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EDUCATION SCIENCE INNOVATION

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Mass spectrometry as a analytical tool for metal complexes in biological samples

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Plan

Metalloproteomics

Omics

Metallomics vs proteomics/peptidomics vs metalloproteomics vs Metabolomics

Metal complex and metallome

Mass Spectrometry

General principle

Analysers and detection

Ion sources: ESI/MALDI/ICP LA-ICP LCM-ICP

Quantification and Speciation

Isotopic mass spectrometry

Biological Metal Complexes

Proteome evolution and metal ligands (Pt, Bi, Li)

Metal complex and metallome, metal sensing and post-translational metal regulation

ICP and microbial metalloproteome

Metalloproteomics

Single Cell ICP-MS

The interface Chemistry / Biology is essential

Two of the three most cited papers of all time[7] report analytical chemistry techniques to study biological systems

R. Van Noorden, B. Maher and R. Nuzzo, Nature, 2014, 514, 550–553.

1. **Protein measurement with the folin phenol reagent**. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. J. Biol. Chem. 193, 265–275 (1951). (305 148 citations)

2. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Laemmli, U. K. Nature 227, 680–685 (1970). (213 005 citations)

3. **A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding**. Bradford, M. M. Anal. Biochem. 72, 248–254 (1976). (155 530 citations)

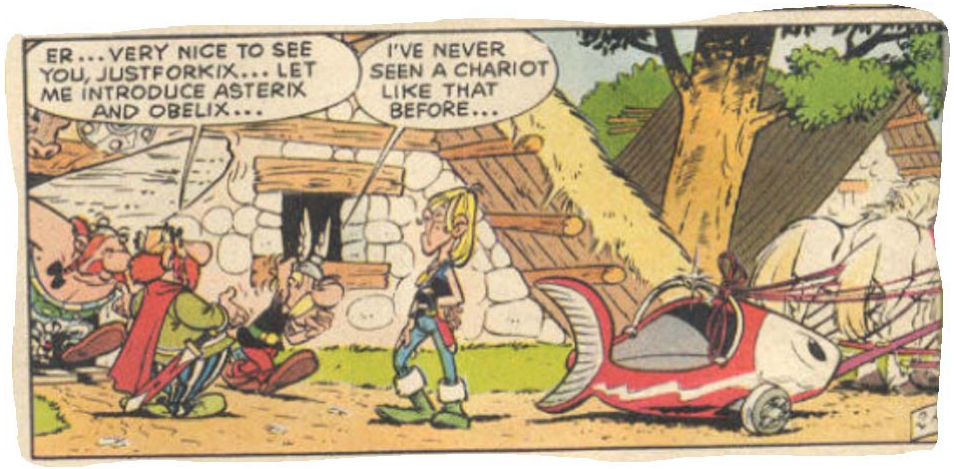
To understand a biological process we often need a better comprehension of the associated chemical envoronment

Omics or Omix

'Omic' sciences are perhaps the best example of a successful integration of chemistry and biology.

D.J. Hare and E.J. New On the outside looking in: redefining the role of analytical chemistry in the biosciences, ***Chem. Commun.,*** 2016, 52, 8918

The 'omic' revolution has been driven by advances in analytical chemistry, from DNA microarray technology to mass spectrometry.

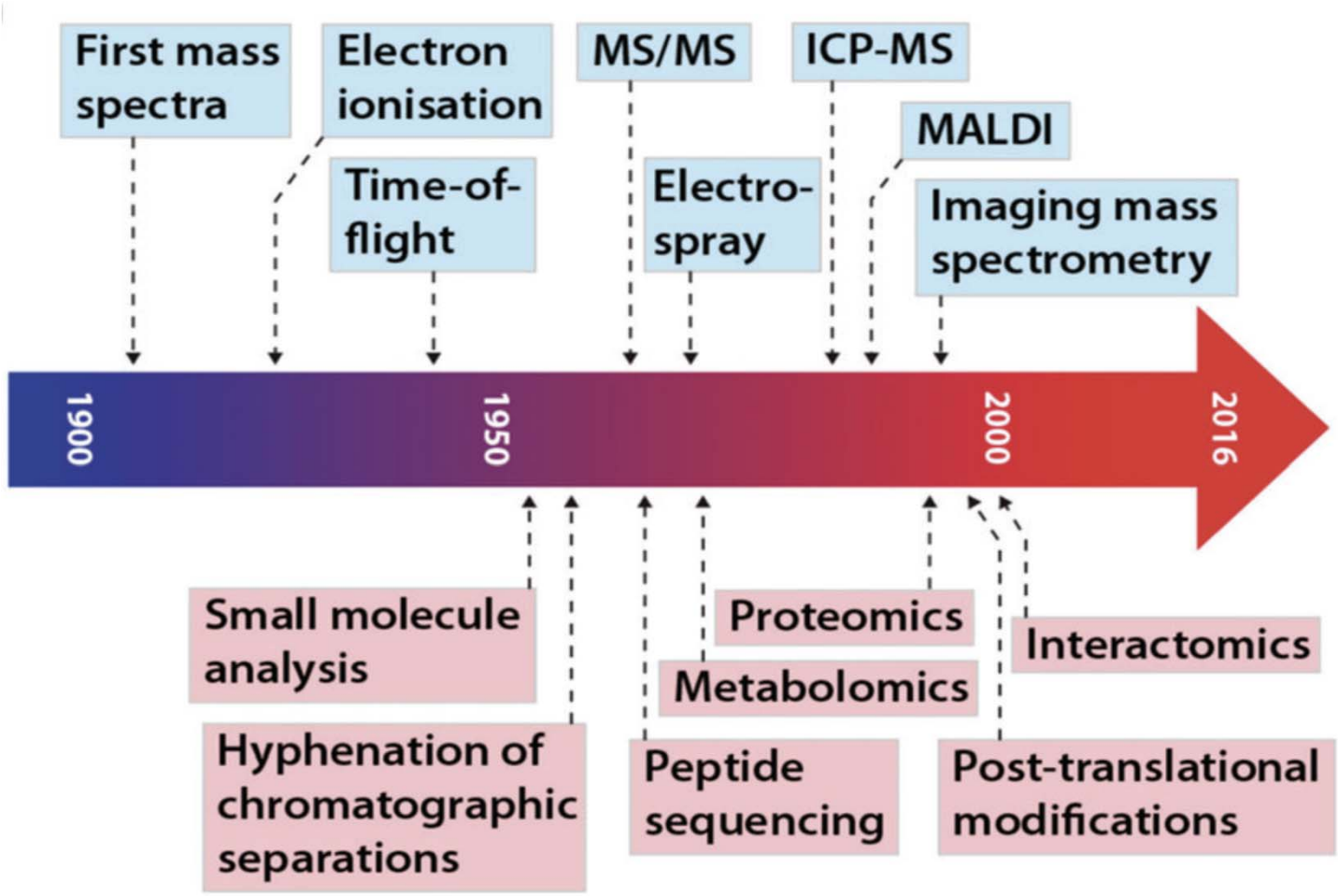


2 divergent lines of enquiry with regard to 'omic' sciences and systems biology :

(1) how can analytical chemistry be improved to better answer key biological questions?

(2) are the right questions being asked that take advantage of the tools at our disposal?

The example of Mass Spec



(1) How much ?

(2) How fast?

(3) What flexibility?

A. Doerr, J. Finkelstein, I. Jarchum, C. Goodman and B. Dekker, Nature Milestones: Mass Spectrometry, Nature Publishing Group, 2015.

Proteomics vs metalloproteomics

Metabolome: Metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes

D. Delneri, et al Curr Opin Biotechnol 12 (2001), pp. 87-91

Metabolomics: Study of chemical processes involving metabolites, ie the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind", the study of their small-molecule metabolite profiles

T.J. Phelps, Curr Op. Biotech, 13 (2002), pp 20-24

Proteome : The set of PROTeins expressed by the genOME of a cell or tissue at a given time and in a given environment.

Wilkins et al., Biotechnol Gene Eng Rev, 1995

Proteomics: Dynamic and quantitative analysis regulation of expression of the gene product that characterizes a given biological process in order to decipher the mechanisms of cellular interactions.

Anderson et Anderson, Electrophoresis, 1998

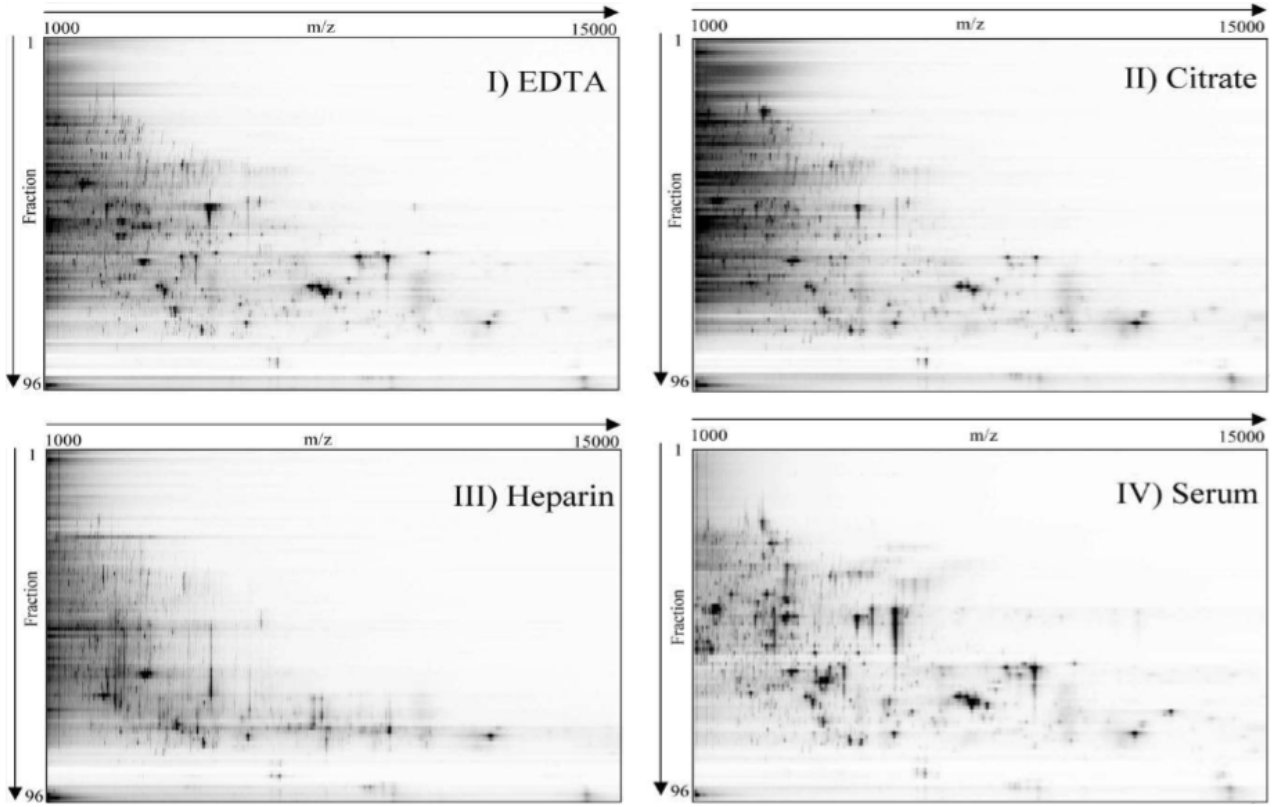
Peptidomics: Analysis and technologies for visualization, quantitation, and identification of the naturally occurring or endogenous peptides, low-molecular-weight proteome (<15 kDa), the "peptidome"

P. Schulz-Knappe et al. Comb Chem High Throughput Screen. 2001 Apr;4(2):207-17

Biological samples are complex

Example of the complexity of peptidome with peptide displays from blood specimens.

4 blood specimens: EDTA plasma, citrate plasma, heparin plasma, and serum



x-Axis : m/z

y-axis : RP-HPLC retention time

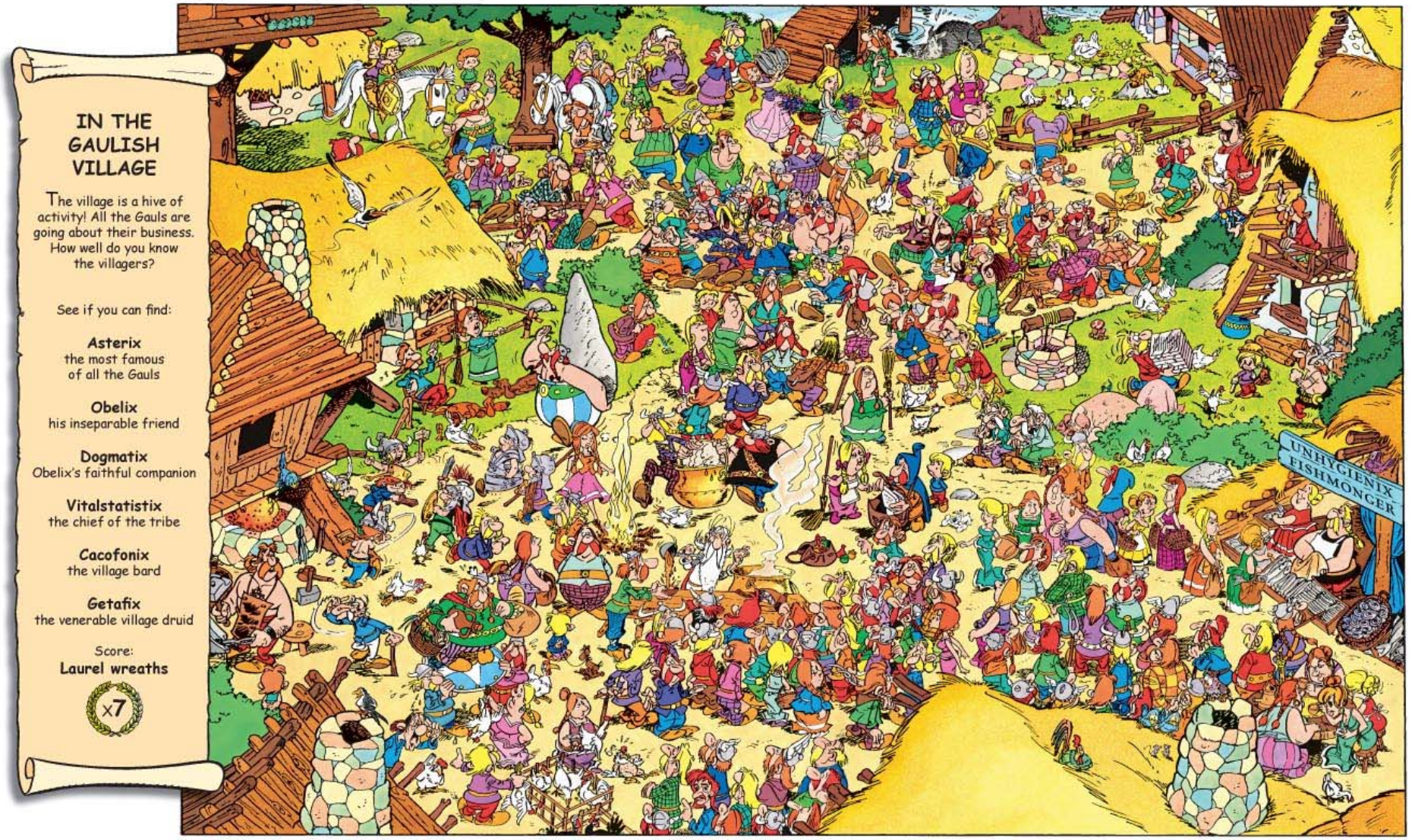
Signal intensity is depicted by color saturation.

Tammen, H., et al Peptidomic analysis of human blood specimens: Comparison between plasma specimens and serum by differential peptide display. Proteomics, 5 (2005),pp 3414–3422

3000-7000 signals are visualized per peptide display: 1500-3000 peptides

METALLOPROTEOMICS

Where is my compound?



Compounds separation

To characterize biological samples separation and purification steps are unperfect yet necessary!

*Soloviev M. **Peptidomics**: divide et impera. Methods Mol Biol. 615 (2010) :pp 3-9.*

Fractionation and separation techniques:
electrophoresis,
chromatography,
phase extraction

Spectrometry:
Mass
Optical
Atomic absorption/emission

Metallomics vs metalloproteomics

Metallome: entirety of metal and metalloid species (the inorganic species-ionome- and protein complexes-metalloproteome) in a cellular compartment, cell, or organism

R.J.P. Williams Coord. Chem. Rev., 216–217 (2001), pp. 583-595

Metallomics: Study (qualitative and/or quantitative) of the metallome, including metal-bound biomolecules

H. Haraguchi Metallomics as integrated biometal science J. Anal. At. Spectrosc., 19 (2004), pp. 5-14

Metalloproteomics: study of the metal-bound proteins, *aka* metalloproteins (structural & functional characterization, identification & quantification)

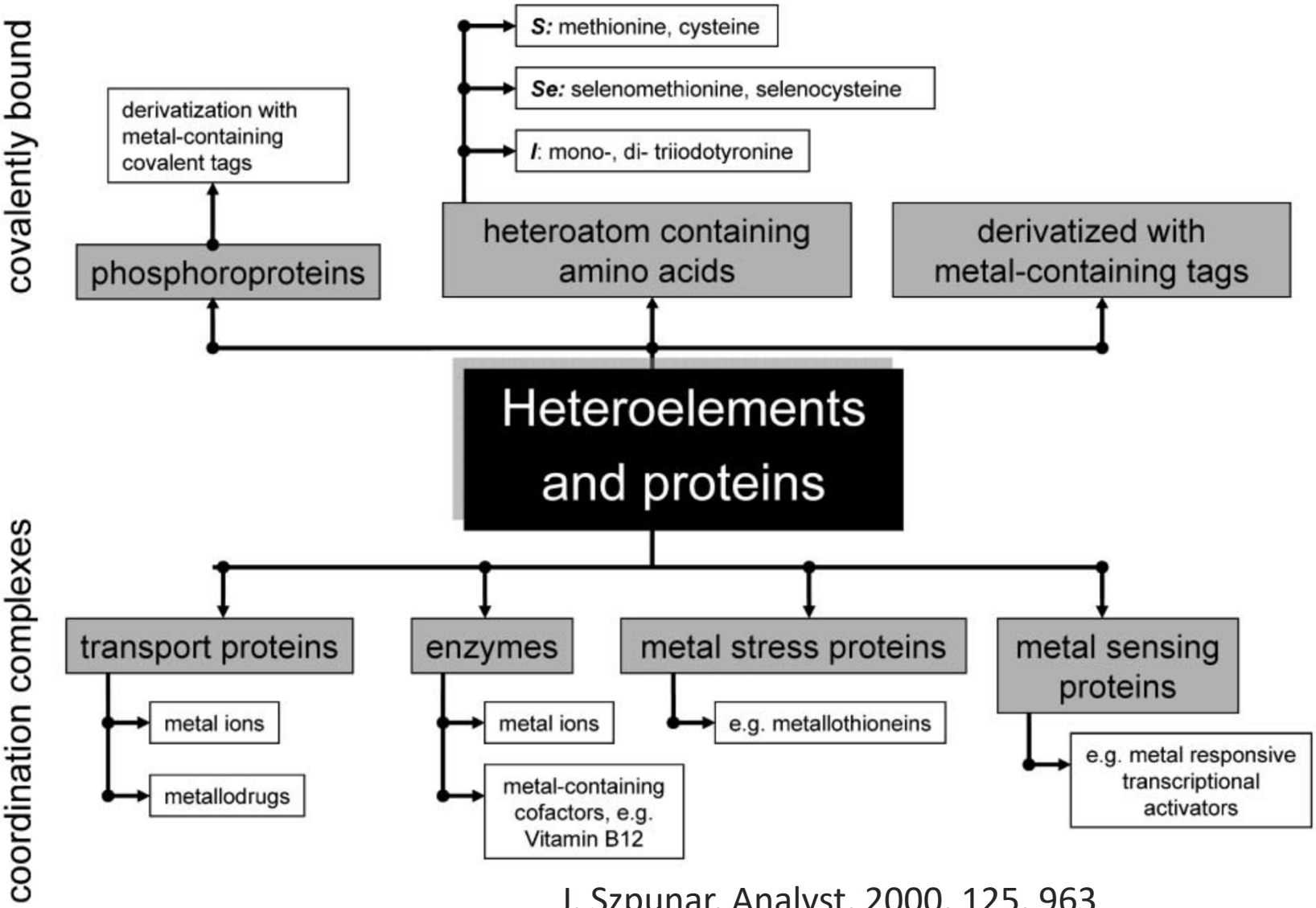
C. Andreini, *et al.* Acc. Chem. Res., 42 (2009), pp. 1471-1479
S. Mounicou, et al. Chem. Soc. Rev., 38 (2009), pp. 1119-1138;
W. Shi, M.R. Chance Cell. Mol. Life Sci., 65 (2008), pp. 3040-3048 and Curr. Opin. Chem. Biol., 15 (2011), pp. 144-148

Metalloproteomics studies

- take into account changes in the amounts expressed in organisms but also
- consider of the global role of all metals/metalloids in the biological system

D.W. Koppenaal, G.M. Hieftje J. Anal. At. Spectrosc. (2007), p. 22
R.A. Hauser-Davis, et al, Ecotox. Environ. Safety, 140 (2017), pp 279-287

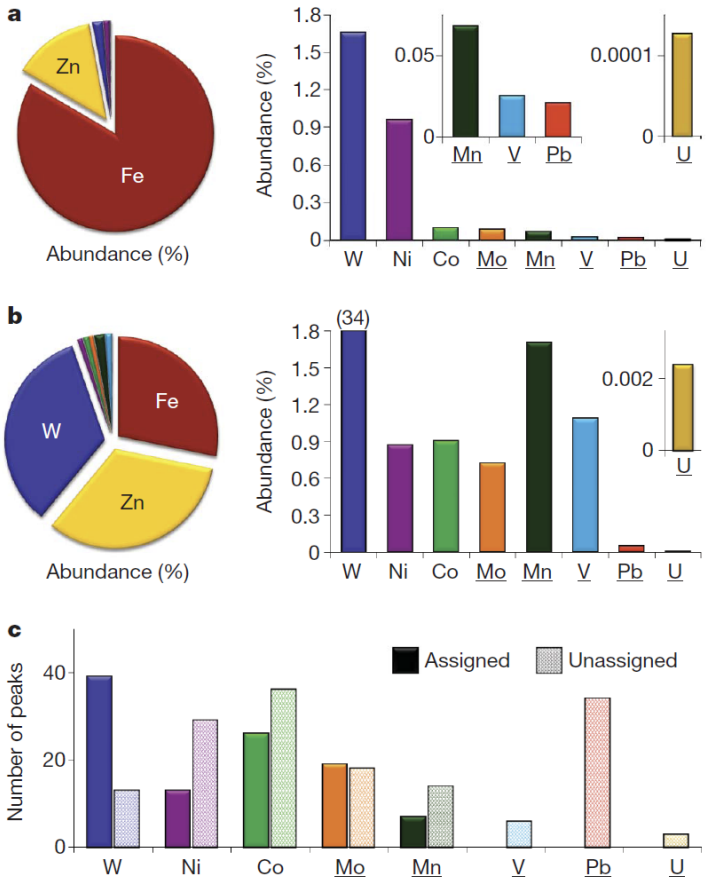
Origins of heteroatoms in proteins and their complexes



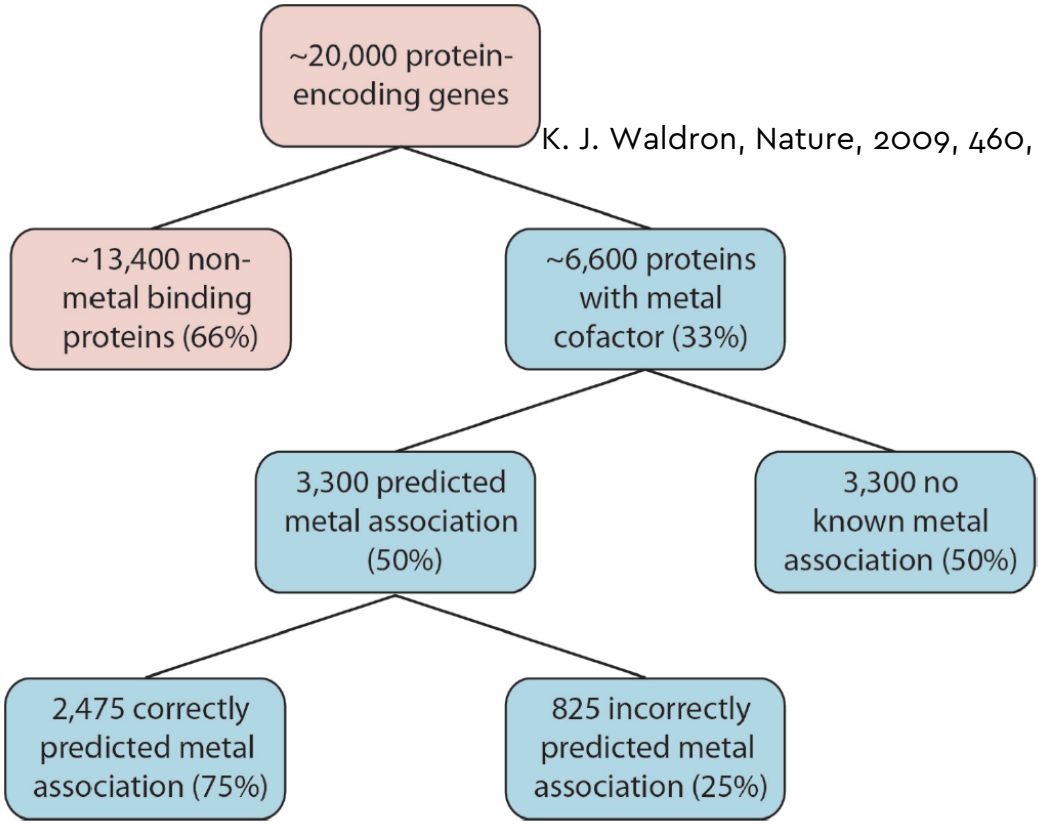
J. Szpunar, Analyst, 2000, 125, 963

Estimated size of the metalloproteome

Example: Study of assimilated metals and metalloproteins from biomass of the extremophile Archae *Pyrococcus furiosus*



A. Cvetkovic, Nature, 2010, 466, 779–782.

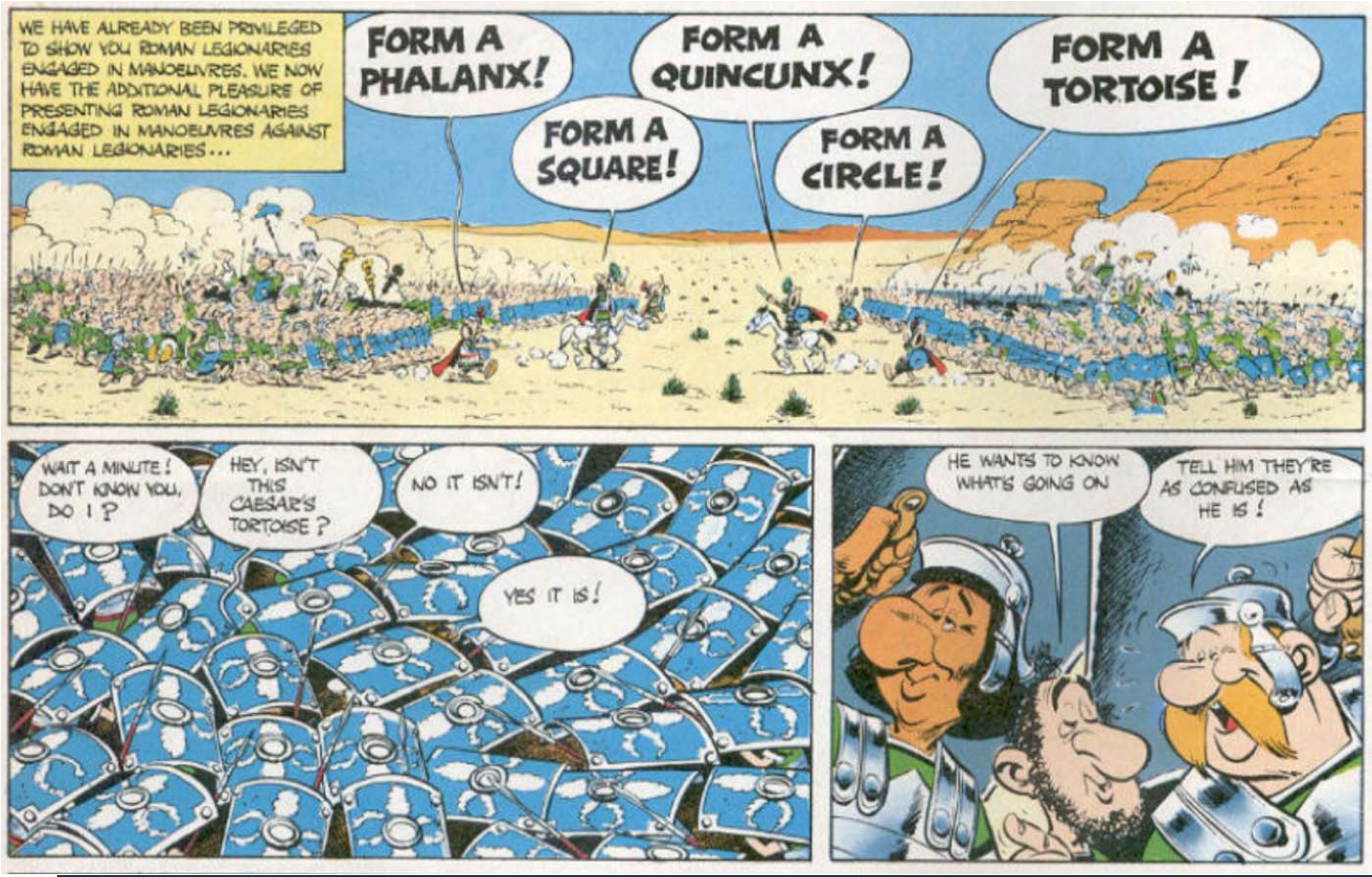


K. J. Waldron, Nature, 2009, 460, 823–830

A. Lothian et al , Front. Aging Neurosci., 2013, 5, 35.

Many metal/protein interactions are unknown or poorly characterized

Metals have a major role in protein function,
this why native metalloproteomes must be characterized.



Whatever the metal (and the form: phalanx, square, quincunx or circle) of the shield,

we need to identify who is hiding behind each fraction

Mass Spectrometry for Biology

STRUCTURAL STUDY OF RECOMBINANT/ENDOGENOUS PROTEINS

- Control the sequence and the purity of protein and peptides
- N-terminal processing and initiation methionine: formylation, acetylation, pyroglutamylation, …
- C-terminal processing : amidation, C-terminal proteolysis, determination of C-terminal sequences (e.g. with enzymatic treatments)
- Identification and localization of post-translational modifications: phosphorylation, glycosylation, polymodifications, new PTms,…
- Localization of disulfide bridges
- Sites of proteolytic or post-translational cleavages

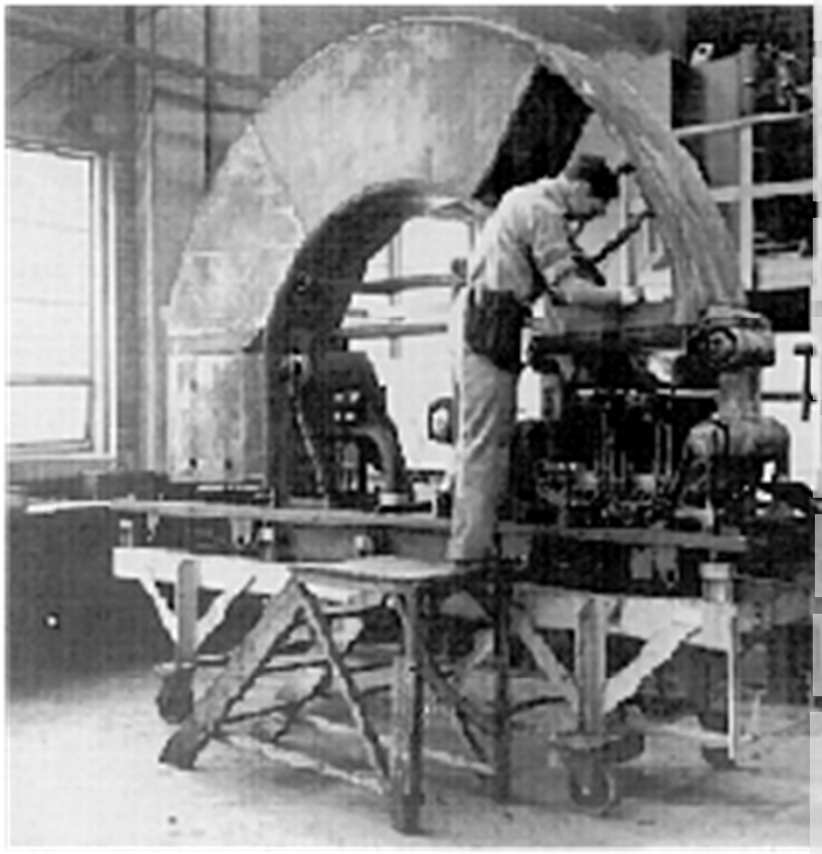
CELLULAR BIOLOGY

- Molecular imaging or nanoSIMS
- Peptide mapping on a body, or a few cells or even a single cell

OMICS:

- Proteome: a given set of PROTeins expressed by the genOME of a cell or a tissue, at a given time and in a given environment
- Peptidome, metabolome, metallome, metalloproteome

Mass Spectrometry(MS)

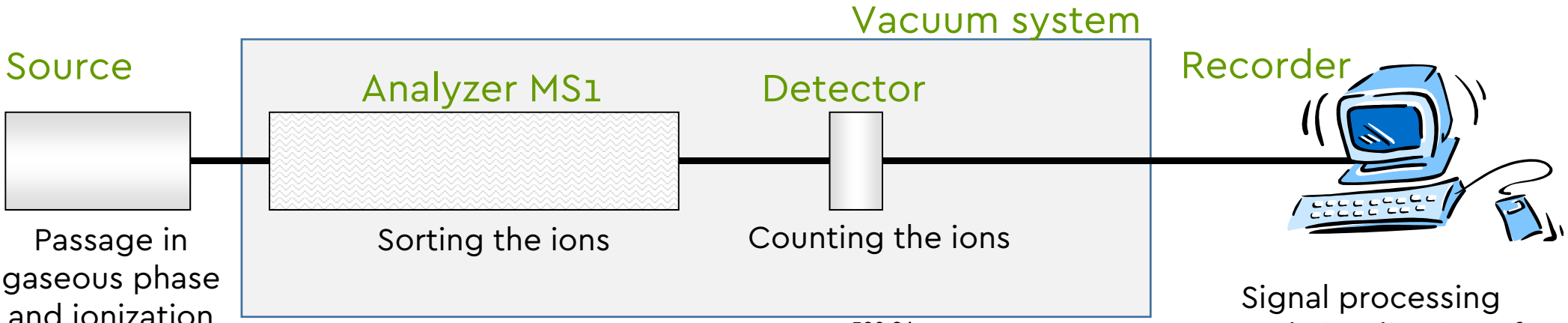


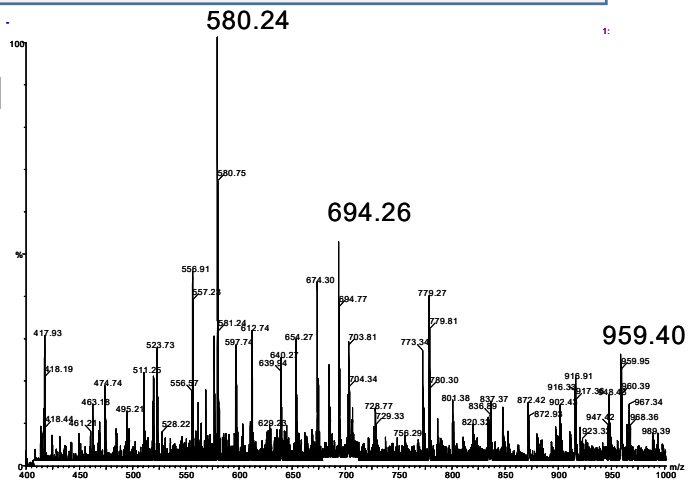
- **1897 : discovery of the electron by Thomson**
- **1912: first mass spectrometer and discovery of non radiactive isotopes**
- **2nd World War: separation of uranium**
- **1960: EI application on the fortuitine**
- **1975: first plasma source (Gray)**
- **1980: ICP MS (Houk and Gray)**
- **1988: ESI and MALDI MS**
- **2002: Nobel Prize in chemistry for Fenn (ESI) and Tanaka (MALDI)**

^ Part of the Calutron mass spectrometer first used for preparative MS
[Yergey, A.L. & Yergey, A.K. "Preparative scale mass spectrometry: A brief history of the calutron." *Journal of the Amer. Soc. for Mass Spectrometry*, 1997, V8 N9:943-953.]

General Principles of MS

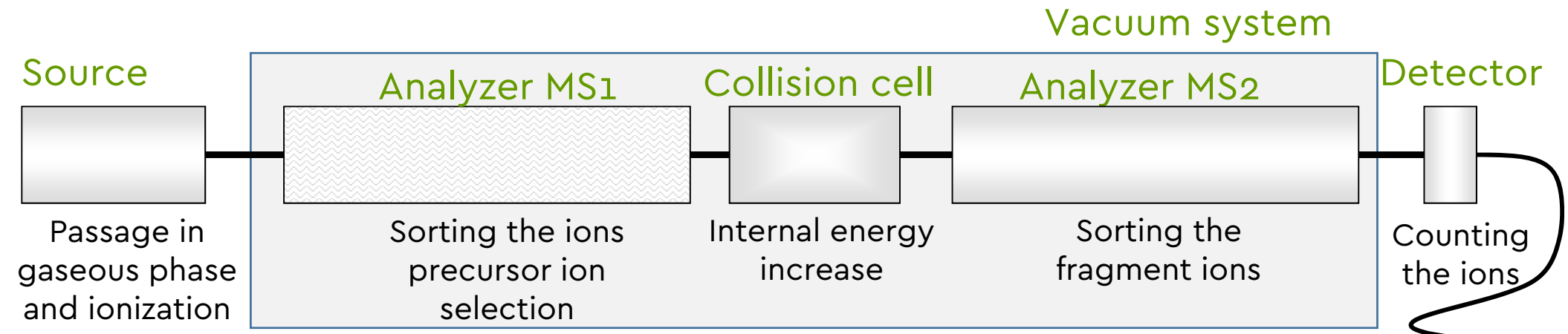
Mass spectrometry: analytical technique based on the separation of gaseous ionized molecules according to the values of the ratio of their mass/charge (m/z).

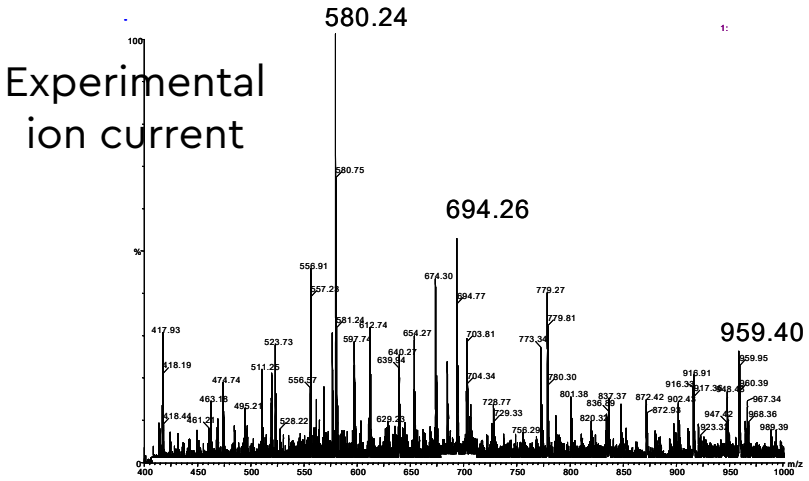


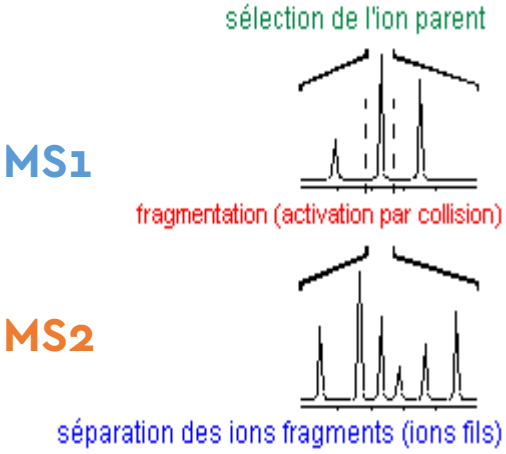


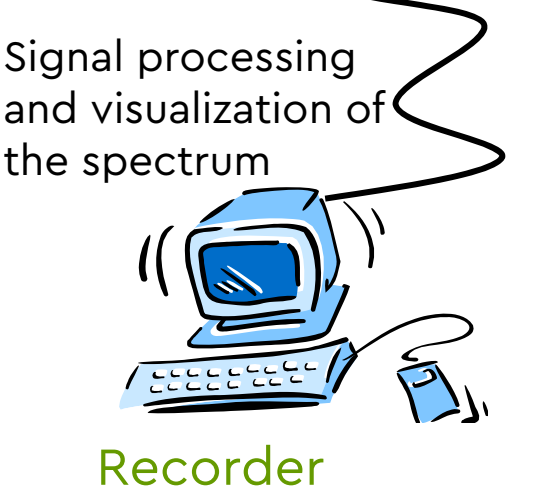
Tandem Mass Spectrometry (MS/MS)

Mass spectrometry: analytical technique based on the separation of gaseous ionized molecules according to the values of the ratio of their mass/charge (m/z).







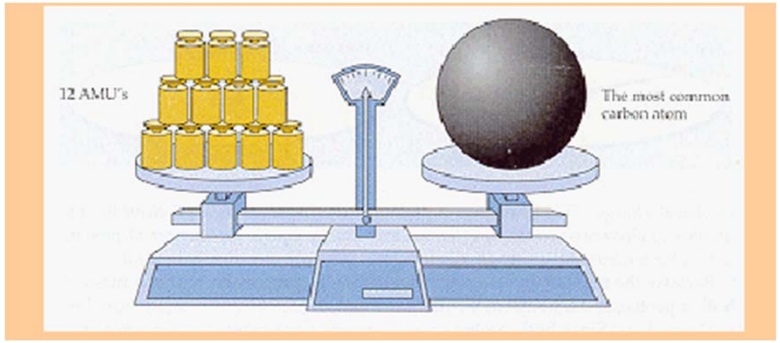


Mass units

Unit of atomic mass (u or AMU)

1/12 of the mass of the isotope carbon 12

1 amu = 1.6605×10^{-27}Kg

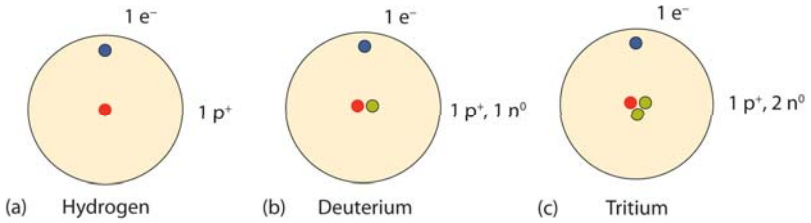


Dalton (Da)

Mass of 1 atom of hydrogen

The molecular masses of the polypeptides are often expressed in kDa.

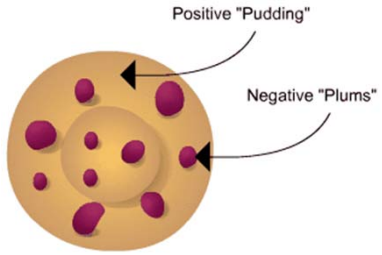
1 Da = 1,008 uma = $1.673911e^{-27}$Kg
proton : 1.672622×10^{-27}Kg=1.007 u
neutron : 1.675×10^{-27}Kg=1.009u
electron : 9.11×10^{-31}Kg=0.0005u



Thomson (Th)

unit used for reporting mass over charge ratios m/z

1 Th = 1 Da/z



Ions types

Molecular Ion

Ion produced when a molecule M introduced into the mass spectrometer loses (M +.) or wins (M-.) an electron.

The molecular ion has a mass equal to that of the neutral molecule of interest and with an odd number of electrons.

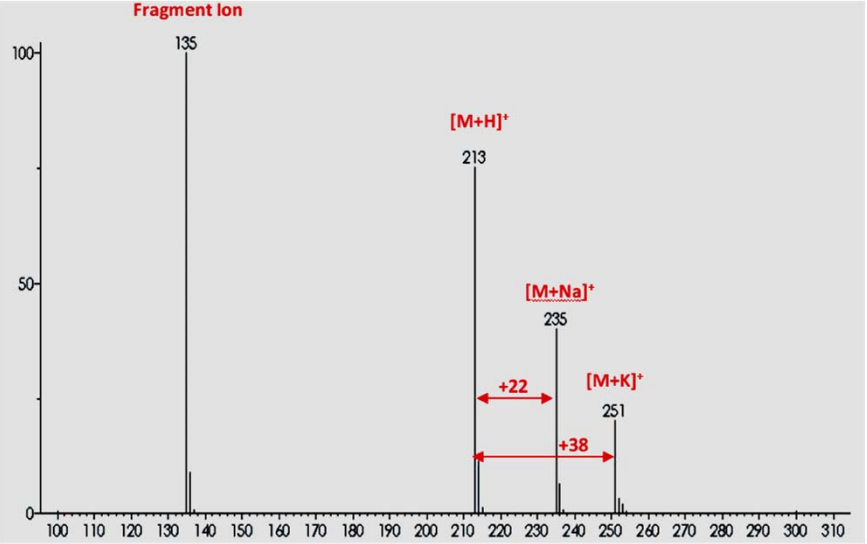
Pseudo-molecular Ion

Ion produced by loss or gain of a proton (H+) by the neutral molecule M.

These ions are of the form $[M + H)]^+$ or $[M - H]^-$.

Adduct

ion obtained by addition of a cation on the neutral molecule M (Na^+, K^+, etc.)

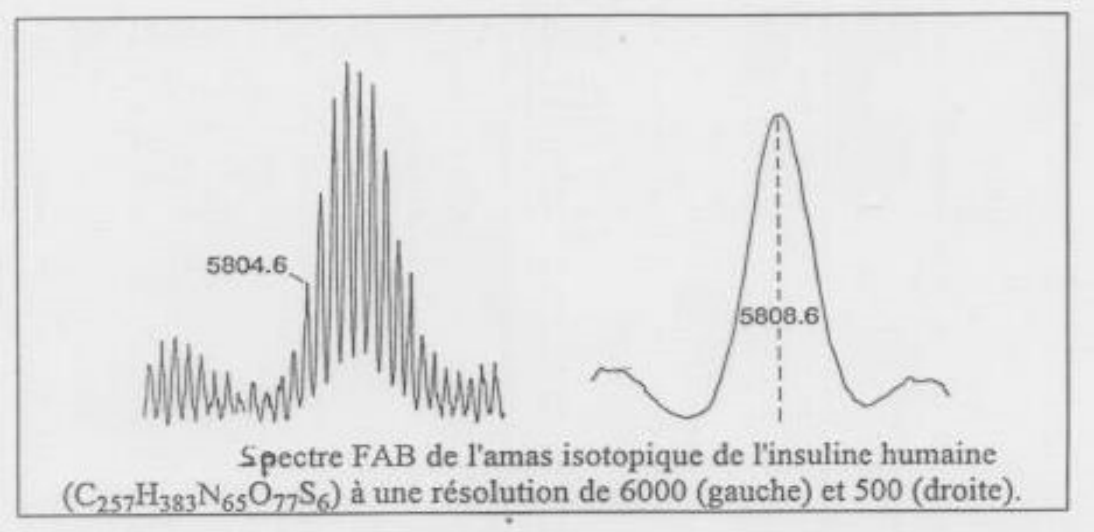


Isotopic masses

Contribution of the different isotopes used in the composition of the polypeptides:

C :	**N** :	Masses M
^{12}C : 98,9%	^{14}N : 99,7%	M+1
^{13}C : 1,1%	^{15}N : 0,3%	M+2…

- 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
- Monoisotopic mass—taking account of the atomic masses of the lighter isotopes
- Average mass—taking account of the relative abundance of natural isotopes



Spectre FAB de l'amas isotopique de l'insuline humaine ($C_{257}H_{383}N_{65}O_{77}S_6$) à une résolution de 6000 (gauche) et 500 (droite).

Relative abundance of monoisotopic masses M, M+1... for a model molecule containing n carbon atoms.

Respective abundances given by the development of the binomial theorem:

$$(0{,}99\ {}^{12}C + 0{,}01\ {}^{13}C)^{n} = \sum_{p=0}^{n} C_n^p\ (0{,}99)^{n-p}\ (0{,}01)^{p}\ [({}^{12}C)^{n-p}({}^{13}C)^{p}]$$

Avec $C_n^p = \dfrac{n!}{(n-p)!p!}$

When n increases, the molecules containing 1, 2, 3... ^{13}C atoms (molecules of masses M + 1, M + 2, M + 3) become majority.

Ex: at 1 kDa the most abundant form of a peptide of is the monoisotopic mass M
at 2,5 kDa, the most abundant form is M + 1
at 3.5 kDa, the most abundant form is M + 2 ...

Exact mass vs. accurate mass

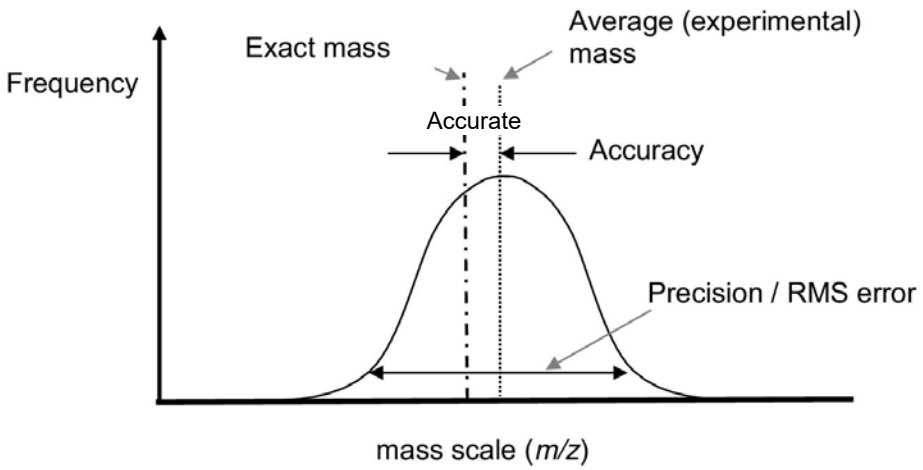
Exact mass—is the calculated mass of an ion (theoretical mass)

- whose elemental formula, isotopic composition and charge state are known.
- using one isotope of each atom involved, usually the lightest isotope (IUPAC definition)

The charge state is relevant as the mass of the electron (0.00055 Da), or multiple charges, may not be negligible in the context of exact mass measurement

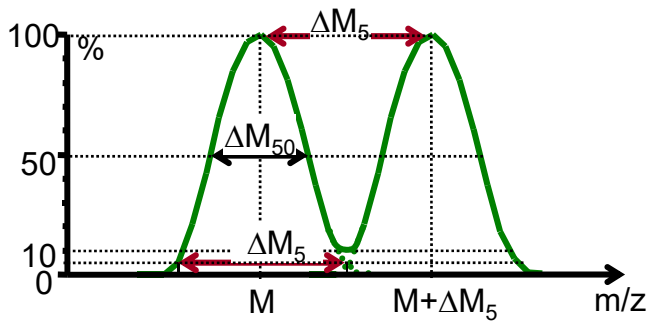
Accurate mass—the experimentally determined mass of an ion

- measured to an appropriate degree of accuracy and precision
- used to determine, or limit the possibilities for, the elemental formula of the ion

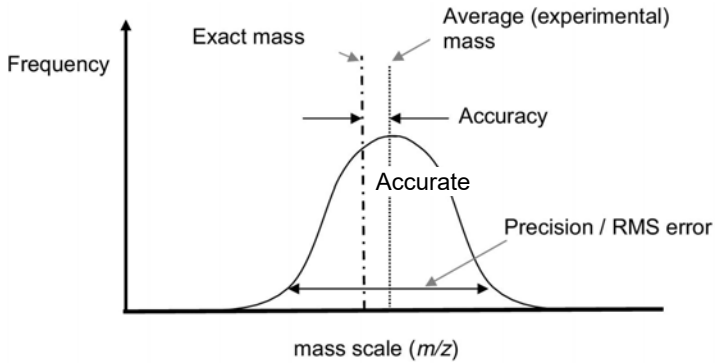


Key parameters of the analyzer

- **Resolution** : It reflects the smallest measurable difference in mass ΔM to a given mass. It is usually expressed by the M/ ΔM report.



- **Mass range**: interval of mass where the analysis is possible, i.e. where the ions are actually transmitted and sorted from the source to the detector.



- **Detectability (sensitivity)**: minimum amount of an analyte that it is possible to detect.

- **Fidelity/precision and mass accuracy**

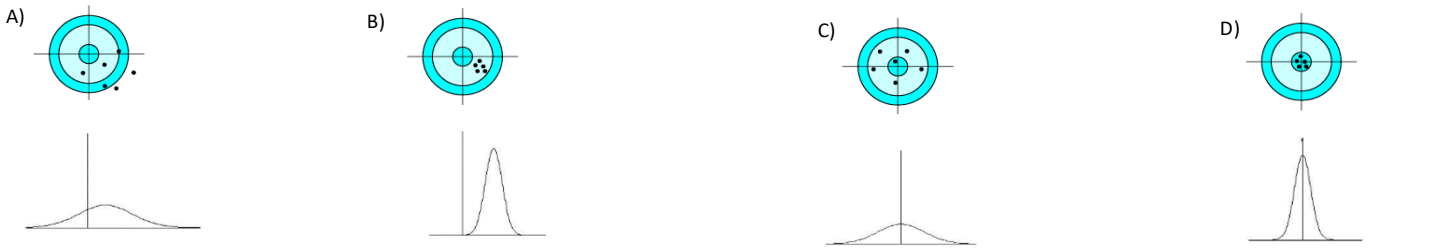
How reliable is your MS?

Accuracy—the proximity of the experimental measurement to the true value (exact mass). A measurement close to the true value is accurate and if not is inaccurate. Normally, *mass measurement error* would be used to describe the accuracy of a single reading.

Precision—the repeatability of the measurement reflecting random errors. When a set of mass measurements of one ion species lie close together, the measurements are precise, and if not the measurements are imprecise.

Repeatability—the short-term precision of multiple replicate experimental measurements made under similar conditions, i.e., the same instrument, operator and over a limited time, normally the same day.

Reproducibility—refers to differences among experimental measurements made under different circumstances i.e., a measurement of the same quantity made by different operators, even different instruments and often with a significant time difference between groups of measurements.



Inaccurate/imprecise | Inaccurate, precise | Accurate, imprecise | Accurate, precise

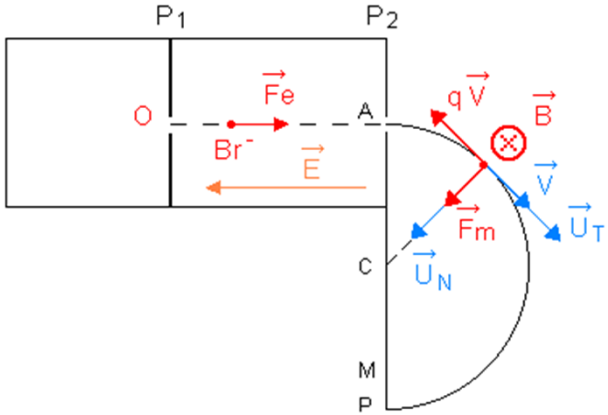
Settings key parameters in MS

- Measurement accuracy
- Resolution
- Mass range
- Limit of detection (sensitivity)
- Dynamic range
- Mode of ionization
- Analysis throughput

- Several types of instruments:
  - Ionization mode,
  - analyzer…

Basic equations of mass spectrometry

$$\frac{1}{2}mv^2 = zV$$ Ion's kinetic E function of accelerating voltage (V) and charge (z).



$F = mv^2 / R$ Centrifugal force

$F = Bzv$ Applied magnetic field

$mv^2 / R = Bzv$ balance as ion goes through flight tube

Combine equations to obtain:

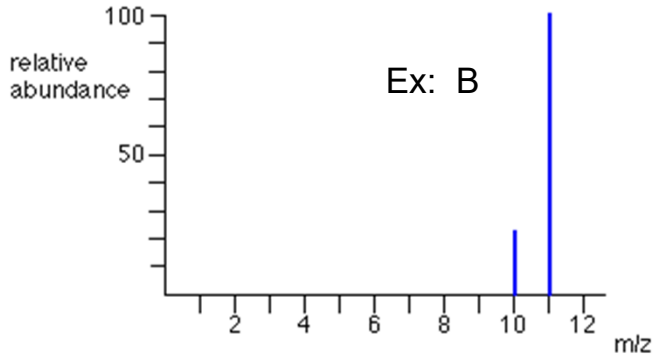
$m / z = B^2 R^2 / 2V$ Fundamental equation of mass spectrometry

Change 'mass-to-charge' (m/z) ratio by changing V or changing B.

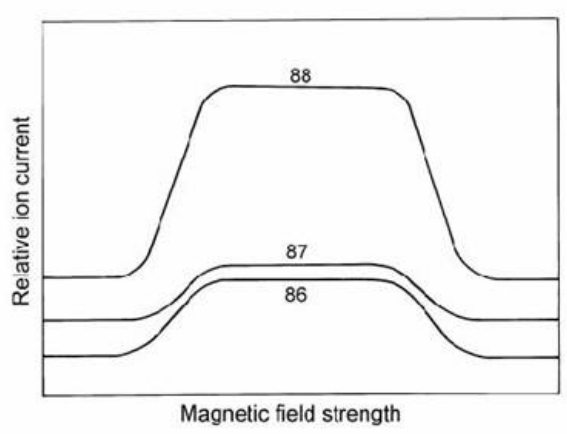
NOTE: if B, V, z constant, then:

$$r \propto \sqrt{m}$$

Electromagnetic sector analyzer

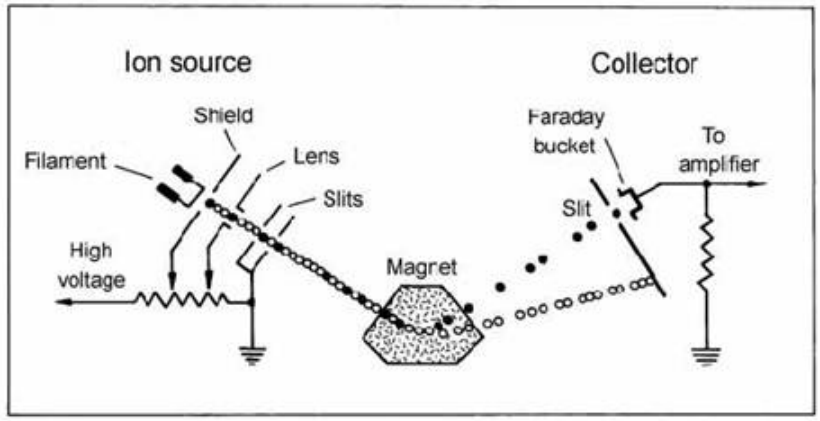


You can scan in B or V to sweep masses across a single detector.



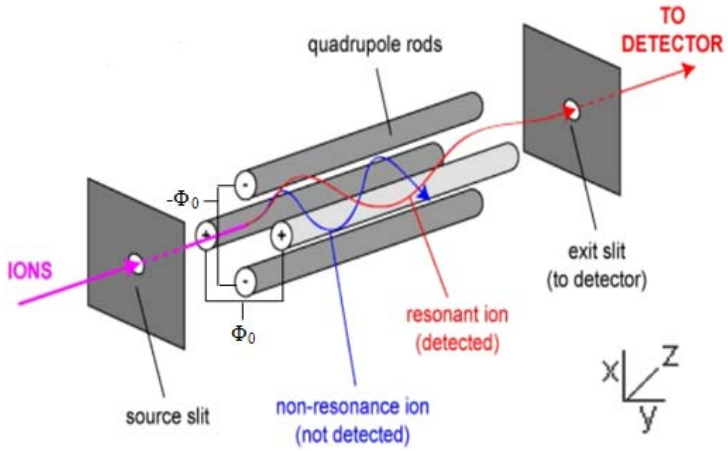
OR

You can put different masses into multiple cups without changing B or V.



<u>Magnetic Sector:</u>
Changes B and V to focus a given m/z into detector.
PRO: turn in geometry means
less 'dark noise',
higher precision & resolution

Quadrupole principle

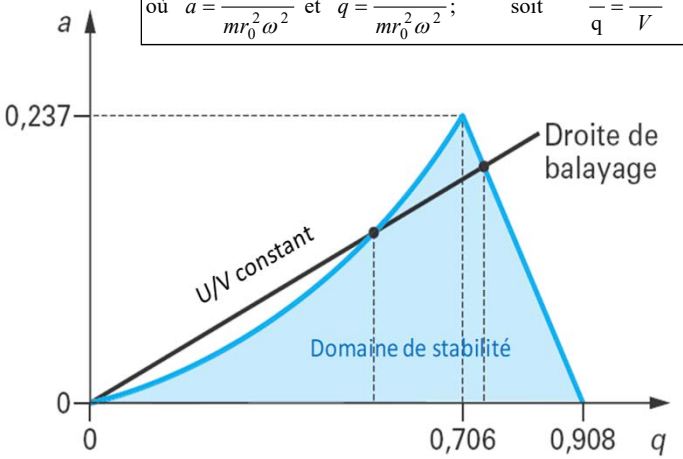


Pour une distance $2r_0$ séparant deux barres diamétralement opposées :

$$\frac{\partial^2 x}{\partial\left(\omega t/2\right)^2} + \left[a + 2q\cos\left(2\left(\omega t/2\right)x\right)\right] = 0$$

$$\frac{\partial^2 y}{\partial\left(\omega t/2\right)^2} - \left[a + 2q\cos\left(2\left(\omega t/2\right)y\right)\right] = 0$$

où $a = \frac{8zeU}{mr_0^2\omega^2}$ et $q = \frac{4zeV}{mr_0^2\omega^2}$; soit $\frac{a}{q} = \frac{2U}{V}$



Quadrupole:
Changes DC and RF voltages to isolate a given m/z ion.
PRO: cheap, fast, easy

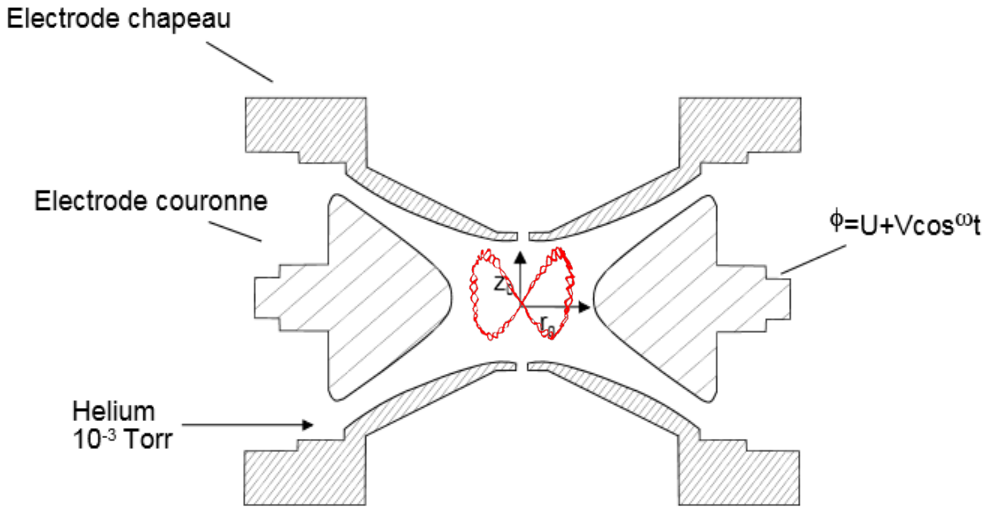
Voltage applied on opposite bars:

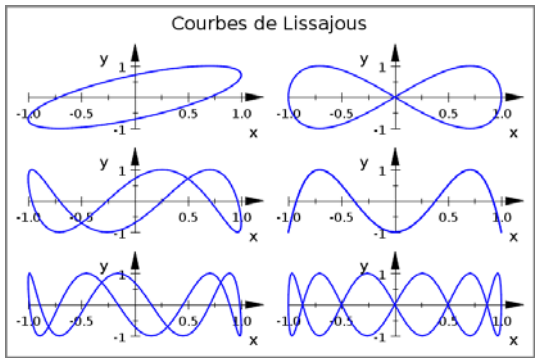
U+Vcos(ωt) and –(U+Vcos(ωt)) (ω : RF env 10^8 Hz)

For (U,V, ω), only ions of one given m/z can go through the whole quadrupole towards the detector

Scanning U and V (U/V and ω constants) ω (U and V constant) to detect a whole m/z range

3D ion trap

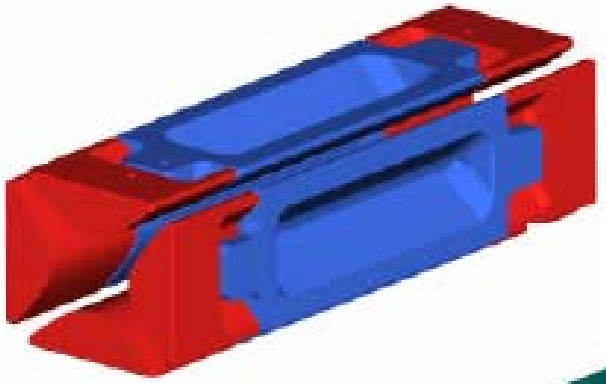


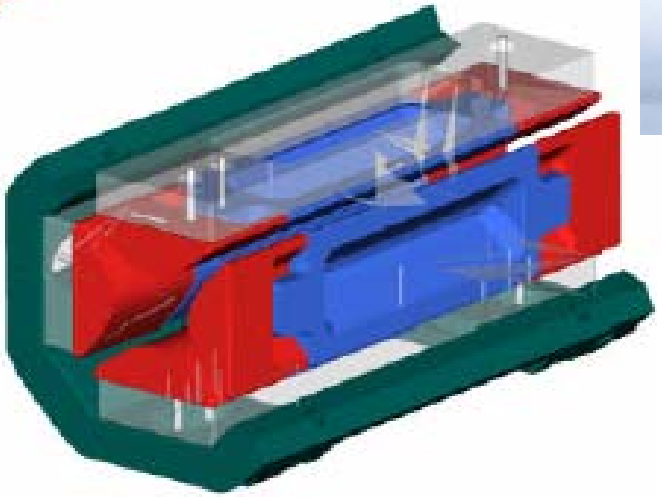


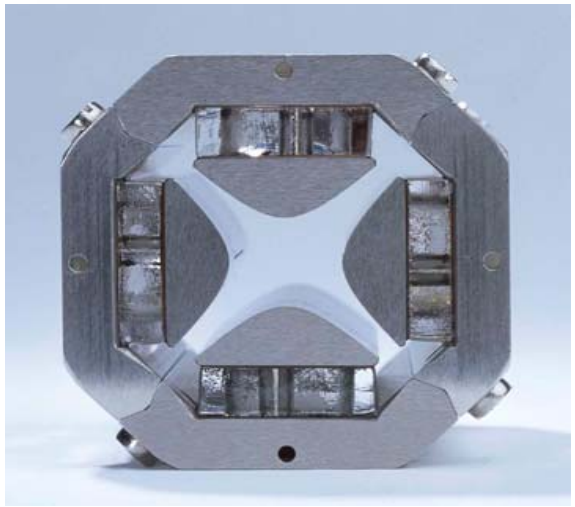
Ions trapping by applying a RF signal (MHz).
Trajectory = Lissajous curve, frequencies $f(m/z)_0$. The m/z of trapped ions is a function of V_o, RF magnitude.
To expulse ions of increasing m/z, we ramp sequentially the magnitude V of RF signal.

Wolfgang Paul, Nobel Prize in Physics in 1989

2D or linear ion trap



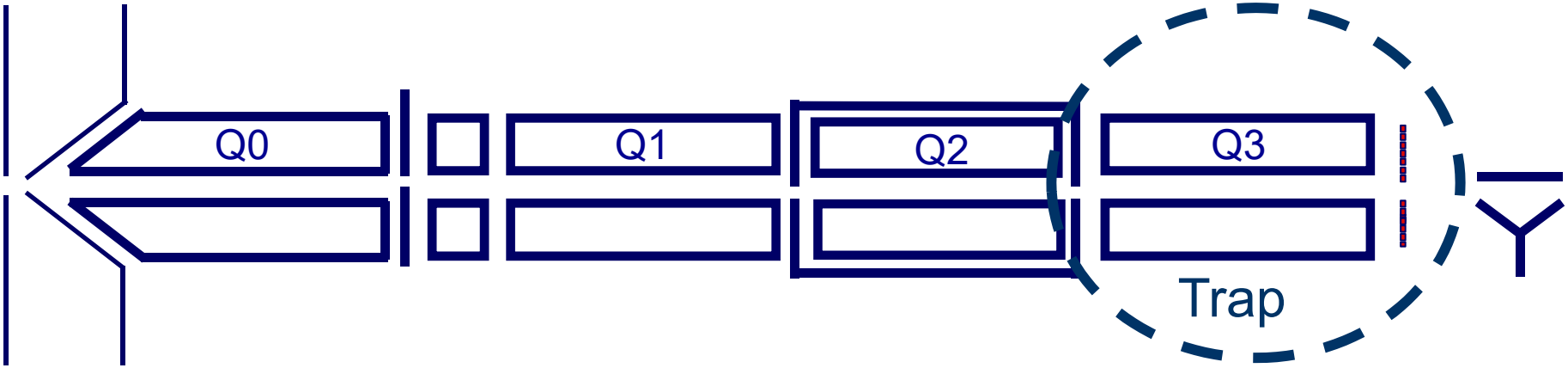


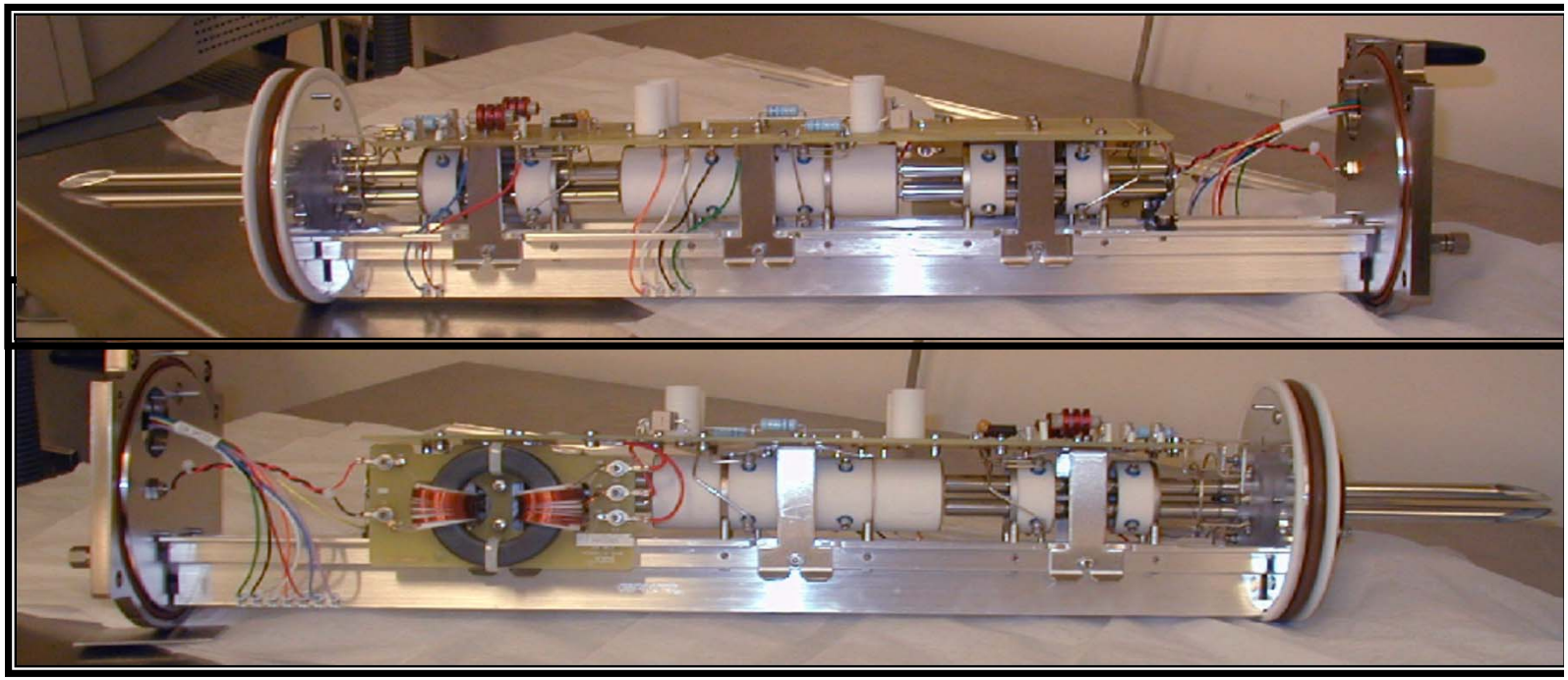


Increase of trapping capacity
Increase of trapping efficiency

Opening to other acquisition modes (triple quad, hybrids instruments…)

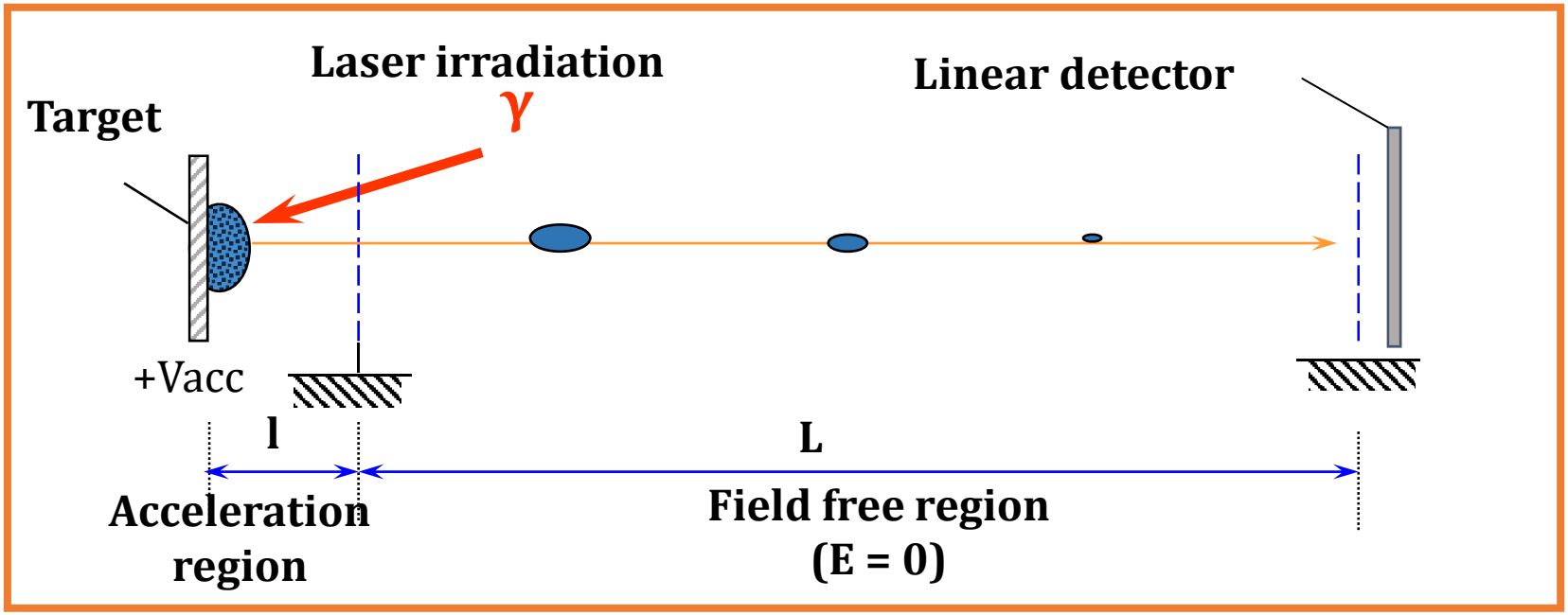
Triple quadrupole and QTrap





Time of Flight analyzer or TOF

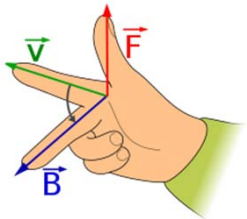
Linear mode

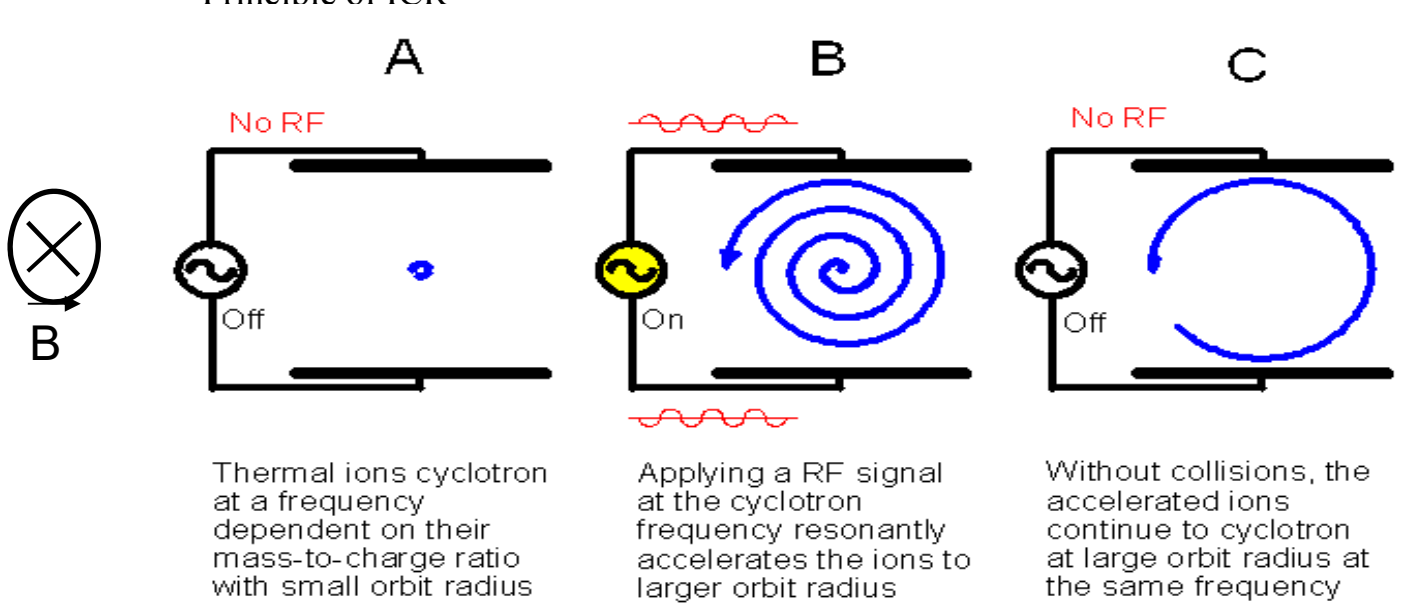


Kinetics: $$E_c = \frac{1}{2}mv^2 = qV = zeV$$

so v_{ion} pis a linear function of $\sqrt{\frac{z}{m}}$ and t_{vol} is proportional to $\sqrt{\frac{m}{z}}$

FTICR : Fourier Transform Ion Cyclotron



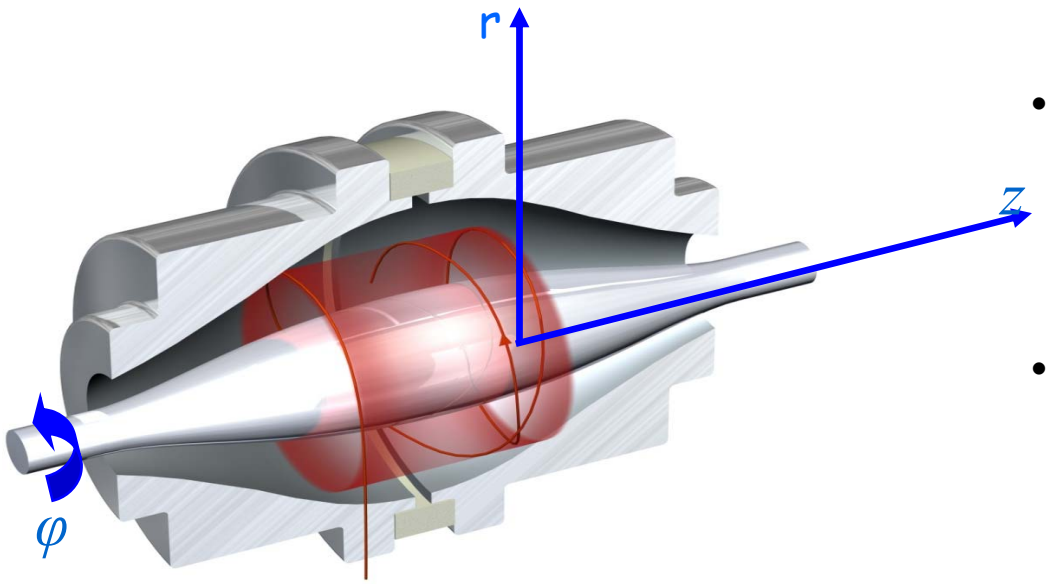


$$F = zevB = \frac{mv^2}{R} \qquad w = \frac{zeB}{m}$$

The ions are alternatively attracted towards the 2 electrodes → their oscillation induces an alternative movement of electrons in the electric cicuit = detected image current

PRO : excellent linearity between the experimental cyclotron frequency and the ratio m/z of the ion
CON : requires a uniform and constant B magnetic field ! (supraconductive cryo-magnets as NMR)

Orbitrap



$$U(r,z) = \frac{k}{2} \cdot \left\{ z^2 - r^2/2 + R_m^2 \cdot \ln(r/R_m) \right\}$$

- Characteristics :
  - Frequency of rotation ωφ

$$\omega_\varphi = \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1}$$

  - Frequency of radial oscillation ωr

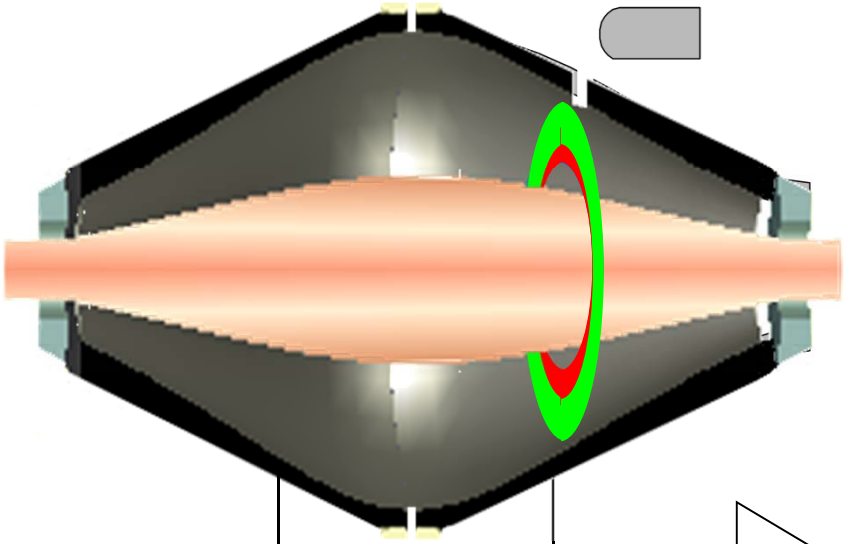
$$\omega_r = \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

  - Frequency of axial oscillation ωz

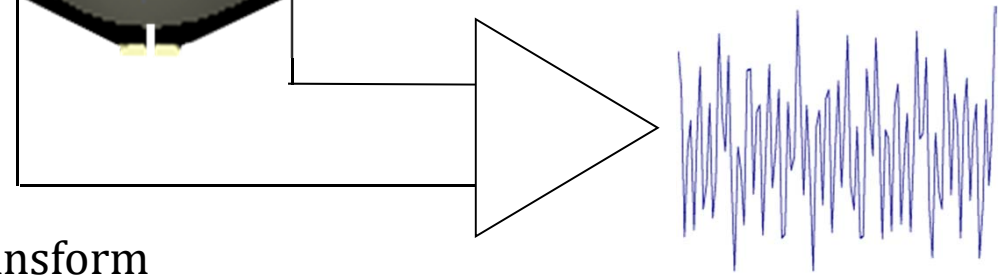
$$\omega_z = \sqrt{\frac{k}{m/q}}$$

A.A. Makarov, *Anal. Chem. (2000), 72(6), 1156-1162*

Ions rings trapping



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



1. Frequency are calculated by Fourier Transform
2. For an optimal sensitivity and resolution, damping of transients should be slow

vacuum precision

A few comparisons

	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	+	+	+	+		+++

Orbitrap

Excellent
Very high
Good
Medium
OK
OK
OK
Seq. MS/MS

+++
++
+++
+

B. Domon; *Science* (2006) 312:212-217

Ionization modes for biology

Type 1: atomic ionization for elemental analysis

ICP or Inductively Coupled Plasma

LA-ICP or Laser-assisted Inductively Coupled Plasma

Type 2: soft ionization of element species for structural analysis

ESI or ElectroSpray Ionization

MALDI or Matrix-Assisted Laser Desorption/Ionization

Some of the main characters in MS stories

Plasma

Gas in which a significant number of atoms are ionized (significant being >1%) that will interact with a magnetic field. Inductive coupling varying between field and the plasma .

Photon

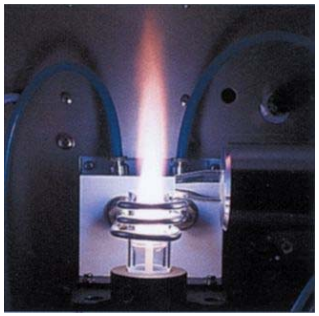
a particle representing a quantum of light or other electromagnetic radiation. A photon carries energy proportional to the radiation frequency but has zero rest mass

Electron

an elementary particle that is a fundamental constituent of matter, having a negative charge of 1.602×10^{-19}C, a mass of 9.108×10^{-31}kg, and spin of ½, and existing independently or as the component outside the nucleus of an atom

Inductively Coupled Plasma ICP

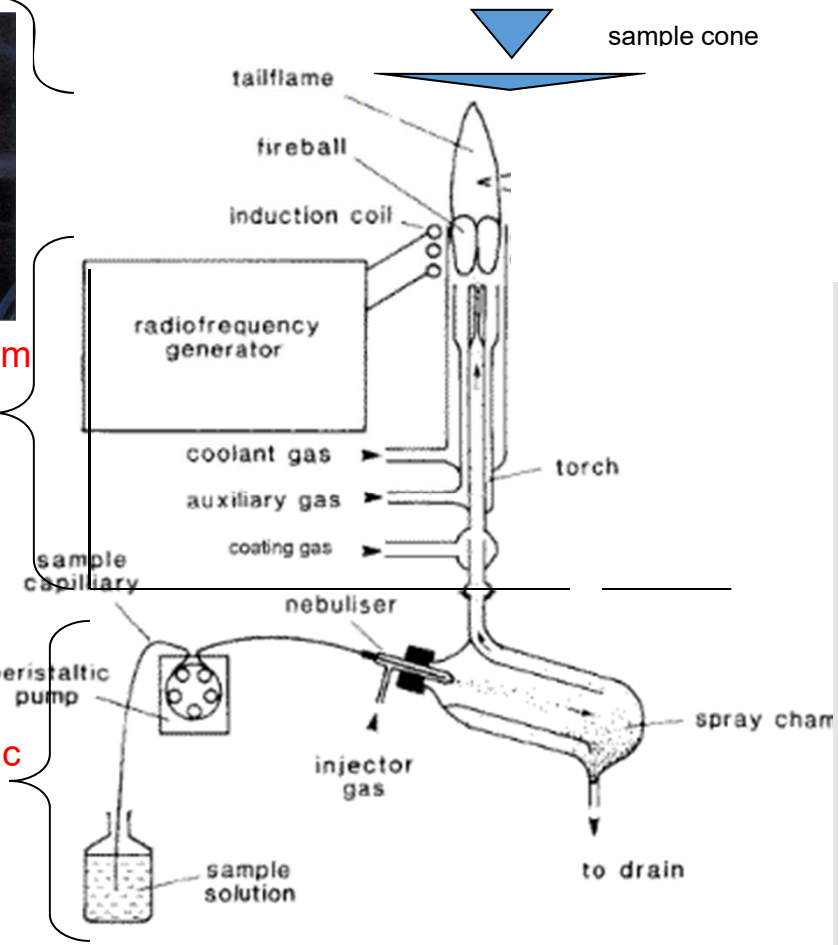
high vacuum
10^{-7} bar



"fore" vacuum
10^{-4} bar

atmospheric pressure

Shared components of all ICPMS machines



- Multi-element analysis technique
- Dissociates a sample into its constituent atoms and ions
- Excites them to a higher energy level
- Emit light at a characteristic wavelength (AES)

1. The sample is nebulized and entrained in the flow of plasma support gas, which is typically Ar.
2. The plasma torch consists of concentric quartz tubes.
3. The inner tube contains the sample aerosol and Ar support gas and the outer tube contains flowing gas to keep the tubes cool.
4. A Radiofrequency (RF) generator produces an oscillating current in an induction coil that wraps around the tubes.
5. The induction coil creates an oscillating magnetic field, which produces an oscillating magnetic field. The magnetic field in turn sets up an oscillating current in the ions and electrons of the support gas (argon).
6. As the ions and electrons collide with other atoms in the support gas, temp increases.

Elements analyzing using ICP

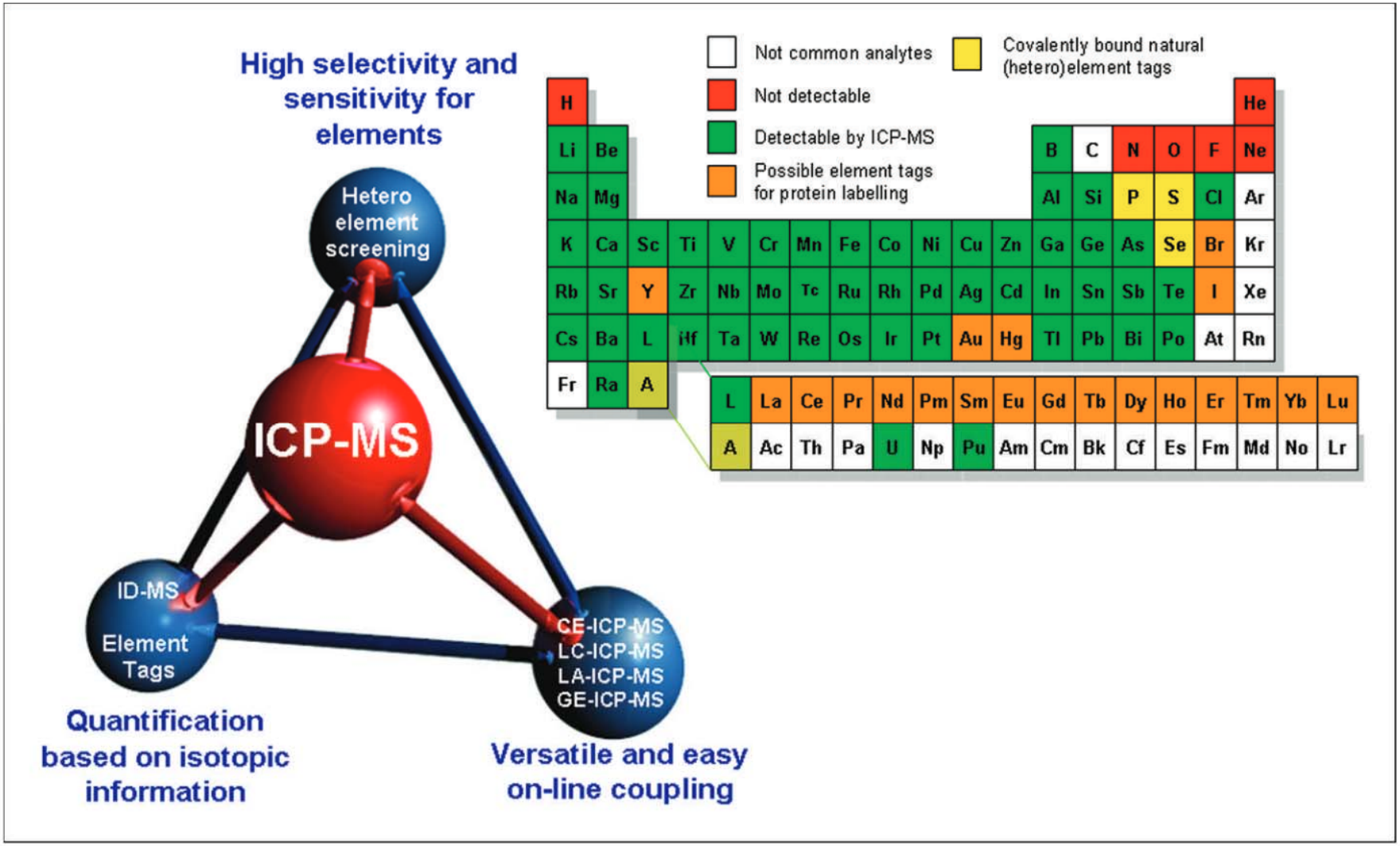


Illustration of the specific features of ICP-MS as a (hetero)element-specific detector.

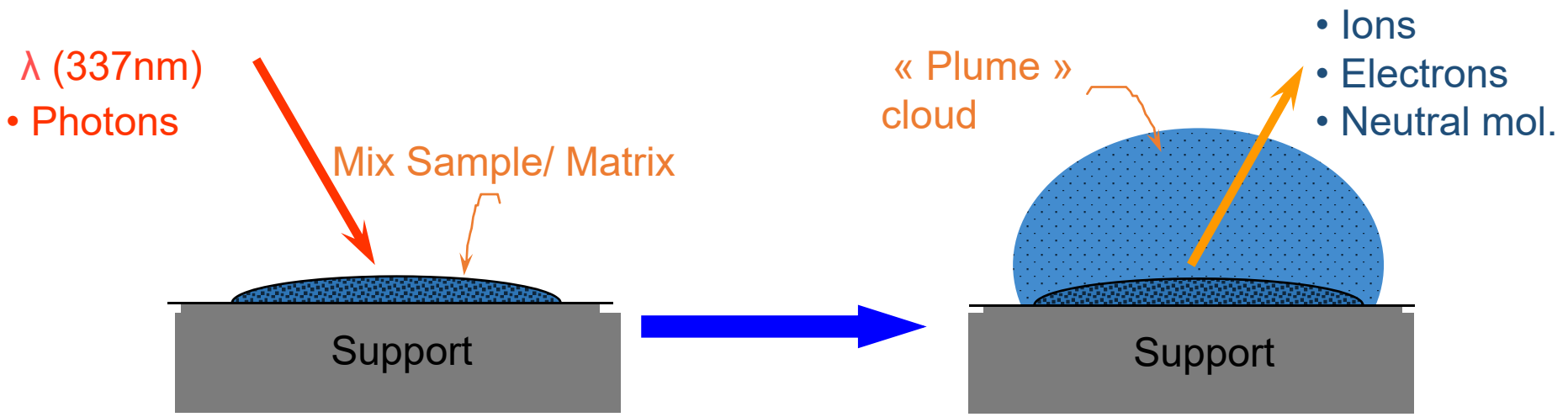
Pröfrock D, Prange A. Appl Spectrosc. 2012 Aug;66(8):843-68.

MALDI : Matrix Assisted Laser Desorption Ionisation (1988)

Principle: transform molecules in solid phase into ions in gaz phase

Incident Particule (Primary Emission)

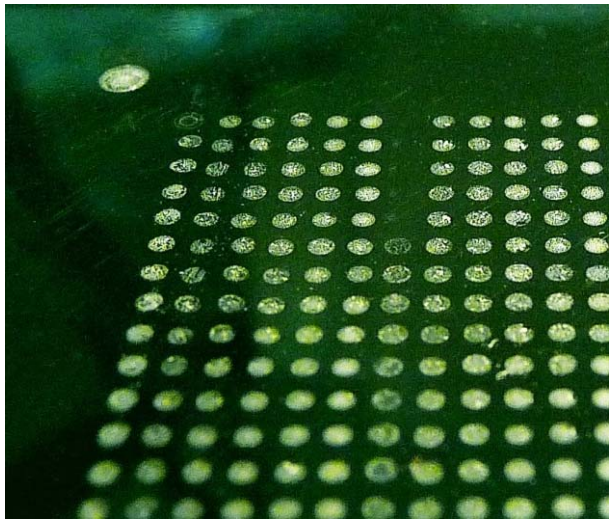
Emitted Particule (Secondry Emission)

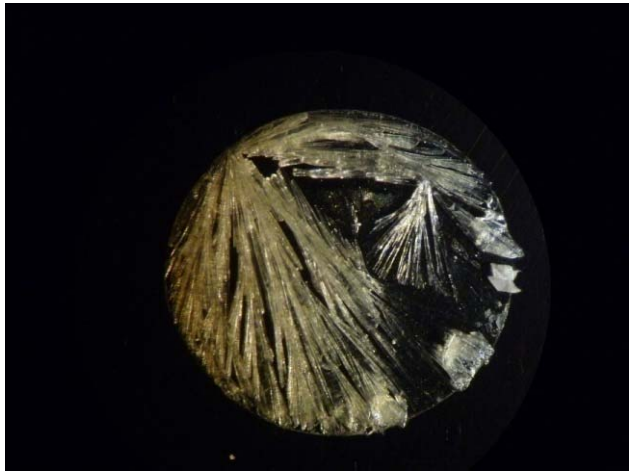


Karas and Hillenkamp Anal.Chem. (1988), 60, 2299

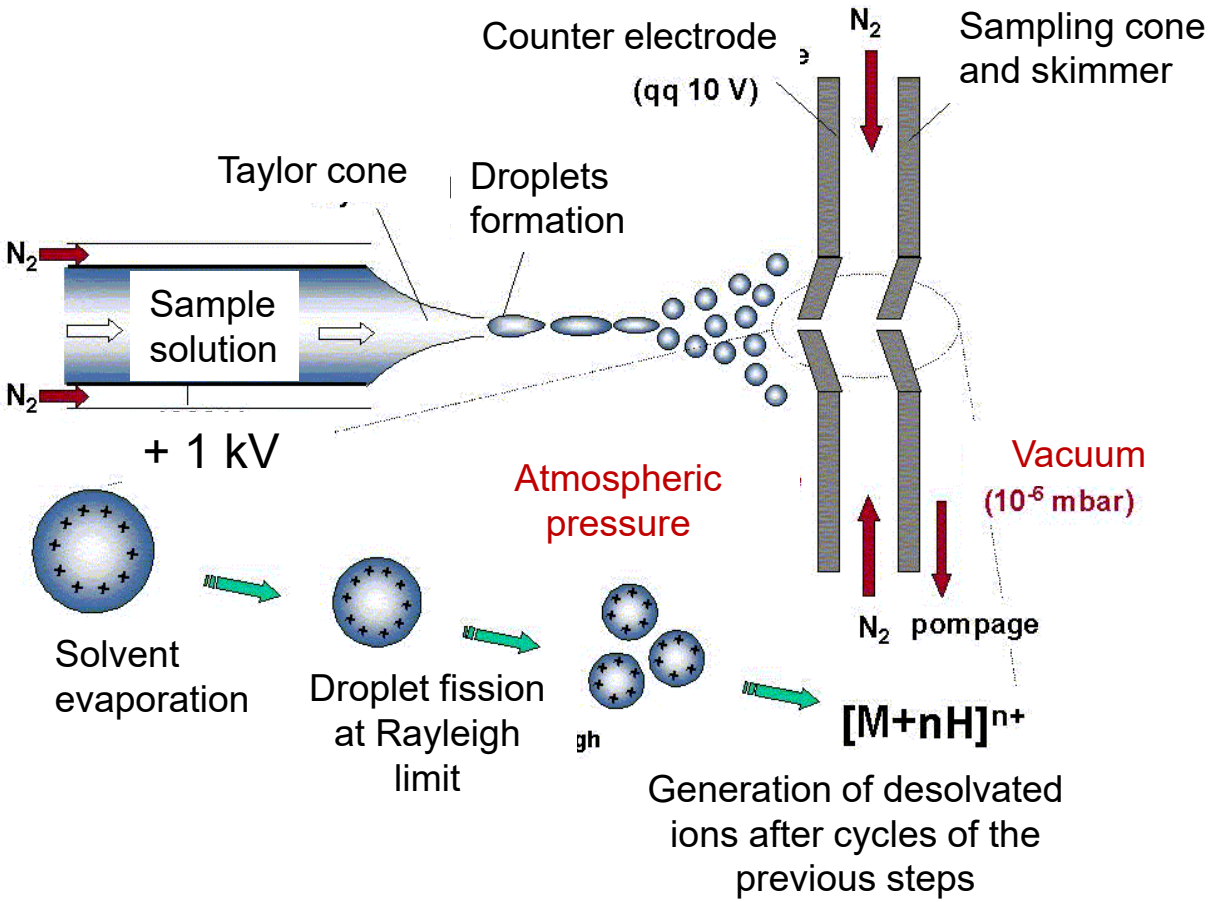
- Absorption of photons by matrix
- Relaxation of internal energy into roto-vibrational energy
- Dissociation of H bonds inter (intra) molecules
- Release of H^+ and Cat^+ , formation of « plume » cloud
- Collisions and ions/molecules reactions -> protons transfert => **$M+H^+$, $M+Cat^+$**

MALDI : Matrix Assisted Laser Desorption Ionisation (1988)

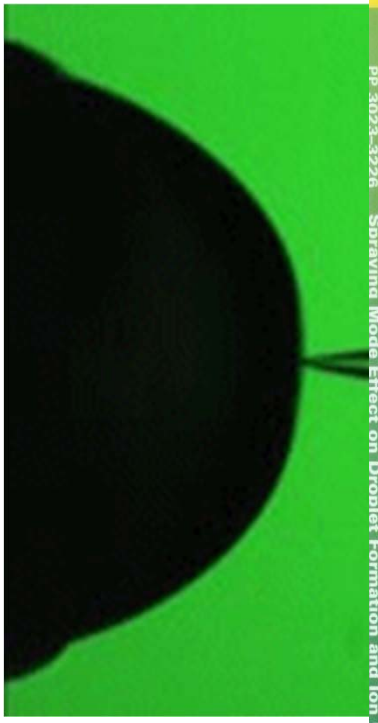




ESI : ElectroSpray Ionization (1988)



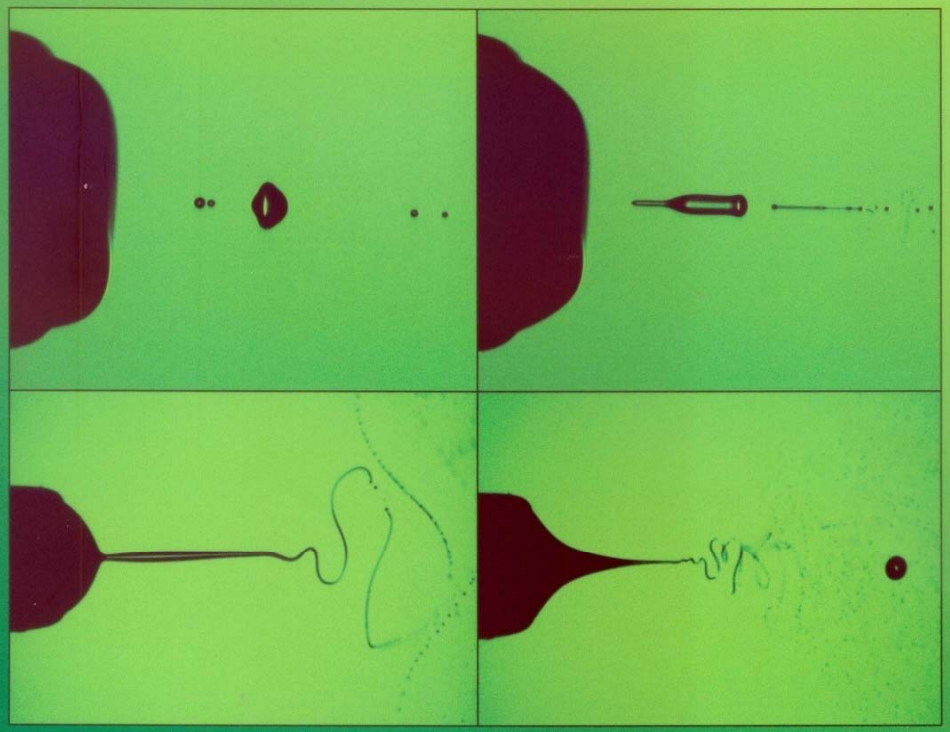
MS analyzer



PUBLISHED BY THE AMERICAN CHEMICAL SOCIETY

analytical chemistry

April 15, 2007



http://pubs.acs.org/ac

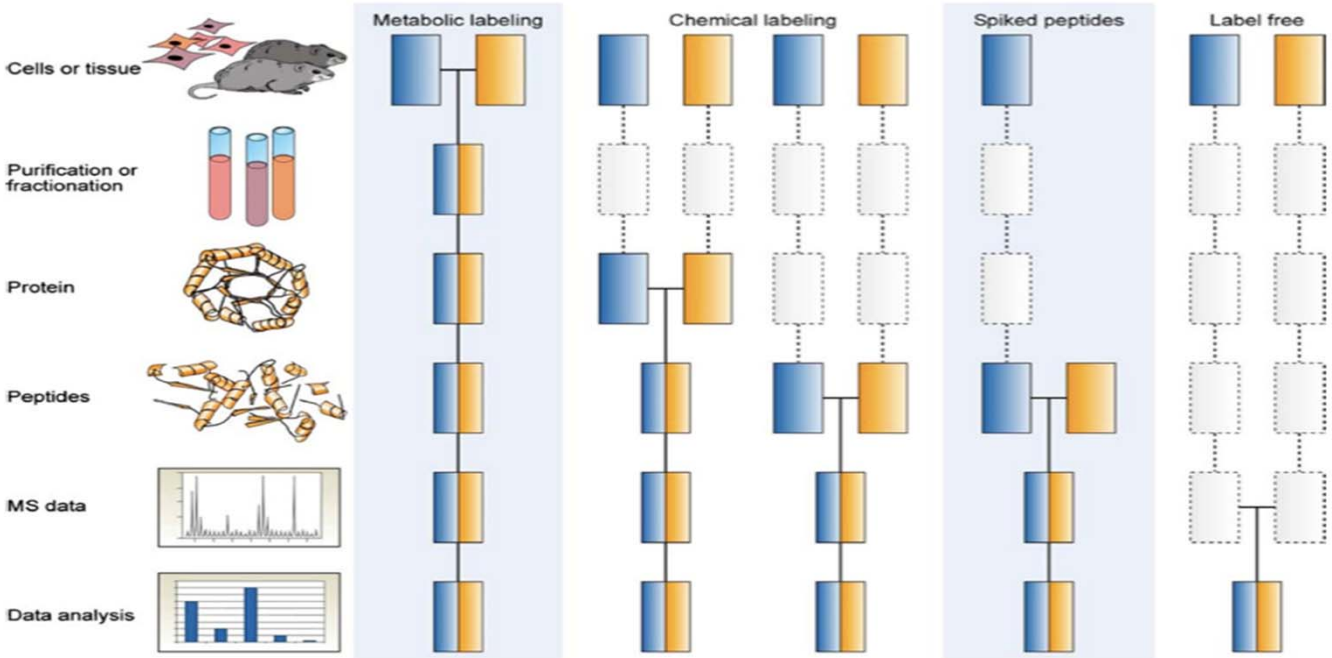
analytical chemistry

pp 3023-3226 Spraying Mode Effect on Droplet Formation and Ion Chemistry in Electrosprays

April 15, 2007

Nemes, P, I Marg
Spraying Mode E
and Ion Chemist
Chemistry, 2007

Quantification in MS



	Application	Accuracy (process)	Quantitative proteome coverage	Linear dynamic range[a]
Metabolic protein labeling	Complex biochemical workflows Comparison of 2–3 states Cell culture systems only	+++	++	1–2 logs
Chemical protein labeling (MS)	Medium to complex biochemical workflows Comparison of 2–3 states	+++	++	1–2 logs
Chemical peptide labeling (MS)	Medium complexity biochemical workflows Comparison of 2–3 states	++	++	2 logs
Chemical peptide labeling (MS/MS)	Medium complexity biochemical workflows Comparison of 2–8 states	++	++	2 logs
Enzymatic labeling (MS)	Medium complexity biochemical workflows Comparison of 2 states	++	++	1–2 logs
Spiked peptides	Medium complexity biochemical workflows Targeted analysis of few proteins	++	+	2 logs
Label free (ion intensity)	Simple biochemical workflows Whole proteome analysis Comparison of multiple states	+	+++	2–3 logs
Label free (spectrum counting)	Simple biochemical workflows Whole proteome analysis Comparison of multiple states	+	+++	2–3 logs

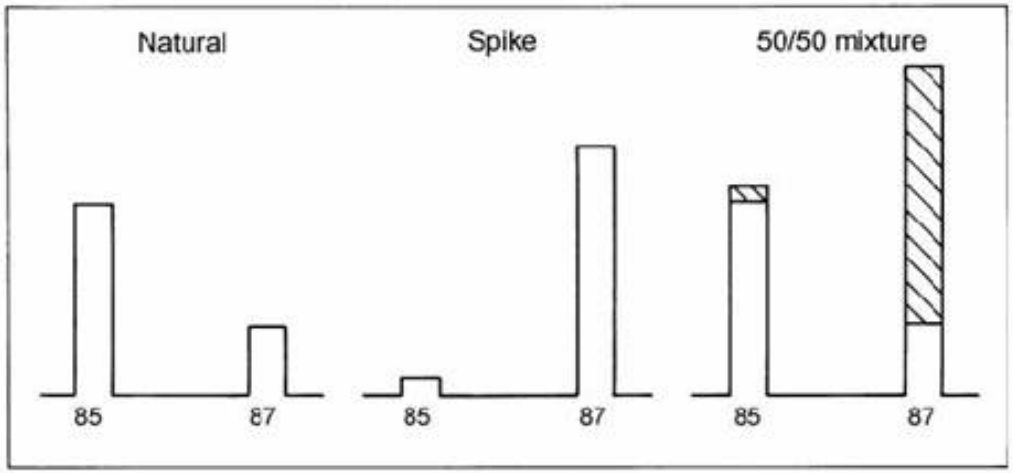
Stable isotopes and their uses

- Most elements have more than one isotope
- E.g. ^{32}S and ^{34}S, or ^{56}Fe and ^{57}Fe
- Can use more than one mass for one element for measurements in ICP-MS
- IDSM: Isotope dilution mass spectrometry, use particular isotope of desired analyte as internal standard in ICP-MS
- Can buy enriched compounds, e.g. ^{67}ZnO, and use as "tracers"

Isotope dilution principle

Isotope dilution is an analytical technique used in combination with mass spectrometry to determine the concentration of element x in unknown samples.

ex: Rubidium (Rb)



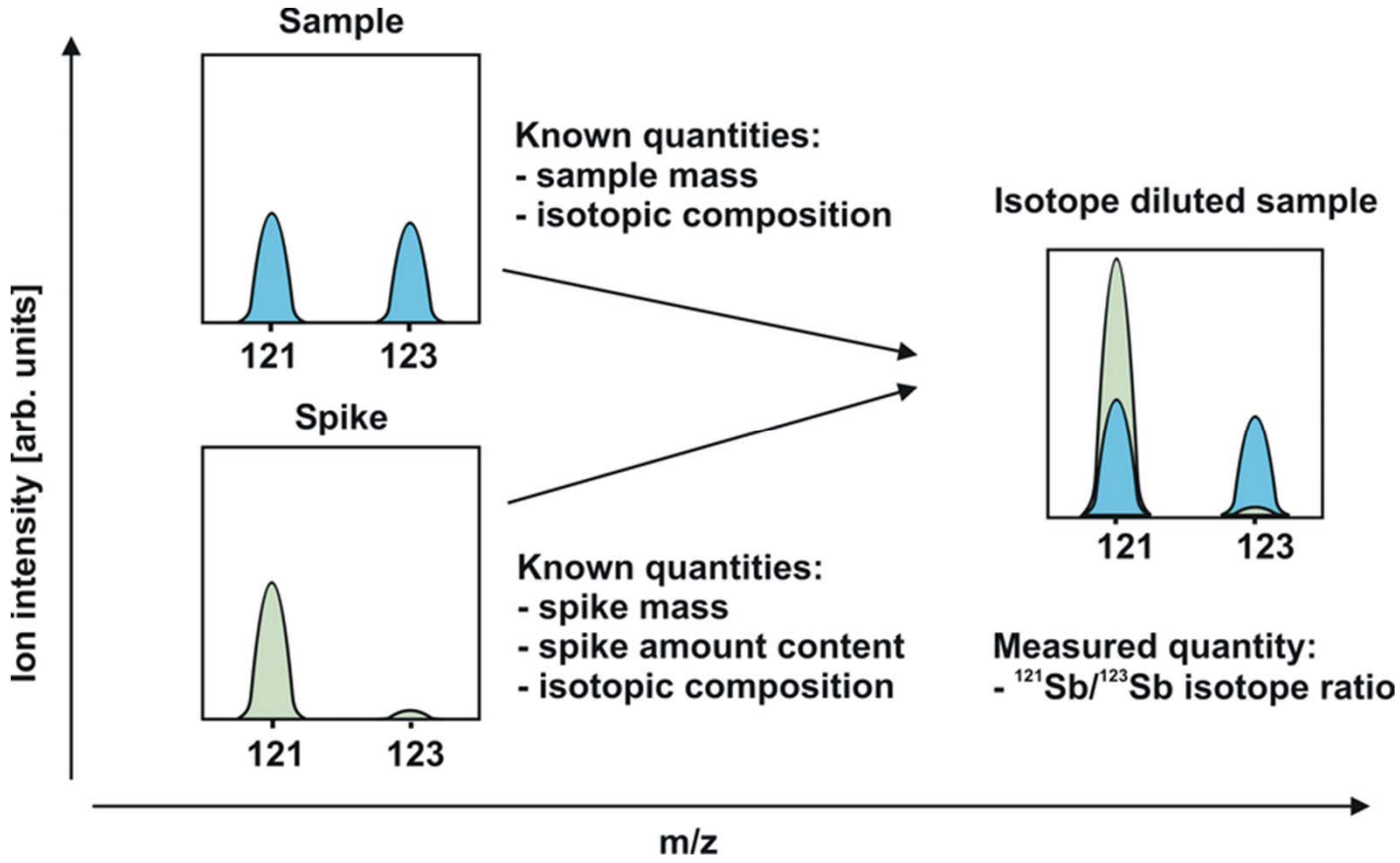
A known amount of "spike" with known elemental concentration and isotopic abundances (what's the diff?)

is added to sample with unknown elemental concentration but known isotopic abundances.

Requirements:

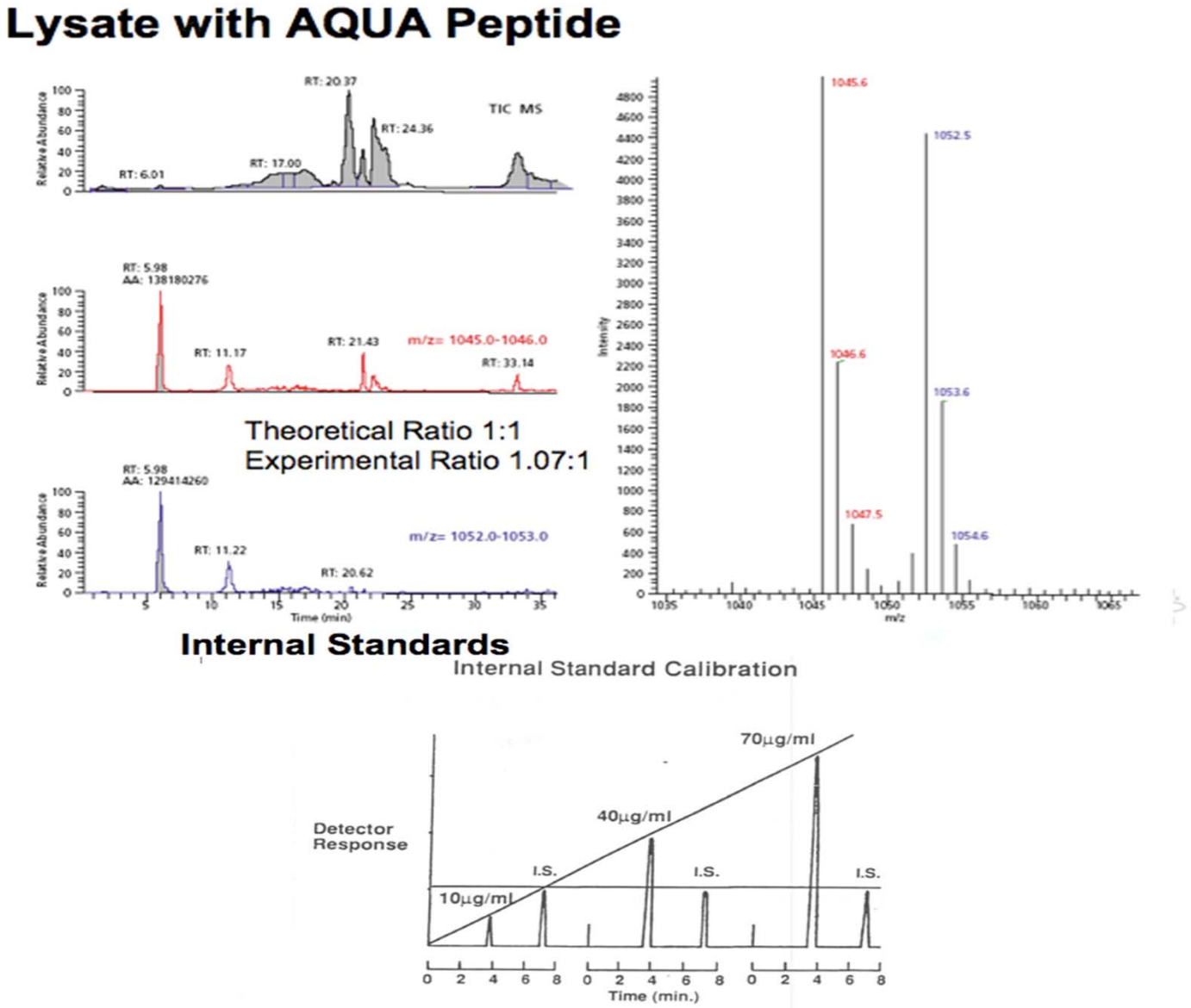
1) The sample has natural (or known) isotopic abundance (usually true).
2) The spike and sample isotopic ratios are different.

Isotopic dilution Mass Spectrometry

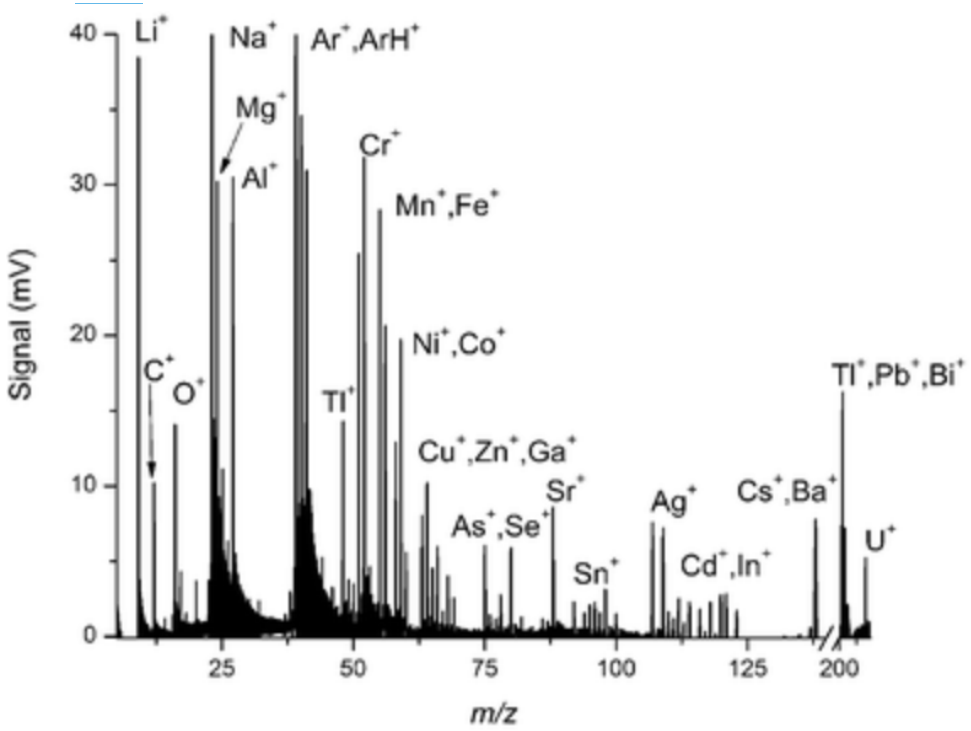


$$w_x = w_{y,b} \cdot \frac{M_x \cdot m_y}{M_b \cdot m_x \cdot a_{x,b}} \cdot \frac{\left(R_y - R_{xy}\right)}{\left(R_{xy} - R_x\right)}$$

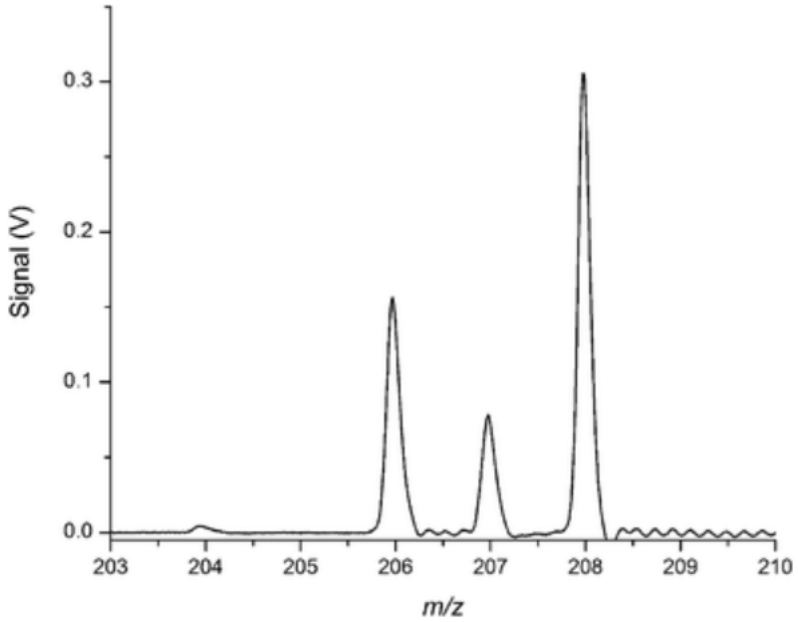
Stable isotope labelling for molecule quantification



Atomic mass spectra



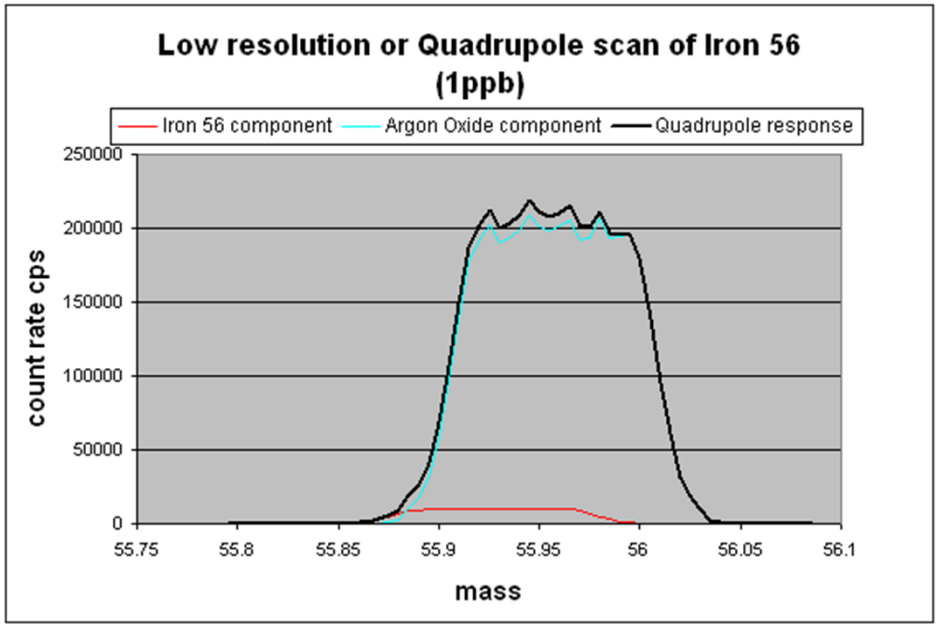
Characteristic ICP mass spectrum of a multi-elemental solution, illustrating the full mass range capabilities from 6Li+ to 238U+.

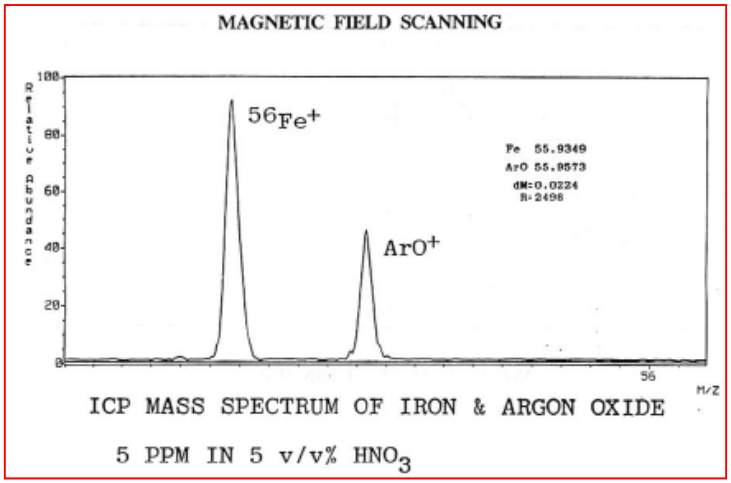


Resolving power of 1500 (FWHM) for $^{208}Pb^+$ is sufficient for baseline resolution of the major lead isotopes.

Duane A. Rogers , Steven J. Ray and Gary M. Hieftje Metallomics, 2009, **1**, 67-77

Low vs. High – resolution ICPMS and Interferences





^{56}Fe

very low concentrations in environmental samples, but high interest

Unfortunately, ^{56}Fe has the same atomic wt as ArO ($^{40}Ar+^{16}O$)

Quadrupole measurement = INTERFERENCE!

HR-ICPMS measurement = can distinguish ^{56}Fe from ArO

NOTE: most elements can be distinguished with a low resolution quadrupole

Speciation

Determining total concentrations of the elements cannot provide the required information about mobility, bioavailability.

Only knowledge about the chemical species of the elements can lead to an understanding of chemical and biochemical reactions involving these species, thus providing information about toxicity or essentiality.

Elements usually interact as parts of macromolecules (proteins, enzymes, hormones, etc.) or according to their oxidation state

Michalke B. Element speciation definitions, analytical methodology, and some examples. Ecotoxicol Environ Saf. 2003 Sep;56(1):122-39.

Chemical species

A chemical species is a specific form of a chemical element, defined as its molecular or complex structure, or oxidation state.

Speciation

Distribution of defined chemical species of an element in a system.

Speciation analysis

Analytical activity of identifying and measuring species, with clear identification of the species (elements and possibly binding partners) as well as exact quantification

Species selective detectors: ESI-MS detection

- **Soft ionization** of element species,
- **Whole molecule** (covalent bonds and stable-element organic molecules) transferred into the gas phase
- Extremely **low flow rates**, LC or CE coupling
- Collision-induced dissociation (**CID**) combined with a MS/MS system can provide further structural information

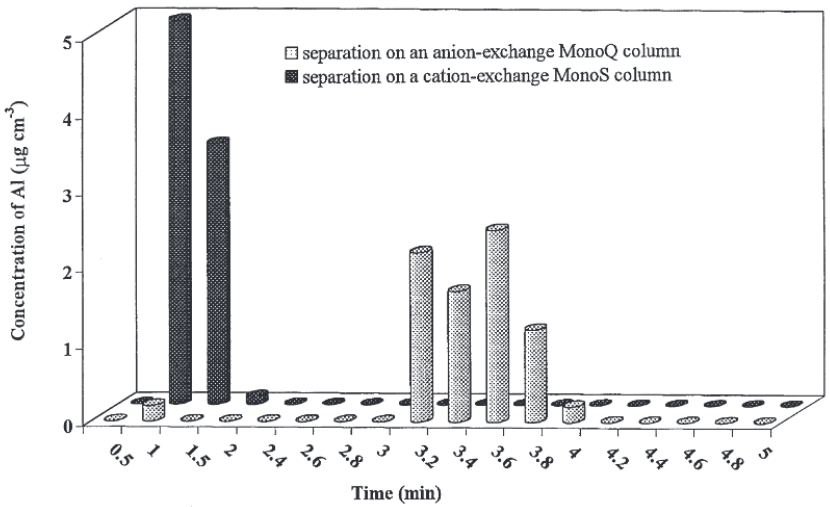
- **Multi-charged ions** from high-molecular-weight element species such as metalloproteins, up to MW=150,000–200,000.
- **Ion–solvent clusters**: native counterions of the metal ion are replaced by H_2O and/or MeOH, independently of the counterion initially present ((e.g., $[Cu(MeOH)]^+$) instead of Cu^{2+} $2Cl^-$)
- Splitting of one species into multiple signals, worsening detection limits and increasing spectral complexity.

- **Electrolytic processes** at the metallic ESI tip needle generation of new species or a transformation of species.

Applications

- Metal complex and metallome
- Proteomics for phospho, seleno or metal binding proteins
- Proteome evolution and metal ligands (Pt, Bi, Li)
- Metal complex and metallome, metal sensing and post-translational metal regulation
- ICP and microbial metalloproteome
- Metalloproteomics
- Single Cell ICP-MS

Anion exchange FPLC ICP MS coupling



Distribution of LMW Al-species in leaf sap of Sempervivum tectorum

aconitic acid : m/z 173 and 129
citric acid : m/z 191

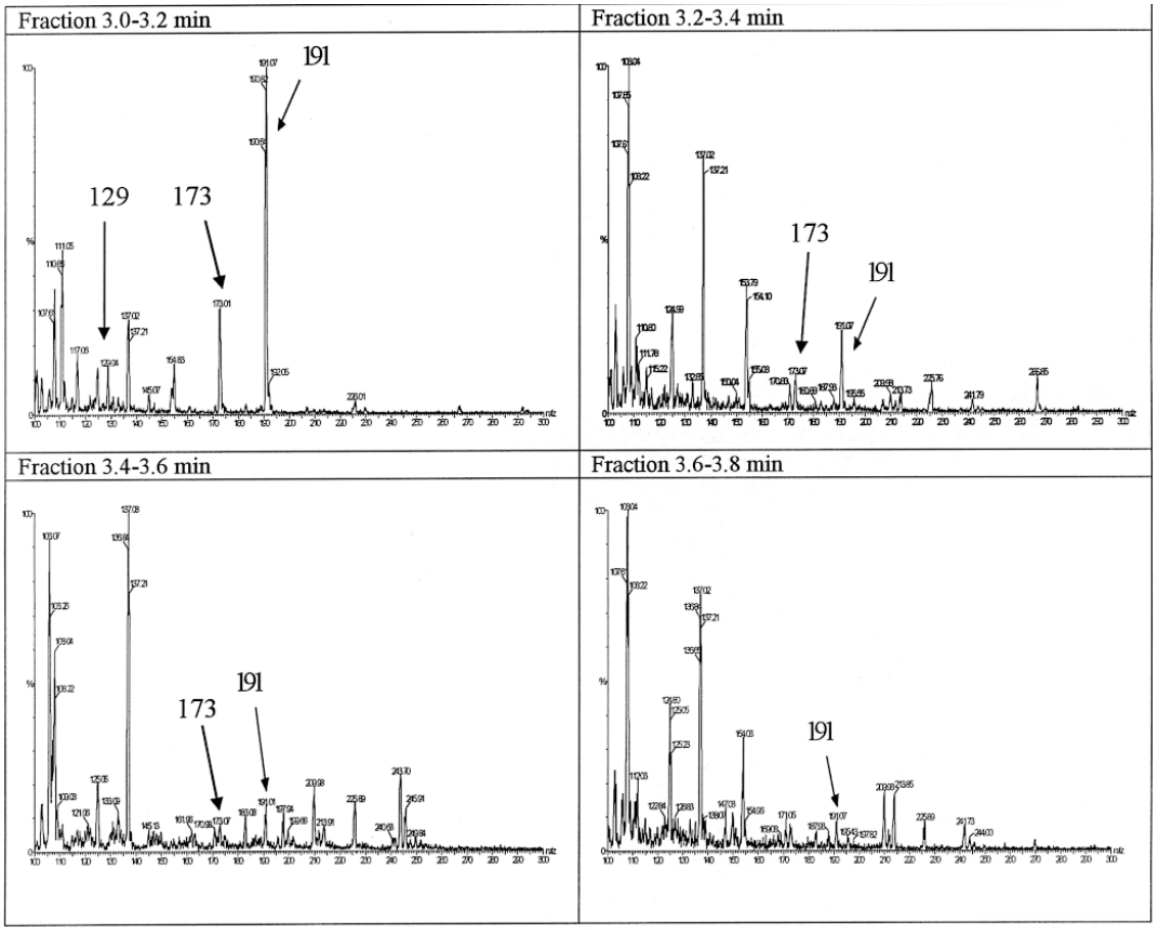


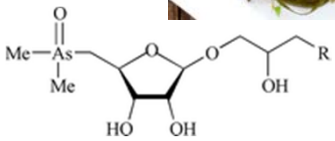
Fig. 2 ESI-MS spectra for *Sempervivum tectorum* in separated fractions of a chromatographic run (anion exchange FPLC) from 3.0–3.8 min

T. Bantan, R. Milacic, B. Mitrovic, B. Pihlar **Combination of various analytical techniques for speciation of low molecular weight aluminum complexes in plant sap** Fresenius' J. Anal. Chem., 365 (1999), pp. 545-552

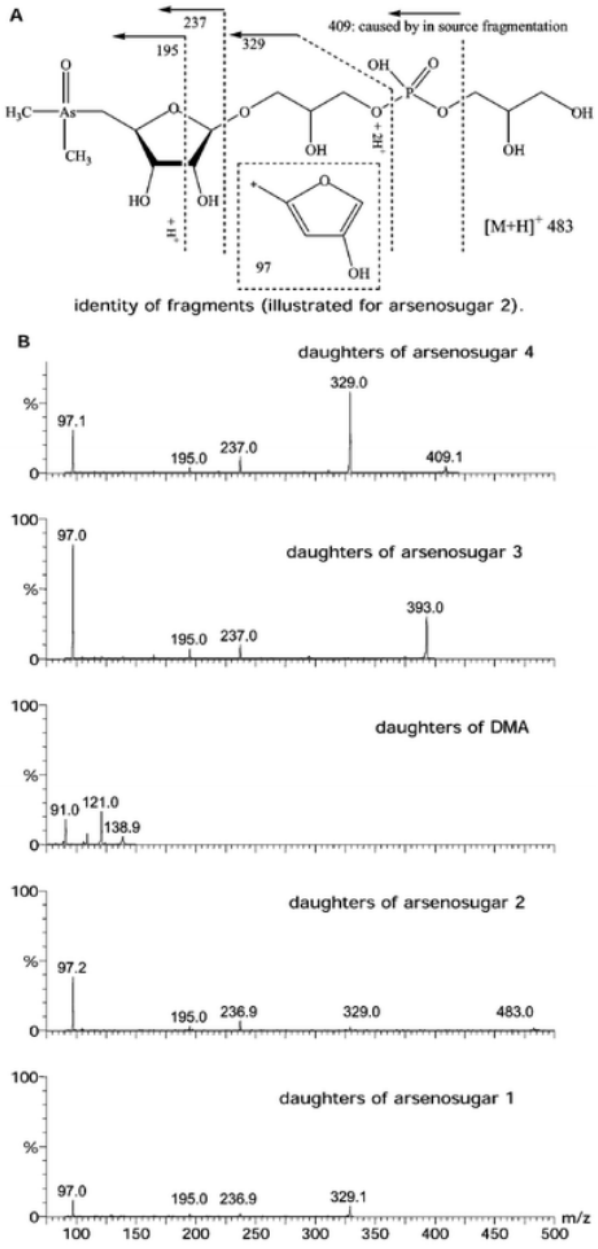
Arsenic speciation in Chinese seaweeds using HPLC-ICP-MS and HPLC-ES-MS

Formulae of the arsenic compounds

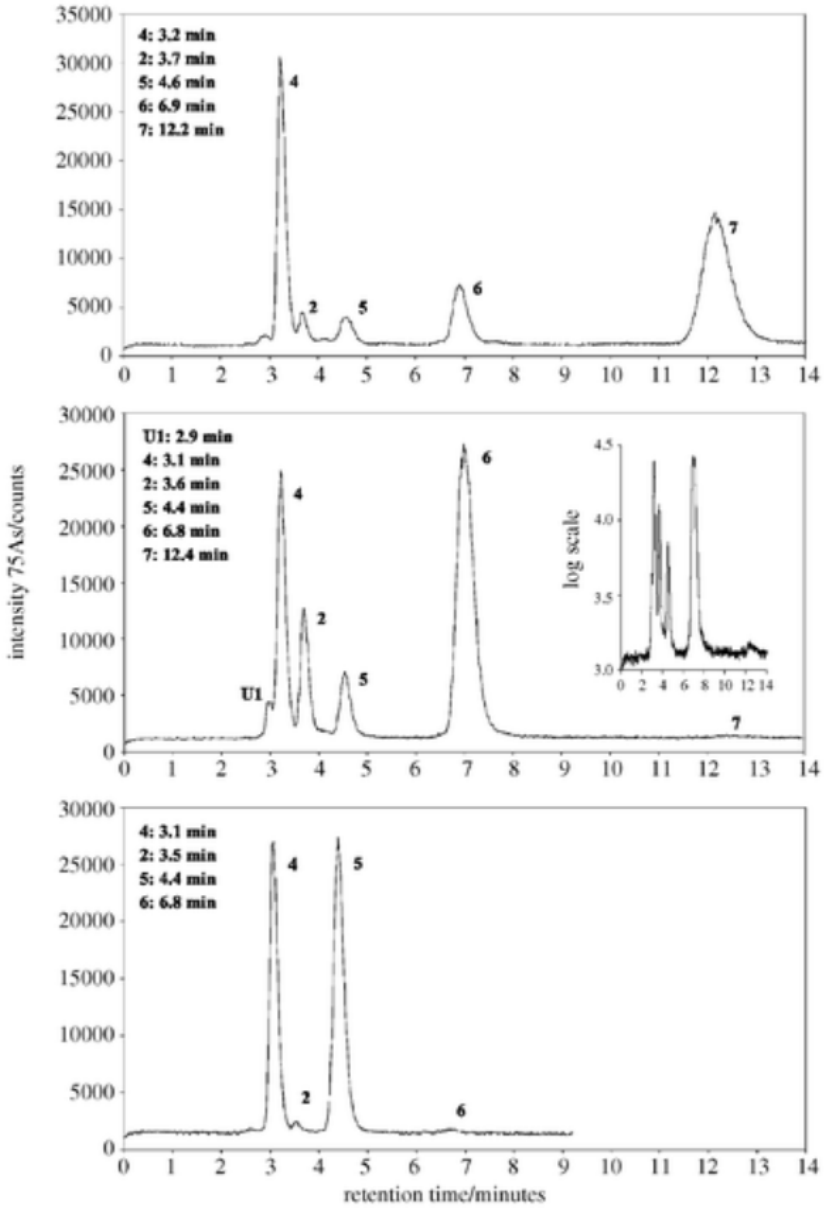
1. Arsenate (As^{v})	$Na_2HAsO_4 \cdot 7H_2O$
2. Dimethylarsinic acid (DMA)	$(CH_3)_2As(O)OH$
3. Arsenobetaine (AsB)	$(CH_3)_3As^+CH_2COOH$



4. Arseno sugar 1	R = OH
5. Arseno sugar 2	$R = OP(O)(OH)OCH_2CH(OH)CH_2OH$
6. Arseno sugar 3	$R = SO_3H$
7. Arseno sugar 4	$R = OSO_3H$
8. Trimethylarsenic oxide (TMAO)	$(CH_3)_3AsO$
9. Tetramethylarsonium ion (TMAs)	$(CH_3)_4As^+I^-$



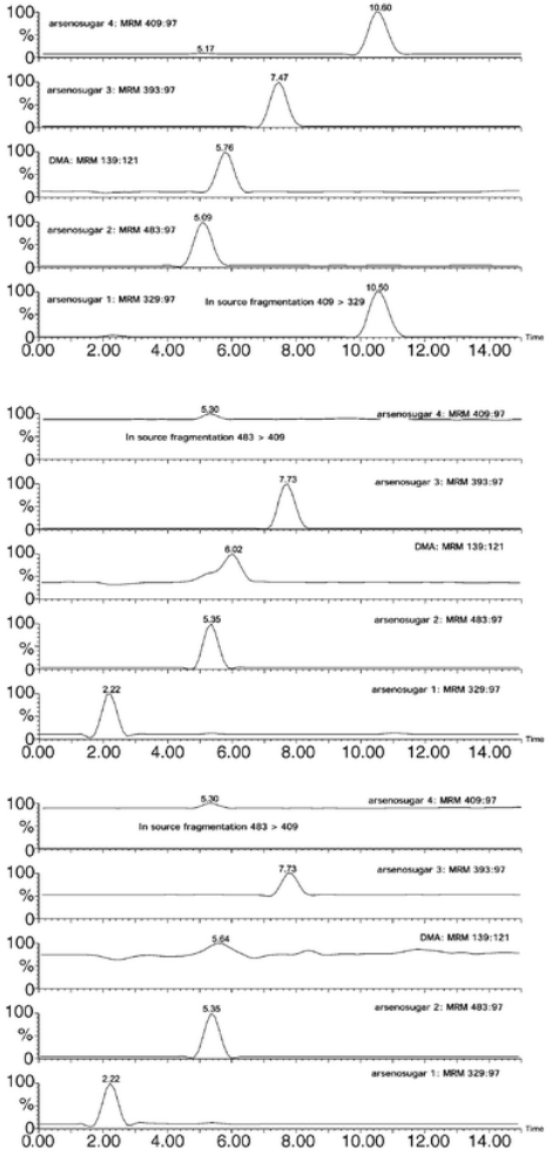
M. Van Hulle, C. Zhang, X. Zhang, R. Cornelis
Analyst, 127 (2002), pp. 634-640



kelp

Laminaria

Porphyra



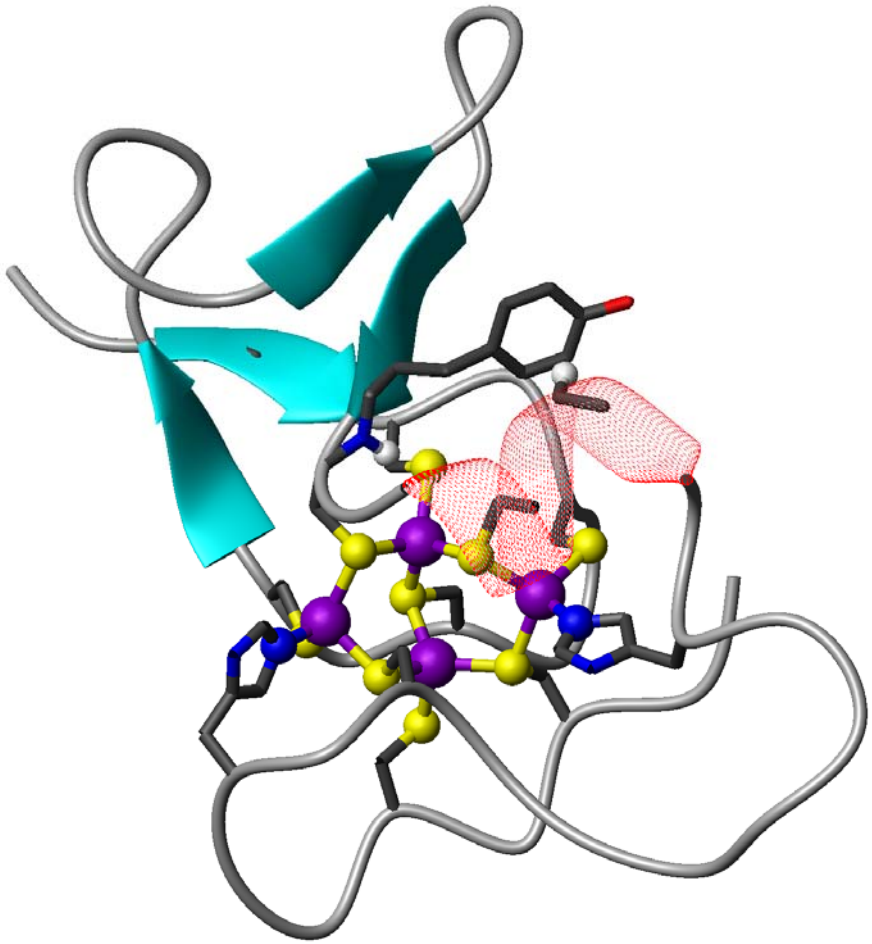
MRM transitions
409 → 97,
393 → 97,
139 → 121,
483 → 97
329 → 97.

Anion-exchange HPLC-ICP-MS

Anion-exchange HPLC-ES-MS in MRM mode

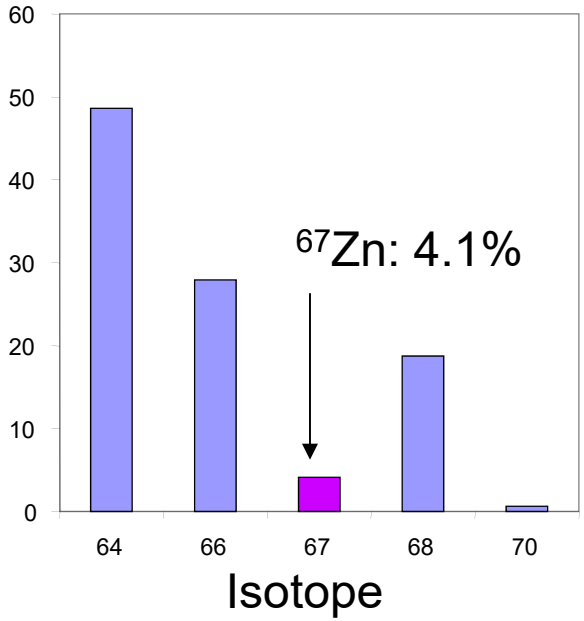
M. Van Hulle, C. Zhang, X. Zhang, R. Cornelis Analyst, 127 (2002), pp. 634-640

Example for use of stable isotopes



- Metal-binding protein with 4 Zn(II)
- Are all four zinc ions exchangeable ?

- Isolated with natural abundance Zn(II):



- Incubated overnight at 37° C with 40 mol equivalents of ^{67}Zn(II) (93% isotopic purity)
- Measured isotopic ratios

Measurement and output
(Thermofinnigan Element2)

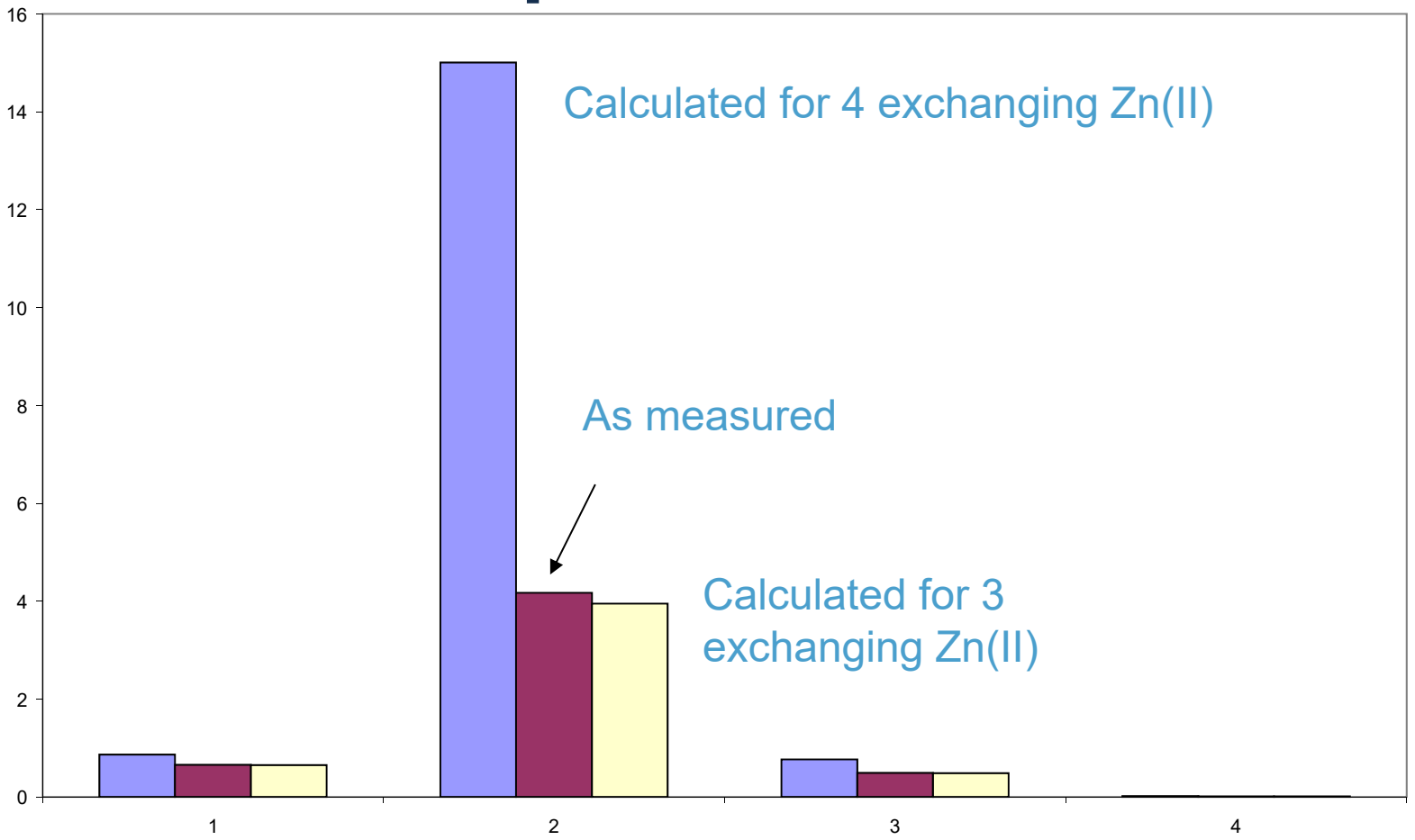
Total Zn and total S were determined using standard addition. For Zn quantification, the sum of the Zn isotopes 64, 66, 67, 68 and 70 was used. S was measured on the ^{32}S isotope. Zn isotopic distribution (64, 66, 67, 68, 70) was determined. All elements and isotopes were measured in Medium Resolution (R = 4000).

Results for sample:

Total S	2.45 mg/L (± 0.2 %)
Total Zn	2.21 mg/L (± 0.6 %)
Ratios:	
^{66}Zn / ^{64}Zn	0.657 ± 0.0028 (n = 7)
^{67}Zn / ^{64}Zn	4.17 ± 0.025 (n = 7)
^{68}Zn / ^{64}Zn	0.490 ± 0.0037 (n = 7)
^{70}Zn / ^{64}Zn	0.01325 ± 0.00007 (n = 7)

➔ S: Zn ratio: 9:4 (as expected; the protein contains 9 sulfurs)

Comparison of experimental and calculated isotopic ratios



➔For each isotopic ratio, results agree best with the scenario for 3 exchanging zinc:

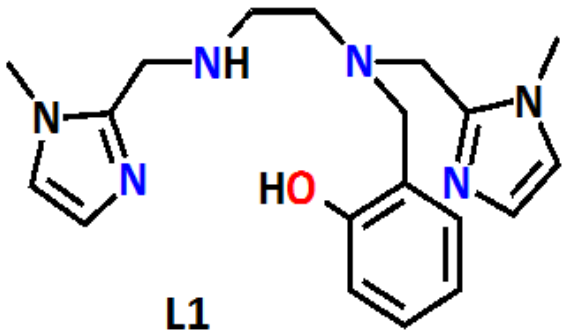
➔Clear demonstration that only 3 out of 4 Zn exchange:

➔The protein has one zinc that is inert towards exchange

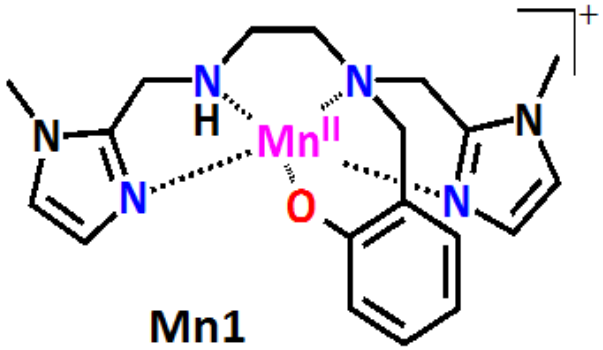
Crohn's Disease and IBD

Antioxidant and anti-inflammatory complex effect mimics of SOD (superoxide dismutase

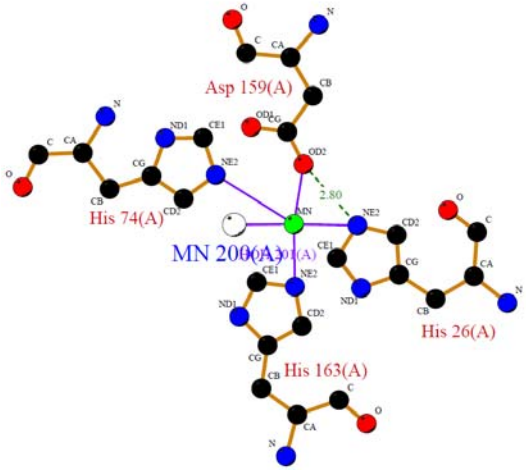
- Activity of SOD : $2O_2^- + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$
- Oxidative stress detoxifying enzyme
- Complex mimics of SOD « Mn1 »



L1

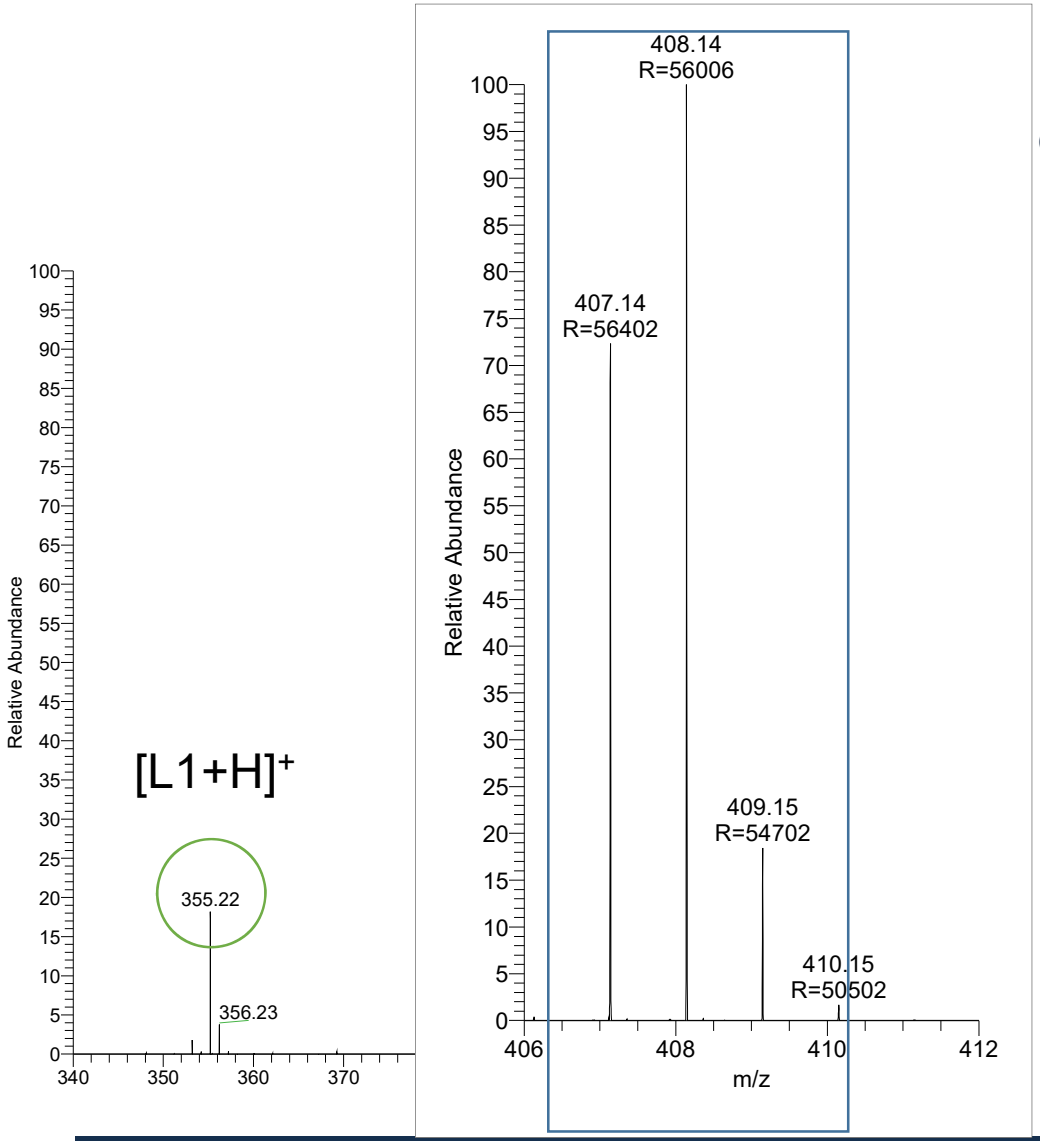


Mn1

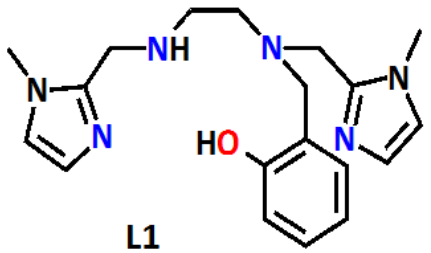


Catalytic site of Mn-SOD

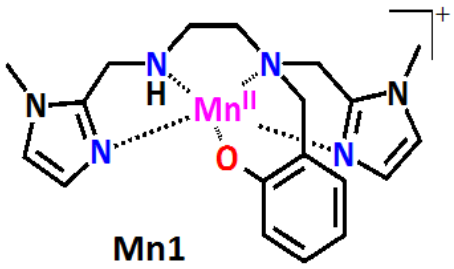
Spectre MS complexe et ligand



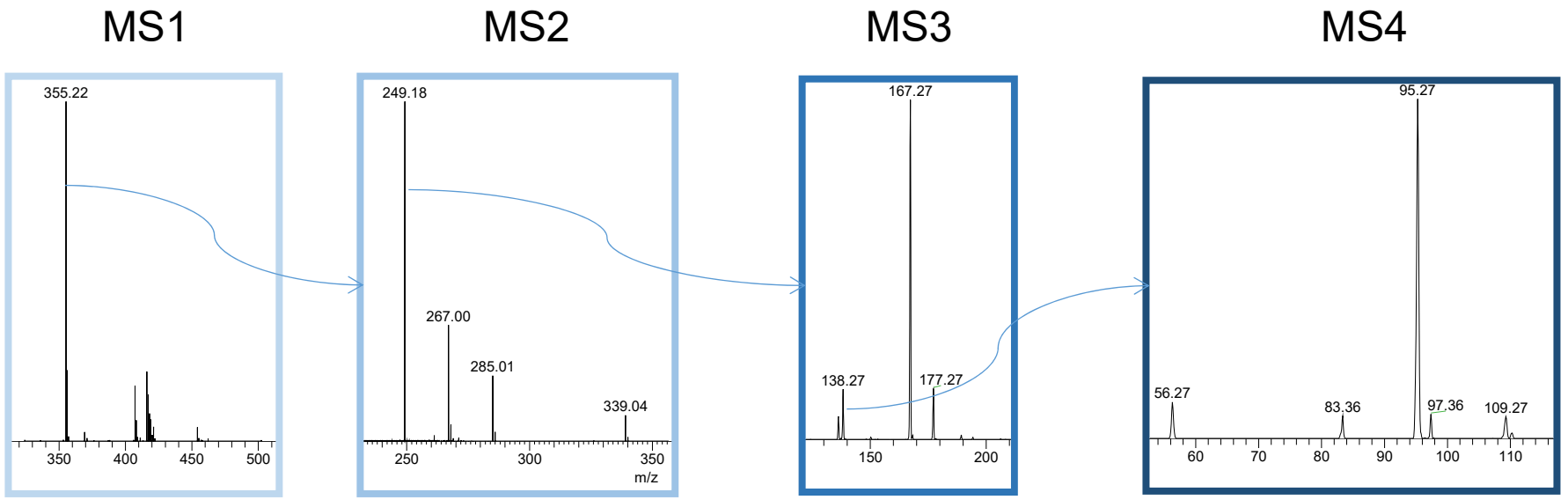
- Exact mass :
  - 354,2163 Da

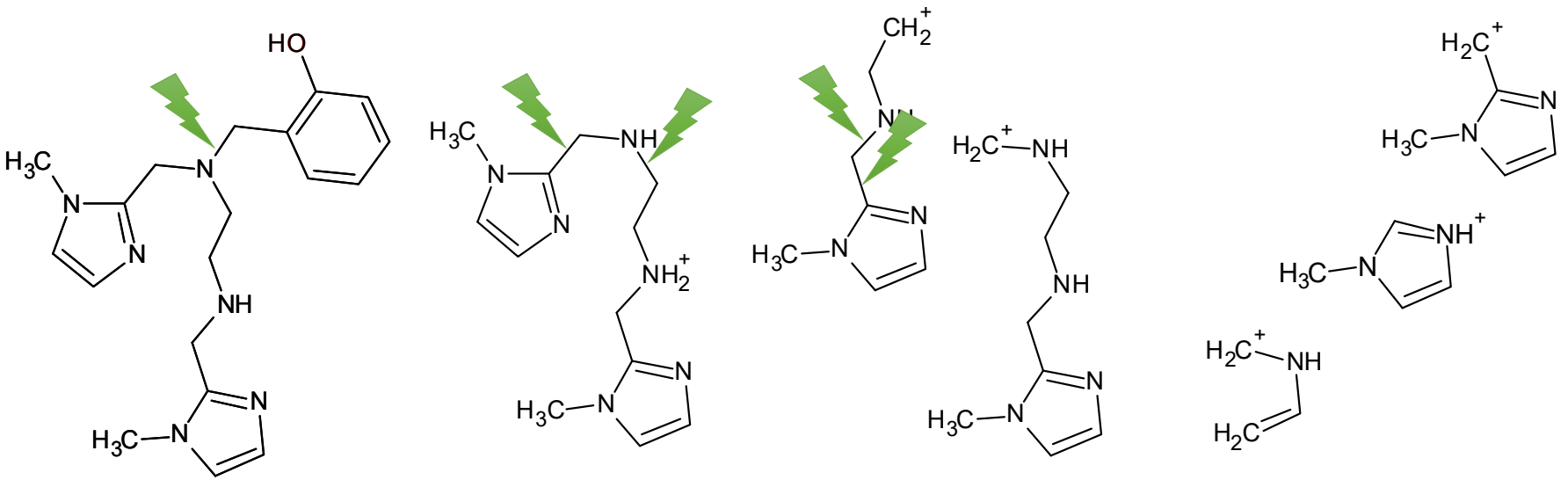


  - 408,1465 Da

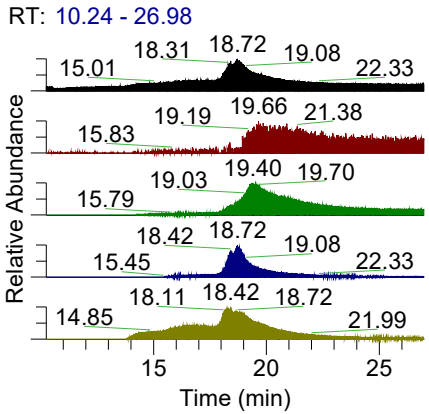


MSn fragmentation signature

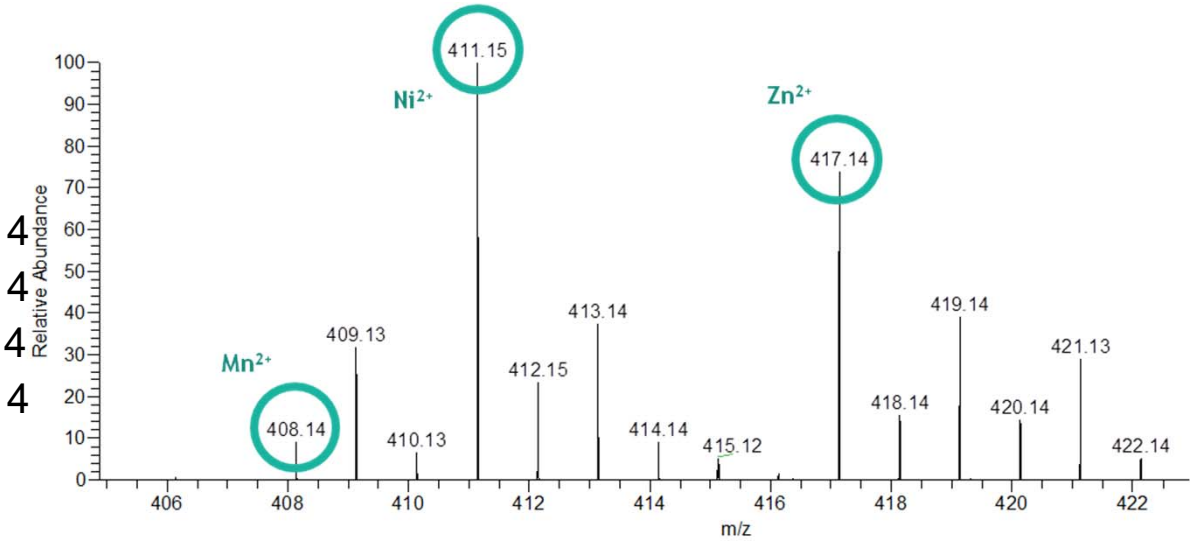




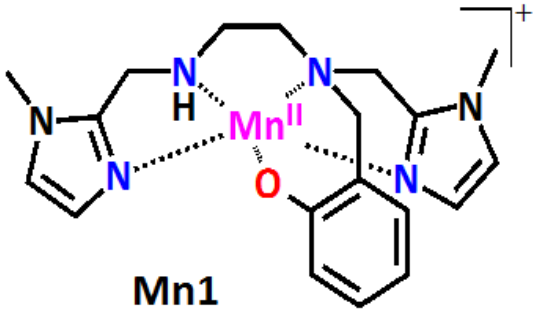
LC-MS : complexe separation

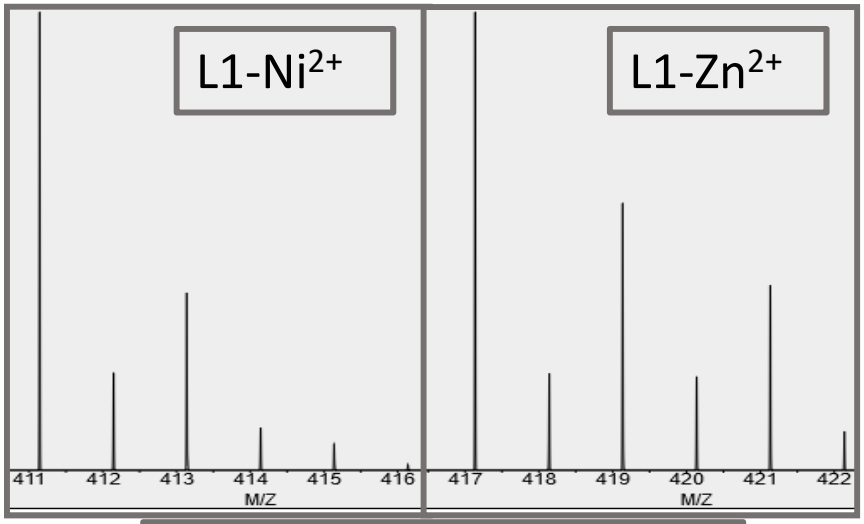


TIC
XIC 407.14
XIC 408.14
XIC 411.14
XIC 417.14



TIC : Total Ion Current
XIC : eXtracted Ion Chromatogram



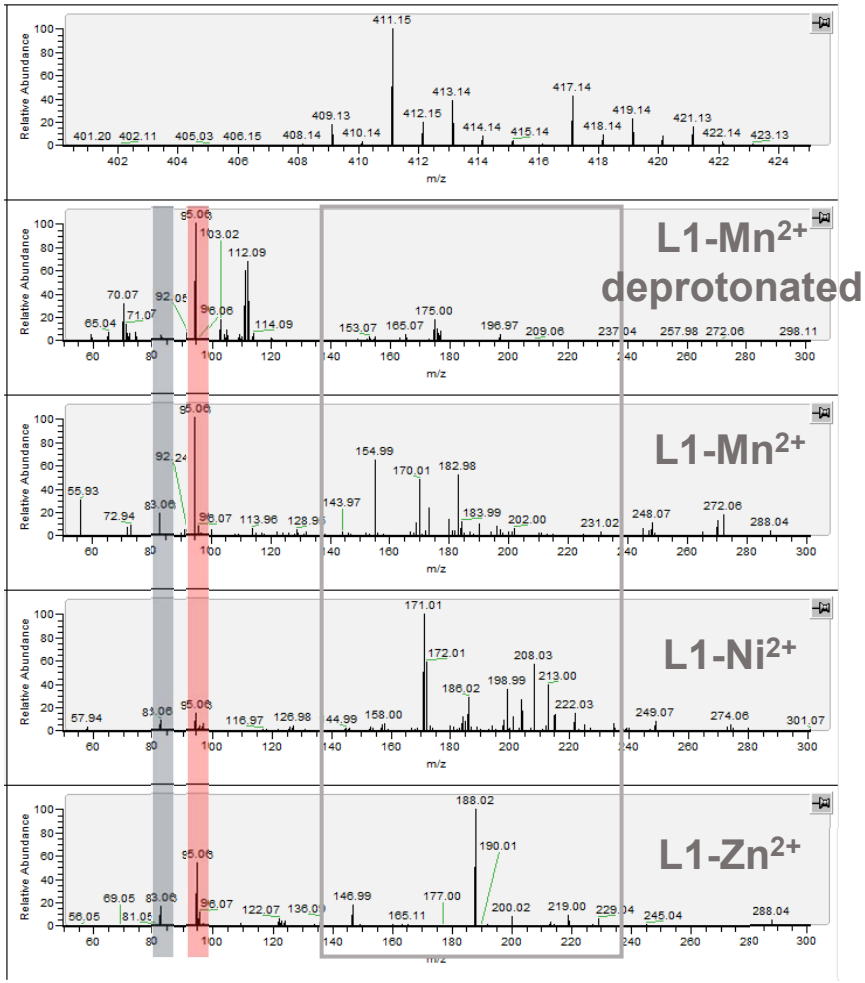


Modélisations L1-cation

m/z 411.14 et 417.14n (+3 and +9 uma respectively)

Isotopes stables majoritaires :
- Mn 55
- Ni 58 (60)
- Zn 64 (66, 68)

MS/MS of the 3 metal complexes



- Common fragments :
  - 95.06
  - 83.06
- Spécific fragments
- Metal exchange in the LC system?

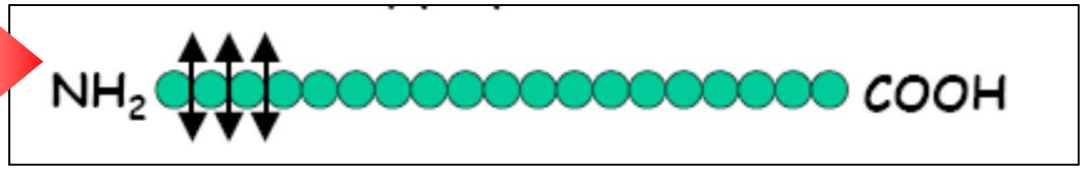
Endopeptidase digestion for bottom-up proteomics

Peptidase

Enzyme that clives polypeptides

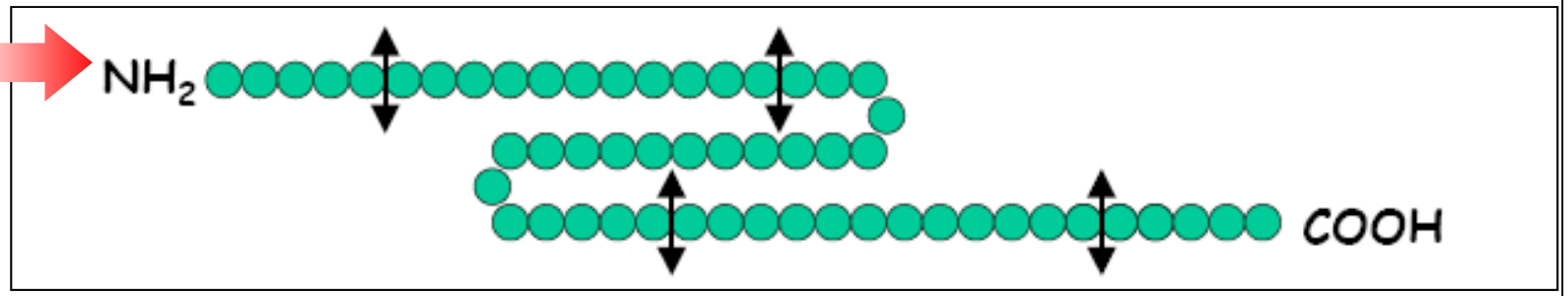
Two typeofde peptidase

Exopeptidase **clives sequentially at the end of the polypeptides**



- **N-ter**
- **C-ter**
- **Both N- and C-ter**

Endopeptidaseclives specifically at defined sites within the sequence of polypeptides



why endoproteases ?

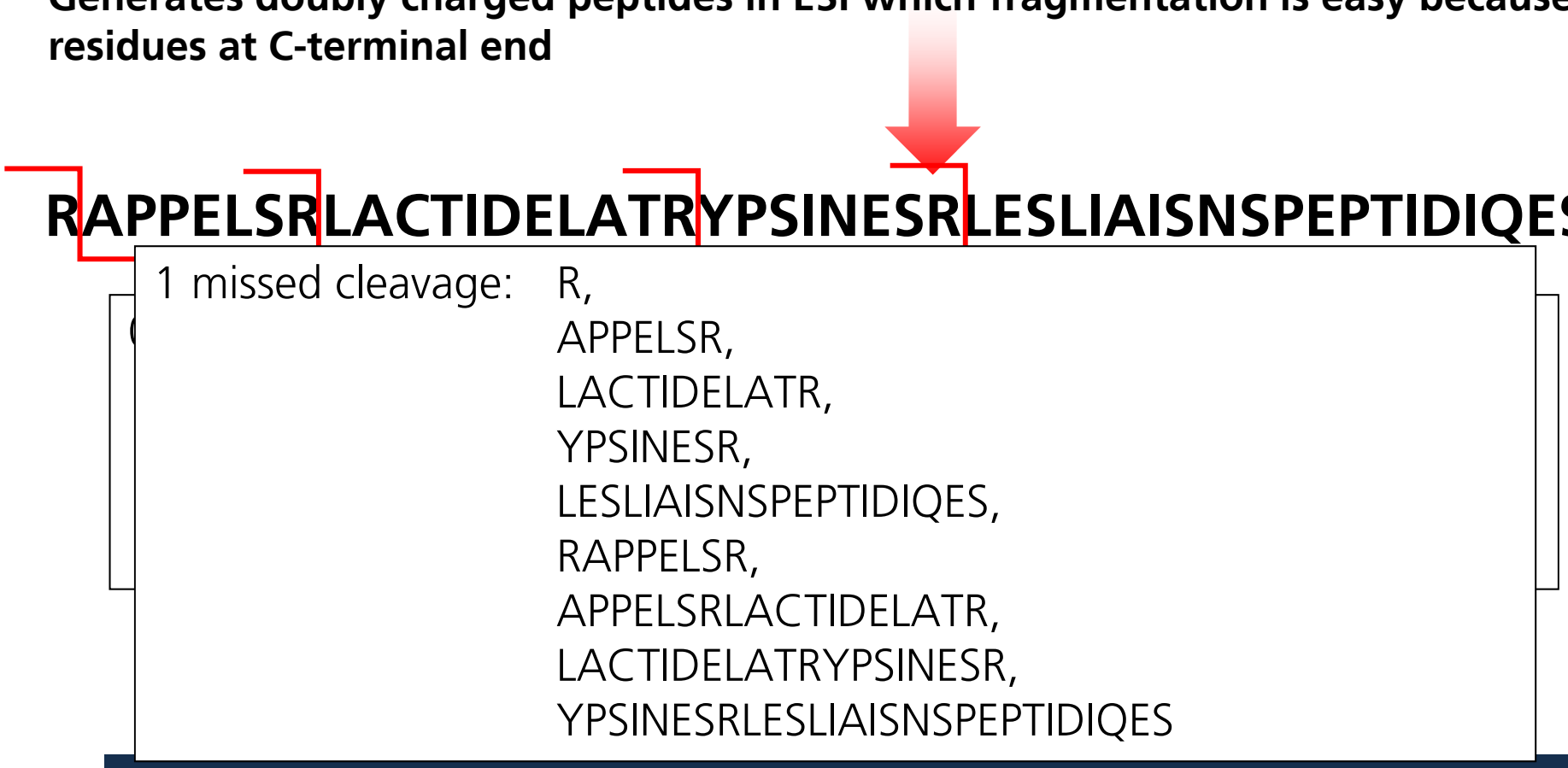
Because of their specificity they generate signature of the amino acids sequence (=fingerprint)

Tryptic digestion

Endopeptidase that clives at C-terminal end of

. lysine (K)
. arginine (R) (unless proline after)

Generates doubly charged peptides in ESI which fragmentation is easy because of the basi residues at C-terminal end

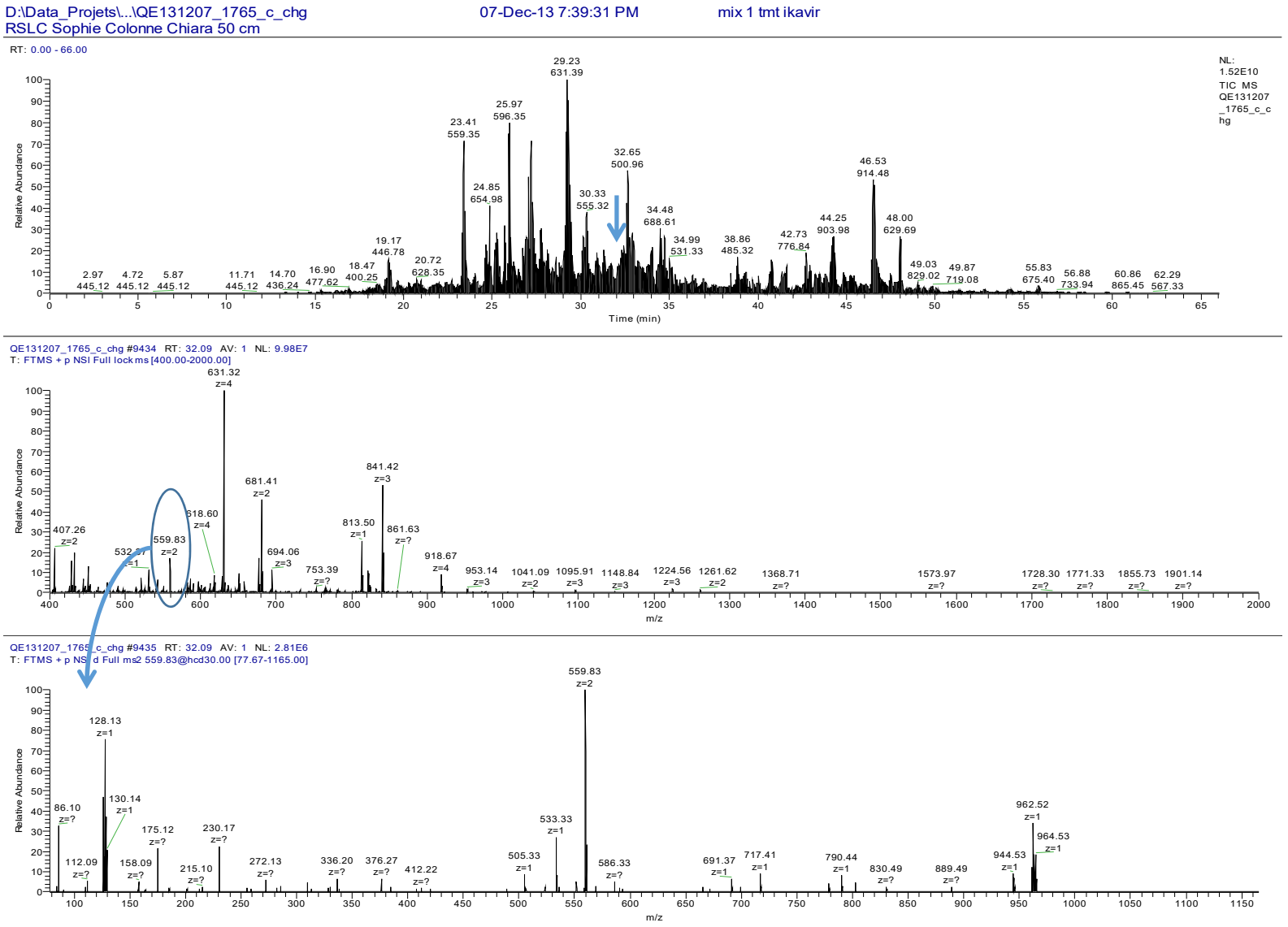


Bottom-up proteomics

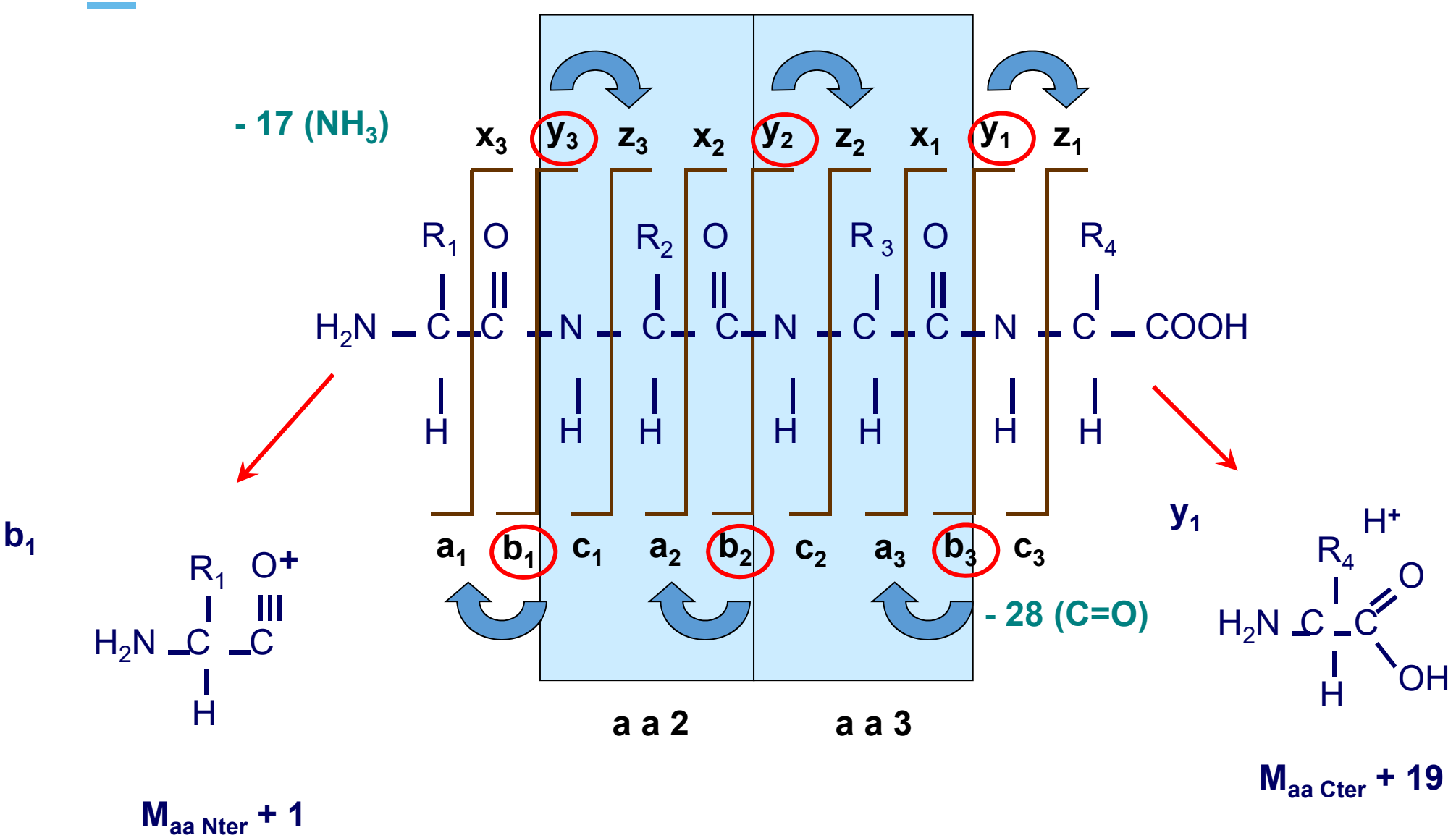
LC

Full scan MS

MS/MS

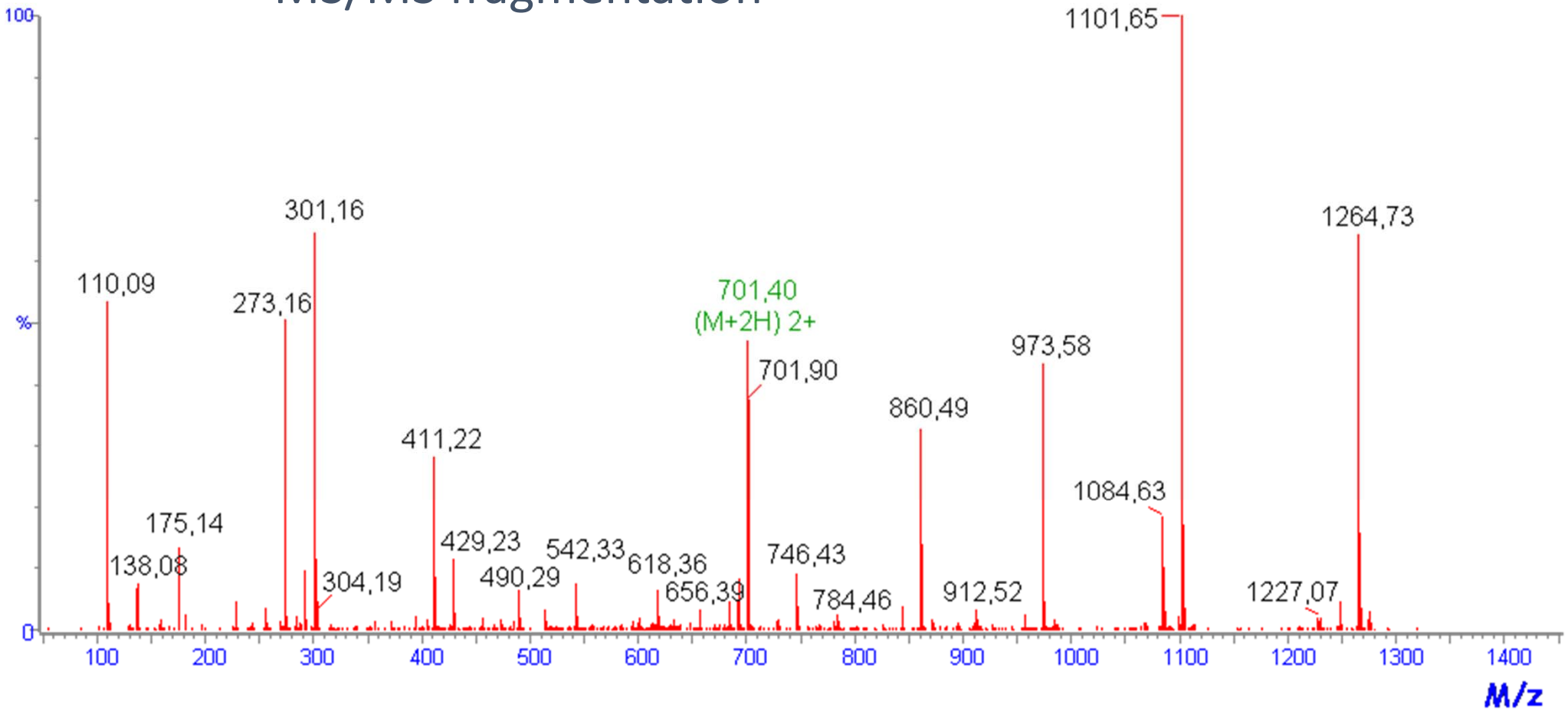


Bottom-up proteomics



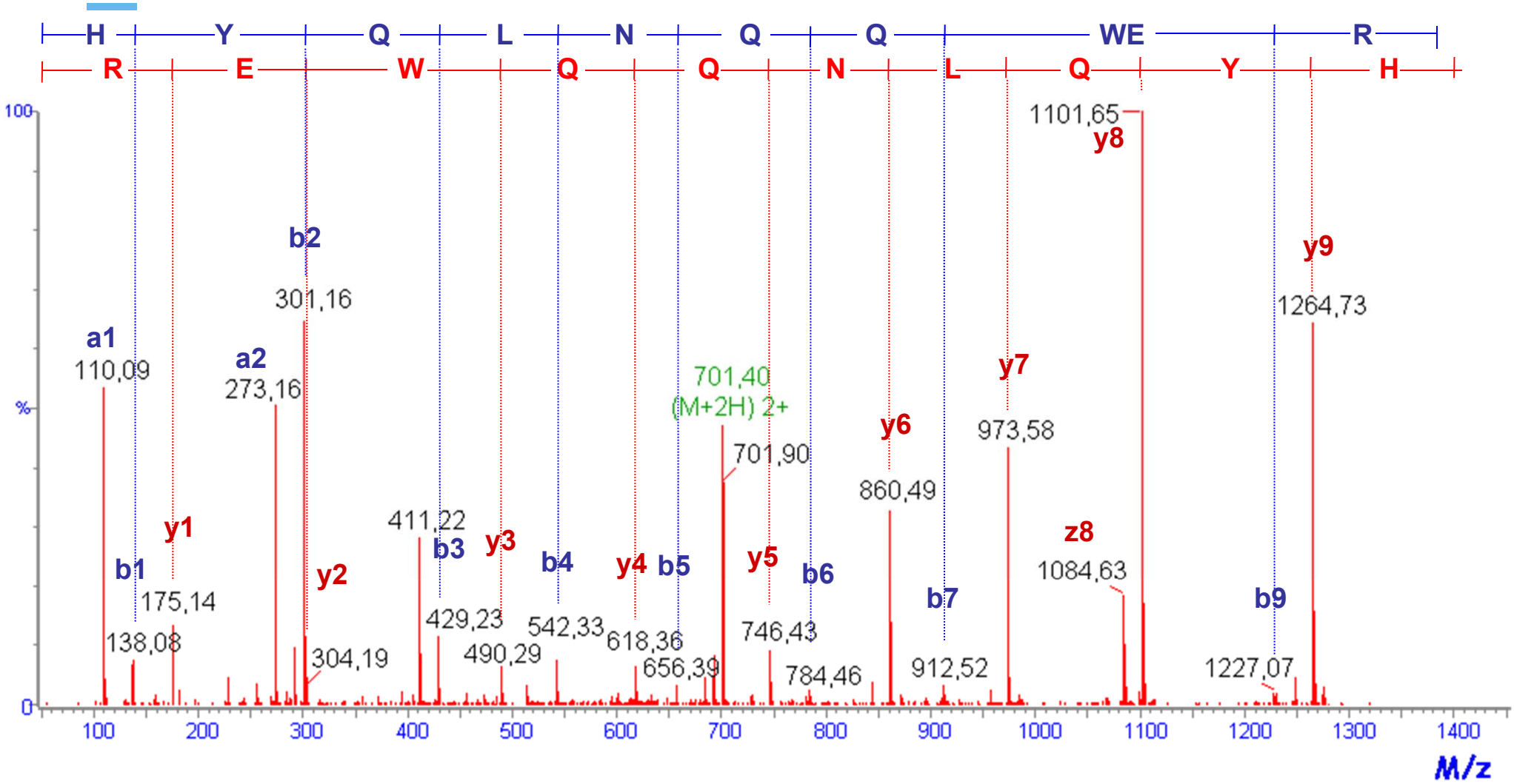
Bottom-up proteomics

MS/MS fragmentation



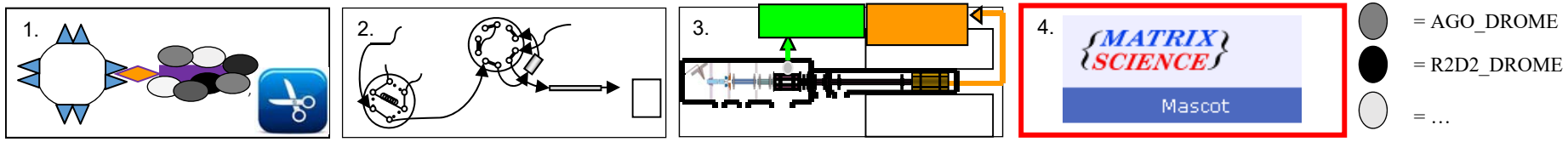
Fragmentation of doubly charged peptide m/z = 701,40 Da

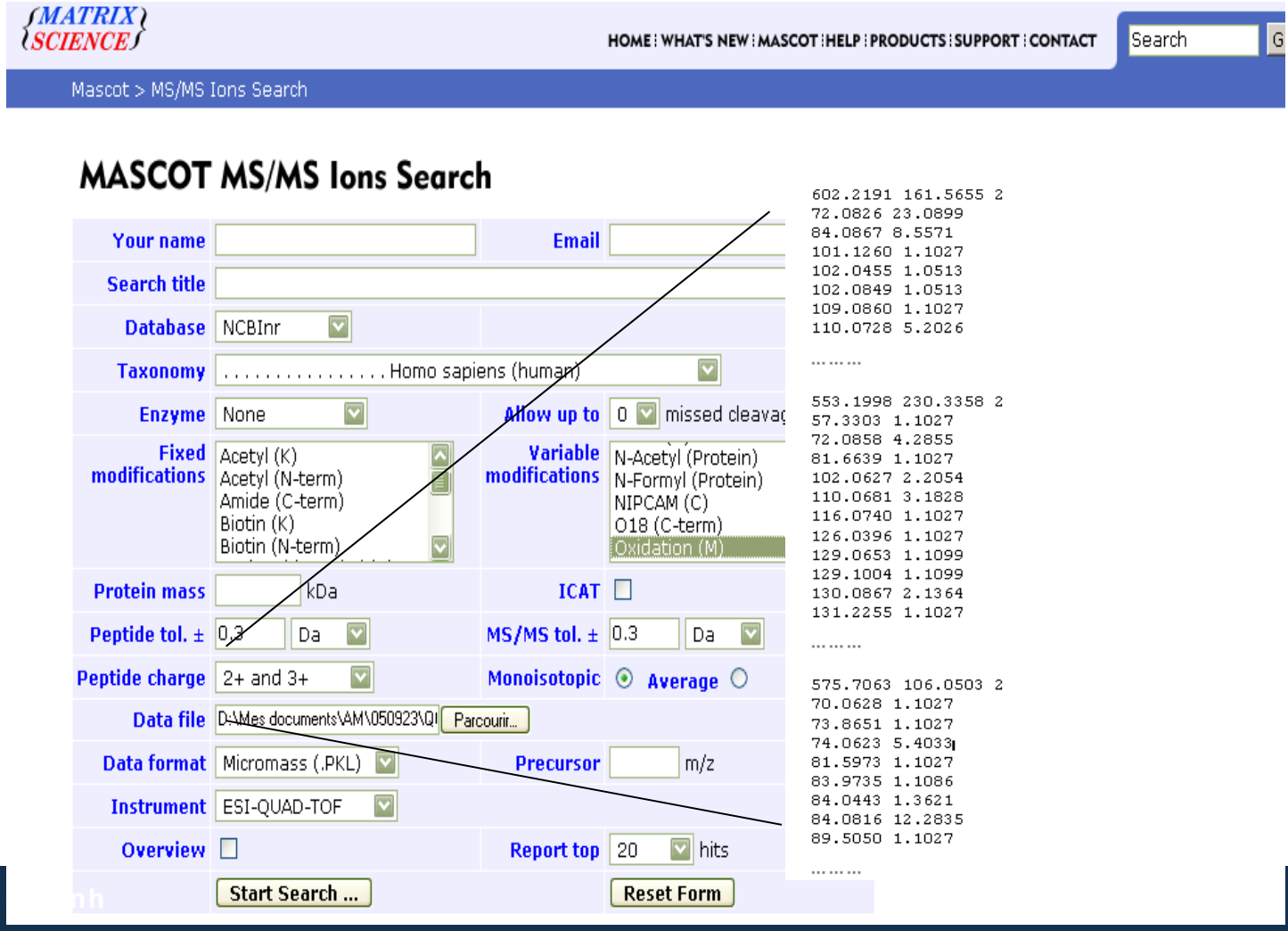
Bottom-up proteomics



Identification of sequence HYQLNQQWER

Analyse protéomique





Quantification in ESI / MALDI MS?

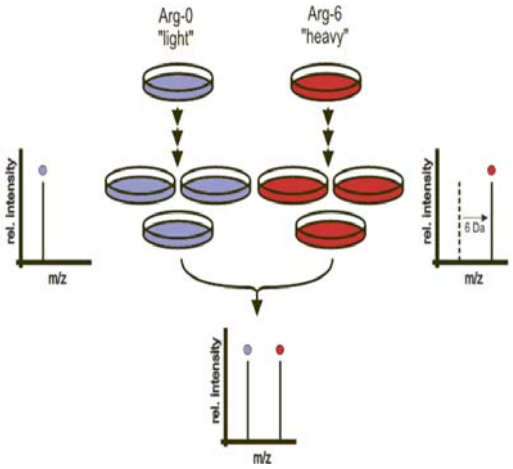
Signal intensity is function of …

- **Amount of analyte**
- Chemical Composition
- Molecular environment, solvents
- Instrument type and state
- …

Different strategies

- Label free
- Chemical labelling
- Metabolic labelling

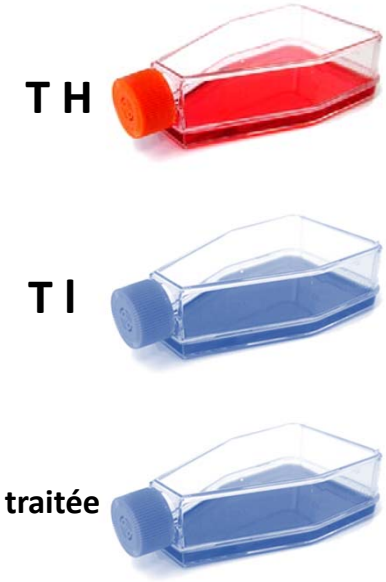
Quantification « SILAC »



SILAC (Stable **I**sotope **L**abeling by **A**mino acids in **C**ell culture**)**

Heavy R/K ($^{13}C/^{15}N$) on control cell culture

Well suited for tryptic digests



Incorporation yield

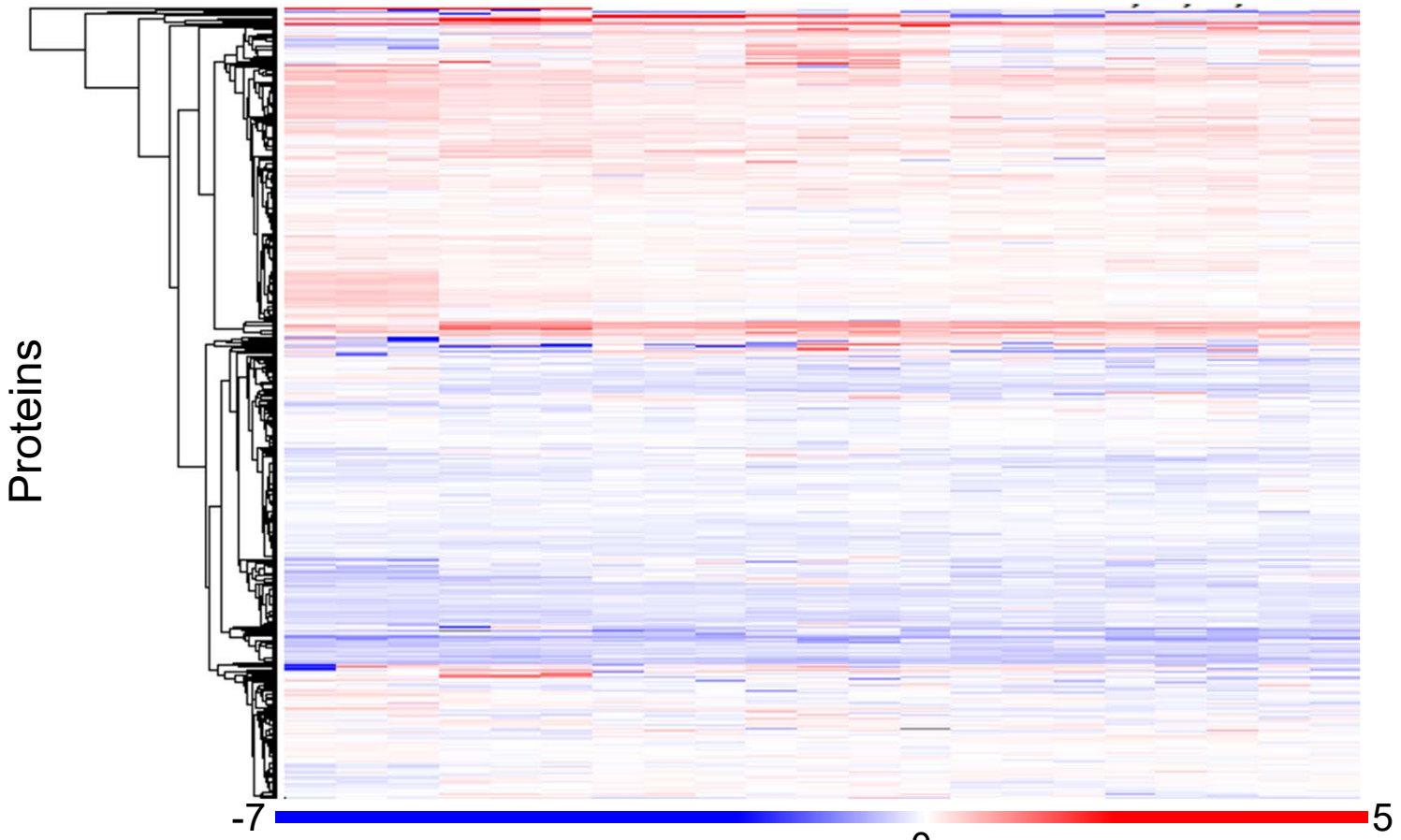
Expression profile of proteome

Light Culture (Arg-0 / Lys-0)		**T* : Heavy Culture (Arg-10 / Lys-8)**
Inflammation	**Traitement**	Non traitée
Non traitée		
Ø	+ $MnCl_2$	
Ø	+ *Mn1*	
+ LPS	Ø	
+ LPS	+ $MnCl_2$	
+ LPS	+ *Zn1*	
+ LPS	+ *Mn1*	

HEATMAP of « SILAC » samples

LPS	+	-	+	-	+	-	+	+
Mn1	+	+	-	-	-	-	-	-
Zn1	-	-	+	-	-	-	-	-
$MnCl_2$	-	-	-	-	-	+	+	-

Samples



Comparison of control vs LPS Mn1

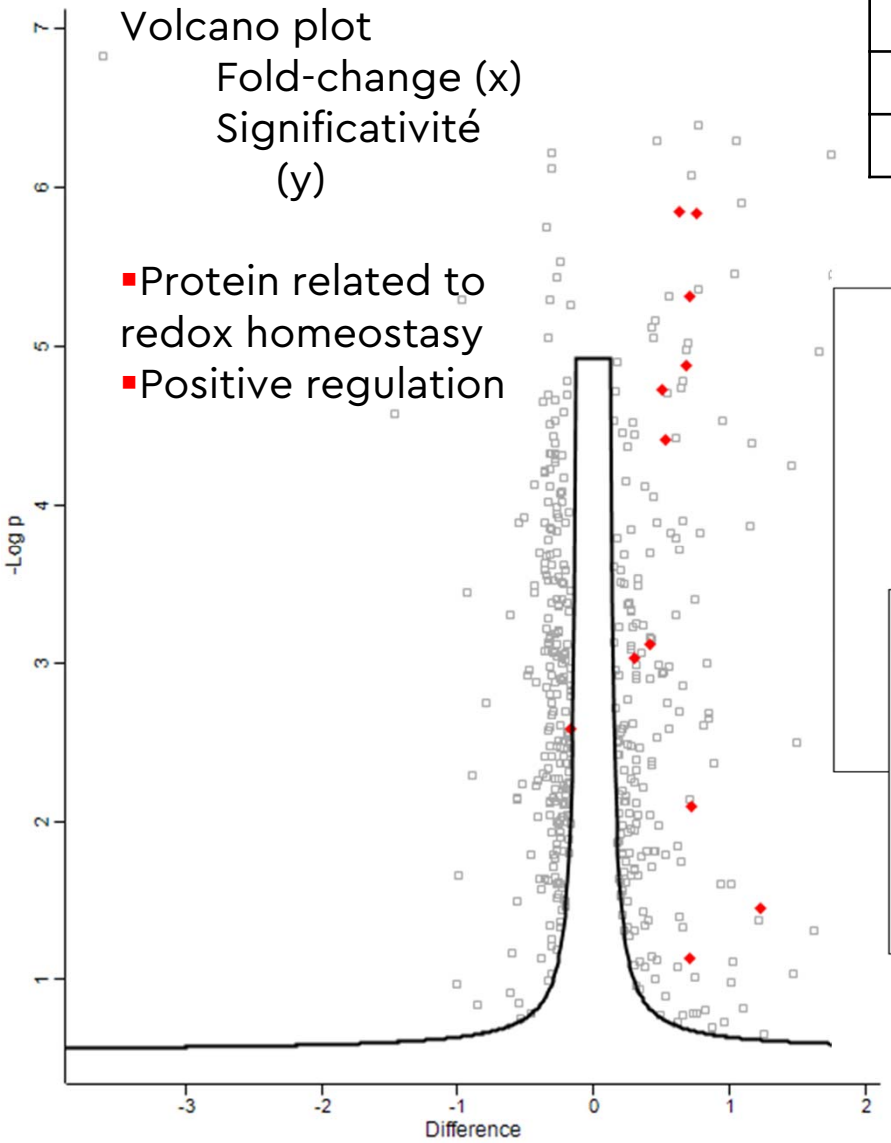
LPS	+	+	+	+	-	-	-
Mn1	-	+	-	-	+	-	-
Zn1	-	-	-	+	-	-	-
$MnCl_2$	-	-	+	-	-	+	-

Volcano plot

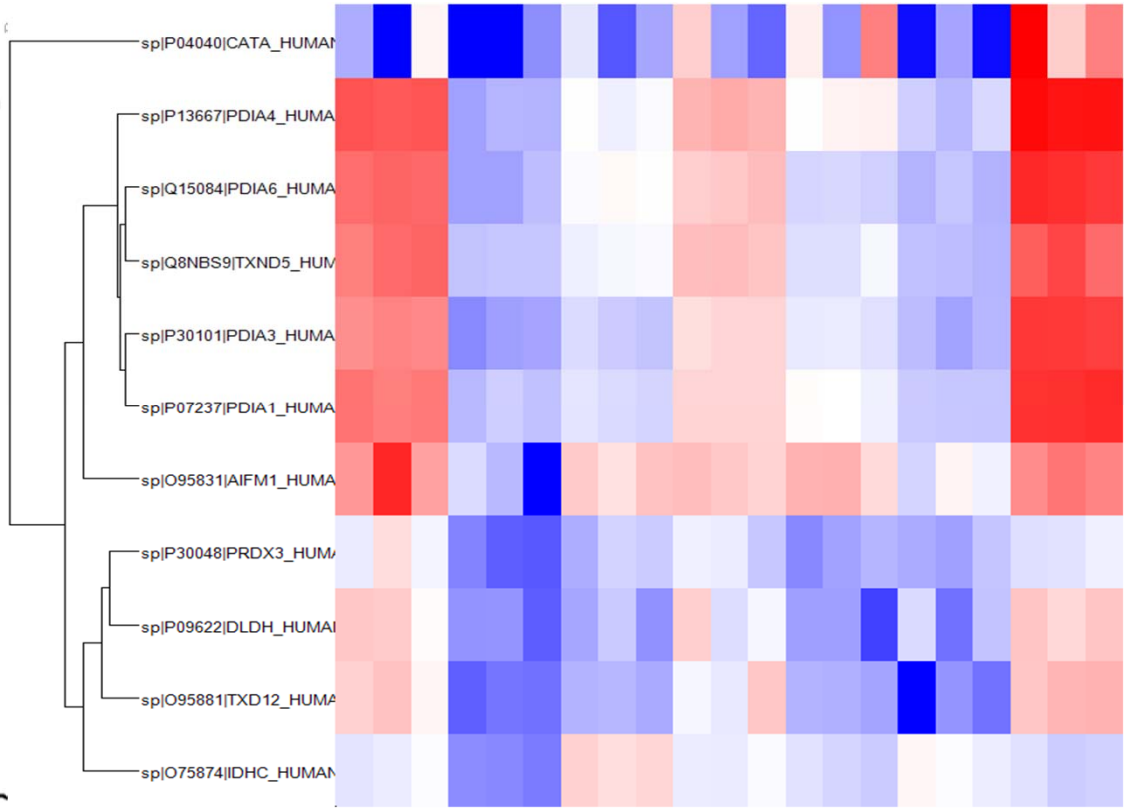
Fold-change (x)

Significativité (y)

- Protein related to redox homeostasy
- Positive regulation



Variations of redox proteins

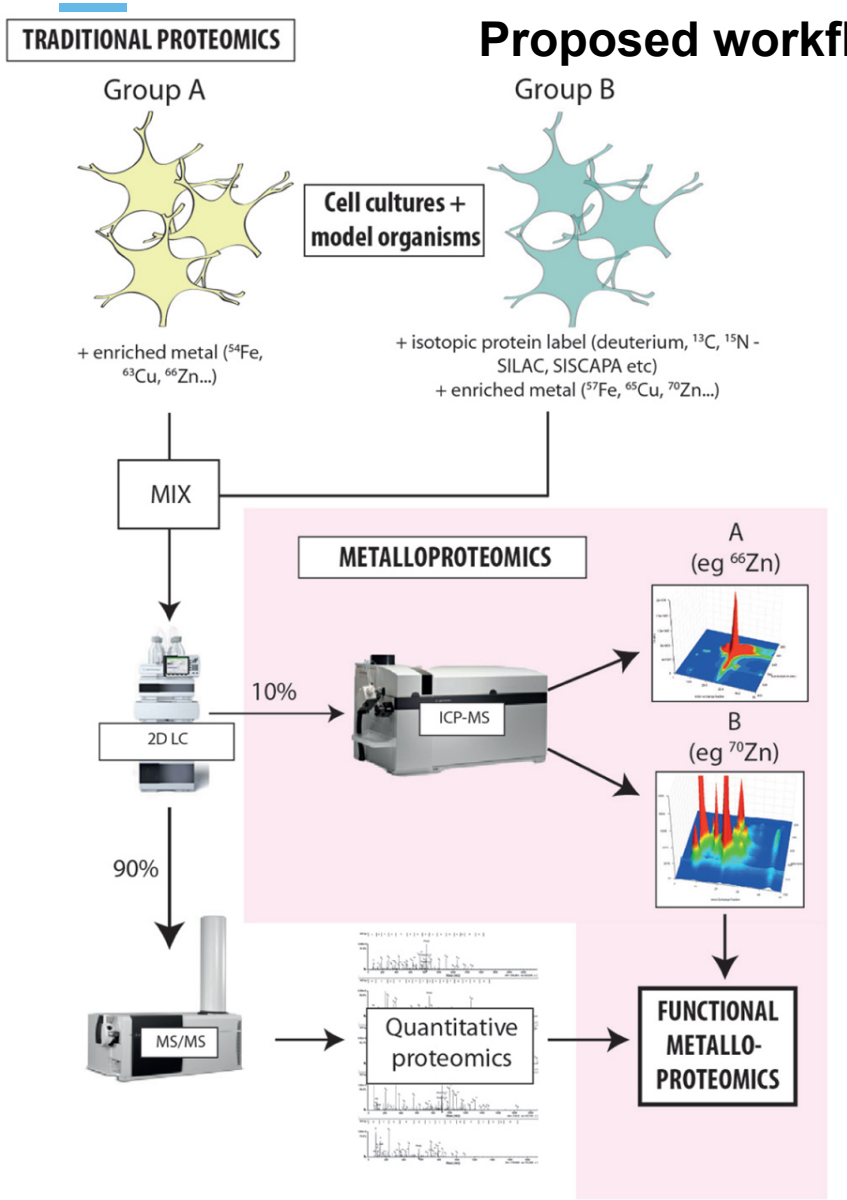


Results and follow up

- Résultats :
  - Signature de fragmentation de Mn1
  - Optimisation de la chromatographie liquide HILIC
  - Effet de Mn1 sur le protéome d'un modèle de MICI
- Application :
  - Identification de Mn1 et de ses métabolites
  - Etude simultanée du complexe et du protéome
  - Etude des effets de Mn1 sur les niveaux protéiques

- *Métabolisme de Mn1 dans un contexte cellulaire*
  - *Evaluation de la toxicité des métabolites*
- *Quantification OcSILAC*
  - *Proportion de cystéines oxydées*
- *ICP and complex speciation in cells*

The combination of techniques



Proposed workflow for integrated metalloproteomics

how metals do carry out biochemical processes in the cell
discover, identify, and characterize metalloproteins.

The workflow
existing isotope labeling techniques used for

addition of isotopically enriched metal salts,

simultaneous analysis of both metals and proteins in individual experimental groups.

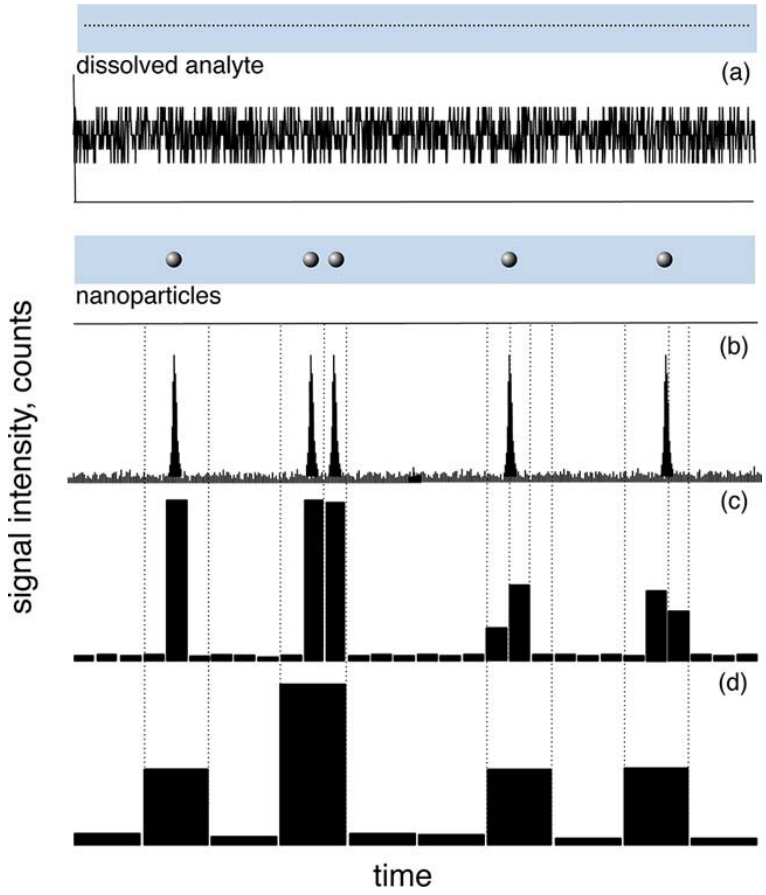
Highly sensitive, isotope-specific ICP-MS detection is used to align metal distribution with quantitative proteomics, directly associating the presence of a protein species with a specific, metal-mediated function.

A. Lothian et al , Front. Aging Neurosci., 2013, 5, 35.

NP-ICP-MS Nano Particules ICP-MS

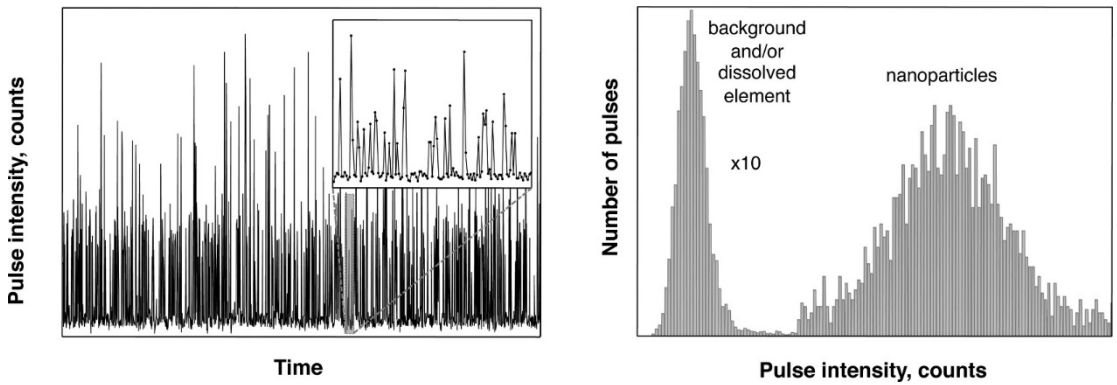
Time resolved ICPMS signals from
a solution (a) and
a nanoparticle suspension (b, c, and d) of the same element.

Frequency of data acquisition: (a and b) × 100, (c) × 3, and (d) × 1 (not in scale).

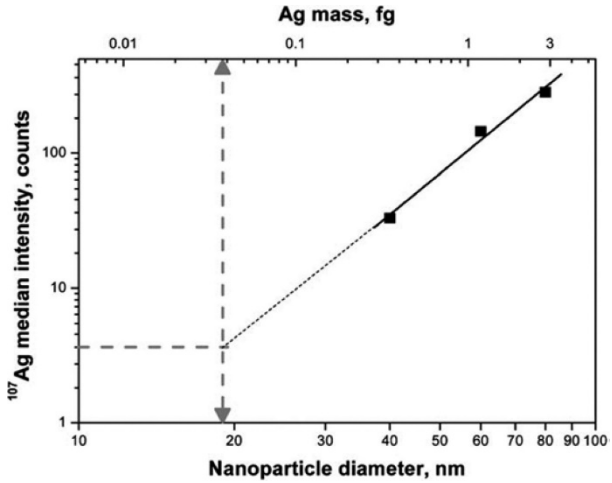


Published in: Francisco Laborda; Eduardo Bolea; Javier Jiménez-Lamana; *Anal. Chem.* **2014,** 86, 2270-2278.
DOI: 10.1021/ac402980q

NP-ICP-MS Nano Particules ICP-MS



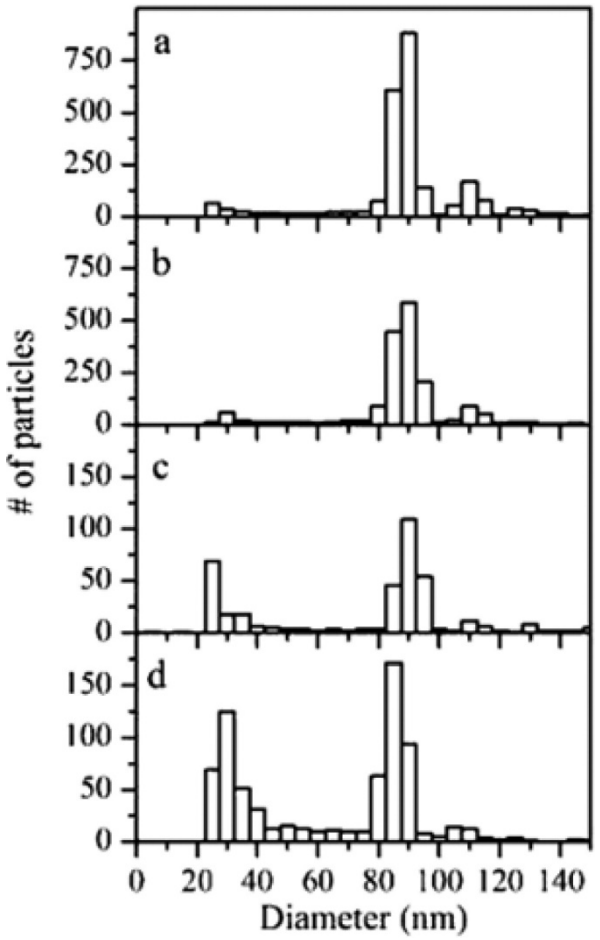
(a) Time scan of a nanoparticle suspension containing dissolved forms of the element contained in the nanoparticle.
(b) Pulse intensity frequency histogram of data from part a.



NP diameter/mass calibration vs 107Ag pulse intensity. Gray dashed lines: limits of detection (3σ criterion).

Francisco Laborda; Eduardo Bolea; Javier Jiménez-Lamana; *Anal. Chem.* **2014,** 86, 2270-2278.

NP-ICP-MS Nano Particules ICP-MS



at 0 h

after 144 h at 1000 µg L^{-1}

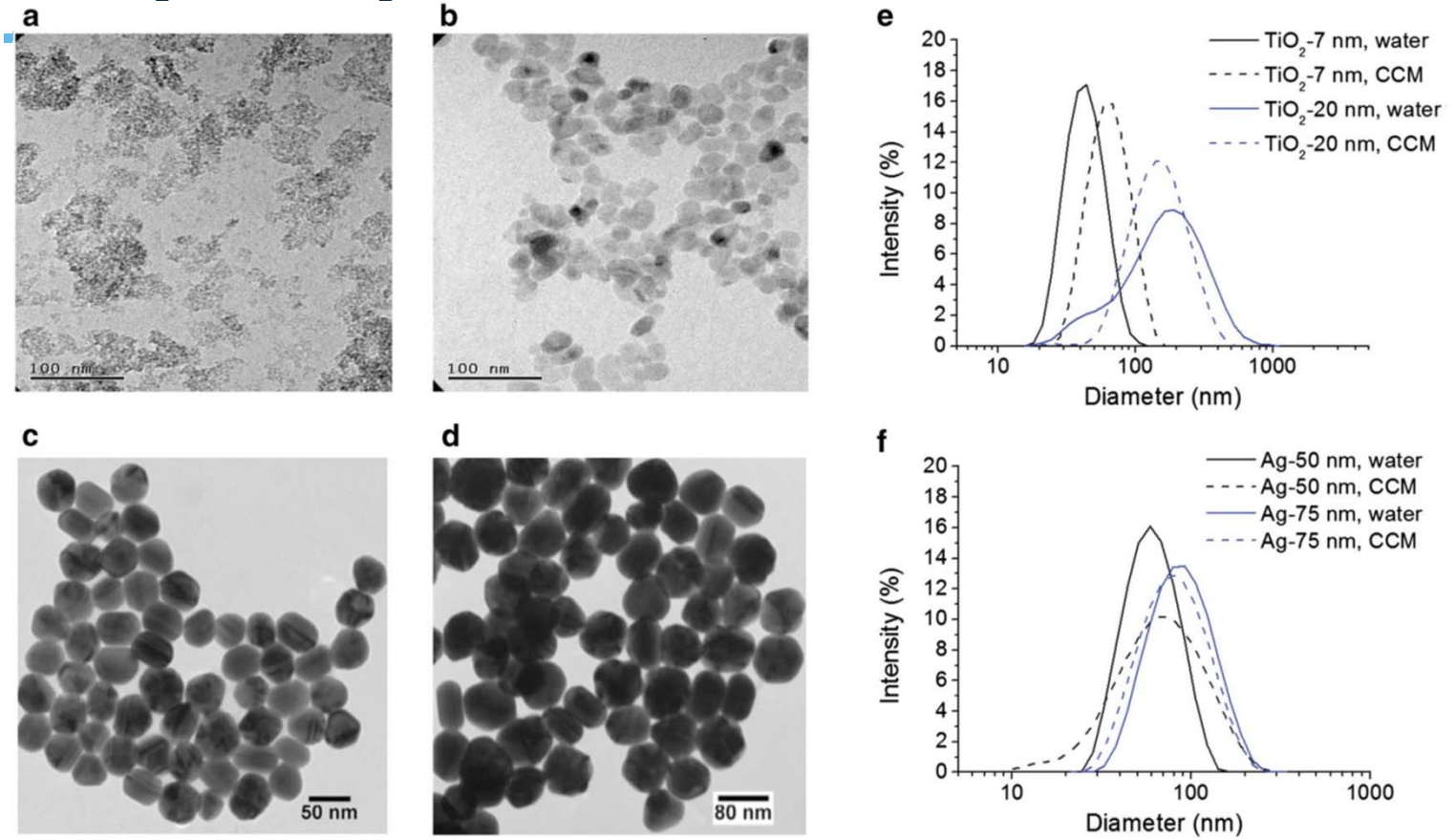
after 144 h at 50 µg L^{-1}

after 144 h at 6 µg L^{-1}

Nanoparticle size distribution of 100 nm silver nanoparticles in algal growth medium before and after incubation at different silver concentrations

Pace, H. E.; Rogers, N. J.; Jarolimek, C.; Coleman, V. A; Gray, E. P.; Higgins, C. P.; Ranville, J. F. Environ. Sci. Technol. 2012, 46, 12272– 12280[

Cellular uptake of NPs in Neuro-2a cells as analyzed by ICP-MS

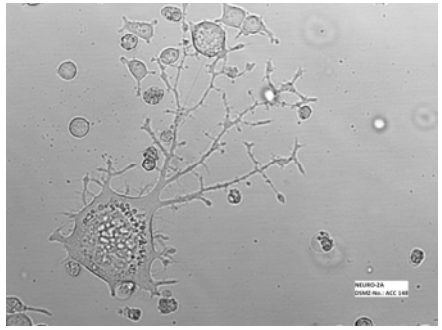


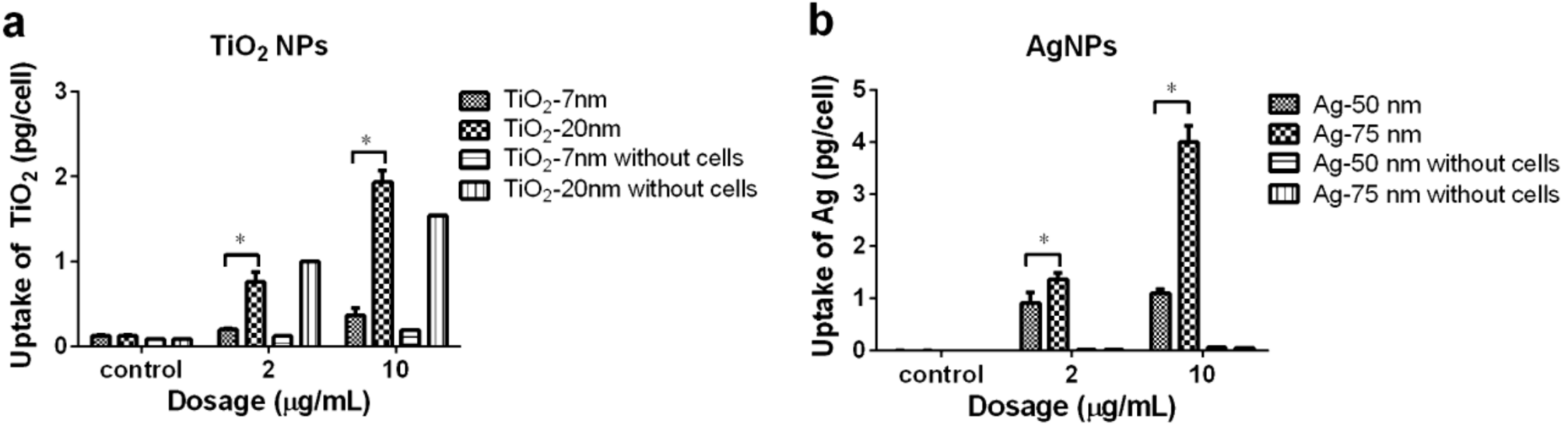
NP Characterization. TEM images of TiO_2 7 nm (**a**); TiO_2 20 nm (**b**); Ag 50 nm (**c**); Ag 75 nm (**d**). Images **c** and **d** were taken from NanoComposix.

Size distribution of TiO_2 (**e**) and Ag NPs (**f**) in water and complete cell culture medium (CCM) as measured by DLS

Cellular uptake of NPs in Neuro-2a cells as analyzed by ICP-MS

Cellular uptake of NPs in Neuro-2a cells as analyzed by ICP-MS for TiO2 NPs (a, 49Ti) and Ag NPs (b, 107Ag).

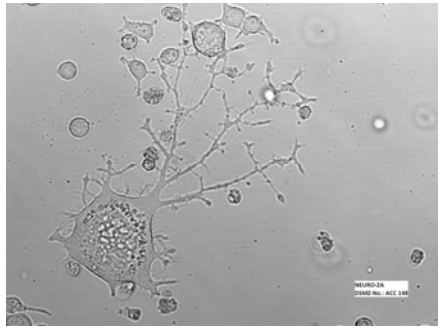




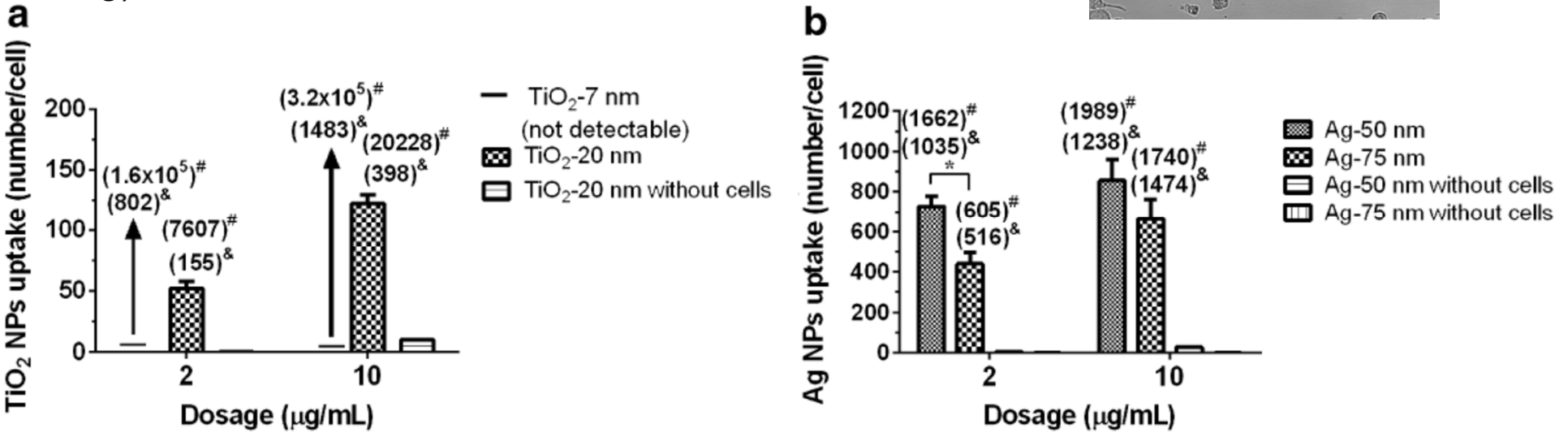
Quadrupole ICP mass spectrometer

TiO2 detected in samples without cells: NPs physically attach to cell culture plates
(*) indicates a significant difference between two treatment groups (student's t test $p < 0.05$)

Cellular uptake of NPs in Neuro-2a cells as analyzed by ICP-MS



Number-based cellular uptake of NPs in Neuro-2a cells as analyzed by SP-ICP-MS for TiO2 NPs (a, 49Ti) and Ag NPs (b, 107Ag).

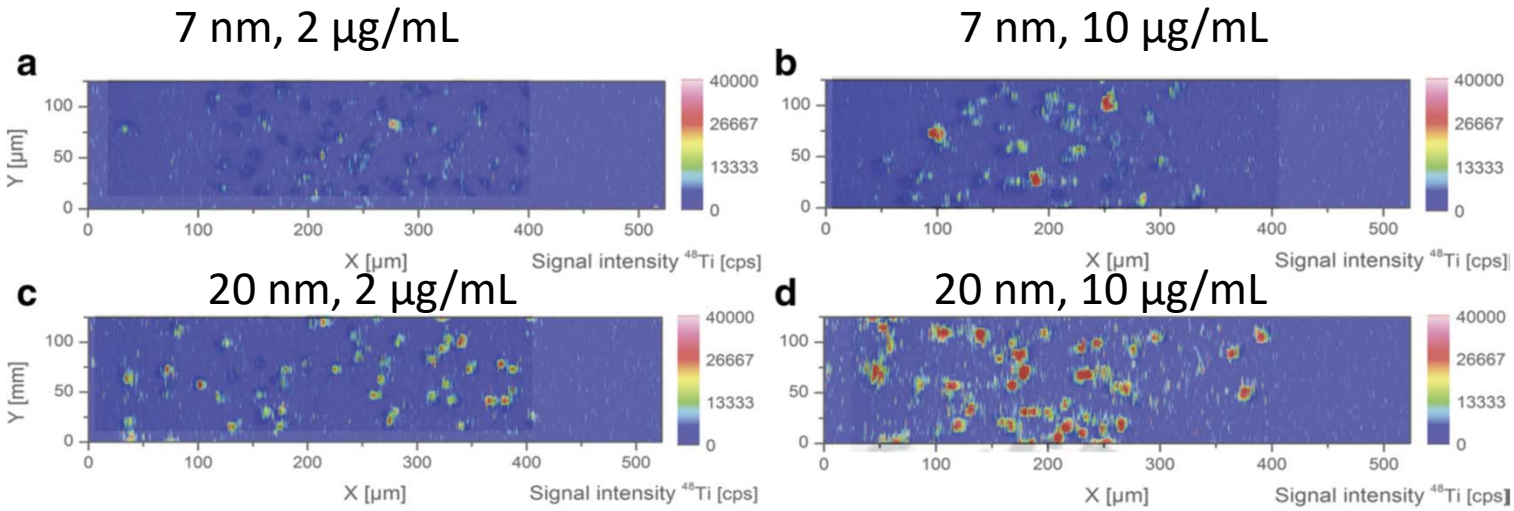


quadrupole ICP mass spectrometer, operated in spike mode (1 min per run, dwell time of 3 ms per reading).
The small TiO2 NPs cannot be detected by SP-ICP-MS because their size was below the limit of detection size (LODsize) of TiO2 (69 nm).

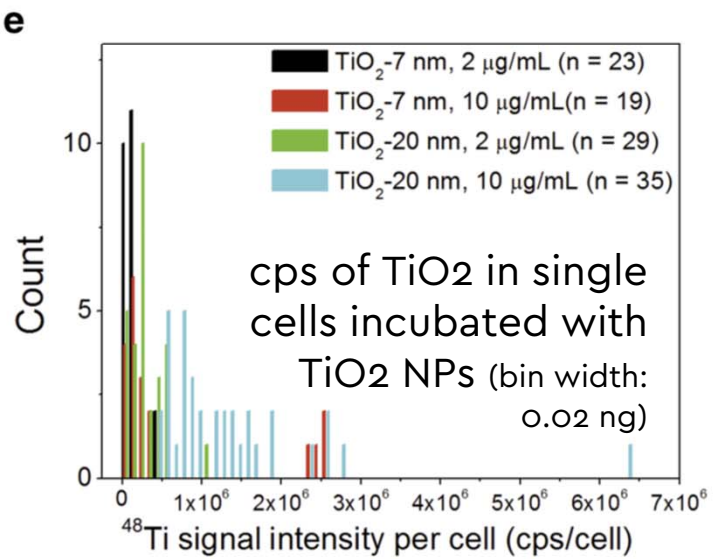
#Calculated number of NPs per cell using ICP-MS (mass) data. &Calculated number of NPs per cell using ICP-MS (mass) data based on DLS size. (*) indicates a significant difference between two treatment groups (student's t-test $p < 0.05$)

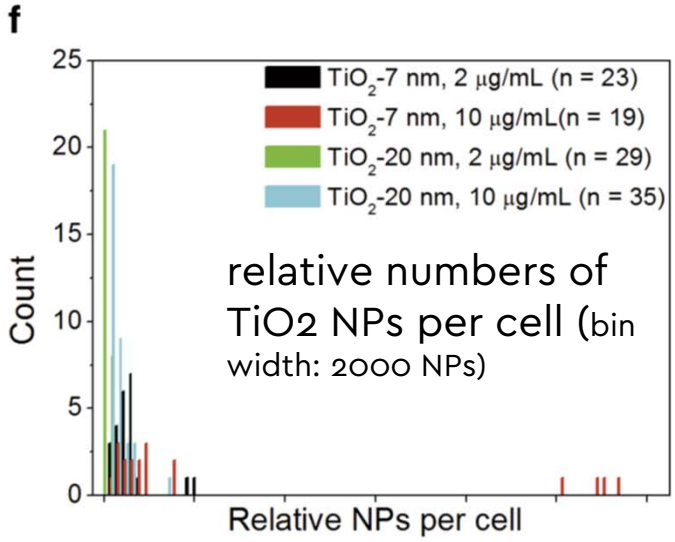
Cellular uptake of NPs in Neuro-2a cells as analyzed by ICP-MS

Cellular uptake of NPs of TiO2 at single cell level in Neuro-2a cells by LA-ICP-MS

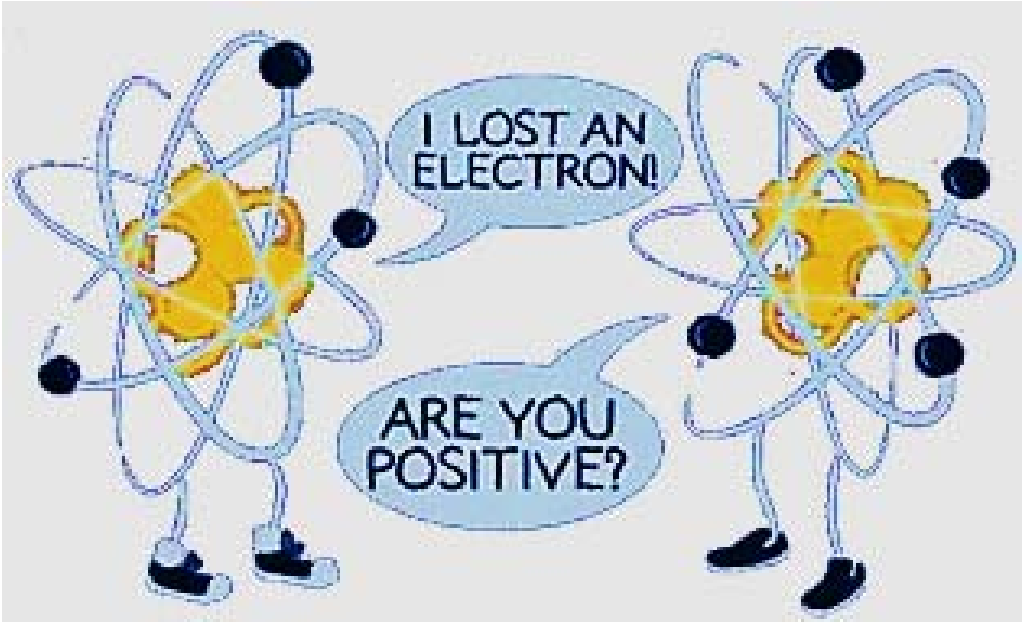


Overlapping contour plots of 48Ti with cell morphology after incubation with TiO_2 NPs





THANK YOU FOR YOUR ATTENTION



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