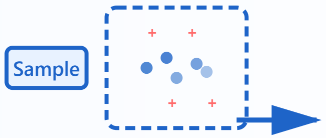
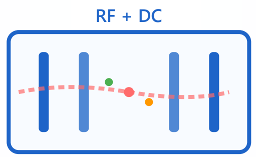
Mass Spectrometry Basics



Ion Generation

ESI / MALDI



Mass Analysis

m/z Separation

Detection

Signal Processing

Resolution & Accuracy

- High resolution: distinguish similar masses
- Mass accuracy: parts per million (ppm)
- Critical for peptide identification

Scan Modes

- Full scan: entire mass range
- Selected ion monitoring (SIM)
- Data-dependent acquisition (DDA)

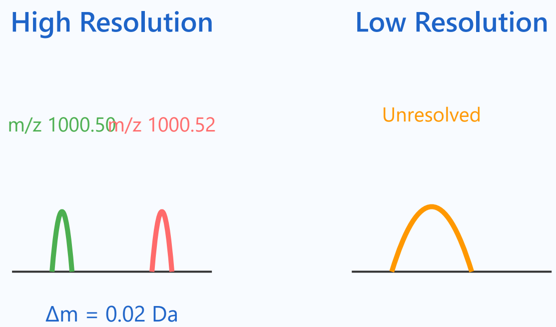
Sensitivity

- Femtomole to attomole detection
- Dynamic range: 3-5 orders of magnitude
- Low abundance protein detection

Applications

- Protein identification
- Quantification
- PTM analysis

1. Resolution & Accuracy



Resolution Formula:

$$R = m / \Delta m$$

where m = mass and Δm = peak width at half maximum (FWHM)

Mass Resolution

The ability to distinguish between ions of similar mass-to-charge ratios. Higher resolution allows separation of peaks that differ by small mass units.

Example:

Orbitrap mass analyzers achieve resolutions >100,000, enabling discrimination between peptides differing by 0.001 Da.

Mass Accuracy

The closeness of measured mass to the true mass, typically expressed in parts per million (ppm). Essential for confident peptide identification.

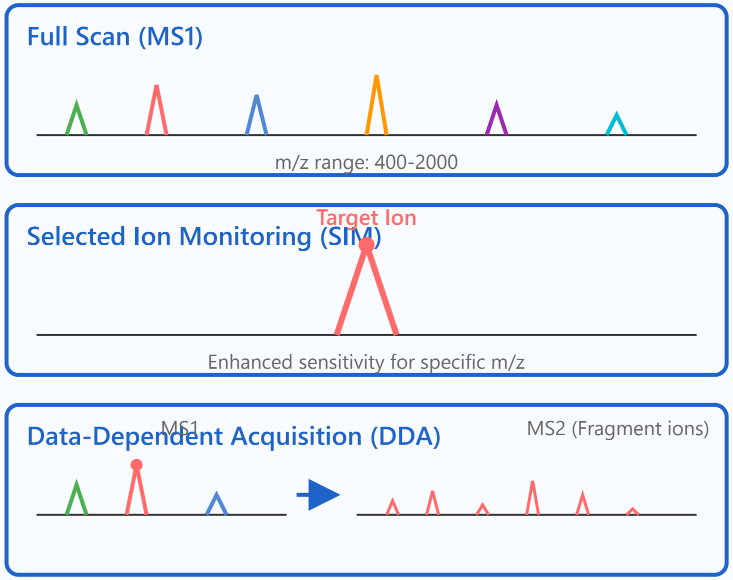
$$\text{Mass Accuracy (ppm)} = [(m_{measured} - m_{theoretical}) / m_{theoretical}] \times 10^6$$

Typical Values:

Modern instruments: <5 ppm accuracy

High-resolution MS: <1 ppm accuracy

2. Scan Modes



Full Scan Mode

Measures all ions across a specified m/z range. Provides comprehensive overview but lower sensitivity for individual ions.

Selected Ion Monitoring (SIM)

Focuses on specific m/z values of interest. Increases sensitivity and detection limit by 10-100 fold for target analytes.

Application:

Targeted quantification of known peptides in complex mixtures, such as biomarker validation studies.

Data-Dependent Acquisition (DDA)

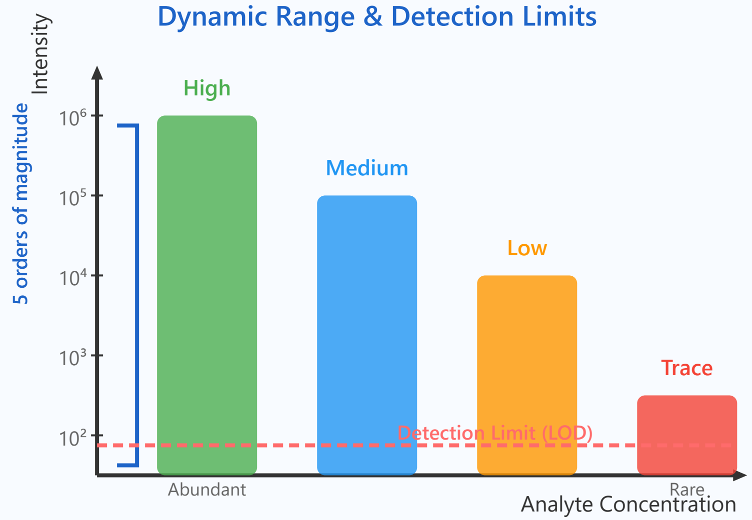
Automatically selects the most abundant ions from MS1 scan for fragmentation (MS2). Enables protein identification through peptide sequencing.

Workflow:

1. Survey scan (MS1)
2. Select top N peaks

3. Fragment selected ions (MS2)
4. Return to survey scan

3. Sensitivity



Detection Sensitivity

Modern MS instruments can detect proteins at femtomole (10^{-15} mol) to attomole (10^{-18} mol) levels, enabling analysis of rare proteins and post-translational modifications.

Practical Context:

1 femtomole = approximately 600,000 molecules
1 attomole = approximately 600 molecules
Sufficient for single-cell proteomics

Dynamic Range

The ratio between the most and least abundant detectable proteins. Typical range: 10^3 to 10^5 (3-5 orders of magnitude).

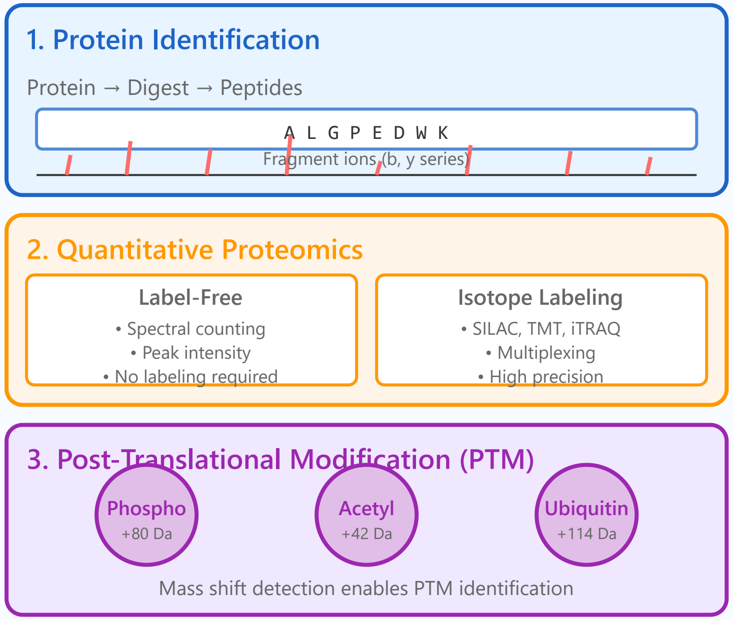
Challenges

• High-abundance proteins (e.g., albumin) can mask low-abundance proteins
• Sample depletion strategies often required
• Ion suppression effects in complex mixtures

Solution:

Fractionation techniques (e.g., HPLC) combined with enrichment methods improve detection of low-abundance proteins by **100-1000 fold**.

4. Applications in Proteomics



Protein Identification

Proteins are digested into peptides, which are analyzed by MS/MS. Fragment ion patterns are matched against databases for identification.

Database Search:

Mascot, SEQUEST, MaxQuant algorithms match experimental spectra to theoretical peptide fragments. Typical identification: >2 unique peptides per protein.

Quantitative Analysis

Compares protein abundance across samples using label-free or isotope labeling approaches. Essential for biomarker discovery and pathway analysis.

PTM Characterization

Detects modifications through characteristic mass shifts. Phosphorylation, acetylation, methylation, ubiquitination are routinely analyzed.

Clinical Application:

PTM analysis in cancer research: Aberrant phosphorylation patterns identify activated signaling pathways and therapeutic targets.