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, report analytical chemistry techniques to study biological systems


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

Oomics 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?

**Proteomics vs metalloproteomics**

**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*

**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


**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

Signal intensity is depicted by color saturation.


3000-7000 signals are visualized per peptide display: 1500-3000 peptides
Where is my compound?

IN THE GAULISH VILLAGE

The village is a hive of activity! All the Gauls are going about their business. How well do you know the villagers?

See if you can find:

Asterix
the most famous of all the Gauls

Obelix
his inseparable friend

Dogmatix
Obelix's faithful companion

Vitalstatistix
the chief of the tribe

Cacofonix
the village bard

Getafix
the venerable village druid

Score:
Laurel wreaths
Compounds separation

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


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


**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)


**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


Origins of heteroatoms in proteins and their complexes

Heteroelements and proteins

- S: methionine, cysteine
- Se: selenomethionine, selenocysteine
- I: mono-, di- triiodothyronine

Covalently bound
- derivatization with metal-containing covalent tags
- phosphoproteins
- heteroatom containing amino acids
- derivatized with metal-containing tags

Coordination complexes
- transport proteins
  - metal ions
  - metallo drugs
- enzymes
  - metal ions
- metal stress proteins
  - e.g. metallothioneins
- metal sensing proteins
  - e.g. metal responsive transcriptional activators

J. Szpunar, Analyst, 2000, 125, 963
Estimated size of the metalloproteome

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

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, pyroglutamylations, ...
- 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 radioactive 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)

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

**Mass units**

**Unit of Atomic Mass (U or AMU)**
1/12 of the mass of the isotope carbon 12
1 amu = 1.6605x10^{-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 : 1.672622x10^{-27}Kg=1.007 u
neutron : 1.675x10^{-27}Kg=1.009 u
electron : 9.11x10^{-31}Kg=0.0005 u

**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.)
Contribution of the different isotopes used in the composition of the polypeptides:

\[ \begin{align*}
\text{C} & : \\
^{12}\text{C} : & 98,9\% \\
^{13}\text{C} : & 1,1\% \\
\text{N} & : \\
^{14}\text{N} : & 99,7\% \\
^{15}\text{N} : & 0,3\% \\
\end{align*} \]

- **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
Relative abundance of monoisotopic masses \( M, M+1 \ldots \) for a model molecule containing \( n \) carbon atoms.

Respective abundances given by the development of the binomial theorem:

\[
(0.99^{12}C + 0.01^{13}C)^n = \sum_{p=0}^{n} C_n^p (0.99)^{n-p} (0.01)^p \left[ (12C)^{n-p}(13C)^p \right]
\]

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

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 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**—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 $\Delta M$ to a given mass. It is usually expressed by the $M/\Delta M$ 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**
**MS General Definitions**

**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.
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

\[ \frac{1}{2} m v^2 = z V \]

Ion’s kinetic E function of accelerating voltage (V) and charge (z).

\[ F = \frac{m v^2}{R} \]  
Centrifugal force

\[ F = B z v \]  
Applied magnetic field

\[ \frac{m v^2}{R} = B z v \]  
balance as ion goes through flight tube

Combine equations to obtain:

\[ \frac{m}{z} = B^2 R^2 / 2V \]  
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.

Magnetic Sector:
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

Voltage applied on opposite bars:
\[ U + V\cos(\omega t) \text{ and } -(U + V\cos(\omega t)) \] \( (\omega : \text{RF env } 10^8 \text{ Hz}) \)

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

Scanning \(U\) and \(V\) (\(U/V\) and \(\omega\) constants) \(\omega\) (\(U\) and \(V\) constant) to detect a whole m/z range
Ions trapping by applying a RF signal (MHz). Trajectory = Lissajous curve, frequencies \( f(m/z) \). The m/z of trapped ions is a function of \( V_0 \), 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...)

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Triple quadrupole and QTrap
**Time of Flight analyzer or TOF**

### Linear mode

- **Target**
- **Laser irradiation** $\gamma$
- **Linear detector**

**Acceleration region**

$+V_{\text{acc}}$

$\mathbf{L}$

**Field free region** ($E = 0$)

- **Kinetics:**

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

so $v_{\text{ion}}$ is a linear function of $\sqrt{\frac{Z}{m}}$ and $t_{\text{vol}}$ is proportional to $\sqrt{\frac{m}{Z}}$
**Analyseurs**

**FTICR : Fourier Transform Ion Cyclotron**

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

\[ F = zevB = \frac{mv^2}{R} \]
\[ w = \frac{zeB}{m} \]

**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

- Characteristics:
  - Frequency of rotation $\omega_\varphi$
    \[
    \omega_\varphi = \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1}
    \]
  - Frequency of radial oscillation $\omega_r$
    \[
    \omega_r = \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}
    \]
  - Frequency of axial oscillation $\omega_z$
    \[
    \omega_z = \sqrt{\frac{k}{m / q}}
    \]

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

Ions rings trapping

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

\[ \omega = \sqrt{\frac{k}{m / z}} \]
## A few comparisons

<table>
<thead>
<tr>
<th>Feature</th>
<th>IT-LIT</th>
<th>Q-Q-ToF</th>
<th>ToF-ToF</th>
<th>FT-ICR</th>
<th>Q-Q</th>
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<td>Additional capabilities</td>
<td>Seq. MS/MS</td>
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<td>Detection of modifications</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
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</table>

**Orbitrap**

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

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 $1.602 \times 10^{-19} \text{C}$, a mass of $9.108 \times 10^{-31} \text{kg}$, and spin of $\frac{1}{2}$, and existing independently or as the component outside the nucleus of an atom
**IONIZATION MODES**

**Inductively Coupled Plasma ICP**

- 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.

**IONIZATION MODES**

**MALDI : Matrix Assisted Laser Desorption Ionisation (1988)**

**Principle:** transform molecules in solid phase into ions in gas phase

**Incident Particule**
*(Primary Emission)*

- \( \lambda \ (337\text{nm}) \)
- Photons

**Mix Sample/ Matrix**

- Absorption of photons by matrix
- Relaxation of internal energy into roto-vibrational energy
- Dissociation of H bonds inter (intra) molecules
- Release of \( H^+ \) and \( \text{Cat}^+ \), formation of « plume » cloud
- Collisions and ions/molecules reactions -> protons transfer \( \Rightarrow M+H^+ \), \( M+\text{Cat}^+ \)

**Emitted Particule**
*(Secondary Emission)*

- Ions
- Electrons
- Neutral mol.

«P l u m e» cloud

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

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IONIZATION MODES

MALDI : Matrix Assisted Laser Desorption Ionisation (1988)
**Ionization Modes**


- Sample solution
- Taylor cone
- Counter electrode (qq 10 V)
- Sampling cone and skimmer
- Atmospheric pressure
- Vacuum ($10^{-6}$ mbar)
- Solvent evaporation
- Droplet fission at Rayleigh limit
- Generation of desolvated ions after cycles of the previous steps

$\text{N}_2$ → $\text{M}+\text{nH}^+ n^+$ → MS analyzer
Spraying Mode Effect on Droplet Formation and Ion Chemistry in Electrospays

Online Concentration and Affinity Separation of Biomolecules Using Multifunctional Particles in CE under Magnetic Field

Comprehensive 2D FFF/LC in the Analysis of Large Molecules
Quantification in MS

<table>
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<tr>
<th>Application</th>
<th>Accuracy (process)</th>
<th>Quantitative proteome coverage</th>
<th>Linear dynamic range*</th>
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<tbody>
<tr>
<td>Metabolic protein labeling</td>
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<td>1–2 logs</td>
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<td>+</td>
<td>2–3 logs</td>
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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 \(\text{x} \) 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

\[ w_x = w_{y,b} \cdot \frac{M_x \cdot m_y}{M_b \cdot m_x \cdot a_{x,b}} \cdot \left( \frac{R_y - R_{xy}}{R_{xy} - R_x} \right) \]
Stable isotope labelling for molecule quantification

Lysate with AQUA Peptide

Theoretical Ratio 1:1
Experimental Ratio 1.07:1

Internal Standards

Internal Standard Calibration

Detector Response

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

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

56Fe very low concentrations in environmental samples, but high interest

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

**Quadrupole** measurement = INTERFERENCE!

**HR-ICPMS** measurement = can distinguish 56Fe 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


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 H₂O and/or MeOH, independently of the counterion initially present ((e.g., [Cu(MeOH)]⁺) instead of Cu²⁺ 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

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\textsuperscript{v})  \( \text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O} \)
2. Dimethylarsinic acid (DMA)  \( (\text{CH}_3)_2\text{As(O)OH} \)
3. Arsenobetaine (AsB)  \( (\text{CH}_3)_3\text{As}^+\text{CH}_2\text{COOH} \)
4. Arseno sugar 1  \( R = \text{OH} \)
5. Arseno sugar 2  \( R = \text{OP(O)(OH)OCH}_2\text{CH(OH)CH}_2\text{OH} \)
6. Arseno sugar 3  \( R = \text{SO}_3\text{H} \)
7. Arseno sugar 4  \( R = \text{OSO}_3\text{H} \)
8. Trimethylarsenic oxide (TMAO)  \( (\text{CH}_3)_3\text{AsO} \)
9. Tetramethylarsonium ion (TMAs)  \( (\text{CH}_3)_4\text{As}^+\text{I}^- \)

M. Van Hulle, C. Zhang, X. Zhang, R. Cornelis
Analyst, 127 (2002), pp. 634-640
Anion-exchange HPLC-ICP-MS


Anion-exchange HPLC-ES-MS in MRM mode

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

kelp
Laminaria
Porphyra
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

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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:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Total S</td>
<td>2.45 mg/L (± 0.2 %)</td>
<td></td>
</tr>
<tr>
<td>Total Zn</td>
<td>2.21 mg/L (± 0.6 %)</td>
<td></td>
</tr>
<tr>
<td>Ratios:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{66}$Zn / $^{64}$Zn</td>
<td>0.657 ± 0.0028 (n = 7)</td>
<td></td>
</tr>
<tr>
<td>$^{67}$Zn / $^{64}$Zn</td>
<td>4.17 ± 0.025 (n = 7)</td>
<td></td>
</tr>
<tr>
<td>$^{68}$Zn / $^{64}$Zn</td>
<td>0.490 ± 0.0037 (n = 7)</td>
<td></td>
</tr>
<tr>
<td>$^{70}$Zn / $^{64}$Zn</td>
<td>0.01325 ± 0.00007 (n = 7)</td>
<td></td>
</tr>
</tbody>
</table>

$\Rightarrow$ 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: \(2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2\)
- Oxidative stress detoxifying enzyme
- Complex mimics of SOD « Mn1 »

Catalytic site of Mn-SOD
Spectre MS complexe et ligand

Complex characterization

Exact mass:
- 354.2163 Da
- 408.1465 Da

[Mn1]+

[L1+H]^+
MSn fragmentation signature

MS1

MS2

MS3

MS4

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**LC-MS : complexe separation**

**TIC**
- XIC 407.14
- XIC 408.14
- XIC 411.14
- XIC 417.14

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

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

**Modélisations L1-cation**
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 type of de peptidase

Exopeptidase clives sequentially at the end of the polypeptides
- N-ter
- C-ter
- Both N- and C-ter

Endopeptidase clives 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 basic residues at C-terminal end

**RAPPELSRLACTIDELATRYPSINESRLESLIAISNSPEPTIDIQES**

1 missed cleavage: R,
APPELSR,
LACTIDELATR,
YPSINESR,
LESLIAISNSPEPTIDIQES,
RAPPELSR,
APPELSRLACTIDELATR,
LACTIDELATRYPSINESR,
YPSINESRLESLIAISNSPEPTIDIQES
Bottom-up proteomics

D:\Data_Projets\QE131207_1765_c_chg
RSLC Sophie Colonne Chiara 50 cm

07-Dec-13 7:39:31 PM mix 1 tmt ikavir

NL: 1.32E10 TIC 55 QE131207

LC

Full scan MS

MS/MS

FrenchBIC 2017 - J. Vinh
Bottom-up proteomics

- 17 (NH₃)

- 28 (C=O)

$M_{aa \text{ Nter}} + 1$

$M_{aa \text{ Cter}} + 19$
Bottom-up proteomics

MS/MS fragmentation

Fragmentation of doubly charged peptide m/z = 701.40 Da
Identification of sequence HYQLNQQWER
## Analyse protéomique

### MASCOT MS/MS Ions Search

<table>
<thead>
<tr>
<th>Your name</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Search title</td>
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</tr>
<tr>
<td>Database</td>
<td>NCBI nr</td>
</tr>
<tr>
<td>Taxonomy</td>
<td>Homo sapiens (human)</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Name</td>
</tr>
<tr>
<td>Fixed modifications</td>
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<tr>
<td></td>
<td>Acetyl (N-term)</td>
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<tr>
<td></td>
<td>Amid (C-term)</td>
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<tr>
<td></td>
<td>Biotin (K)</td>
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</tr>
<tr>
<td>Protein mass</td>
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<tr>
<td>Peptide tol. ±</td>
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<td>Microarray (PKL)</td>
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<tr>
<td>Instrument</td>
<td>ESI-QUAD-TOF</td>
</tr>
<tr>
<td>Overview</td>
<td></td>
</tr>
</tbody>
</table>

### Références

1. Analyse protéomique
2. AGO_DROME
3. R2D2_DROME
4. ...
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 Isotope Labeling by Amino acids in Cell culture)**

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

Well suited for tryptic digests

<table>
<thead>
<tr>
<th>Inflammation</th>
<th>Traitement</th>
<th>T*: Heavy Culture (Arg-10 / Lys-8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non traitée</td>
<td>+ MnCl$_2$</td>
<td>+ MnCl$_2$</td>
</tr>
<tr>
<td>Ø</td>
<td>+ Mnl</td>
<td>Ø</td>
</tr>
<tr>
<td>Ø + LPS</td>
<td>+ Mnl</td>
<td>+ ZnI</td>
</tr>
<tr>
<td>Ø + LPS</td>
<td>+ MnCl$_2$</td>
<td>+ Mnl</td>
</tr>
<tr>
<td>Ø + LPS</td>
<td>+ ZnI</td>
<td>Non traitée</td>
</tr>
<tr>
<td>+ LPS</td>
<td>+ Mn1</td>
<td></td>
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<tr>
<td>+ LPS</td>
<td>+ Zn1</td>
<td></td>
</tr>
<tr>
<td>+ LPS</td>
<td>+ Mn1</td>
<td></td>
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</tbody>
</table>
HEATMAP of « SILAC » samples

<table>
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<th>+</th>
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<th>-</th>
<th>+</th>
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<th>+</th>
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</thead>
<tbody>
<tr>
<td>Mn1</td>
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<td>MnCl₂</td>
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</table>

Samples

Proteins

Log₂(Ratio H/l)
Comparison of control vs LPS Mn1

Volcano plot
Fold-change (x)
Significativité (y)

- Protein related to redox homeostasy
- Positive regulation

<table>
<thead>
<tr>
<th></th>
<th>LPS</th>
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<tbody>
<tr>
<td>Mn1</td>
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<td>MnCl₂</td>
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</tbody>
</table>

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.

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).
(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).

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

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 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

7 nm, 2 μg/mL

Overlapping contour plots of 48Ti with cell morphology after incubation with TiO2 NPs

7 nm, 10 μg/mL

20 nm, 2 μg/mL

relative numbers of TiO2 NPs per cell (bin width: 2000 NPs)

cps of TiO2 in single cells incubated with TiO2 NPs (bin width: 0.02 ng)
THANK YOU FOR YOUR ATTENTION

ESPCI SMBP Paris

- Joëlle Vinh DR CNRS
- Iman Haddad, Emmanuelle Demey IE CNRS
- Giovanni Chiappetta IR CNRS
- Yann Verdier MC VdP
- Shakir Shakir, et Ha Phuong Ta, Post-doc
- Alexandra Emmanuel, Sergio Duque Gonzalez, Nicolas Eskenazi, PhD