Advanced Practical in the Focal Point Programme "Proteins in Biomedicine" VN 185852

(M. Sc. Biochemistry)

A number of „Advanced Practicals" is offered by the Biophysics Department, preferably addressed to Master students of the 1st to 4th semester:

"Time-resolved FTIR spectroscopy 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 (wildtype or mutant) by ion exchange, gelfiltration 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
- Analysis of the spectroscopic data by a global fit.
- Discussion of the obtained infrared spectra and kinetics

Contact: PD Dr. Carsten Kötting

"Vibrational Spectroscopy for biomedical applications"

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 biophysical analysis of cells and tissue for biomarker detection for the early detection of diseases such as cancer, Parkinson or Alzheimer's.

Contents:

- Marker free cancer diagnostics with vibrational spectroscopy by confocal Raman microscopy or FTIR microscopy.

- Subject can be cells from cancer cell lines or urine, tissue from biopsies of lung, colon or bladder or body fluids.
- Data processing and evaluation.

Contact: PD Dr. Carsten Kötting

“Simulations on ligand binding and diffusion pathways in selected drug targets”

Students will be introduced to the field of computational protein modeling and Molecular Dynamics (MD) simulations. During the practical, they will learn the usage of modeling (Moby), MD and TMD simulation (Gromacs) and visualization programs (PyMol), and the theoretical concepts of molecular force fields. Furthermore, they will work on a current research topic, and thus get insight into real research on selected drug target proteins.

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
- Usage of Targeted Molecular Dynamics
- Creation of own force field parameters
- Combining, comparing and assessing computational and experimental results

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:

- ABC transporters of medical interest
- Light-harvesting proteins of algae and cyanobacteria
- Retinal-binding membrane proteins
- Enzymes of microbial pigment biosynthesis
- Enzymes of phytohormon biosynthesis

Contact: Prof. Dr. Eckhard Hofmann

“Spectroscopic study of the molecular mechanism of Cu-ATPases”

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 a selected heavy metal translocating ATPase.

Contents:

- Use of molecular biological and microbiological standard techniques
- Site-specific mutagenesis of genes encoding the protein of interest, such as Cu-ATPase
- Heterologous expression of genes encoding membrane-bound heavy metal transport proteins
- Membrane isolation and membrane protein solubilization
- Chromatographic purification of Cu-ATPase from E. coli
- Biochemical measurement of ATPase activity
- Time-resolved FTIR difference spectoscopy of Cu-ATPase using caged nucleotides
- Kinetic investigation of the reaction mechanism of Cu-ATPase based on time-resolved FTIR data

Contact: PD Dr. Mathias Lübben

“Expression and biophysical 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 function of a selected microbial retinal binding protein.

Contents:

- Use of molecular biological and microbiological standard techniques
- Site-specific mutagenesis of genes encoding the protein of interest, such as Cu-ATPase
- 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
- Measurement of the retinal protein photocycle by optical spectroscopy using a diode-array spectrometer
- Evaluation of the kinetic data using a multiple exponential fitting procedure
- Reconstitution of the retinal protein in phospholipid membrane vesicles and measurement of the photocycle by time-resolved FTIR spectroscopy (fast scan technique)
- Setting up crystallization trials

Contact: PD Dr. Mathias Lübben