NanoInBio

ADVANCES FOR LIFE & MATERIALS SCIENCE

May 31st
June 5th
2016

PROGRAM &

BOOK OF ABSTRACTS


Le Gosier


French West Indies


FWI

ADVANCES FOR LIFE & MATERIALS SCIENCE

NanoInBio 2016

The First Conference On Nanosciences At The Interface Between Materials And Biological Systems

May 31st – June 5th, 2016 - Le Gosier, French West Indies (FWI)

Program & Book of Abstracts

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NanoInBio 2016

The First Conference On Nanosciences At The Interface Between Materials And Biological Systems

Program

Spring School NanoInBio 2016

Tuesday, May 31

Special Lecture

Philippe LECLERE*: Atomic Force Microscopy: a 30-year-old Story in Biology and Materials Science*

Conference NanoInBio 2016

Wednesday, June 1
Thursday, June 2
Friday, June 3
Sunday, June 5

Keynote Lectures

Dennis DISCHER*: Macrophage recognition of 'Self' for Nano- and Micro- applications against solid Tumors*

Georg FANTNER*: Close up and Personal: The Process of Mycobacterial Cell Division*

Andrew PELLING*: Starting Small and Going Big - Cells in Artificial Contexts*

Noriyuki KODERA*: Direct observation of proteins at work by high-speed atomic force microscopy*

Nicholas SPENCER*: Approaches to mimicking cartilage with polymers*

Pierre SCHAAF*: Soft-mechanochemistry – Mechanochemistry inspired by nature*

Keynote Lectures: 40 minutes
Plenary Lectures: 40 minutes
Oral Presentations: 20 minutes

	Tuesday, May 31
08.15 am – 09.00 am	**Welcome Reception & Registration**
	Workshop Session I
09.00 am – 10.00 am	**Philippe Leclere (Keynote Lecturer):** **Atomic Force Microscopy : a 30-year-old Story in Biology and Materials Science**
10.00 am – 10.20 am	**Julien Lopez**: Nanomechanical AFM techniques for biological samples and biomaterials (Asylum Research)
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10.40 am – 11.00 am	**Coffee Break**
11.00 am – 11.30 am	**Pablo Dörig**: FluidFM: Go beyond AFM imaging (Cytosurge AG)
11.30 am – 12.00 pm	**Jean Louis Mansot**: Latest advances in electron microscopies (Université des Antilles)
12.00 pm – 02.00 pm	**Workshop Lunch**
	Workshop Session II **Exhibition Session I**
02.00 pm – 04.00 pm	Demo from Cytosurge AG (Pablo Dörig) Demo from Asylum Research Technology (Julien Lopez) Demo from Bruker nano (Alexander Dulebo) Demo from JPK (Ben Holmes)
04.00 pm – 06.30 pm	**Visit of Memorial Acte Museum**
07.30 pm	**Opening Ceremony & Conference Dinner**

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11.20 am – 11.40 am	Halima Alem: Non-cytotoxic Smart Fluorescent Core/shell Nanoparticles and their further application for cancer drug release.
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12.00 pm – 12.20 pm	Dominika Hobernik: Surface modified dextran nanoparticles as carriers for anti-tumor DNA-vaccines
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05.00 pm – 05.20 pm	Bing Yan: **A Systematic Exploration of Nano-Bio Interactions: the Story of Carbon Nanotubes**
05.20 pm – 06.00 pm	Artium Khatchatouriants: **Bio-AFM and Raman Laboratory : Review of Scientific and Industrial Projects**
07.30 pm	Conference Dinner

	Thursday, June 2
	Conference Session II **Instrumentation & Application Tools**
09.00 am – 09.40 am	**Georg Fantner (Keynote Lecturer):** **Close up and Personal: The Process of Mycobacterial Cell Division**
09.40 am – 10.00 am	**Vincent Martinez**: Microfabricated Hollow Cantilevers Made of SU-8 and Their Potential for in-vitro Neuron Research
10.00 am – 10.20 am	**Juan Pelta**: Biomimetic nanopores fundamental to applications
10.20 am – 10.40 am	**Anny Fis**: Interaction forces of the bacterial translocon: An AFM investigation
10.40 am – 11.00 am	**Coffee Break**
11.00 am – 11.20 am	**Fabienne Quiles**: Multiscale in situ investigation of the action of an antimicrobial peptide on a bacterial nascent biofilm
11.20 am – 11.40 am	**Livie Dorwling-Carter**: The Fluidic Force Microscope: a versatile tool for force-controlled electrophysiology
11.40 am – 12.00 pm	**Oona Freudenthal**: Nanoscale investigation of colistin interactions with model phospholipid membranes by combined infrared and force spectroscopies
12.00 pm – 12.20 pm	**Jan Danko**: In vivo effect of hydroxyapatite ceramics visualized by X-rays
12.30 pm – 02.00 pm	**Conference Lunch**
	Conference Session III **Nanosciences at the frontier with biology**
02.00 pm – 02.40 pm	**Prof. Andrew Pelling (Keynote Lecturer):** **Starting Small and Going Big - Cells in Artificial Contexts**
02.40 pm – 03.00 pm	**Mickael Castelain**: The nanomechanical properties of *Lactococcus lactis* pili are conditioned by the polymerized backbone pilin
03.00 pm – 03.20 pm	**Tien Tuan Dao**: Development of a Nano Osteo-Mimetic System based on Bone Nano-Biomechanics
03.20 pm – 03.40 pm	**Martina J. Baum**: Physico-chemical characterization of a complex shaped ZnO nano-microstructure based polymer composites with potential as mechanically stable antifouling coating
03.40 pm – 04.00 pm	**Coffee Break**
04.00 pm – 04.20 pm	**Aurelien vander Straeten**: Protein-polyelectrolyte complexes for the surface immobilization of biologically active proteins
04.20 pm – 04.40 pm	**Michel Linder**: Nanoscale characterization of liposome-encapsulated curcumin
04.40 pm – 05.00 pm	**Jean-Sébastien Thomann**: Anti-biofouling Properties in Blood of Novel Mesoporous Silica Nanoparticles covered with a Self-assembled Supported Lipid Bilayer
05.00 pm – 05.20 pm	**Tanja Becke**: Single Molecule Force Spectroscopy Reveals Interaction Strength between Streptococcus Pneumoniae TIGR4 Pilus-1 Tip Protein RrgA and Human Fibronectin, Highlighting the Potential Role of Terminal Domains of RrgA
05.20 pm – 05.40 pm	**Frederic Borges**: Interaction between *Lactobacillus rhamnosus* GG and whey proteins: key role of pili
05.40 pm – 06.00 pm	**Peter Massanyi**: Nickel Male Toxicity – Structural and Ultrastructural Alterations
07.30 pm	**Conference Dinner**

	Friday, June 3
	Conference Session IV **Instrumentation & Application Tools**
09.00 am – 09.40 am	**Noriyuki Kodera (Keynote Lecturer):** **Direct observation of proteins at work by high-speed atomic force microscopy**
09.40 am – 10.00 am	**Audrey Beaussart**: Single-cell force spectroscopy of microbes
10.00 am – 10.20 am	**Karel Klepárník**: Implementation of nanotechnologies in single-cell analysis
10.20 am – 10.40 am	**Petr Gorelkin**: Nanopipette Navigation System as a New Tool for Biomedical Application
10.40 am – 11.00 am	**Coffee Break**
11.00 am – 11.20 am	**Olivier Gros**: ESEM sub-micrometer-scale mapping of sulfur as a powerful tool for the study of marine thioautotrophic organisms: case study of a new uncultured epsilon sulfur bacteria colonizing marine mangrove sediment in the Caribbean.
11.20 am – 11.40 am	**Sebastien Jaramillo**: Assessments of nanomechanical properties of biomimetic membranes using AFM Circular Mode
11.40 am – 12.00 pm	**Raphael Tiefenauer**: Complementary Dual Biosensing with Novel Ultrathin Gold Nanohole Films
12.00 pm – 12.20 pm	**Isabel Alves**: Real time monitoring of membrane GPCR reconstitution by plasmon waveguide resonance: on the role of cholesterol
12.30 pm – 01.30 pm	**Conference Lunch**
01.30 pm – 03.00 pm	**Poster Session II** **Exhibition Session II**
	Special NanoInBio Session II
03.00 pm – 03.40 pm	**Scott Guelcher: Injectable, settable, and resorbable nanocrystalline hydroxyapatite/ polyurethane hybrid polymers with bone-like strength**
03.40 pm – 04.00 pm	**Ruby M. Sullan: Nanoscale Interplay Between Bacterial Adhesins and Substrate Properties**
04.00 pm – 04.20 pm	**Coffee Break**
04.20 pm – 05.00 pm	**Ben Holmes: Fast Nanoscale Imaging and Quantitative Nanomechanical Characterization of Cells and Biomaterials with Correlative Atomic Force and Optical Microscopy**
05.00 pm – 05.20 pm	**Ronald Zirbs: Universal melt modification method for the synthesis of core-shell-nanoparticles with ultra-high grafting densities**
05.20 pm – 06.00 pm	**Munisch Chanana: Nano-functionalized Biomaterials and Bio-functionalized Nanomaterials**
07.30 pm	**Conference Dinner**

	Saturday, June 4
	Social Event
07.00 am – 05.00 pm	Special Cruise to Petite Terre Islands (Lagoon and Barrier Reef discovery)
06.30 pm – 07.30 pm	Traditional Music & Dance Show
07.30 pm	Gala Dinner

	Sunday, June 5
	Conference Session V **Nanosciences at the frontier with biology**
09.00 am – 09.40 am	**Nicholas Spencer (Keynote Lecturer):** **Approaches to mimicking cartilage with polymers**
09.40 am – 10.00 am	**Hélène Martin-Yken**: Stress, Drug Resistance and Adhesion: a closer look into the dark side of the wall
10.00 am – 10.20 am	**David Duday**: Degradation of small unilamellar vesicles and their cargo in physiological liquids by cold atmospheric plasma
10.20 am – 10.40 am	**Jérôme Dejeu**: Functionalization of surfaces by peptidic ligands using multivalent host-guest interaction
10.40 am – 11.00 am	**Coffee Break**
11.00 am – 11.20 am	**Lukas Traxler**: Characterization of the Orai-Calmodulin Interaction as Potential Mediator of Calcium-Dependent Orai-Channel Inactivation
11.20 am – 11.40 am	**Kristin Webling**: The complexity of G-protein coupled receptor signaling evaluation
11.40 am – 12.00 pm	**Sandra Posch**: Interplay of domain interactions and unfolding in the force sensing protein von Willebrand factor (VWF)
12.00 pm – 12.20 pm	**Gilmar Salgado**: Using NMR spectroscopy to access ligand binding of G-quadruplex DNA in Xenopus oocytes
12.30 pm – 02.00 pm	**Conference Lunch**
	Conference Session VI **Nanobiotechnology & Nanomedicine**
02.00 pm – 02.40 pm	**Pierre Schaaf (Keynote lecturer):** **Soft-mechanochemistry – Mechanochemistry inspired by nature**
02.40 pm – 03.00 pm	**Michael Timmermann**: Cell-inspired, microwell structures with linear strain-stiffening
03.00 pm – 03.20 pm	**Jana Musilkova**: Impairment of the adhesion, growth and osteogenic differentiation of human osteoblast-like cells on nanofibrous polylactide scaffolds with diamond nanoparticles
03.20 pm – 03.40 pm	**Stefanie Kiderlen**: Mechanotransduction on the Single Cell Level: Investigating Mechanosensitive Genes using Single-Cell Force Spectroscopy combined with Quantitative PCR
03.40 pm – 04.00 pm	**Jana Karpiskova**: Cytocompatibility of Polycaprolactone Nano-Microfibrous Scaffolds Loaded with Amide-Amine Functionalised Carbon Nanoparticles
04.00 pm – 04.20 pm	**Coffee Break**
04.20 pm – 04.40 pm	**Violeta Garcia Romero**: Study of the photocatalytic activity of ZnO nanorods films, in the photodegradation of rhodamine B dye with solar irradiation
04.40 pm – 05.00 pm	**Marcin Kruszewski**: Induction of DNA Damage in HepG2 and A549 Cells Treated with Quantum Dots, Silver and Titanium Dioxide Nanoparticles or Their Binary Mixtures
07.30 pm	**Closing Ceremony & Conference Dinner**

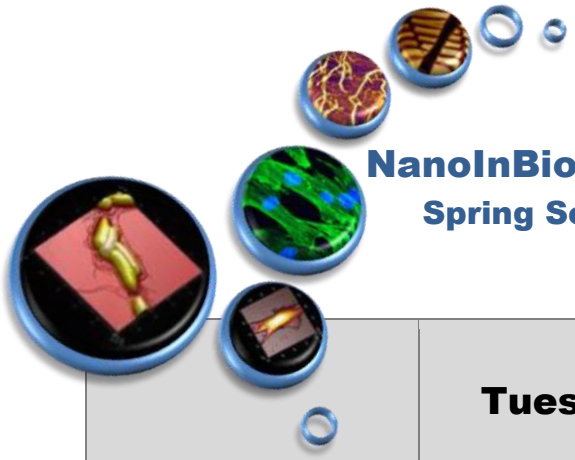
Oral Presentations

Spring School

&

Conference

NanoInBio 2016



NanoInBio 2016
Spring School Program

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Atomic Force Microscopy: a 30-year-old Story in Biology and Materials Science

Philippe LECLERE

Center for Innovation and Research in Materials and Polymers (CIRMAP)
Research Institute for Materials Science and Engineering
University of Mons (UMONS), Place du Parc, 20, B 7000 Mons (Belgium)

philippe.leclere@umons.ac.be

The invention and development of the optical microscope in the seventeenth century revealed the presence of a previously unseen and unimaginable world within and around us. Our lives would not be what they are today if optical microscopy had never existed or if it had not helped us to understand better what we are, how we function, and how we can improve our condition – first in the fields of biology and medicine, and then in many other fields such materials science. Another great step was made with the introduction of transmission and scanning electron microscopy in the 1930s, which was initially integrated with optical microscopy but subsequently developed its own identity and technology and opened up new horizons in human knowledge.

Starting in 1986 (i.e. exactly 30 years ago!), further technological advances led to the development of Atomic Force Microscopy (AFM), which is completely different from its predecessors: instead of being based on lenses, photons, and electrons, it directly explores the surface of the sample by means of a local scanning probe while the use of dedicated software allows the results to be visualized on a screen.

AFM has a number of special characteristics: very high magnification with very high resolution; minimal sample preparation (none of the dyes of optical microscopy, or the vacuum, critical point, or gold sputtering required by scanning electron microscopy); real three-dimensional topographical data that allow us to obtain different views of the samples from a single collected dataset; and the ability to work in a liquid in real time, thus making it possible to study the dynamic phenomena of living specimens in their biological environment and under near-physiological conditions.

Functionalizing the AFM tip has made it possible to obtain “nano-biosensors” that can be used in the field of dynamic biomolecular processes in ways that could not even be imagined just a few years ago. Finally, combining AFM with other microscopic techniques, such as confocal or fluorescence microscopy is now being actively explored, and a number of interesting synergies have been discovered.

Understanding and controlling forces at the nanometer scale is a scientific problem of importance to a wide variety of technological applications of soft materials. Advancement in this field and the development of new nano-materials goes hand-in-hand with ever more sensitive tools and more accurate methods of measuring surface forces. Recent progress in the field of dynamic AFM regarding calibration and dynamic methods of force measurement has set the stage for a critical examination of the physical models commonly used in describing tip–surface interaction. Meaningful interaction models are crucial to our understanding and interpretation, as they are the link between AFM data and the image or map of a physical property of the material surface.

In this lecture, we will describe this 30-year-old story and illustrate the fantastic capabilities of this technique on various examples

Nanomechanical AFM techniques for biological samples and biomaterials

Irène Revenko[a]

[a] *Oxford Instruments Asylum Research Inc., 6310 Hollister Avenue, Santa Barbara, CA, USA*

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Understanding bionanomechanics is of major importance in many fields of research. For example, 'mechanotransduction' offers insight on how cells react at the molecular level to forces applied externally on their membranes and how cells interact with their environment. Numerous pathologies are also linked to changes in the mechanical characteristics of some cells. A vast number of publications show that the physiopathology of cancers involves a change in the viscoelastic properties of cells: The more aggressive types of cancer correspond to the less viscoelastic cells.
Atomic force microscopy (AFM) has emerged as an indispensible technique for measuring mechanical properties of biomaterials and biological samples with high spatial resolution and force sensitivity within physiologically relevant environments in the kPa to GPa elastic modulus range. However, the large diversity of material properties prevents any single AFM technique from providing the most relevant or accurate data for every application. Here we are reviewing a vast number of available techniques and how they apply to all areas of research. In particular we will review and compare the following techniques: Force Curve Measurements, Fast Force Mapping, Nanoindentation, Phase Imaging, Loss Tangent Imaging, AM-FM, Contact Resonance Viscoelastic Mapping, and Force Modulation Imaging. Together the different techniques can be used on any bio related material and measure a wide range of properties including elastic stiffness, loss and storage modulus, viscous damping, adhesion, and hardness. This short review should help determining which technique to choose based on the research goals and the samples.

High-Resolution Imaging of Living Cells by Atomic Force Microscopy

Alexander Dulebo,[a] Andrea Slade,[b] Izhar Medalsy,[b] Shuiqing Hu,[b] James Shaw,[b] and Hermann Schillers[c]

[a] *Bruker Nano Surfaces, Östliche Rheinbrückenstr. 49, Karlsruhe 76187, Germany*
[b] *Bruker Nano Inc., 112 Robin Hill Road, Santa Barbara, CA 93117, USA*
[c] *Institute of Physiology II, University of Münster, Robert-Koch-Str. 27b, Münster 48149, Germany*

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Atomic force microscopy (AFM) provides the unique capability to obtain three-dimensional images of the morphology of individual living cells under physiological conditions without the need for labeling or staining. However, due to their flexible and dynamic nature, visualization of cellular structures by AFM with nanoscale resolution has remained challenging. Microvilli are common structures found on epithelial cells that increase the apical surface thus enhancing the transmembrane transport capacity and also serve as mechanosensors. Changes in both the density and structure of microvilli are associated with various disease states. We have used a newly developed AFM probe, having a unique 17 micron tall tip and 65nm fixed end radius, together with the low piconewton imaging forces enabled by PeakForce Tapping mode to resolve individual microvilli structures on the surface of living cells for the first time by AFM. Our studies also revealed a direct relationship of the observed structure of the microvilli with the interaction force of the AFM probe.

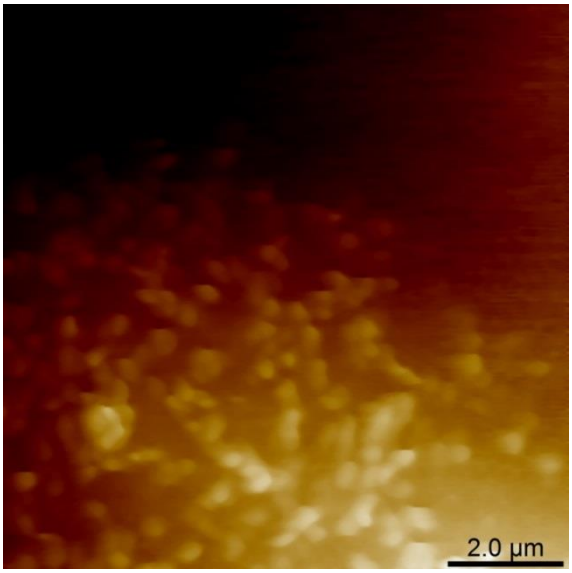

Figure: PeakForce Tapping image (topography) of a live MDCK C11 cell. 10 x 10 µm scan with a vertical force of 80-100 pN force. Z scale is 1.3 µm.

References:
(1) Schillers, H. et al. *Journal of Molecular Recognition* **2015**, *29*, 95.

FluidFM: Go beyond AFM imaging

Pablo Dörig [a]

[a] *Cytosurge AG, Sägereistrasse 25, Glattbrugg, Switzerland*

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Fluid force microscopy (FluidFM) was introduced in 2009[1] and has since widened the horizon of what is possible with AFM. From single cell injection[2] and bacterial adhesion measurements,[3] through to nanolithography in liquid and 3D metal micro printing[4], FluidFM has found very diverse applications.

As product manager at Cytosurge, Pablo Dörig aims to bring these advances into the research labs around the world in the form of comprehensive and powerful platform solutions. He presents a newly introduced standalone system as well as upgrade possibilities for an existing AFM setup. Thanks to intuitive software and a versatile FluidFM probe toolbox unique applications become available to a broader community.

References:

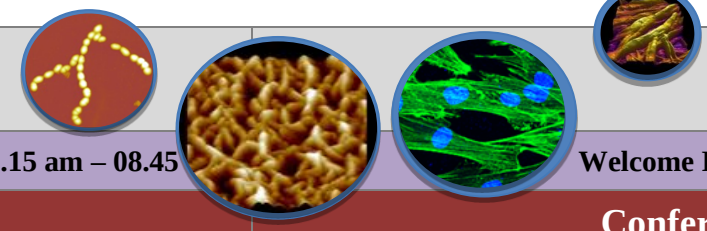
(1) Meister, A.; Gabi, M.; Behr, P.; Studer, P.; Vörös, J.; Niedermann, P.; Bitterli, J.; Polesel-Maris, J.; Liley, M.; Heinzelmann, H.; et al. FluidFM: Combining atomic force microscopy and nanofluidics in a universal liquid delivery system for single cell applications and beyond. *Nano Lett.* **2009**, *9* (6), 2501–2507 DOI: 10.1021/nl901384x.

(2) Guillaume-Gentil, O.; Potthoff, E.; Ossola, D.; Dörig, P.; Zambelli, T.; Vorholt, J. A. Force-controlled fluidic injection into single cell nuclei. *Small* **2013**, *9* (11), 1904–1907 DOI: 10.1002/smll.201202276.

(3) Potthoff, E.; Ossola, D.; Zambelli, T.; Vorholt, J. A. Bacterial adhesion force quantification by fluidic force microscopy. *Nanoscale* **2015**, *7* (9), 4070–4079 DOI: 10.1039/c4nr06495j.

(4) Hirt, L.; Ihle, S.; Pan, Z.; Dorwling-Carter, L.; Reiser, A.; Wheeler, J. M.; Spolenak, R.; Vörös, J.; Zambelli, T. Template-Free 3D Microprinting of Metals Using a Force-Controlled Nanopipette for Layer-by-Layer Electrodeposition. *Adv. Mater.* **2016** DOI: 10.1002/adma.201504967.

NanoInBio 2016
Conference Program



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Macrophage recognition of 'Self' for Nano- and Micro- applications against solid Tumors

Dennis E. Discher [a]

[a] *NanoBioPolymers Labs, University of Pennsylvania, Philadelphia, PA, USA*

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Particles, implants, and cells of any type interact in vivo with the innate immune system, especially phagocytes that try to 'eat' everything. At the same time, 'Self' cells are spared due to a polypeptide found on all cells that marks cells as 'Self', limiting their phagocytic clearance *in vitro* and *in vivo* – even when displayed on nanoparticles (1) and engineered viruses (unpublished). Based on these nano-investigations, an alternative approach to tumor therapy also seems possible by using the innate immune cell's tendency to migrate into injury sites (such as tumors). Three features are required for therapy: 1) highly phagocytic cells that are 2) blinded to 'Self' and 3) activated to 'eat' cancer cells (unpublished). Solid tumors can thus be safely and effectively shrunk by phagocytes, even after chemotherapy has failed, and acquisition of tumor antigens at the same time should increase the chance that acquired immunity develops.

References

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Stretch-Induced Helical Conformations in Poly(L-lysine)/Hyaluronic Acid Multilayers

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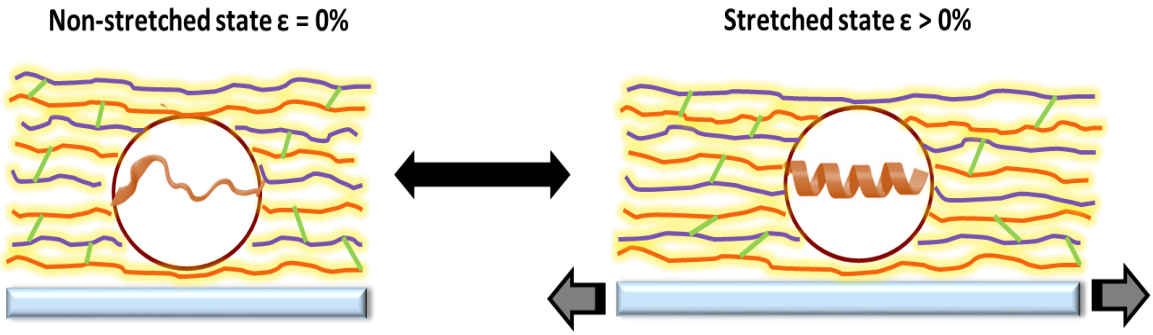
Responsive surfaces have become one of the most active area in the field of materials. To induce a specific response, numerous triggers were reported. Only few studies report about the design of systems that respond to a mechanical stimulus such as stretching. In this study, we investigate the effect of stretching on the secondary structure of cross-linked poly (L-lysine)/hyaluronic acid (PLL/HA) multilayers. We show that stretching these films induces changes in the secondary structure of PLL chains. Our results suggest that not only α- but also 3_{10}-helices might form in the film under stretching. Such 3_{10}-helices have never been observed for PLL so far. These changes of the secondary structure of PLL are reversible, *i.e.* when returning to the non-stretched state one recovers the initial film structure. Using molecular dynamics simulations of chains composed of 20 L-lysine residues (PLL20), we find that these chains never adopt a helical conformation in water.
In contrast, when the end-to-end distance of the chains is restrained to values smaller than the mean end-to-end distance of free chains, a distance domain rarely explored by the free chains, helical conformations become accessible. Moreover, the formation of not only α- but also 3_{10}-helices is predicted by the simulations. These results suggest that it is the change of the end-to-end distance of PLL chains in the stretched film that is at the origin of the helix formation.
Molecular conformation of peptides or proteins is a crucial parameter that can be tuned by stretching mechano-responsive systems. The goal of the next study will be the design of new catalytic materials based on artificial enzymes, whose catalytic activities can be directly controlled through the molecular deformation of active sites by stretching the materials.



Stretching of a polyelectrolyte multilayer induces changes of the secondary structure of the constituting poly (L-lysine).

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Fibrin-coated polylactide nanofibers as carriers for skin cells

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The desirable skin tissue substitute should mimic the functions of original tissue, should enhance tissue regeneration by supporting dermis and epidermis layer formation with well-developed extracellular matrix[1]. The promising approach seems to be fabricating biodegradable nanostructured scaffold which will be slowly resorbed in the organism and finally replaced by new tissue[2]. Nanostructured materials better mimic the nanofibrous component of natural ECM than flat or microstructured surface. These materials enable adsorption of cell adhesion-mediating ECM molecules from body fluids or cell culture media in an appropriate spatial conformation for their binding by the cell adhesion receptors[3]. In our project we focused on a modification of polylactide nanofibrous membranes with cell-degradable fibrin nanocoating, and the subsequent adhesion and growth of human dermal fibroblasts and extracellular matrix deposition by these cells. The nanofibrous membranes were prepared using a Nanospider needleless electrospinning technology. The membranes were coated with fibrin prepared directly on the membranes from human fibrinogen activated by human thrombin[4]. Fibrin regularly formed a coating around individual nanofibers in the membranes, and also created a thin fibrous mesh on several places on the membrane surface. The fibrin cell-free nanocoating remained stable in the cell culture medium for 14 days. However, the cells degraded and reorganized fibrin nanocoating, and the rate of these changes correlated with the degree of cell proliferation. The cell adhesion, spreading and proliferation was enhanced by fibrin nanocoating. Moreover, fibrin nanocoating strongly improved the ECM formation, namely the synthesis of collagen and fibronectin by cells (Fig. 1). The collagen synthesis by cells was enhanced by adding ascorbic acid into the cell culture medium. The ascorbic acid also increased the cell proliferation. Thus, it can be concluded that fibrin coating is a promising modification of nanofibrous membranes for skin tissue engineering, particularly in combination with ascorbic acid.

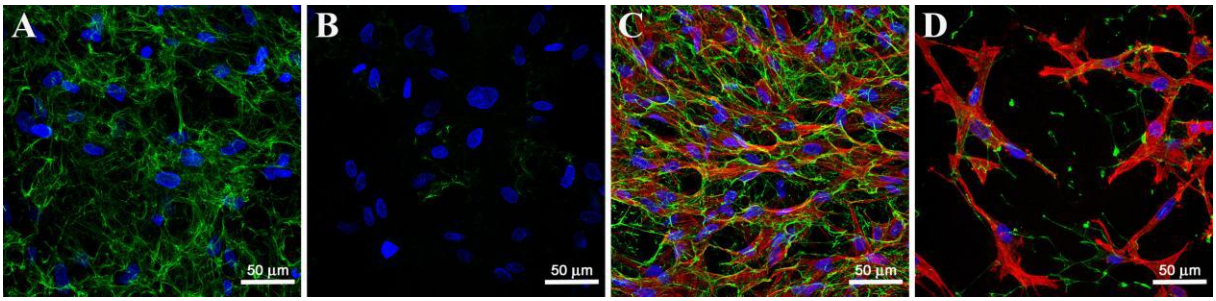

Fig. 1: Collagen I (A, B, green) and fibronectin (C, D, green) produced by dermal fibroblasts on fibrin-coated PLA membranes (A, C) or on non-coated (B, D) membranes on day 7 after cell seeding. Collagen I and fibronectin were stained by immunofluorescence using primary and secondary antibodies (green). Actin cytoskeleton was stained with Phalloidin-TRITC (C, D, red) and cell nuclei with Hoechst #33258 (blue). Leica TCS SPE DM2500 confocal microscope, obj. 40x/1.15 NA oil.

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Atomic force microscopy imaging and indentation measurements to assess murine cartilage properties during development

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In the process of endochondral ossification bones of the appendicular and the axial skeleton are formed from a previous developed cartilaginous template. The growth plate (GP) is a special from of hyaline cartilage which is localized between the epiphysis and diaphysis of long bones during the formation of the skeleton. The GP is mainly responsible for longitudinal bone elongation, due to cell proliferation, differentiation, and matrix production. GP cartilage can be subdivided into several zones with distinct cellular arrangement, function, and matrix composition. Within the proliferative zone of GP cartilage, chondrocytes undergo rapid division accompanied by the establishment of cellular polarity and columnar arrangement [1]. It is increasingly recognized, that the fate of cells, and therefore tissue morphology and function is tightly controlled by the biomechanical properties of their micro-environment. In cartilage biomechanical properties are mainly determined by the composition of its extracellular matrix (ECM). The ECM is mainly composed of a dense collagen network which encloses hydrated proteoglycans (PG) and counteracts their swelling pressure. In the proliferative zone of GP cartilage ECM can be divided into several matrix compartments distinct by their composition. The pericellular matrix (PCM) immediately surrounds chondrocytes. The adjacent territorial matrix (TM) encloses the PCM and clustered chondrocytes defining the columnar chondron. The interterritorial matrix (ITM) is located between the columns (Fig.1). We assume, that the unique columnar arrangement of chondrocytes within the proliferative zone of the GP is driven by structural as well as biomechanical properties of the different matrix compartments. ECM properties might even guide chondrocytes to arrange into a columnar stack and thus, drive the linear elongation of endochondral bones.

We performed atomic force microscopy (AFM) imaging and indentation type AFM measurements, in order to correlate ECM structural and mechanical properties, among different developmental stages of murine tibial GP. AFM images showed a progressive cell flattening and arrangement into columns from embryonic day (E) 13.5 until postnatal week 2. This was correlated with an increasing collagen density and ECM stiffness. At all stages, a marked difference in collagen density and fibril orientation was observed between ITM and TM-PCM accompanied with a roughly two-fold higher stiffness of the TM-PCM. Therefore, we conclude, that local stiffness may force chondrocytes to arrange in columns and drive bone elongation during skeleton development. Furthermore, our results indicate, that AFM is a powerful tool to compare structure-biomechanical properties, and to resolve mechanical properties of distinct matrix compartments. Therefore, our results are of high interest for the comparison with genetically modified mice in order to investigate the role of individual ECM components during cartilage and bone development.

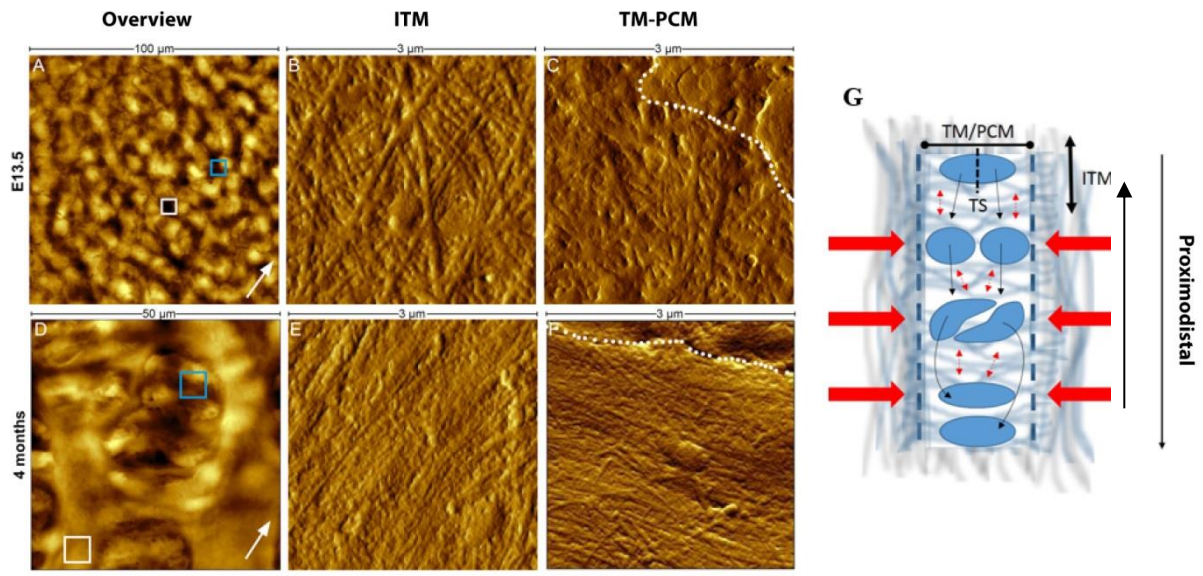

Fig.1: AFM overview and ECM detail images at embryonic and 4 month old mice. The AFM overview images (A, D; white square=ITM, blue square=TM/PCM) show an increasing cell flattening perpendicular to cartilage proximodistal axis (indicated by white arrows) with progressive age. AFM detail images within GP ITM (B, E) and TM/PCM (C, F) reveal a progressive collagen density and fibril orientation during cartilage maturation (dotted line indicate the position of chondrocytes). G: Model for chondrocyte columnar arrangement due to different mechanical properties of the surrounding ECM. Chondrocytes within the soft TM/PCM divide parallel proximodistal axis where the stiff matrix of the ITM resists cell elongation. Consequently, chondrocytes rotate around each other and move into the softer matrix in order to flatten again.

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Lauroyl-gemcitabine loaded lipid nanocapsule hydrogel: feasibility, efficacy and safety for the local treatment of glioblastoma

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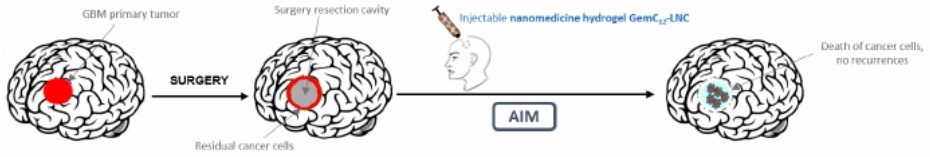
Glioblastoma (GBM) is an aggressive malignant brain tumor characterized by rapid proliferation and propensity to infiltrate healthy brain tissue. Its standard of care therapy includes surgical resection, radiotherapy and chemotherapy with Temozolomide but GBM still remains incurable mainly because of its high invasiveness andchemoresistance to alkylating drugs(1). Among the strategies that have been developed to find a solution to the devastating and incurable effects of GBM there is the local delivery of chemotherapeutic agents (implants, foams, hydrogels, microcarriers) and the use of nanomedicines. Recently, Benoit's group has developed an innovative nanodelivery system formed of lipid nanocapsules (LNC) loaded with the prodrug Lauroyl-gemcitabine (GemC12) which has the unique property of forming a product with gel-like consistency when the drug is incorporated in the LNC structure. This hydrogel does not need the presence of polymers nor UV light irradiation to obtain gelification and can be injected without any loss of viscoelastic properties(2).

Considering that i) 80-90% of GBM recurrences after surgical resection are localized within 2 cm of the original site of the tumor(3) and ii) gemcitabine (Gem) has a MGMT-independent mechanism of action and shows excellent radio-sensitizing properties(4), we have hypothesized to deliver the GemC12-LNC nanomedicine hydrogel directly in the tumor resection cavity in order to avoid recurrences of GBM5.



Hence, we formulated a gel-like GemC12-LNC nanoformulationusing a phase-inversion technique process and evaluated its physiochemical properties, encapsulation efficiency and loading capacity.The viscoelastic properties have been evaluated after extrusion from 30 G needles, demonstrating that the hydrogel is adapted for brain implantation. The GemC12-LNCin-vitro release kinetics from the hydrogel was evaluated in artificial cerebrospinal fluid and showed a sustained and prolonged release of the drug over one month. The invitrocytotoxicity studies on glioma cell lines showed an enhanced cytotoxic activity of GemC12 and GemC12-LNC compared to the parental hydrophilic drug Gem(IC50= 0.18, 0.56 and 12.06 µM, respectively in U87 MG cell line). Invivo anti-tumor efficacy studies have been performed on a subcutaneous human GBM tumor model showing a decrease in the tumor growth in mice treated with GemC12-LNC hydrogel compared to the free drug. Since a significant tumor reduction has been observed after treatment with GemC12-LNC, further efficacy and survival studies are on-going in an orthotopic model of GBM over a longer period of time as well as in a resection model of this tumor. Moreover, short-term and mid-term tolerability studies indicatedthat this system is suitable for local treatment in the brain (Hematoxylin and Eosin, TUNEL assay, Iba-1 immunostaining)(5).

In conclusion, we have demonstrated the feasibility, safety and efficiency of our injectable GemC12-LNC hydrogel for the local treatment of GBM. This system, which has a very simple formulation and combines the properties and advantages of nanomedicines and hydrogels, could be considered as a promising platform for the delivery of GemC12for the local treatment of GBM.

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Non-cytotoxic Smart Fluorescent Core/shell Nanoparticles and their further application for cancer drug release.

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Responsive nanoparticles were obtained by growing poly(N-isopropyl acrylamide) ((PNIPAM)) and poly(ether oxide) derivatives copolymers by a controlled radical polymerization (ARGET-ATRP) from quantum dot (QD) surface [1]. Each step of the synthesis process was monitored by the combination of different techniques like UV measurements, fluorescence and transmission electron microscopy. The influence of the polymerization time or monomer rate on the final photophysical properties of the nanomaterials were studied by fluorescence spectroscopy.
The temperature responsive behavior of the ZnO/(co-)polymer nanohybrids was studied by Dynamic Light Scattering (DLS) and fluorescence spectroscopy. Drug loading and release capacity of poly(ether oxide) derivatives core/shell NPs as well as their cytotoxicity towards cancer cells were evaluated. Results demonstrate the impact of the co-polymer structure on NP properties in view of the application in cancer therapy.

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Polyvalent glycosaminoglycan-based LbL nanofilms: Applications to bone and neural tissues

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The design of advanced biomimetic substrates in the field of cell/biomaterial interactions and more largely, in tissue engineering is a topical challenge. In this respect, layer-by-Layer (LbL) films composed of natural components are promising candidates. We selected chondroitin sulfate A (CSA) as the polyanionic component since it is a natural glycosaminoglycan (GAG) present in the extracellular matrix of bone (1) and neural tissue (2), and poly(L-lysine) (PLL) as the biocompatible polypeptide polycation. It is yet worth mentioning that GAG-containing LbL films are highly hydrated hydrogel-like architectures due to affinity of GAGs for water (3). As a result, they exhibit low mechanical properties and thus are cell-repellent, hindering cell adhesion. To render cytophilic such nanofilms, stiffening post-treatments with crosslinking agents are commonly used. However, conventional synthetic crosslinkers (EDC/NHS or glutaraldehyde) may be cytotoxic in their free form. Thus, we chose genipin (GnP), an iridoid derivative from gardenia fruits, as a natural crosslinking agent much more biocompatible. The treatment with GnP is likely to give rise to a semi-interpenetrating polymer network (semi-IPN) comprised of a crosslinked PLL network through which CSA chains remain free to diffuse (4) (Fig. 1a). We obtained original structural features with (CSA/PLL)n GnP-crosslinked films (Fig. 1b-c) and improved mechanical properties. Then, we applied these LbL nanofilms as (i) favorable biomimetic coatings for the bone biomineralization (Fig. 1d) and (ii) promising biomimetic matrix for the differentiation of neuroblastoma cells (Fig. 1e).

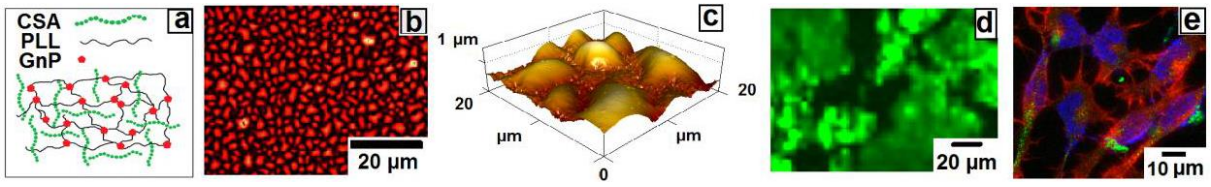

Figure 1: (CSA/PLL)n films crosslinked with genipin (GnP): (a) Semi-IPN structure - (b) Fluorescence observation of (CSA/PLL)12 films (autofluorescence of GnP: red) – (c) AFM picture of (CSA/PLL)12 nanofilms - (d) Raman microspectrocopy of hydroxyapatite-containing nodules after osteoblasts culture atop patterned titanium coated with (CSA/PLL)6 nanofilms – (e) Fluorescence of neurite outgrowth of neuroblastoma cells cultured atop the of (CSA/PLL)6 nanofilms (blue: nucleus, red: actin, green: □-tubulin).

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Surface modified dextran nanoparticles as carriers for anti-tumor DNA-vaccines

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Tumor immune therapy has gained more and more importance over the last few decades. Several systems, like sipuleucel-T (Provenge®), were approved for clinical use (1) whereas many more are still in clinical trials. The purpose of immune therapy is to manipulate the immune system to recognize tumors and to start defeating them. In this regard dendritic cells (DC) which play a key role in adaptive immune responses can be loaded with tumor associated antigens (TAAs). DC internalize antigens and present them to antigen-specific T cells which are then activated and initiate anti-tumor responses. Besides using proteins and peptides an alternative approach to load DC is to use plasmid DNA (pDNA) which encodes for tumor associated antigens.
Dextran based nanoparticles (dbNPs) are biocompatible and biodegradable and are perfectly suited to complex pDNA (2). We used PEG and dextran to modify the surface of fluorescence-labeled particles and analyzed particle uptake/binding in DC and other immune cell subpopulations from murine spleen via flow cytometry. We found that surface modification has an impact on the uptake of dbNPs especially by DC and macrophages. Uptake of dbNPs by these two immune cell types was confirmed using confocal laser scanning microscopy (cLSM). To evaluate the potential of differentially surface-functionalized dbNPs to transfect DC with pDNA all types of nanoparticles were loaded with a luciferase encoding pDNA. We used the well-established DC-like cell line DC2.4 for transfection studies. Transfection by dbNPs could be observed, although transfection efficiencies did not correlate with the results of the uptake studies since non surface modified NPs yielded highest transfection efficiencies. For this reason we had a closer look at the uptake mechanisms of dbNPs by blocking different receptors and analyzing particle uptake by flow cytometry. Especially scavenger receptors as well as Dectin-1, a C-type lectin receptor, seem to play an important role in the receptor mediated part of the uptake mechanism especially for dextran modified dbNPs. We also asked whether surface modification of dbNPs can change the activation state of DC. This question was addressed by analyzing activation markers on the surface of dendritic cells as well as measuring the cytokine pattern in culture supernatants. Again, the influence of surface modification was clearly observable leading to a higher activation of DC in case of particle modification with dextran.
Further work is focused on the optimization of the transfection capacity and DC specificity of dbNPs by functionalization with different targeting structures like antibodies and receptor ligands. Additionally, model antigen and adjuvant encoding pDNA is to be encapsulated in dbNPs and antigen specific T cell response will be analysed after transfection of DC *in vitro* and *in vivo*.

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Poroelastic behavior of living cells confirmed via Wide--bandwidth AFM rheology system

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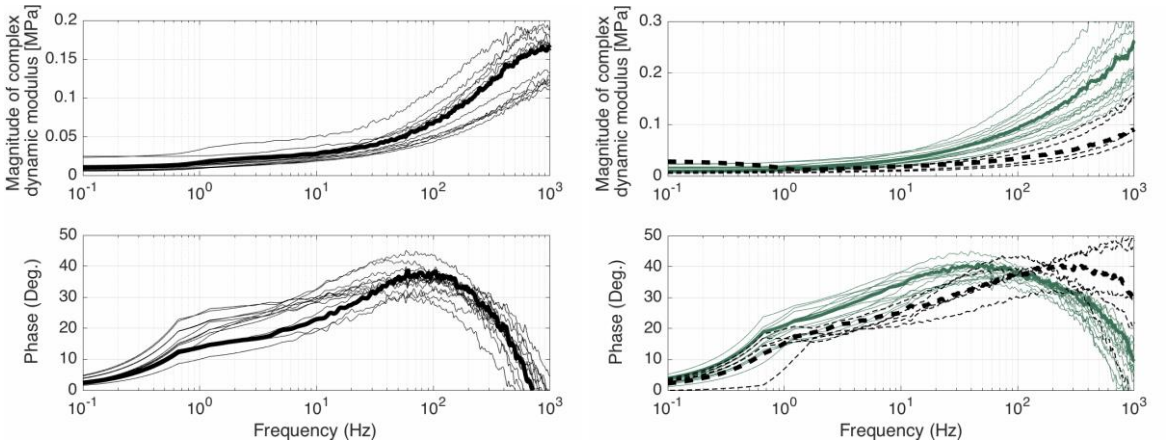
Introduction:
The cytoplasm represents the largest part of the cell by volume and hence its rheology sets the maximum rate at which cellular shape change can occur. To date, most studies have utilized one-phase viscoelastic models to model the time-dependent mechanical behavior of cells (Moeendarbary & Harris, 2014, Wires System Biology). Recently it has been suggested that the poroelastic framework, in which the cell is considered a biphasic system constituted of an interstitial fluid (cytosol) diffusing through a porous elastic solid meshwork (cytoskeleton, organelles, ribosomes), may provide a more adequate description (Moeendarbary Nature Materials, 2013, Charras Nature, 2005). In this framework, the time-dependent mechanics of the cell can be described via a single parameter: the poroelastic diffusion constant D, which scales as D~Eζ2/μD~Kξ"!/!μμ, with E the drained elastic modulus, ζ the cytoplasmic pore size, and μ the cytosolic viscosity. Previously, the Atomic force microscopy (AFM) indentation and force relaxation experiments were utilized to estimate the poroelastic cellular parameters (Moeendarbary et al., 2013). This Work reports the poroelastic behavior of Rat Pulmonary Microvascular Endothelial Cells (RPMECs) detected by a novel wide-bandwidth AFM (W-AFM) rheology system. The wide-bandwidth-AFM with selection of the right AFM tip size enabled measurement of complete nanomechanics of RPMEC live cells for the first time in wide range of frequencies (0.1-1kHz) Not attainable with regular AFM Systems (with achievable frequencies lower than 300 Hz).

Method:
W-AFM experiments were performed to measure the nano-scale complex modulus and phase on an AFM (on MFP-3D-BIO AFM, Asylum Research, Spherical particles D = 5μm, cantilever stiffness = 0.2 N/m, AIO-TL Innovative Solutions) Equipped with a newly designed actuation system similar to actuation system reported in (Nia et al., 2015a) but specially designed for translucent materials (e.g. cells). W-AFM captures both time–independent structural properties of material (i.e. elasticity), as well as time-dependent properties (i.e. Frequency dependent complex dynamic modulus and phase. See Figure1). This technique previously demonstrated poroelastic behavior of cartilage and aggrecan molecules (Nia et al., 2015a, Nia et al., 2015c).

Results and discussion:
All W-AFM results including results shown at Figure 1 and Figure 2 indicate stiffening of the cell with increase in the rate (frequency) of indentation consistent with poroelastic behavior where magnitude of dynamic modulus grows from a plateau in low frequency followed by a rapid increase and a plateau in high frequency. More interestingly, the phase of complex modulus shows a complete peak near the inflection point of the magnitude of complex dynamic modulus which is a clear sign of a poroelastic behaviour.



The high frequency plateau and peak of phase have not been observed before. Increasing the contact area between the AFM tip and cell by increasing the indentation depth clearly shifts the peak angle to lower frequency confirming the length scale dependency of cell rheology that is a major signature for the cell poroelastic nature. The validity of the poroelastic cellular model was further examined by applying osmotic perturbations to change the cytoplasmic pore size (Figure 2). In summary, W-AFM Results verify that cell exhibit a poroelastic response and poroelasticity is a suitable model for predicting the cell rheology under these experimental conditions.

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Bioactive coating system with self-antimicrobial and immunomodulatory properties

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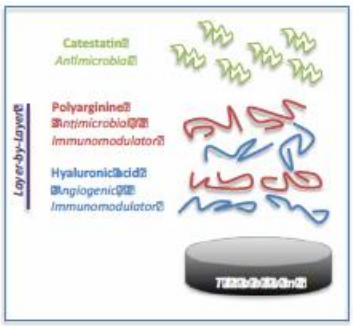
All implantable biomedical systems face several risks once in contact with the host tissue : i) excessive immune response to the implant; ii) development of bacterial biofilms and iii) yeast and fungi infections. A multifunctional surface coating which can address all these issues concomitantly would significantly improve clinical outcomes. We develop here for the first time a coating that address these three issues simultaneously. We hypothesized that polyarginine (PAR), a synthetic highly cationic polypeptide, can act on macrophages to control innate immune response because arginine is an important component of macrophage metabolism. Moreover, PAR is susceptible to act as an antimicrobial agent due to its positive charges. We developed a new polyelectrolyte multilayer films based on PAR and hyaluronic acid (HA). The PAR/HA films have a strong inhibitory effect on the production of inflammatory cytokines released by human primary macrophages subpopulations. This could reduce potential chronic inflammatory reaction following implantation. Next, we show that PAR/HA films were very effective against S. aureus for 24h. The PAR/HA films can be easily further functionalized by embedding antimicrobial peptides, like catestatin (CAT), a natural host defense peptide. This PAR/HA+CAT film proved to be effective as an antimicrobial coating against yeast and fungi. The cytocompatibility of the PAR/HA films was assessed with human umbilical vein endothelial cells (HUVECs). This all-in-one system that limits strong inflammation and prevent pathogen's infections constitutes an original strategy to coat implants in an active way.



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Quantitative accurate mechanical measurements with Atomic Force Microscopy operated in Tapping Mode: AM-FM for biological samples

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Tapping mode Atomic Force Microscopy imaging, also known as amplitude-modulated (AM) atomic force microscopy (AFM) is fast, gentle and provides the high spatial resolution necessary for imaging nanoscale features. However, until recently, mechanical characterization with tapping mode was limited to only qualitative results. In AM-FM mode, a bimodal (dual-frequency) technique (1-2), the first resonant mode is operated in AM, whereas a higher resonant mode is frequency modulated (FM). AM-FM mode delivers high-resolution topographical images, and additionally, it provides quantitative contact stiffness data, from which elastic modulus can be calculated with appropriate models for the tip-sample contact mechanics. Here, we will present a description of AM-FM operations and experimental results on various samples and different environments (air and liquid) such as biomaterials (polymers) and biological samples (DNA, Collagen, cells) will be presented to demonstrate the applicability of AM-FM mode for materials with a wide range of modulus (MPa-GPa). Furthermore, very recent advances in AM-FM imaging will be discussed, such as the use of photothermal excitation instead of the typical piezo activation. In this technique, a blue laser light is used to oscillate the cantilevers, providing the benefit of a very stable environment with reduced frequency shifts during operation and very accurate phase measurements. With the growing demand for mechanical characterization of materials at the nanoscale, the AM-FM technique provides quantitative nanomechanical information while simultaneously offering all the familiar advantages of tapping mode.

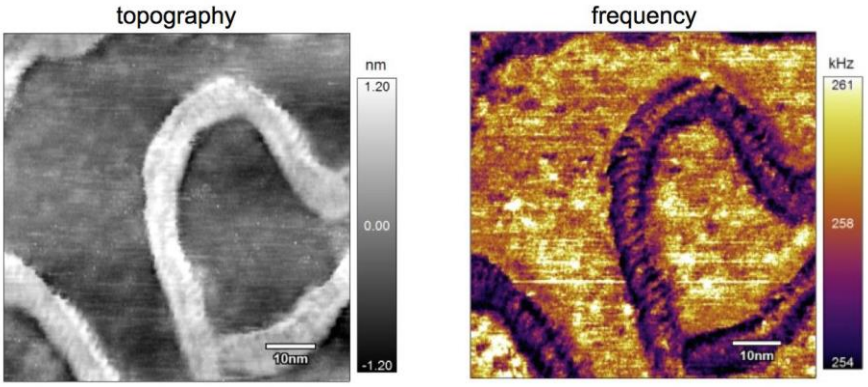

Figure 1: AM-FM image of DNA obtained in buffer. Left: topography image showing details of the double helix. Right: frequency shift of second mode oscillation from which the Young Modulus of the molecule can be derived.

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A Systematic Exploration of Nano-Bio Interactions: the Story of Carbon Nanotubes

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Carbon nanotubes (CNT) are widely used in various industrial sectors, biomedicine, and many consumer products. However, their potential toxicity is a major concern. We discovered that CNTs enter human cells[1], perturb cellular signaling pathways[2], affect various cell functions[3], and cause malfunctions in animals[4,5]. Because the majority of atoms in CNT are on the surface, chemistry modification on their surface may change their biological properties significantly. We modified CNT's surface using nano-combinatorial chemistry library approach[6]. Novel CNTs were discovered to exhibit reduced toxicity[6,7] or re-program cellular signaling machineries[7]. Exploring large sets of bio-assay data with chemoinformatics and computational chemistry, quantitative nanostructure-activity relationship (QNAR) has been established and predictive models built to predict biocompatible CNTs.

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Bio-AFM and Raman Laboratory – Scientific and Industrial Projects Review

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Bio-AFM and Raman laboratory at Tel Aviv University Center for Nanoscience and Nanotechnology provides services to more than 50 research groups from the university campus as well as to several tens of external customers from high tech companies and other academic institutions. In this presentation 4 projects utilizing unique AFM probing will be presented:

1. Differentiation between cancer and normal cells by local AFM probing of the cell elasticity
2. Investigation of the structural and optical properties of diphenylalanine peptide (FF) nanostructures and their phase transitions
3. AFM characterization of human exosomes using AFM for understanding oral cancer research
4. AFM characterization of Copaxone® aggregates

AFM/Raman results and directions for future research will be presented and discussed.

A. Handelman, S. Lavrov, A. Kudryavtsev, A. Khatchatouriants, Y. Rosenberg, E. Mishina, and G. Rosenman, "Nonlinear Optical Bioinspired Peptide Nanostructures", Advanced Optical Materials, 2013, 1, 875-884

NanoInBio 2016
Conference Program

	Thursday, June 2
	Conference Session II **Instrumentation & Application Tools**
09.00 am – 09.40 am	**Georg Fantner (Keynote Lecturer):** **Close up and Personal: The Process of Mycobacterial Cell Division**
09.40 am – 10.00 am	**Vincent Martinez**: Microfabricated Hollow Cantilevers Made of SU-8 and Their Potential for in-vitro Neuron Research
10.00 am – 10.20 am	**Juan Pelta**: Biomimetic nanopores fundamental to applications
10.20 am – 10.40 am	**Anny Fis**: Interaction forces of the bacterial translocon: An AFM investigation
10.40 am – 11.00 am	**Coffee Break**
11.00 am – 11.20 am	**Fabienne Quiles**: Multiscale in situ investigation of the action of an antimicrobial peptide on a bacterial nascent biofilm
11.20 am – 11.40 am	**Livie Dorwling-Carter**: The Fluidic Force Microscope: a versatile tool for force-controlled electrophysiology
11.40 am – 12.00 pm	**Oona Freudenthal**: Nanoscale investigation of colistin interactions with model phospholipid membranes by combined infrared and force spectroscopies
12.00 pm – 12.20 pm	**Jan Danko**: In vivo effect of hydroxyapatite ceramics visualized by X-rays
12.30 pm – 02.00 pm	**Conference Lunch**

Close up and Personal: The Process of Mycobacterial Cell Division.

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Bacterial cell division occurs as a sequence of events for which many processes remain only partially understood. In most bacterial models, FtsZ-ring formation is believed to be the first event leading to division. Nucleoid segregation, septum formation, cytokinesis, and physical cell separation are all successive events in the process of division [1, 2]. To date, many of these processes have been characterized using static methods. Establishing the sequence and possible dependence of these processes however requires time resolved live-cell imaging [3, 4, 5], at high resolution. Here we present a concise time-sequence of events describing division of *Mycobacterium smegmatis*, a non pathogenic cousin of *Mycobacterium tuberculosis*. We used a combination of multi-day time-lapse atomic force microscopy (AFM), time-lapse fluorescence microscopy with real time measurements of the cell separation at timescales down to 10s of milliseconds. Combining the nanoscale 3D information from AFM and the biochemical specificity from fluorescence microscopy we have characterized cell division from the early stage pre-selection of division sites, to assembly and subsequent disassembly of the FtsZ ring, localization of Wag31 and cytokinesis all the way to the rapid cell separation. Contrary to what is believed to be true in many bacterial cell types, cell separation in *Mycobacterium smegmatis* isn't a gradual event, but occurs abruptly within 10s of milliseconds, resembling more a mechanical fracture than a cellular remodeling process. Using mechanical stimulation, we demonstrate that the build-up of mechanical stress governs the time and place of cell separation. By applying additional mechanical stress, we were able to initiate cell separation at times in the cell cycle well before it would occur in the non-stimulated case. These observations suggest a new model for the late stages of cell division in *Mycobacterium smegmatis*, where cell mechanical properties and local stress concentration govern the timing and place of cell separation.

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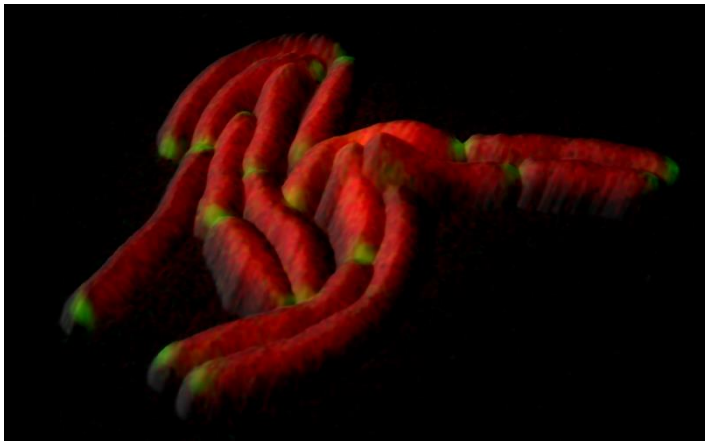

Figure 1: 3D rendering of living M.smegmatis. Height information obtained from AFM is combined with fluorescence information from Wag31 (green) and Histone-like protein to label DNA (red).

Microfabricated Hollow Cantilevers Made of SU-8 and Their Potential for *in-vitro* Neuron Research

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A novel fabrication method was established to produce flexible, transparent and tipless hollow cantilevers made entirely from SU-8 polymer. Microchannels were formed by the etching and removal of an electrochemically deposited sacrificial copper layer. Different cantilever designs were thus envisioned.

Thin cantilevers were operated with optical beam deflection, as in standard atomic force microscopy to perform serial single-cell force spectroscopy (Figure A). As demonstrated previously,[1] cell adhesion forces can be measured with a hollow AFM cantilever by aspiring a single cell and applying negative pressure at the aperture while overcoming and measuring the immobilization or adhesion forces of the cell on the substrate. This method presents significant advantages over the more conventional approach which consists in gluing irreversibly one cell per cantilever.[2]

On the other hand, *thick* cantilevers were operated without force-feedback thanks to their high mechanical flexibility, demonstrating promising outcome for single-cell patterning (Figure B). The latter represents today a key approach to decouple and better understand the role and mechanisms of individual cells in a given population. In particular, the bottom-up approach of engineering neuronal circuits with controlled topology holds immense promises to perceive the relationships between connectivity and function. Numerous techniques such as micropipettes,[3] laser-guided direct writing,[4] optical tweezers,[5] micro-valve,[6] modified inkjet based printers[7] and others based on acoustic field[8] or electrohydrodynamics[9] proved their applicability by positive or additive patterning in order to apprehend some of these problematics. The technology based on thick hollow cantilevers presents the versatility of "additive" patterning by controlled deposition of single cells (Figure C) but also, "subtractive" patterning with the realization of complex and circular neuronal networks with spatial resolution of 5 µm (Figure D) for direct applications in neuron research.

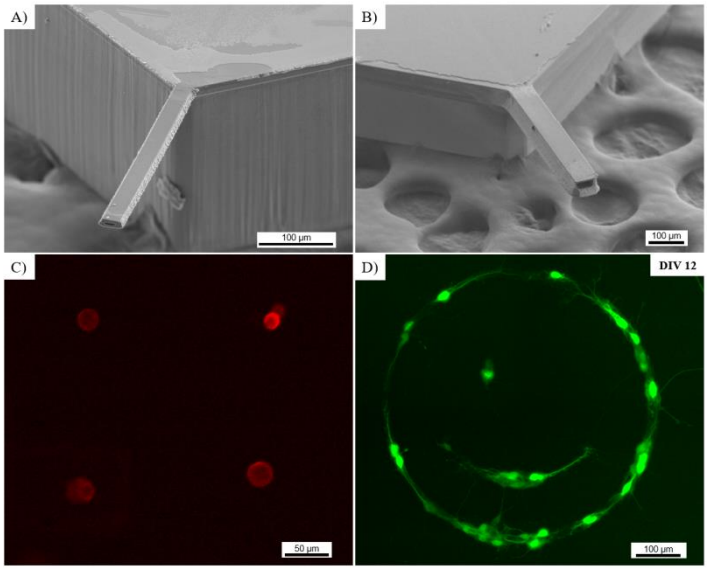

Figure: *Scanning electron microscopy images of a thin in A) and a thick hollow SU-8 cantilever in B) with the aperture defined within the front plane. Optical microscope images of C) a grid of 4 myoblast cells after serial individual deposition and of D) a complex network of primary hippocampal neurons in the form of a smiley 12 days after selective removal. Myoblast cells were fluorescently tagged with a PKH26 dye whereas hippocampal neurons were transfected 4 days after seeding with a GCaMP6s calcium indicator.*

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Biomimetic nanopores fundamental to applications

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We give some examples -protein unfolding, high-resolution size-discrimination of molecules, nanotubes insertion into membrane-, using a single molecule method, with an electrical detection. Macromolecule chains, passing through one nanopore submitted to an electric field induce detectable blockades of ionic current, in the presence of salt solution. These blockades depend on the size, degree of polymerization, conformation, structure of the passing molecule, and the interaction between the molecule and the nanopore.

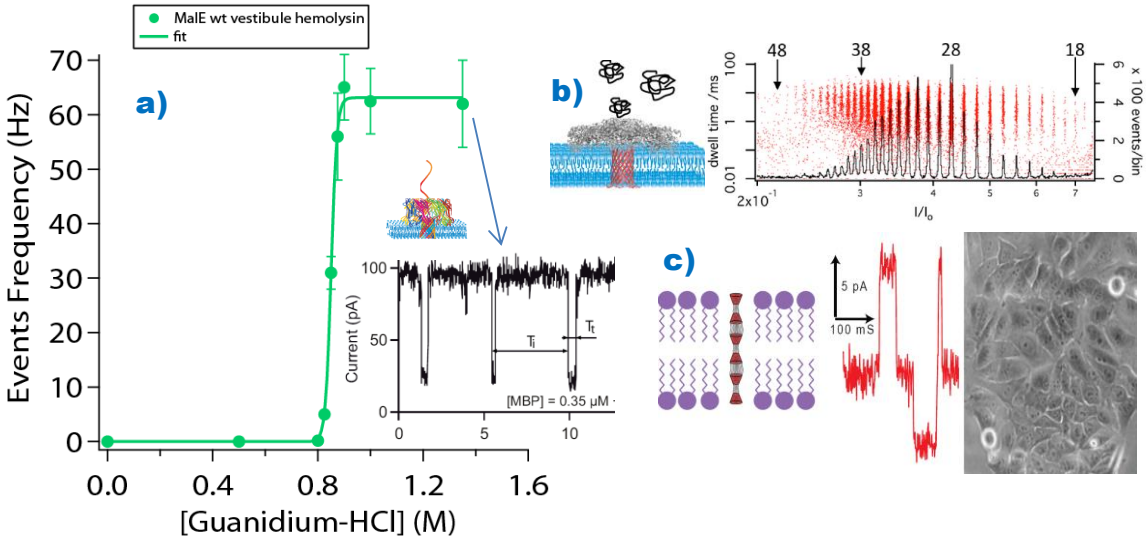

Figure: Protein unfolding (a) and single polymer mass spectrometry (b) through a nanopore coupled with an electric detection, cyclodextrin nanotubes insertion into membrane and cytotoxicity assay (c).

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Interaction forces of the bacterial translocon: An AFM investigation

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Many membrane or secretory proteins are translocated across the endoplasmic reticulum (ER) membrane or the bacterial plasma membrane through a conserved channel called SecYEG complex in bacteria and archaea. In post-translational translocation the SecYEG channel associates with the cytoplasmic SecA-ATPase to transport secretory proteins after completion of their synthesis. The translocation cascade is initiated by the binding of the substrate to the cytosolic chaperone SecB. Next SecA interacts with the SecB and accepts the polypeptide. SecA binds to SecYEG, yet for the transfer of the polypeptide into the channel a full ATP hydrolysis cycle by SecA is required. Once inserted into the channel, the substrate is translocated by a pushing mechanism.

In this work we focused on the binding mechanism of SecA to SecYEG. For our AFM measurements, the translocation channel was reconstituted into planar lipid bilayers suspended by microstructured patterns. For this, a sandwich structure was established that consisted of a mica-supported lipid bilayer containing biotinylated lipids, followed by a streptavidin layer, and SecYEG reconstituted into planar lipid bilayers. This approach attempts to create a native environment that preserves functionality, conformational freedom and lateral mobility of the SecYEG molecules. Force Spectroscopy measurements on the single-molecule level and TREC (simultaneous topography and recognition) imaging required the functionalization of the AFM tips. Single molecular force probes were developed by covalently coupling SecA via a flexible poly(ethylene glycol) (PEG) linker molecules to AFM tips. Utilizing specific ligand-SecYEG interactions we aim at determining interaction forces, energy landscapes, and kinetic rate-constants of SecYEG-SecA recognition.

1.

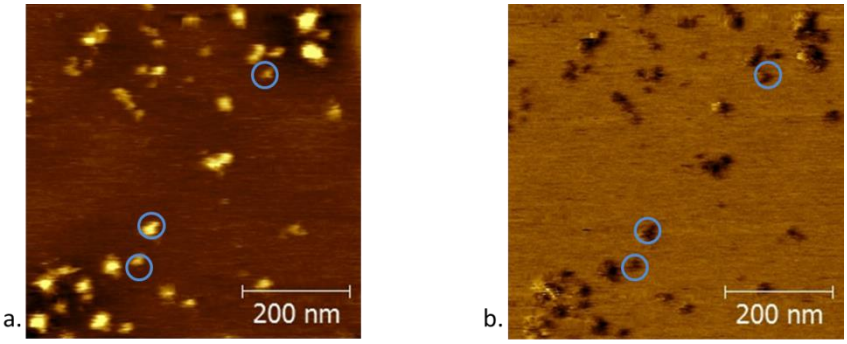

Figure1. Full amplitude TREC. (a) Topography of reconstituted SecYEG molecules into lipid bilayer containing biotinylated lipids, on streptavidin crystal. (b) Recognition image simultaneously recorded with (a).

Multiscale *in situ* investigation of the action of an antimicrobial peptide on a bacterial nascent biofilm

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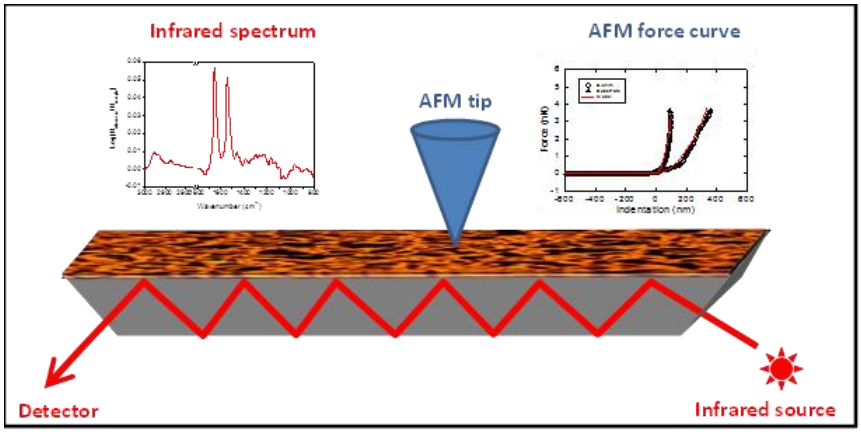
The development of biofilms - complex communities of micro-organisms embedded in an auto-secreted matrix of extracellular polymeric substances - causes significant economic and sanitary problems. Sessile bacteria in biofilms are more resistant to traditional antibiotics with respect to their planktonic counterpart. In this context, antimicrobial peptides (AMP) are considered as promising alternatives.



The purpose of this study was to investigate the action of an AMP against a nascent bacterial biofilm. The activity of a dermaseptin S4 derivative against 6 h-old *Pseudomonas fluorescens* biofilms was assessed at several concentrations by using a combination of Attenuated Total Reflectance - Fourier Transform InfraRed (ATR-FTIR) spectroscopy *in situ* and in real time, fluorescence microscopy using the *Bac*light™ kit, and Atomic Force Microscopy (AFM, imaging and force spectroscopy). After exposure to the peptide at three concentrations, different dramatic and fast changes over time were observed in the ATR-FTIR fingerprints reflecting a concentration-dependent action of the AMP. The ATR-FTIR spectra revealed major biochemical and physiological changes, adsorption/accumulation of the AMP on the bacteria, loss of membrane lipids, bacterial detachment, bacterial regrowth, or inhibition of biofilm growth. AFM allowed estimating at the nanoscale the effect of the AMP on the nanomechanical properties of the sessile bacteria. The bacterial membrane elasticity data measured by force spectroscopy were consistent with ATR-FTIR spectra, and they allowed suggesting a mechanism of action of this AMP on sessile *P. fluorescens*. This study illustrates the advantage to combine ATR-FTIR, AFM, and epifluorescence techniques for *in situ* and in real time monitoring the activity of AMPs against bacteria in a biofilm.

The Fluidic Force Microscope: a versatile tool for force-controlled electrophysiology

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Since its invention in 2009, the Fluidic Force Microscope (FluidFM), a microchanneled Atomic Force Microscope (AFM) allowing force-controlled nanopipette manipulations, has found lots of applications in surface and single cell manipulations[1,2], from injection, isolation or adhesion measurements to nanolithography or 3D printing.

Here, we show the potential of the FluidFM to carry out electrophysiology measurements by patch-clamp and scanning ion conductance microscopy (SICM) experiments. While SICM is a noncontact high-resolution imaging technique allowing limited mechanical contacts on the cell[3], its combination with the FluidFM was shown to offer additional simultaneous quantitative information regarding the forces applied on the cells[4] while allowing a more gentle scan than the conventional glass pipettes thanks to its soft cantilevered probe. Driven by this high-resolution SICM imaging, the probe can then be approached in a region of interest of the cell to perform patch-clamp recordings[5], the gold-standard in electrophysiology research and drug screening as it is the only tool enabling accurate investigations of voltage-gated ion channels (pore-forming membrane proteins regulating the transmembrane ionic flow). Nonetheless, as the procedure to create the gigeaseal (a seal of gigaohms between the membrane and conventional glass pipette) necessary for high-quality measurements is labor-intensive, the conventional patch-clamp suffers from a too low efficiency, triggering the search for alternative automated systems for pharmaceutical companies.
By combining the patch-clamp onto the FluidFM, we were able to offer an alternative approach aiming to control the automatized contact of the probe to the cell membrane by recording their forces of interaction. Such a force-controlled patch clamp tool revealed interesting insights for the studies of contracting cardiomyocytes for which the AFM force control of the FluidFM could not only ensure a constant gentle contact with the contracting cells but also enabled the simultaneous recording of membrane current and force development when contracting[6]. Current investigations focus on the optimization of the setup to improve the seal formation and reach a more reliable patch-clamp protocol.

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Nanoscale investigation of colistin interactions with model phospholipid membranes by combined infrared and force spectroscopies

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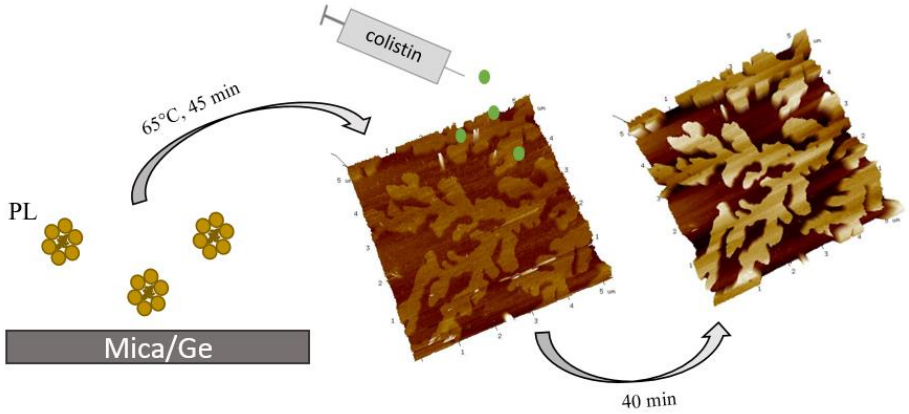
The excessive use of antibiotics has led to a growing interest of microbial resistance[1]. The antimicrobial action of peptides is of interest, as their exact way differ from stereospecific protein receptor-mediated processes and bacteria are less likely to develop resistance against such peptides[2]. Colistin is an antimicrobial peptide of the polymyxin family. Polymyxins are especially renowned for their potential against multi-resistant Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Colistin acts on the external leaflet of Gram negative bacteria outer membrane, more specifically on the negatively charged phospholipids (PL) and lipopolysaccharides (LPS)[3-5]. However, the detailed mechanism of action of colistin still remains unclear.

Langmuir monolayers of PLs as well as supported lipid bilayers are commonly used as model systems to mimic biological membranes. To have a closer look at the mechanism of action of colistin peptide, we have examined the effect of colistin on different PL monolayers at the air/liquid interface via Langmuir isotherms as well as on PL bilayers created by vesicle fusion method in aqueous media via atomic force microscopy (AFM), through imaging and force spectra, and with attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The PLs under our scope were those particularly found at the outer membrane of Gram- bacteria; zwitterionic 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-ethanolamine (DPPE), and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)[6]. The interactions of PLs with colistin at several concentrations (10 µM, 50 µM and 150 µM) have been studied by observations with the above mentioned techniques, over a time period of 1-2 hours.

The effects of colistin (50 µM) on chosen PL monolayer isotherms in comparison to equivalent monolayers on pure water subphase have been observed. Insights into the topographical and elastic changes in the PL bilayers within time after colistin injection are presented via AFM imaging and force spectra. Finally, changes in the PL bilayers ATR-FTIR spectra as a function of time within three bilayer compositions and the influence of colistin on their spectral evolution were examined together with the evolution of the Amide II and νC=O band integrated intensity ratios.

This work shows how three physico-chemical techniques (Langmuir isotherms, AFM and ATR-FTIR) can be used to clarify the importance of PL membrane composition as well as peptide total concentration on the colistin action on PLs. We show that complementary information can be obtained on peptide action on model PL membranes via both, mono- and bilayers.



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In vivo effect of hydroxyapatite ceramics visualized by X-rays

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BACKGROUND

Tissue engineering is the use of combination of cells, biochemical and physio-chemical factors, engineering and biomaterials to improve or replace biological functions (Musumeci *et al*., 2014). The goal of tissue engineering in the craniofacial region is to develop abiological substitute that promotes tissue regeneration (Mao *et al*., 2006). HA implants with appropriate three-dimensional geometry are able to bind and concentrate endogenous bone morphometric proteins in circulation, and may become osteoinductive, and can be effective carriers of bone cell seeds (Le Geros, 2002). Bone marrow mesenchymal stem cells (MMSCs) can differentiate efficiently and robustly into anchorage-dependent cells, such as osteoblasts, chondrocytes, and adipocytes (Bradamante *et al*., 2014). The isolated MMSCs are expanded in culture and finally seeded within or onto a natural or synthetic scaffold that define the shape of the tissue and supports cells during their growth (Abou Neel *et al*., 2014).

MATERIAL AND METHODS

Six male adult Slovak white (Landrace) pigs were used in this study (average weight 35-40 kg). The animals were obtained from a certified breeding company. HA scaffolds (4 pcs with the size for the each one: 4x2x2 mm^3) were grafted in the right lateral mandible body side of each animal. Body of the mandible **30**s exposed, and 4 defects were prepared by the piezotome. The three rostral defects were filled by the HA scaffolds containing MMSCs. The most caudal defect was filled by the HA scaffold alone as a control. X-ray images were prepared in latero-lateral projection immediately at the end of surgery, 3 and 9 weeks after implantation.

RESULTS AND DISCUSSION

Day 0

Latero-lateral projection of the head was done in total anesthesia immediately after the surgery. Implants are radiodense structures with a square shape localized on the right side of the mandible body under the alveolar processes of the molars. Density of implants is well defined and distinguishable compared to surrounding bone tissue. Three weeks after implantation, we can clearly distinguish four implants, and observe a change in density between inserted implants defined by changing the field edge implants in the transition to the bone. This is the result in proliferation of osteocytes between surrounding bone tissue and implants. The change in density is macroscopically observed by X-ray 1-3 implants. On the fourth implant (control), the density is the same as the density of the implants at day 0, and it is well defined from the surrounding bone tissue. Nine weeks after implantation, we observed the absolute change in density of implants 1-4. The 1-3 implants cannot be distinguished from the density of the surrounding bone tissue. The application site of the fourth implant (control) is a more radiopaque island observed compared to the surrounding bone tissue (ventral location to the last molar).

The pigs as an animal model is used frequently in studies. Because they have close similarities to humans in terms of platelet count, clotting parameters, metabolic rate, bone structure and bone remodeling, and mesenchymal stem cells characteristics (Kirchhoff *et al*., 2011). Bone regeneration has been reported to be faster when appropriate scaffolds are combined with MSCs having bone regeneration capability compared to when only synthetic materials are used (Yun *et al*., 2014).

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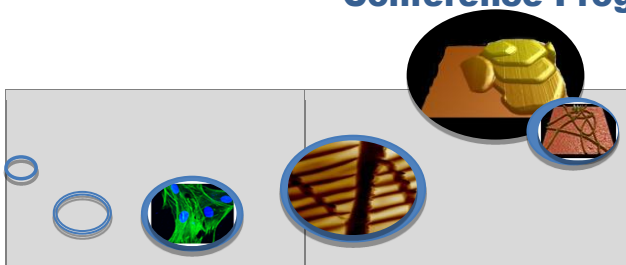
NanoInBio 2016

Conference Program



Thursday, June 2

	Conference Session III **Nanosciences at the frontier with biology**
02.00 pm – 02.40 pm	**Andrew Pelling (Keynote Lecturer) :** **Starting Small and Going Big - Cells in Artificial Contexts**
02.40 pm – 03.00 pm	**Mickael Castelain**: The nanomechanical properties of Lactococcus lactis pili are conditioned by the polymerized backbone pilin
03.00 pm – 03.20 pm	**Tien Tuan Dao**: Development of a Nano Osteo-Mimetic System based on Bone Nano-Biomechanics
03.20 pm – 03.40 pm	**Martina J. Baum**: Physico-chemical characterization of a complex shaped ZnO nano-microstructure based polymer composites with potential as mechanically stable antifouling coating
03.40 pm – 04.00 pm	**Coffee Break**
04.00 pm – 04.20 pm	**Aurelien vander Straeten**: Protein-polyelectrolyte complexes for the surface immobilization of biologically active proteins
04.20 pm – 04.40 pm	**Michel Linder**: Nanoscale characterization of liposome-encapsulated curcumin
04.40 pm – 05.00 pm	**Jean-Sébastien Thomann**: Anti-biofouling Properties in Blood of Novel Mesoporous Silica Nanoparticles covered with a Self-assembled Supported Lipid Bilayer
05.00 pm – 05.20 pm	**Tanja Becke**: Single Molecule Force Spectroscopy Reveals Interaction Strength between Streptococcus Pneumoniae TIGR4 Pilus-1 Tip Protein RrgA and Human Fibronectin, Highlighting the Potential Role of Terminal Domains of RrgA
05.20 pm – 05.40 pm	**Frederic Borges**: Interaction between Lactobacillus rhamnosus GG and whey proteins: key role of pili
05.40 pm – 06.00 pm	**Peter Massanyi**: Nickel Male Toxicity – Structural and Ultrastructural Alterations
07.30 pm	**Conference Dinner**

Starting Small and Going Big - Cells in Artificial Contexts

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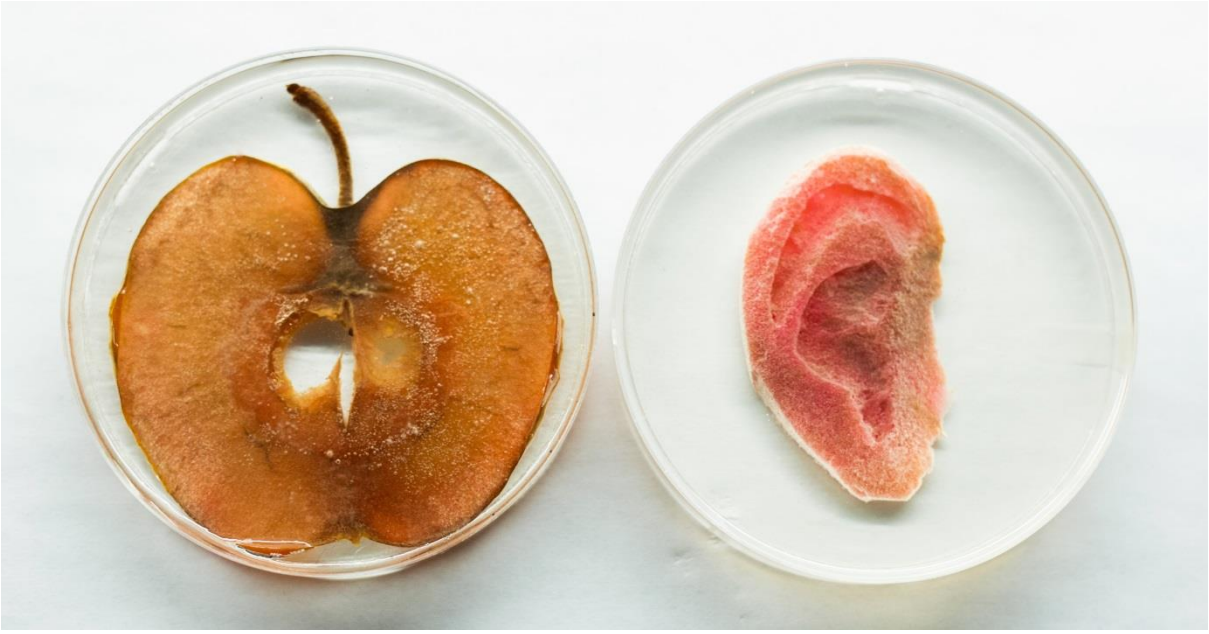
Living cells possess an exquisite ability to sense and respond to physical information in their microenvironment. Although this ability plays a key role in many fundamentally important physiological and pathological processes, it can also be exploited to control and manipulate biological behavior and function. In recent years, the lab has become increasingly interested in created augmented biological systems by exploiting topographical, mechanical and physical cues to direct cellular organization, sorting and complex morphogenesis in three dimensions. This work has also yielded new insights into how cells respond to nano- and micro- scale physical information in highly artificial environments. I will review several projects in which cells are exposed artificial topographies to induce spontaneous cell-sorting in 3D, artificial mechanical stimuli that reveals unexpected physical properties of sub-cellular architecture, and plant-based 3D scaffolds that can be used to create artificial hybrid mammalian tissues. These results may provide insights in how key components of biological and physical feedback loops can be employed to control and govern the life of a cell.



Apple-derived cellulose scaffolds support the growth of mammalian cells and offer a route to creating implantable, biocompatible and low-cost biomaterials for regenerative medicine.

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Bioarxiv doi: http://dx.doi.org/10.1101/039644.

The nanomechanical properties of *Lactococcus lactis* pili are conditioned by the polymerized backbone pilin

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Lactococcus lactis is a major lactic acid bacterium (LAB) present in numerous ecological niches, including soil, plants silages, milk and fermented food. The autochthonous LAB flora in native products is often reinforced by addition of bacterial starters consisting in cocktails of selected LAB. This practice leads to the presence of an important live LAB biota (ca. 109 cfu/g) that is ultimately ingested and delivered to the gastrointestinal tract (GIT). *L. lactis* is able to survive the harsh physicochemical conditions of the GIT and remains metabolically active throughout its transit. *L. lactis* has thus been proposed as a potent probiotic vehicle that could deliver relevant health-promoting molecules *in situ*, notably through close interactions with mucus, a protective gel-forming layer present at the surface of the intestinal mucosa. Adhesion of LAB to mucus, and more particularly to mucins, has been shown to be driven by pili.[1] In *L. lactis*, sortase-dependent pili consist in 3 pilin proteins, namely the backbone-forming major pilin PilB, the cap pilin PilA situated at the distal end of the pilus, and the anchoring pilin PilC involved in the tethering of the pilus to peptidoglycan.[2] Piliated lactococci were previously shown to form thick and aerial biofilms, due to oligomers of the PilB pilin.[2] In this work, Force-Measuring Optical Tweezers experiments were performed to obtain force-extension data from pulling the pili. It reveals under 0-200 pN force range that native pili exhibit an inextensible but highly flexible ultrastructure. Then, a panel of strain derivatives was tested to retrieve the functional role of the different pilins; it shows that the major pilin PilB whose polymerization shapes up the backbone into full-length organelle dictates the nanomechanical properties of such pili. In addition, sortase C that polymerizes PilB subunits is compulsory to obtain a full-length structure. These findings, corroborated with the characterization pili-mediated adhesion properties of *L. lactis* will lead to a better understanding of interactions of this LAB with the mucosal environment in the gastrointestinal tract, further offering novel strategies for medical and food-related applications.

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Development of a Nano Osteo-Mimetic System based on Bone Nano-Biomechanics

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The understanding of bone biomechanics plays an important role in the prevention and diagnosis of bone diseases but also in the optimization of biomaterials dedicated to bone implants and replacement. Human bone has been studied for decades at multi-scale levels considering multiphysical properties such as structural, mechanical and physicochemical ones (1). The objective of this present work is to develop a biomimetic system of the human bone to study its behavior at the nanoscale. Experimental designs were conducted to obtain different mechanical behaviors of different combinations of collagen fibers and mineral concentrations. Bilayer assemblies with different combinations of collagen (100, 7 and 1 µg/mL) and mineral concentrations (from 0 to 8000 µg/mL) were obtained and morphological (fiber surface) and nanomechanical (Young's modulus) properties were measured using medical image processing tool (ImageJ) and Atomic-Force Microscopy (AFM) technique respectively. First, finite element models were performed to simulate different experiments and second for aided design of the osteomimetic system (Fig. 1).

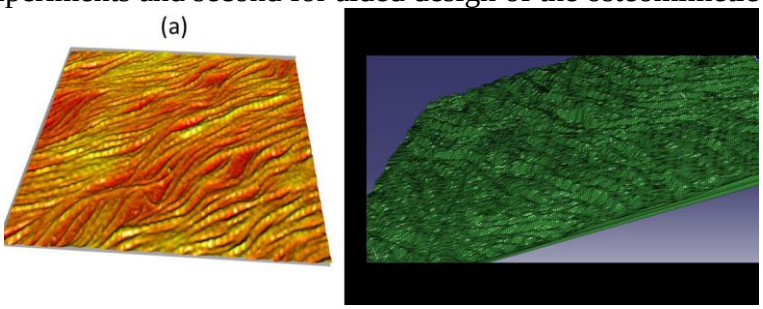

Fig. 1 Illustration of AFM image of the osteomimetic system (a) and its relative mesh model (b).

In fact, AFM image of the experiment was used directly to generate a 3D meshed model of the bone-mimicking system using 'home made' tools (2, 3). Stress-strain relationships were reported and analyzed to optimize the future experiments for the osteomimetic system. This study opens perspectives for exploring bone biomechanics at the nanoscale using the biomimetic system engineering approach. Finally, the coupling between experimental observation and *in silico* modeling will allow for the development of optimal bone-mimetic system leading to a better understanding of bone behavior and knowledge for clinical and industrial applications.

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Physico-chemical characterization of a complex shaped ZnO nano-microstructure based polymer composites with potential as mechanically stable antifouling coating

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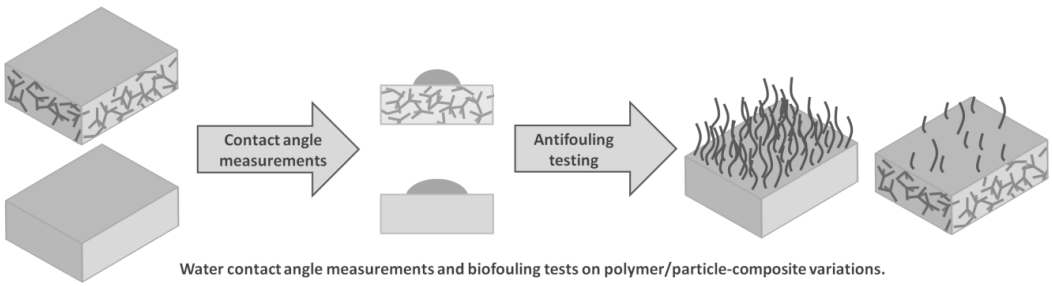
All surfaces exposed to the aquatic environment become colonized by aquatic organisms. This process is widely known as biofouling [1]. The growth of micro- and macroscopic organisms is accompanied by disadvantageous effects for many technical applications, e. g. increasing surface roughness, clogging of heat exchangers in power plants, biocorrosion or the weight gain of ship hulls, which can result in increased fuel consumption of up to 40 %, bringing along an increased greenhouse gas emission as well as increased transport costs [2,3]. Due to the prohibition of the toxic and very potent tributyltin (TBT)-based antifouling paints in 2008, there is huge demand to develop environmentally friendly, mechanically stable and therefore long lasting antifouling coatings, which are applicable at industrial scale.

In this study, we developed a mechanically stable polymer-composite with promising antifouling properties. The combined solvent free two-component polythiourethane (PTU) and tetrapodal shaped ZnO (t-ZnO) were modified and characterized focusing on physico-chemical and mechanical properties. Various filler amounts of t-ZnO (0 wt% t-ZnO, 1 wt% t-ZnO, 5 wt% t-ZnO), incorporated in this polymer composite, were investigated. For the detection of changes of the chemical interactions while polymerization, Raman-spectroscopy, infrared-spectroscopy and thermogravimetric analysis were performed. Influences of the polymer-to-particle variations on the wetting phenomena were evaluated by characterization of the surface topography and contact angle measurements. The potential of the composites as antifouling coating was evaluated by immersion tests in aquarium tanks representing Baltic sea and Pacific Ocean conditions. Keeping in mind that even a material with excellent antifouling properties would never find access to the market of antifouling coatings used for surfaces, exposed to the harsh environmental conditions on ships and off-shore wind turbines, detailed investigation on the mechanical properties of this polymer/particle-composite were performed. For this purpose, tensile testing, hardness measurements and pull-off tests have been carried out.



Water contact angle measurements and biofouling tests on polymer/particle-composite variations.

The detailed analysis by Raman- and infrared-spectroscopy showed no changes due to different filler amounts. These findings were in accordance to the results found by thermogravimetric analysis. The surface free energy characterization by contact angle measurements showed with an increasing filler amount a decreasing surface free energy in general and within the polar fraction in specific. The immersion tests showed for polymer/particle-composites, containing 1 wt% and 5 wt% t-ZnO, strongly reduced and almost no fouling, respectively. Material tests revealed the best mechanical performance on the composite containing 5 wt% t-ZnO.

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Protein-polyelectrolyte complexes for the surface immobilization of biologically active proteins

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The ability to control the immobilization of proteins at interfaces is a major challenge for many applications in biomedical science and biotechnology. The preservation of protein activity can be achieved by their deposition within highly hydrated polyelectrolyte layers. However, protein-polyelectrolyte interactions are very complex due to the polyampholyte nature of proteins, making it difficult for proteins to be integrated in this type of layers. This project aims at guiding protein deposition at interfaces by developing a new approach based on the formation of protein-polyelectrolyte complexes (1), which will stabilize protein conformation and standardize their charge. The obtained complexes will subsequently be integrated into multilayered coatings (Figure 1A).

Lysozyme was chosen to demonstrate the feasibility of such an approach. This globular enzyme (isoelectric point of 11.35) was complexed with either a strong or a weak polyelectrolyte of different molar masses, namely poly(styrene sulfonate) (PSS, Mw=70 000, Mw=7 540 and Mw=1 690), or poly(acrylic acid) (Mw=15 000, Mw=5 100, Mw=2 100). Different protein-polyelectrolyte ratios (*i.e.* different charge ratios) were used to build complexes. At first, complex formation at different pH values and ionic strengths was monitored by turbidimetry, and free and complexed lysozyme fractions were assessed by UV-spectrophotometry. Complexation was shown to be electrostatically driven, as already proposed by Kayitmazer et al. (1). It was also evidenced that complexes are formed for low lysozyme to PSS charge ratios; and that complex formation increased to reach a plateau value at a (-) to (+) charge ratio of about 1.5 at low ionic strength (Figure 1B). When the ionic strength was increased, the plateau was reached at higher (-) to (+) charge ratios. Furthermore, it was shown by dynamic light scattering and by atomic force microscopy (after deposition on silica surfaces) that complex size and morphology highly depend on ionic strength and both polymer type and polymerization degree.

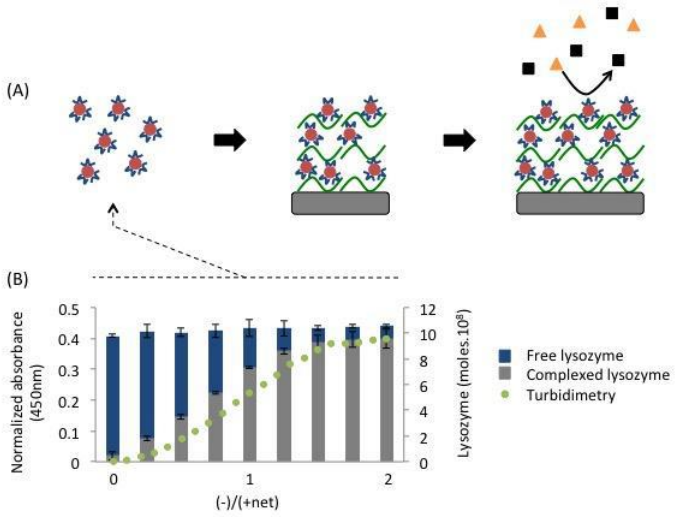

Figure 1 – (A) Scheme of the strategy used to achieve the surface immobilization of biologically active proteins. Step 1: formation of protein-polyelectrolyte assemblies – Step 2: immobilization of these complexes in layer-by-layer assemblies – Step 3: measurement of the biological activity of the immobilized protein. (B) Turbidimetry and UV dosage of free and complexed lysozyme obtained for different poly(styrene sulfonate) (Mw=1690) to lysozyme charge ratios, at pH 7.5 and ionic strength 10mM (HEPES and NaCl).

Secondly, lysozyme-PSS complexes were immobilized into multilayers by a layer-by-layer (LbL) deposition with poly(allylamine hydrochloride) (PAH). Quartz crystal microbalance with dissipation monitoring showed that multilayer growth was linear upon deposition of at least five complex-PAH bilayers when lysozyme was complexed with short PSS chains (Mw=7 540 and Mw=1 690). The adsorption behavior and frequency shifts reached for LbL constructions with the corresponding PSS chains alone were shown to differ from those observed with complexes, suggesting that lysozyme is effectively integrated within the multilayers. The presence of lysozyme in the multilayers built using lysozyme-PSS complexes was further evidenced by X-ray photoelectron spectroscopy.

Lysozyme was thus successfully integrated in multilayers without any chemical modification or harsh solvent, thanks to its complexation with PSS and subsequent layer-by-layer deposition with PAH. In the near future, the obtained multilayers will be extensively characterized, the enzymatic activity of the immobilized protein will be assessed and the developed approach will be extended to other proteins.

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Nanoscale characterization of liposome-encapsulated curcumin

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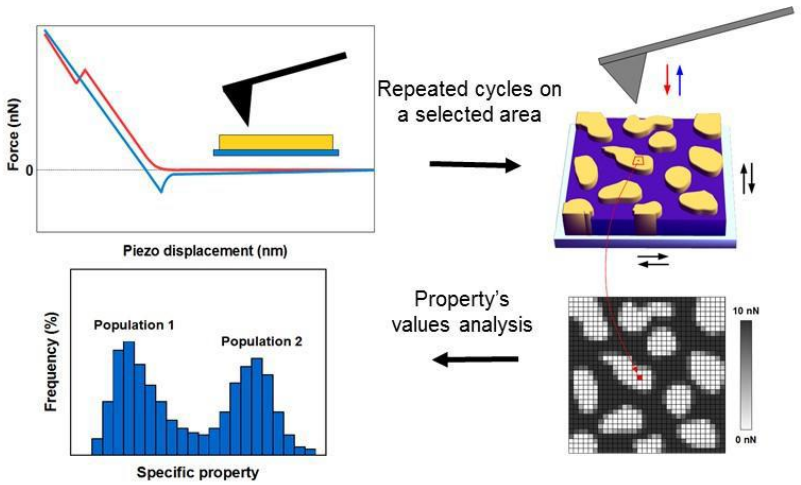
The role of curcumin (diferuloylmethane), for cancer treatment has been an area of growing interest. However, due to its low absorption, the poor bioavailability of curcumin limits its clinical use. In this study, we reported an approach of encapsulation a curcumin by nanoliposome to achieve an improved bioavailability of a poorly absorbed hydrophobic compound (Hasan et al., 2014). Liposomes are currently an important part of biological, pharmaceutical, medical and nutritional research, as they are considered to be among the most effective carriers for the introduction of various types of bioactive agents into target cells (Maherani et al., 2012). The mechanical properties of the membranes are correlated to the fatty acid composition, the morphology, the electrophoretic mobility and the membrane fluidity (Jacquot et al., 2014).

In order to understand the effect of encapsulation of curcumin in nanoliposome and also the position of this active molecule, multiscale characterization of nanoliposome before and after encapsulation of curcumin was studied. Various concentration of curcumin was used. A multiscale approach was employed to study the interaction between curcumin, a hydrophobic drug with potential benefits as antioxidant or anticancerous agent, and a phospholipid bilayer, membrane of a liposome to be employed as a drug carrier to protect, transport and transfer of curcumin. The goal of this study was to evaluate the influence of encapsulated curcumin on the carrier for various amounts of drug loaded and understand interactions at the molecular level, before a potential use as a drug/drug-carrier couple. The innocuity of used curcumin and the phospholipids was a major interest to conceive a drug/drug-carrier couple, as well as their origin and the presence of polar lipids in cells membrane.



Morphological and Nanomechanical Properties of Phospholipids-based Biomembranes

In our study, various curcumin amounts were added to phospholipids to get drug-loaded liposomes. Curcumin's concentration was evaluated thanks to UV-visible measurements. Nuclear magnetic resonance (NMR) was employed to determine curcumin's interactions and localization within the phospholipid membrane of liposomes. X-ray scattering (SAXS) and atomic force microscopy (AFM) experiments were performed to characterize the bilayer structure and organization, as well as its mechanical properties at a nanometer scale.

The results showed that presence of curcumin does not disturb the membrane structure. After the nanoscale characterization of nanoliposome with 110 nm, we observed the minor impact of the addition of small or moderate amounts of curcumin, from 0 to 12 mol%, on the structure and properties of a liposomal carrier, until a certain limit.

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Anti-biofouling Properties in Blood of Novel Mesoporous Silica Nanoparticles covered with a Self-assembled Supported Lipid Bilayer

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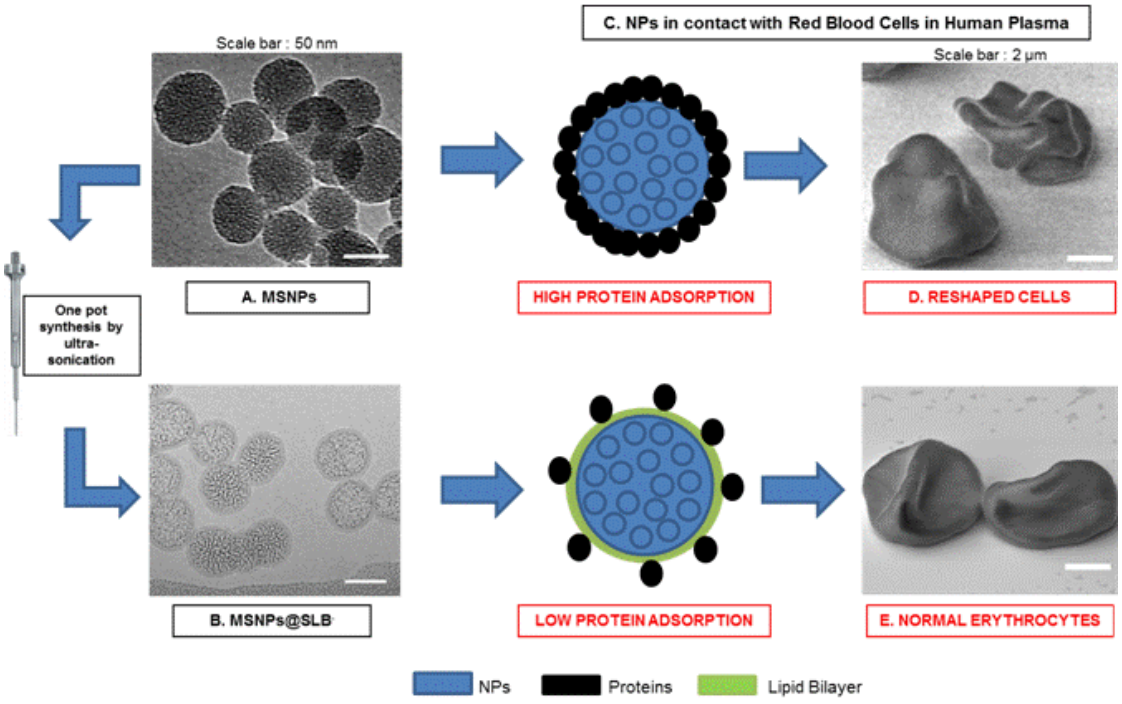

Porous inorganic materials are promising vectors for drug delivery, with numerous materials-specific advantages but also potential toxic effects.[1–3] The materials-specific advantages of the inorganic vectors includes stability within diverse biological environments, flexibility to include any desired drugs or combinations, ability to introduce different image contrast agents and multiple process handles to control the drug-matrix interactions and release kinetics. The potential downsides of the inorganic carriers have been debated in literature including, toxicity due to multiple factors including aggregation induced clogging or abrasive impact causing membrane damage, passive accumulation within tissues due to slow degradation, denaturing protein conformation with potentially adverse immune response. In order to take advantage of inorganic vectors without suffering their drawbacks, an elegant approach consists in giving them an organic appearance, i.e. covering them with an organic shell. The deposition of supported lipid bilayer (SLBs) coatings on mesoporous silica nanoparticles (MSNPs) is thought to significantly enhance the design possibilities of drug molecular release profiles compared with organic-based nanocarriers. MSNPs@SLBs exhibiting defect-free SLBs, good colloidal stability and

biocompatibility is critical to their future exploitation as static or dynamic (stimulus-responsive) gate-keepers in drug delivery systems. To this end, we demonstrate that MSNPs@SLBs constituted of anionic lipid bilayers (SLB^-) coated on cationic MSNPs (<60 nm diameter) exhibit a high stability over several weeks, along with excellent hemato-biocompatibility. The challenge of achieving continuous, defect-free SLBs on highly curved interface of MSPNs as small as 55nm is shown to be overcome by adopting a procedure based on ultrasonication. The defect-free nature of SLBs is confirmed by cryo-TEM measurements.

The SLB^- was found to impart superior anti-biofouling capabilities to the MSNPs in a similar way as pegylated SLB^-. In addition, SLB^- was found to decrease the lysis and the membrane stress of red blood cells (RBCs) induced by bare and positively charged MSNPs in both phosphate buffered saline (PBS) and human plasma, respectively, even at nanoparticle concentrations as high as 1 mg/mL. In addition, SLB^- was found to induce no adverse impact on the integrity of the membranes of peripheral blood mononuclear cells (PBMC) and human bone marrow stromal cells (HS-5). This approach delivers SLBs with high integrity, ease of coating, and promising functionality for application within relevant biological medium, suitable for next steps towards encapsulation of drugs and control over its targeting capabilities.

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Single Molecule Force Spectroscopy Reveals Interaction Strength between *Streptococcus Pneumoniae* TIGR4 Pilus-1 Tip Protein RrgA and Human Fibronectin, Highlighting the Potential Role of Terminal Domains of RrgA

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Gram-positive *Streptococcus pneumoniae* represents a major human pathogen causing serious diseases including pneumonia, meningitis and febrile bacteremia with high mortality rates worldwide. Among other virulence factors, recently discovered surface appendages (pili) are involved in pneumococcal host colonization and invasion.[1] Native *Streptococcus pneumoniae* TIGR4 pilus-1 is composed of an RrgC cell wall anchor protein, multiple covalently linked RrgB backbone subunits and a terminal RrgA adhesion molecule.[2] The pilus tip protein RrgA is a four domain (D1-D4), elongated molecule with multiple binding motifs which was found to interact with specific host components like extracellular matrix molecules (ECMs).[3,4] However the precise role of RrgA and the fundamental molecular mechanisms of respective individual RrgA domains during host factor interplay are not understood in detail. In particular, nothing is known about the underlying interaction forces between RrgA mediated associations and potential consequences regarding their respective role during pneumococcal infection.

In this study, we use single molecule force spectroscopy, a widely used operating mode of the atomic force microscope to directly probe protein-protein-linking, to quantify the interaction forces between the pilus subunits RrgA or RrgB and human fibronectin (Fn). We could show a low affinity and fast dissociation reaction of the tip protein RrgA and Fn with binding forces in the force range of typical receptor-ligand systems whereas the pilus backbone protein RrgB shows no specific binding towards the respective molecule. Interestingly, the Fn interaction seems to be mediated by RrgAs' two distinct, terminal domains (D3 and D4) which bind to Fn in a similar force range as full-length RrgA but apparently display different kinetical behaviours. A specific domain mediated RrgA-Fn interaction was also found using respective ELISA based analysis.

We anticipate these studies to be a starting point for the detailed analysis of the molecular interplay between the pneumococcal type-1 pilus subunits with emphasis on the adhesive tip protein RrgA and various host ECMs like Fn, laminin and collagen I.

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Interaction between *Lactobacillus rhamnosus* GG and whey proteins: key role of pili.

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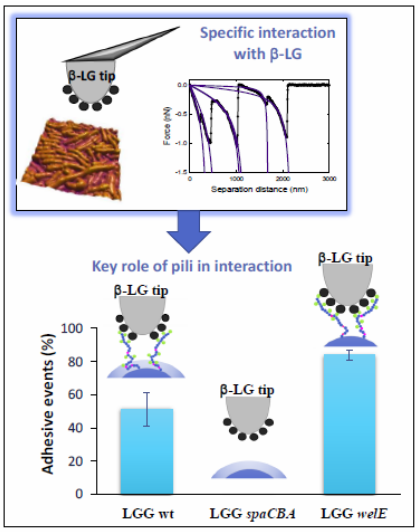
Lactobacillus rhamnosus GG is a well-known probiotic bacteria which possess many demonstrated health effects like prevention and treatment of gastro-intestinal infections and diarrhea, stimulation of immune responses or prevention of allergic symptoms (1). Probiotic bacteria, including *L. rhamnosus* GG, are incorporated in a wide range of foods. To exert their health benefits, probiotic need to be alive during the product shelf-life and during digestion. For this, probiotics encapsulation has a real interest in functional foods by increasing the survival rate of bacteria during storage and product's shelf-life. Interactions between bacteria and food matrices played a key role in the protection of the bacteria in food products, especially in processes such as encapsulation. However molecular mechanisms taking place in this interaction are still unknown. In this work, the interactions between *L. rhamnosus* GG and isolated milk proteins (from whey proteins) were investigated with atomic force microscopy (AFM).



AFM was used in force mode to study interactions between *L. rhamnosus* GG and the isolated whey proteins: β-lactoglobulin (β-LG), α-lactalbumin (α-LA) and bovine serum albumin (BSA). It was clearly demonstrated that *L. rhamnosus* GG is able to specifically interact with the β-LG but not with α-LA and BSA. Two surface mutants of *L. rhamnosus* GG were used to identify the bacterial surface biomolecules involved in interaction with β-LG: a pili depleted strain (*L. rhamnosus* GG *spaCBA*) and an exopolysaccharides depleted strain (*L. rhamnosus* GG *welE*). By comparing the results with the wild type strain, an increase of adhesive events was observed with *L. rhamnosus* GG *welE* whereas no adhesive events occurred with *L. rhamnosus* GG *spaCBA*. These results show that specific interactions with β-LG involved pili at the bacterial surface. To understand interactionbetween pili and β-LG, some force parameters were measured on these two strains: contour length (Lc), adhesion force (Fadh), Kuhn length (lk) and Persistence length (lp). These force parameters models confirmed the key role of pili in interaction with β-LG. This work describes for the first time the specific interaction of *L. rhamnosus* GG pili with β-LG. These results are able to explain the preferential localization of the bacteria in dairy products: around whey proteins and more particularly β-LG. By governing the localization of the bacteria inside the microparticles, this work will also permit some improvement of *L. rhamnosus* GG encapsulation such as loading rate optimization or a better protection of bacteria against outdoor environment (2).

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Nickel Male Toxicity – Structural and Ultrastructural Alterations

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Nickel is a trace element present at low concentrations in agroecosystems. Nickel, however, may have toxic effects on living organisms and is often considered as a contaminant. High quantity of nickel is known to be injurious for animal and human health. Its effects on various aspects of reproduction have been previously described. Animal studies refer that nickel has negative effects on the structure and function of testis, seminal vesicle, and prostate gland, and there is similar report on adverse effect on spermatozoa (1). Nickel is also an essential trace metal that is vital for growth enhancement in very low doses for birds and mammals (2). Animal studies have indicated that nickel may reach the testis, seminal vesicle and prostate gland (3), and there are reports of its adverse effect on spermatozoa (4). Its action on spermatozoa motility, morphology and count, is an important parameter for the evaluation of male fertility.

In this study the effect of nickel as a risk factor of environment on the testicular structure and spermatozoa is reported. In this study the effect of Ni on the testicular structure after an experimental intraperitoneal (i.p.) administration and the effect of in vitro spermatozoa incubation with nickel on the spermatozoa motility and membrane changes are reported. Our findings clearly suggest a negative effect of nickel on the structure as well as on the function of seminiferous epithelium. In experimental groups with nickel a significant ($p<0.001$) decrease of germinal epithelium in comparison with control group was observed. Concentrations from 125 μM Ni/ml in various time periods of culture stimulate spermatozoa motility after 30 minutes ($p<0.001$), but later inhibit spermatozoa motility. After a culture of spermatozoa with addition of 125 μM Ni/ml and 240 minutes a typical Annexin–V fluorescence reaction was detected. Fluorescence was detected in mitochondrial segment of bovine spermatozoa. In spermatozoa exposed to higher nickel concentration the Annexin–V positive reaction was detected also on the spermatozoa head membrane. Nickel in very low concentrations (7.8 μM Ni/ml) stimulates the spermatozoa motility but in higher concentrations (>250 μM Ni/ml) cause decrease of spermatozoa motility in vitro.

Results of this study clearly confirm toxic effect of nickel on male reproductive abilities. All detected results suggest that the nickel has a negative effect on testicular structure affecting mainly the spermatozoa development as well as the steroidogenesis. Previously determined alterations in spermatozoa structure in vivo were completed by detection of the dose dependent effect of nickel on spermatozoa motility and particularly by the detection of fine spermatozoa structural changes associated with nickel toxicity

Financial support: VEGA 1/0857/14, 1/0760/15, APVV-0304-12, KEGA 006/SPU-4/2015

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NanoInBio 2016
Conference Program

	Friday, June 3
	Conference Session IV Instrumentation & Application Tools
09.00 am – 09.40 am	**Noriyuki Kodera (Keynote Lecturer): Direct observation of proteins at work by high-speed atomic force microscopy**
09.40 am – 10.00 am	**Audrey Beaussart**: Single-cell force spectroscopy of microbes
10.00 am – 10.20 am	**Karel Klepárník**: Implementation of nanotechnologies in single-cell analysis
10.20 am – 10.40 am	**Petr Gorelkin**: Nanopipette Navigation System as a New Tool for Biomedical Application
10.40 am – 11.00 am	**Coffee Break**
11.00 am – 11.20 am	**Olivier Gros**: ESEM sub-micrometer-scale mapping of sulfur as a powerful tool for the study of marine thioautotrophic organisms: case study of a new uncultured epsilon sulfur bacteria colonizing marine mangrove sediment in the Caribbean.
11.20 am – 11.40 am	**Sebastien Jaramillo**: Assessments of nanomechanical properties of biomimetic membranes using AFM Circular Mode
11.40 am – 12.00 pm	**Raphael Tiefenauer**: Complementary Dual Biosensing with Novel Ultrathin Gold Nanohole Films
12.00 pm – 12.20 pm	**Isabel Alves**: Real time monitoring of membrane GPCR reconstitution by plasmon waveguide resonance: on the role of cholesterol
12.30 pm – 01.30 pm	**Conference Lunch**
01.30 pm – 03.00 pm	**Poster Session II Exhibition Session II**
	Special NANOinBIO Session II
03.00 pm – 03.40 pm	**Scott Guelcher: Injectable, settable, and resorbable nanocrystalline hydroxyapatite/ polyurethane hybrid polymers with bone-like strength**
03.40 pm – 04.00 pm	**Ruby M. Sullan: Nanoscale Interplay Between Bacterial Adhesins and Substrate Properties**
04.00 pm – 04.20 pm	**Coffee Break**
04.20 pm – 05.00 pm	**Ben Holmes: Fast Nanoscale Imaging and Quantitative Nanomechanical Characterization of Cells and Biomaterials with Correlative Atomic Force and Optical Microscopy**
05.00 pm – 05.20 pm	**Ronald Zirbs: Universal melt modification method for the synthesis of core-shell-nanoparticles with ultra-high grafting densities**
05.20 pm – 06.00 pm	**Munisch Chanana: Nano-functionalized Biomaterials and Bio-functionalized Nanomaterials**
07.30 pm	**Conference Dinner**

Direct observation of proteins at work by high-speed atomic force microscopy

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Direct observation of protein molecules in action at high spatiotemporal resolution is considered to be the most straightforward approach to understand how they operate the functions. To achieve this observation, we have been developing and improving high-speed atomic force microscopy (HS-AFM) (1). Nowadays, HS-AFM can capture an image of biological molecules at sub-100 ms, without disturbing their physiological function. For several proteins, the powerfulness of HS-AFM observation was successfully demonstrated, and great insights into the functional mechanism of the proteins were given (2). Here, we present the recent imaging results obtained by HS-AFM about myosin V and intrinsically disordered proteins (IDPs).

For the study of myosin V, an actin based molecular motor, we developed a new scanning mode called “interactive mode” that allowed us to apply controlled strong tip force onto specific loci of a molecule during imaging of the molecule. With this technique, the trailing head of a two-headed bound myosin V in ADP or in nucleotide-free conditions was mechanically detached from actin filament. After the detachment, the molecule stepped forward, which was very similar to that seen in ATP. Although it is widely believed that the chemical energy from ATP hydrolysis is used for the intramolecular tension generation and the lever-arm swing, our result is strongly against this idea. Alternatively, our result indicates that the chemical energy from ATP hydrolysis is mainly used for the detachment of actin-myosin interaction and that the other mechanical process can be done by thermal energy (manuscript in preparation).

IDPs, proteins having large disordered regions, belong to a new category of proteins and play a number of crucial biological functions (3). We demonstrated that HS-AFM is the only technique capable of directly visualizing the whole structures of IDPs containing dynamically moving ID regions with structural transitions (2). Through the HS-AFM observations of several IDPs, we recently succeeded in the characterization of their unique mechanical properties, from which the number of amino acids contained in ID regions of IDPs could be estimated (submitted). This result will greatly contribute to the structural studies of IDPs lagging so far behind those of conventional ordered proteins.

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Single-cell force spectroscopy of microbes

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Single-cell force spectroscopy (SCFS) is a powerful atomic force microscopy (AFM) modality in which a single living cell is attached to the AFM cantilever to quantify the forces that drive cell-cell and cell-substrate interactions. Although SCFS protocols are well established for animal cells, application of the method to individual bacteria remains challenging, mainly owing to the lack of appropriate methods for the controlled attachment of single live cells on cantilevers. Here, we present a non-destructive protocol for single-bacterial cell force spectroscopy which combines the use of colloidal probe cantilevers and a bioinspired polydopamine wet adhesive.[1] A single living cell is picked up with a polydopamine-coated colloidal probe, enabling us to quantify the adhesion forces between the cellular probe and solid substrates or another live cell (Fig. 1). This novel technique represents a generic platform for studying the molecular mechanisms of cell adhesion, which has already allowed us to decipher the molecular details governing the adhesion of several probiotics and pathogens. We anticipate that the approach will be useful to researchers interested in understanding the molecular bases of cell adhesion in a broad range of microorganisms. Advanced SCFS technologies, in combination with single-molecule assays, could also contribute to the development and screening of anti-adhesion molecules that are capable of inhibiting the adhesion of pathogens,[2] including multidrug resistant organisms or controlling the adhesion of beneficial bacteria.

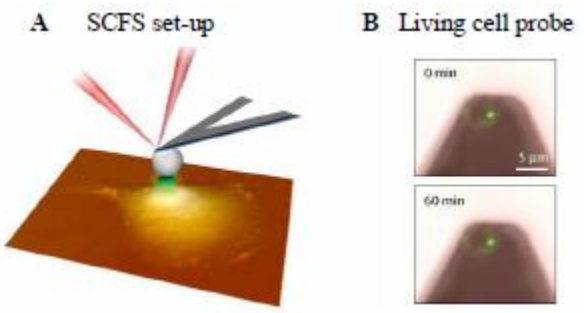

Figure 1. Principle of the SCFS method. (A) The probe is used to measure forces on abotic/biotic/living cells. (B) The viability and positioning of the cell is checked using a LIVE/DEAD fluorescent kit.

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Implementation of nanotechnologies in single-cell analysis

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The state-of-the-art nanotechnologies allow probing of biologically important molecules and their reactions on the surface and in the interior of living cells with nanometer space and picosecond time resolutions. Thus, combinations of specific fluorescent probes and advanced ultrasensitive optical instrumentation gave rise the idea of using individual cells as test tubes. An ability to detect a single cell, organelle or even molecule opens up new possibilities for molecular analysis of biological samples in space and time development. Probably the most advanced example is the DNA sequencing technology enabling reading of nucleotide sequence of a single molecule at a velocity of incorporation of individual nucleotides by DNA polymerase. Moreover, this miniaturized sequencing method provides parallel reading of sequences on millions of nanovials, thus substantially overcoming the throughput of classical sequencing, from months to tens of minutes per human genome.

The objective of the presentation is to show the role of nanotechnologies in analyses of chemical contents of individual cells by fluorescence and electron microscopy. With respect to this, the exceptional properties of highly fluorescent nanocrystals, quantum dots, will be demonstrated. High extinction coefficients together with the wide range of excitation wavelengths, size- and composition-tunable emissions, narrow and symmetric emission spectra, reasonable quantum yields and practically no photobleaching are their main advantages when compared with the conventional organic fluorescent dyes. A relatively easy conjugation of quantum dots with antibodies and their effective uptake into living cells make them promising candidates for imaging of organelles and functional molecules inside cells.

Another example of applications of quantum dots is synthesis of chemical sensors based on Förster resonance energy transfer. This pohenomenon is a photophysical process that occurs between a donor molecule (quantum dot) in the excited state and a covalently linked acceptor in ground state. The absorbed energy of donor is transferred nonradiatively to the acceptor and is emitted at its typical wavelength. If a part of the linker chain is a specific sequence of aminoacids, it can be cleaved by an enzyme and the energy is emitted at the characteristic wavelength of donor. This principle is used for sensitive detections of caspases, signaling enzymes activated during the apoptotic machinery. The detection of apoptosis (programmed cell death) is an important diagnostic method in some pathologies including cancer transformations, neurodegenerative or autoimmune diseases.

Rapidly growing field of the correlative light and electron microscopy proved to be useful for the investigation of relations between cellular structures and their functions. In our arrangement, the cathodoluminescence emission and scanning electron microscopy images are detected simultaneously in the same device and compared. The key point for the development of the method is the synthesis of a stable luminophore with a high electron density providing efficient cathodoluminescence. Nanoparticles of cerium-doped lutetium-aluminium garnet conjugated with antibodies are used for immunostaining of two cellular transmembrane receptor proteins, Fas and Fas-ligand. This demonstrates the potential of the method for the investigation of signaling pathways.

The research was supported by Grant Agency of Czech Republic, grant no. 14-28254S.

Nanopipette Navigation System as a New Tool for Biomedical Application

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Recently we have developed the new system for nanopipette navigation with feedback control. The ability to precisely move the nanopipette to target specific regions such as neuronal processes allows an unprecedented level of control of drug application. The speed of data acquisition positions this as a technology which may be suited to relatively high-throughput application in human neuronal preparations which would greatly facilitate drug discovery. This nanopipette navigation system can be used in combination with other techniques such as microinjection, electrochemical measurement, and patch-clamp recording. This has the potential to open new horizons in medicine and biology and could be of particular value to the pharmaceutical industry.
We have demonstrated unique application of nanopipette as a sensor for local electrochemichal measurements. Nanopipette can be filled with a carbon using butan decomposition in argon atmospher as a result we get a disk-shaped nanoelectrode. We have developed the method of platinum deposition at the tip of nanoelectrode (Figure 1 a). Such probe with platinum can detect various oxidgen consuptions. As a demonstration we have shown the detection of oxygen photosynthesized by plant cell on light (Figure 1 b). Using nanopipette navigation system (Figure 1 c) we can do electrochemical mapping of a living cell surface with nanoscale resolution.

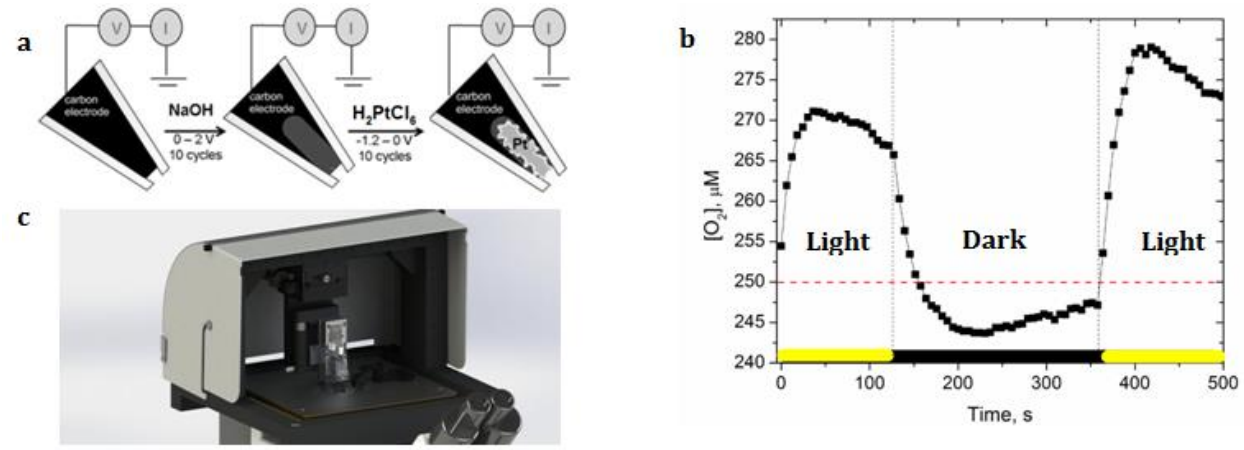

Figure 1. a) Assembled nanopipette navigation system; b) modules of the system.

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ESEM sub-micrometer-scale mapping of sulfur as a powerful tool for the study of marine thioautotrophic organisms: case study of a new uncultured epsilon sulfur bacteria colonizing marine mangrove sediment in the Caribbean.

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Here, we report the first description of an epsilon marine sulfur-oxidizing bacterium from sulfide-rich sediments of marine mangrove in Caribbean. According to TEM, this new strain contains intracytoplasmic large internal granules which appeared "empty" after a conventional preparation of these biological samples.

However, by using an Environmental Scanning Electron Microscope, elemental spectra obtained showed that sulfur was one of the main elements detected from these micro-organisms. Thus, ESEM sub-micrometer-scale mapping of sulfur obtained for this thioautotrophic micro-organism allows concluding that elemental sulfur is the main component of these granules and were probably formed as an intermediate oxidation product (Fig. 1). Intracytoplasmic elemental sulfur granules are a typical feature of sulfur-oxidizing bacteria. Moreover, according to conventional SEM observations, bacterial cells are ovoid, extremely motile by lophotrichous flagella.

Phylogenetic analysis based on the analysis of 16S rDNA confirms that such strain belongs to epsilon bacteria and more precisely to the *Thiovulum* cluster and could be a representative of a new species in this poorly studied genus. Thus, reduced sediments of marine mangrove represent sulfide-rich environment sustaining development of various sulfur-oxidizing bacteria.

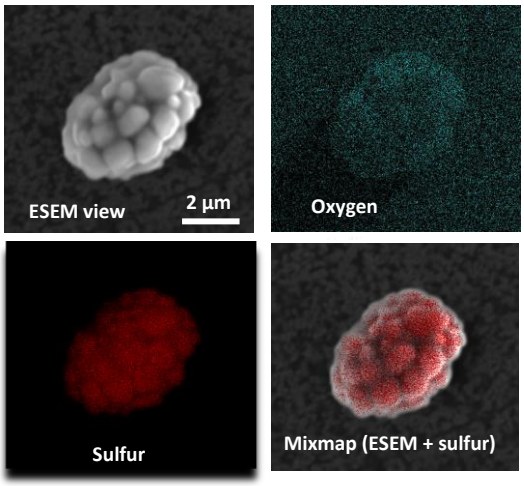

Figure 1: Environmental Scanning Electron Microscopy images and mapping of sulfur recorded on slightly fixed bacterial cells under 130 Pa water vapor atmosphere at 15kV.

Assessments of nanomechanical properties of biomimetic membranes using AFM Circular Mode

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Lipid-based vehicle or liposomes are commonly used in drug delivery system due to its low toxicology, biocompatibility, biodegradability, and simplicity of targeting specific diseased cells (1). The fusion process between liposome and cell membrane is influenced by the nanomechanical properties of the lipid membranes (of both liposome and cell envelope).
Atomic Force Microscopy (AFM) is widely used in characterization of biomembranes. Recently developed circular mode for AFM, has gained interest due its ability to quantify instantaneously the nanomechanical and nanotribological properties of biological materials. It offers high scanning constant velocities and continuous displacement. Thanks to its capability to measure at high frequency, it is possible to measure viscosity of biomembranes which has been reported to be considerably small (2). Thus, the aim of this work is to provide new insights about the fusion mechanism (in the case of drug delivery system) by quantifying the nanomechanical properties of biomembranes using AFM circular mode.
The spring constant of the AFM probes were measured by using the thermal tune method. Beside, the lateral force was calibrated by using an improved method based on the Ogletree's calibration technique (3). The circular displacement was also calibrated with various frequencies (10 – 500 Hz) and voltages (0.01 – 10 V). Supported lipid bilayers (SLB) of Dioleoylphosphatidylcholine (DOPC) were prepared by fusion of vesicles in buffered medium. SLB are often used to mimic bio-membranes due to its simplicity and reproducibility.
Experiments showed that punch-through force, friction force, and viscous constant could be acquired at the same time. The values of punch-through force are considerably higher comparing with recorded values reported in literature (4). The friction force of DOPC bilayers was found to be directly proportional to the sliding velocity, showing its viscous behavior.

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Complementary Dual Biosensing with Novel Ultrathin Gold Nanohole Films

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Simultaneous LSPR and electrical detection using ultrathin gold films with incorporated nanoholes is presented. The sensor is electrochemically accessible and enables label-free sensing in a controlled fashion.

Films with dimensions smaller than the electron mean free path are essential to measure large resistance changes due to small alterations in thickness or close proximity of charged species [1]. Additionally, nanoholes within thin films enable optical sensing based on localized surface plasmon resonance. Our sensor combines both phenomena and can be controlled electrochemically. The chip is fully integrated into a custom-made flow cell. The design of both the flow cell and the chip allows for reproducible and accurate measurements with ideal fluid exchange behavior.

Iodide sensing is one of the possible applications for this device [2]. Most current detection methods are costly and require both a sample pretreatment and a long analysis time [3,4]. However, for determining iodide concentrations of human samples in developing countries or for comprehensive and long-term studies in environmental waters, low cost and simplicity are becoming increasingly important.

Our device takes up on these issues with sensing based on iodide induced electrochemical etching of ultrathin gold films. Iodide anions can be attracted using voltage. The resulting etching can be measured by a change in the impedance of the thin film. As sensing methodology, an amperometric multistep method is presented in buffer as well as in an environmentally relevant fluid (lake water) with limits of detection in the range of 1 μM and 2 μM, respectively.

The versatility of this sensing platform is also shown with binding studies. Attachment of thiolated single-stranded DNA demonstrates this concept. Iodide etching can thereby be used as a controlled cleaning step, enabling the reuse of the device.

As a further development, the use of periodic gold nanowire arrays is a promising approach for higher sensitivity. However, Extreme Ultraviolet Interference Lithography (EUV-IL) is essential for parallel fabrication with high resolution [5,6]. A transfer technique was developed to limit this step to mold making and thus multiplying the number of structures.

We believe this sensor not only enables low cost measurements of iodide concentrations and binding events, but also opens up opportunities for a variety of biosensing applications.

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Real time monitoring of membrane GPCR reconstitution by plasmon waveguide resonance: on the role of cholesterol

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G-protein coupled receptors (GPCRs) are important therapeutic targets since more than 50% of the drugs on the market exert their action through these proteins. To decipher the molecular mechanisms of activation and signaling, GPCRs often need to be isolated and reconstituted from a detergent-solubilized state into a well-defined and controllable lipid model system. Several methods exist to reconstitute membrane protein in lipid systems but usually the reconstitution success is tested at the end of the experiment and often by an additional and indirect method. Irrespective of the method used, the reconstitution process is often an intractable and time-consuming trial-and-error procedure. Herein, we present a method that allows directly monitoring the reconstitution of GPCRs in model planar lipid membranes. Plasmon waveguide resonance (PWR) allows following GPCR lipid reconstitution process without any labeling and with high sensitivity (femtomole quantities of material can be detected) (Figure 1) (1). Additionally, the method is ideal to probe the anisotropy of oriented thin films such as the case of lipid membranes and embedded membrane proteins.

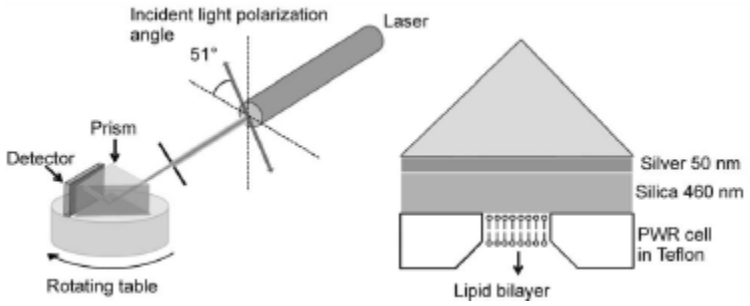

Figure 1. PWR setup. On the left the optical and mechanical components, the incident polarized light beam (a continuous wave He–Ne laser at 632.8 nm) is at 45° and the rotating table allows steps of 1 millidegree. On the right is a detailed view of the prism and the PWR cell sample with the lipid bilayer.

The kinetics of lipid reconstitution of the chemokine CCR5 receptor was investigated by PWR. Additionally the role of the lipid environment especially that of cholesterol on the ligand-receptor interaction was investigated. Maraviroc, an antagonist and anti-HIV was the chosen ligand.

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Injectable, settable, and resorbable nanocrystalline hydroxyapatite/ polyurethane hybrid polymers with bone-like strength

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Introduction: Bone cements utilized for bone fractures at weight-bearing sites, such as intra-articular joints, are subjected to repetitive, dynamic physiological loading from daily activities and must have adequate strength.[1] The performance requirements for non-resorbable poly(methyl methacrylate) (PMMA) are compressive strength >70 MPa, bending modulus >1800 MPa, and bending strength >50 MPa.[2] However, resorbable weight-bearing cements are not currently available. In this study, we designed resorbable nanocrystalline hydroxyapatite (nHA) polyurethane (PUR) inorganic-organic hybrid polymer networks with bone-like mechanical properties exceeding that of PMMA. Remodeling of nHA/PUR hybrid polymers was assessed in a rabbit femoral plug defect model.

Methods: Hydroxyapatite nano-particles (nHA) (<200 nm, Sigma) were reacted with lysine triisocyanate (LTI) to yield a viscous nHA-LTI prepolymer (0-65 wt% nHA). nHA/PUR hybrid polymer networks were synthesized by crosslinking the nHA-LTI prepolymer with either poly(ε-caprolactone) triol or poly(thioketal) diol and an iron acetylamide catalyst. nHA/ Poly(thioketal-urethane) (nHA/PTKUR) hybrid polymer was injected into rabbit femoral plug defects. At 6 and 12 weeks following the surgery, the animals were sacrificed and and the defects analyzed by microCT and histology for new bone formation and cellular infiltration.

Results: The yield strength of the hybrid polymer networks increased from 80 MPa to 113 MPa as the nHA loading increased from 0 wt% to 52 wt% total (65 wt% nHA loading in prepolymer). However, when 52 wt% nHA was blended with the polyurethane as a powder with no prepolymer step, the compressive strength was 91 MPa, which is lower than that of nHA-PUR hybrid polymers with >16 wt% nHA loading. In the rabbit femoral plug defect model, grafts maintained mechanical stability at 12 weeks. Images of histological sections showed evidence of graft resorption, new bone formation near the host bone interface, and integration of the graft with host bone (**Fig 1**).

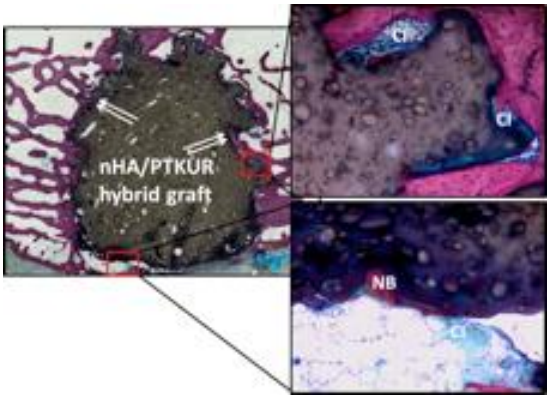

Fig. 1: Cellular infiltration and new bone formation at the interface between host bone and the hybrid bone graft at 12 weeks. Arrows indicate new bone integrating with the graft. Abbreviations. CI: cell infiltration, NB: new bone

Discussion & Conclusions: Covalent bonding of LTI to nHA significantly enhanced the mechanical properties of nHA/PUR hybrid polymers compared to physically blended composites. Furthermore, the compressive strength of the hybrid polymers exceeded that of PMMA bone cement. When implanted in femoral condyle plug defects in rabbits, nHA/PUR hybrid bone grafts resorbed slowly, integrated with host bone, and supported new bone formation. These findings highlight the potential of nHA/PUR bone cements for healing of weight-bearing bone defects.

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Nanoscale Interplay Between Bacterial Adhesins and Substrate Properties

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One key reason why bacteria thrive successfully in diverse and hostile environments is their ability to form biofilms in a wide range of surfaces. Central to this process are surface adhesion molecules (adhesins) that mediate the initial attachment of bacteria to the underlying substrate. In the first part of my talk, I will focus on how (1) the surface appendage (pili) of a probiotic (*L. rhamnosus* GG) and (2) the P1 adhesin of an oral bacterium (*S. mutans)* mediate bacterial adhesion. Using single-molecule and single-cell force spectroscopy, I will show that *S. mutans* exhibit a P1-binding mechanism involving strong forces, cooperativity, and broad specificity.[1] In the case of LGG, the bacteria colonize abiotic surfaces through a pilus nanospring behavior and formation of membrane tethers on host cells.[2] Just as crucial as the adhesins to biofilm formation are the properties of the underlying substrate. I will then discuss my work on how bacterial adhesion depends on the stiffness of the underlying substrate.

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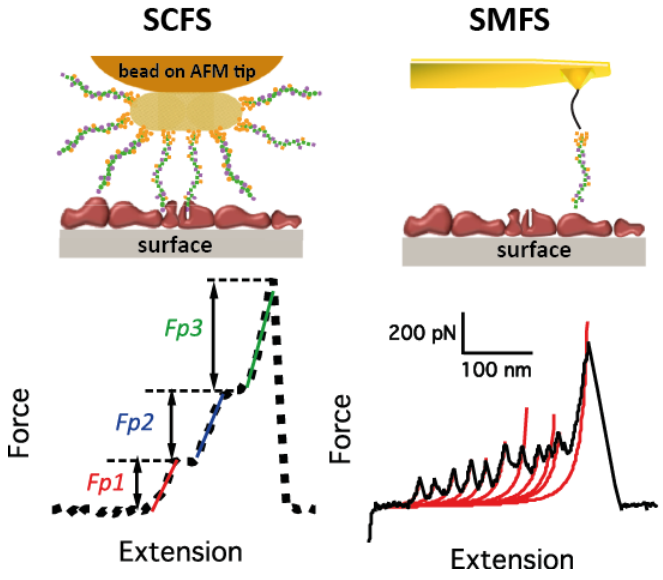

Fast Nanoscale Imaging and Quantitative Nanomechanical Characterization of Cells and Biomaterials with Correlative Atomic Force and Optical Microscopy

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From its first tentative steps 30 years ago Atomic Force Microscopy now walks tall as an integral component of many life-science imaging facilities. Recent technological developments have enabled a suite of mechanical and fast measurements that can be correlated seamlessly with even the most advanced optical microscopy.

JPK ULTRA Speed AFM for instance combines tip-scanning technology and a compact design, allowing AFM AC-mode imaging of approximately 1 frame per second, and can be seamlessly combined with advanced optical methods such as confocal, TIRF, or STED microscopy. The unambiguous correlation between AFM and optical microscopy is achieved by DirectOverlay™. Thus, individual molecule dynamics can now be studied with AFM. We could gain a high-resolution temporal insight into the dynamics of collagen I fibril formation and its characteristic 67 nm banding hallmark.

Mechanical forces in cell and structural biology, for instance cell stiffness related to cell health, or cell-cell interactions for cell differentiation and tissue formation are of paramount importance and our team have developed AFM-based spectroscopic methods to better understand them. Further high speed mechanical mapping will also be discussed. “Quantitative Imaging” (QI™), a fast force-distance curve based measurement developed by JPK acquires topographic, nanomechanical, and adhesive sample properties simultaneously. It can also measure contact point images, Young´s modulus, or even recognition events. To demonstrate the power of QI™, a variety of samples have been investigated. Living cells, polymer surfaces and single biomolecules have been analysed quantitatively, and data compared with conventional force spectroscopy and traditional AFM imaging modes.

Universal melt modification method for the synthesis of core-shell-nanoparticles with ultra-high grafting densities.

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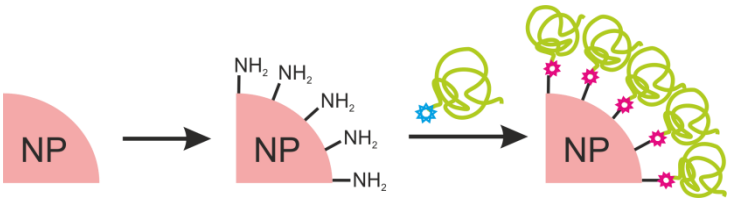

Nanoparticles (NPs) (e.g. superparamagnetic iron oxide, nanodiamond or silica) coated with dense polymer brushes such as poly(ethylene glycol) (PEG); polyisobutylene (PIB); polyvinylpyrrolidone (PVP) or polydimethylsiloxane (PDMS) are used in a wide range of applications, especially in the biomedical field and in the polymer industry. Depending on the polymer, these NPs can assemble into functional materials, responsive structures or be used as innovative filler materials for the production of high impact or functional polymers. Grafting of these polymers to monodisperse NPs remains a challenge mainly because of the conflicting requirements during the exchange process to replace the ligand shell of as-synthesized NPs with irreversibly bound polymer dispersants. Here, we introduce a two-step method to graft a wide range of different polymers (PIB, PDMS, PEG, PVP) and other dispersants (e.g. functional small molecules) from a melt (solvent free) to NPs that were first functionalized with amino moities. Compared with existing approaches, this method yields uniquely dense (~3 chains/nm2) and remarkably colloidally stable NPs.

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Nano-functionalized Biomaterials and Bio-functionalized Nanomaterials

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The inter-combination of "Nano" and "Bio" has gained a substantial interest, not only in the fields of biology and biomedicine, but also in the fields of fundamental and applied material science. Going towards nano-functionalized biomaterials, the incorporation of functional nanoparticles (e.g. magnetic, plasmonic or fluorescent NPs) in biomaterials yields novel nano-in-bio hybrid materials, which exhibit novel properties that go beyond their natural ones. Going towards bio-functionalized nanomaterials, on the other hand, the decoration of biomolecules and biopolymers onto nanoparticles, yields biocompatible and colloidally stable nanosystems with a defined bio-interface.

The present contribution will shed light on both pathways, showing: a) how the incorporation of e.g. superparamagnetic nanoparticles in wood materials, introduces magnetic anisotropy to the wooden scaffold,[1] and b) how biopolymers such as proteins enhance the physico-chemical and colloidal properties of nanoparticles (e.g. gold nanospheres and nanorods), making them highly suitable for biomedical applications.[3-4] Using the diversity offered by both biomaterials (biomolecules, biopolymers) and nanomaterials (organic and inorganic) and their inter-combinations, we aim to create a stronger bridge between the worlds of Nano and Bio.

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NanoInBio 2016
Conference Program

Sunday, June 5

	Conference Session V Nanosciences at the frontier with biology
09.00 am – 09.40 am	**Nicholas Spencer (Keynote Lecturer):** **Approaches to mimicking cartilage with polymers**
09.40 am – 10.00 am	**Hélène Martin-Yken**: Stress, Drug Resistance and Adhesion: a closer look into the dark side of the wall
10.00 am – 10.20 am	**David Duday**: Degradation of small unilamellar vesicles and their cargo in physiological liquids by cold atmospheric plasma
10.20 am – 10.40 am	**Jérôme Dejeu**: Functionalization of surfaces by peptidic ligands using multivalent host-guest interaction
10.40 am – 11.00 am	**Coffee Break**
11.00 am – 11.20 am	**Lukas Traxler**: Characterization of the Orai-Calmodulin Interaction as Potential Mediator of Calcium-Dependent Orai-Channel Inactivation
11.20 am – 11.40 am	**Kristin Webling**: The complexity of G-protein coupled receptor signaling evaluation
11.40 am – 12.00 pm	**Sandra Posch**: Interplay of domain interactions and unfolding in the force sensing protein von Willebrand factor (VWF)
12.00 pm – 12.20 pm	**Gilmar Salgado**: Using NMR spectroscopy to access ligand binding of G-quadruplex DNA in Xenopus oocytes
12.30 pm – 02.00 pm	**Conference Lunch**

Approaches to mimicking cartilage with polymers

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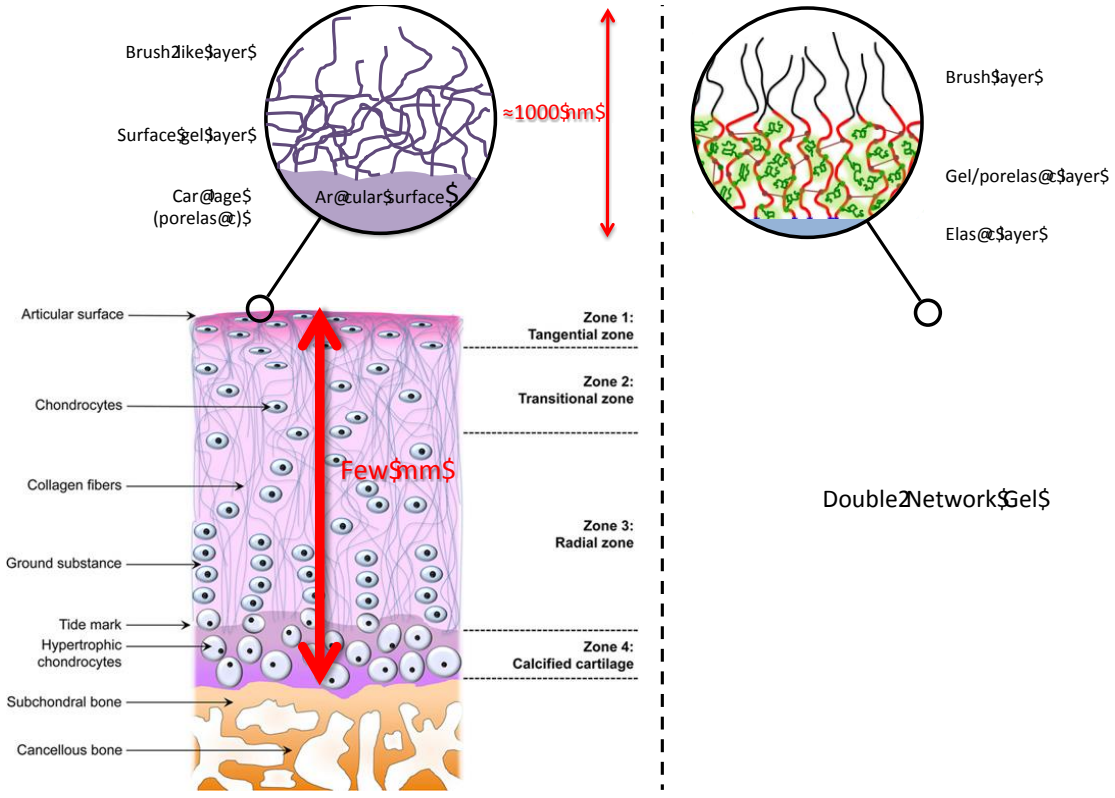
Articular cartilage is an extraordinary material, which allows mammalian joints to function with extremely low friction coefficients for many decades. Moreover it does this in the absence of a blood supply. The precise mechanisms by which cartilage functions are not yet completely understood, but a consensus is beginning to emerge on certain mechanisms that appear to be important for load bearing and lubrication. Of prime importance for lubrication is the surface, and there is at least partial agreement that the surface of cartilage terminates in a very low-modulus gel with many dangling, sugar-rich chain ends. The bulk of the cartilage plays its role in supporting applied load, partially by means of so-called "fluid load support".

There are a number of good reasons for imitating cartilage with polymers. Firstly, by constructing simplified, model systems based on polymers that partially reproduce structures and mechanisms found in cartilage, there is the hope that a better understanding of the natural material will be facilitated. Secondly, an "artificial cartilage" could have important, industrial uses in lubrication, where low loads and aqueous environments are called for (e.g. in a number of medical devices, such as catheters). Finally, with insights gained from such model cartilage studies, there is the possibility that ultimately a material can be developed that functions significantly better, in terms of friction and wear, than the articulating materials currently used in hip and knee implants.



Stress, Drug Resistance and Adhesion: a closer look into the *dark* side of the wall

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Stress conditions and presence of antifungal drugs induce significant changes in the composition and molecular architecture of the cell wall of yeasts and fungi. They particularly alter the nature, repartition and attachment of cell wall proteins to the cell surface. Atomic Force Microscopy (AFM) is a powerful tool to investigate the morphology, nanomechanics and adhesive properties of live microorganisms under physiological conditions. Using recent AFM technological developments, we imaged and measured the biophysical consequences of various stresses on both the baker's yeast *Saccharomyces cerevisiae* and the major human fungal pathogen, *Candida albicans*. We characterized their cell morphology at the nanoscale, focusing on changes in cell surface aspect and characteristics such as roughness, elasticity and adhesive properties. These studies notably revealed dramatic changes occurring at the cell surface upon heat shock[1]. We also investigated the consequences of exposure to osmotic shock and Killer Toxin[2], a small peptide secreted by a "Killer" yeast, which exert cytocidal activity on sensitive strains.

Moreover, we showed that exposure to Caspofungin, an antifungal compound currently used in human health, caused a profound cell wall remodeling evidenced by a dramatic increase in chitin and decrease in beta-glucan content[3]. Remarkably, a low dose of Caspofungin (*i.e.*, 0.5 × MIC) also led to the characteristic expression of adhesive proteins or adhesins on the cell surface of *C. albicans,* a side effect highly relevant considering its medical use. In addition, Single Molecule Force Spectroscopy (SMFS) experiments allowed us to visualize the organization of these adhesins, map them on the cell surface and quantify the adhesion forces, including on cells undergoing mophogenetic differentiation. Combined with molecular biology tools, this approach also enabled us to unravel the particular contribution of previously uncharacterized proteins (PGA22 and PGA59) to *C. albicans* adhesion mechanism[4].

Altogether, our studies establish the great interest of Atomic Force Microscopy, a technology which has no equivalent to explore and quantify the molecular mechanisms occurring at the nanoscale on the cell surface of live fungal cells. In the future we will focus on new approaches using Single Cell Force Spectroscopy with AFM and Optical Tweezers as well as Sheer-Stress Flow Chamber to study adhesion from the molecule scale to the population scale.

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Degradation of small unilamellar vesicles and their cargo in physiological liquids by cold atmospheric plasma

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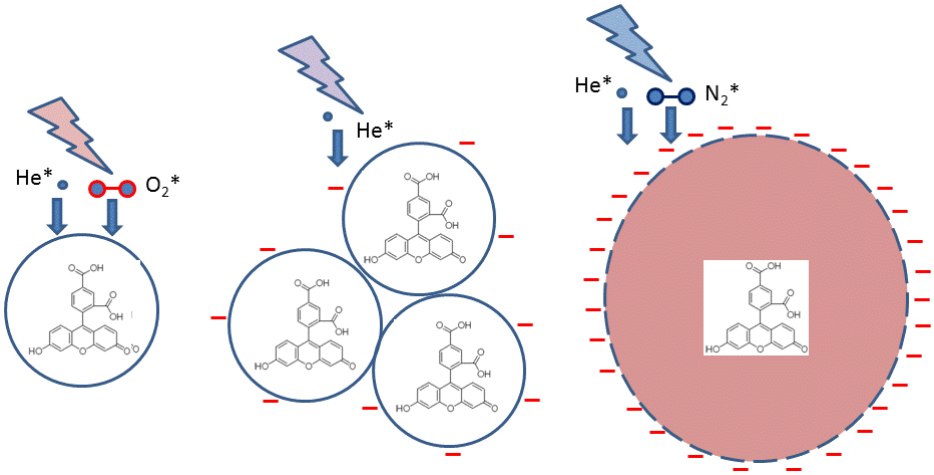
The combination of plasma (ionized gas) and nanoparticles for enhanced permeation of drug into skin is a promising approach which can potentially lead to numerous medical applications [1]. However, the role of plasma in this enhanced permeation process is not understood due to the several effects generated by plasma and the complexity of the skin. Moreover, the integrity of the drug loaded inside the nanoparticle and of the nanoparticle itself has never been investigated when exposed to the plasma. The integrity of these components is mandatory to avoid any toxicity during therapeutic treatment.



Different approaches are available to evaluate the integrity of nanoparticle and the drug loaded inside the nanoparticle but most of them are difficult to apply for nanoparticles in solution treated by plasma due to the number of possible reaction products generated. Several methods have to be combined to obtain a deep understanding of the structural and chemical degradations generated by plasma on small unilamellar vesicles (SUV), which are one of the most used nanocarriers to deliver drug into the body by systemic and sub-cutaneous routes [2]. A very few papers are dealing with treatment of liposomes by atmospheric cold plasma but no one have investigated both simultaneously structural and chemical degradations of liposomes [3, 4].
In this work, different He-based plasma treatments were used to treat the carboxyfluorescein-loaded SUV monodispersed suspensions, in pH buffered saline solution and under physiological relevant conditions like in [5]. Modification of structural and surface charge state for SUVs treated by plasma in solution were studied by dynamic light scattering and zeta potential measurements, respectively. Carboxyfluorescein leakage and degradation both inside and outside the liposomes were evaluated by fluorescence spectroscopy. Chemical modifications on the liposomal membranes were characterized by high resolution mass spectrometry (MALDI-ORBITRAP).

When the concentration of plasma reactive species in solution was above a critical dose, an agglomeration of SUVs as well as an increase of their negative charge state was observed. Above a second higher critical dose, a fusion of SUVs as well as a higher increase of their negative charge state was observed, associated with a carboxyfluorescein leakage. Unfortunately, the fluorescent dye was degraded by the reactive species generated by plasma, mainly HOCl ones. It means than milder or different plasma treatments should be used for future therapeutic applications in order to avoid the cargo degradation under plasma exposure.
OH, O and Cl additions were observed on the unsaturated C=C bonds of the phospholipids mainly due to the reaction of HOCl and reactive oxygen species with the unsaturated C=C bonds. Future studies will deal with milder plasma treatments and a deeper characterization of treated SUVs by using liquid chromatography, X-Ray Scattering and Raman spectroscopy.

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Functionalization of surfaces by peptidic ligands using multivalent host-guest interaction

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Adhesion of cells to the extracellular matrix (ECM) influences their shape, growth, viability, differentiation and migration. Mixed self-assembled monolayers (SAMs) presenting specific ECM components in an inert non-adsorbing interface promote attachment through specific cell adhesion receptors. One of these adhesion ligands is the arginin-glycine-aspartic acid peptide sequence (RGD) which is present in fibronectin (protein of the ECM) and other matrix proteins. In this work, we designed model surfaces with cell adhesion ligands coupled to the substrate through non-covalent host-guest interactions. For this purpose, the adhesion ligands were assembled on a β-cyclodextrin (β-CD) functionalized SAM. Using host-guest multivalent interactions, we studied the influence of the guest valency on the adsorption of guest-RGD conjugates. For this purpose, we synthesized biomolecular systems based on cyclodecapeptide scaffolds exhibiting two different domains: the upper face is functionalized by cell adhesion ligands where as the lower face presents a clustered guest (ferrocene or adamantane) domain with tunable number of guest molecules for the anchoring onto β-CD SAM. The characterization of the multivalent guest scaffold adsorption was investigated thanks to physical methods (quartz crystal microbalance (QCM-D), surface plasmon resonance (SPR) and electrochemistry) allowing the determination of the affinity constants of guest molecules. In order to validate the properties of cell targeting, cell adhesion process on the functionalized surfaces has been analyzed by combining QCM-D and optical microscopy.

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Characterization of the Orai-Calmodulin Interaction as Potential Mediator of Calcium-Dependent Orai-Channel Inactivation

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Calcium is an important second messenger involved in many cellular processes such as cell proliferation, T-cell activation, muscle contraction, egg fertilization, or apoptosis. Calcium entry into non-excitable cells is mainly carried by store-operated channels, whereby Orai was found to be the calcium channel in the plasma membrane and STIM to act as a calcium sensor in the ER and as an activator of Orai channels. A reduction of Ca^{2+} in the ER causes STIM to oligomerize, enabling its accumulation at ER-plasma membrane junctions where it binds directly to Orai to open the channel. Compared with activation, much less is known about the mechanisms underlying Ca^{2+}-dependent inactivation (CDI) processes. It has been proposed that binding of calmodulin (CaM) to a N-terminal segment of Orai1 adjacent to its first transmembrane helix is calcium-dependent and critical for CDI. Hereby it is assumed that CaM acts in concert with STIM1 and the N terminus of Orai1 to evoke rapid CRAC channel inactivation (1, 2). However, the molecular basis of these interactions remains fairly unclear.

In this study, the energy landscape of the interaction between calmodulin and the N-terminal fragment of Orai1 and its homologue Orai3 was explored by Single Molecule Recognition Force Spectroscopy. Both fragments showed a calcium-dependent and highly specific interaction with calmodulin, with higher bond lifetimes for Orai3 than for Orai1. Our data support a 1:2 binding stoichiometry between CaM and Orai1/3, with both the N- and C-lobe of CaM binding one Orai fragment each. Interestingly, both the Orai1 and the Orai3 fragment showed higher unbinding forces and lower dissociation rates than a widely used CaM-binding model peptide which consisted only of Trp, Lys, and Leu residues. This emphasizes the importance of the unique amino acid sequence of the conserved N-terminal Orai segment for high CaM affinity and CDI.

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The complexity of G-protein coupled receptor signaling evaluation

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G-protein coupled receptors are an important drug target with around 50% of the marketed pharmaceuticals acting upon G-protein coupled receptors.

Over the years, accumulating reports have shown that the signaling cascade of G-protein coupled receptors is more complex than previous reported. The concept of biased ligands has emerged, meaning that a ligand can selectively activate one (or a few) signaling pathways when several signaling pathways are possible. Recently, several G-protein independent signaling pathways have been identified to complicate the picture furthere together with a second wave of G-protein signaling from internalized receptors in endosomes, which was formerly regarded as desensitized receptors.

This implicates that using a single second messenger for evaluating the signaling cascade of new ligands as inadequate. Here we present a comparison between a label-free real-time impedance based technique alongside two classical second messenger assays, cyclic adenosine monophosphate measurements and inositol phosphate turnover, for evaluating G-protein coupled receptor signaling for the three galanin receptors. Pros and cons of the two methods are also highlighted.

End-point assays	**Label-free systems**
Biased ligands are difficult to identify	Biased ligands can be identified
Well known systems	
Assay conditions	Normal cell culturing conditions
Overexpression of receptors	Primary cells and endogenous cells
Risk of false negative	Risk of false positive
	Real time
	No labeling required

Interplay of domain interactions and unfolding in the force sensing protein von Willebrand factor (VWF)

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The VWF is a glycoprotein that plays a central role in hemostasis. It is,amongst others, responsible for platelet adhesion at sites of injury via its A1 domain. The adjacent VWF domain A2 is unfolded under shear upon which it exposes a site cleaved by the protease ADAMTS13to prevent thrombosis.It has been shown that the A1/platelet interaction is blocked by mutual A1/A2 binding under lowshear. Nevertheless, the exact shielding mechanism is notyet clarified. We therefore probed the interaction strength between the A1 and A2 domains and the unfolding behavior of A2as an indicator of the shielding capacityutilizing single molecule force spectroscopy (SMFS).

SMFS studies between the isolated domains A1 and A2 revealed specific recognition, underlying a slip bond behavior with a bond life-time of ~1.5s and forces between 50 and 140pN at loading rates ranging from 100 to 60000pN/s.To elucidate the interplay between dissociation of the A1-A2 complex and A2 domain unfolding, we analyzed the elongation of the complex prior to dissociation. In most cases, the measured elongation values (~28nm) were substantially lower than the extension of a fully-unfolded A2 domain (~80nm). Additionally, we used a disulfide bridged A2 domain mutant ([A2]). The introduction of the disulfide bond obstructs the mechanical unfolding of this domain and enhanced A1 binding. Similar complex elongations were observed for both, the A1/A2 and the A1/[A2] interactions. Thus our data indicate, if at all, only a small extent of A2 unfolding before dissociation from A1.

Based on additional investigations using a VWF A1A2 and a VWF A1[A2] construct, we speculate that the linker between A1 and A2 interferes with a direct intramolecular interaction, leading to a preference for intermolecular A1-A2 interactions.Our data are also consistent with the existence of two cooperatively acting binding sites for A2 in domain A1.

Overall, these findings suggest that the platelet binding site in domain A1 is made accessible by the dissociation of domain A1 from A2 and that domain A2 remains largely folded during this process, which keeps it protected against cleavage and degradation.

Using NMR spectroscopy to access ligand binding of G-quadruplex DNA in *Xenopus* oocytes.

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In recent years we have seen the loom of different approaches to obtain information from biological relevant molecules inside living cells (1-5). Notably, NMR spectroscopy is one of the few methods that can probe for atomic structural information in such intricate environment. In this work, we have used multidimensional NMR spectroscopy (SOFAST-HMQC type-spectra) to assess the possibility for DNA repeats of guanines to auto associate into G-quadruplexes (G4) structures inside living *Xenopus laevis* oocytes. G4 sequences are usually found in particular regions such as telomeres and promoters of genes. An important number of human genes contain at least one G4 motif within their promoters. The proto-oncogenes seem particularly enriched with G4 motifs, contrary to tumor suppressor genes. G4 are considered potential anticancer therapeutic targets. Our results clearly demonstrate the folding of G4 structures within the cell environment. In addition, we show for the first time the action of a specific ligand that targets G-quadruplexes directly inside living cells, opening new venues to study ligand binding discrimination in physiological relevant conditions with atomic detail.

The quest for structural information inside living cells using NMR spectroscopy is still very challenging with many perils and rewards to be seized.

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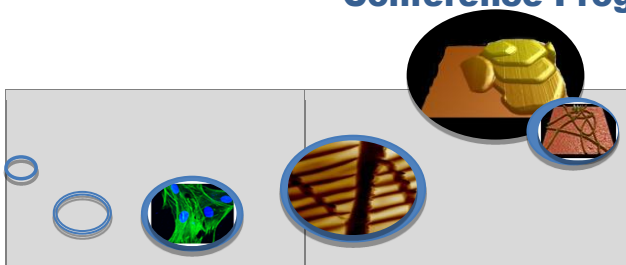
Conference Program



Sunday, June 5

	Conference Session VI **Nanobiotechnology & Nanomedicine**
02.00 pm – 02.40 pm	**Pierre Schaaf (Keynote lecturer):** **Soft-mechanochemistry – Mechanochemistry inspired by nature**
02.40 pm – 03.00 pm	**Michael Timmermann**: Cell-inspired, microwell structures with linear strain-stiffening
03.00 pm – 03.20 pm	**Jana Musilkova**: Impairment of the adhesion, growth and osteogenic differentiation of human osteoblast-like cells on nanofibrous polylactide scaffolds with diamond nanoparticles
03.20 pm – 03.40 pm	**Stefanie Kiderlen**: Mechanotransduction on the Single Cell Level: Investigating Mechanosensitive Genes using Single-Cell Force Spectroscopy combined with Quantitative PCR
03.40 pm – 04.00 pm	**Jana Karpiskova**: Cytocompatibility of Polycaprolactone Nano-Microfibrous Scaffolds Loaded with Amide-Amine Functionalised Carbon Nanoparticles
04.00 pm – 04.20 pm	**Coffee Break**
04.20 pm – 04.40 pm	**Violeta Garcia Romero**: Study of the photocatalytic activity of ZnO nanorods films, in the photodegradation of rhodamine B dye with solar irradiation
04.40 pm – 05.00 pm	**Marcin Kruszewski**: Induction of DNA Damage in HepG2 and A549 Cells Treated with Quantum Dots, Silver and Titanium Dioxide Nanoparticles or Their Binary Mixtures
07.30 pm	**Closing Ceremony & Conference Dinner**

Soft-mechanochemistry – Mechanochemistry inspired by nature

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Mechanotransduction processes play a key role in nature and in particular in cell adhesion. One general way that nature found to transform a mechanical signal into a chemical one is by making use of force induced conformational changes. Such changes can be at the origin of cryptic site exhibition but they can also affect enzymatic activity. We have, over the last years, developed several chemo-mechanoresponsive systems based on mimicking natural processes. We will present here several examples of such systems: We will discuss systems where by stretching one can modulate the binding affinity of a ligand for receptors present on the substrate (1,2). We will also present systems where stretching allows modulating the enzymatic activity of the film. Such systems are either based on exhibiting enzymes through a barrier (3) or on modifying a protein (4) or an enzyme conformation (5) by stretching. In contrary to processes where the application of a mechanical force on a molecule affects its chemical bonds, mechano-transductive processes based on affecting macromolecular conformations require much less energy and new routes to develop such systems will be discussed.

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Cell-inspired, microwell structures with linear strain-stiffening

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The adhesion of a cell to its environment mediates the structural organization of the cell itself and is believed to be directly associated to its mechanical adaptation. We aim to mimic the underlying mechanisms how cells adapt to forces exerted by their environment. In response to force, cells align and cross-link actin fibers upon external stress and connect them to adhesion clusters in a reversible process. The stress-transducing components are biopolymer fibers. We mimic the cytoskeleton´s components in an abstracted model system. The backbone of this structure is a PDMS sample with parallel wells in micrometer dimensions. The walls of the wells resemble biopolymer fibers. Polymer beads inside the wells assure a connection between neighboring walls when the structure is stretched. A feasible surface coating of the walls and beads improves their cross-linking and provides the reversible effect we are aiming for. Such cell-inspired intelligent materials with directional and temporal control of mechanoresponsivity will find many applications, e.g. as biomaterials, but also in more technical applications such as sealing and gaskets.

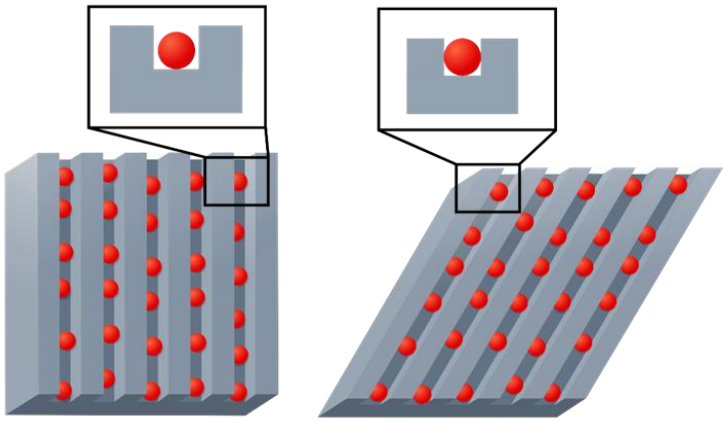

Figure: Schematic of the strain stiffening system with beads (red) inside the PDMS channels (grey). In the un-deformed state (left) the beads do not touch the channel walls. When the system is deformed the beads touch the walls.

Impairment of the adhesion, growth and osteogenic differentiation of human osteoblast-like cells on nanofibrous polylactide scaffolds with diamond nanoparticles

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Carbon nanoparticles are widely studied for their potential supportive effects on the cell adhesion and proliferation. There are many investigations about the interaction of fullerenes, carbon nanotubes, carbon nanofibers, graphene and diamond nanoparticles with various types of cells, but the potential health risk of these nanostructures remains an open issue. Among carbon nanomaterials, nanodiamond seems to be the most promising for biomedical applications. We have investigated the biocompatible character of nanocrystalline diamond films for several years, and we have found that these films have very good biocompatibility with osteoblasts and endothelial cells.[1]
In the present study, we focused on the growth of human osteoblast-like Saos-2 and MG 63 cells on pristine polylactide (PLLA) nanofibers and nanofibers modified with various concentrations of diamond nanoparticles (DNPs; NanoAmando, Nanocarbon Research Institute Co., Ltd. Japan). DNPs were added in concentrations ranging from 0.02 to 0.7 g per 100 ml of the polymer solution. After evaporation of the solvent, the concentration of DNPs ranged from 0.44 to 12.28 wt.%.
We estimated the growth and metabolic activity of cells on the scaffolds, concentration of specific markers of cell adhesion (talin, vinculin), osteogenic cell differentiation (collagen I, alkaline phosphatase and osteopontin,) at the protein level, and also the expression of specific markers of cell adhesion, differentiation, cell cycle regulation and apoptosis at the mRNA level.
We found that the increasing concentration of DNPs in PLLA nanofibrous scaffolds has rather negative effects on the adhesion, growth and osteogenic differentiation of human osteoblasts-like Saos-2 and MG 63 cells. The activity of mitochondrial enzymes, measured by XTT assay in cells on day 3 after seeding, showed a decreasing tendency with increasing concentration of DNPs. Similar results were obtained from LIVE/DEAD staining of the cells, where the number of cells decreased with increasing concentration of DNPs. The gene expression of vinculin and talin in cells cultured on PLLA nanofibers with lower concentration of DNPs remains mainly unchanged in comparison with pristine PLLA. The expression of the osteogenic markers (osteopontin and ALP) but not of type I collagen was significantly lower in all DNP-loaded scaffolds seeded with Saos-2 cells. The osteopontin and ALP expression in MG 63 cells seeded on all tested materials was not detected. In addition, the mRNA expression of the following markers associated with the regulation of cell cycle and apoptosis was also evaluated: cyclin D, involved in regulating cell cycle progression; survivin,, an inhibitor of caspase activation; Bcl-2, anti-apoptotic protein and oncogene; and KLF6 (Krueppel-like factor 6), a transcription factor involved in growth-related signal transduction, cell proliferation and differentiation, development, apoptosis and angiogenesis, postulated as a tumor suppressor. The expression of cyclin D and survivin in cells fall down remarkably with increasing DNP concentration, while the expression of the anti-apoptotic protein Bcl-2 and (KLF6) rose significantly in cells on the scaffolds with lower DNP concentrations (Bcl-2: up to 0.87 wt.%, KLF6: up to 0.44 wt.%), and then decreased. The response obtained in MG 63 cells was weaker.

These results differs from the results obtained in our previous studies, employing DNPs prepared by radiofrequency PACVD method[2,3]. In our present study, the nanoparticles were prepared by a detonation method. The differences in the cell behavior were most probably caused by a different origin and properties of DNPs. The cytotoxicity of DNPs depends on their origin, size, impurities and functionalization. The detonation DNPs used in our present study were of relatively small size (4-5 nm), they were hydrogen-terminated, hydrophobic and of grayish color suggesting the presence of impurities.

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Mechanotransduction on the Single Cell Level: Investigating Mechanosensitive Genes using Single-Cell Force Spectroscopy combined with Quantitative PCR

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Mechanical forces play a key role in proliferation and differentiation of human cells such as mesenchymal stem cells, chondrocytes and osteoblasts. Especially, cells of heavily loaded tissues like cartilage or bone require mechanical stimulation, in order to fulfill their biological function. One reaction of chondrocytes in response to mechanical forces is the increased production of extracellular matrix (ECM) to ensure the compressive strength and structural integrity of the cartilage.[1, 2]

Tissue Engineering aims at repairing pathological tissue and providing functional cell based tissue substitutes. Due to the lack of self-renewal capacities of articular cartilage, cartilage is an attractive target for tissue engineering. So far, tissue engineered cartilage often does not reach the mechanical properties of natural cartilage. The cells produce a fibrous and rigid, instead of a flexible and hyaline matrix, or the cartilage matrix is too soft, to sustain physiological compressional loads. In order to obtain functional tissue engineered cartilage substitutes, it is therefore crucial to identify the forces required to stimulate cells to produce an optimal ECM, and understand the role of mechanical stimulation in cell proliferation and differentiation.[3, 4, 5]

In order to investigate the response of human mesenchymal stem cells and chondrocytes in response to mechanical stimuli, we plan to first stimulate cells using atomic force microscopy based single cell force spectroscopy, which allows for the precise control of the applied forces, and subsequently, analyze the expression levels of selected marker genes using quantitative PCR. In addition, immunohistochemical staining of ECM components, as well as the cultivation of stimulated cells to investigate the long term effects of the mechanical stimuli will be carried out.

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Cytocompatibility of Polycaprolactone Nano-Microfibrous Scaffolds Loaded with Amide-Amine Functionalised Carbon Nanoparticles

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In this study, commercially available activated carbon nanoparticles (CNPs) were functionalized with amide-amine groups varying in alkyl chain lengths and the number of amine groups. Four types of composite scaffolds were prepared via sputtering CNPs into electrospun polycaprolactone (PCL) micro-nanofibres: three of them with three types of functionalized CNPs and one with plain activated CNPs. Using this method, the surface of the particles remained almost entirely accessible. Plain PCL nanofibers and the composite micro-nanofibrous scaffold with plain activated CNPs were used as comparative samples. The structure of the materials was studied using scanning electron microscopy. The specific surface area of the particles and scaffolds was measured via nitrogen and krypton adsorption. Cytocompatibility of the materials was tested using 3T3 mouse fibroblasts. Cell viability and proliferation was measured by MTT assay. During fluorescence microscopy analyses, all scaffolds containing CNPs underwent thermal degradation when irradiated with green light, which could be further used for selective degradation. The scaffolds with functionalised CNPs showed better cytocompatibility than the scaffold with plain CNPs.

The PCL scaffold with plain CNPs was also tested for antibacterial activity using the following bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. However, no antibacterial effect was found.

Study of the photocatalytic activity of ZnO nanorods films, in the photodegradation of rhodamine B dye with solar irradiation

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The growing demand for technologies to decontaminate industrials wastewaters and comply with increasingly demanding standards are driving the development of research that use innovative processes to eliminate persistent organics pollutants than conventional techniques, such as photocatalysis that work with solid nanostructured semiconductors (1) (2) (3) (4) (15).
In this paper we study the photocatalytic activity of zinc oxide nanorods films, in the photodegradation of rhodamine B dye using a single process batch (5) (6) (7) (8). For this, we put on 5 mL of rhodamine B (2 mg/L) solution, exposing it, to different times of solar ultraviolet radiation. Zinc oxide nanorods films were fabricated by spray pyrolysis method from of zinc acetate (0.1M) solutions diluted in water and ethanol used as the precursor solution. The ethanol: water molar ratio (Γ) was varied in the range from 0 to 0.92 and brought to pH 5.5. These solutions were evaporated and deposited on glass substrate at 350 ºC (13).
In the substrate array zinc oxide particles, these are the seeds of which grew the nanorods in a solution of zinc nitrate and sodium hydroxide kept an oven at 90 °C for 1 hour (9) (10) (11) (12) (13) (14). Zinc oxide nanorods films were structurally characterized by X-Ray diffraction. The X-Ray diffraction patterns showing that the nanorods have a structure wurtzite hexagonal with growth preferential direction (002) up and down to substrate. The morphology was watching by high resolution scanning electron microscopy.
The morphology of the ZnO nanorods films obtained from seed with Γequal to 0 correspondents to film P1 is rough, with rods of hexagonally shape very agglomerates on substrate of diameters between 30 and 100 nm and the films thickness is about 3 μm. The morphology of films obtained from Γ equal to 0.31, P9 and 0.92, P10 showing also roughness and high crystallinity.
The films thickness is about 8 μm and the rod are hexagonal shape diameters are between 50 nm and 1 μm, regularly aligned in form vertically on substrate. The optical properties were measures by UV-visible spectroscopy. The values of the energy band gap measured are lower in 0.26 eV, the theoretical value of natural (3.37 eV). We use these films in the photodegradation of dye rhodamine B. the best Photocatalytic activity was found for the a reaction time of 3 hours with 92% average efficiency for films obtained from molar ratios of ethanol:water the 0.92, 0.31, 0.18 and 0.12. These results indicated that there is a correlation direct of the partial molar volume of ethanol with respect to water used in the precursor solution; but also, we observed a positive influence of the surface area, reactive sites and crystalline growth of the nanorods on the photodegradation process.

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Induction of DNA Damage in HepG2 and A549 Cells Treated with Quantum Dots, Silver and Titanium Dioxide Nanoparticles or Their Binary Mixtures

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Objectives: Published toxicity study focuses mostly on single nanoparticle (NP) exposure. However, from the point of view of environmental toxicity this exposure scenario is unrealistic. Thus the aim of this study was to evaluate genotoxicity of binary nanoparticle mixtures as compared with single nanoparticle toxicity.

Methods: Two human cell lines, HepG2 and A549, were exposed for 2 or 24 h to 6 nm CdSe QD, 20 nm AgNPs, 21 nm TiO2NPsor their mixture. Genotoxicity was examined by the comet assay +/- FPG.

Results: QD and AgNPs induced marked damage in all cell lines tested, whereas TiO2NPs induced remarkably lower amount of DNA damage. Each cell line studied had a different pattern of DNA breakage and base damage versus NPs concentration and time of treatment. Themost cases, treatment with NPs mixture revealed simple additive effect. However in some cases a sparing effect was observed. The DNA damage observed in A549 cells after both treatment times did not differ markedly, In contrast, in HepG2 cells longer treatment with AgNPs resulted in decrease of DNA damage. Interestingly, TiO2NPs induced more damage in A549 cells than in HepG2.

Conclusions: Obtained results indicate that the main cause of QD, AgNPs and TiO2NPs toxicity is induction of oxidative DNA damage, and that toxicity of nanomaterials is dependent on cellular context. The effect observed in binary mixtures confirmed similar mode of action.

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