



Project on Imaging of Bacteria treated by anti-biotics with AFM

During the past semesters two nano-students have joined our group to set up sample preparation and measurement protocols to visualize morphological changes on bacteria by tapping mode AFM. This is an on-going collaboration project between our group and Basilea Pharmaceutical and we are looking for students who would like to participate in this project.

Bacterial infections, in particular in hospitals, form a serious problem in health care and new bacteria develop that are resistant against current anti-biotics. One way to target gram-negative class of bacteria is by blocking of enzymes that are responsible for construction and maintenance of the cell wall. Since this cell wall does not occur in mammalian cells this reduces the risk of side effects.

In this project fixed and dehydrated bacteria (some of the living bacteria require a higher safety lab for experimental procedures) are imaged in tapping mode AFM to learn more details on the mechanisms behind certain anti-biotics. Although sub-nanometer resolution is not feasible, the resolution is considerably better than that of optical microscopy, giving interesting new inside on morphological changes.

What you can learn from this project: preparation of bacterial samples for the AFM, atomic force microscopy in combination with optical microscopy, data analysis. Furthermore, communicational skills are important since this is a collaboration with an industry partner.

The project is supervised by Prof. Andreas Engel and the practical work is organized by Dr. Patrick Frederix.

If you are interested in this project we would like to welcome you in to the renovated building of the D-B SSE at Mattenstrasse 26, or you can contact us by e-mail or phone.

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Project on Imaging of fish egg cortex and influence of environmental factors

In the previous semester a nano-student has set up a protocol for mounting and imaging of the egg shell (cortex) of zebra-fish eggs. We now have protocols to image the outer cortex surface *in vivo*, on intact eggs with living fish embryos inside. In addition, after dissection the inner and outer surface of the cortex can be imaged *ex vivo*. This is an on-going collaboration project between our group and the group of Prof. Patricia Holm of the program MGU (Mensch – Gesellschaft – Umwelt), for which we are looking for a student.

The study of fish populations provides information on the quality of the water eco-system. One of the fish populations under investigation by the MGU is the brown trout (Bachforelle), a native inhabitant of Swiss rivers. One question we would like to have answered is, which environment factors influence the development and hatching of eggs of the brown trout. Such environmental factors include water pollution by salts, hormones or nanoparticles. The cortex has been reported to possess pores, but no precise permeability data are available for these pores, and might be elucidated by AFM. Because fish eggs of the brown trout would only be available once a year in spring time, we use fish eggs of the zebra-fish as model system.

What you can learn from this project: sample preparation of fish eggs and cortex dissection for AFM, imaging of the samples and data analysis. Because the cells are imaged *in vivo*, preparation and imaging are not trivial. This means that further protocol optimization can be part of the project. Imaging of specially prepared cortex samples by transmission electron microscopy is another supplementary technique that can be considered.

The project is supervised by Prof. Andreas Engel and the practical work is organized by Dr. Patrick Frederix.

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Project on Imaging glucose consumption of living fibroblast cells by AFM-SECM

We are looking for a student who wants to measure topographical and functional data on living fibroblast cells using a combination of atomic force microscopy (AFM) and scanning electrochemical microscopy (SECM).

AFM has been used by several groups to image living cells. It can be used to obtain height images of the cells with a resolution far better than that of a confocal light microscope. In addition, the cantilever can be used to probe the mechanical properties (elasticity) of the cells under different conditions or to mechanically manipulate the cells. With SECM a local current mediated by redox molecules can be monitored by scanning a small electrode over the surface. One functional signal that can be monitored by SECM is the glucose import by cells by measuring the glucose level in a solution, and has already been applied to living cells (details on how to do this can be explained to those interested). However, with classical SECM topographical information is not available. This is a limitation, because high objects partly block the accessibility of the electrode, and consequently the measured glucose level. As a result, the observed glucose level is convoluted with the topography.

The University of Neuchâtel has, in collaboration with our group, developed cantilevers with electrodes integrated in the tip. In this way we are capable to measure topographical features with functional data simultaneously. When measuring the glucose level around a cell such conductive cantilevers partly compensate for topographical effects. In addition, because the size of our electrodes is in the 100nm range, we will be able to localize glucose importing regions of a cell.

What you can learn from this project:

You will learn to grow your own cells for the experiments. This will be followed by characterization by optical microscopy and AFM. In addition you will learn the principles of SECM and how to carry out AFM-SECM experiments on living cells.

The project is supervised by Prof. Andreas Engel and the practical work is organized by Dr. Patrick Frederix.

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Project on Imaging of the OmpF channel conformation by AFM-SECM

We are looking for a student who wants to measure topographical and functional data on membrane proteins using a combination of atomic force microscopy (AFM) and scanning electrochemical microscopy (SECM). With AFM high resolution images of membrane proteins can be obtained. With SECM a local current mediated by redox molecules can be monitored by scanning a small electrode over the surface.

We have shown that we can measure high resolution images of OmpF on conductive supports like ultra flat template stripped gold or platinum. In addition, we have done first AFM-SECM experiments, which showed a permeability of the OmpF pores for redox molecules (if you are interested in this project I can explain you in more detail how this was done). This indicated that the pores in the supported membrane were in an open conformation at neutral pH. Several functional studies have shown that OmpF closes at acidic pH and is voltage gated. The group of Andreas Engel has shown with AFM that the surface topography of the extracellular side of OmpF changes at low pH or under voltage application.

With combined AFM-SECM experiments we want to directly measure the OmpF gating and correlate this with topographical changes in a single experiment.

What you can learn from this project:

To measure topographical changes on OmpF, you must know how to do high resolution imaging in liquid. In addition, you will learn the principles of SECM and how to do AFM-SECM experiments on membranes containing OmpF, using conductive cantilevers. Modeling of experimental data using finite element simulations (comsol) belong to the options of this project.

The project is supervised by Prof. Andreas Engel and the practical work is organized by Dr. Patrick Frederix.

If you are interested in this project we would like to welcome you in to the renovated building of the D-B SSE at Mattenstrasse 26, or you can contact us by e-mail or phone.

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Project on Imaging of membrane protein by frequency modulation AFM

We are looking for a student on a high resolution imaging project of membrane proteins using frequency modulation atomic force microscopy (FM-AFM). These experiments will be done on a home-built AFM, which uses a highly sensitive Fabry-Perot interferometer to detection the cantilever deflection. With such a sensitive sensor stiff cantilevers can be used that oscillate with amplitudes around 1 nm in oscillation type AFM in liquid. The feedback loop of the AFM operates on a frequency modulation circuit. This means that the phase is kept constant by adjusting the excitation frequency of the cantilever. The change in excitation frequency is the input for the z-piezo regulation.

With FM-AFM a porin from the outer mitochondrial membrane called VDAC could be observed to be present in their native membrane as monomers, dimers, trimers, tetremers and hexamers. We are looking for a motivated student who wants to get high resolution images of other membrane proteins, in particular those that are difficult to image by contact mode AFM.

What you can learn from this project:

You learn to operate an advanced AFM, high-resolution imaging of membrane proteins, including optimization of sample preparation.

The project is supervised by Prof. Andreas Engel and the practical work is organized by Dr. Patrick Frederix.

If you are interested in this project we would like to welcome you in to the renovated building of the D-B SSE at Mattenstrasse 26, or you can contact us by e-mail or phone.

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Project in Single-Molecule Force Spectroscopy on Membrane Proteins

We offer the possibility to do a project on single-molecule force spectroscopy on a membrane channel using atomic force microscopy. The goal of this project is to determine the nano-mechanical behavior of this channel by analyzing its unfolding spectra under different conditions (e.g. ionic strength, pH, ligands, inhibitors). This method allows mapping unfolding barriers in the structure of the membrane protein by using models from polymer physics.

So far, the wildtype membrane channel has been unfolded under different conditions and at different unfolding velocities. The next step will be to unfold engineered variants of the channel to understand the relation between structural changes and unfolding spectra.

The experimental work of this project will include the expression, the purification, the crystallization and the force spectroscopy experiments of the membrane protein. Furthermore, force spectra have to be analyzed, which has been semi-automated. Each step has been established in the lab and protocols do already exist.

What you can learn from this project: biochemistry of protein purification (affinity chromatography, gel electrophoresis), 2D crystallization of a membrane protein, validation of crystals using a transmission electron microscope, atomic force microscopy and spectroscopy, data analysis using IgorPro.

If you are interested in this project we would like to welcome you from November, 2008 after we have moved our lab from the Biozentrum to the renovated building of the D-BSSE at Mattenstrasse 26.

The project is supervised by Prof. Andreas Engel and the practical work is organized by Patrick Bosshart and Dr. Patrick Frederix.

In case of any questions or interest feel free to contact:

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