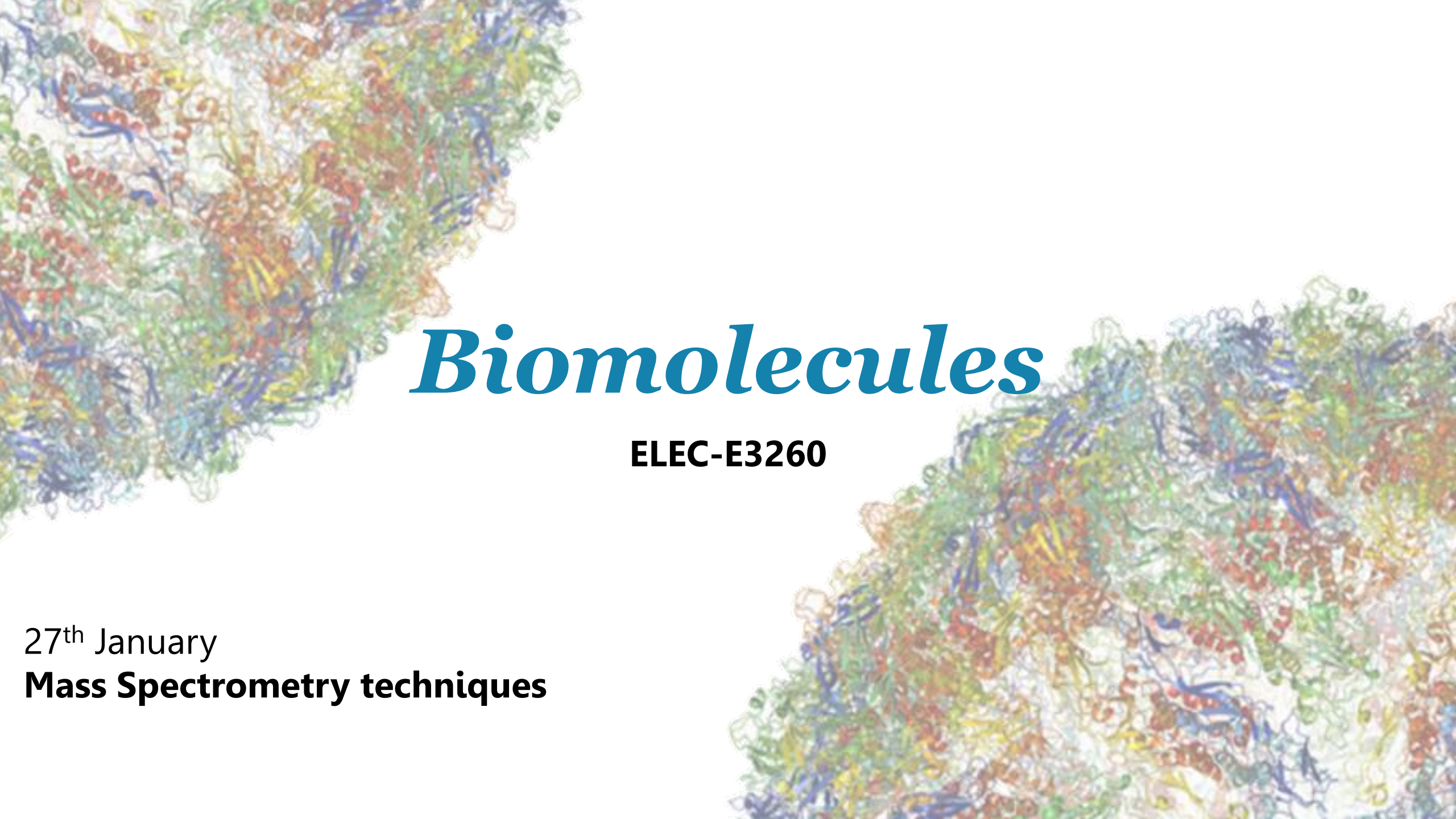


# *Biomolecules*

**ELEC-E3260**

27th January

**Mass Spectrometry techniques**

# Summary

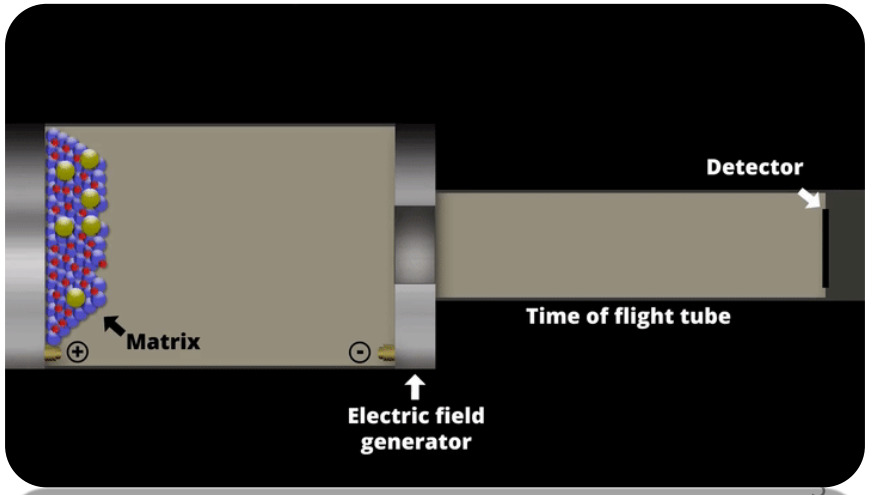
- Brief History
- What is it?
- How is it working?
- Mass Spectrometry and Biomolecules
- Applications, advantages and disadvantages
- In-class activity

# Brief History

- Sir J.J. Thomson: The 'Father' of Mass Spectrometry. 1897-1919 Developed the Mass Spectrograph (with F.W. Aston).
- Nobel Prize of Physics for Thomson in 1906.
- The World's first commercial instrument became available in 1948.
- Time-of-flight (TOF) analysis (Wiley and Maclaren) and quadruple analysis (Paul) were conceived in the early 1950's.
- In 1956, the first biologically important molecules were successfully analyzed.

The MS-2 - marketed by Vickers

Sir J.J. Thomson

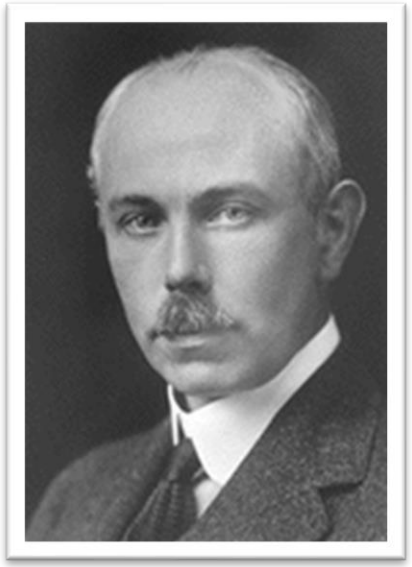


# Some Nobel Prize Pionners For MS

**J.J. Thomson**

**1906 Nobel Prize of Physics**

*For his development of Mass spectrograph*



**Francis William Aston**

**1922 Nobel Prize for Chemistry**

*For his discovery, by means of his mass spectrograph, of isotopes, in a large number of non-radioactive elements, and for his enunciation of the whole-number rule*

**Wolfgang Paul**

**1989 Nobel Prize for Physics**

*For the development of the ion trap technique*

**John Bennet Fenn**

**2002 Nobel Prize for Chemistry**

*For the development of soft desorption ionisation methods (ESI) for mass spectrometric analyses of biological macromolecules*

**Koichi Tanaka**

**2002 Nobel Prize for Chemistry**

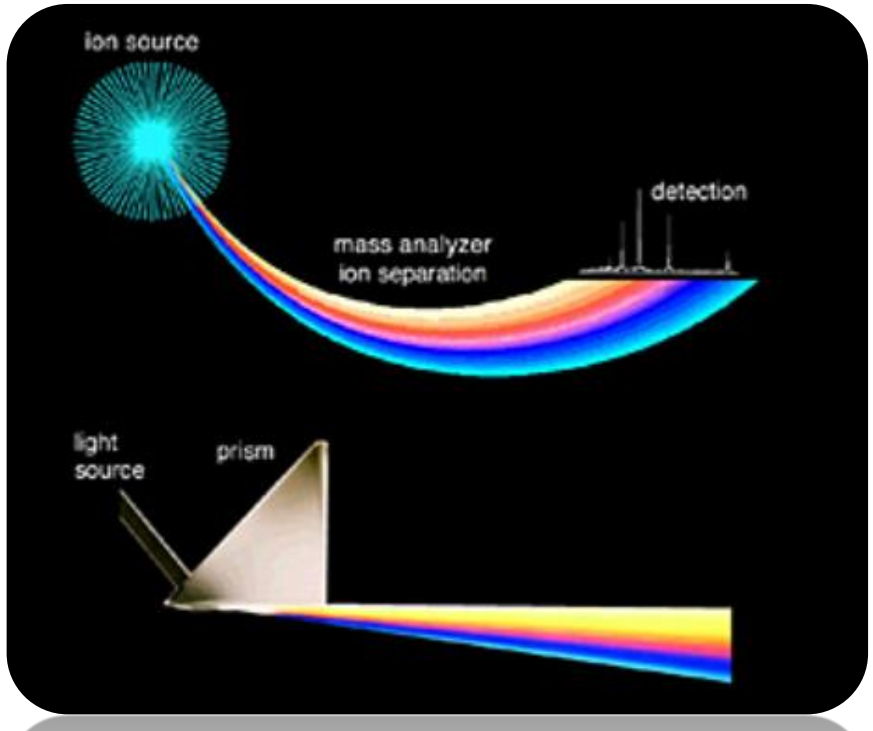
*for the development of soft desorption ionisation methods (MALDI) for mass spectrometric analyses of biological macromolecules*

# What is it?

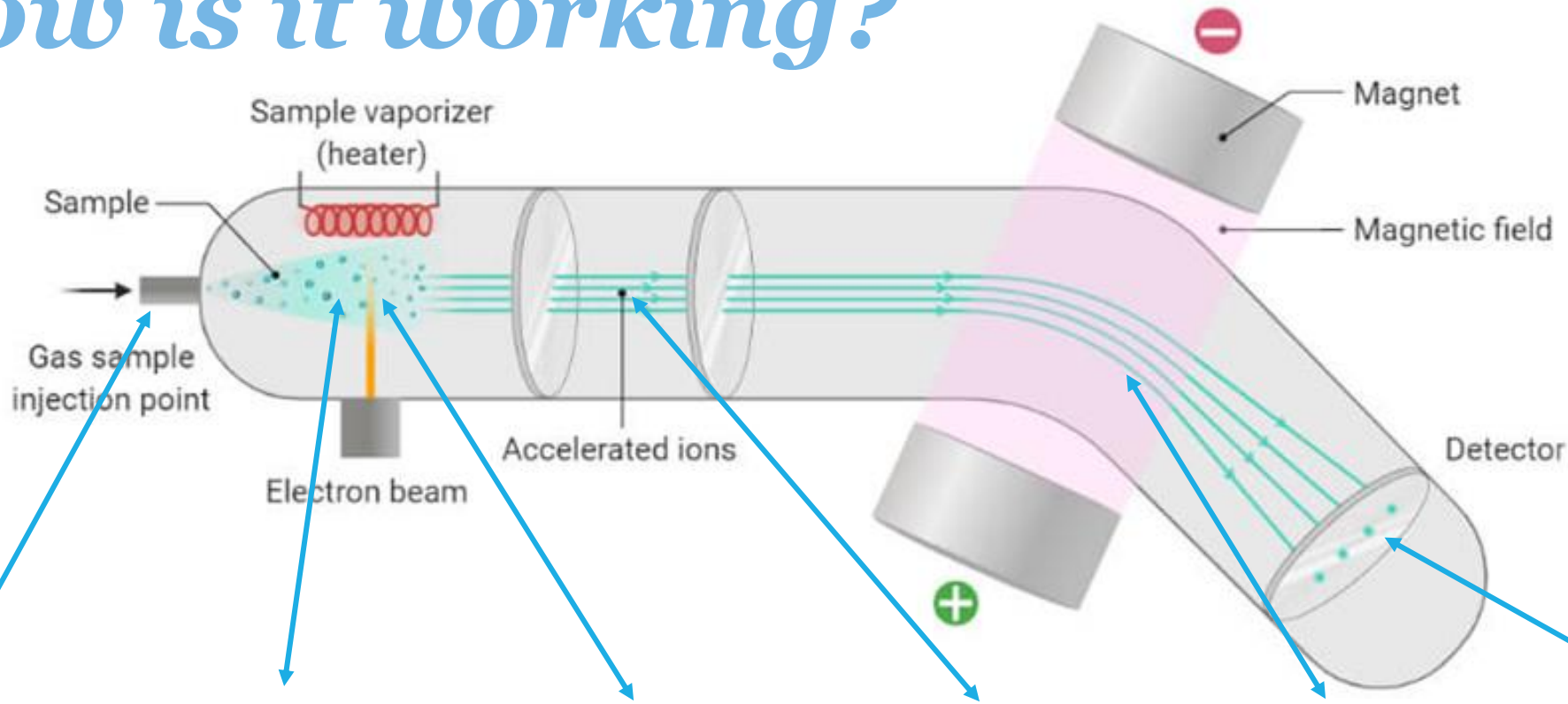
*"Mass spectrometry is the art of measuring atoms and molecules to determine their molecular weight. Such mass or weight information is sometimes sufficient, frequently necessary, and always useful in determining the identity of a species. To practice this art, one puts charge on the molecules of interest, i.e., the analyte, then measures how the trajectories of the resulting ions respond in vacuum to various combinations of electric and magnetic fields."*

- MS can:
  - Quantify known materials
  - Identify unknown compounds
  - Elucidate the structure and chemical properties of different molecules
  - Distinguished different isotopes of the same element

By their mass to charge ratios (m/z or e) and relative abundances



# How is it working?




1 2 3 4 5 6

**Sample Inlet**
Sample Storage

**Vaporization**

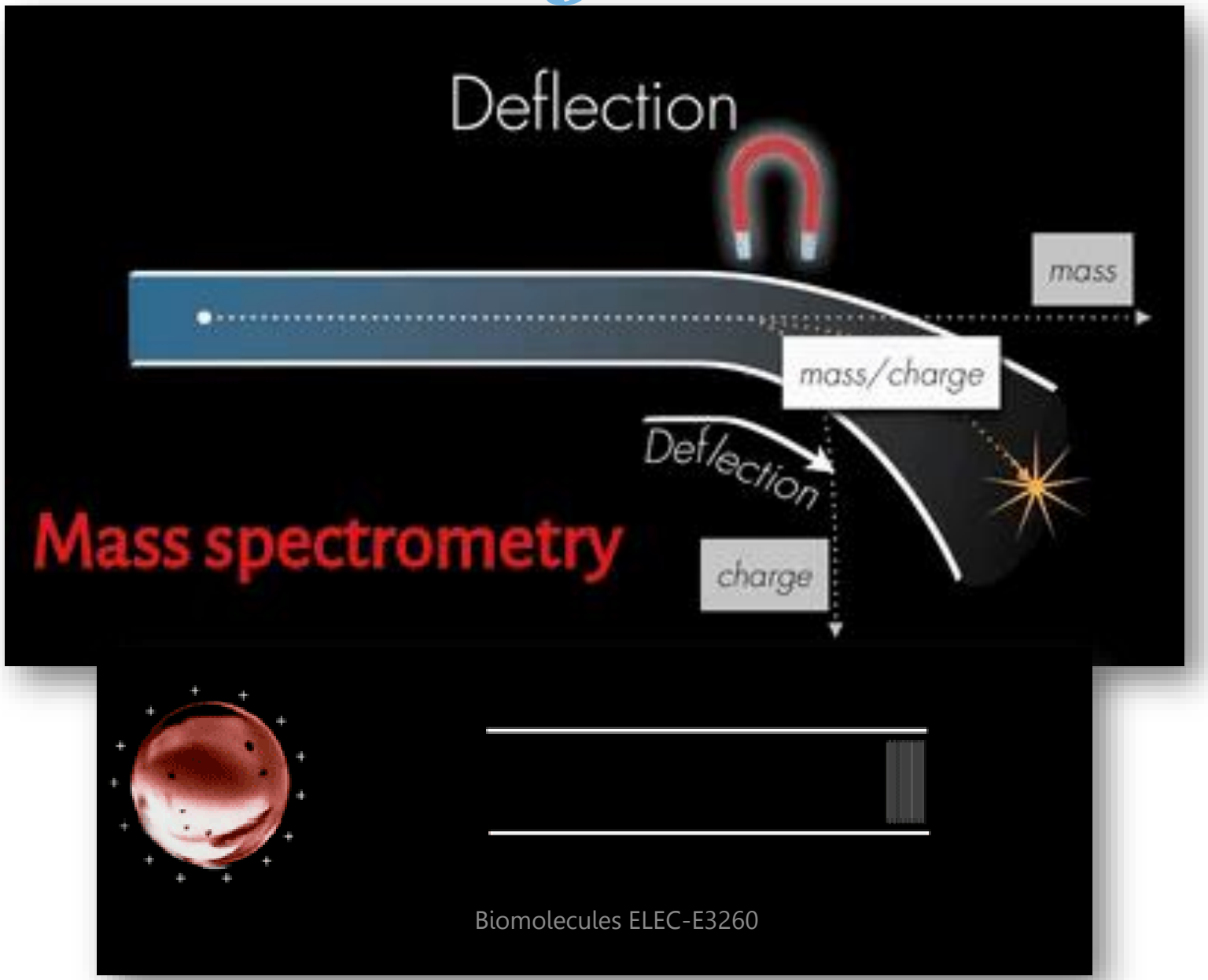
**Ionization**
Molecules break into charged fragments

**Acceleration**
Same Kinetic Energy

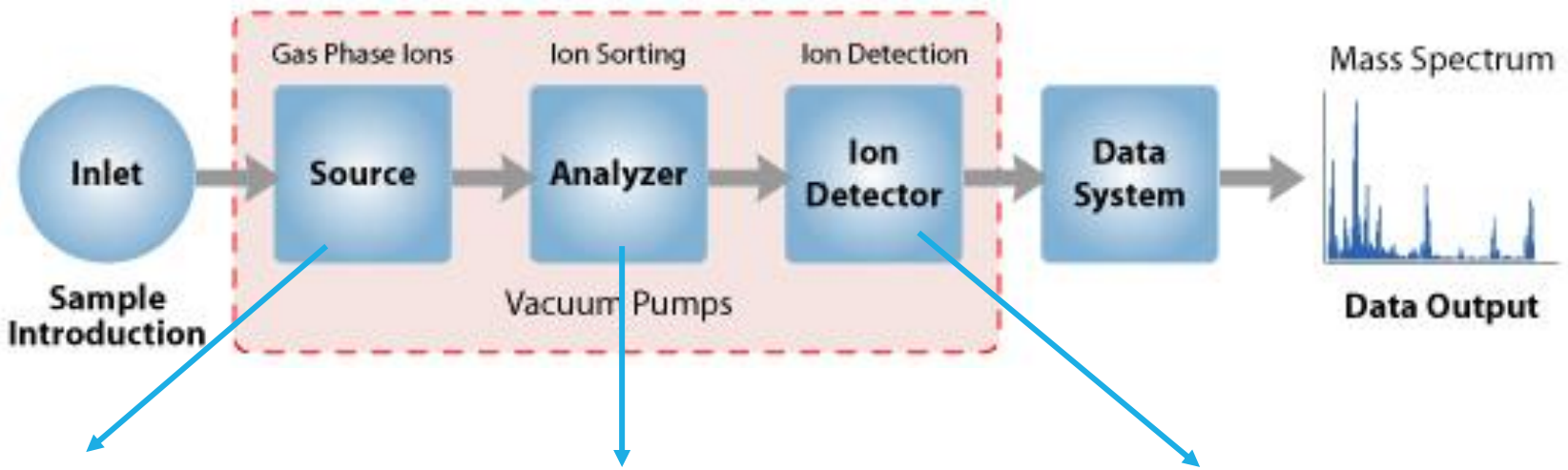
**Deflection**
By a magnetic field due to differences in their masses.

**Detection**
Charge particles -> m/z ratio spectrum

# How is it working?



# What is it ?




**Ion Source:**
For producing gaseous ions from the substance being studied.

**Analyzer:**
For resolving the ions into their characteristics mass components according to their mass-to-charge ratio.

**Detector System:**
For detecting the ions and recording the relative abundance of each of the resolved ionic species..

# Ion Sources

## GAS PHASE METHOD

- ***Electron Ionization (EI):*** Harsh method of molecule fragmentation and ionization and is used for relatively volatile and low molecular weight sample.
- ***Chemical ionization (CI):*** Very soft ionization technique and does not lead to extensive fragmentation.
- ***Direct analysis in real time (DART):*** Ability to analyze materials of different shapes and sizes with no prior sample preparation and in ambient conditions.
- ***Inductively coupled plasma (ICP):*** Ability to ionize almost all elements

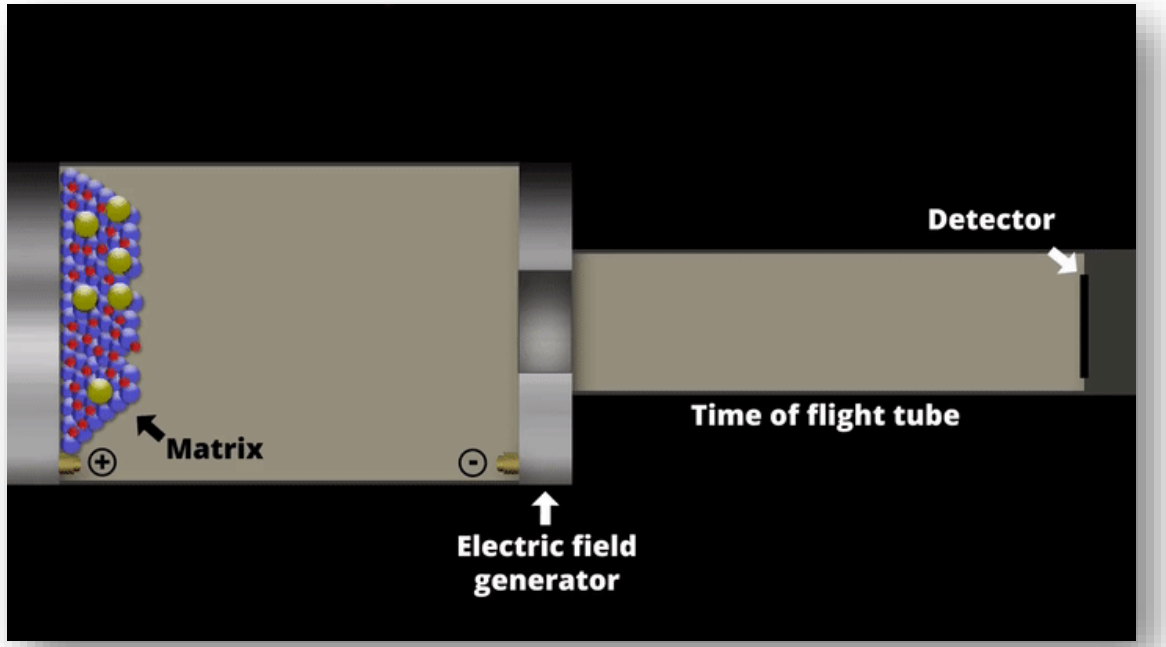
## DESORPTION METHOD

- ***Matrix assisted laser desorption ionization (MALDI):*** One of the major "soft" ionization methods, particularly useful for the analysis of large or labile molecules.
- ***Fast atom bombardment (FAB):*** Soft ionization technique, able to produce positively and negatively charged ions.
- ***Thermal ionization:*** The most common primary ion source and can be focused with electrostatic ion optics for secondary ion MS.
- ***Plasma ionization:*** Commonly used to produce beams of gaseous ions.
- ***Liquid metal ion (LMIS):*** Ion beam with the smallest spot sizes and highest brightness, particularly advantageous in MS imaging.

## SPRAY METHOD

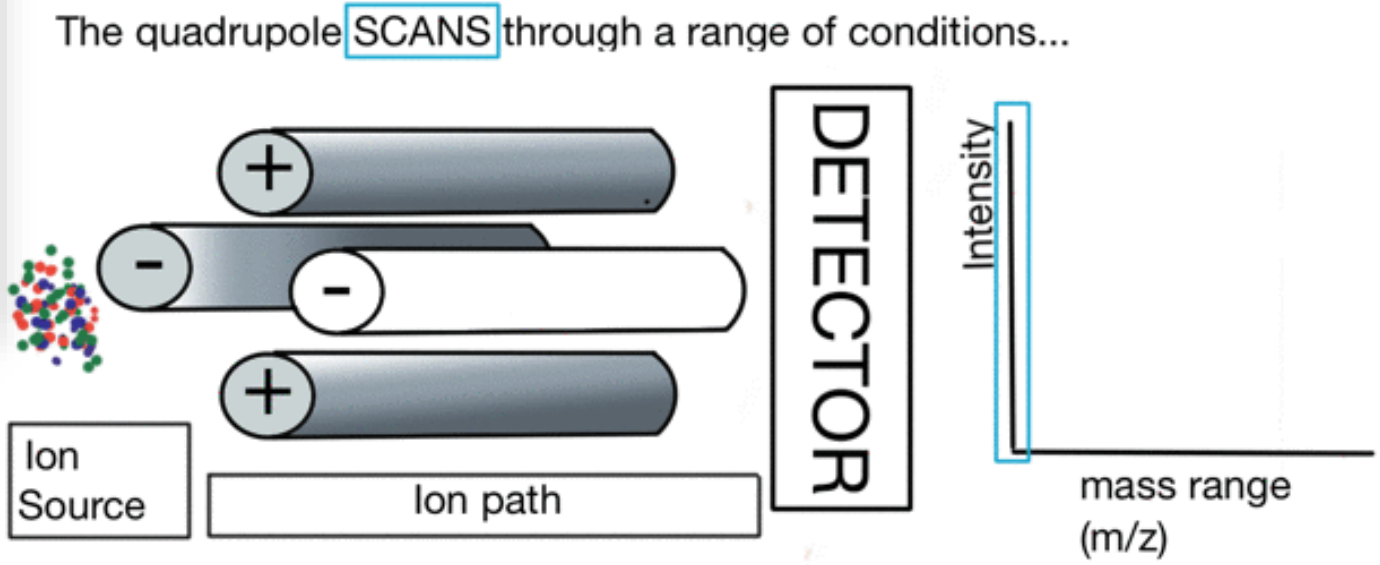
- ***Electrospray ionization (ESI):*** Soft ionization technique suitable for analysis of large molecules and macromolecules.
- ***Desorption electrospray ionization (DESI):*** Droplets are directed to a sample held at ambient pressure.

# MASS ANALYZERS




TOF

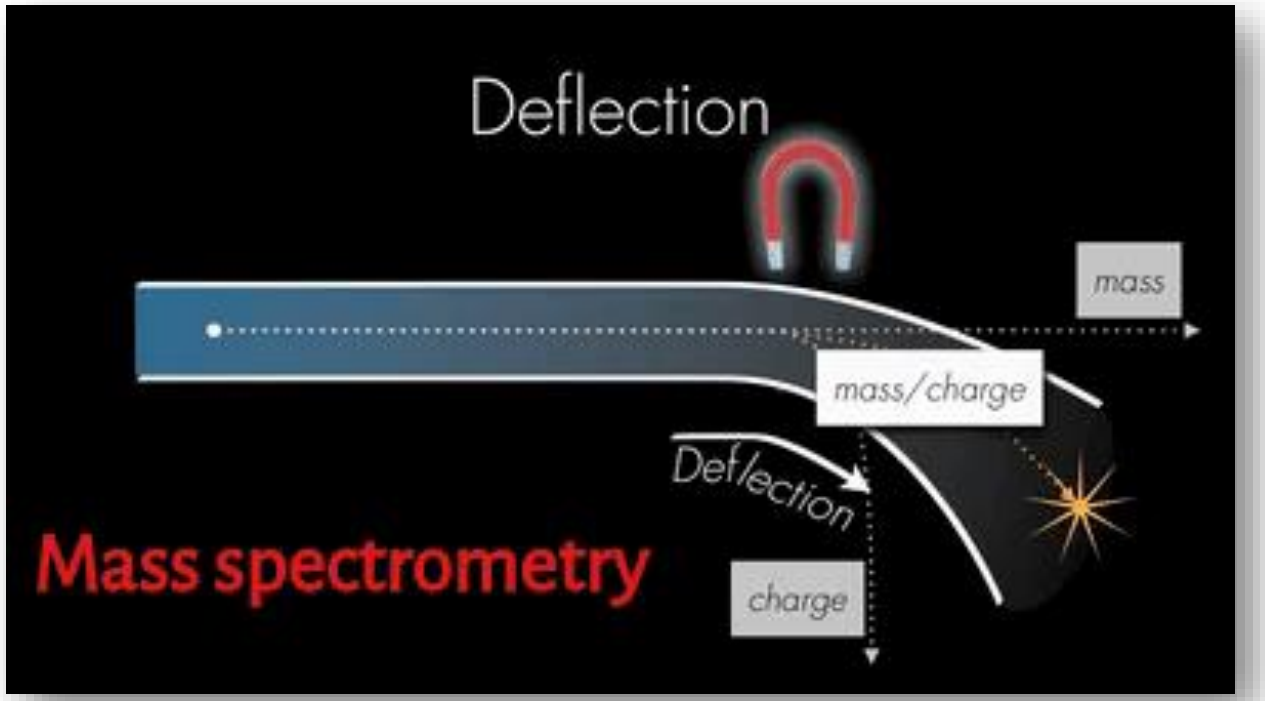
Quadrupole



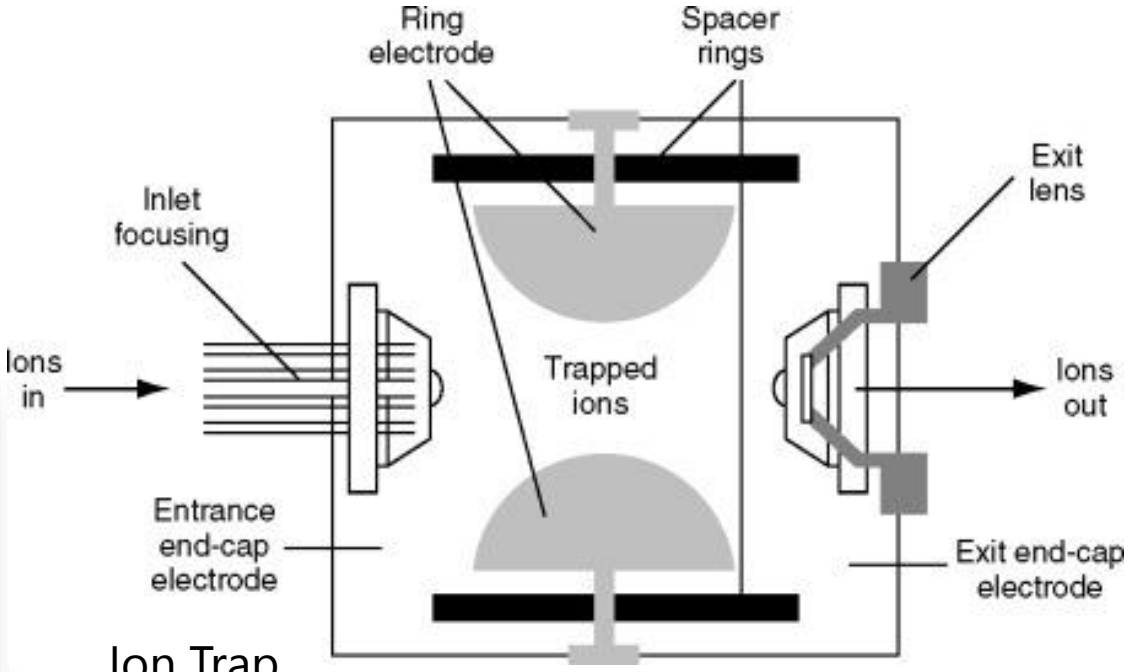

... that lets ions of a specific mass to charge ratio reach the detector.

All the other ions go to waste (over a few milliseconds).

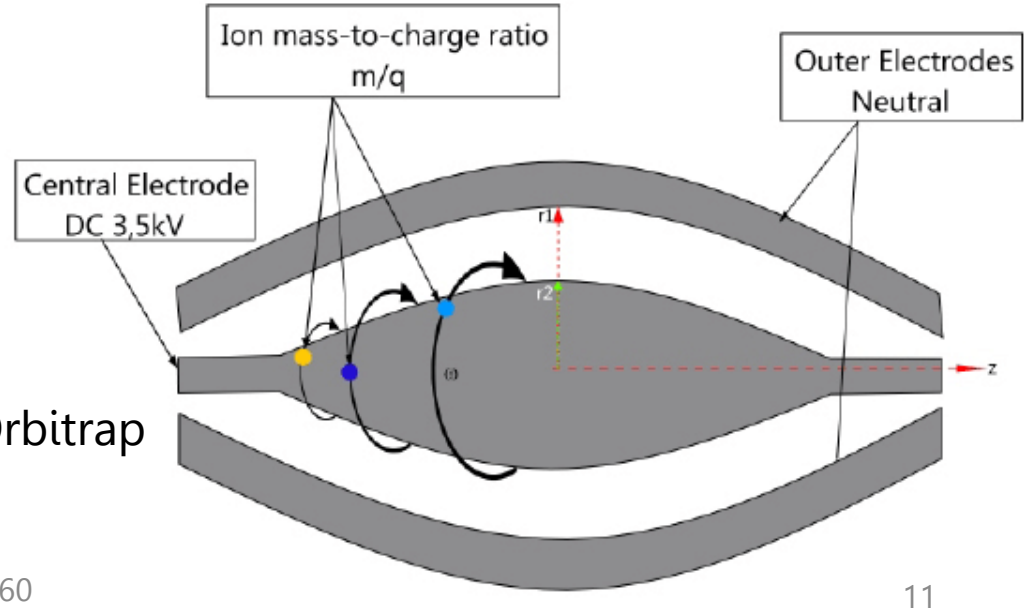
# MASS ANALYZERS



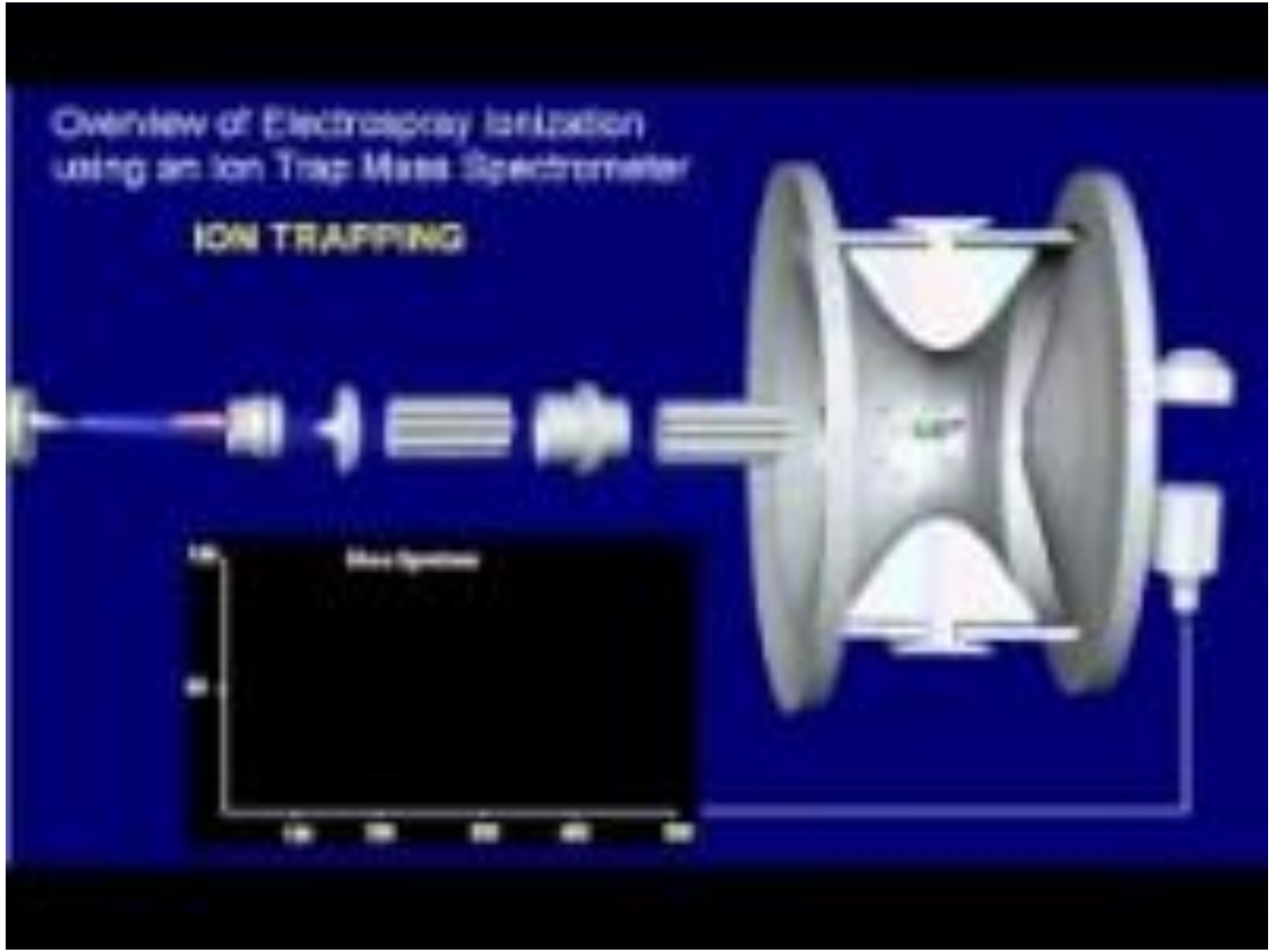

Magnetic Sector



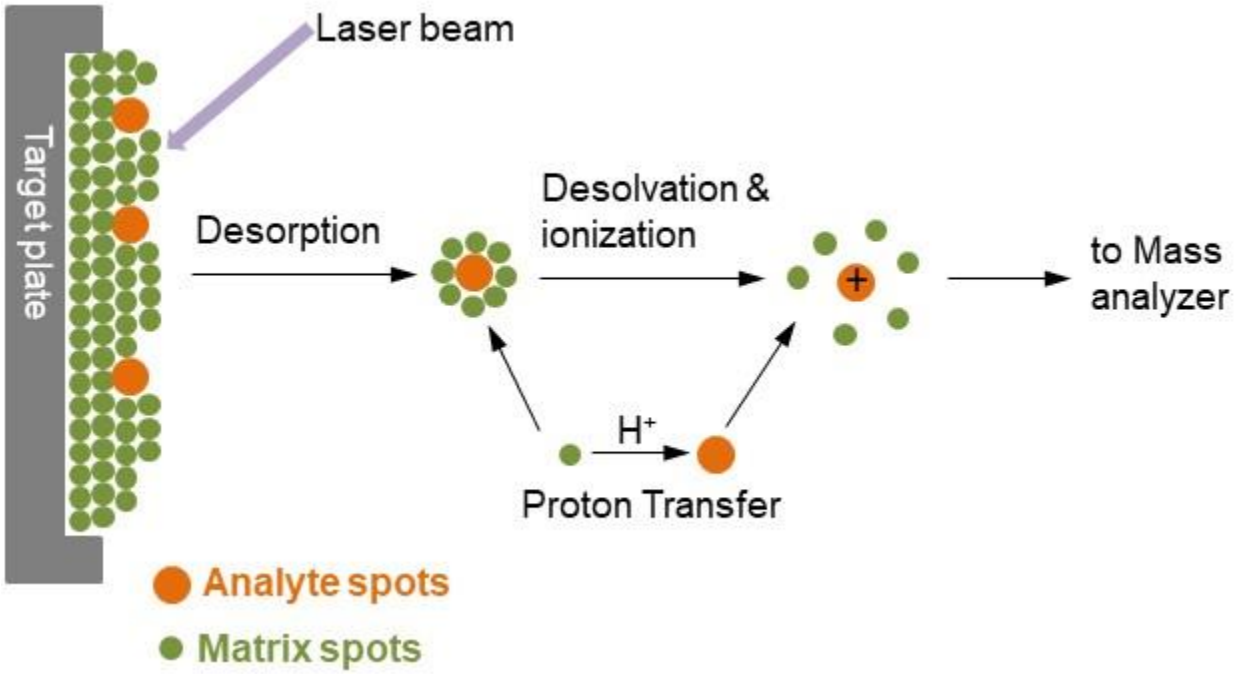

Ion Trap



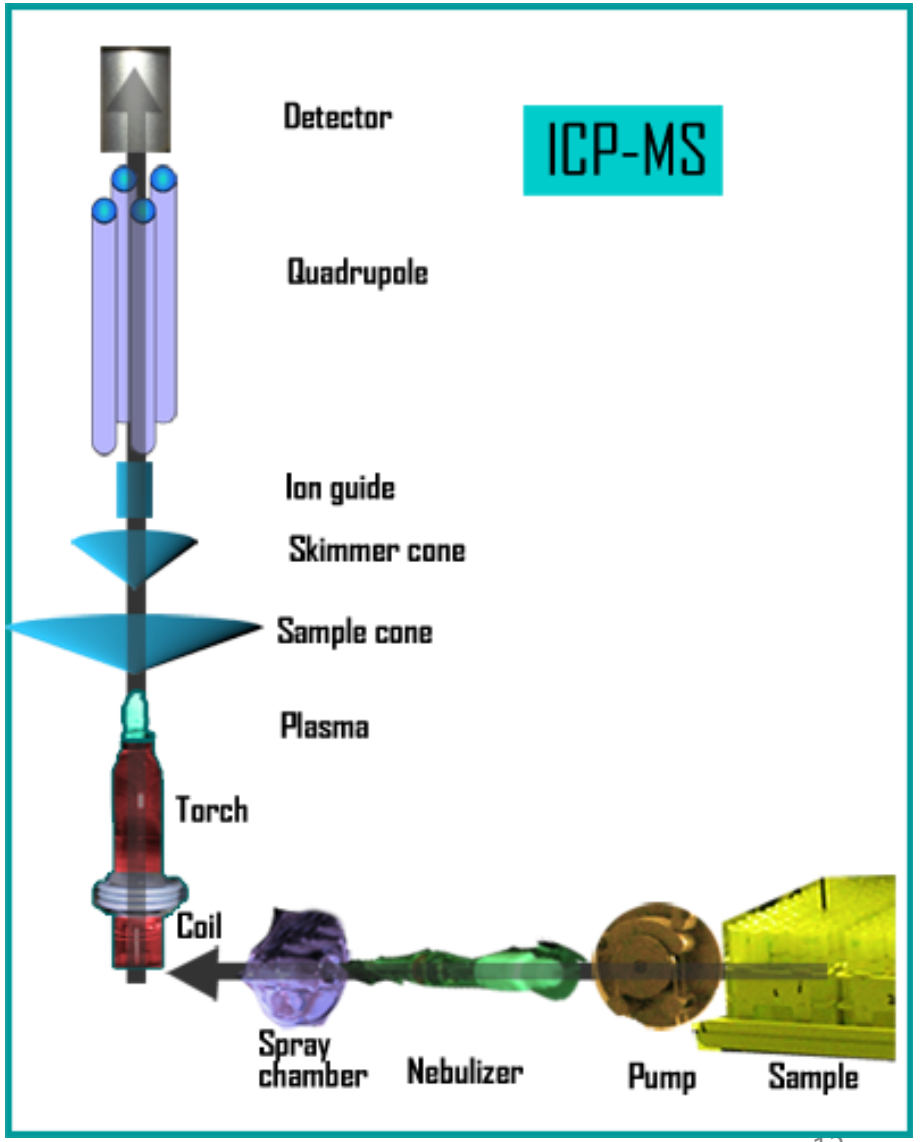

Orbitrap


Overview of Electrospray Ionization
using an Ion Trap Mass Spectrometer
ION TRAPPING

# Pairing ionization techniques with mass analyzers

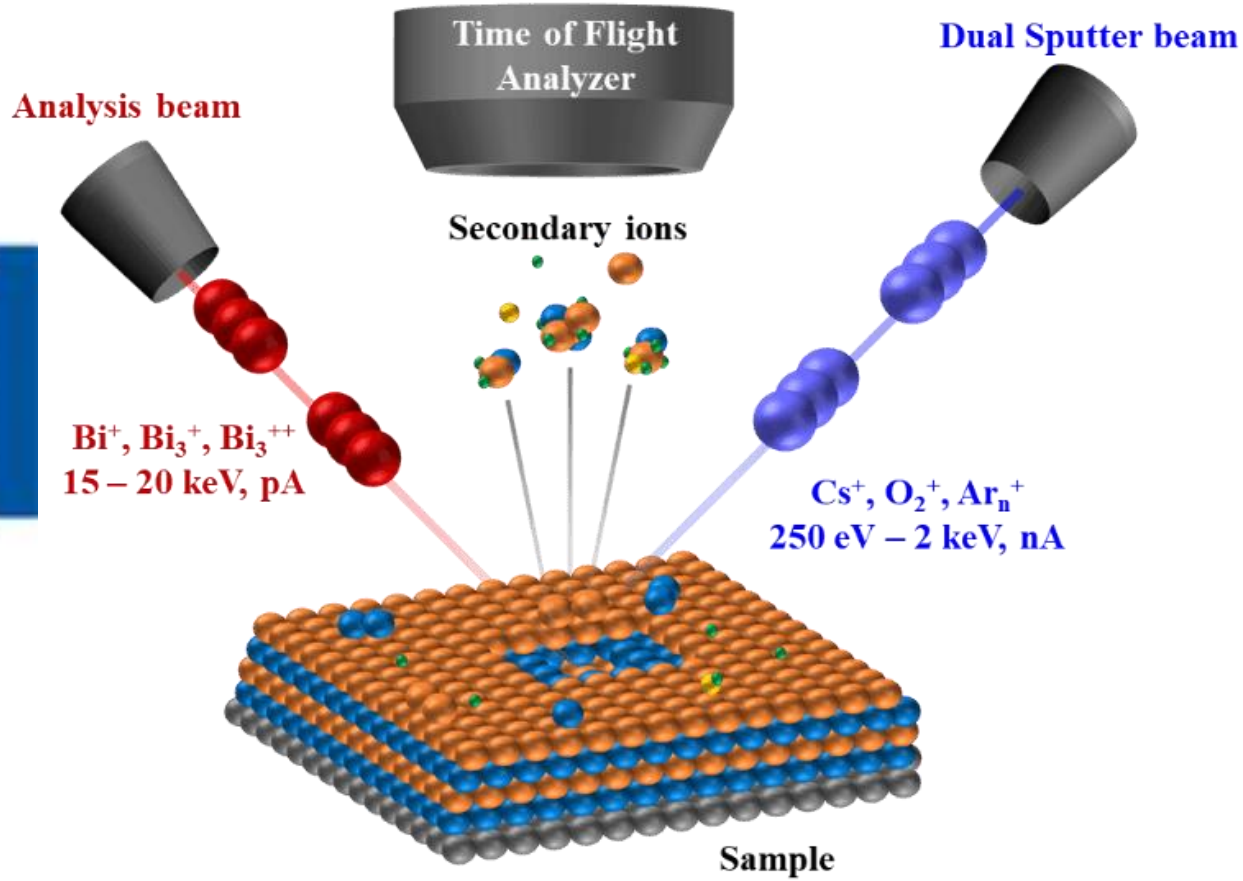



MALDI-TOF



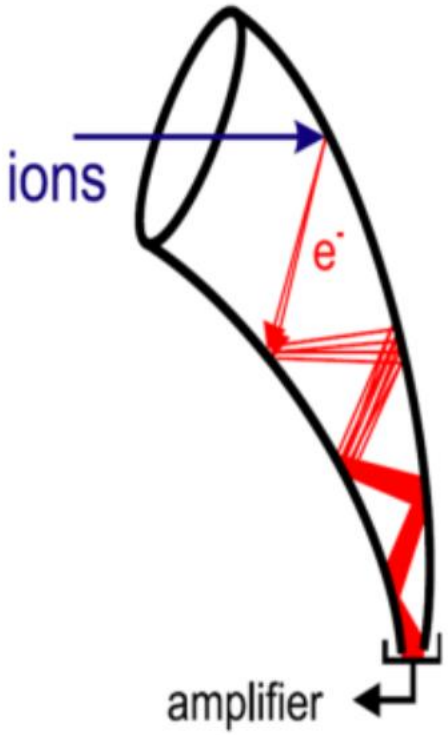
# Pairing ionization techniques with mass analyzers


**DART-MS**

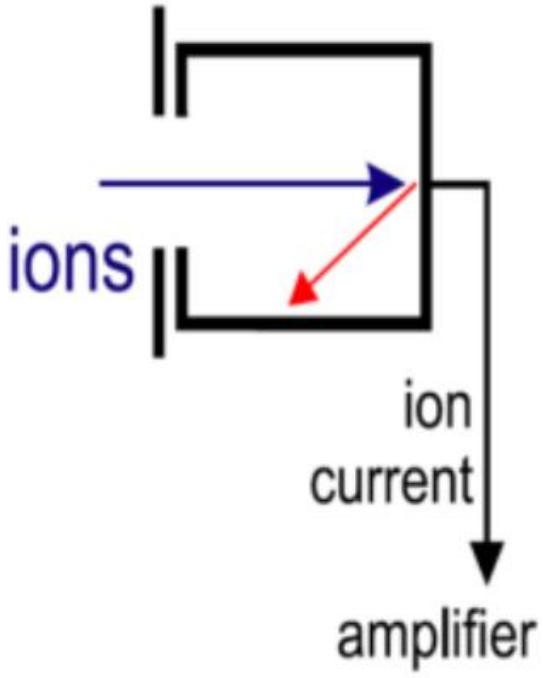



**TOF-SIMS**

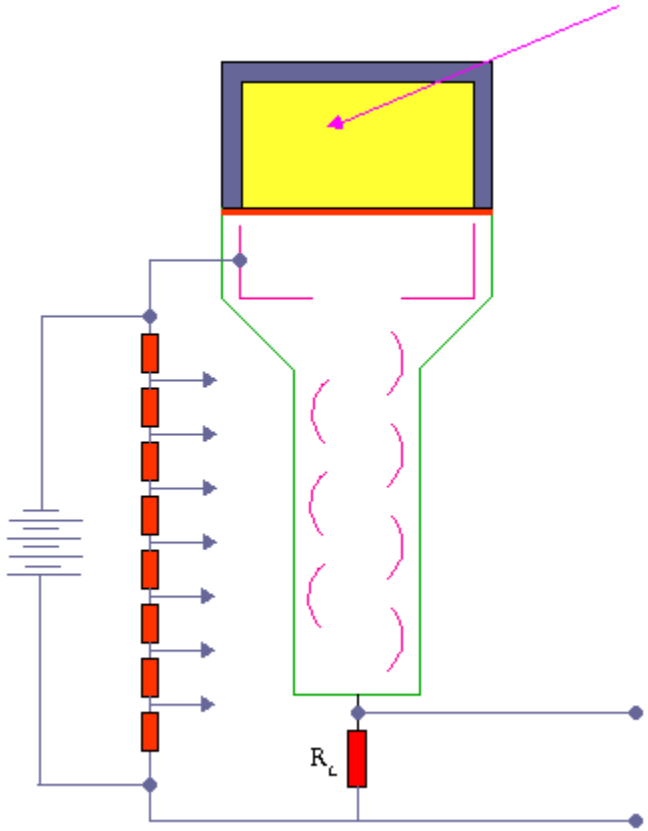
# MASS DETECTORS




Electron Multiplier

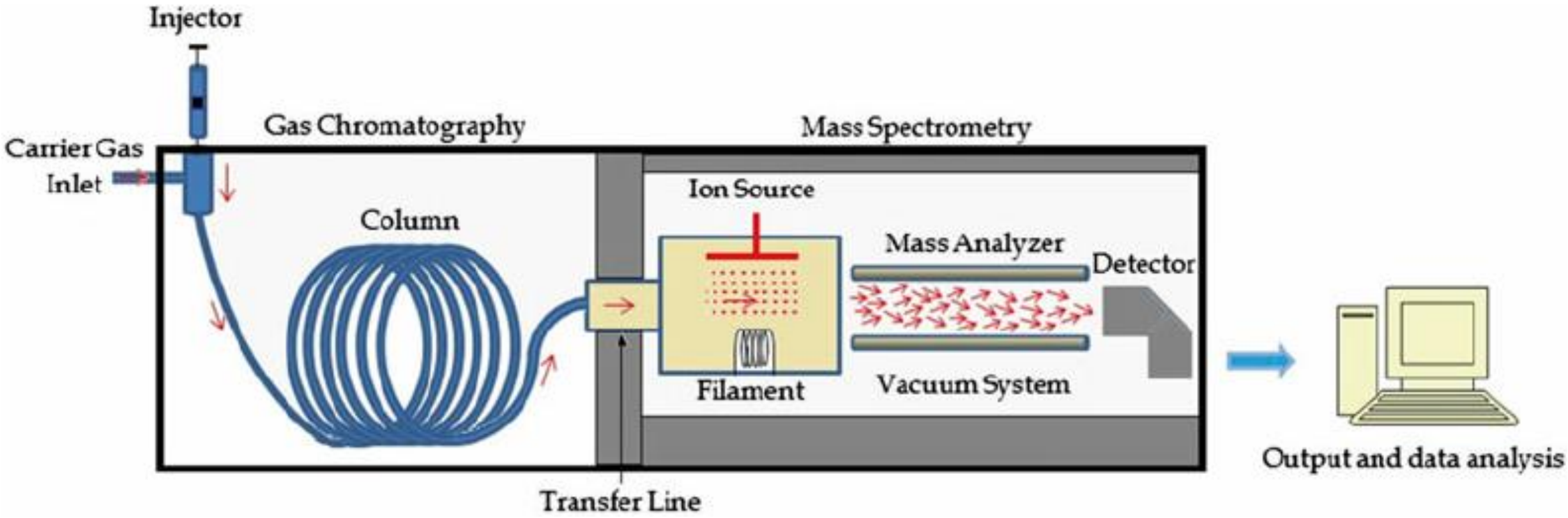



Faraday Cup

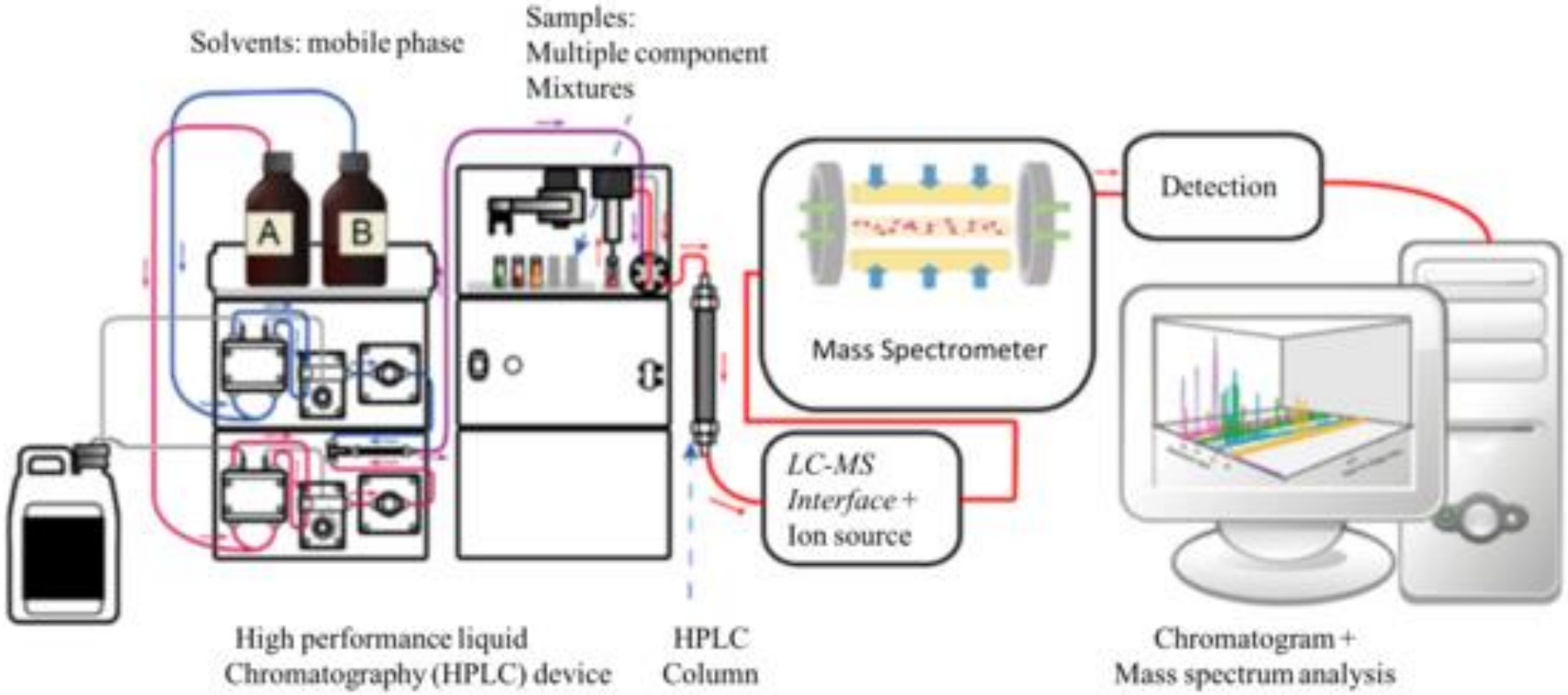



Photomultiplier Conversion Dynode

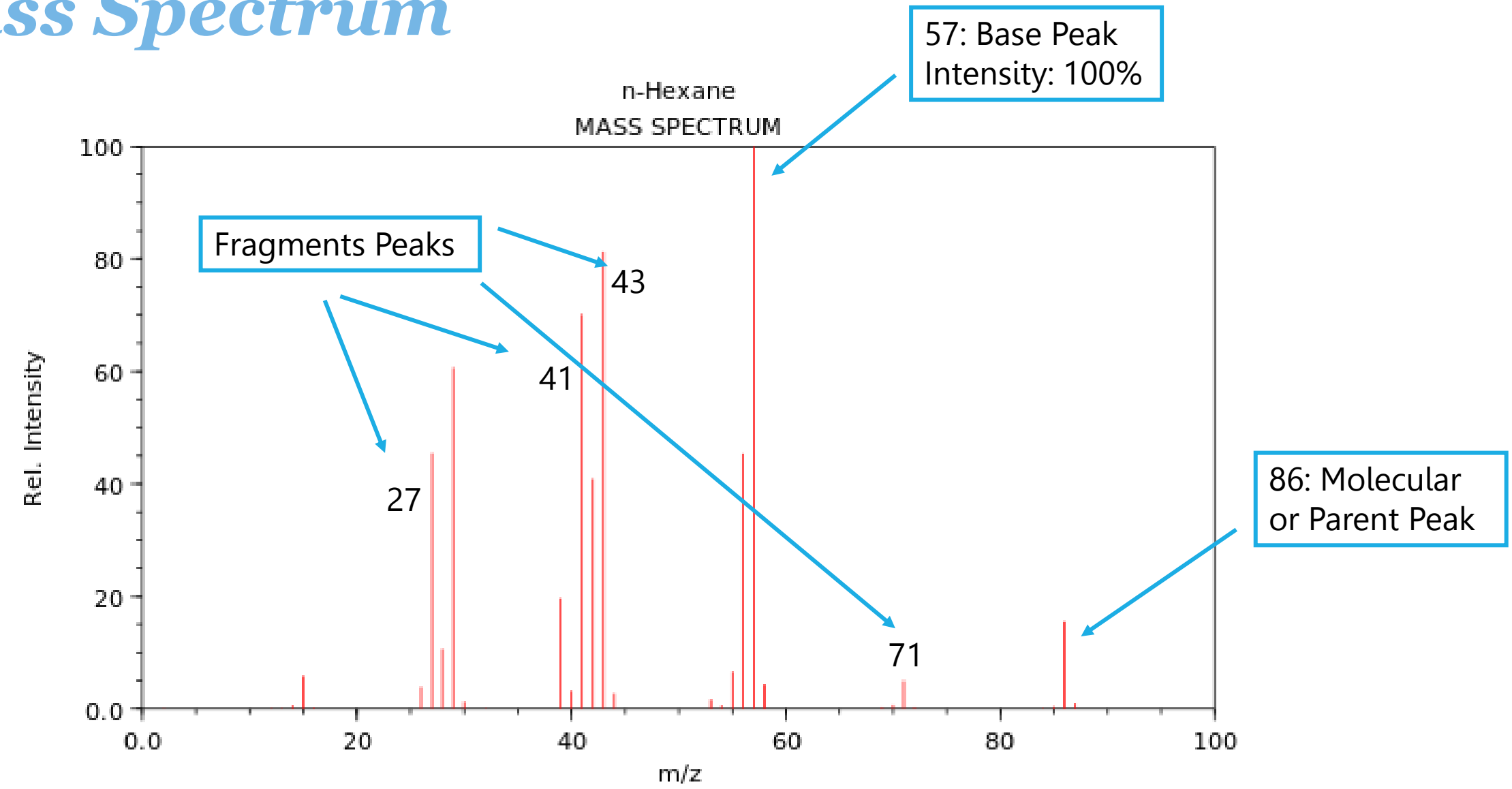
# Combining mass spectrometers with other techniques: GC-MS



# Combining mass spectrometers with other techniques: LC-MS



# Mass Spectrum



# *MS And Biomolecules*

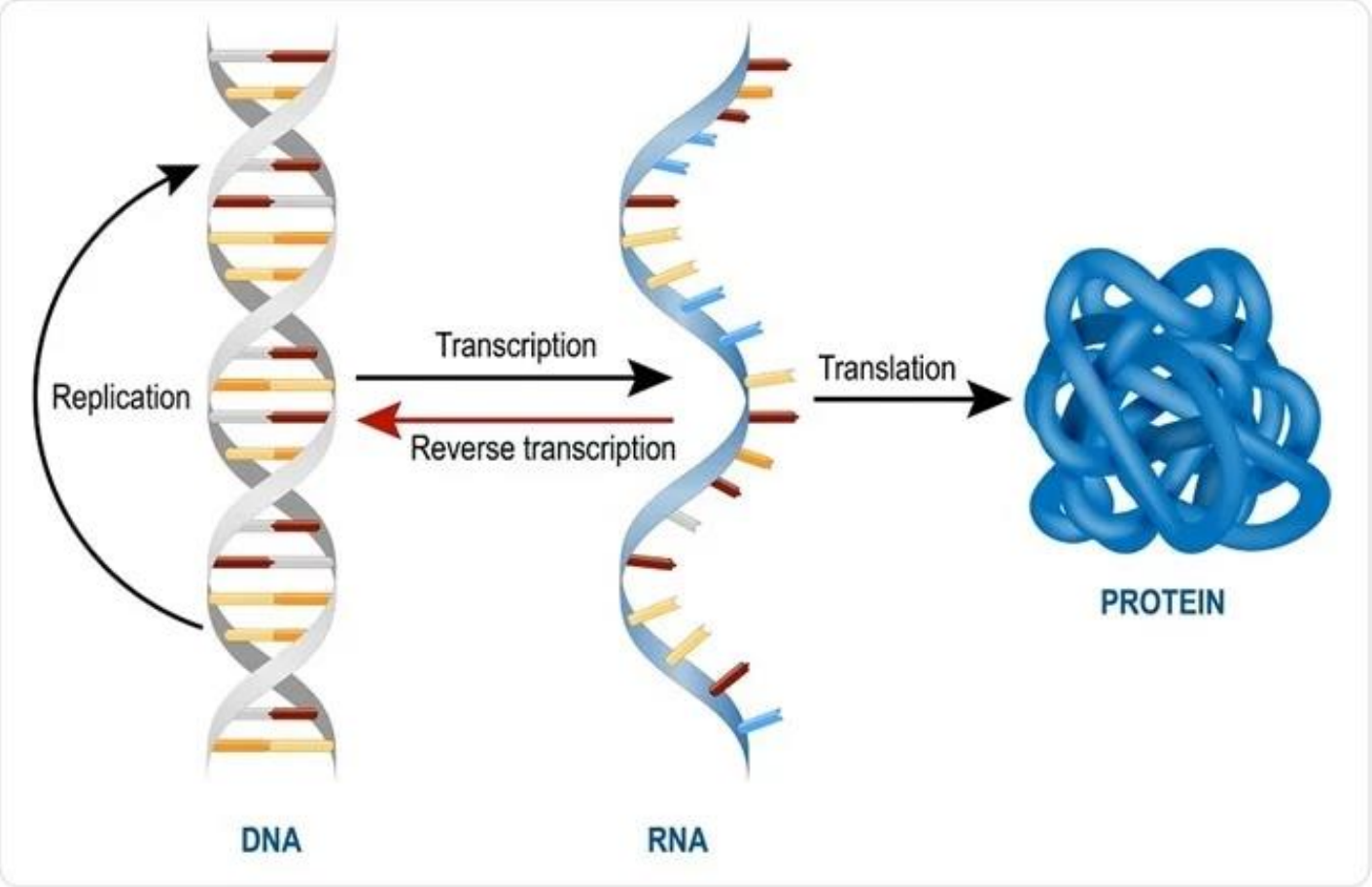
# Proteomics

- Determine protein structure, function, folding and interactions.
- Identify a protein from the mass of its peptide fragments.
- Detect specific post-translational modifications throughout complex biological mixtures using workflows for phosphoproteomics and protein glycosylation.
- Quantitate proteins (relative or absolute) in a given sample.
- Monitor enzyme reactions, chemical modifications and protein digestion.

| *Polypeptide sequence* | *Mass/amu* | *Δ* | *Amino acid* |
|---|---|---|---|
| X-Ser-Gly-Trp-Glu-Asp-Leu-Ile-Lys-Met | 10531 | 131 | Met |
| X-Ser-Gly-Trp-Glu-Asp-Leu-Ile-Lys | 10400 | 128 | Lys/Gln? |
| X-Ser-Gly-Trp-Glu-Asp-Leu-Ile | 10272 | 113 | Leu/Ile? |
| X-Ser-Gly-Trp-Glu-Asp-Leu | 10159 | 113 | Leu/Ile? |
| X-Ser-Gly-Trp-Glu-Asp | 10046 | 115 | Asp |
| X-Ser-Gly-Trp-Glu | 9931 | 129 | Glu |
| X-Ser-Gly-Trp | 9802 | 186 | Trp |
| X-Ser-Gly | 9616 | 57 | Gly |
| X-Ser | 9559 | 87 | Ser |
| X | 9472 | | |

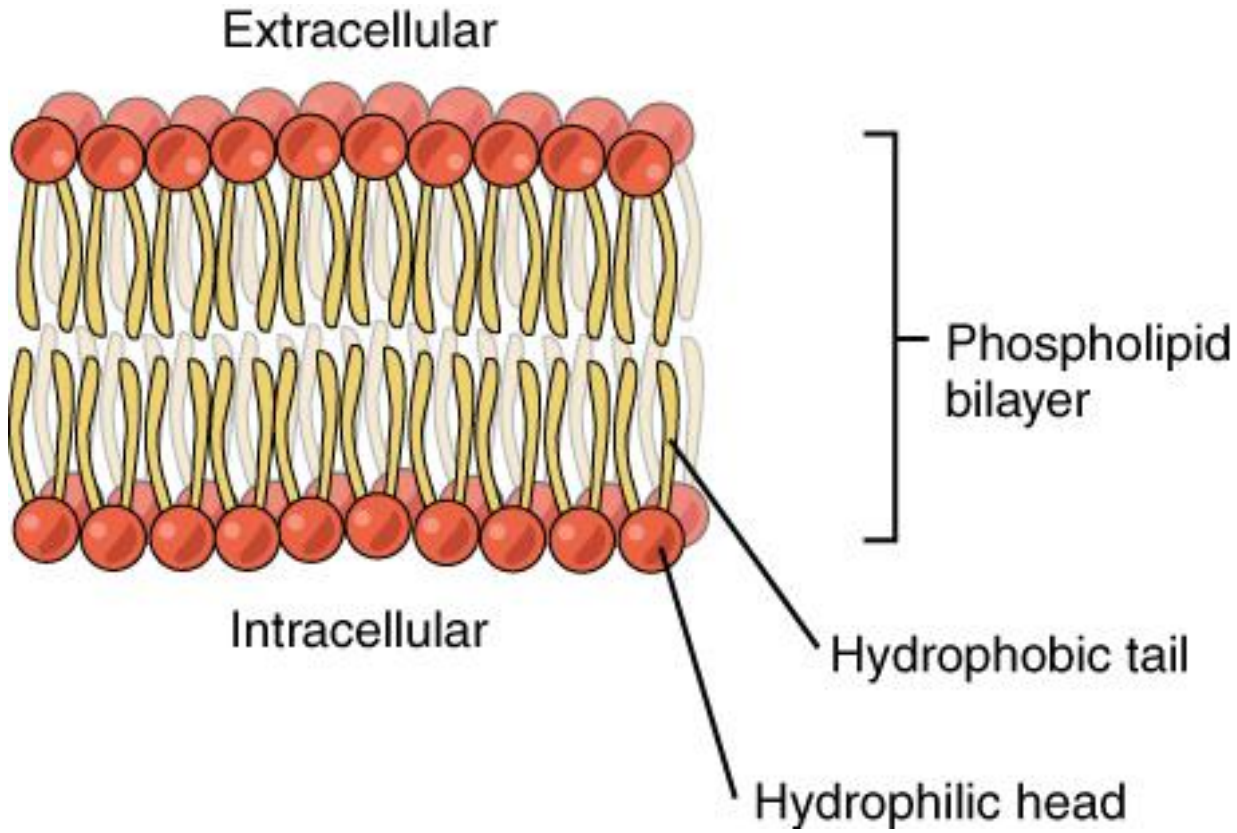
# Oligonucleotides

- Identify the covalent modifications and determining their structure
- Identify their position in the oligonucleotide
- Determination of the molecular weight of oligonucleotides
- Determination of their sequences



# *Lipidomics*

- Determine the molecular weight
- Determine elemental composition
- Determine the position of branching
- Determine nature of substituents in the lipid structure.



# *Applications*

- Quality control
- Identify structures of biomolecules
- Sequence biopolymers
- Determination of the molecular mass of Biomolecules
- Monitoring gases in patients' breath during surgery.
- Identification of drug abuse and metabolites of drugs of abuse in blood, urine, and saliva.
- Analyses of aerosol particles.
- Determination of pesticides residues in food.

# Advantages and Limitations

**Advantages:**

- Extremely sensitive
- Identify unknown components
- Combining with other techniques
- Very precise and rapid
- Very small sample quantities
- Gives the relative molecular mass of every molecule

**Drawbacks:**

- Costly
- Need a skilled technician
- Not a portable system
- Unable to differentiate among isomers of the molecule with the same charge-to-mass ratio
- Chiral columns may be required to separate enantiomer
- Difficult to recognize hydrocarbons that generate parallel ions
- Unable to separate optical and geometric isomers

# *Any Questions ?*

# *In-Class Activity*

Here you have the mass spectrum of the pentane ($C_5H_{12}$), consisting of $CH_3$ and $CH_2$ groups, try to explain me the spectrum obtained.