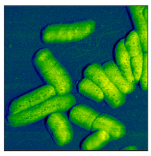


TIME TO SHARE
An Insight Into Bacteria

October 27th, 2020 | 6pm CET | 10:00am PDT - Online Microscopy Symposium

Scope of the symposium

We at Bruker JPK BioAFM are delighted to invite you to join us and a distinguished panel of experts to an international mini-symposium: An Insight into Bacteria.

There is growing recognition that a multidisciplinary approach is crucial to unravelling the complex biomolecular mechanisms involved in the interactions between pathogens and host cells, microbe and membrane, and how these interactions influence the autophagic response.

Each of our speakers has an interdisciplinary background, and during this short symposium will provide exciting new insights into topics such as combining AFM with advanced genetic manipulation to investigate the nanomechanical properties of proteins at the single-cell and single-molecule level and protein involvement in microbial adhesion, mechanosensing and biofilm-associated infections. Other topics include the viscoelastic response of cells to mechanical stress during interactions with pathogens, and high-resolution AFM imaging of live bacteria, their cell envelopes and membrane proteins at molecular resolution, and even the perforation of the outer membrane by immune proteins.

Learn how Atomic Force Microscopy can provide new insights into your life science research!

Program – Tuesday, October 27th, 2020

Chair: Heiko Haschke, Head of Applications, JPK BioAFM, Bruker Nano GmbH

6:00 pm **Welcome address**
Carmen Pettersson, Senior Product Manager, JPK BioAFM, Bruker Nano GmbH

6:10 pm **AFM: From High-Content to High-Throughput Analysis - Applied to Infection**
Prof. Frank Lafont, Director of Research CIIL, Institut Pasteur de Lille, France

6:40 pm **Atomic Force Microscopy: A Window to the Nanomechanics of Proteins**
Marion Mathelié-Guinlet, Louvain Institute of Biomolecular Science and Technology, UCLouvain, Belgium

7:10 pm **AFM on Live Bacteria at Molecular Resolution**
Bart Hoogenboom, Department of Physics and Astronomy and the London Centre for Nanotechnology, University College London, UK

7:40 pm **Open forum discussion**
Heiko Haschke

7:55 pm **Closing**
Carmen Pettersson

Talk abstracts

6:10 pm – AFM from High-Content to High-Throughput Analysis – Applied to Infection

The focus of Frank Lafont's work has been on developing a multidisciplinary approach to combining medicine, biology and biophysics in order to gain a comprehensive understanding of the interactions between pathogens and host cells and tissues, and in particular, how these interactions influence the autophagic response. In biophysics, his focus of interest is on microbe/membrane interactions and the viscoelastic response of cells to mechanical stress upon interaction with pathogenic agents. He has initiated methods such as the Correlative Light Atomic Force Electron Microscopy (CLAFEM) and the stiffness tomography in living cells and now is involved in the automation of AFM. In his talk, he will present an overview of his work in this area.

Speaker: **Prof. Frank Lafont**
Director of Research, CNRS Institut Pasteur de Lille, France

Prof. Frank Lafont *was trained in medicine, biology, and management in Paris. He received his PhD in developmental neurobiology from the Ecole Normale Supérieure, Paris, and did a postdoc in cell biology at the EMBL, Heidelberg, Germany. He lectured on cellular microbiology at the Biochemistry & Medical Center, University of Geneva, and collaborated on Biophysics at EPFL, Lausanne, Switzerland. Lafont is currently Director of Research at the Center of Infection and Immunity of Lille, Pasteur Institute Lille, and is head of the BioImaging Center Lille.*

6:40 pm - Atomic Force Microscopy: A Window to the Nanomechanics of Proteins

Living cells constantly interact with their environment *via* their surface, which exhibits sophisticated functions, most notably mediated by proteins. The spatial organization and functional roles of proteins within the cell or interacting with their surroundings are crucial, yet largely unsolved, questions in cell biology. In the past decades, atomic force microscopy (AFM) has evolved into a multifunctional toolbox enabling the imaging and manipulation of cell surfaces and components with molecular resolution. Combining AFM with advanced genetic manipulation, we have greatly contributed to the elucidation of the nanomechanical properties of proteins within the context of microbial adhesion and mechanosensing. This talk will first focus on the key role of proteins in the overall mechanical behaviour of the bacterial envelope, the first target of various antibiotics. In the pathogen *E. coli*, we unraveled the dual role of the Braun's lipoprotein Lpp in defining cell envelope stiffness and drug sensitivity. We will then address the key role of proteins in the force-driven microbial adhesion

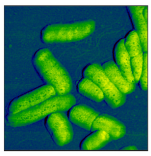
involved in biofilm-associated infections. We recently provided the first quantitative demonstration of a catch-bond in living Gram-positive pathogens using AFM force-clamp spectroscopy. We showed that the dock, lock and latch stress-enhanced interaction between *S. pseudintermedius* SpsD and fibrinogen is extremely strong (~ 2 nN), and the bond lifetime first grows with force (catch bond) before ultimately decreasing (slip bond). Such shear-stress dependent behavior provides pathogens with a mechanism to tightly control its adhesive function during colonization and infection. Finally, we will present the great opportunities offered by the FluidFM, a single-cell manipulation assay combining fluidic force microscopy with force spectroscopy, in the context of *S. cerevisiae* aggregation during yeast mating promoted by specific proteins.

Speaker: Marion Mathelié-Guinlet
Postdoctoral researcher at Louvain Institute of Biomolecular Science and Technology, UCLouvain, Belgium

Dr. Marion Mathelié-Guinlet *received both her multidisciplinary engineering degree from Centrale Lyon, France, and her research master degree in astrophysics from the University of Manchester, UK, in 2014. She received her PhD in nanosciences at the University of Bordeaux (France) in 2017. Combining theoretical knowledge and experimental approaches, she has developed a strong expertise in the (bio-)physics and physico-chemistry of interfaces. She is currently a postdoctoral researcher at the UCLouvain, Belgium, in Yves Dufrêne's group. She is interested in understanding the nanomechanics of proteins at the single-cell and single-molecule level and using atomic force microscopy, in the context of bacterial adhesion and mechanosensing.*

7:10 pm - AFM on Live Bacteria at Molecular Resolution

For a long time, biomolecular-resolution AFM imaging was largely restricted to purified proteins and nucleic acids adsorbed on solid supports, while the imaging of live cells presented various challenges making it difficult to achieve such resolution. The cell envelope of Gram-negative bacteria, however, represents a stable background for AFM imaging at a resolution of the order of 1 nm. Using complementary fluorescence microscopy, it is also possible to ascertain whether the bacteria being imaged are dead or alive. In my talk, I will report on how we use AFM to resolve outer membrane proteins on *E. coli*, as well as the perforation of the outer membrane by immune proteins of the complement system in serum.



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Speaker: Prof. Dr. *Bart Hoogenboom*:

Professor of Biophysics, Department of Physics and Astronomy and the London Centre for Nanotechnology, University College London, UK

Bart Hoogenboom *is a Professor of Biophysics at the Department of Physics and Astronomy (UCL) and the London Centre for Nanotechnology, where he is also lead scientist for its atomic force microscopy facilities. He was initially trained as a solid-state physicist, working on correlated-electron systems and scanning probe microscopy. After his PhD at the University of Geneva, Switzerland, he pioneered atomic-resolution AFM in solution and next gradually shifted his focus to nanoscale biological structures and processes. At UCL, this has led to the first visualisation of the DNA double helix and structural variations thereof in solution; the development of novel nanomechanical and computational approaches to understand the physics of transport selectivity into and out of the cell nucleus via nuclear pore complexes; the understanding of membrane disruption by various natural and engineered antimicrobial peptides and by pore forming proteins employed both by bacteria and by the vertebrate immune system, which has in part involved high-resolution AFM imaging of live bacteria.*

Please don't hesitate to contact us at events.bioafm@bruker.com if you have any questions.