Course
>Protein Purification/Mass Spectrometry<

Lecturer	***Brosch G, Golderer G, Sarg B***
Number	*049009*
Type / ECTS	*VU 2 / 1,5 ECTS*
Dates	***G. Brosch***

- ***10.11.2020*** *(8.30 am)*
- *Institute of Molecular Biology, CCB; room 03.392 (3rd floor)*

G. Golderer

- ***11.11.2020*** *(9 am)*
- *Institute of Biological Chemistry, CCB; room 04.383 (4th floor)*

B. Sarg

- ***12.11.2020*** *(9.30 am)*
- *Institute of Clinical Biochemistry, CCB; room 03.480 (3d floor)*

Background

Protein purification in general aims to isolate one or a few proteins from a complex mixture, usually cells, tissues or whole organisms. Protein purification is vital for the characterization of the function, structure and interactions of target proteins. Separation steps usually exploit differences in protein size, physicochemical properties, binding affinity and biological activity.

In the purification course, histone deacetylase activity from *Aspergillus nidulans* will be purified. Histone acetylation is a posttranslational modification that occurs mainly on histones H3 and H4 and which is implicated in gene regulation. Moreover, this modification represents an essential element of the complex epigenetic “histone code”.

The filamentous fungus *A. nidulans* is an important model organism for the study of genetics and cell biology of lower eukaryotes, but also of biotechnological (e.g. antibiotics) as well as medical (e.g. production of toxins like aflatoxin; aspergillosis) issues.

AIM of the Course

The aim of this course is to gain practice in classical methods of protein biochemistry.

Starting from whole fungal cells (mycelia), it is intended to isolate endogenous histone deacetylase activity by a series of different extraction/purification steps and to finally identify the respective histone deacetylase(s) but also interacting proteins by mass spectrometry.

In detail, cells will be mechanically disrupted and extracted proteins will be separated by a combination of size exclusion-, hydrophobic interaction-, ion exchange-, and affinity chromatography. Histone deacetylase activity will be monitored/followed by an *in vitro* HDAC-Assay. Selected peak fractions will be used for mass spectrometry analysis with subsequent statistical analysis.