Mass Accuracy and Absolute Mass

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Biochemistry & Molecular Genetics

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Masses of elements and their isotopes

- **Mass is defined using the mass of carbon-12 being 12.0000 (exactly)**
- **On this scale,**
  - **^{1}H is 1.007825 and ^{2}H is 2.014102**
  - **^{14}N is 14.003074 and ^{15}N is 15.000108**
  - **^{16}O is 15.994915, ^{17}O is 16.999132 and ^{18}O is 17.999161**
  - **^{31}P is 30.973761**
  - **^{32}S is 31.972071 and ^{34}S is 33.967867**

How is mass defined?

Assigning numerical value to the intrinsic property of “mass” is based on using carbon-12, ^{12}C, as a reference point.

One unit of mass is defined as a Dalton (Da).

One Dalton is defined as 1/12 the mass of a single carbon-12 atom.

Thus, one ^{12}C atom has a mass of 12.0000 Da.

Isotopes

+Most elements have more than one stable isotope.

For example, most carbon atoms have a mass of 12 Da, but in nature, 1.1% of C atoms have an extra neutron, making their mass 13 Da.

+Why do we care?

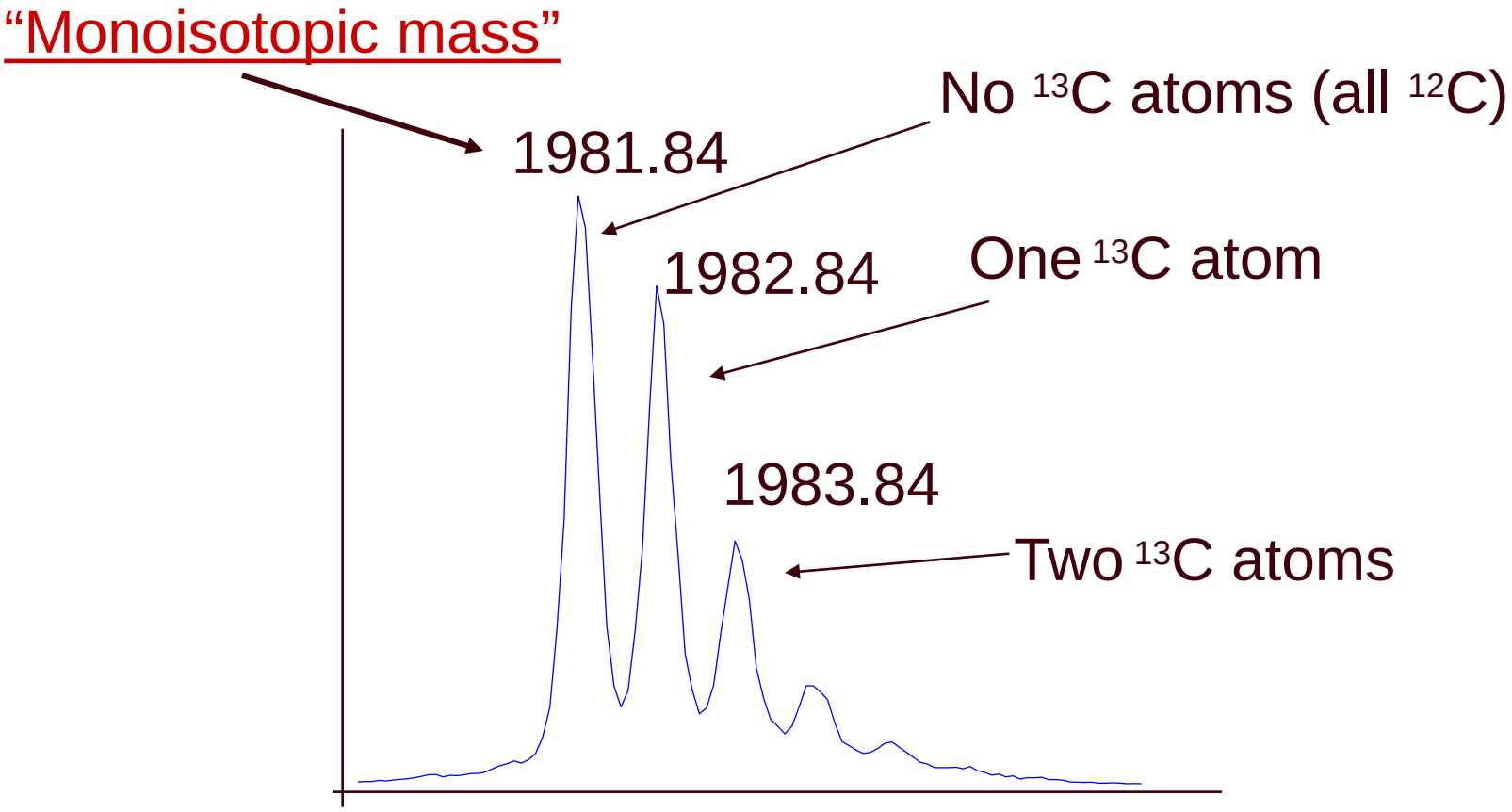
Mass spectrometers can “see” isotope peaks if their resolution is high enough.

If an MS instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.

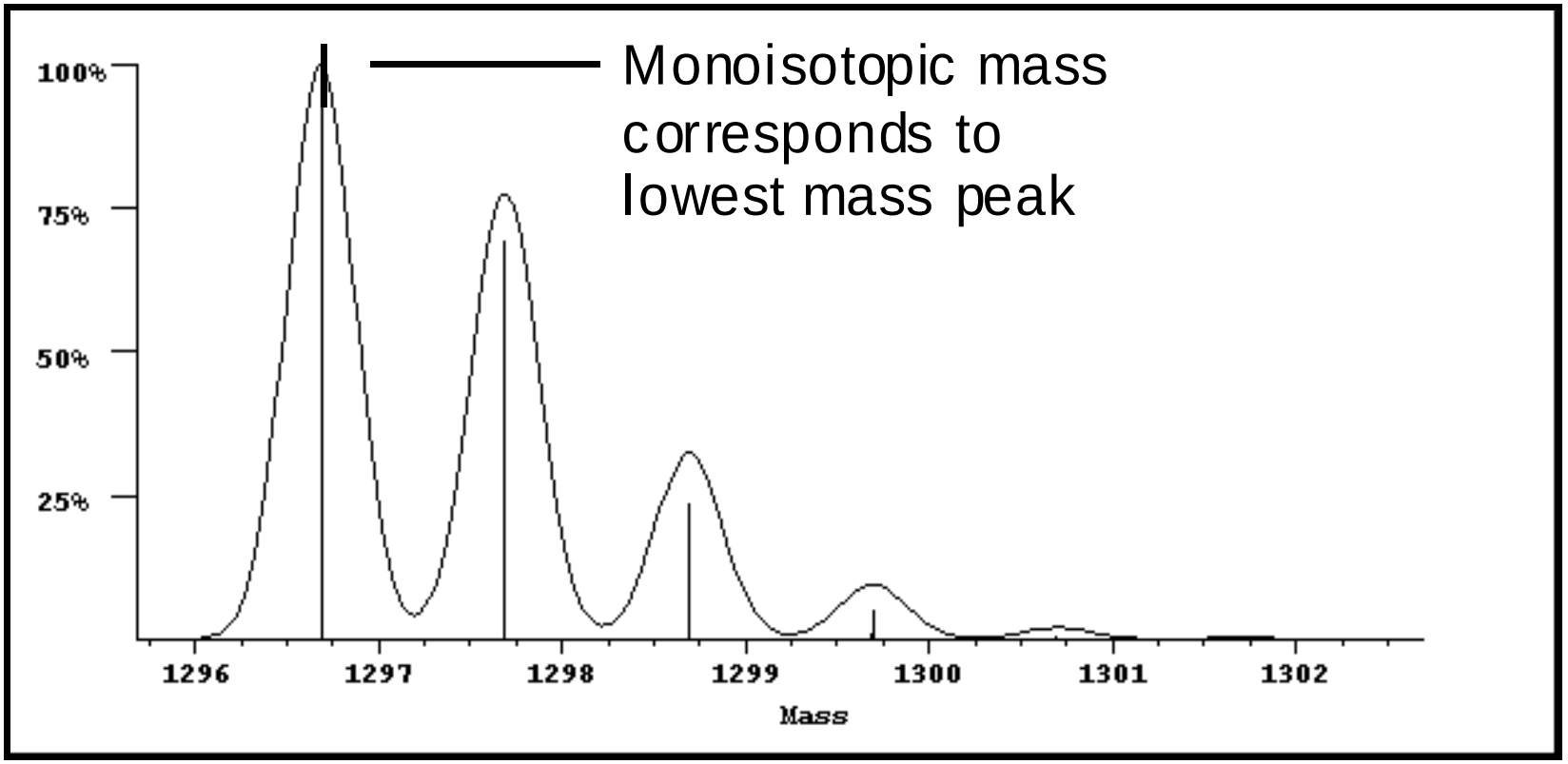
Stable isotopes of most abundant elements of peptides

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

Mass spectrum of peptide with 94 C-atoms (19 amino acid residues)

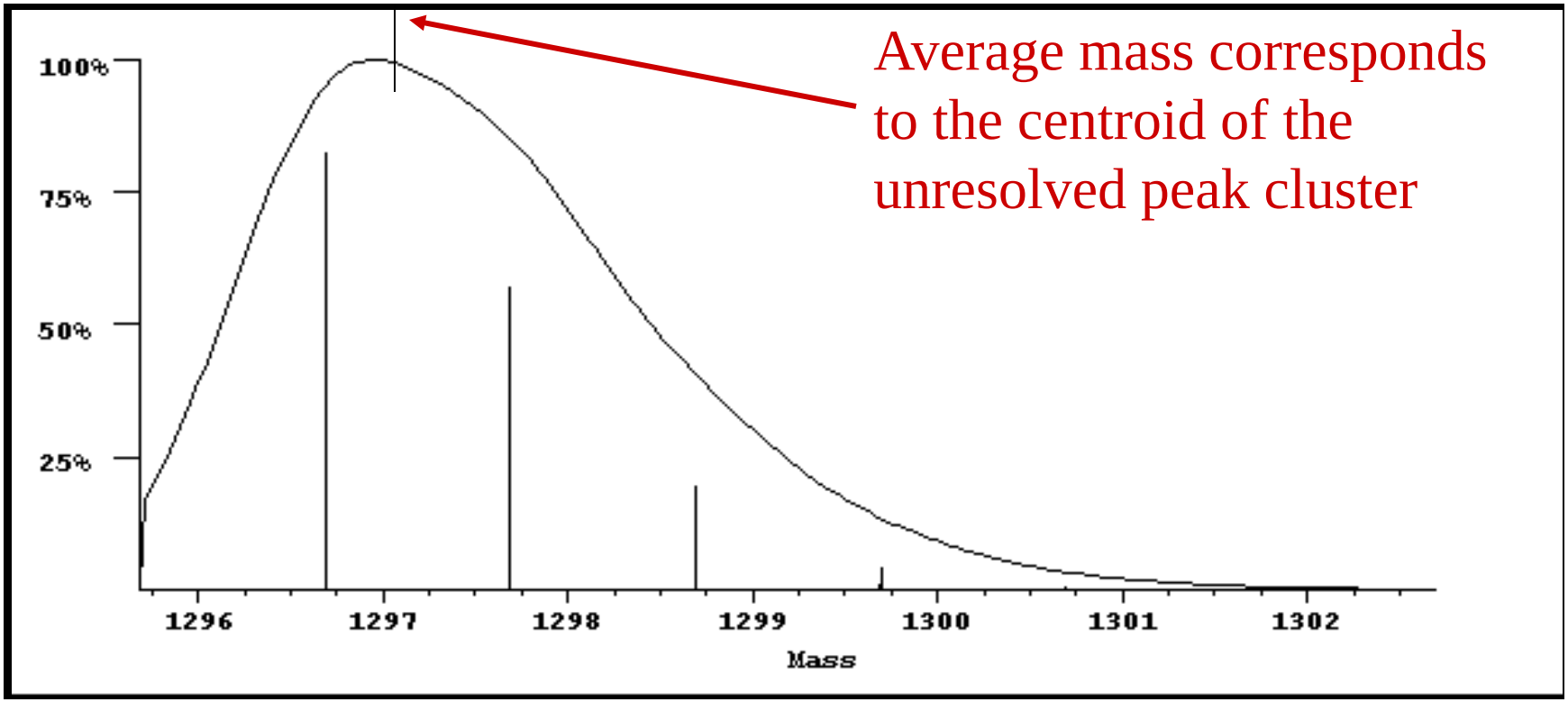


Monoisotopic mass



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

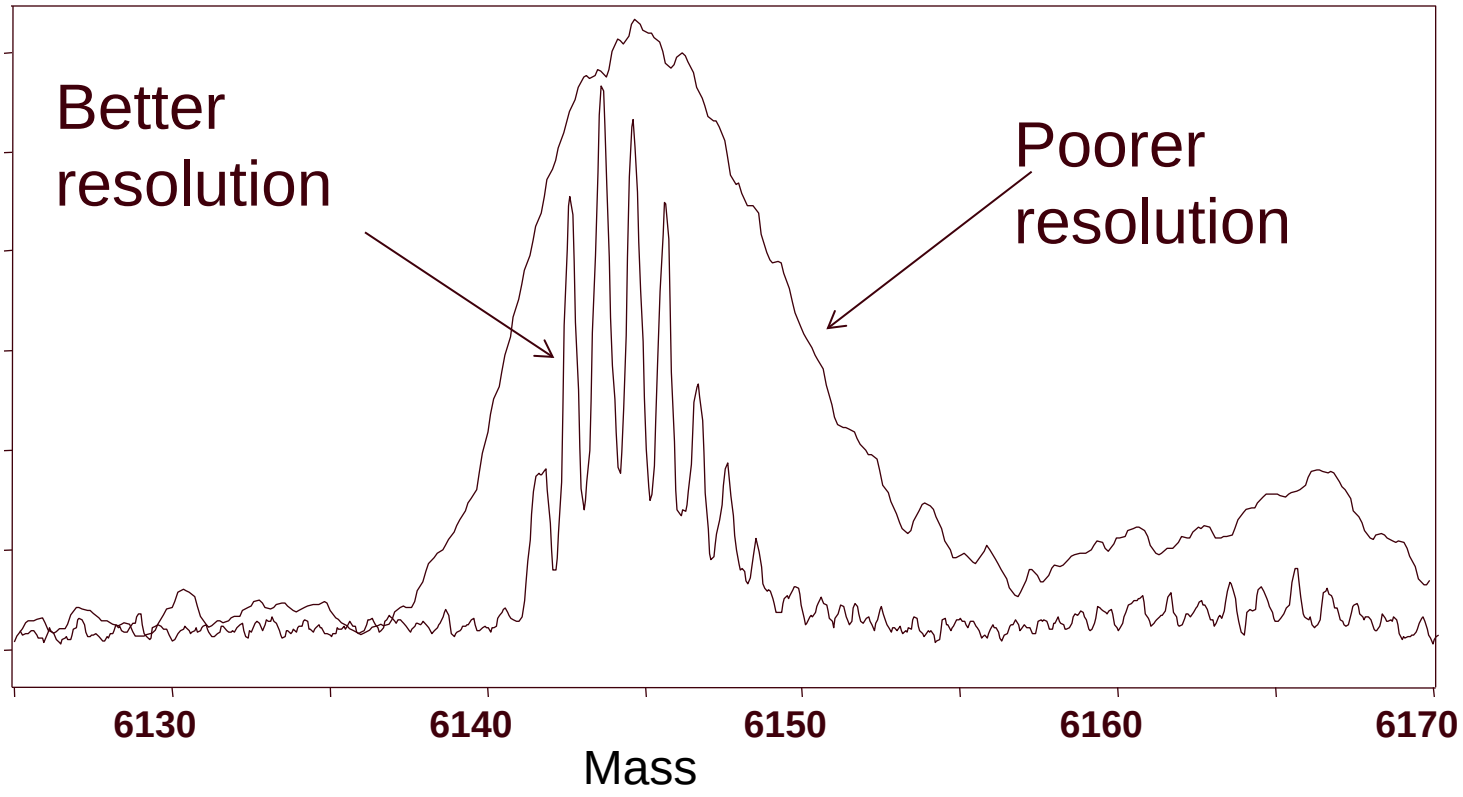
Average mass



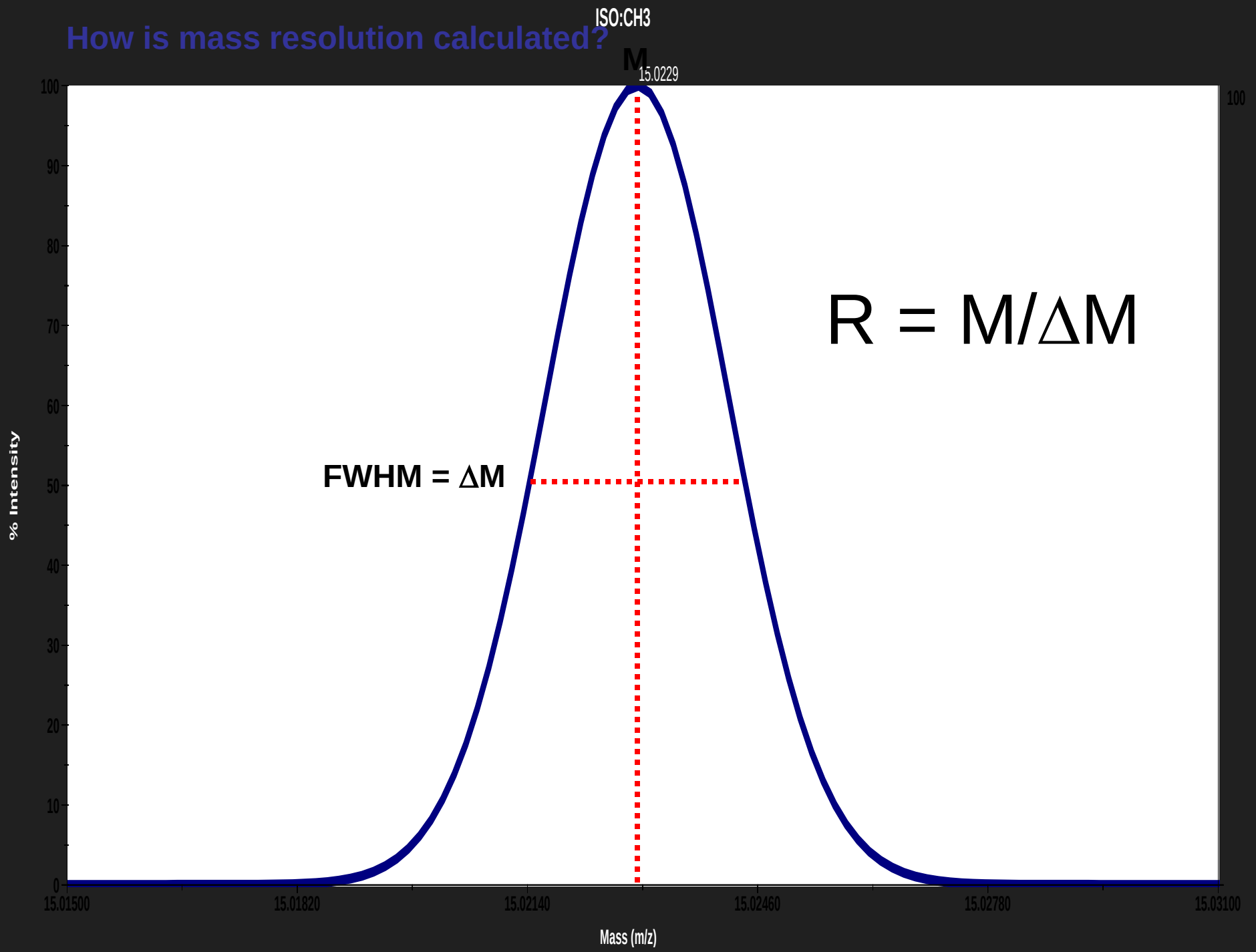
When the isotopes are not resolved, the centroid of the envelope corresponds to the weighted average of all the the isotope peaks in the cluster, which is the same as the average or chemical mass.

What if the resolution is not so good?

At lower resolution, the mass measured is the average mass.

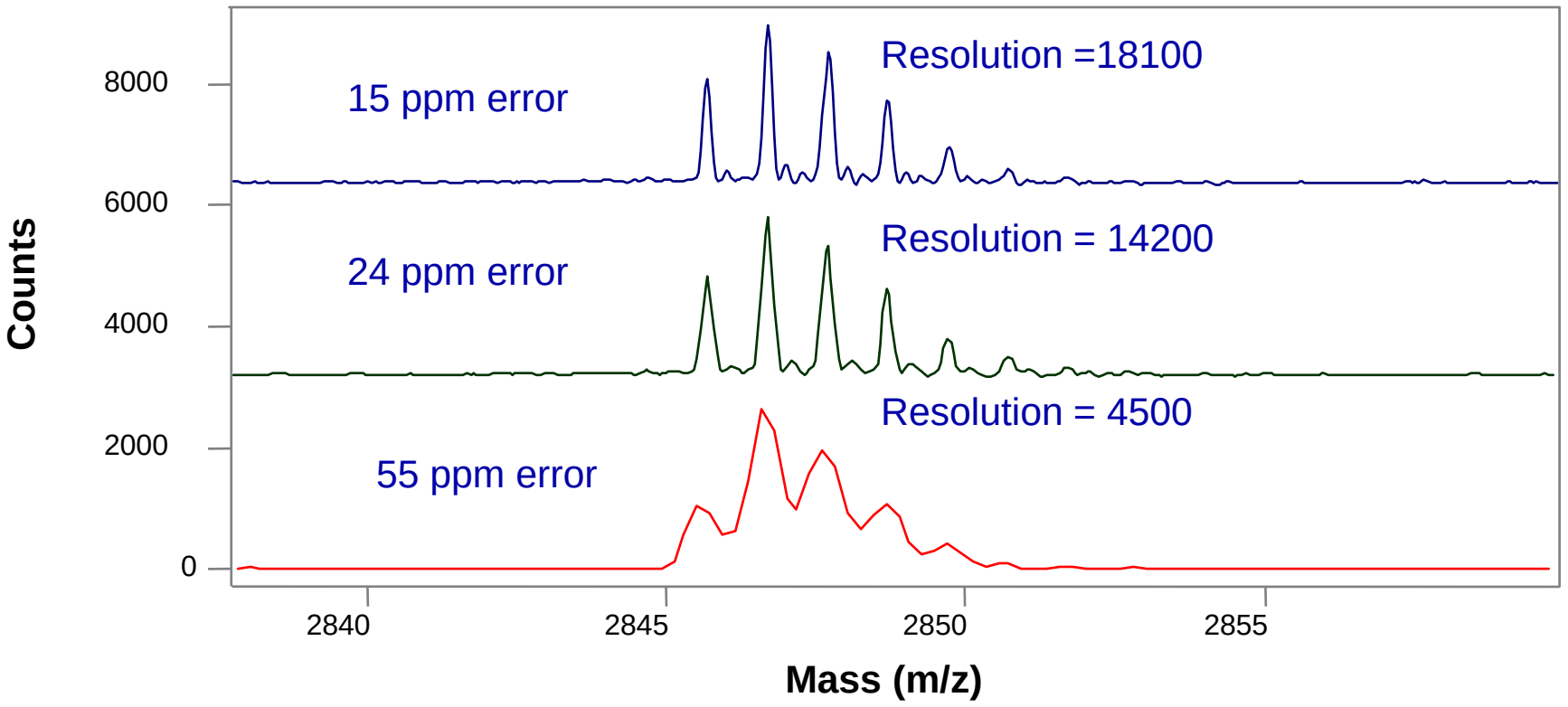


How is mass resolution calculated?



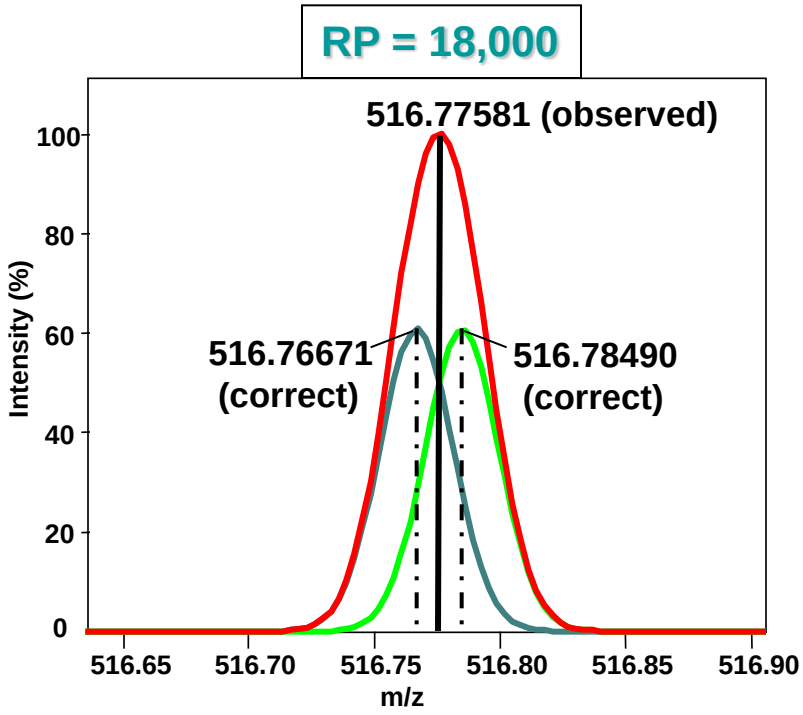
Mass measurement accuracy depends on resolution

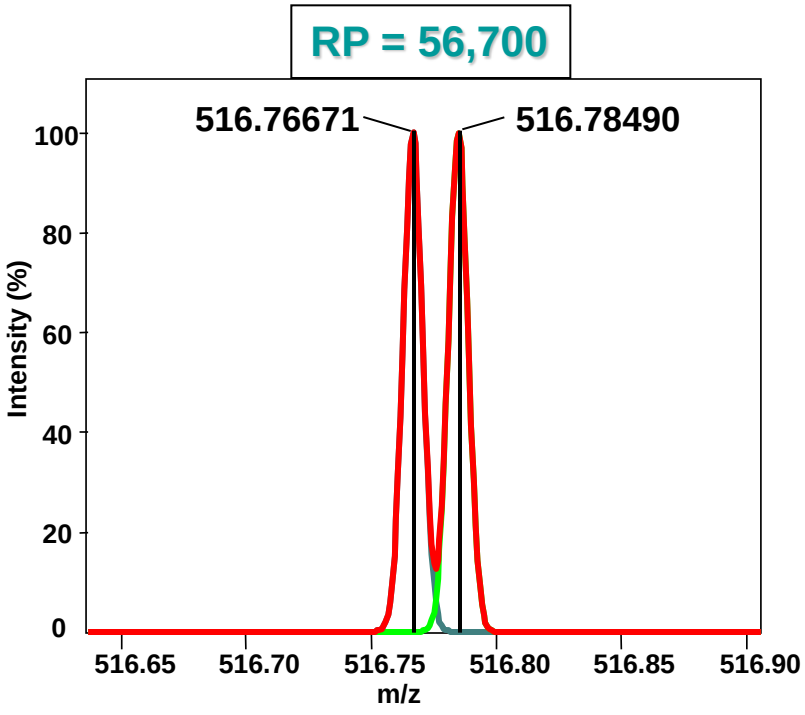
High resolution means better mass accuracy



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**	



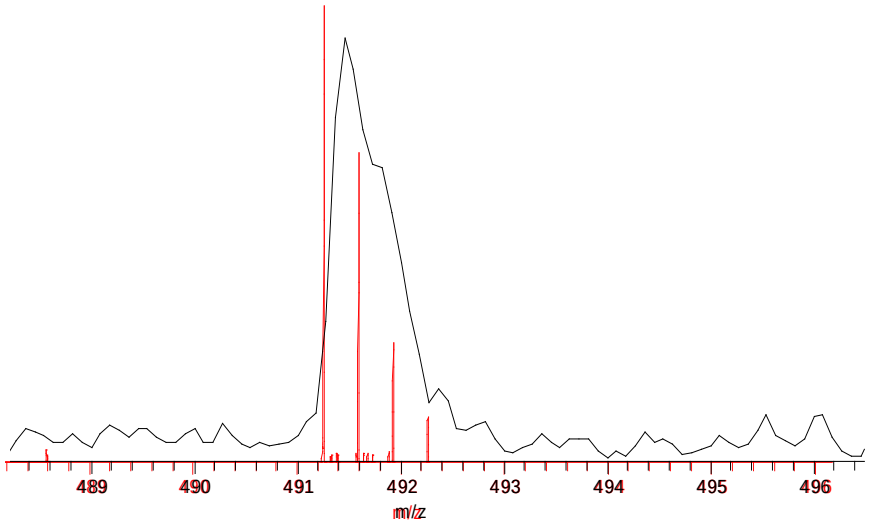


Is Mass Accuracy Important ?

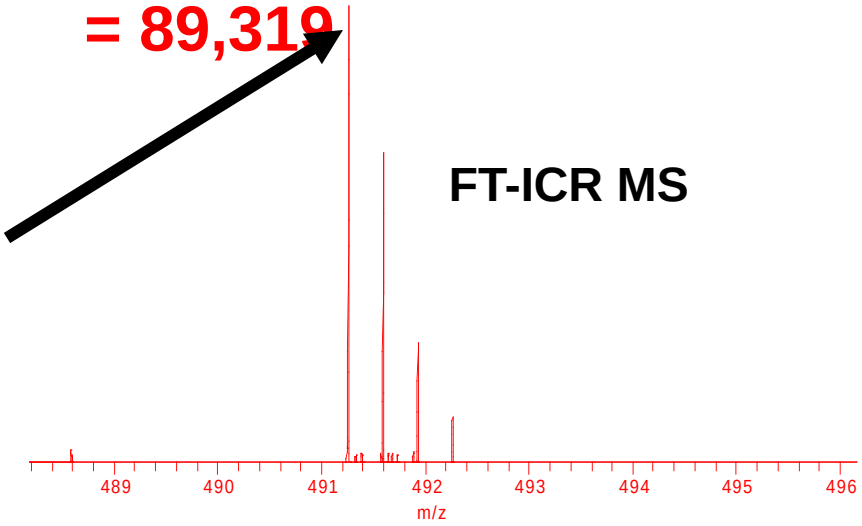
Results for error limit up to 5 ppm

	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_{16} 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_{16} N_{16}
	516.76907	-4.6	-2.4	15.5	C_{48} H_{77} O_{13} N_{10} S_{1}

Mass Resulution = m / Δm $_{50\%}$

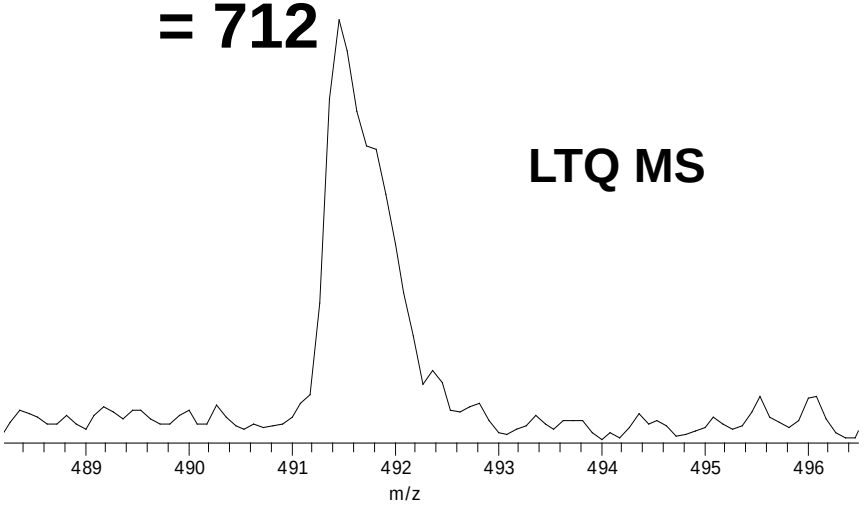


RP = 491.2594 / 0.0055 amu
= 89,319



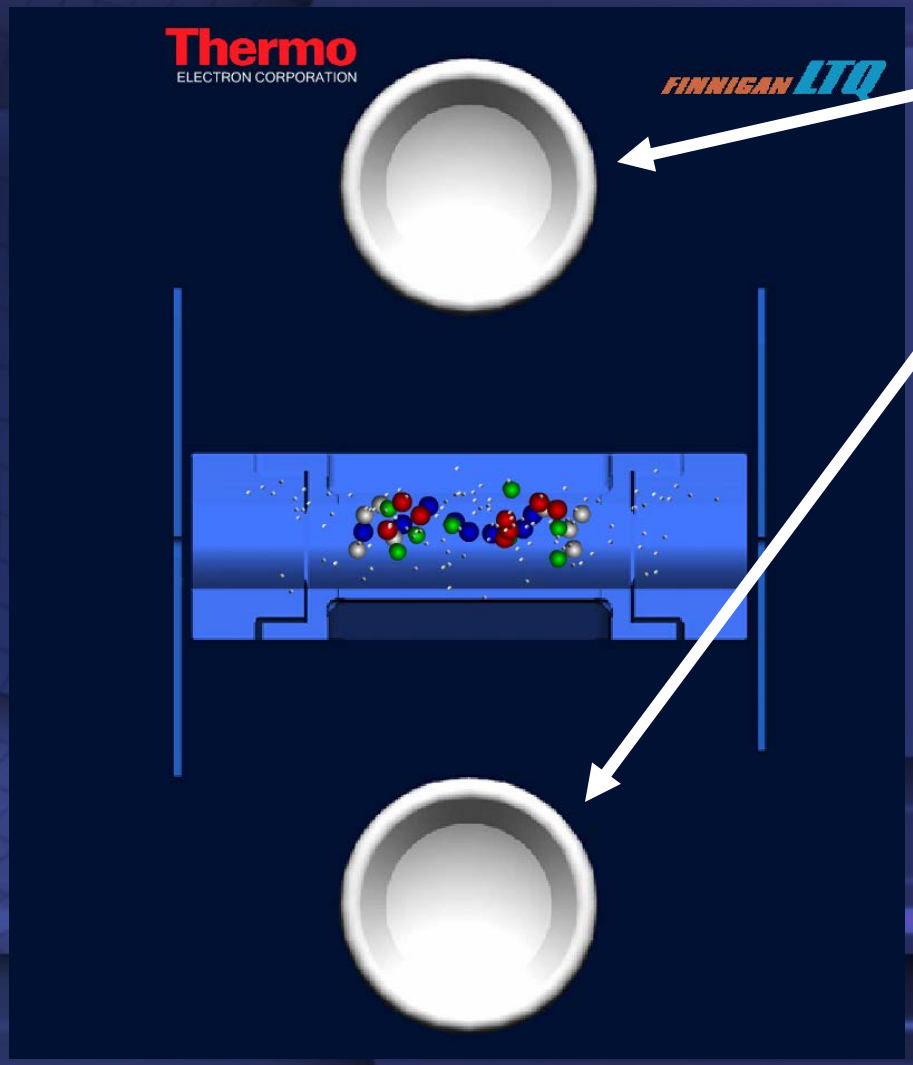
FT-ICR MS

RP = 491.45 / 0.69 amu
= 712



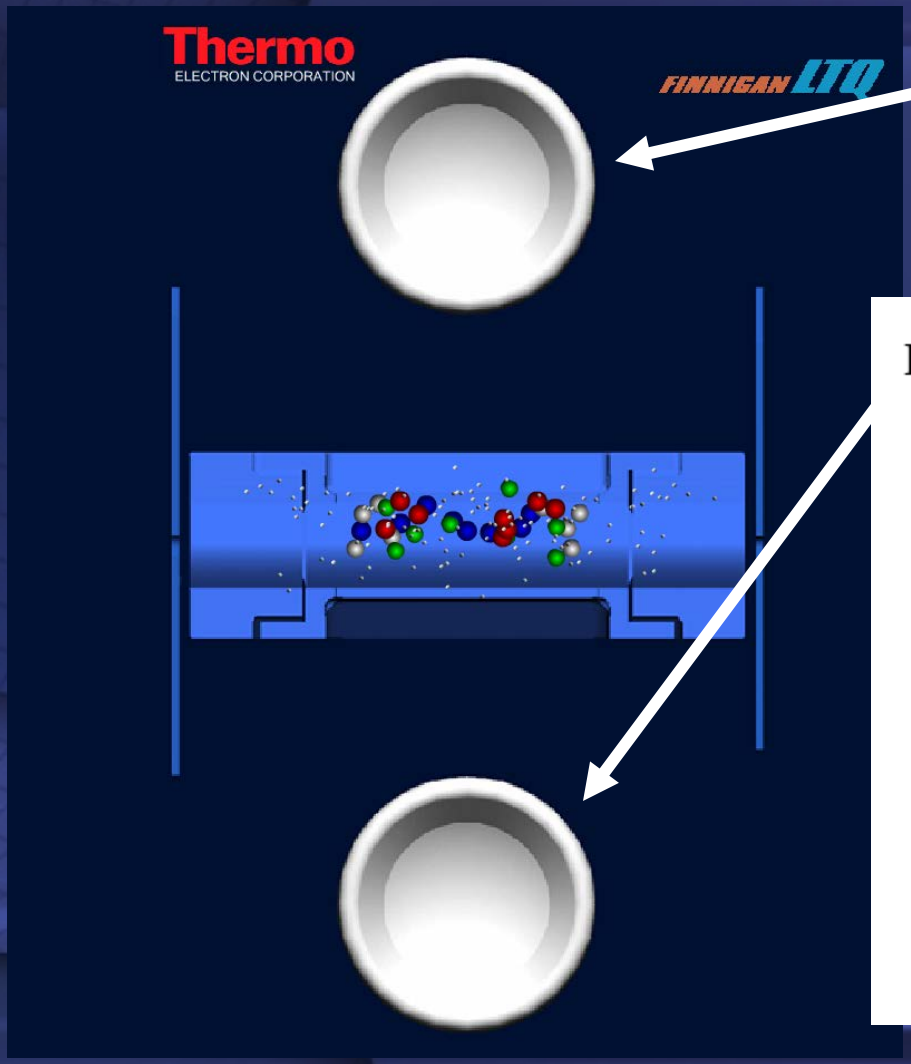
LTQ MS

2D ion trap detection

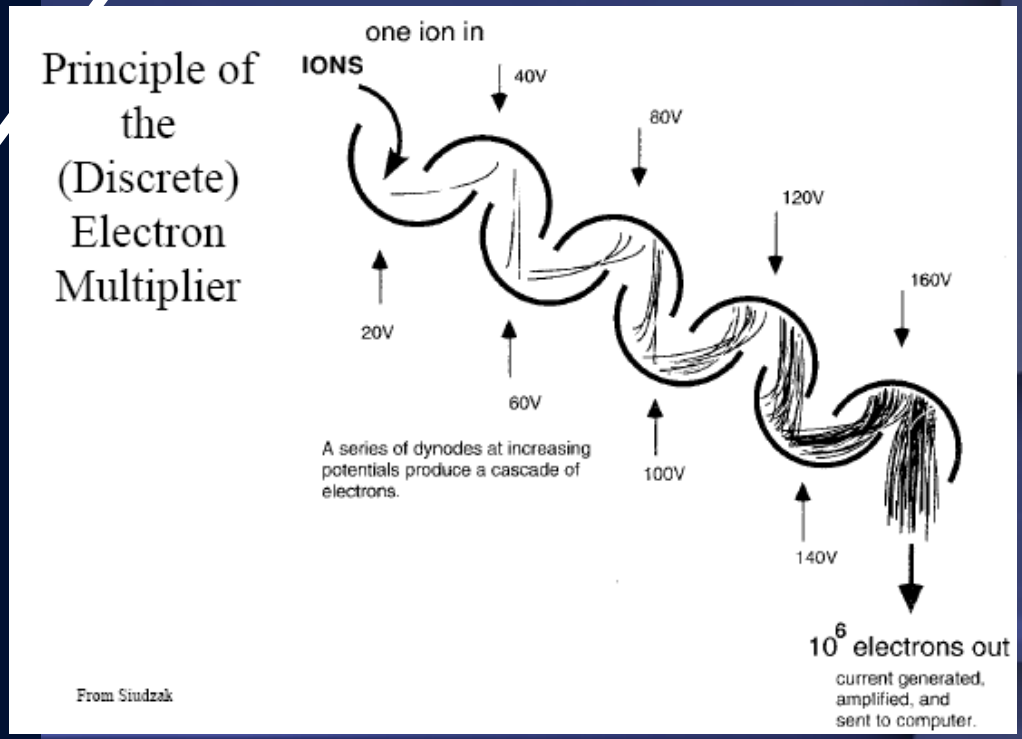


Conversion dynodes

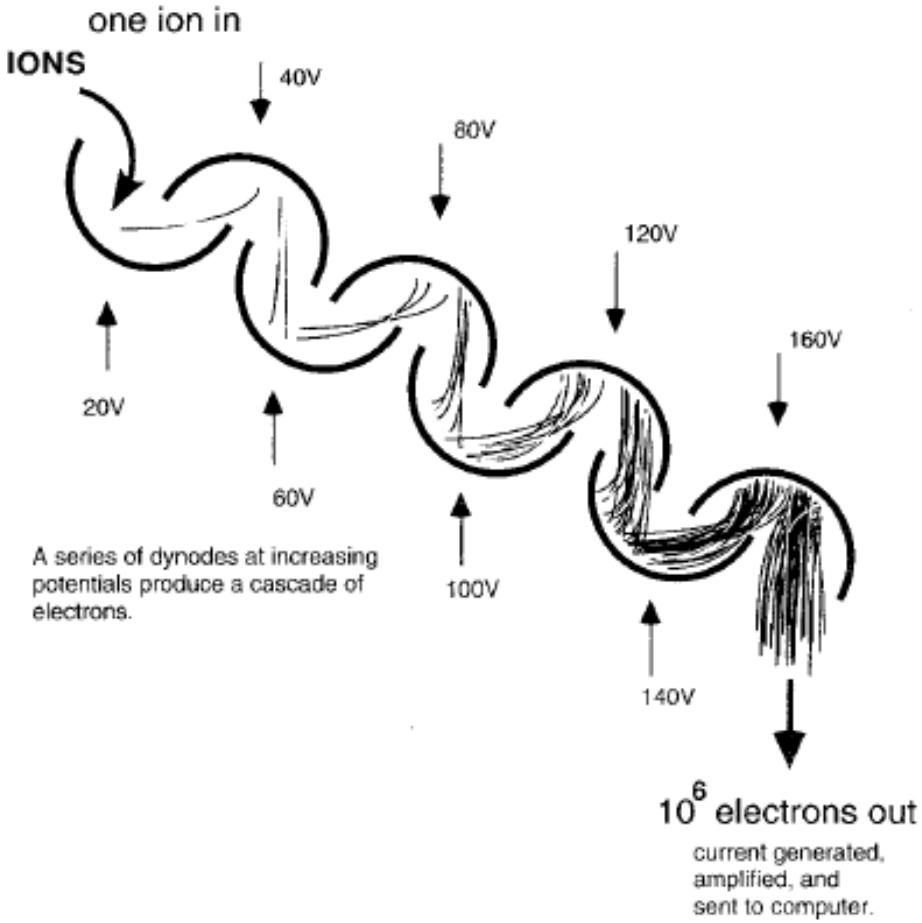
2D ion trap detection



Conversion dynodes (electron multipliers)

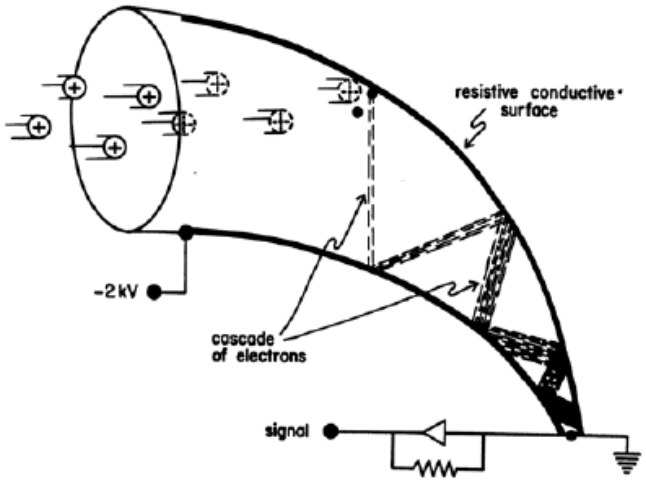


Principle of the (Discrete) Electron Multiplier



A series of dynodes at increasing potentials produce a cascade of electrons.

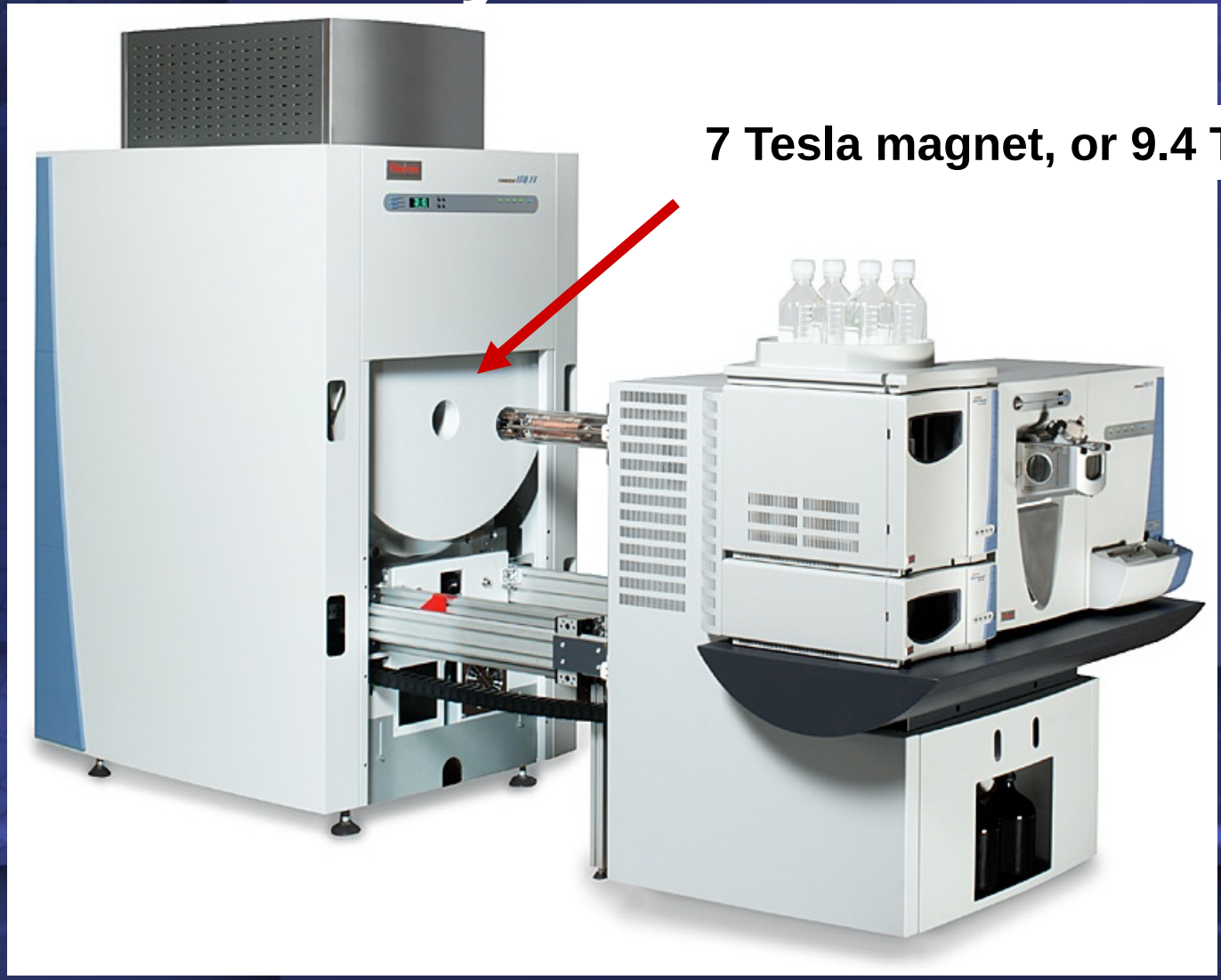
Continuous Dynode Electron Multiplier



From Watson

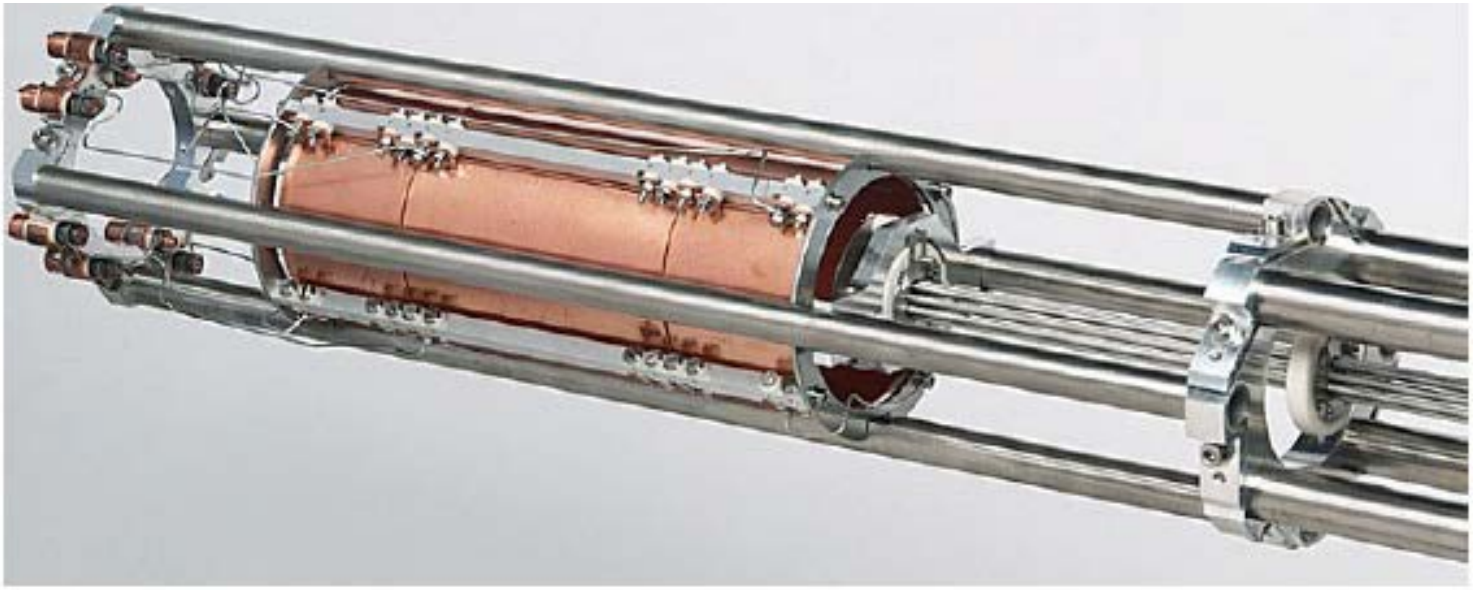
FIG. 13.3. Conceptual diagram of a nonmagnetic electron multiplier; the field gradient along the resistive conductive internal surface of the cornucopia attracts the cascading electrons toward the preamplifier.

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

Penning Trap (ICR cell)



Penning Trap (ICR cell)

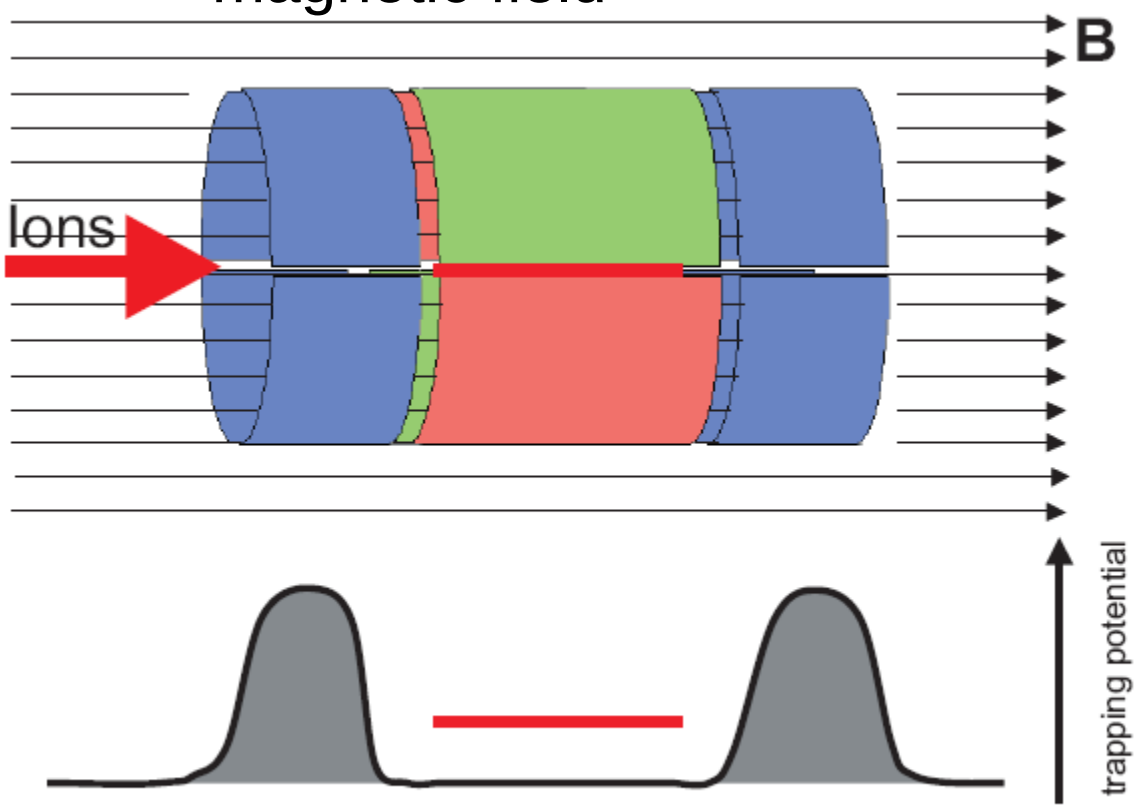
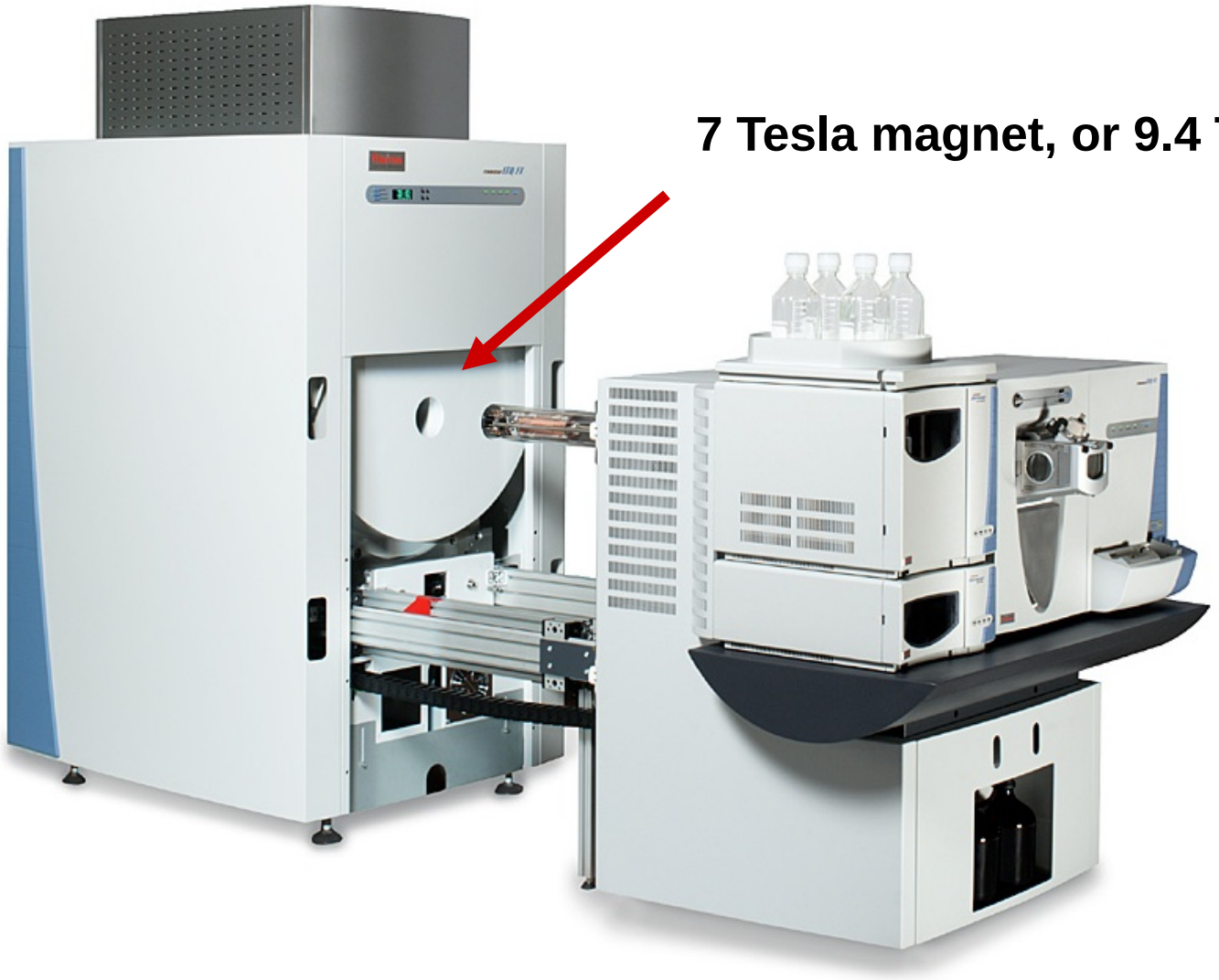


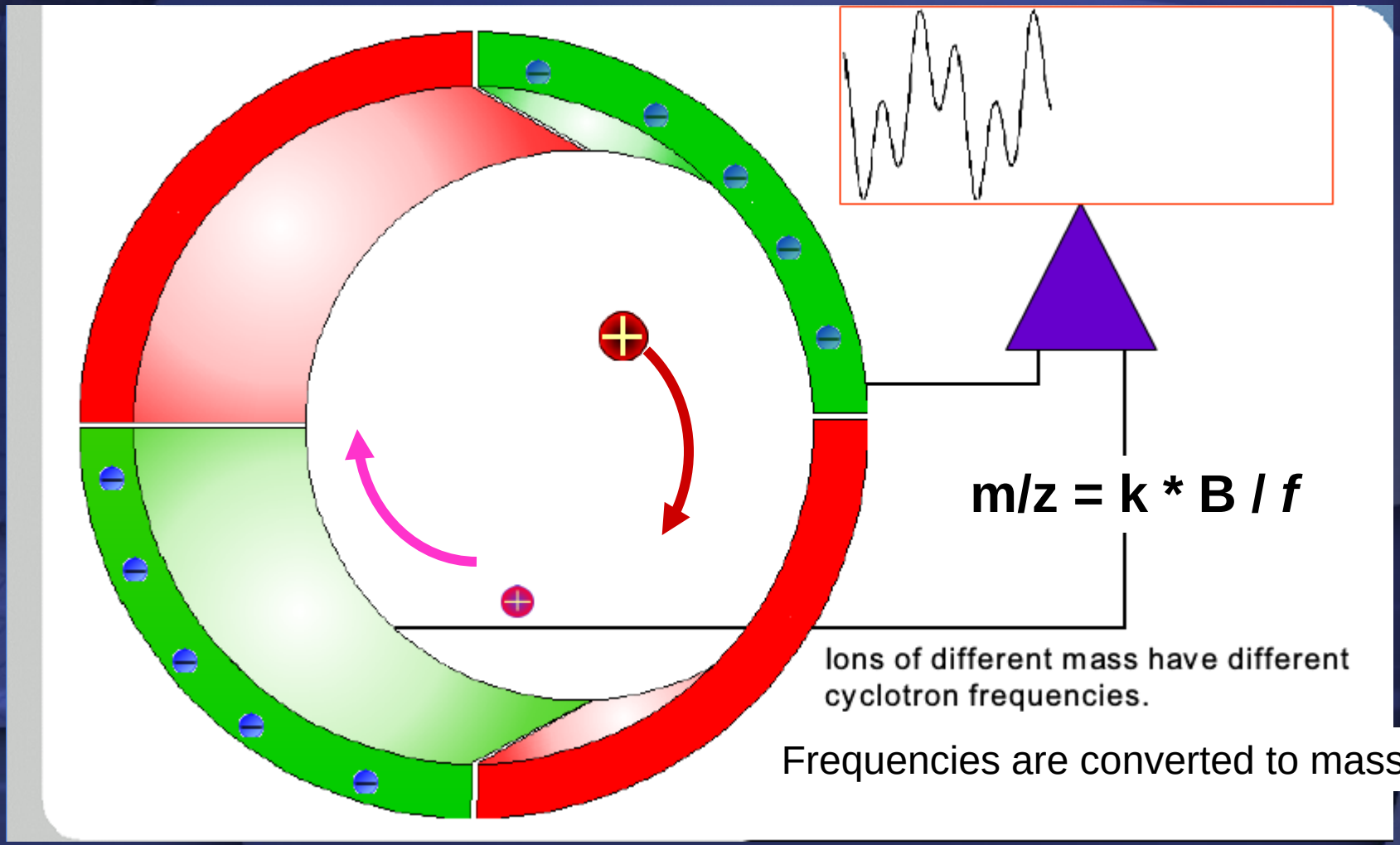
Figure 1-14. Operating principle of the Penning trap

Detecting in the ion trap
Ion cyclotron resonance (ICR)



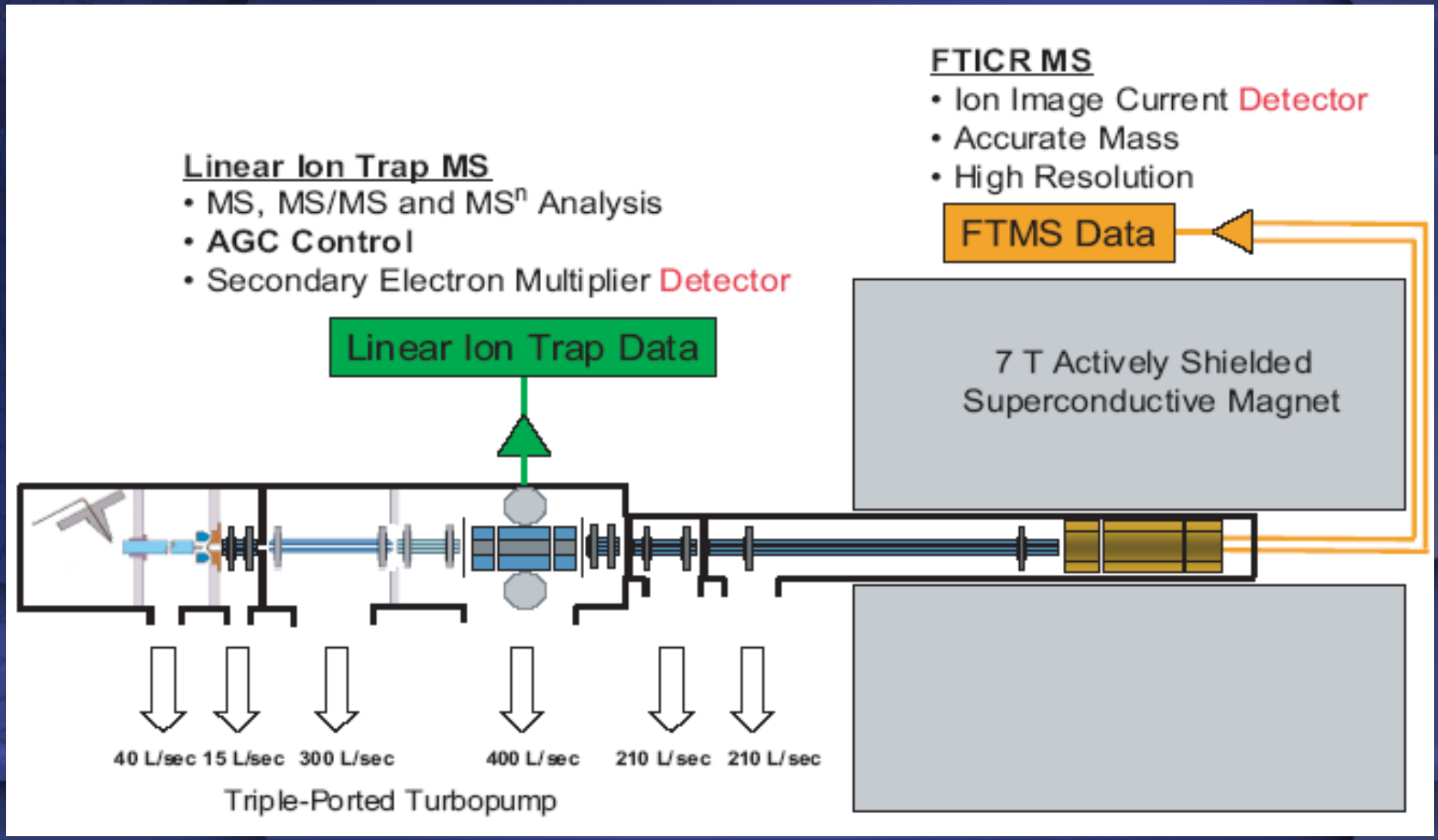
7 Tesla magnet, or 9.4 T or 12 T or 14.5 T

Fourier transform-
Ion cyclotron resonance
FT-ICR MS

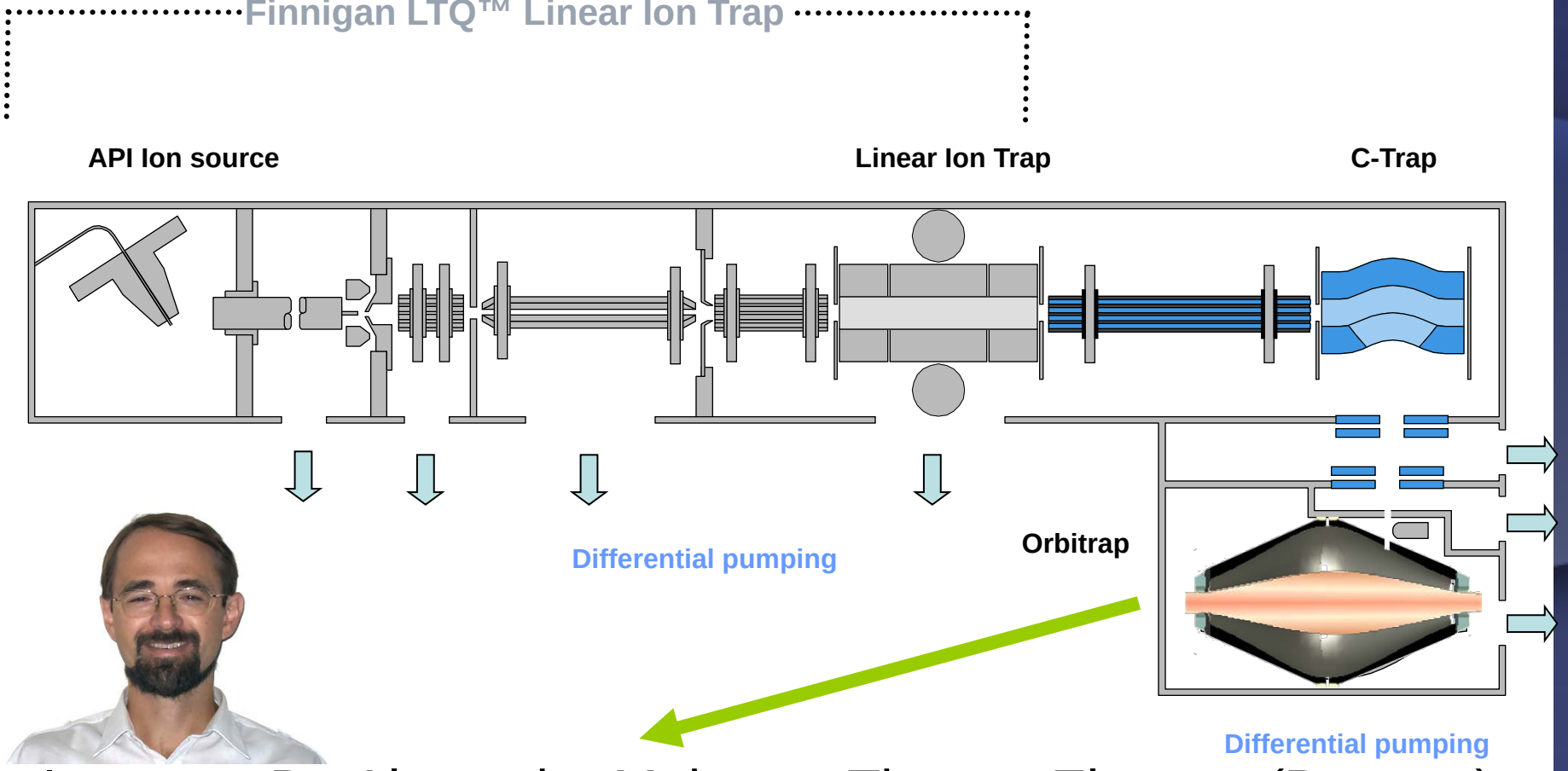


Frequencies are converted to masses.

ThermoFinnigan LTQ-FT



LTQ Orbitrap™ Hybrid Mass Spectrometer



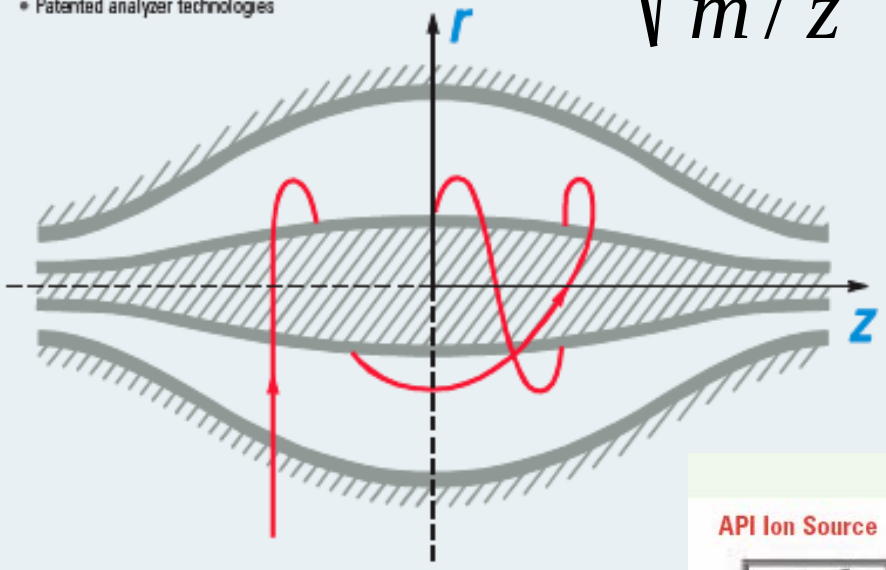
Inventor: Dr. Alexander Makarov, Thermo Electron (Bremen)

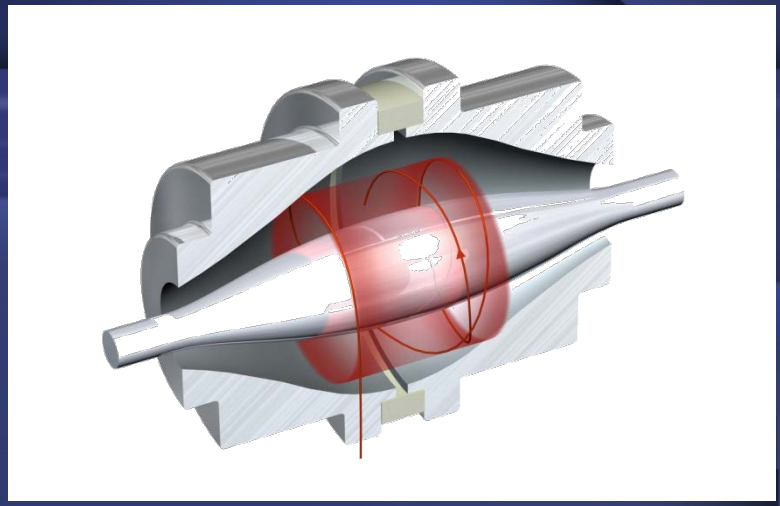
Orbitrap Mass Analyzer

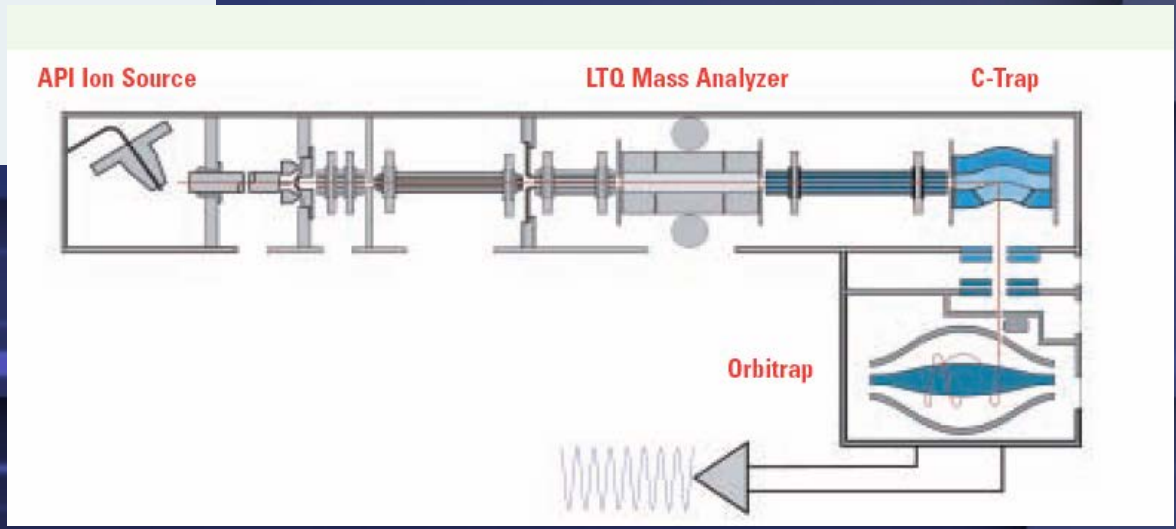
Orbitrap: A Breakthrough Electrostatic Ion Trap

- Highest ion trapping efficiencies
- Large ion capacity
- Stable and robust operation
- Patented analyzer technologies

$$\omega = \sqrt{\frac{k}{m/z}}$$







https://www.youtube.com/watch?v=fqfyyravJkA

https://www.youtube.com/watch?v=zJagpUbnv-Y