Mass spectrometry – gas phase transfer and instrumentation

Matt Renfrow
January 15, 2014

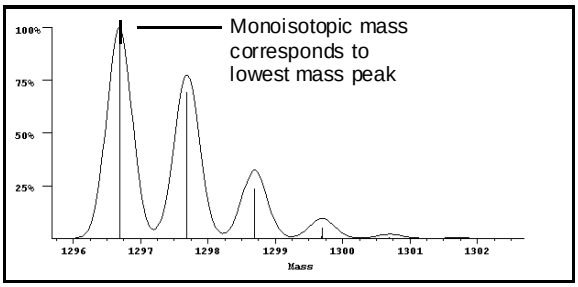
Objectives of the Lecture

1. Make ions
2. Separate/Analyze
3. Detect ions
4. What is mass resolution and mass accuracy?

Stable isotopes of most abundant elements of peptides

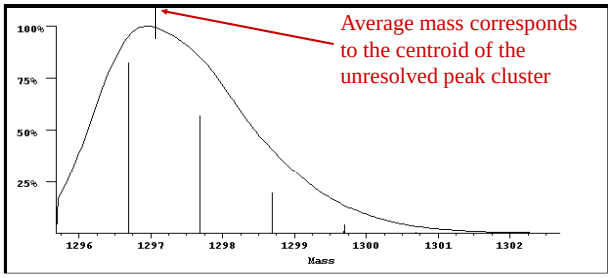
Element	Mass	Abundance
H	1.0078 2.0141	99.985% 0.015
C	12.0000 13.0034	98.89 1.11
N	14.0031 15.0001	99.64 0.36
O	15.9949 16.9991 17.9992	99.76 0.04 0.20

Monoisotopic mass

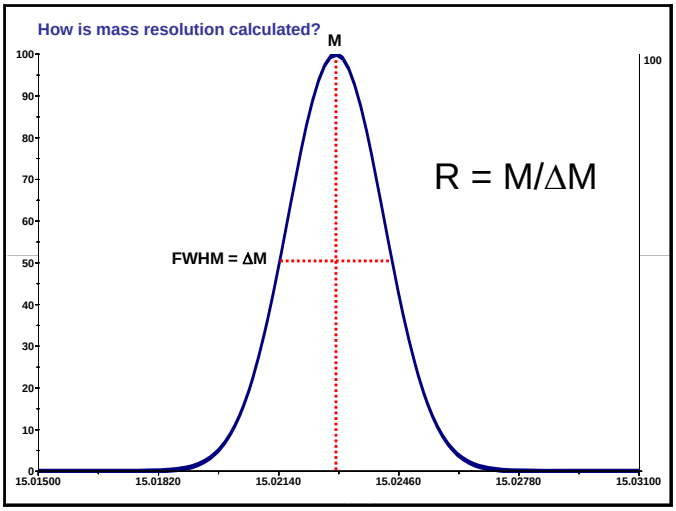


When the isotopes are clearly resolved the **monoisotopic mass** is used as it is the most accurate measurement.

Average mass

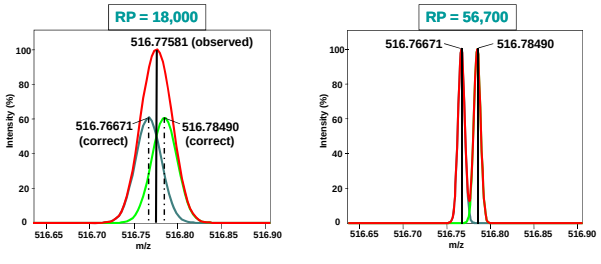


When the isotopes are not resolved, the centroid of the envelope corresponds to the weighted average of all the the isotope peaks in the cluster, which is the same as the average or chemical mass.



Two peptides - same nominal mass - simulation

Peptide mixture:	**[Val^5]-Angiotensin II**	**Lys-des-Arg^9-Bradykinin**
Sequence:	**DRVYVHPF**	**KRPPGFSPF**
Formula:	**$C_{49}H_{69}N_{13}O_{12}$**	**$C_{50}H_{73}N_{13}O_{11}$**
Exact mass:	**$[M+2H]^{2+}$ = 516.76671**	**$[M+2H]^{2+}$ = 516.78490**
Δm (mmu):	**18.2 mmu**	



Is Mass Accuracy Important ?

Results for error limit up to 5 ppm

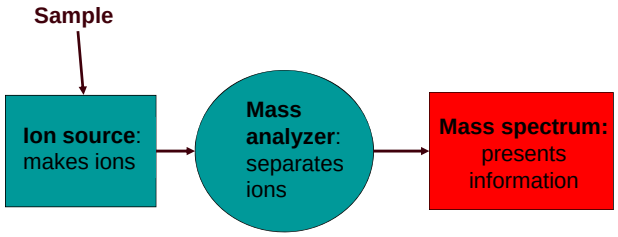
	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
1 ppm (4)	516.76671	0.0	0.0	21.0	C_{49} H_{71} O_{12} N_{13}
	516.76647	0.5	0.2	15.0	C_{49} H_{79} O_{11} N_9 S_2
	516.76638	0.6	0.3	12.0	C_{41} H_{75} O_{14} N_{15} S_1
	516.76705	-0.7	-0.3	11.5	C_{43} H_{77} O_{15} N_{12} S_1
2 ppm (10)	516.76604	1.3	0.7	16.0	C_{48} H_{75} O_{15} N_9
	516.76738	-1.3	-0.7	20.5	C_{51} H_{73} O_{13} N_{10}
	516.76604	1.3	0.7	21.5	C_{47} H_{69} O_{11} N_{16}
	516.76580	1.8	0.9	15.5	C_{47} H_{77} O_{10} N_{12} S_2
	516.76772	-2.0	-1.0	16.5	C_{44} H_{73} O_{11} N_{16} S_1
	516.76773	-2.0	-1.0	11.0	C_{45} H_{79} O_{16} N_9 S_1
5 ppm (23)	516.76805	-2.6	-1.3	25.5	C_{52} H_{69} O_9 N_{14}
	516.76537	2.6	1.3	16.5	C_{46} H_{73} O_{15} N_{12}
	516.76807	-2.6	-1.4	7.0	C_{38} H_{79} O_{14} N_{15} S_2
	516.76513	3.0	1.6	10.5	C_{46} H_{81} O_{14} N_8 S_2
	516.76513	3.1	1.6	16.0	C_{45} H_{75} O_9 N_{15} S_2
	516.76839	-3.3	-1.7	16.0	C_{46} H_{75} O_{12} N_{13} S_1
	516.76479	3.7	1.9	20.0	C_{52} H_{75} O_{11} N_9 S_1
	516.76872	-3.9	-2.0	25.0	C_{54} H_{71} O_{10} N_{11}
	516.76470	3.9	2.0	17.0	C_{44} H_{71} O_{14} N_{15}
	516.76874	-3.9	-2.0	6.5	C_{40} H_{81} O_{15} N_{12} S_2
	516.76446	4.3	2.2	11.0	C_{44} H_{79} O_{13} N_{11} S_2
	516.76897	-4.4	-2.3	12.5	C_{40} H_{73} O_{15} N_{16}
	516.76907	-4.6	-2.4	15.5	C_{48} H_{77} O_{13} N_{10} S_1

$$\text{Mass error} = \frac{\text{theoretical mass} - \text{observed mass}}{\text{theoretical mass}} \times 1{,}000{,}000$$

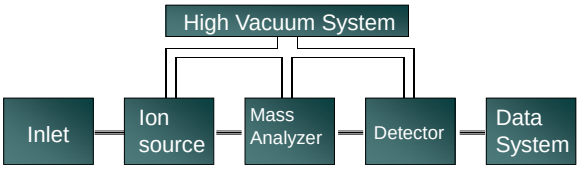
$$m/z = \frac{[M + nH]}{n}$$

$$m/z = \frac{[M - nH]}{n}$$

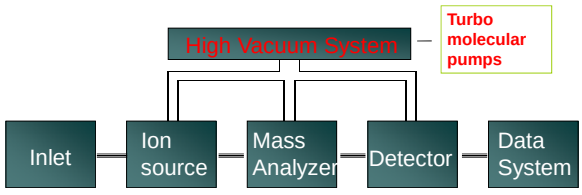
How does a mass spectrometer work?



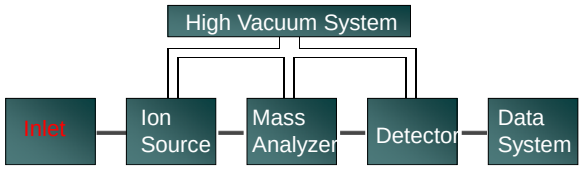
Mass Spectrometer Block Diagram



Mass Spectrometer Block Diagram

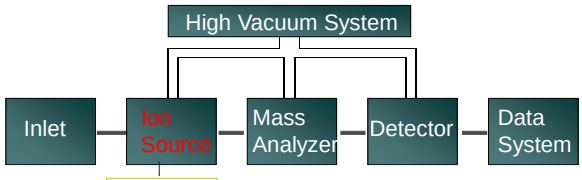


Sample Introduction



HPLC
Flow injection
Charged vapor stream
Sample plate

Ion Source



MALDI
ESI
FAB
LSIMS
EI
CI
DART

Nobel Prize in Chemistry- 2002

For getting proteins and peptides into the gas phase

John Fenn

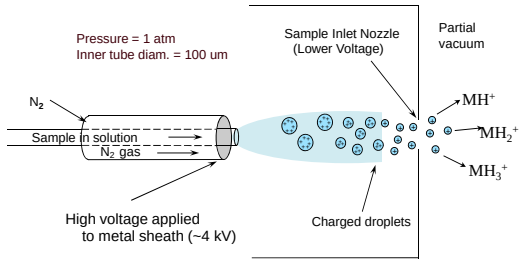
Koichi Tanaka

"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"

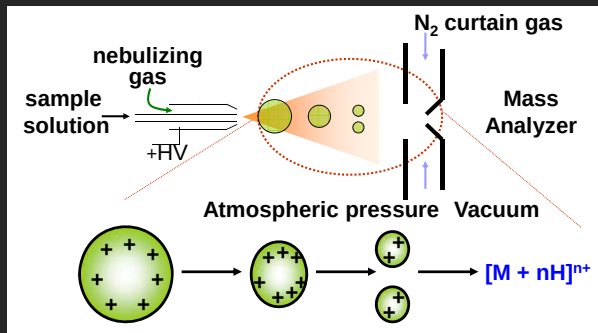
Ion Sources make ions from sample molecules

(Ions are easier to detect than neutral molecules.)

Electrospray ionization:

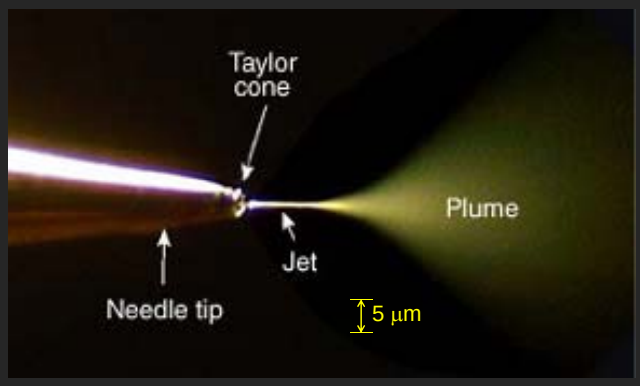


Electrospray Ionization (ESI)

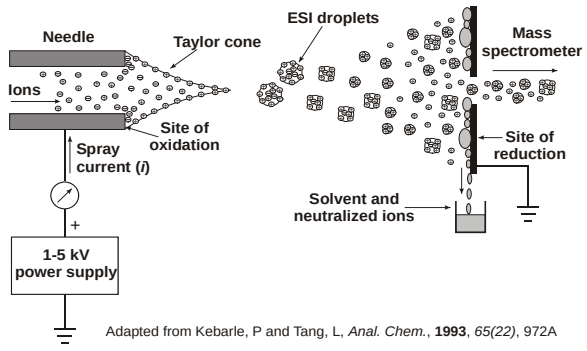


1. Solvent evaporation
2. Coulombic repulsion

Electrospray Ionization (ESI)

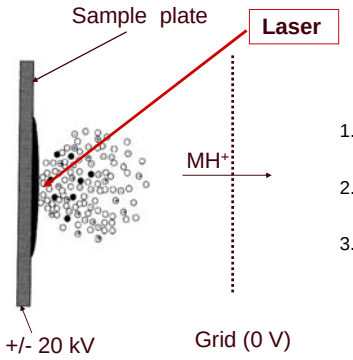


Electrospray Ionization (ESI) Process
(Positive Mode)



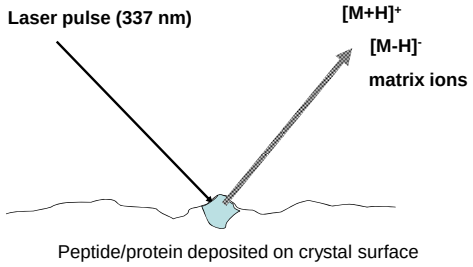
Adapted from Kebarle, P and Tang, L, *Anal. Chem.*, **1993**, *65(22)*, 972A

MALDI: Matrix Assisted Laser Desorption Ionization



1. Sample is mixed with matrix (X) and dried on plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules (M) are ionized by proton transfer: $XH^+ + M \rightarrow MH^+ + X$.

MALDI generation of ions
(Matrix-assisted laser desorption ionization)



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

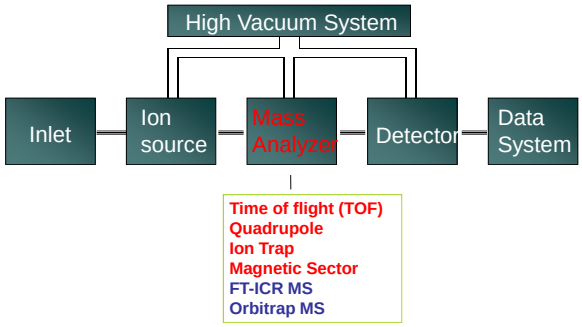
Matrices for MALDI analysis

Peptides/proteins
- 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid)
- α-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.

Mass Analyzer



Mass analyzers separate ions based on their mass-to-charge ratio (m/z)

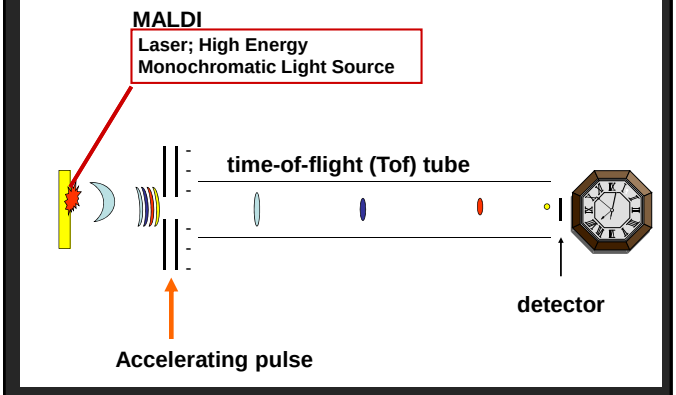
- Operate under high vacuum
(keeps ions from bumping into gas molecules)
- Actually measure mass-to-charge ratio of ions (m/z)

The importance of the mass-to-charge ratio is that according to classical electrodynamics two particles with the same mass-to-charge ratio move in the same path in a vacuum when subjected to the same electric and magnetic fields.

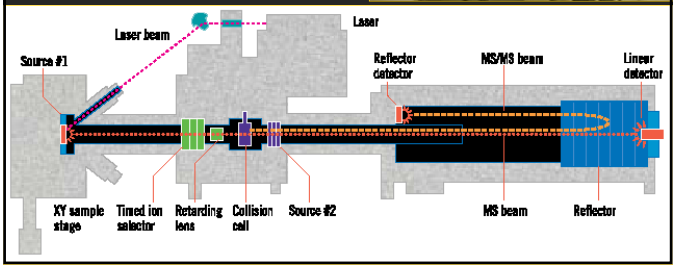
- **F**=*m***a** (Newton's second law of motion)
- **F**=*q*(**E** + v × B) (Lorentz force Law)
- (*m*/*q*)a = **E** + v × **B**

- Key specifications are resolution, mass measurement accuracy, and sensitivity.

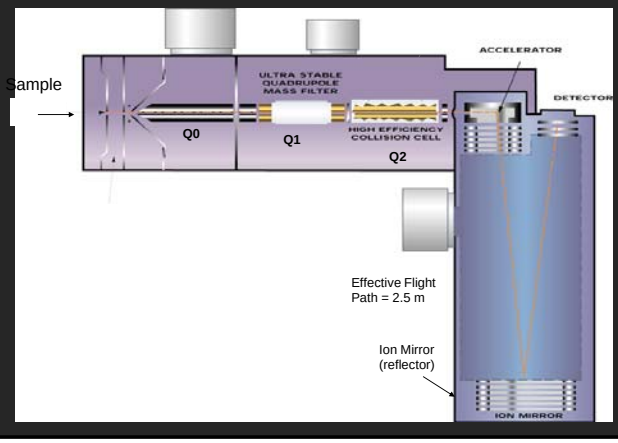
Time-of-flight (Tof) analyzer



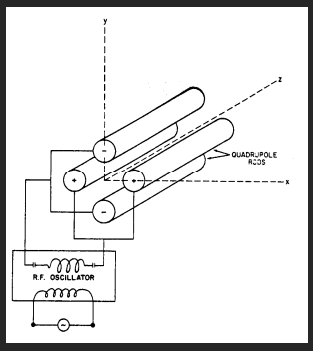
Principals of the MALDI-Tof/Tof 4700



QSTAR™ ESI QQ TOF or MALDI QQ TOF



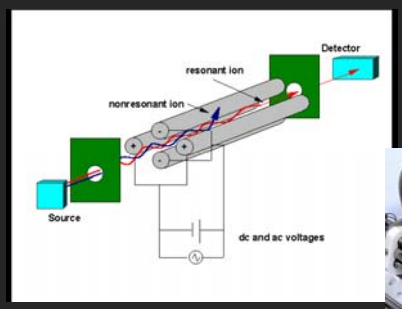
Quadrupole Mass Analyzer



Uses a combination of RF and DC voltages to operate as a mass filter.

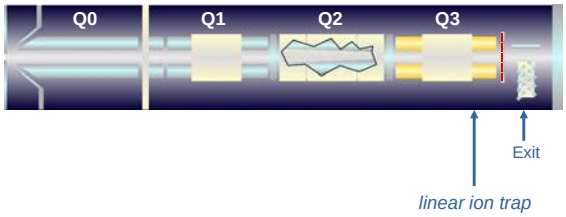
- Has four parallel metal rods.
- Lets one mass pass through at a time.
- Can scan through all masses or sit at one fixed mass.

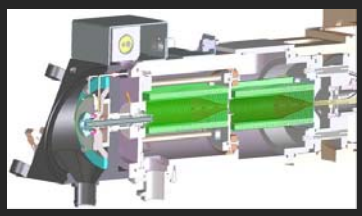
Quadrupole mass filter / ion guide



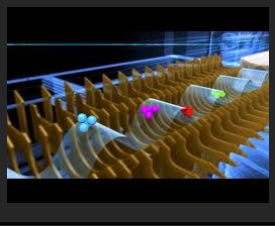
Octapole

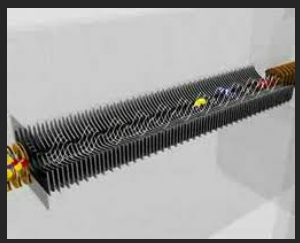
QTRAP: Linear Ion Trap on a Triple Quadrupole

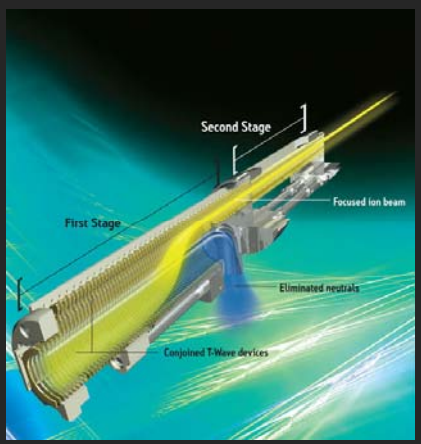




Ion funnel and Ion mobility technology



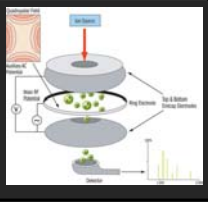
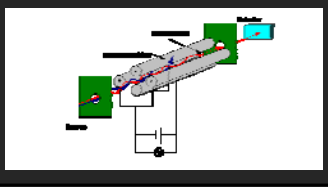




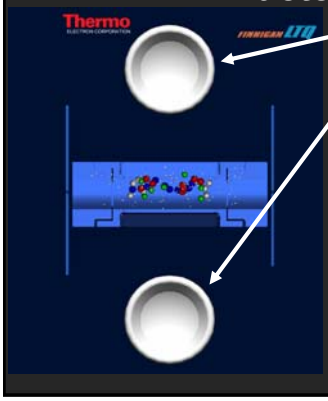
http://www.youtube.com/watch?v=O58VtqPLkiI

3D ion trap and 2D ion trap

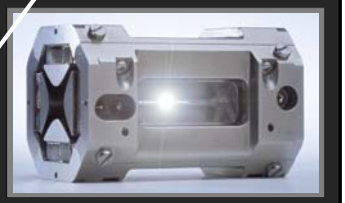


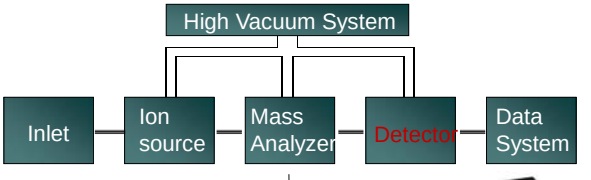
2D ion trap detection



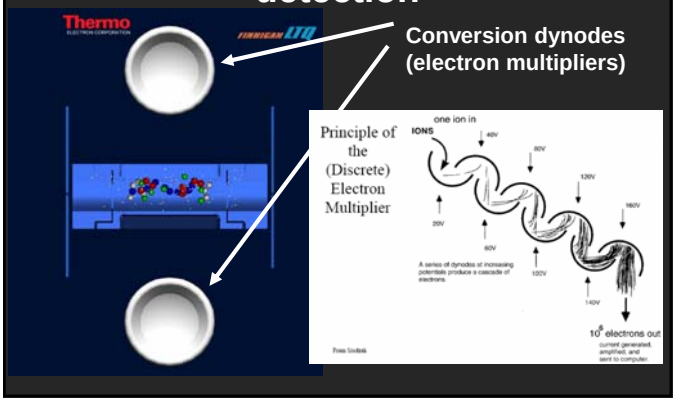
Conversion dynodes

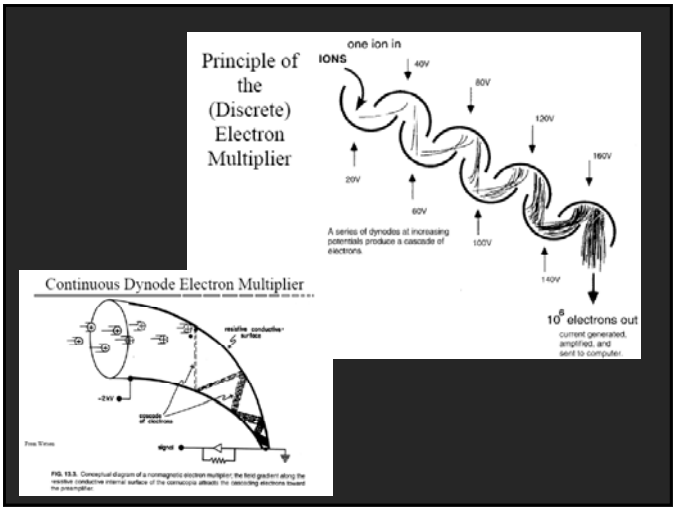


Detector



2D ion trap detection





Qtof

http://www.youtube.com/watch?v=BFuZali-zDk

Triple quad

http://www.youtube.com/watch?v=-iLtW6XQMmw

Triple trap (Qtrap)

http://www.youtube.com/watch?v=VUmcxNLHu4Y
http://www.youtube.com/watch?v=um_ELG02wpY

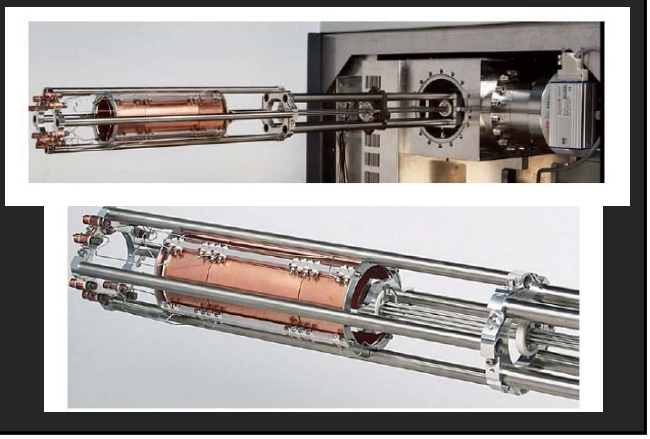
Ion funnel technology

http://www.youtube.com/watch?v=iYwkXXGu3U8

Ion mobility

http://www.youtube.com/watch?v=6EbWdWa2rF0

Penning Trap (ICR cell)



Penning Trap (ICR cell)

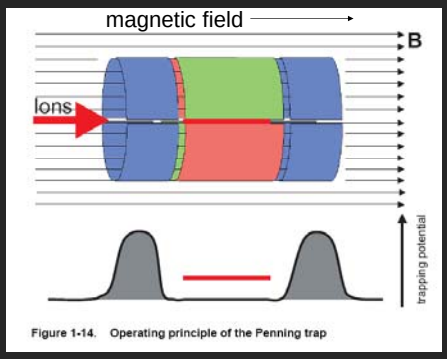
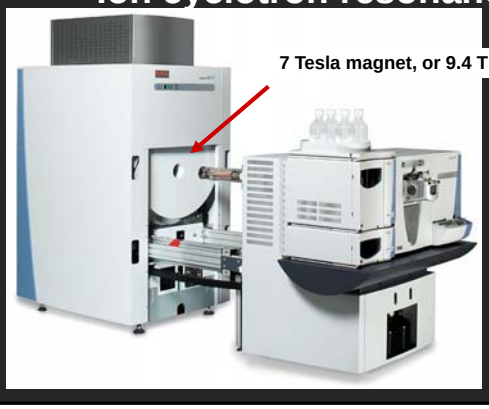


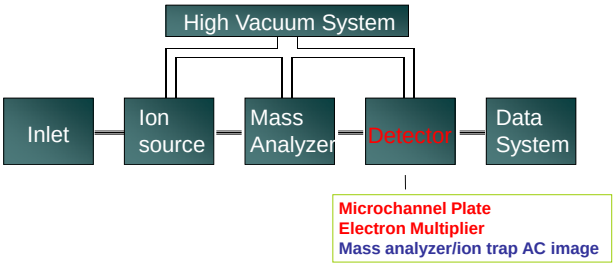
Figure 1-14. Operating principle of the Penning trap

Put the trap in a high magnetic field Ion cyclotron resonance



7 Tesla magnet, or 9.4 T or 12 T or 14.5 T

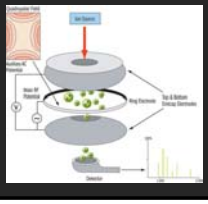
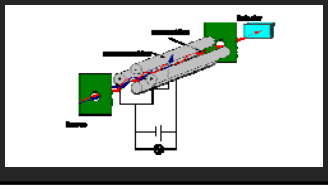
Detector



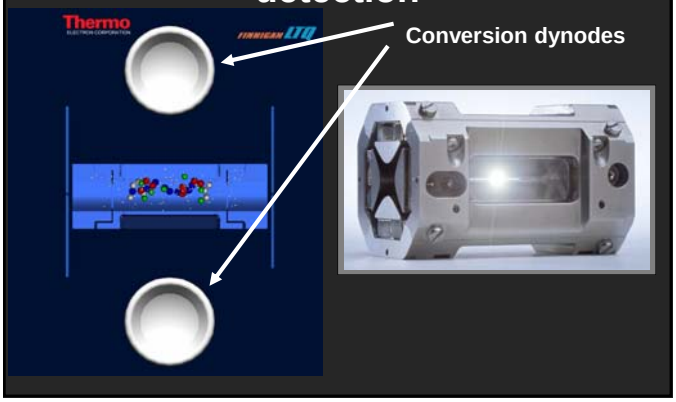
Microchannel Plate
Electron Multiplier
Mass analyzer/ion trap AC image

3D ion trap and 2D ion trap

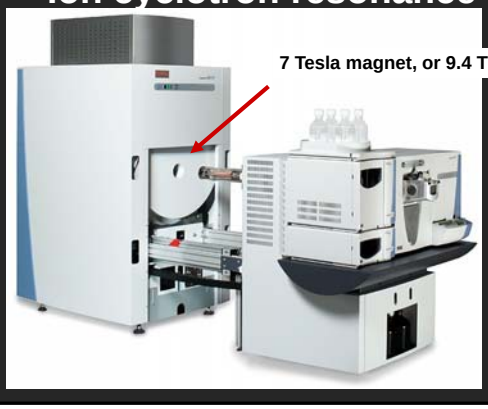


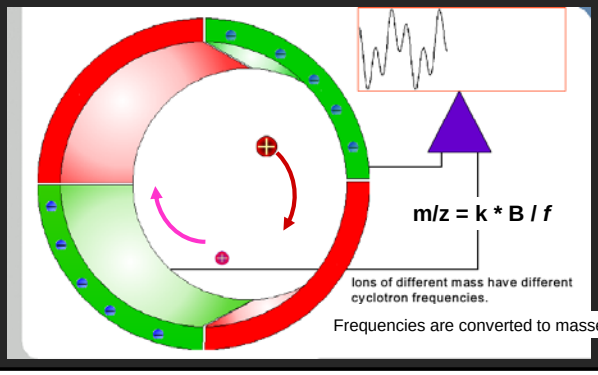
2D ion trap detection



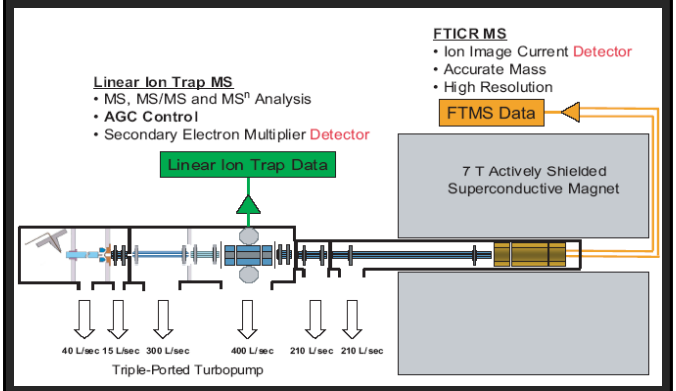
Detecting in the ion trap Ion cyclotron resonance (ICR)



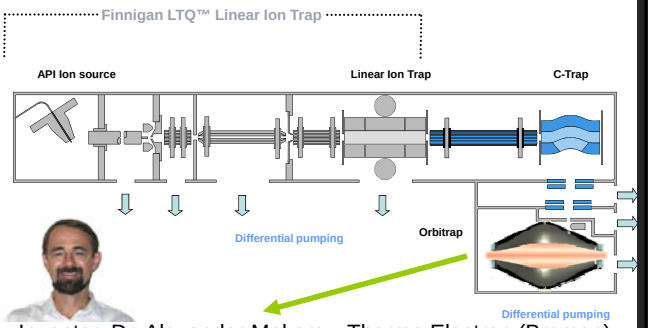
Fourier transform- Ion cyclotron resonance FT-ICR MS



ThermoFinnigan LTQ-FT

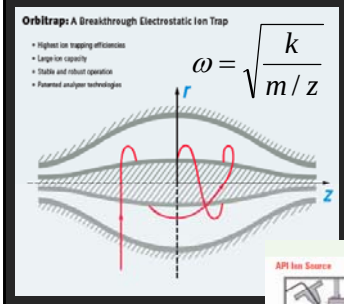


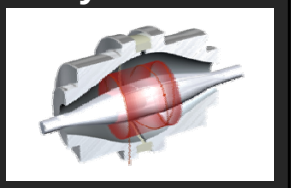
LTQ Orbitrap™ Hybrid Mass Spectrometer

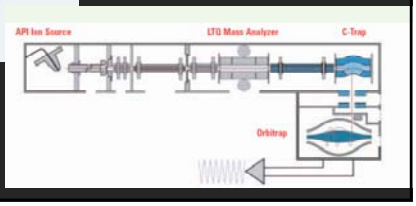


Inventor: Dr. Alexander Makarov, Thermo Electron (Bremen)

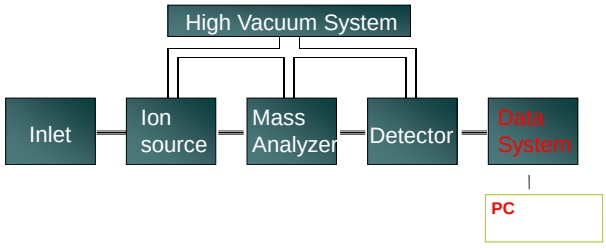
Orbitrap Mass Analyzer





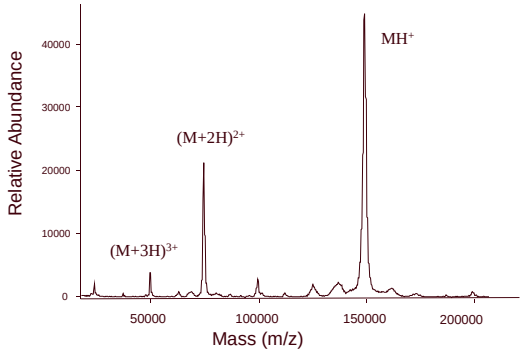


Data System

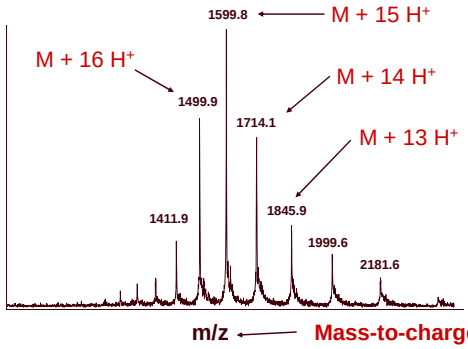


The mass spectrum shows the results

MALDI TOF spectrum of IgG



ESI Spectrum of Trypsinogen (MW 23983)



How do mass spectrometers get their names?

Types of ion sources:

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

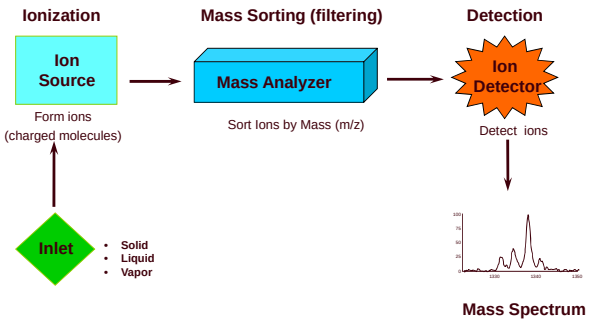
Types of mass analyzers:

- Quadrupole (Quad, Q)
- Ion Trap
- Time-of-Flight (TOF)

-Either source type can work with either analyzer type: "MALDI-TOF," "ESI-Quad."

-Analyzers can be combined to create "hybrid" instruments. ESI-QQQ, MALDI QQ TOF, Q Trap

Summary: acquiring a mass spectrum



Objectives of the Lecture

1. **Make ions**
   ESI, MALDI
2. **Separate/Analyze/Detect ions**
   Tof, ion trap, quadrupole,
   FT-ICR, Orbitrap
   Electron multipliers
3. **What is mass resolution and mass accuracy?**

Slide Acknowledgements

Thermo Electron (Fisher)
Bruker
ABI
Sandler Mass Spectrometry Group
David Agard group
http://www.msg.ucsf.edu/agard/
(Univ. of Calif. San Francisco)

Put it in

- We need Ions (+ or -)
- In the gas phase

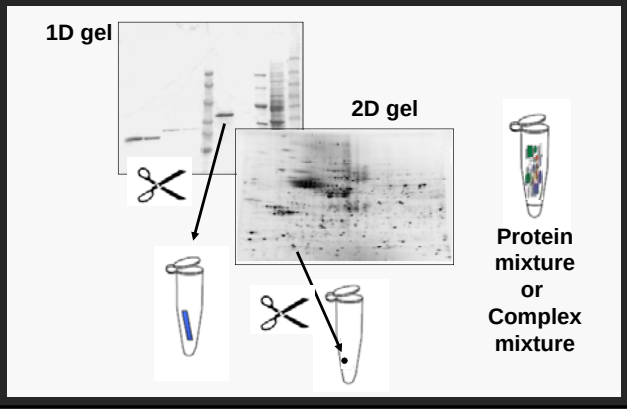
Your machine

- Tof, Tof / Tof
- Quadrupole, Ion trap
- FT-ICR, Orbitrap (high resolution)
- Hybrids

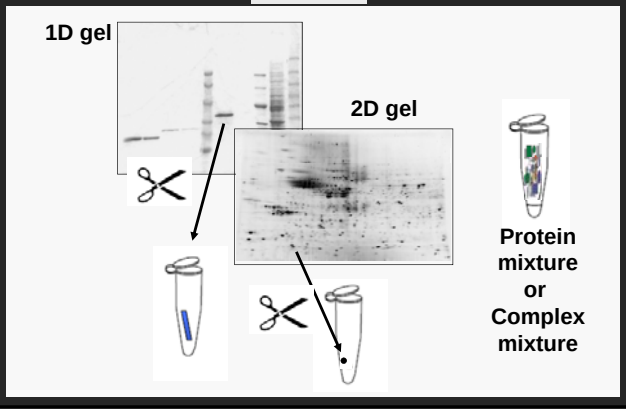
Tell me the RIGHT answer

- How right is it?
  mass resolution and accuracy

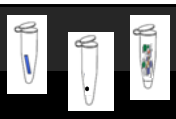
MS of Proteins and Peptides



Put it in your machine and tell me the RIGHT answer



Put it in



- We need Ions (+ or -)
- In the gas phase

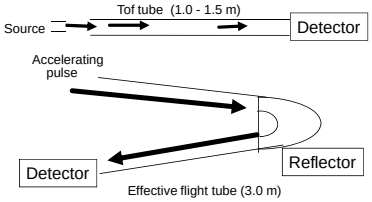
Your machine

- Tof, Tof Tof
- Quadrupole, Ion trap
- FT-ICR, Orbitrap (high resolution)
- Hybrids

Tell me the RIGHT answer

- How right is it?
  mass resolution and accuracy

Time-of-flight (Tof) analyzer



Resolution 2 x 10^4

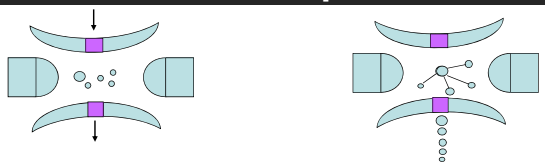
No upper limit of mass

Scan times ~ 1 µsec, good for LC-MSMS

Ions are accelerated so that they have equal kinetic energy. The ions "drift" down a 1 - 1.5 meter tube before striking a photomultiplier detector. "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.
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 3D ion trap