Introduction to mass spectrometry: protein/peptide vaporization and mass analyzers

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Goals of biologists in 2003 (1)

- **To know which proteins are expressed in each cell, preferably one cell at a time**

- **Major analytical challenges**
  - **Sensitivity - no PCR reaction for proteins**
  - **Larger number of protein forms than open reading frames**
  - **Huge dynamic range (10^9)**
  - **Spatial and time-dependent issues**

Goals of biologists in 2003 (2)

- **To know how proteins are modified, information that cannot necessarily be deduced from the nucleotide sequence of individual genes.**

- **Modification may take the form of**
  - **specific deletions (leader sequences),**
  - **enzymatically induced additions and subsequent deletions (e.g., phosphorylation and glycosylation),**
  - **intended chemical changes (e.g., alkylation of sulfhydryl groups),**
  - **and unwanted chemical changes (e.g., oxidation of sulfhydryl groups, nitration, etc.).**

Goals of biologists in 2003 (3)

- **Protein structure and protein-protein interaction**
  - **to determine how proteins assemble in solution**
  - **how they interact with each other**
  - **Transient structural and chemical changes that are part of enzyme catalysis, receptor activation and transporters**

So, what do you need to know?

- **Substances have to be ionized to be detected.**
- **The net charge can be either positive or negative.**
- **The mass-to-charge ratio of an ion (*m/z*) is the most important parameter.**
- **The mass spectrometer is a selective detector (based on mass differences), but all the substances that are present in a sample and can be ionized can be measured.**

What do you need to know?

- **Polyionic buffer salts, particularly phosphate, interfere with ion formation in the electrospray ionization interface.**
- **Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) is very tolerant of the biomedical scientist.**
- **The mass spectrometer is always right.**

Over a 100 years of mass spectrometry

1886 Discovery of “canal rays” by Goldstein
1905 J.J. Thompson introduces the use of low pressure
1919 Francis Aston establishes isotopes of neon (20/22)
1931 Aston discovers U-235/U238 isotopes
1937 Aston notes the mass defect of elements up to fluorine - $e = mc^2$
1938 Hahn/Strassman observe uranium fission
1940 Nier begins isolation of U235 by mass spec
1943 Army takes over - Manhattan project (Lawrence)

Postwar - modern mass spectrometry begins

1952 First meeting of the ASMS

Biomedical Mass Spectrometry

Early work in mass spec concentrated on isotopes and isotope ratios ($^{2}H/^{1}H$, $^{13}C/^{12}C$ and $^{15}N/^{14}N$)

Rittenberg and Schoenheimer established many of the pathways of metabolism using these isotopes

The combination of gas chromatography and mass spectrometry was good for small molecules

BUT what about proteins, peptides and other heat labile molecules?

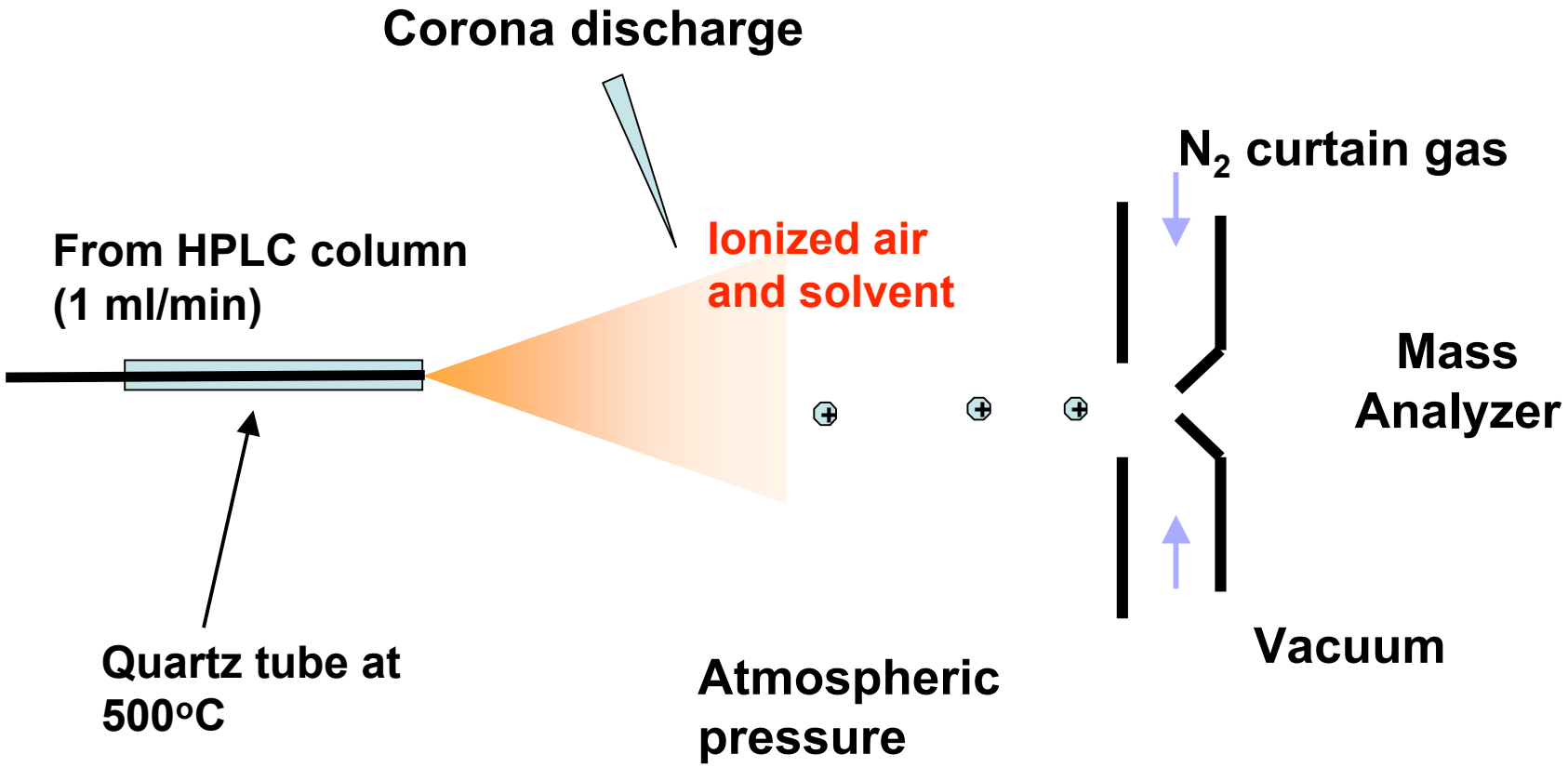
Outline

- **Interfaces and ion sources**
  - Electrospray ionization (ESI)
  - conventional and nanospray
  - Heated nebulizer atmospheric pressure chemical ionization
  - Matrix assisted laser desorption
- **Types of MS analyzers**
  - Magnetic sector
  - Quadrupole
  - Time-of-flight
  - Ion trap/FT-ICR
  - Hybrid

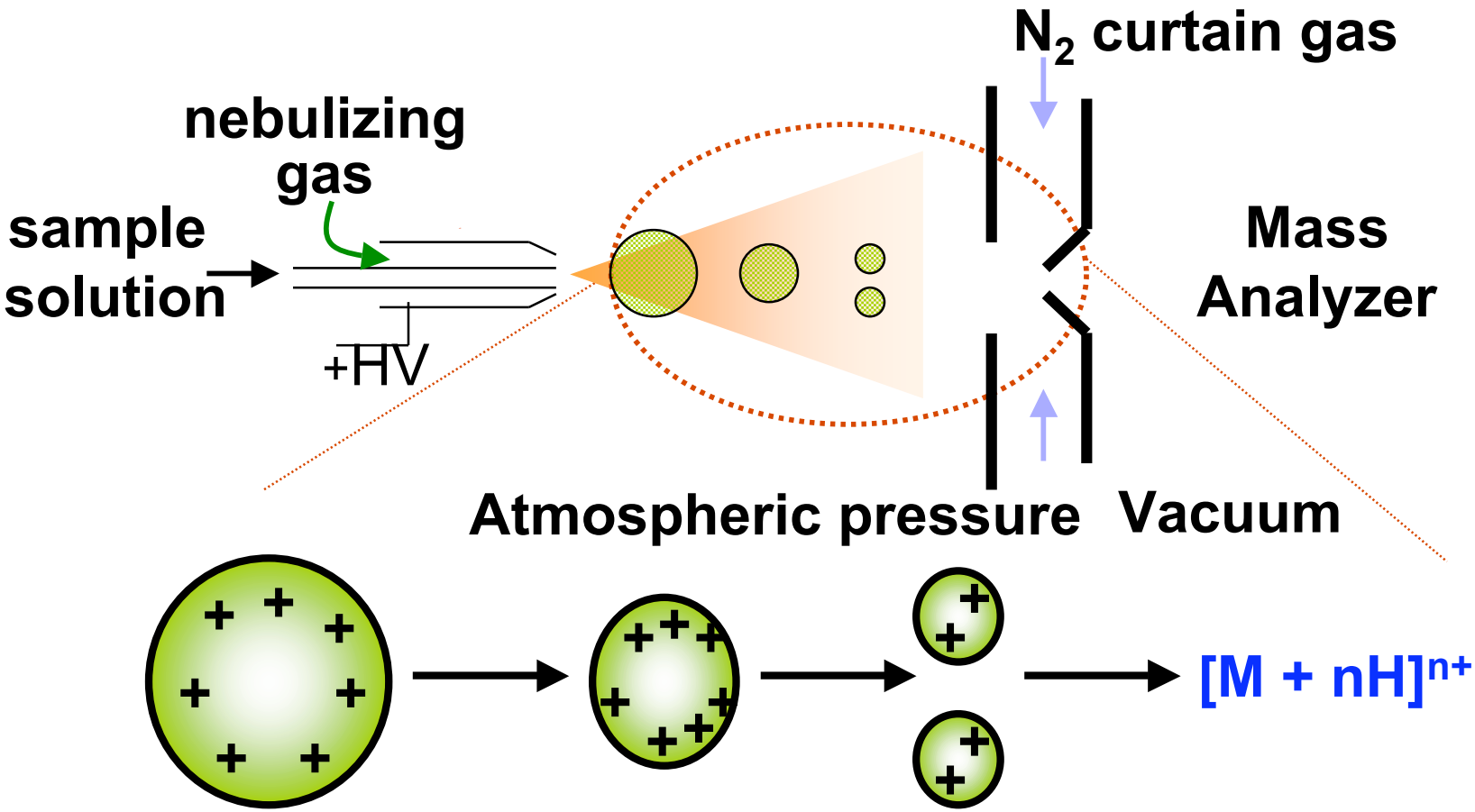
Interfaces and ion sources

- Direct insertion probe - probe heated
- GC-MS (Ryhage, Biemann) volatile derivatives, thermal decomposition, not good for either peptides or proteins
- Field desorption - on carbon fibers
- Sputtering - glycerol matrix for fast atom bombardment (FAB)
  Matrix-assisted laser desorption
- Spraying
  - Thermospray ionization
  - Heated nebulizer atmospheric pressure chemical ionization
  - Electrospray ionization

HN-APCI interface

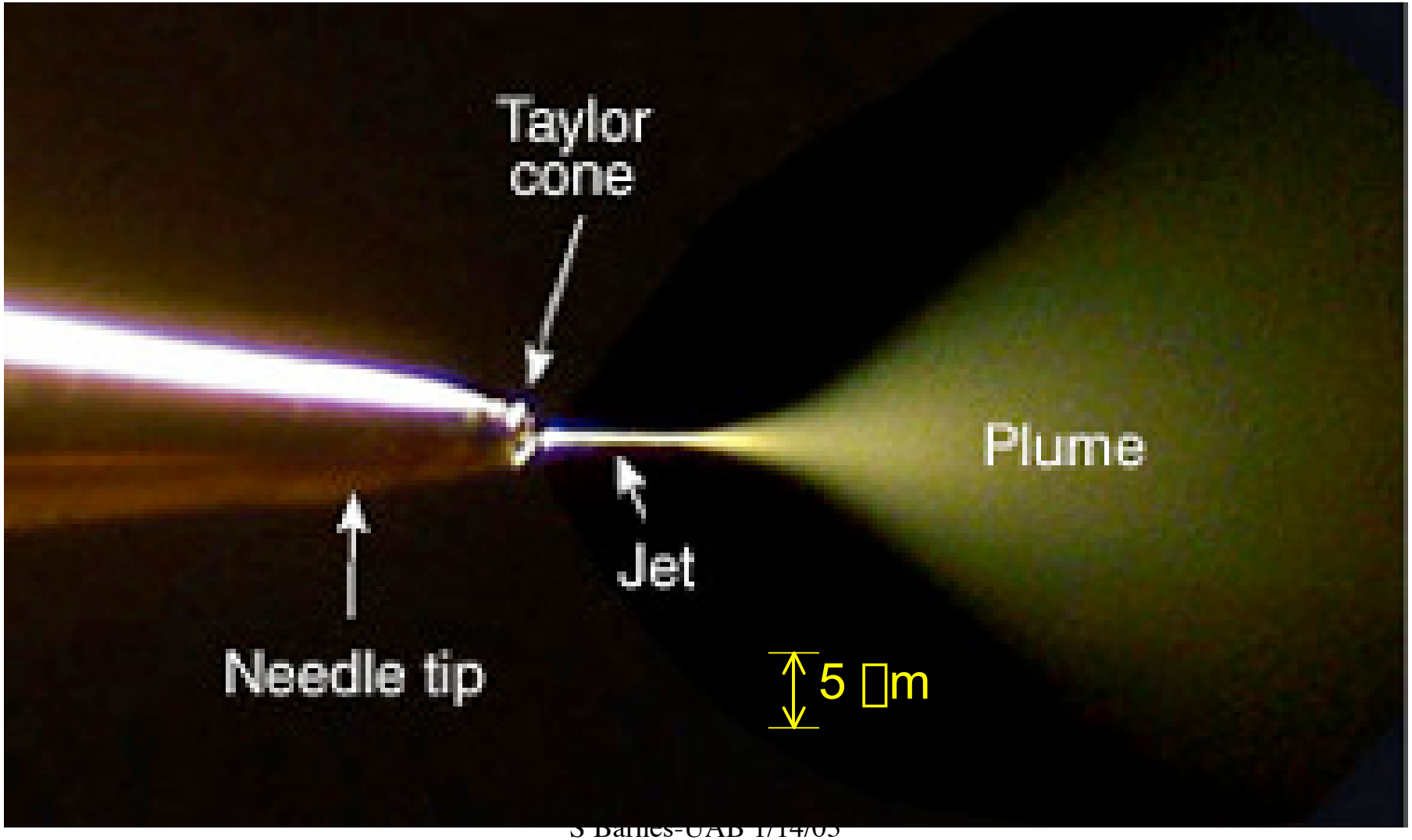


Electrospray Ionization (ESI)



1. Solvent evaporation
2. Coulombic repulsion

NanoElectrospray

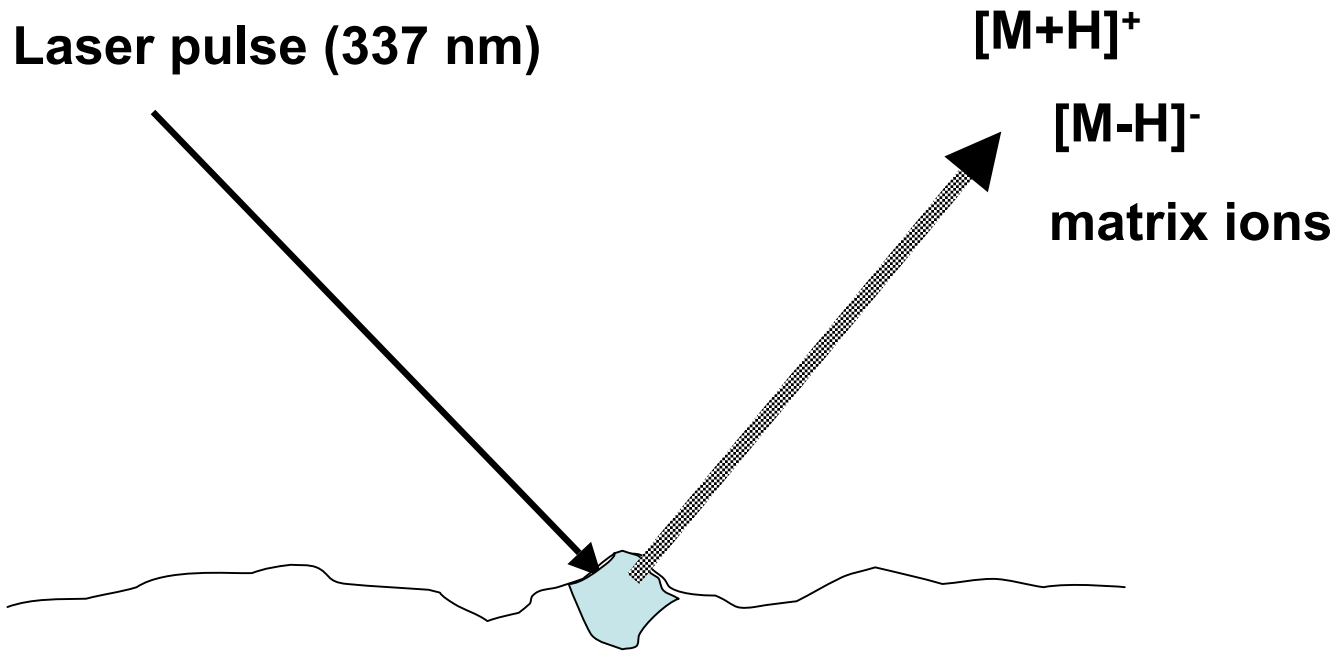


Electrospray ionization

Peptides and proteins can be transferred from solution into the gas phase without degradation by forming a nebulized spray of droplets (a Taylor cone) which are subject to rapid evaporation by warm nitrogen "curtain" gas. Typically, the nebulizing solution contains 30% acetonitrile which lowers the surface tension (and decreases droplet size) and facilitates the evaporation. The solutes are ejected from the surface of the droplet probably by coulombic repulsion. This occurs at atmospheric pressure.

The flow rates that are suitable for ESI interfaces vary from 10 nl/min up to 1 ml/min (latter requires turbo heating). Samples can be introduced by flow injection (no chromatography) or following chromatographic separation.

MALDI generation of ions



Peptide/protein deposited on crystal surface

Sample mixed with a UV-absorbing matrix and is allowed to co-crystallize on the metal target.

Matrices for MALDI analysis

Peptides/proteins

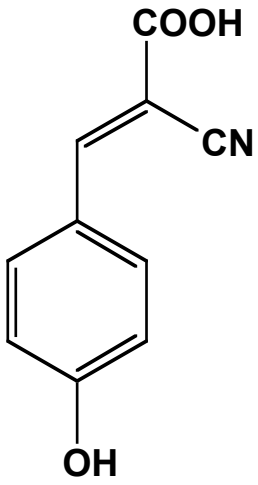
- 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid)
- □-cyano-4-hydroxycinnamic acid (CHCA)
- 2,5-dihydroxybenzoic acid (DHB)
- 2-(4-hydroxyphenylazo)-benzoic acid (HABA)

Oligonucleotides

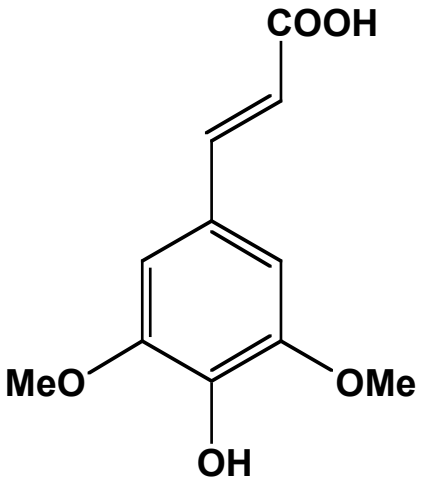
- 2-aminobenzoic acid
- 3-hydroxypicolinic acid (3-HPA)
- 2,4,6-trihydroxyacetophenone (THAP)

The choice of matrix depends greatly on the solute to be analyzed.

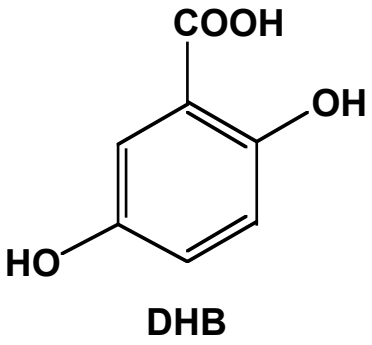
Matrices for MALDI analysis



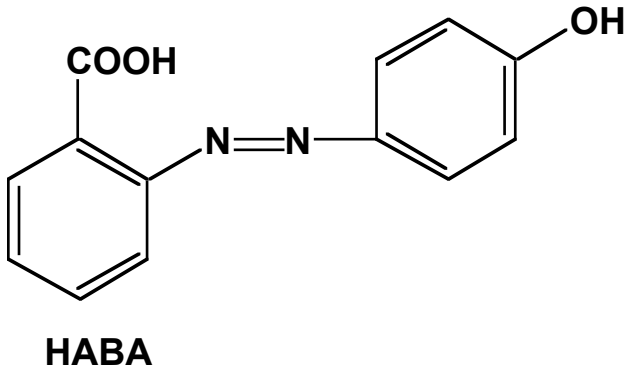
CHCA



Sinapinic acid

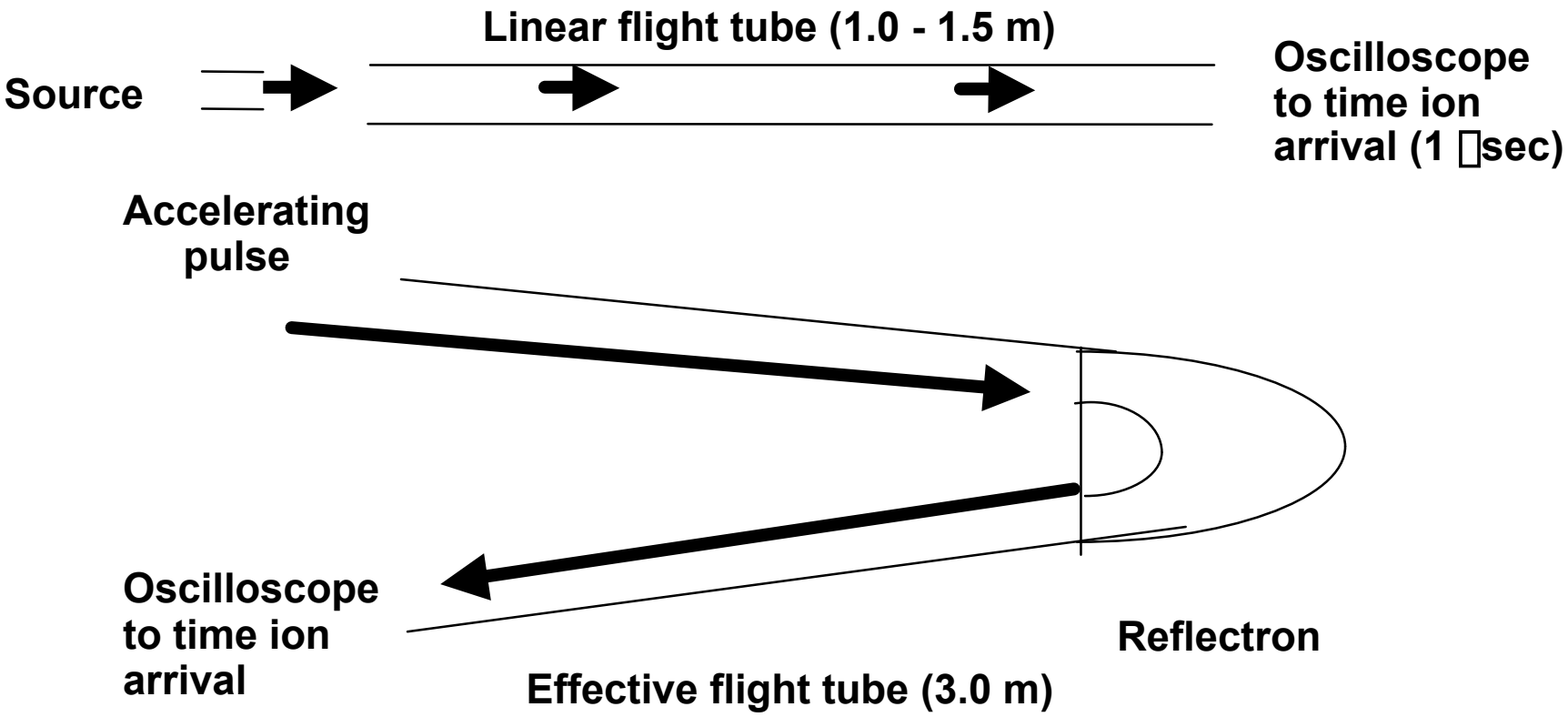


DHB

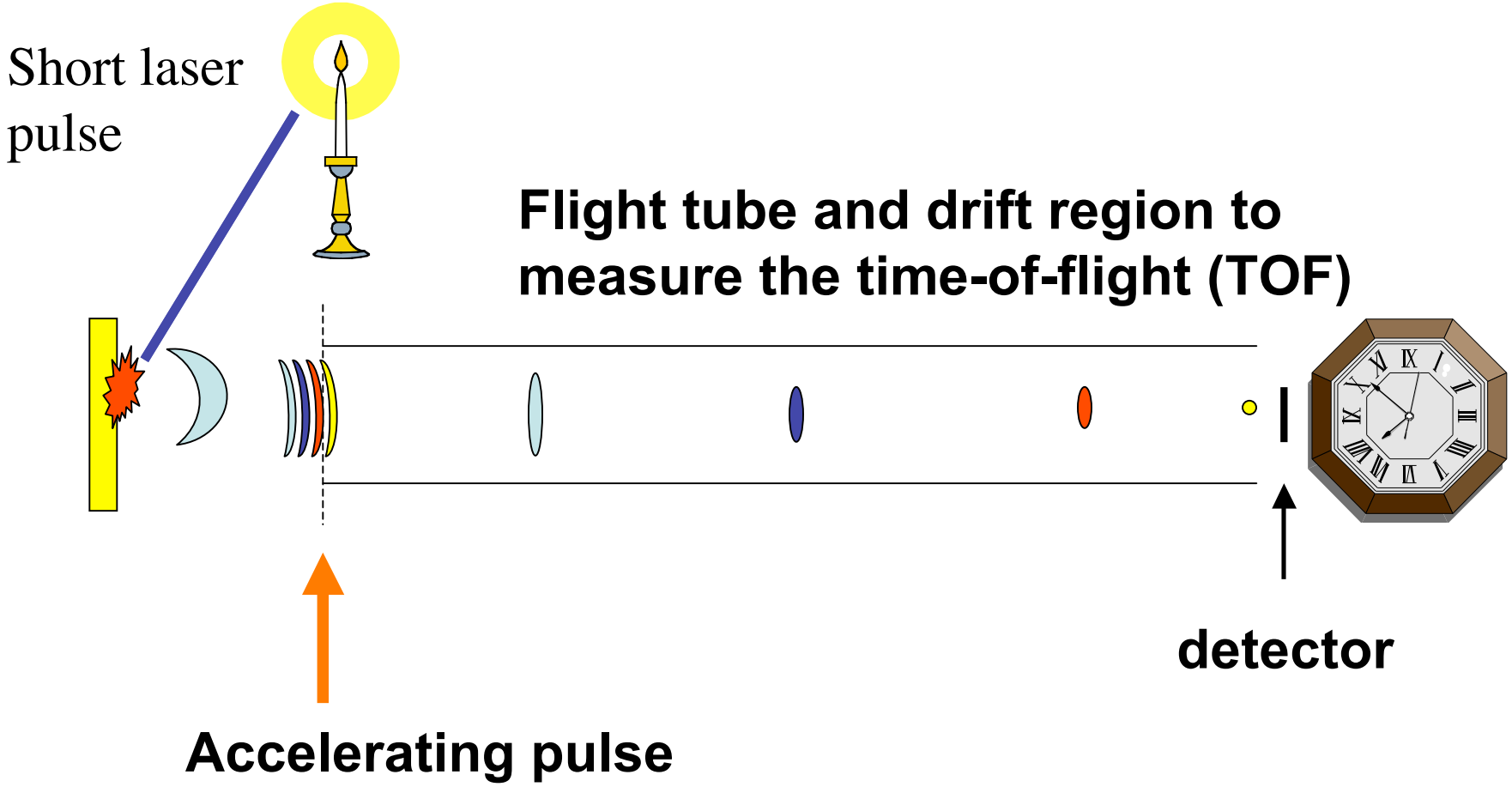


HABA

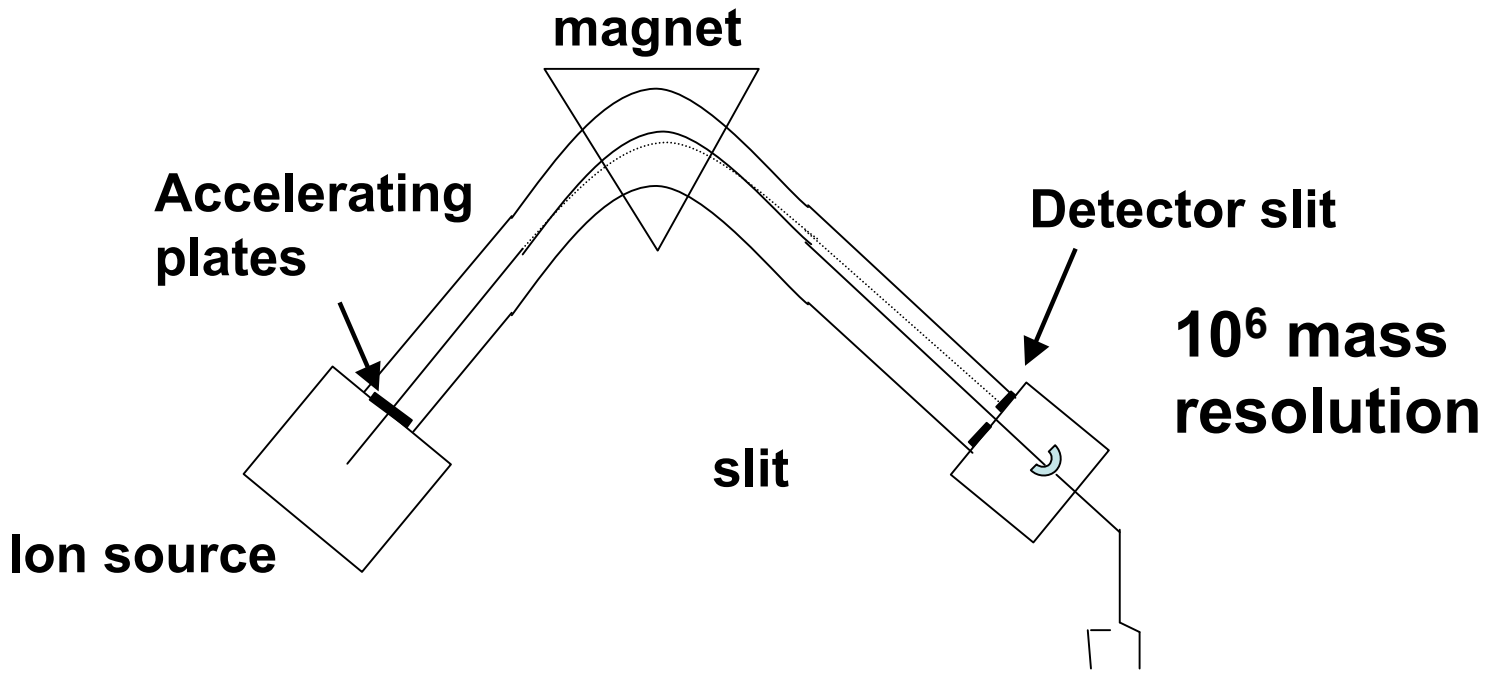
How a TOF instrument works



Matrix-Assisted Laser Desorption Ionization (MALDI)

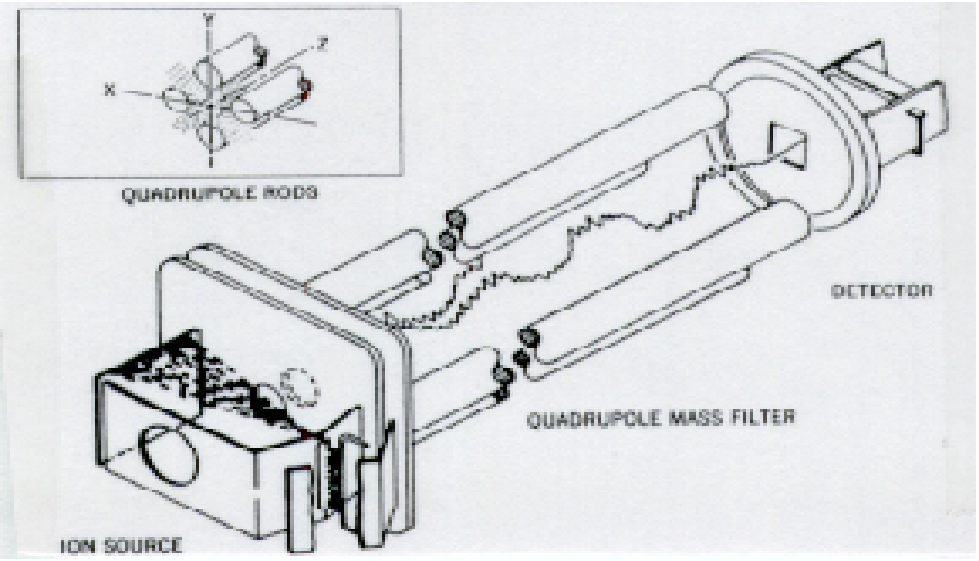


Magnetic sector analyzer



Generated ions are accelerated and are passed around a curved track (the sector) leading to a detector. By increasing the magnetic field applied to the ions, heavier ions with higher momentum can be induced to follow the curved track. A mass spectrum is obtained by applying a magnetic field gradient. Scanning is somewhat slower than in a quadrupole analyzer due to "magnetic reluctance".

Quadrupole analyzer



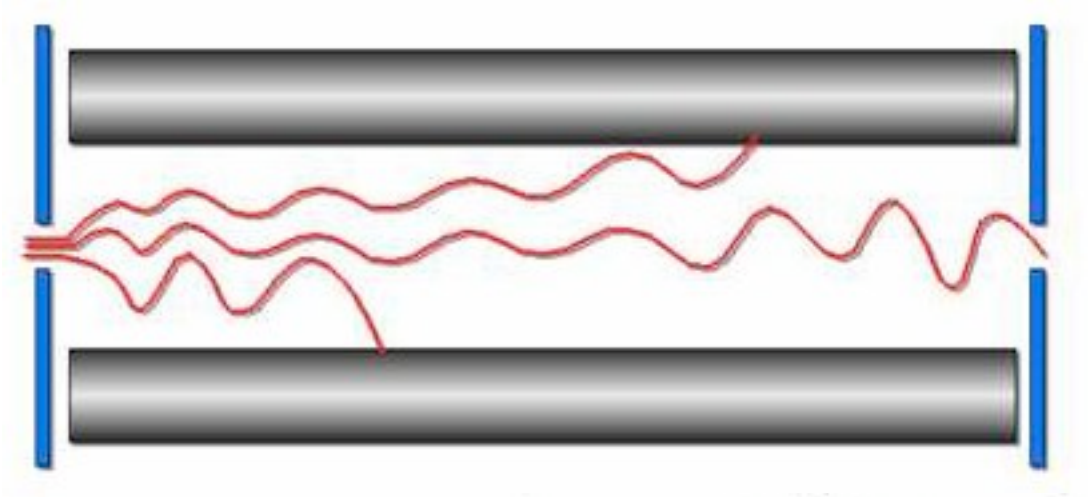
Mass resolution 2 x 10^3

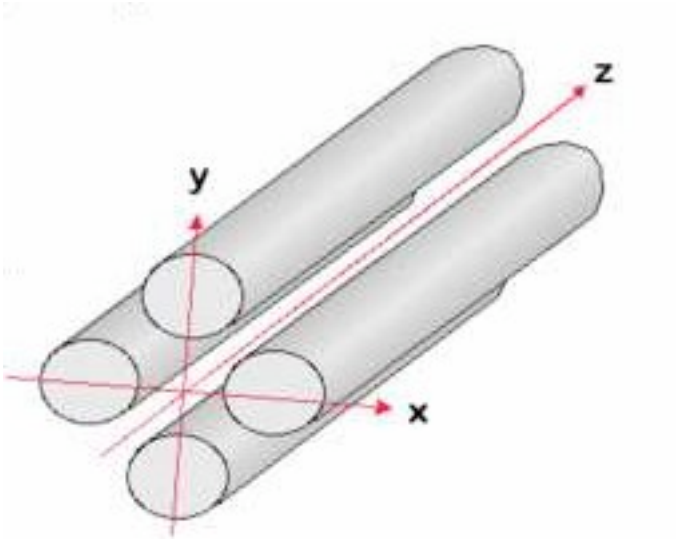
Tolerant of relatively high pressure (10^{-4} torr)

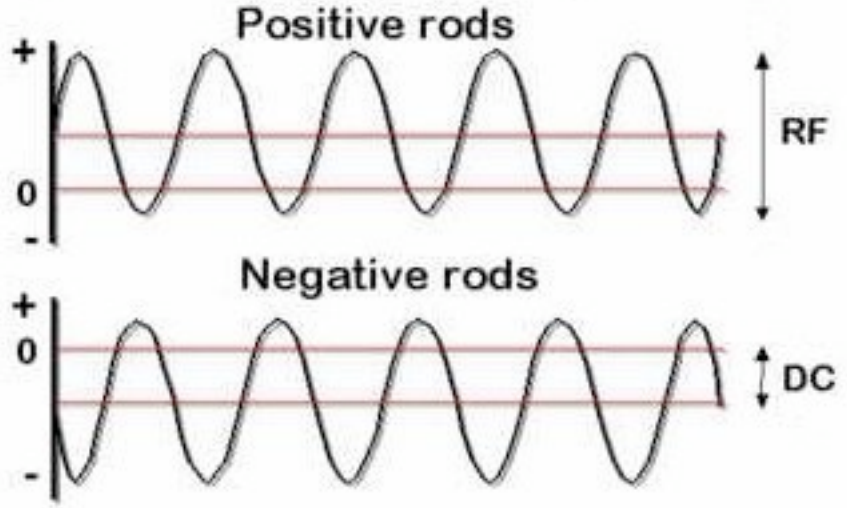
Upper limit for *m/z* is 3,000-4,000

Generated ions are accelerated electrically (5-15V) and passed along the long central axis of four rods arranged symmetrically. By applying combined DC and oscillating RF potentials, the ions drift along irregular flight paths along the rod axis. The DC/RF ratio is held constant and the absolute values of DC and RF are varied. Only ions with a particular *m/z* value have stable trajectories for a given value of DC and RF. If DC is set to 0, then all ions have stable trajectories. *A scan can be accomplished over a period of 10-1000 msec.* Analyzer is tolerant of relatively high pressure (10^{-4} torr).

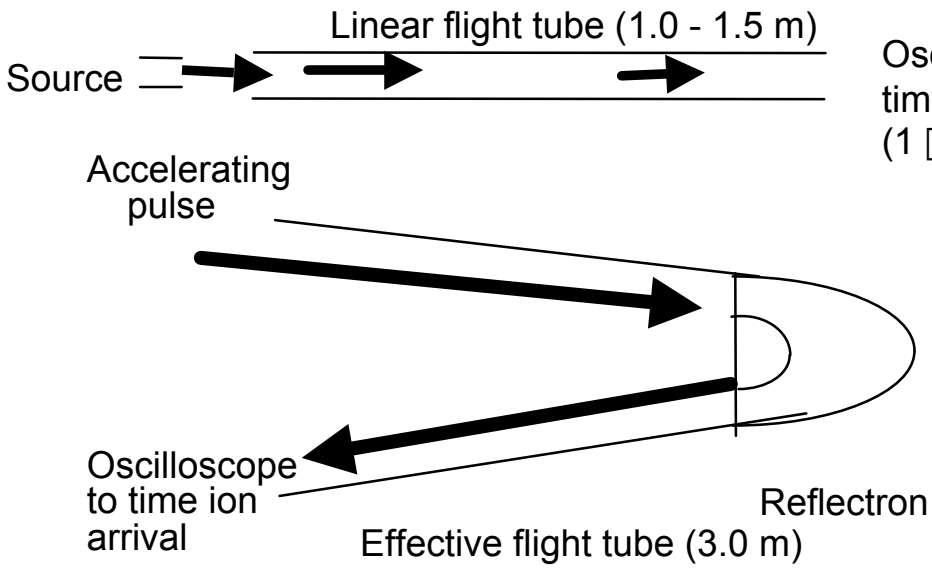
Elements of a quadrupole analyzer







Time-of-flight (TOF) analyzer



Resolution 2 x 10^4

No upper limit of mass

Scan times ~ 1 μsec, good for LC-MSMS

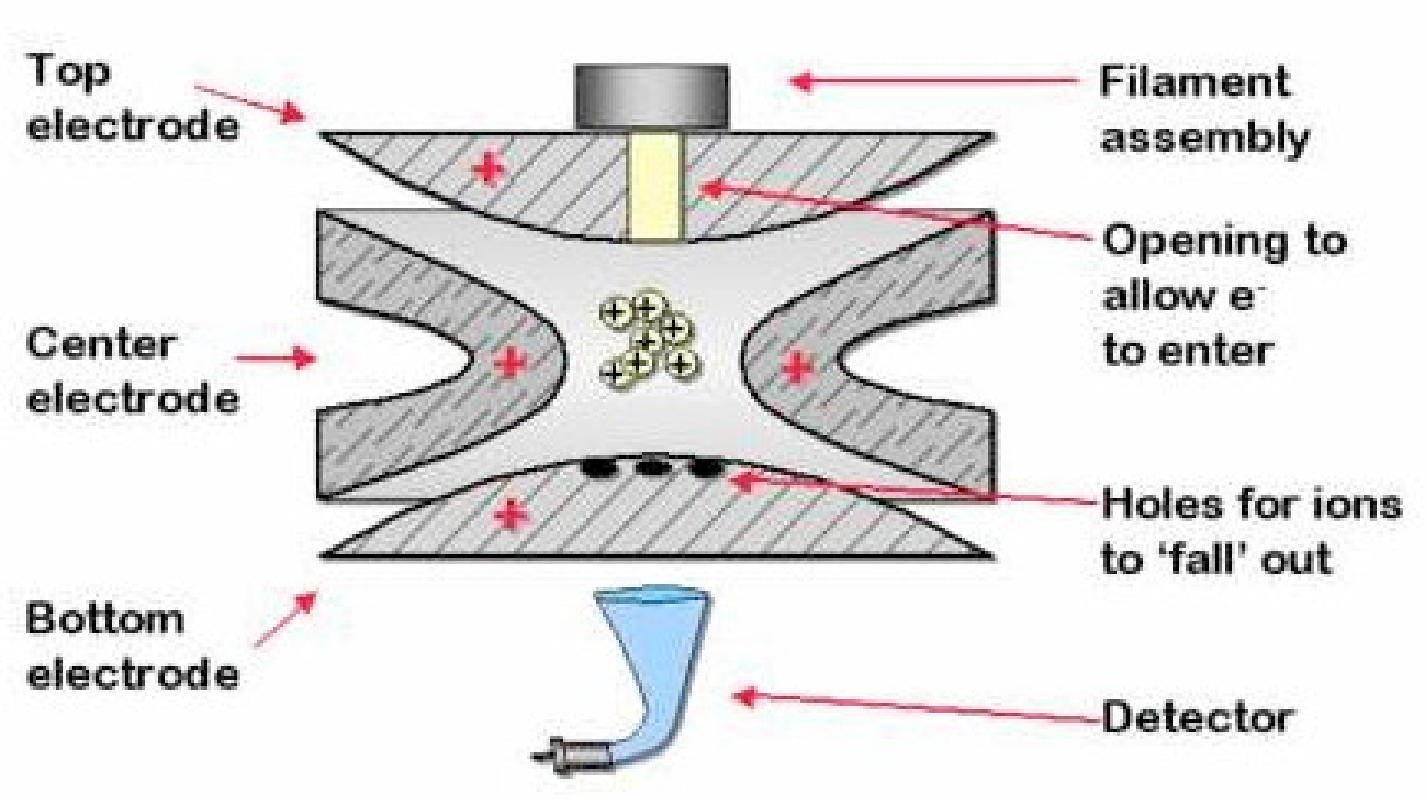
Generated ions are accelerated so that they have equal kinetic energy. They are allowed to "drift" down a 1 - 1.5 meter tube before striking a photomultiplier detector. The "time of flight" (t) depends on the mass of the ion (m), where $t = (m/2eV)^{1/2}.D$

V is the applied potential and D is the flight tube distance. For a given instrument, the flight time varies as the square root of the mass of the ion.

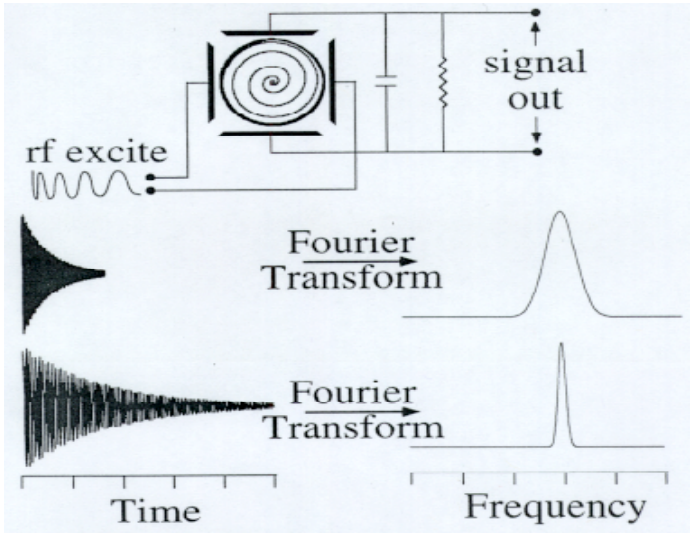
Ion Traps

The ion trap is an energy well - ions with sufficient energy to enter the trap are retained by an energy barrier on the exit side of the trap. The advantage of the ion trap is that it accumulates selected ions prior to their analysis giving it high initial sensitivity (detection limit of approx. 20 fmol). Ions are fragmented by collision with helium gas and their daughter ions analyzed within the trap. Selected daughter ions can undergo further fragmentation, thus allowing MS^n. This is important for structural experiments such as in peptide sequencing. The ion trap has a high efficiency of transfer of fragment ions to the next stage of fragmentation (unlike the triple quadrupole instrument).

Expanded view of ion trap



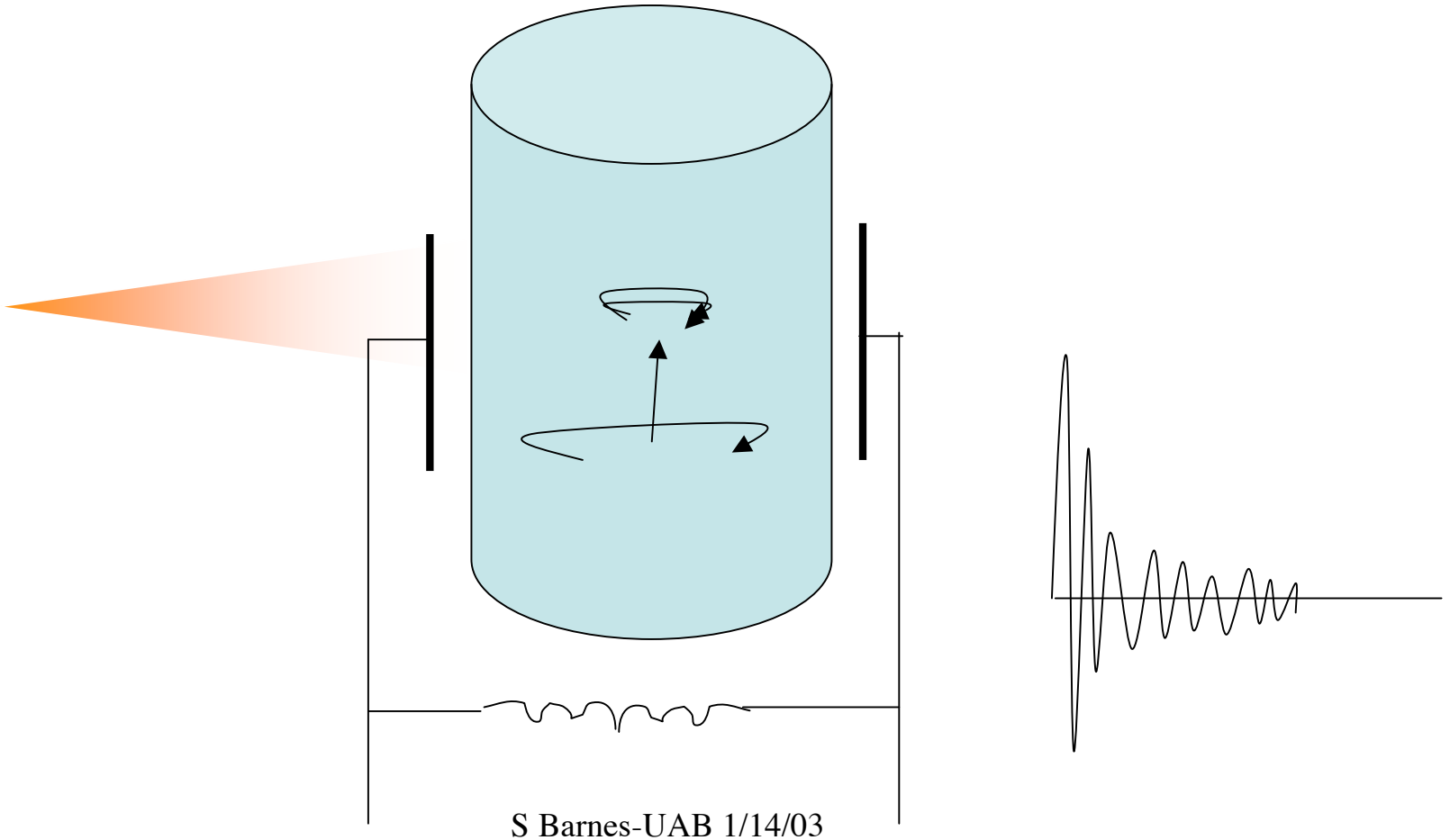
Ion trap and FT-ICR MS



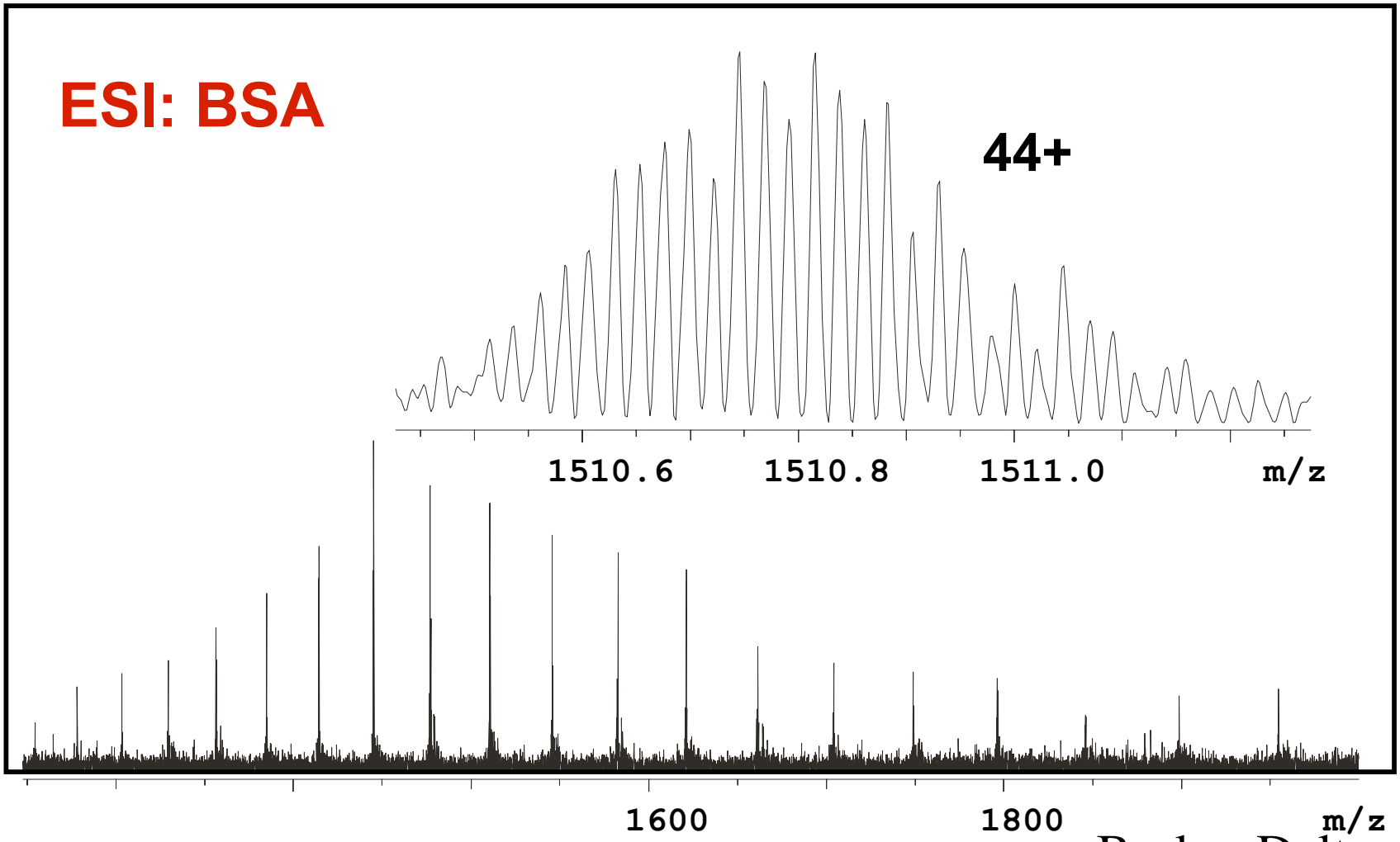
By placing the ion trap within a superconducting magnet, the trapped ions undergo cyclotron gyration and are radially confined. The frequency of the cyclotron radiation is inversely proportional to the *m/z* ratio for an ion and directly proportional to the magnetic field. If an ion is excited at its natural cyclotron frequency, it moves to a higher energy level.

A range of rf components are used to excite a sample. The ions clouds then induce an image current at two or more detection electrodes. The resulting signal when subjected to FT analysis yields an extremely precise measure of ion cyclotron frequencies, and hence *m/z* values, and molecular weights. The sensitivity is substantially enhanced and a 1 to 10^6 mass resolution can be achieved using a 9.4 tesla magnet.

Detection in the FT-ICR cell

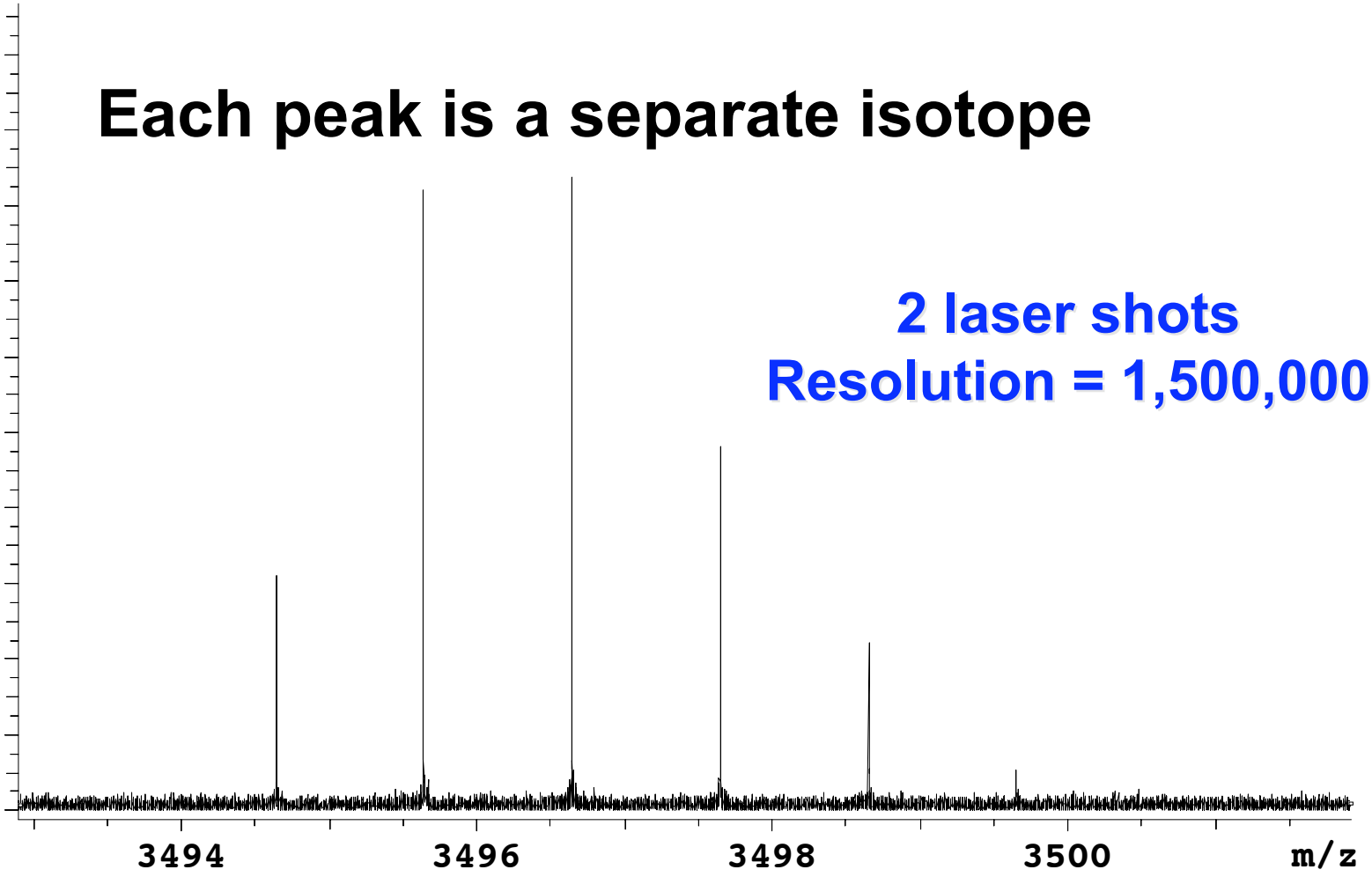


Bovine Serum Albumin (66 kDa)
4.7 T Act. Shielded Magnet



Bruker Daltonics

Insulin B (3,494 Da)
7.0 T Actively Shielded Magnet



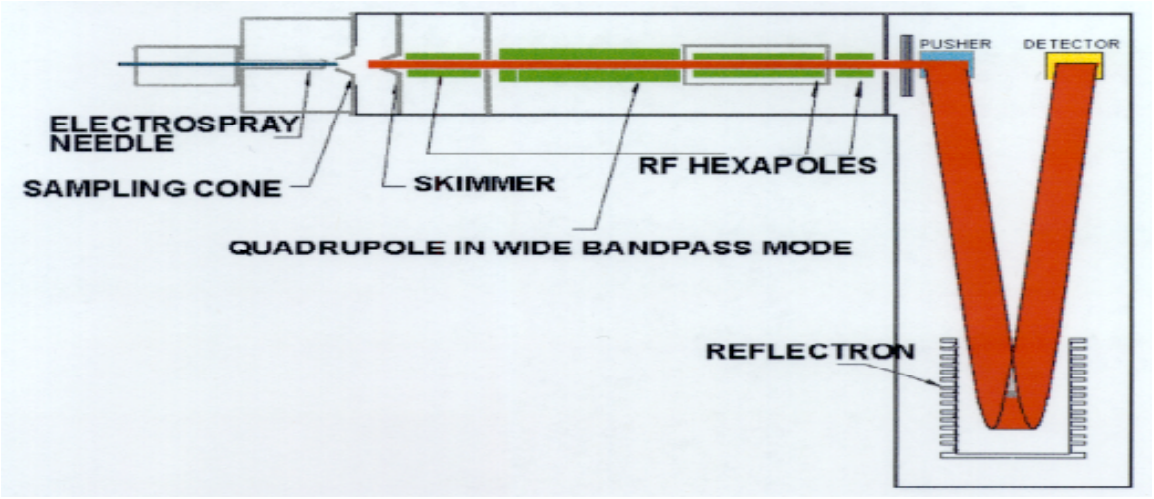
Advantages of High Field FTMS

Which FTMS Performance Factors Increase With Increasing Field?

- **Resolution (B)**
- **Acquisition Speed (1/B)**
- **Maximum Ion Kinetic Energy (B^2)**
- **Radius for a given kinetic energy ($1/B^2$)**
- **Upper mass limit (B^2)**
- **Maximum ion trapping duration (B^2)**
- **Maximum number of trapped ions (B^2)**
- **Quadrupolar axialization efficiency (B^2)**
- **Peak Coalescence (B^2)**

Hybrid Instruments - Qtof

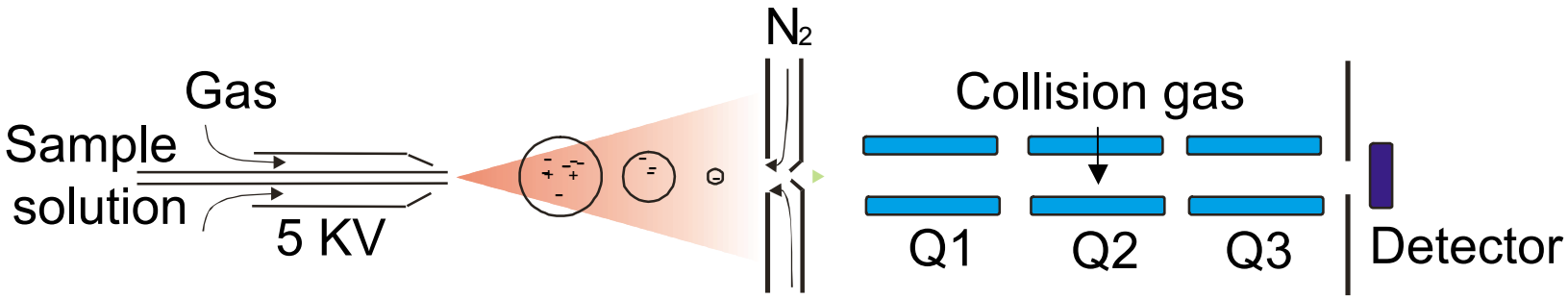
(hybrid quadrupole-orthogonal time-of-fligh)t



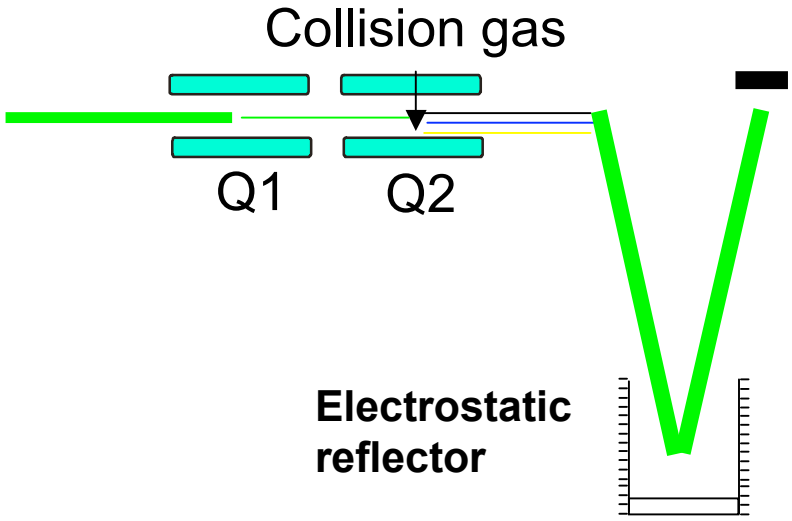
A limitation of the magnetic sector and quadrupole analyzers is that only one ion is measured at a time. Thus while analyzing ions over a mass range of m/z 1-1000 at unit mass resolution, **at any one *m/z* value all the ions at other ions are ignored**. This results in discarding 99.9% of the available information.

For the Qtof, the fragment ions are accelerated orthogonally and **all of them** are detected by the TOF analyzer.

Triple quad versus Q-tof and sensitivity



The quadrupole analyzer (Q3) is slow and insensitive - it's a filter - thus throws away large amounts of data



TOF detector collects all ions generated and yields fmol rather than pmol sensitivity

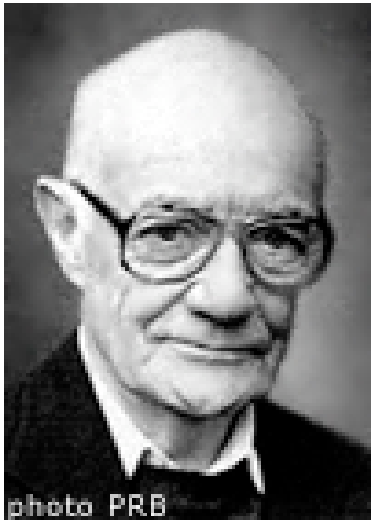
Also gives far greater mass accuracy - from 1000 ppm on the triple quad to 5-10 ppm on the Q-tof

Crucially important for automated interpretation of MS-MS spectra to yield amino acid sequence

Other hybrid instruments

- MALDI-FT-ICR
  - Generates singly charged ions
- MALDI-quadrupole trap
  - High sensitivity and high throughput
- MALDI-TOF-TOF
  - 200-1000 Hz laser leads to highest rate of MS analysis (3,000-4,000 spectra/hr)
  - Also can record novel MS-MS spectra (500/hr)
- Ion trap-FT-ICR
  - Latest instrument - being introduced later this year

Congratulations to the Nobel Laureates - 2002



John Fenn

Koichi Tanaka

"*for the development of methods for identification and structure analyses of biological macromolecules*"
and
"*for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules*"