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HUMBOLDT-UNIVERSITÄT ZU BERLIN

2nd International Workshop – 18 September 2003 in Berlin

Scanning Probe Microscopy in Life Sciences

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Scanning Probe Microscopy in Life Sciences

Organizing Committee

Dr. Andreas Jordan – Center of Biomedical Nanotechnology & Dpt. of Radiation Oncology/CVK, University Clinic Charité
Torsten Jähnke – JPK Instruments AG

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Workshop Location

University Clinic Charité
Campus Virchow-Klinikum, Mittelallee 10, Hörsaal/Lecture Hall 6
Medical Faculty of the Humboldt University at Berlin
Augustenburger Platz 1
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Program

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

9:10 am **Nanomechanics: Opening New Frontiers in Bio Analyses and Diagnostics**
Prof. Dr. Christoph Gerber – NCCR National Center of Competence for Nanoscience, Institute of Physics, University of Basel, and Nanoscale Science Group, IBM Zurich Research Laboratory, Rüschlikon

9:50 am ***Coffee Break***
Poster Session and Demonstration of JPK's NanoWizard™ AFM

10:50 am **Secretion of von Willenbrand Factor Measured and Imaged in Human Endothelial Cells**
PD Dr. med. Stefan Schneider – Clinic and Polyclinic for Skin Diseases, Münster

11:30 am **Characterizing Cell Membranes across Dimensions: From Single Molecule Experiments to Living Cells**
Prof. Dr. Daniel Müller – Biotechnical Center of the Technical University Dresden and MPI of Molecular Cell Biology and Genetics, Dresden

12:10 am ***Lunch***
Poster Session and Demonstration of JPK's NanoWizard™ AFM

2:00 pm **Bioterror and Diagnostic Electron Microscopy / AFM**
Dr. Hans Gelderblom – Robert Koch Institute, Berlin

2:40 pm **The Effect of Lipid Depletion and Microvilli Formation in MDCK Cells, Visualized by Atomic Force Microscopy**
Dr. Kate Poole – MPI of Molecular Cell Biology and Genetics, Dresden

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

4:20 pm **Single Molecule AFM Experiments in NanoBiology: Structure, Mechanics, Kinetics and Application**
Prof. Dr. Dario Anselmetti – Experimental Biophysics & Applied Nanoscience, Bielefeld University

5:00 pm **Probing Mechanosensory Ion Channels in Cochlear Hair Cells by AFM**
Stefan Fink – Hearing Research Center, Tübingen

5:40 pm **Closing**
Torsten Jähnke – JPK Instruments AG, Berlin

Talks

9:10 am – Nanomechanics: Opening New Frontiers in Bio Analyses and Diagnostics

Christoph Gerber

NCCR National Center of Competence for Nanoscience,
Institute of Physics, University of Basel
and
Nanoscale Science Group, IBM Zurich Research Laboratory,
Rüschlikon, Switzerland

Biosensing tools are currently undergo a further stage of development increasing efforts have therefore been put into the development of cantilever-based sensors for the detection of physical phenomena and chemical and biological reaction. Biological and chemical processes are transduced into nanomechanical motion using a microfabricated silicon cantilever array, allowing quantitative and qualitative detection in gaseous and liquid environment. The motion is tracked by optical beam - deflection using a time multiplexed scheme. Miniaturized sensors show fast responses high sensitivity and are suited for parallelization into integrated devices.

We report the first microarray of cantilevers to detect multiple unlabelled biomolecules simultaneously down to picomolar concentrations within minutes. Ligand-receptor binding interactions, such as DNA hybridization or protein recognition, occurring on microfabricated silicon cantilevers generate nanomechanical bending, which is optically detected in-situ. Differential measurements including reference cantilevers on an array of eight sensors can sequence-specifically detect unlabelled DNA targets in 80-fold excess of non-matching DNA as a background and discriminate 3 and 5 overhangs.

Our experiments suggest that the nanomechanical motion originates from predominantly steric hindrance effects and depends on the concentration of DNA molecules in solution. We show that cantilever arrays can be used to mechanically investigate the thermodynamics of biomolecular interactions, and have found that the specificity of the reaction on a cantilever is consistent with solution data. Hence cantilever arrays permit multiple binding assays in parallel, and can detect femtomoles of DNA on the cantilever at a DNA concentration in solution of 10 pM . The significant improvement of functionalisation enables the detection of specific gene fragments within a complete genome suggesting that PCR (Polymerase Chain Reaction) could be circumvented.

The general applicability of the method to biochemical processes was furthermore demonstrated by monitoring continuous label-free detection of cardiac biomarker via measurement of surface stress generated by antigen-antibody molecular recognition.

This underlying nanoactuation mechanism has more wide-ranging implications. The forces involved ~1 nN, are sufficient to operate micromechanical valves and related fluidics devices which would also permit operation of micro and nanomechanical machinery. Since the transductions does not rely on external control systems delivery devices could be triggered directly by signals from single cell, gene expression, or immune responses.

10:50 am – Secretion of von Willebrand Factor Measured and Imaged in Human Endothelial Cells

PD Dr. med. Stefan W. Schneider

Clinic and Policlinic for Skin Diseases
Von Esmarch Str. 58, D-48149 Münster, Germany

The vascular endothelium with its salient location at the interface between blood and tissue plays a pivotal role in the process of blood coagulation and inflammation. The transition into a procoagulatory and proinflammatory state upon stimulation is referred to as endothelial cell activation.

One fundamental characteristic of this activation is the induction of von Willebrand factor (vWF) exocytosis. Human umbilical vein endothelial cells (HUVEC) secrete vasoactive substances such as vWF. This molecule is stored in large (up to 3 μm) cone-like vesicles called Weibel Palade bodies (WPB). By using atomic force microscopy (AFM) we are able to visualize the apical surface topography of fixed human endothelial cells with nanometer resolution. In addition, AFM allows to measure local cell stiffness with a spatial resolution of 100 nm.

In previous studies we showed that endothelial cells have a readily releasable pool of WPB. In resting cells this intracellular vesicle pool can be imaged as plasma membrane protrusions, called humps, with a height of 140 ± 50 nm (±SEM; n = 8) and a diameter of 275 ± 85 nm (±SEM; n = 8). Humps represent areas of docked WPB. Stiffness measurements revealed that humps are characterized by decreased cell membrane stiffness of 30% compared to surrounding cell membrane. Incubation of HUVEC with the actin depolymerization agent cytochalasin D decreased overall cell stiffness and eliminated stiffness differences. Therefore, cell stiffness is mainly due to the subapical actin network.

After stimulation of the cells with hyperosmolaric solutions or histamine these docked WPB immediately fuse with the plasma membrane forming large (diameter: ~500 nm) exocytotic pores and release vWF into the supernatant (measured by ELISA). Appearance of exocytotic pores matches the disappearance of humps.

In addition to confirm that humps and exocytotic pores are docking and fusion sites of WPB followed by vWF release, extracellular and intracellular vWF was immunostained and imaged by laser scanning microscopy on the same single cell. Immunostaining of vWF was found to

be localized next to the exocytotic pores imaged by AFM. The data indicate that human endothelial cells have a readily releasable pool of WPB that allows the instantaneous release of vWF. These distinct areas of exocytosis are characterized by cell membrane protrusions (humps) and decreased cell membrane stiffness due to a missing actin cortical network. Lack of cortical actin at distinct membrane areas is a prerequisite for the release of prothrombotic substances into the blood stream.

11:30 am – Characterizing Cell Membranes across Dimensions: From Single Molecule Experiments to Living Cells

Harald Janovjak, Aliaksei Kedrov, Kate Poole,
Amita Sharma, Fedor Severin & Daniel J. Müller

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The exceptional signal-to-noise ratio of the atomic force microscope (AFM) allows individual proteins to be imaged under physiological relevant conditions at a lateral resolution of 0.4 - 1nm and a vertical resolution of 0.1 - 0.2nm. This capability is reviewed on various membrane proteins. Being applied in molecular and cell biology the AFM can be addressed to many more topics than solely high resolution imaging of native proteins.
Examples discussed are:

Single molecule experiments

Lately, it became possible to observe molecular processes at the single-molecule level using scanning probe microscopy techniques. Examples observing function, variability, and assembly of single proteins are discussed. Recent developments of scanning probe microscopy techniques enable measuring simultaneously multiple biochemical signals on individual macromolecules. Recorded with submolecular resolution these signals can be directly assigned to structural details of individual proteins of a cellular membrane. Examples discussed are the *detection of structural variability and flexibility*, of *surface charges*, and of *electrostatic potentials* of membrane proteins.

Observing membrane protein assembly

In recent approaches we combined above techniques to gain insights into vesicle transport, fusion and signal trafficking of native cell biological systems. First results of the experiments will be presented showing dynamics of membrane protein assembly at molecular resolution.

Characterizing folding and unfolding of native membrane proteins

The recently invented combination of single-molecule imaging and force-spectroscopy enables the controlled manipulation of single proteins to detect their inter- and intramolecular interactions. The experiments allow observing the unfolding pathways and forces of secondary structural elements of biological macromolecules such as α-helices, β-sheets and, most surprisingly, of polypeptide loops. Examples unfolding of antiporters, of bacteriorhodopsin and of human aquaporin are discussed. Direct observations of the unfolding

as well as the folding of individual secondary structure elements are discussed. In future these and forthcoming methods will provide novel molecular biological insights into factors determining structure, stability and function of individual proteins and of their assemblies.

Directing biological assemblies and pathways

We further demonstrate, that the AFM stylus can be used as a 'molecular spinning and knitting machine' to orientate the assembly of individual biological molecules into well-defined, two-dimensional patterns with feature sizes of a few nm to several hundreds of µm. The resulting nanostructured scaffolds are stable for several weeks without loss of orientation or functionality. Our results directly demonstrate the plasticity of biological assemblies, and give insight into the physical mechanisms by which biological systems may be organized by cells *in vivo*. These nanoscaffolds may also serve as platforms on non-biological surfaces to direct molecular and cellular processes.

Observing cytoskeleton assembly

An AFM *in vitro* system is introduced that has been established to study the assembly and dynamics of individual filaments in cell extracts. The approach allows observing the polymerization of filaments, the formation of their networks, to study their length distribution and their formation of different types of junctions and branches. The approach builds an avenue to study *in vitro* assays of complex biological systems.

Observing cellular surfaces

By combining AFM with advanced light microscopy techniques the cell surfaces can be not only directly observed at high resolution, but also their compartments can be identified by specific labeling using modern cell biological techniques. Examples shown are processes of cell surface structuring which are related to the cell function.

2:00 pm – Bioterror and Diagnostic Electron Microscopy / AFM

Hans R. Gelderblom
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Modern, "open" societies are vulnerable from inside and outside and from many angles, one of them being summarized as bioterrorism (BT). BT is defined as intentional release of a health-threatening agent, e.g. smallpox, anthrax, or a deadly toxin, e.g. Clostridium botulinum toxin. BT agents are easily transfered from human to human, pose severe health risks and are associated with a high panic potential. According to the inherent health risk and the likelyhood, that the agent can be effectively "weaponized", BT agents are grouped into three categories A– C (5, 8). The highest Category A contains e.g. anthrax, smallpox, hemorrhagic fevers and the botulinum toxin, all of them difficult to detect, contain or treat.

BT for a number of reasons appears not very likely: e.g. to "weaponize" anthrax spores requires technically demanding methods, and variola major, the etiological agent of smallpox, is thougth not available outside the two WHO Smallpox Collaborating Centres. Nevertheless, the risk of an attack must not be neglected.The damage following a re-introduction of smallpox appears disastrously high considering that the virus would hit an immunologically naive population. Therefore preparations assuming worst-case scenarios are required, including education in clinical and laboratory diagnostics, vaccination policy, and case management. Besides the Cat A – C agents , other more "common" microbes, like influenza, and plant or animal pathogens should be considered as candidate agents for bio- or agriterrorism (3, 6). A distinction between a man-made and a natural incident, however, will become more difficult with these ubiquitous agents.

To cope with a BT attack requires rapid and accurate diagnostics. The detection of an initial BT aerosol will be difficult - the attack will be more likely recognized after first patients are suffering from "unusual" conditions. The potential of electron microscopy (EM) in the rapid lab diagnosis of infectious agents will be discussed (1, 2, 4). Negative staining, a simple preparation method in EM, allows the rapid detection of agents from bacteria down to the smallest virus using the unbiased, "open view" of EM (2, 4, 7, 8). Samples are prepared directly from the patient, after culturing or as "environmental probes", e.g. the contents of an "anthrax letter". EM works as a catch-all-method, is able to detect also multiple infections and agents not considered before, and should be used in front-line diagnostics.

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2:40 pm – The Effect of Lipid Depletion on Microvilli Formation in MDCK Cells, Visualized by Atomic Force Microscopy

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Here, we investigated the effect of the depletion of raft lipids in MDCK cells, combining the atomic force microscopy (AFM) and advanced light microscopy techniques.
Lipid rafts (glycosphingolipid/cholesterol-enriched membrane microdomains) are postulated to play a role in lateral sorting of membrane components into different domains and in signal transduction.
The study of lipid rafts and their function *in vivo* is difficult due to their predicted size (50 nm diameter). The plasma membrane of MDCK cells polarizes, on monolayer formation, into apical and basolateral domains, with asymmetrical distribution of membrane lipids and proteins. The apical surface, which can be observed by AFM, is further divided into microvillar and planar sub-domains.

The metabolic inhibition of either cholesterol, or glyco-sphingolipid, synthesis diminished the number of microvilli formed on the cell surface, although this effect showed non-uniform distribution. The chemical extraction of cholesterol, after the metabolic inhibition of lipid synthesis, led to the loss of all microvilli.
On the surface of these smoother cells much smaller spikes remain, suggesting that the presence of raft domains in the plasma membrane may be required for the stabilization of actin bundling during microvilli formation.

4:20 pm – Single Molecule AFM Experiments in NanoBiology: Structure, Mechanics, Kinetics and Application

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Over the last decade, an almost complete picture of the relation between mechanical single-molecule experiments and biomolecular ensemble experiments has been successfully developped. After a brief introduction, which summarizes the key findings in this biophysical field, latest results of several biological examples will be presented and discussed.

AFM imaging has been applied to bacterial surface layers of *Corynebacterium glutamicum* in order to investigate structural aspects of the 2D protein crystal layers of different bacterial strains [1].

Specific binding of the regulatory protein ExpG to promoter regions represents a central issue of the galactoglucan biosynthesis in the ground bacterium *Sinorhizobium meliloti*, and has been investigated by AFM force spectroscopy with respect to different promoter regions. The measured loading rate dependent binding forces of 50 to 165 pN allowed an estimate of the corresponding thermal off-rates. Additionally, we found evidence of structural properties within the molecular binding potential which allows allocation to different binding mechanisms [2].

Different binding mechanisms between DNA and DNA binding agents can also be identified by probing DNA fragments in AFM force spectroscopy experiments. We investigated the binding of DNA-intercalants as well as of natural and synthetic minor and major groove binders by in-situ overstretching DNA and found characteristic mechanical fingerprints which directly proove the molecular binding and additionally allow identification of the corresponding binding mechanisms. AFM force spectroscopy can therefore be regarded as a efficient and potent single molecule biosensor [3].

References

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5:00 pm – Probing Mechanosensory Ion Channels in Cochlear Hair Cells by AFM

Stefan Fink, Matthias G. Langer, Assen Koitschev, Karsten Löffler,
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In mammals, hearing is mediated by inner and outer hair cells in the inner ear, whereby a mechanical input is transduced into an electrical output. The hair cells consist of rod-like stereocilia at their apical pole arranged in rows of different height essential for the so-called mechanoelectrical transduction. Mechanosensitive ion channels are located within the plasma membrane of individual stereocilia. All stereocilia of a single cell taken together form a hair bundle of specialized structure.

Compared to voltage-gated and ligand-gated ion channels the mechanosensitive ion channel in the mammalian cochlea reveals much faster gating up to 100 kHz and more. It transforms sound into an electrical receptor current with sensitivity to forces in the PicoNewton range. So far mechanoelectrical transduction was investigated stimulating the entire hair bundle of cochlear hair cells rather than single stereocilia. Up to now the only way to get access to single channel events in this configuration is to examine artificial insensitive hair cells induced by accident or experimentally.

Here we use AFM as a nanomanipulator displacing single stereocilia of living outer hair cells of postnatal rats while the current response is measured simultaneously by patch clamp.
The AFM is used in constant height mode applying an increasing force with a superimposed 98 Hz sine to the tips of single stereocilia resulting in a displacement of individual stereocilia. Tip links connecting adjacent stereocilia are supposed to pull directly at the mechanosensitive ion channel in the apical cell membrane. This allows cations to flow from outside into the cell resulting in depolarization of the hair cell.

Combination of AFM and patch clamp is a promising technique for probing mechanical and electrical properties of single mechanosensitive molecules in living cells.

Poster Session

P1 – Morphology and Elasticity Studies of the Tight Junctions at the Blood Brain Barrier in vitro

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‡ Physical Institute, University of Münster, Germany

Clinical studies showed the extraordinary role in cell – cell - interactions of physiological and biological barrier functions. Pharmacologically highly interested and in an outstanding position physiologically important is the functionality of the blood brain barrier, which has not been completely understood yet. Beside clinical investigations the biophysical analysis has still been remained indispensable for the elucidation of the physical properties of the blood brain barrier.
The blood brain barrier shields the brain cells from fluctuations in blood composition by regulating the exchange of substances between the vascular lumen and the central nervous system. This physiological barrier is composed of unique endothelial cells, which are closely connected by tight junctions and line the brain capillary wall and accomplish barrier functions between blood and interstitial fluid environment of the brain at their cell junctions.

Elasticity measurements by employing scanning force microscopy provide an excellent tool to study the viscoelastic properties of living cells under physiological conditions. The scanning force microscopy is a unique method, which allows in an extremely high resolution to distinguish different regions of the cells by surveying the local mechanic properties, which depend on the environmental requirements to the cell. The cell junctions have been surveyed concerning their stiffness and their permeability in dependence on environmental changes.
The quantitative determination of the local properties is achieved by two dimensional mapping of the sample elasticity. While the cantilever tip is raster-scanned across the sample surface, force curves are recorded at each raster point (force mapping). A calculated cell topography is obtained from this array of force curves at different loading forces as well as the local elastic modulus according to the Hertzian model for elastic indentations.
This method combines imaging of the viscoelasic properties and the visualisation of the cell topography and it is gentler than simple imaging modes because no lateral forces and friction occur while the measurement is performed.

P2 – Nano Digital Image Correlation Method for Innovative Sensor Architectures

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With the application of biological materials and processes in micro- and nanotechnological fabrication new experimental issues will arise. The understanding of thermo-mechanical properties of biomaterials will be crucial for successful design of advanced sensors. Moreover, the development and evaluation of interface concepts of biological structures to microelectronic materials such as polymers, metals, ceramics and semi-conducting materials will be a fundamental challenge.

To fulfil this needs new strategies for experimental assessment of ultrathin material layers and interfaces are essential. It has been shown that Scanning Probe Microscopy is the method of choice for the analysis of such layers. In addition to the more or less static analysis of topography or material contrast by the phase signal the authors show, [1], that SPM images can be used for the measurement of deformations on the nano-scale.

The *nanoDAC method* (nano Deformation Analysis by Correlation), allows the determination and evaluation of 2D displacement fields based on SPM data. In-situ SPM scans of the analyzed object are carried out at different thermo-mechanical load states. The obtained topography-, phase- or error-images are compared utilizing grayscale cross correlation algorithms. This allows the tracking of local image patterns of the analyzed surface structure. The measurement results of the nanoDAC method are full-field displacement and strain fields.

The nanoDAC method is suited for measurement of mechanical properties such as fracture properties, Young's modulus, Coefficient of Thermal Expansion, Poisson's ratio. Furthermore the technique should be used for tracking of structures or particles driven by diffusion processes or nanomanipulators. Future generations of SPM equipment will provide modes for observation of dynamic processes. The nanoDAC method will be a useful tool for evaluation of time-dependent processes observed by in-situ SPM techniques.

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P3 – Detection of Potentially Effective Lipid Transfection Reagents

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Introduction: Lipofection is a well-established method of gene delivery performing with usage of different cationic lipids liposome-nucleic acid complex formation. We tried to find potentially effective transfection reagents among a set of newly-synthesized cationic lipid dimers. The method of AFM scanning microscopy was used as a first one for detection of DNA-lipid complex formation under the control of the other independent measurement of liposome's diameter.

Methods: The set of new-synthesized cationic lipid dimers – amphiphilic derivatives of L-aminoacids (glutamine and alanine) as a monomer and two tetrameric ammonium groups conjugated with aliphatic lipid spacer of (DEGA) 3, 4, 7, 11 and 22 links. Lipid film was made by vacuum chlorophorm evaporation, PBS-dissolving suspension preparing and further ultrasonic and extrusion treatment. Plasmid DNA dissolved in water was added to liposome suspension and formation of lipocomplexes was detected by Atomic Force Microscopy (NanoScope III-a, Digital Instruments, Santa Barbara, USA). We used tapping-mode with Si_3N_4 cantilievers with tapping frequency 5 kHz and scan frequency 200-400 kHz. Mica substrate was used for probe drying. The other method of vesicle diameter measurements in water suspension was performed by Dynamic light scattering system (Nicomp-380, Santa Barbara, USA). The effectiveness of transfection was evaluated by time-lapsed fluorescent microscopy (Karl Zeiss, Germany).

Results and Conclusions: Relatively small diameter and stability of complexes were two features needed for successful penetration of DNA-lipid complexes into transfecting cells. The optimal of mole DNA-lipid ratio 1:2.5 was determined in AFM and dynamic light scattering assays. Visualization of transfection process and confirmation of gene delivery – expression of green fluorescent proteins in transfected cells – was made by time-lapsed fluorescent microscopy. One of the set of cationic lipid dimmers DEGA-3 was admitted to be an effective reagent for eucariotic cell's transfection.

P4 – Visualization of Detailed Structures within DNA

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Here we report ultrahigh-resolution scanning tunneling microscopy images of detailed structures within DNA composed of a well-defined base sequence, deposited on substrates using a pulsed injection method. Poly(G–C)–poly(G–C) and poly(A–T)–poly(A–T) have been differentiated by comparing their secondary (helical) structures(fig.1). We have visualized the structure of poly(A–T)–poly(A–T) with a resolution high enough to recognize individual nucleotides. By comparing the size of individual nucleotides (the primary structure) of poly(A–T)–poly(A–T),sequencing of individual nucleotides can be achieved successfully (fig.2). These results validate the Watson and Crick DNA model at the single molecule level 50 years after its discovery.

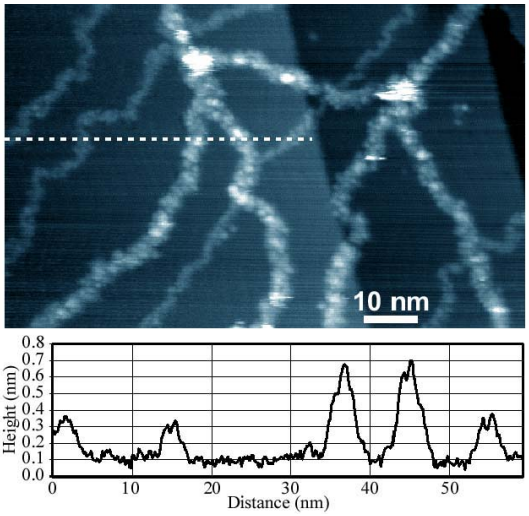

Fig. 1., Fig. 3. STM images and topographic height profile of poly(G-C)-poly(G-C) and poly(A-T)-poly(A-T) obtained after pulsed-injection of a mixed sample solution of poly(G-C)-poly(G-C) and poly(A-T)-poly(A-T) onto clean Cu(111) substrates. Imaging parameters: Vs=-2 V, It=1 pA, scan of 100 × 62 × 1.2 nm^3 and T=90 K.

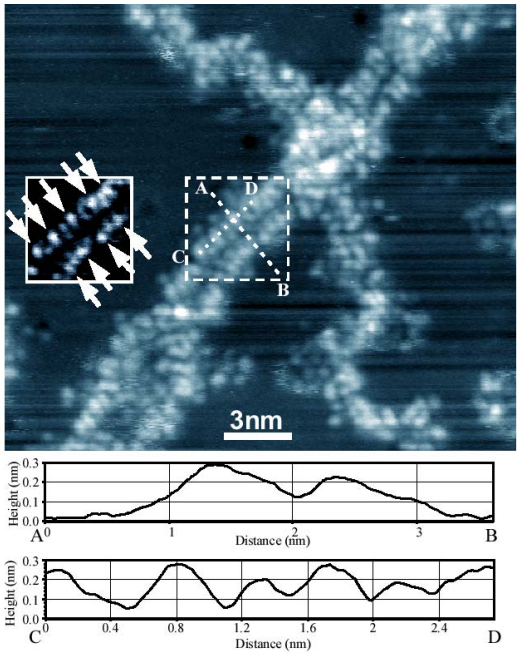

Fig. 2. STM image and topographic height profiles of poly(A-T)-poly(A-T), obtained after pulse injection of the sample solutions onto clean Cu(111) substrates. Imaging parameters: Vs=-1.5 V, It=2 pA, scan of 25 × 22 × 0.4 nm^3 and T=300 K. A contrast-magnified image (z=0.1 nm) is superimposed in the left of the square. White arrows indicate the brighter nucleotides.

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2. Surface Science Volume 432, Issue 3, 20 July 1999, Pages L611-L616.

P5 – AFM Investigations of the Bola Phospholipid PC-C32-PC

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Bipolar lipids with one long alkyl chain may stabilize membranes. For this purpose, the bola phospholipid with a C_{32} alkyl chain was synthesized, namely the lipid dotriacontan-1,1'-diyl-*bis*-[2-(trimethylammonio)ethylphosphate] (PC-C32-PC) [1]. For the pure bolalipid an unusual aggregation behavior was observed. In aqueous samples at room temperature a viscous hydrogel is observed. The gel state can be explained by the existence of a dense three-dimensional network of fibrils which was already found in highly diluted aqueous suspensions (0.03 wt%) by cryo-transmission electron microscopy [2]. The fibrils have a perceptible helical structuring [2]. Partly it can be represented by AFM. AFM images show a parallel arrangement of fibrils with a diameter of about 11 nm in a layer of 7 nm thickness. On a larger scale a network of filaments with 300 - 700 nm diameter was found (Fig 1).

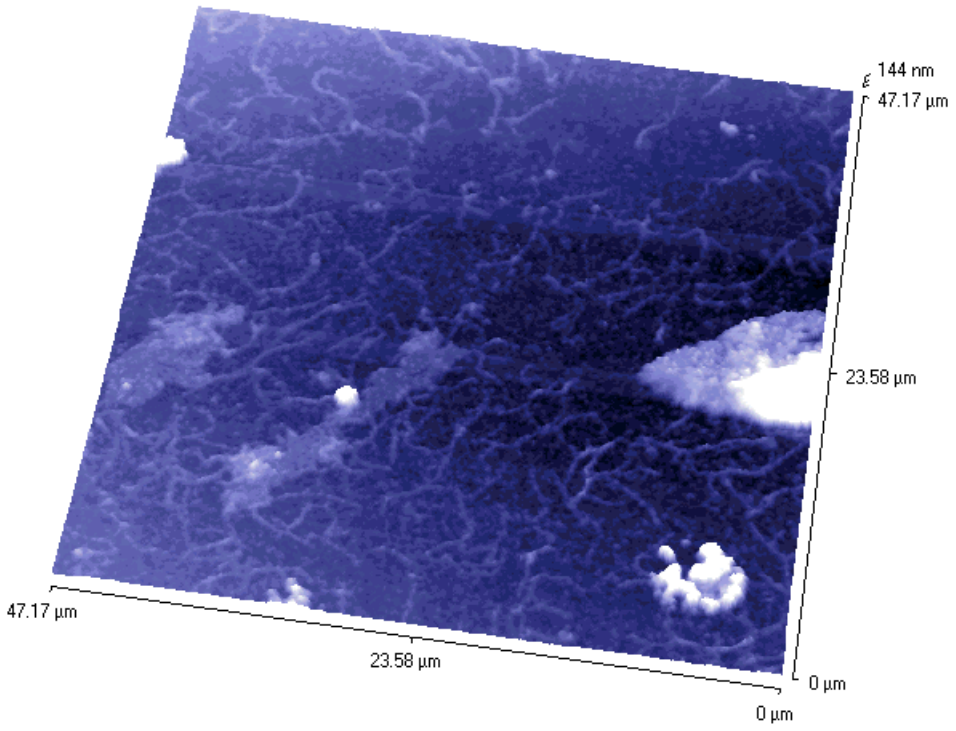

Fig. 1:
AFM image of the bola phospholipid PC-C32-PC from solution on mica showing filaments.

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P6 –
Hollow Polymeric Capsules Covered with S-layers as a Future Template for Receptors and Model to Investigate Polymer-Protein Interactions

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In the last seven years hollow capsules with defined walls made of polyelectrolyte layers (using the layer by layer technique) have been prepared and characterized. Crystalline bacterial cell surface layers (S-layers) are one of the most common outermost cell envelope components of the prokaryotic organism and represent the simplest biological membranes developed during evolution. In this work, we try to crystallize S-layers on the outer surface of microcapsules.
Zeta potential measurements show a slight variation in the potential value for three different types of S-layer deposit on polyelectrolyte covered particles. Comparing these results with the zeta potential for particles without protein, we conclude that they are covered by the S-layer.
TEM micrographs, fluorescence microscopy and laser scanning confocal microscopy experiments on capsules were carried out. They show that different isolated S-layers crystallize on hollow capsules, preferably on negative ones (PSS as outer layer). Adsorption of protein occurs for positive capsules too (PAH as outer layer), however the crucial question about the structure remains. AFM studies on solid support lead to similar conclusions.

A combination of capsules and S-layers could be a suitable model system to study relevant biological interactions, such as membrane-membrane and carbohydrate-protein interactions. Measurements concerning carbohydrate-protein interactions are presented, after the modification of the AFM tip with secondary cell wall polymer. These first results show the coexistence of large adhesion forces with weak non-covalent forces.
This method combines imaging of the viscoelasic properties and the visualisation of the cell topography and it is gentler than simple imaging modes because no lateral forces and friction occur while the measurement is performed.

P7 – Chemical Microspectroscopy on Biomaterials

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Most biomaterials have significant absorption lines in the infrared region, the so called fingerprint region, hence it is very interesting to combine the advantages of normal microscopy and infrared laser spectroscopy. So far, microscopy of living materials requires the use of fluorescing dyes which are very often poisonously. Using our technique it is possible to obtain a chemical landscape of the cells without damage and in a natural environment. In first measurements we use an infrared diode laser (λ = 1,53 – 1,57 μm) to measure the water absorption in rat hepatocytes. With changing osmolarity of the medium we can see a swelling or shrinking of the cells.

P8 – Colloidal Force Microscopy as a Tool for Controlling Layer-by-Layer assembly on Colloidal Particles

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Atomic Force microscope (AFM) is commonly used for imaging a sample surface by positioning a force sensor very close to the sample surface and detecting for variations on the tip as the sample is raster-scanned. However, with the advent of Colloidal Probe Microscope interaction forces between surfaces can be investigated.
In this technique a colloidal particle of known radius and composition is anchored at the end of an AFM cantilever and this well characterised particle is then used as the force probe.
Polyelectrolyte multilayer coating is a novel means to engineer the surface of colloidal particles. A variety of functionalities can be built-in into the multilayer. Such particles meet large interest for diagnostic purposes, as carriers, storage containers or microreaction vessels. A rather important practical problem associated with the fabrication of these coated particles is the control of aggregation during the multi-step process of layer-by-layer deposition. Depending on the nature of the top layer and the conditions of the environment aggregation may occur thus large decreasing the efficiency of the process. To control and to keep the stability of the colloidal dispersion a quantitative understanding of the interparticle interactions is required. To this aim colloidal force microscopy was applied. The aggregation behaviour was studied in parallel by means of fluorescence based flow cytometry (FACS).
Colloidal particles were successfully coated with multilayers of biocompatible polyelectrolytes. Protamine sulfate, Bovin Serum Albumin and dextran sulfate are alternated to form multilayers with various layer numbers. Lipid membranes are assembled onto polyelectrolyte-coated colloidal particles by vesicle adhesion and subsequent spreading.
The layer formation is evidenced by Zeta Potential measurements and flow cytomtery.
The lipid and polyelectrolyte coated particles are anchored onto AFM cantilevers and subsequently used as force sensors. Symmetric (same polyelectrolyte-lipid layer combination on cantilever and substrate) and asymmetric (different polyelectrolyte-lipid layer combination on cantilever and substrate) situations were studied.
Colloidal probe microscopy allowed to quantify adhesion forces as a function of the nature of the top layers and the electrolyte conditions. It was shown that in most cases, although the particles showed repulsion upon approach, a remarkable polyelectrolyte-based adhesion was observed after retraction, once the particle and the substrate layer have been brought into close contact. The adhesion tendency increased with ionic strength. FACS measurements showed a good correlation between the degree of flocculation and the interaction force between the particle and the flat surface. Once the critical steps of the process had been identified it became possible to design optimal conditions for coating.
When the top layers were composed of a lipid bilayer, repulsion upon approach was common. However, with increasing pressure possible fusion events have been detected. They were then followed by adhesion upon retraction. It has also been shown that polyelectrolyte molecules protrude the bilayer with increasing interaction pressure, leading to molecular contacts between the two multilayer systems.

P9 – Applications of Atomic Force Microscopy in Pharmaceutical Research

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Scanning force microscopy (SFM), which is also known as atomic force microscopy (AFM) was developed by Binnig and co-workers in 1986. AFM allows the visualization of conducting as well as non-conducting samples. High lateral and vertical resolution can be achieved in vacuum, in air and even on liquid covered surfaces. In addition, it is not only possible to analyze the topography of a sample with this microscopic approach, but also to investigate other physical properties including frictional forces, softness and viscoelasticity, molecular forces between single ligand/receptor pairs and the charge density on a nanometer scale. All these applications have made this instrument a very useful tool in the pharmaceutical field.

Especially, the ability to operate in liquids opens up the possibility of studying biological processes in their native forms such as aqueous environments in nature. Examples are monitoring dynamics and enzyme degradation of DNA and the measurement of the adhesion forces between ligand receptor pairs. Large biological objects such as whole animal cells as well as smaller structures such as chromosomes, membranes, proteins and nucleic acids have been constantly imaged.

In the presented work, we will give some examples of the applications of this technique on implant materials, lipid-vesicles, nanoparticles, gene delivery systems, classical tablets and high-resolution imaging of red blood cell surfaces.

P10 – Assembly of G-quartet based DNA Superstructures (G-wire) with Gold Nanoparticles

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G-wires are DNA superstructures based on the intermolecular interactions of four Guanine bases. They allow the fabrication of structures reaching the micrometer scale using only short DNA oligonucleotides.

Several sequences were tested for their ability to assemble G-wire structures, and the assembling conditions were optimized regarding buffer composition temperature and time. A labeling of G-wires by gold nanoparticles makes them potentially interesting as building blocks for molecular nanotechnology.

The nanoparticles do not disturb the assembly process, and were detected on the G-wires. For analysis the G-wires were transferred on mica and imaged by SFM.

P11 – From 2D to 3D Molecule Tracking

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2D Molecule Tracking focusses on the spatially and temporally resolved detection of biomolecules in solutions on the nanometer scale. With the help of fluorescence microscopy and fast parallel photon processing units (FPGA-technologies) it is possible to observe the trajectories of small DNA molecules. The 2D observation of single particles and molecules can be an important tool for understanding biochemical processes in cells. We present a concept of a spatially resolving fluorescence detector with the ability to trace single molecules in realtime.

Our Molecule Tracker consists of an open microscope, an image intensifier built by LaVision (Göttingen) and a 2D strip detector based on an adapted nuclear physics device from the Physical Institute of the University of Bonn and configurable parallel hardware. Present research is done aiming at the extension of the 2D detector into a 3D Molecule Tracker. The rigid sample holder is replaced by a piezo table which is movable in XY-direction with nanometer precision. The vertical axis can be adjusted by a Z-piezo attached to the microscope objective. The 3 axis piezo controller will be working with newly developed software based on the parallel image data from the detector. Furthermore the system will be combined with devices for fluorescence lifetime measurements and a spectrometer. This will enable the system to determine multiple physical properties of different fluorophores, such as concentration, diffusion constant, fluorescence lifetime and spectral dispersion *in situ* and *in vivo.* In combination with the high processing speed of the data the Molecule Tracker will be able to measure these quantities in realtime and can therefore be called a *multiparameter system*. With the support of the 3-dimensional piezo control unit an observation of cell compartments by scanning of the sample can be realized as well as the characterization of dynamic processes with a special emphasis on *protein dynamics*. Possible scientific applications include *Live Cell Imaging, FCS, single molecule tracking,* etc.

The enhancement of the apparatus by combining it with *AFM* is considered and is worth to be discussed.

P12 – Scanning Force Microscopy based Lithography Techniques

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The center of our interest is the characterisation and physical/chemical modification of polymer surfaces.

During the development of the Dynamic Plowing Lithography - DPL - several interesting phenomena appeared, opening possible application in life-science. This includes the lithographic preparation of electrically conductive surfaces as well as of piezoactive zones.

Several experiments, as the cutting of a human chromosome and the definition of local hydrophilicity, are diskussed in terms of manipulation, sensor technology and template techniques for life science applications.

P13 – A Novel Total Internal Reflection Fluorescence - Atomic Force Microscopy Combination to Examine Adhesion Properties of Cells under Mechanical Forces Scanning Force Microscopy based Lithography Techniques

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We are working on a combined Total Internal Reflection Fluorescence Microscopy (TIRFM) - Atomic Force Microscopy (AFM) instrument. The setup allows us to scan or apply force to our samples (mostly cells) from the top with the AFM cantilever while examining the responses of the samples with fluorescent markers or media in the evanescent field generated by TIRFM.

At the moment we are interested in the adhesion behavior of fibroblast cells under mechanical loads. Our goal is to examine the relation between mechanical stimuli and biochemical responses in cells. Specifically, we monitor the changes in the adhesion pattern at the interface between the cell and the substrate. Then we apply a mechanical load (or shear force) to the cell using the AFM and observe how the adhesion pattern, including the individual adhesion clusters between cell and substrate, are changed.

These results are analyzed in a modelling scheme which seeks to relate the geometrical cluster distribution in adhesion patterns with the underlying biological processes of the cell.

P14 – Determining Water Structure at Biomolecular Interfaces

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AFM has been used to measure oscillatory forces on approaching a hydrophilic self-assembled monolayer (SAM) of ($COOH(CH_2)_{10}$-SH (Jarvis et al. 2000). The oscillatory forces had a periodicity corresponding to the diameter of a single water molecule and were attributed to the detection of structured water layers in the region under the AFM probe. AFM force measurements were taken using carbon nanotubes as the probe in order to remove the unwanted hydrodynamic damping effect caused by the bulk of the standard tip (O'Shea and Welland 1998). High sensitivity in the force curves was achieved using a magnetic activation technique operated in the resonance-tracking oscillator mode (Dürig et al. 1997). Importantly, by using a constant-dissipation, AFM imaging method (Jarvis et al. 1999), the SAM was imaged on the nanoscale within minutes of performing the oscillatory forces measurements. This allowed the measured forces to be related to the nanoscale surface features of the SAM. Further investigations are underway to see if oscillatory forces corresponding to water structure are present at the surface of biological molecules. In this presentation, we reveal biological molecules that have been surveyed for the presence of oscillatory forces, including membrane proteins and lipid bilayers. We eventually aim to understand the effects of various physiological conditions on water structure (e.g. salt concentration, temperature), the relevant length scales over which hydration forces interact and the influence of water structure on biological process, such as ligand-receptor interactions.

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