8th AFM BioMed Conference

Kraków, Poland
4–8 September 2017
Previous Meetings

AFMBiomed 2017  Cracow (Kraków), Poland
AFMBiomed 2016  Porto, Portugal
AFMBiomed 2014  San Diego, USA
AFMBiomed 2013  Shanghai, China
AFMBiomed 2011  Paris, France
AFMBiomed 2010  Red Island (Rovinj), Croatia
AFMBiomed 2008  Monterey, California, USA
AFMBiomed 2007  Barcelona, Spain
Welcome address

It is our great pleasure to invite you to the 8th International Conference on AFM for Life Sciences and Nanomedicine (AFM BioMed 2017) to be held in Kraków, Poland, from 4th to 8th September 2017. The Conference co-organizers are the Institute of Nuclear Physics of the Polish Academy of Sciences and the Jagiellonian University.

The conference will provide a forum for colleagues from both academia and research institutions throughout the World to exchange results and new ideas on use of atomic force microscopy (AFM) and related techniques in life sciences and medicine ranging from cell or molecular biology to clinical applications. The conference will cover advancements of scientific knowledge from fundamentals to applications. Discussions on present possibilities and future developments of the technique, in view of the future requirements coming from technology and basic research, will be particularly solicited also with dedicated discussion time.

The program of the Conference will be organized into 4 topics dedicated to Cellular Mechanobiology, Bioimaging, Molecular Forces, and Health & Disease.

We cordially invite you to attend AFM Biomed 2017 in Krakow. We hope that you will enjoy not only the scientific atmosphere during the conference, but also a beautiful city with its outstanding cultural heritage and current cultural activities!

Małgorzata Lekka & Marek Szymoński
AFM Biomed Conference, *Krakow 2017*

**Conference Venue**

*Jagiellonian University, Faculty of Law, Krupnicza 33a*

*View from the street*
Conference Chairs

Małgorzata Lekka  
Marek Szymoński  

Organizing Committee

Institute of Physics, Jagiellonian University, Cracow, Poland

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Session Chairs

Martin Guthold  
Wake Forest University, USA  

Alessandro Podesta  
University of Milan, Italy  

Ewa Wójcikiewicz  
Florida Atlantic University, USA  

Wouter H. Roos  
University of Gröningen, Netherlands

Invited Speakers

David Alsteens  
University Catholique de Louvain, Belgium  

James K. Gimzewski  
University of California, Los Angeles, USA  

Martin Guthold  
Wake Forest University, USA  

Sandor Kasas  
École Polytechnique Fédérale de Lausanne, Switzerland  

Andrzej J Kulik  
École Polytechnique Fédérale de Lausanne, Switzerland  

Wiesław Nowak  
Nicolaus Copernicus University, Torun, Poland  

Alessandro Podesta  
University of Milan, Italy  

Manfred Radmacher  
Bremen University, Germany  

Felix Rico  
French National Institute of Health and Medical Research, Aix-Marseille Université, France  

Wouter H. Roos  
University of Gröningen, Netherlands  

Igor Sokolov  
Tufts University, USA  

Ewa Wójcikiewicz  
Florida Atlantic University, USA  

Renato Zenobi  
Swiss Federal Institute of Technology in Zurich, Switzerland
Scientific Program

Monday, 4.09.2017
18:00                Registration and welcome reception

Tuesday, 5.09.2017
09:00 – 09:30 Welcome address

09:30 – 13:00 Session: Bioimaging I
Session chair – Alessandro Podesta, Italy

Invited lecture:
09:30 – 10:00 Wouter H. Roos, Netherlands
“Mechanics and Dynamics at the Sub-Cellular Level”

Oral presentations:
10:00 – 10:20 Anna Rygula, Poland
“Multifunctional imaging of endothelial cells with SNOM, AFM and Raman spectroscopy”

10:20 – 10:40 Maria Ortega, Spain
“Structural Stability of Cu-Azurin in SPM experiments: insights from MD simulations”

10:40 – 11:00 Joanna Raczkowska, Poland
“Bio-interfaces of polymer coatings inducing controlled interactions with cells”

Coffee break 11:00 – 11:30

Invited lecture:
11:30 – 12:00 Andrzej J. Kulik, Switzerland
“Nanoscale chemical analysis - AFM-IR”

Oral presentations:
12:00 – 12:20 Elena Kozlova, Russia
“AFM studies of molecular mechanisms of the arising of topological nanodefects of RBC membranes under the action of toxins and storage of donor blood”

12:20 – 12:40 Sungjio Park, Korea
“Fractal assembly of biomolecules resolved by atomic force microscopy”

12:40 – 13:00 Sevil Ozer, Turkey
“Development of an AFM with Dual Actuation Capability for Biomolecular Measurements”

Lunch 13:00 – 14:30
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14:30 – 18:00  Session: Health & Disease I

Session chair – Ewa Wójcikiewicz, USA

Invited lecture:
14:30 – 15:00  James K. Gimzewski, USA
“Atomic force microscopy as a nanoanalytical tool for exosomes and other extracellular vesicles”

Oral presentations:
15:00 – 15:20  Daniel Navajas, Spain
“Micromechanics of brain tissue measured with atomic force microscopy”
15:20 – 15:40  Marek Haftek, France
“Probing the stratum corneum: cell surface nanomechanical properties change according to the tissue depth”
15:40 – 16:00  Delphine Sicard, USA
“Lung tissue stiffness: study on aging and AFM tip size and geometry effects”

Coffee break 16:00 – 16:30

Invited lecture:
16:30 – 17:00  Sandor Kasas, Switzerland
“Nano-motion based detection of living micro-organisms”

Oral presentations:
17:00 – 17:20  Nuno C. Santos, Portugal
“Identification of the determinants for dengue virus capsid protein binding to host lipid droplets and plasma lipoproteins”
17:20 – 17:40  Marta Targosz-Korecka, Poland
“Glycocalyx degradation coincides with endothelium stiffening during progression of diabetes in db/db mice - an ex vivo AFM nanoindentation study”
17:40 – 18:00  Simone Dinarelli, Italy
“AFM nano-mechanical study of the beating profiles of hiPSC-derived cardiomyocytes beating bodies WT and DM1”

Poster Session I  18:00 – 19:00

19:30  Dinner sponsored by Bruker, in “Kawaleria” Restaurant, ul. Gołębia
Wednesday, 6.09.2017

09:30 – 13:00  Session: Molecular Forces I

Session chair – Martin Guthold, USA

Invited lecture:
09:00 – 09:30  **David Alsteens, Belgium**
“Nanomechanical Mapping of Virus Binding Sites to Animal Cells”

Oral presentations:
09:30 – 09:50  Ana Sancho, Germany
“Intercellular adhesion forces in Endothelial-to-Mesenchymal Transition”

09:50 – 10:10  Arkadiusz Ptak, Poland
“Dynamic Force Spectroscopy: Comparison of competing models of thermally activated unbinding under external force”

10:10 – 10:30  Carlos Alvarez Amo, Spain
“Fundamental High Speed Limits in Single –Molecule and Nanoscale Force Spectroscopies”

10:30 – 10:50  Martin Pesl, Czech Republic
“Simultaneous analysis of cardiomyocyte contractions by Atomic force microscopy and calcium imaging”

Coffee break 11:00 – 11:30

Invited lecture:
11:30 – 12:00  **Wiesław Nowak, Poland**
“Single Molecule AFM Force Spectroscopy – a theoretical perspective”

Oral presentations:
12:00 – 12:20  Ramin Omidvar, Germany
“Reconstitution of initial steps of lectin-driven bacterial uptake into cells”

12:20 – 12:40  Isabell Tunn, Germany
“Coiled coils as mechanical building blocks”

12:40 – 13:00  Svetlana Pleskova, Russia
“Atomic force microscopy in research of bacteria interactions with the blood neutrophils”

Lunch 13:00 – 14:30
14:00 – 15:40  
**Session: Cellular Mechanobiology I**

**Session chair – Wouter H. Roos, Netherlands**

**Invited lecture:**

14:30 – 15:00  
**Ewa Wójcikiewicz, USA**

“Role of pro-inflammatory mediators in cell metastatic progression”

**Oral presentations:**

15:00 – 15:20  
Filomena A. Carvalho, Portugal

“Role of fibrinogen-erythrocyte and erythrocyte-erythrocyte adhesion on cardiovascular pathologies”

15:20 – 15:40  
Vincent Dupres, France

“Influence of autophagy on cell mechanics probed by stiffness tomography and super-resolution microscopy”

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**Visiting and conference dinner at Wieliczka salt mine**

16:30 departure from Krupnicza street 33A (Conference venue)

17:30 – 18:30 visiting salt mine

19:00 – 21:30 dinner
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Thursday, 7.09.2017

09:00 – 13:00  Session: Cellular Mechanobiology II

Session chair – Wouter H. Roos, Netherlands

Invited lecture:

09:00 – 09:30  Manfred Radmacher, Germany
“Measuring viscoelastic properties of cells by AFM”

Oral presentations:

09:30 – 09:50  Massimiliano Galluzzi, China
“AFM experiments and simulations of heterogeneous soft-matter: analysis and biological applications”

09:50 – 10:10 Ignasi Jorba, Spain
“Extracellular Matrix Micromechanics of Regenerating Zebrafish Heart”

10:10 – 10:30 Carlos Guerrero Rodriguez, Spain
“Time-resolved nanomechanical rheology of a single cell under the depolymerization of the actin cytoskeleton”

10:30 – 10:50 Hermann Schillers, Germany
“The unholy alliance of platelets and tumor cells”

Coffee break 11:00 – 11:30

Invited lecture:

11:30 – 12:00 Alessandro Podesta, Italy
“The interaction of room-temperature ionic liquids (ILs) with cells and model biological membranes: investigating the basic mechanisms of ILs cytotoxicity”

Oral presentations:

12:00 – 12:20 Agnieszka Kołodziejczyk, Poland
„Sensing of silver nanoparticles on/in endothelial cells using atomic force spectroscopy”

12:20 – 12:40 Andrea Mescola, Italy
“AFM investigation of mechanical properties of glioblastoma multiforme cells and their relation to motility”

12:40 – 13:00 Janusz Strzelecki, Poland
“Extraction of corneal micromechanics with AFM nanoindentation using ex vivo porcine eye models and intraocular pressure control”

Lunch 13:00 – 14:30
**Invited lecture:**

**14:30 – 15:00**

**Renato Zenobi, Switzerland**

“**Tip-Enhanced Raman Spectroscopy for Nanoscale Chemical Analysis and Imaging**”

**Oral presentations:**

**15:00 – 15:20**

Maria-Astrid Schröter, Germany

“**Structural changes in plasmid DNA caused by radiation and its protection by Ectoine: an AFM analysis**”

**15:20 – 15:40**

Andreas Stylianou, Cyprus

“**Normal and Cancer Associated Pancreatic Fibroblasts Shape Modulation is mediated by Transforming Growth Factor β and Matrix Stiffness**”

**15:40 – 16:00**

Helena Lozano Caballero, Spain

“**Electrical and morphological characterization of bacterial polar flagella**”

**Coffee break 16:00 – 16:30**

**Oral presentations:**

**16:30 – 16:50**

Alexander Dulebo (Bruker), USA

“**Observing cell motility using an AFM – how fast can we go?**”

**16:50 – 17:10**

Sourav Maity, Netherlands

“**Studying viral disassembly by High Speed Atomic Force Microscopy**”

**17:10 – 17:30**

Ewelina Lipiec, Poland

“**Nano-spectroscopic mapping of DNA molecule**”

**17:30 – 17:50**

Maria Starodubtseva, Belarus

“**AFM-based physical-mechanical image of the cell surface**”

**17:50 – 18:10**

Ioana Dobra, France

“**AFM imaging reveals the role of mRNA binding proteins in the formation of pathological protein aggregates**”

**Poster Session II** 18:15 – 19:45
Friday, 8.09.2017

09:00 – 13:00 Session: Health & Disease II

Session chair – Ewa Wójcikiewicz, USA

Invited lecture:
09:00 – 09:30 Martin Guthold, USA
“Molecular Forces and Nanomechanical Measurements in Blood Coagulation”

Oral presentations:
09:30 – 09:50 Sanjay Kumar, USA
“Reversible enzymatic actuation of an intrinsically disordered protein brush”

09:50 – 10:10 Alexandre Berquand, France
“Investigating the influence of LRP-1 silencing on the migratory potential of MDA cancer cells by dynamic cell studies and atomic force microscopy”

10:10 – 10:30 Bartłomiej Zapotoczny, Poland
“Quantification of Fenestrations in Liver Sinusoidal Endothelial Cells by Atomic Force Microscopy”

10:30 – 10:50 Stefanie Kiderlen, Germany
“Young’s Modulus and Mechanotransduction in Actinin 1-Knockout Fibroblasts”

Coffee break 11:00 – 11:30

Invited lecture:
11:30 – 12:00 Igor Sokolov, USA
“Physics of cells and cancer detection with AFM”

Oral presentations:
12:00 – 12:20 Patrick Mesquida, Austria
“Kelvin-probe Force Microscopy to map glycation of proteins”

12:20 – 12:40 Yuri Efremov, USA
“Integrated Spinning Disk Confocal and Atomic Force Microscopy Reveals Anisotropic Indentation Geometry on Living Cells”

12:40 – 13:00 Joanna Zemła, Poland
“Nanomechanics of cells and polymer gels probed with different AFM indenter shapes”

Lunch 13:00 – 14:30
14:30 – 17:00  
**Session: Molecular Forces II**  
*Session chair – Martin Guthold, USA*

**Invited lecture:**  
14:30 – 15:00  
**Felix Rico, France**  
"Molecular to cellular mechanics probed by high-speed force spectroscopy"

**Oral presentations:**  
15:00 – 15:20  
Claire Valotteau, Belgium  
"Molecular mechanisms guiding the adhesion of *Staphylococcus aureus* clumping factor B to the skin protein loricrin"

15:20 – 15:40  
Monika Österberg, Finland  
"Probing forces between stem cells and biomaterials"

15:40 – 16:00  
Thi Huong Nguyen, Germany  
"A New Mechanism of Autoimmune HIT Caused by a Subset of Antibodies"

Coffee break 16:00 – 16:30

**Oral presentations:**  
16:30 – 16:50  
Riina Harjumäki, Finland  
"Interactions of Xenobiotic-free Biomaterials and Cells for Better Tissue Models"

16:50 – 17:10  
Joanna Danilkiewicz, Poland  
"Molecular forces in interaction of bladder cancerous cells with syndecan-1 and syndecan-4"

17:10 – 17:30  
Hamdi Torun, Turkey  
"Miniaturized Magnetic Beads for Singlemolecule Force Measurements"

17:30 – 17:50  
Fidan Sumbul, France  
"Effect of cantilever response time on single molecule unfolding forces"

**Closing session**  
18:00  
*Presentation and poster awards for young scientists*
Invited lectures

I1 Wouter H. Roos, Netherlands
“Mechanics and Dynamics at the Sub-Cellular Level”

I2 Andrzej J. Kulik, Switzerland
“Nanoscale chemical analysis - AFM-IR”

I3 Sandor Kasas, Switzerland
“Nano-motion based detection of living micro-organisms”

I4 James K. Gimzewski, USA
“Atomic force microscopy as a nanoanalytical tool for exosomes and other extracellular vesicles”

I5 David Alsteens, Belgium
“Nanomechanical Mapping of Virus Binding Sites to Animal Cells”

I6 Wiesław Nowak, Poland
“Single Molecule AFM Force Spectroscopy – a theoretical perspective”

I7 Ewa Wójcikiewicz, USA
“Role of pro-inflammatory mediators in cell metastatic progression”

I8 Manfred Radmacher, Germany
“Measuring viscoelastic properties of cells by AFM”

I9 Alessandro Podesta, Italy
“The interaction of room-temperature ionic liquids (ILs) with cells and model biological membranes: investigating the basic mechanisms of ILs cytotoxicity”

I10 Renato Zenobi, Switzerland
“Tip-Enhanced Raman Spectroscopy for Nanoscale Chemical Analysis and Imaging”

I11 Martin Guthold, USA
“Molecular Forces and Nanomechanical Measurements in Blood Coagulation”

I12 Igor Sokolov, USA
“Physics of cells and cancer detection with AFM”

I13 Felix Rico, France
“Molecular to cellular mechanics probed by high-speed force spectroscopy ”
Mechanics and dynamics at the sub-cellular level

Wouter H Roos

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1 Zernike Instituut, Rijksuniversiteit Groningen, Groningen, the Netherlands

Cellular life harbours a fascinating variety of complex processes and we are still at the beginning of our understanding of how the cell manages all these processes. Using Atomic Force Microscopy imaging and force spectroscopy we are now able to make big steps forward in elucidating the mechanisms behind (supra)-molecular cellular processes. In this presentation I show how in my lab we are studying the physics of this fascinating sub-cellular dynamics. I will illustrate this by discussing the mechanics and material properties of viruses and cellular protein nanocages; In particular by revealing the existence of pre-stress in nanoshells, by scrutinizing the interactions between viral RNA and its capsid and by showing how viral infectivity is in essence a mechanical process [1-3]. Furthermore, I will discuss recent studies on viral assembly and disassembly. Finally, I will show our work on extracellular vesicle dynamics, currently a hot topic in nanomedicine as these particles are expected to have great potential in diagnosis and treatment [4,5].

References:
Development of Infrared (IR) tunable laser sources and AFM based detection of thermo-mechanical effect, allows one to measure IR spectra and/or acquire chemical images with spatial resolution of 10 nm. The latest developments increased sensitivity of the method, which allows one to measure monomolecular thin films. In parallel, operation in tapping mode extends the field of applications to polymer and bio-oriented research. Finally, fast measurements were implemented, so spectrum can be acquired within 100 ms.

Talk will be illustrated with the latest results.

References:
Nano-motion based detection of living micro-organisms

S. Kasas¹, ², P. Stupar¹, W. Chomicki¹ and G. Dietler¹

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¹ Laboratoire de Physique de la Matière Vivante, EPFL, 1015 Lausanne, Switzerland
² Plateforme de Morphologie, Université de Lausanne, Lausanne, Switzerland

We recently developed a device that permits to characterize bacterial resistance to antibiotics in minutes instead of days or weeks [1]. The apparatus is based on atomic force microscopy (AFM) technology and has been successfully tested in our laboratory with gram positive and negative bacteria. The technique consists in depositing bacteria [2] onto an AFM cantilever and in monitoring its motion as a function of time as depicted in Figure 1. We recently demonstrated that the technique can also be applied to virtually all living micro-organisms on Earth. Since the working principle of the instrument is very simple miniaturization and massive parallelization can further reduce the screening delays and costs permitting wide implementation of the device in hospitals. During the presentation numerous microbiological applications of the device will be presented.

Figure 1 Living cells such as bacteria (in green) are attached to an AFM cantilever and induce nanometer scale oscillations.

Acknowledgments

We thank the Swiss National Science Foundation (Grant Nr. 200021_144321 and 407240_167137) and the Gebert Rüf Foundation (Grant Nr. GRS-024/14) for support.

References:


Atomic Force Microscopy as a nanoanalytical tool for exosomes and other extracellular vesicles

James K. Gimzewski FRSc

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Scanning probe microscopy opened a new era of microscopic possibilities in the field of biology and medicine over 30 years ago. Recently, attention has turned to a class of subcellular vesicles called exosomes and other extracellular vesicles (EVs) for their potential use as novel circulating biomarkers for diseases such as cancer and several neurodegenerative diseases and for their potential use in mesenchymal stem cell therapies. Historically, exosomes were first discovered in the 80s in mammalian reticulocyte cells, where transferrin receptors (labeled with gold nanoparticles) were found on the outside of small vesicles contained within endosomes and released into the extracellular medium. The presence of transferrin receptors on the outside of these exosomes suggested inversions of the cell membrane, which lead to the currently accepted mechanism for the formation of exosomes. While once thought to be cellular trash, exosomes were discovered to contain protein, m-RNA, and surface receptors that opens a new field of study into their specific physiological functions. Current challenges impeding the use of exosomes in biomarkers and therapy include tumor origin specificity, the number of exosomes that can be obtained from samples, and the need for a renewable cell source for widespread therapeutic application. Nonetheless, analysis of exosomes and other extracellular vesicles in body fluids could in future be the standard diagnostics in medicine.

References:


Nanomechanical Mapping of Virus Binding Sites to Animal Cells

David Alsteens
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Currently, there is a growing need for methods that can quantify and map the molecular interactions of biological samples, both with high-force sensitivity and high spatial resolution. Force-distance (FD) curve-based atomic force microscopy is a valuable tool to simultaneously contour the surface and map the biophysical properties of biological samples at the nanoscale. We 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 an engineered virus and cell surface receptors. Owing to its key capabilities (quantitative mapping, resolution of a few nanometers, and true correlation with topography), this novel biochemically sensitive imaging technique is a powerful complement to other advanced AFM modes for quantitative, high-resolution bioimaging.

Figure. Combination of AFM and fluorescence microscopy to map virus binding sites on living mammalian cells

References: (no more than 5, use Arial 10 pts, single line spacing)
Single Molecule AFM Force Spectroscopy - a Theoretical Perspective

W. Nowak

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Single molecule nanomechanics, critical in many biological functions, is studied using the Atomic Force Microscopy (AFM). It has been known for many years that the AFM recorded Force Spectra (FS), i.e. force-distance curves, may be calculated using theoretical methods, such as the Steered Molecular Dynamics methods (SMD), as well [1]. The qualitative features of FS are usually reproduced well in computer experiments, this helps to understand a mechanism of biomolecule unfolding/stretching and numerous papers have been published (see ref. in [1-5]). However, a quantitative agreement between computed and measured FS is still far from being perfect [2]. Showing results of our numerous simulations of DNA stretching [6] and FS/SMD studies of modular proteins present in axons and synaptic junctions (contactin, neurexin) [3-5] and related to Autism Spectrum Disorders we will discuss how difficult it is to reach a reasonable quantitative matching between standard, fast SMD simulations and typical slow AFM experiments. A faster AFM FS and long-time, slow SMD bring a new hope for biophysics but that new technology is still not so popular. Perhaps, despite all limitations, coarse grained approaches to FS SMD simulations [4] are the most promising and practical path to go.

Acknowledgements: Support from a SCIEX grant, Polish Funds for Science (grants No. N202 262038, 2012/05/N/ST3/03178 and nationwide license for Accelrys software) and National Science Centre (WN) is acknowledged. Calculations were performed at ICNT UMK in Torun and at the Computational Center TASK in Gdansk (PL).

References:
Role of pro-inflammatory mediators in cell metastatic progression

E.P. Wojcikiewicz¹, J. Jaczewska¹, S. Dutta¹

¹ Charles E. Schmidt College of Medicine, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33434

Cancer cell metastasis involves cell detachment from the primary tumor, migration through the extracellular matrix (ECM), the basement membrane of the endothelium, into the bloodstream, and subsequent extravasation into the surrounding tissues. Cell to cell and cell to ECM interactions are altered throughout this complex process. We know that cancer cells are softer than normal cells [1, 2]. This biophysical change likely facilitates cell adhesion and migration. Further evidence is mounting that cell stiffness changes may induce changes in DNA transcription through transcriptional coactivators [3]. Our studies investigated the role of pro-inflammatory mediators in promoting these pro-metastatic biophysical changes in mammary epithelial cells. Under inflammatory conditions, extracellular receptors, Junctional Adhesion Molecule-C (JAM-C) and tumor necrosis factor-alpha (TNF-alpha) are cleaved from the cell surface. Numerous animal studies have revealed that both soluble receptors are pro-inflammatory. Our results indicate that the presence of these soluble receptors leads to an over five-fold decrease in cell stiffness of normal mammary epithelial cells (shown in Fig. 1). In turn, these changes result in enhanced cell adhesion as measured by single cell force spectroscopy. The SCFS measurements reveal adhesion of normal mammary epithelial cells to be comparable to that of very invasive cancer cell lines. Our studies provide detailed insight into how pro-inflammatory mediators initiate biophysical changes, which will be useful in the development of pharmacological agents to selectively target them.

Figure 1. AFM cantilever positioned above normal mammary epithelial cells.

References:

We have investigated thyroid cancer cells and different fibroblast cells by atomic force microscopy. Both cell types were plated on standard Petri dishes and on soft polymer gels, whose stiffness is closer to the normal environment, the tissue, of these cells. For both cell types we have measured the viscoelastic creep response by applying a force step in contact with the cells. Cancer cells were compared to normal cells of the same type, where we have found that on stiff Petri dishes cancer cells appear softer, however on the soft polymer gels the normal cells are much stiffer, so that the difference between the two cell types becomes very minor. This suggests, that normal cells do adopt their stiffness to stiffness of the environment, whereas cancer don't do that. As a consequence, cancer cells tend to be stiffer than normal when being plated on very soft gels.

In addition, we have compared different fibroblast cell lines established from human skin tissue samples. We have investigated normal fibroblasts, scar fibroblasts, and Dupuytren fibroblasts, all from the same patient. Dupuytren fibroblasts, which are differentiated myofibroblasts turned out to be stiffest, whereas scar or normal fibroblasts are softer in that order. Both cell types tend to softer on soft gels, whereas this difference is least prominent for the myofibroblast like Dupuytren cells.

By applying a step in z height, while being in contact, we can determine the viscoelastic creep response of cells.

In summary, this study shows that mechanical properties as measured by AFM are helpful in order to distinguish normal and diseased cells for a large variety of diseases.

References:
The interaction of room-temperature ionic liquids (ILs) with cells and model biological membranes: investigating the basic mechanisms of ILs cytotoxicity

A. Podestà¹, M. Galluzzi ¹, ², S. Asperti¹, A. De Vita¹, L. Marfoni¹, D. Piotti¹, C. Schulte¹, P. Milani³

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²College of Materials Science and Engineering, Shenzhen Key Laboratory of Polymer Science and Technology, Guangdong Research Center for Interfacial Engineering of Functional Materials, Nanshan District Key Lab for Biopolymers and Safety Evaluation, and College of Optoelectronic Engineering, Key Laboratory of Optoelectronic Devices and System of Ministry of Education and Guangdong Province, Shenzhen University, Shenzhen, 518060, PR China

Room-Temperature Ionic Liquids (ILs) [1] are nowadays routinely and massively used as effective and tunable solvents in industrial chemical processes. ILs also represent an alternative to aqueous electrolytes in devices aimed at the conversion and storage of energy (supercapacitors, Grätzel solar cells, and Li-ion batteries), as well as lubricants in micro-electro-mechanical devices. Although the green and sustainable character of ILs is often claimed by virtue of their negligible vapor pressure, controlled miscibility, and low flammability, data on ILs toxicity and biocompatibility are still scarce, and mostly based on the study of the survival rate in populations of micro-organisms exposed to increasing concentrations of ILs. Here we present the results of a study aimed at investigating the basic mechanisms of ILs cytotoxicity. Since the cell membrane is the first (and probably main) target of ILs action, we have used atomic force microscopy to carry out a combined topographic and mechanical analysis of supported DOPC bilayers, as a model of the cell membrane, and of living MDA-MB-231 cells, interacting with ILs. In particular, we have studied the evolution of morphological and mechanical properties of the lipid bilayers and cells upon increasing concentrations of imidazolium-based ILs. We have observed modifications in the breakthrough force and membrane elasticity of DOPC bilayers, likely related to the insertion of the alkyl chains of the ILs cations in the bilayer, an effect already reported in Ref. [2], and known as the “tail-first” mechanism [3]. The analysis performed on living cells showed modifications of elasticity (effective Young modulus) and morphology of cells after exposure to ILs dispersed in their culture medium, which confirmed the primary action of ILs on the membrane and possibly on the outer cytoskeleton components. Our results suggest that imidazolium-based ILs have potentially biologically relevant effects already at concentrations well below those determining the death of the micro-organisms.

References:
Tip-Enhanced Raman Spectroscopy for Nanoscale Chemical Analysis and Imaging

R Zenobi$^1$ and E Lipiec$^2$
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Abstract: Tip-enhanced Raman Spectroscopy (TERS) is a nanoscale chemical analysis and imaging method with a spatial resolution on the order of 10 nm. TERS relies on enhancement of the local electromagnetic field in the vicinity of a plasmonic nanostructure that is scanned over a sample by means of a scanning probe microscope, using either AFM or STM feedback. The local enhancement of Raman scattered light is many orders of magnitude, large enough to render monomolecular films spectroscopically visible that would otherwise be optically too thin to be analyzed with conventional vibrational spectroscopy.

The working principle and experimental realization of TERS will be presented [1]. An important advance concerns the production long-lived silver TERS tips. Thanks to the presence of a chemical protection layer, these live for many weeks as opposed to the typical lifetime of $\approx$1 day for bare Ag tips [2], and can be operated in liquids. The focus of this presentation will be on applications of TERS to the spatially resolved chemical analysis of molecular and biological nanostructures, including self-assembled monolayers [2], a novel class of materials, 2D polymers [3], membranes, and biological nanostructures such as amyloid forming proteins [4].

Figure (right hand side) shows a high-resolution TERS map of Amyloid-β fibrils.

Figure. Distribution of $\alpha$-helices and $\beta$-sheets in Aβ1-42 fibrils: typical spectra of $\alpha$-helices (green) and $\beta$-sheets (blue). AFM image (left) with a TERS map (7.5 nm x 7.5 nm pixel size) overlay (right).

References:


Molecular Forces and Nanomechanical Measurements in Blood Coagulation

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Blood coagulation results in the formation of a blood clot. Blood clots are beneficial in hemostasis as they prevent life-threatening blood loss in the event of injury. However, blood clots can also be harmful when they block healthy flow (thrombosis), being the underlying cause of such diseases as heart attacks, stroke and venous thromboembolism, with the first two diseases accounting for 25% of deaths worldwide.

Blood clots perform the mechanical task of stemming the flow of blood. To improve our understanding of blood clots it is, therefore, important to understand their mechanical behaviour. The main structural and mechanical component of a blood clot is a mesh of microscopic fibrin fibres.

We have developed an AFM/inverted optical microscope-based technique to study the mechanical behaviour of single, microscopic fibres, including fibrin fibres. I will present this technique and the results we have obtained on fibrin fibre mechanical properties, and discuss them in the context of blood clotting and the properties of other microscopic fibres.

References:


Physics of cells and cancer detection with AFM

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I will focus on two topics: what AFM can reveal about physics of cells (properties of the pericellular coat layer and mechanics of the cell body) and imaging of cells to detect cancer. Derivation of physical properties of cells will be demonstrated for human epithelial (cervical, neuronal, and breast) cells, and rodent fibroblasts [1-3]. In particular, I will demonstrate that our AFM approach can distinguish physical properties (length and density) of both the molecular (glycol-proteins and -saccharides) part of the pericellular coat as well as the membrane corrugations (microvilli and microridges). We previously reported on substantial differences in the pericellular coat observed for cervical cells when it becomes cancerous. Based on that we demonstrated that high-resolution imaging of cells may be used for detection of this cancer [4, 5]. Imaging of fixed cells was used, which is clinically preferable over the use of living cells. Here I present a further development, our new work on detection of bladder cancer by imaging cells extracted from human urine. The method is based on the use of artificial intelligence (machine learning) to analyze AFM images collected in PeakForce taping mode. Our preliminary data shows that the accuracy of the method can be higher than 95%.

References:
[3] N. Guz, et al., If cell mechanics can be described by elastic modulus: study of different models and probes used in indentation experiments, Biophys J 107(3) (2014) 564-75.
The mechanical properties of individual proteins, filaments, and supramolecular assemblies provide structural stability and mechanical flexibility to the living cell. Thus, molecular understanding of the mechanics from the single molecule to the whole cell is relevant to understand biological function. High-speed atomic force microscopy (HS-AFM) is a unique technology allowing subsecond, nanometric imaging (1). We have recently adapted HS-AFM to perform high-speed force spectroscopy (HS-FS) to probe protein unfolding at the speed of molecular dynamics simulations (2). This combination provides a unique method to acquire atomistic understanding of biomolecular processes based on experimental results. We have now gone a step further in the adaptation of HS-AFM to probe the mechanics of cells at high frequencies, up to 100 kHz (3). We report the viscoelastic response of different cell types and upon cytoskeletal drug treatments (Fig. 1). At previously inaccessible short timescales, cells exhibit rich viscoelastic responses that depend on the state of the cytoskeleton and the cell type. Microrheology over a wide dynamic range up to the frequency of action of the molecular components provides a mechanistic understanding of cell mechanics.

**Figure 1.** (A) Bright field image of living 3T3-fibroblasts on the HS-AFM setup. (B) Scanning electron micrograph of HS-AFM cantilever (bar = 10 µm) and (C) electron beam deposited spherical tip (bar = 1 µm) (D) Force-time trace with 1 kHz oscillation on a living cell. Maximum force: ~0.5 nN. Red line shows piezo displacement (550 nm + 15-nm amplitude oscillation). (E) Force-indentation loops at different frequencies. (F) Frequency-dependence of the complex shear modulus $G^*(f) = G'(f) + iG''(f)$ of 3T3 cells (N=22). Inset: loss tangent, $\eta = G''/G'$. 

**References:**
**Oral presentations**

O1  Anna Ryguła, Poland  
“Multifunctional imaging of endothelial cells with SNOM, AFM and Raman spectroscopy”

O2  Maria Ortega, Spain  
“Structural Stability of Cu-Azurin in SPM experiments: insights from MD simulations”

O3  Joanna Raczkowska, Poland  
Bio-interfaces of polymer coatings inducing controlled interactions with cells

O4  Elena Kozlova, Russia  
“AFM studies of molecular mechanisms of the arising of topological nanodefects of RBC membranes under the action of toxins and storage of donor blood”

O5  Sungjio Park, Korea  
“Fractal assembly of biomolecules resolved by atomic force microscopy”

O6  Sevil Ozer, Turkey  
“Development of an AFM with Dual Actuation Capability for Biomolecular Measurements”

O7  Daniel Navajas, Spain  
“Micromechanics of brain tissue measured with atomic force microscopy”

O8  Marek Haftek, France  
“Probing the stratum corneum: cell surface nanomechanical properties change according to the tissue depth”

O9  Delphine Sicard, USA  
“Lung tissue stiffness: study on aging and AFM tip size and geometry effects”
O10 Nuno C. Santos, Portugal

“Identification of the determinants for dengue virus capsid protein binding to host lipid droplets and plasma lipoproteins”

O11 Marta Targosz-Korecka, Poland

“Glycocalyx degradation coincides with endothelium stiffening during progression of diabetes in db/db mice - an ex vivo AFM nanoindentation study”

O12 Simone Dinarelli, Italy

“AFM nano-mechanical study of the beating profiles of hiPSC-derived cardiomyocytes beating bodies WT and DM1”

O13 Ana Sancho, Germany

“Intercellular adhesion forces in Endothelial-to-Mesenchymal Transition”

O14 Arkadiusz Ptak, Poland

“Dynamic Force Spectroscopy: Comparison of competing models of thermally activated unbinding under external force”

O15 Carlos Alvarez Amo, Spain

“Fundamental High Speed Limits in Single –Molecule and Nanoscale Force Spectroscopies”

O16 Martin Pesl, Czech Republic

“Simultaneous analysis of cardiomyocyte contractions by Atomic force microscopy and calcium imaging”

O17 Rami Omidvar, Germany

“Reconstitution of initial steps of lectin-driven bacterial uptake into cells”

O18 Isabell Tunn, Germany

“Coiled coils as mechanical building blocks”

O19 Joanna Danilkiewicz, Poland

“Molecular forces in interaction of bladder cancerous cells with syndecan-1 and syndecan-4”
O20 Filomena A. Carvalho, Portugal
“Role of fibrinogen-erythrocyte and erythrocyte-erythrocyte adhesion on cardiovascular pathologies”

O21 Vincent Dupres, France
“Influence of autophagy on cell mechanics probed by stiffness tomography and super-resolution microscopy”

O22 Massimiliano Galluzi, China
“AFM experiments and simulations of heterogeneous soft-matter: analysis and biological applications”

O23 Ignasi Jorba, Spain
“Extracellular Matrix Micromechanics of Regenerating Zebrafish Heart”

O24 Carlos Guerrero Rodriguez, Spain
“Time-resolved nanomechanical rheology of a single cell under the depolymerization of the actin cytoskeleton”

O25 Joanna Zemła, Poland
“Nanomechanics of cells and polymer gels probed with different AFM indenter shapes”

O26 Andrea Mescola, Italy
“AFM investigation of mechanical properties of glioblastoma multiforme cells and their relation to motility”

O28 Janusz Strzelecki, Poland
“Extraction of corneal micromechanics with AFM nanoindentation using ex vivo porcine eye models and intraocular pressure control”

O29 Maria-Astrid Schröter, Germany
“Structural changes in plasmid DNA caused by radiation and its protection by Ectoine: an AFM analysis”
O30 Andreas Stylianou, Cyprus
“Normal and Cancer Associated Pancreatic Fibroblasts Shape Modulation is mediated by Transforming Growth Factor β and Matrix Stiffness”

O31 Helena Lozano Caballero, Spain
“Electrical and morphological characterization of bacterial polar flagella”

O32 Alexander Dulebo (Bruker)
“Observing cell motility using an AFM – how fast can we go?”

O33 Sourav Maity, Netherlands
“Studying viral disassembly by High Speed Atomic Force Microscopy”

O34 Ewelina Lipiec, Poland
“Nano-spectroscopic mapping of DNA molecule”

O35 Maria Starodubtseva, Belarus
“AFM-based physical-mechanical image of the cell surface”

O36 Ioana Dobra, France
“Reversible enzymatic actuation of an intrinsically disordered protein brush”

O37 Alexandre Berquand, France
“Investigating the influence of LRP-1 silencing on the migratory potential of MDA cancer cells by dynamic cell studies and atomic force microscopy”

O38 Bartłomiej Zapotoczny, Poland
“Quantification of Fenestrations in Liver Sinusoidal Endothelial Cells by Atomic Force Microscopy”

O40 Stefanie Kiderlen, Germany
“Young’s Modulus and Mechanotransduction in Actinin 1-Knockout Fibroblasts”
O41  Patrick Mesquida, Austria  
“Kelvin-probe Force Microscopy to map glycation of proteins”

O42  Yuri Efremov, USA  
“Integrated Spinning Disk Confocal and Atomic Force Microscopy Reveals Anisotropic Indentation Geometry on Living Cells”

O43  Hermann Schillers, Germany  
“The unholy alliance of platelets and tumor cells”

O44  Claire Valotteau, Belgium  
“Molecular mechanisms guiding the adhesion of Staphylococcus aureus clumping factor B to the skin protein loricrin”

O45  Monika Österberg, Finland  
“Probing forces between stem cells and biomaterials”

O46  Thi Huong Nguyen, Germany  
“A New Mechanism of Autoimmune HIT Caused by a Subset of Antibodies”

O47  Riina Harjumäki, Finland  
“Interactions of Xenobiotic-free Biomaterials and Cells for Better Tissue Models”

O48  Svetlana Pleskova, Russia  
“Atomic force microscopy in research of bacteria interactions with the blood neutrophils”

O49  Hamdi Torun, Turkey  
“Miniaturized Magnetic Beads for Single Molecule Force Measurements”

O50  Fidan Sumbul, France  
“Effect of cantilever response time on single molecule unfolding forces”
Multifunctional imaging of endothelial cells with SNOM, AFM and Raman spectroscopy

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The aim of this work is to show the capabilities of multifunctional imaging of endothelial cells. The project is based on the spectroscopic techniques, which allow insight into a cell structure and composition, i.e. Raman spectroscopy (RS), atomic force microscopy (AFM) and scanning near-field optical microscopy (SNOM). Multifunctional imaging, which applies all these techniques simultaneously, enables its comprehensive analysis. Whilst SNOM with AFM give structural information about biological samples, Raman spectroscopy completes it with the chemical image.

Studies were carried out on fixed cells of various endothelial cell lines: EA.hy926, HLMVEC and HMEC-1 maintained in physiological buffer. Additionally we attempted to measure live cells by SNOM [1].

References:

Acknowledgments: This study was supported by National Science Centre (UMO:2013/08/A/ST4/00308).
Structural Stability of Cu-Azurin in SPM experiments: insights from MD simulations

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Biological Electron Transfer (ET) is the key step in many basic cellular processes such as respiration and photosynthesis. Nature has developed highly specialized ET proteins capable of transporting charge with unprecedented efficiency. It has been shown that specific mutations of one or more residues in these proteins could cause changes in the ET process which lead to, for instance, an overproduction of reactive oxygen species. Therefore, understanding the mechanisms behind biological ET is key in order to elucidate these changes which result in a malfunctioning of the mitochondria [¹].

In the last decades, the ability of Scanning Probe Microscopy techniques to measure the mechanical or electronic response of a single soft material has enabled to know more about these ET proteins [²]. However, for a complete understanding of the mechanisms behind the response observed in these experiments, theoretical simulations play a key role. Atomistic MD could give information about how proteins are absorbed to surfaces [³] or how they are influenced by a tip. In this work, we have analyzed these two structural properties for a ET protein, the Cu-Azurin, absorbed on gold. We have been able to observe that it is absorbed via two different parts: the cysteines or the hydrophobic patch. Moreover, we have observed that the protein structure remains stable even after the tip establishes physical contact to it (see Figure). These two findings prove the structural stability of this protein in a set of blinking experiments which show a sharp change in the transport behavior of this protein by performing a single-aminoacid mutation. We have also observed with MD simulations that this mutation does not cause significant structural changes on the protein. Therefore, the origin of the transport changes observed must be in the electronical structure of the protein.

References:
Bio-interfaces of polymer coatings inducing controlled interactions with cells

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The main goal of the project was to design and complexly characterize innovative polymer materials with the controlled physicochemical properties for biomedical applications, especially as the diagnostic tools. The possibility of biomedical applications of the material is determined by its interactions with biological material, dependent on physicochemical properties of the surface, such as chemical composition, wettability, topography or elasticity. Performed research showed the strong impact of substrate elasticity on adhesion and proliferation of cancerous cells, extremely sensitive to the change of mechanical properties, even in the narrow range. Examined cancerous cells exhibited strong tendency to proliferate preferentially on the softer substrates [1,2]. Obtained results were used for precise positioning of cells, which perfectly mirror both, gradient and discrete elasticity patterns [3]. This effect was additionally enhanced by simultaneous modification of mechanical and chemical substrate properties, leading to fabrication of isotropic and regular patterns, reproduced by examined cells [4,5]. This approach may be used for precise positioning of cells for numerous applications, ranging from diagnostic to personalized therapy.

This work was financially supported by the project National Science Centre agreement number UMO-2013/09/D/ST5/03859.

Figure. PDMS elasticity patterns (a) for precise positioning of collective groups (b) and individual bladder cancer (HCV29) cells (c)

References:
AFM studies of molecular mechanisms of the arising of topological nanodefects of RBC membranes under the action of toxins and storage of donor blood

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Endogenous and exogenous factors can influence on red blood cells (RBC) and transform their forms. But the molecular mechanism of formation of membrane topological defects which finally lead to the changes of cell shape and deformability is currently unclear. We studied the change of RBC morphology and nanostructure as the result of exogenous intoxication of blood by different far-chemicals - hemin, furosemide, chlorpromazine, zinc [1]. Of particular interest is the study of the change in the nanostructure and shape of cells during long-term blood storage for transfusion [2].

For this we used AFM “NTEGRA Prima” (NT-MDT Co., Russian Federation) in semi-contact mode, cantilevers NSG01 with force constant 5 N/m, tip curvature radius 10 nm were used. The number of scanning points were 512 or 1024 within each line of image.

![AFM images](image)

**Figure 1.** AFM—images of domains with grain-like structures on the membrane surface under the hemin action. Molecular mechanism

In experiments it was established that due to external and internal intoxication of blood the formation of topological nanodefects precedes and this is the cause of changes in cell shapes. The typical diameters of topological nanostructures are 50 -250 nm, depth 10-50 nm (fig. 1). This is comparable with the size of the spectrin matrix. It means that under intoxication and oxidation processes spectrin itself and junctions of spectrin-membrane proteins are violated, the local defects are arisen and as the result the shape of RBC is changed.

References:


Fractal assembly of biomolecules resolved by atomic force microscopy

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Fractal self-assembly is ubiquitous, enabling realization of diverse structural configurations from nanoscale designs to supramolecular architectures1. Biomolecules, serving as starting building blocks, hierarchically intercalate to stimulate growing structural multidimensionality and functional complexity2. Unfolding the underpinnings of fractal macromolecular assembly is of fundamental and applied interest3-5. Here, we examined fractal self-assembly of crude and purified human plasma-derived exosome extracts by high resolution atomic force microscopy as they dried on a mica surface. Nanoscale imaging of plasma-derived exosomes revealed ellipsoidal structures of varying sizes with an average diameter and height of $54 \pm 5$ nm and $0.58 \pm 0.21$ nm, respectively. In the presence of human collagen-based solution, crude plasma-derived exosome extract formed self-assembled nano- and micro-architectures with hierarchical self-similarity producing fractal patterns, whereas purified exosome extract did not generate fractal assembly. The self-assembly architectures developed by crude exosome extract were similar to the canonical fractal assembly of diffusion-limited aggregation of colloids, validated by fractal dimension analysis. The unique physical behavior and fractal nature of plasma-derived crude exosome extract versus purified exosome extract may provide a crucial step in mapping the biological relevance and role at play in cell communication and potential theranostic applications.

References:
Development of an AFM with Dual Actuation Capability for Biomolecular Measurements

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Force spectroscopy using atomic force microscopy has proven to be a primary tool in revealing biomolecular interactions regarding the mechanical folding/unfolding properties of proteins at single-molecule level under external force. Here, we present a novel AFM setup with dual actuation capability employing a conventional piezoactuator and a FeCo core-based electromagnetic actuator integrated to the AFM head [1-3]. In addition to a large piezoactuator, which limits the high and low speeds measurements via the drift and the hydrodynamic effects, the cantilever is directly actuated electromagnetically by attaching a ferromagnetic bead with a diameter of 30 μm. During the electromagnetic actuation, the cantilever base is stationary, which improves the mechanical stability of the system. We have used biotin (on cantilever)/streptavidin (on sample) pairs for the biomolecular experiments.

Figure. (a) Schematic representation of the dually actuated AFM setup, using piezo and/or magnetic actuation method (b) a sample force curve obtained using a magnetic bead attached cantilever actuated using the electromagnet showing the specific biotin-streptavidin interaction with an unbinding force of 285 pN.

References:
Micromechanics of brain tissue measured with atomic force microscopy

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Cells sense and respond to the mechanical properties of their microenvironment. Several diseases including cancer and fibrosis are associated with alterations of tissue stiffness. However, stiffness changes in brain diseases remains poorly known. Alzheimer’s disease (AD) is the most prevalent neurodegenerative disease and the first cause of dementia worldwide, being a considerable public health problem. Preliminary results obtained by Magnetic Resonance Elastography (MRE), a non-invasive technique based in externally exploring the whole organ, suggest that brain tissue stiffness is reduced in AD. Nevertheless, MRE estimation of tissue stiffness is indirect since it requires the use of invers-model based algorithms mapping the 3D structure of the whole organ. To date there are no data providing a direct measure of brain tissue stiffness in AD. We use atomic force microscopy (AFM) to directly measure and compare the local stiffness of the brain cortex of AD-mutant and normal mice.

Mice (6-8 month old) bearing a mutation (APPswe/PSEN1dE9) mimicking human AD and their littermates wild type (N=8 and N=9, respectively) were investigated. After anesthesia and euthanasia by exsanguination, the brain was excised and 200-micron coronal slices were cut with a vibratome. As previously described in detail [1], brain slices were subjected with a 3D printed ring with a compliant mesh (2 mm spacing) of silicone thread (0.2 mm in diameter) and placed on the stage of a custom-built Atomic Force Microscope to measure the local stiffness of the cortex. Force-indentation curves were obtained with a 25 µm diameter polystyrene bead glued to the end of a tipless cantilever (nominal spring constant k=0.01 N/m). The Young’s modulus (E) of the cortex in each animal was computed as the median from 9 randomized force-indentation curves.

E [median (IQR)] of the cortex in AD-mice was considerably reduced as compared with the stiffness of their wild type littermates: 325 (194-513) Pa and 666 (416-985) Pa, respectively. This difference was statistically significant (Mann-Whitney analysis, p-value=0.049).

Mice having the APPswe/PSEN1dE9 mutations, which develop an AD-like phenotype, show a significant decrease in local cortical stiffness. These local measurements by AFM provide a direct evidence of previous indirect estimations by MRE and pave the way for studying the mechanisms determining brain tissue mechanics in AD.

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Probing the stratum corneum: cell surface nanomechanical properties change according to the tissue depth

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During formation of the stratum corneum (SC) barrier, terminally differentiated keratinocytes continue their maturation process within the dead superficial epidermal layer. Morphological studies of isolated human corneocytes have revealed differences between cornified envelopes purified from the profound and superficial SC. Recent studies in patients with atopic dermatitis (AD) suggest the impact of filaggrin mutations on the barrier function that may be related to the composition and structure of corneocytes. We used atomic force microscopy (AFM) to measure the mechanical properties of native human corneocytes harvested by tape-stripping from different depths of normal and pathological SC. Various conditions of data acquisition have been tested and optimized, in order to obtain exploitable and reproducible results. Probing at 200 nN allowed us to investigate the total stiffness of the cells (at 50 nm indentation) and that of the cornified envelopes (at 10-15 nm). Using a softer cantilever, we were able to probe the lipid envelope properties (at 5-10 nm). The obtained data indicated statistically significant differences between the superficial (more rigid) and profound (softer) corneocytes, thus confirming the existence of depth and maturation-related morphological changes within the SC. Preliminary analysis indicated that, generally and at the comparable SC depth, corneocytes from AD patients presented a lower elastic modulus than healthy subjects, independent from the presence or absence of a heterozygous filaggrin mutation. This suggests that the presence of the inflammatory disease, in addition to the level of filaggrin expression, may be responsible for the observed nanomechanical properties of the SC. The proposed approach can be potentially used for minimally invasive evaluation of various skin conditions, especially when coupled with other, complementary analytic methods.
Lung tissue stiffness: study on aging and AFM tip size and geometry effects

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The mechanical properties of lung tissues play important roles in normal tissue function and pathological processes involved in the initiation and progression of pulmonary diseases [1, 2]. In our study, we used AFM micro-indentation to characterize the stiffness of human lung tissue and compare the elasticity of airways, pulmonary micro-arteries and parenchymal areas from the same group of subjects aged between 11 and 60 years old. Our results demonstrated that the elastic modulus of airways (15.57 ± 14.85 kPa, mean ± SD) is significantly higher than vessels (7.15 ± 6.27 kPa) and parenchyma tissues (2.06 ± 1.96 kPa). The elastic modulus of pulmonary arteries is also significantly greater than parenchyma areas. By dividing the original subjects group in two aging groups (“11-30yo group” and “41-60yo group”), we observed that airways, vessels and parenchyma areas from 41-60yo group are significantly stiffer.

We also investigated the effects of AFM tip shape and dimension on the Young’s modulus values of human pulmonary micro-arteries. We performed elastic measurements with three different AFM tips: one pyramidal with 20 nm radius and two spherical with 1 µm and 2.5 µm radii. As presented in the figure below, the elastic modulus of vessels is significantly higher for measurements performed with pyramidal sharp AFM tip. No significant difference of elastic modulus was observed between the two spherical AFM tips.

Together these two results highlighted important parameters such as the age of subjects and the AFM tip size and geometry to characterize appropriately the micromechanical properties of lung tissue.

References:
Identification of the determinants for dengue virus capsid protein binding to host lipid droplets and plasma lipoproteins

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Dengue virus (DENV) infects millions of people worldwide. With no specific treatment available, understanding its replication mechanisms is highly required to identify potential therapeutic targets. Using atomic force microscopy (AFM) based force spectroscopy, combined with additional data from dynamic light scattering, NMR and computational studies, we showed that DENV capsid (C) protein binds specifically to very low density lipoproteins (VLDL) but not to low density lipoproteins (LDL) [1]. DENV C-VLDL binding is similar to DENV C interaction with lipid droplets (LDs), host intracellular structures essential for viral replication [2]. As observed for the DENV C-LDs binding, previously characterized by us [3, 4], DENV C-VLDL interaction is K⁺-dependent, involves DENV C intrinsically disordered N-terminus, and is inhibited by pep14-23, a recently patented peptide drug lead against DENV [4, 5]. As perilipin 3 (DENV C target on LDs [3]) is structurally similar to the VLDL apolipoprotein E (Apo E), we hypothesized that this could be DENV C ligand on VLDL, enabling lipoviroparticles formation. AFM force spectroscopy data using specific antibodies for the Apo E corroborated this hypothesis [6]. The inhibition of DENV C-LDs and/or DENV C-VLDL binding may potentially be used to block DENV life cycle. Our current understanding of DENV C and pep14-23 structure and function [7], paves the way for similar approaches to understand intrinsically disordered protein domains (as the DENV C domain involved in these interactions) and improved peptidomimetics drug development strategies against DENV and related Flavivirus, such as West Nile and Zika viruses.

Glycocalyx degradation coincides with endothelium stiffening during progression of diabetes in db/db mice - an ex vivo AFM nanoindentation study

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The progressive dysfunction of the endothelium in diabetes leads to vascular injury and to the development of the cardiovascular disease. Recent studies have reported endothelium stiffening as an important symptom of the endothelium dysfunction in hyperglycemia. Other studies have shown that the degradation of the glycocalyx, which is a brush-like layer on the endothelium, coincide with the endothelial dysfunction in hyperglycemia. However, a relation between these two early symptoms of endothelial dysfunction has never been investigated in tandem. In this work, mechanical properties of the endothelium and endothelial glycocalyx from mouse aorta were determined simultaneously by means of AFM nanoindentation method. Ex vivo measurements were performed for non-fixed aorta from db/db mice during progression of type 2 diabetes (for 11,12,16 and 19 week old mice) and for age matched db/+ mice as a control. In order to detect and characterize the brush like glycocalyx layer a bi-layer brush model was implemented. This model is well fitted to the morphological phenotype of the endothelial cells and enabled to determine the structural parameters of glycocalyx as well as the apparent elastic modulus of the endothelium layer. We observed a local spatial redistribution of the glycocalyx and its progressive global degradation in the studied period of diabetes. The measured apparent elastic modulus of the endothelial layer increased for regions covered by glycocalyx and, in the same age-dependent way, for the whole endothelium layer. These results may indicate that the degradation of the glycocalyx is tightly related to endothelium stiffening and is a consequence of the endothelial dysfunction caused by the long lasting hyperglycemia.
AFM nano-mechanical study of the beating profiles of hiPSC-derived cardiomyocytes beating bodies WT and DM1

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Myotonic Dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults, characterized by a variety of multisystemic features⁴. Cardiac involvement is prevalent in DM1 and commonly includes defect in conduction system, tachyarrhythmias and dilative cardiomyopathy. Patient-specific induced pluripotent stem cell-derived cardiomyocytes (hiPSC-derived CMs)² represent a powerful in vitro model for molecular, biochemical and physiological studies of disease target cells. Here, we used an Atomic Force Microscope in a peculiar setup to measure the beating profiles of hiPSC-derived CM clusters (Beating Bodies, BBs) from Wild Type (WT) and DM1 patients. We monitored the evolution over time of the frequency and intensity of the beating. We determined the differences between different BBs and over different areas of a single BB, caused by morphological and biomechanical differences.

We exploited the AFM tip to apply a controlled force over the BBs, to carefully assess the biomechanical reaction of the different cell clusters over time, both in terms of beating frequency and intensity (as shown in figure 1). By performing a Fast Fourier Transform (FFT) analysis of the beating profiles over time, we revealed subtle changes in beating synchronicity, highlighting, for the DM1 samples, an instability of the period which was not observed in WT cells.

![Figure 1: Typical time evolution of the beating profile at different forces exerted on the same spot onto a Beating Body](image)

References:
Intercellular adhesion forces in Endothelial-to-Mesenchymal Transition

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Endothelial-to-Mesenchymal Transition (EndMT) is a process involved in cardiovascular development and disease. It is characterized by a phenotypical change where cells show a down-modulation of endothelial markers and an induction of mesenchymal and stem cell markers. Due to this transition, cells become more motile by reorganizing their cytoskeleton and disassembling cell-cell contacts. We present here a new approach to assess intercellular adhesion forces in cell monolayers, after complete maturation of intercellular junctions, by using FluidFM® technology adapted to atomic force microscopy (AFM). Using this technology, we have studied the alteration in cell-cell adhesion forces occurring during EndMT. We introduced a new in vitro model to induce the transition based on lentiviral overexpression of Muscle Segment Homeobox 1 (MSX1) in primary Human Umbilical Artery Endothelial Cells (HUAEcs). Our results showed intercellular adhesion forces of 70 nN and 550 nN in MSX1-overexpressing and control endothelial cells that did and did not undergo the transition, respectively. Additionally, we measured cell height and stiffness by colloidal indentation and observed that cells undergoing EndMT become flatter and stiffer. Biochemical analysis was in agreement with the biomechanical results, showing a decrease in expression of VE-Cadherin and Zonula Occludens 1 upon MSX1 overexpression, indicating a weakening of cell-cell junctions. An increased formation of stress fibers in the actin cytoskeleton and an increase in vimentin content in the intermediate filaments upon upregulation of MSX1 was also observed. In conclusion, this approach allows assessing intercellular adhesion forces in strongly adherent cells, after maturation of junctions and adhesions, even after several days in culture and in a biologically representative setting such as monolayer.

![Figure 1. Decrease of intercellular adhesion forces originated by EndMT.](image)

References:
Dynamic Force Spectroscopy: Comparison of competing models of thermally activated unbinding under external force

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A routinely applied method to study specific interactions, e.g. receptor–ligand bindings, at the single molecule level is the dynamic force spectroscopy (DFS). In the method the most probable (or average) unbinding force is measured over a range of loading rates. Applying a model of thermally activated unbinding (treated as an escape from the potential well) to the DFS data provides a way to determine kinetic and thermodynamic parameters of the interaction potential, i.e. the profile of the energy landscape in the direction of the external force. The first theoretical model – and still the most commonly used – was developed by Evans and Ritchie [1] on the base of Bell’s formula [2]. The model assumes that the dependence of the unbinding force on the loading rate is determined only by the probability of transition over an activation barrier or barriers described with their position. Since the assumption restricts significantly the usefulness of the model and it cannot explain many DFS results, new ones have been developed.

In the paper we compare the models, e.g. by Dudko et al. [3] and by Friddle et al. [4], focusing on their advantages and limitations. To check the applicability of the models, we have used them to fit DFS experimental data for the interaction between concanavalin A and carboxypeptidase Y as an example of very important, from the biological point of view, lectin–carbohydrates interactions. The differences between the results obtained with different models are discussed [5]. We hope that the paper will contribute to better understanding of DFS results and will prevent misinterpretations.

Acknowledgments

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References:
Fundamental High Speed Limits in Single – Molecule and Nanoscale Force Spectroscopies

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Force spectroscopy (FS) is enhancing our understanding of single-molecule, single-cell, and nanoscale biophysical and mechanical properties. FS postulates the proportionality between the interaction force and the instantaneous probe deflection, the well-known Hooke’s law. By studying the probe dynamics through numerical simulations, we show that the total force probed by the tip has two additional contributions: the hydrodynamic (tip’s speed dependent) and the inertial (acceleration dependent). The amplitudes of these terms depend on the inverse of the ratio between the resonance frequency of the cantilever and the frequency at which the interaction is measured. As a consequence, the force–distance curve provides a faithful measurement of the interaction force between two molecules only when the inertial and hydrodynamic components are negligible, i.e., the frequency ratio >1. Otherwise, FS measurements will underestimate the value of unbinding forces unless this effect is taken into account. We also developed a simple but accurate equation to successfully correct this effect. Experiments of biotin – avidin recognition support both the simulations and the theory.

Simultaneous analysis of cardiomyocyte contractions by Atomic force microscopy and calcium imaging

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Abstract Body: The study of dynamic Ca$^{2+}$ changes in in vitro models of cardiomyocytes has become a cornerstone to understand the role of calcium signaling in healthy and diseased hearts$^1$. Atomic force microscopy (AFM) is a highly-sensitive and versatile method that can be integrated with optical microscopy and calcium imaging$^2$. We have implemented a combined set up to measure contractility and calcium waves in human cardiac models. We successfully report the first simultaneous recording of cardiac contractility, though AFM mechanocardiograms (MCG)$^3$ and local calcium waves probed on embryoid bodies. Ensemble empirical mode decomposition (EEMD) filtering was proved the best in terms of signal-to-noise ratio and signal distortions. Caffeine stimulation confirmed the detection capabilities of the used algorithms, measuring the expected physiological response (e.g. calcium duration increased 5.5% and contraction increased 14.5%). The combination of AFM and calcium imaging allows accurate analysis of complex excitation contraction coupling during physiological, disease and drug-induced situations.

References:

Figure. A) AFM contraction profiles B) Relative Calcium transients. t0=signal onset; t100=signal offset; tmax=amplitude; t50I=time to rise 50%; t50r=time to fall 50%.
Reconstitution of initial steps of lectin-driven bacterial uptake into cells

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Abstract: *Pseudomonas aeruginosa* (PA) bacterium, a human opportunistic pathogen, can cause infections and diseases especially in immunocompromised patients [1]. It has been shown that *P. aeruginosa* implements lectin molecules (particularly the galactose-binding LecA) to bind to host cells and promote bacterial engulfment [2]. Amongst various cell receptors, we could previously show that LecA binding to the glycosphingolipid Gb3 is fully sufficient to trigger membrane engulfment in cells and synthetic membranes [3]. Here, we aimed to carry out a detailed biophysical investigation of lectin-driven *P. aeruginosa* uptake on single minimal membrane systems. In detail, we examined the interaction of a lectin-coated cantilever tip with a synthetic giant unilamellar vesicle (GUV) using combined atomic force microscopy and fluorescence microscopy techniques (Figure 1). Giant vesicles containing just some major components of the plasma membrane such as phospholipids, cholesterol and the receptor molecule Gb3 molecules, were immobilized on the substrate via biotin-streptavidin attachment. Bare and lectin-coated cantilevers were kept in contact with vesicles for different time intervals and with different feedback modes, i.e. keeping the piezo height or cantilever vertical deflection constant. Analyzing the recorded force-distance curves provided detailed quantitative information about early steps of “pathogen” binding to the membrane. In later stages, we will build more complex vesicles by adding sphingomyelin and an artificial actin shell to narrow down the gap between synthetic models and native cellular membranes. Our study might lead to a better understanding about the biophysical mechanism(s) of lectin-driven phagocytosis.

References:
Coiled coils as mechanical building blocks

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In nature, coiled coil (CC) protein-folding motifs - consisting of two to seven α-helices wrapped into a superhelix - often occur in proteins with mechanical function, such as myosin or laminin. De novo synthesized CCs can be designed to bind their partner with high specificity and affinity by tuning amino acid sequence. This makes them powerful material building blocks, e.g. acting as reversible dynamic crosslinks in hydrogels for cell culture and tissue engineering [1]. However, the sequence-structure-mechanics relationships that link molecular level mechanical behavior of CC building blocks to macroscopic viscoelastic properties of CC-based materials remain poorly understood.

Towards the goal of elucidating fundamental design principles guiding utilization of CCs as mechanical building blocks, a series of short, synthetic CCs [2] was investigated with AFM-based single molecule force spectroscopy. Loading the CCs in the shear geometry, we show that the rupture forces lie in the range of 20-60 pN and depend on CC length, hydrophobic core packing and helix propensity. Moreover, we are able to tune the helix stability of the CC when inserting histidine metal coordination sites into the terminal repeats, where the force is applied. With this mechanically characterized CC-library at hand, we have developed biomimetic hydrogels where these mechanically tunable CCs are used to crosslink branched poly(ethylene glycol), i.e. starPEG-based hydrogels. These hydrogels entirely consist of mechanically fully characterized components and allow us to establish a direct correlation between the mechanics of a material and its molecular building blocks. Used as extracellular matrix mimics, this series of CC-crosslinked hydrogels will enable us to dissect global and local factors contributing to cellular mechanosensing.

References:
Molecular forces in interaction of bladder cancerous cells with syndecan-1 and syndecan-4

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Syndecans (SDCs) are a four member family of transmembrane heparan and chondroitin sulphate proteoglycans organized as membrane homodimers and higher order oligomers containing cytoplasmic domains connected to cytoskeleton. They play pivotal role in cell-matrix adhesion, growth factors binding, wound healing, ligand dependent activation modulation of primary signaling receptors cell differentiation and tissue regeneration. In particular, syndecan-1 based signaling pathways, coupled with aVb3 integrin, promote cell spreading in human mammary carcinoma [1]. Typically, SDC-1 expression has been reported to be reduced in epithelial cells cancer, including head and neck squamous cell carcinoma [2] and renal cell carcinoma [3]). However, there are also contrary examples such as pancreatic adenocarcinoma where syndecan-1 is up-regulated [4].

The aim of this work is to examine whether unbinding properties of syndecans alter upon the progression of bladder cancer. We have applied dynamic force spectroscopy (DFS) to measure unbinding of single syndecan-1 and syndecan-4 complexes. This interaction was probed directly on a surface of living human bladder cancer cells. Chosen cell lines cover all the stages of cancer progression, starting from non-malignant cell cancer of ureter (HCV29), through grade II (HTB-9) and grade III (HT1376) carcinomas up to transitional cell carcinoma (T24). To quantify the interaction, obtained data were analyzed using Bell-Evans model of thermally activated escape over the energy barrier.

Quantitative results of adhesion force and probability, enriched with the analysis of single cell surface area, provide better insight into the mechanism of syndecans alterations upon bladder cancer progression. In parallel, the dependence of the unbinding force on divalent ions presence has been observed. Lately, it was experimentally shown a regulatory role of syndecans in functioning of stretch-activated ions channels [6]. These findings highlight the importance of syndecans in pathologic cell behavior in different stage of metastasis and the crucial role of divalent ions concentration in kinetics of binding syndecan-4 to its monoclonal antibody.

References:
[7] The studies were financed by the NCN project no UMO-2014/15/B/ST4/04737.
Role of fibrinogen-erythrocyte and erythrocyte-erythrocyte adhesion on cardiovascular pathologies

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Cardiovascular pathologies are the major cause of death worldwide. Erythrocyte aggregation is an indicator of cardiovascular risk, which is influenced by high plasma fibrinogen levels. Our main goals were to understand how fibrinogen-erythrocyte binding influences erythrocyte aggregation and how it constitutes a cardiovascular risk factor in essential arterial hypertension (EAH) and chronic heart failure (CHF).

Differences on cell stiffness, protein-cell interaction and cell-cell adhesion forces were evaluated by AFM-based force spectroscopy with cells from 31 EAH patients, 30 CHF patients and 15 healthy blood donors. The main procedures used were previously described by us [1–3]. Results were correlated with patients’ clinical profiles.

From cell-cell adhesion studies, we concluded that, upon increasing fibrinogen concentration (from 0 to 1 mg/mL), there was an increase in the work and force necessary for erythrocyte-erythrocyte detachment on EAH patients and healthy donors. Nevertheless, higher values from both parameters were obtained for EAH patients, when comparing to healthy donors, at each fibrinogen concentration.

Fibrinogen-erythrocyte (un)binding forces were higher in EAH and in CHF patients, when compared with the control group, despite a lower binding frequency. Ischemic CHF patients showed increased binding forces compared to non-ischemic patients. A 12-month clinical follow-up shows that CHF patients with higher fibrinogen-erythrocyte binding forces, probed by AFM at the beginning of the assessment, had a significantly higher probability of being hospitalized due to cardiovascular complications, pointing out the value of AFM for clinical prognosis [4].

Erythrocyte stiffness studies revealed differences between CHF patients and healthy donors, in terms of erythrocyte elasticity (Young’s modulus) and AFM tip penetration depth into the cells. Erythrocytes from non-ischemic CHF patients presented a higher average stiffness than those from the other groups (ischemic CHF and control). Nevertheless, a significantly higher cell penetration depth at the same applied force was observed for ischemic CHF patients. In conclusion, fibrinogen promotes erythrocyte adhesion, leading to its aggregation, probably by transient simultaneous binding of the protein to two cells, bridging them. Our results may be relevant for potential future drug interventions to reduce aggregation and enhance microcirculatory flow conditions in cardiovascular patients.

Influence of autophagy on cell mechanics probed by stiffness tomography and super-resolution microscopy

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How cells are able to sense their environment and respond to external mechanical forces is fundamental in a wide range of biological processes (cell migration, cell adhesion, differentiation…) but remains poorly understood. In tissue, cells are surrounded by other cells and extracellular matrix, forcing them to adapt to physical constraints by inducing intracellular contraction forces [1]. Consequently, cells in tissue adopt reproducible shape and cytoskeleton organization in response to these physical constraints [2] that influence their fate [3].

In this study, we specifically impose well defined constraints to the cell to study its response in term of cytoskeleton organization and mechanical properties (stiffness, adhesion, viscosity) when the autophagy level is modified, e.g. upon drug treatments, gene knockdown or infection.

To produce single cells that adopt a similar shape, morphology and physiology, human RPE1 cells were grown on coverslips harboring fibronectin-coated patterns [4]. We used the crossbow-shape micropattern that promotes a ‘polar’ intracellular organization of the actin cytoskeleton and endocytic compartments [5, 6].

Innovative coupling between atomic force microscopy (AFM) and super-resolution microscopy (AFM:STED-RESOLFT) is used to probe stiffness properties and morphological changes with unprecedented resolution, with a particular attention to the organization of cytoskeleton components. Herein, we want to unveil important features that influence pathophysiological regulations of cells and tissues related to autophagy and cellular biophysical properties relationships that were so far underestimated.

References:

AFM experiments and simulations of heterogeneous soft-matter: analysis and biological applications

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Atomic Force Microscopy has proven to be a valuable technique to characterize the mechanical and morphological properties of soft materials with nanoscale resolution. The investigation of biological specimens such as tissues, living cells and bacteria is a hot topic for early-diagnostic where mechanical properties can be correlated to a pathophysiological condition. Here, a 3-step method is proposed in order to investigate biological specimens where vertical and lateral heterogeneity hinder a precise quantitative characterization: 1) precise AFM calibration coupling macroscopic rheology on homogenous hydrogels with SNAP procedure,\textsuperscript{1} 2) nano-indentation in force volume mode using spherical colloidal probes\textsuperscript{2} 3) finite element simulations (FEM) of AFM indentation events\textsuperscript{3}. External geometries and material properties in the FEM model construction are directly driven by AFM experiments. Finally, an array of simulated force curves is produced varying unknown parameters, such as internal geometries, multi-layers material properties and interfacial friction: the most comparable simulation is selected minimizing the rms deviation on experimental data. In order to easily perform this analysis from raw AFM data to simulation comparison, a novel standalone software, ‘AFMech Suite’, was built in Matlab environment (software available on e-mail request). Five interacting interfaces were designed for simultaneous calibration, morphology, adhesion, mechanical and simulation analysis. The method was successfully validated on a soft-matter model system, soft hydrogels with hard spherical inclusions of different size. Results of analysis are presented for E. Coli bacteria supported on soft (fig. A, B, C) and hard (fig. D, E, F) hydrogels and for a cell+nucleus system (MDA-MB-231 cancer cell).

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Extracellular Matrix Micromechanics of Regenerating Zebrafish Heart

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After myocardial infarction mammals are unable to regenerate the lost tissue and substitute it with an irreversible scar \cite{1}. However, zebrafish can regenerate its heart after damage through cardiomyocyte dedifferentiation and proliferation \cite{2}. One of the factors that is poorly understood in this process is the role of the extracellular matrix (ECM). We used Atomic Force Microscopy (AFM) to study mechanical changes of the ECM after ventricular resection. We compared the stiffness of the uninjured and regenerating myocardium.

Amputation of 20\% of the myocardium was performed in adult zebrafish. Myocardial stiffness was analysed at 7, 14 and 30 days post amputation (dpa) (n=5) and also in animals with no injury (control, n=5). Hearts were excised, frozen and sliced at 25 µm with a cryostat. Slices were decellularized, with sodium dodecyl disulfate 0.1\% for 5 minutes followed by Triton X-100 1\% for 10 min and NaCl 0.9\% for 20 minutes to remove cellular components. The slices were placed on the stage of a custom-built AFM to measure the Young’s modulus (E) of uninjured and regenerating myocardium. Force-indentation curves were recorded with a spherical tip 4.5 µm in diameter (nominal spring constant k = 0.06 N/m). E decreased in uninjured myocardium at 7 dpa compared to controls, followed by an increase at 14 dpa. ECM stiffness both of uninjured and regenerating myocardium recovered control values at 30 dpa (Fig.1).

Zebrafish heart regeneration is a complex process in terms of ECM mechanics. Micromechanical measurements by AFM provide evidence that there are changes in ECM stiffness in uninjured and regenerating hearts possibly associated with tissue adaptation to help the wound healing.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Stiffness of uninjured (control) and regenerating (wound) heart ECM at 7, 14 and 30 days post-amputation (dpa). Two regions were measured in each heart: uninjured myocardium and wound. N=5. * p<0.05}
\end{figure}

Time-resolved nanomechanical rheology of a single cell under the depolymerization of the actin cytoskeleton

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Single cell stiffness measurements consider the cell as passive and elastic material which reacts instantaneously to an external force. This approximation is at odds with the complex structure of the cell which includes solid and liquid components. Here we develop a force microscopy method to measure the time and frequency dependencies of the Young modulus, the viscosity coefficient, the loss modulus and the relaxation time of a single live cell. Those parameters have different time and frequency dependencies. At low modulation frequencies (0.2-4 Hz), the Young modulus remains unchanged; the loss modulus increases while the viscosity and the relaxation time decrease. We have followed the evolution of a fibroblast cell subjected to the depolymerization of its F-actin cytoskeleton. The Young modulus, the loss modulus and the viscous coefficient decrease with the exposure time to the depolymerization drug while the relaxation time increases. The latter effect reflects that the changes in the elastic response happen at a higher rate than those affecting the viscous flow. The observed behavior is compatible with a cell mechanical response described by the poroelastic model.

Figure. Scheme of the data acquisition in force spectroscopy rheology.

Nanomechanics of cells and polymer gels probed with different AFM indenter shapes.

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Nanomechanics of soft materials like gels and living cells has turned towards instrumentations and methods used for the probing of mechanical properties on the submicron scale. In recent years, nanoindentation measurements, carried out by atomic force microscope (AFM), have been widely applied for differentiating between various cell populations characterized by specific mechanical properties in a quantitative way [1]. This results from an urgent need to measure cellular mechanics as the correlation between elasticity/ rigidity of cells and various diseases has been found. It has already been shown that cancerous cells are softer than the normal ones, additionally, changes in Young’s modulus in vascular diseases, kidney diseases, cardiomyopathies, cataracts, etc. have been observed as well [2].

As the interpretation of elasticity measurements of soft and highly heterogeneous samples is far more complex, here, we present studies on elasticity of soft samples. Homogenous samples made of polyacrylamide gels (PAA) were compared with heterogeneous living cells (HCV29 – non-malignant cell cancer of the ureter and HT1376 – bladder carcinoma), both characterized by similar elastic modulus range. AFM-based elasticity measurements were carried out using probes of various geometries: sharp (cone, pyramid) and blunt (bead). The elastic modulus was calculated using Hertz/Sneddon models, independently of the sample type [4]. Regardless of the probing tip shape, Young’s modulus of PAA gels containing more crosslinking agent was always larger. Also, cancerous cells were softer than their normal counterparts. Depending on the intending probe geometry, the elastic modulus changes as a function of indentation depth as it was previously shown by Pogoda et al. [3]. Elasticity changes were more pronounced for sharp AFM probes where a decrease was observed while for spherical one an increase was obtained. Our results agree with that reported for cervical cancer [5].

References:
[4] The studies were financed by the NCN project no UMO-2014/15/B/ST4/04737.
Sensing of silver nanoparticles on/in endothelial cells using atomic force spectroscopy

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Over the last two decades atomic force spectroscopy (AFS) have attracted huge interest in terms of evaluation of endothelial cells elasticity. Endothelial cells are of particular interest in terms of studying elasticity, due to their location in vivo, where they are subjected to continuous bending and extension by the surrounding (local) environment. Force spectroscopy, also called nanoindentation by atomic force microscope (AFM) probe can be useful in a case of verification of endothelial inflammation state [1] and/or evaluation of different agents effects e.g. hormones, drugs or nanoparticles. Cells elasticity measurements provide information on the mechanical properties which, in the case of endothelium are closely linked to biochemical parameters (NO, PGI₂, Ca²⁺) [2]. Moreover, cells elasticity modulus can be correlated with F-actin distribution in the cortical actin cytoskeleton.

In our work we have employed AFS for evaluation of silver nanoparticles (SNPs) on EA.hy926 endothelial cell line after 24h incubation. Determination of SNPs concentration causing defined levels of cellular viability: 90, 75 and 50% (concentration marked as IC10, IC25 and IC50 respectively) was performed using XTT tests. Cells elasticity modulus was calculated using Hertz-Sneddon model. We have compared elasticity results for two different geometries of AFS probes: spherical and paraboloidal. As the spherical probe a glass ball with 5um in diameter was used. Paraboloidal geometry refers to PFQNM – LC (Bruker) sharp tips with nominal radius of 70nm. Measurements were conducted for live cells in cell culture medium and for fixed cells using “slight” fixation procedure with 3.7% of formaldehyde. Evaluation of silver nanoparticles impact was carried out for different indentation depths and it enabled verification of increased amount of nanoparticles or their agglomerates inside the cells. Obtained results of force spectroscopy were supplemented with measurements of cells morphology and fluorescent labeling of actin cytoskeleton.

A decrease of elasticity in relation to reference cells was observed for application of SNPs at higher concentrations (IC25, IC50). Fluorescent microscopy did not reveal any visible changes of cellular F-actin of cells exposed to SNPs. The presence of nanoparticles inside the cells was confirmed by transmission electron microscopy.

References:
AFM investigation of mechanical properties of glioblastoma multiforme cells and their relation to motility

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Glial tumors are clinically classified in 4 groups according to their malignancy level. Glial tumors belonging to the IV group are called Glioblastoma Multiforme (GBM) and they are among the most aggressive brain tumors. In the recent years the mechanical phenotype of cells has been recognized as a valuable marker of their malignancy level \cite{1-3}. Here we studied by AFM the mechanical behavior of U87MG cells when exposed to a drug which interferes with their cytoskeleton affecting also their migration ability. We found that U87MG cells exposed to the tested drug presented a decreased migration potential which is correlated with an increased stiffness of the cells and with a loss of polarity. By exploiting AFM Dynamic Mechanical Analysis we also characterized the behavior of the cells for different probing frequencies. By exploiting immunofluorescence microscopy we also investigated the effect of the tested drug on the reorganization of the cell cytoskeleton finding a strong increase of the presence of stress fibers.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{AFM_images.png}
\caption{AFM images (error mode) and Young Modulus maps of untreated U87 mg cells and of cells exposed to the tested drug for 24 hours}
\end{figure}

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\cite{3} M. Lekka, Bionanoscience, 6 (2016) 65
Extraction of corneal micromechanics with AFM nanoindentation using ex vivo porcine eye models and intraocular pressure control

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Abstract: The vision impairing diseases such as keratoconus and glaucoma are related to changes in mechanical properties of ocular tissues. Eye biomechanics has been studied with AFM nanoindentation mainly for the cornea, sclera and limbus [1]. However, samples in the form of dissected tissue cuts were usually used, which neither assured physiological conditions nor enabled measurements for different levels of the intraocular pressure (IOP). In this work, we demonstrate AFM nanoindentation of an intact porcine eyeballs for different IOP values (Fig.1). We developed custom 3-D printed ocular holder, and the eye was attached to the IOP control unit. Indentation force curves at the corneal apex were acquired for the IOP of 15, 20, 25 and again 15 mmHg, and the Young’s modulus was extracted using Hertz model. Although the penetration depth of the AFM tip reached only few micrometers (only the corneal epithelium), even small changes of the IOP proved to show a direct influence on measured Young’s modulus. We will also discuss the feasibility of mounting the eye globe in AFM instrument and the advantages of this new approach in studying corneal biomechanics.

Fig. 3. AFM nanoindentation for the measurement of corneal elastic properties. (a) Concept of the experiment. (b) Young modulus histograms of the porcine eye cornea measured with AFM at different IOP levels.

References:
Most ionizing radiation in water ends in an avalanche of low energy electrons which play a dominant role together with OH-radicals in damaging DNA. In the present study we irradiated plasmid DNA with electrons (primary energy 30keV) under physiological conditions, performed with as well as without Ectoine. Ectoine is a compatible solute, synthesized and accumulated in molar concentration within bacteria to withstand osmotic stress or different other stressors [1].

Plasmid DNA (pUC19, 2686 bp) was studied due to its supercoiled isoform which is highly sensitive to radiation damage. In biochemistry gel electrophoresis is applied for structural analysis of DNA. Although it is a standard technique, a reliable discrimination of short fragments caused by radiation is often difficult [2]. AFM is also commonly used for imaging susceptible biomolecules [3] and, since it is based on a single molecule observation, for analysis of contour lengths of linear DNA as well [2,4]. Therefore, in our study the structural changes in plasmid DNA after irradiation with different doses were quantitatively analyzed by means of intermittent contact AFM. The figure shows representative AFM images of electron irradiated pUC19 DNA (bar=200nm). For AFM imaging the DNA was chemically fixed on ultra-smooth mica. As can be clearly seen, with increasing radiation dose the number of undamaged DNA declines and fragmented DNA arises (A, B). In aqueous Ectoine solution (1M) the effect of radiation on DNA is dramatically depressed. Ectoine apparently confers protection even against high radiation: the plasmids remain predominantly in the supercoiled isoform (D). Therefore, we strongly believe that Ectoine is a potent protective substance of DNA against ionizing radiation [5].

References:
Normal and Cancer Associated Pancreatic Fibroblasts Shape Modulation is mediated by Transforming Growth Factor β and Matrix Stiffness

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Abstract: Tumor microenvironment (TME) consists of stromal cells (including fibroblasts and cancer associated fibroblasts-CAFs), the extracellular matrix (ECM) and a myriad of soluble factors in the extracellular milieu, whose importance in cancer progression and metastasis is indisputable. In many types of tumors, including pancreatic cancers, the complex interplay among TME components leads to remodeling and overproduction of tumor ECM, resulting in a desmoplastic reaction. Therefore, desmoplasia is a cancer-specific type of fibrosis, characterized by the presence of CAFs and the accumulation of ECM proteins, such as collagen type I. A crucial role in desmoplasia development is thought to be played by CAFs, while other factors such as transforming growth factor beta (TGF-β) and ECM stiffness are also of great significance [1,2]. However, the mechanism underlying desmoplasia development is not yet fully understood. In the present work, Atomic Force Microscopy (AFM), fluorescence microscopy and image processing techniques were used to investigate the effect of TGF-β and collagen-induced stiffness on pancreatic fibroblasts and CAFs with regards to several cellular morphodynamic characteristics. More specifically, alterations in cell shape, cell spreading and stress fibers orientation were assessed, in the presence or absence of TGF-β, in three different collagen stiffness conditions. Furthermore, real-time PCR was employed to evaluate the expression of specific genes, such as Rac, in the presence and absence of TGF-β and under the influence of different collagen-stiffness conditions. Our results show that TGF-β and collagen stiffness significantly affect CAFs basic morphodynamic characteristics, such as cell elongation, cell spreading, and stress fiber orientation, while this was not the case for normal fibroblasts. Moreover, a significant correlation was revealed between cell spreading and Rac expression in both cell lines. Although more research is needed to elucidate the exact involvement of TGF-β and ECM stiffness in desmoplasia, these findings provide new insights that need to be taken into consideration for understanding of desmoplasia or even for the development of novel therapeutic approaches for treating cancer having TGF-β as a target molecule [4].

References:
Electrical and morphological characterization of bacterial polar flagella

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Bacterial flagella are long, thin and whip-like appendages that allow the bacteria to move towards the nutrients and other attractants. They are mainly composed of many protein subunits called flagellin arranged in several intertwined chains. These flagella can be located in different places depending on the bacterial species. In particular, those located at one extreme of the bacterial cell are referred to as polar flagella1.

In this work, we perform a comparative study of the morphological and dielectric properties of single flagellum belonging to two different bacteria types, namely, Shewanella oneidensis MR-1 and Pseudomonas aeruginosas PAO1. To this end, we used the Atomic Force Microscope (AFM) to perform topographical images of the bacterial cell and its flagellum, and the Electrostatic Force Microscope (EFM), coupled to finite element numerical simulations, to determine its dielectric properties. The dielectric results obtained are in agreement with the results obtained for another protein biological nanostructure, the tail of virus particles3.

Figure: a) Topographical AFM image of the Shewanella oneidensis MR-1 flagellum and b) its cross-sectional profile along the line in (a). (d)-(f) EFM images at different constant tip-sample heights and c) cross-sectional profile along the lines in (d)-(f).

References:
Observing cell motility using an AFM – how fast can we go?

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The actin cytoskeleton is responsible for a wide range of cellular processes including polarity, morphogenesis and migration. Moreover, cytoskeleton dynamics are involved in many cell signaling pathways such as endocytosis and cellular division. Traditional AFM imaging has not been able to compete with optical microscopy techniques in terms of imaging speed when studying living cells. However, recent developments in time-lapse, high-speed AFM imaging and dedicated Bio-AFM probes have now made it possible to observe cytoskeleton dynamics of unlabeled living cells with both nanometer image resolution and an increased temporal resolution that is now relative to many cellular processes (on the order of seconds).

In this work have used high-speed AFM imaging to study the effect of a small molecule clathrin-mediated endocytosis inhibitor Pitstop-2 [1] on global single cell motility. This inhibitor binds at least two members of small GTPase superfamily Ran and Rac1, consequently impairing overall cell motility and nucleocytoplasmic transport. We have observed that 7.5µM Pitstop-2 dramatically inhibits lamellipodial dynamics and causes gradual dismantling of cortical actin network within minutes of its application on living endothelial cells (Ea.hy 926). The effect was fully reversible and cell’s activity was recovered by washing out Pitstop-2 by medium exchange. The results of our high-speed AFM studies were corroborated by light microscopy studies during which the cells were imaged for an initial period of 30 minutes then exposed to Pitstop-2.

![AfM imaging reveals that 7.7µM Pitstop-2 inhibits lamellipodial dynamics and causes gradual dismantling of cortical actin network within minutes of application to living endothelial cells.](image)

Studying viral disassembly by High Speed Atomic Force Microscopy

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The complexity of the molecular processes involved in the virus life cycle has intrigued researchers since a long time. Efficient self-assembly is a remarkable and general feature of virus capsids. The study of these assembly processes will provide essential information to understand the viral life cycle. As this is a highly dynamic process with short-lived intermediates, there is a lack in experimental understanding. Here we present preliminary results of rod-like virus disassembly using High Speed Atomic Force Microscopy (HS-AFM) imaging techniques at a high spatio-temporal resolution.
DNA molecule carries genetic information, enabling a survival of all known living organisms, therefore for decades it is in central interest of many research groups. The topology and chemical structure of DNA is very well defined, therefore it could become a standard sample for nano-spectroscopic methods [1]. High sensitivity and chemical selectivity of Tip-enhanced Raman Scattering (TERS) brought new insights into DNA studies at nanoscale [1, 2]. AFM-TERS was applied in order to follow conformation along single DNA strands [2] and monitor local molecular changes induced upon fixation procedures [1]. High enhancement achieved in gap mode of STM-TERS allowed for DNA mapping presented in figure 1. Our results proved that TERS, which provide molecular information from very small amount of sample located at nanometric distance from plasmonic nanoantenna, is a very efficient tool in studies of single DNA strands. However, under physiological conditions DNA occurs as chromatin packed into chromosomes. Chromosome thickness is of the order of 100 nm therefore, it would not be possible to fully explore their structure and composition by TERS, which could probe just their surface. For chromosome studies we have applied atomic force microscopy combined with infrared spectroscopy (AFM-IR). This technique makes use of AFM cantilever sensitivity to thermal expansion of the sample irradiated by laser tunable in whole medium-infrared spectral range. AFM-IR coupled with Principal Component Analysis (PCA) allowed for deep investigation into chromosome structure and confirmed that chromosome areas containing euchromatin and heterochromatin are distinguishable based on differences in the degree of DNA methylation.

**References:**


AFM-based physical-mechanical image of the cell surface

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phenotyping of cells on base of their mechanical properties using a variety of techniques including atomic force microscopy (AFM) is currently widely discussed [1, 2]. The key element of the cell “mechanical phenotyping” is a formation of the physical-mechanical image of the cell surface, or, more specifically, cell surface layer. Using AFM, the physical-mechanical properties of the cell surface layer of the thickness of a few hundred nanometers and lateral area of some square micrometers can be studied. These properties are described by many parameters depending on the AFM mode, device type, and physical model: the elastic moduli, the parameters of viscoelasticity, the parameters of friction and adhesive forces, and others [2-4]. In general, the physical-mechanical image of the cell surface layer represents the complex of the parameters characterizing the physical-mechanical properties at the microscale (average parameters estimated over the surface areas of a few square micrometers) and nanoscale (parameters of spatial distribution with nanoscale resolution) levels.

In the work, on the base of the AFM-data obtained in a contact mode we analyze the physical-mechanical images of the surface of various cancerous cells (MCF-7, HEp-2c, A549) and normal cells (erythrocytes, thymocytes, fibroblasts, mesenchymal cells) under the normal and pathological conditions including the action of oxidizing agents and ageing. We demonstrate that the behavior of some characteristics of the microscale friction force maps such as averaged spectrograms (obtained by the Fourier transform), the roughness ($R_q$), dependence of the fractal dimension on Z-scale factor ($D_F=f(t)$ [5]) depending on the scan size and resolution is determined by the features of the surface distribution of the cell physical-mechanical properties. Using the examples of human fibroblasts, human epithelial cells (MCF-7, HEp-2c, A549) and thymocytes of different aged rats we show that the complexity of the surface distribution of the physical-mechanical properties at the nanoscale level is related to the values of microscale mechanical parameters (Young’s modulus, friction and adhesive force parameters) and whole cell morphological parameters.

Conclusion. The physical-mechanical image of the cell surface based on AFM-data is a complex characteristic of the mechanical behavior of cells and base of the mechanical phenotype of cell.

References:
AFM imaging reveals the role of mRNA binding proteins in the formation of pathological protein aggregates

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TDP-43 and FUS, two RNA-binding proteins (RBPs) harboring a low complexity domain (LCD), have been recently the subject of increased attention due to their role in neurodegenerative diseases like Amyotrophic Lateral Sclerosis (ALS) and FrontoTemporal Lobar Degeneration (FTLD). In non-pathological conditions, these proteins are mostly found in the nucleus where they participate to mRNA biogenesis and splicing but they are also present in the cytoplasm and participate to mRNA transport and localized translation, which are of critical importance for neuron physiology. In neurons of ALS and FTLD patients, TDP-43 and FUS form insoluble cytoplasmic aggregates that can further spread the disease to other areas of the brain. Documenting the early stages of the formation of TDP-43 or FUS protein aggregates and the role of mRNA stress granules that are considered as critical intermediates for protein aggregation is therefore of interest to understand disease propagation. Here, we developed a single molecule approach via atomic force microscopy (AFM), which provides structural information out of reach by fluorescence microscopy. In addition, the aggregation process can be probed in the test tube without separating the interacting partners which would affect the thermodynamic equilibrium. The results demonstrate that isolated mRNA molecules serve as crucibles to promote TDP-43 and FUS multimerization leading to the formation of mRNA granules containing TDP-43 and FUS aggregates. Interestingly, TDP-43 or FUS protein aggregates can be released from mRNA granules by either YB-1 or G3BP1, two stress granule proteins that compete for the binding to mRNA with TDP-43 and FUS. Altogether, the results indicate that age-related successive assembly/disassembly of stress granules in neurons, regulated by mRNA-binding proteins such as YB-1 and G3BP1, could be a source of protein aggregation.
Reversible enzymatic actuation of an intrinsically disordered protein brush

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Intrinsically disordered protein (IDP) domains are increasingly appreciated to mediate protein-protein interactions that underlie a broad range of cellular processes, including signal transduction, transcription, cytoskeletal assembly, and organelle biogenesis. IDPs are also attractive material building blocks, because these polymers can be synthesized with perfect monodispersity and monomeric precision. Moreover, IDP conformation and function are often gated by specific intracellular stimuli, such as ligand binding and enzymatic modification, raising the exciting possibility that one may be able to create IDP-based sensors and smart materials. Intrigued by these concepts, our laboratory recently developed a polymer brush surface coating system using recombinant IDPs based on the C-terminal sidearm domain of the heavy subunit of the neurofilament complex (rNFH-SA).\textsuperscript{1,2} Using atomic force microscopy (AFM) to measure brush thickness, we successfully swelled and collapsed these brushes by changing ionic strength and pH. We also demonstrated our ability to irreversibly control brush thickness using site-directed proteolytic cleavage. We now describe reversible control of rNFH-SA brush thickness through enzymatic addition and removal of serine phosphates within the sequence. Briefly, we phosphorylated rNFH-SA in vitro via a two-step mechanism in which we used mitogen-activated protein kinase kinase (MKK) to activate mitogen-activated protein kinase 1 (ERK2), and then applied the activated ERK2 to phosphorylate rNFH-SA. SDS-PAGE and mass spectrometry revealed a strongly activity-dependent phosphorylation of rNFH-SA serines. To determine if phosphorylation could be used to modulate the swelling of IDP brushes, we assembled rNFH-SA brushes onto glass surfaces, phosphorylated these layers in situ, and measured brush heights with AFM. Phosphorylation increased the thickness of rNFH-SA brushes, with the magnitude of swelling dependent on pH and ionic strength. This phosphorylation-induced swelling could be fully reversed through alkaline phosphatase-mediated dephosphorylation. Moreover, phosphorylated rNFH-SA brushes could be dramatically condensed upon treatment with micromolar concentration of divalent cations, consistent with a classical chelation mechanism. Together our results illustrate the potential power of multi-site phosphorylation for controlling IDP steric function in biology and biomaterials technology.


Investigating the influence of LRP-1 silencing on the migratory potential of MDA cancer cells by dynamic cell studies and atomic force microscopy

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The low-density lipoprotein receptor-related protein 1 (LRP-1) mediates the clearance of various pericellular molecules such as proteinases involved in cancer progression. As a potential target against cell invasion, LRP-1-dependent endocytosis has been widely studied, and was found to have prognostic value. In addition to this role, LRP-1 is able to regulate other membrane-anchored proteins. Modulating extracellular proteolysis through LRP-1 could thus affect cell-to-matrix adhesion/de-adhesion balance and cytoskeleton dynamics, and could lead to contradictory effects depending on the cellular context. Recent data has indeed provided evidence that cell migration can be decreased by LRP-1 silencing, despite an increase in proteolysis.

Here, we correlated cellular dynamics studies and atomic force microscopy (AFM) experiments carried out on a human breast cancer cell line MDA-MB-231 silenced for LRP-1. Results show that LRP-1 silencing induces changes such as decrease in cell velocity and altered directional persistence, both on gelatin and collagen. Young's modulus determination by AFM shows that these morphological and behavioural alterations are correlated with a significant increase in Young's modulus on gelatin, and collagen. On collagen, the migration speed of silenced cells is significantly decreased, in comparison to their normal counterparts. They seem to exhibit the same tendency on gelatin.

These results show that AFM is a promising non-invasive tool to establish a quantitative link between mechanical and adhesive cellular profiles and the evolution of a cellular disorder such as metastasis.
Quantification of Fenestrations in Liver Sinusoidal Endothelial Cells by Atomic Force Microscopy

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Abstract Body: Over the years evidence accumulated on the unique morphology of liver sinusoidal endothelial cells (LSECs), characterized by lack of basement membrane and presence of fenestrations – transmembrane pores – in their membrane$^{1,2}$. Fenestrations, clustered in sieve plates, are involved in dynamic transport of solutes and particles between the vascular space and the space of Disse. Sensitive to variety of chemical and physical factors, fenestrations in live cells alter their structure rapidly. We apply imaging methods based on Atomic Force Microscopy (AFM), to describe general morphology and characteristic features of LSECs, namely: mean fenestration diameter, porosity, and fenestrations frequency in glutaraldehyde wet-fixed cells$^{3,4}$. Moreover, we show that using force imaging mode visualization of fenestrations in live LSECs is possible. From data on both the topographical and nanomechanical properties of the selected cell areas collected within 1 min, we traced the dynamic rearrangement of the cell actin cytoskeleton connected with the formation or closing of cell fenestrations. We show that AFM allows for describing fenestrations dimension also in live cells (Figure).

We conclude that higher level of fixation and dehydration causes shrinkage of the cell cytoskeleton and consequently reduction of the fenestration diameter (∼180 nm in live cells, ∼140 nm in 1% glutaraldehyde wet-fixed cells and up to ∼100 nm in the fixed dried cells).

Figure. A comparison of sieve plate in live (left) and 1% glutaraldehyde wet-fixed cell (right) measured using force imaging mode.

References:
Young’s Modulus and Mechanotransduction in Actinin 1-Knockout Fibroblasts

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Focal adhesion proteins are crucial mediators for cell adhesion to the substratum, cell signaling and force transduction. They assemble to focal complexes (FC) and focal adhesions (FA), which link the highly dynamic regulated actin cytoskeleton to proteins of the extracellular matrix (ECM) and thereby transfer mechanical forces to the nucleus. This effects the regulation of mechanosensitive genes and other cell responses. Disruption of proteins involved in this mechano-transductive cascade, such as the non-muscle alpha Actinin (Actinin 1 and Actinin 4) in NIH 3T3 fibroblasts results in impaired formation of filopodia, actin stress fibers, cell migration and adhesion [1]. In wild-type condition alpha Actinin 1 and 4 bind the cytoplasmic tails of the beta-subunit of Integrin and crosslink actin filaments [2]. Using atomic force microscope (AFM), we could show a decrease of the Young’s modulus [3] as well as an increasing cell height and cell adhesion area of NIH 3T3 fibroblasts, where Actinin1 is knocked out. A knock-in of Actinin 1 variants, with defects in either their actin- or their integrin-binding site, could not rescue this phenotype. These cells also show a disturbed actin cytoskeleton, since the actin stress fibers are much thinner and non-cell spanning as shown in figure 1.

To answer the question whether the Actinin-deficient cells still transduce mechanical signals, we will analyze expression levels of mechanosensitive genes and genes encoding for focal adhesion proteins using quantitative RT-PCR. For in vivo studies, we will monitor the spatial distribution of the fluorescent labeled mechanosensitive transcription factors MRTF and YAP to investigate their responsiveness to external forces.

![Figure 1](image-url)

Figure 1. Young’s modulus (top) and hight image (bottom) on the of NIH 3T3 fibroblasts, imaged with JPK Nanowizard 4.

References:
[1] Timper et al, submitted manuscript
Kelvin-probe Force Microscopy to map glycation of proteins

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Glycation, that is, the uncontrolled reaction of sugars with proteins, is thought to be a major contributor to the pathological effects of diabetes and tissue dysfunction during natural ageing [1]. At the nanoscale level, glycation has been shown to increase the stiffness of structural proteins of the extracellular matrix such as collagen fibrils [2]. However, it has also been speculated for a long time that glycation could alter the surface charge profile of matrix proteins, with obviously serious consequences on cell-matrix interactions and, ultimately, the health of the whole organism [3].

Here, we show, using Kelvin-probe Force Microscopy (KFM), an AFM-based method to map the surface potential, that exposure to glutaraldehyde does not only increase the stiffness, but also reduces the surface charge of collagen fibrils to a more negative value. Glutaraldehyde is a well-known protein cross-linking agent and it reacts in a similar way as sugars with primary amines of proteins in the first stages of glycation. As KFM is still relatively uncommon in the field of biophysics, the particular aspects in terms of sample preparation, experimental design and signal-processing, as well as the interpretation of KFM data from biological samples will also be discussed at a more general level.

Topography (left) and surface potential (middle) of a collagen fibril determined by KFM (adapted from [4]). Scale bar = 200 nm. Right: Average surface potential of collagen fibrils (GA = exposed to glutaraldehyde solution; control = exposed to water).

References:
Integrated Spinning Disk Confocal and Atomic Force Microscopy Reveals Anisotropic Indentation Geometry on Living Cells

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Linking cell mechanical properties to specific cellular structures is of high interest to many cell biologists. One of most frequently used techniques for assessment of cell mechanical properties is the indentation experiments conducted with the atomic force microscope (AFM), due to AFM's relative ease of operation, its high precision of force measurement, and high spatial resolution. Several mechanical models trying to explain cell mechanical behavior have emerged recently, and the AFM indentation experiments are well suited to test such models. However, without visualization of the cell structure, it is unlikely that indentation experiments will be able to distinguish among these models. Here, we used the AFM in conjunction with a spinning disk confocal (SDC) microscope to directly visualize AFM indentation of living cells (3T3 fibroblasts and MDA-MB-231 breast cancer cells) with high spatial and temporal resolution. With live cell imaging probes to fluorescently label F-actin, microtubules, and membrane, we directly observed structural changes during the indentation process of a living cell with a spherical indenter. We used three protocols to observe the AFM indentation process with the SDC microscope, allowing the capture of either a quasi-static or dynamic picture of the indentation. The presence of apical (perinuclear actin cap) stress fibers correlated with cell stiffness. Stiff 3T3 cells had dense and thick apical stress fibers; this caused an anisotropic indentation geometry. Soft MDA-MB-231 cells lacked apical stress fibers and isotropic indentation geometry was observed. Application of a jasplakinolide derivative-based actin probe (SiR-actin) led to significant increase in cell stiffness, indicating that this probe should be used with caution in studies where mechanical properties of cells are measured. We expect that this integrated imaging and biophysical approach will contribute to a more comprehensive understanding of both normal and altered cell mechanical behavior.

Figure 1. SDC imaging during the AFM indentation of MDA-MB-231 cell. (a) The single optical plane. (b) The reconstructed vertical cross-section along the marked line.
The unholy alliance of platelets and tumor cells

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Background: It is well known that platelets support cancer cells in nearly every step of metastasis [1]. Cancer cell-induced platelet activation is a trick of cancer cells to “hijack” the services of platelets. Activated platelets bind to cancer cells and facilitate tethering and arrest of disseminated cancer cells in the vasculature, enhance invasive potentials and thus extravasation of cancer cells. However, the exact mechanisms how platelets influence blood-borne metastasis remain poorly understood.

Methods: We used a single-cell force spectroscopy (SCFS) approach to quantify the platelet - tumor cell adhesion under control conditions and upon application of inhibitors (approved pharmaceuticals) for cell adhesion molecules. FastTapping and PeakForce QNM were used to follow the dynamics of tumor cell – platelet interaction. Time dependent uptake of platelets by tumor cells was investigated and quantified by confocal laser scanning microscopy (CLSM) and fluorescence activated cell sorting (FACS).
We used human non-small cell lung cancer cells (A549) and freshly isolated human platelets.

Results: We established a method to quantify the adhesion (max. adhesion $F_A$ and detachment work $W_D$) of platelets to tumor cells at the single cell level. Under control conditions we found for $F_A$: 872 pN and for $W_D = 3.7$ fJ. Sigmoidal dose-response fits revealed $IC_{50}$ (half maximal inhibitory concentration) values and max. effects for inhibitors of P-selectin (Tinzaparin®), Integrin $\alpha_{IIb}\beta_3$ (Tirofiban®) and VLA4 (Tysabri®). All $IC_{50}$ values are far below their therapeutical plasma levels and showed a max. decrease of adhesion in the range of 38–67%.
Current research indicates a cloak-formation of platelets around circulating cancer cells. We never observed such cloak formation. FastTapping revealed that platelets disappear into tumor cell membrane upon contact within 15 min. PeakForce QNM showed not only the decrease of platelet size and height on tumor cell membrane until disappearance but also a change of mechanical properties around the spot of the hypothetical uptake.
Confocal fluorescence microscopy of PKH67 and FITC-CD42a stained platelets revealed a dynamin-dependent uptake of platelets. Platelet proteins appear 10 min after contact in the plasma membrane of tumor cells as well as in tumor cell lysosomes. FACS analysis confirms the uptake of platelet-specific proteins in tumor cells within 30 min.

Conclusion: Binding of platelets to tumor cells is a crucial step for the malignancy of cancer. Our findings allow a closer insight into this process and may contribute to the development of new therapeutical strategies to fight cancer.

Molecular mechanisms guiding the adhesion of *Staphylococcus aureus* clumping factor B to the skin protein loricrin

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Abstract: Bacterial pathogens that colonize host surfaces are subjected to physical stresses, such as fluid flow and cell-surface contacts. How bacteria respond to such mechanical cues is an important yet poorly understood issue. *Staphylococcus aureus* uses a repertoire of surface proteins to resist shear stress during the colonization of host tissues, but whether their adhesive functions can be modulated by physical forces is not known. Here we use force nanoscopy to show that the interaction of *S. aureus* clumping factor B (ClfB) with the squamous epithelial cell envelope protein loricrin is enhanced by mechanical force. We find that ClfB mediates *S. aureus* adhesion to loricrin through weak and strong molecular interactions, both in a laboratory strain and in a clinical isolate. Strong forces, by far the strongest measured so far for a bacterial protein and its ligand, are consistent with a high-affinity “dock, lock and latch” binding mechanism involving dynamic conformational changes of the adhesin. We demonstrate that the strength of the ClfB-loricrin bond increases as mechanical force is applied. These findings favour a two-state model whereby bacterial adhesion to loricrin is enhanced through force-induced conformational changes in the ClfB molecule, from a weak-binding folded state, to a strong-binding extended state. This newly described force-sensitive mechanism may provide *S. aureus* with a means to finely tune its adhesive properties during colonization of host surfaces, helping cells to attach firmly under high shear stress and, to detach and spread under low shear stress.
Our aim is to understand what interactions between living cells and extracellular matrix (ECM) components are crucial for successful cell culture. For this purpose we use direct force measurements between living cells and various ECM materials. Knowing these mechanisms better could help us to create improved tissue models for regenerative medicine and drug testing. It is known that stem cells respond to the physical and chemical properties of culturing matrices, but so far little is known about the underlying mechanisms and its outcomes in stem cell behavior. The studied cells were human pluripotent stem cells (line WA07). This cell line has the ability to differentiate into all kinds of cells found in the body, but it is very delicate and challenging to culture and has not been studied with AFM before. For comparison, more robust human hepatocarcinoma cells (HepG2) were also probed. The cells were cultured on the substrate and the biomaterials were coated on silica probes that were used attached to tipless AFM cantilevers and used as colloidal probes (Figure 1). The studied biomaterials were cellulose nanofibers (CNF), laminin, and collagen I and collagen IV. While collagen has been studied previously, with other cell types than here, cellulose nanofibers are rather novel cell culturing materials and there are many questions to answer related to the interaction with cells. We also studied the difference between forces in the presence of living and dead cells to distinguish between specific interactions and interactions due to e.g. surface charge. The measured interactions correlated well with observations during cell culturing. The adhesive forces depended on if the cells were live or dead, but opposite trends were found for the two studied cell lines. However, in all cases the forces were dependent on the time the surfaces were kept in contact and obviously on the biomaterial. We also used surface plasmon resonance to study the adsorption behavior of the same cell lines onto some of the materials. This study shows the applicability of colloidal probe microscopy to get a better understanding of the interactions between cells and various biomaterials. This knowledge is important for tissue engineering.

Figure 1 Schematic of the colloidal probe setup and 1x1 µm AFM image of CNF coated probe.
A New Mechanism of Autoimmune HIT Caused by a Subset of Antibodies

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**Background**: Antibodies recognizing complexes of the chemokine platelet factor 4 (PF4/CXCL4) and polyanions (P) cause the prothrombotic adverse drug reaction heparin-induced thrombocytopenia (HIT) normally in the presence of heparin. In patients with autoimmune-HIT, antibodies activate platelets even in the absence of heparin. The binding mechanism of these antibodies remains unclear.

**Aims**: We used multiple analytical techniques to elucidate the binding mechanism of these antibodies.

**Methods**: ELISA, HIPA, single-molecule force spectroscopy, scanning electron microscopy, isothermal titration calorimetry, and dynamic light scattering were used.

**Results**: Antibodies with binding forces of $\sim$60\,÷\,100\,pN activate platelets in the presence of polyanions, while a subset of antibodies with binding forces $\geq$100\,pN and low thermal off-rates self-clusters PF4 in the absence of polyanions. Their binding to PF4 releases more energy than heparin binding ($\Delta H = -3.5 \pm 0.86 \times 10^7$ cal/mol vs $\Delta H = -7.26 \pm 1.36 \times 10^3$ cal/mpl). The PF4/antibody complexes subsequently allow binding of polyanion- dependent antibodies resulting of large immunocomplexes, which finally induce massive platelet activation.

**Conclusion**: We have discovered a new binding mechanism in autoimmune HIT caused by a subset of antibodies from patients with autoimmune-HIT. Antibody- mediated changes in endogenous proteins triggering binding of otherwise non- pathogenic (or cofactor-dependent) antibodies may also be relevant in other antibody- mediated autoimmune disorders.

**Keywords**: platelet factor 4, polyanions, PF4/Heparin antibody, binding force/energy, thermal off-rate, size, platelet activation

Interactions of Xenobiotic-free Biomaterials and Cells for Better Tissue Models

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The direct interactions between living human pluripotent stem cell (hPSC) and hepatocarcinoma cells HepG2 with different biomaterials were studied using colloidal force microscopy (CPM). Cell interactions with their surrounding extracellular matrix are highly important for normal cell behaviour \textit{in vivo} and \textit{in vitro}. Knowing these mechanisms better could help us to create improved tissue models for regenerative medicine and drug testing. These interactions occur at a nanoscale in liquid environment and are thus difficult to study and still quite poorly known. Atomic force microscope (AFM) has excellent properties for these studies and it has been applied to test cell and biomaterial interactions. Here we test for the first time cell types important for drug industry: hPSC line WA07 owns capability to differentiate into all cells found in humans and HepG2 cells are widely used to test hepatotoxicity of novel drug compounds. Human PSCs offer also great expectations for regenerative medicine. These two cell lines have very different behaviour \textit{in vitro}; WA07 cells are highly sensitive and only few materials are suitable to culture this cell line, on the other hand HepG2 cell line is robust and adapts into various materials. The materials used for these experiments are xenobiotic-free in order to avoid cross-species pathogens, and are already used for culturing either or both of these cell lines. Human collagens I and IV are materials widely studied and used, cellulose nanofibers (CNF), and human laminin-521 (LN-521) are novel materials. Since the hPSCs do not survive as individual cells we applied the biomaterials on the colloidal probe and the living cells were placed on the substrate. We found contact-time dependent attractive forces between all the used cells and biomaterials. The magnitude of the force correlated with the cell attachment seen in cell cultures; LN-521 had strong interaction with the both cell lines, CNF were quite inert and collagens varied with the cell type. In this study we show that the contact-time dependent forces that excellently correlate the \textit{in vitro} cell culture can be shown with CPM. With this method it is possible to test suitability of new materials and explain more detailed the interactions that affects to the cells. With this knowledge it is possible to create better engineered tissues.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure.png}
\caption{Optical microscope image of HepG2 cells cultured for 24 hours on laminin-521 and representative AFM retraction force curves at contact times 1 s, 10 s, and 30 s.}
\end{figure}
Atomic force microscopy in research of bacteria interactions with the blood neutrophils

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Abstract: The neutrophil granulocytes and the proteins of the complement system play an important role in the nonspecific defense of the human organism [1]. Atomic force microscopy (AFM) gives the possibilities to study neutrophil-bacteria, neutrophil-lipopolisaccharide (LPS), complement-bacteria interactions. The changes of neutrophils parameters were found under bacterial (S. aureus, P. mirabilis) and LPS conditions. The character of these changes was different. After interaction with both non opsonized and opsonized bacteria the neutrophils formed classical pseudopodia. After interaction with opsonized bacteria these pseudopodia formed more active and earlier. If the bacteria were used in higher concentrations, then a process of incomplete phagocytosis or neutrophil death by NETosis were observed. Under the influence of LPS the neutrophils initially swell, then death by the apoptosis. In the process of apoptosis, apoptotic bodies were budded. Measurement by FS-spectroscopy showed that during the formation of apoptotic bodies the rigidity of the membrane-cytoskeleton complex increases, whereas before the detachment of the apoptotic bodies, the rigidity of the cell decreases.

This work was supported by the Russian Science Foundation, project 16-14-10179.

Figure. Apoptosis neutrophil granulocytes under LPS condition (70 min co-incubation)

References:
Miniaturized Magnetic Beads for Single-molecule Force Measurements

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We report a novel atomic force spectroscopy technique for biomolecular measurements at single molecular level. We perform the experiments by manipulating the magnetic beads using an electromagnet against a stationary AFM cantilever. Keeping the cantilever stationary and performing the actuation electromagnetically via drastically miniaturized structures (using beads with a diameter of 2.4 μm) provide advantages for stability, dynamics and resolution. We have performed biomolecular pulling experiments using heparin-functionalized magnetic beads to probe the interactions of heparin-FGF2 interaction at single molecule level. We are able to to vary the loading rate with more than five-orders of magnitude (Figure c).

Figure. (a) Structure of the FGF-2 (on cantilever) and Heparin (on magnetic bead) molecular pair (b) a sample force curve with 70 pN/s loading rate showing 60 pN unbinding force, (c) unbinding forces vs loading rates for Heparin-FGF2 pair by 2 different actuation methods (magnetic or piezo) on 2 different AFM setups (custom MANAQA AFM or Bruker Dimension Edge).

References:
Dynamic force spectroscopy (DFS) is a powerful single-molecule approach to measure the forces required for intermolecular bond rupture or protein unfolding. Experimentally, a probe functionalized with a biomolecule is moved at a range of velocities and the resulting unfolding or unbinding forces are recorded\textsuperscript{1,2}. Recent theoretical developments have suggested the contribution of the finite response time of the probe to the measured forces\textsuperscript{3,4}. To assess this effect on the unfolding forces from DFS, we carried out high-speed force spectroscopy\textsuperscript{5} on titin I91 using cantilevers with a range of response times (milli- to microsecond). In agreement with predictions, our preliminary results suggest a non-negligible effect when the unfolding time approaches the response time of the cantilever. This effect may overestimate the unfolding forces of titin I91 by 10-20\% at the highest speed accessible to each cantilever.

References:

Poster Session I

Tuesday, September 5, 2017, 18:00-19:00

P1 Justyna Bobrowska, Poland
   AFM and ToF SIMS investigation of melanoma elasticity and surface properties

P2 Guido Caluori, Czech Republic
   A non-invasive electromechanical system to study cardiac excitation-contraction coupling

P3 Aitziber Eleta Lopez, Spain
   Hydration-dependent buckling and local defects: The molecular surface structure of a plant virus

P4 Benedict Fels, Germany
   Transient receptor potential channel 1 (TRPC1) mediated mechano-signaling in murine pancreatic stellate cells

P5 Cecile Feuillie, Belgium
   Molecular interactions and inhibition of the staphylococcal biofilm-forming protein SdrC

P6 Alessandra Griffo, Finland
   Single molecule force spectroscopy study on modular resilin fusion protein

P7 Bastian Hartman, Germany
   An optical fiber-based force sensor for the detection of cartilage degeneration: comparing sensor sensitivity to atomic force microscopy

P8 Ignasi Jorba, Spain
   Multiscale Nonlinear Mechanics of Lung Extracellular Matrix

P9 Patrick Mesquida, Austria
   Pitfalls of practical KPFM-phase-tuning leading to polarity reversal on biological samples

P10 Arkadiusz Kozioł, Poland
   In vitro enzymatic-induced DASP nanostructure reorganization

P11 Frank Lafont, France
   AFM Automation for Single-Cell Scanning
P12 Martina Maase, Germany
Adequate endothelial response to functional EnNaC-inhibition as a marker for endothelial (dys-)function?

P13 Katarzyna Majzner, Poland
Uptake of fatty acids by a single endothelial cell investigated by Raman spectroscopy supported by AFM mechanical mapping

P14 Daria Malakhova, Czech Republic
Cytoskeletal Structures and Elasticity Measures in Human MSCs

P15 Barbara Orzechowska, Poland
The elasticity of single cells as a disease marker determined by atomic force microscopy

P16 Filippo Pierini, Poland
Development and applications of atomic force microscopy combined with optical tweezers (AFM/OT)

P17 Marco Girasole, Italy
“RBC’s ageing from morphology and membrane structure: an AFM study combined with Raman and Differential Scanning Calorimetry”

P18 Andreas Rohatschek, Austria
Experimental mechanics of collagen molecules

P19 Christina Rösch, Germany
On the Way to Single Molecule Adhesion Forces of a Multidomain Protein by Force Spectroscopy

P20 Christian Spengler, Germany
Bacterial contact formation and breaking: revealing their contact area & contact characteristics on different types of surfaces

P21 Karolina Szafrańska, Poland
Primary vs Immortalized – AFM study of Liver Sinusoidal Endothelial Cells

P22 Attila Gergely Végh, Hungary
Melanoma – endothelial de-adhesion dynamics: crucial step towards brain metastasis
Melanoma is still deadly cancer developed from melanocytes (the pigment-producing cells, located in the skin epidermis). Its incidence rates are rising faster than for any other solid tumors [1]. Ongoing research into cancer provided the evidence that there is no single marker to detect any type of cancer with high sensitivity and certainty. Here, we have combined two advanced techniques, namely, Atomic force microscopy (AFM) and Time-of-flight secondary ion mass spectrometry (ToF SIMS) to examine properties of single melanoma cells, derived from different stages of melanoma progression. This approach allows for investigation of nano-mechanical as well as biochemical properties of the cells, providing a complex information about the cellular state. We compared data obtained for seven melanoma cell lines originating from various stages of melanoma progression (such as primary tumor site, skin and lung metastasis) with primary melanocytes. AFM was used for the elasticity analysis through Young’s modulus determined for each type of cell populations. ToF SIMS allowed for determination of chemical properties of cell surface. High resolution mass spectra were obtained and analyzed based on principal component analysis (PCA) [2,3]. The major advantage of AFM is its ability to work in the fluid conditions. However, SIMS experiments have to be carried out in the high vacuum, therefore, sample preparation protocol for high vacuum conditions was developed and applied [4]. In this way, we have found differences that are significant enough to distinguish cell lines derived from different stages of melanoma progression [5].

References:
[5] The work has been financed by the NCN project no DEC-2013/11/N/ST4/01860. JB is grateful to Polish National Science Center (NCN) for the financial support of the ETIUDA scholarship no DEC-2015/16/T/ST4/00358.
A non-invasive electromechanical system to study cardiac excitation-contraction coupling

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Abstract Body: The prolonged alterations of the two-stages transducing process known as excitation-contraction coupling (ECC) ultimately leads to cardiac tissue remodeling and heart failure1. The use of a highly-sensitive and non-invasive cell-based biosensor can sharpen significantly the evaluation of the in vitro models’ phenotype and novel cardiac drugs2. We have set up and tested an experimental system based on Atomic Force Microscopy (AFM) and MicroElectrode Array-based ElectroPhysiology (MEA-EP), to monitor the ECC components of human pluripotent stem cell-derived cardiac constructs, in the form of an embryoid body (EB)3. The experimental system was tested in basal condition and under drug trials against cardioactive species. The detected response confirmed an immature phenotype, showed multiple pacing sites in the cardiac constructs, and the modulation of the beating rate and force. On the road to implement a complete ECC monitoring system, the proposed combination boosts the informational content of a single analysis, thus leading to refined evaluation of in vitro models and novel cardiac drugs.

Figure. Graphical representation of the combined electromechanical system for ECC monitoring

References:
Hydration-dependent buckling and local defects: The molecular surface structure of a plant virus

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The interaction of water with the coat proteins of viruses is key to understand virus transmission, for mammalian and also for plant viruses. Viruses are known to have highly symmetric structures, often pictured as perfectly symmetric self-assembled structures. However, this knowledge is based on analysis methods that average over many virus particles, ignoring possible local defects.

In this work, Tobacco mosaic virus (TMV), the best known plant virus [1], is used as a simple model towards understanding more complex viruses. Its atomic structure has been very well characterized with several averaging methods, such as XRD and cryo-EM [2, 3]. However, we were able to resolve the TMV helical structure locally by AFM in water with a resolution of 0.2 nm/pixel. Fourier transform analysis of the protein-protein distances gives values of 2.4 nm axially and 3.5 radially, which both fit with the reported TMV standard structure [2]. Furthermore, single particle analysis in a completely hydrated environment reveals various defects of the virus capsid assembly [4, 5]. Such defects must escape standard characterization methods, and AFM is -as of today- the only means to detect them. Upon drying, the complete virus particle exhibits reversible buckling with a very small amplitude of 0.1-0.3 nm, and irregular spacings of 6-12 nm. Thus, AFM is shown to be an appropriate method to study possible defects in the symmetric structure of viruses at different hydration levels.

References:


Pancreatic ductal adenocarcinoma (PDAC) is characterized by abundant deposition of connective tissue surrounding the tumor, primarily formed by activated pancreatic stellate cells (PSCs). The dense stroma leads to altered biomechanics and high tissue pressure, which in turn also activates PSCs. It leads to enhanced secretion of cytokines, growth factors and extracellular matrix compounds, affecting the density of the tumor microenvironment and resulting in a further increase of tissue pressure. This perpetuates the biomechanical activation of PSCs and contributes to tumor progression. Here, we studied the effect of pressure on the function of murine PSCs. We postulate that the putatively mechanosensitive canonical transient receptor potential channel 1 (TRPC1) is involved in the pressure-sensing mechanism and thereby contributes to pressure-induced PSCs activation.

We used primary PSCs isolated from WT and TRPC1-KO mice, pre-incubated for 24h under elevated pressure (100 mmHg). After pressurization, mRNA expressions of different mechanosensitive channels were profiled. In parallel, we monitored cell migration using live cell imaging, measured calcium influx by means of the Mn²⁺ quench technique and the intracellular calcium concentration ([Ca²⁺]) in PSCs. In addition, we measured cytokine secretion in response to elevated pressure conditions. Cell elasticity was determined with atomic force microscopy.

Pressurization leads to reduced mRNA expression of different mechanosensitive ion channels such as Piezo1, Trpm7, Trpv4 and Trpc1. In parallel, we show that TRPC1 channels play a role in mechano-sensing and migration of PSCs in response to elevated pressure. Pressurized WT PSCs migrate 65% faster than untreated cells. In contrast, pressure-incubated TRPC1-KO showed an attenuated effect and were 22% slower than WT PSCs. Secretion of keratinocyte-derived cytokine (KC or CXCL1), CC-chemokine ligand 2 (CCL2) and Interleukin-6 is increased in WT-PSCs after pressurization, but not in TRPC1-KO PSCs. Additionally, pressure incubation leads to increased calcium influx into WT PSCs, which is virtually absent in TRPC1-KO cells. In line with this, [Ca²⁺] rises in WT PSCs by 80% upon pressurization, whereas it falls by 20 % in TRPC1-KO PSCs. Pressurization also alters the mechanical properties of PSCs. Bulk elasticity of the pressurized WT PSCs is higher than in control cells, whereas TRPC1-KO showed no increase in elasticity.

We conclude that TRPC1 channels participate in the mechano-signaling and thereby influence the activation of pressurized PSCs. Knockdown of TRPC1 is sufficient to attenuate the pressure-induced activation and could be a potential target to disrupt the positive feedback of mechanically induced activation of PSCs.
Molecular interactions and inhibition of the staphylococcal biofilm-forming protein SdrC

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*Staphylococcus aureus* forms biofilms on indwelling medical devices using a variety of cell-surface proteins. There is growing evidence that specific homophilic interactions between these proteins represent an important mechanism of cell accumulation during biofilm formation, but the underlying molecular mechanisms are still not well-understood. Here we report the direct measurement of homophilic binding forces by the serine-aspartate repeat protein SdrC and their inhibition by a peptide. Using single-cell and single-molecule force measurements, we find that SdrC is engaged in low-affinity homophilic bonds that promote cell–cell adhesion. Low-affinity intercellular adhesion may play a role in favoring biofilm dynamics. We show that SdrC also mediates strong cellular interactions with hydrophobic surfaces, which are likely to be involved in the initial attachment to biomaterials, the first stage of biofilm formation. Furthermore, we demonstrate that a peptide derived from β-neurexin is a powerful competitive inhibitor capable of efficiently blocking surface attachment, homophilic adhesion, and biofilm accumulation. Molecular modeling suggests that this blocking activity may originate from binding of the peptide to a sequence of SdrC involved in homophilic interactions. Our study opens up avenues for understanding the role of homophilic interactions in staphylococcal adhesion, and for the design of new molecules to prevent biofilm formation during infection.
Single molecule force spectroscopy study on modular resilin fusion protein

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Certain proteins present in Nature have elastic and adhesive domains; they are attractive materials for molecular building blocks because of their ability to create elastic and energy dissipative interfaces between the components of hybrid materials. Examples are nacre and squid beak \cite{1}. In this study, a modular protein construct built from an elastic domain and two adhesive functionalities is investigated on the molecular level. It consists of the resilin like polypeptide (RLP), a rubber like molecule known for its high resilience \cite{2}, having an amphiphilic hydrophobin (HFBI) on one and a double cellulose binding module (dCBM) on the other terminus \cite{3} (Figure 1A). The technique used is the Single Molecule Force Spectroscopy where the idea is to let adsorb few molecules on a cellulose surface, using the affinity CBM-cellulose, and pick up only one of them through the interaction HFBI terminus-hydrophobized tip (Figure 1B). The work of adhesion and the length related to the stretching of RLP in different environments is investigated as well as the rupture force associated to interaction between the HFBI and a hydrophobic surface for which it has affinity. The importance of the single molecule perspective on the development of nanocomposites with enhanced mechanical properties is evident, since only by quantifying the smallest scale events, it is possible to progress towards higher levels of hierarchy.

\textbf{Figure 1.} Schematic representation of the protein studied at different pH (A) and cartoon of the force spectroscopy set up (B). Force distance curves recorded for the protein studied at pH 5 (C) and 11 (D).

\textbf{References:}
\begin{itemize}
\end{itemize}
An optical fiber-based force sensor for the detection of cartilage degeneration: comparing sensor sensitivity to atomic force microscopy

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Osteoarthritis (OA) is one of the most frequent joint diseases worldwide. At an advanced stage, OA causes significant pain accompanied by joint stiffness. At an early stage, cartilage degradation starts at the molecular level, affecting the structural and mechanical properties of the cartilage. These early changes of cartilage can be detected in vitro, e.g., by atomic force microscopy (AFM) [1]. However, in vivo detection of structural and biomechanical changes in early OA remains challenging. Here, we present a novel optical fiber-based force sensor which can potentially be used as a minimally invasive tool during arthroscopy. The sensor is based on a periodic fiber Bragg grating (FBG) inscribed into an optical fiber. When light is sent through the fiber, a part of its intensity is reflected at the FBG. The central wavelength of the reflected spectrum, called Bragg wavelength ($\lambda_B$), depends on the period of the grating. During indentation measurements, the force along the fiber compresses the grating, which results in a characteristic shift of $\lambda_B$. From this shift, the exerted force and the Young’s modulus of the sample can be extracted. Because AFM is capable of detecting the minute biomechanical changes associated with early OA [1], we performed comparative indentation measurements with the FBG sensor and AFM on healthy and enzymatically degraded bovine articular cartilage. The results show that the FBG sensor is capable of detecting changes in the mechanical properties of the cartilage caused by mild collagenase treatment. Whether the FBG sensor has the capability to detect even minor or superficial changes characteristic for early-stage OA has still to be validated.

References:
Multiscale Nonlinear Mechanics of Lung Extracellular Matrix

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A precise knowledge of the mechanical properties of the extracellular matrix (ECM) is critical to further our understanding of the cell-matrix interplay. Atomic force microscopy (AFM) is particularly suitable to study the mechanical properties of ECM at the microscale that cells sense the stiffness of their microenvironment. Nevertheless, although many biological tissues including those of heart and lung are physiologically subjected to stretch, conventional AFM systems do not allow measurement of the stiffness of the sample at different stretch levels. We studied nonlinear micromechanical properties of lung ECM by means of AFM using a novel device fabricated with a 3D printer to stretch the ECM sample during AFM measurements. To compare micro and macroscale mechanics we also probed ECM by means of tensile tests. The study was carried out in lungs (n = 3) excised from healthy Sprague-Dawley rats. For AFM measurements, the left lobe was decellularized with sodium dodecyl sulfate 1% and triton X-100 0.1% and ~10 μm thick sections were cut with a cryostat. An ECM slice was adhered with genipin onto the flexible membrane of the stretching device. Force-indentation (F-δ) curves were recorded with a custom-built AFM at increased stretch levels. Micromechanical Young’s modulus (E) was computed by fitting the Hertz contact model to F-δ data. For tensile tests, peripheral parenchymal strips (~8×1×1 mm) were cut from the right lung lobe with a scalpel. Fresh strips were attached to a servocontrolled actuator (Aurora Scientific) and stress-stretch (σ-λ) curves were recorded. Subsequently, the strips were decellularized and additional tensile tests were performed. The micromechanical Young’s modulus of the strip was defined as E = dσ/dλ. Strip data were analyzed with the Fung’s model which assumes that E increases linearly with stress. Under relaxed conditions, lung ECM exhibited a micromechanical stiffness of 14.03 ± 2.1 kPa (mean ± SD) and raised markedly with stretch. Macromechanical stiffness of the relaxed ECM was one order of magnitude lower (1.83 ± 0.88 kPa) and displayed a weaker stretch dependence. Interestingly, fresh parenchymal tissue strips composed of ECM and lung cells showed similar stiffness than the decellularized strips (relaxed ECM 2.02 ± 1.21 kPa) showing that lung tissue macromechanics is dominated by ECM. We have fabricated a novel device that allowed us to characterize ECM micromechanics with AFM at different levels of sample stretch. To the best of our knowledge, we report the first data of ECM micromechanical nonlinearity. Noteworthy, our device can also be applied to probe mechanical nonlinearity of many soft biological samples including cells. Our data reveal that lung ECM exhibits a marked stretch hardening behavior both at the micro- and macro-scales. Moreover, the higher stiffness obtained by AFM indicates that lung ECM macromechanics is determined by the local intrinsic mechanical properties of ECM as well as the 3D architecture of the lung tissue.
Pitfalls of practical KPFM-phase-tuning leading to polarity reversal on biological samples

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Besides measuring topography at high, spatial resolution, Atomic Force Microscopy (AFM) is known for its capability to record additional surface properties, such as mechanical, magnetic and electrical signals [1]. Kelvin Probe Force Microscopy (KPFM) [2], for example, is often used to map the surface potential in a quantitative way with high sensitivity and spatial resolution. However, inadequate electronic implementation of KPFM can lead to imaging-artifact, which are hard to identify in practice [3,4].

In this paper, specifically the influence of KPFM-phase-tuning, a common procedure necessary for KPFM operation, on quantitative surface potential measurements is investigated. It is shown experimentally, that inadvertent false-tuning of the KPFM-phase in combination with crosstalk reported in the literature [3], can lead to serious imaging-artifact. By measuring the surface potential of a collagen fibril and changing the KPFM-phase, it is demonstrated, that these artifacts can lead not only to higher noise and a scaling-error, but also to a complete sign reversal of the resulting potential (see Figure (a)). Such sign reversal also occurs when the KPFM-feedback controller becomes partially unstable upon phase-tuning (see Figure (b)). It is also shown, that the usual calibration method of applying a known potential to the sample [5], is not suitable for detecting these artifacts. In conclusion, this means that it is difficult or even impossible for a typical AFM-user, to recognize the error. This accounts especially when investigating biological samples, which usually show small KPFM signals. Finally, strategies for avoiding these pitfalls are given.

References:
Diluted alkali soluble pectin (DAPS) fraction might play crucial role in the mechanical and structural properties of fruit tissue. Structure of covalently-linked molecules determine cell wall stiffness [1]. In native conditions, structural modifications of fruit cell wall pectins are controlled by various enzymes that work collectively [2].

In this study following enzymes were used: 1) α-L-Arabinofuranosidase (Araf), 2) endo-Polygalacturonase, 3) Pectate Lyase and 4) α-L-Rhamnosidase to pectins extracted with sodium carbonate in order to evaluate contribution of the each individual enzyme in degradation process. Portion of each enzyme was dosed directly for DASP solution in two concentrations: enz/GalA 1:1,000 and 1:10,000. Height images of pectin molecules were collected with atomic force microscope (AFM) equipped with 10 × 10 µm XY and 2.5 µm Z scanner.

Results demonstrated nanostructural changes depending on enzyme concentration. Figures B and C present disassembling of regular net-like arrangement from native DASP (Fig. A). Mean values of length, height or angles of the structure on images were studied. Characteristic pectic objects were shown as large aggregates (La), short or long molecules (Sm, Lm) and long and linked molecules (Llm).

New provided data let us to better understand the structure and the role of covalently linked pectins in controlling structural and mechanical condition within plant cell walls.

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References:
Over the last few years, biological applications for Atomic Force Microscopy (AFM) have regularly expanded. These applications are at multiple scales from single-cells (both prokaryotes and eukaryotes) to tissue and concern both image and probe mechanical properties at the nanoscale level, in liquid and temperature-/CO2-controlled conditions [1]. Mechanical properties, such as the elasticity, may be linked to various conditions, such as cancer and other diseases [1, 2], or stem cell differentiation [1]. Between-group variance may, however, be relatively small compared to within-group variance, given the great diversity between cells. To ensure statistically significant data, one needs a stable, correctly calibrated system, and a high number of samples for each condition. High-resolution nanomechanical mapping on live samples, in fluid, is relatively slow by itself. A consequent amount of time is also spent when changing samples, approaching the tip, and setting scan parameters properly. Given time limitations for the operators, measurements are often taken with very small sample sizes. To solve this issue, a number of studies have been made on microscopes with a great number of cantilevers, using either cantilever arrays [3] or groups of miniaturized AFM scanners [4] but none of these solutions seem to address challenges linked to scanning on cells. Herein, we present a multi-sample solution for automated measurements on cells, using a custom multi-well plate on a commercially available AFM (FastScan-Bio). Applications on both bacteria and mammalian cells are shown. Each well of the plate can hold its sample in a different condition, so as to develop a comparative analysis. Different levels of automation are implemented to drastically reduce user work time. Such automation tends to be less error-prone and may allow for faster sample swapping and engagement processes. It could significantly increase both the number of conditions and the number of samples per conditions studied, bringing AFM analysis on cells closer to a medium-throughput process.

References:
Adequate endothelial response to functional EnNaC-inhibition as a marker for endothelial (dys-)function?

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An increased membrane abundance of the endothelial Na\textsuperscript{+} channel (EnNaC) is known to cause an increased stiffness of the endothelial cell (EC) cortex (actin-rich layer 50-200 nm underneath the plasma membrane). A stiff cortex in turn results in reduced production of nitric oxide (NO) and hence modulates EC function. On the contrary, functional EnNaC inhibition (amiloride/benzamil) induces cortical softening, i.e. a shift from filamentous (F-)actin to globular (G-)actin within the EC cortex. However, in a clinical trial an association between the attendance of endothelial dysfunction (ED) and an abolished amiloride-induced softening in human ex vivo vessel preparations (non-responder patients) was found. Thus, we postulate a disturbed regulation of (EnNaC-dependent) cortical plasticity within ED. To test this, EC of either wildtype (WT) or ApoE/LDLR\textsuperscript{-/-} mice were employed within an ex vivo approach, whereas the latter represents a reliable model for ED. (i) To quantify the cortical stiffness, patches of aortic rings were fixed onto glass-dishes with the EC facing upwards and studied with an Atomic Force Microscope (AFM). (ii) To test the function of the vessels, aortic rings were studied with a wire myograph. (iii) In vitro the amount of NO secreted by GM7373 EC was determined by chemiluminescence as a marker for endothelial function and (iv) the amount of cortical F-actin in fixed EAhy.926 EC was quantified by applying phalloidin-TRITC. We found, that compared to WT, the basal stiffness is increased in the ApoE/LDLR\textsuperscript{-/-} EC (+17\%) and benzamil (0.1 µM) leads to reduced stiffness exclusively in WT (-17\%), but not in ApoE/LDLR\textsuperscript{-/-} EC. Wire myography-measurements supported the latter finding, as benzamil significantly improved vessel function in WT, but not in ApoE/LDLR\textsuperscript{-/-} vessels. Further, acute functional EnNaC-inhibition with benzamil increased EC NO-secretion (+40\%) and decreased the amount of cortical F-actin (-50\%) in vitro. Hence, we postulate the existence of a vicious circle: Inhibition of EnNaC-function induces a shift from F- to G-actin within the cortical region, leading to decreased cortical stiffness, increased NO-production and thus improved EC function. On the contrary, in the condition of ED (reflected by ApoE/LDLR\textsuperscript{-/-} EC or non-responder patients), the regulation of the (EnNaC-dependent) cortical plasticity is disturbed, as EnNaC-inhibition stays without effect regarding cortical stiffness and consequently vessel function. The loss of an adequate endothelial response to functional EnNaC inhibition can thus be regarded a marker for ED.
Uptake of fatty acids by a single endothelial cell investigated by Raman spectroscopy supported by AFM mechanical mapping

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Eicosanoids are a wide group of biological active products that are involved in many cellular processes associated with activation and proliferation of cells. They're synthesized in endothelial cells (ECs) from 20-carbon PUFAs, namely, arachidonic acid (AA; 20:4 ω-6), eicosapentaenoic acid (EPA; 20:5 ω-3) and docosahexaenoic acid (DHA; 22:6 ω-3). All of those fatty acids have similar cascade reactions in human cells, which involve the same group of enzymes, however, products of those reactions have a slightly different impact on inflammatory response.

By using Raman imaging spectroscopy, and supported by AFM mechanical mapping, it was possible to follow changes induced in the ECs upon various fatty acid uptake \cite{1}. Raman images proved the formation of LDs and showed a complex chemical composition depending on the PUFAs used. In case of AA (20:4) and EPA (20:5) stimulation, an exogenous origin of fatty acids in LDs has been shown.

To establish if exogenous fatty acid was taken up by the cell and stored in LDs, a deuterium labelled PUFA was used. AA-d\textsubscript{8} gave a distinct characteristic bands around 2200–2300 cm\textsuperscript{-1} assigned to the \textsuperscript{=CD} stretching modes. We demonstrated that AA and EPA was taken up by endothelial cells into their newly formed LDs, while docosahexaenoic acid (DHA) was not, even though LDs were formed. In cells incubated with DHA (22:6), that fatty acid was not recognized within LDs, thus an uptake of DHA seems to be controlled, most probably, by other mechanism than observed before for EPA or AA, or DHA is promptly oxidized.

Altogether, the results suggest that formation of LDs in endothelium involve exogenous and endogenous PUFA, and their relative contribution seem distinct for AA, EPA and DHA.

Complementary AFM mechanical mapping measurements revealed that stimulation of endothelial cells with AA affects their stiffness, that could have pathophysiological significance. The impact of AA on cellular stiffness of ECs was investigated for HMEC-1 stimulated with 25 μM AA, in comparison to the control. Stiffness of cells was measured by determining the elastic modulus (Young's modulus). Elasticity parameter was calculated for every force–distance curve by applying the Sneddon's modification of Hertz model.

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Cytoskeletal Structures and Elasticity Measures in Human MSCs

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The most prominent cytoskeletal structures of mesenchymal stem cells MSCs are stress fibers that appear in tissue culture during ex vivo cultivation in adherence to plastic surfaces or bound to extracellular matrix components. When MSCs are expanded for a potential cell therapeutic approach, the occurrence of large bundles of actin filaments, showing mechanosensitive properties, might influence their mechanical properties. These bundles of actin filaments can induce tension forces via focal adhesions to pericellular matrix affecting the overall cellular elasticity [1]. The stress fibers formation, intracellular filament- and mitochondrial organization as well as focal adhesion formation were investigated in MSCs by immunofluorescence microscopy and by laser scanning confocal microscopy. The surface morphology, sub-membrane structure scans and the elasticity measurements were performed by atomic force microscopy.

References:
The elasticity of single cells as a disease marker determined by atomic force microscopy

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Various studies have reported that elasticity of single cells can be used as a biomarker of distinct pathological changes, including cancer \cite{1}. On the other side, elasticity measured by atomic force microscopy (AFM) is not an absolute value. The magnitude of alterations in mechanical properties strongly depends on various conditions such as medium composition or temperature or culture substrate \cite{2}.

AFM-based elasticity measurements can deliver more insights how the presence of neighboring cells affects cellular response to mechanical stimuli. Thus, the main objective of our studies was to quantify single cell elasticity measured in the presence of neighboring cells of the same and different types, i.e. healthy and cancerous cells. As a model system skin cells were chosen, namely, fibroblasts (FBs), keratinocytes (HaCaT) and melanoma cell from radial growth phase (WM35). The obtained results shows that in co-cultures of fibroblasts and keratinocytes, FBs appeared to be more sensitive to the presence of neighboring cells as compared to HaCaT cells. In the co-cultures of fibroblasts and melanoma WM35 cells, both cell types are sensitive to the presence of other cells. These findings show distinct mechanical interaction, which seems to be cell type dependent.

References:
Development and applications of atomic force microscopy combined with optical tweezers (AFM/OT)

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Atomic force microscopy (AFM) is an evolution of scanning tunnelling microscopy that immediately gained popularity thanks to its ability to analyse nanomaterials. Initially, AFM was developed for nanomaterials imaging purposes, however the development of new features made it the most commonly used tool for studying the biophysical properties of biological samples. On the other hand, atomic force microscopy has limited use for examining sub-piconewton forces. Few techniques have been developed to measure forces below the AFM limit of detection. Among them, optical tweezers (OT) stand out for their high resolution, flexibility, and because they make it possible to accurately manipulate biological samples and carry out biophysics experiments without side effects thanks to their non-invasive properties.

The combination of AFM with other techniques in the last decades has significantly extended its capability. The improvement of the AFM force resolution by developing a hybrid double probe instrument based on the combination of AFM and OT has great potential in cell or molecular biology. [1]

We outline principles of atomic force microscopy combined with optical tweezers (AFM/OT) developed by our team underlying the techniques applied during the design, building and instrument use stages. We describe the experimental procedure for calibration of the system and we prove the achievement of a higher resolution (force: 10 fN – spatial: 0.1 nm – temporal: 10 ns) than the stand alone AFM.

We show the use of the hybrid equipment in a number of different biophysics experiments performed employing both AFM and OT probes. The presented studies include the demonstration of simultaneous high-precision nanomanipulation and imaging, the evaluation of single biomolecule mechanical properties and the single cell membrane activation and probing. Finally, we show the further potential applications of our AFM/OT.

![Figure. Stretching of a single DNA molecule by AFM/OT](image)

References:
RBC’s ageing from morphology and membrane structure: an AFM study combined with Raman and Differential Scanning Calorimetry

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Red Blood Cells (RBCs, erythrocytes) are very simple cells which have several peculiarities, such as outstanding mechanical performances, relatively simple architecture, biological relevance and predictable behavior[1]. Due to their physiologically relevant role of oxygen transporters in the bloodstream, RBCs have been extensively studied with a variety of techniques. Since these cells do not bear DNA and do not replicate, their ageing is a particularly important physiological phenomenon, which triggers their turnover in the organism. We studied extensively this pathway, combining several techniques to obtain a complete characterization of the structural, morphological and chemical modifications induced by the ageing pattern. Specifically, we combined the capabilities of Atomic Force Microscopy (AFM) to obtain high resolution topographical images and direct measurements of the membrane roughness[2], Differential Scanning Calorimetry (DSC)[3] to monitor the membrane chemical stability and the evolution of the principal membrane proteins and Raman Spectroscopy[4] to highlight differences associated with conformational and biochemical alterations of the whole cellular architecture. The AFM images evidenced, over time, a marked decrease in the membrane roughness coupled with the appearance of peculiar morphological markers that evolved in time and drove the overall cell shape modifications. DSC showed that the transition temperature and excess heat capacity of Hb unfolding decreased gradually during the entire storage period, while two phases were resolved in the band 3 protein denaturation. A Principal Component Analysis (PCA) performed on the Raman spectra showed a clear separation of the cellular spectra associated to the different ageing times, thus distinguishing the relevant biochemical changes occurring in RBCs. Taken together these label-free characterizations can give an in-depth view of the ageing process, furthermore they can be applied to the study of erythrocytes from pathological donors, allowing a better comprehension of the behavior of these cells.

References:
Experimental mechanics of collagen molecules

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Collagens are the most abundant and structurally the most important proteins of the human extracellular matrix. Therefore, mechanical properties of collagen molecules (tropocollagen) and the progressively larger structures they form are crucial for tissue mechanics and function. While there are a number of studies modeling the mechanical behavior of tropocollagen molecules via molecular dynamics approaches there is little but none experimental data available. Yet, molecular dynamics modeling approaches have strict limitations in terms of time and volume, such that a single tropocollagen molecule cannot be investigated in its entirety. Further, time durations of such models are also limited. This means that experimental validation of predicted behavior such as molecular uncoiling or the interactions between neighboring molecules is needed for validating models and further insight into the molecular mechanics of collagen.

Here, we present an approach to experimentally assess the mechanical behavior of tropocollagen molecules using Atomic Force Microscopy Single Molecule Force Spectroscopy (AFM-SMFS). In our approach, single molecules were tethered to the AFM tip. More precisely, a polyethylene glycol (PEG) based linker system with N-hydroxysuccinimide (NHS) and maleimide (MI) coupling groups (NHS-PEG-MI) was used. Collagen Type III was attached using the thiol group of cysteine, which is part of the primary structure, by MI coupling. Tropocollagen-substrate interactions were investigated by performing AFM-SMFS measurements in PBS buffer on glass. Experiments thus far exhibited high adhesion with peak forces up to 9 nN, which is about 10-fold higher compared to reported adhesion of the amino acid 3,4-dihydroxy-L-phenylalanine (DOPA) \cite{Lee2006}, as well as the adhesion force between 2 tropocollagen molecules as predicted by molecular dynamics \cite{Buehler2006}. Instead of collagen-substrate interactions, future work will expand to investigate collagen-collagen interactions in various conditions.

References:
On the Way to Single Molecule Adhesion Forces of a Multidomain Protein by Force Spectroscopy

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The non-specific adsorption and adhesion of proteins at solid surfaces in aqueous solution is a complex phenomenon involving polar, apolar and electrostatic interactions [1]. To relate the adhesion forces obtained by force spectroscopy to results from MD simulations it is crucial to isolate single molecule interactions in the experiment.

Here, the interaction of the protein bovine serum albumin (BSA) with surfaces is investigated with the aim to reveal the adhesion force of a single protein molecule. Albumin as multidomain, intermediate size protein is able to undergo conformational reorientations upon surface contact [2]. The adhesion force is explored under the influence of several measurement parameters: retract velocity, contact time with the surface, setpoint and retract pause. Additionally, the impact of the substrate material on the adhesion force is tested. For this purpose, smooth, homogenous surfaces with different wettabilities, i.e. silicon, rutile, and HOPG are applied.

To measure protein adhesion BSA is covalently attached to a cantilever via a polyethylene glycol-spacer between tip and protein [3]. The spacer helps isolating the interaction of single albumin molecules with the surface in the single molecule force spectroscopy experiment. Upon cantilever retraction from the surface first tip adhesion and spacer stretching occurs. Protein desorption is attributed to desorption events that occur at tip-sample distances larger than the linker length. Overlap of desorption and unfolding of the protein makes the evaluation of the force distance curves challenging. Here, different approaches of analyzing the data are presented that can give insight to the adhesion of single BSA molecules as well as the extent of protein unfolding in the desorption process.

References:
Bacterial contact formation and breaking: revealing their contact area & contact characteristics on different types of surfaces

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Pathogenic bacteria adhering to surfaces are a nuisance in a wide area of health care applications. We present a combined experimental and computational approach to characterize the adhesion of *Staphylococcus aureus* and other cocci. Single cell force spectroscopy [1] paired with Monte Carlo simulations enabled an unprecedented molecular investigation of the contact formation [2]. Our results reveal that bacteria attach to a surface over distances far beyond the range of classical surface forces via stochastic binding of thermally fluctuating cell wall proteins. Thereby, the cells are pulled into close contact with the surface as proteins of different mechanical properties attach consecutively. This mechanism however, can be manipulated by genetically/ enzymatically/chemically modifying the cell wall proteins [3]. On hydrophobic surfaces, hydrophobic interactions are dominant and many cell wall proteins contribute to the cell's stickiness. On hydrophilic surfaces, contact formation needs a longer time and only few cell wall proteins are involved in this process. Our study furthermore reveals that fluctuations in protein density and structure are much more relevant than the exact form of the binding potential or the contact area [4].

References:

Primary vs Immortalized – AFM study of Liver Sinusoidal Endothelial Cells

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Abstract Body: Liver Sinusoidal Endothelial Cells (LSECs) line hepatic sinusoids, providing filtration of lipoprotein and chylomicron remnants between blood vessels and interior of the liver. Selective transport is supported by the presence of transmembrane pores with size of 50-300 nm, called fenestrations [1]. Therefore, both number and size of fenestrations are responsible for proper functioning of the liver.

Primary LSECs (pLSECs) were isolated from murine liver according to the modified standard procedure [2] and measured using AFM in first 8 days of in vitro culture. We noticed alteration in pLSECs phenotype in 48h after isolation. Therefore, to investigate the possibilities of performing long term experiments, we used immortalized cell line of hepatic endothelial cells (TSECs) [3] for comparison. Then, we investigated both morphology and nanomechanical properties of primary and immortalized cells.

Obtained results indicate that the elasticity parameter of TSECs and freshly isolated LSECs (up to 24h in culture) remain the same, however, TSECs lack porous morphology. We also observe loss of fenestrations during 8 days after isolation and increase in the elasticity parameter. Moreover, we are able to induce fenestrations in pLSECs in 4 and 8 days of in vitro culture using cytochalasin B, while TSECs appear to permanently lose the ability to form fenestrations. These results reveal that TSEC line presents phenotype similar to LSECs in pathological states in comparison to pLSECs isolated from a healthy mice.

References:
Melanoma – endothelial de-adhesion dynamics: crucial step towards brain metastasis

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Metastasis formation is a complex and not entirely understood process. The poorest prognosis and the most feared complications are associated to brain metastases [1]. Melanoma derived brain metastases show the highest prevalence. Due to the lack of classical lymphatic drainage, in the process of brain metastases formation the hematogenous route is of primordial importance [2]. The first and crucial step in this multistep process is the establishment of firm adhesion between the blood travelling melanoma cells and the tightly connected layer of the endothelium, which is the fundamental structure of the blood-brain barrier [3].

Intercellular dynamics might present crucial nanomechanical aspects, therefore direct investigation of intercellular interactions with high accuracy is of primordial importance [4,5]. Using an Asylum Research MFP3D type AFM, de-adhesion properties and dynamics of three melanoma cells types (WM35, A2058 and A375) to a confluent layer of brain micro-capillary endothelial cells (hCMEC/D3) was investigated using single cell force spectroscopy. Cell adhesion depends on multiple and even multivariate individual molecular connections, where the individual players are difficult to identify. Based on simple mechanical assumptions, hereby we present our latest data on comparing the interaction of three different types of melanoma cells, having altered invasive characteristics, with brain endothelial cells. Apparent mechanical properties such as relative elasticity, maximal adhesion force, number, size and distance of individual rupture events showed altered characteristics pointing towards cell type dependent aspects. Our results show that nanomechanical properties can be associated to higher metastatic potential and invasive characteristics may rely on stronger adhesive properties mediated by altered tether formation dynamics.

This work was supported by the National Science Fund of Hungary OTKA K116158, PD115697, PD121130, the GINOP-2.3.2-15-2016-00001 / 00020 / 00034 and GINOP-2.3.3-15-2016-00030 programs. IM Wilhelm and AG Végh were supported by the János Bolyai Fellowship of the Hungarian Academy of Sciences BO/00334/16/8 and BO/00598/14/8 respectively.

References:
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*Electromagnetic AFM Force-Clamp Setup Using a Software-Based Controller*

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P35 Melissa Cäzilie Piontek, The Netherlands

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*Problems of AFM measurements of native blood cells membranes Young’s modulus and interpretation of the results*

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*Theranostics of malignant melanoma with anandamide by QCM-D measurements*

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*Nano-mechanical properties of biopsied bronchial tissues as a marker in obstructive lung diseases*

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*The Effect of Copper Ions on the Aggregation of hIAPP(11-28)*
Effects of cytosine methylation on DNA morphology: An atomic force microscopy study

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Abstract Body: Methylation is one of the most important epigenetic mechanisms in eukaryotes. As a consequence of cytosine methylation, the binding of proteins that are implicated in transcription to gene promoters is severely hindered, which results in gene regulation and, eventually, gene silencing. To date, the mechanisms by which methylation biases the binding affinities of proteins to DNA are not fully understood; however, it has been proposed that changes in double-strand conformations, such as stretching, bending, and over-twisting, as well as local variations in DNA stiffness/flexibility may play a role. Here we investigate, at the single molecule level, the morphological consequences of DNA methylation in vitro. By tracking the atomic force microscopy images of single DNA molecules [1], we characterize DNA conformations pertaining to two different degrees of methylation. Methylation induces no relevant variations in DNA contour lengths, but produces measurable incremental changes in persistence lengths. Furthermore, for the methylated chains, the statistical distribution of angles along the DNA coordinate length is characterized by a double exponential decay, in agreement with what is predicted for polyelectrolytes. The results reported herein support the claim that the biological consequences of the methylation process, specifically difficulties in protein-DNA binding, are at least partially due to DNA conformation modifications [2].

References:


Electromagnetic AFM Force-Clamp Setup Using a Software-Based Controller

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Here we report a force-clamp spectroscopy setup based on a customized AFM [1]. We perform bond lifetime measurements using electromagnetic actuation in a non-contact way between pairs of single molecules functionalized on tip of cantilevers and the surface of magnetic beads [2]. We designed a software-based PID controller to keep the interaction force on the cantilever constant. The controller enables conducting the experiments automatically without any need for manual intervention. High efficiency of this method facilitates the efficient statistical analysis of molecular bond lifetimes. We have performed force-clamp experiments using pairs of heparin/FGF2. In low force regime, the calculated distance to the transition state Δx is 0.099nm, unbinding rate of zero clamping force kₒ is 1.462 s⁻¹; while in the high force regime, Δx is 0.005nm and kₒ is 8.166 s⁻¹.

References:
Biomechanics: a Biomarker for Melanoma Diagnosis?

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Note: The first two authors gave the same contribution to this work

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Melanoma is the most aggressive form of skin cancer and responsible for most skin cancer death [1]. A melanoma starts in melanocyte cells and if it does not spread to the lymph nodes, its excision can be done reducing the death risk to low levels. However, when metastases occur, the risk of mortality will increase since this cancer is very resistant to chemotherapy and radiotherapy. The metastatic process usually grows vertically in the skin, since cancer cells invade the basal membrane, reaching the dermis. It is not clear how a melanocyte becomes a melanoma cell [2] but a deep investigation of this process could be crucial to establish new strategies for diagnosing melanomas at earlier stages. Biomechanical parameters like viscoelasticity and stiffness are envisioned as indicators of melanoma phenotype [3]. However, since melanoma cells can have similar mechanical parameter values when compared with healthy melanocytes, due to pigmentation of cells, this turns out to be difficult [4].

In this study, the viscoelasticity and stiffness of human primary melanoma, the correspondent metastasis and healthy melanocytes were assessed by Atomic Force Microscopy. Moreover, the production of melanin was determined on these three cell types and correlated with their mechanical properties.

References:

Quantitative Characterization of the Specific Interactions of Syndecans–Vitronectin Complexes by means of Dynamic Force Spectroscopy

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Multiple receptors present in a cell membrane mediate a great number of various adhesive with the extracellular matrix (ECM). Syndecans are a family of transmembrane heparan sulphate proteoglycans with ectodomains modified by glycosaminoglycan chains. The extracellular domains can interact with different molecules, including cytokines, proteinases, adhesion receptors, and ECM components [1,2]. Importantly, they can act as co-receptors of other cell surface receptors like growth factor receptors and integrins. In this context, syndecans can induce conformational changes in growth factors and other signaling molecules via their heparan sulfate chains, thus facilitating their receptor interaction [3,4].

The aim of this work is the quantitative characterization of the unbinding processes of syndecans (1 and 4) with corresponding monoclonal antibodies and vitronectin. Single molecule force spectroscopy was performed using Force Robot (JPK) head and a liquid cell filled with TRIS buffer enriched with divalent ions. Dynamic force spectroscopy measurements were carried out at the separation rates ranging from 0.1 µm/s to 19.0 µm/s. The most probable unbinding force was determined for each separation rate. The dependence of the specific adhesion force on the loading rate was analyzed with two thermal activation models: the Bell-Evans and the Dudko-Hummer-Szabo (DHS). DHS model provides information about the position and the height of activation barrier ΔG [5].

References:

Authors thank the National Science Centre of Poland (NCN) for financial support within Project No. UMO-2014/15/B/ST4/04737.
AFM-based single molecule force spectroscopy on bimolecular hybrid DNA/RNA G-quadruplexes

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Non-canonical nucleic acid structures, such as G-quadruplexes, have recently attracted significant research efforts for their potential roles in the regulation of numerous biological processes [1]. There is now evidence that these specific nucleic acid structures modulate gene expression. The formation of hybrid DNA/RNA G-quadruplexes (HQs) where each strand contribute to the structure with two series of guanines, was recently found to form in DNA during transcription [2]. It has been evidenced that the HQ formation competes with intramolecular G-quadruplexe motifs (DQ) and plays a major role in regulating transcription [3]. Given their very recent discovery, only a few studies on the formation of these hybrid structures have been conducted so far. However, the knowledge of their mechanism of formation and their detailed structure is important for understanding their biological function, as well as for manipulating gene expression by targeting HQs.

We propose here to study these hybrid DNA/RNA G-quadruplexe structures by AFM-based single molecule force spectroscopy to obtain detailed information, at the single molecule level, on their formation mechanism and dynamics, their structure and interactions that govern the complex, their mechanical stability and their lability.

References:
Effect of anandamide on keratinocytes treated with different stimulants

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Abstract Body: The discovery of cannabinoid receptors (CB1 and CB2) on eukaryotic cell membranes has launched a new series of studies on the ligand interaction with these receptors in the regulation of several physiological processes such as homeostasis, cell cycle proliferation, differentiation, inflammatory and allergic reactions in the epidermis [1]. The search for new ligands for the cannabinoid receptors, which could be used in therapeutic drug formulations, could be a key extension of scientific knowledge in that field. Many methods of cell receptor detection are known including Western blot and ELISA assays. Label free methods like quartz crystal microbalance (QCM) and atomic force microscopy (AFM) allow to follow the interaction of ligands with receptors on both live and fixed cells in real time [2]. In our studies, we have used three bioactive compounds: bacterial lipopolysaccharide (LPS), pro-inflammatory cytokine tumor necrosis factor (TNF-α) and a plant compound phorbol myristate acetate (PMA) to determine their effect on the immortalized human keratinocyte cell line (HaCaT). In addition, the influence of endogenous cannabinoid anandamide (AEA) on that interactions were evaluated. Several biological tests were performed including cell viability (MTT), membrane integrity (NR and LDH release) and apoptosis. AFM and QCM were also used to study the viscoelastic properties of the cells and the interaction of CB receptors of living cells with AEA. Anandamide treatment of HaCaT cells pretreated with non-cytotoxic concentrations of investigated three compounds revealed the hermetic effect, with proinflammatory action at 1 μM concentration.

References:
Change in the morphometric parameters of blood erythrocytes under FeO·Fe₂O₃ condition estimated by atomic-force microscopy

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Abstract: Nanoparticles of magnetite (FeO·Fe₂O₃) have a wide range of biomedical applications both in vivo and in vitro: increasing the contrast and sensitivity of MRI, targeting drug delivery, specific binding of therapeutic agents, hyperthermia and bioengineering [1]. However, before using for treatment, it is necessary to study the bioinertness of nanoparticles. AFM is a high-resolution method that allows estimating the influence of various nanoparticles on blood cells, including erythrocytes [2]. It gives the possibility both to evaluate the morphology of cells and to carry out morphometric measurements. The morphology and the main morphometric parameters of erythrocytes were observed by AFM before and after incubation with FeO·Fe₂O₃ (0.0018 mg/ml and 0.18 mg/ml) nanoparticles. After incubation with the FeO·Fe₂O₃ (37°C, 60 min), morphology and all morphometric parameters (the volume of erythrocytes, their diameter, the maximum height and the minimum height) changed significantly in both concentrations of nanoparticles (Fig). Thus, poikilocytosis and anisocytosis were the common structural and morphological reaction of erythrocytes to FeO·Fe₂O₃.

This work was supported by the Russian Science Foundation, project 16-14-10179.

Figure. AFM-images of erythrocytes before (a) and after (b) incubation with FeO·Fe₂O₃.

References:
Probing the Nanoscale biophysics for MT1-MMP-expressing metastatic cancer cells

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Abstract Body: During invasive progression of cancer, matrix degrading enzymes is major determinants for matrix remodeling and intrusion of extracellular matrix. The deformability and adhesive properties of cancer cells regulated by these enzymes are also essential for metastatic function. Membrane type-1 matrix metalloproteinase (MT1-MMP) activated in the vicinity of the invading region of cells can stimulate cancer cell migration and proliferation and is thus considered as biomarkers of metastatic potential. In recent, several studies have been conducted to find a significant role of MT1-MMP in cellular mechanics of metastatic cancer. Here, we study the correlation between mechanical property of cancer cells and MT1-MMP expression using a microsphere-attached cantilever of atomic force microscopy and confocal microscopy. Our results demonstrated that the expression levels of MT1-MMP mediate biophysical behavior of cancer cells as dynamic cell adhesion and cortical stiffness by indirectly regulation of focal adhesion molecules and cytoskeleton conformation.

References:
Study on resistance of human nail plates against chemical degradation

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Abstract Body:

Atomic Force Microscopy demonstrated its applicability for studying a large variety of biological systems, especially in the aspect of the condition of molecular components upon treatment with chemical reagents. This issue turns out to be extremely vital in cosmetics since the knowledge whether and how chemical compounds react with tissues the skin is made of would give us better protection against various dermatologic disorders. In presented paper the effect of acetone on plates of human nails was studied.

To this end a set of samples of human nails was prepared, each of which was then treated with acetone for several minutes. Changes in surface morphology of the nails were then studied using Atomic Force Microscopy and Scanning Electron Microscopy, whereas those in chemical passivation was studied using Fourier Transform Infrared Spectroscopy. To obtain reliable measures of spatial evolution of the surface variations, AFM and SEM images were analyzed in terms of allometric scalability brought by fractal geometry (fractal dimension, topothesy, corner frequency) instead of common statistical parameters (roughness, skewness, kurtosis, etc.)

Figure. AFM images of the human nail plates before (left) and after (right) chemical abrasion with acetone.
Location Effect on Formation of Biotin–Streptavidin Complexes on DNA Origami Revealed by Time-Lapse Atomic Force Microscopy

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Abstract: Self-assembled DNA origami has many potential applications in nanotechnology due to its capability of generating nanostructures with a high precision. Each nucleotide on DNA origami providing a unique site from which hierarchical structures can be generated and molecular behavior can be studied at the nanometer scale. However, there is lack of quantitative methods for investigating chemical, physical, and biological variations on DNA origami at molecule resolution. To address this issue, we used time-lapse atomic force microscopy (AFM) to record the reaction process between streptavidin (SA) and biotin modified on DNA origami. We observe that the height of biotin–SA complexes at different locations of DNA origami ranged from 2.0 to 5.0 nm. Importantly, we find that biotin–SA complexes formed fastest at the corner edge, then at the corner and surface, and slowest at the middle surface of DNA origami; at some points, the difference in biotin–SA binding efficiency was more than 90%, with efficiency increasing with incubation times and showing saturation at some sites. The observed differences in binding efficiency suggest distinct microenvironments at different locations of DNA origami. These findings can be generalized to other self-assembling DNA nanostructures and provide insight into the mechanisms of molecular recognition.
Visco-elastic properties of HT-29 under treatment with lactobacillus by atomic force microscopy

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Abstract:

Recent studies have shown that the mechanics of the cell is an effective biomarker of pathological changes¹. A useful technique that has demonstrated to be able to quantify the mechanical properties in living cells is atomic force microscopy² (AFM). Here, we use AFM to measure the viscoelastic moduli of cancer cell line HT-29 cultured in vitro, and the changes in their mechanical properties that suffered when the cells were subjected to different treatments: the habitual drug for cancer treatment (Doxorubicin); and extracellular metabolites produced by Lactobacillus Plantarum + Lactobacillus Casei (Lp+Lc) and Bifidobacterium Longum + Lactobacillus Plantarum (Bl+Lp). Those mixtures of metabolites were studied previously by our group and we found that they act as inhibitors of the HT-29 cell line.

To obtain the mechanical parameters we used the Standard Linear Solid model (SLS), Fractional Zener and Two Exponential Maxwell model. Our results showed that the former two methods had better accuracy in the fitting parameters with the experimental data. Although, each model has different parameters, the resulting behavior was observed to be similar. Furthermore, we found an increase in the relaxation time on cells exposed to Doxorubicin and on those that were treated with Lp+Lc mixture, which is the mixture that has higher inhibition against HT29 cell.

References:
Understanding the Adenovirus Mechanics with Atomic Force Microscopy

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Adenovirus, a human pathogen and a potential therapeutic tool, has an icosahedral shell enclosing the dsDNA genome associated with positively charged proteins VII, V and µ. These proteins are thought to regulate the electrostatic interactions between DNA strands, condensing the genome by a strategy similar to cell histones [1]. Previous studies showed that internal pressure generated upon virus maturation, which entails proteolytic cleavage of proteins VII and µ, contributes to modulate the stability of the viral capsid. This pressure induces stress in the capsid, favoring penton dissociation for the stepwise uncoating cascade [2-3].

To understand the function of core proteins, we are using Atomic Force Microscopy to analyze the mechanical properties of Adenovirus. First, we measure the elasticity and fragility of the particles; then, we carry out mechanical fatigue experiments [4] to assess the stability of the particles. Our results will help us understand the core organization and capsid stability modulation.

Mechanical Properties of extracellular vesicles

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Abstract:
Extracellular vesicles (EVs) are natural nanoparticles which can be found in many body fluids. Due to their importance for cell biology and for nanomedicine, e.g. in intercellular communication and their potential application in diagnosis, EVs are intensively studied. In order to understand the EV’s structure, stability and function it is crucial to have not only an in-depth analysis of the sample’s concentration and the EV’s content, but also of their mechanical behavior. By means of atomic force microscopy (AFM) we study EVs at single particle level in liquid, and thus under near-to physiological conditions. The acquired data provide information about the topography with nanometer-resolution as well as about the EV’s deformability, its stiffness and bending modulus. In addition, our single-particle approach provides insights into the particle to particle variability.
The structural characterization of sodium carbonate soluble fraction of pectin (DASP) extracted from plant cell walls.

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Pectin is a family of biopolymers – heteropolysaccharides – that constitute up to 60% of the plant cell wall, filling the space between the cellulose/xyloglucan network. Structurally and functionally pectin is considered to be the most complex polysaccharide in plant cell walls. Up to now many studies suggested that nanostructure of pectin determines such properties as stiffness and diffusivity of cell walls and plays important role in growth, development and senescence of terrestrial plants. However, the direct relation of physical characteristics of cell wall polysaccharides with macroscopic structural changes and processes, such as postharvest degradation in fruits and vegetables is still a subject of academic studies. The usual way of recognition of individual cell wall polysaccharides involves sequential extraction using different solvents. There are three main fractions of pectin isolated from the cell walls: water soluble pectin (WSP), chelator soluble (CSP) and sodium carbonate soluble (diluted alkali - DASP/SSP) pectin fraction. It was discovered that in fresh fruits and vegetables such as carrot, apples or pears, diluted alkali soluble fraction of pectin (DASP) form regular interlinked network on mica [1]. This regular structure would have a great importance for cell wall integrity and therefore texture and firmness of the whole fruits and vegetables. However, at present neither the mechanism of networking nor molecular structure of this pectin fraction is not known yet.

In this study we've made an attempt to characterize the molecular structure of the DASP fraction by means of AMF imaging methods coupled with image analysis techniques. The structure characterization involved acquisition of topological images of DASP deposited on mica. Pectin were isolated from apple fruit (Malus Domestica, cv. “Golden Delicious”) cell walls during sequential extraction according to the method proposed by Redgwell et al. (1992) with some modifications [2]. Thin films of DASP fraction were uniformly deposited using spin coater. Pectin fraction was observed by a Multimode 8 or Bioscope Catalyst II equipped with a Nanoscope V controller and a piezo scanner AS-12 E(all from Bruker, Billerica, MA, US). The structural characterization of visible objects was provided by means of image analysis procedures developed in our laboratories. These operations were performed using SPIP 6.0.14 software (Image Metrology, Horsholm Denmark), as well as our own developed software, based on Matlab image analysis libraries (MathWorks, Natick, MA, USA). Structural characteristics of DASP fraction were compared with prediction of molecular geometries obtained using the molecular dynamics. This task leads to an answer which components of DASP fraction are responsible for self-assembly mechanism and what conditions are required for it to occur.

Acknowledgments:
This work was funded by The National Centre of Science (NCN) (Project No. 2015/17/B/NZ9/03589, “OPUS” programme).

References:

Mechanical properties of *Bacillus subtilis* cells upon ceragenins treatment

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The atomic force microscopy (AFM), due to its high resolution and ability to assess mechanical properties, has become a powerful technique used for rapid evaluation of bacteria antibiotic resistance [1], observation of real-time nanoscale changes of bacteria morphology and elasticity [2, 3]. In this study, we present the results of nanomechanical characterization of *Bacillus subtilis* cells upon treatment with synthetic cationic lipids (ceragenins) CSA-13 and CSA-131. Results were compared to reference, non-treated cells, and the impacts of ceragenins on the bacteria were correlated with the effects of the human cathelicidin LL-37 treated bacteria. Measurements were conducted using AFM working in spectroscopy mode. Elastic modulus of single bacteria (including real-time stiffness measurements) was calculated based on force-indentation curves and Hertz-Sneddon model applied to those curves. During the elasticity measurements, the adhesive properties of bacteria’s surface were also determined.

We observed that nanomechanical alternations as quantified by bacterial stiffness and adhesion measurements shows that bacteria respond actively to treatment by tuning their surface properties and this response is tightly controlled by the concentration of bactericidal agents. We postulate that nanomechanical responses may be considered as a new way to assess some aspects of antimicrobial activity that evolve in time, especially for molecules targeting bacterial membranes [4].

References:
[4] This work was financially supported by the National Science Centre, Poland, UMO-2012/07/B/NZ6/03504 (to RB). The purchase of JPK instruments used for collection stiffness maps was realized within the frame of the project co-funded by the Malopolska Regional Operational Program Measure 5.1 Krakow Metropolitan Area as an important hub of the European Research Area for 2007-2013 (ML). Additionally, this study was conducted with the use of equipment purchased by Medical University of Białystok as part of the RPOWP 2007-2013 funding, Priority I, Axis 1.1, contract No. UDA- RPPD.01.01.00-20-001/15-00 dated 26.06.2015.
**The spinal cord elasticity in EAE model assessed by atomic force microscopy**

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Multiple sclerosis (MS) is an incurable, progressive neuroinflammatory and neurodegenerative disease. Experimental autoimmune encephalomyelitis (EAE) is one of the widely adopted animal models representing MS. EAE model is known to be well-suited tools for experimentally addressing mechanisms that cause autoimmune pathology of the central nervous system and for studying therapeutic principles for preventing or curing the disease [1].

Histological examination is the basis for diagnosis of autoimmune pathologies. However, in some cases it is not sufficient for delivering complete information concerning the disease. We used atomic force microscopy (AFM) measurements to quantitatively assess the elasticity of spinal cord tissue of mice with EAE by determination of the Young's modulus (i.e. elasticity modulus). Its value describes the magnitude of tissue deformability. In our study, the results obtained for EAE mice were compared with those of control group. The EAE mice showed abnormally low mechanical stability of the spinal cord and the value of Young's modulus marks the severity of the disease. They have low resistance of nerve fibers to deformation, compared to control mice. Histopathological symptoms of axonal damage and demyelination well corresponded with the loss of nerve fiber resistance to deformation, as probed by AFM.

Results of the present study demonstrate that AFM is a useful tool for quantitative analysis of spinal cord neurodegeneration in EAE and that it might supplement the diagnostic methods in SM. The study was supported by a grant K/ZDS/002851 from the Jagiellonian University Medical College to GPF.

Microcontact printing of proteins for surface potential mapping by KPFM

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The understanding of the electrostatic properties of proteins is of immense importance to protein biophysics. For instance, electrostatic properties of proteins determine fundamental functionalities such as association of receptors with charged ligands\textsuperscript{1}, binding in enzymatic reactions and catalysis\textsuperscript{2}, formation of protein-protein and protein-nucleic acid complexes, denaturation at high and low pH values, and aggregation dynamics of amyloid fibres to name a few. In order to investigate these electrostatic properties, micro-patterns of a family of globular proteins were fabricated by microcontact-printing, and surface potential mapping by Kelvin-probe force microscopy (KPFM), a variant of atomic force microscopy (AFM) which spatially resolves surface potential distribution with sub-micron resolution and high sensitivity, was performed. Insulin, BSA and β-lactoglobulin were succesfully patterned on mica and imaged by KPFM. Response to pH and immersions in water was investigated showing a clear reversible shift on surface potential. Similarly, cross-patterning of different proteins on the same substrate for surface potential one-to-one comparison was succesfully achieved. Moreover, the possibility of comparing electrostatic properties of two different proteins using the same substrate as reference would enable to extend the scope in protein biophysics.

![Figure. Patterned BSA by microcontact-printing on mica. A) AFM topographic image and B) surface potential by KPFM.](image)

References:
Problems of AFM measurements of native blood cells membranes Young's modulus and interpretation of the results

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The problems of measuring the membranes stiffness are discussed by many scientists, and the results can differ tens times. The key task is not just to determine the Young's modulus, but it is important to determine the Young's modulus (E) in certain places of membrane. Even in one monolayer values of membranes Young's moduli of different cells can differ substantially one from another. Therefore, it is very important to obtain an image of the red blood cells (RBC) [1].

We used the sedimentation method of RBC monolayer preparation for obtaining an image in a liquid and the Young's modulus measurement [2]. 50 μl of erythrocytes were added to 5 ml of PBS (pH 7.4). 400 μl resulting suspension was applied to the glass with polylysine for 20 minutes. After this we washed glass in PBS and fixed for 10 seconds in a 1% solution of glutaraldehyde, then washed again in buffer and left for 40 or more minutes to form a monolayer.

The glass with formed monolayer was placed into AFM cuvette with buffer for scanning. The value of measured E depends on the of the RBC themselves and on many external experimental factors: on the method of preparation of monolayers, the fixing time, the method of samples washing, the immersion rate of the probe and a number of other parameters. Therefore, in order to compare the results, all measurements must be carried exactly under the same experimental conditions. In our studies, we obtained the following results: the Young’s modulus during long-term blood storage changes 1.5-2 times at the end of the storage period. Under the action of heavy metal ions on E of RBC membrane increases three times with respect to the reference value. When the drug verapamil influenced on blood the value E does not change.

References:
Theranostics of malignant melanoma with anandamide by QCM-D measurements

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Abstract Body: Early diagnosis of malignant melanoma and monitoring of the effectiveness of its treatment is a major challenge for new technologies. Application of Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) may be used for this approach. Nowadays, the distinction between melanoma cells from metastasis, from radial growth phase and melanocytes is possible due to the interaction of specific proteins - lectin - with cell surface - glycoproteins [1]. Lectin-carbohydrate interactions can be found in a wide variety of biological processes, such as cell adhesion and migration, divers infections, host defense, embryogenesis and proliferation as well as apoptosis [2]. Changes in the glycosylation pattern are often associated with various diseases including cancer so that lectins might be also used as therapeutical agents to target and deliver drugs to their site of action [3]. On the other hand, anandamide (AEA) is said to have anticancer activity on melanoma cells [4]

In our studies, the effect of anandamide on melanoma cell lines was determined using several biological tests (activity of selected proteins and enzymes, membrane integrity and apoptosis) as well as nanotechnological methods (like AFM) to study the elastic properties of cells treated with anandamide. Also, the real-time binding between lectin Concanavalin A from Canavalia ensiformis (Jack bean) and glycans present on the surface of melanoma cells was examined with QCM-D, Western blot and immunofluorescence. Cells were seeded onto a quartz crystal sensor coated with polystyrene.

The results show difference in lectin-glycan interactions among the studied non-treated cell types (melanoma cells and melanocytes) demonstrated on the Df plots. In this case the obtained relation between dissipation energy and frequency of sensor vibration from the complex binding measurements serves as a potential biomarker for cancer cells detection (higher signal - higher metastatic potential of cells). The obtained results of metastatic melanoma cells incubated with AEA presents changes in the lectin-glycan interaction observed on the Df plots (lowering the Df relation) what can suggest metastasis inhibition. Several biological tests affirmed the obtained results, what could mine that anandamide could be considered as a new therapeutical agent in the treatment of malignant melanoma.

References:
Nanomechanical properties of biopsied bronchial tissues as a marker in obstructive lung diseases

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Pathological condition of a tissue, often a consequence of abnormal cell behaviors, is frequently manifested not only by histopathological changes but in its mechanical properties variation as well [1-3]. Here, we use atomic force microscopy (AFM) to evaluate alterations in mechanical properties of bronchial wall biopsies originating from patients suffering from obstructive lung diseases that are asthma and chronic obstructive pulmonary disease (COPD) and healthy subjects as well. Each sample was probed in more than 700 locations. Young’s modulus (E) distributions obtained from Hertz model based analysis of collected force-distance curves showed statistically important differences (p<0.05) between studied groups (Figure 1). Data demonstrated the decreased deformability of asthma tissues with respect to control group while COPD tissues revealed higher E values regarding the control group.

![Graph showing Young's moduli values (E) of tissue samples originating from patients suffering from different obstructive lung diseases.](image)

Figure 1. Young’s moduli values (E) of tissue samples originating from patients suffering from different obstructive lung diseases.

Acknowledgements:
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References:
The Effect of Copper Ions on the Aggregation of hIAPP(11-28)

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Abstract Body: Copper ions play a critical role in the aggregation of human islet amyloid polypeptide (hIAPP), which is believed to be closely associated with β-cell death in type II diabetes (T2D). However, the underlying molecular mechanism still remains obscure. In this work, we have used several different experimental approaches to investigate the influence of Cu²⁺ on the aggregation of hIAPP(11-28). Atomic force microscopy measurements and Thioflavin T fluorescence denote that the Cu²⁺ could largely modulate hIAPP aggregation, including the inhibition of hIAPP fibrillation and the promotion of peptide oligomerization. We also present that Cu²⁺ is able to specifically bind to the hIAPP by forming a ring structure, which causes the reduction of Cu²⁺ to Cu⁺ and thus produce reactive oxygen species (ROS). Interestingly, the Amplex Red assay reveals that the hydrogen bubbles water could exhibit ROS-scavenging activity.

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