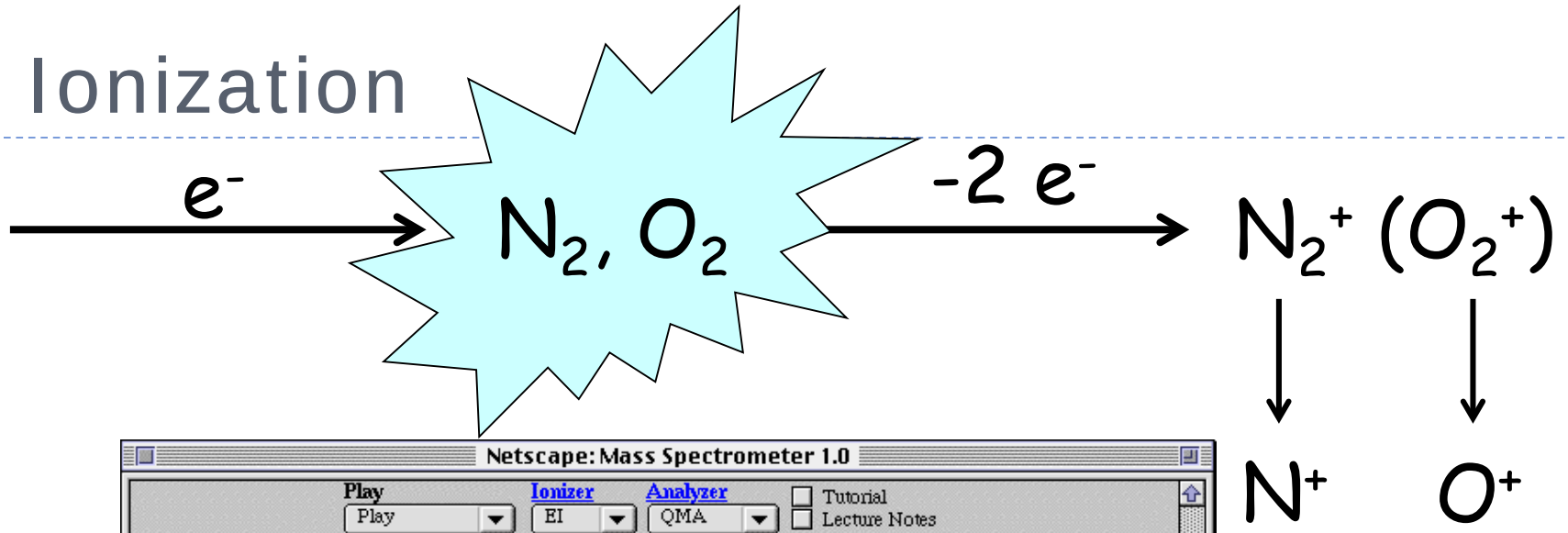
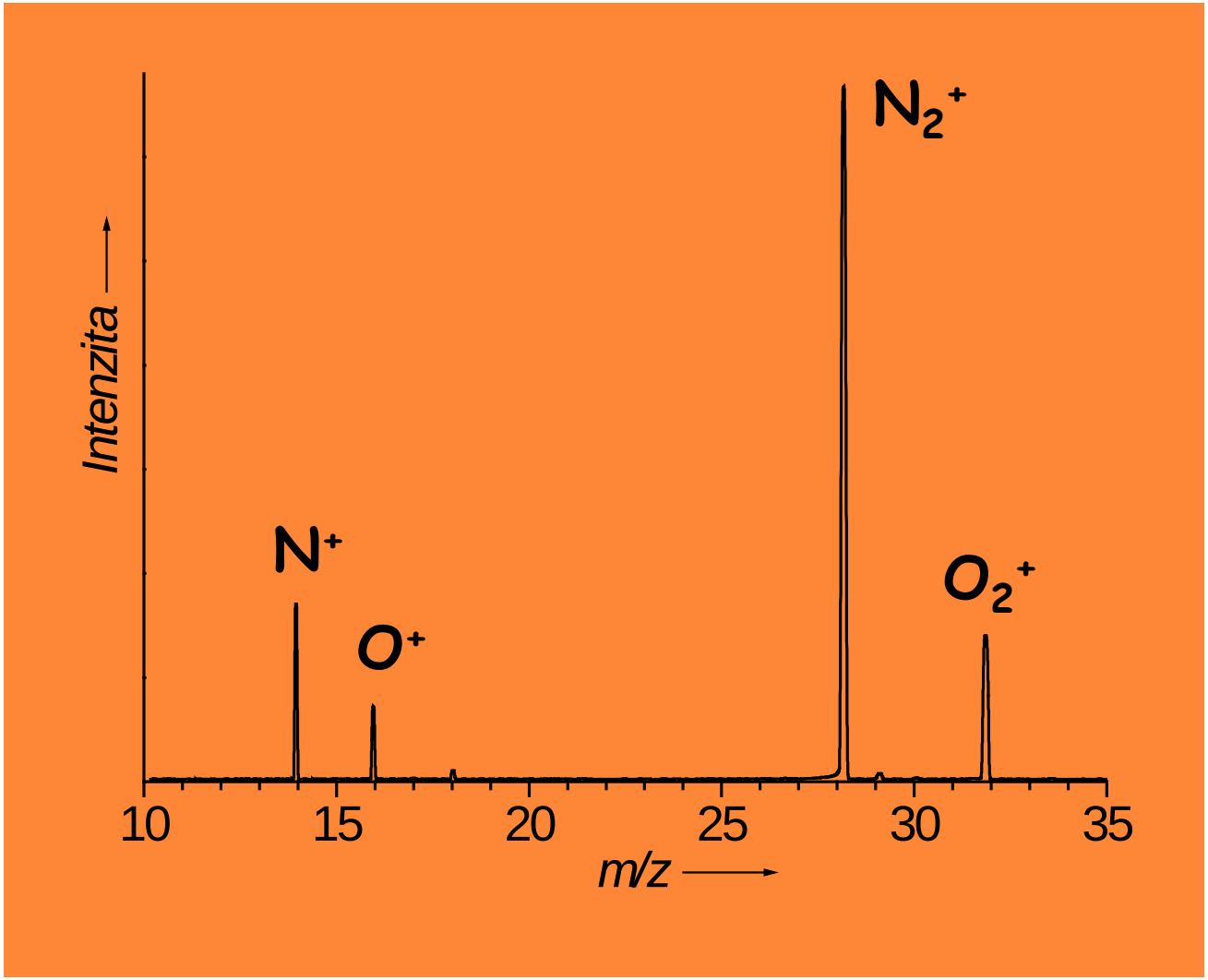
Mass spectrometry Recap

Jana Roithová

Ionization

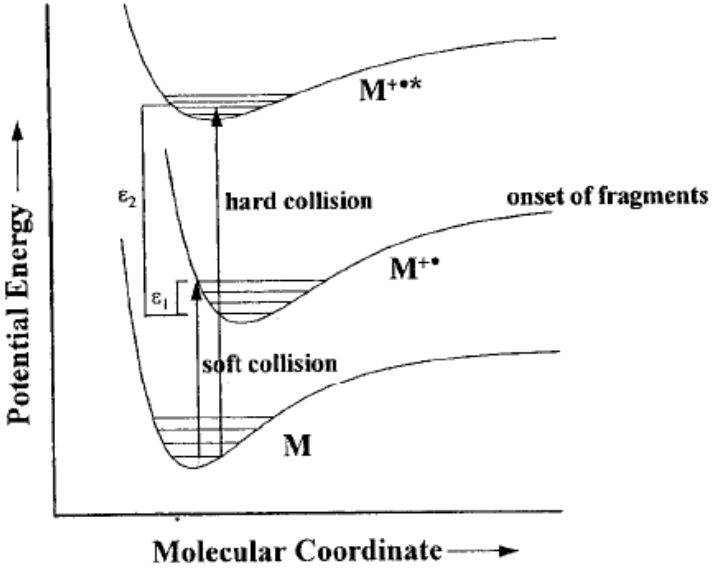

e⁻
N2, O2
-2 e⁻
N2+ (O2+)
N+
O+
Netscape: Mass Spectrometer 1.0
Play
Play
Ionizer
EI
Analyzer
QMA
Tutorial
Lecture Notes
Electron Impact Ionizer
Vacuum pump
© 1997, University of Richmond

Ionization

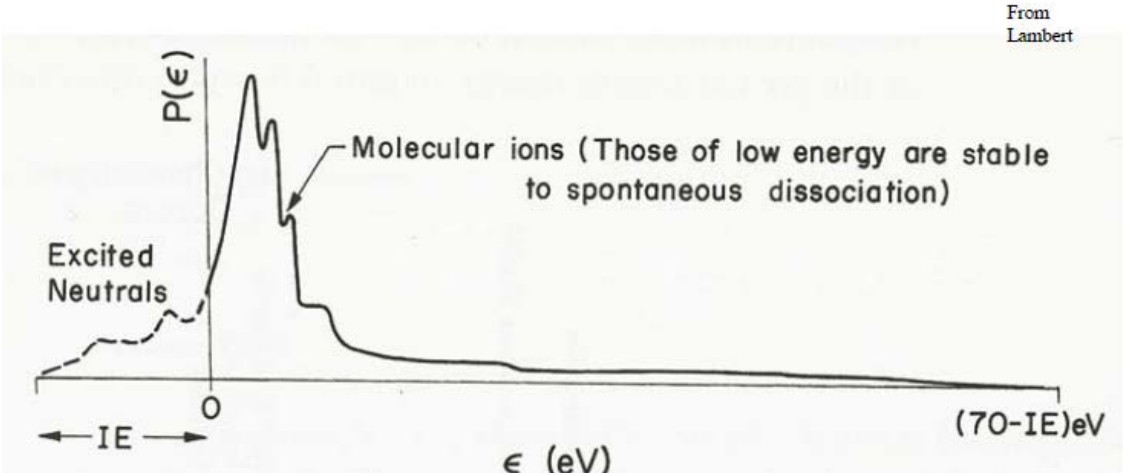


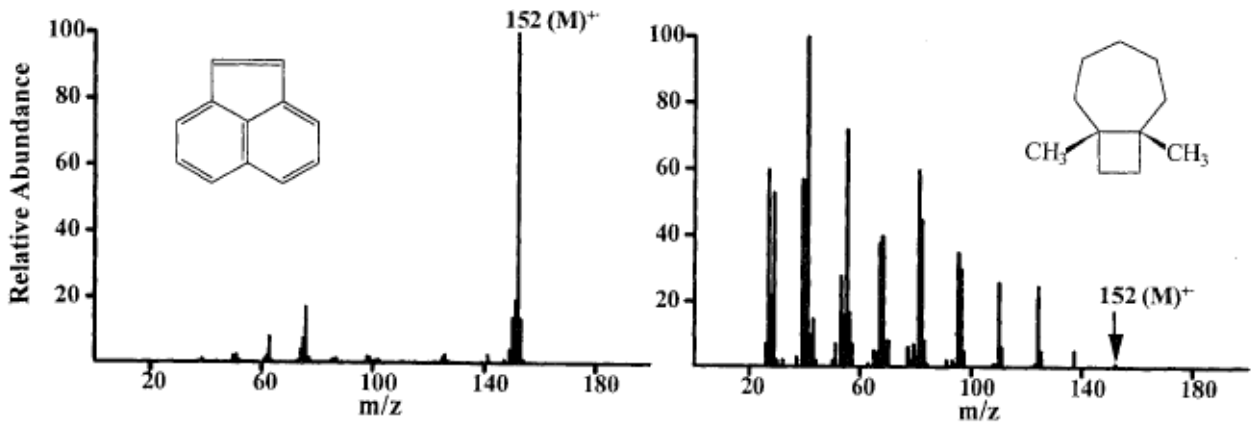
Ionization energy

Adiabatic vs. Vertical *IE*

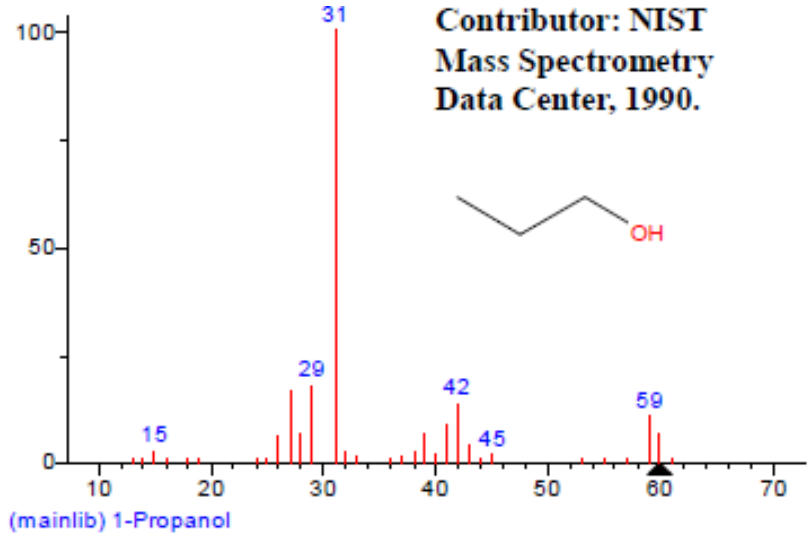


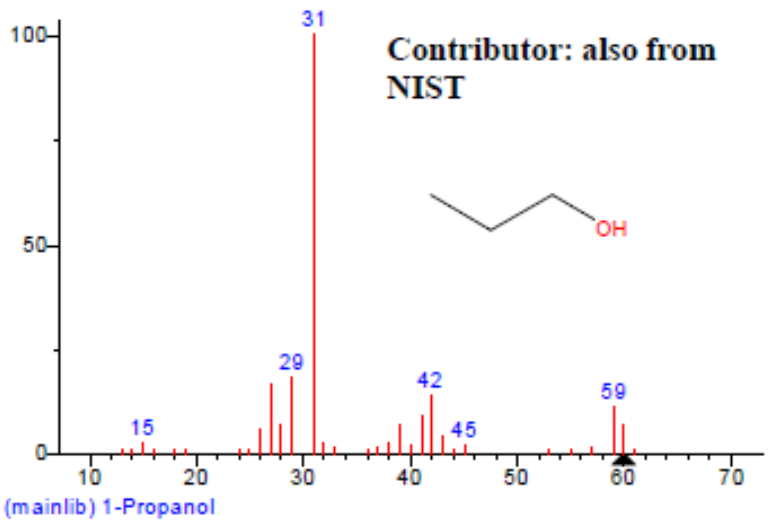
Kinetic energy of electrons: 70 eV

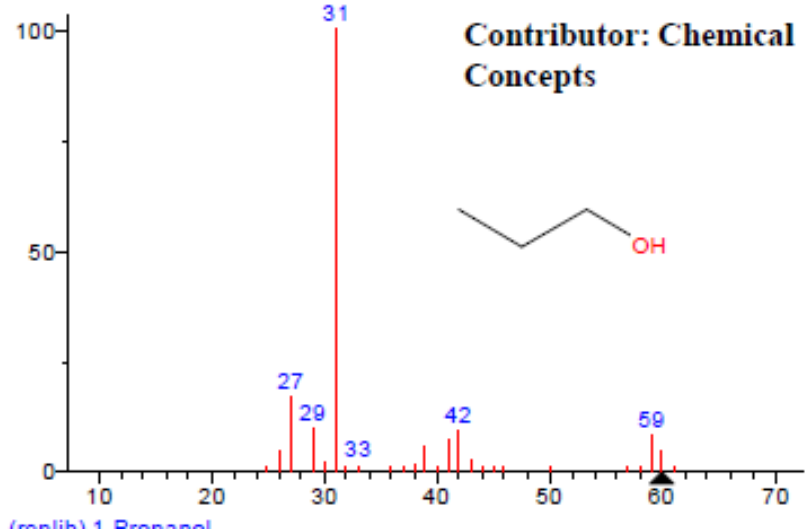


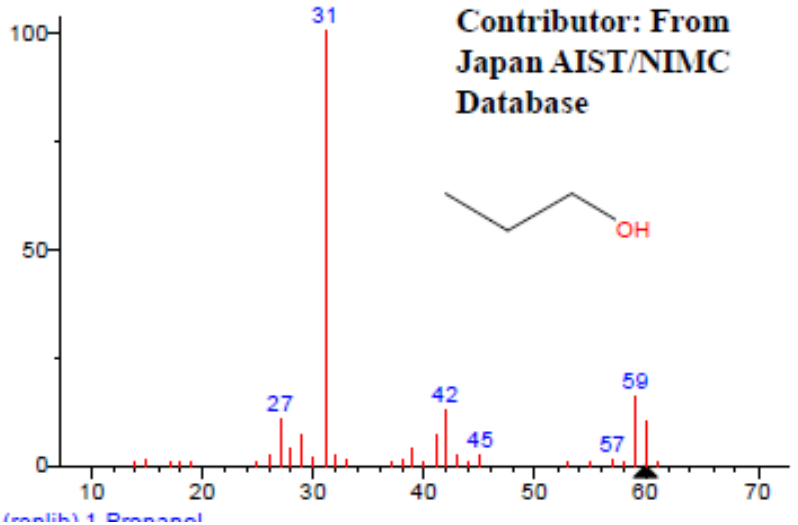


Reproducibility of EI spectra





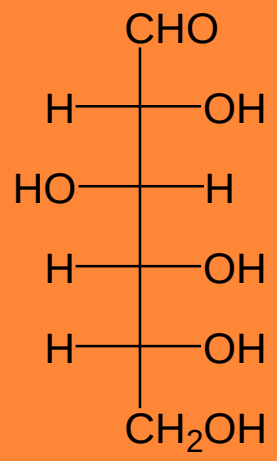


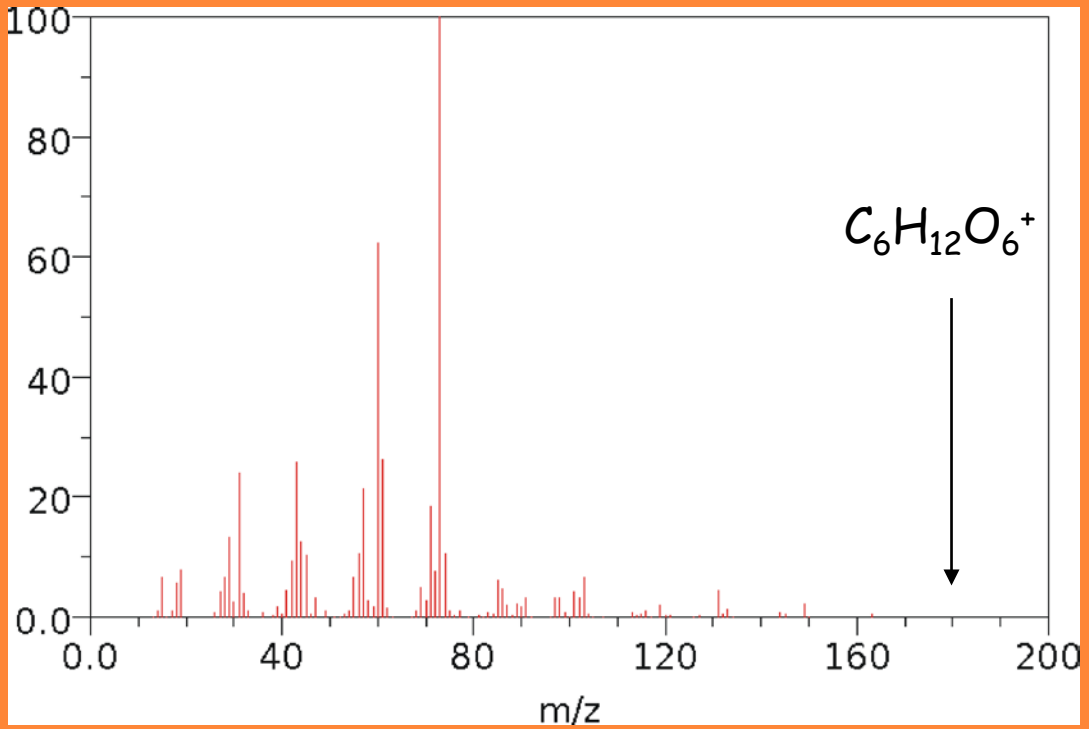


Electron ionization – “hard” ionization

$C_6H_{12}O_6$

M = 180 g/mol



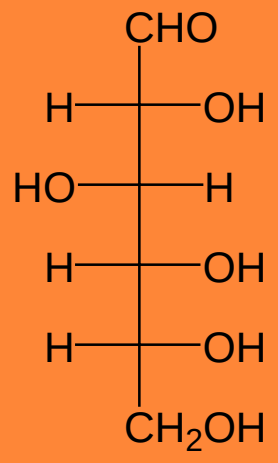


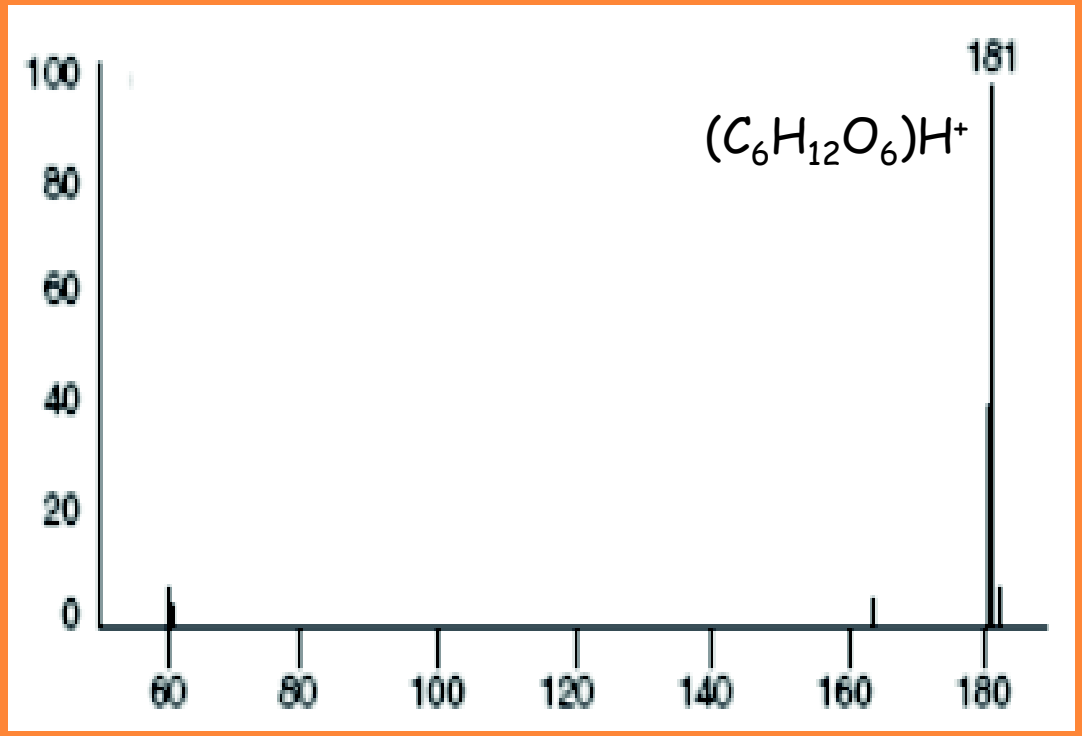
“Soft” ionization techniques

- chemical ionization
  - H_2O → H_3O^+ → protonation of the studied molecule

$C_6H_{12}O_6$

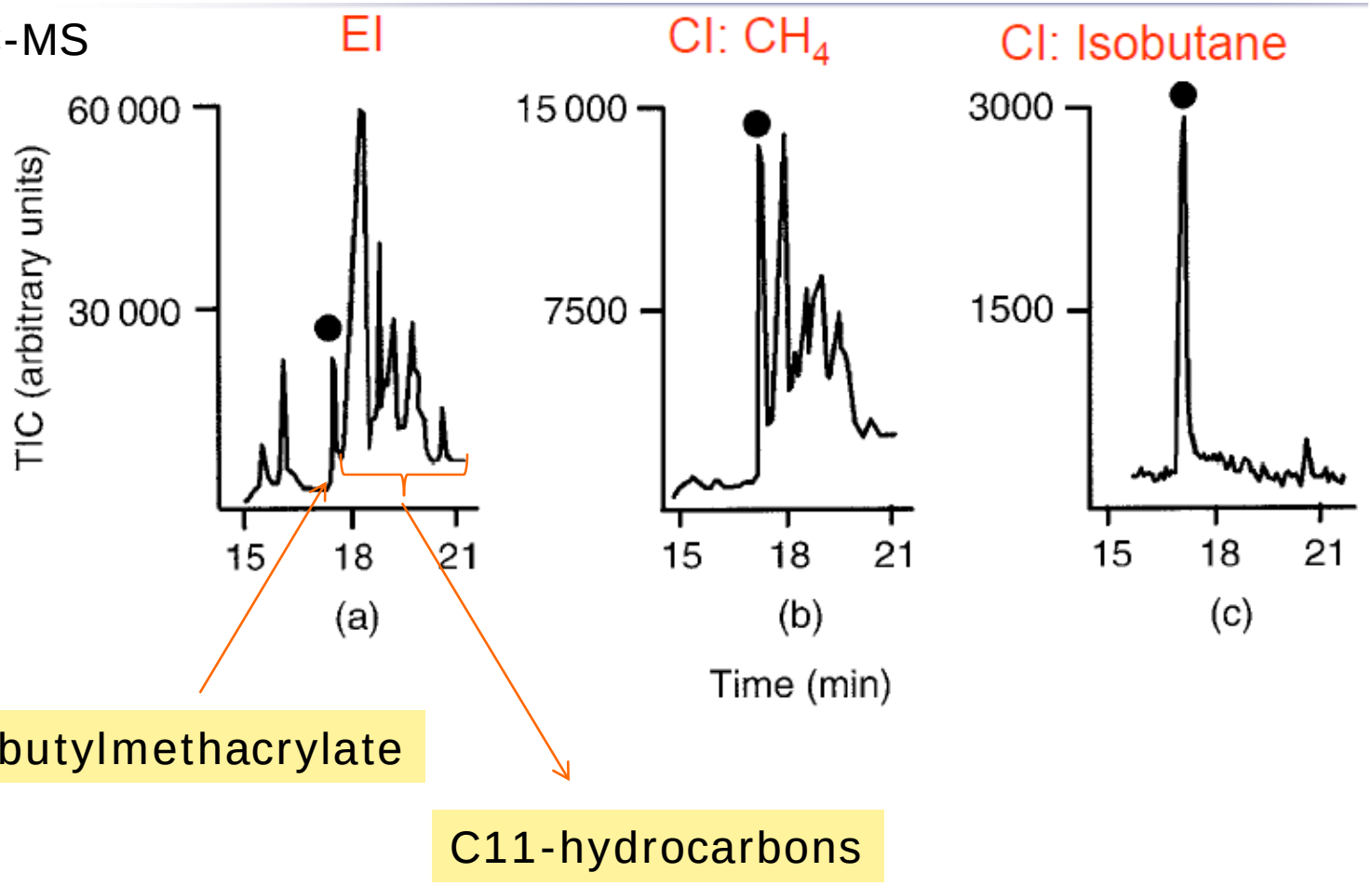
$M = 180\ g/mol$





Selective detection

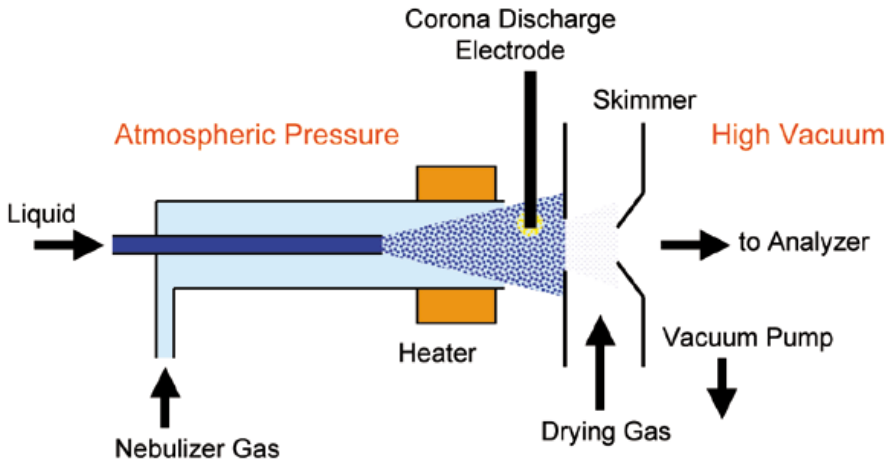
GC-MS



butylmethacrylate

C11-hydrocarbons

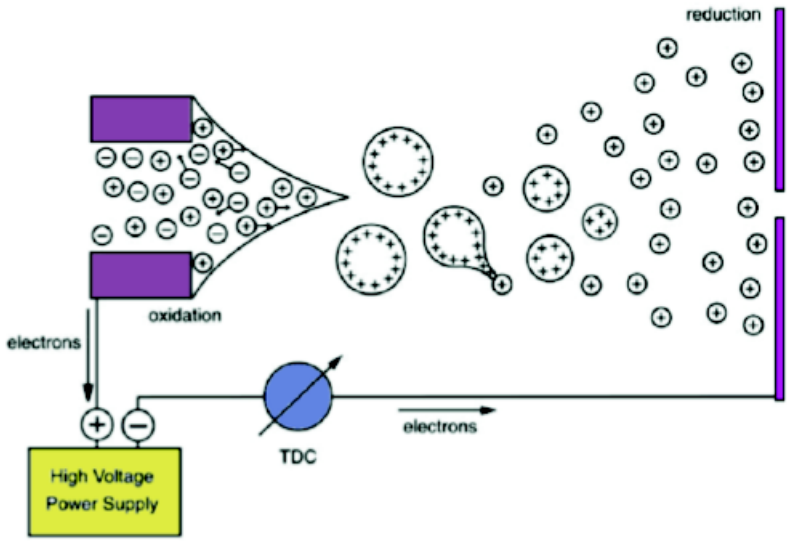
APCI – Atmospheric pressure chemical ionization

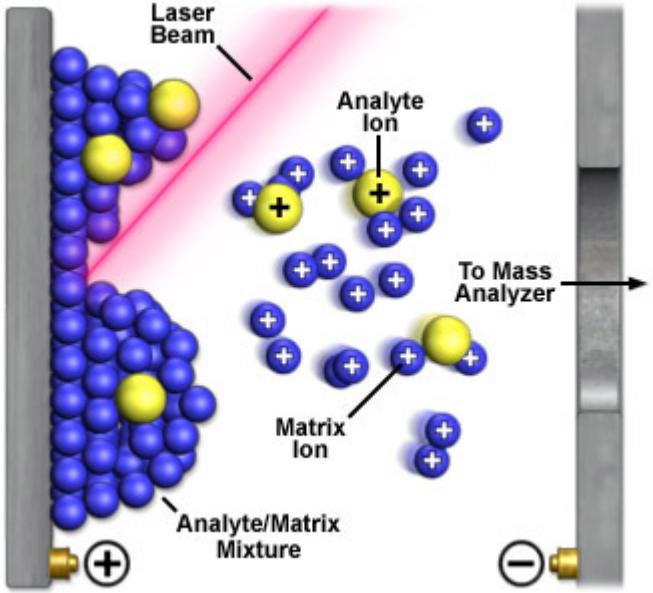


- Connection of MS a LC
- Heat and N2 streem – drops desolvation
- Discharge ionizes solvent molecules (reaction gas)
- Not suitable for thermo-labile samples

Soft ionization – MS opens to biochemistry

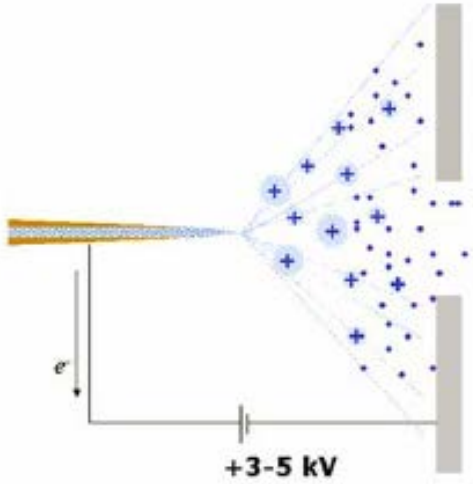
- electrospray ionization
- MALDI

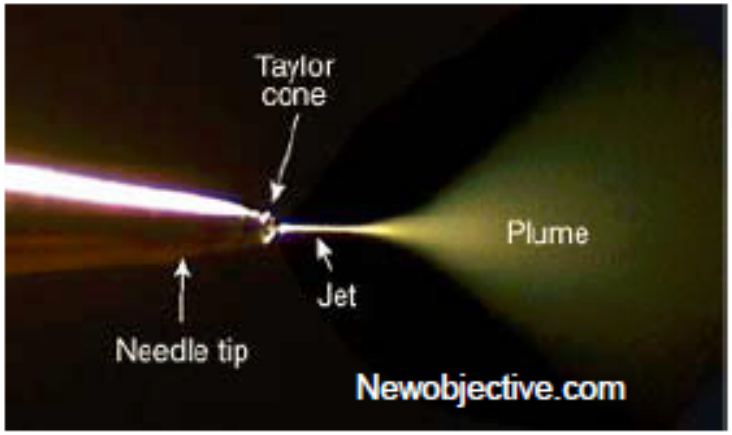


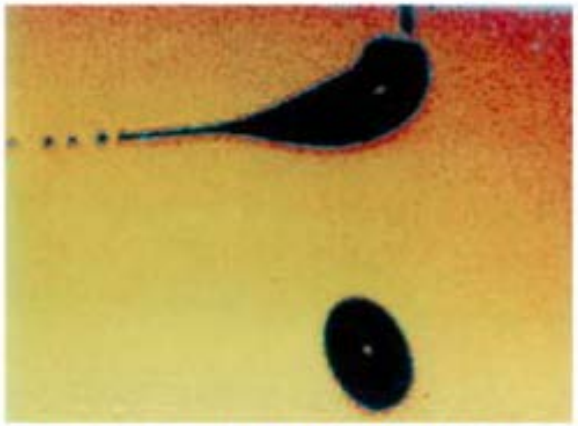


Electrospray ionization

- Atmospheric pressure ionization
- Ionization of large nonvolatile molecules (proteins) without fragmentation
- Solution infused by a capillary
- Coulombic explosions lead to generation of isolated ions (positive or negative)
- Connection to HPLC



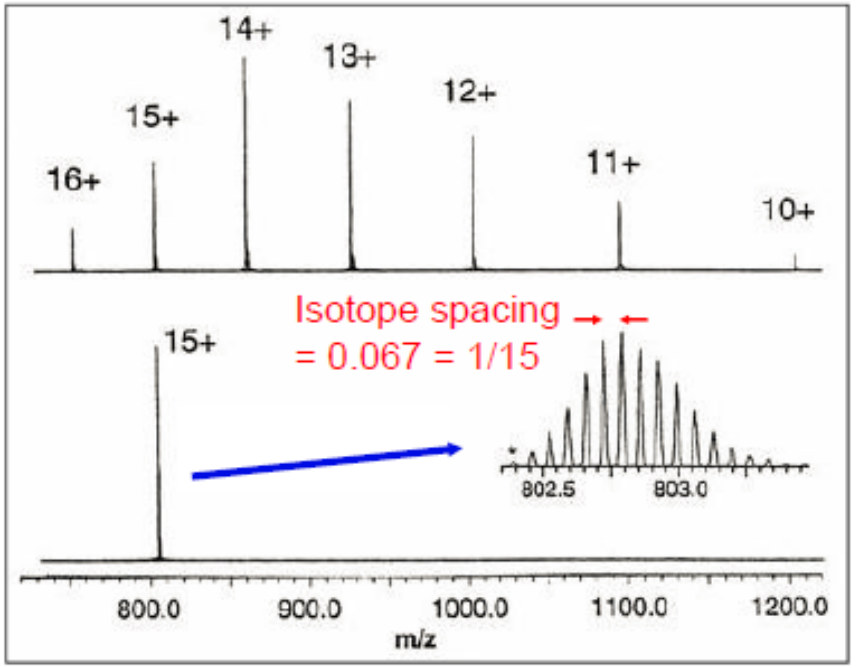




Gomez & Tang, *Phys Fluids*, 1994, 6:404–414

Characteristic ESI-MS spectrum

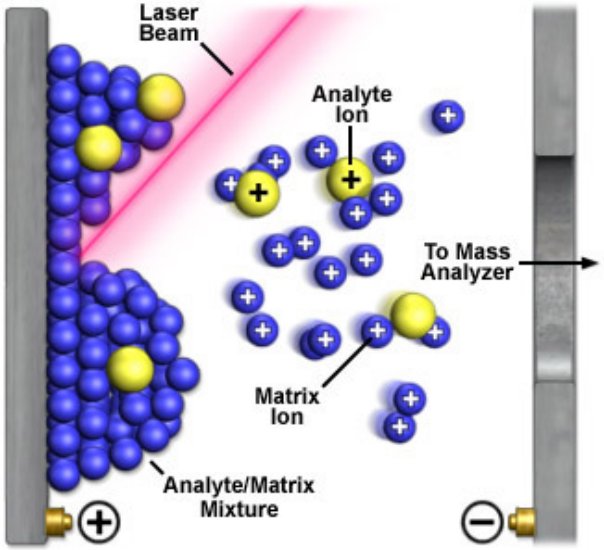
- Multiply charged ions
- Multiple charge → small *m/z* → advantageous for most of the mass analyzers
- *z* can be determined from the spacing of the peaks



ESI-MS of Cytochrome C, ~12,360 Da

From Fig 13-18 Lambert

Matrix assisted laser desorption ionization



- Analyte mixed with a crystalline matrix (e.g., xxx)
- Dry mixture irradiated by a short, intense laser pulse at a wavelengths absorbed by the matrix (usually UV)
- Fast matrix heat-up → sublimation and expansion to the gas phase
- Ionization – proton transfer
- Usually singly charged ions

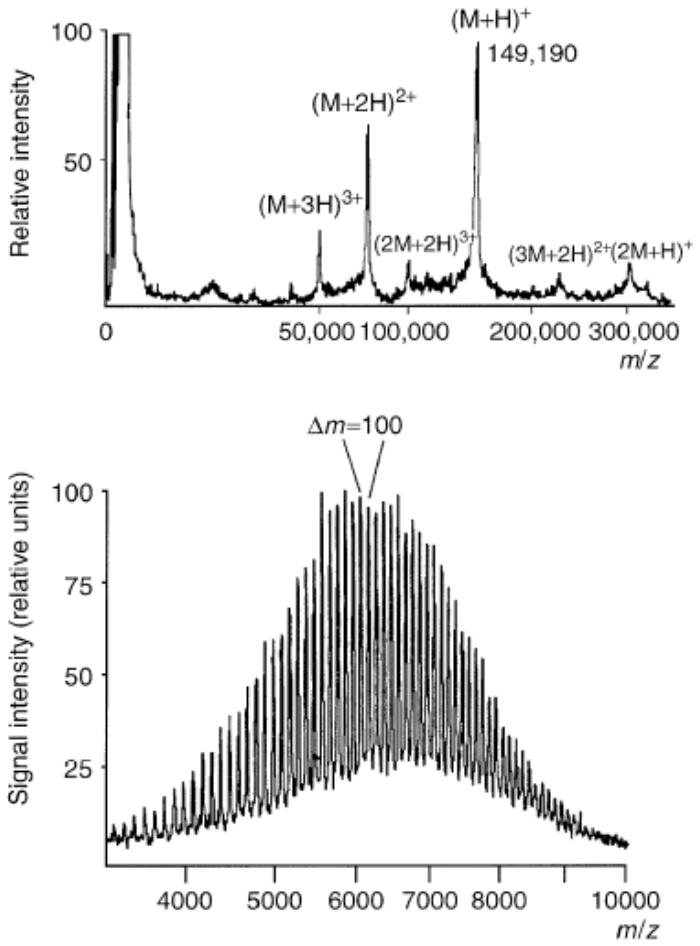
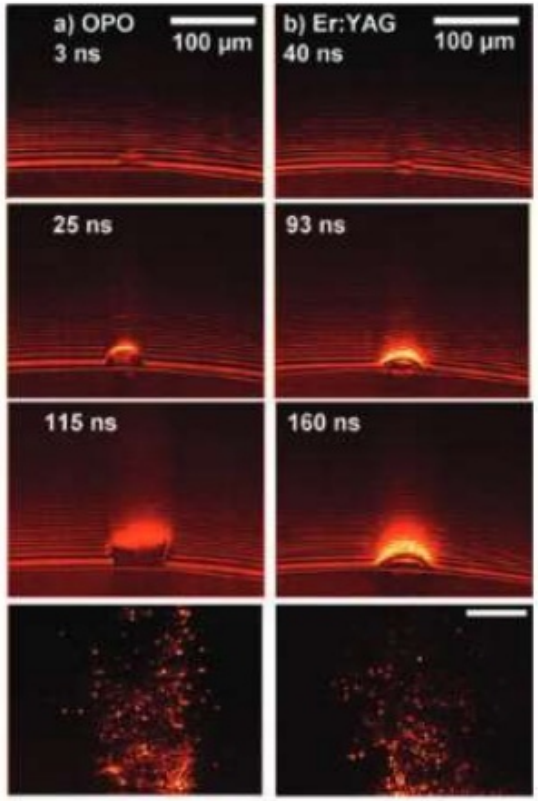


Figure 1.15
The MALDI spectra of a monoclonal antibody (*top*) and poly(methyl methoacrylate) of average mass 7100 Da (*bottom*) (Reproduced (modified) from Ref. 24 and from Finnigan MAT documentation, with permission)



High-speed time-lapse photographs of IR-MALDI plumes with 100-ns pulse width Matrix: glycerol; time resolution 8 ns; spatial resolution 4µm.

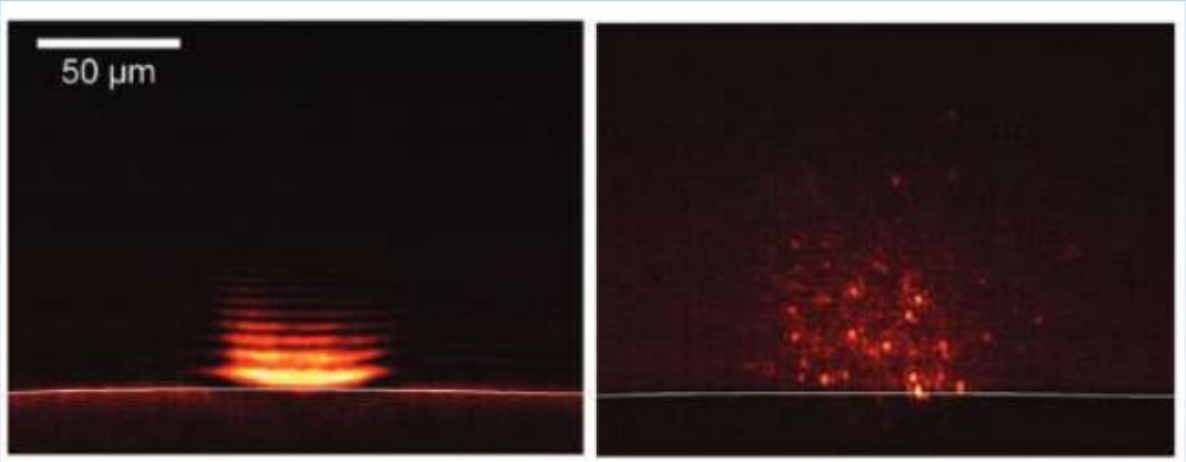
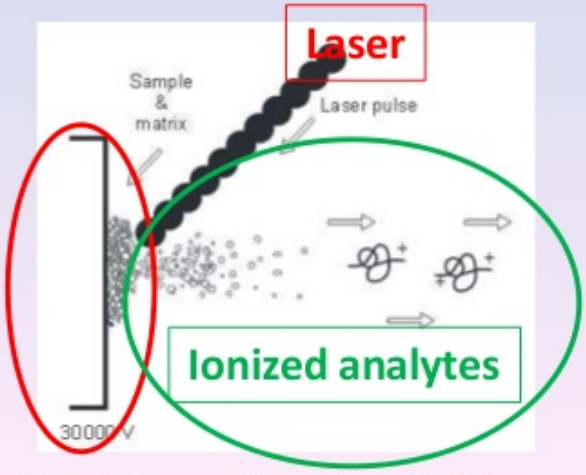


Fig. 13 High-speed photographs of UV-MALDI plumes generated with a frequency-quadrupled Nd:YAG laser of 266 nm wavelength and 8 ns pulse width. Matrix: nitrobenzylalcohol; time resolution 8 ns; spatial resolution 4 µm. Left panel: dark-field image, 45 ns after laser exposure. Right panel: 90° scattered light image, 311 ns after exposure. The thin lowest line indicates the top surface of the glycerol drop; the other striations in the dark-field image are artifacts of optical interference.



Hillenkamp, Franz, and Jasna Peter-Katalinic, eds. *MALDI MS*. John Wiley & Sons, 2007.

New Ionization Techniques: DESI

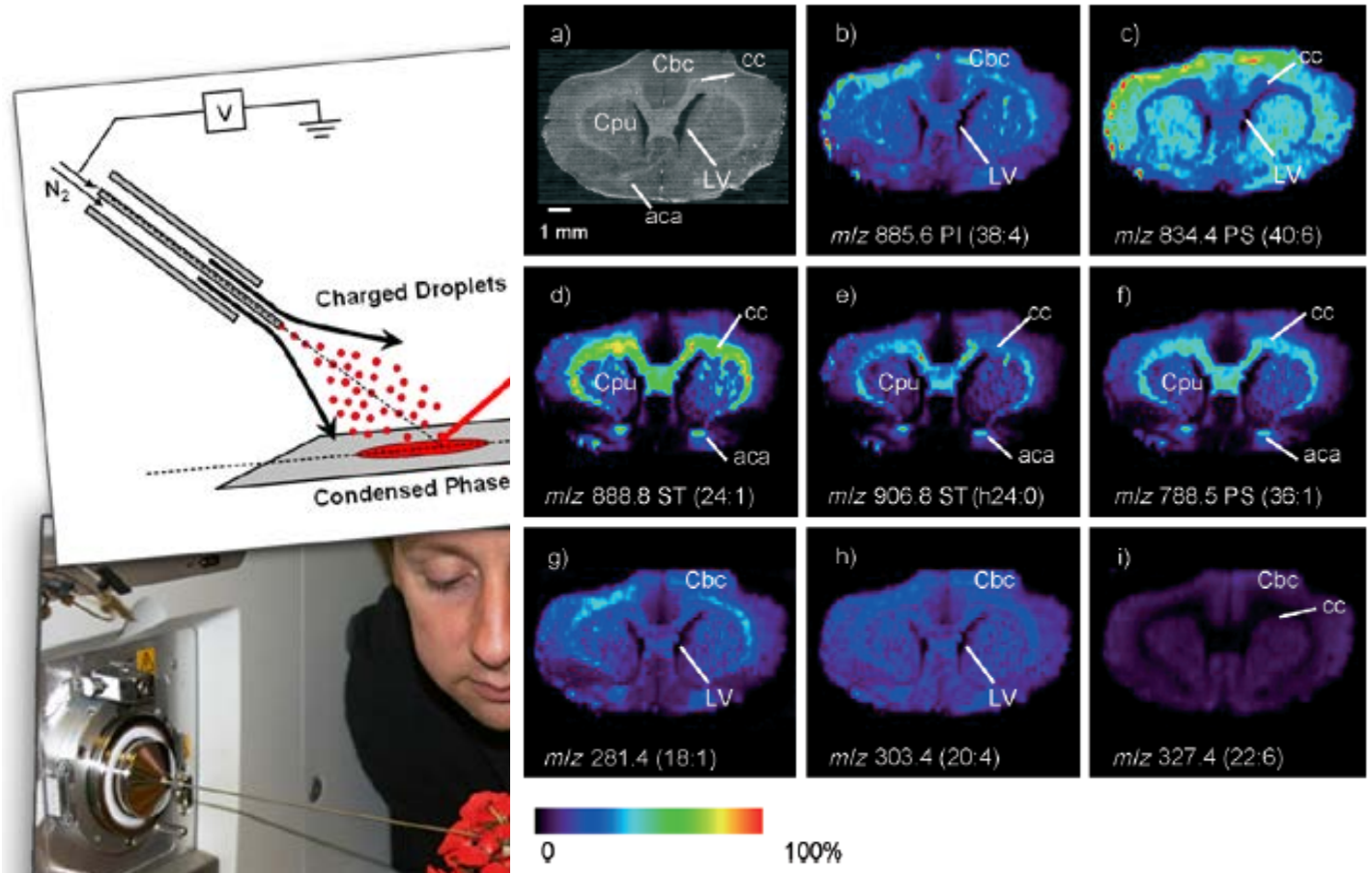
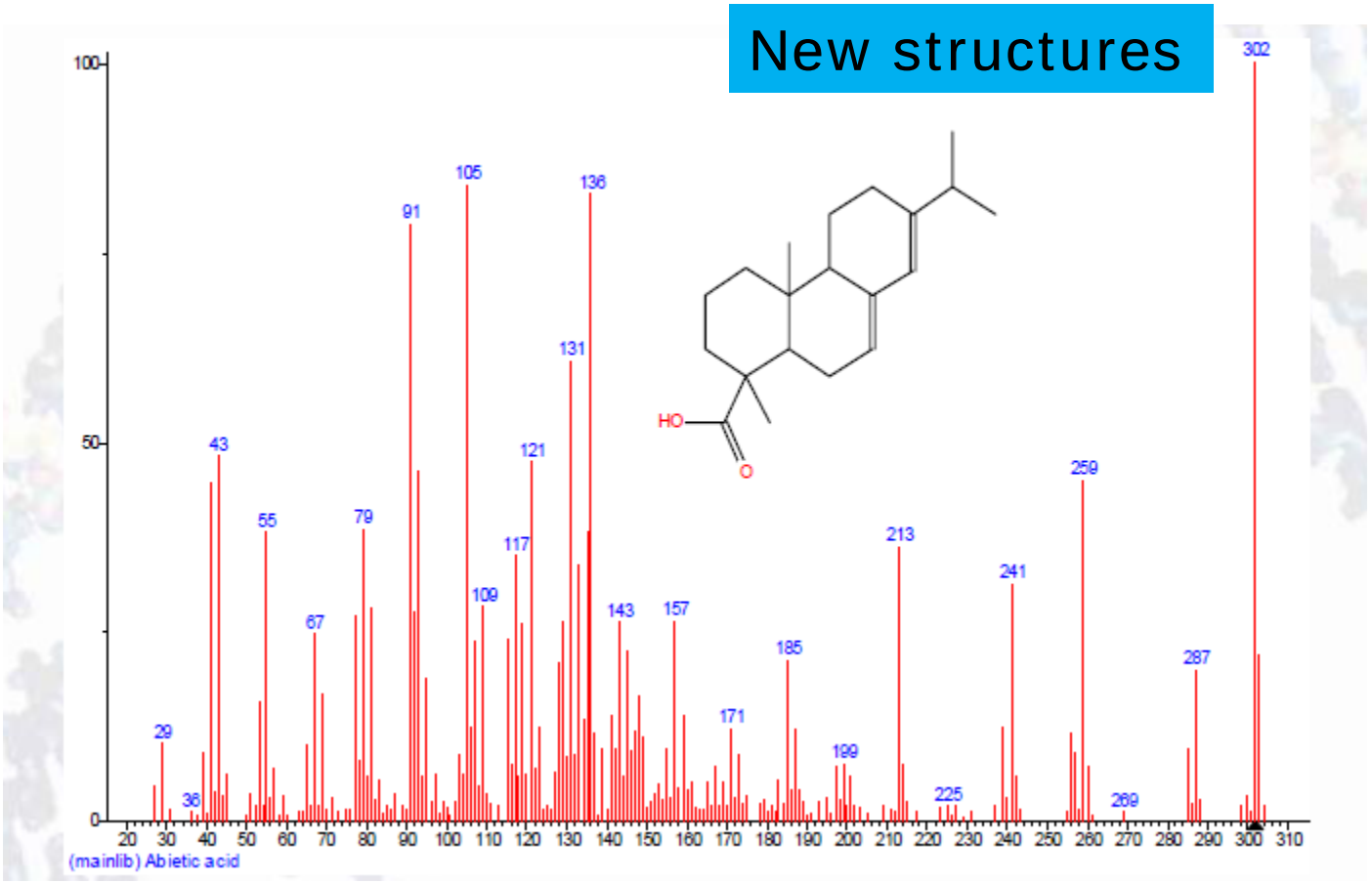


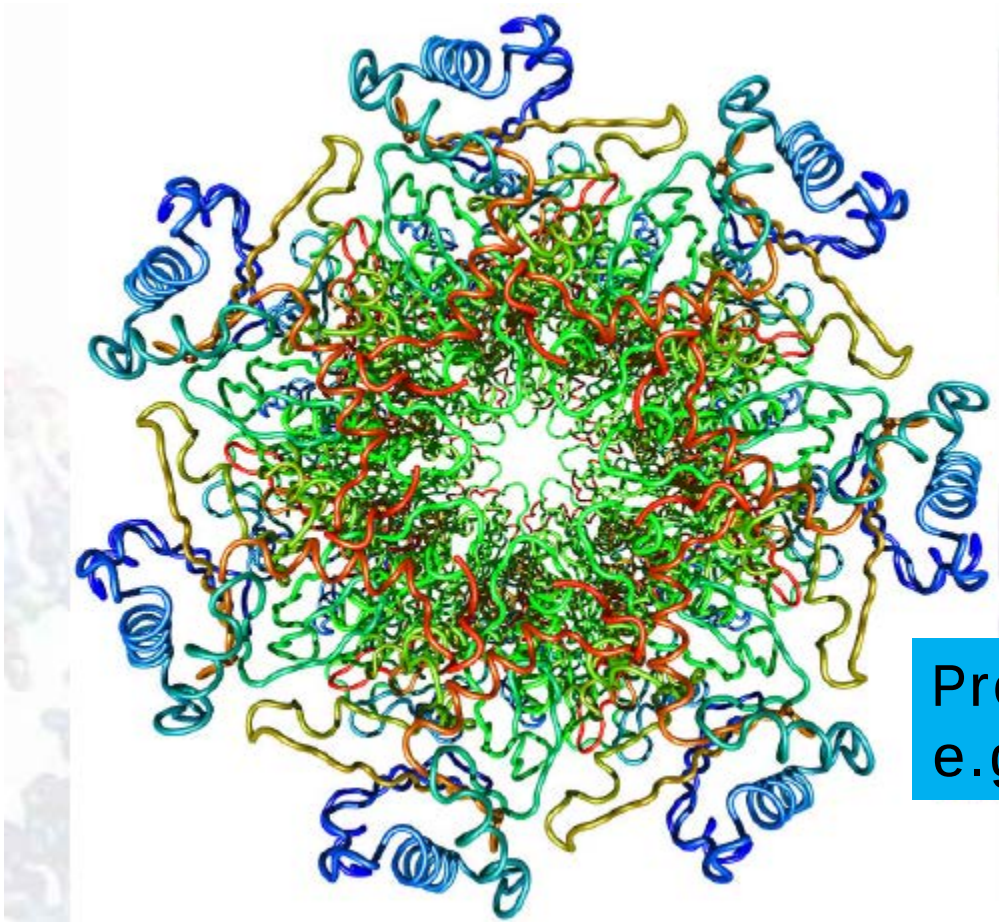
Figure 2. Selected molecular ion $[M-H]^-$ images of specific lipids from analysis of a 13×10 mm^2 area of rat brain tissue section. a) Optical image of the coronal section of the rat brain prior to analysis. cc = corpus callosum; CPu = striatum; Cbc = cerebral cortex; LV = lateral ventricle; aca = anterior part of anterior commissure. b–i) Ion images of PI (38:4; b), PS (40:6; c), ST (24:1; d), ST (h24:1; e), PS (36:1; f), oleate (18:1; g), arachidonate (20:4; h), and

R. G. Cooks: *Science* 2004, *Science* 2006, *Angew. Chem. Int. Ed.* 2006

Examples: Chemistry

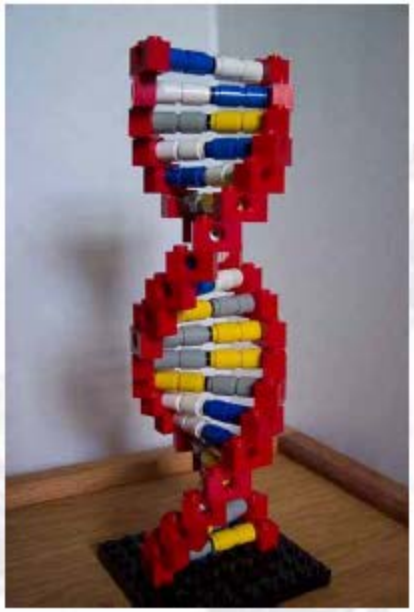


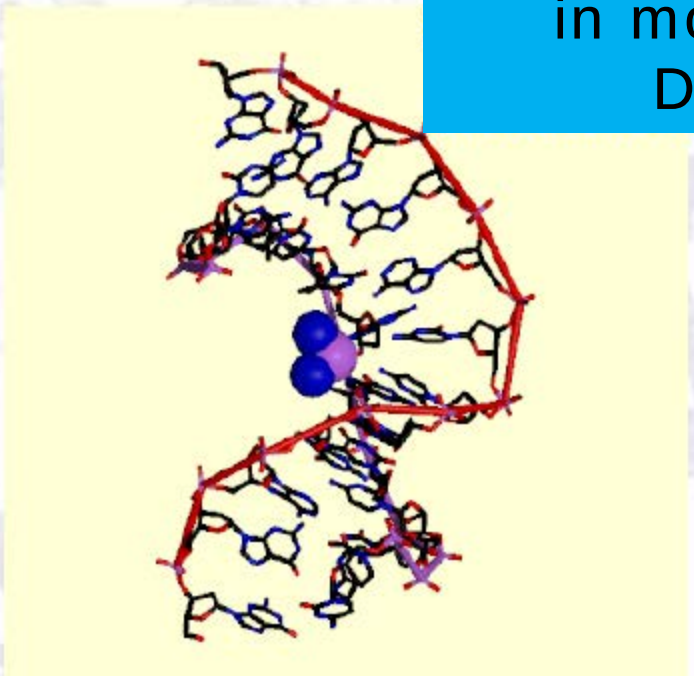
Biology



Protein complexes
e.g. Protease

Biology

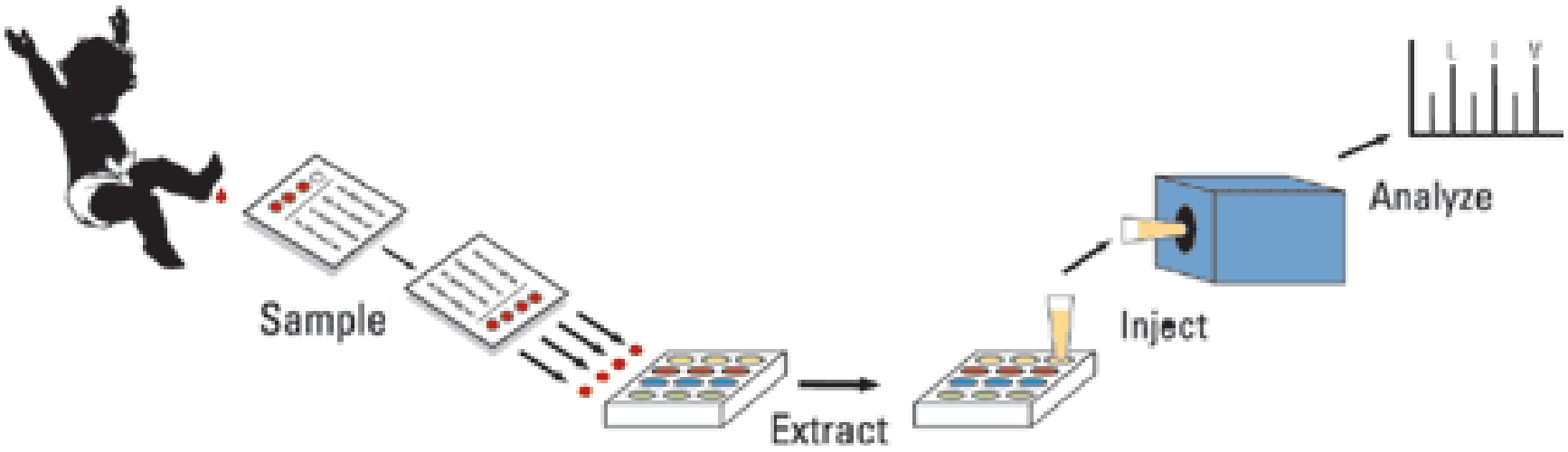




Toxic compounds in modified DNA

Medicine

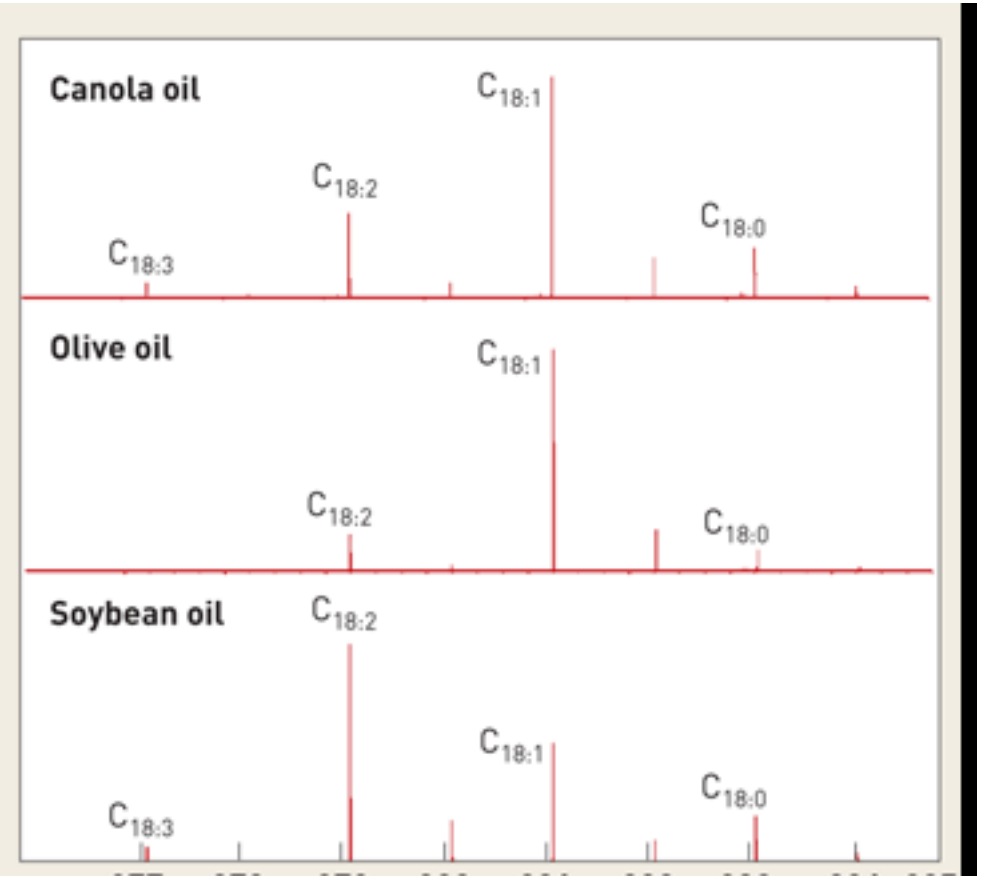
Screening of metabolic diseases of newborns



Environment

Detection of pollutants, aerosol formation monitoring, monitoring of gene mutations, ...

Quality control



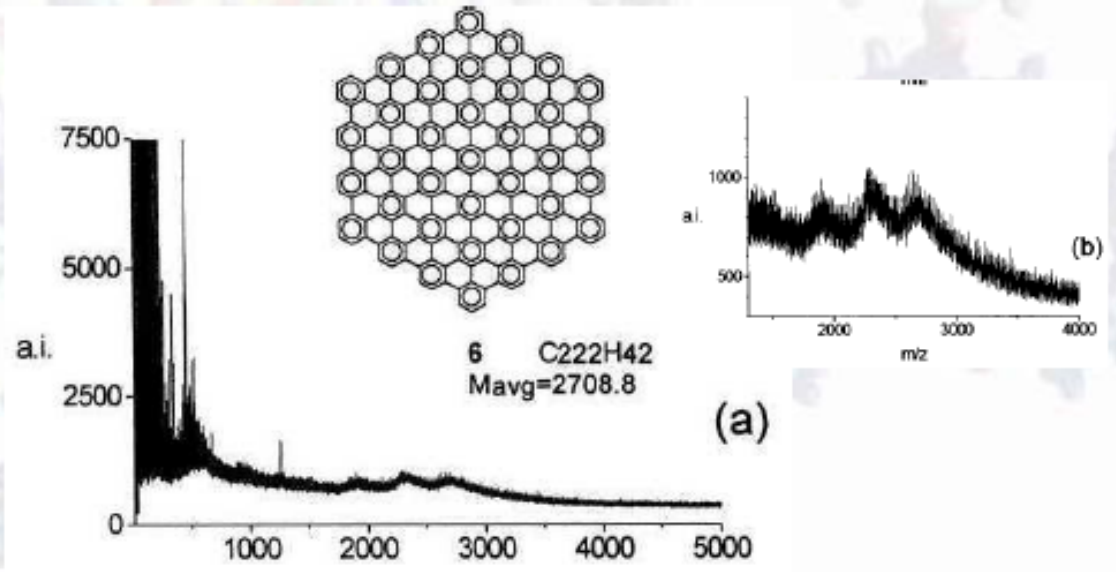
Material science

Anal. Chem. 2000, *72*, 4591−4597

MALDI-TOF Mass Spectrometry of Insoluble Giant Polycyclic Aromatic Hydrocarbons by a New Method of Sample Preparation

Laurence Przybilla, Johann-Diedrich Brand, Kimihiro Yoshimura, Hans Joachim Räder,*,† and Klaus Müllen‡

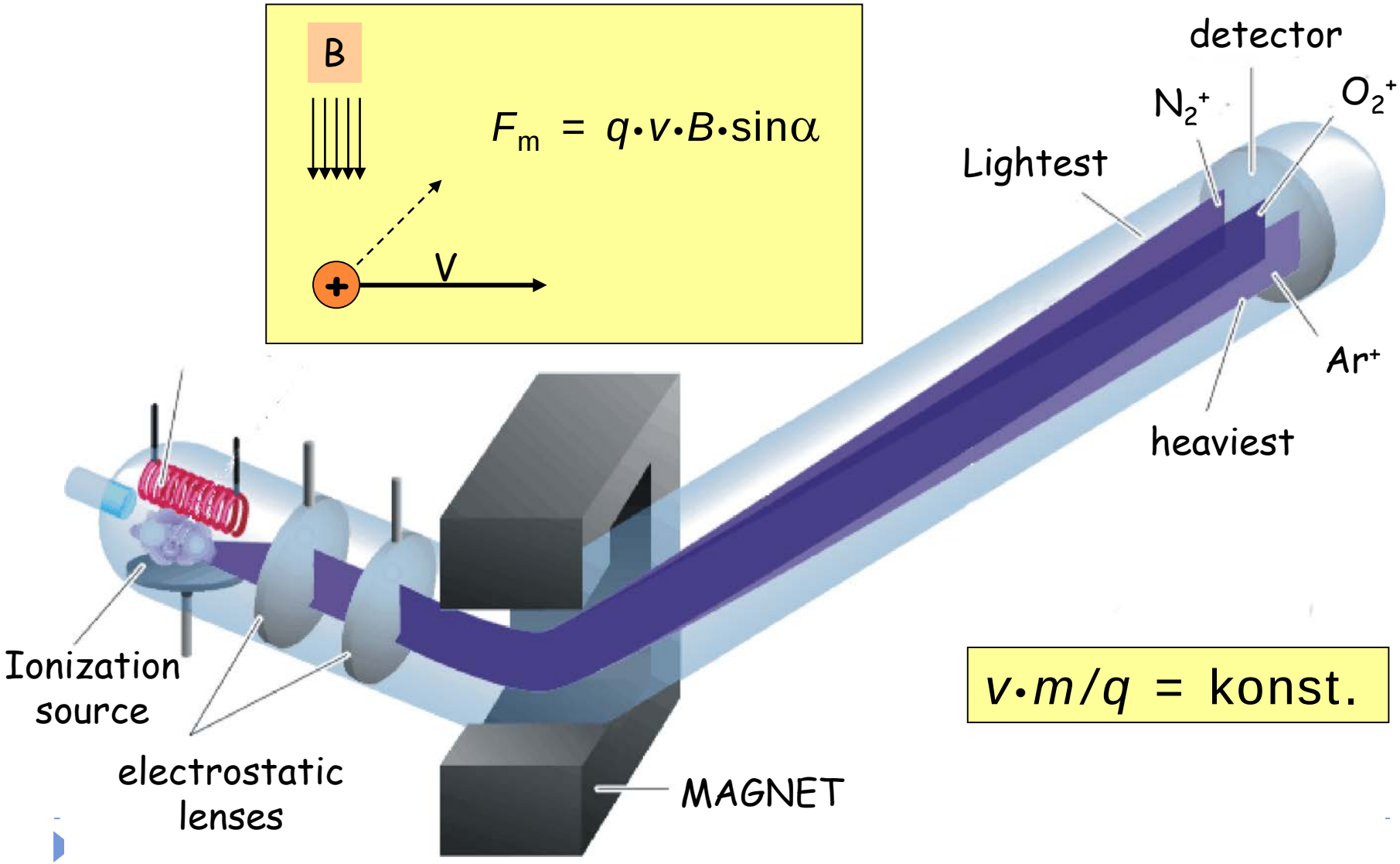
Max-Planck-Institut für Polymerforschung, Ackermannweg 10, D-55128 Mainz, Germany



Mass Analyzers

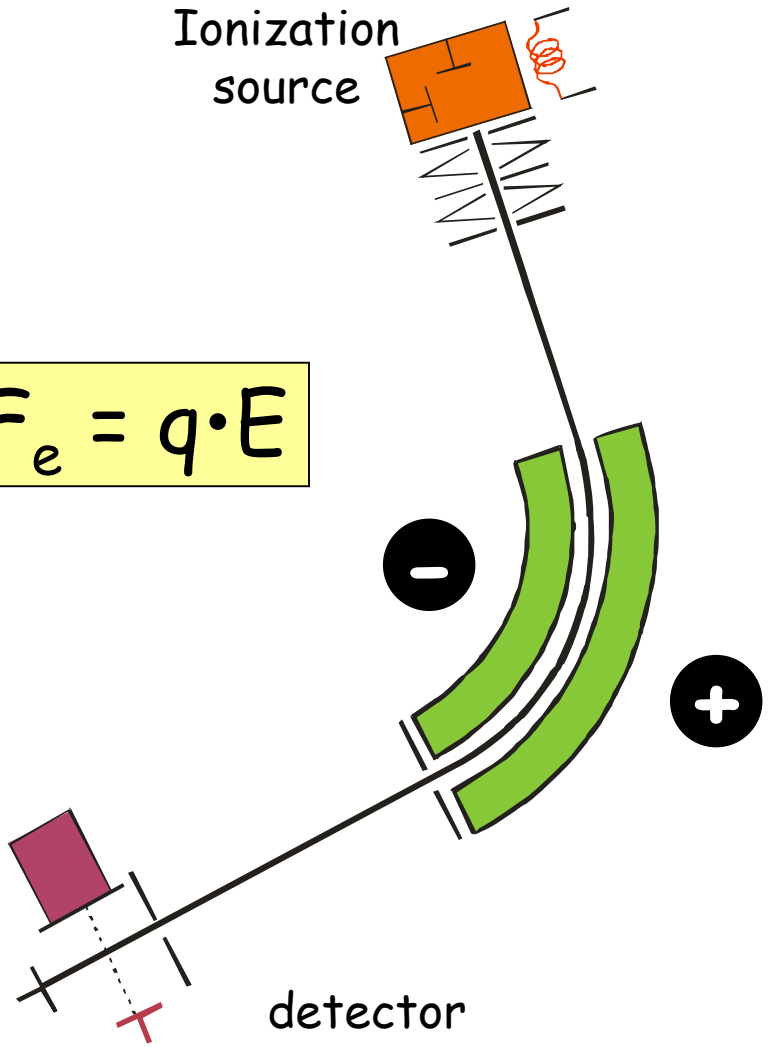
- Magnetic and electrostatic sectors
- Time-of-flight analyzer
- Quadrupoles
- Ion traps
- High resolution analyzers – ICR, Orbitrap

Magnetic field



$v \cdot m/q$ = konst.

Electrostatic field



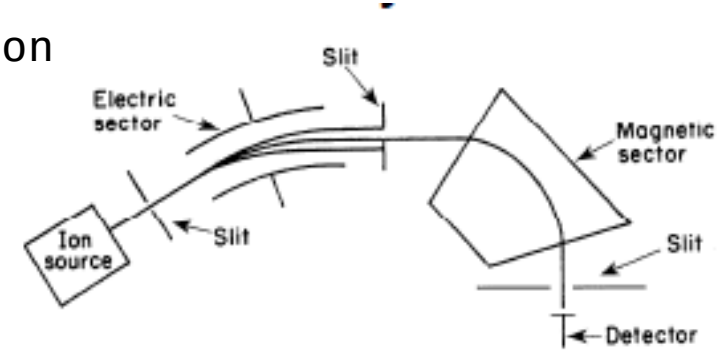
$F_e = q \cdot E$

$v^2 \cdot m/q = konst.$

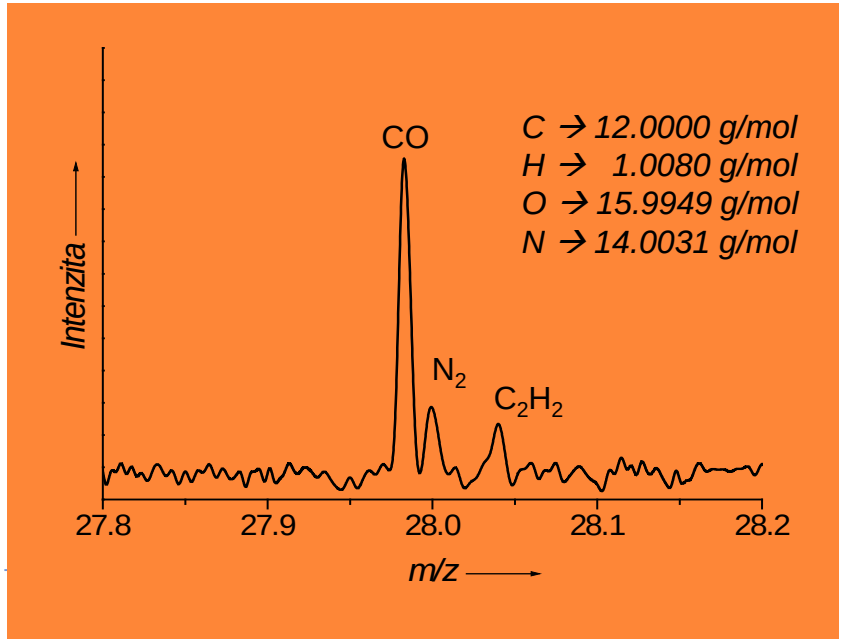
Double-focusing analyzers

- Electrostatic analyzer reduces E_k distribution
- Magnetic analyzer filters m/z

$$R = \frac{mv}{qB} = \frac{\sqrt{2mE_k}}{qB}$$

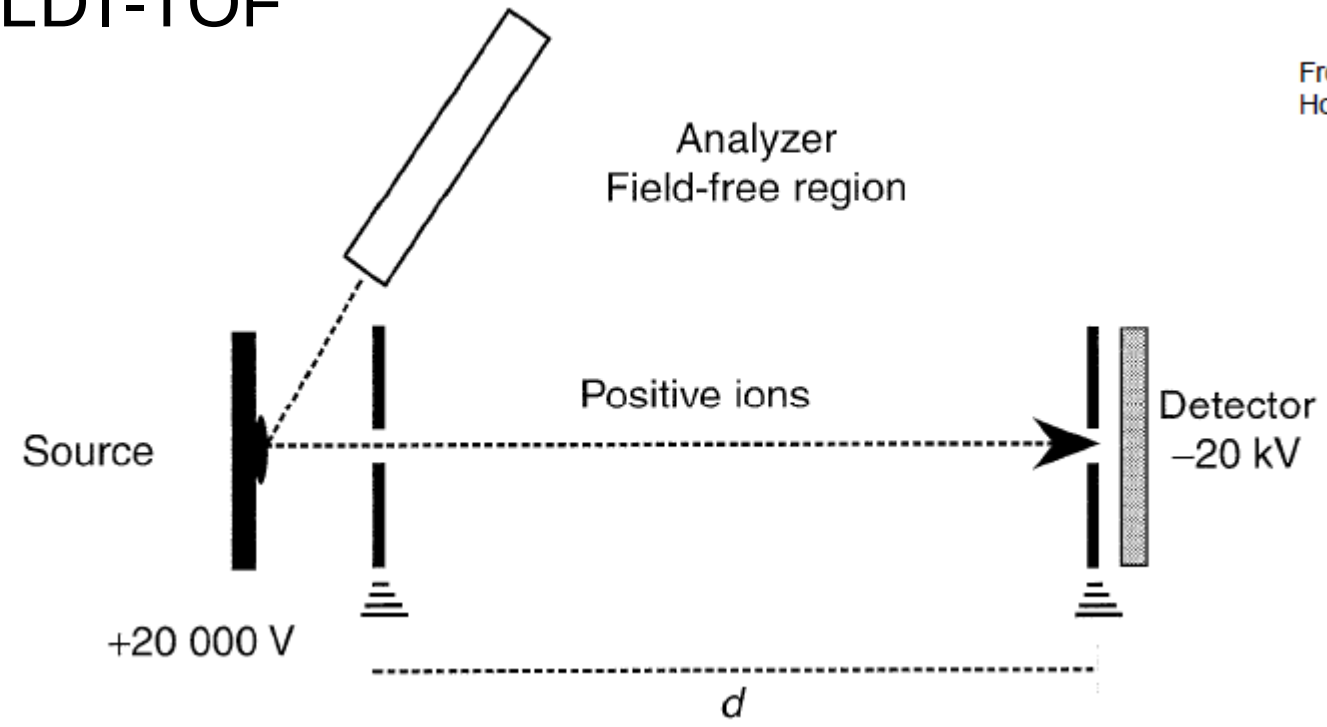


High resolution



Time-of-flight (TOF) analyzers

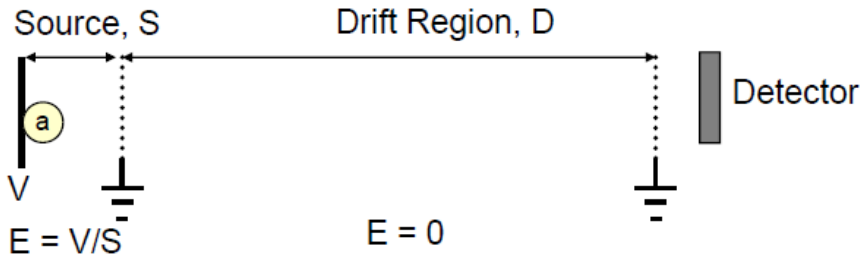
- MALDI-TOF

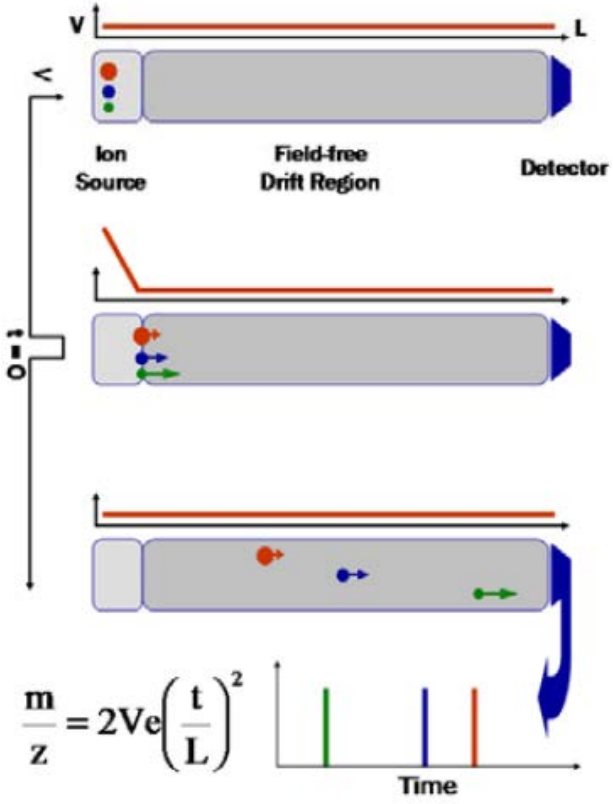


MALDI produces cations that are accelerated towards the analyzer
→ cations fly in a field-free region – time of flight depends on their *m/z*

Time-of-flight (TOF) analyzers

- Ions accelerated by a known potential
- Known flying distance





$$E_k = \frac{1}{2}mv^2 = qV$$

$$zeV = \frac{1}{2}m\frac{x}{t^2}^2$$

$$\frac{m}{z} = 2Ve\left(\frac{t}{x}\right)^2 = Kt^2$$

- Important factors:
  - Time resolution
  - All ions measured simultaneously → faster spectra scanning
  - “Infinite” range of masses
  - cheap

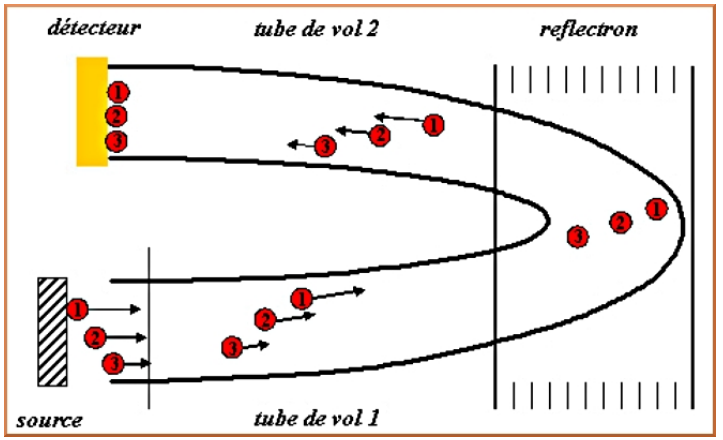
TOF renaissance

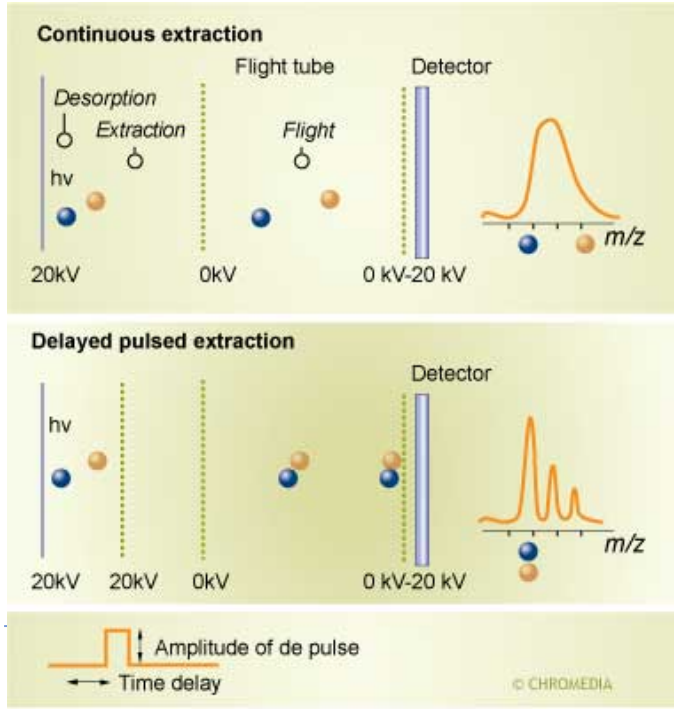
- Reflectrons
  - Series of electrodes creating a linear field with an opposite sign to the initial accelerating field
  - Ions are decelerated and turned to the opposite direction
  - Constructed so that ions are focused to the plane of the detector

  → Ions with different kinetic energy, but the same m/z, fly a different distance → In the end, they have the same time of flight

- Delayed pulsed extraction
  - Extraction of the ions is delayed by 200 – 500 ns
  - During the delay, faster ions move closer to the extraction electrode than the slower ones
    → extraction pulse accelerates the faster ions
    shorter time → final velocities are similar

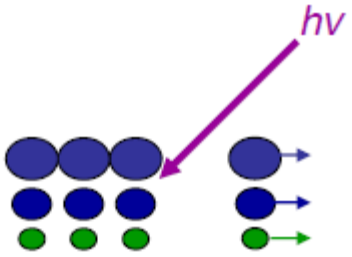
  → Initial distribution of velocities is corrected → the same time of flight





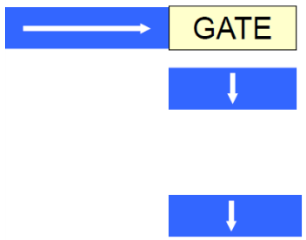
Using TOF analyzers

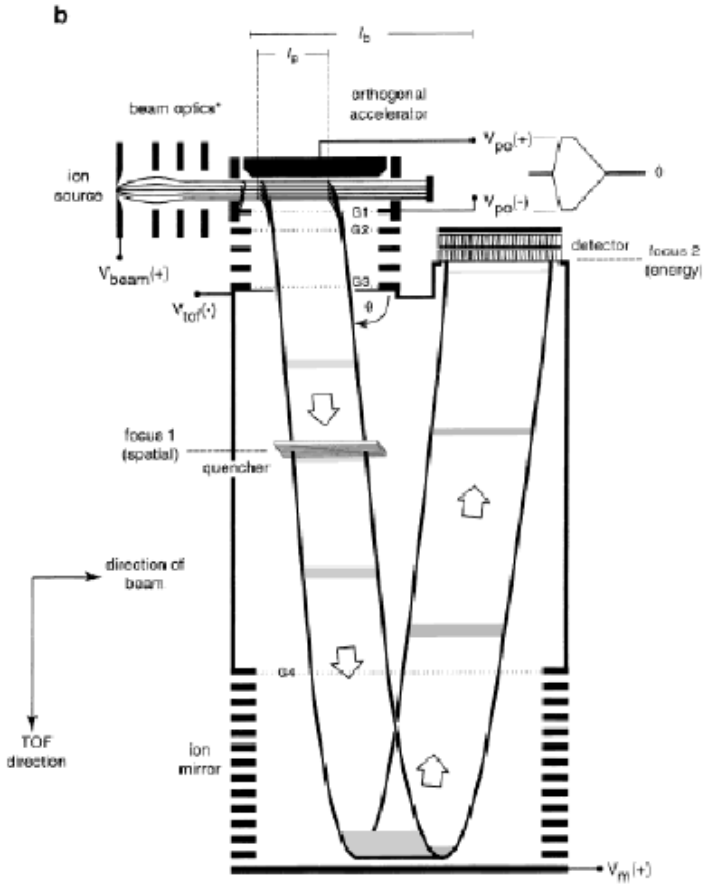
- TOF is an ideal detector for pulse-ionization methods such as MALDI



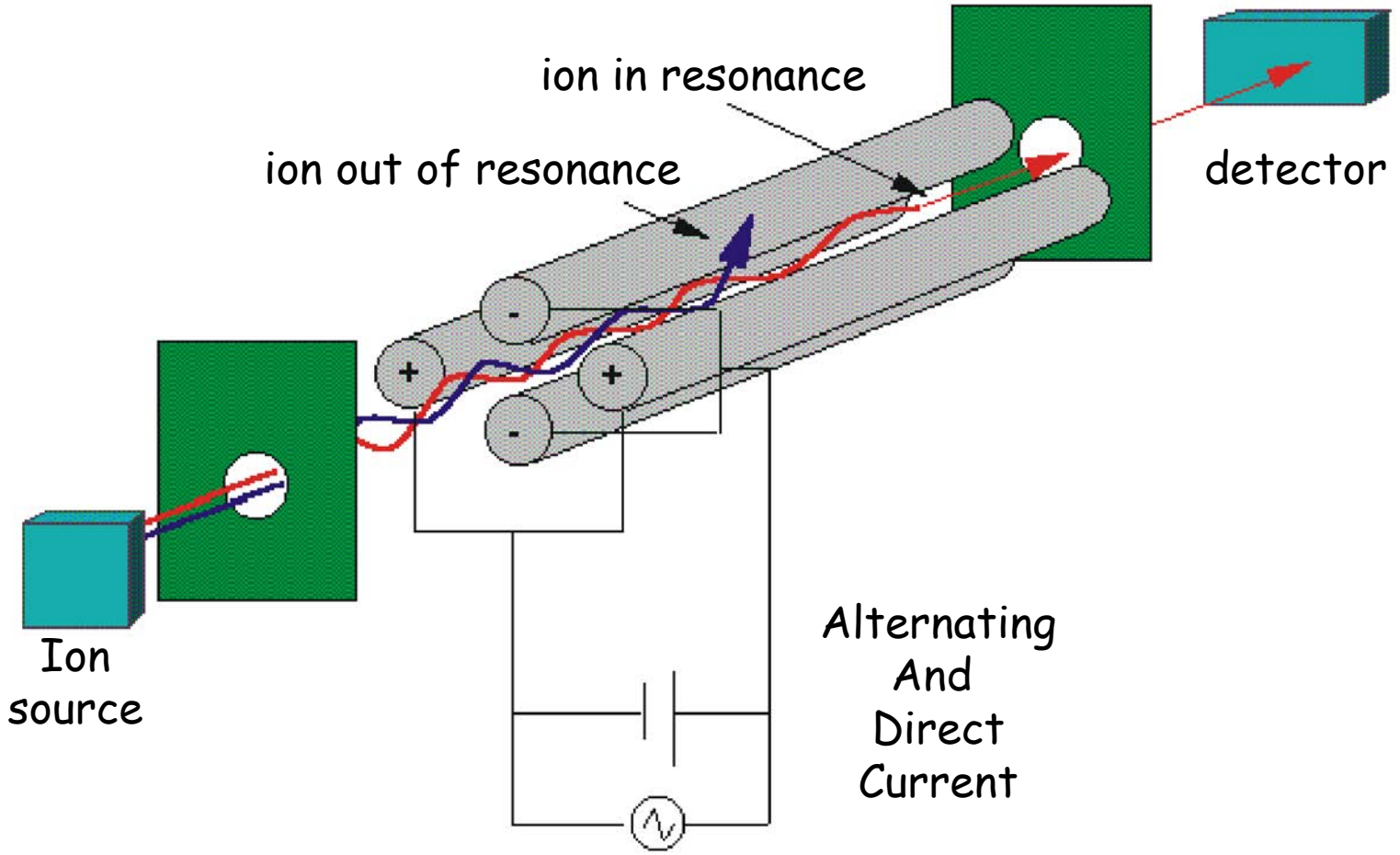
Laser Desorption: Static, solid sample probed with a pulsed laser

- For continuous ionization sources (EI, ESI, etc.) most of the ions are lost with TOF analysis
  - Shorter time of flight → decreasing of the mass range and resolution
  - Orthogonal extraction





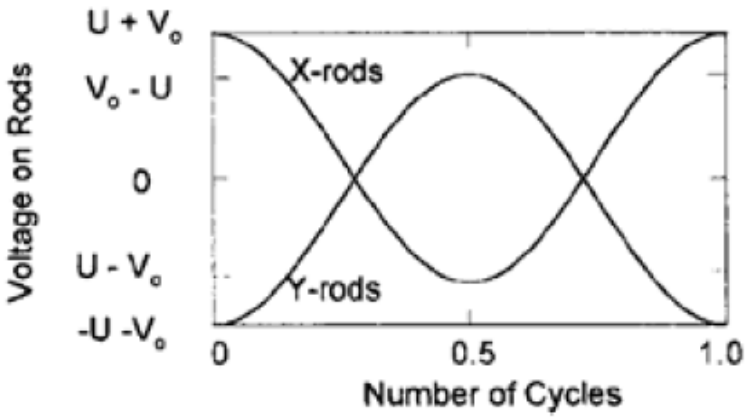
Mass selection using quadrupole

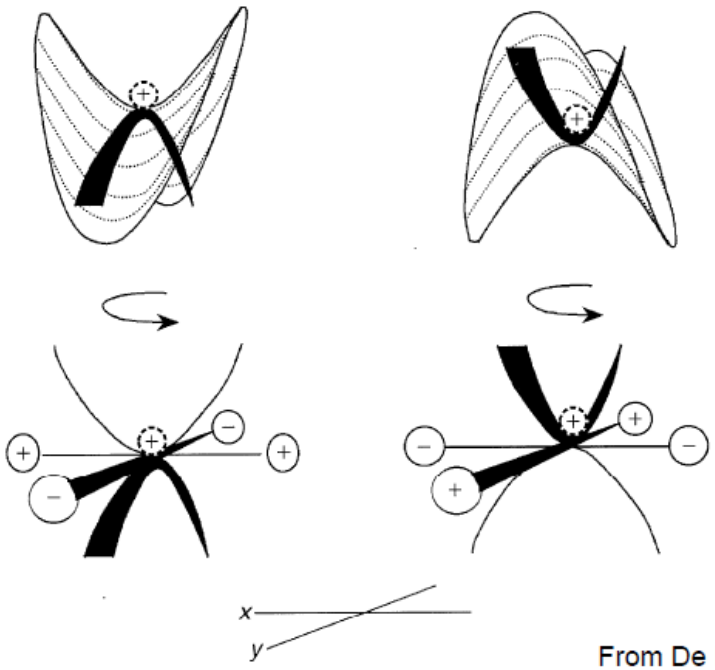


Quadrupoles

Ions are at each moment accelerated

- along one axis to the center
- along the other axis out of the center
- Fast polarity changes create a stable potential well





From De Hoffmann

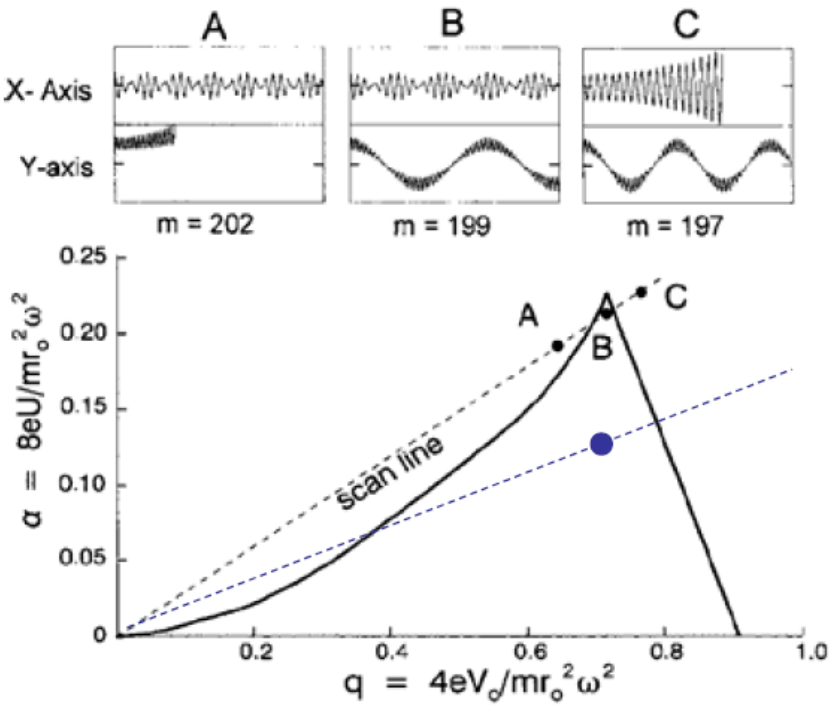
Figure 2.8
A positive ion, represented within a dotted circle, is at the center of quadrupole rods, the potential signs of which are indicated. It goes down the potential 'valley' with respect to the negative rods and acquires some kinetic energy in that direction. However, the potentials quickly change so that the kinetic energy is converted into potential energy and the ion goes back to the center of the rods, as would happen for a ball on a horse saddle that is turned quickly. The name 'saddle field' is an allusion to this phenomenon

Quadrupole as mass analyzer

$\alpha / q = 2U / V_0$

- Scanning along the line → the ratio U/V_0 is kept constant
- Largest resolution:
  - α = 0,237 a q = 0,706
  - Scan with $2U/V_0$ = 0,336 (0,237/0,706)
- Maximum *m/z* ~ 4000
- Resolution ~ 3000
  - Usually used with unit resolution
- Small, light, cheap
- Coupling with chromatography

Triangle of stability

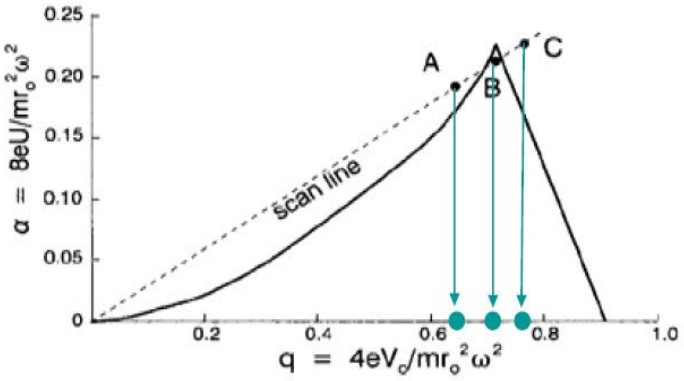


From: Steel and Henchman, *J. Chem. Ed.*, 75(8), 1049, 1998

Figure limited to singly charged ions (hence lack of z in expression)

Quadrupole as an ion guide

- For U = 0, many ions have stable trajectories
- Quadrupoles in rf-mode are important as ion guides (usually denoted as “q”)
- Often higher-number poles used as ion guides (hexapoles, octopoles)

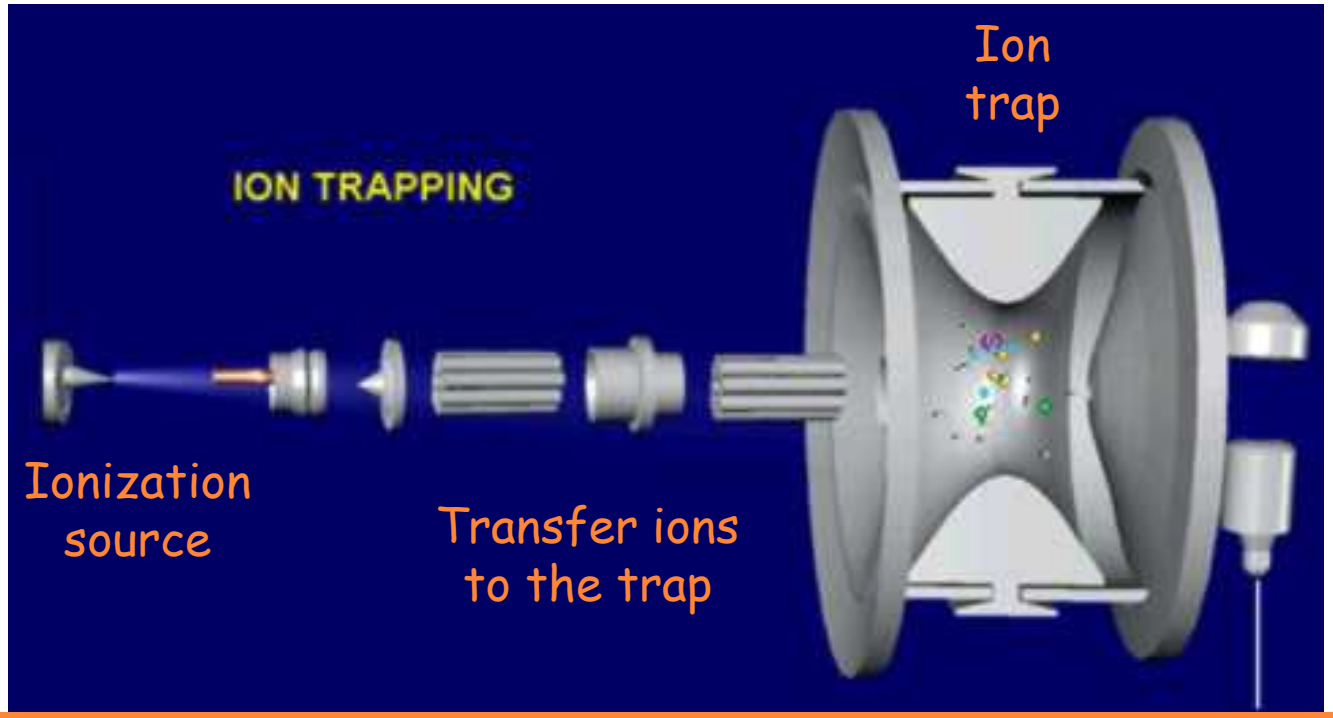


TOF vs. quadrupole

- TOF analyzers
  - Ions are in packets pulsed to the analyzer
  - All ions (all *m/z*) from the packet are analyzed simultaneously
  - *m/z* determined from dispersion of the ions in time
  - Based on static, DC field

- Quadrupoles
  - Continuous inlet of the ions
  - Only ions with specific *m/z* reach the detector
  - *m/z* determined by sequential filtering of ions
  - Based on time-dependent alternating field

Ion traps



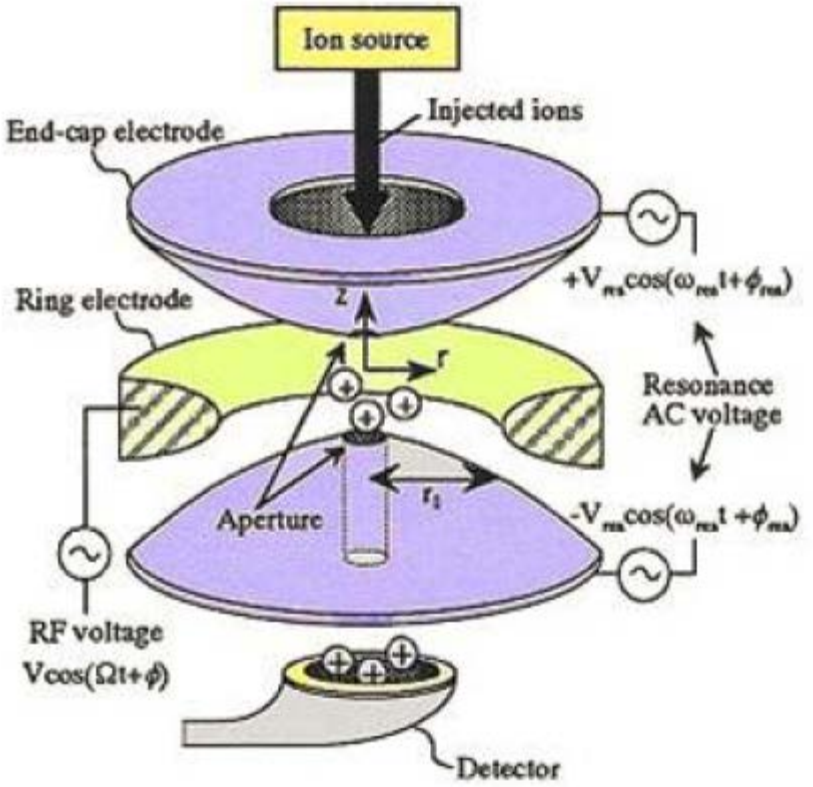
Ions have complicated pathways with frequency proportional to *m/z*

Ions can be analyzed according to *m/z*

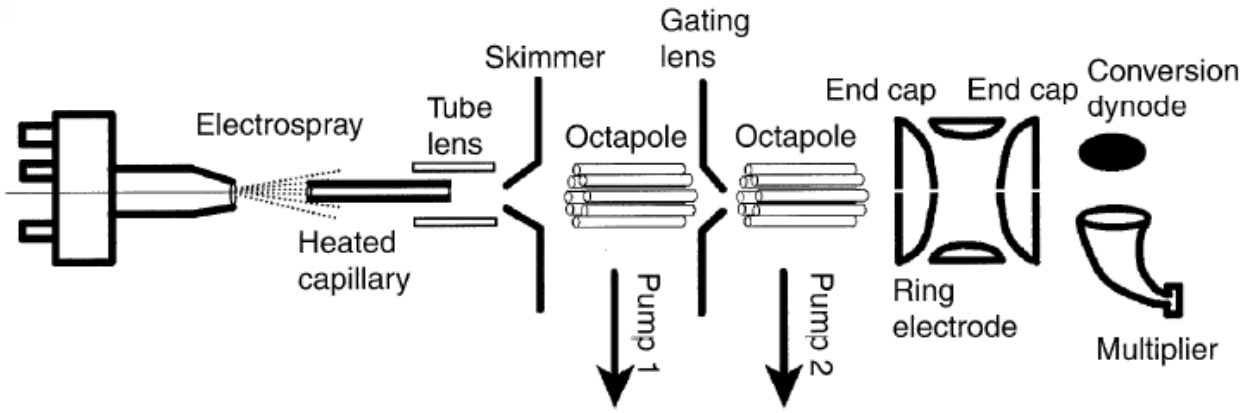
Ions can be mass-selected → up to MS^{12}

Mass-selected ions can be collided with buffer gas and their fragmentations can be studied

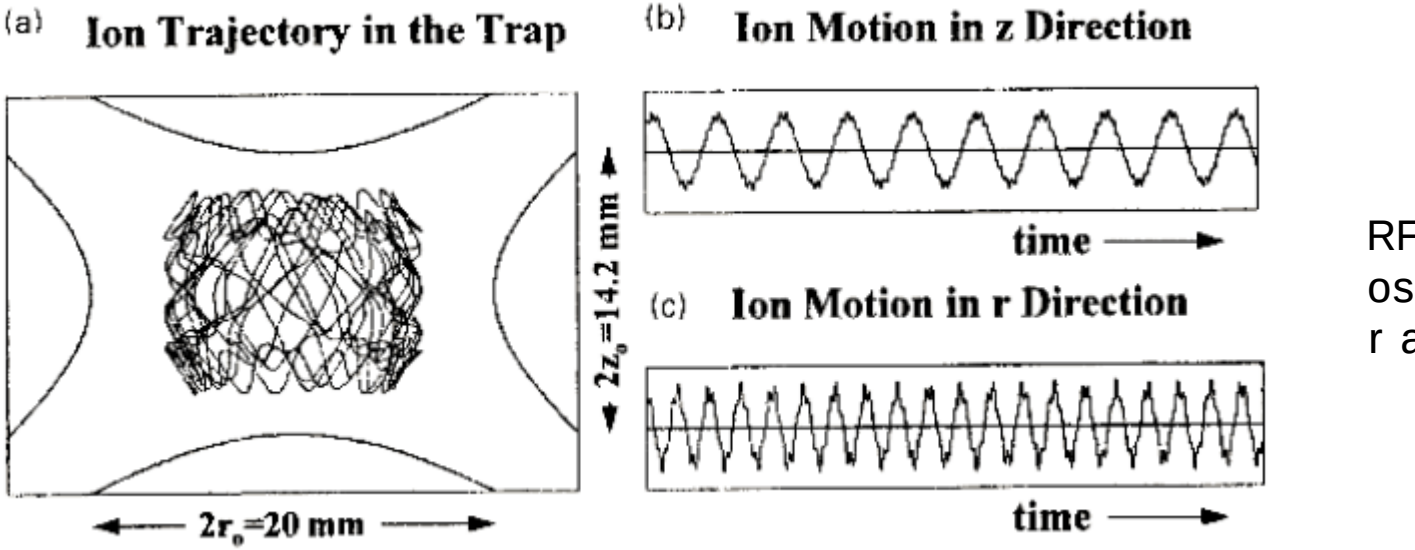
Quadrupole ion traps



- Fundamental RF at the ring electrode
  - Fixed frequency (1,1 MHz), Variable amplitude (do 7 kV)
- AC: voltage with fixed frequency at the end-cap electrodes
  - Resonance excitation for ejection or fragmentation
- Helium pressure ~1 mTorr



Ions motion inside the trap



From Lambert

RF field induces oscilations along r and z axis

Stability diagram

- Stability of ion trajectories affected by combination of AC and DC → mostly DC is set to zero

- For zero DC, stability given by q_Z:

$$q_Z = \frac{8ezV}{m(r_0^2 + 2z_0^2)(2\pi\upsilon)^2}$$

- Stable trajectories up to q_Z = 0.908

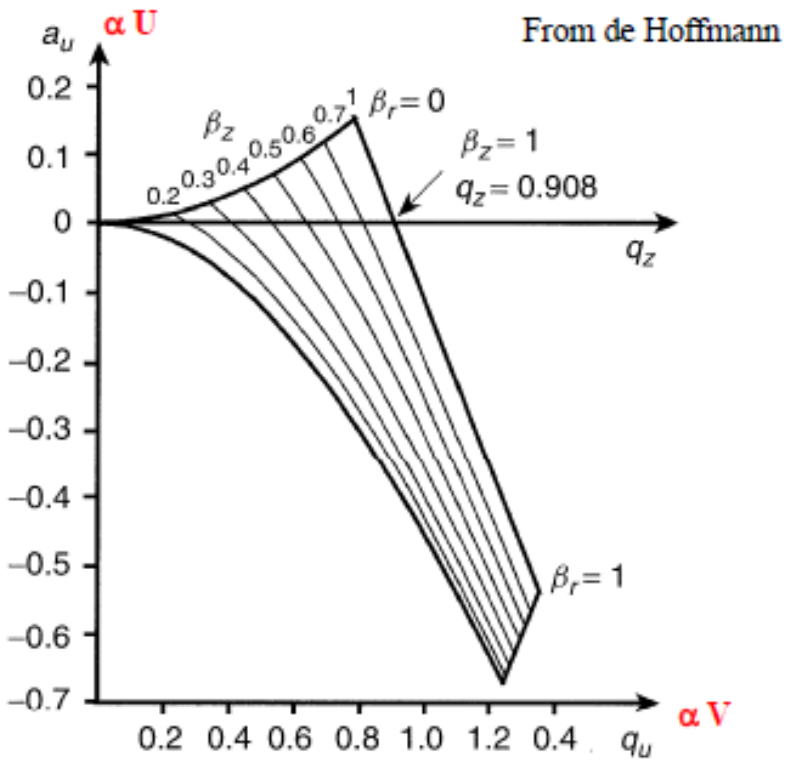


Figure 2.16
Typical stability diagram for a quadrupole ion trap. The value at $\beta_z = 1$ along the q_z axis is $q_z = 0.908$. At the upper apex, $a_z = 0.149998$ and $q_z = 0.780909$. (Data from Ref.12)

Ejection of ions

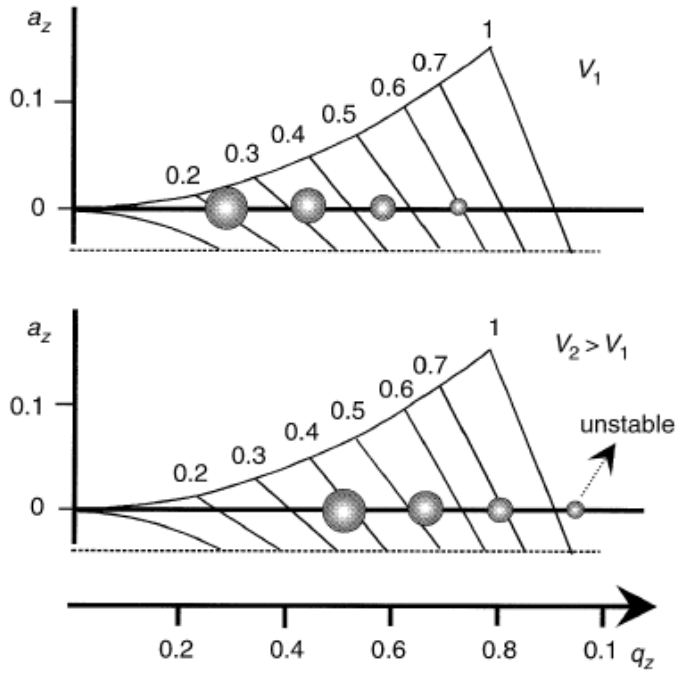


Figure 2.20
At a fixed value of the RF potential V applied to the ring electrode, heavier ions will have lower β_z values and thus lower secular frequencies. If V is increased, β_z values increase for all the ions, as do the secular frequencies. In the example given, the lightest ion now has a β_z value larger than unity and is thus expelled from the trap. The highest mass that can be analyzed depends on the limit V value that can be applied: around 7000–8000 V from zero to peak. For a trap having $r_0 = 1$ cm and operating at a ν frequency of 1.1 MHz, the highest detectable mass-to-charge ratio is about 650 Th

$$q_z = \frac{8ezV}{m(r_0^2 + 2z_0^2)(2\pi\upsilon)^2}$$

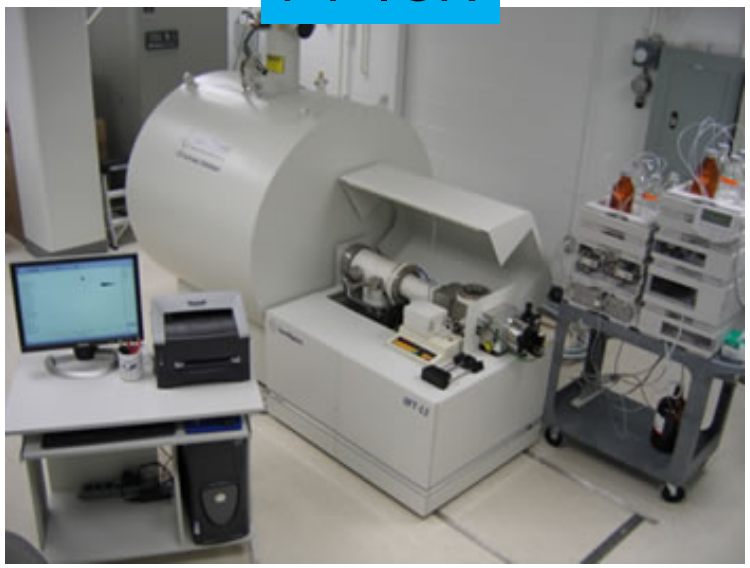
- With increasing V → larger and larger m/z beyond q_Z = 0.908
- Pressure determines the highest V (discharges)

Many other ions traps with similar properties

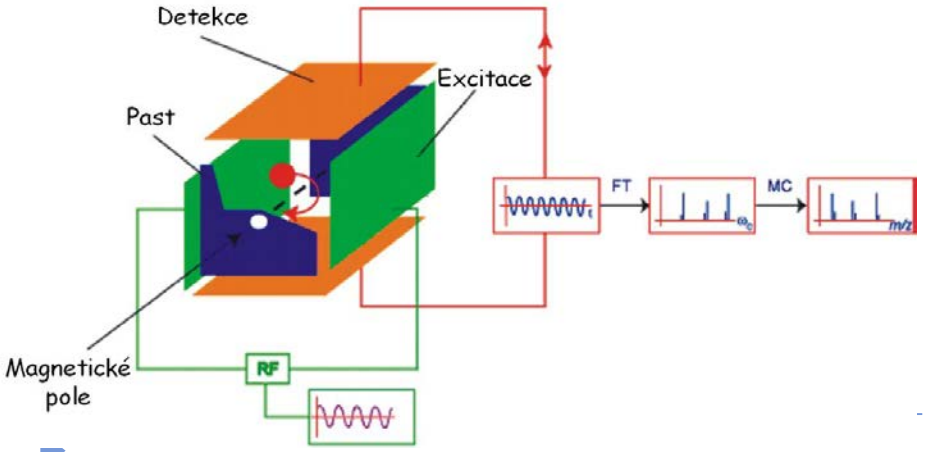
- Linear quadrupole traps
- Higher multipole traps

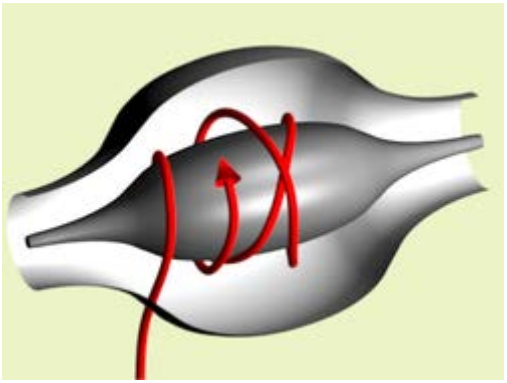
High resolution

FT ICR

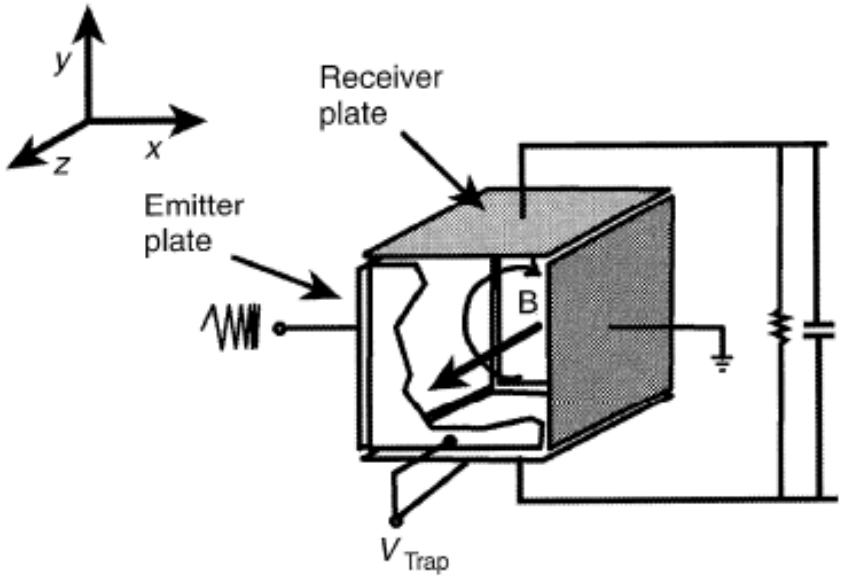


Orbitrap





Ion cyclotron resonance (ICR)



From Hoffmann

Figure 2.50
Diagram of an ion cyclotron resonance instrument. The magnetic field is oriented along the *z*-axis. Ions are injected in the trap along the *z*-axis. They are trapped along this axis by a trapping voltage, typically 1 V, applied to the front and back plates. In the *x*,*y* plane, they rotate around the *z*-axis due to the cyclotronic motion and then go back along the *z*-axis between the electrostatic trapping plates. The sense of rotation indicated is for positive ions. Negative ions will orbit in the opposite direction

FT-MS

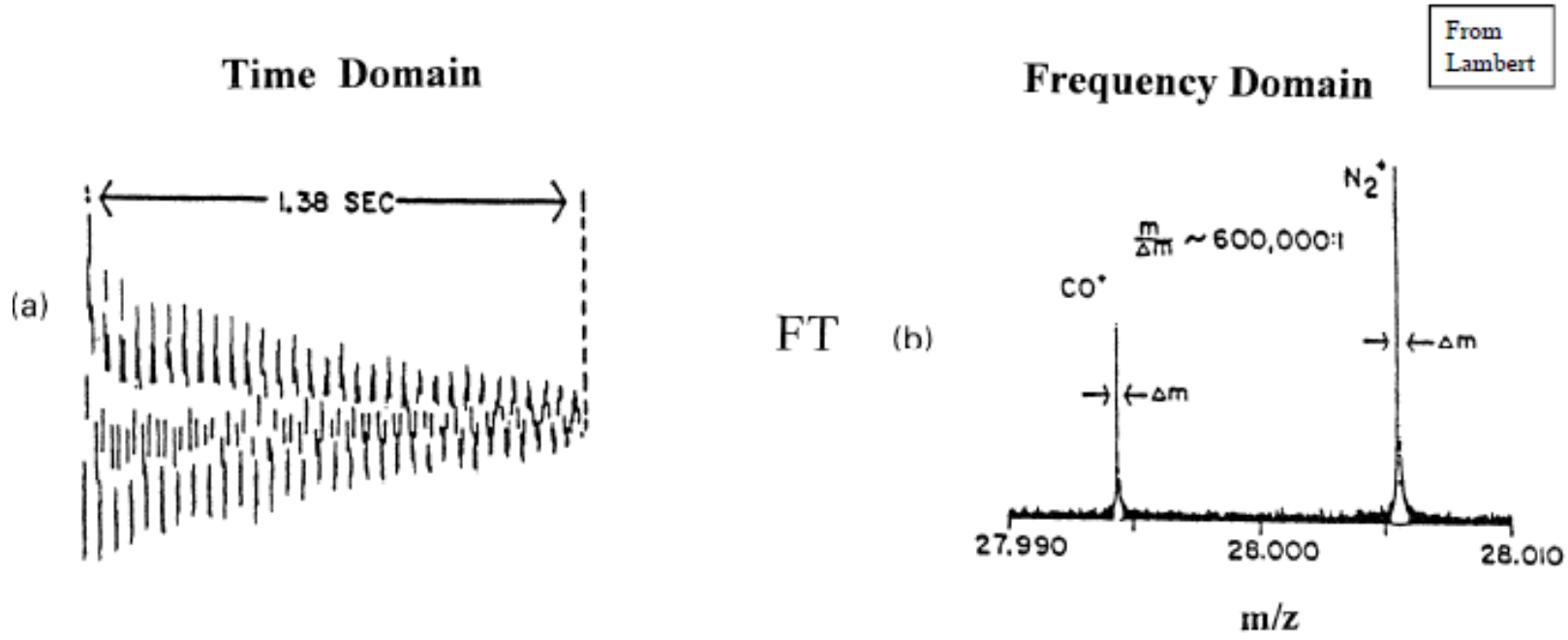


Figure 13-25 (a) Time domain signal (transient) recorded for a mixture of $CO^{+\cdot}$ and $N_2^{+\cdot}$ ions of nominal *m/z* 28 and (b) the corresponding frequency (mass) domain signal. (Courtesy of A.G. Marshall.)

http://www.youtube.com/watch?v=a5aLlm9q-Xc&feature=related

Instruments with highest resolution

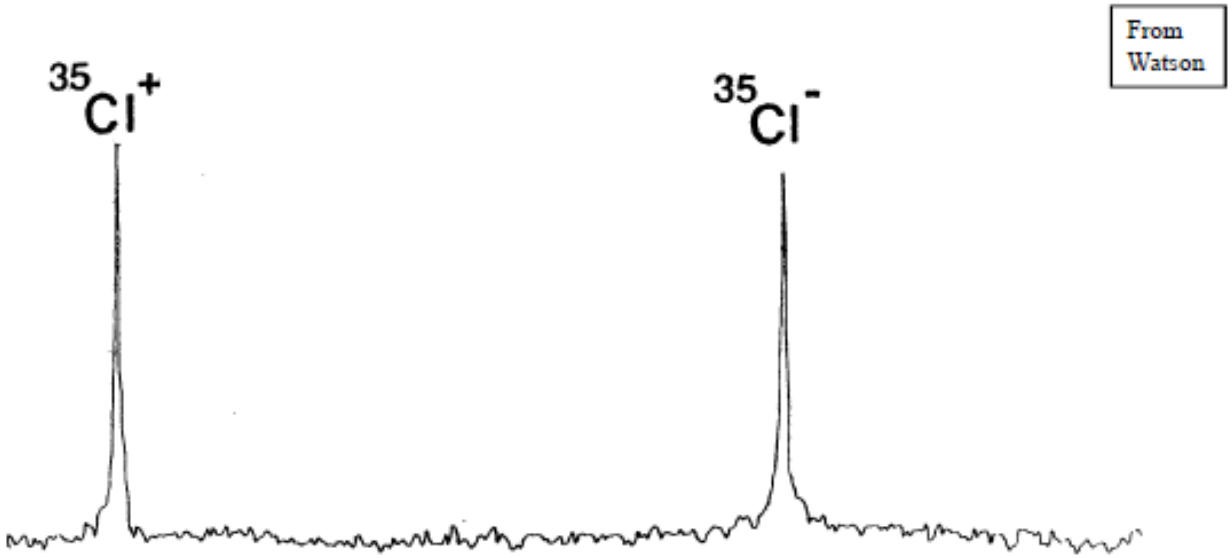
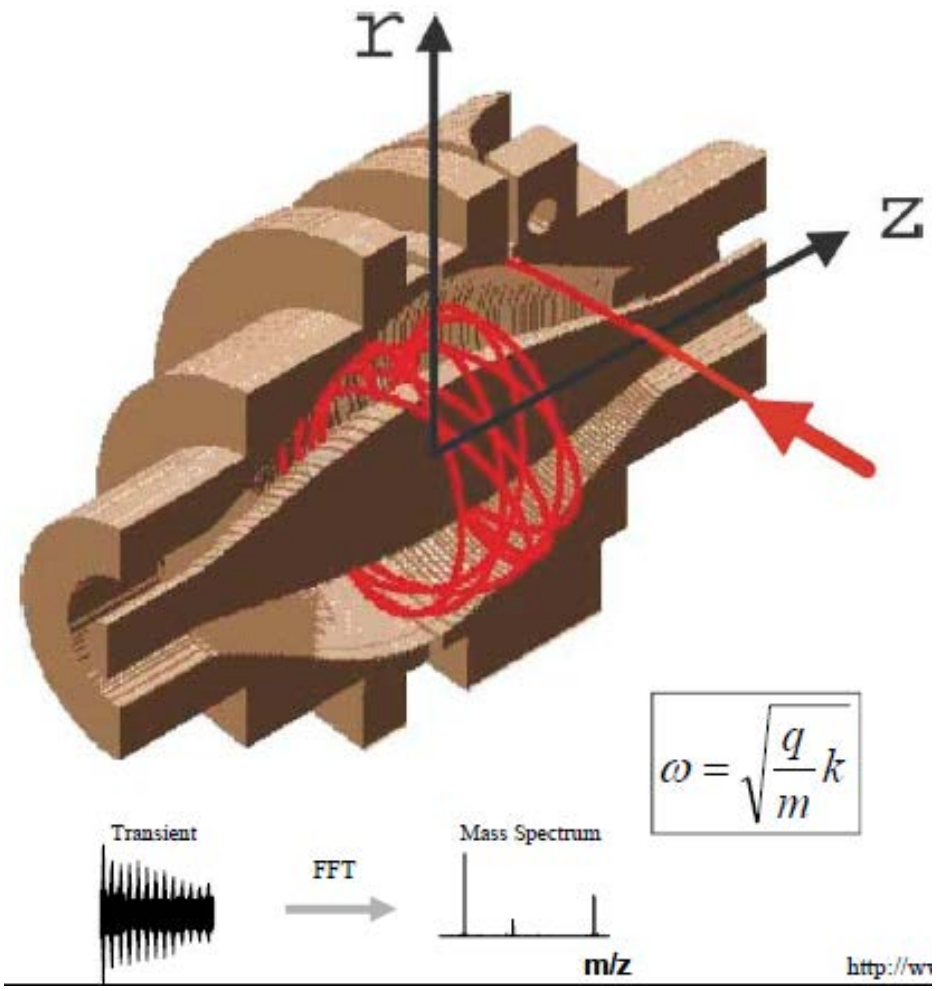


FIG. 4.22. Segment of mass spectrum in region of nominal mass 35 showing a resolution greater than 1,000,000 (FWHM definition) when using FT-MS. The peaks represent the positive and negative ions of ^{35}Cl that have a difference in mass equivalent to the mass of two electrons. The spectrum was obtained using a FT-ICR mass spectrometer with a superconducting magnet (4.7 tesla); the instrument was switched from the positive-ion-detection mode to the negative-ion-detection mode during the scan between the two peaks (Courtesy of Spectrospin AG.)

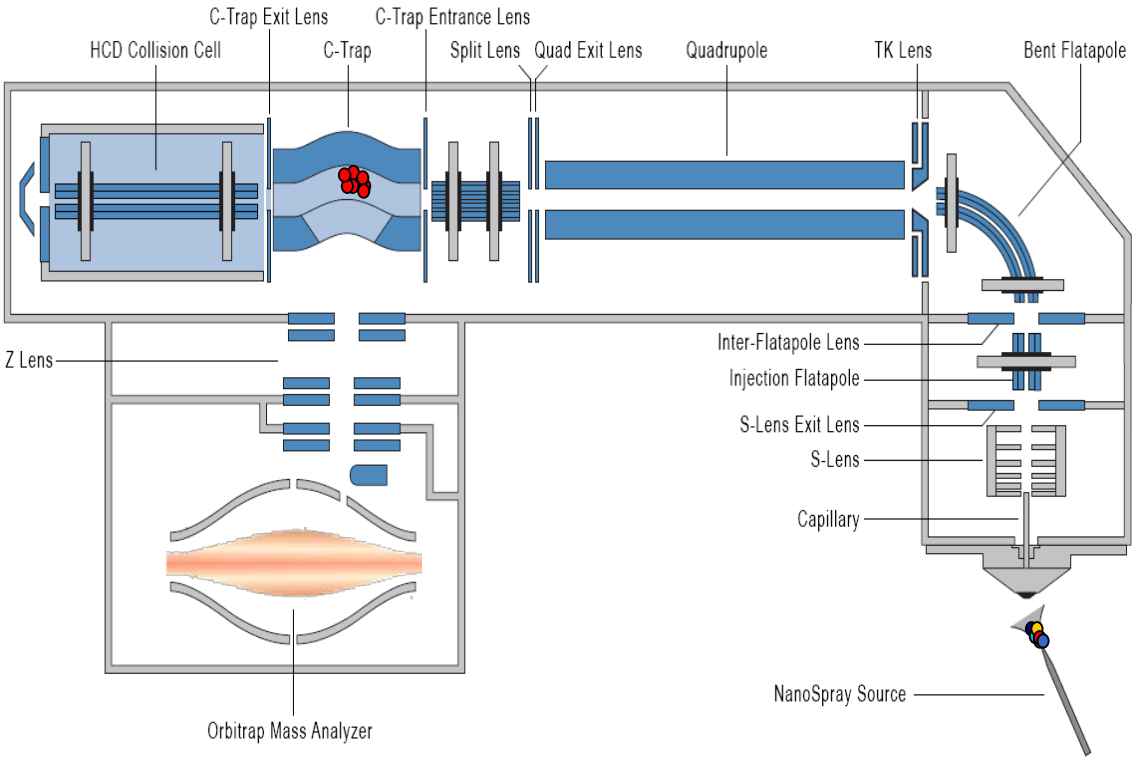
Orbitrap



- Ions introduced perpendicular to the z-axis (red arrow)
- Distance of the entrance point to z = 0 determines potential energy along z-axis

Perspective: The Orbitrap: a new mass spectrometer. Q. Hu, R.J. Noll, H. Li, A. Makarov, M. Hardman, R. Graham Cooks. *J. Mass Spec.*, 40(4): 430 – 443, 2005.

Orbitrap



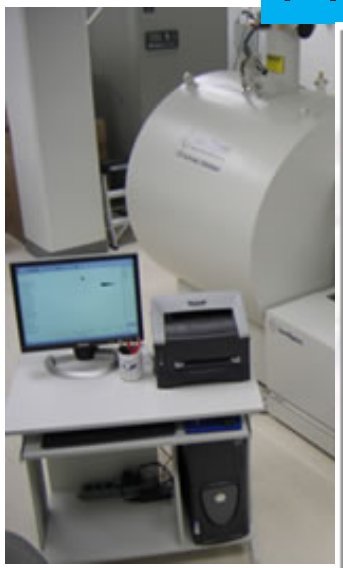
- High precission (1 – 2 ppm)
- High mass resolutions (up to 200 000)
- High dynamic range (~ 5000)

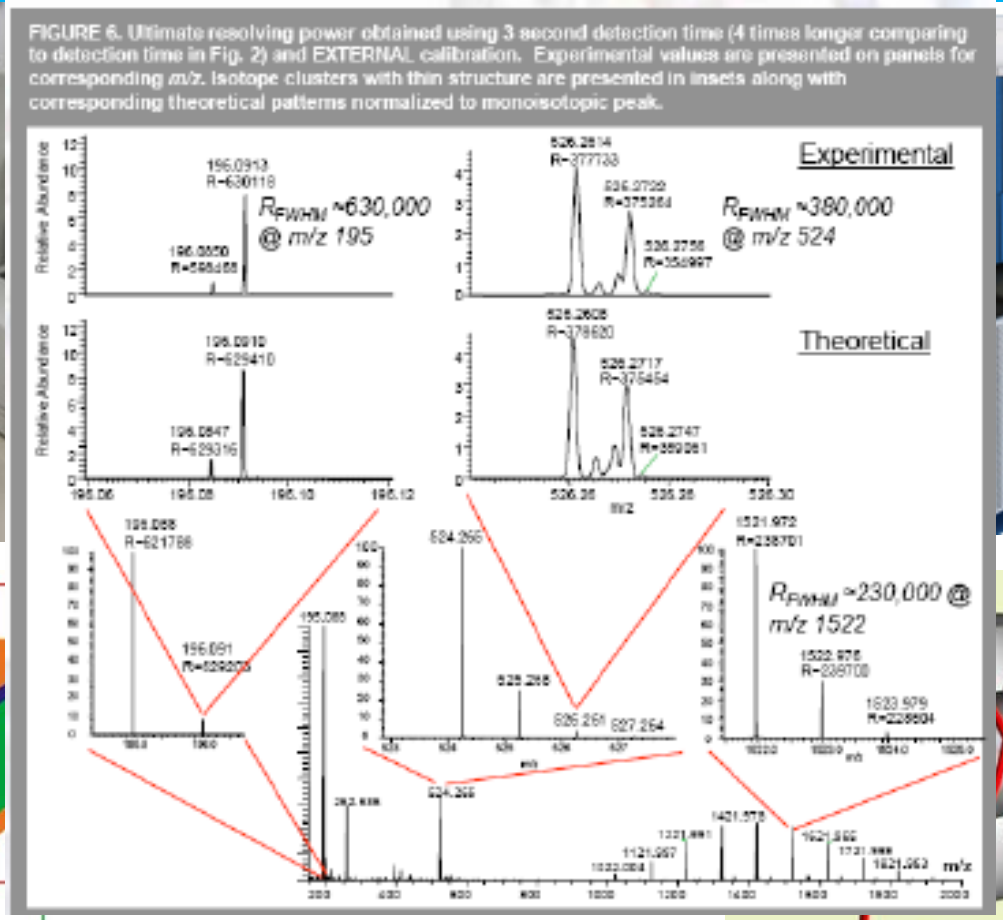
http://planetorbitrap.com/q-exactive

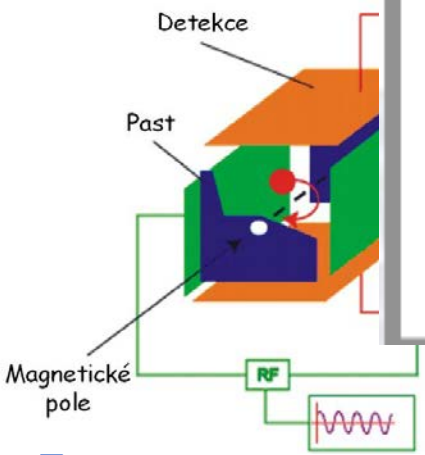
High resolution

FT ICR

Orbitrap







What is the origin of the large success of MS?

- New ionization methods (ESI, direct ESI, MALDI, atd.)
- MS plays an important role in elemental analysis and it will probably grow further
- High resolution analyzers will be standard
- Instruments are getting smaller, user friendlier, and more robust
- Combination of mass spectrometry with ion mobility will be more and more important
- Development in informatics will simplify data evaluation in all fields of MS application
- Combination of different MS techniques with other methods will be developed and could contribute to new applications (optical methods, biochemical methods, EPS)