NANO IN BIO 2022

Nanosciences for Life & Materials Sciences

3rd edition: From May 30 to June 05, 2022

Le Gosier, Guadeloupe (French Caribbean)

NANO IN BIO 2022

Nanosciences for Life & Materials Sciences

3rd edition: From May 30 to June 05, 2022

Le Gosier, Guadeloupe (French Caribbean)

Program

&

Book of

Abstracts

Welcome

Initially planned for 2020, this third edition of NanoInBio had to deal with the very special period we just went through. It is therefore after several postponements that this new edition of the conference launched in 2016 is held. We are very happy to welcome you in Guadeloupe.

We wish you a wonderful stay in Guadeloupe and a very exciting conference !

The NanoInBio Committee

Organizing Committee

Lilia CHTCHEGLOVA, Univ of Linz, Austria

Vincent DUPRES, CIIL CNRS UMR 9017 – INSERM U1019, Université de Lille, Lille, France

Grégory FRANCIUS, LCPME UMR 7564, CNRS - Université de Lorraine, Nancy, France

Jessem LANDOULSI, LRS UMR 7197, CNRS - Sorbonne Universités, Paris, France

Zoya LEONENKO, Univ of Waterloo, Waterloo, Canada

Kislon VOITCHOVSKY, Univ of Durham, Durham, United Kingdom

Scientific Committee

Etienne DAGUE, LAAS UPR 8001 CNRS, Toulouse, France

Karim EL KIRAT, UMR 7338, Université de Technologie de Compiègne, Compiègne, France

Georg FANTNER, EPFL, Lausanne, Switzerland

Pierre SCHAAF, Univ de Strasbourg, Strasbourg, France

Tomaso ZAMBELLI, ETH, Zurich, Switzerland

Local Organizing Committee

Laurence ROMANA, GTSI, Université des Antilles, Pointe-à-Pitre, France

Marc ROMANA, UMRS 1134 INSERM, Pointe-à-Pitre, France

Olivier GROS, UMR 7138 CNRS-UPMC, Université des Antilles, Pointe-à-Pitre, France

Private sponsors of the Conference

BRUKER

Park SYSTEMS

nanosurf

SPECS™

OPTICS11

Quantum Design

NANO IN BIO 2022

Nanosciences for Life & Materials Sciences

Le Gosier, Guadeloupe
(French Caribbean)

3rd edition - From May 30 to June 05, 2022

Supported by Langmuir - ACS Publication & CNRS

Institutional sponsors of the Conference

CNRS

MINISTÈRE DES OUTRE-MER
Liberté
Égalité
Fraternité

LES ÎLES DE GUADELOUPE
Il y a tant d'îles en elles

REGION GUADELOUPE
Archipel d'Avenir

Université des Antilles

LORRAINE Université d'Excellence

Registration & communication

PROGEPI

PROPULS'
Catalyseur de projets

Program

Spring School NanoInBio 2022

Monday, May 30 – Tuesday, May 31

Special Lecture – Conférence Grand public

Dr. Philippe Lavalle, Inserm/University of Strasbourg, France

« Infections à l'hôpital : les biomatériaux innovants pour limiter les maladies nosocomiales »

Conference NanoInBio 2022

Wednesday, June 1 – Sunday, June 5

Keynote Lectures

Loredana Casalis, Synchrotron of Triese, Italy
Jörg Enderlein, University of Göttingen, Germany
Catherine Picart, University of Grenoble, France
Patrick Couvreur, Institut Galien – Université Paris-Saclay, France
Adam Foster, Aalto University, Finland
Teuta Pilizota, University of Edinburgh, United Kingdom
Georg Fantner, EPFL, Lausanne, Switzerland
Núria Gavara, Institute for Bioengineering of Catalonia, Spain
Nicholas Kotov, University of Michigan, United States
Hermann Schillers, University of Münster, Germany
Laia Pasquina-Lemonche, University of Sheffield, United Kingdom

Invited Speakers

Yoo Jin Oh, Johannes Kepler University, Linz, Austria
Paolo Scrimin, University of Padova, Italy
Sergei Kalinin, Oak Ridge National Laboratory, United States
Michel Gringas, University of Waterloo, Canada

Keynote Lectures: 30 minutes
Invited Speakers : 25 minutes
Regular Presentations: 15 minutes

Spring School NanoInBio 2022

The 2-days spring school will focus on the use of modern computer approaches for data analysis, in particular machine learning. Training will be provided using a combination of lectures, worked examples and hands-on sessions with real datasets. The goal is to provide the attendees with practical basic knowledge allowing them to select and use a suitable approach for extracting a desired feature from a dataset.

The school is organised as follow:

Day 1 - morning: Theoretical introduction to **machine learning for data analysis**. Choice of a suitable strategy for a given question, and main steps to enact a given strategy. Basic keys for getting started as a non-specialist. Worked examples.

Day 1 - afternoon: Guided 'hands on' approach to extracting a given feature from a real data set. Hints from experience and discussion of possible variations

Day 2 - morning: Lecture on the advantages and limitations of the main different existing approaches, including obvious pitfalls. How to test a strategy is working properly. Worked examples highlighting the ‘dos’ and ‘don’t’ when implementing and trialling a new strategy.

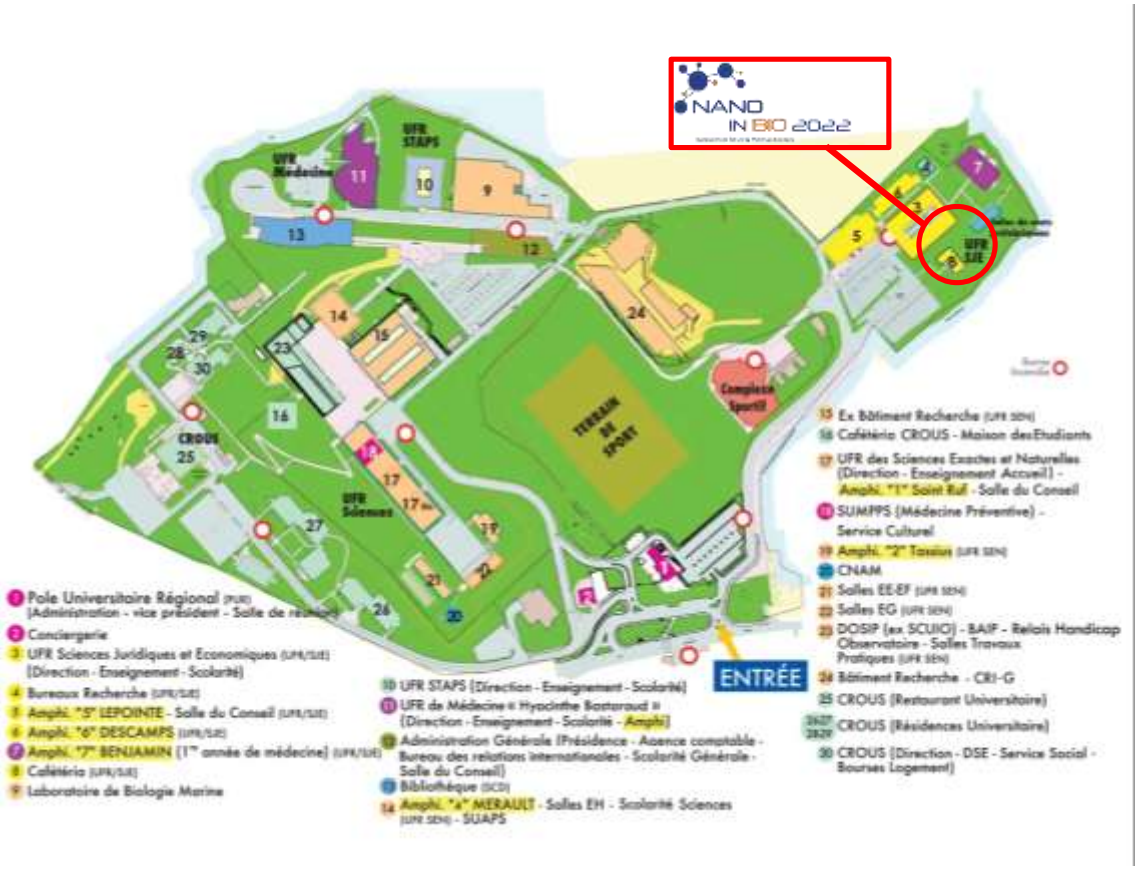
Day 2 - afternoon: Guided 'hands on' approach to extracting a given feature from a real data set (different type to day 1). Hints from experience and discussion of possible variations. Pointers to the main sources of information and resources for using machine learning. Wrap up and conclusion"

Location: The Spring School (lectures and training activities) will take place at the Université des Antilles, Campus of Fouillole, **Amphitheater Lepointe – Building n°5**, 97157 Pointe-à-Pitre.

Schedules: The registration desk will be open on Monday from 8:15 to 9:15 and from 9:00 to 9:15 on Tuesday.
Lunches are included in the fees and will be served from 12:30 to 2:00pm **(University Restaurant)**.
The Spring school will end at 5:30 on Monday and at 4:15 on Tuesday.

On Tuesday, it will be followed by the opening ceremony of the NANOinBIO conference, from 4:30 to 5:00, and by a public conference, from 5:00 to 7:00pm, in French, in the same room (amphitheater Lepointe), about Smart materials for preventing nosocomial infections.

Shuttles from and to the spring school venue:
Departure à 8:15 on Monday and Tuesday at the Creole Beach Hotel, return transfer at the end of the Spring School on Monday (around 5:30pm), and at the end of the Public Conference on Tuesday (around 7:00pm) The conference dinner for the participants staying at the conference venue (Creole beach hotel) will be served at 7:30pm.



May 30	
08.15 am	**Welcome Reception & Registration**
Spring School: Machine learning approaches for data analyses	

9.30 am – 12.30 pm	**Lead: Prof Adam Foster, Aalto University**
	
	Aim: Theoretical introduction to machine learning for data analysis. Basic keys for getting started as a non-specialist. Worked examples.
	
	Program:
	• Introduction
	  ○ Python, Jupyter notebooks, GitHub
	• Tutorials
	  ○ Reading data - parsing files
	  ○ Image processing in Jupyter e.g. FFT
	  ○ *Advanced* - "OpenCV tutorial" - images and movies

12.30 pm	**Lunch Break**

2.00 pm – 5.30 pm	**Lead: Prof Adam Foster, Aalto University**
	
	Aim: Choice of a suitable strategy for a given question, and main steps to enact a given strategy. Guided 'hands on' approach to extracting a given feature from a real data set. Hints from experience and discussion of possible variations
	
	Program:
	• Introduction
	  ○ Machine learning (CNN), reinforcement learning, when and why?
	• Tutorials
	  ○ Sci-kit learning classification on images
	  ○ CNN Good tip vs. bad AFM tip
	  ○ *Advanced* - Deep reinforcement learning toy model (linked to AFM autonomous operation)

May 31	
09.00 am	**Welcome & Registration**
Spring School: Machine learning approaches for data analyses	
9.30 am - 12.30 am	**Lead: Prof Núria Gavara, University of Barcelona** **Aim:** Focus on Atomic Force Microscopy: extracting quantitative information from datasets. Worked example from topographic images to mechanical data. **Program:** • Calibrations • Mechanical models • Data fitting and feature extraction • Looking at data distribution and outliers
12.30 pm	**Lunch Break**
2.00 pm - 4.00 pm	**Lead: Prof Núria Gavara, University of Barcelona** **Aim:** Worked example highlighting the 'dos' and 'don't' when implementing and a machine learning approach. Machine learning for disease diagnostic **Program:** • Classification versus regression, supervised vs unsupervised learning, training vs test datasets • Assessing "success", ROC, statistical descriptors and the issue of generalization • Supervised machine learning algorithms (kNN, SVM, Random Forest, Decision Trees) • Feature selection vs dimensionality reduction
4.15 pm	**Group photos at the University "Campus of Fouillole"**

Major Public Conference at University of the French West Indies	
04.30 pm	**Opening Ceremony**
	Keynote Lecturer : Dr. Philippe Lavalle **(INSERM, Strasbourg)** **Moderator :** Dr. Etienne Dague **(LAAS, Toulouse)**  **Topic: Smart materials for preventing nosocomial infections**
07.30 pm	**Conference Dinner**

Spring School NanoInBio 2022

June 01	
08.15 am – 08.45 am	**Welcome Reception & Registration**
Conference Session I Scanning probe microscopy & force spectroscopy	
09.00 am – 09.30 am	**Loredana Casalis**, Synchrotron of Trieste, Italy **(Keynote lecture)**
09.30 am – 9.45 am	**Pedro de Pablo**, Universidad Autonoma de Madrid, Madrid, Spain
9.45 am – 10.00 am	**Sébastien Lyonnais**, CEMIPAI, Montpellier University, France
10.00 am – 10.20 am	**Massimiliano Berardi**, Optics11 life, Amsterdam, The Netherlands
10.20 am – 10.50 am	**Coffee Break (30 min)**
10.50 am – 11.15 am	**Yoo Jin Oh,** Johannes Kepler University, Linz, Austria **(Invited)**
11.15 am – 11.30 am	**Maria Ines Villalba**, EPFL, Lausanne, Switzerland
11.30 am – 11.45 am	**Sofiane El-Kirat-Chatel**, Université de Lorraine, Villers les Nancy, France
11.45 am – 12.00 pm	**Cécile Formosa-Dague**, Université de Toulouse, France
12.00 pm – 12.20 pm	**Christian Bippes**, Nanosurf AG, Liestal, Switzerland
12.30 pm – 02.00 pm	**Conference Lunch**
Conference Session II Optical microscopies & spectroscopies	
02.00 pm – 02.30 pm	**Jörg Enderlein**, University of Göttingen, Germany **(Keynote lecture)**
02.30 pm – 02.45 pm	**Luca Piantanida**, Boise State University, United States
02.45 pm – 03.00 pm	**Axel Gansmüller**, Université de Lorraine, Vandoeuvre-les-Nancy, France
03.00 pm – 03.15 pm	**Lucie Haye**, Université de Strasbourg, France
03.15 pm – 03.30 pm	**Sophie Lecomte**, University of Bordeaux, France
03.30 pm – 04.00 pm	**Coffee Break (30 min)**
04.00 pm – 04.15 pm	**Hendrik Vondracek**, Elettra Sincrotrone Trieste, Italy
04.15 pm – 04.30 pm	**Kamila Lepicka**, Polish Academy of Sciences, Warsaw, Poland
04.30 pm – 04.45 pm	**Jérémie Mathurin**, Université Paris-Saclay, Orsay, France
04.45 pm – 05.00 pm	**Aurélie Delumeau**, Université des Antilles, France
07.30 pm	**Conference Dinner**

June 02	
Conference Session III Latest advances in materials application & life sciences	
08.40 am – 09.10 am	**Catherine Picart**, University of Grenoble, France **(Keynote lecture)**
09.10 am – 9.25 am	**Eloise Lebaudy**, Centre de Recherche en Biomédecine de Strasbourg, France
9.25 am – 9.40 am	**Jenny Harnett**, University of Edinburgh, UK
9.40 am – 9.55 am	**Yulia Sergeeva**, Faculty of Health and Society, Malmö, Sweden
9.55 am – 10.10 am	**Giovanna Fragneto**, Institut Laue-Langevin, Grenoble, France
10.10 am – 10.40 am	**Coffee Break (30 min)**
10.40 am – 11.10 am	**Patrick Couvreur**, Université Paris-Saclay, France **(Keynote lecture)**
11.10 am – 11.25 am	**Miriam Colombo**, University of Milano, Italy
11.25 am – 11.40 am	**Antoine Combes**, Université de Strasbourg, France
11.40 am – 11.55 am	**Christelle Yacou**, Université des Antilles, Pointe-à-Pitre, France
11.55 am – 12.20 pm	**Paolo Scrimin**, University of Padova, Italy **(Invited)**
12.30 pm – 02.00 pm	**Conference Lunch**
Conference Session IV Simulations and machine learning	
02.00 pm – 02.30 pm	**Adam Foster**, Aalto University, Finland **(Keynote lecture)**
02.30 pm – 02.45 pm	**Magdalena Giergiel**, Jagiellonian University, Krakow, Poland
02.45 pm – 03.00 pm	**Saeid Ekrami**, Université de Lorraine, Nancy, France
03.00 pm – 03.25 pm	**Sergei Kalinin**, Oak Ridge National Laboratory, United States **(Invited)**
03.25 pm – 04.00 pm	**Coffee Break**
04.00 pm – 04.30 pm	**Teuta Pilizota**, University of Edinburgh, United Kingdom **(Keynote lecture)**
04.30 pm – 04.45 pm	**Adrian Martinez-Rivas**, Instituto Politécnico National, Mexico City, Mexico
04.45 pm – 05.00 pm	**Yashasvi Ranawat**, Aalto University, Finland
05.00 pm – 06.00 pm	**Poster Session I – Networking Time**
07.30 pm	**Conference Dinner**

June 03		
Conference Session V Scanning probe microscopy & force spectroscopy		
08.40 am – 09.10 am	**Georg Fantner**, EPFL, Lausanne, Switzerland **(Keynote lecture)**	
09.10 am – 9.25 am	**Xinyu Zhang**, ETH Zurich, Switzerland	
9.25 am – 9.40 am	**William Trewby**, Durham University, UK	
9.40 am – 9.55 am	**Samuel Leitao**, EPFL, Lausanne, Switzerland	
9.55 am – 10.10 am	**Bastian Hartmann**, Munich University of Applied Sciences, Munich, Germany	
10.10 am – 10.25 am	**Peter Hinterdorfer**, Johannes Kepler University Linz, Austria	
10.30 am – 11.00 am	**Coffee Break (30 min)**	
11.00 am – 11.30 am	**Núria Gavara**, Institute for Bioengineering of Catalonia, Spain **(Keynote lecture)**	
11.30 am – 11.45 am	**Javier Lopez-Alonso**, CNRS - Institut Pasteur de Lille, France	
11.45 am – 12.00 pm	**Ophélie Thomas-Chemin**, LAAS-CNRS, Toulouse, France	
12.00 pm – 12.20 pm	**Alexander Dulebo**, JPK BioAFM, Bruker Nano Surfaces, Berlin, Germany	
12.20 am – 12.35 pm	**Andreas Rohtaschek**, TU Wien, Austria	
12.40 pm – 02.00 pm	**Conference Lunch**	
Conference Session VI Latest advances in materials application & life sciences		
02.00 pm – 02.30 pm	**Nicholas Kotov**, University of Michigan, United States **(Keynote lecture)**	
02.30 pm – 02.45 pm	**Davide Prosperi**, University of Milano-Bicocca, Milano, Italy	
02.45 pm – 03.00 pm	**Cédric Vranckx**, Université catholique de Louvain, Belgium	
03.00 pm – 03.15 pm	**Laura Martinez-Vidal**, Universita Vita-Salute San Raffaele, Milan, Italy	
03.15 pm – 03.30 pm	**Luisa Fiandra**, University of Milano-Bicocca, Milano, Italy	
03.30 pm – 03.55 pm	**Michel Gringas**, University of Waterloo, Canada **(Invited)**	
04.00 pm – 04.30 pm	**Coffee Break**	
04.30 pm – 06.30 pm	**Poster Session II – Networking Time**	**International Cooperation workshop**

June 03

Gala Diner

07.30 pm

Caribbean Music & Dance Show

Traditional musics and dances with the famous Guadeloupe Carnival atmosphere by Double Face

Conference & Group photos

June 04	
Social Event	
07.30 am to 05.00 pm	**Mangrove tour by glass bottom boat**  One-day mini ecological cruise type excursion (with meal) in a glass bottom boat, with flippers, mask and snorkel. Supervised by a diving instructor and a biologist guide. Discovery of the mangrove, lagoon and coral reef. The excursion includes the visit of mangroves, Birds Island and Rousseau islet, exploration of the coral reef (diving equipment provided) or swimming. Meals on the beach at Pointe Sables.
07.30 pm	**Conference Dinner**

June 05	
Conference Session VII Special NanoInBio session	
08.40 am – 09.10 am	**Hermann Schillers**, University of Münster, Germany **(Keynote lecture)**
09.10 am – 9.25 am	**Guillaume Berthout**, Anton Paar France, Les Ulis, France
9.25 am – 9.40 am	**Ronald Zirbs**, University of Natural Resources and Life Sciences, Vienna, Austria
9.40 am – 9.55 am	**Halima Alem**, Université de Lorraine, France
9.55 am – 10.10 am	**Marie Dubus**, Université de Reims Champagne Ardenne, France
10.15 am – 10.45 am	**Coffee Break (30 min)**
10.45 am – 11.15 am	**Laïla Pasquina-Lemonche**, University of Sheffield, UK **(Keynote lecture)**
11.15 am – 11.30 am	**Kislon Voitchovsky**, Durham University, UK
11.30 am – 11.45 am	**Fabienne Quiles**, Lorraine University, Villers-les-Nancy (France)
11.45 am – 12.00 pm	**Marc Ropitaux**, Normandie Université, Rouen, France
12.00 pm – 12.15 pm	**Hélene Martin-Yken**, Toulouse Biotechnology Institute, Toulouse, France
12.30 pm – 02.00 pm	**Conluding remarks & Conference Lunch**

Oral Presentations

June 01	
08.15 am – 08.45 am	**Welcome Reception & Registration**
Conference Session I Scanning probe microscopy & force spectroscopy	
09.00 am – 09.30 am	**Loredana Casalis**, « Extracellular vesicles structure-function correlations: cell uptake mechanisms and role as cell biomechanics modulators » **(Keynote lecture)**
09.30 am – 9.45 am	**Pedro de Pablo**, « Physical Virology with Atomic Force Microscopy: seeing and touching viruses and protein cages »
9.45 am – 10.00 am	**Sébastien Lyonnais**, « Analyzing live infectious viruses and bacteria by Atomic Force Microscopy coupled to fluorescence in BSL3 laboratory »
10.00 am – 10.20 am	**Massimiliano Berardi**, « Hydraulic Force Spectroscopy on a Chip »
10.20 am – 10.50 am	**Coffee Break (30 min)**
10.50 am – 11.15 am	**Yoo Jin Oh,** « Single molecule characterization of lectin receptor binding to SARS-CoV-2 spike glycans » **(Invited)**
11.15 am – 11.30 am	**Maria Ines Villalba**, « Studies on the mechanical properties and nanomotion spectroscopy of *B. pertussis*'s antibiotic response and virulence »
11.30 am – 11.45 am	**Sofiane El-Kirat-Chatel**, « Deciphering the role of monosaccharides during phage infection of *Staphylococcus aureus* »
11.45 am – 12.00 pm	**Cécile Formosa-Dague**, « The role of microplastics in microalgae cells aggregation: a study at the molecular scale using AFM »
12.00 pm – 12.20 pm	**Christian Bippes**, « Photothermal excitation for improved biological SPM applications »
12.30 pm – 02.00 pm	**Conference Lunch**

Extracellular vesicles structure–function correlations: cell uptake mechanisms and role as cell biomechanics modulators

L. Casalis,[1] P. Parisse,[1,2] V. Rondelli, [3] P. Brocca, [3] F. Perissinotto [4] and B. Senigagliesi [1,5]

[1]*Elettra Sincrotrone Trieste, Italy*
[2]*IOM-CNR, Trieste, Italy*
[3]*Università degli Studi di Milano, Italy*
[4]*INSERM, Institut Pasteur de Lille, France*
[5]*Università degli Studi di Trieste, Italy*

e-mail: loredana.casalis@elettra.eu

Extracellular vesicles (sEVs) are nano-sized particles, delimited by a lipidic bilayer, which carry bioactive molecules and travel through the body fluids. They play a crucial role in intercellular communication: the molecular cargo, which is specific from the cell of origin, can strongly affect the fate of the recipient cells. Because of that EVs have been proposed as biomarkers for several diseases and as optimal candidates for therapeutic applications. Nonetheless, the understanding of the intricate network of EVs/cell interaction and their specific biological function in relation to vesicle size, origin and composition, is still incomplete. This is due to the tremendous complexity of EVs isolation/purification, biophysical and biochemical characterization, given the small size and the multifunctionality of these particles. Here I will show how, through a combination of multiscale atomic force microscopy and scattering (X-ray, neutron) measurements, we can gain crucial insights into EVs molecular composition as well as into EVs internalization routes by applying the same platform to study the interaction of purified EVs with model membrane systems of variable, complex composition [1]. In a second part of the talk, I will show that EVs can modulate the biomechanical properties of target cells, known to be related to metastatic cancer spreading. In particular I will show that isolated and thoroughly characterized triple-negative breast cancer (TNBC)-derived EVs can directly modify non-metastatic breast cancer cells, by inducing a decrease in cell stiffness, rearrangements in cytoskeleton, focal adhesions and nuclear/cellular morphology [2]. Testing the biomechanical response of cells after EV addition might represent a new functional assay in metastatic cancer framework that can be exploited for future application both in diagnosis and in therapy.

References

[1] F. Perissinotto *et al*., Nanoscale 13 (2021) 5224.
[2] B. Senigagliesi *et al*., bioRxiv, doi: https://doi.org/10.1101/2022.02.28.481921

Physical Virology with Atomic Force Microscopy: seeing and touching viruses and protein cages

P.J. de Pablo[1]

[1]*Física de la Materia Condensada, Unversidad Autónoma de Madrid, 28049, Madrid*
e-mail: p.j.depablo@uam.es

The basic architecture of a virus consists of the capsid, a shell made up of repeating protein subunits, which packs, shuttles and delivers their genome at the right place and moment. Viral particles are endorsed with specific physicochemical properties which confer to their structures certain meta-stability whose modulation permits fulfilling each task of the viral cycle. These natural designed capabilities have impelled using viral capsids as protein containers of artificial cargoes (drugs, polymers, enzymes, minerals) with applications in biomedical and materials sciences. Both natural and artificial protein cages (1) have to protect their cargo against a variety of physicochemical aggressive environments, including molecular impacts of highly crowded media, thermal and chemical stresses, and osmotic shocks. Viral cages stability depends not only on the ultimate structure of the external capsid, which rely on the interactions between protein subunits, but also on the nature of the cargo. During the last decade our lab has focused on the study of protein cages with Atomic Force Microscopy (AFM). We are interested in stablishing links of their mechanical properties with their structure and function. In particular, mechanics provide information about the cargo storage strategies of both natural and virus-derived protein cages (2,3,4). Mechanical fatigue has revealed as a nanosurgery tool to unveil the strength of the capisd subunit bonds (5). This allows to unveil ageing effects on virus structures (6), in a similar way to ageing in materials science.

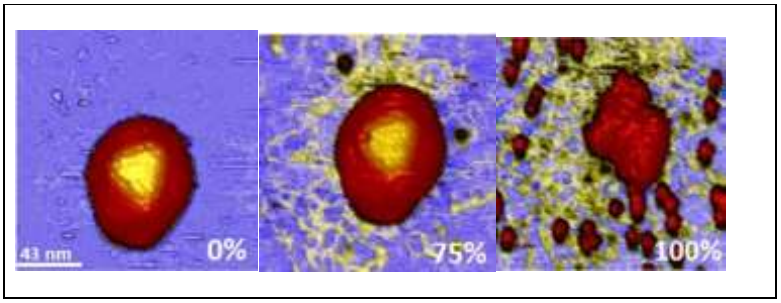


Figure 1: Human adenovirus particle before (left), during (center) and after (right) a fatigue experiment with AFM, showing the dsDNA (yellow) extraction from the virion. Adapted from [5].

References

[1] Llauró et al. Nanoscale, 2016, 8, 9328.
[2] Hernando-Pérez et al. Small, 2012, 8, 2336.
[3] Ortega-Esteban et al. ACS Nano, 2015, 9, 10826, ACS Nano, 2015, 9, 10571.
[4] Jiménez-Zaragoza et al. (2018) https://doi.org/10.7554/eLife.37295.001
[5] Martín-González et al. Nucleic Acids Reseach 2019, (47) 9231,
[6] Martín-González et al. Physical Review X 2021, 11 (2), 021025.

Analyzing live infectious viruses and bacteria by Atomic Force Microscopy coupled to fluorescence in BSL3 laboratory

Sébastien Lyonnais[1], Nathalie Gros[1], AliceTrausch[1], Aymeric Neyret[1], Christine Chable-Bessia[1] and Delphine Muriaux[1,2]

[1]*Centre d'étude des maladies infectieuses et pharmacologie anti-infectieuse CEMIPAI, UAR3725 CNRS, Montpellier University, 34000 Montpellier, France*
[2]*Institut de Recherche en Infectiologie de Montpellier IRIM, UMR9004 CNRS, Montpellier University, 34000 Montpellier, France*
e-mail: sebastien.lyonnais@cemipai.cnrs.fr

Each stage of the life cycle of a pathogen can generate structural, morphological and mechanical modifications linked to diverse aspects of infectivity. Atomic force microscopy (AFM) is a particularly powerful toolbox to explore these aspects on live samples in physiological buffers, from the morphology of viruses and bacteria to their mechanical properties at the nanometric scale and at the single entity level. The latest technologies now allow the physical coupling of a Bio-AFM to an inverted optical microscope to perform correlative imaging, providing a key tool to locate and select the objects of interest in a liquid environment, with increasing resolutions in time and space. We installed such a bio-AFM combo in the level 3 biosafety laboratory of the CEMIPAI facility in Montpellier, as a pilot instrument to analyse pathogenic viruses, bacteria and infected cells in live conditions. We will present in this communication the customization of this Bio-AFM for BSL3 imaging on infectious level 3 samples throughout several examples of qualitative and quantitative AFM imaging of viruses such as SARS-CoV-2 [1], HIV-1 or arboviruses and bacteria such as *brucella* [2]. Our results unambiguously demonstrate the unique perspectives of high resolution AFM to provide direct, fast quantitative and qualitative information on pathogen life cycle and biophysical measurements until now inaccessible with other methods.

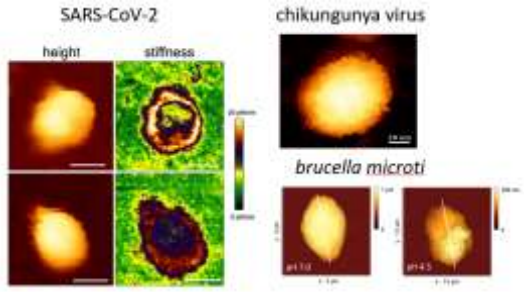


Figure 1: Examples of infectious samples analysed by AFM in BSL3

References

[1] Lyonnais, S., Hénaut, M., Neyret, A. *et al.* Atomic force microscopy analysis of native infectious and inactivated SARS-CoV-2 virions. *Sci Rep* **11,** 11885 (2021).
[2] de la Garza-García JA, et al., Comparative Genome-Wide Transcriptome Analysis of *Brucella suis* and *Brucella microti* Under Acid Stress at pH 4.5: Cold Shock Protein CspA and Dps Are Associated With Acid Resistance of *B. microti*. *Front Microbiol*. **12**, 794535 (2021). Ouahrani-Bettache, S., et al., Lethality of *Brucella microti* in a murine model of infection depends on the *wbkE* gene involved in O-polysaccharide synthesis, *Virulence*, **10**:1, 868 (2021)

Hydraulic Force Spectroscopy on a Chip

M. Berardi,[1,2] K. Bielawski,[1] and B. I. Acka[2]

[1]*Optics11 Life, Hettenheuvelweg 37-39, 1101 BM, Amsterdam, NL*
[2]*LaserLab, Department of Physics and Astronomy, VU University, De Boelelaan 1081, Amsterdam, The Netherlands*
e-mail: massimiliano.berardi@optics11life.com

Microrheology is a powerful, label-free technique to characterize biological matter. Given the variety of shapes, sizes, and mechanical properties of living samples, mechanobiologists developed multiple techniques to comply to their needs. Amongst them, micropipette aspiration is particularly suited for approximately spherical, non-adherent samples [1]. Here, we show the development of an easy to manufacture optofluidic chip that allows to perform multiple dynamic aspiration experiments simultaneously. Thanks to the all-optical readout scheme that we introduced in our previous work [2], dubbed Hydraulic Force Spectroscopy, we can monitor nanoscale displacements continuously, at up to 17 kHz of sampling rate.
The chip consists of four microwells and a perfusion channel. The microwells feature central apertures that can be used to apply a suction pressure on the samples in the Pa-kPa range, both statically and dynamically. Such apertures are monitored by a set of optical fiber positioned at different offsets from the well bottom surfaces (see Figure 1). Light shining through the fibers defines Fabry-Perot cavities between their end facets and the samples at the bottom of the microwells. The fibers are interrogated by a broadband source, which allows to simultaneously monitor the phase variations of all the cavities independently, as they correspond to unique spatial frequencies. By knowing the central wavelength of the excitation source and the refractive index of the medium (typically 1.33), we can extrapolate the displacement of the sample in the channel over time. We show how this measurement device can be used to probe the membrane mechanics of the zebrafish chorion.

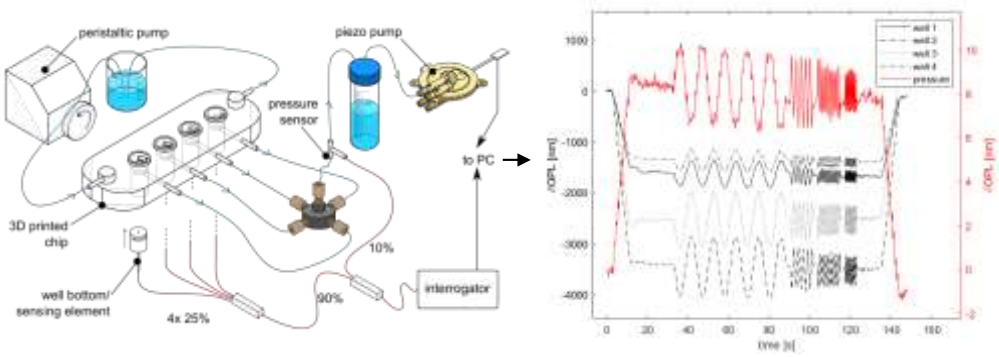


Figure 1: On the left, a simplified scheme of the device, showing the chip connected to both hydraulic and optical lines, with a highlight on one of the sensing elements. On the right, an example of the data collected by the device, after identification of the spatial frequencies corresponding to the sensors and their phase demodulation dOLP is the variation in optical path length as a function of time.

References

[1] González-Bermúdez, B., Guinea, G. V. and Plaza, G. R. (2019) 'Advances in Micropipette Aspiration: Applications in Cell Biomechanics, Models, and Extended Studies', Biophysical Journal. Biophysical Society, pp. 587–594. doi: 10.1016/j.bpj.2019.01.004.
[2] Berardi, M. et al. (2021) 'Optical interferometry based micropipette aspiration provides real-time sub-nanometer spatial resolution', Communications Biology, 4(1). doi: 10.1038/s42003-021-02121-1.

Single molecule characterization of lectin receptor binding to SARS-CoV-2 spike glycans

Y. J. Oh,[1] D. Canena,[1] R. Zhu,[1] L. Hain,[1] M. Klausberger,[2] E. Laurent,[2,3] G. Wirnsberger,[4] N. F. Kienzl,[5] L. Mach,[5] J. M. Penninger,[6,7] D. Markovitz,[8] C. Oostenbrink,[9] P. Hinterdorfer[1]

[1]*Insitute of Biophysics, Johannes Kepler University Linz, Gruberstr. 40, 4020 Linz, Austria*
[2] *University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Molecular Biotechnology, Vienna,Austria*
[3] *University of Natural Resources and Life Sciences, Vienna, Core Facility Biomolecular & Cellular Analysis, Vienna, Austria*
[4]*Apeiron Biologics, Vienna, Austria*
[5] *University of Natural Resources and Life Sciences, Vienna, Department of Applied Genetics and Cell Biology, Institute of Plant Biotechnology and Cell Biology, Vienna, Austria*
[6]*Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna, Austria.*
[7]*Department of Medical Genetics, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada.*
[8]*Division of Infectious Diseases, Department of Internal Medicine, and the Programs in Immunology, Cellular and Molecular Biology, and Cancer Biology, University of Michigan, Ann Arbor, MI 48109, USA.*
[9] *University of Natural Resources and Life Sciences, Vienna, Department of Material Science and Process Engineering, Institute for Molecular Modelling and Simulation, Vienna, Austria*

e-mail: yoo_jin.oh@jku.at

New SARS-CoV-2 variants are continuously emerging with critical implications for therapies or vaccinations. The 22 N-glycan sites of the Spike protein remain highly conserved among SARS-CoV-2 variants, opening an avenue for robust therapeutic intervention. By using a nanomechanical force-sensing approach, we obtained real-time information about the molecular bonds involved in the binding of various carbohydrate-binding proteins, so-called lectins, to viral spike proteins. Out of a lectin library, two lectins, Clec4g and CD209c, were identified to strongly bind to the spike protein of SARS-CoV-2. To study spike binding of lectins at the single molecule level, we applied atomic force microscopy based single molecular force spectroscopy. We quantified unbinding forces and analysed the number of bond ruptures between Clec4g/CD209c and the trimeric spike protein. Multiple bond formations lead to stable complex formation, in which the number of formed bonds enhanced the overall interaction strength and dynamic stability of the lectin/spike complexes. Equipped with suchlike molecular modalities, Clec4g/CD209c are multivalent efficient competitors in SARS-CoV-2 spike binding to cellular ACE2.[1] We also determined the binding capacity of a molecularly engineered lectin cloned from banana, BanLec H84T, which was shown to display broad-spectrum antiviral activity against several RNA viruses.[2] Our studies revealed that H84T-BanLec interacts with the Spike protein of the original viral strain, Wuhan-1 and several variants of concern (Delta, Omicron). Based on our force probing technique, dynamic molecular interaction patterns with accurate rupture force and length distributions were depicted. The complex multiple binding features between the dimeric H84T and trimeric spike protein were analysed with respect to the distribution of the glycosylation sites on the spike. Using high speed AFM, we additionally imaged spike proteins complexed with isolated lectin molecules to visualize oligomeric states and complex formation. Our data obtained by AFM techniques elucidate lectin-spike interactions at the single molecule level and uncover candidate receptors involved in spike binding and SARS-CoV-2 infections. The capacity of lectins to block SARS-CoV-2 viral entry holds promise for pan-variant therapeutic interventions.

References

[1] D. Hoffmann et al., The EMBO journal 40, 19 (2021) e108375.
[2] M. D. Swanson et al., Cell 163, 3 (2015) 746-758.

Acknowledgement: Y.J.O. acknowledges the financial support from the Austrian Science Fund FWF project I 3173, V584-BBL and the Austrian National Foundation for Research, Technology, and Development and Research Department of the State of Upper Austria.

Studies on the mechanical properties and nanomotion spectroscopy of *B. pertussis*'s antibiotic response and virulence

M.I. Villalba[1], L. Venturelli[1], L. Arnal[2], C. Masson[3], G. Dietler[1], M. E Vela[2], O. Yantorno[3], R. Willaert[4] and S. Kasas[1, 5]

[1]*Laboratory of Biological Electron Microscopy (LBEM), Institute of Physics (IPHYS), Faculty of Basic Sciences (SB), Swiss Federal Institute of Technology (EPFL), CH-1015 Lausanne, Switzerland.*
[2]*Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Universidad Nacional de La Plata - CONICET, 1900 La Plata, Argentina.*
[3]*Centro de Investigación y Desarrollo en Fermentaciones Industriales (CINDEFI), Facultad de Ciencias Exactas, Universidad Nacional de La Plata - CONICET, 1900 La Plata, Argentina.*
[4]*Research Group Structural Biology Brussels, Alliance Research Group VUB-UGent NanoMicrobiology (NAMI), 1050 Brussels, Belgium.*
[5]*Centre Universitaire Romand de Médecine Légale, UFAM, Université de Lausanne, 1015 Lausanne, Switzerland.*
e-mail: ines.villalba@epfl.ch

Bordetella pertussis is *a* slowly growing Gram-negative bacteria, which is the agent of whopping cough and it is capable of adopting different states of virulence as a function of the physical-chemical properties of the environment [1]. In this study we explored the nanomechanical properties of both virulent and avirulent *B. pertussis* as exposed to antibiotics. Additionally, we used the Atomic force microscopy (AFM)-based nanomotion detection [2] to study *B. pertussis* in different virulence conditions.

The nanomechanical analysis highlighted that only virulent *B. pertussis* cells undergo a decrease in their cell elastic modulus and height upon antimicrobial exposure, whereas their avirulent counterparts remain unaffected. This study also permitted to highlight different antibiotic react of individual cells as compared to those growing in groups. Furthermore, in the AFM-based nanomotion studies we noticed that *B. pertussis* generates a cantilever movement pattern that depends on its phenotype. More precisely nanometric scale oscillations of *B. pertussis* can be correlated with the virulence state of the bacteria.

In general, these results confirm the usefulness of AFM to study microorganisms and their reaction to different physic-chemical stimulus. The nanomotion spectroscopy analysis indicate a correlation between metabolic/virulent bacterial states and bacterial nanomotion pattern and paves the way to novel rapid and label free pathogenic microorganism detection assays.

References

[1] A.R. Melton, A.A. Weiss, Journal of bacteriology 171 (1989) 6206-6212.
[2] G. Longo, L. Alonso-Sarduy, L. M. Rio, A. Bizzini, A. Trampuz, J. Notz, G. Dietler, S. Kasas, Nature nanotechnology 8 (2013) 522–526.

Deciphering the role of monosaccharides during phage infection of *Staphylococcus aureus*.

Sofiane El-Kirat-Chatel,[1] Baptiste Arbez, Marion Gardette, Christophe Gantzer, Neus Vilà, and Isabelle Bertrand

[1]*Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement (LCPME) CNRS-Université de Lorraine UMR7564, 405 rue de Vandoeuvre 54600 Villers les Nancy.*
e-mail: elkirat1@univ-lorraine.fr

As phages are extensively investigated as novel therapy tools but also as transfer agents for antibiotic resistance genes, thorough understanding of phage-host interactions becomes crucial [1]. Prerequisite for phage infection is its adhesion to the host surface. Here, we used AFM-based single-particle force spectroscopy (SPFS) with phage-decorated tips to decipher the adhesion of phage 187 on living *Staphylococcus aureus* cells (Fig. 1). We found that addition of free N-acetyl-D-glucosamine was able to decrease phage adhesion, suggesting that this monosaccharide plays major role in phage 187 infection of *S. aureus*. Moreover, phage 187 adhesion on monosaccharide-coated model surfaces combined with plaque forming unit counts suggested that a direct link can be established between the propensity to bind to a saccharide and the capability of the latter to inhibit phage infection. Our work demonstrates that SPFS is a powerful platform to screen and predict the molecular target of phages on their host surfaces.

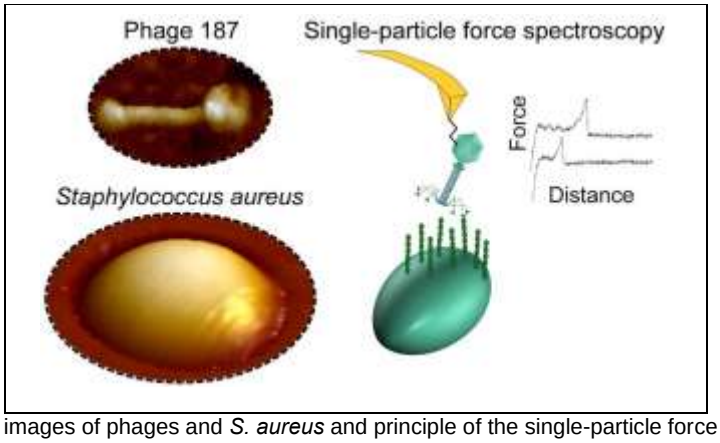


Figure 1: AFM images of phages and *S. aureus* and principle of the single-particle force spectroscopy used to probe the interaction between phages and bacteria.

References

[1] M.R. Clokie, A.D. Millard, A.V. Letarov, S. Heaphy Phages in nature. Bacteriophage 1, (2011), 31-45.

The role of microplastics in microalgae cells aggregation: a study at the molecular scale using AFM

I. Demir-Yilmaz,[1,2] N. Yakovenko,[3] C. Roux,[3] P. Guiraud,[1,4] F. Collin,[3] C. Coudret,[3] A. ter Halle,[3] and C. Formosa-Dague[1,4]

[1]*TBI, Université de Toulouse, INSA, INRAE, CNRS, Toulouse, France.*
[2]*LAAS, Université de Toulouse, CNRS, Toulouse, France.*
[3]*UMR 5623 IMRCP, CNRS, Toulouse, France.*
[4]*Fédération de Recherche Fermat, CNRS, Toulouse, France.*
e-mail: formosa@insa-toulouse.fr

Plastic pollution has become a significant concern in aquatic ecosystems, where photosynthetic microorganisms such as microalgae represent a major point of entry in the food chain. For this reason, an important challenge is to better understand the consequences of plastic pollution on microalgae and the mechanisms underlying the interaction between plastic particles and cell interfaces. In this study, to answer such questions, we developed an interdisciplinary approach to investigate the role of plastic microparticles in the aggregation of a freshwater microalgae species, *Chlorella vulgaris*. First, the biophysical characterization, using atomic force microscopy, of the synthetic plastic microparticles used showed that they have similar properties to the ones found in the environment, with a rough, irregular surface with hydrophobic properties, thereby making them a relevant model. Then a combination of optical imaging and separation experiments showed that the presence of plastic particles in microalgae cultures induced the production of exopolysaccharides (EPS) by the cells, responsible for their aggregation. However, cells that were not cultured with plastic particles could also form aggregates when exposed to the particles after culture. To understand this, advanced single-cell force spectroscopy experiments were performed to probe the interactions between cells and plastic microparticles; the results showed that cells could directly interact with plastic particles through hydrophobic interactions. In conclusion, our experimental approach allowed highlighting the two mechanisms by which plastic microparticles trigger cell aggregation; by direct contact or by inducing the production of EPS by the cells. Because these microalgae aggregates containing plastic are then consumed by bigger animals, these results are important to understand the consequences of plastic pollution on a large scale.

Photothermal excitation for improved biological SPM applications

Christian A. Bippes, Jonathan D. Adams, Patrick L.T.M. Frederix, Pieter van Schendel, and Dominik Ziegler

Nanosurf AG, Gräubernstrasse 12, 4410 Liestal, Switzerland
e-mail: bippes@nanosurf.com

AFM imaging modes known as off resonance modes rely on generating a relative oscillatory motion between the AFM cantilever tip and the sample surface [1]. Typically, such motion is achieved using the Z scanner piezo of the AFM system. This approach, however, is limited in speed due to the dynamics of the Z scanner. In contrast, it is long known that the cantilever can be a significantly faster actuator than the Z scanner [2]. Recently, photothermal excitation has become increasingly popular in AFM due to its ability to directly induce cantilever bending. In photothermal excitation, an intensity-modulated laser is focused at the base of the cantilever and used to drive cantilever bending due to a local heating effect.
Photothermal excitation has three main advantages. First, because it is a local effect, it excites cantilever oscillations in a very clean manner, free from unwanted environmental effects. Second, it can actuate the cantilever over a wide bandwidth. Finally, photothermal excitation provides very stable cantilever actuation. These advantages combine to make a photothermally-excited cantilever an ideal fast actuator for off-resonance AFM imaging modes [3].
Here, we present a newly-designed AFM that offers photothermal excitation and is designed to integrate seamlessly with optical microscopy on inverted optical microscopes. Photothermal excitation is a key functionality of this new instrument and enables several new imaging and measurement methods for biological applications.
In particular, WaveMode is an off-resonance imaging mode in which the relative tip-sample oscillatory motion can be generated through photothermal actuation of the cantilever. It allows higher modulation rates compared to conventional off-resonance modes and the photothermal actuation provides high stability. Due to low lateral imaging forces, WaveMode is particularly suited to imaging often loosely-adhered biological samples. We will show examples of how WaveMode can be applied to imaging of biological material, such as virus capsids bound to intact nuclei.

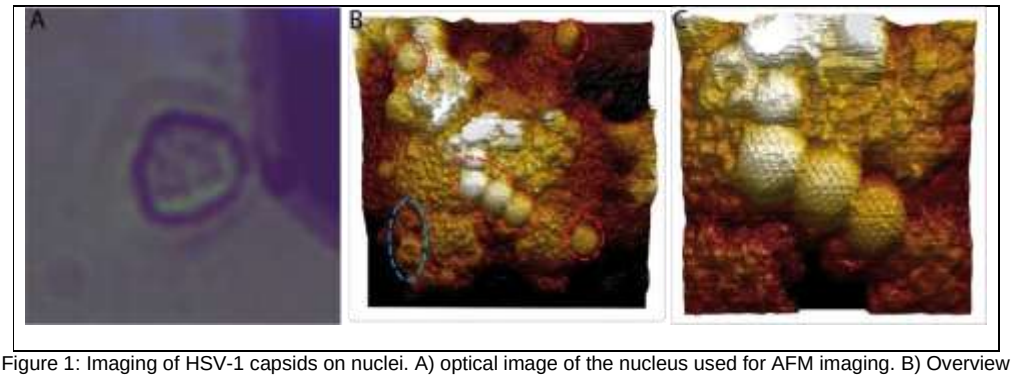


Figure 1: Imaging of HSV-1 capsids on nuclei. A) optical image of the nucleus used for AFM imaging. B) Overview AFM image of the surface of intact rat liver nuclei. HSV-1 capsids (red circles) typically bind to the nucleus through interaction with NPCs (blue circle). Image size: 1350 nm. C) Zoom-in image of a virus capsid assembly with clearly visible structural virus capsid features. Image size: 575 nm. AFM images were acquired on an inverted optical microscope using WaveMode with a modulation frequency of 10 kHz and 600 pN interaction force. Sample courtesy: Alex Evilevitch, Lund University.

References

[1] Rosa-Zeiser *et al.*, Meas. Sci. Technol. 8 (1997) 1333-1338
[2] Manalis *et al.*, App. Phys. Lett. 68 (1996) 871-873
[3] Nievergelt *et al.*, Nat. Nanotechnol. 13 (2018) 696-701

June 01	
Conference Session II Optical microscopies & spectroscopies	
02.00 pm – 02.30 pm	**Jörg Enderlein**, « Metal-Induced Energy Transfer Imaging » **(Keynote lecture)**
02.30 pm – 02.45 pm	**Luca Piantanida**, « Nucleic Acid Memory: Super Resolution Microscopy enhances novel approach to DNA data storage »
02.45 pm – 03.00 pm	**Axel Gansmüller**, « Solid state NMR characterization of photoactive hybrid materials designed for drug delivery »
03.00 pm – 03.15 pm	**Lucie Haye**, « Gold Nanoscluster Loaded Polymer Nanoparticles for SWIR Imaging »
03.15 pm – 03.30 pm	**Sophie Lecomte**, « Near Field microscopy to probe the structures of amyloid peptides involved in Alzheimer diseases »
03.30 pm – 04.00 pm	**Coffee Break (30 min)**
04.00 pm – 04.15 pm	**Hendrik Vondracek**, « Label-Free, Rapid and Facile Gold-Nanoparticles-Based Assay as a Potential Spectroscopic Tool for Trastuzumab Quantification »
04.15 pm – 04.30 pm	**Kamila Lepicka**, « Get closer to the intrinsic properties of Ni^{2+}salen polymer semiconductors accessed by chain isolation inside mesoporous silica channels »
04.30 pm – 04.45 pm	**Jérémie Mathurin**, « Coexistence of multiple prion conformations: morphological and structural analysis by IR nanospectroscopy »
04.45 pm – 05.00 pm	**Aurélie Delumeau**, « Evidence of viable bacteria of public health interest in free-living amoebae isolated from hot springs in Guadeloupe by SEM and LSM »
07.30 pm	**Conference Dinner**

Metal-Induced Energy Transfer Imaging

Jörg Enderlein

Third Institute of Physics – Biophysics, Georg August University, Göttingen, Germany

email: jenderl@gwdg.de

website: https://www.joerg-enderlein.de

Metal-Induced Energy Transfer (MIET) Imaging is a recently developed method [1] that allows for nanometer resolution along the optical axis. It is based on the fact that, when placing a fluorescent molecule close to a metal, its fluorescence properties change dramatically, due to electromagnetic coupling of its excited state to surface plasmons in the metal. This is very similar to Förster Resonance Energy Transfer (FRET) where the fluorescence properties of a donor are changed by the proximity of an acceptor that can resonantly absorb energy emitted by the donor. In particular, one observes a strongly modified lifetime of its excited state. This coupling between an excited emitter and a metal film is strongly dependent on the emitter's distance from the metal. We have used this effect for mapping the basal membrane of live cells with an axial accuracy of ~3 nm. The method is easy to implement and does not require any change to a conventional fluorescence lifetime microscope; it can be applied to any biological system of interest, and is compatible with most other super-resolution microscopy techniques that enhance the lateral resolution of imaging [2-4]. Moreover, it is even applicable to localizing individual molecules [5-6], thus offering the prospect of three-dimensional single-molecule localization microscopy with nanometer isotropic resolution for structural biology. I will also present latest developments of MIET where we use a single layer of graphene instead of a metal film that allows for increasing the spatial resolution down to a few Ångströms [7-9].

References

[1] Chizhik, A. I., Rother, J., Gregor, I., Janshoff, A., & Enderlein, J. (2014). *Nature Photonics* **8**:124-127.

[2] Baronsky, T., Ruhlandt, D., Brückner, B. R., Schäfer, J., Karedla, N., Isbaner, S., ... & Chizhik, A. I. (2017). *Nano Lett.* **17**:3320-3326.

[3] Chizhik, A. M., Ruhlandt, D., Pfaff, J., Karedla, N., Chizhik, A. I., Gregor, I., ... & Enderlein, J. (2017). *ACS Nano* **11**:11839-11846.

[4] Chizhik, A. M., Wollnik, C., Ruhlandt, D., Karedla, N., Chizhik, A. I., Hauke, L., ... & Rehfeldt, F. (2018). *Mol. Biol. Cell* **29**:846-851.

[5] Karedla, N., Chizhik, A. I., Gregor, I., Chizhik, A. M., Schulz, O., & Enderlein, J. (2014). *ChemPhysChem* **15**:705-711.

[6] Isbaner, S., Karedla, N., Kaminska, I., Ruhlandt, D., Raab, M., Bohlen, J., ... & Tsukanov, R. (2018). *Nano Lett.* **18**:2616-2622.

[7] Ghosh, A., Sharma, A., Chizhik, A. I., Isbaner, S., Ruhlandt, D., Tsukanov, R., Gregor, I., Karedla, N., & Enderlein, J. (2019). *Nature Photonics* **13**:860-865.

[8] Raja, S. O., Chizhik, A. I., Schmidt, C. F., Enderlein, J., & Ghosh, A. (2021). *Nano Letters* **21**:8244-8249.

[9] Ghosh, A., Chizhik, A. I., Karedla, N., & Enderlein, J. (2021). *Nature Protocols* **16**:3695-3715.

Nucleic Acid Memory

Super Resolution Microscopy enhances novel approach to DNA data storage

Piantanida L.[1], Dickinson G.D.[1], Mortuza G.M.[2], Clay W.[1], Green C.M.[1,5], Watson C.[1], Hayden E.J.[3], Andersen T.[2], Kuang W.[4], Graugnard E.[1], Zadegan R.[1,6] and Hughes W.L.[1]

[1]Micron School of Materials Science and Engineering, Boise State University, Boise, ID, USA. [2]Department of Computer Science, [3]Department of Biological Sciences, [4] Department of Electrical and Computer Engineering, Boise State University, Boise, ID, USA. Present address: [5]Center for Bio/Molecular Science and Engineering, U.S. Naval Research Laboratory, Washington, DC, USA; [6]Department of Nanoengineering, Joint School of Nanoscience & Nanoengineering, North Carolina A&T State University, USA.
e-mail: lucapiantanida@boisestate.edu

The ever-present connectivity in our lives, and the data storage demands that come with it, is growing exponentially. The projected material supply for silicon-based memory technologies is unable to satisfy future demand, therefore, alternative memory materials are being explored in academia and industry[1]. DNA is analogous to a biological hard drive. It carries and transfers information with exceptional density, stability, and energy efficiency, making it a compelling alternative to current non-volatile information storage technologies.
The Nucleic Acid Memory (NAM) Institute at Boise State exploits DNA as a programmable material to engineer emerging data storage technologies. Here we present our first prototype, digital Nucleic Acid Memory (dNAM), which spatially encodes and retrieves small datasets using only DNA as the material[2]. dNAM is made from DNA origami assembly technique, it is structurally characterized using Atomic Force Microcopy (AFM, Fig.1b) and it is read using Super Resolution Microscopy (SRM, Fig.1c). The origami structure serves as a breadboard where short dye-labelled DNA strands are the fluorescent imager probes (Fig.1a). These probes transiently hybridize with short protruding single strands periodically positioned on the origami design implementing the DNA PAINT technique on its surface. Every time a hybridization event occurs, a blinking signal is recorded. Associating the blinking signal to a “1” and no signal to a “0”, dNAM resembles a molecular version of Lite-Brite toy where distinct patterns of pegs encode different digital data.
Processing the SRM reading with a custom error-correction algorithm, dNAM provides an areal data density of 330 Gbit/cm^2 and is able to use a subset of origami to retrieve the encoded message 100% of the time. Unlike other approaches to DNA-based data storage, reading dNAM does not require DNA sequencing. As such, the research can provide a valuable path to DNA data storage applications for the next-generation of digital memory materials.

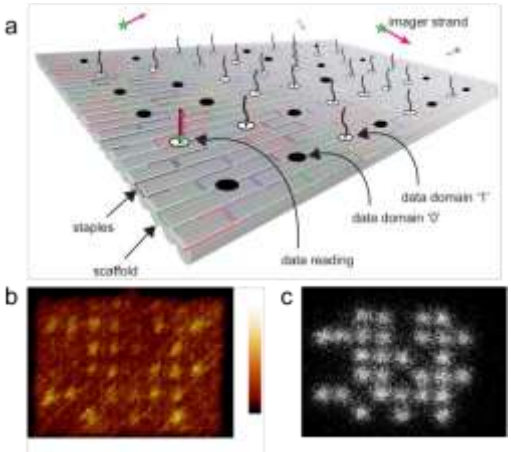


Figure 1: (a) dNAM design, (b) structure (AFM, color scale 2nm) and (c) DNA PAINT reading (SRM).

References

[1] Zhirnov, V., Zadegan R., Sandhu G. et al., **Nature Materials**, 2016, 15(4) 366-370.
[2] Dickinson, G.D., Mortuza G., Clay W., Piantanida L., et al., **Nature Communications**, 2021, 12(1) 2371.

Solid state NMR characterization of photoactive hybrid materials designed for drug delivery

Fabien Deligey[1], Maxime Girardon[2], Sabine Bouguet-Bonnet[1], Andreea Pasc[2], El-Eulmi Bendeif[1], Dominik Schaniel[1], Axel Gansmüller[1]

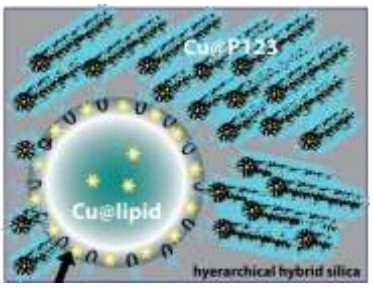
[1] *Université de Lorraine, CRM2, UMR 7036, Vandoeuvre-les-Nancy, France*
[2] *Université de Lorraine, L2CM, UMR 7053, Vandoeuvre-les-Nancy, France*
axel.gansmuller@univ-lorraine.fr

Nowadays, a way of developing novel medicinal compounds focuses on confinement of known active molecules inside nanoparticles. Additionally, the delivery of therapeutic agents can be controlled by photodissociation and photoisomerization processes of photoactive drugs and nanoparticles. Therefore hybrid materials emerge, exhibiting new properties related to nano-confinement. This work shows how the application of Nuclear Magnetic Resonance (to solids and solutions) provides access to the structural and dynamical properties of the confined molecules, that govern the drug release and photophysical properties.

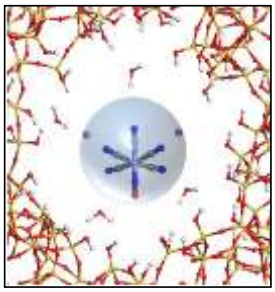
A first example focuses on a system combining a double vectorization of hydrophobic curcumin molecules inside various solid lipid nanoparticles, encapsulated inside a silica matrix (SBA-15) [1]. A joint SS-NMR and Differential Scanning Calorimetry (DSC) characterization strategy provides a picture of the distribution of curcumin inside these very heterogeneous materials. As a consequence, we show that other factors than compartmentalization (in particular, polymorphism and molecular dynamics of host compounds) should also be considered to understand the release properties.

The second example focuses on hydrophilic Sodium NitroPrusside (SNP) complexes, isolated inside a sol-gel nanoporous matrix. From a joint Pair Distribution Function (PDF) and SS-NMR study, the structure and the dynamics of the confined complexes are characterized. Interestingly, temperature and hydration ranges are identified, for which the complex stays associated, although it is in a "liquid-like state". Towards the limit of water absence, movement restrictions of the confined complexes are elucidated providing information on water modulated host / guest interactions that explain their exceptional crystallization properties in confined environment[2,3].

Finally, recent results on photo-NMR and in situ irradiation experiments will be presented, showing the potential of NMR to establish the link between molecular structure and optical properties of the materials[4].



Curcumin in solid lipid nanoparticles @SBA15



Sodium nitroprusside @SiO_2

References

(1) Kim, S.; Stébé, M.-J.; Blin, J.-L.; Pasc, A. PH-Controlled Delivery of Curcumin from a Compartmentalized Solid Lipid Nanoparticle@mesostructured Silica Matrix. *J. Mater. Chem. B* **2014**, *2* (45), 7910–7917. https://doi.org/10.1039/C4TB01133C.

(2) Deligey, F.; Bouguet-Bonnet, S.; Doudouh, A.; Marande, P.-L.; Schaniel, D.; Gansmüller, A. Bridging Structural and Dynamical Models of a Confined Sodium Nitroprusside Complex. *The Journal of Physical Chemistry C* **2018**. https://doi.org/10.1021/acs.jpcc.8b05655.

(3) Bendeif, E.-E.; Gansmuller, A.; Hsieh, K.-Y.; Pillet, S.; Woike, T.; Zobel, M.; Neder, R. B.; Bouazaoui, M.; Hamzaoui, H. E.; Schaniel, D. Structure Determination of Molecular Nanocomposites by Combining Pair Distribution Function Analysis and Solid-State NMR. *RSC Adv.* **2015**, *5* (12), 8895–8902. https://doi.org/10.1039/C4RA11470A.

(4) Angelini, G.; Gansmüller, A.; Pécourneau, J.; Gasbarri, C. An Insight into Cyclocurcumin Cis–Trans Isomerization: Kinetics in Solution and in the Presence of Silver Nanoparticles. *Journal of Molecular Liquids* **2021**, *333*, 116000. https://doi.org/10.1016/j.molliq.2021.116000.

Gold Nanocluster Loaded Polymer Nanoparticles for SWIR Imaging

Lucie Haye,[1] Xavier Le Guével[2], Peremobowei Iyanu Diriwary[3], Andrey Klymchenko[1], Niko Hildebrandt[3] and Andreas Reisch[1]

[1] *Laboratoire de Bioimagerie et Pathologie, Université de Strasbourg, Faculté de Pharmacie, 74 Route du Rhin 67401 ILLKIRCH Cedex, France*
[2] *Institut for Advanced Biosience,Université Grenoble-Alpes (UGA), Allée des Alpes, 38700 La Tronche, France*
[3] *nanoFRET.com, Laboratoire COBRA (Chimie Organique, Bioorganique, Réactivité et Analyse), Université de Rouen Normandie, CNRS, INSA76821, Mont Saint-Aignan, France*
e-mail: lucie.haye@etu.unistra.fr

Fluorescence is widely used today for biomedical imaging from the subcellular to the organism level and for biosensing, but its performance still largely depends on the used fluorescent probes. Various probes emitting in the visible region are available, however, high autofluorescence and low depth of tissue penetration limit in vivo imaging in this wavelength region. A solution to bypass these problems is shifting the window of observation to 1000 – 1700 nm, which is known as Short Wave InfraRed (SWIR, or NIR II). Light at this wavelength can penetrate deeper into tissues due to reduced scattering and absorption[1], and autofluorescence is negligible[2], making it possible to achieve high spatial and temporal imaging resolution. However, probes emitting in this region are rare and creating bright contrast agents is today a crucial challenge to enable the development of SWIR imaging.
Gold Nanoclusters (AuNC) are photostable, exhibit low toxicity and can be tuned to emit in the SWIR.[3] However, they display a limited brightness and their fluorescence emission decreases strongly in aqueous media. Here, we therefore used nanoprecipitation[4] to encapsulate high amounts of AuNCs in polymer nanoparticles (NPs) and so create objects with a very high brightness. In this way we obtained NPs with AuNC contents up to 50 wt% and sizes from 15 to 70 nm. Protection from water by the polymer shell allowed to achieve a very high intensity of emission. At the same time, we observed that increasing the amount of AuNC encapsulated in NPs induced a bathochromic shift associated with an enhanced brightness. These NPs were tested for imaging artificial vessel and quantify their sensitivity and resolution. Analysis of the signal to noise ratio and the overestimation of vessel size showed good sensitivity and resolution up to a depth of 5 mm.

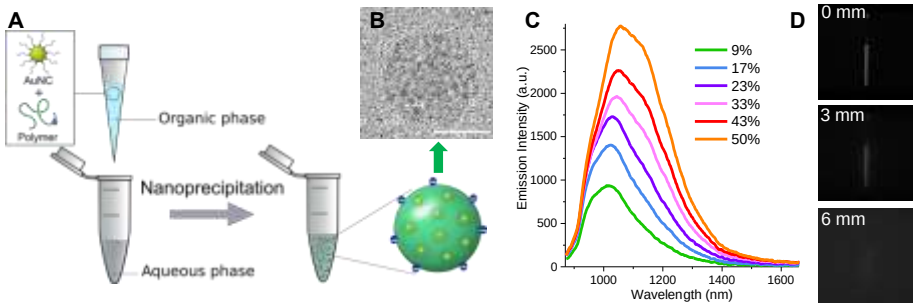


Figure 1: A) Nanoprecipitation scheme B) Cryo-TEM imaging of NP, scale bar 20nm C) Emission spectra of NPs for different AuNC content D) Imaging in a capillary

References

(1) Ximendes, E.; Benayas, A.; Jaque, D.; Marin, R. Quo Vadis, Nanoparticle-Enabled In Vivo Fluorescence Imaging? *ACS Nano* **2021**, *15* (2), 1917–1941. https://doi.org/10.1021/acsnano.0c08349.
(2) Dahal, D.; Ray, P.; Pan, D. Unlocking the Power of Optical Imaging in the Second Biological Window: Structuring NEAR-INFRARED II Materials from Organic Molecules to Nanoparticles. *WIREs Nanomedicine Nanobiotechnology* **2021**. https://doi.org/10.1002/wnan.1734.
(3) Yu, Z.; Musnier, B.; Wegner, K. D.; Henry, M.; Chovelon, B.; Desroches-Castan, A.; Fertin, A.; Resch-Genger, U.; Bailly, S.; Coll, J.-L.; Usson, Y.; Josserand, V.; Le Guével, X. High-Resolution Shortwave Infrared Imaging of Vascular Disorders Using Gold Nanoclusters. *ACS Nano* **2020**, *14* (4), 4973–4981. https://doi.org/10.1021/acsnano.0c01174.
(4) Reisch, A.; Trofymchuk, K.; Runser, A.; Fleith, G.; Rawiso, M.; Klymchenko, A. S. Tailoring Fluorescence Brightness and Switching of Nanoparticles through Dye Organization in the Polymer Matrix. *ACS Appl. Mater. Interfaces* **2017**, *9* (49), 43030–43042. https://doi.org/10.1021/acsami.7b12292.

Near Field microscopy to probe the structures of amyloid peptides involved in Alzheimer diseases

S. Lecomte,[1] S. Bonhommeau,[2] C. Feuillie[1] and M. Molinari[1]

[1]*CBMN, University of Bordeaux-CNRS-INP Pessac, France*
[2]*ISM, University of Bordeaux-CNRS-INP Pessac, France*

e-mail: sophie.lecomte@u-bordeaux.fr

Amyloid toxicity is a subject under intense scrutiny. Many studies link this toxicity to the existence of various intermediate structures prior to the fiber formation and/or their specific interaction with membranes. Our group aim at understanding the process of aggregation of $A\beta_{1-42}$ and Tau proteins involved in Alzheimer disease, and their interaction with membrane. Near-field microscopy techniques are very relevant to probe at the nanoscale the morphology and associated structure with the different species detected during the assembly of the $A\beta_{1-42}$ peptide and Tau protein and their interactions with the membranes. Tip-Enhanced Raman Spectroscopy allows an analysis of the surface of oligomers or fibers at the scale of the single object. We demonstrated with a careful examination of amide I and amide III bands that $A\beta_{1-42}$ fibers are organized in parallel β-sheets while oligomers are organized in anti-parallel β-sheets (Figure 1) [1]. Unlike $A\beta_{1-42}$ which aggregates spontaneously in vitro, Tau requires a co-factor to self-assemble and form fibers. Negatively charged phospholipids (PIP_2 or PS) have demonstrated the ability to initiate the aggregation and fibrillization of Tau protein in vitro. TERS was used to establish the insertion of the co-factor in the structure of the Tau fibers. [2]
The interaction between amyloid peptide and membrane models were investigated using various biophysical techniques and AFM-IR spectroscopy. This work clearly brings to light that the presence of cholesterol in membranes is favorable to the interaction with Aβ peptides in oligomers or aggregates. [3] The behavior of the Tau protein is very different depending on the lipid composition of the membrane. Tau solubilizes membranes (DOPC type) and forms fibers in the presence of PIP_2 [4].
The main limitation of the TERS and AFM-IR techniques is that they are carried out in the air, our new challenge is the development of these methods in liquid media

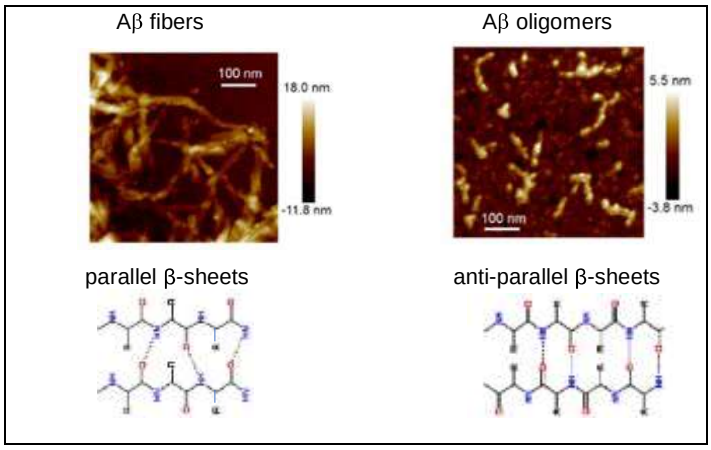


Figure 1: Morphology and structure of $A\beta_{1-42}$ fibers and oligomers.

References

[1] S. Bonhommeau, D. Talaga , J. Humel, C. Cullin, S. Lecomte, Angew. Chem. Int. Ed. 129, (2017) 1797–1800.
[2] D.Talaga, W. Smeralda, L. Lescos, J. Hunel, N. Lepejova-Caudy, C. Cullin, S. Bonhommeau, S. Lecomte Angew. Chem. Int. Ed. 57 (2018) 15738 –15742.
[3] S. Henry, N. B. Bercu, C. Bobo, C. Cullin, M. Molinari, S. Lecomte, Nanoccale,10 (2018), 936-940.
[4] M. Azouz, C. Feuillie, M. Lafleur, M. Molinari, S. Lecomte, Nanoscale Adv,3 (2021) 4244–4253.

Label-Free, Rapid and Facile Gold-Nanoparticles-Based Assay as a Potential Spectroscopic Tool for Trastuzumab Quantification

H.Vondracek,[1,2] A. Alsadig,[1,3] P. Pengo,[2] L. Pasquato,[2] P. Posocco,[4] P. Parisse,[1] and L. Casalis[1]

[1]*Elettra Sincrotrone Trieste S.C.p.A., 34149 Trieste, Italy*
[2]*Department of Chemical and Pharmaceutical Sciences, University of Trieste, 34127 Trieste, Italy*
[3]*Department of Physics, University of Trieste, 34127 Trieste, Italy*
[4]*Department of Engineering and Architecture, University of Trieste, 34127 Trieste, Italy*
e-mail: hendrik.vondracek@elettra.eu

As of today, the reliable bio-functionalization of gold nanoparticles still poses a major challenge towards their diagnostic and therapeutic application. In this direction, we pioneered and optimized several techniques that allow for stable functionalization of particles based on ligand exchange. [1,2] One of this approaches is based on the immobilization of Histidine-tagged proteins by creating a mixed self-assembled monolayer of a top oligo-ethylene glycol (TOEG 3) and alkylthiols terminated with Nitrilotriacetic Acid (NTA). [3] We showed that the optimized mixture offers good dispersibility, protection from non-specific binding and high reliability when immobilizing functional proteins using cobalt (II) chelates.
The focus of this presentation will be on the application of such assay to allow for a facile and quantitative detection of Trastuzumab, a humanized IgG1 monoclonal antibody used against human epidermal growth factor receptor 2 (HER2), overexpressed in breast cancer patients. [4] Since the approach is based on the detection of localized surface plasmon resonance (LSPR) and on particle aggregation induced by Trastuzumab recognition, a simple table-top UV/Vis spectrophotometer is sufficient to quantify the antibody and use the results in order to improve the immunotherapy in terms of personalized treatment. The system was found to be highly specific and sensitive towards clinically relevant concentrations of Trastuzumab (down to 300 ng/mL in buffer and 2 µg/mL in standardized serum). [5]
Furthermore, limitations of this approach and potential alternatives based on DNA directed immobilization (DDI) and Click-based chemistry (Cu(I)-catalyzed azide–alkyne cycloaddition, CuAAC), that are currently being experimented on, will be highlighted.

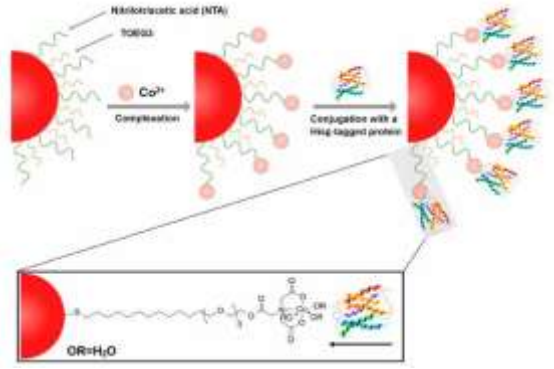


Figure 1: The representation illustrates the attachment strategy of the His-tagged protein onto the surface of the Co(II)NTA/TOEG3 AuNPs. [5]

References

[1] J. Deka, R. Mech, L. Ianeselli, H. Amenitsch, F. Cacho-Nerin, P. Parisse, L. Casalis, ACS Appl. Mater. Interfaces 7 (2015), 7033-7040.
[2] J. Deka, A. Mojumdar, P. Parisse, S. Onesti, L. Casalis, Sci. Rep. 7 (2017), 1-9.
[3] B. Sanavio, D. Scaini, C. Grunwald, G. Legname, G. Scoles, L. Casalis, ACS Nano 4 (2010), 6607-6616.
[4] Hudis, C.A., N. Engl. J. Med. 357 (2007), 39–51.
[5] A. Alsadig, H. Vondracek, P. Pengo, L. Pasquato, P. Posocco, P. Parisse, L. Casalis, Nanomaterials-Basel 11 (2021), 3181.

Get closer to the intrinsic properties of Ni^{2+}salen polymer semiconductors accessed by chain isolation inside mesoporous silica channels

Kamila Łępicka,[1] Piyush Sindhu Sharma,[1] Paweł Borowicz,[1] Gregory Francius,[2] and Alain Walcarius[2]

[1] *Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland*

[2] *Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement, LCPME, UMR 7564 CNRS – Université de Lorraine, 405 rue de Vandoeuvre, Villers-lès-Nancy, F-54600, France*

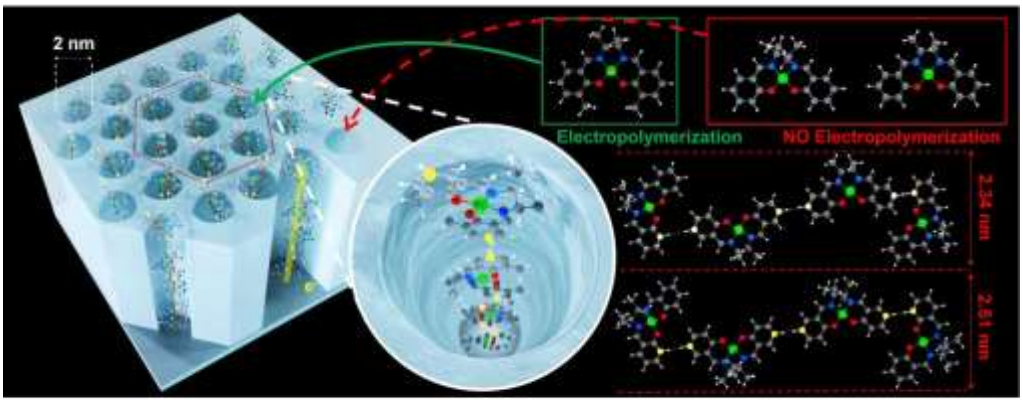
klepicka@ichf.edu.pl

The scientific problem we addressed aims to improve energy transport in Ni^{2+}salen conducting polymers by isolating individual polymer chains inside the silica matrix of vertical nanochannels used to suppress charge carrier trapping observed in the continuous Ni^{2+}salen polymers. With that respect, we prepared molecular wires based on structurally privileged *ortho* substituted Ni^{2+}salen conducting polymer, offering the molecular structure facilitating its linear electropolymerization growth inside the confined space of silica matrix containing 2 nm in diameter channels.

Moreover, we noticed that analogical embedment of *ortho* unsubstituted Ni^{2+}salen polymers was not possible. Thus, we engaged quantum chemical calculations utilizing the density functional to explain this phenomenon. Hence, we succeeded in recognizing structure–property relationships of Ni^{2+}salens governing the local electropolymerization ability inside vertical nanochannels.

Isolation of polymer chains allowed us to get insight into the intrinsic properties of Ni^{2+}salen polymers creating a better understanding of their charge transport and polymerization mechanisms. Prepared molecular wires indicated improved electronic properties evidenced by the electrochemical measurements revealing the energetically favoured charge transport components and the increased ratio between anodic and cathodic charge. These results highlight its promising application in molecular electronics as molecular interconnections transport energy to desired locations with minimum loss.

Furthermore, we applied a holistic physicochemical methodology to evidence a molecular level deposition of the polymer inside the silica nanochannels. Our approach revealed the necessity of using high-resolution X-ray photoelectron spectroscopy combined with a very low-energy argon ion beam sputtering for direct evidence of polymer wires inside silica channels combined with nanomaterial components visualization by Atomic force microscopy and Transmission electron microscopy.



Coexistence of multiple prion conformations: morphological and structural analysis by IR nanospectroscopy

J. Mathurin,[1] A. Deniset-Besseau,[1] A. Dazzi,[2] D. Martin,[2] A. Igel,[2] J. Torent i Mas,[2] V. Beringue,[2] H. Rezaei[2]

[1] *Institut Chimie Physique (ICP), Univ. Paris-Saclay/CNRS, 91405 Orsay, France*
[2] *Université Paris-Saclay, INRAE, UVSQ, VIM, 78350 Jouy-en-Josas, France*
e-mail: jeremie.mathurin@universite-paris-saclay.fr

The prion pathology is based on autonomous structural information propagation towards single or multiple protein conformational changes. Since this last decade, the prion concept referring to the transmission of structural information has been extended to several regulation systems and pathologies including Alzheimer and Parkinson's diseases. The unified theory in Prion replication implies structural information transference from the prion to a non-prion conformer through a mechanism also called improperly, with regards to biophysical considerations, "seeding" phenomenon. Recently, we reported that prion replication is intrinsically source of structural diversification [1], [2]. The coexistence of multiple prion assemblies with different structural and replication propensity questions i) how prion assemblies self-organized, ii) how this diversity is maintained within the same media and iii) how different PrP population escape to best replicator selection process during prion replication.
In this study, heterogeneities in prion assemblies are addressed using AFM-based techniques. AFM measurements show that assemblies have an overall fibrillar shape. However, numerous spherical protrusions are observed, either staked on the fibrils or in the vicinity of the fibres or as isolated objects. To have access to structural information on these objects, it is necessary to determine its secondary structure. This can be done using infrared (IR) spectroscopy. Due to the diffraction limit, classic IR microscopy spatial resolution is limited to few microns and cannot be used to locally studied nanometric size objects. This can be circumvented by using an IR nanospectroscopy technique called AFM-IR. This well-established technique combines an AFM and a tunable IR laser sources to perform sub-micrometric IR analysis [3]. Recently, we used it to study local secondary structure of bacterial amyloid fibres [4]. Applied to prion assemblies, it has revealed at the single assembly's scale an intra-assembly's heterogeneity (Fig. 1). This revealed that prion assemblies, rather than a canonical amyloid assembly, constitute a complex dynamic system far from the equilibrium where at least two different subpopulations coexist through catalytical material exchange.

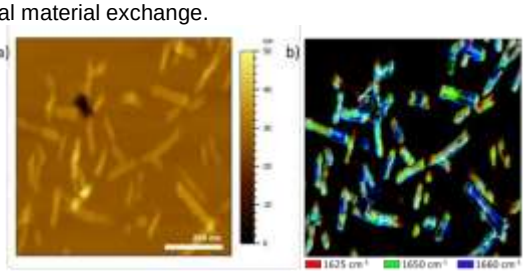


Figure 1: a) AFM topography of an area containing different prion assemblies – b) IR composite image merging IR absorption maps obtained on the same location with wavenumbers associated with different secondary structures

References

[1] A. Igel-Egalon *et al.*, "Heterogeneity and architecture of pathological prion protein assemblies: Time to revisit the molecular basis of the prion replication process?," *Viruses*, vol. 11, no. 5, 2019.

[2] A. Igel-egalon *et al.*, "Crossing Species Barriers Relies on Structurally Distinct Prion Assemblies and Their Complementation To cite this version : HAL Id : hal-03401965 Crossing Species Barriers Relies on Structurally Distinct Prion Assemblies and Their Complementation," 2021.

[3] A. Dazzi, R. Prazeres, F. Glotin, and J. M. Ortega, "Local infrared microspectroscopy with subwavelength spatial resolution with an atomic force microscope tip used as a photothermal sensor," *Opt. Lett.*, vol. 30, no. 18, pp. 2388–2390, 2005.

[4] D. Partouche *et al.*, "Correlative infrared nanospectroscopy and transmission electron microscopy to investigate nanometric amyloid fibrils: prospects and challenges," *J. Microsc.*, vol. 274, no. 1, pp. 23–31, Apr. 2019.

Evidence of viable bacteria of public health interest in free-living amoebae isolated from hot springs in Guadeloupe by SEM and LSM.

Aurélie DELUMEAU[1,2], Youri VINGATARAMIN[2], Isabel MARCELINO[2], Antoine TALARMIN[2] and Olivier GROS[1]

Affiliations:
[1] Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, BP 592 - 97159 Pointe-à-Pitre, Guadeloupe (FWI).
[2]TReD-Path Unit (Transmission, Réservoirs et Diversité des Pathogènes), LEMic (Laboratoire Interactions des Ecosystèmes Microbiens), Institut Pasteur de la Guadeloupe, Morne Jolivière 97183 Abymes, Guadeloupe (FWI).

E-Mails : Aurélie DELUMEAU : aurelie.delumeau@etu.univ-amu.fr; Youri VINGATARMIN ; YVINGATARAMIN@pasteur-guadeloupe.fr; Isabel MARCELINO : IMarcelino@pasteur-guadeloupe.fr; Antoine TALARMIN: ATALARMIN@pasteur-guadeloupe.fr; Olivier GROS : Olivier.Gros@univ-antilles.fr

Free-living amoebae (FLA) are ubiquitous protozoa found in soil and aquatic environment. FLA are also well-known reservoirs and vectors for the transmission of amoeba-resistant bacteria (ARB), most of which are pathogenic to Human and animals. Our recent studies revealed that members of the Vahlkampfiidae family (such as *Naegleria*, *Vahlkampfia* and *Paravahlkampfia*) are abundant in Guadeloupe. Still, little is known about the bacterial microbiome of these FLA. Herein, we aimed to characterize the bacteriome of three species of Vahlkampfiid amoebae isolated from hot springs (frequented by Guadeloupean and tourists) in Guadeloupe. For this, we used cell culture, molecular biology and microscopy methodologies.

Our results showed that the amoebae *Naegleria*, *Vahlkampfia* and *Paravahlkampfia* can naturally harbour viable bacteria of the *Acinetobacter, Cupriavidus, Escherichia* and *Pseudomonas* genera, all being pathogenic to humans. The use of sequential amoebae passaging in different culture medium revealed that the bacteriome of the naturally infected amoebae varies with the *in vitro* passages. Ultrastructural observations (using SEM and STEM and confocal LSM) permitted us to visualize the presence of cytoplasmic intracellular bacteria and their localisation/fate inside different amoebae life stages (dormant cysts vs replicative trophozoite). DAPI staining and FISH assay with universal oligonucleotide probes confirmed the intracellular localization of the endocytobionts and their presence in both replicative and dormant forms of the Vahlkampfiid amoebae.

This is the first study in which the bacterial microbiome of FLA isolated from hot springs in Guadeloupe is studied. We show that viable bacteria of public health interest can be detected and isolated from cysts. As cysts are highly resistant to extreme conditions of temperature, pH, and exposure to chemicals, the presence of both amoebae and ARBs in water bodies in Guadeloupe may represent a health risk.

June 02	
Conference Session III **Latest advances in materials application & life sciences**	
08.40 am – 09.10 am	**Catherine Picart**, « Bioactive Medical Devices for Bone Regeneration » **(Keynote lecture)**
09.10 am – 9.25 am	**Eloise Lebaudy**, « Polyethylene glycol diacrylate/poly epsilon L-lysine hydrogels for wound healing applications »
9.25 am – 9.40 am	**Jenny Harnett**, « Taking inspiration from nature's smartest polymer: the material properties of dense solutions of DNA origami »
9.40 am – 9.55 am	**Yulia Sergeeva**, « Reversible self-assembled monolayers as a multivalent platform for virus detection and inhibition »
9.55 am – 10.10 am	**Giovanna Fragneto**, « Planar lipid bilayers as model biological membranes for structural neutron studies »
10.10 am – 10.40 am	**Coffee Break (30 min)**
10.40 am – 11.10 am	**Patrick Couvreur**, « Advanced nanomedicines for the treatment of severe diseases » **(Keynote lecture)**
11.10 am – 11.25 am	**Miriam Colombo**, « Impact of biofunctionalization in therapeutic application of cutting-edge nanoparticles »
11.25 am – 11.40 am	**Antoine Combes**, « Protein-like Particles through Nanoprecipitation of Mixtures of Polymers of Opposite Charge »
11.40 am – 11.55 am	**Christelle Yacou**, « Valorization of *Sargassum*'s biopolymeric extracts and as precursor for nanoporous materials applied in water pollution treatment, supercapacitors and capacitive deionization »
11.55 am – 12.20 pm	**Paolo Scrimin**, « Hydrolytic Nanozymes: Mimicking the Catalytic Site of Nucleases » **(Invited)**
12.30 pm – 02.00 pm	**Conference Lunch**

Bioactive Medical Devices for Bone Regeneration

Catherine PICART

Research director at CEA / Head of INSERM Unit 1292 Biology and Biotechnology for Health
e-mail: catherine.picart@cea.fr

Biomaterials have since a long time been used to repair broken bones. Bone is a complex environment submitted to various stresses [1], in which bone cells are surrounded by an extra-cellular matrix made of biopolymers and several growth factors. Engineers have developed several strategies to repair bones using a large variety of materials, notably ceramics, metals, polymers and composites [2]. Additive manufacturing techniques are opening new perspectives for the custom-design of implants. The recent development in growth factors and stem cell biology has also led to propose new solutions for repairing bones: their effective implementation depending on their efficacy, safety and capacity to pass the regulatory requirements [3]. In this talk, I will present the context of bone repair using biomaterials and what our team has developed, in close collaboration with B3OA in Paris [4-6] and with Professor Georges Bettega, head of maxillo-facial surgery service at Annecy-Genevois General Hospital [7, 8].

Acknowledgements: ERC Oscodi (GA334966), ERC Regenerbone (GA790435), Association Gueules Cassées : n°21-2016 and 10-2018, ANR OBOE-28-CE17-0016

References

[1] E. Migliorini, A. Guevara-Garcia, C. Albiges-Rizo, C. Picart, Learning from BMPs and their biophysical extracellular matrix microenvironment for biomaterial design, Bone 141 (2020) 115540.
[2] C. Garot, G. Bettega, C. Picart, Additive manufacturing of material Scaffolds for bone regeneration: toward application in the clinics, Adv. Funct. Mat. n/a(n/a) (2020) 2006967.
[3] A. Ho-Shui-Ling, J. Bolander, L.E. Rustom, A.W. Johnson, F.P. Luyten, C. Picart, Bone regeneration strategies: Engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives, Biomaterials 180 (2018) 143-162.
[4] T. Crouzier, F. Sailhan, P. Becquart, R. Guillot, D. Logeart-Avramoglou, C. Picart, The performance of BMP-2 loaded TCP/HAP porous ceramics with a polyelectrolyte multilayer film coating, Biomaterials 32(30) (2011) 7543-54.
[5] R. Guillot, F. Gilde, P. Becquart, F. Sailhan, A. Lapeyrere, D. Logeart-Avramoglou, C. Picart, The stability of BMP loaded polyelectrolyte multilayer coatings on titanium, Biomaterials 34(23) (2013) 5737-46.
[6] R. Guillot, I. Pignot-Paintrand, J. Lavaud, A. Decambron, E. Bourgeois, V. Josserand, D. Logeart-Avramoglou, E. Viguier, C. Picart, Assessment of a polyelectrolyte multilayer film coating loaded with BMP-2 on titanium and PEEK implants in the rabbit femoral condyle, Acta Biomater. (2016).
[7] M. Bouyer, R. Guillot, J. Lavaud, C. Plettinx, C. Olivier, V. Curry, J. Boutonnat, J.L. Coll, F. Peyrin, V. Josserand, G. Bettega, C. Picart, Surface delivery of tunable doses of BMP-2 from an adaptable polymeric scaffold induces volumetric bone regeneration, Biomaterials 104 (2016) 168-81.
[8] M. Bouyer, C. Garot, P. Machillot, J. Vollaire, V. Fitzpatrick, S. Morand, J. Boutonnat, V. Josserand, G. Bettega, C. Picart, 3D-printed scaffold combined to 2D osteoinductive coatings to repair a critical-size mandibular bone defect, Mater Today Bio 11 (2021) 100113.

Polyethylene glycol diacrylate/poly epsilon L-lysine hydrogels for wound healing applications

E.Lebaudy,[1] P. Lavalle[1]

[1] *Inserm UMR_S 1121, Biomaterials and Bioengineering, Centre de Recherche en Biomédecine de Strasbourg, 67000 Strasbourg, France.*
e-mail: eloise.lebaudy@etu.unistra.fr ; philippe.lavalle@inserm.fr

Health-care associated infections (HAIs) are big issues in medical science. They represent nowadays 6% of hospital patients in Europe. One of the major causes of those diseases are the use of medical devices (MDs)[1]. Moreover, the increase of diabetic ulcers shows the importance of HAIs issues. The administration of antibiotics is a conventional method to fight against bacteria but this solution is no more viable in long run because of the emergence of bacteria resistance to antibiotics. Thus, new solutions need to be found in biomaterials science to decrease the infection risks.

Hydrogels with intrinsic antibacterial properties seem to be an interesting way to replace the use of antibiotics and to decrease the number of nosocomial infections[2]. Those gel-like materials could be also interesting for wound healing applications thanks to their high capacity to retain water. They will also establish a platform to load high amounts of various drugs inside their networks and to use these hydrogels as a local delivery system.

In our lab, we have developed new hydrogels composed of polyethylene glycol diacrylate (PEGDA) and poly epsilon L-lysine (PεL). Indeed, polyethylene glycol (PEG) is highly used in the development of hydrogels because of its good solubility in water and its high hydrophilicity[3]. PεL is known for its non-cytotoxicity and its antibacterial and antifungal properties[4]. Under basic pH, amine functions of PεL can react with the acrylate moieties of PEGDA leading to a chemically cross-linked hydrogel.
Various concentrations of both polymers and ratio between acrylate and amine function have been tested and optimized to modulate rheological and mechanical properties of materials.
Then, cells and bacteria behaviours in contact with hydrogels were studied for evaluating the properties of our materials, such as the cytotoxicity, the antibacterial properties, the wound healing properties and the anti-inflammatory properties.

We demonstrated that those hydrogels present strong antibacterial activity against both gram-positive and gram-negative bacteria thanks to their fast and spontaneous degradability. By modifying ratio between reactive functions in the gelation step and concentrations of both polymers, physical properties can be tuned. Thus, elastic modulus and degradation rate can be adjusted. Moreover, these materials are non-cytotoxic and enable to decrease the inflammatory response in LPS-stimulated macrophages. Cell migration is also greatly increased by PεL, which is promising for wound healing applications. Finally, these hydrogels can be easily poured and absorbed in medical compresses for preventing infections in a wound.

PEGDA/PεL hydrogels are thus very promising for medical use and more specifically for wound healing applications thanks to their biocompatibility and their antibacterial and anti-inflammatory properties. These materials could be used as dressings or even as coatings for medical devices to prevent infections and to improve wound healing.

References

[1] H. Nosrati, M. Khodaei, Z. Alizadeh, M. Banitalebi-Dehkordi, International Journal of Biological Macromolecules 192 (2021) 298-322
[2] V.Gribova, F. Boulmedais, A. Dupret-Bories, C. Calligaro, B. Senger, N.E. Vrana, P. Lavalle, ACS Appl. Mater. Interfaces 12 (2020) 19258-19267
[3] W. Cao, C.Gao, Polymer 186 (2020) 122082
[4] N.A Patil, B. Kandasubramanian, European Polymer Journal 146 (2021) 110248

Taking inspiration from nature's smartest polymer: the material properties of dense solutions of DNA origami

J. Harnett,[1] D. Michieletto,[1] and K.Voitchovsky[2]

[1] *School of Physics and Astronomy, University of Edinburgh, EH9 3FD, UK*
[2] *School of Physics and Astronomy*, Durham University, DH1 3LE
e-mail: s2139940@ed.ac.uk

A central question in soft matter is how the microscopic building blocks of a material influence its macroscopic properties, achieving a better understanding of this is vital to the development of novel and smart materials. The ability of DNA to store vast amounts of information using only four different nucleotide bases make it nature's smartest polymer. Despite this, it is only in recent years that scientist have begun to see DNA as more than a genetic storage polymer and start to investigate the interesting capabilities of DNA as material building block. The technique of DNA origami allows precise control over geometry on the nanometer scale, DNA can be folded in a predesigned way by combining a single stranded scaffold of DNA with many smaller staple strands. In this project we use this technique to vary the microscopic topology of DNA, focusing on topologies termed 'chimeric' which display both linear and circular architectures. This class of topologies are expected to display uncommon viscoelastic properties due to special topological constraints, as demonstrated by simulations investigating dense solutions of tadpole topologies [1]. In this project, the viscoelastic properties of high concentrations of chimeric DNA origami are investigated using microrheology. To date, a linear and circular design of DNA origami have been created alongside a tadpole topology. Atomic force microscopy (AFM) confirmed the structures had been successfully created as well as characterising the geometry and topology of the DNA, this is demonstrated in Figure 1.

DNA also exhibits a plethora of topologies in nature: plasmids are a simple ring topology and lambda DNA linear, while kinetoplast DNA exhibits a complex topological structure where a network of interconnected rings forms an 'Olympic Gel' [2]. This project also aims to use AFM to characterise naturally occurring DNA topologies, and image the complex topology of kinetoplast DNA in high resolution.

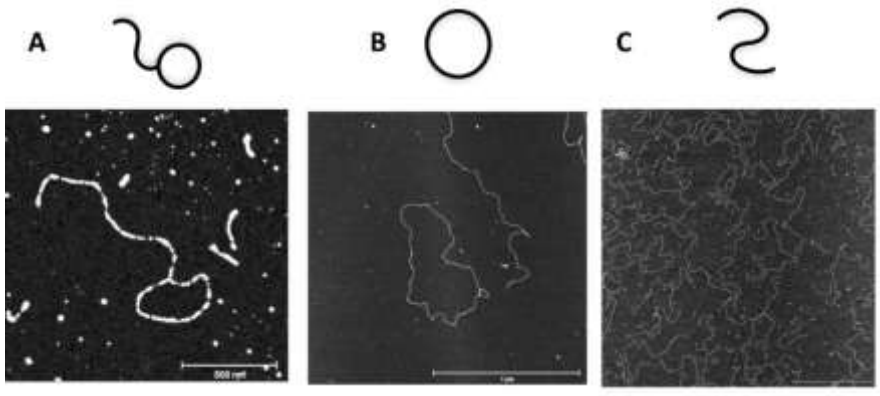


Figure 1: AFM in air of three topologies of DNA origami: Tadpole (A), Circular (B) and Linear (C).

References

[1] Rosa, A., Smrek, J., Turner, M. S., & Michieletto, D. *ACS macro letters* (2020) 743-748.
[2] Chen, J., Rauch, C. A., White, J. H., Englund, P. T., & Cozzarelli, N. R. *Cell* (1995) 61-69.

Reversible self-assembled monolayers as a multivalent platform for virus detection and inhibition

Yulia Sergeeva[1], Sing Yee Yeung[1], Thomas Janssens[1], Tommy Dam[2], Peter Jönsson[2], Börje Sellergren[1]

[1] *Biofilms-Research Center for Biointerfaces (BRCB), Department of Biomedical Sciences, Faculty of Health and Society, Malmö University, 205 06 Malmö, Sweden*

[2] *Division of Physical Chemistry, Department of Chemistry, Lund University, 221 00 Lund, Sweden*

e-mail: yulia.sergeeva@mau.se

Multivalency plays a major role in most biological molecular recognition processes. For instance, multivalent interactions are the driving force in virus attachment to the cell surface at the early stage of the infection process. The development of new drugs with multivalent ligand representation that will inhibit the virus-cell association is considered an advantageous alternative for the treatment of severe viral infections such as Influenza. The design of such drugs includes the synthesis of complex compounds such as dendrimers, fullerenes, polymers, *etc.,* and requires time-consuming multistep organic synthesis. Here we present a new concept for multivalent ligand representation, *reversible self-assembled monolayers (rSAMs)*. These layers are a pH-switchable version of self-assembled monolayers (SAMs). Unlike SAMs, the rSAMs demonstrate lateral mobility and enhanced affinity towards hemagglutinin. The layers mimic the complex multivalent carbohydrate arrays present on the cellular surfaces thus representing an ideal platform for virus detection and the engineering of multivalent virus inhibitors. We also demonstrated that rSAMs functionalized gold nanoparticles can be used for rapid and effective detection of influenza virus detection with a detection limit lower than 0.5 HAU.

References

[1] Mammen, M., Choi, S.-K., Whitesides, G. Polyvalent Interactions in Biological Systems: Implications for Design and Use of Multivalent Ligands and Inhibitors. Angew. Chem., 1998, 110, 2908-2953
[2] Yeung, S.Y., Sergeeva, Y., Dam, T., Jönsson, P., Pan, G., Chaturvedi, V., Sellergren, B. Lipid Bilayer-like Mixed Self-Assembled Monolayers with Strong Mobility and Clustering-Dependent Lectin Affinity. Langmuir, 2019, 35, 24, 8174-8181.A. Martin, B. Dupont, C. March, Journal 163 (2006) 192-156.

Planar lipid bilayers as model biological membranes for structural neutron studies

Giovanna Fragneto[1]
[1]Institut Laue-Langevin, 71 avenue des Martyrs, BP 156, 38042 Grenoble, FR
e-mail: fragneto@ill.fr

Cells, the basic units of living organisms, are well delineated and separated from the external environment by membranes. Capable of both enclosing the cellular constituents and allowing exchanges with the outside world, these membranes are only a few nanometers thick. To study the dynamics and function of these amazing objects, physicists first seek to understand their structure. This involves experiments on model systems, simpler and better controlled than real membranes, and can profit from a probe that is able to access different scales of size and time: thermal neutrons.

Since the pioneering work in the seventies on cell membrane structure by neutron scattering, developments driven by constantly improving neutron instrumentation, coupled with development of measurement and analysis methods, have involved both the optimization of samples towards more biologically relevant model systems and include the use of more complex lipid mixtures up to natural extracts. A natural lipid deuteriation facility has been set-up at the ILL (http://www.ill.eu/L-Lab) and recent results on lipid production and characterisationn will be presented.

Recent developments in the study of the structure of membranes will be presented including neutron and x-ray reflectometry study of the out-of-equilibrium fluctuations of phospholipid membranes induced by the active transmembrane protein bacteriorhodopsin (BR) [1]. A detergent-mediated incorporation method was used to incorporate BR in model planar bilayers and structural modifications induced by light activation were measured.

Furthermore, the use of neutron scattering methods to study the interaction of the spike protein of SARS-CoV-2 virus will be presented [2], including results revealing the different roles of peptides present within the fusion domain and the role of intracellular calcium levels that could provide an indication to where and how the viral and host membranes fuse during SARS-CoV-2 infection [3].

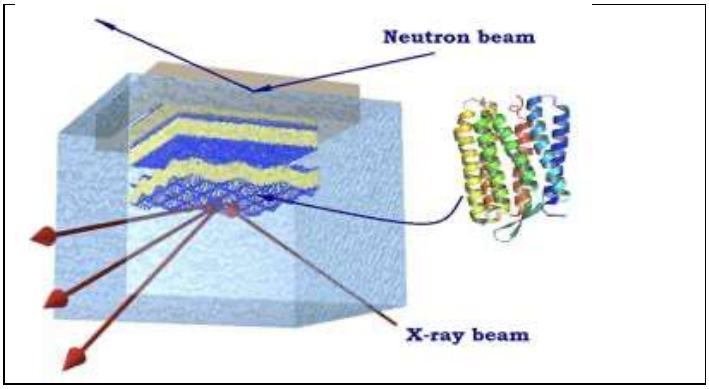


Figure 1: Model system used for study of BR induced fluctuations in model membranes sudied by neutron and synchrotron radiation

References

[1] Insertion and activation of functional Bacteriorhodopsin in a fl oating bilayer by T. Mukhina et al., *JCIS* (2021)
[2] Lipid bilayer degradation induced by SARS -CoV-2 spike protein as revealed by neutron reflectometry, by A. Luchini et al. *Scientific Reports* (2021):
[3] Strikingly Different Roles of SARS-CoV-2 Fusion Peptides Uncovered by Neutron Scattering", by A. Santamaria et al., *J. Am. Chem. Soc.* (2022).

Advanced nanomedicines fort the treatment of severe diseases

Patrick COUVREUR
Université Paris-Saclay, Institut Galien, UMR CNRS 8612, F-92296 Chatenay-Malabry (France)
patrick.couvreur@universite-paris-saclay.fr

The clinical use and efficacy of conventional chemotherapeutics is hampered by the following limitations: (i) drug resistance at the tissue level due to physiological barriers (non-cellular based mechanisms), (ii) drug resistance at the cellular level (cellular mechanisms), and (iii) non-specific distribution, biotransformation and rapid clearance of the drugs in the body. Nanomedicines (ie., drug loaded onto nanocarriers) may overcome some of these limitations. The presentation will show that before entering into clinical trials, the discovery of advanced nanomedicines rests on four scientific pillars: chemistry, physics, cellular and molecular biology as well as, experimental pharmacology. This will be illustrated by the following nanomedicine platforms with the demonstration that interdisciplinarity is the basis for scientific and technological successes:

- The design of *biodegradable doxorubicin-loaded polyalkylcyanoacrylate nanoparticles* for the treatment of the multidrug resistant hepatocarcinoma (a nanomedicine with phase III clinical trials ended) [1].
- The construction of *nanoparticles made of metal oxide frameworks (NanoMOFs)*[2,3], a highly hyperporous material obtained by the complexation of iron oxide clusters with diacids. The nanopores of this material may be finely tuned to the molecular dimension of the drug molecule to be encapsulated.
- The *"squalenoylation"*[4,5], a technology that takes advantage of the squalene's dynamically folded molecular conformation, to link this natural and biocompatible lipid with anticancer drug molecules[6,7] to achieve the spontaneous formation of nanoassemblies (100–300 nm) in water, without the aid of surfactants. Surprisingly, these squalene-based nanoparticles are using the circulating endogenous LDL as "indirect" carriers for targeting cancer cells with high expression of the LDL receptors[8]. The application of the "squalenoylation" concept for the treatment of brain ischemia and spinal cord injury[5] will be discussed, too. And it will be shown that the linkage of squalene to leu-enkephalin can confer to the targeted neuropeptide a significant anti-hyperalgesic effect, devoted of the morphine side effects (ie., addiction, tolerance and resiratory depression)[9]. The possibility to use other terpenes (natural or synthetic) than squalene to design nanoparticles for the treatment of cancer will be discussed, too[10].

The design of "multidrug" nanoparticles, combining in the same nanodevice chemotherapy and imaging properties (ie., "nanotheranostics") or various drugs with complementary biological targets will be also examined[11].

Finally, it will be shown that the construction of nanodevices sensitive to endogenous (ie., pH, ionic strenght, enzymes etc.) or exogenous (ie., magnetic or electric field, light, ultrasounds etc.) stimuli may allow the spatio-temporal controlled delivery of drugs and overcome resistance to current treatments[12].

[1] *L. Barraud et al., J. Hepatology, 42, 736-743 (2005)*
[2] *P. Horcajada et al., Nature Materials. 9, 172-178 (2010)*
[3] *T. Simon-Yarza et al., Angewandte Chemie Int. Edition, 56, 15565 –15569 (2017)*
[4] *P. Couvreur et al., Nano Letters, 6, 2544-2548 (2006)*
[5] *A. Gaudin et al., Nature Nanotechnology, 9, 1054-1063 (2014)*
[6] *A. Maksimenko et al., Proceedings of the National Academy of Science, 111 (2) E217- E226 (2014)*
[7] *L. Kotelevets et al., Cancer Research, 77, 2964-2975 (2017)*
[8] *D. Sobot et al., Nature Communications, 8, 15678 (2017)*
[9] *Jiao et al., Science Advances, 5 : eaau5148 (2019)*
[10] *S. Harisson et al., Angewandte Chemie Int. Edition, 52, 1678-1682 (2013)*
[11] *Dormont F et al., Science Advances, 6 : eaaz5466 (2020)*
[12] *S. Mura et al., Nature Materials, 12, 991-1003 (2013)*

Impact of biofunctionalization in therapeutic application of cutting-edge nanoparticles.

M. Colombo,[1] L. Salvioni,[1] R. Rotem,[1] L. Morelli,[1] and M. Giustra[1]
[1] *NanoBioLab, Department of Biotechnology and Bioscience*
University of Milano-Bicocca, Milan, Italy
e-mail: miriam.colombo@unimib.it

Multifunctional nanoparticles are promising bimodal tracers for noninvasive diagnosis and treatment of cancer and inflammatory diseases in vitro and in vivo. The design of bio-functionalized colloidal nanoparticles needs careful optimization of size and shape, optical and magnetic properties, and efficient conjugation with homing ligands to improve the signal amplification and target selectivity toward malignant cells. One of the greatest challenges in designing nanoparticles functionalized with homing peptides and proteins to optimize molecular recognition resides in the possibility to finely control the ligand orientation on the nanoparticle surface.
To support the research in new drug delivery nanosystems, in the past few years new administration methods of nanoparticles rather than traditional intravenous ones have been explored. This is a highly innovative approach that is nearly unexplored at present. Because of parenteral administration drawbacks, alternative administration routes have been investigated. Among all, the oral and topical administration are the most interesting to obtain a local effect and gain a better patient's compliance.

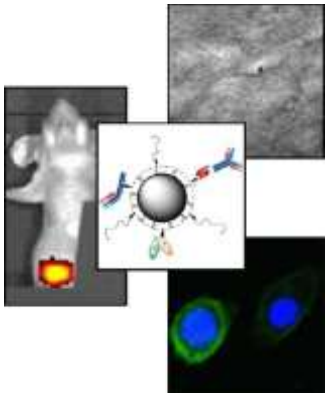


Figure 1: Magnetic nanoparticles functionalized with Trastuzumab or Ab fragments for targeting and treatment of breast cancer cells in vitro and in vivo

References

[1] M. Colombo, et al. Nature Commun. 2016, 7, 13818.
[2] L. Salvioni, et al. Pharmacol. Res. 2016, 110, 122.
[3] M. Colombo, et al. Bioconjug. Chem., 2018, 29, 3817.
[4] V. Codullo, et al.J. Controlled Rel., 2019, 310, 198-208.

Protein-like Particles through Nanoprecipitation of Mixtures of Polymers of Opposite Charge

Antoine Combes, Khanh-Nam Tang, Andrey S. Klymchenko, Andreas Reisch
Université de Strasbourg, CNRS, Laboratoire de Bioimagerie et Pathologies UMR 7021, Strasbourg F-67000, France
antoine.combes@etu.unistra.fr

Polymeric nanoparticles (NPs) have numerous applications in the biomedical field because of their capacity to encapsulate compounds such as drugs[1] or contrast agents[2]. However, using synthetic materials in biological systems generates many challenges as controlling stability, specific interactions or toxicity of NPs [3]. Proteins, in contrast, were designed by nature for this purpose and are a great model to design nano-sized objects for biomedical usage. In this work we used nanoprecipitation of polymers of opposite charge to obtain NPs with both charges present on their surfaces, thus mimicking the surface of proteins[4].

Two series of copolymers of ethyl methacrylate with 1 to 25 mol% of either methacrylic acid or a trimethylammonium bearing methacrylate were synthesized. These carboxylic acid and trimethylammonium bearing polymers are then mixed in different ratios and nanoprecipitated. The influence of the charge fraction, mixing ratio, and precipitation conditions on NP size and surface charge was studied. Using this approach, NPs of less than 25 nm with tunable surface charge from + 40 mV to – 40 mV were assembled.

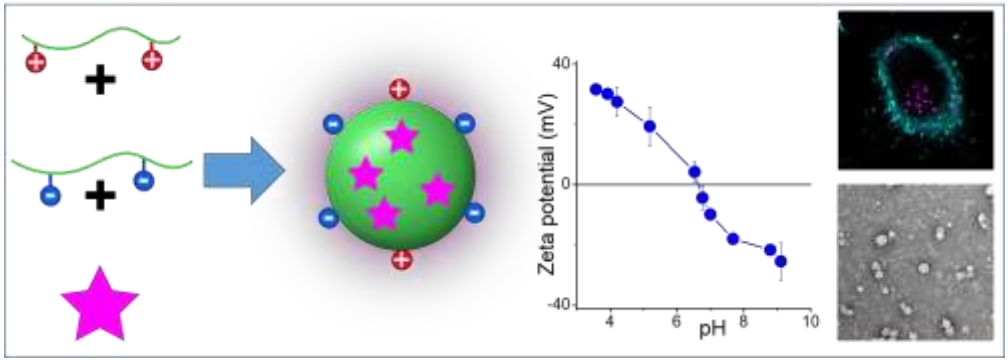


Figure 1: Scheme of NPs formation by nanoprecipitation of oppositely charged polymers with a dye. Zeta potential of NPs depending on pH. Confocal image of NPs incubated with HeLa cells and TEM image of NPs.

The resulting NPs were sensitive to pH, can have an isoelectric point and allowed repeated charge reversal. Especially the most charged polymers, with 25% mol charged groups, made it possible to obtain NPs stable in media of very high salinity (up to 1 M NaCl). Encapsulation of fluorescent dyes yields very bright fluorescent polymer NPs, whose interactions with cells could be studied through fluorescence microscopy. The ratio of charges present on NPs surfaces had a strong influence on their internalization in cells. The obtained results show the potential of the concept of combination of oppositely charged polymers in NPs through nanoprecipitation for the design of NPs with precisely tuned surface properties.

References

[1] I. Brigger, C. Dubernet, P. Couvreur, Adv. Drug Deliv. Rev. 64 (2012) 24-36.
[2] A. Reisch, A.S. Klymchenko, Small 12(15) (2016) 1968-1992.
[3] N. Desai, AAPS J. 14(2) (2012) 282-295.
[4] A. Combes, K-N. Tang, A.S. Klymchenko, A. Reisch, J. Colloid Interface Sci. 607 (2022) 1786-1795.

Valorization of *Sargassum´*s biopolymeric extracts and as precursor for nanoporous materials applied in water pollution treatment, supercapacitors and capacitive deionization

C. Yacou[1], M. Francoeur[1], S. Roche[1], N. Marcin[1], G. Cebrián-Torrejón[1], Y. Alvarez [1], L. Zozo[1], E. Oxybel[1], V. Jeanne-Rose[1], S. Gaspard[1]

[1] *Laboratoire COVACHIM-M2E, EA 3592, Université des Antilles, BP 250, 97157 Pointe-à-Pitre cedex, Guadeloupe, French West-Indies*.

e-mail: christelle.yacou@univ-antilles.fr

Since 2011, substantial amounts of pelagic *Sargassum* algae composed of *Sargassum natans* and *Sargassum fluitans* have washed ashore along the Caribbean basin and Gulf of Mexico. This study aims to propose innovative ways to valorize this available biomass.

At first, the extraction of natural polymeric products from the seaweed was carried out to explore new potential applications [1]. As such, different protocols were proposed consisting of successive extractions with acid and basic aqueous solutions to achieve the best yield of biopolymers (alginic acid and alginate (Figure 1)). Characterization of the extracted molecules was then performed by spectroscopic methods: 1H-NMR, FT-IR and SEM/EDX.

A second-stage valorization was attempted in this study by transforming algae residues (after extraction) into nanoporous carbon matrices. The objective was to evaluate their performance as attractive materials for both environmental and energy storage applications. For comparison purposes, raw seaweed (before extraction) was also used as precursor to produce activated carbon. All samples were prepared either by pyrolysis at different temperature using conventional techniques or by hydrothermal treatment. Their textural properties were evaluated by nitrogen adsorption techniques and their chemical surface characteristics were obtained from X-ray photoelectron and Infra-red spectroscopies. Part of the synthetized samples was used as starting materials for the fabrication of electrodes in capacitive systems (i.e. supercapacitors and water deionization). Depending of the synthesis conditions, activated carbons obtained by pyrolysis of the raw biomass exhibited high surface area up to 1664 m^2/g. A pre-treatment by hydrothermal carbonization followed by an activation step using KOH, allows producing samples with promising textural properties, which have shown remarkable capacitive behaviours by cyclic voltammetry. The biopolymer extracts were also used as binders to prepare the electrodes. Finally, for water treatment application, the synthesis conditions of the nanoporous carbons were optimized and pharmaceutical pollutants adsorption followed by their catalytic degradation were successfully carried out.

(**a**) (**b**)

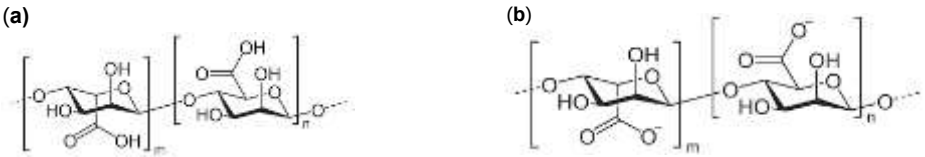


Figure 1. alginic acid (**a**) and alginate (**b**)

Keywords: *Sargassum*, carbon electrode, biopolymer, activated carbon, supercapacitors, capacitive deionization

[1] A. Mohammed, R. Bissoon, E. Bajnath, K. Mohammed, T. Lee, M. Bissram, N ; John, N.K. Jalsa, K.-Y. Lee, K. Ward. Multistageextraction and purification of waste *Sargassum natans* to produce sodium alginate: An optimization approach, 2018, Carbohydrate Polymers 198, 109-118https://doi.org/10.1016/j.carbpol.2018.06.067

Hydrolytic Nanozymes: Mimicking the Catalytic Site of Nucleases

Paolo Scrimin

University of Padova, Department of Chemical Sciences, Padova, Italy
e-mail: paolo.scrimin@unipd.it

As the "molecules of life" DNA and RNA play key roles in reproduction and proteome formation. The study of the mechanism of their cleavage still constitutes a major challenge. Even more challenging is the obtainment of efficient, artificial, cleavage catalysts. Most of the nucleic acids-cleaving enzymes are metalloenzymes, typically featuring two metal ions in the catalytic site.[1] However, mononuclear metalloenzymes are known and even metal-free enzymes, particularly for the cleavage of RNA.[2] In metal ion-based nucleases the metal(s) present in the catalytic site can:[2,3] (a) activate the phosphoryl group towards nucleophilic attack thus acting as Lewis acid(s); (b) increase the leaving group ability (by decreasing its pKa by coordination of the alcoholic oxygen); (c) provide a large fraction of an anionic nucleophile by inducing deprotonation of hydrating water molecules or other coordinated alcoholic groups.

These features of natural enzymes have been exploited by several groups to obtain artificial nucleases.[4] For instance, we have reported that gold nanoparticles (AuNPs) passivated with thiols functionalized with 1,4,7-triazacyclonane (TACN) are, as Zn(II) complexes, among the most active catalysts for the cleavage of the RNA-model substrate 2-hydroxypropyl p-nitrophenyl phosphate (HPNP) and dinucleotides as well. The impressive efficiency and the enzyme-like kinetic profile they show in the catalytic process, induced us to dub them "nanozymes".[5]

Recently we modified the catalytic site of these AuNPs by flanking them with different functional groups, including short peptides featuring key amino acids present in several nucleases' catalytic sites (as arginine and serine), to elucidate the role of the metal complex in the interaction with DNA. The final goal was to turn our nanozymes into proficient catalysts for plasmid DNA cleavage. Here we report our successful results and show how evaluating a catalyst against a model substrate (as bis-p-nitrophenylphosphate, BNP, in the case of DNA) may lead to wrong conclusions on its efficiency against the real biological target.

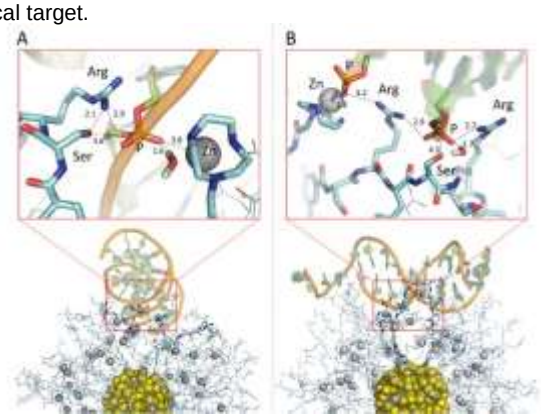


Figure 1: Computer model of two catalytic modes (A and B) for the cleavage of DNA by a gold nanoparticle-based nanonuclease

References

[1] Yang, W.. *Q Rev Biophys* **2011**, *44*, 1–93.
[2] Diez-Castellnou, M.; Martinez, A.; Mancin, F. *Adv. Phys. Org. Chem*, **2017**, *51*, 129–186.
[3] Palermo, G.; Cavalli, A.; Klein, M.; Alfonso Prieto, M.; Peraro, M.; De Vivo, M. *Acc Chem Res* **2015**, *48*, 220–228.
[4] Mancin, F.; Scrimin, P.; Tecilla, P. *Chem Commun* **2012**, *48*, 5545–59.
[5] a) Diez-Castellnou, M.; Mancin, F.; Scrimin, P. *J. Am. Chem. Soc.* **2014**, *136*, 1158–61; b) Gabrielli, L.; Prins, L. J.; Rastrelli, F.; Mancin, F.; Scrimin, P. *Eur. J. Org. Chem.* **2020**, 5044-5055; c) Czescik, J.; Zamolo, S.; Darbre, T.; Rigo., R.; Sissi, C.; Pecina, A.; Riccardi, L.; De Vivo, M.; Mancin, F.; Scrimin, P. *Angew. Chem. Int. Ed.* **2021**, *60*, 1423–1432; d) Czescik, J.; Mancin, F.; Strömberg, R.; Scrimin, P. *Chem European J* **2021**, *27*, 8143–8148; e) Lyu, Y.; Scrimin P. *ACS Catal* **2021**, *11*, 11501−11509.

June 02	
Conference Session IV Simulations and machine learning	
02.00 pm – 02.30 pm	**Adam Foster**, « Structure Discovery in Atomic Force Microscopy » **(Keynote lecture)**
02.30 pm – 02.45 pm	**Magdalena Giergiel**, « Automation of data processing for AFM image classification »
02.45 pm – 03.00 pm	**Saeid Ekrami**, « Molecular dynamics simulation & nanomechanics of hyaluronic acid-based hydrogels »
03.00 pm – 03.25 pm	**Sergei Kalinin**, **(Invited)**
03.25 pm – 04.00 pm	**Coffee Break**
04.00 pm – 04.30 pm	**Teuta Pilizota**, « *Escherichia coli's* life or death at high intracellular pressure » **(Keynote lecture)**
04.30 pm – 04.45 pm	**Adrian Martinez-Rivas**, « Deep learning to classify nanobiomechanical data of cells »
04.45 pm – 05.00 pm	**Yashasvi Ranawat**, « Generalised prediction of hydration layers on surfaces using deep learning »
05.00 pm – 06.00 pm	**Poster Session II – Networking Time**
07.30 pm	**Conference Dinner**

Structure Discovery in Atomic Force Microscopy

Adam S. Foster[1,2]

[1]*Department of Applied Physics, Aalto University, Helsinki Finland*
[2]*WPI Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Kanazawa, Japan*
e-mail: adam.foster@aalto.fi

Atomic force microscopy (AFM) with molecule-functionalized tips has emerged as the primary experimental technique for probing the atomic structure of organic molecules on surfaces [1]. Most experiments have been limited to nearly planar aromatic molecules, due to difficulties with interpretation of highly distorted AFM images originating from non-planar molecules [2]. Here we develop a deep learning infrastructure that matches a set of AFM images with a unique descriptor characterizing the molecular configuration, allowing us to predict the molecular structure directly in a few seconds on a laptop [3]. We apply this methodology to resolve several distinct adsorption configurations and conformations of molecules based on low-temperature AFM measurements. In general, we find high success rates in predicting the atomic and chemical structure of molecules, and the method can also be used for quantitative predictions of electrostatic properties [4]. This approach opens the door to apply high-resolution AFM to a large variety of systems for which routine atomic and chemical structural resolution on the level of individual objects/molecules would be a major breakthrough. We also look at applications of similar approaches to the imaging of biomaterials and AFM imaging in liquids [5].

References

[1] N. Pavliček and L. Gross, Nat. Rev. Chem. **1** (2017) 5
[2] P. Jelinek, J. Phys.:Condens. Mat. **29** (2017) 343002
[3] B. Alldritt, P. Hapala, N. Oinonen, F. Urtev, O. Krejci, F. F. Canova, J. Kannala, F. Schulz, P. Liljeroth, and A. S. Foster, Sci. Adv. **6** (2020) eaay6913
[4] Niko Oinonen, Chen Xu, Benjamin Alldritt, Filippo Federici Canova, Fedor Urtev, Ondřej Krejčí, Juho Kannala, Peter Liljeroth and Adam S. Foster, ACS Nano **16** (2022) 89
[5] Yashasvi S. Ranawat, Ygor M. Jaques and Adam S. Foster, Nanoscale Adv. **3** (2021) 3447

Automation of data processing for AFM image classification

Magdalena Giergiel[1], Bartłomiej Zapotoczny[2], Jerzy Konior[1], Marek Szymoński[1]

[1] *Marian Smoluchowski Institute of Physics, Jagiellonian University*
S. Łojasiewicza 11, 30-348 Kraków, Polska
[2] *Institute of Nuclear Physics, Polish Academy of Science*
Radzikowskiego 152, 31-342 Kraków, Polska
e-mail: m.j.giergiel@gmail.com

Defining simple rules for recognising complex patterns in atomic force microscopy (AFM) images is not an easy task. In many cases, neither algorithms based on the height of the sample nor graphical approaches are efficient enough to classify observed structures. Analysis can be done manually, in which case it is subjective and prone to errors, or a more refined technique is necessary. A particularly useful and fast method is the application of machine learning-based algorithms.

In recent years, convolutional neural networks have revolutionised automated image recognition. We present an example application of this approach to conduct quick AFM image classification. The study of porous structures, called fenestrations - transmembrane pores occurring in the cell membrane of liver sinusoidal endothelial cells (LSECs), provides information on the liver condition. A decrease in the frequency or size of fenestration may be a marker of pathological changes in the liver. So far, a basic methodology for examining the structure of fenestration has been developed [1], but there was no quick and automatic method for analyzing microscopic images of large clusters of LSECs.

It turns out that the accuracy of the machine learning method not only surpasses manual analysis but is also much faster, not sensitive to the image contrast, and fully deterministic [2]. Therefore, the results are more reliable. The presented scheme can be easily modified to different objects of interest, which promotes the use of neural networks as a universal tool for the analysis of many kinds of microscopy images.

References

[1] B. Zapotoczny, K. Szafranska, E. Kus, S. Chlopicki, M. Szymonski. Quantification of fenestrations in liver sinusoidal endothelial cells by atomic force microscopy. Micron, 101, 48–53 (2017). doi:10.1016/j.micron.2017.06.005
[2] M. Giergiel, B. Zapotoczny, I. Czyzynska-Cichon, J. Konior, M. Szymonski, AFM image analysis of porous structures by means of neural networks, Biomedical Signal Processing and Control Volume 71, 103097 (2022), doi: 10.1016/j.bspc.2021.103097

Molecular dynamics simulation & nanomechanics of hyaluronic acid-based hydrogels

Saeid EKRAMI,[1] Erwan ANDRÉ,[1] and Gregory FRANCIUS,[1]

[1]*Université de Lorraine, CNRS, LCPME, F-54000 Nancy, France*
e-mail: saeid.ekrami@univ-lorraine.fr

The demand for biocompatible polymeric materials has increased widely due to their ability as carriers for drugs delivery and therapeutic agents. Understanding their physio-chemical properties at molecular level is of ultimate importance to design smart materials with optimal properties. Among all, hyaluronic acid and its derivatives are well-known biomaterials that are wildly used in medicine [1-3] and cosmetic markets [4]. In particular, hyaluronic acid gels are good candidates for transdermal patch matrices used in phagotherapy. In here, to adjust the mechanical properties of the gel, we investigated the effect of cross-linking on hyaluronic acid through a joint experimental and computational approach. On one hand, we built hyaluronic acid hydrogels cross-linked with 1,4-butanediol diglycidyl ether (known as BDDE) and deciphered their structural and mechanical features by combining infrared spectroscopy, and atomic force microscopy. On the other hand, all-atom molecular dynamics simulation was used to gain more insight into phenomena involved in hydrogel formation at the atomic and molecular level. Those investigations evidenced the weak mechanical properties of the cross-linked hydrogels and connected it to the limitation of BDDE reaction to reduce the mobility of hyaluronic acid chains, in line with the poor effective cross-linking rate. Further investigations will allow the design/adapt of new cross-linking protocol to strengthen mechanical properties of these hydrogels.

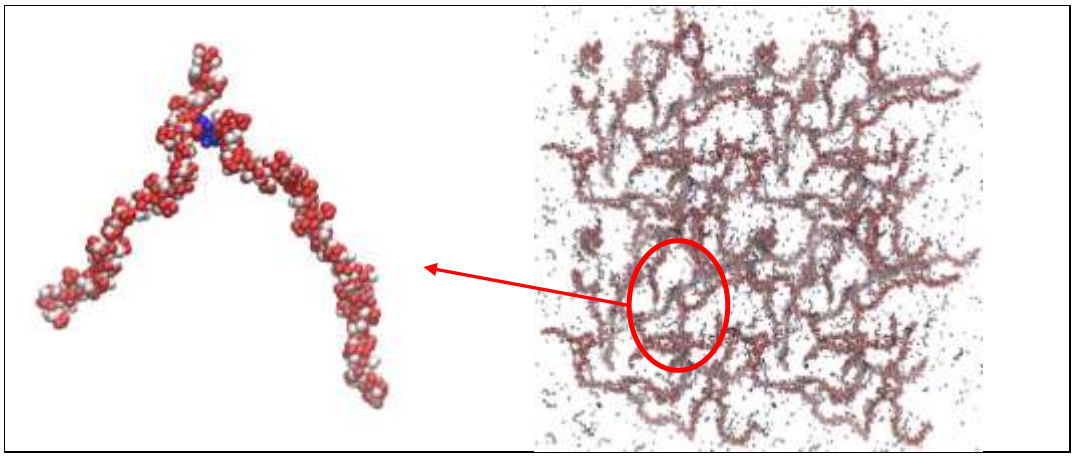


Figure 1: Hyaluronic acid chains, cross-linked by BDDE

References

1. Kogan, G., et al., *Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications.* Biotechnology letters, 2007. **29**(1): p. 17-25.
2. Laurent, T.C. and J.R.E. Fraser, *Hyaluronan 1.* The FASEB journal, 1992. **6**(7): p. 2397-2404.
3. Balazs, E.A. and T. Laurent, *Chemistry, biology and medical applications of hyaluronan and its derivatives*. 1998: Portland.
4. Liu, L., et al., *Preparation and characterization of sponge-like composites by cross-linking hyaluronic acid and carboxymethylcellulose sodium with adipic dihydrazide.* European polymer journal, 2007. **43**(6): p. 2672-2681.

Prof. Sergei Kalinin

Oak Ridge National Laboratory, United States

He is a corporate fellow at the Center for Nanophase Materials Sciences (CNMS) at Oak Ridge National Laboratory. His research interests include atom by atom fabrication, application of machine learning and artificial intelligence in atomically resolved and mesoscopic imaging to guide the development of advanced materials for energy and information technologies, as well as coupling between electromechanical, electrical, and transport phenomena on the nanoscale. He has published more than 500 peer-reviewed journal papers, edited 4 books, and holds more than 10 patents. He is also a member of editorial boards for several international journals, including Nanotechnology, Journal of Applied Physics/Applied Physics Letters, and recently established Nature Partner Journal Computational Materials

Topic: Simulations and machine learning

Escherichia coli's life or death at high intracellular pressure

Teuta Pilizota,[1] Smitha Hegde,[1] and Dario Miroli[1]

[1]*Centre for Synthetic and System Biology, University of Edinburgh, Alexander Crum Brown Road, EH9 3FF, Edinburgh, UK*
e-mail: teuta.pilizota@ed.ac.uk

For most cells, a sudden decrease in external osmolarity results in a fast water influx that can burst the cell. To survive, cells rely on the passive response that has been described as a pressure release valve type of mechanism. Specifically, mechanosensitive channels present in cells open under increased membrane tension and allow the release of cytoplasmic solutes and water.

The gating and the molecular structure of mechanosensitive channels found in *Escherichia coli* have been extensively studied, and recently we have started to understand better the overall dynamics of the whole cellular response. For example, we characterized *E. coli's* passive response to a sudden hypoosmotic shock (downshock) on a single-cell level[1]. We demonstrated that initial fast volume expansion is followed by a slow volume recovery that can end below the initial value[1]. We proposed a theoretical model to explain our observations by simulating mechanosensitive channels opening, and subsequent solute efflux and water flux[1]. The model illustrates how solute efflux, driven by mechanical pressure and solute chemical potential, competes with water influx to reduce cellular osmotic pressure and allow volume recovery.

More recently, we demonstrated that although wild-type cells adapted to osmotic downshocks and resumed growing, a mutant strain that has all seven *E. coli's* mechanosensitive channels deleted, expands but failed to recover[2]. However, the strain did not lyse for hours after expansion. By monitoring the volume dynamics during this long period under high pressure we show that whether or not (and when) the cells burst depends on the cell wall viscoelastic properties, as well as the activity of wall synthesizing enzymes[2]. Understanding this active, self-recovery process can inform the development of materials that better sustain high-stress conditions.

References

[1] Buda R*, Liu Y*, Yang J*, Hegde S*, Stevenson K, Bai F** and Pilizota T**. Dynamics of Escherichia coli's passive response to sudden decreases in external osmolarity. PNAS September 2016, doi:10.1073/pnas.1522185113

[2] Smitha Hegde, Dario Miroli and Teuta Pilizota. Escherichia coli's life or death at high intracellular pressure. *In preparation*

Deep learning to classify nanobiomechanical data of cells

Adrian Martinez-Rivas[1], Cécile Formosa-Dague[2], Kevin Carrillo[3], Vincent Mijoule[4], Ophélie Thomas-Chemin[5], Marie-Véronique Lelann[5], Childérick Séverac[6], Etienne Dague[5]

[1]*CIC, Instituto Politécnico Nacional (IPN), 07738, Mexico City, Mexico*
[2]*TBI, Université de Toulouse, INSA, INRAE, CNRS, Toulouse, France*
[3] *TBS education, 20 Boulevard Lascrosses, 31068 Toulouse, France*
[4]*Amadeus, Sophia-Antipolis, France*
[5]*LAAS-CNRS, Université de Toulouse, CNRS, Toulouse, France*
[6] *RESTORE-CNRS, Université de Toulouse, CNRS, Toulouse, France*
e-mail: nanobiomex@hotmail.com

We are working on automated methodology based on Atomic Force Microscopy (AFM) with minimal human intervention [1-3]. Artificial intelligence to analyse big data is part of this development, so in this work we present, as a proof of concept, the analysis of quantitative data using deep learning (DL) from *Candida albicans (C. albicans)* cells.
Convolutional neural network (CNN or ConvNet) based DL applied to nanobiomechanical data is largely unexplored, hence we present the use of CNN to classify 4000 force-distance (FD) curves from an imaging dataset, obtained by an AFM on live immobilised eukaryotic *C. albicans* cells. The implementation of DL allowed us to analyse the FD curves based on nanomechanical characteristics such as maximum adhesion force, correlated with the degree of adhesiveness of the *C. albicans* cells. Cell adhesiveness is based on nanodomains formed by mannoproteins such as agglutination-like sequences (Als), which are implicated in skin, nail, oral cavity, and vulvovaginal infections (candidaemia). The architecture of the retained CNN is depicted in Figure 1.
Classification of these nanodomains is achieved with a validation accuracy of 99.80% and 100% confidence without the need for image data augmentation or dropout regularisation.

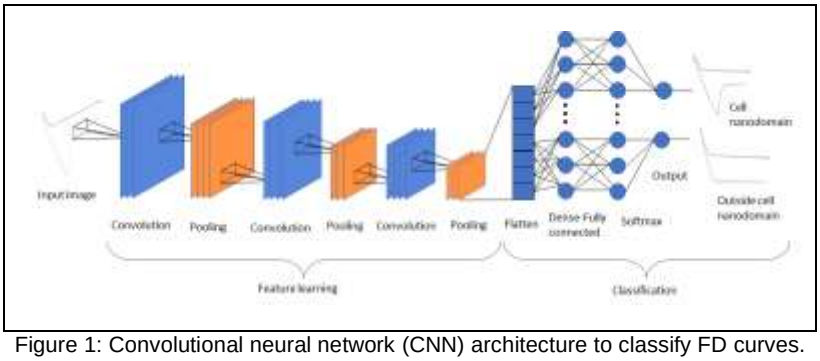


Figure 1: Convolutional neural network (CNN) architecture to classify FD curves.

References

[1] Proa-Coronado, S., Séverac, C., Martinez-Rivas, A. & Dague, E,. Nanoscale Horiz. 5, 131–138 (2020).
[2] Martinez-Rivas. A., Dague, E., Proa, C. S., Séverac, C. & González, Q. G. K. patent number MX2017015624A and PCT.
[3] Séverac, C., Proa-Coronado, S., Formosa-Dague, C., Martinez-Rivas, A. & Dague, E.. JoVE J. Vis. Exp. e61315 (2021).

Generalised prediction of hydration layers on surfaces using deep learning

Yashasvi S. Ranawat,[1] Ygor M. Jaques,[1] and Adam S. Foster[1, 2]

[1]*Aalto University, Finland*
[2]*WPI Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan*
e-mail: yashasvi.ranawat@aalto.fi

Several technological and natural processes are dominated by the mineral-water interactions, for example biominerelisation, corrosion etc, and hence, characterization of such interfaces is vital. Atomic Force Microscopy (AFM) has gained prominence in characterizing such surfaces. However, the complex interplay of the tip with the hydration layers over the surface govern the AFM tip's interaction with the hydration layers, and impede high resolution requirements needed for characterization. We proposed deep learning based approaches[1] to predict, swiftly and robustly, the hydration layers, and demonstrated its application in complementing AFM characterisation for calcite surfaces. Here, we propose a generalised descriptor for surface atoms to predict hydration layers. And, utilising the benefits of transfer learning, demonstrate progressive training on calcite, magnesite, and mica surfaces to build a generalised hydration layer prediction workflow.

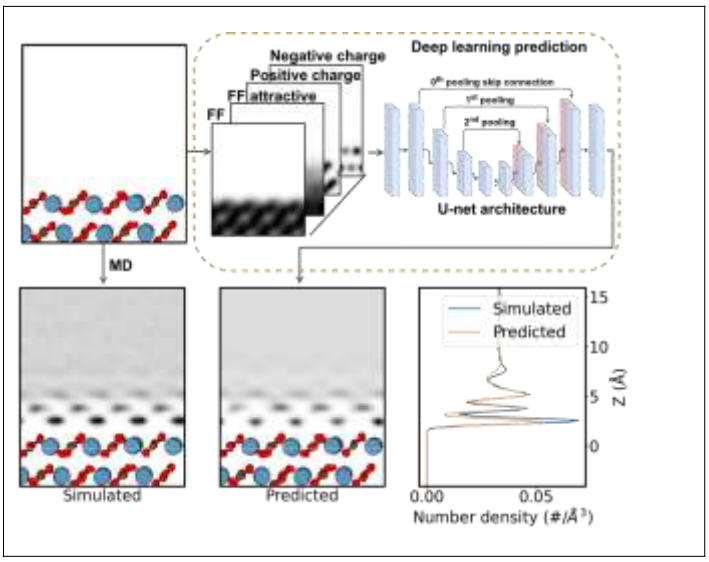


Figure 1: Schematic of the comparison of the molecular dynamics (MD) and the deep learning workflow to simulate/predict hydration layers over a given surface.

References

[1] Ranawat, Yashasvi S., Ygor M. Jaques, and Adam S. Foster. *Nanoscale Advances* 3.12 (2021): 3447-3453.

June 03	
Conference Session V Scanning probe microscopy & force spectroscopy	
08.40 am – 09.10 am	**Georg Fantner**, « Scanning Ion Conductance Microscopy and Spectroscopy » **(Keynote lecture)**
09.10 am – 9.25 am	**Xinyu Zhang**, « Time Resolved 3D force detection of cell adhesion and contractility combining FluidFM and confocal reference-free TFM »
9.25 am – 9.40 am	**William Trewby**, « Direct measurements of nanoscale lipid transport and dynamics »
9.40 am – 9.55 am	**Samuel Leitao**, « Correlative 3D Imaging of Single Cells using Scanning Ion Conductance and Super-Resolution Microscopy »
9.55 am – 10.10 am	**Bastian Hartmann**, « An extended model for the thickness determination of soft layers »
10.10 am – 10.25 am	**Peter Hinterdorfer**, « Two distinct ligand binding sites in monoamine transporters »
10.30 am – 11.00 am	**Coffee Break (30 min)**
11.00 am – 11.30 am	**Núria Gavara**, « Bio-AFM is the new black in biomedical sciences » **(Keynote lecture)**
11.30 am – 11.45 am	**Javier Lopez-Alonso**, « The mechanics of squeezing: automated AFM measurements allows tracking mechanical changes in 2D cell migration »
11.45 am – 12.00 pm	**Ophélie Thomas-Chemin**, « Optimization of AFM measurement conditions for live cell automation »
12.00 pm – 12.20 pm	**Alexander Dulebo**, « High-Speed AFM of Amyloid Fibrils in situ Reveals an “All-or-None” Disaggregation Mechanism »
12.20 am – 12.35 pm	**Andreas Rohtaschek**, « Experimental mechanics of tropocollagen: A molecular fishing trip on the nanoscale »
12.40 pm – 02.00 pm	**Conference Lunch**

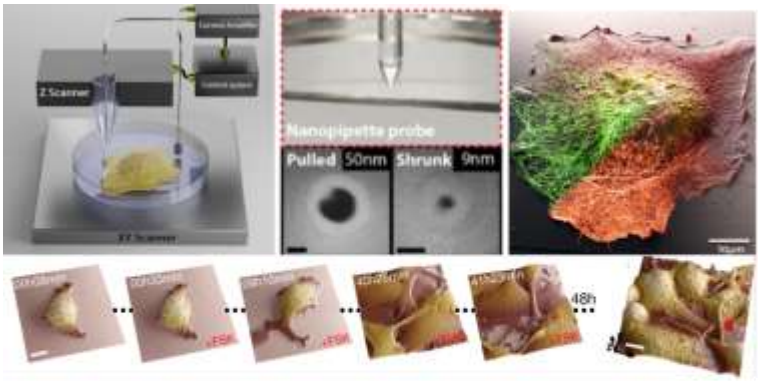
Scanning Ion Conductance Microscopy and Spectroscopy

Georg E. Fantner, Aleksandra Radenovic , Samuel M. Leitao, Vytautas Navikas, Barney Drake

[1] *Institute for Bioengineering, École Polytechnique Fédéral de Lausanne*
EPFL-STI-IBI /Switzerland
e-mail: georg.fantner@epfl.ch

Scanning ion conductance microscopy (SICM) has been around for decades [1], yet it has not received as much attention as other forms of scanning probe microscopy. Recently, this true non-contact technique has kindled renewed interest among biophysicists and biologists because it is ideally suited for label-free imaging of fragile cell surfaces where it achieves exquisite resolution down to the nanometer regime without distorting the cell membrane [2,3]. SICM uses a glass nanopipette as a scanning probe and measures the current through the glass nanopore as a proximity detection of the sample surface. The challenge to harness this technique for time resolved 3D nanocharacterization of living cells lies in the relatively slow imaging speed of SICM. In this presentation I will show how we apply what we have learned from high-speed AFM to the field of SICM. By reengineering the SICM microscope from the ground up, we were able to reduce the image acquisition time for SICM images from tens of minutes down to 0.5s while extending the imaging duration to days [4,5].

SICM, however, is much more versatile than just an imaging tool. I will also discuss our recent results using SICM as a single molecule characterization tool using capillaries with exceptionally small nanopores [6].



References

[1] P. Hansma *et al.*, Science (80-.). **243**, 641 (1989).
[2] P. Novak *et al.*, Nat. Methods **6**, 279 (2009).
[3] Y. E. Korchev *et al.*, Biophys. J. **73**, 653 (1997).
[4] V. Navikas *et al.*, Nat. Commun. **12**, 1 (2021).
[5] S. M. Leitao *et al.*, ACS Nano **15**, 17613 (2021).
[6] L. J. Steinbock *et al.*, ACS Nano **7**, 11255 (2013).

Time Resolved 3D force detection of cell adhesion and contractility combining FluidFM and confocal reference-free TFM

Xinyu Zhang,[1] Nafsika Chala,[2] Dimos Poulikakos[2], Aldo Ferrari[2], Tomaso Zambelli[1]

[1] *1Laboratory of Biosensors and Bioelectronics, Institute for Biomedical Engineering, ETH Zürich, Gloriastrasse 35, 8092 Zürich, Switzerland*
[2] *Laboratory of Thermodynamics in Emerging Technologies, Department of Mechanical and Process Engineering, ETH Zurich, Sonneggstrasse 3, 8092 Zürich, Switzerland*
e-mail: xinyzhang@ethz.ch

Fluid Force Microscopy (FluidFM) combines a hollow microchannel cantilever with conventional AFM and has proven to be a promising tool in biological applications, especially in cell mechanics. [1] FluidFM and Atomic Force Microscopy (AFM) provide abundant force sensitivity in the perpendicular direction but lack information in the x-y lateral direction. Confocal reference-free Traction force microscopy (cTFM) is a powerful tool for the quantification of cellular forces and allows the continuous high-resolution recording of force field without the need of a reference image. [2] By combining these two methods for the first time, a full time-resolved volumetric force detection (4D) is feasible, more specifically contractile force detection by cTFM and z interaction force detection by FluidFM. This opens opportunities in answering biological questions relative to cell mechanics with unprecedented spatial and temporal resolution. How cells interact with their surrounding is an essential topic because the mechanical machinery and cell signaling are fundamental to complex biological processes such as tissue development and crucial to biomaterials design and study of pathology. The generation of cellular forces is mainly based on the actomyosin apparatus and their transmission to the substrates is through integrin-based adhesions. [3] Although FluidFM is proficient in measuring the local elasticity and adhesion strength quantitatively, the broader microscopic traction force information at the basal side, where interaction with the substrate is established, is not available. In this frame, cTFM measuring the traction forces generated by cells provides an additional, all-round perspective. By combining the main features of these two techniques, it is possible to decouple the cell adhesion and contractility during active manipulation of individual cells. To obtain quantitatively the adhesion force of single cells, an individual cell will be pulled from the substrate until detachment. This measurement is commonly referred as Single Cell Force Spectroscopy (SCFS). [4] However, the basal contractile forces during SCFS measurements have not yet been investigated. The simultaneous recording of forces transmitted to the substrate (in and out of plane) could potentially provide more information in how adhesion forces and actomyosin contractility are working together. Furthermore, we are able to indent the cells with the FluidFM probe to investigate the mechanical impedance of single cells. By comparing the impedance difference between control cells and drug treated cells we could infer the role of actin-myosin while applying controlled force.

References

[1] A. Meister et al., “FluidFM: combining atomic force microscopy and nanofluidics in a universal liquid delivery system for single cell applications and beyond.,” Nano Lett., vol. 9, pp. 2501–7, Jun. 2009. [2] A. Martin "Title" in *Book title* (D. Durand, Ed.), Editor, City, 2007, p.10.
[2] M. Bergert et al., “Confocal reference free traction force microscopy,” Nat. Commun. 2016 71, vol. 7, no. 1, pp. 1–10, Sep. 2016.
[3] M. F. Fournier, R. Sauser, D. Ambrosi, J.-J. Meister, and A. B. Verkhovsky, “Force transmission in migrating cells,” J. Cell Biol., vol. 188, no. 2, pp. 287–297, Jan. 2010.
[4] A. V Taubenberger, D. W. Hutmacher, and D. J. Muller, “Single-cell force spectroscopy, an emerging tool to quantify cell adhesion to biomaterials.,” Tissue Eng. Part B. Rev., vol. 20, no. 1, pp. 40–55, Feb. 2014.

Direct measurements of nanoscale lipid transport and dynamics

William Trewby[1] and Kislon Voïtchovsky[1]

[1]*Department of Physics and Astronomy, Durham University, Stockton Road, Durham, DH1 3LE, UK*

e-mail: william.trewby@durham.ac.uk

Lipids are no longer regarded as passive structural components of biological systems, and are now known to drive processes related to protein function, oncogenesis and disease signalling [1,2]. Further, their dynamic role in organelles such as lipid droplets is recognised as being crucial to the proper storage and transport of fats in the body and their dysregulation can lead to severe pathologies including cardiovascular disease [3]. The diverse functions of lipids within the body are largely dependent on their in-plane motion, which governs the ability of biomembranes to restructure, as well as the transport of bound nanostructures and small molecules along and across the lipid layers [4].

Despite the significance of a holistic understanding of lipid dynamics, experimental techniques rarely have access to parameters such as membrane viscosity, friction or diffusive coefficients at a local, nanometer level and over a broad range of timescales. Instead, the reliance on either equilibrium fluctuations or experimentally friendly velocities results in timescales that are typically two orders of magnitude too small for modelling real-life applications.

Here, we develop a novel high-frequency shearing device that can be used in conjunction with atomic force microscopy to provide dynamical information about the in-plane diffusion of molecules and lipids within a membrane. We can access velocities ranging from hundreds of nms^{-1} to mms^{-1}, easily capturing quasistatic (v_{shear} << lipid motion), glassy (v_{shear} > lipid motion) and transitional regimes. Crucially, the technique does not require molecular labels and has contact areas of order ~nm^2, allowing for direct, local energetic measurements on the scale of single proteins. The device allows the impact of membrane and buffer composition to be explored at the nanoscale, and the complex interplay between lipid dynamics, headgroup hydration and experimental timescale to be unpicked. This will have important implications for our understanding of biolubrication, as well as dynamic membrane binding events.

References

[1] S. Beloribi-Djefaflia, S. Vasseur, and F. Guillaumond, Oncogenesis 5 (2016) e189.
[2] N. P. Hessvik and A. Llorente, Cell. Mol. Life Sci. 75 (2018) 193-208
[3] J. A. Olzmann and P. Carvalho, Nat. Rev. Mol. Cell. Biol. 20 (2019) 137-155.
[4] B. Plochberger, C. Röhrl, J. Preiner, ***et al***., Sci. Rep. 7 (2017) 15886.

Correlative 3D Imaging of Single Cells using Scanning Ion Conductance and Super-Resolution Microscopy

Samuel M. Leitao[1], Vytautas Navikas[2], Kristin S. Grussmayer[2], Adrien Descloux[2], Barney Drake[1], Klaus Yserentant[3], Philipp Werther[4], Dirk-Peter Herten[3], Richard Wombacher[4,5], Aleksandra Radenovic[2], and Georg E. Fantner[1]

[1]Laboratory for Bio- and Nano-Instrumentation, Institute of Bioengineering, School of Engineering, Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland.[2]Laboratory of Nanoscale Biology, Institute of Bioengineering, School of Engineering, Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland. [3]College of Medical and Dental Sciences, Medical School & School of Chemistry, University of Birmingham, Birmingham, United Kingdom. [4]Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Heidelberg, Germany. [5]Department of Chemical Biology, Max Planck Institute for Medical Research, Heidelberg, Germany.

samuel.mendesleitao@epfl.ch

The evolution of the 3D morphology is at the center of many relevant biological processes ranging from cellular differentiation to cancer invasion and metastasis. Microscopy techniques, such as super-resolution optical microscopy, electron microscopy, and atomic force microscopy (AFM), have been applied to image the structure of cells in great detail. The major challenge is to obtain 3D information at nanometer resolution without affecting the viability of the cells and avoiding interference with the process. Here, we show a scanning ion conductance microscope (SICM) as an alternative approach for high-speed and long-term nanoscale imaging of mammalian cells [1]. By implementing advances in nanopositioning, nanopore fabrication, microelectronics, and controls engineering we developed a microscopy method that can resolve spatiotemporally diverse 3D processes on the cell membrane at sub-5 nm axial resolution, without probe contact. Additionally, we combine 3D surface data from SICM with volumetric super-resolution optical fluctuation imaging (SOFI), to generate correlative high-resolution information of cell morphology (Figure 1). To demonstrate the capabilities of our method, we employ correlative SICM/SOFI microscopy for visualizing the membrane and cytoskeleton dynamics in live cells with subdiffraction-resolution [2]. We believe that the combination of the multimodal SICM and flexible SOFI approach has the potential to become a routine live-cell imaging modality that will offer new insights into cell-cell interactions, infection, and cancer research.

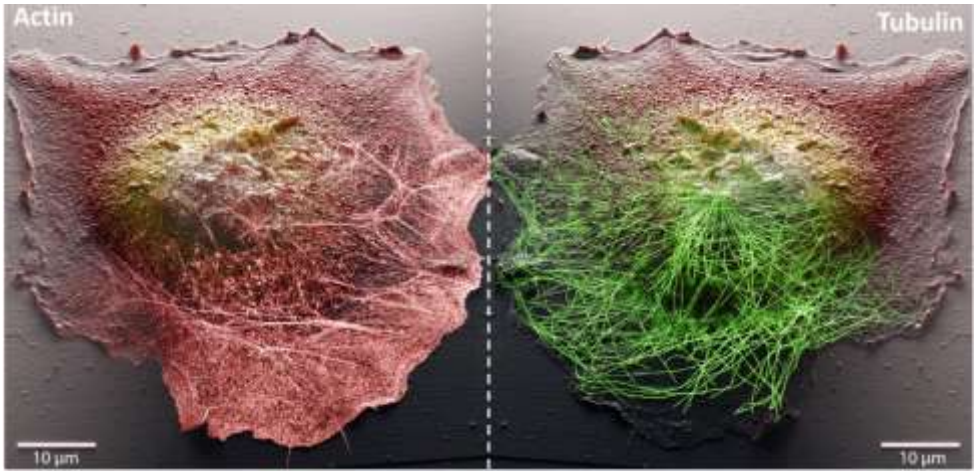


Figure 1: Single-cell correlative SICM/SOFI shows actin distributed within the boundary of the cell (Red) while tubulin displays the canonical cytoskeletal structure (Green).

References

[1] Samuel M. Leitao *et al.* Time-resolved scanning ion conductance microscopy for three-dimensional tracking of nanoscale cell surface dynamics. *ACS Nano* (2021), 15, 11, 17613–17622.
[2] Vytautas Navikas*, Samuel M. Leitao* *et al.* Correlative 3D imaging of single cells using super-resolution and scanning ion-conductance microscopy. *Nature Communications* 12, 4565 (2021).

An extended model for the thickness determination of soft layers

B. Hartmann,[1,2] P. Schmid,[1] A. Aszodi,[3] and H. Clausen-Schaumann[1,2]

[1]*Munich University of Applied Sciences, 80335 Munich, Germany*
[2]*Center for NanoScience (CeNS), LMU, 80539 Munich, Germany*
[3] *Department of Experimental Surgery and Regenerative Medicine, LMU Hospital, 82152 Planegg, Germany*
e-mail: bastian.hartmann@hm.edu

Over the last years, indentation-type atomic force microscopy (IT-AFM) has become an important tool to assess the nano-mechanical properties of a broad variety of samples, from polymers to single cells and the extracellular matrix of different types of tissue. [1-3] To determine the sample's Young's modulus, a variant of the Hertz model depending on the used indenter geometry [4-7] is usually fitted on the force-indentation curve acquired by IT-AFM. The Hertz model gives the relation between the indentation depth and the applied force on the sample by assuming the indented material as a linear elastic infinite half-space, where the Young's modulus does not change in depth.
This model is also used for the indentation of biological samples like tissues (e.g. cartilage), for indentation depths up to a few microns where the material can be assumed to be linearly elastic and other properties like viscoelasticity play a negligible role. Unfortunately, often the Young's modulus is not constant along with the indentation depth in biological samples. Many samples can be approximated as a layered system (cf. Figure 1A) where the top layer is softer than the underlying substrate (e.g. mildly degenerated cartilage). Force-indentation curves of such layered systems show a deviation from the idealized quadratic increase of the force at a certain indentation depth (cf. Figure 1B). This deviation, caused by the influence of the harder substrate in a layered system, cannot be fitted using a single Hertz model [8].
Therefore, we developed an empirical model based on the linear combination of two Hertz models that can be fitted on the full depth range of such force-indentation curves. We evaluated the output of our model numerically by FEM simulations and experimentally by IT-AFM measurements on well-defined layer systems made of polydimethylsiloxane (PDMS, Sylgard 184) using spin coating. With our evaluation, we could find a clear correlation between the fit parameter of our model and the top layer thickness. Thus, our extended model provides an easy way of determining the top layer thickness of synthetic or biological samples.

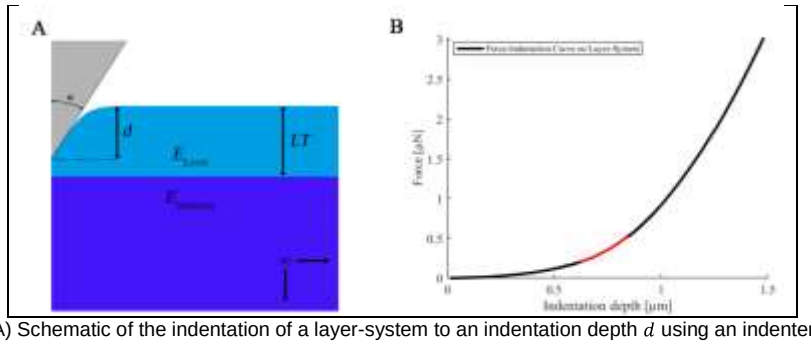


Figure 1: (A) Schematic of the indentation of a layer-system to an indentation depth d using an indenter geometry with a half opening-angle α. The layer has a thickness LT and a Young's modulus of E_{Layer}.The substrate is an infinite bulk material with a Young's modulus $E_{Substrate}$ higher than E_{Layer}. (B) Typical force-indentation curve recorded on a layered system with the indentation depth where the curve deviates from a quadratic increase highlighted in red.

References

[1] D. Docheva, et al., Biochem. Biophys. Res. Commun. 402 (2010) 361-366.
[2] C. Prein, et al., Mat. Biol. 50 (2016) 1-15.
[3] R. Reuten, et al., Nat. Mat. 20 (2021) 892-903.
[4] H. Hertz, J. für Reine Angew. Math. 92 (1881) 156-171.
[5] I.N. Sneddon, Int. J. Engng Sci. 3 (1965) 47-57.
[6] G.G. Bilodeau, J. Appl. Mech. 59 (1992) 519-523
[7] F. Rico, et al., Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 86 (2004) 1777-1793
[8] A. Erben, et al., Adv. Healthcare Mater. 24 (2019) 2000918

Two distinct ligand binding sites in monoamine transporters

R. Zhu,[1] J. Gobl,[1] M. Holy,[2] O. Kudlacek,[2] W. Sandtner,[2] T. Stockner,[2] H. J. Gruber,[1] M. Freissmuth,[2] A. H. Newman,[3] H. H. Sitte,[2] and P. Hinterdorfer[1]

[1] Institute of Biophysics, Johannes Kepler University Linz, Gruberstr. 40, 4020 Linz, Austria
[2] Center of Physiology and Pharmacology, Medical University of Vienna, Waehringerstr. 13a, 1090 Vienna, Austria
[3] Medicinal Chemistry Section, Molecular Targets and Medications Discovery Branch, National Institute on Drug Abuse – Intramural Research Program, 333 Cassell Drive, Baltimore, MD 21224, USA

e-mail: peter.hinterdorfer@jku.at

Controversy regarding the number and function of ligand binding sites in neurotransmitter-sodium symporters arose from conflicting data in crystal structures and molecular pharmacology. We designed novel molecular tools for atomic force microscopy that directly measure the interaction forces between the serotonin transporter (SERT) and the S- and R-enantiomers of citalopram on the single molecule level. Our approach is based on force spectroscopy, which allows for the extraction of dynamic information under physiological conditions inaccessible via X-ray crystallography. Two distinct populations of characteristic binding strengths of citalopram to SERT were revealed in Na^+-containing buffer. In contrast, in Li^+-containing buffer, SERT showed only low force interactions. Conversely, the vestibular mutant SERT-G402H merely displayed the high force population. These observations provide physical evidence for the existence of two binding sites in SERT, i.e. a central (S1) site and a vestibular (S2) site, when accessed in a physiological context. Competition experiments revealed that these two sites are allosterically coupled and exert reciprocal modulation. Interaction forces between the cocaine analogue, MFZ2-12, and the dopamine transporter (DAT) revealed that two populations of binding strength were pronounced in the presence of Zn^{2+} (10 µM), accompanied by an elevated binding activity. These findings are in accordance with a Zn^{2+} induced outward facing conformational change. Absence of Na^+, Zn^{2+}, or mutation at S422A dramatically reduced the population of strong interaction forces, indicating that it originated from the central S1 binding site. Our nanopharmacological approach paves a new avenue to explore transient binding sites in clinically relevant membrane transporters and opens the door to quantitatively address the modulation of interaction forces between ligands and allosterically coupled binding sites.

Acknowledgements. Supported by Austrian Science Fund Grants F35 and P31599-B27, and the National Institute on Drug Abuse-Intramural Research Program, NIH.

Bio-AFM is the new black in biomedical sciences

Núria Gavara[1]

[1]*Serra Húnter programme, Unitat de Biofísica i Bioenginyeria, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona*

e-mail: ngavara@ub.edu

In the last years it has been acknowledged that cellular fate and behaviour are greatly influenced by the mechanical properties of the cells, as well as their environment. The mechanical characterisation of cells and tissues using AFM is no longer a niche technique by those working on biophysical sciences. Instead, it is slowly becoming another method within the toolkit of techniques typically boasted in high-impact papers on basic cell biology or biomedical sciences. While this constitutes a fantastic opportunity for those long working on the field, it has also posed exciting challenges, which i will convey during the talk. From an instrumental side, a massive push has been aimed at building AFMs in combination with advanced light microscopy and super-resolution setups, and to increase the overall user-friendliness of AFM operation. From an experimental and modelling side, new protocols acknowledge the complexity of cellular mechanics, moving beyond the simplest hertzian models at quasi-static regimes. In addition, AFM is morphing into a high-throughput method, thus stressing the need for fully-automated but reliable pipelines to extract mechanical parameters from the thousands of acquired force-distance curves. Finally, the advent of big data and machine learning approaches for disease diagnostic opens new avenues, but raises questions on how we can turn AFM into a more 'multiplex' technique to further its impact in life sciences and biomedicine research.

The mechanics of squeezing: automated AFM measurements allows tracking mechanical changes in 2D cell migration

J. Lopez-Alonso[1], V. Dupres[1], S. Janel[1], L. Redondo-Morata[1], N. Gavara[2,3], F. Lafont[1]

[1] *Université de Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019-UMR9017-CIIL-Centre d'Infection et d'Immunité de Lille, F-59000 Lille, France*
[2] *Unitat de Biofísica i Bioenginyeria, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona,08036 Barcelona, Spain;*
[3] *The Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), 08028 Barcelona, Spain*
e-mail: javier.lopez-alonso@ibl.cnrs.fr

The ability of cells to migrate is critical in both physiological and pathological processes, such as immunity, injury repair, and cancer metastasis. Understanding the core mechanisms that control cell migration from a fundamental biophysical level has the potential of uncovering additional therapeutic targets in disease.

Despite the limitation of 2D systems to study cell migration, they have been extensively applied to uncover fundamental principles of this process [1, 2]. In this context, atomic force microscopy (AFM) reveals itself as a fine technique to study the elastic and viscous characteristics of single cells and their subcellular compartments [3]. Different approaches have been used to study the mechanical properties of motile cells in different stages of their movement by means of AFM [4-6]. However, these studies focused on studying specific areas of the cell over-time, primarily the lamellipodia.

Here, we developed a platform based on automated AFM measurements coupled to optical microscopy (OM) to track mechanical changes over time of single cells migrating in 2D substrates (**Figure 1**). In our experiments, OM images are continuously acquired to estimate the centroid of single cells. This centroid position is used to establish the location in the corresponding AFM measurement. We automatically acquire a force-volume for each cell and time-point, this is an array of force-distance curves which generates spatially resolved mechanical maps of cells. Ultimately, we can correlate cell migration -speed and direction- with the ability of the cell to redistribute its local mechanical properties.

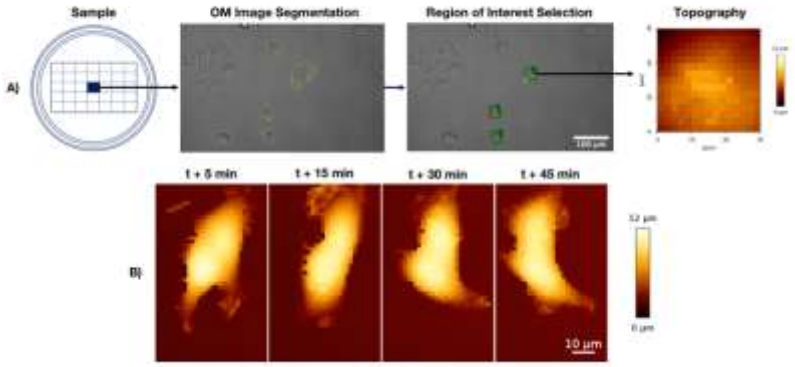


Figure 1. A) AFM automation implementation. Using OM, we automatically segment the image and select the Region of Interest, HeLa cells in this case, where the AFM map will be acquired automatically. acquisition on HeLa cells. B) Time series acquired on GR6 cells. Measurements were acquired for 45 minutes every 5 minutes.

References

[1] H. I.-C. Harn *et al.*, *Exp. Dermatol.*, vol. 24, no. 8, pp. 579–584, Aug. 2015
[2] C. M. Kraning-Rush, S. P. Carey, J. P. Califano, and C. A. Reinhart-King, "Chapter 6 - Quantifying Traction Stresses in Adherent Cells," in *Methods in Cell Biology*, vol. 110, A. R. Asthagiri and A. P. Arkin, Eds. Academic Press, 2012, pp. 139–178.
[3] S. Janel *et al.*, *Nanoscale*, vol. 11, no. 21, pp. 10320–10328, May 2019
[4] C. Rotsch, K. Jacobson, and M. Radmacher, *Proc. Natl. Acad. Sci.*, vol. 96, no. 3, pp. 921–926, Feb. 1999
[5] V. M. Laurent *et al.*, *Biophys. J.*, vol. 89, no. 1, pp. 667–675, Jul. 2005
[6] M. Prass, K. Jacobson, A. Mogilner, and M. Radmacher, *J. Cell Biol.*, vol. 174, no. 6, pp. 767–772, Sep. 2006

Optimization of AFM measurement conditions for live cell automation

O. Thomas- -Chemin, [1] C. Severac, [2] E. Trevisiol, [3,4] E. Dague [1,4]

[1] LAAS-CNRS, Université de Toulouse, CNRS, Toulouse, France
[2] RESTORE Research Center, Université de Toulouse, INSERM, CNRS, EFS, ENVT, UPS, Toulouse, France
[3] TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France
[4] FR FERMAT, Université de Toulouse, CNRS, INPT, INSA, UPS, Toulouse, France
e-mail : othomasche@laas.fr ; edague@laas.fr

The paradigm in bio-AFM is to measure a few dozen of cells and to draw fundamental biophysical conclusions. To apply these results in biology or medicine, it is essential to increase the statistics by measuring of significant number of cells and to automate the measurements. In this context, it is necessary to optimize the measurements, to reduce the results dispersity and to increase the experiment speed. Concerning the dispersity, we hypothesize that cells organized on patterns would have reduced Young's modulus dispersion. We had the same hypothesis in regard to the indenter shape (cone vs. colloidal probe).

Figure 1 shows that randomly shaped cells (A) present Young's moduli more dispersed than cells that have been forced into a square shape (B). The use of patterns therefore makes it possible to increase the homogeneity of the obtained results. We tested the effect of an indenter which probes a larger surface with a 5µm-diameter colloidal probe. The Young's modulus is smaller with the colloidal probe than with the pyramidal probe, but the dispersion is also smaller. There is therefore an advantage to use a colloidal probe in the automation process. To increase the measurement speed, we worked on two parameters, the tip velocity and the number of indentations per cell. We therefore evaluated the impact of the indentation velocity on the resulting mechanical properties. Young's modulus is higher with increasing velocity. But this trend is comparable for cells constrained by square patterns of fibronectin and those not constrained. A high indentation velocity can therefore be applied during automation. Finally, we compared the influence of the number of indentations (1,024; 256; 64 and 16) per cell. There is no significant difference in Young's modulus value between each condition. Automation can therefore be done with a reduced number of indentations per cell.

To conclude, this optimization work opens the door to the implementation of automatic measurements on a large number of cells, which will allow us to probe a large number of cells in a controlled time [1; 2]. This will open the bio-AFM to statistical analyses on populations of cells of interest.

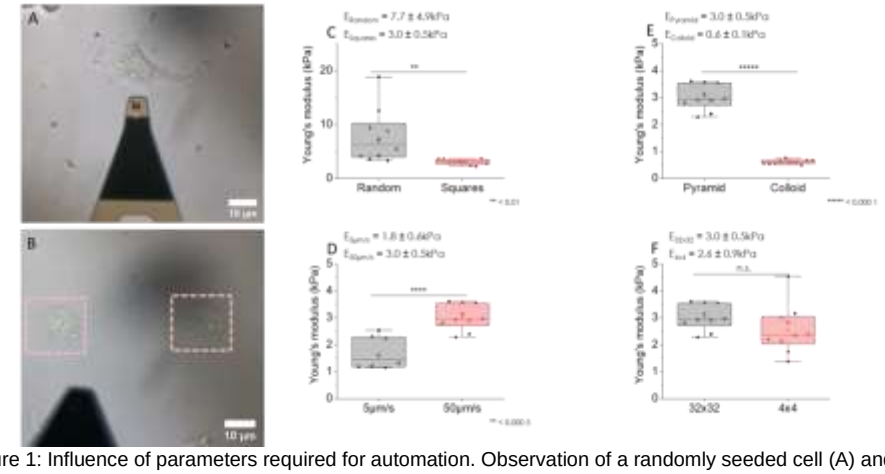


Figure 1: Influence of parameters required for automation. Observation of a randomly seeded cell (A) and a cell having the shape of a square pattern (B). Impact of cell shape (C), velocity (D), indenter geometry (E) and number of measurements per cell (F) on the elastic modulus measured on PC3 cell populations.

References

[1] C. Severac, S. Proa-Coronado, C. Formosa-Dague, A. Martinez-Rivas, E. Dague, E. J. Vis. Exp. 170 (2021)
[2] S. Proa-Coronado, C. Severac, A. Martinez-Rivas, E. Dague, Nanoscale Horizons 5 (2020)

High-Speed AFM of Amyloid Fibrils in situ Reveals an "All-or-None" Disaggregation Mechanism

Alexander Dulebo,[1] Aitor Franco,[2] Adai Colom,[2] Arturo Muga,[2] and Heiko Haschke[1]

[1]*JPK BioAFM, Bruker Nano Surfaces, Am Studio 2D, 12489 Berlin, Germany*
[2]*Biofisika Institute (CSIC, UPV/EHU), 48940 Leioa, Spain*
e-mail: alexander.dulebo@bruker.com

Atomic force microscopy (AFM) is a powerful tool, which allows the comprehensive study of mechanical properties and interactions with nanometer resolution. Recent AFM developments have led to unprecedented imaging rates in fluid, setting new milestones in high-speed scanning capabilities, and enabling the visualization of dynamic biological processes taking place on the sub-20-milisecond scale.

We have used high-speed AFM to study the amyloid fibril destabilization and disassembly in situ. Formation of amyloid fibrils is characteristic for multiple neurodegenerative pathologies, e.g. Alzheimer's and Parkinson's disease [1]. Whereas the human Hsc70-based disaggregase system has been shown to disassemble mature α-synuclein amyloid fibrils, the underlying molecular mechanism has so far remained elusive.

We show that amyloid disassembly is initiated by the destabilization of the fibril ends, followed by fast propagation of protofilament unzipping, and depolymerization along the fibril axis without accumulation of harmful oligomeric intermediates [2]. The biochemical and kinetic characterization of the process suggests that the used chaperone system preferentially clears neurotoxic oligomers and short fibrils, while its activity against large amyloids is substantially hindered. The suggested "all-or-none" disaggregation of individual aggregates mechanism provides a better molecular insight of the chaperone-mediated selective processing of toxic amyloid species and is a crucial milestone in the identification of potential therapeutic targets for treatment of amyloid-related neurodegenerative diseases.

References

[1] I. C. Brás, M. Xylaki, T. F. Outeiro, "Mechanisms of Alpha-Synuclein Toxicity: An Update and Outlook" in *Progress in Brain Research* (Anders Björklund, M. Angela Cenci), Elsevier, 2020, p. 91-129.

[2] A. Franco, P. Gracia, A. Colom, J. D. Camino, J. Á. Fernández-Higuero, N. Orozco, A. Dulebo, L. Saiz, N. Cremades, J. M. G. Vilar, A. Prado, A. Muga, Proc. Natl. Acad. Sci. 118 (2021) e2105548118.

Experimental mechanics of tropocollagen: A molecular fishing trip on the nanoscale

Andreas Rohtaschek[1,*], Patrick Steinbauer[2], Stefan Baudis[2] and Philipp J. Thurner[1]
[1] *Institute of Lightweight Design and Structural Biomechanics (ILSB), TU Wien, 1060 Wien, Austria*
[2] *Christian Doppler Laboratory for Advanced Polymers for Biomaterials and 3D Printing, Institute of Applied Synthetic Chemistry (IAS), TU Wien, 1060 Wien, Austria*
**Recipient of a DOC Fellowship of the Austrian Academy of Sciences at the ILSB*
e-mail: andreas.rohatschek@tuwien.ac.at

Collagens are the most abundant and structurally the most important proteins of the human extracellular matrix. Therefore, mechanical properties of collagen molecules (tropocollagen), and the progressively larger structures they form, are crucial for tissue mechanics and function. While there are a number of studies modeling the mechanical behavior of tropocollagen molecules via molecular dynamics (MD) approaches there is little but none experimental data available. Due to MD limitations experimental validation of predicted behavior such as molecular uncoiling is needed and will also provide further insight into collagen mechanics.
Here, we present an approach to experimentally characterize adhesion forces between tropocollagen molecules and mica.

Maleimide based surface functionalization [1] is used to tether individual collagen type III molecules onto an AFM tip using a N-hydroxysuccinimide (NHS), polyethylene glycol (PEG), maleimide (MI) linker system. Atomic Force Microscopy (AFM) Single Molecule Force Spectroscopy (SMFS) measurements were conducted in water and acetic acid on mica at different approach and retraction speeds (0.1, 0.25, 0.5, 1, 1.5, 2 nm/s) and at different holding times (0, 1, 2, 4s) to investigate Tropocollagen-substrate interactions.

All detected force-distance curves showed similar behavior. In a typical force curve (see Figure 1), unspecific adhesion interactions are witnessed within the first 30 nm from the substrate surface due to tip-substrate interaction. Following this unspecific region, tropocollagen-related interactions are observed with pulling lengths longer than 2 µm and pull-off forces of up to 1.4 nN in acetic acid (0.5 mol/L). More than 38000 force curves were used for the whole analysis.

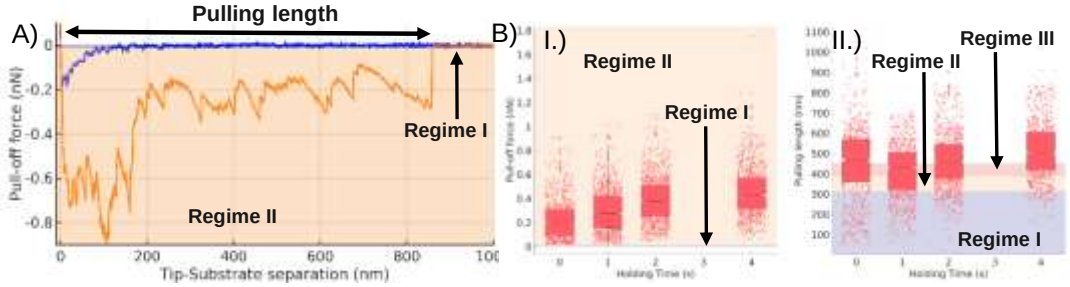


Figure 1: A) Example force-distance curve. B) Analysis of 3346 force curves measured in acetic acid, at 0.25 µm/s approach and retraction velocities and four different holding times (0s, 1s, 2s, 4s). Different force regimes [2] are indicated by background colors. (I.) Pull-off force, (II.) Pulling length. Different force regimes [2] are indicated by background colors: Uncurling of entangled configuration (**Regime I**), Entropic uncoiling of the collagen Polyproline-II helix (**Regime II**), Backbone stretching (**Regime III**)

We report first experimental measurements on collagen molecules reaching deformation-relevant forces. Further, determined median pull-off forces for longer holding times are confirmed by MD simulations of shear-dominant stresses applied which reported a peak force of 621 pN with 12.7% strain [4]. Measured median pulling lengths may indicate even higher strains suggesting even more severe modification, i.e. pulling out of one of the three peptide chains forming the collagen triple helix.

References

1. Kamruzzahan, A *et al, Biocon. Chem.* **17**,1473–81, 2006.
2. Buehler, M. J. et al, *Biophys. J.* **93**, 37–43, 2007.
3. Lee, H. et al, *Proc. Nat. Acad. Sci.* **103**, 12999–13003 2006

Acknowledgements

We thank the Christian Doppler Research Association, Austrian Federal Ministry for Digital & Economic Affairs, National foundation for Research, Technology & Development and the Austrian Academy for Science (ÖAW) for financial support (DOC 25152).

Conference Session VI Latest advances in materials application & life sciences		
02.00 pm – 02.30 pm	**Nicholas Kotov**, « Chirality and Complexity of Engineered Nanostructures » **(Keynote lecture)**	
02.30 pm – 02.45 pm	**Davide Prosperi**, « H-Ferritin: a theranostic nanocarrier for advanced therapy and imaging of breast cancer »	
02.45 pm – 03.00 pm	**Cédric Vranckx**, « Architecture of LL-37-Heparin Layer-by-Layer Antimicrobial Coatings »	
03.00 pm – 03.15 pm	**Laura Martinez-Vidal**, « Micromechanics of bladder cancer and fibrosis »	
03.15 pm – 03.30 pm	**Luisa Fiandra**, « Bioluminescence tracking of nanodrug delivery in tumors by engineered ferritin nanoparticles »	
03.30 pm – 03.55 pm	**Michel Gringas**, « From nano to quantum in bio: the search for quantum effects in neuroscience » **(Invited)**	
04.00 pm – 04.30 pm	**Coffee Break**	
04.30 pm – 06.30 pm	**Poster Session II – Networking Time**	**International Cooperation workshop**

Chirality and Complexity of Engineered Nanostructures

Nicholas A. KOTOV

Department of Chemical Engineering, University of Michigan, Ann Arbor, USA)
e-mail: kotov@umich.edu

The structural and functional complexity of biomimetic materials arises from the spontaneous hierarchical ordering of inorganic building blocks over multiple scales. Empirical observations of complex nanoassemblies are abundant, but physicochemical mechanisms leading to their geometrical complexity remain puzzling, especially for non-uniformly sized components. These mechanisms are discussed in this talk taking an example of hierarchically organized particles with twisted spikes and other morphologies from polydisperse Au-Cys nanoplatelets [1]. The complexity of these supraparticles is higher than biological counterparts or other complex particles as enumerated by graph theory (GT). Complexity Index (*CI*) and other GT parameters are applied to a variety of different nanoscale materials to assess their structural organization. As the result of this analysis, we determined that intricate organization Au-Cys supraparticles emerges from competing chirality-dependent assembly restrictions that render assembly pathways primarily dependent on nanoparticle symmetry rather than size. These findings open a pathway to a large family of colloids with complex architectures and unusual chiroptical and chemical properties. The design principles elaborated for nanoplatelets have been extended to engineering of other complex nanoassemblies. They include polarization-based drug discovery platforms for Alzheimer syndrome,[3] materials for chiral photonics,[5] biomimetic composites for energy and robotics [2,4], CO_2-dispersable catalysis [6] and chiral antiviral vaccines.[7] Yet, the work on the generalization of the engineering principles for chiral biomimetic nanostructures is incomplete; further directions of these efforts will be discussed.

References

[1] W. Jiang, Z.-B. et al, Emergence of Complexity in Hierarchically Organized Chiral Particles, *Science*, **2020**, 368, 6491, 642-648.
[2] Wang, M.; Vecchio, D.; et al Biomorphic Structural Batteries for Robotics. *Sci. Robot.* **2020,** 5 (45), eaba1912.
[3] Jun Lu, et al, Enhanced optical asymmetry in supramolecular chiroplasmonic assemblies with long-range order, *Science*, **2021**, 371, 6536, 1368.
[4] D. Vecchio et al, Structural Analysis of Nanoscale Network Materials Using Graph Theory, *ACS Nano* **2021**, 15, 8, 12847–12859.
[5] L. Ohnoutek, et al, Third Harmonic Mie Scattering From Semiconductor Nanohelices, *Nature Photonics*, **2022**, 16, 126–133.
[6] L. Tang et al. Self-Assembly Mechanism of Complex Corrugated Particles" *JACS,* **2021** 143, 47, 19655–19667.
[7] L. Xu, et al, Enantiomer-Dependent Immunological Response to Chiral Nanoparticles**,** *Nature,* **2022**, 601, 366–373.
[8] Choi W. et al, Chiral Phonons in Microcrystals and Nanofibrils of Biomolecules, *Nature Photonics*, **2022** 16, 366–373.

H-Ferritin: a theranostic nanocarrier for advanced therapy and imaging of breast cancer

Davide Prosperi, Linda Barbieri and Maria Antonietta Rizzuto

NanoBioLab, Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milano, Italy
e-mail: davide.prosperi@unimib.it

The standard clinical protocols for cancer chemotherapy are based on the concept of "maximum tolerated dose" (MTD). However, prolonged time intervals between treatment cycles are required to allow for normal tissue recovery, during which neoangiogenesis and development of therapeutic resistance often occur. Recent discoveries in tumor biology suggest alternative strategies toward tumor eradication using targeted approaches that disengage the neoplastic microenvironment. This latter new concept, referred to as "metronomic" chemotherapy, redefines the therapeutic regimen to aim for prolonged responses rather than short-term tumor regression. In contrast to MTD regimens, low-dose metronomic (LDM) chemotherapy is characterized by the administration of a cytotoxic agent at lower, less toxic dose given at regular, more frequent time intervals. However, the clinical impact of LDM chemotherapy is still under debate. The main disadvantages are low drug accumulation at the tumor and thus poor effectiveness against advanced-stage metastatic tumors. With the aim to improve the potential of metronomic paradigm, we investigated the use of H-ferritin (HFn) nanocages [1,2] in delivering continuative low doses of cytotoxic doxorubicin (DOX) to a highly invasive breast cancer model [3]. Mice bearing 4T1 cells were treated with placebo, free DOX or HFn-DOX for 21 days at about 1/3 of the standard clinical dosage. Our results demonstrate that LDM treatment with HFn-DOX is able to strongly affect the progression of the tumor and to alter the neovascularization process. Moreover, HFn-DOX allows overcoming cardiotoxicity, one of the most severe side effects of DOX chemotherapy. In addition, the same HFn nanocage was demonstrated to stably incorporate Mn(II) ions providing targeted imaging contrast enhancers for magnetic resonance imaging (MRI). Taking advantage of combined HFn capacity as drug delivery system and as contrast agent for MRI suggest its potential development as a future theranostic agent for the next generation cancer detection and treatment.

References

[1] K. Fan, C. Cao, Y. Pan, D. Lu, D. Yang, J. Feng, L. Song, M. Liang, X. Yan, Nat. Nanotechnol. 7 (2012) 459-464.
[2] M. Bellini, S. Mazzucchelli, E. Galbiati, S. Sommaruga, L. Fiandra, M. Truffi, M. A. Rizzuto, M. Colombo, P. Tortora, F. Corsi, D. Prosperi, J. Controlled Release 196 (2014) 184-196.
[3] S. Mazzucchelli, M. Bellini, L. Fiandra, M. Truffi, M. A. Rizzuto, L. Sorrentino, E. Longhi, M. Nebuloni, D. Prosperi, F. Corsi, Oncotarget 8 (2017), 8383-8396.

Architecture of LL-37–Heparin Layer-by-Layer Antimicrobial Coatings

C. Vranckx,[1] O. Cornu,[2] and C. Dupont-Gillain[1]

[1]Institute of Condensed Matter and Nanosciences, Bio- and Soft Matter, Université catholique de Louvain, Place Louis Pasteur, 1 bte L4.01.10, B-1348 Louvain-la-Neuve
[2]Neuro-Musculo-Skeletal Pole, Experimental and Clinical Research Institute, Université catholique de Louvain, 1200 Brussels, Belgium
e-mail: cedric.vranckx@uclouvain.be

The last decades have seen the number of antimicrobial-resistant bacteria rising, and a lack in the development of new therapeutic options to deal with it, which could have catastrophic consequences. The World Health Organization has declared antimicrobial resistance as one of the biggest threats to humanity. Moreover, nosocomial diseases are linked, in more than 50% of cases, to the presence of a medical device (catheters, implants, etc.). To address this issue, more and more studies are being carried out to improve the biomaterial surface properties in order to fabricate antimicrobial coatings.

Among these strategies, the use of antimicrobial peptides (AMPs) such as LL-37 seems an interesting avenue for study. These peptides are broad spectrum antimicrobial compounds which demonstrate potential as novel therapeutic agents. In order to achieve a bactericidal effect on Gram-positive and negative bacteria, the minimal inhibitory concentration (CMI) values were reported from 1 to 10 µM of LL-37. However, controlling peptide immobilization and their subsequent release or appropriate exposure is largely recognized as challenging due to their polyampholyte nature and anisotropic charge distribution. In order to create nanostructured antibacterial coatings, layer-by-layer (LbL) assembly method can be used, with the benefit that the peptide is not submitted to covalent binding to the substrate, but with limitations linked to the polyampholyte nature and anisotropic charge distribution of peptides.

To favor its assembly, LL-37 was complexed with heparin, a polyanion, to obtain a negatively-charged peptide-polyelectrolyte complex (PPC). These PPCs were incorporated into multilayers using chitosan as polycation. As a matter of comparison, bare LL-37 was directly assembled into thin films with heparin, *i.e.,* using a more classical LbL assembly approach. Multilayer growth was monitored using quartz-crystal microbalance (QCM), and LL-37 adsorbed in the multilayer was quantified by bicinchoninic acid assays (BCA). This work aims first at studying the architecture and organization of LL-37-based multilayered films. Second, it also targets the quantification of LL-37, heparin and chitosan to determine whether the amount of LL-37 in the multilayers is sufficient to get antibacterial properties.

QCM results show a better growth of multilayers incorporating PPCs than those with bare LL-37. After five bilayers, the total adsorbed mass is almost three times higher. However, this hydrated mass does not disclose the amount of LL-37 in the film. BCA assays show that, after the adsorption of 25 bilayers, the quantity of LL-37 is much higher when bare LL-37 is assembled rather than PPCs (Figure 1). It seems that the PPCs film architecture is completely different from the one obtained with bare LL-37. The PPCs-based multilayers are more hydrated but contain less LL-37. However, in both cases, the amount of LL-37 in the multilayers is sufficient to reach the CMI.

Very importantly, our approach establishes a way to design antimicrobial coatings with different architectures and organizations. It will therefore be beneficial to modify interfaces with antimicrobial peptides in order to find new strategies against the increasing number of antimicrobial-resistant bacteria.

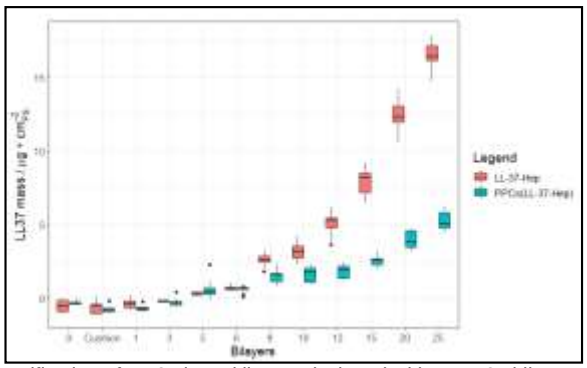


Figure 1: BCA quantification of LL-37 in multilayers designed with up to 25 bilayers of LL-37 or PPCs

Micromechanics of bladder cancer and fibrosis

L. Martinez-Vidal[1,2], M. Chighizola[3], M. Berardi[4,5], E. Alchera[1], I. Locatelli[1], F. Pederzoli[1,2], C. Venegoni[1], R. Lucianò[6], P. Milani[3], A. Podestà[3], K. Bielawski[4], A. Salonia[1,2] and M. Alfano[1]

[1]*Division of Experimental Oncology/Unit of Urology; IRCCS Ospedale San Raffaele, Milan, Italy 20132;*
[2]*Università Vita-Salute San Raffaele, Via Olgettina, 60, Milan, Italy 20132;*
[3]*C.I.Ma.I.Na and Dipartimento di Fisica "Aldo Pontremoli", Università degli Studi di Milano, Milano, Italy 20133;*
[4]*Optics11, Amsterdam, The Netherlands;*
[5]*LaserLab, Department of Physics and Astronomy, VU University, Amsterdam, The Netherlands.*
[6]*Pathology Unit, IRCCS Ospedale San Raffaele, Milan, Italy 20132*

e-mail: martinezvidal.laura@hsr.it

Modification of the extracellular microenvironment represents a hallmark of cancer, as both biochemical and biophysical features of the extracellular matrix (ECM) are modified during tumorigenesis. Among the different ECM characteristics, stiffness plays an important role on several key aspects of tumor growth and invasion (i.e., durotaxis). In this preliminary study, we aimed to establish mechanical fingerprints of the tissue layers of healthy bladder, and those associated to fibrotic bladder and bladder cancer.

For this purpose, rat bladders were isolated from untreated, x-ray irradiated and orthotopic tumor-bearing animals (bladders instilled with AY-27 cancer cell line). Tissue stiffness was measured using Atomic Force Microscopy and the Piuma nanoindenter (Optics11, Amsterdam). Micromechanical maps of the bladder wall in health and disease were associated with histological analysis, ultrasound and photoacoustic in vivo imaging.

We found that the healthy bladder wall was a mechanically inhomogeneous tissue. Therefore we investigated the existence of a correlation with the stiffness distribution and the different anatomical bladder layers. We observed a gradient of increasing stiffness from the internal to the outer layers: the urothelium (Young's modulus (YM) around 20 kPa); to the lamina propria (YM 100 kPa); which gradually decreased when reaching the outer layer, the muscle layer (approx. 70 kPa). Using the stiffness map of the healthy bladder as reference, we observed stiffening in fibrotic tissues, characterized by a YM up to 300 kPa that correlated with increased deposition of dense ECM. In the model of orthotopic tumor there was a gradient in the mechanical properties of the tumor-bearing bladder, with the maximum stiffness measured in bladder wall below the tumor (YM 200-300 kPa), which gradually decreased with increased distance from the tumor, reaching YM 20-50 kPa at the maximum distance from the tumor. For both pathological conditions the stiffness gradient from urothelium to muscle was maintained, and appreciated by the two different nanoindentation techniques.

This study highlights the intrinsic mechanical heterogeneity of the different bladder layers, laying out eventual hints for bladder reconstruction purposes. By providing high resolution micromechanical maps, we here reported an alteration of the mechanical properties of bladder tissue in presence of fibrosis and tumor. Such mechanical fingerprints can pave the way for future clinical diagnostic and prognostic tools.

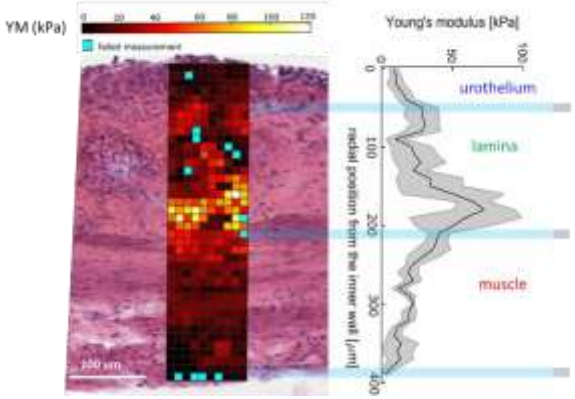


Figure 1: Micromechanical map of the healthy bladder wall paired to haematoxylin-eosin staining. The graph on the right shows the mean and standard deviation for each row of the mechanical map (n=10)

Bioluminescence tracking of nanodrug delivery in tumors by engineered ferritin nanoparticles

L. Fiandra,[1] M. Bellini,[1] B. Riva,[1] V. Tinelli,[1] M. Colombo[1] and D. Prosperi[1,2]

[1]*Dep. of Biotechnology and Biosciences, University of Milano-Bicocca, P.zza della Scienza 2, 20126 Milan, Italy*
[2]*Nanomedicine laboratory, ICS Maugeri S.p.A. SB, via S. Maugeri, 10-27100 Pavia, Italy*
e-mail: luisa.fiandra@unimib.it

Besides the large amount of studies aimed to the development of theranostic nanosystems, several nano-based imaging techniques have been specifically directed to determine the efficiency of nano-drug delivery to the cancerous tissues.[1,2] In this scenario, the identification of a highly sensitive method to check the delivery of administered nanodrugs into the tumor cells represents a crucial step of the preclinical studies aimed to develop new nanoformulated therapies. We have already demonstrated the ability of Ferritin (HFn) nanocages to act as a powerful tool for cancer therapy thanks to its interaction with the Transferrin Receptor 1 (TfR1).[3,4] Nevertheless, so far, no data have been reported about the employment of ferritin nanoparticles for bioluminescence imaging (BLI), which has been proved to be a more advantageous detection approach respect to fluorescence and other imaging technologies.

Here, we show the great efficiency of a bioluminescent HFn-based nanoparticle, where the luciferin probe is conjugated on nanoparticle surface by means of a disulfide containing linker (Luc-linker@HFn). Once Luc-linker@HFn is internalized into luciferase$^+$ 4T1 cells, the reductive environment due to glutathione in the cytoplasm leads to linker disulfide bridge disruption, resulting in a cyclization with consequent release of luciferin (Figure 1A). A rapid onset of BLI into the cells exposed to Luc-linker@HFn, followed by a long-lasting luminous signal, was observed *in vitro* and *in vivo* (Figure 1B). The prolonged imaging of luciferase$^+$ tumors makes Luc-linker@HFn a highly efficient tool for the monitoring of drug delivery to the cancerous tissues. Other main advantages of this nanodevice are: 1) the in-situ generation of a free luciferin molecule, which allows a quantitative BLI production due to luciferase activity; 2) the use of a self-immolative linker able to discriminate between nanoparticles confined in the endo-lysosomal pathway and those escaped from endosomes into the cell cytoplasm; 3) the use of an external reporter, which does not impair the interaction of HFn with TfR1, making this nanoconjugate an ideal system to check the translocation of encapsulated antitumor drugs to their effective cytosolic or nuclear targets.

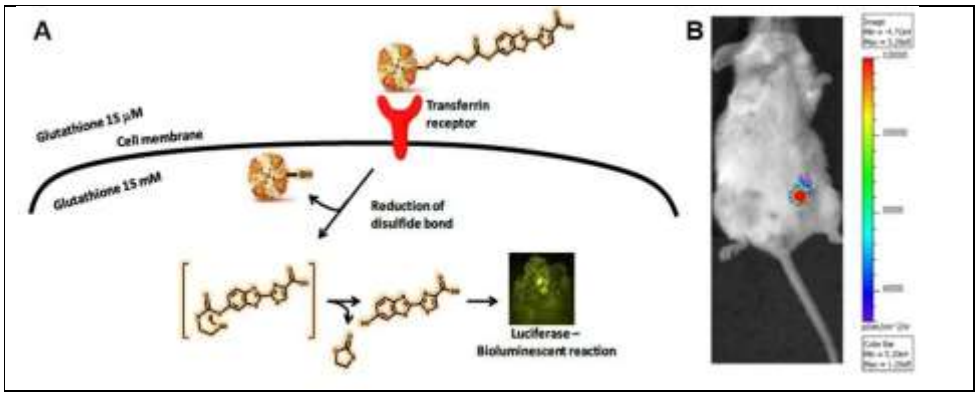


Figure 1: A) Luc-linker@HFn is recognized by transferrin receptor and internalized into luciferase$^+$ cell, where the glutathione reduces the disulfide bridge between HFn and the linker. Released luciferin is now allowed to react with luciferase producing BLI. B) BLI in luciferase$^+$ orthotropic tumor.

References

[1] H. Zhou, W. Qian, F. M. Uckun, et al. ACS Nano 9 (2015) 7976-7991.
[2] T. Tagami, W.D. Foltz, M.J. Ernsting, et al. Biomaterials 32 (2011) 6570-6578.
[3] M. Bellini, S. Mazzucchelli, G. Galbiati, et al. J Control Release 196 (2014) 184-196.
[4] S. Mazzucchelli, M. Bellini, L. Fiandra et al. Oncotarget. 8 (2017) 8383-8396.

From nano to quantum in bio: the search for quantum effects in neuroscience

Michel J.P. Gingras,[1] Tony Fong,[2] John G. Mielke,[2] and Zoya Leonenko[1,3]
[1]*Department of Physics and Astronomy, University of Waterloo, Waterloo, Ontario, N2L-3G1, Canada*
[2]*School of Public Health Sciences, University of Waterloo, Waterloo, Ontario, N2L-3G1, Canada*
[3]*Department of Biology, University of Waterloo, Waterloo, Ontario, N2L-3G1, Canada*
e-mail: gingras@uwaterloo.ca

The past ten years or so have seen the steady growth of a new interdisciplinary field of science, quantum biology. A key appeal of this field is that it brings and interfaces together ideas, concepts, and theoretical and experimental methods from the fields of biology, quantum physics, biochemistry and biophysics to address a number of long-standing puzzling problems and phenomena in biology. Up to recently, the topics of photosynthesis, magneto-reception in avian navigation and olfaction have attracted the largest share of attention and interest from experimentalists and theorists alike. In the past four years, a new context where quantum mechanics may play an unexpected role has been put forward, and is gaining momentum; neuroscience. The overarching question in the field of quantum biology is whether some of the quintessential foundational aspects of quantum mechanics, such as coherence, entanglement, particle-wave duality, indistinguishability postulate (e.g. fermion/boson spin statistics) play an essential role for biological processes above and beyond the constraints that these concepts impose at the molecular bonding and molecular structure length scale. In this talk, I will review the new theoretical ideas that have been put forward in the fledgling field of quantum neuroscience, what experimental results exist that suggest some nontrivial manifestation of quantum mechanical effects may be at play and, finally, discuss our interdisciplinary efforts at the University of Waterloo to explore the existence of novel and heretofore overlooked quantum effects in neuroscience. By the nature of the problems at stake in this area, I will argue that novel experiments at the crossover nanometer length scale, between the molecular and the cellular scale, have a natural role to play to advance this field and secure, or not, its legitimacy.

June 05	
Conference Session VII **Special NanoInBio session**	
08.40 am – 09.10 am	**Hermann Schillers**, « Development of a method to quantify cellular mechano-response on the single cell level » **(Keynote lecture)**
09.10 am – 9.25 am	**Guillaume Berthout**, « Nanomechanical characterization of soft biological materials: case study on cornea and heart tissue »
9.25 am – 9.40 am	**Ronald Zirbs**, « Antimicrobial Polypeptoids: Synthesis & Biological Tests of Sequence-Specific Folded 3D-Structured Antimicrobial Polymers »
9.40 am – 9.55 am	**Halima Alem**, « 3D bio-printing of living systems for food industries and medical applications »
9.55 am – 10.10 am	**Marie Dubus**, « Decellularized Wharton's jelly as an antibacterial and immunomodulatory scaffold for tissue engineering »
10.15 am – 10.45 am	**Coffee Break (30 min)**
10.45 am – 11.15 am	**Laïla Pasquina-Lemonche**, « The peptidoglycan architecture of the Gram-positive bacterial cell wall and its destruction by antibiotics » **(Keynote lecture)**
11.15 am – 11.30 am	**Kislon Voitchovsky**, « Targeted detection of membrane nanoparticles in unprocessed saliva using vibrating microcantilever »
11.30 am – 11.45 pm	**Fabienne Quiles**, « An *in situ* nanoscale investigation of $A\beta_{1-42}$ and model membranes to decipher the early mechanism involved in Alzheimer's disease »
11.45 pm – 12.00 pm	**Marc Ropitaux**, « 4D THz microscopy of biological materials at atomic scale »
12.00 pm – 12.15 pm	**Hélene Martin-Yken**, « Force Spectroscopy Characterization of Neurotoxins Binding »
12.30 pm – 02.00 pm	**Concluding remarks & Conference Lunch**

Development of a method to quantify cellular mechano-response on the single cell level

H.Schillers[1] and D. Ciechańska[1]

[1]*Institute of Physiology II, University Hospital Münster, Germany*
e-mail: Hermann.Schillers@uni-muenster.de

All cells in our body, from blood cells to osteocytes, are constantly exposed to varying mechanical forces. Cells sense these mechanical changes and respond to them by intracellular signalling and generation of force using myosin II motorproteins, a process called mechanoresponse. The actomyosin gel of the cell cortex, located underneath the plasma membrane, plays a crucial role in sensing and in generating mechanical forces. The excellent spatial and temporal resolution, the application of precisely controlled force and the possibility to measure living cells in nearly physiological conditions offered by Atomic Force Microscopy (AFM)[1] enabled us to develop a new approach that quantifies mechanical responses on the single cell level. By operating AFM in force-clamp mode, a low (200pN) and constant force was applied to a living cell and its mechanoresponse was recorded as cell height changes for 300s. The measured signal (height versus time curve) consists of two components of mechanoresponse: cell pushes against (active) and relaxes upon (passive) the force applied by the cantilever. To quantify these mechanics, we established a mathematics-based data processing method that includes the active part of cells mechanoresponse. Three cell lines (EA.hy925, A549 and MDA-MB-231) were studied and the medians of total push heights for these cell lines were found within the range of 319nm to 848nm, whereas the dish bottom, measured as control, were stable at around 131nm. The recordings represent height over time curves at constant force, which allow calculating the work and the power of the mechanoresponse. For the measured cell lines the values of work are between 63.8 and 169.6 ·10-18J and power in the range of 0.22 to 0.59 ·10-18 W. Our approach of quantifying the mechanoresponse of living cells allows investigating the behaviour of cells in a mechanically variable environment.

References

[1] H. Schillers, C. Rianna, J. Schape, T. Luque, H. Doschke, M. Walte, J.J. Uriarte, N. Campillo, G.P.A. Michanetzis, J. Bobrowska, A. Dumitru, E.T. Herruzo, S. Bovio, P. Parot, M. Galluzzi, A. Podesta, L. Puricelli, S. Scheuring, Y. Missirlis, R. Garcia, M. Odorico, J.M. Teulon, F. Lafont, M. Lekka, F. Rico, A. Rigato, J.L. Pellequer, H. Oberleithner, D. Navajas, M. Radmacher; Standardized Nanomechanical Atomic Force Microscopy Procedure (SNAP) for Measuring Soft and Biological Samples. Scientific reports 2017, 7 (1), 5117.

Nanomechanical characterization of soft biological materials: case study on cornea and heart tissue

Guillaume Berthout[1] Jiri Nohava[2] and Jean-Philippe Jehl[3]

[1]*Guillaume Berthout, Anton Paar France S.A.S., Les Ulis, France*
[2]*Anton Paar TriTec SA, Corcelles, Switzerland*
[3]*Institut Jean Lamour, Université de Lorraine, France*
e-mail: guillaume.berthout@anton-paar.com

With the development of new medical techniques and devices, more detailed knowledge of mechanical properties of soft tissues is required. Although some limited research has been done in this area, the information currently available is not sufficient for good understanding of the biomechanical function of these tissues on a local level. Nanoindentation is one of the few tools that can investigate very locally (~hundreds of µm) mechanical properties of soft and hard tissues. This abstract will present application of this method to characterize the effects of crosslinking on human cornea to prevent the keratoconus disease as well as on localized characterization of elastic modulus of heart tissue.

Crosslinking by ultraviolet light is often used for treatment of corneal diseases such as keratoconus in order to stabilize the corneal tissue by crosslinking of the collagen fibers [1]. It is has been shown that the crosslinking treatment leads to a stiffening of the central corneal tissue. However, knowledge of lateral extent of the corneal stiffening as well as a systematic study of the mechanical response of human cornea is still missing. In our study we measured the stiffness (elastic modulus) of the anterior surface of healthy and crosslinked human corneas by instrumented indentation using a spherical indenter. The results show that the stiffness of the central and paracentral cornea increased almost two times after the crosslinking but the stiffening effect rapidly decreased towards the periphery of the radiation field. These new insights into the understanding of the biomechanical response of corneal crosslinking shall contribute to a better understanding and an optimization of this perspective medical treatment.

In this study, we focus on myocardium (cardiac tissue) and more precisely (i) cardiomyocytes that are connected end to end by gap junctions (muscular fibers), allowing concerted contractile activity and (ii) the extracellular matrix, which surrounds the muscular fibers as a mortar. Results of instrumented indentation experiments in a liquid environment of five pigs' heart are presented (n=5). We demonstrate it is possible to reveal the variations in elasticity according to the local orientation of structure of the tissue. Understanding these properties contribute to develop models used in simulated surgical procedures, specialized neo-tissue engineering or imaging-based differentiation between healthy and pathological tissues based on their response to external stimuli.

References

[1] G. Wollensak, E. Spoerl, T. Seiler, Am. J. Ophthalmol. 135 (2003) 620–627.

Antimicrobial Polypeptoids: Synthesis & Biological Tests of Sequence-Specific Folded 3D-Structured Antimicrobial Polymers

R. Zirbs,[1] E. Petrovska-Siebert[1]

[1]*University of Natural Resources and Life Sciences, Vienna (BOKU Wien)*
Department of Nanobiotechnology, Institute of Biologically inspired materials
Muthgasse 11,1190 Vienna
e-mail: Ronald.zirbs@boku.ac.at

The increasing number of antimicrobial resistances and the resulting global emergence of superbugs, as well as the lack of new therapeutic molecules, pose major challenges to the global health system. Therefore, the development of alternative classes of antibiotics is urgently needed. Antimicrobial peptides (AMPs) are naturally occurring peptides that play an important role as part of the innate immune system of various organisms and represent a new class of molecules that have been discovered as potentially antibiotically active substances. Peptoids represent a class of peptidomimetics that, compared to antimicrobial peptides, have higher structural stability and resistance to degradation by proteases.

This work describes the synthesis and testing of peptidomimetic analogues of the naturally occurring amphibian antimicrobial peptides aurein 1.2 and magainin-II based on highly simplified sequence-specific polypeptoids. The use of an optimised solid-phase submonomer synthesis and five representative monomers (one simple mimick per amino acid class (hydrophobic, weakly hydrophobic, hydrophilic, positively charged, negatively charged)) enables a time- and cost-efficient synthesis of novel antimicrobial molecules. The obtained sequence-specific polypeptoids were analysed and their antimicrobial properties were investigated by peptoid-induced calcein leakage assays and antimicrobial assays on three different bacterial strains. Initial results show successful membrane activity and indicate that it is possible to produce active molecules using this concept.

These findings and results represent the starting point for the design and synthesis of polypeptoids that mimic naturally occurring antimicrobial polypetides (AMPs) as promising potential antibiotics with improved properties to fight multidrug-resistant germs.

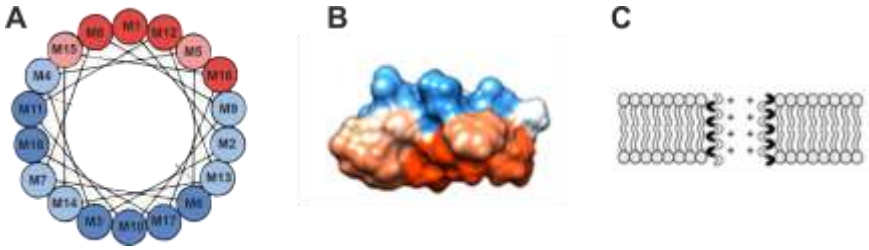


Figure 1: A: Exemplary representation of a helical polypeptide (red= hydrophilic, light red= slightly hydrophilic; blue= hydrophobic; light blue= slightly hydrophobic); B: 3D representation of a single molecule (aureine) with strongly anisotropic surface hydrophobicity[1] (e.g. blue= hydrophobic, red= hydrophilic); C: Oversimplified interaction of anisotropically charged helical molecules with a lipid membrane leads to membrane rupture.

References

[1] D.K. Rai, S. Qian, Sci. Rep.7 (1) (2017), 1–10.

3D bio-printing of living systems for food industries and medical applications

Zakaria Baka[1], Claire Godier[1], Abhik Mallick[1], Varvara Gribova[2] Fabienne Quilès[3], Grégory Francius[3], Jenifer Burgain[4], Claire Gaiani[4,5], Joël Scher[4], Eric Gaffet[1], Halima Alem[1,5]*

[1]*Université de Lorraine, CNRS, IJL, F-54000 Nancy, France.*

[2]*CRBS, Centre de Recherche en Biomédecine de Strasbourg, INSERM UMR 1121, Biomatériaux et Bioingénierie, 67000 Strasbourg, FRANCE*

[3]*Université de Lorraine, CNRS, LCPME, F-54600 Nancy, France.*

[4]*Université de Lorraine, LIBio, F-54000, France*

[5]*Institut Universitaire de France*

e-mail: halima.alem@univ-lorraine.fr

3D bioprinting is considered as a promising technology to build *living-like* models. 3D constructs containing different cell types can be generated which is crucial to simulate the heterogeneity and complexity of the human cells microenvironment. As, 3D bioprinting is a computer assisted process that generates 3D structures with a controlled architecture and a high reproducibility, it makes it an interesting alternative to other cell culture approach. This presentation will then merge different works that we are conducting in collaboration with interdisciplinary teams to bring a new approach through the development of an innovative approach-based 3D bio-printing to lead to 3D structures that can be used for food industries or cancer therapies. In one hand, we will present our 3D bio-printing process able to lead efficiently to the production of the encapsulated bacteria. Each capsule contains at least 10^8 CFU/ml (figure 1). The bacteria in the capsules were viable up to seven days and can survived the harsh gastrointestinal fluid environment in vitro. This bioprinting method for encapsulating probiotic bacteria for their specific delivery in the gut can than become a revolutionary method for industrial applications. In another hand 3D structures were obtained to mimic the ovarian cancer for further application in cancer on chip devices by their implementation in microfluidic device. To get closer to the cancer tissue structure, ovarian cancer cells (SKOV-3) were bio-printed with cancer-associated fibroblasts (CAFs) to create the stroma cells (Figure 1). The hydrogel composition was first optimized to ensure good printability at 37°C while applying minimal extrusion pressure. The tumor model was then printed and cell viability was assessed using different technics including Live-Dead assay, WST1 assay and Alamar-Blue assay. Annexin V/PI assay and flow cytometry were also performed to quantify the apoptotic cells within the bioprinted structures. We have shown that the tumor-like model can be maintained alive up to 7 days and its transfer in a high-throughput microfluidic system was achieved. This work aims then to present the strength of 3D bio-printing process to lead to model structure that can be used in different field as foods or pharmaceutic industries.

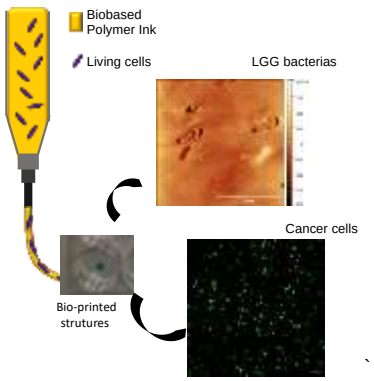


Figure 1: 3D bio-printed structures (bacteria and cancer cells)

Decellularized Wharton's jelly as an antibacterial and immunomodulatory scaffold for tissue engineering

M. Dubus[1], L. Scomazzon[1], J. Chevrier[1], A. Baldit[2], J. Braux[1], M. Colin[1], C. Mauprivez[1] and H. Kerdjoudj[2]

[1]*Université de Reims Champagne Ardenne, EA 4691, Biomatériaux et Inflammation en Site Osseux (BIOS), Reims, France.*
[2]*LEM3-UMR-7239, CNRS - Arts et Métiers ParisTech; ENIM, Université de Lorraine, Metz, France.*
e-mail: marie.dubus@univ-reims.fr

Of all biologic matrices, decellularized tissues have emerged as a promising tool in the field of regenerative medicine. Few empirical clinical studies have shown that human Wharton's jelly (WJ) of the human umbilical cord promotes wound closure and reduces wound-related infections. It was in this scope that we investigated if decellularized (DC)-WJ could be used as an engineered biomaterial. Following the full removal of cell membranes and nuclei moieties from Wharton's jelly (WJ) tissue (Figure 1), no major alterations in the ECM components (*i.e*. collagen, GAG content and growth factors), physical (*i.e*. porosity and swelling) and mechanical (*i.e*. linear tensile modulus) properties were noticed [1]. Interestingly, an increase in macromolecules and growth factors release was observed for DC-WJ, assuring thus a suitable bioactive matrix for cell maintenance upon recellularization. Based on the *in vitro* biocompatibility and stromal cell homing capabilities, DC-WJ provided an ideal substrate for stromal cells adhesion and colonization. Few empirical clinical studies have been using WJ to treat infected wounds. Therefore, we evaluated the antibacterial effect of DC-WJ on gram positive and gram negative strains. Surprisingly, in comparison with devitalized (DV)-WJ, our results showed bacteriostatic and antiadhesive effect of DC-WJ on both Gram positive and negative strains (*S. aureus, S. epidermidis* as well *E. coli* and *P. aeruginosa)*. Although DC-WJ activated the neutrophils and monocytes in comparable magnitude than DV-WJ, macrophages modulated their phenotypes and polarization states from the resting M0 phenotype to the hybrid M1/M2 phenotype in the presence of DC-WJ. M1 phenotype was predominant in the presence of DV-WJ. Finally, the subcutaneous implantation of DC-WJ showed a total resorption after three weeks of implantation without any sign of foreign body reaction. Used as a membrane for guided bone regeneration, few bone regeneration evidence was found at the marginal area of the rat calvarial defect after eight weeks of implantation. The limited bone regeneration could be attributed to the immunomodulatory properties in favour of M2 phenotype but also to lack of sufficient mechanical strength [2] that lead to the membrane collapse into bone defect area. These significant data shed light on the potential regenerative application of DC-WJ in providing a suitable biomaterial for soft tissue regenerative medicine and an ideal strategy to prevent wound-associated infections. An increase in the mechanical features of DC-WJ in hydrated conditions is in progress.

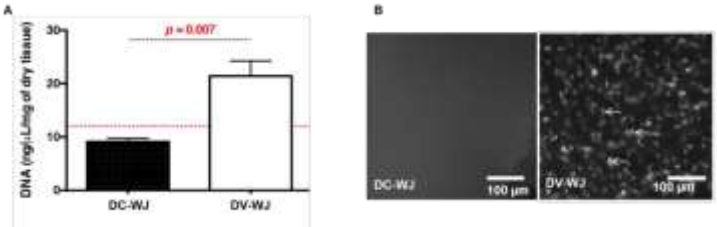


Figure 1: A: Nuclei moieties quantification in DC-WJ and DV-WJ, indicating a significant decrease in DNA content within DC-WJ (red dashed line indicates the limit of detection of the used kit; n=6, Mann & Whitney test). B: Fluorescence microscopy observation of DAPI stained nuclei of DC-WJ and DV-WJ (scale bars = 100 µm), white arrows indicating the presence of nuclei inside DV-WJ.

References

[1] Dubus, M.; Scomazzon, L.; Chevrier, J.; Ledouble, C.; Baldit, A.; Braux, J.; Gindraux, F.; Boulagnon, C.; Audonnet, S.; Colin, M.; et al. Antibacterial and Immunomodulatory Properties of Acellular Wharton's Jelly Matrix. *Biomedicines* **2022**, *10*, 227.
[2] A. Baldit, M. Dubus, J. Sergheraert, H. Kerdjoudj, C. Mauprivez, R. Rahouadj, Biomechanical tensile behavior of human Wharton's jelly. *Journal of the Mechanical Behavior of Biomedical Materials* **2022**, 126, 104981.

Acknowledgements: This work was partially supported by 3D4MED Interreg project.

The peptidoglycan architecture of the Gram-positive bacterial cell wall and its destruction by antibiotics

Laia Pasquina-Lemonche[1], Bartlomiej Salamaga[2], Oliver Meacock[1], William M Durham[1], Ethan Garner[3], Simon J Foster[2], Jamie K Hobbs[1]

[1]Department of Physics and Astronomy, University of Sheffield, United Kingdom

[2]School of Biosciences, University of Sheffield, United Kingdom

[3]Department of Molecular and Cellular Biology, Harvard University, United States

e-mail: l.pasquinalemonche@sheffield.ac.uk

The primary structural component of the bacterial cell wall is peptidoglycan which is crucial for survival and division. Peptidoglycan (PG) is a heterogeneous macromolecule composed of glycan chains (sugars) crosslinked with small peptides. PG is tens of nanometres thick in Gram-positive bacteria and acts as a semi-permeable barrier with the environment. This component of the cell is also one of the major targets by cell wall antibiotics such as penicillin, methicillin and vancomycin. The architecture of PG has been studied with great detail (on the order of 1 nm) by combining microbiology, advanced microscopy and image analysis techniques.

In this project, we applied several imaging modes of atomic force microscopy (AFM) to interrogate the morphology of this heterogeneous hydrogel. The bacterial species of study were *Staphylococcus aureus* and *Bacillus subtilis* which are both Gram-positive species with distinct cell shape (*cocci* vs *rod*). Unprecedented molecular resolution was obtained using tapping to image the external surface of live cells and peakForce™ tapping to study both the internal and external surface of purified PG. Then, quantitative image analysis methods were developed to automatically study the orientation of glycan chains in AFM images, [1] obtaining robust conclusions when comparing different samples. The results were that contrary to established theories, the PG is not a homogeneous impenetrable wall, it is a highly porous heterogeneous hydrogel with four different architectures [2].

Once the architecture of PG from healthy cells was well characterised, the same techniques were applied to study and quantify the effect of antibiotics to the PG morphology. We used *Staphylococcus aureus* wild type, together with different mutants to decipher the role of PG modification enzymes during cell death [3]. The results corroborate the model that for the cell to survive there must be an equilibrium between synthesis and hydrolysis of PG, this equilibrium is disrupted when antibiotics are applied, holes spanning the whole the cell wall thickness develop and eventually lead to cell death. In the talk we will also discuss how these approaches can be used more widely as a tool for understanding the role of cell wall synthesis and hydrolysis enzymes in bacterial life and death and how the image analysis techniques can be applied to a broad range of AFM images from different samples.

References:

[1] O. Meacock*, L. Pasquina-Lemonche*, W. M Durham, E. Garner, S. J Foster, J. K Hobbs (in preparation).

[2] L. Pasquina-Lemonche*, J. Burns*, *et al.* Nature, 582, 294-297 (2020).

[3] B. Salamaga*, L. Kong*, L. Pasquina-Lemonche*, Lucia Lafage*, M. v. und zur Muhlen*, *et al.* PNAS (2021).

*The authors share first authorship

Targeted detection of membrane nanoparticles in unprocessed saliva using vibrating microcantilever

K Voitchovsky,[1] and C. Cafolla,[1]

[1]*Durham University, Physics Department, Durham UK*
e-mail: kislon.voitchovsky@durham.ac.uk

Membrane Nanoparticles (MNPs) are small (40 -150 nm) vesicles ubiquitously present in bodily fluids. They vehicle information between distant cells and have an enormous potential for early diagnosis of numerous diseases [1] including cancer, and for tailored therapeutic applications. Unsurprisingly, MNP research and technology is anticipated to be worth in excess of €1 billion/year by 2022 [2].
Current approaches for MNPs characterisation rely on complicated, expensive and time-consuming bio-assays [1]. Part of the problem comes from the fact that most (if not all) characterisation procedures require MNPs to be extracted, purified and concentrated from bodily fluid, necessitating relatively large quantities of raw fluid. As a result, studies requiring MNP quantification can vary significantly depending on the details of the MNPs extraction procedure used with no accepted standard to date. To make the matter worse, it is not clear whether the extraction and purification can themselves influence the properties and composition of the isolated MNPs which may degrade during the process. There is hence an urgent need to develop techniques able to characterise MNPs *in-situ*, directly is raw bodily fluid samples. Ideally such techniques should also be quick from sample collection to quantitative results, relatively inexpensive, have inbuilt references, and be able to function on small volumes of fluid.
Here we test the idea of a using microcantilevers to detect MNPs directly in raw saliva and with sample volumes smaller than 0.1 mL. Microcantilevers have long been used for bio-detection with excellent sensitivity [3,4], but usually with the cantilever in air to prevent biofouling and poor signal to noise. Having the cantilever immersed in saliva could by-pass all the questions related to sample preparation and guarantee full exposure to all the MNPs even if not volatile. However, the added complication of working directly in complex bio-fluids renders reliable measurements more challenging.
Using a particular operating mode, microcantilevers functionalised with streptavidin, are used to detect small quantities of synthetic lipid vesicles dissolved in raw saliva. 0.5% of the vesicles' lipids are biotinylated, allowing for strong, irreversible binding to the cantilever's streptavidin. Preliminary results suggest a detection sensitivity better than 1 microgram/ml of vesicles in raw saliva, a concentration two orders of magnitude smaller than the natural MNP concentration in blood [5]. Significantly, the approach can be upscaled, parallelised and its sensitivity further improved. The key conditions underpinning detection in complex biofluids are examined.

References

[1] S. Fais, *et al. ACS Nano* **10** (2016) 3886–3899.
[2] Biomarkers Market Analysis & Trends - Forecast to 2025. *Research and Markets* (2016).
[3] H. P. Lang, M. Hegner, & C. Gerber *Materials Today* **8** (2005) 30–36.
[4] T. Braun, *et al. Nat Nanotechnol* **4** (2009) 179–185.
[5] I. Helwa, *et al. PLoS ONE* **12** (2017) e0170628.

An *in situ* nanoscale investigation of $A\beta_{1-42}$ and model membranes to decipher the early mechanism involved in Alzheimer's disease.

Fabienne Quilès[1], David Siniscalco[1], Mounir Tarek[2] Catherine Malaplate[3], Thierry Oster[3], Lynn Pauron[3] and Grégory Francius[1]

[1] *Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et pour l'Environnement (LCPME), UMR 7564, Université de Lorraine-CNRS, Villers-lès-Nancy, France.*
[2] *Laboratoire de Physique et Chimie Théorique (LPCT), UMR 7019, Université de Lorraine-CNRS, Vandoeuvre-lès-Nancy, France*
[3] *Unité de Recherche Animal et Fonctionnalités des Produits Animaux (URAFPA), Université de Lorraine, Vandoeuvre-lès-Nancy, France*
e-mail: fabienne.quiles@univ-lorraine.fr

Alzheimer's disease (AD) is a widespread chronic pathology inducing loss of cognitive capacities. This degenerative disease is characterized by the presence of fibrils and amyloid plaques in the brain. These plaques are predominantly composed by amyloid β–peptides (Aβ) generated after a proteolytic cleavage of the amyloid precursor protein (APP) through the so called pathologic amyloidogenic pathway. The accepted mechanism of Aβ accumulation suggests that Aβ monomers are expelled from the membrane before becoming neurotoxic upon oligomerization. Such aggregates target cellular membrane to induce cell death.[1]

Our Molecular Dynamics simulations indicated that contrary to this amyloidogenic hypothesis, the nascent Aβ peptide produced upon APP cleavage remain inserted in the hydrophobic domain of model membranes. These results point to the possibility of a novel scenario in which Aβ peptides can at least partially directly aggregate within the membrane while bound to it to form neurotoxic oligomers and fibrils.

To check this hypothesis we focused our research on $A\beta_{1-42}$, which is considered to be one of the main components of amyloid plaques. We used Atomic Force Microscopy (AFM) and InfaRed spectroscopy in attenuated total reflexion mode (IR-ATR) to localize $A\beta_{1-42}$ peptides and to study their occurrence/amount in or near supported lipid bilayers (SLB) composed of DPPC and POPC. All measurements were performed in aqueous medium at controlled temperature. The interaction of $A\beta_{1-42}$ with the SLB was studied in two ways: $A\beta_{1-42}$ was either added after or during the building of the SLB. These SLBs were monitored by AFM in the same area during 2 hours to ensure the observation of the $A\beta_{1-42}$ interaction with the model membranes. IR-ATR spectroscopy was combined to AFM to evaluate the biochemical evolution of SLB and $A\beta_{1-42}$ components, and to provide conformational and quantitative information on $A\beta_{1-42}$. In agreement with the modelling predictions, using the two techniques we have observed clearly the retention of Aβ peptide within the membrane and studied its dependence on the membrane composition. In presence of the peptide, the SLB structure appeared highly disturbed and their mechanical properties weakened. These results support the hypothesis that toxicity of Aβ peptides might occur despite their permanent presence within the membrane.

[1] E.N. Cline, M.A. Bicca, K.L. Viola, W.L. Klein J Alzheimers Dis 64 (2018) S567-S610.

4D THz microscopy of biological materials at atomic scale

Marc Ropitaux,[1] Manon Salvetti,[2] Emmanuel Cadel,[1] Félix Sauvage,[3] Virginie Nachbaur,[1] Laurence Chevalier,[1] Jonathan Houard,[1] Jérémy Bellien,[4] Thomas Duflot,[4] Patrice Lerouge,[2] Arnaud Lehner,[2] Angela Vella[1].

[1]*Normandie Université, UNIROUEN, INSA Rouen, CNRS, Groupe de Physique des Matériaux, 76000, Rouen, France.*
[2] *Normandie Université, UNIROUEN, Laboratoire Glyco-MEV UR 4358, Structure Fédérative de Recherche Normandie Végétal FED 4277, Innovation Chimie Carnot, 76000 Rouen, France*
[3]*Laboratory of General Biochemistry and Physical Pharmacy, Faculty of Pharmaceutical Sciences, Ghent University, 9000 Ghent, Belgium.*
[4]*Normandie Université, UNIROUEN, INSERM U1096, CHU Rouen, Department of Pharmacology, Rouen, France.*
e-mail: marc.ropitaux1@univ-rouen.fr

From medical imaging to structural biology, the spatial characterization of biological molecules and drug delivery nanosystems is a major issue to further understanding their functions and actions. Today, many questions are much easier to apprehend thanks to many technics and methodologies allowing to view and turn images in 3D and even to slice and segment the different elements of the images [1,2,3]. However, these technics give little information about the chemical composition of the sample. To go further, 3D spatial characterization technics should therefore approach the so-called 4D technics by adding information on the local chemical composition as the fourth dimension.

Tomographic Atom Probe (TAP) can meet this need for 4D microscopy in biology. TAP is a 4D imaging technique based on the controlled field evaporation of atoms from a nanometric needle-shaped sample under a strong electric field. TAP combines sub-nanometer spatial resolution with high chemical sensitivity across the entire periodic table and its isotopes [4].

Moreover, current developments of this technique, such as the use of pulsed UV laser illumination or intense THz pulses to control the field evaporation process, bring groundbreaking perspectives into biomaterial studies [5,6,7]. In addition, the recent atomic scale tomographic analysis of biomaterials using TAP has been facilitated by the development of a new sample preparation methodology [8]. This method is based on replacing the water surrounding biological molecules with an amorphous silica matrix obtained by sol-gel process. The silica glass structure provides a solid support that allows conventional sample preparation procedures for TAP, without distorting the original and functional protein structure.

We will present and discuss how to obtain a matrix suitable for biological samples, their detection during samples nano-machining and their studies in TAP.

References

[1] C. Bonnaud, C.A. Monnier, D. Demurtas, C. Jud, D. Vanhecke, X. Montet, R. Hovius, M. Lattuada, B. Rothen-Rutishauser, A. Petri-Fink, ACS Nano 8 (2014) 3451-3460.
[2] J.P. Renaud, A. Chari, C. Ciferri, W.T. Liu, H.W. Rémigy, H. Stark, C. Wiesmann Nature Reviews Drug Discovery 17 (2018) 471.
[3] S.G. Wolf, Y. Mutsafi, T. Dadosh, T. Ilani, Z. Lansky, B. Horowitz, S. Rubin, M. Elbaum, D. Fass, eLife 6 (2017) e29929.
[4] B. Gault, A. Chiaramonti, O. Cojocaru-Mirédin, P. Stender, R. Dubosq, C. Freysoldt, T. Li, M. Moody, J.M. Cairney, Nature Review 51 (2021).
[5] A. Vella, J. Houard, L. Arnoldi, M. Tang, M. Boudant, A. Ayoub, A. Normand, G. Da Costa, A. Hideur, Science Advances 7 (2021) eabd7259.
[6] D.E. Perea, J. Liu, J. Bartrand, Q. Dicken, S.T. Thevuthasan, N.D. Browning, J.E. Evans, Scientific Report 6 (2016) 22321.
[7] J. Houard, L. Arnoldi, A. Ayoub, A. Hideur, A. Vella, Applied Physics Letter 117 (2020) 15.
[8] G. Sundell, M. Hulander, A. Pihl, M. Andersson, Small 15 (2019) 1900316.

Force Spectroscopy Characterization of Neurotoxins Binding

H. Martin-Yken[1,2], Dague E.[2] and E. Trevisiol [1,2]

[1]*TBI, Toulouse Biotechnology Institute INSA UMR CNRS 5504 UMR INRA 792,135 Avenue de Rangueil 31077 TOULOUSE CEDEX 4*

[2]*LAAS-CNRS, 7 avenue du Colonel Roche BP 5420031031 Toulouse cedex 4*

e-mail: helene.martin@insa-toulouse.fr

Keywords: Emerging toxins, global warming, force spectroscopy, atomic force microscopy, neuronal receptors.

Brevetoxins and Ciguatoxins are emerging marine neurotoxins responsible for frequent food poisonings. Produced by the microalgae *Karenia brevis* for brevetoxins and *Gambierdiscus sp.* for ciguatoxins, they respectively cause Neurotoxic Shellfish Poisoning syndrome and Ciguatera, the most frequently encountered seafood poisoning worldwide, with between 300,000 and 500,000 victims per year (1). The geographical areas of presence of these microalgae are increasingly expanding due to global warming and degradation of marine ecosystems. These toxins strongly bind voltage-dependent sodium channels (VGSCs) that play a critical functional role in the initiation and propagation of the action potential in excitable cells.

However, the molecular mechanism behind this binding has never been elucidated. We started to characterize these interactions using force spectroscopy, an approach new to this field, which allows us to have access to fine measurements (of the order of a picoNewton) of force intensities as well as to the tearing profiles of the molecular interactions between the toxins and their receptors. This project constitutes, to our knowledge, the first attempt to study the binding of toxins to these receptors by AFM.

We successfully functionalized AFM tips with purified Brevetoxin and recorded the interaction forces with two different mammalian neuronal cells lines, SHY5Y and Neuro2A. In addition, to get a clearer view of the pure molecular interactions taking place, we also used an abiotic model, membrane vesicles rich in VGSCs produced by Dr. R. Araoz at CEA Saclay. Finally, we also have shown that addition under the functionalized AFM tip of a patented therapeutic molecule can significantly reduce the interaction between Brevetoxin and VGSCs, proving *in vitro* its role as a binding inhibitor.

Our next step is to use purified Ciguatoxins produced by the Louis Malardé Institute (French Polynesia) which share some structural features with Brevetoxins. In the medium term, this strategy could be extended to other toxins, marine or not, also acting on voltage-gated sodium channels. Moreover, the key role of VGSCs sodium channels which bind a wide variety of toxins and drugs (2) makes them a major target for the search of selective molecules with very different application areas such as the medical field (anaesthesia) or defence (paralytics/anti-paralytics).

References

[1] Chinain M., Gatti C., Martin-Yken, H., Roué M., Darius H.T. Ciguatera poisoning: an increasing burden for Pacific Island communities in light of climate change. 2021 In: Climate change and marine and freshwater toxins, 2nd edition,Chapter 10, 369-428; DOI: 10.1515/9783110625738-010

[2] Nicholson and Lewis, Marine Drugs, 200 Ciguatoxins: Cyclic Polyether Modulators of Voltage-gated Iion Channel Function. Mar. Drugs 2006, 4(3), 82-118; DOI: 10.3390/md403082

Posters

Authors	Title	Poster #
Sofiane El-Kirat Chatel	Bio-inspired multi-enzymatic surfaces for antibiofilm protection	**1**
Ngutyet-Thanh Ha-Duong	Gold Biomineralization on plant virus	**2**
Beguem Dikecoglu	Human IgG monoclonal antibody binding to microbial polysaccharide poly-N-acetylglucosamine	**3**
Paul Emile Contarini	The first giant bacteria from the Campylobacterota phylum displays apparent compartmentalization of DNA in membrane-bound organelles according to LSM and TEM analyses	**4**
Yoan Debaud	Carbon nanoparticles from local biomass for tribological applications	**5**
Irem Demir	Probing the interactions between air bubbles and (bio)-interfaces at the molecular scale using FluidFM technology	**6**
Marie Dubus	*In vivo* bone regeneration with a bio-inspired coated membrane	**7**
Amine Filali	Electrosynthesis of free-standing composite films of chitosan – iron oxide nanoparticles	**8**
Vladislav Genevskiy	Antimicrobial patterned electrodes based on NIL technology	**9**
Francesca Goudou	Chlordecone and β-hexachlorocyclohexane interaction with acidic and basic functionalized activated carbon by molecular modelling and molecular dynamics simulation	**10**
Lisa Hain	SARS-CoV-2 Spike interaction using Surface Plasmon Resonance	**11**
Miryana Hémadi	Eradicating bacterial infections by nanomedecine	**12**
Lucie Hochvaldova	Silver modified surfaces as a platform for basic research and other medical applications	**13**
Zoya Leonenko	Nanoscale biophysics to study molecular mechanism of Alzheimer's disease	**14**
Verena Maier	Nanomechanical Properties of the Articular Cartilage in a Murine Osteoarthritis Model Investigated by Atomic Force Microscopy	**15**
Georges Minatchy	Determination of the mechanical properties of red blood cells in sickle cell disease by means of AFM technique	**16**

Margit Németh	Self-sensing cantilevers for biological sample characterization in liquid	**17**
Nicolo Tormena	Bio-inspired active nano-membrane	**18**
Carolina Paba	Biophysical analysis of small extracellular vesicles (sEVs) and their interaction with model plasma membrane	**19**
Nicolò Paracini	Structure of nanoparticle-supported lipid bilayers	**20**
Fabio Priante	Carbon nanotube penetration of cell membranes	**21**
Fabienne Quilès	Nanoscale surface modification by alkanethiol self-assembly onto ZnSe: the role of substrate preparation on bacterial attachment	**22**
Sarra Gaspard	Adsorption of vitamin B12 on sugarcane-derived activated carbon: Fractal isotherm and kinetics modelling, electrochemistry and molecular modelling studies	**23**
Hannah Seferovic	The effect of hinge region flexibility on the binding kinetics of human IgG subclasses	**24**
Tiago dos Santos	Quantitative Assessment of the Comparative NP-Uptake Efficiency in a range of biological fluids	**25**
Lucie Válková	*In vitro* anticancer photothermal therapy using noble metal nanolayers	**26**
Tomaso Zambelli	Forces during Cell Membrane Puncturing Detected by FluidFM Coupled with Fluorescence Lifetime Imaging Microscopy	**27**
Laurence Romana	Nano mechanical characterization techniques: a tool to predict macro mechanical behavior of polymer samples	**28**
Ngutyet-Thanh Ha-Duong	Virus-based nano-bio-hybrid materials for hyperthermia	**29**
Sofiane El-Kirat Chatel	Deciphering the Role of Surface Interactions in the Antibacterial Activity of Layered Double Hydroxide Nanoparticles by AFM	**30**

Bio-inspired multi-enzymatic surfaces for antibiofilm protection

Sofiane El-Kirat-Chatel,[1] Baptiste Arbez, Grégory Francius, and Fabienne Quilès

[1]*Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement (LCPME) CNRS-Université de Lorraine UMR7564, 405 rue de Vandoeuvre 54600 Villers les Nancy.*
e-mail: elkirat1@univ-lorraine.fr

Surface protection strategies against biofilms remain challenging as protection efficacy tends to decrease promptly over time. Current approaches rely on the two following principles: antiadhesion to prevent microbial colonization; and antibacterial effect to kill attached cells. Existing antiadhesion strategies have specific efficacy targeted to certain species, certain bacterial adhesive processes or certain media and thus have a limited range of action. This limitation can lead to biofouling over time reducing the antiadhesion efficacy even more. Due to their "one-shot" nature, some antimicrobial surface treatments can also have limited long-term action. However, antimicrobial enzymes have already been considered as an interesting alternative since their activity is "re-usable" as long as the catalytic site is active [1]. In the present work, we focused on a bio-inspired multi-enzymatic system, a supramolecular nanomachine made of several enzymes anchored on a protein scaffold. Our system was made of a protein with several receptors sites and which serves as a skeleton onto which antibacterial enzymes were attached, namely, lysozyme and lysostaphin that both degrade peptidoglycans in bacterial cell wall (Fig. 1). DNase, an enzyme that cleaves extracellular DNA was also docked on the system as an "antiadhesion" and "antifouling" agent. Enzyme anchoring was achieved by the use of interactions between ligands on the enzymes and receptors on the scaffold. Atomic force microscopy (AFM)-based single-molecule force spectroscopy was performed with tips functionalized with enzymes of interests and showed these interactions were highly specific and reversible. Enzyme proportions and relative positions on the scaffold were achievable because of the high specificity of ligands-receptors interactions. *Micrococcus luteus* (Gram +), *Staphylococcus aureus* (Gram +), and *Escherichia coli* (Gram -) viability was showed to be reduced in presence of the enzyme-scaffold system thus revealing its antimicrobial effect. The antimicrobial effects were lessened when enzymes were grafted without a protein scaffold which demonstrates that the scaffold improved the orientation and spatial conformation of enzymes and led to an optimized antimicrobial activity. The presented bio-inspired system combined antimicrobial enzymes as well as antiadhesion agents capable of killing Gram positive and Gram negative bacteria. The reversibility of ligands-receptors links used to dock the enzymes gave promising insights into renewing enzymes without grafting a new scaffold and thus insuring sustainable antimicrobial effects over time.

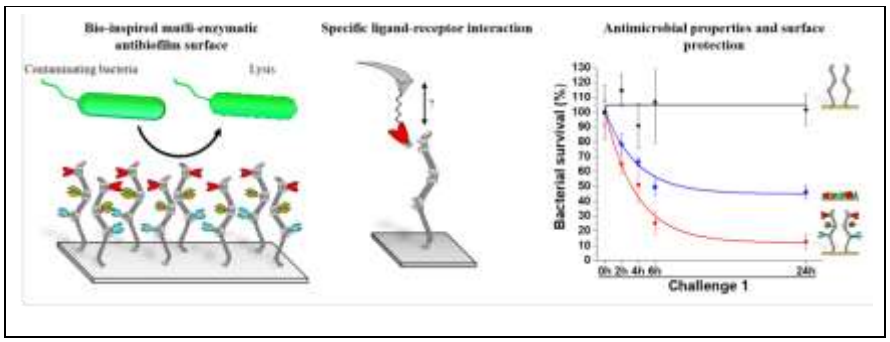


Figure 1: Principle of the bio-inspired antibiofilm strategy, AFM-based measurement to demonstrate the specific ligand-receptor interaction and antimicrobial efficiency of functionalized surfaces.

References

[1] Beaussart, C. Retourney, F. Quilès, R. Dos Santos Morais, C. Gaiani, H.P. Fiérobe and S. El-Kirat-Chatel. Supported lysozyme for improved antimicrobial surface protection. Journal of Colloid and Interface Science, 582, (2021),764-772.

Gold Biomineralization on plant virus

C. Moreira da Silva[1], D. Alloyeau[2], T. Moravec[3], A. Vernet[4], C. Brugidou[4], NT. Ha-Duong[1],

[1]*Université de Paris, ITODYS, CNRS, UMR 7086, 15 rue J-A de Baïf, F-75013 Paris, France*
[2]*Université de Paris, MPQ, CNRS, UMR 7262, 10 rue A. Domon et L. Duquet, F-75013 Paris, France*
[3]*Institute of Experimental Botany of the Czech Academy of Sciences, Prague, Czech Republic*
[4]*PHIM Plant Health Institute, Univ Montpellier, IRD, CIRAD, INRAE, Institut Agro, Montpellier, France*

e-mail: thanh.haduong@u-paris.fr

Nanomaterials sciences show an increasing interest in bottom-up synthesis of functional structures based on plant virus as 3D nanoscale templates, to biomimetically synthetize complex functional nanostructures with remarkable physicochemical properties and a wide variety of applications: catalysis, energy conversion, and especially nanomedecine and biology because of their lack of pathogenicity in humans and animals [1]. Because of their well-defined and highly organized symmetric structures, high robustness over wide ranges of temperature, pH, buffer, and in the presence of organic solvents, viral capsid proteins then provide a 3D scaffold for the precise placement of plasmon materials yielding hierarchical hybrid materials [2]. Two ways are possible to obtain plasmonic nanostructures onto capsid: grafting pre-formed nanoparticles or biomineralization.

In this work, we proposed to decorate tobacco mosaic virus (TMV) and rice yellow mottle virus (RYMV) capsid by biomineralization, in order to construct *in vitro* and in mild conditions gold nanoparticles (AuNP) assemblies. TMV is one of the most exploited plant virus in nano-bio-technology. TMV is a rigid, rod-shaped virus with helical symmetry. The TMV capsid is a 300-nm-long rod composed of 2130 identical protein subunits of 17.5 kDa forming a right-handed helical array around the RNA (16.3 subunits per turn). The diameter of the native TMV helix is 18 nm while the core is 4 nm-wide [3]. RYMV capsid is a quasi-spherical capsid with icosahedral symmetry (T=3), with a diameter of 29 to 32.2 nm [4].

For the synthesis, TMV or RYMV were incubated in mixture of acetic acid and tetrachloroauric acid ($HAuCl_4$), at room temperature. A reductant agent (sodium borohydride or ascorbic acid) was added to the solution, followed immediately by the addition of ethanol. Samples were collected after each cycle of addition of $HAuCl_4$ and ascorbic acid for absorption spectroscopic and TEM analysis [4] (Fig 1). The size, morphology, monodispersity of AuNP and gold assembly on virus will be studied according to the experimental conditions (concentrations of reactant, number of cycle, nature of reductant…) and in situ TEM observations directly in liquid media will be performed to unravel the nucleation and growth mechanisms.

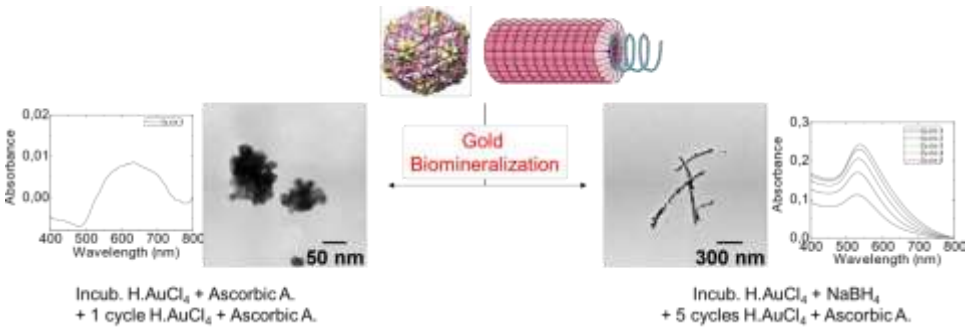


Fig 1. Scheme of gold biomineralization procedure on TMV and RYMV

References

[1] (a) Capek, I. **Adv Colloid Interface Sci.** 2015, *222*, 119-134. (b) Culver, J.N.et al. **Virology** 2015, 479-480, 200-212. (c) Li, F.& Wang, Q. **Small** 2014, 10, 230-245. (d) Liu, Z.et al. **Chem Soc Rev** 2012, 41, 6178-6194.
[2] Petrescu DS., Blum AS., **WIREs Nanomed NanobiotechnoI**. 2018;10:e1508.
[3] R. Hull, Chapter 3 - Architecture and Assembly of Virus Particles, in: R. Hull (Ed.), Plant Virology (Fifth Edition), Academic Press, Boston, 2014, pp. 69-143
[4] Qu C, et al. **Structure,** 2000, 8, 1095

Human IgG monoclonal antibody binding to microbial polysaccharide poly-N-acetylglucosamine

B. Dikecoglu,[1] R. Zhu,[1] Y. J. Oh[1], C. Cywes-Bentley[2], G. B. Pier[2], P. Hinterdorfer[1]

[1]*Institute of Biophysics, Johannes Kepler University, Gruberstrasse 40, 4020 Linz, Austria*
[2]*Division of Infectious Diseases, Department of Medicine, Brigham and Women`s Hospital/Harvard Medical School, Boston, Massachusetts 02115*
e-mail: beguem.dikecoglu@jku.at

Poly-N-acetylglucosamine (PNAG) has been identified as surface polysaccharide antigen expressed by many microbial pathogens during human and animal infection. The group of Gerald Pier developed human IgG monoclonal antibodies (MAbs) with quantitative difference in effector functions due to differences in epitope binding and affinity. IgG MAbs that bound to PNAG show different activities with respect to their complement-depositing activity. [1,2] Here, we used atomic force microscopy based single molecule force spectroscopy (SMFS) to determine interaction forces of different human IgG MAb subclasses (F598 IgG1 and F628 IgG2) with purified PNAG polysaccharide, N-acetylglucosamine (GlcNAc), and PNAG on the surface of biofilm-forming Staphylococcus aureus bacterial cells. In order to measure forces between specific interaction pairs, a conventional AFM cantilever was upgraded into a specific sensor by flexible attachment of a sensor molecule (i.e. MAb) to localize the complementary target molecule (i.e. PNAG) on the sample surface using well-established protocols. [3] Consistent with previous activity studies [2], we found that F598 Mab showed stronger interactions with PNAG than F628 MAb. With further experimental validation, we aim at deciphering nanomechanical details of antibody-antigen binding and dissociation on the single molecular level.

References

[1] Maira-Litran, T., A. Kropec, C. Abeygunawardana, J. Joyce, G. Mark III, D. A. Goldmann, and G. B. Pier. 2002. Immunochemical properties of the staphylococcal poly-N-acetylglucosamine surface polysaccharide. Infect. Immun. 70:4433–4440
[2] Kelly-Quintos C, Cavacini LA, Posner MR, Goldmann D, Pier GB. 2006. Characterization of the opsonic and protective activity against Staphylococcus aureus of fully human monoclonal antibodies specific for the bacterial surface polysaccharide poly-N-acetylglucosamine. Infect Immun. 74:2742-2750.
[3] Ebner, A., Wildling, L., Kamruzzahan, A. S. M., Rankl, C., Wruss, J., Hahn, C. D., ... & Gruber, H. J. 2007. A new, simple method for linking of antibodies to atomic force microscopy tips. Bioconjugate chemistry, 18(4), 1176-1184.

Acknowledgment

The authors acknowledge the financial support from the Austrian Science Fund (FWF) project FWP303140000.

The first giant bacteria from the Campylobacterota phylum displays apparent compartmentalization of DNA in membrane-bound organelles according to LSM and TEM analyses.

Contarini PE.[1*], Hermant C.[1], Elisabeth N.H.[2], Gros O.[1,3] and Volland J-M.[4,5]

1 Institut de Systématique, Evolution, Biodiversité, Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles. Guadeloupe, France
2 Department of Energy Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National, Laboratory. Berkeley, CA, USA.
3-Centre Commun de Caractérisation des Matériaux des Antilles et de la Guyane. Université des Antilles, UFR SEN, Guadeloupe, France.
4 Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, USA.
5 Laboratory for Research in Complex Systems, Menlo Park, CA, USA.

e-mail: paulemile.contarini@gmail.com

Bacteria and archaea are the most diverse and abundant organisms on all ecosystems including marine mangroves. With only a small fraction of them isolated in culture, we remain grossly ignorant of their biology. While most model bacteria and archaea are small and simple, some exceptional members of sessile filamentous sulfur-oxidizing gamma-proteobacteria *Thiomargarita magnifica* reach up to 20 mm in length and have complex cellular organization[1].

Using DNA and membrane labeling, laser scanning microscopy, electron microscopy an single-cell genomics, we characterized *Thiovulum imperiosus,* a new Campylobacterota from mangrove swamps. Single cells have an average diameter of 50 μm and a highly polyploid 2.2 Mb genome. They display an apparent compartmentalization of their genomic material in membrane-bound organelles distributed throughout the cytoplasm. Such compartmentalization is analogous to the one recently described in *T. magnifica*. *Thiovulum imperiosus* challenges traditional concepts of bacterial cells and could represent a new model to investigate gain of complexity in Prokaryotes.

Reference:

1- A centimeter-long bacterium with DNA compartmentalized in membrane-bound organelles. Volland *et al.,* 2022 (BioRxiv doi.org/10.1101/2022.02.16.480423)

Carbon nanoparticles from local biomass for tribological applications

Y. Debaud[1], P. Thomas[1], T. Césaire[1], Y. Bercion[2], M. Dubois[3], H. Farhat[3]

[1]*Groupe de Technologie des Surfaces et Interfaces (GTSI EA24 32), Université des Antilles, Faculté des Sciences Exactes et Naturelles, Pointe-à-Pitre (FRANCE)*
[2]*Centre Commun de Caractérisation des Matériaux des Antilles et de la Guyane (C3MAG), Université des Antilles, Faculté des Sciences Exactes et Naturelles, Pointe-à-Pitre (FRANCE)*
[3]*Équipe Matériaux Inorganiques, Institut de Chimie de Clermont-Ferrand, Université Clermont-Auvergne, Clermont-Ferrand (France)*
email : yoan.debaud@univ-antilles.fr

Friction and wear phenomena are the main causes of the decrease in performances and durability of mechanical systems. Manufacturing techniques for metal parts such as gears, ball bearings, segments, etc., must meet the growing economic demands of the market, such as improved material durability, performance increase, and reduced maintenance costs and energy consumption. The use of tribology, defined as the science that studies the phenomena of friction, wear and lubrication, is essential and constitutes an important economic issue.
It is the role of lubrication to minimize friction and protect surfaces from wear. The strategy put in place to fight these phenomena is to introduce a lubricant between the sliding surfaces. This lubricant can be solid, liquid or gaseous, and must prevent direct contact between surfaces.

My thesis project focuses on the synthesis of new friction reducer additives from local biomass, in order to produce ecofriendly lubricants. These new carbon phases are obtained using the spray-pyrolysis technique[1] with sugarcane from local biomass. It consists in nebulizing a solution of saccharose in the form of micro-droplets, transported to a tubular oven heated at temperatures ranging from 800°C to 1000°C.

The first results have permitted to see the effects of the different synthesis conditions, i.e. oven temperature, catalyst concentration and carrier gas pressure, using scanning electron microscopy to observe the spherical shape and the porosity of the particles. This part concerns the tribological properties of our particles, measured with a sphere/plan contact tribometer. The effect of an annealing process on these particles is also studied, just as the effect of fluorination realized in collaboration with the Chemical Institute of Clermont-Ferrand.

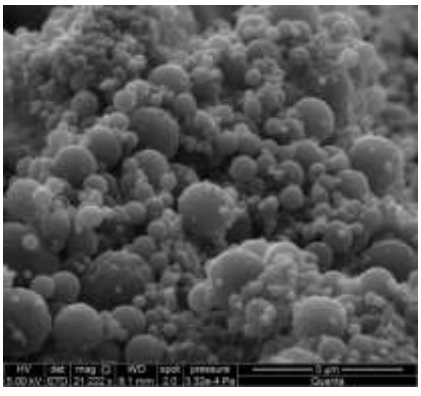


Figure 1: SEM observation of synthesized particles

References

[1] Maria E. Fortunato, Massoud Rostam-Abadi, and Kenneth S. Suslick, “Nanostructured Carbons Prepared by Ultrasonic Spray Pyrolysis” in *Chemistry of Materials Communication*

PROBING THE INTERACTIONS BETWEEN AIR BUBBLES AND (BIO)-INTERFACES AT THE MOLECULAR SCALE USING FLUIDFM TECHNOLOGY

Irem DEMIR[1,2,*], Ines LÜCHTEFELD[3], Claude LEMEN[1], Etienne DAGUE[2,4], Pascal GUIRAUD[1,4], Tomaso ZAMBELLI[3], and Cécile FORMOSA-DAGUE[1,4]

[1] TBI, Université de Toulouse, INSA, INRAE, CNRS, Toulouse, France

[2] LAAS, Université de Toulouse, CNRS, Toulouse, France

[3] Laboratory of Biosensors and Bioelectronics, Institute for Biomedical Engineering, ETH Zurich, Switzerland

[4] Fédération de Recherche Fermat, CNRS, Toulouse, France

demir@insa-toulouse.fr

Understanding the molecular mechanisms underlying bubble-(bio)surfaces interactions is currently a challenge that if overcame, would allow to understand and control the various processes in which they are involved. Atomic force microscopy is a valuable tool to measure such interactions, but it is limited by the large size and instability of bubbles that can be attached on surfaces or on AFM cantilevers. To overcome these challenges, we here develop a new method to probe more accurately the interactions between bubbles and (bio)-interfaces by taking advantage of the fluidic force microscopy technology (FluidFM) that combines AFM with microfluidics. In this system, a micro-sized channel is integrated into an AFM cantilever and connected to a pressure controller system, thus creating a continuous and closed fluidic conduit that can be filled with a solution, while the tool can be immersed in a liquid environment [1]. An aperture at the end of the cantilever allows liquids to be dispensed locally. In this study, we use FluidFM in an original manner, to produce microsized bubbles of 8 µm in diameter, directly at the aperture of the microchanneled FluidFM cantilevers. For that, as shown in Figure 1 instead of liquid, the cantilever is filled with air and immersed in a liquid environment. By applying a positive pressure inside the cantilever, we succeeded in forming bubbles of controlled size directly at its aperture. Because the same pressure is maintained in the cantilever during the experiment, the dissolution of the gases from the bubble is compensated, which allows keeping the size of the bubble constant over time. After the characterization of the bubbles produced using this method, their interactions with hydrophobic surfaces were probed, showing that bubbles behave like hydrophobic surfaces. Thus, they can be used to measure the hydrophobic properties of microorganisms' surfaces, but in this case the interactions are also influenced by electrostatic forces. Finally, we developed a strategy to functionalize their surface, thereby modulating their interactions with microorganisms' surfaces**.** This new method provides a valuable tool to understand bubble-(bio)surfaces interactions but also to engineer them.

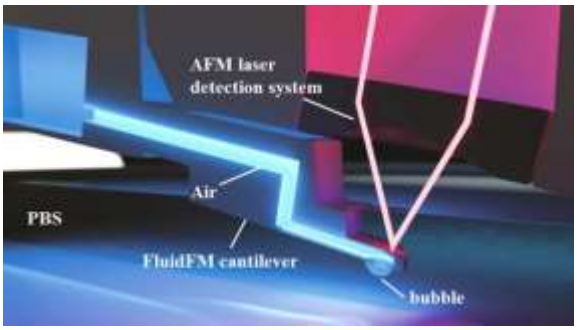


Figure 1: A tipless FluidFM microchanneled cantilever with a circular aperture of 8-µm diameter, connected to a pressure controller, is filled with air and immersed in liquid. By applying a positive pressure, an air bubble can be formed at the aperture of the cantilever.

References

[1] Meister, *et al*. Nano Letters 9.6 (2009): 2501-2507

In vivo bone regeneration with a bio-inspired coated membrane

M. Dubus[1], L. Scomazzon[1], L. Abou Nassif[1], A. Baldit[2], A. Beljebbar[3], J. Braux[1], C. Mauprivez[1] and H. Kerdjoudj[2]

[1]*Université de Reims Champagne Ardenne, EA 4691, Biomatériaux et Inflammation en Site Osseux (BIOS), Reims, France.*
[2]*LEM3-UMR-7239, CNRS - Arts et Métiers ParisTech; ENIM, Université de Lorraine, Metz, France.*
[3]*UMR CNRS 7369, BioSpectroscopie Translationnelle BioSpecT, Université de Reims Champagne Ardenne, Reims, France.*
e-mail: marie.dubus@univ-reims.fr

Guided bone regeneration (GBR) is one of the most attractive technique for restoring oral bone defects, where an occlusive membrane is positioned over the bone graft material, providing space maintenance required to seclude soft tissue infiltration and to promote bone regeneration. However, bone regeneration is in many cases impeded by a lack of an adequate tissue vascularization and/or by bacterial contamination. Therefore, a multifunctional membrane that kills bacteria and drives bone healing is urgently sought. Herein, we used simultaneous spray coating of interacting species (SSCIS) process to develop a bone inspired coating made of calcium phosphate nano particles and chitosan/hyaluronic acid nanofilm. The resulting hybrid coating was built on one side of a GBR membrane and the observation by scanning electron microscopy revealed the presence of mineral structures with rod-*like* shapes; These mineral structures were exposed on the membrane surface and occupied some space within the thickness of the membrane, suggesting the diffusion and the complexation of species within the collagenous fibers. The chemical composition of the hybrid coating analyzed by Confocal Raman spectroscopy mapping revealed the presence of dicalcium phosphate dehydrate phase. *In vitro* studies revealed that hybrid coating possesses excellent bioactivity and capability of inducing an overwhelmingly positive response of stromal cells and monocytes in favour of bone regeneration [1-3]. Furthermore, the antibacterial experiments showed that the hybrid coating provides contact-killing properties by disturbing the cell wall integrity of Gram-positive and Gram-negative bacteria. Its combination with stromal cells, able to release antibacterial agents and mediators of the innate immune response, constitutes an excellent strategy for fighting bacteria [4]. A preclinical *in vivo* study was therefore conducted in rat calvaria bone critical size defect (5 mm of diameter) and the newly formed bone was characterized eight weeks post implantation. µ-CT reconstructions showed that hybrid coated membrane favoured bone regeneration, as we observed a two-fold increase in bone volume/total volume ratios *vs*. uncoated membrane (p<0.005). The histological characterizations revealed that hybrid membrane was almost completely degraded, leading to the presence of a newly formed bone composed of mineralized collagen (Masson's Trichrome and Von Kossa staining). This newly formed bone was marked by the presence of osteoblasts, osteocyte lacunae, bone marrow and blood vessels, confirmed by CD31 positive cells (i.e. endothelial cell marker). CD68 positive cells (i.e. macrophage marker) were mainly localized in bone marrow. Confocal Raman spectroscopy analysis on freshly explanted specimen revealed the presence of bone specific bands, and mineral-to-collagen ratios (i.e. carbonate-to-collagen and phosphate-to-collagen) were higher in the newly formed bone in the presence of hybrid membrane *vs.* uncoated membrane. An increase in the mineral/matrix ratios in the presence of hybrid membrane could result in mature bone with an increase in the bone mechanical strength. Moreover, confocal laser scanning microscopy and second harmonic generation imaging showed a lamellar-*like* collagen assembly in the presence of hybrid membrane. Despite an increase in the stiffness of the newly formed bone with hybrid membrane (*vs*. uncoated membrane), the obtained values of stiffness were lower than those for native bone (approximately 5 times less). These significant data shed light on the regenerative potential of such bioinspired hybrid coating, providing a suitable environment for bone regeneration and vascularization, as well as an ideal strategy to prevent bone implant-associated infections.

References

[1] Rammal et al., ACS Appl. Mater. Interfaces. 2017, 9, 12791−12801.
[2] Rammal et al., Int. J. Mol. Sci. 2018, 19, 3458.
[3] Dubus et al., Colloids Surf. B Biointerfaces. 2019, 181, 671.
[4] Dubus et al., Biomater. Sci. 2020, 8, 5763.

Acknowledgements: This work was partially supported by "la région Grand Est" (ERMES project).

Electrosynthesis of free-standing composite films of chitosan – iron oxide nanoparticles

Amine Filali,[1] Guofeng Lu,[1] Magdalena Kaliszczak,[1] Manuel Dossot,[1] Liang Liu,[1] Joana Vazramos,[2,3] Sylvie Bégin,[2] Stéphane Le Calvet,[3] Grégoire Herzog[1], and Grégory Francius[1]

[1] Université de Lorraine, CNRS, LCPME, F-54000 Nancy, France
[2]Institute for Chemistry and Processes for Energy, Environment and Health 25 Rue Becquerel, 67087 Schiltigheim2,France
[3]Institut de physique et de chimie des Matériaux de Strasbourg, 23 Rue du Lœss Bâtiment 69, 67200 Strasbourg, France
gregoire.herzog@cnrs.fr

The usage of renewable and abundant polymers as like chitosan free standing films is an attempt to inhibit viral transmission. The combinaison with iron oxide raspberry-shaped nanostructures (RSNs) are suitable for the adsorbtion and decontamination of the virus using different modes (irradiation, ROS and photothermia).

We report here the electrodeposition of chitosan (CS) films at the interface between two-immiscible electrolyte solutions. The CS film formation was investigated under varying conditions of current, times and CS molecular weight. When a current was applied, CS adsorbed at the interface between the aqueous solution and the organic solution. The presence of terephthaldehyde (TPA) in the organic phase allowed the cross-linking of CS polymeric chains, giving rise to CS films as attested by Raman spectroscopy. CS deposition was examined under different deposition conditions of (i) current (1, 2, 5 mA), (ii) time (2000 and 4000 s) and CS molecular weight (high, medium, low). After the deposition, the CS films were characterized ex-situ using atomic force microscopy, scanning electron microscopy, raman spectroscopy and profilometry. AFM images shows that the roughness morphology is correlated to the deposition conditions, Chitosan polymer with high molar mass (310-375 $Kg.mol^{-1}$) allows us to obtain more homogeneous and thinner films. The objectif is to generate porous films CS/ RSNs that allowed the contact with the viral particles for their deactivation by photothermia and to avoid agglomerates for a homogeneous distribution in the films.

The photothermy measurements iron oxide raspberry-shaped nanostructures showed interesting property in photothermy efficiency that increase until 20 °C, Further study also is needed to investigate antibacterial activity of CS/ RSNs.

Antimicrobial patterned electrodes based on NIL technology

V. Genevskiy,[1,2] N. Nilsson,[2] K. Thulin,[2] Z. Lopes-Da-Silva,[3] S. Shleev,[1] and T. Ruzgas[1]

[1]*Department of Biomedical Science and Biofilms Research Center for Biointerfaces, Malmö University, 205 06 Malmö, Sweden*
[2]*Obducat Technologies AB, 223 63 Lund, Sweden*
[3]*Faculty of Odontology, Department of oral biology, Malmö University 214 21, Malmö, Sweden*
e-mail: vladislav.genevskiy@mau.se

Surface biofouling of industrial and medical devices remains a central challenge. The adhesive irreversibility of tenacious bacterial agglomerates known as biofilm, represents an arduous enemy to eradicate. In medical industry in particular, therapy resilience predisposes the relapse of infections over long periods of time. Indwelling devices such as catheters, vascular access devices and airway tubes are well known to be one of the main leading causes of nosocomial infection onset, setting a challenging task for the healthcare systems around the globe [1], [2]. Traditional methods to solve implant-associated infections involve the use of antimicrobial or antibiotic agents released by the implant itself or administered as a drug, however, this approach often results in uncontrolled overuse, side-effects and, on a larger scale, leads to antimicrobial resistance [3], [4].
A safer and more sustainable approach may rely on surface topographies. In the last decade a great interest was focused on antimicrobial surfaces inspired by nature. Animals spanning different kingdoms, from cicadas to sharks, have shown astonishing strategies in preventing the early adhesion of bacteria by developing specialised patterns on their bodies [5], [6]. The surface pattern at the micro and nano scale can hamper the early bacterial colonisation, delaying or preventing the biofilm development.
Here we show the versatility of nanoimprint lithography (NIL) utilising the IPS® technology from Obducat Technologies AB over the traditional patterning techniques for biomedical applications. The parallel replication of 3D patterns across a large length scale, combined with the capability to print on a variety of polymers and flexible non-planar substrates, makes NIL an ideal candidate for both research and industrial applications in this field. Our aim is to propose a cost-effective way of pattern-fabrication on flexible substrates and subsequently, study the impact that spacing, and aspect ratio of the architectures can have on the bactericidal properties.
In fact, by depositing a thin layer of metal on top of the pattern, the surface is turned into a sensing electrode. This step allows us to carry out non-destructive electrochemical measurements (open circuit potential, OCP) enabling the detection of an early biofilm formation and the evaluation of the anti-microbial efficacy of certain patterns over the others.

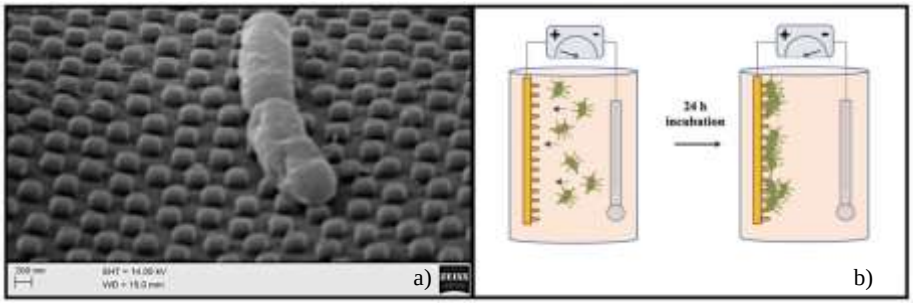


Figure 1. a) SEM micrograph of a NIL-patterned electrode incubated with a E. coli for 24h. b) Schematic representation of the OCP measurement.

We gratefully acknowledge financial support from the Knowledge Foundation, viz “ComBine” Industrial PhD school and “Biobarriers” profile at the Biofilms Research Center for Biointerfaces.

References

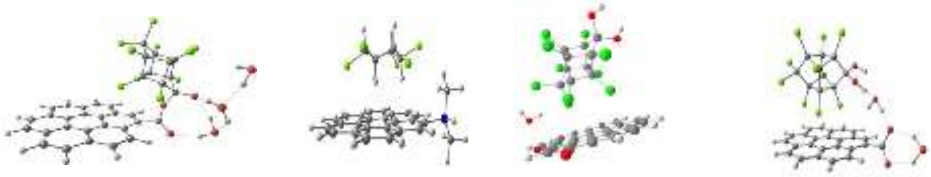
[1] P. Dadgostar, *IDR*, vol. Volume 12, pp. 3903–3910, Dec. 2019.
[2] F. Prestinaci, P. Pezzotti, and A. Pantosti, *Pathogens and Global Health*, vol. 109, no. 7, pp. 309–318, Oct. 2015.
[3] I. Banerjee, R. C. Pangule, and R. S. Kane, *Adv. Mater.*, vol. 23, no. 6, pp. 690–718, Feb. 2011
[4] H. van D. Belt, D. Neut, W. Schenk, J. R. van Horn, H. C. van D. Mei, and H. J. Busscher, *Acta Orthopaedica Scandinavica*, vol. 71, no. 6, pp. 625–629, Jan. 2000.
[5] S. Rostami *et al. Applied Surface Science*, vol. 544, p. 148828, Apr. 2021.
[6] E. P. Ivanova *et al. Small*, vol. 8, no. 16, pp. 2489–2494, Aug. 2012.

CHLORDECONE AND β-HEXACHLOROCYCLOHEXANE INTERACTION WITH ACIDIC AND BASIC FUNCTIONALIZED ACTIVATED CARBON BY MOLECULAR MODELLING AND MOLECULAR DYNAMICS SIMULATION

F. Goudou[2], A. Duro[2], K. Melchor-Rodríguez[1], C. Carmenate-Rodríguez[1], A. Ferino-Pérez[1], J. J. Gamboa-Carballo[1], B. Minofar, S. Gaspard[2], U. J. Jáuregui-Haza[3].

[1]INSTEC, University of Havana, Avenida Salvador Allende, 1110, Quinta de los Molinos, Plaza de la Revolución, A.P. 6163, La Habana, Cuba.
[2]Laboratoire COVACHIM M2E, EA 3592, Université des Antilles, 97157 Pointe à Pitre Cedex, Guadeloupe.
[3]Instituto Tecnológico de Santo Domingo, Ave. de los Próceres, Santo Domingo, República Dominicana
[4] Institute of Microbiology of the Czech Academy of Sciences · Laboratory of Structural Biology and Bioinformatics
e-mail:francesca.goudou@univ-antilles.fr

A molecular modeling study of the influence of acidic [1-3] and basic [4] surface groups (SG) of activated carbon (AC) model on chlordecone (CLD) and β-hexachlorocyclohexane (β-HCH) adsorption is presented, in order to help understanding the adsorption process considering pH and hydration effect. A coronene molecule, with the functional groups under study in the edge, were used as a simplified model of AC. Multiple Minima Hypersurface methodology was employed to study the interactions of CLD and β-HCH with SGs on AC using PM7 semiempirical Hamiltonian. A further re-optimization of obtained structures was done for pesticide-AC complexes by means of Density Functional Theory. The Quantum Theory of Atoms in Molecules was used to characterize the interaction types using the Nakanishi criteria. As results, the interactions are governed by dispersive interactions of chlorine atoms of the pollutants with the graphitic surface and by electrostatic interactions with COO^- and O^- acidic groups and water molecules. For oxygenated basic SGs, like pyrone, chromene and ketone, no interactions have been shown at acidic pH for both pollutants whereas dispersive interactions have been found at neutral and basic pH. For nitrogenous basic SGs, the results showed a greater association of both pesticides with the primary amine in comparison with the pyridine, secondary and tertiary amine in the absence and presence of water molecules, and this behavior increase at acidic pH conditions where the amines and pyridine are protonated. As conclusion, significant associations of acidic SGs with CLD suggest a chemical sorption at slightly acidic and neutral pH conditions. On the other hand, the interactions of both pollutants with basic SGs on AC are similar with the physisorption process. Finally, an increase in carboxylic SGs content is suggested to enhance CLD and β-HCH adsorption onto AC. Molecular dynamics simulation is as well employed to understand experimental findings.



Donor-acceptor interaction **Dispersive interaction** **Electrostatic interaction**

References

[1] J.J. Gamboa-Carballo *et al.*, Theoretical Study of Chlordecone and Surface Groups Interaction in an Activated Carbon Model under Acidic and Neutral Conditions. J. Mol. Graph. Mod. 65 (2016) 83-93.
[2] K. Melchor-Rodríguez *et al.*, Theoretical study on the interactions between chlordecone hydrate and acidic surface groups of activated carbon under basic pH conditions. J. Mol. Graph. Model. 81 (2018) 146-154.
[3] K. Melchor-Rodríguez *et al.*, Chlordecone adsorption on functionalized activated carbons: Computational chemistry as a tool for understanding the adsorption process. Quim. Nova. 44(2) (2021) 172-179.
[4] K. Melchor-Rodríguez et al., Role of Basic Surface Groups of Activated Carbon in Chlordecone and β-Hexachlorocyclohexane Adsorption: A Molecular Modelling Study. Molecules. 26 (2021) 6969.

SARS-CoV-2 Spike interaction using Surface Plasmon Resonance

L. Hain,[1] M. Klausberger,[2] E. Laurent,[2,3] G. Wirnsberger,[4] N. F. Kienzl,[5] L. Mach,[5] J. M. Penninger,[6,7] D. Markovitz,[8] P. Hinterdorfer,[1] and Y.J. Oh[1]

[1]Institute of Biophysics, Johannes Kepler University Linz, Gruberstr. 40, 4020 Linz, Austria
[2]University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Molecular Biotechnology, Vienna, Austria[3]University of Natural Resources and Life Sciences, Vienna, Core Facility Biomolecular & Cellular Analysis, Vienna, Austria
[4]Apeiron Biologics, Vienna, Austria
[5] University of Natural Resources and Life Sciences, Vienna, Department of Applied Genetics and Cell Biology, Institute of Plant Biotechnology and Cell Biology, Vienna, Austria
[6]Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna, Austria.
[7]Department of Medical Genetics, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada.
[8]Division of Infectious Diseases, Department of Internal Medicine, and the Programs in Immunology, Cellular and Molecular Biology, and Cancer Biology, University of Michigan, Ann Arbor, MI 48109, USA.

e-mail: lisa.hain@jku.at

The main entry route of the SARS-CoV-2 is mediated by the viral Spike protein, which binds to the angiotensin converting enzyme 2 (ACE2) expressed on host cells.[1] To be able to understand the molecular interactions of the viral Spike protein with ACE2 in detail, reliable quantification of binding kinetics and affinity is of key importance. Surface plasmon resonance (SPR) has developed towards a major tool for probing the interactions between an analyte and a ligand. In this study, we characterized the molecular interactions between the SARS-CoV-2 Spike protein and various cognitive receptors in addition to ACE2 by SPR. This allowed us to elucidate auxiliary modes of viral binding and to characterize novel therapeutic approaches against Covid-19.

First, we investigated the binding affinity between soluble ACE2 receptors and the *wildtype* SARS-CoV-2 Spike protein and confirmed that the Spike receptor binding domain (RBD) is the major protein entity involved in the ACE2 interactions. Kinetic rate constants between ACE2 and trimeric *wildtype* spike and a spike glyco-mutant with an ablation of a glycan (N234) close to the receptor binding domain have been investigated. Additionally, kinetic profiles of the *wildtype* RBD and RBD proteins harbouring mutations characteristic for several emerged variants (N501Y, E484K) were determined.

For a pharmacological application, we tested different lectins, which are highly specific for carbohydrates of Spike protein. Two lectins, Clec4g and CD209c, were identified to strongly bind to glycans within the Spike RBD. The interactions between Spike and human/murine Clec4g and CD209c were characterized by deriving the dissociation equilibrium constant K_D, using a bivalent binding model.[1] We also determined the binding capacity of a molecularly engineered lectin cloned from banana, H84T BanLec, which was shown to have broad-spectrum antiviral activity against several RNA viruses.[2] Our studies with *wild type*, Omicron and Delta spike and spike glycomutant N234Q revealed that H84T BanLec binds to the glycans that are located outside of the spike RBD variants and glycomutants.

In conclusion, we applied SPR using different binding models to assess binding of the ACE2 receptor as well as various lectins to different locations within the SARS-CoV-2 spike protein. Our studies contribute to the understanding of molecular binding mechanisms for developing new strategies to prevent virus entry to host cells and for characterizing new therapeutics against Covid-19.

References

[1] D. Hoffmann et al., The EMBO journal 40, 19 (2021) e108375.
[2] M. G. Lloyd et al. Scientific reports 12,1 (2022) 1641.

Acknowledgement: L.H acknowledges the financial support from the ÖAW fellowship STIP13202002 and the Austrian Science Fund FWF project I3173.

Eradicating bacterial infections by nanomedecine

Miryana Hémadi,[1] Zhendong Peng,[1] Yun Luo,[2] Claire Mangeney,[2] Christine Gravier-Pelletier,[2] Sergio Moya,[3] John S. Lomas,[1] Laurent Royon,[4] Romain Briandet.[5]

[1] Université de Paris, CNRS-UMR 7086, Interfaces, Traitements, Organisation et DYnamique des Systèmes (ITODYS), Paris, France

[2] Université de Paris, CNRS-UMR 8601, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Paris, France

[3] CIC biomaGUNE, Paseo de Miramón 182, 20014 Donostia-San Sebastián, Spain

[4] LIED Laboratory - IUT Paris Pajol, Université de Paris, Paris, France.

[5] Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, Jouy-en-Josas, France.

e-mail: hemadi@u-paris.fr

The failure of conventional treatments of bacterial infections and biofilms has become a major human health problem [1]. We are working on the design of new hybrid multifunctional nanomaterials for the eradication of bacteria and, therefore, biofilms by drug targeting and photothermia (PT) [2-5].
This involves the construction of assemblies of different building blocks, namely gold nanoparticles (NPs) of several shapes (nanorods, nanostars and spheres) with active molecules, notably enzymes, bactericides and inhibitors. The activity of these composites will be enhanced by means of NIR laser irradiation to trigger local heating. Photothermal stimulation of the nanomaterial will disrupt the biofilm and release the active molecules which go on to attack the planktonic bacteria whose membranes are already weakened by PT. The nanomaterials are characterized by several techniques (XPS, SEM, FTIR, DLS, etc.) and their light-to-heat conversion parameters determined in the presence and absence of biofilm. A laser sheet and a high-speed camera are used to investigate the mechanism of nanomaterial displacement in the biofilm by Particle Image Velocimetry. Moreover, the internalization of the gold NPs inside the biofilm and their interaction with bacteria are tracked by Raman spectroscopy.
This approach exploits technologies in material biosciences, from the engineering of bacterial inhibitors to the design of colloidal nanoplatforms, to discover ways of penetrating biofilm structure. The employment of stimulable nanomaterials carrying relevant antibacterial agents will open up new perspectives in the field.

References

[1] C. Sahli, S. Moya, J.S. Lomas, C. Gravier-Pelletier, R. Briandet, M. Hémadi. **Theranostics, 2022,** 12, 2383.
[2] H. Belkahla, R. Boudjemaa, V. Caorsi, D. Pineau, A. Curcio, J.S. Lomas, P. Decorse, A. Chevillot-Biraud, T. Azaïs, C. Wilhelm, H. Randriamahazaka, M. Hémadi. **Nanoscale Advances**, **2019**, 9, 5755-5768
[3] J. Hai, N. Serradji, L. Mouton, V. Redeker, D. Cornu, J.M. El Hage Chahine, P. Verbeke, M. Hémadi. **PLoS One, 2016**, 11(2):e0150031.
[4] J. Hai, H. Piraux, E. Mazario, J. Volatron, N.T. Ha-Duong, P. Decorse, J.S. Lomas, P. Verbeke, S. Ammar, C. Wilhelm, J.M. El Hage Chahine, M. Hémadi. **J Mater Chem B. 2017**; 5: 3154-62
[5] H. Belkahla, E. Mazarío, A. Plan Sangnier, J.S. Lomas, T. Gharbi, S. Ammar, O. Micheau, C. Wilhelm, M. Hémadi. **Theranostics, 2019,** 9, 2383.

Silver modified surfaces as a platform for basic research and other medical applications

L. Hochvaldova,[1] L. Válková,[2] M. Mistrik,[3] Z. Skrott,[3] L. Kvítek[1] and A. Panáček[1]

[1]*Department of Physical Chemistry, Faculty of Science, Palacky University, Olomouc, Czech Republic*
[2]*Department of Biophysics, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic*
[3]*Laboratory of Genome Integrity, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic*

e-mail: lucie.hochvaldova@upol.cz

Silver nanoparticles are in most of the cases prepared in colloidal form. Later, they can be coated on different surfaces (e.g., cultivation plates, medical devices) and form 2D films. Plasmonic silver nanoparticles were chosen for localized thermal therapy for their strong localized surface plasmon resonance (LSPR) and their tuneable photothermal properties. If the frequency of the incident light (laser) matches with the frequency of the LSPR, light is absorbed more efficiently and the energy can be easily converted to the heat. Desired wavelength, therefore the surface plasmon, is therefore selectively changed via adjusting the structure and morphology of the silver nanoparticles.

Plasmonic properties of silver nanoparticles can be used in light-assisted therapeutic methods (e.g., photothermal, photodynamic therapy), which use the physical properties of light and its transformation to heat to cause selective hyperthermia and irreversible damage of living cells and the treatment of various diseases (antibacterial, anticancer therapy). Photothermal effect in combination with silver modified surfaces can be used to trigger heat-shock and to inflict thermal protein damage in subcellular compartments, which causes rapid heat-shock chaperone recruitment and ensures the engagement of the ubiquitin–proteasome system, which provides insights into the spatiotemporal response to thermal damage relevant for degenerative diseases.(1)

Overall, presented methodology of silver nanoparticles coated onto various surfaces can be used to enhance absorption properties, scattering and conversion of energy to heat, with use in photothermal/photodynamic therapy and the treatment of the bacterial/cellular infection or in studies of microthermal-induced protein damage in cells, which could be used in screening strategies, diagnostics and studies of molecular pathways, which are helpful in research of hallmarks of various diseases and has a broad applicability in biomedicine.

This work was supported by the students grant nr. DSGC-2021-0120 "Light-assisted in vitro therapy using plasmonic materials" funded under the OPIE project "Improvement of Doctoral Student Grant Competition Schemes and their Pilot Implementation", reg. no. CZ.02.2.69/0.0/0.0/19_073/0016713.

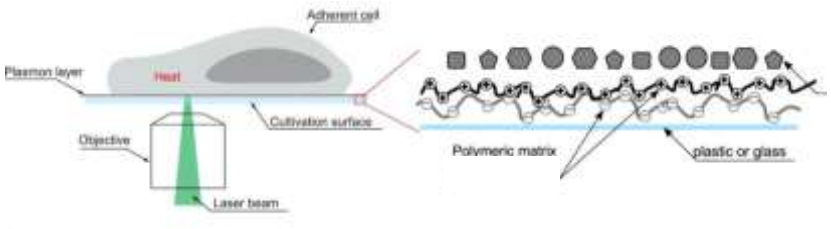


Figure 1: Schematic of the modification of the cultivation plate and its activation by laser (1)

References

[1] M. Mistrik et al. Microthermal-induced subcellular-targeted protein damage in cells on plasmonic nanosilver-modified surfaces evokes a two-phase HSP-p97/VCP response. Nat Commun. 2021;12(1).

Nanoscale biophysics to study molecular mechanism of Alzheimer's disease.

Z.Leonenko[1,2], M.Robinson[2], N.Mei[2], C.Filice[2] and E.Drolle[2]

[1]*Department of Physics and Astronomy,*
[2]*Department of Biology,*
University of Waterloo

e-mail: zleonenk@uwaterloo.ca

Alzheimer's disease (AD) is a neurodegenerative disease characterized by dementia and memory loss for which no cure or prevention is available. Amyloid toxicity is a result of the non-specific interaction of toxic amyloid oligomers with the plasma membrane.
We studied amyloid aggregation and interaction of amyloid beta (1-42) peptide with model lipid membranes using atomic force microscopy (AFM), Kelvin probe force microscopy and surface Plasmon resonance (SPR). Using AFM-based atomic force spectroscopy (AFS) we measured the binging forces between two single amyloid peptide molecules [1.3]. Using AFM imaging we showed that oligomer and fibril formation is affected by surfaces, presence of metals and inhibitors. We demonstrated that lipid membrane plays an active role in amyloid binding and toxicity: changes in membrane composition mimicking neuronal membrane in health and AD, the presence of cholesterol and melatonin are discussed [2-6]. We discovered that membrane cholesterol creates nanoscale electrostatic domains which induce preferential binding of amyloid peptide [4], while membrane melatonin reduces amyloid-membrane binding [7]. Using AFS we showed that novel pseudo-peptide inhibitors effectively prevent amyloid-amyloid binding on a single molecule level, to prevent amyloid toxicity [1.3]. These findings contribute to better understanding of the molecular mechanisms of Alzheimer's disease and aid to the developments of novel strategies for cure and prevention of AD.

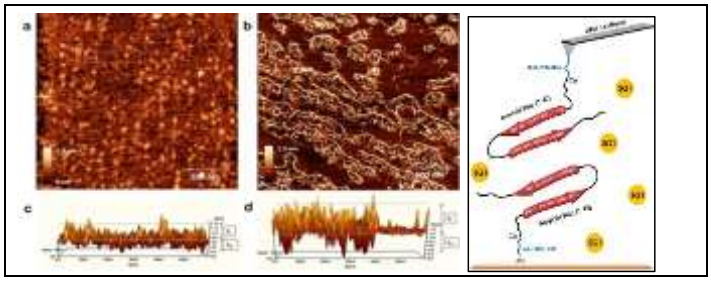


Figure 1: AFM images amyloid binging to membrane and AFS binding of two amyloid peptides [4,3].

References

[1]. M.Robinson, J.Lou, B.Mehrazma, A.Rauk, M.Beazely, Z.Leonenko, Pseudopeptide Amyloid Aggregation Inhibitors: in silico, single molecule and cell viability studies. *Int.Journal Molecular Sciences*, 2021, *22*(3), 1051
[2]. E.Drolle, K.Hammond, A.Negoda, E.Pavlov, Z.Leonenko, Changes in lipid membranes may trigger amyloid toxicity in Alzheimer's disease. *PLOS ONE*, 2017, 12(8), e0182194.
[3]. B.Mehrazma, M.Robinson, S.K.A.Opare, A.Petoyan, J.Lou, F.T.Hane, A.Rauk, Z.Leonenko. Pseudo-peptide amyloid-β blocking inhibitors: molecular dynamics and single molecule force spectroscopy study. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* 1865 (11), 1707-1718.
[4]. E.Drolle, R.M.Gaikwad, Z.Leonenko, Nanoscale electrostatic domains in cholesterol-laden lipid membranes create a target for amyloid binding. *Biophysical Journal,* 2012, 103(4), L27-L29.
[5]. E.Drolle, F.Hane, B.Lee, Z.Leonenko, Atomic force microscopy to study molecular mechanisms of amyloid fibril formation and toxicity in Alzheimer's disease. *J. of Drug Metabolism Reviews*, 2014, 46(2): 207-223.
[6]. E.Drolle, N. Kučerka, M.I.Hoopes, Y.Choi, J. Katsaras, M. Karttunen, Z.Leonenko, Effect of melatonin and cholesterol on the structure of DOPC and DPPC membranes, *Biochimica & Biophysica Acta: Biomembranes*, 2013, 1828 (9): 2247-2254.
[7]. E. Drolle, A. Ollagnier, E. Finot, Z. Leonenko (2016). Surface Plasmon Resonance Imaging to Study the Molecular Mechanism of Alzheimer's Disease. Proceedings, *All Sensors*. ISBN: 978-1-61208-523-4.

Nanomechanical Properties of the Articular Cartilage in a Murine Osteoarthritis Model Investigated by Atomic Force Microscopy

V. Maier,[1] S. Stöckl,[2] L. Fleischhauer,[1] D. Muschter,[2] S. Grässel[2], and H. Clausen-Schaumann[1]

[1]*Munich University of Applied Sciences, Lothstr. 34, 80335 Munich, Germany*
[2]*Department of Orthopaedic Surgery, ZMB im Biopark1, University of Regensburg, Am Biopark 9, 93053 Regensburg, Germany*
e-mail: verena.maier@hm.edu

Osteoarthritis (OA) is the most frequent musculoskeletal disease worldwide and its prevalence is rising due to the Western life style including increasing age [1]. For diagnosis of OA, methods such as radiography or arthroscopic evaluation of the joint surface are frequently used. However, to understand the pathophysiology of OA development, additional information on the micro- and nanoscale is needed, where the pathophysiological processes leading to OA and the subtle changes occuring in early OA can first be detected. During OA progression, measurable changes in the nanomechanical properties of the articular cartilage tissue occur before changes in the cartilage morphology can be detected. The biomechanical properties on the micro- and nanometer scale are therefore a powerful marker for the early onset of cartilage degradation. Indentation-type atomic force microscopy (IT-AFM) can detect and monitor these changes of the biomechanical properties at the nanometer scale already in the early stages of OA in both animal model systems and in patient samples. [2, 3, 4]

Recently, the role of the sensory neuropeptides substance P (SP) and alpha calcitonin gene-related peptide (αCGRP) in OA development and progression has come into focus. In a recent study, we have used histology, nano-CT and IT-AFM to identify and compare the structural and biomechanical changes in murine articuar cartilage upon aging and surgical OA induction in wild-type (WT), SP knock out (KO) and αCGRP KO mice. [5] In addition to a protective effect against age-related OA pathology by SP and αCGRP, we found considerable differences of the cartilage nanomechanical properties between WT and KO, both age-related and upon OA induction. To further elucidate the effects of SP and αCGRP on cartilage biomechanics and OA, we have now investigated the nanomechanical properties in a surgical murine OA model system with intra-articular injected bone marrow derived mesenchymal stem cells overexpressing SP and αCGRP in WT mice. Our poster will present the first results of this IT-AFM study.

References

[1] F. Berenbaum, I. J. Wallace, D. E. Lieberman, and D. T. Felson, “Modern-day environmental factors in the pathogenesis of osteoarthritis,” Nat. Rev. Rheumatol., vol. 14, no. 11, pp. 674–681, 2018.
[2] Stolz, Martin; Gottardi, Riccardo; Raiteri, Roberto; Miot, Sylvie; Martin, Ivan; Imer, Raphaël et al. (2009): Early detection of aging cartilage and osteoarthritis in mice and patient samples using atomic force microscopy. In: Nature nanotechnology 4 (3), S. 186–192. DOI: 10.1038/nnano.2008.410.
[3] Hartmann, Bastian; Marchi, Gabriele; Alberton, Paolo; Farkas, Zsuzsanna; Aszodi, Attila; Roths, Johannes; Clausen-Schaumann, Hauke (2020): Early Detection of Cartilage Degeneration: A Comparison of Histology, Fiber Bragg Grating-Based Micro-Indentation, and Atomic Force Microscopy-Based Nano-Indentation. In: International journal of molecular sciences 21 (19). DOI: 10.3390/ijms21197384.
[4] Loparic, Marko; Wirz, Dieter; Daniels, A. U.; Raiteri, Roberto; Vanlandingham, Mark R.; Guex, Geraldine et al. (2010): Micro- and nanomechanical analysis of articular cartilage by indentation-type atomic force microscopy: validation with a gel-microfiber composite. In: Biophysical journal 98 (11), S. 2731–2740. DOI: 10.1016/j.bpj.2010.02.013.
[5] Muschter, Dominique; Fleischhauer, Lutz; Taheri, Shahed; Schilling, Arndt F.; Clausen-Schaumann, Hauke; Grässel, Susanne (2020): Sensory neuropeptides are required for bone and cartilage homeostasis in a murine destabilization-induced osteoarthritis model. In: Bone 133, S. 115181. DOI: 10.1016/j.bone.2019.115181.

Determination of the mechanical properties of red blood cells in sickle cell disease by means of AFM technique

Georges Minatchy [1], Presenting author Laurence Romana [1], Grégory Francius [2], Marc Romana [3]

[1] Groupe de Technologie des Surfaces et des Interfaces - Université des Antilles - 97 159 Pointe à Pitre - Guadeloupe - France - French West Indies

[2] LCPME - UMR 7564, 405 rue de Vandoeuvre, F-54600 Villers-lès-Nancy, France

[3] UMR S_1134, Université des Antilles, Pointe-à-Pitre, F-97100 France, F-97100, France - French West Indies

Sickle cell disease (SCD) is the most frequent genetic disease in the West Indies and in France. This disorder is caused by polymerization of the abnormal hemoglobin S which results from the substitution of an acid glutamic by a valine at codon 7 of the beta globin chain. Polymerization of deoxygenated hemoglobin S induces formation of long stiff rod-like fibers which force the red blood cells to take over a wide variety of irregular shape. SCD patients exhibit life-threatening complications such as chronic anemia and vaso-occlusion events due to reduced sickle red blood cell (RBC) deformability and increased RBC adherence to endothelial cells [1]. In this study, the mechanical properties of the red blood cells are investigated by atomic force microscopy. For this purpose, the cells are immobilized on glass lamella coated with poly-L-lysine in order to increase RBC adherence. All the experiments are performed in presence of phosphate buffered saline (PBS). Red blood cells were imaged with silicon nitride probe with nominal spring constant of 0.06 N/m whereas the mechanical measurements were performed with silicon tips colloidal particle with nominal spring constant of 0.035 N/m. All force/deformation curves were analyzed with PUNIAS (Protein Unfolding and Nano-Indentation Analysis Software), a custom-built semi-automatic processing and analysis software. To calculate the young modulus, we used Sneddon's modification of the Hertz model for the elastic indentation of a flat and soft sample by a stiff sphere [2]. Four different groups of patient (n=5) were analyzed, AA, AS, SS and SS with hydroxyurea treatment. More than 150 mechanical tests have been performed on each patient sample in order to have reliable statistical data. The results show that the SS red blood cells are stiffer than AA cells, whereas we do not observed any difference between AS and SS cells. We also observed a decrease of the young modulus for the patients treated with hydroxyurea.

REFERENCES

[1] Microelasticity of red blood cells in sickle cell disease, J. L. Maciaszek, B. Andemariam, and G Lykotrafitis, J. Strain Anal. Eng. Des. 46(5), 368–379 (2011)

[2] Elasticity measurement of living cells with an atomic force microscope: data acquisition and processing, Carl P. and Schillers H., Pflugers Arch - Eur J Physiol, 457:551–559 (2008)

ACKNOLEDGEMENT

The authors would like to thanks the Fond Social Européen and La Région Guadeloupe for their financial support.

Self-sensing cantilevers for biological sample characterization in liquid

M. Németh[1], H. Seferovic[1], E. Sitter[1], V. Stavrov[2], D. Bernhard[3], C. Schwalb [4] , A. Ebner[1], M. Leitner[1]

[1] *Institute of Biophysics, Department of Applied Experimental Biophysics, Johannes Kepler University, A-4020 Linz, Austria*
[2] *AMG Technology Ltd, Botevgrad, Bulgaria*
[3] Zentrum für Medizinische Forschung, *Johannes Kepler University, A-4020 Linz, Austria*
[4] *Quantum Design Microscopy GmbH, 645293, Darmstadt, Germany*

e-mail: margit.nemeth@jku.at

Atomic force microscopy (AFM) is one of the well-suited high-resolution microscopy techniques used for nano-scale microscopy and characterization. In case of conventional state-of-the-art AFM optical deflection detection of the cantilever is used for high-resolution imaging, but this method has disadvantages regarding stability, and it is very difficult to automate as well as the fusion with other analysis techniques are limited due to the lot of optical components. The other drawback of the optical beam deflection (OBD) that it is not possible to work with them in turbid liquids, these setups are limited to dry state and transparent liquids.

Self-sensing cantilever can overcome the above-mentioned limitations of the traditional AFM readout method and the biggest preference of these cantilevers that they have the same resolution and sensitivity as the traditional optical readout cantilevers. The self-sensing cantilevers were used with all-electric AFM system.

The aim of our experiments is to investigate biological samples in physiological-relevant environment. In most cases it means non-transparent or scattering liquids as the circumstances of the measurement.

During our experiment we started to optimize measuring biological samples in non-transparent liquid. We imaged fixed human smooth muscle cells (SMC) with traditional AFM in dry state and rehydrated in transparent liquid and we compared these results with measurements in physiological environment - meaning cell culture media, self-sensing cantilevers and all-electric-AFM. Additionally, we investigated thrombocytes and erythrocyte ghost cells with the all-electric AFM setup under different conditions from dry state to different transparent and non-transparent liquids.

References

[1] M. Leitner, H. Seferovic, S. Stainer, B. Buchroithner, CH. Schwalb, A. Deutschinger, A. Ebner ; Atomic Force Microscopy Imaging in Turbid Liquids: A Promising Tool in Nanomedicine. Sensors (2020)

Bio-inspired active nano-membrane

N. Tormena,[1] K. Voitchovsky,[1] and T. Pilizota 3[2]

[1]*Department of Physics, Durham University, South Road, Durham DH1 3LE, UK.*
[2]*Systems and Synthetic Biology Centre and School of Biological Sciences, University of Edinburgh, Alexander Crum Brown Road, Edinburgh EH9 3FF, UK*
e-mail: nicolo.tormena@durham.ac.uk

Bacterial membranes play a crucial role in many physiological and pathological conditions, from providing a physical barrier sustaining the integrity of the bacterium to controlling membrane trafficking, shape changes including growth and underpinning drugs resistance. Biochemically, these membranes comprise of a hydrophobic core, formed by a self-assembled double layer of phospholipids, and a hydrophilic surface made by the lipid headgroups. Within this double layer, sugars and proteins are bound or embedded and mediate specific signals and responses to external stimuli. The composition of these membranes is constantly evolving and can actively and precisely respond to changes in the environment through complex chemical and mechanical interactions between proteins and lipids. However, our understanding of this communication within lipid membranes is currently limited by the ability to track in real time the nanoscale changes they undergo when subject to any external stimulus.
A promising approach is to simplify the problem by using a model biological membrane, which consist of an artificial, well-controlled membrane where the contribution of the different elements to the overall response of the system can be distinguished. Currently, limited information is available for model bacterial cell membranes. Here, we build a model membrane system for *E Coli*, aiming to embed proteins and investigate the resulting mechanical interactions when exposed to external stimuli. We combine atomic force microscopy and differential scanning calorimetry to identify the simplest lipids combination that mimics the membrane's transition temperature while broadly retaining the main elements of its molecular composition. The model system structure and properties are compared to previous bacterial cell membrane analysis showing an excellent agreement. This opens up new possibilities for molecular-level biological studies where it can work as a platform to incorporate specific bioactive proteins and study their functions in the context of the surrounding lipids.

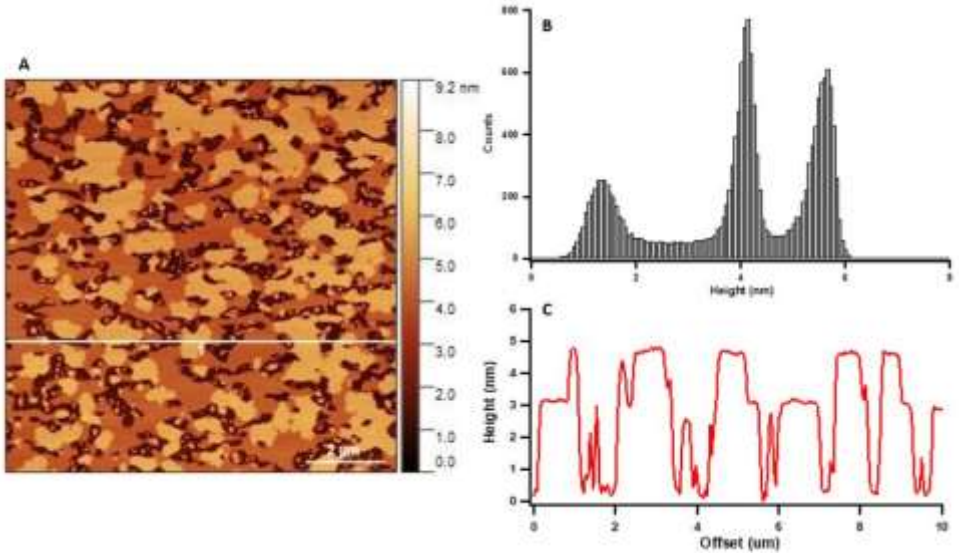


Figure 1: Temperature induced phase separation in a model membrane made by POPE – DPPG (3:1 molar ratio) in X1 MOPS saline buffer. (A) AFM topographical image of the membrane; (B) Histogram of the heights distribution in the membrane; (C) Profile traced on the AFM image graphically showing the height difference of the lipid phases.

Biophysical analysis of small extracellular vesicles (sEVs) and their interaction with model plasma membrane

(C. Paba)[1],V. Dorigo[2], B. Senigagliesi[3], H. Vondracek[4], V. Rondelli[5], P.Parisse[6], L.Casalis[4,*]

[1]University of Trieste, Trieste, Italy, [2]Hochschule Fresenius, Idstein, Germany , [3]SISSA, Trieste, Italy, [4]Elettra Sincrotrone Trieste, Trieste, Italy, [5] University of Milan, Milan ,Italy, [6]IOM-CNR, Trieste, Italy, [4,]Elettra Sincrotrone Trieste, Trieste, Italy,*
e-mail: carolina.paba@phd.units.it

Small extracellular vesicles (sEVs) are nanometer-sized vesicles (30-200 nm) that are released from the vast majority of cells both in pathological or physiological conditions. These biological nanoparticles have shown potential for cancer diagnostics/therapeutics as they travel in bodily fluids (blood, saliva) to carry biological information between distant cells. However, because of their high heterogeneity (e.g. origin, composition, size), the processes of EV biogenesis, release and uptake are still subject of debate, as well as the involved targeting cell receptors and internalization pathways (e.g. cell membrane fusion and endocytosis).

Here we propose an assay based on Atomic Force Microscopy, to reveal purified EVs' structure-function correlations, obtained from two breast cancer cell lines with different aggressiveness. Their interaction with lipid model membrane systems with variable composition (e.g. mixing of glycolipids, sphingolipids, cholesterol) and in different environmental conditions (e.g. temperature, pH, buffer) has been tested; functional molecules such as tetraspanin proteins have been added to clarify their role in EV uptake. Preliminary AFM analysis, performed with sEVs isolated from human umbilical cord-derived mesenchymal stromal cells (UC-MSCs) as a reference sample, revealed different interaction mechanisms of EVs with lipid model membranes depending on the adopted isolation method, salt concentration, and cholesterol percentage. In both cases, a preferential interaction with raft-like lipid domains has been observed. These results lead to a better understanding of both the EVs uptake mechanisms by recipients cells and the relevant parameters involved in the EVs' selective targeting in tumor microenvironments.

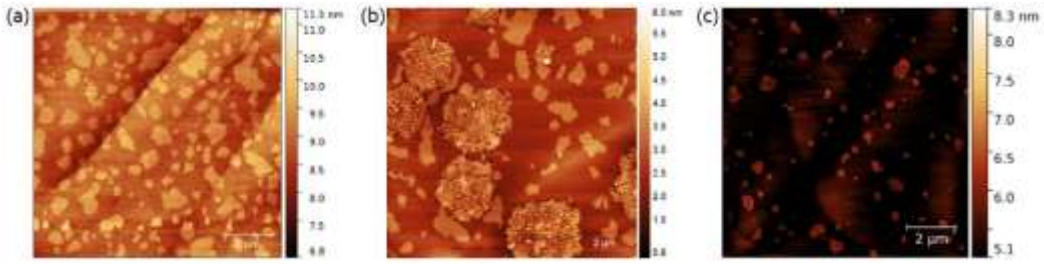


Figure 1: AFM topographic images of a DOPC : SM : Chol (2 : 1 : 0.17) SLB before (a) and after the addition of sEVs from UC-MSC cell line (b). We also report the SLB enriched with CD63 protein (c).

References

[1] Uribe, Johana, et al. ACS Biomaterials Science & Engineering. 2020, 6.7: 3945-3956.
[2] E Van Der Pol, et al. Journal of Thrombosis and Haemostasis. 2010, 8(12):2596{2607.
[3] Shivani Sharma, et al. ACS nano. 2010, 4(4):1921{1926.
[4] Clotilde Théry, et al. Journal of extracellular vesicles. 2018, 7(1):1535750.
[5] Edwin Van der Pol, et al. Pharmacological reviews. 2012, 64(3):676{705.
[6] Yáñez-mó, et al. Journal of extracellular vesicles. 2015, 4.1: 27066.

Structure of nanoparticle-supported lipid bilayers

Nicolò Paracini[1], Max Wolff [2], Philipp Gutfreund[3], Rebecca Welbourn[4], Maritè Cardenas[1]
[1] *Biofilms: Research Center for Biointerfaces: Malmö University, Malmö, SE,*
[2] *Uppsala University, Uppsala, SE,*
[3] *Intitute Laue Langevin, Grenoble, FR*
[4] *ISIS neutron and muon source, Didcot, UK.*
e-mail: nicolo.paracini@mau.se

Supported lipid bilayers (SLBs) are robust and widespread models of biological membranes used in applications ranging from biophysical studies of membrane function to biosensing. Recent years have seen interest growing towards the development of non-planar SLBs formed on nano-patterned surfaces which impart a degree of curvature on the lipid bilayer. We present an accessible approach to create large arrays of spherical nanoparticles that are suitable and re-usable substrates for the formation of curved SLBs. We use a modified Langmuir-Schaefer deposition to transfer large monolayers of silica nanoparticles onto silicon substrates suitable for characterisation with both real-space and scattering techniques [1]. Formation of supported lipid bilayers (SLB) onto the nanoparticle arrays can be monitored in real time by QCMD whilst the structure of the nanoparticle array and the deposited SLB is probed by fluorescence microscopy, X-ray and neutron scattering techniques. In particular, specular and off-specular neutron reflectometry (NR) and grazing incidence neutron and X-ray small angle scattering (GISANS, GISAXS) are shown to yield accurate information on the structure of the SLB and the nanoparticle array along the normal to the interface, as well as providing information on the average in-plane correlation distances within the samples. Whilst GISAXS measurements provided data over an extend-ed Q range at the solid/air interface, NR and GISANS measurements were key to probe the structure and coverage of the lipid bilayer at the buried solid/liquid interface.

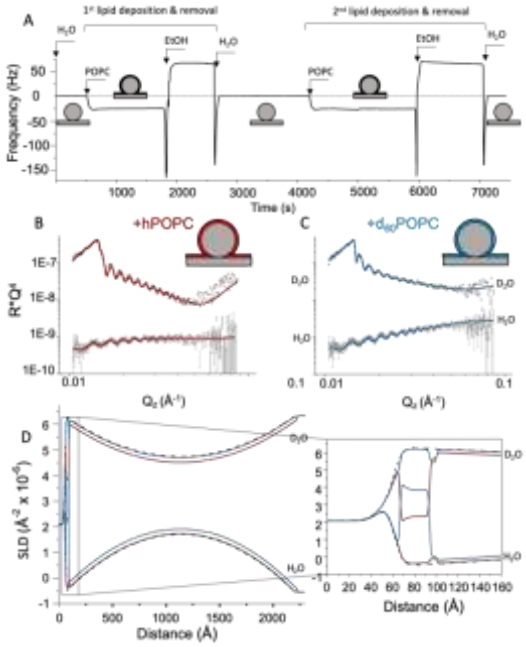


Figure 1: QCMD (A) and neutron reflectometry (B, C and D) characterisation of the formation of nanoparticle-supported curved lipid bilayers on an ordered array of silica nanoparticles

References

[1] Vorobiev A., Paracini N., Cardenas M., Wolff M., Π- GISANS: probing lateral structures with a fan shaped beam. Scientific Re-ports | (2021) 11:17786 | https://doi.org/10.1038/s41598-021-97112-x

Carbon nanotube penetration of cell membranes

F. Priante,[1] Y. M. Jaques,[1] T. Fukuma,[2] and A. S. Foster [1, 2]

[1]*Department of Applied Physics, Aalto University, Helsinki FI-00076, Finland*
[2]*WPI Nano Life Science Institute (WPI-Nano LSI), Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan*
e-mail: fabio.priante@aalto.fi

The possibility of non-destructively measuring living cells and their internal structures with nanoscale resolution would allow for a much greater understanding of numerous biological phenomena. To realize this goal, the use of carbon nanotube (CNT)-terminated tips in atomic force microscopy (AFM) has been shown to hold promise [1].

However, even in the very first step of the proposed “nanoendoscopic” process, consisting in the penetration of the plasma membrane, it is not always easy to give a precise interpretation of the experimental signal, which is a convolution of cell dynamics, tip shape and buffer solution.

Here, we use coarse-grained (CG) molecular dynamics simulations to investigate the effect that different widths, thicknesses and functionalization of the CNT tip can have in the penetration process. To simulate the membrane and the solvent dynamics we use the Martini 2 Force Field [2], while the CNT is simulated with a Martini-compatible force-field [3] which is more accurate in reproducing multi-layer graphene mechanical properties.

We carry out the membrane penetration by steering the CNTs downwards with constant velocity, then extracting the system's free energy using umbrella sampling, finally obtaining simulated force profiles. Additionally, we probe the possible role of the membrane skeleton mesh size [4] by emulating its effect as a position restraint on the lipids at the membrane’s edge. We directly compare our results to experimental measurements using an equivalent setup and outline the successes and limitations of the model.

References

[1] - Singhal, R., Orynbayeva, Z., Kalyana Sundaram, R., Niu, J., Bhattacharyya, S., & Vitol, E. et al. (2010). Multifunctional carbon-nanotube cellular endoscopes. *Nature Nanotechnology*, *6*(1), 57-64.
[2] - Marrink, S., Risselada, H., Yefimov, S., Tieleman, D., & de Vries, A. (2007). The MARTINI Force Field: Coarse Grained Model for Biomolecular Simulations. *The Journal Of Physical Chemistry B*, *111*(27), 7812-7824.
[3] - Ruiz, L., Xia, W., Meng, Z., & Keten, S. (2015). A coarse-grained model for the mechanical behavior of multi-layer graphene. *Carbon*, *82*, 103-115.
[4] - Morone, N., Fujiwara, T., Murase, K., Kasai, R., Ike, H., & Yuasa, S. et al. (2006). Three-dimensional reconstruction of the membrane skeleton at the plasma membrane interface by electron tomography. *Journal Of Cell Biology*, *174*(6), 851-862.

Nanoscale surface modification by alkanethiol self-assembly onto ZnSe: the role of substrate preparation on bacterial attachment

F. Quilès,[1] E. Yunda,[1,2] D. Horwat,[2] R. Gago,[3] J. Ghanbaja,[2] and H. Alem[2,4]

[1] *Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement, LCPME, UMR 7564 Université de Lorraine-CNRS, 54600 Villers-lès-Nancy, France*
[2] *Institut Jean Lamour, IJL, UMR 7198 Université de Lorraine-CNRS, 54000 Nancy, France*
[3] *Instituto de Ciencia de Materiales de Madrid, 28049 Madrid, Spain*
[4] *Institut Universitaire de France*
e-mail: fabienne.quiles@univ-lorraine.fr

Biofilms are communities of microorganisms embedded in a self-produced matrix of extracellular polymeric substances and adherent to each other and/or a surface. They are widespread on biological tissues, in the environment, and on surfaces as medical and food equipment, water distribution systems etc. The understanding of biofilms formation and their further control require to understand the initial stages of their formation – bacterial attachment. Among the methods used for the detection/monitoring of bacterial attachment, infrared spectroscopy in the attenuated total reflection mode (ATR-FTIR) is a powerful tool owing to a possibility to obtain quantitative and qualitative molecular information on the attached bacteria *in situ* and in real time [1]. Nonetheless, this method requires the use of substrates transparent in the infrared spectrum and possessing a high refractive index, e.g. zinc selenide (ZnSe). Among other parameters, bacterial adhesion is influenced, by the surface properties of the substrate. Thus, by controlling chemical properties of ATR-FTIR-suitable substrates, the understanding the initial steps of the bacterial attachment can be leveraged.

In this work, we explored the possibility of the surface functionalization of a ZnSe ATR crystal with alkanethiol self-assembled monolayers (SAMs) for the *in situ* monitoring of the bacterial attachment. Three methods to prepare the ZnSe surface prior to functionalization with the alkanethiol were performed: (i) exposure to ozone/UV [2], (ii) exposure to H_2O_2 followed by HCl aqueous solutions [3], and (iii) deposition of a gold [4] thin film onto ZnSe followed by exposure to ozone/UV. An amino-terminated alkanethiol was used to form the SAMs. The variations in the surface morphology, composition, and their correlation with the kinetics of the amino-terminated alkanethiol self-assembly of obtained SAMs were examined by the combination of atomic force and electronic microscopies, and elastic Rutherford backscattering and ATR-FTIR spectroscopies. We have then monitored the first steps of biofilm formation on the functionalized ZnSe using as model bacterium *Lactobacillus rhamnosus* GG (LGG). The ATR-FTIR fingerprints of LGG recorded *in situ* and in real time revealed that the organization of the molecular groups in bacterial cells was remarkably different on gold-coated ZnSe, which could be related to the organization of the SAM on this substrate. The average degree of LGG attachment obtained by epifluorescence measurements was similar on all the ZnSe functionalized surfaces, indicating the versatility of the applied protocol for ZnSe surface treatment prior to SAM grafting. The outcomes of this study increase the understanding of alkanethiol self-assembly onto ZnSe, and advance the possibilities of infrared spectroscopy in studying bacterial attachment and growth on chemically controlled surfaces.

References

[1] E. Yunda, F. Quilès, Biofouling 35 (2019), 494-507.
[2] A. R. Noble-Luginbuhl, R. G. Nuzzo, Langmuir 2001, 17, 3937-3944.
[3] C. S. Riccardi, D. W. Hess, B. Mizaikoff, Analyst 2011, 136, 4906-4911.
[4] C. D. Bain, E. B. Troughton, Y. T. Tao, J. Evall, G. M. Whitesides, R. G. Nuzzo, 111 (1989) J. Am. Chem. Soc. 321-335.

Adsorption of vitamin B12 on sugarcane-derived activated carbon: Fractal isotherm and kinetics modelling, electrochemistry and molecular modelling studies

R. Ranguin[1], M. Chaker. Ncibi[2], C. Jean-Marius[1], F. Brouers[3], G. Cebrián-Torrejón[1], A. Doménech-Carbó[6] , J-D. Maréchal[7], U. Jauregui-Haza[4, 5], S. Gaspard[1]

[1] Laboratoire COVACHIM M2E, EA 3592. Université des Antilles et de la Guyane. BP 250, 97157 Pointe à Pitre Cedex. Guadeloupe, French West-Indies.

[2] International Water Research Institute, Mohammed VI Polytechnic University, Green City Ben Guerir 43150, Morocco

[7]Insilichem, Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain

[3] Emeritus professor of Faculty of Applied Physics, Liege University, Liège 4000, Belgium.

[4] Instituto Superior de Tecnologías y Ciencias Applicadas, Universidad de la Habana, Cuba.

[5] Instituto Tecnológico de Santo Domingo, Dominican Republic.

[6] Departament de Química Analítica. Universitat de València. Dr. Moliner, 50, 46100 Burjassot (València) Spain.

e-mail: sarra.gaspard@univ-antilles.fr

Adsorption of vitamin B12 (VB12) on sugarcane-derived activated carbon (AC) was investigated to develop a nano-hybrid material [1] able to degrade [2] highly toxic and recalcitrant chlordecone (CLD). The study aims at the remediation of this pesticide as it is a major contaminant of Caribbean water and soils. For that reason, the AC form used in the experiments was produced from locally available sugarcane bagasse. The experimental kinetic and isotherm data of VB12 adsorption on AC were assessed using several models. The results showed that the BG is the most suitable one and involve a heterogeneous adsorption mechanism on the surface and providing the maximum adsorption capacity. An electrochemical analysis, based on cyclic voltammetric studies confirm the interactions between VB12 and the AC, and the entrapment of VB12 inside the porous system to form a new hybrid material. Finally, molecular modelling allows to better ascertain the most stable VB12-AC interactions at the atomic scale. This results confirm that CLD could be able to enter into large micropores and mesopores of AC as previously described [1], [2]

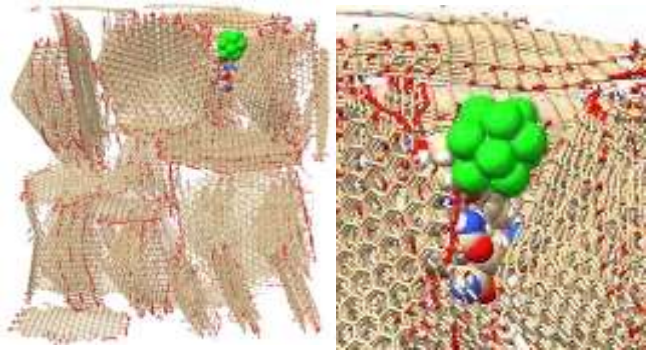


Figure 1:- Docking of chlordecone molecule in an AC pore with previously inserted vitamine B12

References

[1] Ranguin, R., Ncibi, M.C., Cesaire, T., Lavoie, S., Jean-Marius, C., Grutzmacher, H.J., Gaspard, S.; Development and characterisation of a nanostructured hybrid material with vitamin B12 and bagasse-derived activated carbon for anaerobic chlordecone (Kepone) removal (2020) Environmental Science and Pollution Research, 27 (33), pp. 41122-41131.

[2] Ronald Ranguin, Axelle Durimel, Reeka Karioua, Sarra Gaspard , Study of chlordecone desorption from activated carbons and subsequent dechlorination products formation by reduced cobalamin, Environmental Science and Pollution Research, 2017, (24) 25488-25499

The effect of hinge region flexibility on the binding kinetics of human IgG subclasses

Hannah Seferovic[1*], Patricia Sticht[1], Sebastian Diethör[1], Florian Weber[2], Christian Wechselberger[3], Rong Zhu[1], Wei Chen[4], Javier Chaparro-Riggers[4], David Bernhard[3], Birgit Plochberger[2], and Peter Hinterdorfer[1]

[1]*Institute of Biophysics, Johannes Kepler University, Linz, Austria*
[2]*Center of Excellence Medical Engineering, University of Applied Sciences Upper Austria, Linz, Austria*
[3]*Division of Pathophysiology, Medical Faculty, Johannes Kepler University, Linz, Austria*
[4]*Biotherapeutic Design & Discovery, Pfizer Inc., South San Francisco, USA*
e-mail: hannah.seferovic@jku.at

For an effective immune response, antigen-recognition as well as coupling of T-cell surface receptors to ligands on the surface of antigen-presenting cells (APCs) is required. One of these ligands on APCs is the transmembrane protein CD40. In immunological pathways, the stimulation of CD40 is triggered by helper T-cells via CD154, found as a trimer in T-cell membranes. Simultaneous coupling of CD154 to CD40 receptors leads to a clustering of CD40 in the APC membrane, initiating a downstream response in the cell [1]. Cancer cells block certain host immune mechanisms, leading to immune tolerance. By utilizing special therapeutic antibodies, the immune tolerance can be disrupted by blocking certain checkpoints or stimulating receptors mimicking ligands [1]. We investigated the binding behaviour of a set of human IgG monoclonal antibody subclasses to CD40 using single molecule force spectroscopy (SMFS) and studied the CD40 cluster formation in cell membranes in the presence of the different IgG subclasses, as well as CD154, with fluorescence imaging. IgG subclasses, such as IgG1, IgG4 and two isoforms of the human IgG2 (IgG2A and IgG2B), differ in the disulphide-connectivity of their hinge region, which determines the antibodies' flexibility. Previous studies showed that IgG2B is less flexible and has the most compact structure compared to the other IgG subclasses [2]. Furthermore, all antibody subclasses trigger B-cell activation when coupled to CD40, if stabilized additionally via the Fcγ receptor on the APC's surface, with the exception IgG2B, which does not require the extra support of the Fcγ receptor [2],[3]. Using SMFS we monitored ruptures between single bonds of the IgG-CD40 and CD154-CD40 complexes and derived kinetic rates of the interactions. No significant difference was found in kinetics of bond formation and bond lifetime of the IgG subclasses carrying the same binding paratope, showing that the antibodies' hinge flexibility has little effect on its monovalent binding behaviour. Furthermore, all IgG subclasses coupled with their two Fab arms simultaneously to CD40. Using fluorescence imaging, a fast formation of CD40 clusters in the membrane was observed upon addition of CD154 and IgG2B, whereas cluster formation was less pronounced in the presence of IgG1. CD40 clusters and antibody associates were well correlated.

References

[1] Lee CS *et al.*, British journal of clinical pharmacology **vol. 76,2** (2013)
[2] Liu X *et al*, Nature Communication **vol. 10,4206** (2019)
[3] White AL *et al.*, Cancer cell **vol. 27,1** (2015)

Acknowledgment

The authors acknowledge the financial support from the Austrian Science Fund (FWF) project FWP30314000.

Quantitative Assessment of the Comparative NP-Uptake Efficiency in a range of biological fluids

Tiago dos Santos[1,2], Péter Hamar[3], Zsombor Lacza[3], P. Granja[1,2]

[1] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

[2] Biomaterials for Multistage Drug & Cell Delivery, INEB, Portugal

[3] Institute of Clinical Experimental Research, Semmelweis University, Budapest, Hungary

e-mail: Tiago.f.santos@ineb.up.pt

Nanoparticles potentially provide a powerful tool for specific treatments of diseases, acting as a drug delivery transport. However, a deep understanding and control of how nanoparticles interact with biological systems is a key driver to assure the safe implementation of nanomedicine[1, 2]. The overall idea of this project was to provide new leads in the development of such a new field, finding tools for various biomedical applications. What the biological cell actually "sees" when interacting with a nanoparticle will influence the mechanism of internalization. Thus, whether the cell is presented with the bare particle or the particle dispersed in a biological medium, thus covered by a protein corona, results in different uptake behaviour. [3, 4]

For this project, multiple cell lines were used and the ultimate goal was to control and quantify uptake of a series of negatively charged carboxylate modified polystyrene of different size, (20nm, 40nm, 100nm, 200nm, 500nm, 1um) understanding the endocytic pathways required for NPs internalization and their final sub-cellular destination, when NPs were dispersed in different biological fluids (no serum, Foetal Bovine Serum, Human Albumin, Proprietary Blood Fraction)

We found that internalization of nanoparticles is highly size dependent for all cell lines studied, with the different cell types showing very different uptake efficiencies for same materials. Moreover, our studies showed that, in a physiological relevant environment, highly complex protein coronas are established and modulate biological effects at the nanoparticle-cell interface in an innovative way, which has important implications for nanomedicine.

References

1. dos Santos, T., et al., *Quantitative Assessment of the Comparative Nanoparticle-Uptake Efficiency of a Range of Cell Lines.* Small, 2011. **7**(23): p. 3341-3349.

2. dos Santos, T., et al., *Effects of Transport Inhibitors on the Cellular Uptake of Carboxylated Polystyrene Nanoparticles in Different Cell Lines.* Plos One, 2011. **6**(9).

3. O'Connell, D.J., et al., *Characterization of the bionano interface and mapping extrinsic interactions of the corona of nanomaterials.* Nanoscale, 2015. **7**(37): p. 15268-15276.

4. Salvati, A., et al., *Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface.* Nature Nanotechnology, 2013. **8**(2): p. 137-143.

In vitro anticancer photothermal therapy using noble metal nanolayers

Lucie Válková,[1] Lucie Hochvaldová,[2] Karolína Simkovičová,[2] Aleš Panáček,[2] Hana Kolářová[1]

[1]*Department of Medical Biophysics, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, 775 15, Olomouc, Czech Republic*
[2]*Department of Physical Chemistry, Faculty of Science, Palacký University, 17. listopadu 12, 771 46, Olomouc, Czech Republic*
e-mail: lucie.valkova@upol.cz

Photothermal therapy (PTT) is a promising method in cancer treatment based on the absorption of electromagnetic radiation after irradiation of photothermal agent with a suitable wavelength resulting in the local production of heat causing irreversible damage of cancer cells.
In this work, noble metal nanoparticles of different sizes, shapes and therefore optical properties were synthetized and deposited on 96-well plate to evaluate antitumor efficacy of photothermal therapy. To induce the photothermal effect, laser sources of different powers and wavelengths corresponding to the plasmon band of the metal were used. Important part of the study was determination of the influence of the irradiation on HeLa cell line. Doses of irradiation were non-toxic to the cells, however, in combination with noble metal nanoparticles caused significant decrease in cell viability through photothermal effect. Biophysical methods such as spectroscopic determination of cell viability were used to determine the photothermal efficacy. This relatively non-invasive and novel technique based on physicochemical properties of plasmonic materials is offered as an effective and promising tool in the fight against cancer diseases.

Acknowledgement: This work was supported by the students grant nr. DSGC-2021-0120 "Light-assisted in vitro therapy using plasmonic materials" funded under the OPIE project "Improvement of Doctoral Student Grant Competition Schemes and their Pilot Implementation", reg. no. CZ.02.2.69/0.0/0.0/19_073/0016713.

Forces during Cell Membrane Puncturing Detected by FluidFM Coupled with Fluorescence Lifetime Imaging Microscopy

Elaheh Zare-Eelanjegh[1], Ines Lüchtefeld[1], Mi Li[2], and Tomaso Zambelli[1]

[1]*Laboratory for Biosensors and Bioelectronics, Institute for Biomedical Engineering, ETH Zurich, Switzerland, 8092*

[2]*State Key Laboratory of Robotics, Shenyang Institute of Automation, Chinese Academy of Sciences, Shenyang, Liaoning Province, P.R.China, 110016*

e-mail: ztomaso@ethz.ch

Understanding the interactions between biochemical and mechanical cues in the cellular behaviors is a key for revealing numerous biological functions and development of major diseases. This necessitates creating tools enabling in situ monitoring and quantifying such small forces in single-cell levels while preserving cell viability and avoiding influences on the ongoing cellular events. Mechanosensory complexes at the cellular level are such a demanding situation in which the dynamic interconnectivity of the cytoskeleton, nuclear envelope, and nucleoskeleton is yet to be understood. Fluorescence lifetime imaging microscopy (FLIM) is advantageous for probing such molecular environments using fluorophores report the membrane tension changes through their fluorescence lifetime changes [1].

FluidFM a force-controlled micropipette configured on an atomic force microscope (AFM) enables to manipulate intact cells with simultaneous screening of molecular responses [2]. FluidFM micro-manipulation of single cells includes but not limited to quantitative injection of impermeable molecules and extraction of biomarkers, transcriptomes, and other biomolecules into and from cytosol (or nucleus) of living cells. This provides noticeable evidence on the single cell dynamics and therefore numerous biological processes in a spatially defined fashion.

FluidFM combined with FLIM allows for mechano-chemical manipulation of intact cells along the parallel in situ mapping of cellular responses over single cells. Thanks to the versatility of FluidFM tips and gentleness of cantilever-membrane contact, cell viability is preserved and influences on the ongoing cellular events is minimized. Thereby, I will elaborate FluidFM combined with FLIM-based sensory systems to address the dynamic mechanosensory of essential components of the linker of nucleoskeleton and cytoskeleton (LINC) complex by in situ 3D stress mapping over the nuclear envelope.

References

[1] R. Datta et al., “Fluorescence lifetime imaging microscopy: fundamentals and advances in instrumentation, analysis, and applications”, J. of Biomedical Optics 25:071203 (2020)
[2] A. Meister et al., “FluidFM: combining atomic force microscopy and nanofluidics in a universal liquid delivery system for single cell applications and beyond”, Nano Lett. 9:2501 (2009)

Nano mechanical characterization techniques: a tool to predict macro mechanical behavior of polymer samples

T. Pertin, G. Minatchy, P. Bilas, A. Flory and L. Romana

Groupe de Technologie des Surfaces et des Interfaces, Université des Antilles- Campus de Fouillole 97 159 Pointe à Pitre – Cedex – Guadeloupe- French West Indies

Email address: t.pertin@live.fr

Durability of polymers is often characterized by changes in mechanical properties with ageing time. These changes are mostly due to surface cracks, oxidation process and/or rearrangement of crystalline structure induced by the combined effects of UV radiation, temperature and humidity. Multi scale mechanical approach has been developed to study the early stage degradation. In addition, nanomechanical results are correlated to results from more conventional techniques such as Infra-red spectroscopy and Raman nanomechanical. The final aim of this work is to develop a fast characterization nanomechanical tool to predict polymer durability

In this work, commercial thick PP/PE copolymer samples were aged up to 44 days using an artificial aging chamber. Chemical degradation was monitored by infrared spectrometry in Attenuated Total Reflection mode (ATR) and spectrums were analyzed. Scanning Electron Microcopy (SEM) was conducted to study surface topography of the samples. Nanomechanical properties were determined by continuous stiffness measurement (CSM) with indentation depths of 2 µm to 10 µm. Two setups were used to measure the nanomechanical properties: normal setup for which the indents were performed either on the exposed/unexposed faces or cross setup for which the indents were performed perpendicularly to the exposed face. Elastic modulus at macroscale was determined by Dynamical Mechanical Analysis (DMA).

The results show that oxidation of the samples occurred after 3 days of aging and increased progressively up to 32 days. SEM observation showed apparition of surface cracks between 6 and 10 days of aging. More cracks propagation through the sample were observed as aging time increases. Elastic modulus, E_{nano}, measured by nanoindentation on the exposed faces, increases from (1.35±0.04) GPa at 0 days of aging to (3.6±0.2) GPa at 44 days. Cross setup measurements showed an exponential decrease of the Young's modulus with depth. The perturbation zone reached a maximal depth of 1300 µm at 44 days. Furthermore, a Voigt model was used to calculate the Young's modulus of the bulk sample using nanoindentation data. This calculated parameter, $E_{macro\ fit}$, was then compared to the macro Young's modulus, E_{macro}, obtained from DMA. A good correlation was observed at macro and nano scales as long as macroscopic defects such as cracks appeared. This work shows that nanoindentation is a suitable technique to detect early stage degradation of polymers and therefore a powerful tool to predict durability of polymeric materials.

ACKNOLEDGEMENT

The authors would like to thanks the Fond Social Européen and La Région Guadeloupe for their financial support.

Virus-based nano-bio-hybrid materials for hyperthermia

NT. Ha-Duong[1], S. Darwish[1], HA. Nguyen[2], HN. Pham[3]

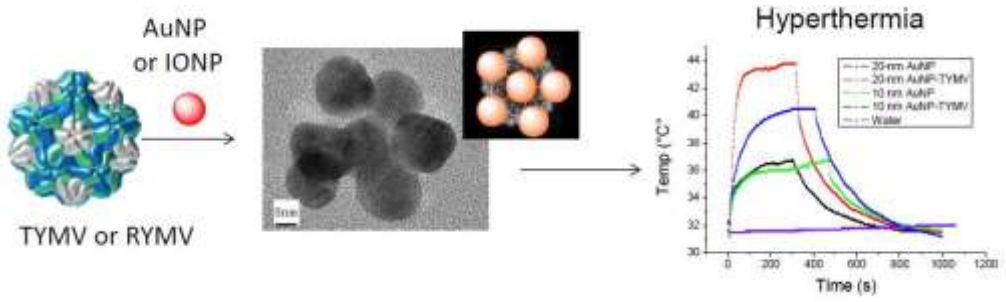
[1]*Université de Paris, ITODYS, CNRS, UMR 7086, 15 rue J-A de Baïf, F-75013 Paris, France*
[2]*Phenikaa University, PHENA Institute, Yen Nghia, Ha Dong, Hanoi, Vietnam*
[3]*Institute of Materials Science, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Hanoi, Vietnam.*

e-mail: thanh.haduong@u-paris.fr

The ability to construct three dimensional architectures via nanoscale engineering is important for emerging applications of nanotechnology in sensors, catalysis, controlled drug delivery, microelectronics, and medical diagnostics [1]. Our strategy consists to use two plant viruses, Turnip yellow mosaic virus (TYMV) and Rice yellow mottle virus (RYMV) as scaffold, because of their low cost of production, monodispersity, and robustness. TYMV and RYMV are icosahedral viruses with an average diameter of 28 nm. Their capsid consists of a self-assembly of 180 chemically identical protein subunits [2,3], and TYMV surface is known to be easily functionalized [4].

In the first part of this work, I will describe the synthesis and characterization of new nano-bio-hybrid materials, which are soluble and stable in solution. Gold nanoparticles (AuNP) of different sizes (5, 10 and 20 nm) were grafted on TYMV capsid, according to two strategies. After purification, the resulting nano-bio-hybrids were characterized by different technics. For example, dynamic light scattering (DLS) confirmed the grafting through the hydrodynamic size increase by comparing AuNP alone to AuNP-TYMV (up to 33, 50 and 68 nm for 5, 10 and 20 nm sized AuNP, respectively) or capsid alone (28 nm). Transmission electronic microscopy (TEM) observations revealed that NP were arranged with 5-fold symmetry, in agreement with their grafting around icosahedral capsids. Moreover, UV-vis absorption spectroscopy showed a red-shift of the plasmon absorption band on the grafted AuNP spectrum (530 nm) compared to that of the non-grafted one (520 nm) [4]. Similarly, we grafted onto the virus capsid iron oxide nanoparticles (IONP) synthetized by the polyol process, and then characterized the objects, specifically their magnetic properties.

In the second part, I will present some results on hyperthermia experiments in solution. We observed enhancement of the temperature for a solution of Np-virus, under an excitation wavelength of 808 nm or a magnetic field, compared to a solution containing the ungrafted NP. The solution can reach a temperature over than 40°C, which makes our nano-bio-hybrid objects particularly interesting as hyperthermia agents.



References

[1] (a) Capek, I. **Adv Colloid Interface Sci.** 2015, 222, 119-134. (b) Culver, J.N.et al. **Virology** 2015, 479-480, 200-212. (c) Li, F.& Wang, Q. **Small** 2014, 10, 230-245. (d) Liu, Z.et al. **Chem Soc Rev** 2012, 41, 6178-6194.
[2] Canady M.A. et al. **Nat Struct Biol**, 1996, 3, 771-781.
[3] Brugidou et al. **Virology**, 2002, 297, 98
[4] (a) Barnhill HN et al. **Bioconjug Chem**, 2007, 18, 852-859 (b) Zeng Q et al. **Bioconjug Chem**, 2011, 22, 58-66P. Morris, M. Jones, Phys. Rev. Lett. 70, 21 (2000)
[5] Nguyen, HA et al. ***RSC Adv.***, 2019,**9**, 32296-32307

Deciphering the Role of Surface Interactions in the Antibacterial Activity of Layered Double Hydroxide Nanoparticles by AFM

Sofiane El-Kirat-Chatel,[1] Jazia Awassa, Samantha Soulé, Damien Cornu, and Christian Ruby

[1]*Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement (LCPME) CNRS-Université de Lorraine UMR7564, 405 rue de Vandoeuvre 54600 Villers les Nancy.*
e-mail: elkirat1@univ-lorraine.fr

As pristine zinc-based layered double hydroxides (LDHs) are extensively investigated as efficient antibiotic-free antibacterial agents [1], understanding the mechanisms of interactions between LDHs and bacterial surfaces becomes of great importance. In the present study, the role of surface interactions in the antibacterial activity of zinc-based LDH nanoparticles was investigated (Fig. 1). Synthesized ZnAl LDH nanoparticles showed a strong antibacterial effect against *Staphylococcus aureus* and caused serious cell wall damages as revealed by growth test and atomic force microscopy (AFM) imaging, respectively. Further, we developed an original approach to functionalize AFM tips with LDH nanoparticles in order to probe their interactions with live *S. aureus* cells by mean of AFM-based single-particle force spectroscopy (SPFS). The force spectroscopy analysis revealed that antibacterial ZnAl LDH nanoparticles possessed a specific recognition to S. aureus cells with high adhesion frequency and remarkable force magnitudes. Such finding provided a first insight about the antibacterial mechanism of Zn-based LDHs by direct surface interactions through which they are able to recognize and adhere to bacterial membranes which lead to their damage and subsequent growth inhibition.

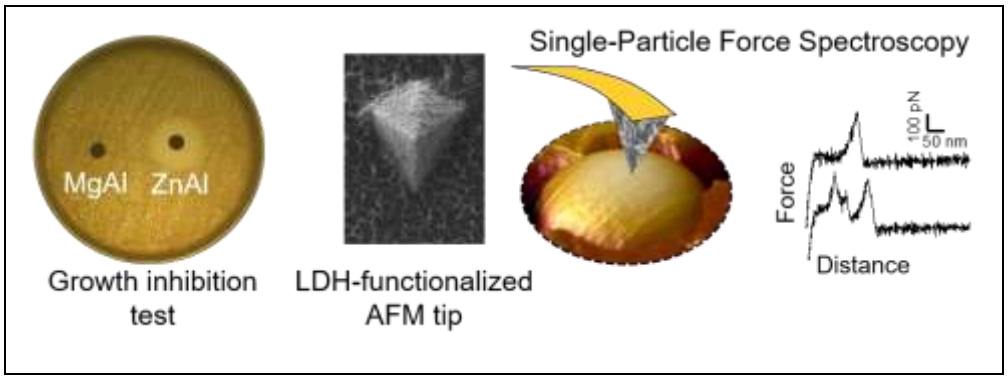


Figure 1: After growth inhibition tests, AFM tips were functionalized with LDH and used to record force-distance curves on bacterial cells.

References

[1] J. Awassa, D. Cornu, S. Soulé, C. Carteret, C. Ruby and S. El-Kirat-Chatel. Divalent Metal Release and Antimicrobial Effects of Layered Double Hydroxides. Applied Clay Science, 216, (2022),106369.

List of Participants

Spring School

&

Conference

NanoInBio 2022

Name		Email
Adams	Jonathan	adams@nanosurf.com
Alem	Halima	halima.alem@univ-lorraine.fr
Berardi	Massimiliano	massimiliano.berardi@optics11life.com
Berthout	Guillaume	guillaume.berthout@anton-paar.com
Billote-Cabre	Catherine	Catherine.Billotte@etu.univ-antilles.fr
Casalis	Loredana	loredana.casalis@elettra.trieste.it
Cesaire	Thierry	thierry.cesaire@univ-antilles.fr
Colombo	Miriam	miriam.colombo@unimib.it
Combes	Antoine	antoine.combes@etu.unistra.fr
Contarini	Paul Emile	paulemile.contarini@gmail.com
Couvreur	Patrick	patrick.couvreur@u-psud.fr
Dague	Etienne	edague@laas.fr
De Pablo	Pedro	p.j.depablo@uam.es
Debaud	Yoan	yoan.debaud@gmail.com
Delumeau	Aurélie	aurelie.delumeau@etu.univ-amu.fr
Demir-Ylmaz	Irem	iremdemirsu@gmail.com
Dikecoglu	Berguem	beguem.dikecoglu@jku.at
Dubus	Marie	marie.dubus@hotmail.com
Dulebo	Alexander	confregis.bioafm@bruker.com
Dupres	Vincent	vincent.dupres@ibl.cnrs.fr
Duro	Allan	Allan.duro@etu.univ-antilles.fr
Ekrami	Saeid	saeid.ekrami@univ-lorraine.fr
El Kirat-Chatel	Sofiane	elkirat1@univ-lorraine.fr
Enderlein	Jörg	joerg.enderlein@phys.uni-goettingen.de
Fantner	Georg	georg.fantner@epfl.ch
Fiandra	Luisa	luisa.fiandra@unimib.it
Filali	Amine	mohammedaminefilali1@gmail.com
Flory	Anny	anny.flory@univ-antilles.fr
Formosa-Dague	Cécile	formosa@insa-toulouse.fr
Foster	Adam	adam.foster@aalto.fi
Francius	Grégory	gregory.francius@univ-lorraine.fr
Gansmuller	Axel	axel.gansmuller@univ-lorraine.fr
Gaspard	Sarra	sarra.gaspard@univ-antilles.fr
Gavara	Núria	ngavara@ub.edu
Genevskiy	Vladislav	vladislav.genevskiy@mau.se
Giergiel	Magdalena	magdalena.rusaczonek@gmail.com
Gingras	Michel	gingras@uwaterloo.ca

Name		Email
Goudou	Francesca	francesca.goudou@univ-antilles.fr
Gros	Olivier	Olivier.gros@univ-antilles.fr
Ha Duong	Ngutyet-Thanh	thanh.haduong@u-paris.fr
Hain	Lisa	lisa.hain@jku.at
Harnett	Jenny	s2139940@ed.ac.uk
Hartmann	Bastian	bastian.hartmann@hm.edu
Haye	Lucie	lucie.haye@etu.unistra.fr
Hemadi	Myriana	hemadi@u-paris.fr
Hinterdorfer	Peter	peter.hinterdorfer@jku.at
Hochvaldova	Lucie	lucie.hochvaldova@upol.cz
Hoff	Brice	h.brice.personnel@gmail.com
Jean-Marius	Corine	corine.jean-marius@univ-antilles.fr
Jeanne-Rose	Valérie	valerie.jeanne-rose@univ-antilles.fr
Kalinin	Sergei	sergei2@ornl.gov
Kerdjoudj	Halima	halima.kerdjoudj@univ-reims.fr
Kotov	Nicholas	kotov@umich.edu
Lavalle	Philippe	philippe.lavalle@inserm.fr
Lebaudy	Eloise	eloise.lebaudy@etu.unistra.fr
Lecomte	Sophie	s.lecomte@cbmn.u-bordeaux.fr
Leitao	Samuel	samuel.mendesleitao@epfl.ch
Leonenko	Zoya	zleonenk@uwaterloo.ca
Lepicka	Kamila	klepicka@ichf.edu.pl
Lopez Alonso	Javier	Javier.lopez-alonso@ibl.cnrs.fr
Lyonnais	Sébastien	sebastien.lyonnais@cemipai.cnrs.fr
Maier	Verena	verena.maier@hm.edu
Marinez-Rivas	Adrian	nanobiomex@hotmail.com
Martinez-Vidal	Laura	martinezvidal.laura@hsr.it
Martin-Yken	Hélène	helene.martin@insa-toulouse.fr
Mathurin	Jérémie	jeremie.mathurin@universite-paris-saclay.fr
Narayanin	Stacy	Stacy.narayanin@univ-antilles.fr
Nemeth	Margit	margit.nemeth@jku.at
Oh	Yoojin	yoo_jin.oh@jku.at
Oxybel	Elodie	elodie.oxybel.971@gmail.com
Paba	Carolina	carolina.paba@phd.units.it
Paracini	Nicolò	nicolo.paracini@mau.se
Pasquina Lemonche	Laila	l.pasquinalemonche@sheffield.ac.uk
Piantanida	Luca	lucapiantanida@boisestate.edu
Picart	Catherine	catherine.picart@cea.fr

Name		Email
Pilizota	Teuta	teuta.Pilizota@ed.ac.uk
Priante	Fabio	fabio.priante@aalto.fi
Prosperi	Davide	davide.prosperi@unimib.it
Quiles	Fabienne	fabienne.quiles@univ-lorraine.fr
Ranawat	Yashasvi	yashasvi.ranawat@aalto.fi
Ranguin	Ronald	ronald.ranguin@univ-antilles.fr
Roche	Sandra	sroche01@hotmail.fr
Rohatschek	Andreas	andreas.rohatschek@tuwien.ac.at
Romana	Laurence	laurence.romana@univ-antilles.fr
Romana	Marc	marc.romana@inserm.fr
Ropitaux	Marc	marc.ropitaux1@univ-rouen.fr
Santos	Tiago	tiago.f.santos@ineb.up.pt
Scher	Joel	joel.scher@univ-lorraine.fr
Schillers	Hermann	schille@uni-muenster.de
Scrimin	Paolo	paolo.scrimin@unipd.it
Seferovic	Hannah	hannah.seferovic@jku.at
Sergeeva	Yulia	yulia.sergeeva@mau.se
Serralta	Marine	marine.serralta@anton-paar.com
Thomas	Philippe	philippe.thomas@univ-antilles.fr
Thomas-Chemin	Ophélie	othomasche@laas.fr
Tormena	Nicolo	nicolo.tormena@durham.ac.uk
Trewby	William	william.trewby@durham.ac.uk
Válková	Lucie	luc.valkova@gmail.com
Villalba	Maria Ines	ines.villalba@epfl.ch
Vingataramin	Youri	yvingataramin@pasteur-guadeloupe.fr
Voitchovsky	Kislon	kislon.voitchovsky@durham.ac.uk
Vondracek	Hendrik	hendrik.vondracek@elettra.eu
Vranckx	Cédric	cedric.vranckx@uclouvain.be
Yacou	Christelle	christelle.yacou@univ-antilles.fr
Zambelli	Tomaso	ztomaso@ethz.ch
Zhang	Xinyu	xinyzhang@ethz.ch
Zirbs	Ronald	ronald.zirbs@boku.ac.at
Zozo	Léa	Lea.zozo@outlook.com

<table>
<tr><th>May 30th</th><th>May 31st</th><th>June 1st</th><th>June 2nd</th><th>June 3rd</th><th>June 4th</th><th>June 5th</th></tr>
<tr><td>9.30am – 12.30pm
Spring School</td><td>9.30am – 12.30pm
Spring School</td><td>9am – 12.20pm
Conference session I: Scanning probe microscopy & force spectroscopy</td><td>8.40am – 12.20pm
Conference session III: Latest advances in materials application & life sciences</td><td>8.40am – 12.35pm
Conference session V: Scanning probe microscopy & force spectroscopy</td><td rowspan="5">7.30 am – 5pm :
Social Event</td><td>8.40am – 12.30pm
Conference session VII: Special NanoInBio session</td></tr>
<tr><td colspan="5">Lunch</td><td>Lunch</td></tr>
<tr><td>2pm – 5.30pm
Spring School</td><td>2pm – 4pm
Spring School</td><td>2pm – 5pm
Conference session II: Optical microscopies & spectroscopies</td><td>2pm – 5pm
Conference session IV: Simulations and machine learning</td><td>2pm – 4.15pm
Conference session VI: Latest advances in materials application & life sciences</td><td rowspan="3"></td></tr>
<tr><td rowspan="2"></td><td rowspan="2">4.30pm – 7.30pm
Conférence Grand Public</td><td rowspan="2"></td><td rowspan="2">5pm – 6pm
Poster session I</td><td>4.30pm – 6.30pm
Poster session II & International Cooperation workshop</td></tr>
<tr><td>7.30 pm:
Gala dinner</td></tr>
</table>