Mass Spectrometry- based proteomics

Part A: Instruments & Chromatography

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FZI Core Facility Proteomics - Quantitative Proteomics

Instrument Platforms:
3 TIMS-TOF Pro
2 Exploris 480 FAIMS
1 TIMS-TOF SCP

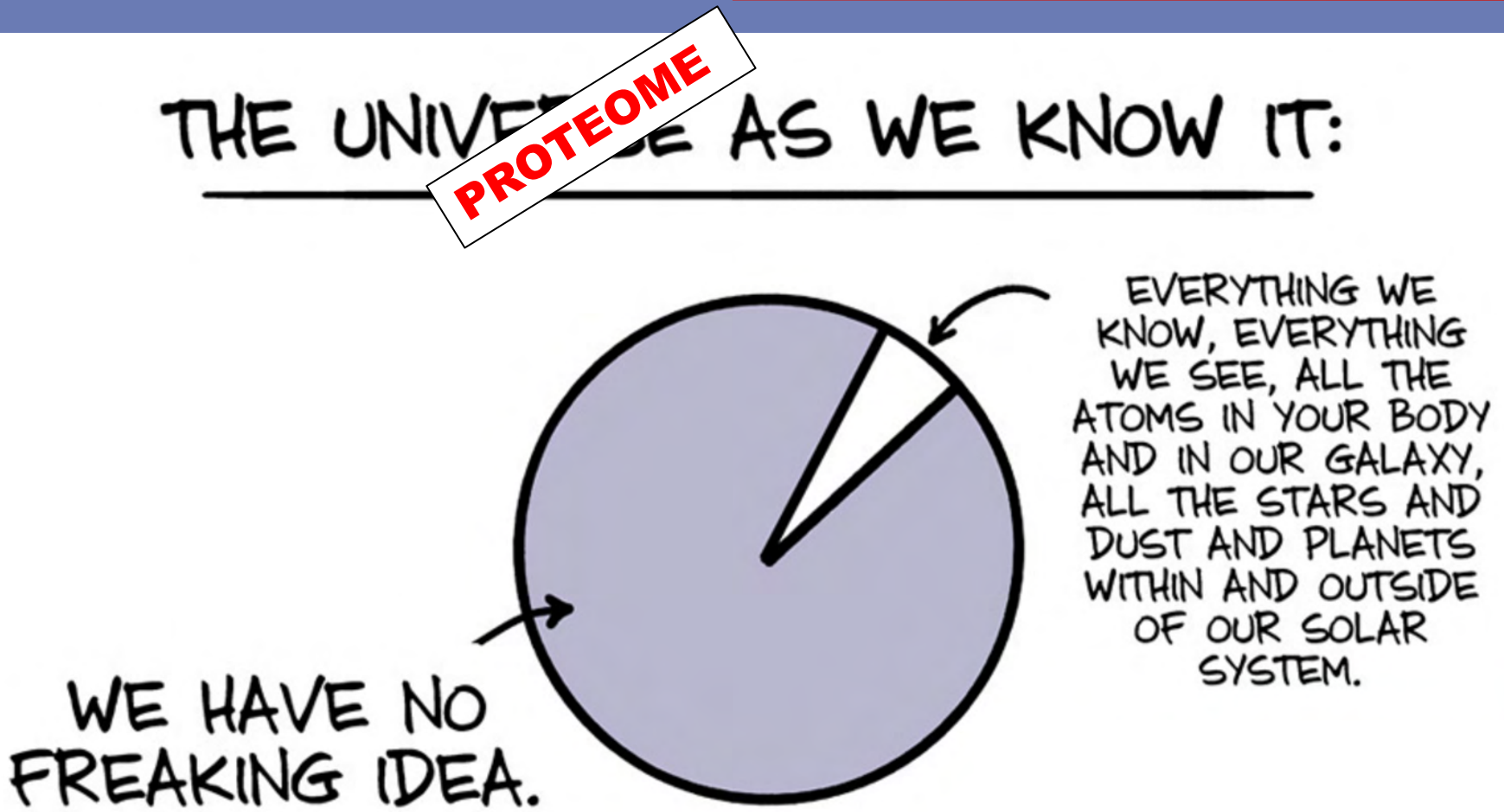
PEAKS X+

Spectronaut™

MaxQuant

DIA-NN

Current state of the art!



http://phdcomics.com/noidea/

Why Proteomics ?

DNA: what could be

RNA: what it is trying to be

Protein: what it is

Black Swallowtail – larva and adult butterfly

Why not transcriptomics?

Topics

- Liquid Chromatography
- Introduction to mass spectrometry
- Ion Sources
- Analyzers
- Hybrid instruments

Life is not easy – challenges for proteomics

Full human proteome

13k genes expressing ~13k proteins

↓

Trypsin digest (after K/R): ~6 mln peptides

↓

At m/z accuracy 1ppm: 1000's of candidates

(+ PTMs, Splice Variants etc..)

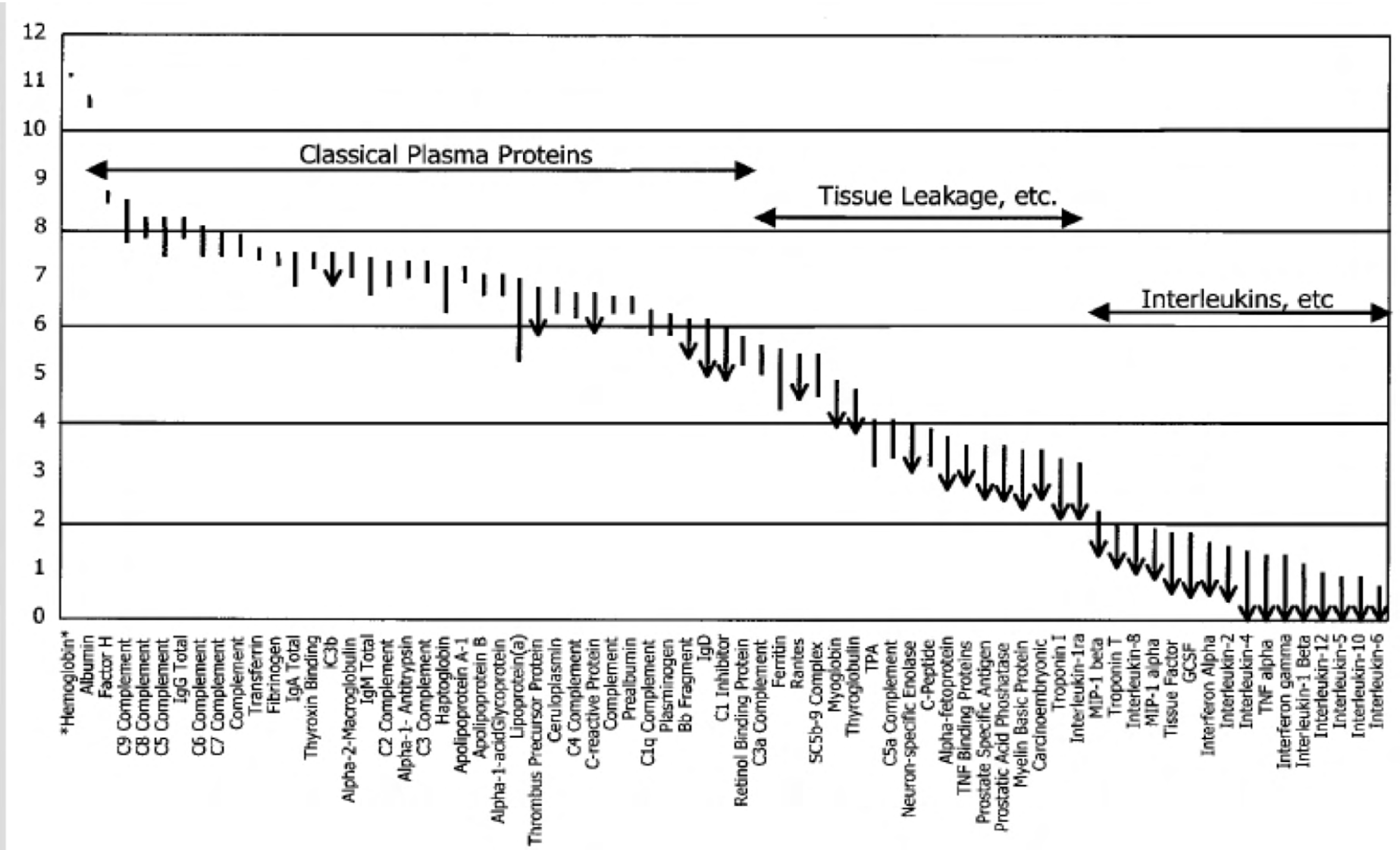
One copy / cell corresponds to

$$\frac{1}{6.02 * 10^{23}} = 1.66 * 10^{-24}\ moles$$

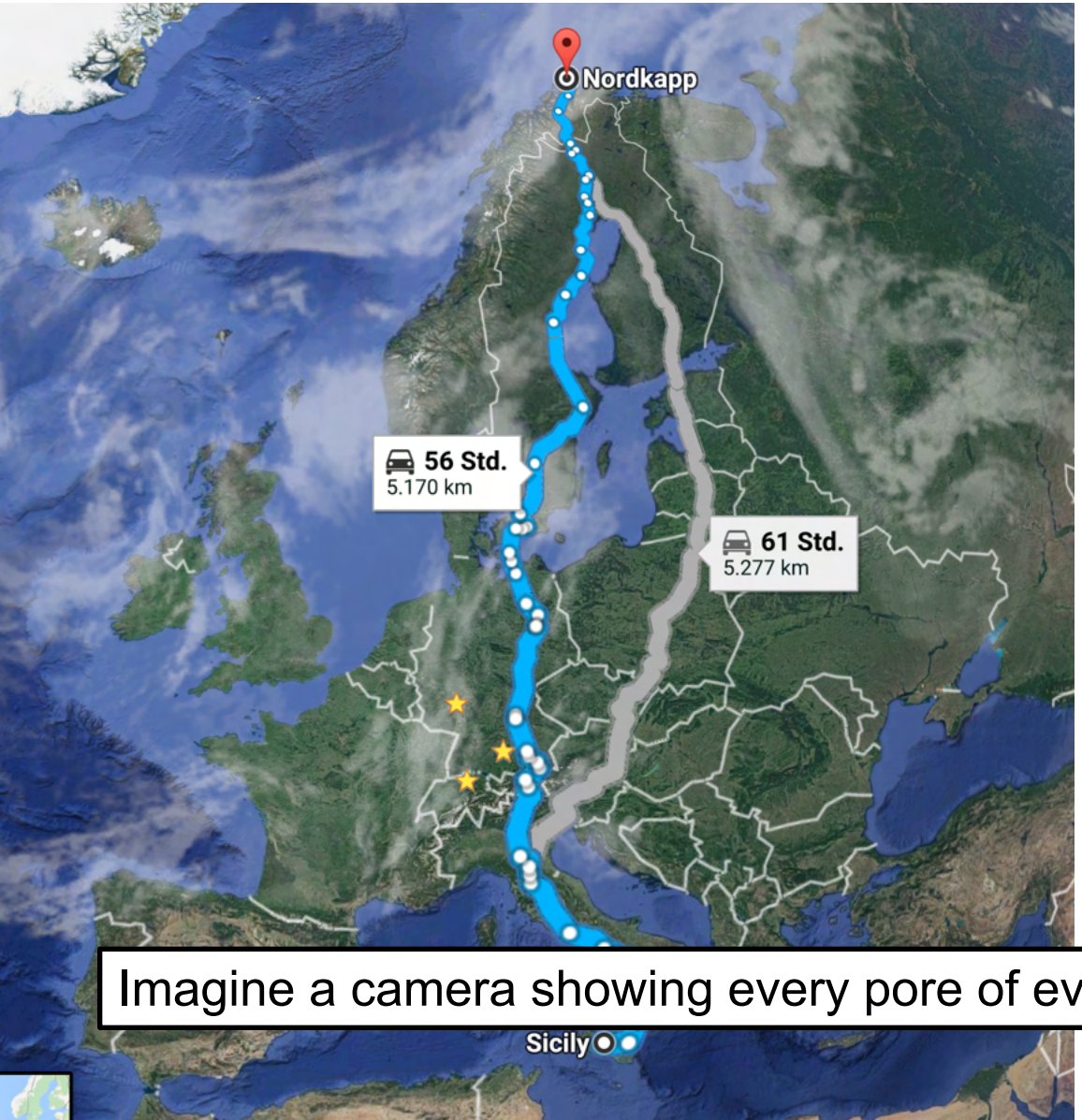
For a net Detection Limit of 1 femtomole (*i.e.* 10^{-15} moles):

$6*10^{8}$ cells required to detect a single copy

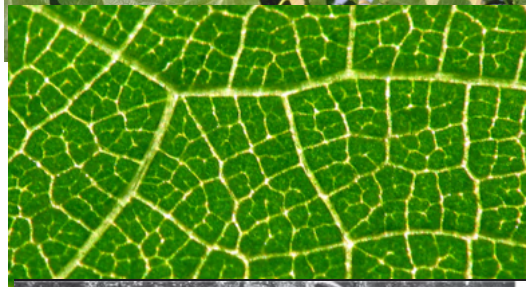
Dynamic range of the human plasma proteome



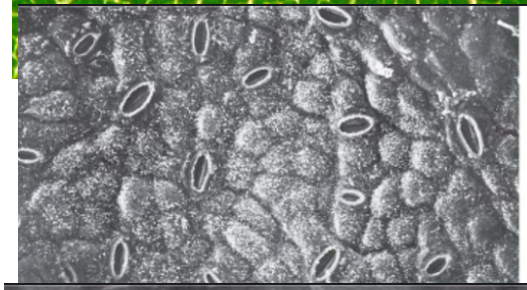
From Nordkap to Sicily : 5000 km



50cm



5 mm

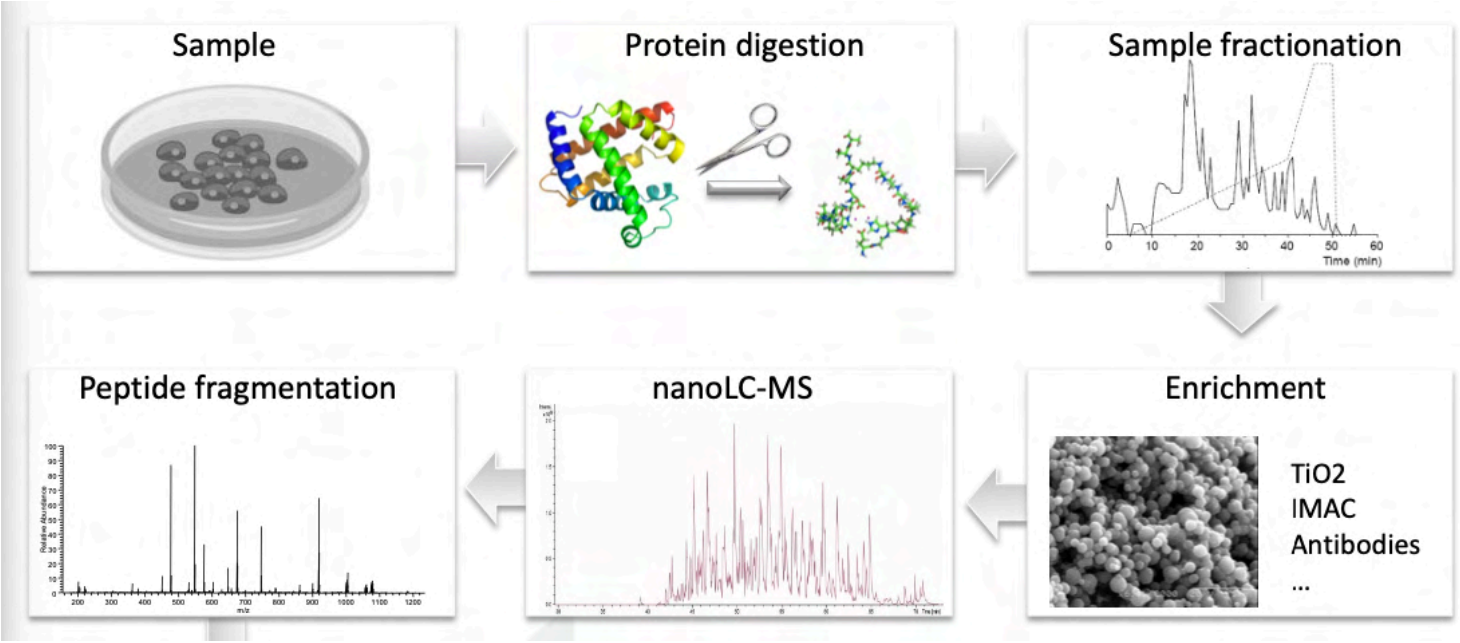


25 µm

5 µm

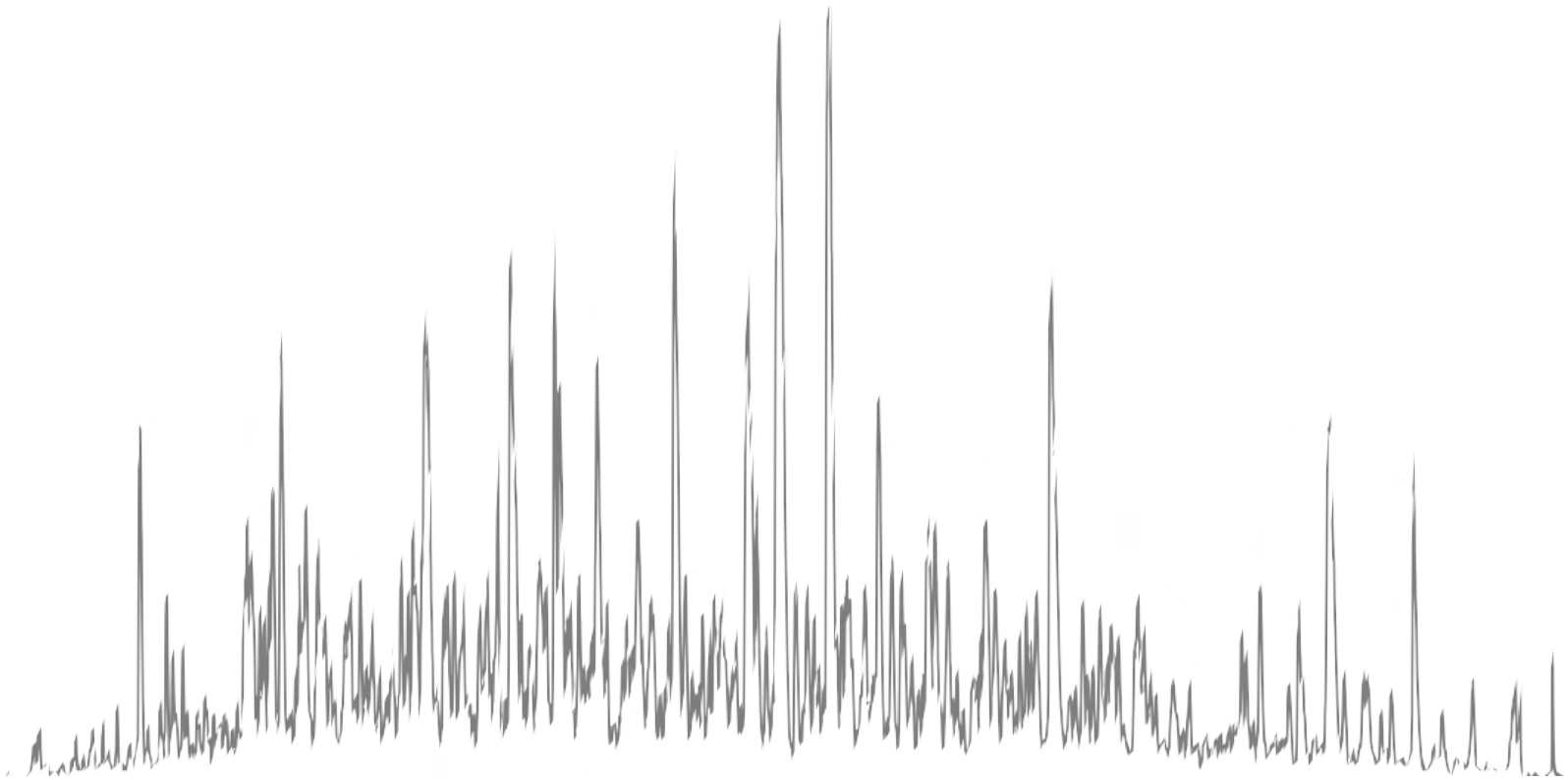
Imagine a camera showing every pore of every leaf in Europe!

Standard proteomics approaches



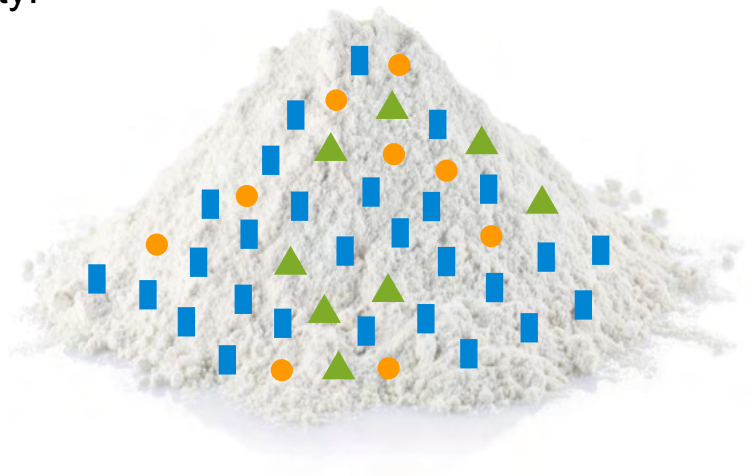
Data processing

Basics of liquid chromatography



Application of HPLC
Example

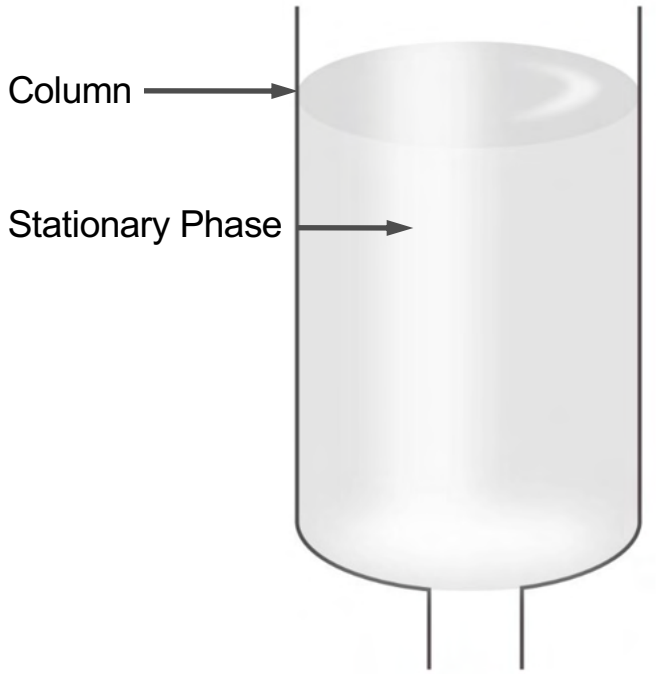
- In the pharmaceutical industry, for example, controlling the quality of active ingredients (■) in drugs is vital.
- HPLC is used to identify drug impurities (● ▲) that may occur during synthesis or by decomposition of the active ingredient.
- Quality control ensures patient safety.



Application of HPLC

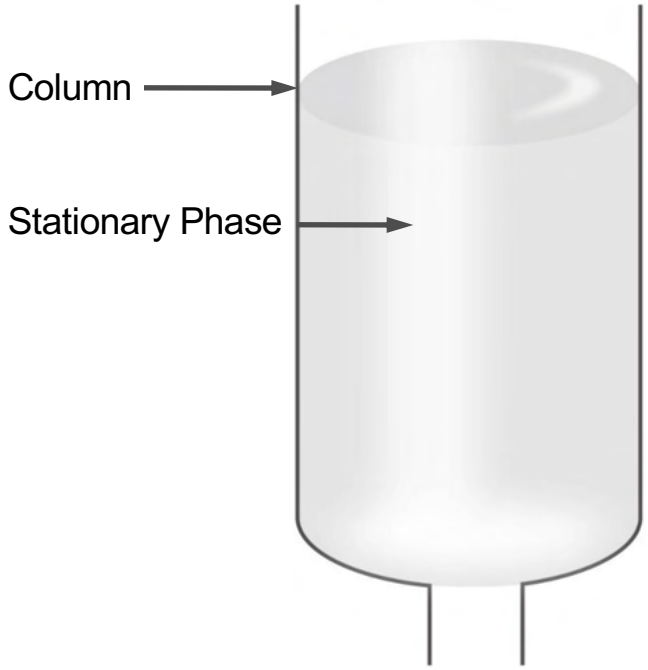
Step 1: Tablet Dissolution and Substance Release

Application of HPLC
Step 2: Separation



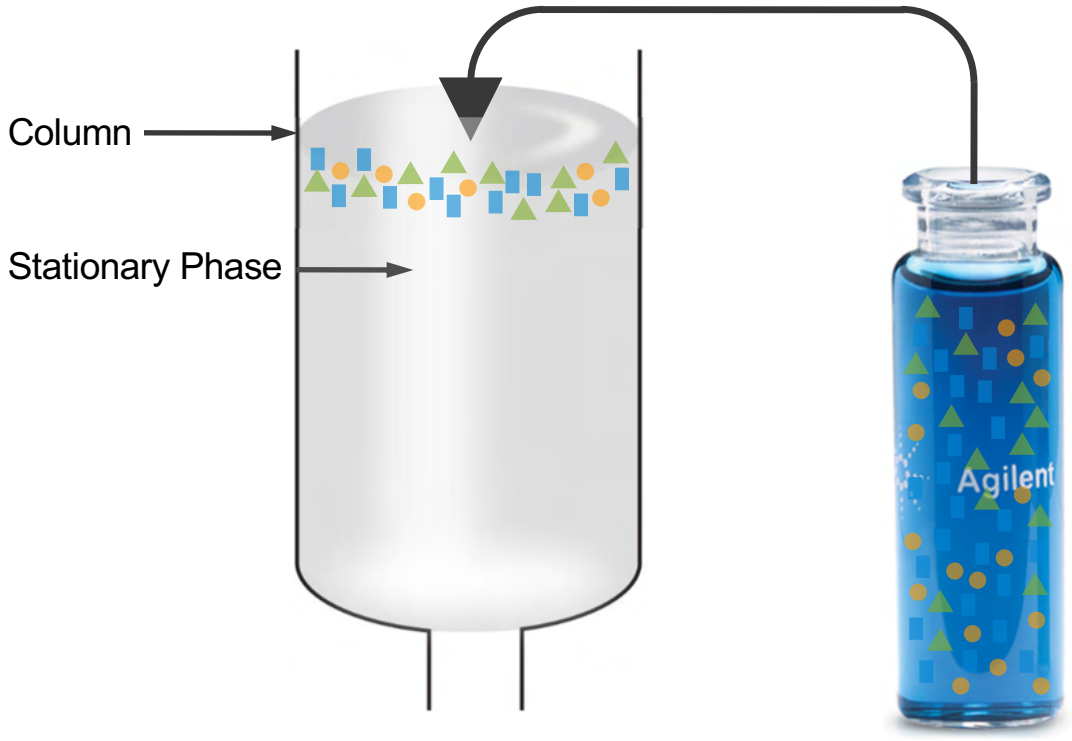
- Substance separation occurs in the separation column.
- The column consists of a metal tube filled with silica gel for what is known as the stationary phase.

Application of HPLC
Step 2: Separation

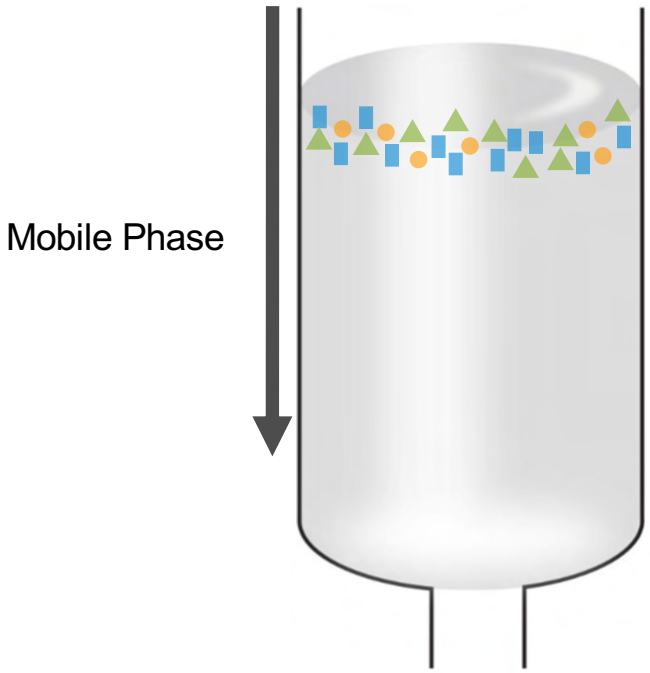


- The dissolved substances are injected onto the separation column.

Application of HPLC
Step 2: Separation



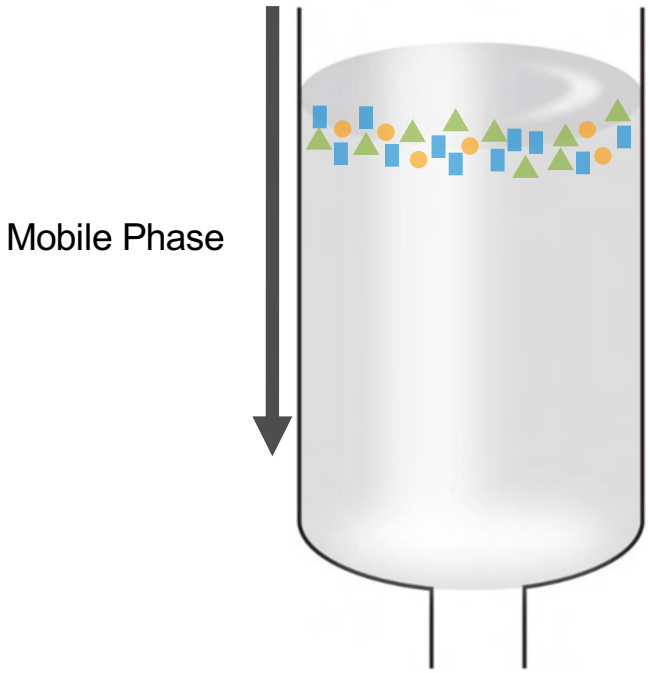
Application of HPLC
Step 2: Separation



Pressure is applied to force liquid (for example, a mixture of water and methanol) through the column.

This called the mobile phase.

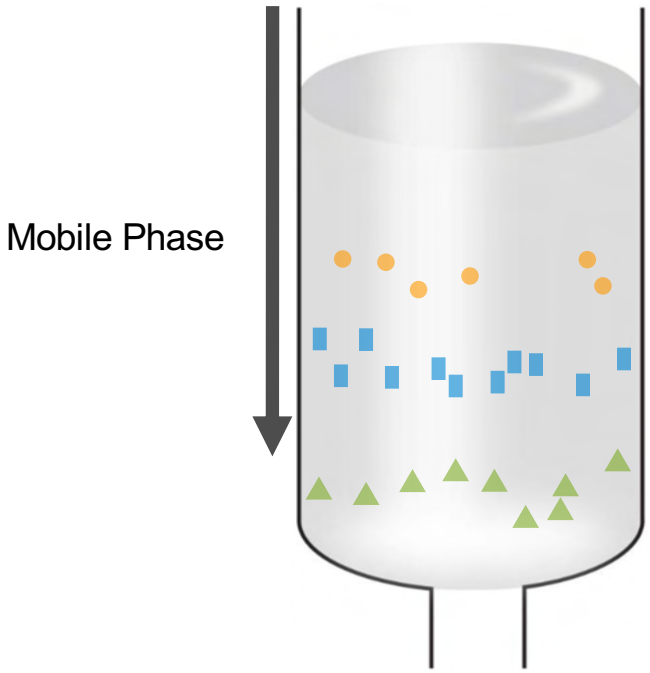
Application of HPLC
Step 2: Separation



The liquid (mobile phase) flows through the silica gel (stationary phase) and carries the substances with it.

Different components travel at different rates through the column.

Application of HPLC
Step 2: Separation

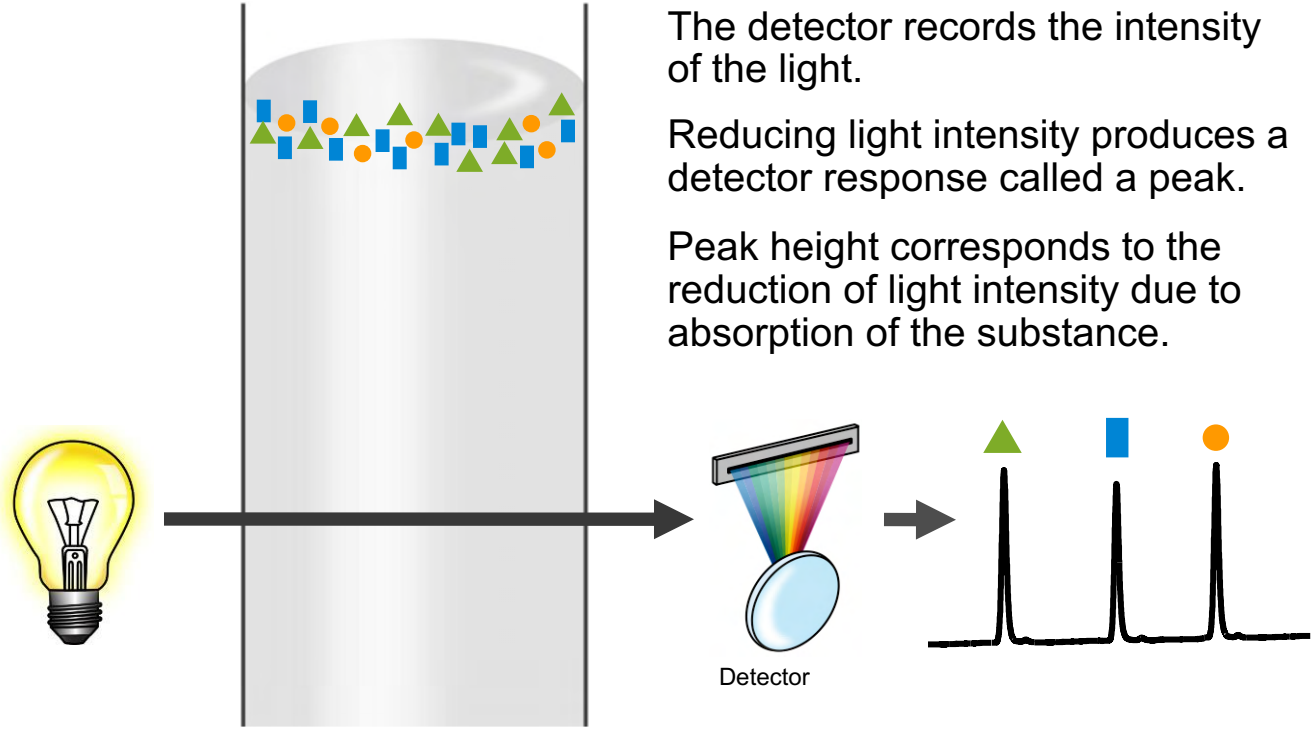


This means the substances reach the end of the column at different times.

Application of HPLC
Step 3: Quantitative Determination of Substances

- After separation, an ultraviolet light is used to measure the amount of substance present in a sample.
- More or less light is absorbed depending on the quantity of the substance.
- The amount of light absorbed is proportional to the quantity of the substance, which means twice the amount of substance will absorb twice the amount of light.

Application of HPLC
Step 3: Quantitative Determination of Substances



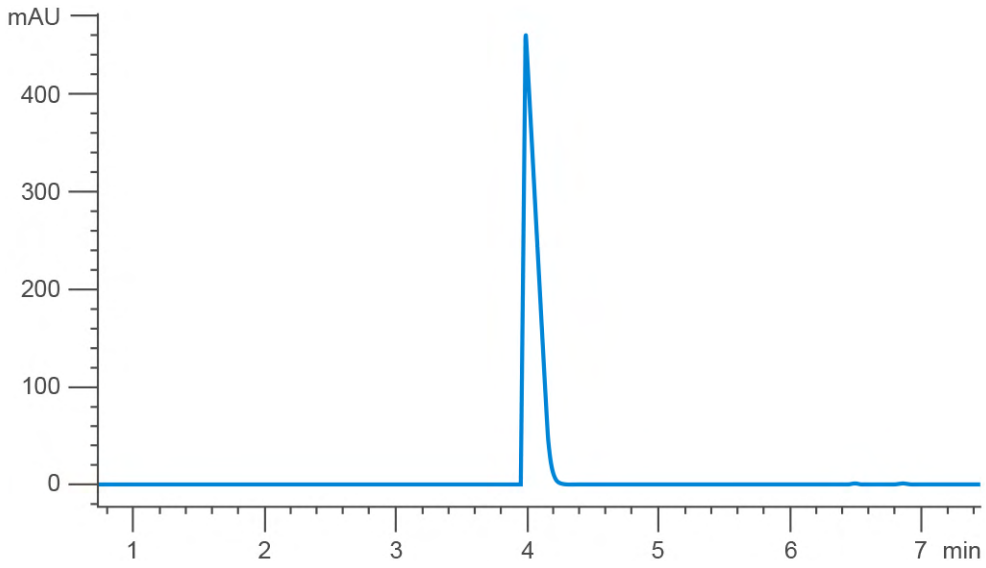
The detector records the intensity of the light.

Reducing light intensity produces a detector response called a peak.

Peak height corresponds to the reduction of light intensity due to absorption of the substance.

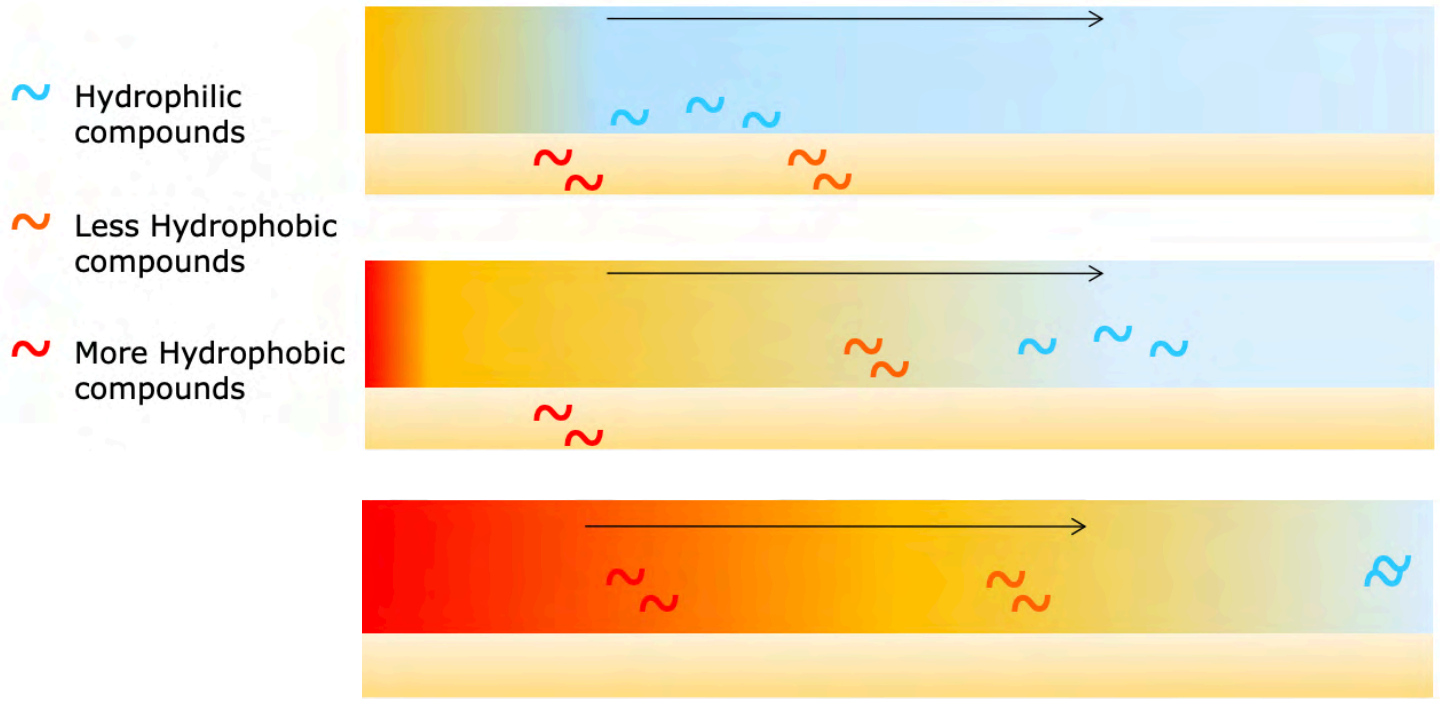
Application of HPLC
Step 3: Quantitative Determination of Substances

- This chromatogram shows the detector signal measured over time.
  The quantative amount of the substance is determined by peak height.



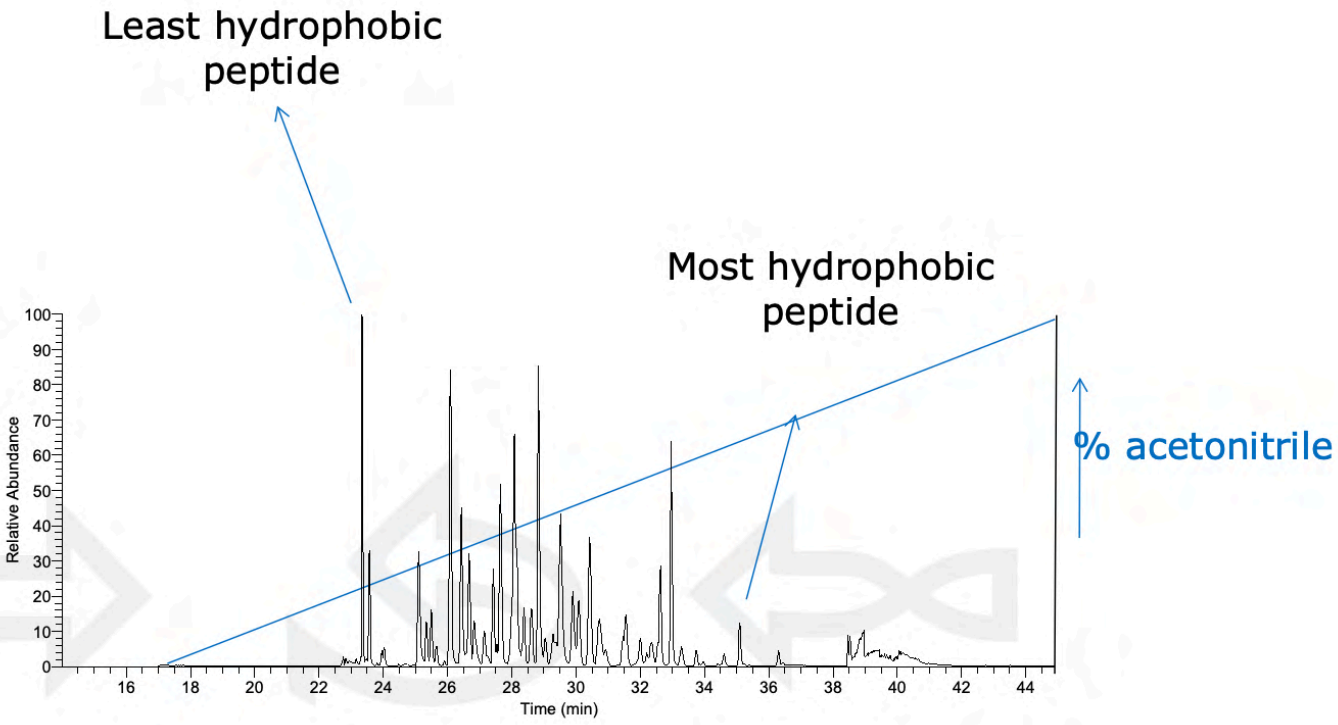
Mechanisms of separation in RP-LC

Elution from column by increasing the organic contents of the solvents, for example with acetonitrile.

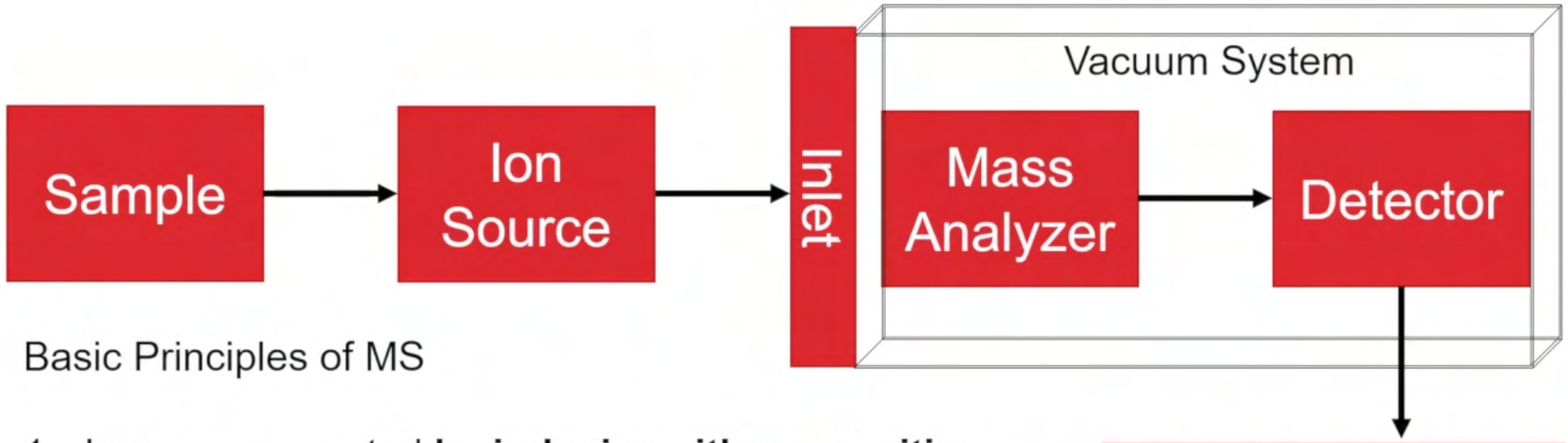


Reversed phase LC-MS

separation based on hydrophobicity using a gradient of increasing [ACN]



Principles of Mass Spectrometry

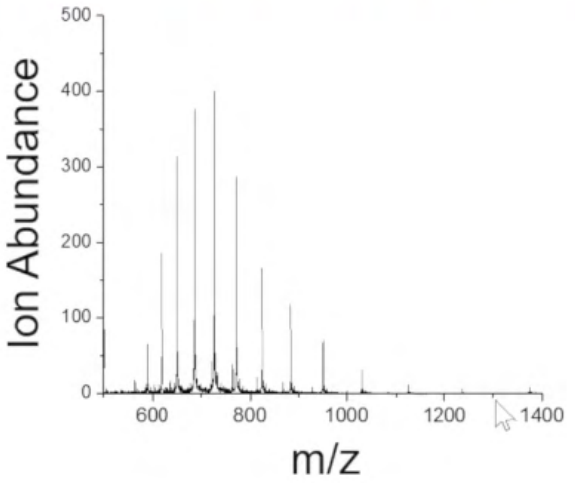


Basic Principles of MS

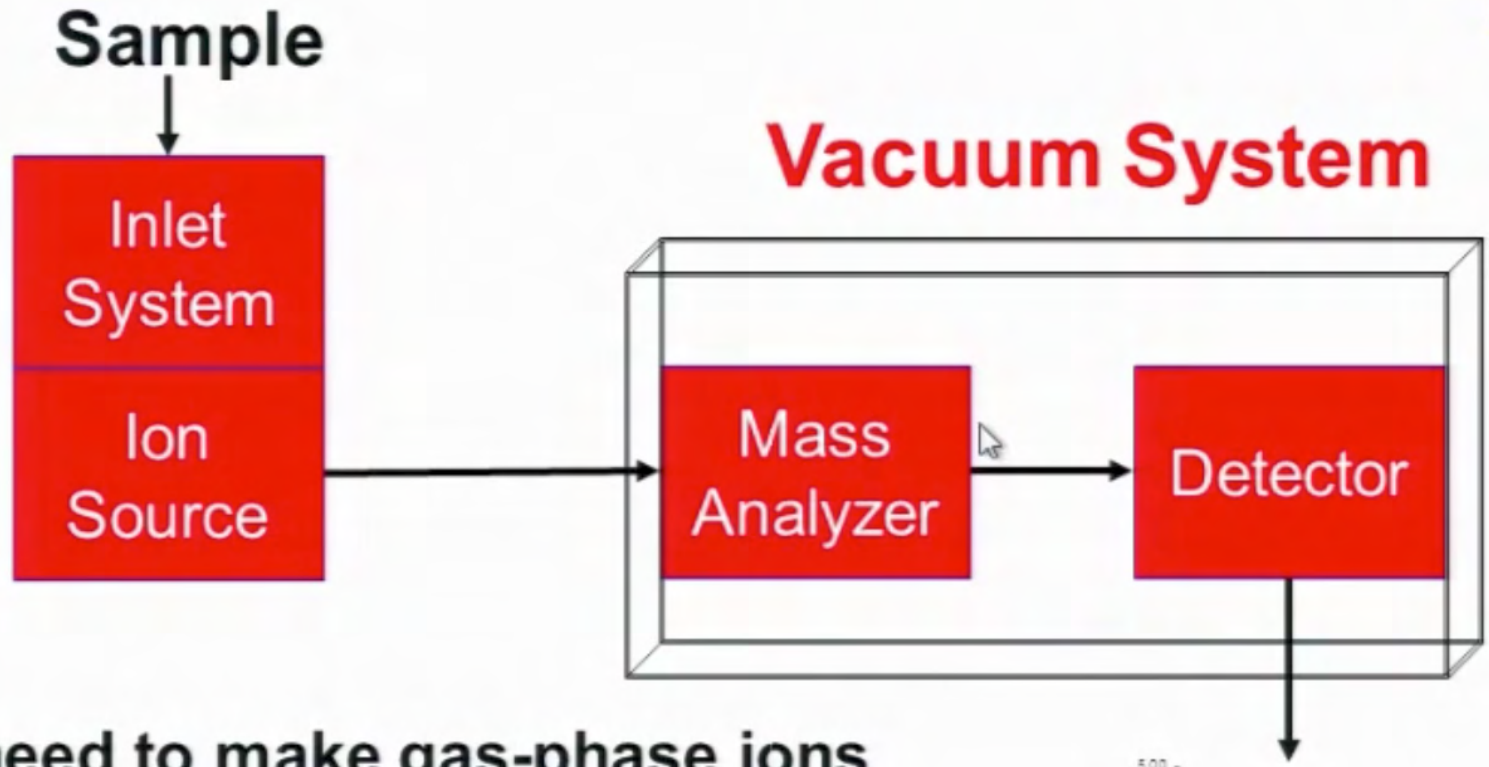
1. Ions are generated **by inducing either a positive or negative charge** in a neutral species.

2. Once formed, **ions are electrostatically directed** into a mass analyzer, where they are separated according to **mass-to-charge ratio (*m/z*).**

3. A mass spectrometer determines the abundance and *m/z* of each compound present, which creates a mass spectrum.



Step 1: Generating Ions

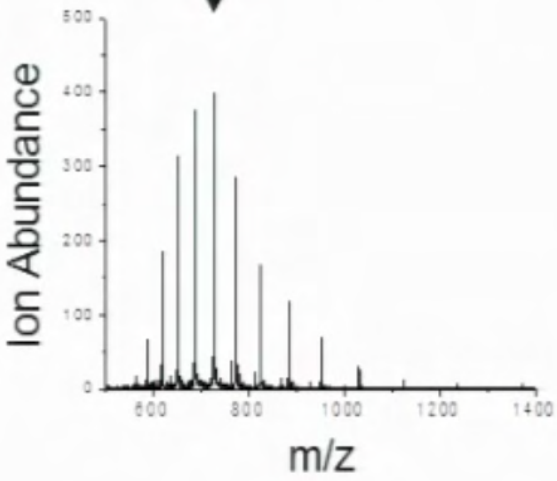


We need to make gas-phase ions

could be called. . .
mass-to-charge (m/z) spectrometry

Ions are analyzed at low pressures

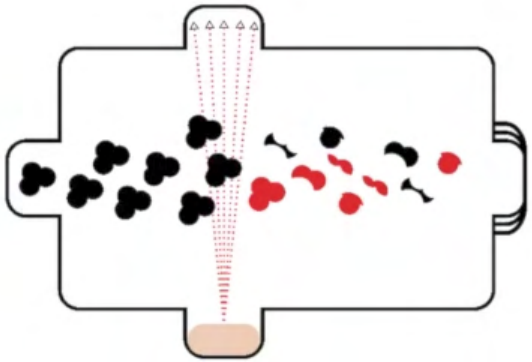
Mass spectrometers are very sensitive



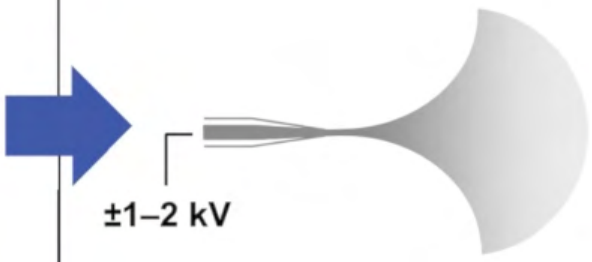
Ionization techniques for Proteomics

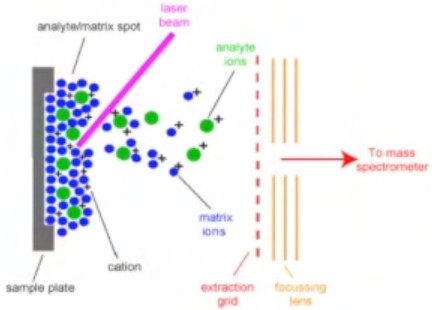
Ion Source → Mass Analyzer → Detector

Gas phase techniques
Electron Ionization (EI)
Chemical Ionization (CI)



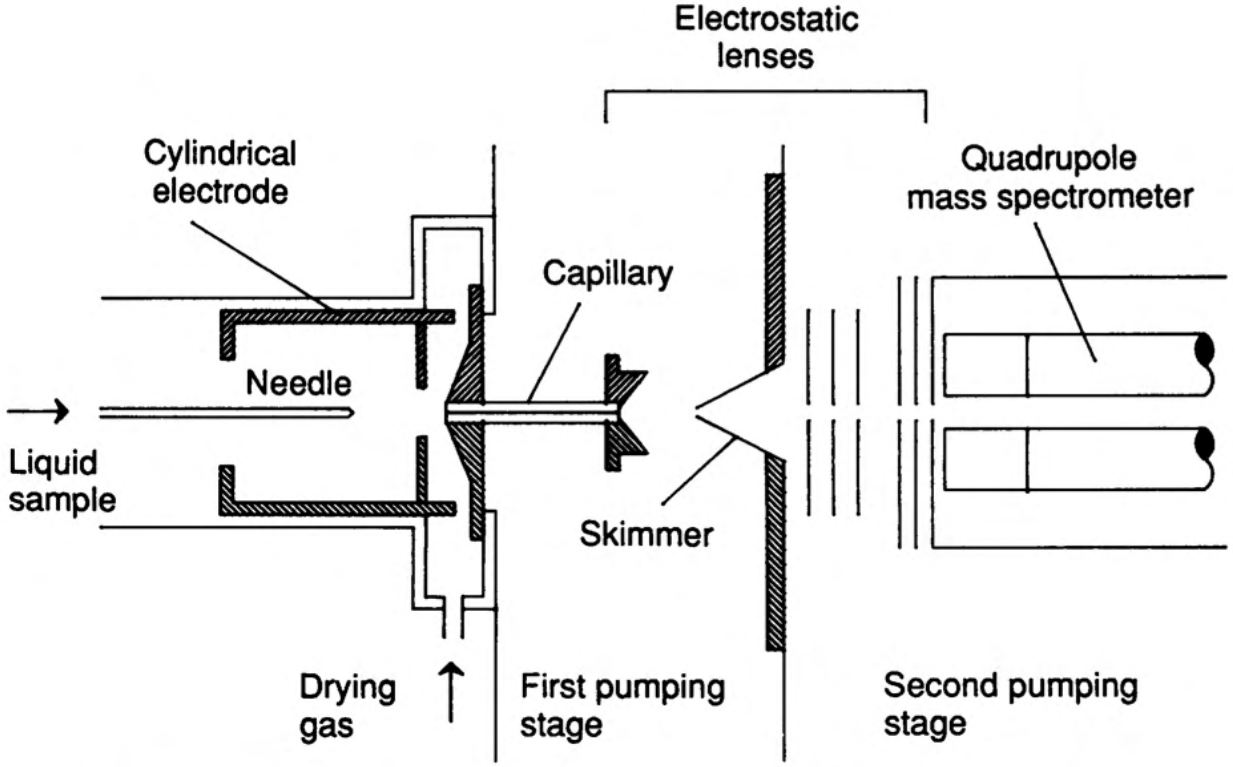
Desorption techniques
Matrix Assisted Laser Desorption Ionization (MALDI)
Electrospray Ionization (ESI)





Electrospray Ionization Fenn et al. 1989

Electro Spray Ionization – soft ionization



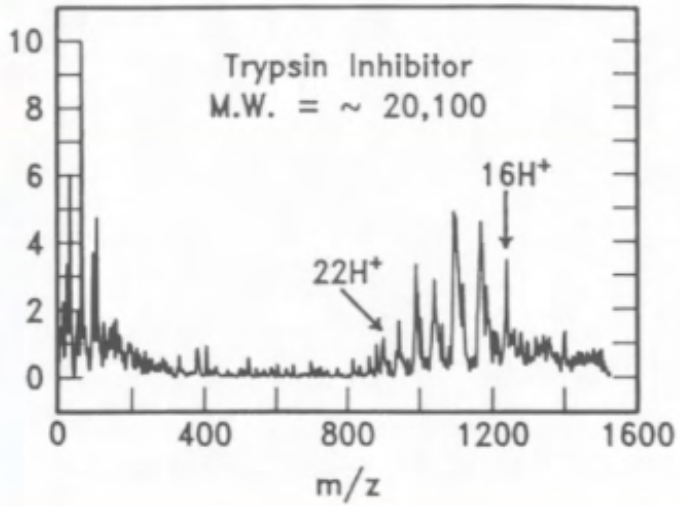
John Fenn (1970's – 1986)
- PhD-student Matthias Mann

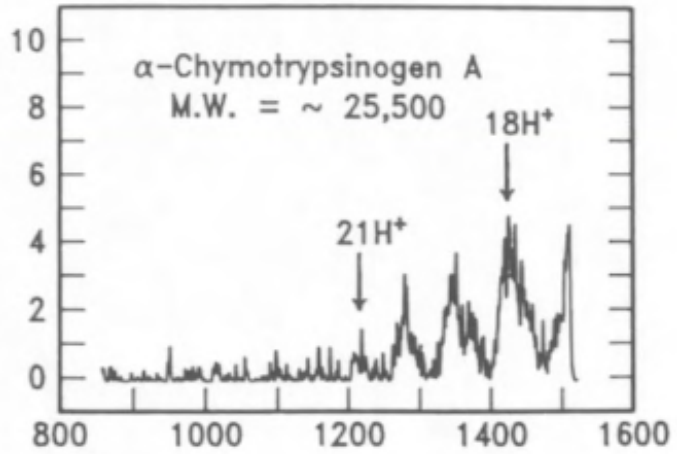
Fig. 1. Sketch of the ion desolvation process. Small, charged droplets produced by the electrospray evaporate, generating a high electric field at the droplet surface. Analyte molecules that were dissolved in the droplet can attach to charges and be lifted into the gas phase by this field.

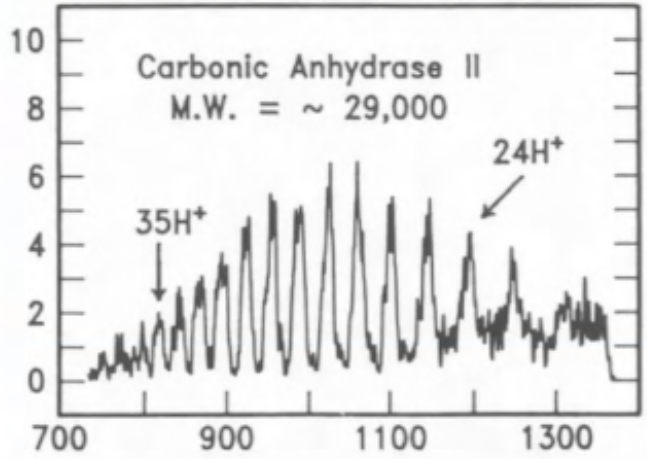
6 OCTOBER 1989

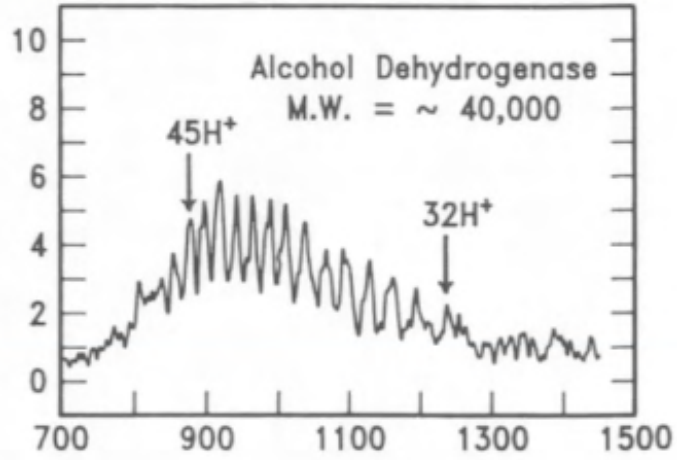
Electrospray ionization of small proteins

John B. Fenn
(Nobel prize, 2002)

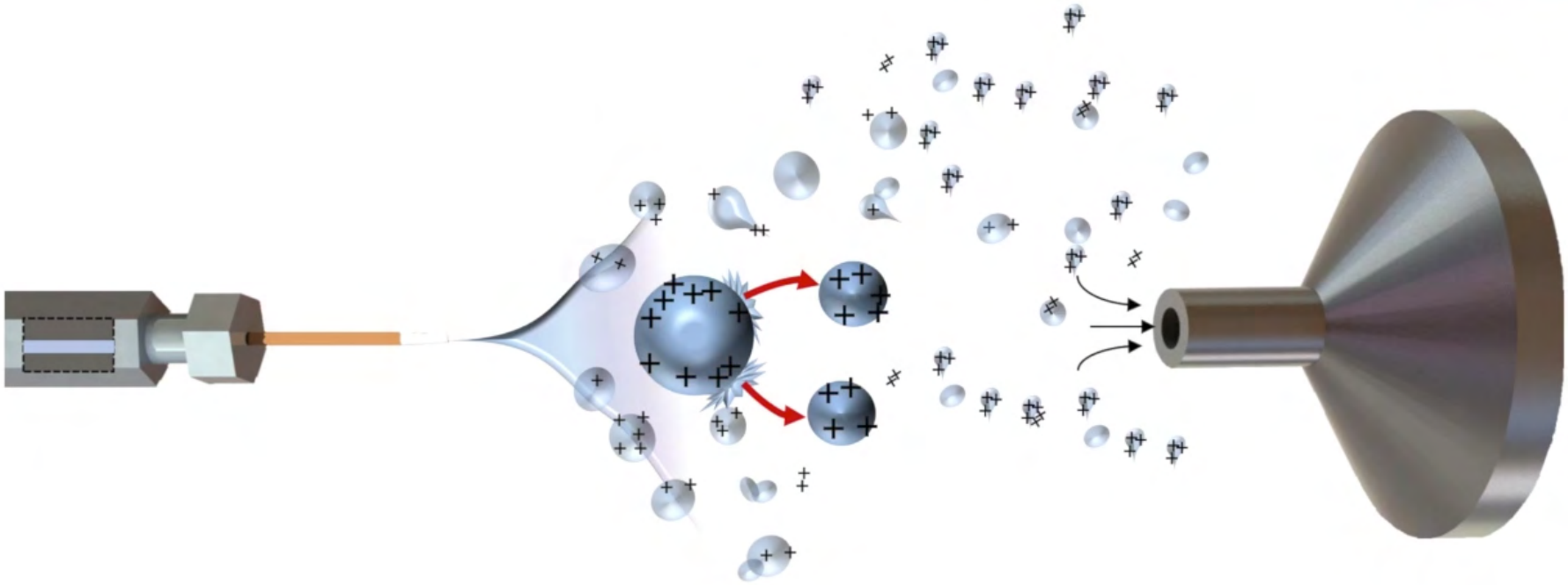






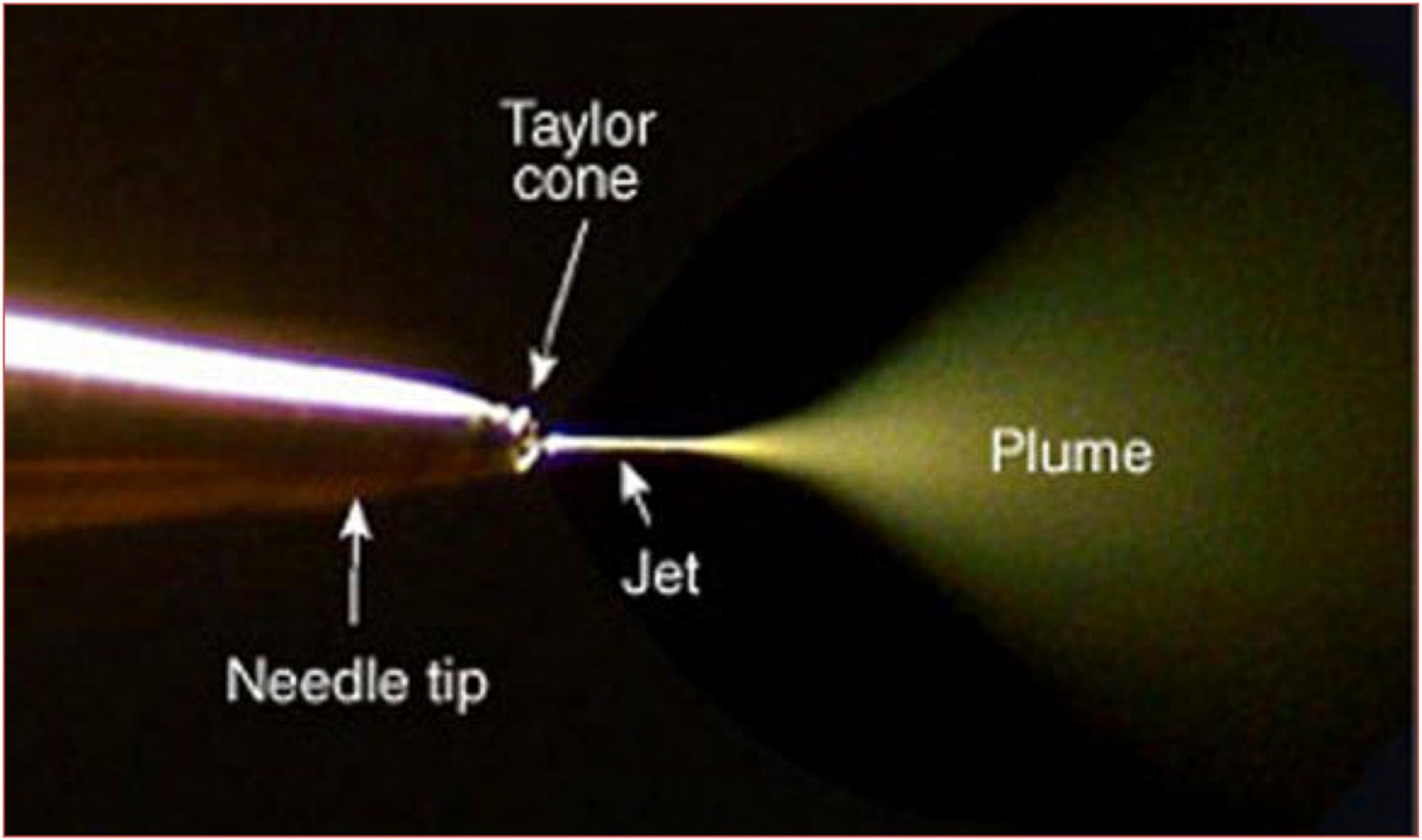


Electrospray Ionization

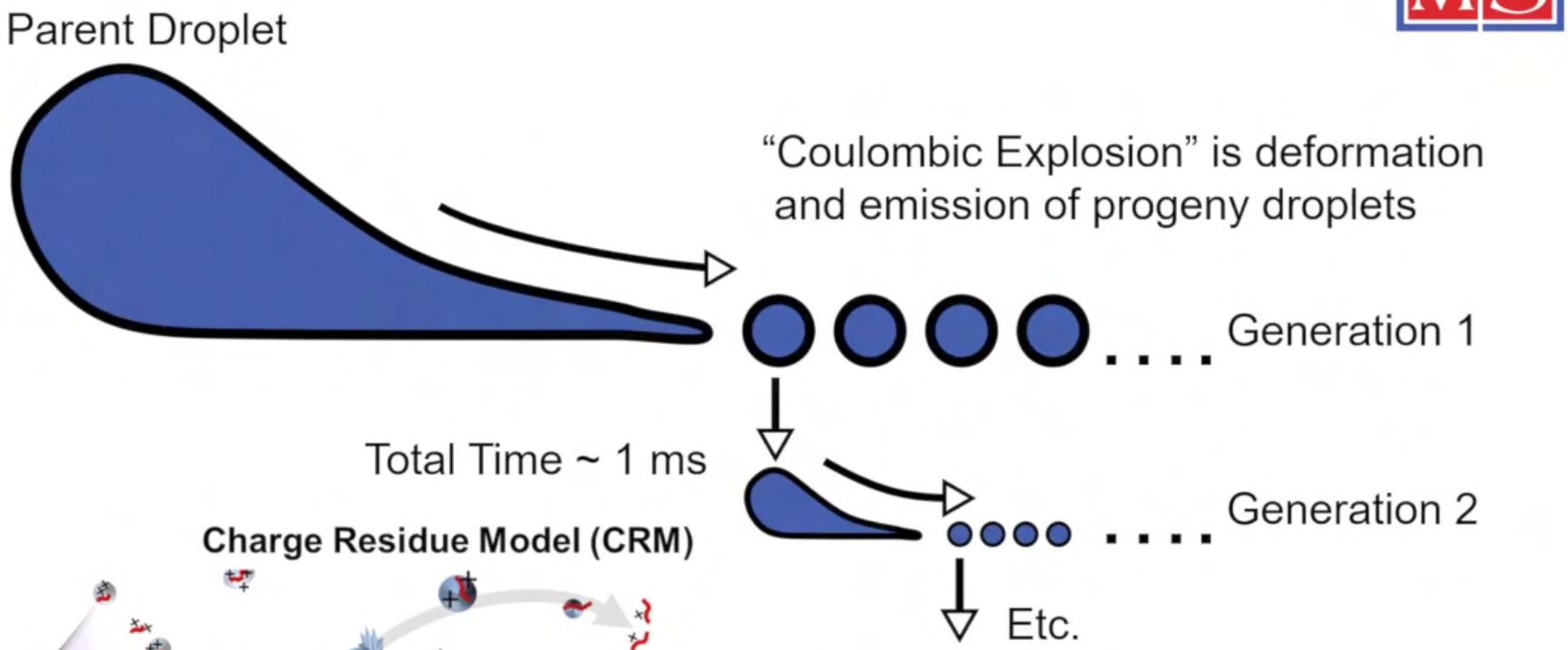


- Considered the softest ionization technique, allowing large molecules and even non-covalent clusters to be analyzed
- Enables direct coupling of liquid separations to mass spectrometry
- Detection limits femtomole to subattomole

Taylor Cone



ESI Process: ion generation



Ions do not require an "ultimate droplet" and can be emitted directly to gas phase

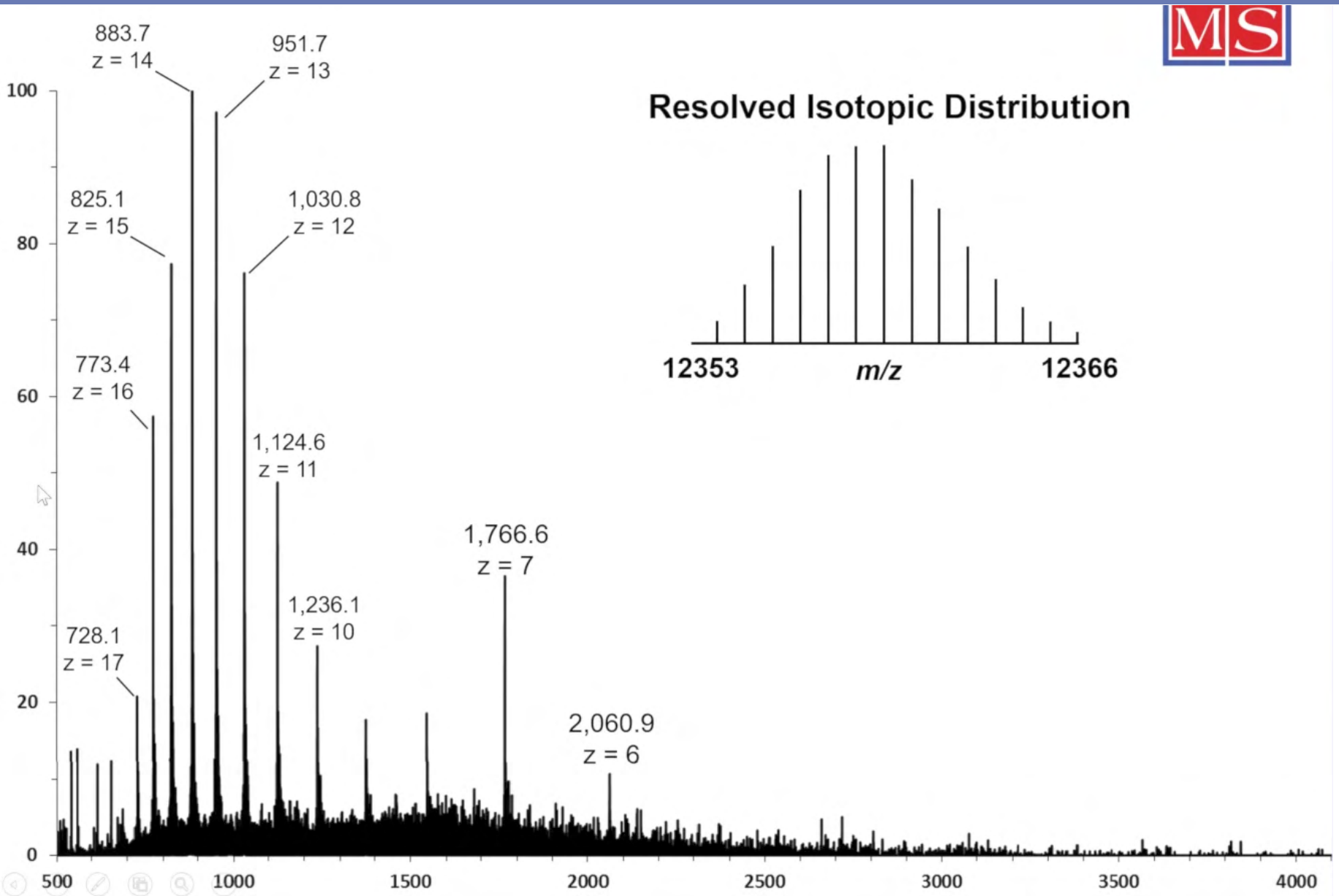
Physiochemical properties explain differences in ion abundance.

Dole, M.; Mack, L.L.; Hines, R.L., *J. of Chem. Physics*, **1968**, *49*(5), 2240-2249

Iribarne, J.V.; Thomson, B.A., *J. of Chem. Physics*, **1976**, *64*(6), 2287-2294

N.B. Cech, C.G. Enke, *Mass Spectrom. Rev.*, **2001**, 20, 362-387.

ESI (typically) generates multiple charge states



Why are we using nanospray?

Normal Flow

50-200 µl/min

Nanoflow

50-400 nl/min

-> Higher ionization and sampling efficiency!

Dark facts about ion sources..

Exact Mechanisms of ESI and MALDI remain unknown !
(but there are good hypotheses)

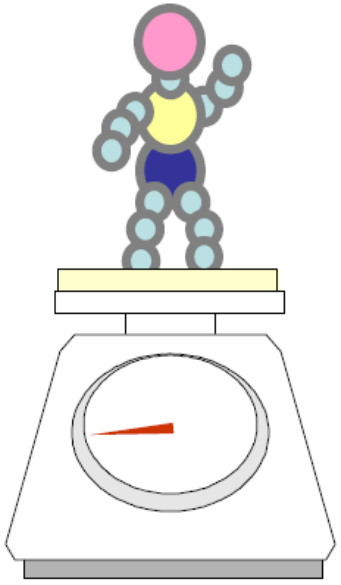
ESI:

- Latest sources reach up to 50% ioinization efficiency for low-flow nanospray
- Only for low-flow nanospray, there is a chance to ionize all analyte molecules
- Not all peptides ionize equally – difference up to 100-fold, making absolute quantification difficult

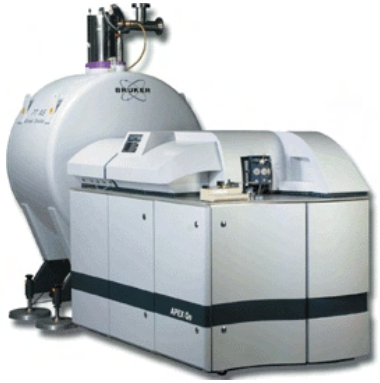
MALDI

- Ionization efficiency very low – between 0.001% and 0.1%
- Ionization highly dependent on matrix preparation – makes absolute quantification impossible

What is a mass spectrometer?



A molecular scale,
e.g. based on the measurement of time

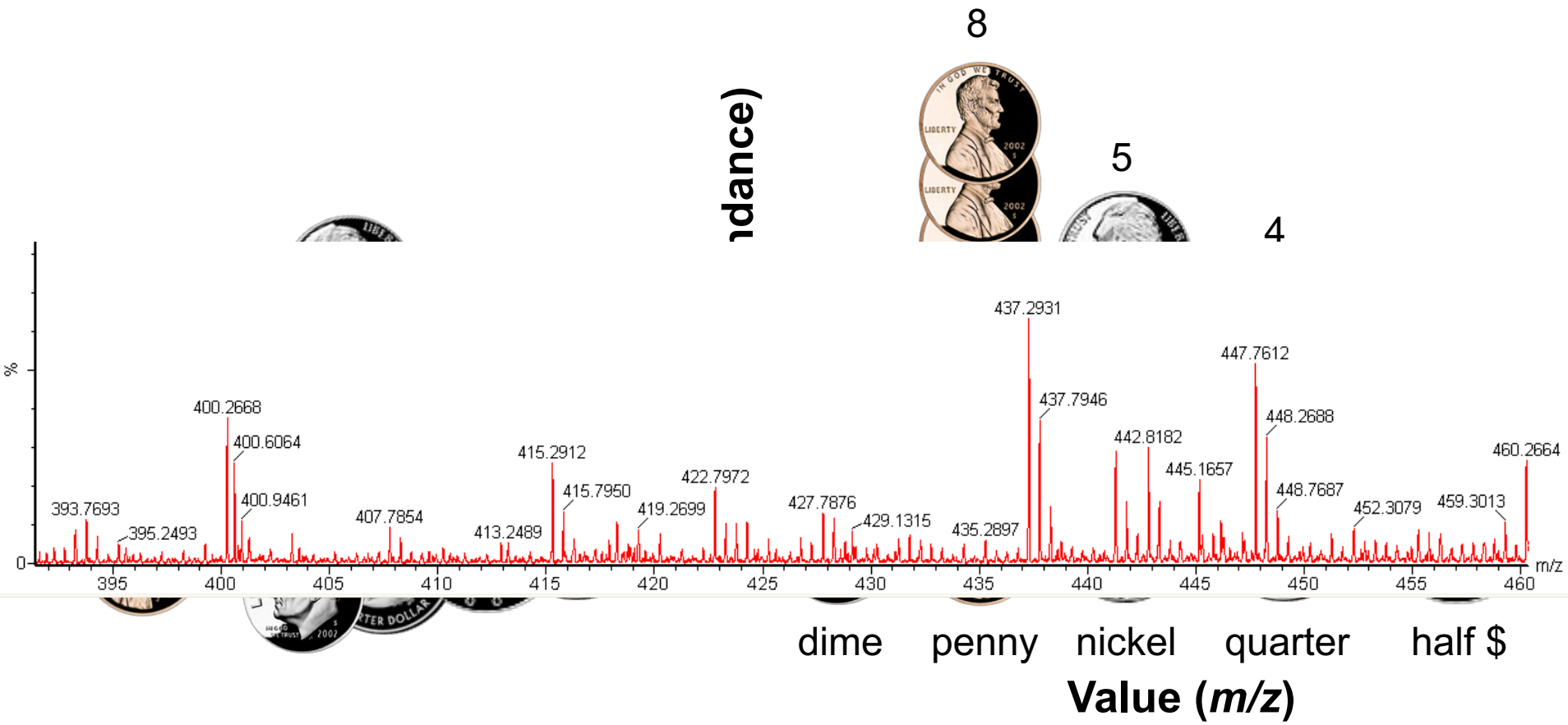


High Mass Accuracy

Delta Mass = 10 g / 1000 kg = 10 ppm

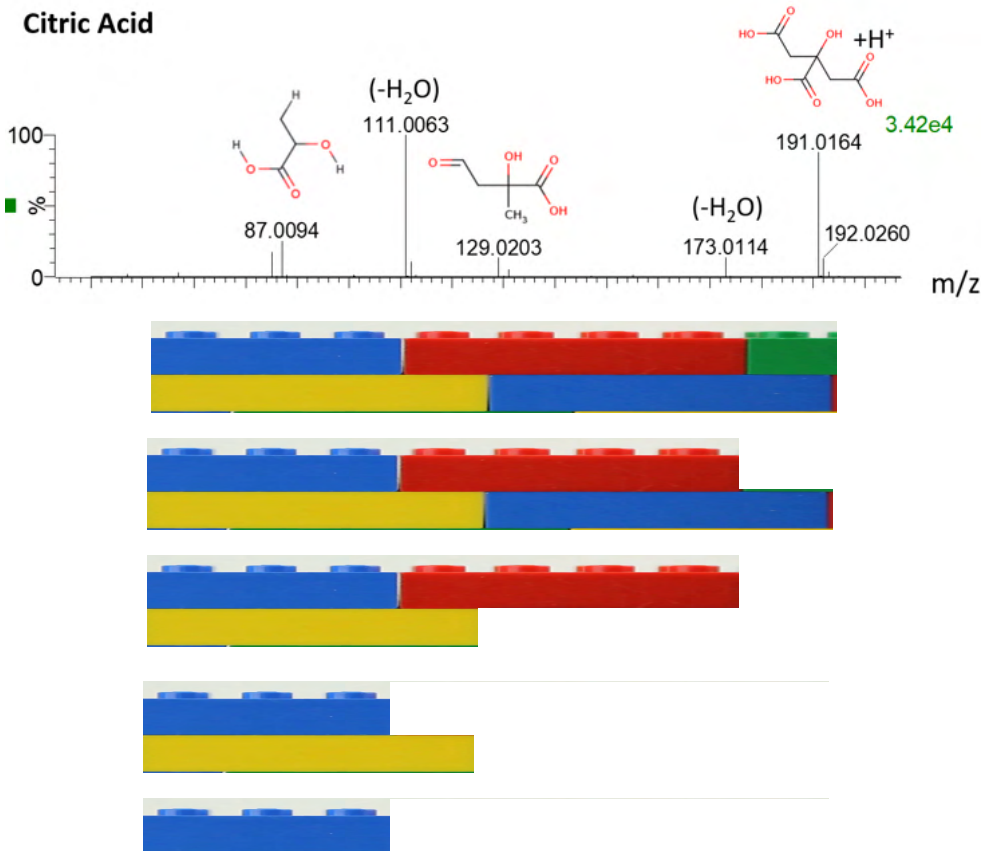
Basic Concept of Mass Analysis

- **Sorting and counting**
  - *Pocket change (mixture of coins)*
  - *Penny, dime, nickel, quarter, half $*
  - *Sorting change by value or size*
  - *Mixture of molecules*
  - *Molecules of different weight, size*
  - *Separation by mass spectrum*



Mass spectrometry basic principles: fragmentation (MS/MS) spectrum

- **Ions can be fragmented in MS**
- **Product, daughter or fragment ions**: are generated from the fragmentation or decomposition of a **precursor or parent ion.**
- **Fragmentation spectrum:** is the pattern of fragment ions plotted as intensity Vs m/z.
  - Synonyms: **MS/MS, MS2 MS^2 or MS^n**



[Gomez-Zepeda]

Mass spectrometry basic principles: fragmentation (MS/MS) spectrum

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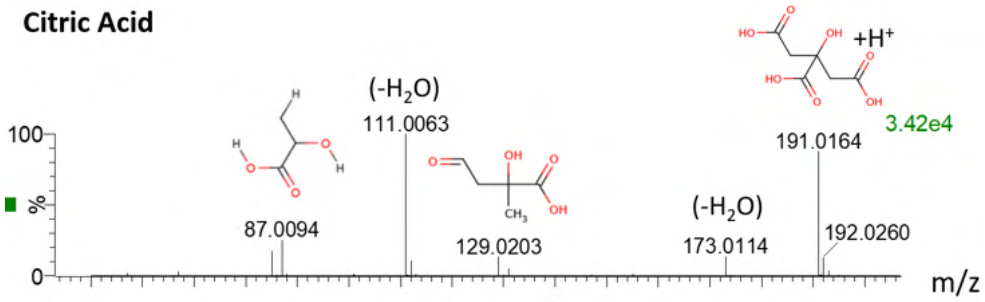
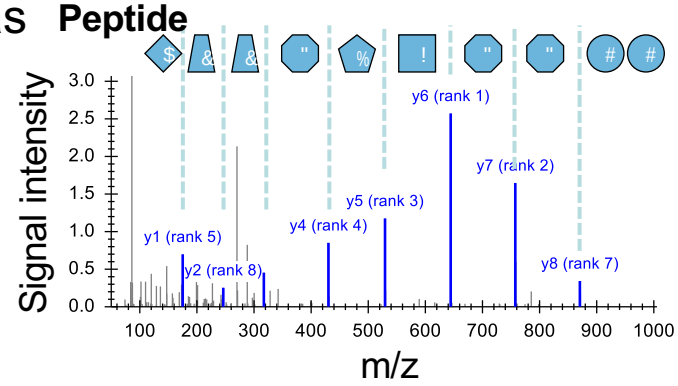
  - Synonyms: ! "# ! "$% ! "&%% ! "' ! '(% ! "*
  -) *+(' , - ./01*%2 3 '1* . -14(%5/6748-(*9 (in the given instrumental conditions)
  - Identification of molecules
  - Structural elucidation





[Gomez-Zepeda]

The origins of mass spectrometry

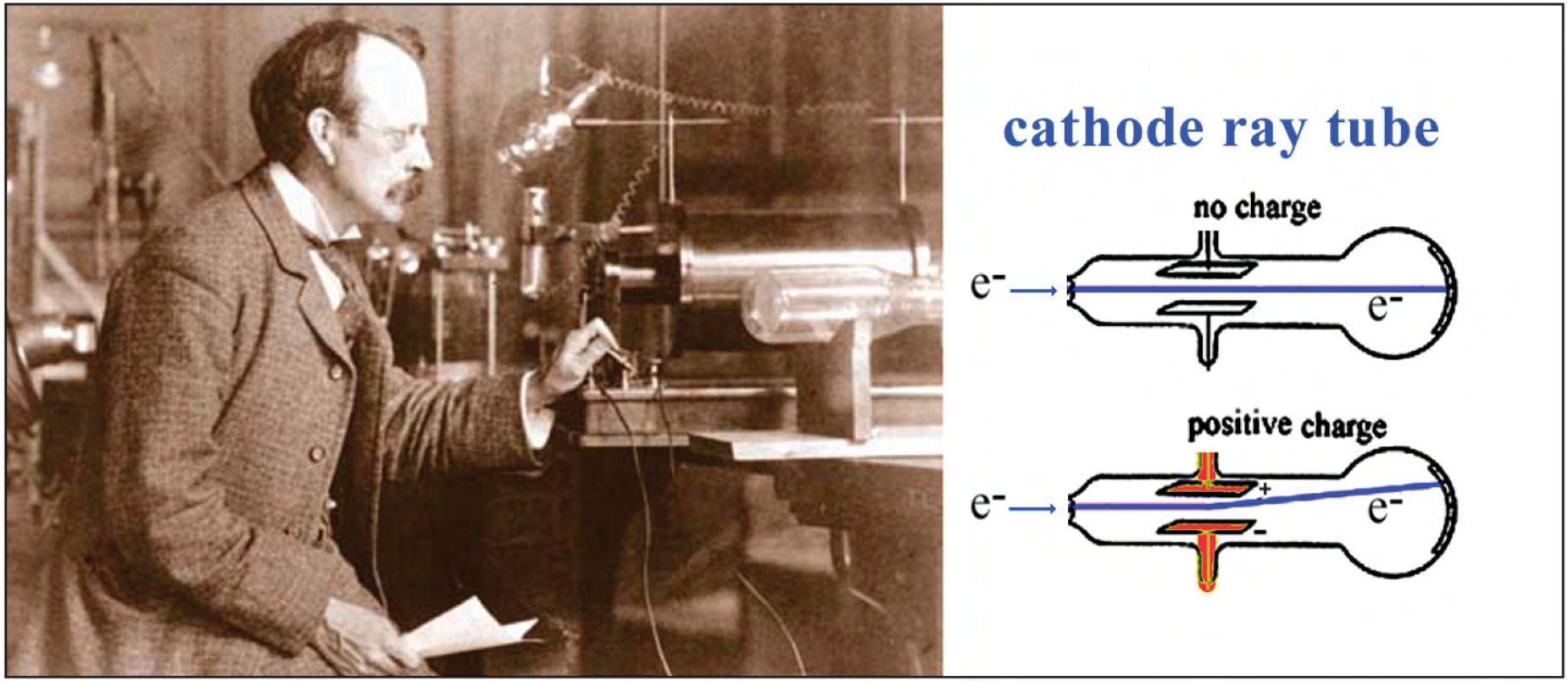
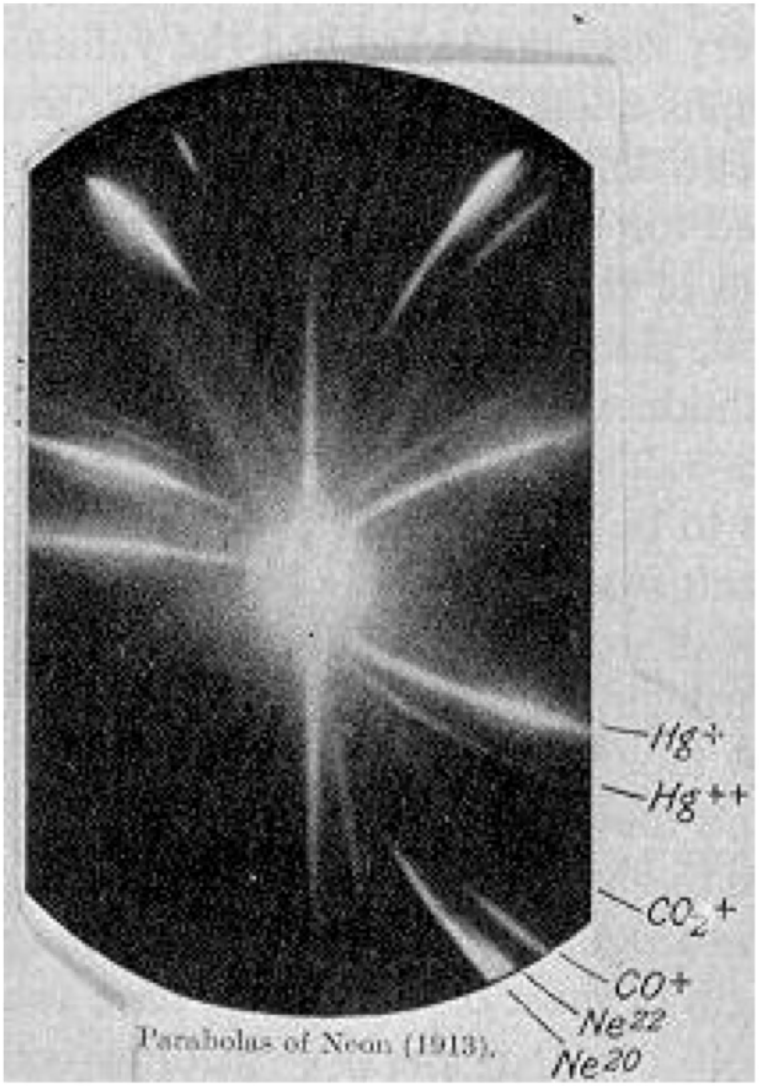
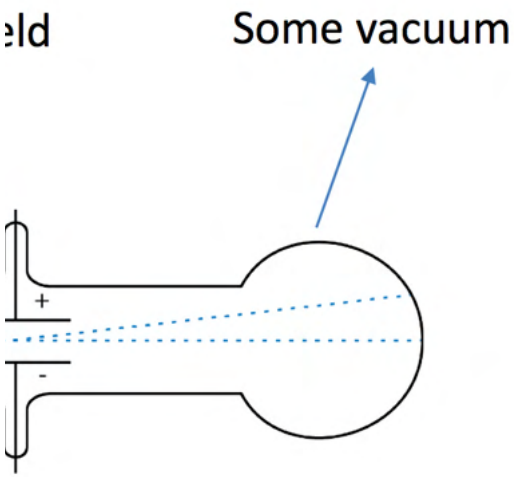


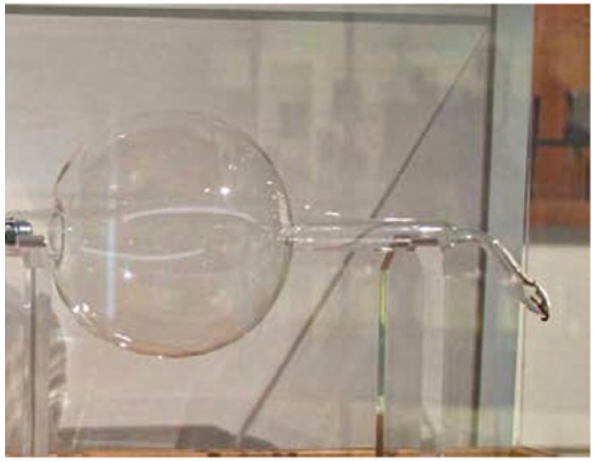
FIGURE 1: J. J. Thomson and a cathode ray tube used to perform some of the first *m/z* measurements. Deflection of the electron was observed once the electric field was turned on.

The origins of mass spectrometry

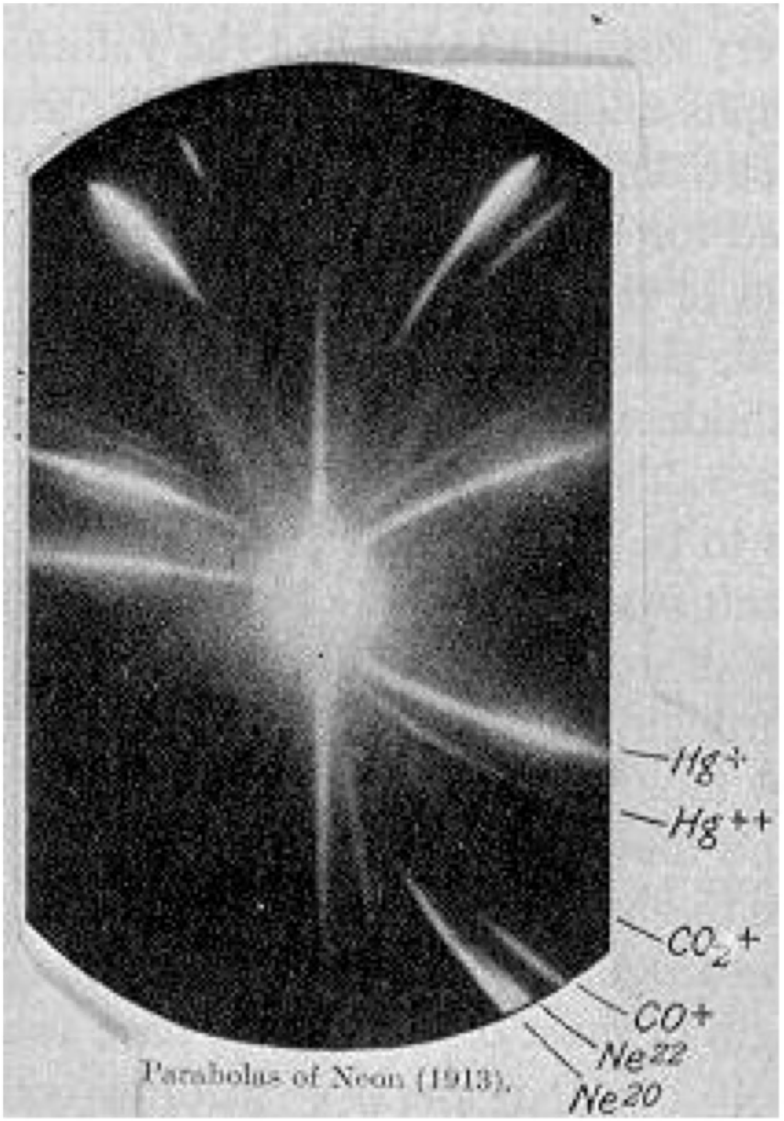
J.J Thompson 1914







Lessons from a „simple“ experiment



In the presence of a magnetic field:

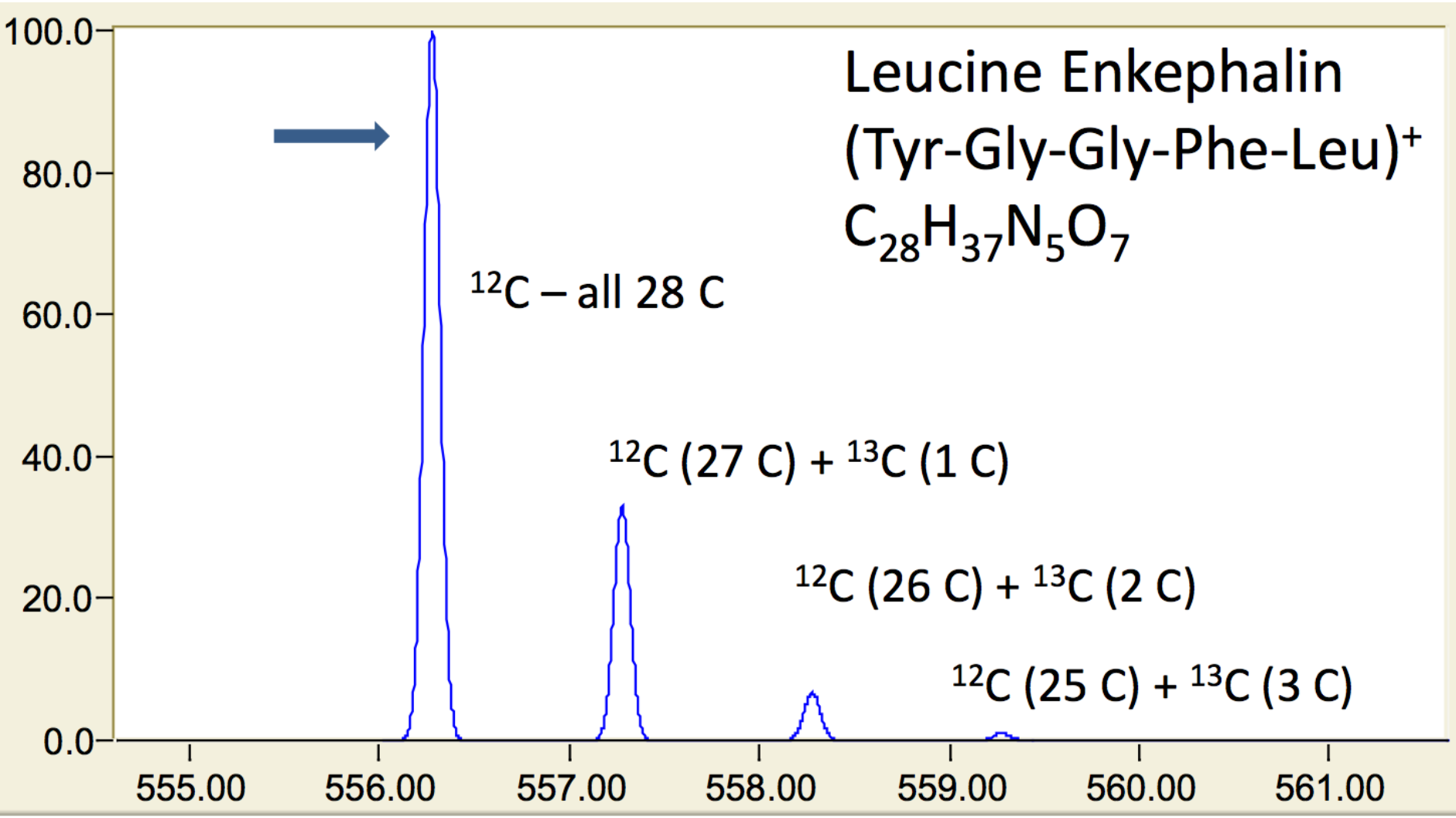
- Different elements respond differently
- Different charge states respond differently:
  we measure m/z (unit: Thompson)
- Different elemental composition repond differently
- Isotopes can be resolved

Andromeda Galaxy 1888

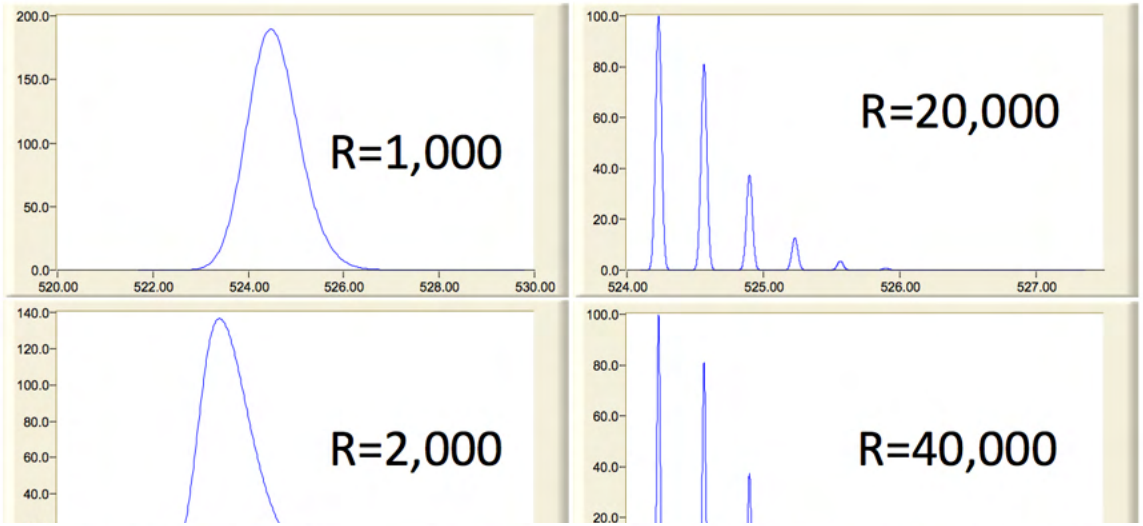
Common Stable Isotopes and Relative Abundances

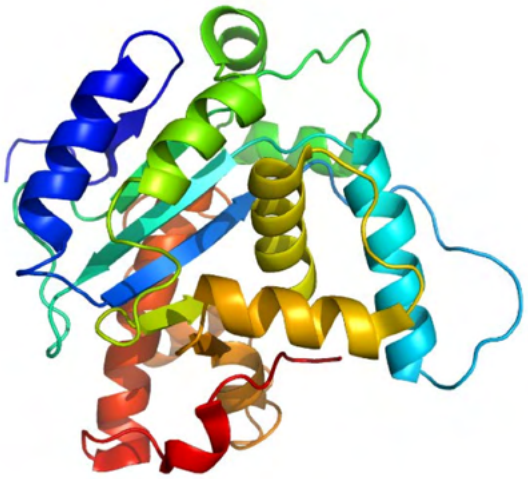
Element	Isotope	Mass	Abundance
Hydrogen	H(1)	1,007825	99,990
	H(2)	2,014102	0,015
Carbon	C(12)	12,000000	98,900
	C(13)	13,003355	1,100
Nitrogen	N(14)	14,003074	99,630
	N(15)	15,000109	0,370
Oxygen	O(16)	15,994915	99,760
	O(17)	16,999131	0,038
	O(18)	17,999159	0,200
Sulfur	S(32)	31,972072	95,020
	S(33)	32,971459	0,750
	S(34)	33,967868	4,210
	S(36)	35,967079	0,020

Isotopic Patterns

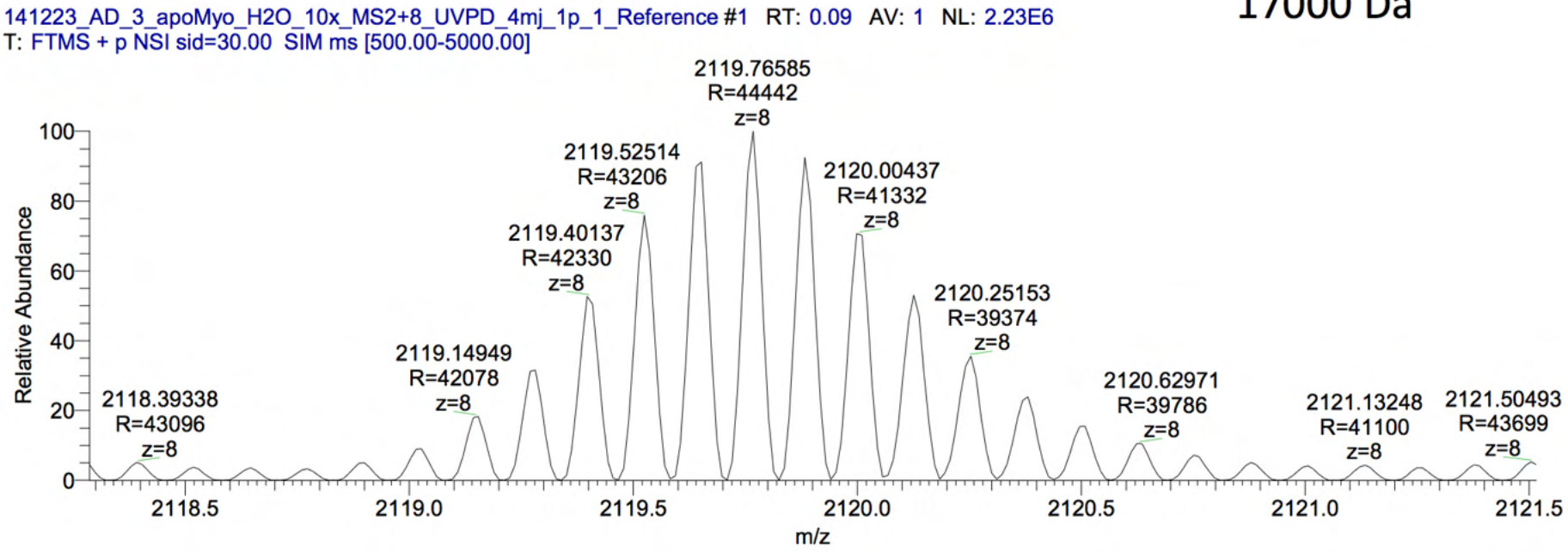


Effects of Instrument resolution



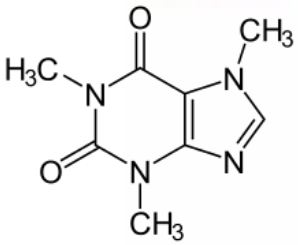


17000 Da



Which molecules can we analyze by mass spectrometry?

Hydrogen
1Da



Caffeine
194Da

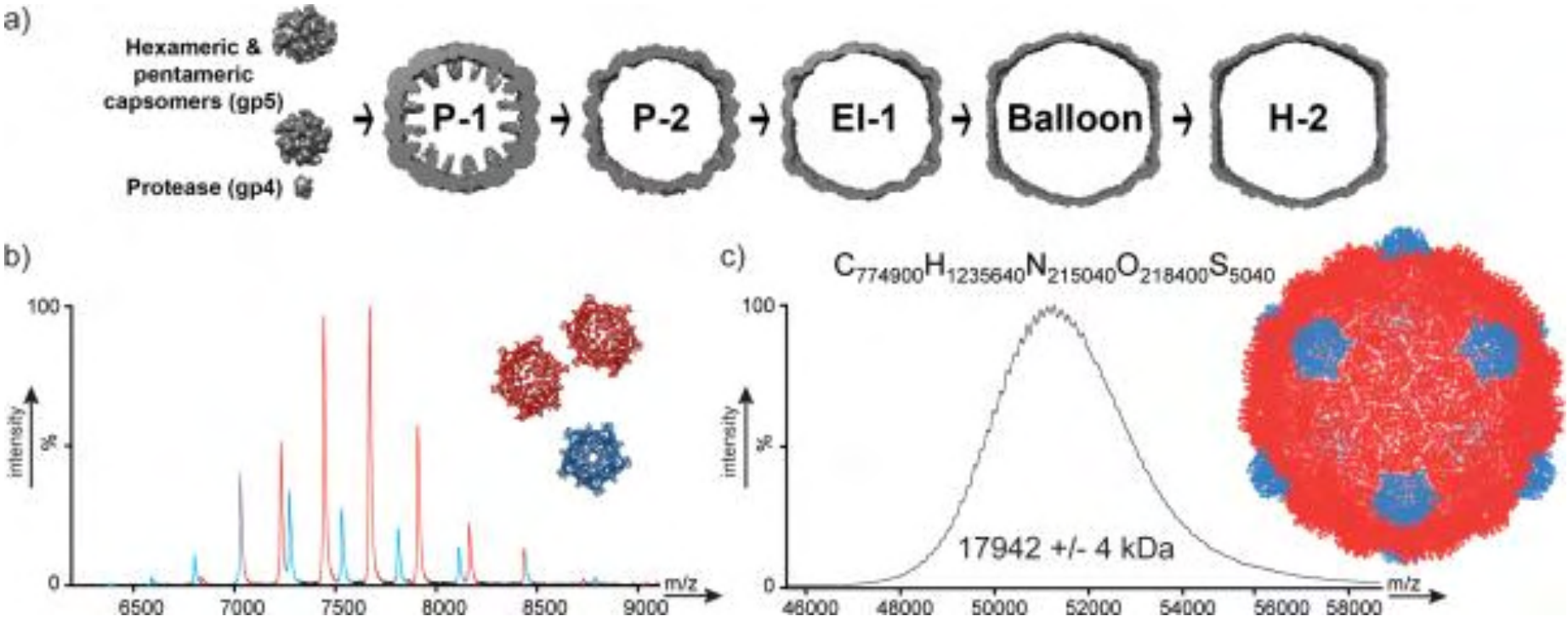
Monoclonal Antibody
150 kDa

Caffeine
194 g/mol

Drugs /Small Molecules
100 - 1000 Da
Relatively stable

Proteins
1 – 1000 kDa
Relatively labile
Solvent clusters

Monitoring Viral Capsid assembly my native MS

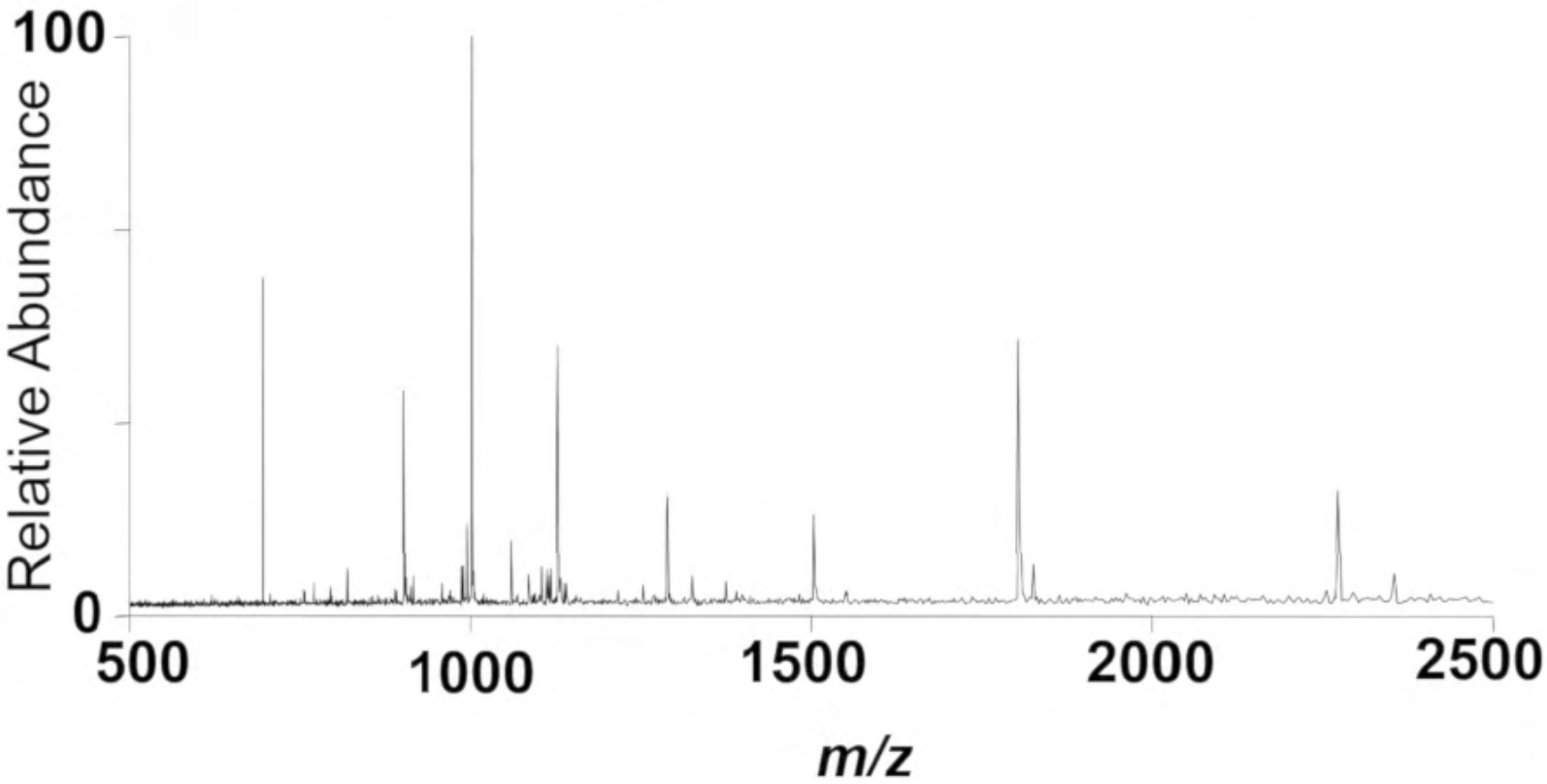


$C_{774900}H_{1235640}O_{218400}S_{5040}$

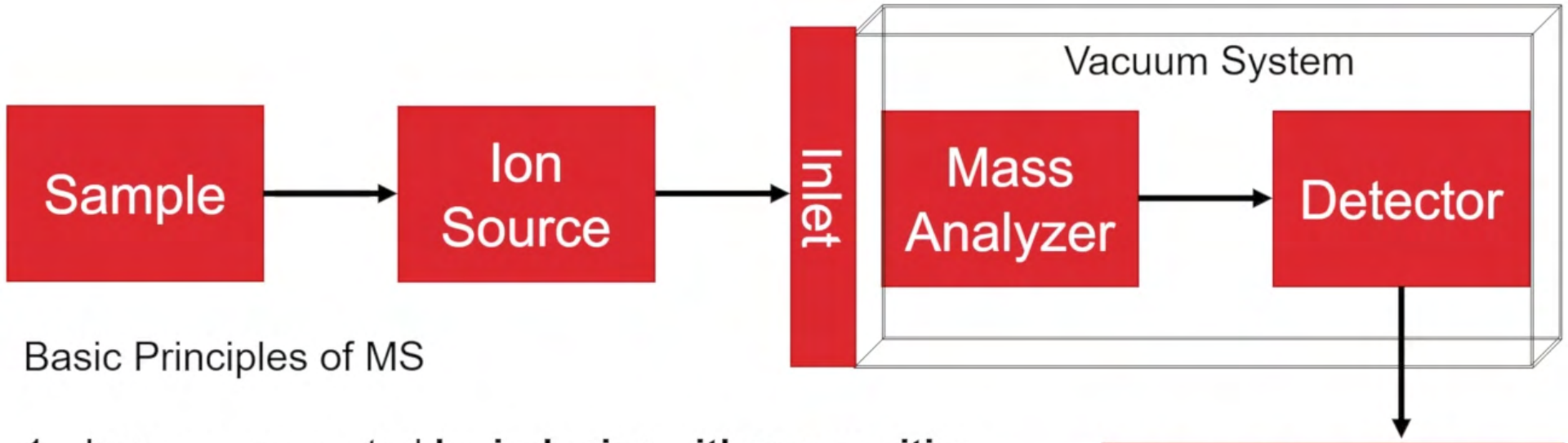
Angewandte Chemie International Edition
Volume 52, Issue 14, pages 4020-4023, 28 FEB 2013 DOI: 10.1002/anie.201210197
http://onlinelibrary.wiley.com/doi/10.1002/anie.201210197/full#fig1

What information is contained within a mass spectrum?

- The minimum number of components in a sample
- The mass-to-charge ratio of each component in a sample
- The relative abundance of each species
- The amount of each component in a sample (via Internal Standards)
- Structural information of each component (via MSMS)



Principles of Mass Spectrometry

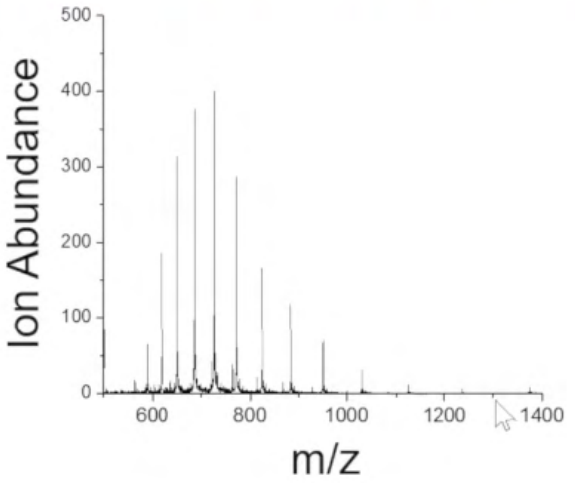


Basic Principles of MS

1. Ions are generated **by inducing either a positive or negative charge** in a neutral species.

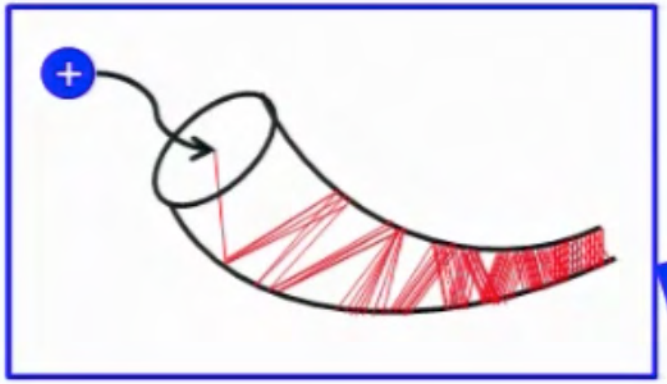
2. Once formed, **ions are electrostatically directed** into a mass analyzer, where they are separated according to **mass-to-charge ratio (*m/z*).**

3. A mass spectrometer determines the abundance and *m/z* of each compound present, which creates a mass spectrum.

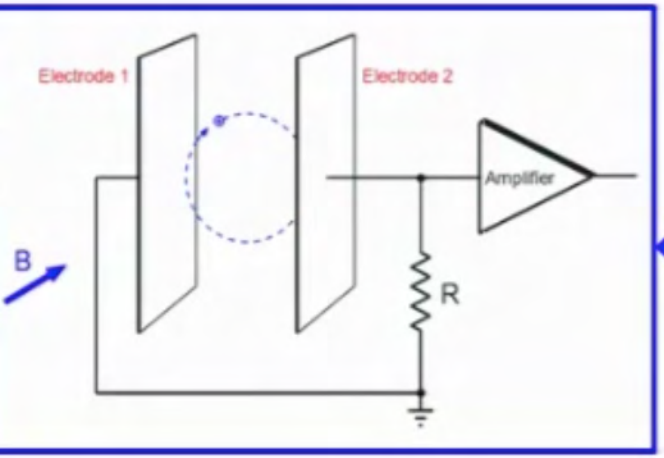


Detectors used for mass spectrometry

Ion Source → Mass Analyzer → Detector

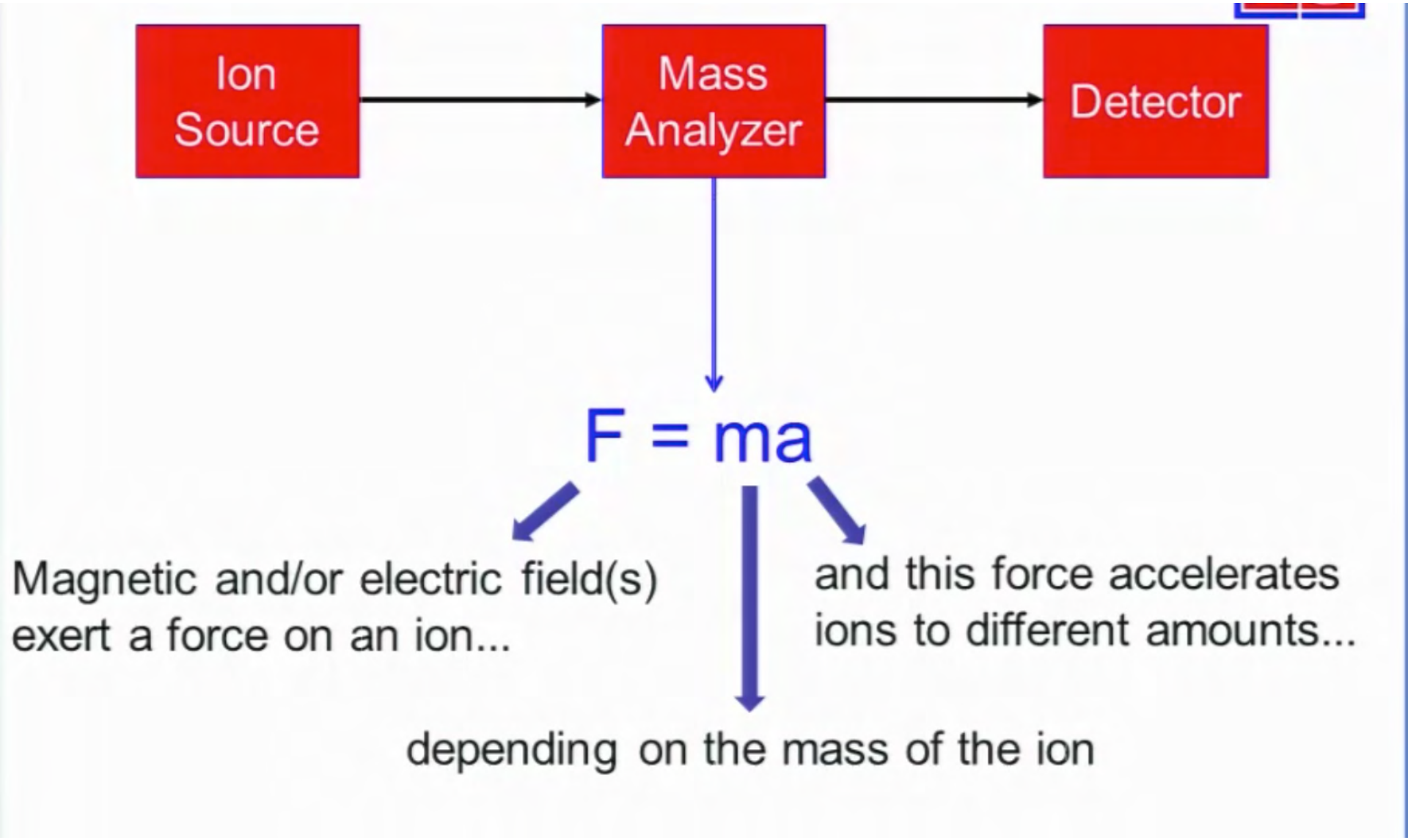


- Electron Multiplier/Microchannel Plate
  - converts ion signal into current
  - ion produces cascade of electrons that leads to "multiplication" of the signal



- Image Detection
  - ions induce current on nearby plates
  - non-destructive detection

Basic Principles of Mass Analyzers



Mass Analyzers

- Separate ions by their mass-to-charge (m/z) ratio
- Mass analyzers use physics to separate ions (F = m * a)

Mass accuracy and mass resolution (or resolving power)

Mass Accuracy: how close to the true m/z of the ion

Parts-per-million (ppm) – a relative measure

$$\frac{(\text{measured m/z} - \text{theoretical m/z})}{\text{theoretical m/z})} * 10^6 = \text{ppm}$$

Millimass units (mDa or mmu) - an absolute measure

1 mmu = 0.001 u
1 mDa = 0.001 Da

Definitions

Mass Resolution:
Two peaks at similar but slightly different m/z are „resolved“ when detection of one does not significantly interfere with detection of the other

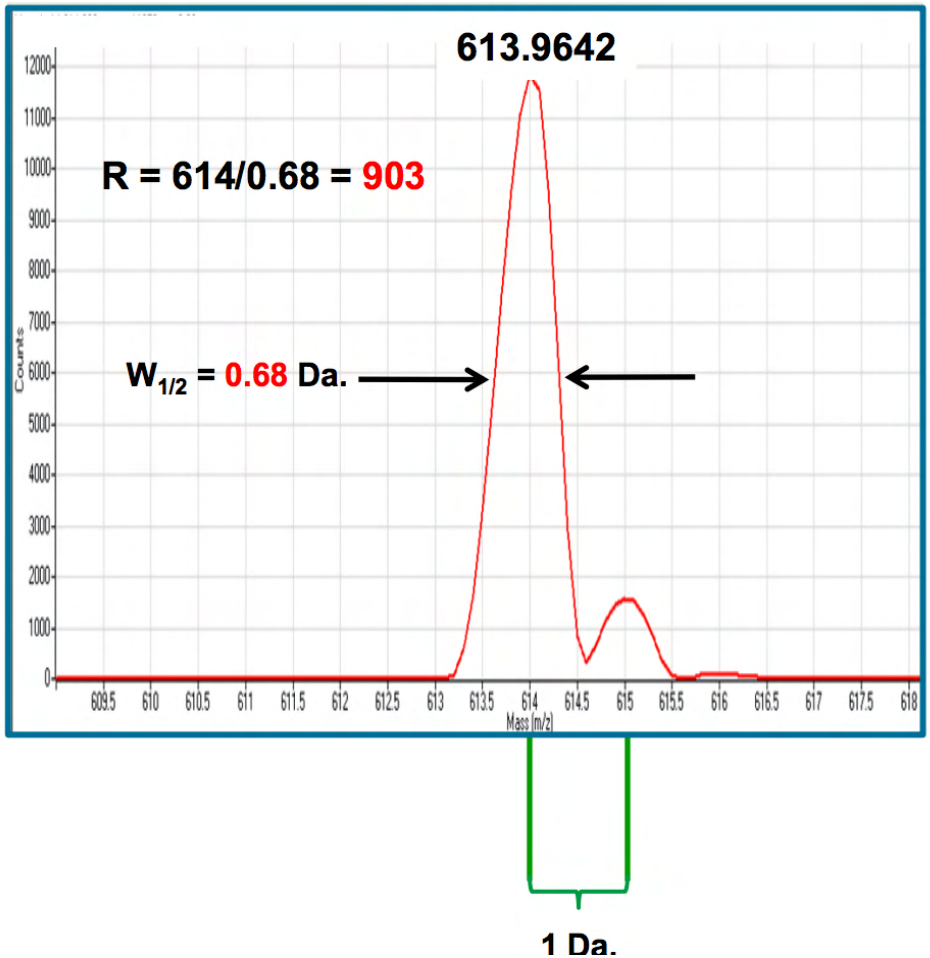
Resolving Power: M/ΔM

M: - mass ion ion(s) of interest
ΔM: - width of m/z peak at half-maximum
OR
- m/z difference between two resolved peaks

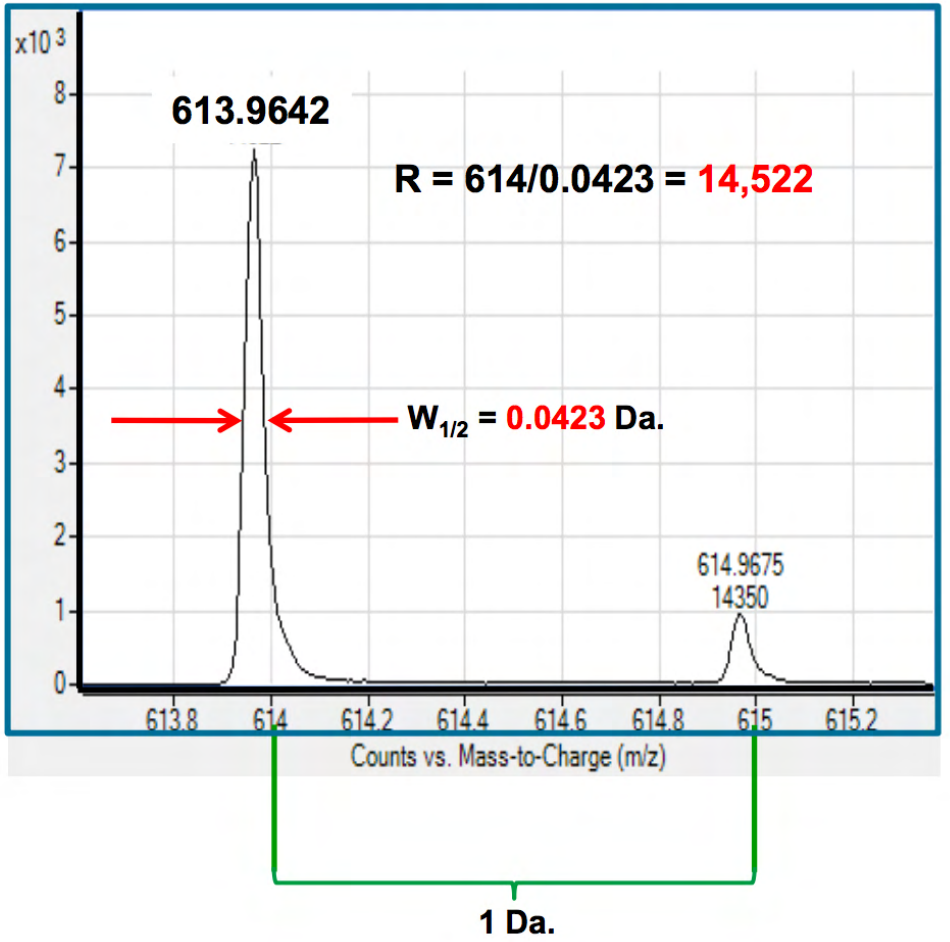
Resolving Power and Mass accuracy

m/z = 613.964203

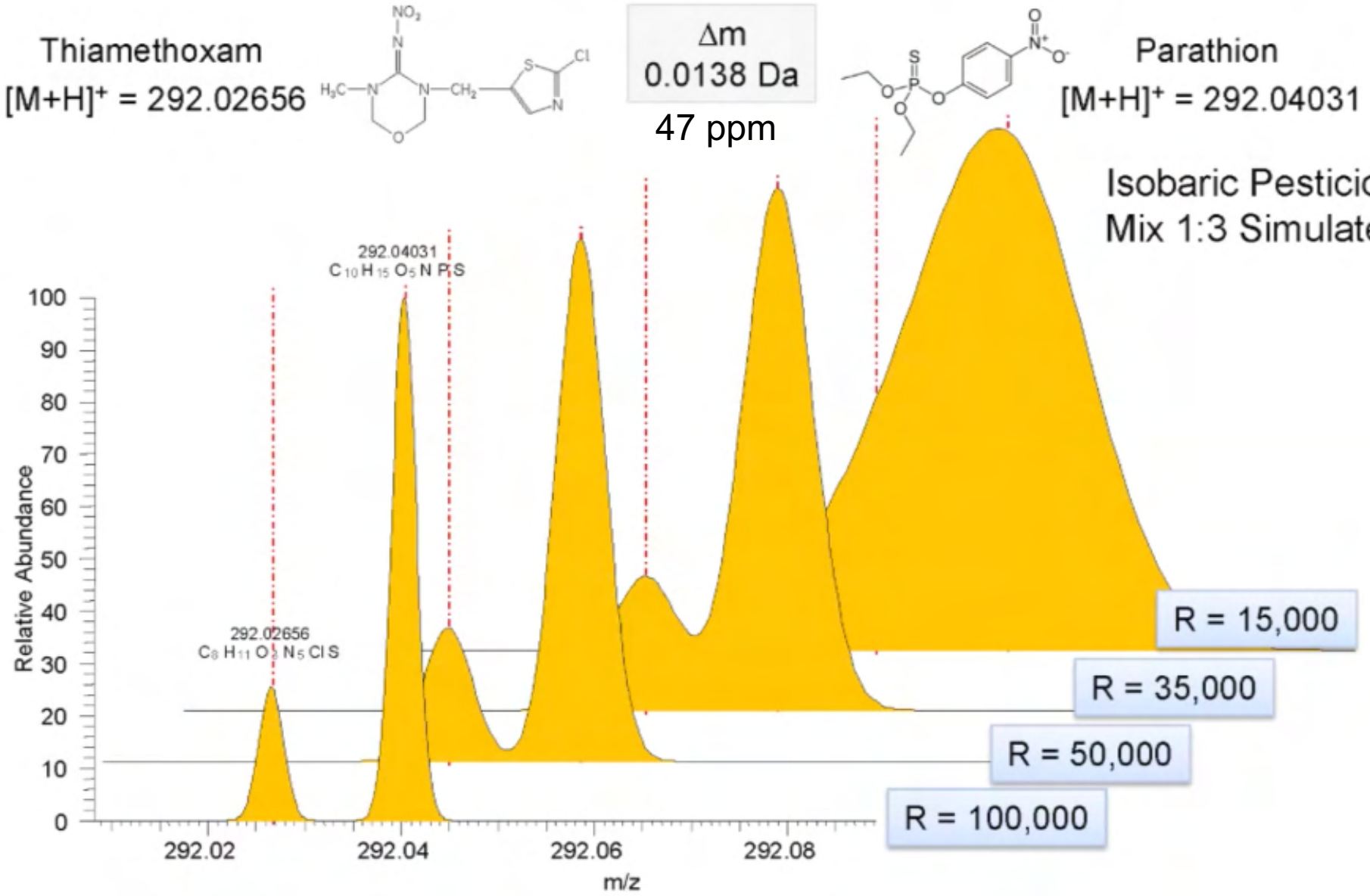
SQ, TQ, IT



TOF, Q-TOF

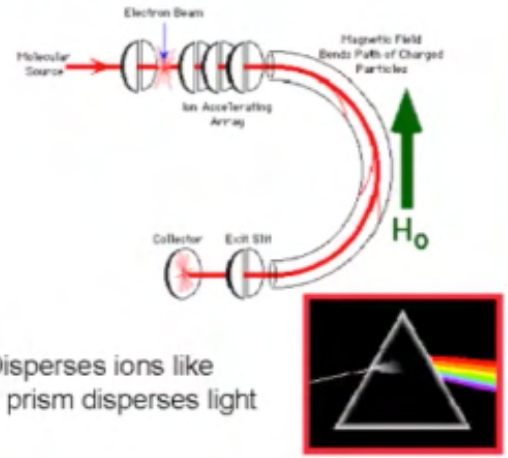


Interference at different resolutions



Types of Mass Analyzers

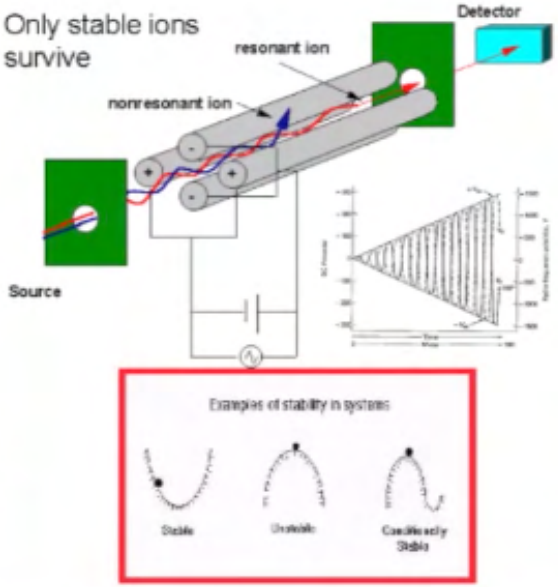
Beam: Magnetic sector



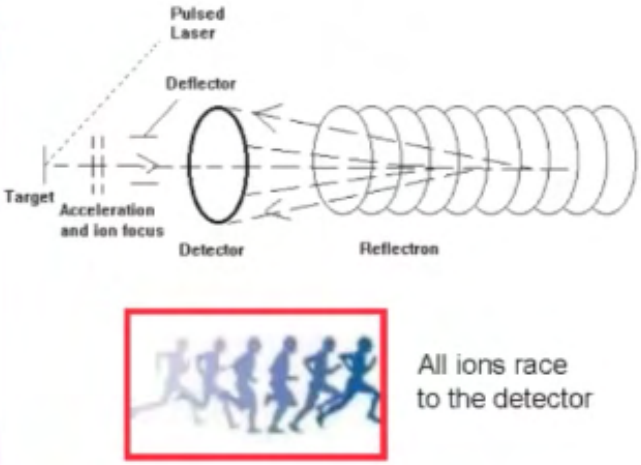
Disperses ions like a prism disperses light

Quadrupole

Only stable ions survive



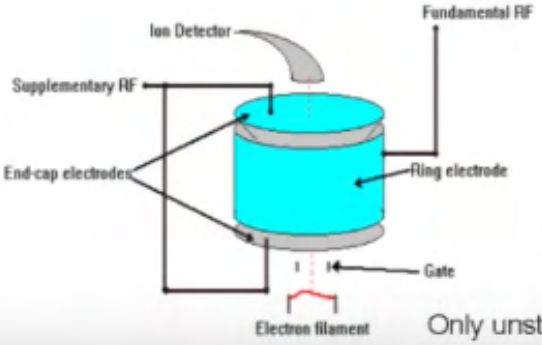
Time-of-flight



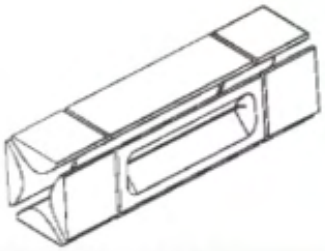
All ions race to the detector

Traps: RF traps

Paul



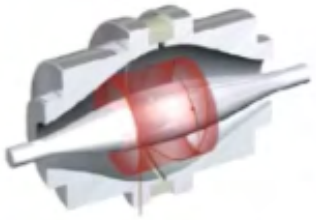
Linear



Only unstable ions get to the detector

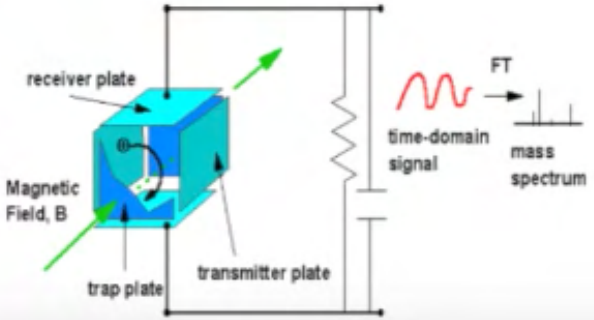
Fourier-transform

Orbitrap



All ions ring together

ICR



The Zoo of Mass Analyzers - some basic stats

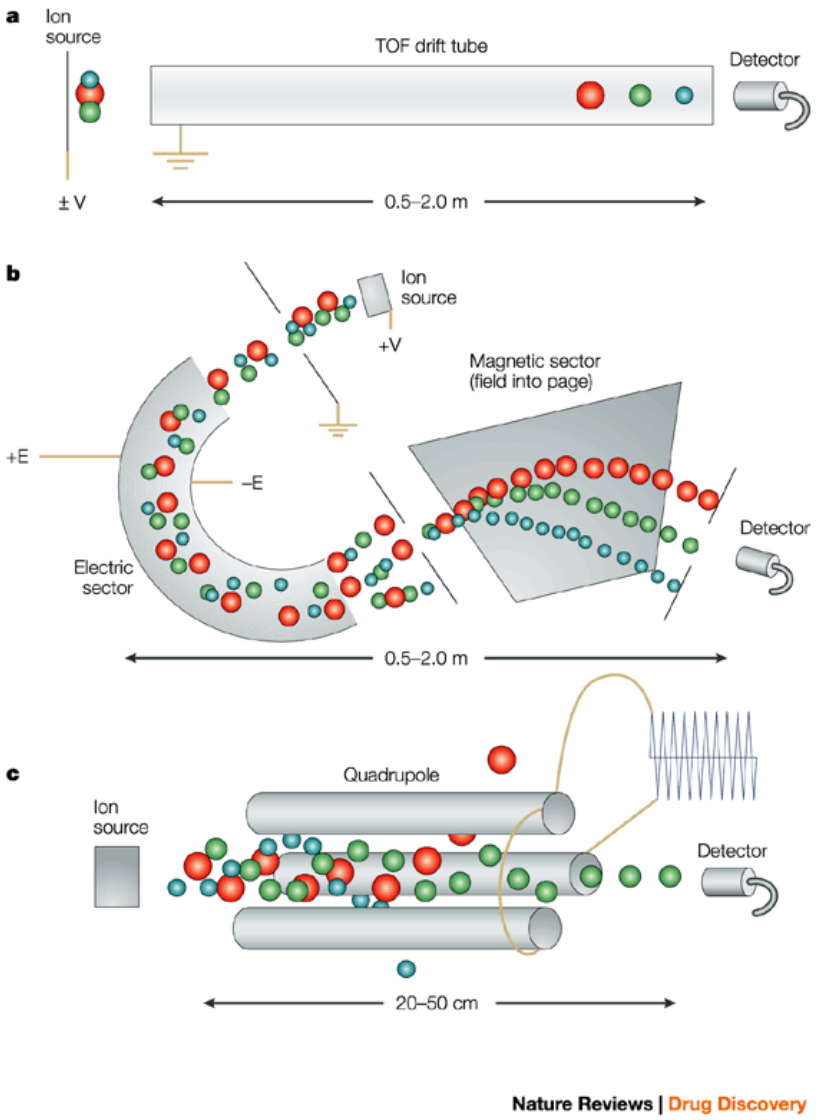
- Magnetic Sector Analyzer (MSA)
  – High resolution, exact mass, original MA
- Time-of-Flight Analyzer (TOF)
  – High resolution, fast, no upper m/z limit
- Quadrupole Analyzer (Q)
  – Low (1amu) resolution, fast, cheap

Beam Type

- Ion Trap Mass Analyzer
  – Fair resolution, all-in-one mass analyzer
- Ion Cyclotron Resonance (FT-ICR)
  – Highest resolution, exact mass, costly
- Orbitrap Mass Analyzer
  – High resolution, exact mass,

Trap Type

Beam Type Mass Analyzers

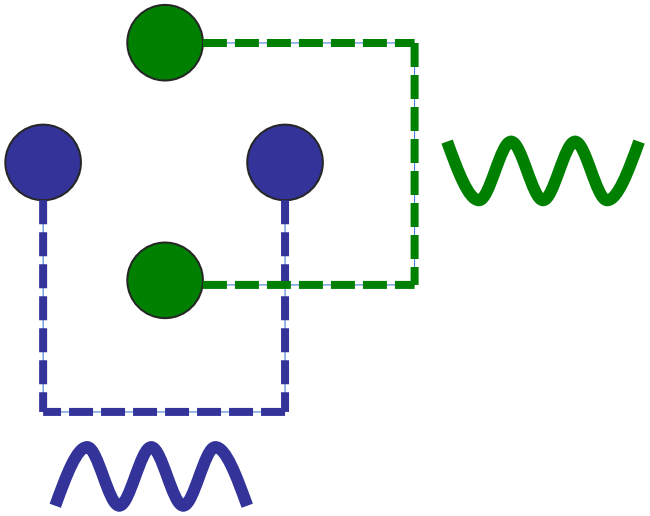


Time-Of-Flight (TOF)

Magnetic Sector

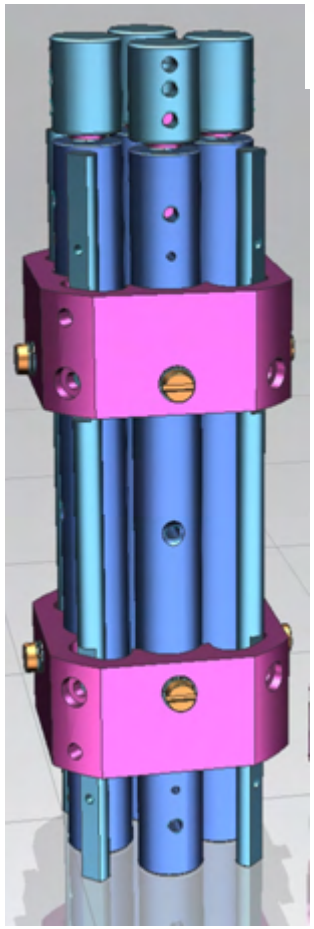
Quadrupole

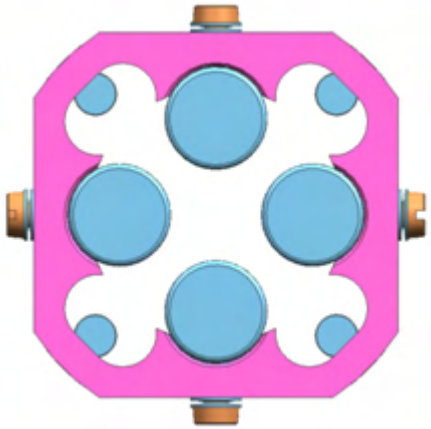
The Quadrupole Mass Analyzer



- In a quadrupole instrument, electric fields are used to separate ions according to mass, as they pass along the central axis of four parallel, equidistant rods (poles) which have (DC) and alternating (RF) voltages applied to them.

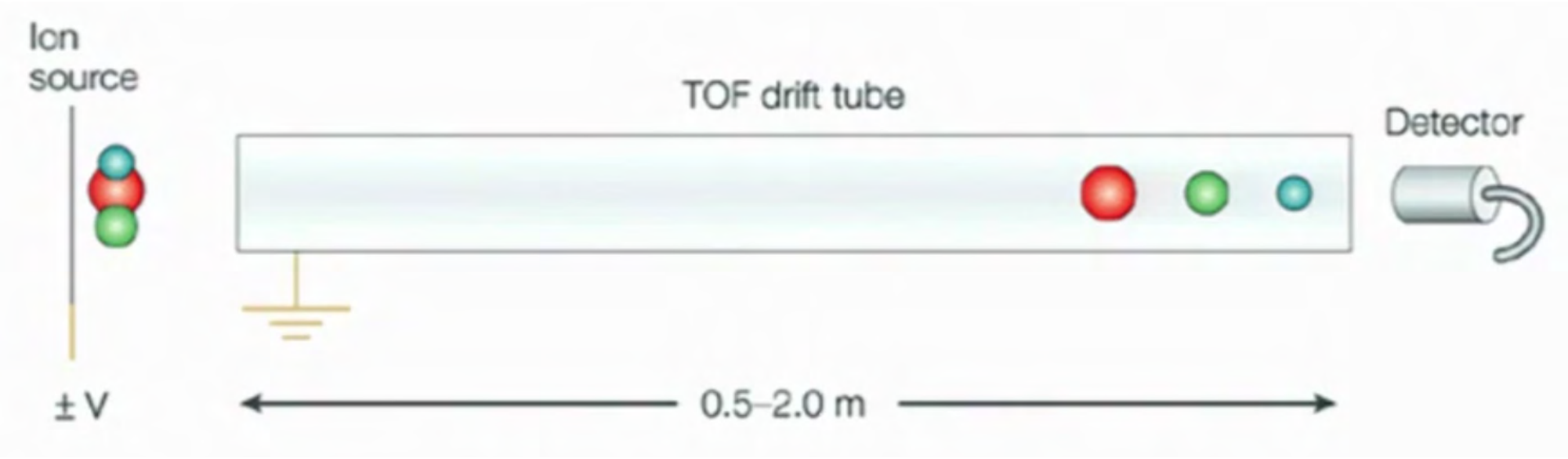
How does a quadrupole look like in real life?





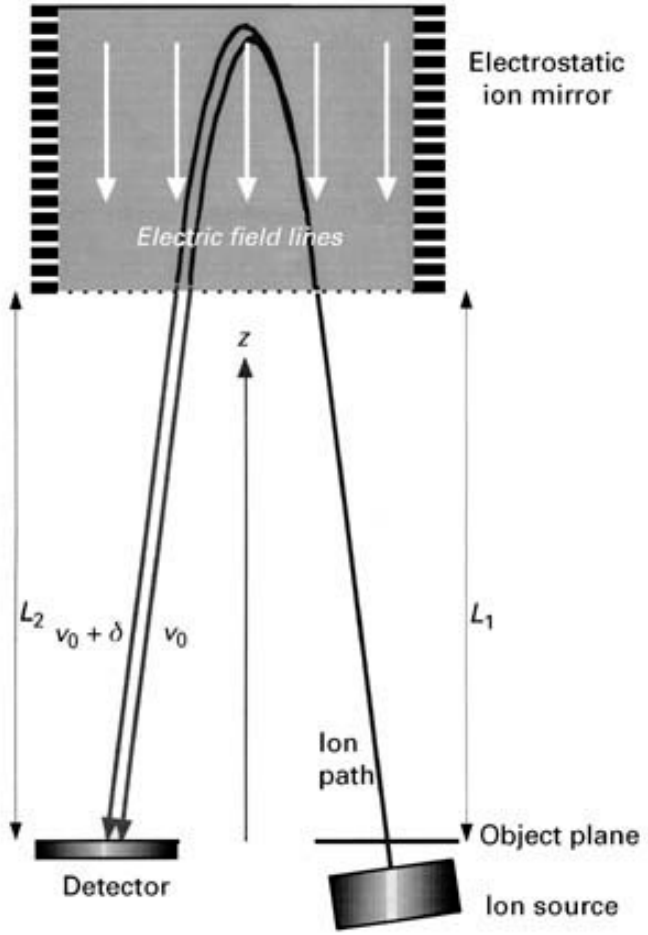
- Xevo TQ-XS
  - **12 kV_{p-p} of RF at 1.2 MHz**
  - **1 kV of DC**
- The radius of the rods is 6 mm.
- A change of just 1μm is enough to shift the mass by 0.8 Da.
- Rods constructed out of Molybdenum

TOF Principle



- Ions are given the same amount of energy through a pulse (Energy is proportional to charge and the applied potential. $E=z*e*V$, z is number of charges, e is the amount of charge on an electron, V is volts)
- Ions then move at the speed determined by their mass ($E=0.5*m*v^2$, v for velocity (L/t)) i.e. velocity goes down as mass goes up
- Distance to finish line is established and stopwatch (t) is accurate to 1 nanosecond or better

Time of Flight (TOF) – Pros and Cons



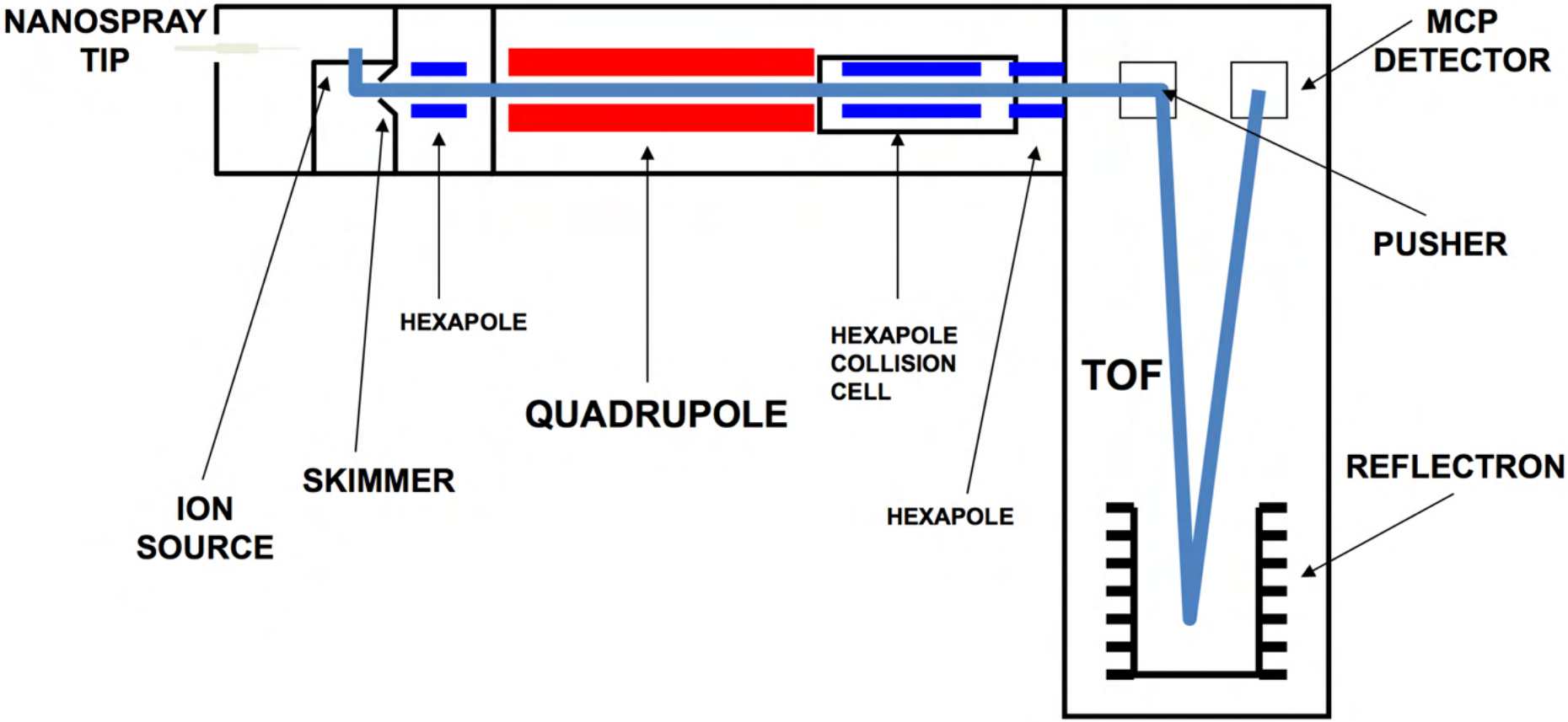
$m/z \propto t^2$

+ Very high mass range
+ *Both* high resolution and high sensitivity
+ Mass accuracy
+ High scan speed
+ Mechanically simple

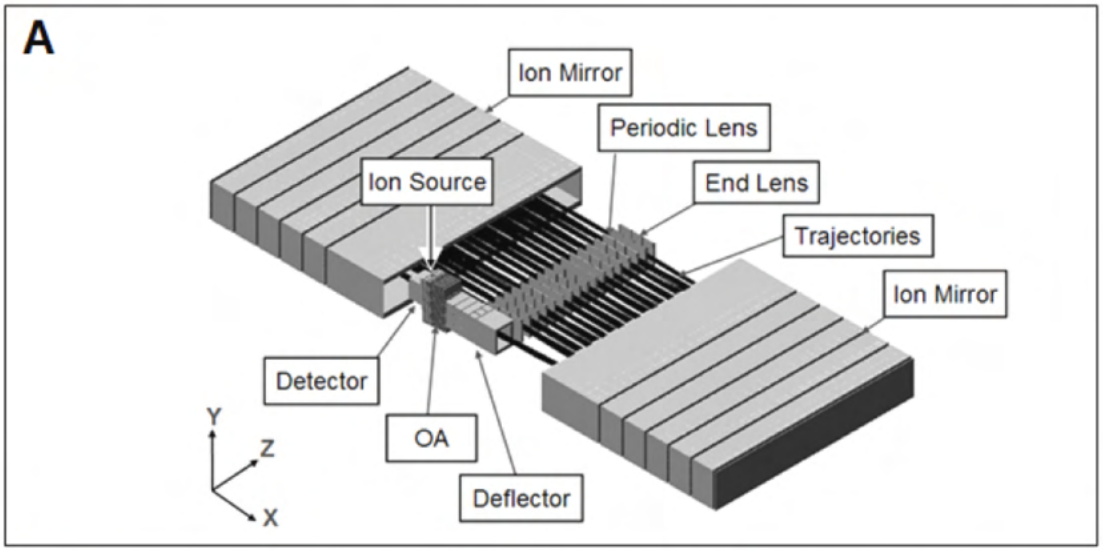
- High vacuum critical
- Demanding high voltage/ pulsed/ high precision electronics
- Expensive

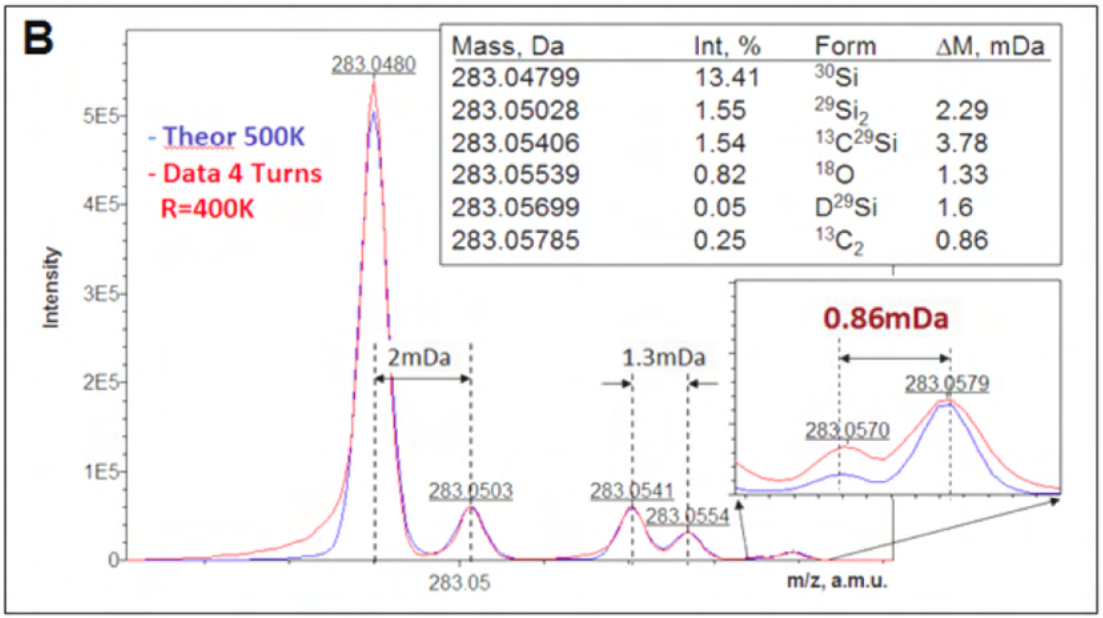
Bruker, Waters-Micromass, JEOL, Analytica

Schematic of a Q-TOF Instrument



Multipass-TOF at 400k Resolution (JEOL)

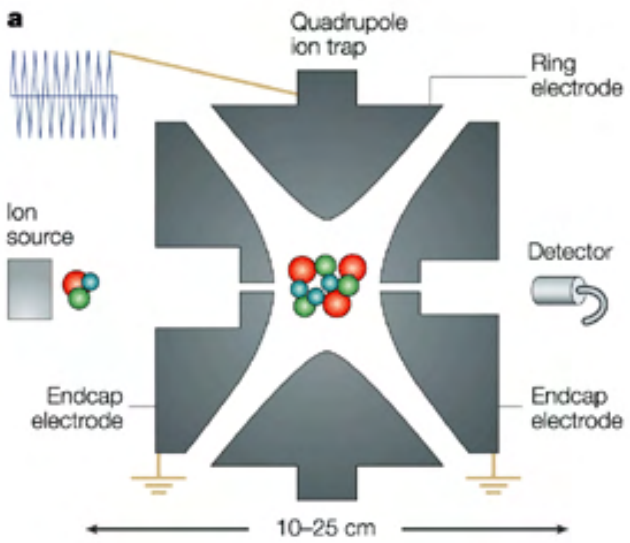




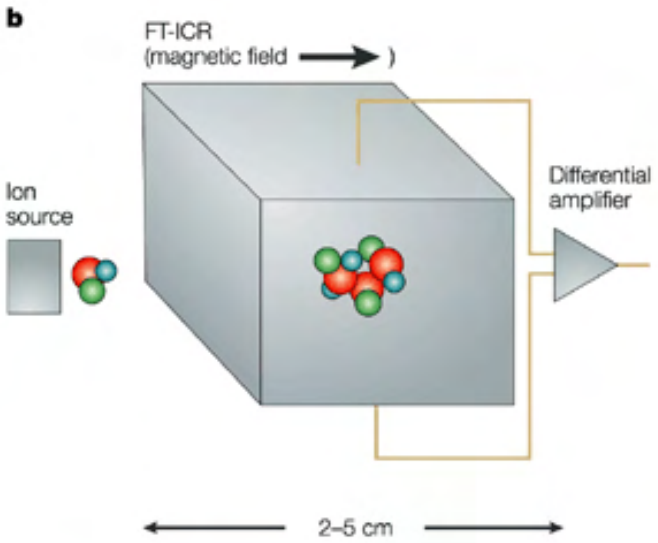
Mass, Da	Int, %	Form	ΔM, mDa
283.04799	13.41	^{30}Si	
283.05028	1.55	$^{29}Si_2$	2.29
283.05406	1.54	$^{13}C^{29}Si$	3.78
283.05539	0.82	^{18}O	1.33
283.05699	0.05	$D^{29}Si$	1.6
283.05785	0.25	$^{13}C_2$	0.86

***Journal of Applied Solution Chemistry and Modeling*, 2017, *6*, 1-22**

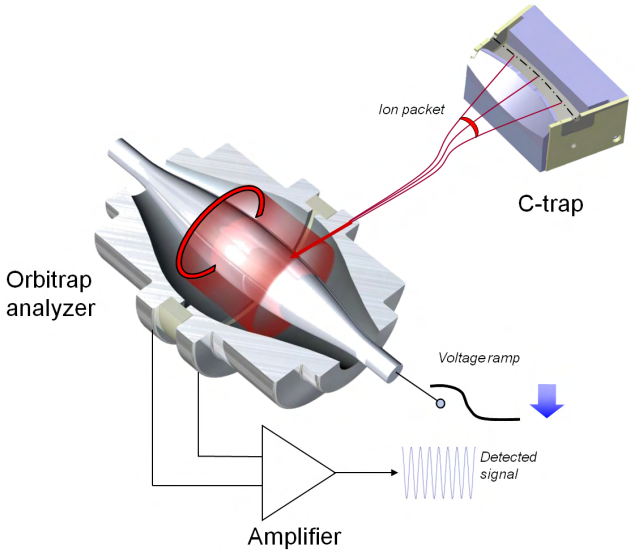
Trapping Analysers



Ion Traps

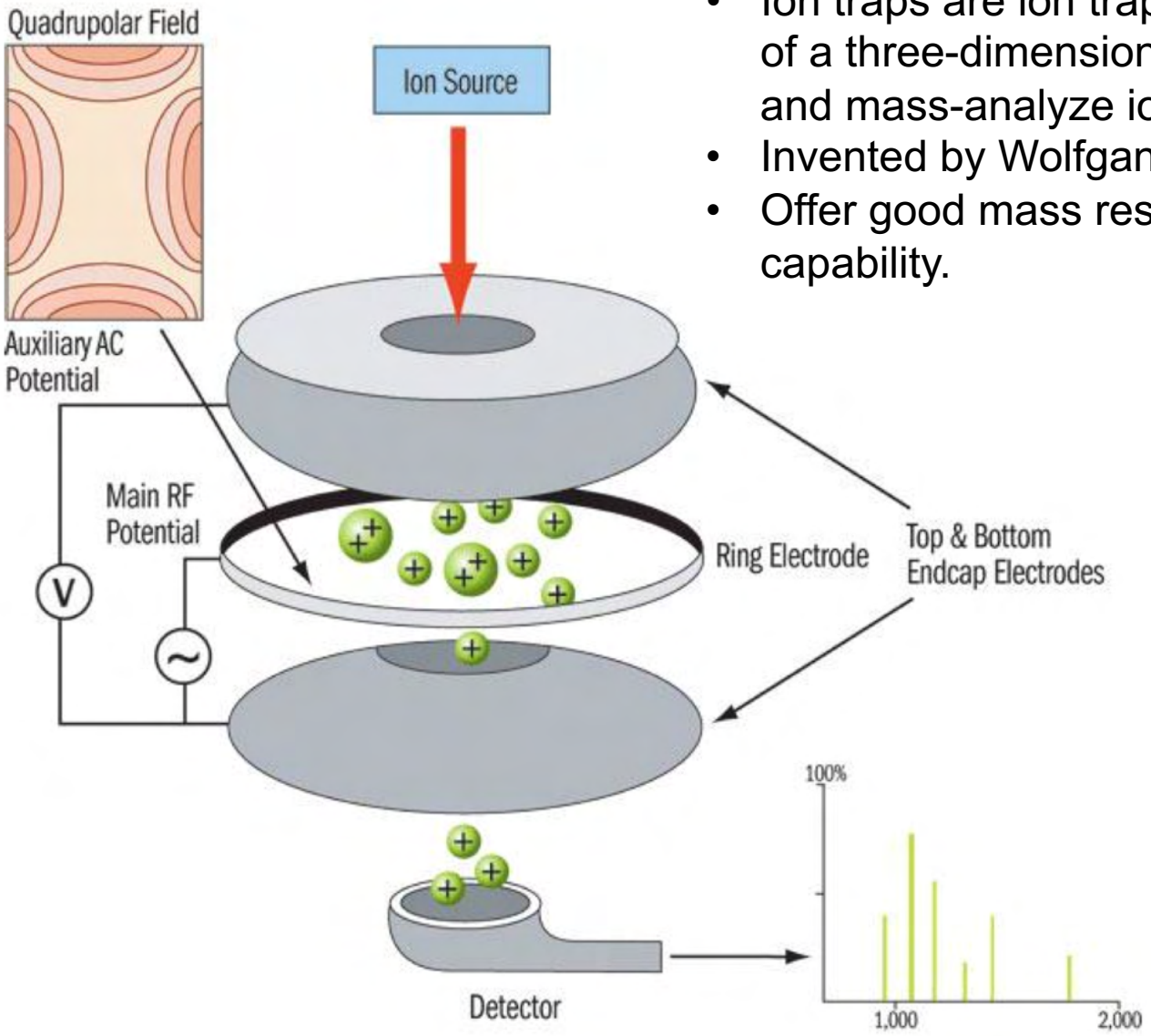


Fourier-Transform Ion Cyclotron Resonance (FT-ICR)



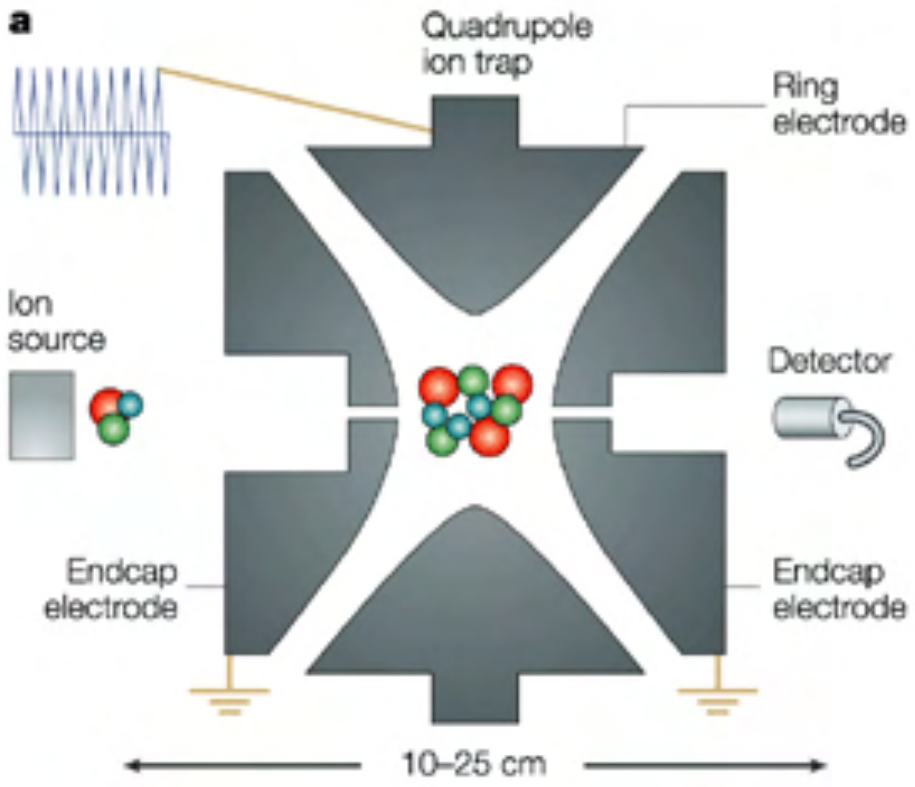
Orbitrap

Ion Traps



- Ion traps are ion trapping devices that make use of a three-dimensional quadrupole field to trap and mass-analyze ions
- Invented by Wolfgang Paul (Nobel Prize 1989)
- Offer good mass resolving power, and MSn capability.

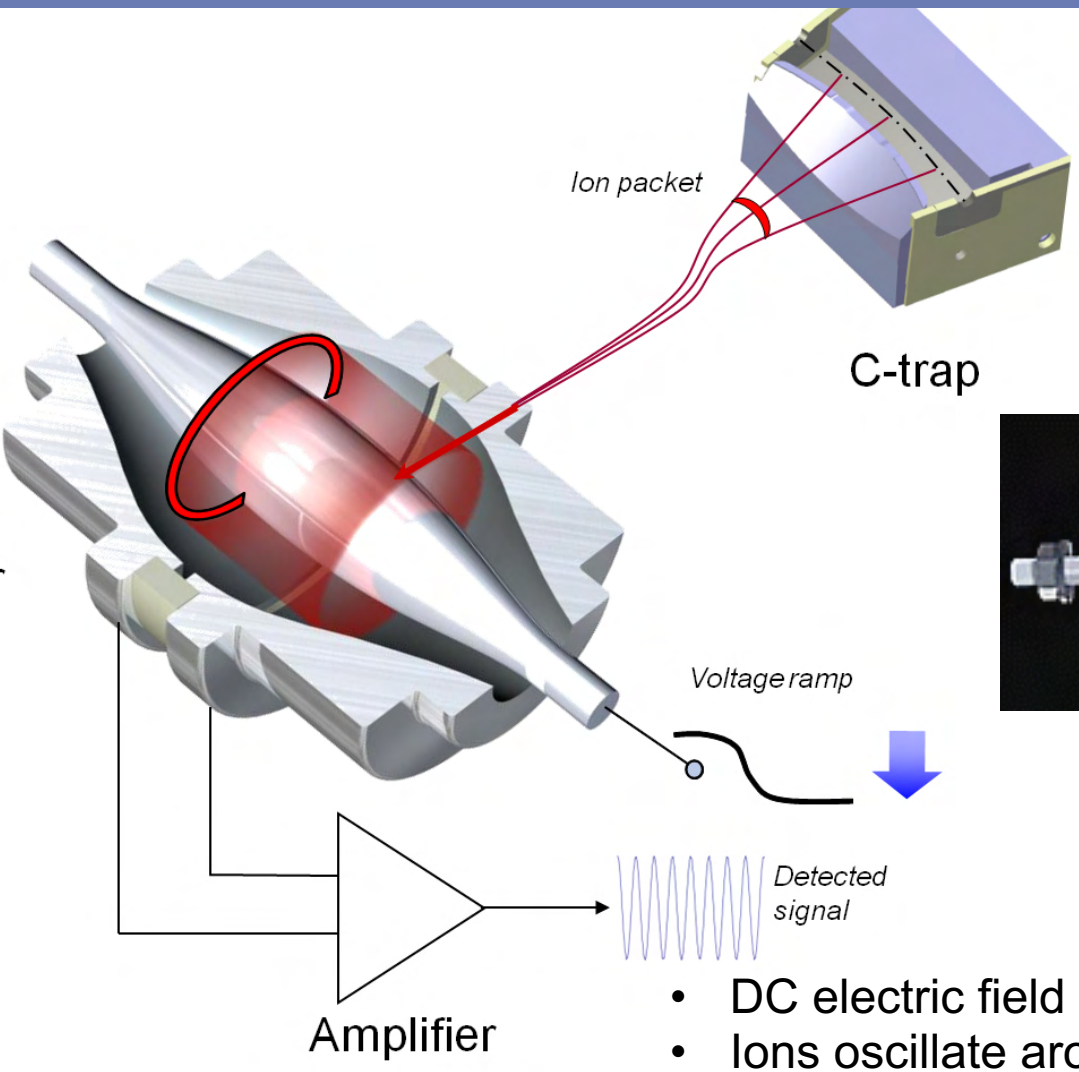
3D Quadrupole Ion Trap

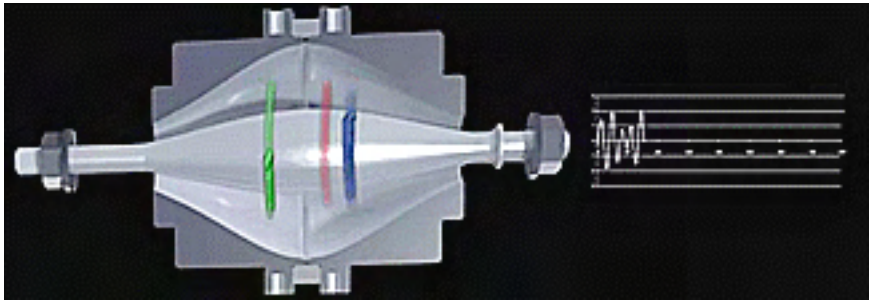


- RF voltage applied to ring electrode creates 3D trapping field that traps a range of m/z ions
- Ions are mass selectively „untrapped“ and detected

- Low mass accuracy and resolving power
- Excellent at MS/MS

Orbitrap – principle of operation

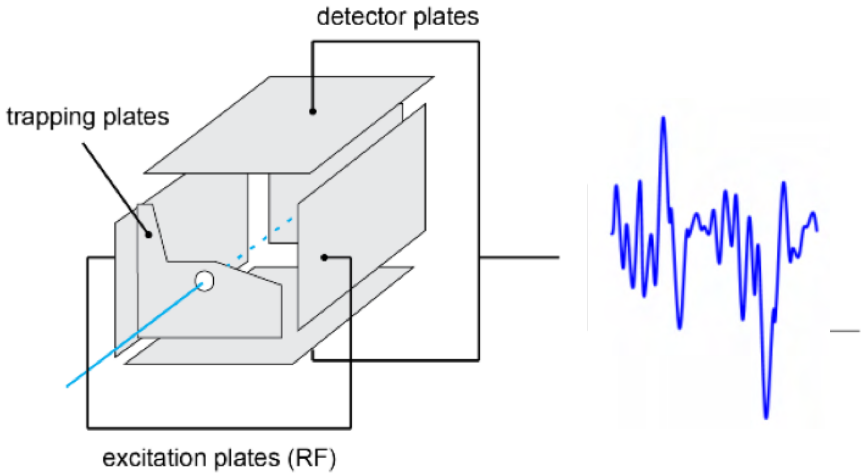




- DC electric field causes trapping of ions
- Ions oscillate around center electrode and along z-axis
- Ions are non-destructively detected via oscillations
- High mass accuracy and resolving power

FT- ICR and Orbitrap - Comparison

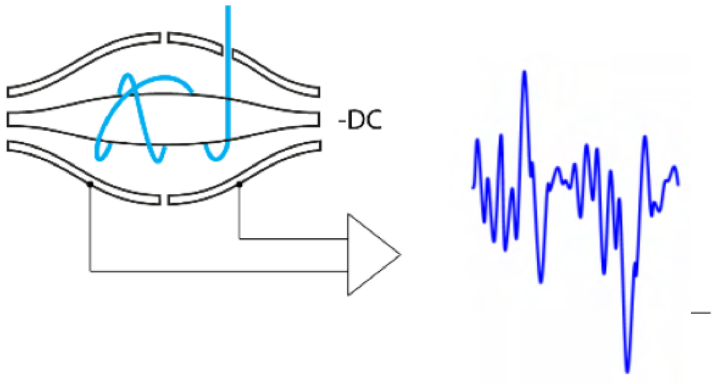
FT-ICR



Ions trapped by massive magnets (max. 6 T)

Excitation plates make ions rotate; detector plates record passage.

Orbitrap

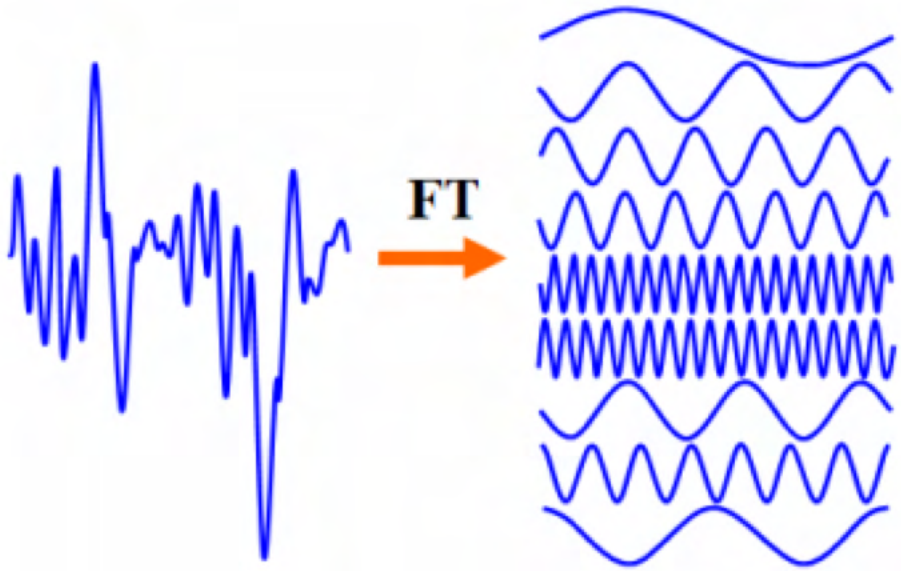


Ions rotate around central spindle.

The central spindle attracts (like gravity); the ions their kinectic energy keeps them in orbit.

Fourier Transformation

Amplitude is intensity; frequency m/z.



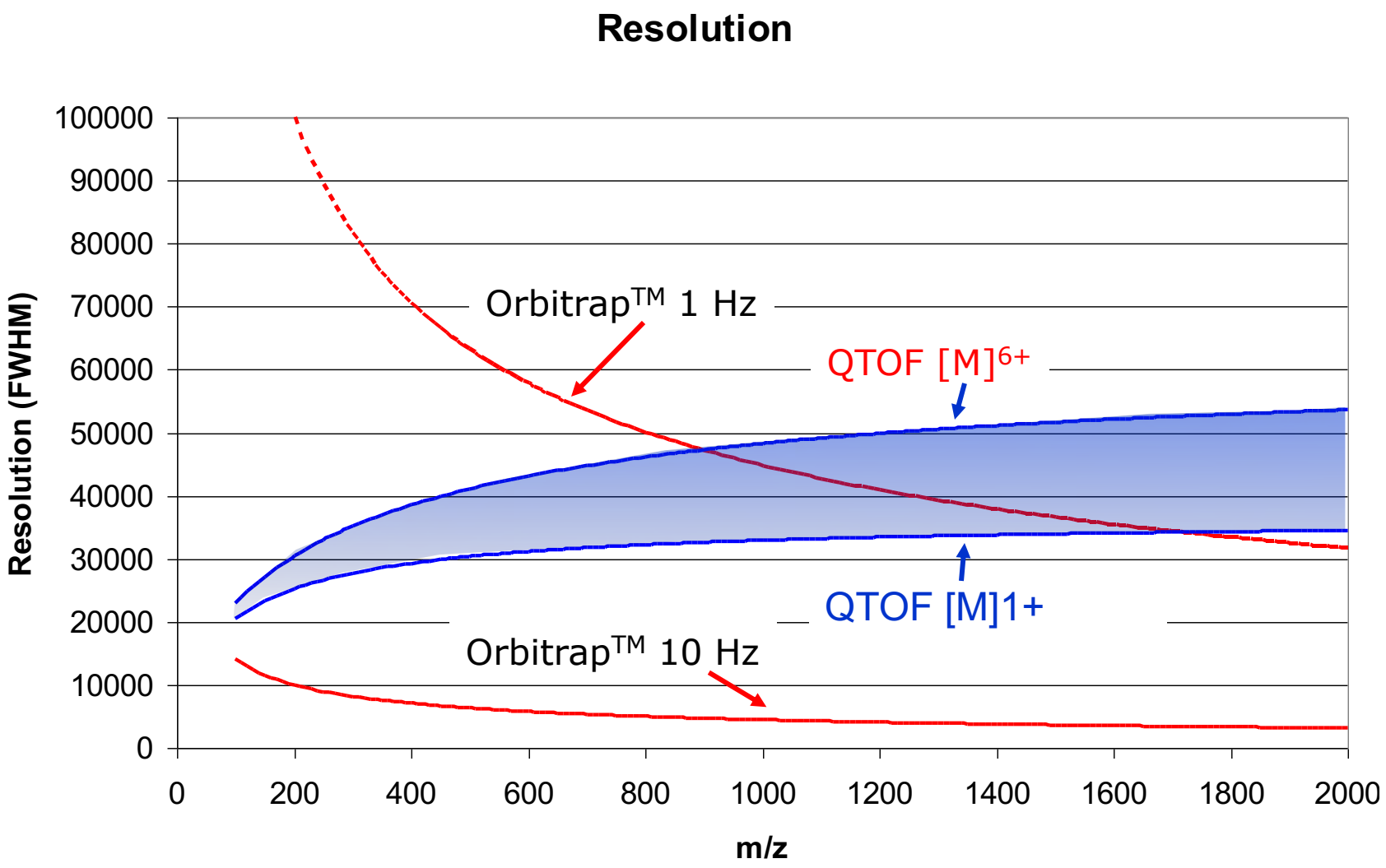
Fourier Transform is used to deconvolute the complex signal to its individual components.

Comparing Apples and Oranges?

- **Oa-TOF analyser (Q-TOF)**
  - Destructive analyser, measures charge (ADC)
  - Resolution increases with m/z
  - Fast acquisition rates (intrinsically many KHz)
  - High charge capacity/unit time

- **Electrostatic trap analyser (Orbitrap)**
  - Non destructive analyser, measures charge
  - Resolution decreases with m/z
  - Slower acquisition rate (up to 20 Hz)
  - Medium charge capacity/spectrum (1 - 2 million)

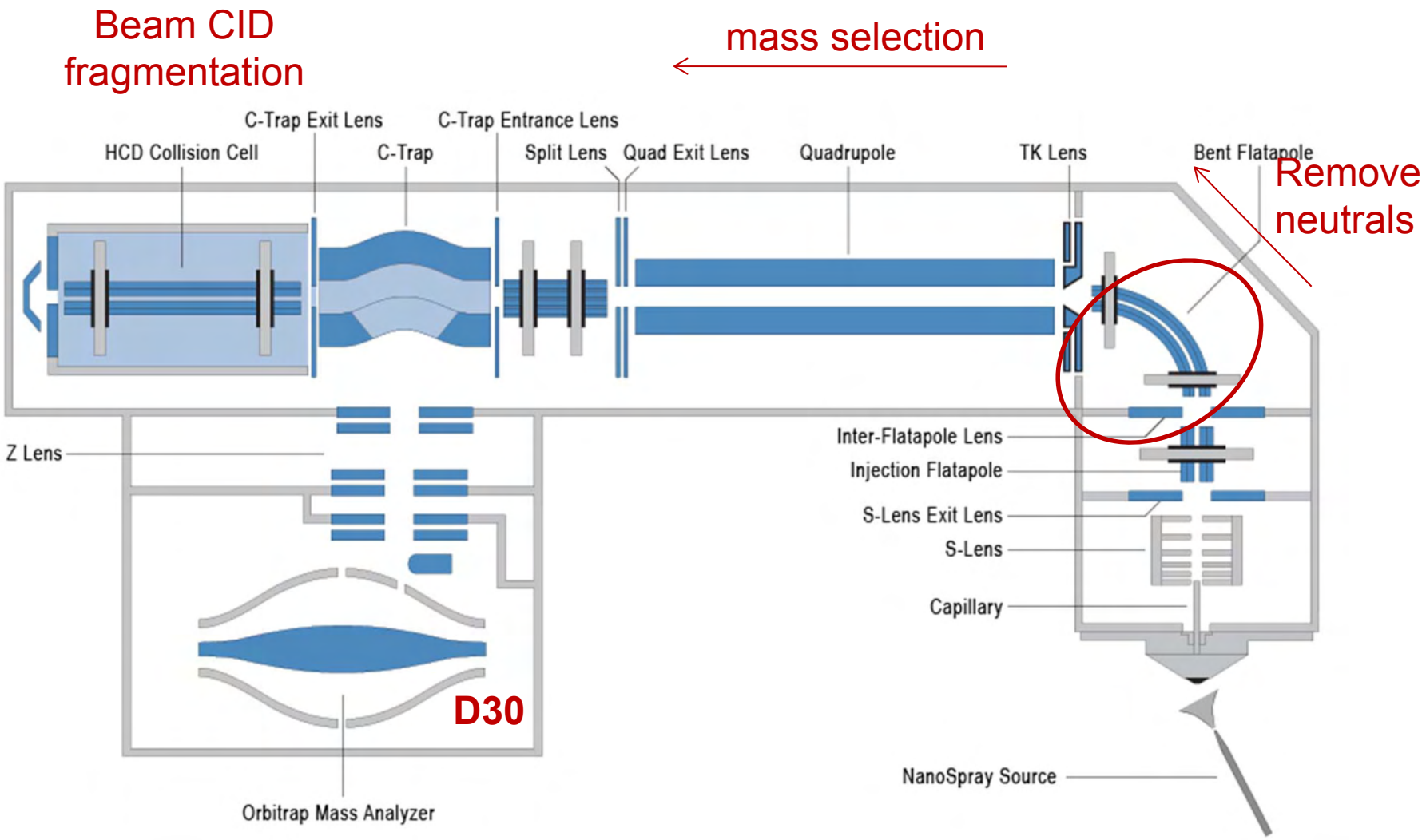
Resolution for Q-TOF & Orbitrap™



Figures of merit of mass analyzers

- Resolving Power
- Transmission / Sensitivity
- Mass Accuracy
- Dynamic Range
- Acquisition Speed
- Quantification Accuracy & Precision
- Mass range of detection
- Simplicity
- Ease of Use & Robustness

Schematic of a Q-Exactive Instument

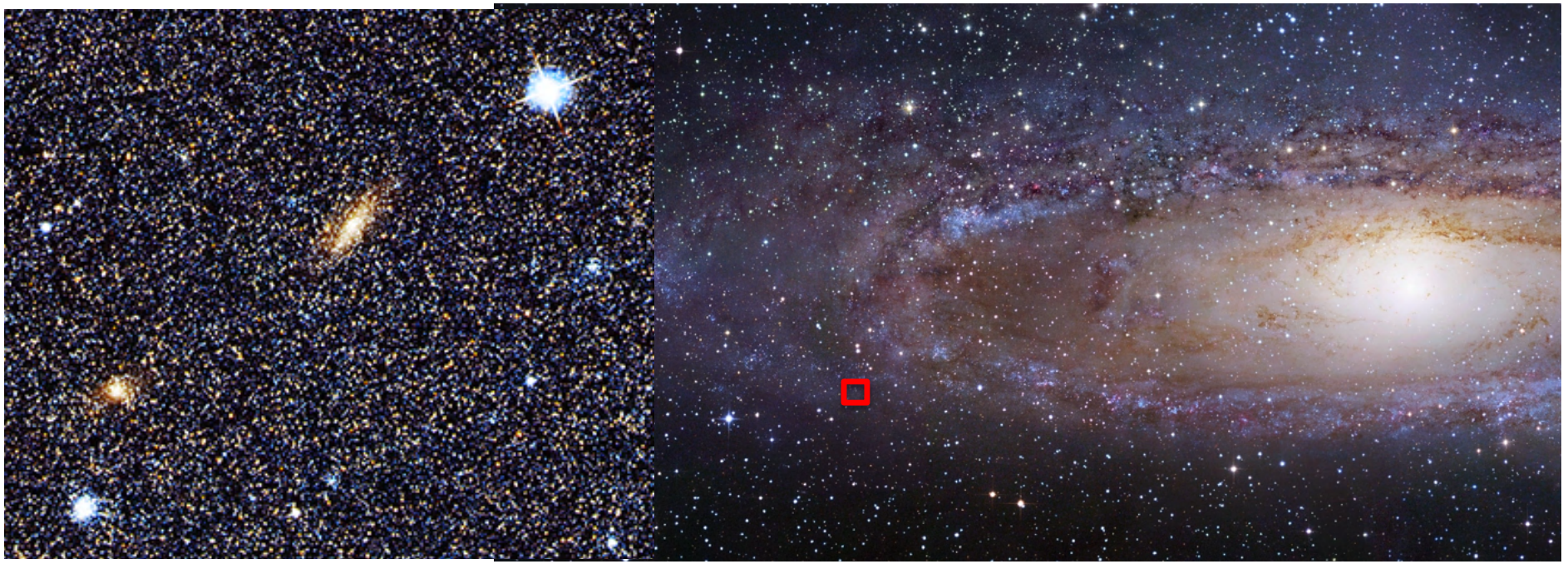


New HF-X: up to 40 MS/MS per second

Summary : Mass Analyzers

- Measuring the masses of components in a sample requires the samples to be ionized first – „mass-to-charge“ spectrometry
- Mass analyzers work on the basic principle F = m * a
- Mass analyzers come in two general types: beam type and trapping type
- Two or more mass analyzers can be combined to provide enhanced measurement capabilities

Conclusions:



Andromeda Galaxy 2015

- Mass spectrometry has evolved into an essential tool that can support most types of biological questions
- Instrumentation continues to evolve rapidly – improving performance and reliability of MS-based workflows
- Future progress will (hopefully) improve robustness, quantification accuracy, dynamic range, quality of PTM and top-down protein analysis

Source: NASA Gigapixel panorama of the andromeda galaxy