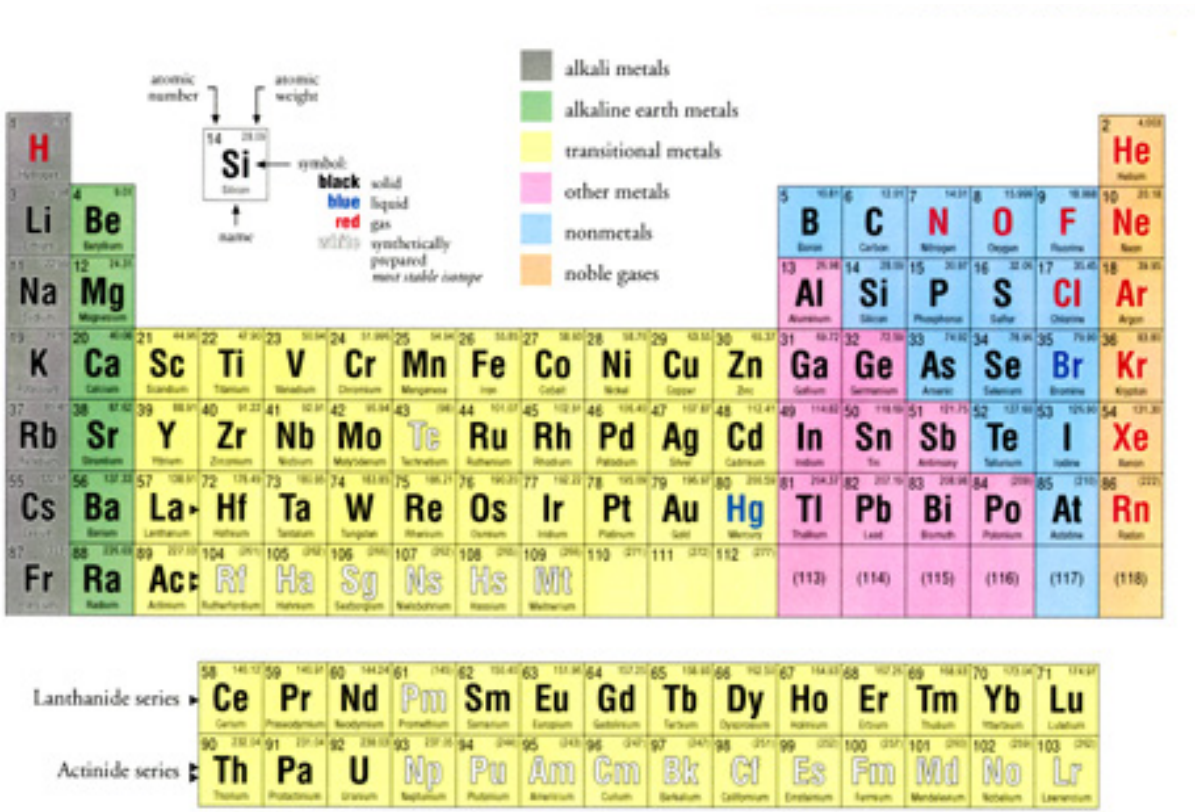
MS Principles

- Different elements can be uniquely identified by their mass



What does a mass spectrometer do?

1. It measures mass better than any other technique.

2. It can give information about chemical structures.

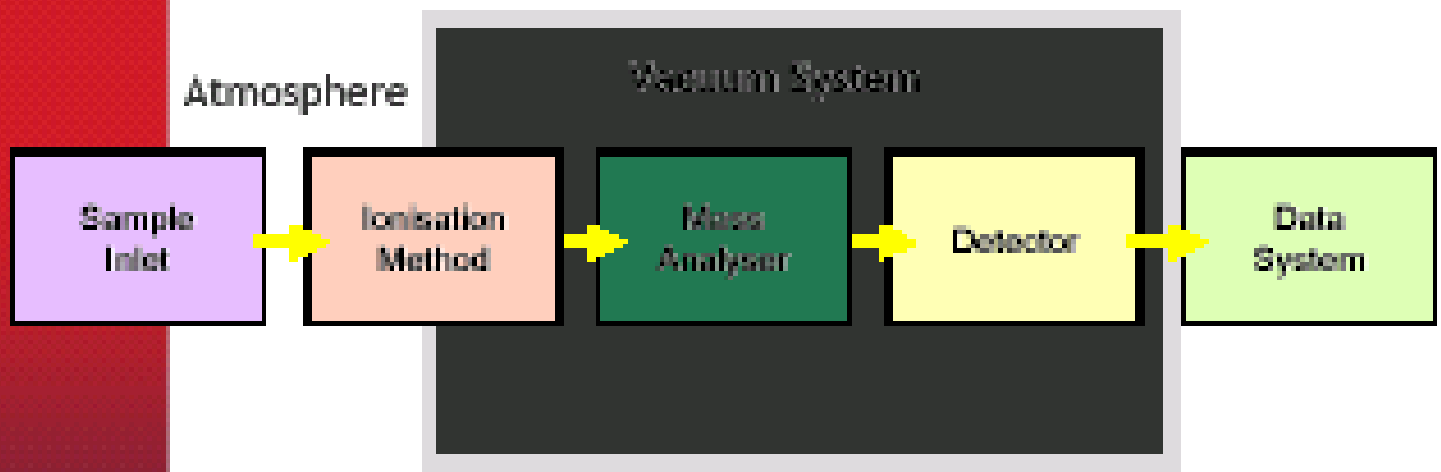
What are mass measurements good for?

To identify, verify, and quantitate: metabolites, recombinant proteins, proteins isolated from natural sources, oligonucleotides, drug candidates, peptides, synthetic organic chemicals, polymers

Applications

- Determination or confirmation of chemical structure of drugs and drug metabolites (MS-MS)
- Detection/quantitation of impurities
- Detection/quantitation of drugs and their metabolites in biofluids and tissues
- High throughput drug screening
- Analysis of liquid mixtures (LC-MS)

Components of a Mass Spectrometer

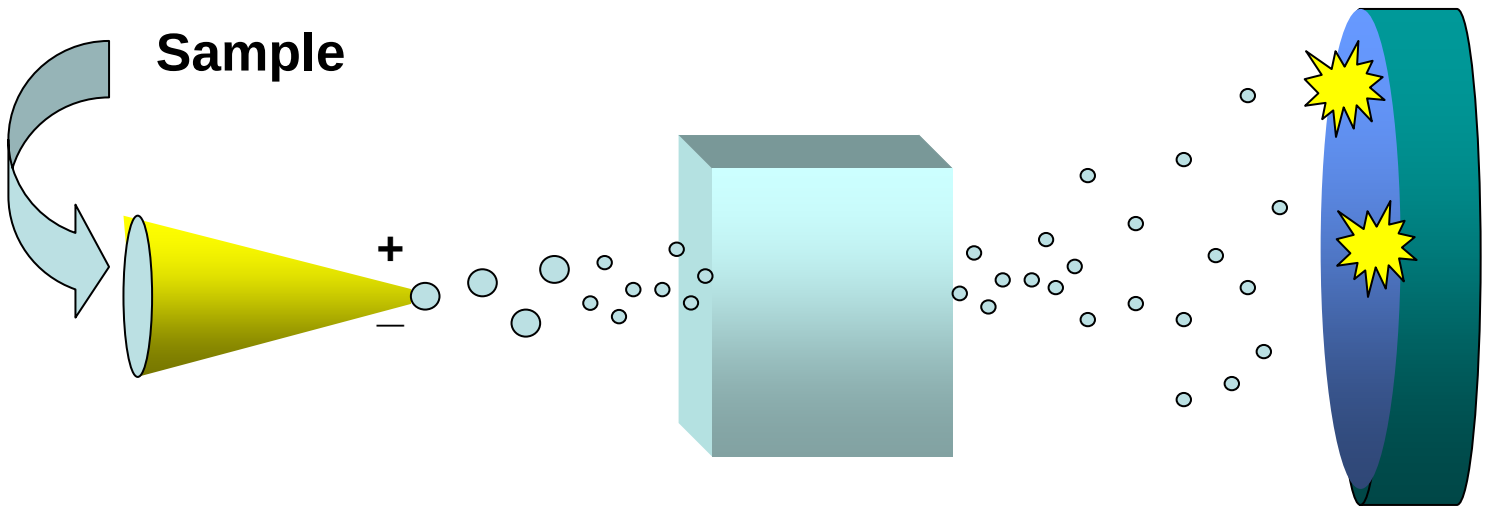


Basic features of Mass Spectrometry

- Mass spectrometry can only analyse charged molecules, i.e. ions
- Charged species are separated on the basis of their m/z ratio

- Molecules are ionised within the Ion Source
- Ions are separated into the Analyser

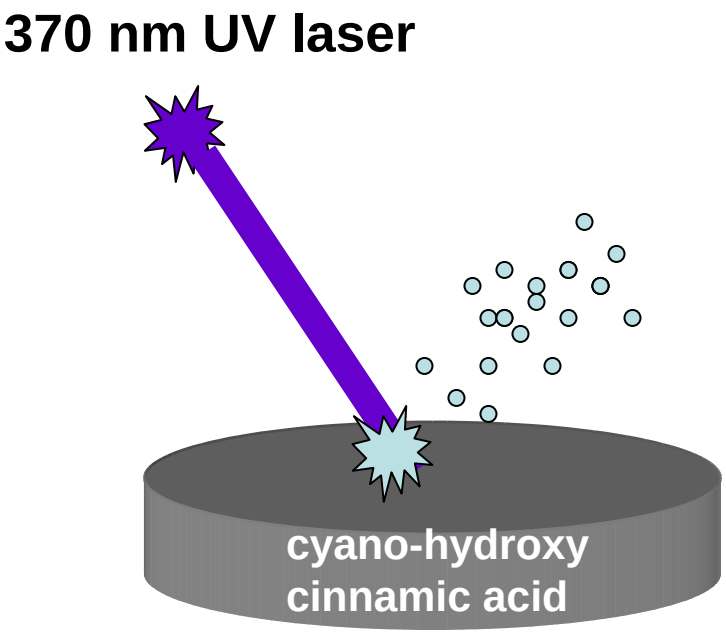
Mass Spec Principles



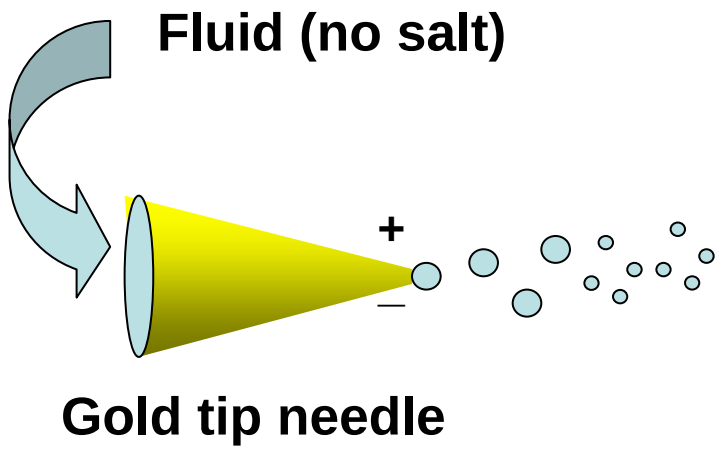
Different Ionization Methods

- Electron Impact (EI - Hard method)
  - small molecules, 1-1000 Daltons, structure
- Fast Atom Bombardment (FAB - Hard)
  - peptides, sugars, up to 6000 Daltons
- Electrospray Ionization (ESI - Soft)
  - peptides, proteins, up to 200,000 Daltons
- Matrix Assisted Laser Desorption (Soft)
  - peptides, proteins, DNA, up to 500 kD

Soft Ionization Methods

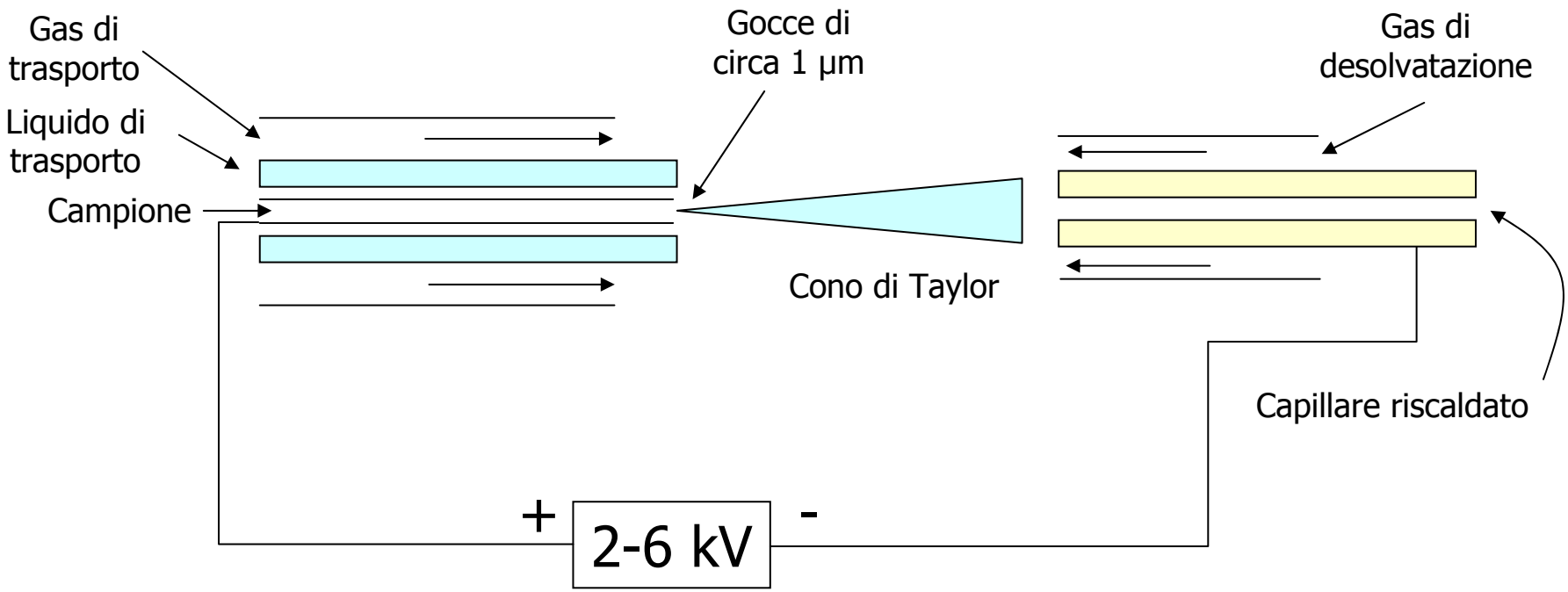


MALDI

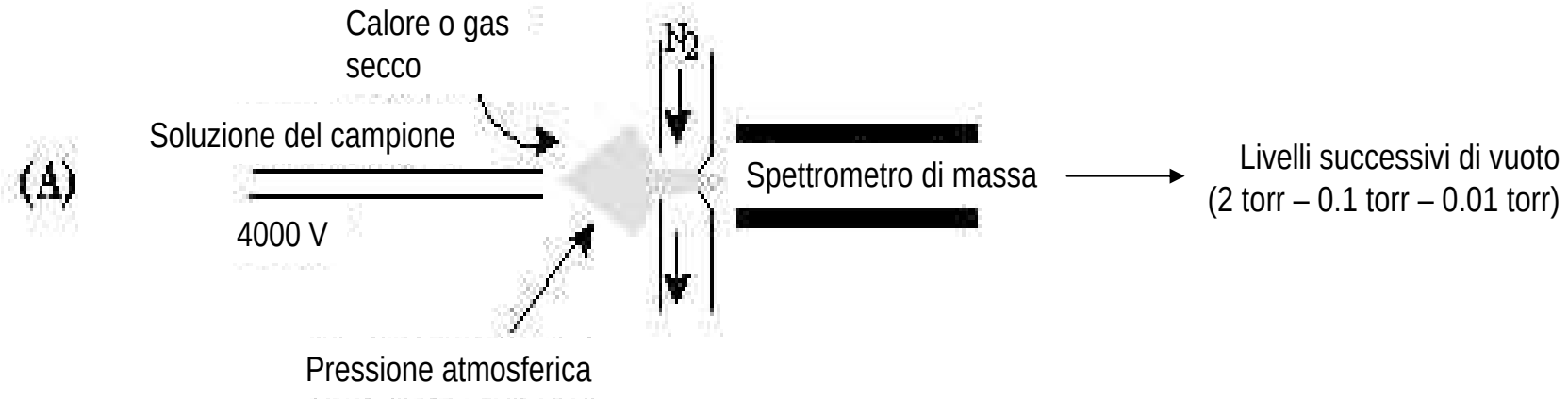


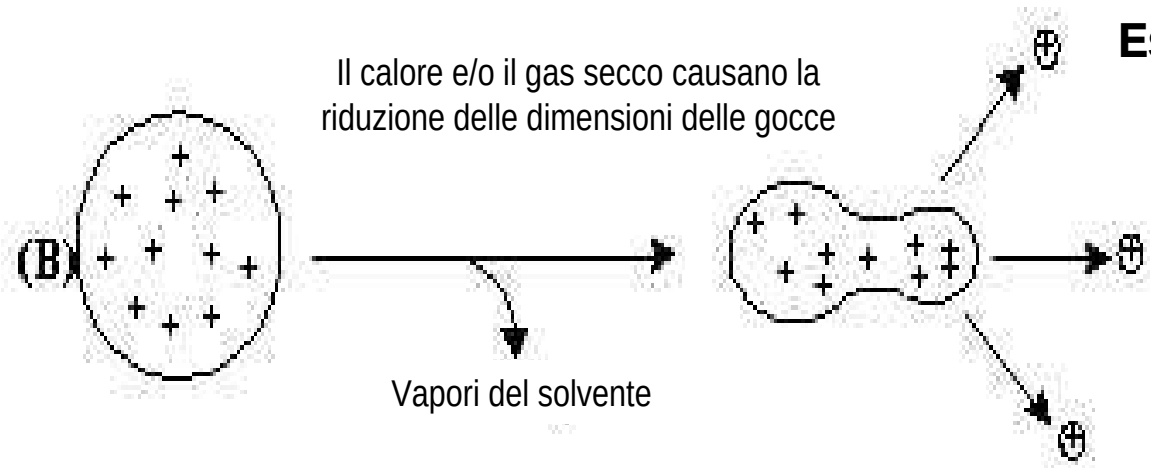
ESI

Sorgente elettrospray



Meccanismo di deplezione degli ioni





Esplosione Coulombiana

Limite di Rayleigh: Limite di dimensioni della goccia al quale si prevede che le molecole cariche vengano espulse.

$M + nH^+ \rightarrow [M + nH]^{n+}$ (polypeptides)

low pH favors protonated cations

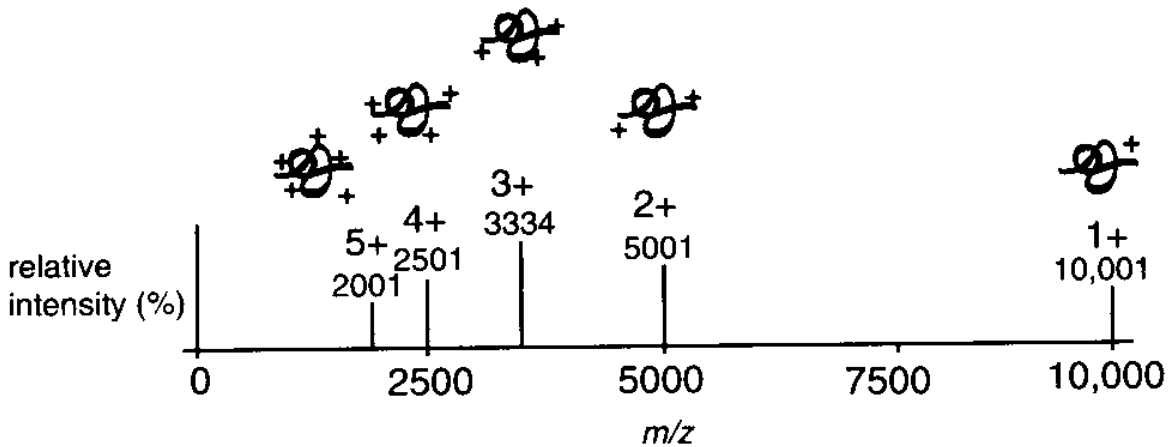
$[M - nH]^{n-}$ (nucleic acids)

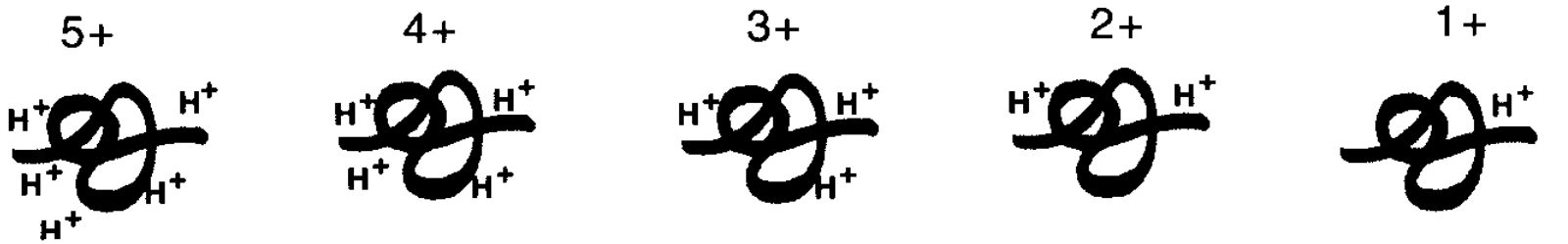
high pH favors
less common (corona discharge problem)

$[M+cat]^+$ $[M+anion]^-$

can help or hurt with adduct formation
too much salt interferes

Una delle caratteristiche dell'ESI è la possibilità di generare ioni multicarica. Per molti composti il numero di cariche è proporzionale alla grandezza del composto in esame per cui il rapporto m/z per le molecole che arrivano all'analizzatore è dell'ordine di 500-2000. Inoltre il numero di cariche assunte dalla molecola dipende sia dalla presenza di gruppi basici che dal pH del solvente.

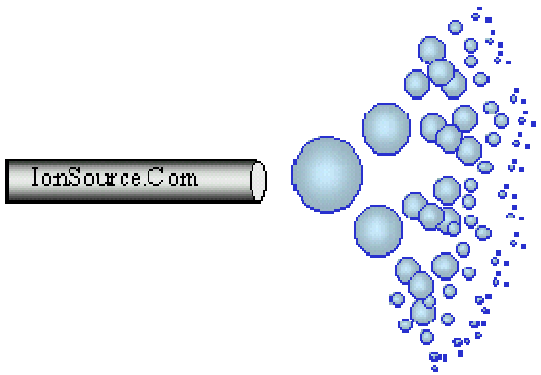


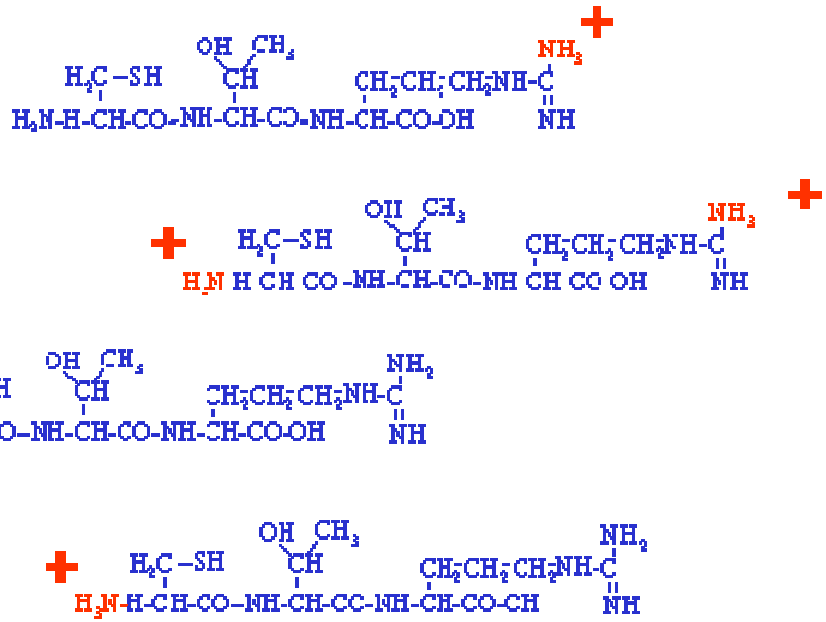


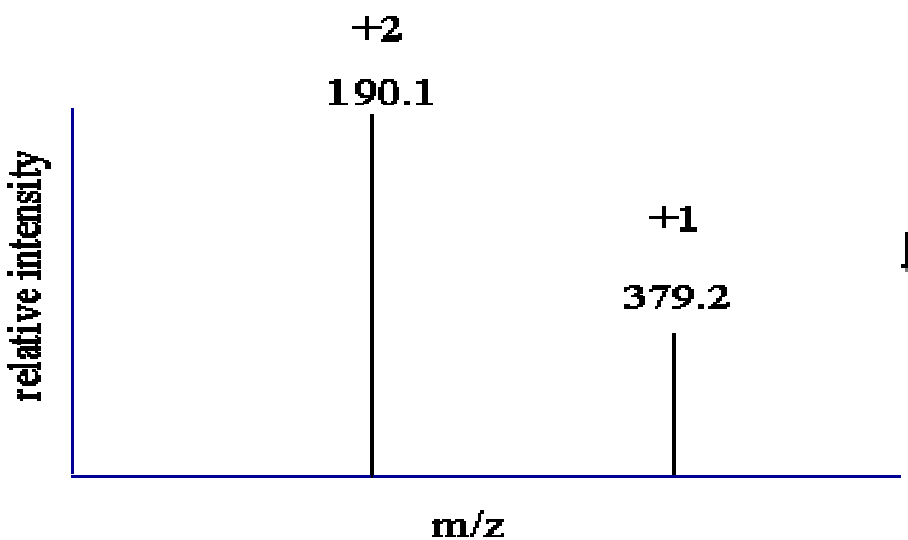
$[M + 5H]^{5+}$ $[M + 4H]^{4+}$ $[M + 3H]^{3+}$ $[M + 2H]^{2+}$ $[M + 1H]^{1+}$

$m/z = 10{,}005 \div 5$ $m/z = 10{,}004 \div 4$ $m/z = 10{,}003 \div 3$ $m/z = 10{,}002 \div 2$ $m/z = 10{,}001 \div 1$

$m/z = 2001$ $m/z = 2501$ $m/z = 3334$ $m/z = 5{,}001$ $m/z = 10{,}001$

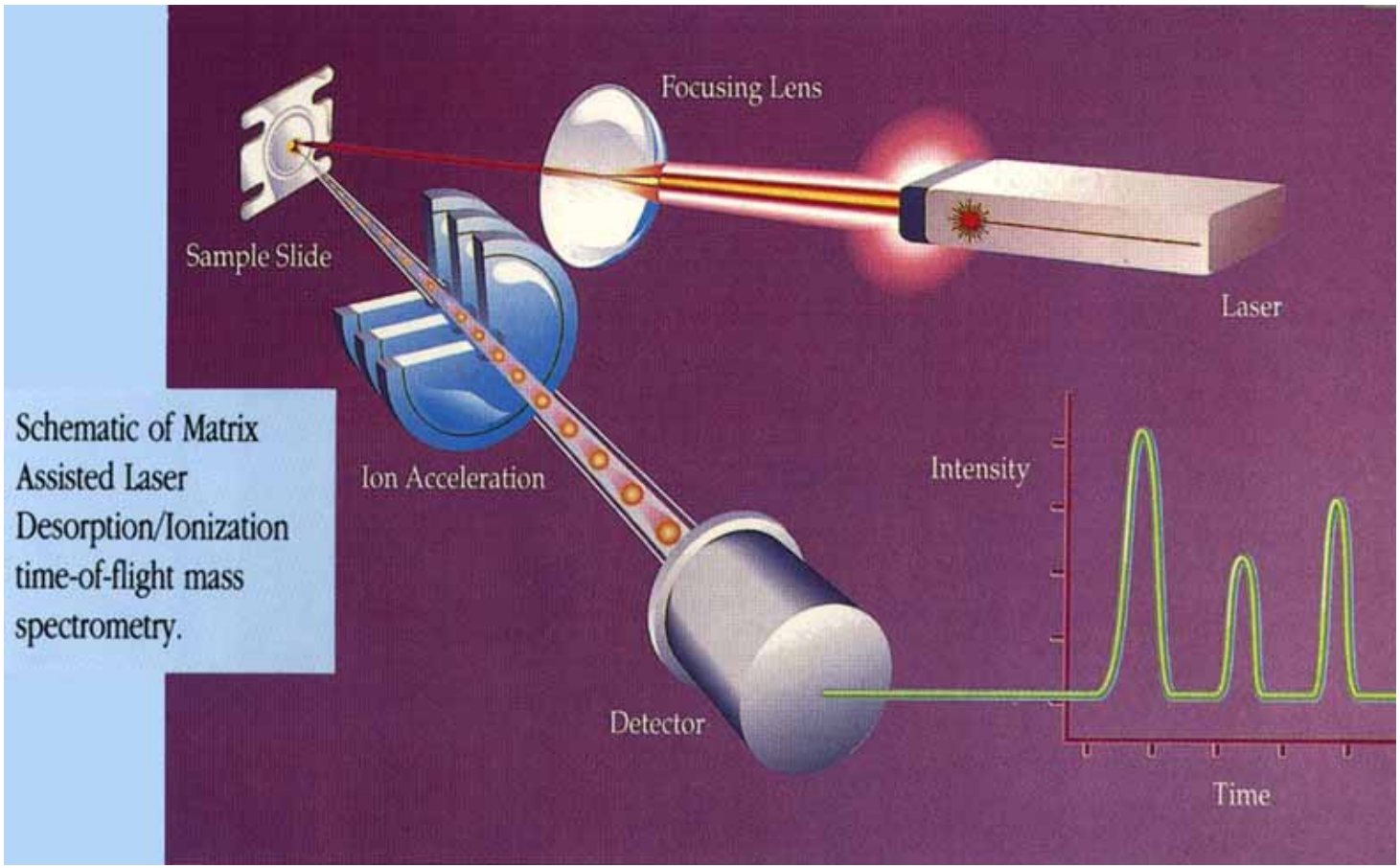






Qual è la massa

MALDI



Schematic of Matrix Assisted Laser Desorption/Ionization time-of-flight mass spectrometry.

Sorgenti ioniche a desorbimento

Desorbimento/ionizzazione laser assistito da matrice (MALDI)

- L'analita viene codepositato su un bersaglio con una matrice, scelta per favorire la formazione degli ioni di analita in fase gas

- Il bersaglio viene colpito da un impulso laser che riscalda la matrice e provoca la ionizzazione e il desorbimento di ioni prevalentemente monocarica

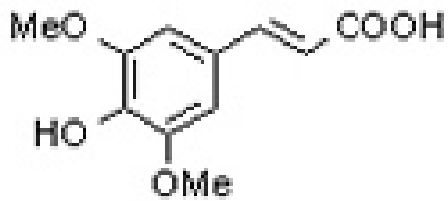
The most commonly used substances

Matrix	Wavelength	Comments
2,5-Dihydroxy benzoic acid (DHB)	337	
DHB + 10% 5-methoxy salicylic acid	337	**used for masses >20 kDa**
Nicotinic acid	266 nm	
4-Hydroxy picolinic acid	337	**used for oligonucleotides**
Glycerol	2.94μm	**liquid matrix**

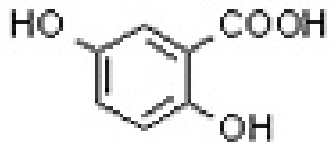
U. Bahr, M. Karas, F. Hillenkamp, *Fresenius J. Anal. Chem.* **348**, 784 (1994).

La matrice assorbe energia dall'irradiazione della luce laser, si riscalda e può agire conseguentemente come donatore di protoni formando ioni pseudomolecolari di tipo $[M+H]^+$. Per composti con una elevata affinità per i cationi si possono formare anche addotti di tipo $[M+Na]^+$, $[M+K]^+$.

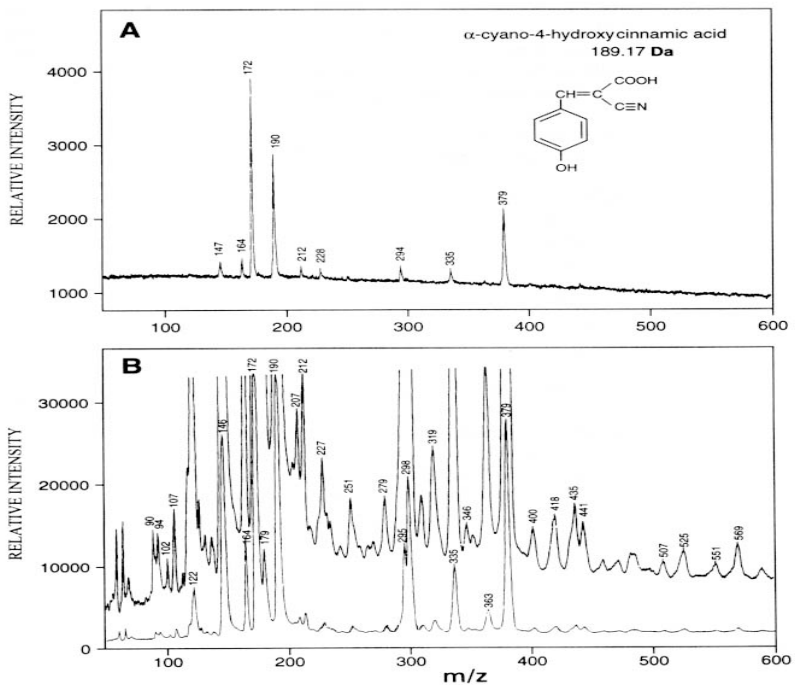
alpha-cyano-hydroxycinnamic acid (CHCA)



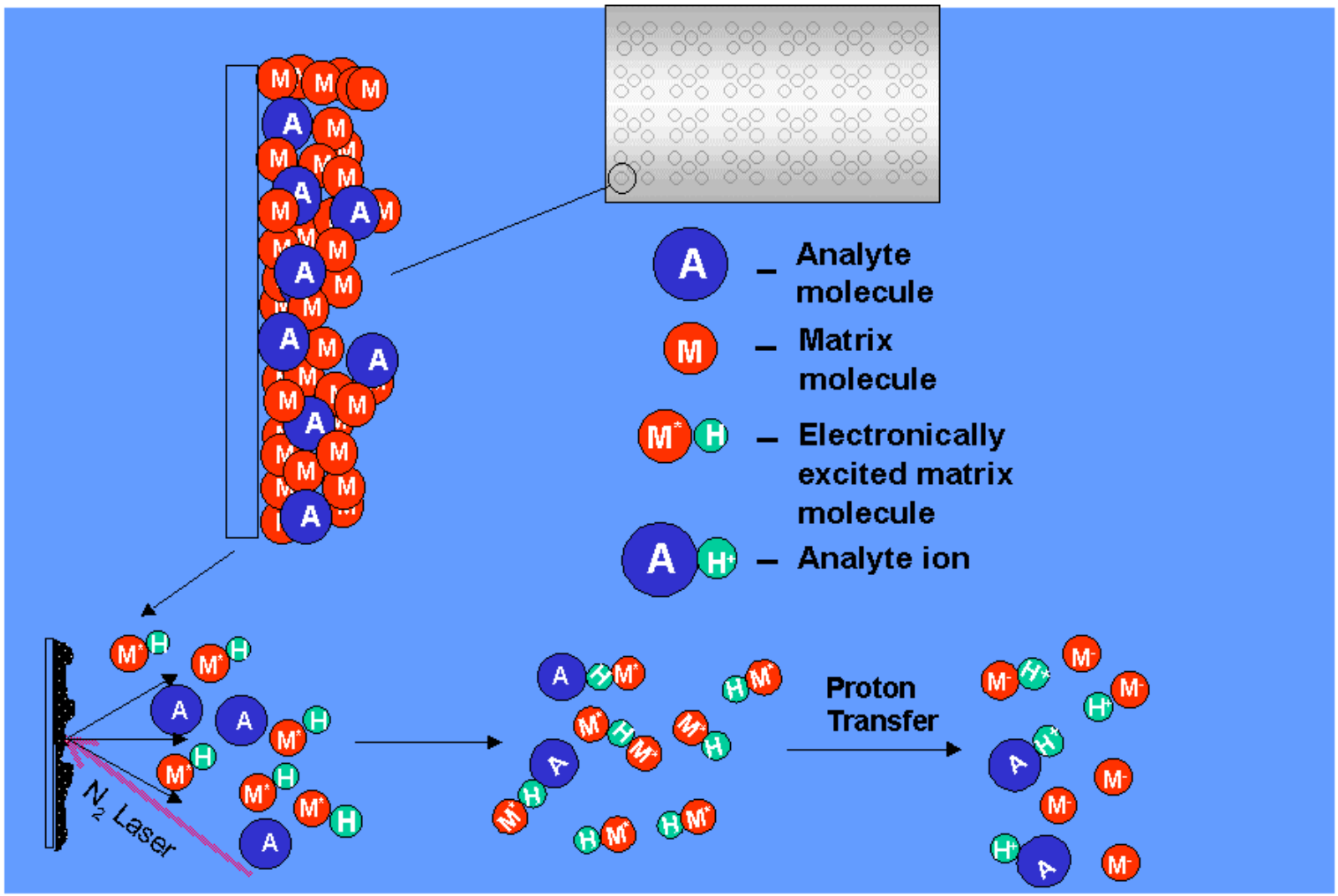
sinapinic acid (SA)



2,5-dihydroxybenzoic acid (DHB)



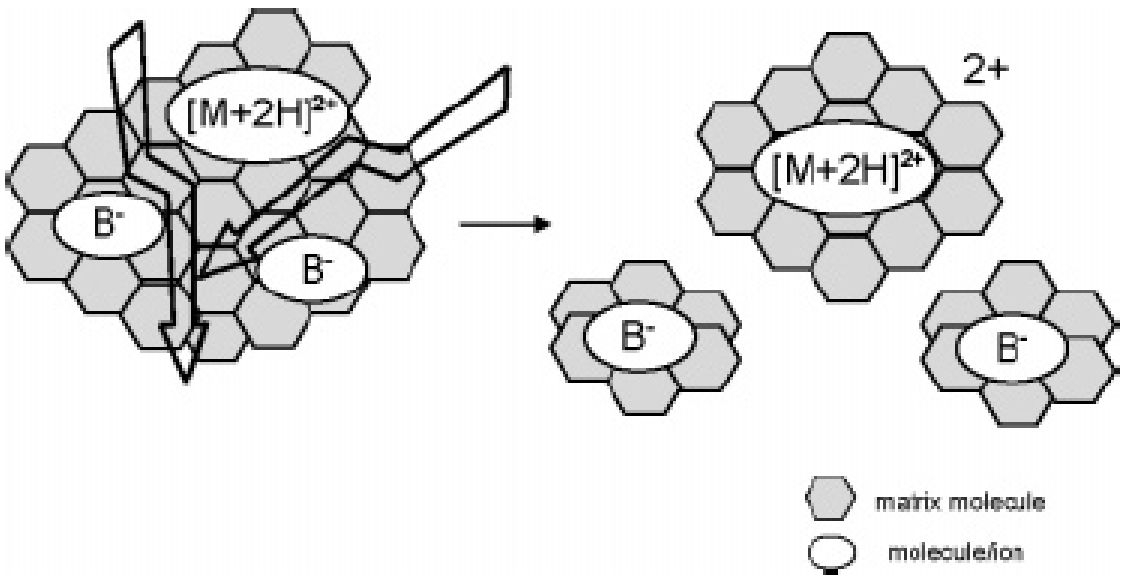
La ionizzazione Maldi prevede l' irradiazione con una luce laser (λ 337 nm) di una piccola superfice (100 μm di diametro) in cui è posto il campione, cristallizzato con una matrice, con conseguente riscaldamento dell'area di irradiazione e e vaporizzazione delle molecole organiche .



The principle of matrix assistance

– Absorption of energy from the laser light

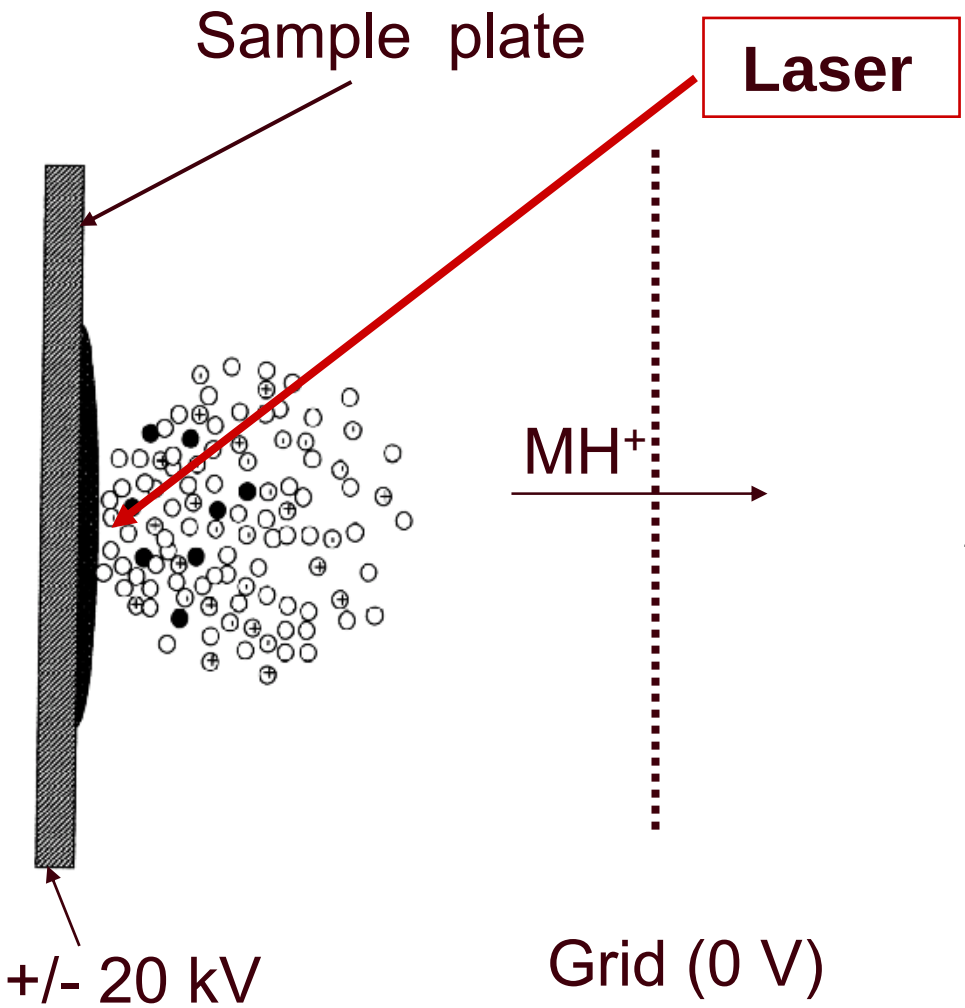
The matrix molecules absorb the energy from the laser light and transfer it into excitation energy of the solid system. Thereby inducing an instantaneous phase transition of a small molecular layers of the sample into a gaseous species.



The principle of matrix assistance

- Ionization of the biomolecules
  - **An active role of the matrix in the ionization of the analyte molecules by photoexcitation or photoionization of matrix molecules, followed by proton transfer to the analyte molecules is likely, though not proven unequivocally to date.**
  - **In practice, success or failure of a matrix depends on properties other than acting as an energy transfer mediator**
  - **Unfortunately, none of the many theories so far proposed can fully describe the chain of events.**

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: $XH^+ + M \rightarrow MH^+ + X$.

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?

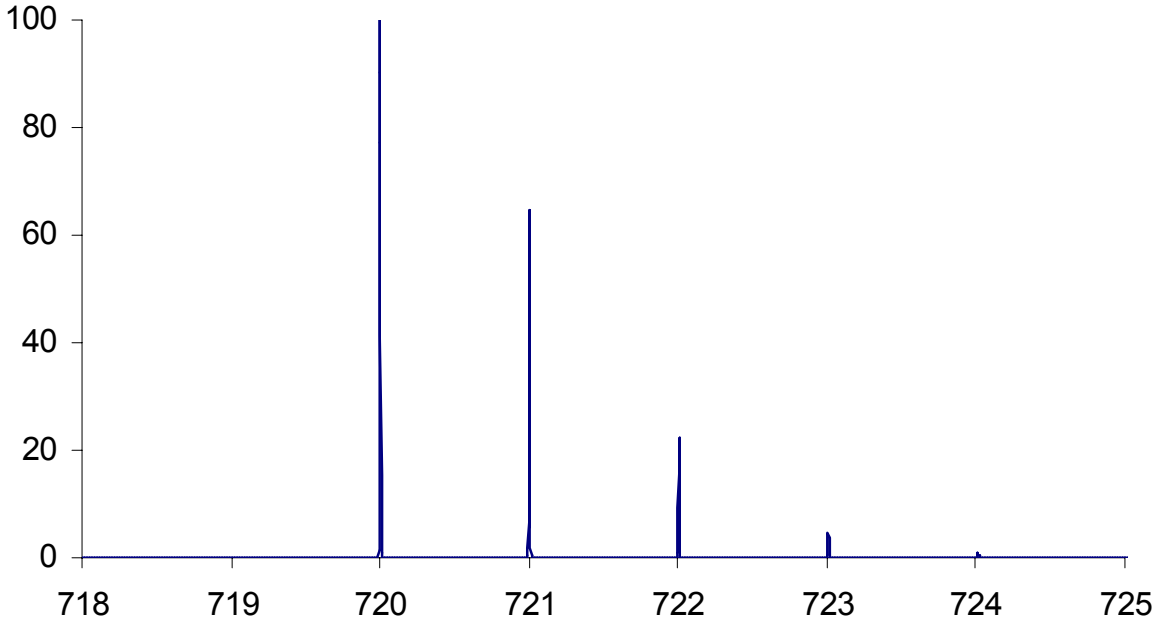
Mass spectrometers can “see” isotope peaks if their resolution is high enough.

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

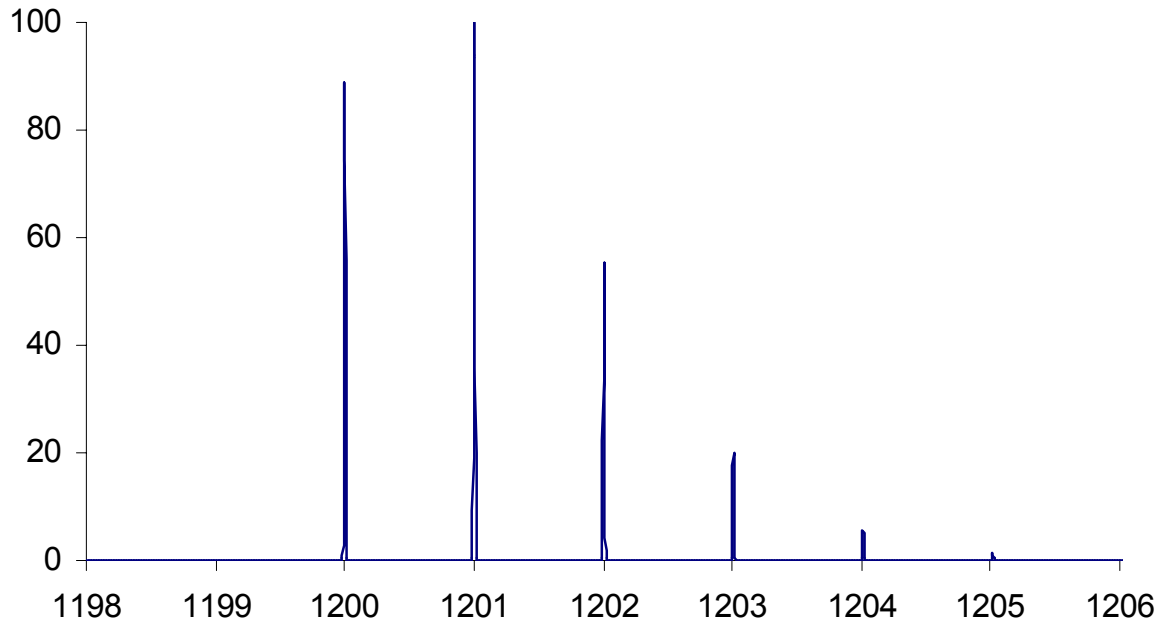
Stable isotopes of most abundant elements of peptides

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

C_{60}

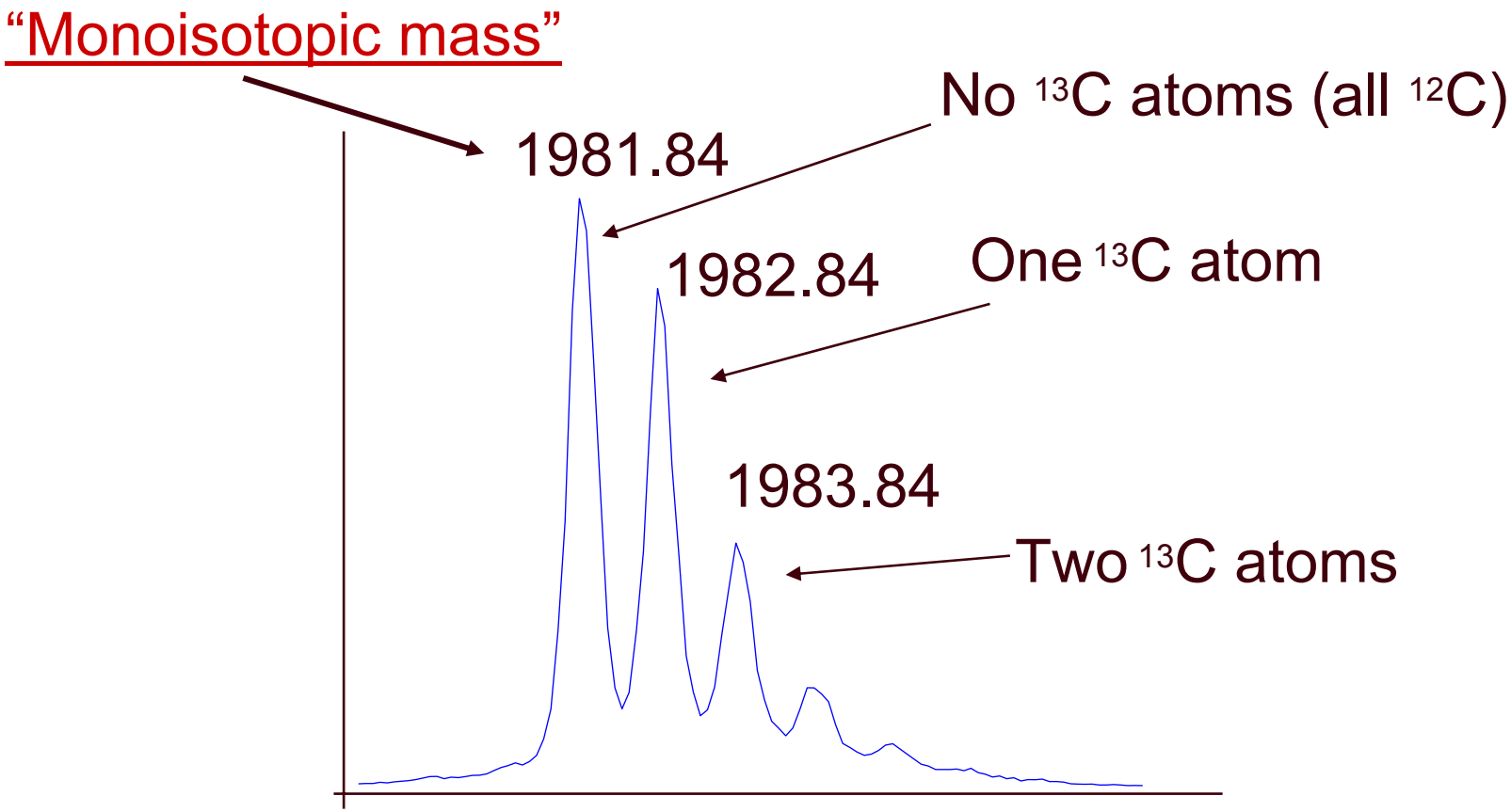


C_{100}

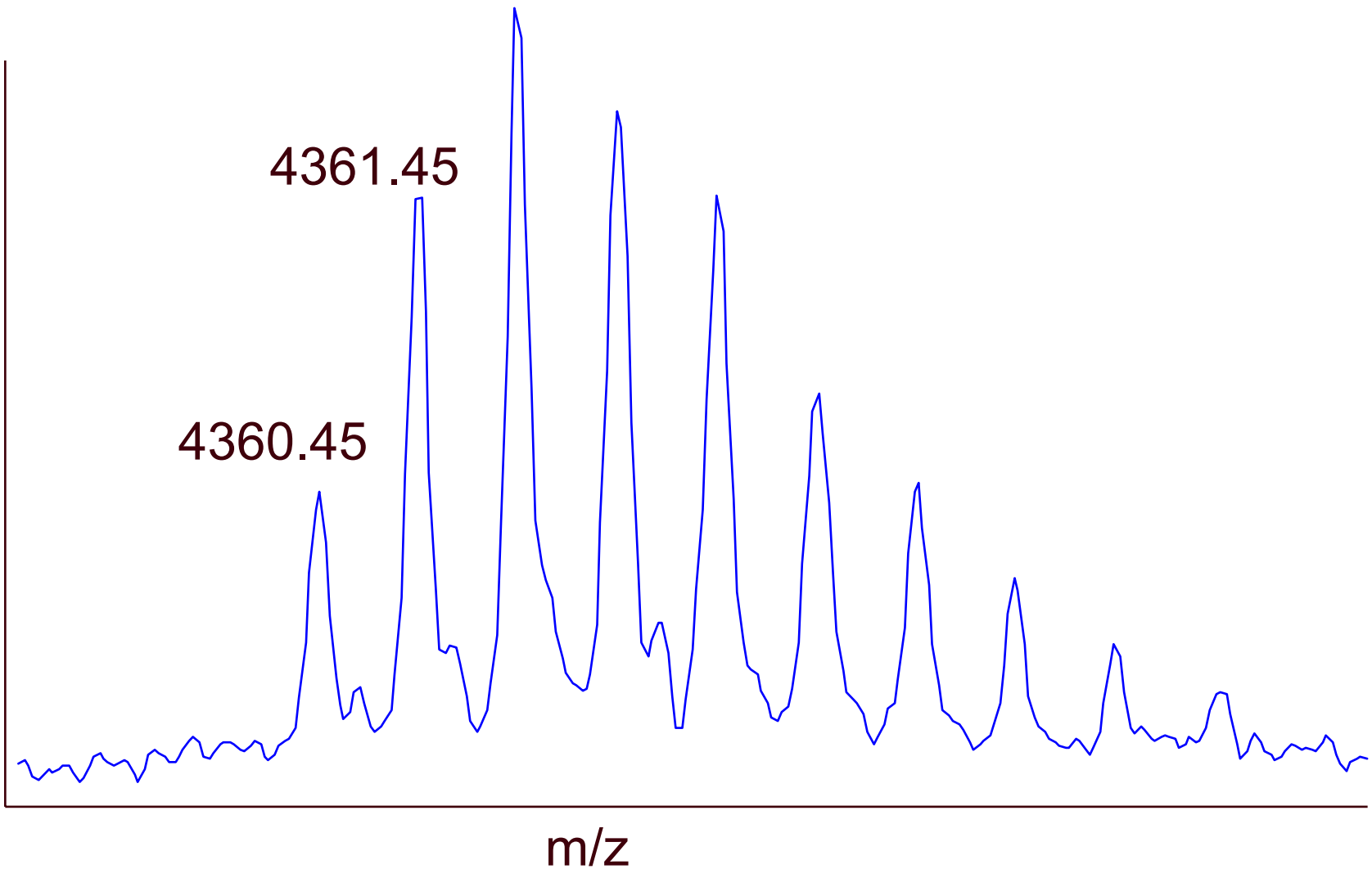


http://www.sisweb.com/mstools.htm

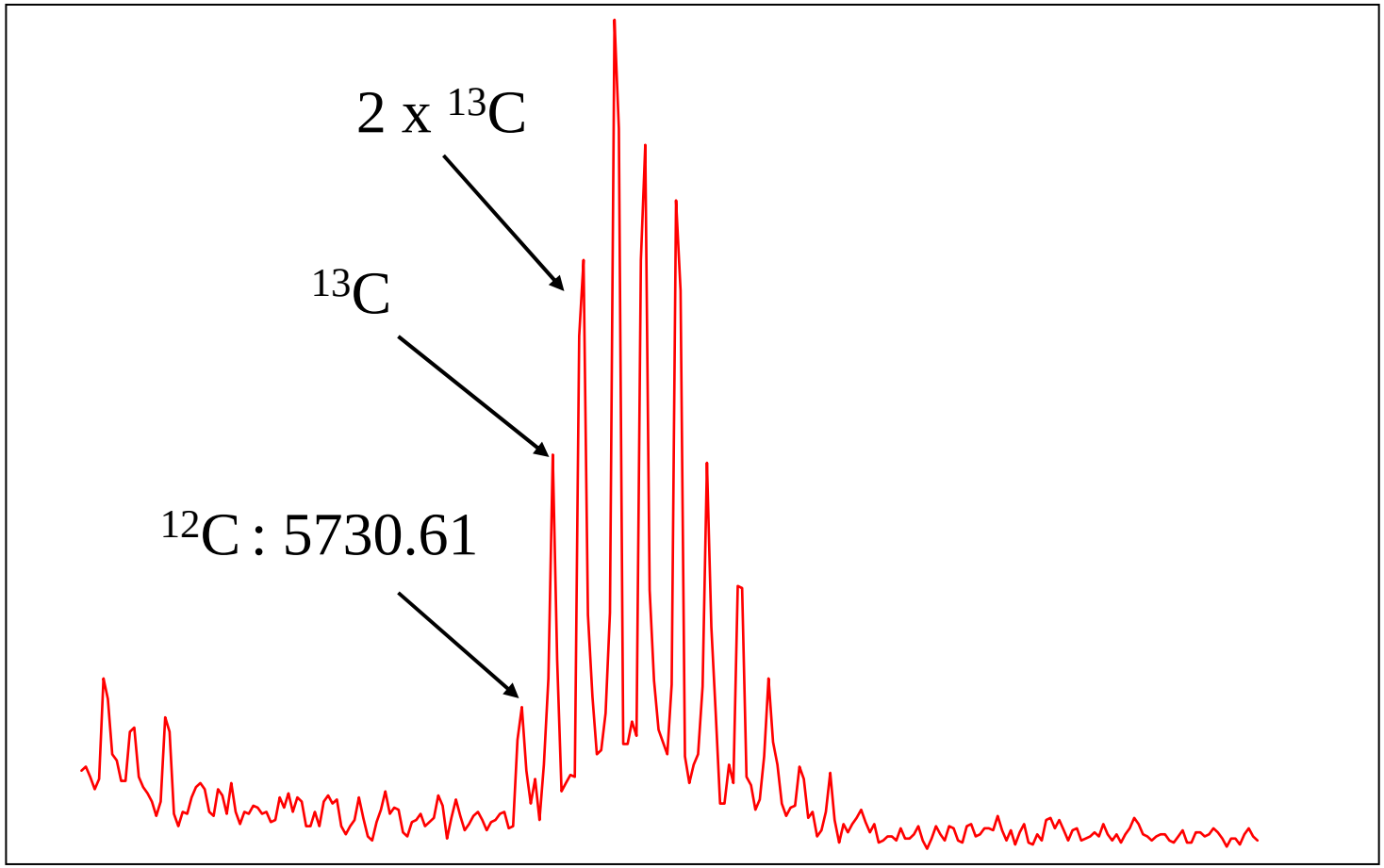
Mass spectrum of peptide with 94 C-atoms (19 amino acid residues)



Isotope pattern for a larger peptide (207 C-atoms)

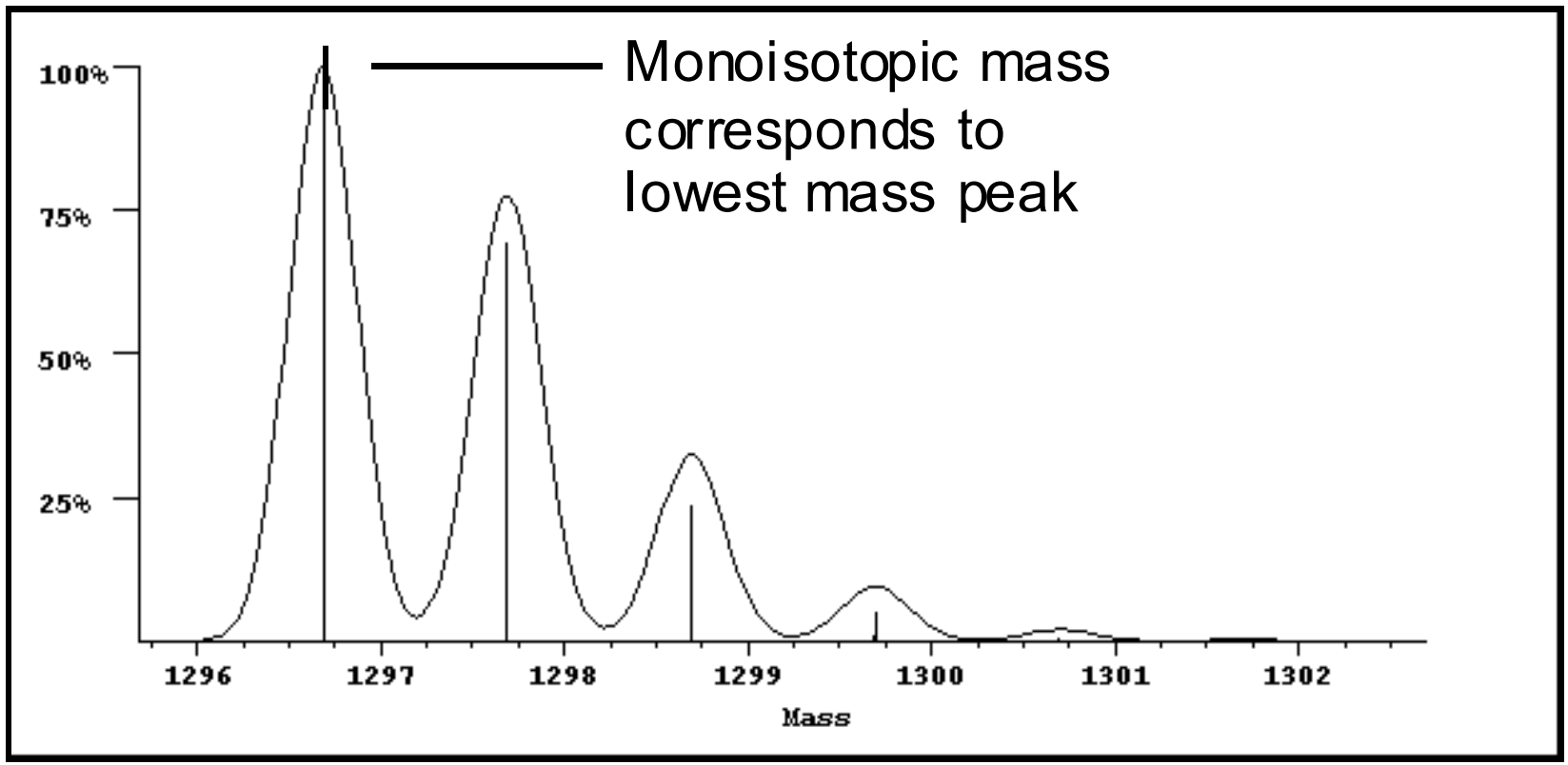


Mass spectrum of insulin



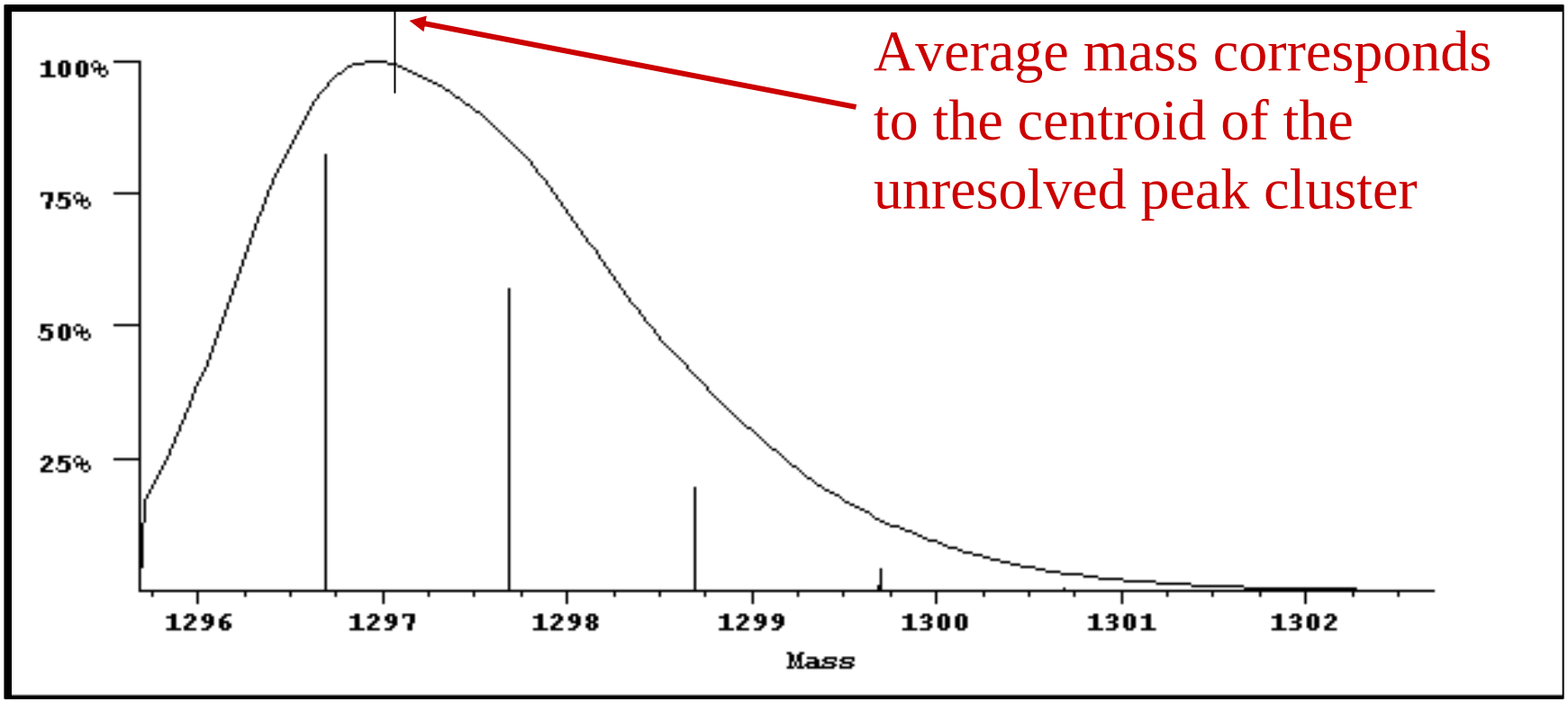
Insulin has 257 C-atoms. Above this mass, the monoisotopic peak is too small to be very useful, and the average mass is usually used.

Monoisotopic mass



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

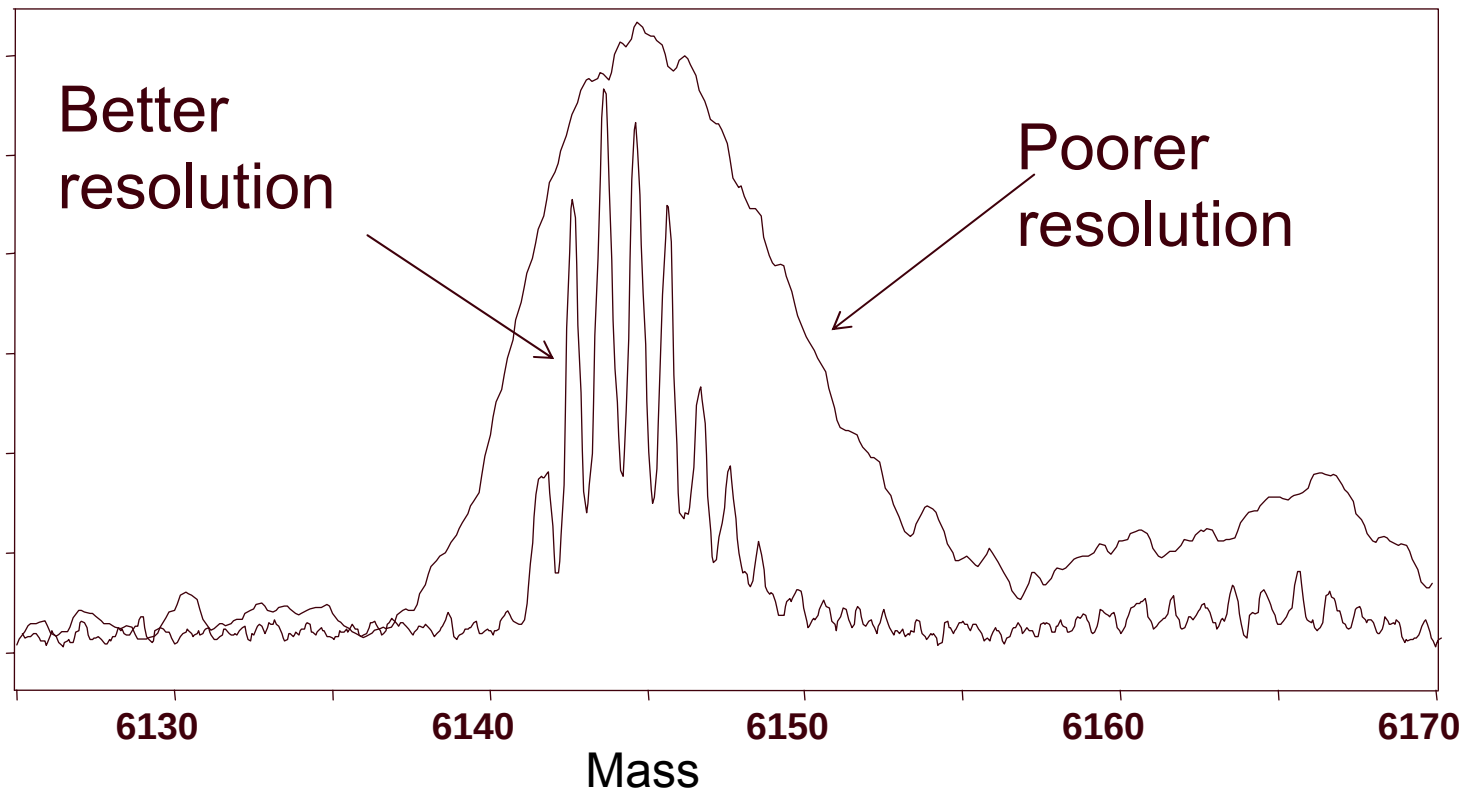
Average mass



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.



Risoluzione

La capacità di uno spettrometro di massa di differenziare le masse è generalmente espressa dalla risoluzione *R* definita come:

$$R = m/\Delta m$$

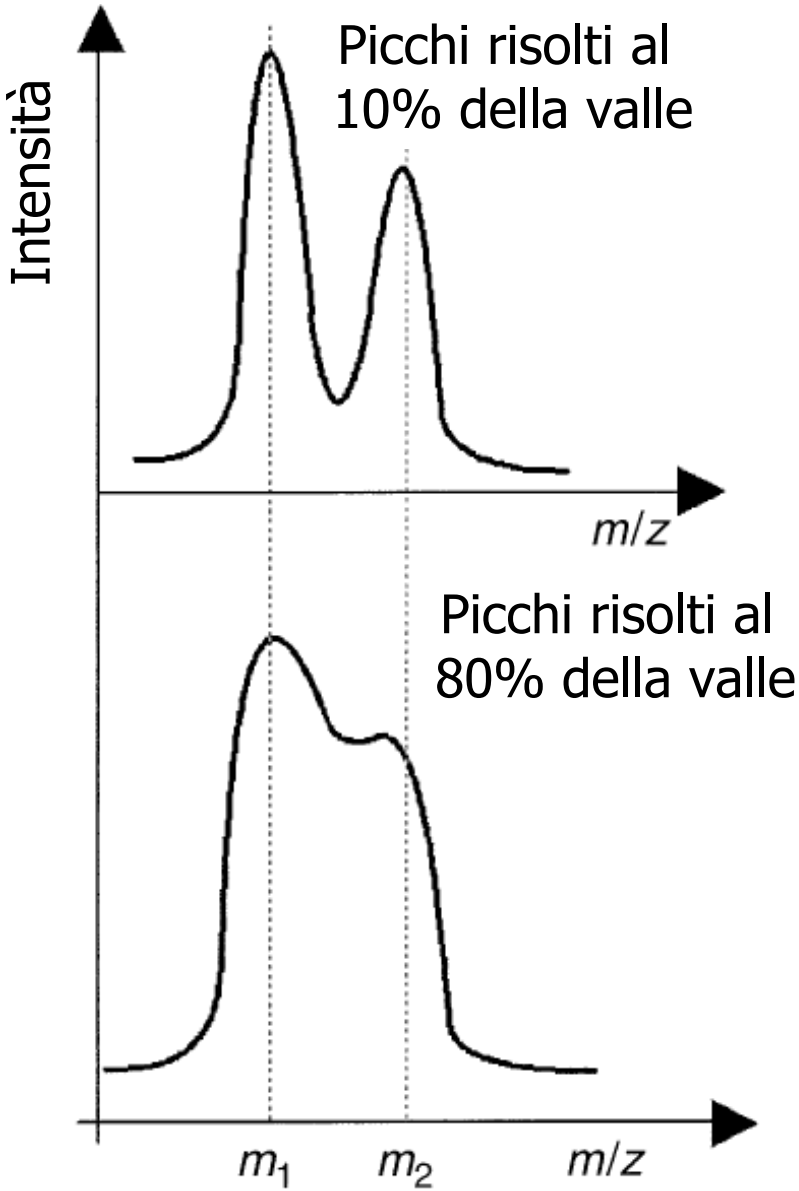
dove Δm è la differenza di massa tra due picchi adiacenti risolti e *m* è la massa nominale del primo picco (o la media delle masse dei due picchi).

Due picchi sono considerati risolti se l'altezza della valle tra di essi è inferiore ad una certa percentuale dell'altezza del picco meno intenso (di solito il 10%).

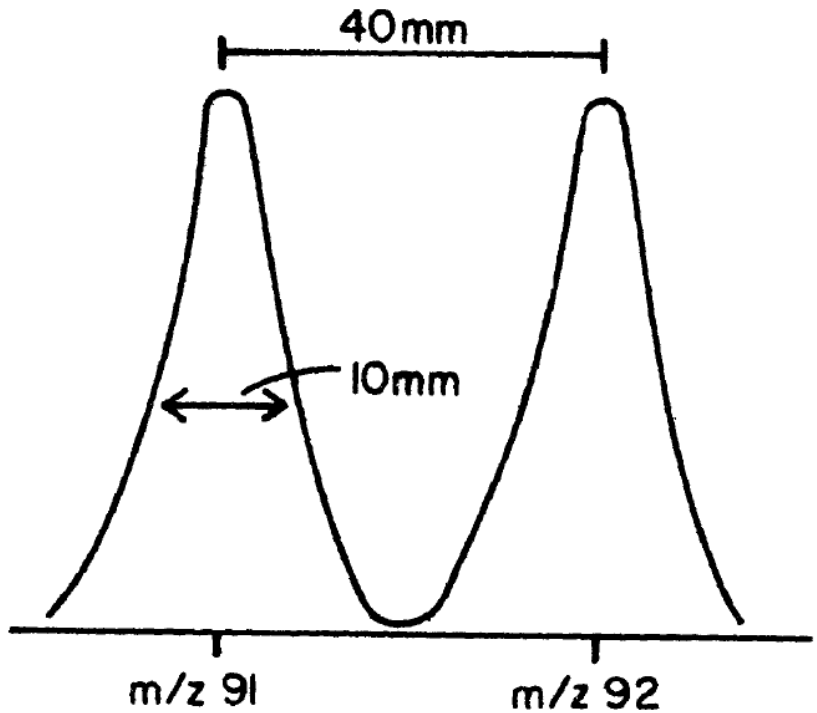
Uno spettrometro con una risoluzione di 4 000 risolverà due picchi con valori di *m/z* 400,0 e 400,1 (o di 40,00 e 40,01).

Gli spettrometri commerciali hanno *R* che variano circa tra 500 e 500 000.

Risoluzione

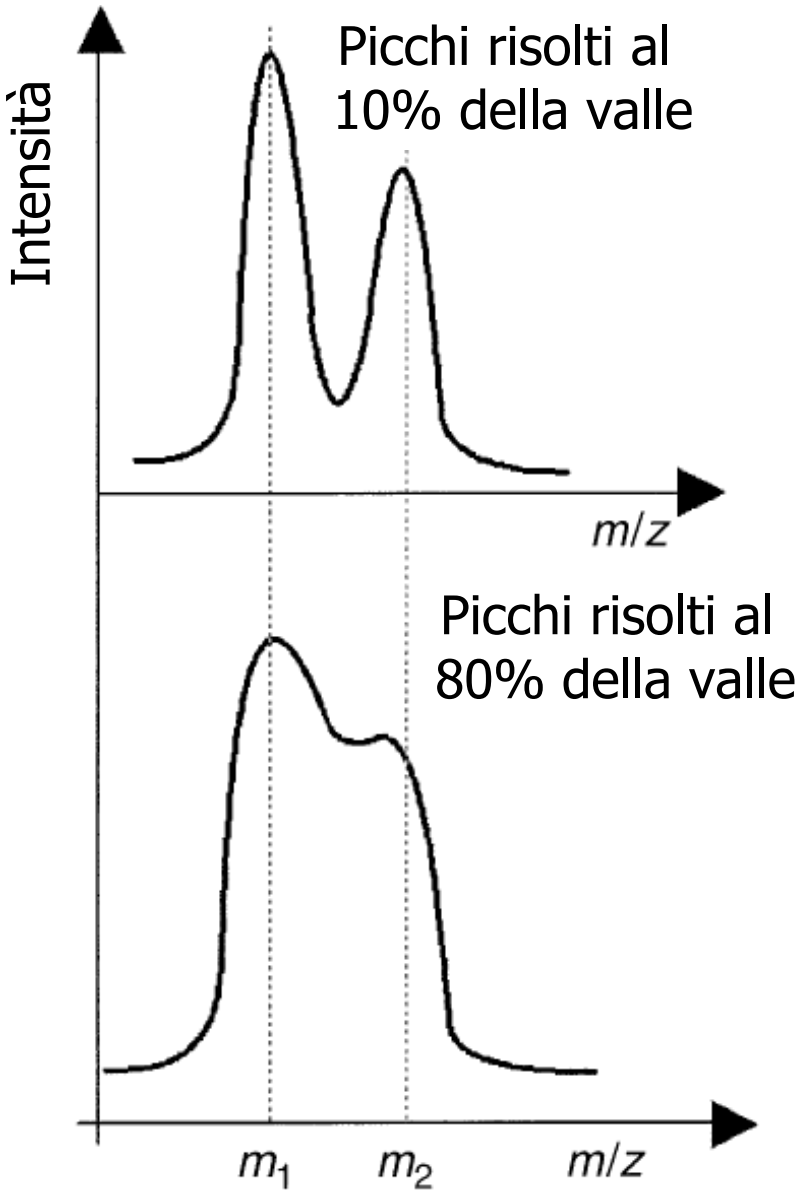


Definizione alternativa

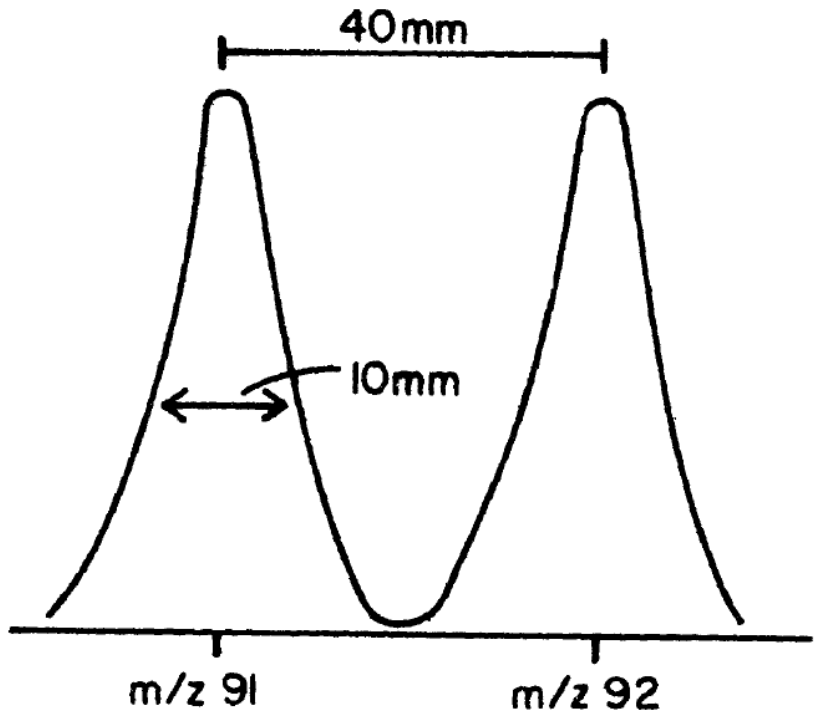


La risoluzione di un picco isolato si può anche definire come larghezza δm del picco al x% dell'altezza. Spesso si prende x = 50%, e δm è la larghezza a metà altezza.

Risoluzione



Definizione alternativa

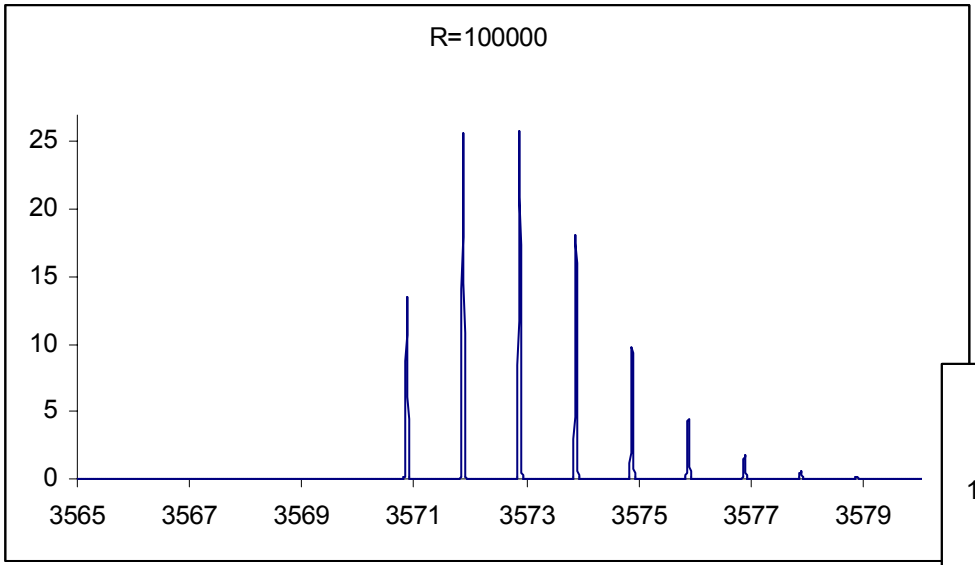


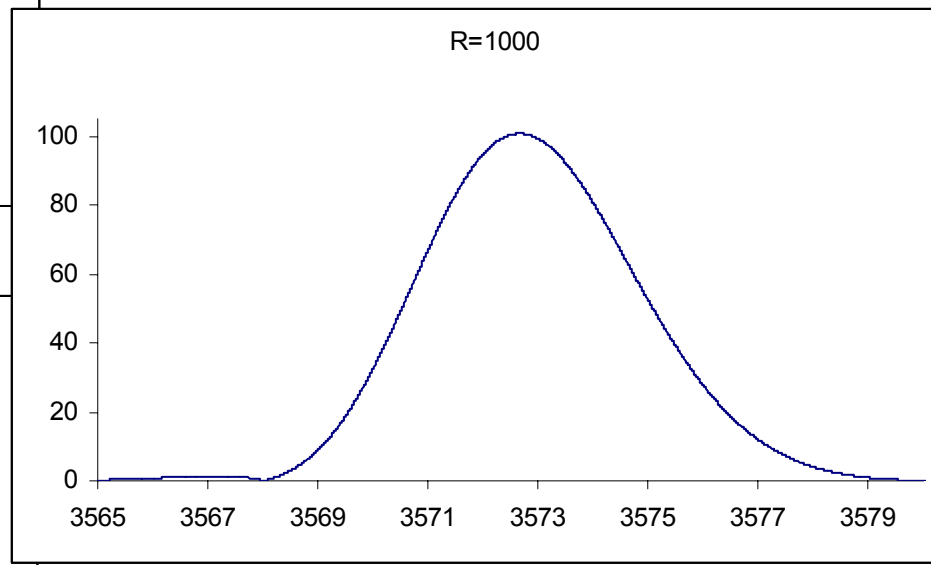
La risoluzione di un picco isolato si può anche definire come larghezza δm del picco al x% dell'altezza. Spesso si prende x = 50%, e δm è la larghezza a metà altezza.

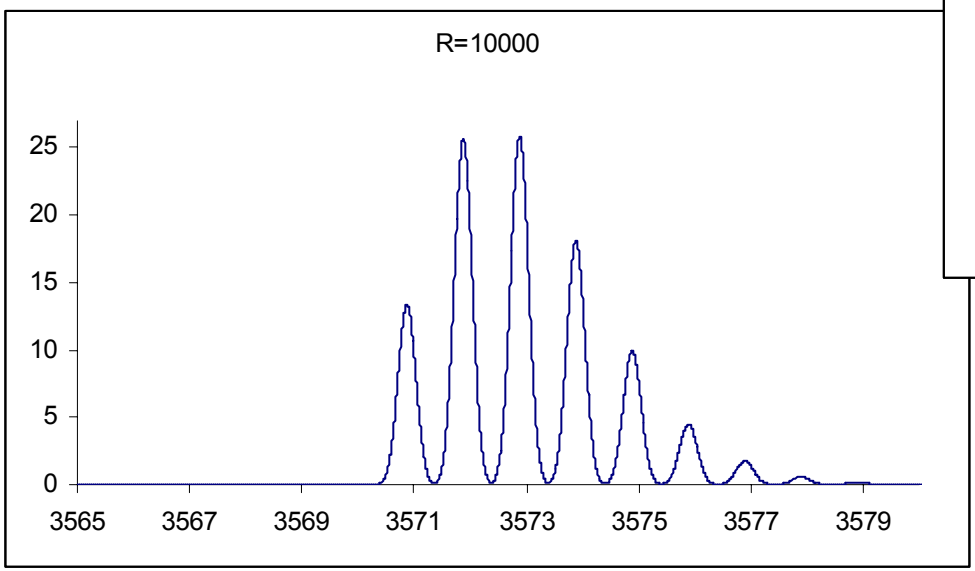
Effects of resolution on A_{50}^{+}

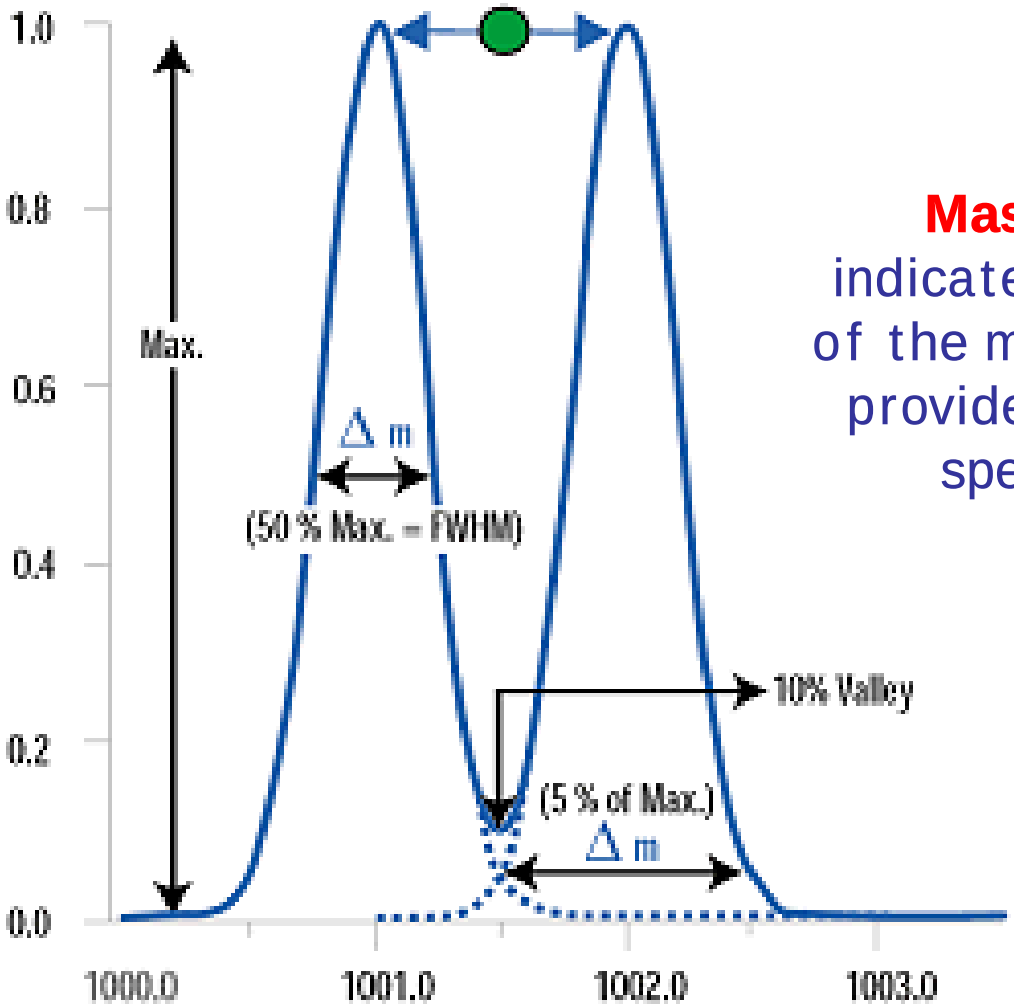
Elemental composition:

C H N O









Mass accuracy
indicates the accuracy of the mass information provided by the mass spectrometer

Mass accuracy is the difference observed between the theoretical mass and the measured mass: **Dm accuracy = m_{real} – $m_{measured}$** expressed in parts per million (ppm): **ppm = 10^6 * Δm accuracy / $m_{measured}$**
i.e.: theoretical mass: 1000, measured mass: 999.9 error: 100 ppm

Mass accuracy:

- elemental composition of organic ion
- mixture analysis (telling what's what)

Peak at m/z = 102, $^{12}C_5{}^{1}H_{10}{}^{16}O_2{}^{+}$ or $^{12}C_6{}^{1}H_{14}{}^{16}O^{+}$?

z = 1 m = 102.06808 102.104465

Accuracy $= \Delta m/m = 1/R = 3.6 \times 10^{-4} \times 10^{6} =$ 360 ppm

many other possible formulas, usually need ± 10 ppm or better

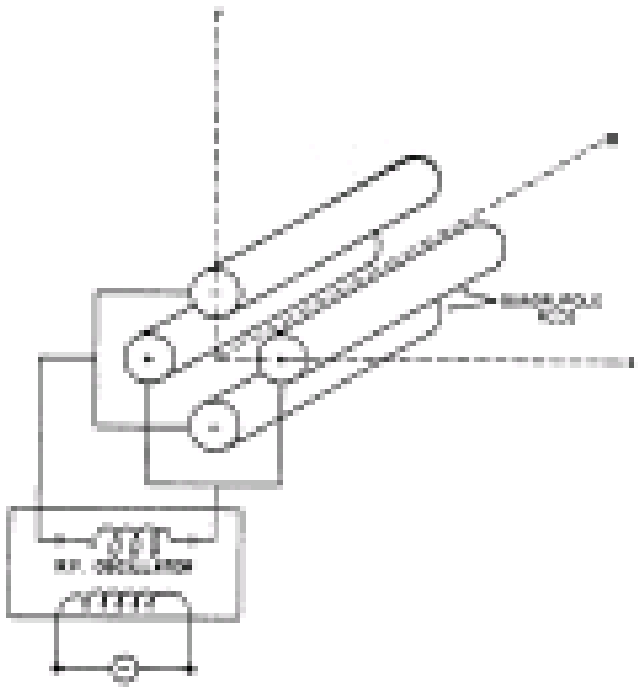
Classi di analizzatori di massa

- A settore magnetico
- A doppia focalizzazione
- A quadrupolo (lineare)
- Trappola ionica a quadrupolo
- A tempo di volo

Quadrupoles

- Use a combination of RF and DC as a mass filter

Quadrupole

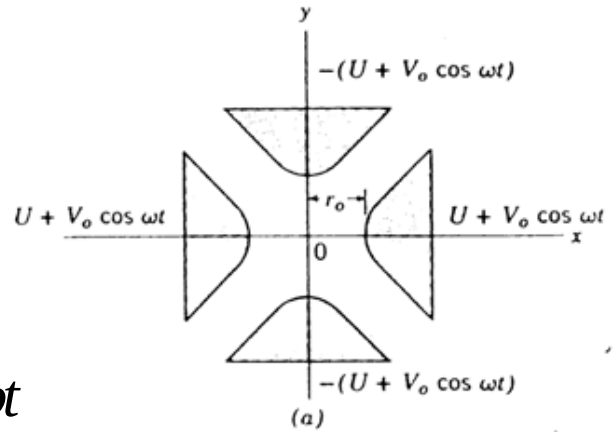


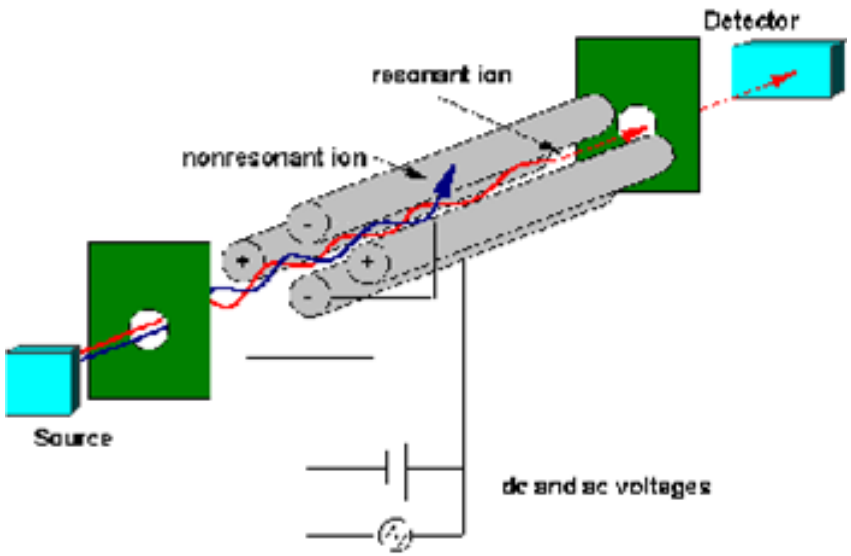
- Consists of four parallel rods (poles)
- Sorting of the ions depend on the ratio of RF:DC
- MS/MS using two quadrupole filters - triple quadrupole

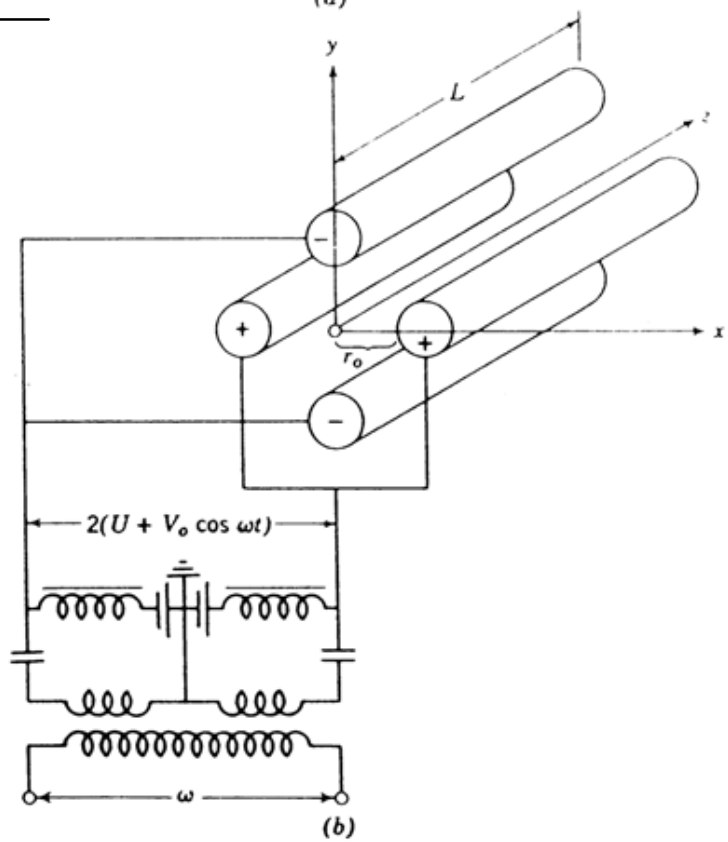
Analizzatore di massa a quadrup

Potenziale nel quadrupolo

$$\Phi = \frac{1}{2}(U - V\cos\omega t)\left(\frac{x^2 - y^2}{r_0^2}\right) + \frac{U + V\cos\omega t}{2}$$







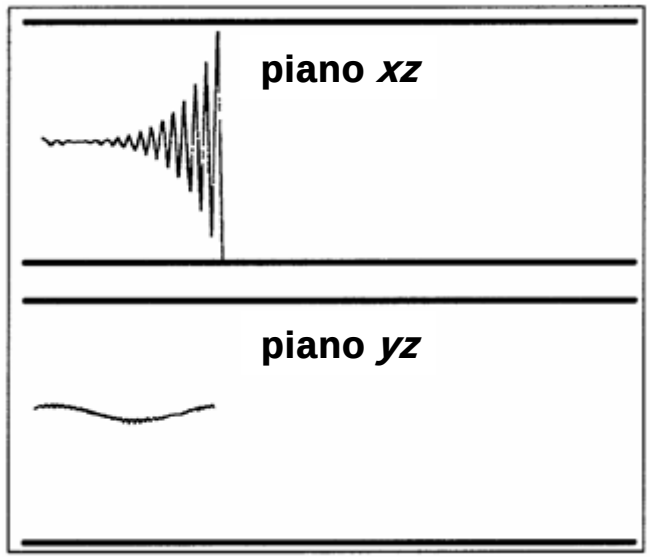
Quadrupoles have variable ion transmission modes

mass scanning mode

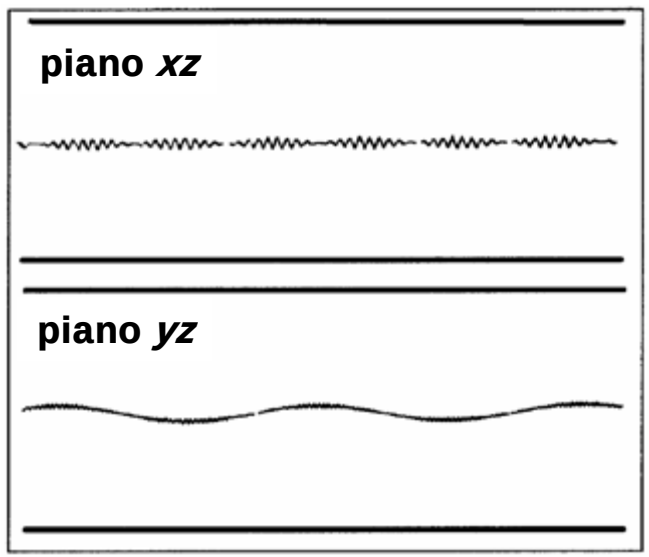
single mass transmission mode

Traiettorie dello ione

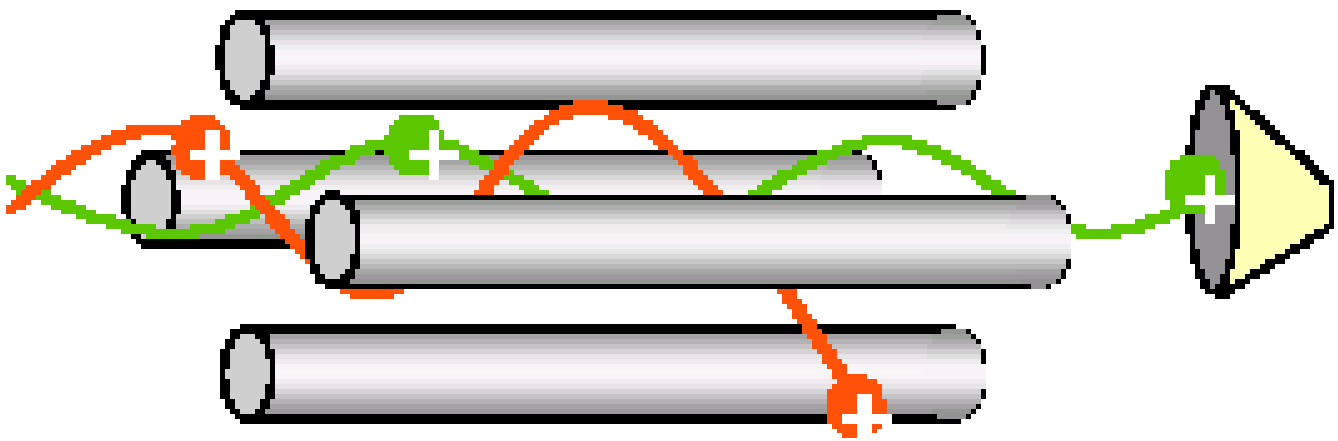
Traiettoria instabile lungo *x*, stabile lungo *y*



Traiettoria stabile sia lungo *x* che lungo *y*



Quadrupole Mass Analyser



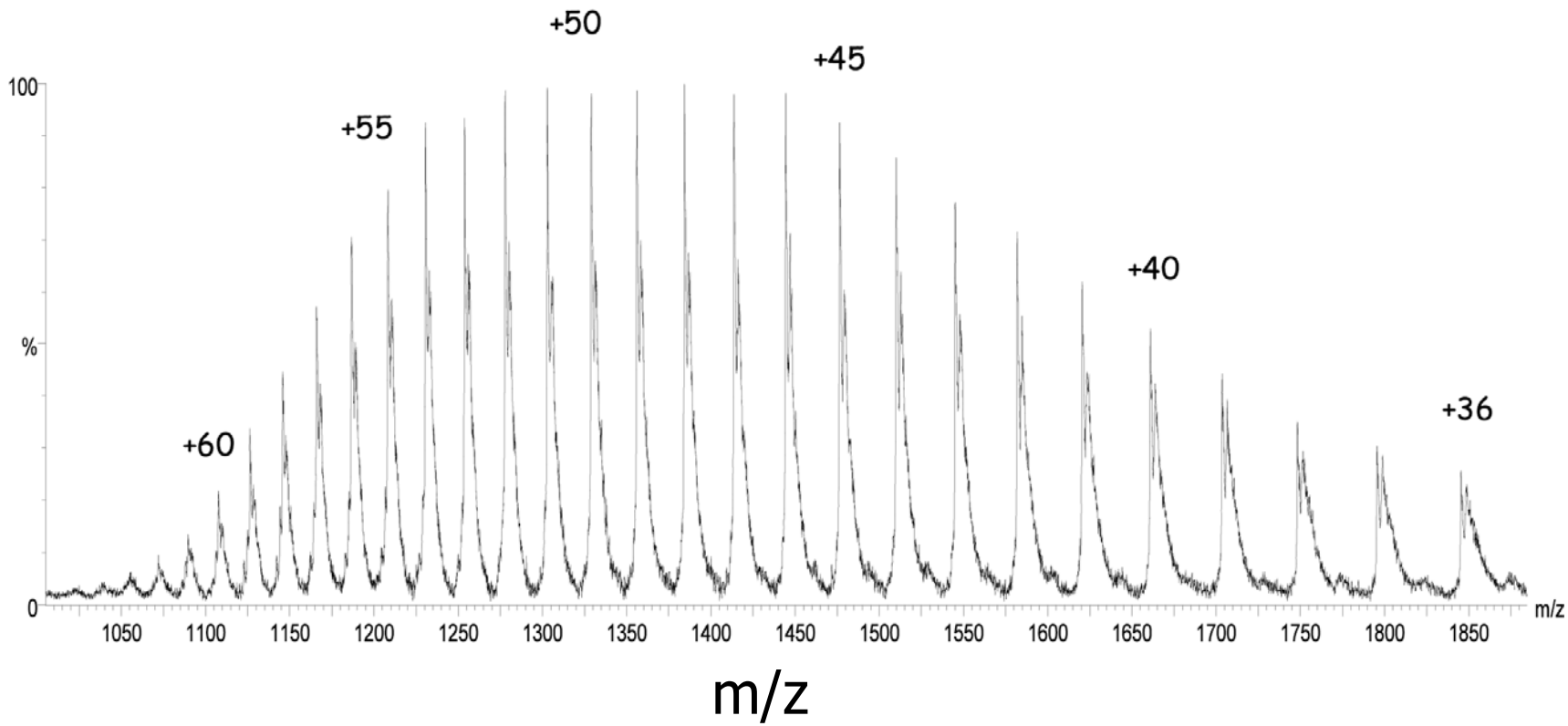
The ● ion is transmitted along the quadrupole in a stable trajectory Rf field. The ● ion does not have a stable trajectory and is ejected from the quadrupole.

Analizzatore di massa a quadrupolo

- Massimo valore *m/z* ~ 4 000
- Risoluzione ~ 3 000
  - I quadrupoli sono strumenti a **bassa risoluzione**
  - Si lavora normalmente alla risoluzione di una unità di massa.
- Leggero, di dimensioni contenute
- Facile da accoppiare alla cromatografia
- Efficiente trasmissione degli ioni
- Necessaria una elevata precisione nell'allineamento delle barre degli elettrodi

Lo spettro di massa

Spettro di massa multicarica (ESI/qTOFMS)



m/z

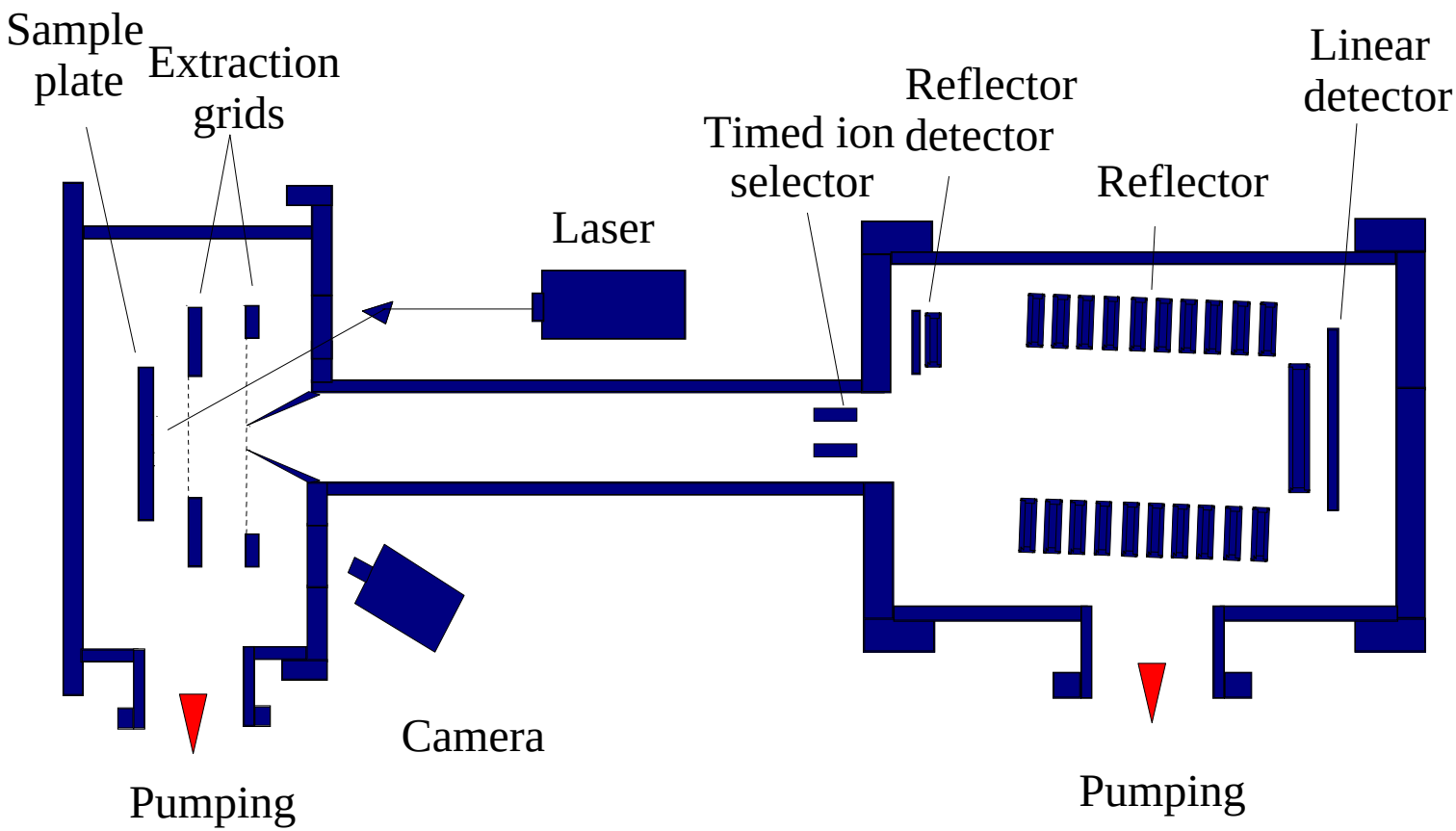
Albumina del siero bovino: M_r = 64 000

How do we achieve superior mass resolution?

Reflector TOF Mass Analyzer

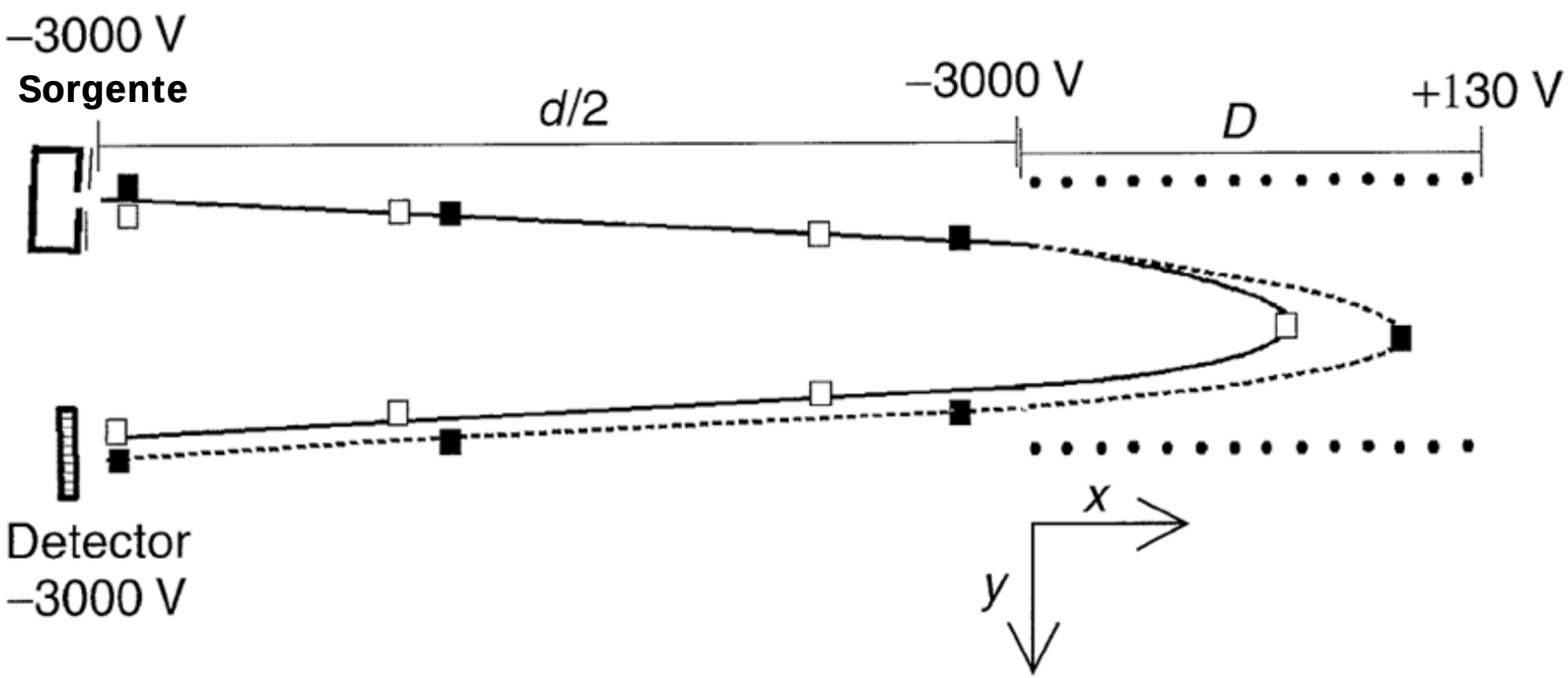
Delayed Extraction on a MALDI source

Voyager-DE STR MALDI TOF



Reflectron TOF

Metodo per correggere la distribuzione di velocità, diminuendo Δt

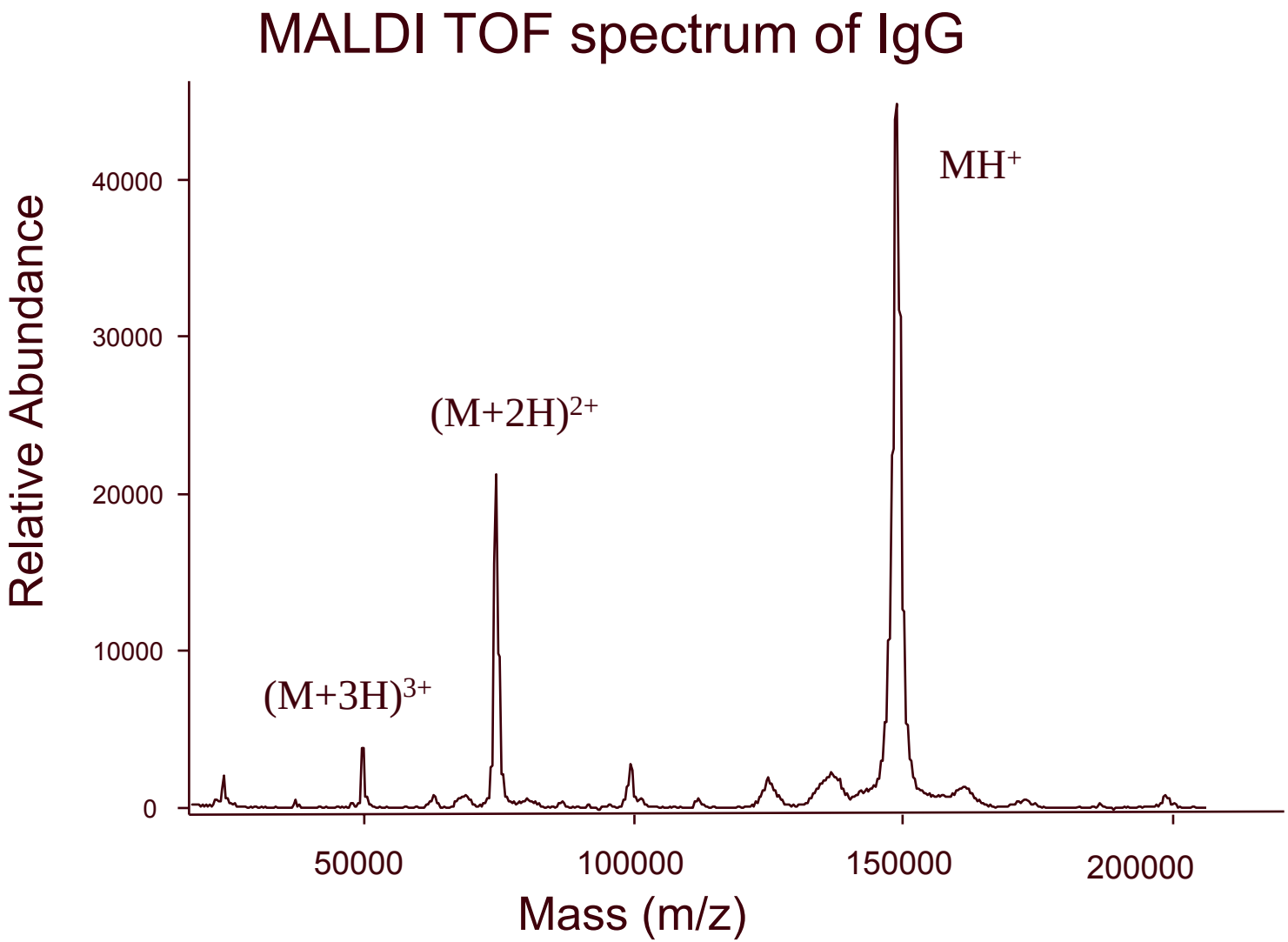


Reflectron TOF a stadio singolo

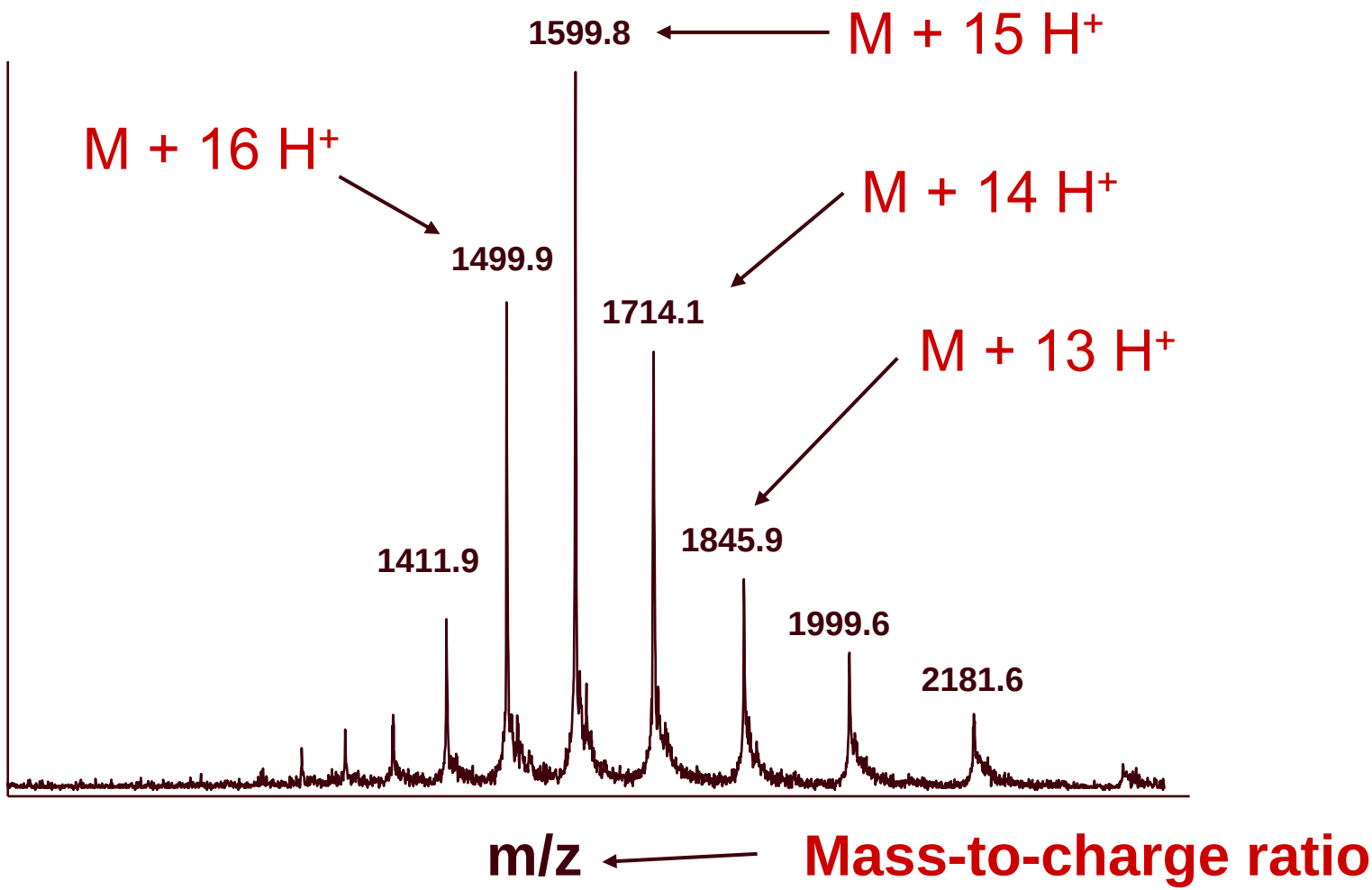
Analizzatore di massa a tempo di volo

- Massimo valore *m/z* > 200 000
- Risoluzione fino a 10 000 – 20 000.
- Il reflectron consente di:
  - Ridurre le dimensioni
  - Aumentare la risoluzione
- Ideale accoppiamento con sorgenti pulsate
- Efficiente trasmissione degli ioni

The mass spectrum shows the results



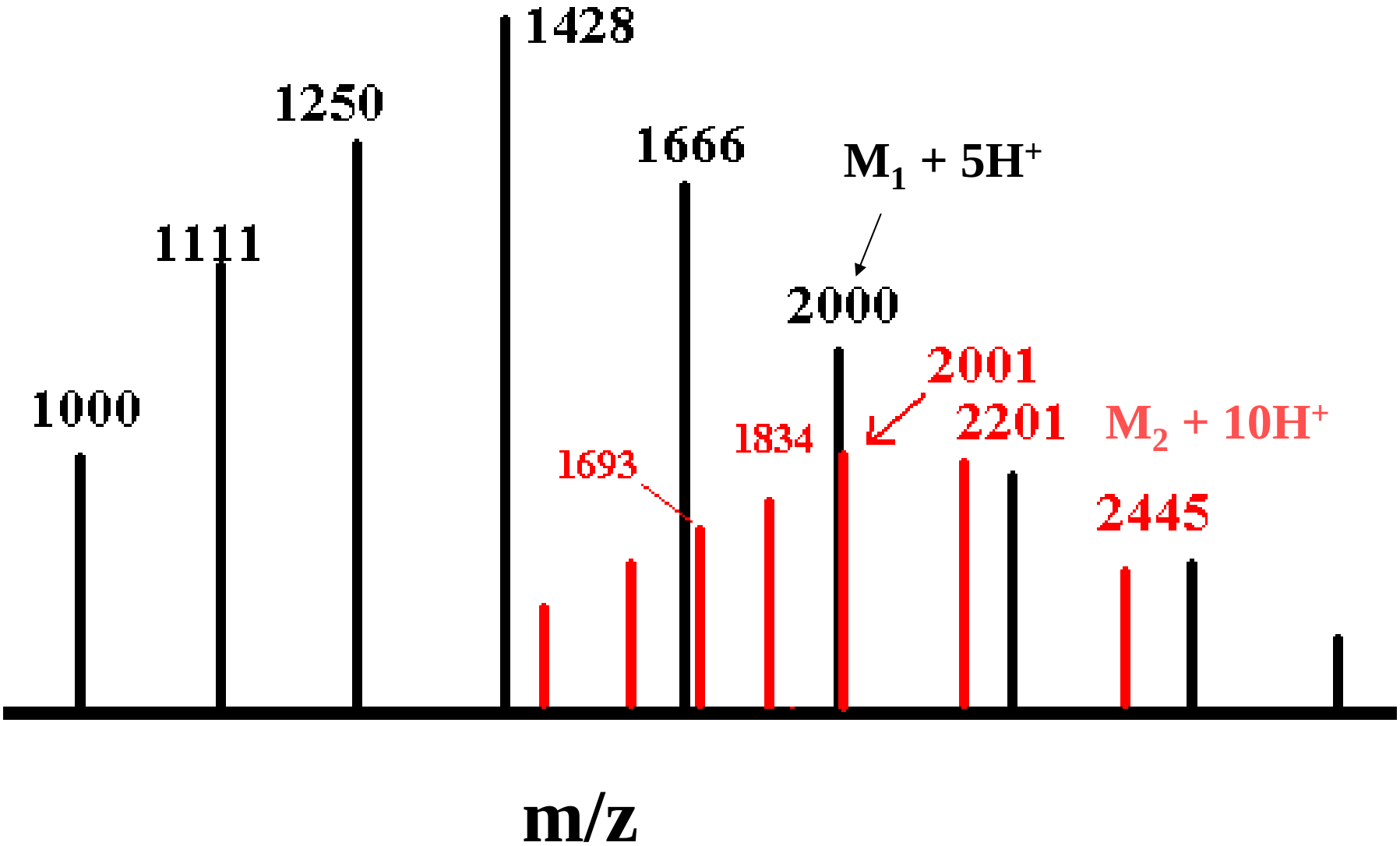
ESI Spectrum of Trypsinogen (MW 23983)



MIXTURE TWO PROTEINS

$M_1 = 10{,}000$ $M_2 = 22{,}000$

*USE INTENSITY ENVELOPE TO ID RELATED IONS!!

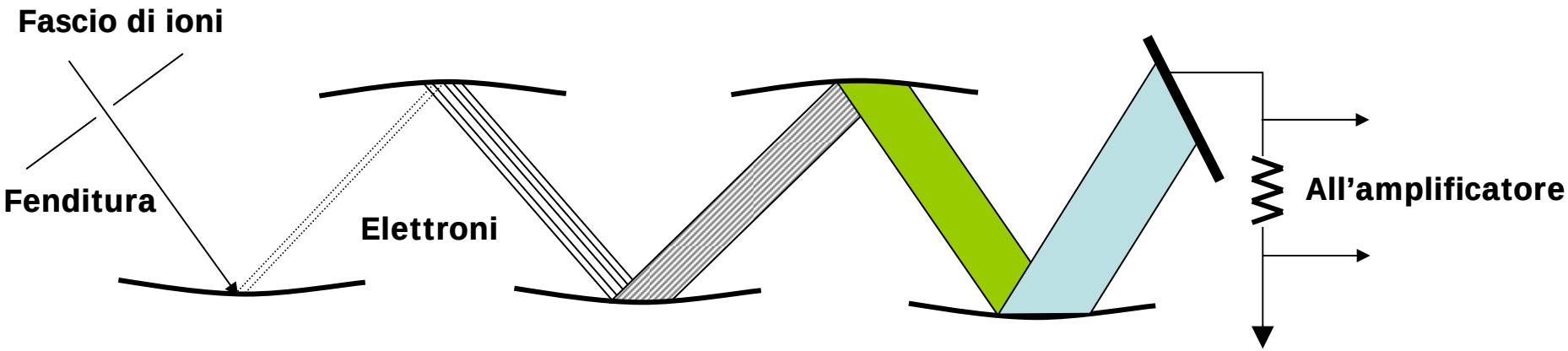


Rivelatori di ioni

- Lastre fotografiche
  - Ad AgBr. Sono state i primi rivelatori di ioni
- Coppa di Faraday
  - Un semplice elettrodo collettore schermato
  - Economico, poco sensibile
- Rivelatori a scintillazione
  - A cristalli di materiale fosforescente
- Moltiplicatori di elettroni
  - A dinodi separati
  - A dinodo continuo

Moltiplicatori di elettroni

Moltiplicatore a dinodi separati



Moltiplicatore a dinodo continuo