Protein mass spectrometry and proteomics

L. Dayon

Spring Semester 2026

EPFL

Course outline

1. Proteomics and mass spectrometry

Introduction to protein analysis and proteomics; Reminders in mass spectrometry; Why proteomics and mass spectrometry?; Ionization sources, analysers, and detectors used in proteomics; Latest generation of mass spectrometers used in proteomics

2. Mass spectrometry-based proteomic strategy and workflows

Bottom-up versus top-down strategies; Data-dependent acquisition (DDA) and data-independent acquisition (DIA) approaches; Sample preparation

Lab visit of the Proteomics Laboratory at Nestlé Research (EPFL Innovation Park)

3. Quantitative proteomic workflows

Label-free methods; Labelling-based techniques; Other quantitative techniques

4. Proteomic bioinformatics

Databases; Identification of protein; Quantification of proteins; Bioinformatics tools; Practical examples

5. Applications to biology, clinical research, and beyond

What strategy?; Experimental design & randomization; Biomarker discovery; Industrialized and population proteomics; Forensics; Targeted mass spectrometry-based approaches; Other biological applications of mass spectrometry; Advanced innovations (single-cells, 4D proteomics, multi-omics) and emerging technologies; Limitations and ethical consideration

Additional support/information on Separations techniques in proteomics

What are you hoping to get out of this course?

Course outline

- 1. Introduction

And more…

Introduction to protein analysis and proteomics; Reminders in mass spectrometry; Why proteomics and mass spectrometry?; Ionization sources, analysers, and detectors used in proteomics; Latest generation of mass spectrometers used in proteomics

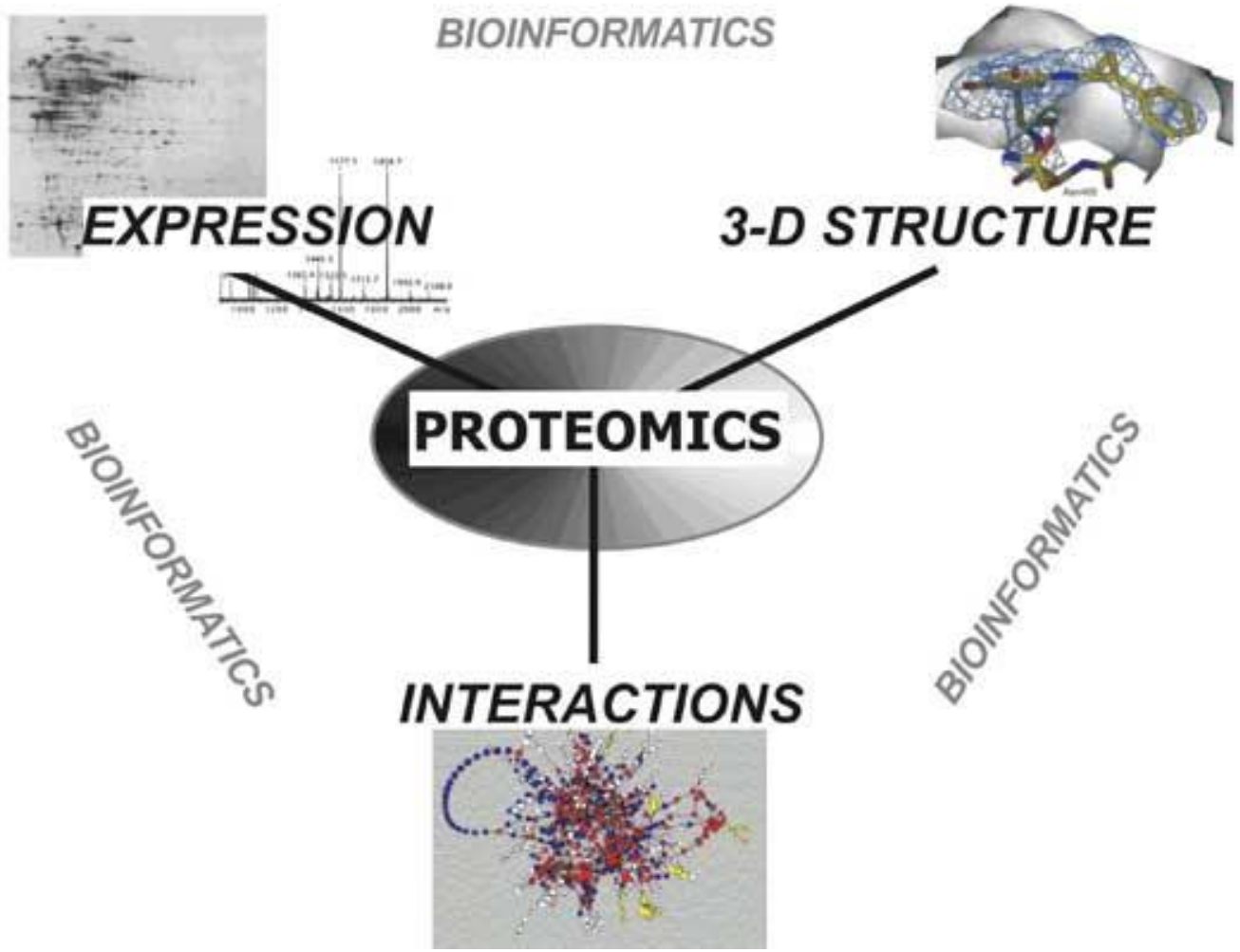
What are your ideas about Proteomics?

When was coined the word "proteomics"?

1.1. Introduction to protein analysis and proteomics

Proteomics is the large-scale study of proteins/proteomes

A proteome is a set of proteins produced in an organism, system, or biological context.



Genomics and proteomics

SAME GENOME

DIFFERENT PROTEOMES

"The proteome is not constant; it differs from cell to cell and changes over time. To some degree, the proteome reflects the underlying transcriptome. However, protein activity (often assessed by the reaction rate of the processes in which the protein is involved) is also modulated by many factors in addition to the expression level of the relevant gene."

Source: https://www.ebi.ac.uk/training/online/course/proteomics-introduction-ebi-resources/what-proteomics

Proteome complexity and analytical challenges

• One gene can encode more than one protein (even up to 1000). The human genome contains about 21000 protein-encoding genes, but the total number of proteins in human cells is estimated to be between 250000 to one million

• Proteins are dynamic. Proteins are continually undergoing changes, *e.g.*, binding to the cell membrane, partnering with other proteins to form complexes, or undergoing synthesis and degradation. The genome, on the other hand, is relatively static

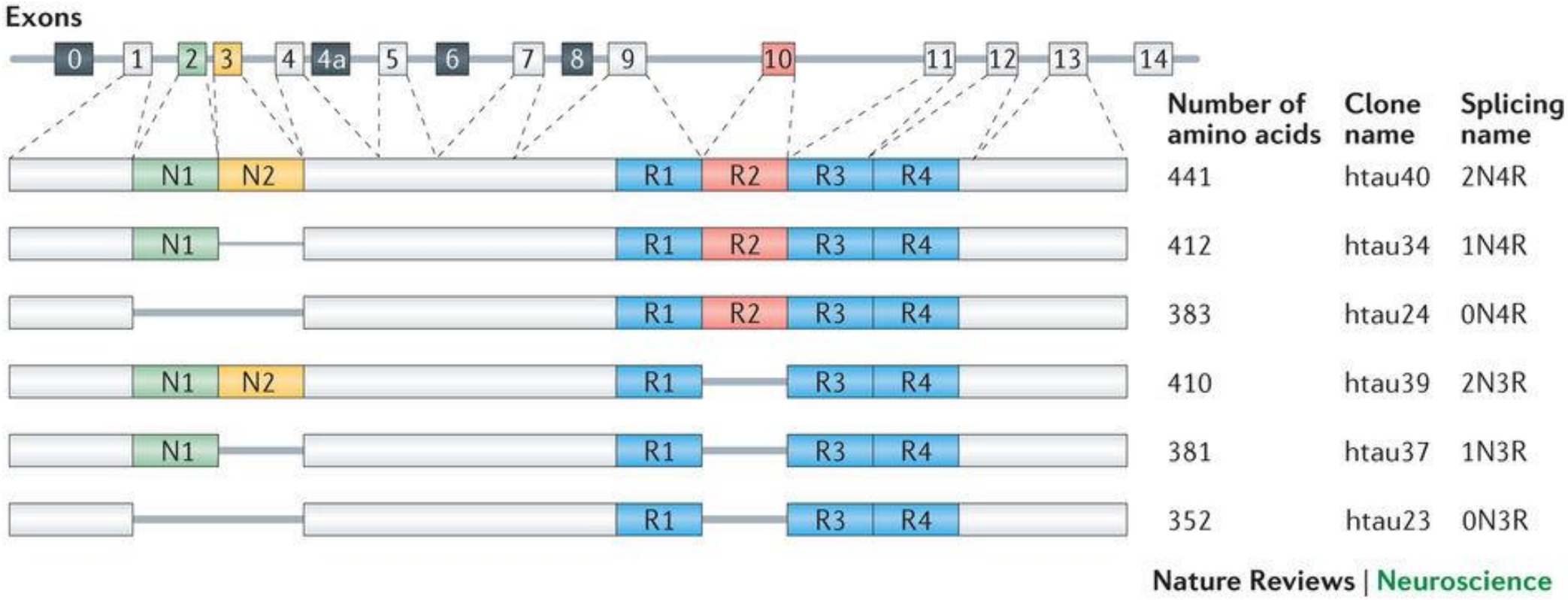
• Proteins are co- and post-translationally modified. As a result, the types of proteins measured can vary considerably from one person to another under different environmental conditions, or even within the same person at different ages or states of health. Additionally, certain modifications can regulate the dynamics of proteins

• Proteins exist in a wide range of concentrations in the body. For example, the concentration of the protein albumin in blood is more than a billion times greater than that of interleukin-6, making it extremely difficult to detect the low abundance proteins in a complex biological matrix such as blood

Source: http://proteomics.cancer.gov

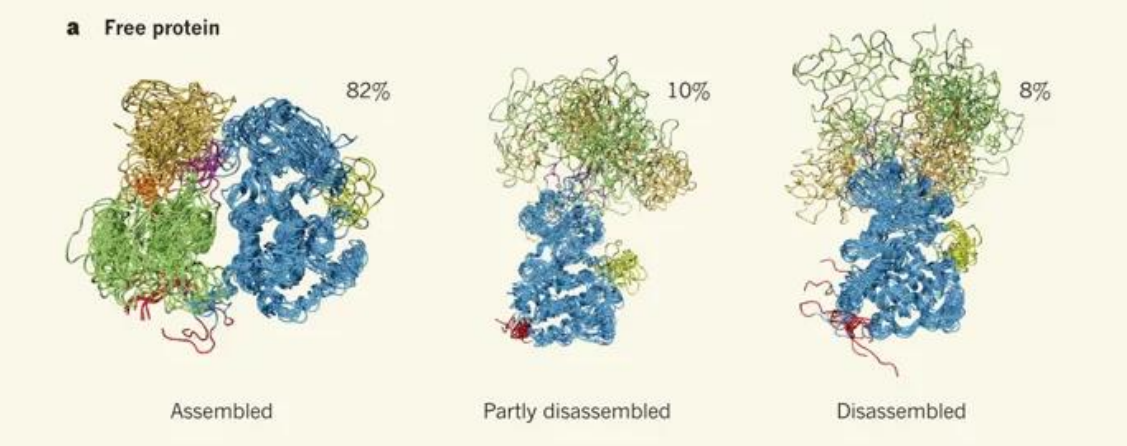
One gene can encode more than one protein

6 tau isoforms in adult human brain, generated by alternative splicing



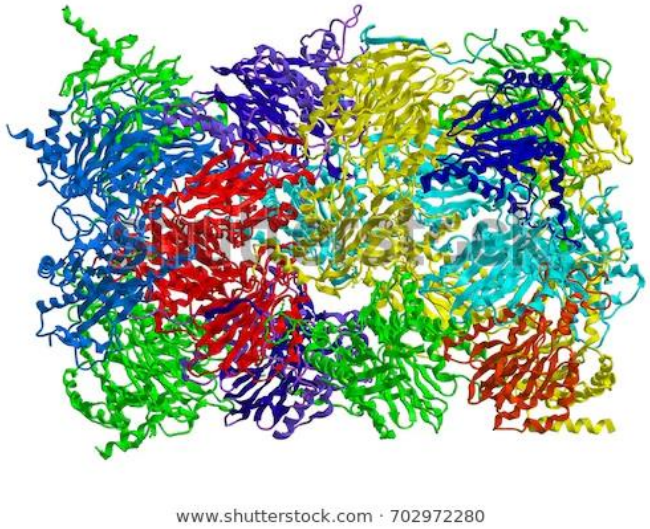
Nature Reviews Neuroscience 17, 22–35 (2016)

Proteins are dynamic

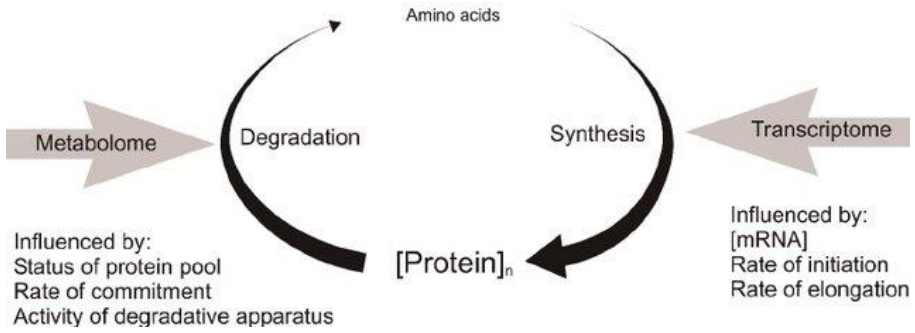


Nature, 468, 1046–1048(2010)

The multidomain enzyme Hck can adopt several conformational states in solution, ranging from a compact 'assembled' state to partially assembled and disassembled states



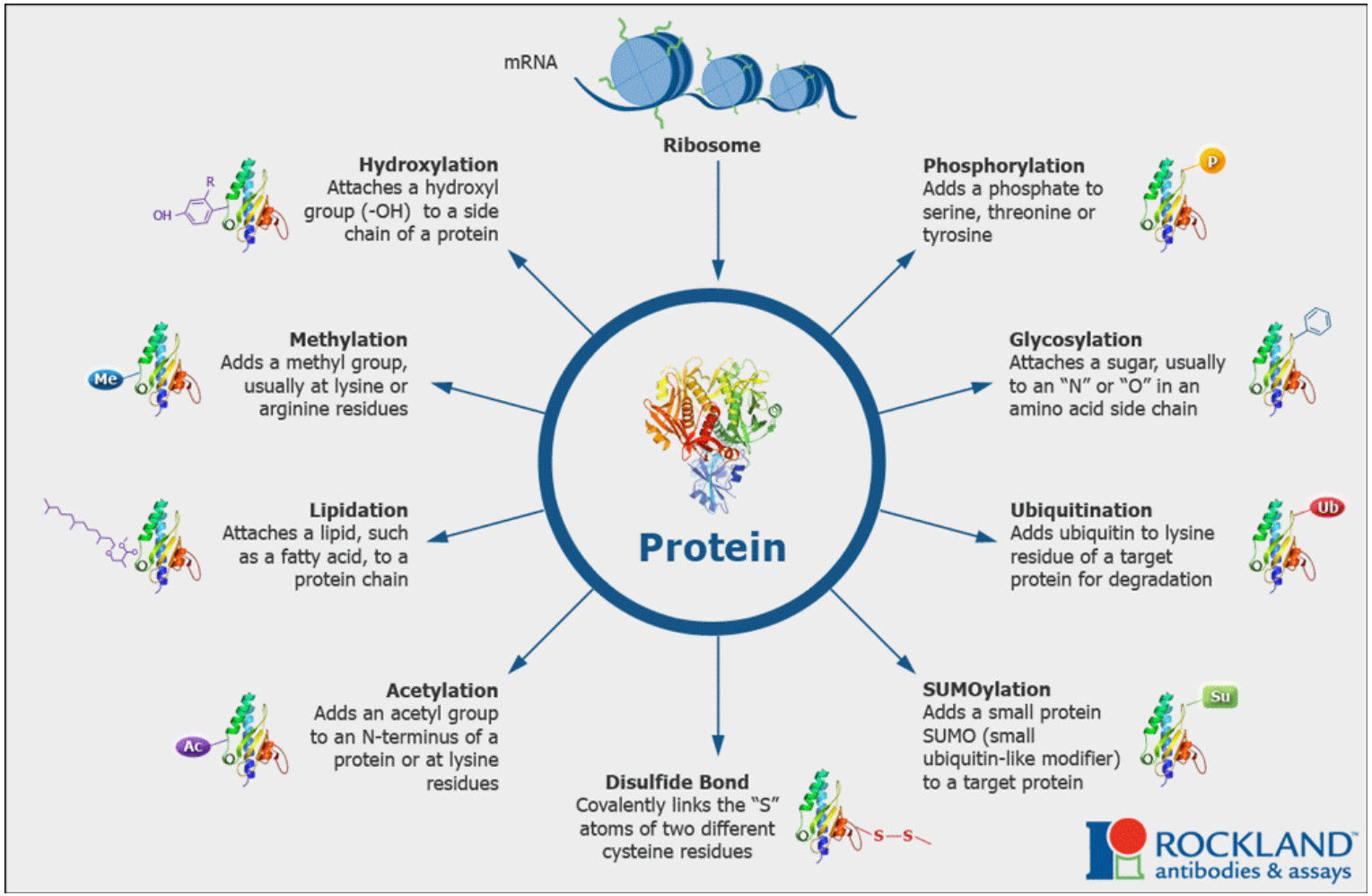
Molecular structure of proteosome - protein complex that degrades unneeded or damaged proteins by proteolysis



DOI: 10.1093/bfgp/3.4.382

A simple model of **protein turnover**. The amount of any protein in the cell is the outcome of the opposing processes of synthesis and destruction. The rate of **synthesis** is most closely linked to the **transcriptome** and the rate of **degradation** is linked to the **metabolome**

Proteins are post-translationally modified



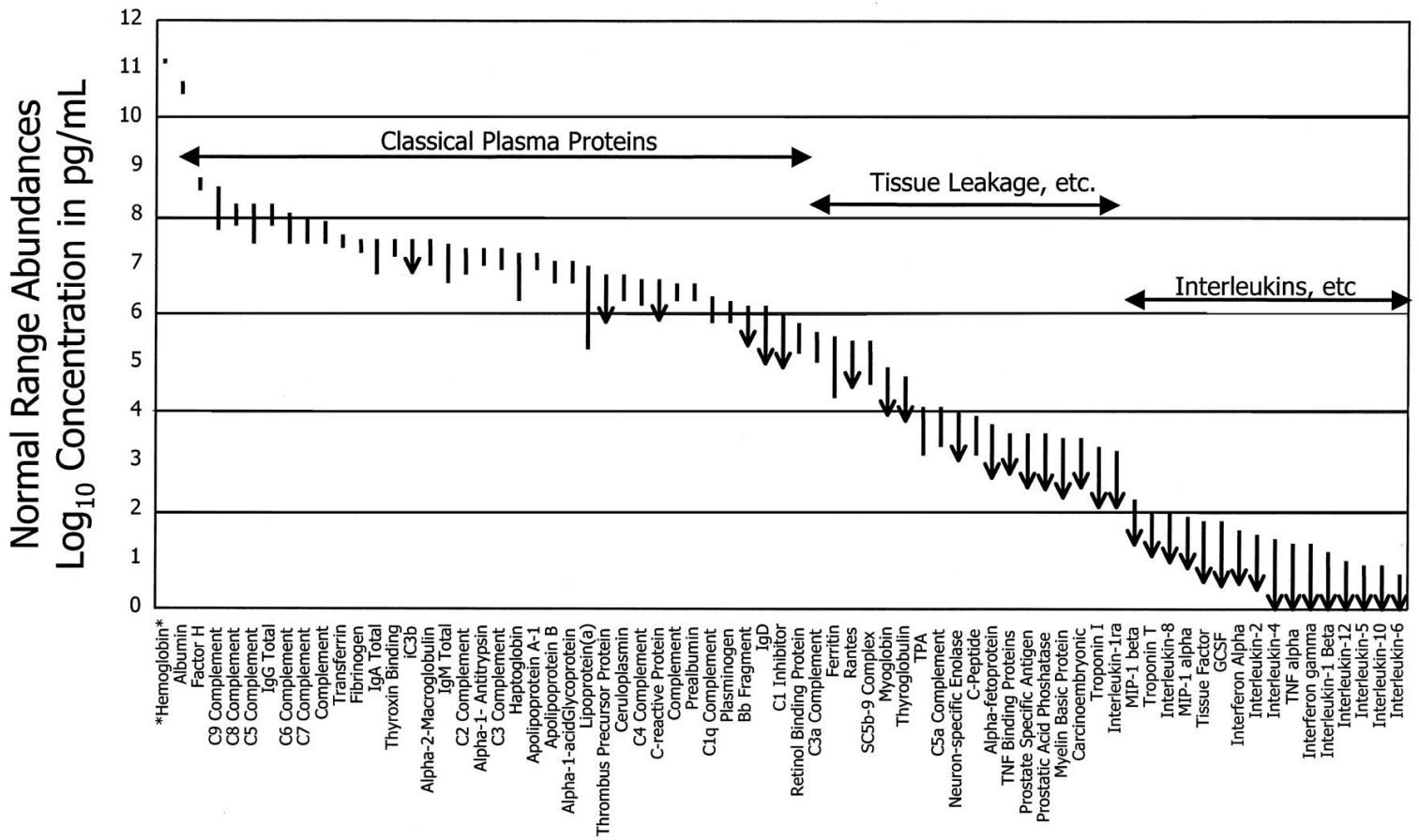
https://rockland-inc.com/post-translational-modification-antibodies.aspx

Experimental

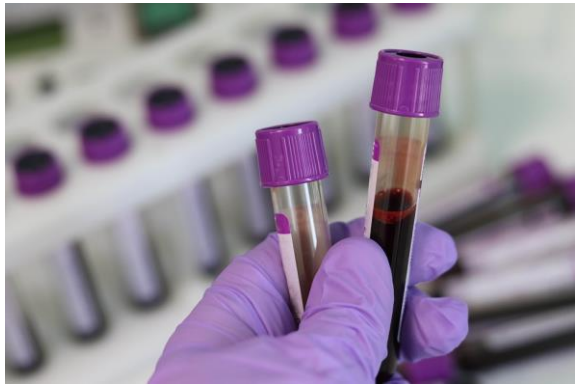
Frequency	Modification
58383	Phosphorylation
6751	Acetylation
5526	N-linked glycosylation
2844	Amidation
1619	Hydroxylation
1523	Methylation
1133	O-linked glycosylation
878	Ubiquitylation
826	Pyrrolidone Carboxylic Acid
504	Sulfation
450	Gamma-Carboxyglutamic Acid
413	Sumoylation
305	Palmitoylation
178	Myristoylation
152	ADP-ribosylation
147	C-linked glycosylation
81	Farnesylation
65	Nitration
62	S-nitrosylation
56	Geranyl-geranylation
55	Citrullination
55	Formylation
53	Deamidation
37	S-diacylglycerol cysteine
34	GPI anchoring
33	Bromination
19	FAD
7208	Others
82182	Total Characterized
89390	Total Processed

http://selene.princeton.edu/PTMCuration/

Proteins exist in a wide range of concentrations in the body



https://doi.org/10.1074/mcp.R200007-MCP200



Reference concentrations for 70 selected proteins in human plasma

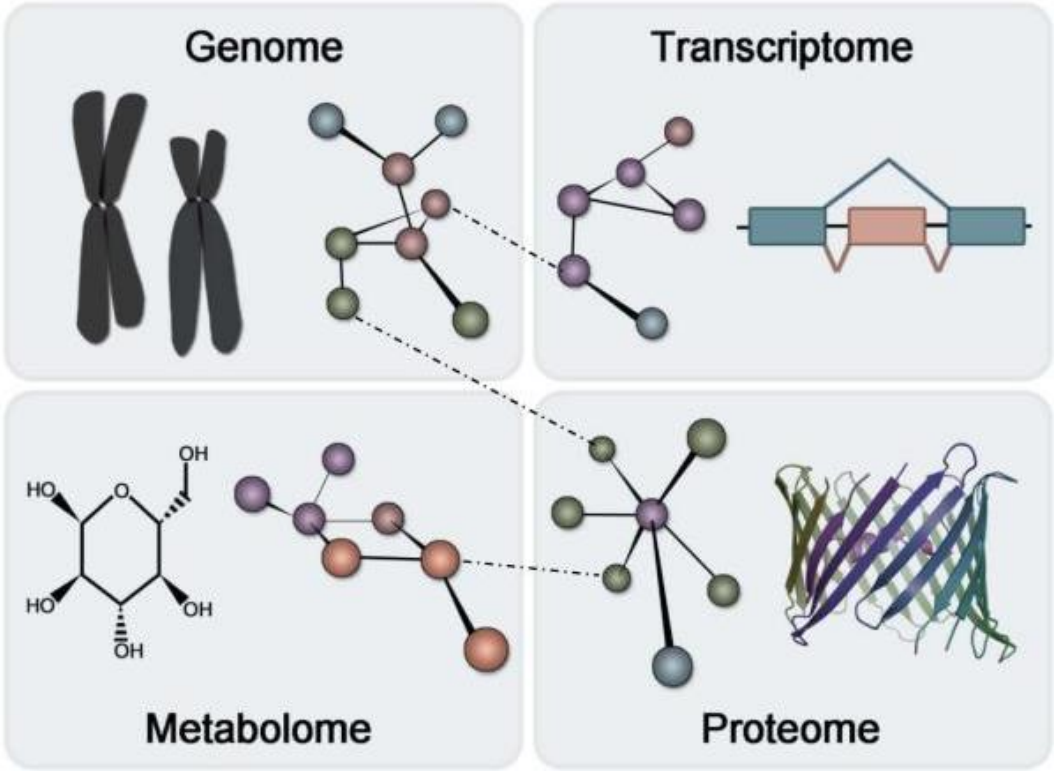
Protein synthesis and the central dogma

- DNA encodes mRNA, and mRNA, protein

DNA $\xrightarrow{\text{transcription}}$ mRNA $\xrightarrow{\text{translation}}$ protein

- But whereas the nucleotide sequence of a gene determines the sequence of its mRNA product, and whereas an mRNA's sequence determines the amino acid sequence of the resulting polypeptide, there is no trivial relationship between the concentration of a transcript and the concentration(s) of the protein(s) derived from a particular locus

http://dx.doi.org/10.1016/j.cell.2016.03.014



Circ Cardiovasc Genet. 2011 Oct 1; 4(5): 576.

From DNA to protein

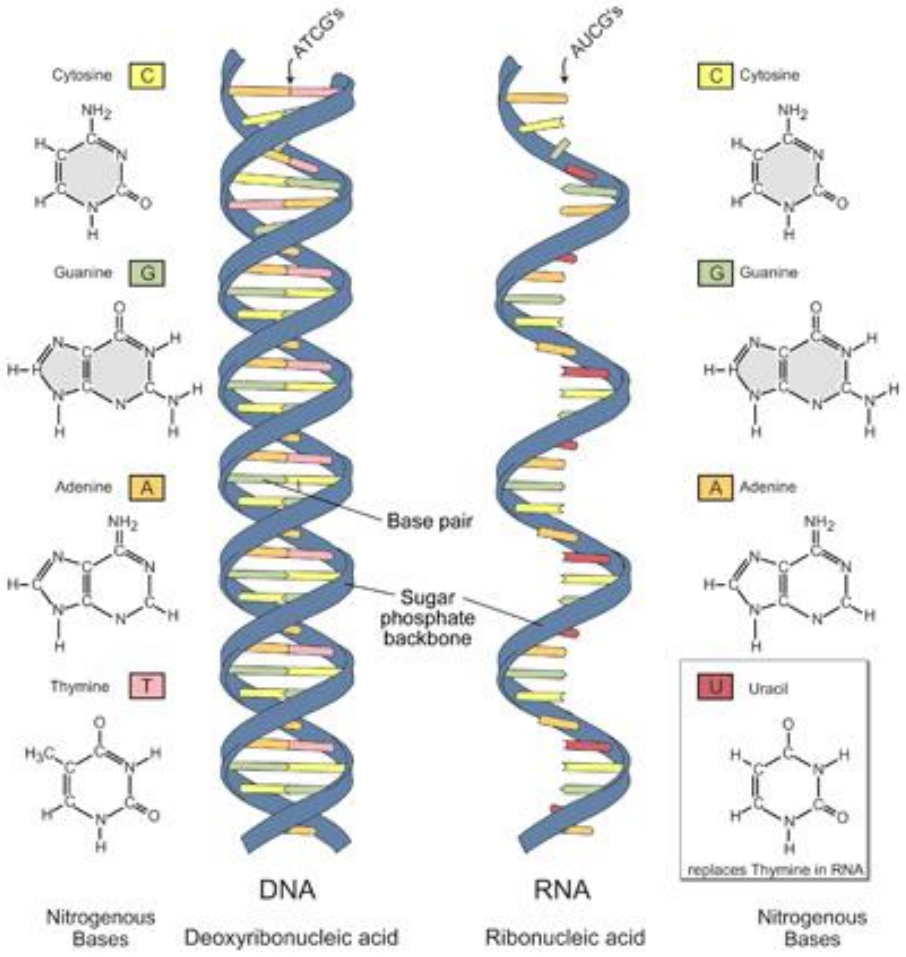
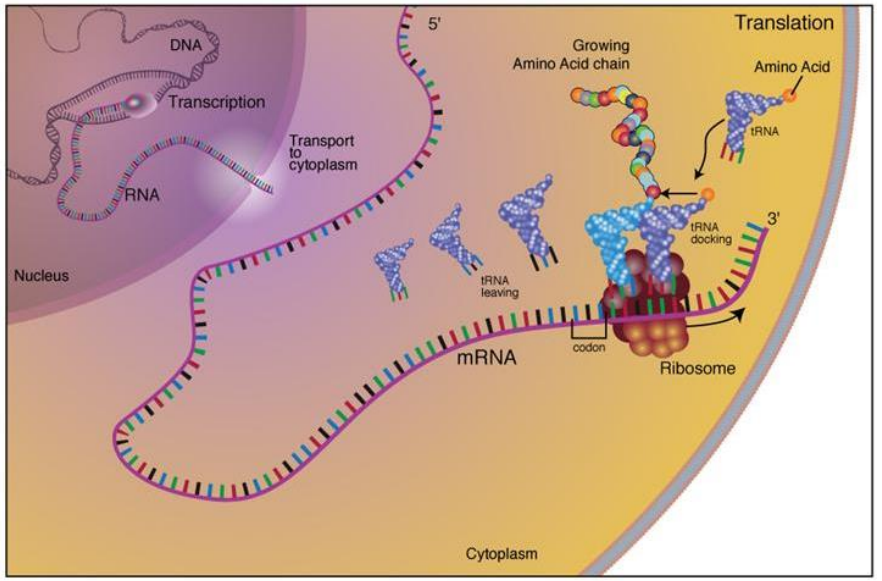
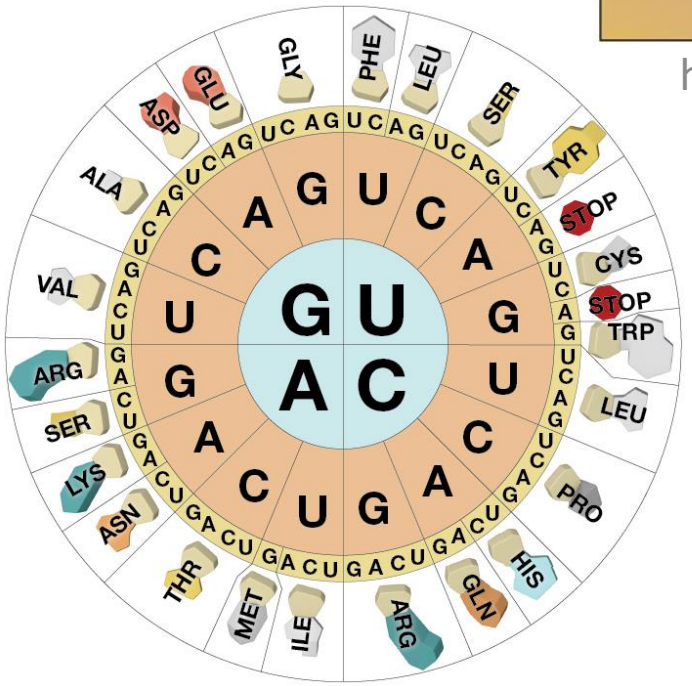


Image adapted from: National Human Genome Research Institute.



https://www.genome.gov/glossary/

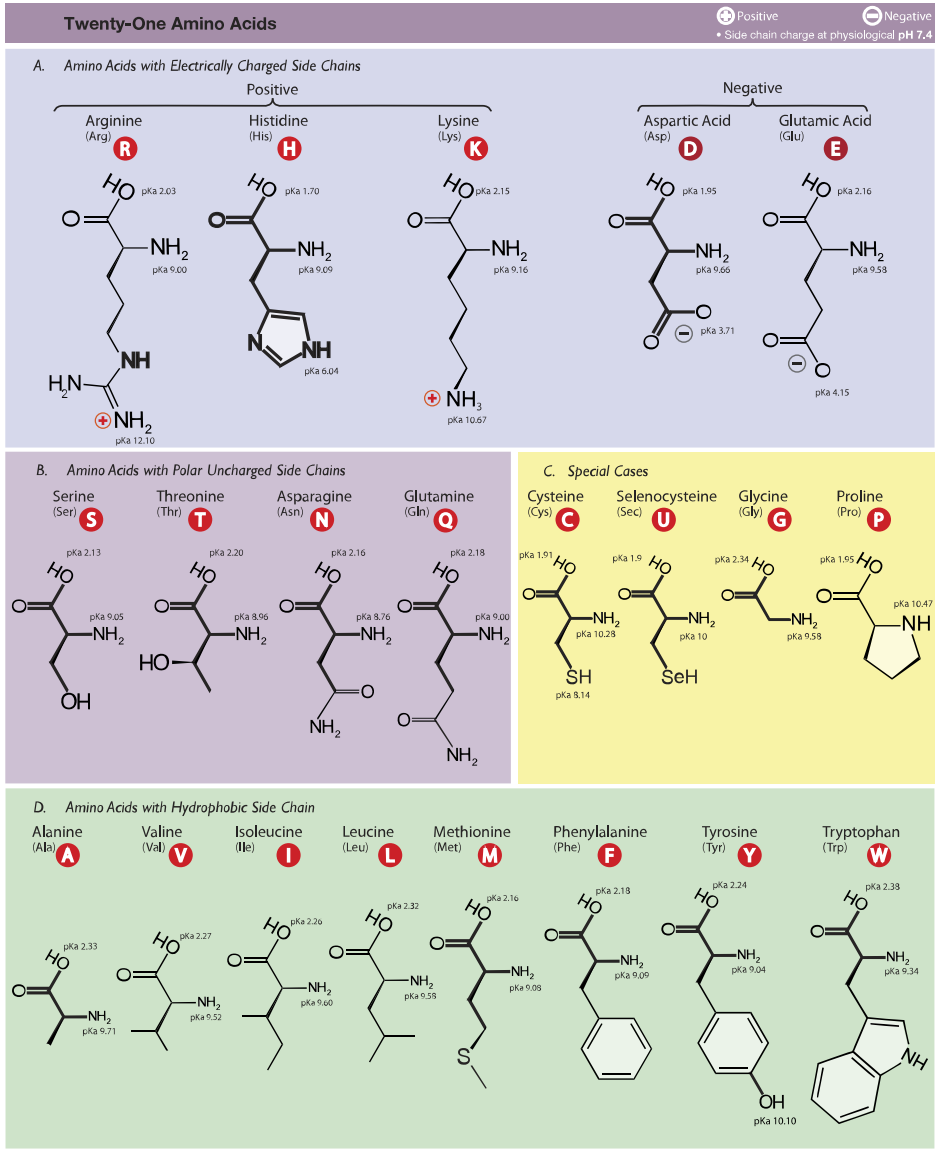


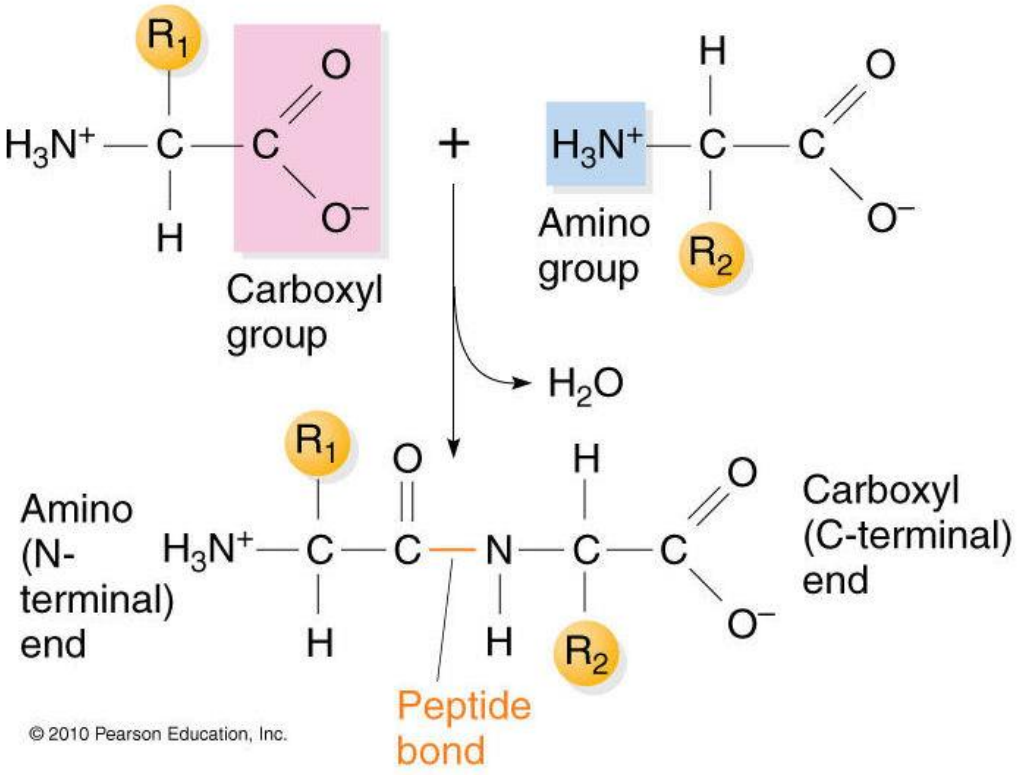
"Each group of three consecutive nucleotides in RNA is called a codon, and each codon specifies either one amino acid or a stop to the translation process"

Source: Molecular Biology of the Cell. 4th edition.

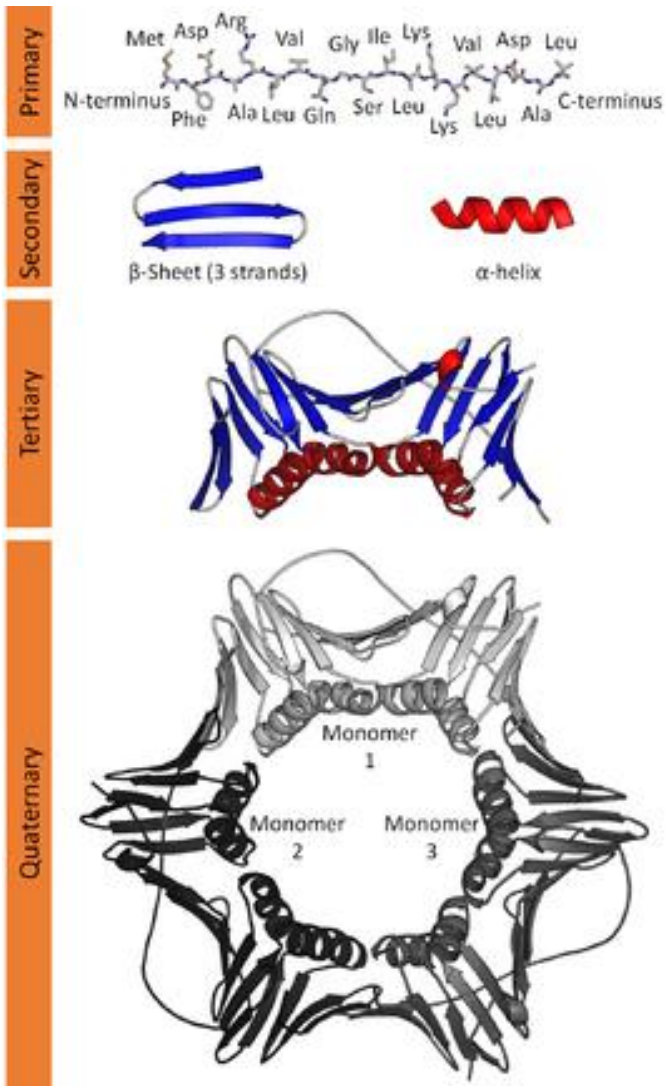
http://learn.genetics.utah.edu/content/basics/dnacodes/

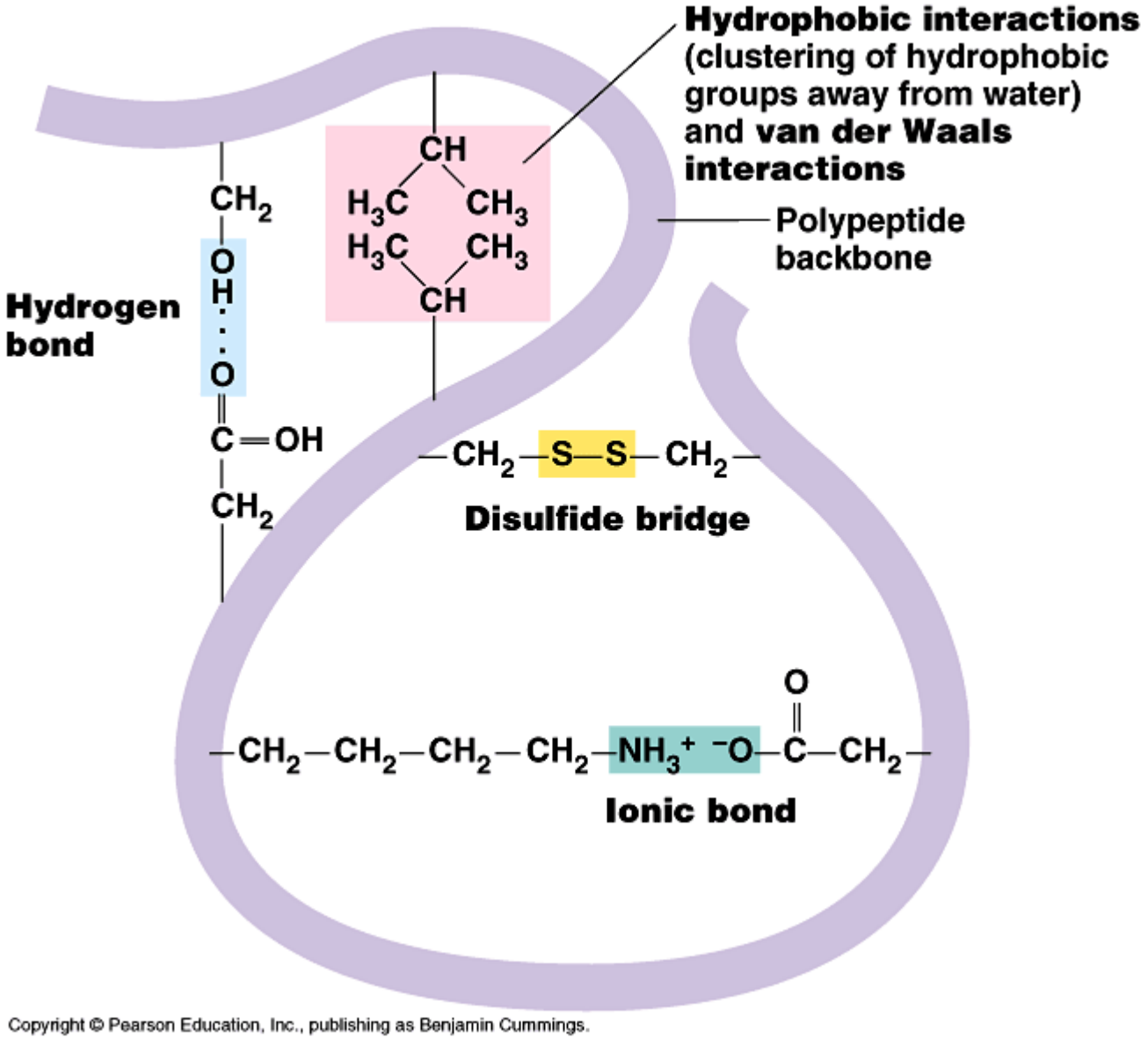
Amino-acids: constituents of proteins





Protein structure





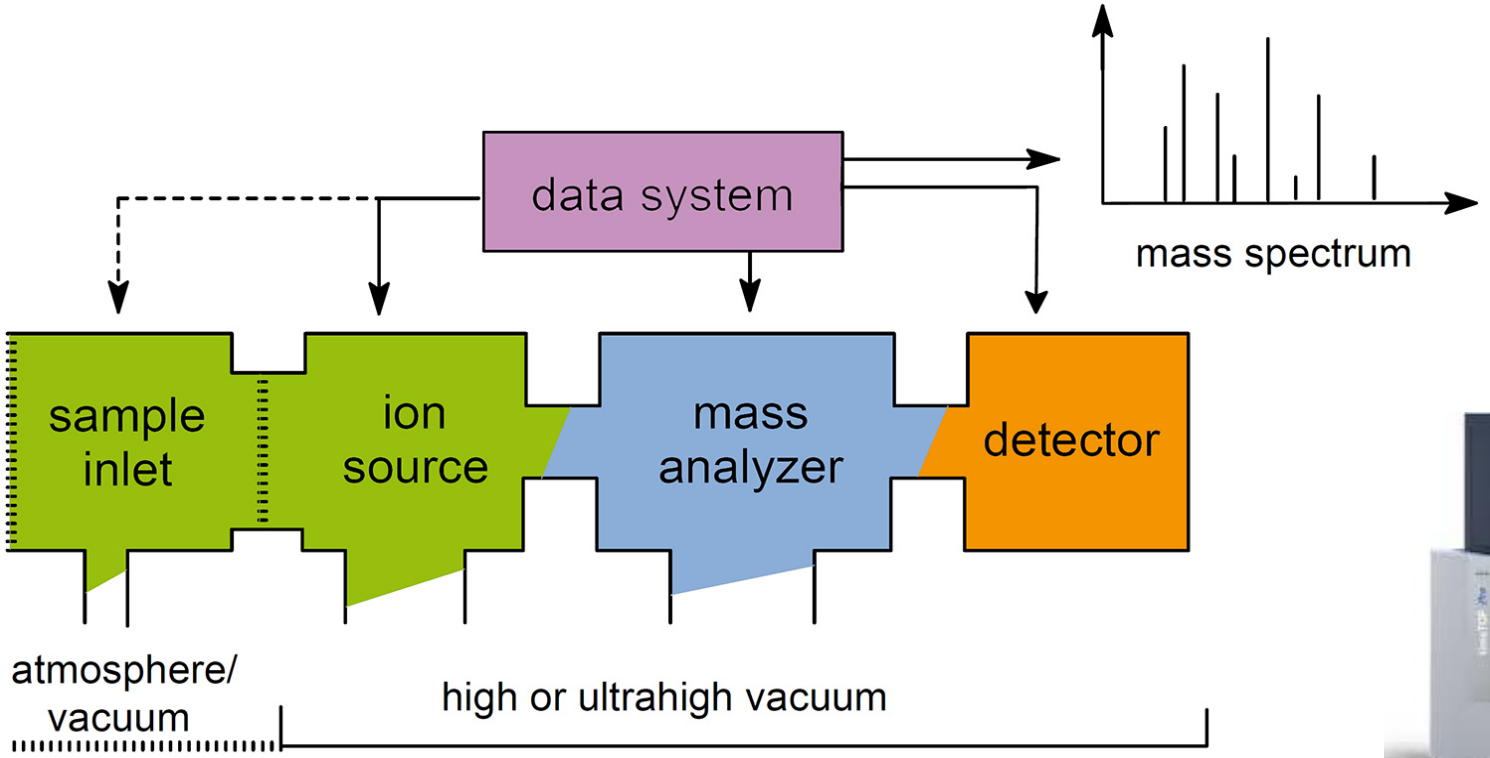
https://en.wikipedia.org/wiki/Protein_structure

Of importance for the mass spectrometrists: molecular weight (*MW*) of amino acids

Amino Acid	Single-Letter Code	Residue MW (amu)	Amino Acid MW (amu)
glycine	G	57.02	75.03
alanine	A	71.04	89.05
serine	S	87.03	105.04
proline	P	97.05	115.06
valine	V	99.07	117.08
threonine	T	101.05	119.06
cysteine	C	103.01	121.02
isoleucine	I	113.08	131.09
leucine	L	113.08	131.09
asparagine	N	114.04	132.05
aspartic acid	D	115.03	133.04
glutamine	Q	128.06	146.07
lysine	K	128.09	146.11
glutamic acid	E	129.04	147.05
methionine	M	131.04	149.05
histidine	H	137.06	155.07
phenylalanine	F	147.07	165.08
arginine	R	156.10	174.11
tyrosine	Y	163.06	181.07
tryptophan	W	186.08	204.09

Quiz time – 3 quick questions

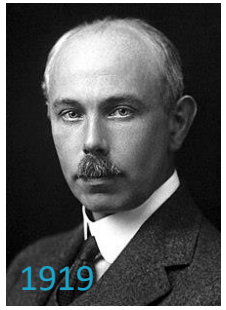
1.2. Reminders in mass spectrometry

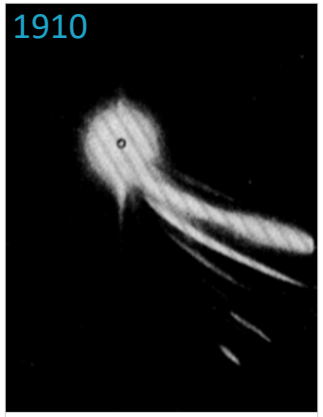


1000 mbar 10^{-5} to 10^{-6} mbar 10^{-6} to 10^{-9} mbar

DOI: 10.1007/978-3-319-54398-7

A brief history





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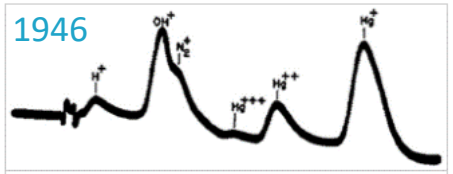
J.J. Thomson captured the parabolas of deflected rays on a photographic plate. Reproduced with permission from *Proc. Roy. Soc. A* ***89***, 1–20 (1913), J.J. Thomson, 'Bakerian Lecture: rays of positive electricity'.



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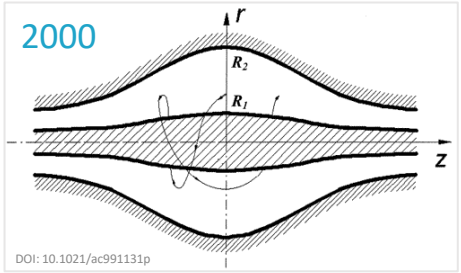
The velocitron developed by Cameron and Eggers could resolve mercury ions with one, two or three positive charges, but not their isotopes. Reprinted with permission from Cameron, A.E. & Eggers, D.F., ***Rev. Sci. Instrum. 19,* 605–607 (1948).**



…

Elsevier

John Hipple in 1943. Image reproduced from *Encyclopedia of Mass Spectrometry: Vol. 9: Historical Perspectives, Part A: The Development of Mass Spectrometry* (Keith A. Nier, Alfred L. Yergey & P. Jane Gale), Newnes, 2015, p. 112, with permission from Elsevier.

In 2002, William Henzel, John Stults and Colin Watanabe (pictured left to right) won the Distinguished Contribution in Mass Spectrometry Award for their work on peptide mass fingerprinting. Reproduced from Robinson, C. & Gross, M., Focus on proteomics in honor of the 2002 Distinguished Contribution in Mass Spectrometry Award to W. J. Henzel , J. T. Stults , and C. Watanabe , *J. Am. Soc. Mass Spectrom.* ***14***, 929–930 (2003), with kind permission from Springer Science and Business Media.

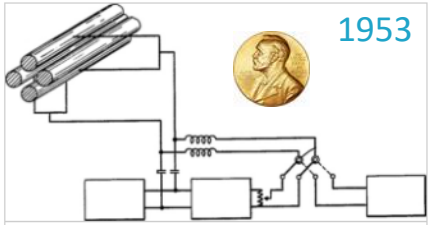
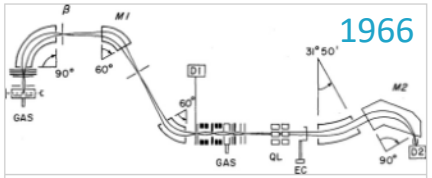


Diagram of Wolfgang Paul's patent for a quadrupole mass filter. Image adapted from US patent 2,939,952 A (1953).



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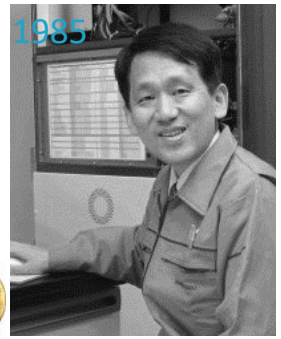
A schematic of the tandem mass spectrometer developed by Futrell and Miller. Reprinted with permission from Futrell, J.H. & Miller, C.D., Tandem mass spectrometer for study of ion-molecule reactions, *Rev. Sci. Instrum.* ***37***, 1521 (1966).



Of protons or proteins 1988

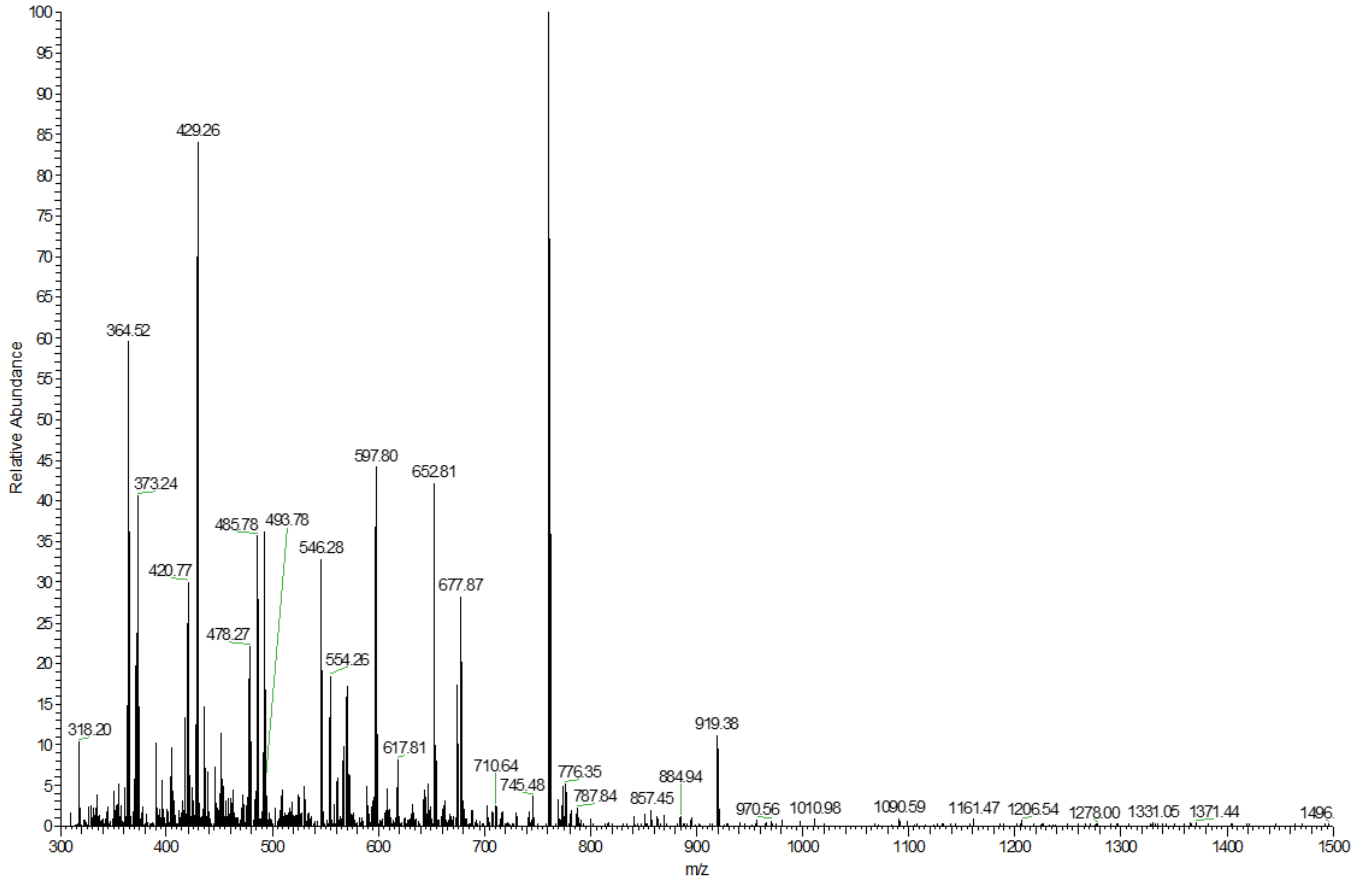
C.K. Meng, M. Mann, and J.B. Fenn

MILESTONES TIMELINE	
1910	The beginnings (Milestone 1)
1929	Development of ionization methods (Milestone 2)
1939	Environmental analysis (Milestone 3)
1946	Time of flight (Milestone 4)
1949	Trapping mass analyzers (Milestone 5)
1953	Quadrupole and triple-stage quadrupole mass filters (Milestone 6)
1956	Small-molecule analysis (Milestone 7)
1959	Separations (Milestone 8)
1959	Peptide sequencing (Milestone 9)
1961	Inductively coupled plasma mass spectrometry (Milestone 10)
1962	Imaging mass spectrometry (Milestone 11)
1963	Carbohydrate analysis (Milestone 12)
1966	Tandem mass spectrometry (Milestone 13)
1966	Metabolomics (Milestone 14)
1968	Electrospray ionization (Milestone 15)
1975	Medical applications (Milestone 16)
1978	Selected reaction monitoring (Milestone 17)
1985	Matrix-assisted laser desorption/ionization (Milestone 18)
1991	Structural biology applications (Milestone 19)
1993	Proteomics (Milestone 20)
1995	Post-translational modification analysis (Milestone 21)
1999	Interactome analysis (Milestone 22)

https://www.nature.com/milestones/milemassspec/timeline/index.html

Few definitions

- A mass spectrum (ions separated according to their mass and detected in proportion to their numbers)
- *m* is the mass of the ion
- Dalton (Da) or mass unit (u): 1/12 of the mass of ^{12}C = 1.66054×10^{-27} kg))
- *m*/*z* (“thomson (Th)” or dimensionless)



Note: Some mass spectrometrists use the unit thomson [Th] (to honor J. J. Thomson) instead of the dimensionless quantity m/z. Although the thomson is accepted by some journals, it is not a SI unit. In particular mass spectrometrists in the biomedical field of mass spectrometry tend to use the dalton [Da] (to honor J. Dalton) instead of the unified atomic mass [u]. The dalton also is not a SI unit.

DOI: 10.1007/978-3-319-54398-7

Mass: definition

- **Average mass**—the mass of an ion of a known empirical formula, which is calculated by summing the relative atomic mass of each atom present, the so-called weighted average

- **Nominal mass**—the mass of an ion or molecule calculated using the mass of the most abundant isotope of each element rounded to the nearest integer value and equivalent to the sum of the mass numbers of all constituent atoms

- **Accurate mass**—the experimentally determined mass of an ion measured to an appropriate degree of accuracy and precision used to determine, or limit the possibilities for, the elemental formula of the ion

- **Exact mass**—is the calculated mass of an ion whose elemental formula, isotopic composition and charge state are known, *i.e.*, it is the theoretical mass. The IUPAC definition constricts the definition to using one isotope of each atom involved, usually the lightest isotope

https://doi.org/10.1016/j.jasms.2010.06.006

Note: The mass number is the sum of the total number of protons and neutrons in an atom, molecule, or ion. The mass number of an isotope is given as superscript preceding the elemental symbol, *e.g.*, ^{12}C.

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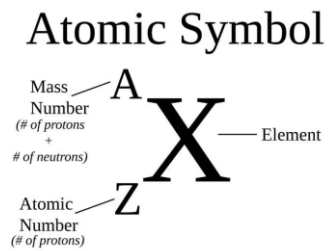
Atomic mass of selected elements



HYDROGEN

Isotope	Atomic mass (Da)	Isotopic abundance (amount fraction)
^{1}H	1.007 825 0322(6)	[0.999 72, 0.999 99]
^{2}H	2.014 101 7781(8)	[0.000 01, 0.000 28]

CARBON

Isotope	Atomic mass (Da)	Isotopic abundance (amount fraction)
^{12}C	12(exact)	[0.9884, 0.9904]
^{13}C	13.003 354 835(2)	[0.0096, 0.0116]

NITROGEN

Isotope	Atomic mass (Da)	Isotopic abundance (amount fraction)
^{14}N	14.003 074 004(2)	[0.995 78, 0.996 63]
^{15}N	15.000 108 899(4)	[0.003 37, 0.004 22]

OXYGEN

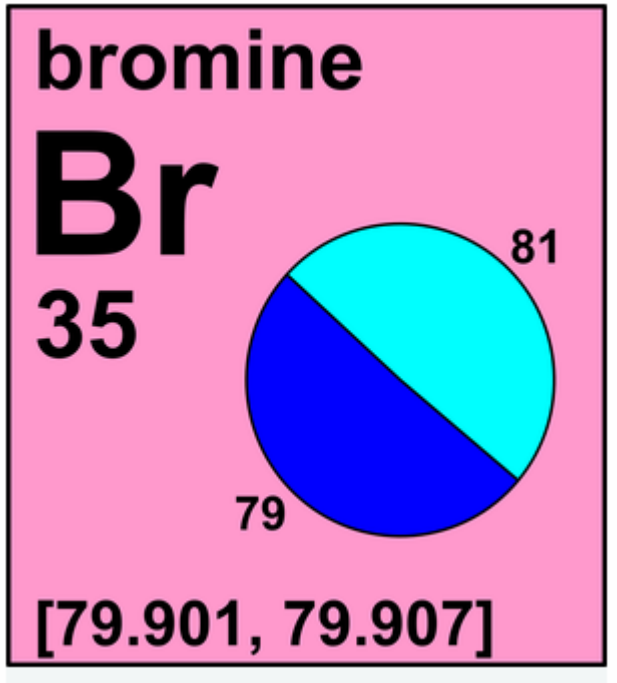
Isotope	Atomic mass (Da)	Isotopic abundance (amount fraction)
^{16}O	15.994 914 619(1)	[0.997 38, 0.997 76]
^{17}O	16.999 131 757(5)	[0.000 367, 0.000 400]
^{18}O	17.999 159 613(5)	[0.001 87, 0.002 22]

SULFUR

Isotope	Atomic mass (Da)	Isotopic abundance (amount fraction)
^{32}S	31.972 071 174(9)	[0.9441, 0.9529]
^{33}S	32.971 458 91(1)	[0.007 29, 0.007 97]
^{34}S	33.967 8670(3)	[0.0396, 0.0477]
^{36}S	35.967 081(2)	[0.000 129, 0.000 187]

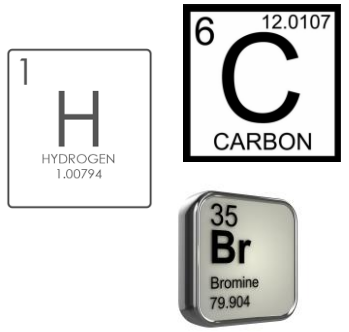
BROMINE

Isotope	Atomic mass (Da)	Isotopic abundance (amount fraction)
^{79}Br	78.918 338(7)	[0.505, 0.508]
^{81}Br	80.916 288(6)	[0.492, 0.495]



http://ciaaw.org/index.htm

About mass and isotopes



- Average mass

  average mass of CH_3Br = 12.0107 + 3 × 1.00794 + 79.904 = 94.93852 Da

- Nominal mass

  nominal mass of CH_3Br = 12 + 3 × 1 + 79 = 94 u

- Monoisotopic mass

  monoisotopic mass of CH_3Br = $^{12}C^{1}H_3{}^{79}Br$ = (12.0000 + 3 × 1.007825 + 78.918338) = 93.94181 u

- Exact mass of an isotopic species

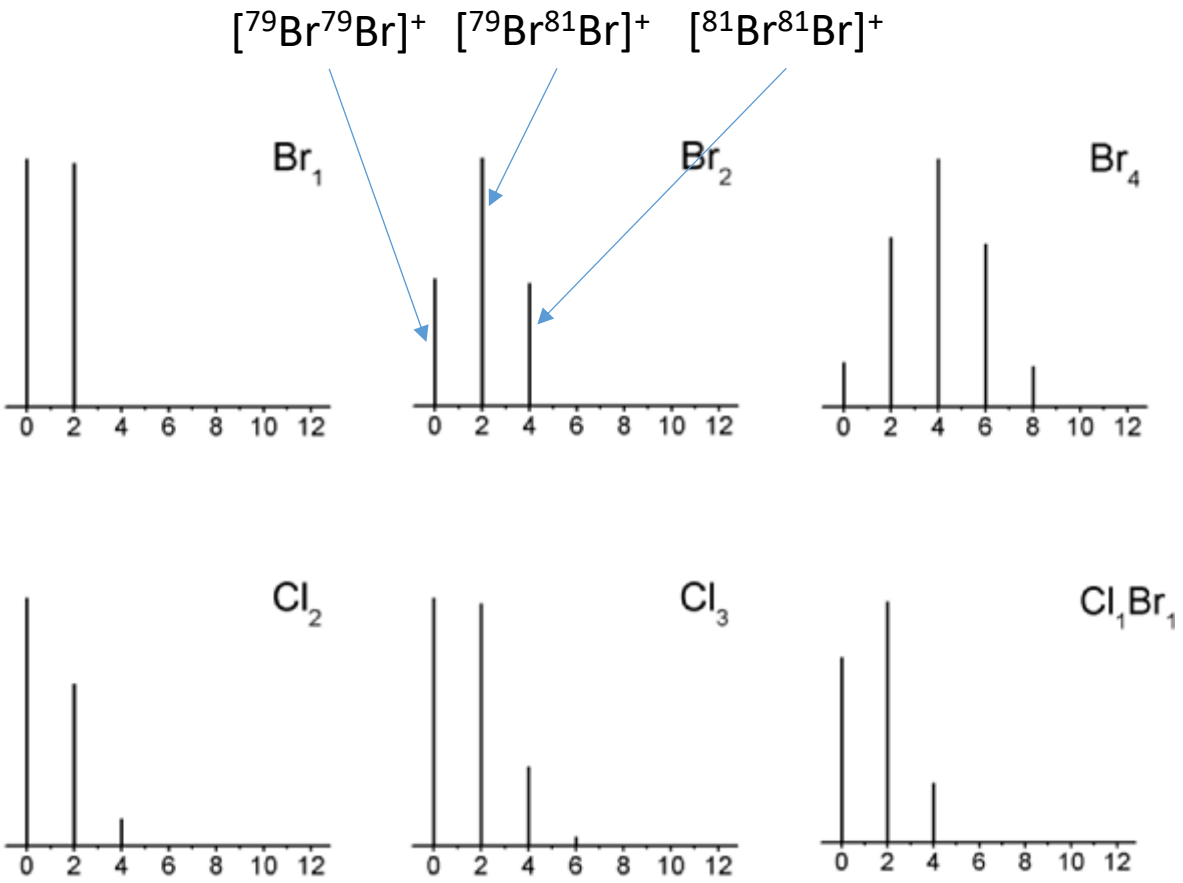
  exact mass of H_2O = 2 × 1.007825 + 15.99491 = 18.01056 u

  exact mass of D_2O = 2 × 2.014105 + 15.99491 = 20.02312 u

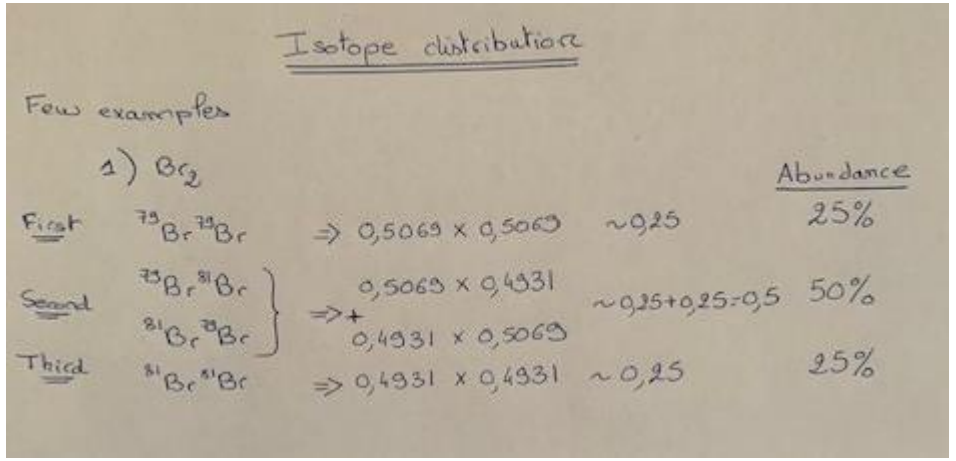
Q1: Insulin ($C_{257}H_{383}N_{65}O_{77}S_6$): average, nominal, and monoisotopic mass?

A- 5803.638 u B- 5807.570 Da C- 5801 u

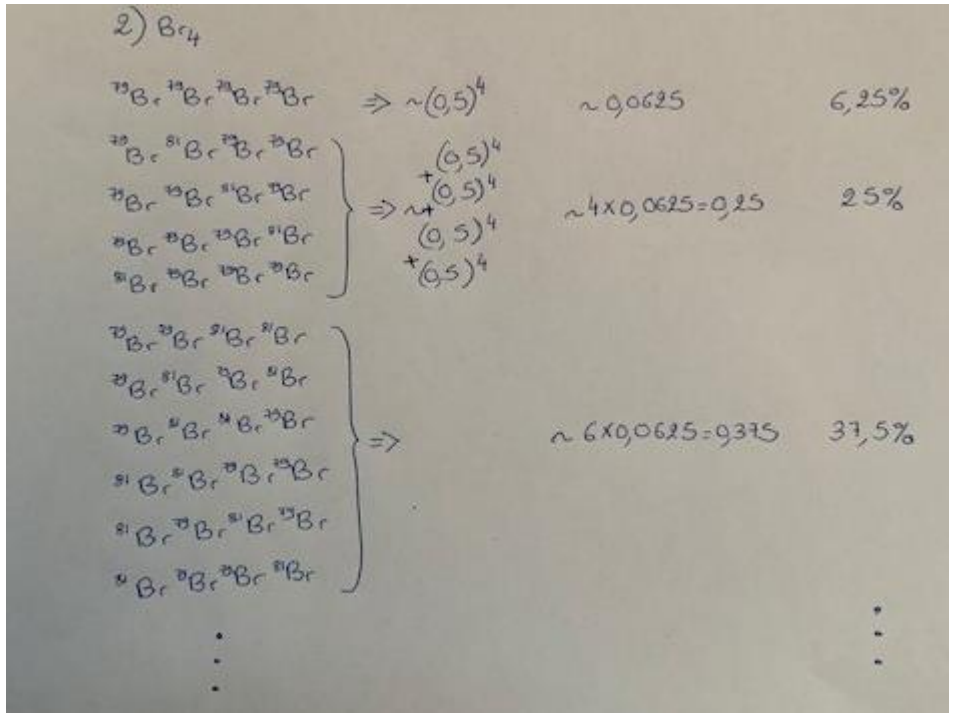
About mass and isotopes



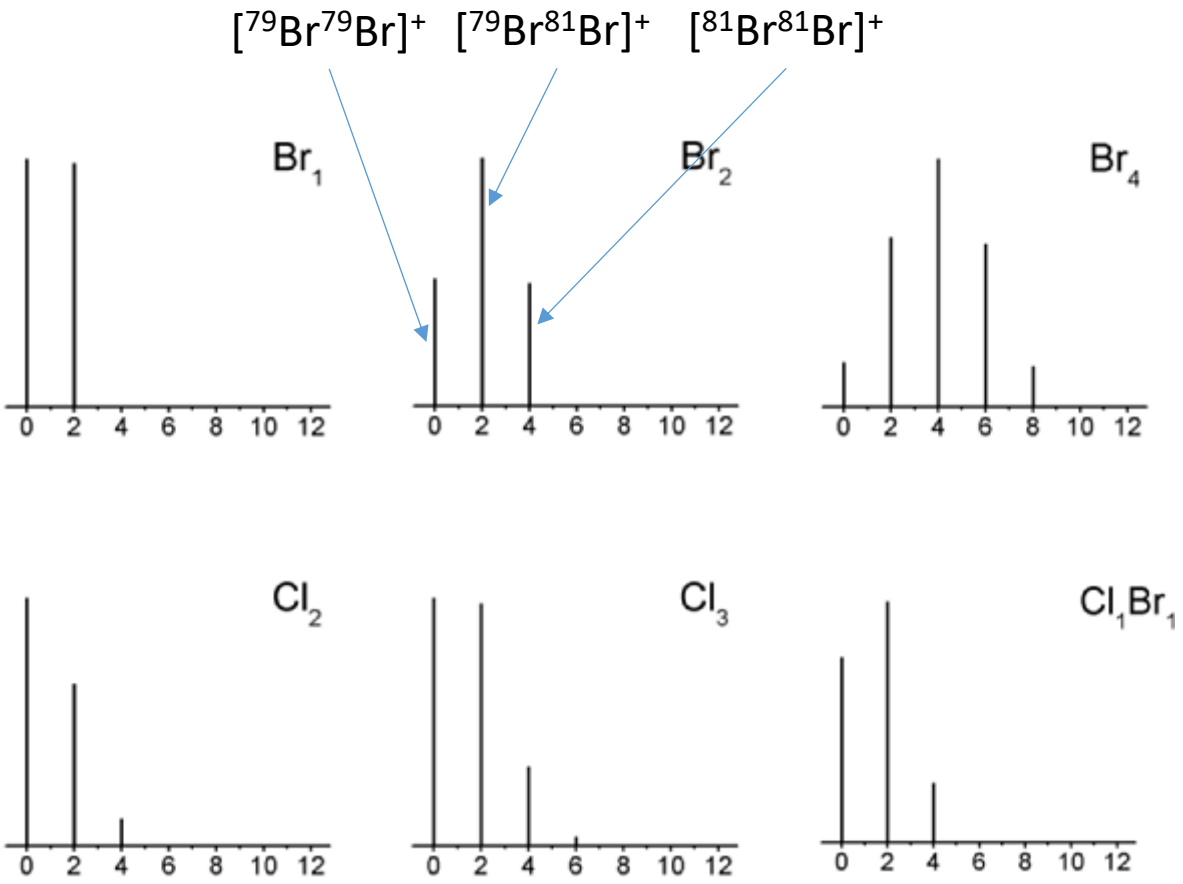
DOI: 10.1007/978-3-319-54398-7

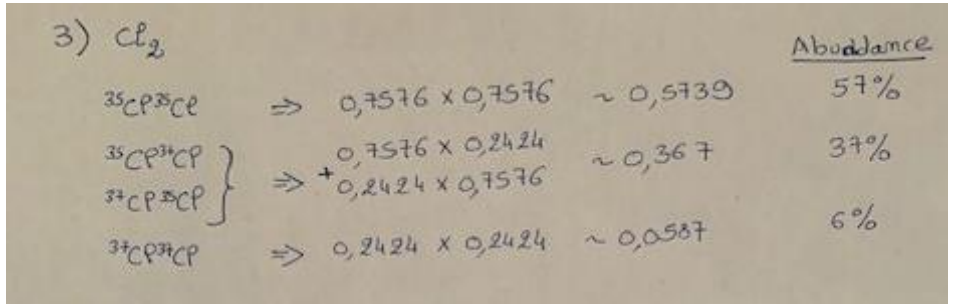


Isotope	Abondance
^{79}Br	50.69%
^{81}Br	49.31%



About mass and isotopes





Isotope	Abondance
^{35}Cl	75.76%
^{36}Cl	Trace
^{37}Cl	24.24%

DOI: 10.1007/978-3-319-54398-7

Elemental composition of peptides/proteins

Isotope	Abondance
^{1}H	**99.9885%**
^{2}H	0.0115%

Isotope	Abondance
^{12}C	**98.93%**
^{13}C	1.07%

Isotope	Abondance
^{14}N	**99.636%**
^{15}N	0.364%

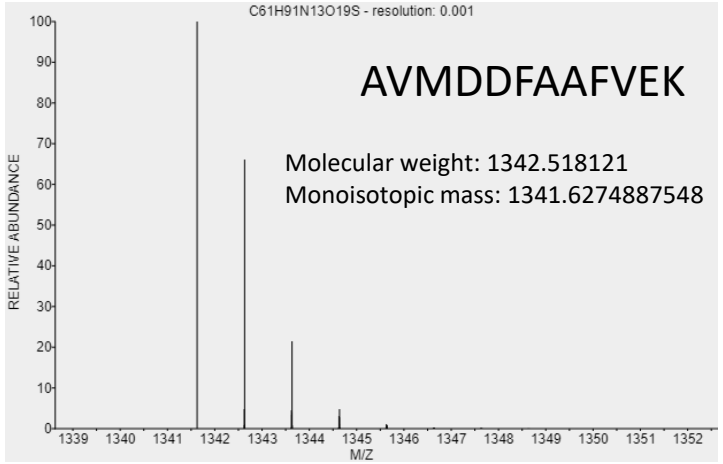
Isotope	Abondance
^{16}O	**99.757%**
^{17}O	0.038%
^{18}O	0.205%

Isotope	Abondance
^{32}S	**94.93%**
^{33}S	0.76%
^{34}S	4.29%
^{36}S	0.02%

Q2: What is the main isotope for formula C_1, C_{10}, C_{100}?

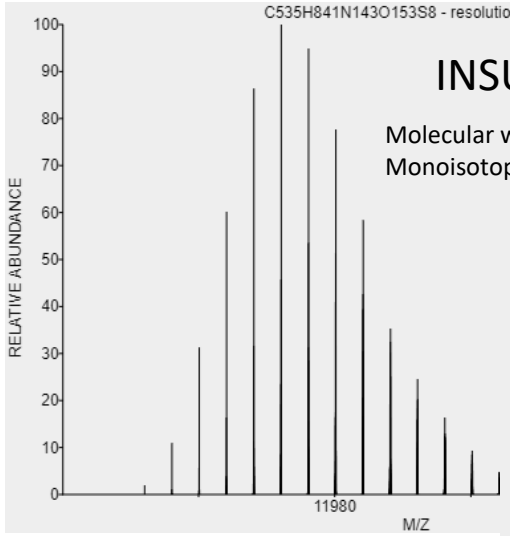
http://www.chemcalc.org/

Isotopic distribution of peptides/proteins



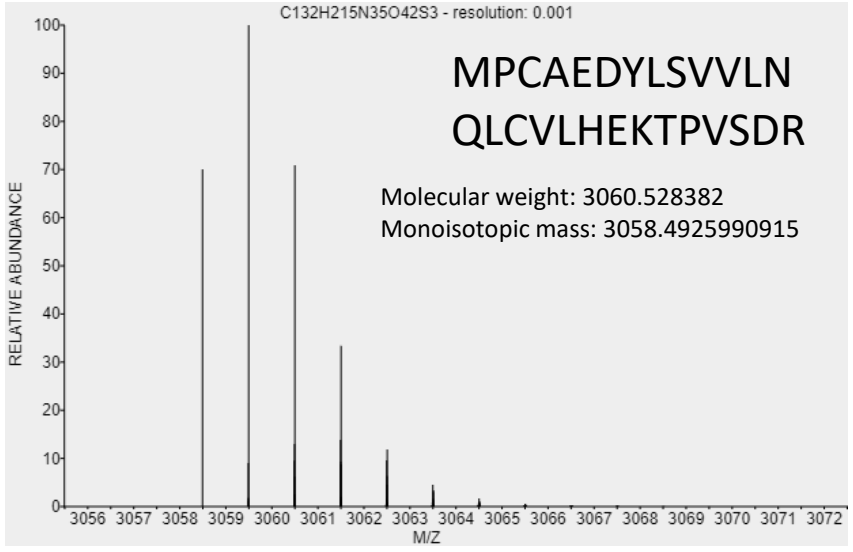
AVMDDFAAFVEK

Molecular weight: 1342.518121
Monoisotopic mass: 1341.6274887548



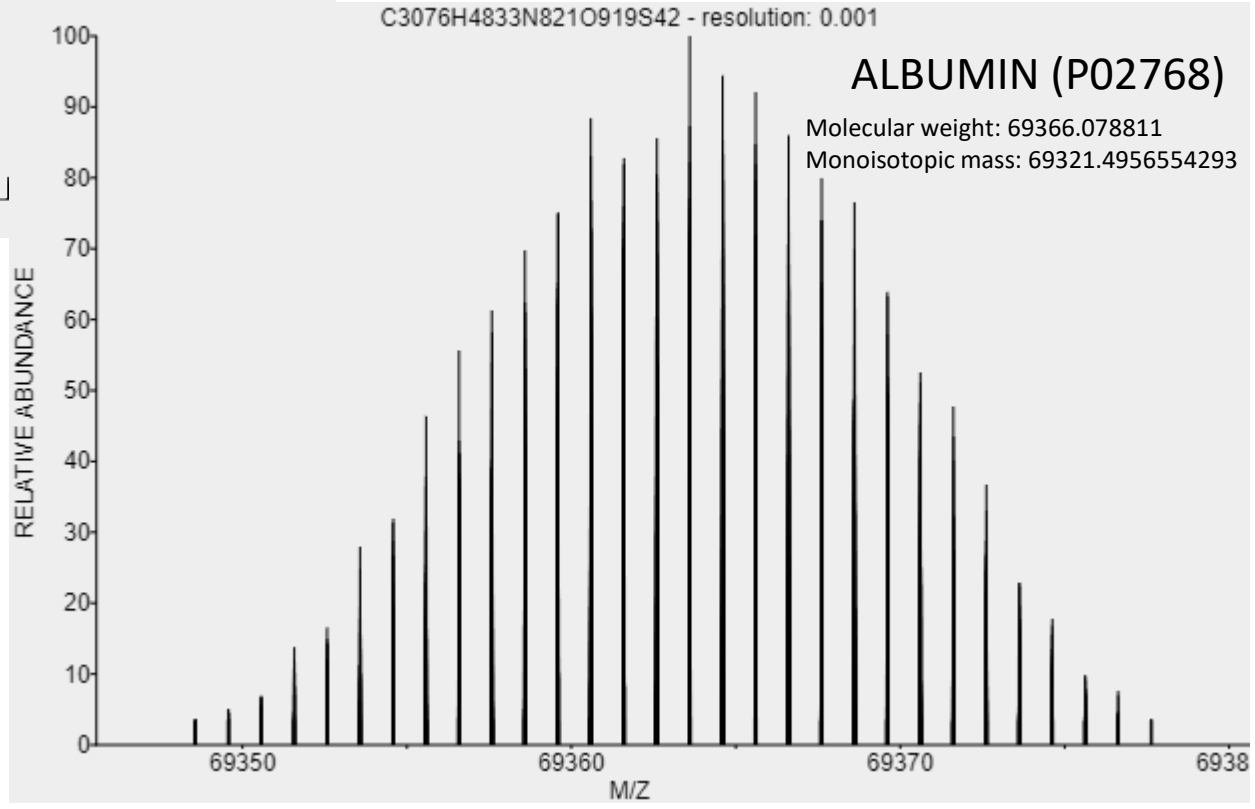
INSULIN (P01308)

Molecular weight: 11980.80769
Monoisotopic mass: 11973.0189394716



MPCAEDYLSVVLN
QLCVLHEKTPVSDR

Molecular weight: 3060.528382
Monoisotopic mass: 3058.4925990915

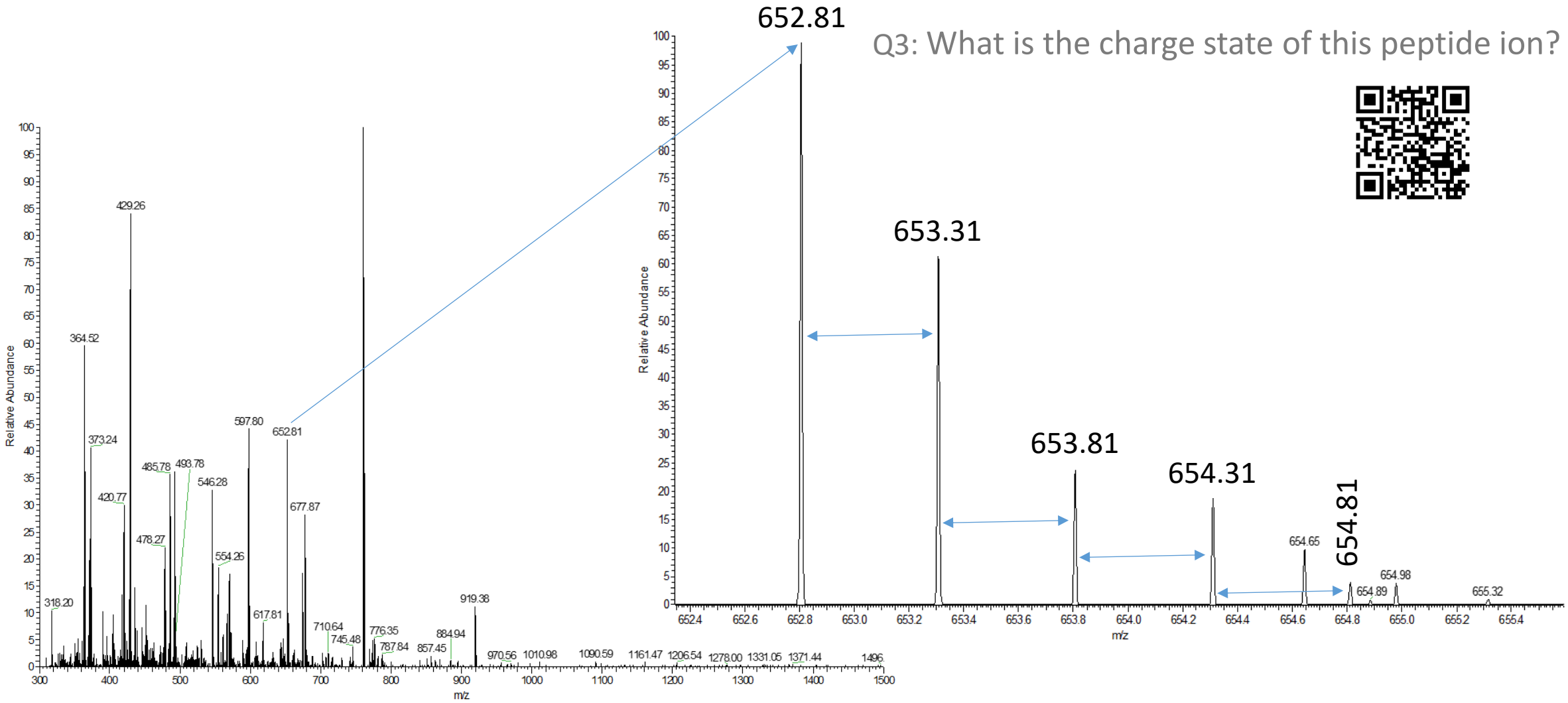


ALBUMIN (P02768)

Molecular weight: 69366.078811
Monoisotopic mass: 69321.4956554293

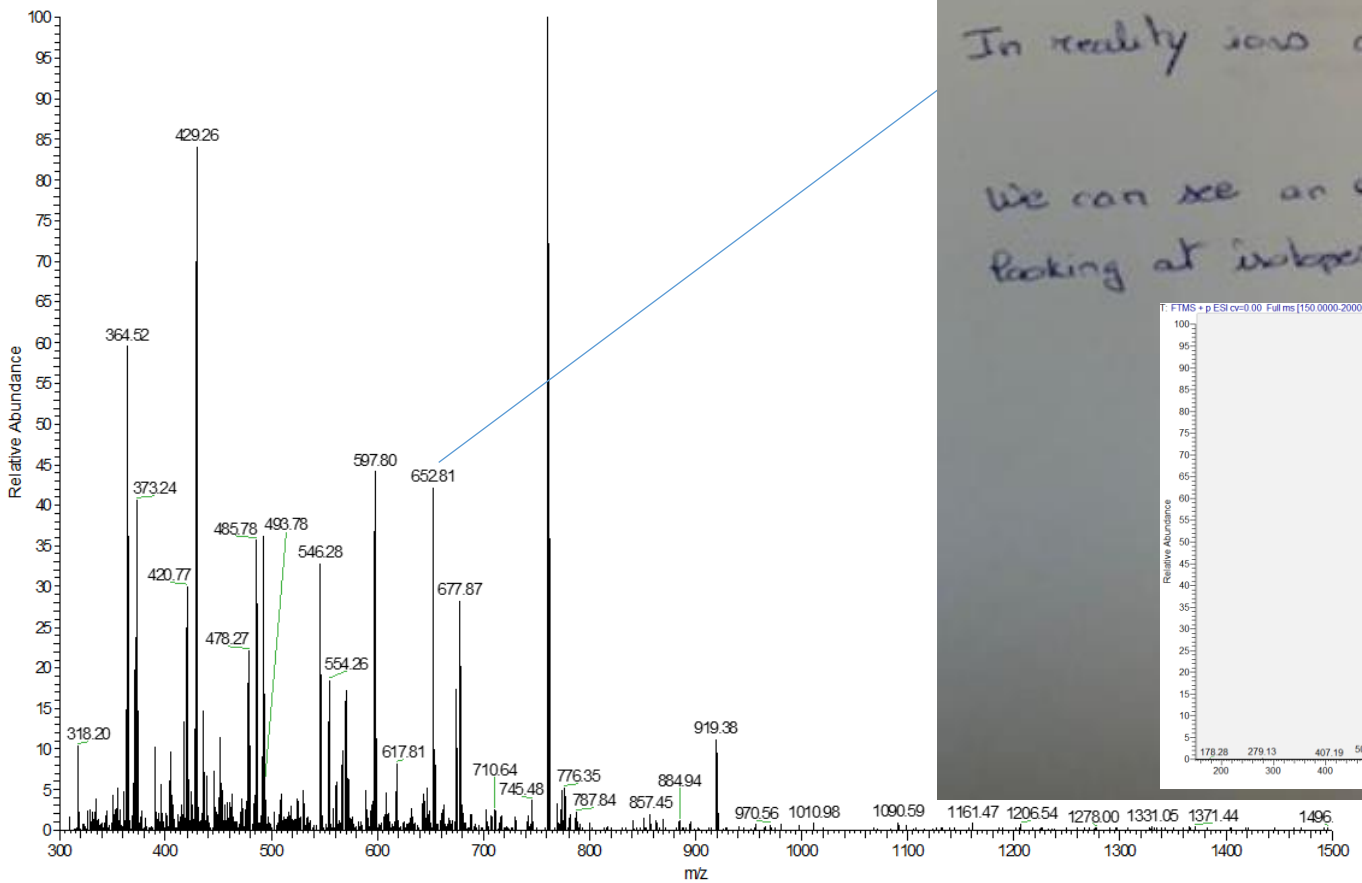
http://www.chemcalc.org/list/atomgroups

About mass, isotopes, and charges



Q3: What is the charge state of this peptide ion?

About mass, isotopes, and charges

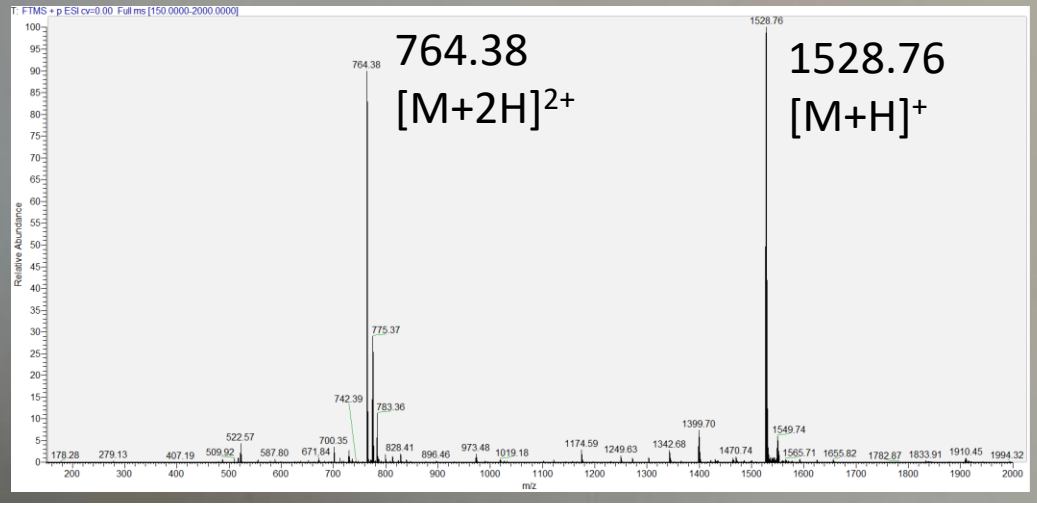


Mass spectrometry measures the mass of charge particules => m/z

An analyte, noted M, is ionized [M+H]⁺ = MH⁺

In reality ions can even be multi-charged [M+2H]²⁺ [M+3H]³⁺

We can see an effect on the mass spectrum, specially when looking at isotopes and the distance between them.

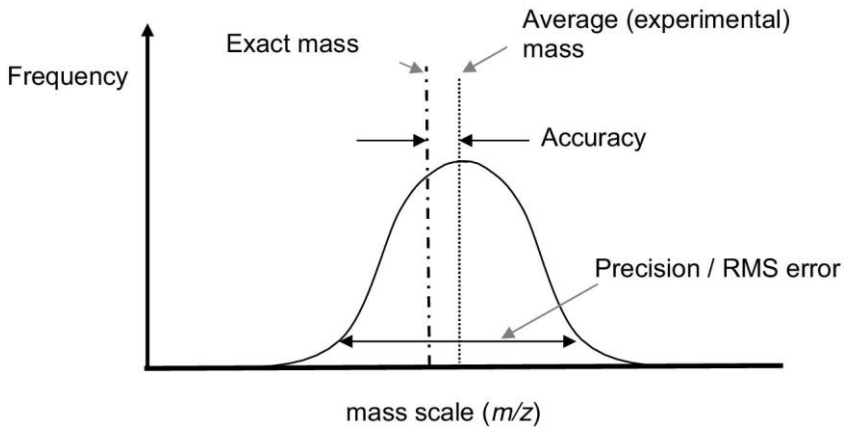


About mass accuracy

- The difference between the measured value (accurate mass) and the true value (exact mass) is the “accuracy”

- Mass measurement error (or accuracy) of a single reading will be:

$$\Delta m_i = (m_i - m_a)\mathrm{inDa} = (m_i - m_a) \times 10^3 \mathrm{inmDa} = \frac{(m_i - m_a)}{m_a} \times 10^6 \mathrm{inppm\,(partspermillion)}$$

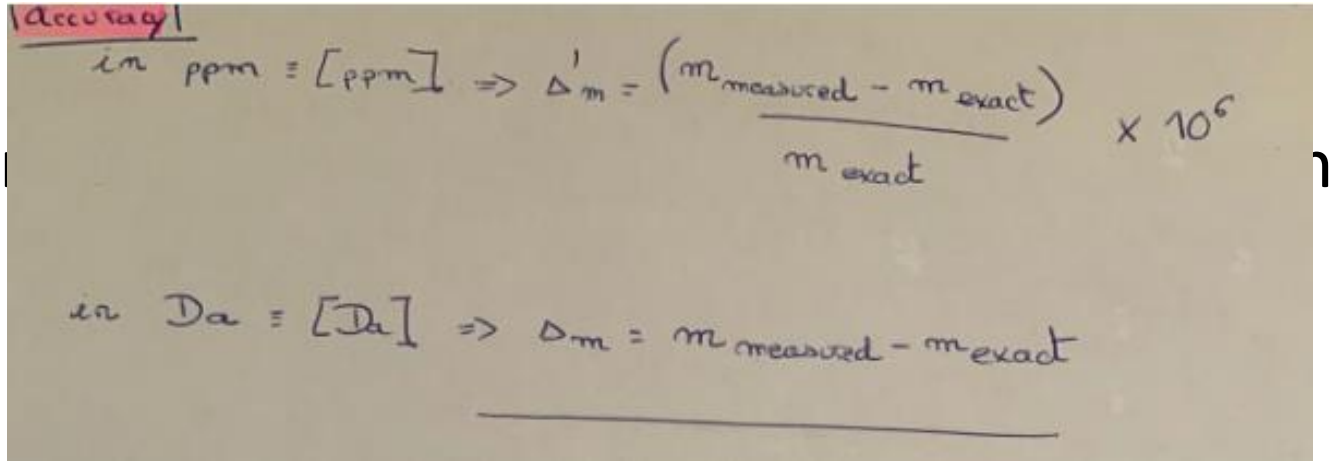


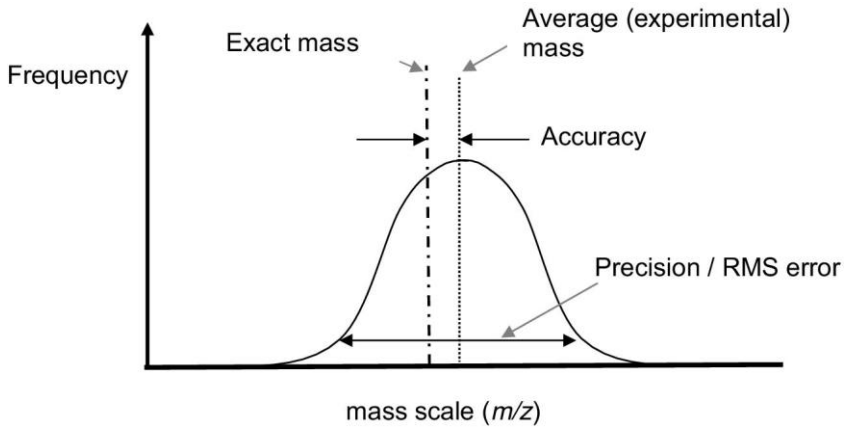
Q4: My mass spectrometer achieves an accuracy of 1 ppm. What is my Δm at m/z = 1000?

https://doi.org/10.1016/j.jasms.2010.06.006

About mass accuracy

- The difference between the measured value (accurate mass) and the true value (exact mass) is the “accuracy”
- Mass measu ng will be:





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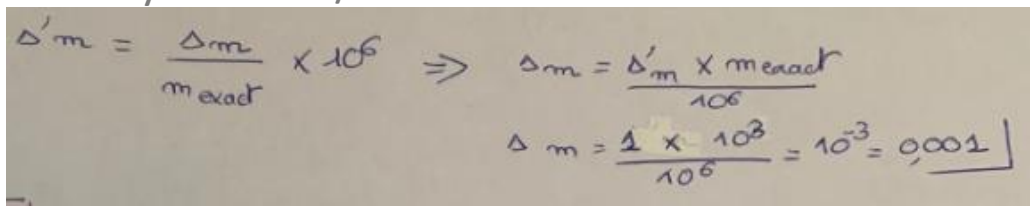
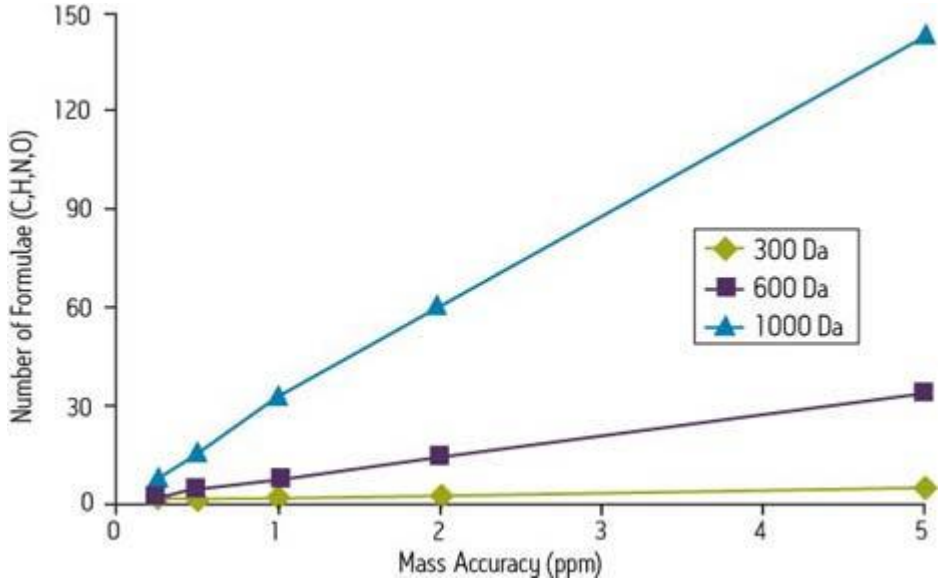
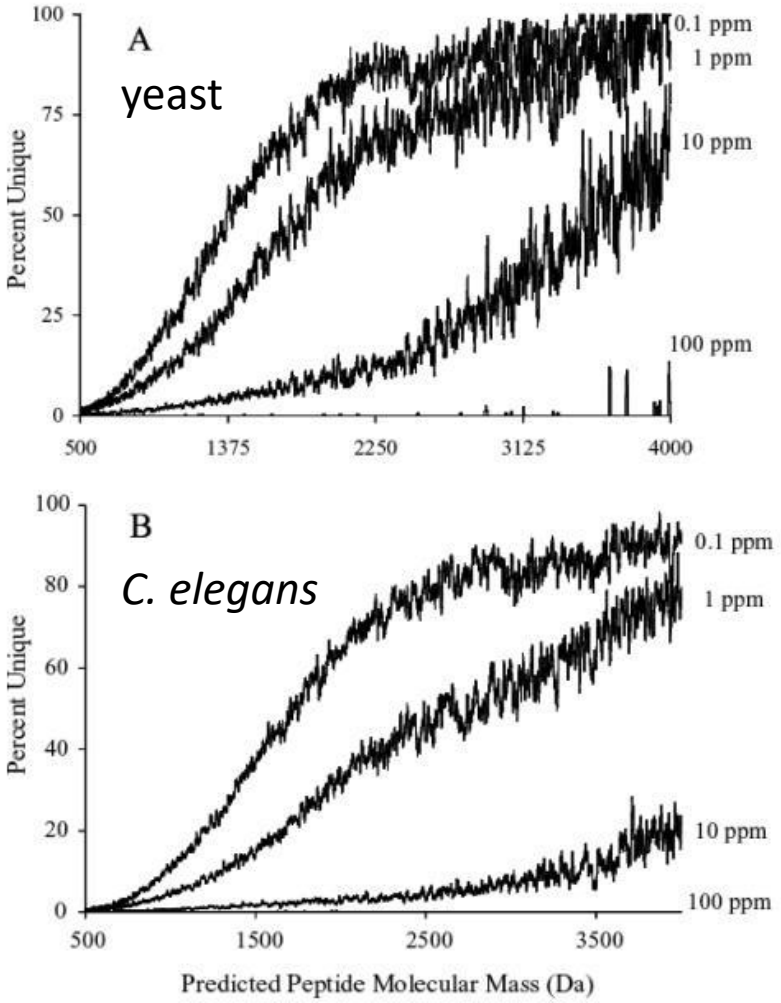


https://doi.org/10.1016/j.jasms.2010.06.006

Why is mass accuracy needed?

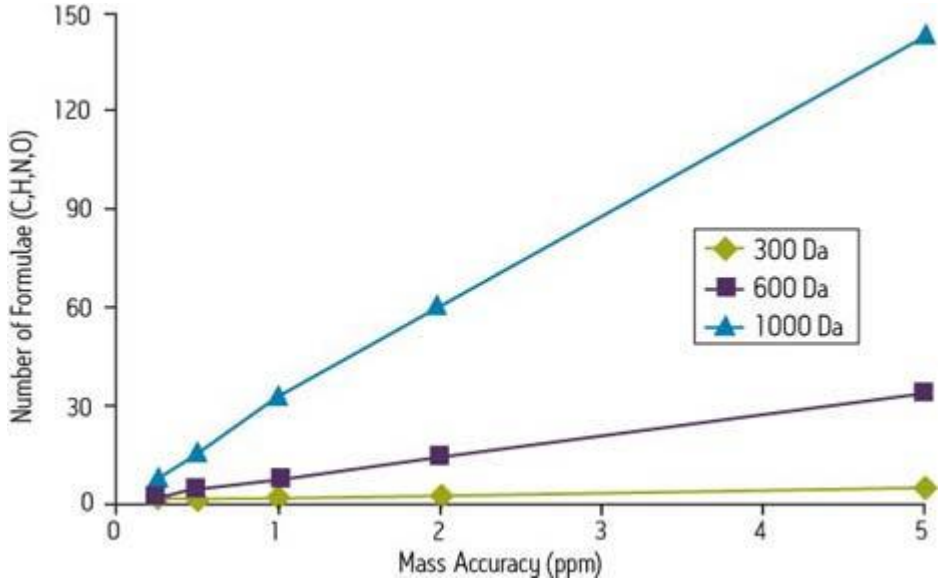


Effect of increasing mass accuracy for unambiguous identification of compounds (Quenzer, T.L., Robinson, J.M., Bolanios, B., Milgram, E. and Greig, M.J., Automated accurate mass analysis using FTICR mass spectrometry, Proceedings of the 50th Annual Conference on Mass Spectrometry and Allied Topics, Orlando, FL, 2002)



Anal. Chem. 2000;72:3349

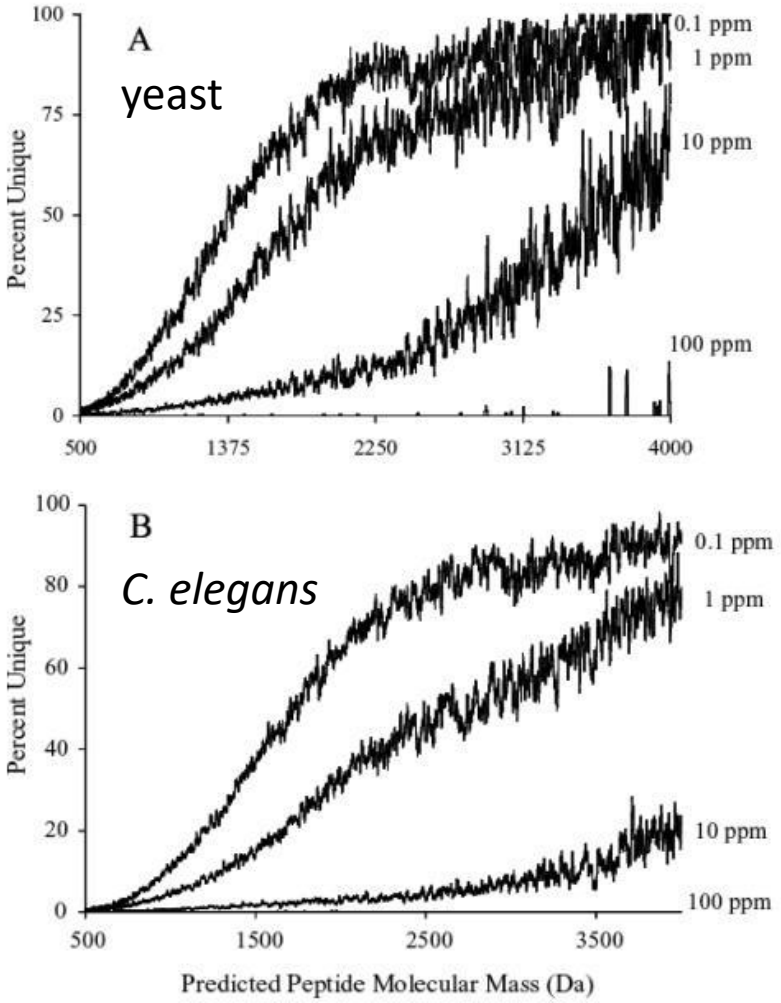
Why is mass accuracy needed?



More accuracy ⇐> Less possible formulae

Less accuracy ⇐> More possible formulae

N.B.: for pictamics, proteins and peptides are composed of amino acids. That actually constraints the possibilities of masses.



Anal. Chem. 2000;72:3349

About mass resolution

- For two peaks of equal height with masses m1 and m2, when there is overlap between the two peaks to a stated percentage of either peak height (10 % is recommended), then the resolution is defined as:

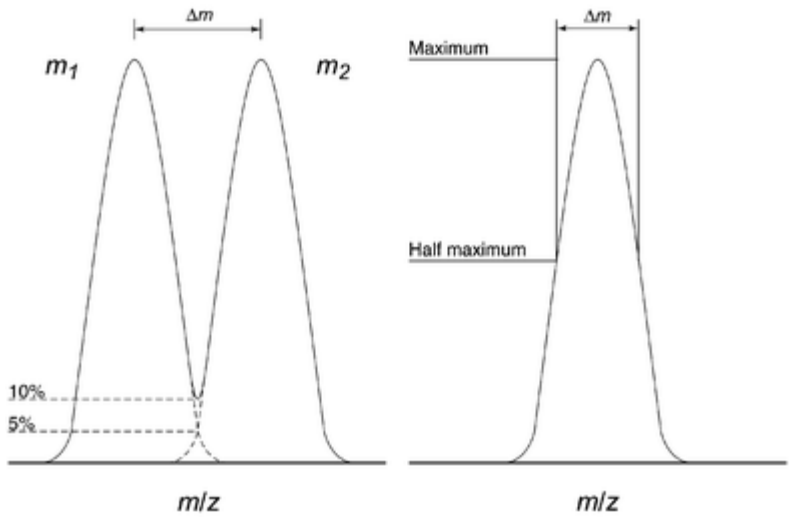
$$\frac{m_1}{m_1 - m_2}$$

The percentage overlap (or 'valley') concerned must always be stated

- For a single peak made up of singly charged ions at mass m in a mass spectrum, the resolution may be expressed as:

$$\frac{m}{\Delta m}$$

where Δm is the width of the peak at a height which is a specified fraction of the maximum peak height (for example, 50 %)



Analyst, 2005, 130, 18-28

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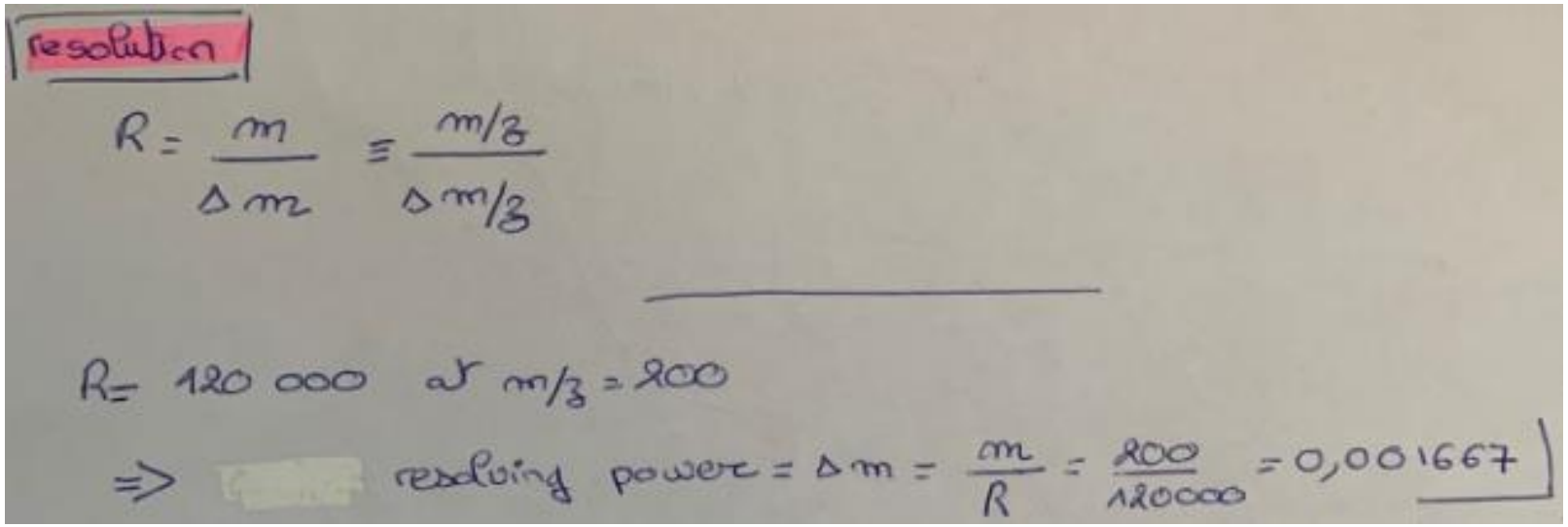
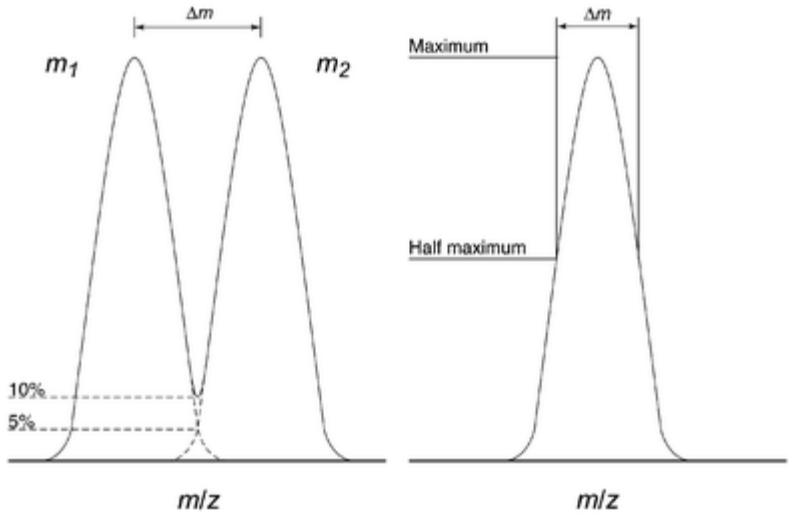
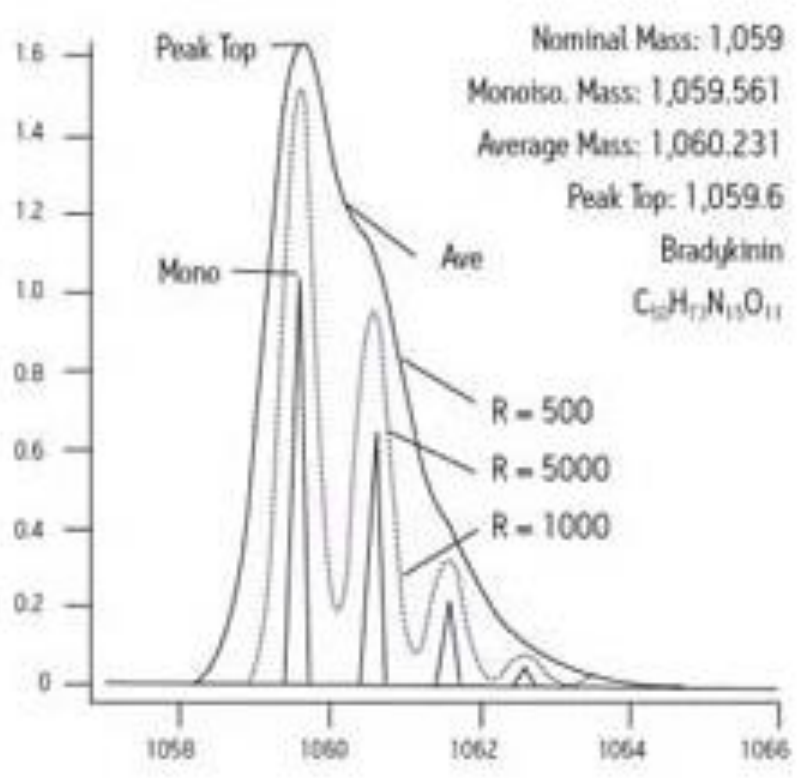


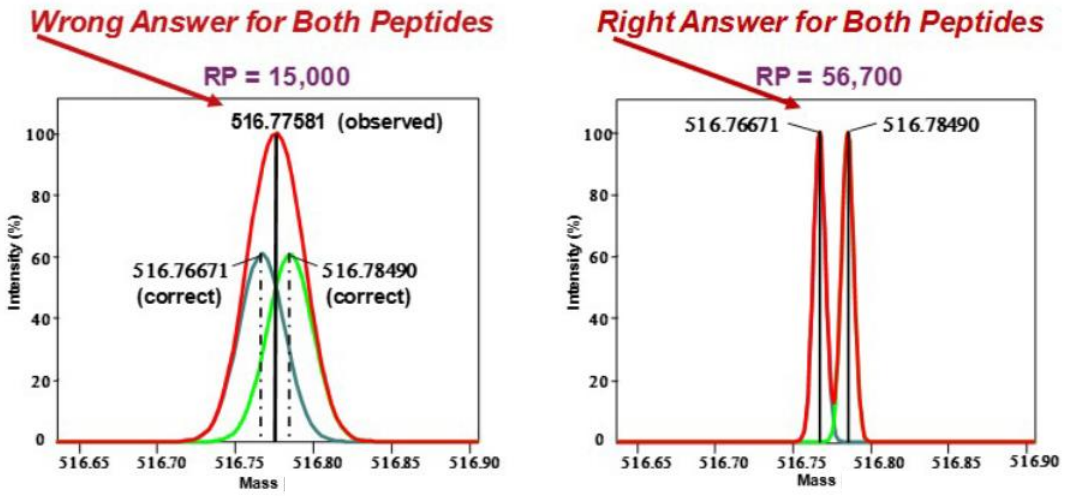


Analyst, 2005, 130, 18-28

Why resolution is needed?



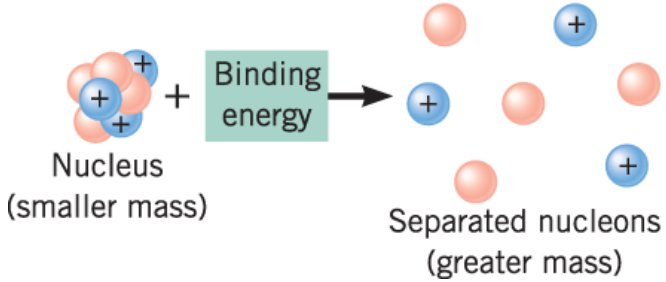
An Overview of Peptide and Protein Analysis by Mass Spectrometry, S. Carr and R. Annan, in Current Protocols in Protein Science, J. Wiley and Sons (1996)



Peptide mixture:	**[Val^5]-Angiotensin II**	**Lys-des-Arg^9-Bradykinin**
Sequence:	**DRVYVHPF**	**KRPPGFSPF**
Formula:	**C49H69N13O12**	**C50H73N13O11**
Exact mass:	**$[M+2H]^{2+}$ = 516.76671**	**$[M+2H]^{2+}$ = 516.78490**

Joshua J. Coon, et al. ASMS 2012

Mass defect in mass spectrometry

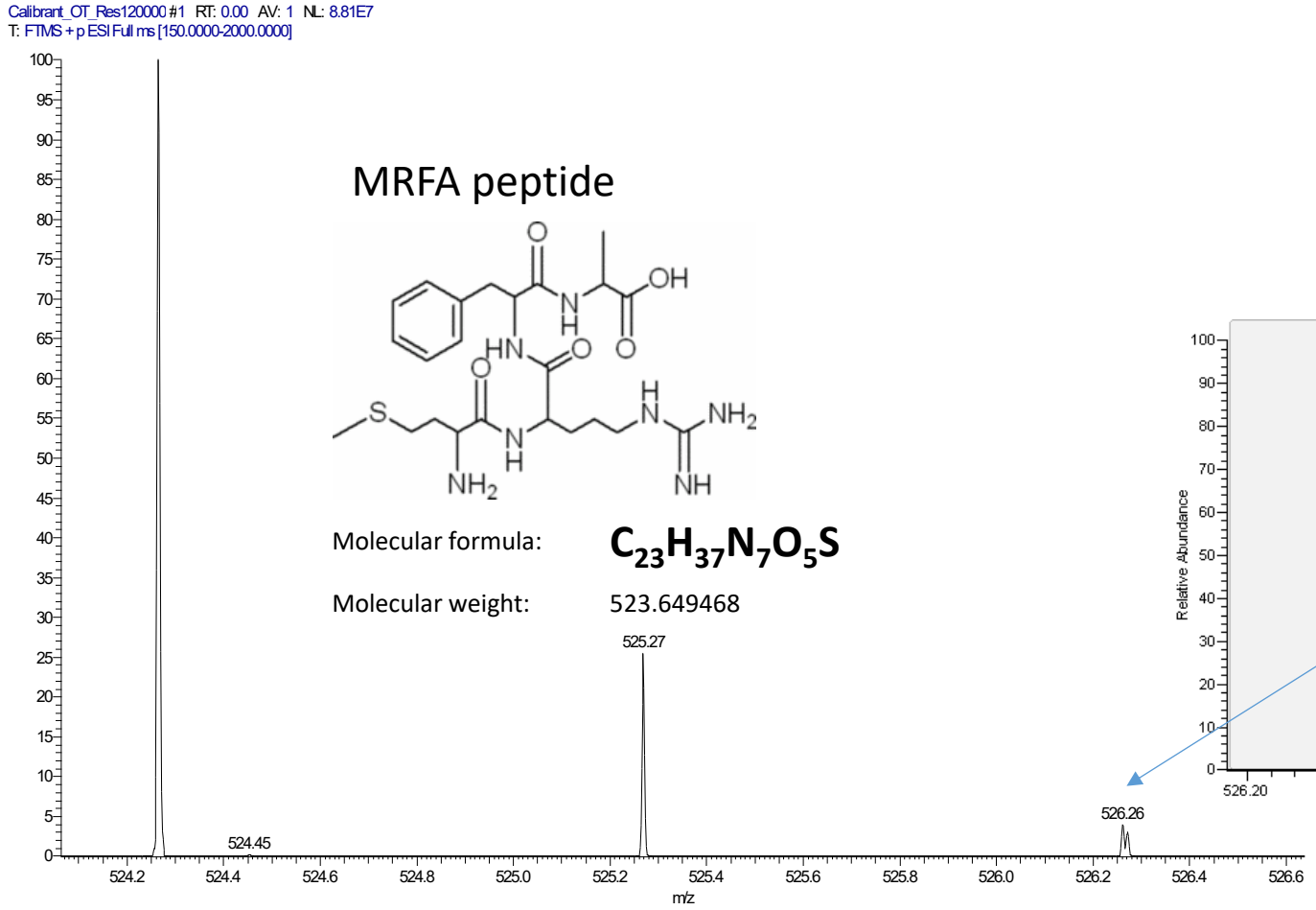


The term mass defect is defined as the difference between the exact mass and the nominal mass. It describes the fact that the exact mass of an isotope or a complete molecule is lower than the corresponding nominal mass. In case of ^{16}O, for example, the isotopic mass is 15.994915 u, being 0.005085 u deficient as compared to the nominal value
The term mass deficiency is also used to describe this deviation

Most isotopes are more or less mass deficient with a tendency towards larger mass defects for the heavier isotopes. Thanks to these small deviations from integer values the accurate mass of an ion depends on its elemental composition, i.e., accurate mass measurements can be used to identify the elemental composition of an ion

DOI: 10.1007/978-3-319-54398-7

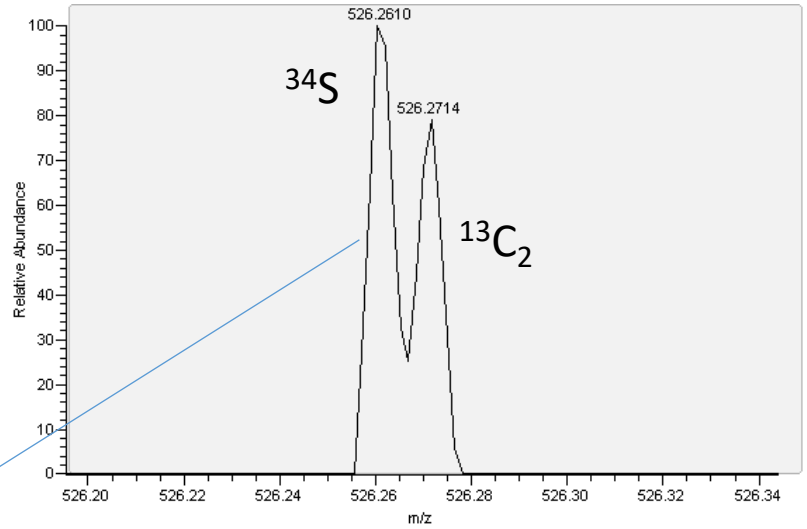
Mass defect at high resolving power

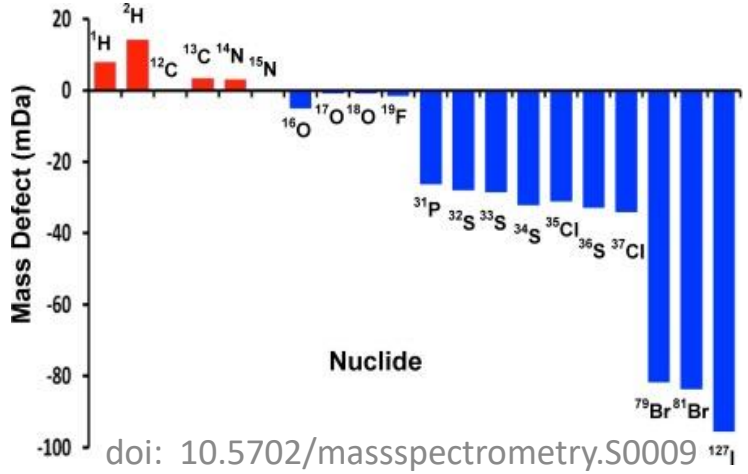


MRFA peptide

Molecular formula: $C_{23}H_{37}N_7O_5S$

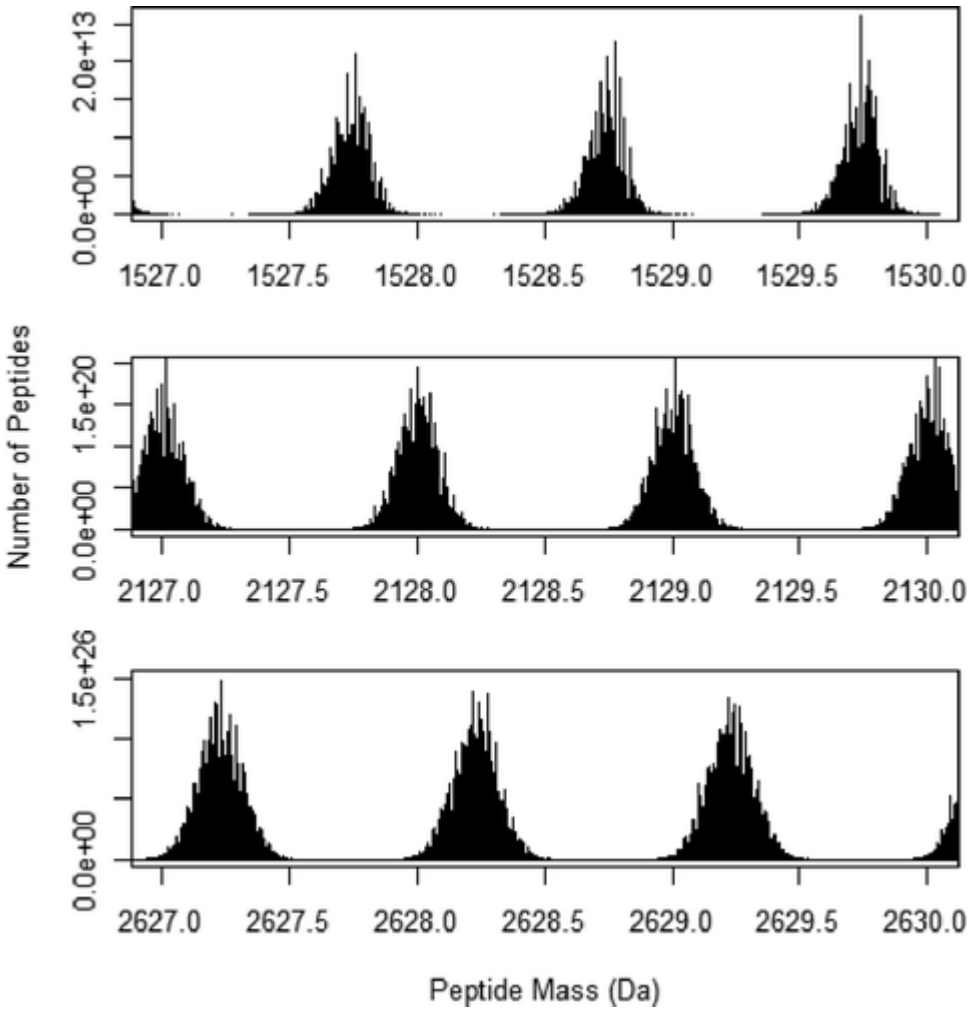
Molecular weight: 523.649468

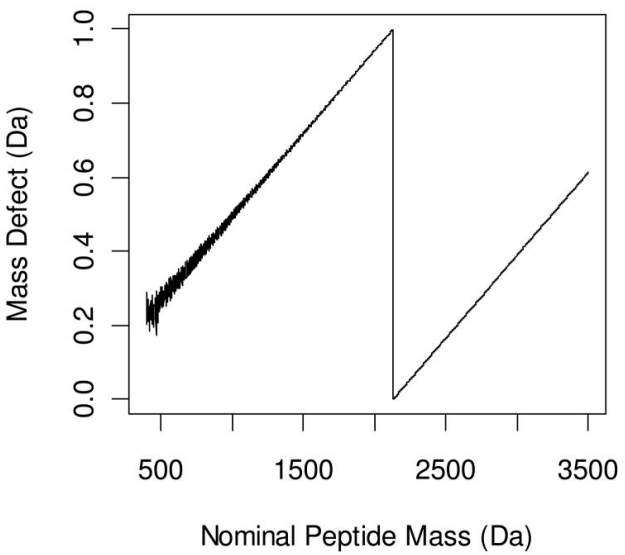


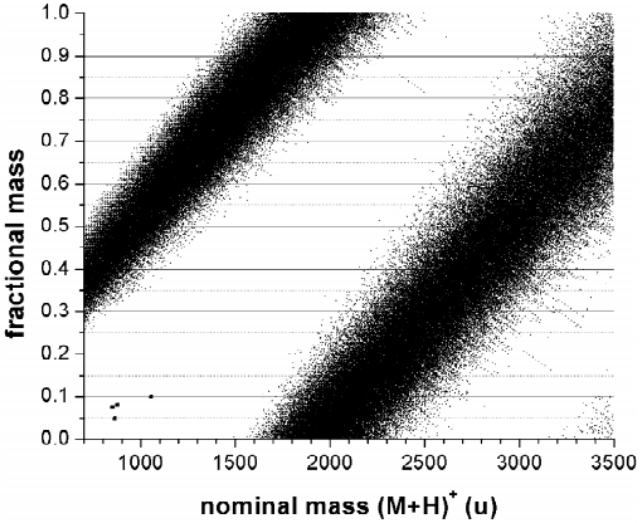


doi: 10.5702/massspectrometry.S0009

Specific mass defect for tryptic peptides







doi: 10.1021/ac203255e

doi: 10.1016/S1570-0232(02)00550-0

1.3. Why proteomics and mass spectrometry?

MS has become the method of choice for the detection of proteins from complex samples. The introduction of effective laser desorption ionization time-of-flight (MALDITOF)MS and electrospray ionization (ESI) tandem mass spectrometry (MS/MS) have enabled this evolution. In addition, the concept of correlating MS data with genomic sequence databases has revealed to be perfectly suited to identify proteins

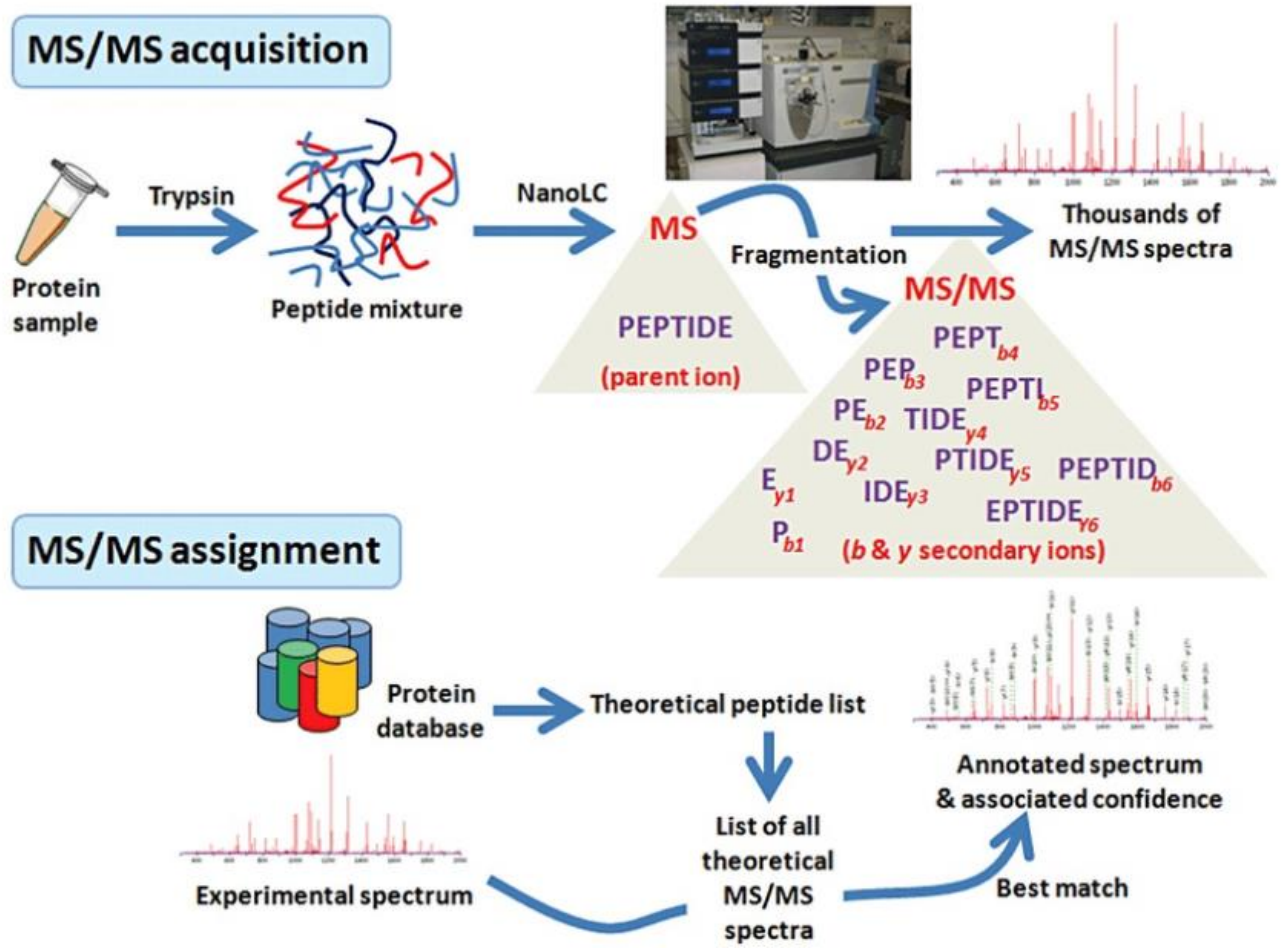

John B. Fenn

Koichi Tanaka

2002 Nobel Prize in Chemistry "for the development of methods for identification and structure analyses of biological macromolecules"

Get molecular and sequence information

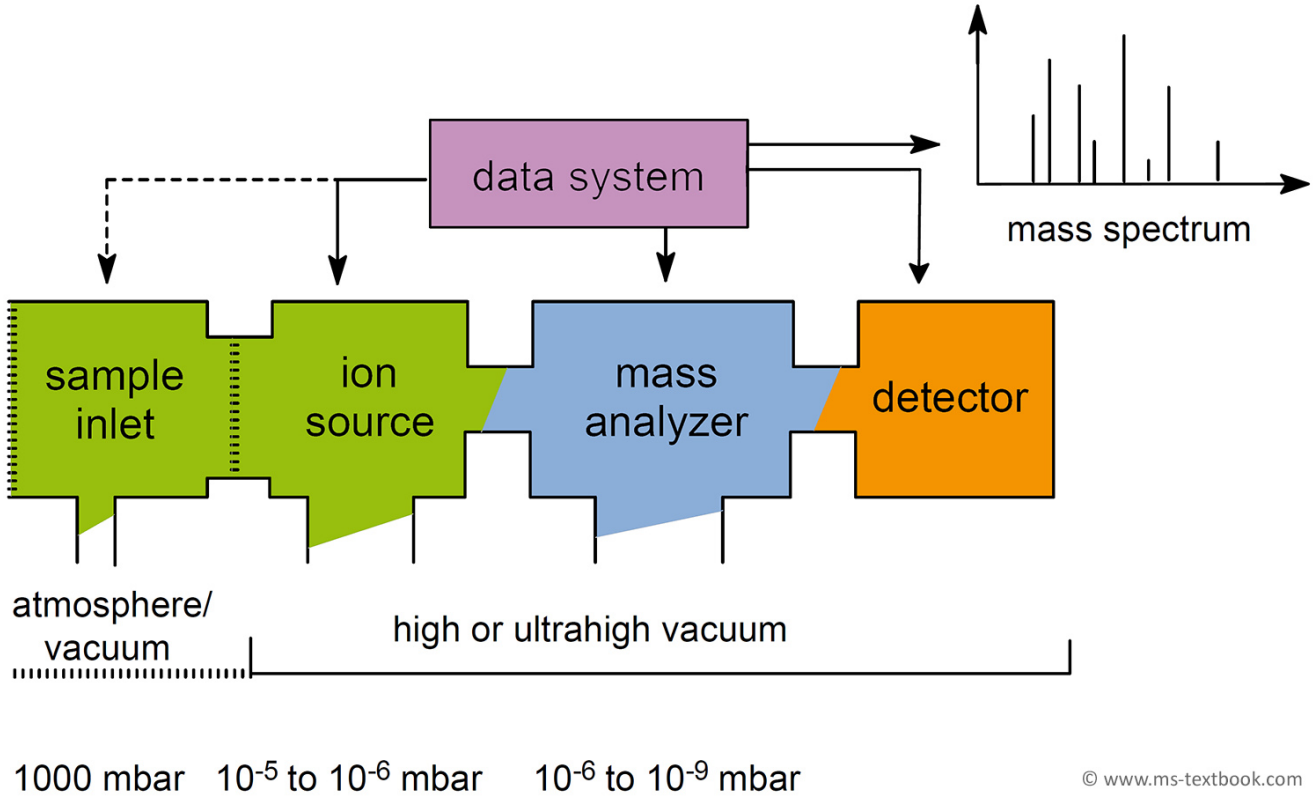
- Need soft transfer to the gas phase of protein/peptide ions (formation of stable ions)
- Fragment ions to obtain sequence information
- Use theoretical knowledge (protein database) to match spectra



Environmental Microbiology (2013) 15(1), 12–23

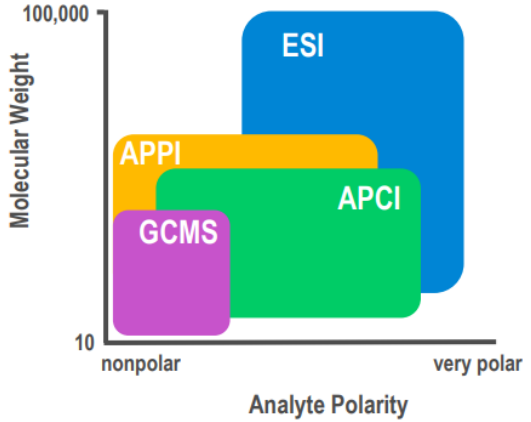
1.4. Ionization sources, analyzers, and detectors used in proteomics

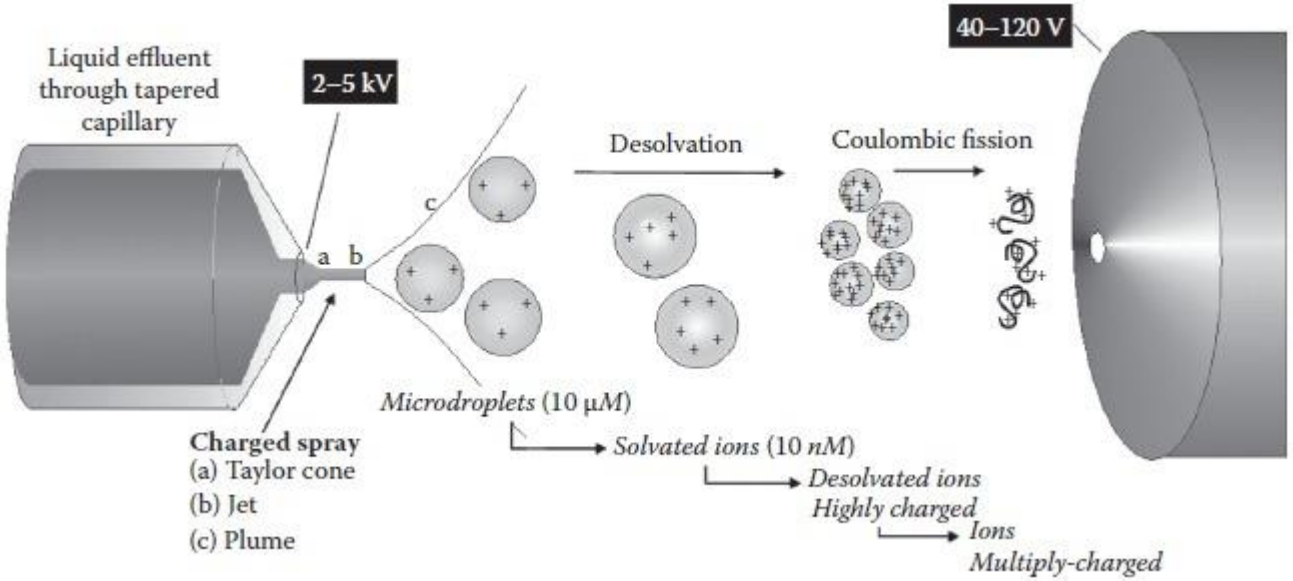
A mass spectrometer consists of an ion source, a mass analyzer and a detector which are operated under high vacuum conditions. A closer look at the front end of such a device might separate the steps of sample introduction, evaporation and successive ionization or desorption/ionization. More recent systems will have some data system used to collect and process data from the detector



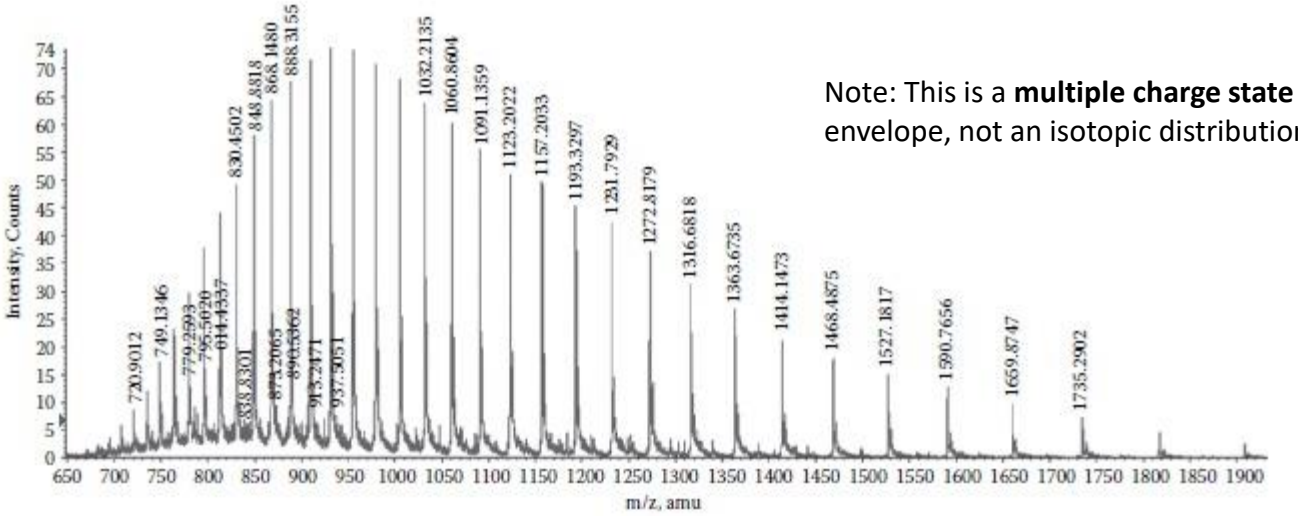
DOI: 10.1007/978-3-319-54398-7

Electrospray ionization (ESI)





At the open end of the capillary, the electric field causes charge separation in the liquid and finally deformation of the meniscus into a cone. When a certain field strength is reached, the starts ejecting a fine jet of liquid from its apex towards the counter electrode. The jet carries a large excess of ions of one particular charge sign. Such a jet cannot remain stable for an elongated period, but breaks up into small droplets. Due to their charge, these droplets are driven away from each other by Coulombic repulsion. Overall, this process causes the generation of a fine spray

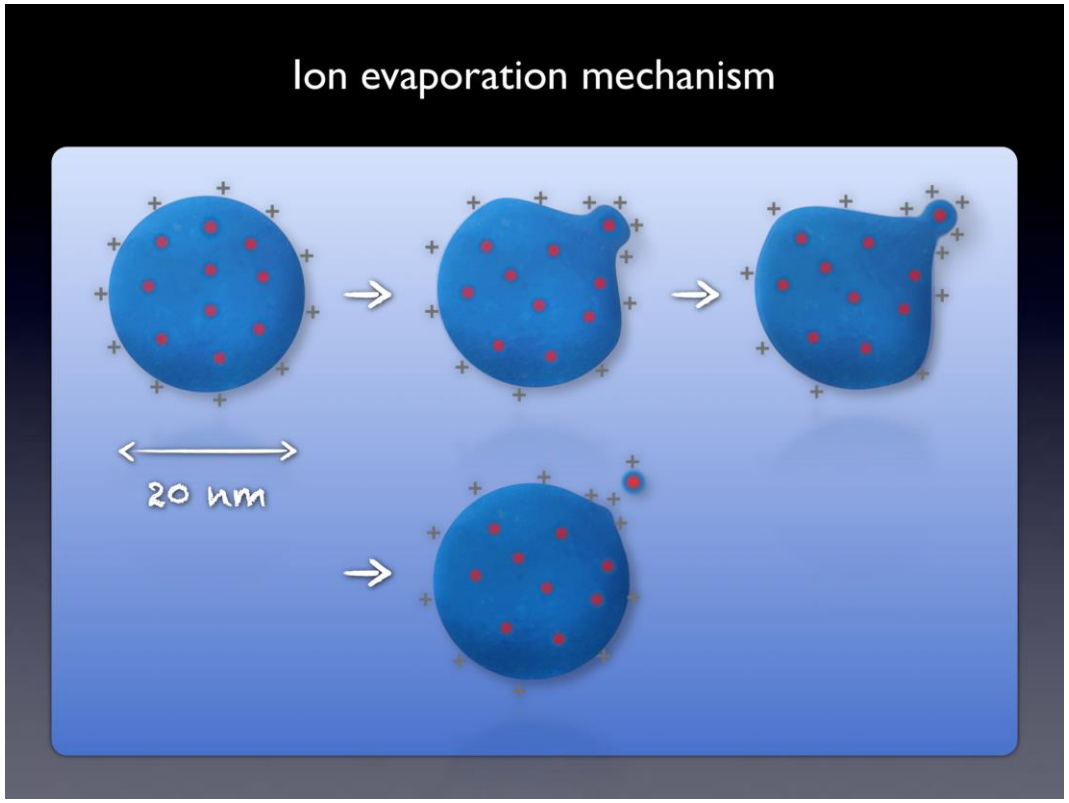


Note: This is a **multiple charge state** envelope, not an isotopic distribution!

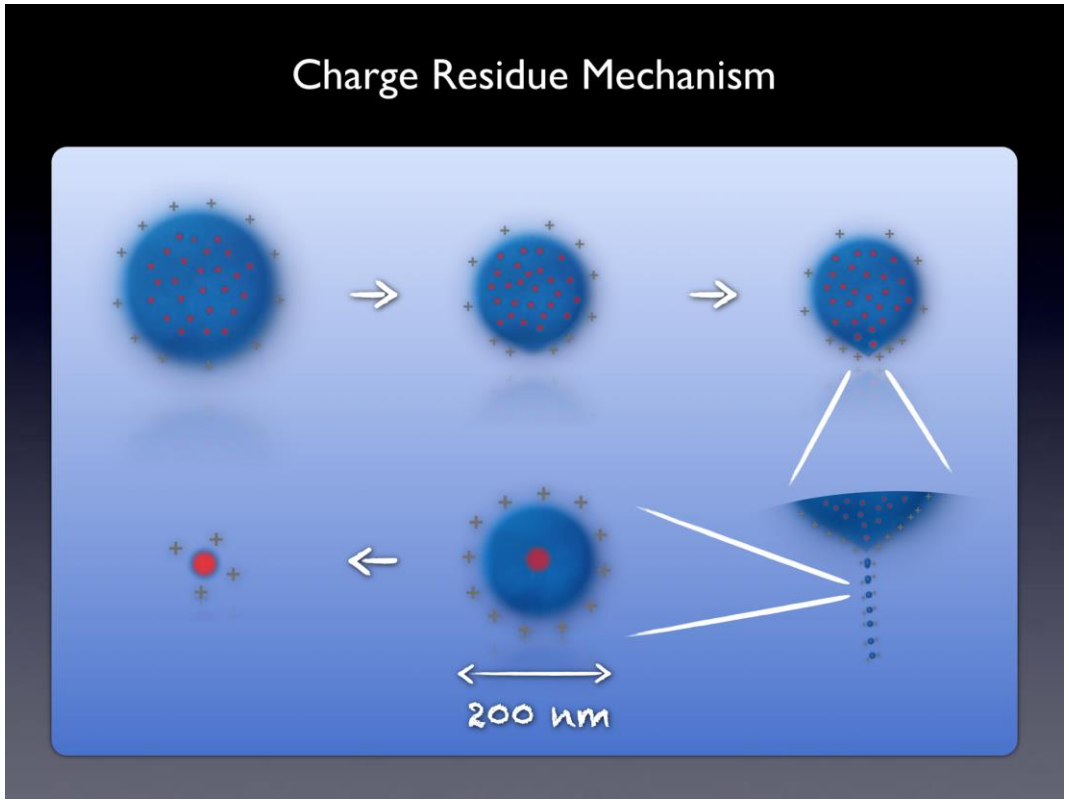
DOI: 10.1007/978-3-319-54398-7

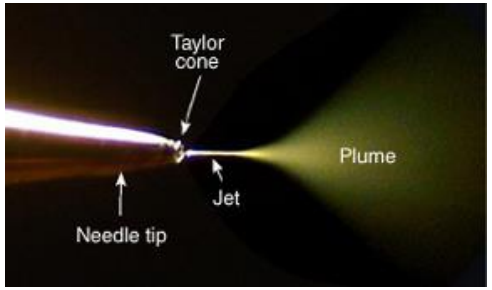
https://www.ncbi.nlm.nih.gov/books/NBK56011/

Electrospray ionization (ESI) mechanism



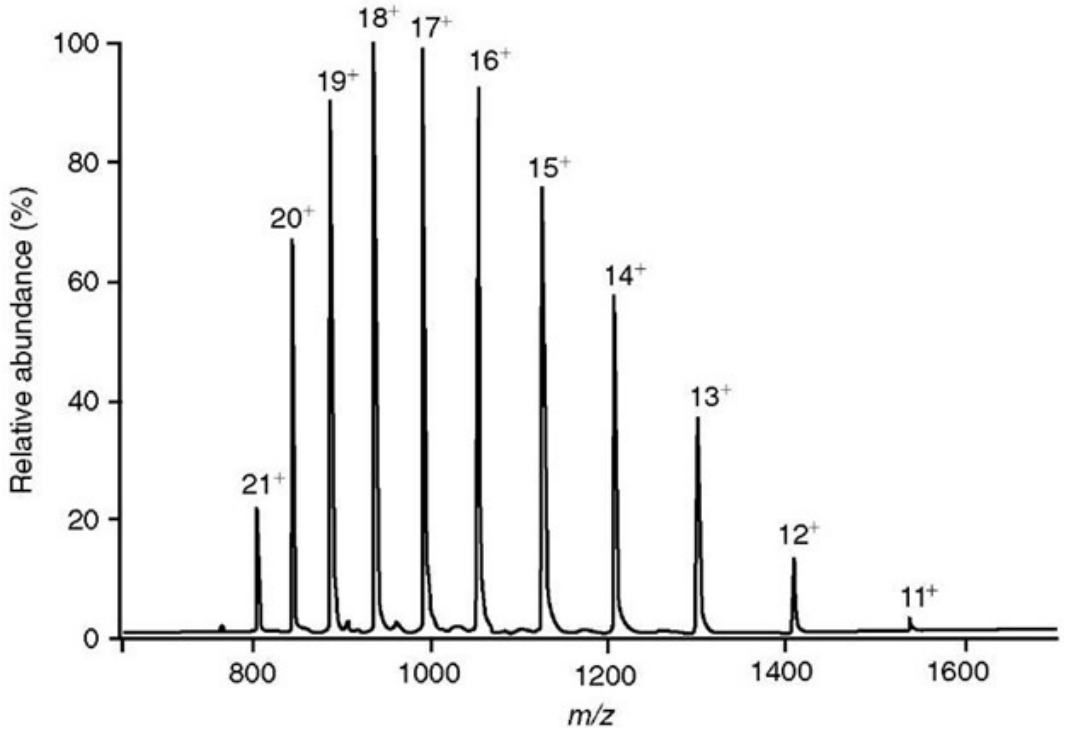
doi: 10.1074/mcp.R111.009407



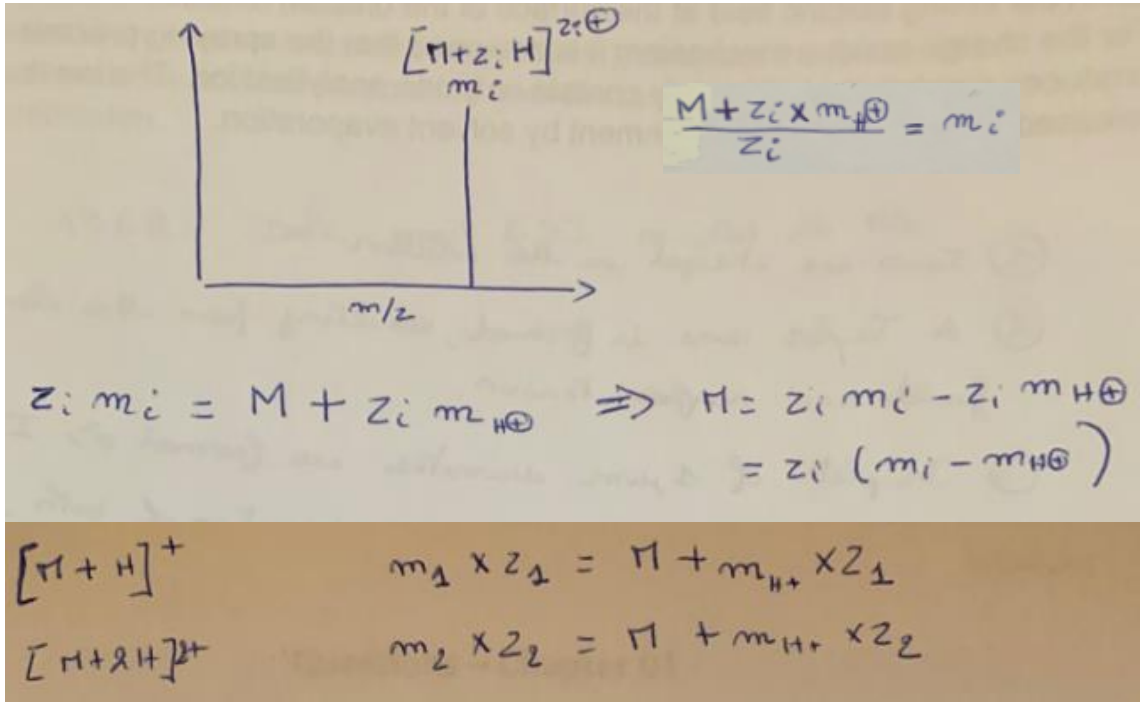


http://www.newobjective.com

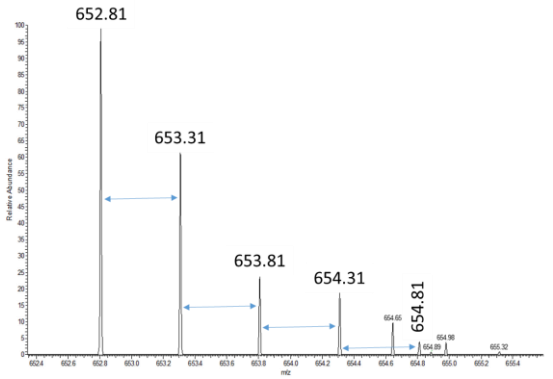
Multiply charged ions in ESI and deconvolution



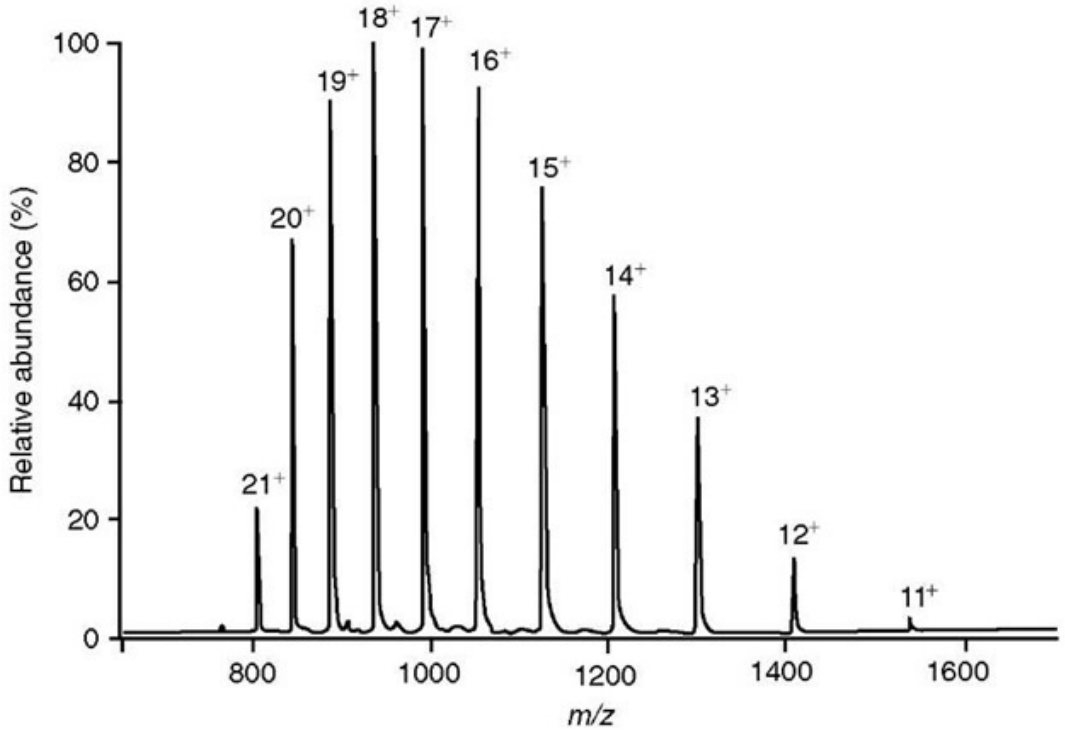
http://what-when-how.com/proteomics/mass-spectrometry-proteomics/



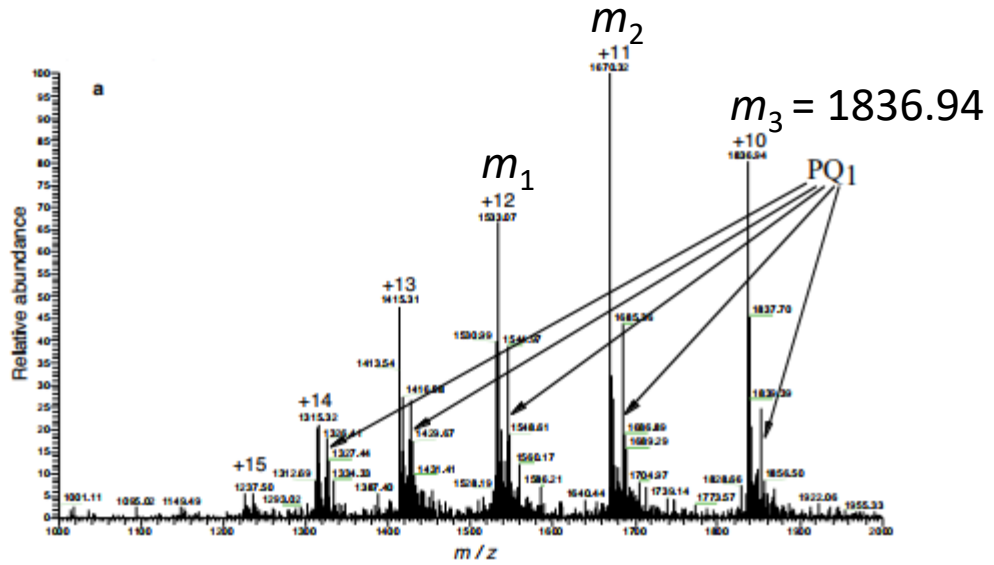
Q5: How to obtain the *MW (knowing m_i and z_i)?*

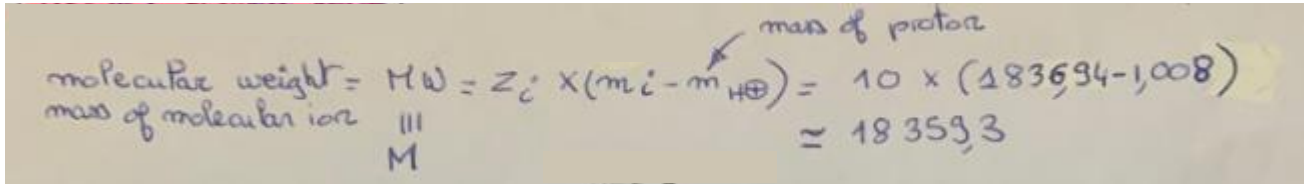


Multiply charged ions in ESI and deconvolution



http://what-when-how.com/proteomics/mass-spectrometry-proteomics/



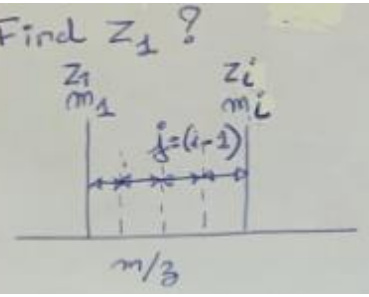


Q5: How to obtain the *MW (knowing m_i and z_i)?*

Thèse EPFL, n° 3525 (2006)

Multiply charged ions in ESI and deconvolution

Find z_1 ?



$$m_1 \times z_1 = M + m_{H^\oplus} \times z_1 \quad (1)$$

$$m_i \times z_i = M + m_{H^\oplus} \times z_i \Leftrightarrow m_i \times (z_1 - j) = M + m_{H^\oplus}(z_1 - j) \quad (2)$$

$$z_i + j = z_1$$

(1) in (2) gives:

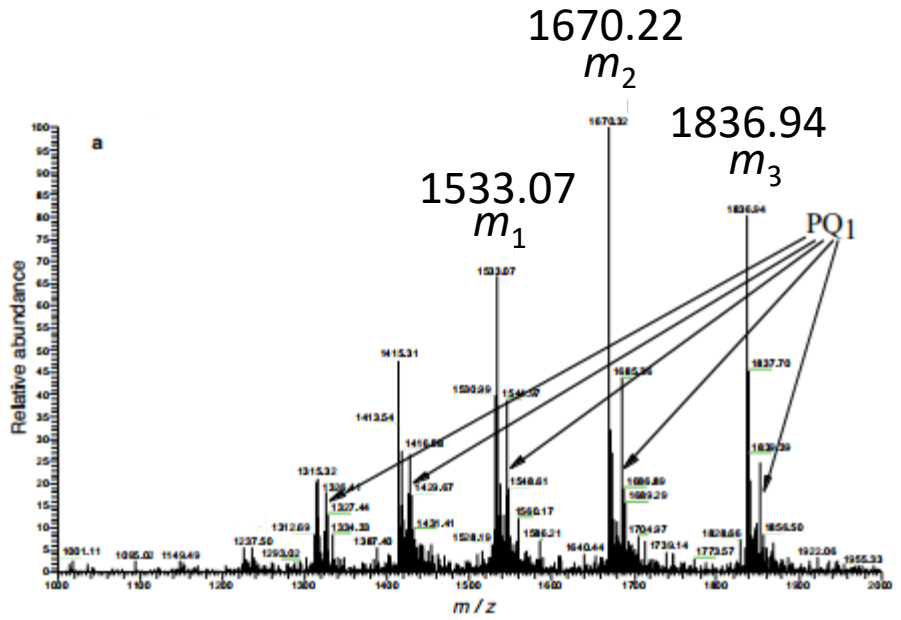
$$m_i (z_1 - j) = m_1 \times z_1 - m_{H^\oplus} \times z_1 + m_{H^\oplus}(z_1 - j)$$

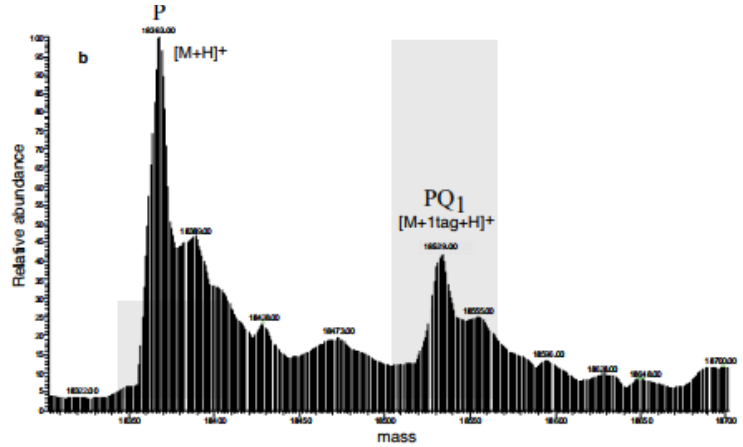
$$m_i z_1 - m_i j = m_1 z_1 - m_{H^\oplus} z_1 + m_{H^\oplus} z_1 - m_{H^\oplus} j$$

$$z_1 (m_i - m_1) = j (m_i - m_{H^\oplus})$$

$$\boxed{z_1 = \frac{j (m_i - m_{H^\oplus})}{(m_i - m_1)}}$$

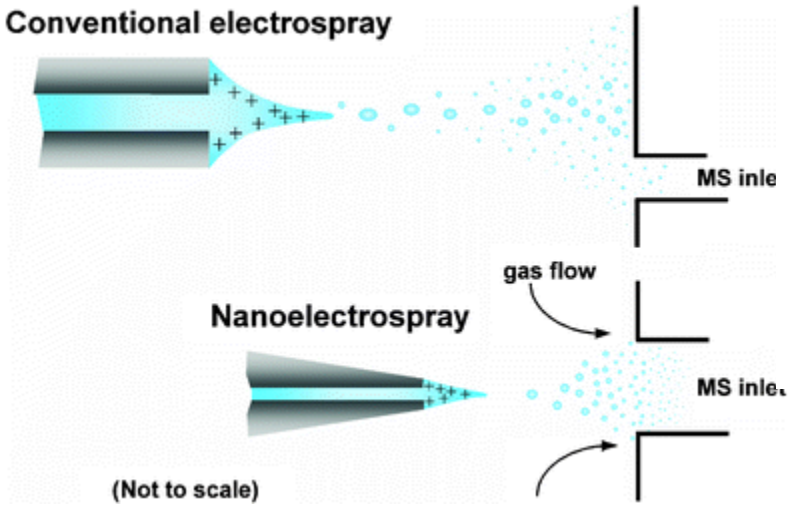
$$z_1 \simeq \frac{2 \times (1836{,}94 - 1{,}008)}{(1836{,}94 - 1533{,}07)} \simeq 12$$



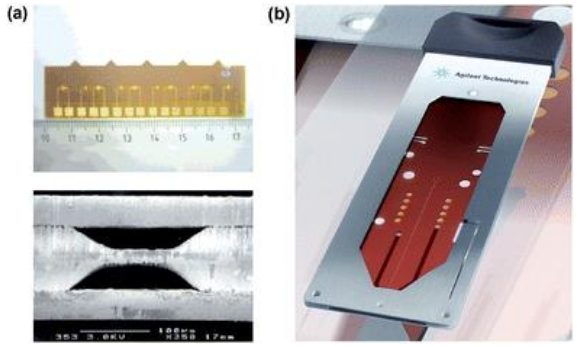


Thèse EPFL, n° 3525 (2006)

Micro-spray



DOI: 10.1039/B601468B



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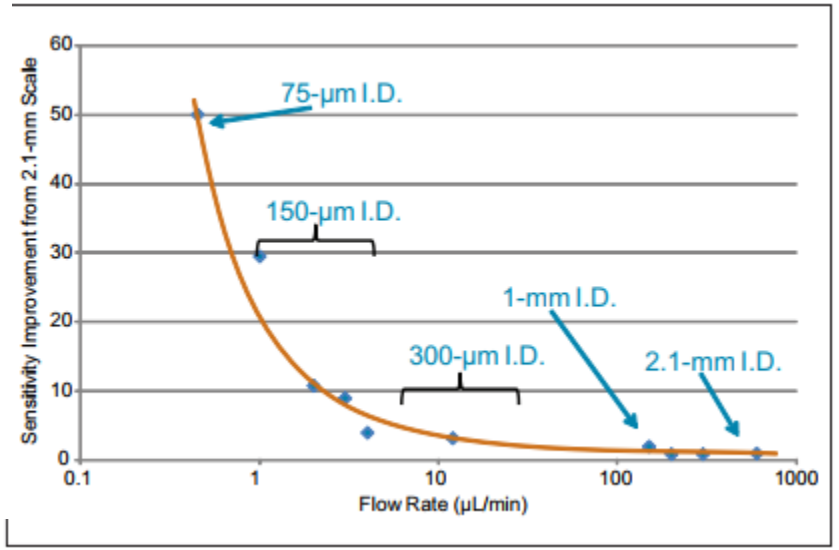
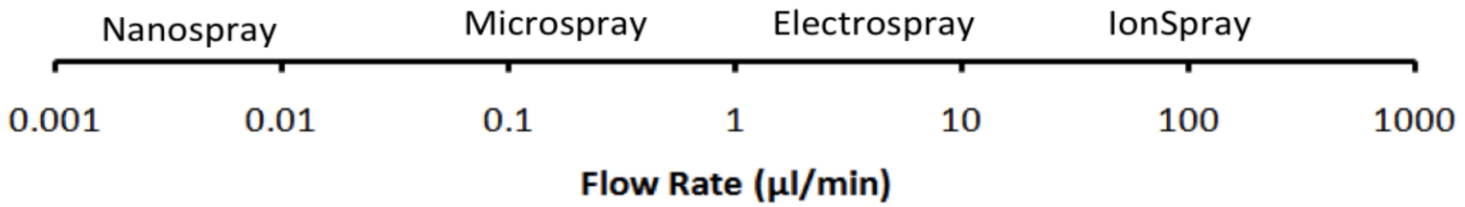
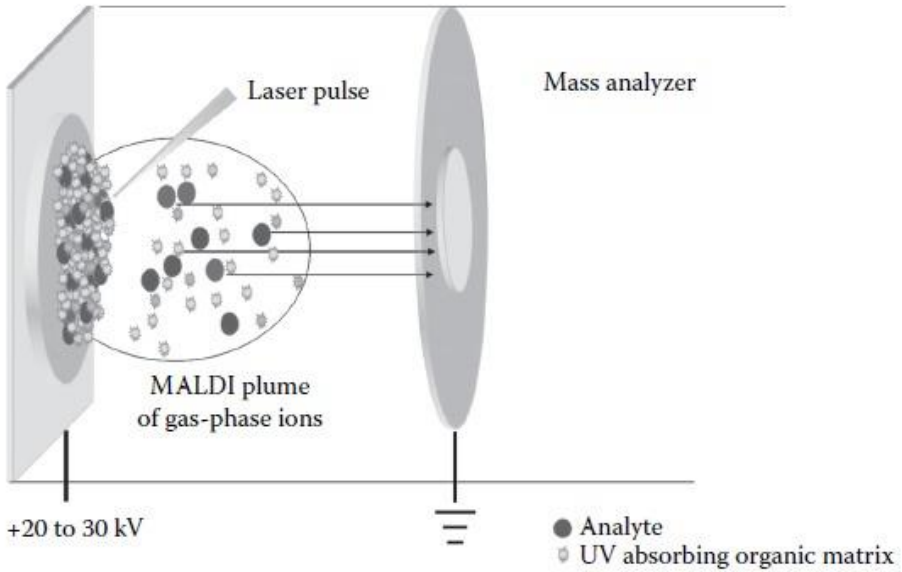


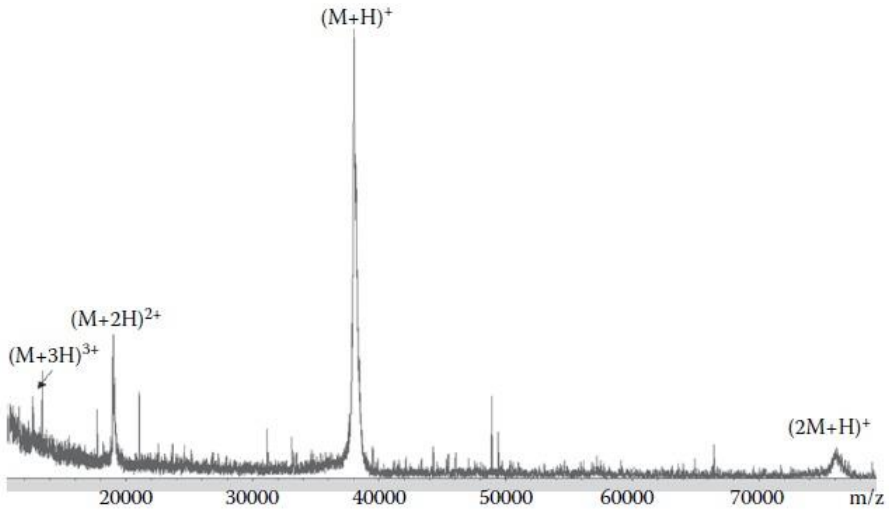
http://www.waters.com/webassets/cms/library/docs/720004967en.pdf



http://blog.waters.com/what-is-microflow

Matrix-assisted laser desorption/ionization(MALDI)





https://www.ncbi.nlm.nih.gov/books/NBK56011/

The sample for analysis by MALDI MS is prepared by mixing or coating with solution of an energy-absorbent, organic compound called matrix. When the matrix crystallizes on drying, the sample entrapped within the matrix also co-crystallizes. The sample within the matrix is ionized in an automated mode with a laser beam. Desorption and ionization with the laser beam generates singly protonated ions from analytes in the sample

doi: 10.3389/fmicb.2015.00791

Typical MALDI spectra include mainly the **monocharged** molecular species by protonation in positive ion mode

MALDI is more tolerant of salts and complex mixture analysis than ESI

Gas Phase Protonation

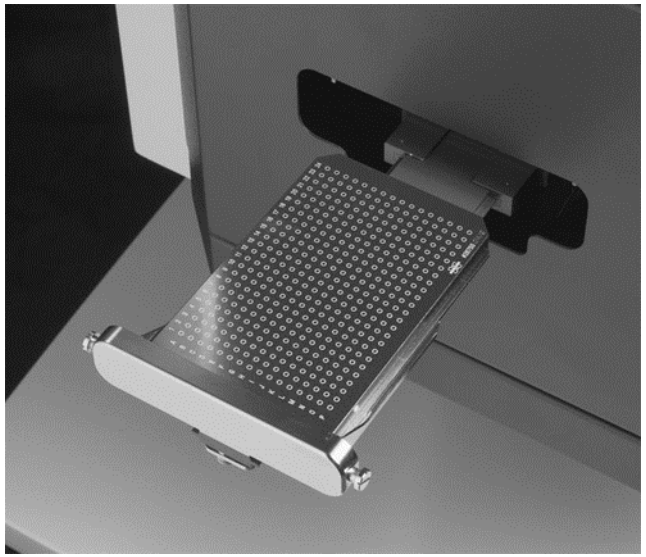
$$A \xrightarrow[-\text{Matrix}]{+\,[\text{Matrix} + H]^+} AH^+$$

Precharged Analyte (Lucky Survivor)

$$AH_n^{n+} + {}_nX^- \longrightarrow AH^+ + X^- + {}_{n-1}HX$$

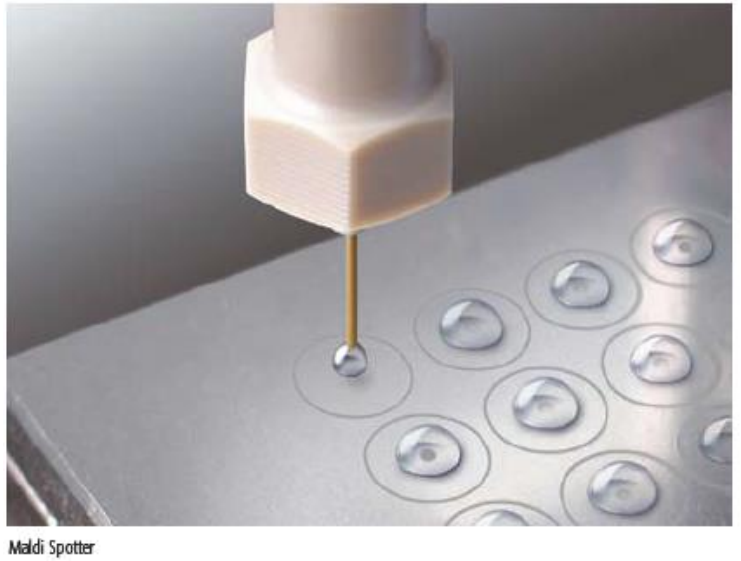
https://www.ssi.shimadzu.com/service-support/faq/maldi/index.html

MALDI target



Example of MALDI plate

Manual spotting
(Sample is mixed with matrix
and applied to the metal plate)

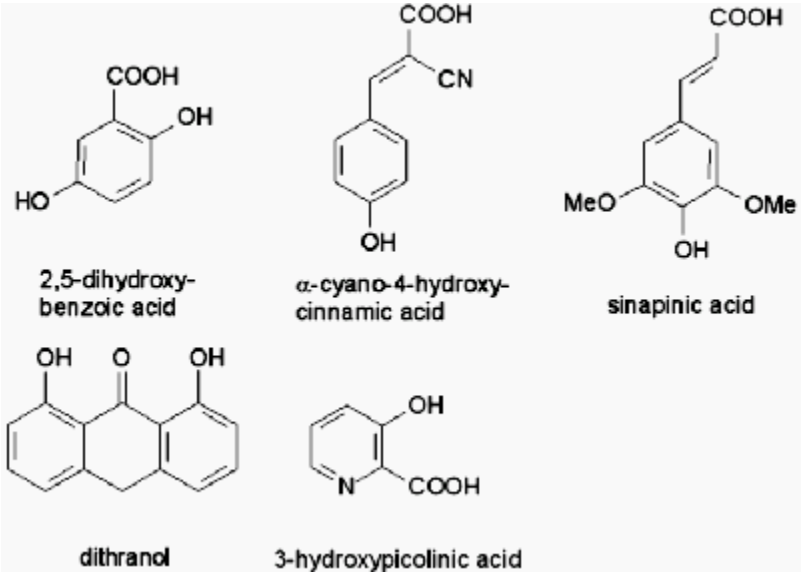


LC MALDI
(e.g., sample then matrix)

MALDI matrices

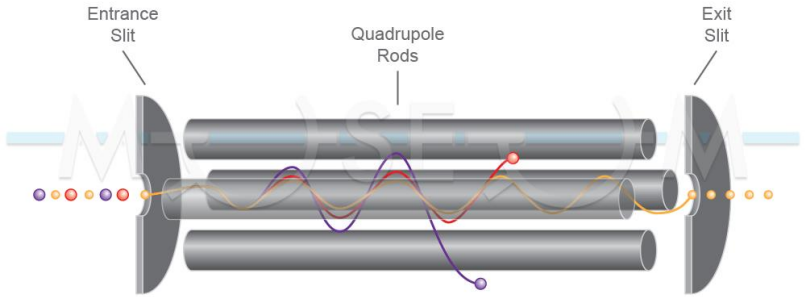
Analyte	Matrix	Abbreviation
Peptides/proteins	α-Cyano-4-hydroxycinnamic acid	CHCA
	2,5-Dihydroxybenzoic acid (gentisic)	DHB
	3,5-Dimethoxy-4-hydroxycinnamic acid (sinapic)	SA
Oligonucleotides	Trihydroxyacetophenone	THAP
	3-Hydroxypicolinic acid	HPA
Carbohydrates	2,5-Dihydroxybenzoic acid	DHB
	α-Cyano-4-hydroxycinnamic acid	CHCA
	Trihydroxyacetophenone	THAP
Synthetic polymers	Trans-3-indoleacrylic acid	IAA
	Dithranol	DIT
	2,5-Dihydroxybenzoic acid	DHB
Organic molecules	2,5-Dihydroxybenzoic acid	DHB
Inorganic molecules	Trans-2-(3-(4-tert-Butylphenyl)-2methyl-2-propenyliedene)malononitrile	DCTB
Lipids	Dithranol	DIT

Peptides Proteins

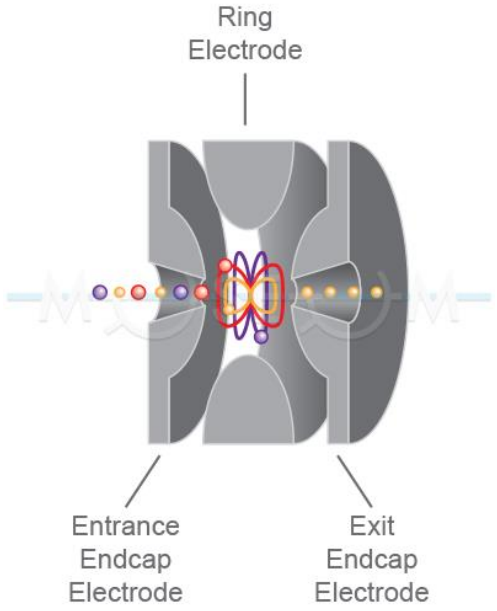


DOI: 10.1007/978-3-319-54398-7

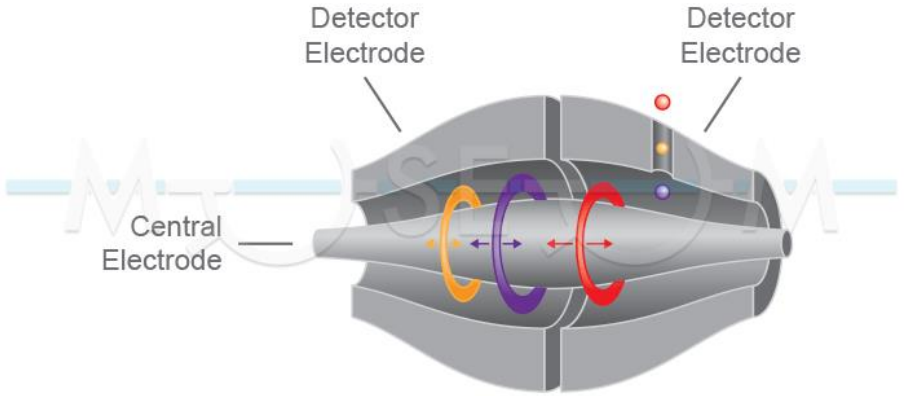
Mass analysers in proteomics



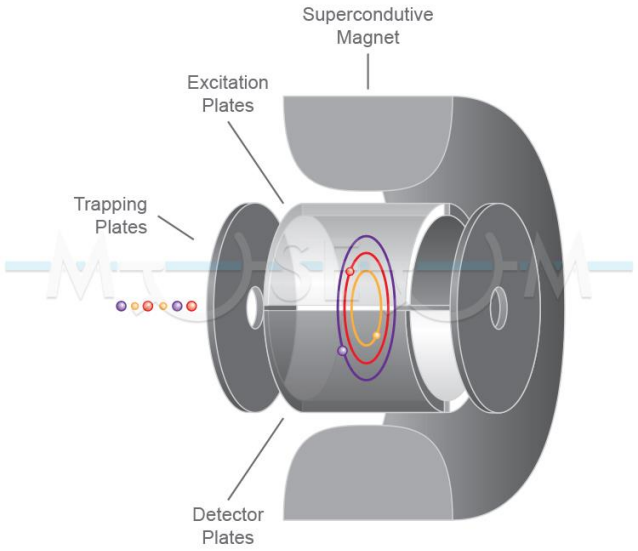
Quadrupole (Q)



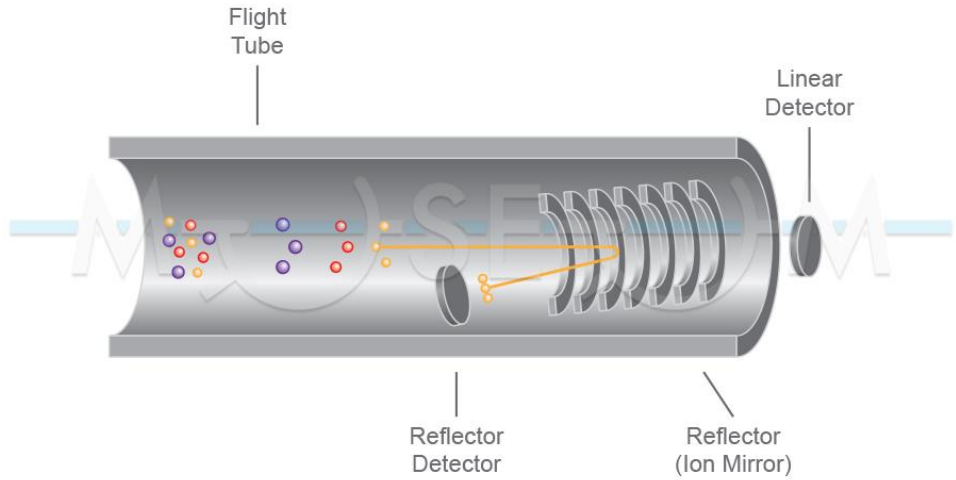
Ion Trap (IT)



OrbiTrap (OT)

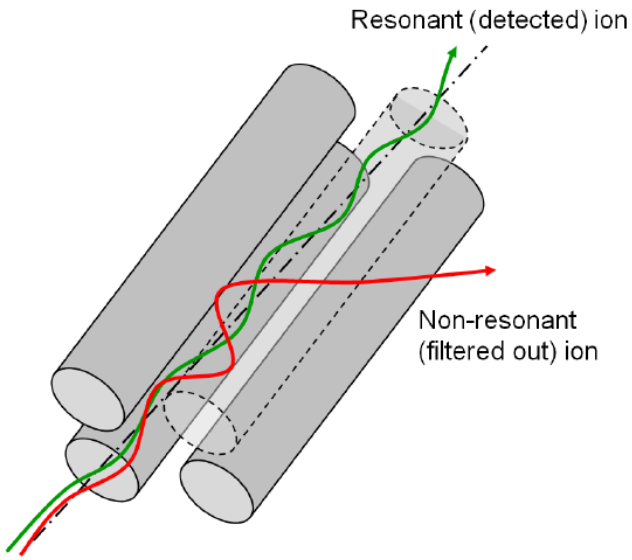


Fourier Transform Ion Cyclotron Resonance (FT-ICR)



Time Of Flight (TOF)

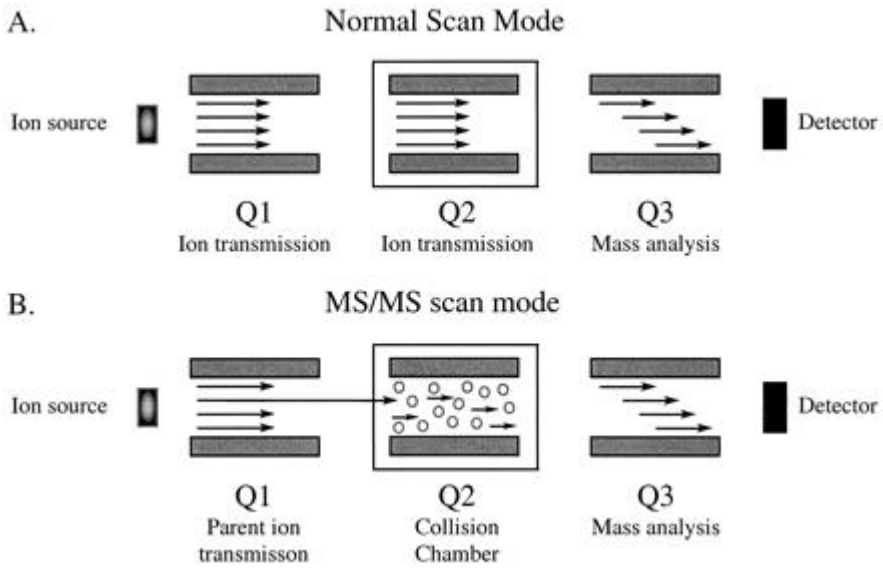
Quadrupole (Q)



Each opposing rod pair is connected together electrically, and a radio frequency (RF) voltage with a DC offset voltage is applied between one pair of rods and the other. Ions travel down the quadrupole between the rods. Only ions of a certain mass-to-charge ratio will reach the detector for a given ratio of voltages: other ions have unstable trajectories and will collide with the rods. This permits selection of an ion with a particular m/z or allows the operator to scan for a range of m/z-values by continuously varying the applied voltage

https://en.wikipedia.org/wiki/Quadrupole_mass_analyzer

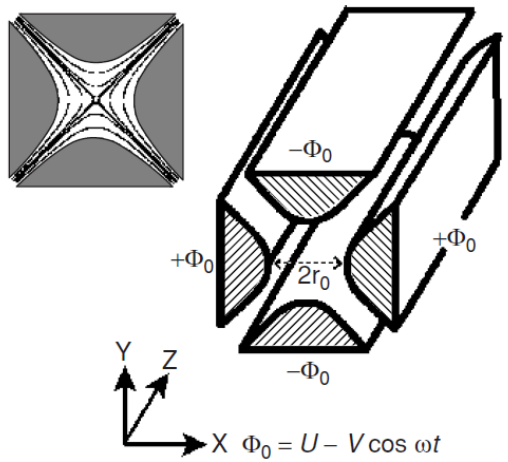
Q is most common due to low scan times, compact design, and lower cost

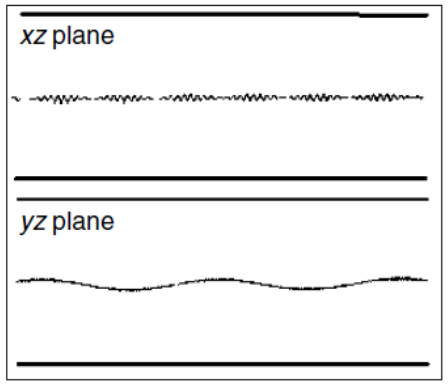


Paul R. Graves, and Timothy A. J. Haystead Microbiol. Mol. Biol. Rev. 2002;66:39-63

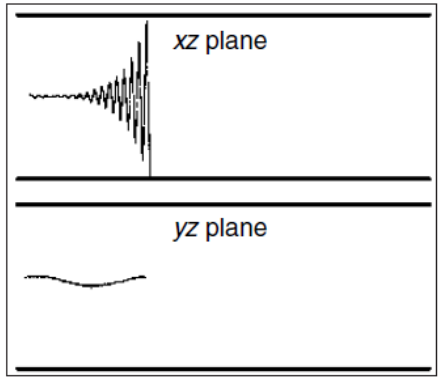
https://youtu.be/8AQaFdI1Yow

Quadrupole (Q)



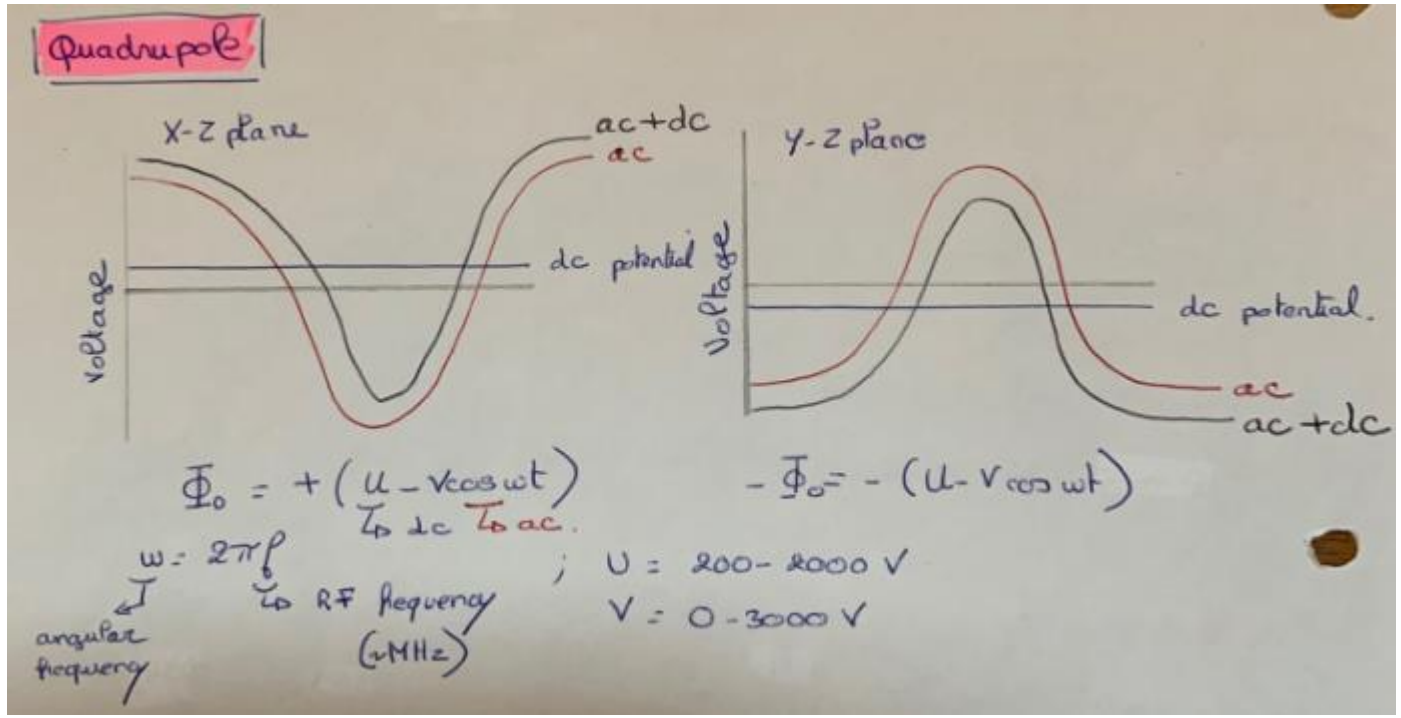


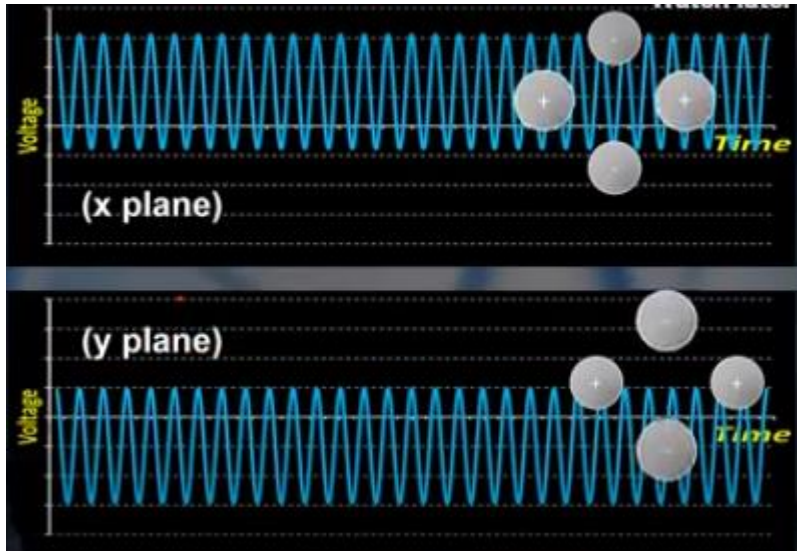
Stable along both *x* and *y*



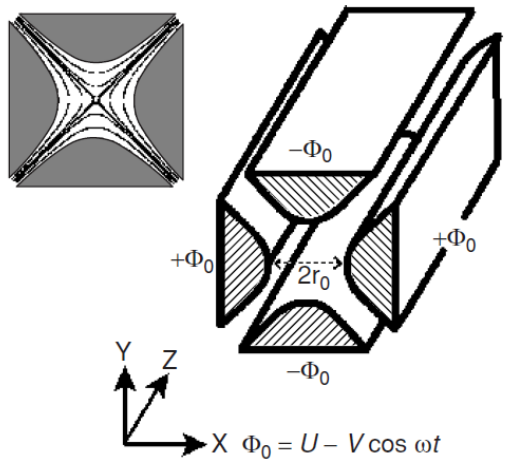
Stable along *y*, unstable along *x*

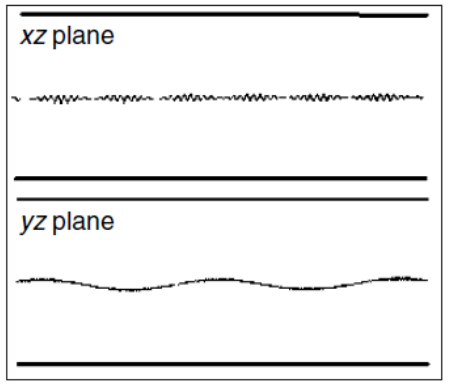
ISBN: 978-0-470-03310-4



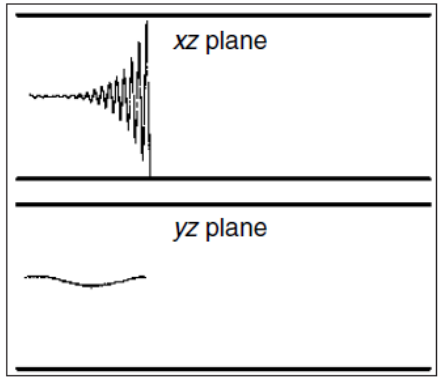


Quadrupole (Q)



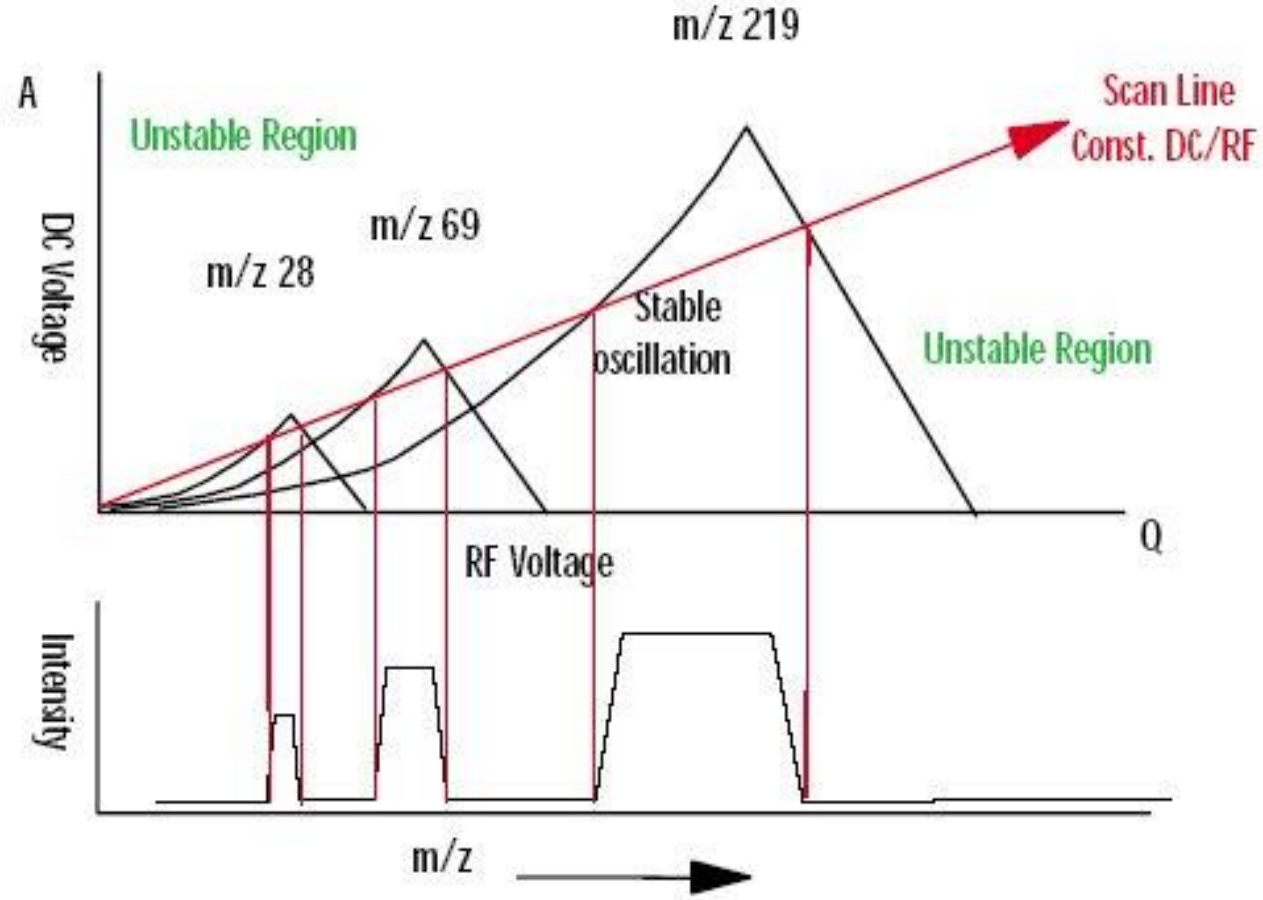


Stable along both *x* and *y*



Stable along *y*, unstable along *x*

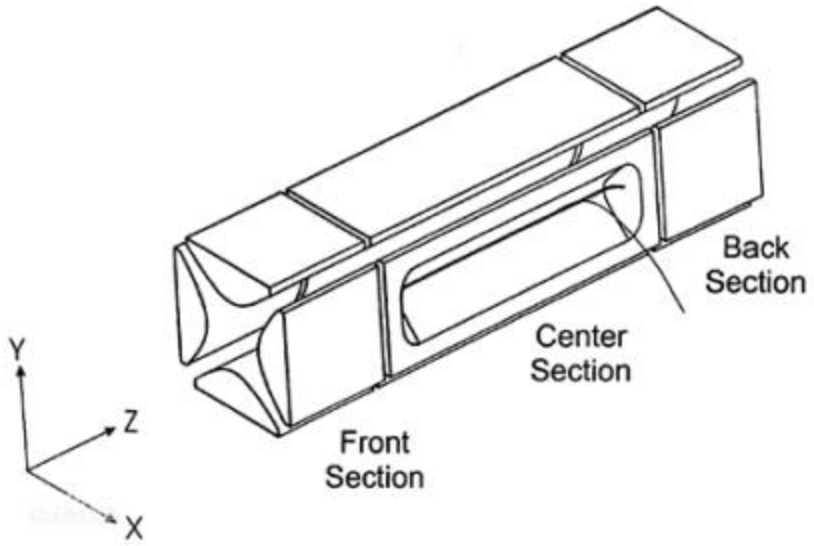
ISBN: 978-0-470-03310-4



http://hdl.handle.net/2047/d10016740

Ion trap (IT)

Quadrupole ion trap is also called 3-dimension ion trap. The QIT mass spectrometer uses three electrodes to trap ions in a small volume. It consists of a cylindrical ring electrode and two end-cap electrodes. A mass spectrum is obtained by changing the electrode voltages to eject the ions from the trap. The end-cap electrodes contain holes for the introduction of ions from an external ion source and for the ejection of ions toward an external detector



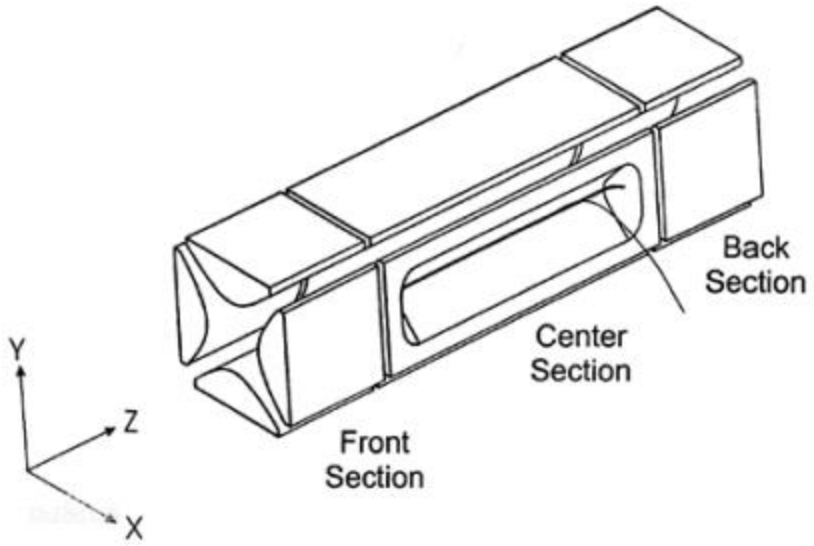
https://www.creative-proteomics.com/blog/index.php/several-types-of-mass-analyzer/

By creating a potential well for the ions, the **linear ion trap** can be used as a mass filter or as a trap. The linear ion trap uses a set of quadrupole rods to confine ions radially by a two-dimensional radio frequency (RF) field. And a static electrical potential can confine the ions axially. They are confined by application of appropriate RF and DC voltages with their final position maintained within the center section of the ion trap. The RF voltage is adjusted, and multi-frequency resonance ejection waveforms are applied to the trap to eliminate all but the desired ions in preparation for subsequent fragmentation and mass analysis

https://youtu.be/EF_gW7jHEbY

Ion trap (IT)

Quadrupole ion trap is also called 3-dimension ion trap. The QIT mass spectrometer uses three electrodes to trap ions in a small volume. It consists of a cylindrical ring electrode and two end-cap electrodes. A mass spectrum is obtained by changing the electrode voltages to eject the ions from the trap. The end-cap electrodes contain holes for the introduction of ions from an external ion source and for the ejection of ions toward an external detector



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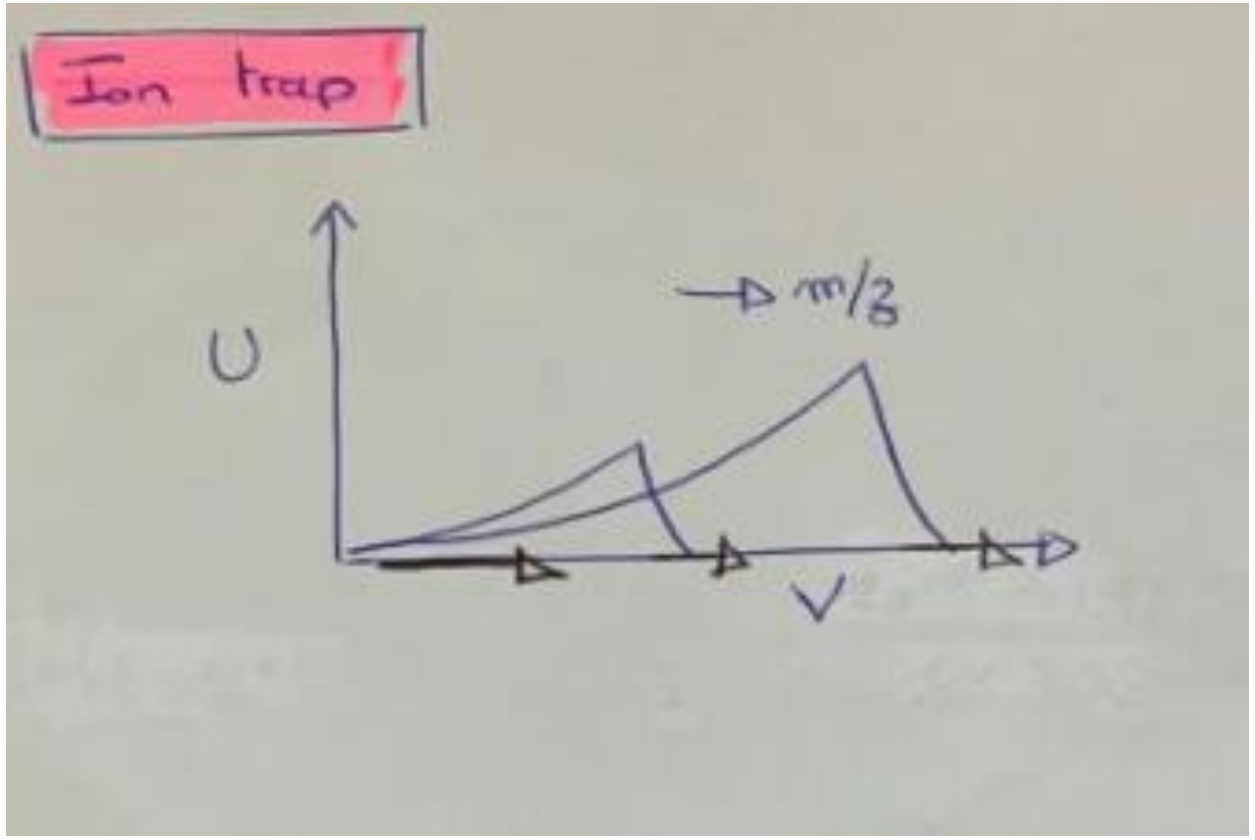
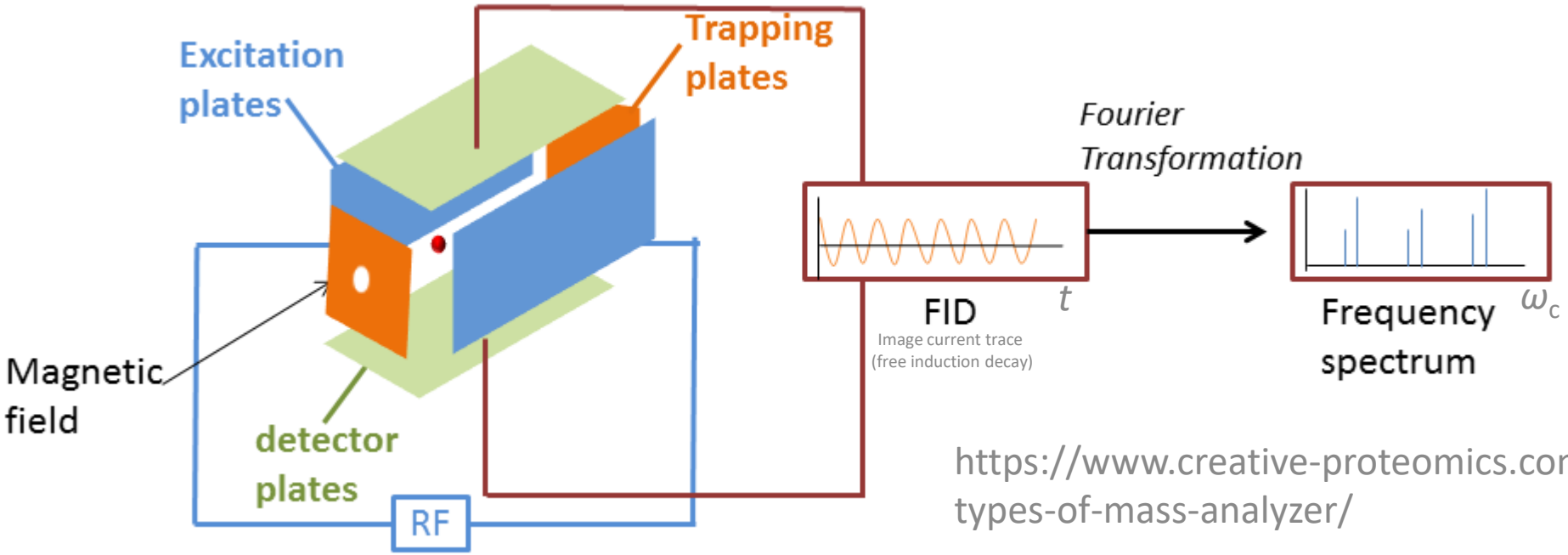


https://youtu.be/EF_gW7jHEbY

Fourier transform ion cyclotron resonance (FT-ICR)



$$\omega_c = \frac{qB}{m}$$

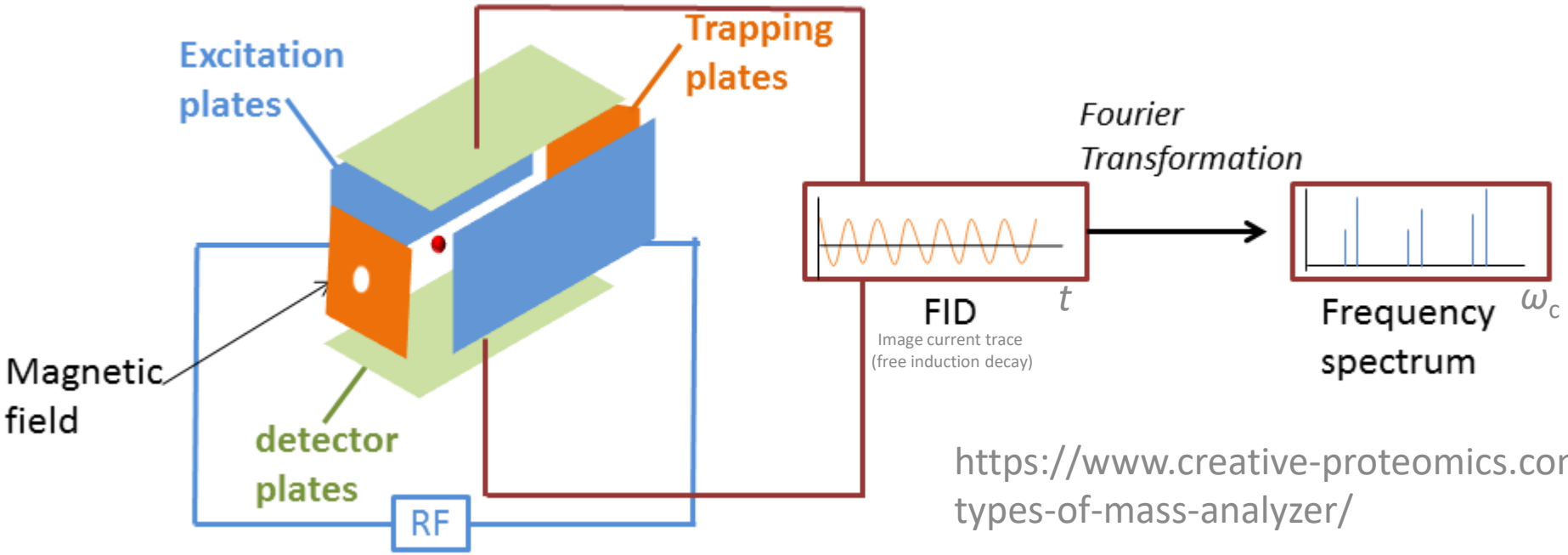
q = ion charge
B = magnetic field strength
m = ion mass
ω_c = angular cyclotron frequency

https://www.creative-proteomics.com/blog/index.php/several-types-of-mass-analyzer/

The Fourier transform ion cyclotron resonance mass spectrometer consists of three main sections: excitation plates, trapping plates, detector plats, and they consist a cell
Ions which are affected by a magnetic field move at a cyclotron frequency. After a radio frequency voltage at the same frequency of cyclotron frequency is applied, the ions absorb energy and accelerate to a larger orbit radius than their original path. After excitation, the cyclotron radius of ions still remains the larger state. And as the ions around approach to the top and bottom plate, the electrons travel from top to bottom. The motion of electrons between these two plates produces a detectable current. The decay over time of the image current resulting after applying a short radio-frequency sweep is transformed from the time domain into a frequency domain signal by a Fourier transform

https://youtu.be/iQ3tSjoiQJ8

Fourier transform ion cyclotron resonance (FT-ICR)

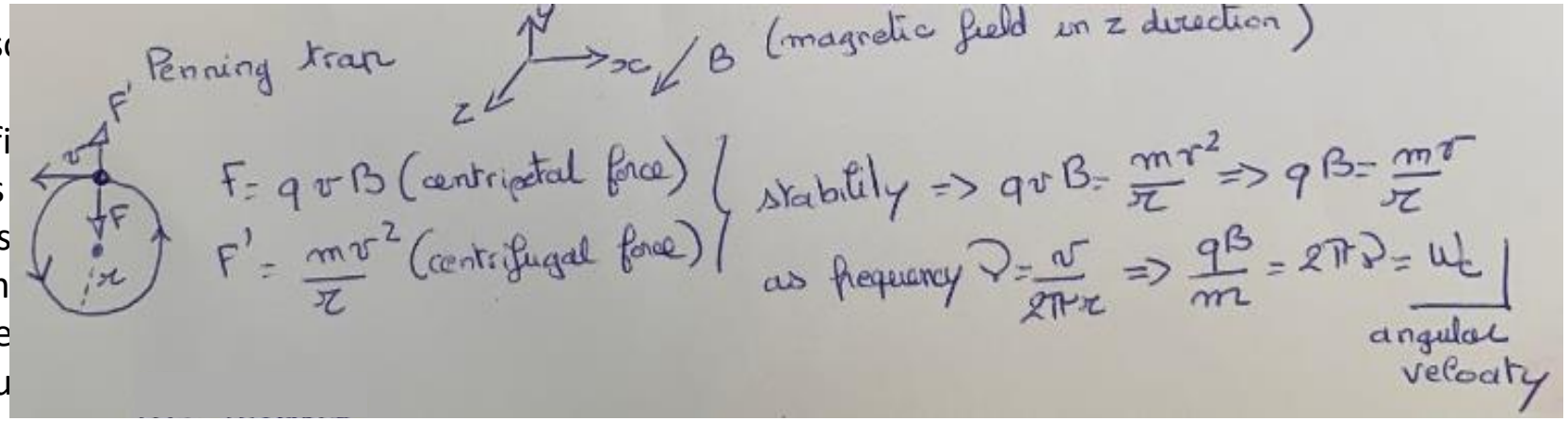


$$\omega_c = \frac{qB}{m}$$

q = ion charge
B = magnetic field strength
m = ion mass
ω_c = angular cyclotron frequency

https://www.creative-proteomics.com/blog/index.php/several-types-of-mass-analyzer/

The Fourier transform ion cyclotron resc
detector plats, and they consist a cell
Ions which are affected by a magnetic fi
cyclotron frequency is applied, the ions
excitation, the cyclotron radius of ions s
the electrons travel from top to bottom
decay over time of the image current re
into a frequency domain signal by a Fou



https://youtu.be/iQ3tSjoiQJ8

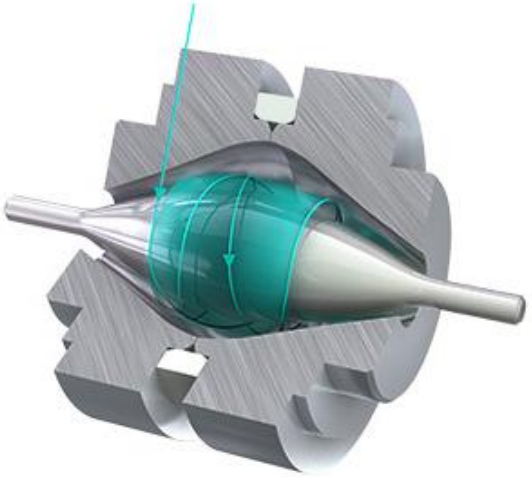
Orbitrap (OT)

Orbitrap is the newest addition to the family of high-resolution mass analyzer

In orbitrap, moving ions are trapped in an electrostatic field. The electrostatic attraction towards the central electrode is compensated by a centrifugal force that arises from the initial tangential velocity of ions. The electrostatic field which ions experience inside the orbitrap forces them to move in complex spiral patterns

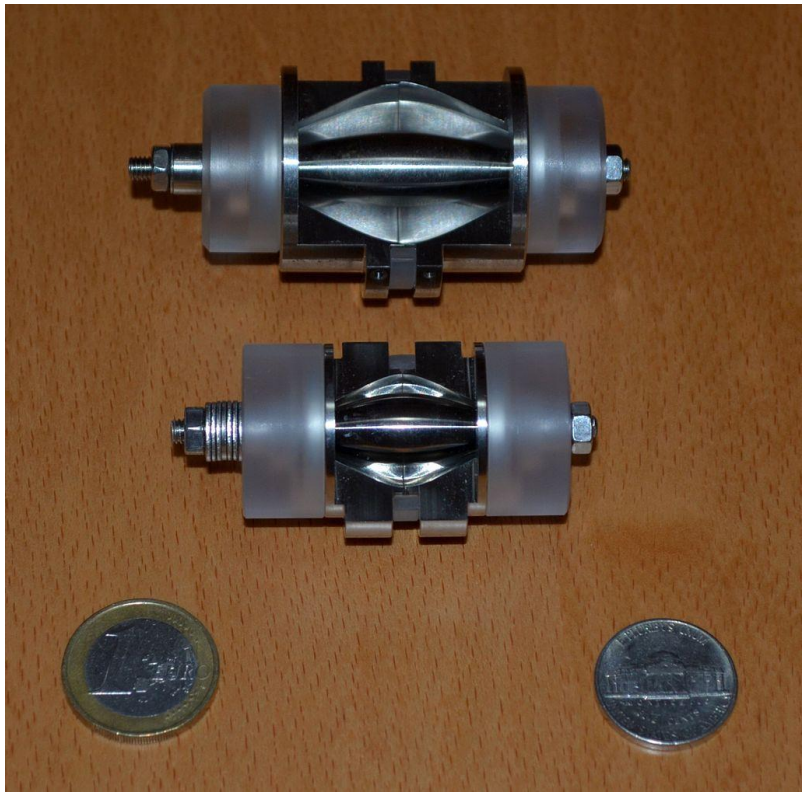
The axial component of these oscillations is independent of initial energy, angles and positions, and can be detected as an image current on the two halves of an electrode encapsulating the Orbitrap

A Fourier transform is employed to obtain oscillation frequencies for ions with different masses, resulting in an accurate reading of their *m/z*



https://www.creative-proteomics.com/blog/index.php/several-types-of-mass-analyzer/

Orbitrap (OT)



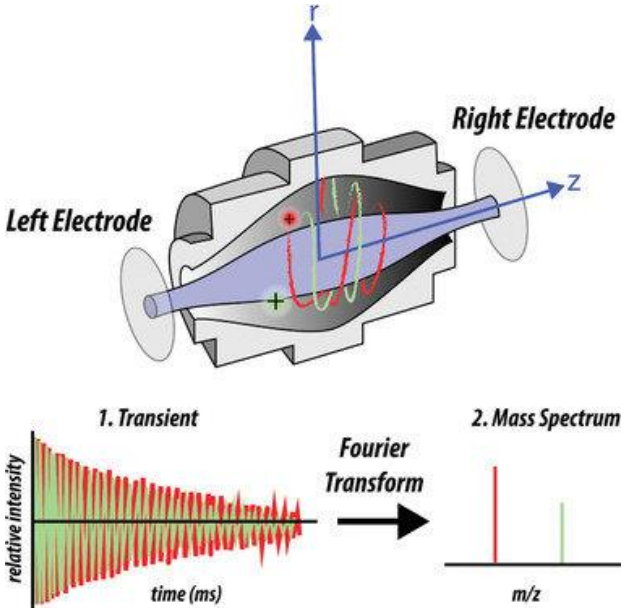
Photograph by Thermo Fisher Scientific

The axial oscillation frequency follows the formula $\omega = \sqrt{\frac{k}{m/z}}$

Where ω = oscillation frequency

k = instrumental constant

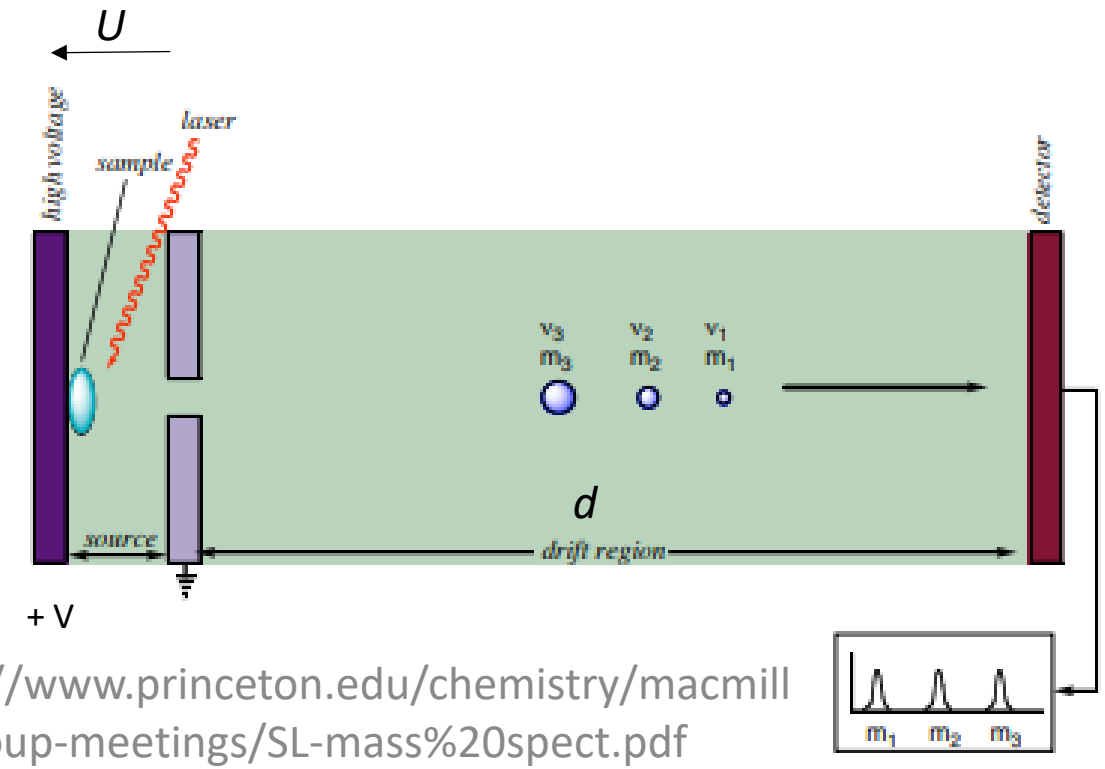
m/z



DOI: 10.1002/pmic.201600113

https://www.youtube.com/watch?v=K1VSYjuw6os

Time of flight (TOF)



https://www.princeton.edu/chemistry/macmillan/group-meetings/SL-mass%20spect.pdf

Time of flight analyzer consists of a pulsed ion source, an accelerating grid, a field-free flight tube, and a detector

The flight time needed by the ions with a particular mass to charge (*m/z*), accelerated by a potential voltage, to reach the detector placed at a distance, can be calculated from a formula.

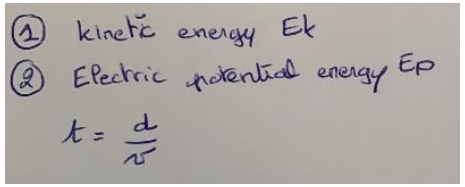
Pulsing of the ion source is required to avoid the simultaneous arrival of ions of different *m/z* at the detector

Typical flight times are 1-30 µs

$$E_p = qU \qquad E_k = \frac{1}{2}mv^2$$

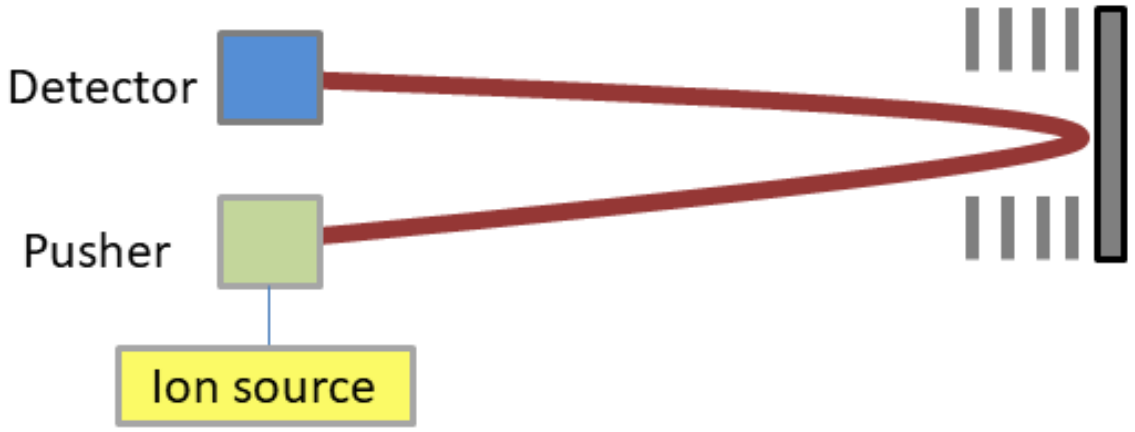
$$qU = \frac{1}{2}mv^2 \quad \Rightarrow \quad qU = \frac{1}{2}m\left(\frac{d}{t}\right)^2$$

$$t = \frac{d}{\sqrt{2U}}\sqrt{\frac{m}{q}} = k\sqrt{\frac{m}{q}}$$



https://youtu.be/V-B5VIP_AQE

Time of flight (TOF) with reflectron



https://www.creative-proteomics.com/blog/index.php/several-types-of-mass-analyzer/

At high masses, not all the ions of the same *m/z* values reach their ideal velocities

To fix this problem, often a reflection which consists of a series of ring electrodes with high voltage is added to the end of the flight tube

Because of the high voltage, an ion is reflected in the opposite direction, when it into the reflection. For the ions of same *m/z* value, faster ions travel further than the slower ones into the reflections. In this way, both the slow and fast ions of the same *m/z* value reach the detector at the same time

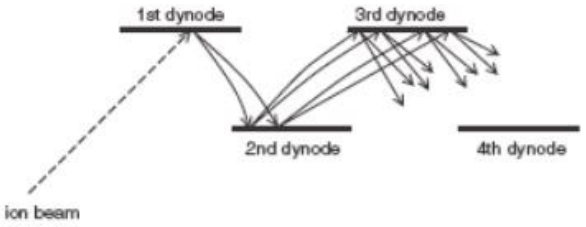
The reflection increases resolution by narrowing the broadband range of flight times for a single *m/z* value

Summary and comparison of mass analysers

	Speed	Resolution	Accuracy	Sensitivity	Dynamic range	Cost
Quadrupole	Fast	Limited (100-1000)	100 ppm	Very good	1×10^7	$
Fourier transform ion cyclotron resonance	Limited	Highest (10000-1000000)	1-5 ppm	Limited	$1 \times 10^3 – 1 \times 10^4$	$$$$
Ion trap	Fast	1000-10000	50-100 ppm	Very Good	$1 \times 10^2 – 1 \times 10^4$	$$
Orbitrap	Average	10000-150000	2.5 ppm	Good	1×10^4	$$$$
Time of flight	Very fast	1000-40000	5-150 ppm	Good	1×10^6	$$$

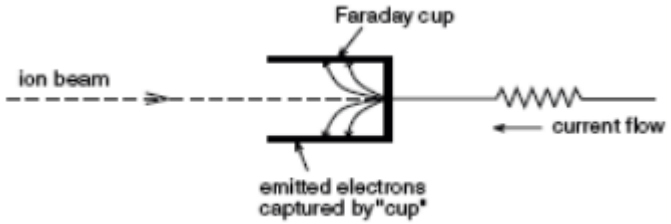
Adapted from https://application.wiley-vch.de/books/sample/3527329242_c01.pdf

Detectors

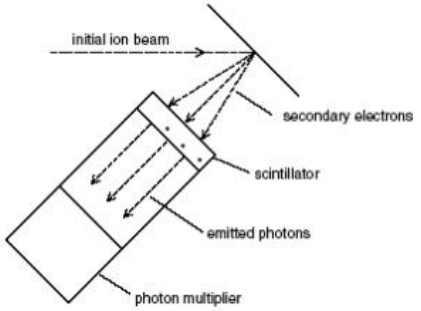


- **Electron multipliers**: An incident ion beam causes two e^- to be emitted from the first dynode. These electrons are accelerated to the second dynode where each causes two more electrons (four in all) to be ejected. These in turn are accelerated to a third dynode and so on.

- **Faraday cup**: Ions travelling at high speed strike the inside of the metal (Faraday) cup and cause secondary e^- to be ejected. This production of electrons constitutes a temporary flow of electric current until the electrons have been recaptured. The Faraday cup detector is simple and robust and is used in situations in which high sensitivity is not required.

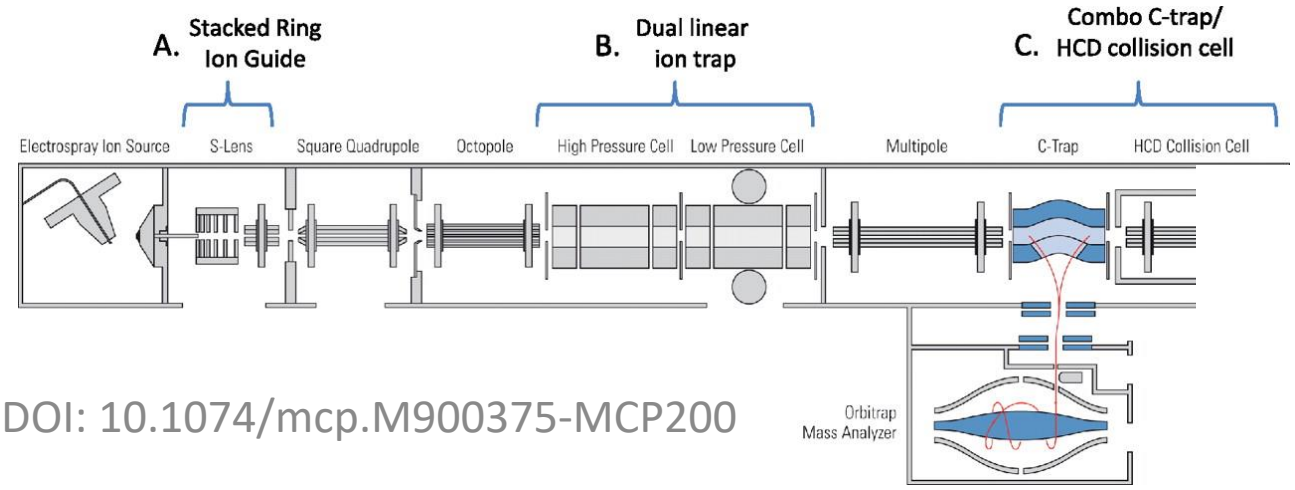


- **Scintillator** ('Daly' detector): A fast ion causes electrons to be emitted and these are accelerated towards a second ‘dynode’ . In this case, the dynode consists of a substance (a scintillator) which emits photons (light). The emitted light is detected by a photomultiplier and is converted into an electric current. Since photon multipliers are very sensitive, high gain amplification of the arrival of a single ion is achieved.



https://www.princeton.edu/chemistry/macmillan/group-meetings/SL-mass%20spect.pdf

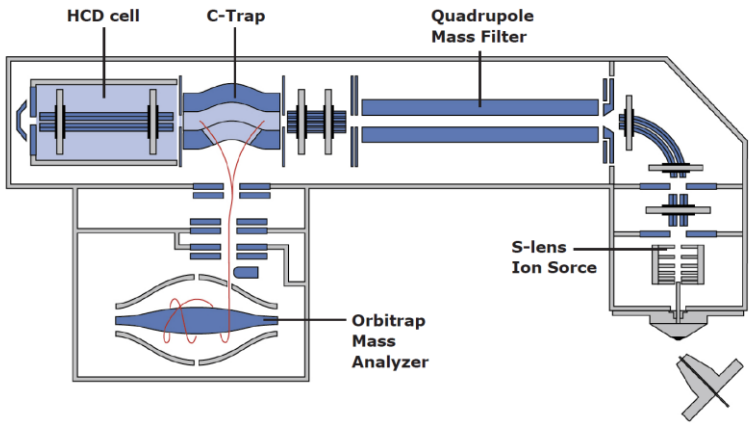
Hybrid instruments



DOI: 10.1074/mcp.M900375-MCP200

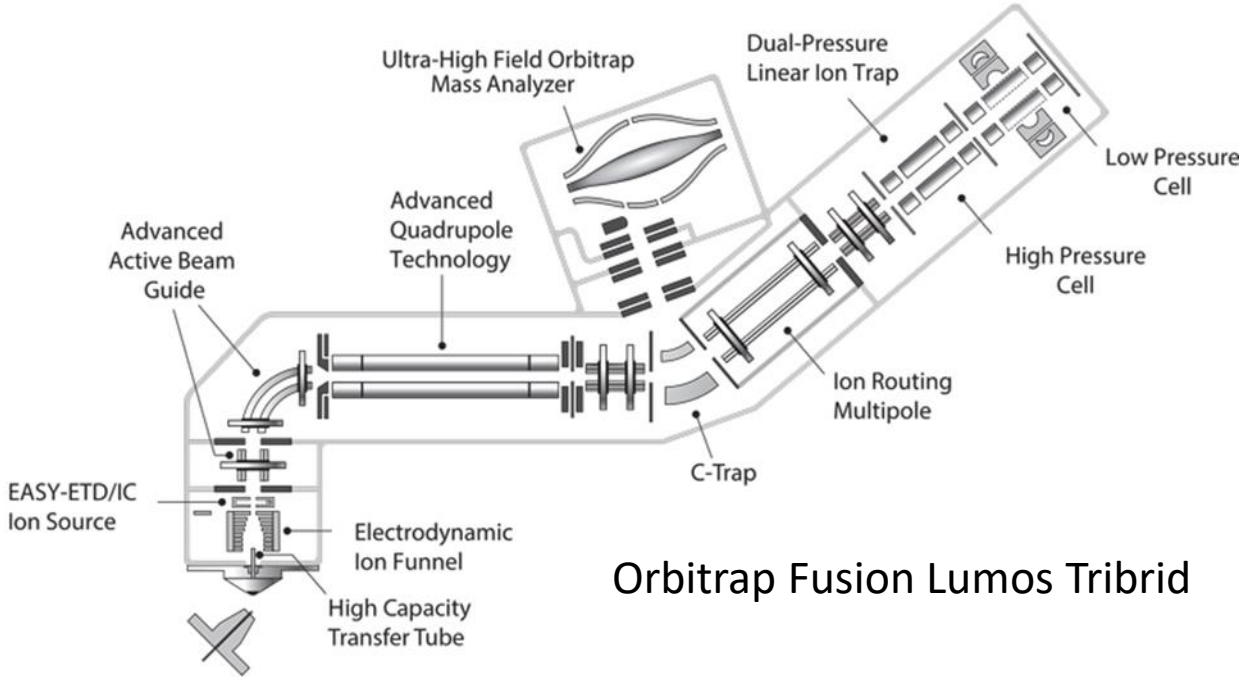
LTQ-OT

https://www.youtube.com/watch?v=KjUQYuy3msA



Q-Exactive

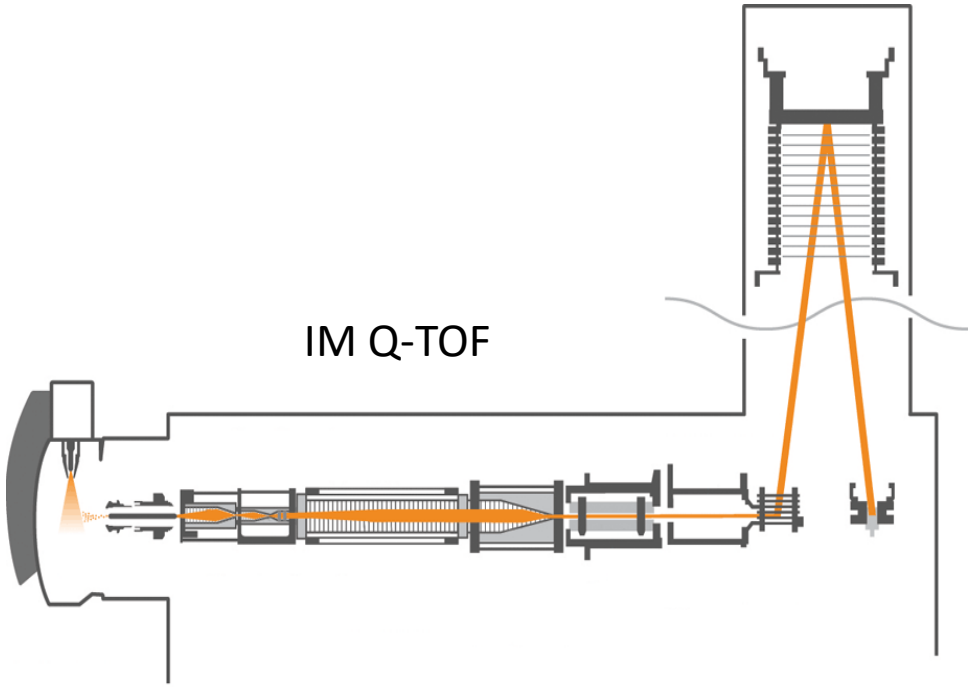
http://planetorbitrap.com/q-exactive#tab:schematic



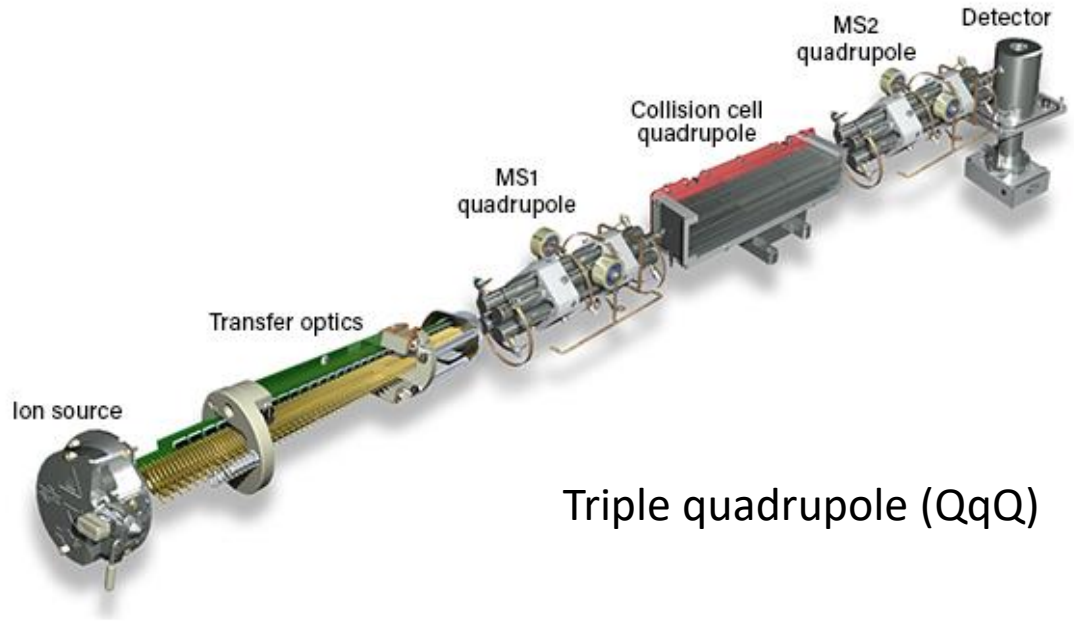
Orbitrap Fusion Lumos Tribrid

http://planetorbitrap.com/orbitrap-fusion-lumos#tab:schematic

Hybrid instruments



https://www.agilent.com/en/newsletters/accessagilent/2014/may/ims6560

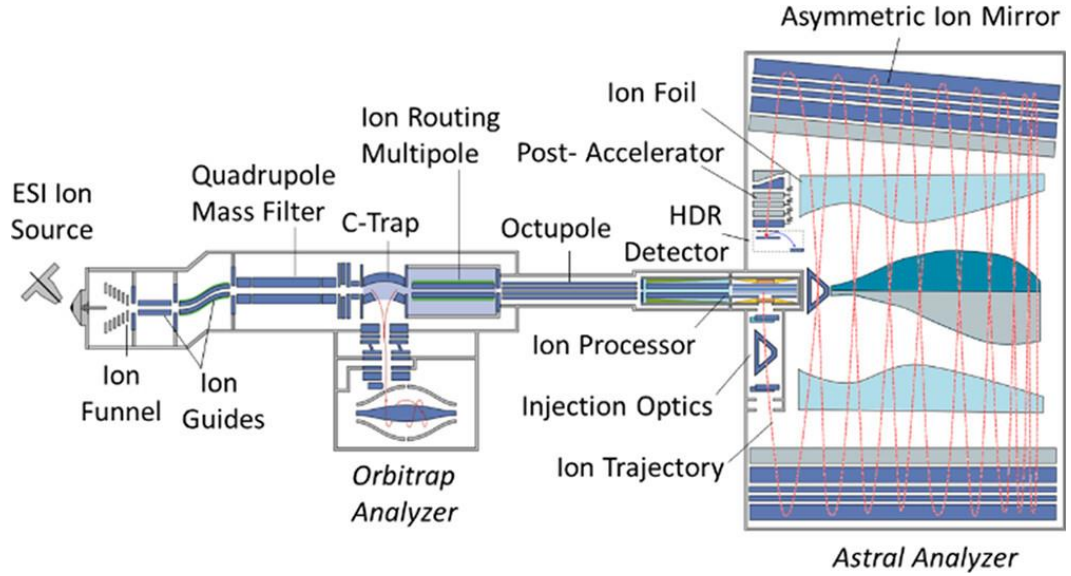


Triple quadrupole (QqQ)

http://blog.waters.com/the-gold-standard-for-quantitative-measurement

Hybrid instruments – Latest Generation

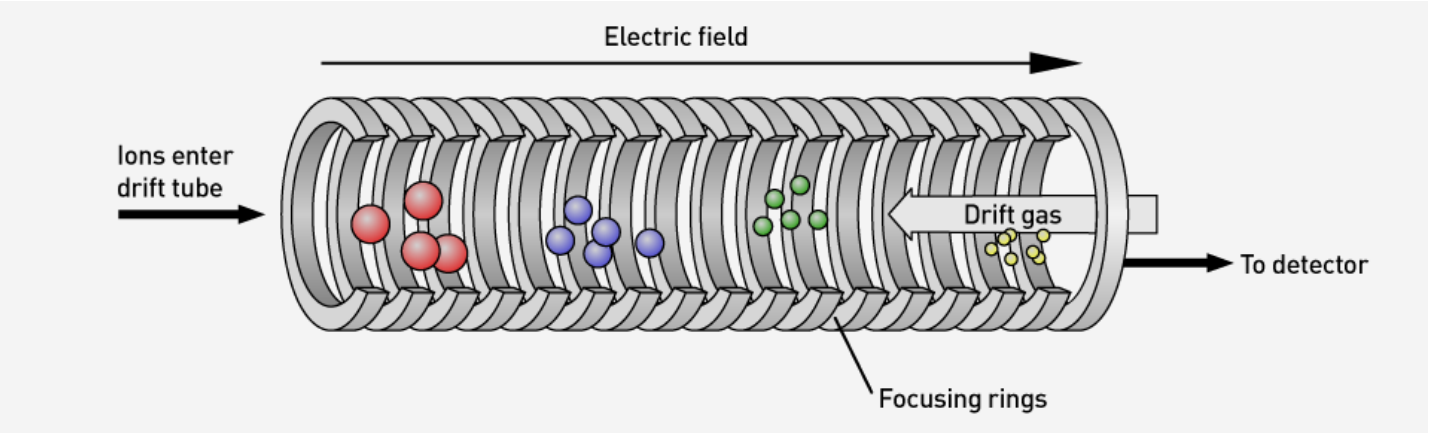
https://www.bruker.com/en/products-and-solutions/mass-spectrometry/timstof/timstof-pro-2.html



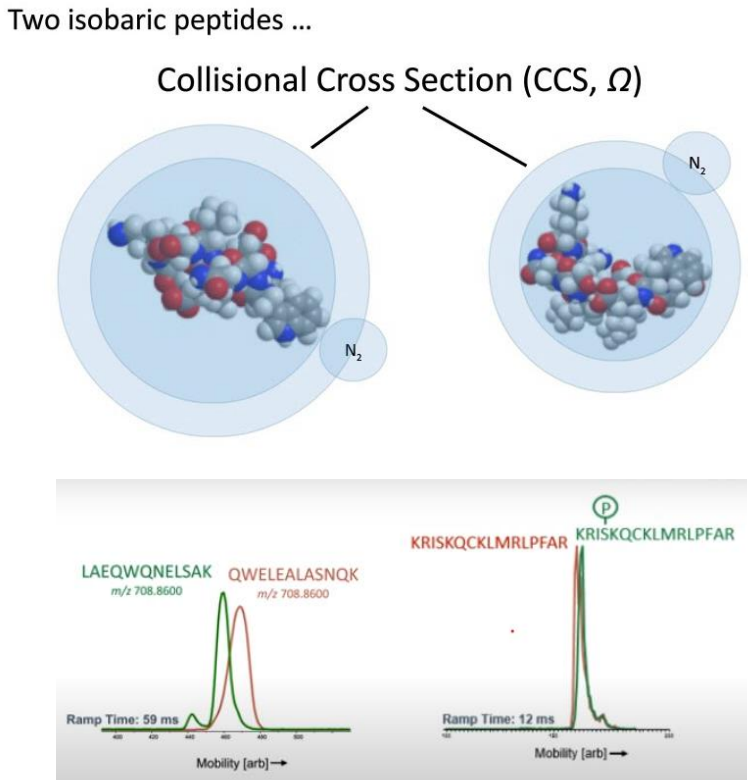
https://doi.org/10.1021/acs.analchem.3c02856

Hybrid instruments – Latest Generation

- Ion mobility spectrometry



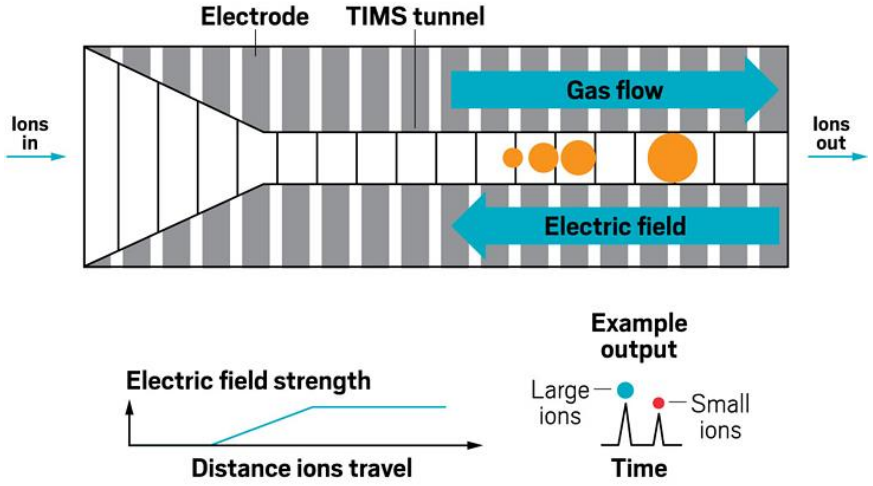
https://www.analyticon.eu/files/analyticon/inhalte/Technologien_Ionen-Mobilit%C3%A4ts-Spektrometrie/Ion_mobility_spectrometry_diagram_en.png



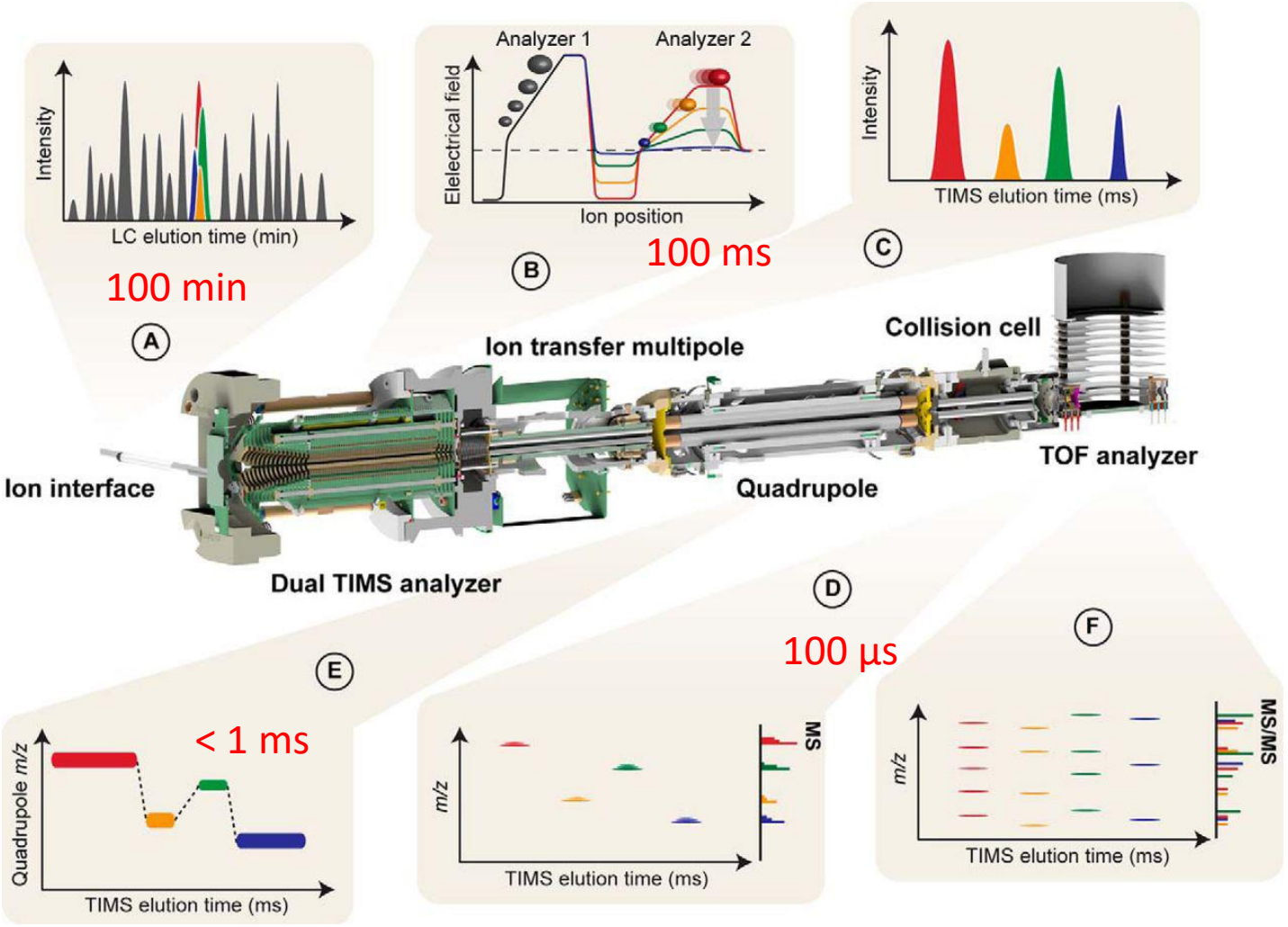
Bruker Life Sciences Mass Spectrometry
Meier *et al.*

Hybrid instruments – Latest Generation

- Trapped ion mobility spectrometry (TIMS)-TOF instruments



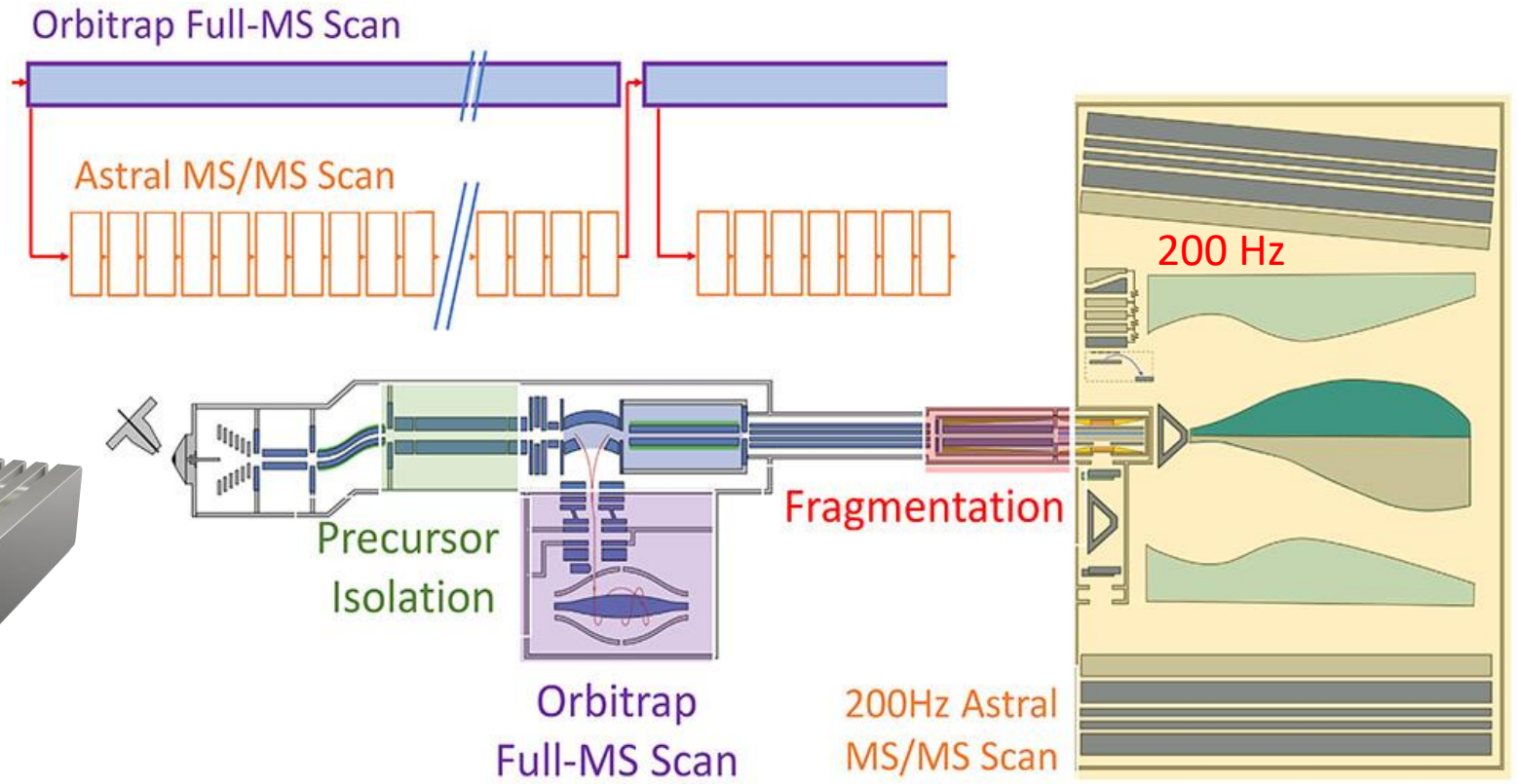
https://cen.acs.org/analytical-chemistry/mass-spectrometry/Resolving-power-people-Ion-mobility/98/i20



10.1074/mcp.TIR118.000900

Hybrid instruments – Latest Generation

- Parallelized Acquisition of Orbitrap and Astral Analyzers



10.1021/acs.analchem.3c02856

Some performances of hybrid instruments

Characteristics and performances of commonly used types of mass spectrometers. Check marks indicate available, check marks in parentheses indicate optional. +, ++, and +++ indicate possible or moderate, good or high, and excellent or very high, respectively. Seq., sequential.

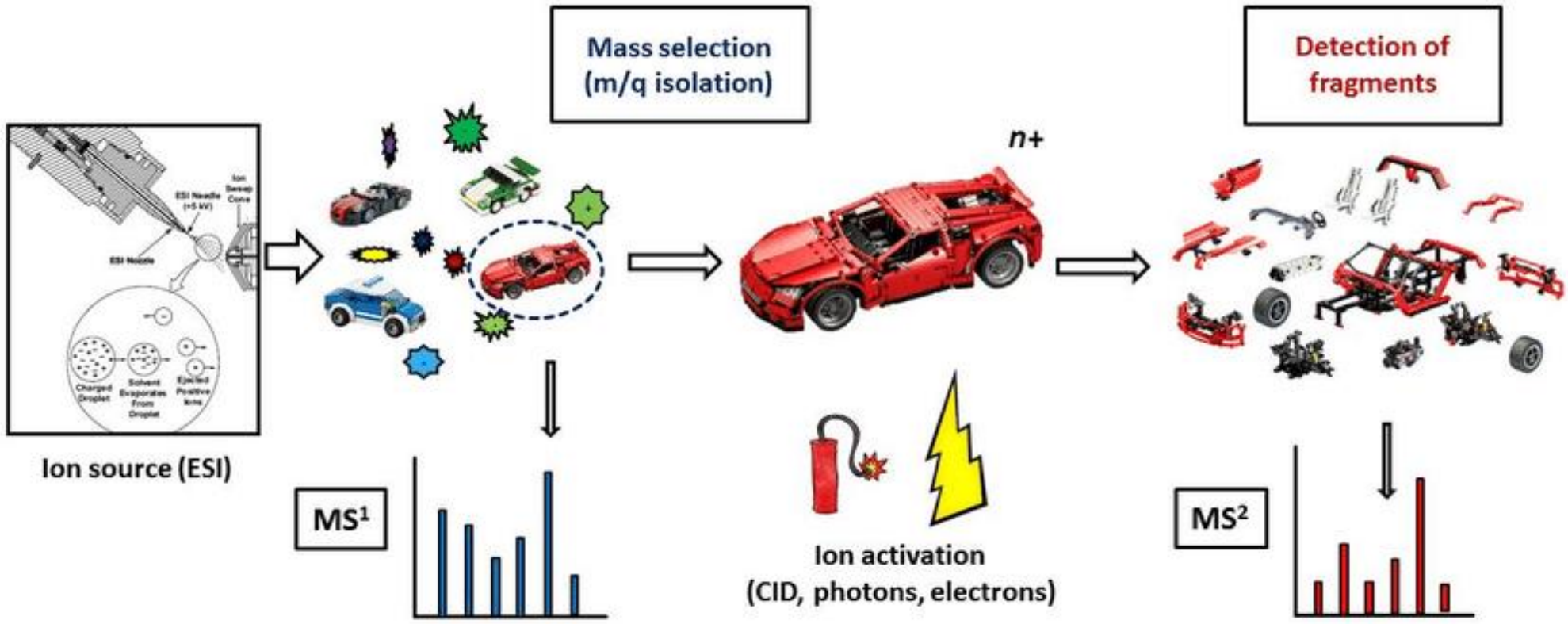
	IT-LIT	Q-Q-ToF	ToF-ToF	FT-ICR	Q-Q-Q	QQ-LIT
Mass accuracy	Low	Good	Good	Excellent	Medium	Medium
Resolving power	Low	Good	High	Very high	Low	Low
Sensitivity (LOD)	Good		High	Medium	High	High
Dynamic range	Low	Medium	Medium	Medium	High	High
ESI	✓	✓		✓	✓	✓
MALDI	(✓)	(✓)	✓			
MS/MS capabilities	✓	✓	✓	✓	✓	✓
Additional capabilities	Seq. MS/MS			Precursor, Neutral loss, MRM		
Identification	++	++	++	+++	+	+
Quantification	+	+++	++	++	+++	+++
Throughput	+++	++	+++	++	++	++
Detection of modifications	+	+	+	+		+++

LTQ-OT Tribrid OT

Q6: Any idea?

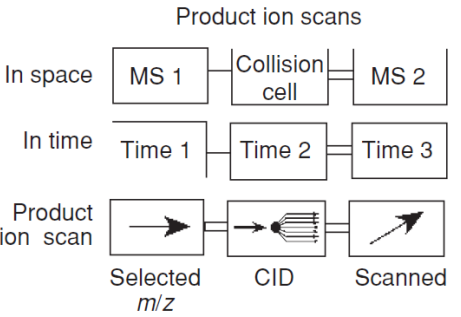
DOI: 10.1126/science.1124619

MS and tandem MS (MS/MS)

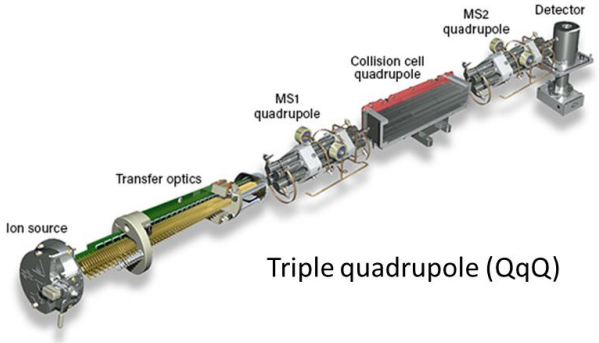


Ković, Miloš. (2016). Photon and electron action spectroscopy of trapped biomolecular ions - From isolated to nanosolvated species. . 10.13140/RG.2.2.20901.91365.

MS/MS in space and in time

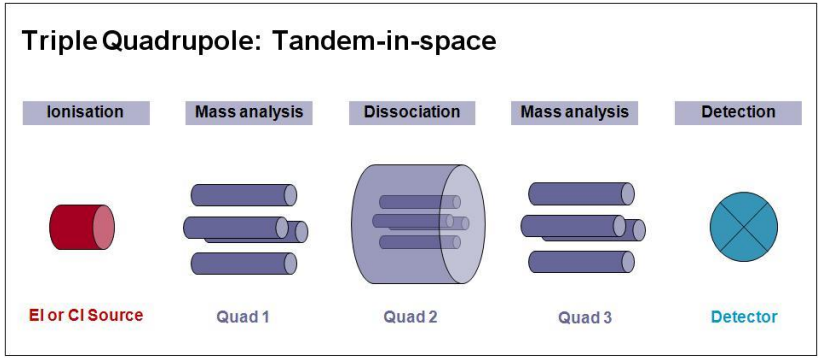


- MS/MS in space: perform tandem mass spectrometry by the coupling of two physically distinct instruments. Common space instruments have two mass analyzers, allowing MS/MS experiments to be performed. A frequently used instrument of this type uses quadrupoles as analyzers. MS^n spectra requires *n* analyzers to be combined



Triple quadrupole (QqQ)

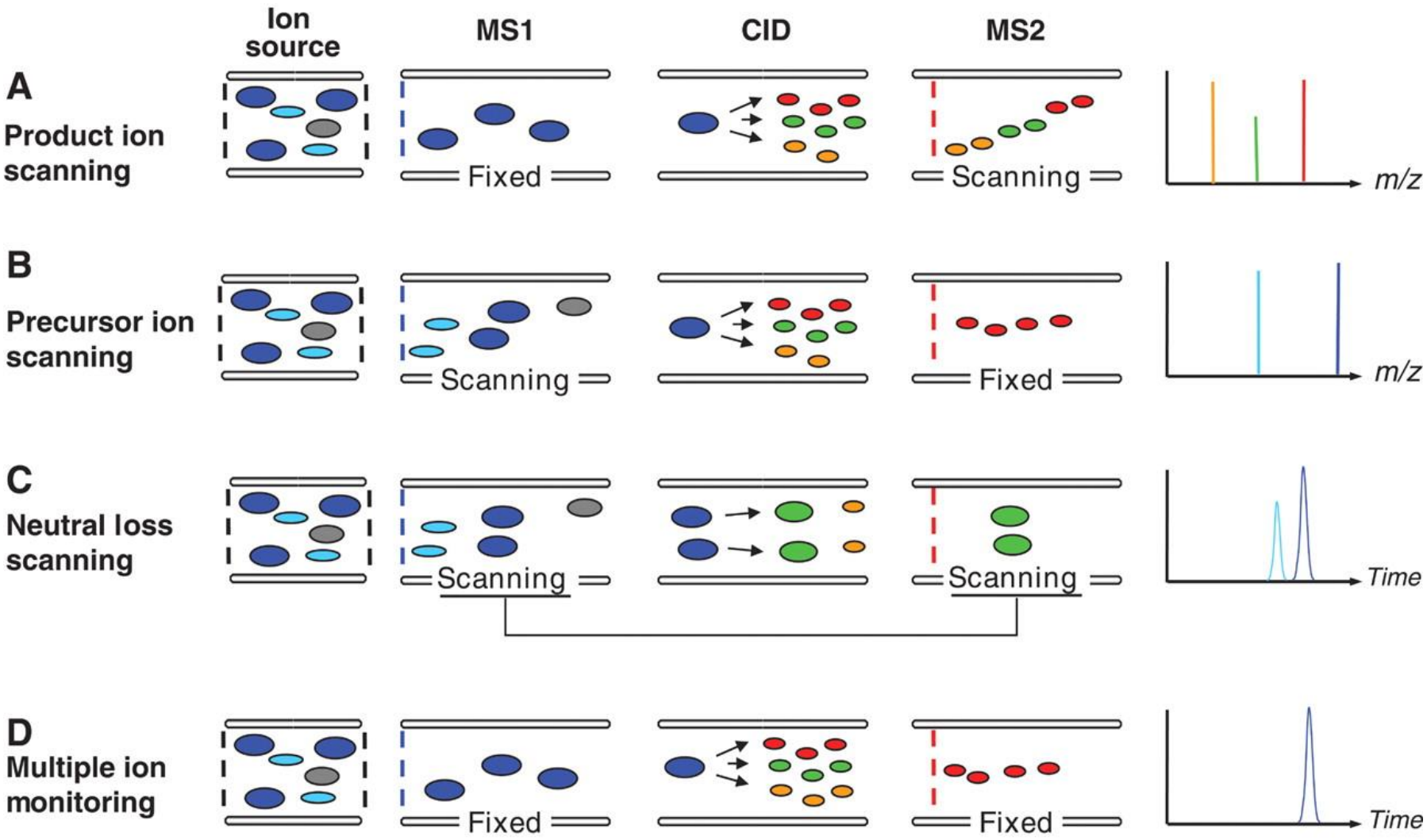
http://blog.waters.com/the-gold-standard-for-quantitative-measurement



https://www.biologie.hu-berlin.de/de/gruppenseiten/oekologie/meth/massspec/mass_sp

- MS/MS in time: perform an appropriate sequence of events in an ion storage device. Tandem mass spectrometry can be achieved through time separation with a few analyzers such as ion traps, orbitrap and FTICR, programmed so that the different steps are successively carried out in the same instrument

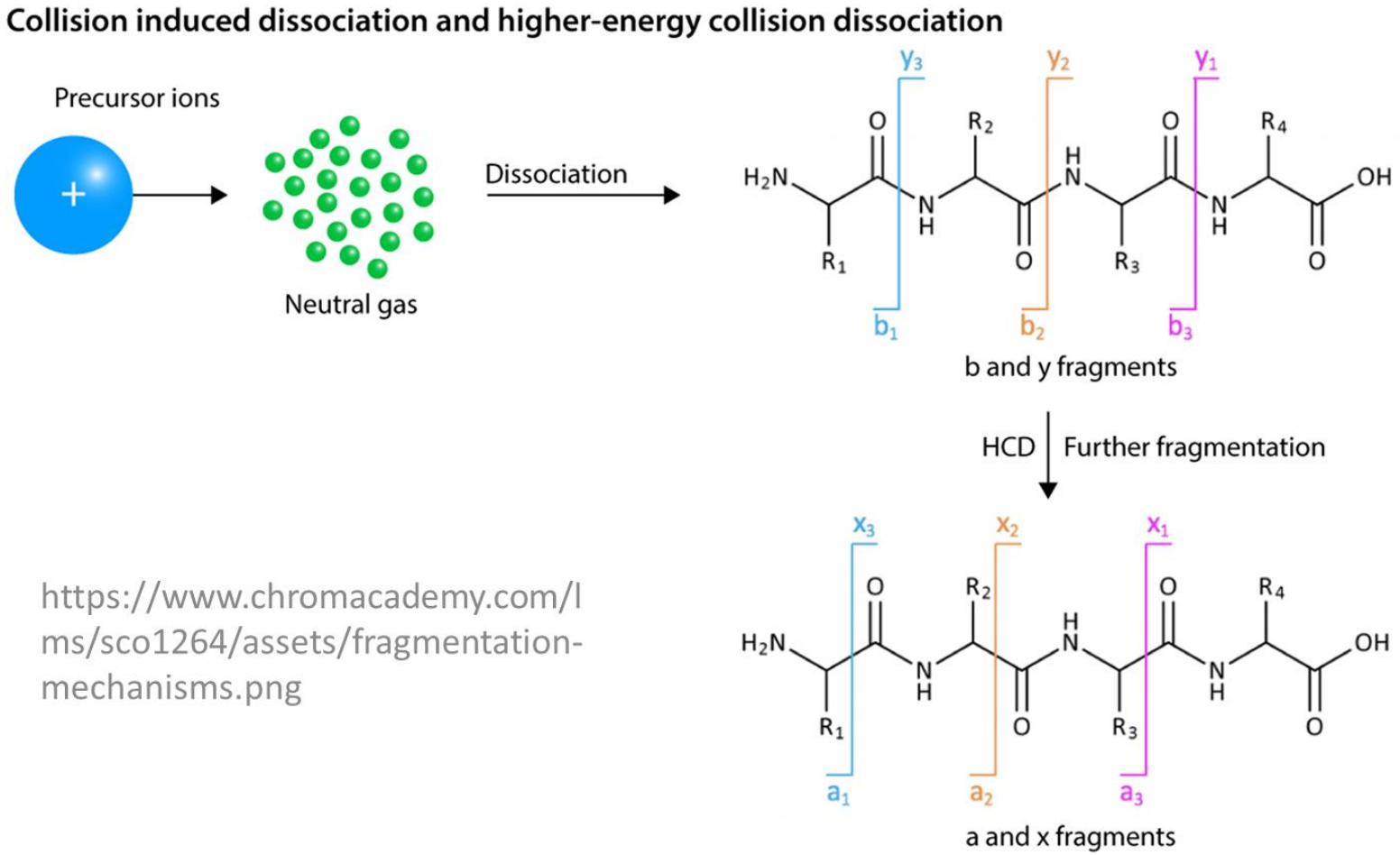
Types of MS/MS experiments



DOI: 10.1126/science.1124619

Types of fragmentations used in proteomics

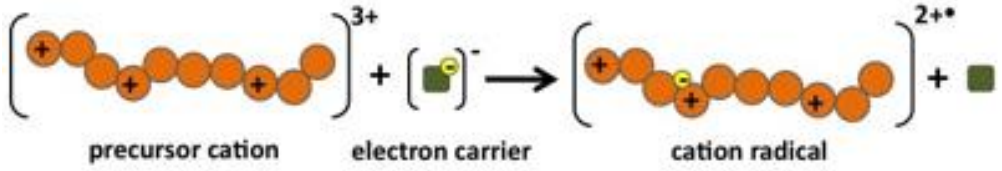
- Collision-induced dissociation (CID): the precursor ion undergoes repeated collisions with a chemically inert collision gas (*e.g.*, Ar, He, N_2). B- and y- ions are generated

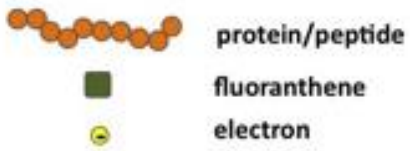


https://www.chromacademy.com/lms/sco1264/assets/fragmentation-mechanisms.png

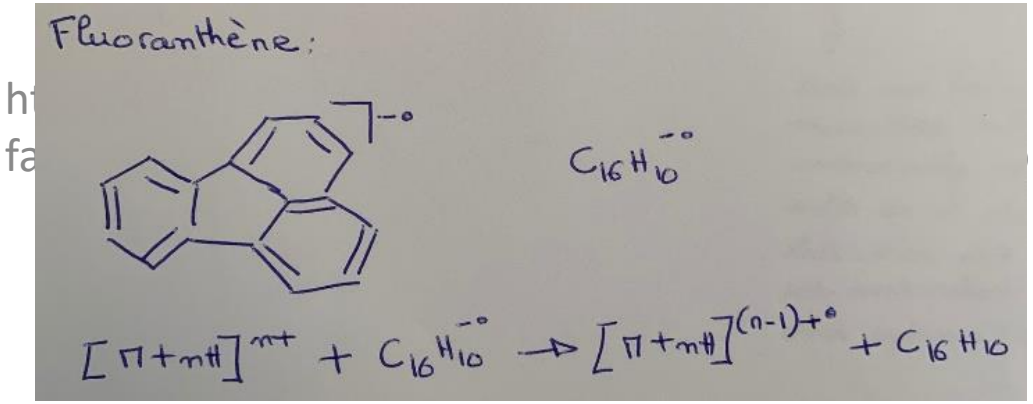
Types of fragmentations used in proteomics

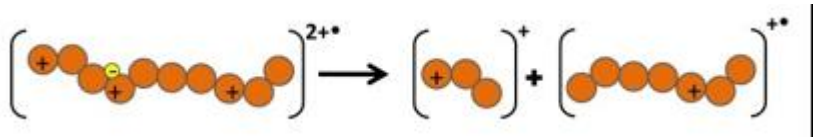
- Electron transfer dissociation (ETD) is a popular peptide fragmentation technique in mass spectrometry. It requires multiply-charged gas-phase cations (z > 2) and therefore it is typically limited to an electron spray ion source. It leaves labile bonds from post-translational modifications intact



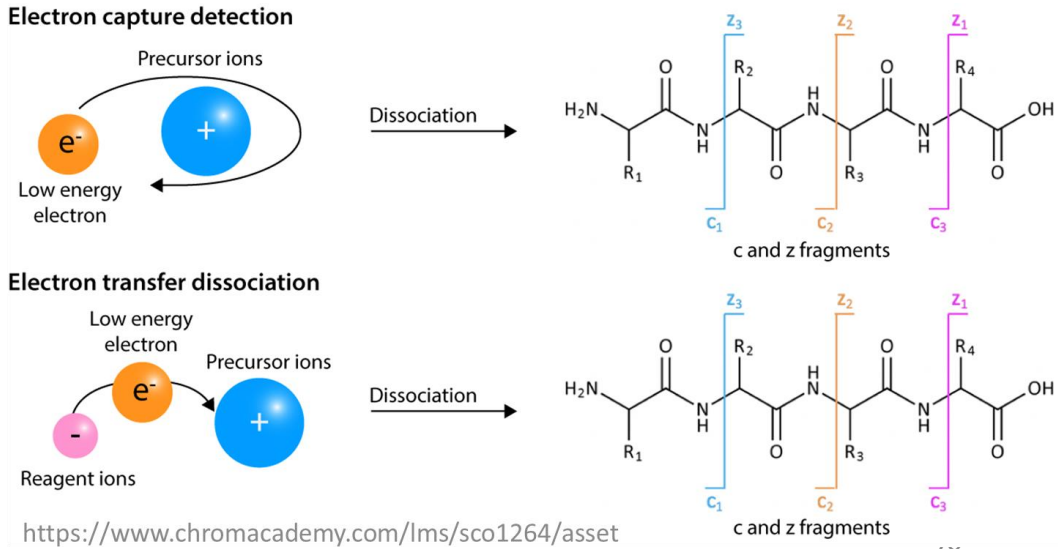


Capture of an electron from the anion yields an unstable cation radical



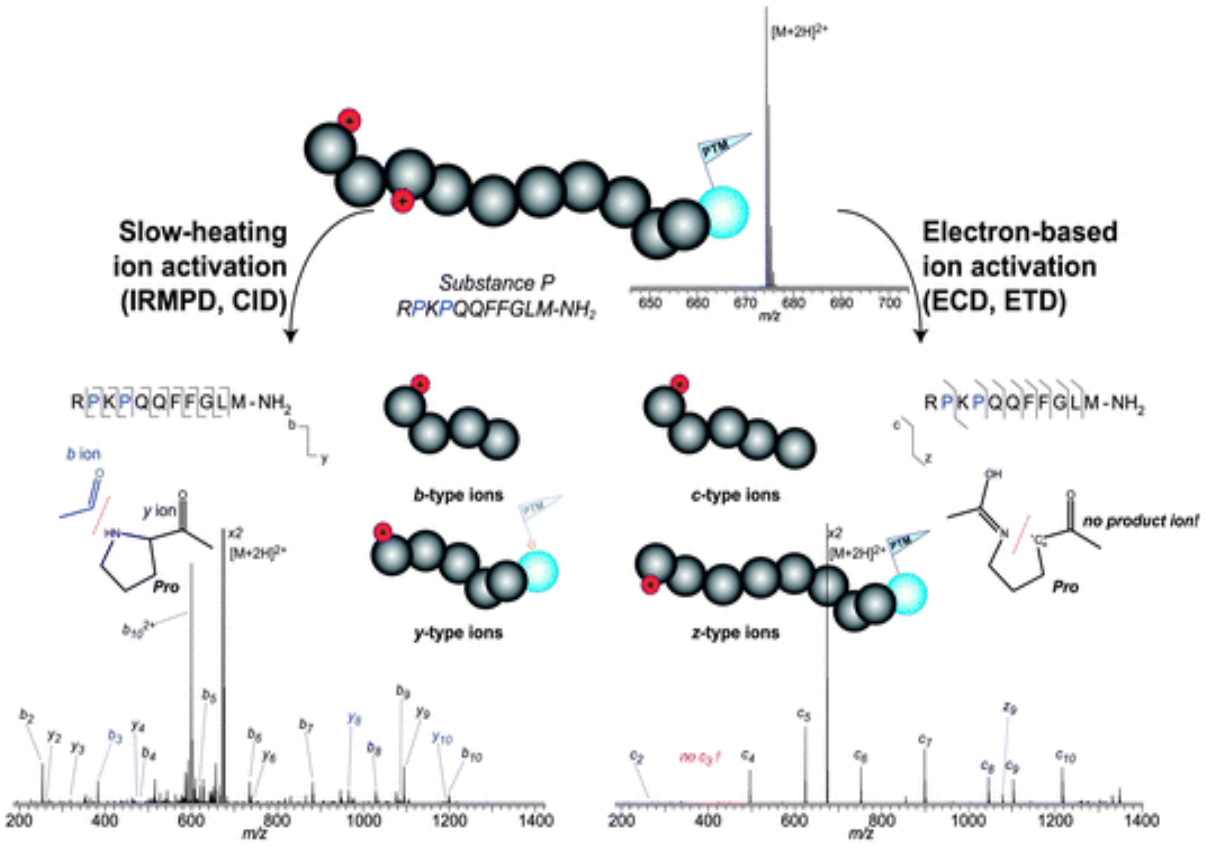


Cation radical then breaks into two fragments, usually one c ion and one z ion

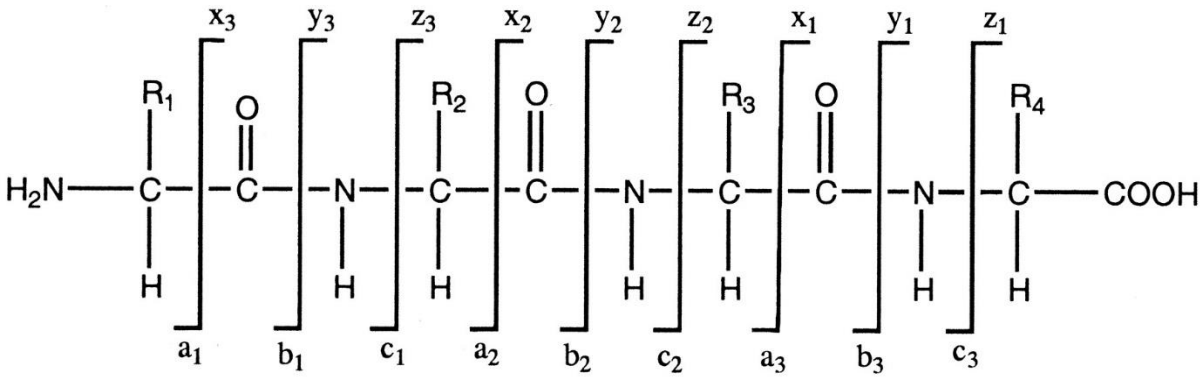


https://www.chromacademy.com/lms/sco1264/assets/fragmentation-mechanisms.png

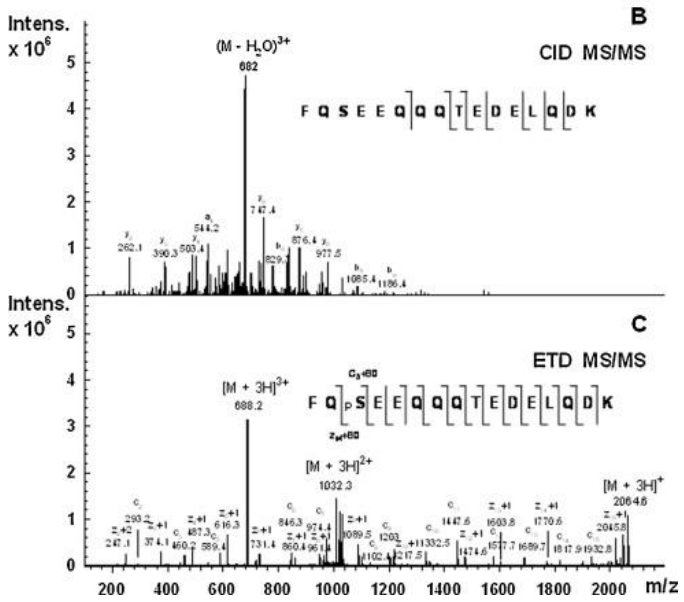
MS and tandem MS (MS/MS)



DOI: 10.1039/C3CS35477F



Paul R. Graves, and Timothy A. J. Haystead Microbiol. Mol. Biol. Rev. 2002;66:39-63



https://doi.org/10.1016/j.aca.2014.10.053

To conclude and for next chapters: mass spectrometers and their use in proteomics

instrument	*m*/*z* range	scan rate (Hz)	mass resolution	mass accuracy (ppm)	dynamic range	sensitivity	MS_n compatibility	ion source	ion mobility	ideal applications	release
LIT											
TFS Velos Pro	15–4000	125 000 Da/s	0.075–3.0 fwhm	0.1–1.5 Da	>1 × 10_6	femtomole	rCID,ETD	ESI, APCI, APPI	FA1MS*	1, 4, 9, 10	2011
QqQ											
Agilent 6495D	5–3000	18 700 Da/s	0.4–23 fwhm	0.1 Da	>6 × 10_6	atto- to femtomole	bCID	ESI, APCI		1, 4, 9, 10	2023
SCIEX Triple Quad 7500	5–2000	20 000 Da/s	3100	0.1 Da	>1 × 10_6	atto- to femtomole	bCID	ESI		1, 4, 9, 10	2020
Shimadzu 8060NX	2–2000	30 000 Da/s	0.5 fwhm	0.1 Da	1 × 10_7	atto- to femtomole	bCID	ESI, APCI		1, 4, 9, 10	2020
TFS TSQ Altis	5–2000	15 000 Da/s	0.2–2.0 FWHM	0.2–2.0 Da	>1 × 10_6	atto- to femtomole	rCID,bCID	ESI, APCI	FAIMS (Pro Duo)*	1, 4, 9, 10	2019
Q-TOF											
Agilent 6545XT	20–30 000	50	50 000	<0.8$_i$	1 × 10_5	femtomole	bCID, ECD*	ESI, APCI, MALDI*	SLIM*	1, 4, 6, 9, 10	2017
Bruker maXis II	50–20 000	50	80 000	<0.8$_i$	1 × 10_5	attomole	bCID, ETD	ESI, APCI, APPI		1, 4, 6, 10	2014
SCIEX IripleTOF 6600+	5–40 000	100	35 000	<0.5$_i$	1 × 10_5	atto- to femtomole	bCID	ESI, APCI		1, 4, 6, 10	2019
Waters Select Series MRT	50–20 000	10	200 000	<0.2$_i$	1 × 10_5	femtomole	bCID	MALDI, ESI		1, 4, 6, 9, 10	2021
Waters Xevo G3	20–100 000	30	40 000	<1$_i$	1 × 10_4	femtomole	bCID	ESI, APCI		1, 3, 4, 6, 7, 9, 10	2022
TOF-TOF											
Bruker rapifleX	50–8000	50–100	45 000	<3$_e$; <1$_i$	1 × 10_4	femtomole	bCID	MALDI, ESI		1, 4, 6	2015
Q-IMS-TOF											
Waters Synapt XS	20–64 000	30	75 000	<$_i$	1 × 10_4	femtomole	bCID, ETD	MALDI, ESI	TWIMS (25 cm)	4, 6, 7	2019
Waters Select Series Cyclic IMS	20–64 000	50	100 000	<0.5$_i$	1 × 10_5	attomole	bCID, ECD, SID	ESI, MALDI	TWIMS (>100 cm)	1, 3, 4, 5, 6, 7, 9, 10	2019
IMS-Q-TOF											
Agilent 6560	20–20 000	50	42 000	<2$_e$; <1$_i$	1 × 10_5	femtomole	bCID, in-source CID	ESI, MALDI*	DT-IMS (80 cm)	4, 6, 7	2021
Bruker timsTOF fleX	50–20 000	150	60 000	<2$_e$	1 × 10_5	atto- to femtomole	bCID	ESI, MALDI	TIMS (v. 4)	1, 3, 4, 6, 7, 9, 10	2019
Bruker timsTOF SCP	50–20 000	120	60 000	<2$_e$	5 × 10_4	zepto- to attomole	bCID	ESI	TIMS (v. 3)	1, 2, 3, 4, 5, 6, 7, 9, 10	2021
Bruker timsTOF HT	50–20 000	150	60 000	<2$_e$	1 × 10_5	atto- to femtomole	bCID	ESI	TIMS (v.4)	1, 3, 4, 6, 7, 9, 10	2022
Bruker timsTOF Ultra	50–20 000	300	60 000	<2$_e$	5 × 10_4	zepto- to attomole	bCID	ESI	TIMS (v. 4)	1, 2, 3, 4, 5, 6, 7, 9, 10	2023
Q BIT-TOF											
SCIEXZenoTOF 7600	40–40 000	133	42 000	<2$_e$; <1$_i$	1 × 10_5	atto- to femtomole	bCID, rCID, EAD	ESI, APCI		1, 3, 4, 7, 9, 10	2021
Qq-FTICR											
Bruker solariX FTICR	100–10 000	1	10 000 000	0.25$_i$	2 × 10_3	atto- to femtomole	bCID, ETD, ECD, SORI	ESI, MALDI		4, 6	2009
LIT-Orbitrap											
TFS Orbitrap Elite Hybrid	50–4000	12	240 000	<3$_e$; <1$_i$	5 × 10_3	atto- to femtomole	bCID, rCID, ETD	ESI, APCI, APPI	FAIMS*	1, 2, 3, 4, 5, 7, 8, 9, 10	2012
Q-Orbitrap											
TFS Q Exactive HF	50–6000	18	240 000	<3$_e$; <1$_i$	5 × 10_3	femtomole	bCID	ESI, APCI, APPI	FAIMS*	1, 4, 6, 7, 8, 9, 10	2014
TFS Q Exactive UHMR	350–80 000	22	200 000	<3$_e$; < 1$_i$	1 × 10_5	femtomole	bCID, in-source CID	ESI, APCI, APP!	FAIMS*	4, 6	2018
TFS Orbitrap Exploris	40–8000*	40	480 000	<3$_e$; 1<$_i$	1 × 10_5	femtomole	bCID, in-source CID	ESI, APG, APPI	FAIMS (Pro Duo)*	1, 4, 6, 7, 8, 9, 10	2019
Q-Orbitrap-LIT											
TFS Orbitrap Ascend Tribrid	40–16 000*	50	1 000 000*	< 3$_e$; 1<$_i$	5 × 10_3	atto- to femtomole	bCID, rCID, ETD*, UVPD*, PTCR*	ESI, APCI, APPI	FAIMS (Pro Duo)*	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	2022
Q-Orbitrap-Astral											
TFS Orbitrap Astral	40– 2000	200	80 000	<5$_e$	>1 × 10_4	zepto- to attomole	bCID	ESI, APCI, APPI	FAIMS (Pro Duo)*	1, 2, 3, 4, 5, 7, 8, 9, 10	2023

Instrument models are grouped by their primary instrumental components including mass analyzers and ion mobility cells,. Mass accuracy: e with external calibration, i with internal calibration. BIT, branched ion trap; bCID, beam-type collision-induced dissociation; rCID, resonant collision-induced dissociation; ETD, electron transfer dissociation; ECD, electron capture dissociation; EAD, electron-activated dissociation; SORI, sustained off-resonance irradiation; UVPD, ultraviolet photodissociation; PTCR, proton transfer charge reduction; APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; FAIMS, high-field asymmetric waveform ion mobility spectrometry; SLIM, structures for lossless ion manipulation; DT-IMS, drift tube-ion mobility spectrometry. Ideal applications: **(1) biofluids, (2) immunopeptidomics, (3) deep and unbiased proteomics, (4) post-translational modifications, (5) single-cell proteomics, (6) native proteomics, (7) spatial and temporal proteomics, (8) cross-linking MS, (9) clinical proteomics, (10) large-scale proteomics. (*) Upgrade required.**

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