Proteomics Course

LECTURE-20
Fundamentals of mass spectrometry

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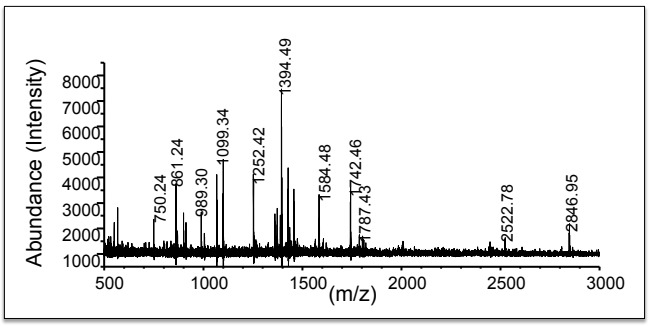
Lecture outline

1. Fundamental of Mass Spectrometry
   - Role of MS and basic concepts
2. Ionization Sources
3. Mass Analyzers
4. Tandem Mass Spectrometry

MS basic concepts

Mass spectrometry

- Mass spectrometry - technique for production of charged molecular species, and their separation by magnetic and electric fields based on mass to charge ratio



Unique features of MS

- Molecular specificity
- Detection sensitivity
- Versatility and wide applicability
- Analysis of complex samples

Basic principle of MS

- Ionization
- Separation and mass analysis
- Detection and generation of mass spectrum

General properties of MS

- Sensitivity, resolution and accuracy vary
- Sensitivity drops off as mass increases
- Ion sources generate positive, negative ions & neutrals

Mass spectrometer measures m/z

+1 charge state: $[M+H]$

+2 charge state: $[M+2H]^{++}$

+3 charge state: $[M+3H]^{+++}$

Multiple charge states in ESI

- Many charge states in protein
  - many possible proton acceptors in equilibrium with solution
- Multiple charge states are useful

Average and monoisotopic masses of amino acids

Amino acid	3LC	SLC	Average	Monoisotopic
Glycine	Gly	G	57.0519	57.02146
Alanine	Ala	A	71.0788	71.03711
Serine	Ser	S	87.0782	87.02303
Proline	Pro	P	97.1167	97.05276
Valine	Val	V	99.1326	99.06841
Threonine	Thr	T	101.1051	101.04768
Cysteine	Cys	C	103.1388	103.00919
Leucine	Leu	L	113.1594	113.08406
Isoleucine	Ile	I	113.1594	113.08406
Asparagine	Asn	N	114.1038	114.04293
Aspartic acid	Asp	D	115.0886	115.02694
Glutamine	Gln	Q	128.1307	128.05858
Lysine	Lys	K	128.1741	128.09496
Glutamic acid	Glu	E	129.1155	129.04259
Methionine	Met	M	131.1926	131.04049
Histidine	His	H	137.1411	137.05891
Phenyalanine	Phe	F	147.1766	147.06841
Arginine	Arg	R	156.1875	156.10111
Tyrosine	Tyr	Y	163.1760	163.06333
Tryptophan	Trp	W	186.2132	186.07931

Top down approach

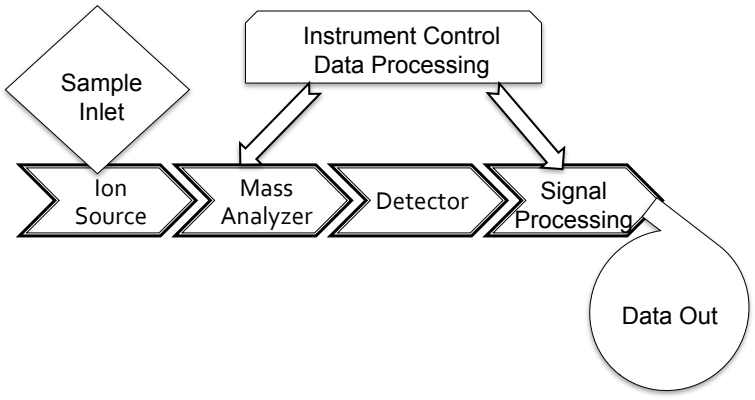
- An analytical approach of separating and analyzing intact proteins
- Top-down involves direct analysis of intact proteins, without previous proteolytic digestion

Bottom-up approach

- Bottom up - analytical approach of separating & analyzing peptides following proteolytic digestion of a sample
  - digesting a protein mixture into short peptides with a protease
  - analyzing the peptide mixture by MS

Parts of mass spectrometer

Basic components of a mass spectrometer



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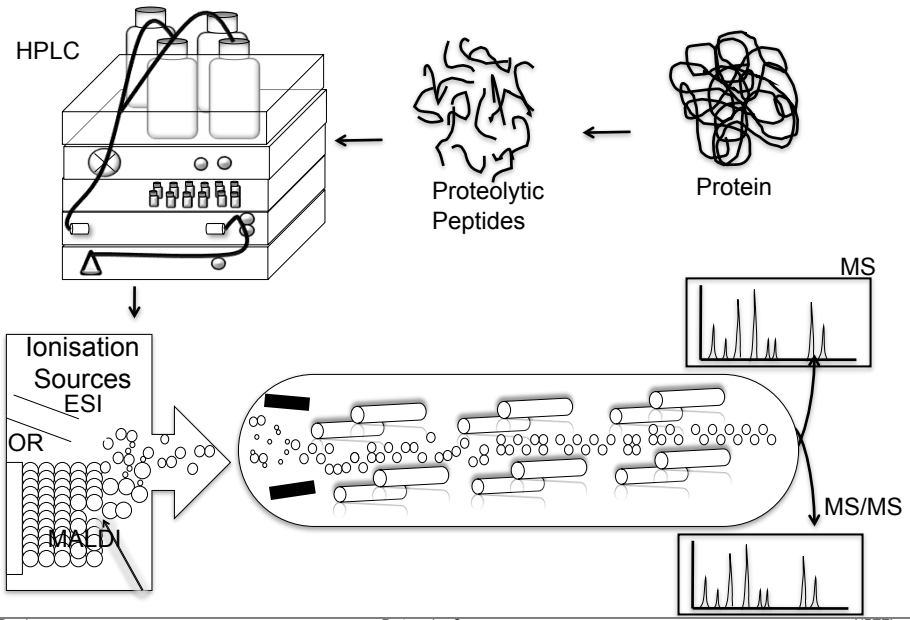
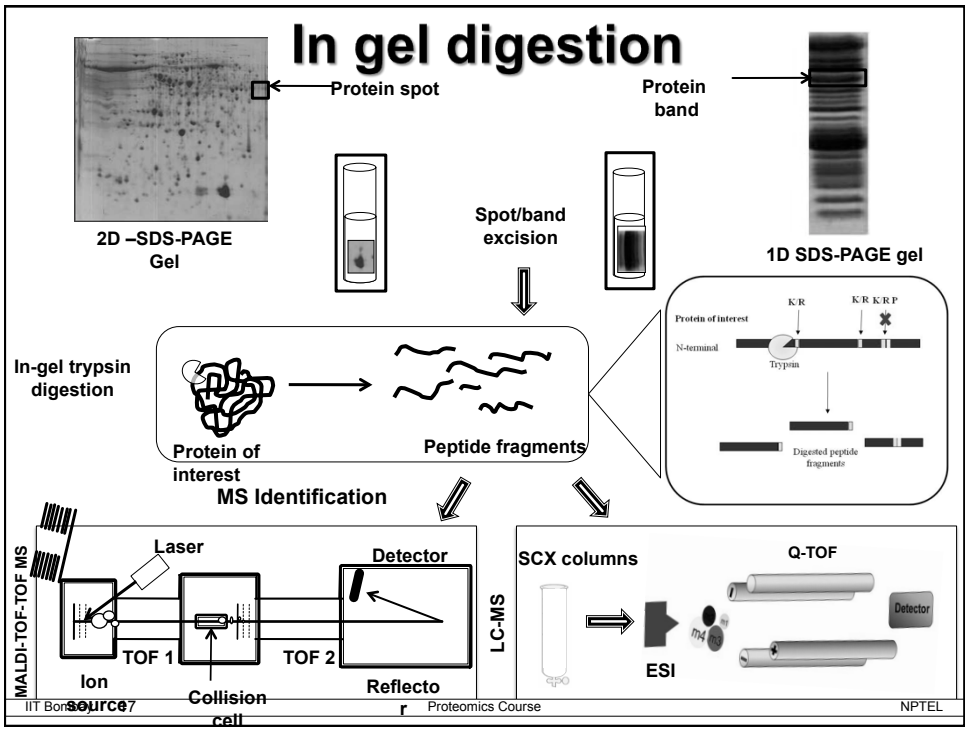
MS components

- Sample introduction
- Sample ionization
- Sample transfer to high vacuum region
- Ion mass-to-charge filtering
- Ion detection
- Data acquisition and analysis

MS experiment – work-flow





Ionization sources

Gas phase	Solution Phase	Solid Phase
Electron ionization	Electrospray	Matrix-assisted laser desorption
Chemical ionization (CI)	Atmospheric-pressure PI	Plasma desorption
Photoionization (PI)	Atmospheric-pressure CI	Fast Atom Bombardment

Need for soft ionization methods

- In MS due to ionization generally large molecules are broken into several random fragments
- Non-selective fragmentation
- Very difficult to interpret
- Therefore, need for soft ionization methods

Properties of Ionization source

- High ionization efficiency
- Stable ion beam
- Minimum background ion current
- Less cross-contamination in successive samples

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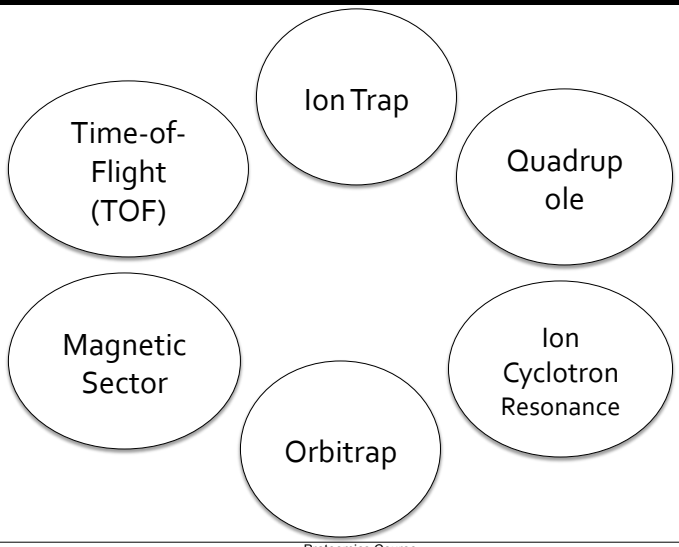
Ionization Sources:
Animation

Mass Analyzer

- Disperses all ions based on mass-to-charge ratio
- Focuses all mass-resolved ions at a single focal point

Types of mass analyzers



Mass analyzers: desirable features

- Mass range: the maximum allowable m/z ratio amenable to analysis
- Resolution: ability to separate two neighboring mass ions
- Adaptability

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Mass analyzers: desirable features (2)

- Efficiency: transmission multiplied by the duty cycle
- Mass accuracy: how far is the measured mass from the actual mass
- Linear dynamic range
- Speed of spectra acquisition per unit time

Mass analyzers: desirable features (3)

- Sensitivity: minimum concentration of a compound that the instrument can detect
- Mass Stability

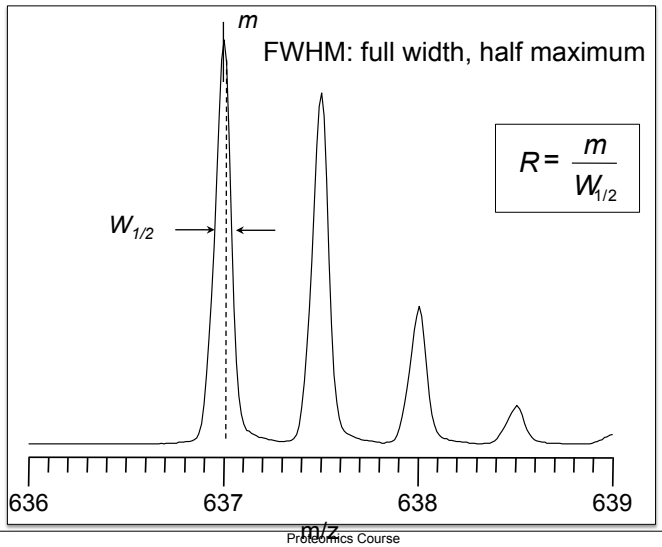
Mass resolution

- Ability of a mass spectrometer to resolve different molecular species with similar but distinct masses
- Mass resolution is the dimensionless ratio of m/z value of a peak divided by its width at half maximum intensity

High resolution is desirable

- Accurate mass measurements
- Resolve an isotopic cluster when charge state of high-mass compounds is to be determined
- Enhance the accuracy of quantification

Mass resolution



Mass accuracy

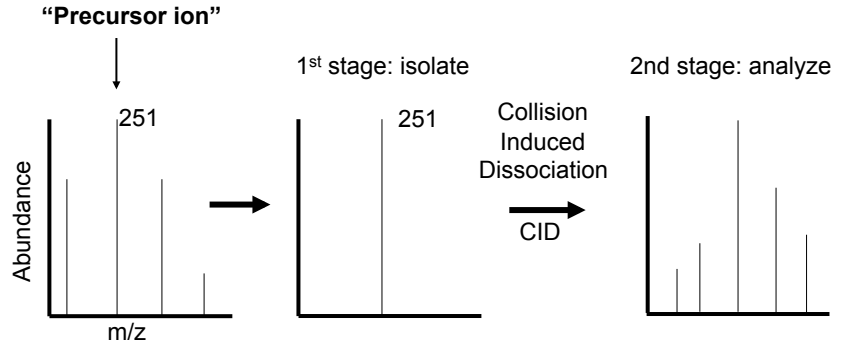
- Mass accuracy - how close a mass measurement is to its true (theoretical or exact) value
- Expressed in parts-per-million (ppm)

$$\text{Parts per million (PPM)} = \frac{[\text{Mass}_{theor} - \text{Mass}_{exp}]}{\text{Mass}_{theor}} \times 10^6$$

Mass Analyzers:
Animation

Tandem MS

Two consecutive stages of mass analysis to detect fragment ions



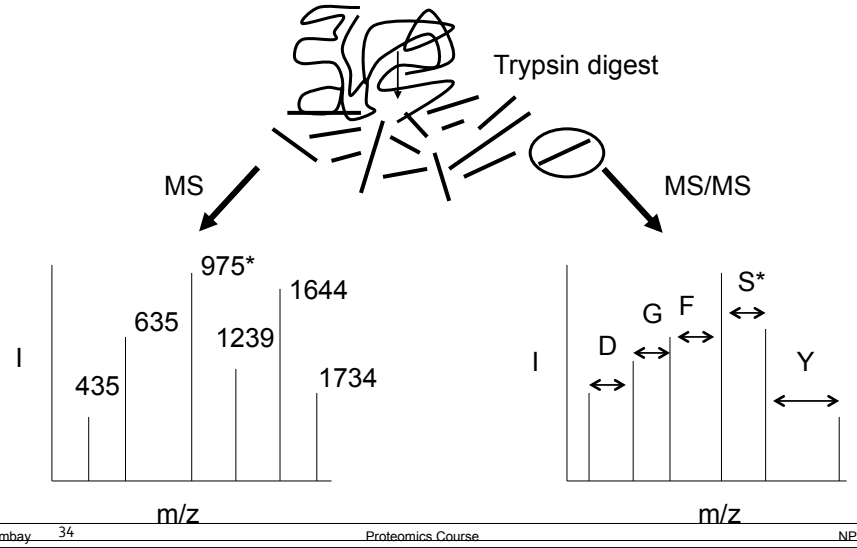
Fragmentation methods

CID	IRMPD	ECD
ETD	EI	CI

MALDI-TOF Applications

PSD	ISD	CID

MS vs. MS/MS



Tandem MS:
Animation

Summary

- Fundamental of Mass Spectrometry
  - Role of MS and basic concepts
- Ionization Sources
- Mass Analyzers
- Tandem Mass Spectrometry

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