

**TWO-DAY COURSE, Saturday and Sunday
12 Peptides and Proteins in Mass Spectrometry**

Instructors



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Over the past two decades, mass spectrometry has become the key technology for the characterization of proteins. This course is designed as an introduction for researchers needing to expand their knowledge of the use of mass spectrometry-based methods for the identification, characterization, and quantification of peptides and proteins. Background material in basic protein chemistry will be provided along with a review of mass analyzers, acquisition types, and ionization sources used for protein and peptide analysis. Real-world examples will be used to illustrate protein sample preparation strategies, characterization of intact proteins, characterization of post-translational modifications (with emphasis on glycosylation and phosphorylation), identification of proteins via database searching, direct identification of proteins from gels and complex mixtures, and quantitative differential protein expression studies. The role of MS-based methods in interdisciplinary efforts to solve complex biomedical problems will also be addressed. Additionally, there will be tutorials on the use of open source proteomic software tools for interpretation of proteomic datasets. Problem sets will be used to emphasize practical aspects of comprehensive protein characterization, including peptide sequencing and protein identification by database searching.

Topics covered in the course include:

- **Sample preparation methods for proteins characterization and proteomics:** A review of general concepts with detailed protocols for key types of analyses.
- **LC/MS of intact proteins:** Analysis of proteins by ESI-LC/MS to determine protein molecular weight, construct integrity, presence of post-translational or other modifications.
- **MALDI of intact proteins and peptides:** Analysis of proteins and peptides by MALDI-MS and its role alongside ESI-LC/MS, including imaging MS and other *in situ* desorption ionization approaches.
- **MS/MS of peptides:** Fragmentation behavior of peptides using collision-induced dissociation (CID) or electron-transfer dissociation (ETD); de-novo peptide sequencing.
- **Capillary LC/MS and LC/MS/MS:** Why use capillary LC systems for LC/MS?; typical separation conditions for peptide analysis; data-dependent and data-independent acquisition strategies for peptide MS/MS; multidimensional separations.
- **Protein identification by database searching:** Topics include - choosing a database, selecting modifications and other search parameters, false discovery rates, and protein inference.
- **Phosphorylation analysis:** Fragmentation behavior of phosphopeptides using CID and ETD; Phosphosite localization and determination of phosphorylation stoichiometry; Phosphopeptide affinity isolation strategies including IMAC and TiO₂.
- **Glycoprotein analysis:** Review of protein glycosylation; Fragmentation behavior of glycopeptides and isolated glycans; Glycan site localization; Glycomics
- **Quantitative analysis:** Stable isotope labeled and label-free methods for relative protein quantification; Multiple-reaction monitoring (MRM) for targeted protein quantification
- **Bioinformatics tools for proteomics:** Overview of available software tools for analysis and interpretation of proteomics datasets.
- **Top-Down and Native Protein MS:** Overview of methods for biophysical characterization and PTM mapping in intact proteins.

Prerequisite: Hands on experience in mass spectrometry and/or protein chemistry