

Modular Advanced Practical in the Focal Point Programme "Proteins in Biomedicine"
VN 185750

(B. Sc. Biochemie)

A number of "Modular Advanced Practicals" is offered by the Biophysics Department, preferably addressed to Master students of the 1st semester:

"Expression, Purification and FTIR spectroscopic investigation of GTPases"

Students will acquire an overview on advanced applications to address issues in an ongoing research project. They will be introduced to independent laboratory work and gain insights to recent research topics in biochemical and biophysical analysis of protein function with the focus on FTIR spectroscopy.

Contents:

- Heterologous expression of a GTPase of the Ras superfamily or a heterotrimeric GTPase
- Purification of the protein by ion exchange, gel filtration and or affinity chromatography
- Nucleotide exchange from GDP to caged-GTP, control of the exchange by HPLC
- Start of the reaction by an XeCl excimer laser flash and time resolved FTIR of the purified protein
- Discussion of the obtained infrared spectra and kinetics

Contact: [PD Dr. Carsten Kötting](#)

"Protein crystallography"

Students will acquire knowledge about strategies for the structural characterization of proteins with protein crystallography. They will be able to apply these strategies to the purification and characterization of selected proteins. They will be able to discuss results of these experiments in the context of current research in written and oral form.

Contents:

The course covers modern methods in molecular biology, protein biochemistry, protein crystallization, X-ray diffraction and generation of atomic models of proteins. The experimental results are processed with computational methods and are

analyzed utilizing our 3D graphic workstations. These experiments are always complemented by a biophysical characterization of the proteins of interest.

Depending on the interest of the applicant, the focus of the projects can be more on wet lab work or computational work. Topics are subprojects of our current research projects.

Examples are:

1. ABC transporters of medical interest
2. Light-harvesting proteins of algae and cyanobacteria
3. Retinal-binding membrane proteins
4. Enzymes of microbial pigment biosynthesis
5. Enzymes of phytohormon biosynthesis

Contact: [Prof. Dr. Eckhard Hofmann](#)

“MD simulations on selected transmembrane proteins”

Students will acquire an overview on advanced applications to adress issues in an ongoing research project. They will be introduced to independent computational research and gain insights to recent research topics in theoretical simulations of selected transmembrane proteins. The respective content of the project depends on and is taken from the current research at the Department of Biophysics.

Contents:

- Proton conduction in hydrogenases
- Protein-internal water molecules dynamics
- Basics of Molecular Dynamics simulations: Molecular Mechanics, force field concept
- Usage of MD, visualization and modeling programs
- Combining, comparing and assessing computational and experimental results

Contact: [PD Dr. Carsten Kötting](#)

“Expression and spectral characterization of microbial retinal proteins”

Students will acquire an overview on advanced applications to adress issues in an ongoing research project. They will be introduced to independent laboratory work and gain insights to recent research topics in biochemical and biophysical analysis of the function of a selected microbial retinal protein.

Contents:

- Safety instructions

- Preparation of fermentation media
- Transformation of the Escherichia coli expression strain
- Expression of microbial rhodopsin in Pichia pastoris or Escherichia coli
- Membrane preparation and detergent solubilization
- Chromatographic purification using affinity and gel filtration techniques
- Identification of the purified protein by Western blotting
- Measurement of light-driven proton pumping of E. coli expressing the microbial retinal protein

Contact: [PD Dr. Mathias Lübben](#)

“Expression and biochemical characterization of heavy metal translocating ATPases”

Students will acquire an overview on advanced applications to address issues in an ongoing research project. They will be introduced to independent laboratory work and gain insights to recent research topics in biochemical and biophysical analysis of the function of a selected heavy metal translocating ATPase.

Contents:

- Safety instructions
- Preparation of fermentation media
- Transformation of the Escherichia coli expression strain
- Expression of a heavy metal translocating ATPase in Escherichia coli
- Membrane preparation and detergent solubilization
- Chromatographic purification using affinity and gel filtration techniques
- Identification of the purified protein by Western blotting
- Lipid reconstitution of the ATPase
- Measurement of ATPase and investigation of metal activation

Contact: [PD Dr. Mathias Lübben](#)

“Purification, biochemical and biophysical characterization of a G protein coupled receptor”

Students will acquire an overview on advanced applications to address issues in an ongoing research project. They will be introduced to independent laboratory work and gain insights to recent research topics in biochemical and biophysical analysis of the function of a G protein coupled receptor (GPCR).

Contents:

- Safety instructions

- Infection of insect cells with a baculovirus harbouring the gene encoding the GPCR of interest
- Expression of the GPCR in insect cells
- Membrane preparation and detergent solubilization
- Purification using affinity chromatography
- Measurement of biological activity by radioligand receptor assay in the central isotope lab (RUBION)

Contact: [PD Dr. Mathias Lübben](#)