

Methods in Life Science - Protein Expression and Purification – Learning Outcomes

(What students should be able to explain, draw, calculate, predict, annotate etc. about the content presented in the lecture)

- The need for purified recombinant proteins arises from the fundamental nature of proteins in life sciences and medicine.
 - Proteins as a subject of life sciences
 - Proteins as targets and possible therapeutics in the treatment of various diseases
- General workflow of recombinant protein expression
 - Cloning of expression vectors; elements necessary for functional expression vectors
 - Choice of expression host(s); procaryotic host strains, yeast, insect cells, mammalian cells
 - The nature of the target protein and the demands towards the recombinant protein dictate the choice of a suitable expression host system.
 - Advantages and disadvantages / strengths and weaknesses of different expression host systems
- Principle of the CIPP purification strategy
 - Capture, intermediate purification and polishing
 - Parameters in protein purification: speed, capacity, recovery and resolution
- Use of protein characteristics for purification
 - Binding affinities → affinity purification; with or without affinity purification tags; examples for tags
 - Charges on protein surfaces → anion or cation exchange chromatography
 - Hydrophobic protein surfaces → hydrophobic interaction chromatography and salting-out / reversible precipitation
 - Size / shape → size-exclusion chromatography or gradient centrifugation
- General principle of column chromatography
 - Columns, solid phase, liquid phase, semi-automated chromatography systems
 - How are proteins bound to and eluted from different kinds of columns?