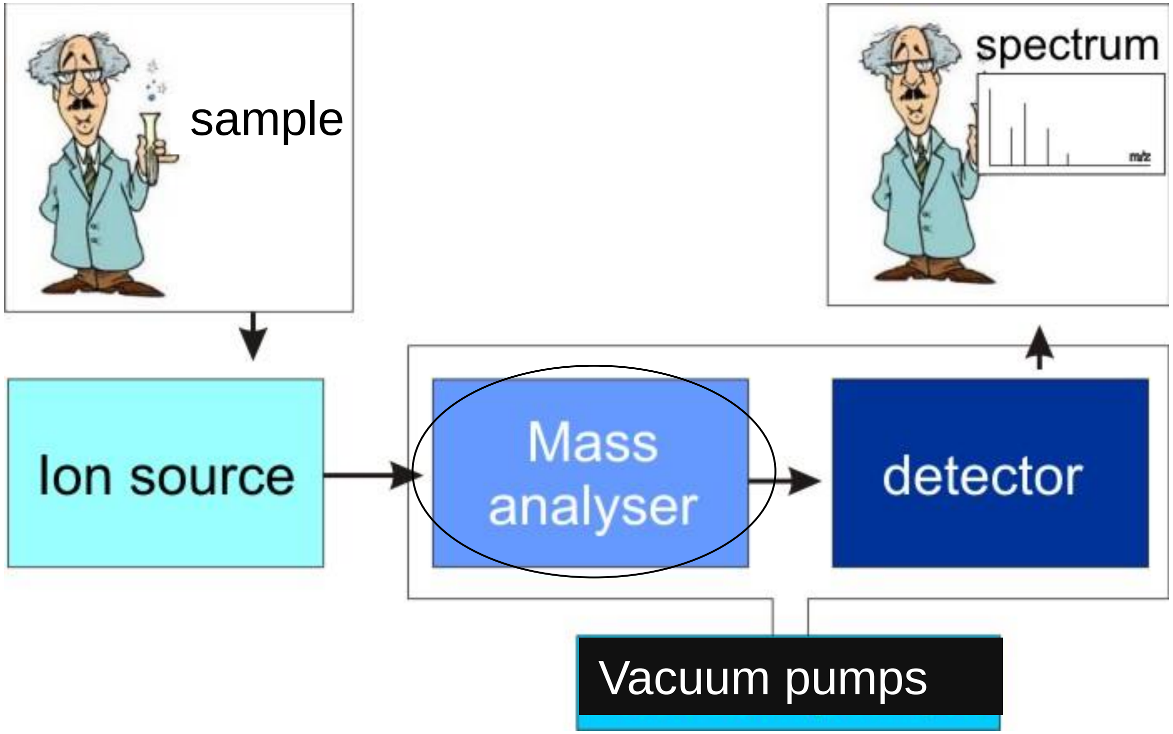
ION ANALYZERS

Mass analyser



- Mass analysers - separate the ions according to their mass-to-charge ratio

MASS ANALYSER

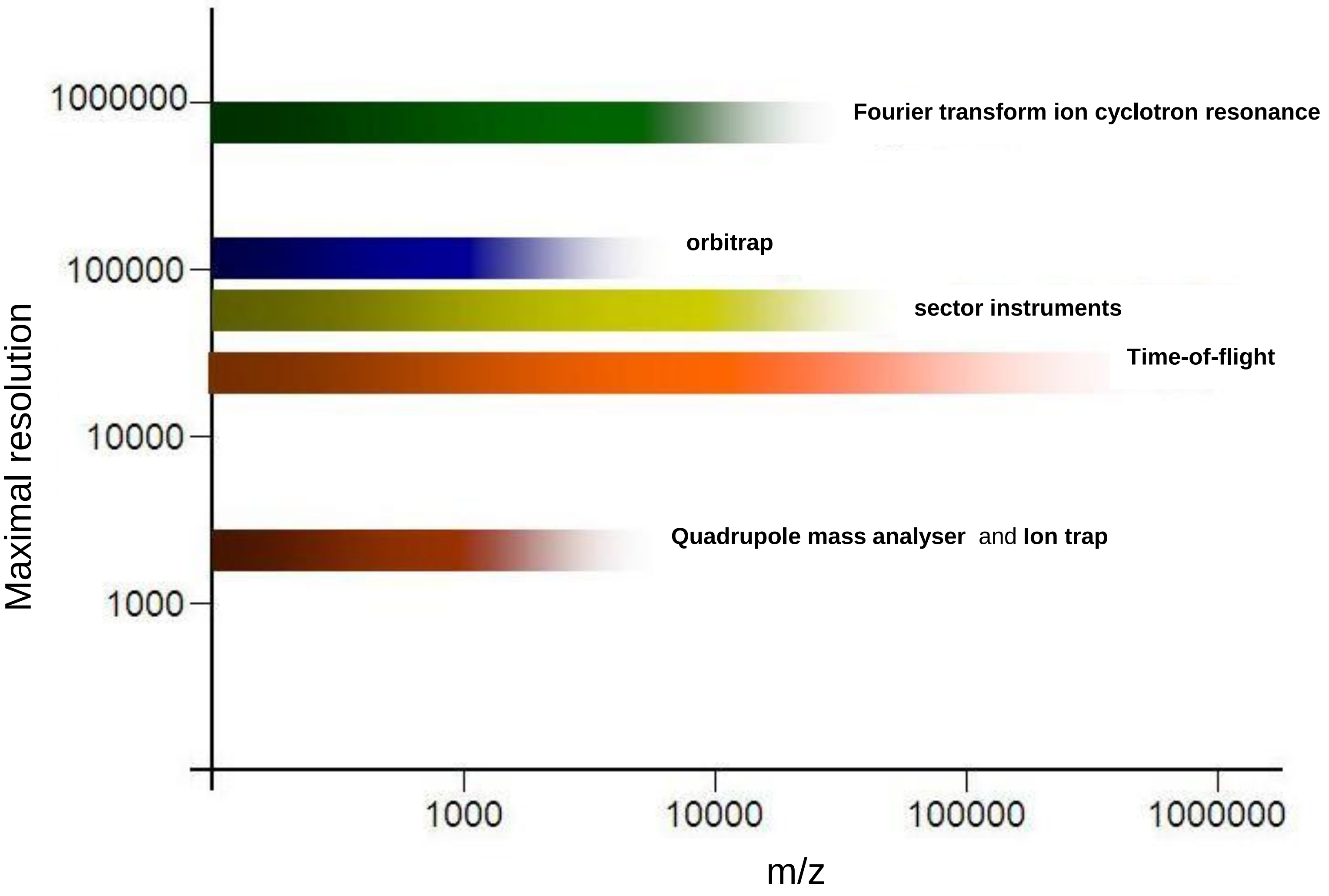
Separate the ions according to their mass-to-charge ratio in space or time

- Magnetic Sector (MAG)
- Electrostatic Sector (ESA)
- **Time-of-flight** (TOF)
- **Quadrupole mass analyser** (Q)
- **Ion trap (IT)**
  - **Three-dimensional quadrupole ion trap (3D)** (QIT)
  - **Linear ion trap (2D)** (LIT)
- **Fourier transform analzyers**
  - **Fourier transform ion cyclotron resonance** (FT-ICR-MS)
  - **Orbitrap** (FT-Orbi, FT-O)

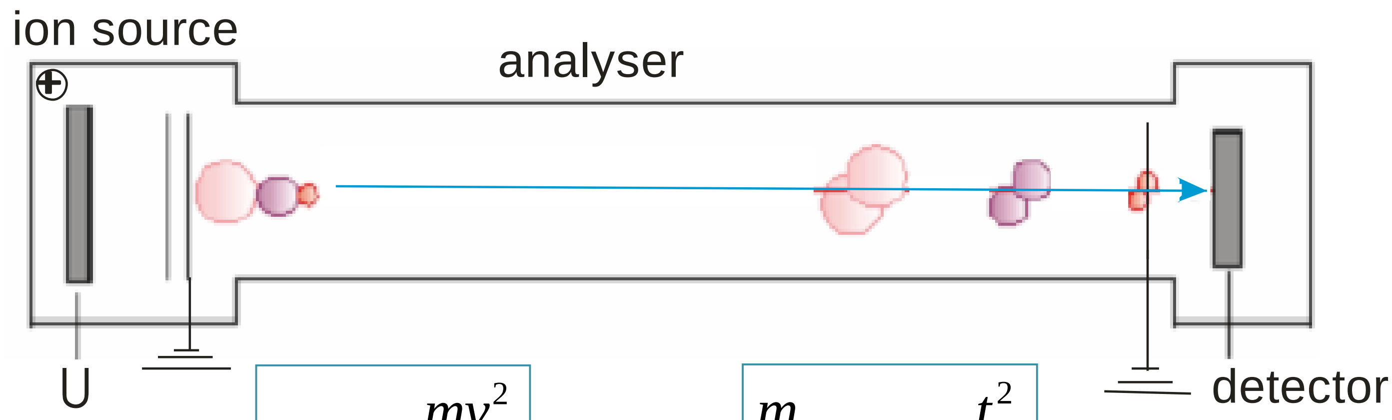
- Tandem mass spectrometry (MS/MS or MS^n)
  - fragmentation of analyte

- **Ion mobility –** separate and identify ionized molecules in the gas phase based on their mobility in a carrier buffer gas
  - Based on an ion's **mass, charge, size** and **shape**

MASS ANALYSERS



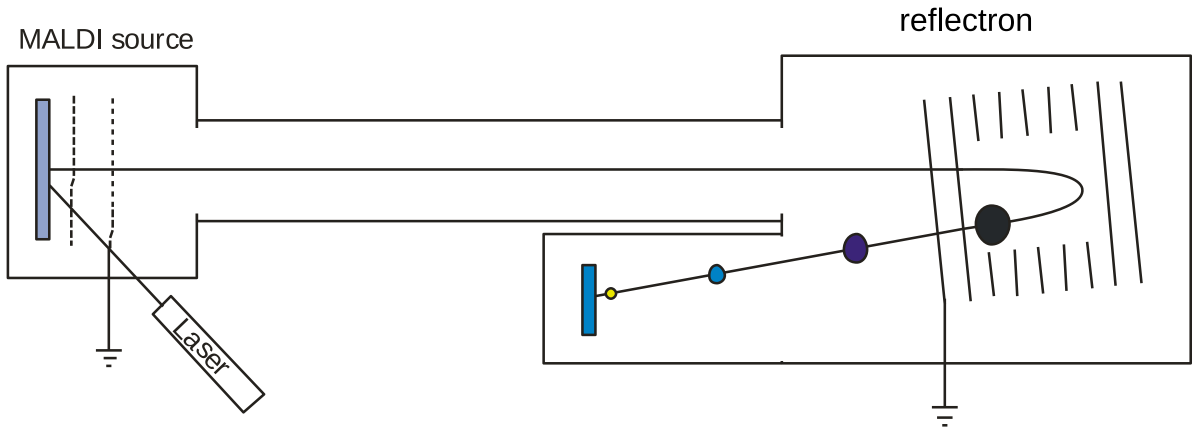
TIME-OF-FLIGHT (TOF)



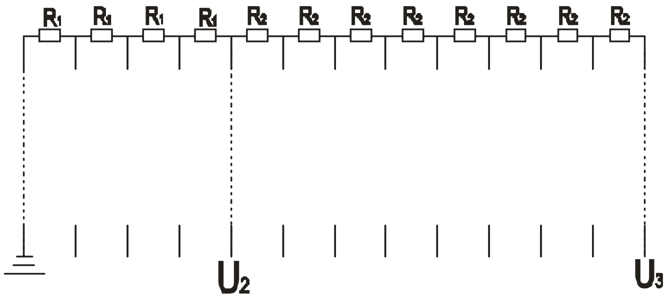
$$\boxed{zeU = \frac{mv^2}{2}} \longrightarrow \boxed{\frac{m}{z} = 2eU\frac{t^2}{L^2}}$$

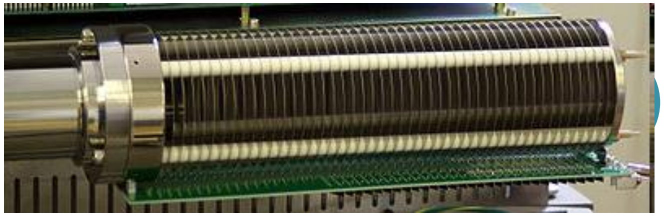
- Ions are accelerated by an electrostatic field - travel over a drift path to the detector
  - Measuring the flight time for each ion allows the determination of its mass
- Resolution depends on the length of the path
- Major advantages are
  - The extremely high transmission
  - The detection of all masses (all spectrum for each puls)
  - The theoretically unlimited mass range
- Suitable for MALDI (MALDI-TOF instruments)
- Can be use for accurate mass spectra

TOF WITH REFLECTRON



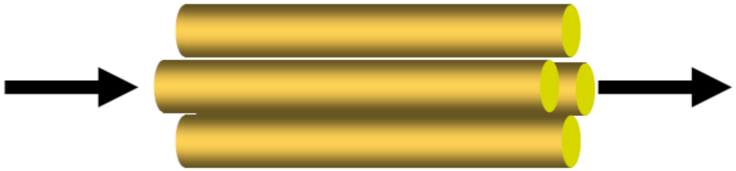
- The reflectron uses an electrostatic field to reflect the ion beam toward the detector.
  - Ring electrodes
- Advantage – better resolution
  - Longer path of ions
  - Focusing of ions in reflectron
- Disadvantage
  - Not suitable for protein – too long pass for large molecules

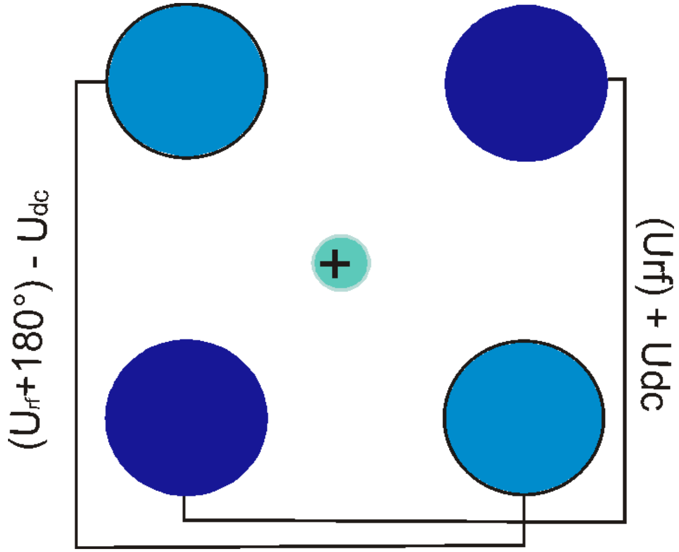





BRUKER
ultraflexXtreme

QUADRUPOLE MASS ANALYSER (Q)

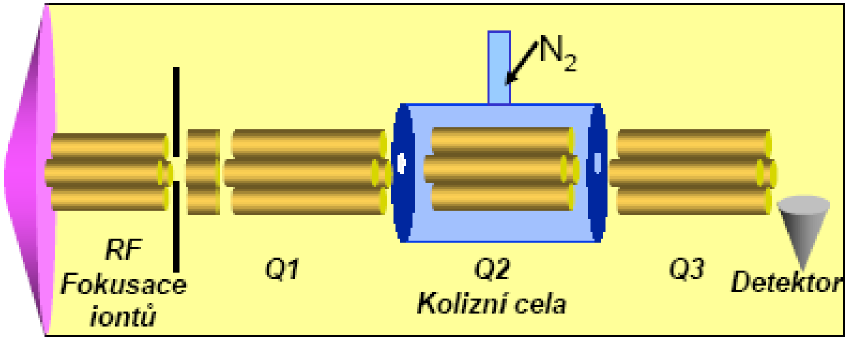


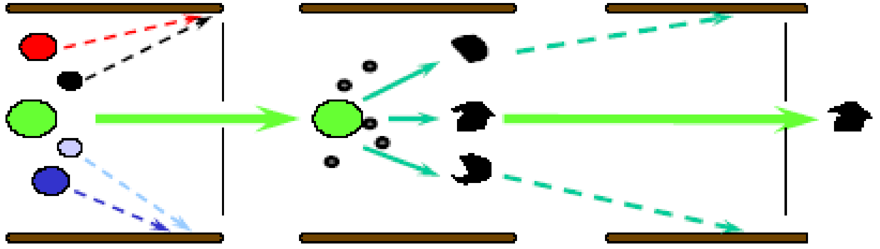


- Use oscillating electrical fields to selectively stabilize or destabilize the paths of ions passing through a radio frequency (U_{RF}) quadrupole field created between 4 parallel rods
  - Only the ions in a certain range of m/z are passed through the system at any time
- Limits m/z 2000 – 4000
- Low resolution spectra (not for accurate mass measurement)
- One Q can not be use for MS/MS

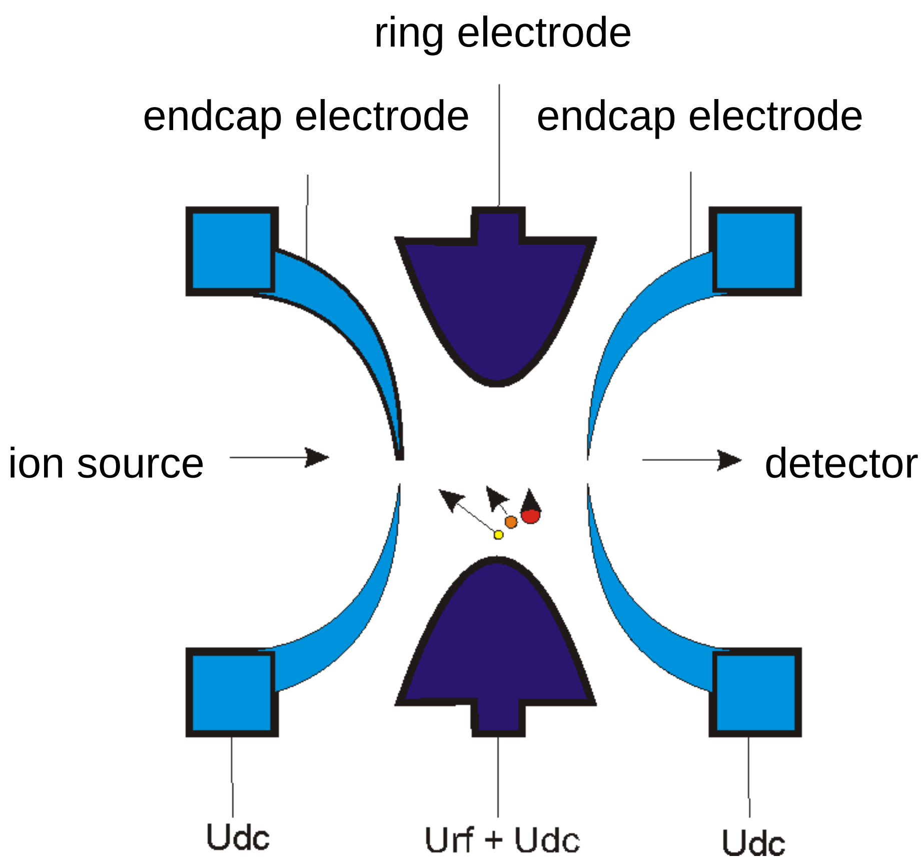
COLLISION-INDUCED DISSOCIATION (CID) IN COLLISION CELL

- QqQ
  - Q1 mass analyser can isolate one m/z (precursor ion)
  - **Q2 as a collision cell** - they collide with a gas (He, Ar) - they are fragmented.
  - Q3
    - Scan all fragment – identification of compound
    - Scan one or a few ions – **quantitative analysis**
- **CID**
  - **1-100eV (low energy)**
  - **multiple collisions**
  - **Instruments QQQ, IT, Q-TOF, …**
  - Proteomic - primarily *b*- and *y*-type of fragment





THREE-DIMENSIONAL (QUADRUPOLE) ION TRAP (IT)



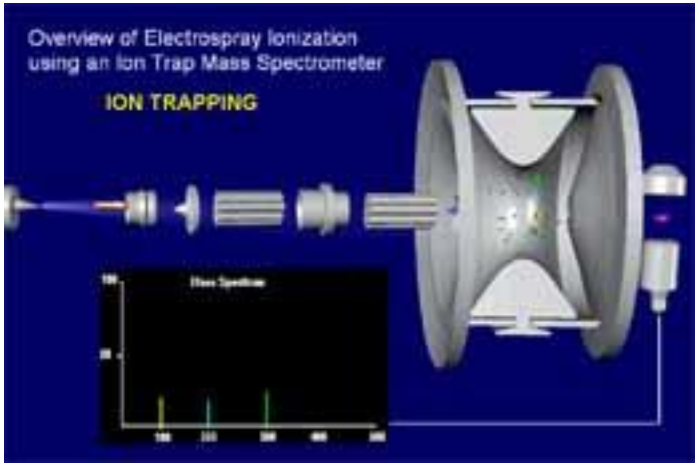
- The ions enter into the trap through the inlet and they are trapped through action of the three hyperbolic electrodes.
- The ions are in a stable oscillating trajectory
- The ions are ejected in order of increasing *m/z* by a gradual change in the potentials


Thermo
LCQ FLEET

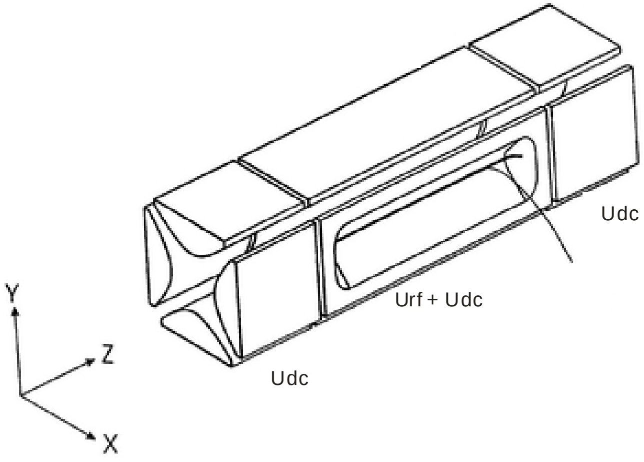
Ion trap (IT)

- Possibility MS/MS (CID) (in real applications MS^3)
  - Rule 30:70 – ions at low 30% of m/z range are not stabile in ion trap – lose information
- Limits m/z 2000 – 4000
- Low resolution spectra (not for accurate mass measurement)
- **Lineat ion trap**
  - **2D ion trap** (LIT) better sensitivity, resolution, capacity and scanning faster

3D

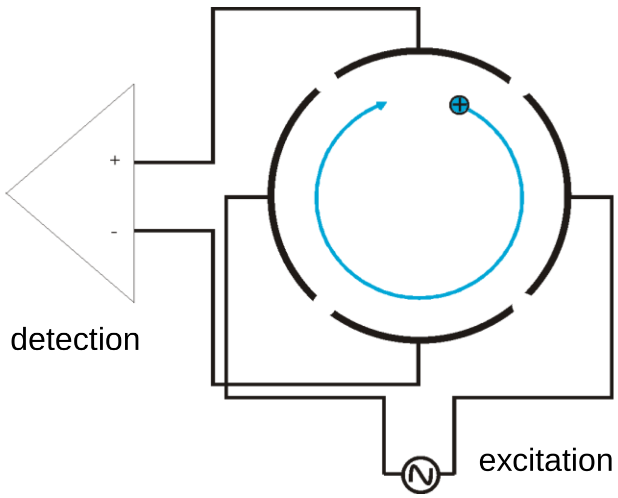


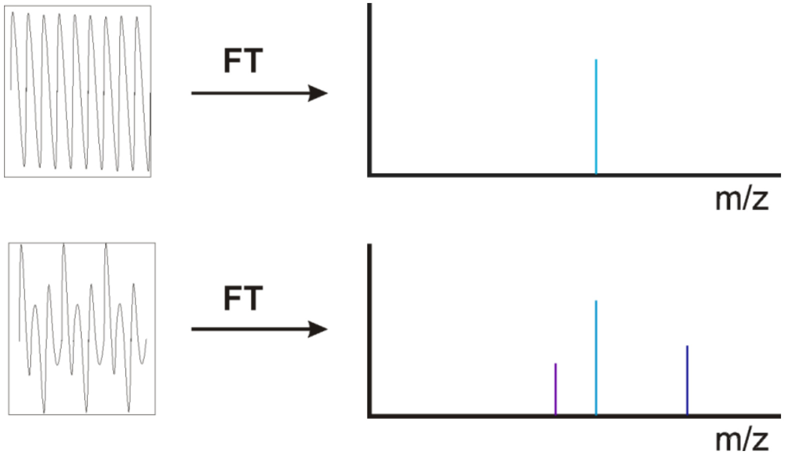
2D



Fourier transform ion cyclotron resonance (FT-ICR -MS)

- Based on the circular movement of charged particles in a strong magnetic field (cyclotron movement)
  - The cyclotron frequency depends directly on the mass-to-charge ratio of the ions

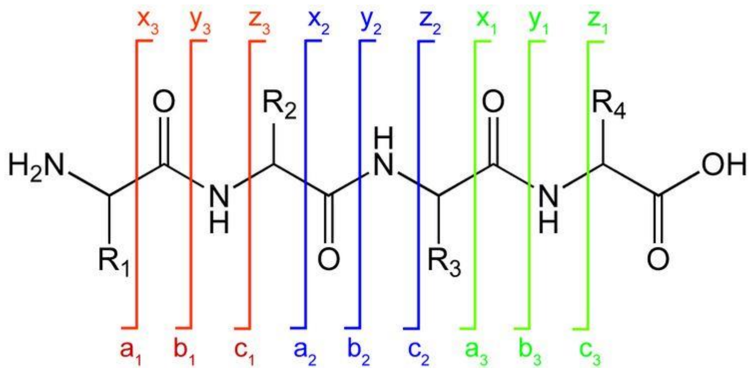




$$\omega = \frac{v}{r} = \frac{Be}{m/z}$$

- Detector electrodes measure the electrical signal of ions which pass near them over time, producing a periodic signal

Fourier transform ion cyclotron resonance (FT-ICR-MS)



- Advantage
  - High accuracy (about 1 ppm)
  - High resolution (900 000)
  - Possible measured of MS^n (CID, ECD, IRMPD)

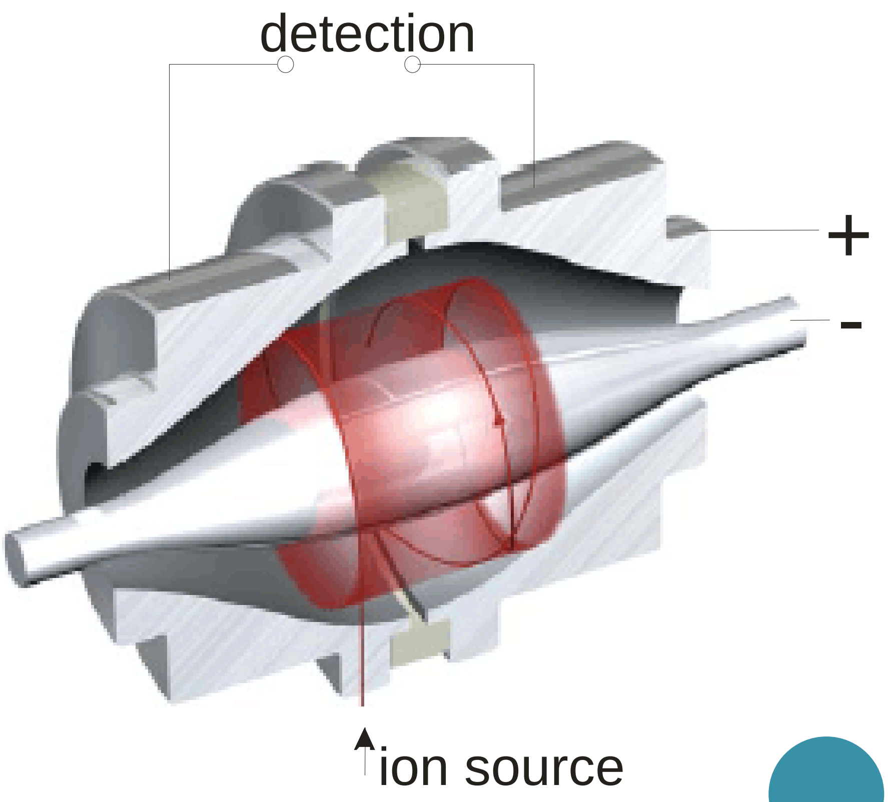
  - MS/MS on FT-ICR for proteomic
    - Electron capture dissociation (ECD)- by capturing a thermal electron
      - Proteomic aplicatio- primarily *c*- and *z*- type of fragments

$[M+nH]^{n+} + e^{-} \rightarrow [[M+nH]^{(n-1)+}]^{*} \rightarrow$ Fragments

    - Infrared multiphoton dissociation (IRMPD) - by IR laser
      - Proteomic application- primarily *b*- and *y*- type of fragment

ORBITRAP

- Similar principle to FT-ICR-MS
- The Orbitrap is an ion trap – but there are not RF or magnet fields!
- Ions in orbitrap
  - Moving around a central electrode
  - **Moving in *z* axis**
  - Detector electrodes measure the electrical signal of ions



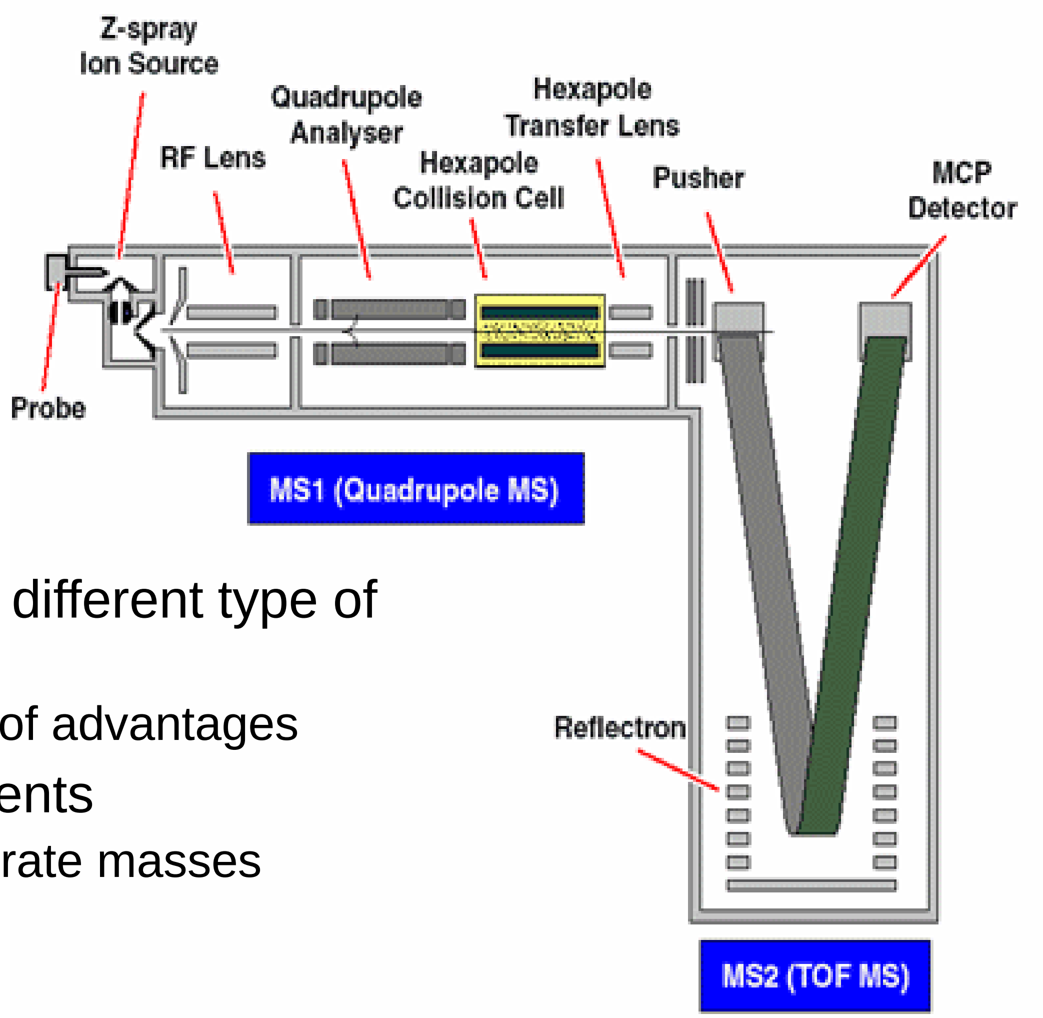
ORBITRAP

- Advantage
  - High accuracy (about 1 ppm)
  - High resolution (our instrument 100 000)
    - New generation of instrument 250 000
  - Does not need magnet
    - the most expensive part of ICR instrument

- Electron-transfer dissociation
    - ETD does not use free electrons but employs radical anions (e.g. fluoranthene, anthracene, azobenzene,.....)
    - Proteomic - c- and z-type of fragments (similar to ECD)

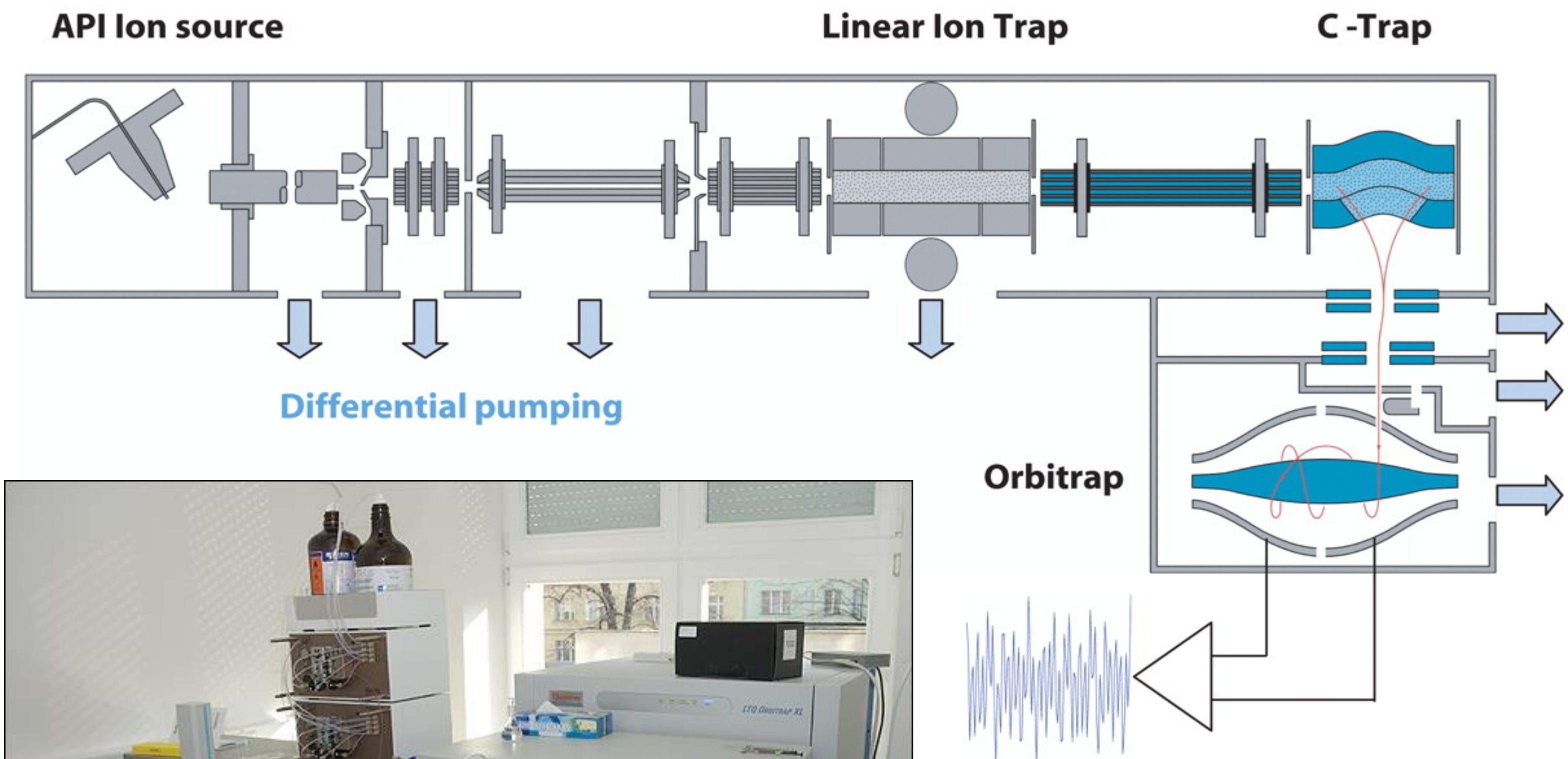
$[M+nH]^{n+} + Y^{-\bullet} \rightarrow [M+nH]^{(n-1)+\bullet} \rightarrow$ Fragments

HYBRID MASS SPECTROMETERS



- Combination of different type of analysers
  - Combination of advantages
- Q-TOF instruments
  - MS/MS, accurate masses
- LIT-Orbitrap
- Q-Trap
- ……..

Linear Ion Trap Orbitrap Hybrid MS

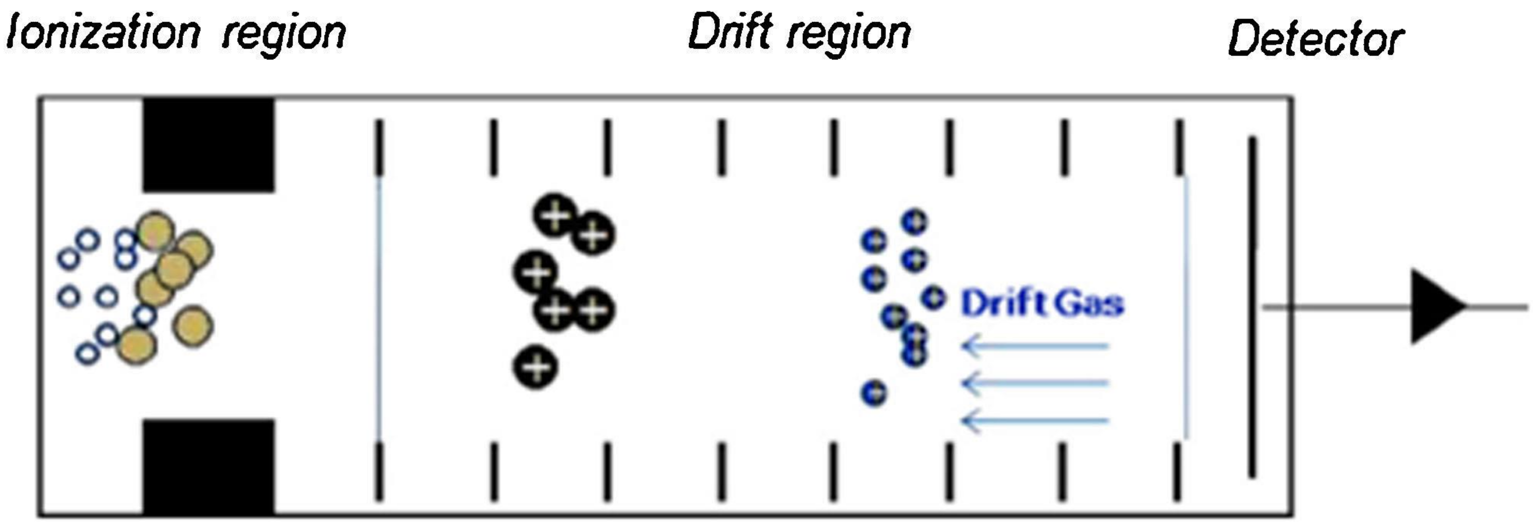


Ion mobility

- **Ion mobility –** separate ions in the gas phase based on their mobility in a carrier buffer gas
  - Based on an ion's **mass, charge, <u>size</u>** and **<u>shape</u>**
- **Ions migrate in an electrical field against the direction of a carrier gas**
  - Ambient or reduced pleasure
    - Buffer gas - He, Ar, N_2 or CO_2
  - Separation of ions in gas phase
- **Ion mobility techniques coupled with MS**
  - Drift Tube Ion Mobility Spectrometry (DT-IMS)
  - Differential Mobility Spectrometry (DMS), Field Asymmetric ion Mobility Spectrometry (FAIMS)
  - **Traveling Wave Ion Mobility Spectrometry** (TWIMS)

IMS-MS

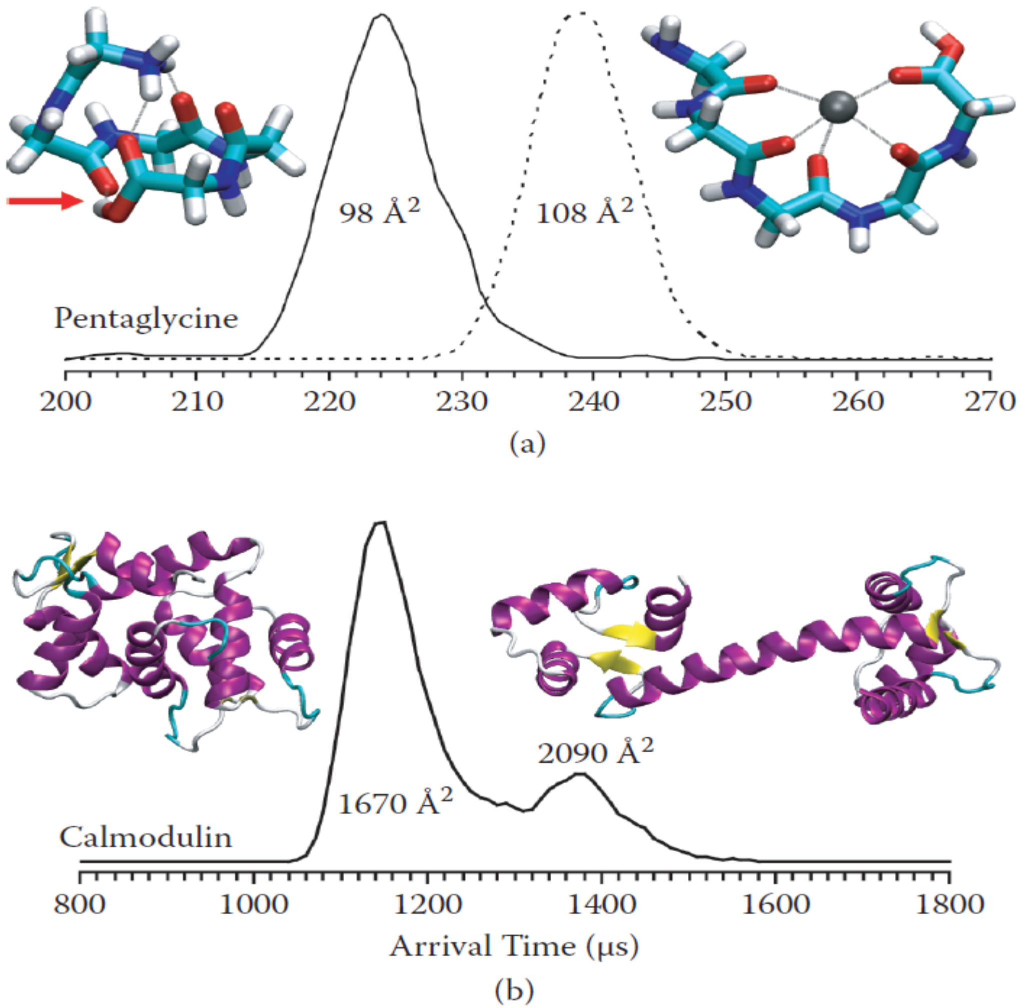
- $v = K\,E$
  - V….. drift velocity, K….. ion mobility, E…..electric field



- Collision cross section
  - Size and shape of ion

$$K = f\left(\frac{1}{\Omega}\right)$$

Example



$[GGGGG + Na]^+$ ———

$[GGGGG + H]^+$ - - - - - - -