LC-MS Based Metabolomics

Analysing the METABOLOME

1. Metabolite Extraction

2. **Metabolite detection (with or without separation)**

3. Data analysis

Metabolite Detection

- GC-MS: Naturally volatile or made volatile (any organic- flavors, sugars, lipids, acids)
- NMR – any compound containing hydrogen
- HP Liquid - Chromatography + detector

  Comon detectors-
  - UV-detector (phenolics)
  - MASS SPECTROMETER (MS) as detector (LC-MS)

Metabolite Detection

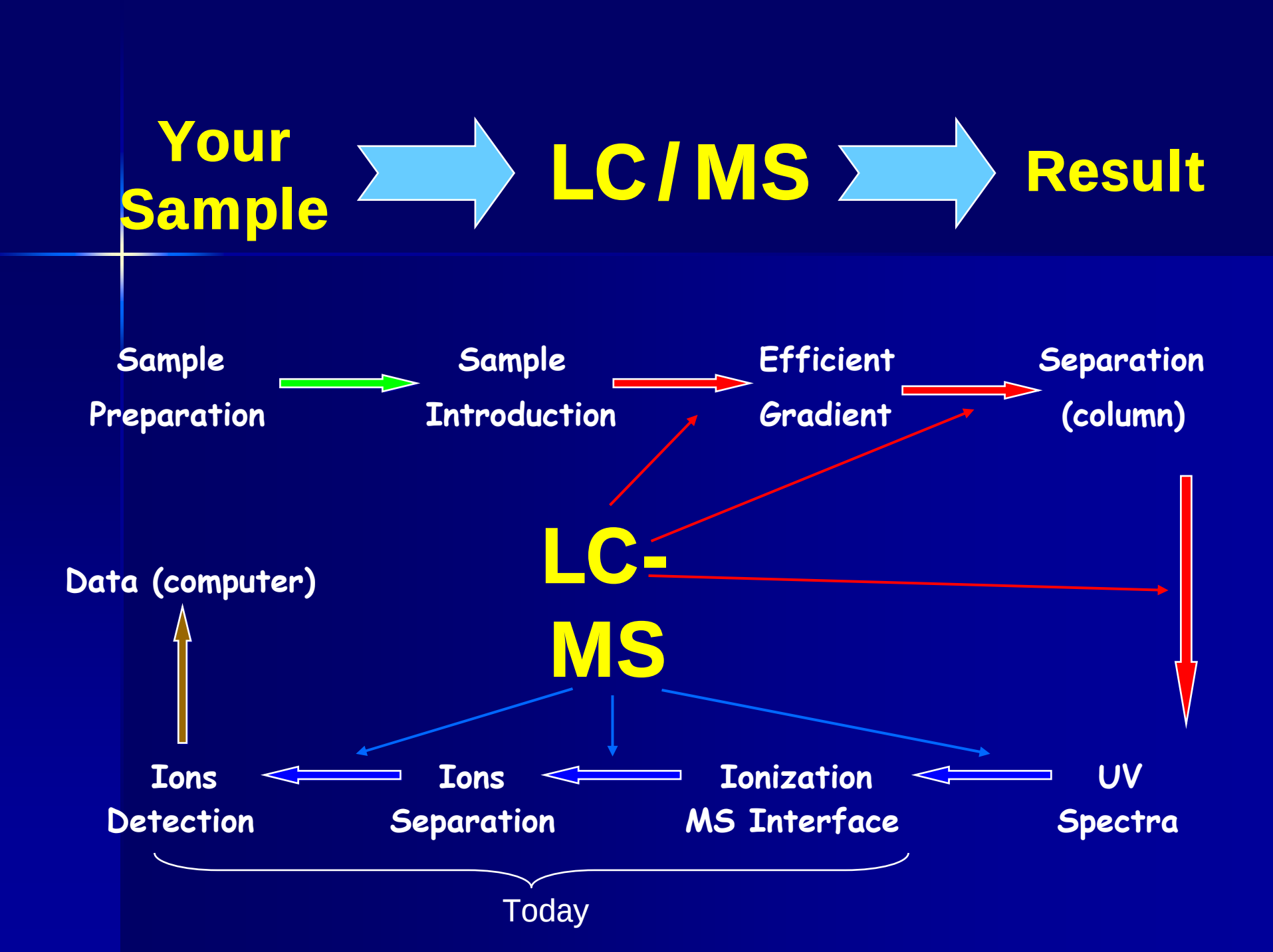
MASS SPECTROMETER (MS) as detector (LC-MS):

Compounds that are not well characterized by other methods:

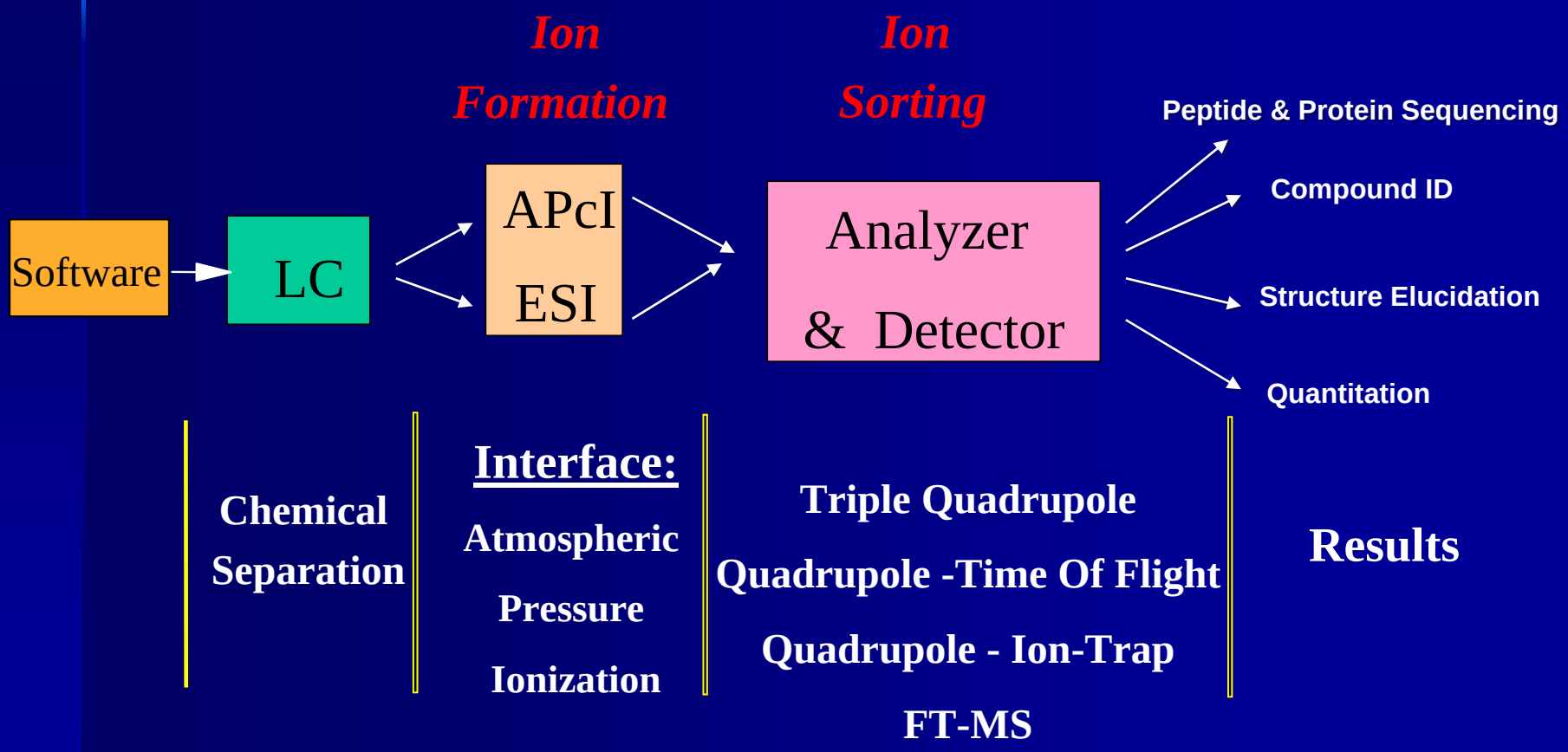
Non volatile

High molecular weight

Too sensitive to heat to be analyzed by GC


Your Sample
LC / MS
Result
Sample Preparation
Sample Introduction
Efficient Gradient
Separation (column)
LC-MS
Data (computer)
Ions Detection
Ions Separation
Ionization MS Interface
UV Spectra
Today

Components in LC-MS



Mass Spectrometer

1. Breaks up constituents into molecular ions and other fragments

2. The ions then pass through an electric and/or magnetic field that separates them according to their mass-to-charge ratio (m/z)

3. Measures masses

Mass Spectrometer

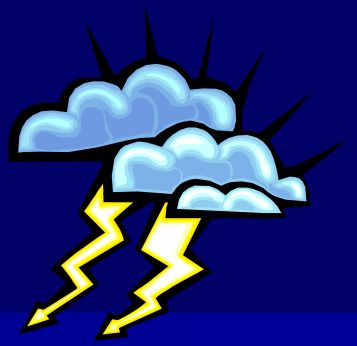
4. Universal detection method
    * compared to UV/VIS (PDA), fluorescence etc.
    * more specific than NMR
5. More sensitive for most compounds
6. Structural information on metabolite
    * fragmentation pattern
    * accurate mass
7. For both LC and GC

Technology of LC-MS and LC-MS-MS

- Interfaces- Ionization (elimination of solvent and generation of gas-phase ions)
- e.g. Z Spray

- Analyzers – Quadrupoles (Q) and Time of Flight (TOF)

LC-MS Interfaces



In MS-

* Measuring the mass of a huge variety of compounds, in a huge variety of matrices

* Need range of methods to IONISE all the different compounds

→ Alternative Ionization Modes

Alternative Ionization Modes

- EI or CI, Electron (impact) OR Chemical Ionization (in GC-MS)

- Gas-phase ionization methods
- Small volatile molecules are heated and enter the gas phase

Not always suitable:

- Difficult to get large or involatile molecules into the gas phase

- Laser desorption
  - Matrix-assisted laser desorption ionization (MALDI)
- Particle bombardment
  - Fast atomic bombardment (FAB)
  - Secondary ion mass spectrometry (SIMS)
- Field desorption Ionization

Alternative Ionization Modes

• EI or CI, Electron (impact) OR Chemical Ionization (in GC-MS)

• Gas-phase ionization methods
• Small volatile molecules are heated and enter the gas phase

Not always suitable:

• Difficult to get large or involatile molecules into the gas phase
• Heating the non-volatile molecules degrades them

Alternative Ionization Modes

Ionization for Non-Volatiles:

Early ones-

- Particle bombardment
  - Fast atomic bombardment (FAB)
  - Secondary ion mass spectrometry (SIMS)
- Field desorption Ionization
- Thermospray ionization

Alternative Ionization Modes

Ionization for Non-Volatiles:

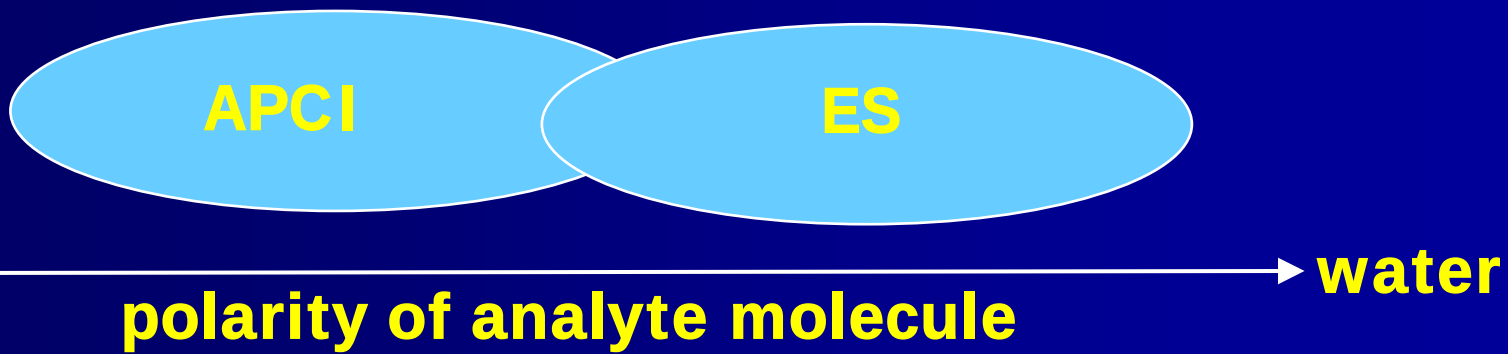
Early ones-

- Particle bombardment -
- Fast atomic bombardment (FAB)
- Secondary ion mass spectrometry (SIMS)
    - single experiments, background signal from matrix
    -
- Field desorption - complex, single experiments at once

- Thermospray - temprature degrades sample

Alternative Ionization Modes

- Atmospheric Pressure Ionization (API), in LC-MS
  - Electrospray Ionisation (ESI): polar and semi-polar
  - Atmospheric Pressure Chemical Ionization (APCI): less polar



Atmospheric Pressure Ionisation (API) Techniques

ESI and APCI differ in…

- **How ions are generated**
  - **ESI - solution phase ionization**
  - **APCI - gas phase ionization**
- **Analyte compatibility**
  - **ESI - polar compounds and large biomolecules**
  - **APCI - less polar, smaller compounds (relative to those ionized by ESI) that have some volatility**
- **Flow rate compatibility**
  - **ESI - 0.001 to 1 mL/min**
  - **APCI - 0.2 to 2 mL/min**

Ionization Methods

- *Electrospray (ESI)*
- *Atmospheric Pressure Chemical Ionization (APCI)*
- *Laser Desorption (MALDI)*
- *Chemical Ionization*

"Soft" Ionization

ESI, APCI and MALDI can be used with LC

- *Fast Atom Bombardment (FAB or SIMS)*
- *Electron Impact*

"Hard" Ionization

EI ionization can be used with GC

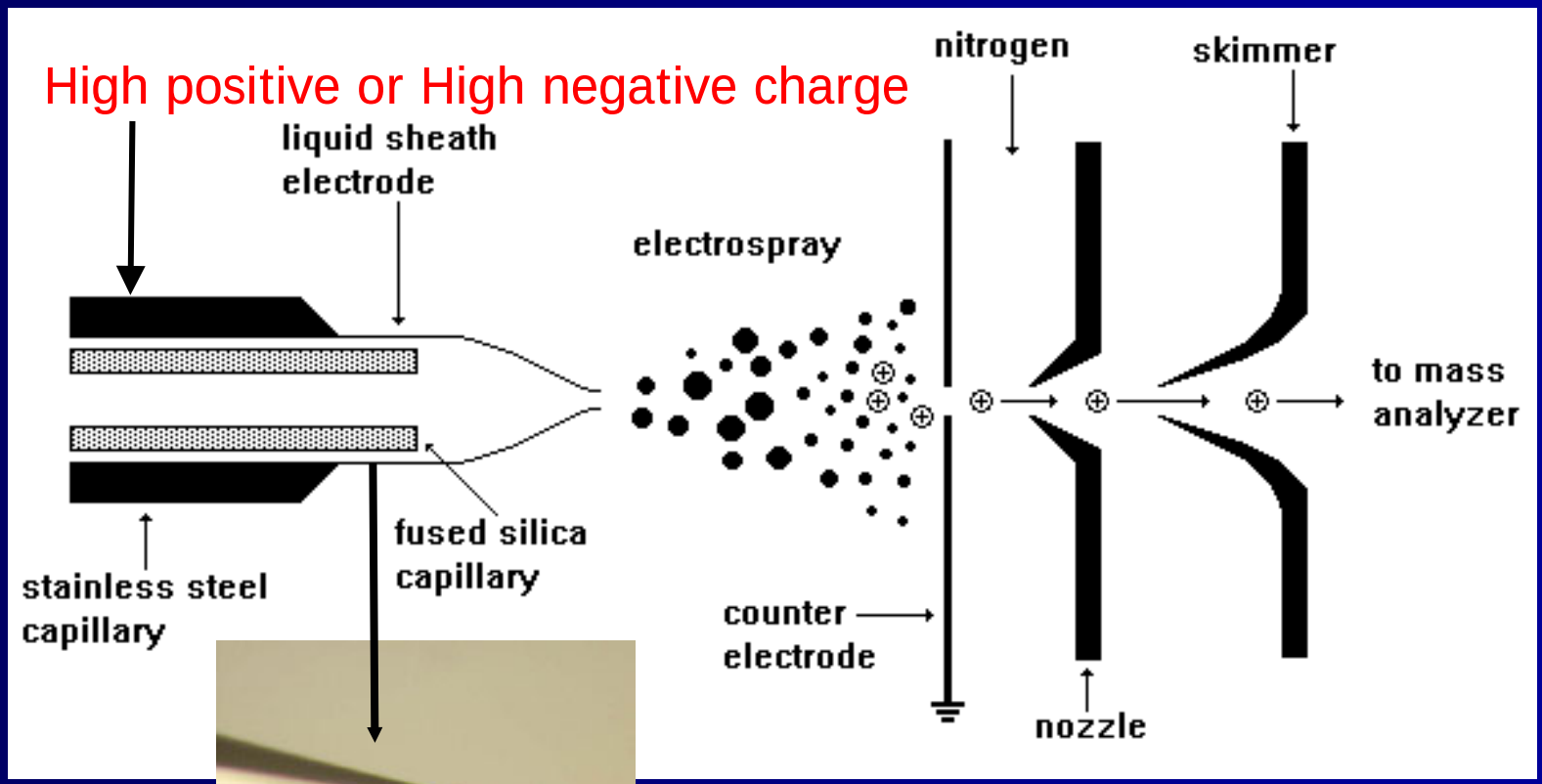
How do the analytes become charged?

- While in EI, loss of an electron producing a radical molecular ion

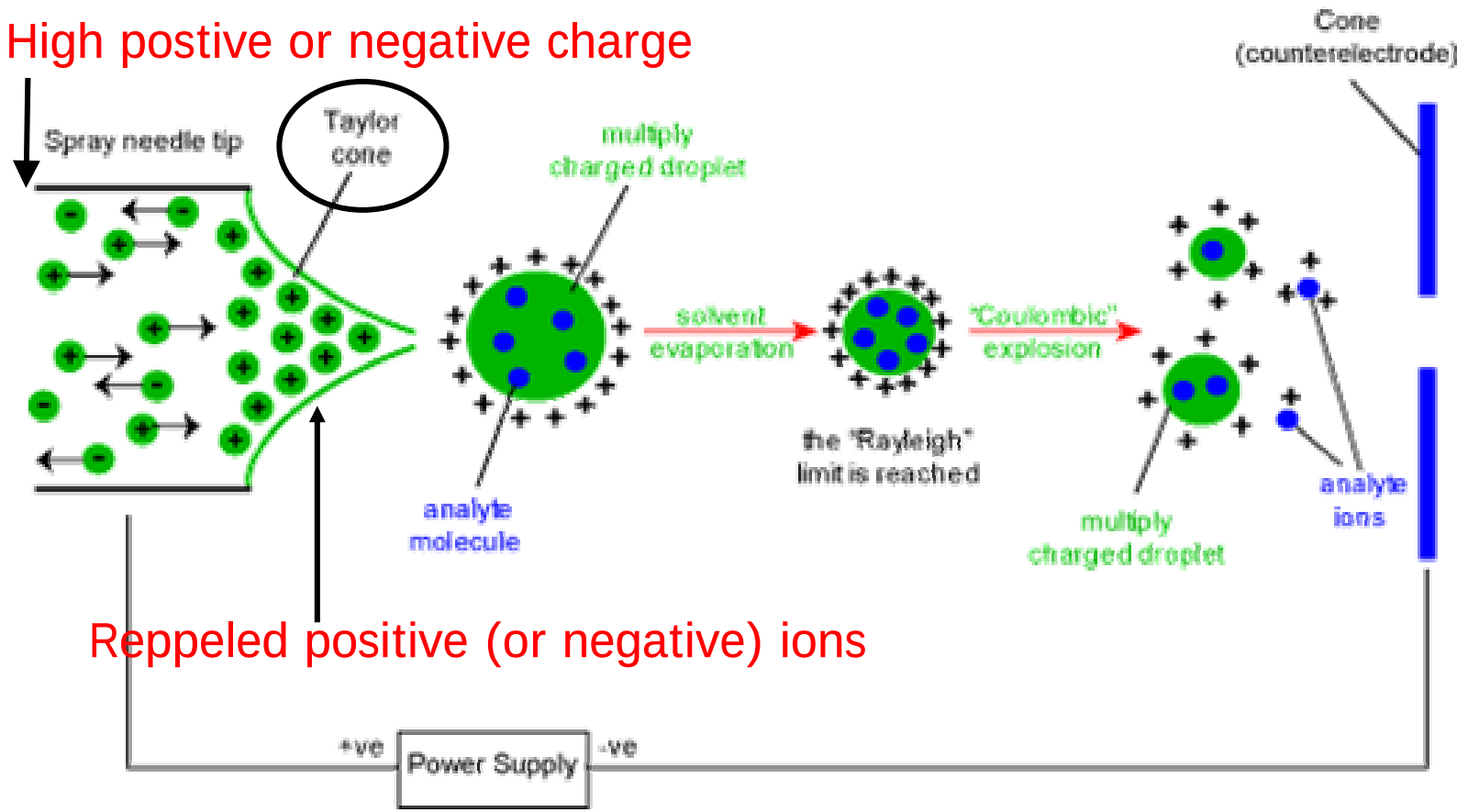
- In soft ionisation techniques, analyte molecules are:
protonated $[M + H]^+$
or:
de-protenoated $[M - H]^-$

- Could also be sodiated, potassiated etc.. (adducts)

Ion Formation in ESI



How do the analytes become charged?



Positive or Negative Modes?

The formation of positive or negative ions depends on the sign of the applied electrical field

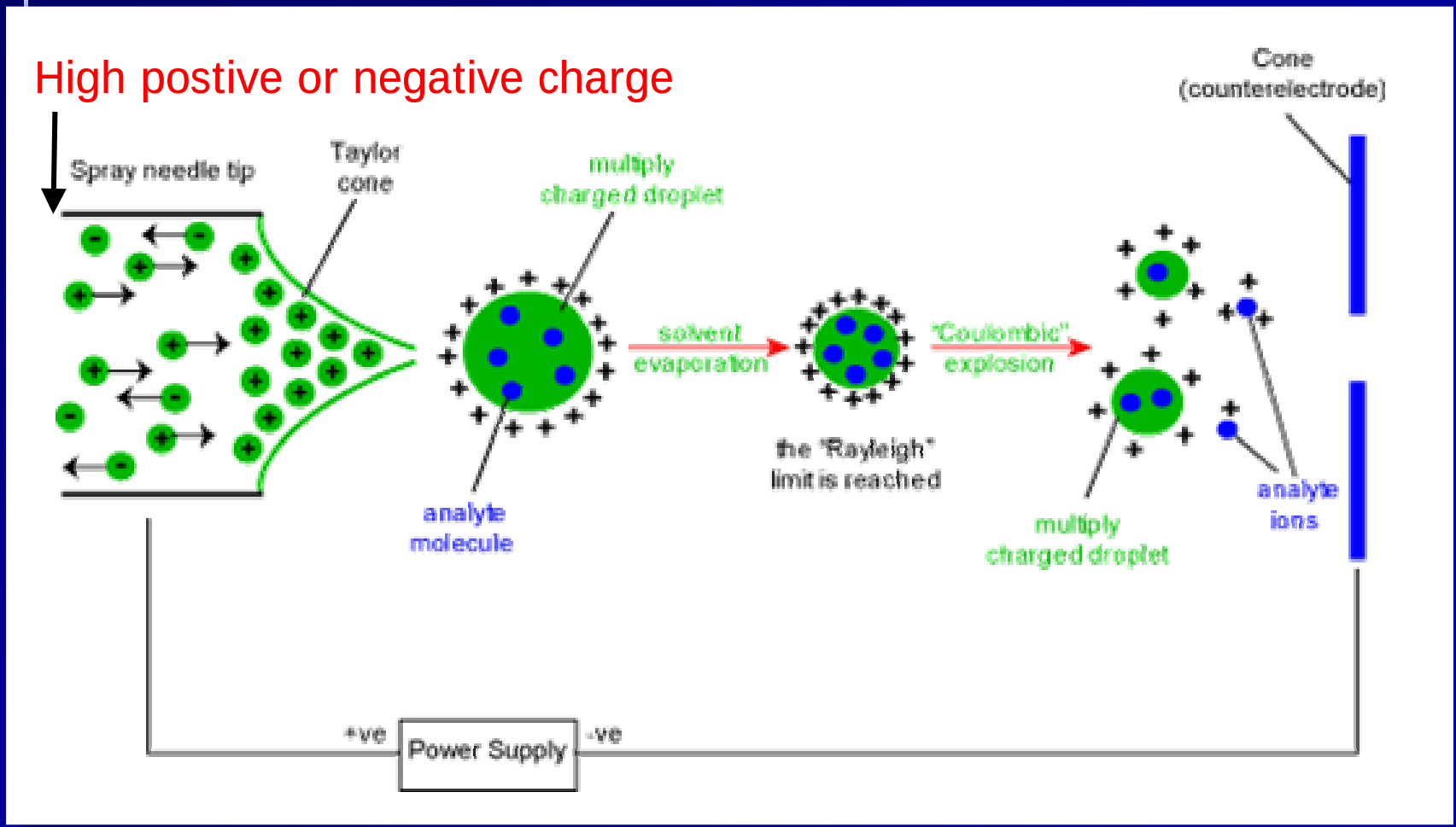
ES+: $(M+H)^+$

Good ionization of basic compounds (get proton) E.g. amino, amide, ester, aldehyde/keto functional groups (formic acid in sample solution to help ionize)

ES-: $(M-H)^-$

Acidic Compounds (give proton) E.g. organic acids, containing OH (ammonium buffer in sample solution to help ionize)

Solvent Loss in ESI & Ion Formation



Electrospray Ionization ESI

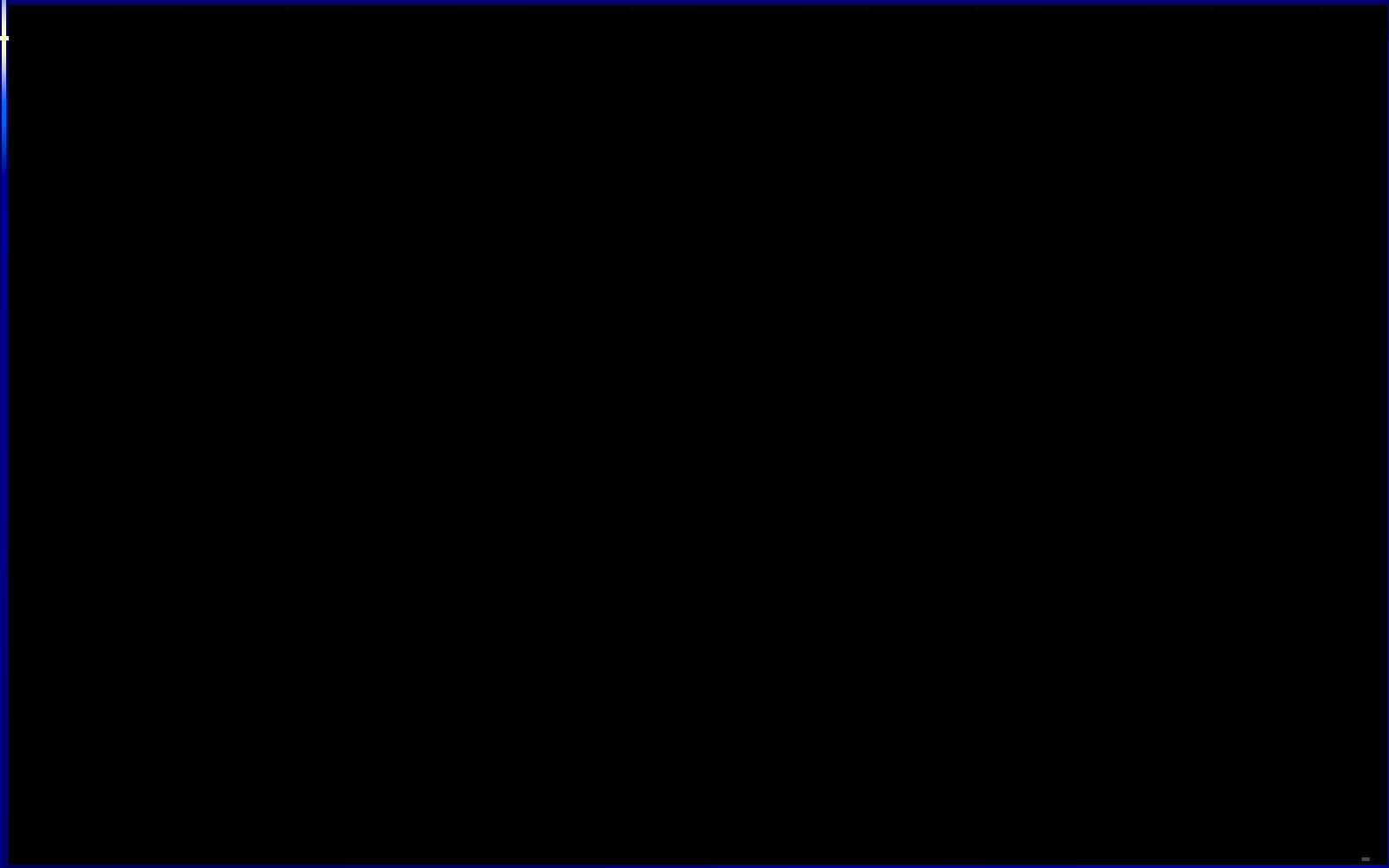
Typical ES Positive Ion Samples

- Peptides and proteins.
- Small polar compounds.
- Drugs and their metabolites.
- Environmental contaminants (e.g. pesticides / pollutants).
- Dye compounds.
- Some organometallics.
- Small saccharides.

Typical ES Negative Ion Samples

- Some proteins.
- Some drug metabolites (e.g. glucuronide conjugates).
- Oligonucleotides.
- Some saccharides and polysaccharides.

Electrospray Theory



Summary ESI

ESI is an atmospheric pressure ion source

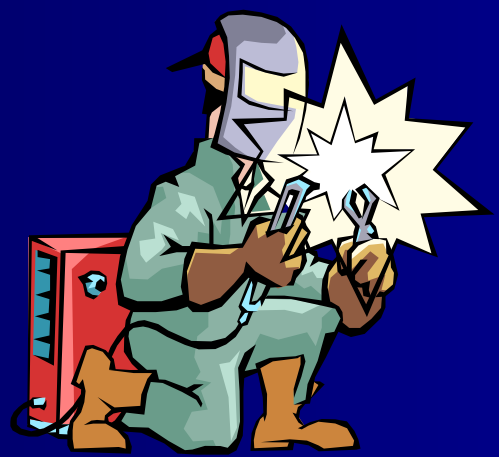
Small molecules singly chraged

High MW samples become multiply chares (e.g. proteins)

MWs of 150,000 Da (amu) cab be measured accurately

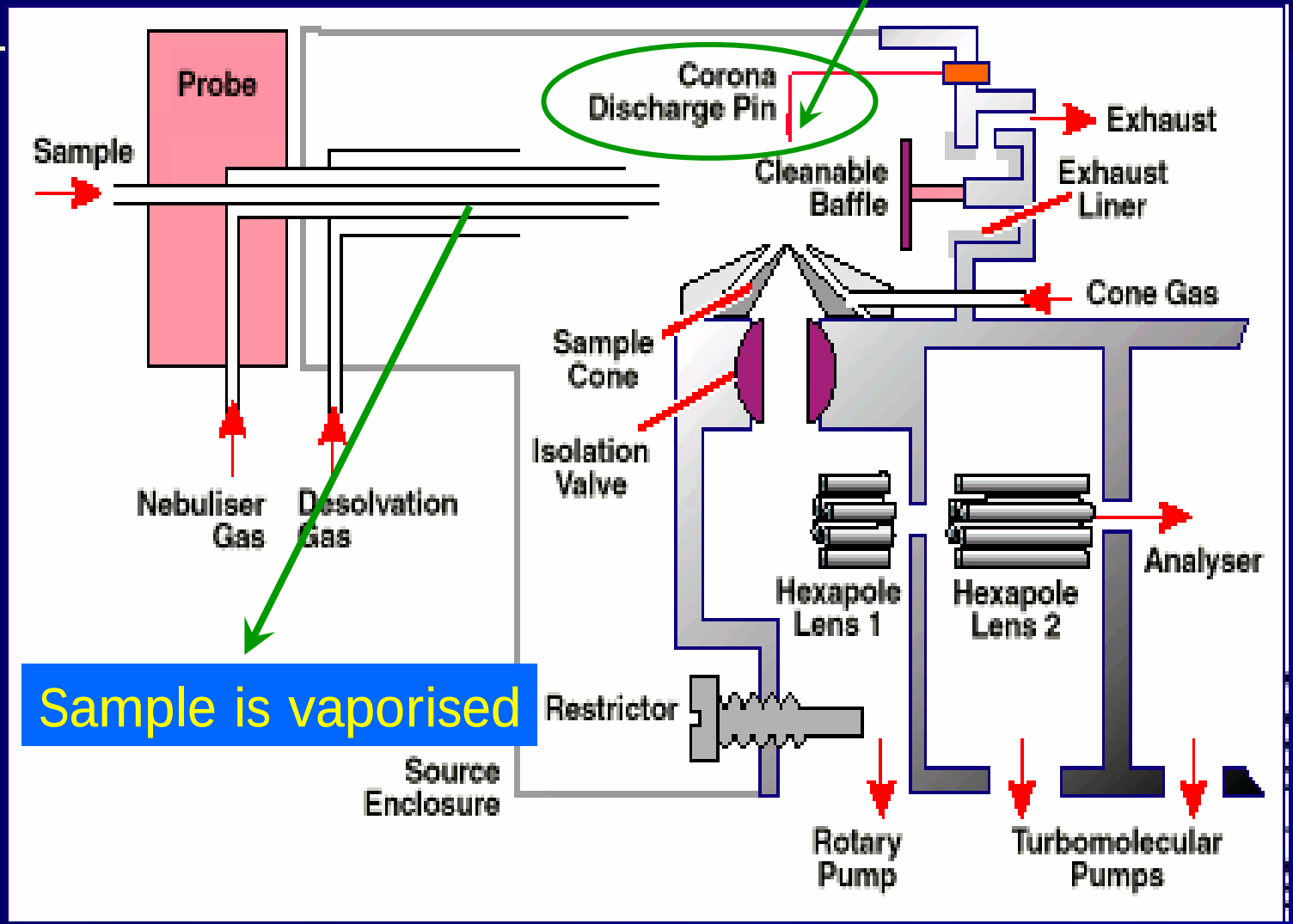
Atmospheric Pressure Chemical Ionization - APCI

Atmospheric Pressure
Ionization Interface



APcI Source

Ionization of solvent
& solvent transfers the charge to analyte



APcI Theory

Atmospheric Pressure Chemical Ionisation (APcI)

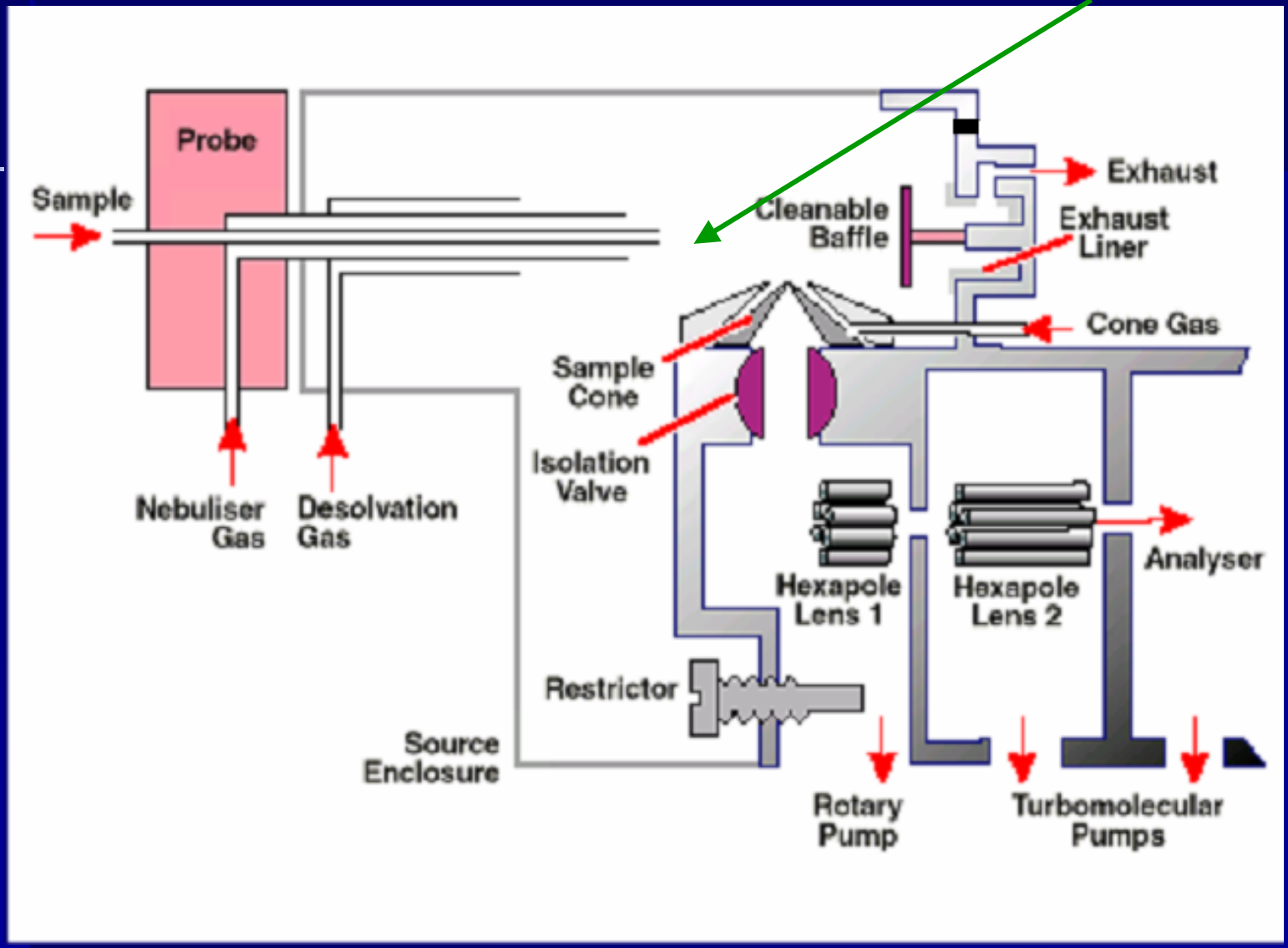
Low molecular weight (<1000 Da)

Singly charged species

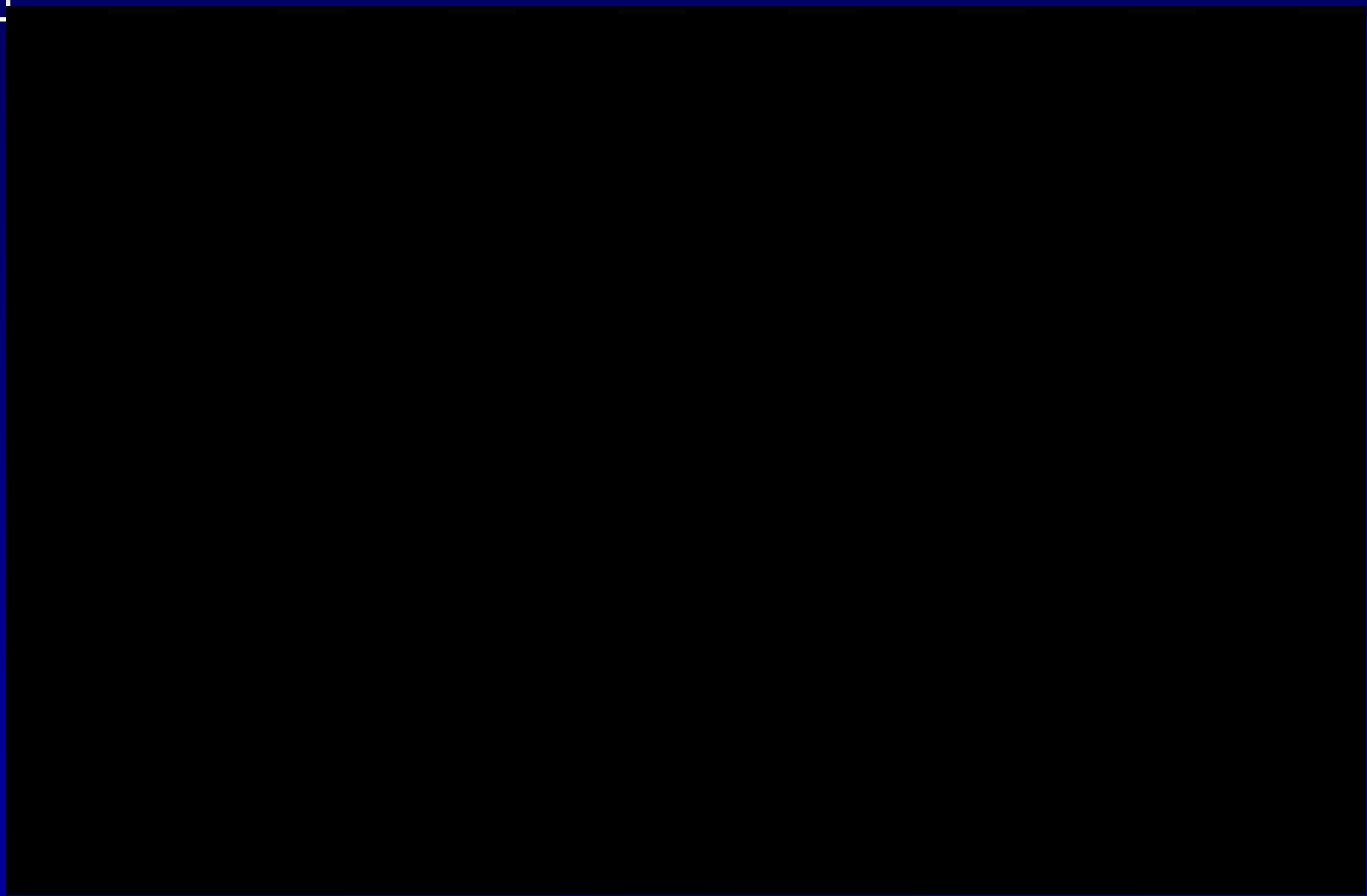
ESI vs APcI

Technique	Flow Rate (ml/min)	MW Range	Species Produced
ESI	0.001 – 0.3	<200,000 Da	$(M+H)^+$ $(M-H)^-$ $(M+nH)^{n+}$
APcI	0.2 – 2.0	<1000 Da	$(M+H)^+$ $(M-H)^-$

Z *SPRAY*™ Source

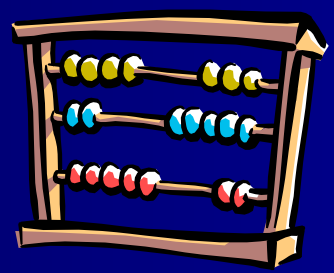


Z-Spray Interface

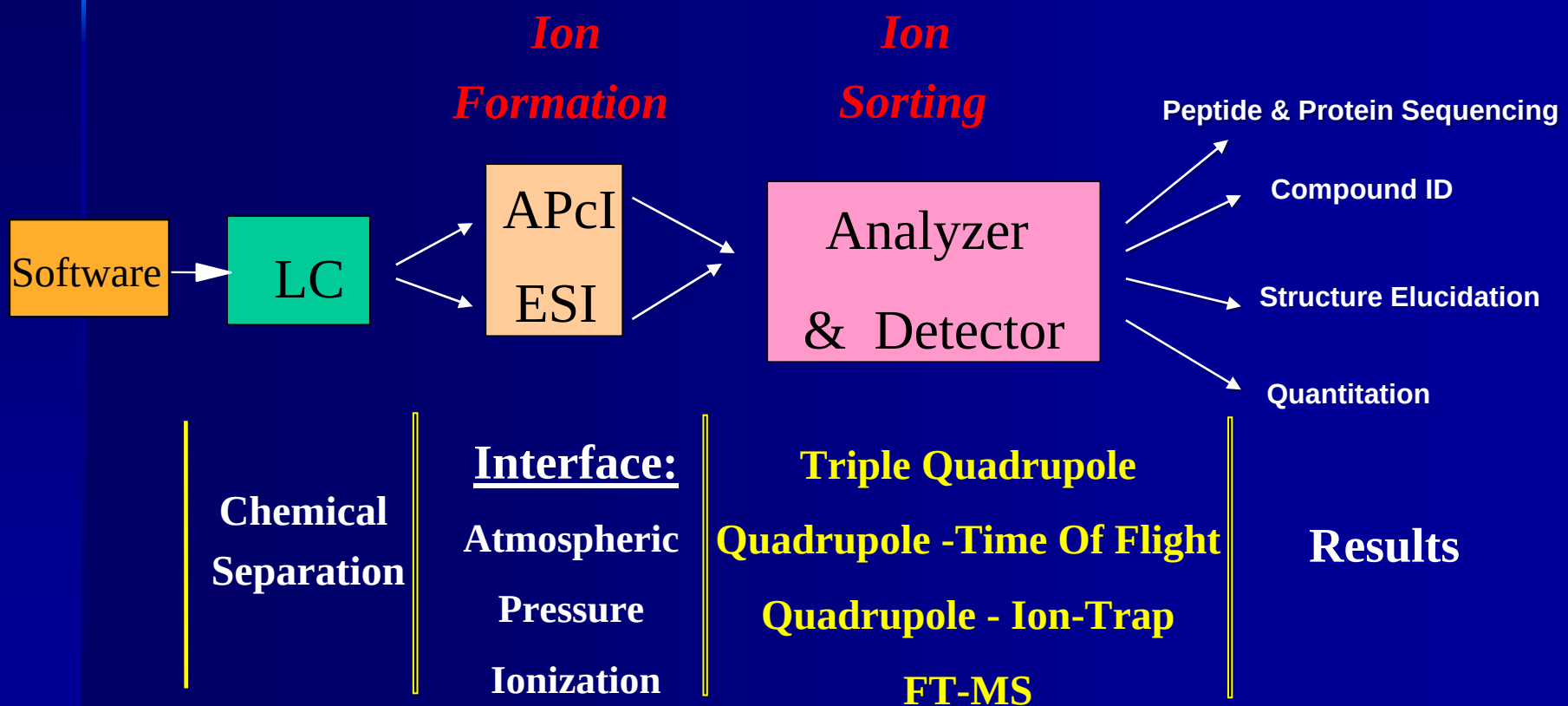


Mass Analyzers

Ion Sorting

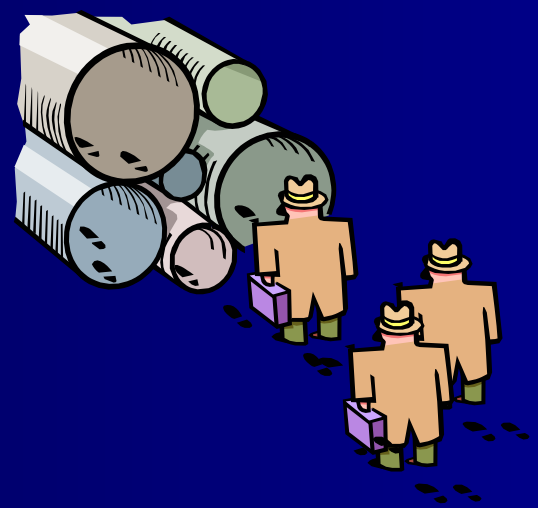


Components in LC-MS

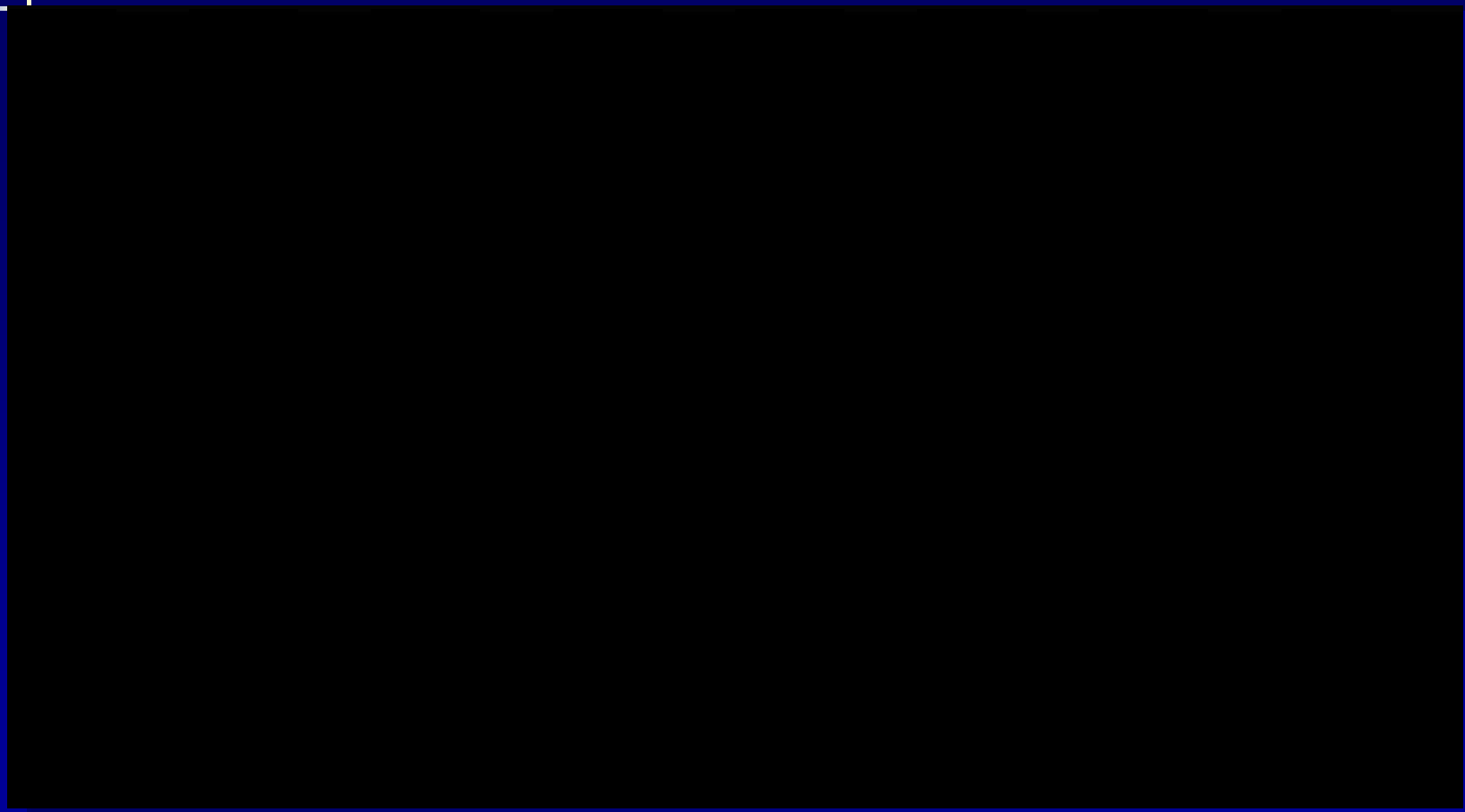


Quadrupole and Tandem Quadrupole

Ion Separation Analyzers

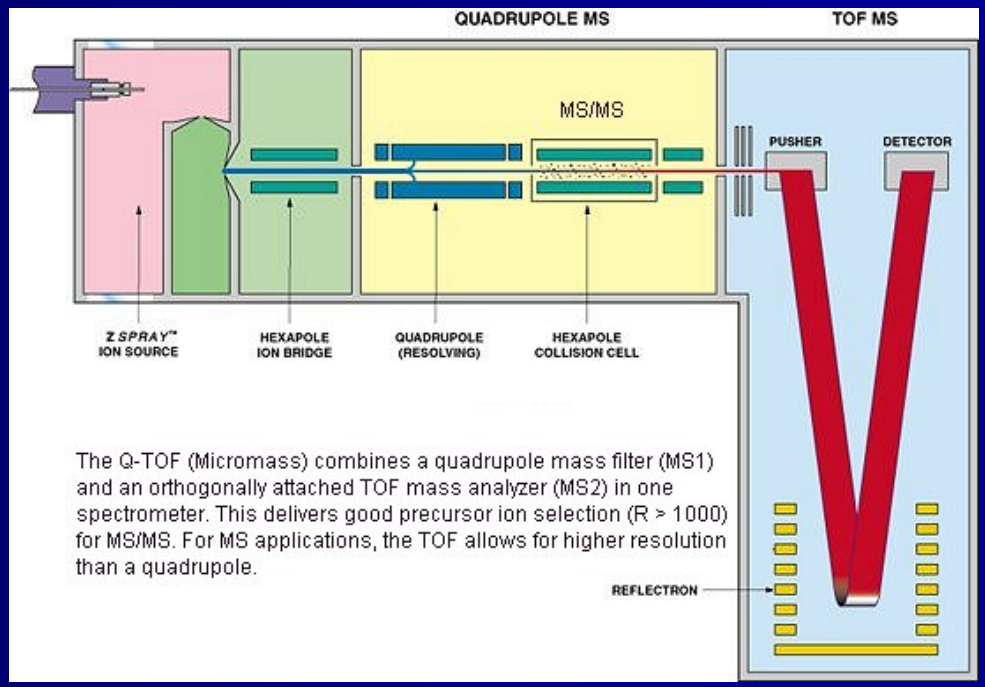


Quadrupole Theory



Benefits of Time-Of-Flight MS

- high mass resolution (up to 10 or 5 ppm)
- exact mass



Resolution & Accuracy of a Mass-spectrometer

Resolution

Resolution, (or Resolving Power) of a mass sectrometer:

A measure of its ability to separate adjacent ions

At higher resolution, small differences may be detected.

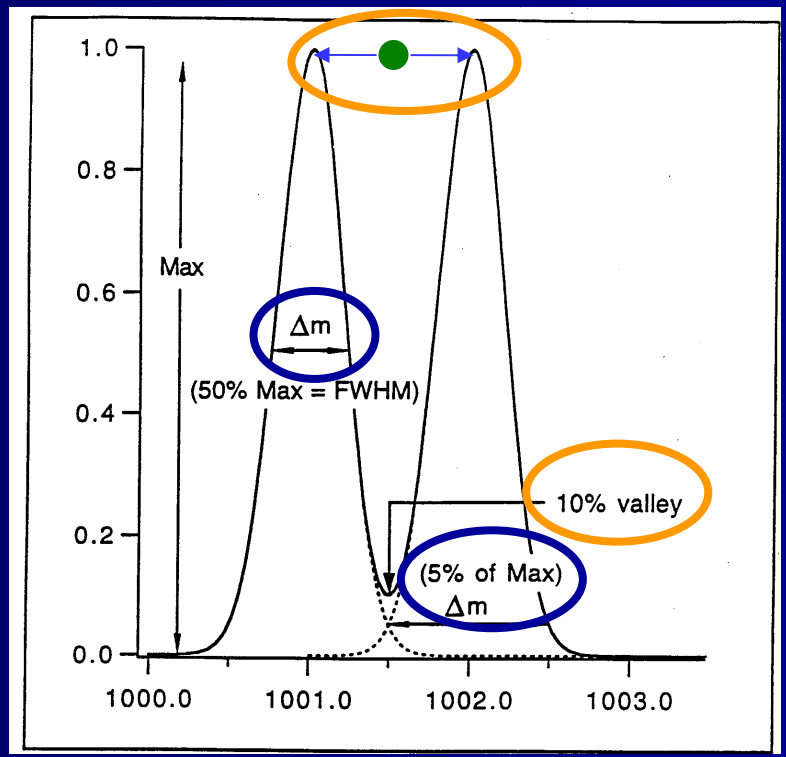
Determining Resolution

Single Ion method

Full Width at Half Maximum (50%, FWHM)

or at 5% of the peak height

$$R = \frac{m}{\Delta m}$$



Double Ion method

2 adjacent ion peaks with a 10% valley max

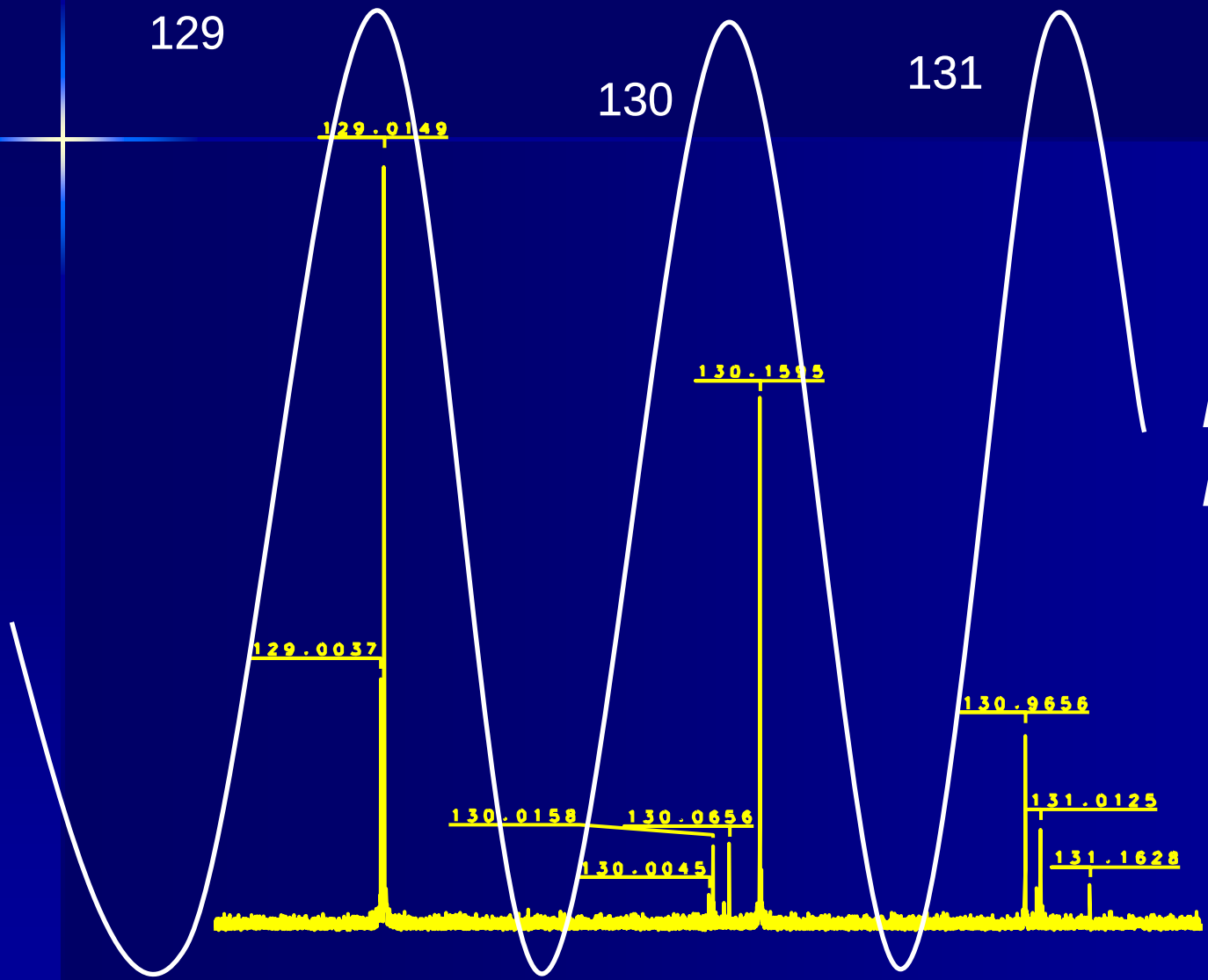
Mass Analyzers

- *Ion Cyclotron (FT-ICR-MS)*
- *Time of Flight (TOF)*
- *Magnetic Sector*
- *Quadrupole Ion Trap*
- *Quadrupole*

"High Resolution" Instruments

"Low Resolution" Instruments

High Resolution vs. Low Resolution

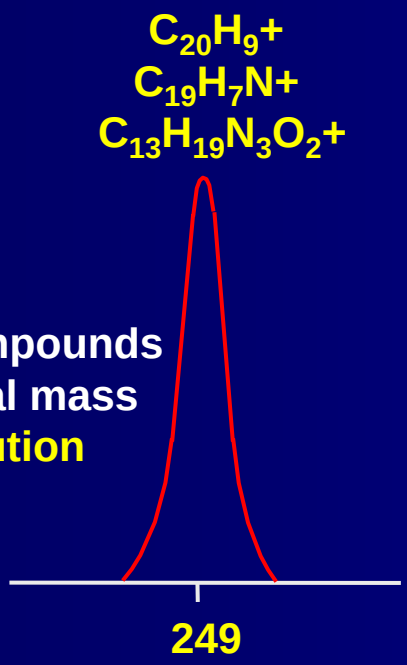


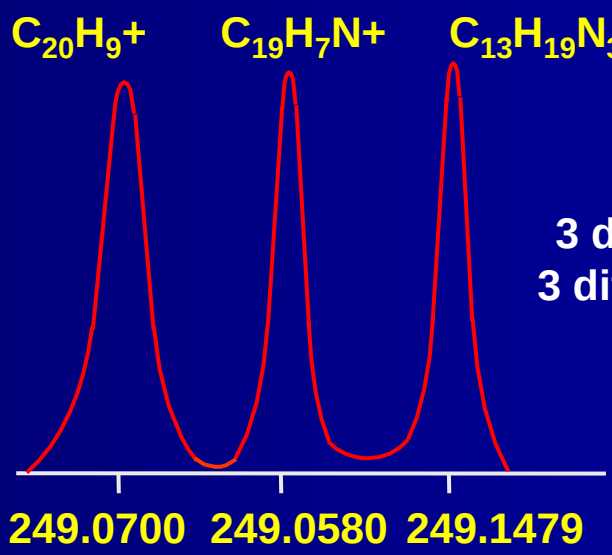
Low Resolution

High Resolution

Resolution

3 different compounds
Same nominal mass
Low resolution





3 different compounds
3 different exact masses
High resolution

Mass Analyzers

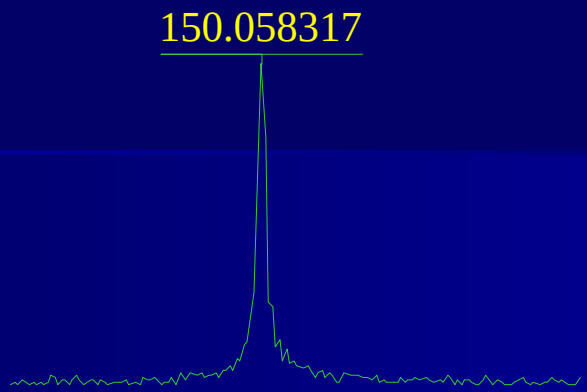
	Resolving Power	*Mass Accuracy*
•Ion Cyclotron (FT-ICR-MS)	*200,000*	*<1ppm*
•Time of Flight (TOF)	*20,000*	*3-10ppm*
•Magnetic Sector	*60,000*	*2-5ppm*
•Quadrupole Ion Trap	*1,000*	*n/a*
•Quadrupole	*1,000*	*n/a*

Mass Accuracy- FTMS

- Instrument Calibration -

Calibrant	Exact Mass	Experimental Mass	Difference (ppm)
Alanine	90.0550	90.054998	0.0175
Lysine	147.1128	147.112783	0.1173
Glutamate	148.0604	148.060420	0.1353
Methionine	150.0583	150.058317	0.1109
Tyrosine	182.0812	182.081184	0.0866
Tryptophan	205.0972	205.097192	0.0380

Mass Accuracy Determining Empirical Formula, Structural Elucidation



Methionine

$C_5H_{12}NO_2S$

0.06 ppm

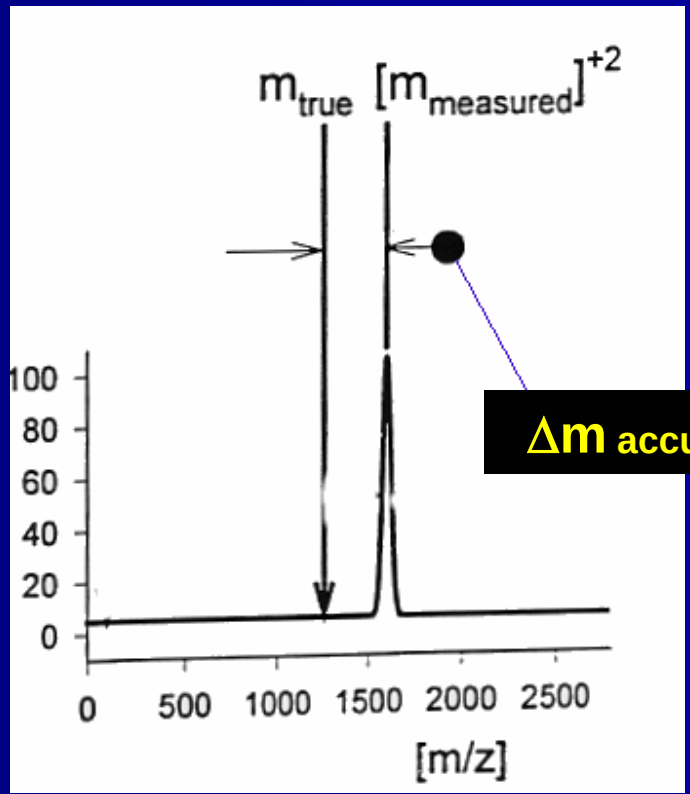
#	12C	1H	16O	14N	31P	32S	23Na	39K	mass	error
1	5	12	2	1	0	1	0	0	150.0583257	5.826e-08
2	1	15	2	2	1	1	0	0	150.0586364	2.128e-06
3	3	15	0	2	0	1	0	1	150.0587525	2.902e-06
4	9	11	0	0	1	0	0	0	150.0592883	6.473e-06
5	3	15	0	1	2	0	1	0	150.0571936	7.486e-06
6	5	14	0	1	2	0	0	0	150.0595989	8.543e-06
7	4	16	1	0	1	0	0	1	150.0570349	8.544e-06
8	3	10	1	4	0	1	0	0	150.0569831	8.889e-06
9	2	16	3	0	2	0	0	0	150.0569188	9.318e-06

Mass Accuracy

Ability of a mass analyzer to assign the mass of an ion close to its true value (exact mass)

$\Delta m_{accuracy} = m_{real} - m_{measured}$

$\underline{\text{In ppm}} = 10^6 * \Delta m_{accuracy} / m_{measured}$



Mass Accuracy

High mass accuracy (exact mass measurement) is usually associated to high resolution analyzers

Unknown compound determination
Exact mass helps to define its atomic composition

Scan Speed (or rate)

- The rate at which we can acquire a mass spectrum, (mass units/sec).
- Is an essential acquisition parameter for MS
- Will affect the amount of information (qualitative and quantitative) that can reasonably be attained with a given mass analyzer.

Mass Analyzers

Analyzer	Range amu	Accuracy amu	Resolution	Speed amu/s
Quad	< 4000	0.1	1000-2000	4000
Ion Trap	< 20 000	0.1	1000-2000	4000
TOF	1 000 000	0.0001	500-10 000	1 000 000

Next Class

Data after ion detection in LC-MS