NANO IN BIO 2024

4th Edition: From April 14 to April 20, 2024

Le Gosier, Guadeloupe (French Caribbean)

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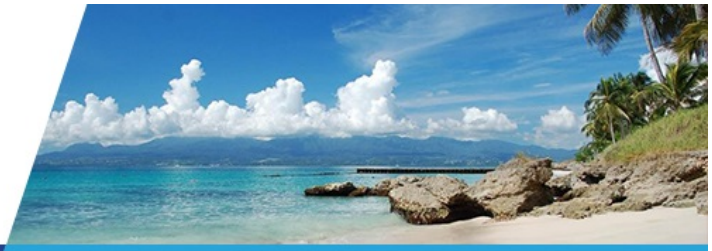

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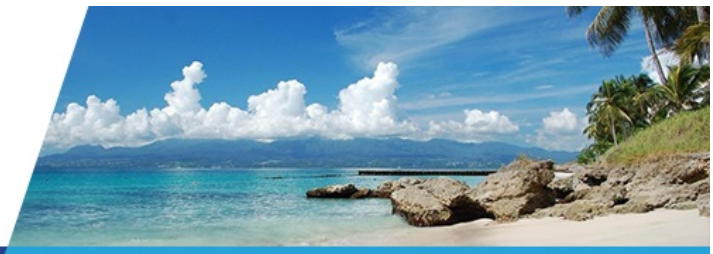

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Program

Spring School NanoInBio 2024

Sunday, April 14

Electrochemical and ionic processes in biological systems at the nanoscale

Ulrich Keyser, University of Cambridge, United Kingdom
Georg Fantner, EPFL, Lausanne, Switzerland
Mingdong Dong, Aarhus University, Denmark

Conference NanoInBio 2024

Monday, April 15 – Saturday, April 20

Guest Speakers

Philippe Leclère, University of Mons, Belgium
Igor Sokolov, Tufts University, United States
Sungsu Park, Sungkyunkwan University, South Korea
Elena Ferapontova, Aarhus University, Denmark
Valeria Rondelli, University of Milan, Italy
Frank Caruso, University of Melbourne, Australia
David Alsteens, Université catholique de Louvain, Belgium
Nicole Robb, University of Warwick, United Kingdom
Mingdong Dong, Aarhus University, Denmark
Bing Xu, Brandels University, United States
Takeshi Fukuma, Kanazawa University, Japan

Experts

Ulrich Keyser, University of Cambridge, United Kingdom
Georg Fantner, EPFL, Lausanne, Switzerland
Loïc Jierry, University of Strasbourg, France
Yuri Korchev, Imperial College of London, United Kingdom

Guest Speakers: 30 minutes
Experts: 30 minutes
Oral Presentations: 20 minutes

	April 14
08.15 am – 09.15 am	**Welcome Reception & Registration**
	Spring School: Electrochemical and ionic processes in biological systems at the nanoscale
9.30 am – 11.00 am	**Prof. Ulrich Keyser** (Cambridge University, UK)
11.00 am – 12.30 pm	**Prof. Georg Fantner** (EPFL, Switzerland)
12.30 pm	**Lunch Break**
2.00 pm – 4.30 pm	**Prof. Mingdong Dong** (iNANO, Aarhus University, Denmark)
4.45 pm	**Group photos at Parc du Calvaire in downtown Le Gosier** **(Free Time)**    

	April 15
08.15 am – 08.45 am	**Welcome Reception & Registration**
	Conference Session I The bio/non-bio interface
08.45 am – 09.00 am	**Introduction – Grégory Francius**
09.00 am – 09.30 am	**Philippe Leclère**, University of Mons, Belgium **(Keynote lecture)**
09.30 am – 9.50 am	**Béatrice Labat**, University of Rouen Normandie, Evreux, France
9.50 am – 10.10 am	**Régis Badin**, University of Lorraine, Nancy, France
10.10 am – 10.30 am	**Luca Puricelli**, Area Science Park, Trieste, Italy
10.30 am – 11.00 am	**Coffee Break (30 min)**
11.00 am – 11.30 am	**Igor Sokolov**, Tufts University, United States **(Keynote lecture)**
11.30 am – 11.50 am	**Lucie Klopffer**, University of Lorraine, Nancy, France
11.50 am – 12.10 pm	**Andreas Stylianou**, EUC Research Centre / European University Cyprus, Cyprus
12.30 pm – 02.00 pm	**Conference Lunch**
	Conference Session II Instrumentation for bio- & nanotechnologies
02.00 pm – 02.30 pm	**Sungsu Park**, Sungkyunkwan University, South Korea **(Keynote lecture)**
02.30 pm – 02.50 pm	**Etienne Dague**, LAAS-CNRS, Toulouse, France
02.50 pm – 03.10 pm	**Katarzyna Matczyszyn**, Wroclaw University of Science and Technology, Poland
03.10 pm – 03.30 pm	**Jordyn Ann Howard**, University of Lyon 1, Villeurbanne, France
03.30 pm – 04.00 pm	**Coffee Break (30 min)**
04.00 pm – 04.20 pm	**Juan Felipe Salazar Ariza**, University of Lyon 1, Villeurbanne, France
04.20 pm – 04.40 pm	**Thomas Hackl**, TU Wien, Vienna, Austria
04.40 pm – 05.10 pm	**Marco Portalupi**, Nanosurf AG, Liestal, Switzerland
05.10 pm – 06.30 pm	**Poster Session I – Networking Time**

	April 16
08.30 am – 09.00 am	**Registration**
	Conference Session III **Bio- and nano-electrochemistry & bio applications**
09.00 am – 9.30 am	**Ulrich Keyser**, University of Cambridge, United Kingdom **(Expert)**
9.30 am – 9.50 am	**Kamila Łępicka**, Polish Academy of Sciences, Warsaw, Poland
9.50 am – 10.10 am	**Ronald Zirbs**, University of Natural Resources and Life Sciences, Vienna, Austria
10.10 am – 10.30 am	**Allan Duro**, Université des Antilles, Pointe-à-Pitre, France
10.30 am – 11.00 am	**Coffee Break (30 min)**
11.00 am – 11.30 am	**Elena Ferapontova**, Aarhus University, Denmark **(Keynote lecture)**
11.30 am – 11.50 am	**Menglin Chen**, Aarhus University, Denmark
11.50 am – 12.10 pm	**Stacy Melyon**, Université des Antilles, Pointe-à-Pitre, France
12.30 pm – 02.00 pm	**Conference Lunch**
	Conference Session IV **Bio- and nano-materials for health & nanomedicine**
02.00 pm – 02.30 pm	**Valeria Rondelli**, University of Milan, Italy **(Keynote lecture)**
02.30 pm – 02.50 pm	**Xavier Fernàndez-Busquets**, University of Barcelona, Spain
02.50 pm – 03.10 pm	**Caterina Medeot**, University of Trieste, Italy
03.10 pm – 03.30 pm	**Audrey Malardé**, Université of Lorraine,Vandoeuvre les Nancy, France
03.30 pm – 04.00 pm	**Coffee Break (30 min)**
04.00 pm – 04.30 pm	**Frank Caruso**, University of Melbourne, Australia **(Keynote lecture - Visio)**
04.30 pm – 04.50 pm	**Malobi Seth**, Université of Lorraine, Metz, France
04.50 pm – 05.10 pm	**Bernardo Albuquerque Nogueira**, International Iberian Nanotechnology Laboratory, Braga, Portugal
05.10 pm – 05.30 pm	**Yue Xu**, University of Waterloo, Canada

	April 17
	Social Event
07.00 am – 05.30 pm	**Marie-Galante Day Tour** **Discover Marie-Galante in 10 exciting stages.** History, distilleries, restaurants and heavenly beaches await you.  Immersion in the history of Habitation Murat, where you will discover the history of slavery as well as life in the XVIII century and the Rum history.  Visit a local distillery and enjoy a Rum Masterclass to learn about rum. You will enjoy it with perfectly paired local appetizers. White rum up to 59° and old rum from 40° to 42°.  Enjoy a meal of your choice at the Aux Plaisirs des Marins restaurant of the Catimini Restaurant in St Louis. Then farniente, swimming and snorkeling at wild beach of 3 Ilets or Anse Canot.

	April 18	
	Conference Session V Nanomedicine, Nano-Micro Biology	
08.40 am – 09.10 am	**David Alsteens**, Université catholique de Louvain, Belgium **(Keynote lecture)**	
09.10 am – 9.30 am	**Philippe Lavalle**, University of Strasbourg, France	
9.30 am – 9.50 am	**Yoann Roupioz**, Université Grenoble Alpes, France	
9.50 am – 10.10 am	**Fabienne Quiles**, University of Lorraine, Nancy, France	
10.20 am – 10.50 am	**Coffee Break (30 min)**	
10.50 am – 11.20 am	**Nicole Robb**, University of Warwick, United Kingdom **(Keynote lecture)**	
11.20 am – 11.40 am	**Peter Hinterdorfer**, Johannes Kepler University, Linz, Austria	
11.40 am – 12.10 pm	**Olivier Gros**, Université des Antilles, Pointe-à-Pitre, France **(Expert)**	
12.30 pm – 02.00 pm	**Conference Lunch**	
	Conference Session VI Bio- and nano-electrochemistry & bio applications	
02.00 pm – 02.30 pm	**Mingdong Dong**, Aarhus University, Denmark **(Keynote lecture)**	
02.30 pm – 02.50 pm	**Petr Gorelkin**, ICAPPIC Limited, London, United Kingdom	
02.50 pm – 03.10 pm	**Sebastian Sittl**, University of Bayreuth, Germany	
03.10 pm – 03.30 pm	**Cécile Formosa-Dague**, INSA-University of Toulouse, France	
03.30 pm – 04.00 pm	**Coffee Break (30 min)**	
04.00 pm – 04.30 pm	**Yuri Korchev**, Imperial College of London, United Kingdom **(Expert)**	
04.30 pm – 04.50 pm	**Isalyne Drewek**, University of Mons, Belgium	
04.50 pm – 05.10 pm	**Serena Danti**, University of Pisa, Italy	
05.20 pm – 06.30 pm	**Poster Session II – Networking Time**	**International Cooperation workshop**

	April 19
	Conference Session VII **The bio/non-bio interface**
08.40 am – 09.10 am	**Bing Xu**, Brandeis University, United States **(Keynote lecture)**
09.10 am – 9.40 am	**Loïc Jierry**, University of Strasbourg, France **(Expert)**
9.40 am – 10.00 am	**Pierre Schaaf**, Insitut Charles Sadron, University of Strasbourg, France
10.00 am – 10.20 am	**Burkhard Bechinger**, University of Strasbourg, France
10.20 am – 10.50 am	**Coffee Break (30 min)**
10.50 am – 11.20 am	**Maryam Tabizian**, McGill University, Montreal, Canada **(Expert)**
11.20 am – 11.40 am	**Jeremy Lakey**, Newcastle University, United Kingdom
11.40 am – 12.00 pm	**Pilar Rivera Gil**, Universitat Pompeu Fabra, Barcelona, Spain
12.00 pm – 12.20 pm	**Patrick Mesquida**, King's College London, United Kingdom
12.30 pm – 02.00 pm	**Conference Lunch**
	Conference Session VIII **Instrumentation for bio- & nanotechnologies**
02.00 pm – 02.30 pm	**Takeshi Fukuma**, Kanazawa University, Japan **(Keynote lecture)**
02.30 pm – 02.50 pm	**Claire Godier**, University of Lorraine, Nancy, France
02.50 pm – 03.10 pm	**Angela C. Debruyne**, UGent, Ghent, Belgium
03.10 pm – 03.30 pm	**Maximilian Seuss**, Bruker Nano GmbH, Berlin, Germany
03.30 pm – 04.00 pm	**Coffee Break (30 min)**
04.00 pm – 04.30 pm	**Tomaso Zambelli**, ETH Zurich, Switzerland **(Expert)**
04.30 pm – 04.50 pm	**Pawel Karpinski**, Wroclaw University of Science and Technology, Wroclaw, Poland
04.50 pm – 05.10 pm	**Nicolo Tormena**, University of Durham, United Kingdom
05.10 pm – 05.30 pm	**Zahra Ayar**, EPFL, Lausanne, Switzerland

April 19

Gala Diner

07.30 pm

Caribbean Music & Dance Show




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

Gala Dinner

	April 20
	Conference Session IX **Special NanoInBio session**
08.40 am – 09.10 am	**Georg Fantner**, EPFL, Lausanne, Switzerland **(Expert)**
09.10 am – 9.30 am	**James Flewellen**, University of Edinburgh, United Kingdom
9.30 am – 9.50 am	**Ariane Boudier**, University of Lorraine, Nancy, France
9.50 am – 10.10 am	**Wojciech Chrzanowski**, The University of Sydney, Australia
10.10 am – 10.40 am	**Coffee Break (30 min)**
10.40 am – 11.10 am	**Stefano Leporatti**, CNR Nanotech-Istituto di Nanotecnologia, Lecce, Italy
11.10 am – 11.30 am	**Xavier Bellanger**, University of Lorraine, Nancy, France
11.30 am – 11.50 am	**Tomasz Antosiewicz**, University of Warsaw, Poland
11.50 am – 12.10 pm	**NANOinBIO Awards & Closing Ceremony**
12.30 pm	**Conference Lunch**

April 20	
Major Public Conference (in French) **Santé et environnement: quelles solutions apportées par les sciences ?**	
6.00 pm – 8.30 pm	**Keynotes Lecturers :** Jacqueline Deloumeaux (ARS, CHUG) Jean Sébastien Nicolas (ARB-IG) Jérôme Roch (ADEME) **Moderators :** Lucie Largitte (Université des Antilles) Kanell Ambroise (ARB-IG) **Topic: Health and the environment: what solutions can science offer?**

Oral Presentations

	April 15
08.15 am – 08.45 am	**Welcome Reception & Registration**
	Conference Session I **The bio/non-bio interface**
08.45 am – 09.00 am	**Introduction – Grégory Francius**
09.00 am – 09.30 am	**Philippe Leclère** (University of Mons, Belgium) « Towards quantitative mapping of physical and chemical properties of soft materials using unsupervised data clustering methods: When IA meets materials » **(Keynote lecture)**
09.30 am – 9.50 am	**Béatrice Labat** (University of Rouen Normandie, France) « Biomimetic LbL assembly for 2D/2.5D bone matrix modeling »
9.50 am – 10.10 am	**Régis Badin** (University of Lorraine, France) « Environmental Atomic Force Microscopy as a tool to probe single food particle properties over storage and glass transition »
10.10 am – 10.30 am	**Luca Puricelli** (Area Science Park, Trieste, Italy) « AFM-based investigation of cellular membrane – extracellular vesicles interaction: exploring the role of membrane biophysical properties »
10.30 am – 11.00 am	**Coffee Break (30 min)**
11.00 am – 11.30 am	**Igor Sokolov** (Tufts University, United States) « AFM-based OuterOmics: Progress in Cell Phenotyping and Cancer Detection Using Machine Learning » **(Keynote lecture)**
11.30 am – 11.50 am	**Lucie Klopffer** (University of Lorraine, France) « Deep Learning and 3D Holographic Microscopy to assess bacterial adhesion's dynamics under shear rate in microfluidic systems »
11.50 am – 12.10 pm	**Andreas Stylianou** (EUC Research Centre / European University Cyprus, Cyprus) « Nanomechanical Fingerprints of pulmonary fibrosis »
12.30 pm – 02.00 pm	**Conference Lunch**

Towards quantitative mapping of physical and chemical properties of soft materials using unsupervised data clustering methods: When IA meets materials !

Philippe Leclère

Laboratory for Physics of Nanomaterials and Energy – LPNE
Research Institute for Materials science and Engineering
University of Mons – UMONS
Avenue Victor Maistriau, 19, B 7000 Mons (Belgium)
e-mail: philippe.leclere@umons.ac.be

Over the past few decades functional materials have replaced existing materials in many applications from aerospace to cosmetics. With these novel materials impacting every part of our lives, they have become ubiquitous. Mechanical property mapping can provide critical insights into the fundamental processes at the local scale that lead to deformation phenomena in these materials or their degradation upon external mechanical or electrical stress.

This talk will focus on the last cutting-edge developments of scanning probe microscopies and spectroscopies (SPM) for the characterization of materials surfaces and interfaces in soft biological and polymeric materials (i.e. polymer blends, hydrogels, cosmetics, …).

Beyond surface imaging, we will highlight the abilities of SPM to characterize the properties of materials with a particular attention on the quantitative mapping of nanomechanical properties such as adhesion, indentation, Young modulus, storage modulus, loss modulus using various recent SPM-based techniques (*PeakForce Tapping, nanoDynamic Mechanical Analysis)*.

In this context, Machine Learning has been perceived as a promising tool for the design and discovery of novel materials for a broad range of applications. For instance, we propose adapted protocols for the data analysis (i.e. validation of the data acquisitions, data clustering, …), expecting to help the scientific community to better understand the key parameters in the optimization of the behaviour of materials not only for fundamental aspects but also for industrial applications.

We will shortly discuss computational methods and ML algorithms dealing with data clustering (such as *K-Means* or *Automatic Gaussian Mixture Model*) that can be used to detect the different domains and (inter)phases in materials by partitioning the recorded data (i.e. the observables) into clusters according to their similarities.

This algorithmically driven approach will enable analyze materials with more complex architectures and/or other properties, opening new avenues of research on advanced materials with specific functions and desired properties leading to the creation of functional, more reliable and ideally eco-responsible materials.

Biomimetic LbL assembly for 2D/2.5D bone matrix modeling

H. Ozanne, F. Gaudière, L. Moubri, L. Abou-Nassif, A. Echalard, S. Morin-Grognet, H. Atmani, G. Ladam, B. Labat

PBS UMR CNRS 6270 – Group BioMMAT (Biomaterials and Matrix Models for Tissue Adaptation), University of Rouen Normandie, 55 rue St Germain, CS40486, 27004 Evreux Cedex, France.
e-mail: beatrice.labat@univ-rouen.fr

Conventional cell culture systems are often far away from the physiology of targeted cells microenvironment, leading to a loosening of cell phenotype over time, because they mismatch the natural cell niche properties. Cell microenvironment is a multi-signal and multi-scale system that controls all cell processes such as adhesion, migration, proliferation and differentiation. Thus, biomimetic cell culture systems are required to provide more relevant microenvironments. In that context, we develop biomimetic matrix for bone cells with the aim of tissue repair or tissue modeling.

At the 2D scale, biomimicry is provided by an extracellular matrix (ECM)-like thin film coating, using the Layer-by-Layer (LbL) method [1]. In previous works, we thoroughly characterized a polyvalent LbL system composed of Poly-L-Lysine (PLL), a cationic polypeptide known to favor cell adhesion, and Chondroitin Sulfate A (CSA), a natural anionic glycosaminoglycan (GAG) found in connective tissues [2-3]. Not only does this LbL system provide a biomimetic interface to the cells, but it also offers a nano-micrometric topography favorable to cell adhesion and the subsequent cell processes [4].

At the 2.5D scale, a simple cell-instructive microenvironment that controls pre-osteoblasts is developed, combining adjustable stiffness and surface features that mimic bone topography by using sandpaper grits as master molds with two stiffness formulations of polydimethylsiloxane (PDMS). The subsequent replicas perfectly conform the grits and reproduce the corresponding negative relief with cavities separated by convex edges. The replicas then were post-treated with the same LbL assembly as for 2D coatings. The topographical features, alternating concave and convex structures, drive pre-osteoblasts organization and morphology. Strikingly, curvature orchestrate the commitment of pre-osteoblasts cells MC3T3-E1 into two co-existing sub-populations: (i) active osteoblasts able to produce a dense collagenous matrix that ultimately mineralizes in the cavities, and (ii) quiescent cells hosted on the edges, resembling to bone lining cells, that synthetize a very thin immature collagen layer with no mineralization (Fig. 1). This promising culture system opens new avenues to advanced tissue-engineered modelling and can be applied to pre-cellularized bone biomaterials [5].

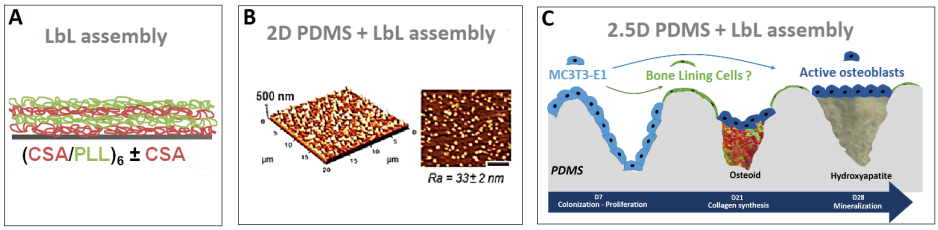

Figure 1: Pre-osteoblasts fate atop 2D or 2.5D PDMS coated with LbL assembly. (A) CSA/PLL System; (B) Corresponding AFM imaging; (C) Curvature-dependence of MC3T3-E1 fate atop concave-convex 2.5D PDMS

References

[1] G. Decher, Science 277 (1997) 1232–1237.
[2] K. Abdelkebir, F. Gaudière, S. Morin-Grognet, G. Coquerel, B. Labat, H. Atmani, G. Ladam, Soft Matter 7 (2011) 9197–9205.
[3] B. Labat, N. Buchbinder, S. Morin-Grognet, G. Ladam, H. Atmani, J.-P. Vannier, Acta Biomater. 135 (2021) 383–392.
[4] F. Gaudière, S. Morin-Grognet, L. Bidault, P. Lembré, E. Pauthe, J.-P. Vannier, H. Atmani, G. Ladam, B. Labat, Biomacromolecules, 15 (2014) 1602-1611.
[5] H. Ozanne, L. Moubri, L. Abou-Nassif, O. Thoumire, A. Echalard, S. Morin-Grognet, H. Atmani, G. Ladam, and B. Labat, Adv Healthc Mater, 13(3) (2023), e2302222.

Environmental Atomic Force Microscopy as a tool to probe single food particle properties over storage and glass transition

Régis Badin*†[1], Claire Gaiani[1], Stephane Desobry[1], Sangeeta Prakash[2], Bhesh Bhandari[2], and Jennifer Burgain[1]

[1]Laboratoire d'Ingénierie des Biomolécules (LIBio), 54000 Nancy – Université de Lorraine – France
[2]School of Agriculture and Food Sustainability, University of Queensland, Brisbane, QLD 4072 – Australia

Abstract

Powders are widely used in the food industry, mainly thanks to their ease of use, transport, and for their extended storage properties. Powder techno-functionalities (such as reconstitutability, flowability) are highly dependent on particle surface, as it is the first layer in contact with other surfaces (wall material, particles in the sample) and with the environment. Environmental conditions are known to highly impact powder properties, especially with glass transition, which completely modifies particle surface features. In this work, environmental AFM was used to probe the *in-situ* effects of glass transition on single particle surface properties by variating relative humidity (RH) and temperature. Maltodextrin powders of different DE values (low, intermediate, and high) were used as a model matrix. Humidity ramps (from 20 to 80% RH) and temperature ramps (from 20 to 50∘C) were performed. It was proved that glass transition and RH drive particle surface properties. Indeed, surface topography measurements revealed that glass transition was always accompanied by a large global surface smoothing and a surface roughness value drop, whatever the DE value. On the other hand, force-spectroscopy experiments proved that particles in the glassy state were relatively hard with a high Young modulus and an heterogenous distribution. An increase of the RH led to a progressive surface softening, while reaching glass transition led to a large decrease of the Young modulus, with a homogenization of its distribution. These results were useful to show that glass transition significantly impacts particle surface properties and could be useful to optimize maltodextrin-based formulations.

Keywords: Atomic Force Microscopy (AFM), Food powders, Maltodextrins, Glass transition, Surface topography, Young modulus

*Speaker
†Corresponding author: regis.badin@univ-lorraine.fr

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AFM-based investigation of cellular membrane – extracellular vesicles interaction: exploring the role of membrane biophysical properties

Luca Puricelli[1], Beatrice Senigagliesi[2*], Carolina Paba[2,3**], Elena Ferraguzzi[2,3], Pietro Parisse[4], Loredana Casalis[2]

[1]Area Science Park - Padriciano, 34149 Trieste - Italy
[2]Elettra Sincrotrone Trieste, 34149 Basovizza (TS), Italy
[3]Department of Physics, University of Trieste, 34127 Trieste, Italy
[4]IOM-CNR, 34149 Basovizza (TS), Italy
**Current address: IINS, Bordeaux Neurocampus, 33076 Bordeaux Cedex, France.*
*** Current address: NanoSurf AG, Gräubernstrasse 14, 4410 Liestal, Switzerland*

e-mail: luca.puricelli@areasciencepark.it

In the last decades, the research interest on biophysical topics for theranostic purposes has been growing. In this context, AFM has proved to be a valuable tool, allowing for the acquisition of simultaneous topographic and mechanical maps of biological systems on different spatio-temporal scales, ranging from cells and tissues to biological macromolecules.
In particular, keen attention has been recently dedicated to the analysis of cellular membranes models and their interaction with small Extracellular vesicles (s-EVs) [1], given the similarities of the latter to viral particles and their well-known capability to influence the fate and properties of recipient cells depending on their origin and the transferred cargo [2]. Nevertheless, a complete understanding of s-EVs fusion process with recipient cells and the role of cell membrane's biophysical properties in this framework is still lacking.
In order to get a more detailed picture of this phenomenon, here we will present our results on AFM-based analysis of topographical and compositional key features of cellular membranes model and their interaction with breast cancer derived-EVs (see fig.1, adapted from reference [1]). We will finally show our preliminary force-spectroscopy measurements on cellular membrane models to quantify the mechanical and fluidity properties of these systems on the nanoscale and their changes induced by EVs, given the demonstrated correlation between mechanical properties and the patho-physiological state of many biological specimens. Altogether our results underline the importance of understanding fusion mechanisms of EVs in breast cancer framework, since it represents the first step for potential applications in the theranostic field.

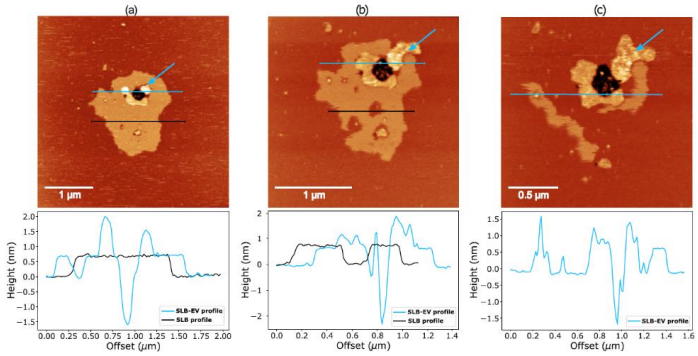

Figure 1. Time-resolved AFM topographic images of EVs (from MDA-MB-231 cell line) interacting with DOPC/SM 2:1 (m/m) supported lipid bilayer with 17 mol% Chol and corresponding height profiles, acquired at 27 ◦C in Tris buffer 10 mM, with a time-lapse of 10 minutes (from a to c).

References

[1] C. Paba et al., Journal of Colloid and Interface Science (2023), Volume 652, Part B, pp. 1937-1943.
[2] B. Senigagliesi et al., Biomolecular Concepts (2022), vol. 13, no. 1, pp. 322-333.

AFM-Based OuterOmics: Progress in Cell Phenotyping and Cancer Detection Using Machine Learning

Igor Sokolov[1]

[1] *Department of Mechanical Engineering, Biomedical Engineering, Tufts University, Medford, MA 02155, USA;*
e-mail: igor.sokolov@tufts.edu

Recent developments of sub resonance tapping AFM have demonstrated the ability of simultaneous recording up to fourteen different physical and mechanical properties of cells [1, 2]. These multidimensional (14D) images carry the amount of information comparable to the bioinformatic "omics", such as genomics, that are currently used in biomedical and clinical practices. We have suggested to call this approach "OuterOmics" given its focus on the outer cell surface.

Here, I describe the recent developments done in my group in applications of OuterOmics for the identification of cell phenotype, in particular, cell malignancy [3-5]. We employ a machine learning (ML / AI) approach, which presents unique challenges in data preparation. While the volume of data from each AFM image is substantial enough to consider deep learning, the relatively slow nature of AFM limits the number of measurements, a common issue known as the problem of small data sets. We will discuss our solutions to this challenge and propose several templates.

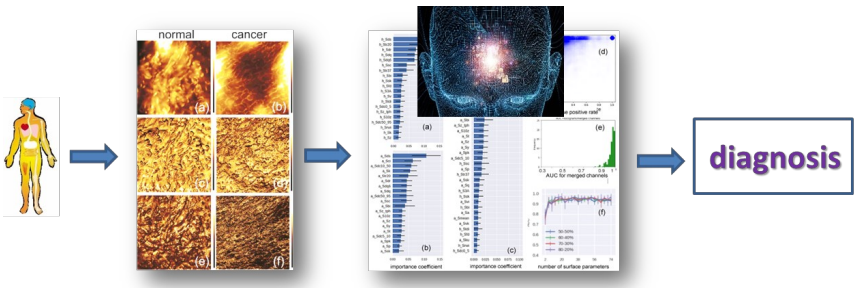

Figure 1: A schematic of detection of cancer using OuterOmics combined with machine learning.

Data reliability, another major challenge in ML, is crucial in AFM where it translates into repeatability. Our findings indicate that sub-resonance tapping and ringing modes yield high repeatability. Next, we propose a special cell fixation protocol to preserve the cell surface. And lastly, we propose to use imaging in air. Employing human epithelial colorectal cancer cells as a model, our approach demonstrated superior accuracy compared to traditional cell mechanics measurements and even single-cell DNA tests.

The described approach will be demonstrated on human cervical cells, colorectal cells, bladder cells, as well as using the actual patient samples to detect the presence of active bladder cancer. In the latter case, a result demonstrated 94% accuracy in identification of active bladder cancer when using cells extracted from patient's urine, surpassing the 80% accuracy rate of conventional cystoscopy (visual inspection of bladder) [6].

A general template for ML analysis specific to AFM will be suggested. Special attention will be given to the analysis of the statistical significance of the obtained results, an important feature that is often overlooked in papers dealing with machine learning. A simple method for finding statistical significance will also be described.

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Deep Learning and 3D Holographic Microscopy to assess bacterial adhesion's dynamics under shear rate in microfluidic systems

L. Klopffer[1,2], L. Mathieu[1,4], C. Pradalier[3], S. Becker[2] and N. Louvet[2]

[1] *Université de Lorraine, CNRS, LCPME, F-54500 Nancy, France*
[2] *Université de Lorraine, CNRS, LEMTA, F-54500 Nancy, France*
[3] *GeorgiaTech Europe, IRL 2958, F-57070 Metz, France*
[4] *EPHE, PSL, UMR CNRS 7564, LCPME, F-54500 Nancy, France*

e-mail : lucie.klopffer@univ-lorraine.fr

Pioneer bacterial adhesion events play a crucial role in biofilm formation and need further understanding to appreciate bacterial colonization and consider appropriate control strategies. In this context, our study aims at implementing 2 complementary approaches based on Deep Learning and Holographic Digital Microscopy. Our objective is to monitor in 3D space and time the dynamics of wall transfer and adhesion of a model bacteria in a home-made microfluidic system under different hydrodynamics (from 0 to 1250 s^{-1}). This device is mounted under an epifluorescence microscope equipped with a CCD camera and fed 2 hours with a *Shewanella oneidensis MR-1* suspension (stationary phase; 10^7 cells/mL). From images recorded, Deep Learning analysis allows massive data and information extractions of the bacteria dynamics at the wall i.e. their attachment/detachment frequencies, residence time, orientation and movement. The Holographic Digital Microscopy technic enables to access bacteria dynamics in 3 dimensions and appreciate bacteria transfer mechanisms at the wall, trajectories or speed in the bulk and near the wall.

Our preliminary Holographic Microscopy results showed that about 10% of the bacteria in the inoculum are initially motile with an average speed of 40 µm/s. While bacteria trajectories are relatively straight in the bulk, close to the wall we observe that bacteria spend a long time to swim with complex trajectories. Moreover, Deep Learning analysis revealed that the orientation of the pioneering adhered bacteria at the wall under static conditions follow random distribution, while bacteria subjected to hydrodynamic constrains were mostly oriented parallel to the flow. Quite unexpectedly, we also observed that most of the adhered bacteria presented a very short residence time at the wall, i.e. < 10 seconds, which could question the capability for further colonization. To this end, we have characterized the attachment / detachment frequencies and measured the increased surface colonization over time. The hydrodynamics significantly impacted this bacterial colonization kinetics with notably better colonization speed and level obtained for wall shear rate close to 125 s^{-1}. These two approaches help to better characterize the behavior and dynamics at the very early steps of adhesion of pioneer bacteria at the wall.

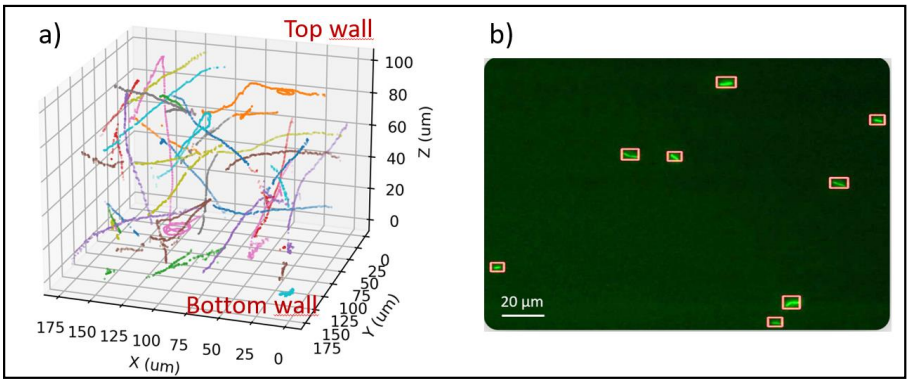

Figure 1: a) Example of *S.oneidensis* swimming trajectories in the microfluidic device used under static condition (Holographic Microscopy)
b) Example of *S.oneidensis* bacteria detection at the bottom wall of the microfluidic device used under flow at the beginning of the adhesion experiment (Deep Learning)

Nanomechanical Fingerprints of pulmonary fibrosis

A. Stylianou[1,2], K. Polemidiotou[1,2], V. Gkretsi[1,2], F. Mpekris[3], A. Zachariades[4], T. Stylianopoylos[3]
[1]European University Cyprus, Cyprus, [2]E.U.C. Research Centre, Cyprus
[3] University of Cyprus, Cyprus, [4]Nicosia Lung Center, Cyprus
e-mail: an.stylianou@euc.ac.cy

The efficacy of standard pulmonary fibrosis therapies varies due to intra- and inter- patient variability and due to the absence of specific biomarkers that can characterize the state of a specific fibrotic lung. Given that only some patients respond to a particular treatment and only a few drugs have been approved for pulmonary fibrosis, personalized, patient-specific treatments are urgently needed to identify biomarkers able to predict and/or diagnose pulmonary fibrosis, tissue response to treatment or even monitor treatment outcome . As fibrosis progression is associated with changes in tissue content and structure, as well as modifications in mechanical properties and mechano-cellular phenotype,, the field of mechanobiology emerges as a very promising research area. . In this study, we tested the hypothesis that Atomic Force Microscopy (AFM) can identify unique and novel nanomechanical fingerprints (NMFs) for the characterization and diagnosis of the fibrosis state as well as treatment monitoring, as it has been previously demonstrated in other pathological conditions [1,2]. The NMFs that characterize the state of a particular fibrotic tissue were explored and evaluated as possible biomarker by using murine models of pulmonary fibrosis at different time points (Fig. 1A,B). Then, the unique NMFs were studied using biopsies from mice with pulmonary fibrosis receiving treatment with Pirfenidone, an approved pulmonary fibrosis drug . The AFM data were supported by histopathological staining with picrosirius red, polarized microscopy and real-time PCR analysis of collagen gene expression. Finally, the capabilities of the techniques and methods to assess NMFs in human tissue biopsy samples were investigated (Fig.1C). The results showed that AFM-based measurements are sensitive enough to capture small alterations on the NMFs during pulmonary fibrosis progression. Also, alterations of NMFs were correlated with alterations in collagen type I content as shown by gene expression analysis. Our findings provide the first evidence that novel AFM-based Nanomechanical Biomarker for pulmonary fibrosis staging and treatment monitoring can be established and have the potential to classify individuals to possible responders to a particular treatment, which is especially valuable and easily transferable to the clinic as it can be used in parallel to standard biopsy procedures.

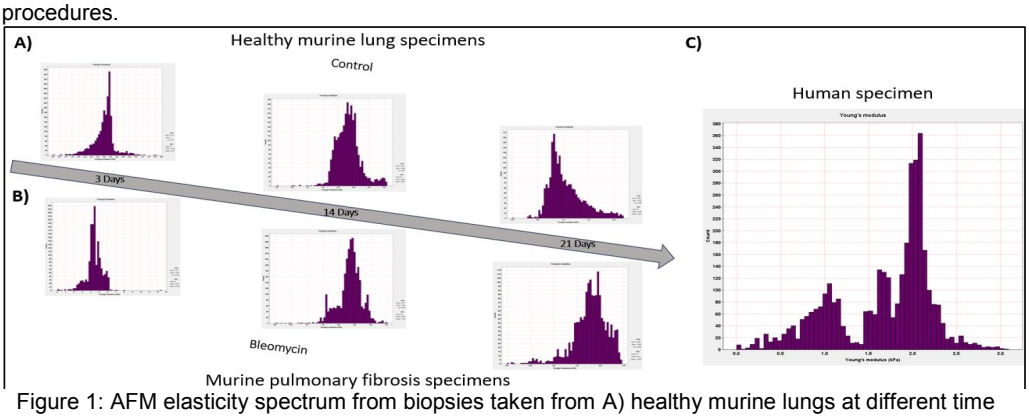

Figure 1: AFM elasticity spectrum from biopsies taken from A) healthy murine lungs at different time points, B) murine lungs with pulmonary fibrosis and C) a patient with pulmonary fibrosis.

Acknowledgement

This work was funded by the Research and Innovation Foundation (RIF) for the projects "MechanoLung" (EXCELLENCE0421/0263).

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	April 15
	Conference Session II Instrumentation for bio- & nanotechnologies
02.00 pm – 02.30 pm	**Sungsu Park** (Sungkyunkwan University, South Korea) « Revolutionizing Cancer Research with 3D Microfluidic Devices: Simulating Drug Resistance and Immune Dynamics » **(Keynote lecture)**
02.30 pm – 02.50 pm	**Etienne Dague** (LAAS-CNRS, Toulouse, France) « Automated generation of large-scale mechanome datasets using bio-AFM technology, followed by machine learning analysis for the classification of prostatic cell lines »
02.50 pm – 03.10 pm	**Katarzyna Matczyszyn** (Wroclaw University of Science and Technology, Poland) « Bioimaging of DNA, proteins and myelin with the use of carbon nanodots »
03.10 pm – 03.30 pm	**Jordyn Ann Howard** (University of Lyon 1, Villeurbanne, France) « Application of a Chitosan-Based Chelating Polymer as a Novel Therapy Against Lead and Cadmium Ingestion »
03.30 pm – 04.00 pm	**Coffee Break (30 min)**
04.00 pm – 04.20 pm	**Juan Felipe Salazar Ariza** (University of Lyon 1, France) « Chitosan based hydrogel for iron (III) chelation in biological conditions »
04.20 pm – 04.40 pm	**Thomas Hackl** (TU Wien, Vienna, Austria) « Atomic Force Microscopy based Charge Mapping in Water »
04.40 pm – 05.10 pm	**Marco Portalupi** (Nanosurf AG, Liestal, Switzerland) « Developments in photothermal excitation for AFM sample imaging, characterization, and manipulation »
05.10 pm – 06.30 pm	**Poster Session I – Networking Time**
07.30 pm	**Conference Dinner**

Revolutionizing Cancer Research with 3D Microfluidic Devices: Simulating Drug Resistance and Immune Dynamics

Sungsu Park

School of Mechanical Engineering
Sungkyunkwan University (SKKU) – Korea
e-mail: nanopark@skku.edu

In this presentation, I unveil two cutting-edge microfluidic devices engineered to revolutionize our understanding of cancer by simulating the accelerated development of drug resistance and the complex interplay between cancer cells and the immune system. The first device is a sophisticated 3D microfluidic cell culture platform that utilizes concentration gradients and microfabricated habitats to effectively replicate the dynamic tumor microenvironment (TME) observed in chemotherapy patients. The second innovation, a novel multi-inlet spheroid generator (MSG), allows for the precise introduction of drug-containing solutions or cells into spheroids through a side inlet, significantly mitigating mechanical stress on the spheroidal structure. Demonstrating their clinical relevance, I will show how these devices can assess drug efficacy in patient-derived cancer (PDC) cells and finely tune the stromal cell ratio within TME-analogous spheroids. The findings I will share underscore the immense potential of these microfluidic platforms to enhance the high-throughput screening (HTS) of anticancer drugs and to offer a more faithful representation of the TME, thus setting the stage for groundbreaking advancements in cancer therapy and research.

Key Words: 3D Microfluidic Devices, Cancer Drug Resistance, Tumor Microenvironment (TME), High-Throughput Screening (HTS), Patient-Derived Cancer Cells (PDC), Immune-Cancer Cell Interaction

Bio: Professor Sungsu Park received his Ph.D. from Department of Food Science of Cornell University in 1999 and worked as a postdoctoral researcher at several institutes: Department of Bioengineering of Tokyo Institute Technology (1999), Department of Environmental and Agricultural Engineering of Cornell University (2000), and Department of Physics at Princeton University (2001-2003). In 2004, he joined as an assistant professor at Chemistry and Nano Science Department of Ewha Womans University in Seoul, Korea and promoted to a full professor in 2011. He moved to School of Mechanical Engineering of Sungkyunkwan University in 2014. His major research interests are focused on organ on a chip, tumor on a chip and 3D printed paper fluidic device for molecular diagnostics and point of care (POC) testing.

Automated generation of large-scale mechanome datasets using bio-AFM technology, followed by machine learning analysis for the classification of prostatic cell lines.

Ophélie Thomas - - Chemin,[1] Childérick Severac,[2] Abderazzak Moumen,[1] Adrian Martinez-Rivas,[3] Christophe Vieu,[1] Marie-Véronique Le Lann,[1] Emmanuelle Trevisiol[4] and Etienne Dague[1]

1LAAS-CNRS, Université de Toulouse, CNRS, Toulouse France
2RESTORE Research Center, Université de Toulouse, INSERM, CNRS, EFS, ENVT, UPS, Toulouse, France
3CIC, Instituto Politécnico Nacional (IPN), 07738, Mexico City, Mexico
4TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France
e-mail: edague@laas.fr

Mechanobiological measurements hold promise in distinguishing between healthy and pathological cells. However, the commonly employed technology for such measurements, atomic force microscopy (AFM), is hindered by its low throughput and lack of standardization. In this study, we have refined AFM mechanical measurements on cell populations and devised a technology that integrates cell patterning and AFM automation. This innovation has the capacity to gather data on hundreds of cells.

We conducted mechanome analysis by capturing 16 force curves (FCs) and identifying seven features per FC on each cell. These features were then utilized in a fuzzy logic algorithm trained to differentiate between non-malignant and cancerous cells. Our training dataset comprised up to 120 cells per cell line. We tested this approach on prostate non-malignant (RWPE-1) and cancerous (PC3-GFP) cell lines. Despite the high degree of similarity in measurements across the dataset (ranging from 82% to 100% similarity), our method correctly classified 73% of cells (194 cells per cell line), demonstrating its efficacy.

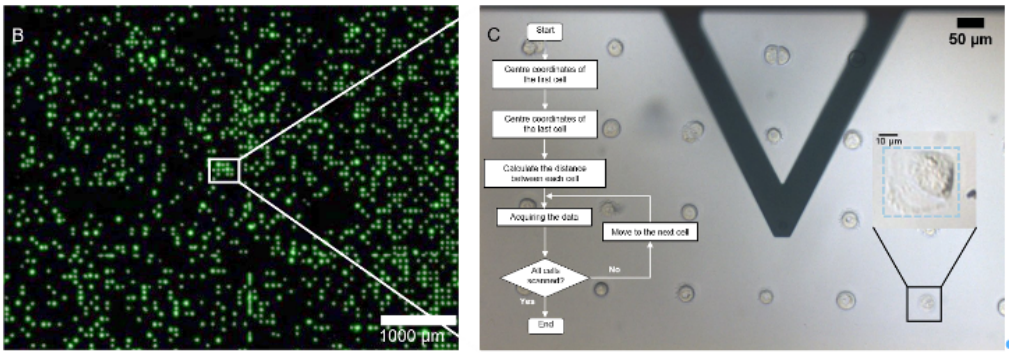

Figure 1: Fluorescence image of PC3-GFP cells having adhered to the fibronectin patterns. (**C**) Steps for automating AFM measurements on an array of cells.

Bioimaging of DNA, proteins and myelin with the use of carbon nanodots.

Katarzyna Matczyszyn[1] , Dominika Benkowska-Biernacka,[1] and Sebastian G. Mucha[2]

[1] *Faculty of Chemistry, Wroclaw University of Science and Technology, Wyb.Wyspianskiego 27, 50-370 Wrocalw, Poland*
[2] *Laboratoire Charles Coulomb, Université de Montpellier (CNRS), Campus Triolet, Place Eugène Bataillon, Montpellier 34095, France*
e-mail: Katarzyna.matczyszyn@pwr.edu.pl

The presented research focuses on the development of novel fluorescent probes characterized by high brightness and biocompatibility. Carbon nanodots (CNDs) were synthesised from different precursors (including phloroglucinol and folic acid), and their structural and optical properties were established. Our CNDs exhibited notable fluorescence capabilities under both linear and non-linear (two-photon) excitation, making them well-suited for imaging purposes [1,2].
The objective of the study is to develop new probes for the detection and visualisation of biomolecules such as DNA, BSA and myelin [1,3].
We aimed to comprehend membrane-mediated processes by observing morphological changes in lipidic mesophases, emphasizing the importance of non-invasive imaging. Confocal fluorescence microscopy and two-photon excited fluorescence microscopy were employed to investigate the three-dimensional distribution of folic acid-derived CNDs within bioinspired myelin figures [1, 4].
The research showcased that CNDs effectively function as fluorescent markers, facilitating the imaging of various forms and components within phospholipid-based myelin figures' multilamellar microstructures.
In summary, the presented studies proved the utility of CNDs synthesised by us for selective detection of proteins both in the linear and non-linear regime as well as showed our abilities of successful introduction and characterization of folic acid-derived CNDs as exceptional fluorescent markers for non-invasive imaging of myelin figures. This advancement enables the visualization and examination of structural changes in lipidic mesophases. The research outcomes hold promising applications in enhancing our understanding of membrane-related processes and could contribute to the advancement of imaging techniques in the realms of biology and medicine.

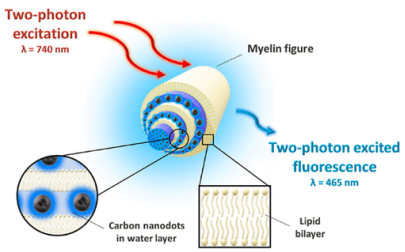

Figure 1: Scheme of doping the myelin figures with folic acid-based carbon nanodots as presented in [1].

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Application of a Chitosan-Based Chelating Polymer as a Novel Therapy Against Lead and Cadmium Ingestion

Presenting author (J.A. Howard),[1,2] H. Kuznietsova,[3] N. Dziubenko,[3] A. Aigle,[1] M. Natuzzi,[1] E. Thomas,[4] V. Lysenko,[2] L. David,[6] T. Brichart,[2] F. Lux,[2,5] and O. Tillement[2]

[1]*MexBrain, Villeurbanne, France*
[2]*Institute of Light and Matter, UMR5306, University of Lyon 1, Villeurbanne Cedex, France*
[3]*Corporation Science Park, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine*
[4]*LAGEPP, UMR5007, University of Lyon 1, Villeurbanne Cedex, France*
[5]*Institut Universitaire de France (IUF)*
[6]*University of Lyon 1, INSA de Lyon, Université Jean Monet, CNRS, UMR5223 Ingénierie des Matériaux Polymères (IMP) Villeurbanne Cedex, France*
e-mail: jordyn.howard@mexbrain.com

Heavy metals present a threat to human health, even at minimal concentrations within the body. Consumption of low-level contaminated food and water is the major source of exposure to lead and cadmium (non-smokers). [1] This low-level, chronic exposure exists in all countries, even when established regulatory limitations for certain food products are respected, and is therefore a concern for all humans. Lead and cadmium have been shown to accumulate within organs like the kidneys and liver and have been associated to cardiovascular disease and kidney dysfunction, therefore presenting a most immediate concern for patients already suffering from renal dysfunction due to the vicious cycle lead and cadmium instill in the system that leads to a faster clinical decline. [1-3] Chelation therapy is the only true therapy that has been established for an excess of heavy metals, however, the costs (adverse effects, trans-metalation, not acceptable for children, elderly, or pregnant women) greatly outweigh the benefit as a therapy for chronic exposure. Currently, only subtle recommendations of dietary intake and reducing direct exposure to the source are offered to chronic patients. There is a great need for novel therapeutic strategies to address and prevent the absorption and accumulation of these metals within chronically exposed humans. In this work, a DOTAGA-functionalized chitosan polymer is evaluated and shown to be a promising candidate as a therapeutic strategy.[4-5] Mice studies show that the size of this polymer allows for its retention within the digestive tract, while still excreted in a timely manner, and its functionalization provides a protective effect against lead and cadmium exposure within foodstuff (shown by the biochemical and hematological analyses as well as the histopathology observations of the mice organs after sacrifice), when compared to control mice. Specifically, the mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and white blood cell count (WBC) were normalized, the blood lead level was mitigated, and there were no signs of tubular injury unlike the controls who exhibited diminished interstitial nephritis and glomerulo-nephritis. The ongoing work includes the optimization of this polymer for lead and cadmium as well as other toxic heavy metals.

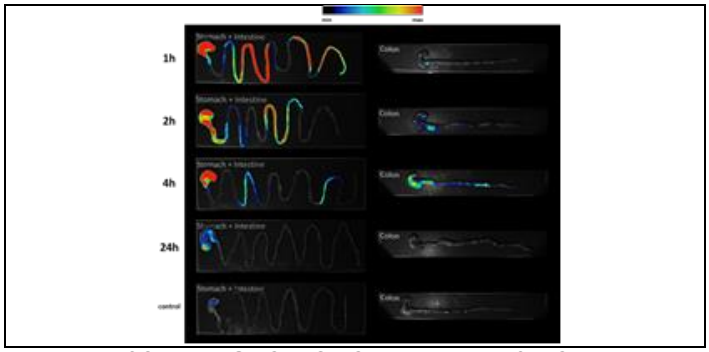

Figure 1: Visualization of Chitosan@DOTAGA Over 24 Hours After Oral Administration in Mice

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Chitosan based hydrogel for iron (III) chelation in biological conditions.

Juan Felipe Salazar Ariza,[1,2] François Lux,[1,3] Fernande Da Cruz-Boisson,[2] Ruben Vera,[4] Olivier Tillement.[1] Alexandra Montembault,[2] Laurent David,[2]

[1] Institut Lumière-Matière, UMR 5306, Université Lyon1-CNRS, Université de Lyon, Villeurbanne Cedex, France.
[2] IMP, CNRS UMR 5223, Univ Claude Bernard Lyon 1, Univ Lyon, 15 bd Latarjet, 69622 Villeurbanne, France.
[3] Institut Universitaire de France (IUF), 75231 Paris, France.
[4] Université Claude Bernard Lyon 1, Centre de Diffractométrie Henri Longchambon, 43 boulevard du 11 Novembre 1918, 69622 Villeurbanne, France.
e-mail: juan.salazar-ariza@univ-lyon1.fr

Despite iron's crucial physiological role in multiple life-sustaining biological processes, it has garnered attention as a therapeutic target for various diseases, particularly neurological conditions, owing to its involvement in oxidative stress and inflammation[1–3]. When iron homeostasis is compromised, excess iron becomes loosely bound to proteins and small molecules, participating in redox reactions and generating reactive oxygen species (ROS) [1,3]. Despite the potential induction of various diseases by excess iron, the body lacks an effective mechanism for its elimination [4]. Iron homeostasis relies solely on regulating proteins (transferrin, ferritin, and hepcidin), which become overwhelmed during iron overload crises [5]. In this study, we developed a chitosan-based polymer with robust iron (III) chelating capabilities by grafting the potent iron (III) chelator DFO, achieving a substitution degree of 3.8±0.2%. Through blending with low-degree acetylation (DA) chitosan, a physical hydrogel formed in aqueous media without the need for a cross-linking agent. The functionalization with DFO resulted in reduced xerogel crystallinity due to steric hindrance from the large DFO moiety, leading to a higher swelling capacity when exposed to aqueous solutions compared to an unmodified chitosan xerogel. This chelating biomaterial exhibited superior iron chelation properties at physiological pH compared to bare chitosan gels, with chelation capacity correlating with the amount of DFO present within the gel. Furthermore, in the presence of other cations such as copper (II) and zinc (II), the material demonstrated a preference for chelating iron (III).

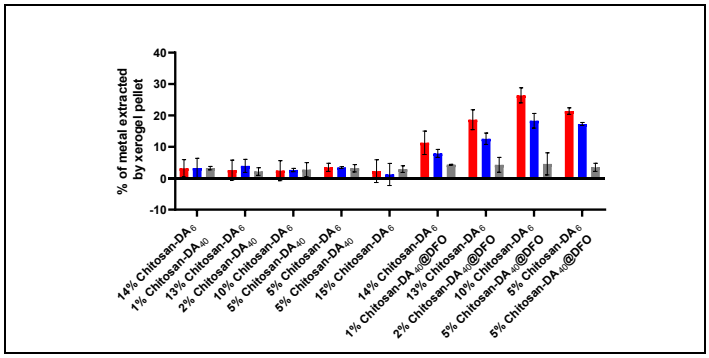

Figure 1: Extraction of ●Iron (III), ■copper (II) and ▲zinc (II) by xerogel pellet after 90 hours in 20 ml of a multi metal solution with a concentration of 3.58 µM for each metal, 12.9 µM of EDTA and 35 mM of Na_2SO_4 in HEPES 10 mM (pH:7.4). The measurements were obtained using ICP-MS

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Atomic Force Microscopy based Charge Mapping in Water

T. Hackl,[1] P. Mesquida,[1,2] M. Poik,[1] and G. Schitter[1]

[1]*Automation and Control Institute (ACIN), TU Wien, A-1040 Vienna, Austria*
[2]*Department of Physics, King's College London, London WC2R 2LS, United Kingdom*
E-Mail: hackl@acin.tuwien.ac.at

Measuring nanoscale surface charges or the related surface potential at the solid-liquid interface is of high importance in many areas, ranging from colloidal systems to biomolecular interactions. Classical methods, like dynamic light scattering or streaming-potential measurements, either rely on hydrodynamic models and do not provide spatial resolution or, like Kelvin-probe force microscopy (KPFM), cannot be operated in aqueous environments. Here, the application of a dc-bias between the scanning cantilever probe and the sample induces various parasitic effects when operated in water (i.e. electromigration and electrolysis). With the recent development of AC-KPFM [1-3], this dc-bias is replaced by a high frequency ac-voltage or, in the case of SH-KFPM [4], completely omitted. Thus, paving the way for nanoscale surface potential measurements in liquid environments.
Figure 1 shows the schematic principle of AC-KPFM, with its use of two high-frequency ac-voltages, whose controlled amplitude-modulation results in a measurement of the local sample surface potential underneath the cantilever tip. In [2] its operation in aqueous solution is demonstrated, by measuring the surface potential of micropatterned, functionalized alkanethiol layers, which expose ionized amino- and carboxy-groups. The charge of these groups (i.e. de-/protonation) is reversibly altered by changing the pH of the solution ($COOH \leftrightarrow COO^-$/ $NH_3^+ \leftrightarrow NH_2$). Their potential is then quantified via relation to the uncharged CH_3 groups. The thiol-monolayers are structured by standard micro-contact printing on a flat gold-covered substrate, ensuring no topographical features and pure electrostatic nature of the measured signal. Since the sample is immersed in aqueous media, an electric double layer (EDL) forms itself at the solid-liquid interface. It is found that arrangement of counter-ions (i.e. the Stern-layer) near the surface impacts the measured potential and forms a deterministic tip-sample distance dependence, when compared to measurements performed in air.
The results show, that AC-KPFM enables the measurement of surface potentials in aqueous media and the investigation of the EDL with high spatial resolution, which is not possible with classical atomic force microscopy (AFM) methods. With the more recent development towards heterodyne detection [3] its spatial resolution is further increased, enabling quantitative determination of surface potentials of nanoscale samples, such as collagen fibrils. With that, many applications in biology or related fields are conceivable, such as the investigation of collagen glycation or histone acetylation.

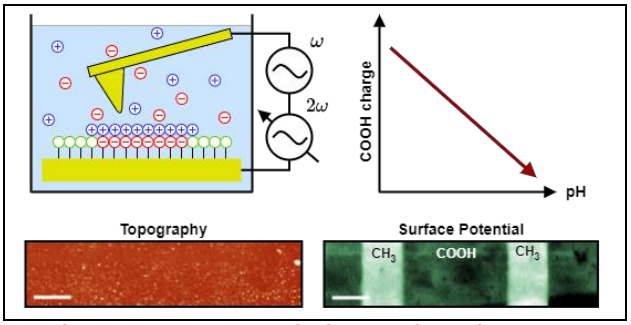

Figure 1: Illustration of the working principle of AC-KPFM for surface potential measurements in aqueous solutions. Altering the pH of the solution results in de-/protonation of the ionizable thiol monolayer, which is measured by AC-KPFM. (scale bar: 10µm) [2]

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Developments in photothermal excitation for AFM sample imaging, characterization, and manipulation

M. Portalupi[1], J.D. Adams[1], Ch.A. Bippes[1], A. Axt[1], P.J.A. van Schendel[1], H. Gunstheimer[1,2], B.W. Hoogenboom[1], D. Ziegler[1]

[1]*Nanosurf AG, Gräubernstrasse 12-14, CH-4410 Liestal, Switzerland*
[2]*Institute of Microstructure Technology (IMT), Karlsruhe Institute of Technology, Karlsruhe, Germany*
e-mail: portalupi@nanosurf.com

Atomic force microscopy (AFM) is a powerful and multifunctional instrument capable of imaging, characterization, and manipulation through interaction of the tip and the sample. In most AFM systems, a light source is used to detect the cantilever motion using the beam deflection method. Including a second light source in the AFM for actuating the cantilever through the photothermal effect has proven in recent years to be a valuable addition to the AFM instrument. Beyond exciting the cantilever at its resonance frequency, photothermal excitation offers numerous additional ways of actuating the cantilever. These include low-frequency manipulation of cantilever deflection and temperature, actuation at sub-resonance frequencies, and excitation of higher eigenmodes, opening new approaches for using the AFM.

In this presentation, we will provide an overview of new developments and methods in using photothermal excitation in AFM for imaging, characterization, and manipulation of samples at the nanoscale. These include off-resonance imaging techniques that make use of photothermal excitation for overcoming traditional speed limits in off-resonance imaging [1], mass and mechanical property measurements of cells and particles [2, 3], and manipulation of samples through photothermal control of the tip temperature.

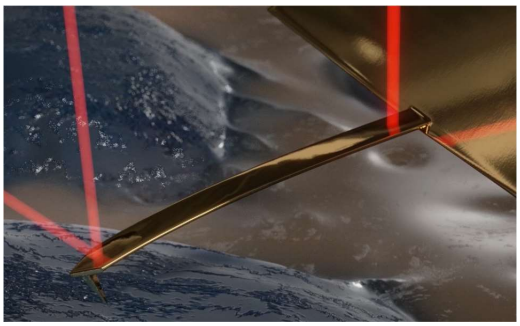

Figure 1: schematic representation of photothermal actuation for direct cantilever bending, through a second, intensity-modulated light source directed towards the base of the cantilever.

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	April 16
08.30 am – 09.00 am	**Registration**
	Conference Session III **Bio- and nano-electrochemistry & bio applications**
09.00 am – 9.30 am	**Ulrich Keyser** (University of Cambridge, United Kingdom) « Single-molecule analysis of RNA using nanopores: From structure to disease diagnostics » **(Expert)**
9.30 am – 9.50 am	**Kamila Łępicka** (Polish Academy of Sciences, Warsaw, Poland) « Donor-Acceptor-Donor type polymer semiconductor layers: Charge transport and conductivity features studies »
9.50 am – 10.10 am	**Ronald Zirbs** (University of Natural Resources and Life Sciences, Vienna, Austria) « Sustainable Food Packaging Using Modified Kombucha-Derived Bacterial Cellulose Nanofillers in Biodegradable Polymers »
10.10 am – 10.30 am	**Allan Duro** (Université des Antilles, France) « Study of interactions between biological receptors and pesticides using *in silico* methods»
10.30 am – 11.00 am	**Coffee Break (30 min)**
11.00 am – 11.30 am	**Elena Ferapontova** (Aarhus University, Denmark) « Rationalizing Interfacial Design of Electrocatalytic Biosensors Relying on Electrical Properties of DNA » **(Keynote lecture)**
11.30 am – 11.50 am	**Menglin Chen** (Aarhus University, Denmark) « Electrohydrodynamics based functional nanofibers for wireless electrical neuromodulation »
11.50 am – 12.10 pm	**Stacy Melyon** (Université des Antilles, France) « The potential of Sargassum *natans* and *fluitans* for new biomaterials for water depollution and corrosion protection »
12.30 pm – 02.00 pm	**Conference Lunch**

Single-molecule analysis of RNA using nanopores: From structure to disease diagnostics

Ulrich F. Keyser[1]

[1]*Cavendish Laboratory, University of Cambridge*, UK
e-mail: ufk20@cam.ac.uk

Rapid identification of RNA molecules is a major challenge in biotechnology. This is driven by the discovery of RNAs that control cellular function ranging in length from a few to 1000s of nucleotides. Here we design three-dimensional nucleic acid constructs that enable the identification of short and long RNA molecules and nanopore readout.
First, we describe the identification of transcript isoforms at the single-molecule level using solid-state nanopore microscopy. We refold target RNA into RNA identifiers with designed sets of complementary DNA strands. Each reshaped molecule carries a unique sequence of structural (pseudo)colours. The sequence of structural colours of RNA identifiers enables simultaneous identification and relative quantification of multiple RNA targets without prior amplification. RNA IDs discriminate circular and linear transcript isoforms in a one-step, enzyme-free reaction in a complex human transcriptome using single-molecule read-out [1]. We will show recent results on analysing transcription termination [2] and introduce a methodology to count CTG repeats in RNA.
In the second part, we use designed DNA identifier that allows the multiplexed identification of short RNA molecules. We demonstrate the power of the approach by identifying common viruses and their variants with a nanopores microscope [3]. Finally we show bacterial disease identification with single-base pair resolution with advanced RNA:DNA nanotechnology and solid-state nanopore sensing [4].

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Donor-Acceptor-Donor type polymer semiconductor layers: Charge transport and conductivity features studies

Kamila Łępicka,[1] and Gregory Francius,[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*

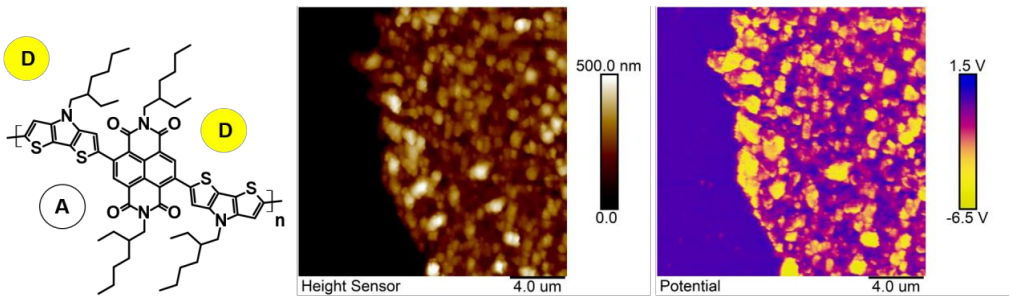
klepicka@ichf.edu.pl

A new alternated donor-acceptor-donor, DAD type polymer semiconductor layers reviling ambipolar properties, i.e., electrochemical activity in both the positive and negative potential range, should afford the conductivity extending over a very wide voltage range, highly desirable for devising and fabricating materials capable of energy transducing and storage. Energy transport of such polymer semiconductors is triggered by current or voltage. It can be considered efficient if it results in the formation of recognizable isoenergetic states throughout the active polymer layer. However, this conduction process is usually interrupted by the presence of so-called defects [1], i.e., conjugation breaks, short conjugation ranges, and chemically altered polymer units. Notably, the limited ability of electrons and holes transport is directly connected to the semiconductor stability problem caused by charges trapped [1] in the bulk or on the surface without being sufficiently released.

The scientific problem aimed to be solved by our research consists of improving the surface conductivity and the charge transport in the bulk of ambipolar polymer semiconductor layers by the targeted tuning of ambipolar polymer layers molecular structure for application in miniaturized energy storage and energy transducing systems dedicated to biocompatible devices.

With that respect, we combined the electrochemical control studies with Electrostatic Force Microscopy, EFM, and Raman spectroelectrochemistry measurements to distinguish the most stable and conducting microstructures of the ambipolar layers fabricated by electropolymerization under distinct conditions.

Significantly, with the EFM surface potential measurements, we could correlate the surface potential and, thus, the electronic states of the local structures on the surface of differently structured DAD-type ambipolar layers. Hence, we recognized the charge-trapping features characteristic of the specific structures of DAD-type polymer semiconductor layers.



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Sustainable Food Packaging Using Modified Kombucha-Derived Bacterial Cellulose Nanofillers in Biodegradable Polymers

Mikhail Koreshkov[1], Yuuki Takatsuna[1], Alexander Bismarck[2], Ines Fritz[3], Erik Reimhult[1] and Ronald Zirbs[1]*

[1] *Department of Bionanosciences, Institute of Biologically Inspired Materials, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria. *e-mail: **ronald.zirbs@boku.ac.at***
[2] *Polymer and Composite Engineering (PaCE) Group, Institute of Material Chemistry and Research, Faculty of Chemistry, University of Vienna, Vienna, Austria*
[3] *Department of Agrobiotechnology, Institute of Environmental Biotechnology, University for Natural Resources and Life Sciences (BOKU), IFA-Tulln, Tulln, Austria*

Plastic pollution from single-use food packaging is a major environmental challenge. Biodegradable alternatives would have to demonstrate mechanical and barrier properties that rival those of fossil-based polymers. One of the most promising modification strategies for biodegradable composites is the addition of bacterial cellulose (BC) as a filler material, but BC's hydrophilic nature prevents its homogeneous dispersion within hydrophobic polymer matrices, leading to poor nanocomposite properties. We show that chemically modifying bacterial nanocellulose using lactic acid oligomers and adding this filler material to poly-L-lactic acid (PLLA) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) sufficiently reduces oxygen permeability, increases the biodegradation rate and results in only minor changes in mechanical properties. This superior performance compared to standard composites using cellulose fillers was correlated to improved distribution of the modified BC. We used conventional polymer processing methods to produce nanocomposites with modified bacterial nanocellulose derived from agricultural waste. These offer a sustainable solution for food packaging.

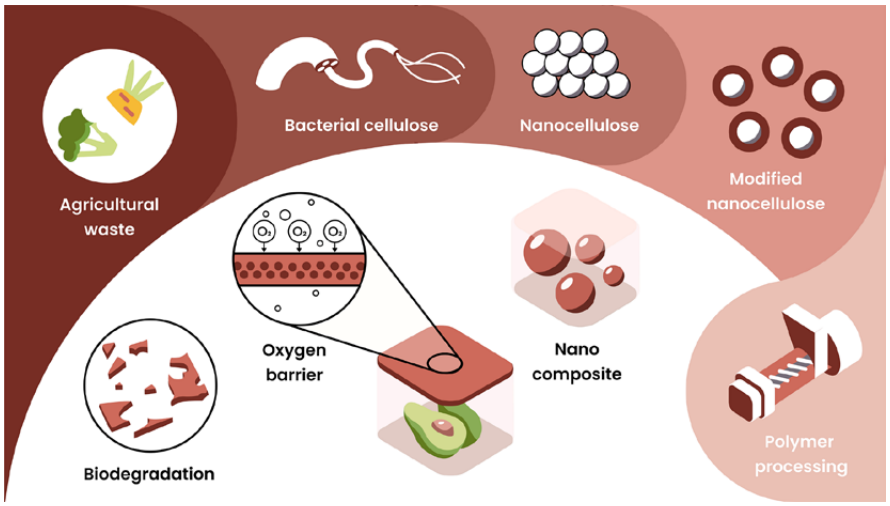

Figure 1: Addressing plastic pollution with sustainable food packaging!
Modified bacterial cellulose enhances biodegradable composites, reducing oxygen permeability, accelerating biodegradation, and preserving mechanical properties.

Study of interactions between biological receptors and pesticides using *in silico* methods

A. Duro,[1] F. Goudou,[1] G. Cebrian-Torrejon,[1] U. Jauregui-Haza,[2] and S. Gaspard[1]
[1]*COVACHIM M2E, département de chimie, Campus de la Fouillole, 97157 Pointe-à-Pitre Cedex*
[2] *INTEC, Av. de Los Próceres 49, Santo Domingo 10602, République dominicaine*
e-mail: allan.duro@univ-antilles.fr , sarra.gaspard@univ-antilles.fr

Nowadays, molecular modeling is used to predict the toxicity of xenobiotics. Due to issues related to animal protection, the 3R principle (refine, reduce, replace) involving new means of toxicological studies has emerged, notably *in silico* methods [1]. This technique makes it possible to construct and visualize the structure of macromolecules such as membranes, biological receptors, and surface proteins at the molecular scale. These molecular structures are obtained from characterization results obtained by X-ray crystallographic studies generally published in the RCSB PDB database or more recently alphafold which integrates the artificial intelligence [2]. In addition, through in silico studies, it is also possible to describe toxicodynamics by studying molecular interactions between the surface of biological receptors and xenobiotics, in order to understand their mechanism of action in living organisms.

As part of this communication, the aim is to review the literature on existing studies concerning molecular interactions between receptor surfaces and xenobiotics, like pesticides for example. This presentation will focus on a case study at the heart of the actuality in French West Indies: the Chlordecone (CLD), $C_{10}Cl_{10}O$ [C.A.S : 143.50.0], an organochlorine pesticide that has been extensively used, for years, in the French West Indies.

The massive use of this compound has contributed to widespread pollution of ecosystems (soil, waterways, seas, food chain, mangroves) in banana production areas in Guadeloupe and Martinique. Likewise, even if the use of CLD has been forbidden since 1993, the population is still widely exposed to this toxic compound having neurotoxic, endocrine disruptor and carcinogenic properties. [5–7]. To date, there are only two *in silico* studies on the receptor interactions of the thyroid and endocrine systems with CLD, published by the same Chinese team [6,7].

Prospects for studies interactions of the CLD with other receptors carried out as part of a doctoral thesis will be presented.

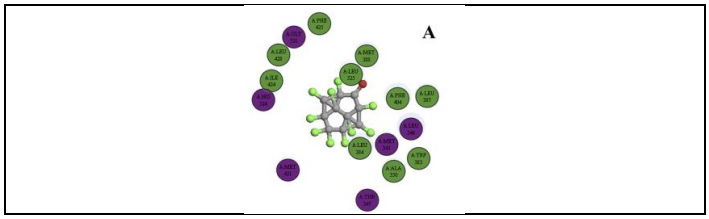

Figure 1: Estrogen receptor (ERα) and chlordecone interaction (Discs in green and purple indicate residues contributing to van der Waals force and electrostatic forces. Oxygen, chlorine and carbon atoms in chlordecone are respectively represented by balls in red, green and gray.) [6]

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Rationalizing Interfacial Design of Electrocatalytic Biosensors Relying on Electrical Properties of DNA

Elena E. Ferapontova[1]

[1] *Interdisciplinary Nanoscience Center (iNANO), Faculty of Natural Sciences, Aarhus University, Gustav Wieds Vej 1590-14, 8000 Aarhus C, Denmark*
e-mail: elena.ferapontova@inano.au.dk

Exceptional bio-recognition and electrical properties of nucleic acids (NAs) are craved in bioelectronics, electrochemical biosensor and actuator systems, and for the development of sensitive and accurate, yet inexpensive bioanalytical platforms. Recent progress achieved in understanding electrochemical and interfacial behaviours of NAs at electrodes enables tuning NA surface state and electrical properties at an almost atomic level [1], to precisely control the bio-recognition event and its electrochemical read-out, either via reactions of NA with redox indicators, capable of specific interactions with single and double stranded DNA [2], or via varied electrochemical signals from the redox-labelled NA [3]. Electrochemical assays with aptamers similarly depend on their interfacial and electronic properties [4]. In addition, 1:1 stoichiometry of bio-recognition makes ultra-low concentration analysis challenging, and requires signal amplification, e.g., via electrocatalytic schemes, while non-specifically adsorbing media components demand antifouling bio-interfaces. To rationalize interfacial design of electrocatalytic biosensors relying on electrical properties of DNA, all possible electrode reactions proceeding in DNA at polarised interfaces must be considered [1,5].
Here, I discuss the interfacial state and structure of the electrode-tethered DNA, and electrode reactions possible in such systems, with a particular emphasis on DNA-mediated long-range electron transfer [6] and reactions proceeding in redox-labelled DNA duplexes [7]. I report our research on electrocatalytically amplified assays for cancer biomarker Human Epidermal growth factor Receptor-2 and thrombin, both avoiding interference from serum components by careful designs of the antifouling interface and enabling electrocatalytic signal amplification either by ferricyanide/methylene blue [8] or covalent G4-hemin complex/oxygen redox couples [9-11]. General aspects and specific approaches for a single nucleotide polymorphism detection and analysis of ultra-low DNA and RNA concentrations by electrocatalytic genosensors [12-14] and perspectives of fast and reliable diagnostic devices for liquid biopsy analysis will be outlined.

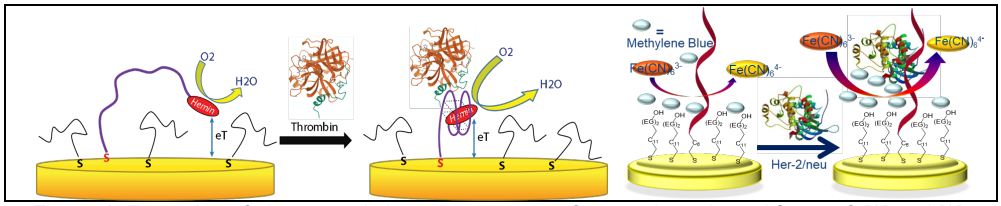

Figure 1: Examples of electrocatalytic aptamer assays for proteins adapted from ref. [8] and [9].

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Electrohydrodynamics based functional nanofibers for wireless electrical neuromodulation

Menglin Chen[1]

[1]Department of Biological and Chemical Engineering, Aarhus University, Aarhus, Denmark
e-mail: menglin@bce.au.dk

Alongside the widely studied pathways of biochemical regulation by chemokines, cytokines and growth factors, one often-overlooked but significant influence over the behavior of biological systems is electrical signalling. Voltage gradients among all somatic cells (not just excitable nerve and muscle) control cell behavior, and the ionic coupling of cells into networks via electrochemical synapses allows them to implement tissue-level patterning decisions, which is called developmental bioelectricity. Electrical modulation is therefore a potential target for many new therapies for a range of diseases and biological functions.

The significance of the overall fibrillar and porous nanoscale topography of the extracellular matrix in promoting essential cellular processes has led to consideration of biomaterials with nanofibrous features. Of the many methods for fabricating fibers with micrometer and nanometer diameters, electrohydrodyanmics (EHD) based spinning is simplest, most straightforward and cost-effective. We have pioneered various new techniques to enable artificial, biomimetic, nanofibrous scaffold substrates to mediate cell behavior using electrical stimulation[1-4]. Our current research focuses on advancing EHD technologies to explore multi-dimensional nano-biointerfaces that synergise the nanostructural induction and the bioelectrical/biochemical signalling to affect cellular behaviours, for biomedical applications in neural and cardiac stimulation and tissue engineering.

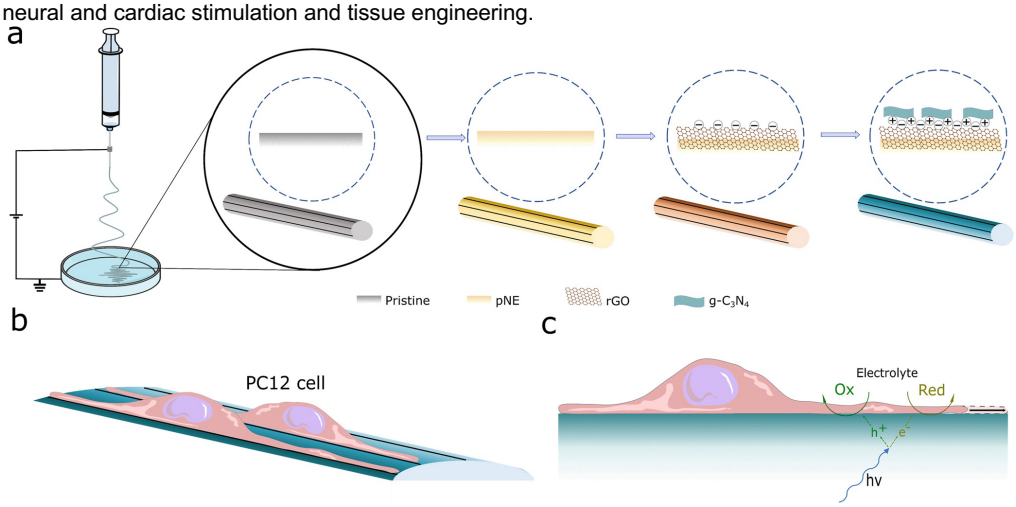

Figure 1: Effect of photocatalytic stimulation initiated by g-C3N4 decorated nanogrooved scaffolds on neurite outgrowth and the proposed photovoltaic mechanism.

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The potential of Sargassum *natans* and *fluitans* for new biomaterials for water depollution and corrosion protection

S.Melyon, W.B. Mainviel, M. Sénard , M. Francoeur , P. Reig Rodrigo , L. Brelle , M. Sylvestre , G. Cebrián Torrejón, S. Gaspard

COVACHIM M2E laboratory, EA 3592, Université des Antilles, BP 250, 97157 Pointe à Pitre Cedex. Guadeloupe, French West-Indies.
e-mail: narayanin.s@hotmail.fr

Finding new methods and procedures to make the most of Sargassum seaweed, in order to mitigate its negative impacts and turn it into an opportunity, has become a major objective for all territories in the Caribbean Basin and Gulf of Mexico.
Sargassum is rich in bioactive chemicals. This study concentrated on extracting natural chemicals from *natans* and *fluitans* Sargassum utilizing various extraction methods. Various empirical methods including maceration, traditional methods like Soxhlet extraction, and modern procedures like as microwave-assisted extraction (MAE) were used on sargassum. All samples will be subjected to molecular analysis and bioactivity assessments for antibacterial, antifungal, anticancer, antidiabetic, and antioxidant properties.

Corrosion studies were conducted using the electrochemical Tafel method to evaluate the mat erial's ability to prevent corrosion. Furthermore, our better extract, chloroform maceration was analyzed using Scanning electron microscopy (SEM) for textural study, and NMR characterization permit to show the presence of fatty acid in the chloroform extract.

However, to further enhance the value of this seaweed, biochar has also been synthesized from sargassum extraction residues. Two types of residues are currently used: ethanolic extraction residues and MAE residues. Microwave pyrolysis was used to optimize the various synthesis parameters using a design of experiment with factors (pyrolysis time and microwave power). Characterization analyses such as pHpzc, infrared, Boehm titration, textural analysis by scanning electron microscopy (SEM) and, as well as caffeine pollutant adsorption capacity tests on biochar were carried out. The initial comparison of caffeine adsorption for water depollution, reveals that raw sargassum has a capacity of 212.07 mg/g, but sargassum residues obtained from MAE and ethanolic extraction have capacities of 352 mg/g and 394 mg/g, respectively. Adsorption of a pesticide, chlordecone on a microwave prepared nanocarbon materials is studied.

	April 16
	Conference Session IV **Bio- and nano-materials for health & nanomedicine**
02.00 pm – 02.30 pm	**Valeria Rondelli** (University of Milan, Italy) **(Keynote lecture)** « Disclosing the unknown deep inside biomembranes »
02.30 pm – 02.50 pm	**Xavier Fernàndez-Busquets** (University of Barcelona, Spain) « Encapsulation in liposomes strongly increases the antileishmanial effect *in vitro* of the aggregated protein dye YAT2150 »
02.50 pm – 03.10 pm	**Caterina Medeot** (University of Trieste, Italy) « Evaluation of anticancer efficacy of 2C inhibitor-loaded Lipid Nanoparticles (LNPs) in a 2D melanoma models »
03.10 pm – 03.30 pm	**Audrey Malardé** (University of Lorraine, France) « A new synthetic copper transporter for the treatment of menkes disease »
03.30 pm – 04.00 pm	**Coffee Break (30 min)**
04.00 pm – 04.30 pm	**Frank Caruso** (University of Melbourne, Australia) **(Keynote lecture - Visio)** « Bio-Nano Science – Insights for Nanomedicine »
04.30 pm – 04.50 pm	**Malobi Seth** (University of Lorraine, Metz, France) « Fabrication of ZnO nanowires on 3D-printed Ti-6Al-4V towards mechano-bactericidal activity »
04.50 pm – 05.10 pm	**Bernardo Albuquerque Nogueira** (International Iberian Nanotechnology Laboratory, Braga, Portugal) « Development of a gold nanoparticle – covalent organic polymer (COP) composite for the detection of saxitoxins »
05.10 pm – 05.30 pm	**Yue Xu** (University of Waterloo, Canada) « Trehalose Sugar Protects Lipid Membrane Against Amyloid-β Toxicity in Alzheimer's Disease »
07.30 pm	**Conference Dinner**

Disclosing the unknown deep inside biomembranes

Valeria Rondelli

Department of Medical Biotechnology and Translational Medicine
University of Milan – Italy
e-mail: valeria.rondelli@unimi.it

Cell membranes are complex objects made by several different molecular species. One of their most significant complexities is compositional asymmetry, key factor claimed to be associated to functional and structural roles. Nonetheless, membranes asymmetry is often hard to be reproduced in mimics. Experimental models, bearing forced membrane leaflets asymmetry in the form of dispersed aggregates in solution or of single supported bilayers, have been developed to be suitably investigated by complementary techniques such as scattering and reflectometry of neutrons and X-rays and calorimetry, to link the thermotropic behavior of membrane mimics to their structuring on the colloidal and the nanoscale. The possibility to create and study customized systems mimicking different cell membrane portions is the way to the detailed structural investigation of a variety of specific molecule-membrane interactions, being also potentially predictive of the fate of extracellular bodies, macromolecules and nanodrugs intended to cross the extracellular medium and eventually enter cells.

Encapsulation in liposomes strongly increases the antileishmanial effect *in vitro* of the aggregated protein dye YAT2150

L. Román-Álamo,[1,2] Y. Avalos-Padilla,[1,2] I. Bouzón-Arnáiz,[1,2] V. Iglesias,[1,2,3] J. Fernández-Lajo,[4] J.M. Monteiro,[4] L. Rivas,[4] R. Fisa,[5] C. Riera,[5] D. Andreu,[6] C. Pintado-Grima,[3] S. Ventura,[3] E.M. Arce,[7] D. Muñoz-Torrero,[7] and X. Fernàndez-Busquets[1,2,8]

[1]*Barcelona Institute for Global Health (ISGlobal), Hospital Clínic-Universitat de Barcelona, Barcelona, Spain*
[2]*Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain*
[3]*Institut de Biotecnologia i de Biomedicina, and Universitat Autònoma de Barcelona, Spain*
[4]*Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain*
[5]*Faculty of Pharmacy and Food Sciences, University of Barcelona, Spain*
[6]*Pompeu Fabra University, Barcelona, Spain*
[7]*Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Spain*
[8]*Nanoscience and Nanotechnology Institute (IN2UB), University of Barcelona, Spain*
e-mail: xfernandez@ibecbarcelona.eu

The problems associated with the drugs currently used to treat leishmaniasis, including resistance, toxicity, and the high cost of some formulations, call for the urgent identification of new therapeutic agents with novel modes of action. The aggregated protein dye YAT2150 has been found to be a potent antileishmanial compound, with a half-maximal inhibitory concentration (IC_{50}) of approximately 0.5 μM against promastigote and amastigote stages of *Leishmania infantum*. The encapsulation in (immuno)liposomes (Figure 1) of YAT2150 significantly improved its *in vitro* IC_{50} to 0.37 and 0.19 μM in promastigotes and amastigotes, respectively, and increased the half-maximal cytotoxic concentration in human umbilical vein endothelial cells to >50 μM. YAT2150 became strongly fluorescent when binding intracellular protein deposits in *Leishmania* cells. This fluorescence pattern aligns with the proposed mode of action of this drug in the malaria parasite *Plasmodium falciparum*, the inhibition of protein aggregation. In *Leishmania major*, YAT2150 rapidly reduced ATP levels, suggesting an alternative antileishmanial mechanism. To the best of our knowledge, this first-in-class compound is the only one described so far having significant activity against both *Plasmodium* and *Leishmania*, thus being a potential drug for the treatment of co-infections of both parasites.

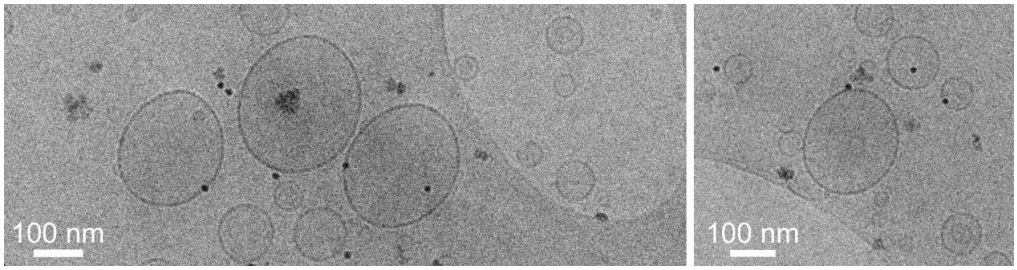

Figure 1: Cryo-transmission electron microscope images of immunoliposomes encapsulating YAT2150.

Funding

This work was supported by grants (i) *Fundació La Marató de TV3*, Ref. 201811 (XF-B); (ii) PID2021-128325OB-I00 (XF-B) and PID2020-118127RB-I00 (DM-T), funded by *Ministerio de Ciencia e Innovación/Agencia Estatal de Investigación* (MCIN/AEI/10.13039/501100011033), which included ERDF funds; (iii) *II Premis Innovació Campus Clínic 2022, Hospital Clínic de Barcelona* (XF-B); and (iv) *Generalitat de Catalunya*, Spain (http://agaur.gencat.cat/), grant numbers 2021-SGR-00635 (SV) and 2021-SGR-00357 (DM-T). Work at Pompeu Fabra University was supported by "La Caixa" Banking Foundation (https://fundacionlacaixa.org/, grant HR17-00409), and by grant AGL2017-84097-C2-2-R and the "María de Maeztu" Program for Units of Excellence in R&D from the Spanish Ministry of Science, Innovation and Universities. Work at the *Centro de Investigaciones Biológicas* was supported by grants from MCIN *Subdirección General de Redes y Centros de Investigación Cooperativa*-FEDER RD16/0027/0010 and CSIC PIE 201620E038. ISGlobal and IBEC are members of the CERCA Programme, *Generalitat de Catalunya*. We acknowledge support from the MCIN through the *"Centro de Excelencia Severo Ochoa 2019-2023"* Program (CEX2018-000806-S). This research is part of ISGlobal's Program on the Molecular Mechanisms of Malaria which is partially supported by the *Fundación Ramón Areces*.

Evaluation of anticancer efficacy of 2C inhibitor-loaded Lipid Nanoparticles (LNPs) in a 2D melanoma models

Caterina Medeot,[1,2] Denis Scaini,[3] Pietro Parisse,[4] Loredana Casalis,[2] and Serena Bonin[1]

[1]*Department of Medical Science – DSM, University of Trieste, 34149 Trieste, Italy;*
[2]*NanoInnovation Laboratory - Elettra Sincrotrone Trieste S.C.p.A., in Area Science Park, 34149 Basovizza, Italy;*
[3]*IkerBasque Foundation for Science, 48013 Bilbao,Spain; University of Basque Country, Faculty of Pharmacy, 01006 Vitoria-Gasteiz, Spain;*
[4]*Consiglio Nazionale delle Ricerche, Istituto Officina dei Materiali (IOM), 34149 Trieste, Italy.*
e-mail: caterina.medeot@phd.units.it

Melanoma is the most aggressive among skin cancers, with a poor prognosis when it invades the dermis [1]. A 15% increase has been recorded in melanoma incidence in the las decade, with 100,000 new cases yearly in Caucasian. This scenario is expected to worsen further in the coming years [2].
Considering the actual increasing incidence, there is a rising demand for finding innovative drug delivery systems to pave the way for new treatment opportunities. Given the urgency, nanoscale systems can play an important role in improving actual treatment approaches [3]. Amongst nanomaterials, lipid nanoparticles (LNPs) are the most promising for drug delivery applications. Due to their natural origin, they are highly biocompatible, biodegradable, and minimally toxic. This potential is further enhanced by their ability to mimic the structure of cell membranes.
In this study, our objective was to set up a drug delivery system to treat advanced melanoma by utilizing bioengineered LNPs to encapsulate a deubiquitinating enzyme inhibitor (DUBs), known as 2C, synthetized in the chemical laboratories at the University of Trieste [4]. DUBs are a heterogeneous family of isopeptidases involved in proteome homeostasis and can also modulate the activity of proteins involved in DNA repair [5]. Moreover, studies suggested that a dysregulation of some of them can lead to cancer proliferation. For this reason, DUBs can be considered as targets for the design and development of novel cancer drug treatments.
According to our objectives, we initially evaluated the anticancer efficacy of 2C at serial concentration directly on A375 melanoma cell line. The results obtained from viability assay, conducted at 3, 6, and 24-hour intervals, revealed evident effects already after 3 hours. At the highest concentration (100 µM), the percentage of viable cells falls below 20%. Furthermore, after 24 hours, the percentage of surviving cells remains barely above 40% across all concentrations. To confirm the efficacy of 2C inhibitor on melanoma cancer cells, we loaded the inhibitor in LNPs synthetized using the NanoAssemblr Ignite microfluidic device. It gives numerous advantages, including reproducibility, controlled and rapid synthesis, and the possibility of scaling-up directly to a major production. Indeed, by mixing DMPC, DMPG, and cholesterol, we successfully produced empty LNPs around 159.7 ± 50.6 nm, used as control, and 2C (100 µM) - loaded LNPs with an average hydrodynamic size of 120.2 ± 29.4 nm. Subsequently, cells were exposed to both LNPs, and their viability was assessed at 3, 6, and 24-hour time intervals. Upon comparing the results of the 2C-loaded LNPs with the activity of the free drug, the former gave clear effects only after a 24-hour interval. Thus, it is possible that LNPs give delayed effects and a higher control over the pharmacokinetic and distribution profile of the drug.
With these results, we introduce an innovative delivery system for potential treatment of advanced melanoma stages. The advantage of using LNPs for drug delivery purposes instead of a free drug or other nanomaterials is the possibility to increase local drug concentration directly in specific regions to avoid systematic side effects (that are present in normal chemotherapy) and to have a more controlled release.

Funding
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A new synthetic copper transporter for the treatment of menkes disease

Audrey Malardé[1],Jean-Marc Alberto[1], Benjamin Creusot[2,3], Igor Clarot[2,3], Ariane Boudier[2,3,4], François Feillet[1,5]

[1] Unit INSERM U1256 NGERE, University of Lorraine, Vandoeuvre les Nancy
[2] CITHEFOR UR 3452, University of Lorraine, Vandoeuvre les Nancy
[3] *Université de Lorraine, CNRS, LRGP Nancy,*
[4] *Institut Universitaire de France, (IUF)*
[5] *CHRU de Nancy – Hôpitaux de Brabois,* Vandoeuvre les Nancy

e-mail: audrey.malarde@univ-lorraine.fr

Menkes disease is a rare genetic disorder of copper metabolism affecting 1 in 300,000 male newborns in Europe with a life expectancy of 3 years. This disease is linked to a deficiency of copper transporter (ATP7A) present in the intestine and in the blood-brain barrier (BBB) inducing a severe copper deficiency with a combined deficiency of essential cuproproteins. This deficiency leads to multisystem symptoms (depigmentation of the skin and hair, osteoporosis, vascular and urinary diverticula) and as severe neurodegeneration with epilepsy. Currently, treatment is based on a parenteral injection of a histidine-copper complex which don't cross the BBB and consecutively has no neurological efficacy. This is why we designed a new synthetic copper transporter, in the form of a nanocluster (CuNC), which can cross the BBB with the aim of treating the neurological damage of the disease. These nanoclusters were synthesized, characterized and tested in a mouse model of Menkes disease (ATP7A^{Moblo} mice). They are characterized by a size of 0.7 nm in diameter, with a metallic core surrounded by biodegradable ligands. Subcutaneous injections of nanocluster into Moblo mice from 5 days of life or during the antenatal period saw their life expectancy considerably increased, in correlation with a restoration of the activity of the impacted cuproproteins. Indeed, tyrosinase, a cuproprotein responsible for the production of melanin, showed its activity restored by a darkening of the fur and the increase in the production of black pigment responsible for this darkening. This has also been proven for the activity of cytochrome C oxidase in the brain, showing a restoration of the activity of the brain mitochondrial respiratory chain. From a functional point of view and in the case of a structure-function approach, neurobehavioral tests (horizontal scale, Open Field) showed a drastic improvement in locomotion and coordination of movements in mice having received the injection. All of these results allowed the obtention of orphan drug designation from the European Medicine Agency (EMA) and the establishment of a pharmaceutical form of the nanocluster currently being tested in the study mice with the aim of launching clinical trials in 2024.

Bio-Nano Science – Insights for Nanomedicine

F. Caruso

Department of Chemical Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia

e-mail: f.caruso@unimeb.edu.au

Self-assembly technologies have been used extensively to engineer a diverse array of particles for biological applications, including therapeutic delivery. This presentation will focus on our research on the development of particles and their interactions with biological barriers. Aspects of the physicochemical properties of the particles, post-assembly biological modification, ligand targeting, and the formation of protein coronas will be covered. Particle variants based on metal-ligand complexation will also be highlighted. Our studies are aimed at obtaining detailed knowledge of complex bio-nano interactions to aid in the rational design of nanoengineered materials for applications including HIV, hearing loss, and cancer targeting.

Fabrication of ZnO nanowires on 3D-printed Ti-6Al-4V towards mechano-bactericidal activity

Malobi Seth,[1,2] Samantha Soulé,[1] and Laurent Weiss[2]

[1]*LCPME, Université de Lorraine, CNRS, 54600, Villers-lès-Nancy, France*
[2] *LEM3, Université de Lorraine, CNRS, 57073, Metz, France*
e-mail: malobi.seth@univ-lorraine.fr

3D-printed porous Ti alloys have emerged as the most popular choice for orthopaedic implants due to their biocompatibility, corrosion resistance, mechanical capabilities close to the bone ones, and ease of personalization[1]. Despite the benefits, its success has been adversely affected by periprosthetic bacteria-related infections leading to implant failure and gradual loosening of implants from the fracture-site[2]. Whereas various coatings have been developed to overcome these issues on implants, it becomes a challenge while developing porous bone scaffolds with complicated internal geometry.

At certain times, the physical killing of bacteria is an efficient mechanism as it does not contribute to antimicrobial resistance or environmental pollution. Known as mechano-bactericidal activity, the mechanism relies on stretching and perturbation of bacterial cell membrane upon contact with the nanostructured surface under normal forces resulting in cell lysis and ultimately death[3].

In this study, we have developed a coating of ZnO nanowires on 2D plates and 3D lattices of Ti-6Al-4V made by selective laser melting. The unmelted particles of the as-prepared Ti-6Al-4V were removed by chemical etching before coating deposition. The coating involves the deposition of ZnO nanoparticles and subsequent growth of nanowires by hydrothermal method. The fabrication parameters such as precursor composition, deposition/ growth time, and temperature were adjusted to obtain optimized coating. SEM characterization showed that the nanowires have grown perpendicularly from the surface and the formation of ZnO was confirmed by Raman spectroscopy and XRD. The antibacterial activity was evaluated in submerged conditions and also in just-dried conditions. Epifluorescence microscopy supported by colony counting assay exhibited that the mechano-bactericidal effect took place in just-dried conditions where no viable cells of *E. coli* were detected on the coated surface (Fig. 1), citing a stark difference from submerged conditions. It is believed that as the nanowires dried with the evaporation of water, the capillary forces came into action to rupture the cell wall of the bacteria, in accordance with previous reports[4]. Further, the nanowires were doped with osteogenic metals such as strontium to promote bone regeneration and enhance long-term antibacterial activity. Overall, the mechanical effect of ZnO nanowires and the long-term chemical effect could be an effective tool to combat bacterial contamination of biomedical implants.

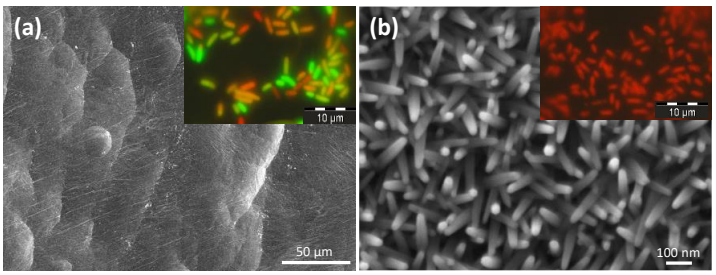

Figure 1: SEM images of (a) Ti-6Al-4V after etching and (b) after ZnO growth. Inset shows the epifluorescence images of the viable (stained green with SYTO 9) and the non-viable *E. coli* cells (stained red with Propidium Iodide) just after 5 min of contact with bacterial suspension

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Development of a gold nanoparticle – covalent organic polymer (COP) composite for the detection of saxitoxins

B. Albuquerque Nogueira,[1] V. Silva,[1] M. Chaves de Sousa,[1] A. Álvarez,[1] M. Santos,[1] L. M. Salonen,[1,2] B. Espiña,[1] and L. Rodriguez-Lorenzo[1]

[1]*Water Quality Group, International Iberian Nanotechnology Laboratory (INL), Av. Mestre José Veiga s/n, 4715-330 Braga, Portugal*
[2]*CINBIO, Universidade de Vigo, Department of Organic Chemistry, 36310 Vigo, Spain*
e-mail: bernardo.a.nogueira@inl.int

Harmful algal blooms (HABs), which represent an excessive accumulation of algae in aquatic environments, pose a significant and pressing concern, exacerbated by climate change, with profound ecological and socio-economic implications. These blooms give rise to dense populations of algae that may release toxins, detrimental to both aquatic ecosystems and to human health. Specifically, the accumulation of these biotoxins, particularly in seafood, has considerable economic impact in aquaculture. Consequently, the development of novel, efficient, cost-effective early monitoring methods is imperative to ensure optimal aquaculture practices and, ultimately, human safety [1].
In this communication, an innovative organic–plasmonic hybrid composite designed for surface-enhanced Raman scattering (SERS) detection of HAB toxins will be presented. The main goal of this novel system is to sense trace amounts of toxins in water during the early stages of HAB outbreaks, thereby enabling the anticipation of the aforementioned health and economical effects. The system comprises gold nanoparticles (GNPs) and a covalent organic polymer (COP) specifically designed for preferential adsorption of saxitoxin family compounds [2], a relevant class of toxins associated with HABs [3].
Characterization of the newly developed hybrid composites for toxin detection has been conducted through SEM, TEM, as well as crystallographic and spectroscopic techniques. The optimization of the diverse composites has been achieved using toxin molecular analogues. The preliminary encouraging results of toxin detection will be presented in this communication.

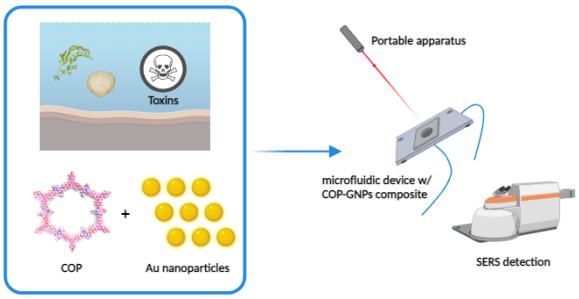

Figure 1: Schematic representation of the microfluidic COP–GNP system designed to detect trace amounts of HAB toxins by SERS.

Acknowledgments
The authors acknowledge the financial support of the project “Pacto da Bioeconomia Azul”, with the reference n.º C644915664-00000026, co-funded by Component C5 – Capitalisation and Business Innovation under the Portuguese Resilience and Recovery Plan, through the NextGenerationEU Fund. This work benefitted also from financial support through the EEA grant ATLANTICLAM (PT-INNOVATION-0097) is acknowledged. L.R.-L. acknowledges funding from FCT (Fundação para a Ciência e Technologia) for the Scientific Employment Stimulus Program (2020.04021.CEECIND). L.M.S. acknowledges financial support from the Spanish Ministry of Science and Innovation through the Ramón y Cajal grant RYC2020-030414-I.

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Trehalose Sugar Protects Lipid Membrane Against Amyloid-β Toxicity in Alzheimer's Disease

Yue Xu*[1], Danielle Mcrae[1], Carina Filice[2], and Zoya Leonenko†[1,2,3]

[1]Department of Physics and Astronomy, University of Waterloo – Canada
[2]Department of Biology, University of Waterloo – Canada
[3]Waterloo Institute for Nanotechnology, University of Waterloo – Canada

Abstract

The amyloid-β peptide (Aβ1-42) is one of the main pathogenic factors in Alzheimer's disease and is known to induce damage to the lipid membrane (1). Trehalose, a naturally existing disaccharide, has been shown to protect plant cellular membranes in extreme conditions and has been attracting attention in neurodegeneration research due to its ability to reduce Aβ misfolding (2). We hypothesize that trehalose can also protect the neuronal membrane from amyloid toxicity. In this work, we studied the protective effect of trehalose against Aβ-induced damage in model lipid membranes (DPPC/POPC/Cholesterol in mass ratio of 4:4:2), used to mimic neuronal membranes. We used atomic force microscopy (AFM), Kelvin Probe Force Microscopy (KPFM), Black lipid membrane (BLM) and Localized Surface Plasmon Resonance (LSPR) techniques.
Our AFM and KPFM results demonstrated that trehalose modifies the properties of model lipid membranes and monolayers (both topography and electrical surface potential), especially in combination with NaCl. Our BLM data show that Aβ induced damage to membranes and led to ionic current leakage across membranes due to the formation of various defects and pores. The presence of trehalose reduced the ion current caused by Aβ peptides' damage to membranes. Our LSPR results revealed that trehalose reduced the binding of Aβ to lipid membranes, indicating the protective effect through suppression on Aβ-membrane interaction.
These findings suggest that trehalose sugar can be useful in protecting neuronal cellular membranes against amyloid toxicity, and thus, this study may contribute to the development of membrane-targeted preventive approaches to overcome AD.

Keywords: Alzheimer's Disease, Amyloid, beta, Trehalose, Sugar, Membrane, Atomic Force Microscopy, Surface Plasmon Resonance

*Speaker
†Corresponding author: zleonenk@uwaterloo.ca

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	April 18
	Conference Session V Nanomedicine, Nano-Micro Biology
08.40 am – 09.10 am	**David Alsteens** (Université catholique de Louvain, Belgium) « Deciphering the role of Glycans as attachment factors in viral infection using AFM » **(Keynote lecture)**
09.10 am – 9.30 am	**Philippe Lavalle** (University of Strasbourg, France) « New easy to make hydrogels of ε-poly(L-lysine) and polyethylene glycol diacrylate with intrinsic antibacterial, antifungal and anti-inflammatory properties »
9.30 am – 9.50 am	**Yoann Roupioz** (Université Grenoble Alpes, France) « Bio-probe Engineering for Bacteria detection by Surface Plasmon Resonance imaging »
9.50 am – 10.10 am	**Fabienne Quiles (**University of Lorraine, Nancy, France) « The effect of anthropic drug release in river on bacterial biofilms: a multiscale analysis »
10.20 am – 10.50 am	**Coffee Break (30 min)**
10.50 am – 11.20 am	**Nicole Robb** (University of Warwick, United Kingdom) « Rapid virus detection using single-particle imaging and machine learning » **(Keynote lecture)**
11.20 am – 11.40 am	**Peter Hinterdorfer** (Johannes Kepler University, Linz, Austria) « Distinct binding mechanisms of SARS-CoV-2 Spike variants viewed on the single-molecule level »
11.40 am – 12.10 pm	**Olivier Gros** (Université des Antilles, France) **(Expert)** « How free-living sulfur-oxidizing bacteria from Guadeloupe's marine mangroves challenge and shift the traditional concepts of bacteriology »
12.30 pm – 02.00 pm	**Conference Lunch**

Deciphering the role of glycans as attachment factors in viral infection using AFM

D. Alsteens,[1]

[1] *Nanobiophysics lab, Université catholique de Louvain, Croix du sud 4-5, bte L7.07.07, 1348 Louvain-La-Neuve, Belgium*
e-mail: david.alsteens@uclouvain.be (Arial, centred, 9pts)

During the last three decades, a series of key technological improvements turned atomic force microscopy (AFM) into a nanoscopic laboratory to directly observe and chemically characterize molecular and cellular biological systems under physiological conditions. I will present the key technological improvements that enable us to apply AFM as analytical laboratory to observe and quantify living biological systems at the nanoscale. I will report the use of advanced FD-based technology combined with chemically functionalized tips to probe the localization and interactions of chemical and biological sites on single native proteins and on living cells at high-resolution. I will present how an atomic force and confocal microscopy set-up allows the surface receptor landscape of cells to be imaged and the virus binding events within the first millisecond of contact with the cell to be mapped at high resolution (<50 nm). I will also highlight theoretical approaches to contour the free-energy landscape of early binding events between virus and cell surface receptors.

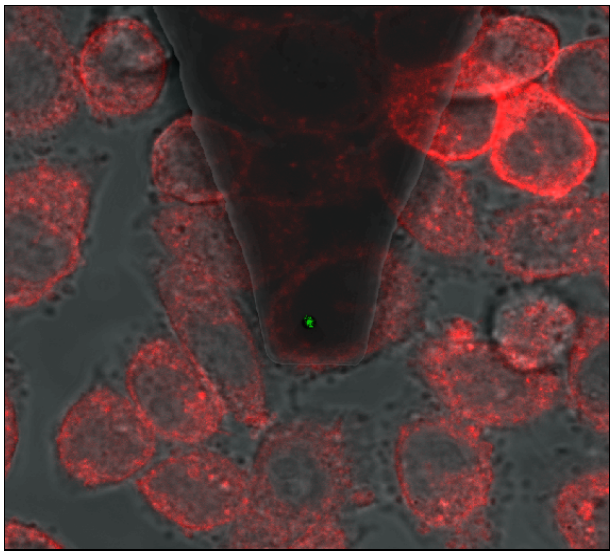

Figure 1: Combination of AFM and fluorescence microscopy image showing an AFM tip functionalized with a single virus while mapping virus binding sites on living mammalian cells

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New easy to make hydrogels of ε-poly(L-lysine) and polyethylene glycol diacrylate with intrinsic antibacterial, antifungal and anti-inflammatory properties

Philippe Lavalle,[1,2] Eloïse Lebaudy,[1] Skander Hathroubi[2], Benoit Frisch[1] and Nihal Engin Vrana[2] (Arial, centred, 11pts)

[1]*Inserm UMR_S 1121, CNRS EMR 7003, Université de Strasbourg, "Biomaterials and Bioengineering", Strasbourg, France*

[2]*SPARTHA Medical, Strasbourg, France*

e-mail: philippe.lavalle@inserm.fr

Nosocomial infections linked to the use of biomedical devices like implants lead to major problems like implant failures and other major concerns for patients. Furthermore, the steady increase in bacterial resistance to antibiotics necessitates the search for new solutions to fight against these infections.
In this study, we developed an innovative hydrogels based on association of a polymer, poly(ethylene glycol) diacrylate, and a natural polypeptide, ε-poly(L-lysine). These hydrogels, built at specific polymer/polypeptide ratio, demonstrate strong antibacterial properties against both Gram-positive and Gram-negative bacteria. No cytotoxicity towards eukaryotic cells was observed. This antimicrobial activity is related to the slow degradation of the hydrogels in the medium and the release of ε-poly(L-lysine), the active antimicrobial ingredient. Additionally, we demonstrated that the hydrogel surface prevents bacterial adhesion through contact-killing mechanism. Finally, to obtain a cumulative antifungal property, thereby introducing a novel feature to the hydrogels of clinical importance, we have successfully loaded the hydrogels with poly(L-arginine) composed of 10 arginine residues (PAR10). Moreover, the addition of PAR10 significantly reduces the pro-inflammatory activity of macrophages by reducing their polarization into M1 pro-inflammatory macrophages. Finally, to validate the effectiveness of these hydrogels, in vivo experiments were conducted using a mouse model with a *S. aureus* infected wounds. The hydrogels, poured into medical meshes, allow to treat the wounds with a significant reduction in infection around the infected area.

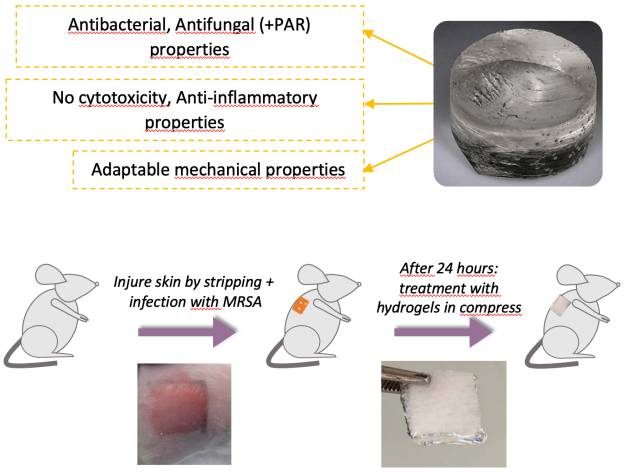

Figure 1: Hydrogels designed and used to treat wound infections

Bio-probe Engineering for Bacteria detection by Surface Plasmon Resonance imaging

Y. Roupioz,[1] E. Pardoux,[1,2] T. Livache.,[1] D. Botruyn,[2] R. Mathey,[1] M. Stalet,[1] and L. Leroy[1]

[1]*Univ. Grenoble Alpes, CNRS, CEA, Grenoble INP, IRIG, SyMMES, 38000 Grenoble, France*
[2]*Univ. Grenoble Alpes, CNRS, UMR 5250, ICMG FR2607, 38000 Grenoble, France*
e-mail: yoann.roupioz@cea.fr

Microbiological analyses are carried every day worldwide to ensure food safety and healthcare. In these domains, the main challenge remains the fast specific detection of pathogens, present at very low (few bacteria per gram or ml) in the sample.
The standard microbiological protocol for bacterial detection in agronomic fields remains the microbial culture on selective media, which may take days to identify pathogens and specifically confirm their presence in the sampled foodstuff. Such delay is mainly due to the requirement of an enrichment phase allowing bacterial amplification before running the characterization assay. This time lag is a real bottleneck for setting up more performing and cheaper assays. Besides food safety, similar issues are also shared for the diagnosis of bacterial infections, leading to sepsis. The main differences are the fact that samples (mostly blood) from healthy patients are sterile and that, in case of sepsis, even less bacteria might be present in 20-50 millilitres of blood. But once again, the time lap required for bacteria detection/identification is crucial. Thus, there is a strong need to develop new techniques to identify pathogenic bacteria in a shorter time.

To achieve this goal, Surface Plasmon Resonance imaging (SPRi) technology has been successfully used for the specific detection of bacterial populations growing on microarrays functionalized with antibodies targeting bacterial strains [1] (*Listeria*, *Salmonella*, *E. coli*, *Cronobacter*, etc). This strategy of simultaneous bacterial growth monitoring and bacterial strain specific detection enabled the detection of only few bacteria per millilitre, within few hours. Interestingly, the time-delay is directly linked to the bacterial concentration of the processed sample and allows quantitative assessment of the initial concentration by comparison to calibration experiments [2]. Our quantitative results are consistent with the expected doubling time of bacteria reported in the literature. More recently, we also engineered peptide microarrays for the universal detection of bacteria (presence/absence), without any prerequisite on the identity of the contaminating bacteria. Interestingly, this peptic based probing strategy gave access to new insights regarding bacteria/surfaces interactions [3].

All these aspects will be presented in this talk, along with recent references published in the literature.

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The effect of anthropic drug release in river on bacterial biofilms: a multiscale analysis

Fabienne Quiles*†[1], Aleksandra Umanets[2], Olena Rzhepishevksa[2], Sofiane El-Kirat-Chatel[3,4], Grégory Francius[3], and Madeleine Ramstedt[2]

[1]Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement – Université de Lorraine, Centre National de la Recherche Scientifique - CNRS – France
[2]Department of Chemistry, Umeå University – Sweden
[3]Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement – Centre National de la Recherche Scientifique - CNRS, Université de Lorraine – France
[4]Chimie Biologie des Membranes et des Nano-objets – Centre National de la Recherche Scientifique - CNRS, Université de Bordeaux (Bordeaux, France) – France

Abstract

A new group of pollutants has emerged in the form of pharmaceuticals. Once they reach natural ecosystems, the effects of many of the pharmaceuticals released in rivers on the aquatic ecosystem is poorly described. Diclofenac (DCF) is commonly detected in wastewater treatment plant effluents. Epilithic biofilms are early in the food chain. Microorganisms in biofilms are less susceptible to toxic compounds as biocides including antibiotics. Can biofilms be a reservoir for DCF? Is DCF toxic for sessile bacteria? Can biofilms contribute to DCF degradation? To answer these questions, we used a fundamental approach to investigate the role a bacterial biofilm on the fate of DCF.
Biofilms formed with a bacterial strain isolated from a Swedish river were exposed to DCF. The effects on the physiology, the morphology, nanomechanics, drug accumulation/degradation in the planktonic and sessile form were investigated. Infrared spectra during drug exposure provided chemical information on biofilm physiology, possible drug accumulation/degradation and its effect on sessile bacteria exposed to the drug in situ and in real time. At selected exposure times, epifluorescence microscopy and AFM provided information on the bacterial morphology and nanomechanics. The metabolite pattern was also investigated to provide information on possible degradation pathways and products.
This multidisciplinary study gives information on the interactions between a drug and a bacterial biofilm. Knowledge of the chemical composition and structure of biofilms, and how biofilms are altered by exposure to pharmaceuticals, plays a role in understanding the overall ecological consequences of pharmaceuticals released by human activities.

Keywords: drugs in river, biofilm, AFM, infrared spectroscopy

*Speaker
†Corresponding author: fabienne.quiles@univ-lorraine.fr

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Rapid virus detection using single-particle imaging and machine learning

Nicole Robb

Warwick Medical School – Interdisciplinary Biomedical Research Building
The University of Warwick – United Kingdom
e-mail: Nicole.Robb@warwick.ac.uk

The increasing frequency of viral outbreaks, such as the COVID-19 pandemic, underscores the urgent need for rapid and adaptable viral diagnostic methods. I will present a novel method that combines a universal labelling strategy, single-particle fluorescence microscopy and deep learning to detect and classify viruses, with potential application for pandemic preparedness. We have previously described a novel, calcium-mediated interaction of the surface of enveloped viruses with DNA, which we use for the rapid fluorescent labelling and optical detection of viruses. We then employ wide-field fluorescence imaging, advanced image analysis, and a custom convolutional neural network to analyse images of cation-mediated labelled enveloped viruses. The assay achieves labelling, imaging, and virus identification in less than 5 minutes, without requiring lysis, purification, or amplification steps. We have been able to differentiate SARS-CoV-2 from negative clinical samples, as well as from other common respiratory pathogens such as influenza and seasonal human coronaviruses, with high accuracy. For example, in a clinical validation study using patient samples, our deep learning-based approach achieved a high overall accuracy of 97.1%, demonstrating its potential for rapid and accurate viral detection in clinical settings. Single-particle imaging combined with deep learning therefore offers a promising alternative to traditional viral diagnostic methods.

Distinct binding mechanisms of SARS-CoV-2 Spike variants viewed on the single-molecule level

Rong Zhu[1], Daniel Canena[1], Mateusz Sikora[2,3], Miriam Klausberger[4], Hannah Seferovic[1], Ahmad Reza Mehdipour[2,5], Lisa Hain[1], Elisabeth Laurent[4,6], Vanessa Monteil[7], Gerald Wirnsberger[8], Ralph Wieneke[9], Robert Tampé[9], Nikolaus F. Kienzl[10], Lukas Mach[10], Ali Mirazimi[7,11], Yoo Jin Oh[1], Josef M. Penninger[12,13], Gerhard Hummer[2,14], Peter Hinterdorfer[1]

[1]Department of Experimental Applied Biophysics, Johannes Kepler University Linz; Linz, Austria.
[2]Department of Theoretical Biophysics, Max Planck Institute of Biophysics; Frankfurt am Main, Germany.
[3]Faculty of Physics, University of Vienna; Vienna, Austria.
[4]Department of Biotechnology, Institute of Molecular Biotechnology, University of Natural Resources and Life Sciences, Vienna; Vienna, Austria.
[5]Center for Molecular Modeling, University of Ghent; Ghent, Belgium.
[6]Core Facility Biomolecular & Cellular Analysis, University of Natural Resources and Life Sciences, Vienna; Vienna, Austria.
[7]Department of Laboratory Medicine, Unit of Clinical Microbiology, Karolinska Institute and Karolinska University Hospital; Stockholm, Sweden.
[8]Apeiron Biologics; Vienna, Austria.
[9]Institute of Biochemistry, Biocenter, Goethe University Frankfurt; Frankfurt, Germany.
[10]Department of Applied Genetics and Cell Biology, Institute of Plant Biotechnology and Cell Biology, University of Natural Resources and Life Sciences, Vienna; Vienna, Austria.
[11]National Veterinary Institute; Uppsala, Sweden.
[12]Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA); Vienna, Austria.
[13]Department of Medical Genetics, Life Sciences Institute, University of British Columbia; Vancouver, British Columbia, Canada.
[14]Institute of Biophysics, Goethe University Frankfurt; Frankfurt am Main, Germany.

e-mail:peter.hinterdorfer@jku.at

Recent waves of COVID-19 correlate with the emergence of the Delta and the Omicron variant. In this study, we combined high-speed atomic force microscopy with single molecule recognition force spectroscopy to investigate, at single molecule resolution, the interaction dynamics of trimeric Spike with its essential entry receptor ACE2. We report that the Spike trimer undergoes rapid conformational changes on surfaces, resulting in arc-like movements of the three receptor binding domains (RBDs) that collectively screen a circular range of almost 360° degrees. Acting as a highly dynamic molecular caliper, it thereby forms up to three tight bonds through its RBDs with ACE2 expressed on the cell surface. The Spike of both Delta and Omicron (B.1.1.529) Variant enhance and markedly prolong viral attachment to the host cell receptor ACE2, as opposed to the early Wuhan-1 isolate. Delta Spike showed rapid binding of all three Spike RBDs to three different ACE2 molecules with considerably increased bond lifetime when compared to the reference strain, thereby significantly amplifying avidity. Intriguingly, Omicron (B.1.1.529) Spike displayed less multivalent bindings to ACE2 molecules, yet with a ten time longer bond lifetime than Delta. Delta and Omicron (B.1.1.529) Spike variants enhance and prolong viral attachment to the host, which likely not only increases the rate of viral uptake, but also enhances the resistance of the variants against host-cell detachment by shear forces such as airflow, mucus or blood flow. We uncovered distinct binding mechanisms and strategies employed by circulating SARS-CoV-2 variants to enhance infectivity and viral transmission.

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How free-living sulfur-oxidizing bacteria from Guadeloupe's marine mangroves challenge and shift the traditional concepts of bacteriology.

O. Gros[1] and J-M Volland[2]

[1]Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, 97110 Pointe-à-Pitre, France
[2]Department of Molecular, Cellular, and Developmental Biology. University of California, Santa Barbara, California, U.S.A.

e-mail: olivier.gros@univ-antilles.fr

In the Caribbean, the marine edge of the mangrove ecosystem is characterized by the presence of *Rhizophora mangle* trees living in coastal intertidal zones. In such marine environments, the anaerobic degradation of organic matter by sulfate-reducing bacteria results in the production of sulfides and sustains the development of various sulfur-oxidizing bacteria. Some of these marine bacteria are exceptional, pushing back the limits of what was thought possible in bacteriology. Thus, *Thiomargarita magnifica*, the largest bacterial cell that has a maximum cell length of 20,000 micrometers has been described in 2022 [1]. This bacterium, in addition to this extraordinary length displays a huge polyploidy of more than half a million copies of a very large genome with compartmentalization of its genomic material and ribosomes in active organelles bound by membranes. Our current data suggest that at least 4 other genera of mangrove bacteriaalso harbor high polyploidy and DNA compartmentalization in similar organelles, suggesting that cellular complexity has been underestimated within Prokaryotes.

Aiming at unveiling other bacterial species with unusual or complex features, we collected and analyzed two new veil-forming *Thiovulum* species (Campylobacterota) from mangrove [2]. By moving collectively or individually within the veils, these bacteria generate a macroscopic flow across the nutrient gradient (sulfides, oxygen, CO_2, etc.). More recently, we observed a third species which form large white "clouds" of cells that swim collectively like « shoals of sardines » through the water column above the sediments. These new *Thiovulum* species present a maximum speed of 2.5 mm/s for a bacterial cell length of 5µm. With a relative speed of 500 body length per second, they are the fastest organisms ever described.

These few examples suggest that marine mangrove may hold other exceptional microbiological treasures that are sure to advance Science.

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	April 18	
	Conference Session VI Bio- and nano-electrochemistry & bio applications	
02.00 pm – 02.30 pm	**Mingdong Dong** (Aarhus University, Denmark) « Reversible Crystallization of Amyloid Proteins » **(Keynote lecture)**	
02.30 pm – 02.50 pm	**Petr Gorelkin** (ICAPPIC Limited, London, United Kingdom) « Correlative SICM: Nanomechanical Insights into Alzheimer's-Related Amyloid Aggregates on Living Cells »	
02.50 pm – 03.10 pm	**Sebastian Sittl** (University of Bayreuth, Germany) « Contactless calibration of micro-channeled AFM cantilevers for fluidic force microscopy »	
03.10 pm – 03.30 pm	**Cécile Formosa-Dague** (INSA-University of Toulouse, France) « Probing adhesion forces between biofilms and anti-biofouling filtration membrane surfaces using FluidFM technology »	
03.30 pm – 04.00 pm	**Coffee Break (30 min)**	
04.00 pm – 04.30 pm	**Yuri Korchev** (Imperial College of London, United Kingdom) « Nanopipette sensors for Scanning Ion Conductance Microscopy » **(Expert)**	
04.30 pm – 04.50 pm	**Isalyne Drewek** (University of Mons, Belgium) « Self-Assembling Bisbenzimidazole Derivatives: A Pioneering Approach to Bacterial Flocculation »	
04.50 pm – 05.10 pm	**Serena Danti** (University of Pisa, Italy) « Electrospun bacterial cellulose nanofibers coated with chitin nanofibrils for eardrum repair »	
05.20 pm – 06.30 pm	**Poster Session II – Networking Time**	**International Cooperation workshop**
07.30 pm	**Conference Dinner**	

Reversible Crystallization of Amyloid Proteins

Mingdong Dong[1]

1) Institution, country Interdisciplinary Nanoscience Center (iNANO), Aarhus University

2) Department of Biology - Center for Electromicrobiology, Aarhus University

E-mail dong@inano.au.dk

Amyloid self-assembly is a complex phenomenon with implications in both degenerative human disorders and materials science. In this context, various structures such as amyloid fibers, particles, and crystals exist, with crystal amyloids being the most stable energetically. However, achieving control over amyloid assembly and reversibly manipulating the crystallization process pose significant challenges. This study aims to explore the reversible formation of macroscopic crystals by examining the effects of temperature, pH, ionic strength, and solvents. Furthermore, in situ microscopy will be utilized to investigate the dynamic process of amyloid disassembly. The reversibility of self-assembly under external stimuli provides valuable insights into the mechanisms of amyloid formation, emphasizing the importance of considering the influence of external factors on crystal formation. Ultimately, the investigation of reversible amyloid crystallization holds promise for the development of innovative strategies in biomaterial design.

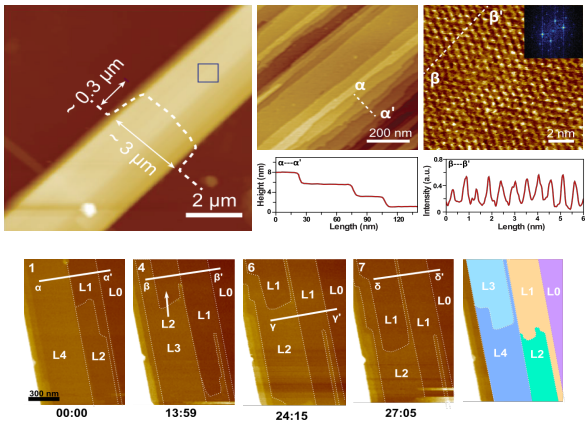

Figure 1: Disassembly of macroscopic crystal studied by in situ AFM

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Correlative SICM: Nanomechanical Insights into Alzheimer's-Related Amyloid Aggregates on Living Cells

P. Gorelkin,[1,2] V. Kolmogorov, [1] A. Erofeev, [1,2] Y. Korchev[3]

[1]*ICAPPIC Limited, London, United Kingdom*
[2]*National University of Science and Technology "MISiS", Moscow, Russia*
[3]*Imperial College London, London, United Kingdom*
e-mail: pg@icappic.com

The scanning ion-conductance microscopy (SICM) probe, a versatile micro- or nanopipette, has emerged as a powerful tool for nanoscale assays on cell surfaces. In this study, we present a novel approach using SICM to investigate the mechanical properties of living cells [1], particularly focusing on the impact of β-amyloid (Aβ) peptide aggregates associated with Alzheimer's disease (AD). By combining SICM with fluorescence and nanomechanical imaging, we achieved high-resolution imaging of uneven cell surfaces [2], enabling the observation of fine dendritic segments and synaptic connections.

Our investigation delves into the alterations of cell mechanical properties induced by drugs targeting various cytoskeletal components, providing valuable insights into the dynamic changes occurring at the nanoscale [3,4]. Notably, we employed low-stress SICM for real-time, long-term nanomechanical mapping, offering a rapid and effective means to observe biological events, particularly those associated with AD.

Alzheimer's disease is characterized by the aggregation of Aβ peptide on neuronal cell surfaces, leading to a cascade of cellular deviations. In this context, we utilized correlative SICM to comprehensively study cell topography, Young's modulus mapping, and confocal imaging of Aβ aggregates formation on living cell surfaces. Additionally, we assessed reactive oxygen species levels within single cells using platinum nanoelectrodes [5,6].

Our findings showcase the effectiveness of correlative SICM, combined with topography mapping and confocal imaging, for Patch-Clamp recordings from living cells with evident Aβ aggregates on their surfaces. The study not only establishes the capability of SICM in studying the cytotoxicity mechanisms of Aβ aggregates but also highlights its potential in unraveling the intricate connection between amyloid formation and local mechanical properties of living cells. This research contributes to our understanding of Alzheimer's disease pathology and opens avenues for future studies investigating therapeutic interventions at the nanoscale [7].

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Contactless calibration of micro-channeled AFM cantilevers for fluidic force microscopy

Sebastian Sittl,[1] Nicolas Helfricht,[1] and Georg Papastavrou[1]

[1] *Department of Chemistry II,*
University of Bayreuth,
Universitätsstr. 30, 95440 Bayreuth, Germany
e-mail: sebastian.sittl@uni-bayreuth.de

Atomic force microscopy (AFM) is a very powerful tool for imaging the surface topography of various samples, yet also a staple technique for determining interaction and adhesion forces between colloidal particles, liquid droplets, and living cells, alongside many more.[1][2] Almost 15 years ago, a new addition to the AFM, called FluidFM, facilitated such experiments, especially for the mentioned applications.[3] FluidFM combines AFM and nanofluidics by means of a microchanneled cantilever that bears an aperture instead of a tip at its free end. Thereby, single colloids or cells can be aspirated and immobilized to the cantilever by applying a suitable pressure.[2]
An accurate calibration of the cantilever spring constant and the (inverse) optical lever sensitivity (InvOLS) is essential for a quantitative evaluation of forces using AFM.[4] While there is an abundance of research on the topic of spring constant calibration, the standard method for the InvOLS has not changed significantly in the last 30 years. Usually, it is determined by ramping the tip against a hard, non-deformable substrate and evaluation of the cantilever's response in the so-called constant compliant region, where the captured photodiode signal is in a linear relationship to the actual physical deflection of the cantilever.[5]
However, for soft samples, like liquid droplets or cells, determining the optical sensitivity provides a significant challenge due to a lack of this constant compliance region. Hence, the photodiode can't be related to the cantilever. Here, we propose an alternative method rooted in hydrodynamic principles, utilizing the internal microfluidic channel of a FluidFM-cantilever and an external pressure controller to deflect the cantilever linearly. Our method incorporates a quantitative model based on the thrust equation, a well-established concept in avionics, and finite element simulations, to describe the cantilever's physical deflection in dependence of the externally applied pressure.
This approach enables a contact-free calibration of the (inverse) optical lever sensitivity (InvOLS) in less than a minute while demonstrating equal accuracy compared to the classical approach.[6]

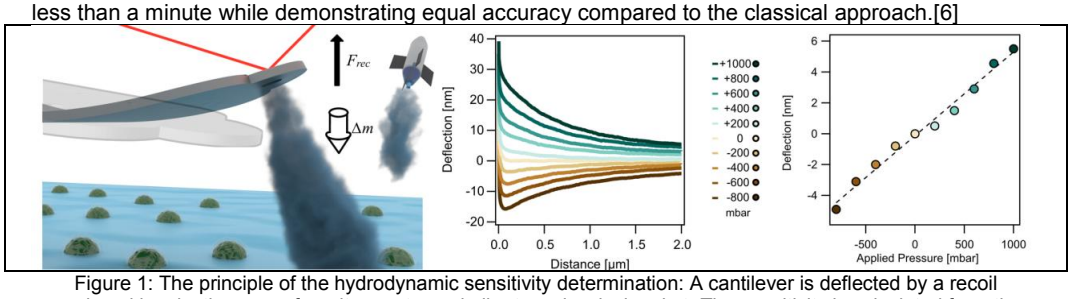

Figure 1: The principle of the hydrodynamic sensitivity determination: A cantilever is deflected by a recoil produced by ejecting mass from its aperture, similar to a classical rocket. The sensitivity is calculated from the linear dependency of the deflection on the applied pressure.

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Probing adhesion forces between biofilms and anti-biofouling filtration membrane surfaces using FluidFM technology

Abigail Burato Rosales[1], Christel Causserand[1], Clémence Coetsier[1,3] and Cécile Formosa-Dague[2,3]

[1]*Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INPT, UPS, France*
[2]*TBI, Université de Toulouse, INSA, INRAE, CNRS, France*
[3]*Fédération de Recherche Fermat, CNRS, France*
e-mail: formosa@insa-toulouse.fr

Biofouling results from biofilm development on filtration membranes' surfaces leading to performance loss and increased operating costs in water filtration. Therefore, it is pertinent to understand the molecular basis of the interactions between biofilms and membranes to develop new anti-fouling strategies. To access these interactions, atomic force microscopy (AFM) is a well-suited technology able to measure adhesion forces at piconewton level. However, the available methods only allow probing interactions between single cells and surfaces which is not representative of a full biofilm. Thus, we developed an original method combining AFM and microfluidics (FluidFM) to probe adhesion forces between biofilms and filtration membranes modified with an anti-biofouling agent, vanillin [1]. For that, the strategy involves growing bacterial biofilms on micrometer-sized polystyrene beads that could be aspirated on microfluidic AFM cantilevers and used as probes in force spectroscopy experiments. The results obtained showed that after membrane modification, there was a significant decrease in adhesion forces, work of adhesion, and percentage of adhesion between biofilms and membranes. Comparing these results to the ones obtained using classic single-cell force spectroscopy experiments [2], we could observe significant differences in force curve shape, percentage of adhesion, and work of adhesion, highlighting the difference in behaviour of single cell and biofilms. In particular, biofilm force curves and work of adhesion demonstrated the presence and complex unfolding of polymeric matrix from the biofilm. This new method showed the potential of vanillin-coated membranes to reduce biofouling and opens up several possibilities in biofouling studies. Indeed, this method can be used in different environmental conditions (temperature, medium, pH), and different stages of biofouling can be studied by adapting the biofilm growing conditions on the beads, allowing the development of new types of anti-biofouling surfaces.

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Nanopipette sensors for Scanning Ion Conductance Microscopy

Y. Korchev [1,2]

[1]Department of Medicine, Imperial College London, London, United Kingdom
[2]Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Kanazawa, Japan
e-mail: y.korchev@imperial.ac.uk

Molecular Biology has advanced we know much about the individual molecular components that make up living cells down to the level of the individual atoms. The challenge, however, is to fully understand the functional integration of these components. This requires determining how the molecular machines that make up a living cell are organized and Molecular Biology has advanced we know much about the individual molecular components that make up living cells down to the level of the individual atoms. The challenge, however, is to fully understand the functional integration of these components. This requires determining how the molecular machines that make up a living cell are organized and interact together not at the atomic length scale but on a nm scale. To do this we need to develop and applying nanoscale techniques for the visualization and quantification of cell machinery in real-time and on living cells. This will lead to detailed, quantitative models of sub-cellular structures and molecular complexes under different conditions for both normal and diseased cells. This approach ultimately requires the development of novel biophysical methods.

Scanning ion conductance microscopy and a recently developed battery of associated innovative methods (Fig.1) are unique among current imaging techniques, not only in spatial resolution of living and functioning cells, but also in the rich combination of imaging with other functional and dynamical interrogation methods [1-4]. There are significant advances to deliver nanotechnological solutions to biosensing that are affordable, integrated, fast, capable of multiplexed detection and monitoring, and crucially to offer high selectivity for the specific detection of trace levels of analyte in biological fluids.

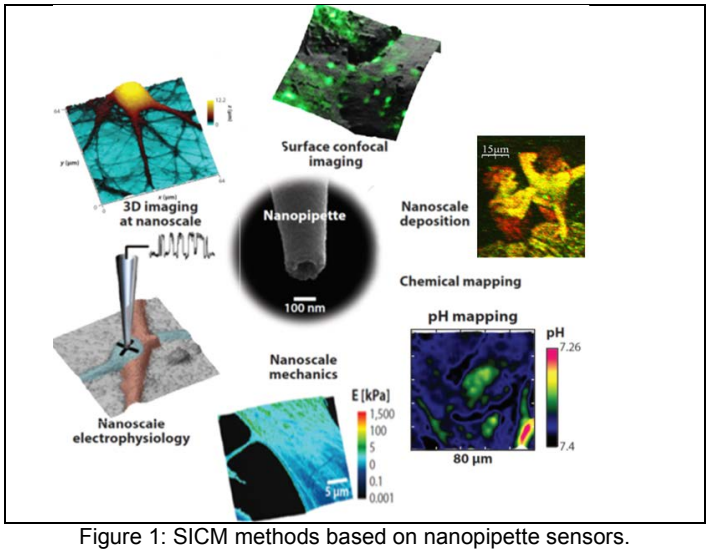

Figure 1: SICM methods based on nanopipette sensors.

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Self-Assembling Bisbenzimidazole Derivatives: A Pioneering Approach to Bacterial Flocculation

I.Drewek,[1,2], A.Pietka[1,2], C.Chipot[3], L.Van Melderen[4], M. Surin[5], P.Leclère[6], R.Wattiez[2], D.Stanicki[1] and S.Laurent[1,7]

[1]*General, Organic and Biomedical Chemistry Laboratory, University of Mons, B-7000 Mons, Belgium*
[2]*Proteomics and Microbiology Laboratory, University of Mons, B-7000 Mons, Belgium*
[3]*Laboratoire International Associé, Unité de Recherche n°7019, Université de Lorraine, F-54506 Nancy, France*
[4]*Cellular and Molecular Microbiology Laboratory, University of Brussels, B-6041 Gosselies, Belgium*
[5]*Chemistry of Novel Materials Laboratory, University of Mons, B-7000 Mons, Belgium*
[6]*Physics of Nanomaterials and Energy Laboratory, University of Mons, B-7000 Mons, Belgium*
[7]*Center for Microscopy and Molecular Imaging, B-6041 Gosselies, Belgium*

e-mail: isalyne.drewek@umons.ac.be

Bacterial flocculation is a biological process that has gathered increasing attention in recent years due to its diverse applications, spanning from wastewater treatment to biodegradation or biocatalytic processes. During this phenomenon, bacteria aggregate into visible flocs, facilitating the straightforward recovery of cells. While this process can occur naturally, flocculating agents are generally used to improve the flocculation efficiency[1]. The primary mechanism of action of these agents relies on electrostatic interactions between the bacterial membrane (negatively charged) and the flocculating agent (positively charged)[2]. However, this specific mechanism of action, coupled with elevated costs and toxicity, presents challenges that hinder the widespread industrial application of current flocculating agents[1].

In recent investigations, our research team has highlighted the intriguing biological activity exhibited by specific bisbenzimidazole derivatives. These compounds display substantial efficacy against both Gram-positive and Gram-negative bacterial strains, as evidenced by the formation of flocs within minutes when the bacterial suspension is exposed to a low concentration of a bisbenzimidazole compound. This phenomenon indicates a bacterial flocculation process initiated by these derivatives.

Since the structure of our compounds shows no indications of a conventional flocculation mechanism (no presence of positive charge in the molecule), we opted to conduct additional investigations. Initially, a range of microbiological studies (*e.g.* microscopic examinations, biological characterizations of the flocs) revealed that the observed flocculation did not emerge from a biological process but rather from physicochemical factors. The literature widely acknowledges that numerous heterocyclic structures have the ability to self-assemble in solution, forming supramolecular arrangements[3]. Through AFM studies, we successfully illustrated the compounds' ability to create fibre networks in solution through a molecular self-assembly process. Consequently, we were able to establish a correlation between the formation of this network and the onset of the flocculation phenomenon, this offering a new class of rapid and effective bacterial flocculating agents.

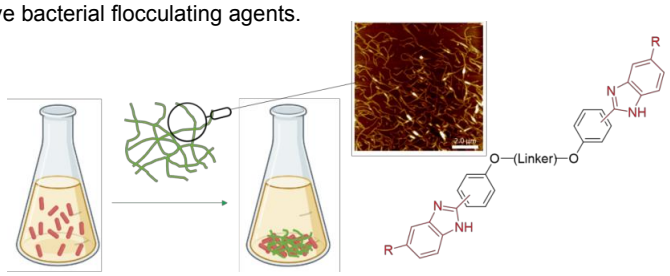

Figure 1: Bacterial flocculation is induced by bisbenzimidazole derivatives' self-assembly.

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Electrospun bacterial cellulose nanofibers coated with chitin nanofibrils for eardrum repair

S. Danti,[1] B. Azimi,[1] and Ipsita Roy[2]

[1] *Department of Civil and Industrial Engineering, University of Pisa, 56122 Pisa, Italy*
[2] *Department of Materials Science and Engineering, University of Sheffield, Sheffield, United Kingdom*
e-mail: serena.danti@unipi.it

Repairing perforations in the tympanic membrane (TM) resulting from chronic otitis media presents a significant challenge in otologic surgery due to persistent infection and inflammation hindering natural healing processes and compromising the durability of graft materials. This study investigates an innovative tissue engineering approach employing nanotechnology to fabricate a functional TM patch aimed at facilitating optimal wound healing and tissue regeneration [1]. We propose the utilization of a bio-based patch composed of nanoscale materials derived from carbohydrates, specifically bacterial cellulose (BC) electrospun fibers obtained through bacterial fermentation, and electrosprayed chitin nanofibrils (CNs) sourced from fishery byproducts. Both materials are processed using eco-friendly solvents, namely, 1-Butyl-3-methylimidazolium acetate ([Bmim]OAc) for BC and a water/acetic acid solution for CNs. The use of ionic liquids, despite their non-volatile nature, offers a solution to the challenges posed by the poor solubility of cellulose in traditional solvents.
BC was sourced from xylinus Gluconacetobacter. The rheological properties of the 3% w/w BC/solvent system were investigated to achieve the desired viscosity suitable for BC electrospinning. An electrospinning setup was established, employing a rotating collector partially submerged in a container filled with distilled water to eliminate solvents from the newly formed fibers. The stationary bath was strategically positioned to intersect with the rotating collector, set at 50 rpm with a ground charge and positioned 9 cm away from the needle tip (21G×3/4"). A flow rate of 0.3 mL/h and a voltage of 23 kV were applied. CNs were utilized at a concentration of 0.52 w% in an aqueous solution of acetic acid and distilled water (50:50 w/w). The resulting CN/BC meshes underwent comprehensive morphological, physicochemical, and mechanical analyses. Cytotoxicity assessments were conducted using L929 mouse fibroblasts, while in vivo irritation tests were performed on rabbit skin. Finally, in vitro regenerative evaluations were carried out using human dermal keratinocytes (HaCaT cells) and human umbilical vein endothelial cells (HUVECs).
Through optimization of electrospinning parameters and the incorporation of dimethylsulfoxide (DMSO) as a co-solvent, we achieved the fabrication of continuous and uniformly partially aligned BC fibers, measuring 228 ± 77 nm in size, and exhibiting a cellulose type II crystalline structure. The resulting BC electrospun fiber mesh displayed a thickness of 90 ± 14 µm and a Young's modulus of 2.48 ± 0.62 MPa. To augment the immunomodulatory and regenerative characteristics of the BC fiber mesh, CNs were electrosprayed onto its surface. The inclusion of CNs elicited indirect antimicrobial effects by upregulating the expression of beta-defensin-2 in HaCaT cells and facilitated the modulation of pro-inflammatory cytokines, thereby positioning the developed patch as a promising contender for supporting the wound healing process in TM perforations. In vitro cytocompatibility evaluations using mouse L929 fibroblasts, HaCaT cells, and HUVECs affirmed the biocompatibility of both uncoated and CN-coated BC fiber meshes. In vivo assessments conducted on rabbit skin revealed no signs of irritation after 24 hours.
The integration of electrospun BC nanofibers with electrosprayed CNs presents a compelling strategy for the creation of an environmentally friendly and sustainable TM patch, offering potential solutions to the existing challenges associated with conventional graft materials. This innovative biomaterial holds promise for significantly enhancing the outcomes of myringoplasty procedures, delivering optimal regenerative capabilities alongside functional support for TM healing.

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	April 19
	Conference Session VII The bio/non-bio interface
08.40 am – 09.10 am	**Bing Xu** (Brandeis University, United States) « Intracellular peptide assemblies » **(Keynote lecture)**
09.10 am – 9.40 am	**Loïc Jierry** (University of Strasbourg, France) « Enzyme-Assisted Self-Assembly of Peptide Nanofibers: From Nanostructure Resolution to Supramolecular Hydrogel Applications » **(Expert)**
9.40 am – 10.00 am	**Pierre Schaaf** (Insitut Charles Sadron, University of Strasbourg, France) « Enzyme-assisted self-assembly in host gels under the control of reaction-diffusion processes »
10.00 am – 10.20 am	**Burkhard Bechinger** (University of Strasbourg, France) « Nanosized supramolecular assemblies of histidine-rich designer peptides with multiple biological functions »
10.20 am – 10.50 am	**Coffee Break (30 min)**
10.50 am – 11.20 am	**Maryam Tabizian** (McGill University, Montreal, Canada) « Nanosystem-Enabled Delivery and Regeneration Strategies for Enhanced Therapeutic Efficiency » **(Expert)**
11.20 am – 11.40 am	**Jeremy Lakey** (Newcastle University, United Kingdom) « Creating a bio-nano interface using the Caf1 superpolymer »
11.40 am – 12.00 pm	**Pilar Rivera Gil** (Universitat Pompeu Fabra, Barcelona, Spain) « NanoTarg : a nanotechnological platform for cancer management »
12.00 pm – 12.20 pm	**Patrick Mesquida** (King's College London, United Kingdom) « Nanofibrils on PDMS : Linking the electrostatic and mechanical properties of collagen »
12.30 pm – 02.00 pm	**Conference Lunch**

Intracellular peptide assemblies

Bing Xu

Department of Chemistry, Brandeis University, 415 South St., Waltham, MA 024542, USA
e-mail: bxu@brandeis.edu
URL: http://people.brandeis.edu/~bxu/

Subcellular compartmentalization is a key feature of eukaryotic cells. Although selectively targeting subcellular organelles holds many exciting possibilities for biomedicine, it remains an underdeveloped area. Enzyme-instructed self-assembly (EISA) integrates enzymatic reactions and self-assembly, enabling the creation of complex nanostructures from single molecules for targeting subcellular organelles. This talk discusses the use of EISA to generate intracellular peptide assemblies for targeting mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, and nucleus. Particularly, we will showcase the use of EISA to generate in-situ peptide assemblies inside cells as nanomedicines for developing therapeutics that can overcome drug resistance and immunosuppression in cancer therapy.

Enzyme-Assisted Self-Assembly of Peptide Nanofibers: From Nanostructure Resolution to Supramolecular Hydrogel Applications

Loïc Jierry*[1,2]

[1]Institut Charles Sadron UPR22-CNRS / University of Strasbourg – Centre national de la recherche scientifique - CNRS (France) – France
[2]Université de Strasbourg – Université de Strasbourg, CNRS – France

Abstract

Nature controls many biological mechanisms through localized self-assembly processes. Cellular adhesion is, for example, initiated by focal adhesions which result in the formation of actin fibers that play an essential role in cell motility; centrosomes initiate the formation of microtubule spindles that control cellular division. Yet, developing localized molecular self-assembly processes leading to the growth of nanostructures exclusively near a surface, or at an interface, and with a precise control over its structure and resulting properties is still a challenge. In 2004, a new way to initiate the self-assembly of low molecular weight hydrogelators (LMWH) has been reported: an enzyme is used as a trigger able to transform a precursor compound into an efficient LMWH.(1) In the last seven years, we have spatially localized different enzymes on various kind of materials to control the self-assembly process in a spatiotemporal way (Fig. 1a and 1b).(2) Based on this approach, we have designed original flow reactors allowing the production of enantiopure chemicals using a catalytically-active hydrogel supported on polymer foams.(3) Supramolecular hydrogel able to self-sustain its own growth through an autocatalytic way was demonstrated as well.(4) In addition, this presentation will also focus on our recent developments about the near-atomic nanostructure resolution of a model tripeptide and the impact of the assembly initiation process (Fig. 1c).(5)

Keywords: Enzyme, assisted self, assembly, self, assembled peptide nanofiber, supramolecular hydrogel, near, atomic nanostructure resolution

*Speaker

Enzyme-assisted self-assembly in host gels under the control of reaction-diffusion processes

Pierre Schaaf*[1,2], Jean-Yves Runser[3,4], Bernard Senger[5], and Loïc Jierry[4]

[1]Institut Charles Sadron (ICS) – université de Strasbourg, Institut de Chimie du CNRS, Centre National de la Recherche Scientifique, Matériaux et Nanosciences Grand-Est, Réseau nanophotonique et optique, Réseau nanophotonique et optique – 23 rue du Loess, BP 84047, 67034 STRASBOURG Cedex 2, France

[2]Centre de Recherche en Biomédecine de Strasbourg (CRBS) – université de Strasbourg, Institut National de la Santé et de la Recherche Médicale – 1 rue Eugène Boeckel - CS 60026 - 67084 Strasbourg Cedex, France

[3]Institut Charles Sadron – Centre National de la Recherche Scientifique, Matériaux et Nanosciences Grand-Est – France

[4]Institut Charles Sadron – Centre National de la Recherche Scientifique – France

[5]Centre de Recherche en Biomédecine de Strasbourg – université de Strasbourg, Institut National de la Santé et de la Recherche Médicale – France

Abstract

Enzyme-assisted self-assemblies are ubiquitous in cellular processes. They also offer the possibility to design new materials since they allow spatio-temporally tuning the self-assembly. We report recent results relative to the self-assembly of Fmoc-FFY (Fmoc: 9-Fluorenylmethoxycarbonyl; F: phenylalanine; Y: tyrosine) resulting from the diffusion of Fmoc-FFpY (p: phosphate group) into a host gel containing alkaline phosphatase (AP) free to diffuse. The interplay between the diffusion of Fmoc-FFpY into the gel, the enzymatic transformation of Fmoc-FFpY into Fmoc-FFY and the self-assembly of Fmoc-FFY leads to a non-monotonous self-assembly profile perpendicularly to the interface. Such a profile is explained by the fact that the self-assembly process is a nucleation process starting when the Fmoc-FFY concentration exceeds a critical nucleation concentration. This then leads to a Liesegang type process characterized by the succession of maxima and minima of self-assembled structures.(1) When the enzyme concentration becomes very small the "continuous" self-assembly profile is replaced by the presence of individual self-assembly globules. These globules can originate either from local Fmoc-FFY fluctuations or the presence of small enzyme globules.(2) All these observations are supported by a model that will be described.
(1) Runser J.Y. et al. Non-monotonous enzyme-assisted self-assembly profiles resulting from reaction-diffusion processes in host gels, J. Coll. Interf. Sci. 620 (2022) 234

2) Runser J.Y. et al. Transition from continuous to microglobular shaped peptide assemblies through a Liesegang-like enzyme-assisted mechanism, J. Coll. Interf. Sci. 633 (2023) 876

Keywords: self, assembly, peptide, gel, enzyme triggered

*Speaker

sciencesconf.org:nanoinbio2024:506302

Nanosized supramolecular assemblies of histidine-rich designer peptides with multiple biological functions

B.Bechinger,[1,2] R. Manca[1], J. Raya[1], A. Saad[1], J. Jiang[1], M.Lointier[1], L.Vermeer[1], D. Fenard[3,4], A. Kichler[5], A.Galy[3,6]

[1]*University of Strasbourg / CNRS, Chemistry Institute UMR7177, F-67000 Strasbourg*
[2]*Institut Universitaire de France, F-75005 Paris*
[3]*Généthon / INSERM UMR S951, F-91002 Evry*
[4]*Sangamo Therapeutics France, Allée de la Nertière, F-06560 Valbonne (present address)*
[5]*University of Strasbourg / CNRS, Faculty of Pharmacy UMR7199, F-67401 Illkirch*
[6]*INSERM US35, Department, F-91100 Corebeil-Essonnes*

e-mail: bechinge@unistra.fr

A family of designed histidine-rich peptides will be presented which can assemble in a number of different aggregation states. Interestingly, whereas sequences that occur in soluble form exhibit considerable antimicrobial activities, nucleic acid transfection is best by peptides that form large complexes of tuneable size with nucleic acids (DNA, siRNA, mRNA…) and these also exhibit strong cell penetrating activities for large proteins, peptide vaccines, adeno associated viruses and nanodots. Due to the presence of four histidines their membrane interactions are strongly pH dependent. The delivery of cargo by these peptides is complex, involving many steps, which we investigated on a structural and biophysical level.

More recently, vectofusin-1, a member of the same family of LAH4 peptides has been shown to spontaneously self-assemble into helical oligomers, spherical aggregates, that further assemble into annular and extended nanofibrils and hydrogels as a function of phosphate concentration and in a pH-dependent manner. This bears considerable interest for the design of biomaterials.

Importantly, the peptide has a strong capacity to enhance the gene transfer by lentiviral vectors into the cell interior. Thereby, the fibres formed by this relatively short sequence have therapeutic applications ranging from monogenic and infectious diseases to cancer, by enhancing transduction levels of target cells and reducing the amount of lentivirus for greater safety and reduced costs. Vectofusin-1 associates with viral particles and promotes the entry of several retroviral pseudotypes into target cells when added to the culture medium, without cytotoxicity. The vectofusin-1 fibrils have a unique coiled-coil α-helical structure whereas most other viral transduction enhancers form β-amyloid fibrils and are investigated by solid-state NMR and other biophysical approaches. Our observations define vectofusin-1 as a member of a new class of α-helical lentiviral transduction enhancers. Its coiled-coil fibril formation is reversible which bears considerable advantages in handling the peptide in conditions of gene therapy protocols.

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Nanosystem-Enabled Delivery and Regeneration Strategies for Enhanced Therapeutic Efficiency

Maryam Tabrizian[1,2]

[1]Department of Biomedical Engineering, McGill University, 37775 University St., Montreal H3A 2B4
[2]Faculty of Dental Medicine and Oral Health Sciences, 2001 McGill College Ave, Montreal, Quebec H3A 1G1

Nanosystems possess a number of excellent physicochemical properties that make them well suited for the challenging task of targeting, repairing and regenerating various tissues [1,2]. In recent decades, a comprehensive design of nanosystems with high specificity, sensitivity and selectivity has been achieved through a rigorous control of their physicochemical properties and an understanding of the nano-bio interface [3,4]. This progress has allowed them to be used in manifold contexts, such as improving the biopharmaceutical properties of encapsulated drugs by modulating their release kinetics, improving physicochemical stability and reducing toxicity. In addition, their ability to specifically target or induce immunomodulatory effects by surface modification or by coupling ligands to promote internalization by endocytosis offers multiple therapeutic opportunities [5,6]. The aim of this talk is to provide the audience with some our strategies for the development of nanosystems, such as core-shell, polymer- and lipid-based nanosystems for osteogenesis, targeting atherosclerosis, immunomodulation against melanoma as well as nanocoating to camouflage the islet transplant used for the treatment of type 1 diabetes **(Fig.1)**. For all these examples, the rationale behind the design strategy, a thorough characterization of the physicochemical properties, such as their composition, formulation, functionalization and size and charge of nanosystems, along with their biological behavior *in vitro*, (cellular uptake, cellular toxicity, immune response), and in mouse model will be presented.

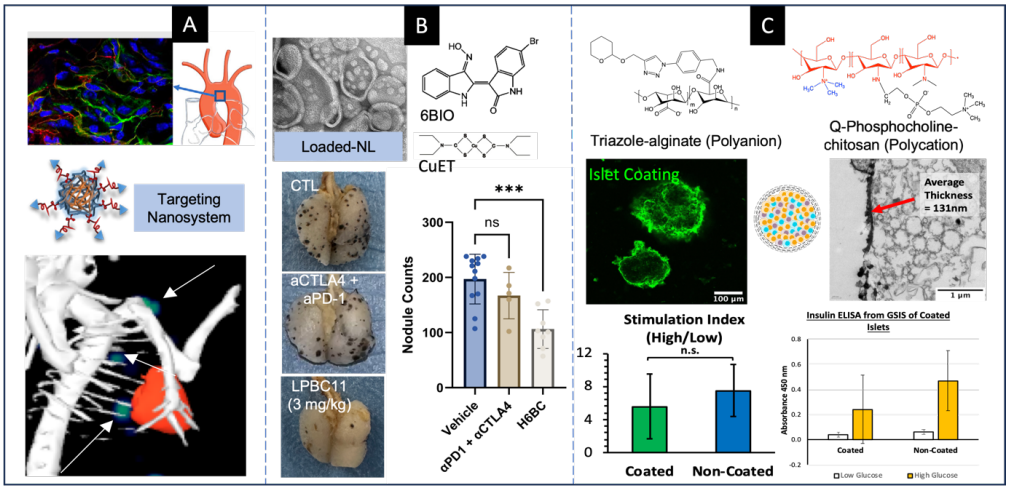

Figure 1. **A)** Peptide-functionalized polymer nanosystem with targeting ability to atherosclerotic regions as demonstrated by ex-vivo and live animal fluorescence imaging. **B)** 6BIO/CuEt-loaded nanoliposmes with dual action, and the demonstration of its ability to reduce metastatic cancer region. **C)** Layer-by Layer deposition of Triazole-alginate and quaternized-Phosphocholine grafted chitosan as conformal coating for immunoprotecting pancreatic islets. The graphs show that nanocoating does not significantly affect insulin secretion.

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Creating a bio-nano interface using the Caf1 superpolymer

J. H. Lakey,[1] H. Waller,[2] E.K., Corbin[2] and D.T. Peters [2]

[1]*Biosciences Institute, Medical School, Newcastle University UK*
[2]*MarraBio Ltd, Cookson Building Framlington Place, Newcastle NE2 4HH*
e-mail: jeremy.lakey@ncl.ac.uk

In vivo, eukaryotic cells grow within, and are supported by the nanoscale extra-cellular matrix (ECM) both physically and via its biochemical signals. There is a great demand for cells to be grown *in vitro* for the biochemical products they release (antibodies, vaccines etc), regenerative medicine (stem cells, tissue culture) laboratory grown meat and research (cancer studies, immunology etc). To do this effectively the ECM has to be recreated either in 2D or 3D formats. Artificial ECM comes in many forms and ranges from complex animal extracts (Basement membrane extract e.g. Matrigel, Geltrex) to chemically synthesised peptides. We are developing the protein Caf1 as an economic and flexible toolkit for artificial ECM manufacture [1].

Naturally produced by *Yersinia pestis*, Caf1 forms polymers, of up to 250 non-covalently linked individual 15 kDa monomer subunits, measuring up to 1.5 um in length and 2 nm in diameter. The polymer is used to prevent recognition and phagocytosis of the pathogen by host macrophages – in effect forming a molecular "cloaking device" that surrounds the bacterial cell. Previously we have shown that the Caf1 polymers can be produced recombinantly in *E. coli*, and used to coat cell culture surfaces, where in its natural form it retains its native properties, forming a Teflon-like "non-stick" surface that mammalian cells do not adhere to or interact with. This allows bioactive peptide motifs, such as cell adhesion proteins of the ECM or growth factor molecules to be inserted into the Caf1 protein at various positions within the monomer structure. This provides us with the ability to exquisitely define what bioactive signals will be transmitted to cells. We have utilized this system to create functional mimics of ECM proteins such as fibronectin and laminin as well as growth factors such as BMP2 and VEGF(2). My presentation will describe these and recent results with a range of cell types which show that engineering the cell protein interface at the nanoscale can provide significant improvements in the economics and reliability of cell culture.

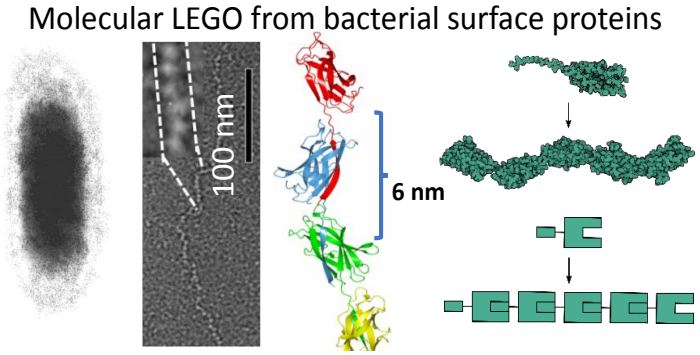

Figure 1: Left to right, bacterial cell with Caf1 coat, electron micrograph of Caf1 polymers, high resolution structural model of Caf1 and schematic of how Caf1 monomers assemble into polymers.

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NanoTarg: a nanotechnological platform for cancer management

Pilar Rivera Gil
Integrative Biomedical Materials and Nanomedicine Lab,
Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona, Spain
e-mail: pilar.rivera@upf.edu

We present a nanotechnological platform consisting of 3 main building blocks *i.e.,* a cancer target, a monoclonal antibody towards this target, and a nanoformulation. The main working principle of this platform is based on tumor targeting. We specifically recognize Zip4, a tumor target present only in the epithelium of tumoral cells. Based on this recognition, the platform can target and controlled the delivery of an active ingredient.
To validate the technology, we first proved *in vitro* and *in vivo* specific tumor targeting and now we are validating the efficiency of targeting the chemotherapeutics *vs.* its non-targeted form. One big advantage of this platform is its versatility since the 3 building blocks can be used all together or independently. Furthermore, the nanoformulation is itself composed of different building blocks that can be assembled differently yielding different properties to the nanomaterial. For example, in one configuration the nanomaterial has photothermal properties for melanoma treatment, in other configuration it has SERS sensing properties. We will present this platform and examples for its biomedical applications.

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Nanofibrils on PDMS: Linking the electrostatic and mechanical properties of collagen

E.Gachon,[1,2] Z.Bai,[2] and P.Mesquida[2]

[1]CEA Cadarache, Zone Cité des Énergies BIAM, Bâtiment 1900, Saint-Paul-lez-Durance 13108, France
[2]Department of Physics, King's College London, London WC2R 2LS, United Kingdom
e-mail: patrick.mesquida@kcl.ac.uk

Collagen is a fibrillary protein that provides strength, mechanical stability and shape to connective tissue in vertebrates. It is the basic component of the extracellular matrix and forms a scaffold to which cells need to attach. There is evidence that the mechanical properties and the electrical surface charge of fibrils influences cell behaviour in the matrix and, possibly, stem cell differentiation. Understanding these properties is not only important from a fundamental, biophysical or medical perspective but will also be of great value in the targeted design of collagen-based scaffold materials in tissue engineering.
In this study, single collagen fibrils were stretched along their entire length by depositing them on a highly stretchable foil of polydimethylsiloxane (PDMS). Kelvin-probe Force Microscopy (KPFM) was then performed on strained fibrils to probe their electromechanical response (Fig. 1). Native fibrils and fibrils exposed to glutaraldehyde, which is a typical protein cross-linking agent for cell cultures, were compared. The results show that their surface potential increases towards more positive values for up to 10% strain and then decreases again at even higher strains [1]. We interpret this phenomenon as breaking of cross-links, which exposes positive charges at the surface of collagen fibrils. This trend correlates with the stiffness of collagen fibrils, where fibrils strain-stiffen for strains up to roughly 15%, and then strain-soften for greater strains. The change in charge described here could affect the interaction of collagen with cell-adhesion proteins and the calcification of fibrils, thereby ultimately affecting collagen-cell interactions and cell behaviour.
Using the same experimental approach, individual collagen fibrils were deposited on a pre-strained PDMS foil [2]. By releasing the PDMS foil from its initial strain, the attached collagen fibrils spontaneously buckled. AFM imaging was then used to determine the shapes of individual, buckled fibrils. The data obtained allows calculation of the fibrils' tensile moduli using the well-known column- buckling theory from mechanical engineering without the need for force measurements. Comparison of our calculated moduli with data obtained by AFM nanoindentation and more sophisticated techniques show that our results are in good agreement. The great advantage of our approach, however, is that it is much easier to use and can be implemented by any lab to quickly determine the mechanical properties of a large number of fibrils without requiring specially built instrumentation.

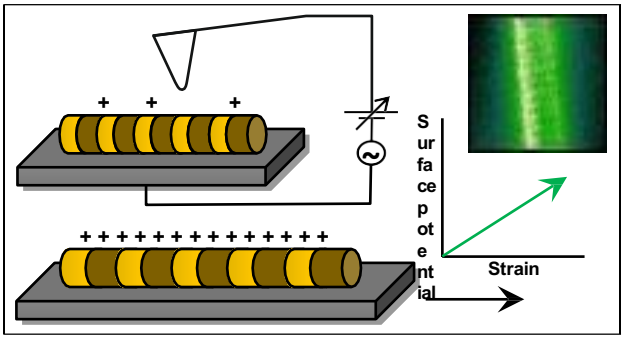

Figure 1: Measuring the electrostatic surface potential of collagen fibrils attached to PDMS (from [1]).

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	April 19
	Conference Session VIII Instrumentation for bio- & nanotechnologies
02.00 pm – 02.30 pm	**Takeshi Fukuma** (Kanazawa University, Japan) « Biomaterial Interfaces and Intracellular Phenomena Investigated by 3D Atomic Force Microscopy » **(Keynote lecture)**
02.30 pm – 02.50 pm	**Claire Godier** (University of Lorraine, France) « Innovative combination of 3D bio-printing and microfluidic to create PDAC-on-chip for anticancer drug development »
02.50 pm – 03.10 pm	**Angela C. Debruyne** (UGent, Ghent, Belgium) « Live microscopy of multicellular spheroids with multi-modal near-infrared nanoparticles reveals differences in oxygenation gradients »
03.10 pm – 03.30 pm	**Maximilian Seuss** (Bruker Nano GmbH, Berlin, Germany) « Visualizing Molecular Dynamics with High-Speed Tip-Scanning Atomic Force Microscopy »
03.30 pm – 04.00 pm	**Coffee Break**
04.00 pm – 04.30 pm	**Tomaso Zambelli** (ETH Zurich, Switzerland) « How a cell reacts to an internally growing mechanical stimulus » **(Expert)**
04.30 pm – 04.50 pm	**Pawel Karpinski** (Wroclaw University of Science and Technology, Poland) « Optical manipulation of nanoparticles for heating, cooling and rheological measurements »
04.50 pm – 05.10 pm	**Nicolo Tormena** (University of Durham, United Kingdom) « Impact of the substrate on the phase transitions properties in supported lipid bilayers »
05.10 pm – 05.30 pm	**Zahra Ayar** (EPFL, Lausanne, Switzerland) « Toward a faster and smarter SICM with temporal resolution optimization through a deep-learning technique »

Biomaterial Interfaces and Intracellular Phenomena Investigated by 3D Atomic Force Microscopy

Takeshi Fukuma[1]

[1]*Nano Life Science Institute (WPI-NanoLSI), Kanazawa University*
e-mail: fukuma@staff.kanazawa-u.ac.jp

Frequency modulation atomic force microscopy (FM-AFM) was traditionally used in ultrahigh vacuum for atomic- or molecular-scale studies. In 2005, we developed in-liquid FM-AFM and enabled operating FM-AFM in liquid with atomic resolution [1,2]. Since then, this method has been used for subnanoscale imaging of various biological systems, including proteins, lipid bilayers and DNAs. Meanwhile, we also noticed a serious limitation of the AFM in studying nanobio interfaces. Surface structures of biological systems and water thermally fluctuate to produce 3D non-uniform distribution. Therefore, nanobio interfaces have a nanoscale vertical extent and hence cannot be visualized by conventional 2D-AFM. To overcome this limitation, in 2010, we developed 3D-AFM and enabled subnanoscale 3D imaging of hydration structures and flexible molecular features [3]. So far, this method has been used for investigating interfacial structures of various minerals and organic, polymeric, and biological systems. However, its applications to biomaterial interfaces have hardly been explored.

Recently, we started to use 3D-FM-AFM for investigating biomaterials such as cellulose/chitin nanocrystals [4,5] and peptide self-assembled monolayers [6]. For cellulose, we successfully imaged molecular-scale surface structures of cellulose nanocrystals (CNCs) in water and hydration structures formed on them. From the results, we found the existence of nanoscale defects and identified crystal polymorph and orientation of the facet terraces. We also performed molecular-resolution imaging of chitin nanocrystals, which are another polysaccharide derived from crab shells, and found they have much fewer defects and show almost no flat terraces due to the wide variation in the chain rotation angle. In both cases, the stable formation of hydration shells on their surfaces was confirmed. For peptides, we investigated graphite-binding dodecapeptide (GrBP5) self-assembled layers on HOPG. This self-assembly is potentially useful for controlling biomolecule-electrode interfaces in bioelectronic devices such as sensors. We clarified molecular-scale details of the self-assembling process by in-situ FM-AFM imaging. Combined with simulation, we proposed the atomistic model of the peptide layer and hydration structures on it.

During the 3D imaging of hydration structures, the water distribution is heavily disturbed by the tip insertion. However, as the original structure is recovered by the self-organization of water, we can observe nearly intrinsic hydration structures. Therefore, the internal structures of other 3D structures having a self-organizing capability may be also visualized by 3D-AFM. We recently started to explore this possibility by imaging various 3D self-organizing systems. Examples include hydration structures, ionic liquids, surfactant layers, swollen polymers, chromosomes, and living cells. Among them, we named the method for imaging live cells “nanoendoscopy AFM” [7,8]. In this method, we insert a thin needle probe into a living cell to perform 2D- or 3D-AFM imaging. Owing to the self-organizing capability of a cell, its structure and functions are recovered once the probe is extracted. So far, we have successfully visualized the whole cell structures, nucleus, actin fibers and focal adhesions. We also confirmed that such measurements do not significantly damage the cells by the fluorescent assay and cell division tests. The growth process of the focal adhesions and their associated actin stress fibers were directly visualized in 3D inside living cells. In addition, the nuclear elasticity changes with the cancer progression and cell cycle were quantitatively measured by directly indenting the nucleus with a needle probe. These measurements highlight the unique capability of this newly developed AFM method.

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Innovative combination of 3D bio-printing and microfluidic to create PDAC-on-chip for anticancer drug development

C. Godier[1], Z. Baka[2], L. Lamy[3,4], L. Bolotine[3,4], E. Gaffet[1], H. Alem[1]*

[1] *Université de Lorraine, CNRS, IJL, F-54000 Nancy, France*
[2] *EA* 3452/CITHEFOR, Faculté de Pharmacie, Université de Lorraine, F-54000 Nancy, France
[3] *Université de Lorraine, CNRS, CRAN, F-54506 Vandoeuvre-les-Nancy, France*
[4] *Département Recherche, Institut de Cancérologie de Lorraine (ICL), F-54519 Vandœuvre-lès-Nancy, France*
e-mail: halima.alem@univ-lorraine.fr

Cancer is a public health issue that represents the second cause of death in the world. In particular, Pancreatic Ductal Adenocarcinoma (PDAC) shows a 5-years survival rate of less than 10%. The discovery of new treatments or the understanding of the limits of the current used therapeutics is then more than urgent. Indeed, the research for effective anti-cancer drugs relies on relevant *in vitro* models. Three-dimensional cell cultures offer appealing alternatives owing to their high capacity to mimic *in vivo* cell-cell interactions and the gradients of oxygen and nutrients. In this domain, 3D bioprinting emerges as a promising strategy for creating reliable and resilient 3D tumor models. Its capacity to construct 3D structures encompasses diverse cell types with controlled distribution and precise architectural arrangements which make it a powerful process to lead to biomimetic PDAC. Furthermore, the integration of microfluidic technology offers the capability to mimic dynamic *in vivo* conditions, giving rise to what is commonly referred to as "cancer-on-chip" platforms.

In this work, 3D bioprinting is used to create a 3D pancreatic tumor model. This involved the encapsulation of pancreatic cancer cells (Panc-1) and cancer-associated fibroblasts (CAFs) in a gelatin-alginate based hydrogel. The bio-printed tumor models were then characterized by assessing the viability and morphology of the encapsulated cells. Cell membrane integrity was confirmed up to 14 days post-bioprinting. The combination of complementary characterizations led to confirm that we have developed a relevant preclinical and reliable PDAC model.

Then, to strengthen the robustness of this model, we introduced dynamic conditions by coupling it to a microfluidic system. The objective of this approach was to mimic *in vivo* conditions as closely as possible, thus providing a more realistic platform for the evaluation of treatments. To monitor the behavior of the model towards drugs, gemcitabine, a first-line chemotherapy agent in the treatment of PDAC was used put in contact with our 3D bio-printed structure. The comparison of the results obtained from the model with established drug efficacy was performed using multiple parameters, including LIVE/DEAD cell viability assays, assessment of metabolic activity by AlamarBlue method, demonstration of apoptosis by flow cytometry using Annexin V/PI, as well as immunological staining to show proliferation (Ki67) at low concentration and apoptosis (Caspase 3) at important concentration of gemcitabine. This integration of 3D bioprinting into preclinical 3D models, combined with dynamic conditions using microfluidics, offers an innovative approach for a better understanding of tumor mechanisms and drug testing, opening new perspectives in anti-cancer research.

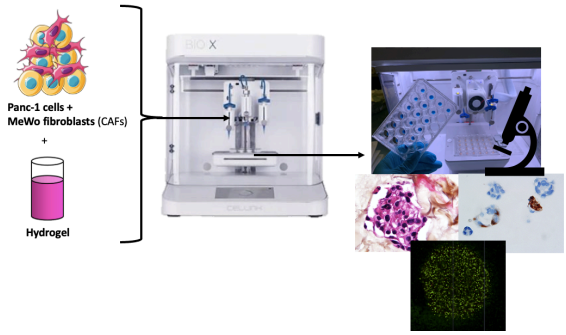

Figure 1: 3D bio-printing process and characterization of the developed PDAC model

Live microscopy of multicellular spheroids with multi-modal near-infrared nanoparticles reveals differences in oxygenation gradients

A. Debruyne,[1] I. Okkelman,[1] N. Heymans,[1] M. Nobis[2], S. Borisov[3], and Dmitriev R.[1]

[1] *UGent, Corneel Heymanslaan 10, building MREB3, 2th floor, 9000 Ghent, Belgium*
2 VIB Center for Cancer Biology, KU Leuven, 3000 Leuven, Belgium
[3]*Graz University of Technology, Stremayrgasse 9, Graz, 8010 Austria*
e-mail: angela.debruyne@ugent.be

The tumor microenvironment supports cell survival, promotes local invasion, and metastatic dissemination. While nutrient and O_2 (hypoxia) availability are important contributors to the 'niche' environment and lead to metabolic cell heterogeneity, their quantitative, real-time, and non-destructive analyses are rarely assessed.

To address this, we produced a deep cell-penetrating O_2-sensitive nanoparticle probe[1], which can measure oxygenation in live cells and spheroids via intensity ratio-based or phosphorescence lifetime imaging (PLIM) microscopies. This is based on the phosphorescence quenching of the near-infrared O_2-sensitive metalloporphyrin, encapsulated with a red light-emitting reference dye into biocompatible methacrylate-based polymer nanoparticles (Figure 1). O_2 probe provided efficient cell and multicellular spheroids (during formation) staining, with no observed cytotoxicity.

After initial O_2 probe testing with multiple cell lines including cancer HCT116, PANC-1, HEK-293T, MCF-7, SKOV-3 and non-cancer human dental pulp stem cells (DPSC), we applied it to analysis of oxygenation gradients in multicellular spheroids and observed size-dependent oxygenation of HCT116 spheroids. Next, the spheroid growth medium's viscosity (0-5% dextran addition) affects their oxygenation gradients and we found that increased viscosity led to a more hypoxic core formation. Furthermore, comparison of different spheroid formation methods leads to differences in morphology, cell viability and oxygenation.

Most surprisingly, we found that with time and culture conditions, some spheroids (HCT116 and DPSC cells) could gradually produce an "inverted" oxygenation gradient with elevated oxygenation at the core, compared to the cells at the periphery. This contrasted with the expected gradual decrease of $[O_2]$ towards the core due to diffusion limits ('direct' gradient). The performed multi-parameter microscopy of spheroids with inverted gradients, analysis of cell death, redox, temperature, proliferation and other parameters, helped to understand this phenomenon. Thus, two-photon FLIM imaging revealed that spheroids with such a gradient also displayed a gradual strong gradient in NAD(P)H autofluorescence lifetimes, demonstrating a decrease of OXPHOS function in the core compared to the periphery.

Presented O_2 microscopy helps probing heterogeneity of spheroid oxygenation, effects of growth medium conditions, presence of drugs, size, and time in the culture. This approach, which can be multiplexed with FLIM and other advanced microscopies, can help visualise 3D microenvironment and dynamic changes in cell metabolism in basic studies of cancer cells.

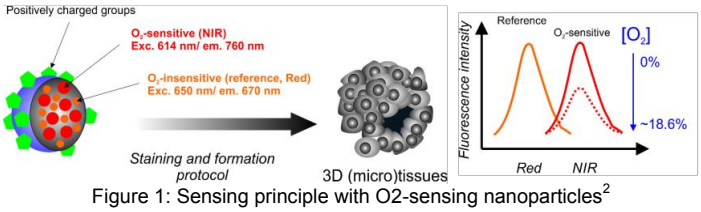

Figure 1: Sensing principle with O2-sensing nanoparticles[2]

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Visualizing Molecular Dynamics with High-Speed Tip-Scanning Atomic Force Microscopy

M. Seuss,[1] A. Körnig,[1] J. Barner,[1] and T. Henze[1]

[1]*JPK BioAFM, Bruker Nano GmbH, Am Studio 2D, 12489 Berlin, Germany*
e-mail: Maximilian.Seuss@bruker.com

Biological systems exhibit very high structural and functional dynamics on molecular scales. Understanding the principles of the kinetics behind structural changes at that scale is of critical importance when studying samples ranging from single membrane proteins to complex macromolecular systems, in order to accurately develop novel therapeutic applications. We have used high-speed tip-scanning atomic force microscopy (AFM) with a kilohertz linerate to visualize molecular dynamics by enabling temporal resolution on the sub-100-milisecond scale. The use of a tip-scanning AFM, as compared to a sample-scanning system, enables high-resolution correlation experiments with advanced optical techniques. We will give two examples in which high-speed tip-scanning AFM was applied for studying of structural transitions and biomolecular dynamics in samples, containing triangular DNA origamis and photosensitive surfactants.

DNA origami structures serve as a functional template in multiple artificial and native molecular systems. We studied the development of order in 2D DNA triangular Rothemund lattices. By mobilizing the DNA origami adsorption on mica with varying buffer composition we looked at the temporal dependence between lattice order development and Na+ ion content in the studied sample with a temporal resolution of 1 frame/s.

We monitored the structural photosensitive transition of photosensitive surfactants under external light-induced deformation. By simultaneous high-speed AFM measurements and switching the external wavelength illumination from 365 nm to 546 nm and vice versa, we could monitor and induce a reversible structural transition within the studied sample in real-time.

How a cell reacts to an internally growing mechanical stimulus

Tomaso Zambelli,[1] Xinyu Zhang,[1] Lasse Hyldgaard Klausen,[2] Anne-Kathrine Kure Larsen,[2] Mingdong Dong[2]

[1] *Laboratory of Biosensors and Bioelectronics, ETH Zurich, 8092 Zurich, Switzerland*
[2] *Interdisciplinary Nanoscience Center (iNANO), Aarhus University, 8000 Aarhus C, Denmark*
e-mail: ztomaso@ethz.ch

To maintain internal stability and protect cellular components from external fluctuations and ensuring optimal conditions for biochemical reactions, cells must function as closed systems restricted by the cell membrane and control their shapes throughout their life cycles. The interplay between cellular mechanical forces and interactions with the extracellular matrix (ECM) dictates cell shape, influencing critical processes such as cell migration, tissue homeostasis, and cellular differentiation. Therefore, the quantification of cellular forces plays a crucial role in determining cell states. One strategy involves assessing the dynamic changes in cytoskeletal elasticity. This assessment can be performed through techniques such as indentation using Atomic Force Microscopy (AFM), pulling via micropipette aspiration and more recently manipulating the cytoskeleton using optical tweezers [1]. However, these techniques focus on the local measurements of cytoskeleton and could not address the origin of the intracellular force as a whole. Alternatively, another approach centers on bidirectional cell-extracellular matrix (ECM) interactions. This can be achieved by measuring extracellular traction forces using traction force microscopy (TFM) [2] or studying cell-ECM adhesions with single cell force spectroscopy obtained by FluidFM (FFM) [3]. However, these methods rely on extracellular anchoring, thus they are limited by the presence focal adhesion and actomyosin clutch and provide only a partial representation of intracellular forces.

In this study, we employ an intracellular protein crystal setup, focusing on the mechanical quantification of the impact of such crystals on the cytoskeleton. For the first time, the cytoskeleton is experimentally considered as a three-dimensional elastic network. The phenomenon of protein crystallization within living cells has been frequently observed as a natural assembly process over the past decades. [4] Crystalline states of recombinant proteins are also reported to grow in animal cells, in which metazoan specific kinase PAK4 crystalizes within the cytosol in the presence of its potent endogenous inhibitor Inka1. (Inka-PAK4) [5] The Inka-PAK4 aggregates and spontaneously nucleates resulting in the formation of long rod-shaped crystals. It offers engineering flexibility through different designed plasmids during the transfection procedure. [6] [7] Inka-PAK4 in this work is treated as a form of intracellular stress, with rigid ends protruding internally onto the cell cytoskeleton.

The interactions of Inka-PAK4 crystals with the cytoskeleton are quantified with different techniques, elucidating the wholistic elastic behavior of the actin cortex. Firstly, the growth and the contact of the intracellular crystal with the cytoskeleton are recorded with time lapses. We then compared the states of the crystals with intact and depolymerized actin cortex. The intracellular forces during crystal growth are also interpreted with confocal reference free imaging (cTFM) [8]. Interestingly, we observe a “soft” feature of the Inka-PAK4 crystal manifesting as bending and fracture. This phenomenon is further investigated through live actin imaging upon depolymerization treatment via the FFM technique [3]. In order to quantify the modulus of the “soft” crystal, we propose a convenient method that measures the flexus modulus of the protein crystal rod with FFM.

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Optical manipulation of nanoparticles for heating, cooling and rheological measurements

P. Karpinski,[1] K. Bruchal[1] and V. Paschoal[1]

[1]*Faculty of Chemistry, Wroclaw University of Science and Technology, Wyb. Wyspianskiego 27, 50-370 Wroclaw, Poland*
e-mail: pawel.karpinski@pwr.edu.pl

Optical trapping is a widely used technique allowing for remote and precise manipulation of particles and measurement of the forces acting on them. Optical forces can be enhanced when particles resonantly interact with light, like in case of e.g. plasmonic and high-index dielectric nanoparticles. These particles have the cross section for the light-matter interaction much larger than their physical size. This makes them perfect nanoantennas for bio-sensing, SERS, local temperature measurements, and heat-therapy. It also allows for efficient transfer of spin and orbital angular momentum of light for realization of fast nanorotors. We show that the circularly polarized Laguerre-Gaussian beam allows simultaneous rotation of a gold nanorod about its symmetry axis and revolution around the beam's symmetry axis.[1] We also show that the trapped Silicon nanorods can be rotated at high frequencies comparable to plasmonic particles. Analysis of strong Raman scattering of Si and rotation motion of Si nanorod allows us to estimate the temperature increase of the trapped particle and its surroundings.[2]
On the other hand, strong interaction of resonant nanoparticles with light makes their optical trapping in 3D challenging and limited to a narrow size range due to the strong radiation pressure. Therefore, we developed a novel optical trapping configuration based on counter-propagating laser beams generated through focus splitting in a uniaxial birefringent crystal and reflection from a mirror. We demonstrate this application by trapping and rapid rotation of gold nanorods that are impossible to trap in 3D using conventional laser tweezers.[3]
Another class of nanoparticles, which we investigate, are nanocrystals doped with rare earth ions. These particles exhibit strong luminescence with long excitation life times. The luminescence from thermally coupled excited states of lanthanide ions e.g. Erbium can be used to measure temperature and optically trapped nanocrystal can be used as precise local thermometer. Additionally, efficient anti-Stokes luminescence in Ytterbium inos can lead to laser induced refrigeration and local cooling. The optical cooling of small particles in biological systems may allow for measurements of the specimen properties and its response to low temperature stress.
Optical trapping of resonant and rare earth doped nanocrystals can be used as a platform, which allows one to investigate micro-rheological properties of complex fluids in different spatial arrangements and at different temperatures with both heating and cooling. The complex fluid might be a biological fluid, such as cytoplasm, or artificial e.g. an active fluid like a polymer network with azo-benzene groups attached to its chain or chromonic liquid crystals with addition of azo-benzene dye.

Acknowledgements
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Impact of the substrate on the phase transitions properties in supported lipid bilayers

Nicolò Tormena,[1] Teuta Pilizota[2], and Kislon Voitchovsky[1]

[1]*University of Durham, Physics Department, Durham, UK*
[2]*University of Edinburgh, Biology Department, Edinburgh, UK*
e-mail: nicolo.tormena@durham.ac.uk

Bacterial membranes are critical to the survival of organisms, from providing a physical barrier between the inside and outside of the cell, to controlling membrane trafficking, organism reshaping and growth, and underpinning drugs resistance. Membranes comprise a phospholipid bilayer where proteins and sugars are embedded. It is well known that the activity of the proteins and their response to external stimuli depend on the biophysical properties of the lipid bilayer such as thickness, elasticity, lipid packing and molecular mobility (1). The effect of physical contact with the surroundings are less well understood. Bacterial membranes are always in close contact with various structural filaments such as cytoskeleton tubules or peptidoglycan chains which can all influence the properties of the bilayer.
Here, we use a minimal model system for E. coli's inner membrane to study the effects of external interactions on the membrane's biophysical properties. Using a combination of atomic force microscopy (AFM) and differential scanning calorimetry (DSC), we comparatively track the phase transition kinetics of our model E. coli membrane when supported and unsupported. The results show that only part of the kinetics depends on the cooling/heating, with the main influence coming from contact with a support in AFM experiments. The presence of a contacting substrates not only shifts the transition temperature but can also arrest the transition and induce a global re-arrangement of the lipid species. The result is a phase transition that follows classical nucleation on short timescales but is subsequently dominated by a spinodal decomposition of the membrane components on a longer term. Our work highlights the importance of external membrane contacts to control the composition and biophysical properties of bacterial membranes.

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Toward a faster and smarter SICM with temporal resolution optimization through a deep-learning technique

Z. Ayar[1], M. Penedo[1], B. Drake[1], J. Shi[1], S. M. Leitao,[1] and G. Fantner[1]

[1] *Laboratory for Bio- and Nano-Instrumentation, Institute of Bioengineering, School of Engineering, Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne 1015, Switzerland*

e-mail: zahra.ayar@epfl.ch, georg.fantner@epfl.ch

Scanning ion conductance microscopy (SICM) is a powerful high-resolution technique for live imaging of cellular morphology and micro-dynamics of cell structures. SICM provides high precision in lateral and axial resolution[1]; however, studying cell dynamics is also dependent on temporal resolution. The temporal resolution in SICM is highly influenced by the low scanning rate, making capturing the rapid dynamics of cells a potential challenge. The scanning rate in SICM is affected by many factors, including the mechanical design and resonant frequency of the piezoelectric actuator for Z-range control and inherent delay in the electrical circuit[1,2]. To address this issue and increase the scanning rate, advanced technological improvement is required, which causes a cost overload due to the equipment upgrade. Here, we propose a novel approach using a deep-learning-based image processing technique that avoids increasing hardware costs. In this method, a percentage (e.g., 30 %) of lines randomly selected are skipped, and a deep learning model, which has been trained on the SICM data, is used to reconstruct the images. The model performance has been monitored and tested using different metrics, such as peak signal-to-noise ratio (PSNR), structural similarity index measure (SSIM), mean squared error (MSE), edge detection, and Pearson's correlation coefficient to ensure the quality of image reconstruction. This technique can effectively reduce the scanning time and, as a result, leads to a higher temporal resolution while keeping the image quality. This novel software-based strategy allows for capturing the cell dynamics more effectively with higher temporal resolution without significant hardware improvement.

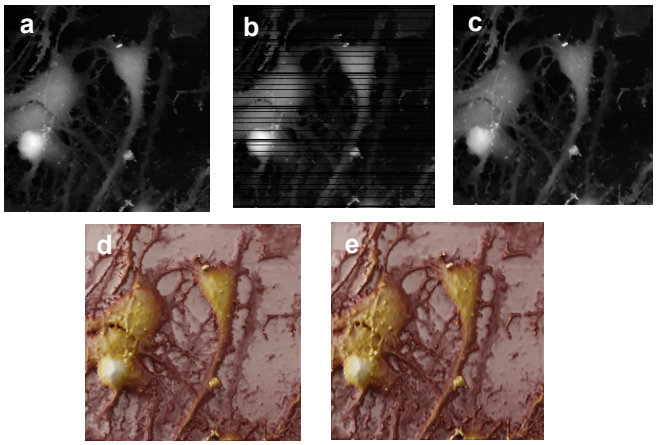

Figure 1: Reconstruction of SICM image with 30% skipped lines; a) Original image, b) Skipped line image (Masked), c) Reconstructed image, d) 3D model of the original image, e) 3D model of the reconstructed image

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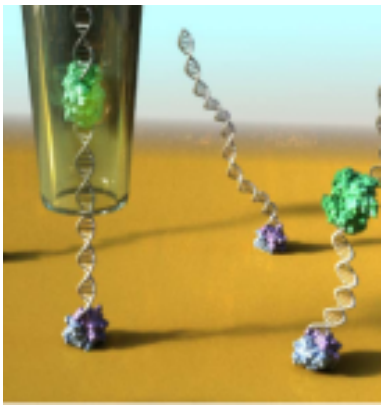
	April 20
	Conference Session IX **Special NanoInBio session**
08.40 am – 09.10 am	**Georg Fantner** (EPFL, Lausanne, Switzerland) « Time resolved Scanning Ion Conductance Microscopy » **(Expert)**
09.10 am – 9.30 am	**James Flewellen** (University of Edinburgh, United Kingdom) « Interfacing bacteria with an integrated circuit to develop a bioengineered, reprogrammable biosensor »
9.30 am – 9.50 am	**Ariane Boudier** (University of Lorraine, France) « Dynamics of molecular interactions between Concanavalin A and gold nanoparticles »
9.50 am – 10.10 am	**Wojciech Chrzanowski** (The University of Sydney, Australia) « Extracellular vesicles as the next-generation multifunctional nanotherapeutics for tissue regeneration »
10.10 am – 10.40 am	**Coffee Break (30 min)**
10.40 am – 11.10 am	**Stefano Leporatti** (CNR Nanotech-Istituto di Nanotecnologia, Lecce, Italy) « Atomic Force Microscopy as Diagnostic Tool in the War Against Cancer: Overview and Insight in the Future »
11.10 am – 11.30 pm	**Xavier Bellanger** (University of Lorraine, France) « Developing the next generation of anti-staphylococci bacteriophage-based biomaterials »
11.30 am – 11.50 am	**Tomasz Antosiewicz** (University of Warsaw, Poland) « Plasmonic quantification of reaction rates and structural changes »
11.50 am – 12.10 pm	**NANOinBIO Awards & Closing Ceremony**
12.30 pm	**Conference Lunch**

Time resolved Scanning Ion Conductance Microscopy

Georg E. Fantner, Barney Drake, Samuel M. Leitao, Zhara Dulabi, Jialin Shi, Aleksandra Radenovic, Vytautas Navikas

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. 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 [2]. 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 [3].



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. We term this method scanning ion conductance spectroscopy (SICS) [4]. Using capillaries with exceptionally small nanopores, we can detect and manipulate single molecules in a repeatable and high throughout manner. Compared to other nanopore sensing techniques SICS has inherent temporal and spatial control of the DNA translocation through the nanopore. This greatly increases the SNR and enables detection of even single base gaps in a dsDNA strand. The ability to read the same molecule multiple times makes this technique well suited for biophysics and diagnostic applications.

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Interfacing bacteria with an integrated circuit to develop a bioengineered, reprogrammable biosensor

James L. Flewellen,[1] Amritha Janardanan,[1] Urte Puodziunaite,[1] Soner Sonmezoglu,[2] Stefano Sonedda,[3] Michel Maharbiz,[3] and Teuta Pilizota[1]

[1]*Centre for Engineering Biology, University of Edinburgh, Edinburgh, United Kingdom*

[2]*Department of Electrical and Computer Engineering, Northeastern University, Boston, MA, USA*

[3]*Department of Electrical Engineering and Computer Sciences, University of California, Berkeley, CA, USA*

e-mail: james.flewellen@ed.ac.uk

Motile bacteria constantly sense their environment to provide input for navigation. Via their chemotactic network, these microorganisms can sense specific chemicals down to nanomolar concentrations on second-time scales. In some species, the sensing input is translated into mechanical output in the form of controlling the direction of rotation of the bacterial flagellar motor. The input pathways of the chemotactic network can be bioengineered to create sensitivity and specificity to chemicals not normally in the repertoire of the wild-type organism. This opens up the potential for the bacterial flagellar motor to be used as a biosensor[1] of unrivalled sensitivity and speed for a truly vast array of chemical inputs.

However, to realise this potential, a fundamental change is required in detecting the flagellar motor rotation signal at speed and at scale. Current techniques are optical in nature, requiring large and complex equipment and limited to tens of cells per instance. Here, we demonstrate a means of detecting flagellar motor rotation electrically by interfacing live bacteria with a custom-designed integrated circuit (IC), along with our work in developing this technology for commercial applications.

Our bioelectronic interface device has motile bacteria attached to the surface of the IC, with each sensing bacterium proximal to a set of micron-sized electrodes. To detect the rotation signal, the electrodes are probed with a high-frequency voltage and the impedance change due to rotating flagella is measured. A custom microfluidic channel facilitates the delivery of liquid samples to the IC chamber. Microstamping of molecular adhesives in distinct patterns can better align bacteria to the electrodes, and our technique is scalable to have 100s to 1,000s of cells per device, which can be made small and readily portable. In the formulation of our device, the hardware (IC and associated microfluidics) remains a constant platform, while the 'software' (i.e. the bacteria) can be reprogrammed to new chemical sensitivities via bioengineering techniques. This technology is a step change in bioelectronic interfacing and has the power to revolutionise whole-cell biosensors and their application for rapid detection of trace chemicals in liquid environments, from environmental monitoring through to medical diagnostics.

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Dynamics of molecular interactions between Concanavalin A and gold nanoparticles

S. Lahouari[1,2], J. Gouyon[1,3], G. Francius[3], F. Picaud[4], R. Albino dos Reis[5], A. B. Seabra[5], I. Clarot[1,2] and A. Boudier[1,2,5]

[1] *Université de Lorraine, CITHEFOR F-54000 Nancy, France*
[2] *Université de Lorraine, CNRS, LRGP F-54000 Nancy, France*
[3] *Université de Lorraine, CNRS, LCPME F-54000 Nancy, France*
[4] *Université de Franche-Comté, UR SINERGIES F-25030 Besançon*
[5] *Federal University of ABC, Center for Natural and Human Sciences, Santo André 09210-580, SP, Brazil*
[6] *: Institut Universitaire de France (IUF), France*
e-mail: ariane.boudier@univ-lorraine.fr

Gold nanoparticles (AuNP) modified by lectins, *e.g.* concanavalin A, ConA, are studied, mainly for bacterial lipopolysaccharide (LPS) detection, thanks to ConA carbohydrate-recognition sites. AuNP are often functionalized with ConA using linkers or thiol-gold bonds [1-2]. Based on the unspecific capacity of AuNP and more generally of NP, to bind proteins [3], the present work describes the mechanism behind the binding of ConA and AuNP thanks to an integrative approach, based on both experimental and theoretical studies.

A direct interaction was realized, adding ConA to citrate-stabilized AuNP in a sodium citrate medium. The resulting objects were characterized using UV-Visible and fluorescence spectrometries, dynamic light scattering (DLS), atomic force (AFM), and transmission electron microscopies (TEM). Molecular dynamic simulations were used to improve the experimental observations and give *in silico* explanations on the chemical mechanistic responsible for the AuNP and ConA interactions.

Citrate-stabilized AuNP were synthesized and characterized (3.3 ± 0.8 and 6.5 ± 0.4 nm for core and hydrodynamic diameters, respectively, 513 ± 1 nm for the surface plasmon resonance (SPR) band, and -30.2 ± 9.1 mV for the surface charge). A hydrodynamic diameter of 18,4 ± 2,4 nm was measured for AuNP-ConA complex without any modification of the gold core diameter. The AuNP-ConA showed a SPR shift of + 3 ± 1 nm *vs* AuNP, confirming a change in the AuNP electronic environment. A dynamic phenomenon was highlighted: AuNP and ConA continually interact without the formation of a stable complex, after the calculation of the values of Stern-Volmer constants by studying the quenching of the tryptophane fluorescence of ConA. This corroborated with molecular dynamics simulations demonstrating that AuNP and ConA did not form a stable complex during the whole simulations, while interacting quite strongly. Indeed, AuNP diffuse continuously around ConA and explore several interaction sites of the protein without any specific coupling. The dynamic contribution in the structuration of the AuNP-ConA complex was attributed to the non-displacement of the citrate ions adsorbed at the surface of the AuNP by ConA, contrary to what was modelled when reacting albumin with AuNP.

Parallel calculations by both further interpretation of the fluorescence quenching of the tryptophane of ConA with AuNP and molecular dynamics, revealed low-energy bindings between AuNP and Con A. Binding forces between ConA and AuNP were addressed by Single Molecule Force Spectroscopy (SMFS) using AFM. Interactions forces between AuNP and ConA grafted onto AFM probe in the range of 50-150 pN were significantly weaker than the ones measured between the same ConA probe and mannose grafted substrate (*ie* 50-350 pN). The latter was typical values reported for multiple ConA-mannose interactions [4,5]. Lastly, adding high concentration of NaCl in the medium induced a spacing between ConA and AuNP, highlighting the presence of electrostatic bindings.

A better understanding of the mechanisms of the formation of the AuNP-ConA complex was brought. The contribution of a dynamic complex characterized by low-energy binding was highlighted. Further chemical process to anchor the protein to the gold core will be developed to study its interactions with LPS in biological samples such as in plasma or cerebrospinal fluid.

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Extracellular vesicles as the next-generation multifunctional nanotherapeutics for tissue regeneration

Wojciech Chrzanowski*[1]

[1]The University of Sydney – Australia

Abstract

The high rate of respiratory failure from chronic injuries reaches an epidemic level. Unfortunately, no current interventions unequivocally restore the lost lung function associated with acute and chronic injuries. Since both types of injuries have characteristic degeneration of lung tissue, inflammation and remodelling of the airways, this propelled stem cell-based therapies, and more recently extracellular vesicle (EV) therapies, into the scientific and public spotlight. Increasing evidence reveals that immunomodulatory and reparative properties of stem cells arise from EVs they secrete. EV-based therapies have been lauded 2nd generation of stem cell therapies. We propose a novel approach for active lung repair, where we maximise the beneficial effects of stem cells but producing specialised EVs and delivering them to lungs using our innovative aerosolisation technology. We have shown that specialized EVs effectively reduce inflammatory mediators and package key molecular signals that play a central role in tissue repair. Therefore, targeted delivery of specialised EVs to lungs represents an innovative solution, which will facilitate active and effective repair of the injured epithelium. Results of this project will provide fundamental knowledge of how stem cell-derived EVs control tissue repair, which is crucial to devising new and personalised therapies. The proposed solution seeks to provide a new treatment regimen with better therapeutic outcomes and could lead to an essential change of the way lung injuries are treated. This could have significant societal and economic consequences, including reduced mortality, accelerated recovery, and overall improved quality of life.

Keywords: nanomedicine, multifunctional nanoparticles, extracellular vesicles, tissue regeneration

*Speaker

Atomic Force Microscope as Diagnostic Tool in the War Against Cancer: Overview and Insight in the Future

Stefano Leporatti[1]

[1]CNR Nanotec-Istituto di Nanotecnologia, Lecce, 73100, Lecce (Italy)
e-mail: stefano.leporatti@nanotec.cnr.it

Atomic Force Microscopy has been employed worldwide as standard tool for investigating biological samples in fluid environment [1], but recent integration with always more sophisticated optical modules like Fluorescence Microscopy, Laser Scanning Confocal, Super-resolution Microscopy and others has paved the way towards a better comprehension of hidden pathways and mechanisms in the cellular nanoworld to develop an effective nano-weapon against the evil of the Century.
In this lecture I will overview recent development of Atomic Force Microscopy with integrated Optical microscopy Tools for enhancing the investigation of Morpho-mechanical features in cancer cells [2]. Super-resolution Microscopy and a perspective outlook in the future of cancer research will be further discussed.

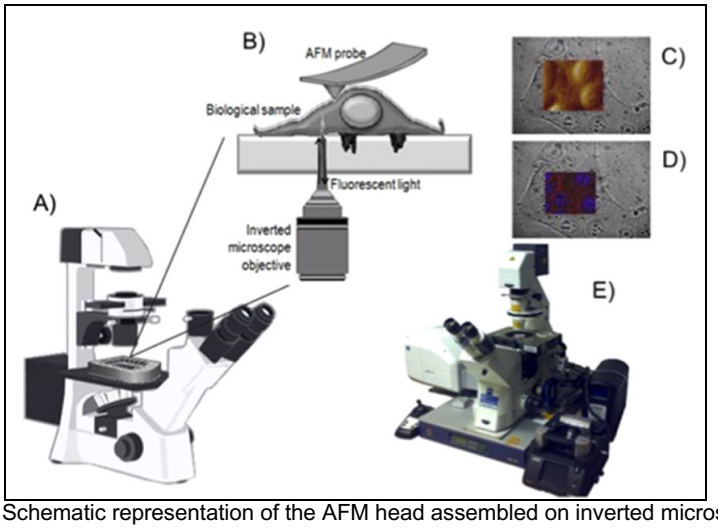

Figure 1: (A) Schematic representation of the AFM head assembled on inverted microscope. In the magnification (B), the simultaneous acquisition of AFM and -fluorescence images is represented. (C and D) The images acquired with an hybrid system CAT (Confocal Atomic Force Tirf microscope) (E) that was allocated in the laboratories of CNR (Lecce, Italy). [Cascione M. et al. 2017].

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Developing the next generation of anti-staphylococci bacteriophage-based biomaterials

E. CENRAUD, A. SCHOLLHAMMER, S. EKRAMI, G. FRANCIUS, E. ANDRE, F. QUILES and X. BELLANGER

Université de Lorraine, CNRS, LCPME, F-54000 Nancy, France
e-mail: xavier.bellanger@univ-lorraine.fr

The increasing number of therapeutic failures due to antibiotic resistance is of global concern. In this context, new alternative treatments such as the therapeutic use of bacterial viruses, also called bacteriophages or phages are being considered. Although phage therapy seems to be a promising solution, there are still significant knowledge gaps on fundamental and practical aspects related to its implementation modalities. Through this project, we aim to propose a prophylactic phage therapy approach, focused on human and animal skin infections caused by *Staphylococcus aureus.* Hydrogels are safe and biocompatible materials already used as patches or sprays for wound healing, or deposit as coatings on the surface of medical devices. Our strategy is to develop different hydrogels based on large polyions such as hyaluronate or poly(allylamine), which have intrinsic antimicrobial activity, and to load them with phages for a synergistic bactericidal effect, with virus release triggered by the presence of bacteria.

In the present work, 8 model *S. aureus* phages from different families were individually loaded by co-incubation into pre-established multilayer hydrogels composed of alternating layers of hyaluronic acid and poly(allylamine). All but one of the phages appeared to enter the hydrogel by passive diffusion at concentrations of 10^6 up to 10^8 plaque-forming units/mL of gel, representing a concentration factor of 5 to 100 times that of the initial phage suspension.

We have simultaneously characterized the physical and chemical properties of the phages: hydrophobicity, charge, size, geometry. These properties are variable from phage to phage, even within phylogenetically close viruses. Among these properties, the phage negative charge, and, to a lesser extent, the phage size/geometry, seemed to be the main factors determining their behavior in hydrogels. Evaluation of the bactericidal activity of our hydrogels, with or without phage loading, is ongoing, but the preliminary results offer promising insights for the development of effective phage-loaded biomaterials.

The incorporation of phages into these biomaterials could improve infection control, minimize the impact of *Staphylococcus*-related healthcare-associated infections, and ultimately improve the quality of the patient care while addressing a pressing global healthcare issue.

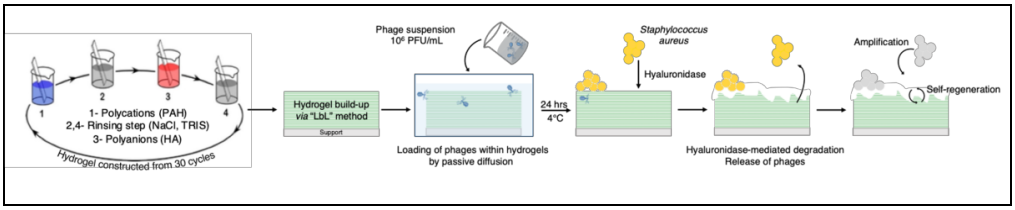

Figure 1: Schematic of the hydrogel construction, bacteriophages loading procedure and their release triggered by *S. aureus* hyaluronidase production. The antimicrobial property of this hydrogel is intrinsically due to the positively charged poly(allylamine), which can disrupt the integrity of the negatively charged bacterial cell membrane, and extrinsically enhanced by the action of bacteriophages.

Plasmonic quantification of reaction rates and structural changes

T.J. Antosiewicz,[1] D. Świtlik, [1] F.A.A. Nugroho,[2] S. Acimovic,[2] M. Kall,[2] and C. Langhammer[2]

[1]*University of Warsaw, Faculty of Physics, Pasteura 5, 02-093 Warszawa, Poland*
[2]*Department of Physics, Chalmers University of Technology, 412 96 Göteborg, Sweden*
e-mail: tomasz.antosiewicz@fuw.edu.pl

Localized surface plasmon resonance (LSPR) biosensing utilizes metal nanoparticles for high-end multiplexing and miniaturization and high-throughput label-free molecular interaction. Such sensors can be employed for real-time analysis, especially when combined with high-stability optofluidic systems. It is thus possible to obtain time-resolved measurements of changes in composition, size and shape of nanobiological objects and layers to understand their properties and functionality, quantify kinetics, and optimize their performance. Despite the relative simplicity of LSPR, most state-of-the-art solutions require assumptions on the physical processes being sensed in order to yield quantitative information. Alternatively, intricate modeling or multiparameter measurements are required to disentangle conformational or thickness changes of biomolecular layers from complex interfacial refractive index variations. Here, we discuss the use of plasmonic sensors in two cases: for studying antibody-antigen reaction kinetics at equilibrium over long time scales and present a dual-band nanoplasmonic ruler for simultaneous measurements of thickness and refractive index variations.

First, we discuss the development and use of an ultrastable single-molecule LSPR sensing platform optimized for quantifying antibody−antigen interaction kinetics over very long time scales. Such measurements, central to the immune response, are relevant for analysing antibody−antigen interactions for in vivo and in vitro diagnostics, and development of therapeutic substances. Utilizing gold nanorods, we measure equilibrium fluctuations of the PEG/anti-PEG interaction and next leverage time and frequency domain analysis to demonstrate that reversible adsorption of monovalently bound anti-PEG antibodies is the dominant factor affecting the LSPR fluctuations. This offers an alternative to established methods for determination of interaction rates and is particularly well suited to analyze molecular systems whose properties change during interaction phases, e.g. mass transport limitations or a varied effective association rate constant.

Second, we introduce a dual-band plasmonic nanoruler constructed of intermixed arrays of two types of plasmonic nanoparticles with spectrally separated resonance peaks. Employing electrodynamic simulations and model experiments we demonstrate the ability to perform real-time simultaneous measurements of thickness and refractive index variations in uniform and heterogeneous layers with sub-nanometer resolution. This way we can follow nanoscale changes of the structure of entities deposited on the sensors. This is demonstrated by quantifying the degree of lipid vesicle deformation at the critical coverage prior to rupture and supported lipid bilayer formation. Generally, the proposed approach provides a flexible and generic route for multimodal nanoplasmonic optical sensing.

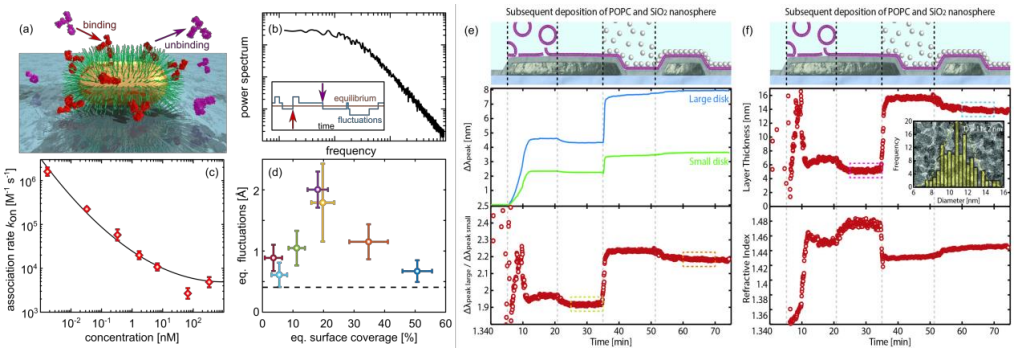

Figure 1: (a-d) Equilibrium fluctuation study of antibody-antigen interactions. (e-f) Simultaneous measurement of thickness and refractive index of lipid vesicle to bilayer structural change.

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Posters

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From Tip to Ship: Characterisation of New Marine Antifouling Coatings

Benjamin Devenish (Durham University)[1], Professor Kislon Voïtchovsky (Durham University),[1] and Robin Heath (AkzoNobel)[2]

[1]*Ogden Centre For Fundamental Physics, Durham University, Lower Mountjoy, South Road, Durham, DH1 3LE*
[2]*AkzoNobel, Stoneygate Lane, Felling, Gateshead, NE10 0EX*
e-mail: benjamin.devenish@durham.ac.uk

Biofouling is a well documented problem on marine structures, such as offshore platforms and large shipping vessels. Fouling of these surfaces can increase costs as well as reduce the operational lifetime of structures[1], both major issues in marine industries. Current antifouling strategies rely on biocides, such as cuprous oxides, which kill fouling organisms coming in close contact with the coated surfaces during the early stages of biofouling[2]. While effective, this method is known to have detrimental impacts on marine ecology, especially in busy water ways[3]. There is thus a need for new, novel antifouling strategies that do not rely on biocides. One possible solution entails physical methods, whereby the function of the antifouling coating comes from the inability of fouling organisms to attach to the protected surface due to its mechanical and physical properties, such as low surface energies and water contact angles[4]. However, most existing strategies cannot easily be scaled up for routine use in shipping or marine industries. In this research, we investigate a promising new strategy for a polymer-based antifouling coating that combines particular mechanical properties together with nanoscale control of the surface hydrophobicity. This strategy can easily be scaled up, however its specific mode of action and evolution with aging are not yet fully understood. Using Atomic Force Microscopy (AFM) in solution, we characterise differences between candidate coatings, quantifying nanoscale structural and viscoelastic properties that we aim to correlate with the coatings' antifouling performance. These results form a platform for the rational development of functional antifouling strategies to be deployed on a larger scale.

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Dairy powders: in situ characterization of particle surface features

C. Gaiani[1*], S. Bakalis[2], A. Filali[1], M. Andersen[2], K. Malmos[3], J. Burgain[1]
[1]Université de Lorraine, LIBio, F-54000 Nancy, France.
[2]Department of Food Science, University of Copenhagen, DK-1958 Frederiksberg, Denmark
[3]Arla Foods amba Aarhus, Denmark
e-mail: claire.gaiani@univ-lorraine.fr

One part of the AMUSE project (funding from Innovation Fund Denmark) is related to *in situ* measurements of milk powders surface evolution. The overall aim of AMUSE project is to ensure growth of exports of dairy powders, through a validated platform able to predict shelf life under real supply chains. For this purpose, real industrial powders were characterized at the nanoscale level in order to add data and develop mathematical models from the surface to the particle and the powder bulk scales.

Dynamic *in situ* Atomic Force Microscopy (AFM) was used to follow particle surface topography (i.e. rugosity), softness (i.e. young modulus measurements) during storage events (i.e. T°C, humidity). Results obtained by AFM were crossed with XPS analysis, surface fat extraction and classical physicochemical characterisation of milk powders. With this *in situ* technique it was possible to follow the same area at the particle surface under various temperatures ramps (from 10 to 50°C) and/or relative humidities ramps (from 20 and 75%). Two milk powders were analysed : whole milk and skim milk powders (WMP and SMP respectively).

Particle surface evolution was followed for the two powders during storage events. Coupling AFM and XPS enable us to precisely identify lactose crystallisation event and/or surface fat evolution. It appears that crystal apparition was impacted by the nature of the powder (SMP or WMP), the temperature (under or above the glass transition temperature), the ramp kinetics (regular increase or increase by plateau). In comparison, crystallisation measurements with the dynamic vapor sorption (DVS) equipment could not be characterized so finely.

The AFM equipment equipped with a climatic chamber (T°C and RH control) and developed by the team was able to successfully mimic storage conditions (in the ranges: 10-50°C and 20-75RH%). This tool provides essential clues to better link particle surface evolution and functional properties. For the first time, *in situ* measurements were performed on milk powders and linked to storage events.

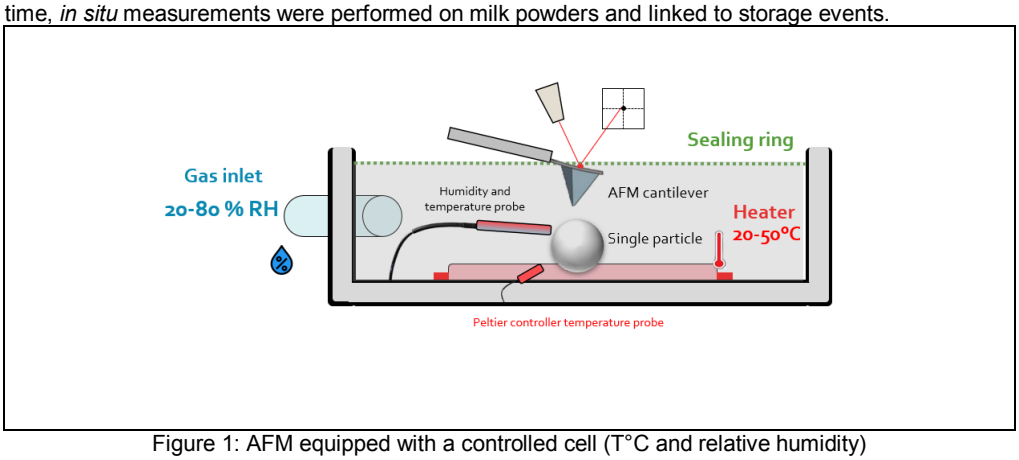

Figure 1: AFM equipped with a controlled cell (T°C and relative humidity)

Funds : AMUSE has received a three-year funding from Innovation Fund Denmark (January 1st, 2022 - December 31st, 2025).

Biofouling impact on the real time measurement of a portable conductivity sensor for water distribution systems

Fabienne Quilès,[1] Laurence Mathieu[1,2], Nathan Dijoux[1], Mariela Brites-Helu[1] Mathieu Anton[3], Hélène Lacroute[3], Grégoire Herzog[1]

[1]Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement, LCPME, UMR 7564, Villers-lès-Nancy, France
[2]EPHE, PSL, UMR CNRS 7564, LCPME, F-54500 Nancy, France.
[3] HYDRAO Smart and Blue, F-38000 Grenoble, France
e-mail: fabienne.quiles@univ-lorraine.fr

Monitoring of water quality is a concern for general public as well as for drinking water suppliers to provide good quality drinking water to customers. Conductivity tells us about the quality of water because it is a measurement of pollutants and salinity in the water, and unusual conductivity ranges are often indications of water pollution.

In this context, a compact instrumentation to be installed directly in small diameter pipes close to the end consumers is developed in this work. The sensitivity of electrochemical sensors is strongly dependent on the experimental conditions. One of the biggest challenges for in-situ monitoring is the biofouling of the sensor surface over time. Biofouling (bacterial adhesion to surfaces and further biofilm formation) is responsible for the deterioration of sensors' response over time.

As a first approach, the impact on the water conductivity measurement of bacterial adhesion and growth of a pure bacterial strain, *Pseudomonas fluorescens* that may be present in the drinking water distribution, was investigated through a combination of *ex situ* and *in situ* physical chemistry methods and microbiology analysis. Biofouling at different extend was obtained by exposure of the sensors to bacteria suspended in minimum media to mimic the poor nutrient status of drinking water. Series of conductimetry measurements at regular intervals for 24 hours were performed in presence or not of bacteria. After the measurements, both planktonic bacteria from the liquid medium and on the sensor surface were analysed. Such a monitoring process provides an *in situ* and in real-time information of the sensor integrity status as a function of the biofilm age and maturation.

Optical Nanomotion Detection to Discriminate Between Fungicidal and Fungistatic Effects of Antifungals on Single-Cell *Candida albicans*

Vjera Radonicic[1,2,3], Charlotte Yvanoff [1,3], Maria Ines Villalba[3,4], Bart Devreese[2,5], Sandor Kasas[3,4,6], Ronnie Willaert[1,2,3]

1. *Research Group Structural Biology Brussels, Vrije Universiteit Brussel, 1050 Brussels, Belgium*
2. *Alliance Research Group VUB-UGent NanoMicrobiology (NAMI), Vrije Universiteit Brussel, 1050 Brussels, Belgium*
3. *International Joint Research Group VUB-EPFL NanoBiotechnology & NanoMedicine (NANO), Vrije Universiteit Brussel, 1050 Brussels, Belgium*
4. *Laboratory of Biological Electron Microscopy, Ecole Polytechnique Fédérale de Lausanne (EPFL), University of Lausanne, 1015 Lausanne, Switzerland*
5. *Laboratory of Microbiology, Department of Biochemistry and Microbiology, Ghent University, 9000 Ghent, Belgium*
6. *Centre Universitaire Romand de Médecine Légale, UFAM, Université de Lausanne, 1015 Lausanne, Switzerland*

e-mail: vjera.radonicic@vub.be

Candida albicans is an emerging pathogen that poses a significant challenge due to its multidrug-resistant nature, leading to global healthcare expenses [1]. There are two types of antifungal agents, fungicidal and fungistatic, with distinct mechanisms of action against fungal pathogens. Fungicidal agents kill fungal pathogens, whereas fungistatic agents inhibit their growth. The growth could be restored once the agent is removed and favorable conditions are established once again. Recognizing this difference is crucial as it influences treatment selection and infection prognosis [2]. Our innovation involves the development of a microfluidic chip with an array of microwells designed to capture cells, conduct rapid Antifungal Susceptibility Testing (AFST), and differentiate between fungicidal and fungistatic drugs using optical nanomotion detection (ONMD). By trapping yeast cells in microwells, we facilitate a quick exchange of growth medium with antifungal agents (fluconazole and caspofungin), enabling ONMD measurements on individual cells before and after treatment. Fluconazole primarily acts by inhibiting ergosterol synthesis, disrupting fungal membrane integrity, impairing fungal growth, and conferring its fungistatic properties [3]. On the other hand, caspofungin, as a fungicidal antifungal agent, inhibits fungal cell wall synthesis by disrupting the cell wall glucan formation through the non-competitive inhibition of an enzyme complex 1,3-β-D-glucan synthase, leading to cell death [4]. Dynamic ONMD measurement during exposure to both antifungals followed by growth medium allowed to discriminate between the fungicidal and fungistatic antifungals. This label-free and straightforward approach holds promise for further development into a user-friendly device for rapid AFST, potentially becoming a routine procedure in hospitals worldwide, including both developed and developing nations.

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

Tiago Santos*†[1], Peter Hamar[2], Zsombor Lacza[2], and Pedro Granja[1]

[1]I3S/INEB – Portugal
[2]Semmelweis University [Budapest] – Hungary

Abstract

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.

Keywords: Protein corona, nanomedicine, flowcytometry, nanoparticles

*Speaker
†Corresponding author: tiago.f.santos@ineb.up.pt

HDL affinity for diverse lipid environments: Insights from ester and ether lipids

Florian Weber[1,2], Mariana Amaro[4], Markus Axmann[2], Martin Hof[4], Herbert Stangl[3], Taras Sych[1], Erdinc Sezgin[1] and Birgit Plochberger[2]

1 Science for Life Laboratory, Department of Women's and Children's Health, Karolinska Institutet, 17165 Solna, Sweden
2 University of Applied Sciences Upper Austria, Department of Medical Engineering, 4030 Linz, Austria
3 Medical University of Vienna, Center for Pathobiochemistry and Genetics, Department of Medical Chemistry, Vienna, Austria
4 J. Heyrovský Institute of Physical Chemistry of the Academy of Sciences of the Czech Republic, 182 00 Prague, Czech Republic
e-mail: florian.weber2@fh-linz.at

High-Density Lipoprotein (HDL) particles are one of several different transporters for lipophilic substances in the body. Besides the receptor-mediated cargo exchange, a direct, membrane-controlled process[1-5] is observed. Understanding the biophysical properties of the target membrane lipids (i.e. chain length, degree of saturation and binding of the glycerol region to the fatty acids) is essential to control the interaction between lipoproteins and the target membrane. We employed fluorescence microscopy and correlation spectroscopy, as well as General Polarization and time-dependent fluorescence shift analysis of membrane-incorporated Laurdan to examine HDL particle interaction with liquid disordered (L_d), liquid ordered (L_o), as well as gel phases with varying lipids. With phase-separated supported lipid bilayers with solely ester glycerol lipids (brain Sphingomyelin (bSM), DOPC, and Cholesterol (Chol) in a 2:2:1 molar ratio), HDL particles showed a pronounced preference for interaction with the L_d (DOPC) phase. We compared L_d phase lipids with gel phase lipids, and varied the linkage of the glycerol region as well as the fatty acid chain length. Notably, HDL particle interactions were weakest with ester gel phase lipids like 16:0 DPPC and were significantly enhanced with ether gel phase lipids, such as 16:0 DietherPC. This trend was weaker but consistent even when the fatty acid chain lengths was increased to 18:0 DietherPC, indicating a nuanced interplay between lipid structure and HDL particle affinity. Vesicles solely composed of ester lipids showed reduced cargo transfer compared to those with 25% ether lipids. We observed that a reduced glycerol region mobility in ester lipids corresponds to a lower HDL particle interaction. However, the high interaction with ether gel phase lipids, particularly 16:0 DietherPC, suggests a more complex underlying mechanism in HDL particle-lipid interactions. In summary, our study reveals intricate dynamics in HDL particle interactions with lipid membranes, highlighting the significant influence of lipid composition. We provide foundational insights for further scientific exploration into lipid-HDL particle interactions.

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Application of e-FluidFM in electrophysiology measurements

Mengjia Xu,[1] Livie Dorwling-Carter,[1] Edin Sarajlic,[2] Tomaso Zambelli,[1]

[1]*Laboratory of Biosensors and Bioelectronics, ETH Zürich, Switzerland*
[2]*SmartTip BV, Enschede, The Netherlands*
e-mail: xumen@ethz.ch

FluidFM is a force-controlled pipette, comprising a standard AFM with microchanneled cantilevers,[1] which makes it a versatile tool from single-cell biology to metal microprinting.

By inserting an electrode in the FluidFM channel and a reference electrode in the physiological solution of the petri dish (e-FluidFM), we combined AFM and scanning ion conductance microscopy (SICM) into a single technology.[2] This enabled simultaneous measurements of the ionic current and the cantilever deflection (force). We took advantage of the force signal to increase the scanning rate yet avoided unwanted interaction between the tip and cell membrane. Later we employed nanopore cantilevers with 15 nm diameter openings to study the effect of the aperture edge on the ion current imaging.[3] On the other side, we measured the ionic current of the electrical double layer, which gives us access to the local properties (e.g. surface charge) of the samples using the phenomenon of ion current rectification.[4]

When it comes to another highlighted electrophysiological property, the membrane potential of cells, the patch clamp technique remains the gold standard for measurements. Our e-FluidFM setup can be directly utilized as the patch clamp setup.[5] However, pyramidal tips seem not able to allow a stable and controlled tight seal interface between the cells and the probes (gigaseal), indispensable for an accurate measurement of the membrane potential. We are now testing different probe geometries, including cylindrical probes,[6] funnel probes and nanosyringes, as well as surface modifications. We are also combining the e-FluidFM with membrane potential dyes to acquire the force-controlled calibration curve between the fluorescent intensity and the absolute potential values.

References

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List of Participants

Summer School

&

Conference

NanoInBio 2024

Name		Email
Albuquerque Nogueira	Bernardo	bernardo.a.nogueira@inl.int
Alsteens	David	david.alsteens@uclouvain.be
Antosiewicz	Tomasz	tomasz.antosiewicz@fuw.edu.pl
Ayar Dulabi	Zahra	zahra.ayar@epfl.ch
Badin	Régis	regis.badin@univ-lorraine.fr
Beaumale	Jérôme	jerome.beaumale@bruker.com
Beaussart	Audrey	audrey.beaussart@u-bordeaux.fr
Bechinger	Burkhard	bechinge@unistra.fr
Bellanger	Xavier	xavier.bellanger@univ-lorraine.fr
Boudier	Ariane	ariane.boudier@univ-lorraine.fr
Caruso	Frank	fcaruso@unimelb.edu.au
Casalis	Loredana	loredana.casalis@elettra.trieste.it
Cenraud	Emilie	emilie.cenraud@univ-lorraine.fr
Chrzanowski	Wojciech	wojciech.chrzanowski@sydney.edu.au
Ciofani	Gianni	gianni.ciofani@iit.it
Coffinier	Yannick	yannick.coffinier@univ-lille.fr
Dague	Etienne	edague@laas.fr
Danti	Serena	serena.danti@unipi.it
Debruyne	Angela	angela.debruyne@ugent.be
Devenish	Benjamin	benjamin.devenish@durham.ac.uk
Dong	Mingdong	dong@inano.au.dk
Drewek	Isalyne	isalyne.drewek@umons.ac.be
Dupres	Vincent	vincent.dupres@ibl.cnrs.fr
Duro	Allan	Allan.duro@etu.univ-antilles.fr
El Kirat-Chatel	Sofiane	sofiane.el-kirat-chatel@u-bordeaux.fr
Fantner	Georg	georg.fantner@epfl.ch
Ferapontova	Elena	elena.ferapontova@inano.au.dk
Fernandez Busquets	Xavier	xfernandez@ibecbarcelona.eu
Ferraguzzi	Elena	elena.ferraguzzi@phd.units.it
Flewellen	James	james.flewellen@ed.ac.uk
Formosa-Dague	Cécile	formosa@insa-toulouse.fr
Francius	Grégory	gregory.francius@univ-lorraine.fr
Fukuma	Takeshi	fukuma@staff.kanazawa-u.ac.jp
Gaiani	Claire	claire.gaiani@univ-lorraine.fr
Genedy	Hussein	hussein.genedy@univ-angers.fr
Godier	Claire	claire.godier@univ-lorraine.fr
Gorelkin	Peter	pg@icappic.com
Gros	Olivier	Olivier.gros@univ-antilles.fr

Name		Email
Hackl	Thomas	hackl@acin.tuwien.ac.at
Hinterdorfer	Peter	peter.hinterdorfer@jku.at
Howard	Jordyn Ann	jordynann11@gmail.com
Jackman	Richard	r.jackman@ucl.ac.uk
Jierry	Loïc	ljierry@unistra.fr
Karpinski	Pawel	pawel.karpinski@pwr.edu.pl
Keyser	Ulrich	ufk20@cam.ac.uk
Khanji	Aya	aya.khanji@univ-lorraine.fr
Klopffer	Lucie	lucie.klopffer@univ-lorraine.fr
Korchev	Yuri	y.korchev@imperial.ac.uk
Labat	Béatrice	beatrice.labat@univ-rouen.fr
Lakey	Jeremy	jeremy.lakey@ncl.ac.uk
Largitte	Lucie	lucie.largitte@univ-antilles.fr
Lavalle	Philippe	philippe.lavalle@inserm.fr
Leclère	Philippe	Philippe.LECLERE@umons.ac.be
Lepicka	Kamila	klepicka@ichf.edu.pl
Leporatti	Stefano	stefano.leporatti@unisalento.it
Liepins	Andis	andis@applyit.lv
Malardé	Audrey	audrey.malarde@univ-lorraine.fr
Matczyszyn	Katarzyna	katarzyna.matczyszyn@pwr.edu.pl
Medeot	Caterina	caterina.medeot@phd.units.it
Melyon	Stacy	narayanin.s@hotmail.fr
Menglin	Chen	menglin@bce.au.dk
Mesquida	Patrick	Patrick.mesquida@kcl.ac.uk
Oh	Yoojin	yoo_jin.oh@jku.at
Olulana	Abimbola Feyisara	a.adedeji@sheffield.ac.uk
Park	Sungsu	nanopark@skku.edu
Portalupi	Marco	portalupi@nanosurf.com
Potiron	Grégory	
Puricelli	Luca	luca.puricelli@elettra.eu
Quiles	Fabienne	fabienne.quiles@univ-lorraine.fr
Radonicic	Vjera	vjera.radonicic@vub.be
Rivera Gil	Pilar	pilar.rivera@upf.edu
Robb	Nicole	nicole.robb@physics.ox.ac.uk
Rondelli	Valeria	valeria.rondelli@unimi.it
Roupioz	Yoann	yoann.roupioz@cea.fr
Salazar Ariza	Juan	juan.salazar-ariza@univ-lyon1.fr
Santos	Tiago	tiago.f.santos@ineb.up.pt

Name		Email
Schaaf	Pierre	schaaf@unistra.fr
Scher	Joel	joel.scher@univ-lorraine.fr
Seth	Malobi	malobi.seth@univ-lorraine.fr
Seuss	Maximilian	maximilian.seuss@bruker.com
Sittl	Sebastian	sebastian.sittl@uni-bayreuth.de
Sokolov	Igor	igor.sokolov@tufts.edu
Stylianou	Andreas	an.stylianou@euc.ac.cy
Sviderskaya	Elena	esviders@sgul.ac.uk
Tabrizian	Maryam	maryam.tabrizian@mcgill.ca
Tormena	Nicolo	nicolo.tormena@durham.ac.uk
Voitchovsky	Kislon	kislon.voitchovsky@durham.ac.uk
Weber	Florian	florian.weber2@fh-linz.at
Xu	Mengjia	xumen@ethz.ch
Xu	Yue	y549xu@uwaterloo.ca
Xu	Bing	bxu@brandeis.edu
Zambelli	Tomaso	ztomaso@ethz.ch
Zirbs	Ronald	ronald.zirbs@boku.ac.at

April 14	April 15	April 16	April 17	April 18	April 19	April 20
9.30am – 12.30pm Spring School	**8.45am – 12.20pm** Conference session I: The bio/non-bio interface	**9am – 12.10pm** Conference session III: Bio- and nano-electrochemistry & bio applications	**7 am – 5.30pm :** Social Event	**8.40am – 12.10pm** Conference session V: Nanomedicine, Nano-Micro Biology	**8.45am – 12.20pm** Conference session VII: The bio/non-bio interface	**8.40am – 12.10pm** Conference session IX: Special NanoInBio session
Lunch				Lunch		
2pm – 4.30pm Spring School	**2pm – 5.10 pm** Conference session II: Instrumentation for bio- & nanotechnologies	**2pm – 5.30pm** Conference session IV: Bio- and nano-materials for health & nanomedicine		**2pm – 5.10pm** Conference session VI: Bio- and nano-electrochemistry & bio applications	**2pm – 5.30 pm** Conference session VIII: Instrumentation for bio- & nanotechnologies	
	5.10pm – 6.30pm Poster session I			**5.20pm – 6.30pm** Poster session II	**7.30 pm:** Gala dinner	