Proteomics Course

LECTURE-22
Liquid chromatography-Mass spectrometry (LC-MS/MS)

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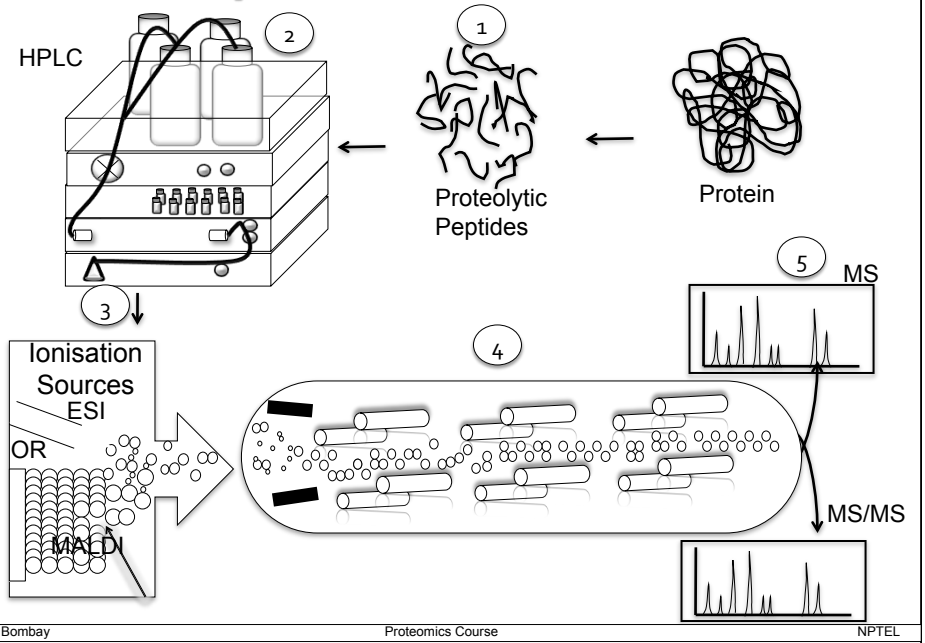
Lecture outline

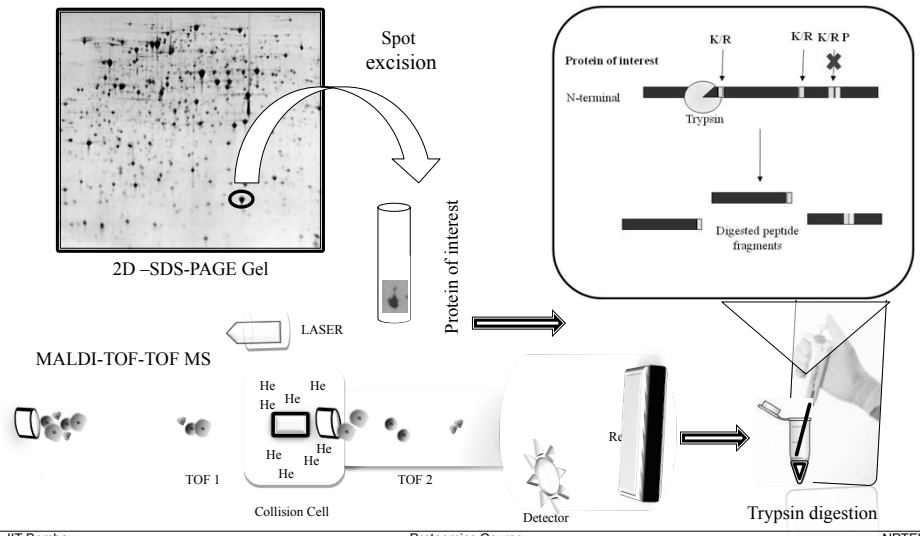
- Mass spectrometry work-flow
- Liquid chromatography
- In-gel digestion
- Ionization source
- Mass analyzers
- Tandem mass spectrometry

MS experiment – work-flow



(1) In-gel digestion

In-gel digestion



In-gel digestion:
Animation

(2) Separation technology – Liquid chromatography (LC)

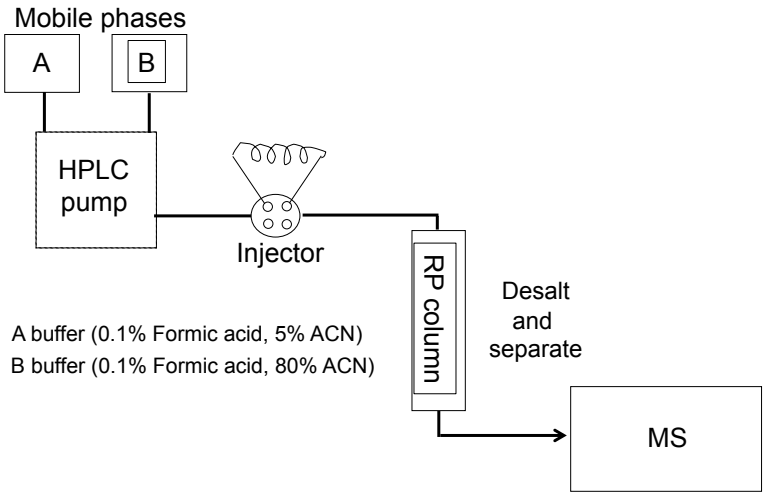
Liquid chromatography (LC)

- Separate mixture components on basis of differences in affinity for stationary & mobile phase
- Removes undesired impurities
- Increased sensitivity, detection of low level proteins
- Separates peptide mixture

Reversed phase (RP) chromatography

- Based upon hydrophobic binding interaction between
  - peptides/proteins (mobile phase)
  - immobilized hydrophobic ligand (stationary phase)

RP-HPLC configuration

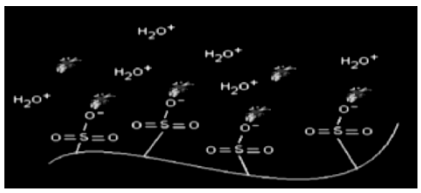


RP-HPLC with ESI

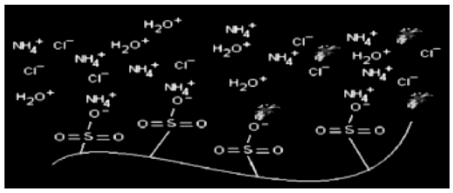
- RP is used with ESI
  - due to compatibility of RP's acidic aqueous & polar mobile with ESI
- In-line RP-HPLC is useful
  - desalting peptides before ESI
  - no need for off-line desalting

Strong cation exchange (SCX) resin

- Silica based cation exchange stationary phase
- Sulfonic acid cation-based exchange ligand
- Ligand covalently bound to polymer coated silica



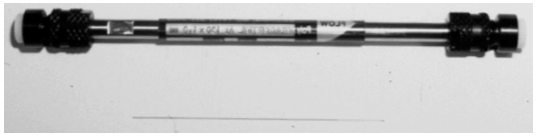
Retention



Elution

Microcapillary HPLC columns

- Microcapillary HPLC's low flow rate is more sensitive than standard RP-HPLC
- Microcapillary HPLC columns prepared using fused silica capillary



Column packing

Multidimensional separations

- Multidimensional separations
  - Size exclusion chromatography (SEC)
  - Ion exchange chromatography (IEX)
  - Capillary electrophoresis (CE)
  - Reversed-phase (RP)
  - Affinity chromatography

Multidimensional approaches coupled with MS

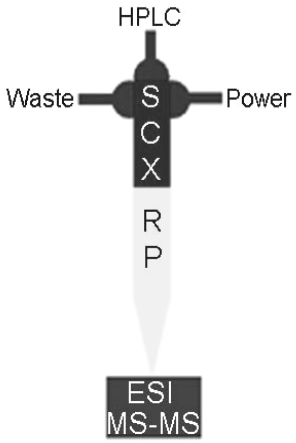
I. SEC → RP

II. RP → CE
SEC → CE

III. IMAC → RP
Avidin → RP

IV. SCX → RP

MUltimensional Protein Identification Technology (MudPIT)



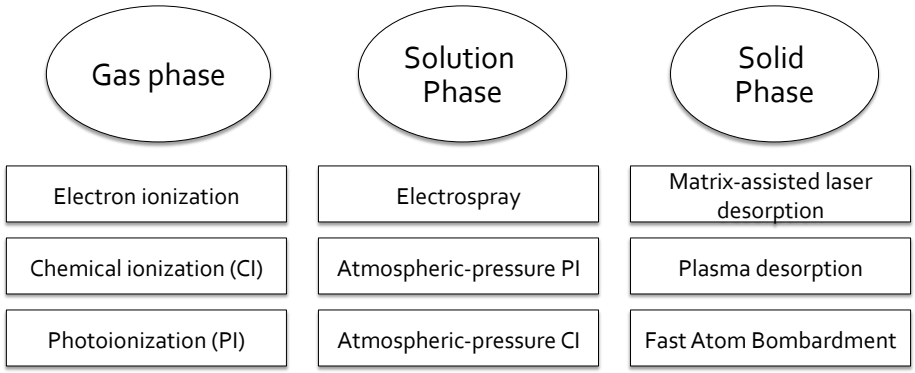
SCX – separation by charge

RP (C18) – separation by hydrophobicity

Liquid chromatography: *Animation*

(3) Ionization sources

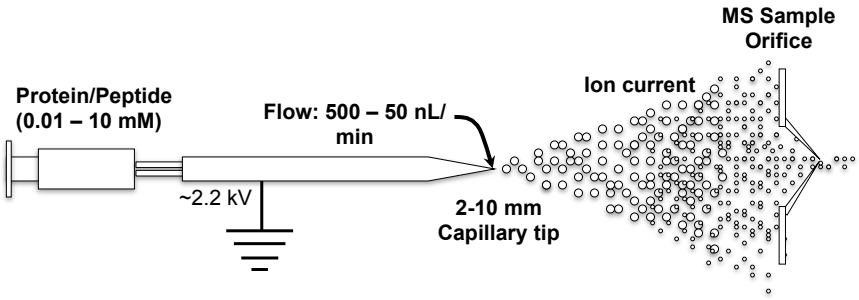
Ionization sources



Electrospray ionization (ESI)

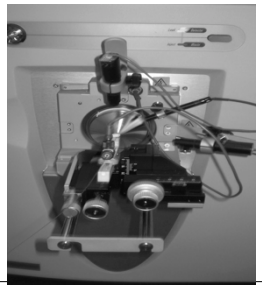
- ESI requires sample of interest to be in solution
- To ionize samples high voltage is applied to high conductively coated needle
- Distinguishing feature of ESI
  - its ability to produce multiply charged ions

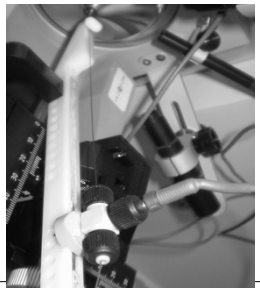
ESI



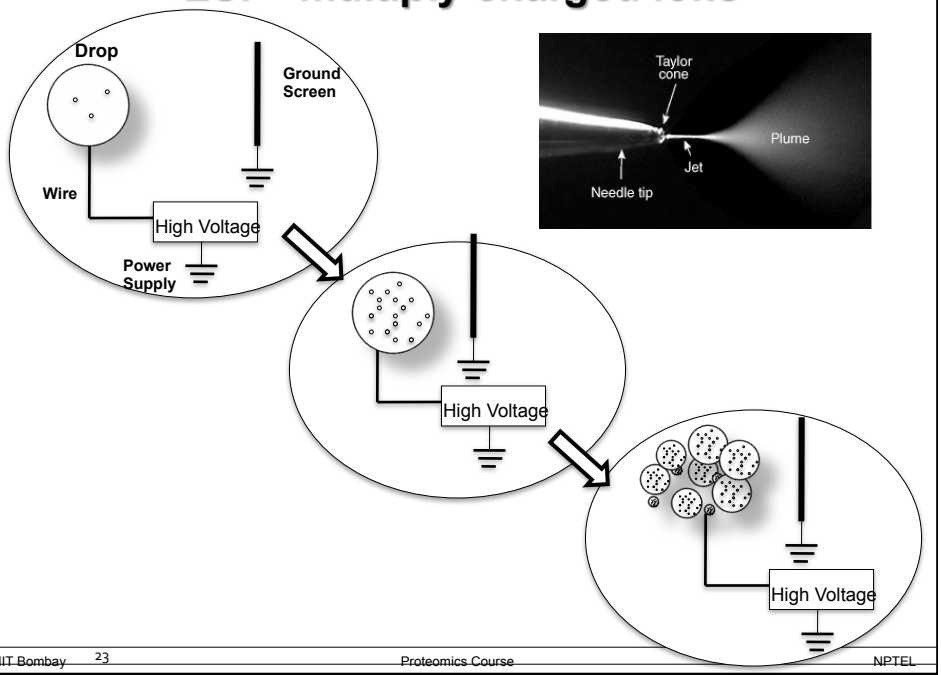
Electrospray ionization (ESI)

- Desolvation of ions occurs at atmospheric pressure and mass analyzer is maintained at lower pressure
- During movement, evaporation reduces droplet size
- Ions when enter into MS, droplets are dried using a stream of inert gas



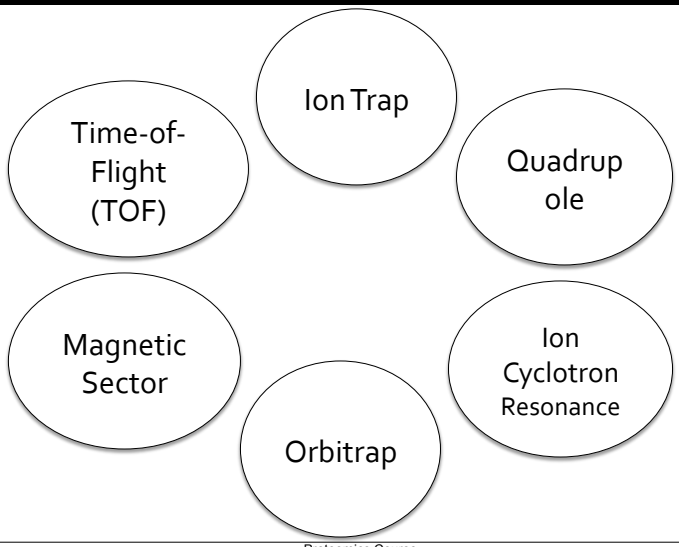


ESI – multiply charged ions

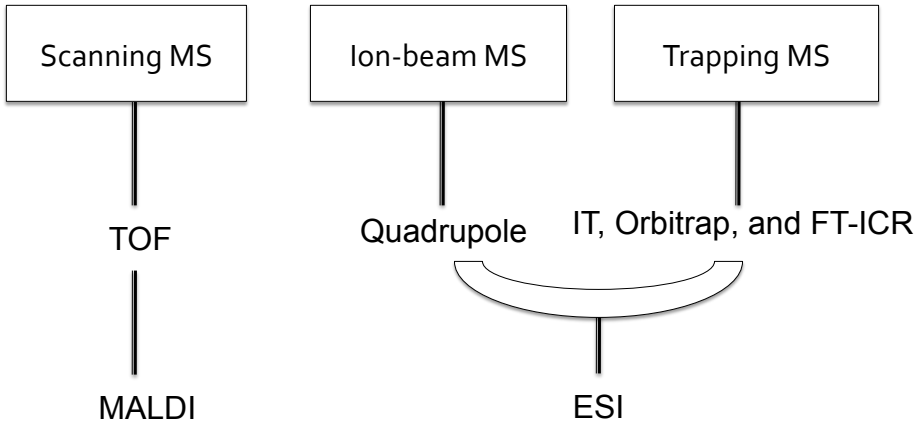


(4) Mass analyzers

Types of mass analyzers

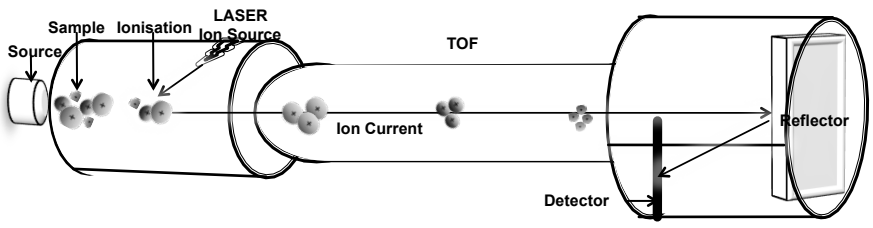


Mass analyzers: categories



Time of Flight (TOF)

TOF



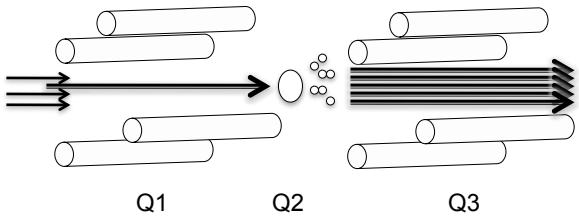
Quadrupole

Quadrupole

- Quadrupole (Q) – set of 4 parallel metallic rods
- Radio frequency mode
- Scanning mode
- Neutral loss scan and precursor ion scanning mode

Triple quadrupole mass spectrometer (TQ)

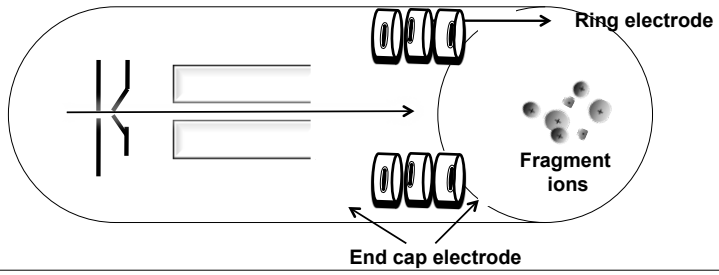
- TQ – 3 arrangements similar to quadrupole



Ion Trap

Ion Trap

- Consist of a chamber surrounded by a ring electrode and two end-cap electrodes
- Voltage applied to ring electrode determines which ion remain in the trap



Fourier transform ion cyclotron resonance

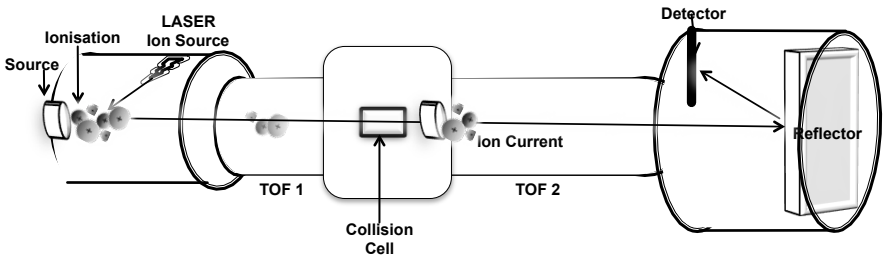
Fourier transform ion cyclotron resonance

- Uses cyclotron motion (cyclotron frequency) to resolve ions
- Most complex, difficult to operate
- Highest resolution, mass accuracy and sensitivity
- Multiple tandem experiments feasible
- MS/MS of very large ions feasible

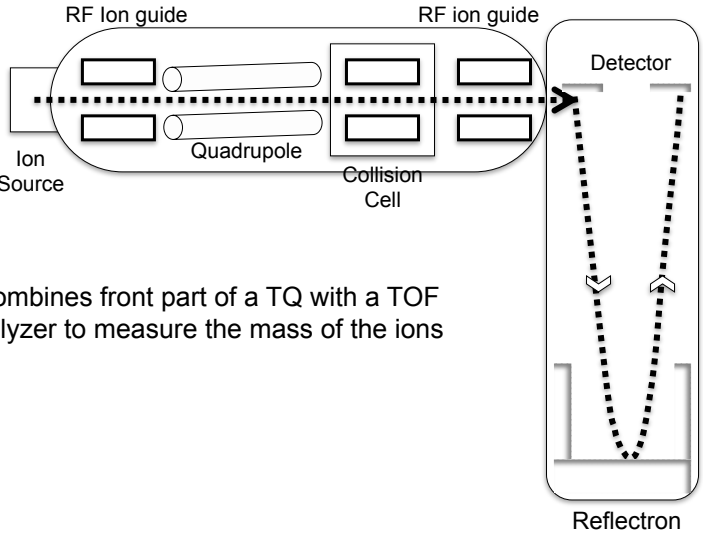
(5) Hybrid-MS & MS configuration comparison

MALDI TOF-TOF

- MALDI can be coupled to tandem TOF-TOF or hybrid Q-TOF analyzers, separated by collision cell
- Much higher sensitivity than TQ and single TOF



Q-TOF



• Combines front part of a TQ with a TOF analyzer to measure the mass of the ions

MS: concepts review
Animation

Performance comparisons of MS instruments

Instrument	Resolution	Mass Accuracy	Sensitivity	Scan Rate
LIT/LTQ (Linear Ion Trap)	2000	100 ppm	Femtomole	Fast
TQ (Triple Quadrupole)	2000	100 ppm	Attomole	Moderate
LTQ-Orbitrap	100,000	2 ppm	Femtomole	Moderate
LTQ-FTICR	500,000	< 2 ppm	Femtomole	Slow
Q-TOF	10,000	2-5 ppm	Attomole	Moderate, Fast

Ref: Annu. Rev. Biomed. Eng. 2009. 11:49–79

Summary

- Mass Spectrometry work-flow
- In-gel digestion
- Liquid chromatography
- Ionization source
- Mass analyzers
- Tandem mass spectrometry

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- Timothy D. Veenstra, John R. Yates. Proteomics for Biological Discovery. Chapter 1. Mass Spectrometry: The Foundation of. Proteomics DOI: 10.1002/0470007745.ch1

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