

HPLC-MS/MS analysis of a complex protein sample

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Duration: 2-3 days

Number of participants: 2-3

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HPLC (high performance liquid chromatography) is often used to separate the peptides in a complex peptide mixture. Hereby, a liquid digested protein sample is being passed over a stationary phase packed into a column using a flow of liquid solvent (mobile phase). Each peptide in the sample interacts slightly differently with the stationary and the mobile phase. If the interaction with the stationary phase is weak, the peptide elutes early, and if the interaction is strong, the peptide is retarded and elutes lately.

HPLC is usually combined with MS/MS (tandem mass spectrometry). The eluted peptides are ionized and subsequently analyzed by their mass to charge ratios (m/z). By applying an inert gas, the peptides are fragmented alongside their peptide backbone. Later on, the tandem mass spectra can be assigned to peptides derived from a protein database.

Please contact me in advance, if you wish to bring some of your own protein samples for analysis.

Day 1: Introduction; digestion of a complex protein sample

Day 2: HPLC-MS/MS run of the digested protein sample

Day 3: Data analysis and evaluation