Proteomics and Protein Mass spectrometry in Biology. BIOPL4832 (also PLPA/PLBR 4832) (1 credit)

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When: 10/2/09 - 10/30/09 - MWF 01.25-02.15 pm (12 lectures); **Where:** Warren Hall 260

This course will provide an advanced introduction to proteomics and mass spectrometry and its application in (plant) biology.

1. Introduction to proteomics and mass spectrometry
a. History and development of the field of proteomics and mass spectrometry
b. HUPO and The Protein Standard Initiative (PSI) and proteomics/spectral depositories (e.g. PRIDE)
c. Overview of the literature and list of reviews (What should you read?)

2. Mass spectrometry of proteins and peptides
a. Biological Mass Spectrometry – Introduction and overview
b. Ionization techniques (MALDI, ESI)
c. Mass analyzers (TOF, Quadropole/hexapole, ion traps, Orbitrap)
d. Popular MS instruments

3. Identification of proteins by peptide mass finger printing (MALDI-TOF MS)
a. Preparing the proteins/peptides (proteolysis, extraction, spotting, matrices and crystallization)
b. Linear and Reflectron Mode; delayed extraction
c. Examples of spectra and features (Mass Resolution/Accuracy, Isotope envelopes, Spectral quality)
d. Bioinformatics (databases, software, significance/False Positive Rates)

Fall break - no class Monday 12 October

4,5. Identification of proteins by Tandem MS
a. Off-line (direct infusion) and On-Line MS/MS - survey Scan and DD MS/MS scans and their features
b. Examples of spectra and features (Mass Resolution/Accuracy, Isotope envelopes, Spectral quality)
c. Bioinformatics of MS/MS data (databases, software, significance/False Positive rates)
d. MudPit analysis (on-line LC based multi-dimensional separation)
e. 'Bottom-up' versus 'Top-down' proteomics by FT-MS

6. Strategies for identification of (complex) proteomes
a. General considerations (goals, proteome complexity, fractionation, Mass spectrometers - $$ and time)
b. Protein fractionation techniques (gel based techniques, chromatography)
c. Available sequence data (genomic, EST, homology based identification)
d. Plant Proteomics databases

7. Discussion selected proteomics papers

8. Characterization of post-translational modifications (PTMs)
a. Common PTMs induced by sample preparation
b. Phosphorylation and others
c. N-terminal modifications

10. Comparative quantitative proteomics I
a. Soluble or membrane proteomes -
b. 2-DE Gel based methods
c. Stable Isotope based methods (ICAT, 15N-labeling, iTraq)

11. Comparative quantitative proteomics II
a. More Stable Isotope based methods (formaldehyde, heavy water during trypsination, etc)
b. Label-free quantification (spectral counting and MRMs)
c. Absolute quantification methods and considerations (e.g. AQUA)

12. Identification and Characterization of protein complexes (interactions)
a. Immuno-affinity purification
b. Epitope (TAP) Tagging
c. Nearest neighbor analysis by Crosslinking and MS
d. Analysis of large complexes within specialized mass spectrometers