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3rd International Workshop – 13 October 2004 in Berlin

Scanning Probe Microscopy in Life Sciences

Poster Award 2004

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Scanning Probe Microscopy in Life Sciences

Organizing Committee
Dr. Andreas Jordan – Center of Biomedical Nanotechnology & Dpt. of Radiation Oncology/CVK
Charité, Universitätsmedizin Berlin
Torsten Jähnke – JPK Instruments AG

Workshop Office
Ms. Zsuzsa Konczos
Tel.: +49 (0)30 5331 12073
Fax: +49 (0)30 5331 1202
E-mail: konczos@jpk.com
www.spm-workshop.jpk.com

JPK Instruments AG
Bouchéstrasse 12
12435 Berlin, Germany
www.jpk.com

Workshop Location
Charité - Universitätsmedizin Berlin
Campus Virchow-Klinikum, Hörsaal/Lecture Hall 3
Lehrgebäude, Forum 3
Medical Faculty of the Humboldt University at Berlin
Augustenburger Platz 1
13353 Berlin, Germany

Program

9:00 am **Opening**
Dr. Andreas Jordan – Center of Biomedical Nanotechnology & Dpt. of Radiation Oncology/CVK, Charité, University Medicine Berlin

9:10 am **Advanced Dynamic Scanning Probe Microscopies**
Prof. Dr. Harald Fuchs – Department of Physics, Interface Physics Group, Münster University

9:50 am **Propulsive Forces Genreated by Fast Moving Cells**
Prof. Dr. Josef Käs – Department of Soft Matter Physics, Leipzig University

10:30 am ***Coffee Break***
Poster Session and Demonstration of JPK's NanoWizard® AFM

11:00 am **SPM Studies of Membrane Assemblies**
Dr. Anthony Coleman – Institut de Biologie et Chimie de Protéines, Université de Lyon

11:40 am **SPM and Fluorescence on Biological Samples**
Dr. Thomas Jovin – MPI of Biophysical Chemistry, Göttingen

12:20 am ***Lunch***
Poster Session and Demonstration of JPK's NanoWizard® AFM

2:00 pm **Mechanical Experiments with Bio-Molecules: From AFM Based Single Molecule Force Spectroscopy to Force Based Bio-Chips**
Dr. Hauke Clausen-Schaumann – National Research Center for Environment and Health, Neuherberg

2:40 pm **Structural Calorimetry of Supported Membranes by Temperature Controlled AFM**
Prof. Dr. Hans-Albert Kolb – Department of Biophysics, Hannover University

3:20 pm ***Coffee Break***
Poster Session and Demonstration of JPK's NanoWizard® AFM

4:00 pm **Implementation of AFM in the Characterization of pharmaceutically relevant Nano-Systems**
Prof. Dr. Udo Bakowsky – Department of Pharmaceutical Technology and Biopharmacy, Marburg University

4:40 pm **Poster Award and Closing**
Torsten Jähnke – JPK Instruments AG, Berlin

Talks

9:10 am – Advanced Dynamic Scanning Probe Microscopies

Harald Fuchs

Physikalisches Institut
Westfälische Wilhelms-Universität Münster and
Center for Nanotechnology (CeNTech)
D-48149 Münster, Germany
E-mail: fuchsh@uni-muenster.de
http://www.uni-muenster.de/Physik/PI/Fuchs
http://www.centech.de

Nanoscale Sciences are strongly driven by Scanned Probe Techniques which allow us to investigate and manipulate surfaces down to the atomic scale. While the imaging capabilities of techniques such as STM, SFM, SNOM etc. dominated the application of these methods at their early development stages, the physics of probe-sample interactions, and the quantitative analysis of elastic, electronic and magnetic surface and transport properties are becoming now of increasing interest.

On recent progress in dynamic force microscopy/spectroscopy (SFM/SFS) as applied to molecular layers such as OMBE-films and LB-films will be reported. In particular, Dynamic Force Spectroscopy (DFS) was introduced allowing us to understand quantitatively dissipative and non-dissipative processes in dynamic force microscopy. With this technique the adhesion strength in technical compounds such as metallic layers on polymers can be locally and quantitatively investigated.

Scanning Near Field Optical Microscopy (SNOM) opens the perspective to apply optical imaging and spectroscopy techniques on soft matter far below the classical diffraction limit. On recent progress using a novel SNOM technique based on an aperture scattering type probe exhibiting a lateral optical resolution of 1-10 nm will be reported. The influence of the geometrical shape of a novel aperture like SNOM probe on its imaging properties of photonic nano structures will be discussed.

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A. Naber, D. Molenda, U.C. Fischer, H.-J. Maas, C. Höppener, N. Lu, H. Fuchs
Enhanced light confinement in a near-field optical probe with a triangular aperture
Phys. Rev. Lett. 89, 210801-(1-4) (2002)

9:50 am – Propulsive Forces Generated by Fast Moving Cells

Josef Käs

Universität Leipzig
Abteilung Physik der weichen Materie,
Linnéstr. 5, D-04103 Leipzig, Germany

Cell motility is a fundamental component of many biological phenomena in nature, such as immune response, wound healing, and metastasis. Mechanisms of force generation for cell migration, like actin polymerization and/or molecular motors, have been described in various hypotheses, but quantitative force measurements to elucidate this issue hardly exist.

Here we present a direct measurement of the forward force generated at the leading edge of the lamellipodium and at the cell body of a migrating fish keratocyte. In addition, we determined the forward force of locomoting keratocyte fragments.

We attached a polystyrene bead to a cantilever-tip of an atomic force microscope (AFM) and positioned it in front of a cell. The cell crawled underneath the bead and therefore pushed the cantilever up. The forward force was calculated using the detected upward force (vertical deflection) in an elastic "wedge model".

The results for the propulsive forces are in the lower nN range, which agrees with expectations of previous studies. In a more illustrative picture this means that cells are able to push several hundredfold of their own weight out of the way.

11:00 am – SPM Studies of Membrane Assemblies

Anthony W. Coleman and Adina Lazar

Institut de Biologie et Chimie des Protéines
CNRS UMR 5086, Lyon, France
aw.coleman@ibcp.fr and a.lazar@ibcp.fr

The biological membranes, both at the cellular level and in their various forms as barriers, form a range of structures in which the organisation varies in size from the nanoscopic, for cells to the microscopic, for the skin. In all cases the organisation is complex and serves to maintain highly different physical conditions on each side of the barrier. All biological membranes are multi-component with both lateral and transversal divergences in their composition. With regard to cell membranes, not only is there a highly divergent nature in the lipid constituents, but also three or even four types of proteins, integral, anchored, peripheral and circulatory proteins may associate with membranes. To complicate even further the matters, dephasing in the membrane lipids may occur to create rafts in which one species separates out at the nanoscopic level often occurs. To add to the problems of the study of membrane assemblies the whole system is in a dynamic state and responds to external stimuli to reorganise. Finally it should be noted that once extracted from their natural environment many membrane components refuse to reorganise in a convenient way to study them separately.

In reality only SPM techniques can provide detailed knowledge of the structuring and behaviour of membrane assemblies!

Three cases will be treated:

Firstly the assembly of a multi-protein complex NADPH oxidase and how the use of the information available in the z-dimension from SPM allows study of how the complex assembles or mis-assembles.

Secondly the assembly of various natural and synthetic amphiphiles into structures ranging from vesicles through lipid nanoparticles (SLNs) to supported lipid bilayers (SLBs) and even to more complex assemblies.

Thirdly the action of the circulatory enzyme GM_2AP on, its substrate, the ganglioside GM_2 and how the enzyme modifies membrane assembly.

We acknowledge the financial support of the FRM and Delta Proteomics.

11:40 pm – Self-assembly of α-synuclein Imaged by Temperature-Controlled *in situ* AFM

Thomas Jovin
Wolfgang Hoyer, Dmitry Cherny, Gudrun Heim, and Vinod Subramaniam‡

Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry
Am Fassberg 11, D-37077 Goettingen, Germany
‡ Biophysical Engineering Group, Faculty of Science and Technology
University of Twente, P. O. Box 217, 7500 AE Enschede, The Netherlands

The self-association of peptides and proteins into aggregated amyloid structures is characteristic of various neurodegenerative conditions, notably Alzheimer's and Parkinson's diseases (AD, PD). Typical amyloid fibrils are 10-20 nm in width and feature an extended cross-β secondary structure. The major component of Lewy bodies, the neuronal inclusions of PD is the protein α-synuclein (AS).

We have studied the binding of aggregation-promoting agents such as polyamines and metal ions to AS by biochemical and spectroscopic techniques and by high-resolution microscopy (scanning probe, e.m.). In particular, we have used temperature controlled *in situ* tapping mode AFM to continuously monitor the self-assembly of α-synuclein in solution, into nanostructures of various morphologies.

On mica surfaces, elongated sheets 1.1(±0.2) nm in height, presumably representing individual β-sheet structures, were formed within a few minutes in a substrate-governed process. In the presence of the cellular polyamines spermidine or spermine, 5.9(±1.0) nm high spheroidal structures were preferentially formed, sharing characteristics with similar structures previously reported for other amyloidogenic proteins and linked to neurotoxicity. At elevated temperatures, protofibril growth from spheroids or preformed aggregates was observed.

The findings demonstrate that temperature controlled *in situ* AFM constitutes a valuable technique for the analysis of biologically relevant protein assembly, allowing the elucidation of underlying mechanistic features and the identification of key structural, biochemical, and microenvironmental factors.

Some References

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2:00 pm – Mechanical Experiments with Bio-Molecules: From AFM Based Single Molecule Force Spectroscopy to Force Based Bio-Chips

Hauke Clausen-Schaumann

GSF – Forschungszentrum für Umwelt und Gesundheit,
Ingolstädter Landstr. 1,
D-85764 Neuherberg, Germany

Structural and mechanical parameters of bio-molecules are closely correlated to each other and to biological function, and single molecule force spectroscopy has provided new insights into the mechanisms governing biological phenomena at the molecular level (1).

Arguments based on the fluctuation-dissipation-theorem predict, that the force resolution in a given bandwidth is limited by viscous damping (2). Therefore, attempts have been made, to reduce the size of micro-mechanical force sensors. Viani et al. have demonstrated that the thermal noise of an AFM cantilever spring can be reduced by a factor of five, if the size of the cantilever is reduced by one order of magnitude (3). However, a further miniaturization of micro-fabricated cantilevers faces tremendous technological challenges.

Therefore, we have introduced a new approach, where a single molecular bond with a known rupture force – i.e. a short DNA duplex – is used as a force sensor to measure the rupture force of an unknown bond. With this differential setup, where the sample is directly compared to a known reference, it was for the first time possible to detect single nucleotide mutations in DNA, based on bond rupture forces (4). In addition, this differential setup is compatible with a chip based assay format, which allows for the characterization of large numbers of sample molecules in parallel. Conjugates of sample and reference bonds can be connected to a chip surface on the sample side and to a second, parallel chip surface on the reference side. When the two surfaces are separated, the weaker bond in each conjugate fails and a fluorescence label can be used to localize the bond rupture site.

Compared to standard biochip formats, which rely on the measurement of thermodynamic parameters, such as on- and off-rates, force based biochips have a number of advantages. The overall assay times can be reduced, because thermodynamic equilibrium is not required. Assay times are not determined by off-rates and by rebinding. Assay conditions can be optimized for all analytes simultaneously, by selecting appropriate reference molecules. Finally, in multiplexed sandwich immuno assays, the secondary antibodies can be coupled to the second chip surface via force sensor molecules. This allows for local application of analyte

specific secondary antibodies onto the corresponding primary antibody spots on the first chip surface. Unlike in conventional multiplexed sandwich assays, where a cocktail of secondary antibodies is applied and where cross-reactions and non-specific binding often lead to false positive results, here cross talk between the different channels can be effectively eliminated (5-7).

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2:40 pm – Structural Calorimetry of Supported Membranes by Temperature Controlled AFM

O. Enders, A. Ngezahayo, M. Wiechmann, F. Leisten, and H.-A. Kolb

Institute of Biophysics, University Hannover, Germany
email: kolb@biophysik.uni-hannover.de

Atomic force microscopy at high temperature resolution ($\Delta T < 0.1$ K) provided a quantitative structural calorimetry of the transition from fluid (L_α)- to the gel ($P_{\beta'}$)- phase of mica-supported dimyristoylphosphatidylcholine (DMPC) bilayers. Besides a determination of the main transition temperature (T_0) and the van't Hoff transition enthalpy (ΔH_{vH}), the structural analysis in the nm-scale at T close to T_0 of the ripple phase for the first time allowed an experimental estimation of the area of postulated cooperative units from small lipid domains. Thereby, the corresponding transition enthalpy (ΔH) of single molecules could be determined.

The lipid organization and the corresponding parameters T_0 and ΔH_{vH} (ΔH) were modulated by heptanol or external Ca^{2+}. Heptanol shifted T_0 to lower values causing a fluidization of the membrane while the presence of Ca^{2+} increases T_0. Surprisingly, the size of the cooperative unit was not significantly affected by the presence of 1 mM heptanol. But in contrast to theoretical considerations, it cannot be excluded that the cooperative unit depends on the specific phase state of DMPC.

The observed linear relationship of ΔH_{vH} and T_0 was discussed in terms of a change in heat capacity. The experimental approach appears to be suitable to evaluate the proposed structure and modulation of lipid rafts at molecular level.

4:00 pm – Implementation of AFM in the Characterization of pharmaceutically relevant Nano-Systems

Prof. Dr. Udo Bakowsky

Institut für Pharmazeutische Technologie und Biopharmazie
Philipps Universität Marburg, Ketzerbach 63, D-35032 Marburg
E-mail: bakowsky@staff.uni-marburg.de

The scanning force or atomic force microscopy (AFM) developed by Binnig and co-workers (1986) belongs to the new, rapidly growing family of scanning probe microscopes. The AFM allows the observation of conducting and non-conducting samples with a high lateral and vertical resolution, in vacuum, air or liquids. Additionally, the microscope can be used to analyze some physical properties such as friction and softness or the charge density on a nanometric scale. All these possibilities have made this instrument very useful in biological applications including the study of whole cells as well as smaller structures such as chromosomes, membranes, proteins and nucleic acids. Molecular resolution on "life-science materials" has been demonstrated on purple membranes of *Halobacterium halobium*, gap junctions, fibrinogen and plasmid-DNA. The ability to operate in liquids opens up the possibility of studying biological processes as they occur in native, aqueous environments in real time. Examples for this include monitoring dynamics and enzyme degradation of DNA and the measurement of the adhesion forces between ligand receptor pairs. We will illustrate the described potential of the atomic force; especially, for the pharmaceutical technology. These will include nanoscalic 3-dimensional structures vesicles, immunoliposomes, polymeric nanoparticles of various compositions and also surface modified implant materials. We used this technique for analyzing DNA and plasmids as well as DNA/lipoplexes or polyplexes. Furthermore, force measurements between single ligand-receptor pairs, and living human cells surface interactions were characterized.

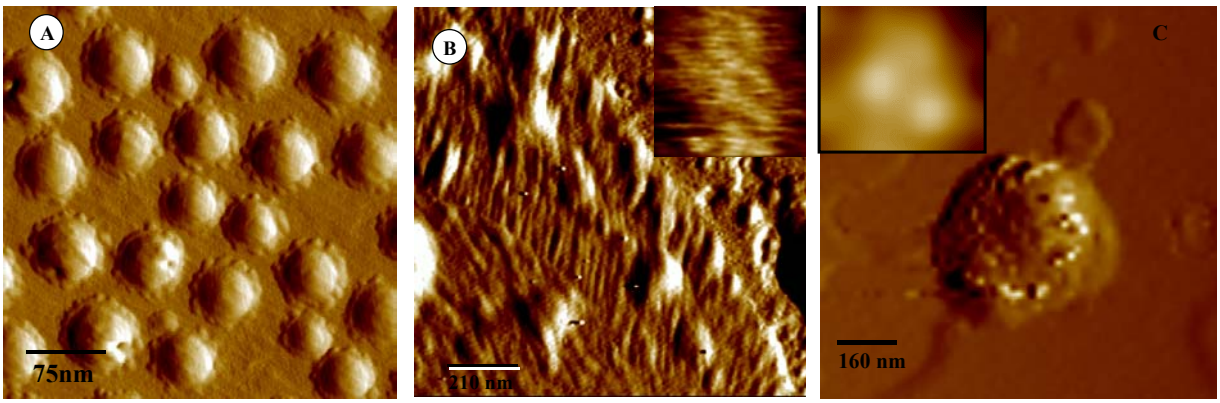

Figure: A) PLGA/chitosane nanoparticles coated with plasmid DNA, B) plasmid DNA pCMVβ 7.5 kbp measured in buffer, insert 10x10nm, C) PEG/DPPC/Cholesterol liposomes surface modified with anti-eSel mAb IgG antibody, insert: single IgG molecule 12.5x12.5nm

Poster Session

P1 – Investigation and Manipulation of Biofilms by Scanning Force Microscopy

Asta Richter and Ronald Ries

Department of Engineering Physics
University of Applied Sciences Wildau (Berlin)
Bahnhofstrasse 1, D-15745 WILDAU, Germany
Email: richter@pt.tfh-wildau.de

Biofilms differ from conventional thin films because the specimens with a special shape and size are much bigger than individual atoms or molecules and move only slowly or not at all over the substrate surface compared to the diffusion of molecules. In addition, the bacteria grow on the substrate by absorbing nutrient. Cell division occurs after the bacteria reach a certain size, thus forming networks and clusters. Biofilms are grown on different materials with various surface morphology and are investigated by scanning force microscopy in detail. The growth patterns, coverage and adherence of the biofilms are shown to depend critically on the substrate roughness, the substrate material and type of the micro-organisms.

A pulsed nitrogen laser has been applied to the samples and the interaction of the laser beam with the biofilm and the underlying substrate has been studied. Because of the inhomogeneity of the biofilms, the ablated areas are different. With increasing number of laser pulses more biofilm material is removed but there appears also damage of the substrates which are metals, metallic films and polymer materials in the case reported here. Careful investigation of the PEEK polymer surface within the fringes of the laser radiated spot indicate typical depressions with the same size as the E.-coli bacteria. This is a new type of light amplification via biological material which results in a damage of the polymer surface with a replica of the bacterium.

Manipulation and dissection of the bacteria were investigated with the sharp silicon tip in the scanning force microscope for E. coli biofilms on polymer substrates PEEK. During the process of nano-cutting, different cut or scratch patterns are observed which are mainly distinguished by the cut depth for a fixed force. Forces between 10 μN and 150 μN have been applied to perform these cuts. Due to the different elastic properties of the polymer and the bacterium the cut depth for a fixed force varies in both materials in a contrary way. The cut depth is larger in PEEK than in the bacterium for applied forces below 32 μN and vice versa. A complete cut of the bacterium could be realised with a threshold value of 50 μN.

P2 – Copper as Modulator of Prion Protein Binding

S. Kunze [a], J. Metze [a], W. Bodemer [b]

[a] Institute for Bioprocessing and Analytical Measurement Techniques e.V.
Rosenhof, D-37308 Heilbad Heiligenstadt
[b] German Primate Center, Department of Infectionpathology
Kellnerweg 4, D-37077 Göttingen

Prion diseases or Transmissible Spongiform Encephalopathies (TSE) are a major concern to public health since they are in contrast to all other neurodegenrative disorders transmissible and infectious. There is also no definite answer on the "protein only hypothesis" which implies that an abnormal isoform of the cellular prion protein (PrP) represents the infectious entity. Most experimental data favour prions as an unconventional pathogen replicating without any coding of nucleic acids. Structural characteristics like amyloid formation are shared by many neurodegenerative diseases but TSEs of many species are also transmissible and a tight species barrier does not exist. There is ample evidence that the conformational transition of PrP molecules from benign into malignant forms is an essential mechanism to form amyloids. Although a wealth of data on the abnormal PrP designated PrP^{res} or PrP^{sc} exists, the physiological function of PrP is not well understood. Due to its copper binding capability a regulatory function of the cellular prion protein (PrP^{c}) for heavy metals is assumed.

This binding of heavy metals to the prion protein was utilized for PrP immobilization on a solid support in order to monitor binding events by Atomic Force Microscopy (AFM). For this study we have used recombinant prion protein comprising amino acids 23–208, which was synthesized in bacteria. Its quality was repeatedly monitored by Western Blot and two-dimensional gel electrophoresis.

PrP was immobilized on mica without and with addition of different heavy metals ions. Whereas the addition of copper(II) to the PrP solution resulted in an enhanced PrP immobilization, nickel(II) reduced the surface coverage with prion protein. Apart from measuring in liquid, dried PrP preparations were also imaged in air. This high resolution data provided for the first time a distribution of geometrical parameters of the PrP precipitates. Our findings point not only to interaction of recombinant PrP molecules with heavy metals ions and followed immobilization but also allowed to perform determination of single molecule particle volumes.

P3 – AFMing of Forisomes

Magnus S. Jäger[1], Katja Uhlig[1], Hauke Clausen-Schaumann[2], Wilfrid Bleiß[3], Claus Duschl[1]

[1] Fraunhofer Institute for Biochemical Engineering (FHG-IBMT)
Invalidenstraße 42, D-10115 Berlin, Germany
magnus.jaeger@ibmt.fraunhofer.de
[2] National Research Center for Enviroment and Health (GSF)
Ingolstaedter Landstraße 1, D-85764 Neuherberg, Germany
[3] Humboldt University Berlin
Unter den Linden 6, D-10099 Berlin, Germany

Ellipsoid protein complexes named forisomes are ATP-independent motorproteins derived from legumes (Fabaceae). They protect the plant from the loss of photoassimilates by blocking its vessels when they are injured. Forisomes occur in two different states between which they can be switched depending on calcium concentration or pH (electrotitration): in the Ca-free, neutral pH elongated state they are 30 µm long and 3 µm thick whereas upon Ca application or pH shift their length contracts to 20 µm and their diameter expands to 7 µm.

To micromanipulate the forisomes we used laser tweezers. This method enables us to position them exactly and to cut them in slices by means of an UV laser. The visualisation and analysis of the protein complexes in both states was done with an AFM and an electron microscope. In order to get information about the elasticity of forisomes in both states, we took force-distance measurements by pressing the lever on the forisomes. Thus, we calculated Young's modulus. Furthermore, we compare the different imaging resolutions between two tips.

The importance of forisomes for biotechnology lies in their application in microchips e. g. as valves in a fluidic system regulating a flow.

Figure Forisomes (plant protein complexes, length 30 µm) arranged between electrodes (black) for electrotitration of pH.

P4 – Preliminary Study of Tip-Enhanced Raman Spectroscopy (TERS) and AFM for Single-Molecule DNA Investigation

Akiko Rasmussen and Volker Deckert

ISAS – Institute for Analytical Sciences Dortmund
D-44139 Dortmund, Germany

Raman spectroscopy is a powerful analytical tool to obtain chemical information of samples. However, the signal intensity of normal Raman spectroscopy is weak. One possible improvement is to use surface enhanced Raman scattering (SERS) method, where rough metal surfaces or colloids are used to obtain electromagnetic and chemical enhancement. A new approach, TERS is a unique technique which combines SERS with scanning probe microscopies [1, 2]. In this method, a sharp metal edge or a metal particle at the very end of a AFM or STM tip is used, as illustrated in figure below.

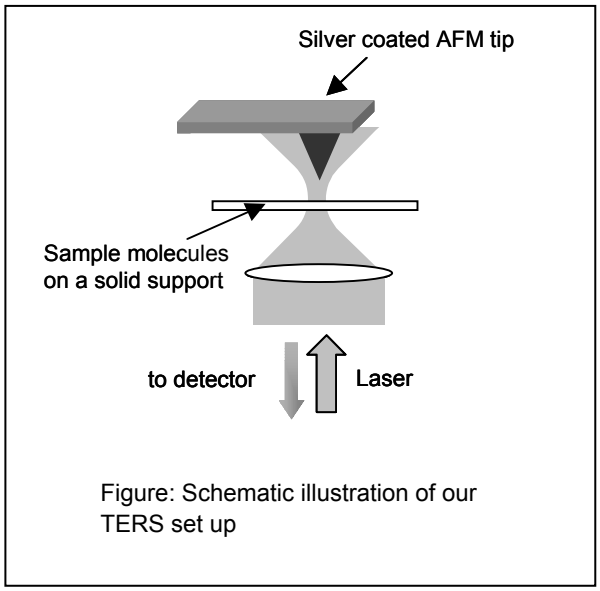

Figure: Schematic illustration of our TERS set up

Coupling AFM with Raman spectroscopy allows to obtain the high resolution topography and chemical information simultaneously. The great advantage of TERS is that only the very small area of molecules close to the tip apex will experience the field enhancement. Since AFM tips with an apex diameter of 2 to 10 nm are commercially available, the lateral resolution of the spectroscopic investigations are predicted to be in a similar range.

So far, SERS spectra of various components of DNA molecules have been acquired according to our requirements. The next step is AFM topographical analysis of DNA immobilised on solid surface, which will be then possible to perform TERS studies on DNA by scanning the specifically designed AFM probe along an isolated DNA molecules.

References

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P5 – Characterization of Silver Enhancement Methods for Nanoparticle-based DNA Microarrays

Grit Festag, Robert Möller, Andrea Csàki, Wolfgang Fritzsche

Institute for Physical High Technology Jena
Biotechnical Microsystems Department, Germany

DNA-microarrays - as highly parallel and miniaturized DNA detection methods – offer a large potential for medical and environmental applications. Beside standard fluorescence techniques, gold nanoparticles can be used as interesting alternative labeling methods. For parallel readout, optical and electrical detection methods were described. Metal deposition on the nanoparticles leads to the amplification of the signal and allows the optical readout by simple desktop scanners.

A variety of silver enhancement methods are established in electron and light microscopy. For characterizing we non-specifically immobilized 30 nm gold nanoparticles on silicon oxide and enhanced them by different commercial and homemade silver solutions. Individual nanoparticles can be visualized and particle growth by metal deposition was followed up over a stepwise enhancement procedure using scanning force microscopy (AFM). Particle heights were measured and the distribution of the enhanced particles was observed. We could demonstrate that the examined silver enhancement methods considerably differ in enhancement velocity, homogeneity, and specificity.

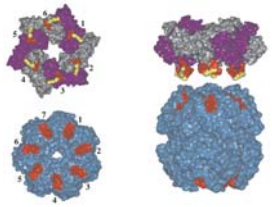
P6 – AFM Investigations on ATP-dependent Proteolytic Machineries

Iris Asen, Reinhard Guckenberger, Kornelius Zeth

MPI for Biochemistry
Am Klopferspitz 18a, D - 82152 Martinsried, Germany

Protein folding and controlled degradation of misfolded or incompletely synthesized proteins are very important regulatory mechanisms within cells of eukaryotic and prokaryotic organisms.

The folding state of proteins is monitored by an energy-dependent quality control network of ATP-dependent proteases and molecular chaperones. Chaperones of the AAA+ superfamily like ClpA and ClpX are connected to the peptidase ClpP to form proteolytic machines.



For studying the structure and function of biomolecular macromolecules, surface-sensitive techniques like scanning probe microscopy after immobilization on flat biocompatible surfaces is an established technology. As a key requirement, any general strategy will have to fully preserve the biological activity of molecules and to minimize any adverse effects in terms of their structure and function. To comply this, ClpA, ClpB and ClpP were immobilized on different surfaces and investigated with AFM in buffers, similar to physiological conditions [2]. Imaging was executed in tapping mode with a Nanoscope III AFM microscope in solution.

P7 – Scanning Probe Devices Will Aid Surgeons to Diagnose Diseases and Repair the Human Body

Martin Stolz[1], Werner Baschong[1], Ueli Aebi[1,*], Raphael Imer[2], Urs Staufer[2], Riccardo Gottardi[3], Roberto Raiteri[3], Patricia Scandiucci de Freitas[4], Beat Goepfert[4], Dieter Wirz[4], A.U. Daniels[4], Mark R. VanLandingham[5], Niklaus F. Friederich[6]

[1]M.E. Müller Institute for Structural Biology, Biozentrum University of Basel, Switzerland
[2]Institute of Microtechnology, University of Neuchâtel, Switzerland
[3]Department of Biophysical and Electronic Engineering, University of Genova, Italy,
[4]Laboratory for Orthopedic Biomechanics, Felix Platter Hospital, University of Basel, Switzerland,
[5]Army Research Laboratory, Aberdeen Proving Ground, MD 21005, USA
[6]Department of Orthopeadic Surgery & Traumatology, University of Basel, Switzerland

*Corresponding author: Ueli Aebi, Phone: +41 61 267 2260, Fax: +41 61 267 2109
E-mail: Ueli.Aebi@unibas.ch

The atomic force microscope (AFM) has opened completely new vistas for analyzing biological matter in its native environment, thereby providing a means to directly correlate structural with functional states, for example, at the level of single molecules, living cells, and even entire tissues. Moreover, the AFM does not only give us the "eyes" for imaging biological matter from the mm to the μm and nm scale, but it also provides us with the "fingers" to measure and manipulate biological matter at these different length scales. The AFM which is based on piezoelectric actuators has far greater dimensional sensitivity compared to conventional clinical indentation testing devices. This technical improvement allows for higher-resolution indentation testing data and bears the promise of an early detection of diseases (Stolz, 2004a and b; Stolz 2003; Hunziker 2002).

As illustrated in Fig. 1, for performing IT AFM within the knee joint the scan head of the AFM is inserted under arthroscopic control into the knee and stabilized by two sets of liquid-inflatable balloons, similar to those used for heart angioplasty. By filling the balloons with saline solution the head is stabilized against the surfaces within the knee joint. For recording the biomechanical properties of cartilage a regular sharp tip (nanometer) or micrometer-sized spherical tip is mounted in a holder that is attached at the distal end of a piezoelectric actuator tube (piezo). For recording the force-displacement curves the AFM tip is driven by the piezo into and out of the cartilage surface. After each loading/unloading cycle the AFM tip then is moved to its next position.

Added values: First, conventional indentation testing devices perform mechanical testing at the mm to cm scale, so they cannot assess tissue properties at the cellular to molecular level, i.e. at the scale were the biological metabolism occurs and also where most diseases start.

Second, the initial key application of the arthroscopic AFM is in the quality control of transplanted autologous cartilage tissue as well as of tissue-engineered constructs (both approaches are already available to the clinician) where it is of major interest to trace the development of the transplanted or tissue-engineered cartilage over time in terms of mechanical stability and biocompatibility. Third, unlike any other applied technique used for assessing the morphological and biomechanical state of articular cartilage the AFM enabled us to directly image, measure and manipulate the tissue *in situ* by employing multifunctional tips.

Fig. 1 Illustration of a prototype arthroscopic AFM. The instrument is inserted into the knee cavity under arthroscopic control.

The arthroscopic AFM might be the beginning of a new generation of nanotools, designed for minimally invasive interventions of body parts, such as the detection of vulnerable plaques in the coronary arteries by a catheter-based approach. In that perspective, we are still living in the "stone age" of scanning probe-based clinical tools. However, we believe that scanning probe devices will eventually help surgeons to more effectively detect diseases and to repair the human body (Stolz, 2004a).

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P8 – Kontrollierte Biochemische Synthese auf Metall-/Halbleiter-Nanostrukturen

H. Sailer[1], T. Altebäumer[1], D.P. Kern[1], M. Raschke[2], E. Nibbering[2], C. Lienau[2], R. Ehlich[3], F. Pouthas[3], H. Hörber[3]

[1] Inst. f. Angewandte Physik, Universität Tübingen
[2] Max Born Institut Berlin , [3] EMBL Heidelberg

Ziel dieses Projekts ist die kontrollierte Synthese kurzer DNA-Stränge auf metallischen Nanostrukturen. Die Synthese soll durch optische Anregung spezifischer Plasmonenresonanzen der Nanostruktur an chemisch auf diesen Strukturen gebundenen Ausgangsmolekülen induziert und mit nanoanalytischen Methoden charakterisiert werden. Die Untersuchung grundlegender physikalischer Mechanismen der Molekül/Substrat-Wechselwirkung ist ein wesentlicher Aspekt dieses Projekts. Die Stabilität und die vermuteten elektrischen Eigenschaften machen DNA zu einem idealen Modellsystem zur Studie von Übergängen in Molekül-Leitersystemen. Ziel ist die Optimierung der Synthese kurzer DNA-Stränge auf den Nanostruktur-Oberflächen. Dies geschieht insbesondere durch die geeignete Wahl von Material und Geometrie der Nanostrukturen. Im Fall von Metall-Dots läßt sich die Plasmonenresonanzfrequenz durch die Größe, die Elliptiziät und den Abstand der Dots im sichtbaren Wellenlängenbereich beliebig einstellen. Die so gewonnenen Erkenntnisse gewinnen vor allem dadurch an Bedeutung, dass sie die sich in letzter Zeit rasch entwickelnden DNA-Chip-Technologien maßgeblich beeinflussen könnten.

Im Rahmen dieses Projekts wurden Gold-Dot Arrays mit Dots verschiedenen Durchmessers und Abstands auf Glassubstrat durch Verwendung von hochauflösender Elektronenstrahl-Lithografie, Lift-Off und Ionenstrahlätzen hergestellt. Für die Zukunft sollen passende Hochdurchsatzverfahren entwickelt werden. Die Geometrie der Dots wurde mittels AFM und REM analysiert. Die Charakterisierung der Plasmonenresonanz der Gold-Dots soll durch spektroskopische Untersuchungen im optischen Nahfeld mittels eines eigens hierfür konzipierten Meßplatzes erfolgen. Ferner befindet sich ein optisch kontrolliertes STM im Aufbau. Hierdurch sollen die Elektronentransfereigenschaften der Molekül-Dot-Komplexe im Hinblick auf die Einstellung der optimalen Linkerlänge mittels Tunnelspektroskopie im Laserlicht charakterisiert werden.

Ein besonderes Augenmerk liegt bei dem Projekt auf möglichen biomedizinischen Anwendungen. Effiziente biochemische Mechanismen und Reaktionen auf den Trägersubstraten sollen unter Ausnutzung spezieller Eigenschaften von Nanostrukturen entwickelt werden, die eine kostengünstige und flexible Massenproduktion erlauben.

P9 – Mechanical Properties of Polyelectrolyte DNA/PAH Multilayer Microcapsules Studied by Atomic Force and Confocal Microscopy

O.V.Lebedeva, B.S.Kim and Olga I. Vinogradova

Max Planck Institute for Polymer Research
Ackermannweg 10, 55128 Mainz, Germany

We have suggested a novel approach to probe mechanical properties of multilayer microcapsules. The combination of atomic force and confocal laser scanning microscopes allows one to perform the simultaneous measurements of the mechanical properties and shape of microcapsules during the compression. The method has been used to explore DNA-based multilayer microcapsules.

Capsules were produced by electrostatic layer-by-layer (LbL) assembly of four pairs of negatively charged DNA and positively charged polyallylamine hydrochloride (PAH) layers on colloidal particles with subsequent dissolution of the template core.

Load (force) vs deformation curves were measured with the Molecular Force Probe (MFP) 1D AFM (Asylum Co, Santa Barbara, CA). The MFP was used in combination with an inverted optical microscope Olympus IX70 with a high resolution immersion oil objective (60×), especially adjusted for the MFP 1D. Confocal laser scanning microscopy images were taken with a commercial confocal unit FV300 (Olympus, Japan) used in combination with an inverted fluorescence microscope Olympus IX70. A high-resolution (60×) bright (NA=1.45) immersion oil objective was used.

We have found that DNA/PAH capsules are soft and their highly porous shell is permeable for water even in a short time scale of AFM compression experiment. The data analysis, however, suggests that Young's modulus of DNA/PAH capsules is similar to that of multilayers studied before.

P10 – Visualization of Extracellular Polymeric Substances-Mediated Attachment of *Acidithiobacillus Ferrooxidans* to Pyrite

K. Harneit, G. Meyer, Z. Keresztes*, T. Gehrke, W. Sand

Universität Hamburg, Biozentrum Klein Flottbek, Abt. Mikrobiologie
Ohnhorststr. 18, D-22609 Hamburg

*Hungarian Academy of Science, CRIC, H-1025 Budapest

Bioleaching of metalsulfides like pyrite (FeS_2) or chalcopyrite ($CuFeS_2$) by *Acidithiobacillus ferrooxidans* and other chemolithotrophic leaching bacteria is an economically important process used for metal recovery (1, 2). *Acidithiobacillus ferrooxidans* dissolves minerals mainly via the contact mechanism (3), which requires attachment and biofilm formation.
The extracellular polymeric substances (EPS) produced by the bacteria mediate their attachment to minerals by rendering the cell surface electropositive (in contrast to the electronegative pyrite at the physiological pH 2). Besides, the EPS carry the oxidative agent iron (III) ions in a complexed form. The iron(III)ions chemically react with sulfide minerals forming iron(II)ions, other soluble metalions and thiosulfate. The bacteria reoxidize the iron(II)ions to iron(III)ions for metabolic purposes, thereby regenerating the oxidative agent.

Atomic force microscopy (AFM) and fluorescence microscopy (FM; after staining with acridine orange) is used to visualize attachment and biofilm formation by iron(II) grown cells of *A. ferrooxidans* strain R7 on pyrite coupons. Most cells with EPS attached to the pyrite surface after 24 h of incubation. A net-like structure was visible with AFM and FM. After 48 h the coupon was covered by a smooth surface layer (biofilm), which could be visualized by AFM. By FM a monolayer of attached cells was detectable. Cells depleted of their EPS by centrifugation hardly attached to pyrite within the first 24 h. After 48 h the cells had formed a structure on the pyrite surface comparable to the biofilm formed by cells with EPS after 24 h. Therefore it may be assumed that cells regenerate their EPS within 24h.
Cell-free EPS of *A. ferrooxidans* also attach to a pyrite surface. Compared to an untreated surface patches of thin EPS layers are visible after 24 h. As the biofilm of *A. ferrooxidans* turns the mineral surface into a more hydrophilic one, the cells or their cell-free EPS may be used as flotation reagents in froth flotation for pyrite exclusion.

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P11 – Active Force Generation of Motile Cells

Claudia Brunner

Universität Leipzig
Abteilung Physik der weichen Materie,
Linnéstr. 5, D-04103 Leipzig, Germany

Cellular motility is a ubiquitous component of prokaryotic and eukaryotic cells, spanning varied functions from the immune system, to the developing brain, to the grave invasiveness of cancer.

Various individual components, such as molecular motors and actin filament polymerization have been explored to explain cell movement, but the cell as a whole system is not well understood. Experiments on living cells are necessary to understand the properties and abilities of their polymer networks functioning together as one system.

The atomic force microscope is an excellent tool to determine the mechanical forces which are responsible for cell movement. With a polystyrene bead glued on a commercial AFM-tip, living cells can be safely measured. We have probed fast moving keratocytes and directly measured the cell extension forces, allowing us to compare extension forces with the cells' velocities and other properties.

P12 – AFM Investigation of Tubular J-Aggregates

Thorsten Ollek, Stefan Kirstein

Institut für Physik
Humboldt Universität zu Berlin

The J- aggregates of cyanine dyes are characterized by an optical absorption spectrum that is significantly shifted towards the red compared to a monomer spectrum.. Additionally, strong fluorescence is observed which is nearly in resonance with the lowest energy state. These properties are due to excitonic coupling of the dyes and a typical feature of J-aggregates. In this work we use amphiphilic cyanine dyes that form tubular aggregates. The dyes are termed "amphi-PIPES" (amphiphiles with pigment interaction performing energy migration). It is known from cryo-TEM investigations that one of these dyes (C8O3) forms tubular aggregates with diameters of 10 nm and length of several hundred μm. Some of the tubules are bundled to form helically twisted threads [1].

It is a challenging task to adsorb the tubular aggregates on solid substrates in a way that they are not destroyed by the strong adhesion forces. Therefore, the aggregates of C8O3 are adsorbed on several substrates with different surface energy and the morphology of the aggregates was visualized by means of AFM. For transparent substrates the absorption spectra are recorded and for all samples fluorescence spectra were measured with the help of an optical microscope and an attached CCD spectrometer.

It was found that the structures can be preserved best if adsorbed on a thin film of poly(vinyl alcohol). The fluorescence of the samples was only slightly disturbed by the adsorption process and the helical structure of the bundles was clearly resolved. The size of the aggregates as obtained from a height analysis was comparable to the data obtained by cryo-TEM.

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P13 – The Role of DNA-Polyethyleneimine Complexes Formation for Enhancing Transfection Efficiency in Gene Therapeutic Vectors Delivery

E. Anashkina, A.Kaloshin, A. Soloviev, E. Filinova

Russian N.N. Blokhin Memorial Cancer Research Centre
Moscow, Russia

Background: Efficiency of non-viral vectors for gene therapy is still a matter of the great efforts for experimental molecular genetics. In order to reach higher efficiency for suicide gene vectors delivery we have studied DNA-PEI complexes formation and showed highly expressed transfection effect dependence on DNA-PEI molar ratio different for plasmid size and each PEI modification.

Material and Methods: Plasmid vectors of fluorescence: pIRES-EGFP, pIRES-YFP, and dual fluorescence-antibiotic resistance: pIRES2-GFP selection (Clontech) - were used for cloning of Tymidine Kinase Herpes Simplex Virus Type I (HSV-TK-I-GCV) suicide gene acceptable for Gancyclovir-dependant elimination of transfected cells in tumors. PEI 12.5 kDa and PEI 30-40 kDa (Serva) mol. weight, respectively, were studied as an instrument for adaptation of transfection method. The evaluation of optimal DNA-PEI molar ratios for complexes formation efficiency was achieved using 1% agarose gel electrophoresis, Atomic Force Scanning Probe Microscopy (NanoScope III-a, Digital Instruments, Santa Barbara, USA) and Dynamic Light Scattering Particle Sizing System (PSS Nicomp-380, Santa Barbara, USA). Transfection effect percentage evaluation in vitro in breast adenocarcinomas HBL-100 (St-Petersburg), MDA-MB-435S and ovarian carcinoma Skov-3 (Cleveland, USA) was made with help of fluorescent microscopy (Leica DM RXA2 with Olympus DP70 Digital Camera, Germany) and flow cytometry (FACSCalibour, BD).

Results: Polyethyleneimine was admitted as effective cationic transfection agent for in vitro applications more then 10 years ago. However there are no standard protocols of the method and molar ratios for vectors of different sizes and types of PEI. Electrophoretically we have demonstrated binding for control pIRES-FP vectors of 5.2-5.3 kb in Mol/% ratio DNA:PEI 1:0.8 for 0.05% sol. PEI 12.5 kDa and 1:0.12 for 0.05% sol. PEI 30-40 kDa, i.e. 1 mkg plasmid DNA binded 0.8 Mol/% and 0.12 Mol/% PEI. Cloned HSV-TK gene is increasing plasmid size for 1134 bp. Data repeated for HSV-TK-I-containing vectors of 6.5-6.6 kb, the ratio were 1: 1.2 for PEI 12.5 kDa and 1:0.2 for PEI 30-40 kDa, respectively. An optimal size of DNA-PEI complexes formation measured by AFM scanning on solid substrate and

scattering in liquid dissolved cell medium mixtures was settled as 1100-2000 nm for PEI 12.5 kDa and 1800-3700 nm for PEI 30-40 kDa with variations in vector-size dependence mode. The optimal complexes formation size correlated with the highest 20-30% levels of transfection efficiency achieved for the similar deviations of 5.2 -6.5 kb plasmid size. In spite of clumsy movement ability PEI 30-40 kDa complexes have lower cytotoxicity and the same or higher transfection level could be got with more vector mass/% for the same square of transforming cell culture.

Conclusions: Low activity of modern virus gene therapeutic systems seems to be due to the following restrictions: poor targeting and low level of in vivo transfection, unsafety of administration for retrovirus systems, insufficient product expression in tissue. Non-viral vectors transfection systems provide several advantages able to overcome them: there are possible tissue-specific targeting in DNA-PEI-PEG complexes conjugated with cancer-specific monoclonal antibodies, significantly higher and regulating gene expression, controlled levels of transfection and expression. For further in vivo models we foresee the necessity of changing of PEI group for poly-L-Lysine component with less toxicity and the same safety of DNA intracellular transport.

P14 – Colloidal Force Microscopy to Probe Supported Membrane Systems and Cells

Stefano Leporatti [1], Anja Gerth [2], Sergio E. Moya [3], Guido Köhler [1], Bernd Kohlstrunk [4], Sunna Hauschildt [2] and Edwin Donath [1]

[1] University of Leipzig, Institute of Medical Physics and Biophysics,
[2] University of Leipzig, Institute of Biology II, Immunobiology
[3] University of Cambridge, Nanoscience
[4] University of Leipzig, Institute of Experimental Physics I, Soft Matter Physics

Scanning Force Microscopy was employed to study biomimetic polyelectrolyte multilayer lipid composite structures and cell membranes. In both cases the interaction of the lipid bilayer with a polyelectrolyte support, either the multilayer or the cytoskeleton, is essential for the properties and the function of the bilayer membrane. The interaction was studied by means of colloidal force microscopy. A colloidal particle was glued to the end of an AFM microcantilever and subsequently used as force sensor. The advantage of this technique is that the particle surface can be coated with a variety of materials thus providing great flexibility in the design of the interacting interfaces.

Lipid bilayer membranes adsorbed on polyelectrolyte multilayer supports showed a number of interesting features. Quite often upon interaction with the colloidal probe sudden jump-in events termed as kinks were observed. These instabilities are correlated to a remarkable adhesion (jump-out of contact) after retraction. To explore the nature of these events lipid membranes adsorbed onto thiol-modified gold plates were studied as model systems. The interaction with the support can systematically varied changing the interfacial properties of the thiol surface. Negatively and positively charged thiols combined with different lipids involving cholesterol have been tested against a standard microcantilever (Si_3N_4). These studies revealed that the observed kinks are related to a break-through of the tip into the bilayer. The likeliness of this event depended on the strength of the lipid support interaction. Upon retraction lipid thethers have been pulled of. Their extension and breaking force were quantified. Kinks observed when studying the interaction of polyelectrolyte multilayer lipid composites against a sphere where identified as break-through events of underlying polymer blobs across the bilayer. Lipid thethers of higher stability were found.

When the interaction with cells was studied the picture was even more complex. As an example macrophages obtained from human peripheral blood mononuclear cells were investigated.It was found that cells stimulated by lipopolysaccharide (LPS) showed a much larger adhesion (100 nN up to 1-2 μN) upon retraction compared with non-stimulated cells as well as changed deformation behaviour related to the physiological state of the cell.

P15 – High-resolution Imaging of Mouse Sperm Morphology by Atomic Force Microscopy and Scanning Electron Microscopy

Sang-Joon Cho[1], Soo-Kyong Choi[1], Ki-Pyung Jun[1], Myung Chan Gye[2]

[1] Chung Moon Soul Bio Information and Electronics Center
Korea Advanced Institute of Science and Engineering, Daejeon 305-701, Korea
[2] Department of Life Science, College of Natural Sciences
Hanyang University, Seoul 133-791, Korea

In the past cell biological research, various cell preparation techniques such as fixing and dying methods have been applied in order to overcome the technical limitations. We, however, don't fully understand the effects of these sample preparation techniques to the cells. Using Atomic Force Microscopy (AFM), cells can be imaged directly requiring little or no sample pre-treatment and even in most native physiological media of aqueous solutions. The unique operating method and data format also let AFM be successfully employed in combination with other techniques such as optical and electron microscopy (EM), SNOM, patch clamp and other biochemical methods.

The sperm cell is one of the simplest forms of eukaryotic cell containing only half of the whole chromosome but it shows us its complex nature of a biological system. Nature even designs sperm cells between species differently, probably by optimizing its design for optimal function for thousand years. The sperm head consists of acrosome containing degradative enzyme and nucleus containing a haploid set of chromosomes. The rest of the sperm parts are a middle piece containing mitochondria for energy and a tail for movement. By understanding the mechanism of this biological system, it could provide the possible blueprint of nano- or micro-biomimetic robot for drug delivery.

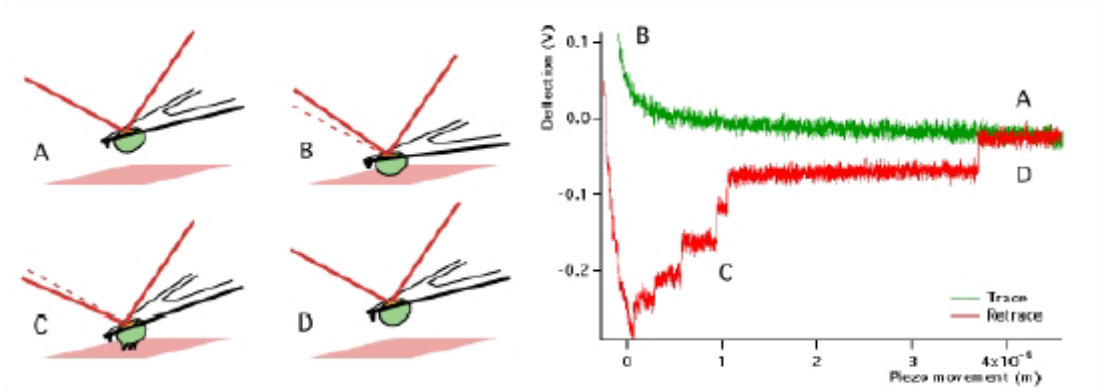
AFM is ideal tool to investigate the surface structure of sperm cell because a sperm head is flat and can be attached on the slide glass firmly after it is fixed or dried. We have studied the surface structure of mouse epididymal spermatozoa by using AFM and SEM. AFM and SEM pictures are compared and possible sample damages caused by SEM are demonstrated.

P16 – Gastrulation of Zebrafish Embryos
A Study of Cell Adhesion using AFM

Pierre-Henri Puech[1,*], Anna Taubenberger[1], Florian Ulrich[2]
Carl-Philipp Heisenberg[2], Daniel J. Müller[1]

[1] BioTec/TU-Dresden – Tatzberg 45, 01307 Dresden, Germany
[2] Max-Planck Institute for Molecular Cell Biology and Genetics
Pfotenhauerstr. 108, 01307 Dresden, Germany
* e-mail : puech@mpi-cbg.de

Wnt signaling molecules play a central role for regulating tissue morphogenesis in a wide variety of developmental processes. Although the molecular and cellular mechanisms by which Wnt signaling functions in morphogenesis are not yet fully understood, the regulation of cell adhesion is likely to be involved. Therefore, to test if Wnt signaling is required for proper cell adhesion during development, we have used Atomic Force Microscopy (which enables the quantification of forces within the expected range for protein-protein and cell-cell interactions) to compare the mechanical and adhesive properties of cells taken from Zebrafish wild-type and Silberblick/Wnt11 mutant embryos.



The present "cell force AFM" technique (see Figure) consists in gently grafting a cell on an extremely soft cantilever, minimizing its possible trauma, and using it as a probe for adhesion on a substrate that has been coated with a bio-molecule.
Here, our preliminary results from experiments on fibronectin using gastrulation stage Zebrafish single cells will be shown and analysed in terms of detachement force(s) and work. An attempt to characterize their elasticity will be also presented.

P17 – AFM versus histological Analysis of Porcine Crystalline Lens Structure: a preliminary Investigation

A. Antunes[1,*], C. A. M. Rigonati[2], A. M. V. Safatle[3], M. I. Borella[2], P. S. M. Barros[3], S. L. Morelhão[1]

[1]Instituto de Física-Universidade de São Paulo-Brazil
[2]Instituto de Ciências Biomédicas- Universidade de São Paulo-Brazil
[3]Faculdade de Medicina Veterinária e Zootecnia-Universidade de São Paulo-Brazil
*antunes@if.usp.br

We performed a preliminary study of crystalline lens using AFM. The lens is a soft tissue compost by 90 % of proteins with structure and shape well defined. The lenses are formed by a large number of fiber cell aligned, which are distributed of manner to execute your main function that is focusing of the light. The peculiar arrangement is accompanied of distribution distinct of index refraction in all tissue. The relation between a loss transparency by a disease, p. e., and structural model it is not known completely. Others scanning techniques has been applied for characterization of ocular tissues [1], however few studies has used AFM [2]. This technique offers an advantage of sample minimal sample preparation in relation to others morphological techniques. Two decades ago, AFM was developed [3] and its application in situation-problem involving biological tissue is wide today. This is a first time that AFM is employed to provide information about crystalline lens. Mammals crystalline lenses were removed after two-hours post-mortem and maintained in solution fixative even use in this experiment. The sections of 1mm-thickness of lens were analyzed. The AFM imaging was performed using a Digital Instruments Nanoscope in the Atomic Force and Tunnelling Microscopy Laboratory. We results are compatible with histologic information. The epithelium layer and fiber distribution (figure below) were obtained. AFM imaging provide information about surface topography of porcine crystalline lens showing estimative of dimension the fiber in the distinct shells of the tissue. All images were acquired by tapping-mode AFM. We too measured a profundity of separation among the fiber in each shell. Understanding, in details, the internal structure of the crystalline lens can be very helpful for developing preventive treatments, drugs for diseases and to extend to older ages the focusing capability of the lens.

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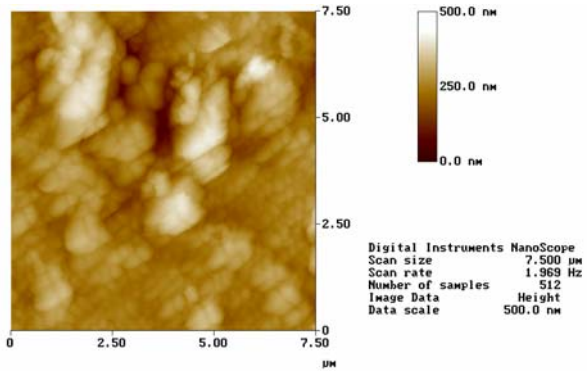

P18 – Flexible, actin-based Ridges on the Surface of Melanoma Cells Co-localize with Beta1 Integrin

Kate Poole and Daniel Müller

BioTechnological Center, University of Technology Dresden
Tatzberg 49, D-01307 Dresden, Germany

Using a combination of laser scanning confocal microscopy and atomic force microscopy, we have identified flexible, actin-based structures on the surface of cells derived from the vertical growth phase of melanoma progression. These flexible structures, lacking on the surface of mature melanocytes, were observed on the surface of all 4 melanoma cell lines tested.

Further investigation revealed that the β1 integrin colocalizes with these actin based structures on the surface of the cell. The β1 integrin is known to be upregulated in melanoma cells, in comparison with melanocytes, and plays a role in attachment to the extracellular matrix, invasion and metastasis.

This approach has allowed us to identify a common feature within cells isolated from the vertical growth phase of melanoma development, despite the known variation in genetic composition of melanoma.

P19 – Molecular Cues Influence the Morphodynamics of Fibroblasts on 2D Collagen Surfaces

Kate Poole[1]*, Khaled Khairy[2]*, Jens Friedrichs[1]*, David A. Cisneros[1], Jonathon Howard[2] and Daniel Mueller[1]

* These authors contributed equally to this work
[1] BioTechnological Center, University of Technology Dresden
01307 Dresden, Germany.
[2] Max Planck Institute for Molecular Cell Biology and Genetics
01307 Dresden, Germany

We have investigated the structural features of collagen that are required for directional motility of mouse dermal fibroblasts, by analysing cell movement on 2-dimensional collagen surfaces. The surfaces were prepared with aligned microfibrils of collagen type I, oriented in a predefined direction, as characterized by atomic force microscopy. These collagen-coated surfaces were generated with or without the characteristic 67 nm D-periodic banding. Phase contrast video microscopy was used to monitor the movement of MDF cells attached to surfaces coated with aligned collagen.

Quantative analysis of cell morphodynamics showed a strong correlation of cell elongation and motional directionality with the orientation of D-periodic collagen microfibrils. Neither directed motility, nor cell body alignment, was observed on aligned collagen lacking D-periodicity. As such, we show that directional motility on aligned collagen type I fibrils is not merely due to contact guidance and we present evidence suggesting a biological function for the 67 nm periodicity.

P20 – Multiple Analysis of Single Cell Traces using a Combination of AFM, IRM and TIRF Microscopy

I. Westphal[1], H. Clausen-Schaumann[2], M. Jäger[1], B. Morgenstern[1], U. Joos[1], J. Kriegsmann[1], G. Pilarczyk[1], C. Duschl[1]

[1]Fraunhofer Institute for Biomedical Engineering
Invalidenstrasse 42, D-10115 Berlin, Germany
[2]GSF-FZ Environment and Health, Ingolstaedter Landstrasse
D-85764 Neuherberg, Germany
ines.westphal@ibmt.fhg.de

During the locomotion on artificial surfaces, adherent cells release tubular filaments with regularly ordered branches and patches. These highly ordered cell traces are approximately 100 μm in length and 100 nm in diameter. As recently shown, cell traces are coated by lipid bilayer and contain cytoplasm, organelles, cytoskeleton and focal adhesion proteins, such as talin and vinculin. Cell-specific surface receptors like major histocompatibility complex (MHC), cluster of differentiation (CD 71) and integrins (beta 1) are also found in the cell trace membrane.

Our microscopic investigations using Concanavalin A-549 and Phalloidin-488 show the cell trace to be enveloped with proteoglycans and to contain fibrillar actin. The sub-wavelength dimension of the cell trace makes the investigation of the trace topography by light microscopy difficult. Our aim is to correlate the intensity of the fluorescence signal with sub-structures like patches, tubes and vesicles. Therefore, we use atomic force microscopy for the measurement of the varying trace morphology. Thus, the combination of both microscopic techniques provides unprecedented information on the traces' molecular constituents while avoiding ambiguities due to dye concentration and epitope binding characteristics.

P21 – Nanofibers of Organic Molecules

L. Kankate, F. Balzer, H. Niehus,
R. Frese[1], C. Maibohm[1], H.-G. Rubahn[1]

Humboldt-Universität zu Berlin, Institut für Physik / ASP
Newtonstr. 15, D-12489 Berlin, Germany
[1] SDU Odense, Fysisk Institut, Campusvej 55
DK-5230 Odense M, Denmark

Nanoaggregates assembled from organic light-emitting molecules are promising candidates as building blocks of new electronic and optoelectronics devices like organic light emitting diodes (OLEDs) or organic field effect transistors (OFETs). In biology possible applications rely on biosensitive near field detection of guided light along individual nanofibers or nanofiber-arrays.

One of the most appropriate class of molecules to grow these organic fibers are rod like para-phenyl oligomers, *p*-*n*P. In the past we have shown the dipole assisted self assembly of nanoscopic *para*-hexaphenyl (*p*-6P) aggregates on muscovite mica by vacuum deposition [1]. Up to a millimetre long, mutually parallel aligned single crystalline fibers have been grown which show heights and widths of a few ten to a few hundred nanometers. The fibers emit blue light after UV excitation and waveguiding within the aggregates has been demonstrated [2].

For application purposes an independent means of controlling the fibers widths, heights and lengths is essential. Introducing an ultrathin gold layer allows variation of the mean fiber height by a factor of three, whereas the fibers widths become more uniform than for growth on a plain mica surface. The total luminescence intensity as well as the mean length and orientation of the fibers show a complex dependence on the Au layer thickness, which relates to its morphology. Finally we present first results on fiber growth of another class of rod-like molecules on mica, i.e. the thiophene molecules α-quaterthiophene and α-sexithiophene.

[1] F. Balzer and H.-G. Rubahn, Appl. Phys. Lett. 79 (2001) 3860.
[2] F. Balzer, V.G. Bordo, A.C. Simonsen, and H.-G. Rubahn, Phys. Rev. B 67, 115408 (2003).

P22 – Adsorption of DNA Molecule and DNA Patterning on Si Substrate

Shin-ichi Tanaka, Masateru Taniguchi and Tomoji Kawai

The Institute of Scientific and Industrial Research
Osaka University, 8-1, Mihogaoka, Ibaraki
Osaka 567-0047, Japan, CREST JST

The DNA molecule is a candidate electrical material for molecular devices. However, in order to realize a DNA molecular device, it is necessary to combine characteristics of DNA with semiconductor technology.

DNA molecule can be selectively adsorbed to SiO_2 surface in SiO_2/SiH pattern, which is fabricated using photolithography, by adding $MgCl_2$ to DNA solution, and DNA patterning is made on Si substrate. Since DNA molecule can be adsorbed to Si substrate through Mg^{2+}, the adsorption of DNA molecule in SiO_2/SiH pattern is influenced by the concentration of $MgCl_2$ and the difference of chemical property between SiO_2 surface and SiH surface. The optimum concentration of $MgCl_2$ in which DNA is selectively adsorbed to SiO_2 surface was 0.1 mM. The development of a DNA device combined with silicon technology can be expected by applying this patterning.

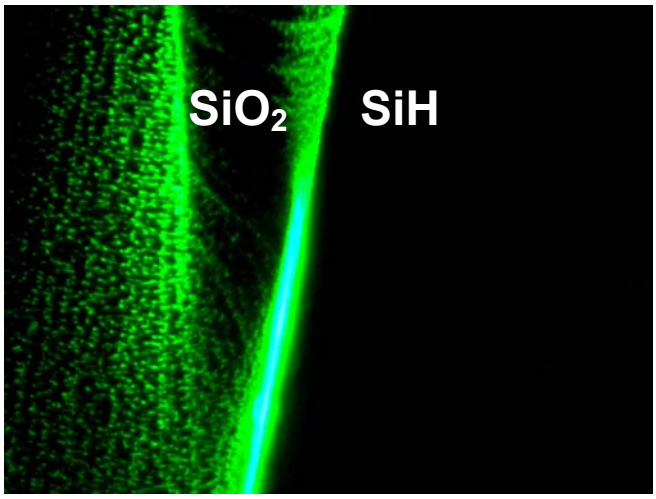

Figure Fluorescence microscope images of DNA (Poly(dG-dC)·Poly(dG-dC)) stained by Yo-Pro (1 μM in SiO_2/SiH pattern (×2000). Green color is DNA-(Yo-Pro) complex.

P23 – DNA/PAH Multilayers: Interaction, Adhesion Properties and Morphology

Haofei Gong, Javier Garcia-Turiel, Krasimir Vasilev and Olga I. Vinogradova

Max Planck Institute for Polymer Research
Ackermannweg 10, 55128 Mainz, Germany

The alternate deposition of polycations and polyanions on charged surfaces leads to a building up of films called polyelectrolyte multilayers. These films have received considerable attention during the last decade due to their potential applications in various fields, ranging from electroluminescent diodes to biomaterial coatings.

We study here the adhesion properties, morphology, and growth of the DNA/PAH multilayers assembled on the glass surface. The main methods are Surface Plasmon Resonance Spectroscopy (SPR) and the Atomic Force Microscopy (AFM). The imaging was performed with commercial AFMS, while interaction and pull-off forces were measured with the home-made AFM-related setup. Regular oscillations in the pull-off force were observed for multilayers assembled both with and without drying. Strong adhesive forces were detected on the PAH surface of DNA/PAH multilayer.

On the polyanion surface of DNA, the force-distance curves show repulsions on approach and weak attractions on retract. The film growth shows two distinct regimes. One is from a PEI prelayer to a third bilayer. At this stage the adhesive forces increase slowly. Starting from forth bilayer, the pull-off forces increase dramatically. The SPR results show a nonlinear film growth at the first several layers, consistent with the pull-off force trends. AFM images show clear DNA chain aggregations on the surface of DNA and porous morphology on the surface of PAH.

Our results are important for various applications, such biosensors, biochips, and more.

P24 – AFM-Imaging of Structures Linked to the Actin Cytoskeleton in de-roofed Cells

Clemens Franz and Daniel Müller

Biotechnologisches Zentrum, TU Dresden und Max-Planck-Institute for Cellular Biology and Genetics, Dresden

Given the possibility to image samples under physiological conditions, atomic force microscopy (AFM) is well-suited for the investigation of biological samples and has been successfully employed to generate high-resolution images of both fixed and living cells. However, because it is a surface imaging technique, usually only structures on the cell surface can be imaged by AFM, whereas inner cellular structures, such as the cytoskeleton, are not accessible.

We have employed a cell de-roofing method during which the apical cell membrane is removed, exposing intracellular structures so that they can now be imaged by AFM. After de-roofing actin-containing structures, such as focal adhesion complexes and actin filaments underlying the basal cell membrane were identified using a fluorescent marker for actin filaments. Using a setup allowing combined fluorescent microscopy and AFM, these fluorescently-labelled actin structures were subsequently imaged by AFM. The AFM images have a much higher resolution compared with the corresponding light microscopic images.

This technique demonstrates the usefullness of combined fluorescent microscopy and AFM, in which fluorescent labelling techniques serve to identify protein complexes which can then imaged at superior resolution using AFM.

P25 – AFM Studies on Membrane Phospholipids in Presence of Drugs

Maria Tomoaia-Cotisel[1], Gheorghe Tomoaia[2], Daniela-Vasilica Pop[1]
and Aurora Mocanu[1]

[1] "Babes-Bolyai" University of Cluj-Napoca
Faculty of Chemistry and Chemical Engineering
Physical Chemistry Department,
[2] Iuliu Hatieganu University of Medicine
Orthopedic Surgery, 3400 Cluj-Napoca, Romania

Phase behavior and surface structure of dipalmitoyl phosphatidyl choline (DPPC) spread as Langmuir nanolayers at the air/water interface in the absence and in the presence of two drugs in the aqueous phase, such as procaine (P) or deferoxamine (DFO), at a drug concentration of 10^{-3} and 10^{-6} mole dm^{-3}, respectively, have been investigated using Langmuir-Blodgett (LB) self-assembly technique and atomic force microscopy (AFM).

The LB self-assembled films were transferred from nanolayers at the air/water interface onto solid substrates, like glass optically polished, at different controlled surface pressures before collapse and at advanced collapse, by using vertical transfer and horizontal deposition method. In the presence of these drugs, the stability of DPPC films is highly increased as it is seen from high collapse pressures of mixed nanolayers at the air/water interface. Depending on the lateral surface pressure, highly ordered structures and less organized features have been directly evidenced. In addition these observations reveal some specific molecular interactions between these biologically relevant biocompounds in agreement with thermodynamic studies. These may include the interaction among DPPC molecules and between DPPC and drug molecules in addition to the interaction between the underlying substrate and the film forming molecules.

The different micro- and nano-structures evidenced by AFM measurements on LB mixed films of DPPC and P and of DPPC and DFO are in substantial agreement with the molecular structure of these biocompounds. The data indicate that both procaine and deferoxamine can penetrate and interact with membrane dipalmitoyl phosphatidyl choline stabilizing the membrane phospholipids at both internal and external membrane interfaces.

P26 – Probing Different Origins of Potential Barriers Stabilizing the Membrane Proteins Halorhodopsin and Bacteriorhodopsin

David A. Cisneros[1], Dieter Oesterhelt[2] and Daniel J. Müller[1]

[1] BIOTEC, University of Technology, Dresden, Germany
[2] Max-Planck-Institute of Biochemistry, Martinsried, Germany

Single-molecule atomic force microscopy (AFM) and force-spectroscopy was applied to detect molecular interactions stabilizing the structure of halorhodopsin (HR), a light-driven chloride pump from the cell membrane of *Halobacterium salinarum*.

The force spectra revealed unfolding pathways of single HR molecules. While in distinct unfolding pathways single structural elements such as transmembrane α-helices or polypeptide loops unfolded in separate steps, other pathways showed these elements unfolding cooperatively. Because of the high structural and sequence similarities between HR and the light-driven proton pump bacteriorhodopsin (BR) we compared their unfolding pathways and polypeptide regions that established energetic barriers against unfolding.

The unfolding pathways of both proteins showed a high similarity for almost every structural domain. Correlating the structural position of the potential barriers of both proteins showed that they were located at structurally identical positions. This finding suggests that different amino acid compositions can establish structurally indistinguishable potential barriers. It is concluded, that these barriers may rather result from comprehensive interactions of all amino acids within a structural region than from specific interactions. However, one additional potential barrier located within a short segment of helix E was detected for HR. This additional barrier correlated with a Pi-bulk interaction which disrupts helix E.