Linz Winter School 2016

Date: Tue, Jan 26-2016 until Thu, Jan 28-2016
Location: JKU Life Science Center Upper Austria, Institute for Biophysics
Gruberstraße 40, 4020 Linz, Austria

Program

Tuesday, Jan 26: Morning Talk Session

(Chair: Peter Hinterdorfer)
Institute for Biophysics, Gruberstraße 40, Seminar-Room, Basement

08.30	Meeting point at Julius Raab Heim, Lobby, Julius Raab Straße 10, 4040 Linz and transfer to Gruberstraße by tram/bus
09.15 – 09.30	Registration
09.30 – 09.45	Welcome & Introduction
09.45 – 10.15	*Molecular Recognition Force Microscopy/Spectroscopy* Peter Hinterdorfer
10.15 – 10.45	*High-Speed AFM* Johannes Preiner
10.45 – 11.15	**Coffee Break**
11.15 – 11.45	*Functionalization of AFM tips with native proteins or with His6-tagged fusion proteins* Hermann J. Gruber
11.45 – 12.15	*Force Spectroscopy Experiments and Analysis* Andreas Karner
12.15 – 12.35	*The Physics of TREC imaging* Sandra Posch
12.35 – 12.50	*AFM Functional Imaging on Melanoma Cells* Lilia A. Chtcheglova
12.50 – 14.00	**Lunch Break** **Mensa "OÖ Gebietskrankenkasse"**

Tuesday, Jan 26: Afternoon Talk Session

(Chair: Gerald Kada)
Institute for Biophysics, Gruberstraße 40, Seminar-Room, Basement

14.00 – 14.30	*Introduction round of attendees*
14.30 – 14.50	*Application of combined AFM and fluorescence microscopy: Localization of cellular membrane receptors and stimulation of T cells* Rong Zhu
14.50 – 15.10	*Characterization of bacterial surfaces by scanning probe microscopy* Yoo Jin Oh
15.10 – 15.40	*Introduction to AFM with Keysight Technologies* Gerald Kada
15.40 – 16.10	**Coffee Break**
16.10 – 16.25	*Scanning Microwave Microscopy: nanoscale complex impedance imaging, modeling, and biological applications* Enrico Brinciotti
16.25 – 16.55	*Single Molecule Biology - Studying Movements and Meetings within the Plasma Membrane* Gerhard Schütz
16.55 – 17.25	*STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation* Martin Muik
17.25	*Arrangement of 6 groups for the demo session on Wednesday and of 4 groups for the CBL demo session on Thursday*

<u>Wednesday, Jan 27</u>: Demo Session

Institute for Biophysics, University of Linz, Gruberstraße 40: 4th and 1st floor

09.15 *Meeting point, Institute for Biophysics, Gruberstraße 40, 4th floor, Seminar Room (449)*

09.30 – 13.00 and 14.30 – 16.30 *Demos*

Attendees will be divided into groups of 3-4 participants and switched to the next demo every 30 min.

Lunch break from 13.00 – 14.30 at Mensa "OÖ Gebietskrankenkasse".
Coffee break from 11.00 – 11.30 and 15.30 – 16.00 in the Seminar-Room 449, 4th floor.

DEMO-1: Self Sensing (Room 415)
(Michael Leitner/ Boris Buchroithner)

DEMO-2: Single Molecule Force Spectroscopy (Room 413)
(Sandra Posch/ Anny Fis)

DEMO-3: Topography and Recognition Imaging (TREC) (Room 417)
(Rong Zhu/ Melanie Köhler)

DEMO-4: High Speed AFM (Room 403)
(Andreas Karner/ Jürgen Strasser)

DEMO-5: Bacterial Imaging (Room 416)
(Yoo Jin Oh/ Silviu Sorin Tuca)

DEMO-6: High frequency imaging of nanoscale structures (Room 405)
(Giulio Campagnaro/ Giorgio Badino)

DEMO-7: Combined Optical and Force Microscope (Room 406)
(Lilia Chtcheglova/ Constanze Lamprecht)

DEMO-8: Association kinetics between receptor and ligands using Quartz Crystal Microbalance (QCM) (Room 415)
(Lukas Traxler / Joan Ahiable)

DEMO-9: Patch Clamp and FRET Microscopy (1st floor Room 101 and 102)
(Martin Muik/ Rainer Schindl)

19.00 **Social Event**
Meeting point "Weinstadl"
Tram station: St. Magdalena (tram number 1 or 2)
Griesmayrstraße 18, 4040 Linz

Thursday, Jan 28: Hands-on Session and CBL Demo Session

Center for Advanced Bioanalysis (CBL), Gruberstraße 40: 3rd floor
Institute for Biophysics, University of Linz, Gruberstraße 40: 4th floor

09.30 – 13.00 and **Hands-on Session: attendees´ samples**
14.00 – 17.00 Attendees´ samples investigation and practical AFM training according to time schedule displayed in the Seminar-Room 449, 4th floor.

10.00 – 11.00 and **CBL Demo Session** (in parallel to the hands-on session) according to
11.30 – 12.30 the time schedule below.

Lunch break from 12.45 – 14.00 at Mensa "OÖ Gebietskrankenkasse".
Coffee break from 11.00 – 11.30 and 15.30 – 16.00 in the Seminar-Room 449, 4th floor.

CBL Time Schedule (4 groups)

10.00 Meeting point CBL, 3rd floor **First and second group**
10.00 – 11.00 Introduction and Demos:

- Single molecule fluorescence microscopy
  (Jan Hesse)
- Rapid prototyping of microfluidic chips using thiol-ene-based soft lithography
  (*Thomas Haselgrübler and Roland Hager*)

11.30 Meeting point CBL, 3rd floor **Third and forth group**
11.30 - 12.30 Introduction and Demos:

- Single molecule fluorescence microscopy
  (Jan Hesse)
- Rapid prototyping of microfluidic chips using thiol-ene-based soft lithography
  (*Thomas Haselgrübler and Roland Hager*)

Molecular Recognition Force Microscopy/Spectroscopy

Peter Hinterdorfer

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In molecular recognition force microscopy (MRFM), ligands are covalently attached to atomic force microscopy tips for the molecular recognition of their cognitive receptors on probe surfaces. A ligand-containing tip is approached towards the receptors on the probe surface, which possibly leads to formation of a receptor-ligand bond. The tip is subsequently retracted until the bond breaks at a certain force (unbinding force). In force spectroscopy (FS), the dynamics of the experiment is varied, which reveals a logarithmic dependence of the unbinding force from the loading rate. These studies give insight into the molecular dynamics of the receptor-ligand recognition process and yield information about the binding pocket, binding energy barriers, and kinetic reaction rates. Applications on isolated proteins, native membranes, viruses, and cells will be presented. We have also developed a method for the localization of specific binding sites and epitopes with nm positional accuracy. A magnetically driven AFM tip containing a ligand covalently bound via a tether molecule is oscillated at a few nm amplitude while scanning along the surface. In this way, topography and recognition images are obtained simultaneously.

High-speed AFM

Johannes Preiner

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The function of proteins is determined by their structure and is generated when changing it dynamically. These nano-mechanical dynamics cannot be captured by available techniques such as X-ray crystallography, NMR spectroscopy, and electron or optical microscopy, since they are either ensemble techniques, carried out under non-physiological (vacuum) conditions, or do not have the proper spatial resolution. With commercially available AFMs, only very slow processes could be recorded, since it requires minutes to form an acceptable image at high spatial resolution, far beyond the timescale at which biological processes occur. Recent developments on tapping mode AFM, conducted in the lab of T. Ando (reviewed in (1, 2)) have led to an AFM capable of recording images of 100x100 pixel^2 within 40 ms at sub-nanometer resolution in aqueous solutions. In order to achieve this high scan rate, various parts involved in an AFM such as cantilevers, scanners, and electronic devices had to be modified to obtain efficiently small response times of every single component. In this way it is possible to generate movies consisting of many successive images (40 ms-intervals) of biological specimens and their dynamics(3, 4, 5).

Besides the technical aspects of HS-AFM, this lecture will give an overview on HS-AFM experiments conducted in our lab, especially on the interaction of antibodies with antigenic epitopes on bacterial and viral surfaces (6). Antibodies, also named Immunoglobulins are key for the immune system. Via their Fab arms IgGs can bind two neighboring epitopes resulting in higher avidity and slower dissociation as compared to monovalent Fabs. We demonstrate that IgG molecules do not remain stationary on surfaces of regularly spaced epitopes but exhibit “bipedal” random walking. Their mobility depends on symmetry and spacing of the antigens; monovalent Fabs do not move. We identified steric strain as the main reason for short-lived bivalent binding. On collision, the randomly walking antibodies form transient clusters. Such aggregates might serve as docking sites for the complement system and/or phagocytes.

[1] Ando T, *et al.* (2007) High-speed atomic force microscopy for observing dynamic biomolecular processes. *J Mol Recognit.*

[2] Ando T, Uchihashi T, & Fukuma T (2008) High-speed atomic force microscopy for nano visualization of dynamic biomolecular processes. *Progress in Surface Science* 83(7-9):337-437.

[3] Kodera N, Yamamoto D, Ishikawa R, & Ando T (2010) Video imaging of walking myosin V by high-speed atomic force microscopy. *Nature* 468(7320):72-76.

[4] Uchihashi T, Iino R, Ando T, & Noji H (2011) High-speed atomic force microscopy reveals rotary catalysis of rotorless F1-ATPase. *Science* 333(6043):755.

[5] Shibata M, Yamashita H, Uchihashi T, Kandori H, & Ando T (2010) High-speed atomic force microscopy shows dynamic molecular processes in photoactivated bacteriorhodopsin. *Nat Nano* 5(3):208-212.

[6] Preiner J, *et al.* (2013) Antibody movement on regular antigen clusters: Fab arms are made for walking. *submitted*.

Functionalization of AFM tips with native proteins or with His_6-tagged fusion proteins

Rong Zhu[a], Jürgen Strasser[a], Elena Pohl[b], Robert Tampé[c], Andreas Ebner[a], Peter Hinterdorfer[a], and Hermann J. Gruber[a *]

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Flexible attachment of a probe molecule (e.g. an antibody) to the AFM tip converts the tip into a biospecific sensor by which can localize and identify cognate target molecules on the sample surface when using dynamic force microscopy modes. Moreover, the biophysical details of the interaction between a tip-bound ligand molecule and complementary receptor molecules on the sample surface can be studied by force spectroscopy. Best results were obtained if the probe molecules were tethered to the apex of the AFM tip via a single polyethylene glycol chain (PEG) with a maximal length of 6-10 nm. The standard procedure requires four steps: (i) generation of NH_2 groups on the tip surface, (ii) reaction of the NH_2 groups with one end of a PEG chain, (iii) generation of SH groups on the protein, and (iv) reaction of the SH group with the free-tangling end of the PEG chain.[1]

Step (iii) is inconvenient and it requires considerable amounts of protein. Therefore, a new heterobifunctional linker (aldehyde-PEG-NHS) was devised which allowed coupling native proteins via their endogenous NH_2 groups (antibodies have 80-90 NH_2 groups per molecule[2]). As a consequence, minute amounts of protein are sufficient for tip functionalization by this method.[2] It can also be used for attachment of small molecules, as exemplified by a commercial NH_2 derivative of ATP.[3] In spite of this significant improvement, the new linker aldehyde-PEG-NHS has adverse aspects: (1) It must be used at high concentration in order to suppress crosslinking of adjacent NH_2 groups on the tip surface by the two ends of the linker, and (2) its synthesis is inconvenient and has low yield. Meanwhile, all problems are solved with a new linker (acetal-PEG-NHS) which is easy to use for tip functionalization with unmodified (native) proteins.[4]

Due to the abundance of NH_2 groups on proteins, the above method is widely applicable, the only disadvantage being the random attachment site of the linker on the protein. As an alternative, antibodies or other glycoproteins can be linked to hydrazide-functionalized AFM tips via their small carbohydrate groups after periodate treatment. Another option for site-directed linkage is oriented attachment of His_6-tagged proteins to AFM tips. The aminosilanized tip is reacted with maleimide-PEG-NHS, subsequently tris-NTA is attached to the free-tangling end of the PEG chain on the AFM tip. In the presence of 200 µM Ni^{2+}, the His_6 tag is stably bound to tris-NTA via three Ni^{2+} ions on the time scale of a day, even in presence of EDTA, providing for the easiest method of AFM tip functionalization with engineered proteins. It has extensively been used to study the interaction of nuclear shuttle proteins by force spectroscopy.

1. Kamruzzahan et al. (2006) *Bioconjugate Chem. 17*, 1473-1481.
2. Dorner et al. (1967) *J. Exp. Med. 125*, 823-831.
3. Ebner et al. (2007) *Bioconjugate Chem. 18*, 1176-1184.
4. Wildling et al. (2011) *Bioconjugate Chem. 22*, 1239-1248.
5. Rangl et al. (2013) *Angew. Chem. 52*, 10356-10359.

Force spectroscopy experiments and analysis

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In force spectroscopy experiments, the binding of ligands immobilized on AFM tips toward surface bound receptors (or vice versa) is studied by applying force to the receptor-ligand complex until the bond breaks at a measurable rupture force[1]. The interaction forces of such ligand-receptor pairs are measured in force-distance cycles using a ligand carrying tip and a target surface with firmly attached receptor molecules. As a typical example, a force distance cycle of tip bound human rhinovirus serotype 2 and its binding partner a very-low density lipoprotein receptor fragment is shown in Figure 1. At a fixed lateral position, the tip vertically approaches the surface and is subsequently retracted. During this cycle, the cantilever deflection which is direct proportional to the force is continuously measured and plotted versus piezo movement. The characteristic nonlinear force–distance profile is determined by the elastic properties of the flexible tether, whereas the strength of the interaction (termed "rupture force") is governed by the type of receptor-ligand pair. The specificity of ligand-receptor binding is usually demonstrated by blocking experiments with free ligands injected into the solution to block the receptor sites on the surface.

When ligand-receptor binding is viewed on the single-molecule level, the average bond lifetime at zero force is given by the inverse of the kinetic off-rate constant. Therefore, ligands will dissociate from receptors even without any force applied to the bond, driven by thermal impulses. This stochastic nature results in a distribution of rupture forces (Figure 2). If molecules are pulled apart very fast, the bond will resist and require measurable force for detachment. In the thermal activation model, the dissociation rate of a complex in solution is described by an Arrhenius dependency on the barrier height. A force acting on a binding complex deforms the interaction energy landscape and lowers the activation energy barrier. This description together with the stochastic description of the unbinding process predicts the rupture force distribution. The maximum of each force distribution (termed "unbinding force") scales linearly with the logarithm of the loading rate[2] (Figure 3), i.e. the effective force increase, which can be deduced as df/dt at rupture, being equal to the product of pulling velocity and effective spring constant. In force spectroscopy experiments, the variation in the pulling speed applied to specific ligand-receptor bonds will lead to detailed structural and kinetic information of the interaction. Length scales of energy barriers are obtained from the slope of the spectroscopy plot (i.e. force versus loading rate) and extrapolation to zero forces yields kinetic off-rate for the dissociation of the complex in solution.

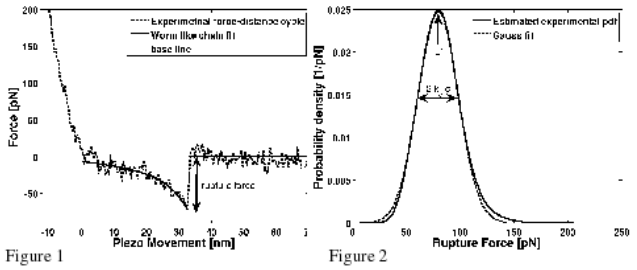

Figure 1

Figure 2

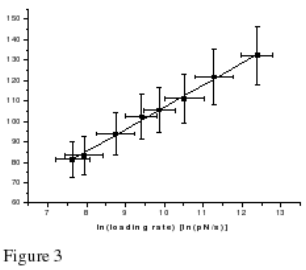
Figure 3

[1] C. Rankl, F. Kienberger, L. Wildling, J. Wruss, H. Gruber, D. Blaas, P. Hinterdorfer. Multiple receptors involved in human rhinovirus attachment to live cells. PNAS 105 (2008), 17778-83.
[2] J. Preiner, H. Janovjak, H. Knaus, D. Cisneros, A. Kedrov, F. Kienberger, D. Mueller*, P. Hinterdorfer. Insertion and folding free energy of membrane proteins derived from single-molecule force measurements. Biophysical Journal 93 (2007), 930-7.

The Physics of TREC imaging

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Simultaneous topography and recognition imaging (TREC) allows the investigation of receptor distributions on natural biological surfaces under physiological conditions[1]. Based AFM in combination with a cantilever tip carrying a ligand molecule, it enables to sense topography and recognition of receptor molecules simultaneously with nanometer accuracy [2-10]. Here, we discuss optimized handling conditions and guide through physical properties of the cantilever-tip-sample ensemble [9], which is essential for the interpretation of the experimental data gained from this technique. In contrast to conventional AFM methods TREC is based on a more sophisticated feedback loop, which enables to discriminate topographical contributions from recognition events in the AFM cantilever motion. The features of this feedback loop were investigated through a detailed analysis of topography and recognition data obtained on a model protein system. Single avidin molecules immobilized on a mica substrate were imaged with an AFM tip functionalized with a biotinylated IgG. A simple procedure for adjusting the optimal amplitude for TREC imaging is described by exploiting the sharp localization of the TREC signal within a small range of oscillation amplitudes. This procedure can also be used for proving the specificity of the detected receptor-ligand interactions. For understanding and eliminating topographical crosstalk in the recognition images we developed a simple theoretical model, which nicely explains its origin and its dependence on the excitation frequency.

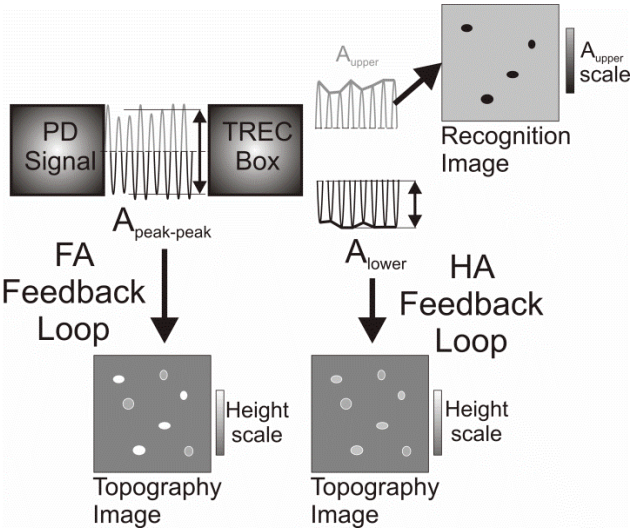

Figure 1: The principle of TREC imaging and the two possible feedback mechanisms. In case of molecular recognition between the ligand coupled to the AFM cantilever tip and a receptor on the sample, the cantilevers oscillation signal, coming from the photodiode (PD) contains information about the samples topography (at the lower part of the oscillation, black), and information about the recognition process (at the upper part of the oscillations, grey). The recognition image is constructed from the envelope of the upper part (A_{upper}) of the oscillation, and recognition spots on the sample are usually displayed as dark spots. Depending on the used feedback parameter, i.e. $A_{peak-peak}$ or A_{lower}, topographical features recognized by the ligand on the tip, exhibit their true height (HA feedback loop) or an increased height (FA feedback loop), since in the latter case the feedback tries to compensate for the additional amplitude reduction in the top peaks of the oscillations due to the stretching of the linker molecule.

[1] P. Hinterdorfer, and Y. F. Dufrene, Nat Methods **3** (2006).
[2] A. Ebner *et al.*, Chemphyschem **6** (2005).
[3] C. M. Stroh *et al.*, Biophys J **87** (2004).
[4] C. Stroh *et al.*, Proc Natl Acad Sci U S A **101** (2004).
[5] J. Tang *et al.*, Nano Letters **8** (2008).
[6] A. Ebner *et al.*, Nanotechnology **19** (2008).
[7] L. Chtcheglova *et al.*, Biophys J (2007).
[8] L. Chtcheglova *et al.*, Pflügers Archiv European Journal of Physiology (2008).
[9] J. Preiner *et al.*, Nanotechnology **20** (2009).
[10] J. Preiner *et al.*, Nano Letters **9** (2009).

AFM Functional Imaging on Melanoma Cells

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One of challenging tasks in molecular cell biology is to identify and localize specific binding sites on biological samples with high spatial accuracy. This routinely can be achieved by immunofluorescence techniques, however, the lateral resolution is limited by ~ 200 nm and no information about cell topography can be obtained. TREC technique permits to record AFM topography images and simultaneously localize specific binding sites with a lateral accuracy of several nm, as shown on isolated protein systems such as avidin-biotin, chromatin, and isolated RBC membranes. Contrarily to these systems, a cell surface represent a more complex composition, organization, and processing in space and time.

In the present study TREC technique was applied to B16 mouse melanoma cells in order to visualize the stress inducible heat shock protein 70 (Hsp70). Beside its normal cytosolic localization, Hsp70 is also known to be present at the extracellular leaflet of the plasma membrane of most tumor but not corresponding healthy cells. Apart from an immunostimulatory effect, a function of Hsp70 localized at the tumour cellular surface remains elusive.

Application of combined AFM and fluorescence microscopy: Localization of cellular membrane receptors and stimulation of T cells

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Combined atomic force microscopy (AFM) and fluorescence microscopy has become a powerful tool for the investigations in the micro- and nano-world, especially for studies in cellular biology. Localizing cellular membrane receptors using conventional fluorescence microscopy is fast and simple, yet the resolution is limited to ~300 nm due to the Rayleigh criterion. This limitation can be overcome by using simultaneous topography and recognition imaging (TREC), an AFM based technique to localize cellular membrane receptors with ~10 nm resolution. Here we used this technique to determine the distribution of a GPI anchor protein derived from the decay acceleration factor fused to GFP, which was reported to be a highly effective lipid raft marker. The fluorescence and the recognition images revealed micro-domains of the GPI-GFP molecules at the same location on the T24 cell membrane, showing the capability of both techniques for specific detection. The recognition image further revealed nano-domains of GPI-GFP, which is beyond the resolution of conventional fluorescence microscopy. Using the same technique, we also measured the distribution of CD4-YFP molecules on the T24 cell membrane, which revealed interesting nano-domains. Furthermore, fluorescence guided force spectroscopy showed reasonable correlation between the binding probability and the expression level of CD4-YFP on the cell membrane. At last, we used the combined setup for the study of the activation of the T cell (Jurkat cell was used here) by anti-CD3 antibody functionalized cantilever tips via monitoring the calcium concentration in the cell with the fluorescence indicator Fura-2. From the experiments, we found that the Jurkat cells are much more active in HBSS with Ca^{++} & Mg^{++} and with 10% FCS at 37°C than in PBS at room temperature. The cantilever tip functionalized with non-specific goat IgG did not activate the Jurkat cell, in contrast to tips containing anti-CD3 antibody. Force-distance curve measurements at different force loading rates allowed the calculation of the energy landscape of the interaction between the anti-CD3 antibody tip and the Jurkat cell. Recognition imaging provided the information of the distribution of CD3 molecules on the Jurkat cell.

Characterization of bacterial surfaces by scanning probe microscopy

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The bacterial cell wall plays a significant role in maintaining cellular structure and resisting turgor pressure. It changes during growth and division and also opens a pathway to transfer information from the outer environment into the cell, suggesting that the cell wall is dynamic and its mechanical properties are of significant importance. Biological scanning probe microscopy (BIO-SPM) is the tool of choice for detailed microbial studies because it allows for studying living microbial organisms in their natural environment at the nano-scale. As the microbial outer membrane interacts with the extra-cellular environment or other surfaces directly, characterizing its membrane structures and binding capacities provides crucial information for understanding fundamental processes such as bacterial adhesion, surface recognition, and initial attachment to abiotic or biotic surfaces. Bio-SPM is also capable of measuring the cell wall stiffness. Analyses of AFM force-indentation curves yield physical properties of the cellular surface such as Young's modulus, internal turgor pressure, and the stretching modulus of the bacteria [1]. In addition, several sample preparation methods and experimental details for high-resolution bacterial AFM imaging [2] will be given.

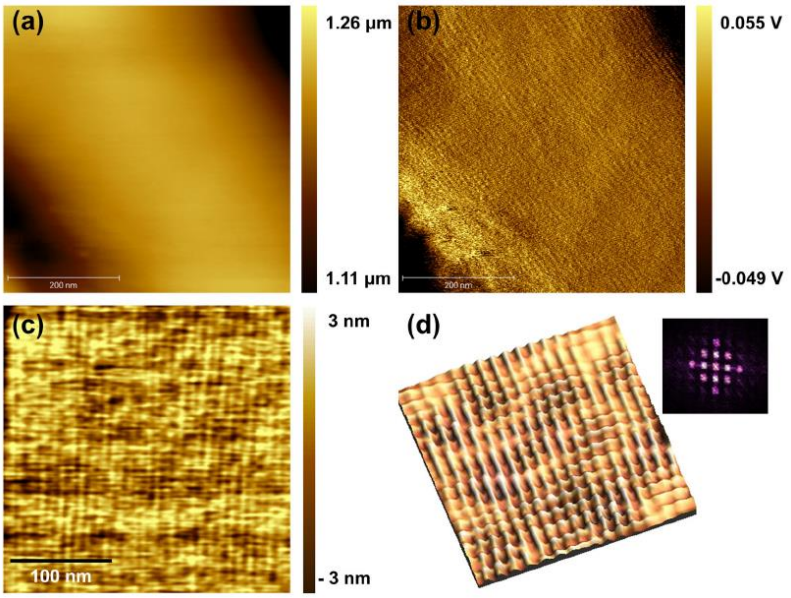

Fig.1. AFM images of *T. forsythia* wild-type bacteria: (a) Topography, (b) amplitude image, (c) magnified topographic image, and (d) reconverted FFT 3D image of topography

[1] Y. J. Oh, Y. Cui, H. Kim, Y. Li, P. Hinterdorfer, and S. Park, Biophysical Journal 103, 1666 (2012)
[2] Y. J. Oh, G. Sekot, M. Duman, L. Chtcheglova, P. Messner, H. Peterlik, C. Schäffer, and P. Hinterdorfer, J. Mol. Recognit. 26, 542 (2013)

Introduction to AFM with Keysight Technologies

Dr. Gerald Kada

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Webpage: www.keysight.com/find/nano

The unique combination of characterization and modification of molecules on solid surfaces with high resolution (nanometer and sub-nanometer scale) in all environments (air, gases, or solution) makes Atomic Force Microscopy (AFM) the technique of choice for nanotechnology measurements in biology, material science and polymer applications.
The lecture will provide an introduction to AFM measurement techniques, different operating modes and options for system setup, focusing on biological and single molecule studies.
Examples will demonstrate IMAGING of biological species ranging from cells to small molecules, PROBING single molecule properties like recognition or protein unfolding, and COMBINATION with other complementary techniques like fluorescence microscopy or nearfield microwaves.

1. Fast scanning on soft samples, up to seconds per frame imaging.

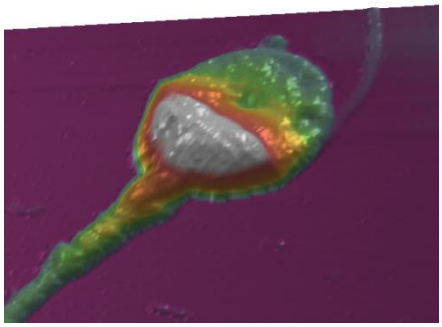
2. Topography and recognition: We will also present recent advances in the unique AFM technology called TREC, a technique that enables measuring real-time, simultaneous Topography and RECognition in a single scan.

3. Single Molecule Force Spectroscopy: from intermolecular forces to protein unfolding

4. AFM in combination with inverted light microscopy for simultaneously gathering of light, fluorescence, and probe data of living cells.

5. Scanning *nearfield* Microwave Microscopy (SMM) and its potential in biology

9500 Quick Scan AFM



Sperm Cell

Scanning Microwave Microscopy: nanoscale complex impedance imaging, modeling, and biological applications

Enrico Brinciotti

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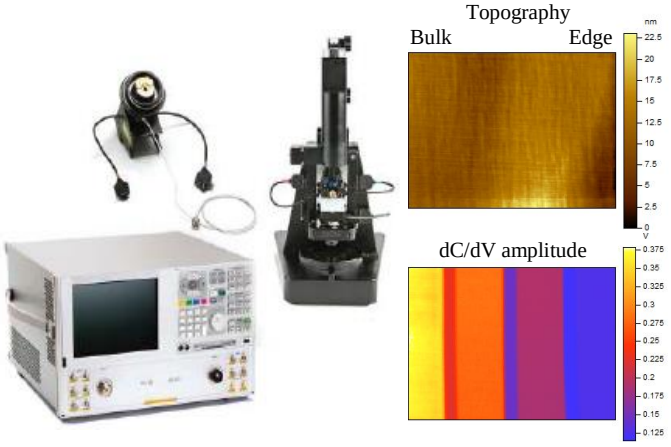
Scanning Microwave Microscopy (SMM) is a recent development in nanoscale imaging technique that combines the lateral resolution of Atomic Force Microscopy (AFM) with the high measurement precision of microwave analysis at GHz frequencies. It consists of an AFM interfaced with a Vector Network Analyzer (VNA). SMM allows measuring complex materials properties for nano-electronics, materials science, and life science applications with operating frequencies ranging between 1 MHz and 20 GHz. Here we present the basic working principles of SMM and its advanced applications. In particular, the capabilities of the SMM include: calibrated capacitance and resistance measurements with a noise level of 1 aF [1]; a 2D mapping workflow to acquire roughly 20.000 C-V curves during one image [3]; calibrated complex impedance imaging of semiconductor [2], dielectric [6, 7], and biological [8] samples; point wise C-V (capacitance-voltage) spectroscopy curves allowing for oxide quality characterization, interface traps, and memory effects of novel materials. Recently, calibrated complex impedance images of cells and bacteria have been obtained with the SMM [8]. Experimental investigations are complemented by finite element radio-frequency modelling using the 3D architecture of the probe and the sample, done with the Keysight software EMPro [4, 5, 9].



Left panel:
SMM experimental setup. The AFM is interfaced with a Vector Network Analyzer (VNA), probing the electromagnetic properties of the sample under test.

Right panel:
Topography and dopant density (dC/dV) image of a semiconductor dopant sample with different dopant concentrations for quantitative and calibrated measurements.

References:
[1] H. P. Huber et al, Calibrated nanoscale capacitance measurements using a scanning microwave microscope, Rev. Sci. Instrum. 81, 113701 (2010); [2] H. P. Huber et al., Calibrated nanoscale dopant profiling using a scanning microwave microscope, J. Appl. Phys. 111, 014301 (2012); [3] M. Moertelmaier et al., Continuous capacitance-voltage spectroscopy mapping for scanning microwave microscopy, Ultramicroscopy, 136, 2013, 67-72. [4] M. Kasper et al., Electromagnetic Simulations at the Nanoscale: EMPro Modeling and Comparison to SMM Experiments. Agilent AppNote Aug. 2013. [5] Oladipo et al., Three-dimensional finite-element simulations of a scanning microwave microscope cantilever for imaging at the nanoscale. APL 103, 2013, 213106-1 to 4. [6] G. Gramse et al, Calibrated complex impedance and permittivity measurements with scanning microwave microscopy, Nanotechnology, 25, 145703 (2014). [7] G. Gramse and E. Brinciotti et al, Quantitative sub-surface and non-contact imaging by scanning microwave microscopy, (2014), submitted. [8] Tuca et al, Calibrated Complex Impedance of CHO Cells and E-coli Bacteria in Air Using Scanning Microwave Microscope, in preparation. [9] P. Fernandez-Medina et al., Transmission and reflection mode scanning microwave microscopy (SMM): experiments, calibration, and simulations, (2014), submitted.

Single Molecule Biology - Studying Movements and Meetings within the Plasma Membrane

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In the last decade, single molecule detection has refashioned light microscopy by opening up the nanocosm towards optical investigations [1]. The life sciences profited particularly from these new techniques, as cells contain complex and dynamic structures at the nanoscale which are difficult to study with alternative methods. In my lecture, I will show examples how to obtain insights into the organization of the cellular Nanocosm by single molecule experiments.

- We use single molecule switching microscopy (PALM, (d)STORM) to obtain localization maps at a resolution below the diffraction limit [2].
- Brightness analysis allows us to study stable or transient molecular associations *in vivo* [3,4]. By "thinning out clusters while conserving the stoichiometry of labeling" (TOCCSL) we can virtually dilute the probe directly in the cell, without affecting the fluorescence labeling of single clusters [5]. Essentially, an analysis region is created within the cell by photobleaching; this region is devoid of active probe. Brownian diffusion or other transport processes lead to reentry of active probe into the analysis region. At the onset of the recovery process, single spots can be resolved as well separated, diffraction-limited signals. Standard single molecule microscopy then allows for characterizing the spots in terms of their composition and mobility.
- With *in vivo* micropatterning of plasma membrane proteins we can measure molecular interactions [6]. Cells transfected with a fluorescent fusion protein ("prey") are grown on micropatterned surfaces functionalized with specific antibodies to the extracellular domain of a membrane protein ("bait"); the fluorescence copatterning is used as readout for the bait-prey interaction. We used the micropatterning approach in combination with single molecule tracking to quantify the influence of a glycosylphosphatidylinositol-anchored protein (GPI-AP) – a typical marker of liquid ordered phases – on its molecular environment directly in the live cell plasma membrane [7]. We found that the captured proteins merely acted as bulky obstacles to the diffusion of other membrane constituents, but did not influence their membrane environment over distances past their actual physical size.

[1] U. Kubitscheck, *fluorescence microscopy – from principles to biological applications* (Wiley-Blackwell Weinheim Germany, 2013).
[2] E. Klotzsch, A. Smorodchenko, L. Löfler, R. Moldzio, E. Parkinson, G. J. Schütz, and E. E. Pohl, Proc Natl Acad Sci U S A **112**, 130 (2015).
[3] M. Brameshuber *et al.*, J Biol Chem **285**, 41765 (2010).
[4] A. Anderluh, E. Klotzsch, A. W. Reismann, M. Brameshuber, O. Kudlacek, A. H. Newman, H. H. Sitte, and G. J. Schütz, J Biol Chem **289**, 4387 (2014).
[5] M. Moertelmaier, M. Brameshuber, M. Linimeier, G. J. Schütz, and H. Stockinger, Appl Phys Lett **87**, 263903 (2005).
[6] M. Schwarzenbacher *et al.*, Nat Methods **5**, 1053 (2008).
[7] E. Sevcsik, M. Brameshuber, M. Folser, J. Weghuber, A. Honigmann, and G. J. Schutz, Nat Commun **6**, 6969 (2015).

STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation

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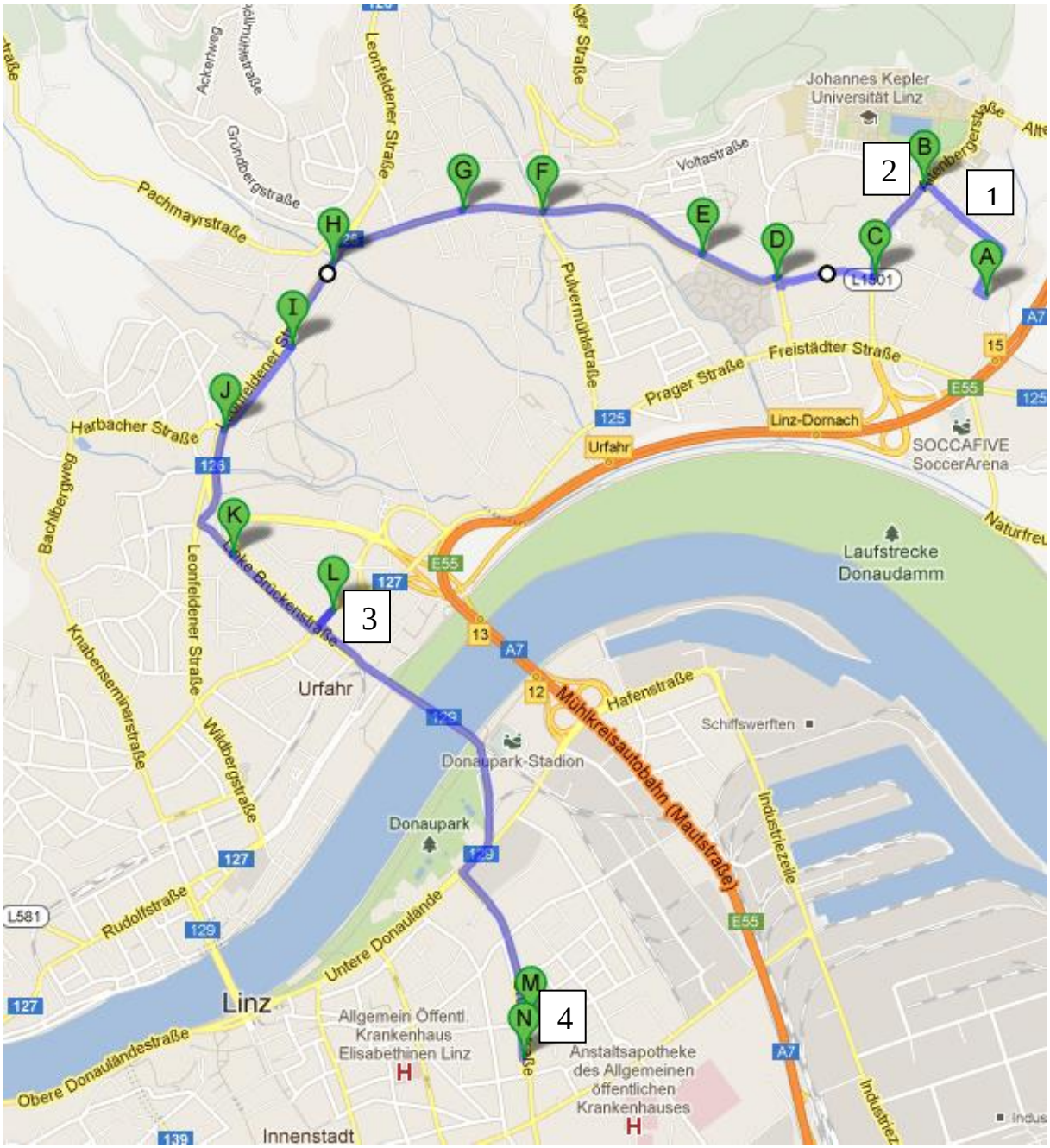
STIM1 and ORAI1 are key players of the Ca2+ release-activated Ca2+ (CRAC) current that plays an important role in T cell activation and mast cell degranulation. CRAC channel activation occurs via a physical interaction of ORAI1 with STIM1 when ER Ca2+ stores are depleted. Here we show, utilizing a novel STIM1-derived FRET sensor, that STIM1 when activating ORAI1 rearranges via a C-terminal, intramolecular transition into an extended conformation. This intramolecular rearrangement of STIM1 does not require a functional CRAC channel suggesting interaction with ORAI1 as sufficient for this conformational switch. Extended conformations could be also engineered by mutations within coiled-coil domains in the cytosolic portion of STIM1 revealing their involvement in the intramolecular transition. These mutants exhibit strongly facilitated interaction with ORAI1 inducing constitutive CRAC currents, even in the absence of store depletion. The STIM1-derived FRET sensor represents a widely applicable tool for correlating conformational rearrangements within STIM1 cytosolic portion to its interaction with ORAI1 and with other STIM1-associated proteins or drugs.

Suggested path from Julius **Raab Heim (Linz, Julius-Raab-Straße 10) to the Institute for Biophysics and CBL, Gruberstraße 40, 4020 Linz.**



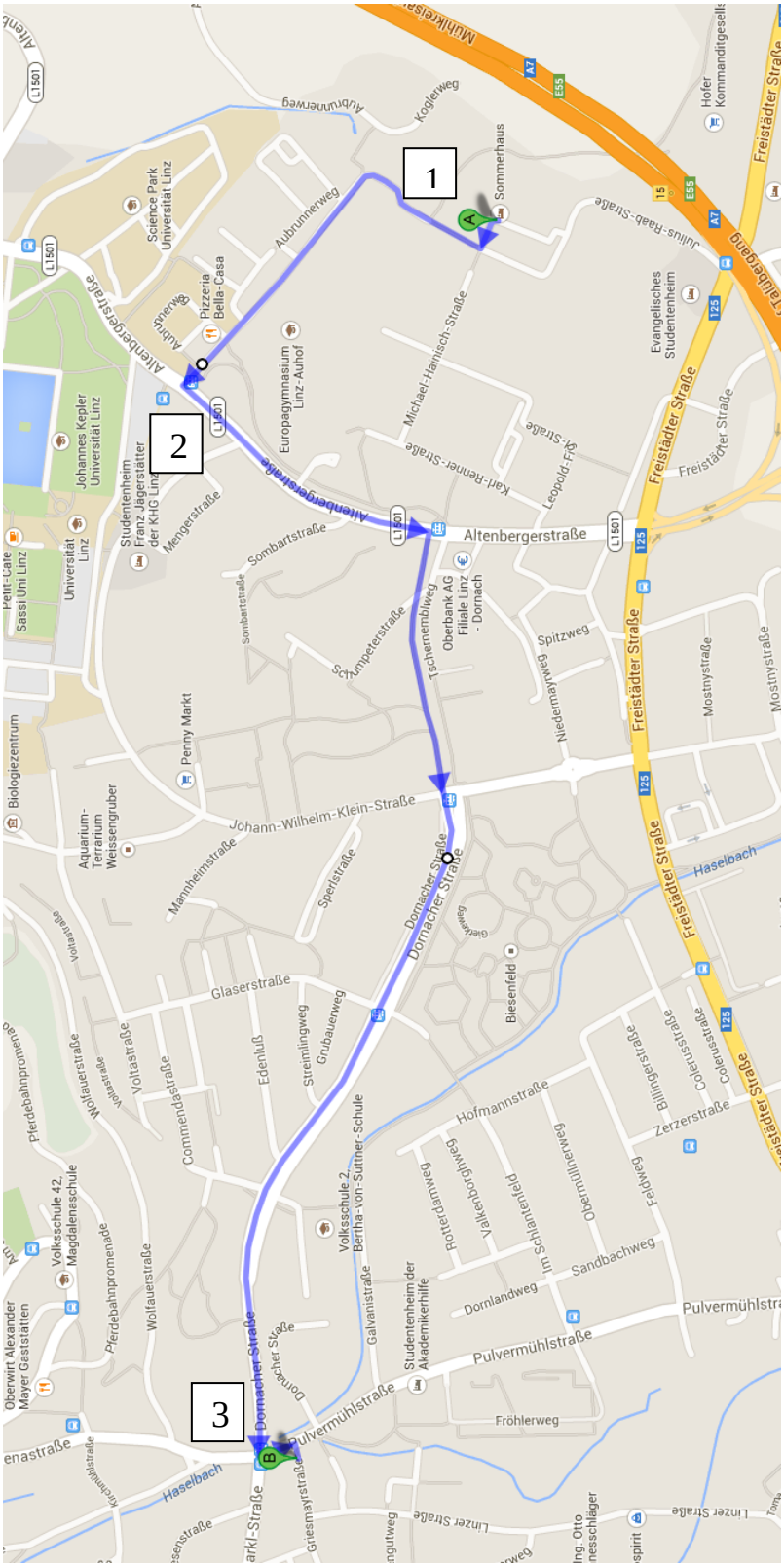
1: Walk from Raab-Heim (A) to Tram-Station “Universität” (B)

2: Take Tram 1 or 2 to Tram-Station “Linke Brückenstraße” (L)

3: Take the Bus Nr. 12 or Nr. 25 to Bus-Station “Kaplanhofstraße” (M)

4: Walk about 160 m south to Gruberstraße 40, JKU Building, Institute for Biophysics (N)

“Weinstadl”
Tram station: St. Magdalena (tram number 1 or 2)
Griesmayrstraße 18, 4040 Linz



1. Walk from Raab-Heim to Tram-Station “Universität”
2. Take Tram 1 or 2 to Tram-Station “St. Magdalena”
3. Walk about 70 m south to Pulvermühlstraße/ Griesmayrstraße