparticles placed inside a nanofluidic channel. To investigate this effect, we have nanofabricated open nanochannels decorated with gold (good scatterer) and platinum (good absorber) nanoparticles of well-defined sizes using electron beam lithography to systematically study the size dependence of the optical contrast of the particles in the channel. As key result, depending on the composition and size of the nanoparticle, the channel cross-section dimensions and the surrounding medium, we find that particles either appear as bright spots or as dark spots in a dark field microscope, and that bright ones can be turned dark by altering the surrounding conditions. Furthermore, we have identified a point of contrast inversion, at which a particle becomes invisible.

This behavior can be understood theoretically by describing the light scattered from a nanoparticle in a nanochannel as the sum of the scattering contributions from each object plus an interference term, in which the channel and particle scattering contributions are multiplied. Furthermore, the polarizabilities of the nanoparticle and its surrounding medium determine whether the interference term is positive or negative, and hence whether the particle appears bright or dark. Hence, tailoring nanoparticle and nanochannel dimensions we can drape our particles in an invisibility cloak, or more importantly, “uncloak” nanoparticles. We predict this effect to find application in, for example sensing, where a small change in surrounding properties can induce a large optical contrast .



Nanoparticles nanochannel lw.png

Nanodiamonds for nanoscale thermometry: applications and limits

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Prof. Anna Ermakova [1]

1. *Hasselt University, Royal Belgian Institute for Space Aeronomy*

A lot of biological processes are strongly connected to temperature variation. Such temperature changes can result from different factors, for example, mitochondria activity or inflammations. On the other hand, externally initiated temperature fluctuations influence biochemical reactions within living systems. Therefore, precise measurement of intracellular temperature with a high special resolution is essential to understanding cell biophysics.

There are a few systems for intracellular thermometry: fluorescent molecules and proteins (depend also on pH level or viscosity), quantum dots (might be toxic), and fluorescent nanodiamonds. Nanodiamonds are inert and non-toxic materials that can be functionalised for cellular applications. Their size down to 5-10 nm allows us to use them as sensors inside living cells without harmful effects. Nanodiamonds might contain different optically active defects; now, the most used colour centres are Nitrogen-Vacancy or Silicon-Vacancy. Both of these defects provide extremely stable fluorescence, that is, temperature or magnetic-dependent. The fluorescence spectra of nanodiamonds are determined only by the type of colour centres without reference to nanodiamonds' size or environment. Nanodiamonds with colour centres are unique quantum sensors that can be operated at body temperature. Nitrogen-Vacancy centres can be used to detect magnetic fields or temperature by applying laser and microwave fields. Silicon-Vacancy Centre allows pure all-optical thermometry. Noninvasively confocal measurements can perform all sensing experiments.

Currently, we have evaluated different colour centres in nanodiamonds for real intracellular thermometry. The application potential of nanodiamonds for temperature sensing will be presented. The weak points of thermometry with nanodiamonds will be shown, and ways to overcome them will be discussed.

Reversible quantum-dot cellular automata digital circuits with ultralow energy dissipation

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Mr. Mohammed Alharbi [1], ***Dr. Gerard Edwards*** [1], ***Dr. Richard Stocker*** [2]

1. *Liverpool John Moores University,* ***2.*** *University of Chester*

Introduction:

Quantum-dot cellular automata (QCA) are a promising nanoscale computing technology that exploits the quantum mechanical behaviour of electrons in quantum dots. QCA can achieve higher speed, lower power, and smaller areas than conventional complementary metal-oxide-semiconductor (CMOS) technology. Developing QCA circuits in a logically and physically reversible manner can provide exceptional reductions in energy dissipation. The main challenge of reversible QCA design is to maintain reversibility at the physical level. Current QCA designs are either irreversible or logically reversible; however, physical reversibility is a crucial requirement to increase energy efficiency. This study introduces a novel logically and physically reversible QCA 4:1 multiplexer circuit with ultralow energy dissipation.

Methods:

We propose a novel logically and physically reversible design method for developing a QCA 4:1 multiplexer circuit. This method comprises two main stages: reversibly developing the circuit at the logical level (synthesis) and then reversibly developing the circuit at the physical level (layout) based on QCA-interconnected devices. To achieve **time-synchronisation** characteristics, we included the universal, standard, and efficient (USE) clocking scheme. For **wire junctions**, we used the multilayer crossover method, wherein three different layers are used to prevent crosstalk interference between the crossing wires.

Results:

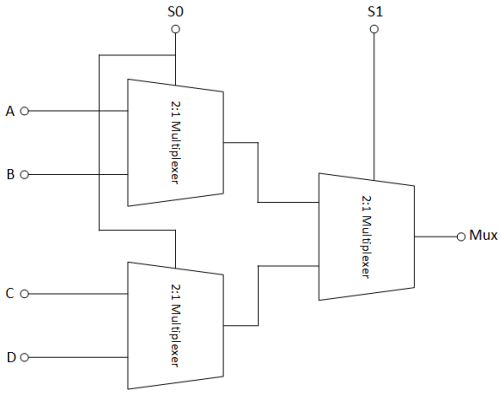
The simulation results confirm that the proposed logically and physically reversible design method can generate QCA circuits that dissipate ultralow energy. Compared to the most energy-efficient equivalent circuits presented in the literature, the proposed QCA 4:1 multiplexer has an improved energy dissipation by 97%.

Conclusion:

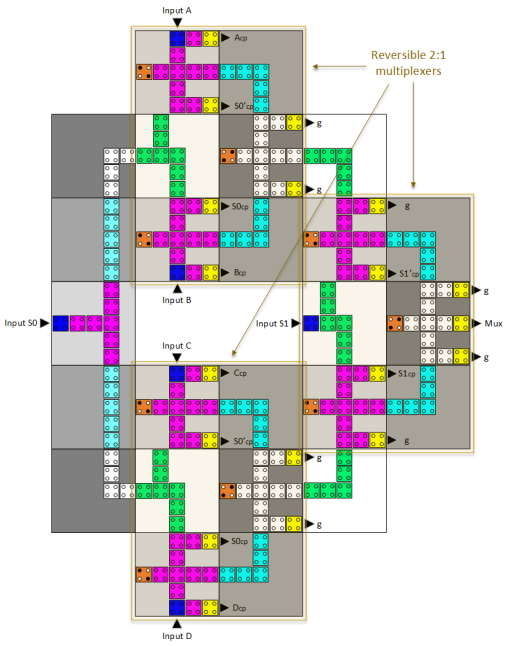
This study introduces a novel method for designing QCA circuits that is both logically and physically reversible. Using the proposed method, the first logically and physically reversible QCA circuits for a 4:1 multiplexer circuit are developed. The simulation results validated the efficiency of the proposed method for generating QCA digital circuits with extremely low energy dissipation.

Keywords:

Quantum-dot cellular automata (QCA); Reversible; Energy dissipation; Universal, standard, and efficient clocking scheme (USE)



4-to-1 multiplexer schematic.png



4-to-1 multiplexer layout.png

Bringing the power of DNA sequencing to metabolite detection

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Prof. Andrew Fraser [1], ***Dr. June Tan*** [1]

1. *University of Toronto*

Metabolites are at the heart of biology and medicine. They are critical biomarkers that report on our health, and errors in the regulation of metabolite levels cause diseases like diabetes and phenylketonuria. Despite this central importance of metabolites, it is still very challenging to analyse and quantify them — this is a key hurdle in many areas of biology. This is because metabolites are biochemically diverse: sugars, amino acids, hormones, and vitamins, are very different molecules. The challenge for any metabolomics platform is to be able to analyse this huge range of molecules. Currently, mass spectrometry (MS) is the workhorse of metabolomics. Complex mixtures of diverse metabolites can be analysed on the same platform and MS has been applied to biomarker discovery, and metabolome profiling: it is the gold standard. However, MS is still slow, and produces complex data that are often hard to interpret.

Here we present a new platform that can read metabolite or drug levels with DNA sequencing and thus bring the power of DNA sequencing to metabolomics. We term the technique smolSeq for 'Small MOLecule Sequencing'. At the core of our smolSeq platform is structure-switching aptamers (SSAs). Aptamers are short oligonucleotides that can bind specific targets with high affinity – when they do so, they undergo a major conformational change. This can be used to generate a readout — typically this is either a change in fluorescence or in conductance. We use a different readout — DNA sequence. Each SSA recognises a specific target and releases a unique stretch of DNA sequence when it sees its target — by capturing and sequencing these released 'DNA barcodes', we can measure precisely the concentrations of different targets in a complex mixture.

I will show the SSA metabolite sensors deliver quantitative outputs, that they can be multiplexed to read many metabolites in parallel, that the signal can be highly amplified, and that we can discover these sensors rapidly. We believe that this is the first new platform for metabolomics in many years and want very much to discuss the possibilities with the single molecule sensor community.

Deciphering the Mechanisms of λ-DNA Translocation through Nanopipettes

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Mr. Alejandro Colchero [1], ***Dr. Isabel Pastor*** [1], ***Dr. Felix Ritort*** [2]

1. *University of Barcelona, Department of Condensed Matter Physics,* ***2.*** *Small Biosystems Lab, Departament de Física de la Materia Condensada, Facultat de Física, Universitat de Barcelona, 08028 Barcelona, Spain*

Nanopores have emerged as a promising tool for the realm of single-molecule techniques, specifically in the study of DNA and RNA. These tiny pores hold the potential to detect and scrutinize the properties of molecules as they translocate through them. Nanopores have proven to be a highly sensitive and versatile approach to studying biomolecules, offering insights into their structure and function. Nevertheless, during the translocation of a single molecule through a nanopore, many forces are involved, and the balance of these forces is dependent on the experimental conditions.

In this work, we present our findings on the translocation of λ-DNA (48.5 kbp) molecules through quartz nanopipettes with diameters ranging from 15 to 50 nm. We carried out these experiments under a range of voltages, salts (LiCl, KCl, NaCl and MgCl2), and salt concentrations (1 to 4M). We have studied how the time distributions and the current blockades are affected by the different experimental conditions. Finally, we have investigated how the different conformations in which λ-DNA can translocate through our nanopipette, and how these are influenced by the experimental conditions.

Thermoplasmonics based control of nano scale fluid flows for structure assembly

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

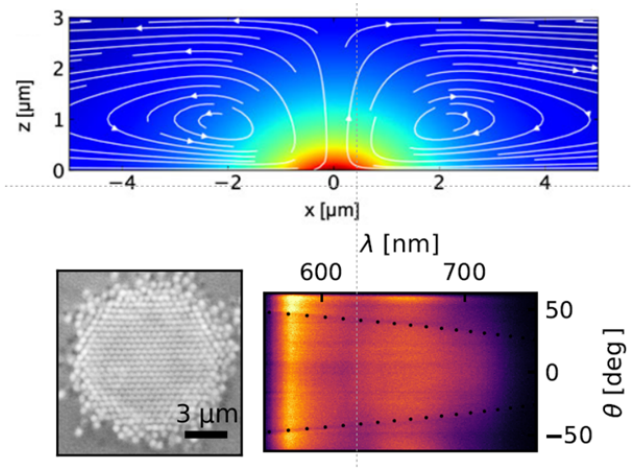
Mr. Desmond Joseph Quinn [1], ***Dr. Diptabrata Paul*** [1], ***Prof. Frank Cichos*** [1]

1. *Leipzig University*

Flows in micro/nano fluidic chips are conventionally driven by external pressures. This is useful for moving bulk liquids, but precise manipulation and assembly of individual particles and molecules requires precise local control of fluid flow. We use thermoplasmonically generated fluid flows and study the controlled manipulation and non-equilibrium assembly of colloids in micro/nano fluidic environments.

Our fluid sample is bounded on one side by a gold film that is resonantly heated by a focused laser spot. This generates thermos-osmotic flows that arise due to temperature induced differences in interaction energies at the gold-water interface. In addition, added depletant molecules create a concentration profile due to the temperature gradient and push the particle towards the heated region.

We observed that colloidal nanoparticles get attracted to the heated region and assemble into ordered structures. These structures show an emergent photonic stopband which was measured and fit with theoretical expectations. The non equilibrium nature of assembly enabled reconfigurable structures. The control of the spatial position of the heating, the heating strength, and duration will pave the way for more complex assemblies of structures.



S3ic abstract.png

Infrared Nanoimaging of Hydrogenated Perovskite Nickelate Memristive Devices

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Prof. yohannes abate [1]

1. *The University of Georgia*

Solid-state devices made from correlated oxides such as perovskite nickelates are promising for neuromorphic computing by mimicking biological synaptic function. However, comprehending dopant action at the nanoscale poses a formidable challenge to understanding the elementary mechanisms involved. Here, we perform operando infrared nanoimaging of hydrogen-doped correlated perovskite, neodymium nickel oxide (H-$NdNiO_3$) devices and reveal how an applied field perturbs dopant distribution at the nanoscale. This perturbation leads to stripe phases of varying conductivity perpendicular to the applied field, which define the macroscale electrical characteristics of the devices. Hyperspectral nano-FTIR imaging in conjunction with density functional theory calculations unveil a real-space map of multiple vibrational states of H-NNO associated with OH stretching modes and their dependence on the dopant concentration. Moreover, the localization of excess charges induces an out-of-plane lattice expansion in NNO which was confirmed by in-situ - x-ray diffraction and creates a strain that acts as a barrier against further diffusion. Our results and the techniques presented here hold great potential to the rapidly growing field of memristors and neuromorphic devices wherein nanoscale ion motion is fundamentally responsible for function.

Single-molecule detection of protein phosphorylation using chemical labeling

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Mr. Moon Hyeok Choi [1], ***Dr. Mike Filius*** [1], ***Prof. Chirlmin Joo*** [1]

1. *Delft University of Technology*

Post-translational modification (PTM) is a key mechanism that increases proteomic diversity after translation. It is an important chemical variant of proteomics involved in activation, interaction, and localization with other molecules, such as proteins and nucleic acids. Among abundant PTMs (glycosylation, acetylation, and phosphorylation), we have proposed a detection method for phosphorylation. Our objective is particularly to detect the number and location of phosphorylation sites within a protein, which can help determine its structural characteristics. To this end, we combined organic chemistry and single-molecule FRET assays.

Directing Surface Functionalization of Gold Nanorods for Improved Optical Biosensors

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Dr. David Botequim [1], Dr. Rui Oliveira-Silva [2], Dr. Vanda V. Serra [1], Prof. Ana S. Viana [1], Prof. Peter Zijlstra [3], Prof. Duarte M. F. Prazeres [2], Prof. Sílvia M. B. Costa [1], Dr. Pedro M. R. Paulo [1]

1. *Centro de Química Estrutural, Institute of Molecular Sciences,* ***2.*** *Institute for Bioengineering and Biosciences,* ***3.*** *Eindhoven University of Technology*

The strong interaction of gold nanoparticles with light, through collective oscillations of free electrons in the metal, has been explored in many different ways to develop optical detection schemes for biochemical sensors. Generally, the detection scheme requires that target species interact with the particle's surface, which is accomplished by its modification with molecular recognition units. The motivation behind the development of directed functionalization strategies, as opposed to indiscriminate surface functionalization, is based on a more rationale use of the particle's surface by directing the target species to its more sensitive regions (plasmon hot-spots), while leaving other regions available for complementary functionalities, such as colloidal stabilization or anti-fouling coatings. In this contribution, we will discuss selected examples from our research on directed surface functionalization of gold nanorods towards improved optical biosensors. The pursuit of such strategies has contributed to enhance single-molecule detection of proteins,[1] and to the development of brighter fluorescent nanoprobes.[2] Recently, we have explored a new photoinduced plasmon-assisted strategy to target hot-spots in gold nanorods for modification with bioreceptor units. The biotin-functionalized gold nanorods respond specifically to streptavidin binding in the nanomolar range with a total peak wavelength shift of ca. 4 nm, thus, providing a model plasmonic biosensor (Fig. 1).

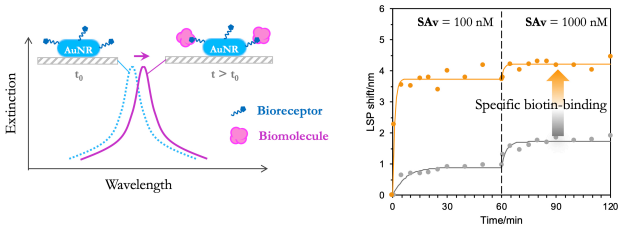


Figure 1.jpg

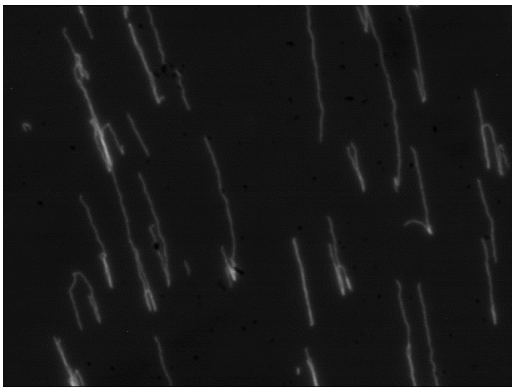
Combing of single DNA molecules over interfaces having dual characteristics

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

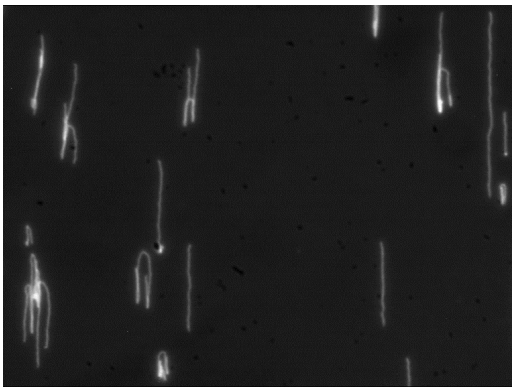
Dr. Hemendra Yadav [1]

1. *Central University of Punjab*

DNA combing is the most powerful technique at present to study replication dynamics. No other technique provides such comprehensive information regarding fork velocity, fork directionality and origin of replication as this technique. The technique is used for studying DNA replication, repair mechanisms and G quadruplexes. Still, this technique has got some drawbacks such as complexity associated with it and low throughput nature. Interfaces made by both surface coatings were obtained having high hydrophobic character as well as affinity towards negatively charged molecules such as DNA. Different range of deposition efficiencies were observed i.e. from 12% to 28%. XPS analysis of the interface made by two surface coatings showed variations in terms of disappearance of some peaks, including shift in Binding Energy of C-C back bone peak and variations in FWHM. FTIR analysis showed that absorption bands was represented by various vibrational modes related to NH_2, CH_3, CH_2, and Si-O bonds. The spectral region was characterized by three peaks related to the asymmetric stretching mode of CH_3, CH_2 and symmetric stretching mode of CH_2. In relation to Si, the deformation mode of Si-CH_2 was observed and asymmetric stretching modes of Si-O-Si were detected. Both the XPS and FTIR analysis thus proved that the developed interfaces had dual characteristics i.e. hydrophobicity and affinity towards negatively charged molecules. Thus a surface was developed which had high affinity for combing of single DNA molecules with enhanced retention after post wash conditions. DNA ssb protein interaction was observed on combed DNA fibers. EcoRI restriction fragments were observed on combed DNA fibers. Cisplatin DNA adduct formation were observed on glass slides. We tried to resolve all the problems associated with DNA combing to make it simple, reproducible and easy to use. Also, many groups need combing protocols where DNA extracted from few cells can be used for combing DNA for diagnostics and studying DNA repair mechanisms. Combed DNA can be used for studying DNA replication dynamics, DNA damage response, loop extrusions and measuring telomere length. The surface can be used for multiple other uses for coating of nanoparticles and as biosensors for environmental samples



8-2.png



8-3.png

DETECTION THE ICE FORMATION PROCESSES USING MULTIPLE HIGH ORDER ULTRASONIC GUIDED WAVE MODES

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Dr. Regina Rekuviene [1], ***Dr. Vykintas Samaitis*** [1], ***Dr. Audrius Jankauskas*** [1], ***Prof. Liudas Mažeika*** [1], ***Prof. Virginija Jankauskaitė*** [2], ***Ms. Laura Gegeckienė*** [2], ***Dr. Abdolali Sadaghiani*** [3], ***Dr. Shaghayegh Saeidiharzand*** [4], ***Prof. Ali Koşar*** [4]

1. *Ultrasound Research Institute, Kaunas University of Technology,* ***2.*** *Department of Production Engineering, Kaunas University of Technology,* ***3.*** *Faculty of Engineering and Natural Sciences, Sabanci University,* ***4.*** *Faculty of Engineering and Natural Sciences, Sabanci University, Turkey*

Icing brings significant damage to aviation and renewable energy installations. Air-conditioning, refrigeration, wind turbine blades, airplane and helicopter blades often suffer from icing phenomena, which cause severe energy losses and impair aerodynamic performance. The icing process is a complex phenomenon with many different causes and types. Icing mechanisms, distributions, and patterns are still relevant research topics. The adhesion strength between ice and surfaces differs in different icing environments. This makes the task of anti-icing very challenging. There are a significant number of different studies proposing many anti-icing methods and techniques. The proposed techniques for various icing environments must satisfy different demands and requirements (e.g., efficient, lightweight, low power consumption, low maintenance and manufacturing costs, reliable operation). There are two major groups of anti-icing methods: passive and active. Active techniques have high efficiency, but at the same time quite high energy consumption and require intervention in the structure's design. The main effect of passive methods is to delay ice formation and growth or reduce the adhesion strength between the ice and the surface. These methods are time-consuming and depend on forecasting. There is some quite promising information on ultrasonic ice mitigation methods that employ UGW (Ultrasonic Guided Wave). These methods are novel and have the characteristics of low energy consumption, low cost, lightweight, and easy replacement and maintenance. The objective of this work was to identify the ice formation processes and its progress by employing ultrasonic guided wave technique. Throughout this research the universal set-up for acoustic measurement of ice formation in a real condition (temperature range from $+24^0$ C to -23^0 C) was developed. Ultrasonic measurements were performed by using high frequency 5 MHz transducers in a pitch-catch configuration. The selection of wave modes suitable for detection of ice formation phenomenon on copper metal surface was performed. Interaction between the selected wave modes and ice formation processes was investigated. It was found that selected wave modes are sensitive to temperature changes. It was demonstrated that proposed ultrasonic technique could be successfully used for the detection of ice layer formation on a metal surface.

Optical near-field electron microscopy (ONEM)

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Mr. Ilia Zykov [1]***, Prof. Thomas Juffmann*** [1]

1. *University of Vienna*

In ONEM [1], a sample is probed non-invasively using light, and the resulting near-field interference patterns are converted into an electron current using a photocathode. The emitted electrons are then imaged with nanometric resolution using an aberration-corrected Low Energy Electron Microscope (LEEM). For sample-photocathode distances much smaller than the optical wavelength (z□λ) this allows for label-free, non-invasive superresolution microscopy of interfaces. After introducing the basics of this new imaging concept, we will show the first results of this new microscopy technique.

[1] Marchand, R., Šachl, R., Kalbac, M., Hof, M., Tromp, R., Amaro, M., van der Molen, S. J. & Juffmann, T., Optical Near-Field Electron Microscopy. Physical Review Applied 16, 014008 (2021)

Investigations on electrochemical, spectroelectrochemical and piezoelectrical analysis on heavy metal ions interactions with receptor layers based on DNA strands

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Dr. Robert Ziółkowski [1], ***Mrs. Anna Szymczyk*** [1], ***Prof. Marcin Olszewski*** [2], ***Prof. Elżbieta Malinowska*** [1]

1. *Chair of Medical Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland,* ***2.*** *Chair of Drug and Cosmetics Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Koszykowa 75, 00-664 Warsaw, Poland*

In recent years nucleic acids (NA) gained considerable attention as selective receptors of metal ions. This approach is characterized by many advantages including the possibility of adjusting NA sequences in new aptamers selection. This in turn offers convenience of elaborating new detection mechanisms and its integration in ready to use biosensors which can be characterized by exceptionally low detection limits and fast responses. All these properties, together with low power consumption, make DNA biosensors an ideal candidates as detection elements of portable analytical devices. However, all the interactions which are responsible for biosensor's analytical signal generation have to be thoroughly investigated and confirmed. Depending on the mechanism of detection used, during biosensor's development, and appropriate physico-chemical properties changes of the receptor layer are responsible for the analytical signal. Because of the above it is important to be able to assign all interactions which took place to the appropriate change for which they are responsible for. That is why engagement of various analytical techniques in their analysis could offer significant in-depth look into not interactions itself, but also into their dependencies on environmental conditions or even the influence of the measurement itself on the changes which can occur in the receptor layer. The presented study is an investigation on physicochemical changes which occur in the DNA receptor layer and its qualitative and quantitative description by chosen, various, electrically dependent analytical techniques.

Experimental Measurement of Information-Content in Mutational Ensembles

Thursday, 23rd November - 13:30: (Poster Aera) - Poster

Dr. Alvaro M. Monge [1], ***Dr. Danny Incarnato*** [2], ***Dr. Anna Alemany*** [3], ***Dr. Marco Ribezzi*** [4], ***Dr. Maria Manosas*** [1], ***Dr. Felix Ritort*** [1]

1. *Small Biosystems Lab, Departament de Física de la Materia Condensada, Facultat de Física, Universitat de Barcelona, 08028 Barcelona, Spain,* ***2.*** *Faculty of Science and Engineering, Molecular Genetics, University of Groningen,* ***3.*** *Scar Lab, Dpt. Anatomy and Embryology Leiden University Medical Center LUMC Building 2, Einthovenweg 20, 2333 ZC Leiden,* ***4.*** *Protein Engineering and Single-Molecule Tech, Paris*

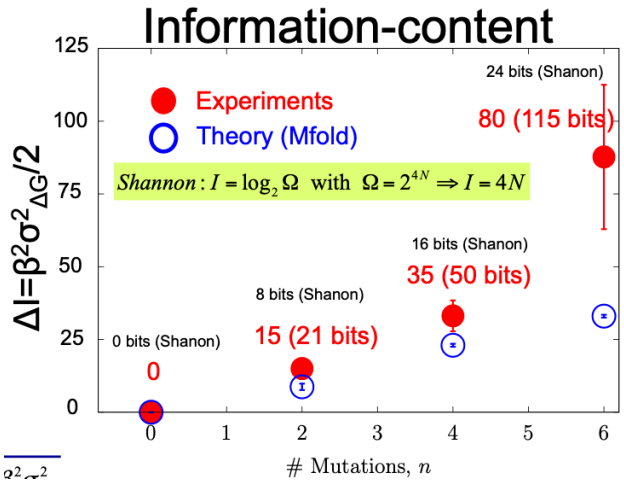
Biology is noisy at all levels, from molecules to cells, tissues, organs, communities, and ecosystems [1]. While thermodynamic processes in the ordinary matter are driven by free-energy minimization, living matter delineates a fascinating evolutionary state governed by information flows across all organizational levels [2]. While we know how to measure energy and entropy in physical systems, we have poor general knowledge about measuring information content. Recent developments in stochastic thermodynamics of feedback systems and single-molecule experiments [3,4] show that information content equals free energy differences between disordered populations. A fluctuation theorem for mechanical work and information content is applied to a mutational ensemble of DNA hairpin folders with different base pair mutations. The thermodynamic information-content correlates but is higher than the Shannon entropy of the mutational ensemble.

1. F. Ritort, *The noisy and marvelous molecular world of biology,* Inventions, ***4***(2) (2019) 24

2. T. Dobzhanski, F. J. Ayala, G. L. Stebbins & J. W. Valentine, *Evolution,* W. H. Freeman, 1977

3. M. Ribezzi-Crivellari and F. Ritort, *Large work extraction and the Landauer limit* in the *Continuous Maxwell Demon,* Nature Physics **15** (2019) 660–664

4. M. Rico-Pasto, R. K. Schmitt, M. Ribezzi-Crivellari, J. M. Parrondo, H. Linke, J. Johansson and F. Ritort, *Dissipation reduction and information-to-measurement conversion in DNA pulling experiments with feedback,* Physical Review X **11** (2021) 031052



Ic.png

Quantum Advances in Magnetic Resonance: From Nanoscale Resolution to Hyperpolarised MRI

Thursday, 23rd November - 14:30: (Auditorium) - Oral

Prof. Martin Plenio [1]

1. *Ulm University*

Nuclear Magnetic Resonance (NMR), one of the most widely used spectroscopic techniques in biology and the life sciences, has a broad range of applications spanning from chemical analysis and drug discovery to medical imaging. However, it is inherently limited by its low sensitivity. This limitation is rooted in the weak nuclear spin nuclear spin polarisation in thermal equilibrium, typically amounting to a few parts per million, and the constraints of inductive NMR signal detection.

Here, I discuss how both of these limitations can be overcome through the utilisation of quantum technologies. Specifically, I will demonstrate how optically detected magnetic resonance, employing colour centers in diamond, offers a means for the detection of NMR signals with chemical shift resolution at the nano- and micon-scale. Furthermore, I delve into how quantum control can facilitate nuclear spin hyperpolarisation, resulting in signal increases of over 10,000-fold.

Despite these advancements, NMR signals often remain weak and noisy. Therefore, I will also introduce signal processing methods capable of extracting the maximum of information from measured time series. The combination of these methods holds the potential to enable the metabolic profiling of even single cells. They can also provide access to metabolic imaging, for example for cancer in standard clinical MRI scanners.

[online] - Quantum entangling living systems

Thursday, 23rd November - 15:10: (Auditorium) - Oral

Prof. Vlatko Vedral [1]

1. *University of Oxford*

A growing body of (usually circumstantial bits of) evidence suggests that biological processes could be utilising quantum coherence, superpositions, and even, in some cases, quantum entanglement to perform some tasks with higher efficiency. I will first briefly summarise the existing body of knowledge, including two of the most famous examples of biological processes: photosynthesis and magneto-reception. I will then present our recent observations of the vacuum Rabi splitting in a living bacterium strongly coupled with the electromagnetic field, as well as the energy shifts induced in superconducting qubits via their coupling to tardigrades. The main challenge of all of the quantum bio work is to experimentally obtain a handful of parameters believed to be important for describing the interplay between coherence (within the system) and noise (arising due to the interaction with the system's environment). I will present single molecule spectroscopy experiments we are currently undertaking in our laboratory to obtain a better understanding of quantum effects in biomolecules. Finally, I will discuss how these experiments can be scaled-up, having the long-term aim of creating quantum entanglement between two living systems.

Interfacing coherent qubits with biological targets

Thursday, 23rd November - 16:25: (Auditorium) - Oral

Prof. Peter Maurer [1], ***Dr. uri zvi*** [1]

1. *University of Chicago*

Quantum metrology enables some of the world's most sensitive measurements with potentially far-reaching applications in the life sciences. Although the ultrahigh sensitivity of qubit sensors has spurred the imagination of researchers, implementation in actual devices that enable monitoring cellular processes or detecting diseases still remains largely elusive. Overcoming limitations that hold back wider application of quantum technology in the life sciences, requires advances in both fundamental science and engineering. In this talk, I will discuss our research group's recent results on addressing one of these long-standing research challenges, namely, how to interface highly coherent quantum sensors with biological target systems. My discussion will start with the development of a novel biocompatible surface functionalization architecture for highly coherent diamond crystals. I will then continue with discussing a new approach to engineering spin coherence in core-shell structured diamond particles, which can be readily chemically modified and delivered to intact biological systems. Finally, I will conclude my talk with an outlook on a novel class of molecular qubit sensors that will overcome many of the fundamental challenges associated with current diamond-based quantum sensors. The unifying theme of these advances are the convergence of techniques from single-molecule biophysics, material science, and quantum engineering. Specific applications of the developed sensing platforms to questions in the life sciences will be discussed throughout this talk.

Protein Conformational Dynamics by using Optical Nanotweezers

Thursday, 23rd November - 16:52: (Auditorium) - Oral

Dr. Cuifeng Ying [1]

1. *Advanced Optics and Photonics Laboratory, Department of Engineering, School of Science and Technology, Nottingham Trent University, Nottingham NG118NS, United Kingdom*

Single-molecule techniques have made incredible progress in unravelling the fundamental mechanisms by which biomolecules execute their functions through diverse conformations, aspects often hidden in ensemble measurements. For meaningful insights from single biomolecules, an ideal technique should enable extended data collection from the same molecule without modifying it. Exploiting localised surface plasmonic resonance, plasmonic nanostructure can confine the optical field within tens of nanometres, generating a gradient force that enables to trap single proteins in the hotspot. By monitoring the shift introduced by the trapped protein, plasmonic nanotweezer offers a rapid, simple technique for studying biochemical processes in real-time.[1] This presentation will introduce our recent progress in protein characterisation using optical nanotweezers. We will demonstrate how we connected and experimentally validated the optical trapping signal with the conformation of trapped proteins. We will showcase the capabilities of optical nanotweezers in wide range applications, including folding and unfolding dynamics of calmodulin, enzyme kinetics and pathway of adenylate kinase,[2] in-situ iron loading of single ferritin,[3] and protein disassembly kinetics. This talk will also share our primary results in monitoring the conformational dynamics of intrinsically disordered proteins in solution.

1. Gordon, R. Future Prospects for Biomolecular Trapping with Nanostructured Metals. ACS Photonics 9, 1127–1135 (2022).

2. Ying, C. et al. Watching single unmodified enzymes at work. ArXiv Prepr. ArXiv210706407 (2021).

3. Yousefi, A. et al. Optical Monitoring of In Situ Iron Loading into Single, Native Ferritin Proteins. Nano Lett. 23, 3251–3258 (2023).

Probing photothermal unfolding and refolding of single proteins using plasmon-enhanced fluorescence

Thursday, 23rd November - 17:09: (Auditorium) - Oral

Ms. Martina Russo [1], ***Mr. Roy Teeuwen*** [2], ***Mrs. Marloes Pennings*** [2], ***Mr. Sjoerd Nooteboom*** [2], ***Prof. Peter Zijlstra*** [2], ***Dr. Lorenzo Albertazzi*** [3], ***Mr. Luc Brunsveld*** [2]

1. *Department of Applied Physics, Eindhoven University of Technology,* ***2.*** *Eindhoven University of Technology,* ***3.*** *Molecular Biosensing. Department of Applied Physics and Science Education, Eindhoven University of Technology.*

Introduction

Proteins play a crucial role in biological processes and their function is inherently linked to their temperature-dependent conformation. Since protein misfolding and abnormal folding are major contributors to numerous diseases, various methods have been explored to investigate protein unfolding. However, existing techniques do not allow to selectively control the conformation of given proteins, leaving unperturbed other biomolecules in the same environment. The objective of our research is to develop a novel method to probe selective single-molecule protein unfolding and refolding in real-time, paving the way to the study of complex biomolecular interactions.

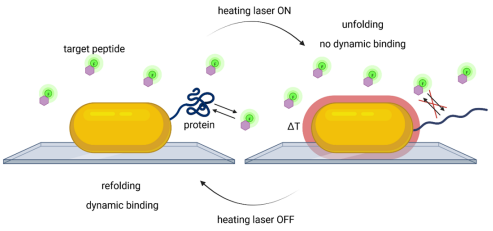
Methods

To accomplish this, we will employ photothermal heating of plasmonic nanoparticles. Plasmonic nanoparticles will be employed as nano-source of heat to locally control the temperature and thus the conformation of surface-bound proteins. Photothermal heating of gold nanorods will induce a local temperature increase [1] able to unfold the conjugated proteins. By delivering fluorescent ligand to functionalized nanorods, binding events can be recorded, including when they cease or resume due to thermal unfolding or refolding [2]. To monitor nanoparticle temperature, we employ a phase-sensitive camera from Phasics Inc., which relies on interferometric measurements of thermal-induced refractive index variations [3].

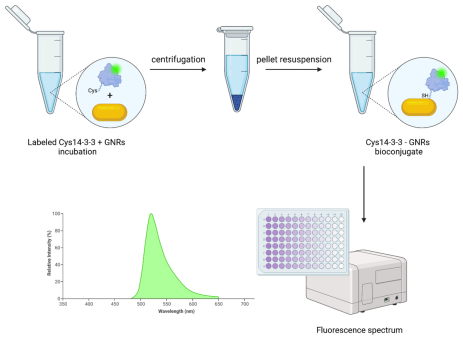
Results and discussion

Our preliminary work involved circular dichroism measurements to determine the folding temperature and validate the spontaneous refolding of the protein of interest. 14-3-3 protein was selected as candidate of biological interest, due to its involvement in cell signaling and neurodegenerative diseases. The conjugation of cysteine-modified 14-3-3 to nanorods surfaces via thiol-gold chemistry was characterized in ensemble fluorescence measurements. Nanoparticles are incubated with fluorescently labelled 14-3-3 protein; centrifugation and resuspension steps are performed to remove free protein and the pellet of functionalized nanoparticles is collected. Fluorescence spectroscopy allowed for characterization of the bioconjugate specificity and quantification of conjugated proteins. In the advanced stage of our research, we will apply this innovative approach to explore the interactions of proteins with chaperone systems, with the ultimate goal to characterize the cooperativity between multiple species.

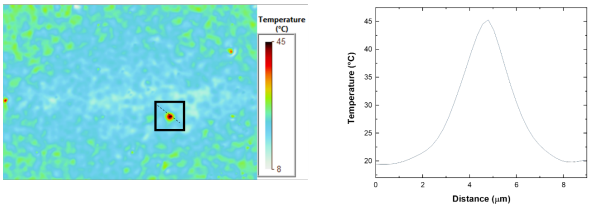
1. S. W. Nooteboom, P. Zijlstra, *Small* **18** (2022).
2. S. Khatua, P. Zijlstra, *ACS Nano* **8** (2014).
3. G. Baffou et al., *ACS Nano* **6** (2012).



Photothermal unfolding and refolding.png



Proteing-nanoparticles functionalization and characterization workflow.png



Temperature map measured with phase sensitive camera.png

Direct identification of single amino acids in nanopores

Thursday, 23rd November - 16:25: (Room 607) - Oral

Prof. Jiandong Feng [1]

1. *Zhejiang University*

Biomolecular sequencing is a key driving force of nanopore research. Protein sequencing using nanopores is faced with a great challenge due to the lack of physical resolution needed to discriminate single amino acids in a peptide chain. Our group develops novel nanopore structures, nanofluidic manipulation approaches and biophysical understandings that promote the nanopore resolution. We recently demonstrated the direct experimental identification of single amino acids free in solution using a sub-nanometer engineered MoS_2 pore. With untra-confined regions of sensitivity, MoS_2 nanopores provide a sub-1 Dalton resolution for discriminating the chemical group difference of single amino acids, and 16 out of 20 types of natural amino acids can be identified in a same nanopore. MoS_2 nanopore system also allows the direct discrimination of isomers and post-translational modifications of individual amino acids. We believe that nanopores with sub-nanometer sensing region may pave the way for future single-molecule chemical recognition and de novo protein sequencing.

Monocrystalline Gold Double Wire Gratings: An Evolutionary Algorithm-Optimized Platform for Innovative SERS Sensing

Thursday, 23rd November - 16:42: (Room 607) - Oral

Mr. Amro Sweedan [1], ***Dr. Mariela Pavan*** [1], ***Mr. Enno Schatz*** [2], ***Dr. Henriette Maaß*** [2], ***Ms. Ashageru Tsega*** [1], ***Dr. Vered Tzin*** [1], ***Dr. Katja Höflich*** [3], ***Mr. Paul Mörk*** [4], ***Dr. Thorsten Feichtner*** [4], ***Dr. Muhammad Bashouti*** [1]

1. *Ben Gurion University of the Negev,* ***2.*** *NanoStruct GmbH, Friedrich-Bergius-Ring,* ***3.*** *The Ferdinand-Braun-Institut gGmbH, Leibniz-Institut für Höchstfrequenztechnik (FBH),* ***4.*** *University of Würzburg*

Achieving reliable and quantifiable performance in large-area surface-enhanced Raman spectroscopy (SERS) substrates poses a formidable challenge, demanding substantial signal enhancement while ensuring response uniformity and reproducibility. Conventional SERS substrates are typically made of inhomogeneous materials with random resonator geometries and distributions. As a consequence, they exhibit multiple or broadened plasmonic resonances, undesired absorptive losses, and inhomogeneous field enhancement. These limitations diminish the signal strength and hamper reproducibility, making it difficult to conduct comparative studies with high sensitivity. This study introduces an innovative approach that utilizes monocrystalline gold flakes to fabricate plasmonic double-wire resonators with nanometer-level precision using focused ion-beam lithography (Figure 1). Inspired by biological evolution strategy, the double-wire grating substrate (DWGS) geometry was evolutionary optimized to enhance both excitation and emission processes involved in generating SERS peak signature of each analyte. The use of monocrystalline material minimizes absorption losses while enhancing the shape fidelity during the nanofabrication process. We numerically and experimentally evaluated not only the DWGS double resonance concept, but also a single wire grating substrate (SWGS) with single resonance, along with a planar non-structured gold substrate (Figure 2-3). For the experiments, all three substrates (SWGS, DWGS and non-structured) were realized using both monocrystalline and polycrystalline materials. Our experimental results depicted in Figure 3, reveal that the monocrystalline DWGS outperforms all other tested SERS substrates in performance, consistently exhibits reproducible large-area surface enhancement factors up to several 10^6 and detection capability for sub-monolayer coverage. DWGS demonstrates notable repeatability (RSD=5.6%), reproducibility (RSD=6.6%), and large-area homogeneity over areas $>10^4$ μm^2. This advancement overcomes several technological challenges, including reusability without a noticeable loss in performance, as well as long-term stability on the shelf up to at least one year under ambient conditions, requiring no special handling. Lastly, we successfully tested the DWGS on eight different use cases, spanning from chemisorbed, physiosorbed, plant extract and even gaseous species to proteins and DNA strains (Figure 3-4). These findings establish SERS based on DWGSs as an auspicious Raman spectroscopy methodology, which can be upscaled by nano-imprint lithography and extended to new applications by utilizing the grating as electrodes in electrochemical environments and sensing technology.

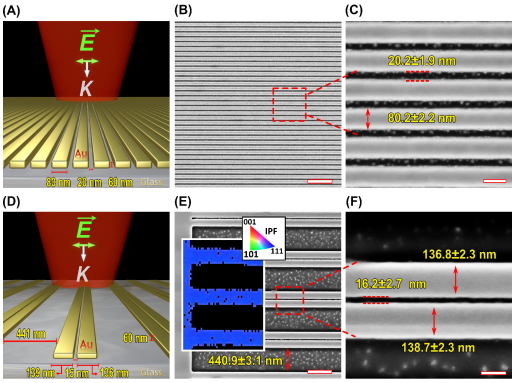


Fig 1 schematic model sem and ebsd of swgs and dwgs.jpg

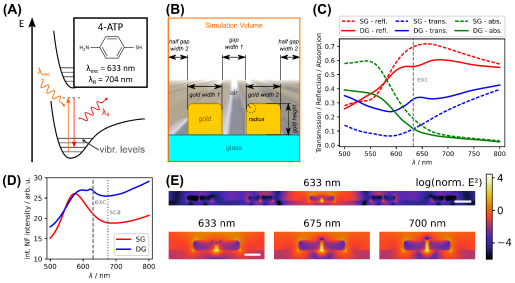


Fig 2 numerical optimization for dwgs and swgs.jpg

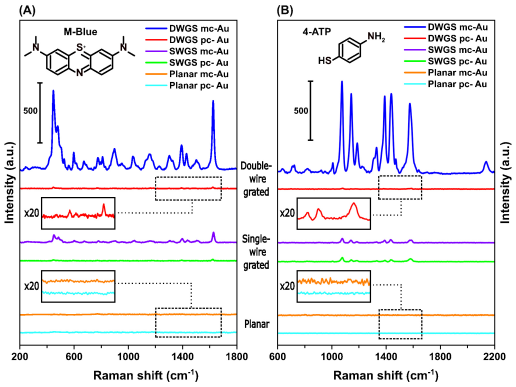


Fig 3 dwgs vs swgs vs planar polycrsyalline pc or monocrystalline mc gold.jpg

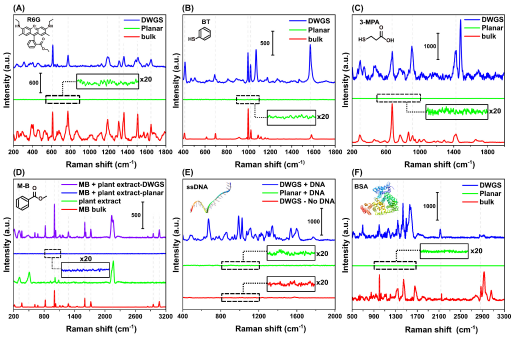


Fig 4 dwgs potential future applications . a-solution b-c gas phase d extract e-f biomolecules.jpg

Exploring nanopore squeezing for rapid mechanical characterization of DNA origami virus-like nanoparticles

Thursday, 23rd November - 16:59: (Room 607) - Oral

Dr. Kun Li [1], ***Mr. Arjav Shah*** [2], ***Prof. Patrick Doyle*** [3], ***Prof. Slaven Garaj*** [4]

1. *Singapore-MIT Alliance for Research and Technology Centre,* ***2.*** *Department of Chemical Engineering, Massachusetts Institute of Technology,* ***3.*** *Department of Chemical Engineering, Massachusetts Institute of Technology,,* ***4.*** *Department of Physics, National University of Singapore*

Studying the mechanical properties of small biological nanoparticles, typically below 100 nm in size, provides valuable insights into their structural characteristics and functional mechanisms. However, current techniques characterizing nanomechanical properties of individual particles, such as nanoindentation, atomic force microscopy, optical tweezers, etc., although proven effective, are hampered by complex procedures and limited throughput, thereby constraining their broader applications. In this study, we introduce a new, efficient method: nanopore squeezing, involving electrophoretic pulling of soft particles through smaller nanopores while monitoring the corresponding ionic current blockage. As a proof of concept, we investigate the squeezing behavior of two distinct 3D wireframe DNA origami particles, both with similar contour sizes (~30 nm) but different rigidities, through nanopores of varying diameters (ranging from 8 to 40 nm). We discovered three distinctive translocation regimes: (1) Elastic Regime: At low voltages, particles translocate slowly through the nanopore due to creep deformation of the overall wireframe structure, with translocation speed exhibiting weak voltage scaling. (2) Plastic Regime: At high voltages, particles rapidly collapse and pass the nanopore akin to free translocation, with a linear increase in speed corresponding to voltage maintaining constant constraint mobility (μ_c). (3) Transient Regime: Occurring at an onset voltage (V_{on}), this regime marks a transition between the two aforementioned translocation mechanisms. Furthermore, we observed the correlations between μ_c, V_{on} and nanopore diameter (D_p), emphasizing their sensitivity to the mechanical properties of the particles. Utilizing our findings in conjunction with deep learning protocols, we achieved impressive accuracy (up to 96%) in distinguishing between similarly sized but structurally distinct particles. This pioneering method has promising applications in diagnostics, particularly for identifying and characterizing viruses, exosomes, and other biological nanoparticles, advancing our understanding of these essential biological entities.

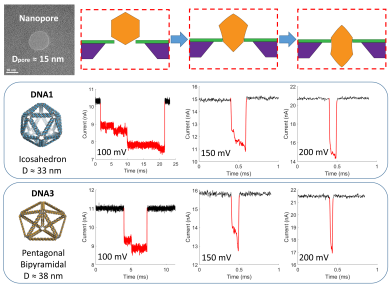


Figure1 concept sketch of nanopore squeezing and raw events.png

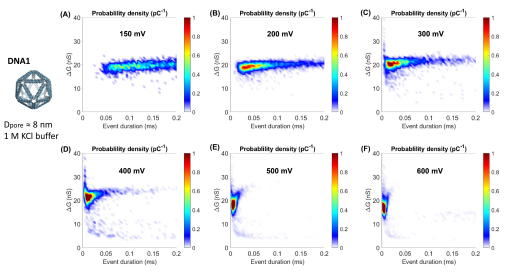


Figure2 voltage dependence in the distributions of translocation duration.png

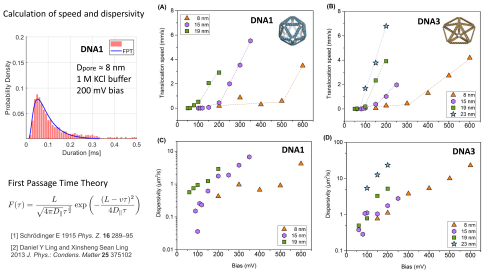


Figure3 voltage dependence in translocation speed and speed fluctuation.png

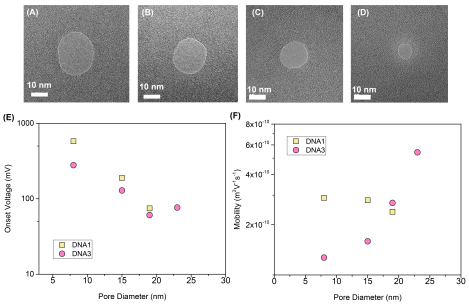


Figure4 pore-size dependence in onset voltage and constraint mobility.png

Event-based vision sensor unleashes unforeseen potential for super-resolution fluorescence imaging

Thursday, 23rd November - 16:25: (Room 608) - Oral

Dr. Clément Cabriel [1], ***Dr. Tual Monfort*** [2], ***Dr. Christian Specht*** [3], ***Prof. Ignacio Izeddin*** [1]

1. *Institut Langevin, ESPCI Paris, PSL University, CNRS,* ***2.*** *Sorbonne Université, INSERM, CNRS, Institut de la Vision. National Hospital of Ophthalmology (CHNO) of Quinze-Vingts, INSERM,* ***3.*** *DHNS, Inserm U1195, Université Paris-Saclay*

Single-molecule localization microscopy (SMLM) enables crucial insights into cellular structures and processes to be revealed at the single-molecule level. However, SMLM is often hampered by limited temporal resolution and the fixed frame rate of the acquisition. We present a new approach to SMLM data acquisition and processing based on an affordable event-based sensor. This type of sensor reacts to changes in light intensity, rather than integrating photons during the exposure time of each frame. Each pixel works independently and returns a signal only when an intensity change is detected. Compared with video acquisition using traditional cameras, the event-based sensor provides higher temporal resolution and throughput on the positions of blinking molecules. We demonstrate event-based SMLM super-resolution imaging on biological samples with spatial resolution on a par with the performance of EMCCD or sCMOS cameras, while registering only the on and off switching of blinking molecules. We use event-based SMLM to perform very dense single-molecule imaging, where frame-based cameras experience significant limitations.

Event-based sensors, also known as neuromorphic vision sensors, operate differently from conventional cameras. Inspired by the human eye, these sensors consist of asynchronous pixels that respond to changes in brightness with remarkable temporal precision. With their low energy consumption, reduced data volume, rapid response times, and cost-effectiveness, event-based sensors hold considerable potential in the field of biological imaging, particularly for capturing the asynchronous and dynamic behavior of SMLM fluorophores.

We have successfully implemented event-based single-molecule localization microscopy, called Eve-SMLM. This novel approach not only produces super-resolution images of quality and resolution comparable to traditional scientific cameras but also overcomes the limitations of high-density imaging, where images of individual fluorophores overlap. While conventional cameras fail in such scenarios, Eve-SMLM excels by selectively detecting ON and OFF transitions, extracting crucial information, and performing efficient temporal resampling.

Eve-SMLM marks a paradigm shift for single-molecule microscopy and its applications. This new approach could be the key to studying multi-scale processes in space and time, which are ubiquitous in biology. The study illustrates the transformative potential of event-based detection in super-resolution fluorescence imaging, offering new prospects in biology, medical applications, materials science, and beyond.

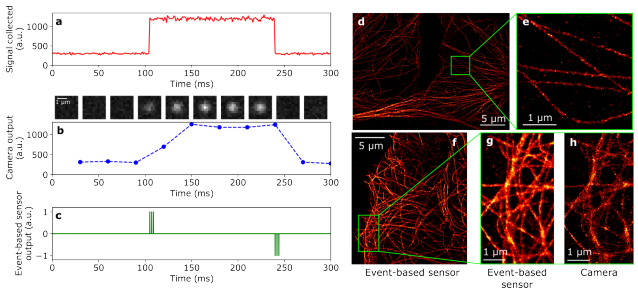


Figure combined.png

Sensing the nanoscale dynamics and dimensionality of complex environments by 3D localization microscopy of carbon nanotubes in the SWIR domain.

Thursday, 23rd November - 16:42: (Room 608) - Oral

Mr. Quentin Gresil [1], ***Dr. Somen Nandi*** [1], ***Dr. Laurent Cognet*** [1]

1. *LP2N - CNRS, Institut d'Optique, Université de Bordeaux - Talence (France)*

Elucidating how the nanometric structure of complex environments shapes the mesoscopic motion of the nano-objects within them is a fascinating but still open question. Due to a lack of knowledge of nanoscale details, diffusion properties are often described as anomalous. This applies to many systems, such as diffusion near interfaces, or in structured and porous materials (rocks, granular media, polymer gels, microfluidic systems, etc.) or in biology (mammalian tissues, plants, etc.). A particularly interesting structure is the extracellular space of the brain, which is heterogeneous and tortuous, and where ions, nutrients and signaling molecules diffuse for proper function of the brain.

To this end, in recent years we have developed a new strategy based on single-particle localization microscopy in the SWIR of single-walled carbon nanotubes (SWCNTs) diffusing in complex environments [1]. We now show that 3D tracking is essential to link the anomalous diffusion often observed at the macroscale to the local microscopic topologies of the edifices. To this end, we introduce three-dimensional tracking of SWCNTs by modifying the microscope's point spread function (PSF) using a customized double-helix phase mask [2] operating in the SWIR. We then use a Bayesian Gaussian mixture model [3] to spatially segment the 3D trajectories, enabling us to measure pure diffusion regimes in nanoscale reconstructed volumes. We will present applications to the study of brain extracellular space, but this strategy is applicable to many other optically accessible structures.

References

[1] Godin et al, Nat Nano 12 (2017) 238–243

[2] S. Pavani et al., PNAS 106 (2009) 2995-2999

[3] S. J. Roberts et al., IEEE TPAMI 20(11) (1998) 1133-1142

Quantum optics meets microscopy – An ultra-sensitive resonator microscope for nano- and life sciences

Thursday, 23rd November - 16:59: (Room 608) - Oral

Dr. Florian Steiner [1], ***Dr. Rute Fabiana Martins Fernandes*** [1], ***Ms. Ines Amersdorffer*** [1], ***Dr. Thomas Hümmer*** [1]

1. *Ludwig-Maximilians-University Munich, Department of Physics, Munich, Germany*

Isolated nanoscale systems provide only weak interaction with light due to their small size and therefore often escape direct observation in conventional light microscopy. This limits insights into individual nanosystems and slows down research in the fields of nanotechnology, material science, drug design, and pharmaceutical diagnostics.

To overcome these limitations, our group continuously developed optical micro-resonators, a technology pioneered in quantum optics [1]. In these resonators, light passes a sample up to 100.000 times and thereby enhances weak absorption signals tremendously. By means of micro-cavities with a small mode waist a scanning microscopy approach, i.e. ultra-sensitive spatially resolved absorption measurements near the diffraction limit, can be performed [2]. By optimizing the mechanical stability and by developing integrated electronics, extinction cross sections of down to 1 nm^2 can be imaged in real time.

The potential of this new type of microscopes is illustrated by imaging of individual carbon nanotubes [3], 2D-materials [4,5], and label free imaging of ultrathin biological sections [6]. Further developments are heading towards label-free single-molecule sensing as a complementary technology to state-of-the-art fluorescence spectroscopy techniques. This will open up new possibilities to get insights into structure-function relationships of isolated nanosystems from material sciences to life sciences.

References

1. D. Hunger et al., New J. Phys. 12, 065038 (2010)
2. M. Mader et al., Nat. Commun. 6, 7249 (2015)
3. T. Hümmer et al., Nat. Commun. 7, 12155 (2016)
4. M. Förg et al., Nat. Commun. 10, 3697 (2019)
5. F. Sigger, I. Amersdorffer et al., J. Phys. Chem. Lett. 13 (44), 10291 (2022)
6. J. Noe et al., Imaging & Microscopy 4 (2022)

Quantum Biology: past, current and future perspectives

Thursday, 23rd November - 17:25: (Auditorium) - Oral

Prof. Jim Al-Khalili [1]

1. *University of Surrey*

In this talk I wish to stay faithful to the title and divide it into three parts. After a brief introduction to the field, I will first trace the history of quantum biology back to its origins almost a century ago and the influence of Niels Bohr and highlight some of the key landmarks along its sometimes controversial past, up to it emerging in the 21st century as one of the most exciting current areas of interdisciplinary research. I will then talk a little about some of the recent work at Surrey with a focus on my own interests in the quantum tunnelling of H-bond protons between DNA nucleotide bases and their role in point mutations. Finally, I will look ahead briefly at where I see the biggest challenges in the field and where progress is likely to be made in the near future. I will end with a challenge to the community to answer the fundamental question, first laid out by Schrödinger in his book *What Is Life?* of whether life not only knows about quantum mechanics, but whether it has evolved the ability to utilise it in order to have, or prevent it from having, a functional role in biology that is distinct from its role in inanimate matter of equivalent complexity.

Using nanopores to watch enzymes at work

Friday, 24th November - 09:00: (Auditorium) - Oral

Prof. Jens Gundlach [1]

1. *University of Washington*

My group has been at the nexus of developing nanopore sequencing and establishing nanopores as a new tool for single-molecule biophysics. Much of our work is based on the engineered protein pore MspA. Here, I will show the stunning capabilities of using nanopores to observe enzyme mechanics in real-time as these enzymes move along DNA or RNA. We easily achieve ten times better position and time resolution than optical tweezers, while simultaneously measuring the exact nucleotide sequence in the enzyme. I will show hereto unseen detail of the motion of helicases, DNA and RNA polymerases,
reverse transcriptases, etc. Besides establishing decisive kinetic enzyme models, our method reveals many surprisingly properties of these enzymes.

Fluorescence-based single-molecule DNA sensors

Friday, 24th November - 09:40: (Auditorium) - Oral

Prof. Achillefs Kapanidis [1]

1. *University of Oxford*

Single-molecule fluorescence imaging has revolutionised the way we study biological molecules, mechanisms and cells. This family of methods had sprouted in large part from the quest to perform ultrasensitive sensing, and its remarkable development over the past 20 years has indeed fuelled much progress in the fields of sensors and nanosystems applications.

Over the past decade, my group has been developing several sensing methods that leverage sensors made of molecules of labelled single-stranded or double-stranded DNA; these methods were highly assisted by the availability of robust high-throughput wide-field imaging. I will discuss examples of how we use single-molecule DNA sensors to detect pathogenic viruses and bacteria, as well as DNA sequences. I will first discuss how our discovery of a novel interaction between calcium ions and the surface of many enveloped viruses led to rapid detection and identification of viruses (such as influenza and SARS-CoV-2) using imaging and deep-learning. I will then discuss our efforts to detect pathogenic bacteria and their antimicrobial resistance status rapidly using a combination of microfluidics, microscopy and machine-learning. Finally, I will discuss ongoing efforts to connect different DNA sequences to their functional properties in terms of DNA-protein interactions at the single-molecule level; these efforts feature a novel short-read single-molecule DNA sequencing method that relies on the binding of short DNA molecules to a surface-immobilised gapped DNA. Our methods are general and will be helpful for the study of many molecular and cellular systems.

Structurally adaptable DNA origami nanoactuators as size-selective nanopore sensors

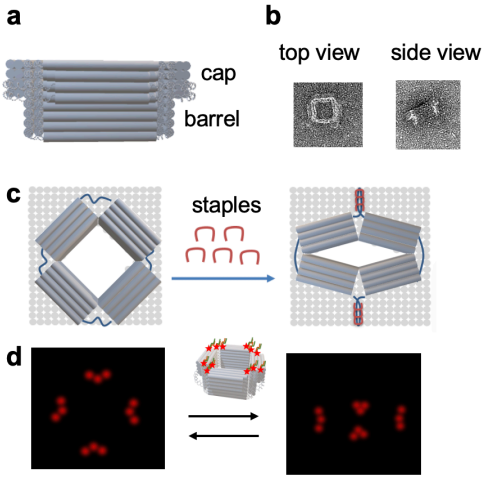
Friday, 24th November - 10:50: (Auditorium) - Oral

Dr. Sabina Caneva [1], ***Dr. Ze Yu*** [1]

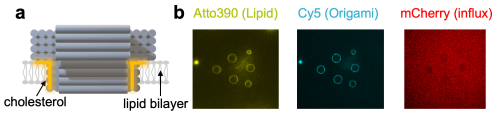
1. *TU Delft*

Protein nanopores are the most important molecular gateways in the biological world. They pierce the cell membrane and play a crucial role in regulating the passage of ions and small molecules. Delivering beneficial macromolecules, such as molecular therapeutics, across the cellular membrane is, however, an important problem since protein channels are typically narrow (~3 nm) and with fixed diameters. Synthetic nanopores with larger diameters, which can be engineered to embed in the cell membrane, could make significant headway in enabling transport of bulky, clinically-relevant molecules. Yet, it is crucial that these nanopores feature a controlled gating mechanism. Due to material and design constraints of the current approaches, nanopores that can insert in biomembranes while also enabling size-selective transport and on-demand gating remain elusive. We have realized the first class of structurally-adaptable DNA based nanopores that can insert in lipid bilayers and undergo large scale conformational changes in response to molecular triggers. Our approach combines the structurally precise 3D nanoscale objects that can be made through DNA origami nanotechnology with machine-inspired component design to generate nanopores with compliant mechanisms, featuring highly specific gating triggers. Two classes of square actuators are designed and assembled, the first type featuring a corner locking mechanism, and the second adopting an adjustable strut mechanism. Using AFM and TEM imaging we confirm the pre-programmed shape change, where the nanopore area can be modulated between 60 nm^2 and 30 nm^2 (Figure 1a.b).

We complement our mechanistic study with dynamics and reveal the real-time conformational changes via DNA PAINT (Figure 1c,d). Furthermore, the interaction with lipid bilayers is tested in a cDICE (continuous droplet interface crossing encapsulation) system, where the DNA nanoactuators are directly integrated during liposome formation. We confirm localization of the actuators at the membrane via confocal fluorescence imaging, and demonstrate size-selective translocation of Dextran molecules ranging in size from 70 kDa to 2 MDa (Figure 2a,b). Such mechanically actuated nanopores could find use in next generation drug delivery systems and synthetic cell research.



Scaneva figure1.png



Scaneva figure2.png

Optimizing single molecule barcodes for the NP-FET

Friday, 24th November - 11:07: (Auditorium) - Oral

Mr. Aderik Voorspoels [1], ***Ms. Juliette Gevers*** [1], ***Dr. Sybren Santermans*** [1], ***Dr. Nihat Akkan*** [1], ***Dr. Koen Martens*** [1], ***Dr. Kherim Willems*** [1], ***Prof. Anne S Verhulst*** [1], ***Prof. Pol Van Dorpe*** [1]

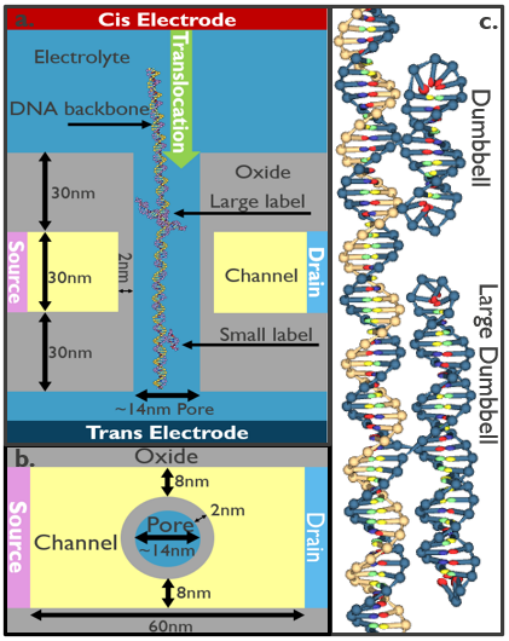
1. *Imec*

DNA barcodes (Bell & Keyser, 2016; Chen et al., 2018) are a promising vehicle for DNA data storage or as intermediary within omics (Martens et al., 2022), whereby the readout is based on a nanopore. An important design requirement for these barcodes is readability at high throughput. The voltage sensing based NP-FET (nanopore-FET), a novel technology, promises increased sampling frequency and better parallelization, and therefore a boost in the efficiency and throughput of reading these barcodes. To seize this opportunity, we present an optimized DNA barcode design for this novel reader.

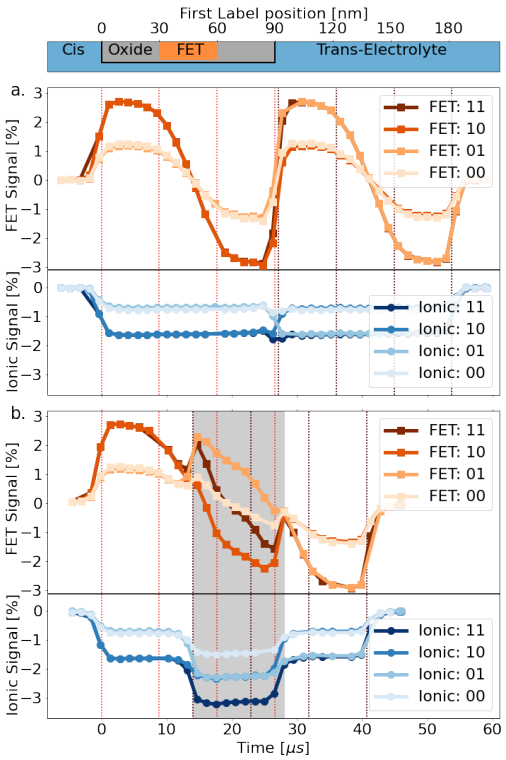
Dumbbell-labelled DNA has strong potential for barcoding applications as it offers high designability through the principles of DNA origami. Coarse-grained molecular dynamics (MD) are used to investigate the conformational fluctuations of dumbbell-labelled DNA. We then predict the NP-FET translocation signals for differently sized dumbbells and different conformations seen in MD using continuum modelling TCAD (technology computer aided design). Furthermore, the TCAD model is calibrated to experimental measurements of the ionic current through a solid-state nanopore (10-15 nm in diameter).

To design a barcode, we select two dumbbells (to encode 0 and 1) which have conformationally dependent signal ranges that do not overlap. We find that a small dumbbell of ~5nm (14 base pairs) and a larger dumbbell of ~10 nm (30 base pairs) are suitable building blocks for a barcode which will be read inside a 14 nm nanopore. Separating these dumbbells by ~90 nm allows us to detect the two labels individually. At the experimentally measured translocation speed of 3,4 mm/s, this results in a throughput of ~39 kb/s.

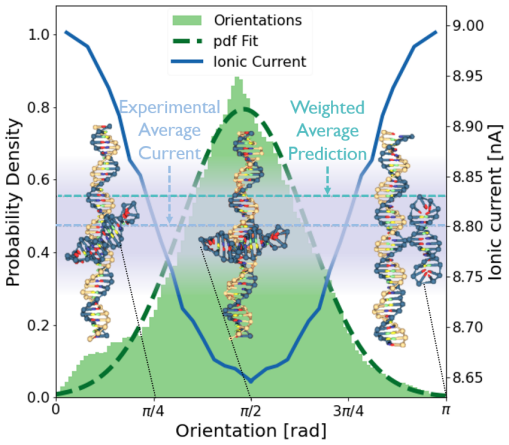
The combination of experimentally informed TCAD and MD thus provides a holistic picture of NP-FET signals and the variability thereof. Using this information to design DNA barcodes enables us to achieve optimal label density and thus speed of readout. Exploiting the higher sampling frequencies and improved signal size of the NP-FET, these barcodes are expected to be readable without the need to control the translocation speed. Therefore, this design and the voltage sensing principle behind the NP-FET are expected to provide increased throughput for DNA barcoding applications.



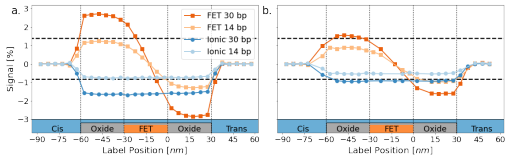
Sketch of the npfet and labels.png



Signals of barcodes in npfet.png



Dumbbellorientation vs ioniccurrent.png



Signal of labels in npfet.png

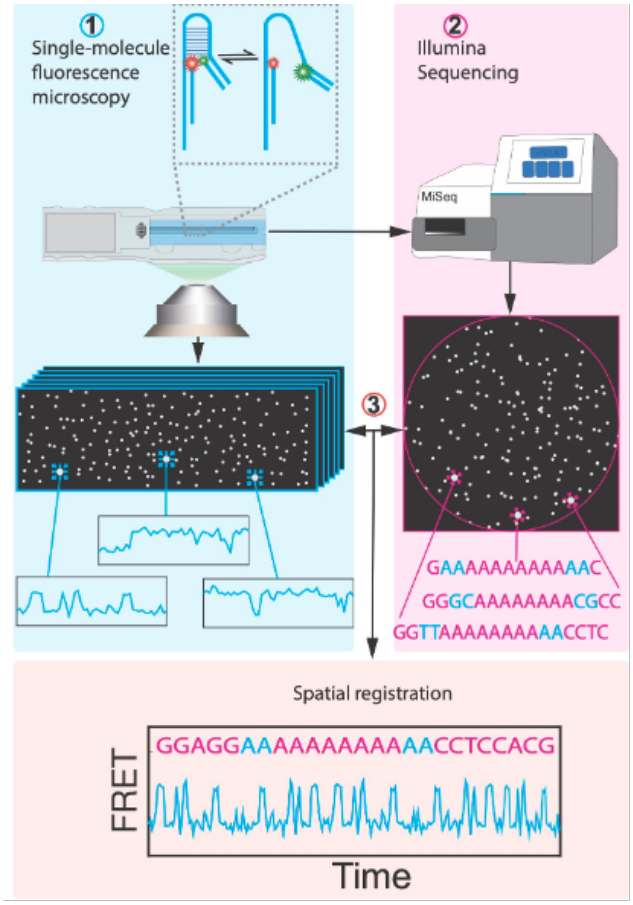
[online] - Massively multiplexed single-molecule fluorescence microscopy

Friday, 24th November - 11:24: (Auditorium) - Oral

Prof. Sebastian Deindl [1]

1. *Uppsala University*

Cells critically rely on a wide range of genome-processing and nucleic acid-interacting proteins. Single-molecule fluorescence microscopy has long been appreciated as a powerful tool to study the often-complex structural dynamics that enable the biological function of these sophisticated protein machines. The sequence of their nucleic acid substrates will inevitably shape their energetic and dynamic landscapes, and massively multiplexed single-molecule observations of their complex dynamics as a function of sequence will therefore greatly facilitate an in-depth mechanistic understanding of key genome transactions. Given the inherently low throughput of single-molecule techniques, this cannot be achieved with currently existing methods. We have therefore developed a novel high-throughput platform that combines, for the first time, single-molecule measurements of complex dynamics with next-generation sequencing. This platform enables the comprehensive profiling of sequence-dependent processes at the single-molecule level. Here I will discuss examples of how we have leveraged this platform to gain new insights into the sequence-dependent mechanisms of fundamental nucleic acid transactions.



Picture 1.png

Revealing the dynamics of Pif1 helicase when it collides with a G-quadruplex embedded in dsDNA using single-molecule assays.

Friday, 24th November - 11:41: (Auditorium) - Oral

Dr. Jessica Valle Orero [1], ***Dr. Martin Rieu*** [2], ***Dr. Phong Lan Thao Tran*** [2], ***Dr. Alexandra Joubert*** [3], ***Dr. saurabh Raj*** [2], ***Prof. Jean-Francois Allemand*** [2], ***Prof. Vincent Croquette*** [4], ***Prof. Jean-Baptiste Boule*** [3]

1. *ENS, the American University of Paris,* ***2.*** *ENS,* ***3.*** *Museum National d'Histoire Naturelle,* ***4.*** *ENS, ESCPI*

G-quadruplexes (G4) are secondary structures formed by guanine-rich DNA and RNA sequences. G4 motifs are found in different regions of the genome, such as at telomeres, promoters, or replication origins. These structures can be very stable and may act as a roadblock for replication forks or transcription bubbles. Cells have developed specialized proteins that can either stabilize or remove these structures. Among those, the Pif1 helicase has served as a model helicase capable of removing these G4 structure in vitro and has been shown to prevent G4-linked genomic instabilities. To date, our mechanistic knowledge of G4-helicase interaction is largely inferred from *in vitro* biophysical studies of G4s in a single-stranded DNA context. The interaction between Pif1 and G4 structures have already been studied using a single-molecule approach, which fail to capture the helicase dynamics during translocation and interaction with the G4 in a double stranded context. We have developed dsDNA assays where we induced very stable G4 structures (c-Myc G4, lifetime > 1hour), and observe in real time Pif1 opening the duplex and resolving the G4. Our results show that the helicase can reduce the lifetime of G4 from hours to seconds, but moreover, once the G4 is resolved Pif1 can resume translocation without changing state from a monomer to dimer, contrarily to previous reports. We also observed that in presence of roadblocks, including G4s, Pif1 exhibits a strand switching behavior. Our quantitative analysis allows us to build a model that characterizes Pif1 dynamics under a dsDNA-G4 context. Our model will be especially pertinent in studying the role of protein motors when removing stable structures during replication.

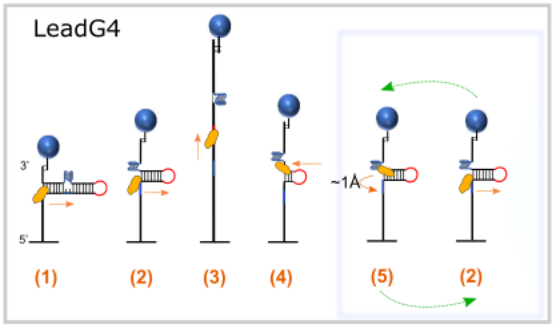


Fig.1 Sketch of Pif1 activity when encountering a G4 structure on the leading strand. The helicase travels on the lagging strand, passing the loop, onto the leading strand (1-4), until it collides with the G4 (5); since the opposite strand is in the vicinity (~1Å) the helicase manages to strand switch (2), and recycle again through the hairpin.

Fig1 pif1strandswitching valle v2.png

SensPIV - Imaging flow fields and chemical gradients using nanoparticle based sensors

Friday, 24th November - 10:50: (Room 607) - Oral

Prof. Klaus Koren [1]

1. *Aarhus University*

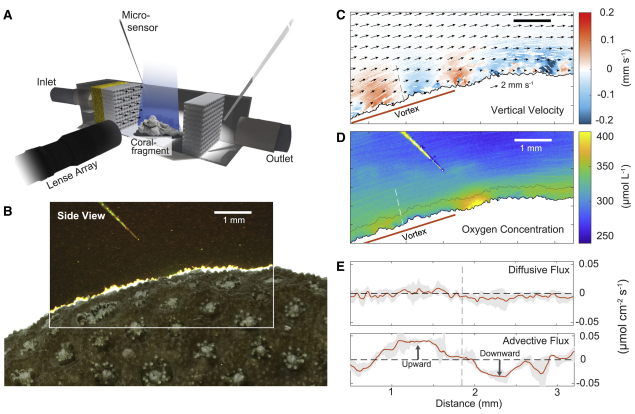
Transport of oxygen (O_2) is essential for life on Earth as it sustains aerobic organisms respiring O_2. Biological respiration leads to the local depletion of O_2 while diffusive and advective transport processes replenish the O_2 required for proper biological functioning. From individual cells to whole organisms, O_2 transport unfolds across micrometer to millimeter length scales and can change within milliseconds in response to fluid flow and organism behavior. The spatio-temporal complexity of these processes makes the accurate assessment of O_2 dynamics via currently available methods difficult or unreliable. Here, we combined microscale O_2 imaging methods with particle tracking to determine O_2 concentrations and flow fields simultaneously. This new method - '*SensPIV*' - now enables non-invasive measurements of O_2 dynamics across a wide-range of spatial scales at unprecedented accuracy and speed [1].

In essence SensPIV enables the tracking of individual O_2 sensitive sensor nanoparticles to reconstruct the flow field and O_2 gradients even in complex biological systems like around reef building corals. Using SensPIV we have shown, see figure, that corals actively mix the stagnant water layer around them to transport O_2 from areas of production (photosynthesis by the symbionts) to areas of O_2 consumption (the mouth opening of the coral) [2].

In this contribution I will present SensPIV, a single particle tracking based method for dual parameter sensing, and elaborate on current advances in this methodology. Furthermore I will present recent studies using this approach to better understand complex and changing chemical microenvironments in biological systems.

[1] S. Ahmerkamp, F. M. Jalaluddin, Y. Cui, D. R. Brumley, C. O. Pacherres, J. S. Berg, R. Stocker, M. M. M. Kuypers, K. Koren and L. Behrendt, *Cell Reports Methods*, 2022, **2**, 100216.

[2] C. O. Pacherres, S. Ahmerkamp, K. Koren, C. Richter and M. Holtappels, *Curr. Biol.*, 2022, **32**, 4150-4158.e3.



Senspiv-coral-flow-o2.png

Single-molecule plasmonic biosensor for continuous sensing

Friday, 24th November - 11:07: (Room 607) - Oral

Mr. Livio Oliveira de Miranda [1], ***Prof. Peter Zijlstra*** [1], ***Dr. Mathias Dolci*** [1], ***Dr. Khulan Sergelen*** [2]

1. *Molecular Biosensing. Department of Applied Physics and Science Education, Eindhoven University of Technology.,* ***2.*** *BioMed X Institute*

Introduction

New biosensor technologies have become of great interest in a wide range of areas such as health care, food control, and environmental monitoring. Nevertheless, there is a need for label-free sensing methodologies that provide real-time information on biochemical processes in the environment of interest [1, 2].

Methods

To perform continuous sensing, we develop a fluorophore-free optical read-out based on a plasmonic nanoparticle-on-film construct. The particle-film interaction can be tailored by the particle size and the particle-film spacing [2], which depend on the molecular design. The plasmonic particles as well as the metallic thin film are functionalized with ssDNA capture probes, promoting a DNA sandwich construct in the presence of a specific target (Figure 1). Complementary regions of 8-10nt provide bound-sate lifetimes on the order of a second [3, 4], resulting in reversible DNA interactions and switching between particle-bound and unbound states in the presence of the specific target analyte. The reversibility of the system provides a “reset” of the sensor, enabling continuous analyte monitoring. The events are dynamically detected using dark-field microscopy on a large number of particles simultaneously.

Results and discussion

The frequency of binding events is proportional to the analyte concentration, resulting in a femtomolar limit-of-detection (Figure 2). Moreover, kinetic fingerprinting reveals stochastic single-molecule interactions governed by Poisson statistics and thus exponentially distributed (Figure 3). We foresee that the proposed biosensor will pave the way for the development of continuous biosensors not only for DNA but also for peptides and proteins.

[1] Zijlstra, P. *et al.* ACS Phys. Chem Au, 3, 2, 143–156 (2023)

[2] Prins, M *et al.* Nat Commun 13, 6052 (2022)

[3] Zijlstra, P. *et al.* J. Phys. Chem. C 123, 42, 25801–25808 (2019)

[4] Zijlstra, P. *et al.* Nanoscale 12, 4128 (2020)

[5] Jungmann, R *et al. Nano Lett.* 10, 11, 4756–4761 (2010)

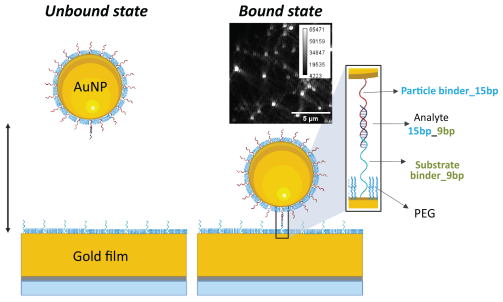


Figure 1. dna sandwich single molecule plasmonic biosensor assay concept.png

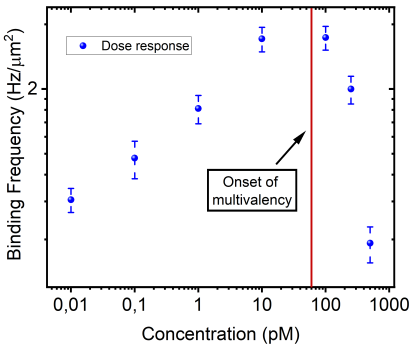


Figure 2. dose response dna sandwich assay..png

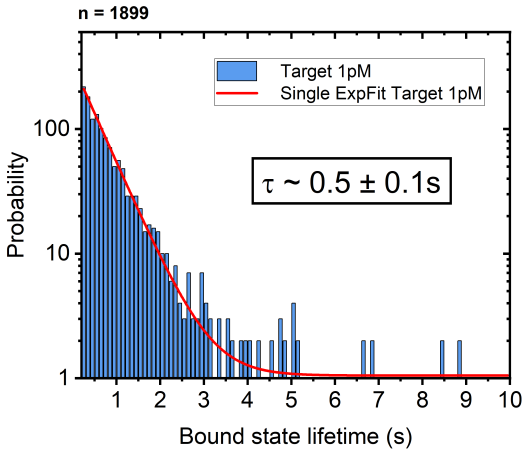


Figure 3. kinetic fingerprinting of 9bp complementary dna sandwich target at 1pm.png

Development of single-atom (Ba2+) sensors for NEXT experiment

Friday, 24th November - 11:24: (Room 607) - Oral

Prof. Zoraida Freixa [1]

1. *University of the Basque Country (UPV-EHU)*

This contribution is on behalf of the NEXT Collaboration.

(https://next.ific.uv.es/next/experiment/collaboration.html)

The NEXT Collaboration is an international and interdisciplinary collaboration aiming to explain the imbalance between matter and antimatter in the observed Universe (so-called baryogenesis). Baryogenesis could be explained if there was an elemental particle able to transform into its own antiparticle with a slight preference for the particle nature. The only elemental particle suspicious to have this behavior is the neutrino, a neutral subatomic elemental particle with a non-zero mass. Thus, establishing neutrino's nature is one of the most important standing questions in Particle Physics and Cosmology.

NEXT proposes to demonstrate the nature of neutrino by continuously monitoring a rare nuclear disintegration process of ^{136}Xe (double beta decay) that simultaneously generates two neutrinos. Since particles and antiparticles are known to annihilate mutually, eventually, one would expect the observation of a neutrinoless event.

NEXT pursues observing a ββ0v decay using a large chamber of ^{136}Xe gas (called Xe-TPC). A 100 Kg Xe-TPC (NEXT-100) is currently under construction at the Laboratori Subterrani de Canfranc in Spain (Figure 1), and a tone-scale Xe-TPC is envisaged for 2026. In a ^{136}Xe matrix, a double beta disintegration will produce two electrons and the daughter atom (Ba^{2+}). Within the previous stages of the NEXT experiment, physicists have been able to detect events with a signature compatible with a neutrinoless double beta decay, but it has not been possible to ascertain that they were not due to spurious events or cosmic radiation. It has been established that the only definite confirmation would be the synchronous detection of the daughter atom, Ba^{2+}. Inside the Xe-TPC, submitted to an electric field, whereas the ejected electrons start a trajectory towards the anode, the charged Ba^{2+} atoms are drifted towards the cathode. Therefore, the most logical strategy consists of using an optically transparent cathode covered by a monolayer of luminescent chemical sensors able to signal the single atom of Ba^{2+} produced inside the chamber. Such an experiment requires chemical and technological development beyond the state of the art that can only be brought to success by a multidisciplinary team.

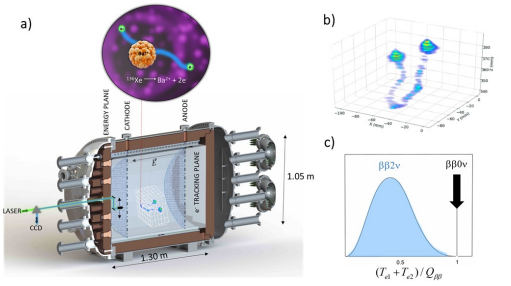


Figure 1. **a)** Rendering of the NEXT-100 detector (1.27 m³ fiducial volume). It will be holding a mass of 97 kg of xenon gas enriched at 90% in ^{136}Xe, and operating at 15 bar. **b)** Traces reconstruction of electrons ejected in ββ0ν candidate, showing 2 energy blobs at the extremes. **c)** Energy distributions of the ejected electrons in ββ decays.

Figure1 nextexperiment.jpg

Light-guiding nanowires for single molecule detection with TIRF-level sensitivity

Friday, 24th November - 11:41: (Room 607) - Oral

Mx. Rubina Davtyan [1], ***Prof. Nicklas Anttu*** [2], ***Ms. Julia Valderas Gutiérrez*** [1], ***Prof. Fredrik Höök*** [3], ***Prof. Heiner Linke*** [1]

1. *Lund University,* ***2.*** *Åbo Akademi University,* ***3.*** *Chalmers University of Technology*

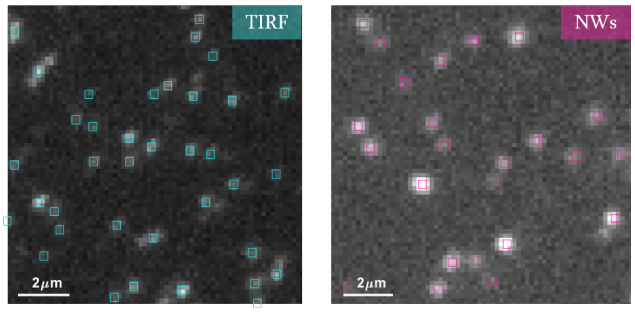
Semiconductor nanowires have been demonstrated to significantly improve the limit of biomarker detection, with the ability to detect concentrations as low as 100 fM, and with evidence of single molecule detection [1]. This is achieved through the interplay of several optical phenomena, namely waveguiding properties of nanowires, excitation enhancement, and directional emission from nanowire tips [1][2]. Due to these optical properties and large surface-to-volume ratio, nanowires offer detection advantages in low molecular concentrations and serve as surfaces for monitoring molecular kinetics and binding dynamics with widefield illumination microscopy.
In this study, we perform a direct benchmark against single-molecule TIRF detection on planar glass using the same biotin-streptavidin assay, flow channel, and experimental conditions. Using arrays of vertically standing GaP nanowires of 100 nm diameter and 1 µm spacing to detect fluorescently labeled streptavidin, we demonstrate that nanowires offer TIRF-level single molecule detection using only epifluorescence microscopy. We illustrate that nanowire arrays with a lateral spacing larger than the diffraction limit not only enable the detection of nanowire-bound single molecules but also serve as a way to characterize molecular concentrations by direct localization and digital counting of bright nanowires. Additionally, we introduce an image enhancement and analysis framework optimized for tackling nanowire-enhanced fluorescence microscopy data to achieve accurate results and improve the limit of detection demonstrating the results on both synthetic and experimental data.

Figure caption:

Figure 1. TIRF vs nanowire enhanced widefield microscopy images of Alexa647 labeled streptavidin (StvA647) bound to biotinylated BSA (bBSA).

References:

[1] J. Valderas-Gutiérrez, et al, ACS Appl. Nano Mater. 5, 9063–9071(2022)
[2] D. Verardo et al, Nanomaterials 11(1), 227 (2021)



Tirfvsnws fluorescence microscopy images.png

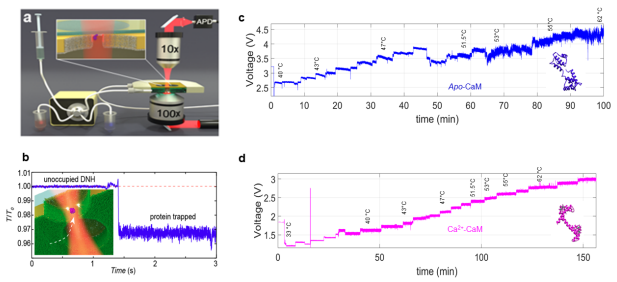
Probing the Thermal Unfolding and Refolding Trajectory of Single Calmodulin Proteins using Plasmonic Optical Tweezers

Friday, 24th November - 10:50: (Room 608) - Oral

Ms. Edona Karakaci [1], ***Dr. Cuifeng Ying*** [2], ***Dr. Esteban Bermundez Urenia*** [3], ***Prof. Reuven Gordon*** [4], ***Prof. Michael Mayer*** [1]

1. *Adolphe Merkle Institute, University of Fribourg, Chemin des Verdiers 4, CH-1700, Switzerland,* ***2.*** *Advanced Optics and Photonics Laboratory, Department of Engineering, School of Science and Technology, Nottingham Trent University, Nottingham NG118NS, United Kingdom,* ***3.*** *Centro de Investigación en Ciencia e Ingeniería de Materiales and Escuela de Física, Universidad de Costa Rica, San José, 11501, Costa Rica,* ***4.*** *Department of Electrical and Computer Engineering, University of Victoria, Victoria, British Columbia V8P 5C2, Canada*

Studying the conformational dynamics of label-free single proteins is essential to understanding their function fully. Ensemble measurements for analyzing protein dynamics are limited to averaged information from a protein population. They cannot capture heterogeneity regarding post-translational modifications, folding states, mutations, or other variations. Existing single-molecule techniques for determining conformational changes of proteins in real-time require labeling or other modifications, which can alter the native state and function of the protein under observation. In contrast, Plasmonic Optical Tweezers have proven to be a powerful tool for monitoring a single unmodified protein trapped within a plasmonic hotspot with an impressive temporal resolution of 40 microseconds.[3]. This work reports the first thermal unfolding and refolding trajectory of a single calmodulin protein using the double-nanohole (DNH) plasmonic optical tweezer (Fig. 1a-d). Calmodulin, a compact and soluble protein primarily composed of alpha helices, is a calcium-dependent protein activator. The binding of calcium has an impact on both the conformation and stability of calmodulin. Our work demonstrates successfully trapping a single calmodulin molecule (17 kDa) within a plasmonic double nanohole. We reveal that individual apo-calmodulin molecules thermally unfold and refold in distinct steps, driven by conformational fluctuations within specific protein domains. These changes occur in response to variations in the hotspot temperature (Fig. 1c, d). Importantly, the conformational fluctuations of individual calmodulin domains result in observable changes in transmission, a phenomenon that persists for up to two hours. This extended observation period is achieved without the limitations associated with the photobleaching of fluorescent labels or contamination often encountered in scanning probe tip-based force spectroscopy. The findings are consistent with the single-molecule fluorescence spectroscopy (smFS) study and indicate that Plasmonic Tweezers have solid use in investigating protein folding processes.



Abstract figure thermal unfording of calmodulin.png

Cavity-enhanced ultrafast sensing of single nanosystems

Friday, 24th November - 11:07: (Room 608) - Oral

Mr. Shalom Palkhivala [1], ***Dr. Larissa Kohler*** [1], ***Prof. David Hunger*** [1]

1. *Karlsruhe Institute of Technology*

Introduction. The study of the optical, physical and dynamical properties of single unlabelled nanosystems is of interest in many branches of science. In biophysics and chemistry, for example, most processes occur in solution, necessitating sensors which function in water. We demonstrate an open-access optofluidic platform for the high-speed label-free sensing of nanoparticles in aqueous suspensions.

Methods. The heart of the sensor is a fibre-based Fabry-Perot microcavity integrated into a microfluidic system. By modulating the cavity length and measuring the cavity resonance shift and peak transmission change due to the interaction of the cavity field with a nanoparticle, the particle can be detected and characterised. Simultaneous monitoring of several transverse modes allows the three-dimensional tracking of a single nanoparticle in the cavity as well. To increase the bandwidth of our sensor, we implement a cavity-locked detection scheme, allowing sensitive measurements several orders of magnitude faster than other current techniques. Additionally, orthogonal polarisation modes are interrogated separately to yield rotational information of anisotropic particles.

Results. Using a modulated cavity, the Brownian diffusion of single silica nanospheres in water was tracked in three dimensions [1] with spatial and temporal resolutions of down to 8 nm and 0.3 ms. Furthermore, gold nanorods were investigated with a locked cavity stabilised to 4% of the resonance linewidth at a finesse of 50000. This enabled a detector-limited measurement bandwidth of 100 MHz, so that both rotational and translational dynamics could be resolved. Autocorrelation analysis allows determination of the size of individual nanorods, accurate to about 2 nm, and nanorods having lengths 20 nm and 25 nm could thus be differentiated. Finally, polarisation-based measurements allow us to track the orientation of a single nanorod with 10 ns temporal resolution.

Discussion. In this work, we demonstrate an optofluidic microresonator as an open-access and high-bandwidth sensor, and use it to investigate the physical and dynamical properties of single nanoparticles suspended in fluids. In the next step, we shall apply this technology to investigate the diffusional and structural dynamics of biological nanosystems, such as the folding of DNA “origami”.

[1] Kohler, L. *et al. Nat Commun* **12**, 6385 (2021).

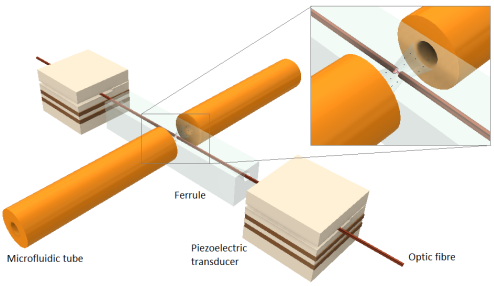


Fig. 1 - setup of the optofluidic cavity.png

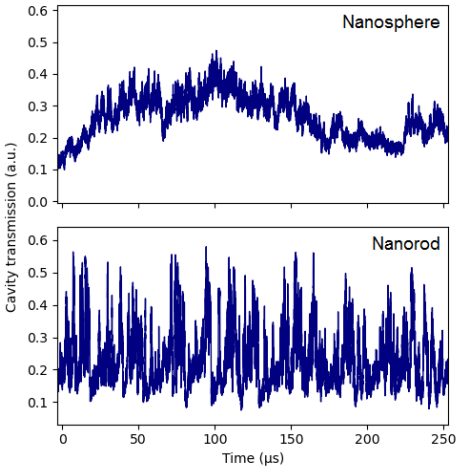


Fig. 2 - comparison of high-bandwidth signals from a nanosphere and a nanorod.png

Nanofluidic Scattering Spectroscopy

Friday, 24th November - 11:24: (Room 608) - Oral

Mr. Björn Altenburger [1], ***Prof. Christoph Langhammer*** [1]

1. *Chalmers university of technology*

At the center of heterogeneous catalysis are nanostructures with a high surface to volume ratio of specific materials that can assist a chemical reaction by improving its rate or tuning its selectivity by altering the energy landscape. These catalysts come in a broad spectrum of sizes and shapes, so that dedicated single particle studies are needed to detect particles with exceptional performance and avoid erroneous structure-function correlations.

Various techniques for single particle catalysis have been developed, including methods like fluorescence and electron microscopy, X-ray diffraction and scattering and plasmonic sensing, which detect electrons or photons that report either on molecules formed and consumed or on changes to the catalyst particle and its surrounding. However, none of these methods provide direct single particle activity information without plasmonic enhancement effects or fluorescence, impacting thereby the studied reaction itself, the range of catalyst materials and the reactant concentrations.

As a response, we are developing an optical microscopy technique that can quantitatively measure catalytic activity of a single nanoparticle without the limitations of existing single particle methods. The core of this method are nanofluidic channels that can accurately control the transport of reagents to and from a single catalytically active particle localized inside such channels. Furthermore, the light scattering properties of nanochannels render them highly sensitive to refractive index changes of the fluid inside them. Hence, when a catalytic reaction alters the molecular composition of the fluid in the channel, its light scattering characteristics change and reveal in this way the catalytic performance of the nanoparticle.

In this presentation, we focus on the possibility to spectrally resolve changes in the light scattered from a nanochannel. We illustrate this on measurements of dye molecules, extracting so the wavelength-dependent refractive index of the dyes. Via the Kramers-Kronig transformation, we transform the obtained refractive index spectrum into a compound-specific absorption spectrum, allowing us to not only identify the compound in the nanochannel via its spectral fingerprint, but also its local concentration. We project our results on single particle catalysis on the example of the decomposition of Allura Red with $NaBH_4$ over Au catalyst nanoparticles.

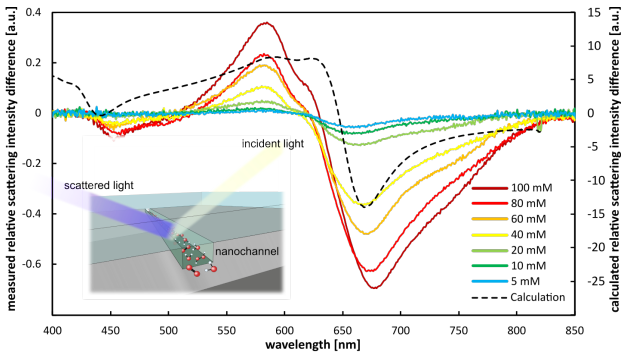


Figure 1. Measured relative scattering intensity difference spectra for the dye Brilliant Blue at different concentrations. The spectra shown are the averaged response of a 25 µm long channel with a 200 nm by 200 nm geometrical cross section embedded in SiO2. For the calculated spectrum, the absorbance spectrum of Brilliant Blue was transformed via the Kramers-Kronig relation into the refractive index spectrum, from which then the scattering spectrum was calculated. The inset shows the basic principle, where the incident light is scattered on the nanochannel and thereby spectrally modified.

Abstract image.png

High-throughput nanoplasmonic microarray for spatiotemporal-resolved single-cell secretion monitoring

Friday, 24th November - 11:41: (Room 608) - Oral

Dr. Yen-Cheng Liu [1], ***Mr. Saeid Ansaryan*** [1], ***Dr. Xiaokang Li*** [2], ***Dr. Eduardo R. Arvelo*** [3], ***Ms. Augoustina Maria Economou*** [1], ***Dr. Christiane Sigrid Eberhardt*** [4], ***Prof. Camilla Jandus*** [4], ***Prof. Hatice Altug*** [1]

1. *EPFL,* ***2.*** *Centre hospitalier universitaire vaudois,* ***3.*** *University of Wisconsin-Madison,* ***4.*** *University of Geneva*

Cell secretion is a precisely regulated dynamic process closely related to physiology and disease progression. Dissecting secretomic heterogeneity provides a better understanding of disease mechanisms, and a powerful tool for single-cell secretomic analysis helps realize such studies. Conventional single-cell analysis methods provide only semi-quantitative endpoint data, limiting our insight into dynamic secretion behavior. Most current efforts for quantitative analysis rely on fluorescence bioassays, involving tedious labeling and complex handling, compromising time resolution. Therefore, there is an unmet need to analyze the secretomes of many single cells in real-time without using molecular tags for signal interrogation.

In this work, we present a high-throughput and ultrasensitive nanoplasmonic biosensor integrated with microwell compartment arrays for monitoring the secretome of living single cells in real-time on a large scale without labels. The nanoplasmonic substrate utilizes gold nanohole arrays (AuNHA), which feature extraordinary optical transmission (EOT). The EOT spectrum shifts highly sensitively in response to localized refractive index changes upon analyte binding on its surface (Figure 1). High-throughput configuration is achieved by synchronizing the movement of the motorized stage with predefined microwells containing single cells and capturing images. Signal interrogation can be achieved by measuring resonance shifts caused by secretome release from each cell or by shining the chip with a narrowband light source at one flank of the EOT peak and monitoring the resulting intensity change caused by the peak shift upon surface binding.

Using spectroscopic scanning, we observed interleukin-2 (IL-2) secretion by EL4 cells in response to chemical stimuli or stimuli with an inhibitor. Spatiotemporal sensorgrams were constructed from time-resolved spectroscopic images, revealing resonance shifts over time. Hundreds of single cells were studied, showing significant IL-2 secretion differences between experimental and control groups (Figure 2). With the intensity imaging approach, we validated the system by tracking immunoglobulin G (IgG) secretion from engineered hybridoma cells for over 12 hours with minute-level resolution. We extracted spatial distribution information of secreted products around cells and observed diverse secretory patterns (Figure 3). In conclusion, this high-throughput nanoplasmonic microarray enables single-cell secretion analysis with resolved spatiotemporal profiles, enhancing single-cell functionality characterization.

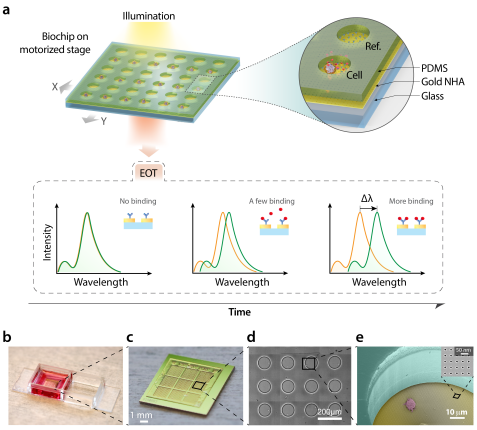


Figure 1. Schematic representation of the integrated biochip and the measurement principle for real-time secretion analysis with the nanohole arrays. (a) The transmitted light presents the extraordinary optical transmission (EOT) resonance peak, which responds to the binding events of secreted analytes by the cells on the functionalized nanohole array surface. By measuring the resonance shift (ΔΔ) dynamically from the acquired spectrum, the binding events of the secrete analytes can be quantified in a label-free manner. (b) The picture of the chamber slide for measurement with the biochip immersed in specific cell medium. (c) Illustration of the microwell array with single EL4 cells dispensed in the wells. (d) Colored SEM image of a single EL4 cell in the microwell on AuNHA and a Zoomedin SEM image of the nanoholes.

Fig.1 schematic illustration and microcompartments.png

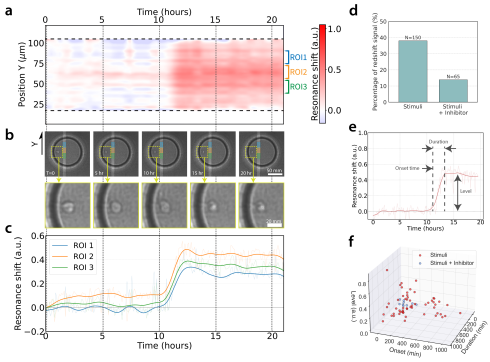


Figure 2. Real-time spectroscopic imaging and bright-field imaging of single-cell secretion and statistical analysis. (a) Spatiotemporal sensorgram of a single EL4 cell secreting IL-2 cytokine upon chemical stimulation. (b) Snapshots of one EL4 cell in the microwell at various time points (top) and the zoom-in views of the cell (bottom). (c) Three temporal sensorgram curves obtained by averaging the resonance shift information along the Y-axis within the ROIs indicated in Figure 2a and 2b. (d) Percentage of single cells showing IL-2 secretion signal for both experimental group (with stimuli) and control group (with stimuli + inhibitor). (e) Temporal sensorgram obtained from a single EL4 cell secreting IL-2 cytokine annotated with the definition of onset, duration, and level of secretion. (f) 3D scatter plot of all secreting single EL4 cells with chemical stimuli and with stimuli and inhibitor.

Fig.2 single-cell secretome analysis with spectroscopic scanning.png

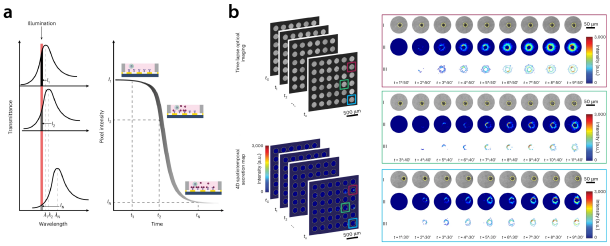


Figure 3. Single-cell secretome analysis with intensity imaging approach. (a) Working principle: by shining an LED on the gold nanohole array substrate, the interactions between analytes and receptors change the nearby surface's refractive index, causing the resonance peak to shift towards red. The sCMOS camera captures time-lapse images with sub-minute temporal resolution to convert spectral shifts into pixel intensity changes over time. This yields a 1D binding event sensogram for each camera pixel in real-time. Efficient light transmission through the patterned gold surface enables high-contrast optical imaging for simultaneous analysis of cell secretion and morphology. (b) Time-lapse optical images of the single-cell microwell array are analyzed via machine learning to track cell positions, monitor morphological changes, and generate 4D spatiotemporal secretion maps. These maps illustrate variations in camera pixel intensity due to IgG binding, resulting in spectral redshifts. Additionally, three hybridoma cell secretion profiles, accompanied by optical images, depict progressive increases in secretion intensity and coverage over time. Yellow lines indicate cell boundaries tracked by the machine-learning protocol.

Fig.3 single-cell secretome analysis with intensity imaging approach.png

Scaning Ion Conductance Spectroscopy

Friday, 24th November - 13:30: (Auditorium) - Oral

Prof. Aleksandra Radenovic [1]

1. *Ecole Polytechnique Fédérale de Lausanne*

In this talk, I will describe a novel single-molecule method where we engineer precise spatial and temporal control into the single-molecule experiment. We use a glass nanopore mounted on a 3D nanopositioner to spatially select molecules, deterministically tethered on a glass surface, for controlled translocations. By controlling the distance between the nanopore and the glass surface, we can actively select the region of interest on the molecule and scan it a controlled number of times and at a controlled velocity. Decreasing the velocity and averaging thousands of consecutive readings of the same molecule increases the signal-to-noise ratio (SNR) by two orders of magnitude compared to free translocations. We applied our method to various DNA constructs, achieving down to single nucleotide gap resolution. The spatial multiplexing combined with the sub-nanometer resolution could be used in conjunction with micro-array technologies to enable the screening of DNA, improving point-of-care devices, or enable high-density, addressable DNA data storage.

Label-free direct detection and sizing of single proteins and bioparticles

Friday, 24th November - 14:10: (Auditorium) - Oral

Prof. Vahid Sandoghdar [1]

1. *Max Planck Institute for the Science of Light Erlangen*

TBD

Authors Index

Conferences, Events & Workshops