



Institut Jacques Monod



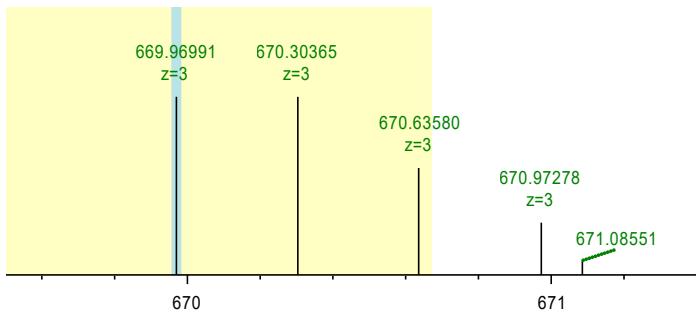
université
PARIS
DIDEROT
PARIS 7



Production of omics data: Proteomics

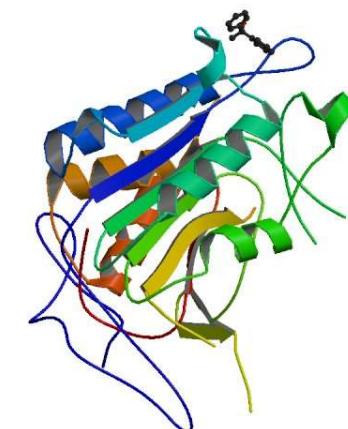
Thibaut Léger, PhD

IJM proteomics facility

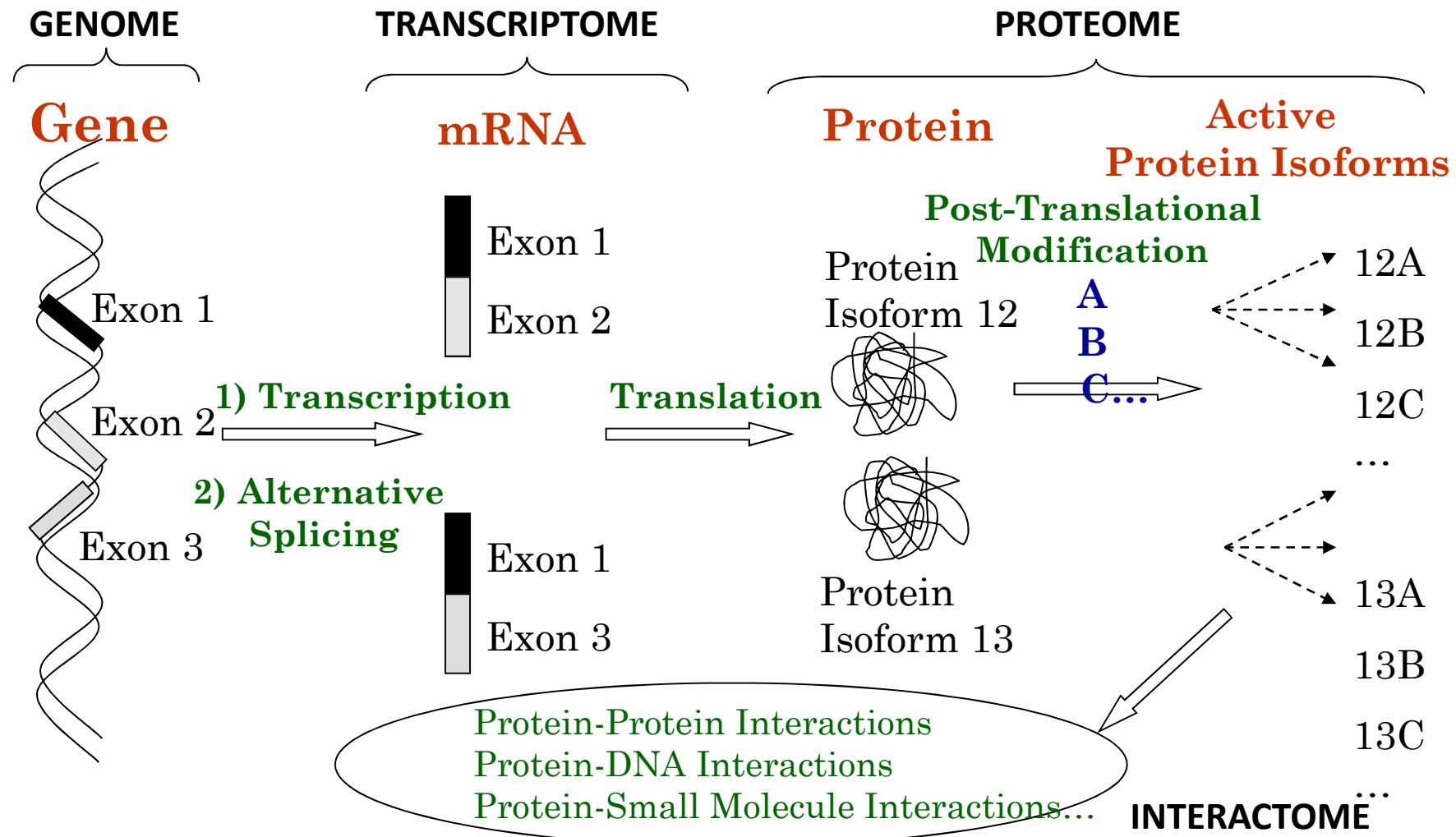


DUBii

05th feburay 2019



CONCEPT



GENOMICS vs PROTEOMICS

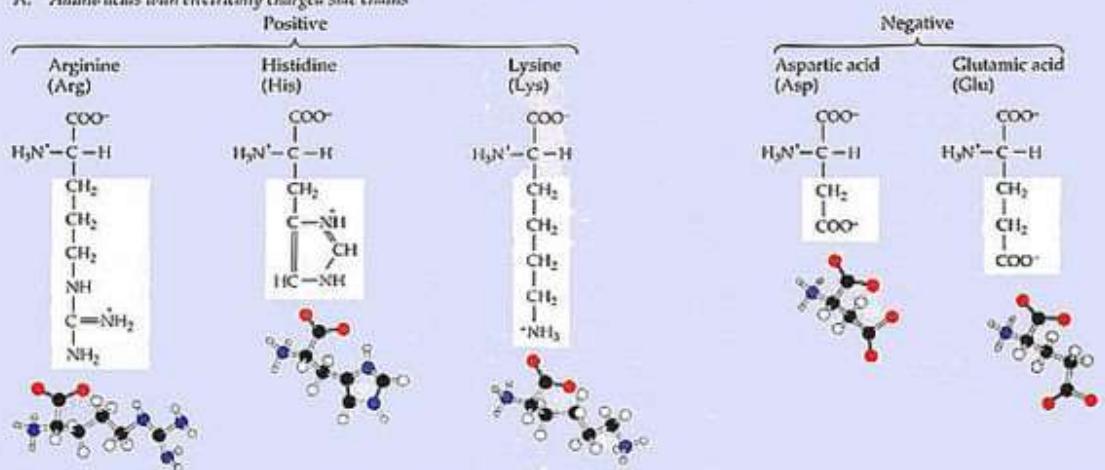
Genome (DNA)

- Static (no change with time)
- Can be amplified (PCR)
- Little sample complexity
- (*4 base pairs, very similar, same order of concentration*)
- Good solubility

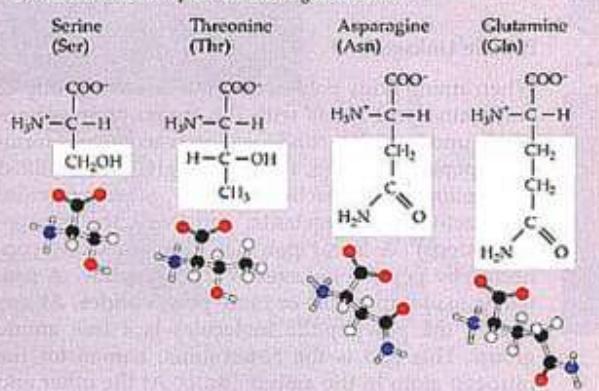
Proteome (proteins)

- Dynamic
- (*highly variable with time; many proteomes for one genome*)
- Cannot be amplified
- High sample complexity (wide variety of physical and chemical properties; concentrations can differ by 9 orders of magnitude)
- Various solubility; some proteins are insoluble in water

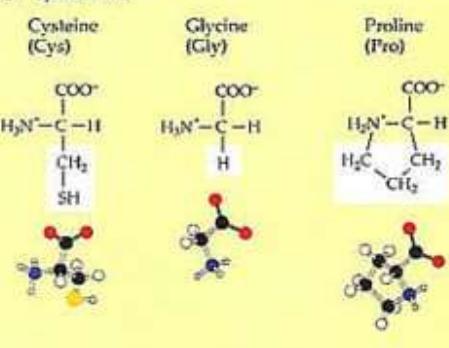
A. Amino acids with electrically charged side chains



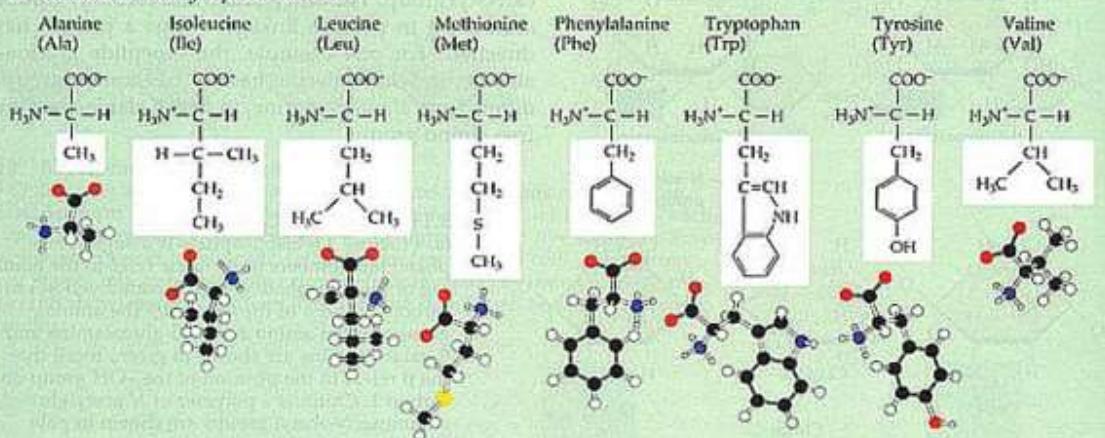
B. Amino acids with polar but uncharged side chains



C. Special cases



D. Amino acids with hydrophobic side chains



<u>Alanine</u>	A, Ala	71.079
<u>Arginine</u>	R, Arg	156.188
<u>Asparagine</u>	N, Asn	114.104
<u>Aspartic acid</u>	D, Asp	115.089
<u>Cysteine</u>	C, Cys	103.145
<u>Glutamine</u>	Q, Gln	128.131
<u>Glutamic acid</u>	E, Glu	129.116
<u>Glycine</u>	G, Gly	57.052
<u>Histidine</u>	H, His	137.141
<u>Isoleucine</u>	I, Ile	113.160
<u>Leucine</u>	L, Leu	113.160
<u>Lysine</u>	K, Lys	128.17
<u>Methionine</u>	M, Met	131.199
<u>Phenylalanine</u>	F, Phe	147.177
<u>Proline</u>	P, Pro	97.117
<u>Serine</u>	S, Ser	87.078
<u>Threonine</u>	T, Thr	101.105
<u>Tryptophan</u>	W, Trp	186.213
<u>Tyrosine</u>	Y, Tyr	163.176
<u>Valine</u>	V, Val	99.133

Proteomics?

- Proteomics is the large-scale study of proteomes, it means all proteins from a cell, an organelle, a tissue, an organ or from an organism at a one point, under specific conditions.
- Proteomics is at the crossroads of biochemistry, analytical chemistry and bioinformatics.

⇒ Proteins can be modified by different biological or chemical processes; The different variants of proteins are called now:

Proteoforms

Nat Methods. 2013 Mar;10(3):186-7. doi: 10.1038/nmeth.2369.

Proteoform: a single term describing protein complexity.

Smith LM, Kelleher NL; Consortium for Top Down Proteomics

PROTEOMICS GOALS

- Identification of all proteins in a proteome
- Search for new, hypothetical or predicted proteins
- Analysis of differential expression between 2,3,... different conditions (protein up- or downregulation)
- Identification of post-translational modifications
- Characterization of proteins by function, pathway, cellular location, etc.
- Study of protein-protein interactions

Proteomics techniques

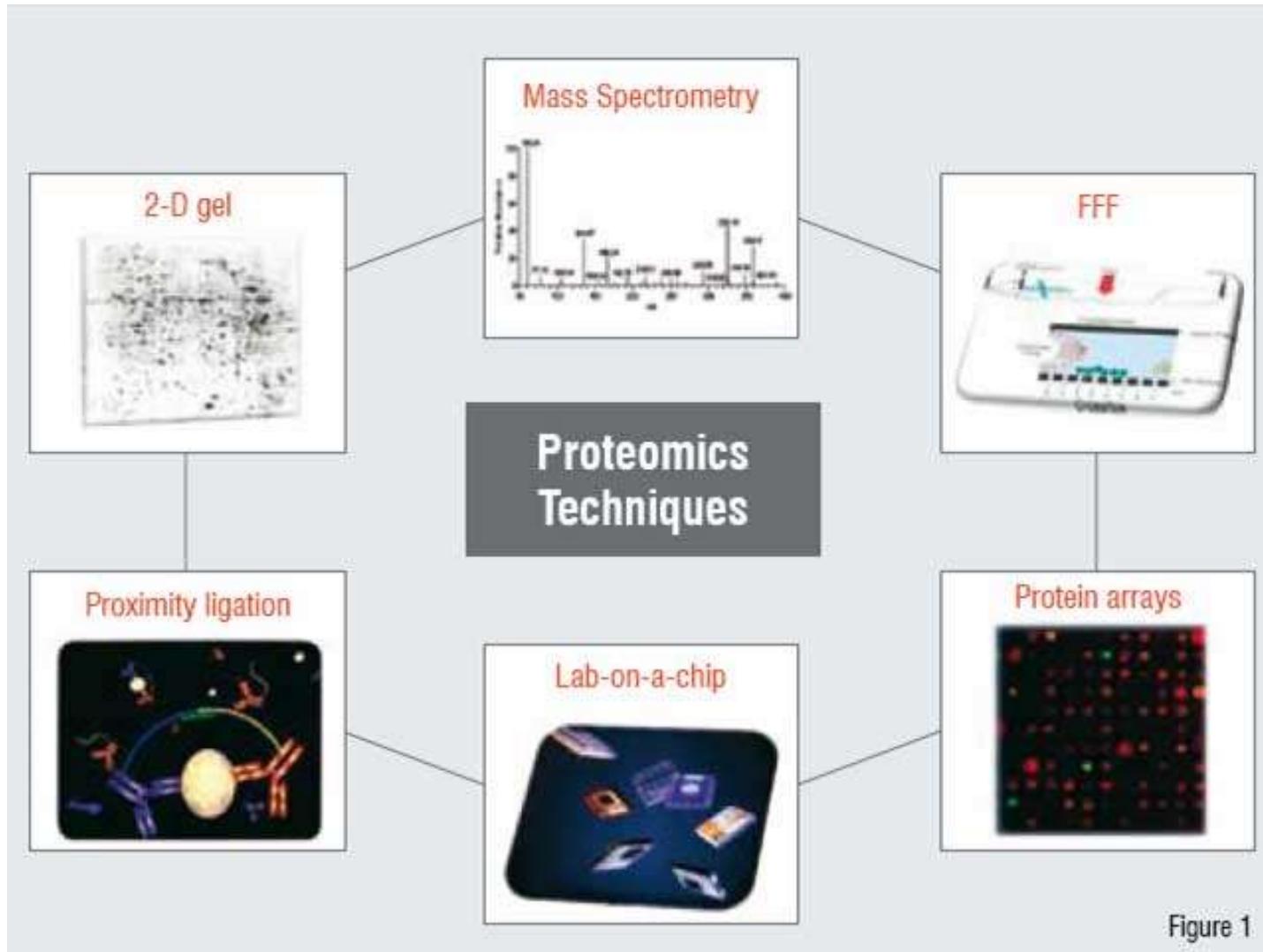


Figure 1



HUMAN PROTEOME MAP

Statistics

Organs/cell types	30
Genes identified	17,294
Proteins identified	30,057
Peptide sequences	293,700
N-terminal peptides	4,297
Splice junctional peptides	66,947
Samples	85
Adult tissues	17
Fetal tissues	7
Cell types	6

Welcome to ProteomicsDB!

ProteomicsDB is a joint effort of the Technische Universität München (TUM) and the Max-Planck-Institut für Biochemie (MPI-B) to support the study of the human proteome and its use across the scientific community.



Browse proteins

Explore the human proteome protein by protein.

Status

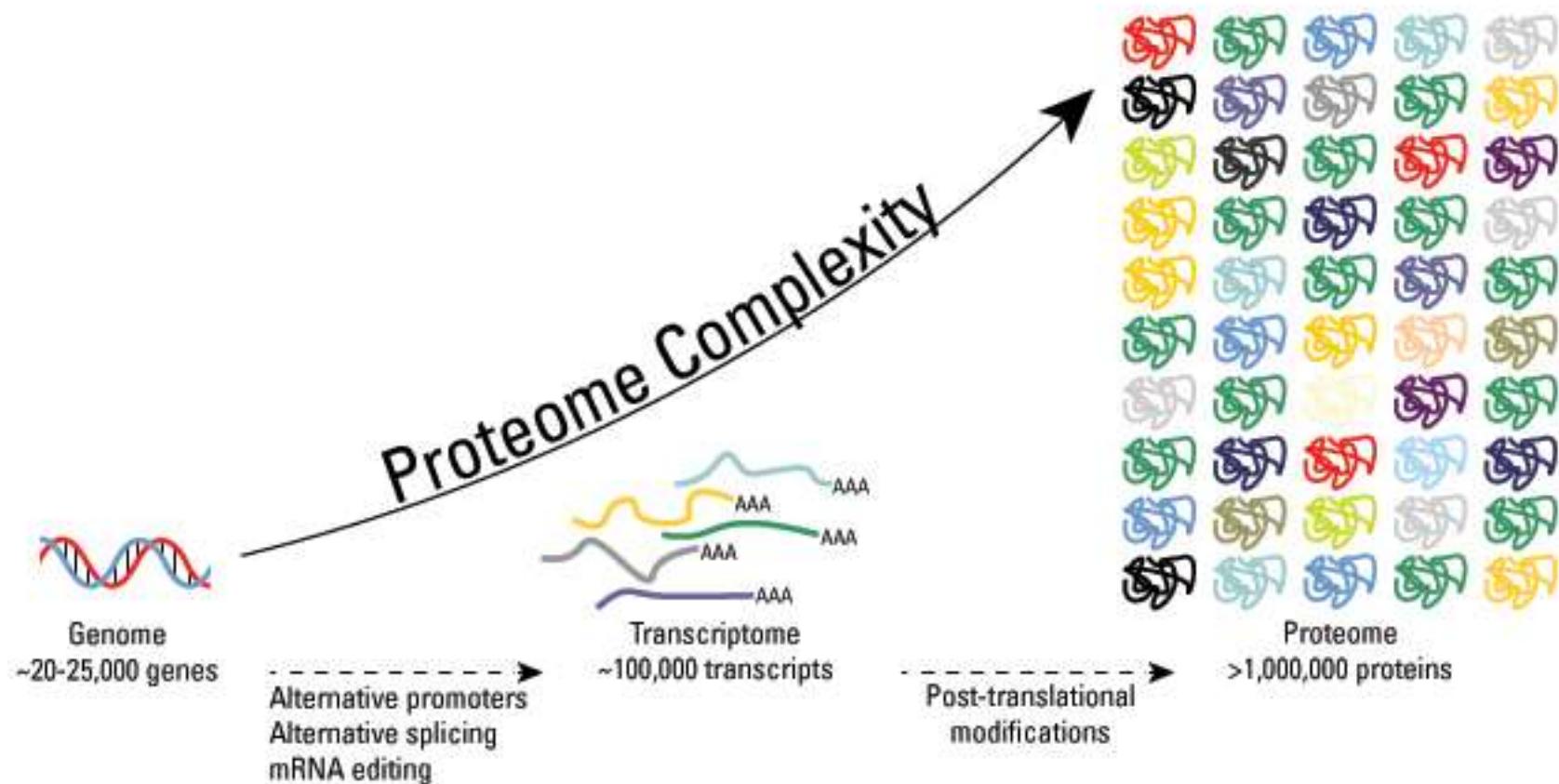
Human Proteome

Coverage:	80%
Proteins:	15721 of 19629
Isoforms:	11353 of 86771
Unique Peptides (Isoform):	113944
Unique Peptides (Gene):	455289
Spectra:	43237800

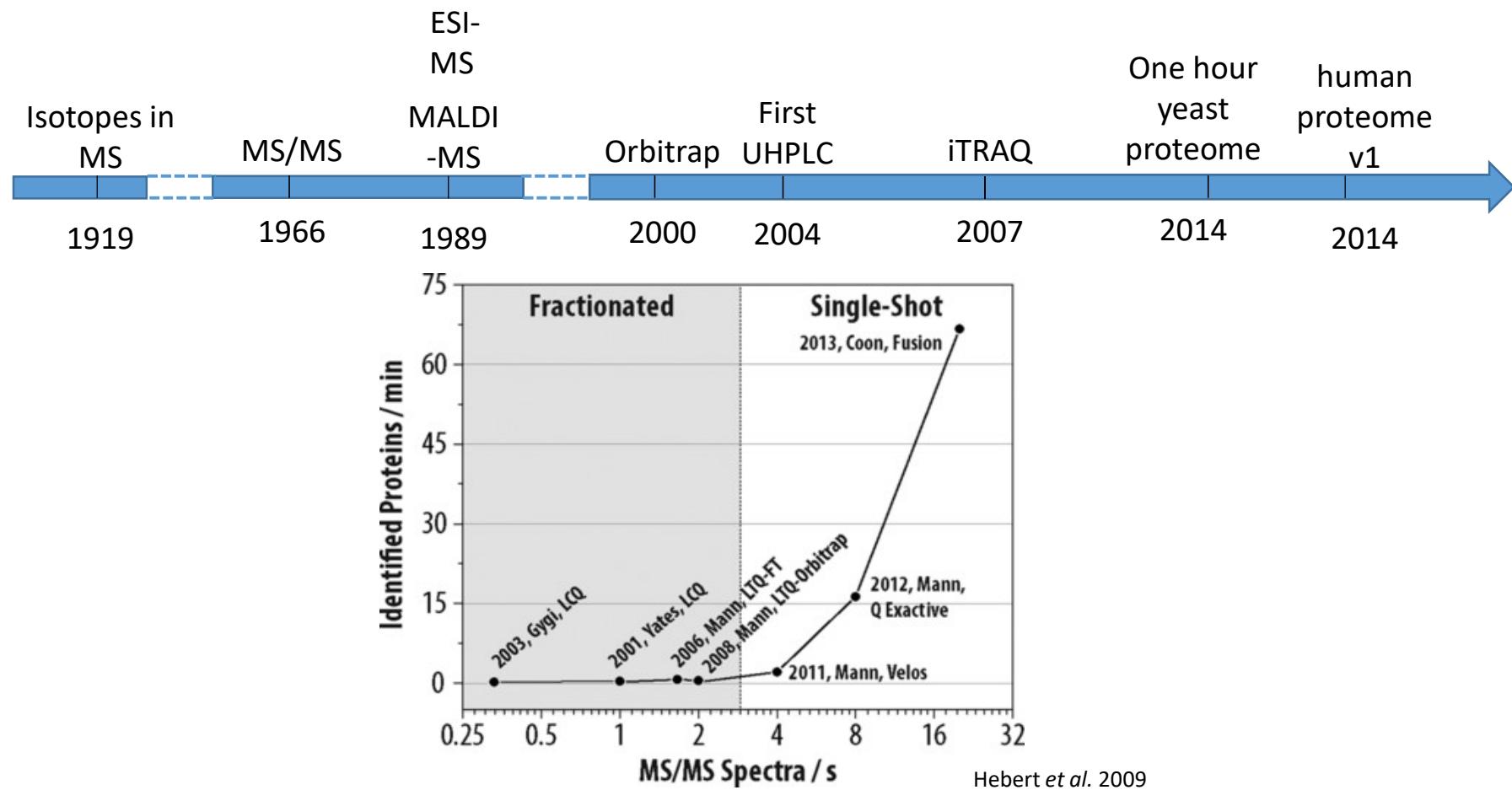
Repository

Registered Users:	533
Projects:	75
Experiments:	397
Files:	19459
Data Volume:	7.84 TB

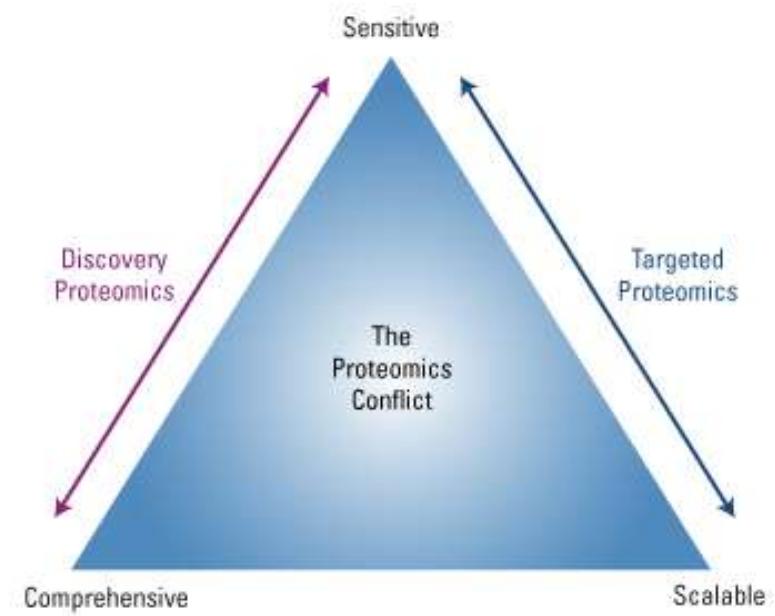
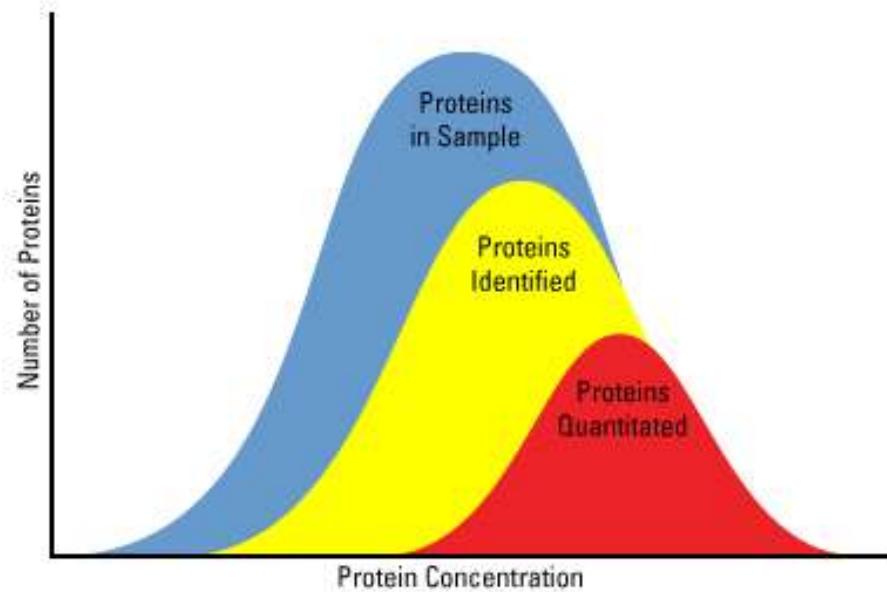
Problem of proteome complexity



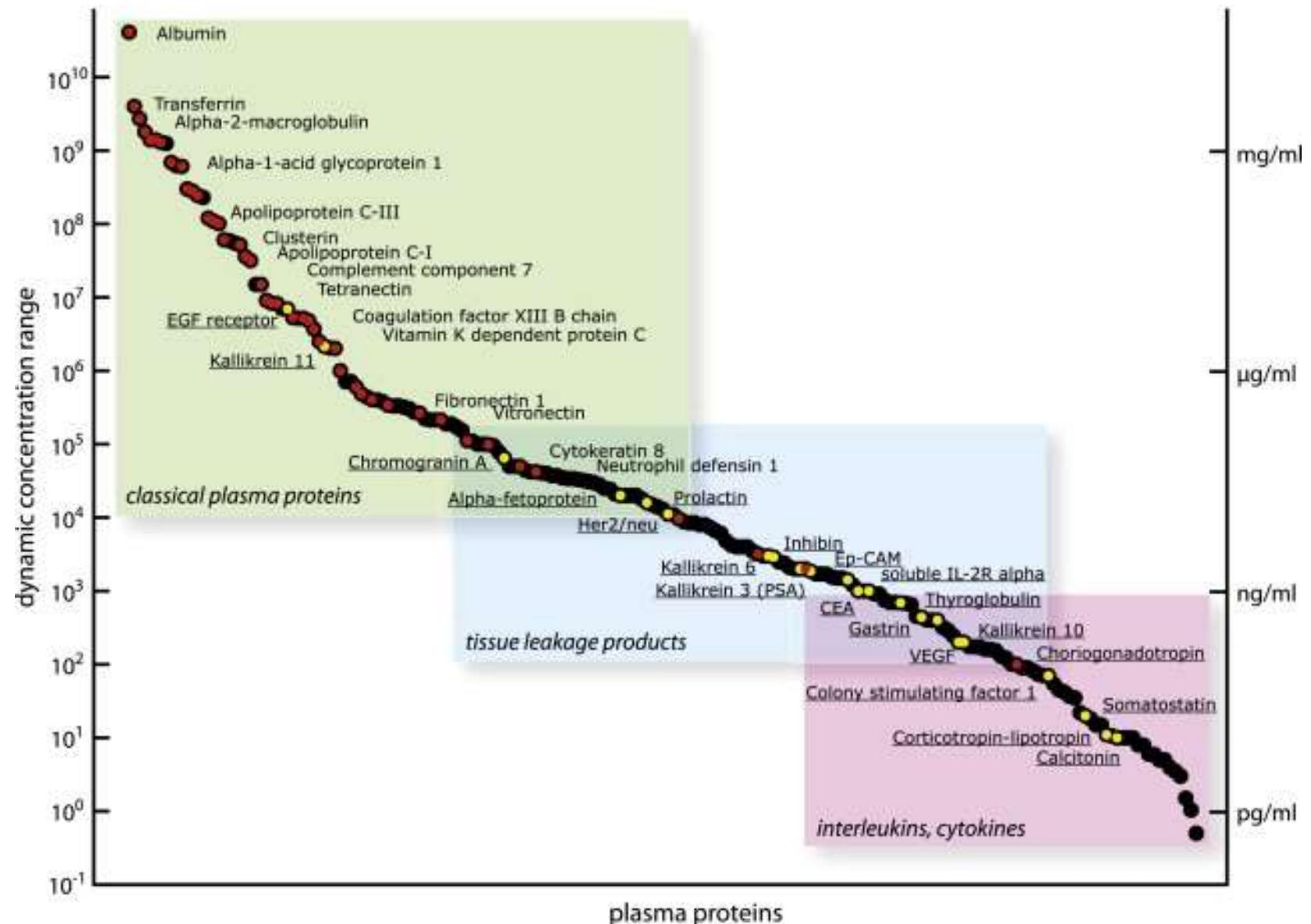
Evolution of proteomics performances



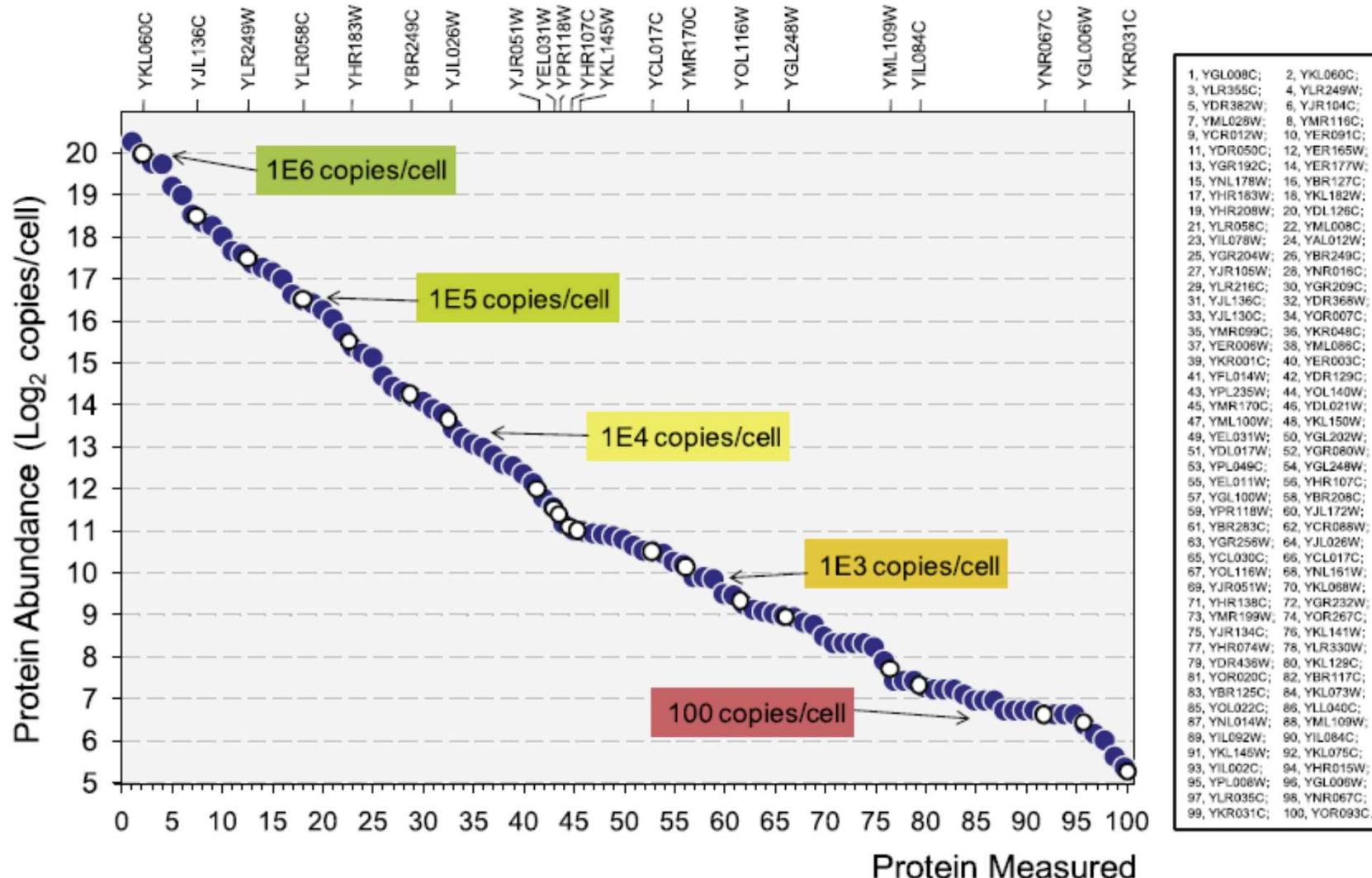
Inherent dilemma linked to proteomics



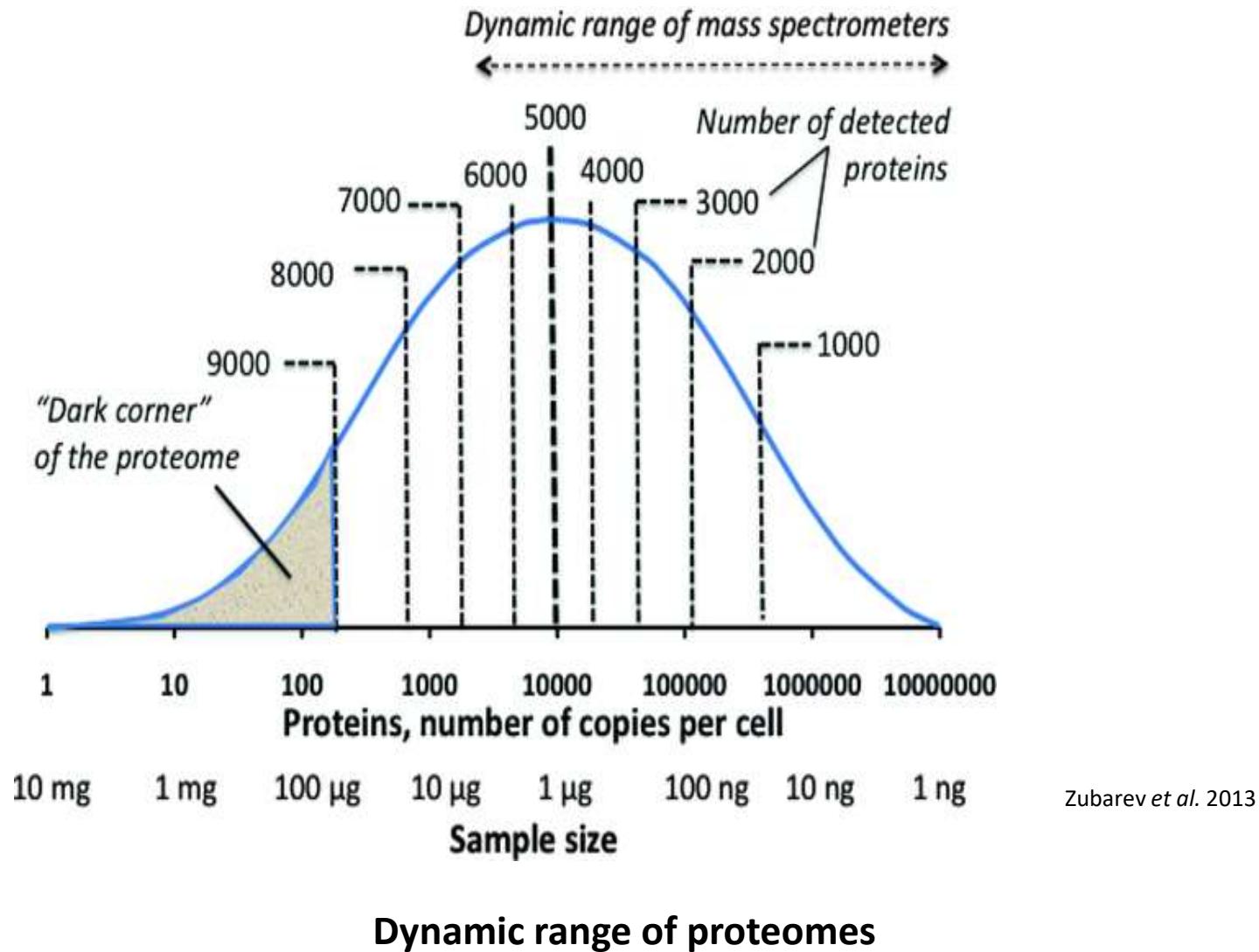
Dynamic range in human plasma



Dynamic range for *Saccharomyces cerevisiae* proteome



Proteomics and proteome coverage



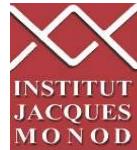
Key questions in proteomics

- What is the protein content of my biological sample?
=> problem of **identification**
- What is the abundance of my protein of interest?
=> **quantification problem**
- Relative question: What are the protein abundance variations of the proteomes studied?
- What are the partners of my protein of interest?
- Are there any signature proteins related to a particular biological process?
=> **biomarkers identifications and quantifications**

MISSIONS

- **Identification and quantification of proteins by mass spectrometry**
 - Proteins in gel (1D or 2D)
 - Simple or complex protein samples (sub-proteomes, co-IP,...)
 - Proteins in solution (top-down and bottom-up approaches)
 - Identification, characterization (mutations, PTMs,...), quantification
- **Quality control** (recombinant peptides or proteins)
- **System maintenance**
- **Formation and training** (Master 1 & 2, Doctoral schools)
- **Quality: ISO9001 certification**
- **R&D:**
 - **Biological projects and methodological developments**
 - **Biomarkers and bioinformatics** (proteolysis in pathology)
 - **Mass spectrometry imaging** (Julie Le Faouder, Pr P. Bedossa, Hosp. Beaujon, Clichy)
- **Valorisation (congress and publications)**

Functional organization chart of the Jacques Monod Institute



Institut Jacques Monod

Unité mixte de recherche du CNRS (UMR7592) et de l'Université Paris Diderot

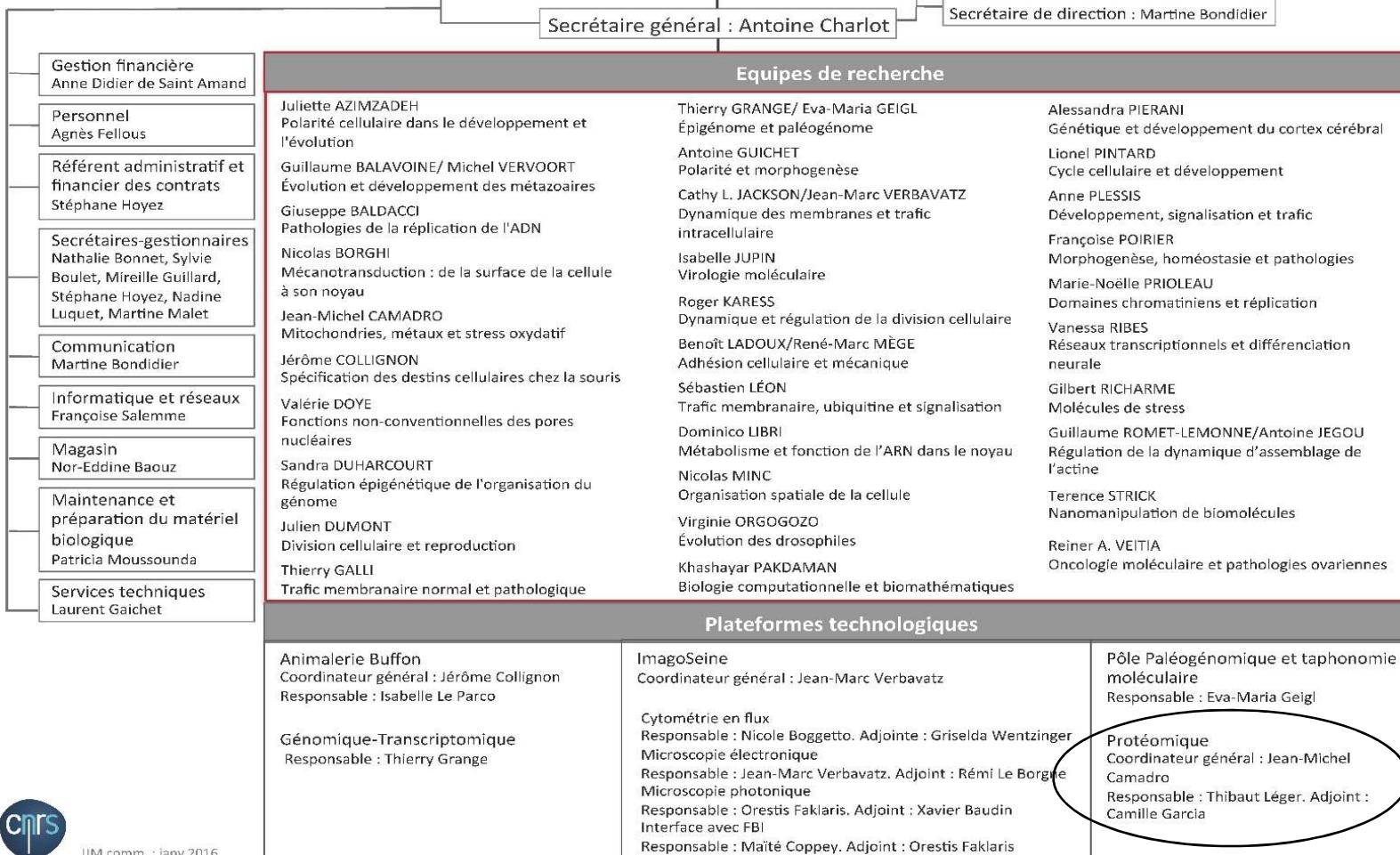
Directeur adjoint : Roger Karess

Directeur : Giuseppe Baldacci

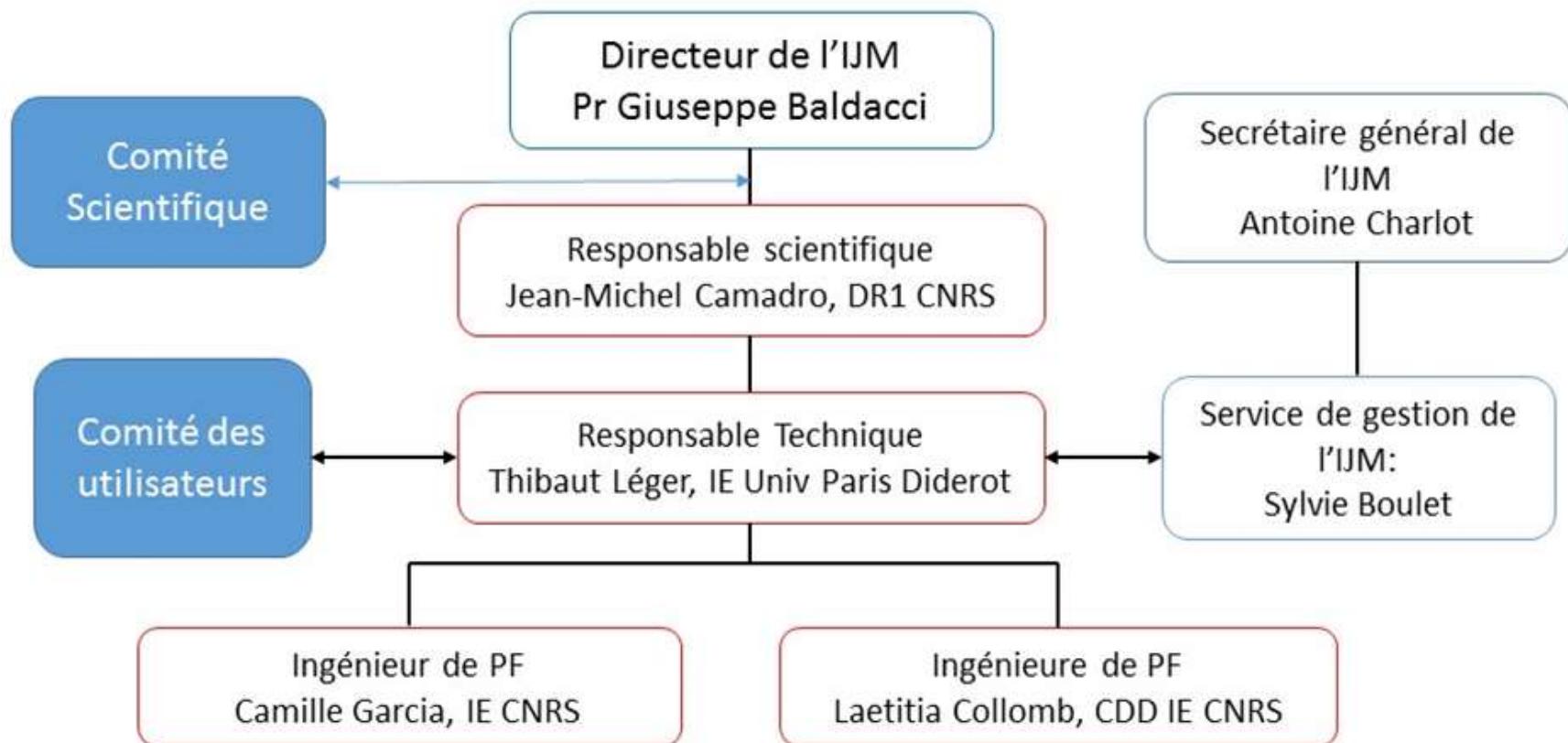
Prévention et sécurité : Laurent Gachet

Secrétaire général : Antoine Charlot

Secrétaire de direction : Martine Bondidier



Functional organization chart of the proteomics facility



A collaborative environment

85 research teams (136 researchers, feb 2014-feb 2016) :

Paris Diderot:

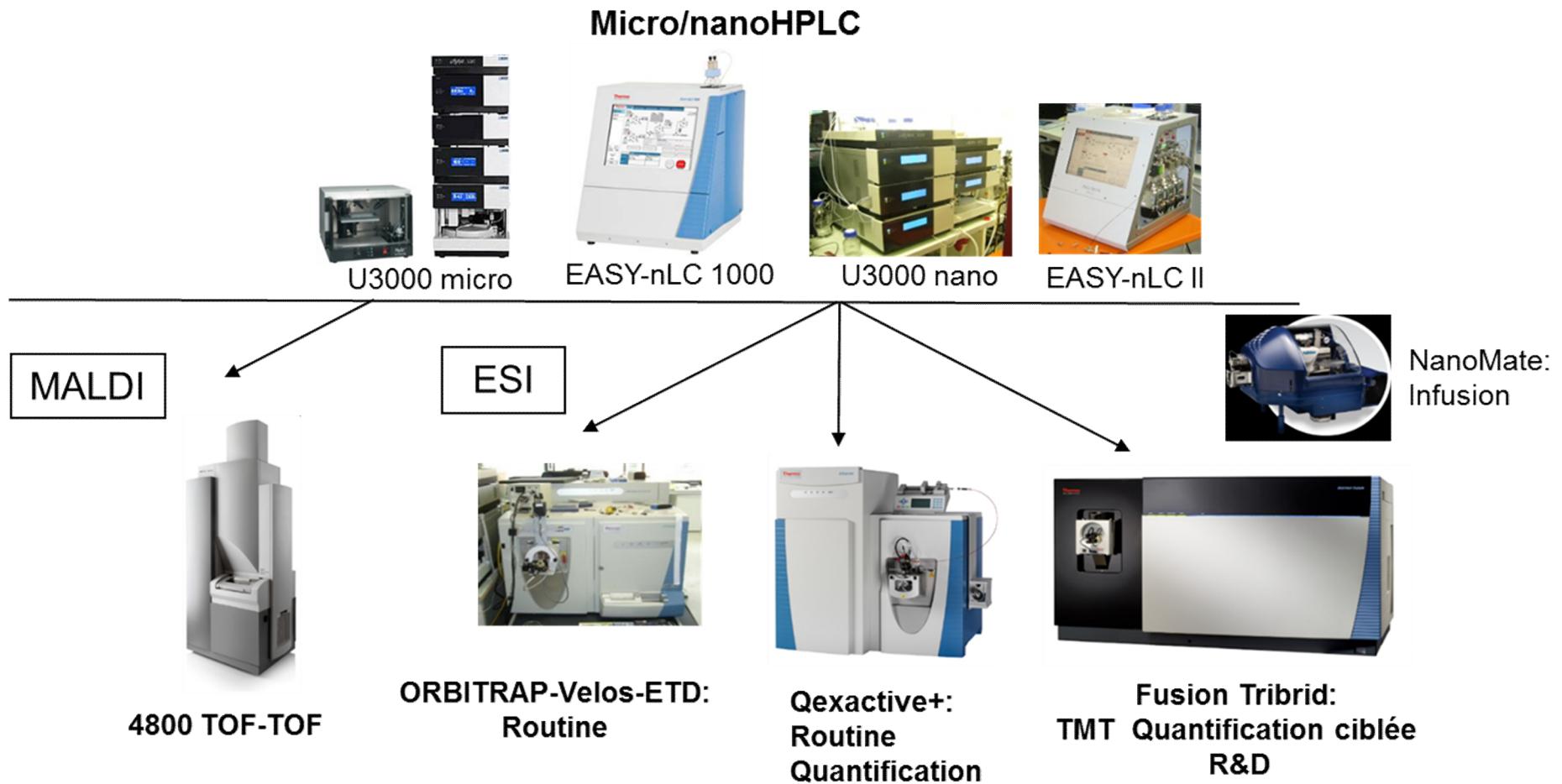
- UFR SdV (IJM, BFA, CEDC)
- UFR of medicine
- UFR of chemistry

Other universities (UMPC, Paris Descartes, La Rochelle...),
Laboratories CNRS, INSERM, INRA...

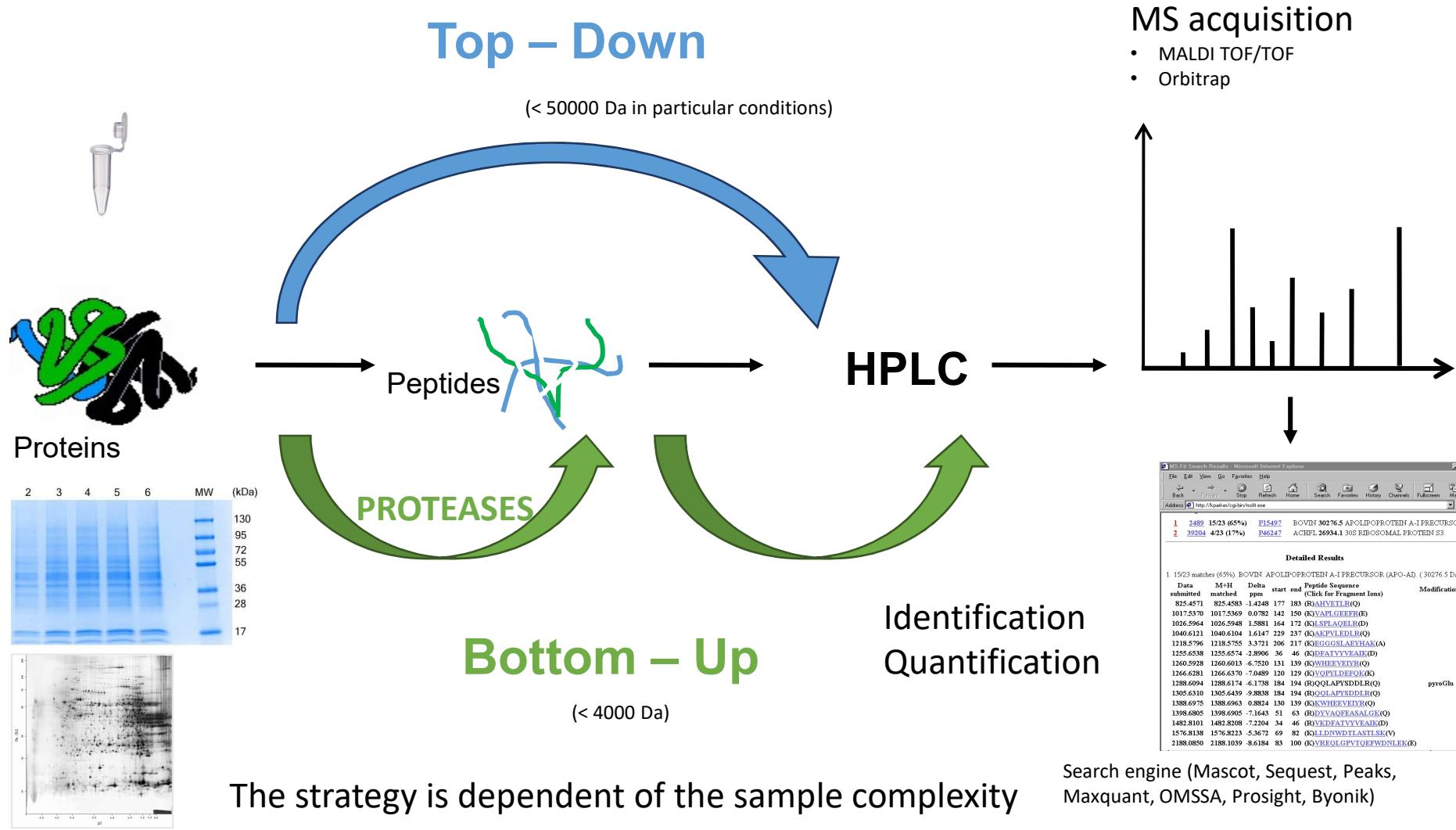
Research teams: Institut Curie, Institut Pasteur, Institut Gustave
Roussy...

Private companies: Généthon, Neoneuro, Agrobio/thales

Instrumentations



Proteomics workflows



BOTTOM-UP PROTEOMICS: PRO'S AND CON'S

Advantages

- Less sophisticated instrumentation and expertise
- High throughput
- More info about proteins with “extreme” phys.-chem. properties (hydrophobic, Hi/Low MW, acidic/basic)

Disadvantages

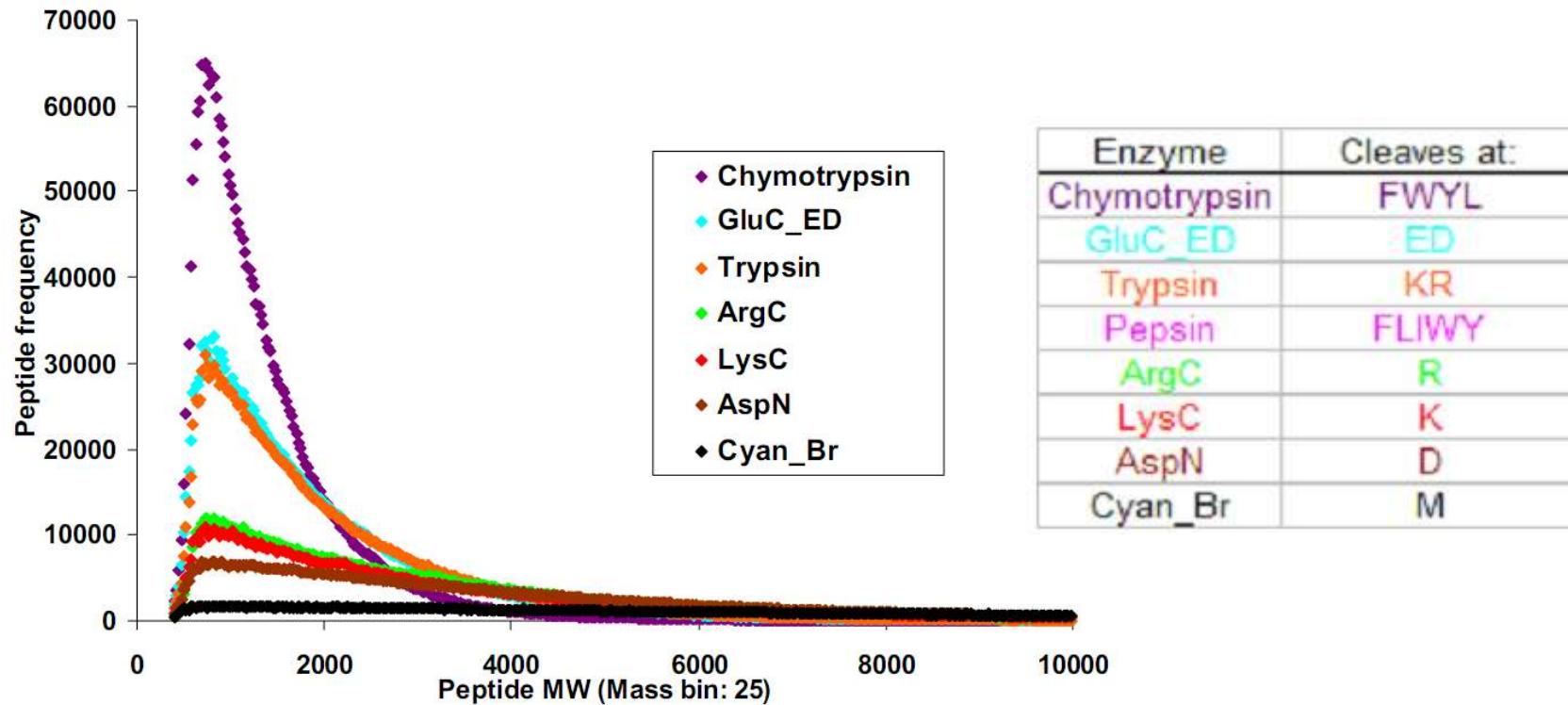
- Confidence in protein ID strongly depends on restriction criteria (subjective; potential bias)
- Since protein ID is often done by 1-2 peptides, PTM and isoform information is often lost

Cleavage rules of proteases

Enzyme or Reagent	Cleaves where?	Exceptions
Trypsin	C-terminal side of K or R	if P is C-term to K or R
Trypsin	(C-term to K/R, even before P)	C-terminal side of K or R
Trypsin (higher specificity)	C-terminal side of K or R	if P is C-term to K or R; after K in CKY, DKD, CKH, CKD, KKR; after R in RRH, RRR, CRK, DRD, RRF, KRR
Lys C	C-terminal side of K	
CNBr	C-terminal side of M	
Arg C	C-terminal side of R	if P is C-term to R
Asp N	N-terminal side of D	
Asp N + N-terminal Glu	N-terminal side of D or E	
Glu C (bicarbonate)	C-terminal side of E	if P is C-term to E, or if E is C-term to E
Glu C (phosphate)	C-terminal side of D or E	if P is C-term to D or E, or if E is C-term to D or E
Chymotrypsin	(C-term to F/Y/W/M/L, not before P, not after Y if P is C-term to Y) C-terminal side of F, L, M, W, Y	if P is C-term to F, L, M, W, Y, if P is N-term to Y
Chymotrypsin (C-term to F/Y/W/, not before P, not after Y if P is C-term to Y)	C-terminal side of F, Y, W	if P is C-term to F, Y, W, if P is N-term to Y
Trypsin/Chymotrypsin (C-term to K/R/F/Y/W, not before P, not after Y if P is C-term to Y)	C-terminal side of K, R, F, Y, W	if P is C-term to K, R, F, Y, W, if P is N-term to Y
Pepsin (pH 1.3)	C-terminal side of F, L	
Pepsin (pH > 2)	C-terminal side of F, L, W, Y, A, E, Q	
Proteinase K	C-terminal side of A, C, G, M, F, S, Y, W	

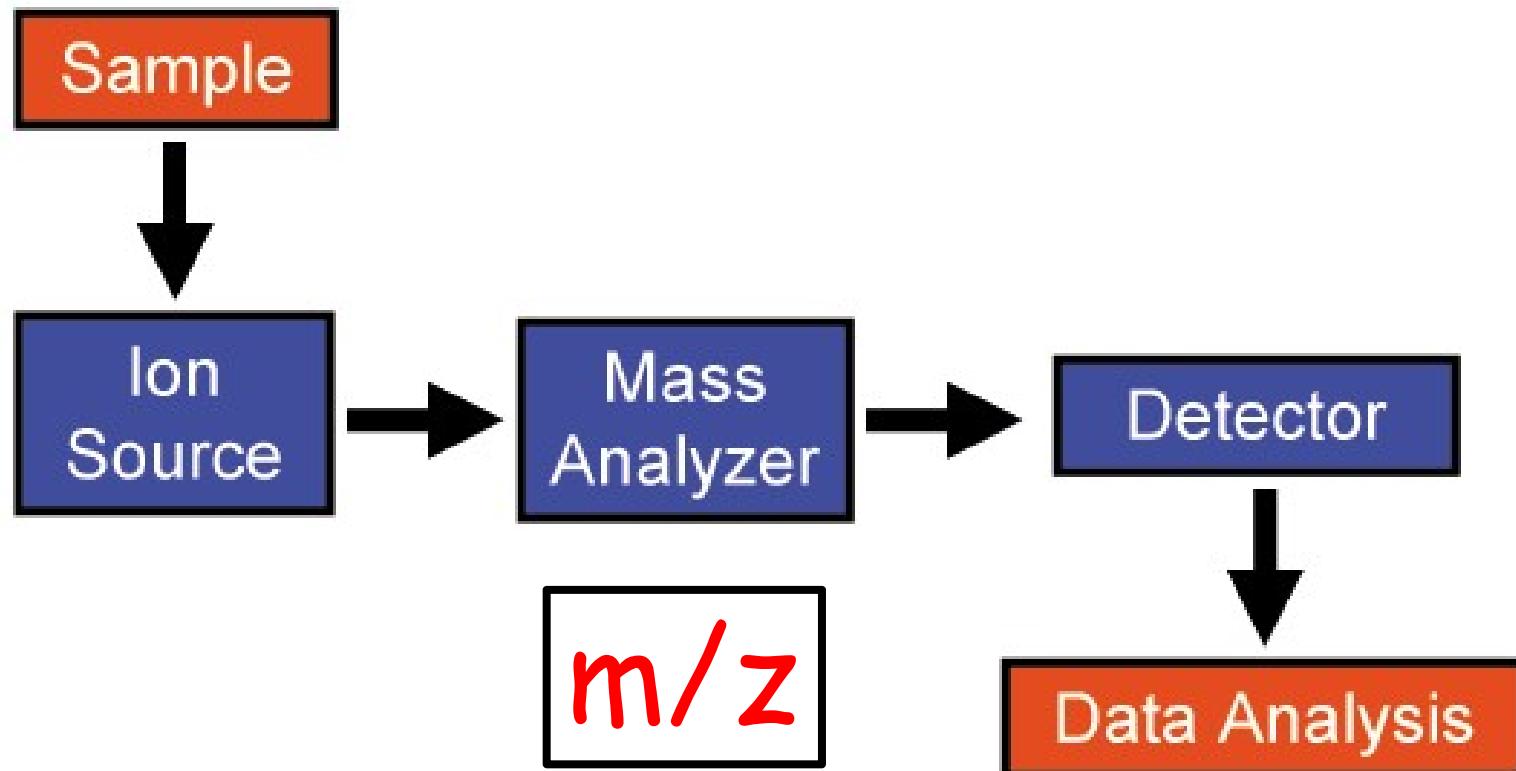
PEPTIDE LENGTH AND NUMBER OF PEPTIDES GENERATED DEPENDING ON ENZYME USED FOR DIGESTION

Other enzymes with more or less specific cleavage:

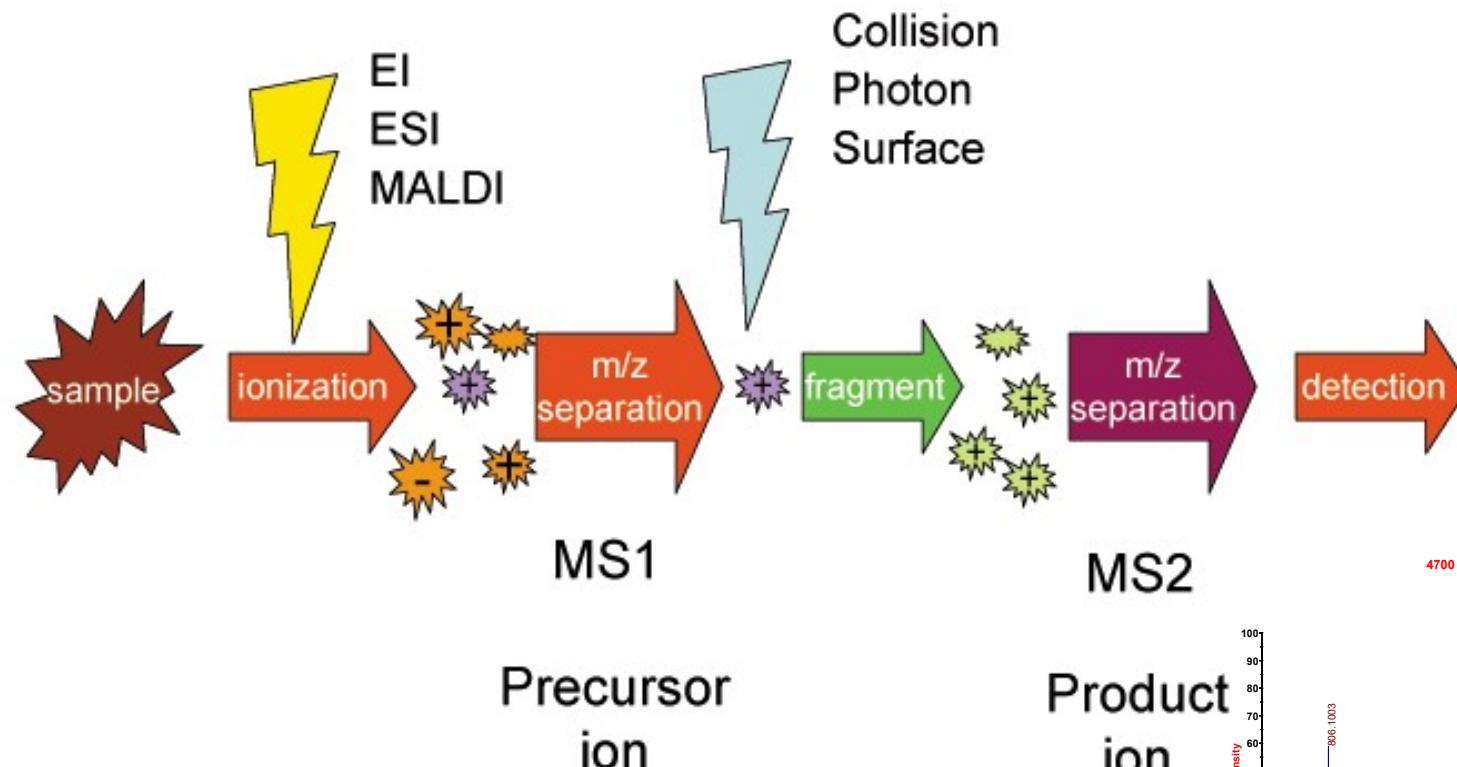


*Advantages of a new proteomic approach that uses accurate mass measurements, LC retention time, isoelectric point and dual enzymatic digestion. Petritis K. et. al., Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99352; ASMS'2007 poster presentation
http://www.chem.agilent.com/Library/posters/Public/Petritis_ASMS_2007.pdf*

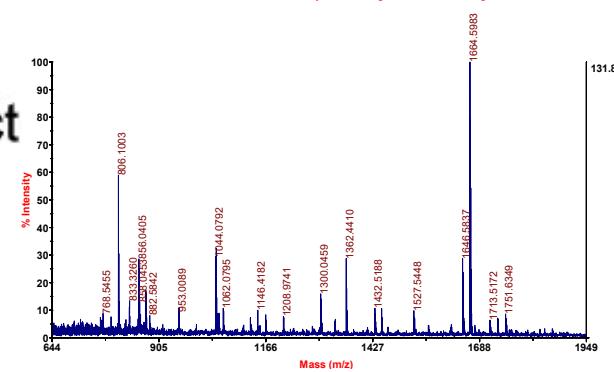
What is MS?



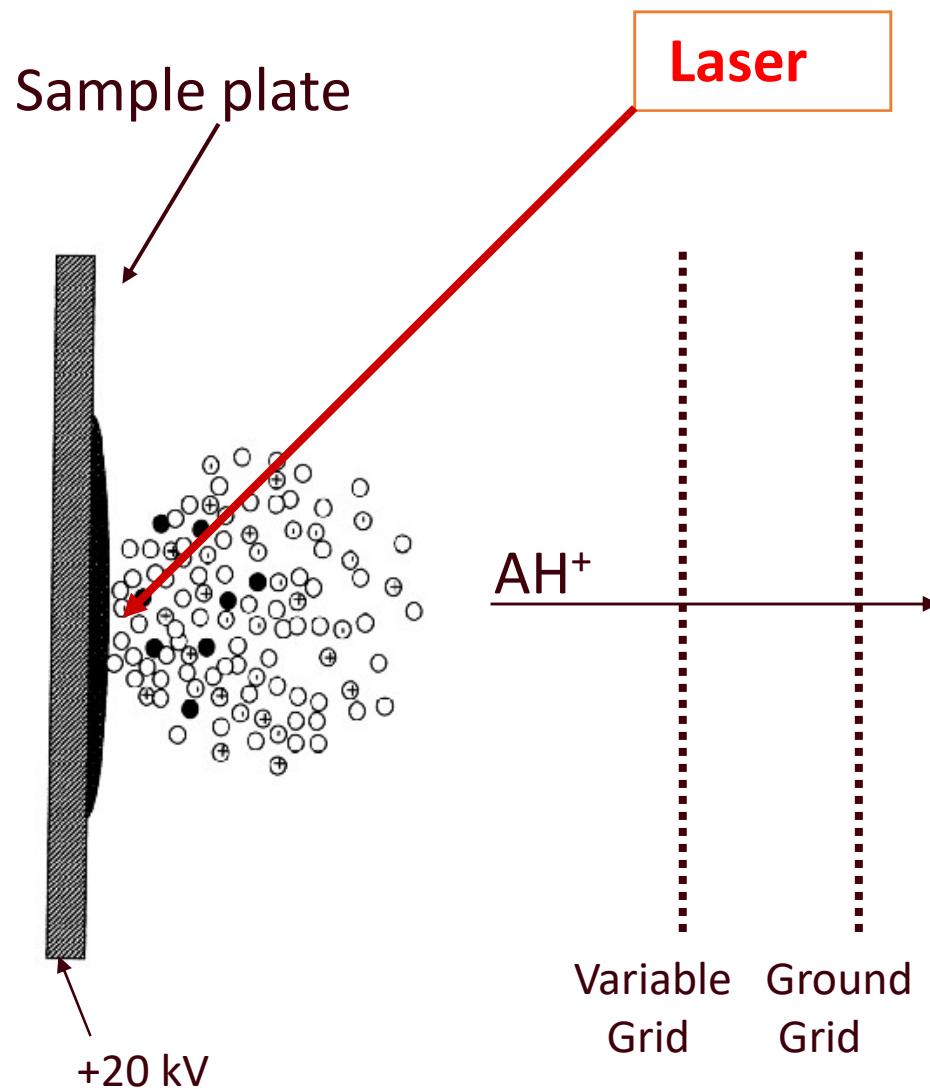
MS and MS/MS



4700 Reflector Spec #1 MC[BP = 1664.6, 132]

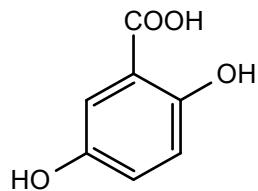


MALDI ionization (Matrix Assisted Laser Desorption Ionization)

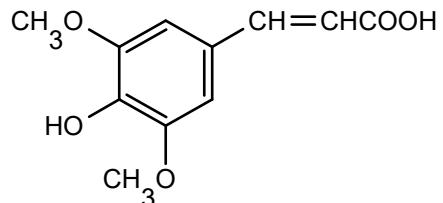


1. L'échantillon (A) est mélangé avec un excès de matrice (M) et séché sur la plaque MALDI
2. Le flash Laser ionise les molécules de matrice
3. Les molécules d'échantillon sont ionisées par transfert de protons de la matrice:
 $MH^+ + A \rightarrow M + AH^+$.

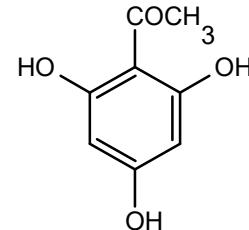
MALDI-TOF Matrix



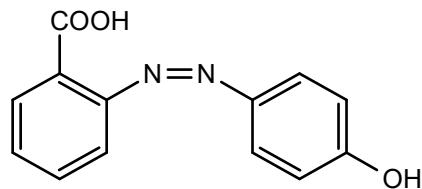
2,5-dihydroxybenzoic acid
(2,5-DHB)



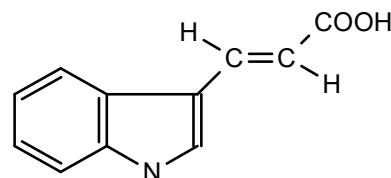
Sinapinic acid (3,5-Dimethoxy-4-hydroxy cinnamic acid)



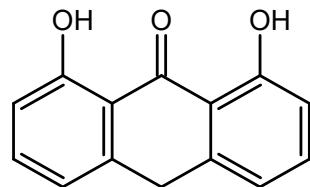
2,4,6-trihydroxy acetophenone (THAP)



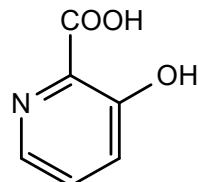
2-(4-hydroxyphenylazo)-benzoic acid
(HABA)



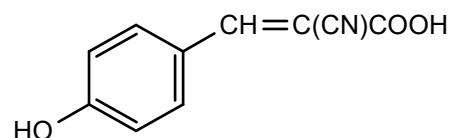
trans-3-indoleacrylic acid



Dithranol



3-hydroxypicolinic acid (3-HPA)

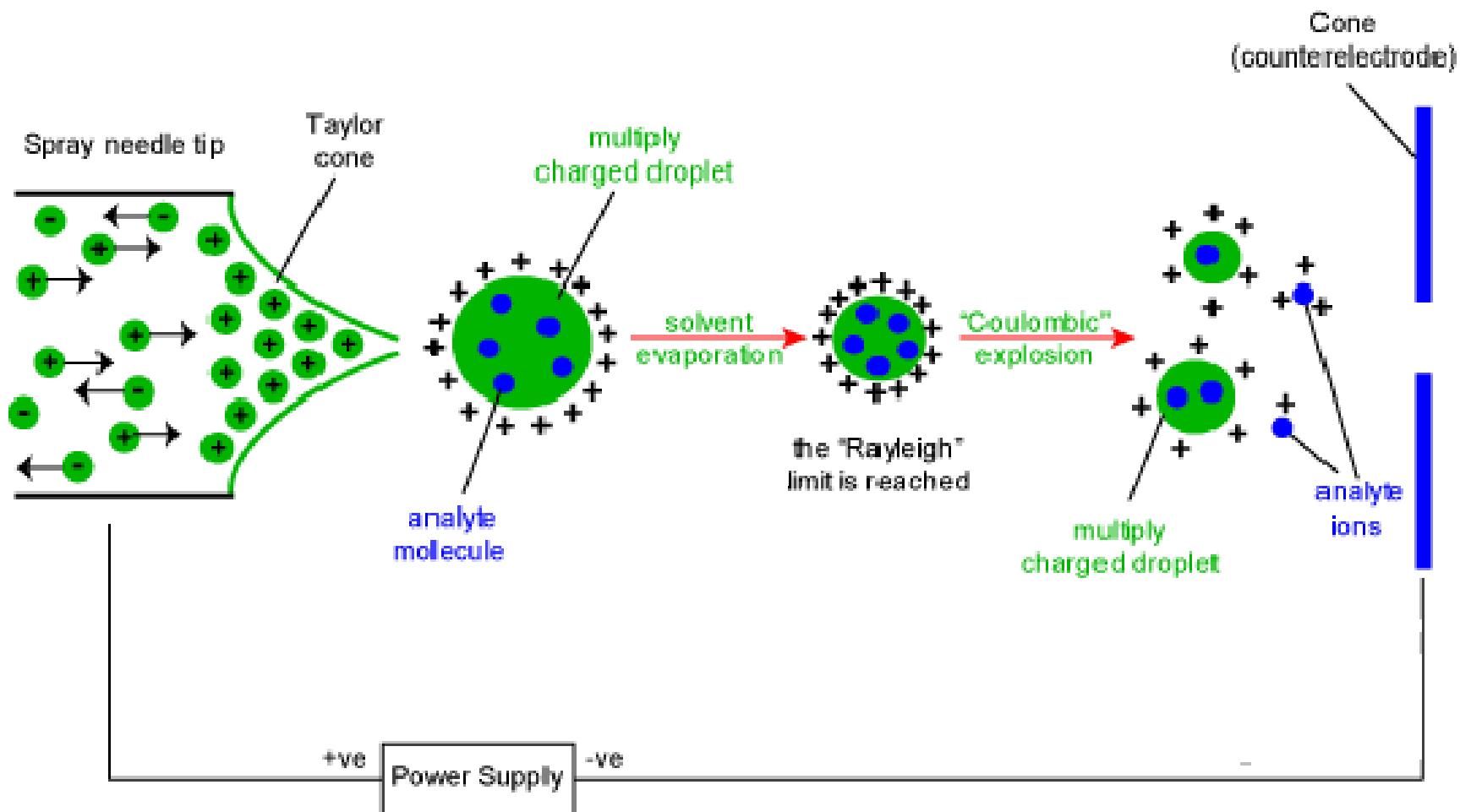


α-cyano-4-hydroxycinnamic acid

Matrix choices

α -Cyano-4-hydroxy-cinnamic acid (CHCA)	Peptides<10kDa
Sinapinic Acid	Proteins >10kDa
2,5-Dihydroxybenzoic acid (DHB)	Neutral carbohydrates, Synthetic Polymers
“Super DHB”	Proteins, Glycosylated proteins
3-Hydroxypicolinic acid	Oligonucleotides
HABA	Proteins, Oligosaccharides

Ionization by electrospray



Electrospray and nanospray sources

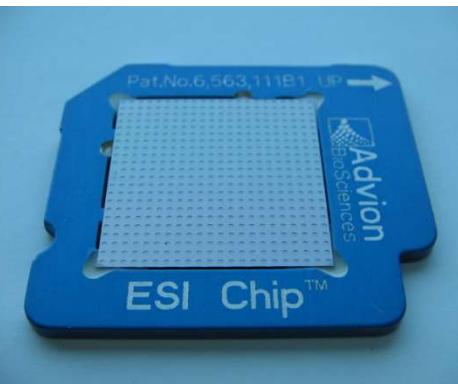
Electrospray



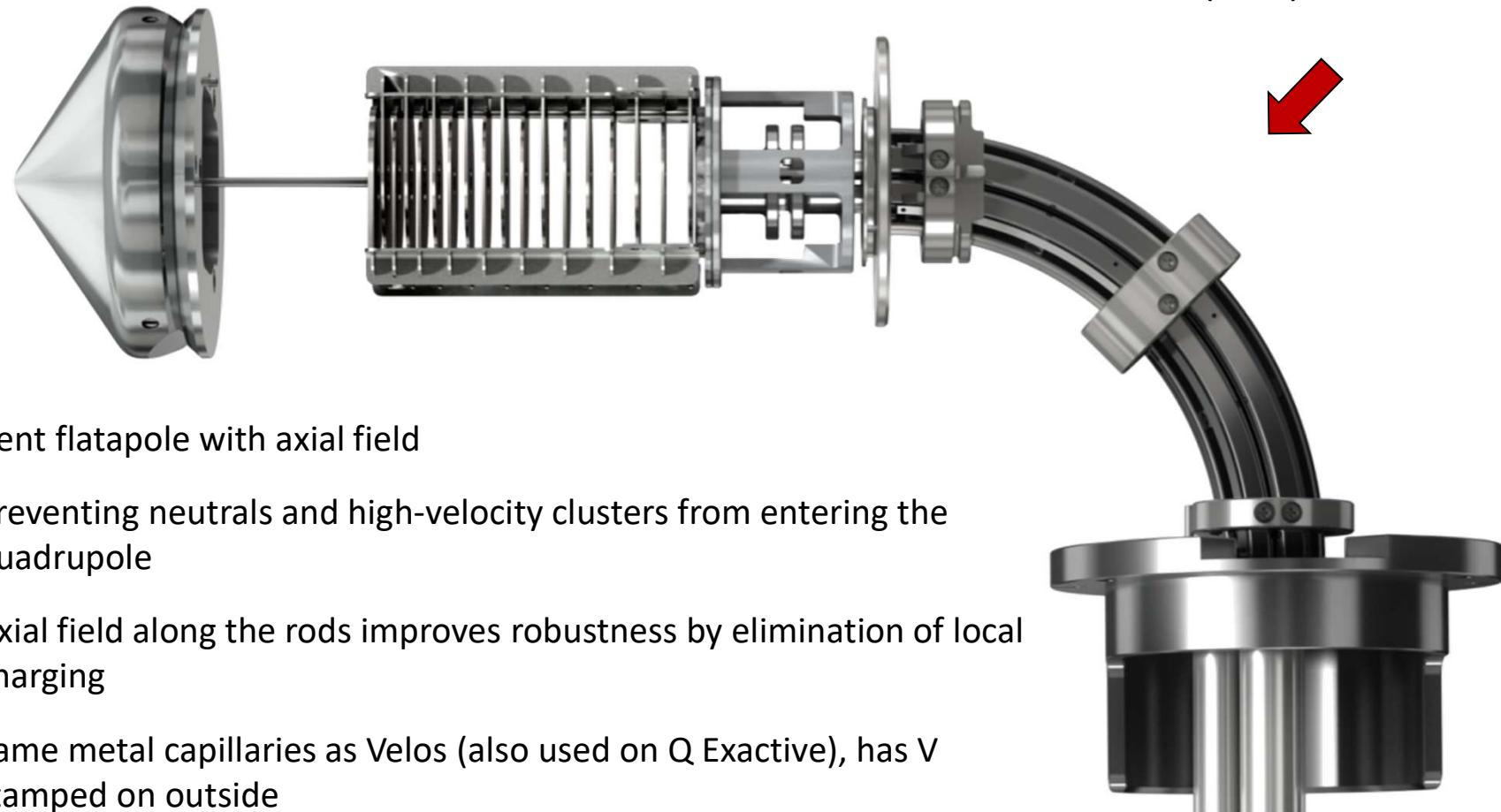
nanospray



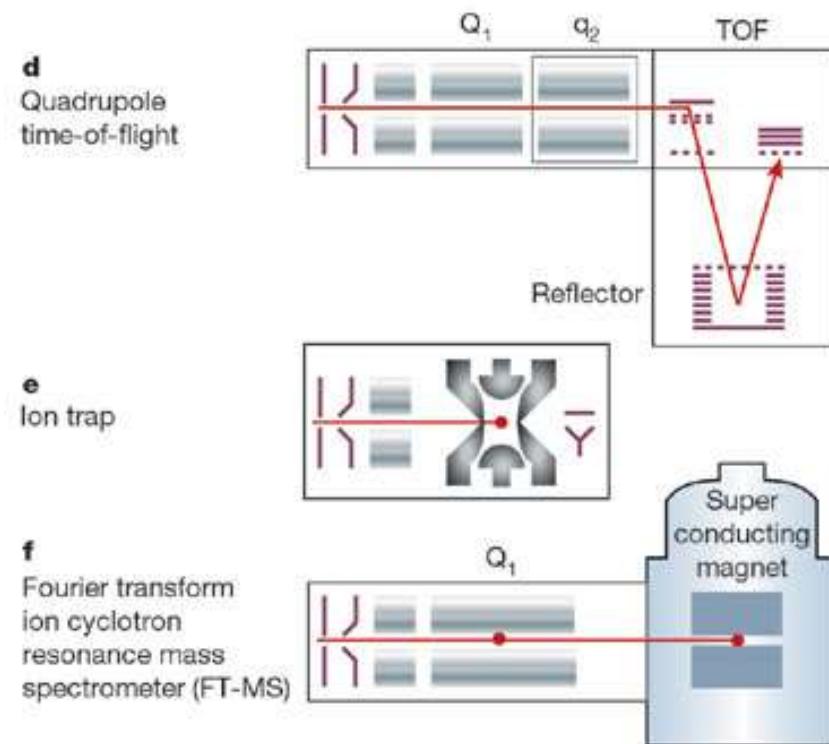
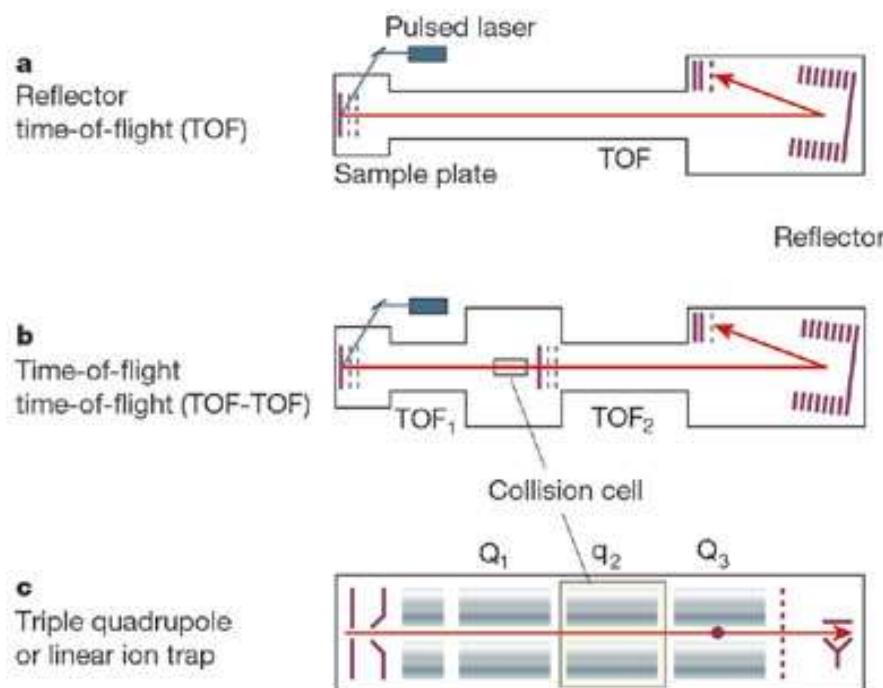
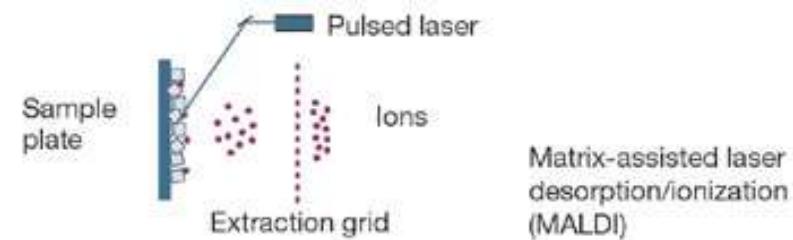
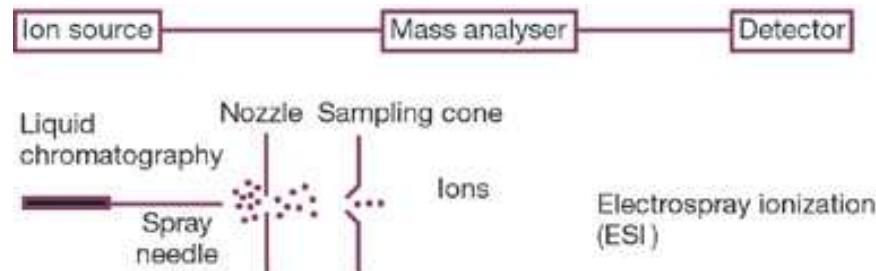
nanospray



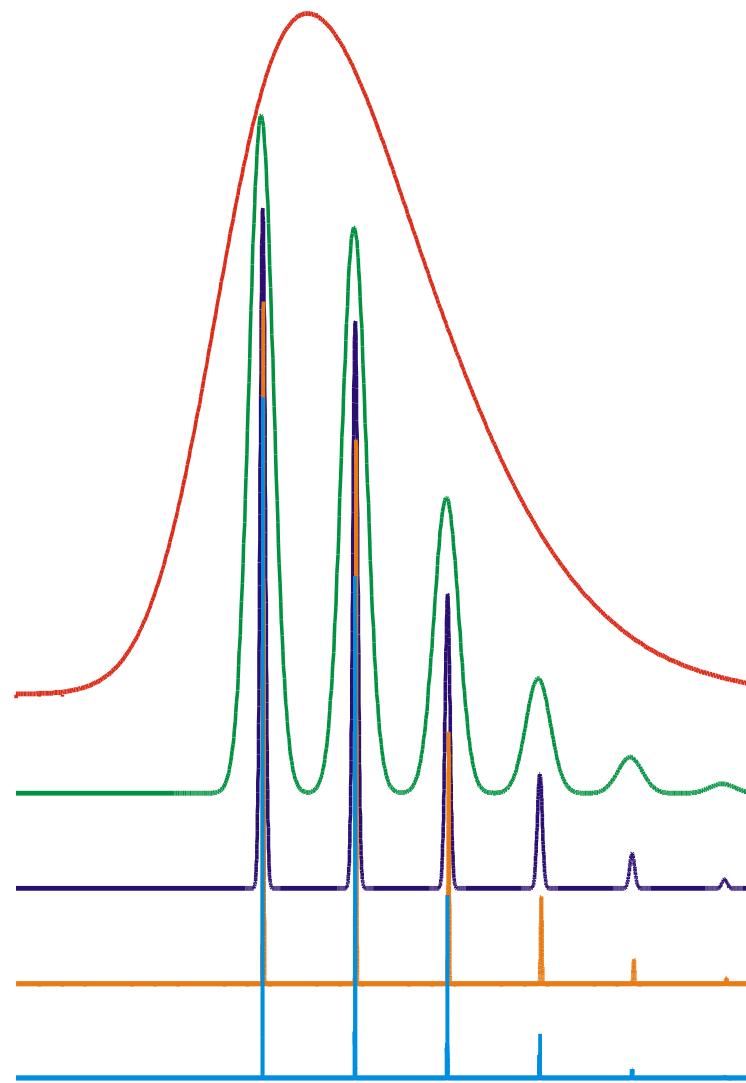
“Active Beam Guide” transmission



Different instrumental design



Importance of spectral resolution

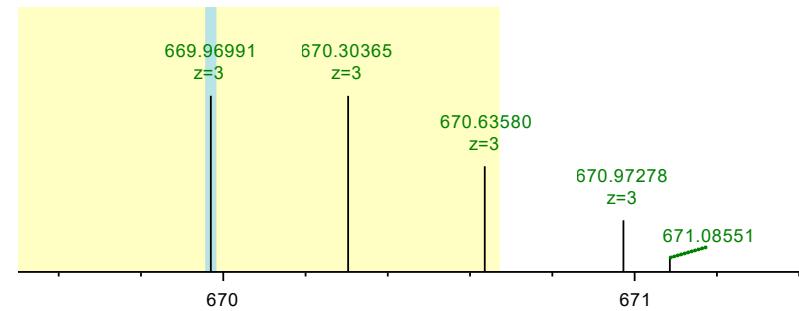


Resolution

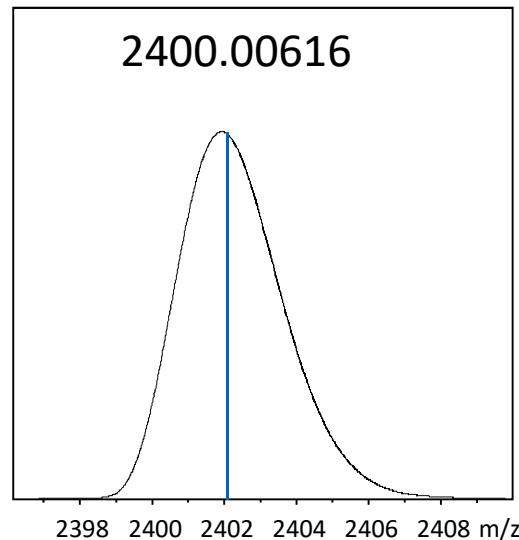
- | | |
|------------------|------------------------------|
| 1.000 | linear TOF w/o DE |
| 5.000 | reflector TOF w/o DE |
| 25.000 | reflector TOF with DE |
| 125.000 | FTMS wideband mode |
| 1.000.000 | FTMS high-res mode |

Natural abundance of atoms isotopes in proteins

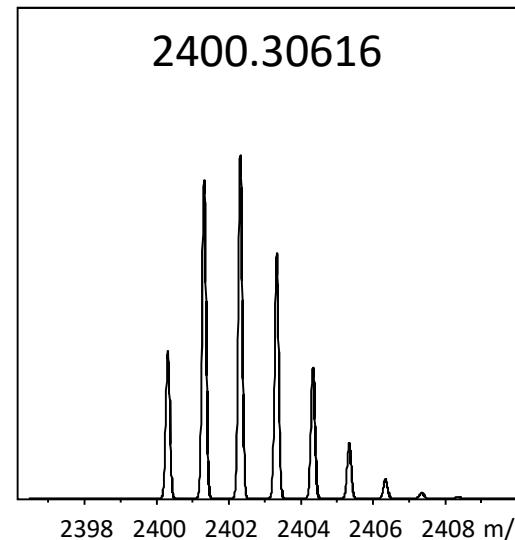
Name	Symbol	Mass (Da)	Abundance (%)
Hydrogen	H	1.007825	99.9885
Deuterium	H	2.014102	0.0115
Carbon	C	12.000000	98.9300
	C	13.003355	1.0700
Nitrogen	N	14.003074	99.6320
	N	15.000109	0.3680
Oxygen	O	15.994915	99.7570
	O	16.999132	0.0380
	O	17.999160	0.2050
Phosphorus	P	30.973762	100.0000
Sulfur	S	31.973762	94.9300
	S	32.971458	0.7600
	S	33.967867	4.2900
	S	35.967081	0.0200



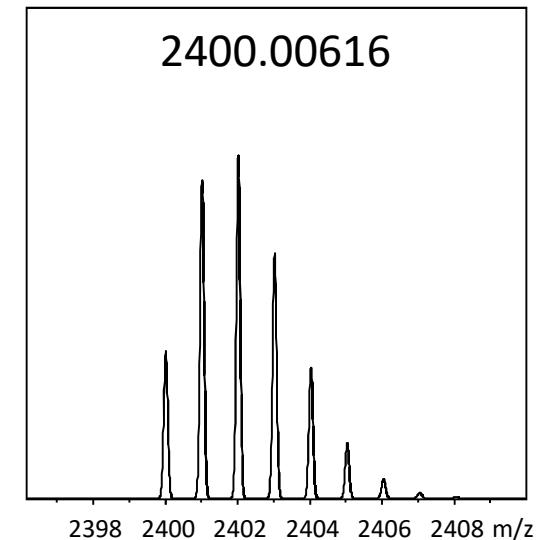
Resolution and mass accuracy



Poor resolution
High mass accuracy



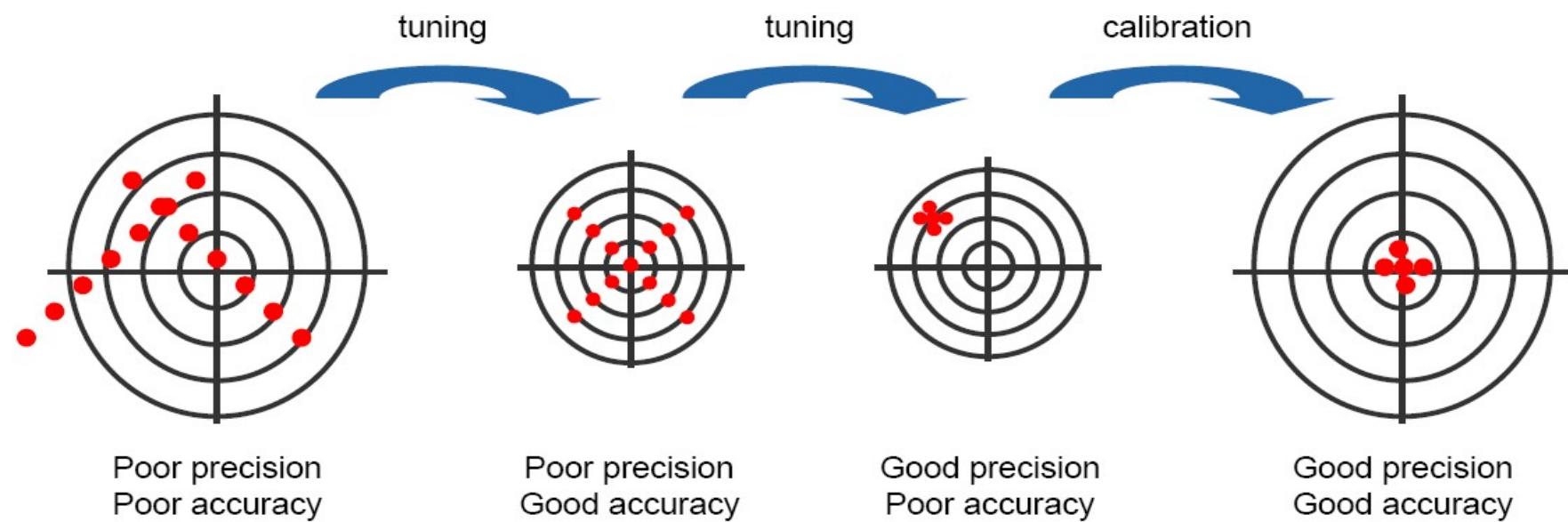
High resolution
Poor mass accuracy



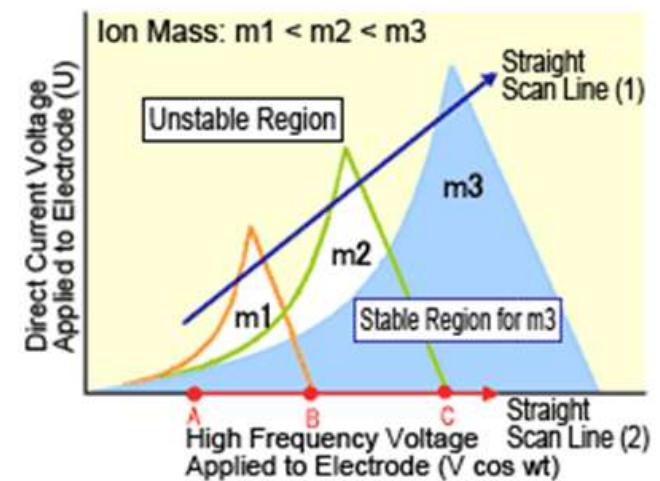
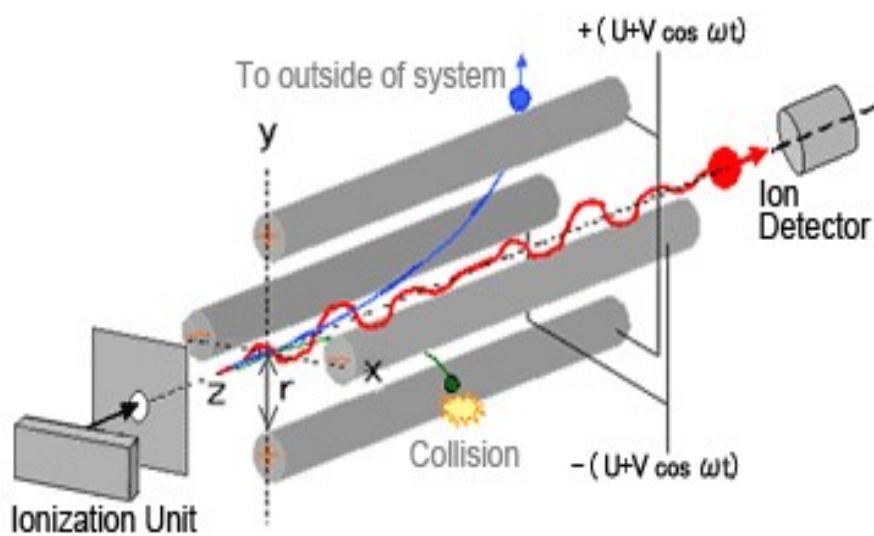
High resolution
High mass accuracy

High resolution makes it easier to achieve high mass accuracy – but high mass accuracy does not necessarily require high resolution! High resolution is only mandatory to avoid overlapping peaks.

Accuracy and precision in mass



Quadrupole analysers



$$\varphi_0 = U - V \cdot \cos(2\pi f t)$$

$$\varphi = \varphi_0 \cdot (x^2 - y^2) / r_0^2$$

Quadrupole analysers

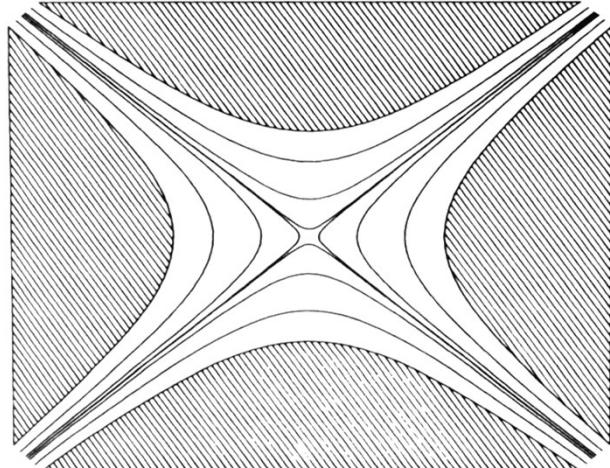
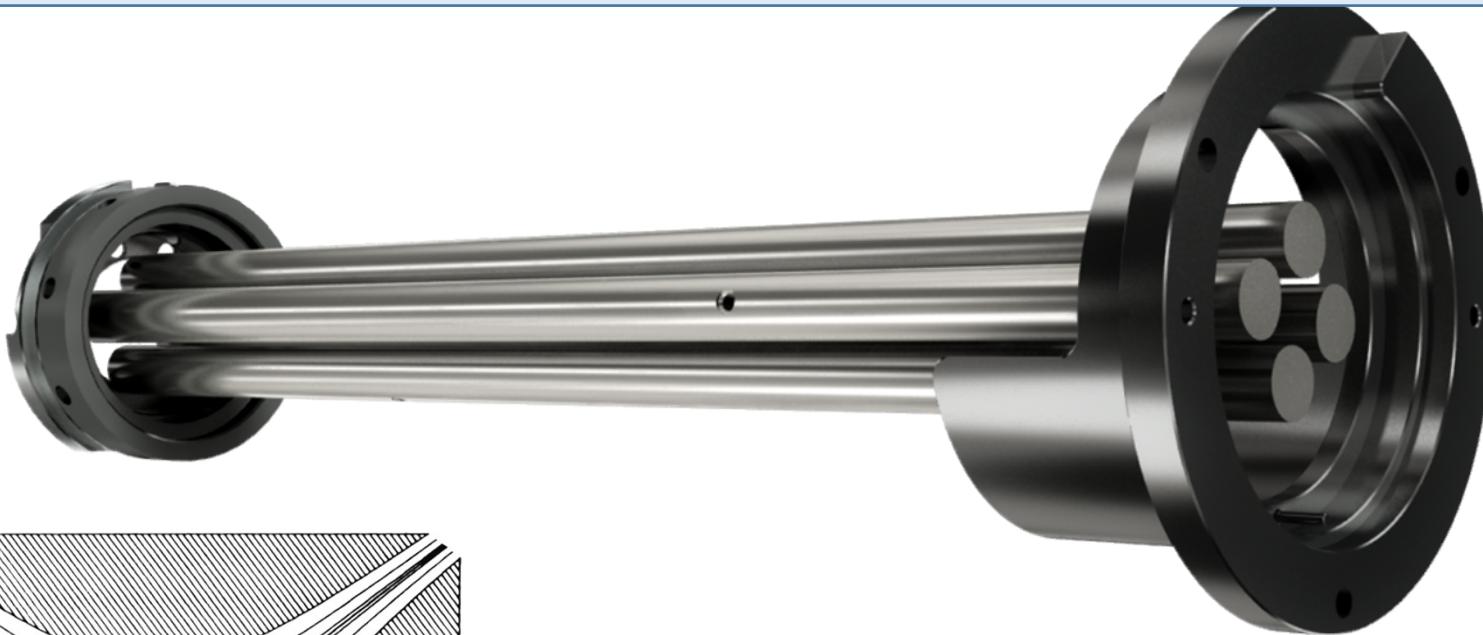


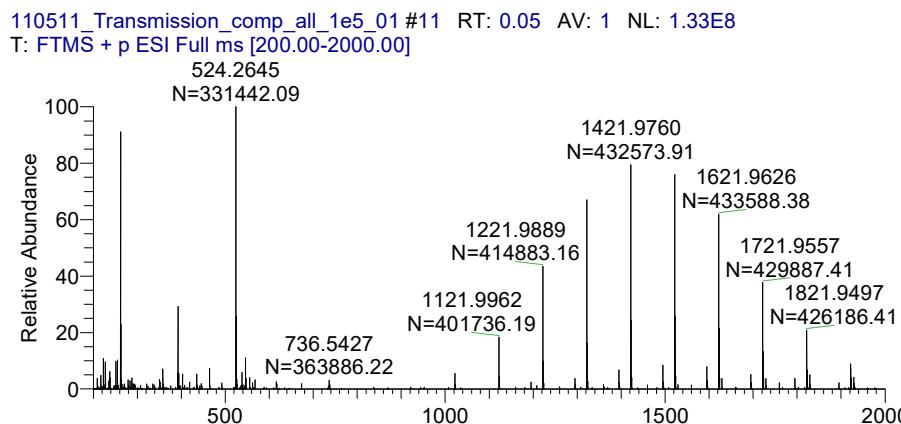
Figure 2.4. Hyperbolic equipotential contours in an ideal two-dimensional quadrupole field.



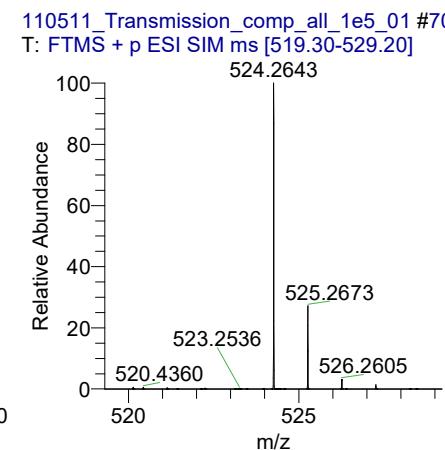
The **Quadrupole Mass Filter** on the Orbitrap Fusion is used to select a specific mass range for transmission to the ion optics and detectors downstream. Its high ion transmission at isolation widths down to **0.4 amu** improves sensitivity and selectivity.

* Raymond E. March, Richard J. Hughes. "Quadrupole Storage Mass Spectrometry." Wiley Interscience, 1989.

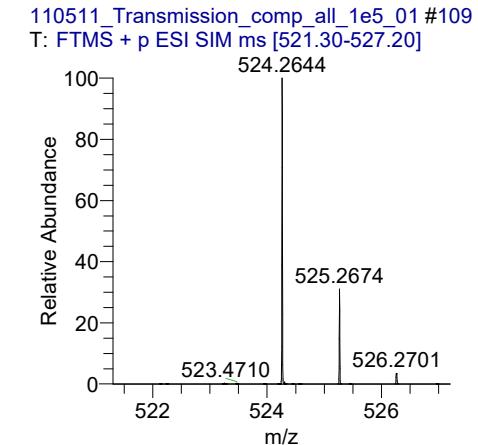
Isolation Width: Full MS to 1 amu (MRFA)



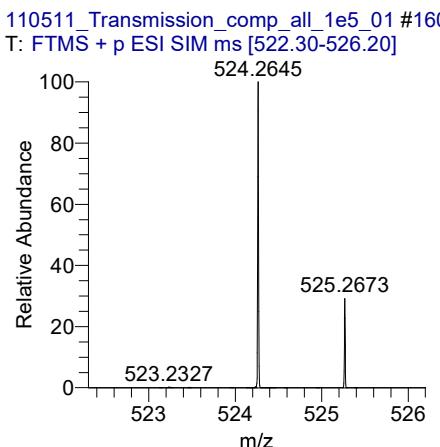
IsoW= 1800



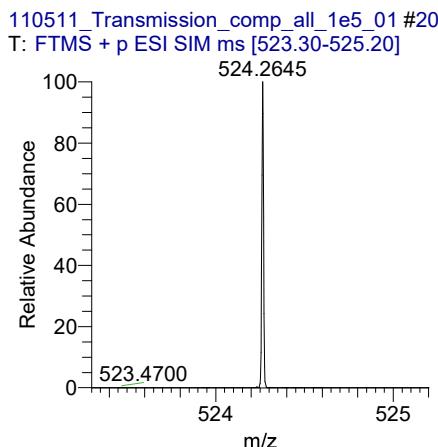
IsoW= 10



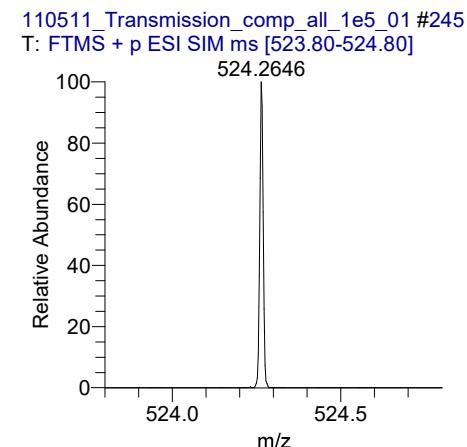
IsoW= 6



IsoW= 4

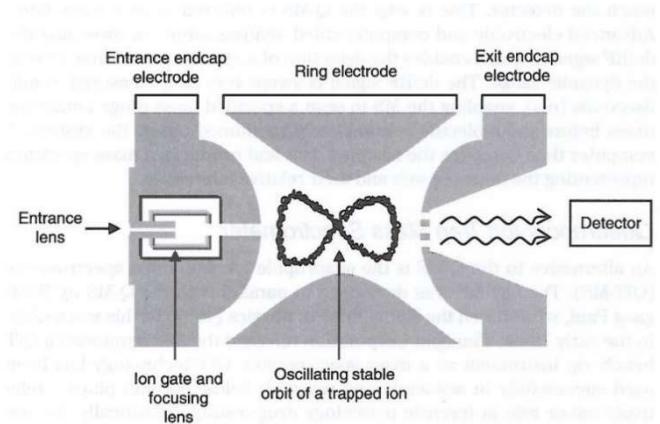


IsoW= 2

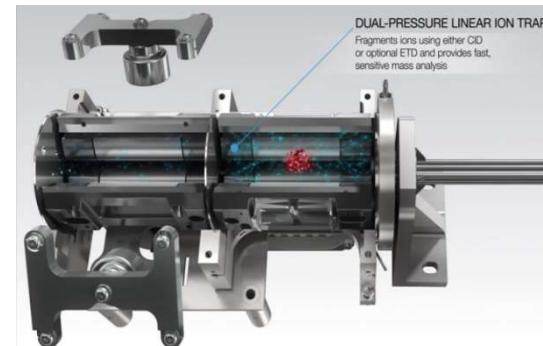
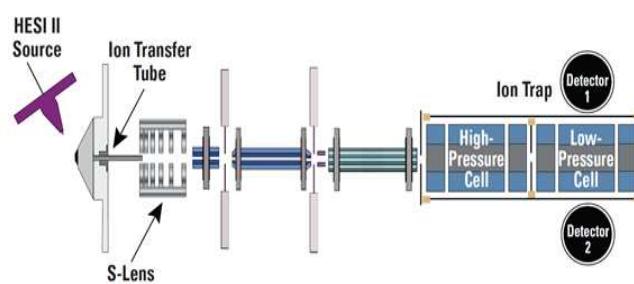
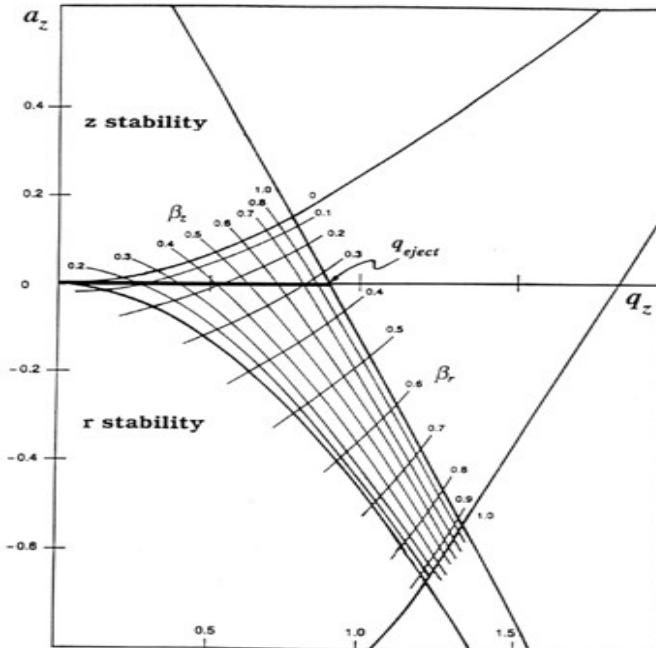


IsoW= 1

Different instrumental design

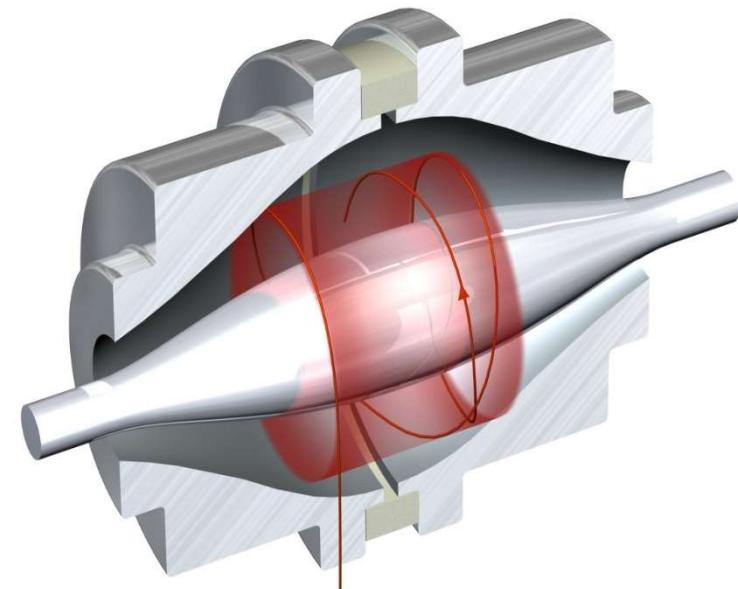
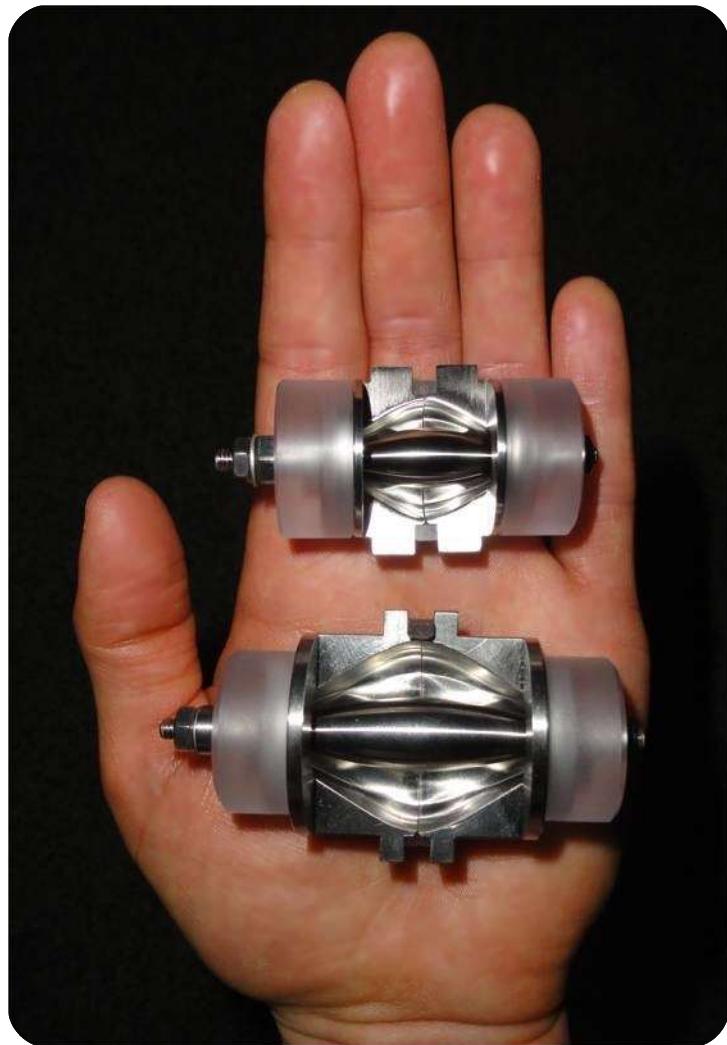


Trappe tridimensionnelle (Paul)



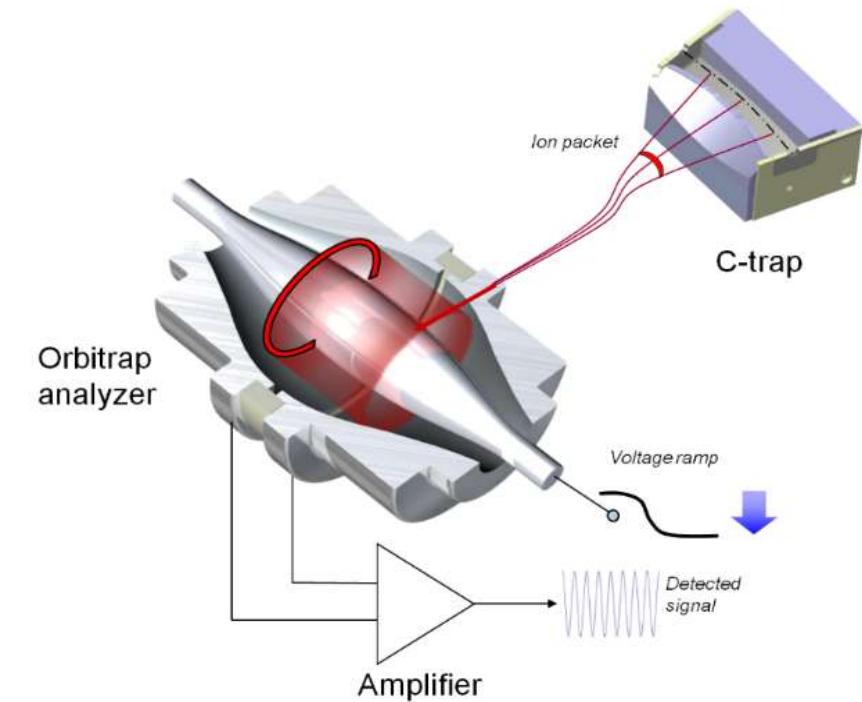
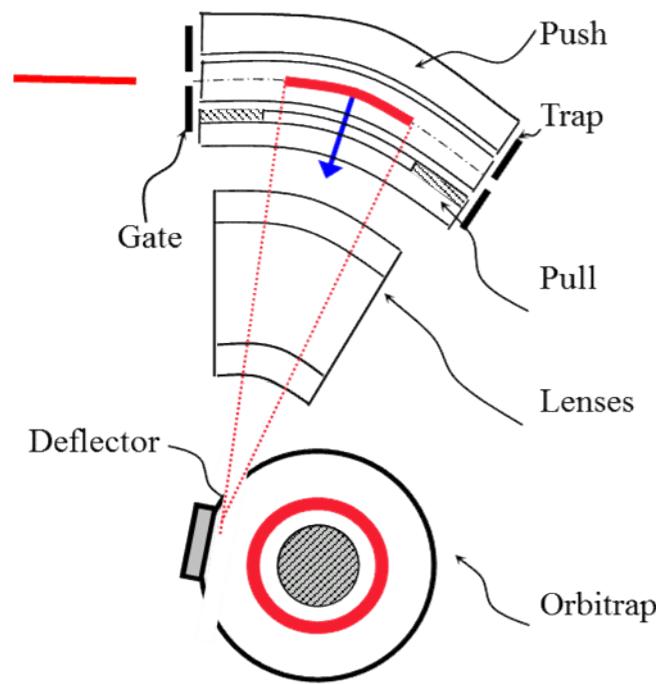
Trappe linéaire (double pression)

the orbitrap cell



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

C-trappe



Developpement of the orbitrap family

2007

LTQ Orbitrap XL and Discovery

2008

LTQ Orbitrap XL ETD

2009

LTQ Orbitrap Velos



2011

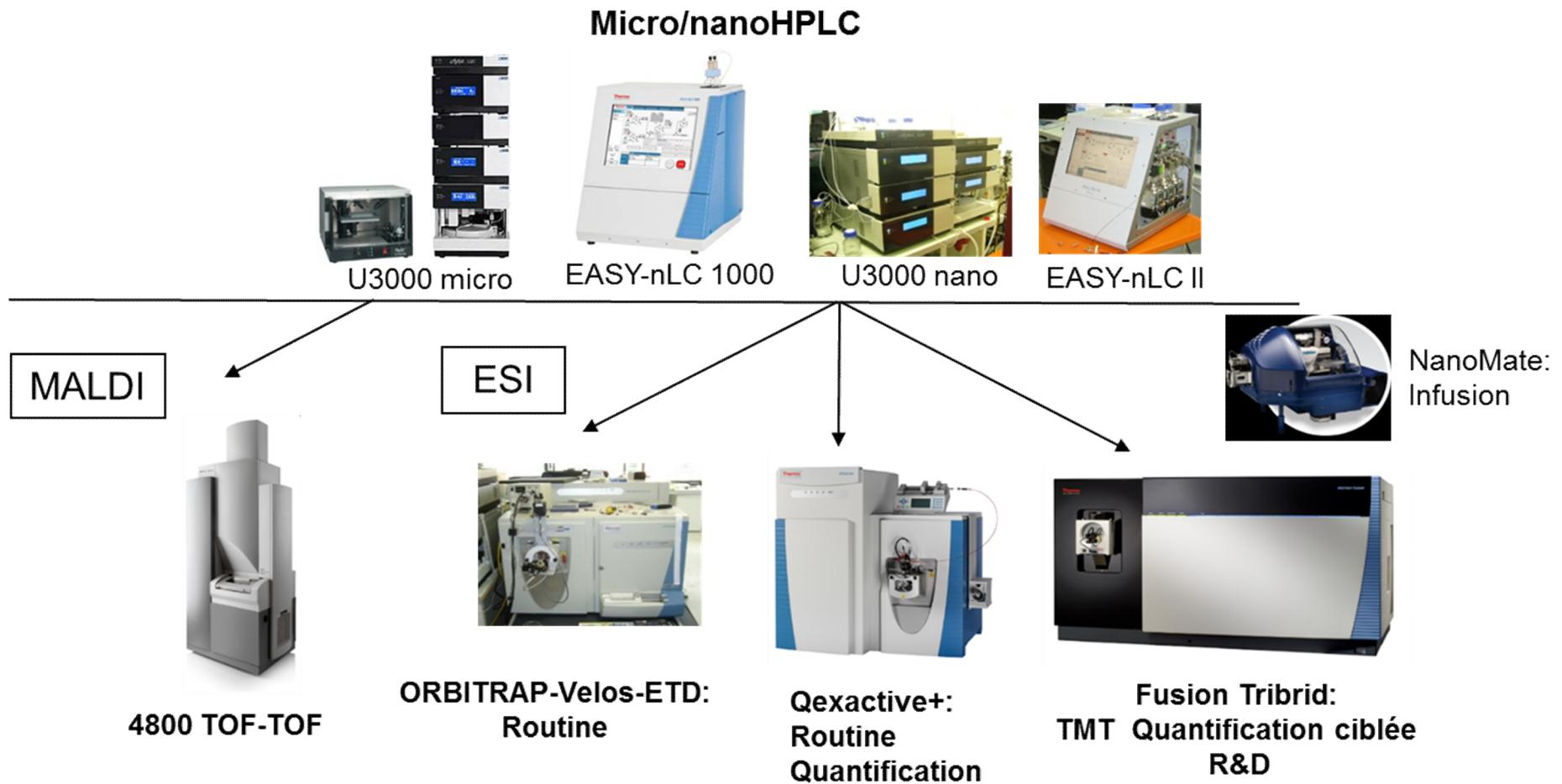
LTQ Orbitrap Velos Pro
Orbitrap Elite



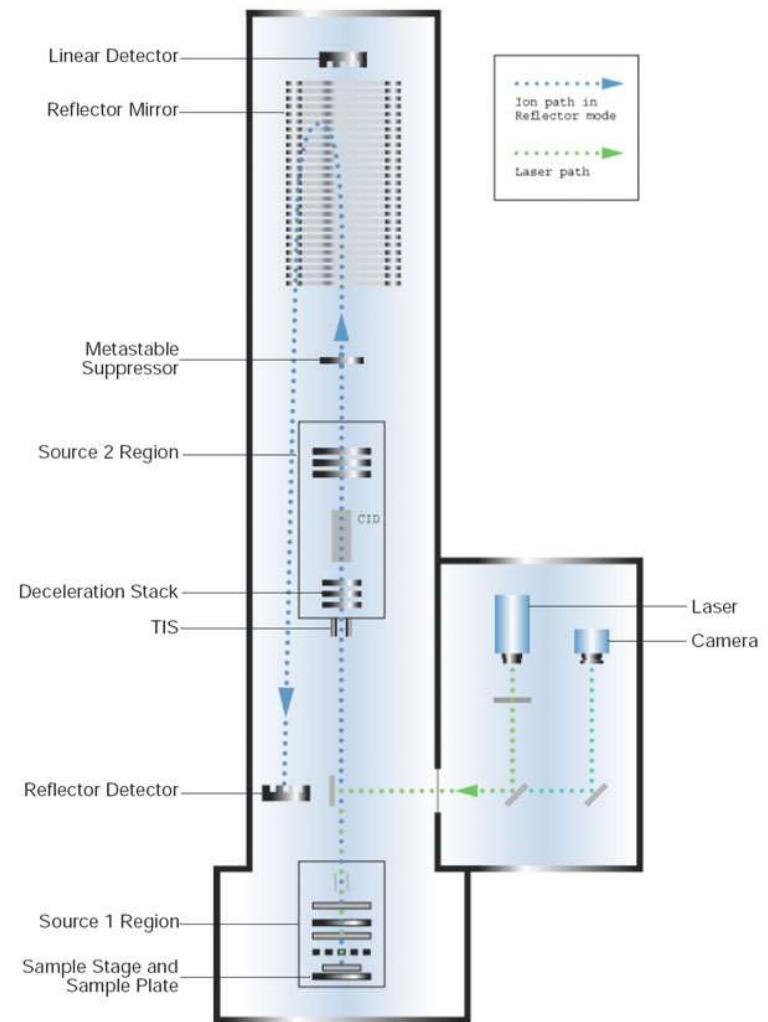
2013

Orbitrap Fusion Tribrid

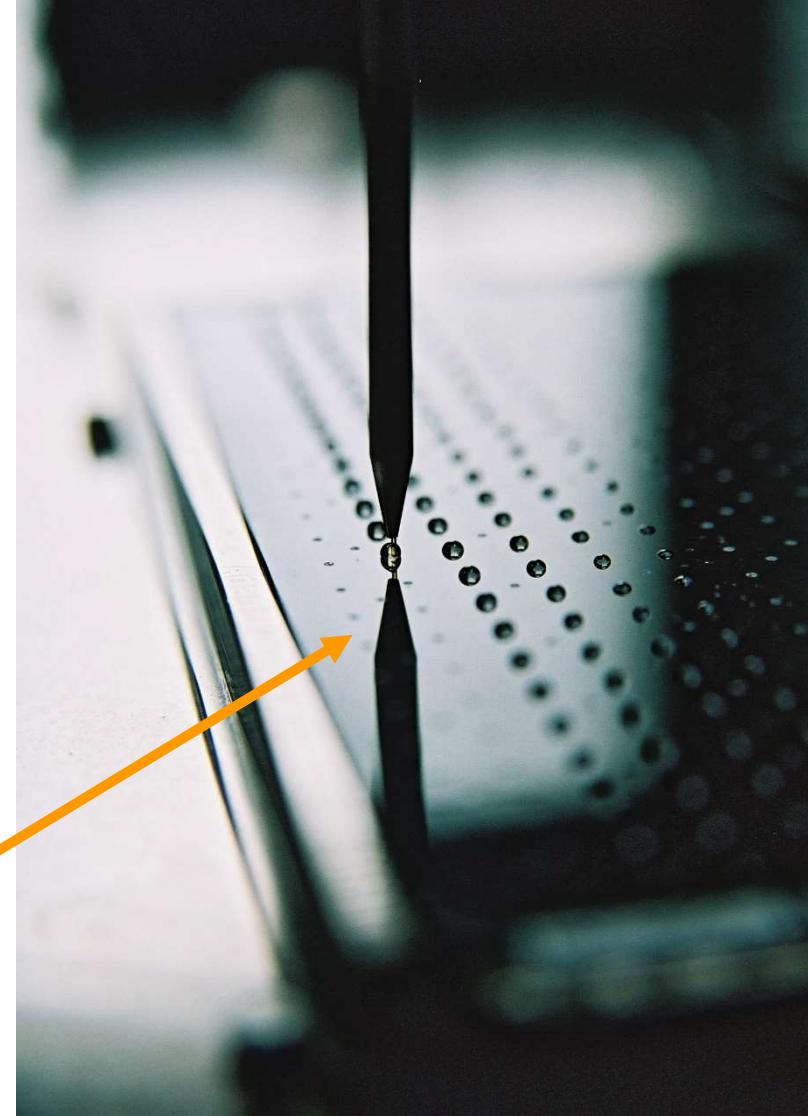
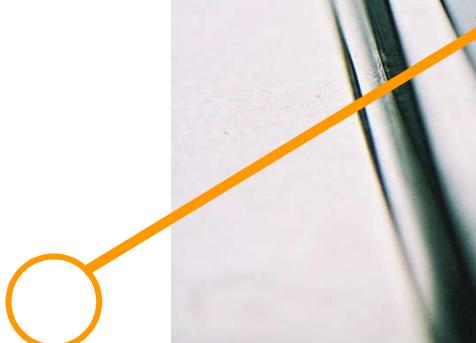
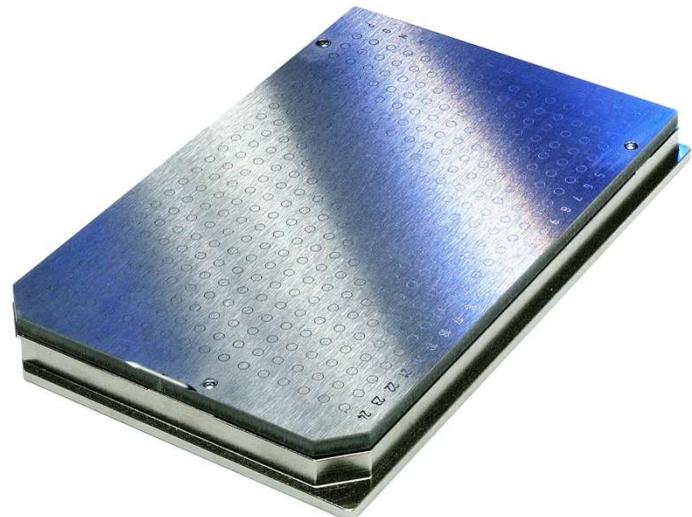
Instrumentations



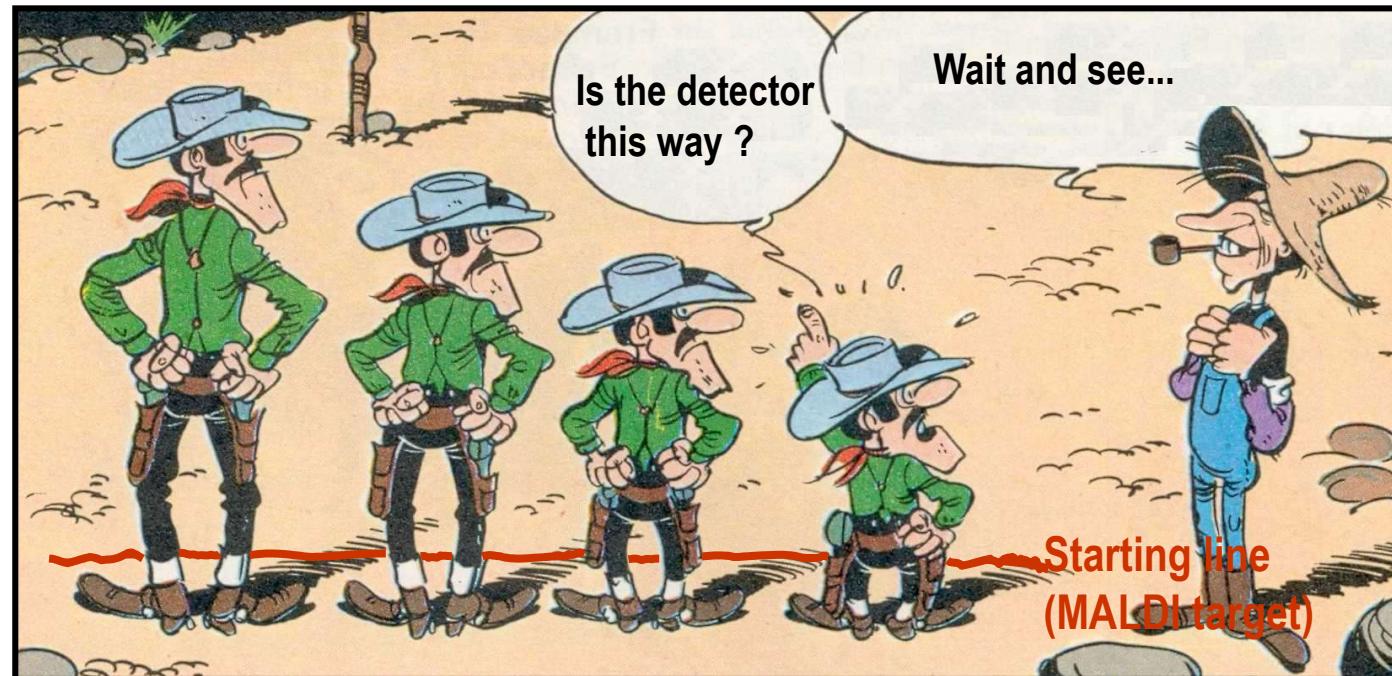
MALDI-TOF/TOF



Samples on MALDI plate



Time of flight – principles (TOF)

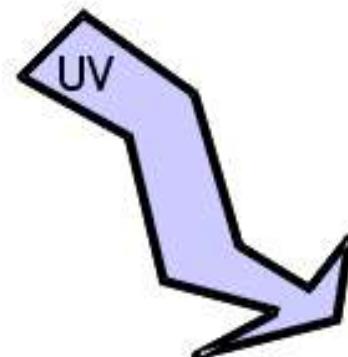


Remember : Mass of an ion is measured in the Dalton units !

Start !

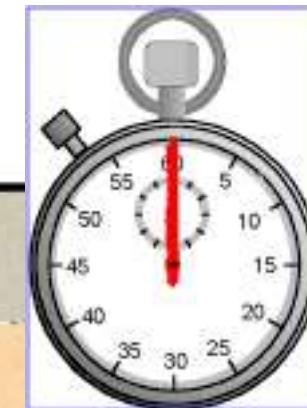
Laser

The desorption event



Induced by the
laser impuls

Start →



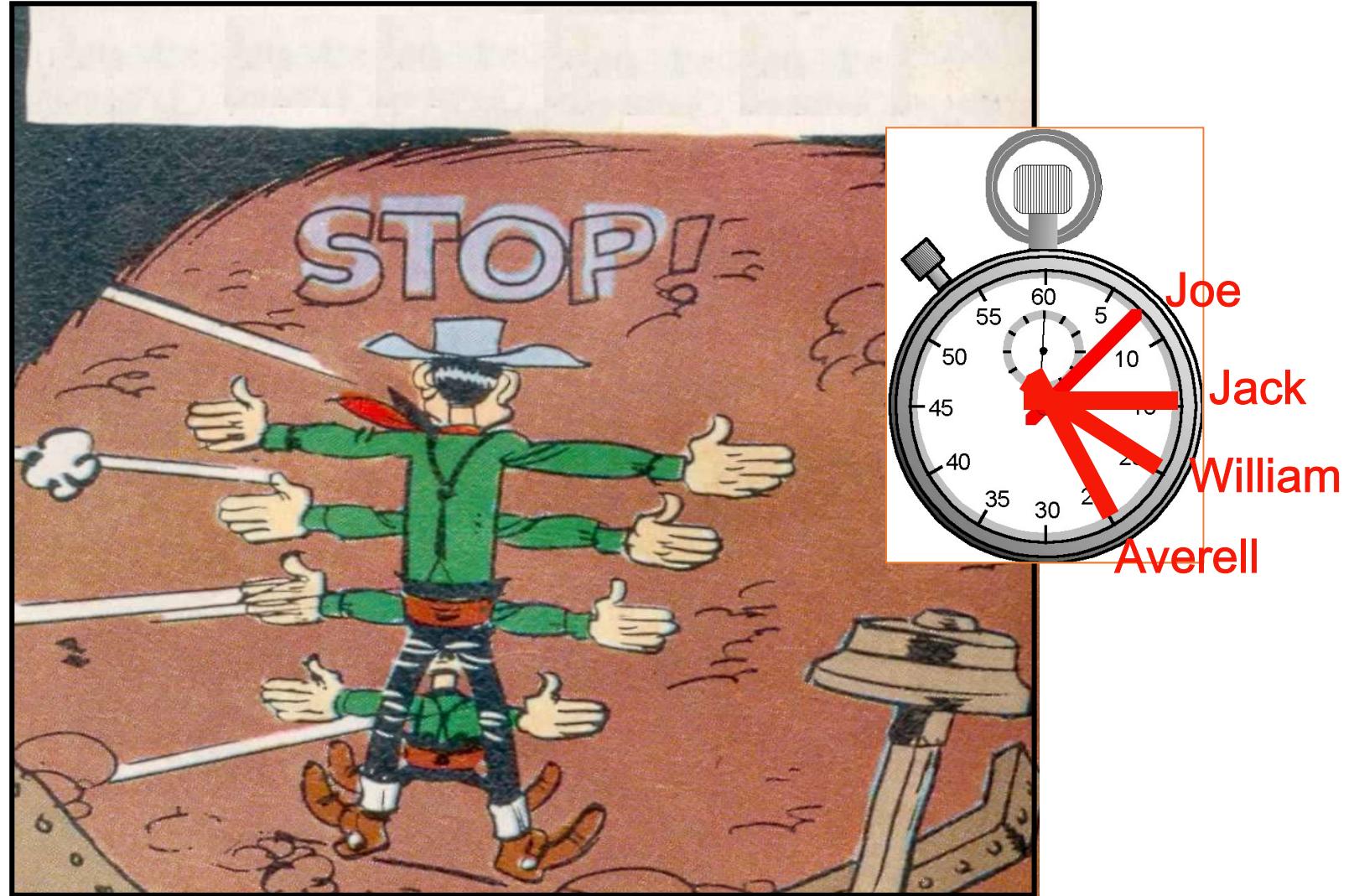
Ions in the time of flight (TOF)



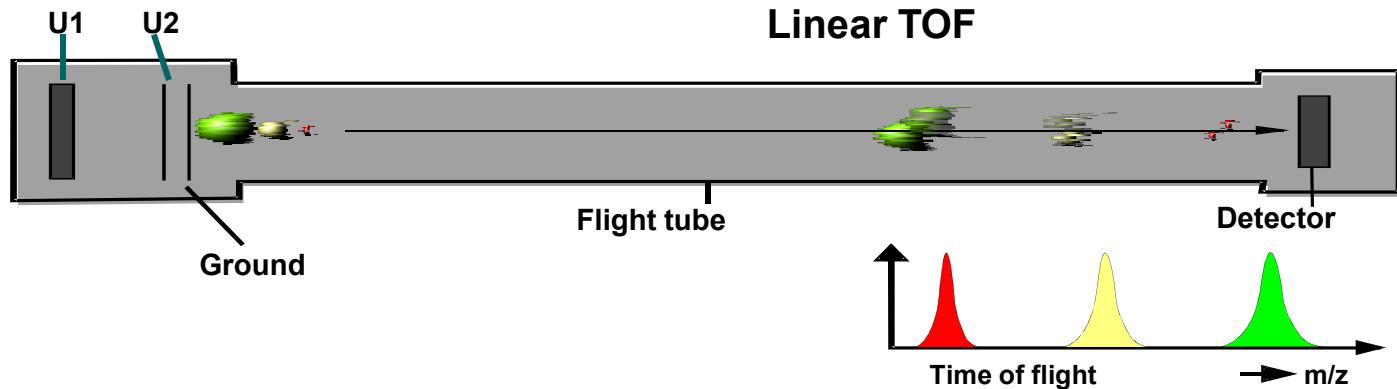
Increasing MW



Ions in the detector



Ions analysis in linear mode



Electric field : $E_c = qU = 1/2 mV^2$ Identical for all ions

($V=L/t$ L : tube length)

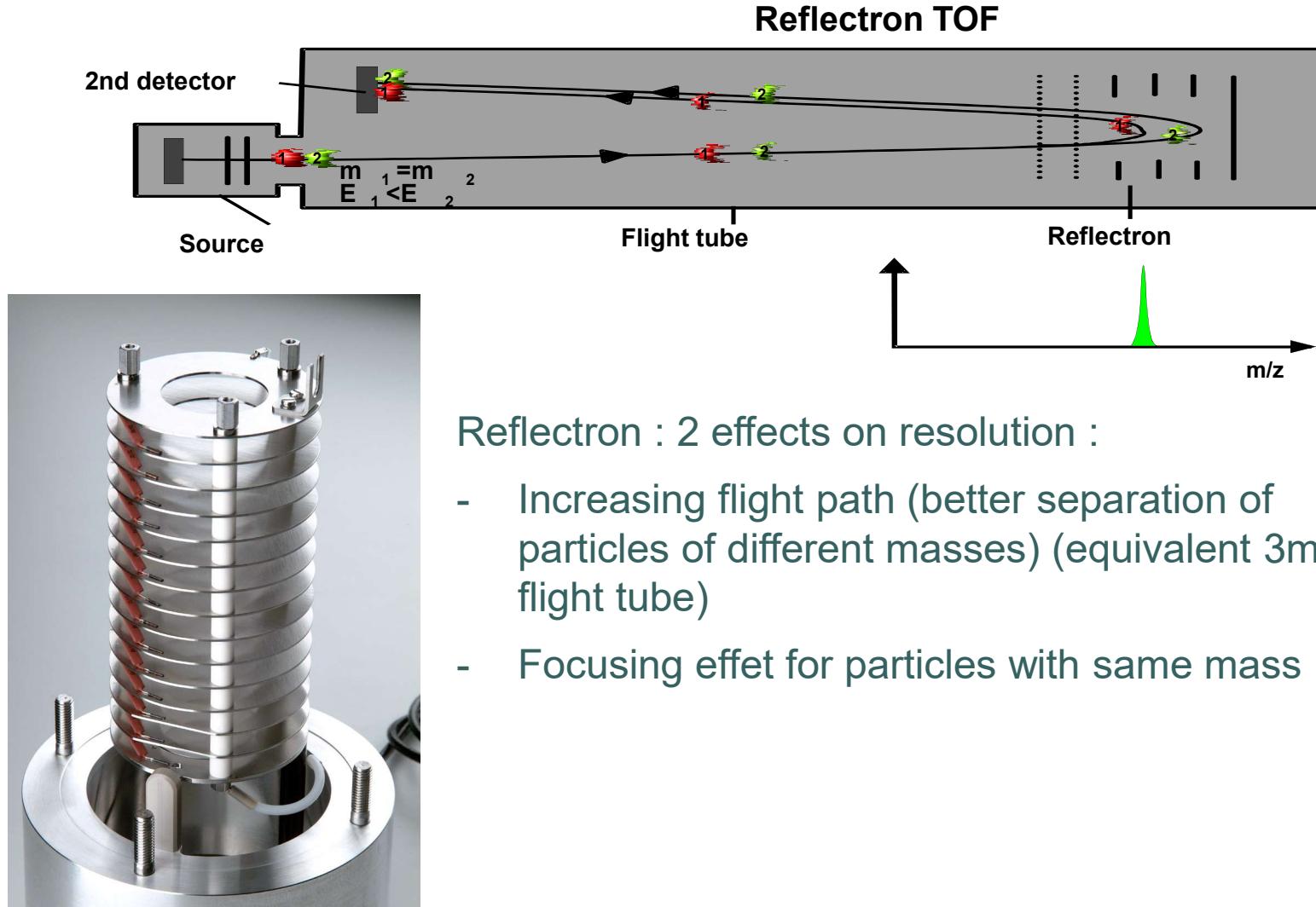
⇒ Simple relation $t^2 = mL^2/2qU = \text{Constante} \times m/z$

Light Corrections :

$$t^2 = Am^2 + Bm + C \quad (\text{A : initial desorption } E_c \quad C : \text{Extraction Delay})$$

=> Simple Quadratic equation

Ions analysis in reflectron mode



Reflectron : 2 effects on resolution :

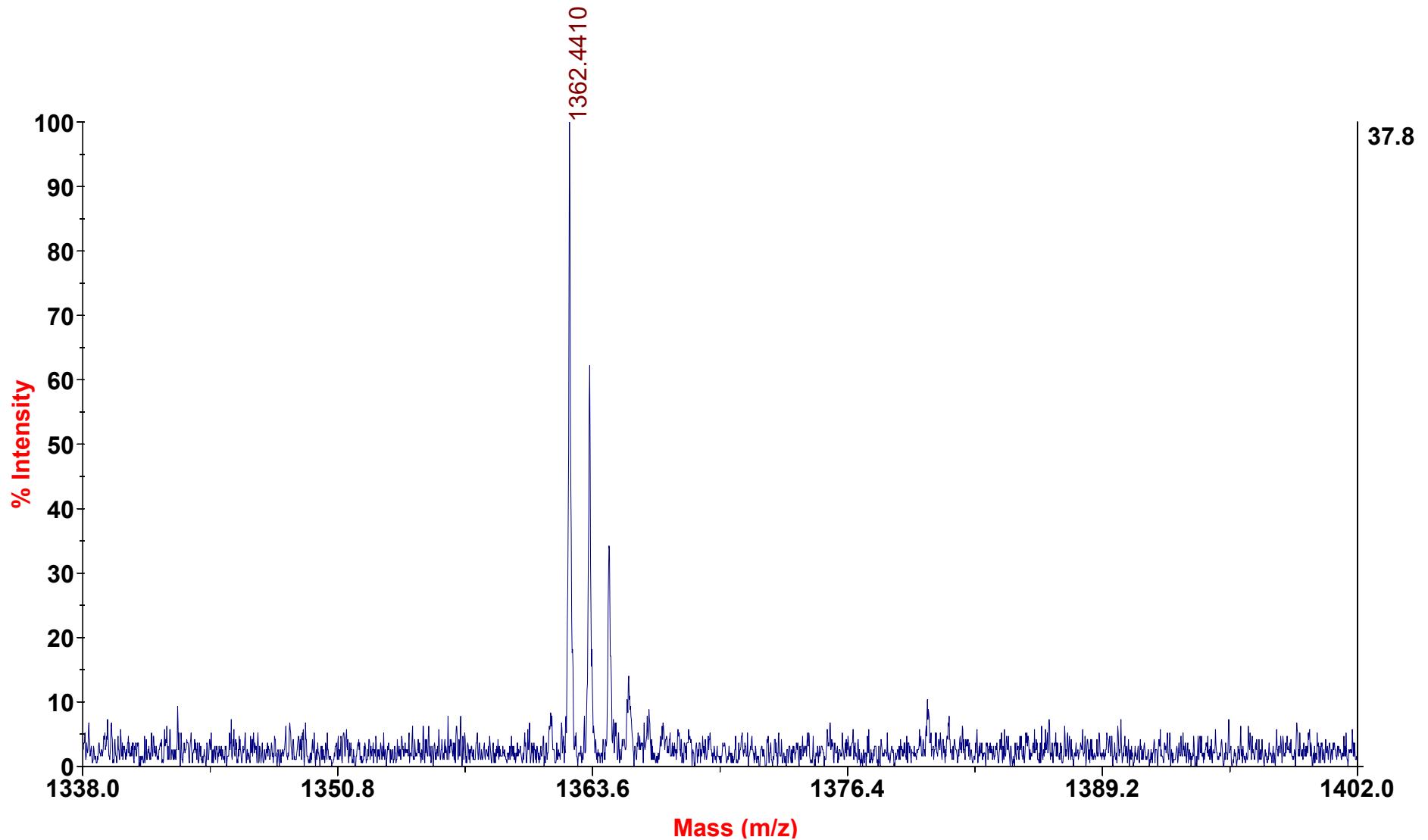
- Increasing flight path (better separation of particles of different masses) (equivalent 3m flight tube)
- Focusing effect for particles with same mass

Key questions in proteomics

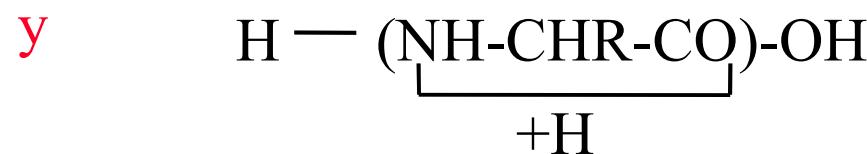
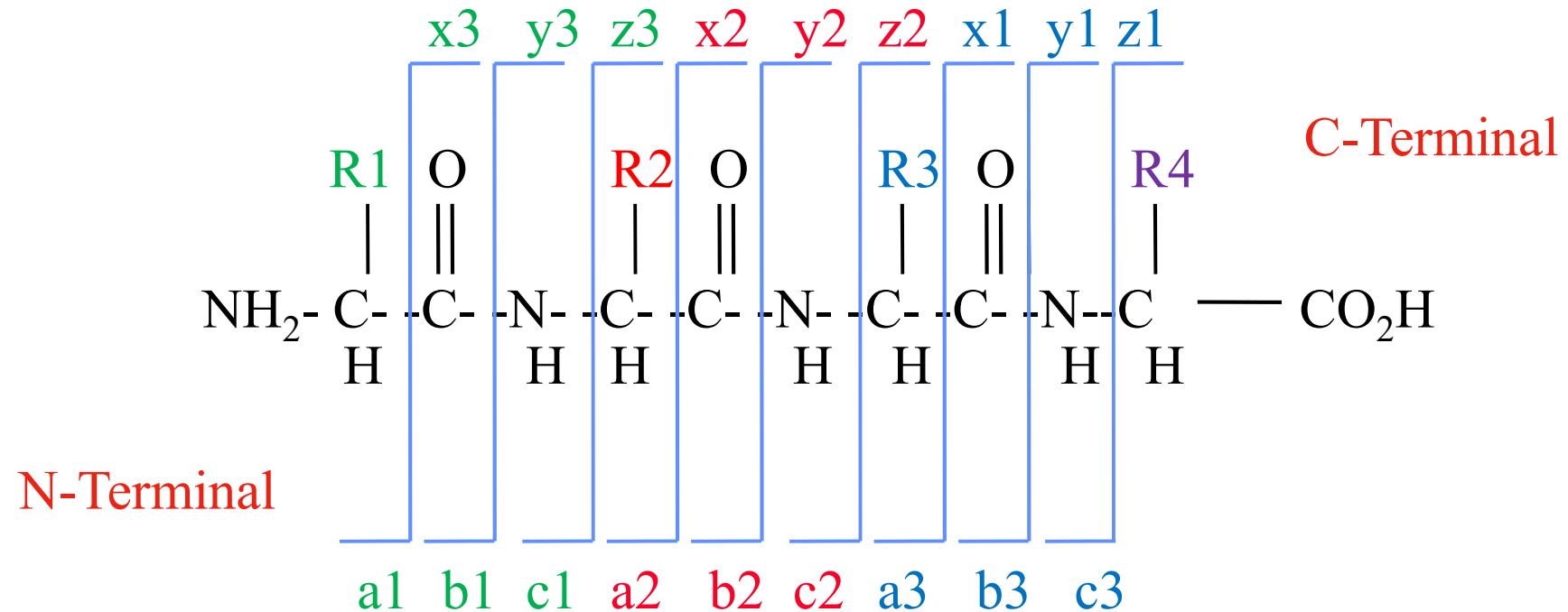
- What is the protein content of my biological sample?
=> problem of **identification**
- What is the abundance of my protein of interest?
=> quantification problem
- Relative question: What are the protein abundance variations of the proteomes studied?
- What are the partners of my protein of interest?
- Are there any signature proteins related to a particular biological process?
=> biomarkers identifications and quantifications

Ion precursor selection

4700 Reflector Spec #1 MC[BP = 1664.6, 132]

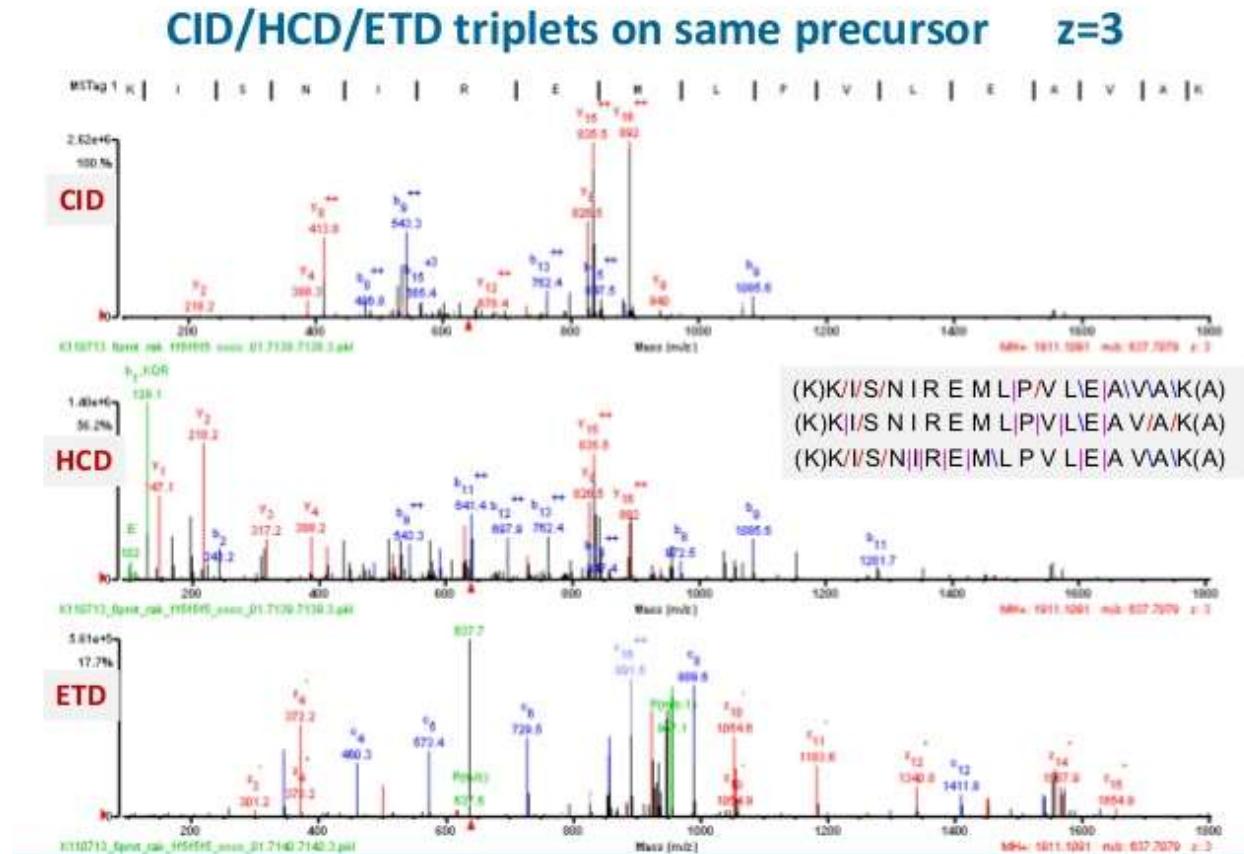


MS/MS fragmentation for peptides

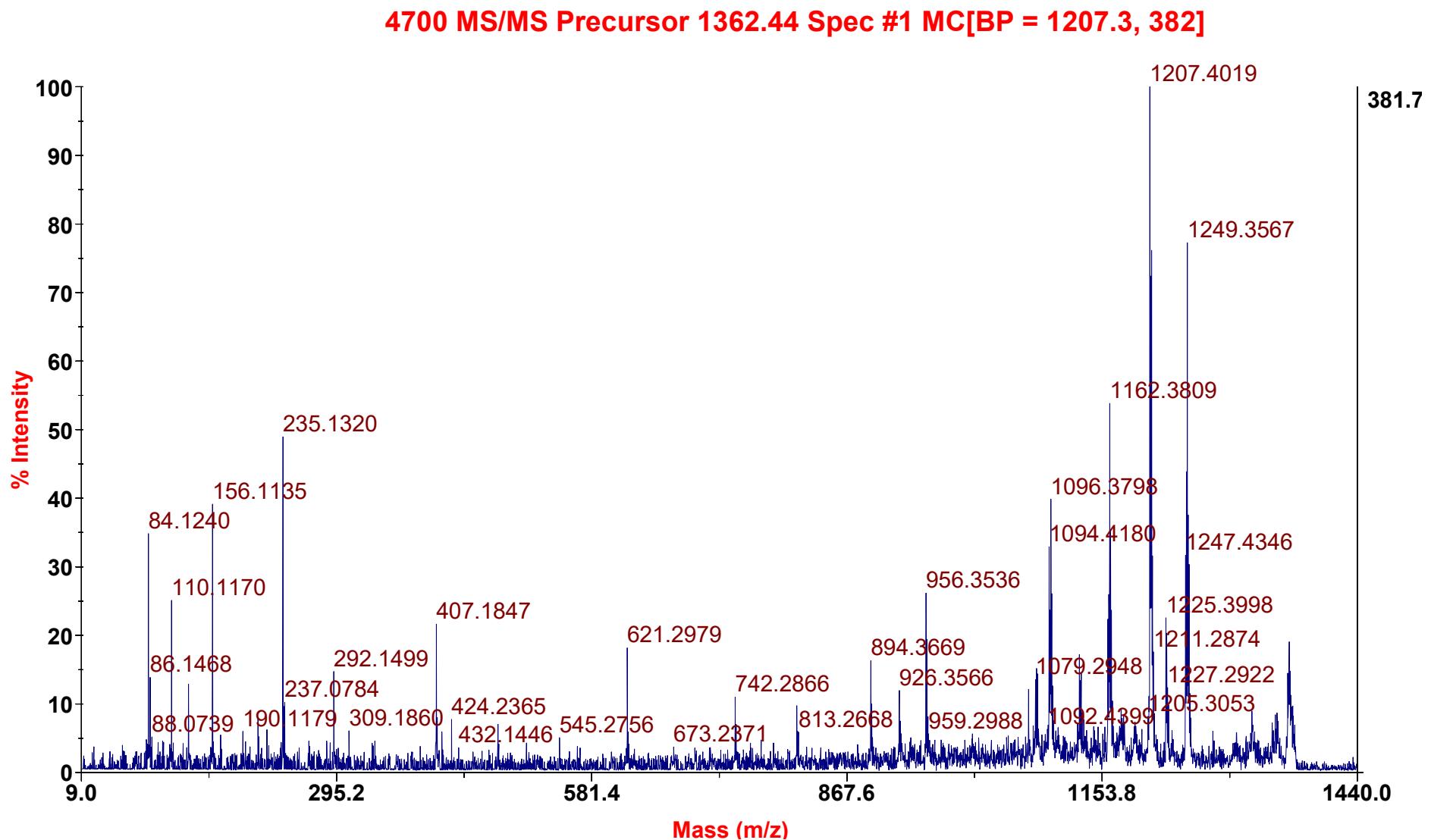


MS/MS fragmentation for peptides

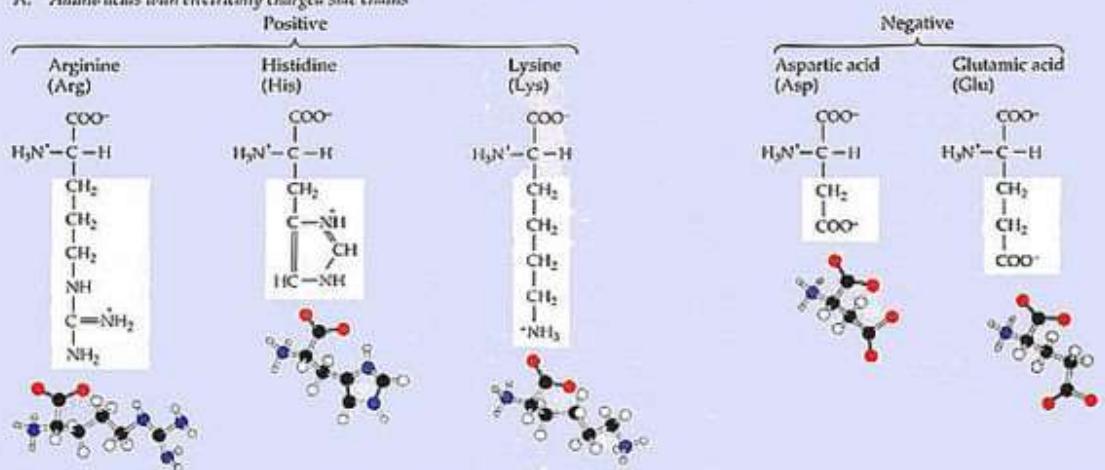
DISSOCIATION INDUIITE PAR COLLISION (CID)
HIGHER ENERGY COLLISIONAL DISSOCIATION (HCD)
ELECTRON TRANSFER DISSOCIATION (ETD)



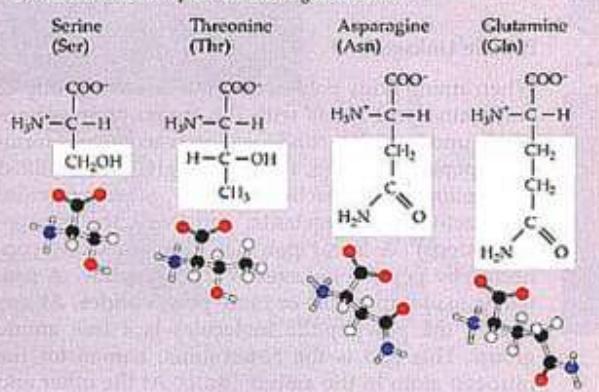
MS/MS spectrum of the precursor 1362.44 m/z



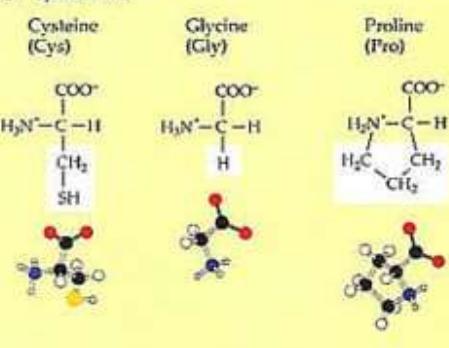
A. Amino acids with electrically charged side chains



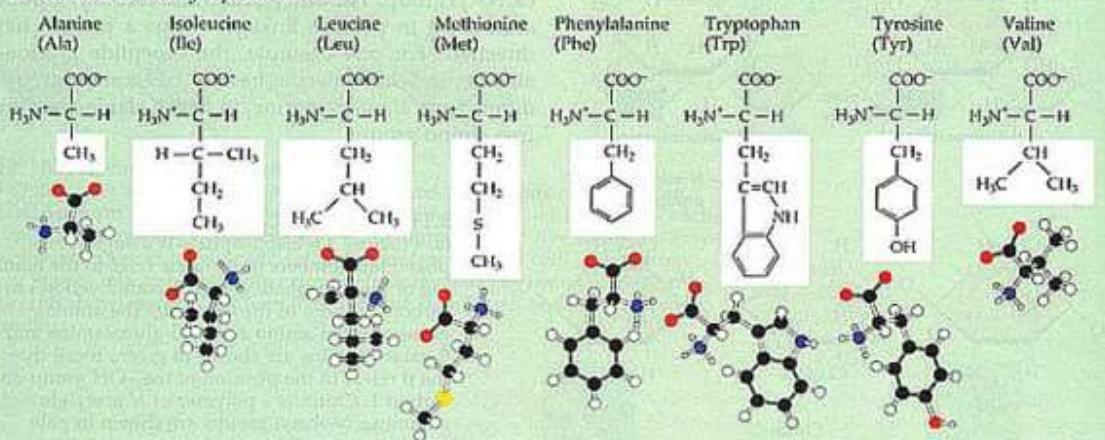
B. Amino acids with polar but uncharged side chains



C. Special cases



D. Amino acids with hydrophobic side chains



<u>Alanine</u>	A, Ala	71.079
<u>Arginine</u>	R, Arg	156.188
<u>Asparagine</u>	N, Asn	114.104
<u>Aspartic acid</u>	D, Asp	115.089
<u>Cysteine</u>	C, Cys	103.145
<u>Glutamine</u>	Q, Gln	128.131
<u>Glutamic acid</u>	E, Glu	129.116
<u>Glycine</u>	G, Gly	57.052
<u>Histidine</u>	H, His	137.141
<u>Isoleucine</u>	I, Ile	113.160
<u>Leucine</u>	L, Leu	113.160
<u>Lysine</u>	K, Lys	128.17
<u>Methionine</u>	M, Met	131.199
<u>Phenylalanine</u>	F, Phe	147.177
<u>Proline</u>	P, Pro	97.117
<u>Serine</u>	S, Ser	87.078
<u>Threonine</u>	T, Thr	101.105
<u>Tryptophan</u>	W, Trp	186.213
<u>Tyrosine</u>	Y, Tyr	163.176
<u>Valine</u>	V, Val	99.133

MS/MS spectra interpretation

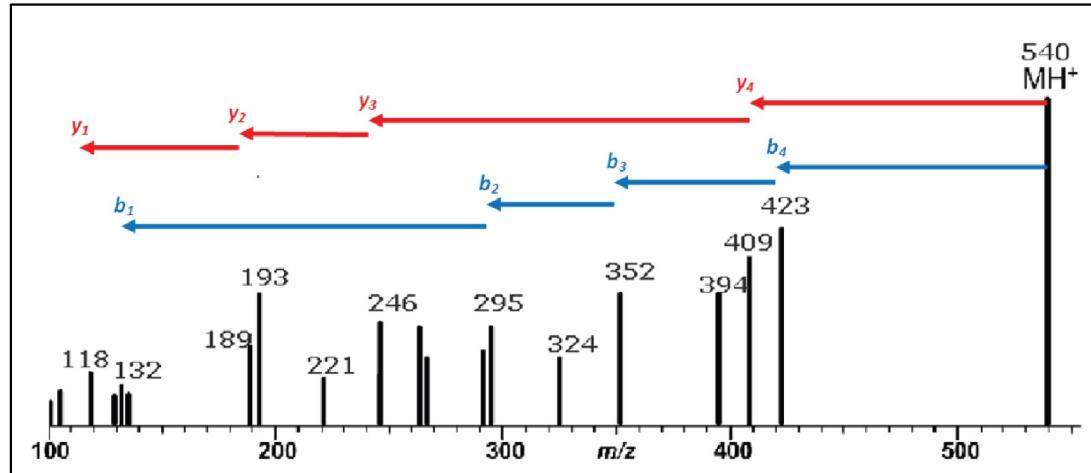


Table 1

Ion	<i>m/z</i>	Neutral loss (from previous ion in the series)	Amino Acid Residue
Precursor [M+H] ⁺	540		
<i>y</i> ₄	409	131	M
<i>y</i> ₃	246	163	Y
<i>y</i> ₂	189	57	G
<i>y</i> ₁	118	71	A
<i>b</i> ₄	423	117 (99+18)	V
<i>b</i> ₃	352	71	A
<i>b</i> ₂	295	57	G
<i>b</i> ₁	132	163	Y
<i>a</i> ₄	395?		
<i>a</i> ₃	324		
<i>a</i> ₂	267		
<i>a</i> ₁	104		

MYGAV

User AA Formula 1: C2 H3 N1 O1

Elemental Composition: C24 H38 N5 O7 S1

MH+1(av) MH+1(mono)

540.6627 540.2486

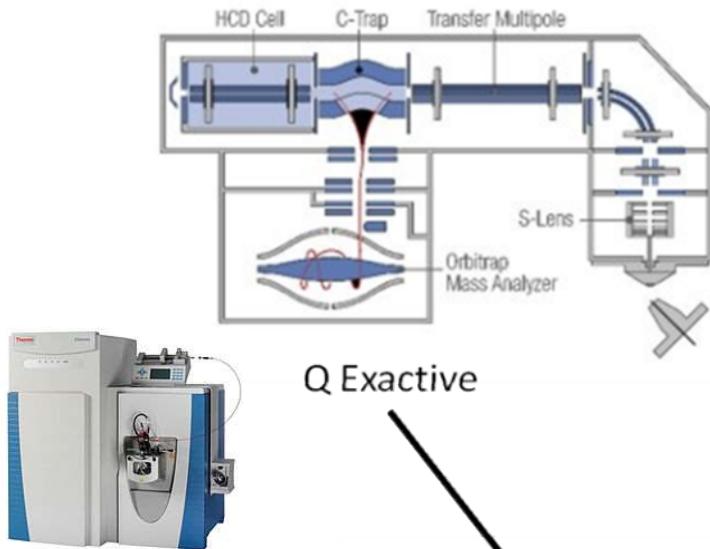
[–] Main Sequence Ions

b			y	
---	1	M	5	---
295.1111	2	Y	4	409.2082
352.1326	3	G	3	246.1448
423.1697	4	A	2	189.1234
---	5	V	1	118.0863

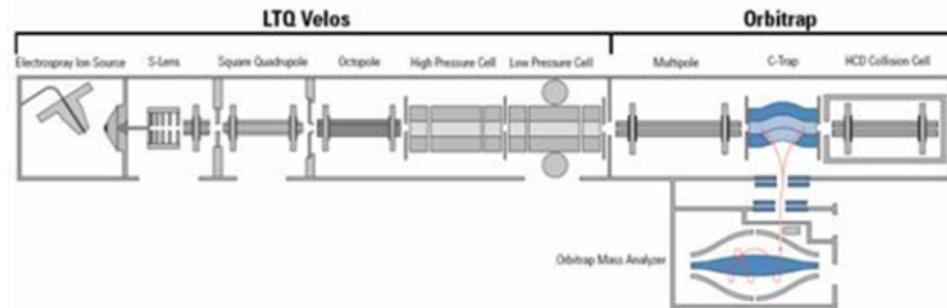
Current post-translational modifications (PTMs)

Acids & amides (E/D/Q/N)	Pyroglutamic acid (Q)	-17.0306	Deamidation (Q/N)	+0.9847
	Carboxylation (E/D)	+44.0098		
Hydroxyl groups (S/T/Y)	Phosphorylation	+79.9799	Sulphation	+80.0642
Carbohydrates (S/T/N)	Pentoses	+132.1161	Deoxyhexoses	+146.1430
	Hexosamines	+161.1577	Hexoses	+162.1424
	N-acetylhexosamines	+203.1950	Sialic acid	+291.2579
Sulphydryls (C)	Disulphide bond	-2.0159	Oxidation	+15.9994
	Cysteinylation	+119.1442	Glutathionylation	+305.3117

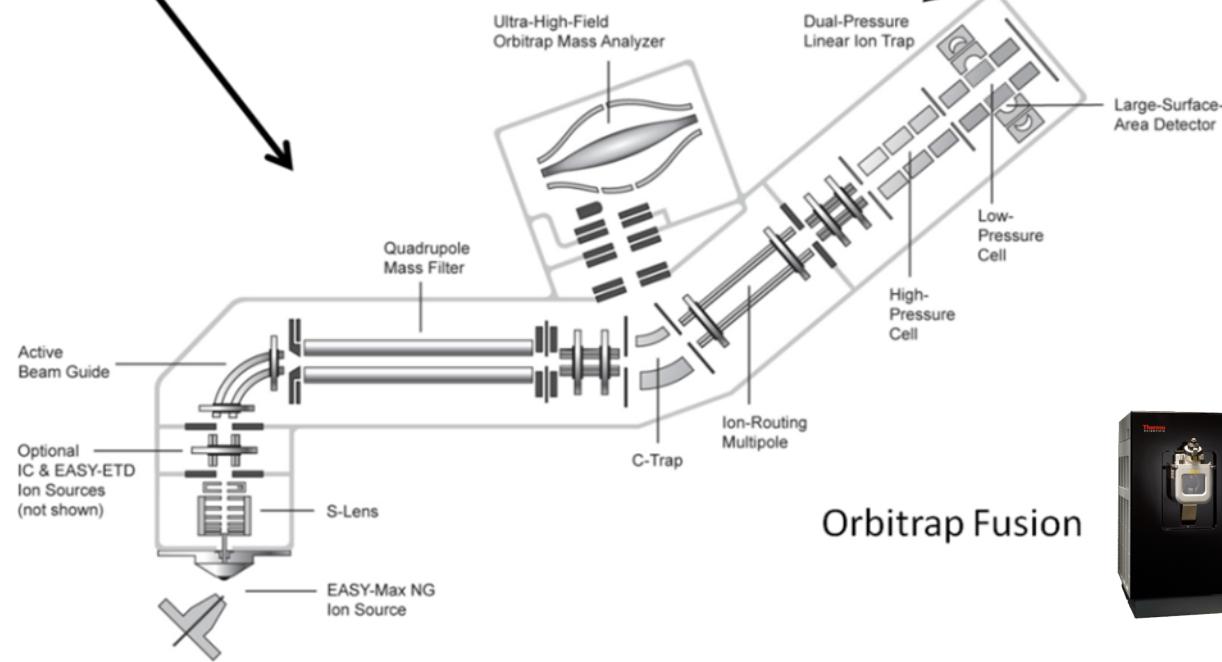
Orbitrap mass spectrometers



Q Exactive



Orbitrap Velos

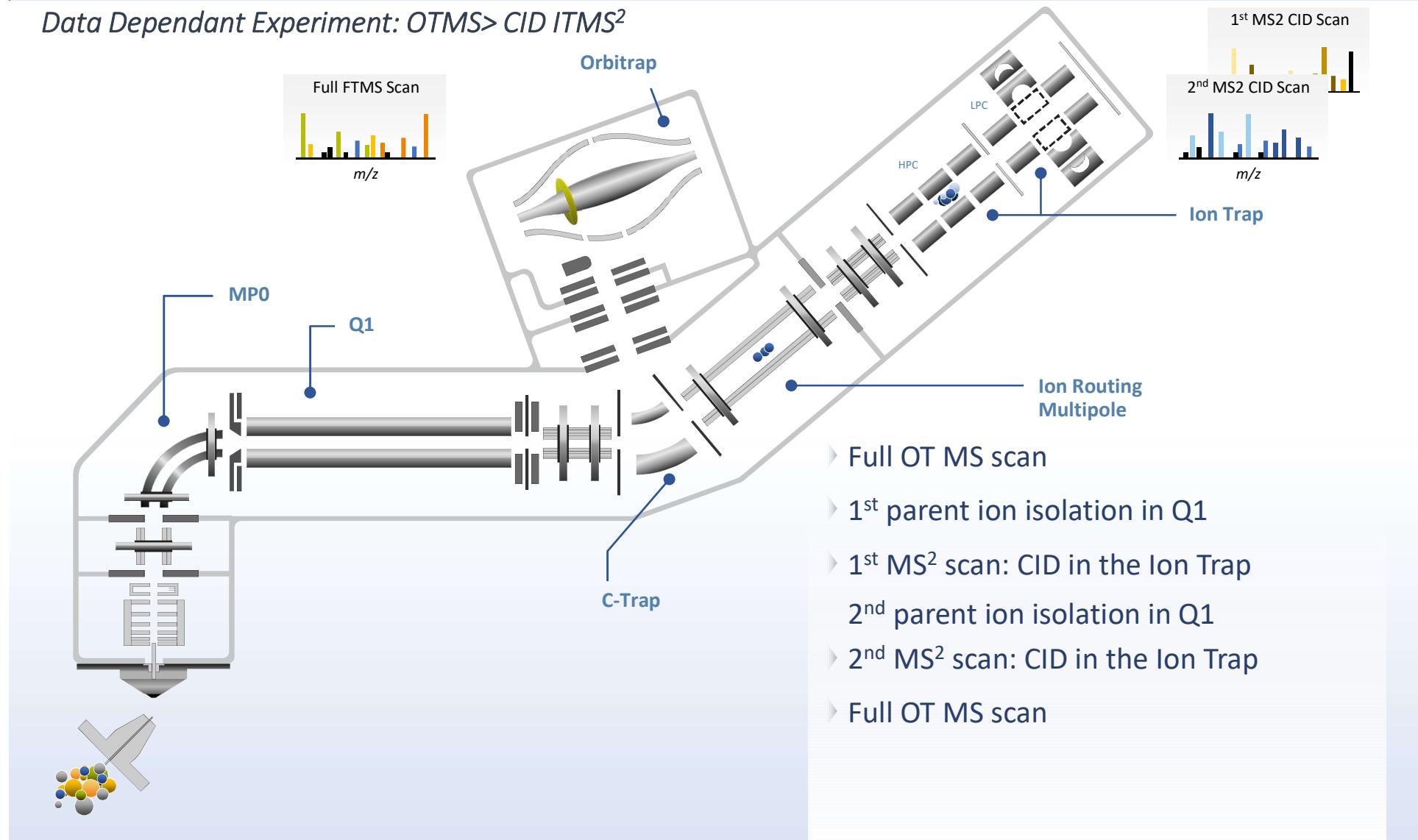


Orbitrap Fusion

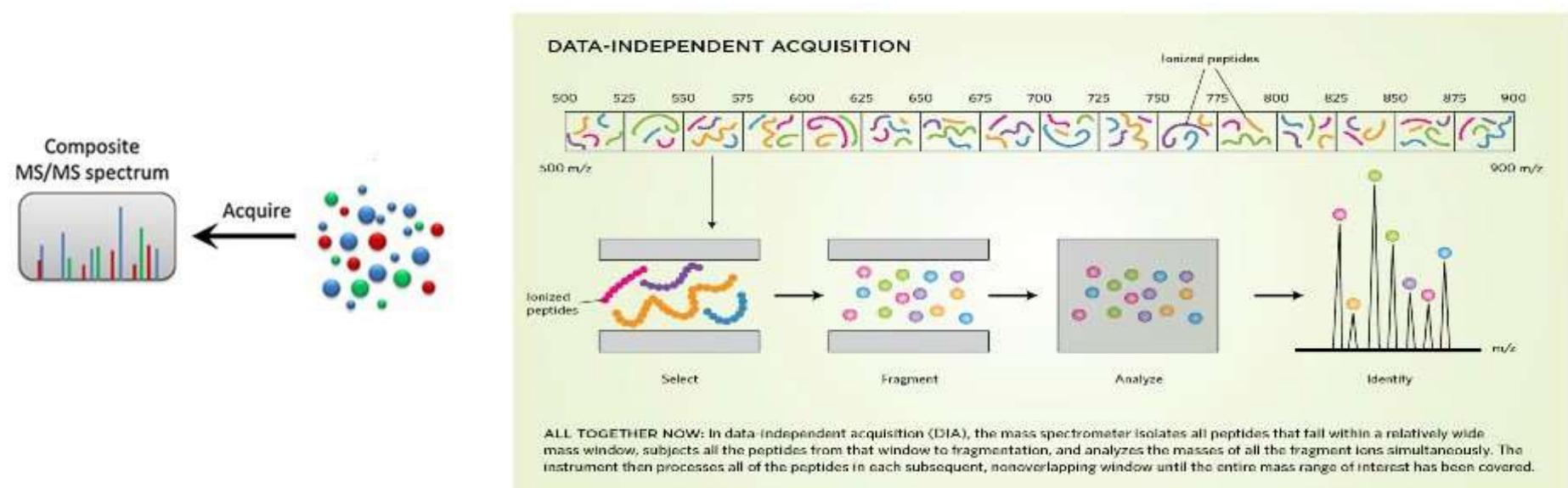
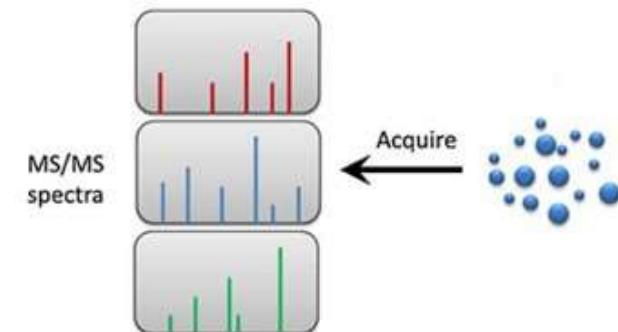
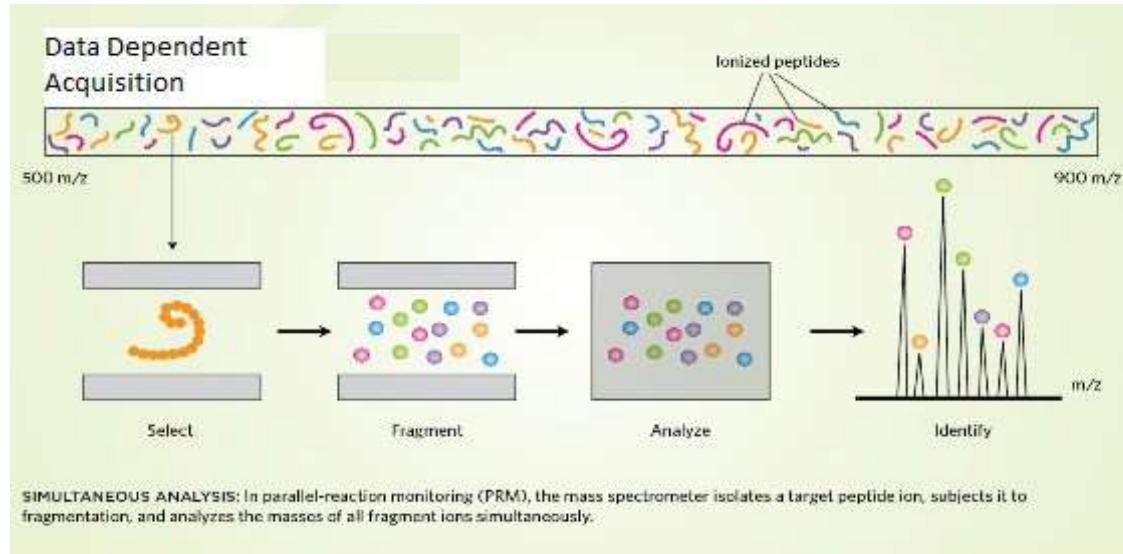


MS and MS/MS spectra generation

Data Dependant Experiment: OTMS> CID ITMS²



DDA versus DIA



Data Independent Acquisition: DIA

(A) DIA

Orbitrap			...			
MS/MS	m/z	m/z	m/z	m/z	m/z	m/z
Full scan	500- 520- 540-	840- 860- 880-		520 540 560	860 880 900	
R=30K						

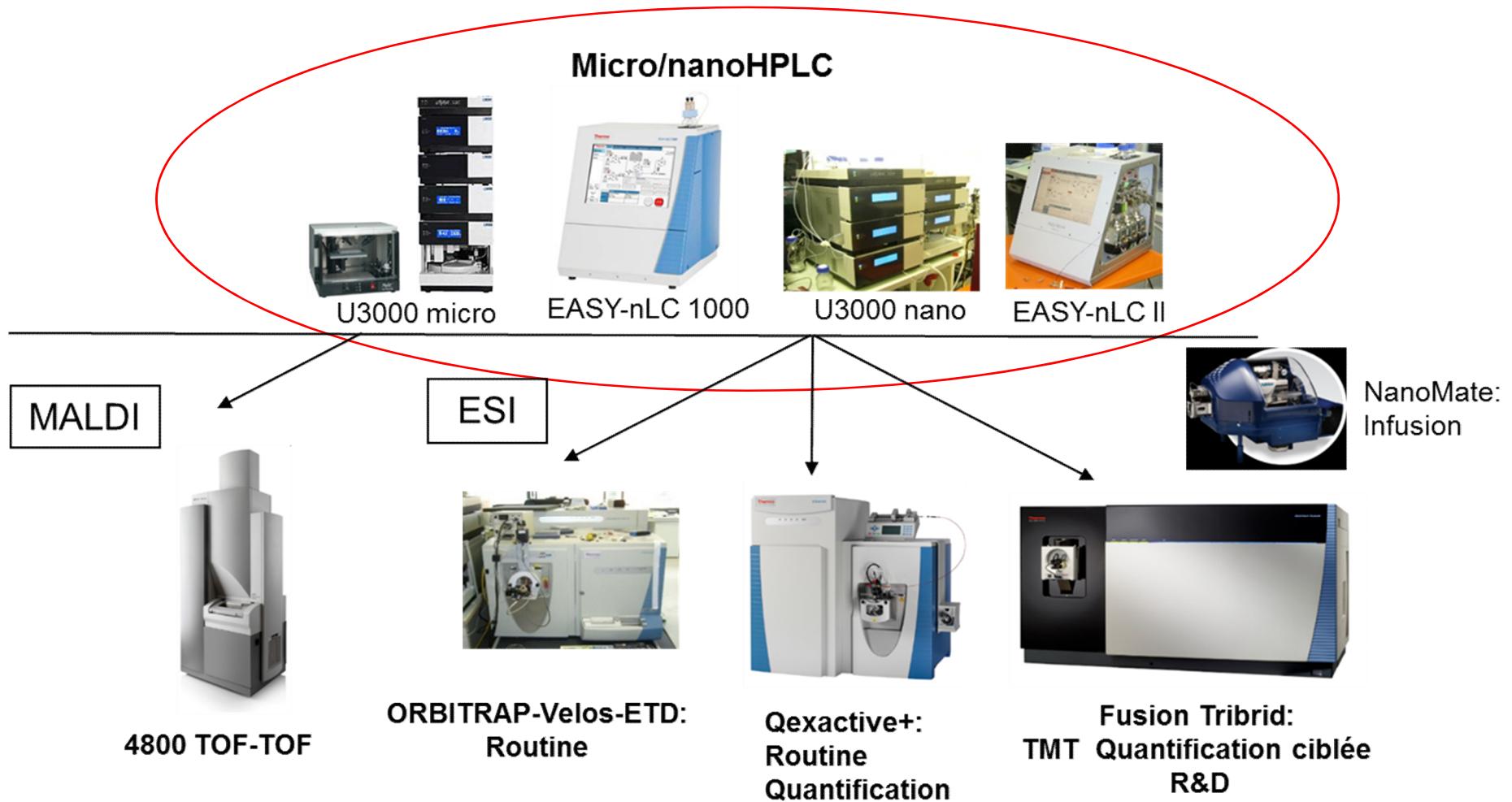
(B) WiSIM-DIA

Orbitrap						
MS						
SIM scan	m/z 400-600	m/z 600-800	m/z 800-1000			
R=240K						
Ion Trap						
MS/MS	m/z m/z	m/z m/z	m/z m/z			
Full scan	400- 412- 412 424	568- 580- 592- 580 592 604	600- 612- 612 624	768- 780- 792- 780 792 804	800- 812- 812 824	968- 980- 992- 980 992 1004

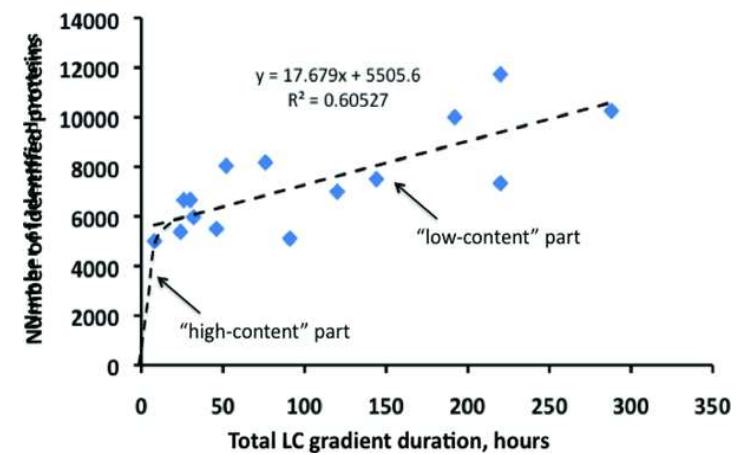
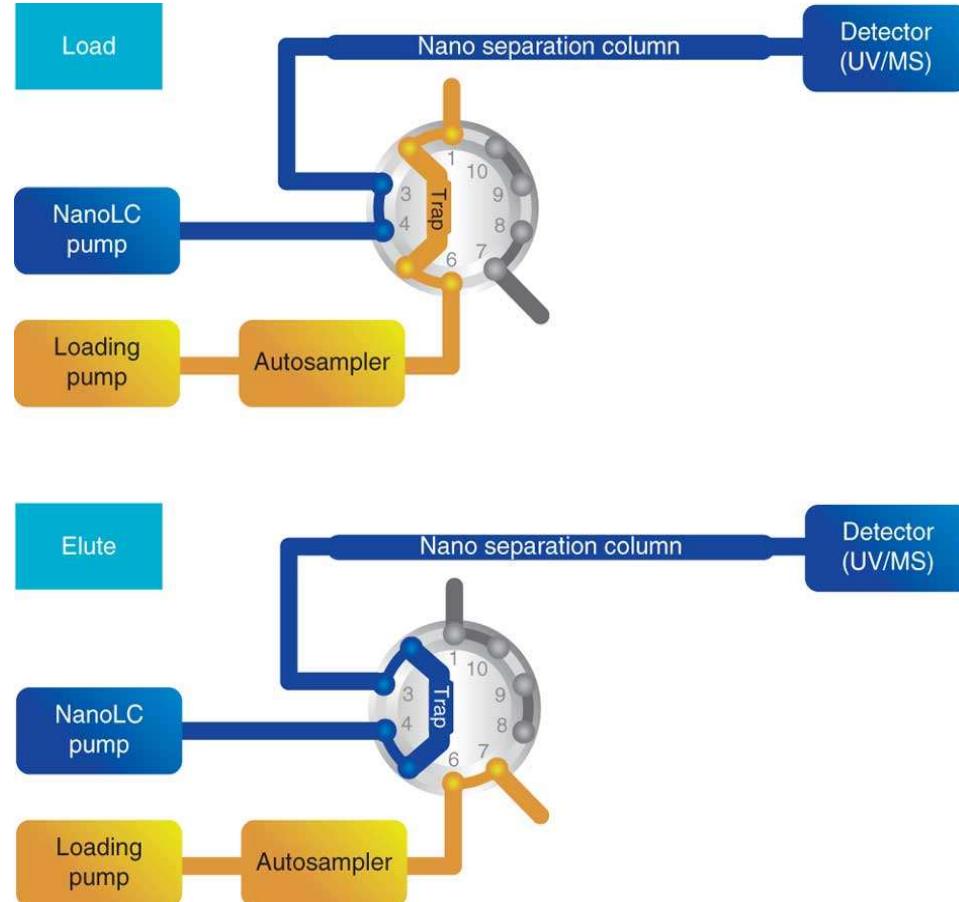
(C) Full MS-DIA

Orbitrap										
MS										
Full scan	m/z 400-1000									
R=240K										
Ion Trap										
MS/MS	m/z m/z									
Full scan	400- 403- 403 406	514- 517- 517 520	520- 523- 523 526	634- 637- 637 640	640- 643- 643 646	754- 757- 757 760	760- 763- 763 766	874- 877- 877 880	880- 883- 883 886	994- 997- 997 1000

Contribution of nano-HPLC



Peptides separation by nano-LC

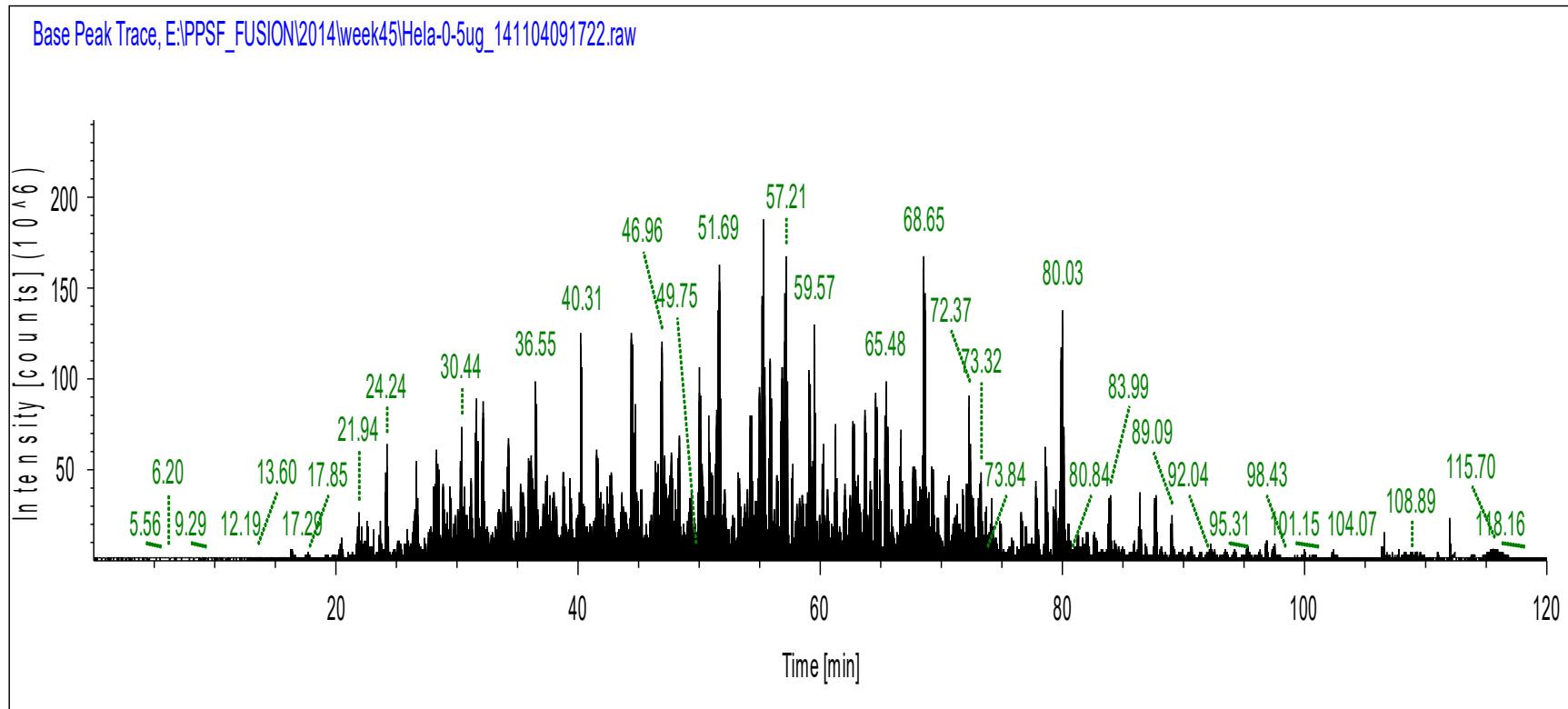


Peptides separation nano-LC

- It is impossible to resolve all species in a proteomics sample using only one separation method
- Multidimensional separation - two or more independent (“orthogonal”) separation techniques coupled together for the analysis of a single sample.

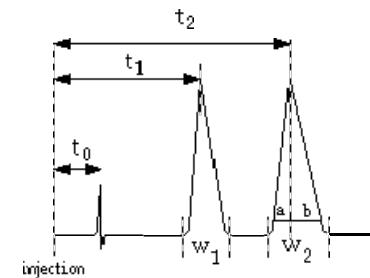
Separation method	Separation by:
Reversed phase	Hydrophobicity
Ion exchange, IsoElectroFocusing (IEF)	Net charge, Isoelectric point
Size exclusion, SDS Gel Electrophoresis	Size, molecular weight
Affinity chromatography	Specific functional groups

Total ion current (TIC) Hela tryptic digest (0.5 µg of total proteins)

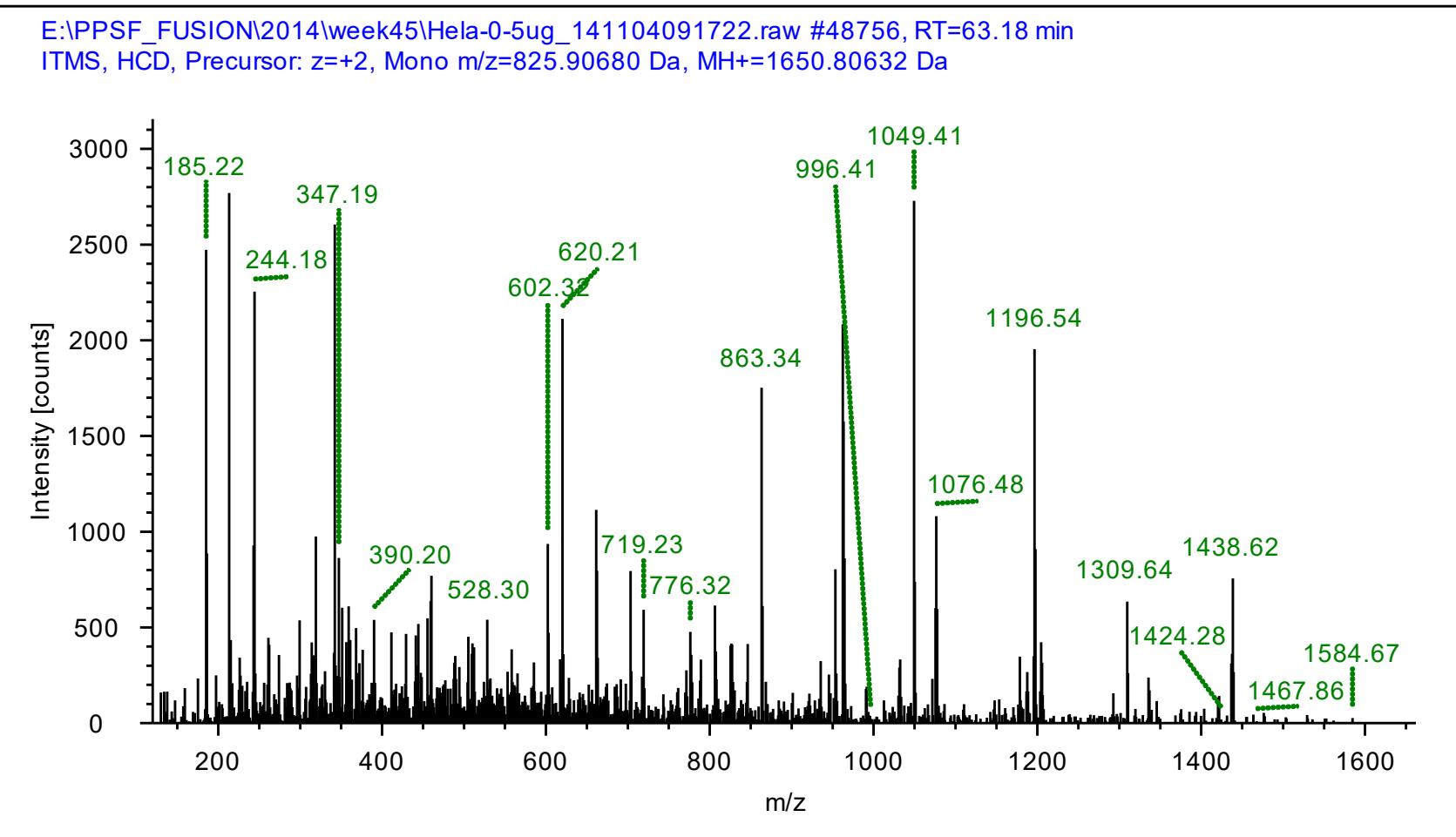


$$R = \frac{2(t_2 - t_1)}{(w_2 - w_1)}$$

with t_1 and t_2 the retention time and w_1 and w_2 peak widths at mid-height



90000 MSMS in 2h gradient (C18 RPC)



Proprietary MS data formats

Company	Extension	File type
Agilent	.D (folder)	Agilent MassHunter, Agilent ChemStation, or
Bruker		Bruker BAF/YEP/TDF data format
Agilent/Bruker	.YEP	instrument data format
Bruker	.BAF	instrument data format
Bruker	.FID	instrument data format
Bruker	.TDF	timsTOF instrument data format
ABI/Sciex	.WIFF	instrument data format
ABI/Sciex	.t2d	4700 and 4800 file format
Waters	.PKL	MassLynx peak list format
Thermo	.RAW*	Thermo Xcalibur
PerkinElmer		PerkinElmer TurboMass
Micromass**/Waters	.RAW* (folder)	Waters MassLynx
Chromtech		Finnigan ITDS file format; MAT95 instrument
Finnigan***	.DAT	data format
VG		MassLab data format
Finnigan***	.MS	ITS40 instrument data format
Shimadzu	.QGD	GCMSSolution format
Shimadzu	.qgd	instrument data format
Shimadzu	.lcd	QQQ/QTOF instrument data format
Shimadzu	.spc	library data format
Bruker/Varian	.SMS	instrument data format
Bruker/Varian	.XMS	instrument data format
ION-TOF	.itm	raw measurement data
ION-TOF	.ita	analysis data
Physical Electronics/ULVAC-PHI	.raw*	raw measurement data
Physical Electronics/ULVAC-PHI	.tdc	spectrum data

Open MS data formats

JCAMP-DX

This format was one of the earliest attempts to supply a standardized file format for data exchange in mass spectrometry. JCAMP-DX was initially developed for infrared spectrometry. JCAMP was officially released in 1988. JCAMP was found impractical for today's large MS data sets, but it is still used for exchanging moderate numbers of spectra.

ANDI-MS or netCDF

The Analytical Data Interchange Format for Mass Spectrometry is a format for exchanging data. ANDI was initially developed for chromatography-MS data and therefore was not used in the [proteomics](#) gold rush where new formats based on [XML](#) were developed.

mzData

mzData was the first attempt by the [Proteomics Standards Initiative](#) (PSI) from the [Human Proteome Organization](#) (HUPO) to create a standardized format for Mass Spectrometry data. This format is now deprecated, and replaced by mzML.

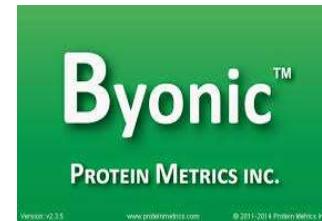
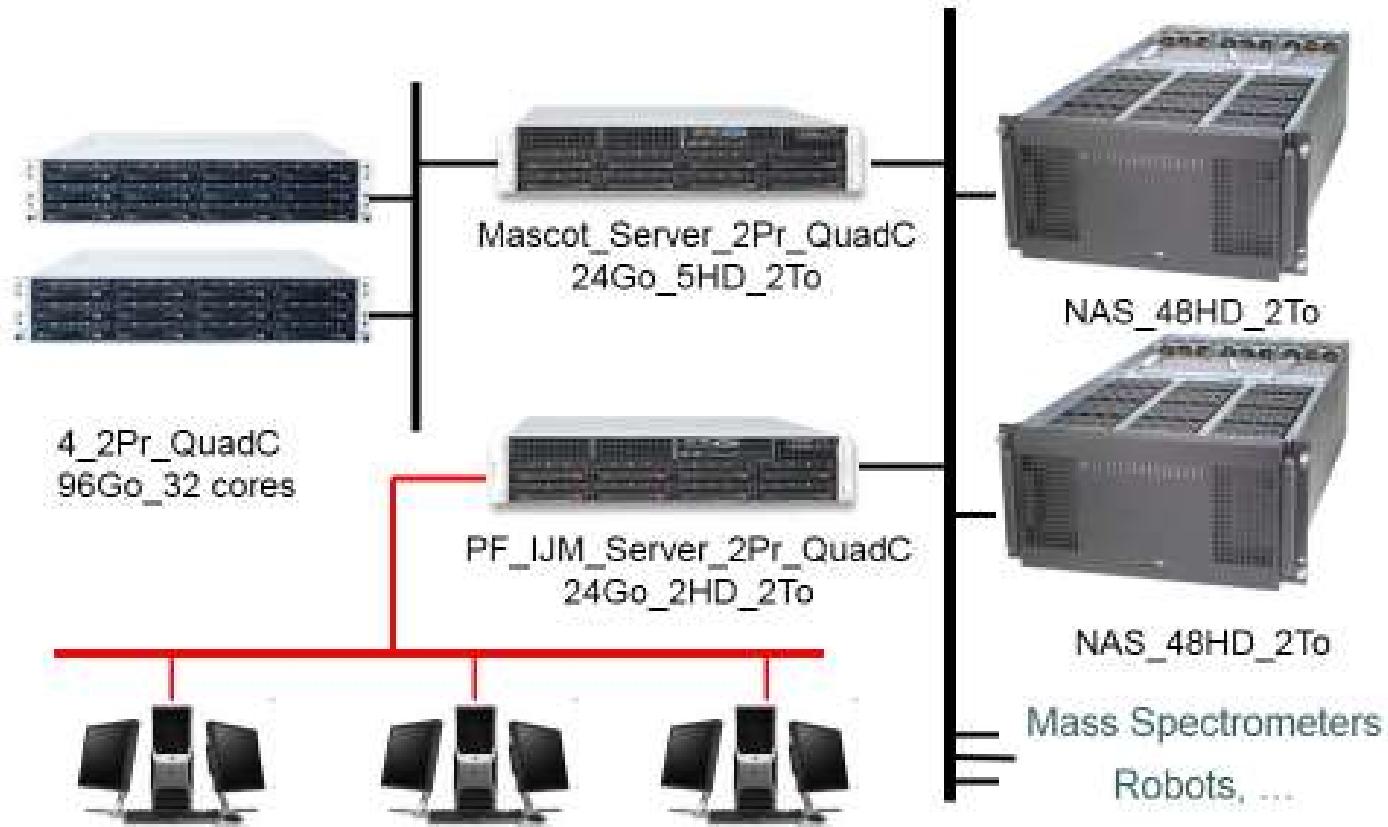
mzXML

mzXML is a [XML](#) (eXtensible Markup Language) based common file format for [proteomics](#) mass spectrometric data. This format was developed at the Seattle Proteome Center/Institute for Systems Biology while the HUPO-PSI was trying to specify the standardized mzData format, and is still in use in the proteomics community.

mzML

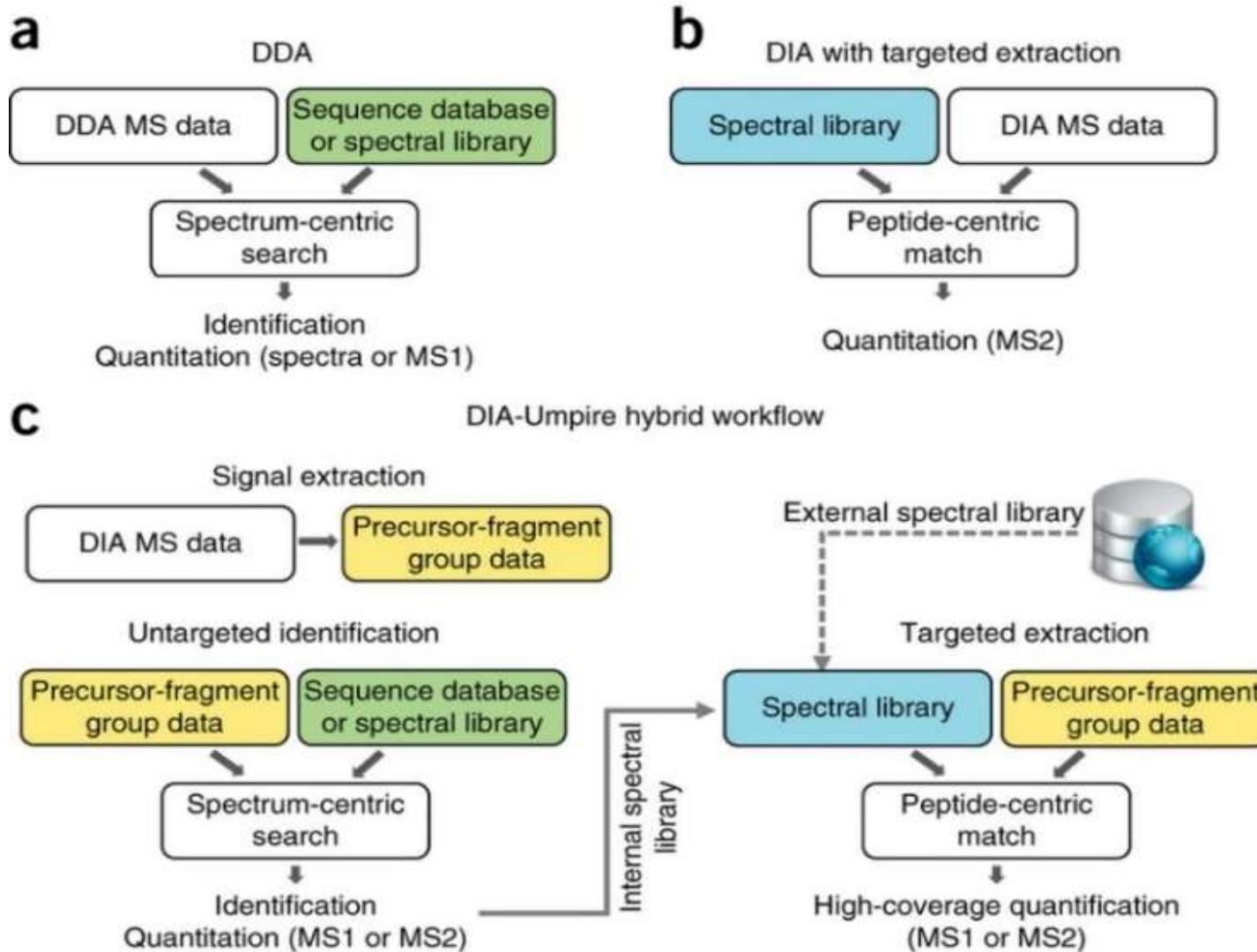
As two formats (mzData and mzXML) for representing the same information is an undesirable state, a joint effort was set by HUPO-PSI, the SPC/ISB and instrument vendors to create a unified standard borrowing the best aspects of both mzData and mzXML, and intended to replace them. The first specification was published in June 2008. This format was officially released at the 2008 [American Society for Mass Spectrometry](#) Meeting, and is since then relatively stable with very few updates. On 1 June 2009, mzML 1.1.0 was released. There are no planned further changes as of 2013.

Saving data and servers



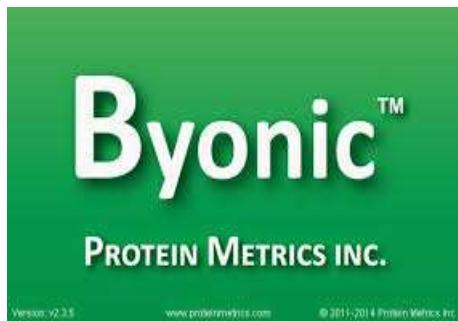
{MATRIX}
SCIENCE}

Search engine



Search engines and validation of peptides and proteins identifications

$$FDR (\%) = \frac{(number\ of\ false\ positive\ peptides) \times 2}{total\ number\ of\ peptides\ (positives + false\ positive)}$$



{*MATRIX*}
{*SCIENCE*}



Critical importance of mass accuracy for database searches

Expressed as Da or as ppm ($10 \text{ ppm} = 0,001\%$ $1 \text{ ppm} = 0,0001\%$)

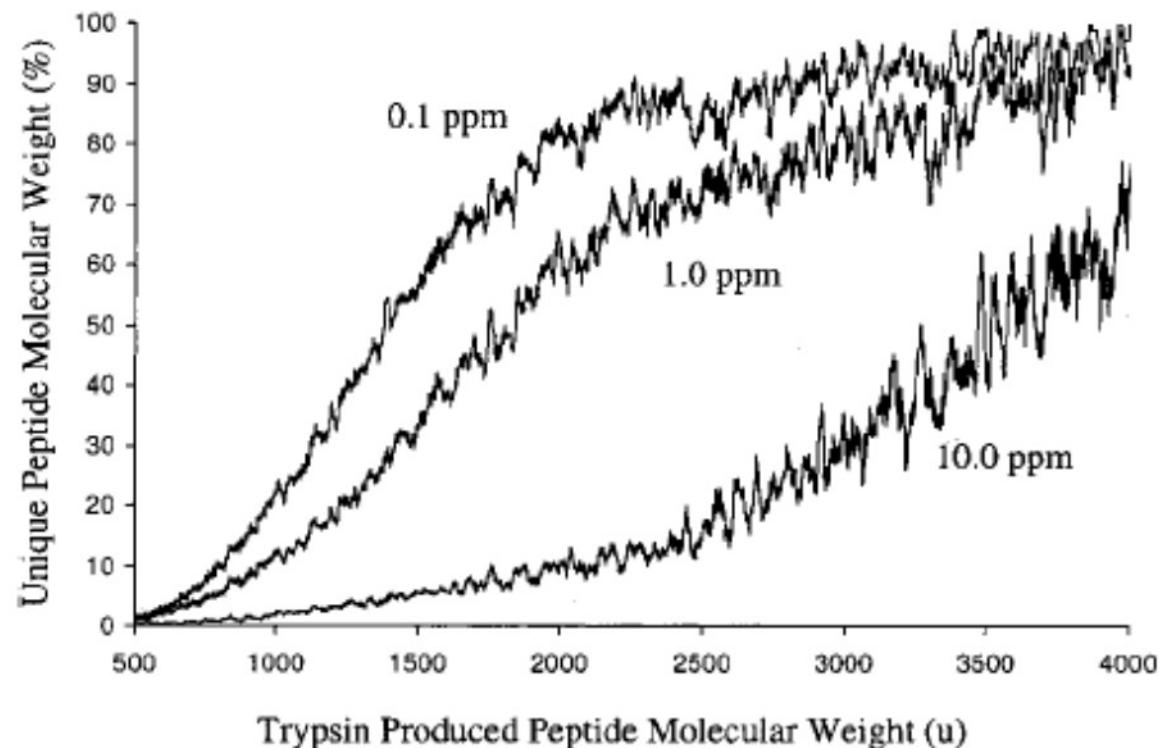


Figure 1. All possible unique peptide molecular weights after digestion of all yeast proteins in the National Center for Biotechnology Information at a mass accuracy of 0.1, 1.0, and 10.0 ppm.

A database search engine : Mascot

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Mascot database search > Access Mascot Server > MS/MS Ions Search

MASCOT MS/MS Ions Search

Your name _____ Email _____

Search title _____

Database(s) Invertebrates_EST
Human_EST
Fungi_EST
Environmental_EST
SwissProt

Enzyme Trypsin
Allow up to 1 missed cleavages

Quantitation None

Taxonomy All entries

Fixed modifications --- none selected --- > < Acetyl (K)
Acetyl (N-term)
Acetyl (Protein N-term)
Amidated (C-term)
Amidated (Protein C-term)
Ammonia-loss (N-term C)
Biotin (K)
Biotin (N-term)
Carbamidomethyl (C)
Carbamyl (K)
Carbamyl (N-term)

Variable modifications --- none selected --- > <

Display all modifications

Peptide tol. ± 1.2 Da # ¹³C 0 MS/MS tol. ± 0.6 Da

Peptide charge 2+ Monoisotopic Average

Data file Parcourir... Aucun fichier sélectionné.

Data format Mascot generic Precursor m/z

Instrument Default Error tolerant

Decoy Report top AUTO hits

Start Search ... Reset Form

MATRIX SCIENCE

MASCOT MS/MS Ions Search

Your name

Email

Search title

Database(s) Invertebrates_EST
Human_EST
Fungi_EST
Environmental_EST
SwissProt

Enzyme Trypsin

Allow up to 1 missed cleavages

Quantitation None

Taxonomy All entries

Fixed modifications All entries
... Archaea (Archaeobacteria)
... Eukaryota (eucaryotes)
.... Alveolata (alveolates)
..... Plasmodium falciparum (malaria parasite)
..... Other Alveolata
.... Metazoa (Animals)
..... Caenorhabditis elegans
..... Drosophila (fruit flies)
..... Chordata (vertebrates and relatives)
..... bony vertebrates
..... lobe-finned fish and tetrapod clade
..... Mammalia (mammals)
..... Primates
..... Homo sapiens (human)
..... Other primates
..... Rodentia (Rodents)
..... Mus.
..... Mus musculus (house mouse)
..... Rattus

Variable modifications Acetyl (K)
Acetyl (N-term)
Acetyl (Protein N-term)
Amidated (C-term)
Amidated (Protein C-term)
Ammonia-loss (N-term C)
Biotin (K)
Biotin (N-term)
Carbamidomethyl (C)
Carbamyl (K)
Carbamyl (N-term)

Peptide tol. ± 0.6 Da

Peptide charge Average

Data file

Data format

Instrument Default

Error tolerant

Decoy

Report top AUTO hits

Start Search ...

Reset Form

MASCOT MS/MS Ions Search

Your name

Email

Search title

Database(s) Invertebrates_EST
Human_EST
Fungi_EST
Environmental_EST
SwissProt

Enzyme Trypsin
Trypsin
Trypsin/P
Arg-C
Asp-N
Asp-N_ambic
Chymotrypsin
CNBr
CNBr+Trypsin
Formic_acid
Lys-C
Lys-C/P
LysC+AspN
Lys-N
PepsinA
semiTrypsin
TrypChymo
TrypsinMSIPI
TrypsinMSIPI/P
V8-DE
V8-E

Allow up to

Quantitation

Taxonomy All entries

Fixed modifications --- none selected ---
Display all modifications

Variable modifications --- none selected ---

Peptide tol. ± 1.2 Da # ¹³C 0 MS/MS tol. ±

Peptide charge 2+ Monoisotopic Average

Data file Parcourir... Aucun fichier sélectionné.

Data format Mascot generic

Precursor m/z

Instrument Default

Error tolerant

Decoy

Report top AUTO hits

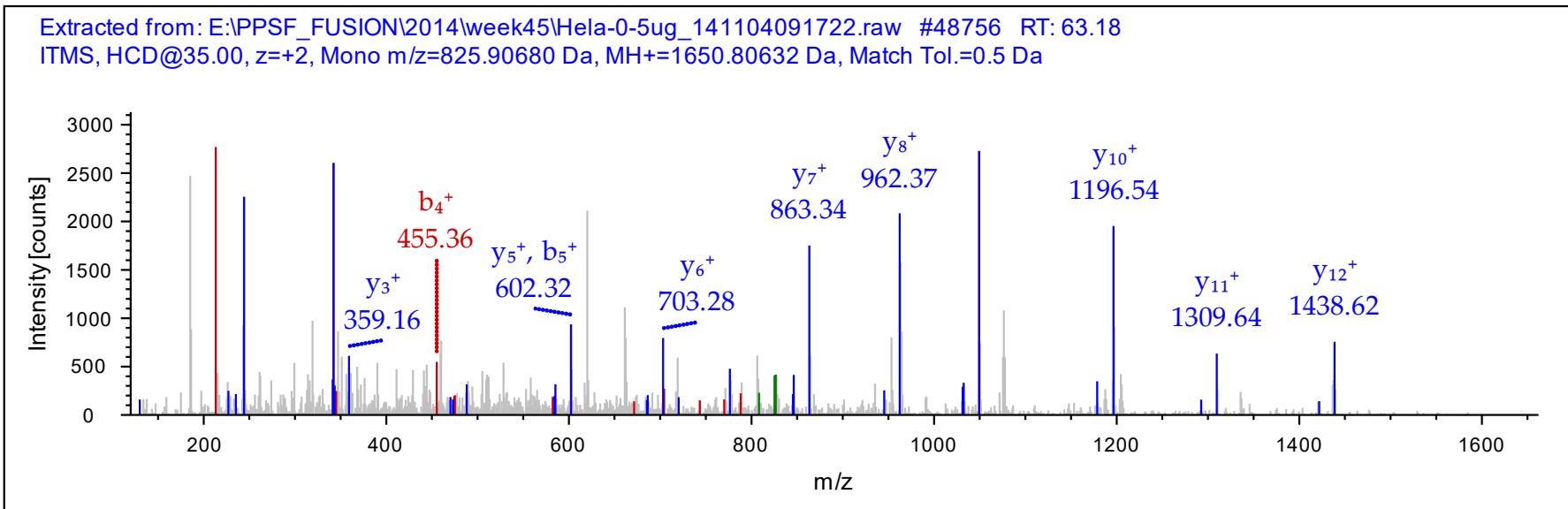
Start Search ...

Reset Form

Search engine output formats

File name	File content
Processed peak lists	Heavily processed form of mass spectrometry data, usually derived from raw data files via various (semi-) automatic steps, e.g.: centroiding, deisotoping and charge deconvolution. These files are formatted in plain text, with typical formats like dta , pkl , ms2 or mgf .
Search engine output files	These files contain the data and metadata generated by the software (called search engines) used for performing the identification and quantification of peptides and proteins. Each search engine has its own specific output file format. The outputs are typically formatted in either plain text or XML. mzIdentML - provides a common format for the export of identification results from any search engine. mzQuantML - provides a common format for the export of quantification results from any search engine. mzTab - represents both identification and basic quantification results. To allow a full representation of the processed results in the PRIDE database and in the PX tool, the search engine output files need to be converted to PRIDE XML. PRIDE Converter and PRIDE Converter 2 are the two tools developed by the PRIDE team to make this conversion possible.
Protein/peptide identifications	Proteomics mass spectra can be matched to peptides or proteins, resulting in identifications for those spectra. Typically a spectrum is considered to have been identified if the score attributed to a peptide or protein match qualifies against an <i>a priori</i> or <i>a posteriori</i> defined threshold. In the case of fragmentation spectra, the initial identification will consist of a peptide sequence; subsequent steps will derive a list of proteins from the identified peptides. The protein assembly step can be a discernible process with its own input and output files, or it can be implicit in the overall identification software.

31700 MS/MS spectra interpreted!!!



Sequence: VIELFSVCTNEDPK, C8-Carbamidomethyl (57.02146 Da)

Charge: +2, Monoisotopic m/z: 825.90680 Da (+0.95 mmu/+1.15 ppm), MH+: 1650.80632 Da, RT: 63.18 min,

Identified with: Sequest HT (v1.3); XCorr:4.48, Ions matched by search engine: 0/0

Fragment match tolerance used for search: 0.5 Da

Fragments used for search: b; b-H₂O; b-NH₃; y; y-H₂O; y-NH₃

Protein references (1):

- Lymphokine-activated killer T-cell-originated protein kinase OS=Homo sapiens GN=PBK PE=1 SV=3 - [TOPK_HUMAN]

5448 identified proteins

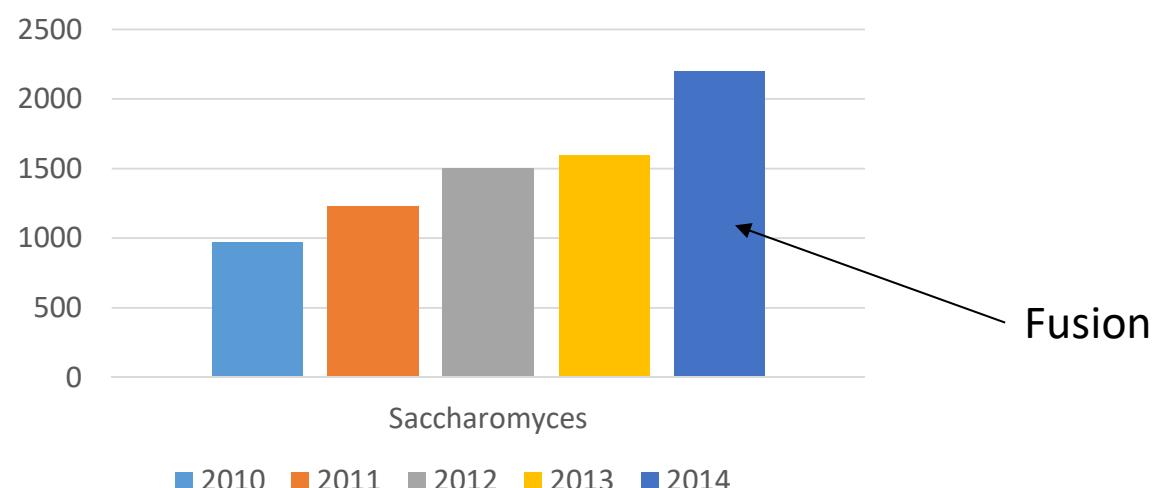
100 ▶		Q96KB5	Lymphokine-activated killer T-cell-originated protein kinase OS=Homo sapiens				41.78	60.87 %	1	12	12	13	322	30		
		A2	Sequence		# PSMs	# Proteins	# Protein Groups	Protein Group Accessions		Modifications		ΔCn	q-Value	PEP	XCorr	Charge
			=	=	=	=	=			=	=	=	=	=	=	
+	1		IcDVGVLPLDENMTVTDP...		1	1	1	Q96KB5		C2(Carbamidomethyl); C22...	0.0000	0	1.17e-07	5.10	3	
+	2		VIELFSVcTNEDPK		1	1	1	Q96KB5		C8(Carbamidomethyl)	0.0000	0	5.96e-07	4.48	2	
+	3		SVLcSTPTINIPASPFMQK		1	1	1	Q96KB5		C4(Carbamidomethyl)	0.0000	0	3.75e-05	3.24	3	
+	4		AFTEANDGSLcLAMEYGGK		1	1	1	Q96KB5		C11(Carbamidomethyl)	0.0000	0	9.89e-05	3.22	2	
+	5		INPIcNDHYR		1	1	1	Q96KB5		C5(Carbamidomethyl)	0.0000	0	0.000356	3.09	3	
+	6		SLHHPNIVGYR		1	1	1	Q96KB5			0.0000	0	0.00076	2.86	3	
+	7		SLNDLIEER		1	1	1	Q96KB5			0.0000	0	0.00187	2.85	2	
+	8		ASQDPFPAAIILK		1	1	1	Q96KB5			0.0000	0	0.00016	2.71	2	
+	9		TFDESDFDDEAYYAALGTRP...		1	1	1	Q96KB5		Q32(Deamidated)	0.0000	0.001	0.0153	3.34	4	
+	10		TFDESDFDDEAYYAALGTRP...		1	1	1	Q96KB5		N23(Deamidated); Q32(De...	0.0000	0.001	0.0198	2.40	3	
+	11		VALNMAR		1	1	1	Q96KB5			0.0000	0.001	0.00852	2.14	2	
+	12		EAVEENGVITDK		1	1	1	Q96KB5			0.0000	0.004	0.048	2.78	2	
+	13		DRPSAAHIVEALETDV		1	1	1	Q96KB5			0.0000	0.006	0.0779	3.58	3	
		Accession	Description				Score	Coverage	# Proteins	# UniquePeptides	# Peptides	# PSMs	# AAs	MW [kDa]		
+	101	A0AVT1	Ubiquitin-like modifier-activating enzyme 6 OS=Homo sapiens GN=...				43.86	14.83 %	1	12	12	14	1052	11.1		
+	102	O00116	Alkyldihydroxyacetonephosphate synthase, peroxisomal OS=Homo...				40.54	25.08 %	1	11	11	11	658	7.7		
+	103	Q12802	A-kinase anchor protein 13 OS=Homo sapiens GN=AKAP13 PE=1...				32.11	9.14 %	1	11	11	13	2813	30.0		
+	104	O43684	Mitotic checkpoint protein BUB3 OS=Homo sapiens GN=BUB3 PE=...				38.40	44.51 %	1	11	11	11	328	3.3		
+	105	O60832	H/ACA ribonucleoprotein complex subunit 4 OS=Homo sapiens GN...				32.86	24.71 %	1	11	11	11	514	5.1		
+	106	P19525	Interferon-induced, double-stranded RNA-activated protein kinase...				34.26	24.68 %	1	11	11	11	551	6.1		
+	107	Q8N3D4	EH domain-binding protein 1-like protein 1 OS=Homo sapiens GN=...				43.89	11.36 %	1	11	11	13	1523	16.1		
+	108	P60228	Eukaryotic translation initiation factor 3 subunit E OS=Homo sapie...				41.87	32.13 %	1	11	11	12	445	5.1		
+	109	P62495	Eukaryotic peptide chain release factor subunit 1 OS=Homo sapien...				53.24	37.30 %	1	11	11	16	437	4.1		
+	110	P15170	Eukaryotic peptide chain release factor GTP-binding subunit ERF3A...				45.47	36.27 %	1	11	11	12	499	5.1		

Ready

4681/4776 Protein Group(s), 5448/18724 Protein(s), 25168/152137 Peptide(s), 31700/190665 PSM(s), 88527/88527 Search Input(s)

History of standard identifications

Mass Spectrometer	HPLC	Gradient Time (min)	Column	Species	Mascot (Protein/Peptide)	Sequest (Protein/Peptide)	
Velos	EasynLC Proxeon	75	10	<i>Saccharomyces cerevisiae</i>	972/3912	1111/4884	
		120	25	<i>Saccharomyces cerevisiae</i>	1234/5245	1402/5948	
	RSLC	240		<i>Saccharomyces cerevisiae</i>	1198/4583	1422/6072	
		120	50	<i>Saccharomyces cerevisiae</i>	1505/8317	1638/8339	
				<i>Candida glabrata</i>	1598/7097		
				<i>Saccharomyces cerevisiae</i>	2135/7337		
				<i>Candida albicans</i>	2049/7676	2135/7337	
	EasynLC 1000	240		<i>Saccharomyces cerevisiae</i>	2202/16726	2350/11897	
				<i>Candida albicans</i>			
Fusion	EasynLC 1000	120	50				



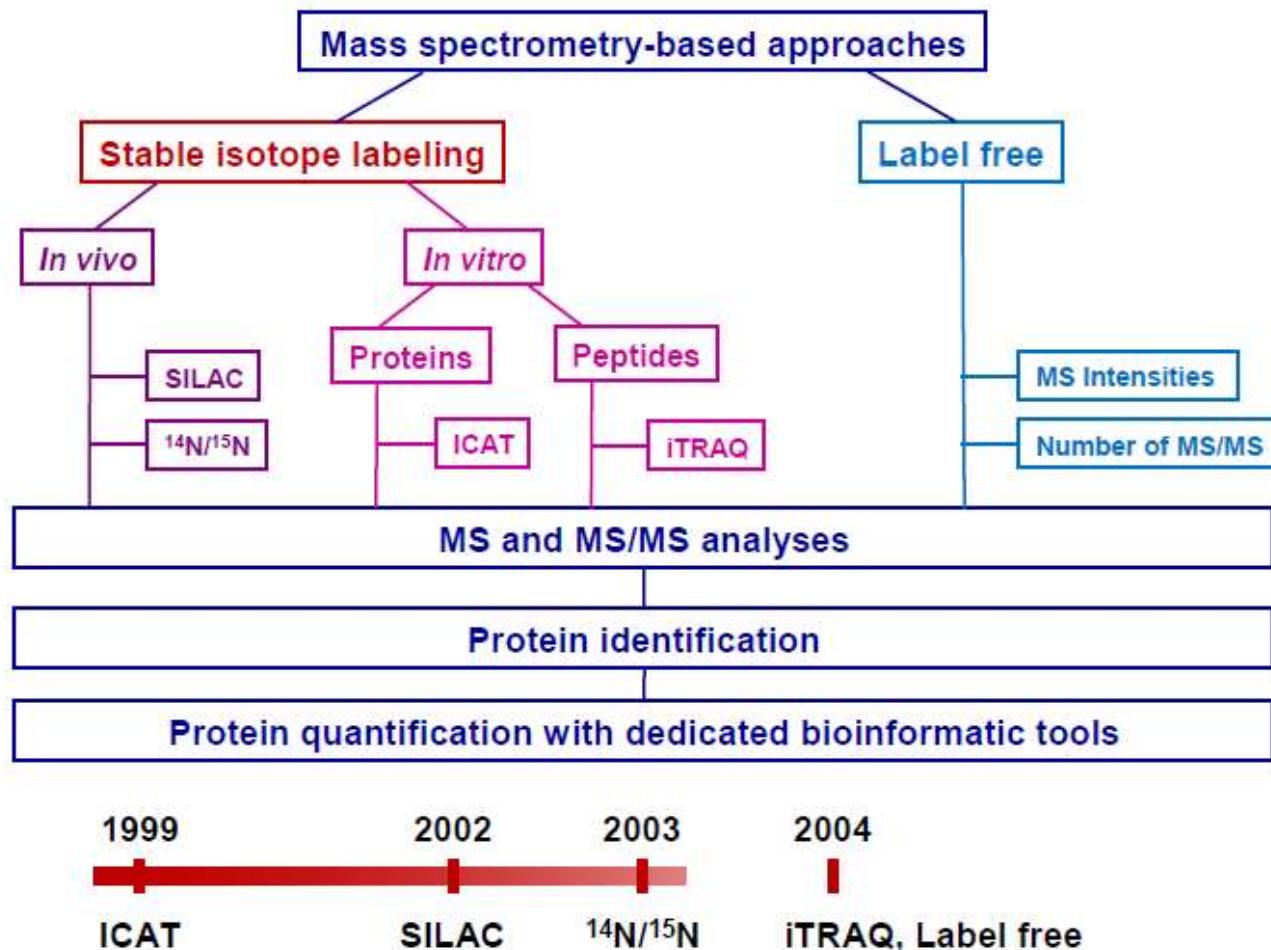
Key questions in proteomics

- What is the protein content of my biological sample?
=> problem of identification
- What is the abundance of my protein of interest?
=> **quantification problem**
- Relative question: What are the protein abundance variations of the proteomes studied?
- What are the partners of my protein of interest?
- Are there any signature proteins related to a particular biological process?
=> biomarkers identifications and quantifications

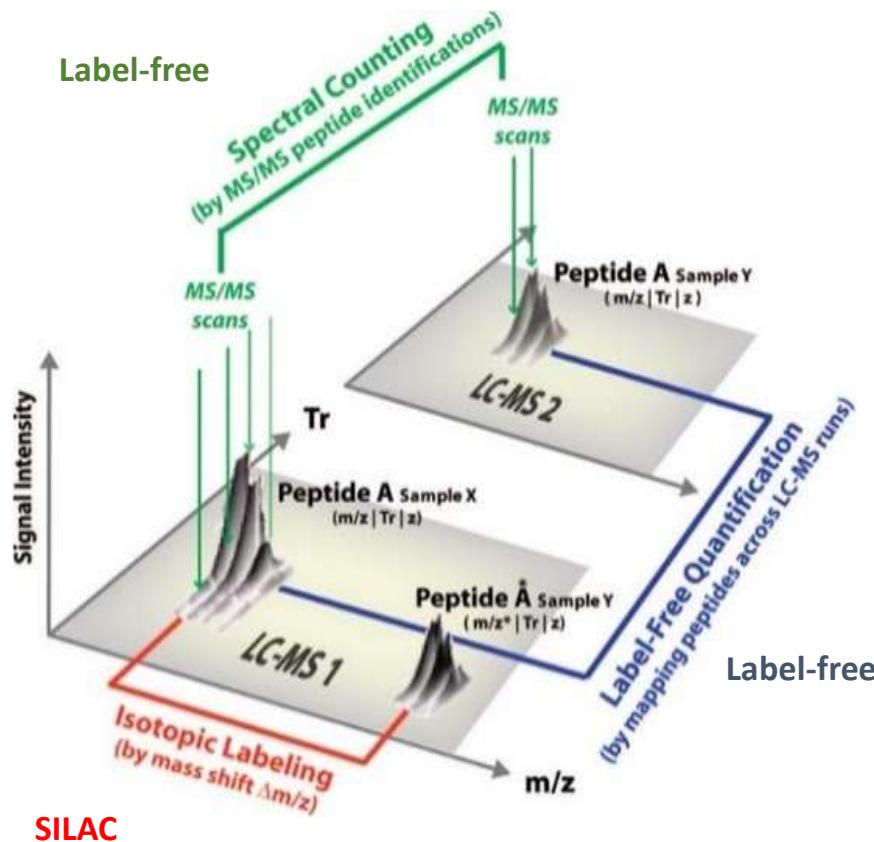
Quantitative proteomics

- Relative quantification
 - Stable isotopes labelling
 - Label-free
 - Metabolic labeling
- Absolute quantification

Quantitative proteomics

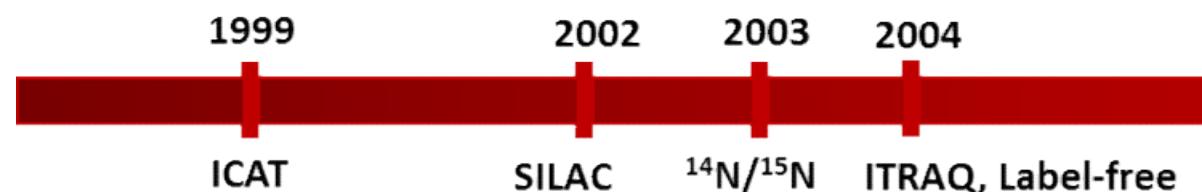


Quantitative proteomics in bottom-up

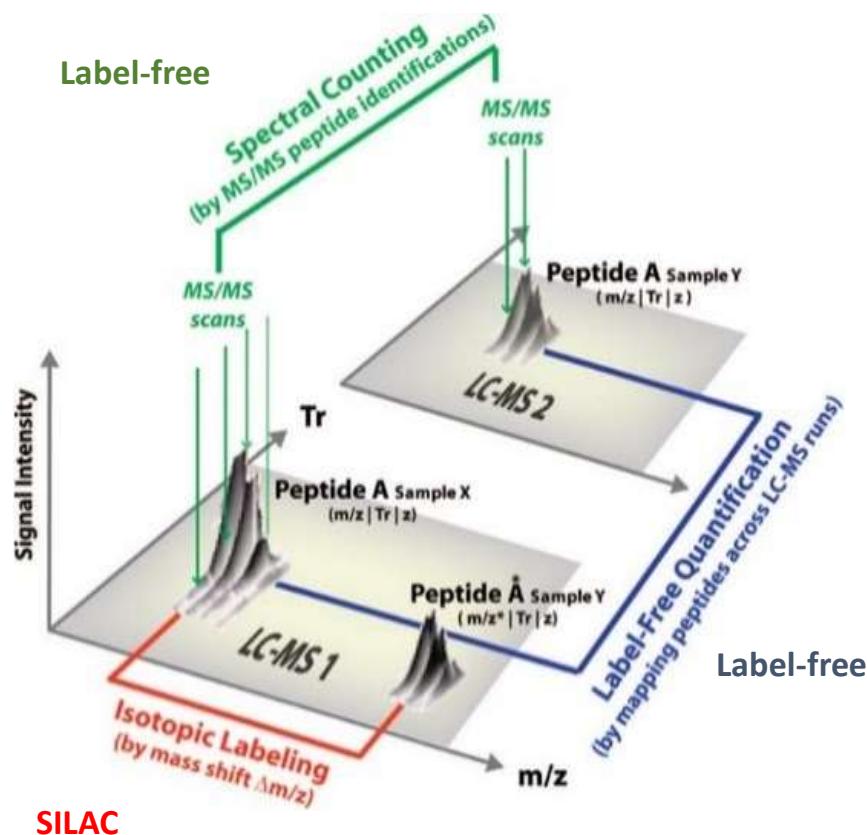


Advantages/Limitations:

- Label-free:
- Metabolic Labeling (SILAC, $^{14}\text{N}/^{15}\text{N}$ – ^{13}C labeling)
- Chemical labeling (TMT, iTRAQ)



Quantitative proteomics: label-free

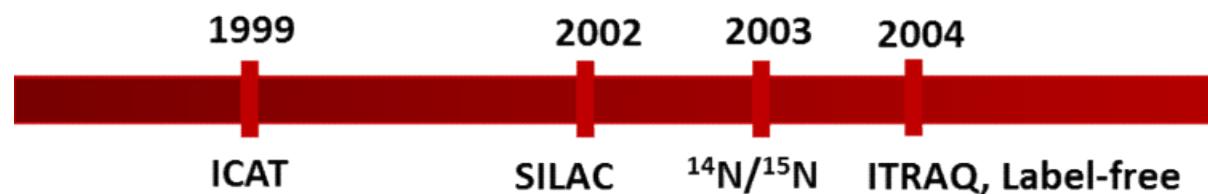


Advantages/Limitations:

Label-free:

- Simplicity
- Number of identifications
- Reproducibility between runs
- Number of samples to run

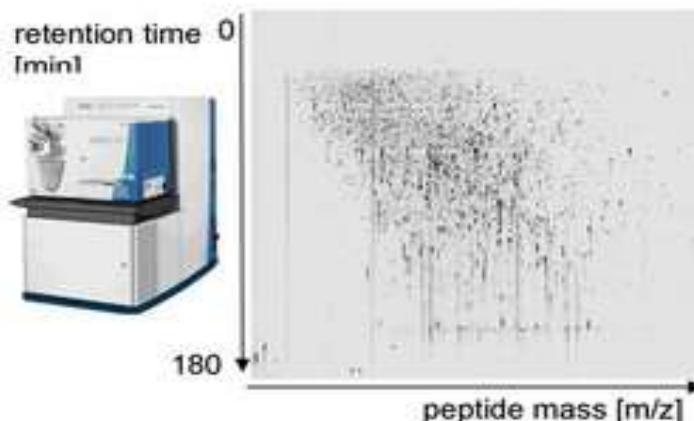
Review for Label-free and yeasts:
Leger et al. Methods Mol Biol (2016)



Quantitative proteomics without labeling

Quantification label-free basée sur les intensités MS

A: LC-MSMS

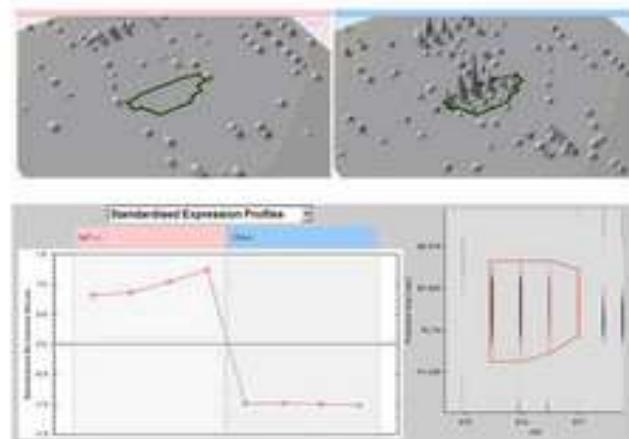


B: alignment



Progenesis QI
for proteomics

C: quantification



D: identification

MASCOT MS/MS Ions Search

User name:	<input type="text" value="jwahab"/>	Client:	<input type="text"/>
Search title:	<input type="text" value="Protein-protein interaction_Unknown_Proteins_070904_070904"/>		
Database:	<input type="text" value="UniprotKB-PI"/>		
Taxonomy:	Ascomycota (Prokaryotic)		
Enzyme:	Trypsin		
Fixed modifications:	<input checked="" type="checkbox"/> Methionine sulfoxide <input checked="" type="checkbox"/> Carbamidomethyl (-S-C(=O)-NH2)		
Variable modifications:	<input checked="" type="checkbox"/> Oxidized methionine <input checked="" type="checkbox"/> Phospho (S) [T] [Y] <input checked="" type="checkbox"/> Phospho (T) <input checked="" type="checkbox"/> Phospho (S) <input checked="" type="checkbox"/> Proline imidation (-NH-C(=O)-NH2)		
Quantification:	None		
Peptide id. s:	<input type="text" value="100"/>	Ident. s:	<input type="text" value="100"/>
Peptide charge:	2+ and 3+		
Data file:	<input type="text" value="Proteome Database 7.2.00"/>		
Data format:	<input checked="" type="checkbox"/> FASTA format		
Instrument:	<input type="text" value="ESI-TOFMS"/>		
Decoy:	<input type="checkbox"/>		
<input type="button" value="Start Search..."/>		<input type="button" value="Search Form"/>	

Quantitative proteomics without labeling : results

Experiment Design

Condition	WT	1003	1006	1215	1443
Replicates	3	3	3	3	3

Proteins

Protein building options

Protein grouping **Group similar proteins**

Protein quantitation **Using only features with no protein conflicts**

Accession	Peptides	Score	Anova (p)*	Fold	Tags	Description	Average Normalised Abundances				
							WT	1003	1006	1215	1443
HSP71_YEAST	57 (18)	4959.38	5.84e-005	2.40	●	Heat shock protein SSA1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SSA1 PE=1 SV=4	1.23e+007	7.42e+006	9.72e+006	9.87e+006	5.13e+006
EFT1_YEAST	69	4650.11	7.26e-004	2.16	●	Elongation factor 2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=EFT1 PE=1 SV=1	3.40e+007	2.39e+007	2.89e+007	3.28e+007	1.58e+007
FAS1_YEAST	74 (71)	4506.21	9.72e-003	2.33	●	Fatty acid synthase subunit beta OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=FAS1 PE=1 SV=2	1.03e+007	6.55e+006	8.51e+006	1.01e+007	1.53e+007
EF3A_YEAST	58 (44)	3816.91	7.06e-006	3.32	●	Elongation factor 3A OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=YEF3 PE=1 SV=4	2.88e+007	1.29e+007	2.33e+007	2.72e+007	8.66e+006
METE_YEAST	46	3373.13	1.85e-006	13.40	●	5-methyltetrahydropteroylglutamate--homocysteine methyltransferase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=MET6 PE=1 SV=4	4.34e+006	5.55e+006	3.77e+006	5.16e+006	5.05e+007
HSP104_YEAST	53	3190.19	6.84e-004	2.29	●	Heat shock protein 104 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=HSP104 PE=1 SV=4	6.351e+006				
HSP75_YEAST	40 (1)	3062.55	1.21e-006	28.16	●	Heat shock protein SSB1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SSB1 PE=1 SV=4	5.105e+004				
HSP7F_YEAST	39 (32)	2658.69	2.58e-004	2.01	●	Heat shock protein homolog SSE1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=HSP7F PE=1 SV=4	6.350e+006				
ENO1_YEAST	31 (15)	2367.12	6.07e-005	2.19	●	Enolase 1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=ENO1 PE=1 SV=4	6.146e+007				
ATPA_YEAST	32	2341.09	3.17e-006	2.59	●	ATP synthase subunit alpha, mitochondrial OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=ATPA PE=1 SV=5	6.366e+006				
SYLC_YEAST	37	2176.12	1.52e-006	2.01	●	Leucine--tRNA ligase, cytoplasmic OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SYLC PE=1 SV=4	6.182e+006				
HXKA_YEAST	29 (28)	2162.35	3.17e-004	2.88	●	Hexokinase-1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=HXKA PE=1 SV=4	7.461e+006				
ALDH6_YEAST	30	2091.58	4.85e-004	2.15	●	Magnesium-activated aldehyde dehydrogenase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=ALDH6 PE=1 SV=4	6.250e+006				
ATPB_YEAST	28	2015.82	4.45e-006	2.39	●	ATP synthase subunit beta, mitochondrial OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=ATPB PE=1 SV=2	6.405e+006				
G3P1_YEAST	31 (21)	1986.15	8.75e-005	4.16	●	Glyceraldehyde-3-phosphate dehydrogenase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=G3P1 PE=1 SV=3	6.179e+007				
HSP74_YEAST	26 (12)	1750.55	0.04	2.68	●	Heat shock protein SSA4 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=HSP74 PE=1 SV=4	5.524e+005				
PUR92_YEAST	28 (22)	1725.94	6.21e-007	7.07	●	Bifunctional purine biosynthesis protein OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=PUR92 PE=1 SV=2	6.963e+006				
ADH1_YEAST	24 (17)	1689.13	5.88e-004	2.62	●	Alcohol dehydrogenase 1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=ADH1 PE=1 SV=2	7.103e+007				
HSP26_YEAST	18	1538.64	2.83e-006	2.31	●	Heat shock protein 26 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=HSP26 PE=1 SV=2	7.843e+006				
SAH1_YEAST	27	1535.76	2.79e-006	3.51	●	Adenosylhomocysteinase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SAH1 PE=1 SV=2	6.131e+007				
PCKA_YEAST	20	1515.31	3.42e-009	9.67	●	Phosphoenolpyruvate carboxykinase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=PCKA PE=1 SV=2	6.625e+005				

Accession HSP71_YEAST

Description Heat shock protein SSA1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SSA1 PE=1 SV=4

Peptides 57 (18)

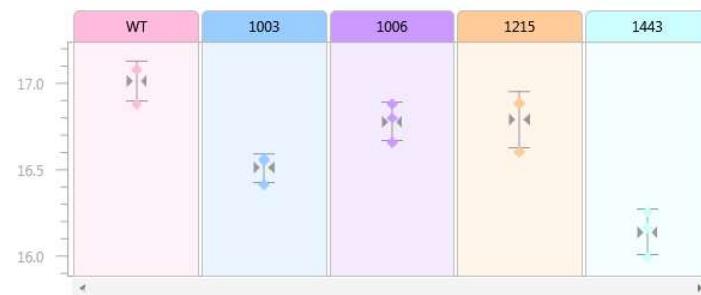
Score 4959.38

Anova 5.84e-005

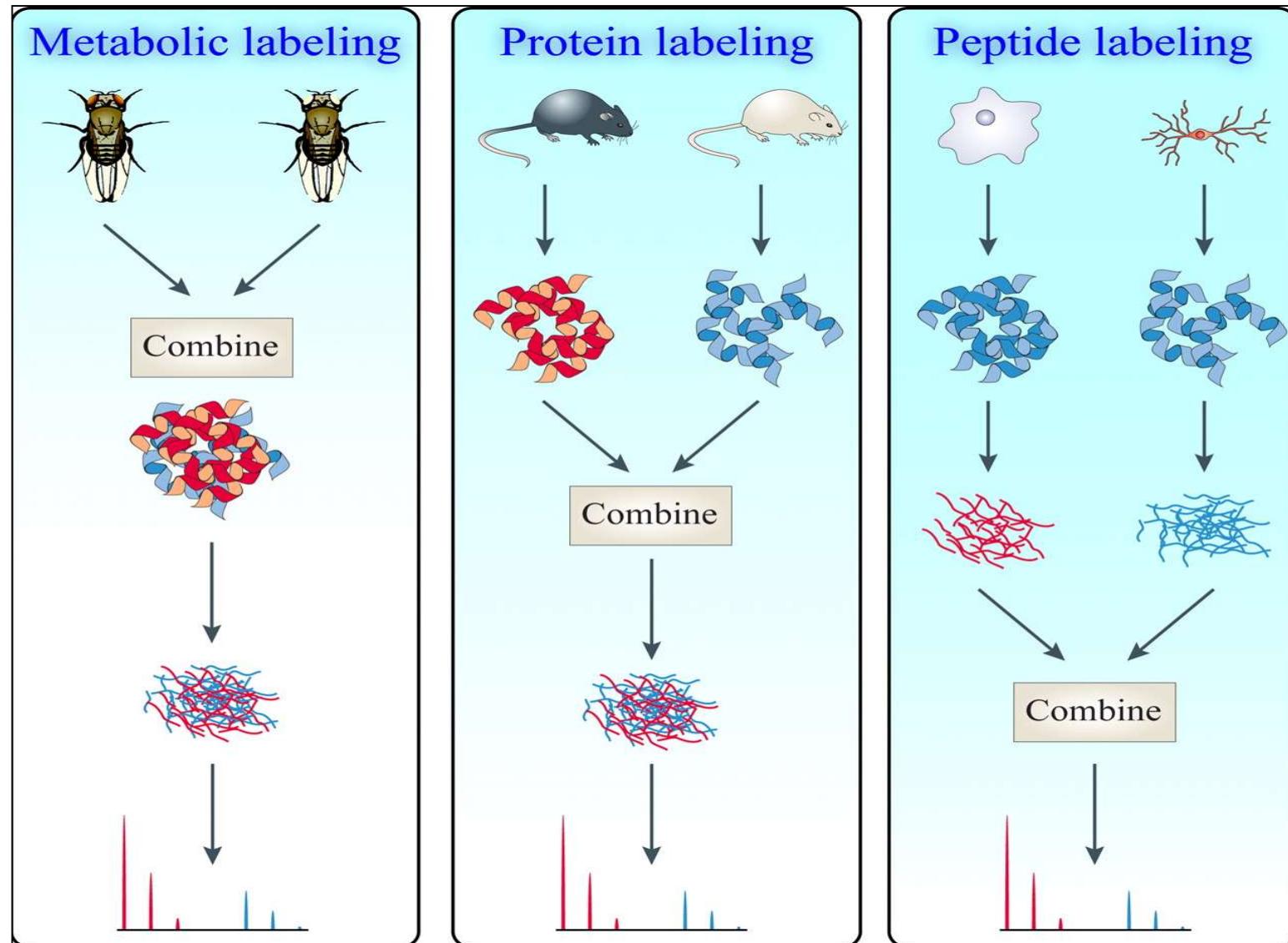
Fold 2.40

● Anova p-value ≤ 0.05

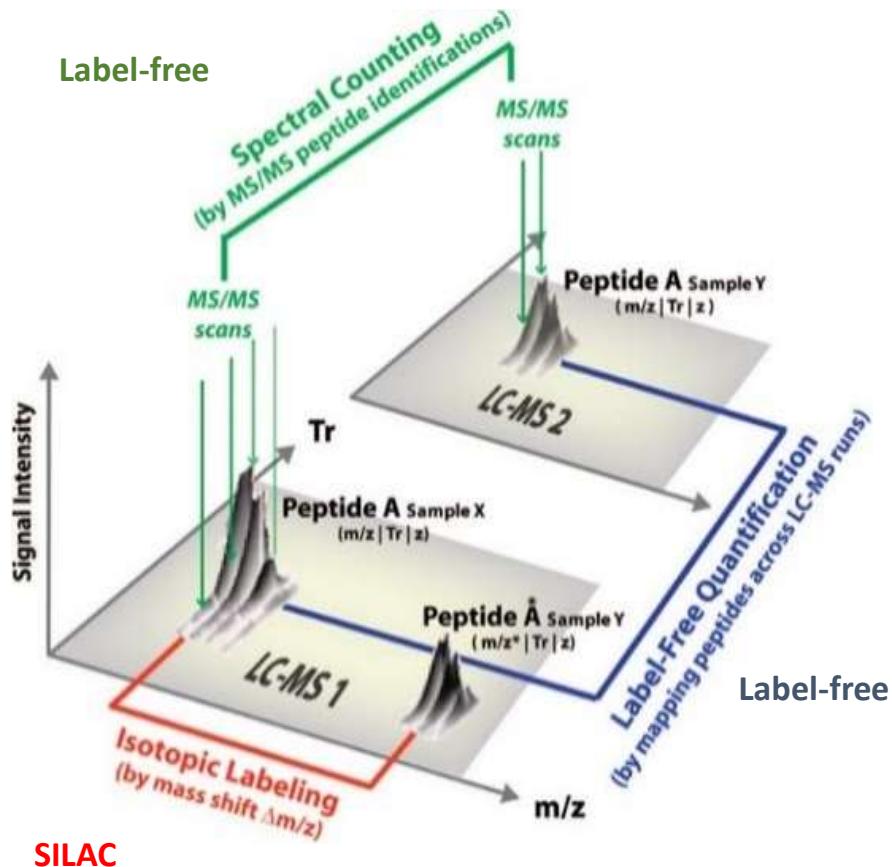
● Max fold change ≥ 2



Quantitative proteomics with labeling



Quantitative proteomics: metabolic labeling



SILAC

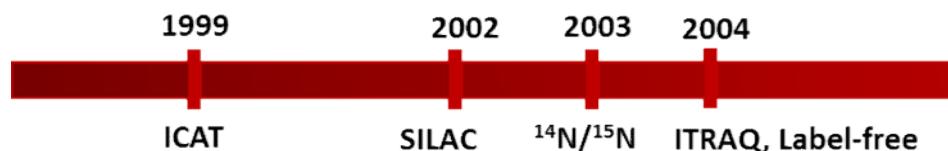
Advantages/Limitations:

SILAC:

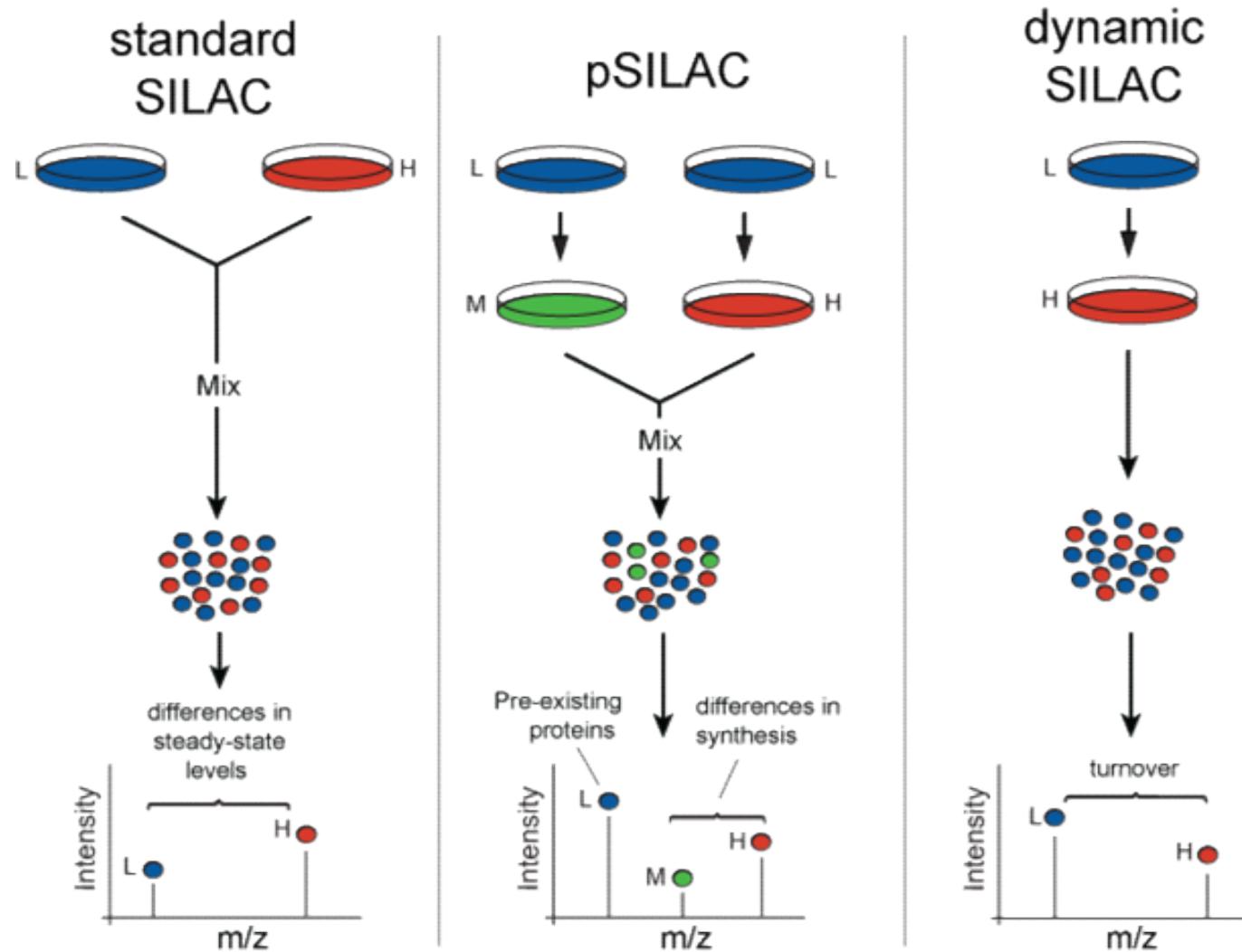
- Multiplexing
- Reproducibility
- **2 peaks instead of 1 to analyze by the MS (for 2 samples)**
- Less identifications
- Partial labeling
- Arginine/proline conversion (use of mutants)
- Trypsin exclusively

$^{14}\text{N}/^{15}\text{N} - ^{13}\text{C}$ labeling:

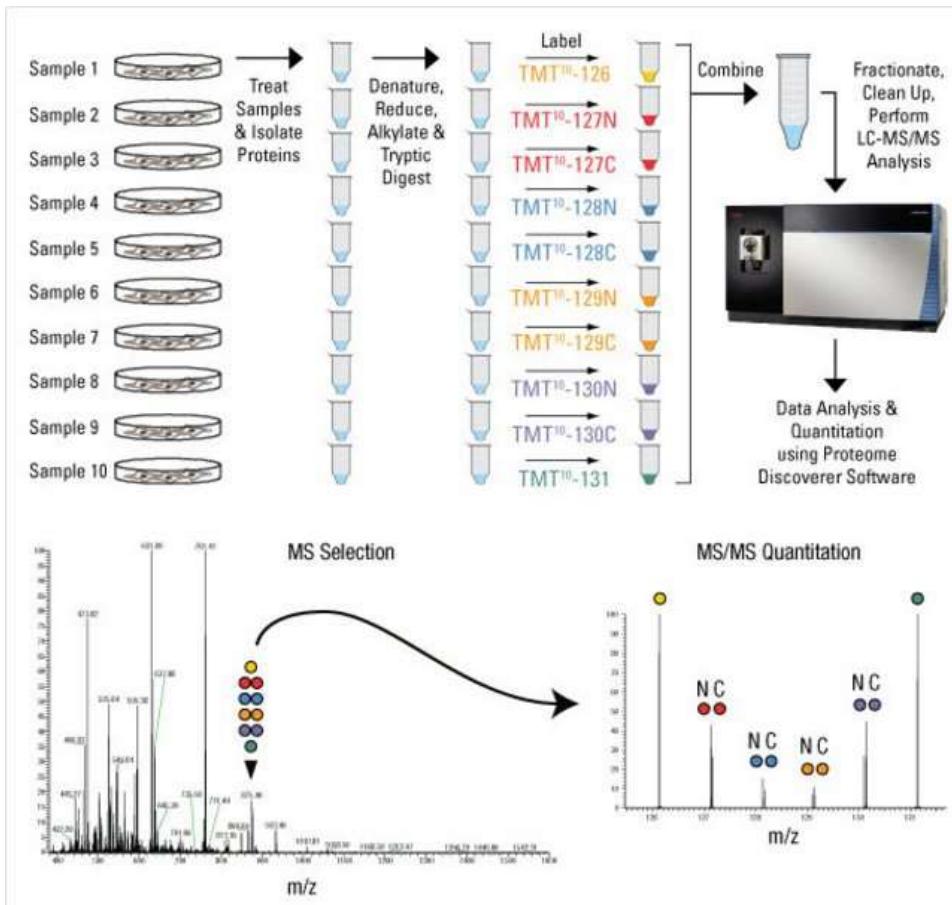
- Multiplexing
- Reproducibility
- **2 peaks instead of 1 to analyze by the MS (for 2 samples)**
- Less identifications and quantifications
- Partial labeling
- **Variable mass shift between heavy and light forms**



SILAC approaches



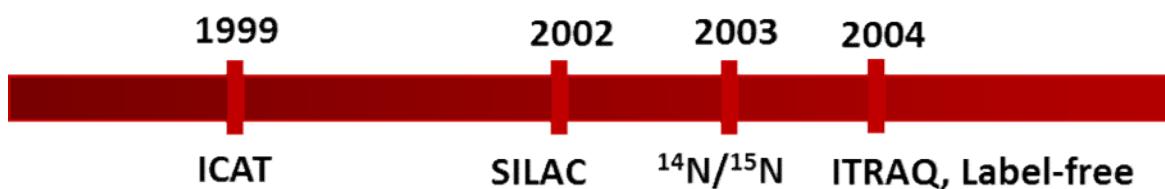
Quantitative proteomics: chemical labeling



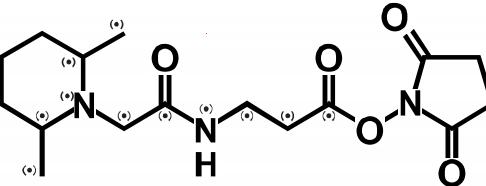
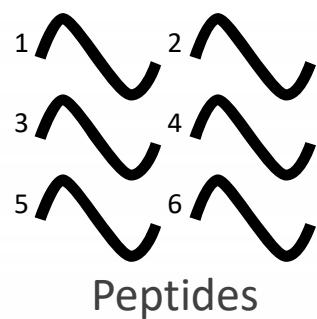
Advantages/Limitations:

□ Chemical labeling (TMT, iTRAQ)

- Multiplexing (until 11plex)
- Reproducibility
- Quantification in MS2 or MS3
- **1 peak instead of N (for N samples) to analyse in MS**
- Amount of materials for the peptide labeling
- Need of resolution in MS2 for quantifications
- Incomplete labeling
- Less identifications and quantifications



TMT labeling : principles

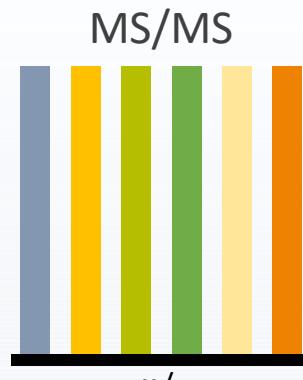


LC-MS/MS

Full MS



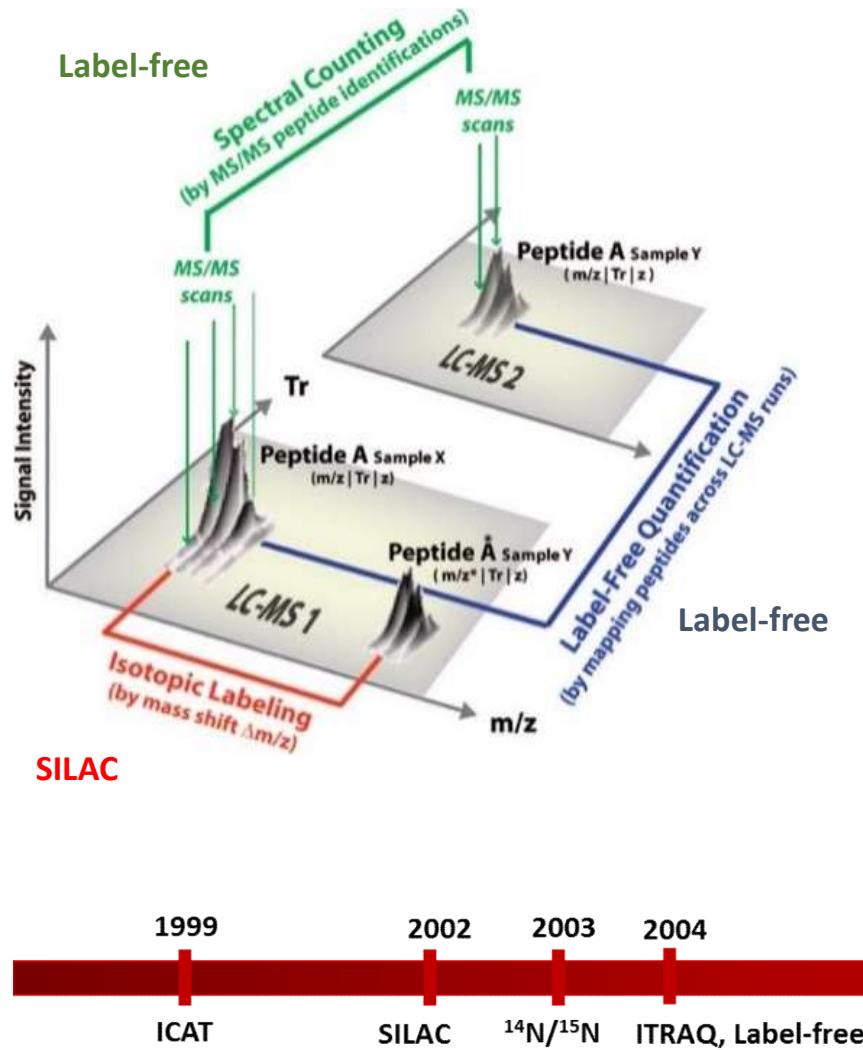
One Signal



6 Reporter Ions

HCD FRAGMENTATION

Quantitative proteomics in bottom-up



Advantages/Limitations:

- Label-free:
 - Simplicity
 - Number of identifications
 - Reproducibility between runs
 - Number of samples to run
 - SILAC:
 - Multiplexing
 - Reproducibility
 - **2 peaks instead of 1 to analyze by the MS (2 samples)**
 - Less identifications
 - Partial labeling
 - Arginine/proline conversion (use of mutants)
 - Trypsin exclusively
 - $^{14}\text{N}/^{15}\text{N} - ^{13}\text{C}$ labeling:
 - Multiplexing
 - Reproducibility
 - **2 peaks instead of 1 to analyze by the MS (2 samples)**
 - Less identifications and quantifications
 - Partial labeling
 - **Variable mass shift between heavy and light forms**
 - Chemical labeling (TMT, iTRAQ)
 - Multiplexing
 - Reproducibility
 - **1 peak instead of N to analyse in MS (N samples)**
 - Amount of materials for the peptide labeling
 - Need of resolution in MS2 for quantifications
 - Incomplete labeling
 - Less identifications and quantifications
- A blue bracket on the right side of the diagram groups the SILAC, $^{14}\text{N}/^{15}\text{N}$, and TMT/iTRAQ methods under the heading "Metabolic labeling".

Quantitative proteomics without labeling : export



Accession	Peptide count	Unique peptides	Confidence	Anova (p)	q Value	Max fold ch Power	Highest mean	Lowest mean	Mass	Description	Normalized abundance									Patient
											Sain	1845007-F1	1845007-F3	1845007-F5	1845007-F7	1845007-F9	1845007-F11	1845007-F2	1845007-F4	
P40197	12	12	545.23	2.48E-07	4.74E-05	32.042418	1	Patient	Sain	60.921	Platelet glycoprotein V OS=Homo sapiens	1447.8857	898.47877	2114.249	3517.982	2506.9091	1186.7152	33178.605	73995.781	
P02776	2	2	134.77	2.63E-07	4.74E-05	78.817355	1	Patient	Sain	10.838	Platelet factor 4 OS=Homo sapiens	3812.4369	3755.8358	1044.3911	2939.4867	3862.0883	1110.5549	333829.66	68071.51	
Q13201	6	6	221.4	5.33E-07	6.40E-05	72.663027	1	Patient	Sain	138.023	Multimerin-1 OS=Homo sapiens	160.48528	939.93933	155.7663	732.57482	572.39752	196.60688	75519.303	23299.12	
P04114	336	334	28302.18	7.99E-06	0.0006593	2.1384481	1	Patient	Sain	515.283	Apolipoprotein B-100 OS=Homo sapiens	14117253	10601958	8472023.6	9982572.2	10056625	11898407	24415094	1744463	
P07996	42	42	2678.71	9.16E-06	0.0006593	143.08362	1	Patient	Sain	129.3	Thrombospondin-1 OS=Homo sapiens	11604.367	10346.75	4059.3393	5652.5533	2121.1343	2367.1543	2303007.2	83175.08	
Q15485	7	7	289.57	1.42E-05	0.0007087	24.427918	0.9999997	Patient	Sain	33.98	Ficolin-2 OS=Homo sapiens	8778.176	6132.5206	10104.107	8194.6312	8690.2929	1737.9715	185819.2	105024.9	
P10720	3	3	208.63	1.49E-05	0.0007087	36.552808	0.9999997	Patient	Sain	11.545	Platelet factor 4 variant 4 OS=Homo sapiens	32881.787	11758.269	1451.6395	5448.2604	4187.5362	4196.5766	5646497.96	239272.0	
Q12884	2	2	70.47	1.58E-05	0.0007087	Infinity	0.9999996	Patient	Sain	87.657	Prolyl endopeptidase FAP	0	0	0	0	0	0	961.55592	106.477	
Q15061	1	1	3.67	3.71E-05	0.0014832	23.811179	0.9999905	Sain	Patient	74.843	WD repeat-containing protein	567513.12	751475.75	852125.74	1189728.8	625225.18	78135.037	46681.126	36585.061	
P04075	8	8	371.85	4.58E-05	0.0016488	4.2937733	0.9999814	Patient	Sain	39.395	Fructose-bisphosphate aldehyde	33219.801	26324.171	28730.699	26696.908	33816.665	32024.353	128602.25	168571.1	
P09486	10	9	463.23	6.25E-05	0.0020451	6.1746973	0.999953	Patient	Sain	34.61	SPARC OS=Homo sapiens	9522.5101	37758.131	36721.019	20388.641	15117.423	28228.93	299004.24	97804.24	
P10124	1	1	70.16	7.11E-05	0.002134	27.244302	0.9999321	Patient	Sain	17.641	Serglycin OS=Homo sapiens	67.740323	0	958.26875	53.15204	160.31231	238.84011	105180.09	42426.24	
P02775	6	6	388.98	8.21E-05	0.0022557	28.012451	0.9998996	Patient	Sain	13.885	Platelet basic protein OS=Homo sapiens	61607.9	109352.83	105910.89	67216.894	60865.635	22752.188	3524260.5	1692399.1	
Q9H1K0	2	2	12.94	8.77E-05	0.0022557	1.8919044	0.9998799	Patient	Sain	88.815	Rabenosyn-5 OS=Homo sapiens	871721.79	1194024.5	1103071.3	1121288.3	1020557.4	1361492.9	1506333.1	1757466.1	
P35542	4	4	338.41	0.0001236	0.0029659	16.656374	0.9997122	Patient	Sain	14.737	Serum amyloid A-4 protein	192227.76	102064.74	9416.0169	8023.3014	46690.651	107524.48	1543185.2	1741696.1	
P08188	6	6	223.58	0.0001847	0.0041561	9.1914461	0.9992657	Patient	Sain	22.574	Neutrophil gelatinase-associated	2265.4484	1473.9176	6203.72	3676.0015	7827.3304	4603.3796	66633.817	26485.80	
P02144	4	4	209.23	0.0002392	0.0050651	6.5032553	0.9987201	Patient	Sain	17.173	Myoglobin OS=Homo sapiens	2754.3333	2223.1201	1035.6898	1813.5104	1462.164	5472.3528	17977.389	20813.2	
P05067	5	5	179.76	0.000297	0.0059397	18.947351	0.9980148	Patient	Sain	86.888	Amyloid beta A4 protein C	408.20926	53.510036	338.118	494.28658	258.47482	516.31147	8561.0101	2161.775	
Q9NPH3	7	7	223.36	0.0003417	0.0064734	2.9354965	0.9973949	Patient	Sain	65.377	Interleukin-1 receptor activator	19269.326	23550.392	17918.705	19071.463	29165.24	20448.453	74326.28	82743.30	
P22352	7	7	322.73	0.0005632	0.0101356	2.3655577	0.9936247	Patient	Sain	25.537	Glutathione peroxidase 3	356078.09	500176.63	411199.92	354265.74	608490.2	620677.21	1139291.1	1214566.1	
A0A075B610	1	1	30.88	0.0005981	0.0102519	15.884721	0.9929515	Sain	Patient	12.806	Immunoglobulin lambda	14017.761	25223.891	6493.2483	70640.84	15600.774	36585.067	4641.3232	1487.298	
P05155	33	33	2513.9	0.0007389	0.0120898	5.4023431	0.9900925	Patient	Sain	55.119	Plasma protease C1 inhibitor	741421.19	592478.75	377555.54	465885.04	2331779.9	7791080.5	11109177	1051176	
A0A0C4DH2	2	2	100.38	0.0013344	0.0208833	4.8653639	0.9766266	Patient	Sain	12.999	Immunoglobulin heavy chain	50500.146	64330.439	38938.111	121804.39	27881.723	32180.678	24057.435	17302.86	
P14780	6	6	207.75	0.0014988	0.0221418	62.06206	0.972746	Patient	Sain	78.408	Matrix metalloproteinase	794.97907	136.39171	262.24899	313.84328	0	555.64619	58928.664	9636.858	
P02649	27	27	1941.9	0.0015378	0.0221418	2.4710215	0.971824	Patient	Sain	36.132	Apolipoprotein E OS=Homo sapiens	2552396.1	1536331.8	853676.88	1079240.3	1188651.7	132757.52	2857655.7	2177433	
Q3C1V8	1	1	14.2	0.0019728	0.0268284	5.551993	0.9615339	Patient	Sain	25.917	Brain-specific homeobox	119.76184	56.50442	16.821931	8.5075688	3888.8817	85.190432	6077.0842	3199.583	
P02652	11	11	995.34	0.0020124	0.0268284	2.4014067	0.9606031	Patient	Sain	11.168	Apolipoprotein A-II OS=Homo sapiens	34056410	26771849	14632439	15663194	42268641	26890744	89512485	4323663	
P08571	12	12	937.27	0.0025308	0.0320335	1.6971067	0.9485492	Patient	Sain	40.051	Monocyte differentiation	797874.17	434082.26	49942.38	477652.53	733837.28	418725.29	352767.33	318079.3	
P26927	31	31	1222.76	0.0025808	0.0320335	3.3123536	0.9474046	Patient	Sain	80.268	Hepatocyte growth factor	190652.76	40328.557	155598.93	60550.701	75682.634	141408.77	141623.19	380083.0	
P02655	6	6	819.16	0.0030504	0.0361789	4.7206762	0.9368237	Patient	Sain	11.277	Apolipoprotein C-II OS=Homo sapiens	128181.8	751583.4	312951.49	1013499	603757.76	1691499.6	4859804.1	137623	
P02671	8	8	369.69	0.0031158	0.0361789	191.8384	0.9353762	Patient	Sain	94.914	Fibrinogen alpha chain	1792.4021	4843.2609	2616.5938	746.39304	685.49376	8280.2447	1254362.7	107899	
P02763	17	17	1378.36	0.0036234	0.0407586	2.8533872	0.924364	Patient	Sain	23.497	Alpha-1-acid glycoprotein	67476432	40320887	40796528	53919933	106057189	35895348	25642029	2274357	
Q14831	1	1	23.81	0.0038979	0.0425178	2.9787874	0.9185746	Patient	Sain	102.185	Metabotropic glutamate receptor 5	26519.187	83466.172	72384.114	34456.805	83227.769	36550.192	8908.5356	31759.88	
P02647	32	32	2367.65	0.0040205	0.0425648	2.2688435	0.9160271	Patient	Sain	30.759	Apolipoprotein A-I OS=Homo sapiens	105663566	161836184	96430549	75237801	158864474	104736598	166481100	27802701	
P01876	21	21	1557.26	0.0044316	0.0455765	3.5420267	0.9076475	Patient	Sain	37.631	Ig alpha-1 chain C region	10958465	3390608.1	19371147	11129440	8232536	4863588.4	3403586.2	5212718	
Q9UNW1	8	8	227	0.005259	0.0525841	1.7900311	0.8915219	Sain	Patient	55.016	Multiplex inositol polyphosphate	62640.486	55052.459	58667.71	30459.856	63293.849	79838.921	34467.455	30358.52	
Q16853	2	2	81.52	0.0054293	0.0528194	6.1436713	0.8883207	Sain	Patient	84.568	Membrane primary amine	24451.336	3442.0472	3308.1744	4995.8869	4135.7996	9790.8939	236.61601	194.7495	
P01833	8	8	219.66	0.006944	0.0656448	9.1555604	0.8614401	Sain	Patient	83.232	Polymeric immunoglobulin	14801.731	38882.249	8643.925	36538.175	31852.344	72666.846	1025.7634	2483.330	
P27169	28	27	2064.1	0.0071604	0.0656448	1.8486226	0.8578184	Patient	Sain	39.706	Serum paraoxonase/arylesterase	3861076.2	5186138.5	2149700.4	5390878.2	2346566.5	3471134.2	5925120.8	6280114	
P60174	1	1	28.75	0.0074	0.0656448	2.916777	0.8538651	Patient	Sain	30.772	Triosephosphate isomerase	4225.6237	1794.3666	1066.6861	1210.1584	3775.1162	2981.76	10398.334	4675.589	
P00736	41	39	3067.37	0.0074771	0.0656448	1.8466701	0.8526056	Sain	Patient	80.067	Complement C1r subcomponent	5434650.2	5169551.9	3906709.7	4169170.8	3458132.5	4641351.5	4547911.2	2638367	
P09871	37	37	258.95	0.0085558	0.0726349	1.93603	0.8835606	Sain	Patient	76.635	Complement C1s subcomponent	5789892.8	382940.1	5033975.1	5033975.1	2280238.9	1956631.4	2302798		
A0A0B4J1V0	3																			

Quantitative proteomics without labeling : export

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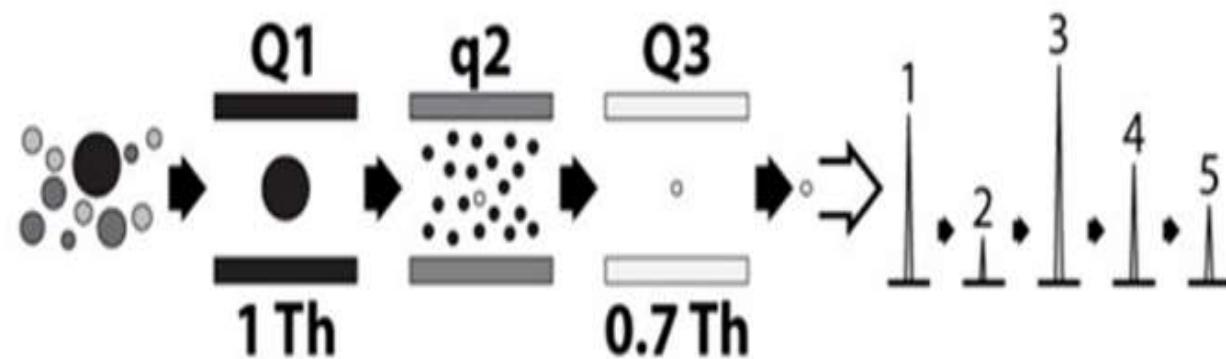
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1 Protein IDs Majority protein IDs → Peptide counts (all) → Peptide counts (razor+unique) → Peptide counts (unique) → Fasta headers → Number o
2 C1_00060W_A>C1_00060W_A>10→10→10→10→>C1_00060W_A translated using codon table 12 (512 amino acids) Verified ORF; (orf19.6109) Transcriptional
3 C1_00070W_A>C1_00070W_A>12→12→12→12→>C1_00070W_A translated using codon table 12 (362 amino acids) Verified ORF; (orf19.6105) Mevalonate dip
4 C1_00110W_A>C1_00110W_A>14→14→14→14→>C1_00110W_A translated using codon table 12 (540 amino acids) Verified ORF; (orf19.6099) Chaperonin-con
5 C1_00140W_A>C1_00140W_A>5→5→5→5→>C1_00140W_A translated using codon table 12 (1018 amino acids) Verified ORF; (orf19.6092) Kelch repeat
6 C1_00150C_A>C1_00150C_A>1→1→1→1→>C1_00150C_A translated using codon table 12 (622 amino acids) Verified ORF; (orf19.6091) Beta-arrestin-
7 C1_00160C_A>C1_00160C_A>4→4→4→4→>C1_00160C_A translated using codon table 12 (400 amino acids) Verified ORF; (orf19.6090) Putative nucle
8 C1_00170W_A>C1_00170W_A>9→9→9→9→>C1_00170W_A translated using codon table 12 (579 amino acids) Verified ORF; (orf19.6086) Putative 2-iso
9 C1_00180W_A>C1_00180W_A>3→3→3→3→>C1_00180W_A translated using codon table 12 (200 amino acids) Verified ORF; (orf19.6085) Ribosomal prot
10 C1_00210C_A>C1_00210C_A>2→2→2→2→>C1_00210C_A translated using codon table 12 (384 amino acids) Verified ORF; (orf19.6082) Ortholog(s) ha
11 C1_00220W_A>C4_04530C_A>C1_00220W_A>5;1>5;1>5;1>>C1_00220W_A translated using codon table 12 (544 amino acids) Verified ORF; (orf19.6081) Gl
12 C1_00320W_A>C1_00320W_A>2→2→2→2→>C1_00320W_A translated using codon table 12 (261 amino acids) Uncharacterized ORF; (orf19.6076) Ortholo
13 C1_00330C_A>C1_00330C_A>2→2→2→2→>C1_00330C_A translated using codon table 12 (182 amino acids) Uncharacterized ORF; (orf19.6075) Putativ
14 C1_00340W_A>C1_00340W_A>4→4→4→4→>C1_00340W_A translated using codon table 12 (248 amino acids) Verified ORF; (orf19.6074) Essential prot
15 C1_00380C_A>C1_00380C_A>7→7→7→7→>C1_00380C_A translated using codon table 12 (745 amino acids) Uncharacterized ORF; (orf19.6071) Ortholo
16 C1_00400W_A>C1_00400W_A>7→7→7→7→>C1_00400W_A translated using codon table 12 (382 amino acids) Uncharacterized ORF; (orf19.6068) Putativ
17 C1_00410C_A>C1_00410C_A>13→13→13→12→>C1_00410C_A translated using codon table 12 (542 amino acids) Uncharacterized ORF; (orf19.6066) Hexadec
18 C1_00420W_A>C1_00420W_A>7→7→7→7→>C1_00420W_A translated using codon table 12 (323 amino acids) Uncharacterized ORF; (orf19.6065) RNA pol
19 C1_00440W_A>C1_00440W_A>11→11→11→11→>C1_00440W_A translated using codon table 12 (478 amino acids) Uncharacterized ORF; (orf19.6063) Putativ
20 C1_00450C_A>C1_00450C_A>2→2→2→2→>C1_00450C_A translated using codon table 12 (150 amino acids) Uncharacterized ORF; (orf19.6062.3) Mitoc
21 C1_00460W_A>C1_00460W_A>1→1→1→1→>C1_00460W_A translated using codon table 12 (106 amino acids) Verified ORF; (orf19.6062) Putative TIM23
22 C1_00480C_A>C1_00480C_A>4→4→4→4→>C1_00480C_A translated using codon table 12 (751 amino acids) Uncharacterized ORF; (orf19.6060) YEF3-su
23 C1_00490C_A>C1_00490C_A>2→2→2→2→>C1_00490C_A translated using codon table 12 (119 amino acids) Verified ORF; (orf19.6059) Putative gluta
24 C1_00500C_A>C1_00500C_A>3→3→3→3→>C1_00500C_A translated using codon table 12 (342 amino acids) Uncharacterized ORF; (orf19.6058) Putativ
25 C1_00560W_A>C1_00560W_A>2→2→2→2→>C1_00560W_A translated using codon table 12 (390 amino acids) Verified ORF; (orf19.6052) Putative co-ch
26 C1_00590W_A>C1_00590W_A>14→14→14→14→>C1_00590W_A translated using codon table 12 (426 amino acids) Uncharacterized ORF; (orf19.6047) Transla
27 C1_00610W_A>C1_00610W_A>3→3→3→3→>C1_00610W_A translated using codon table 12 (590 amino acids) Verified ORF; (orf19.6045) Phosphatidylse
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Quantification output formats

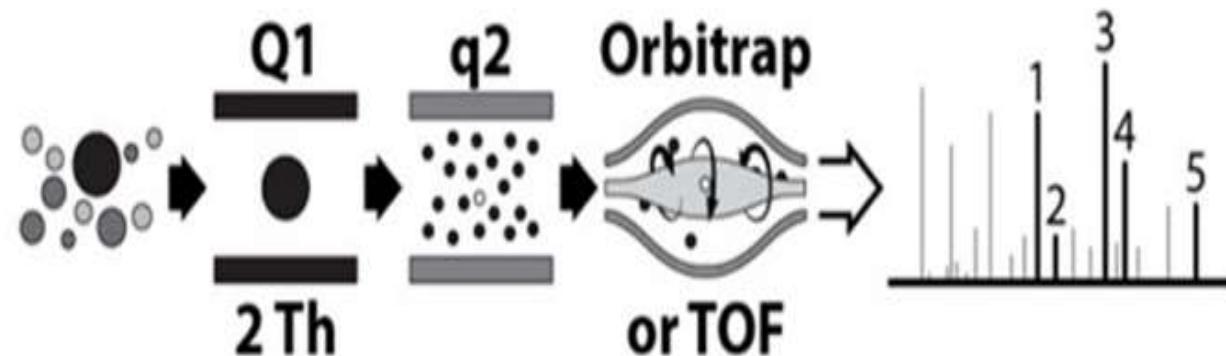
File name	File content
Protein/peptide quantification	Protein/peptide expression values can also be obtained from an MS--based proteomics experiment and then this data and metadata is used for performing the quantification analysis of peptides and proteins.
Metadata	A term used to describe data that provides additional information about a particular data set. This information can include how, when and where the data set was generated and what standards were used. In the proteomics context the addition of metadata such as peptide and protein identifications and quantification of their expression values gives meaning to a simple collection of mass spectra output files.

Targeted proteomics : PRM mode

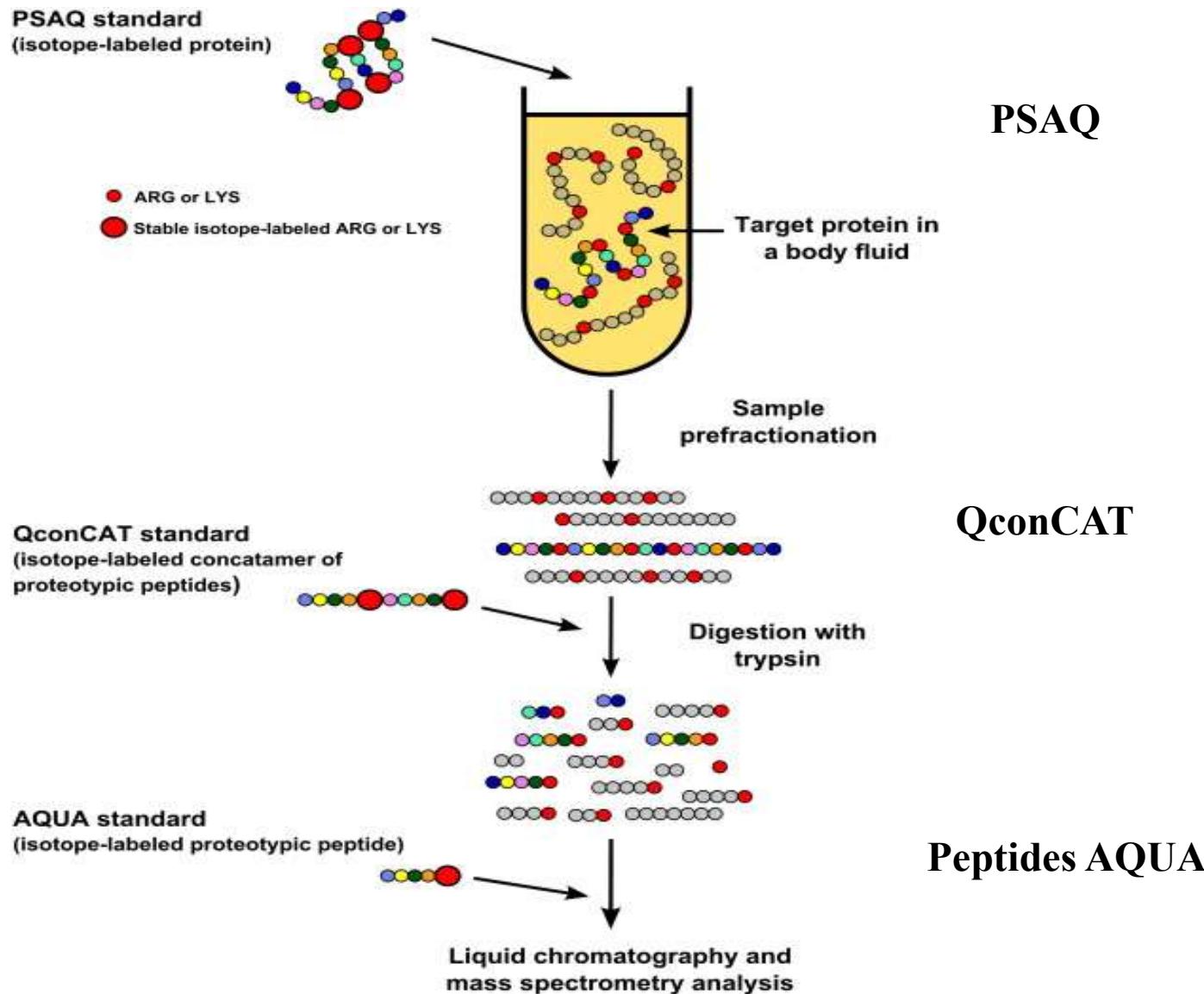
SRM



PRM



Absolute quantification

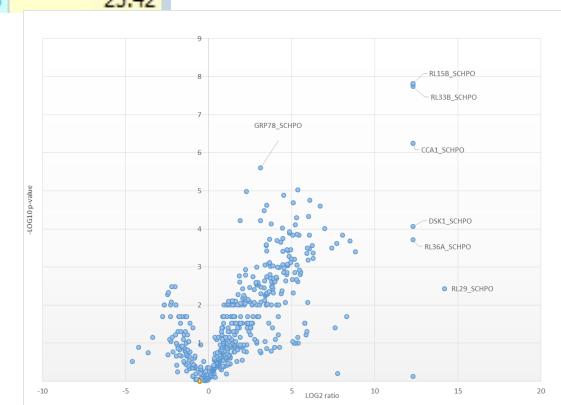
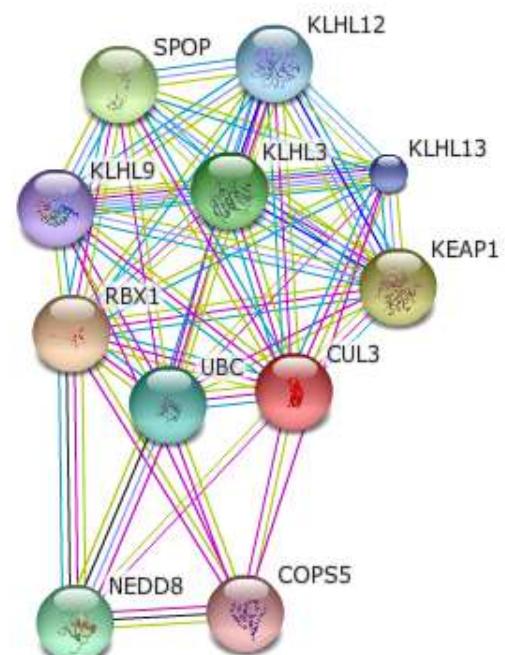


Key questions in proteomics

- What is the protein content of my biological sample?
=> problem of identification
- What is the abundance of my protein of interest?
=> quantification problem
- Relative question: What are the protein abundance variations of the proteomes studied?
- **What are the partners of my protein of interest?**
- Are there any signature proteins related to a particular biological process?
=> biomarkers identifications and quantifications

Co-immunoprecipitation

		Accession	Description	Score A3	Score B3	Score C3
1	<input type="checkbox"/>	Q13618	Cullin-3 OS=Homo sapiens GN=CUL3 PE=1 SV=2-[CUL3...		1172.08	547.08
2	<input type="checkbox"/>	Q86VP6	Cullin-associated NEDD8-dissociated protein 1 OS=Homo...	0.00	394.42	0.00
3	<input type="checkbox"/>	P62877	E3 ubiquitin-protein ligase RBX1 OS=Homo sapiens GN=R...		251.21	123.68
4	<input type="checkbox"/>	P07437	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV...		199.36	136.63
5	<input type="checkbox"/>	Q9Y2M5	Kelch-like protein 20 OS=Homo sapiens GN=KLHL20 PE=...		164.62	78.37
6	<input type="checkbox"/>	Q9P2N7	Kelch-like protein 13 OS=Homo sapiens GN=KLHL13 PE=...		158.68	86.54
7	<input type="checkbox"/>	P68371	Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE...		150.00	143.23
8	<input type="checkbox"/>	Q9P2K6	Kelch-like protein 42 OS=Homo sapiens GN=KLHL42 PE=...		149.87	62.55
9	<input type="checkbox"/>	P05141	ADP/ATP translocase 2 OS=Homo sapiens GN=SLC25A5 P...		148.12	57.72
10	<input type="checkbox"/>	Q92905	COP9 signalosome complex subunit 5 OS=Homo sapiens...		142.70	29.32
11	<input type="checkbox"/>	Q99627	COP9 signalosome complex subunit 8 OS=Homo sapiens...		135.68	37.43
12	<input type="checkbox"/>	P68363	Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B P...	37.89	135.00	103.98
13	<input type="checkbox"/>	Q9P2J3	Kelch-like protein 9 OS=Homo sapiens GN=KLHL9 PE=1S...		131.61	110.90
14	<input type="checkbox"/>	P12236	ADP/ATP translocase 3 OS=Homo sapiens GN=SLC25A6 P...		131.31	78.50
15	<input type="checkbox"/>	Q96M94	Kelch-like protein 15 OS=Homo sapiens GN=KLHL15 PE=...		130.71	35.01
16	<input type="checkbox"/>	Q53G59	Kelch-like protein 12 OS=Homo sapiens GN=KLHL12 PE=...		127.87	23.09
17	<input type="checkbox"/>	P49411	Elongation factor Tu, mitochondrial OS=Homo sapiens GN...		112.52	119.93
18	<input type="checkbox"/>	Q9P2G9	Kelch-like protein 8 OS=Homo sapiens GN=KLHL8 PE=2S...		110.71	28.11
19	<input type="checkbox"/>	Q7L5N1	COP9 signalosome complex subunit 6 OS=Homo sapiens...		106.43	25.42

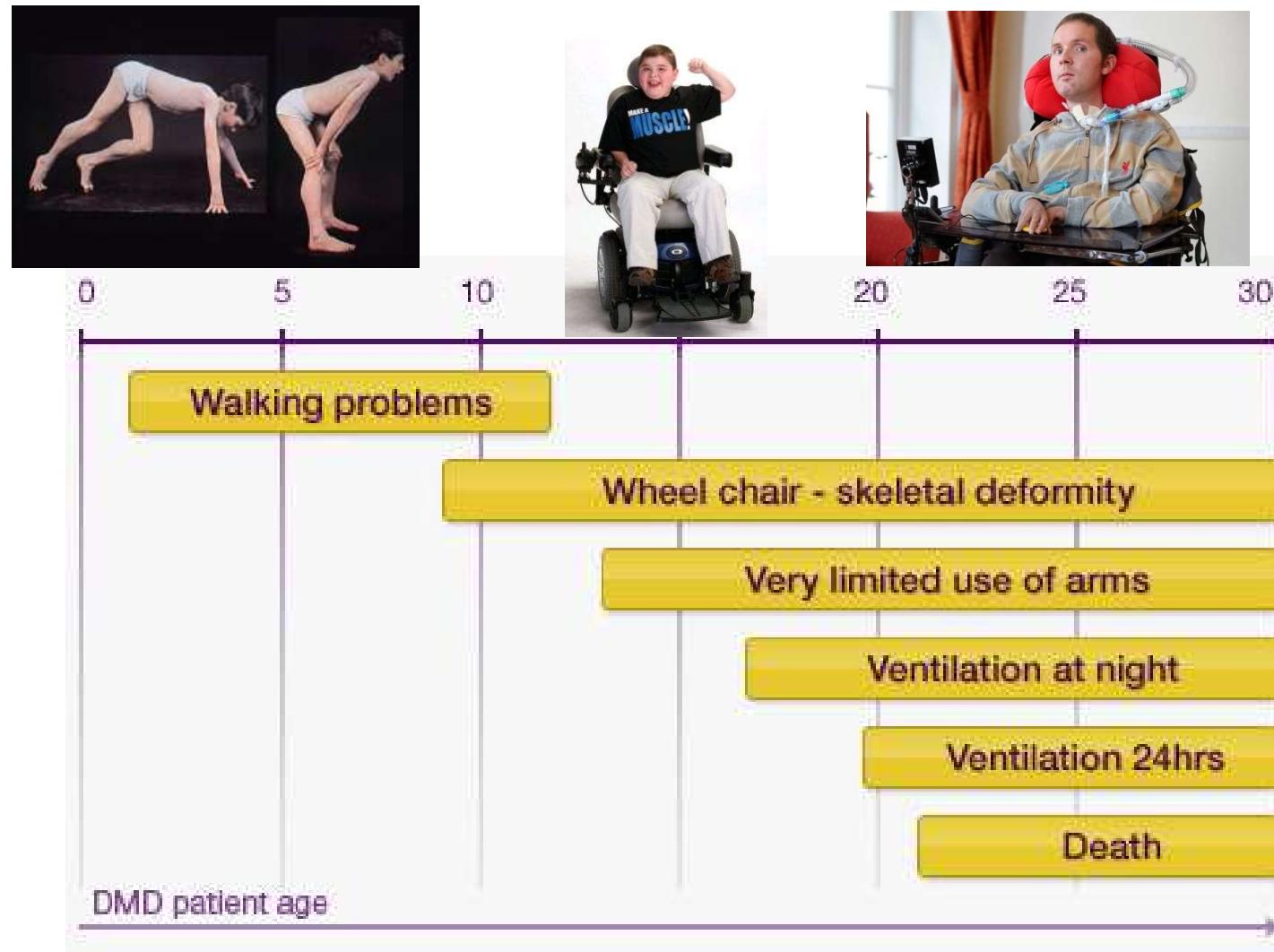


Ilektra Kouranti (HEGP)

Key questions in proteomics

- What is the protein content of my biological sample?
=> problem of identification
- What is the abundance of my protein of interest?
=> quantification problem
- Relative question: What are the protein abundance variations of the proteomes studied?
- What are the partners of my protein of interest?
- Are there any signature proteins related to a particular biological process?
=> biomarkers identifications and quantifications

Biomarkers: applications to Duchenne dystrophy



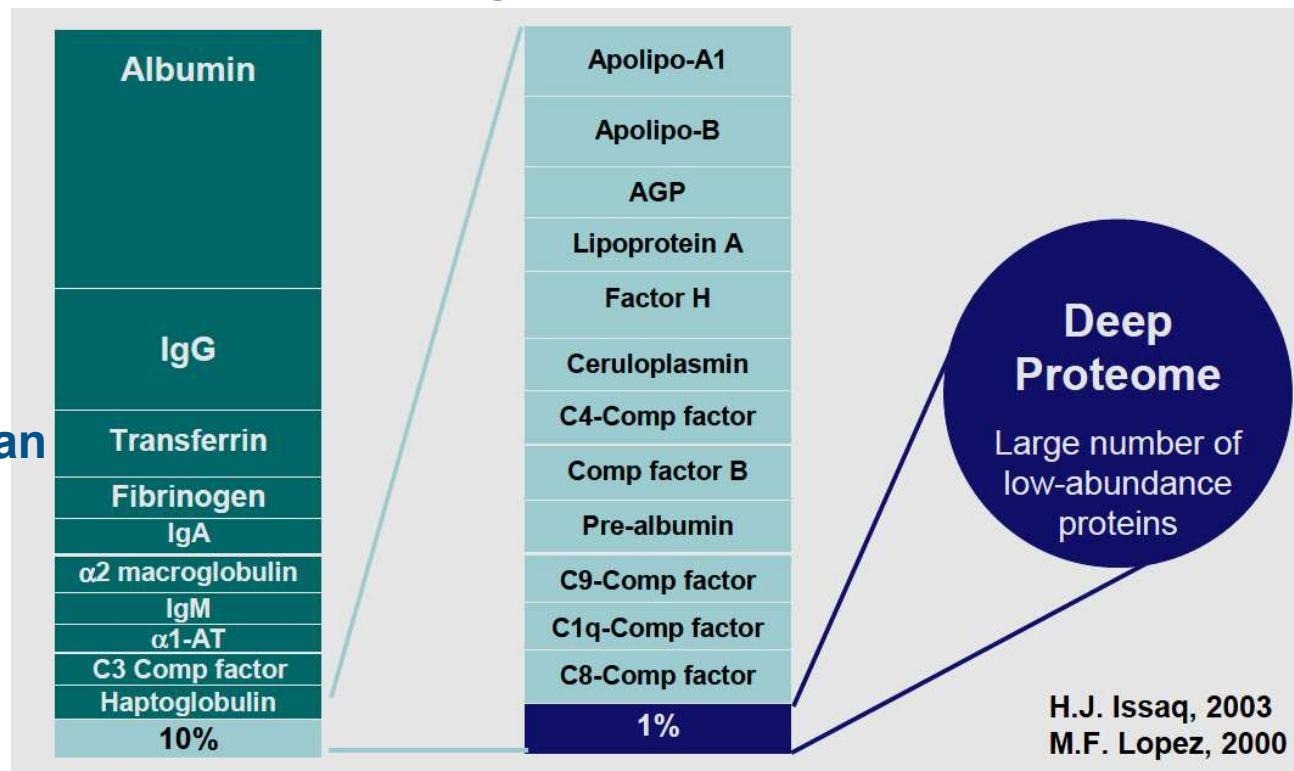
- Death of DMD patients usually occurs ~ 30's

Serum: a “tricky” fluid for Mass Spectrometry

- Serum : Mixture of proteins with different ranges of proteins concentration (from mg/ml to pg/ml)

- 99% of serum proteome = 20 major proteins

- 1% remaining = more than one thousand proteins



- *Albumin* : ~40 mg/ml (60% of serum proteome)
- *C-reactive protein*: ~1 µg/ml (40 000 times less than albumin)
- *FGF-9* : ~400 pg/ml (100 000 000 times less than albumin)

Serum: Depletion of high abundance proteins

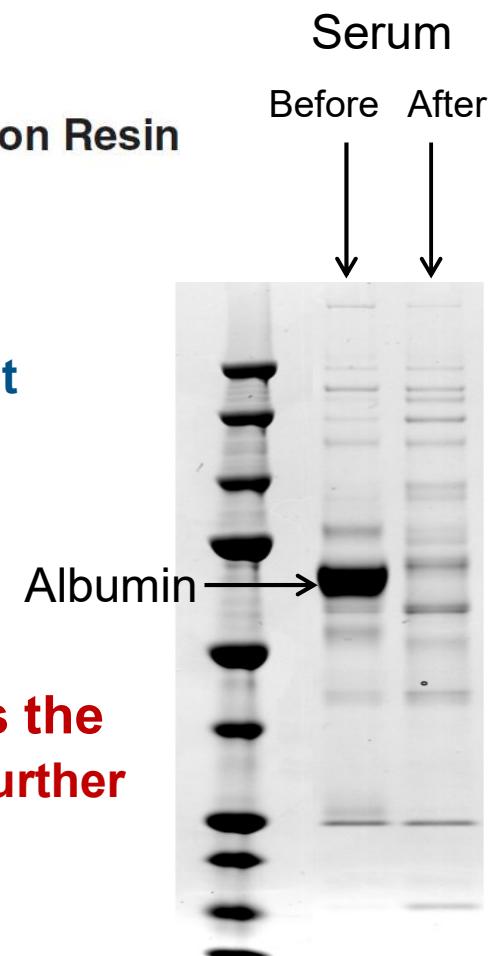
Proteome Purify™ 12

R&D Systems

Human Serum Protein Immunodepletion Resin

- Antibody based column raised against the 12 most abundant proteins in serum
- Reduction of albumin by > 90%

Depletion of high abundance proteins gave us the highest number of identifications: selected for further analysis



Biomarkers: applications to Duchenne dystrophy

Description	No. of peptides	Score	ANOVA (<i>p</i> -value)	Fold change DMD/Healthy
Titin	23	1469.0	3.88E-06	37.4
Uromodulin	13	777.4	3.96E-03	5.5
Cubilin	10	576.8	2.55E-03	-2.3
Nuclear transport factor 2	5	356.9	1.05E-04	5.8
TNF-receptor superfamily member 16	4	308.7	4.07E-05	3.3
Myosin-1	3	265.3	8.66E-04	39.4
Fibulin-2	3	256.7	1.75E-03	2.9
β-galactosidase	6	253.7	1.23E-03	-2.4
Complement C1r subcomponent-like protein	5	235.8	3.47E-05	2.7
Aminopeptidase	3	213.7	2.71E-03	2.4

From: “Proteomics profiling of urine reveals specific titin fragments as biomarkers of Duchenne muscular dystrophy”, J. Rouillon, A. Zocevic, T. Leger, C. Garcia, J-M. Camadro, B. Udd, B. Wong, L. Servais, T. Voit, F. Svinartchouk, 2014 Neuromuscular Disorders

Biomarqueurs: applications à la dystrophie de Duchenne

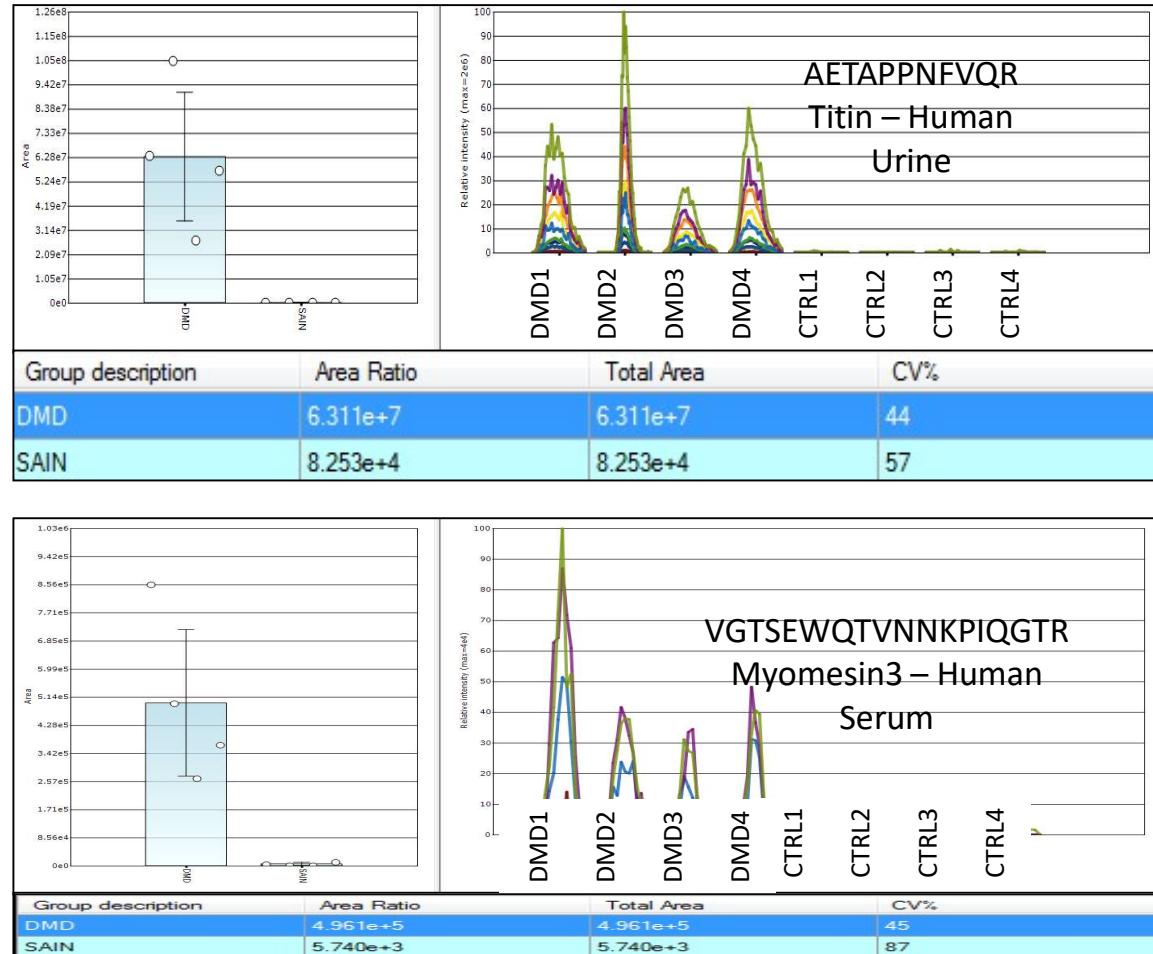
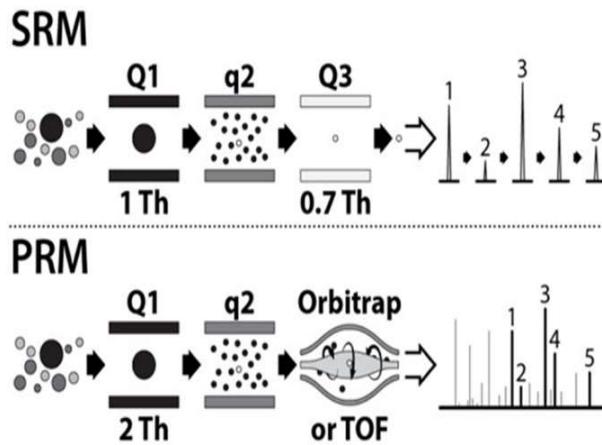
No. accession	Description	Localization	Peptides	Score	ANOVA (P-value)	Fold change
MYG_HUMAN	Myoglobin	Cytoplasm	4	195	2.7e-03	234.8
MYOM2_HUMAN	MYOM2	Myofibril	10	390	9.8e-05	100.1
MYOM3_HUMAN	MYOM3	Myofibril	11	491	1.5e-05	49.7
TPIS_HUMAN	Triosephosphate isomerase	Cytoplasm	3	128	2.3e-03	48.4
AATC_HUMAN	Aspartate aminotransferase	Cytoplasm	3	75	4.7e-04	45.7
KCRM_HUMAN	CK-M	Cytoplasm	15	849	2.9e-05	39.8
MYH7_HUMAN	Myosin-7	Myofibril	11	520	2.2e-05	38.3
ENO1_HUMAN	β-enolase	Cytoplasm	4	178	7.4e-05	34.8
G6PI_HUMAN	Glucose-6-phosphate isomerase	Cytoplasm/Secreted	4	130	1.6e-03	29.5
CAH3_HUMAN	Carbonic anhydrase 3	Cytoplasm	5	182	8.6e-05	23.9
FLNC_HUMAN	Filamin-C	Myofibril	4	145	4.3e-04	19.4
ALAT1_HUMAN	Alanine aminotransferase 1	Cytoplasm	4	127	3.0e-05	15.6
ALDOA_HUMAN	Fructose-bisphosphate aldolase A	Cytoplasm	15	729	9.3e-05	14.2
KPYM_HUMAN	Pyruvate kinase PKM	Cytoplasm	16	845	1.1e-05	12.8
TITIN_HUMAN	Titin	Myofibril	14	495	1.9e-03	10.8
VINC_HUMAN	Vinculin	Cytoplasm/Membrane	2	74	7.2e-05	10.3
PYGM_HUMAN	Glycogen phosphorylase, muscle form	Cytoplasm	8	257	6.1e-04	9.9
LDHA_HUMAN	L-lactate dehydrogenase A chain	Cytoplasm	8	378	9.1e-04	9.5
HPT_HUMAN	Haptoglobin	Secreted	29	1867	1.5e-04	7.6
HBD_HUMAN	Haemoglobin subunit δ	Cytoplasm	3	100	5.1e-03	6.2
LDHB_HUMAN	L-lactate dehydrogenase B	Cytoplasm	10	598	2.4e-05	5.4
HBB_HUMAN	Haemoglobin subunit β	Cytoplasm	7	552	8.0e-03	3.6
HBA_HUMAN	Haemoglobin subunit α	Cytoplasm	7	407	5.3e-03	3.4
TPM2_HUMAN	Tropomyosin βchain	Myofibril	5	170	2.0e-02	2.6



Rouillon, J., Zocevic, A., Poupiot, J., Amor, F., **Léger, T.**, Garcia, C., Camadro, J.M., Wong, B., Cosette, J., ML Coenen-Stass, A., McClorey, G., C Roberts, T., JA Wood, M., Servais, L., Voit, T., Richard, I., Svinartchouk, F. (2015). Serum proteomic profiling reveals specific MYOM3 fragments as biomarkers of Duchenne muscular dystrophy with applications for the follow-up of gene therapy treatment in a mouse model of muscular dystrophies. – *Human Mol. Genetics*

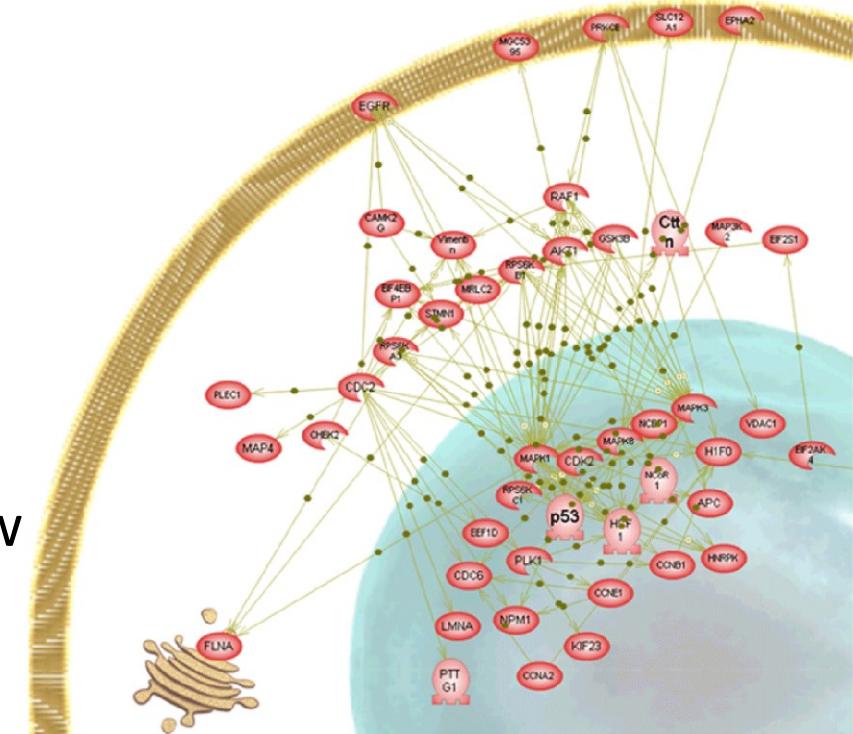
Jeremy Rouillon; Aleksandar Zocevic; **Thibaut Léger; Camille Garcia; Jean-Michel Camadro;** Bjarne Udd; Laurent Servais; Thomas Voit; Fedor Svinartchouk. (2014). Proteomics profiling of urine reveals specific titin fragments as biomarkers of Duchenne muscular dystrophy. *Neuromuscular disorders.*

Protéomique ciblée de type PRM



Why study PTMS?

- Cells can rapidly respond to stimuli and perturbations
- Important cellular mechanisms are tightly controlled
- Often, diseases (e.g. cancer) are due to aberrantly activated proteins
 - Protein expression is much too slow for quick adaption
 - PTMs are crucial regulator
 - MS-based proteomics allows to analyze complex networks of post-translationally modified proteins



PTMs *in vivo*

- **Phosphorylation** (Ser, Thr, Tyr; +80 Da)
 - Phosphorylation is one of the most important PTMs
 - A key event in signaling
 - Catalyzed by kinases/phosphatases
 - **Glycosylation** (Asn, Ser, Thr)
 - marks proteins for degradation
 - s for degradation
 - **Glycation** (Asn, Ser, Thr)
 - marks proteins for degradation
 - s for degradation
 - **Ubiquitination** (Lys; +114 Da)
 - marks proteins for degradation
 - **Proteolytic cleavage**
 - **Acetylation** (N-termini and Lys +42 Da)
 - often combined with removal of protein initial Met
- Others: oxidations, methylations, sumoylations, glutathionylations...

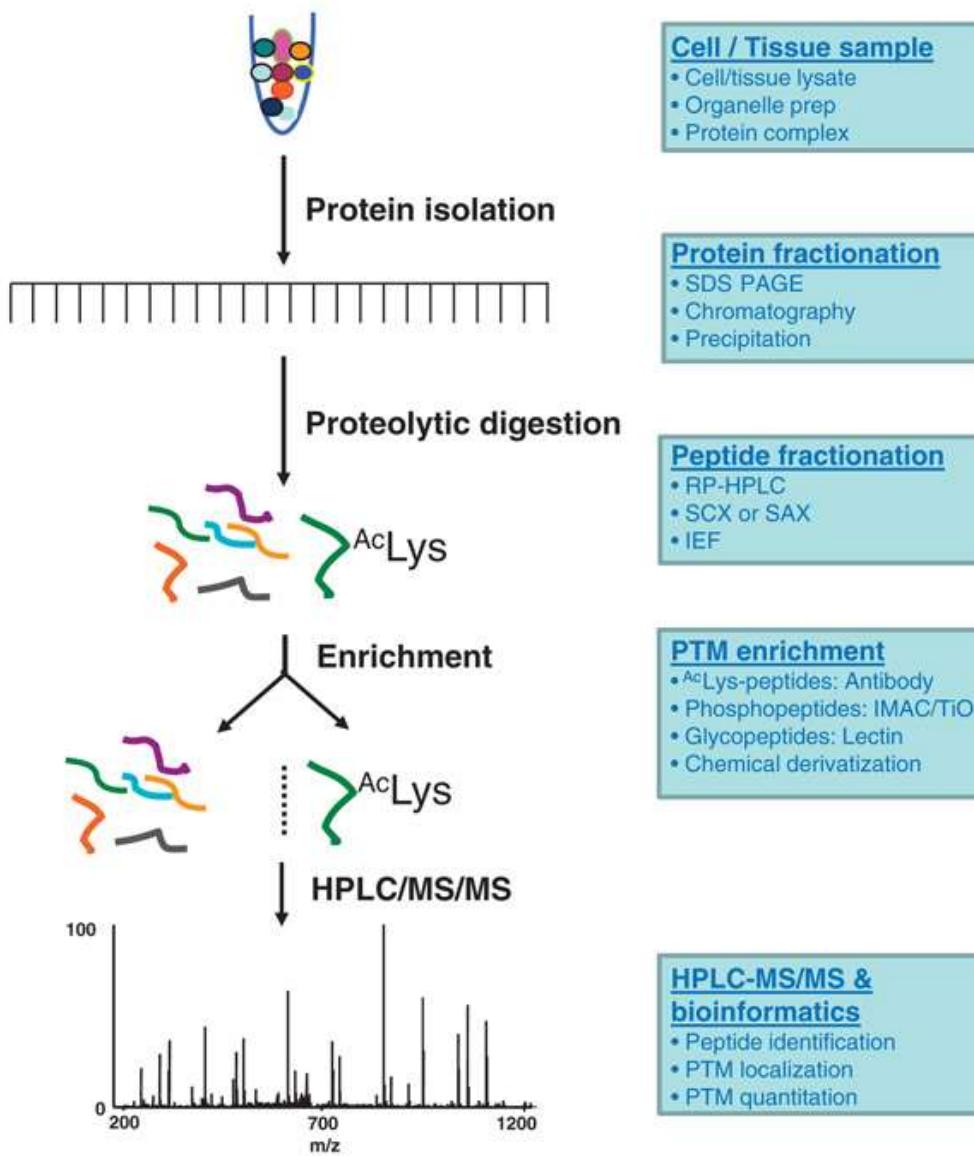
Caractérisation des PTMs: techniques

Techniques for detection and identification of PTM substrates

Method	<i>In vitro</i> or <i>in vivo</i>	Case studies	Advantages	Disadvantages
Radioactive isotope labeling	<i>In vitro</i> or <i>in vivo</i>	³² P (pSer, pThr, pTyr) ³ H, or ¹⁴ C for AcLys or MeK	Reagents accessible	Inconvenience/hazard low sensitivity
Western blotting	<i>In vitro</i> or <i>in vivo</i>	pTyr, AcLys or MeK	Good affinity	Moderate sensitivity
Peptide/protein array	<i>In vitro</i>	pSer/Thr/Tyr, AcLys or MeK	Rapid, global scale	Possibly non-specific, low sensitivity, requires verification
MS-proteomics	<i>In vitro</i>	pSer/Thr/Tyr, AcR or MeK	Specific, global scale	Need enrichment methods

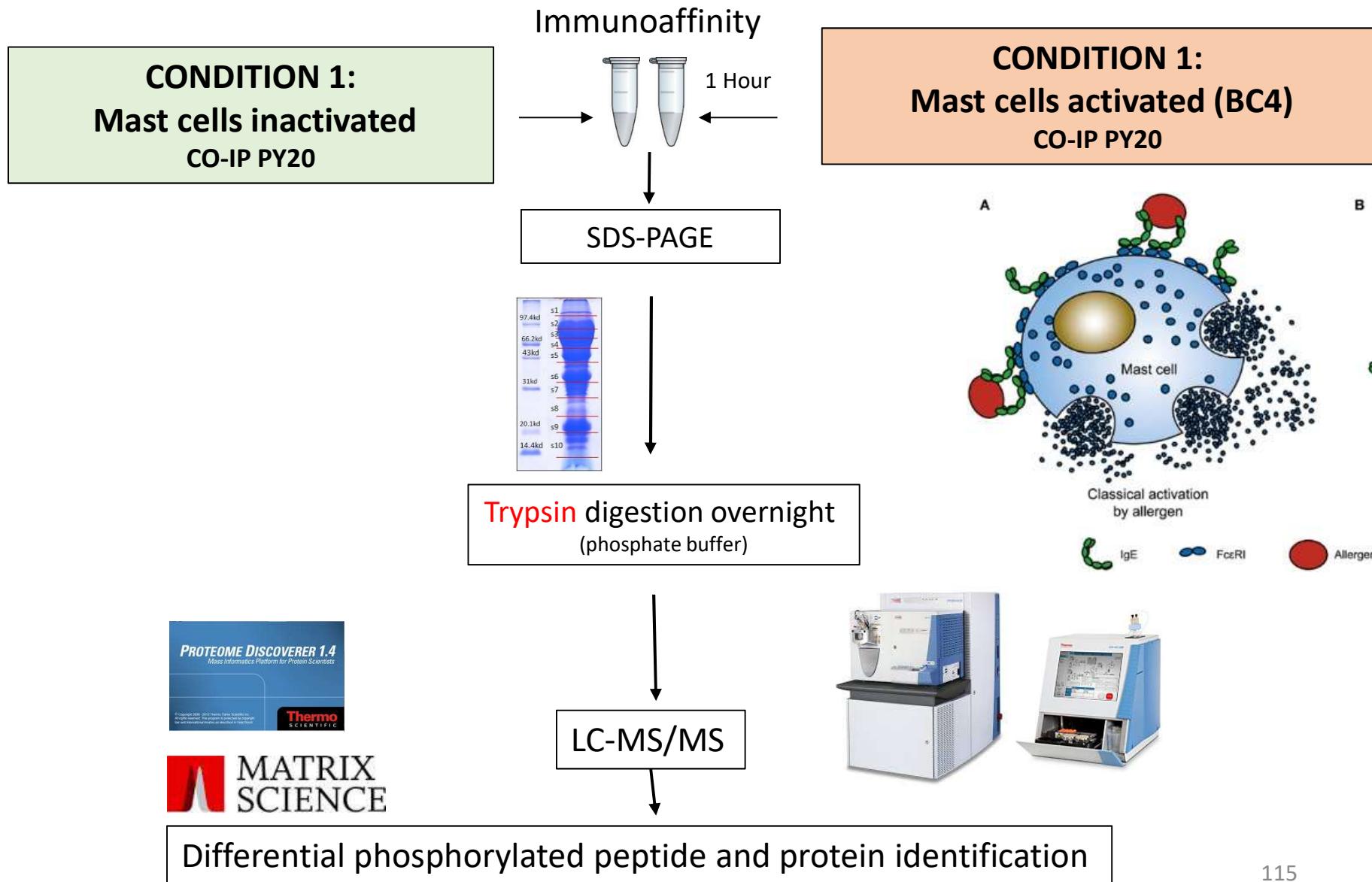
AcR, MeK, pSer, pThr, and pTyr, represent acetylated arginine, methyllysine, phosphorylated serine, threonine, and tyrosine residues, respectively.

Workflow pour la caractérisation PTMs

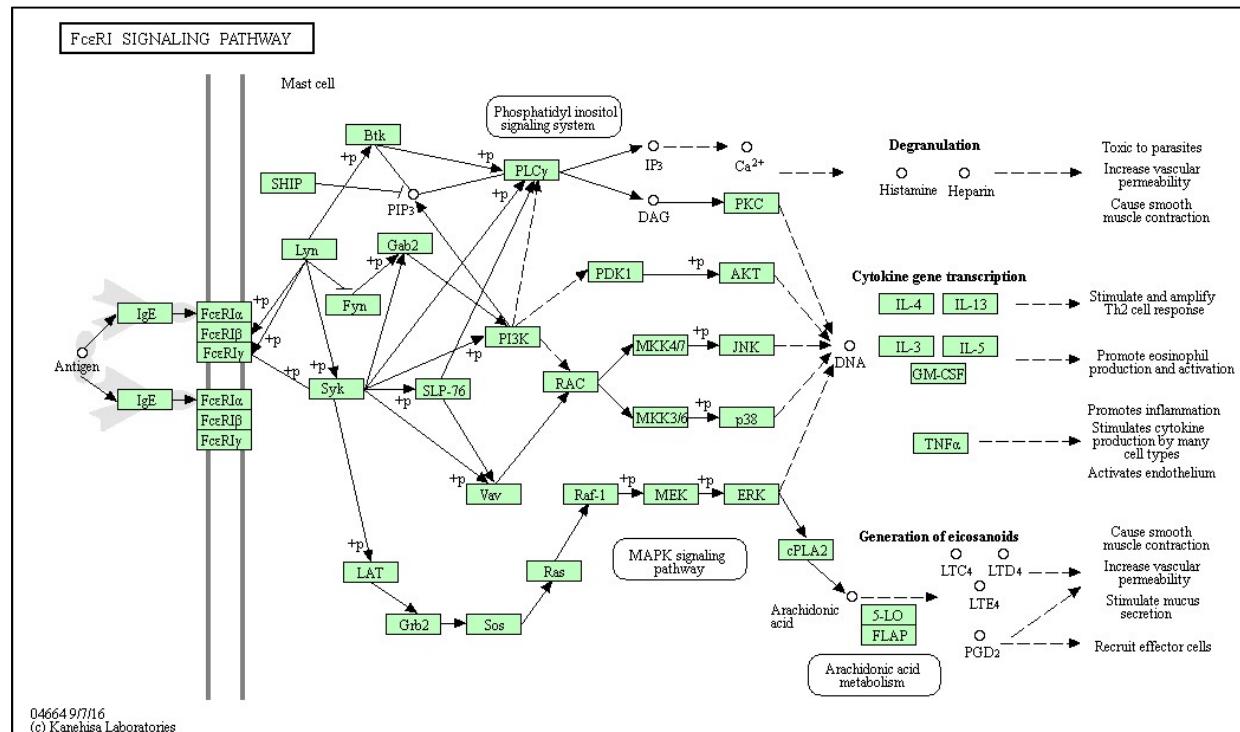


Proteomics. 2009 Oct; 9(20): 4632–4641.[\[CrossRef\]](#)

Quantitative proteomics and phosphorylations



93b	U9JIM8U	Protein associated with glycosphingolipid-enriched microtransducer activity	membrane ; response to stimulus	/b3, iu	b4,b5	45,9	4,81				
953	P20411	High affinity immunoglobulin epsilon receptor subunit gamma Cnaf transducer activity cell surface; membrane ; to stimulus; transport	Pf11628	573.65	352.21	9.8	6.00				
963	Q64725	Tyrosine-protein kinase SYK OS=Rattus norvegicus GN=Syk Plnf transducer activity cleav.; organelle lumen ; to stimulus; transport	Pf00017; Pf00069; Pf07714	835.28	323.20	71.5	8.15				
964	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications	MH+[Da]	phosphoRS Site Probabilities	A4	IonScore A4	Exp Value A4
965	LLTLEDNEILGSGNFGTVK	2	1	1	Q64725		1906.57726		High	107	1.50125E-10
966	DSEQTIVLIGSK	4	1	1	Q64725		1305.65435		High	84	2.49757E-08
967	EIINGTYAISGGR	7	1	1	Q64725		1237.61919		High	55	2.38967E-05
968	ADENYYK	3	1	1	Q64725		902.38894		High	46	0.000100972
969	NVLLVTQHYAK	8	1	1	Q64725		1285.72683		High	44	0.000137239
970	ISDFGLSK	6	1	1	Q64725		866.46208		High	42	0.000234007
971	LIATTAHEK	7	1	1	Q64725		983.55124		High	37	0.000403124
972	LRNYYYDVN	4	1	1	Q64725		1318.64326		High	37	0.001486192
973	YLEESNFVHR	4	1	1	Q64725		1293.62261		High	36	0.00176677
974	MGCPPGCPR	4	1	1	Q64725	M1(Oxidation); C3(Carbamidon)	1047.41679		High	35	0.000734469
975	GSEVTAMLEK	2	1	1	Q64725		1064.52982		High	34	0.002136328
976	GSEVTAMLEK	4	1	1	Q64725	M7(Oxidation)	1080.52370		High	34	0.002154235
977	EVYLDRK	2	1	1	Q64725		922.49926		High	33	0.001493862
978	ALRADENYYK	2	1	1	Q64725		1242.61182		High	30	0.007003353
979	VLTVPCK	5	1	1	Q64725	C6(Carbamidomethyl)	944.52278		Medium	25	0.020099256
980	GSEVTAMLEKGER	1	1	1	Q64725	M7(Oxidation)	1422.68938		Medium	25	0.027162273
981	LRNYYYDVN	6	1	1	Q64725	Y4(Phospho)	1398.61077	Y(4): 93.9; Y(5): 5.7; Y(6): 0.4	Medium	24	0.023646873
982	TGPFPEDLKENLIR	2	1	1	Q64725		1531.81073		Medium	22	0.025122601
983	GSEVTAMLEKGER	2	1	1	Q64725		1406.69446		Medium	20	0.067444564
984	KPNRFPGVQPK	1	1	1	Q64725		1364.77961		Medium	19	0.035807446
985	LLTLEDNEILGSGNFGTVKK	1	1	1	Q64725		2035.07124		Low	19	0.093821803
986	WYAPECINYFK	1	1	1	Q64725	C6(Carbamidomethyl)	1490.67815		Low	17	0.171057347
987	NYYYDVN	2	1	1	Q64725		1049.45671		Low	16	0.121475657
988	MGCPPGCPR	1	1	1	Q64725	C3(Carbamidomethyl); C7(Car)	1031.42131		Low	15	0.108098357
989	MPWFHGNISR	1	1	1	Q64725	M1(Oxidation)	1260.59497		Low	13	0.348397318
990	YLQQRN	1	1	1	Q64725		821.42674		Low	12	0.268819476
991	NYLQQLGFLPSVAHNR	1	1	1	Q64725	Y2(Phospho)	1598.76055	Y(2): 99.9; S(9): 0.1			Low
992	Crk-like protein OS=Rattus norvegicus GN=Crk PE=1 SV=1 - protein binding m; cytosol; membrane					development	Pf00017; Pf00018; Pf07653	234.55	96.32	33.8	6.74
1005	P60868	40S ribosomal protein S20 OS=Rattus norvegicus GN=Rps20 F:tural molecule activity ism; cytosol; ribosome				metabolic process	Pf00338	172.28	36.35	13.4	9.94



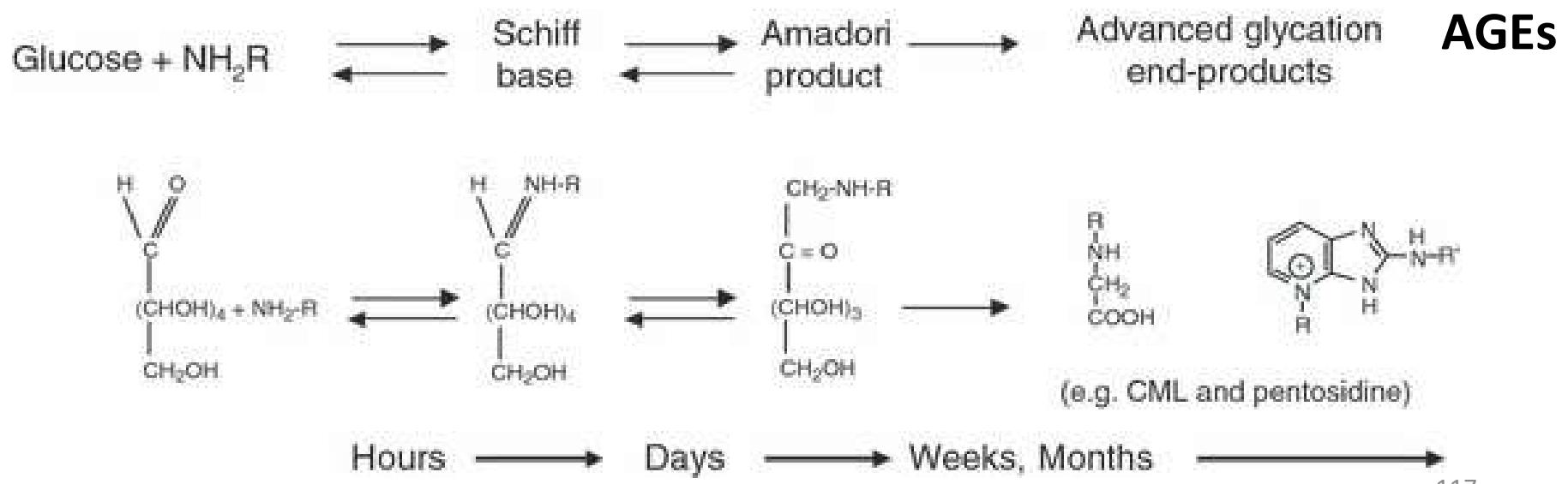
Glycation: principles



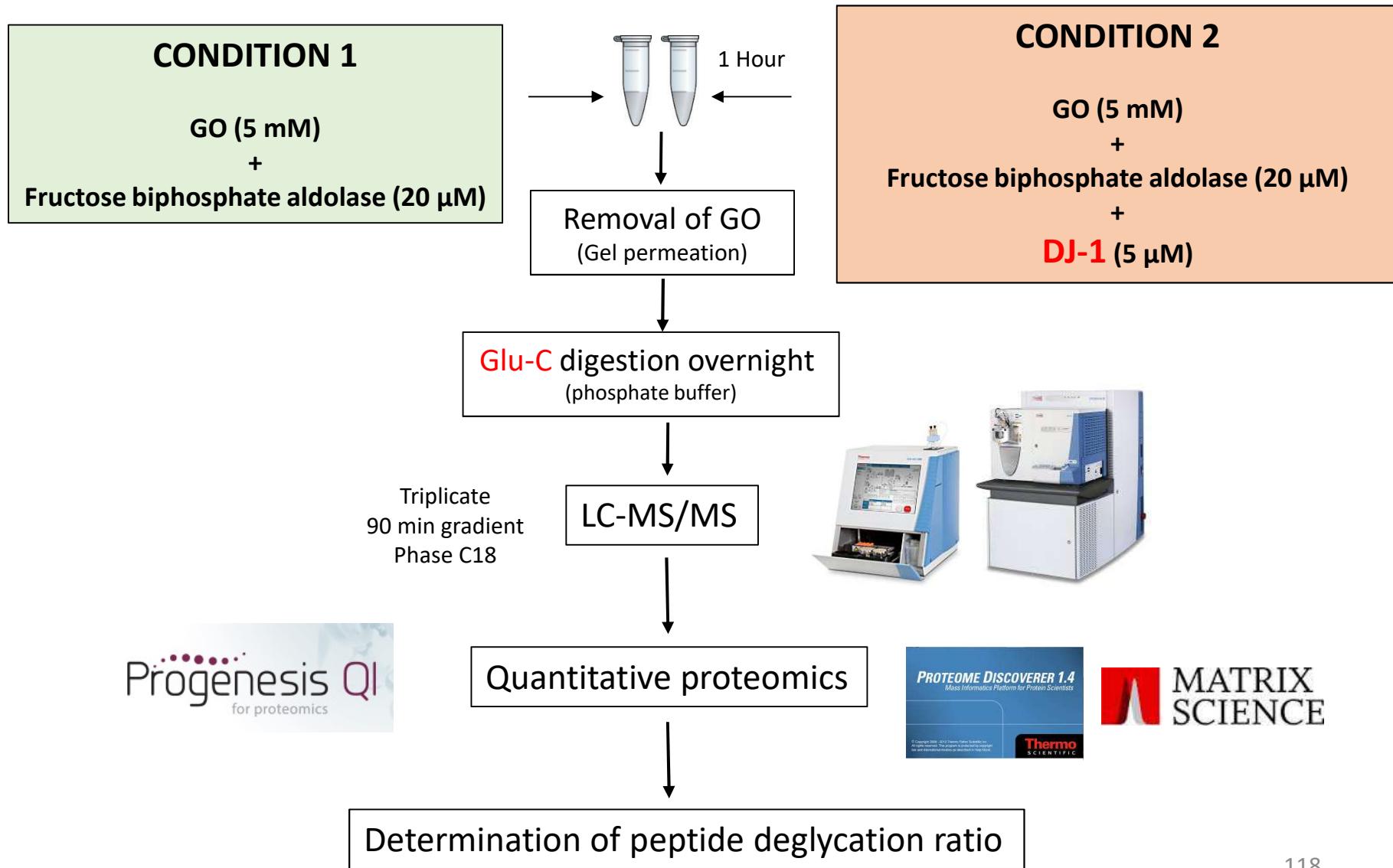
- Glycation is an inevitable nonenzymatic covalent reaction between proteins, amino-lipids or nucleic acids and endogenous reducing sugars or dicarbonyls (methylglyoxal, glyoxal) that results in protein inactivation.
- The reaction between carbonyl groups and amino-acids was discovered by **Louis Camille Maillard** in 1912
- AGE carbonyl adducts alter the aromatic and gustatory properties of biomolecules present in cooked food products
- As important in cells that oxidation for protein and DNA damages



L. C. Maillard, C.R. Acad. Sci. 154, 66 (1912).

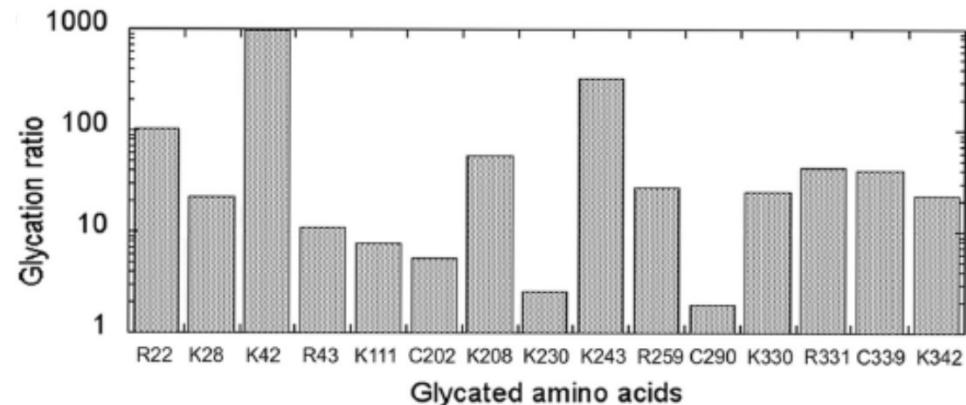


Mass spectrometry analysis of fructose bisphosphate aldolase deglycation by DJ-1



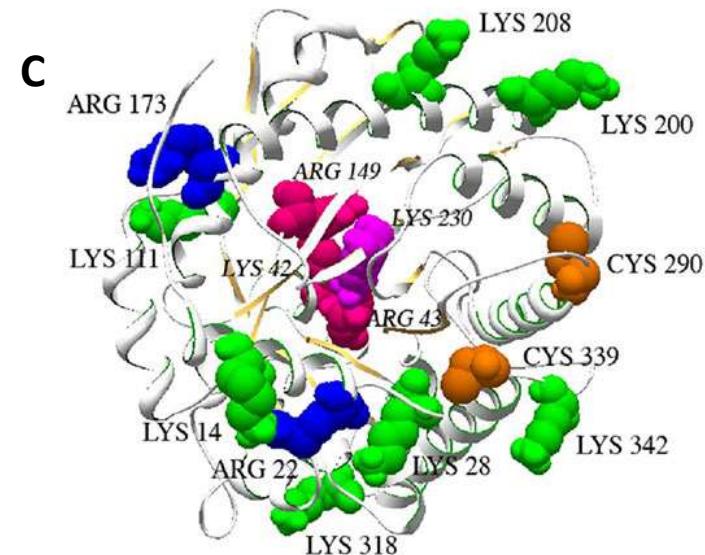
Mass spectrometry analysis of fructose bisphosphate aldolase deglycation by DJ-1

A



B

Sequence of glycated peptide	Modification and position in peptide	Amino acid	Glycation ratio	Analysis of variance	Mascot score
IAHRIVAPGKGILAADE	Glyoxal (Arg ⁴)	Arg ²²	102	3.70E-05	23.93
LSDIAHRIVAPGKGILAADE	Glyoxal (Lys ¹³)	Lys ²⁸	22	1.71E-06	40.01
STGSIAKRLQSIGTENTEE	Glyoxal (Lys ⁷)	Lys ⁴²	980	1.12E-05	43.52
STGSIAKRLQSIGTENTEE	Glyoxal (Arg ⁸)	Arg ⁴³	11	2.73E-03	38.45
KGVVPLAGTNGE	Glyoxal (Lys ¹)	Lys ¹¹¹	7.7	0.000105	75.07
LKRCQYVTE	Glyoxal (Cys ⁴)	Cys ²⁰²	5.4	3.14E-05	22.75
KVLAAYVKALSD	Glyoxal (Lys ¹)	Lys ²⁰⁸	56	0.00995	47.56
GTLLKPNMVTPGHACTQKYSHEE	Glyoxal (Lys ⁵)	Lys ²³⁰	2.6	7.51E-05	22.41
	Glyoxal (Lys ¹⁸)	Lys ²⁴⁸	330	1.02E-02	27.07
IAMATVTALRRTVPPAVTGVTFLSGGQSEEE	Glyoxal (Arg ¹¹)	Arg ²⁵⁹	27	0.000572	27.79
ASINLNAINKCPLLKWPWALTFSYGRAL	Glyoxal (Cys ¹¹)	Cys ²⁹⁰	1.9	0.0286	21.28
YVKRALANSLACQGKYTPSGQAGAAASE	Glyoxal (Lys ³)	Lys ³³⁰	25	0.0016	45.9
	Glyoxal (Arg ⁴)	Arg ³³¹	43	5.41E-05	22.83
	Glyoxal (Cys ¹²)	Cys ³³⁹	40	0.00195	38.72
	Glyoxal (Lys ¹⁵)	Lys ³⁴²	23	2.43E-06	62.1

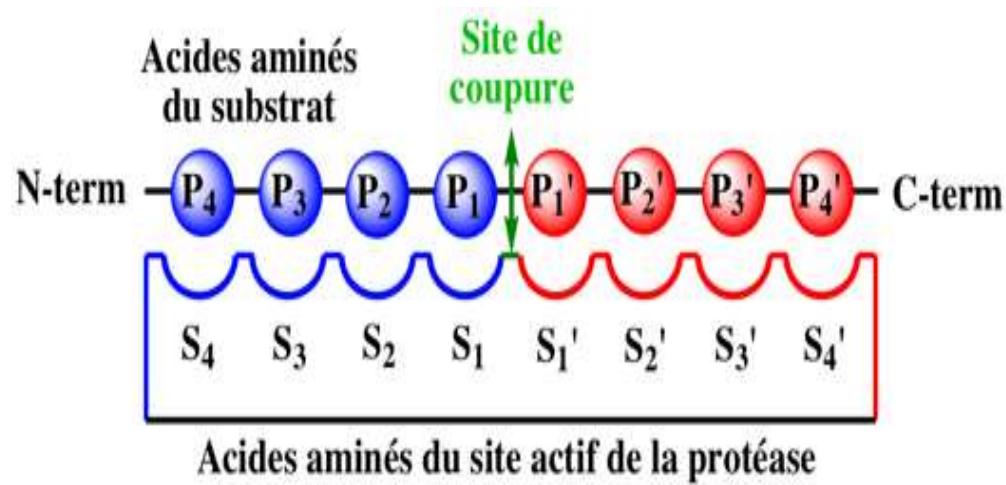


DJ-1 and protein glycation

- **The discovery of the deglycase activity of DJ-1 is a major advance in glycation research**
- Whereas there are mechanisms to degrade or export reactive carbonyl compounds (aldoketoreductases, glyoxalases, ...) are relatively efficient, FN3Ks display more moderate functions in electrophile stress resistance.
- Compared with FN3Ks, **DJ-1 appears to be an overachiever:**
 - (i) its specific activity is 20,000-fold higher
 - (ii) it deglycates cysteines, arginines, and lysines
 - (iii) it deglycates all proteins tested
 - (iv) it operates immediately after glycation onset
 - (v) it releases innocuous products
 - (vi) it does not require any co-factors
 - (vi) DJ-1/ mice display Parkinson disease-type abnormalities
- Complementary actions of these enzymes

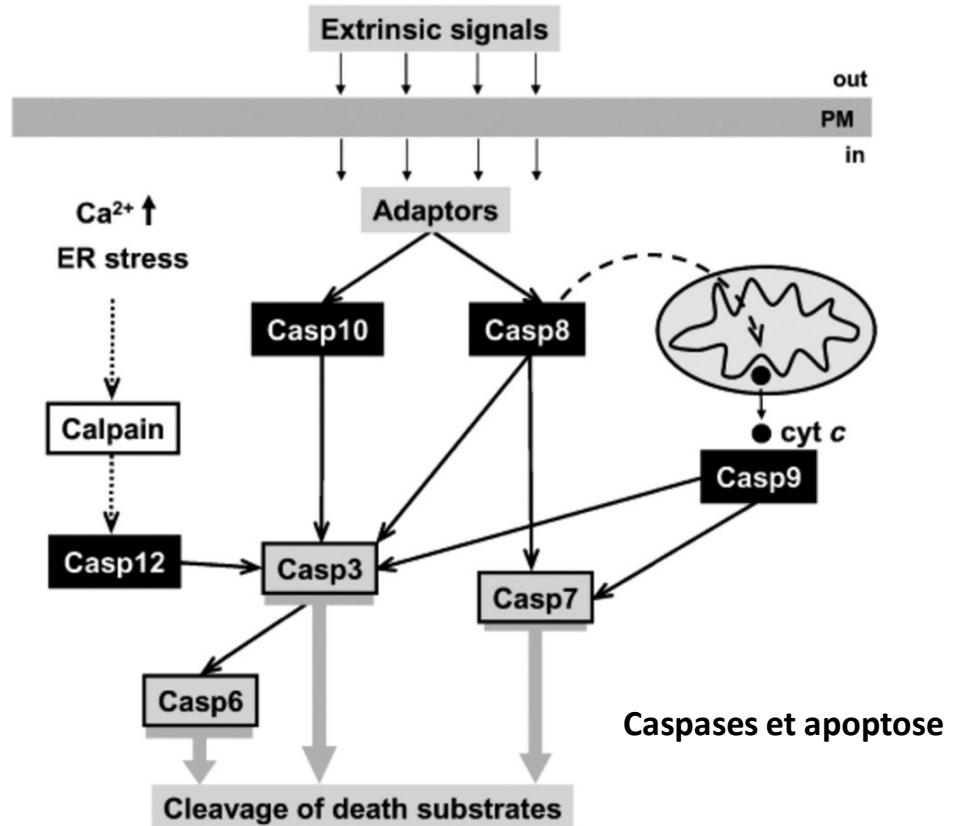
Proteolytic cleavages as PTMs

- Enzymes hydrolysant des liaisons entre acides aminés
- Classification des protéases : aspartate-, cystéine-, glutamate-, métallo-, sérine-, thréonine-, et les asparagine- protéases)
- Autres classifications



E. Jaspard (2013)

Biological functions of proteases



Vachova *et al.* 2007

□ Variété des protéases

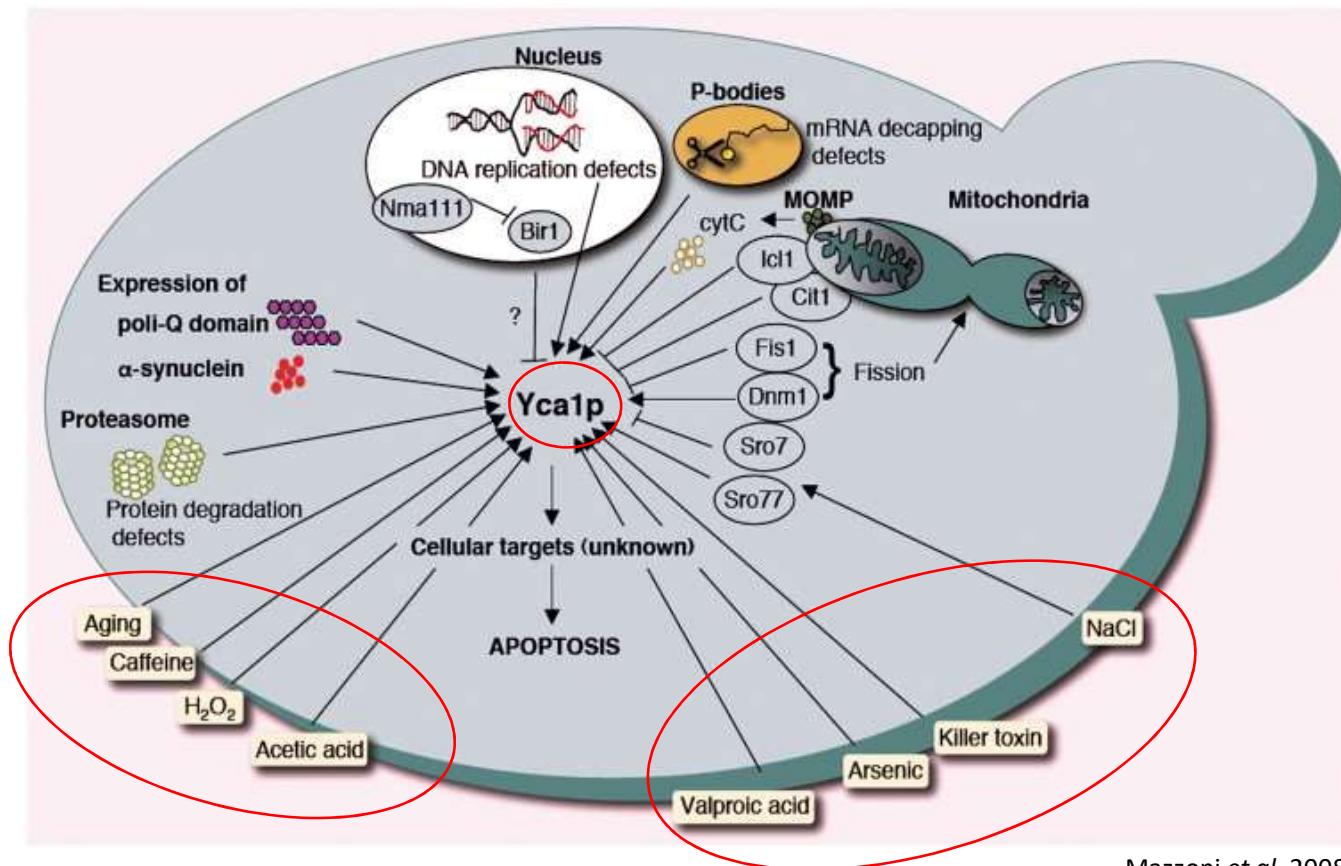
□ Régulations de processus biologiques

- Turnover des protéines
- Dégradation des protéines non correctement repliées
- Adressage cellulaire
- Activation de protéines

□ Dérégulations associées à des états pathologiques

□ Organisme modèle et étude des protéases

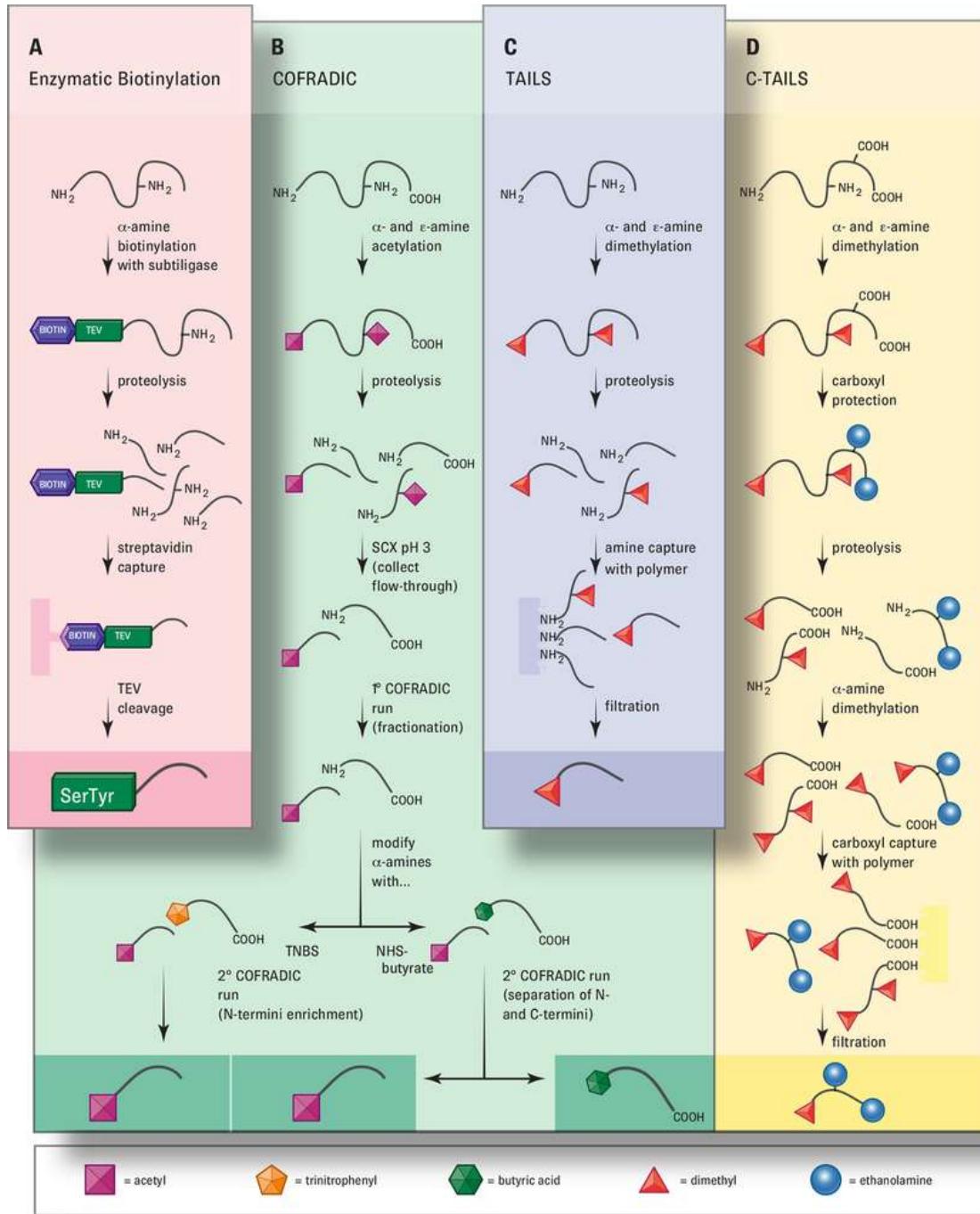
Mca1p activation and apoptosis release



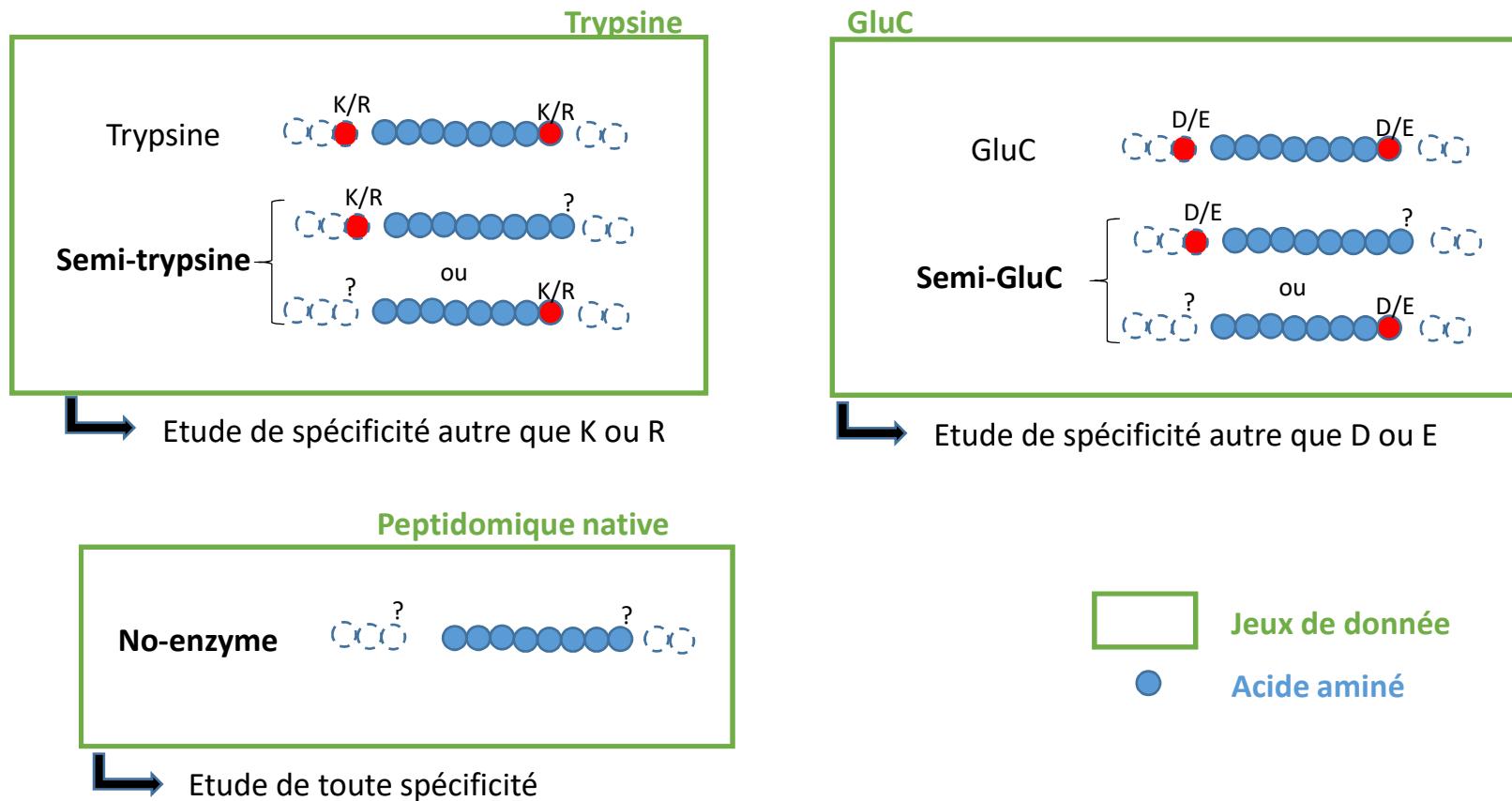
Mazzoni et al. 2008

- Activée par de nombreuses molécules dont la molécule de quorum-sensing farnésol
- Pas d'informations sur la spécificité de clivage de ces substrats (coupures suspectées au niveau des résidus K et R)
- Un seul substrat caractérisé *in vitro* pour la métacaspase de *S. cerevisiae* (Gapdh).

Terminomics

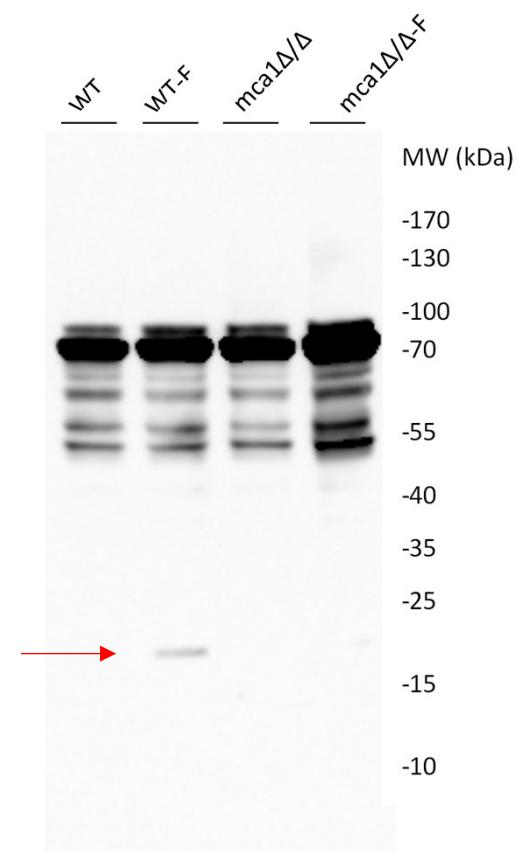


Recherche de la spécificité de clivage de Mca1p



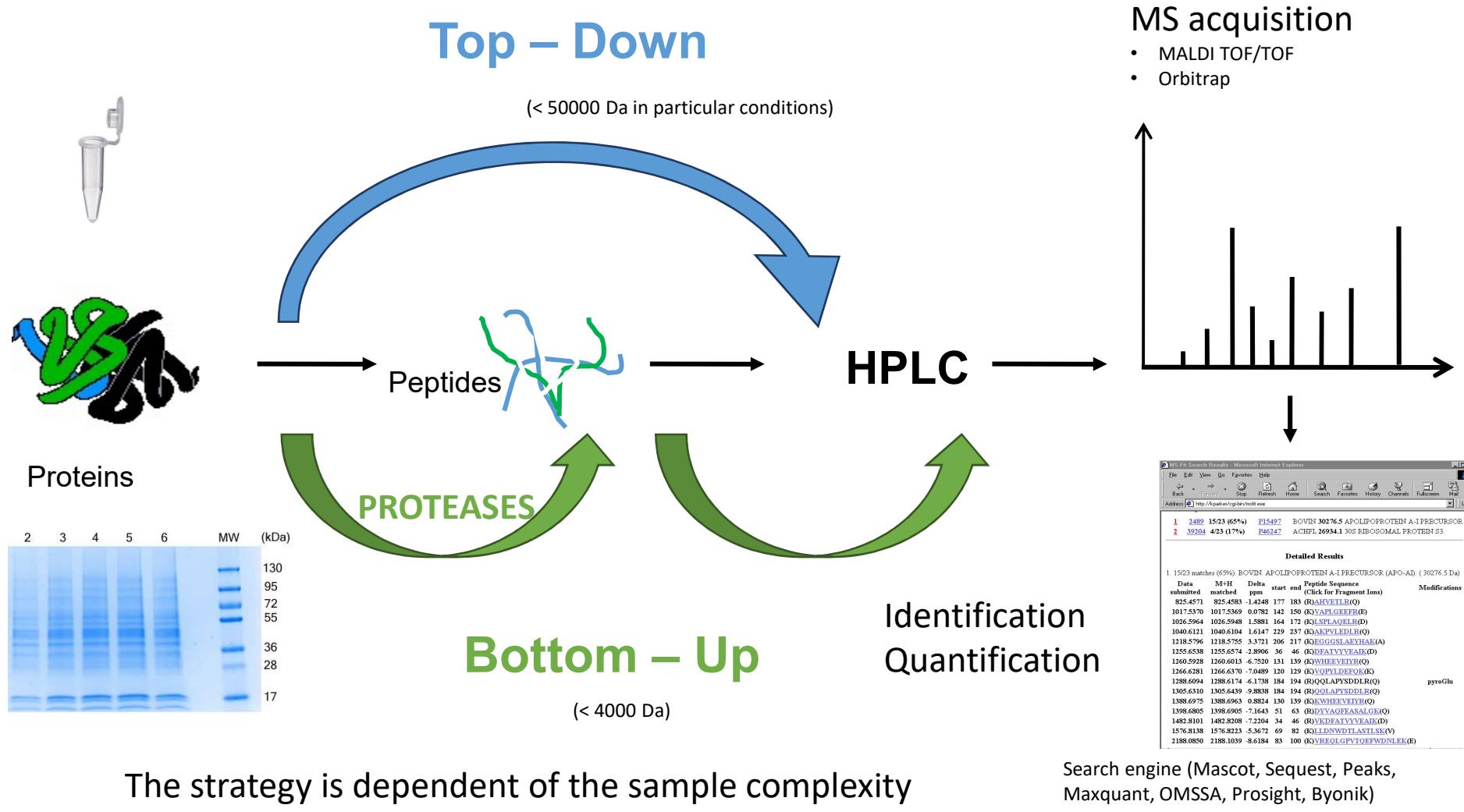
Substrats potentiels de Mca1p et protéines de réponse aux stress

Accession	Description	Experiment	Sequences
orf19.778	PIL1, composant de l'éisosome	Native	WGEDNEDDISDVTDK
orf19.7350	RCT1 Protéine induite par le fluconazole	Native	YDPKRSSNQGSSSNDEQQDR
Orf19.4309	GRP2, Methylglyoxal réductase	GluC	K.EKPNFTLSVINPVYVFGPQAFE
Orf19.2340	CDC48; ATPase microsomale	GluC	R.FALGNNSNPSALRE R.GQFSSFRFNE
orf19.2483	RIM1; protéine liant l'AND simple brin	GluC	K.VGSLVHVD
orf19.2644	QCR2; Ubiquinol-cytochrome-c réductase	GluC	R.GLGNPLFYNE
orf19.1435	TEF1; Facteur d'elongation	GluC	HALLAYTLGVK K.SGKVTGKTLLE
orf19.6515	HSP90; Protéine chaperon essentielle	GluC	K.LVDAPAAIRTGQFGWSANME
Orf19.6367	SSB1; Protéine de choc thermique (HSP70)	GluC	R.LIGRAFDDE
orf19.4980	HSP70; Protéine chaperon de famille HSP70	GluC	K.LVSDFFNGKE K.RTLSSSAQTSIE
orf19.1065	SSA2; Protéine chaperon de famille HSP70	GluC	K.RTLSSSAQTSIE R.LIGDAAKNQAAMNPANTVFD
Orf19.5928	RPP2B; protéine ribosomale acide	GluC	R.LQALLKDLE

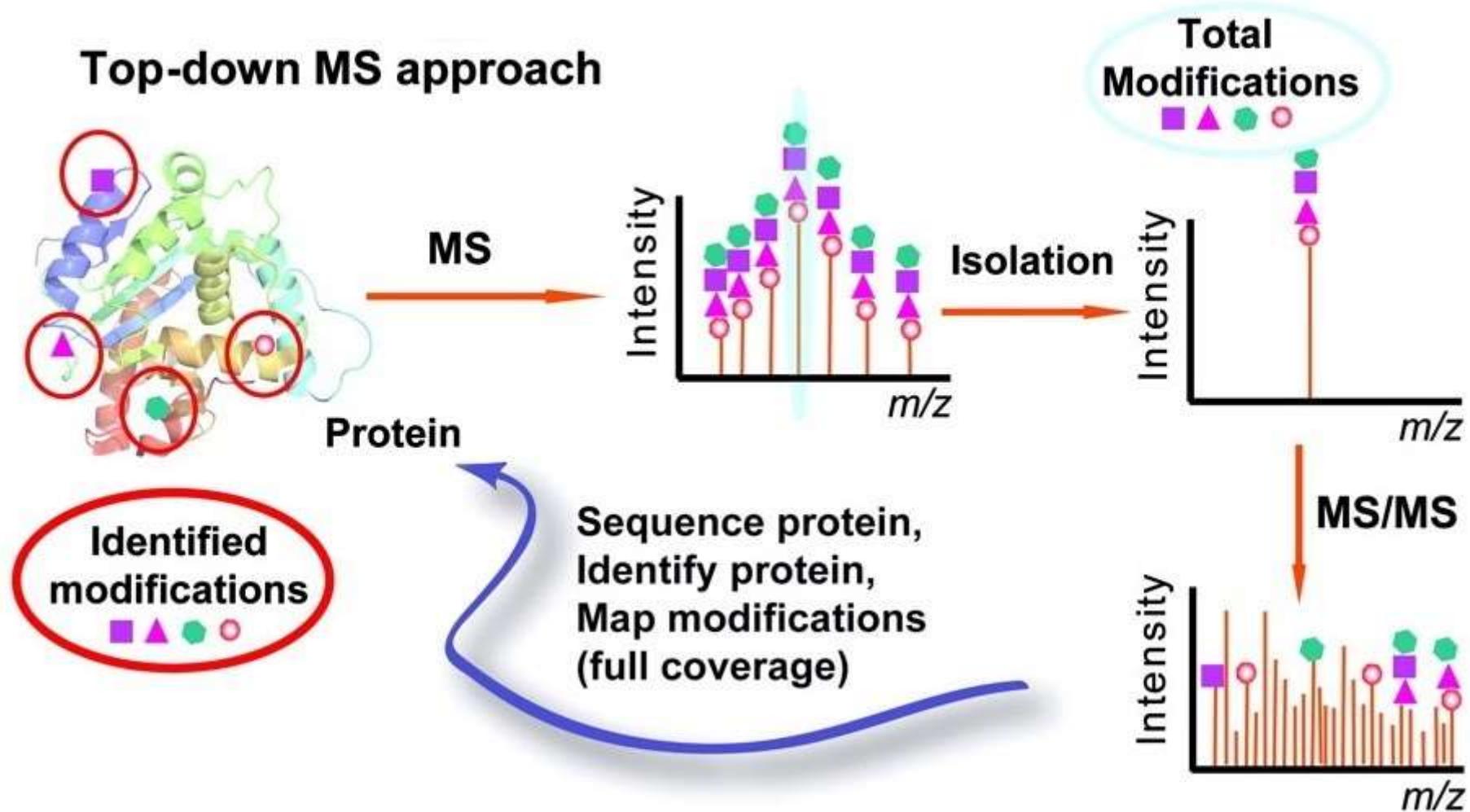


→ 77 substrats potentiels de Mca1p (pour 62 protéines), dont 13 validés dans des conditions de sélection les plus drastiques

Proteomics workflows



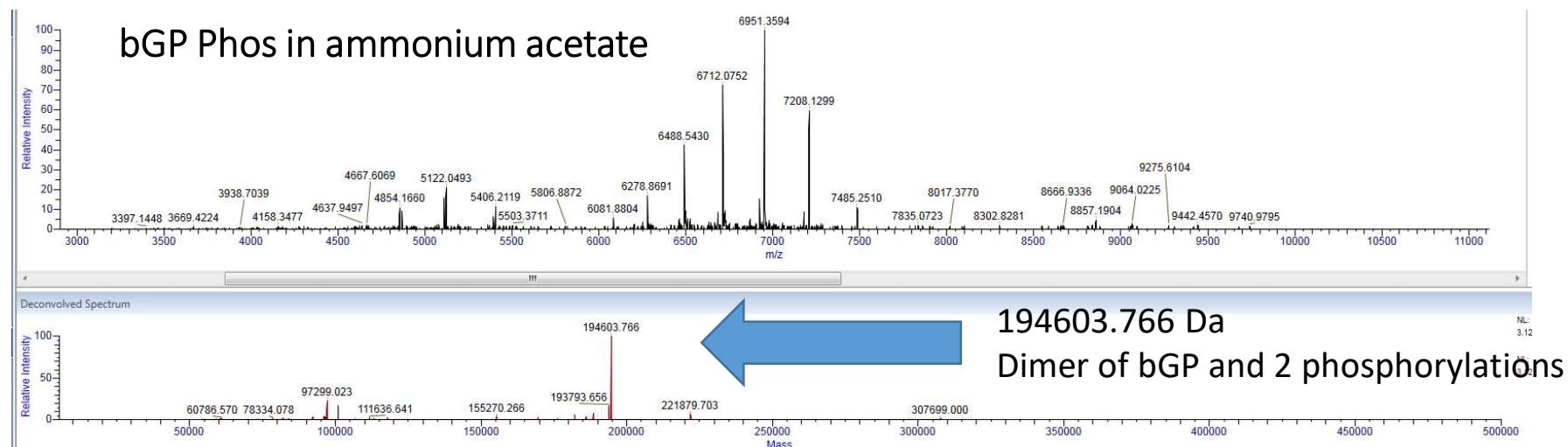
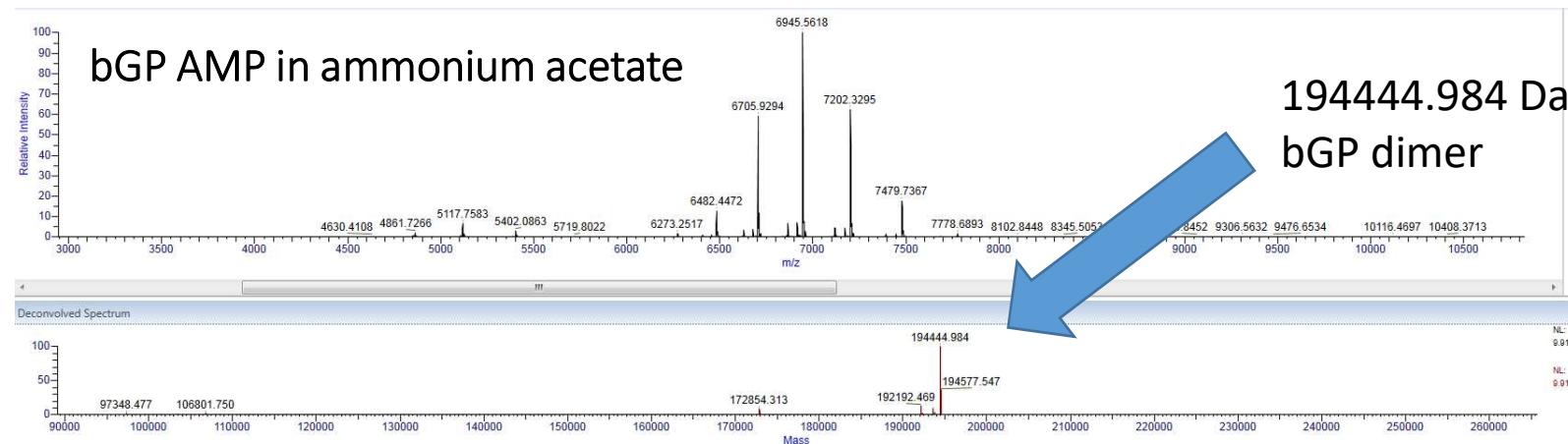
TOP DOWN proteomics for PTMs characterization



Challenges in TOP-DOWN proteomics

Challenges	Innovations
1. Protein solubility Conventional surfactant (e.g. SDS) not compatible with MS	Develop new top-down MS compatible surfactant
2. Proteome complexity Intact protein chromatography underdeveloped	Develop novel multi-dimensional chromatography for intact protein separation
3. Proteome dynamic range Difficulty in detecting low abundant proteins	Develop novel nanomaterials for enriching low abundant proteins
4. Protein MS data interpretation Software for top-down proteomics underdeveloped	Develop user-friendly and versatile software interface

Analysis in intact protein mode: human brain glycogen phosphorylase

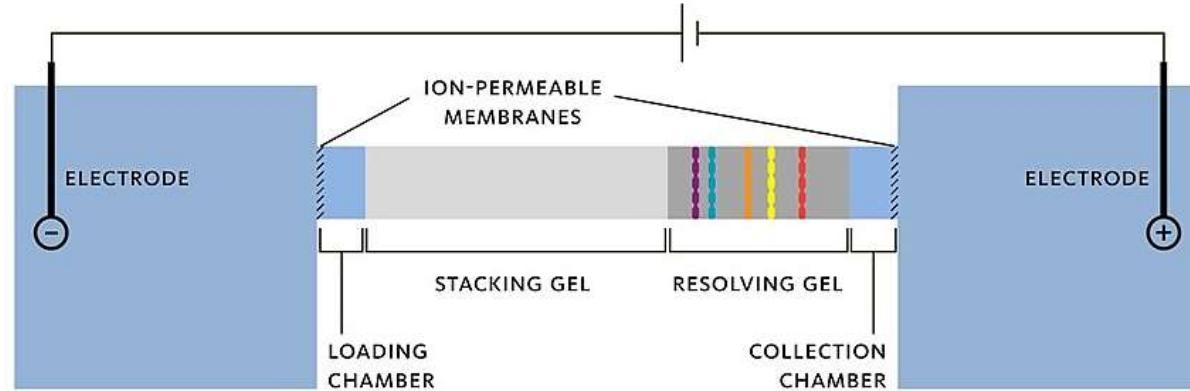


Crystal structure of human brain glycogen phosphorylase. Cécile Mathieu, Ines de la Sierra-Gallay, Romain Duval, Ximing Xu, Angélique Cocaign, Thibault Léger, Jean-Michel Camadro, Catherine Etchebest, Ahmed Haouz, Jean-Marie Dupret, Fernando Rodrigues-Lima. Under review.

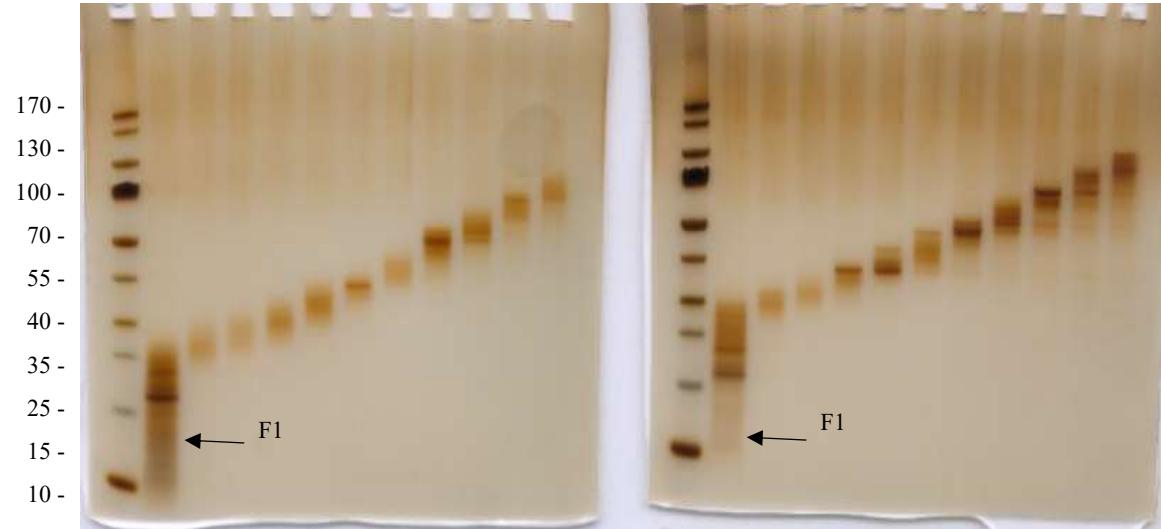
Analysis in intact protein mode



Système Gelfree (Expedeon)



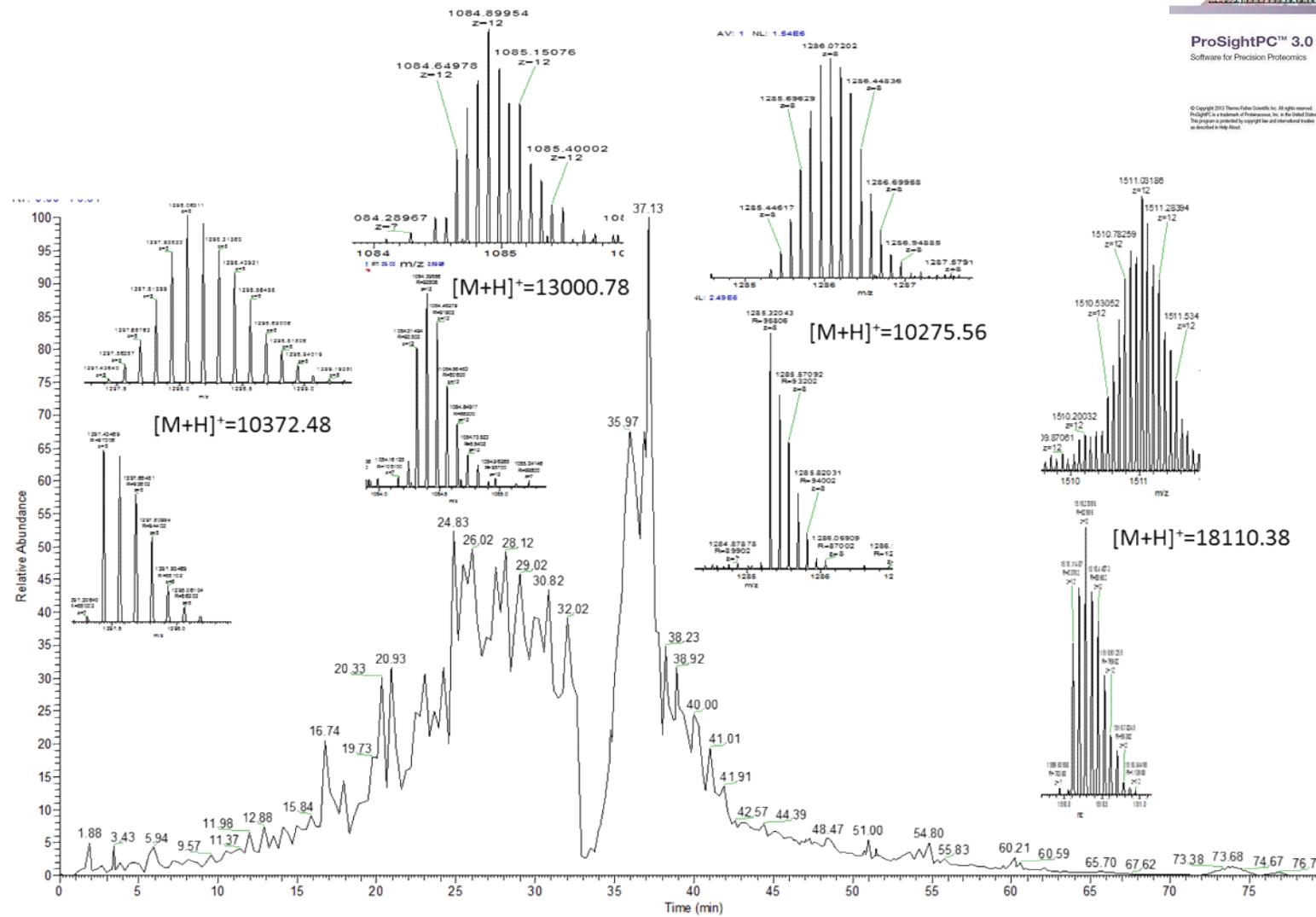
CTRL SDS-PAGE



NanoLC-MS/MS
(Colonne pepswift)



Analysis in intact protein mode

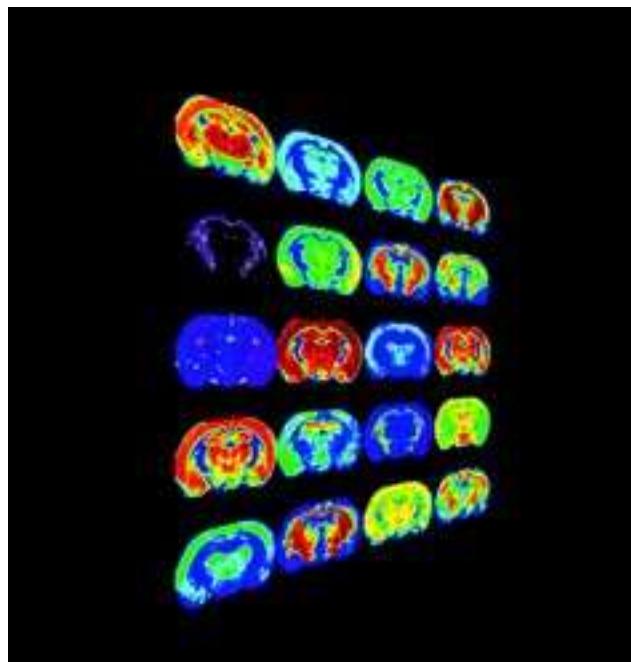


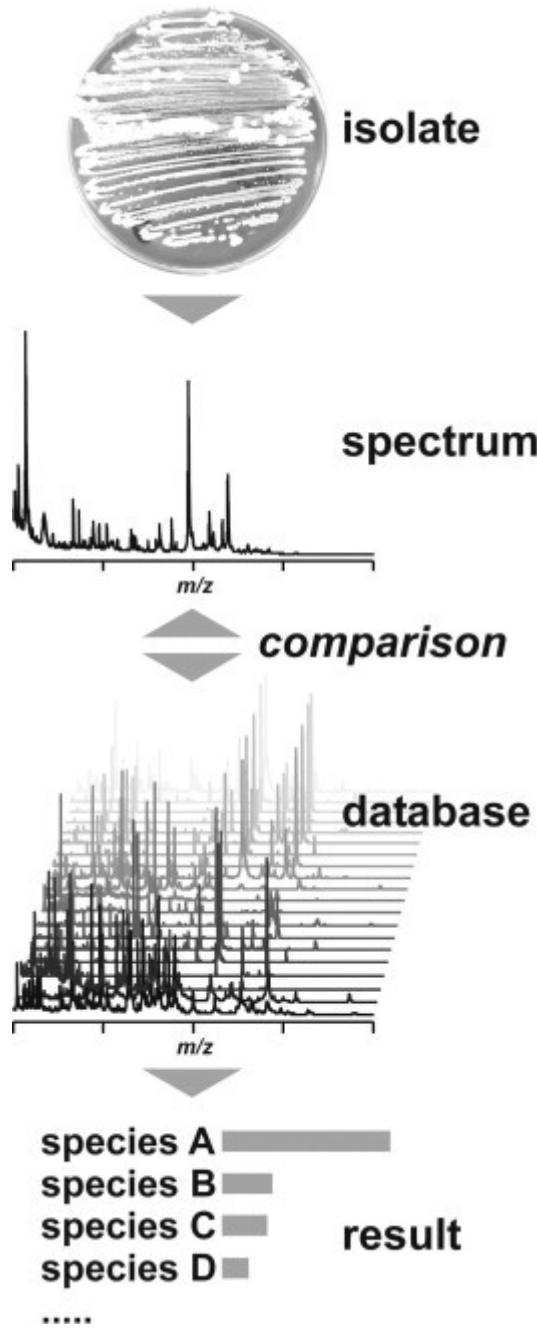
ProSightPC™ 3.0
Software for Precision Proteomics

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ProSight®PC is a trademark of Proteinaceous, Inc. in the United States and other countries.
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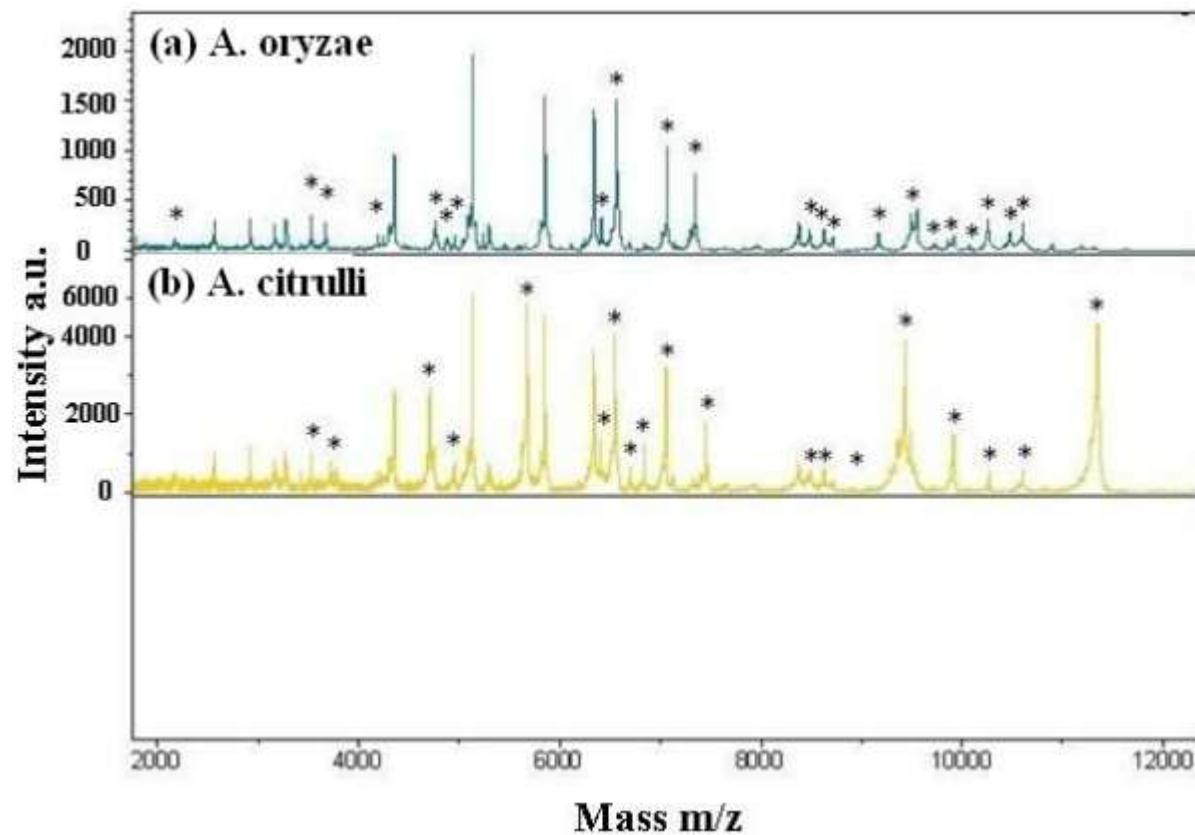
Thermo
SCIENTIFIC

Emerging MS technologies

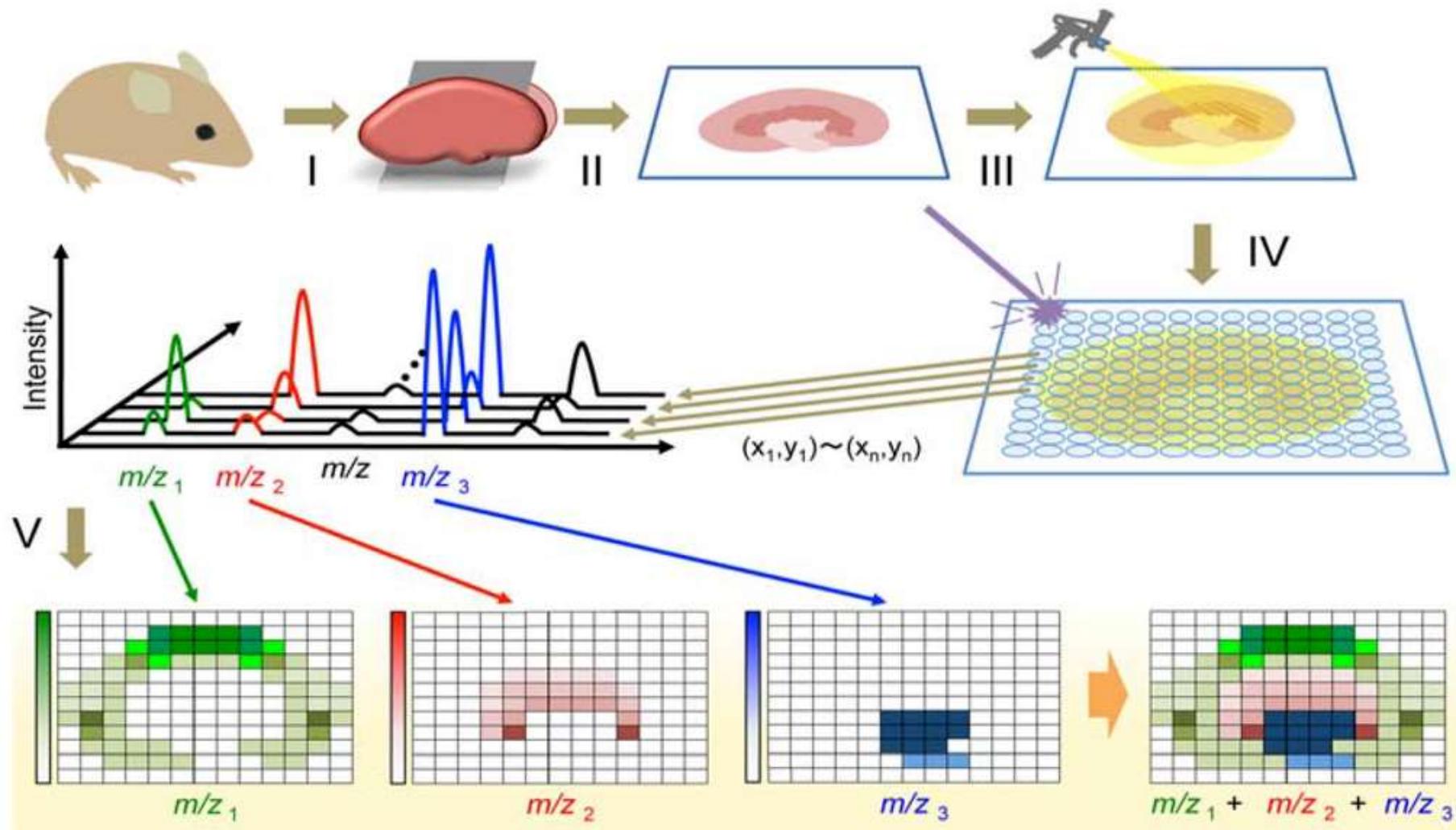




MS identification in Bacteriology (Biotype)



Mass spectrometry imaging



I Sacrifice and organ dissection

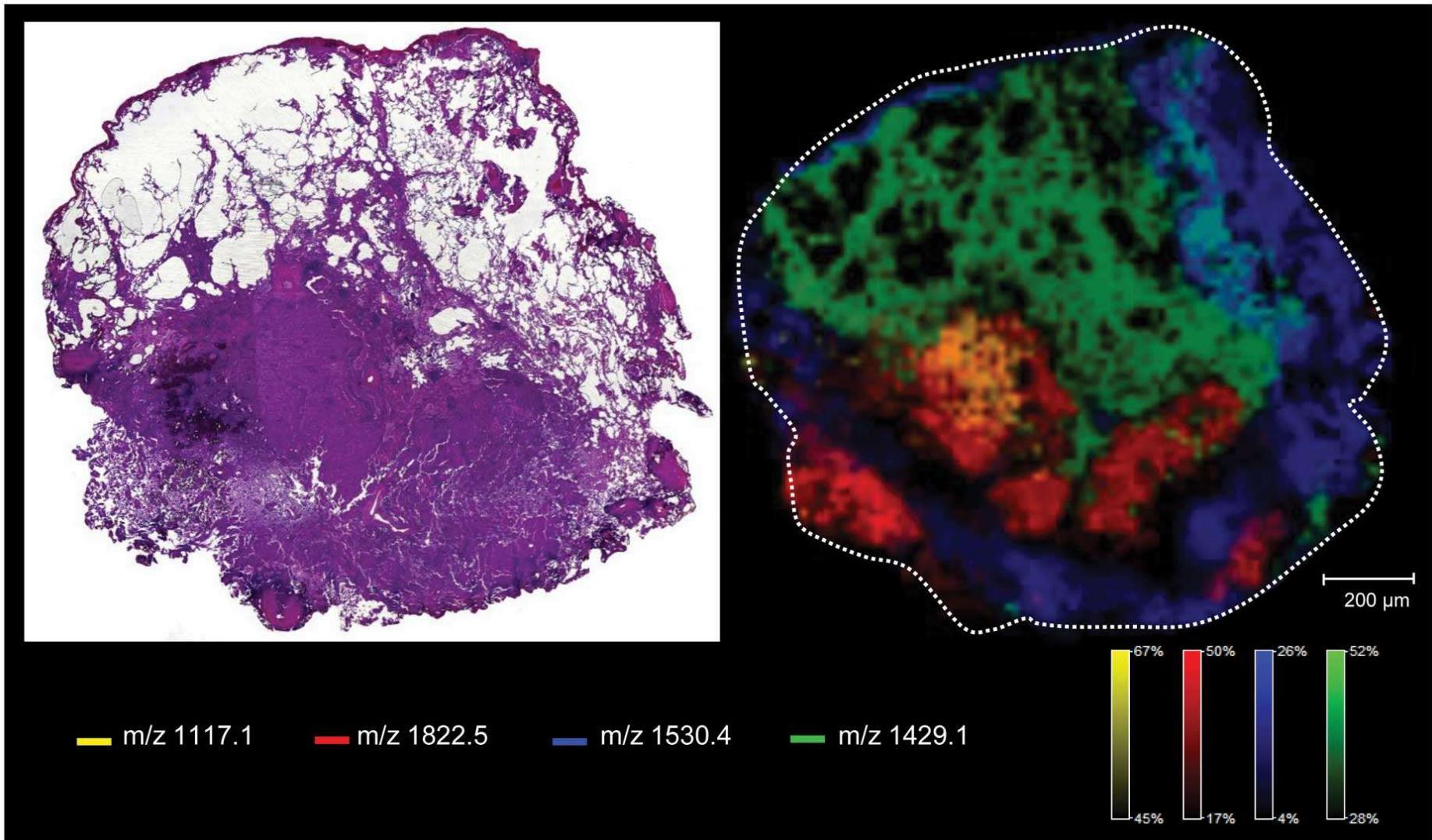
III Matrix deposition

V Reconstruction of intensity image

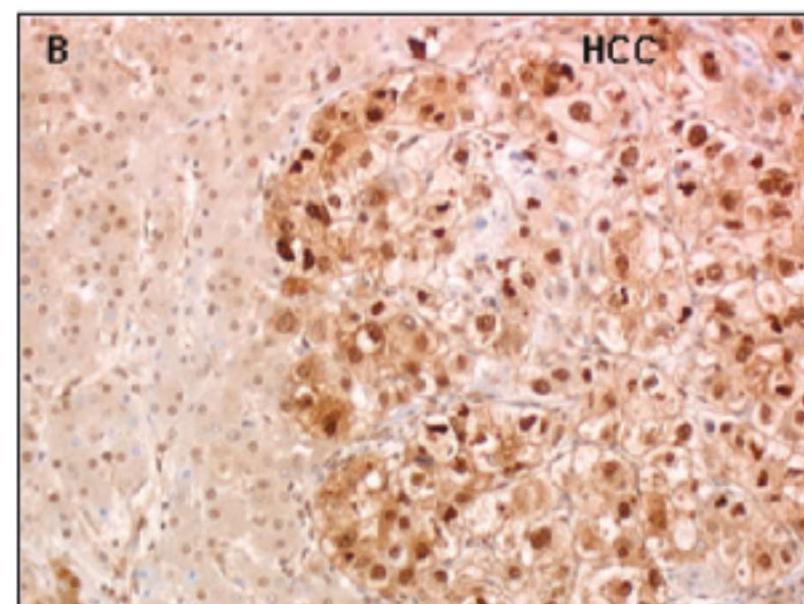
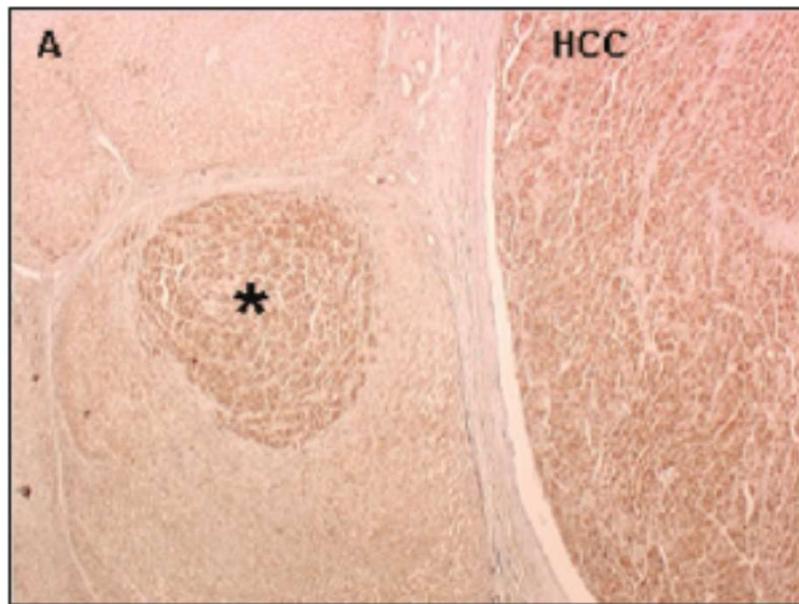
II Cryosectioning and moving to ITO glass slide

IV MALDI laser 2D scanning

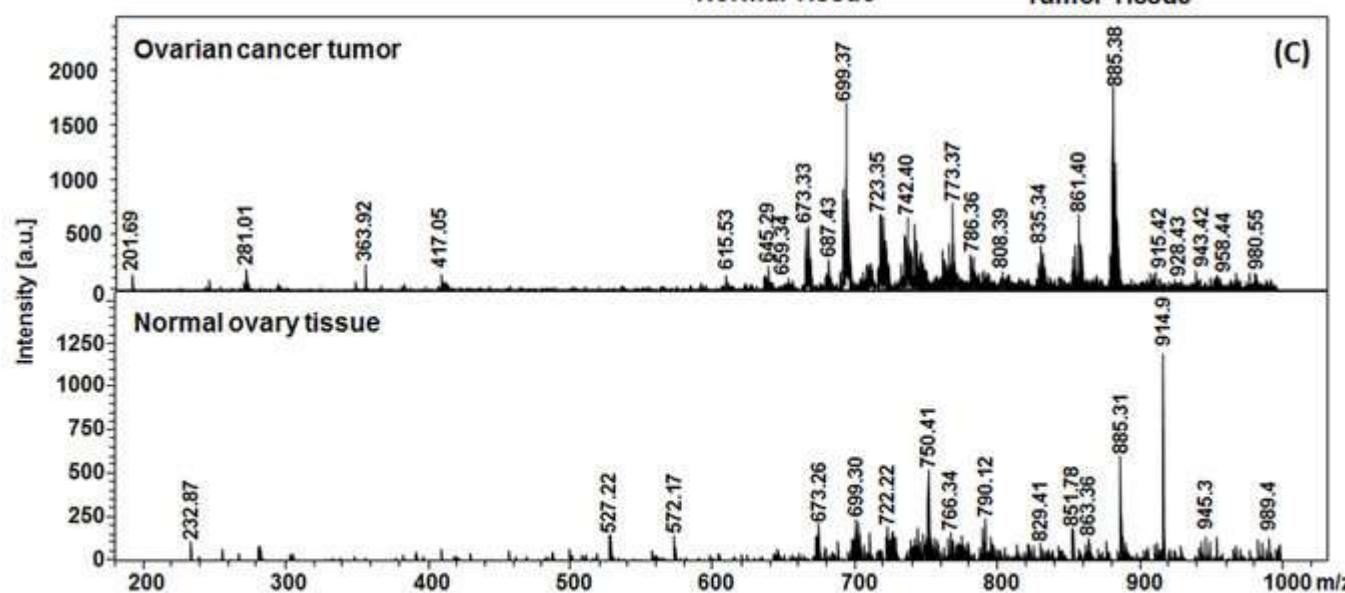
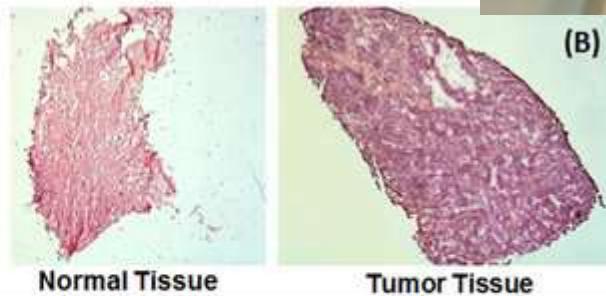
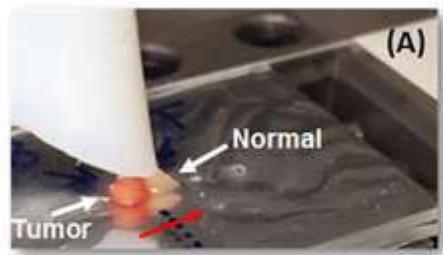
Mass spectrometry imaging



Immunohistochemical validation



SPIDERMASS





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PARIS 7

Plateforme de protéomique

/



Jean-Michel Camadro
DR CNRS, PhD
Responsable scientifique



Thibaut Léger
IE UP7, PhD
Responsable technique



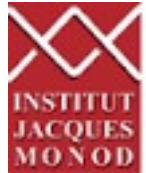
Camille Garcia
IE CNRS



Laetitia Collomb
CDD IE CNRS

Merci pour votre attention

<http://www.ijm.fr/plateformes/spectrometrie-de-masse>



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	Tarf A. Origine des crédits : université Paris Diderot, CNRS	Tarif B. Origine des crédits = autres académiques	Tari C. Origine des crédits : privé
Découpe de spots sur gel	0,50 €	0,50 €	0,50 €
Digestion	10,00 €	15,00 €	25,00 €
Dessalage	2,00 €	3,00 €	5,00 €
Dépôt sur cible Maldi	0,50 €	1,00 €	2,00 €
Pré-fractionnement nano-LC-Maldi	60,00 €	80,00 €	180,00 €
Analyse nanoESI-Orbitrap	120,00 €	180,00 €	280,00 €
Analyse nanoESI-Orbitrap + ETD	140,00 €	200,00 €	300,00 €
ESI : infusion manuelle (heure)	80,00 €	100,00 €	150,00 €
ESI : infusion automatisée (tip)	10,00 €	15,00 €	30,00 €
Maldi	5,00 €	10,00 €	30,00 €
Acquisition LC-Maldi	60,00 €	80,00 €	180,00 €
Retraitements des données	- €	- €	50,00 €
Destruction des échantillons	- €	3,00 €	10,00 €

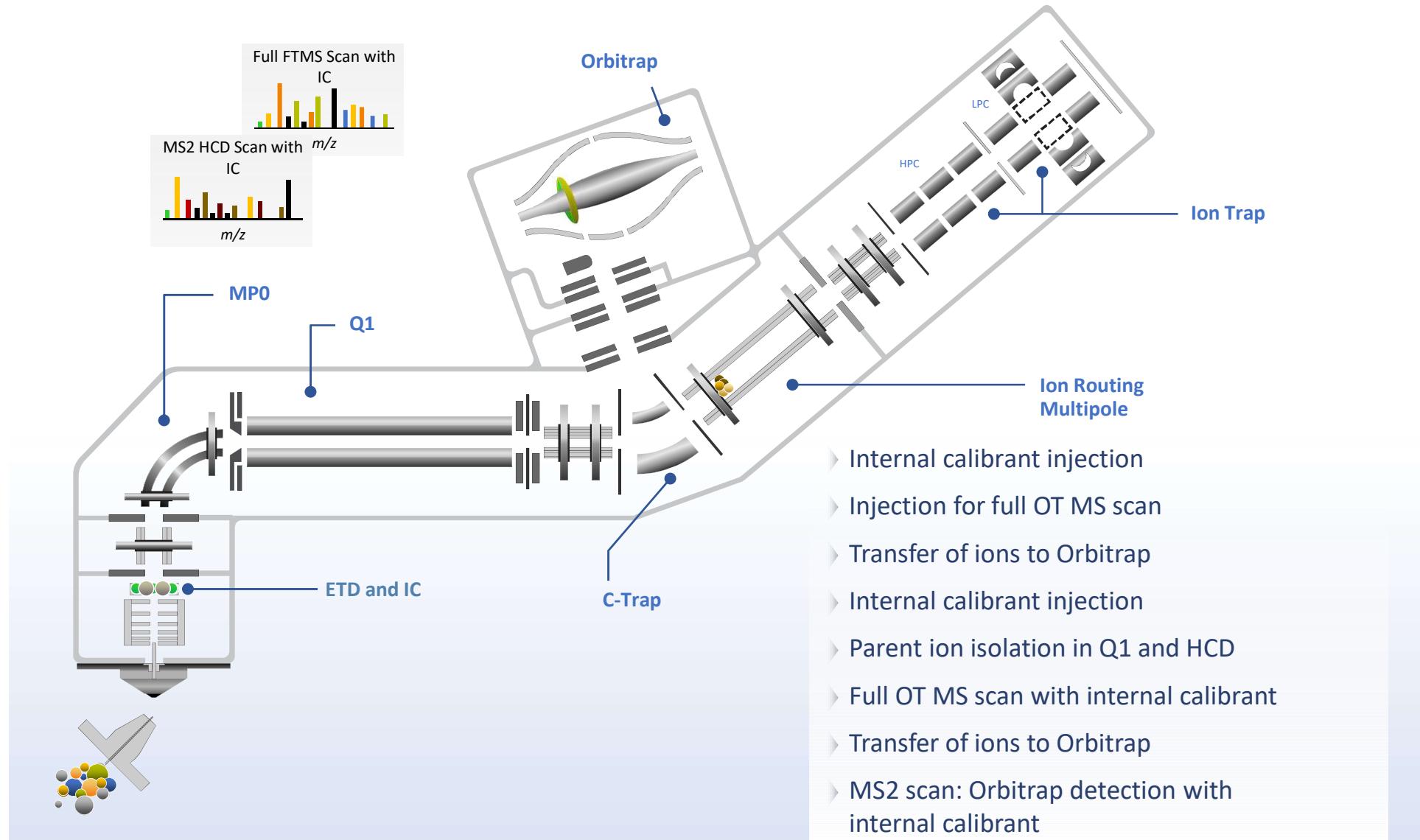
Valorisation

- Léger T., Garcia, C., Mathieu Videlier, M., and Camadro, J.M. Label-free quantitative proteomics in Yeast. –Methods Mol Biol. (2016).
- Léger T., Garcia, C., and Camadro, J.M. The metacaspase Mcalp restricts O-glycosylation in farnesol-induced apoptosis in Candida albicans. Mol Cell Proteomics 2016.
- Botebol H., Lesuisse E., Sutak R., Six C., Lozano JC., Schatt P., Vergé V., Kirillovsky A., Morrissey J., Léger T., Camadro J.M., Gueunegues A., Bowler C., Blain S., and Bouget F.Y. (2015) A central role for ferritin in the day/night regulation of iron homeostasis in marine phytoplankton – PNAS.
- Clabaut A, Grare C, Léger T, Hardouin P, Broux O. (2015). Variations of secretome profiles according to conditioned medium preparation: the example of human mesenchymal stem cell derived adipocytes – Electrophoresis
- Rouillon, J., Zocevic, A., Poupiot, J., Amor, F., Léger, T., Garcia, C., Camadro, J.M., Wong, B., Cosette, J., ML Coenen-Stass, A., McClorey, G., C Roberts, T., JA Wood, M., Servais, L., Voit, T., Richard, I., Svinartchouk, F. (2015). Serum proteomic profiling reveals specific MYOM3 fragments as biomarkers of Duchenne muscular dystrophy with applications for the follow-up of gene therapy treatment in a mouse model of muscular dystrophies. – Human Mol. Genetics.
- Tavernier, N., Bernasconi-Noatynska, A., Gotta, M., Schwager, F., Panbianco, C., Léger, T., Van Hove, L. and Pintard, L. (2015) Cdk1 phosphorylates SPAT-1/Bora to trigger PLK-1 activation and drive mitotic entry in early *C. elegans* embryos. – J Cell Biol.
- Floch AG, Tareste D, Fuchs P, Chadrin A, Naciri I, Léger T, Schlenstedt G, Palancade B, Doye V. (2014) Nuclear pore targeting of the yeast Pom33 nucleoporin depends on karyopherin- and lipid-binding. - J Cell Sci.
- Léger, T., Garcia, C., Ounissi, M., Lelandais, G. and Camadro, JM. (2015). The Metacaspase (Mca1p) has a Dual Role in Farnesol-induced Apoptosis in Candida albicans. - Mol Cell Proteomics.
- Richarme, G., Mihoub, M., Dairou, J., Linh Chi Bui, Léger, T., and Lamouri, A. (2014). Parkinsonism-associated protein DJ-1/Park7 is a major protein deglycase. – Journal of biological chemistry.
- S. Laouirem, J. Le Faouder, T. Alexandrov, D. Mestivier, T. Léger, X. Baudin, M. Mebarki, V. Paradis, J.M. Camadro, P. Bedossa. (2014) MALDI imaging mass spectrometry reveals novel ubiquitin modification as a biomarker of cirrhosis progressing to hepatocellular carcinoma Journal of Pathology.
- J. Rouillon; A. Zocevic; T. Léger; C. Garcia; JM. Camadro; B. Udd; L. Servais; T. Voit; F. Svinartchouk. (2014). Proteomics profiling of urine reveals specific titin fragments as biomarkers of Duchenne muscular dystrophy. Neuromuscular disorders.
- Le Faouder, J., Laouirem, S., Alexandrov, T., Ben-Harzallah, S., Léger, T., Albuquerque, M., Bedossa, P., and Paradis, V. (2014). Tumoral heterogeneity of hepatic cholangiocarcinomas revealed by MALDI imaging mass spectrometry. Proteomics.
- Rebours, V., Le Faouder, J., Laouirem, S., Mebarki, M., Albuquerque, M., Camadro, J.M., Léger, T., Ruszniewski, P., Levy, P., Paradis, V., et al. (2014). In situ proteomic analysis by MALDI imaging identifies ubiquitin and thymosin-beta4 as markers of malignant intraductal pancreatic mucinous neoplasms. Pancreatology
- Bretes, H., Rouviere, J.O., Léger, T., Oeffinger, M., Devaux, F., Doye, V., and Palancade, B. (2014). Sumoylation of the THO complex regulates the biogenesis of a subset of mRNPs. Nucleic acids research.

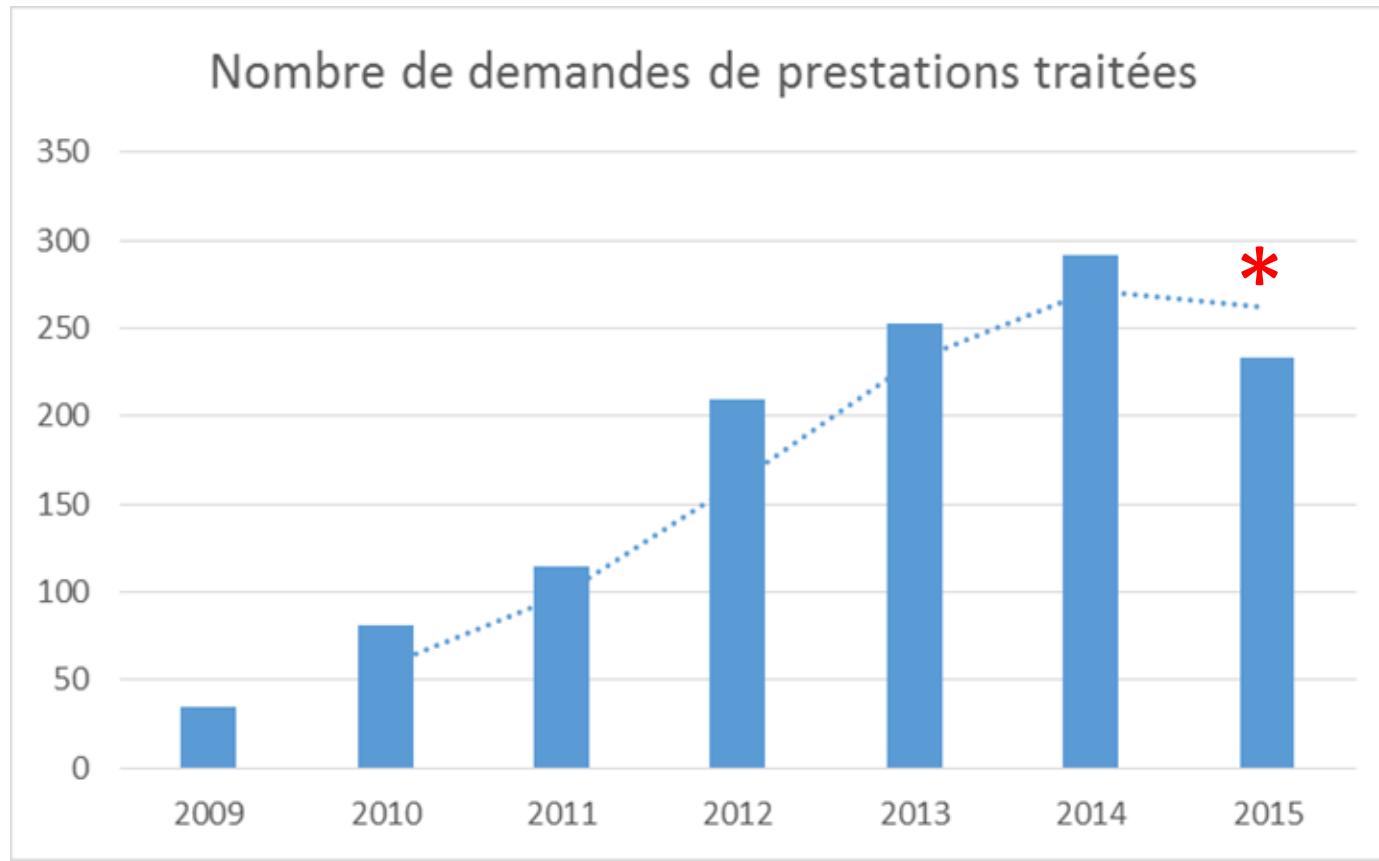
Congrès :



Génération des spectres MS et MS/MS

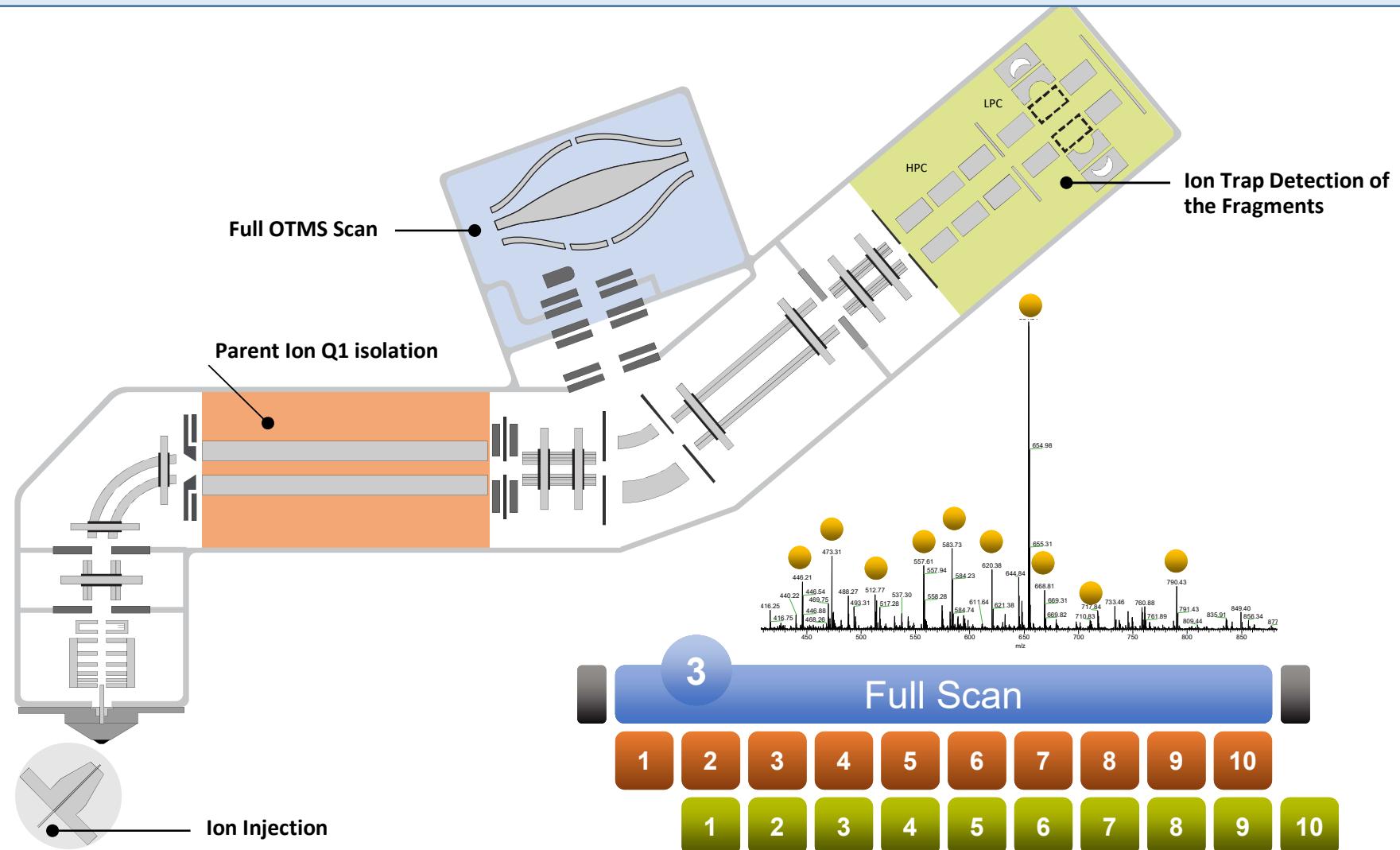


Activités de la plateforme

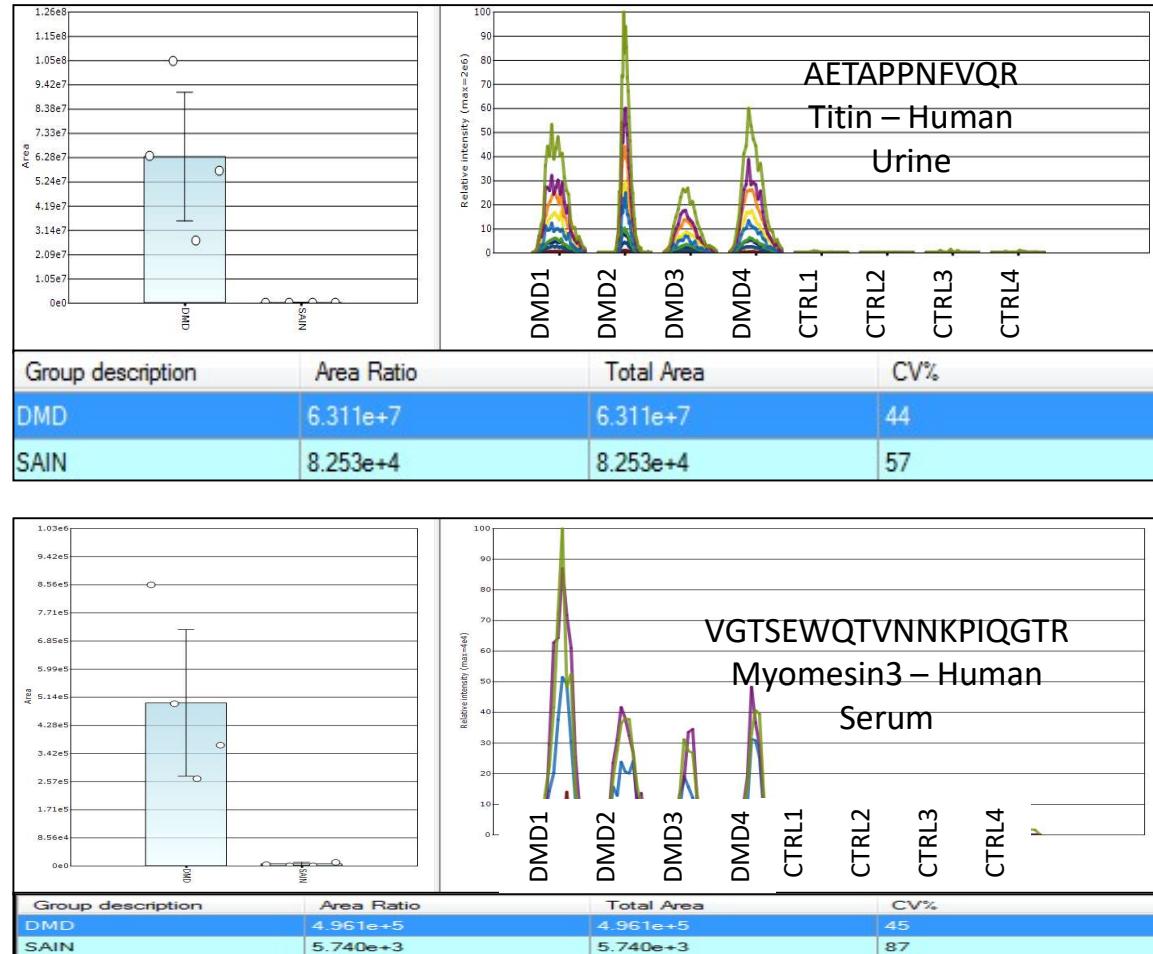
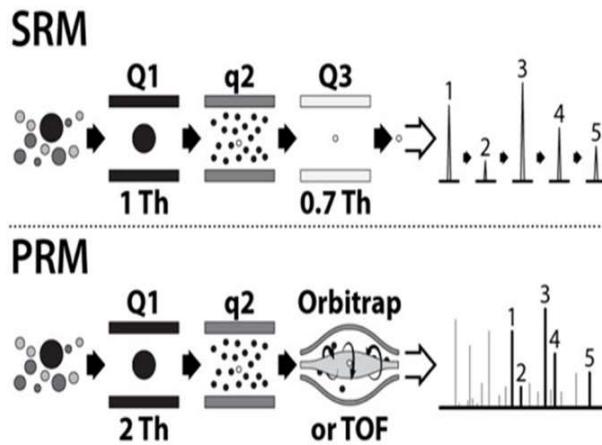


- * Chaque demande de prestation peut concerner de quelques à plusieurs dizaines d'échantillons.
- * L'évolution récente des demandes va vers de l'analyse de séries de plus en plus importantes (études de cohortes)

Ion Trafficking and Dynamic Scan Management



Protéomique ciblée de type PRM



C-trappe

