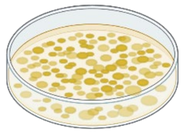
Bottom-Up Proteomics

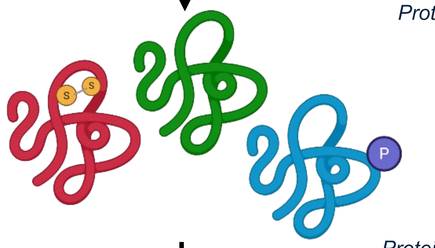
Mass Spectrometry and Proteomics Facility
University of Notre Dame

Sample Preparation & Digestion



Biological Sample

Proteins are extracted while removing cellular debris. Proteins are quantified via BCA assay or similar.



Proteins

Proteins are denatured with heat, detergent, and reducing agents to break disulfide bonds.

Denatured Proteins

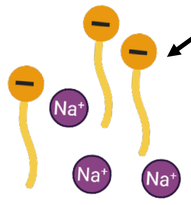
Denatured proteins alkylated to stop the reformation of disulfide bonds.

Alkylated Proteins

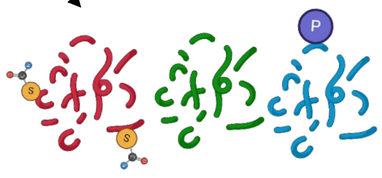
Proteins are digested into peptides with a protease such as trypsin, which reliably cleaves after Arg and Lys.

Digested Proteins

Peptides are cleaned-up to remove detergents and salts which suppress signal and contaminate the instruments.

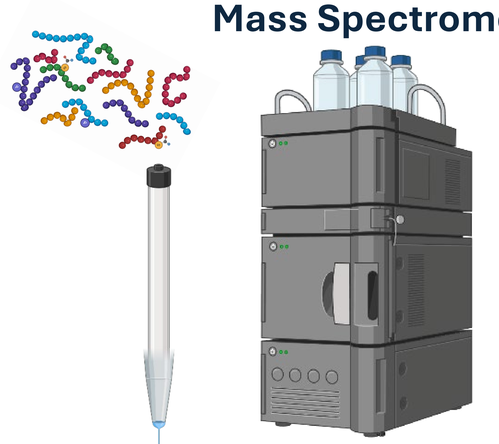


Salts & Detergents



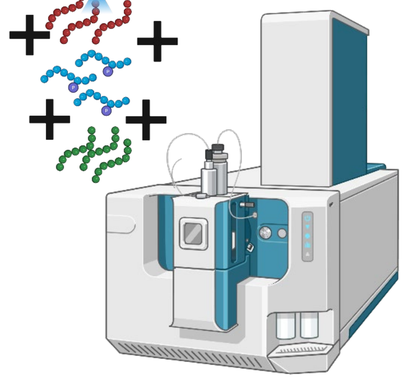
Peptides

Liquid Chromatography & Mass Spectrometry



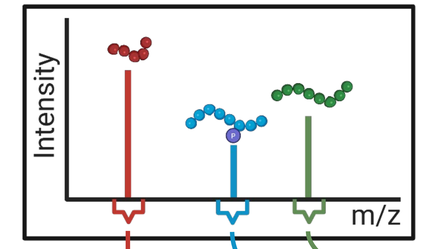
Reversed Phase LC

Peptides are separated based on hydrophobicity on the reversed phase column and ionized via electrospray ionization as they elute.



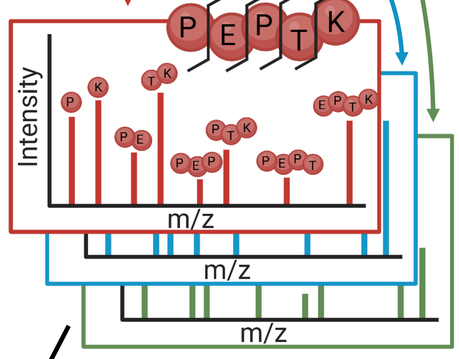
Tandem Mass Spectrometer

Mass/charge (m/z) for each peptide ion is measured in an MS1 spectra, showing peaks for each peptide eluting from the LC column at any given time.



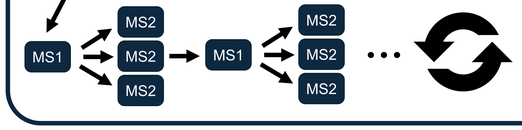
MS1 Spectrum

A peptide ion is isolated in the instrument and fragmented. The m/z of the fragments are measured in an MS2 spectrum. The amino acid sequence of the peptide results in a unique fragmentation pattern. The instrument then repeats the process with the next peptide in the MS1 spectrum, generating an MS2 spectrum for each peptide peak from the MS1 spectrum

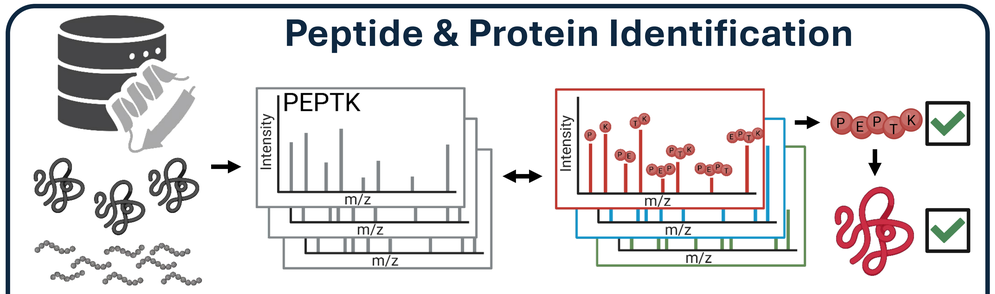


MS2 Spectra

The instrument takes a fresh MS1 spectrum, isolates the new peptide ions, generates MS2 spectra, and repeats the process for the length of the LC separation.



Peptide & Protein Identification



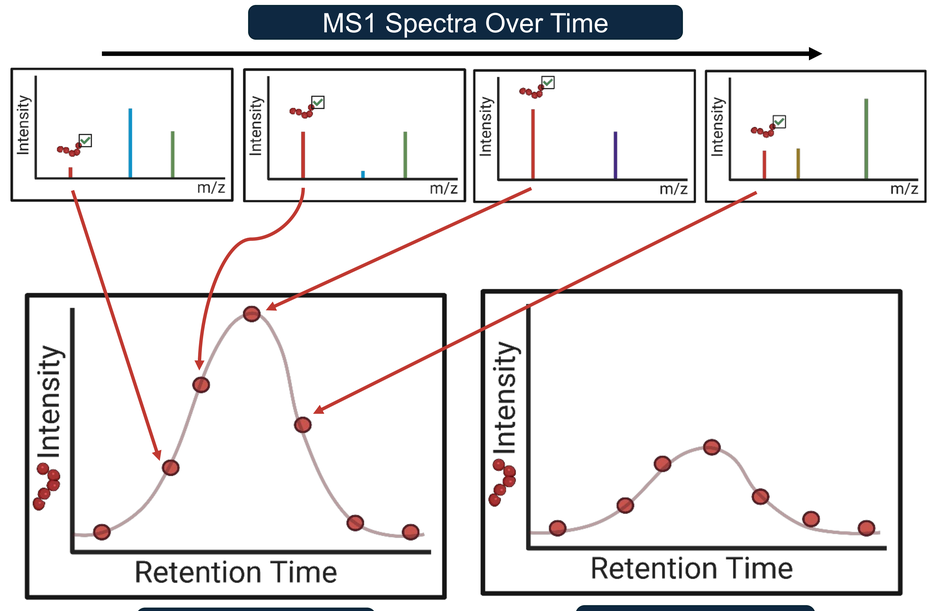
Protein Database (fasta) → Theoretical Peptide Spectra ↔ Experimental MS2 Spectra → ID'd Peptide/ Protein

The protein database is digested in silico, *and the fragmentation patterns and intensities are predicted for each theoretical peptide to generate theoretical spectra.*

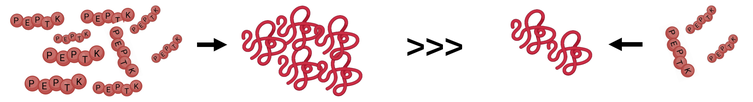
Theoretical spectra and experimental spectra are compared and scored. After statistical validation and controls for false discovery rate, matches are identified. ID'd peptides are mapped back to proteins.

Label Free Quantification (LFQ)

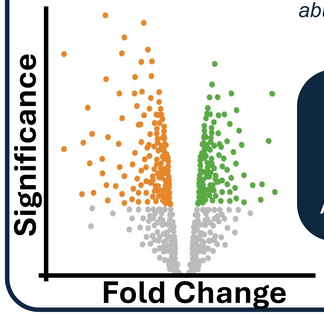
After a peptide is identified and validated, the intensity of its signal in all the MS1 spectra throughout the run is plotted against time creating an extracted ion chromatogram (XIC).



XIC Sample 1 | XIC Sample 2



The peak area from the XIC for any given peptide can be calculated and compared to the area for that same peptide in another sample. After normalization steps these areas are used as proxies for peptide abundance. The best performing peptides from a protein are used to calculate relative abundance of that protein across samples.



Volcano Plot for Differential Abundance

This same quantification can be performed for each detected protein. Repeated measurements of different samples allow for the statistical quantification of change between samples. This can be plotted as a volcano plot with fold change on one axis and significance on the other where each data point is a protein. Potentially interesting proteins are those that are in the top corners of the plot, showing large and significant difference between samples

Some figures made with BioRender

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