

P22 – AFM-Studies on Solid Supported Lipid-bilayers With Bound Proteins

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Proteins are involved in all processes which are necessary for living, e.g. they are responsible for growth and cleavage of cells, which makes them an interesting topic for science and medical application. Small GTPases, like Ras, are GTP-binding proteins involved in cellular processes, e.g. vesicle transport, cell cycles, nuclear import and signal transduction. GTPases act as molecular switches, which cycle between a GDP-bound inactive and a GTP-bound active state regulated through different factors. In oncogenic mutants of Ras this regulation is disturbed. Most small GTPases are associated with membranes within the cell. The association with the lipid membrane is achieved by posttranslational added lipidanchors at N- or C- terminus, mostly containing farnesyl-residues.

In this study our first AFM-studies on solid supported lipid-bilayers with bound proteins are shown. Considering the protein Ras, so far most investigations were done without membrane. We want to study the protein in its natural environment bound to a lipid-bilayer, in order to get a more detailed view of the function of GTPases within the cell. Furthermore we want to observe the interaction between GTPases and its interacting proteins. Therefore the aim of the project is to combine spectroscopic methods like trFTIR-Spectroscopy with imaging methods like Atomic Force Microscopy and Confocal Raman Microscopy. By means of time-resolved Fourier-transform infrared (trFTIR) spectroscopy detailed information on reaction mechanisms of proteins can be gained^[1]. Especially the method of Attenuated Total Reflection-FTIR-Spectroscopy (ATR-FTIR) enables measurement of proteins in a more *in vivo* like environment, e.g. Ras bound to a lipid-bilayer^[2]. However, ATR-FTIR can only detect the overall changes on the surface without spatial resolution. In order to attain the space-resolved information, e.g. on the distribution of the proteins on a membrane, this method can be combined with Atomic Force Microscopy (AFM), which allows the characterization of the three-dimensional topography and the physical properties of a surface. A further step is possible with Tip Enhanced Raman Scattering (TERS)^[3], a technique where the AFM tip provides the surface enhanced effect and molecular information on nanometre scale can be obtained.

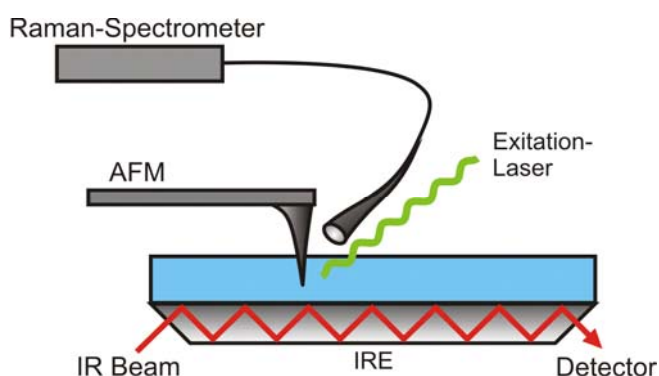


Figure 1: Scheme for the combination of time-resolved Fourier-transform Infrared Spectroscopy (trFTIR) with space-resolved methods, like Atomic Force Microscopy (AFM) and Tip Enhanced Raman Scattering (TERS)

[1] Kötting, Gerwert, *Proteins in Action Monitored by Time-Resolved FTIR Spectroscopy*, ChemPhysChem, 2005, 6, 881-888

[2] Güldenhaupt et al., *Secondary Structure of Lipidated Ras at a Membrane Bilayer*, FEBS J., 2008, 275, 5910-5918

[3] Bailo, Deckert, *Tip-enhanced Raman scattering*, Chem. Soc. Rev., 2008, 37, 921 – 930



AFM-STUDIES ON SOLID SUPPORTED LIPID BILAYERS WITH BOUND PROTEIN

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ABSTRACT

Proteins are involved in all processes which are necessary for life, e.g. they are responsible for growth and cell proliferation, which makes them an interesting topic for science and medical application. Small GTPases, like Ras, are GTP-binding proteins involved in cellular processes, e.g. vesicle transport, nuclear import and signal transduction. GTPases act as molecular switches, which cycle between a GDP-bound inactive and a GTP-bound active state, regulated through different factors. In oncogenic mutants of Ras this regulation is disturbed. Most small GTPases are associated with membranes within the cell. The association with the lipid membrane is achieved by post-translational added lipid anchors at N- or C-terminus, mostly containing farnesyl-residues. In this study our first AFM-work on solid supported lipid-bilayers with bound proteins are shown. Considering the protein Ras, so far most investigations were done without the cell. By means of time-resolved Fourier-Transform Infrared (FTIR) Spectroscopy detailed information on reaction mechanisms of proteins can be gained [1]. Especially the method of Attenuated Total Reflection Fourier-FTIR (ATR-FTIR) Spectroscopy enables measurement of proteins in a more natural environment, e.g. Ras bound to a lipid-bilayer [2]. However, ATR-FTIR can only detect the overall changes on the surface without spatial resolution. In order to attain the space-resolved information, e.g. on the distribution of the physical properties of a surface, this method can be combined with Atomic Force Microscopy (AFM), which allows the characterization of the three-dimensional topography and the

ATTENUATED TOTAL REFLECTION FOURIER-TRANSFORM INFRARED SPECTROSCOPY

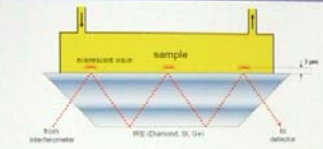


Fig. 1: Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy (ATR-FTIR). ATR-FTIR enables measurement of proteins in a more natural environment, e.g. Ras bound to a lipid bilayer. A flow through system as shown above is used for the experiments. Important for this method is an internal reflection element (IRE) to our studies germanium IREs of 30 x 20 x 2 mm were used. The infrared beam is led through the IRE. By total reflection evanescent waves are built up, which can interact with the sample. The beam that leaves the IRE is recorded at the detector of the spectrometer and shows the absorbance characteristics of the sample on the surface of the IRE.

PREPARING SOLID SUPPORTED LIPID-BILAYER

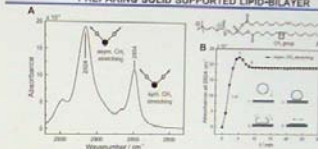


Fig. 2: Monitoring the preparation of a solid supported lipid bilayer (SSLB) via Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy (ATR-FTIR). The SSLB was prepared by vesicle spreading of small unilamellar vesicles (SUVs) consisting of Phosphatidylcholine (PC) onto the hydrophilic surface of a germanium IRE. A: The absorbance band of the amide I (C=O stretching vibration of PC) (1640 cm⁻¹) and used as a marker band for PC binding. B: The absorbance band of the amide I (C=O stretching vibration of PC) (1640 cm⁻¹) and used as a marker band for PC binding. C: The absorbance band of the amide I (C=O stretching vibration of PC) (1640 cm⁻¹) and used as a marker band for PC binding. The banding kinetic shows a fast process which yields a stable SSLB after 10 min. Additionally a signal overhead of approx. 3 times is observed, which is indicated for the rupture of already bound vesicles [5].

AFM-STUDY ON FORMATION OF LIPID-BILAYER ON GERMANIUM IRE

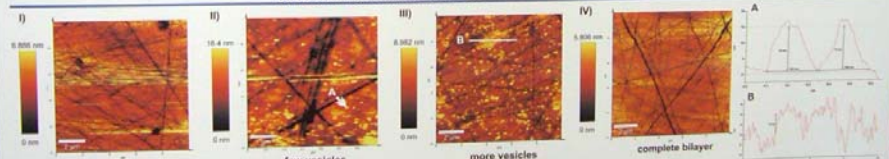


Fig. 3: Comparison of germanium IREs before and after formation of solid supported lipid bilayer (SSLB). AFM images (AC Mode) were obtained from germanium IREs under buffer. Germanium IREs have an uneven surface covered with grooves of different width. These grooves derive from the preparation of the IRE. The grooves are still visible after formation of a SSLB. I) Height image of germanium IRE in buffer, 10 x 10 micron area. II) Height image of germanium IRE in buffer incubated with PCPC-vesicle solution (1:100), 5 x 5 micron area. III) Height image of germanium IRE in buffer incubated with PCPC-vesicle solution (1:50), 10 x 10 micron area. IV) Height image of germanium IRE in buffer after formation of SSLB, 10 x 10 micron area. A) Expected height of a lipid bilayer. B) Expected height of a lipid bilayer. C) Expected height of a lipid bilayer.

ATR-FTIR INVESTIGATION OF MEMBRANE BOUND RAS PROTEIN

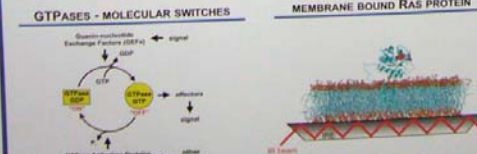


Fig. 4: GTPases are molecular switches. The GTPase cycle between a GTP-bound state, associated with the "ON" state, and a GDP-bound state, associated with the "OFF" state. This cycling mechanism is a key feature in several signal transduction pathways and its malfunction is involved in many diseases. The signaling is stopped through GTP hydrolysis to GDP [4].

AFM-STUDIES OF MEMBRANE BOUND PROTEINS

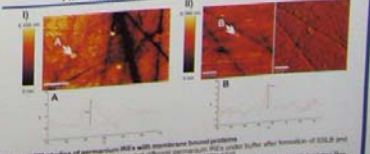


Fig. 5: AFM studies of germanium IREs with membrane bound proteins. AFM images (tapping mode) were obtained of germanium IREs under buffer after formation of SSLB and incubation with membrane bound proteins. The formation of the SSLB was monitored by ATR-FTIR. I) Height image of germanium IRE in buffer after formation of SSLB (PCPC) and incubation with buffer. II) Height image of germanium IRE in buffer after formation of SSLB (PCPC) and incubation with membrane bound proteins. A) Expected height of a lipid bilayer. B) Expected height of a lipid bilayer.

OUTLOOK

Aim of the project is to combine spectroscopic methods like tFTIR-Spectroscopy with imaging methods like Atomic Force Microscopy and Confocal Raman Microscopy. ATR-FTIR can only detect the overall changes on the surface without spatial resolution. In order to attain the space-resolved information, e.g. on the distribution of the proteins on a membrane, the method can be combined with Atomic Force Microscopy (AFM), which allows the characterization of the three-dimensional topography and the physical properties of a surface. With Tip Enhanced Raman Scattering (TERS), a technique where the AFM tip provides the surface enhanced effect, molecular information on nanometre scale can be obtained [6]. The combination of these methods is an important step towards vibrational spectroscopy in living cells.

REFERENCES

- [1] Kölling, Oweini, Proteins in Action: Molecular Biology of Proteins by Time-Resolved FTIR Spectroscopy. ChemPhysChem, 2005, 6, 991-999.
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