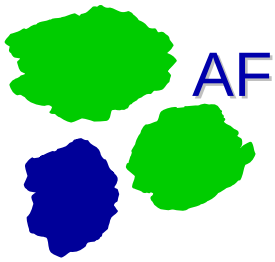


AFM **BioMed Conference**
Red Island Croatia 2010

Third International Meeting on AFM in Life Sciences and Medicine
Red Island, Croatia, May 12-15, 2010

Program

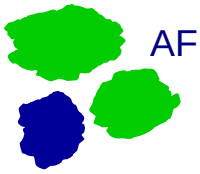
&

Book of Abstracts

AFM BioMed Conference
Red Island Croatia 2010

Third International Meeting on AFM in Life Sciences and Medicine
Red Island, Croatia May 12-15, 2010

Published by



AFM **BioMed Conferences**

http://www.afmbiomed.org

For Publisher
Vesna Svetličić

Editor
Pierre Parot / Jean-Luc Pellequer

Circulation
150 Copies

Printing
Denona, Zagreb

Organizing Committee

Vesna Svetličić
Ruđer Bošković Institute Zagreb, Croatia

Pierre Parot
CEA/DSV, Marcoule, France

Jean-Luc Pellequer
CEA/DSV, Marcoule, France

Simon Scheuring
Institut Curie, Paris, France

Local Organizing Committee

Renato Batel	Ruđer Bošković Institute Rovinj, Croatia
Nenad Smodlaka	Ruđer Bošković Institute Rovinj, Croatia
Amela Hozić Zimmermann	Ruđer Bošković Institute Zagreb, Croatia
Maja Dutour Sikirić	Ruđer Bošković Institute Zagreb, Croatia
Tea Mišić Radić	Ruđer Bošković Institute Zagreb, Croatia
Galja Pletikapić	Ruđer Bošković Institute Zagreb, Croatia
Vera Žutić	Ruđer Bošković Institute Zagreb, Croatia
Zoran Mandić	University of Zagreb, Croatia
Vesna Svetličić	Ruđer Bošković Institute Zagreb, Croatia

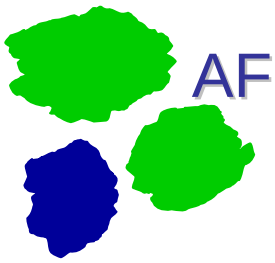
.

Scientific Committee

Paolo Facci	S3-INFM-CNR, Modena, Italy
Sanjay Kumar	UC Berkeley, USA
Gil Lee	University of Dublin, Ireland
Pierre-Emmanuel Milhiet	CNRS, Montpellier, France
Daniel Navajas	Universitat Barcelona, Spain
Hans Oberleithner	University of Münster, Germany
Pierre Parot	CEA, Marcoule, France
Jean-Luc Pellequer	CEA, Marcoule, France
Simon Scheuring	Institut Curie Paris, France
Chanmin Su	Veeco Santa Barbara, USA
Vesna Svetličić	Ruđer Bošković Institute, Zagreb, Croatia

Main sponsors of the Conference

Direction des Sciences du Vivant



AFM BioMed Conference
Red Island Croatia 2010

Third International Meeting on AFM in Life Sciences and Medicine
Red Island, Croatia, May 12-15, 2010

Program

Training Day and Courses

Tuesday, May 11th

Special lectures

Daniel NAVAJAS: Mechanical properties of living cells
Pierre-Emmanuel MILHIET: Membrane reconstituted Systems

Conference Schedule

Wednesday, May 12th
Thursday, May 13th
Friday, May 14th
Saturday, May 15th

Keynote Lecture: 45 minutes
Plenary and Invited Lectures: (25+5) minutes
Selected Lectures: (15+5) minutes
Selected Short Oral Presentations: (8+2) minutes

Tuesday, May 11th

Training Day and Courses **Special lectures** **Daniel NAVAJAS: Mechanical properties of living cells** **Pierre-Emmanuel MILHIET: Membrane reconstituted Systems**
From 16:00 **Attendees arrive** **Registration and Rooming**
20:00 **Welcome Party**

Wednesday, May 12th

8:15	**Opening Ceremony** **Danica RAMLJAK** Ruđer Bošković Institute, Zagreb, Croatia

	Session I: Interaction & Recognition **Gil LEE** University of Dublin, Ireland
8:25	*Chair's introduction*
8:30	***Invited Lecture*** **Fishing single molecules on live cells** **Yves DUFRÊNE,** Université de Louvain-la-Neuve, Belgium
9:00	**A fiber force probe for soft materials in fluids** **Babak SANII,** *Lawrence Berkeley National Lab, Berkeley CA, USA*
9:20	**AFM Force spectroscopy reveals the complex dynamics of single protein molecules** **Jasna BRUJIC,** *New York University NY, USA*
9:40	**Mapping single interactions between DC-SIGN and *Candida albicans*-associated molecular patterns by AFM force spectroscopy** **Joost TE RIET,** *Radboud University Nijmegen, The Netherlands*
10:00	Break (30 minutes)
10:30	**Tailored 2D and 3D cell culture substrates for AFM-based single-cell force spectroscopy** **Clemens FRANZ,** *Karlsruhe Institute of Technology, Germany*
10:50	**AFM and living cells: from immobilization to single molecule force spectroscopy** **Etienne DAGUE,** *Université de Toulouse, France*
11:10	**Shear force microscopy with active and passive micromachined AFM probes** **Artūras ULČINAS,** *University of Technology, Kaunas, Lithuania*
11:30	***Plenary Lecture*** **Topography and Nanomechanics of Live Neuronal Growth Cones Analyzed by Atomic Force Microscopy** **Gil LEE,** *University of Dublin, Ireland*

12:10	**Lunch**
13:30	**Poster session A**

Wednesday, May 12th

Session II: Nanoecology & Nanotoxicology **Vesna SVETLIČIĊ** Ruđer Bošković Institute, Zagreb, Croatia	
14:55	*Chair's introduction*
15:00	***Invited Lecture*** **Natural and manufactured nanoparticles in the environment** **Jamie LEAD**, *University of Birmingham, UK*
15:30	**Heterotrophic bacteria and Synechococcus interactions with organic matter continuum: an AFM study of microscale microbial ecology** **Francesca MALFATTI,** *Scripps Institution of Oceanography, La Jolla CA, USA*
15:50	**The size related toxicity of cerium oxide nanoparticles** **Ruth MERRIFIELD,** *University of Birmingham, UK*
16:10	**Seawater at nanoscale: Organic assemblies imaged by AFM** **Tea MIŠIĆ RADIĆ,** *Ruđer Bošković Institute, Zagreb, Croatia*
16:30	Break (30 minutes)
17:00	**Dynamic of proteo-nucleic complexes** **Eric LESNIEWSKA,** *University of Bourgogne, Dijon, France*
17:20	***Plenary Lecture*** **Marine ecology at nanoscale** **Vesna SVETLIČIĆ,** *Ruđer Bošković Institute, Zagreb, Croatia*
17:50	Break (25 minutes)

Wednesday, May 12th

Selected short oral presentations of sessions I, II, III **Simon Scheuring** Institut Curie, France	
18:15	**AFM investigation of self assembling amphiphilic peptide** **Michele ALDERIGHI,** *University of Pisa, Italy*
18:25	**External conditions influencing the elasticity of the cerebral endothelial cells** **György VÁRÓ,** *Biological Research Center of the HAS, Szeged, Hungary*
18:35	**Atomic force microscopy-based molecular recognition of fibrinogen receptor in human erythrocytes** **Filomena CARVALHO,** *Universidade de Lisboa, Lisbon, Portugal*
18:45	**AFM imaging of extracellular polymer release by marine diatom** **Galja PLETIKAPIĆ,** *Ruđer Bošković Institute, Zagreb, Croatia*
18:55	Break (15 minutes)
19:10	**Screening estrogenic compounds using force spectroscopy** **Céline ELIE-CAILLE,** *Institut Femto-st, Besançon, France*
19:20	**Bimodal atomic force microscopy imaging of proteins** **José LOZANO,** *Instituto de Microelectrónica de Madrid, Spain*
19:30	**Analysis of the Bacterial Cell Employing a Novel Lysis System and Atomic Force Microscopy** **Douglas DENNIS,** *Morehead State University KY, USA*
19:40	**Changes in the Mechanical Properties of *Bacillus anthracis* by Germinants and Antimicrobial Peptide Chrysophsin-3** **Paola PINZÓN-ARANGO,** *Worcester Polytechnic Institute, MA, USA*
19:50	**Internal motion of proteins under force leads to nonexponental unfolding kinetics** **Eric VANDEN-EIJNDEN** *New York University, New York, NY, USA*

20:30	**Dinner**

Thursday, May 13th

Session III: AFM Bio I **Pierre-Emmanuel MILHIET** CNRS, Montpellier, France	
8:25	*Chair's introduction*
8:30	***Invited Lecture*** **Quantifying domain patterns and domain textures in lipid bilayers** **Adam COHEN SIMONSEN,** *University of Southern Denmark, Odense, Denmark*
9:00	**Annular oligomers in the transthyretin amyloid fibrillogenetic pathway captured with AFM** **Ricardo PIRES,** *Semmelweis University,Budapest, Hungary*
9:20	**AFM nano-guided bacterium adhesion to the host cell for biophotnic-based measurements of the dynamics of cytoskeleton and signaling molecules upon infection** **Yann CICZORA,** *Institut Pasteur de Lille, France*
9:40	**Direct correlation of structures and nanomechanical properties of multicomponent lipid bilayers** **Shan ZOU,** *Steacie Institute for Molecular Sciences, Ottawa, Canada*
10:00	Break (30 minutes)
10:30	***Plenary Lecture*** **Nanomechanics of living cells: applications in respiratory medicine** **Daniel NAVAJAS,** *Universitat Barcelona, Spain*
10:50	**Nanoscale investigation of the interaction between ionic detergents and supported lipid membranes** **Karim EL KIRAT,** *Université de Technologie de Compiègne, France*
11:10	**Atomic force microscopy as a valuable tool to study amyloid aggregation and aggregate-membrane interaction** **Annalisa RELINI,** *University of Genoa, Italy*
11:30	***Plenary Lecture*** **AFM Imaging of Purified Transmembrane Proteins Reconstituted into Artificial Membranes** **Pierre-Emmanuel MILHIET,** *CNRS, Montpellier, France*

12:10	**Lunch**
13:30	**Poster Session B**

Thursday, May 13th

Session IV: Trends in Theory & Technologies **Chanmin SU** Veeco Santa Barbara, USA	
14:55	*Chair's introduction*
15:00	***Invited Lecture*** **Direct Visualization of Dynamic Processes on Biomolecules with High-Speed AFM** **Takayuki UCHIHASHI,** *Kanazawa University, Japan*
15:30	**First FluidFM applications in biology: from single-virus dispensing to cell-organellae transplantation** **Tomaso ZAMBELLI,** *ETH Zurich, Switzerland*
15:50	**Precision force spectroscopy: A new window on the dynamics of unfolding and refolding membrane proteins** **Thomas PERKINS,** *University of Colorado, Boulder CO, USA*
16:10	**Principles for using high-resolution topographical surfaces obtained in AFM for assembling molecular complexes: test case of the Tobacco mosaic virus (TMV)** **Minh Hieu TRINH,** *CEA Marcoule, Bagnols sur Cèze, France*
16:30	Break (30 minutes)
17:00	**Near-field IR microspectroscopy as a tool to discriminate nano-molecular alterations at cell or near-cell level in biomedical research** **Hubert POLLOCK,** *Lancaster University, UK*
17:20	**Quantifying the dielectric constant of biomembranes and lipids bilayers with atomic force microscopy on insulating substrates** **Laura FUMAGALLI,** *Institute for BioEngineering of Catalonia, Barcelona, Spain*
17:40	**Expanding the temporal and spatial scales in scanning force microscopy** **Miklós KELLERMAYER,** *Semmelweis University, Budapest, Hungary*
18:00	**Using microcantilevers to count biological bonds** **Todd SULCHEK,** *Georgia Institute of Technology, Atlanta GA, USA*
18:20	Break (15 minutes)

Thursday, May 13th

Selected short oral presentations of sessions IV, V, VI **Jean-Luc PELLEQUER** CEA Marcoule, France	
18:35	**AFM in drug discovery and development – Application opportunities and technology needs** **Johannes KINDT,** *Veeco GmbH, Mannheim, Germany*
18:45	**How to make a fresh start with carbon nanotube probes?** **Sophie MARSAUDON** *Université Bordeaux 1, France*
18:55	**Insulated nanoneedle probes for combined atomic force and scanning electrochemical microscopy (AFM-SECM)** **Mehdi YAZDANPANAH,** *University of Louisville KY, USA*
19:05	**Detection of populations of amyloid-like protofibrils with different physical properties** **Ranieri ROLANDI,** *University of Genoa, Italy*
19:15	Break (15 minutes)
19:30	**Characterization of self-oscillating Soft Imaging by means of photothermal excitation** **Massimo VASSALLI,** *Institute of Biophysics, Genova, Italy*
19:40	**Functionnalized Si(111) surfaces with tunable surface chemistry: New atomically flat platforms for the anchoring of bio-molecules for AFM characterization purposes** **Catherine HENRI de VILLENEUVE,** *Ecole Polytechnique, France*

20:30	**Dinner**

Friday, May 14th

Session V: AFM Bio II **Paolo FACCI** S3-INFM-CNR, Modena, Italy	
8:25	*Chair's introduction*
8:30	***Invited Lecture*** **Quantifying the dynamics of nucleosomes with AFM** **John van NOORT,** *Leiden University, The Netherlands*
9:00	**A combined imaging and force spectroscopy approach reveals the mechanical structure of viral nano-particles** **Wouter ROOS,** *Vrije Universiteit, Amsterdam, the Netherlands*
9:20	**Imaging bacterial cell death induced by antimicrobial peptides in real time using high speed AFM** **Georg FANTNER,** *Massachusetts Institute of Technology, Cambridge MA, USA*
9:40	**Measurement of DNA morphological parameters at highly entangled regime on surfaces** **Annalisa CALÒ,** *Istituto per lo Studio dei Materiali Nanostrutturati, Bologna, Italy*
10:00	Break (30 minutes)
10:30	**An integrated AFM–polarized Raman micro-spectroscopy instrument for investigations of biological nanomaterials** **Banyat LEKPRASERT,** *The University of Nottingham, UK*
10:50	**Imaging the nanoscale organization of peptidoglycan in living *Lactococcus lactis* cells** **Guillaume ANDRE,** *Université Catholique de Louvain, Belgium*
11:30	***Plenary Lecture*** **Unravelling lipid-protein interaction in model bilayers by AFM** **Paolo FACCI,** *S3-INFM-CNR, Modena, Italy*

12:10	**Lunch**

Friday, May 14th

14:00 **18:00**	**Large Poster Session C** **NETWORKING & SOCIAL EVENTS**

19:00 **20:00**	**Best Short presentations and Posters Awards** **&** **Keynote Lecture** **AFM as an essential tool in cell and molecular biology studies** **Dennis DISCHER** *University of Pennsylvania, Philadelphia PA, USA*

21:00 **23:00**	**Conference Gala dinner** Announcement and invitation to the next AFM BioMed Conference

Saturday, May 15th

Session VI: Nanomedicine **Hans OBERLEITHNER** University of Münster, Germany	
8:25	*Chair's introduction*
8:30	***Invited Lecture*** **Mechanics of Cancer versus Normal Cells: Fundamentals and Possible Applications** **Igor SOKOLOV,** *Clarkson University, Potsdam NY, USA*
9:00	**Investigating Angiotensin II AT-1 receptor bias signaling with AFM** **Elie SIMARD,** *Université de Sherbrooke, Canada*
9:20	**Combining atomic force microscopy with micro-electrode arrays for studying the mechano-electrical behavior of cardiac myocytes** **José SAENZ,** *University of Genova, Italy*
9:40	**Substrate elasticity dominates integrin-ligand availability during cellular adhesion and spreading** **Ilia LOUBAN,** *Max-Planck-Institute f. Metals Research, Stuttgart, Germany*
10:00	Break (30 minutes)
10:30	**Parallel AFM imaging and force spectroscopy using 2-dimensional probe arrays for applications in cell biology** **André MEISTER,** *Centre Suisse d'Electronique et de Microtechnique, Neuchâtel, Switzerland*
10:50	**Monitoring of mechanical properties of serially passaged bovine articular chondrocytes by atomic force microscopy** **Michal WOZNIAK,** *Faculty of Materials Science and Engineering, Warsaw, Poland*
11:10	**AFM Based Force Spectroscopy:** **A New Tool to Probe *Ex Vivo* Vascular Endothelium Rigidity** **Yannick MIRON,** *Université de Sherbrooke, Canada*
11:30	***Plenary Lecture*** **Nanophysiology of the vascular endothelium** **Hans OBERLEITHNER,** *University of Münster, Germany*

12:10	**Lunch**
13:30	**Poster Session D**

Saturday, May 15th

Session VII: **Opening Round table about future of AFM in Nanomedicine** **Frank LAFONT** Institut Pasteur de Lille, France	
14:25	*Chair's introduction*
14:30	***Plenary Lecture*** **Mechanobiological regulation of tumor and stem cell biology in the central nervous system** **Sanjay KUMAR,** *University of California, Berkeley CA, USA*
15:00	**Identification of cancerous cells using AFM** **Malgorzata LEKKA,** *The Henryk Niewodniczański Inst. of Nuclear Physics, Poland*
15:30	Break (30 minutes)
16:00	**AFM in health and diseases: identification of technological gaps toward new applications for specific needs?** **Frank LAFONT,** *Institut Pasteur de Lille, France*
16:20	**European Cooperation in AFM for Nanomedicine** **Jean-Luc PELLEQUER,** *CEA Marcoule, France*
16:40	***End Meeting Plenary lecture*** **Past, Present and Future of AFM in NanoMedicine and Life Sciences** **Simon SCHEURING,** *Institut Curie, France*
	Conference ends

SESSION I

Interaction & Recognition

Chair: Gill LEE
University of Dublin, Ireland

Invited Lecture

Fishing single molecules on live cells

Yves F. Dufrêne

Unité de Chimie des Interfaces, Université Catholique de Louvain, Croix du Sud 2/18, B-1348 Louvain-la-Neuve, Belgium

Although much is known about the structure and biosynthesis of microbial cell envelope constituents, the three-dimensional organization, assembly, and interactions of the individual components remain poorly understood. What is the spatial arrangement of cell surface receptors, clustered or homogeneous? What are the adhesive and mechanical properties of cell surface proteins and polysaccharides, and how are they related to function? Traditionally, these questions have been difficult - or impossible - to address owing to the lack of high-resolution single-cell and single-molecule probing techniques. With its ability to observe and force probe single live cells to molecular resolution, atomic force microscopy (AFM) techniques offer new opportunities in molecular microbiology [1,2]. While high-resolution imaging is a powerful tool for visualizing the architecture of cells in buffer solution, force spectroscopy offers a means to analyze the localization, interactions and elasticity of their individual constituents. In this talk, I will discuss recent progress we have made in the area.

[1] Y.F. Dufrêne, Nat. Rev. Microbiol., 6 (2008), 674-680.
[2] D.J. Müller, J. Helenius, D. Alsteens, Y.F. Dufrêne, Nat. Chem. Biol., 5 (2009), 383-390.

A fiber force probe for soft materials in fluids

Babak Sanii and Paul D. Ashby*

Molecular Foundry, Lawrence Berkeley National Lab, Berkeley, CA 94618 USA
bsanii@lbl.gov; pdashby@lbl.gov

Performing AFM on soft materials in fluids (e.g., living cells) is challenging due to their ready deformation by the tip. The thermal force-noise of the cantilever is the principal limitation to reducing sample deformation, and minimizing a cantilever's cross-section drastically reduces its noise. However, the minimum size of a usable cantilever is currently limited by the conventional deflection detection scheme, which requires a large surface area for laser specular reflection. We introduce a non-interferometric optical approach to determine the position of nanowires with high sensitivity and bandwidth. Its physical origins and limitations are determined by Mie scattering analysis. This enables a dramatic miniaturization of detectable cantilevers, with attendant reductions to the fundamental minimum force noise in highly damping environments. We measure the force-noise of a 81±9nm radius Ag2Ga nanowire-cantilever in water at 6±3fN/ Hz.

Reference:
[1] B. Sanii and P.D. Ashby, High sensitivity deflection detection of nanowires, Phys. Rev. Lett. (2010).

AFM Force spectroscopy reveals the complex dynamics of single protein molecules

Herbert Lannon, Ted Mariallis, Maxime Clusel, **Jasna Brujic**

New York University, 4 Washington Place, 10003 New York, USA

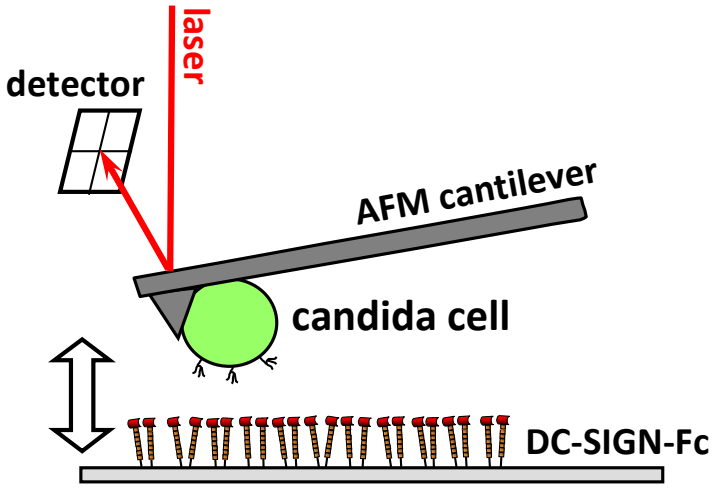
Ensemble studies of protein unfolding are typically modeled as two state reactions with a well-defined rate constant. Here we examine whether this view also holds at the single molecule level. We have developed force-clamp spectroscopy to follow the end-to-end length of single small proteins during their folding reaction. We first measure the kinetics of unfolding of the protein ubiquitin under a constant force and discover a surprisingly broad distribution of unfolding rates that follows a power law with no characteristic mean. The structural fluctuations that give rise to this distribution reveal the architecture of the protein's energy landscape. Following models of glassy dynamics, this complex kinetics implies large fluctuations in the energies of the folded protein, characterized by an exponential distribution with a width of 5-10 kbT. Our results predict the existence of a ``glass transition" force below which the folded conformations interconvert between local minima on multiple time-scales. Both the unfolding as well as the folding pathways captured by force-clamp spectroscopy are much more complex than the two state model that is commonly used to interpret such data in classical protein biochemistry. Our results point to the necessity of using statistical mechanics to fully describe the folding of proteins under a stretching force.

Mapping single interactions between DC-SIGN and *Candida albicans*-associated molecular patterns by AFM force spectroscopy

Joost te Riet[1,2], Inge Reinieren-Beeren[1], Sylvia Speller[2], Alessandra Cambi[1] and Carl G. Figdor[1]

[1]*Department of Tumor Immunology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500HB Nijmegen, The Netherlands*
[2]*Department of Scanning Probe Microscopy, Institute for Molecules and Materials, Radboud University Nijmegen, P.O. Box 9010, 6500GL Nijmegen, The Netherlands*

The fungus *Candida albicans* is the most common cause of mycotic infections in immunocompromised hosts. Little is known about the initial interactions between *Candida* and immune cell receptors, such as DC-SIGN, because detailed characterization at the structural level is lacking. Understanding these processes will ultimately provide relevant information to develop novel treatments. The C-type lectin DC-SIGN expressed on dendritic cells (DCs) recognizes specific *Candida*-associated molecular patterns. DC-SIGN binds to *Candida* via *N*-linked mannan present in the cell wall of *Candida*. However, the exact binding epitope is not yet determined. Furthermore, the regulation of binding by DC-SIGN oligomerization is still unknown. Here, we exploit atomic force microscope force spectroscopy (AFM-FS) to gain better insight in the carbohydrate recognition profile of DC-SIGN. We demonstrate that slight differences in the *N*-mannan structure of *Candida* glycosylation mutants can be detected by AFM-FS, giving insight in the single bond affinity of the DC-SIGN-*Candida* interaction. Furthermore, biophysical Bell parameters determined for different carbohydrate-protein interactions exhibit a similar dynamical response to forces (*i.e.* dynamical affinity) for this type of interactions. More detailed knowledge on the binding pocket of DC-SIGN-*Candida* will definitely lead to a better understanding of the carbohydrate recognition of C-type lectins.

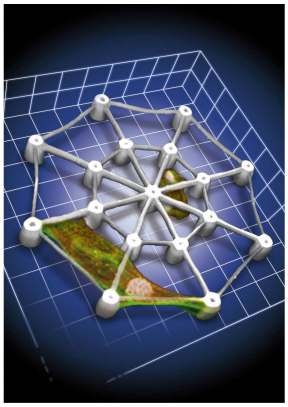


Tailored 2D and 3D cell culture substrates for AFM-based single-cell force spectroscopy

Clemens M. Franz

Young Scientist Group Nanobiology DFG-Center for Functional Nanostructures
Karlsruhe Institute of Technology Wolfgang-Gaede-Str 1a
76131 Karlsruhe Germany

AFM-based single-cell force spectroscopy (SCFS) is a sensitive method to measure cellular adhesion forces across dimensions from the single-molecule level to that of the entire cell. In SCFS experiments rigid surfaces homogeneously coated with components of the extracellular matrix (ECM) are commonly used as adhesive substrates. However, in tissues cells are often surrounded by a well-structured and flexible scaffold of ECM macromolecules. Analyzing the adhesive interactions between cells and structured microenvironments is therefore important for understanding how cells perform their tissue-specific function. Microstructured ECM substrates may mimic the cellular environment more closely and ensure that results from adhesion experiments can be applied for understanding physiological interactions between cells and ECM. We have developed different 2D surface patterning methods and analyzed the effect of pattern configuration on cell adhesion formation using combined fluorescence microscopy and AFM. Furthermore, we have used hetero-functional ECM micropatterns to directly compare the affinity of specific adhesion receptors to different ECM proteins or to the same ECM protein offered in different topographies. To furthermore test the effect of substrate 3-dimensionality and flexibility on cellular forces, we have produced elastic 3D cell culture scaffolds by direct laser writing into a bio-compatible photoresist. These 3D scaffolds contain flexible beam elements of submicron thickness which can be rhythmically deformed by single beating cardiomyocytes. To obtain a quantitative measure of the involved cellular contraction forces, the cell culture substrates can be calibrated using the AFM cantilever as a micro-indenter. In conclusion, SCFS performed on micropatterned, multi-functional 2D and 3D adhesion matrices provides novel insights into mechanisms of receptor-mediated ECM adhesion.



References

1. F.Klein, T. Striebel, J. Fischer, Z. Jiang, C.M. Franz, G. von Freymann, M. Wegener and M. Bastmeyer**.** *Elastic Fully Three-dimensional Microstructure Scaffolds for Cell Force Measurements.* Adv. Mat. **22**, 1-4 (2010)
2. C.M. Franz and P.H. Puech. *Atomic force microscopy – a versatile tool for studying cell morphology, adhesion and mechanics.* Cell. Mol. Bioeng. **1**, 289-300 (2008)
3. C.M. Franz, A. Taubenberger, P.H. Puech, and D.J. Muller. *Studying Integrin-Mediated Cell Adhesion at the Single-Molecule Level Using AFM Force Spectroscopy.* Sci. STKE 4**06** (2007)
4. J. Friedrichs, A. Taubenberger, C.M. Franz, and D.J. Muller. *Cellular Remodelling of Individual Collagen Fibrils Visualized by Time-Lapse AFM.* J. Mol. Biol. **372,** 594-607 (2007)

AFM and living cells: from immobilization to single molecule force spectroscopy

Etienne Dague

CNRS ; LAAS ; 7 avenue du colonel Roche, F-31077 Toulouse, France
Université de Toulouse ; UPS, INSA, INP, ISAE ; LAAS, F-31077 Toulouse, France
* edague@laas.fr

Exploring living cells at the nanoscale is now possible thanks to Atomic Force Microscopy conducted in liquid. Our researchs on different micro-organisms (*Lactococcus lactis*, *Escherichia coli*) but also on eukaryotic cells (*Saccharomyces cerevisiae* or human fiboblasts) aims at measuring parameters such as cell wall elasticity and to correlate the result with a function (adhesion, viability, mobility). For example we've demonstrated that *E. coli* cell wall becomes stiffer when it's damaged by a thermal treatment[1] (figure 1).

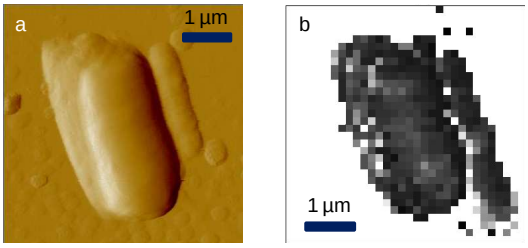


Figure 1: *E. coli* as seen by AFM. On the left (a), the deflection image recorded in contact mode, in PBS. On the righ (b) the elasticity map of the cell. The brighter the pixel, the higher the elastic modulus.

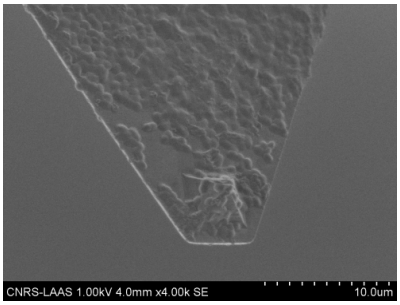


Figure 2: Lactoprobe

With *Lactococcus lactis* with have designed a cell probe (figure 2) and measured the interactions between a single bacteria and mucin, the main glycoprotein of the intestinal mucus[2]. Moreover a dynamic study tends to demonstrate that we are probing single molecule interactions as the force is a function of the loading rate. This approach has been used to compare different strains known for their different ability to macroscopically adhere to glass.

Finally, I will present a new biofunctionalization strategy for AFM tips that we have developed on the couple GST-antiGST. Figure 3A shows the result of single molecule interactions between the tip functionalized with the antibody directed against GST and a surface covered by GST. Figure 3B shows the result of the same experiment when free antiGST antibodies are added in the solution.

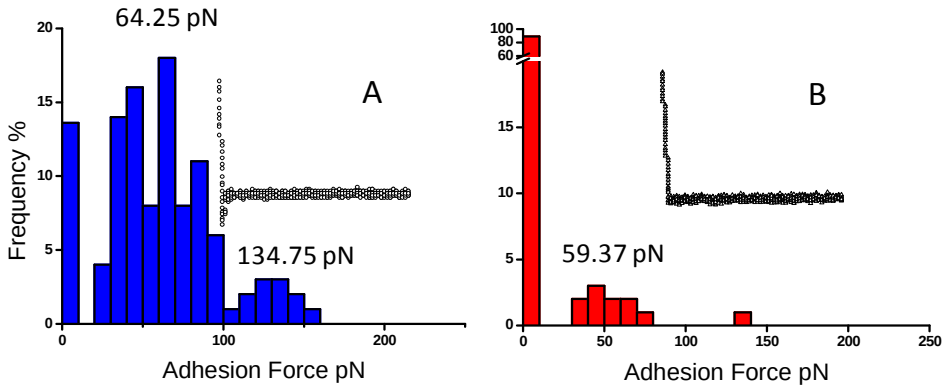


Figure 3: Single molecule force spectroscopy between GST on a surface and antibody antiGST on the AFM tip.
A: the histogram repartition of the interactions forces between GST and anti-GST and a typical force curve.
B: the force repartition when the GST surface is saturated with free antibody and a typical force curve

1. Cerf, A.; Cau, J.-C.; Vieu, C.; Dague, E., Nanomechanical properties of dead or alive single-patterned bacteria. *Langmuir* **2009,** 25, (10), 5731-5736.
2. Dague, E.; Le, D. T. L.; Zanna, S.; Loubière, P.; Marcus, P.; Mercier-Bonin, M., Probing in vitro interactions between *Lactococcus lactis* and mucins using AFM. *Langmuir* **Submitted**.

Shear force microscopy with active and passive micromachined AFM probes

Artūras Ulčinas, Giovanni Valdre[*] and Massimo Antognozzi[**]

Research Centre Microsystems and Nanotechnologies, Kaunas University of Technology, Kaunas, Lithuania
[*] *Laboratory of Biomaterials and Applied Crystallography, Department of Earth and Geo-Environmental Sciences, University of Bologna, Bologna, Italy*
[**] *H. H. Wills Physics Laboratory, University of Bristol, Bristol, UK*

Shear force microscopy (ShFM) is an important force microscopy technique, offering true non-contact operation and ability to investigate the interfacial phenomena such as lubrication and friction on the nanoscale with unparalleled sensitivity. Applications of this technique to study biological systems were limited, mostly due to the lack of reliable detection system to sense the force on the probe in liquid, and difficulty with using micromachined probes with carefully controlled properties in vertical orientation. Recently developed innovative ShFM based on scattered evanescent wave detection [1] enables to directly monitor the displacement of the probe tip in air and liquid, thus opening the way to use variety of commercial or custom nanomechanical force probes. Here, we report applications of this technique for surface imaging and investigation of viscoelastic properties of confined fluids using mechanically driven (“active”) and thermal noise actuated (“passive”) micromachined probes, and discuss its suitability for high resolution non-contact imaging and shear force spectroscopy of biomolecules and hydration shells around them.

[1] M M. Antognozzi, A. Ulcinas, L. Picco, S.H. Simpson, P.J. Heard, M.D. Szczelkun, B. Brenner, M.J. Miles, A new detection system for extremely small vertically mounted cantilevers. Nanotechnology 19 (2008) 384002.

SESSION I

Plenary Lecture

Topography and Nanomechanics of Live Neuronal Growth Cones Analyzed by Atomic Force Microscopy

Ying Xiong[1], Emily Gryzwa, [1] Aih Cheun Lee[2], Daniel M. Suter[2], and **Gil U Lee**[1,3]

[1] *School of Chemical and Biomedical Engineering, Purdue University, West Lafayette, Indiana, USA*
[2] *Department of Biology, Purdue University, West Lafayette, Indiana, USA*
[3] *School of Chemistry and Chemical Biology, University College, Dublin, Ireland*

Neuronal growth cones are motile, hand-like structures located at the end of axons that read extracellular guidance information and translate it into directional movement. Despite of their important role in the development and regeneration of the nervous system, relatively little quantitative information is available regarding their three-dimensional morphology or mechanical properties. Aplysia bag cell neuron growth cones are a model system for the study of cytoskeletal dynamics and biomechanics due to their large size and distinct cytoplasmic regions. In this presentation we will describe the used the atomic force microscope (AFM) to obtain high-resolution images of the peripheral (P) domain, transition (T) zone, and central (C) domain of live *Aplysia* growth cones. The average height of these regions was calculated from contact mode AFM images to be 183 ± 33, 690 ± 274, and 1322 ± 164 nm, respectively, which was consistent with data derived from dynamic mode images of live and contact mode images of fixed growth cones. Nanoindentation measurements revealed that the elastic moduli of the P-domain and T-zone ruffling region are significantly higher than those measured in the C domain. High-resolution images of the P-domain suggest that the higher elastic modulus results from a dense meshwork of actin filaments in lamellipodia and actin bundles in filopodia. The nanomechanical properties of the *Aplysia* growth cone have been interpreted with the cytoskeletal organization of the subcellular regions and related to their proposed function in growth cone motility.

SESSION II

Nanoecology & Nanotoxicology

Chair: Vesna SVETLIČIĊ
Ruđer Bošković Institute, Zagreb, Croatia

Invited Lecture

Natural and manufactured nanoparticles in the environment

Jamie Lead

GEES, University of Birmingham
Birmingham, B15 2TT, UK. E: j.r.lead@bham.ac.uk

Nanoparticles (NPs) are defined as between 1 and 100 nm in size and may be produced naturally by microbial processes, weathering and chemical hydrolysis, as an incidental by-product of combustion and other industry and deliberately by human activity for specific purposes. This talk will discuss the uses of AFM, within a multi-method approach, to understanding the properties and characteristics of both natural and manufactured NPs. The formation of nanoscale films of natural NPs, influencing fate, behaviour and ecotoxicology of pollutants (including manufactured NPs) will be described. Further discussion of AFM, within a multi-method approach, will be given for the physico-chemical characterisation of manufactured NPs, including accuracy and underpinnings of this approach to nanotoxicology.

Heterotrophic bacteria and *Synechococcus* interactions with organic matter continuum: an AFM study of microscale microbial ecology.

Francesca Malfatti and Farooq Azam

Scripps Institution of Oceanography, University California San Diego, La Jolla, USA

Marine bacteria are important players in biogeochemical cycles in the ocean. They intimately interact with the size continuum of organic matter, that includes truly dissolved molecules, colloids up to organisms. Using atomic force microscopy (AFM) we discovered that a substantial, and variable, fraction (on average 30% ±17.8 with a range 0% to 55%) of "free-living" bacteria in our samples from California coastal and open ocean environments were intimately associated with other bacteria at nm-µm scale. Twenty-one percent to 43% bacteria, including *Synechococcus* (a major primary producer), were conjoint. Moreover, a substantial fraction (4-55%) of bacteria was connected by pili and gels into cell-cell pairs or occurred in networks of up to 20 cells. We followed the dynamic of heterotrophic bacteria interacting with organic matter and conjoint heterotrophic bacteria-*Synechococcus* during microcosm bloom experiments. Using AFM we imaged the colloidal fraction of the organic matter as well as measured viscosity changes during the phytoplankton bloom. This latter measurement relies on the dampening of the resonance frequency of the cantilever because of increase in viscosity. We will discuss our results in the context of how marine bacteria interact with organic matter and with other bacteria and the biogeochemical consequences of these interactions for ocean carbon cycle.

The size related toxicity of cerium oxide nanoparticles.

R.C. Merrifield, P. Cole, J.R. Lead

University of Birmingham, Birmingham B15 2TT, UK

Cerium oxide nanoparticles have been shown to increase the efficiency of diesel engines, therefore these particles could quite easily enter the environment either into the air as particulates or into the water systems. We show that commercially available powders have a large size distribution when dispersed in algae media and have a range in size from 3 to hundreds of nanometers. Our synthesised cerium oxide nanoparticles of sizes 5, 7, 10, and 35nm exhibit narrow size distributions. We exposed the fresh water algae (Pseudokirchneriella subcapitata) to the bulk, commercially brought nanoparticles and our synthesised ceria participles to determine the EC50 of the algae. Samples of the algae were taken at several points over the 72 hour exposures for AFM analysis, where the size and shape of the algae cells were recorded. In the exposures to the larger synthesised partials, the bulk and the commercial ceria nanoparticles, it was observed that the ceria coated the algae. The EC50 for these samples showed no significant effect on the growth rate of the algae. In contrast the algae exposed to the 5nm synthesised nanoparticles changes the algae cell shape and showed rapid signs of degradation with the apperence of dips in the algae surface. This research highlights the importance of size on the toxicity of cerium oxide nanoparticles and the uncertainty in the size of mass produced commercial nanoparticles.

Seawater at nanoscale: Organic assemblies imaged by AFM

Tea Mišić Radić, Vesna Svetličić, Vera Žutić

Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia

The present study introduces atomic force microscopy (AFM) as a direct imaging technique for characterization of marine biopolymer assemblies and understanding molecular mechanism of biopolymers associations in seawater, not accessible by other techniques.

A nutrient enrichment experiment ("Phosphorus limited carbon fixation, cycling and persistence and role of aggregation in the processes") performed from October 2007 until February 2008 in the Northern Adriatic (Piran, Slovenia) gave us the opportunity to follow the stages in self-assembly of exopolymers produced during the diatoms dominated phytoplankton bloom. Direct deposition of whole seawater on freshly cleaved mica followed by rinsing is the procedure that causes the least impact on the original structures of biopolymer assemblies in seawater.

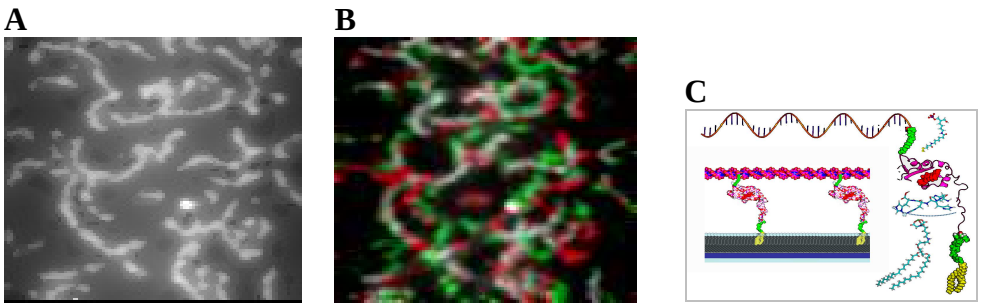
Topographic images revealed main structural organizations spanning from single fibrils and globules to their associations and gel networks, but also vesicles and nanobubbles. Although these findings apply directly to the organic structures observable in the northern Adriatic Sea, they also form basis for advancing research on biopolymer associations in the ocean using AFM.

Dynamic of proteo-nucleic complexes

M. Ewald[1], D. Carriou[1], E. Bourilot[1], M. Branowska[1], **E. Lesniewska**[1],
N. Kodera[2], T. Ando[2],
A. Laisné[3], J.-L. Leroy[3], D. Pompon[3]

[1]*Institute Carnot Bourgogne UMR CNRS 5209, University of Bourgogne, Dijon, France.*
[2]*Department of Physics, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan.*
[3]*CGM-FRE3144 and ICSN-UPR2301 CNRS, Gif-sur-Yvette, France*

Designing realistic nanofabrication approaches leading to reliable network of functional nanostructures in the size range of one to few tenth of nanometer remains today a real challenge. This project combines classical and high-speed atomic force microscopy (AFM) to synthetic biology. Commercially available AFMs require at least 30 – 60s to capture an image, whereas many interesting biological processes occur at a much higher rate. High-speed AFM imaging, because "direct and real-time visualization" can provide straightforward and prompt answers to understand biomolecular processes. The capability of this new microscope has been demonstrated by studying the dynamic of reversible molecular interactions between synthetic proteo-nucleic (p-DNA) structures. In such structures, the protein domain is used to control interaction with the surface providing a tight attachment while preserving lateral diffusion and the DNA domain to link protein parts together and to control self-assembly of objects by reversible lateral interactions. Extension of high-speed AFM imaging approach is now considered for the analysis of the self-assembly mechanism of DNA I-motif tiles. This non-classical tetrameric form of DNA can self-assemble to form wire structures by a mechanism critically dependant on pH and monomer concentration. Low-speed AFM in solution evidenced that such self-organization can lead to a large variety of structural organizations with in some cases long distance order. The major goal is now to transpose the concepts of p-DNA technology for the analysis of mechanisms of I-wire formation and structure stabilization under geometric constraints. This involves the characterizations of kinetic parameters, the analysis of the length distributions and of the contribution of different parameters (pH, strand concentration, temperature and geometry).



Dynamic analysis of a population of transiently interacting p-DNA constructs. **A.** Left, static image of the population. **B.** Right, superposition of two frames (red and green) at 100 ms interval showing immobile (white) and moving (red and green) segments. **C.** Structure of b5-based p-DNA building block

[1] D. Pompon D and A. Laisné. (2007) Biochem. Soc. Transact. 35 :495-497.
[2] J.L. Leroy. (2009) Nucl. Acids Res. 37, 4127-4134.
[3] T. Ando, T. Uchihashi, N. Kodera, D. Yamamoto, M. Taniguchi, A. Miyagi & H. Yamashita (2007) J. Mol. Recognit. 20, 448-458.
[4] M-C. Giocondi, D. Yamamoto, E. Lesniewska, P-E. Milhiet, T. Ando, C. Le Grimellec. (2009) Biochem. et Biophys. Acta, in Press
[5] A. Laisné, E. Lesniewska, D. Pompon. (2009) Nucl. Acids Res. (submitted).

SESSION II

Plenary Lecture

Marine ecology at nanoscale

Vesna Svetličić

Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia

This study highlights the capacity of AFM to become a tool in marine ecology. Marine ecology describes the interactions of marine species with their biotic and abiotic environments. The biotic environment includes interactions with other living organisms. The abiotic environment includes aspects of the physical habitat.

The two intriguing processes which occur in the Adriatic Sea at very different length scales will be presented. First: an abiotic process of rapid sol-gel transition within the water column resulting in a giant gel formation. Second: a biological process of extracellular polymer release by marine diatom.

Selected short oral presentations of sessions I, II, III

Chair: Simon Scheuring
Institut Curie Paris, France

AFM investigation of self assembling amphiphilic peptide

M. Alderighi[1], A. Battisti[1], F. Fuso[2], M. Allegrini[2], R. Solaro[1]

[1]*Chemistry and Industrial Chemistry department - via Risorgimento 35- 56126 University of Pisa, Italy*
[2]*Physics department - Largo Pontecorvo 3 - 56127 University of Pisa, Italy*

Nanoscience and nanomedicine are strongly driven by scanning probe microscopy which allows investigation and manipulation down to the atomic scale. The Atomic Force Microscope (AFM) can be exploited as a powerful tool to explore and understand aggregation processes of a particular type of peptides under different conditions. Among self-assembling peptides, the class of amphiphilic peptides is of special interest: This family of peptides has both hydrophilic and hydrophobic properties. This unique structural feature allows for the formation of stable aggregates under particular conditions (pH, concentration, temperature and time).
In this work the self-aggregation behavior of a particular amphiphilic peptide PA (Fig.1) has been studied with light scattering, viscosimetry and AFM microscopy both in air and in liquid environment.

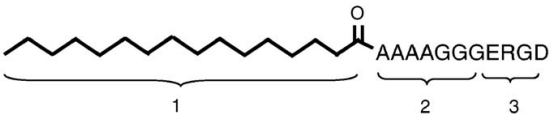


Fig. 1 Schematics of the amphiphilic peptide studied

Experimental

AFM. Scanning of peptide aggregates was performed in tapping modes by using a commercial AFM (Multimode with Nanoscope IV controller, Veeco Instruments) equipped with scanners E and J. Measurements in fluid environment were performed by using a MTFML cell (Veeco Metrology, Inc.). Samples imaged in air were scanned by using RTESP probes (Veeco Instruments) with 40 N/m elastic constant and 300 kHz resonance frequency.

Light Scattering. A PerkinElmer LS 50 spectrofluorimeter equipped with a xenon pulsed lamp was used for light scattering measurements. The excitation wavelength was 320 nm. The scattered light was collected at 90° with respect to the excitation beam and analyzed in the range 310 - 330 nm; micro sample cells of 10mm×2 mm have been used.

Viscosimetry. The viscosity measurements were performed with an automated Micro Viscosimeter AMVn (Anton Paar GmbH) equipped with a 500 µl capillary tube. The results were expressed as relative viscosity ($\eta_{rel} = \eta/\eta_0$, where η and η_0 are the viscosities of sample solution and solvent, respectively).

Hydrogel formation. The PA hydrogels were prepared by adding water solution containing different concentration of $CaCl_2$ to PA water solution at pH 7.4 (1:1v/v).

Hydrogel stiffness measurement with AFM.

Stiffness measurement were performed in liquid by AFM equipped with PicoForce stage, allowing for closed–loop scans in the Z direction (J–type scanner) and thus endowing a precise and reliable movement along the z axis, useful during elasticity measurements. To measure the elastic properties of the peptide gel a special cantilever (elastic constant K=4.5 N/m) with, mounted as probe a borosilicate sphere with a diameter of 10 µm, was used. This special cantilever was provided by Novascan Technologies, Inc. (Ames, IA Iowa).

In synthesis, the aggregation behavior of a specific amphihilic peptide has been studied. The cac has been evaluated between $7 \cdot 10^{-6}$M and $2 \cdot 10^{-4}$M showing a rather fast (tens of minutes) aggregation dynamics. Morphology of the aggregates has been analyzed by AFM on deposited samples, demonstrating the transition from nanosized, rod-like shapes to compact and tightly interconnected morphologies as a function of time. Moreover, AFM in liquid has been used to evaluate the stiffness of freshly-prepared peptide hydrogels through force vs. distance curves acquired at the local scale during the tip approach onto hydrogel specimens. Results demonstrate the material is rather stiff, being the stiffness related to the amount of Ca^{++} ions present in the preparation solution. This suggests that Ca^{++} ions are effectively involved in promoting inter-peptides links, leading to the formation of robust networks suitable for biological scaffolds.

External conditions influencing the elasticity of the cerebral endothelial cells

György Váró, Gergely A. Végh, István Krizbai, Imola Wilhelm, Csilla Fazakas, Krisztina Nagy, Zsolt Szegletes

Institute of Biophysics, Biological Research Center of the HAS, Szeged, Hungary 6726

Cerebral endothelial cells form the blood-brain-barrier, which blocks the passage of different chemicals from the blood stream to the brain, except that necessary for living. In the following study cerebral endothelial cells were investigated by atomic force microscope. Beside imaging the cells force measurements were also conducted on them. The change of the elastic Young's modulus on the external conditions was investigated. First the reliability of the force measurements was estimated by two ways. One was measuring for long time (hours) at the same place of the cell. This gives the temporal stability of the force measurement. Another set of forces were measured from one edge of the cell across the nucleus to the opposite edge in many points, resulting the spatial dependeence of the cell elasticity. For similar task was also applied the technique of force volume mapping. The measurements were performed on three types of cerebral endothelial cells. Single living cells on a collagen coated surface, confluent living cell culture and fixed cells.

The spatial dependence of the elastic modulus had a small error and showed that the nuclear part of the cell is softer. The value of the Young's modulus increases toward the edge of the cell, compared to the nuclear region. The temporal stability in short time period was remarkable. On long term, in several cases, instead of a stabile elasticity it shows a long period oscillation. We investigated when these oscillation occur and clarified the conditions, which produces it. The error of the elastic modulus on the fixed cells increased, due to offline data processing

By comparing the three different types of cell cultures, the confluent cells appeared to be the softest. The single cells had about two-three times larger Young' modulus, while the fixed cells were more than one order of magnitude harder.

The effect of some chemicals on the cerebral endothelial cells was investigated. One of the studied chemical was the mannitol, a cell-impermeable non-toxic alcohol, successfully used in clinical treatments for reversible opening of the blood-brain barrier in hyperosmotic concentration. It was observed a ten fold decrease in the Young's modulus and a remarkable decrease in the volume of the cell. These point to an action mechanism of the mannitol on the cells (1). In another study the depletion of the extracellular calcium disrupted the connection between the confluent cells by a decrease in the volume of the cells and a change in their shape These changes were reversible (2).

The above study indicates that the atomic force microscope is a powerful tool in drug testing by being able to study cells in their native environment.

This work was supported by the National Science Fund of Hungary OTKA K 81180.

References:
1. Z. Bálint, I.A. Krizbai, I. Wilhelm, A.E. Farkas, Á. Párducz, Z. Szegletes and G. Váró (2007) Changes induced by hyperosmotic mannitol in cerebral endothelial cells: an atomic force microscopic study. *Eur. Biophys. J.* 36. 113-120.
2. I. Wilhelm, A.E. Farkas, P. Nagyőszi, G. Váró, Z. Bálint, G.A. Végh, P.O. Couraud, I.A. Romero, B. Weksler and I.A. Krizbai (2007) Regulation of cerrebral endothelial cell morphology by extracellular calcium. *Phys. Med. Biol*. 52. 6261-6274

Atomic force microscopy-based molecular recognition of fibrinogen receptor in human erythrocytes

Filomena A. Carvalho[1*], Simon Connell[2], Gabriel Miltenberger-Miltenyi [1,3], Sónia Vale Pereira[3], Alice Tavares[4], Robert A.S. Ariëns[5], Nuno C. Santos[1]

[1]*Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal*
[2]*School of Physics and Astronomy, University of Leeds, Leeds, United Kingdom*
[3]*GenoMed Diagnosticos de Medicina Molecular, Lisbon, Portugal*
[4]*Serviço de Imuno-Hemoterapia, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Lisbon, Portugal.*
[5]*Division of Cardiovascular and Diabetes Research, Leeds Institute for Genetics, Health and Therapeutics, University of Leeds, Leeds, United Kingdom*
[*]*filomenacarvalho@fm.ul.pt*

The established hypothesis for the mechanism of erythrocyte hyperaggregation is due to an increase in plasma adhesion proteins, particularly fibrinogen. Fibrinogen (Fg) is a blood-borne glycoprotein comprised of three pairs of distinguishable polypeptide chains with three potential integrin-binding sites, but can also interact with cells through non-integrin receptors. Fibrinogen-induced erythrocyte aggregation was considered to be caused by non-specific protein binding to erythrocytes membranes. In contrast, platelets are known to have a fibrinogen integrin receptor expressed on the membrane surface (membrane glycoprotein complex $\alpha_{IIb}\beta_3$).

We showed that there is also a single-molecule interaction between Fg and an unknown receptor on RBC membrane, with a lower but comparable affinity relative to platelet binding (fibrinogen-erythrocyte and -platelet average (un)binding forces were 79 and 97 pN, respectively). In order to evaluate the similarity between the erythrocyte receptor for fibrinogen and the glycoprotein $\alpha_{IIb}\beta_3$ platelet receptor, force spectroscopy studies were conducted, using Fg-functionalized atomic force microscope (AFM) tips and RBC or platelets, in different experimental conditions: (i) in the presence of calcium or EDTA (to evaluate if the binding between fibrinogen and erythrocyte membrane receptor is calcium-dependent); (ii) with blood cells isolated from a Glanzmann Thrombasthenia (a rare hereditary bleeding disease caused by $\alpha_{IIb}\beta_3$ deficiency) patient; and (iii) in the presence and absence of eptifibatide, at different concentrations. Eptifibatide is a cyclic heptapeptide that inhibits specifically and reversibly the binding of fibrinogen to the $\alpha_{IIb}\beta_3$ receptor in platelets. It is clinically used for the treatment of platelet hyperaggregation.

On all the experiments, blood cells were deposited on poly-L-lysine treated glass slides. Force spectroscopy measurements were performed using fibrinogen functionalized AFM silicon nitride tips that were first silanized *via* a short linker of glutaraldehyde. Rupture forces were measured at the same pulling speed and loading rate on all experiments.

From the results we could conclude that the erythrocyte receptor is influenced by calcium and eptifibatide, but not as severely as the platelet receptor. Its inhibition by eptifibatide indicates that it is a $\alpha_{IIb}\beta_3$-related integrin. Results obtained for a Glanzmann thrombastenia patient show impaired fibrinogen-erythrocyte binding. Correlation with genetic sequencing data demonstrates that one of the units of the fibrinogen receptor on erythrocytes is a product of the expression of the β_3 gene, found to be mutated in this patient.

AFM imaging of extracellular polymer release by marine diatom

Galja Pletikapić, Tea Mišić Radić, Amela Hozić Zimmermann, Vesna Svetličić, Martin Pfannkuchen[1], Vera Žutić

Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia
[1]Center for Marine Research, Ruđer Bošković Institute, Rovinj, Croatia

Extracellular polysaccharide production by marine diatoms is a significant route by which photosynthetically produced organic carbon enters the trophic web and may influence the physical environment in the sea [1]. Visualization of supramolecular organization of diatom extracellular substance (EPS) release is a key step towards understanding the relationship that links diatom EPS and large scale phenomenon of gel formation in the Northern Adriatic Sea [2]. This study highlights the capacity of AFM for investigating diatom extracellular polysaccharides with a subnanometer resolution. Here we address a ubiquitous marine diatom *Cylindrotheca closterium*, we have isolated from the Northern Adriatic seawater and its EPS at the single cell level using AFM. A congeneric diatom, *Cylindrotheca closterium* [3] was used for comparative studies-taxonomy and EPS release. For AFM imaging a suspension of live cells was pipetted directly onto mica substrate. The diatom cells and EPS stayed firmly attached to the mica surface after rinsing with water enabling stable high resolution imaging in air. Cells used for AFM experiments were recovered from both logarithmic and stationary growth phase. In logarithmic growth phase we visualized almost no EPS release, while during stationary growth phase EPS was found on approximately 25% cells. The interconnected polysaccharide fibrils were mainly extending from cell rostra and seemed attached to cells (Fig. 1). Single fibrils and three dimensional networks of fibrils were also found free in bulk culture medium. Extracellular fibrils were compared with a biofilm formed on mica sheets in the culture medium and with extracted and purified exopolysaccharides. The biofilm appeared as a dense gel structure, while isolated exopolysaccharides were present both as single fibrils and networks, depending on concentration used for sample preparation.

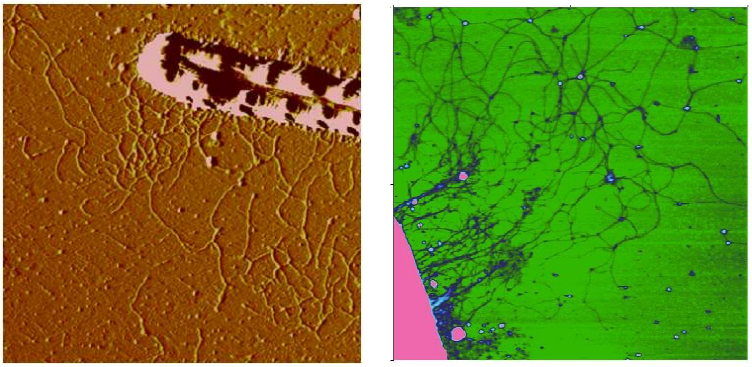


Figure 1. Extracellular polysaccharide release by marine diatom *Cylindrotheca closterium* visualized by AFM in air. Contact mode (5µm x 5µm).

References:
[1] G.J.C. Underwood and D.M. Paterson, The importance of extracellular carbohydrate production by marine epipelic diatoms. Adv. Bot. Res. **40** (2003) 184-240.
[2] V. Žutić and V. Svetličić: Interfacial processes, The Handbook of Environmental Chemistry, Vol. 5 Part D, Marine Chemistry, *Springer*, Heilderberg, 2000, pp. 149-165.
[3] Obtained from the Provasoli-Guillard Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences.

Screening estrogenic compounds using force spectroscopy

C. Elie-Caille[1], A. Berthier[2], W. Boireau[1], R. Delage-Mourroux[2], E. Lesniewska[3]

[1]*Institut Femto-st, UMR6174-CNRS, UFC, CNRS, ENSMM, Besançon Cedex, France*
[2]*Estrogènes, Expression Génique et Pathologies du Système Nerveux Central, EA3922, IFR 133, UFC, Besançon Cedex, France*
[3]*Laboratoire Interdisciplinaire Carnot de Bourgogne UMR CNRS 5209, University of Bourgogne, Dijon Cedex, France*

Estrogens, and particularly Estradiol-17-β (E2), are female hormones involved in development, growth and maintenance of reproductive tissues. These hormones interact with Estrogen Receptors (ER), which are transcription factors activated by ligand binding. These activated nuclear receptors modulate expression of estrogen-responsive genes after the interaction with palindromic DNA sequences called estrogen response element (ERE). Classically, the identification of new potential estrogenic compounds was based on cellular or animal models. However, the ER/DNA interaction being the initial step of the estrogenic genomic mechanism, a prescreening of interesting molecules could be processed upstream, in order to reduce time and money consumption. Surface Plasmon Resonance (SPR) biosensors, which allow the real-time biomolecular interaction detections, has been recently proposed for this prescreening step [1]. Nevertheless, the binding of ER to the ERE is not sufficient to induce target gene expression. Indeed, this receptor must first dimerise and then, dimers must have the right conformation to interact with the transcriptional machinery. Thus, the agonist or antagonist ligand's nature could be determined studying the ER dimerisation. We propose here to use force spectroscopy to screen new potential estrogenic compounds or to detect derivative estrogenic molecules (like phytoestrogens [2], paraben…), employing an ER modified tip interacting with a ER covered gold surface. Indeed, interactions of these compounds with ER have an impact on the conformational state of ER. Thus, the agonist or antagonist nature of the bonded compounds allows or not receptor dimerisation. We show in this work that such interactions between ER monomers appeared, and was revealed, in the presence of Estradiol-17-b (E2), through a particular, specific and reproducible pattern of force curves, for which modelling would help in understanding ER conformational modification.

References :

[1] Berthier A., Elie-Caille C., Lesniewska E., Delage-Mourroux R., Boireau W. (2008). Nanobioengineering and characterization of a novel estrogen receptor biosensor. Sensors, 8, 4413-4428.

[2] Berthier, A., Girard, C., Grandvuillemin, A., Muyard, F., Skaltsounis, A. L., Jouvenot, M. and Delage-Mourroux, R. (2007) Planta Med 73, 1447-51.

Bimodal atomic force microscopy imaging of proteins

José R. Lozano, Elena T. Herruzo, Christian Dietz and Ricardo Garcia

Instituo de Microelectrónica de Madrid, CSIC, Isaac Newton, 8, (28760) Tres Cantos, Madrid, Spain

We develop a dynamic atomic force microscopy (AFM) method based on the simultaneous excitation of the first two flexural modes of the cantilever. The instrument, called bimodal AFM, opens up additional channels (amplitude and phase of the 2nd flexural mode) which can be used for imaging with enhanced lateral resolution with respect to amplitude modulation AFM (AM-AFM). Bimodal AFM allows us to resolve the structural components of antibodies in both monomer and pentameric forms. The instrument operates in both high and low quality factor environments, i.e., air and liquid, so that the imaging of biomolecules can be carried out in their natural media.

Bimodal AFM is studied in great detail by means of theoretical and numerical methods. The theoretical approach also allows us to estimate the forces applied on the sample during bimodal AFM operation. The calculated forces lie below 120 pN, an essential fact when imaging proteins. This is due to the enhanced sensitivity of the 2nd mode phase channel, able to detect changes while the cantilever tip vibrates far away from the sample.

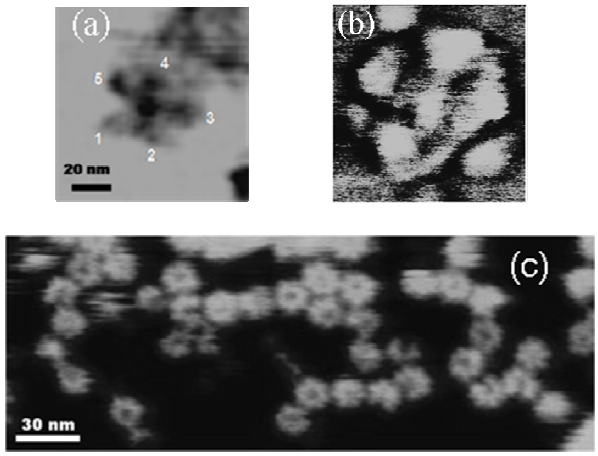


Fig. 1. (a)Bimoda AFMl topography image of an isolated IgM antibody in water and (b)Bimodal phase image in air. (c)Topography image with Bimodal AFM imaging of GroEl chaperonins in buffer.

[1] Patil S, Martinez N F, Lozano J R and Garcia R 2007, *J. Mol. Recognit.* 20 516–23
[2] Lozano J R and Garcia R 2008 *Phys. Rev. Lett.* 100 076102–4
[3] N F Martínez et al 2008 *Nanotechnology* 19 384011

Analysis of the Bacterial Cell Employing a Novel Lysis System and Atomic Force Microscopy

Douglas Dennis, Tiffany Stacy and Jacob Jordan

Department of Biology and Chemistry, Morehead State University, Morehead, KY 30251

A novel bacterial lysis procedure that results in the generation of intact subcellular structures has been developed. The procedure is rapid and does not rely on the intervention of enzymes, chaotropic chemicals, or harsh physical conditions. Because of this, it is thought that the structures generated are realistic representations of interior cell surfaces, offering an unparalleled opportunity for high resolution atomic force microscopy. Structures obtained by this procedure are intact outer membrane ghosts, intact cell ghosts, spheroplasts that possess a unique curvilinear network on their surface, cell ghosts possessing helical constrictions, cell ghosts with stippled surfaces, and cells in which the outer membrane has lysed revealing a multi-layered peptidoglycan surface. The results of high-resolution atomic force microscopy on all of these structures, and the implications for bacterial cell wall structure will be presented.

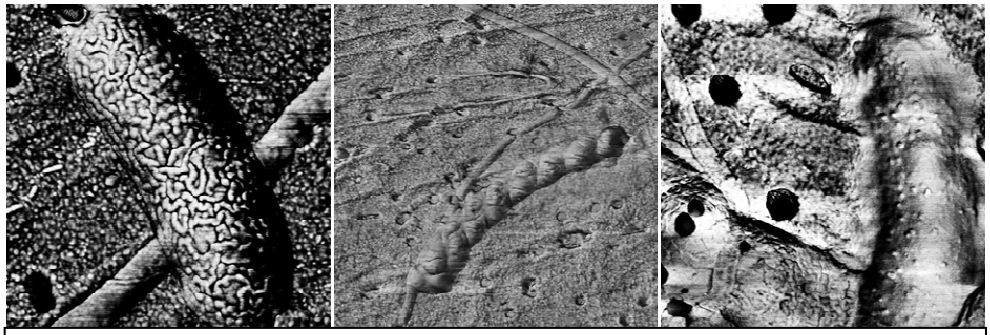


Fig 1. AFM phase images of intact cellular products released by osmotic lysis. A) Spheroplast ghost exhibiting curvilinear structures on surface. Height = ~240 nm, 1.9 μm^2 scan. B) Spiral ghost. Height = ~80 nm. 3.5 μm^2 scan. C) Stippled cell ghost exhibiting round raised structures. Height = 100 nm. 2.0 μm^2 scan.

Changes in the Mechanical Properties of *Bacillus anthracis* by Germinants and Antimicrobial Peptide Chrysophsin-3

Paola Pinzón-Arango[1], Ramanathan Nagarajan[2], and Terri Camesano[1]

[1]*Department of Chemical Engineering, Worcester Polytechnic Institute, 100 Institute Road, Worcester MA 01609;*
[2]*U. S. Army Natick Soldier Research, Development & Engineering Center, Molecular Sciences and Engineering Team, Natick, MA 01760*

Bacterial spores such as *Bacillus anthracis* are one of the most resistant life forms known due to their nanoscale multi-compartmental structure and morphology. During germination, the thick surface coats of spores are degraded, leaving a virulent, vegetative cell with a thinner membrane that is susceptible to the entrance of antisporal agents. However, during the virulent state, these organisms are capable of developing life-thratening infections, such as pulmonary anthrax. Atomic force microscopy (AFM) was used to characterize changes in the surface elasticity of *B. anthracis* spores before and after exposure to L-alanine and/or inosine. The Hertz model was applied to calculate the Young's modulus of the spores. Dormant spores had the highest modulus (197 ± 81 MPa), due to the numerous protein layers that protect the core. Elasticity of spores significantly decreased after exposure to L-alanine and inosine (23.5 ± 14.8 MPa) and vegetative *B. anthracis* had the lowest Young's moduli (12.4 ± 6.3 MPa) due to the degradation of the spore coat. *B. anthracis* spores were also treated with the antimicrobial peptide chrysophsin-3 and the elasticity of peptide-treated spores increased compared to the untreated spores. The Young's modulus of vegetative *B. anthracis* exposed to 500 µg/mL increased significantly from 12.4 MPa to 84.12 MPa. The ability of chrysophsin-3 to kill *B. anthracis* spores was also tested and our results suggest that spores treated with a mixture of germinants and peptide results in the killing of ~90% of spores. AFM images of the killed spores indicated that chrysophsin-3 lyses vegetative *B. anthracis* (Figure 1). The antimicrobial peptide chrysophsin-3 in conjunction with germinants can be potentially used for the development of antisporal agents, due to their powerful method for spore deactivation.

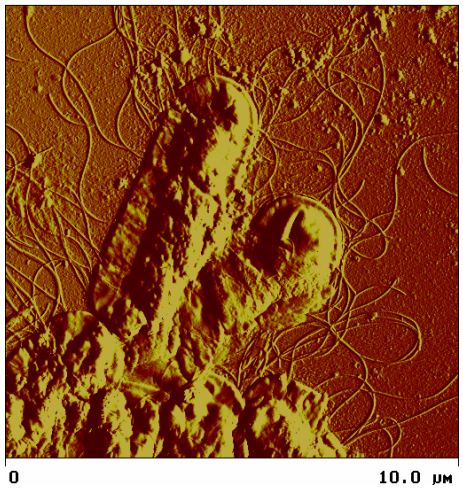


Figure 1: Vegetative *B. anthracis* exposed to 500 µg/mL chrysophsin-3 for 2 hours. Imaged in air in tapping mode.

Internal motion of proteins under force leads to nonexponentai unfolding kinetics

Eric Vanden-Eijnden

Courant Institute of Mathematical Science New York University, New York, NY, USA

Single molecule force spectroscopy experiments using the AFM have revealed a diversity of unfolding and refolding pathways of the small protein ubiquitin on multiple timescales. The observed heterogeneous trajectories are a signature of the complex protein folding landscape. Since the AFM measurements are limited to a single reaction coordinate of the protein's end-to-end length, the microscopic mechanism by which folding occurs remains unknown. In order to gain access to the structural features of the protein's response to a constant stretching force we employ molecular dynamics simulations. While standard numerical calculations are obliged to pull on the proteins with forces that are orders of magnitude larger than those used in vitro or encountered in vivo, we bridge the timescale gap between experiments and simulations using novel sampling methods to accelerate the process without modifying the free energy landscape. These numerical experiments indeed reveal conformational dynamics that is different to that observed at high forces. The thermal motions of the protein exert forces on the hydrogen bonds between the beta-sheets in directions different from the one of the applied force. We argue that this mechanism is consistent with the nonexponential kinetics observed in the experiments.

SESSION III

AFM Bio I

Chair: Pierre-Emmanuel MILHIET
CNRS, Montpellier, France

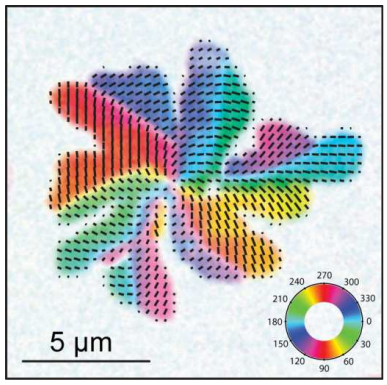
Invited Lecture

Quantifying domain patterns and domain textures in lipid bilayers

Adam Cohen Simonsen

MEMPHYS – Center for Biomembrane Physics, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

Lipid bilayers are formed by self-assembly and constitute the structural backbone of complex biomembranes. The lipid composition of biomembranes is a key factor for regulating biological function through modulation of a wide range of physical membrane properties. One example is the lateral pressure profile and another is the formation of lipid domains and laterally differentiated regions in the plane of the membrane. An improved understanding of the spatial organization of biomembranes is crucial for establishing the relationship between membrane structure and function. The use of simplified model membranes has advanced our understanding of how domain formation is governed by the lipid composition and thermodynamic parameters. In this talk I will review our recent efforts to quantitatively characterize membrane domains patterns and domain textures based on scanning probe and optical microscopy of supported model membranes. We have focused on a detailed characterization of domains in simple systems as a foundation for understanding more complex membranes. I will outline the major challenges related to domains in biological membranes which are often far from thermodynamic equilibrium and driven by various energy sources in addition to exchange of components.



Texture of a gel domain [2]

[1] U. Bernchou, J. H. Ipsen, and A. C. Simonsen. Growth of Solid Domains in Model Membranes: Quantitative Image Analysis Reveals a Strong Correlation between Domain Shape and Spatial Position. J. Phys. Chem. B. 113(20):7170–7177, 2009.
[2] U. Bernchou, J. R. Brewer, H. S. Midtiby, J. H. Ipsen, L. A. Bagatolli, and A. C. Simonsen. Texture of lipid bilayer domains. J. Am. Chem. Soc. Commun., 131(40):14130+, 2009.

Annular Oligomers in the Transthyretin Amyloid Fibrillogenetic Pathway Captured with AFM

Ricardo H. Pires[1,2], Árpád Karsai[3], Maria J. Saraiva[2,4], Ana M. Damas[2,4], Miklós S. Z. Kellermayer[1,3]

[1]*Institute of Biophysics and Radiation Biology, Semmelweis University, Tűzoltó u. 37-47, 1094 Budapest IX, Hungary*
[2]*Instituto de Biologia Molecular e Celular, Universidade do Porto, R. Do Campo Alegre 823, 4150-180, Porto, Portuga*
[3]*Dept. Biophysics, University of Pécs, Szigeti ut 12, Pécs H7624, Hungary*
[4]*Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, L. Prof. Abel Salazar, 2, 4099-003 Porto, Portugal*

Transthyretin (TTR) is a human serum-circulating protein involved in the transport of thyroxine and co-transport of retinol. The presence of TTR amyloid fibrils is associated with severe neurological and systemic disorders. Cytotoxicity in amyloidoses has often been attributed to the formation of ion channels by annular oligomers. However, the role of such annular in amyloid fibrilogenesis remains to be understood.

Here we report the early events leading to the formation of TTR amyloid protofibrils and fibrils, which we have followed using AFM in liquid. We observed that the formation of WT TTR protofibrils in mildly acidic conditions is preceded by the appearance of annular oligomers with 16 ± 2 nm (s.d.) in diameter, 1.1 ± 0.5 nm (s.d.) in height and containing a 6 nm ± 3 nm (s.d.) substructure. We show that annular oligomers display the tendency to associate laterally, forming axial structures with 2.6 ± 0.6 nm in height. Subsequently, amyloid protofibrils positive for thioflavin and Congo red appeared. Height analysis of these protofibrils revealed a periodicity of 9 and 16 nm, a mean height of 3.2 ± 0.8 nm (s.d.) and an amplitude of 1.0 ± 0.3 nm (s.d.). Annular oligomers are therefore likely to be an “on pathway” intermediate in amyloid fibrillogenesis. To probe the protofibrils' underlying structure, we evoked their disassembly by solvent exchange. Strikingly, a distinct type of annular oligomers appeared with a diameter of 7.2 ± 0.8 nm (s.d.) and a height of 4.8 ± 0.7 nm (s.d.).

To further probe structural details, we performed dynamic force spectroscopy on native TTR and on protofibrils at different growth stages. Force curves revealed a ~4 nm periodicity. Conceivably, this pattern corresponds to successive structural transitions related to the sequential unfolding of the ß-strands within the TTR monomer. Force spectra of TTR protofibrils also revealed a time-dependent increase in the length of the manipulated structure, indicating that the axial association between monomers stabilizes with time. Thus, stabilization of intermonomeric contacts appears to be a much slower process than that of mere assembly, possibly involving additional structural rearrangements of the monomer within the protofibrils.

In sum, our results indicate that annular oligomers are directly involved in the fibrillation of TTR via an associative mechanism followed by structural rearrangements giving rise to a double helical structure as suggested earlier for the TTR protofilament.

AFM nano-guided bacterium adhesion to the host cell for biophotnic-based measurements of the dynamics of cytoskeleton and signaling molecules upon infection

Yann Ciczora, Sébastien Janel and Frank Lafont

Cellular Microbiology of Infectious Pathogens – Center for Infection and Immunity of Lille, Institut Pasteur de Lille,
F-59019 Lille, France; CNRS UMR8204, F-59021 Lille, France; INSERM U1019, F-59021 Lille, France; Univ. Lille Nord-de-France, F-59000 Lille

For biologists AFM gives the opportunity on the same sample and with a single scan to obtain the topography of the cell surface, to locate a given receptor, to determine the interaction force between this receptor and its ligand and the membrane elasticity where the interaction occurs[1].
However, in this work, we used the AFM to nano-manipulate bacteria in order to process samples for biophotonic measurements. Using specific growing conditions, AFM cantilever preparation and coating procedures, it was possible to adsorb non-covalently living bacteria onto the tip of an AFM cantilever. With this method, the same living bacteria could be engaged on several parts of the cell surface with the control of the contact force between the bacteria and the cell surface at the selected binding sites.
By coupling a video microscope to the AFM, we followed the infection kinetic of the bacteria onto 3 different cell surface compartments. First, we observed the recruitment of a membrane inserted GPI-anchor protein (a GFP-GPI fusion protein) at *Yesinia pseudotuberculosis* entry sites.
Secondly, we observed the recruitment of cytoplasmic ubiquitin proteins at the cell surface during the bacterium adhesion step. As bacteria entry is impaired, we could uncouple adhesion and invasion steps while recording the dynamics of GFP-tagged ubiquitinated proteins.
Thirdly, using AFM coupled to TIRF video microscope, it was possible to follow the dynamics of the actin cytoskeleton upon adhesion of the bacterium coated on the tip applying different forces with the AFM. We could demonstrate that bacteria can induce actin network reorganization not only at the entry site but also at distance from it. This opens new avenue in investigating specific pathways during the host cell signalling response upon pathogen invasion.

[1] Roduit C, *et al.* (2008) Elastic membrane heterogeneity of living cells revealed by stiff nanoscale membrane domains. *Biophys J* 94(4):1521-1532.

Direct Correlation of Structures and Nanomechanical Properties of Multicomponent Lipid Bilayers

Ruby May A. Sullan[1,2], James K. Li[2], and **Shan Zou**[1*]

[1]*Steacie Institute for Molecular Sciences, National Research Council Canada, 100 Sussex Drive, Ottawa, ON K1A 0R6, Canada*
[2]*Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, ON M5S 3H6, Canada*

Quantification of the mechanical stability of lipid bilayers is important in establishing the composition-structure-property relations, and shed light on understanding functions of biological membranes. A direct correlation of the self-organized structures has been demonstrated in phase-segregated supported lipid bilayers consisting of dioleoylphosphatidylcholine / egg sphingomyelin/cholesterol (DEC) in the absence and presence of ceramide (DEC-Ceramide) with their nanomechanical properties using AFM imaging and force mapping. Direct incorporation of ceramide into phase-segregated supported lipid bilayers formed ceramide-enriched domains, where the height topography was found to be imaging setpoint dependent. In contrast, liquid ordered domains in both DEC and DEC-Ceramide presented similar heights regardless of AFM imaging settings. Owing to its capability for simultaneous determination of the topology and interaction forces, AFM-based force mapping was used in our study to directly correlate the structures and mechanical responses of different coexisting phases. We have designed an experiment to directly probe and quantify the nanomechanical stability and rigidity of the ceramide-enriched platforms that play a distinctive role in a variety of cellular processes. Our force mapping results have demonstrated that the ceramide-enriched domains require both methyl β-cyclodextrin (MbCD) and chloroform treatments to weaken their highly ordered organization, suggesting a lipid packing different from typical gel states. Our results also show the expulsion of cholesterol from the sphingolipid/cholesterol- enriched domains as a result of ceramide incorporation. This work provides quantitative information on the nanomechanical stability and rigidity of coexisting phase-segregated lipid bilayers with the presence of ceramide-enriched platforms, indicating that generation of ceramide in cells drastically alters the structural organization and the mechanical property of biological membranes.

References:
1. R.M.A. Sullan, J.K. Li, S. Zou, *Langmuir* **25** (2009) 7471-7477.
2. R.M.A. Sullan, J.K. Li, S. Zou, *Langmuir* **25** (2009) 12874-12877.
3. Ira; Zou, S.; Carter, D.; Vanderlip, S.; Johnston, L. *J. Struct. Biol.* **2009**, *168*, 78-89.

SESSION III

Plenary Lecture

Nanomechanics of living cells: applications in respiratory medicine

Daniel Navajas

Biophysics & Bioengineering Unit. School of Medicine, University of Barcelona and Institute for Bioengineering of Catalonia

Mechanical properties of cells play a critical role in many essential biological functions including migration, contraction, differentiation and gene expression. Moreover, cells feel and actively respond to adhesive forces and deformations exerted by the adjacent cells and the extracellular matrix. The cell mechanical behavior is increasingly recognized as a key determinant of normal cell function and of its alterations under pathological conditions. However, knowledge of the mechanical behavior of the cell remains largely incomplete. Atomic force microscopy (AFM) allows 3-D manipulation of cells and molecules with nanometric resolution with simultaneous measurement of the applied force with pN sensitivity. These are the displacement and force scales suitable for probing single molecules and cells. Moreover, measurements can be carried out in liquid with controlled environmental conditions and the cell response to pharmacological agents can be monitored in real time. AFM is therefore a powerful tool for probing the mechanical behavior of living cells and its alterations in disease. Chronic obstructive pulmonary disease (COPD) is associated with an abnormal inflammatory response of the lung to noxious particles or gases characterized by a specific pattern of inflammation involving neutrophil pulmonary infiltration. The mechanical properties of neutrophils regulate their passage through the pulmonary capillary bed. Abnormal cell stiffening slows down the capillary passage, promoting neutrophil pulmonary sequestration and subsequent transendothelial infiltration. Methods for probing cell mechanics with AFM are discussed and alterations in neutrophil mechanics associated with COPD are investigated. Neutrophil mechanics was measured in patients with advanced hypoxemic COPD before and after bilateral lung transplantation, and compared with measurements taken in healthy nonsmokers. Neutrophils isolated from venous or arterial blood were plated on poly(HEMA) coated cover slips to avoid cell spreading. Cell stiffness was probed with a custom-built AFM attached to an inverted optical microscope. The surface of the cell was indented with a spherical polystyrene bead attached to the end of the cantilever. Young's modulus was computed by fitting the Hertz contact model of a spherical punch to the force-indentation curves. Young's modulus of neutrophils isolated from COPD patients was significantly greater than that of neutrophils obtained from healthy nonsmokers. Neutrophil stiffness decreased after lung transplantation showing no significant differences with respect to healthy nonsmokers. Our findings indicate that increased neutrophil stiffness in COPD patients may be related to the abnormal inflammatory response of the lung. Neutrophil improvement in very severe COPD patients after lung transplantation suggests decreased inflammatory pulmonary and systemic responses. AFM is a useful technique for assessing neutrophil mechanical abnormalities induced by inflammatory diseases.

Acknowledgements: Ministerio de Sanidad y Consumo, PI081908, CIBERES and CIBERBBN.

Nanoscale investigation of the interaction between ionic detergents and supported lipid membranes

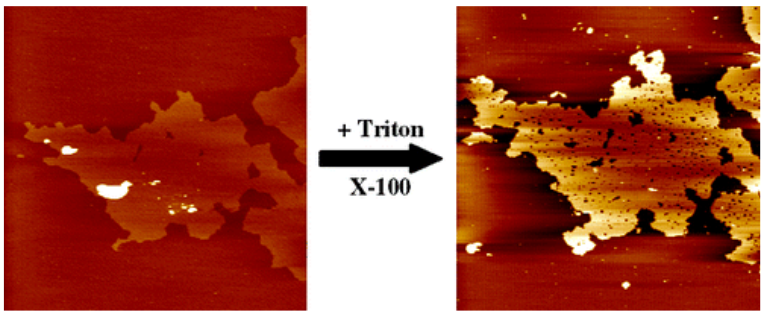
Karim El Kirat[1] and Sandrine Morandat[2]

[1]*Laboratoire de Biomécanique et Génie Biomédical, UMR-CNRS 6600,*
[2]*Laboratoire de Génie Enzymatique et Cellulaire, UMR-CNRS 6022,*
Université de Technologie de Compiègne, BP 20529, 60205 Compiègne Cedex, France

Biomembranes are not homogeneous, they present a lateral segregation of lipids and proteins which leads to the formation of functional domains in membranes, also called "rafts". These rafts are particularly enriched in sphingolipids, cholesterol (Chol) and glycosylphosphatidylinositol anchored proteins. Many studies have been based on the assumption that rafts can be isolated from cells as detergent-resistant membrane (DRMs). To obtain the DRMs, the cell membranes have to be treated with non-ionic detergents at 4°C. Despite the huge body of literature on DRMs, the mechanisms governing their resistance at the nanometer scale still remain an important matter of debate.

Owing to their conical molecular structure, detergents molecules produce a positive, spontaneous curvature of membranes. Therefore, alone in solution, detergents form small spherical structures with a strongly curved surface (micelles). The solubilization of vesicles in solution was described with a three-stage model in an all-or-none mechanism. During the first stage, when the detergent is added at low concentration, the detergent molecules are partitioned between the aqueous medium and the phospholipid bilayers. This progressive insertion of individual molecules of detergent provokes the swelling of the vesicles. At stage II, when increasing detergent concentration, some mixed micelles of lipid-detergent dissociate from the detergent-saturated membranes thus resulting in a decrease of the turbidity recorded at 450 (or 540) nm. For higher detergent concentrations, at stage III, vesicles are completely solubilized and all the phospholipid molecules reside in curved, mixed lipid-detergent micelles.

In this study, the solubilization of homogeneous supported lipid bilayers (SLB) or of SLB exhibiting phase segregation was followed by time-lapse atomic force microscopy (AFM). To this end, we have prepared different types of bilayers: dipalmitoylphosphatidylcholine (DPPC) alone, dioleoylphosphatidylcholine (DOPC)/DPPC 1:1 (mol/mol), DOPC/sphingomyelin (SM) 1:1 (mol/mol), DOPC/SM/Chol 2:1:1 (mol/mol/mol) or 4:3:1 (mol/mol/mol). In this work, AFM permitted to explore, at the nanometer scale, the differences between detergents belonging to the same family of non-ionic detergents (Triton X-100 (TX-100), *n*-octyl β-D-glucopyranoside (OG) and Tween-20 (Tw20)). AFM finally pointed out major differences between TX-100, OG and Tw20 mediated solubilizations of biomembranes, even though they have an indistinguishable behavior at the macroscopic level. Moreover, our results confirm the requirement for a minimum amount of Chol mixed with SM to provide a good resistance to DRMs' solubilization by TX-100.



Atomic force microscopy as a valuable tool to study amyloid aggregation and aggregate-membrane interaction

Annalisa Relini, Ranieri Rolandi, Alessandra Gliozzi

Department of Physics, University of Genoa

Aggregation of normally soluble proteins into ordered fibrillar structures named amyloid fibrils is a process involved in a number of severe pathological conditions, including Alzheimer's and Parkinson's disease and systemic amyloidoses. Under appropriate conditions, even disease-unrelated proteins can undergo amyloid aggregation, forming amyloid fibrils and cytotoxic prefibrillar aggregates, with the same properties as those involved in pathologies.
We AFM to obtain detailed information about the steps of the aggregation process of different amyloidogenic proteins, such as the N-terminal domain of the bacterial hydrogenase maturation factor Hypf-N, beta2-microglobulin, acylphosphatase, apolipoprotein A-I, hexokinase, calcitonin. We have characterized the morphology of the intermediates that precede fibril formation, such as spherical oligomers [2], beaded protofibrils, rings, and we have investigated the ultrastructure of mature amyloid fibrils. In addition, from the analysis of AFM images using a polymer theory-based approach, we obtained physical parameters, such as bending modulus and Young's modulus, which are related to the mechanical properties of fibrils or protofibrils.
Finally, to get insight into the mechanism of aggregate cytotoxicity, we have used AFM to study the interaction between amyloid aggregates and artificial or natural membranes, such as supported lipid bilayers of different composition and membrane rafts extracted from neuroblastoma cells and deposited on solid support. In the case of rafts, by measuring force-distance curves and imaging the raft sample before and after exposure to proteases, we identified the domains observed in the sample as fluid proteolipid components of the raft fraction [2]. Membranes were imaged before and after incubation with protein aggregates. We have analyzed the changes in membrane morphology induced by prefibrillar protein aggregates and we have compared them with the effects of monomeric proteins and mature amyloid fibrils.

[1] S. Campioni, B. Mannini, M. Zampagni, A. Pensalfini, C. Parrini, E. Evangelisti, A. Relini, M. Stefani, C. M. Dobson, C. Cecchi, and F. Chiti. A causative link between the structure of aberrant protein oligomers and their toxicity. Nature Chem. Biol. 6 (2010), 140-147.

[2] C. Cecchi, D. Nichino, A. Pensalfini, C. Bernacchioni, A Relini, A Gliozzi, G. Liguri, and M. Stefani. A protective role for lipid raft cholesterol against amyloid-induced membrane damage in human neuroblastoma cells. Biochim Biophys. Acta **1788** (2009) 2204.

SESSION III

Plenary Lecture

AFM Imaging of Purified Transmembrane Proteins Reconstituted into Artificial Membranes

Pierre-Emmanuel Milhiet, Bastien Seantier, Christian Le Grimellec
Centre de Biochimie Structurale, UMR5048 CNRS, UMR554 INSERM, Montpellier, France
pem@cbs.cnrs.fr

Manuela Dezi and Daniel Levy
Institut Curie, UMR168 CNRS, Paris, France

Laura Picas, M. Teresa Montero and Jordi Hernandez-Borrell
Departament de Fisicoquímica, Facultat de Farmàcia, Universitat de Barcelona, and Institut de Nanociència i Nanotecnologia de la Universitat de Barcelona

Besides its important contribution in the field of lipid microdomains, AFM is the only microscope that allows imaging of proteins embedded in biological membranes with a subnanometer resolution under physiological conditions. In the difficult context of the structural analysis of membrane proteins (they represent about 25% of the open-reading frames of the human genome but only ~200 structures are available in the protein data bank), AFM represents a very attractive technique but requires the reconstitution of detergent-solubilized and purified membrane proteins within artificial supported membranes.
In this talk we will introduce some technical developments allowing high resolution imaging of membrane proteins reconstituted in supported lipid bilayers (SLBs), namely the direct incorporation of proteins within SLBs preformed on mica and destabilized by low cmc detergents [1,2] and the transfer of membrane patches obtained from two-dimensional crystallization trials performed at the air-water interface. Both techniques requires very small amount of purified proteins. Development of these techniques in the context of membrane-inspired biosensors will be discussed.

References

[1] P.E. Milhiet, F. Gubellini, A. Berquand, P. Dosset, J.L. Rigaud, C. Le Grimellec and D. Lévy, Biophysical J 91 (2006), 3268-3275.
[2] Picas, L., Teresa Montero, J.L. Vazquez-Ibar, Seantier, B., Milhiet, P.E. and Hernández-Borrell, J. BBA Biomembranes (2010), in press.

SESSION IV

Trends in Theory & Technologies

Chair: Chanmin SU
Veeco Santa Barbara, USA

Invited Lecture

Real Time Imaging of Protein Conformational Change using High-Speed AFM

Takayuki Uchihashi and Toshio Ando

Physics Department, Kanazawa University, Japan

Biological molecules show their vital activities only in aqueous solutions. It had been one of dreams in biological sciences to directly observe biological macromolecules such as proteins and nucleotides at work under a physiological condition, because such observation is straightforward to understanding their dynamic behaviors and functional mechanisms. Atomic force microscopy (AFM) can visualize molecules in liquids at high resolution. However the imaging rate of conventional AFM is too low to capture dynamic biological processes. The imaging rate is limited by slow mechanical response of the cantilever and scanner. In addition, large tip-sample interaction would disturb weak protein-protein interactions and sometimes leads to destruction of fragile proteins. Two key techniques are required to realize high-speed AFM for biological research; fast feedback control to maintain a weak tip-sample interaction force and a technique to suppress mechanical vibrations of the scanner. Various efforts have been carried out in the past decade to materialize high-speed AFM. The current high-speed AFM can capture images on video at 30-60 frames/s, without significantly disturbing week biomolecular interaction.[1,2] Our recent studies demonstrate that this new microscope can reveal biomolecular processes such as hand-over-hand movement of myosin V along actin tracks[3] and photo-induced conformational change of bacteriorhodopsin[4]. The capacity of nanometer-scale visualization of dynamic processes in liquids will innovate on biological research.

[1] T. Ando, N. Kodera *et al.*, *Proc. Natl. Acad. USA* **98** (2001) 12468
[2] T. Ando, T. Uchihashi and T. Fukuma, *Prog. Surf. Sci.* **83**(2008) 337
[3] N. Kodera *et al.* (in preparation)
[4] M. Shibata, H. Yamashita, T. Uchihashi, H. Kandori and T. Ando, *Nature Nanotech.* (in press)

First FluidFM applications in biology: from single-virus dispensing to cell-organellae transplantation

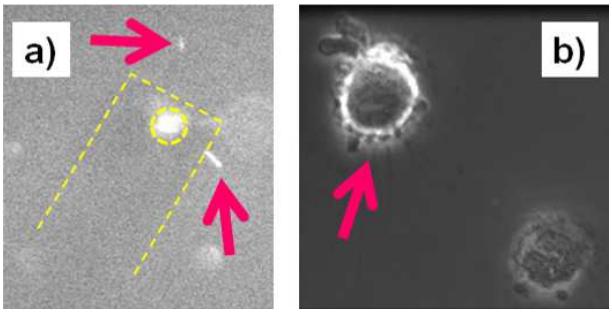
Tomaso Zambelli,[1] Pablo Dörig,[1] Philipp Stiefel,[2] Pascal Behr,[1,3] Edin Sarajlic,[3,4] Daniel Bijl,[3,4] Michael Gabi,[1,3] János Vörös,[1] Julia Vorholt,[2] Jason Mercer[5]

[1]*Laboratory of Biosensors and Bioelectronics, D-ITET, ETH Zurich, Switzerland*
[2]*Institute of Microbiology, D-BIOL, ETH Zurich, Switzerland*
[3]*Cytosurge GmbH, Zurich, Switzerland*
[4]*SmartTip BV, Enschede, The Netherlands*
[5]*Institute of Biochemistry, D-BIOL, ETH Zurich, Switzerland*

Glass micropipettes are the typical instrument for intracellular injection, patch clamping or extracellular deposition of liquids into viable cells. The micro pipette is thereby slowly approached to the cell by using micro manipulators and visual control through an optical microscope. The method is usually performed by extensively trained and experienced operators. During this process, however, the cell is often mechanically injured which leads to cell death and failure of the experiment. To overcome these challenges and limitations of this conventional method we developed in the framework of a collaboration with the CSEM SA (Neuchâtel, CH) the FluidFM technology, an evolution of standard AFM microscopy combining nanofluidics with conventional BioAFM. [1]

The instrument is composed of custom made AFM cantilevers encompassing an integrated microfluidic channel. The microchannel ends at a well defined aperture located at or in the vicinity of the apex of the AFM probe tip while the other end is connected to a reservoir. The reservoir is thereby also used to precisely control the pressure in the liquid channel. At the same time, the force control system of the AFM microscope allows a very precise control of the force exerted on the sample by the modified AFM probe tip. The instrument can therefore also be regarded as a very sophisticated, multifunctional micropipette with force feedback. Thanks to the instruments versatility, interactions like gentle contact, deep indentation or membrane penetration can therefore be well defined by the user. Liquids can be released or extracted through the hollow probe tip whenever necessary. Highly localized biochemical stimulation or manipulation of single living cells under physiological conditions becomes essentially practicable using our instrument.

To demonstrate the potential of FuidFM we are now trying to answer true biological open questions. In this presentation we will concentrate on two applications: The first one deals with viruses and in particular with the phenomena connected with their entry into a cell. We are taking advantage of our technology combined with confocal microscopy to dispense single viruses onto a selected cell in order to investigate the molecular factors involved in localized cytoskeletal perturbation and signalling during vaccinia virus binding and subsequent entry (blebbing effect, [2]). The second one is related with the extraction of organellae from yeast cells and their subsequent transplantation into neighbour cells.



a) Two fluorescent viruses ejected from a microchanneled cantilever. b) The arrow indicates a blebbing cell after being individually infected with the fluidFM.

We would like to thank Stephen Wheeler from the LBB Workshop for technical help.

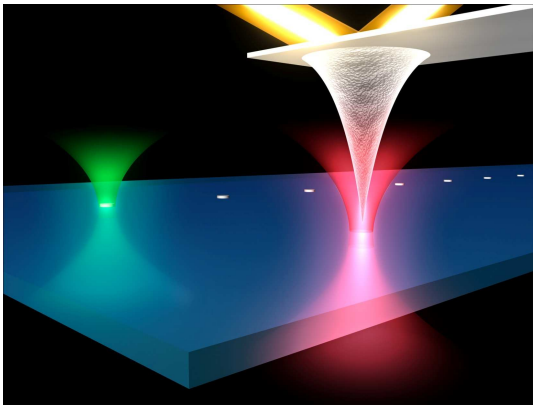
[1] A. Meister, M. Gabi, P. Behr, P. Studer, J. Vörös, P. Niedermann, J. Bitterli, J. Polesel-Maris, M. Liley, H. Heinzelmann, T. Zambelli, Nano Letters **9** (2009) 2501.
[2] J. Mercer, A. Helenius, Science **320** (2008) 531.

Precision force spectroscopy: A new window on the dynamics of unolding and refolding membrane proteins

Gavin M. King[1], Allison B. Churnside[2], and **Thomas T. Perkins**[3]

JILA, National Institute of Standards and Technology and University of Colorado at Boulder, Boulder, CO, USA
[1]*Present address: Department of Physics and Astronomy, University of Missouri-Columbia, Columbia, MO, USA*
[2]*University of Colorado Department of Physics,*
[3]*University of Colorado Department of Molecular, Cellular, and Developmental Biology, Boulder, CO, USA*

Single-molecule force spectroscopy studies have produced rich insights into the unfolding of individual proteins and nucleic acid structures. In a typical force spectroscopy experiment, an AFM tip is coupled to a surface-adsorbed protein by pressing the tip into it. Force-extension curves are then generated by retracting the tip at a constant velocity using a piezoelectric (PZT) stage. Force is measured by cantilever deflection. Extension, or more precisely tip-sample separation, is deduced from the PZT stage position used to control the vertical tip position. Thus, this deduced extension is sensitive to the vertical mechanical drift of the AFM assembly (~10 nm/min). We have previously developed an ultrastable AFM in which the tip and the sample positions are independently measured by, and stabilized with respect to, a pair of laser foci in three dimensions. These lasers establish a local reference frame that is insensitive to long-term mechanical drift of the AFM assembly. This new measurement of position is complementary to the cantilever deflection sensing, which measures force. We have now extended the ultrastable AFM capabilities into liquid and can routinely mechanically unfold proteins at slow pulling velocities (2 nm/s), which allows averaging to increase precision. We can also stop pulling altogether and hold the molecule at constant force while independently measuring tip-sample separation (σ = 0.2 nm, Δf = 1-50 Hz). Alternatively, we can stabilize tip-sample separation and measure force (σ = 5 pN, Δf = 1-50 Hz) over 100s of seconds. Using these techniques, we are studying the unfolding and re-folding of bacteriorhodopsin (BR), a model transmembrane protein.



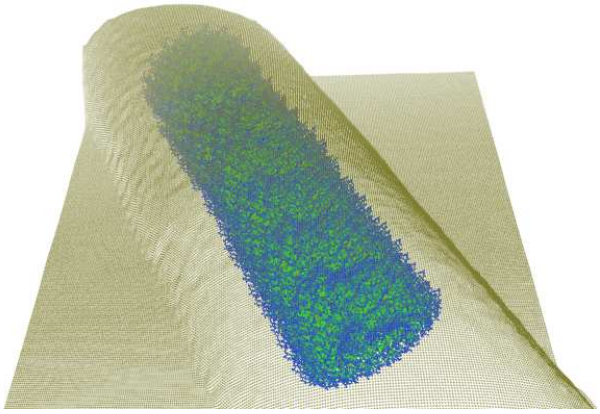
In an atomic force microscope (AFM), force is measured by a laser beam (yellow in this artist's rendition) bouncing off the diving-board like cantilever. To make an ultrastable AFM, two other lasers (green and red) were added to measure the three dimensional position of the tip and a reference mark in the sample.

Principles for using high-resolution topographical surfaces obtained in AFM for assembling molecular complexes: test case of the Tobacco mosaic virus (TMV)

Minh-Hieu Trinh, Michael Odorico, Shu-wen W. Chen, Pierre Parot, Jean-Luc Pellequer

CEA Marcoule, IBEB, Department of Biochemistry and Nuclear Toxicology,
F-30207 Bagnols sur Cèze, France

One of the challenges in determining the structure of biological macromolecules is their size and flexibility. Promising techniques for studying large and flexible molecules are emerging from the near-field microscopy. Under appropriate conditions, atomic force microscopy (AFM) is able to obtain high-resolution topographic surface of single molecules. Using topographical surfaces, we developed a computational protocol for building the complete structure of larges proteins or supercomplexes using their own components.
The difficulty in fitting molecular models at the atomic resolution in AFM-based surface topographs is the complexity of the search in the six dimensions. We use an exhaustive search engine (docking program DOT) to test all the possibilities for positioning individual domains of a molecule/complex using fine translations and rotations in the reel space. A best fit score has been devised to identify multiple solutions to the problem of molecular assembly. Our method allows the investigation of the flexibility range of large molecular complexes at the single molecule level.
We have applied this protocol to the tobacco mosaic virus particle which is an assembly of 2130 identical sub-units around a single stranded RNA. Using high-resolution topographs of the TMV on mica and appropriate surface erosion to take into account the finite shape of the AFM tip, we were able to assemble a long particle using a single disk of proteins as docking domains.



Two-dimension topographic AFM image in white and the assembled structure of a large section of the tobacco mosaic virus (blue and green).

Acknowledgement
This work was supported in part by a grant from the Agence Nationale de la Recherche (ANR-07-PCVI-0002-01)

Near-field IR microspectroscopy as a tool to discriminate nano-molecular alterations at cell or near-cell level in biomedical research

Frank L. Martin, Matt J. German, Nigel J. Fullwood, Azzedine Hammiche and **Hubert M. Pollock***

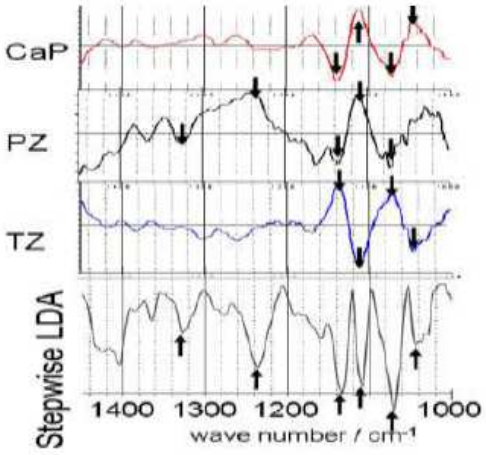
**Biomedical Sciences Unit, Lancaster University, Lancaster LA1 4YQ, England*

For some years, conventional methodologies such as synchrotron Fourier-transform infrared (FTIR) microspectroscopy have been employed for cell-by-cell characterization. Potentially, through the generation of an infrared (IR) spectrum of a received sample, a "biochemical-cell fingerprint" may be generated, and such applications have immense potential in disease diagnosis and characterization. Meanwhile, there have been useful recent developments in the area of "near-field" technology and in its application to the imaging or localised spectroscopy of solid state, organic and biological samples [1]. Novel approaches that exploit the photothermal effect have now been successfully used to achieve chemical resolution combined with sub-wavelength spatial imaging, giving localised PhotoThermal Micro-Spectroscopy (PTMS). Combined with multivariate analyses such as principal component analyses (PCA) that allow for the reduction of large spectroscopic datasets towards cluster analysis, there is now the possibility to derive from a cell, or a sub-cellular compartment, a biomolecular signature of what represents a normal state. It is then possible to rapidly discriminate IR spectra that deviate from this "normal" state, and to identify the wavenumbers, and thus the nano-molecular alterations, that are responsible for such deviation.
A major obstacle to diagnosing of cancers earlier has been the difficulty in differentiating between molecular species within large populations of cells. To characterise the very small proportion of cancerous cells in a typical biopsy is a "needle in a haystack" problem. In the absence of a brilliant light source such as a synchrotron facility, PTMS allows individual types of suspicious cells to be selected at the bench-top. Thus any diluting effects derived from an IR signature of normal adjacent cells are eliminated, and differences in spectral abnormalities are greatly enhanced. Conventional FTIR spectroscopy, especially when run in transmission mode, may generate misleading results as a consequence of the interrogation of IR opaque material such as condensed chromatin: in contrast, PTMS allows for the generation of true absorption IR spectra. The multivariate data analyses enable identification of the molecular groups responsible for these differences. We have employed this approach to generate a spectroscopic fingerprint for susceptibility-to-adenocarcinoma in the prostate; to monitor cell cycle distributions in a mammary cell line; and to discriminate different categories of exfoliative cervical cytology. The same techniques may be used to identify a new type of marker for stem cells, again pointing to the wide applicability of this tool to address unresolved questions in other areas of biomedical research such as regenerative medicine.

[1] F L Martin and H M Pollock, in: Oxford Handbook of Nanoscience and Technology **2** (A V Narlikar and Y Y Fu, eds.), 2009, pp. 285-336 ;
http://www.lancs.ac.uk/depts/physics/research/condmatt/micro/pages/microtherm.htm

Processed spectral data acquired from epithelial cells lining glandular elements of prostate tissue from one patient, acquired using synchrotron FTIR microspectroscopy. The spectra were collected from particular tissue regions (peripheral zone, ●, black; transition zone, ■, blue; and cancerous zone, ▲, red). With processing by PCA-LDA (principal component analysis / linear discriminant analysis), the spectra clearly separate into clusters (not shown here).

Three loadings plots are shown, corresponding to the cluster vectors for the three tissue types, and processed using PCA-LDA. The bottom curve shows the result of analysing the same data using a new stepwise LDA method. Important spectral wavenumbers appear as either peaks or troughs in the three PCA-LDA plots, and as troughs in the stepwise LDA plot. As shown by the arrows, the agreement is satisfactory. The cancerous zone (CaP) is distinguished by the wavenumbers 1135 cm^{-1} (C-O ring vibrations of nucleic acid "sugars"), 1110 cm^{-1} (phosphate species such as adsorbed $H_2PO_4^-$, also C=O stretching and bending of ketones, C-H in-plane vibrations of polyimide aromatic rings), 1075 cm^{-1} (PO_2^- symmetric stretching vibrations of nucleic acids and phospholipids), and 1050 cm^{-1} (ribose-phosphate main-chain vibrations).



Quantifying the dielectric constant of biomembranes and lipids bilayers with atomic force microscopy on insulating substrates

Laura Fumagalli[1,2], Georg Gramse[1,2], Aurora Dols[1,2], Daniel E. Ferrer[1], Martin A. Edwards[1] and Gabriel Gomila[1,2]

[1]*Institute for BioEngineering of Catalonia (IBEC) and* [2]*University of Barcelona c/ Martí i Franquès 1, 08028 Barcelona, Spain*

The dielectric constant is a fundamental parameter of cell bioelectricity, as it quantifies the intrinsic dielectric behaviour of the plasma membrane at low frequencies (< 1MHz) in processes such as membrane potential formation, action potential propagation or ion membrane transport. It also determines the cell response to electric fields employed in bioelectrical techniques, such as dielectrophoresis, impedance spectroscopy or electroporation. Furthermore, probing the dielectric constant can be a route to nanoscale compositional mapping of biomembranes. However, very little is known about the dielectric constant of biomembranes, since it cannot be laterally resolved by standard dielectric characterization techniques, limited to micrometer-scale spatial resolution. Although many scanning probe microscopy (SPM) techniques have been developed to measure electrical properties at the nanoscale [1], e.g impedance, capacitance, surface potential, polarization forces and piezoelectric response, the *quantification* of the low-frequency dielectric constants of biomembranes by AFM has been demonstrated only very recently [2,3] for the case of using conductive supports.

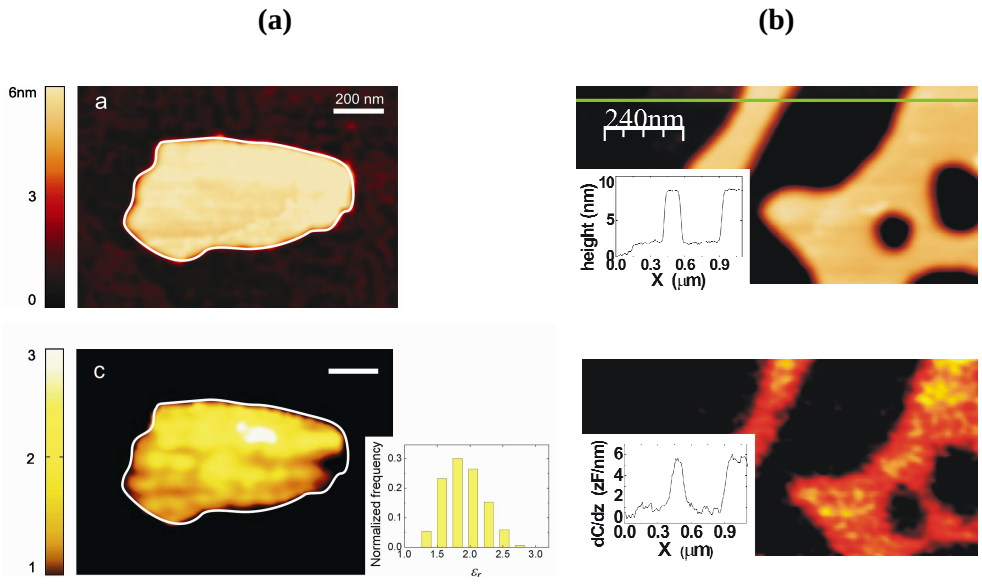


Fig. 1 (a) Topography and dielectric image of a patch of Purple Membrane on graphite [2], showing a dielectric constant of ~ 2. (b) Topography and capacitance gradient images of a freeze-dried DPPC lipid bilayer on mica. The electric signal shows an excellent spatial resolution (<50 nm) thanks to the high sensitivity of the AC-EFM measurements (<1 zF/nm).

Here we extend these previous results to the more challenging and interesting case of biomembranes on millimeter-thick insulating supports, such as mica and glass cover slips, conventionally used in the study of biomembranes at the nanoscale. To this end we have implemented a state-of-the-art ac electrostatic force microscope (ac-EFM) and developed large scale finite-element calculations in order to map and quantify at the nanoscale level the dielectric constants of supported biomembranes on insulating substrates [4]. Results obtained on lipid bilayers and protein monolayers will be presented as examples of application of this technique.

References: [1] S. Kalinin and A. Gruverman, eds., Scanning Probe Microscopy, 'Electrical and electromechanical phenomena at the nanoscale' (New York, Springer, 2007). [2] L. Fumagalli, G. Ferrari, M. Sampietro and G. Gomila, *Nano Lett*, **9** (2009) 1604. [3] G. Gramse, I. Casuso, J. Toset, L. Fumagalli and G. Gomila, *Nanotechnology* **20** (2009) 395702. [4] L. Fumagalli, G. Gramse, D. E. Esteban, M. A. Edwards and G. Gomila (submitted).

Expanding the temporal and spatial scales in scanning force microscopy

Miklós S.Z. Kellermayer, Zsolt Mártonfalvi, Balázs Kiss, Ünige Murvai, Attila Nagy*, Árpád Karsai*, Pasquale Bianco*, András Kengyel*, Tamás Huber*, Margit Benke*, Brennan Decker* and László Grama*

Dept. Biophysics and Rad. Biol. Semmelweis University, Budapest Hungary
**Dept. Biophysics, University of Pécs, Pécs Hungary*

The atomic force microscope is a high-resolution scanning-probe instrument which has become an important tool for cellular and molecular biophysics in recent years. However, it lacks the time resolution and functional specificities offered by other methods. An important aim of our research is to expand the spatial and temporal scales of AFM either by combining it with fluorescence or by employing alternative scanning schemes on unique biomolecular systems.
To simultaneously exploit the advantages of AFM and fluorescence, we developed a spatially and temporally synchronized total internal reflection fluorescence and atomic force microscope system (**Figure 1.a**). The instrument is a stage-scanning device in which the mechanical and optical axes are co-aligned to achieve spatial synchrony. At each point of scan the sample topography (AFM) and fluorescence (photon count or intensity) information are simultaneously recorded. The tool was tested and validated on various cellular (monolayer cells in which actin filaments and intermediate filaments were fluorescently labeled, **Figure 1.a**) and biomolecular (actin filaments and titin molecules) systems. Using the technique correlated sample topography and fluorescence images can be recorded, soft biomolecular systems can be mechanically manipulated in a targeted fashion, and the fluorescence of mechanically stretched titin can be followed with high temporal resolution.
To enhance the temporal resolution of AFM for the purpose of following fast biomolecular events, we applied a simply modified technique called scanning force kymography (**Figure 1.b**). Using the method we monitored the growth, on mica surface, of individual amyloid fibrils with near-subunit (~1 nm) spatial and subsecond (~300 ms) temporal resolution. Amyloid fibril assembly was polarized and discontinuous. Bursts of rapid, up to 300 nms^{-1} growth phases that extended the fibril by ~8 nm or its integer multiples were interrupted with pauses. Amyloid assembly may thus involve fluctuation between a fast-growing and a blocked state in which the fibril is kinetically trapped because of intrinsic structural features. The employed scanning force kymography method may be adapted to analyze the assembly dynamics of a wide range of linear biopolymers.

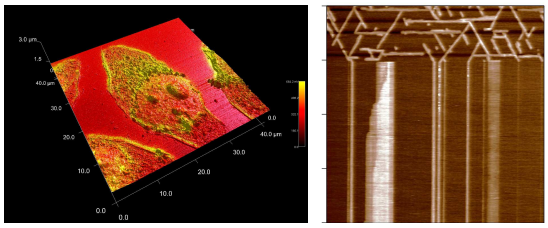


Figure 1. a. TIRF/AFM image of a HeLa cell labeled for actin. **b.** Kymographic recording of Aß25-35 fibrils on mica. The spatial domain image (top) converts to temporal-domain kymogram (bottom).

Using microcantilevers to count biological bonds

Todd Sulchek

Georgia Institute of Technology, Atlanta, GA, USA

This talk will describe our method of using single molecule dynamic force spectroscopy to determine the binding strength of antibody-protein complexes as a function of interaction valency in a direct and simple measurement. It is well known that the adhesion force increases with an increasing number of biological bonds. However, because the rupture force of biological bonds will vary due to their stochastic nature, it is problematic to definitively determine the number of bonds, or the valency, of a particular interaction. We show that the compliance of nanomechanical polymer tethers correlates with the number of biological bonds and can therefore be used to count the number of bonds formed. Mechanical work will disrupt these bonds and can be used to quantify the overall kinetics of dissociation. The ability to form, count and dissociate biological bonds with nanomechanical forces provides a powerful method to study the physical laws governing the interactions of the biological molecules.

Selected short oral presentations of sessions IV, V, VI

Chair: Jean-Luc Pellequer

CEA Marcoule, France

AFM in drug discovery and development – Application opportunities and technology needs

[1]**Kindt, Johannes** H, [2]Lauer, Matthias E.

[1]Veeco GmbH,,Dynamostr. 19, 68165 Mannheim, Germany
[2]F. Hoffmann-La Roche Ltd., Discovery Technologies, Molecular Structure Research, CH-4070 Basel, Switzerland

For over a decade AFM was explored as a research tool in the Pharmaceutical Industry. The performed applications are diverse and cover the broad experimental capabilities of AFM. Once a biologically active molecule is selected for further development the solid state properties of the material are in focus. In addition to well established methods that characterize the bulk properties of the material AFM is about to become a routine method for surface analysis of both API and excipients. In particular the characterization of API polymorphism and API – excipient physico-chemical compatibility were addressed with AFM methods. In addition powder particle characterizations were performed in order to clarify unexpected milling properties or powder flow ability of newly discovered compounds. So far, the AFM has not made the leap to the status of a «Standard Tool» in this area. However, we expect that recent and future advances in AFM technology will enable routine applications of AFM in pharmaceutical industry

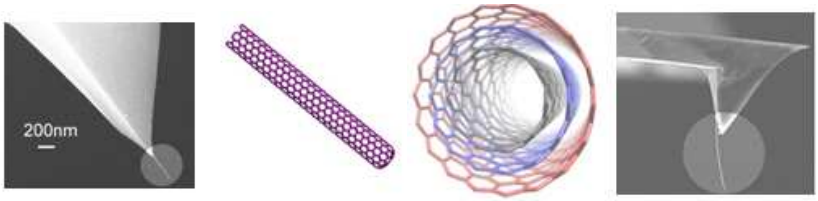
The class of problems for which the AFM can bring unique capability to drug development will be explored. Furthermore, a recently completed study of an assay to profile API-excipient miscibility and stability will be presented in detail. Considerations for AFM tool and application design for pharmaceutical applications will also be discussed.

How to make a fresh start with carbon nanotube probes?

Sophie Marsaudon, Julien Buchoux, Anne Marie Bonnot*, Cattien V. Nguyen** Jean-Pierre Aimé

*Chimie Biologie des Membranes et Nano-objets, Université Bordeaux 1, Bordeaux, France- *Institut Néel, Grenoble, France- *NASA Ames research center, CA, USA*

Carbon nanotubes are man-made nanosized cylinders made of carbon atoms discovered in 1991.With their small size, their excellent mechanical properties and their high aspect ratio, carbon nanotubes solve many tip related difficulties and problems. Spectacular demonstrations of carbon nanotube tips contributions have been published since 1996 on various samples including antibodies, fibrils, DNA,... Despite the nanotube tips high intake, the use of such carbon nanotube tips hasn't spread as much as expected. Two main reasons explain why the development of carbon nanotube tips isn't as large as expected: the difficult control of the nanotube length orientation and attachment to tip, and the complex mechanical nanotube behavior.
With the latest synthesis progress and the large literature on nanotube tips specific properties, it may be the time now for their development. Two strategies for nanotube tip fabrication will be presented: either direct growth of single walled nanotubes (made of one cylinder) on commercial tips, or synthesis of multi walled nanotubes (many cylinders with the same axis) on a filament followed by manual attachment to a tip (see figure). Examples of applications will be given.



From left to right : scanning electron microscopy images of a 600nm long single walled nanotube on an AFM tip , a model of a single walled nanotube; model of a multiwalled nanotube and scanning electron microscopy image of a 10µm long multiwalled nanotube.

Reference:
S. Marsaudon, C. Bernard, , D. Dietzel, C. V. Nguyen, A. M. Bonnot, J. P. Aimé, R. Boisgard,. "Carbon nanotubes as scanning Microscopy tips, understanding carbon nanotube tips mechanical properties and consequent use for imaging", chapter 4 in "Applied Scanning Probe Methods", pp 137-181, Vol 8-10, edited by B. Bhushan, H. Fuchs and M. Tomitori, Springer – Verlag, Heidelberg.(2008).

Insulated nanoneedle probes for combined atomic force and scanning electrochemical microscopy (AFM-SECM)

Romaneh Jalilian*, Olga S. Ivanova**, Jose E. Rivera,* Francis P. Zamborini**, **Mehdi M. Yazdanpanah***

**NaugaNeedles LLC, Louisville, KY*
***Department of Chemistry, University of Louisville, Louisville, KY*

The combination of atomic force microscopy (AFM) and scanning electrochemical microscopy (SECM), known as AFM-SECM, is highly desirable in order to correlate surface chemical information (reactivity, porosity, catalytic activity, etc.) obtained by SECM with high resolution topographic information obtained by AFM. The addition of AFM to SECM allows better control and knowledge of the distance between the tip and the sample and improved spatial resolution for determining the location of the electrochemical information. To make this possible, it is necessary to fabricate conductive AFM probes that are electrically-insulated everywhere except at the very end of the tip. There are no such commercially available probes, requiring scientists to fabricate custom probes, usually requiring several fabrication steps and high cost.
In this work, we report a commercially viable method for low-cost fabrication of AFM-SECM probes. The first step involves the fabrication of conductive high aspect ratio AFM probes by growing metallic silver-gallium (Ag2Ga) nanoneedles on an AFM tip (*NeedleProbes* TM) [1]. Next, we mount the NeedleProbes onto an alumina substrate, wire and then insulate them with non-conductive glue (except the cantilever and area close to it). We then coat the entire device with parylene followed by a hydrophobic monolayer layer of perfluorodecyltrichlorosilane (FDTS) to decrease the wetting property of the parylene, thereby decreasing liquid diffusion into pinholes. Finally, we cut the insulated nanoneedle to expose a conductive region at the end. We will report three simple methods for exposing the end of the nanoneedle. To enhance the electrochemical stability of the probes, we coat the very end of the probe with Pt by galvanic exchange of Ag2Ga with PtCl42- by dipping the very end of the needle into a solution of K2PtCl4 in water. Due to cylindrical geometry of the nanoneedles, a stable meniscus forms between the end of the needle and the liquid surface that results in the formation of a sharp Pt tip at the end of the coated needle.
The probes fabricated as described above performed well electrochemically when tested with cyclic voltammetry as the working electrode with a Pt counter and Ag/AgCl reference electrode in an aqueous solution containing 5 mM Ru(NH3)63+ dissolved in 0.1 M KClO4. The completely insulated nanoneedles displayed a leakage current less than 10 pA and no discernable Faradaic current from the Ru(NH3)63+/2+ wave. Exposing the end of the probe by cutting led to characteristic sigmoidal voltammograms for several tips with steady-state currents ranging from 50 pA to nA values, as expected for electrodes on the nano/micro scale. Future studies will explore the use of these probes for scanning cardiomyocyte cells in buffer solutions.

[1] www.nauganeedles.com

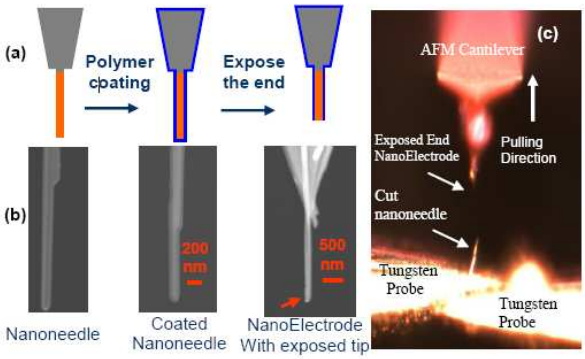


Figure 1. Nanoneedles are used as templates to fabricate more complex devices. (a) Schematic of the fabrication steps. (b) SEM images of the device in different stages. (c) Optical image of a coated nanoneedle that is being cut and exposed at the end.

Detection of populations of amyloid-like protofibrils with different physical properties

Ranieri Rolandi[*], Annalisa Relini[*,§], Silvia Torrassa[*], Riccardo Ferrando[*], Silvia Campioni[†], Fabrizio Chiti[†,§], Alessandra Gliozzi[*]

[*]*Department of Physics, University of Genoa, and Consorzio Nazionale Interuniversitario per le Scienze Fisiche della Materia (CNISM) Via Dodecaneso 33, 16146 Genoa, Italy*
[†]*Department of Biochemical Sciences, University of Florence, Viale Morgagni 50, 50134 Florence, Italy*
[§] *Consorzio Interuniversitario "Istituto Nazionale Biostrutture e Biosistemi" (I.N.B.B.), Viale delle Medaglie d'Oro, 305, 00136 Roma, Italy*

Amyloid aggregation is involved in several fatal diseases, such as Alzheimer's disease, Parkinson's disease and systemic amyloidoses. HypF-N (MW 10452 Da) is the N-terminal domain of a bacterial hydrogenase maturation factor. This protein undergoes amyloid aggregation in the presence of trifluoroethanol (TFE) and its prefibrillar aggregates are toxic to cultured cells. Therefore, it represents a useful model to study amyloid formation and aggregate toxicity [1].
We used tapping mode atomic force microscopy to study the morphology of the amyloid protofibrils formed at fixed conditions by self-assembly of HypF-N. Although all protofibrils in the sample share a beaded structure and similar values of height and width, an accurate analysis of contour length and end-to-end distance and the comparison of experimental data with theoretical predictions based on the worm-like chain model show that two different populations of protofibrils are present. These populations are characterized by different persistence lengths, bending rigidity and Young's modulus. Fluorescence quenching measurements on earlier globular intermediates provide an independent evidence of the existence of different populations. The finding that differences in mechanical properties exist even within the same sample of protofibrils can be correlated to the presence of different sub-populations of pre-fibrillar aggregates with potentially different toxicity [2] . This study describes a strategy to discriminate between such different sub-populations that are otherwise difficult to identify with conventional analyses.

[1] A. Relini, C. Canale, S. Torrassa, R. Rolandi, A. Gliozzi, C. Rosano, M. Bolognesi, G. Plakoutsi, M. Bucciantini, F. Chiti and M. Stefani. Monitoring the process of HypF fibrillization and liposome permeabilization by protofibrils. *J. Mol. Biol*. 338 (2004), 943-957.

[2] S. Campioni, B. Mannini, A. Pensalfini, M. Zampagni, C. Parrini, E. Evangelisti, A. Relini, M. Stefani, C. M. Dobson, C. Cecchi, F. Chiti. A causative link between the structure of aberrant protein oligomers and their toxicity. *Nature Chem. Biol.* 6 (2010), 140-147.

Characterization of self-oscillating Soft Imaging by means of photothermal excitation

Massimo Vassalli[1], Paolo Paoletti[2] , Michele Basso[3], Valerio Pini[4], Bruno Tiribilli[2]

[1]*Institute of Biophysics, National Research Council (IBF-CNR), Genova, Italy*
[2]*Istitute of Complex Systems, National Research Council (ISC-CNR), Firenze, Italy*
[3]*Systems and Informatics Dept., University of Firenze, Firenze, Italy*
[4]*BioNanoMechanics Lab, Nat. Center for Microelectronics IMM-CNM (CSIC), Madrid, Spain*

Since its invention in 1986, atomic force microscopy (AFM) has been widely used as a tool for investigating material characteristics at a nanoscale level and its improvement has rapidly led to reliable and reproducible methods of imaging of biological specimens. Such soft samples can be imaged in liquid without severe distortions by using dynamic mode AFM, where the cantilever is typically excited near its resonance frequency by a dither piezo and the measuring system senses the amplitude of the oscillations during the scan. Besides its simple implementation scheme, largely used in commercial instruments, this working mode (AM-AFM) presents some drawbacks limiting the effectiveness of the measurement procedure. As an important enhancement, several self-driven (SD-AFM) excitation schemes have been proposed, in which the excitation signal is provided by a positive feedback of the cantilever deflection that induces self-sustained oscillations, whose frequency is related to the tip-sample distance and used as feedback signal. Although such operating mode has the potential of achieving higher resolution, the complex nature of the frequency shift signal makes it difficult to be used in most imaging applications. Recently, some novel methods (AT-AFM) have been proposed with the goal of combining the benefits of the above classes of dynamic AFMs [1], [2]. These approaches are based on some feedback mechanisms providing the cantilever excitation, similarly to SD-AFM, although the measured quantity is still the oscillation amplitude as in AM-AFM.
In this work we report a detailed experimental characterization of transient behavior and imaging performance of AT-AFM, compared with standard AM-AFM. Moreover, an additional degree of freedom provided by the presence of a variable saturation threshold in control loop is exploited to design a novel control algorithm (ATC-AFM) which greatly decreases the tip-sample mean interaction force yet achieving better resolutions. This soft imaging mode has been tested in air and in water by using photothermal excitation of the cantilever [3], thus obtaining a stable and reproducible dynamical characterization.

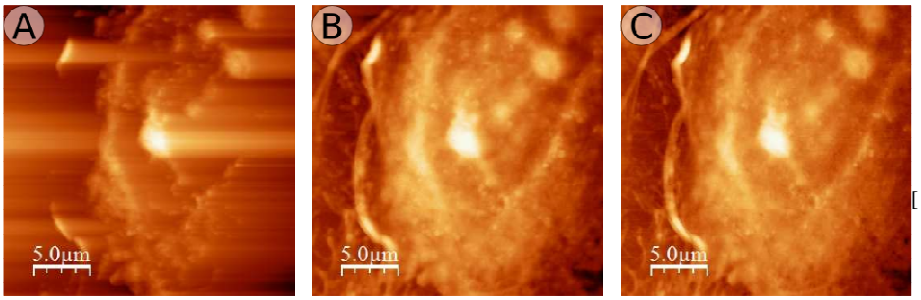


Image of biological sample in different conditions. A] Standard AM-AFM with an amplitude threshold of 90% of the free amplitude (soft). B] Standard AM-AFM with an amplitude threshold of 78% of the free amplitude (hard). C] Image in ATC-AFM with an amplitude threshold of 90% of the free amplitude (soft).

[1] M.Basso, P.Paoletti, B.Tiribilli, M.Vassalli, *Modelling and analysis of autonomous micro-cantilever oscillations*, Nanotechnology, 19, 475501 (2008)
[2] G. Prakash, S. Hu, A. Raman, and R. Reifenberger, *Theoretical basis of parametric-resonance-based atomic force microscopy*, Phys. Rev. B 79, 094304 (2009)
[3] V.Pini, B.Tiribilli, C.M.C.Gambi, M.Vassalli, *Dynamical characterization of vibrating AFM cantilevers forced by photothermal excitation*, Phys. Rev. B 81, 054302 (2010)

Fonctionnalized Si(111) surfaces with tunable surface chemistry: New atomically flat platforms for the anchoring of bio-molecules for AFM characterization purposes

C. Henry de Villeneuve, J. S. Andresa, P. Allongue, L. Touahir, A. Moraillon, A. C. Gouget-Laemmel, J.-N. Chazalviel and F. Ozanam

LPMC - UMR 7643 CNRS – Ecole Polytechnique – France

The anchoring of bio-molecules on atomically flat surface is key issue for the investigation of bio-interactions at molecular scale by AFM. Very few substrates answer to the prerequisites (flatness, inertness, adapted interaction strength to immobilize bio-molecules) for the implementation of the technique which explains that mica remains currently the substrate mostly used even though the electrostatic immobilization of the bio-molecules on its surface originates some limitations.
In this talk, we will present our works dealing with the preparation of atomically flat functionalized Si(111) surfaces which open interesting alternative routes for bio-molecules anchoring. The Si(111) surfaces are hydrogenated and prepared atomically flat by anisotropic chemical etching in NH_4F solution[1] and then after functionalized by grafting a monolayer of alkyl chains bearing carboxylic (-COOH, -COOR) end-groups. By coupling AFM characterizations at molecular scale and quantitative FTIR measurements we have developed and optimized protocols which allow i) the preparation of COOH functionalized Si surfaces with well controlled chemical composition[2], ii) the conversion of the COOH groups into succimidyl activated ester allowing iii) the subsequent covalent coupling of amino modified entities through amidation (fig.1). At each step, particular attention has been paid on the control of surface composition and on the preservation of the well defined atomic structure of the initial H-Si(111) surface. These functionalized Si surfaces exhibiting flat terraces separated by atomic steps are well suitable platforms for AFM characterizations of individual biological recognition events and/or surface interaction/adsorption phenomena. Compare to other substrates, the physico-chemical properties of the surfaces can be adjusted allowing various kinds of bio-molecules anchoring (covalent, electrostatic…), variable density of anchoring sites, tuning of the bio-molecules environment... First results concerning the covalent anchoring of DNA strands on these surfaces will be presented.

References:
[1] M.L Munford and al. Sens. Mater (2001) 13, 259
[2] A. Faucheux and al. Langmuir (2006) 22, 153

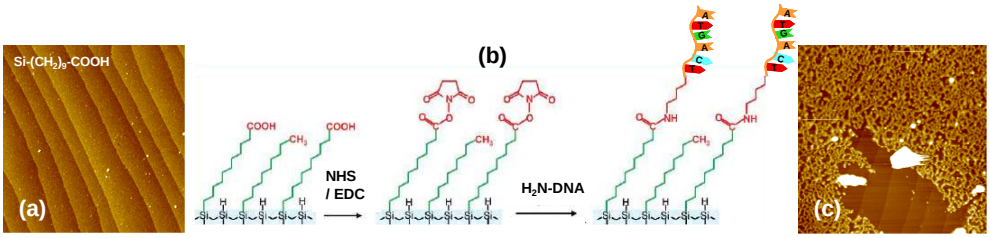


Figure 1: (a) AFM image showing the atomic stair-like structure of a Si(111) surface functionalized with alkyl chains bearing COOH end-groups. The flat terraces are ~ 100 nm large, separated by parallel atomic steps 3.1Å height and homogeneously covered by an acid monolayer (3 10^{14} acid chains/cm^2). (b) Multi-step protocol for the covalent linkage of amino modified DNA single strands. (c) AFM image showing the anchorage of a DNA monolayer on a NHS-activated surface.

SESSION V

AFM Bio II

Chair: Paolo FACCI
S3-INFM-CNR, Modena, Italy

Invited Lecture

Quantifying the dynamic of nucleosomes with AFM

John van Noort

Physics of Life Processes Huygens Laboratories, Leiden University, Leiden, The Netherlands

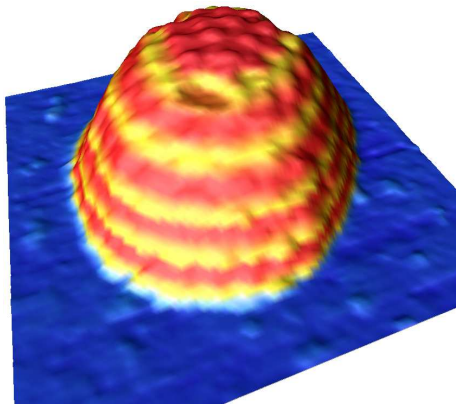
In the past decade it has become clear that all processes involving DNA are regulated in eukaryotes by modulation of its packaging into chromatin. In chromatin an octamer of histone proteins tightly wraps 1.7 turns of DNA, forming a nucleosome. About 75% of all eukaryotic DNA is wrapped into nucleosomes, which sterically hinder its accessibility by transcription factors, repair enzymes and other factors that interact with DNA. Occlusion of DNA by nucleosomes can de lifted by ATP-dependent remodeler enzymes, which are DNA based molecular motors that translocate nucleosomes along the DNA. Here I will discuss how nucleosomes move over DNA and how we used Atomic Force Microscopy to unravel the mechanics of such dynamics.
There is an intricate interplay between the DNA sequence itself and the position of the nucleosomes on the DNA. Using the 601 nucleosome positioning element for reconstitution of nucleosomes we show that we obtain a homogeneous population of well positioned nucleosomes. In the same field of view we resolved the substructure of the 1.7 MD remodeler complex RSC, featuring a large cavity that can encompass the nucleosome. By imaging, careful quantification and monte-carlo modelling of the reaction products after ATP dependent remodelling, we show that the processivity of translocation is strongly modulated by DNA sequence and we propose a kinetic model that describes the reaction cycle.
Using a pair of nucleosomes reconstituted on the DNA of the Mouse Mammory Tumor Virus, we show that a brief exposure to elevated temperatures is sufficient to move nucleosomes along the DNA. In fact the nucleosomes approach and invade each other's DNA. With AFM we show that such nucleosomes loose the H2A-H2B histone, but retain the possibility to stack on each other, which induces higher order structure in chromatin. Overall, analysis of nucleosome positions and sizes reveals the physical mechanisms that regulate DNA accessibility in model chromatin.

A combined imaging and force spectroscopy approach reveals the mechanical structure of viral nano-particles

Wouter H. Roos, Marian Baclayon, Gijs J. L. Wuite

Natuur- en Sterrenkunde, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, the Netherlands

Force spectroscopy measurements are becoming increasingly popular to study the material properties of macromolecular assemblies such as viral shells (capsids) [1]. Here we present AFM nanoindentation studies on the mechanical properties of various viral capsids. Combining these measurements with high resolution imaging of the viral nano-particles before and after indentation it can be observed how single capsomeres (the viral structural units) are removed out of the icosahedral capsid lattice [2]. We furthermore biochemically remove the 12 vertices, the pentons, of the herpes capsids (see figure) and test the effects on the material properties of an icosahedron with missing vertices. In the light of changes in the particle spring constant and breaking force we discuss the influence of the icosahedral corner points on the mechanical stability of the particle. In addition we show how finite element modelling and molecular dynamics simulations on the deformation of viral shells by AFM cantilever tips can give additional essential insights into the viral material properties. In particular accurate values of the shell's Young's modulus and the molecular basis of irreversible deformation of viruses are extracted from the comparison of experimental and modelling results.



AFM image of the icosahedral shell of a herpes simplex virus particle. Individual capsomeres and the holes left by the removed pentons can be observed.

[1] W. H. Roos, G. J. L. Wuite, Nanoindentation studies reveal material properties of viruses, Adv. Mat., **21** (2009) 1187

[2] W. H. Roos, K. Radtke, E. Kniesmeijer, H. Geertsema, B. Sodeik, G. J. L. Wuite, Scaffold expulsion and genome packaging trigger stabilization of Herpes Simplex Virus capsids, Proc. Natl. Acad. Sci. U. S. A., **106** (2009) 9673

Imaging Bacterial Cell Death Induced by Antimicrobial Peptides in Real Time using high speed AFM

Georg E. Fantner[†‡], Roberto J. Barbero[*], David S. Gray[†], Angela M. Belcher[†*].

[†]*Biomolecular Materials Group, Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge MA, 02139 USA*
[†]*Biomolecular Materials Group, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge MA, 02139 USA*
[†] *fantner@mit.edu*

Antimicrobial peptides (AMP) are a promising class of antimicrobial agents in the battle against bacteria that have built up resistance to conventional antibiotics. The mechanism by which these peptides kill bacteria is still poorly understood. The interaction of the peptides with the bacterial cell walls has been suggested to be responsible for the bactericidal effect (*1-3*). Thus far, this interaction has not been visualized nor is there much data on the kinetics of this interaction. Traditional Atomic Force Microscopy (AFM) is a useful tool for observing the changes in the cell wall of living cells (*4*), but due to it's slow image acquisition speed it can not provide insight into the dynamics of the mechanism. We have used custom-built AFM components based on small cantilevers (*5*) to image for the first time the bactericidal action of antimicrobial peptides in real time. With this system, we investigated the activity of the chimeric AMP CM15 with nanometer spatial- and seconds temporal-resolution on live E.coli cells. We observed rapid changes in surface morphology of the cells after injection of the AMP (see figure 1), with a response time that differs between individual monoclonal bacteria in the same image (see figure 2). Using combined AFM and fluorescence microscopy, we correlated the change in cell morphology to cell wall permeability and cell death (*6*).

The results of this study show that high-speed atomic force microscopy can reveal distinctively different behavior of individual cells than compared to the behavior of bulk cells. In the case of the antimicrobial peptides, the presence of an incubation-phase gives new indications for the mechanism by which the antimicrobial peptide attacks the bacterium. We believe that this technique will enable a whole new method of characterizing and studying the effectiveness of synthetic antimicrobial peptides.

1. B. Bechinger, *Biochimica Et Biophysica Acta-Biomembranes* **1462**, 157 (Dec 15, 1999).
2. G. Francius, O. Domenech, M. P. Mingeot-Leclercq, Y. F. Dufrene, *Journal of Bacteriology* **190**, 7904 (December 15, 2008, 2008).
3. R. E. W. Hancock, *Current drug targets - Infectious disorders* **2**, 79 (2002).
4. Y. F. Dufrene, D. J. Muller, *Microbial Imaging* **34**, 163 (2005).
5. G. E. Fantner *et al.*, *Ultramicroscopy* **106**, 881 (Jun-Jul, 2006).
6. G. E. Fantner, R. J. Barbero, G. S. Gray, A. M. Belcher, *Nature Nanotechnology* **In press**, (2010).

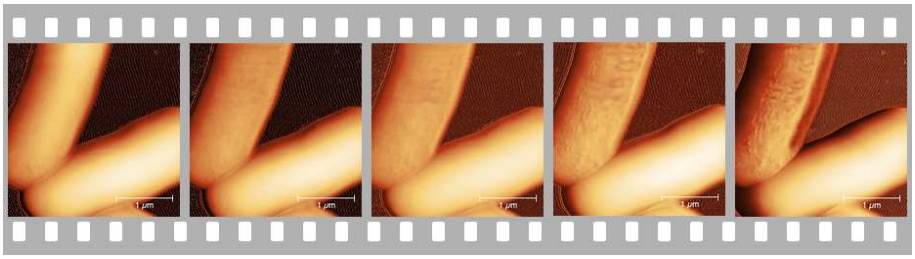


Figure 1: Bacterial response to antimicrobial peptides: The upper bacterium is attacked by the AMP (bacterial surfaces develop a disturbance of the smooth surface) but the lower bacterium still resists the antibiotic. 7 seconds / image

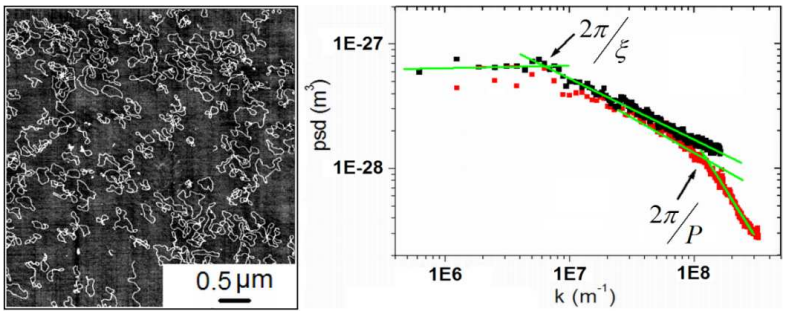
Measurement of DNA morphological parameters at highly entangled regime on surfaces

Annalisa Calò, Pablo Stoliar, Eva Bystrenova, Francesco Valle, Fabio Biscarini

CNR, Istituto per lo Studio dei Materiali Nanostrutturati (ISMN), Via Gobetti 101 - 40129 Bologna (Italy)

The morphology of circular DNA deposited from solution on the mica surface is analyzed from the power spectrum density (PSD) of the AFM images (1-2). DNA exhibits a multi-affine behaviour with two correlation length scales, the shorter one (about 50 nm) which approximates well the persistence length P (3-4) and a longer one (ξ) which can be related to the intermolecular distance. We verify this assumption exploring a broad range of concentration C from isolated molecules to highly entangled networks. As P remains constant within the C range, ξ exhibits a decay increasing C. Applying a diffusion based model in which ξ scales as $\xi \approx D^{-0.25} \cdot C^{-0.5}$ we extracted DNA diffusion coefficient D ≈ 2•10-7 cm2/s. This value is consistent with a high molecular weight plasmid DNA supercoiled in the depositing solution.

1. A. Calò, P. Stoliar, E. Bystrenova, F. Valle, F. Biscarini *J. Phys. Chem. B* 2009, 113, 4987.
2. Biscarini, F.; Samorì, P.; Greco, O.; Zamboni, R. Phys. Rev. Lett. 1997, 78, 2389.
3. Rivetti, C.; Walker, C.; Bustamante, C. J. Mol. Biol. 1998, 280, 41.
4. Rivetti, C.; Guthold, M.; Bustamante, C. J. Mol. Biol. 1996, 264, 919.



An integrated AFM–polarized Raman micro-spectroscopy instrument for investigations of biological nanomaterials

Banyat Lekprasert[1], Victoria Sedman[2], Clive Roberts[2] and Ioan Notingher[1]*

[1]*School of Physics and Astronomy, The University of Nottingham,*
[2]*Laboratory of Biophysics and Surface Analysis, School of Pharmacy*
University of Nottingham, Nottingham NG7 2RD

An integrated instrument based on atomic force microscopy (AFM) and confocal polarized Raman micro-spectroscopy has been developed for investigations of biosamples at the nanoscale. The system is based on an inverted microscope to enable simultaneous high resolution topography images by the AFM as well as chemical properties of the samples by Raman spectroscopy. Polarized Raman spectra allow further analyzing of conformation and orientation of molecules in samples. We will report on using this instrument to investigate diphenylalanine nanotubes (FF-nanotubes)[1] , particularly molecular structure and orientation within the tubes. These nanotubes have potential in many applications, including patterning or drug delivery. The AFM images deliver an accurate measurement of the size of the investigated nanotube while the Raman mappings reveal the distribution of the selected chemical bond. The molecular conformation of the FF-nanotubes can be evaluated from the set of polarized Raman spectra. This integrated instrument offers great potential for advancing the understanding of molecular interactions in the nanotubes. Such understanding is needed for tailoring the properties of the nanotubes for practical applications.

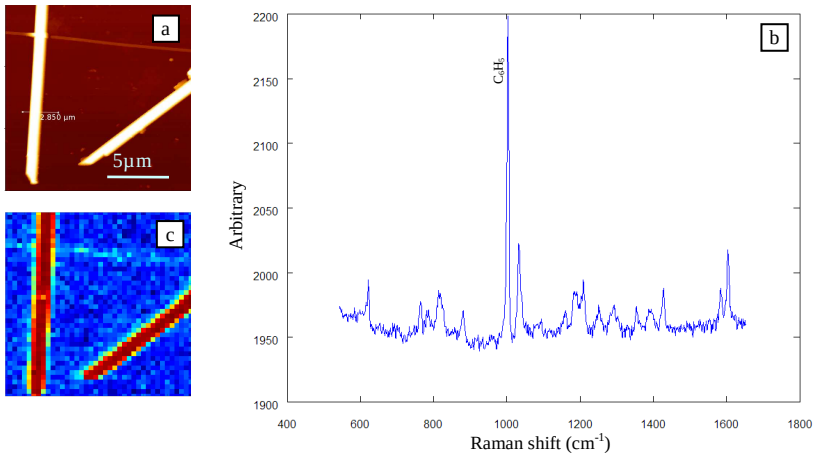


Figure 1: Simultaneous AFM and Raman imaging of FF nanotubes.
a) AFM image of a 1µm FF tube.
b) Typical Raman spectra obtained from a nanotube.
c) Raman imaging of the tube in a) was built by using the intensity of the 1000 cm^{-1} assigned to C_6H_5 breathing vibration of the phenyl group.

[1] C. H. Görbitz, The structure of nanotubes formed by diphenylalanine, the core recognition motif of Alzheimer's β-amyloid polypeptide, Chem. Commun. (2006) 2332-2334

Imaging the nanoscale organization of peptidoglycan in living *Lactococcus lactis* cells

Guillaume Andre[1], Saulius Kulakauskas[2], Marie-Pierre Chapot-Chartier[2], Benjamine Navet[1], Marie Deghorain[3], Pascal Hols[3], and Yves F. Dufrêne[1*]

[1]*Institute of Condensed Matter and Nanosciences - Bio & Soft Matter, Université Catholique de Louvain, Croix du Sud 2/18, B-1348 Louvain-la-Neuve, Belgium*
[2] *INRA, UMR1319 Micalis, Domaine de Vilvert, F-78352 Jouy-en-Josas, France*
[3]*Unité de Génétique, Institut des Sciences de la Vie, Université catholique de Louvain, Croix du Sud 5/6, B-1348 Louvain-la-Neuve, Belgium*

Bacterial cell walls constitute the frontier between the cells and their environment, and therefore play several key functions as supporting the internal turgor pressure of the cell, protecting the cytoplasm from the outer environment, imparting shape to the organism, acting as a molecular sieve and controlling interfacial interactions, i.e. molecular recognition, cell adhesion and aggregation[1,2]. Understanding these functions requires elucidation of the molecular architecture of bacterial cell walls. Particularly, the spatial organization of peptidoglycan, the major constituent of bacterial cell walls, is an important, yet still unsolved issue in microbiology[3].

Here, we show that the combined used of atomic force microscopy and cell-wall mutants is a powerful platform for probing the nanoscale architecture of cell wall peptidoglycan in living Gram-positive bacteria. Using topographic imaging, we find that *Lactococcus lactis* wild-type cells display a smooth, featureless surface morphology, while mutant strains lacking cell wall exopolysaccharides (WPS^-) show 25 nm-wide periodic bands attributed to peptidoglycan. Next, using the recognition properties of LysM[4], we use single-molecule recognition imaging to demonstrate that peptidoglycan in the mutant cells localizes as discrete lines that are parallel to the bands observed in the topographic images.

The single-cell experiments presented here open new avenues for understanding the architecture and assembly processes of peptidoglycan during the cell cycle of Gram-positive bacteria. The use of mutant strains lacking outer cell wall components is a powerful approach to reveal the organization of inner cell wall layers directly in live cells, thus without using any fixation or staining procedures.

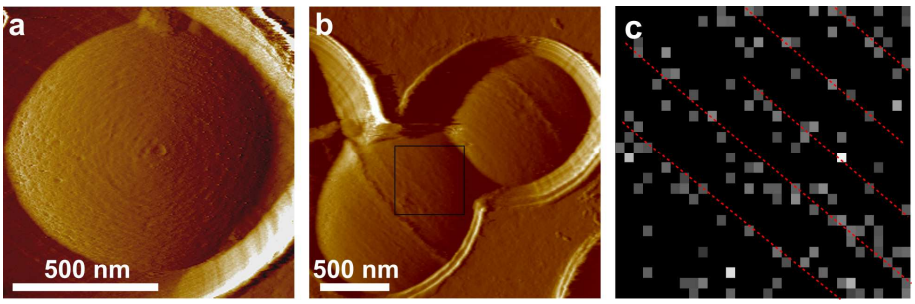


Figure 2 : AFM imaging of the organisation of peptidoglycan in living *L. lactis* cells. AFM deflection images of the (a) polar region and of (b) dividing cells of a WPS^- mutant of *L. lactis*. (c) Single-molecule recognition imaging of peptidoglycan recorded with a LysM tip in the square area shown in (b).

1. Beveridge, T.J. & Graham, L.L. Microbiol Mol Biol Rev **55**, 684-705 (1991).
2. Sleytr, U.B. & Beveridge, T.J. Trends Microbiol **7**:253-260 (1999).
3. Vollmer, W. & Seligman, S.J. Trends Microbiol **18**:59-66 (2010).
4. Andre, G. *et al.* J Bacteriol **190**:7079-7086 (2008).

SESSION V

Plenary Lecture

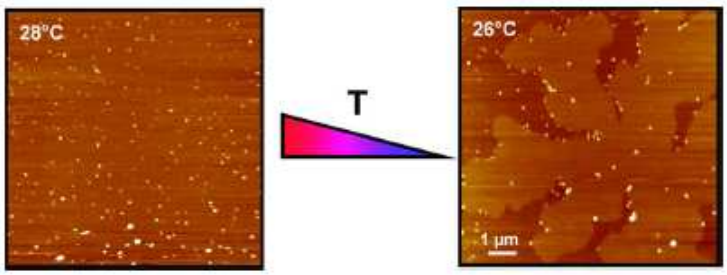
Unravelling lipid-protein interaction in model bilayers by AFM

Andrea Alessandrini[1,2], Heiko M. Seeger[2], **Paolo Facci**[2]*

1 Department of Physics, University of Modena and Reggio Emilia, Modena, Italy,
2 Center S3 CNR-Istituto di Nanoscienze, Modena, Italy.

Atomic force microscopy is particularly suited for the investigation of biological and artificial model membranes at surfaces. Parameters such as ionic strength, pH, temperature, molecular composition can be easily tuned and controlled throughout the experiments. Nucleation and kinetics of domain formation [1, 2] as well as molecular 2d diffusion and clustering [3] can be readily followed by the technique. We report an atomic force microscopy study on the lateral spatial redistribution of an integral membrane protein reconstituted in supported lipid bilayers (SLBs) subjected to a thermally induced phase transition. KcsA proteins were reconstituted in proteoliposomes of POPE/POPG (3:1, mol/mol), and SLBs, including the proteins, were then obtained by the vesicle fusion technique on mica. By decreasing the temperature, the lipid bilayer passed from a liquid disordered (ld) phase, in which the proteins are homogeneously distributed, to a coexistence of solid ordered (so) and ld domains with the proteins preferentially distributed in the ld domains. The inhomogeneous distribution eventually led to protein clustering. The obtained results are discussed, along with functional data obtained on BLM equivalent model systems, in light of the role that the lipid/protein interaction can have in determining the function of integral membrane proteins such as selected ion channels [4].

1. H. M. Seeger, G. Marino, A. Alessandrini, P. Facci "Effect of Physical Parameters on the Main Phase Transition of Supported Lipid Bilayers" Biophys. J., **97** (2009) 1067.
2. H. M. Seeger, A. Di Cerbo, A. Alessandrini, P. Facci "Supported lipid bilayers on mica and silicon dioxide: comparison of the main phase transition behaviour", 2010, submitted.
3. H. M. Seeger, C.A. Bortolotti, A. Alessandrini, P. Facci "Phase transition-induced protein redistribution in lipid bilayers" J. Phys. Chem. B **113** (2009) 16654.
4. H. M. Seeger, L. Aldrovandi, A. Alessandrini, P. Facci "KcsA functionality is affected by the physical state of the hosting lipid bilayer", 2010, submitted.



KcsA molecules are excluded from the so phase and segregated in the ld one upon decreasing T

Keynote Lecture

AFM as an essential tool in Cell and Molecular Biology studies

Dennis E. Discher

Biophysical Eng'g. Lab, University of Pennsylvania, Philadelphia, PA
discher@seas.upenn.edu

Cells generate force and are also exposed to external forces, but the extent of molecular remodeling is not yet clear. Do proteins within cells unfold under force and change in tertiary structure, and/or do the proteins dissociate from each other with changes in quaternary structure due to stress? We have developed a suite of nano-mechanical and chemical approaches to address these questions. Coupled AFM nano-mechano-chemical schemes with purified proteins [1] establish a general methodology for cell studies, and the strong temperature dependence of biomolecular transitions must be appreciated – not only for the special challenges it presents at the single molecule scale [2]. The nanomechanical probing has been further used to characterize the compliance of substrates that cells adhere to and apply stresses to (in proportion to substrate compliance) [3, 4]. This fact together with the entire set of experimental/computational methods can be extended to help identify – within living cells – proteins and their sites that indeed unfold and dissociate under stress [5]. How these processes contribute to the collective self-organization of the cytoskeleton is a topic of ongoing interest [6].

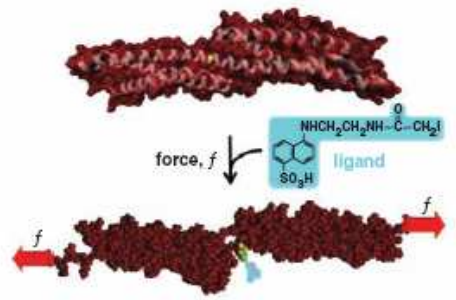


Figure 1: Force-induced changes in protein structure are hypothesized to expose novel binding sites for ligands. This example of a molecular dynamics simulation shows that Cysteine1167 in β-spectrin exposes 0 $Å^2$ surface area (of 224 $Å^2$) until forced extension exposes Cysteine's thiol group for reaction.

References:

[1] P. Carl, C. Kwok, G. Manderson, D.W. Speicher, and D.E. Discher. Forced unfolding modulated by disulfide bonds in the immunoglobulin domains of a cell adhesion molecule. ***Proc. Natl. Acad. Sci. USA*** 98: 1565-1570 (2001).
[2] R. Law, G. Liao, S. Harper, G. Yang, D.W. Speicher, and D.E. Discher. Pathway shifts and thermal softening in temperature-coupled forced unfolding of spectrin domains. ***Biophysical Journal*** 85: 3286-3293 (2003).
[3] A. Engler, S. Sen, H.L. Sweeney, and D.E. Discher. Matrix elasticity directs stem cell lineage specification. ***Cell*** 126: 677-689 (2006).
[4] A.E.X. Brown, R. Litvinov, D.E. Discher, P. Purohit, J. Weisel. Multiscale mechanics of fibrin polymer: Gel stretching with protein unfolding and loss of water. ***Science*** 325: 741-744 (2009).
[5] C.P. Johnson, H-Y. Tang, C. Carag, D.W. Speicher, and D.E. Discher. Forced unfolding of proteins within cells. ***Science*** 317: 663-666 (2007).
[6] A. Zemel, F.Rehfeldt, A.E.X. Brown, D.E. Discher, and S.A. Safran. Optimal matrix rigidity in the self-polarization of stem cells. ***Nature Physics*** (to appear, 2010).

SESSION VI

Nanomedicine

Chair: Hans OBERLEITHNER
University of Münster, Germany

Invited Lecture

Mechanics of Cancer versus Normal Cells: Fundamentals and Possible Applications

Igor Sokolov

Department of Physics, Nanoengineering and Biotechnology Laboratories Center (NABLAB), Clarkson University, Potsdam, NY 13699, USA

Humans are still far from conquering cancer with traditional biochemical methods. There is a hope that physical sciences could bring new insights and provide new ways to attack on cancer. With the development of nanoscience and nanotechnology, scientists have obtained a new set of instruments to study physics of cancer at nanoscale. Atomic force microscopy (AFM) allows collecting unique information about interactions near cell surface, in particular, studying surface properties of viable cells in the physiological environment. In this talk, I will describe the study of mechanical properties of human epithelial cervical, both cancerous and normal, cells. We found quantitative differences between normal and cancerous human cervical epithelial cells by considering a brush layer on the cell surface. These brush layers, which consist mostly of microvilli, microridges, and cilia are important for interacting with the environment. After taking into account the brush, the difference in mechanics of cancerous and normal cells was found to be statistically insignificant. However, the bush lengths and densities turned out to be significantly deferent. Moreover, a small and dense brush was found to be specific only to cancerous cells. To understand the nature of this brush, electron microscopy, specific dye staining, confocal microscopy, etc. were used. However, the nature of this layer is still not completely understood, and will be discussed in this talk.

The discovery of the specific physical feature of cancerous cells opens not only a new way to look at fundamental changes associated with cancer, but also show a possible non-traditional way for cancer detection. Here I will describe a few possible methods of this kind. The accuracy of the detection of cervical cancer with these just pure physical methods seems to be substantially higher than when using the existing biochemical and medical methods. This high sensitivity and selectivity for cancer makes it interesting to perform further statistical tests.

Investigating Angiotensin II AT-1 receptor bias signaling with AFM

Elie Simard, Jeffrey J. Kovacs[*], Robert Lefkowitz[*] and Michel Grandbois

Département de pharmacologie, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Canada
[*]Department of medicine and Immunology/Howard Hughes Medical Institute, Duke University Medical Center, USA

With the emergence of new drug categories such as reverse antagonist or bias ligand, developing sensitive and specific assays to detect subtle cellular events has become an important focus in pharmacology. We have previously used of AFM for real-time monitoring of angiotensin II-induced contractile response in individual cells providing a new perspective on cellular events such as contractility. AT-1 receptor is known to activate a variety of signalling pathways through the heterotrimeric G protein Gq which leads to an increases in intracellular calcium levels with associated cellular contraction in a variety of cellular models. However, AT-1 receptor can also activate a Gq independent pathway involving β-arrestin-dependent signalling through MAPK ERK1/2. In the present study, we use AFM-based force measurements to delineate the contribution of these distinct signalling pathways in cell contraction using the AT-1 receptor bias agonist SII, which is known to signal solely through the β-arrestins. We first establish the Ang II contractile response of HEK 293 cells overexpressing AT-1 receptor using phase contrast microscopy and real-time AFM measurements. In these experiments, the AFM tip is kept in contact with the cell body for 30 min while force data were recorded. As published previously, 1 nM Ang II triggered a cell contraction of approximately 400 nm followed by cell spreading. To identify Gq and β-arrestin-dependent individual contribution, we conducted similar experiments with the AT-1 receptor ligand, SII, specific for the β-arrestin signalling pathway. Surprisingly, no significant cells body contraction was observed by AFM, however intracellular activity could be detected as indicated by the fluctuation in the AFM signal. To further determine the possible contribution of β-arrestin in the contractile response, we measured the mechanical response induced by AngII in cells knock down for type 1 and/or 2 β-arrestins. In this experiment we observed an attenuation of the contractile response. Taken together, our data suggest a supporting role of β-arrestins signalling in Ang II-induced contractile response and that β-arrestin signalling is not sufficient to trigger a contractile response by itself. In conclusion, this study demonstrate how AFM-based force measurement can be used to study of cellular processes involving contraction of the cell body, intracellular reorganisation associated with the movement of the intracellular organelles and cytoskeleton or general cellular morphological activity.

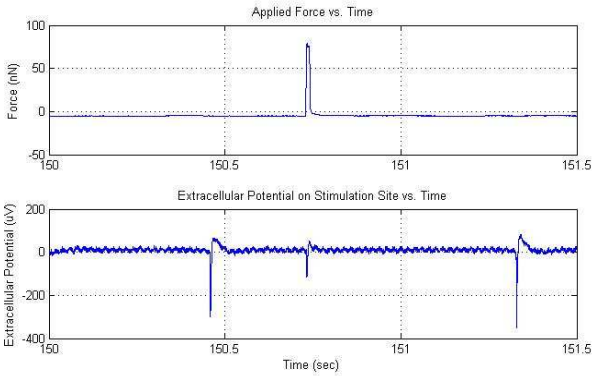
Combining Atomic Force Microscopy with Micro-Electrode Arrays for Studying the Mechano-Electrical Behavior of Cardiac Myocytes

Jose F. Saenz*, M. Tedesco, S. Martinoia, R. Raiteri

Department of Biophysical and Electronic Engineering – DIBE, University of Genova, Via Opera Pia 11A, 16145, Genova - Italy
** E-mail: jose.saenz@unige.it*

In the present work we propose a new analytical platform based on the combination of Atomic Force Microscopy (AFM) with Micro-Electrode Arrays (MEA) for performing precise-and-local mechanical stimulations on living cells while measuring in situ and in real time changes in their extracellular electrical activity and mechanical properties. We applied this combined set-up to mechanically stimulate a single cardiac myocyte (CM) and monitor the induced electrophysiological response. Such approach allows investigation, at the single cell level, of cell cardiac electro-mechanics and Mechano-Electrical Feedback (MEF) phenomenon which is involved in the adjustment of heart rate, the initiation of arrhythmias, and the re-setting of disturbed heart rhythm by 'mechanical' first aid procedures [1]. Despite all the work done in the exploration of the dynamical electro-mechanical properties of the myocardium at the organ and tissue level, the signal transduction in MEF and other effects and mechanisms of beating CMs are not completely understood yet[2, 3]. In order to elucidate the (sub)cellular mechanisms and processes of signal transduction in the cardiac MEF phenomenon, AFM looks particularly promising since it allows the application of controlled low forces, the possibility to measure the mechanical properties at the point of stimulation and a minimal disruption to the membrane. On the other hand, recording of extracellular potentials from contracting CMs without interfering with cell motility or producing undesirable side effects is possible when the cells are grown on a glass surfaces with integrated microelectrodes such as in the MEA technology [4].

We have combined a commercial Agilent 5500 AFM with MEA instrumentation so that the AFM body sits onto a custom made MEA connector while both are positioned onto an inverted light microscope. In this way it is possible to check with accuracy the position of the AFM tip over the MEA and the cell culture. The electrical and mechanical noise in the developed platform is comparable with the noise in the standard configuration of both instruments. In initial experiments we studied monolayers and patterned cultures of CMs from rat embryos. First we characterized the mechanical and electrical spontaneous activity of contracting cells. Then we applied controlled pulses of force, peak force in the range 1nN to 150nN and duration in the range 10ms to 100ms, onto the membrane of cells sitting on top of or near to recording electrodes. We could clearly observe induced electrophysiological responses, especially when applying force pulses greater that 40nN. By controlling the delay between the spontaneous electrical activity and the induced, we observed that the waveforms of the evocated activity are in agreement with the possible activation of stretch-activated ion channels. By analyzing the force vs. distance information of the AFM stimulation we estimated the elasticity of the cell membrane related with the cycle of the spontaneous electrical activity and the induced electrophysiological changes. Preliminary results suggest that the electrical response of CMs to nano-mechanical stimulation is related to the localization f the indentation site and changes in the local mechanical properties of the membrane-cytoskeleton structure.



1. Ravens, U., Mechano-electric feedback and arrhythmias. Progress in Biophysics and Molecular Biology, 2003. 82(1): p. 255-266.
2. Kohl, P., C. Bollensdorff, and A. Garny, Effects of mechanosensitive ion channels on ventricular electrophysiology: experimental and theoretical models. Experimental physiology, 2006. 91(2): p. 307-321.
3. Azeloglu, E.U. and K.D. Costa, Crossbridge cycling gives rise to spatiotemporal heterogeneity of dynamic subcellular mechanics in cardiac myocytes probed with atomic force microscopy. Am J Physiol Heart Circ Physiol, 2009: p. 00427.2009.
4. Egert, U. and T. Meyer, Heart on a Chip — Extracellular Multielectrode Recordings from Cardiac Myocytes in Vitro, in Practical Methods in Cardiovascular Research. 2005. p. 432-453.

Substrate elasticity dominates integrin-ligand availability during cellular adhesion and spreading

Ilia Louban[1,2], Janosch A. Deeg[1,2], Daniel Aydin[1,2], Catharina Cadmus[1,2], Christine Selhuber-Unkel[3], Roberto Fiammengo[1,2] and Joachim P. Spatz[1,2]

[1]*Max-Planck-Institute f. Metals Research, Dept. of New Materials & Biosystems; Heisenbergstr. 3, D-70569 Stuttgart,Germany*
[2]*University of Heidelberg, Dept. of Biophysical Chemistry; Im Neuenheimer Feld 253 D-69120 Heidelberg, Germany*
[3]*Niels Bohr Institute, Optical Tweezers Group, Copenhagen, Denmark*

Properties of the cellular environment e.g. biophysical and biochemical signals play a crucial role in regulating the integrity and functionality of cells and tissues. Compliance of the extracellular matrix (ECM), for example, could be sensed by cells, which results into various biological responses such as changes in proliferation or gene expression. To understand the interplay between crucial parameters like ECM compliance and cellular adhesion-ligand availability artificial ECM analogs are needed, where the environmental stimuli could be altered fully *independently* from each other. These alterations should include several orders of magnitude and thereby reveal functional limits of the investigated cell type.

Here, we present a substrate system acting as a two-dimensional space of environmental parameters, offered to the cells, featuring the biophysical and biochemical properties of connective tissues.

The Young's moduli E_Y of poly(ethylene glycol)-diacrylate (PEG-DA) based hydrogel substrates span more than four orders of magnitude ($0.6\ kPa < E_Y < 6\ MPa$). Since PEG-DA substrates are protein repellent, they were decorated by quasi hexagonally ordered, extended gold nanoparticle arrays, manufactured by block copolymer micellar nanolithography (BCMN). To provide bioactivity in terms of cell adhesion c(RGDfK) peptide, which is specific for $\alpha_V\beta_3$ integrins, was immobilized on the nanoparticles. The interparticle spacing and, hence, spacing of integrin binding sites ΔL could be precisely tuned, *independently* of the substrate rigidity ($20\ nm < \Delta L < 160\ nm$).

This system was used to investigate the behavior of fibroblasts as a function of changes within two-dimensional parameters space (ΔL; E_Y). To this end, cell spreading area and cell-substrate interaction forces were determined by phase contrast microscopy and single cell force spectroscopy (SCFS), respectively. During the experiments both environmental parameters were varied simultaneously. Results from these experiments were determined as a function of hydrogel stiffness and integrin-ligand spacing. They revealed two tactile set points, thresholds in cellular sensing behavior, at $E_Y \approx 8\ kPa$ and $\Delta L \approx 70\ nm$, after 6, 12, and 24 hours of adhesion, respectively.

Moreover, according to the hierarchical phase model in cellular behavior [1], classes of functional protein modules dominate the behavior of spreading cells during a certain phase. From the biophysical point of view, progression in phase transition can be expected as trajectories in multidimensional parameter space. With the presented substrate system we could show that the mechanosensing signal, originating from soft environment, stops phase transitions. In contrast, disturbed integrin clustering, that originates from high spacing of binding sites most likely does not. Therefore, substrate elasticity was identified to be the dominant parameter in cellular sensing processes.

Parallel AFM imaging and force spectroscopy using 2-dimensional probe arrays for applications in cell biology

André Meister, Mélanie Favre, Stefan Dasen, Gabriel Gruener, Réal Ischer, Thomas Overstolz, Joanna Bitterli, Peter Vettiger, Martha Liley, Harry Heinzelmann

CSEM, Centre Suisse d'Electronique et de Microtechnique SA, Neuchâtel, Switzerland

Atomic force microscopy (AFM) is increasingly used in cell biology to study individual cells: to characterize a cell's morphology or to measure its mechanical or adherence properties using force spectroscopy. Today's commercial AFMs use single cantilevers to probe the sample surface. However, AFM based analyses involving living cells are usually extremely time consuming, due to cell dynamics and the need for a large number of identical experiments for statistical reasons. In order to be routinely utilized in R&D e.g. for cell based screening, the method has to become faster and easier to use.

We describe here an instrument to manipulate 2-dimensional cantilever arrays. The deflections of all cantilevers in the array are measured in parallel using a Michelson interferometer. An interferogram captured by a CMOS camera is analyzed using dedicated software to determine the deflection of each cantilever. A special instrumental platform has been developed, that includes the optical read-out, micro- and nanopositioning stages, as well as a fluid chamber to keep the cells in a liquid environment at 37°C. The 2-dimensional AFM arrays were developed and fabricated at CSEM. Cantilevers with and without tips were fabricated: the former to analyze the topography and mechanical properties of cells, and the latter to measure intercellular interaction forces.

First experimental measurements have demonstrated the use of the platform to operate 2D cantilever arrays in air and in liquid. The system was first tested by parallel topographical imaging on fixed and dehydrated cells in air. The 35 topography images shown in the Figure were taken simultaneously by the cantilever array. Individual cells can be distinguished in the inset. The platform was then tested for parallel force spectroscopy on living 3T3 cells in a liquid environment. All force curves were taken simultaneously. The bottom curve in the graph was taken on a hard substrate.

One targeted application of the new system is in oncology, especially research into cancer metastasis. Analysis of intracellular adhesion is relevant to the release of metastatic cells from the primary tumor. Studies of mechanical cell stiffness allow to analyze how reagents can affect the softness of the metastatic cells, the softness of metastatic cells being related to their invasive character. These developments aim to open new research possibilities in the field of metastasis, from fundamental cancer research to pharmacology.

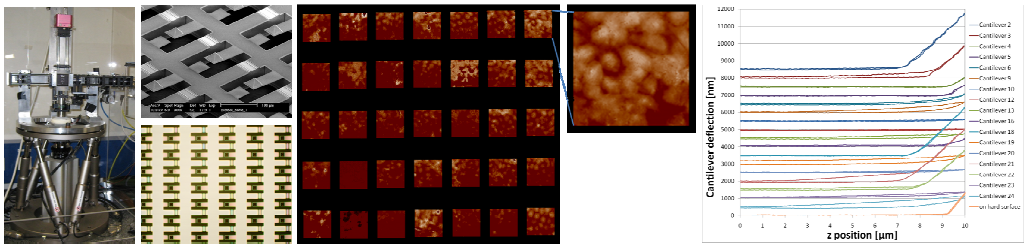


Figure: From left to right: Instrumental platform, AFM probe arrays, parallel imaging on fixed cells, and parallel force spectroscopy on living cells.

Monitoring of mechanical properties of serially passaged bovine articular chondrocytes by atomic force microscopy

Michal J. Wozniak[1,2], Naoki Kawazoe[2,3], Tetsuya Tateishi[2], Guoping Chen[2,3] and Krzysztof J. Kurzydlowski[1]

[1]*Faculty of Materials Science and Engineering, Warsaw University of Technology, 141 Woloska Str., 02-507 Warsaw, Poland*
[2]*Biomaterials Center, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan*
[3]*International Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science,1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan*

Chondrocytes are highly specialized cells responsible for the production and maintaining the integrity of the cartilaginous extracellular matrix (ECM), which contributes strongly to the viscoelasticity of the cartilage tissue. Chondrocytes change their morphology and dedifferentiate during in vitro expansion of the culture. They change gene expression pattern from chondrocyte-specific to type I collagen, which is normally expressed by fibroblasts. In this study, we investigated the effect of the passage number of the living Bovine Articular Chondrocytes (BACs) on their elasticity using AFM. The BACs were isolated from a knee joint of a 6-week-old calf and sub-cultured 5 times to obtain serially passaged chondrocytes (generations P1-P5). AFM images of the living cells are shown in Fig.1A. Freshly isolated chondrocytes (P0) show typically round morphology. Serially passaged on uncoated polystyrene tissue culture dishes cells (P2 and P4) progressively change their morphology to fibroblast-like spindles. The values of Young's modulus, calculated using Hertz model from data obtained by AFM, are given in Fig. 1B. Statistical analysis of the data shows a significant difference among the mean moduli of all groups of chondrocytes, with exception of P3 and P4 passages for which no significant difference is observed.

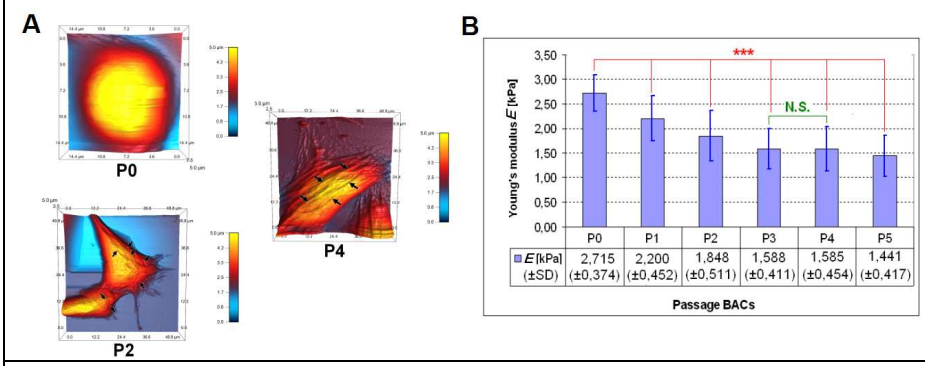


Figure 1. A) AFM images (merged: height and illumination) of living, freshly isolated P0 (scan size 15 x 15 µm) and serially passaged P2 and P4 (scan size 50 x 50 µm) chondrocytes in PBS solution. The Z-scale of images is equal 5 µm. The P0 cells show a chondrocyte-like, round morphology; cells P2 and P4 show a fibroblast-like, spindle morphology. B) Average Young's modulus values calculated for the indentation depth up to 400 nm. Data are presented as mean ± standard deviation. For a single generation of cells, n = 1000 (20 cells, 50 force curves collected for each cell). Results of the parametric Tukey-Kramer test: *** - $p < 0.001$, N.S. - no significant difference.

The dedifferentiation of chondrocytes (phenotype variation), evaluated by changes in cell morphology, was accompanied by the decrease in cell stiffness. The changes in the mechanical properties of cells are most evident for the first two passages (P0-P2) and less significant for the next three generations of cells (P3-P5).

Acknowledgement: This work was supported in part by the WPI Initiative on Materials Nanoarchitectonics and in part by the NEDO Japan. Michal J. Wozniak is awarded the Fellowship for Young Doctors Center for Advanced Studies Warsaw University of Technology.

References: 1. M.J. Wozniak et al., Micron 40 (2009) 870–875. 2. M.J. Wozniak et al., Soft Matter, in review

AFM Based Force Spectroscopy: A New Tool to Probe *Ex Vivo* Vascular Endothelium Rigidity.

Yannick Miron, Charles M. Cuerrier and Michel Grandbois

Département de Pharmacologie, Université de Sherbrooke

Mechanical properties of individual cells are a crucial component of several biological processes involving cell motility, cell contraction and force-mediated cell signalling. Several studies have been conducted using the AFM to quantify cell mechanical properties, making it a valuable tool in the field of single cell biomechanic. In this context, endothelial cells have been widely studied because of there biomechanically responsive abilities. Indeed, in their physiological environment, endothelial cells are continuously exposed to forces from the blood flow mediated shear stress and friction due to the cellular components of the blood plasma. Up to now, most AFM experiments on individual cells have been conducted on cultured cells *in vitro*. In this study, we performed AFM-based force experiments to characterize the elastic properties of endothelial cells in mouse aortas *ex vivo*. The aortas endothelium can be exposed to allow AFM experiments to be conducted on single endothelial cells. Prior to the AFM experiments, the structural integrity of the endothelium was assessed by immunofluorescence detecting the von Willbrand factor as an endothelial cells marker. Then, we conducted elasticity assays on the endothelium by doing indentation force curves to evaluate the young modulus which was found to be 3.35±0.40 kPa. Measurements performed as a control on endothelial cell *in vitro* (1.06±0.03 kPa) were in a similar range value. Additionally, our data are consistent with previously published endothelial cells Young moduli varying from 0.5 to 10 kPa depending on the nature of the cell substrate or which region of the cell was probed. In conclusion, we show that AFM-based force experiments can be used not only to characterize the mechanical properties of cultured cell models *in vitro* but also to evaluate the mechanical state of cells composing a living tissue *ex vivo*. Such experiments may prove to be crucial in elucidating the relationship existing between cell mechanics within the endothelium and the physiological process in blood vessels.

SESSION VI

Plenary Lecture

Nanophysiology of the vascular endothelium

Hans Oberleithner

Institute of Physiology II, University Münster, Germany

A scenario is reported that should demonstrate the application of advanced nanotechniques in the field of cardiovascular research.
Dietary sodium and potassium contribute to the control of the blood pressure. Endothelial cells are targets for aldosterone which activates sodium channels in the apical plasma membrane[1]. The activity of these channels is negatively correlated with the release of nitric oxide (NO) and therefore determines endothelial function [2]. A mediating factor between channel activity and NO release is the mechanical stiffness of the cell's plasma membrane, including the submembranous actin network (the cell's "shell"). Mechanical stiffness of living endothelial cells grown in vitro can be quantified with picoNewton resolution using the atomic force microscope as a nanosensor. Changes of plasma sodium [3] and potassium [4], within the physiological range, regulate the viscosity of this shell and thus control the shear-stress dependent activity of the endothelial NO synthase located in the shell's "pockets" (caveolae). High plasma sodium gelates the shell of the endothelial cell while it is fluidized by high potassium. Accordingly, this concept envisages that communications between extracellular ions and intracellular enzymes occur at the plasma membrane barrier while 90% of the total cell mass remains uninvolved by these changes. In conclusion, endothelial cells are highly sensitive to extracellular sodium and potassium. This sensitivity may serve as a physiological feedback mechanism to regulate local blood flow. Understanding the nanomechanical dynamics of endothelial cells using AFM techniques opens new perspectives in cardiovascular medicine.

References

1. Kusche-Vihrog K, Sobczak K, Bangel N, Wilhelmi M, Nechyporuk-Zloy V, Schwab A, Schillers H, Oberleithner H (2008). Aldosterone and amiloride alter ENaC abundance in vascular endothelium. Pflugers Arch 455: 849-857.
2. Kusche-Vihrog K, Callies C, Fels J, Oberleithner H (2009). The epithelial sodium channel (ENaC): Mediator of the aldosterone response in the vascular endothelium? Steroids: epub ahead of press.
3. Oberleithner H, Riethmuller C, Schillers H, MacGregor GA, de Wardener HE, Hausberg M (2007). Plasma sodium stiffens vascular endothelium and reduces nitric oxide release. Proc Natl Acad Sci U S A 104: 16281-16286.
4. Oberleithner H, Callies C, Kusche-Vihrog K, Schillers H, Shahin V, Riethmuller C, MacGregor GA, de Wardener HE (2009). Potassium softens vascular endothelium and increases nitric oxide release. Proc Natl Acad Sci U S A 106: 2829-2834.

SESSION VII

Opening Round table about future of AFM in Nanomedicine

Chair: Frank LAFONT
Institut Pasteur de Lille, France

Plenary Lecture

Mechanobiological regulation of tumor and stem cell biology in the central nervous system

Sanjay Kumar

Department of Bioengineering, University of California, Berkeley, USA

One of the most exciting breakthroughs in cell biology over the past decade is the recognition that micromechanical inputs to cells from the solid-state extracellular matrix (ECM), such as those encoded in ECM geometry, topography, and elasticity, can influence cell and tissue physiology and pathology in profound and specific ways. This connection between mechanics and biology (mechanobiology) bears direct relevance to the pathogenesis of diseases of the nervous system in which cells alter their structure, motility, or compliance, including neuronal and glial tumors and neurodegenerative disorders, and suggests that specific cell behaviors may be engineered by directly manipulating the underlying molecular systems. I will discuss efforts my colleagues and I have taken to harness the potential of mechanobiological crosstalk between cells and the ECM to understand and manipulate tumor and stem cell biology in the nervous system. I will describe studies in which we elucidate the role of physical cues from the ECM in driving cell structure, cytoskeletal organization, cell migration, and proliferation in malignant brain tumors. I will also discuss our efforts to engineer the differentiation trajectories of neural stem cells by manipulating the biophysical properties of the ECM and the mechanotransductive signaling pathways that enable cells to mechanically communicate with the ECM.

Identification of cancerous cells using AFM

Malgorzata Lekka[1], Piotr Laidler[2]

[1]*The Henryk Niewodniczański Institute of Nuclear Physics Polish Academy of Sciences, Poland*
[2]*Chair of Medical Biochemistry, Collegium Medicum of the Jagiellonian University, Poland*

The determination of mechanical properties of living cells and their capabilities of being an indicator of cancer transformation has become possible with the development of such local measurements techniques as an atomic force microscopy, magnetic or optical tweezers. From the first publication showing the capability of the atomic force microscopy to measure mechanical properties of a single living cell in conditions close to natural one, several publications have been reported the application of AFM as a technique having potential possibility to detect the pathological changes at the single cell level. The correlation between the cell ability to deform, different diseases, and the internal organization of cytoskeleton has been known for a long time. The noticeable change of cancer cell stiffness has been already observed by AFM for bladder cells, where non−malignant cells were one order of magnitude stiffer than cancerous ones. These results highlighted the AFM capability to detect cancer through mechanical analysis. Thus, when oncogenic transformation introduces large changes, cancer cells can be easily detected by both AFM and histomorphological analysis. However, when cells do not differ significantly, their stiffness distributions can overlap, and the detection becomes difficult. Moreover, samples collected from patients can be affected by other factors influencing cell stiffness. Therefore, the number of measured cells should be significantly high, especially for samples from patients where i) the possibility of repeating the experiment and ii) the probability of obtaining identical cancerous cells are rather low. Thus, to apply the cell stiffness as an indicator of cancer alterations, its value should be determined with high cells statistics where for each single cell, stiffness values origin separately from many indentations taken at different positions. This includes the stiffness variations within a single cell and within the non−homogenous population of cancerous cells in patient's sample. Nevertheless, the elasticity measurements at a single cell level have a potential to be applied as an alternative approach used for the cancer diagnosis.

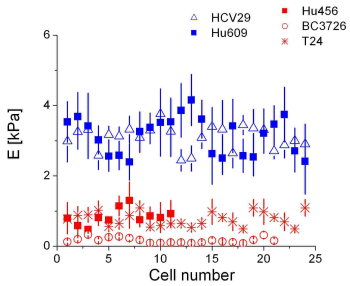


Figure 1. Deformability of human bladder cell lines.
Non-malignant cells (blue) were stiffer than the malignant ones (red) indicating changes in the cytoskeleton organization.

AFM in health and diseases: identification of technological gaps toward new applications for specific needs?

Frank Lafont

Institut Pasteur de Lille, France

AFM has entered in maturity as a technique to address biological issues. Its validation in investigating molecular interactions, morphology, adhesion and stiffness make it a valuable tool to investigate for instance cell biology, infectious and disease-related topics. However, AFM remains still confidential in the toolbox of physicians and pharmacists as well as quality control officers in food industry. Will the future of AFM turn out to consider it as an important component of the toolkit in diagnosis, control quality and investigation methods in global health and biomedicine fields?
As an attempt beginning to answer this question, we will discuss how coupling AFM to other techniques could boost its use for more versatile needs in nanomedicine.

European Cooperation in AFM for Nanomedicine

Jean-Luc Pellequer

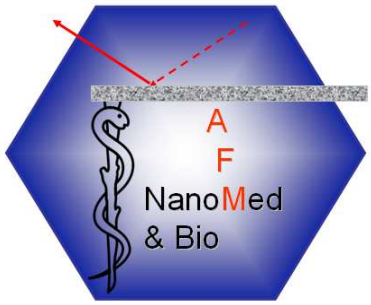
CEA Marcoule, iBEB, Department of Biochemistry and Nuclear Toxicology,
30207 Bagnols sur Cèze, France
Email: jlpellequer@cea.fr

Atomic Force Microscopy (AFM) has become an enabling platform in nanotechnology. It has provided a great impact in Life Sciences and is becoming indispensable also in NanoMedicine. NanoMedicine is an emerging area, which focuses in imaging, early diagnosis, pathological tissue analysis, and drug delivery. Although significant efforts have been devoted to enhancing the performance of AFM, full exploitation of its capabilities has been hampered by the uncoordinated relationship between researchers active in fundamental sciences, and users in the biomedical field. In addition, due to the swift development of AFM, Life science scientists depend on collaboration with experts in physical sciences to utilize the power of AFM instrumentation. In 2009, a European Cooperation in Science and Technology (COST) action has been deposited to the European Science Foundation. This action aims at bundling the expertises of the most active European AFM laboratories with the biomedical scientific environment into a network to foster further enhancement of AFM instrumental development, and explore and support its extensive applications in Life Sciences and Nanomedicine.

An interdisciplinary COST Action is believed to provide a key strategic platform and community to bundle these research activities. The COST Action will provide the conditions required for facilitating personal interactions throughout Europe and for increasing collaborations in the AFM community, ranging from physicists to clinicians.

The Action envisions an establishment of a dynamic network of AFM scientists including the major AFM centres in Europe. These AFM research centres have different focuses or specialization, such as AFM imaging, AFM manipulations, AFM nanomechanics, AFM nanodiagnostics, and so on. These centres will provide a diverse training environment for physician-scientists based on their needs, thus introducing new aspects of technological knowledge for their research. This training strategy will provide the basis for medical scientists to becoming AFM experts. As they return to their clinical research facilities, they can build up their own AFM laboratories. By interacting with medical scientists, the range of applications of AFM would be significantly enhanced in Nanomedicine and biology. By exploring new research fields of AFM, one may also identify current limitations and propose solutions to overcome them by integrating the network expertise.

Currently, the proposal has passed several rounds of evaluation and the final result is awaiting. Perspective on the action will be presented and discussed.



End Meeting Plenary Lecture

Biological AFM: Where we come from – where we are – (where we go)

Simon Scheuring

Institut Curie, U1006 INSERM, UMR168 CNRS, 26 rue d'Ulm, 75005 Paris, France
Email: simon.scheuring@curie.fr

Biological AFM is a fast growing and advancing field. This lecture has as objective to conceptualize the state of the art and retrace achievements of biological AFM, as presented by past and present research, and wishes to give a (subjective) outlook where biological AFM may go in the upcoming years. The following areas of interest are discussed: High-resolution imaging, cell imaging, single molecule force spectroscopy, cell mechanical measurements, combined AFM instrumentation, and AFM instrumentation. Of these topics, particular representative examples are discussed, each of them standing for a variety of achievements by a variety of groups.

Communications & Posters

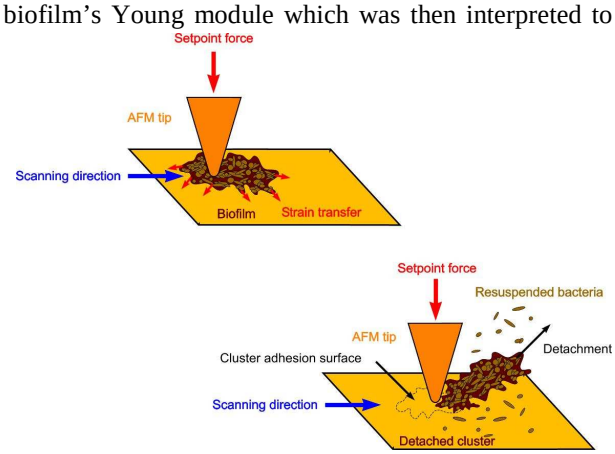
Quantification of the detachment force for water drinking biofilm using Atomic Force Microscopy

Yumiko Abe[1], Pavel Polyakov[1], Salahédine Skali-Lami[2] and **Grégory Francius**[1]

[1]*Laboratory of Physical Chemistry and Microbiology for the Environment, Nancy University, CNRS UMR7564, 405 rue de Vandoeuvre, Nancy University, F-54600 Villers-lès-Nancy, France*
[2]*Laboratory of Energetics and Applied Theoretical Mechanics, Nancy University, CNRS UMR7563, 2 avenue de la Forêt de Haye, F-54504 Vandoeuvre-lès-Nancy, France*

Around 99% of the world's population of bacteria is found in the form of a biofilm at various stages of their growth. Biofilms are observed in many medical, industrial, and domestic environments where the growth of biofilms is detrimental in most cases. There has been a large volume of studies carried out to better understand the growth of biofilms. However, their detachment behavior is much less explo ed despite the increasing interest in effective removal of undesired biofilms. Most studies use a flow-cell reactor in which biofilm had been developed to measure the detachment force of a biofilm by gradually increasing the applied hydrodynamic forces. However, such method utterly lacks a detailed control on the magnitude and the direction of forces. In this study, we propose AFM methods to estimate the shear modulus of a biofilm using a 1-month-old drinking water biofilm as an example. A biofilm matrix is the result of lateral reinforcement of cellular adhesion after the initial contact between a bacterial cell to a surface. In addition, biofilms are known to have viscoelastique properties; hence, in order to mechanically remove a biofilm, a lateral force which exceeds the shear module of the biofilm must be applied.

We tested two different approaches to better understand the applicability of AFM to quantify the shear modulus, or detachment force, of a biofilm. First method can be referred to as a mechanical detachment of biofilm by the movement of the cantilever while scanning. Several scans of the same surface were collected while increasing the applied force each time. Then, the deflection data were analyzed to calculate the lateral force exerted upon the biofilm, and the surface area occupied by each aggregate (given that the drinking water biofilm is highly discontinuous) was calculated based on the height image. Based on these data, the shear modulus applied to the biofilm surface can be obtained. Second method involves the force spectroscopy to directly quantify the biofilm's Young module which was then interpreted to calculate the shear modulus. The results from two methods revealed that the shear moduli obtained from these two methods were in good agreement. We also observed that a drinking water biofilm is heterogeneous in terms of shear moduli. Despite a rather complex interpretation of the data, the presented AFM methods to estimate the shear modulus can be performed rapidly and are capable of providing the special distribution of shear moduli.



Unfolding single cell-adhesion proteins from *Candida albicans*

David Alsteens[1], Vincent Dupres[1], Peter N. Lipke[2] and Yves F. Dufrêne[1]

[1]*Unité de Chimie des Interfaces, Université Catholique de Louvain, Croix du Sud 2/18, B-1348 Louvain-la-Neuve, Belgium*
[2]*Brooklyn College, Brooklyn, New York*

Microbial infection is generally initiated by the specific adhesion of the pathogens to host tissues. A prominent example is found in *Candida albicans*, the most common agent causing opportunistic infections in immunocompromised patients. Adhesion of *C. albicans* yeast cells to host tissues is mediated by a family of cell-surface proteins known as the agglutinin-like sequence (Als) proteins. Als proteins possess four functional regions (Fig. 1a), i.e. an N-terminal immunoglobulin (Ig)-like region, which initiates cell adhesion, followed by a threonine-rich region (T), a tandem repeat (TR) region that participates in cell-cell aggregation, and a stalk region projecting the molecule away from the cell surface.With its ability to observe and manipulate single molecules at work, atomic force microscopy (AFM) has provided a wealth of novel opportunities in life sciences [1-3]. Here we used AFM to measure the forces required to sequentially unfold single Als proteins, both on purified molecules and on live cells [4]. Soluble Als Ig-T-TR6 fragments terminated with a His-tag were attached at low density on gold surfaces modified with nitrilotriacetate groups, and picked up by their terminal Ig domain using AFM tips modified with Ig-T fragments (Fig. 1b). Remarkably, force extension curves showed sawtooth patterns with well-defined force peaks, each peak corresponding to the force-induced unfolding of an individual TR domain (Fig. 1c). Urea altered the shape of the unfolding peaks, reflecting a loss of mechanical stability of the TR domains due to hydrogen bond disruption. Next, single Als proteins were localized and unfolded directly on live cells. Force-extension curves obtained on cells expressing six repeats displayed sawtooth patterns similar to those found on isolated proteins, while cells expressing no repeat were unable to bind the AFM tip. The unfolding probability increased with the number of repeats and was correlated with the level of cell-cell adhesion, suggesting these modular domains may play a role in fungal adhesion. The modular and flexible nature of Als conveys both strength and toughness to the protein, making it ideally-suited for cell adhesion. These single molecule measurements open new avenues for understanding the mechanical properties of adhesion molecules from mammalian and microbial cells, and may help us to elucidate their potential implications in diseases like inflammation, cancer and infection.

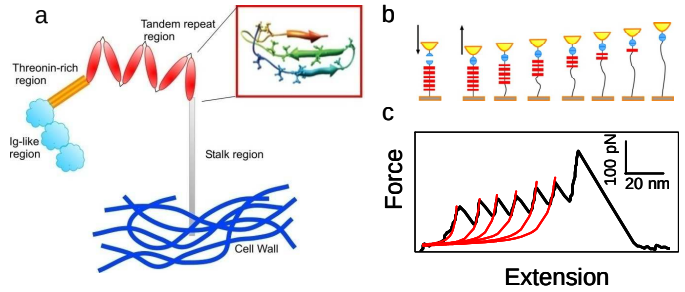


Figure 1. (a) Representation of an Als5p molecule projecting outward from the *C. albicans* cell wall by means of the stalk region. (b) Principle of the unfolding experiments. Ig-T-TR_6 fragments were attached on a gold surface and stretched via their Ig domains using an Ig-T-tip. (c) Force-extension curves obtained by stretching single Ig-T-TR_6 showed periodic features well-described by the worm-like-chain model (red line) and reflecting the sequential unfolding of the TR domains.

[1] D.J. Müller, J. Helenius, D. Alsteens, Y.F. Dufrêne, Nat. Chem. Biol., **5** (2009), 383-390.
[2] D.J. Müller, M. Krieg, D. Alsteens, Y.F. Dufrêne, Curr. Opin. Biotechnol., **20** (2009), 4-13
[3] Y.F. Dufrêne, Nature Reviews Microbiology, **6** (2008), 674-680.
[4] D. Alsteens, V. Dupres, S.A. Klotz, N.K. Gaur, P.N. Lipke, Y.F. Dufrêne, ACS Nano, **3** (2009),1677-1682.

Using microfluidics to allow the ex-vivo study of blood soluble microparticles

B.A. Ashcroft, J. de Sonneville, Y. Yuana, S. Osanto, R. Bertina, M.E. Kuil, T.H. Oosterkamp

Leiden University, Kamerlingh Onnes Laboratory, P.O. Box 9504, 2300 RA Leiden, The Netherlands

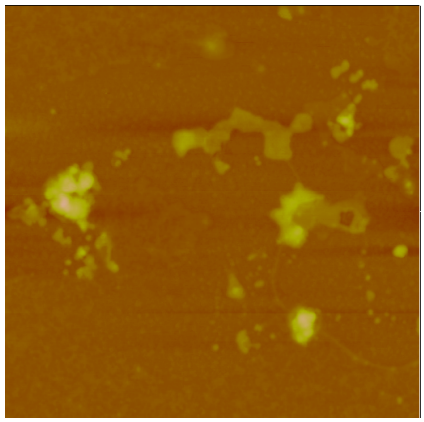
A novel method has been developed that allows the specific capture and analysis of an emerging biomarker of microvesticles. Microvesticles could be valuable biomarkers of cancer, and are taken from blood plasma, urine and other body fluids, but recent work has shown that the majority of the particles are too small to use fluorescence-activated cell sorting. The described method uses microfluidics to achieve a very small interaction over a coated mica surface, that is then analyzed using atomic force microscopy. This method allows the capture of both greater numbers of particles as well as a larger variety of particles than comparable methods. In addition, the method could be used ex vivo, removing the need for ultracentrifugation and washing, which can potentially disturb the microvesticles and cause systematic errors. Last, the method detects and counts biomarkers that are too small to be detectable and analyzed using other methods, and can even give additional information about the state of the microvesticles.

Quantifying the structure of natural aquatic nanoparticles (<5 nm) by field flow fractionation and atomic force microscopy

Mohammed Baalousha and Jamie R. Lead

School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, B15 2TT, United Kingdom

It is well recognized that natural nanoparticles, in particular those smaller than 25 nm, are of prime importance in trace element partitioning. Understanding the role of natural nanoparticles in the environment requires a thorough quantification of their size and structure. Here, we present a methodology and initial results based on verifying the assumptions of Stokes-Einstein relationship by comparing the sizes from two different measurements. Field flow fractionation (FlFFF) is a separation technique based on hydrodynamic principles in which particles are separated according to their diffusivity and from which a corresponding hydrodynamic diameter (*d*) distribution can be calculated by applying Stokes-Einstein equation. AFM measures nanoparticle topography/height (*h*) on the basis of the repulsive or attractive forces between the sample and the tip. If the ratio *d/h* is 1, then nanoparticles fulfil the Stokes-Einstein assumption of hard spheres. Results from three different sites show a ratio of 1 in one site and higher values at the two other sites. The ratio *d/h* increased with the decrease in ionic strength, suggesting that variations between *d* and *h* might be related to structural/shape variations at the nanoscale level. The ratio *d/h* deviates more from a value of 1 in the presence of a surface coating probably of organic matter, as demonstrated by AFM images. The methodology offer an opportunity to quantify the structure (sphericity and permeability) of nanoparticles relevant to environmental processes such as trace pollutant chemistry in a simple parameter which could be incorporated into, for example, speciation codes defining metal chemistry. Current work focuses on applying AFM in different modes (contact, tapping and noncontact) and in dry and liquid modes to obtain the most representative size measurement of natural nanoparticles.



AFM tapping mode image (2x2 μm) of natural colloidal particles lake water (Vale Lake, Birmingham, UK)

Combining AFM with hollow cantilevers for electrophysiological measurements

Pascal Behr,[1,2] Pablo Dörig,[1] Edin Sarajlic,[2,3] Daniel Bijl,[2,3]Michael Gabi,[1,2] Janos Vörös,[1]
Tomaso Zambelli[1]

[1]*Laboratory of Biosensors and Bioelectronics, ETH Zurich, Switzerland*
[2]*Cytosurge GmbH, Löwenstrasse 42, 8001 Zurich, Switzerland*
[3]*SmartTip B.V., Enschede, The Netherlands*

Biomedical experiments on single cells such as intracellular injections, patch clamp or extracellular deposition of liquids are typically carried out manually using tapered glass micro pipettes. The cell is thereby slowly approached by the micropipette using micro manipulators under optical control. During this process, even when carried out by skilled operators, the cell is often mechanically injured. Such perturbations frequently lead to cell death and therefore failure of the experiment. To overcome these drawbacks we propose a technique based on a combination of micro fluidics and atomic force microscopy known as FluidFM technology [1]. This approach is further motivated by the fact that AFM techniques are already widely used in the field of nano-biotechnology [2]. Additionally, the direct manipulation of single cells using AFM has already been successfully reported in earlier works [3].

Our instrument is composed of custom made hollow AFM cantilevers with different tip geometries and opening sizes. The cantilevers have an integrated micro-fluidic channel that ends at a well-defined aperture located at or in the vicinity of the tip apex. The other end is connected to a pressure controlled reservoir. The force control system of the underlying AFM allows for a very precise regulation of the force applied by the hollow AFM probe to e.g. the membrane of a cell. It even becomes possible to discriminate between a “gentle contact” and a controlled perforation of the cell membrane under investigation. Highly localized biochemical stimulation or direct manipulation of single viable cells under physiological conditions essentially becomes practicable using the FluidFM technology. The feasibility of our approach has been demonstrated earlier by selectively injecting single living cells in vitro with a membrane impermeable dye [1].

In this work we present how standard AFM microscopy in combination with hollow cantilevers can be used for electrophysiological experiments (see figure 1). The high resolution imaging capabilities and the force feedback of the underlying AFM opens the door for numerous new experiments: Investigations such as spatially coherent patch clamping while minimizing mechanical perturbations of the investigated cells are pictured to become practicable using our technique.

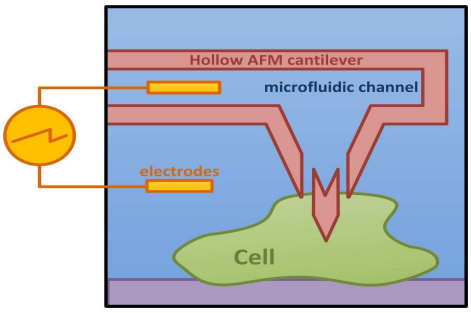


Fig 1: Schematics of a combined AFM-patch-clamp experiment. Using hollow cantilevers it is possible to use the same probe for acquiring AFM images and measuring ion channel activities.

[1] A. Meister et al., Nano Letters, **2009** 9 (6), 2501-2507
[2] D. J. Müller et al., Nature Nanotechnology 3 **2008**, 261-269
[3] M. Currie et al., Biochem. and Biophys. Research Comm., **2007** 355 (3), 632-636

Morphology and nanomechanics of surface-adsorbed liposomes

Tamás Bozó, Ünige Murvai, László G. Kovács, Pál Gróf, Miklós S. Z. Kellermayer

Dept. Biophysics and Rad. Biol. Semmelweis University, Budapest Hungary

Liposomes, owing to their unique biocompatible and biosimilar properties, are increasingly used as drug delivery systems and biomembrane models. Morphological features of liposomes are usually charaterized by light and electron microscopy. While light microscopy lacks resolution and electron microscopic techniques generate artifacts due to fixation and staining,, atomic force microscopy (AFM), beside its high spatial resolution on unfixed samples, enables the nanomanipulation of nano- and submicron-sized vesicles. Furthermore, AFM may provide information about local structural, chemical and physical properties of domains or membrane-bound molecules in the vesicles.

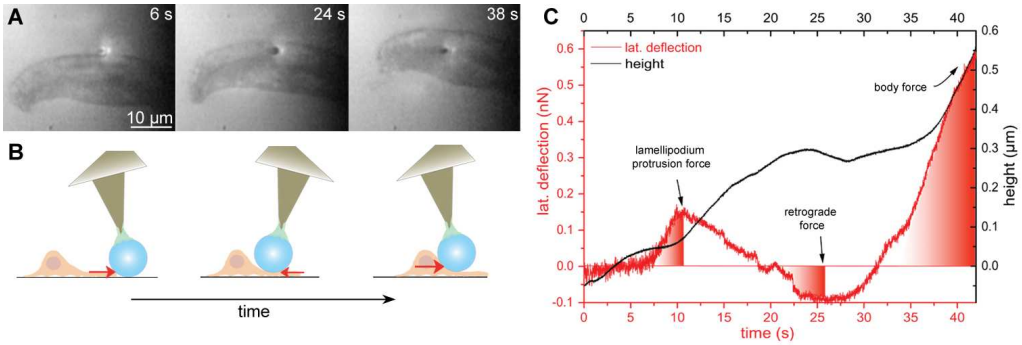
In the present work we investigated the structure and nanomechanics of small unilamellar vesicles immobilized on glass and mica surfaces. Liposomes were prepared by extrusion of hydrated DPPC film through a polycarbonate membrane with 100 nm porosity. Liposomes were attached to either cleaned glass or freshly-cleaved mica surface by incubating an aliquot of the sample for 5 minutes and washing away the unbound vesicles. The surface-adsorbed liposomes were investigated by using tapping-mode AFM under aqueous buffer conditions. Vesicles appeared as flat circles with an average diameter of 160 nm and height of 5 nm. While the diameter corresponds well to size measurements with dynamic light scattering, the flat shape deviates from the presumed spherical appearance. We hypothesize that the flattening of the vesicles was caused by adhesion forces arising upon surface adsorption. To explore nanomechanical properties, a calibrated cantilever was first pushed into the vesicle surface with ~1 nN force, then the cantilever was pulled away with a mean velocity of 1000 nm/s. In the nanomechanical traces constant-force plateau appeared which may be explained by the generation of membrane nanotubes. The force most likely reflects the adhesion force arising between the vesicle and the surface, considering that pulling a nanotube from a completely flattened vesicle can occur only at the expense of desorption from the substrate surface. In sum, the AFM techniques employed here provide a unique insight into the structural and nanomechanical features of liposomes.

Origin and spatial distribution of forces in motile cells

Claudia Brunner[1], Michael Gögler[1], Daniel Koch[1], Allen Ehrlicher[1], Thomas Fuhs[1], Charles W. Wolgemuth[2], and Josef A. Käs[1]

[1]*Division of Soft Matter Physics, Department of Physics, University of Leipzig, Linnéstrasse 5, 04103 Leipzig, Germany* cbrun@physik.uni-leipzig.de
[2]*Department of Cell Biology and the Center for Cell Analysis and Modeling, University of Connecticut Health Center, Farmington, Connecticut 06030 USA*

A fundamental step in cell migration is the advancement of the cell's leading edge. It is generally accepted that this motion is driven by actin polymerization against the plasma membrane but this has not been directly measured.
Here we present precise force measurements using a newly established AFM-technique combined with high resolution imaging and lamellipodium feature tracking analysis. Our AFM-based technique uses the vertical and lateral deflection of a modified cantilever and allows direct measurements of the forces exerted by the cell [1]. We measure the maximum forces which are generated at the leading edge of the lamellipodium, retrograde forces within the lamellipodium, and the cell body. Through selective manipulation of molecular components by addition of different drugs, such as Jasplakinolide, Cytochalasin D, and ML-7 the measured forces and velocity changes can be compared.
We resolve that the force generating mechanism at the leading edge is indeed actin polymerization, and we directly measured a force attributed to the retrograde flow within the lamella, which critically demonstrates that the protrusion forces are decoupled from the cell body and are generated exclusively at the leading edge.
Protrusion and retrograde flow in the central lamellipodium are thus driven by polymerization and depolymerization forces, whereas the lamellipodial wings and the forces that pull the cell body along rely heavily on contractile actin-myosin interactions. The traction forces in the wings significantly contribute to the local retrograde flow and are the origin of strong forces that advance the cell body [2].
To improve the temporal and spatial sensitivity, a combination with optical tweezers to stabilize the setup, promises possible measurements of forces on slower and softer cells, like neurons, fibroblast etc to complete the phase space of motile cells and to understand the underlying mechanisms nature invented.



SFM-Force measurement of a migrating keratocyte. A: Interference reflection microscopy images: the cell pushes against the cantilevered bead (bright spot) and finally lifts it up. B: Sketch of the experiment, the cell pushes against the cantilever; the lateral deflection corresponds directly to the force. C: Lateral deflection signal (red) reflects the forces the cell exerts in different regions of the cell during the measurement, vertical deflection signal (black) corresponds to height .

References*:*

[1] C. A. Brunner, A. Ehrlicher, B. Kohlstrunk, D. Knebel, J. A. Kaes, M. Goegler, Cell migration through small gaps, *European Biophysical Journal*, **35**, (2006) 713-719
[2] M. Goegler; C. A. Brunner; A. Ehrlicher; D. Koch; T. Fuhs; C. W Wolgemuth; J. A Kaes, Origin and spatial distribution of forces in motile cells (in preparation)

Multiscale morphology of organic semiconductor thin films controls the adhesion and viability of human neural cells

Bystrenova E[1], Tonazzini I[1], Chelli B[1], Greco P[1*], Stoliar P[1], Calò A[1], Lazar A[1‡], Borgatti F[1], D'Angelo P[1], Martini C[2] and Biscarini F[1]

[1]*CNR-Institute for Nanostructured Materials Studies; Via Gobetti 101, Bologna, 40129, ITALY*
[2]*Department of Psychiatry, Neurobiology, Pharmacology and Biotechnology; University of Pisa; Via Bonanno 6, Pisa, 56126, ITALY*

Current Addresses:
[*]*Scriba Nanotecnologie Srl, Via Gobetti 52/3, 40129 Bologna, ITALY*
[‡]*INSERM-UPMC UMRS 975-CRICM, Escurolle Neuropathology Laboratory, La Salpetriere Hospital, Bd de l'Hôpital 47, 75651 Paris Cedex 13, FRANCE*

Cell-substrate interactions determine cell adhesion, motility, growth, proliferation, and fate. They can influence cell differentiation and commitment, as well as intracellular signaling. Their technological impact concerns transducers, sensors, cell carriers, scaffolds for tissue growth. We investigate how multi-scale morphology of functional thin films affects the *in vitro* behavior of human neural astrocytoma 1321N1 cells. Pentacene thin film morphology is precisely controlled by means of the film thickness Θ (here expressed in monolayers, ML). Fluorescence and atomic force microscopy allows us to correlate the shape, adhesion and proliferation of cells to the morphological properties of pentacene films controlled by: saturated roughness σ, correlation length ξ and fractal dimension d_f. At early incubation time, cell adhesion exhibits a transition from higher to lower values at Θ ≈10 ML. This is explained with a model of conformal adhesion of the cell membrane onto the growing pentacene islands. From the model fitting of the data we show that the cell explores the surface with a deformation of the membrane whose minimum curvature radius is 90(±45) nm. The transition in the adhesion around 10 ML arises from the saturation of ξ accompanied by the monotonic increase of σ, which leads o a progressive decrease of the pentacene local radius of curvature and hence to the surface area accessible to the cell. Cell proliferation is also enhanced for Θ < 10 ML, and optimum morphology range for cell deployment and growth is for σ≤6 nm, ξ>500 nm, d_f >2.45. Characteristic time of cell proliferation is τ≈10±2hours. The trend of viability mimics the one of adhesion, although the reasons might be entirely different. Here we highlight that there is optimum viability is achieved for ultra-thin films. This finding hints that a technology capable of controlling the multiscale morphology of ultra-thin films is attractive also for engineering substrates for cell research, sensing interfaces and tissue engineering.

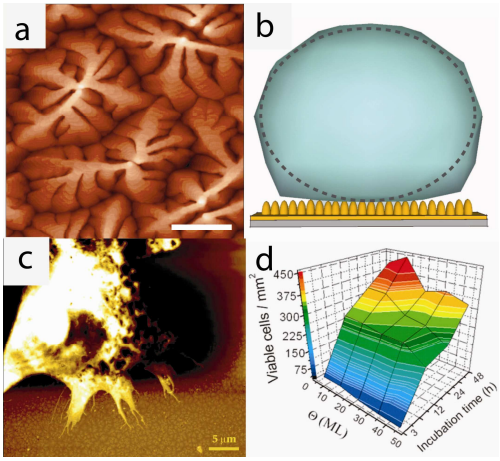


Figure 1. a) AFM images of typical pentacene thin films grown on silicon dioxide surface. b) Schematic drawings of the cell sitting on a rough pentacene surface. c) Typical AFM image of an astroglial cell grown on pentacene thin film (6 ML) for 48h under standard conditions and fixed in 4% paraformaldehyde. d) Human 1321N1 astroglial cell viability and proliferation characteristics. Cells were incubated for different time on pentacene thin films with different thickness and tested by Trypan blue exclusion test.

Watching membrane-mediated protein-protein interaction in action

Ignacio Casuso*[1], Pierre Sens[2], Felix Rico[1], Simon Scheuring[1]

[1]*Institut Curie, U1006 INSERM, UMR168 CNRS, Centre de Recherche, Paris, F-75248 France*
[2]*UMR Gulliver CNRS-ESPCI 7083, 10 rue Vauquelin, Paris, F-75231 France*

Ignacio.Casuso@curie.fr

Membrane proteins are, due to their amphiphilic nature, restricted to the membrane plane. They diffuse within the membrane, form oligomers, and are densely packed in supramolecular assemblies. Although numerous theoretical and numerical studies have explored membrane-mediated interactions between membrane proteins, direct experimental measure of in-membrane-plane interaction potentials have not been obtained until now. Here, we show high-speed atomic force microscopy (HS-AFM) image sequences of native purple membranes (PM) of Halobacterium salinarum, containing ATP-synthase c-rings, in bacteriorhodopsin (bR) array edge regions. PM borders have a lipid membrane brim where bR and c-rings diffuse. C-rings formed dimers that temporary dissociated. C-ring dimers were rather static and revealed subdiffusive motion, while dissociated monomers diffused freely. Direct imaging analysis of the c-rings center-to-center distance probability distribution allowed the calculation and modeling of an in-membrane-plane energy landscape that presented repulsion at 80Å, most stable dimer association at 103Å (-3.5kBT strength), and dissociation at 125Å (-1kBT strength) center-to-center distance. We present first experimental data of non-labeled membrane protein diffusion analysis and the corresponding in-membrane-plane interaction energy landscape. We conclude that membrane protein diffusion is assembly and size dependent and that the attractive range of a membrane protein is of several kBT and reaches to a radius of about 50Å within the membrane plane.

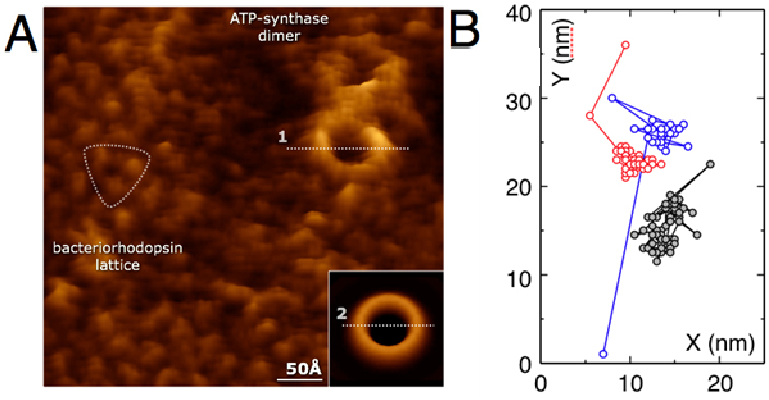


Fig. A) Two ATP-synthase c-rings on the edge lipid region of the bR arrays. B) Particle tracking of the motion of the ATP-synthase dimer.

Atomic Force Microscopy study of binding STAT3 protein to DNA

Sergey Chasovskikh, Olga Timofeeva*, Anatoly Dritschilo

Department of Radiation Medicine and Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, D. C. 20007*

Signal transducer and activator of transcription 3 (STAT3) proteins modulate various physiological functions including cell-cycle regulation, apoptosis and cell survival by regulating gene expression. Upregulation of phospho-STAT3 activity or increases of unphosphorylated STAT3 levels have been observed in cancers. Both forms of STAT3 are able to regulate gene transcription and contribute to tumor progression. The phosphorylated STAT3 forms a dimer and binds to IFNγ-activated sequences (GAS) on DNA. It is not clear yet whether unphosphorylated STAT3 binds to its own DNA site or regulates gene expression through interaction with other transcription factors. Atomic Force Microscopy (AFM) allows visualization of structural relationships in the interaction between protein and DNA. In this study we used AFM to analyze binding of unphosphorylated STAT3 to DNA to better understand the mechanism of STAT3-dependent transcriptional regulation. We investigated the effect of Hel2A-pen small molecules on STAT3 dimerization and STAT3-DNA interactions.

We found that full sized STAT3 protein binds to GAS element as a dimer. We observed that the C-terminally truncated 67.5 kDa STAT3 isoform can binds to the ends of the hairpin arms of the cruciform structure on DNA containing short unpaired AT-rich sequences. Full length STAT3 proteins bind to the four-way junction region in the cruciform structure and DNA nodes. Statistical analyses of the volume distributions of STAT3 molecules in DNA-STAT3 complexes reveals that the 67.5 kDa STAT3 isoform binds predominantly as monomers or dimers to cruciform structures. We found that Hel2A-pen small molecules increase oligomerization STAT3 in solution and on the plasmid DNA.

Secondary DNA structures, such as cruciforms, can create new protein binding sites and potentially block the movement of the transcription-elongation complex. Our data suggest that STAT3 might be involved in regulation of gene activity through recognitions of secondary structures DNA.

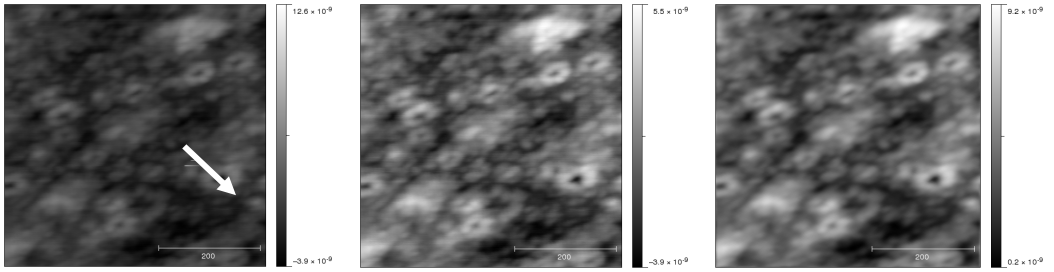
Accordingly, the effects of Hel2A-pen small molecules on the STAT3-DNA interactions may offer a novel therapeutic target for gene regulation.

Enhancement of molecular features by stripe noise reduction in AFM images

Shu-wen W. Chen, Michael Odorico, Jean-Marie Teulon, Pierre Parot, Jean-Luc Pellequer

CEA Marcoule, iBEB, Department of Biochemistry and Nuclear Toxicology, 30207 Bagnols sur Cèze, France

The immediate obstacle of characterizing objects in AFM images is stripe noises. Different sample and operational conditions lead to various types of stripe noises. The present work demonstrates two categories of stripe noises often encountered in AFM images for investigating a system of biomolecules. We characterize them based on their intensity values relative to that of the environment. The first type of noise will be denoted as generic stripes while the second one addressed as abrupt stripes. We have found that the generation of the two noises is correlated with contents of the studied sample. To demonstrate this, we have developed two techniques of noise reduction with respect to the two kinds of noises. For the first one, we simply adopted the subtraction strategy to remove it from the frequency domain of the image. Regarding the second category of noise, we have developed a methodology which detects noise locations and re-assigns an intensity value to the noise location. The consequence of this image treatment is enhancing the molecular feature of the system studied.



Abrupt noise is clearly seen in the raw AFM image on the left (arrow). After removing it, only the classical generic strip noise left in the middle image. The final image shows the results after both types of noises removed, on the right. The scale bar is in pixel.

Acknowledgement
This work was supported in part by a grant from the Agence Nationale de la Recherche (ANR-07-PCVI-0002-01)

Label-free optical imaging of membrane patches for atomic force microscopy

Allison B. Churnside[1], Gavin M. King[2], and Thomas T. Perkins[3]

JILA, University of Colorado and National Institute for Standards and Technology, Boulder, CO
[1]University of Colorado Department of Physics, [2]Currently with University of Missouri Department of Physics, Columbia, MO, [3]University of Colorado Department of Molecular, Cellular, and Developmental Biology

In atomic force microscopy (AFM), it can be difficult and time-consuming to locate sparsely distributed regions of interest. Typically, the tip is scanned until the desired object is located. This process can mechanically or chemically degrade the tip, and damage fragile biological samples. Protein assemblies can be detected using the backscattered light from a focused laser beam. We previously used a pair of focused laser beams scattering off an AFM tip and a fiducial mark on the substrate to stabilize an AFM. In the present work, we used one of these foci to optically image patches of purple membranes. These rapidly acquired optical images were inherently registered with the subsequent AFM images, with minimal misregistration (~200 nm). This registration arises because the tip position is referenced to the same laser focus used to generate the optical image. Thus, this label-free imaging over a large area ($30\times30\ \mu m^2$) efficiently locates sparsely distributed membrane patches for AFM study and thereby minimizes unnecessary tip-sample interaction.

Protein Immobilization Methods for Biological Atomic Force Microscopy: from monolayers to resolved molecules

Rocío de Miguel [a], Carlos Marcuello [a], Marta Martínez-Júlvez [a,b], Carlos Gómez-Moreno [a,b], Anabel Lostao [a,†]

[a]*Instituto de Nanociencia de Aragón (INA), Universidad de Zaragoza, 50018- Zaragoza, Spain*
[b]*Departamento de Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza 50009- Zaragoza, Spain*
† Fundación Aragón I+D (ARAID), Aragón, Spain

AFM has been increasingly used in biological sciences and it is now established as a versatile tool to address the structure, properties and functions of biosystems. AFM is unique in providing 3-D images of biological structures of all kinds in their native environment with nanometer resolution. A crucial prerequisite for successful, reliable biological AFM is that the samples need to be well attached to a solid flat surface using appropriate and non destructive methods. Our group has designed different procedures for immobilizing proteins for several AFM studies: topography imaging, molecular recognition imaging and Force Spectroscopy. The methods have been optimized to minimize nonspecific adhesion with the tip. We have also developed procedures to control the quantity and functionality of the bound molecules. These methods range from electrostatic adsorption to covalent binding to achieve separated resolved molecules or monolayers. In the case of protein-ligand recognition studies, the rupture forces are often obscure by the lack of molecular mobility, nonspecific adhesive bindings or an incorrect orientation of a molecule over the other. Our group has been using a linker molecule to increase the mobility of the molecule at the tip with success [1]. Typically, the immobilization of the molecules is carried out in a non-oriented manner. In some cases, this could be problematic for imaging, but can be very negative in Force Spectroscopy experiments, where an incorrect orientation of the molecule in the sample over the one at the tip makes the binding does not take place or occurs in a very small percentage of approaches, which is quite common in these experiments. In this work we introduce the factor of protein orientation to measure intermolecular forces in flavoprotein complexes. The enzyme FNR catalyses the transfer of two electrons from two independent Ferredoxin molecules, previously reduced by Photosystem I, to $NADP^+$ in the photosynthetic chain. This reaction requires formation of a complex that allows the optimal orientation between the redox centers of both molecules for the subsequent electron transfer. We have developed two strategies of oriented immobilization of FNR on mica. On one hand, we have attached FNR oriented to its protein redox partner that will be also oriented bound to the AFM tip [2]. On the other hand, a double histidine mutant in FNR will favour the proper orientation in the surface to the $NADP^+$ substrate that will be attached to the tip. In summary, we have developed some new strategies to control the immobilization of different proteins on substrates and tips in order to optimize imaging and Force Spectroscopy experiments.

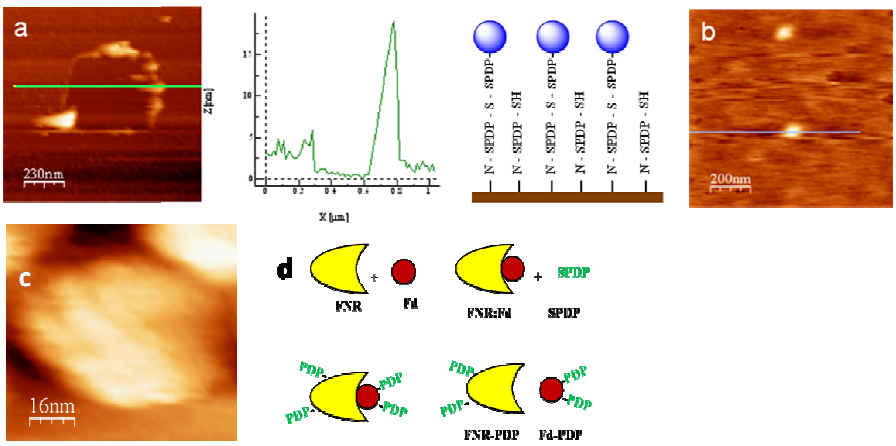


Figure 1. Streptavidin covalently bound a) to APTES-mica through glutaraldehyde to form a monolayer and b) to thiolated mica to get resolved molecules. c) Ferritin molecule in acetate buffer. d) Orientation strategy to achieve FNR be mostly oriented to its protein partner on mica meanwhile Fd will be oriented to FNR at the tip.

[1] J. Sotres, A. Lostao, L. Wildling, A. Ebner, C. Gómez-Moreno, HJ. Gruber, P. Hinterdorfer, AM. Baró, Unbinding molecular recognition force maps of localized single receptor molecules by AFM, ChemPhysChem, **9** (2008) 590.
[2] A. Lostao, J.Sotres, R. de Miguel, M. Martínez-Júlvez, C. Gómez-Moreno and A.M. Baró, AFM applied to the Study of single Flavoprotein Complexes, Flavins and Flavoproteins, **16** (2008) 589.

Controlled displacement of microorganisms by fluidFM

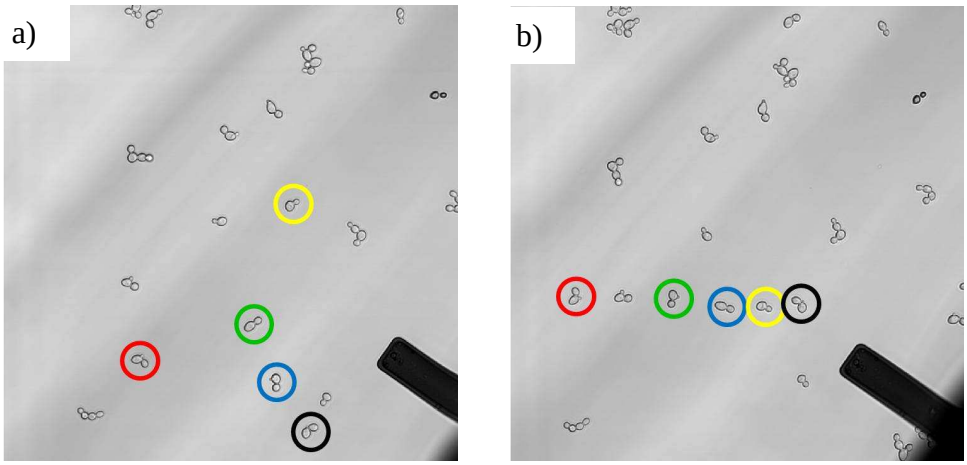
Pablo Dörig,[1] Philipp Stiefel,[2] Edin Sarajlic,[3,4] Daniel Bijl,[3,4] Pascal Behr,[1,3] Michael Gabi,[1,3] János Vörös,[1] Julia Vorholt,[2] Tomaso Zambelli[1]

[1]*Laboratory of Biosensors and Bioelectronics, D-ITET, ETH Zurich, Switzerland*
[2]*Institute of Microbiology, D-BIOL, ETH Zurich, Switzerland*
[3]*Cytosurge GmbH, Zurich, Switzerland*
[4]*SmartTip BV, Enschede, The Netherlands*

The development of the fluidFM [1] technology, combining the precise AFM force feedback with nanofluidics via an incorporated microchannel directly in the cantilever, opens novel strategies for the spatial manipulation of biological objects. The microchannel in the cantilever ends with a submicron aperture at the apex of the pyramidal tip, and with a reservoir on the other side. By fixing the chip against the hollow probeholder, a continuous fluidic pipeline is obtained connecting the tip aperture with a syringe or a pressure controller. Therefore, the fluidFM can be immersed in liquid environment while a pressure can be applied to the solution inside the channel.

In this study, we demonstrate a unique way of spatial manipulation of various cell types including myoblasts, neurons, yeast, and bacteria. We take advantage of the principal features of the fluidFM technology, the force feedback, for a safe and quick approach onto the biological objects and the possibility to apply an underpressure to "grasp" the cells, whereby different tip apertures can be used. The object is then lifted and moved to a predefined new position where the force feedback is activated again to approach the surface and a short overpressure pulse is administered to release the object.

Two different cantilever designs were used for these experiments, one having a blunt and the other a tube shaped tip. Manipulations were carried out with a low cost, custom BioAFM (skeleton-fluidFM) and custom LabView software.



*Budding yeast cells **a)** before and **b)** after spatial manipulation with the fluidFM.*

We would like to thank Stephen Wheeler from the LBB Workshop for technical help.

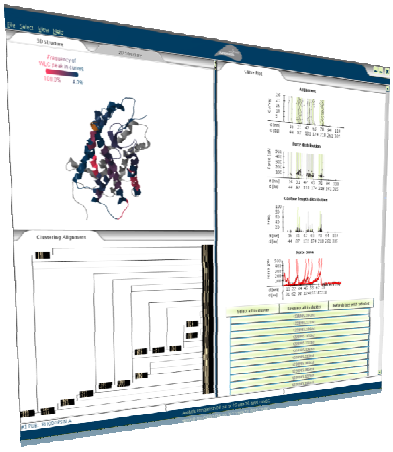
[1] A. Meister, M. Gabi, P. Behr, P. Studer, J. Vörös, P. Niedermann, J. Bitterli, J. Polesel-Maris, M. Liley, H. Heinzelmann, T. Zambelli, Nano Letters **9** (2009) 2501.

Fully Automated Analysis of Force-Distance Curves

Frank Dressel[1], **Bill Andreopoulos**[1], Dirk Labudde[2]

[1] *Biotechnological Centre TU Dresden, c/o nanometis, Tatzberg 47-51, 01307 Dresden, Germany;*
[2] *University of Applied Sciences Mittweida, Technikumplatz 7, 09648 Mittweida, Germany*
E-mail: f.dressel@nanometis.com

Force spectroscopy allows scientists to examine properties of biomolecules like the stability, dynamic properties or forces within globular and membrane proteins (see for example [1]). However, the potential of force spectroscopy techniques does not end here. Force spectroscopy is successfully applied to further measurements like binding forces of antibodies [2], stretching a diverse set of biomolecules like DNA [3] or inferring information about cell-cell adhesion forces [4]. Nowadays, the devices for force spectroscopy are getting more and more automated. Today, it is possible to measure thousands of force-distance curves per hour in a fully automated manner. In contrast, the state of the analysis of the experimental data is far from it. The exhausting analysis is mostly based on manual peak finding and annotation of the force-distance curves. The manual annotation is time-consuming and error-prone. Up to now, there is no fully automated analysis software available, which could deal with the huge amount of data and support the scientist with objective and faster results.



For this reason, we developed software that is able to analyze up to 40.000 force-distance curves per hour fully automated. The analysis includes filtering, finding signals (WLC) and alignment of the curves with high accuracy. Moreover, the scientist is served with a wide range of tools for further data analyses.

This is the first time, scientists are provided with a technology to analyze high-throughput force spectroscopy experiments on biopolymers in a fully automated manner.

Our technology is free to use for academics within the context of collaboration projects. Additionally, a free online version will be available soon.

References:

[1] M Kessler, K E Gottschalk, H Janovjak, D J Muller, H E Gaub, Bacteriorhodopsin folds into the membrane against an external force., J Mol Biol, 357 (2), 2006

[2] T A Sulchek et al., Dynamic force spectroscopy of parallel individual Mucin1-antibody bonds., Proc Natl Acad Sci U S A, 102 (46), 2005

[3] F M Hameed, G V Soni, H Krishnamurthy, G V Shivashankar, Probing structural stability of chromatin assembly sorted from living cells., Biochem Biophys Res Commun, 2009

[4] M Benoit, H E Gaub, Measuring cell adhesion forces with the atomic force microscope at the molecular level., Cells Tissues Organs, 172 (3), 2002

Probing the mechanical behavior of the yeast plasma-membrane sensor Wsc1 using single-molecule AFM

Vincent Dupres[1], Jürgen J. Heinisch[2], Yves F. Dufrêne[1]

[1]*Unité de Chimie des Interfaces, Université catholique de Louvain, Belgium*
[2]*Universität Osnabrück, Fachbereich Biologie/Chemie, AG Genetik, Germany*

Understanding how proteins respond to mechanical force and how this response could be related to function is of fundamental importance in current cell biology. During the past two decades, atomic force microscopy (AFM) has emerged as a versatile technique for probing biological systems in their native state, going from single molecules to live cells [1-5]. Moreover, there have been rapid advances in employing single-molecule force spectroscopy (SMFS) for studying the mechanical behavior of proteins. Yet, mechanical studies on cell surface proteins embedded in their native cellular environment, thus in their fully functional state, remain very challenging.

Here, we present in vivo measurements of the mechanical behavior of a cell surface sensor using single-molecule AFM. We focus on the yeast wall stress component sensor Wsc1, a plasma membrane protein that is thought to function as a rigid probe of the cell wall status. We first detected individual histidine-tagged sensors on living yeast cells using AFM tips carrying nitrilotriacetate groups. We then showed that Wsc1 behaves like a linear nanospring that is capable of resisting high mechanical force and responding to cell surface stress [6] (Fig. 1).
We also studied the relationship between the structure of Wsc1 and its mechanical properties using mutagenesis, and found that glycosylation of Wsc1 substantially contributes to its linear spring properties.

Then, we mapped the distribution of individual sensors on living yeast cells and found that they form clusters of ~ 200 nm. We showed that clustering of Wsc1 is strongly enhanced in deionized water or at elevated temperature, suggesting its relevance in proper stress response.

The combined method of genetic design and single-molecule measurements used in this study has great potential for investigating how proteins respond to forces in cells, and how mechanosensing and mechanotransduction events proceed *in vivo*.

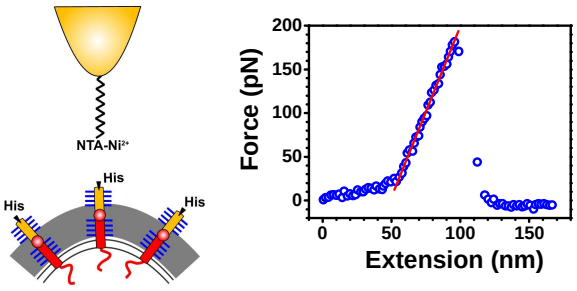


Fig.1. Detection of single Wsc1 sensors on live cells and representative force-extension curve showing linear spring behavior.

[1] A. Engel and D.J. Müller, Nat. Struct. Biol., **7** (2000) 715
[2] P. Hinterdorfer and Y.F. Dufrêne, Nat. Methods, **3** (2006) 347
[3] D.J. Müller and Y.F. Dufrêne, Nat. Nanotechnol., **3** (2008) 261
[4] Y.F. Dufrêne, Nat. Rev. Microbiol., **6** (2008) 674
[5] A. Engel and H.E. Gaub, Annu. Rev. Biochem., **77** (2008) 127
[6] V. Dupres *et. al.*, Nat. Chem. Biol., **5** (2009) 857

Towards nanoscale electrical characterisation of single bacteria

Martin A. Edwards,[1] Daniel Esteban-Ferrer,[1] Laura Fumagalli,[1,2], Antonio Juárez,[1,3] Gabriel Gomila[1,2]

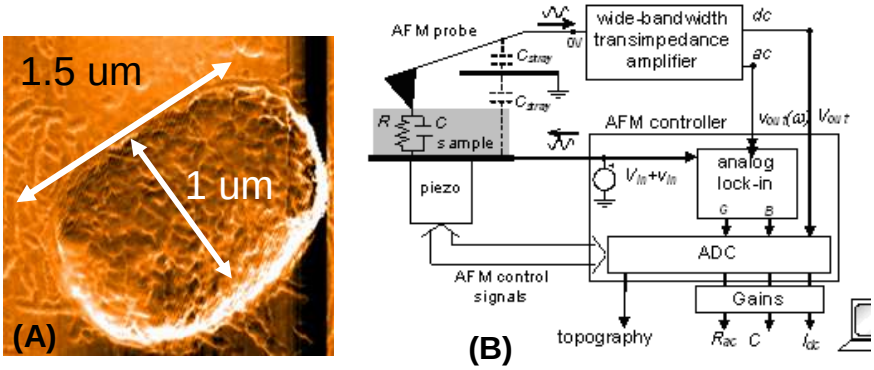
[1]*Institut de Bioenginyeria de Catalunya (IBEC), Barcelona (Spain)*
[2]*Departament d'Electrònica Universitat de Barcelona, Barcelona (Spain)*
[3]*Departament de Microbiologia, Universitat de Barcelona*

Individual cells within clonal microbial cultures exhibit remarkable phenotypic heterogeneity, i.e. in spite of having the same genetic content their response to the same environment provides different observable characteristics related to morphology, development, biochemical and physiological properties, and behaviour. The heterogeneity at the single-cell level is typically masked in conventional studies of microbial populations, which rely on data averaged across thousands or millions of cells in a sample. Considerable effort has been devoted to perform methodological advances enabling the investigation of processes governing cell-to-cell variability in single bacteria [1]. To cope with this methodological issue in recent years some groups have started to develop nanoscale techniques and methods, in what is being named nano-microbiology [2-4]. Until now, almost all investigations in this emerging field of research have been performed with the use of the force measuring capabilities of conventional atomic force microscopes in order to gain nanoscale insight on morphological, adhesion and mechanical properties of single bacteria cells.

In spite of these results, much still remains to be explored in order to better understand the single bacteria cell variability. One avenue of investigation that we are pursuing is performing electrical AFM measurements on single bacteria cells; addressing amongst others: surface charges, dielectric constants, conductivity, etc. which would allow one to differentiate between different phenotypes, *in vivo*, without the need for labelling, or otherwise complex sample preparation. Thus the molecules can be viewed in an unperturbed environment and potentially processes may be followed on a temporal basis. Furthermore, the electrical properties are often intrinsic to the function of the molecules in the cellular environment and so their measurement can prove critical to understanding their mode of operation.

Within our group an AFM equipped with a custom, high-bandwidth, low-noise, and low-current amplifier has been developed capable of performing of nanoscale dielectric spectroscopy as well as other electrical AFM measurements (e.g. electrostatic force microscopy)[5]. This work presents a brief outline of the instrumentation and techniques followed by results obtained within the group looking at the application of electrical modes of AFM operation to single bacteria.

Crucial to the interpretation of electrical AFM measurements is a suitable theoretical framework to convert the current/force vs. position data into a quantity of interest, e.g. dielectric constant, surface charge, etc. of the sample beneath the tip. To this end a finite element modelling scheme will be presented, which allows one to quantitatively interpret electrical AFM measurements.



(A) Topographic image of single *Samonella enterica* (*S.* Typhimurium) SV5015 (B) Schematic of instrumentation set-up for high-resolution electrical AFM.

[1] S. V. Avery, Nat. Rev. Microbiology 4, 577 (2006)
[2] Y. F. Dufrene, Nat. Rev. Microbiology 2, 451 (2004)
[3] D. Alsteens et al.,Nanosc. Res. Lett. 2, 365–372 (2007)
[4] Y. F. Dufrene, The Analyst 133, 297–301 (2008)
[5] L. Fumagalli et al., Nanotech. 17, 4581, (2006)

Bacterial surface appendages strongly impact on nanomechanical and electrokinetic properties of *Escherichia coli* cells subjected to osmotic stress

Grégory Francius,[1*] Pavel Polyakov,[1] Jenny Merlin,[2] Yumiko Abe,[1] Jean-Marc Ghigo,[3] Christophe Merlin,[1] Christophe Beloin,[3] Jérôme F.L. Duval[2*]

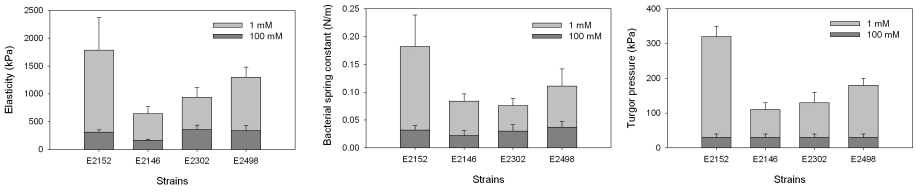
[*]*Corresponding authors*

[1]*Laboratory of Physical Chemistry and Microbiology for the Environment, Nancy University, CNRS UMR7564, 405 rue de Vandoeuvre, Nancy University, F-54600 Villers-lès-Nancy, France*

[2]*Laboratory of Environment and Mineral Processing, Nancy University, CNRS UMR7569, B.P. 40, F-54501 Vandoeuvre-lès-Nancy, France*

[3]*Institut Pasteur, Unité de Génétique des Biofilms, CNRS URA 2172, 25-28 rue du Dr Roux, 750724 Paris cedex 15, France*

The physicochemical properties and dynamics of bacterial cell envelopes, which possibly involve protruding cell surface appendages anchored at the cell wall, play a major role in bioadhesion and infection processes. In this study, the morphological, nanomechanical and electrohydrodynamic properties of the Gram-negative *Escherichia coli* K-12 mutant cells were thoroughly investigated as a function of bulk medium ionic strength (KNO_3 salt) using atomic force microscopy (AFM) and electrokinetics (electrophoresis). The studied cells were differing according to genetic alterations controlling the production of different surface appendages, ranging from none to either short rigid Ag43 adhesins, or longer and more flexible surface appendages (type 1 *fimbriae* or F conjugative *pilus*). From the analysis of the spatially resolved force-indentation curves, it is shown that the elasticity (Young Modulus) and Turgor pressure of *E. coli* cells are not only depending on bulk salt concentration but also on the presence/absence and nature of cell surface appendage. In 1 mM KNO_3 solution, cells with no visible surface appendages or cells surrounded by short Ag43 adhesins exhibit large Young moduli and Turgor pressures, with values in the range ~1.3-1.8 MPa and ~100-300 kPa, respectively. Under similar ionic strength condition, a dramatic ~50% to ~70% decrease of these nanomechanical parameters was evidenced for cells with longer surface appendages (F-*pilus*, type 1 *fimbriae*). Qualitatively, such dependence of nanomechanical behavior on bacterial cell envelope organization remains when increasing medium salt content to 100 mM, even though, quantitatively, differences are marked too much smaller extent. Additionally, for a given surface appendage, it is found that the magnitude of the nanomechanical parameters decrease significantly when increasing bulk salt concentration. This effect is ascribed to a bacterial exoosmotic water loss resulting in a combined contraction of bacterial cytoplasm together with an electrostatically-driven shrinkage of the surface appendage. The former process is demonstrated upon AFM analysis of the bacterial morphology, while the latter, inaccessible upon AFM imaging, is inferred from electrophoretic data interpreted according to advanced soft particle electrokinetic theory. Altogether, AFM and electrokinetic results clearly demonstrate the intimate relationship between structure/flexibility and charge of bacterial envelope and propensity of bacterium and surface appendage to contract under hypertonic conditions.



AFM application in research of interaction Neutrophils with Er/Yb nanoparticles

Ekaterina N. Gorshkova, Svetlana N. Pleskova, Elza R. Mikheeva

N.I. Lobachevsky Nizhny Novgorod State University, Russia

Er/Yb nanoparticles are a new type of fluorophores which can be applied in biological investigations (For example these stuffs can be used for indicting of extracellular transport). But it is necessary to determine its bioharmlessness before using this substance in vivo. The main goal of this research was to establish the possibility of nanoparticles toxicity. We used neutrophils as the test system because this kind of cells participates in the immune reactions. For neutrophils testing we used the method of scanning probe microscopy. Atomic force microscope SOLVER BIO™ (NT-MDT, Zelenograd, Russia) includes optical inverted microscope and measuring AFM head was established on it. The research was made under vital condition by DNP (Veeco, USA) probe in the semicontact mode [1]. Er/Yb was added after series of control scanning. The treatment of images was made on TopoSpm software (Veeco, USA). The neutrophils have been scanning for 45 minutes. We did not see any considerable changes in the cell morphology during this time. The observation within 60-120 minutes after edition of the Er/Yb at entering various concentration of $1{,}66 \cdot 10^{-3}$M, $8{,}25 \cdot 10^{-5}$M has shown that the neutrophils morphology was significantly changed. The viability of cells was estimated by using of postmortal dye propidium iodide (PI). As a result of incubation with Er/Yb in concentration of $1{,}66 \cdot 10^{-3}$M the destruction of cells with nucleus visualization was registered near 100%; 73% lethality was registered after entering Er/Yb into concentration $8{,}25 \cdot 10^{-5}$M. The estimation of neutrophyl's membrane rigidity was made by method of power curves which is based on elasticity indicator of Yung module counted on Hertz model [2]. For measurement we used MSCT (Veeco, USA) probe. Firstly, we measured rigidity of membrane without influence of Er/Yb nanopowder, it was 26,46 ± 2,49 kPa. Secondly, we measured rigidity of membrane with influence of Er/Yb. This value has decreased to 19,07 ± 3,34 kPa. For reduction of toxic effect nanoparticles was incubated with blood whey (37ºC, 60 min). As a result we had a decrease of membrane rigidity only to 23,98 ± 3,46 kPa. This fact shows the protective action of blood whey proteins.

Acknowledgement: The work was supported by RFBR grant №09-04-97068-r_Volga_region_a and CRDF (RUXO 001NN-06)

[1] S.N.Pleskova, M.B.Zvonkova, Y.Y.Gushina, The investigation of neutrophyl granulocytes morphological properties by AFM, Morphology 127 (2005), P.60-62 (by Russian)
[2] D.V.Lebedev, A.P.Chuklanov, A.A.Bukharev , O.S.Druzhinina, The measurement of Yung module of biological objects in liquid condition by atomic force microscope special probe, Letters to journal of theoretical physics, 35 (2009) 8, P. 54-61 (by Russian)

Atomic force microscopy det ection of DNA single nucleotide polymorphisms

Ierardi V. [1], Giacopelli F. [2,3], Ravazzolo R. [2,3], Valbusa U. [1]

[1]*Nanomed labs, CBA, Physic Dept, Genova University, Italy*
[2]*Dept of Pediatrics and CEBR, Genova University, Italy*
[3]*Molecular Genetics Laboratory, Gaslini Institute, Genova, Italy*

The analysis of effect of single-nucleotide-polymorphisms (SNPs) in the human genome is greatly contributing to increase the comprehension of the relation between genetic factors and diseases. Sequence analysis of genomic DNA in different individuals reveals positions at which variations, involving individual base-substitutions, can occur. The single nucleotide polymorphisms (SNPs) are highly abundant and can have different consequences at the phenotypic level. Several attempts were made to apply Atomic Force Microscopy (AFM) imaging to detect and map SNP sites in DNA strands. This study was performed using AFM techniques to identify the SNPs present in a portion of the human SPP1 gene promoter, encoding osteopontin.

The osteopontin gene has multiple functions, mainly at the level of immune response, inflammation and cancer, which makes very interesting the search for molecular variants eventually associated to functional variation. Analysis of polymorphic variants associated to pathological conditions becomes more powerful if it can identify different combinations of them in haplotypes. The aim of this work is the set up of a diagnostic method able to directly identify a haplotype using AFM techniques. The method is based on the knowledge of different SNPs in a fragment of the gene, that can be combined in different haplotypes. Namely, knowing the location of SNPs in the DNA sample under investigation, we are able to find a correspondence among the DNA sample in exam and the possible haplotype combination. In this way it is possible to classify an unknown DNA sample in one of the known haplotype classes of the osteopontin promoter. Such method may be applied to the analysis of association between DNA variants and haplotypes and diseases. Moreover, having knowledge of possible haplotypes in a gene fragment, this approach is also able to detect the presence of new DNA variants.

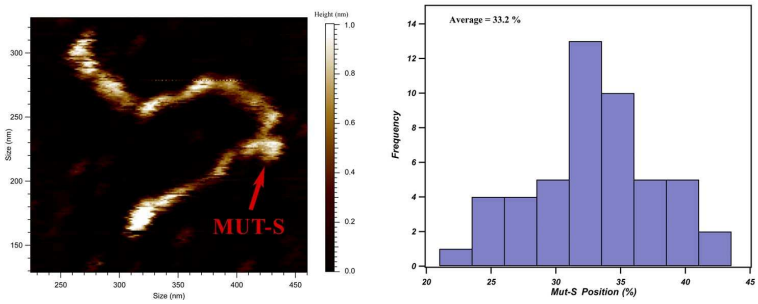


Fig. 1: AFM Tapping mode image of a DNA strand functionalized with Mut-S a mismatch repair protein and histogram of the Mut-S position in heteroduplex-DNA strands.

In order to achieve this aim we have used the Mut-S proteic marker, a mismatch repair protein that allows us to tag mismatches in heteroduplexe DNA strands. The heteroduplex-DNA strands are obtained by mixing a wild-type homoduplex DNA and a mutant DNA to subject it to a thermal denaturation and renaturation. After the heteroduplexes formation, the Mut-S label is added to recognize the SNP sites in heteroduplex-DNA strands by means of the AFM. As first step toward the detection of the all polymorphic sites in the osteopontin gene fragment, we apply AFM imaging to the detection and mapping of a DNA strand with one single mutation. In Tapping Mode AFM image (Fig.1) the presence of a hetroduplex molecule at the level of a known SNP is clearly shown. In Fig1 is displayed the histogram of the Mut-S position as well. The average Mut-S position is in full agreement with actual Mut-S position. The next step will be analyses on DNA strands with multiple SNPs.

Structural transitions in individual desmin intermediate filaments

Balázs Kiss, Árpád Karsai, Miklós S.Z. Kellermayer

Department of Biophysics and Radiation Biology, Semmelweis University Budapest, H-1094 Budapest, IX. Tűzoltó u. 37-47. Postal address: H-1444 Budapest, Pf. 263, Hungary

Desmin filaments form the intermediate filament system in muscle cells where they are thought to be important in determining mechanical integrity and elasticity. The molecular basis of desmin's elasticity is not fully understood. In the present work we mechanically manipulated desmin filaments polymerized from purified monomers, by using single-molecule atomic force microscopy (AFM).

Desmin, purified from chicken gizzard, was polymerized by the addition of either $MgCl_2$ or NaCl. For mechanical manipulation desmin filaments, adsorbed to mica or silanized glass surface, were captured with the tip of a flexible AFM cantilever. The filaments were then stretched by moving the cantilever away from the surface. Mechanically manipulated desmin displayed complex force responses. We identified four fundamental types of mechanical behavior: a) initial transition, b) force plateau c) plateau bumps and d) non-linear elasticity. a) The initial transition trace was the most frequently observed force pattern characterized by two discrete 20-60 pN force steps. This may correspond to unbinding and removal of individual coiled-coil desmin dimers from the filament surface. b) Force plateaus are characterized by constant force as a function of extension and resemble polymer desorption processes by protofilaments longer than 60 nm. c) Plateau bumps were superimposed on force plateaus in 16-nm steps. Conceivably, these force transitions appear as a result of unzipping or peeling protofilaments away from the surface of the desmin filament. d) Non-linear force curves often followed in tandem to form a sawtooth pattern. The non-linear curves were fitted with the wormlike chain model of entropic elasticity to obtain the persistence length (measure of bending rigidity) of the mechanically manipulated chains. The mean persistence length acquired from force measurement experiments was ~0.4 nm, which is far below previous measurements for intermediate filaments (~1 µm). Considering that the persistence length was similar to that of unfolded protein molecules (e.g., mechanically unfolded titin), it is conceivable that the non-linear force curves reflect the behavior of unfolded desmin monomers/protofilaments. To independently assess the entropic elasticity of unperturbed desmin, we analyzed the shape fluctuations of surface-adsorbed filaments. Based on this shape analysis the persistence length of desmin filaments is ~0.45 µm, and the calculated Young modulus is 3.7 MPa. The obtained quantitative measures of desmin elasticity may provide a basis for estimating desmin-associated mechanical features at the muscle fiber level.

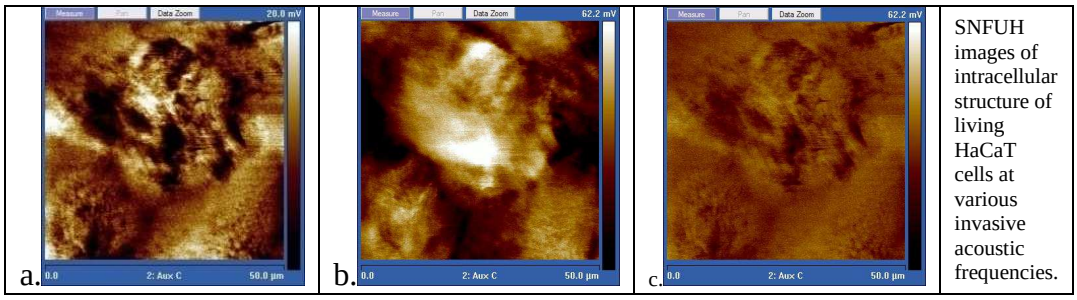
Living cells investigated by ultrasound holography

E. Lesniewska[1], M. Ewald[1], E. Bourillot [1], C. Heu[2], C. Elie-Caille[2], L. Tétard [3], T.G. Thundat [3], C. Le Grimellec [4], P.E. Milhiet [4]

[1]*Institute Carnot Bourgogne UMR CNRS 5209, University of Bourgogne, Dijon, France*
[2]*Institut FEMTO-ST CNRS UMR 6174, CLIPP, University of Franche-Comté, Besançon, France*
[3]*Nanoscale science & devices group, Oak Ridge National Laboratory, Oak Ridge, TN, USA*
[4]*Centre de Biochimie Structurale INSERM U554, CNRS UMR5048, University of Montpellier, Montpellier, France*

Atomic force microscopy has been extensively used in cell biology [1-2]. Imaging techniques [3] that can visualize local populations of nanoparticles at nanometer resolution within the structures of cells are therefore important. We show that living cells can be probed using a nondestructive imaging method, called scanning near field ultrasonic holography (SNFUH). This method developed for study in liquid, has provided depth information as well as spatial resolution at the nanometer scale using a resonant frequency of about 5 MHz. Calibration has been performed on samples of buried structures made by e-beam lithography and ionic machining or reactive ion etching. Different materials, forms, shapes, periodicity, depths have been used to adjust the resonant frequency and understand the acoustic image formation (problem in contrast understanding). On different biological specimens such as bilayer, we could distinguish the different phases: a liquid phase in the background, and a gel phase gel in clear contrast on the image. The acoustic answer is revealed by a positive contrast. Proteins incorporated in bilayers were detected in inverted contrast. Scanning near field ultrasonic holography is a useful technique for probing the interactions of engineered nanomaterials in biological systems, which will greatly benefit areas cellular biology. We have developed a non-invasive and innovative tool of characterization for biology: make an adaptation of the acoustic microscope to study aqueous hence: a huge potential for biological samples in terms of resolution and information. The transverse generation of wave of high frequency seems an alternative which it is interesting to exploit within the biomolecular film framework. Adaptation of acoustic mode AFM microscope on High Speed (HS-AFM for non-contact AFM mode imaging will be performed.

[1] E. Lesniewska, P.E. Milhiet, M.C. Giocondi, C. Le Grimmellec. AFM imaging of cells and membranes. Article in AFM in Cell Biology by B.P. Jena and H. Hörber, Series Methods in Cell Biology 68, 39 (2001) 39.
[2] S. Lydataki, E. Lesniewska, M.K. Tsilimbaris, A. Bron, I.G. Pallikaris. Corneal tissue observed by means of atomic force microscopy. Methods Mol. Biol. 242 (2004) 69.
[3] L. Tetard, A. Passian, K.T. Venmar, R. M. Lynch, B.H. Voy, G. Shekhawat, V.P. Dravid, T. Thundat. Imaging nanoparticles in cells by nanomechanical holography. Nature Nanotechnology 3 (2008) 501.



SNFUH images of intracellular structure of living HaCaT cells at various invasive acoustic frequencies.

Influence of subsurface composition on the adhesion of bacteria and the adsorption of proteins

Peter Loskill, Yvonne Schmitt, Hendrik Hähl and Karin Jacobs

Saarland University, Saarbrücken, Germany

Biofilms are of special importance in various fields of the everyday life. Their initial formation is composed of two crucial steps: the adsorption of proteins and the adhesion of bacteria. These are complicated processes that depend on many factors.
So far, most studies focused on surface chemistry, hydrophobicity and surface roughness - factors that influence mainly the short-range interactions.
Our studies concentrate on the impact of long-range interactions, in particular van der Waals forces, which can be tuned by the use of tailored substrates.
To characterize the processes, we follow two pathways: One way is to characterize protein adsorption on a fundamental level via ellipsometry. Another is to directly probe bacterial adhesion by AFM - force spectroscopy.
As model systems we use *Staphylococcus aureus* bacteria and proteins like amylase, lysozyme and bovine serum albumin.
The results of our experiments show that protein adsorption kinetics as well as bacterial adhesion are dependent on the subsurface composition of the substrate [1,2].
Hence it is of great importance for the design of anti-adhesive surfaces to consider not only the lateral but also the vertical composition of the substrate.

[1] A. Quinn et al., Protein adsorption kinetics in different surface potentials, Europhysics Lett. **81** (2008) 56003
[2] M. Bellion et al., Protein adsorption on tailored substrates: Long-range forces and conformational changes, J. Phys.: Condens. Matter **20** (2008) 404226

Direct monitorization of the oligomerization properties of proteins by *in situ* Atomic force microscopy: the case of the ferric uptake regulator FurA analyzed under different redox conditions in a physiological environment

Anabel Lostao [a,†], María Luisa Peleato[b], Andrés González [b], Carlos Gómez-Moreno[a,b] and María F. Fillat[b]

[a]*Instituto de Nanociencia de Aragón (INA), Universidad de Zaragoza, 50018- Zaragoza, Spain*
[b]*Department of Biochemistry and Molecular and Cell Biology and Biocomputation and Complex Systems Phyics Institute (BiFi), Universidad de Zaragoza, 50018- Zaragoza, Spain*
[†] *Fundación Aragón I+D (ARAID), Aragón, Spain*

The knowledge of the oligomerization states of the proteins in different physiological conditions can bring significant information about the role they play in the cellular life. The quantification of different oligomeric species is often quite difficult to perform. There are useful techniques that can be effective in some cases as gel filtration chromatography, mass analysis or some forms of electrophoresis. However, these techniques do not give good results with many proteins. This work proposes Atomic force microscopy (AFM) as an alternative technique to analyze the oligomerization states of proteins. To achieve this purpose is needed to immobilize the protein on a flat substrate in the physiological conditions of study, preferably by adsorption; choose a incubation concentration that allows to resolve each oligomer individually; and obtaining high-resolution images that can distinguish each monomer. In some cases may be necessary to make a volumetric analysis of the features but if the images have a good resolution will be enough to zoom on each feature to determine the type of oligomer.

In this work we have used AFM to unravel protein-protein interactions in relevant conditions that give us important data of protein dynamics. AFM allows single-molecule imaging and monitorization of the status of the ferric uptake regulator FurA under different redox conditions in a physiological environment. Fur proteins are global prokaryotic transcriptional regulators. Functional studies of FurA from the cyanobacterium *Anabaena* sp. PCC 7120 evidenced the influence of the redox environment in the activity of the regulator and its ability to aggregate through disulphide bridges. The estimated FurA average diameter was of 4 ± 1 nm. In the absence of reducing agents FurA is mainly associated as trimers; being 40^{o} the prevalent angle α conformed by protein monomers. Reducing conditions induces trimer rearrangment to protein monomers and a major fraction of FurA dimers. Disruption of the dimeric assemblies and appearance of higher order aggregates, namely trimers and tetramers are induced by oxidation with diamide or hydrogen peroxide. The homogeneity of the angles exhibited by the trimeric particles, as well as the occurrence of dimers in the presence of DTT, suggests the participation of relatively specific hydrophobic interactions maintaining the dimer. Direct visualization of the regulator under liquid phase at molecular resolution unravels the importance of non-polar interactions in FurA dynamics and shows that in *Anabaena* disulphide bridges are not essential for the dimerization of FurA.

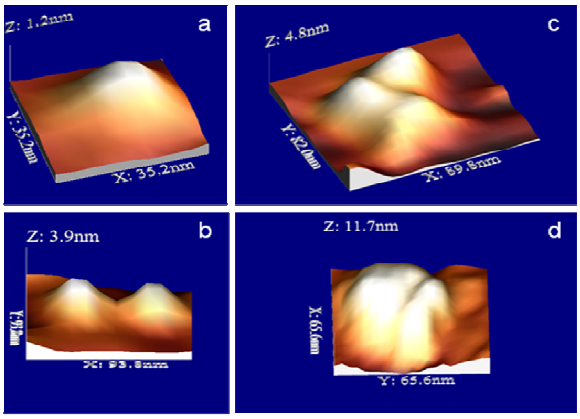


Figure 1. AFM features zoomed to observe some FurA aggregates in detail, showing a) a monomer, b) a dimer, c) a trimer and d) a tetramer. Images obtained using Jumping mode in Bis-Tris 10 mM, KCl 40 mM, $MnCl_2$ 0,1 mM, $MgCl_2$ 1mM, pH 7,5, at different redox conditions using DTT, H_2O_2 or Diamide.

[1] A. Lostao, M.L. Peleato, C. Gómez-Moreno, M.F. Fillat, Oligomerization properties of FurA from the cyanobacterium *Anabaena sp*. PCC 7120: Direct visualization by in situ atomic force microscopy under different redox conditions, submitted to BBA (BBAPRO-09-467).

Initial Bioadhesion on Medical Glass Packaging Material Investigated by Scanning Force Microscopy

Anne Lueders[1], Christine Mueller[1], Holger Roehl[2], Christiane Ziegler[1]

[1]*Department of Physics and Research Centre OPTIMAS; University of Kaiserslautern; D-67663 Kaiserslautern;*
[2]*Schott AG; Corporate Research and Technology Development; D-55122 Mainz*

Glass is one of the most important materials in medical agent and drug storage. Therefore, glass has to fulfil special requirements to assure that there is no alteration of the agent. On the one hand it is important to minimize the structural change of the glass surface, which can influence the agent dilution and on the other hand a strong agent adsorption should be avoided. During adsorption the structure of an agent can change reversibly or irreversibly. This comes along with a decrease of the agent concentration. To compensate this effect there is an overfilling of the agent. Especially for low dose, expensive or toxic ingredients, agent adsorption should be prevented.

Nearly the half of agents that are stored in glass packaging like vials and injections are based on a biological structure. This reveals why it is necessary to investigate the adsorption of proteins as a starting point for further investigations of the more complex agent adsorption process. The adsorption of proteins is a very common effect, it appears as soon as a protein comes in contact with a surface. Scanning force spectroscopy is a useful method for investigations on protein adsorption. Besides the topographic differences before and after protein adsorption, there is the opportunity to measure adhesion forces up to the pico-Newton regime. The measurement of the adhesion force depends on the pH-value, the contact time, the chosen surface and protein. The topographic information obtained via force maps can be combined with force spectroscopy to specify the surface coverage.

The focus of this presentation is on the adsorption behavior of albumin and lysozyme, as model proteins for weak and strong proteins, as well as fibrinogen, as an important clotting protein. Conventional and SiO_2 covered borosilicate glass were used as medical glass packaging.

The adhesion force between the proteins and both glass surfaces shows time dependence. After an increase of adhesion force with contact time, saturation could be detected. The adhesion process follows a second order kinetics. By investigating the pH-dependency, the electrostatic force could be identified as the dominating force of the interaction. Furthermore, a quantitative difference between both glasses with respect to protein adsorption was observed.

Research of the semiconductor nanoparticles influence on the neutrophil granulocytes by AFM

Elza R. Mikheeva, Svetlana N. Pleskova, Ekaterina N. Gorshkova

N.I. Lobachevsky Nizhny Novgorod State University, Russia

Semiconductor nanoparticles (quantum dots) are perspective products of nanotechnology for bioimaging and medical diagnostics, due to their unique properties. However, the information about of quantum dots toxicity in the system with biological objects is actual known [1 – 5]. Therefore the question about biosafety and bioavailability nanocrystals is priority and the possibility of creation the systems for example using of blood cells allow to express and economy estimate (diagnostics) to influence of quantum dots. In the presented work interaction between the neutrophil granulocytes and semiconductor quantum dots has been investigated. The neutrophil granulocytes are the blood cells which responsible for nonspecific immunity and are the first barrier on a way of the antigens penetration. Human neutrophils were isolated from venous blood of healthy volunteers by centrifugation through ficoll-verografin density gradient (Pharmacia, Sweden) using densities of 1.077 and 1.116 g/ml. The quantum dots CdSe/CdS coated with mercaptoacetic acid (MAA) have been received in laboratory of V.R.Zlomanov (Lomonosov Moscow State University) and has been used at a concentration of 0.06 mg/ml. The cells morphology was investigated by atomic force microscopy (Solver Bio ™ NT-MDT, Russia). Neutrophils were incubated in Petri dishes and have been scanning for 45 minutes. We did not see any changed in the morphology of cells. After control scanning CdSe/ZnS-MAA QDs was added and studied in real time regime during 4 h. We have been observed the changes in the cells morphology: height of cells was decreased significantly in the field of a nucleus (more then 60%); the cells formed atypical pseudopodia; the membranes of cells became thinner. The rigid of cells membrane has been investigated too. For the estimation of neutrophil membranes rigidity has been used the kind of AFM: FS-spectroscopy. The rigid of native cells was 26.46 ± 2.49 kPa, but after addition of quantum dots it was decrease for 10.18±1.56 kPa.

Acknowledgement: The work was supported by RFBR grant №09-04-97068-r_Volga_region_a and CRDF (RUXO 001NN-06)

References

[1] A. Shiohara, A. Hoshino, K. Hanaki, K. Suzuki, K. Yamamoto, On the cytotoxicity caused by QD, Microbiol. Immunol. 48 (2004), P. 669 – 675.

[2] A.V.Kolesnichenko, M.A.Timofeev, M.V.Protopopova, Toxicity of the nanomaterial – 15 years of researches, Russian nanotechnology 3 (2007) 3, P. 54 – 61. (by Russian)

[3] A. Nel, T. Xia, L. Madler, N. Li Toxic Potential of Materials at the Nanolevel, Science 311 (2006), P. 622 – 627.

[4] J.M. Tsay, X. Michalet, New Light on Quantum Dot Cytotoxicity, Chemistry & Biology 12 (2005), P. 1159 – 1161.

[5] A.M. Derfus, Warren C. W. Chan, S.N., Bhatia Probing the cytotoxicity of semiconductor Quantum Dots, Nano latters. 4 (2003), P. 11 – 18.

Cytochrome c-induced aggregation of zwitterionic biomembranes observed by Atomic Force Microscopy.

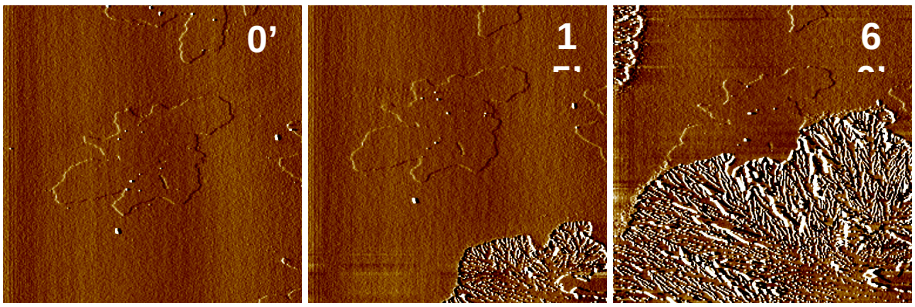
Sandrine Morandat[1] and Karim El Kirat[2]

[1]*Laboratoire de Génie Enzymatique et Cellulaire, UMR-CNRS 6022,*
[2]*Laboratoire de Biomécanique et Génie Biomédical, UMR-CNRS 6600,*
Université de Technologie de Compiègne, BP 20529, 60205 Compiègne Cedex, France

The interaction of cytochrome c (cyt c) with fluid/gel neutral supported lipid membranes was investigated by time-lapse atomic force microscopy (AFM). AFM revealed the random formation of depressed areas in fluid membranes promoted by cyt c. These depressions corresponded to the desorption of fluid bilayer patches induced by cyt c. By contrast, the gel domains were never desorbed but they were progressively thickened in the presence of the protein. These results suggest that cyt c molecules might intercalate between the mica and the lipid bilayer. Furthermore, our data show that the net positive charge of cyt c molecules plays a pivotal role but it is not the sole factor responsible for cyt c insertion in the membrane.

Considering the pivotal influence of cyt c in these important cellular processes, the nature of cyt c interactions with model lipid membranes has attracted much interest. The forces driving the interaction of cyt c with negatively charged membranes are mainly electrostatic. Indeed, the cyt c molecules expose a cluster of lysine residues (called site A and surrounding the heme edge) which is involved in the electrostatic interaction with negatively charged lipids. Cyt c molecules also present a second electrostatic binding site that interacts with protonated acidic phospholipids by hydrogen bonding (site C). Another important feature of cyt c is also its ability to interact with phospholipids via hydrophobic forces. Indeed, cyt c can be found inserted, at least partially, into the inner mitochondrial membrane. This membrane-inserted form of cyt c may have a biological role in both electron transfer and in apoptosis.

This work presents the time-lapse imaging of cyt c interaction with zwitterionic supported model membranes at the nanometer scale. The model bilayers were composed of dioleoylphosphatidylcholine (DOPC)/dipalmitoylphosphatidylcholine (DPPC) (1:1, mol/mol) or DPPC alone. These neutral membranes were chosen to favor the hydrophobic-driven interaction with cyt c. In this work, we have studied the influence of two parameters on the behaviour of cyt c: the lipid molecular packing and the protein charge. Moreover, we have compared the interaction between cyt c and model lipid membranes with another comparable small basic protein: egg yolk lysozyme. Finally, by measuring the force necessary to punch the supported bilayers with the AFM tip, we have evidenced the weakening of lipid phases.



Effect of beta-sheet-breaker peptide on epitaxially grown Aß25-35 amyloid fibrils

Ünige Murvai., Miklós S.Z. Kellermayer

Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

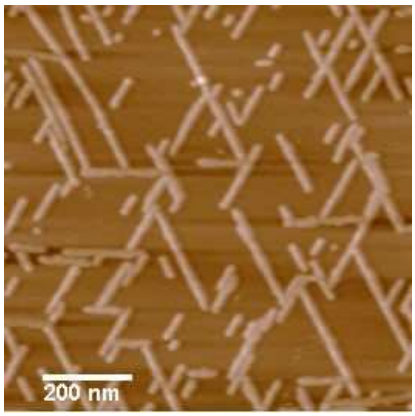
Amyloid fibrils are filamentous aggregates deposited in extracellular tissue in various neurodegenerative and protein misfolding disorders. Amiloid β peptides form self-associating fibrillar structures possessing predominantly cross-β conformation. The structural properties of amyloid fibrils are hard to investigate because of the insoluble aggregate formation that precludes the use of standard structural methods such as x-ray crystallography and solution NMR. The molecular structure of β-amyloid fibrils and the exact mechanism of amyloidogenesis and fibrillogenesis have not been fully elucidated yet. Atomic force microscopy is a useful tool for the visualization of single amyloid fibrils and for measuring the forces that hold the fibril-structure together.

It has been shown that beta-sheet-breaker (BSB) peptides may interfere with amyloid fibril assembly. Although BSB peptides are prospective therapeutic agents in amyloidosis, there is ambiguity about the mechanisms and generality of their action.

In the present work we analyzed the effect of the BSB peptide LPFFD on the growth kinetics, morphological and mechanical properties of amyloid ß25-35 (Aß25-35) fibrils assembled in an oriented array on mica surface. Aß25-35 is thought to represent the biologically active, toxic fragment of the full-length beta peptide. Growth kinetics and morphological features were analyzed by using *in situ* AFM in the presence of various concentrations of LPFFD. We found that the addition of LPFFD slightly altered the assembly kinetics of Aß25-35 fibrils. Already formed fibrils did not disassemble in the presence of high concentrations of LPFFD.

The mechanical stability of the fibrils was explored with force spectroscopy methods. The nanomechanical behavior of Aß25-35 fibrils is characterized by the appearance of force staircases which correspond to the force-driven unzipping and dissociation of several protofilaments. In the presence of LPFFD single-plateau force traces dominated.

The effects of LPFFD on Aß25-35 fibril assembly and stability suggest that inter-protofilament interactions were slightly weakened. Complete disassembly of fibrils, however, was not observed. Thus, LPFFD may not be considered as a BSB peptide with generalized beta-sheet breaking properties.



Scanning probe microscopy investigation of magnetite micro- and nano-particles interaction with rat pulmonary phagocytes *in vivo*

Ekaterina V. Nikolaeva, Alexander I. Rastegaev, Vladimir Ya. Shur, Marina P. Sutukova*, Larisa I. Privalova*, Boris A. Katsnelson*

Ural center for joint usage "Modern nanotechnologies", Ural State University, Ekaterinburg, Russia
**Medical research center for prophylaxis and health protection in industrial workers, Ekaterinburg, Russia*

Rapid development of nanotechnologies no doubt will result in wide usage of nanomaterials and products containing nanomaterials in the everyday life. One of the most important questions on the way of introduction the nanotechnology products is their safety for environment and human being. Investigation of the interactions between nanoobjects, in particular nanoparticles, with biological cells is one of the main nanotoxicology problems.

In this work we present application of the scanning probe microscopy (SPM) methods for investigation of magnetite interaction with alveolar macrophages of rats. Micro- and nanoparticles of magnetite Fe_3O_4 with nominal sizes 10 nm, 50 nm and 1 μm (produced by "IVA" Ltd, Russia) were used for the experiments. All experiments were carried out on outbred white female rats with the initial body weight of 150-220 g. We have studied *in vivo* micro- and nanoparticles interaction with alveolar macrophages. Intratracheal instillation of nanoparticles 1 ml aqueous suspension (2 mg/ml) was made to the rats. Bronchoalveolar lavage fluid (BALF) taken 24 hours after instillation was then analyzed by SPM. 8 to 10 animals were in each exposed (interaction with micro-and nanoparticles) and control groups. Samples for SPM were prepared by the following way: a 3 μl drop of the cell suspension was placed on the fresh cleavage of mica; after 1 minute incubation the drop was removed by the filter paper; the sample was then dried by the nitrogen.

SPM measurements were performed using the probe nanolaboratory NTEGRA Prima (NT-MDT, Russia) and the NSG01 probes with the tip height 15 μm and the tip curvature radius less than 10 nm. Statistical treatment and analysis of the SPM data has been done using the specialized software SPIP (Image Metrology, Denmark) and SIAMS Photolab (SIAMS, Russia).

Mainly, the semi-contact atomic force microscopy (sc-AFM) was utilized to visualize the surface morphology of the BALF cells (both alveolar macrophages and neutrophil leucocytes) of exposed and control groups. The characteristic "pits" were observed at the surface of the cells from the exposed groups. It was shown that the typical transverse sizes of these pits depend on the size of the magnetite particles used. It should be stressed that for the cells of control group the number of the pits at the surface was negligible. Consequently it was concluded that these pits on the cell surface are associated with the micro- and nanoparticles incorporated inside the cells. It may be assumed that pits visible with the sc-AFM are produced by invagination of the plasma membrane which is the 1st step of particles' engulfment by phagocytizing cells.

The statistical analysis on distribution of the pits of different sizes at the surface of cells belonging to different exposed groups was carried out in order to disclose the peculiarities of the phagocytosis of nanoparticles of different size.

This research was carried out thanks to the financial support from the part of the Russian Federal Agency of Science and Innovations.

Design, study and applications of a biomimetic cellular cortex

Lea-Laetitia Pontani, Cecile Sykes*, Jasna Brujic

Center for Soft Matter Research, Department of Physics, New York University, New York
**Laboratoire Physico-Chimie Curie, UMR 168, Institut Curie, Paris*

The cell cytoskeleton is a composite and versatile structure that confers the cell their complex mechanical properties. The actin cortex, in particular, assembles in a dynamic way under the membrane and provides the necessary forces for cell shape changes and motility: actin polymerization allows the growing filaments to push the membrane forward while molecular motors produce the contractile forces. Biomimetic systems are useful tools to study individually the cellular modules in a simplified environment. Such an experiment was performed to reproduce the actin cortex. The necessary ingredients for actin polymerization were introduced inside a liposome and the reaction was localized next to the membrane thanks to the grafting of the activator directly onto the membrane lipids, thus mimicking the cellular cortex architecture. After the polymerization was triggered, we managed to obtain an actin gel assembling into a shell under the membrane. The mechanical properties of such a system were addressed through the characterization of their dynamics while spreading onto a surface. The results were in good agreement with those obtained on cells. Those simplified systems were also used in a physiological context: the internalization of the Shiga toxin in cells. We found that this toxin was internalized in a system as simple as the liposomes containing a reconstituted actin cortex, thus underlying the critical role of the actin cortex in this process. This bottom-up approach will be extended to the study of cell-cell interactions. As a first step, the adhesive forces in a cellular assembly can be deciphered through the study of biomimetic liposomes packing.

Insertion of lactose permease in lipid planar bilayers mimicking *Escherichia coli* membrane

Laura Picas[a], Carme Suárez-Germà[a], Oscar Domènech[a], M. Teresa Montero[a,b], Jordi Hernández-Borrell[a,b]

[a]*Departament de Fisicoquímica, Facultat de Farmàcia UB, 08028-Barcelona.*
[b]*Institut de Nanociència i Nanotecnologia de la Universitat de Barcelona (IN^{2}UB), 08028-Barcelona, Spain*

Phosphatidylethanolamine (PE) and phosphatidylgycerol (PG) are the main component of the *Eschericia coli* inner membrane. In previous studies we used atomic force microscopy (AFM) and force spectroscopy (FS) to study the topographic and nanomechanical properties of supported lipid bilayers (SLBs) of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and POPE and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) (3:1, mol/mol). In the present report the study has been extended for completeness to asymmetric SLBs obtained by the Langmuir-Blodgett (LB) method. Thus we have have prepared SLBs with the proximal leaflet extracted at 30 mN m^{-1} and the proximal leaflet extracted at 25 mN m^{-1}. The composition of the SLBs: both leaflets with the same composition (POPG/POPG), and with the proximal leaflet of POPE and distal leaflet of POPG or POPE:POPG (3:1, mol/mol). The topography of the SLBs adquired in liquid wer compared with the topography of the monolayers adquired in air. Breakthrough (F_y) and adhesion forces (F_{adh}) of SLBs have been extracted from force curves. The values obtained are discussed in terms of possible implication of the nanomechanical properties of the SLBs on membrane protein insertion. We report the insertion of a transmembrane protein, lactose permease (LacY) from *Escerichia coli* (*E. coli*), in supported lipid bilayers (SLBs) of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG), in biomimetic molar proportions. We provide evidence of the preferential insertion of LacY in the fluid domains. Analysis of the self-assembled protein arrangements showed that LacY: (i) is inserted as a monomer within fluid domains of SLBs of POPE:POPG (3:1, mol/mol), (ii) has a diameter of approx. 7.8 nm; and (iii) keeps an area of phospholipids surrounding the protein that is compatible with shells of phospholipids.

Using of AFM for morphological and visco-elastic characteristics of living cells under hydrogen peroxide conditions

Svetlana N. Pleskova, Elza R. Mikheeva, Ekaterina N. Gorshkova

N.I. Lobachevsky Nizhny Novgorod State University, Russia

It is great number of hydrogen peroxide (H_2O_2) physiological effect [1, 2]. They are especially important for professional phagocytes, because NADH-oxidase system generated reactive oxygen forms in the respiratory burst [3]. We use atomic force microscopy (AFM) for estimation the influence H_2O_2 on the human blood neutrophils.

The neutrophils have been isolated from blood healthy volunteers and investigated under vital conditions by DNP probe (Veeco, USA) in the semicontact mode, using atomic force microscope SOLVER BIO™ (NT-MDT, Zelenograd, Russia). First of all we have scanning the cells in the control (about 2 h) and have seen that cells didn't change morphology under probe condition just long time. Secondary we have added H_2O_2 in different concentrations (from 3 mM till 25 mM). We can't use bigger concentrated hydrogen peroxide, because the concentrations after 25 mM decompose very quickly and forms bubble of oxygen, which lift the cantilever and prohibit the scanning process.

Under H_2O_2 condition cells have loosed their height from 2,1 ± 0,1 mkm till 1,3 ± 0,2 mkm (H_2O_2 in concentration 5 mM). We saw three kind of cells death: (1) necrosis – cells has loosed height and destructing; (2) mummification – cells hadn't change morphology for a long time scanning and cells were extremely rigid (increased the pressing force of a probe in 6,5 times exceeding the influence on the native cells didn't cause cells' destruction and/or modification); (3) blebbing – under surface of cells numerous bubbles have been formed and cells lost their nucleus. The first and second type of cells death by scanning probe microscopy has been observed; the third type can be studied only by optical microscopy.

Acknowledgement: The work was supported by RFBR grant №09-04-97068-r_Volga_region_a and CRDF (RUXO 001NN-06)

References

[1] I.V. Klyubin, K.M. Kirpichnicova., I.A Gamaley, Hydrogen peroxide-induced chemotaxis of mouse peritoneal neutrophils // Europ. J. Cell Biol. 70 (1996) p. 347 – 351.

[2] I. Bejarano, M.P. Terron, S.D. Paredes, C. Barrida, A.B. Rodriguez, J.A. Pariente, Hydrogen peroxide increases the phagocytic function of human neutrophils by calcium mobilization // Mol. Cell. Biochem. 296 (2007) p. 77 – 84.

[3] Y. Kasahara, K. Iwai ., A. Yachie, K. Ohta, A. Konno, H. Seki, T. Miyawaki, N. Taniguchi, Involvement of reactive oxygen intermediates in spontaneous and CD95 (Fas/APO-1)-mediated apoptosis of neutrophils // Blood. 89 (1997) p. 1748 –1753.

Production and characterization of epoxy-free colloidal probes for SPM studies

Alessandro Podesta[1], Marco Indrieri[1], Gero Bongiorno[2], Davide Marchesi[2], Paolo Milani[1,2]

[1]*CIMaINa and Dipartimento di Fisica, Università degli Studi di Milano, via Celoria 16, 20133 Milano, Italy.*
[2]*Micro and Nano-Fabrication Platform, Fondazione Filarete, viale Ortles 22/4, 20139 Milano, Italy.*
E-mail: alessandro.podesta@mi.infn.it

Attaching micrometer-sized spherical particles to the cantilevers of atomic force microscopes (AFMs) allows producing colloidal probes for a wide range of applications, such as the characterization of nanoscale surface interactions or the test of nanomechanical properties of thin films and tissues. One of the main advantages of using colloidal probes instead of commercial AFM tip is that they have a well defined geometry, and their size can be controlled and characterized accurately; this allows in turn applying well consolidated models for describing the interaction of the colloidal probe with the surface under investigation. Different techniques have been developed with the aim of attaching microspheres to cantilevers [1-2]. Typically, these techniques require using glue (epoxy resin) or glycerol. This might be a potential source of contamination of the surface of the colloidal probe. Furthermore, when the probe is used in liquid environment, in particular in organic solvents, a certain amount of the glue might be dissolved and re-adsorb on the substrate under investigation; this would be particularly unpleasant for biological samples like cells. Here we report a simple method for the production and characterization of colloidal probes. In particular we have improved the high-temperature sintering technique reported by Bonaccurso [1], developing a simple method that does not require adhesives like glues or glycerol.
We have also addressed the problem of performing a reliable and accurate characterization of the probe radius, a task that is at present typically accomplished by scanning electron or optical microscopy, or by making a section analysis of AFM inverted tip images acquired on suitable gratings. These methods are often either time-consuming or poorly accurate, and lack of statistical strength. We present a procedure based on the statistical analysis of AFM maps containing hundreds of independent inverted probe images, where the interacting part of each colloidal probe is modeled as a spherical cap. This method allows characterizing with ~1% accuracy the radius of curvature (and other geometrical properties) of the region of the probe interacting with the surface. We discuss the sources of errors in the determination of the tip radius, highlighting the role of defected calibration gratings (broken spikes), pixelization of AFM images, etc…

[1] Y. Gan, *Review of Scientific Instruments*, 2007, 78, 081101.

[2] Veeco Metrology group, Support Note 226, Revision B.

Investigation of the structural properties and oligomerization state of the complex enzyme glutamate synthase by atomic force microscopy

A. Podesta'[1], M.Indrieri[1], G. Caprini[2], S. Paravisi[2], M.A. Vanoni[2], P. Milani[1]

[1]*Dipartimento di Fisica and CIMAINA, Universita' degli Studi di Milano, Via Celoria 16, 20133 Milano, Italy*
[2]*Dipartimento di Scienze Biomolecolari e Biotecnologie, Via Celoria 26, 20133 Milano, Italy*
alessandro.podesta@unimi.it

Glutamate synthases (GltS) are complex iron-sulfur flavoproteins, found in bacteria, yeast and plants, where they play an essential role in ammonia assimilation processes [1]. In order to unravel the complex biological functionalities of this class of enzymes, their structure and oligomerization behavior in solution must be determined. Crystallization experiments of the model NADPH-GltS holoenzyme, consisting of two subunits (α subunit, 164 kDa and β subunit, 52 kDa), have been so far unsuccessful. In order to overcome this limitation, a combined effort based on different microscopy and spectroscopic techniques has been initiated. Recently, the synergic use of cryo-electron microscopy, small angle x-ray scattering and molecular modeling provided for the first time a molecular model for the GltS holoenzyme, which is a 1.2 MDa $(\alpha\beta)_6$ complex that exists in solution in equilibrium with the catalytically active αβ protomer, in the 1-20 mg/ml concentration range explored by these techniques [2].
Here we present the complementary results of a quantitative atomic force microscopy (AFM) study of the structure and the oligomerization behavior of NADPH-GltS. By combining high-resolution AFM to suitable quantitative metrological protocols, we could characterize the oligomerization state of GltS αβ holoenzyme and of its α subunit, in different ionic strength conditions, in the low concentration regime that is typical of kinetic assays. Our findings are in substantial agreement with the results of cryo-electron microscopy and small angle x-ray scattering, but provide insights on the behavior of the system at low protein concentrations, where oligomeric forms intermediate between the $(\alpha\beta)_6$ hexamer and the αβ protomer are detected. Comparison of the experimental AFM topographies to those simulated on the basis of the three-dimensional structures of the GltS hexamer and of the isolated α subunit, proved to be a powerful guide for the interpretation of AFM images, and provided additional insights on the structure of the observed species.
This work highlights the relevance of a combined multi-technique experimental approach for the investigation of the structure of biomolecular complexes, and emphasizes the single-molecule capability of AFM in this framework.

[1] Vanoni, M. A., Curti, B. (2005) Structure-function studies on the iron-sulfur flavoenzyme glutamate synthase: an unexpectedly complex self-regulated enzyme, *Arch Biochem Biophys* 433, 193-211.
[2] Cotteville, M. et al. (2008) The subnanometer resolution structure of the Glutamate synthase 1.2-MDa hexamer by cryoelectron microscopy and its oligomerization behavior in solution: functional implications, *J. of Biological Chemistry* 283(13), 8237.

AFM studies of macrophages

Roduit Charles[1], Giovanni Dietler[1], Bhaskar Saha[2] and Sandor Kasas[1,3]

[1]*Institut de Physique des Systèmes Biologiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
[2]*National Centre for Cell Science, Ganeshkhind, Pune 411007, India*
[3]*Département de Biologie Cellulaire et de Morphologie, Université de Lausanne, Lausanne, Switzerland*
Département de Biologie Cellulaire et de Morphologie, UNIL, Switzerland

Macrophages plays central role in the immune system. As shown in recent study [1], the mechanical properties of the substrate provide information that is integrated by the cells. Investigating the mechanical properties of macrophages can provide a better understanding of the immune system. Our laboratory is interested by looking in the cells mechanical properties at a very high resolution level. We recently developed a new imaging mode allowing detecting stiffness changes inside a substrate, which we called "Stiffness Tomography" [2].

The difficulty to image the macrophages by AFM comes from their non adherent and versatile properties. We overcome these limitations by incubating the macrophages in substrate coated with high concentration of Poly-L-Lysine. We post-processed our AFM force-spectroscopy images with OpenFovea [3] to reveal the stiffness properties of the cell.

The figure 1 shows the post-processing result of force-spectroscopy scan from the fixed macrophages. Some interesting features of 0.3-0.4 µm size are observed on these fixed cells. The stiffness tomogaphy reveals that these features are on the top of columns that come from deeper in the cell.

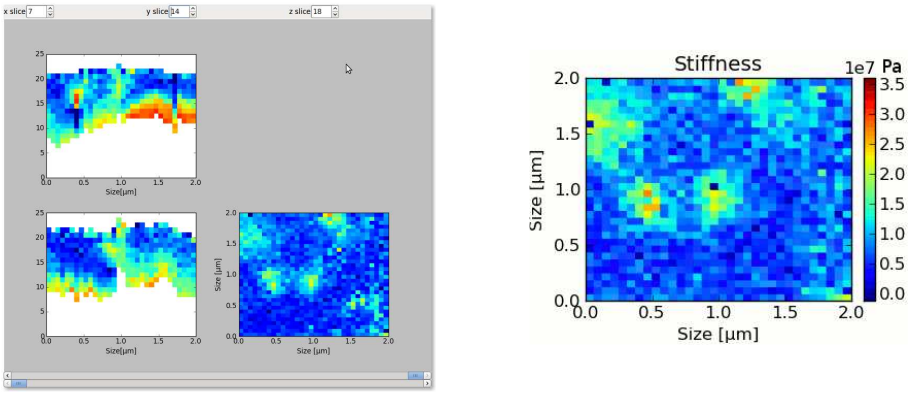


Figure 1: Stiffness tomography of a Macrophage showing details of columns under the membrane.

1. Salaita, K. et al. Restriction of Receptor Movement Alters Cellular Response: Physical Force Sensing by EphA2. Science 327, 1380-1385 (2010).
2. Roduit, C. et al. Stiffness Tomography by Atomic Force Microscopy. Biophysical Journal 97, 674-677 (2009).
3. see http://www.freesbi.ch/openfovea

AFM and dynamic light scattering study of amorphous calcium phosphate formation in the presence of monomeric and dimeric cationic surfactants

Suzana Šegota, Atiđa Selmani*, Katja Magdić, Darija Jurašin, Nada Filipović-Vinceković, Vesna Svetličić, **Maja Dutour Sikirić**

Ruđer Bošković Institute, Bijenicka 54, Zagreb, Croatia
**University of Zagreb, Horvatovac 102 a, Zagreb, Croatia*

A major problem encountered in the application of bone bioimplant materials is their slower rate of resorption in comparison to the rate of new bone formation. It was shown that the rate of new bone formation coincides more closely with the resorption rate of poorly crystalline or amorphous calcium phosphate (ACP) cements and ceramics [1]. But these materials still exhibit problems of mechanical strength, injectability, and application techniques. It is known that the highly hydrate spherulites of ACP grow by aggregation of much smaller primary particles and are subsequently joined together by secondary aggregation into chainlike structures [2]. Therefore coating of ACP primary particles with surfactants could be the solution to some of these problems. In recent years, dimeric surfactants, consisting of two single-chain ionic surfactants linked by a spacer at head level, have attracted considerable attention due to their superior properties in comparison to conventional surfactants. In this work we have compared the influence of monomeric dodecyltrimethlyammonium bromide, DTAB, and corresponding dimeric dimethylene-1,2-bis(dodecyldimethlyammonium bromide), 12-2-12, on the transformation of ACP. AFM was successfully applied to study amorphous calcium phosphate formation in each step of aggregation process.
In this work it was found that in the presence of phosphate ions DTAB forms only spherical micelles. 12-2-12 forms two kinds of micelles, spherical micelles at lower concentrations and larger non spherical micelles at higher concentrations. Combination of AFM and dynamic light scattering (DLS) enables to determine the size of both, primary particles and their secondary aggregates. The largest ACP primary particles were formed in the presence of both kinds of 12-2-12 micelles as confirmed by AFM. However, DLS measurements have shown that the smallest ACP aggregates are obtained in the presence of both DTAB and 12-2-12 micelles. These findings could be explained by zeta potential measurements which show that charge of ACP particles becomes more positive with increasing surfactants concentration resulting in inhibition of aggregation at higher surfactant concentrations.

[1] P. Frayssinet, J.L. Trouilelet, N. Rouquet, E. Azimus, and A. Autefage, Osseointegration of macroporous calcium phosphate ceramics having a different chemical composition, Biomaterials, **14** (1993) 423
[2] Lj. Brečević, V. Hlady, H. Füredi-Milhofer, Influence of gelatin on the precipitation of amorphous calcium phosphate, Colloids Surf. 28 (1987) 301

Friction, wear and funicity of β- and κ- casein monolayers studied by lateral force microscopy

Javier Sotres, Olof Svensson and Thomas Arnebrant

Biomedical Laboratory Science and Technology, Faculty of Health and Society, Malmö University

Caseins, the main protein component in milk, are flexible proteins with a segregated structure therefore being highly amphiphilic and surface active [1]. Caseins act as natural emulsifiers in milk but are also used as emulsifiers or dispersants in many applications [2, 3]. Caseins are present in milk in four major fractions varying in composition mainly in the hydrophilic part. The frictional and cohesive properties of self-assembled casein layers are of both fundamental and applied interest, in particular with respect to any influence by the different structures. In the present work the friction, wear and funicity properties of β- and κ-casein monolayers on hydrophobic surfaces immersed in ionic solutions have been studied by means of lateral force microscopy. For this purpose, nanometer-sized tips were employed to scratch a given area of the sample. Despite of the uncertainty in the probe size, using common imaging tips allows both to apply the high pressures needed to exert wear on these layers, and to visualize the topography of the scratched area while simultaneously monitoring the lateral forces exerted on the tip. The performance of these experiments on both β- and κ- casein monolayers revealed several important properties of these systems. First, it is shown how they can support pressures up to hundreds of MPa exerted by a model hydrophilic particle, i.e. the tip, before rupture and/or removal while still exhibiting a highly frictional behaviour. It is also shown that, although the friction while sliding is similar for both protein layers, the cohesion of those made up of β-casein is higher than those made up of κ-casein. Moreover, the cohesion of both systems increases with the ionic strength of the solution. Finally, the funicity of these systems has been characterized by scratching the same area of given samples several times. These experiments reveal two different diffusion processes of the proteins along hydrophobic surfaces: one where the monomers cover the surface in a very short time scale (faster than the AFM characteristic acquisition time), and a slower process where the monomers rearrange themselves increasing the stability/cohesion of the system.

References

[1] M.R. Ginger and M.R. Grigor, Comparative aspects of milk caseins, Comp. Biochem. Physiol. B **124** (1999) 133.

[2] E. Dickinson, Milk protein interfacial layers and the relationship to emulsion stability and rheology, Coll. Surf. B **20** (2001) 197.

[3] Y. Ai and D. Wei, Preparation of Hydrophilic Polystyrene Microspheres with Casein Molecules on the Surface, J. Macrom. Sci. **45** (2008) 456.

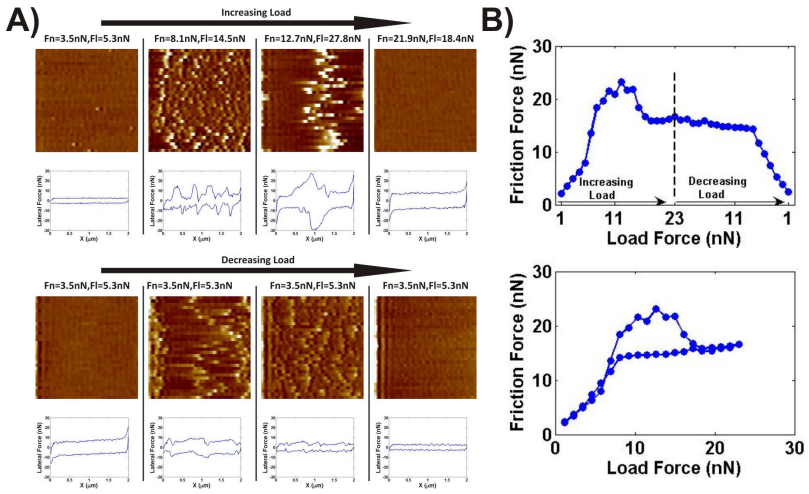


Fig. A) Topography images, along with examples of lateral force profiles, corresponding to different load forces of the scratch process of a β-casein monolayer on top of a hydrophobic silica surface in water. Images in the upper row correspond to the loading process. The first image corresponds to the sliding of the tip along the protein layer. This behaviour ends abruptly when the layer is indented for the first time. Right after this moment (second image) a new regime, with higher friction coefficient, starts. The third capture shows the protein aggregates generated during the scratching process. Due to its high affinity for the surface, these aggregates give raise to a high increase in the friction of the system. The final image in the upper row corresponds to the clean silica surface after removal of all the proteins. In a similar way, four images from the unloading process are shown in the lower row, starting from the clean silica surface at the higher load (left), going through the proteins diffusing back on the scratched area (two middle images), and finally, the sliding along the recovered protein monolayer (right). **B)** Two different representations of the friction vs. load forces acting on the tip corresponding to the same scratching process as the images shown in A).

High resolution AFM imaging to understand heterogeneous lipoprotein assembly and structure

Todd Sulchek

Georgia Institute of Technology, Atlanta, GA, USA

Traditional biochemical analytical techniques often suffer from a common problem—they provide an average, or "ensemble" view of the complexes. Thiscan be a particular problem if the ensemble average masks important structural or biochemical information. Here, we present our work in understanding heterogeneous lipoprotein assemblies formed from the apolipoproteins apoE4 and apoA1 fragments and various phospholipids. We show that chromatography separation techniques often do not produce homogenous particle sizes. This has important implications for techniques requiring homogeneity, such as X-ray crystallography. We also present a strategy to achieve 90+% particle monodispersity, which is sufficient to obtain crystals of lipoproteins.

Characterisation of mechanical unfolding trajectory of HaloTag 7 protein by dynamic force spectroscopy

Yukinori Taniguchi and Masaru Kawakami

School of Materials Science, Japan Advanced Institute of Science and Technology (JAIST), 1-1 Asahidai, Nomi, Ishikawa, Japan

Atomic force microscopy-based dynamic force spectroscopy is a powerful tool to probe the dynamics of protein (un)folding. In this method, typically tandemly-arranged multidomain protein is tethered between the substrate and cantilever to stretch and obtain the force-extension profile, and unfolding forces of each folded domain are recorded. For attaching a protein molecule to the substrate/cantilever, various approaches have been proposed so far. Physical (non-specific) adsorption is one of the commonly used methods. However, in this case a protein molecule has to strongly bind to the substrate or cantilever, while the other end has to bind to the other surface at the same time. This contradicting request is one of the serious reasons for the low hitting rate in the force measurement. Biological tags such as His-tag are not effective for this study, due to their typical weak interaction force (their rupture forces are typically 100-200 pN). The immobilisation of a protein using gold-thiol bond is often used because of its strong (its mean rupture force is ~1.4 nN) and specific formation between gold coated substrate/cantilever and sulfur atoms of cysteine residues in the protein. Usually the cysteine residue is inserted/mutated to be located only at the N or C terminal of the protein. However, this method cannot be used for protein molecules if they have intrinsic cysteine residues.

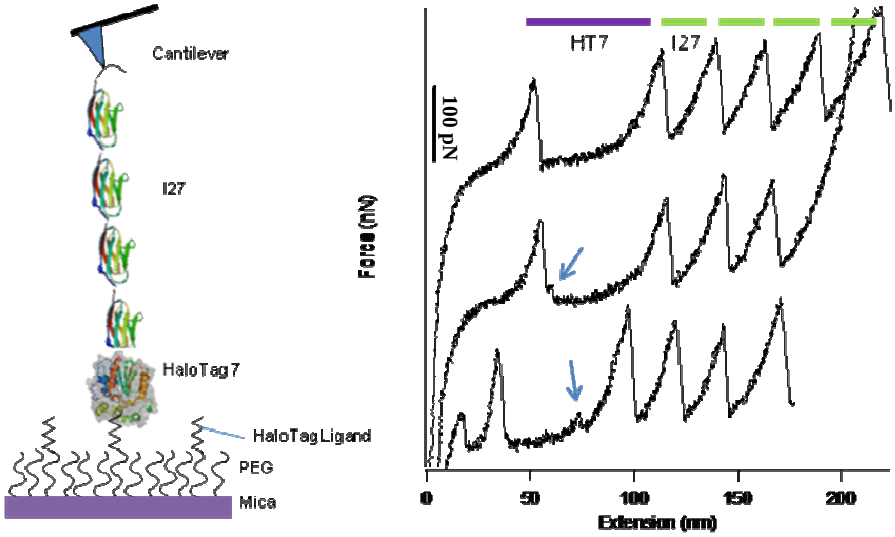


Figure: A cartoon representation of the experimental setup (left) and typical force-extension profiles of HT7-I27$_4$ (right). Infrequently small peaks were observed (arrows). Bar represents 100 pN.

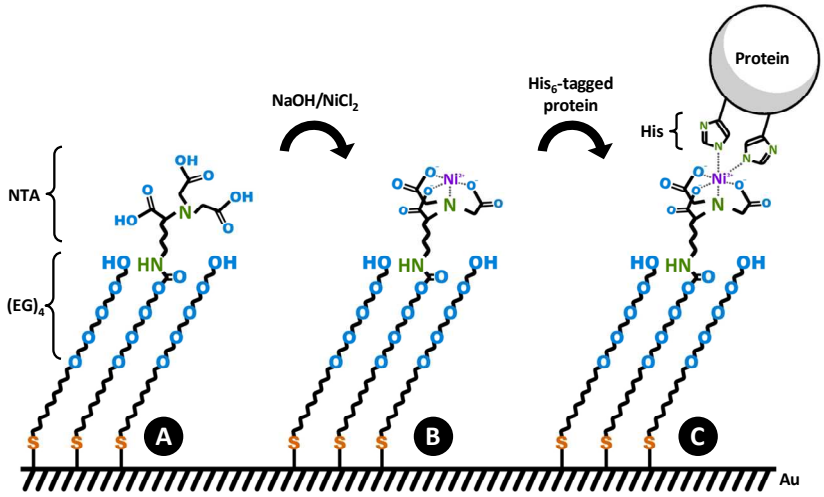
In this study, we have introduced a commercial HaloTag technique to immobilise a tandem protein. A covalent bond is formed between a catalytically inactive derivertive of haloalkane dehaloganase, HaloTag 7 (HT7) protein, and its ligand. HaloTag ligand was linked covalently to mica substrate using a PEG-linker. HT7 was expressed as a fusion protein in which four I27 domains are connected to the C-terminal of HT7, and immobilised to the substrate. We performed dynamic force spectroscopy to characterise the mechanical unfolding trajectory of the HT7. The force curve showed a characteristic sawtooth pattern, in which one of unfolding force peak has contour length increment of ~70 nm and the unfolding force of ~100 pN. The value of 70 nm is consistent with the length calculated from the number of amino acids between the C-terminal of HT7 and Asp106, the site of covalent bond with HaloTag ligand. In addition, in some force curves two different types of small rupture event were detected; one was found at ~30 pN immediately after the unfolding event of HT7 and the other was at ~40 pN in the rising phase of the next force peak. These small peaks suggest the existence of unfolding intermediate states of HT7 domain. The new immobilising method demonstrated in this study would become a useful tool for the study of cysteine containing proteins by single molecule force spectroscopy.

Measuring nanopatterns of His-tagged proteins by AFM

Joost te Riet[1,2], Inge Reinieren-Beeren[1], Alessandra Cambi[1], Sylvia Speller[2]and Carl G. Figdor[1]

[1] *Department of Tumor Immunology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500HB Nijmegen, The Netherlands*
[2] *Department of Scanning Probe Microscopy, Institute for Molecules and Materials, Radboud University Nijmegen, P.O. Box 9010, 6500GL Nijmegen, The Netherlands*

The last decade, proceedings in patterning technologies have made it possible to construct molecular structures at the nanoscale. An interesting application of patterning proteins for immuno-cell-biology would be to mimic nano- and micro-clusters of cell adhesion molecules (CAMs) as observed on the cellular membrane, such as DC-SIGN (dendritic cell-specific ICAM-3-grabbing non-integrin) and ALCAM (activated leukocyte cell adhesion molecule) clusters. By mimicking these structures, it is possible to investigate the purpose of CAM clustering on regulating the adhesion by cells. In this study, we investigated the chemistry needed to construct nanopatterns of DC-SIGN and ALCAM by atomic force microscopy (AFM) in self-assembled monolayers (SAMs) step-by-step. Therefore, AFM assisted nanografting is exploited to construct these nanostructures. To specifically immobilize proteins in an oriented fashion on a substrate N-nitrilotriacetic acid-histidine (NTA-His_6) chemistry is exploited. The patterned SAMs consist of two components, one being an NTA-thiol which specifically binds to a His-tag of the protein, and the other a multi-ethylene glycol (EG)-thiol. With single molecule accuracy, we demonstrate by AFM that the His-tagged proteins DC-SIGN-His and ALCAM-His can be specifically bound to an NTA-containing SAM.



Biotin-(Strept)avidin bonding revisited using Dynamic Force Spectroscopy

Jean-Marie Teulon, Yannick Delcuze, Michael Odorico, Pierre Parot and Jean-Luc Pellequer

CEA Marcoule, iBEB, Department of Biochemistry and Nuclear Toxicology, 30207 Bagnols sur Cèze, France

Life at the cellular level can be described as more or less durable interactions between biological molecules involved in processes spanning a wide range of strength and complexity. The duration of the molecular interaction between those molecules depends on the frequency of their encounter, the structure of the partners, and mostly the number of simultaneous bonds they can form. Among the innumerable pairs of ligand – receptor, one special couple composed of biotin, a water soluble vitamin (vitamin H), and avidin or streptavidin (its bacterial equivalent), became a paradigm.
The biotin-(strept)avidin complex exhibits the possibility of forming multiple bonds due to the tetrameric structure of the (strept)avidin. In this case, multiple bonds lead to avidity and explain the exceptionally high affinity of this complex. Indeed, the streptavidin - biotin system has one of the largest free energy of association yet observed for noncovalent binding of a protein and small ligand in aqueous solution (K_{assoc} = 10^{14}). The complex is extremely stable over a wide range of temperature and pH.
We present force-displacement curves of multiple parallel bonds between biotin and avidin. Using Dynamic Force Spectroscopy (DFS) formalism, we analyzed the rupture of these multiple bonds using the YIELDFINDER II software. Our results indicate that most published results on this system encompass various level of multiple binding.

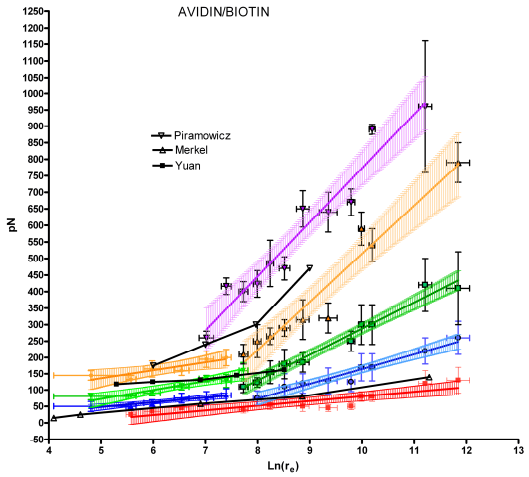


Figure: Experimental most probable rupture forces with the confidence interval (95% of the linear regression) vs the experimental loading rate of multi-bonds interaction between avidin and biotin. Solid black lines indicate the distribution of previously published most probable rupture forces (according to Bell-Evans theory).

■ Yuan C., A. Chen, P. Kolb and V. T. Moy (2000) Biochemistry 39(33): 10219-23.
▼: de Odrowaz Piramowicz M., P. Czuba, M. Targosz, K. Burda and M. Szymonski (2006) Acta Biochim Pol 53(1): 93-100.
▲: Merkel R., P. Nassoy, A. Leung, K. Ritchie and E. Evans (1999) Nature 397(6714): 50-3.

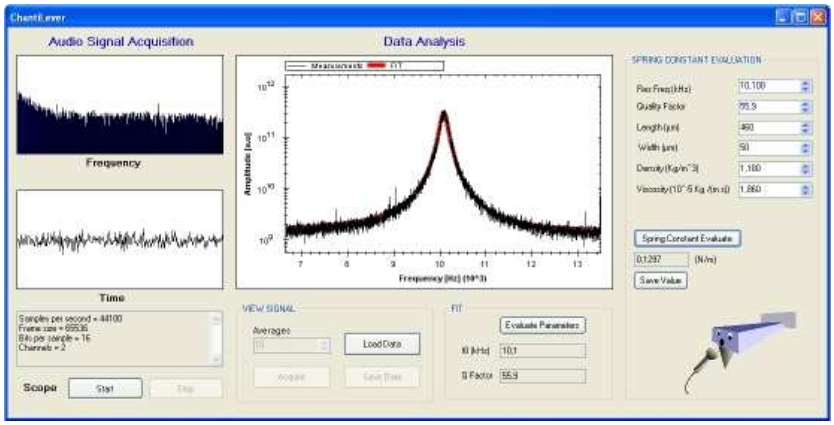
A sound card application for cantilever calibration

Bruno Tiribilli[1], Paolo Paoletti[1] , Massimiliano Papi[2], Valerio Pini[3], Francesca Sbrana[4], Massimo Vassalli[5]

[1]*Istituto dei Sistemi Complessi, Consiglio Nazionale delle Ricerche, (ISC-CNR) Sesto Fiorentino, Italy*
[2]*Istituto di Fisica, Universitá Cattolica S. Cuore, Largo Francesco Vito 1, I-00168, Roma, Italy.*
[3]*BioNanoMechanics Laboratory, Nat. Center for Microelectronics IMM-CNM (CSIC) Madrid, Spain*
[4]*Università di Genova Dip. Ingegneria Biofisica ed Elettronica (DIBE) Genova, Italy*
[5]*Istituto di Biofisica, Consiglio Nazionale delle Ricerche, (IBF-CNR) Genova, Italy*

"Chantilever" is a simple software application that, using the standard PC sound card, acquires the deflection signal of an AFM probe subjected to thermal noise. The power spectral density is obtained from an FFT algorithm and a fitting procedure is applied for evaluating the peak frequency and the Q-factor. These parameters together with the length and width of the probe allow calculating the spring constant according to the Sader method [1].
Alternatively, when the geometry of the probe is unknown, the thermal noise method can be applied [2] to obtain the spring constant with reasonable accuracy.
Finally if both calibration method can be applied to the same probe, the software additionally provides the calibration of the deflection signal (nm/V) without the need to push the probe tip against an hard surface [3].
We report the results of some tests performed with our home-made AFM on rectangular soft cantilevers by means of a standard 44 kHz/16 bit sound card and high quality 192 kHz/24 bit board.
"Chantilever" has been developed using C# programming language and it runs natively on Windows® platforms using .NET framework. The software is distributed free of charge under GPL open source license.

Binary and source code download will be provided soon at the webpage: http://www.fi.isc.cnr.it/chantilever.php



Picture. Screenshot of the running "Chantilever" application

[1] J. E. Sader, W. M. James, and Paul Mulvaney Rev. Sci. Instrum. 70, 3967 (1999).
[2] S. M. Cook, T. E. Schaffer, K. M. Chynoweth, M. Wigton, R. W. Simmonds and K. M. Lang, Nanotechnology 17, 2135 (2006)
[3] - M. J. Higgins, R. Proksch, J. E. Sader, M. Polcik, S. Mc Endoo, J. P. Cleveland, and S. P. Jarvis, Rev. Sci. Instrum. 77, 013701 (2006)

Changing of cellular mechanics during aging

J. Zahn[1,2], I. Louban[1,2], M. Bissinger [1,2], R. Kemkemer [1], J.P. Spatz [1,2]

[1]Max-Planck-Institute for Metals Research, Dept. of New Materials & Biosystems; Heisenbergstr. 3, D-70569 Stuttgart, Germany
[2]University of Heidelberg, Dept. of Biophysical Chemistry; Im Neuenheimer Feld 253, D-69120 Heidelberg, Germany

Aging is closely correlated with drastic changes (e.g. genetic, molecular) on the cellular level. Many details about aging from the molecular side of view are already known, e.g. changes in gene expression or the reduction of telomeres protecting DNA (Nobelprize 2009). How the cytoskeleton changes its structure and function and how aging alters the mechanical properties of cells are still under investigation.

For our experiments fibroblasts from human donors differing in age were chosen. Their age lies in the range between 10 and 54 years. Initial experiments demonstrated an age-specific difference in mechano-sensitivity. Senescent cells react faster on mechanical stimulation than cells from young donors. To correlate that age-specific difference in mechano-sensitivity to mechanical cell properties the Young-modulus of the cells was measured using atomic force microscopy (AFM). Large cell areas (100x100µm²) were mapped with 5x5µm (per pixel) resolution to get an insight of the local cell properties. At each point a force-distant curve was acquired and the Young-modulus was calculated via Hertz fit.

A significant difference for the Young-Modulus values of young and senescent cells was found. As the most common and best preserved structural protein of cells actin was chosen as structure of interest. The whole amount of actin as well as the amount of G-actin & F-actin was determined. A decrease of the amount of actin in senescent cells was shown. The ratio G/F actin seems to be independently from age. To analyze these further cells were transfected with siRNA to decrease the amount of actin aiming for the value for senescent cells. Using this method it was shown that the elasticity of the transfected cells is in the same range as the elasticity of senescent cells.

In conclusion, our results suggest on a genetic level as well that the expression of actin is decreases with aging and thus softening of the cells takes place. This may also explain the faster mechano-response of the older cells since a smaller amount of actin needs to be reorganised.

Author Index

Direction des Sciences du Vivant

AFM **BioMed Conferences**

http://www.afmbiomed.org

Post-Doctoral and PhD Positions are published on:

http://www.afmbiomed.org/opencms/afmbiomed/jobsevents/postdocs.html