



3-Day Workshop

Day 1

Lecture: Intro to DIGE, MALDI-TOF
Laboratory: DIGE, gel IEF, spot picking and digestion, MALDI-TOF peptide mixtures

Day 2

Lecture: Intro to ProteoSep, Electrospray
Laboratory: DIGE 2nd dimension, DIGE analysis, MALDI intact protein, ESI intact protein

Day 3

Lecture: Intro to ESI data analysis
Demo: ProteoSep (protein LCMS)
Computation: Intact protein mass data, deconvolution, Protein database searching

Schedule:

Three day workshops are held every few months. Please see our web site at http://cbs.umn.edu/mass_spec/workshop.html for dates and fee structure. Space in the workshops is limited so please register early.

Contacts:

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Mass Spectrometry Consortium for the Life Sciences & Proteome Analysis Core Facilities

Proteomics Workshops

University of Minnesota
Twin Cities



Sponsored by the Department of Biochemistry, Molecular Biology & Biophysics

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Proteomics Workshop

The Proteomics Analysis Core (PAC) and the Mass Spectrometry Consortium for the Life Sciences (MSCLS) are managed by the Department of Biochemistry, Biophysics and Molecular Biology at the University of Minnesota and provide support, training and equipment for the separation, analysis, and identification of proteins / peptides using a variety of proteomic and mass spectrometric technologies. The "Proteomics Workshop" is a teaching and training course designed to familiarize the investigator with the capabilities of the facilities as well provide a technical foundation for further proteomic experimentation.



PAC

The PAC has equipment to aid an investigator with the separation of complex protein mixtures and distinguishing

differential expression of proteins between two samples. Among the available technologies are traditional **2D SDS-PAGE**, **DIFFERENTIAL IN GEL ELECTROPHORESIS (DIGE)**, and **PROTEOSEP** (2D liquid chromatofocusing) along with analysis software to support each method. The PAC also includes the ability to robotically excise proteins from gels, proteolytically digest samples and spot the peptide mixture on targets in preparation for mass spectrometry.

DIFFERENTIAL IN-GEL ELECTROPHORESIS allows an investigator to separate a pair of samples on a single gel in order to quantitate differences in protein expression. The samples representing two different treatments are labeled with either the fluorescent dye Cy3 or with Cy5 along with a third dye, Cy2, to ensure every protein is uniquely represented. This allows quantitative comparative analysis of protein amounts similar to DNA array technology. After two-dimensional resolution, the gel image is captured on a high-resolution laser scanner and the results are analyzed with a Decyder software package. Decyder allows easy analysis of gels and has the capability of combining data from multiple gels. The advantages of DIGE technology over standard 2D – electrophoresis are the sensitivity, linear range of use and the ability to identify slight shifts in mobility that indicate important post translational modifications.

PROTEOSEP is an alternative to traditional gel electrophoresis that utilizes separation in a liquid phase that allows an investigator access to full length proteins for analysis and identification. The virtual gel allows a quantitative comparison of differential protein expression. **PROTEOSEP** is not limited by protein molecular mass and allows increased ability to identify membrane proteins.

MSCLS

The goal of MSCLS is to help researchers identify and characterize peptides and proteins and in special cases quantitate relative amounts of protein in two samples.



MASS SPECTROMETRY offers both high **SENSITIVITY** (with protein detection from picomole – femtomole amounts of material) and **SPECIFICITY** (which is inherent in tandem mass spectral data, in general). Multiple ionization modes are available to researchers for the production of ions from molecules in solution and their introduction into the mass spectrometer in the gas phase. Different types of mass spectrometers offer specific types of data including, but not limited to, mass-specific sensitivity enhancement scan methods. One and two-dimensional HPLC systems (ranging from analytical to capillary modes) are present for the separation of complex mixtures. The ability to choose from among multiple ionization methods, multiple mass spectrometers and multiple LC applications is important to fit any project.

ESI-mass spectral data from **INTACT PROTEINS** can provide mass values for each individual protein present in the sample for species that differ by a minimum of about 5-10 Da. The lowest detectable mass difference is dependent on the mass spectrometer chosen for analysis, protein concentration and ease of ionization for the molecules of interest with the existing buffer system. This is important to detect post-translational modifications. Data acquisition of intact protein and **DATA DECONVOLUTION** will be covered in the workshop. Intact protein mass spectrometry by MALDI-TOF (time of flight) mass spectrometry will be shown also.

WORKSHOP FORMAT

The workshops will involve lectures, hands-on experimentation and discussion on critical aspects of proteomic analysis including: Experimental design, Sample Preparation, Sample separation, Sample Analysis, Protein preparation for MS identification, Mass spec data collection and Mass spec data analysis. Workshops will also allow practical applications including DIGE, ProteoSep concepts and preparation of materials for MS analysis. Individuals will collect MS data utilizing instruments that will allow MS and MS/MS and the analysis and interpretation of data will be reviewed.

The **MSCLS** will demonstrate how to collect peptide mass spectra using both **MALDI** and **ESI** techniques on multiple mass spectrometers and how to interpret mass spectral data from proteins and peptides. **TANDEM MASS SPECTRAL** data on **PEPTIDE FRAGMENTS** (that are generated after collision of a peptide with an inert gas inside a mass spectrometer) provide data from which peptide sequence and consequently protein identification can be obtained. Inherent in peptide tandem mass spec data is the identification of suspected post-translational modifications and amino acid location for advanced protein characterization. Techniques for providing relative quantification of proteins in solution using isotope tagging methods (including **ICAT®**, isotope coded affinity tags) will be reviewed in the context of current applications.

Multiple database searching strategies will be covered including searches for proteins which are in public databases and searching for proteins from unsequenced genomes, in particular, *de novo* peptide sequencing combined with homology-based searches against available sequences. Methods for interpreting quantification results and protein identification of ICAT data will be reviewed.

