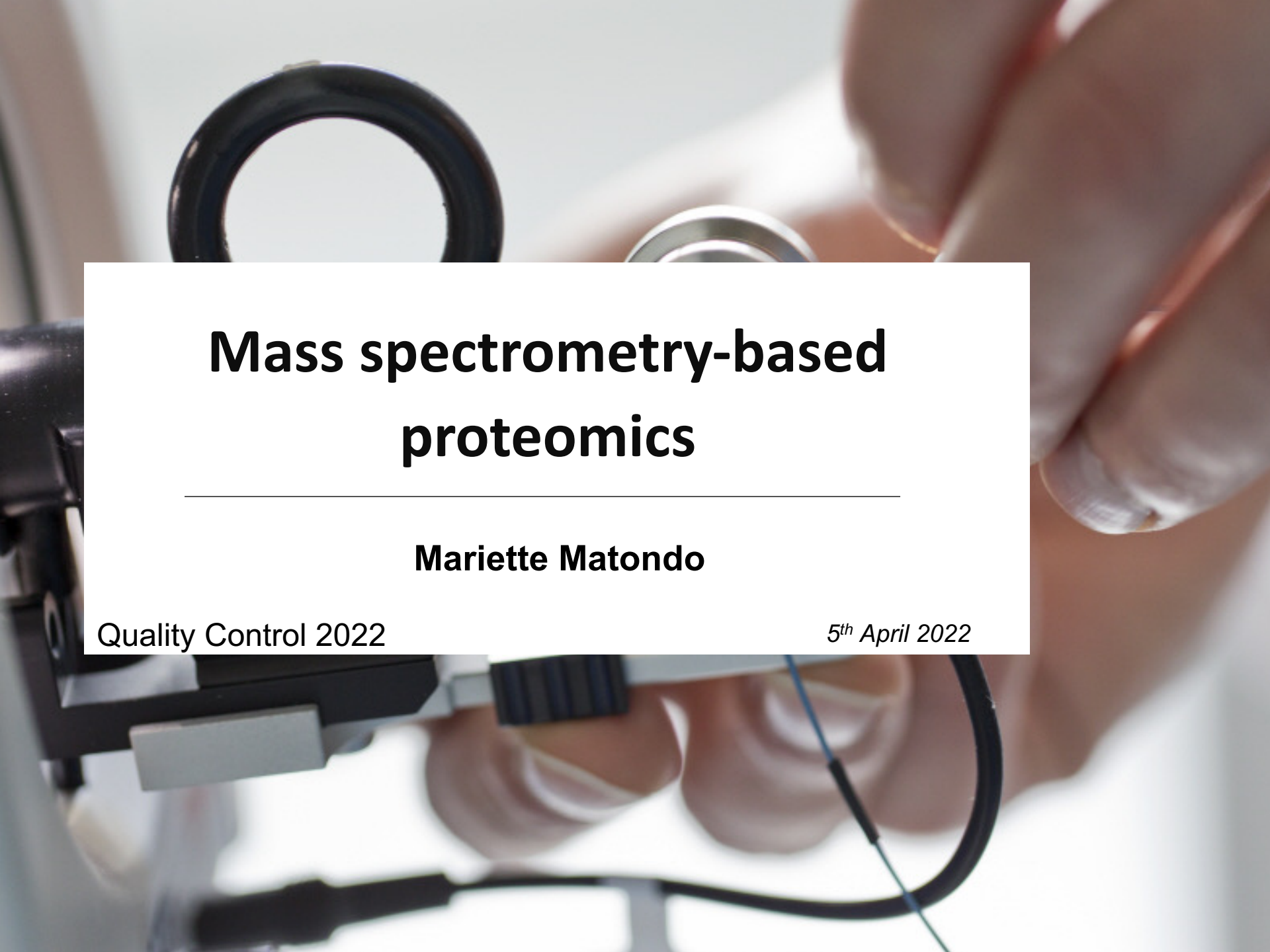


Mass spectrometry-based proteomics

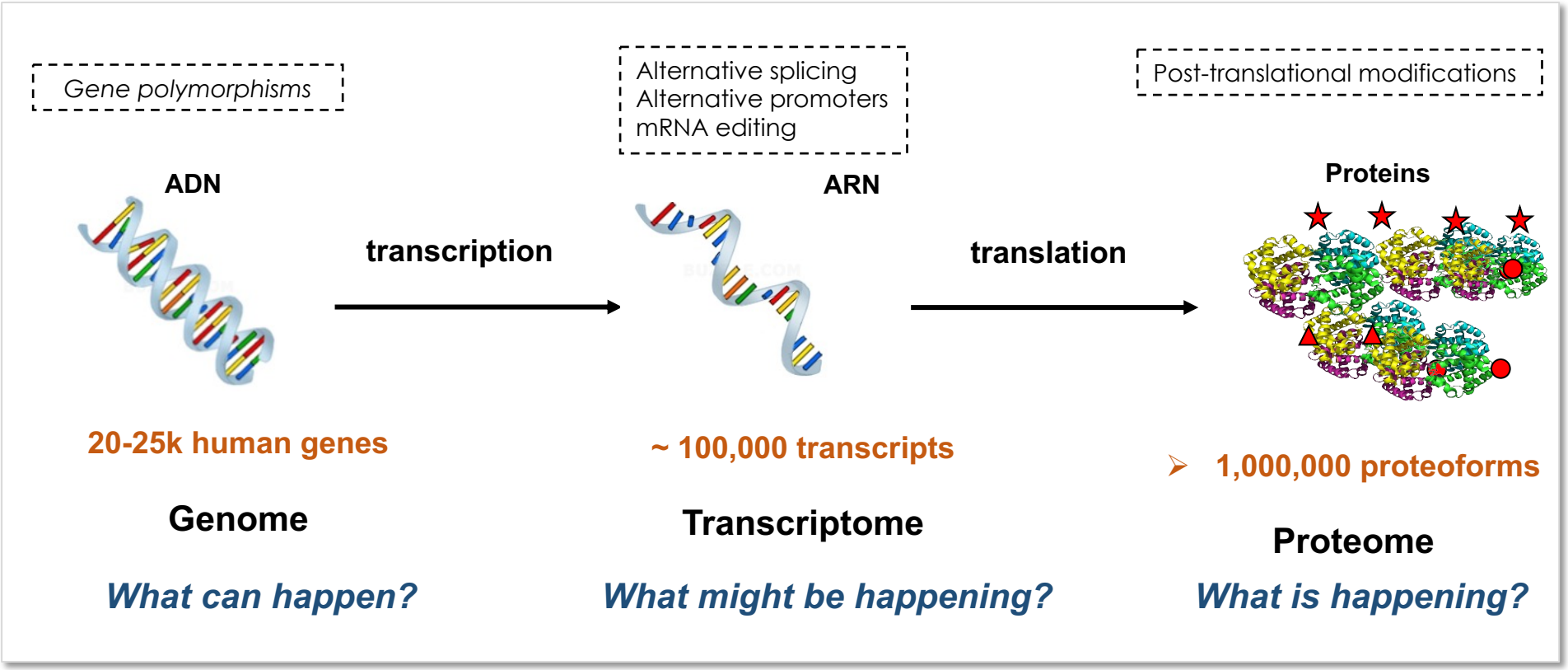
Mariette Matondo

Quality Control 2022

5th April 2022

Definition & Goals of Proteomics

- From **genomics, transcriptomics** to **proteomics**



What is a proteoform?

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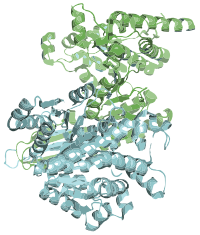
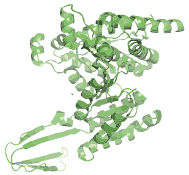
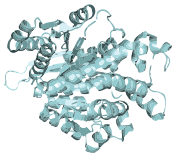
- A distinct molecular form of a protein product arising from a single gene

Cannonical Sequence (UniProtKB)

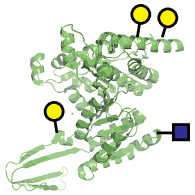


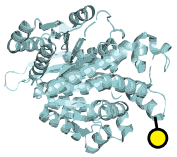
Endogenous proteolysis, mRNA splicing, Mutations, SNPs

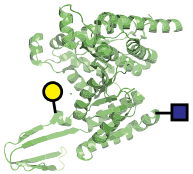


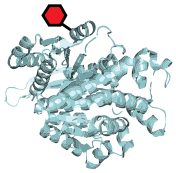


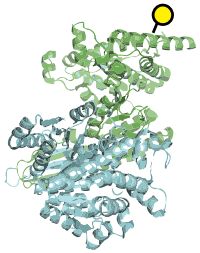
Site specific features:
Govern activity, localization, interactions, half-life

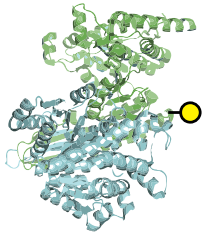












Smith, L.S., Kelleher, N.L., & The Consortium for Top-Down Proteomics, *Nat. Methods*, **2013**, *10*, 186–187.

Analyzing Protein Structure and Function

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Temporal interactions

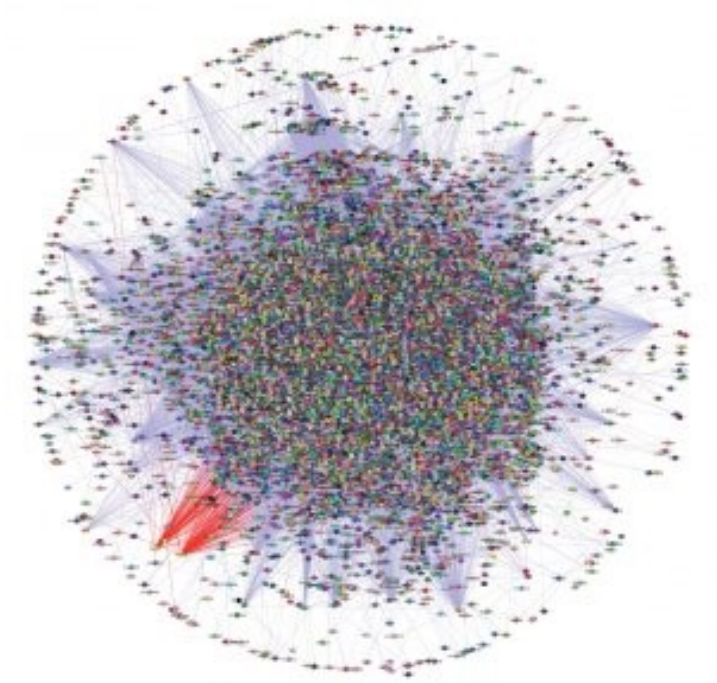
Cryo-electron microscopy
Cross-linking mass spectrometry
& Molecular Modeling

Copy numbers & Composition

Quantitative mass spectrometry
Quantitative immunoblotting

Atomic Structure

Cryo-electron microscopy
& Atomic modeling
X-ray crystallography
Nuclear magnetics resonance (NMR)



Protein interactions

Mass spectrometry
Cross-linking
Two-Hybrid System

Molecular architecture

Cryo-electron microscopy
& Atomic modeling
Saxs

Residue Positions

Mass spectrometry (HDX, LiPs, XL)

Complex shape and symmetry

SAXS
Cryo-electron microscopy

What does a mass spectrometer do?

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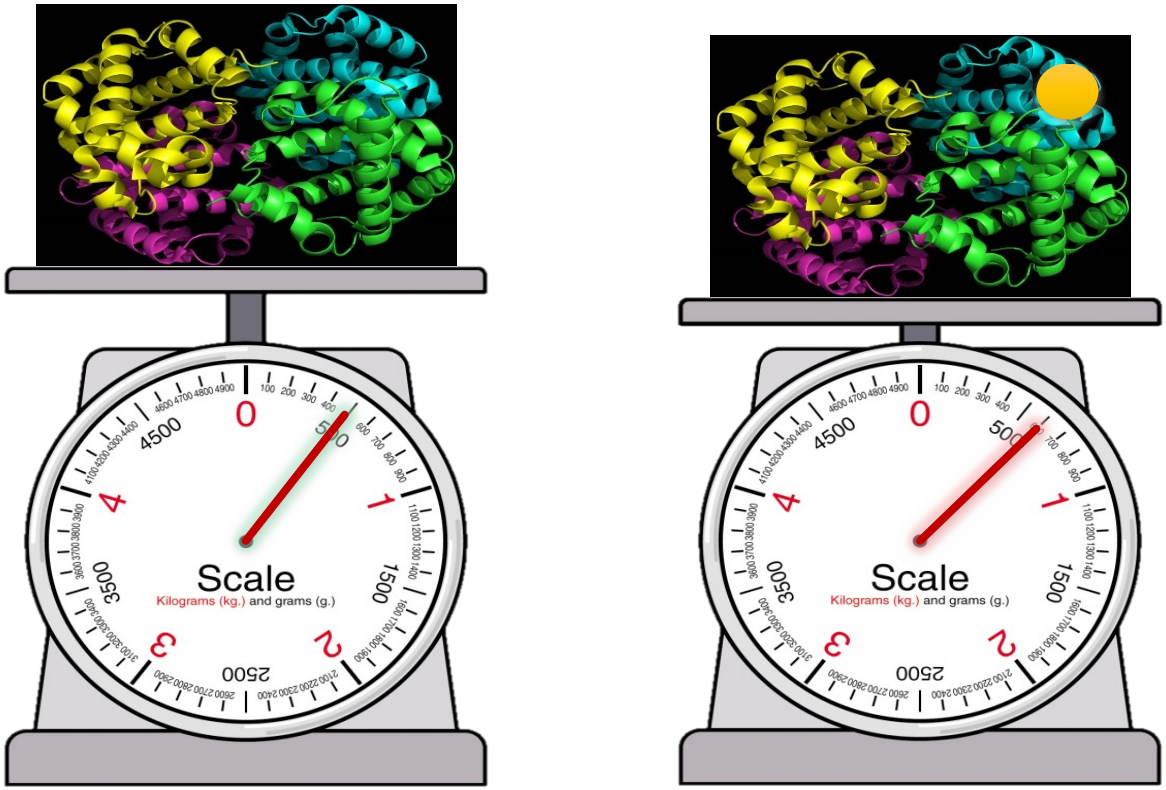
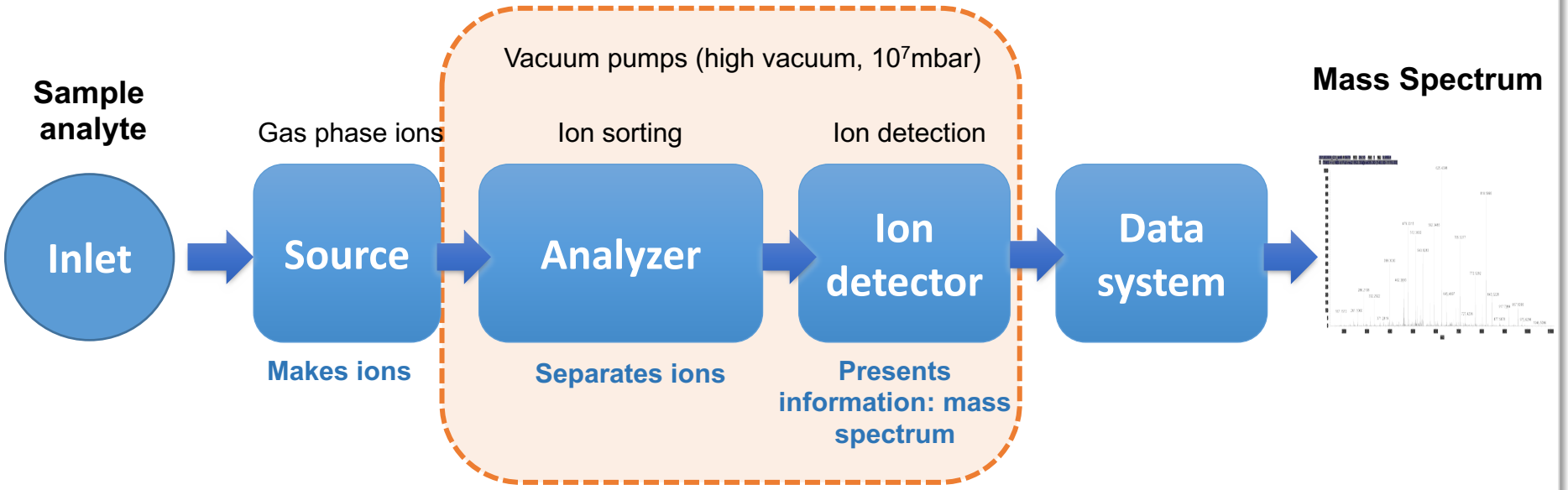
Measuring Intact Proteins (Simple idea!)



It measures masses with high accuracy.
It can give information about chemical structures

Mass Spectrometer

- A **mass spectrometer** is a device for measuring the **mass-to-charge ratio** of ionized molecules.



- **ESI**
- DESI
- **MALDI**
- APCI
- Etc …

- Quadrupole
- Time of Flight (ToF)
- Ion Trap (Linear, 3D)
- Orbitrap
- FT-ICR, Etc …

Multiple Fragmentation mode:
CID, HCD, ETD, ECD, EThCD, UVPD, IRMP,…

Ionisation techniques

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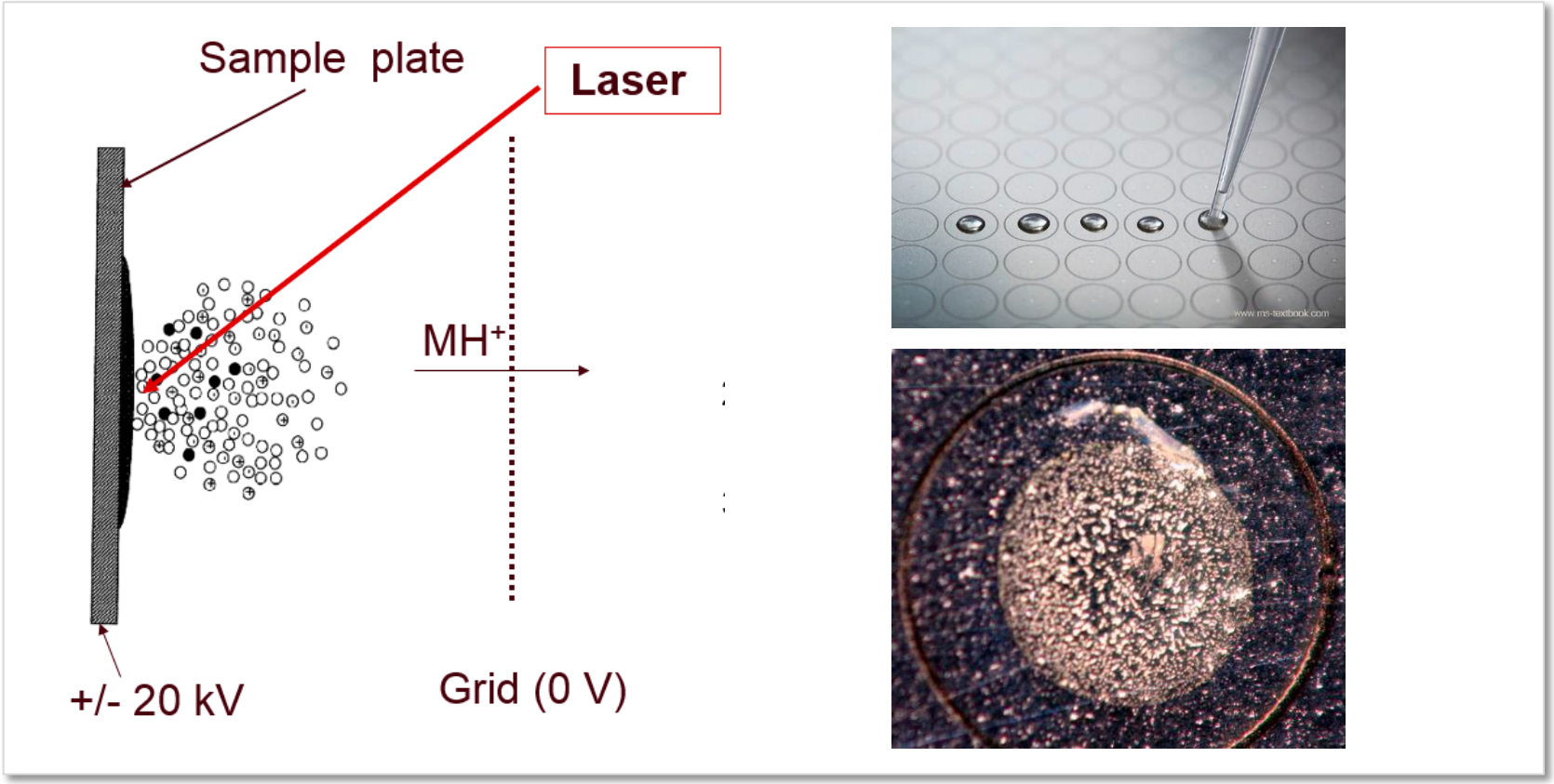
- **For biomolecules** (thermolabile): MALDI or electrospray

MALDI (Matrix Assisted Laser Desorption Ionisation)

The sample is mixed with a matrix , dried on plate and MH+ ions are formed after laser shot



MALDI ionization is appropriate for imaging

Ionisation techniques

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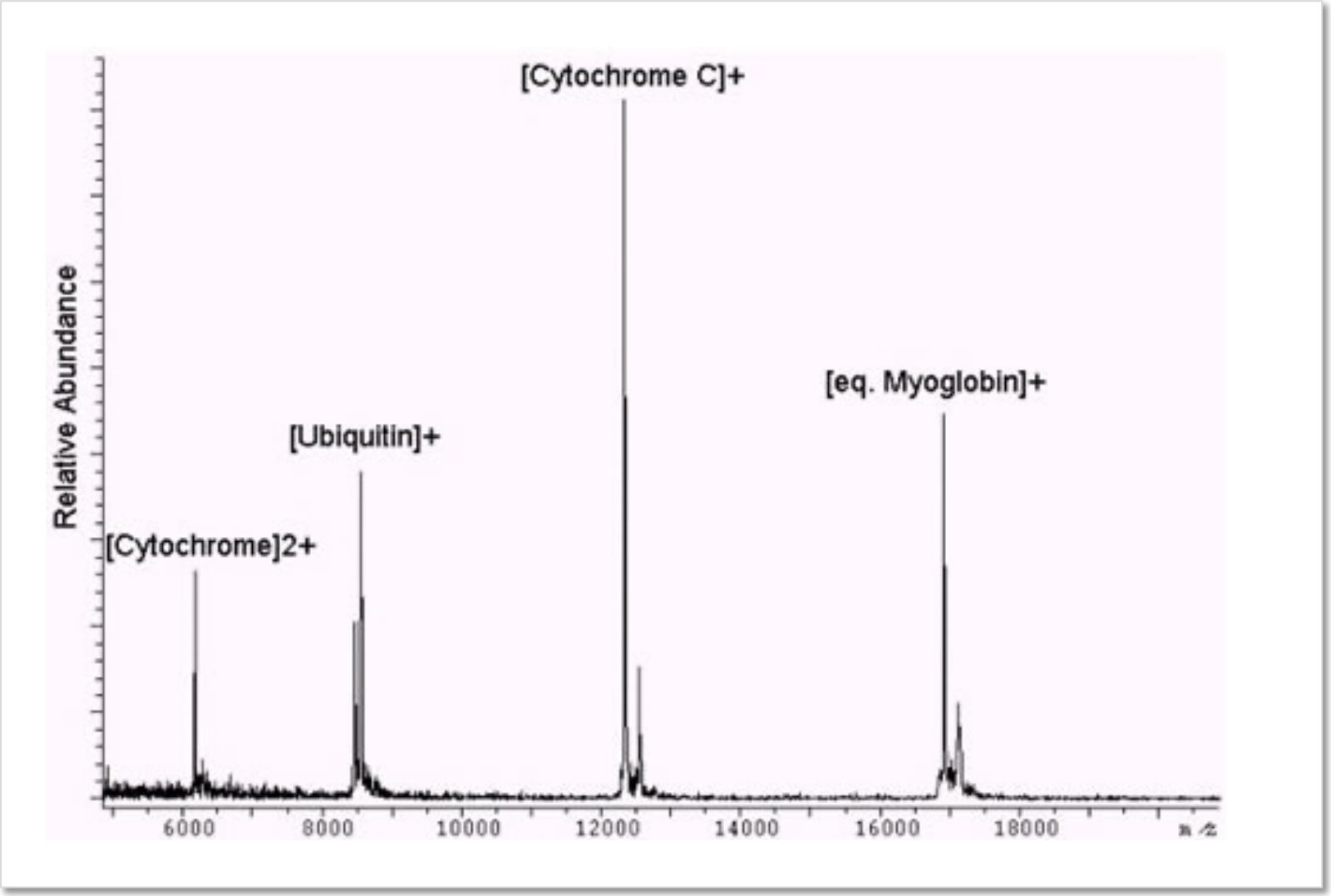
- **Example of MALDI-TOF spectrum**

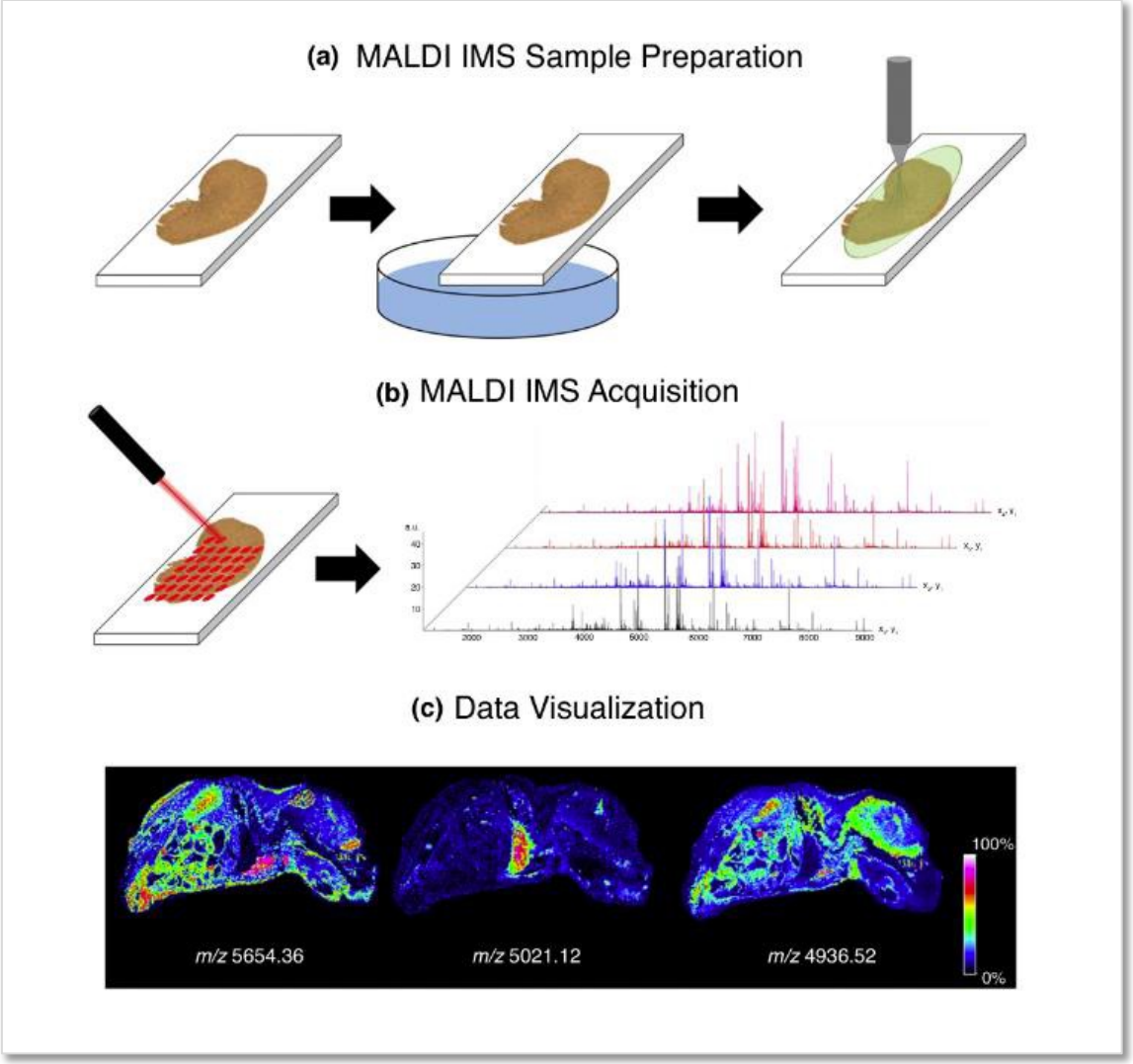


No protein identification using only molecular weight (MW)

TOF: Time Of Flight

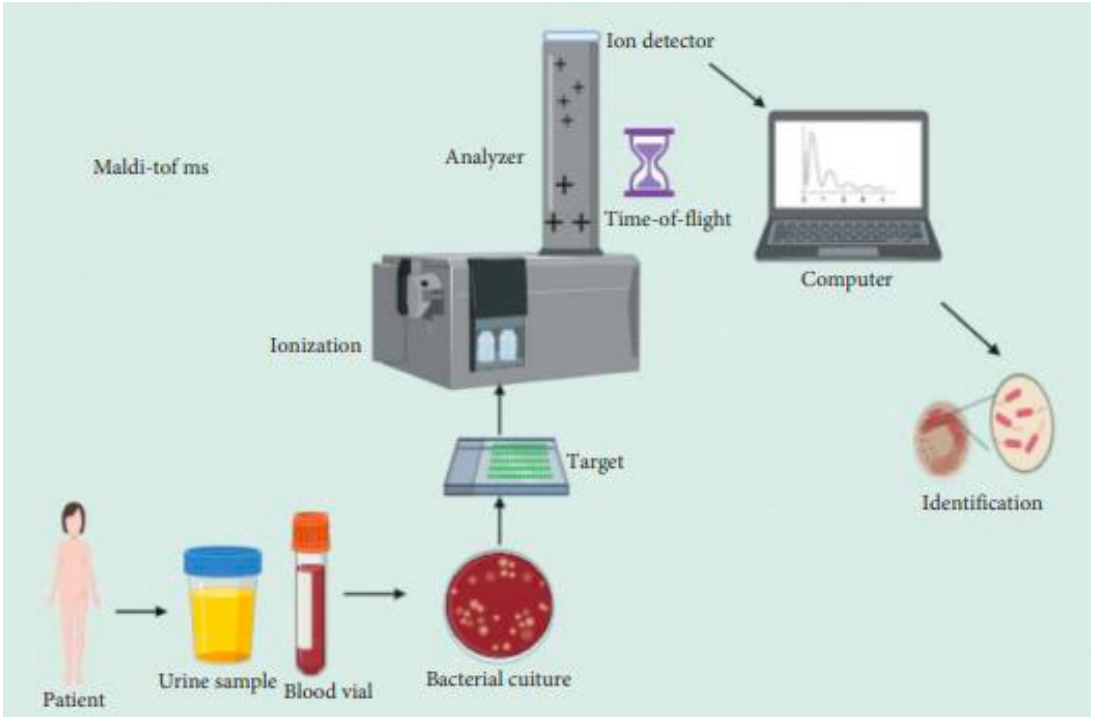
Major application of MALDI: Imaging

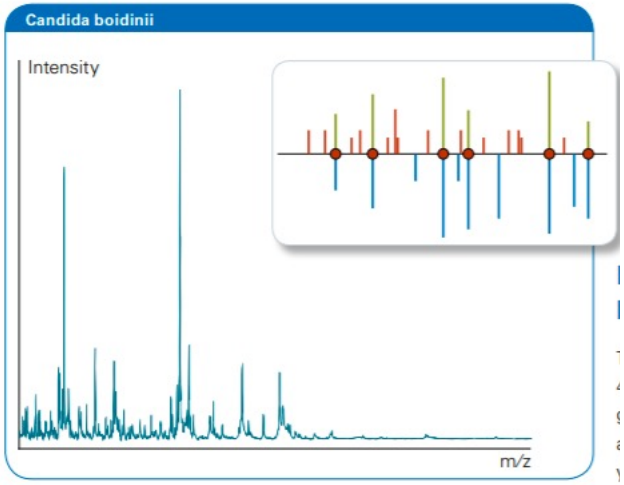
- Tissue sample is sectioned, washed to remove interfering salts and lipids, and coated homogenously with a MALDI matrix.
- The sample is loaded into the instrument and the section is irradiated by a laser, moving a defined lateral distance which dictates the spatial resolution of the image.
- A mass spectrum is generated at each pixel location.
- Ion intensities for a selected mass range are then plotted in a coordinate system within the sampled tissue area, creating an ion image.



*From, D. J. Ryan, et al., Current Opinion in Chemical Biology, **2019**(48), 64-72.*

Major application of MALDI: discrimination of microorganisms





MALDI-TOF MS spectrum

- Bacterial or fungal growth is isolated from plated culture media and applied directly onto the MALDI plate.
- Samples are then overlaid with matrix and dried.
- MALDI-TOF MS spectrum is matched against a reference library to lead to an identification.

its concentration dependant

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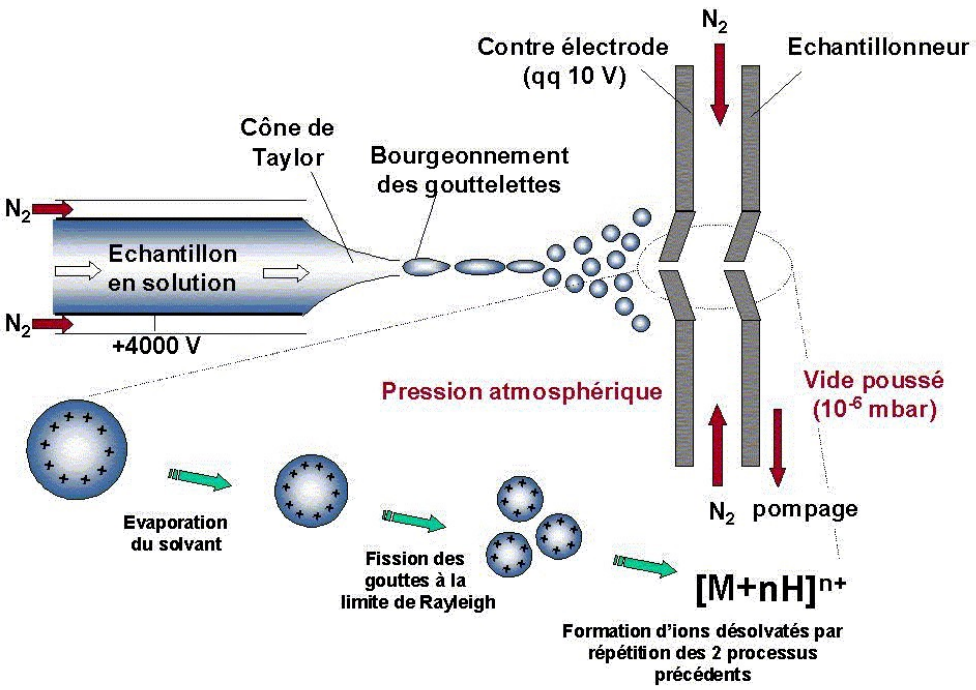
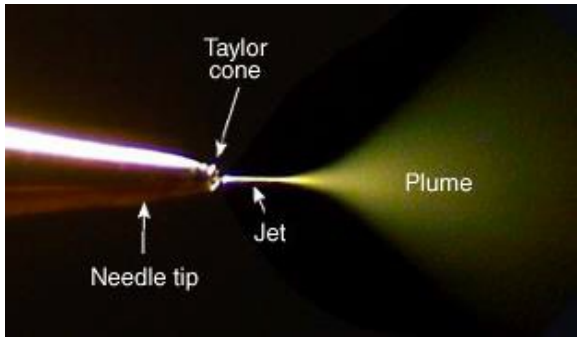
- **For biomolecules** (thermolabile): MALDI or electrospray

Electrospray (ESI)

- Ions are preformed in solution and multiply charged ions$(M+nH+)^{n+}$ are formed after droplet desolvation
- ESI can be direcly coupled to liquid chromatography (LC)





Nobel Prize in Chemistry (2002)

Institut Pasteur

John FENN

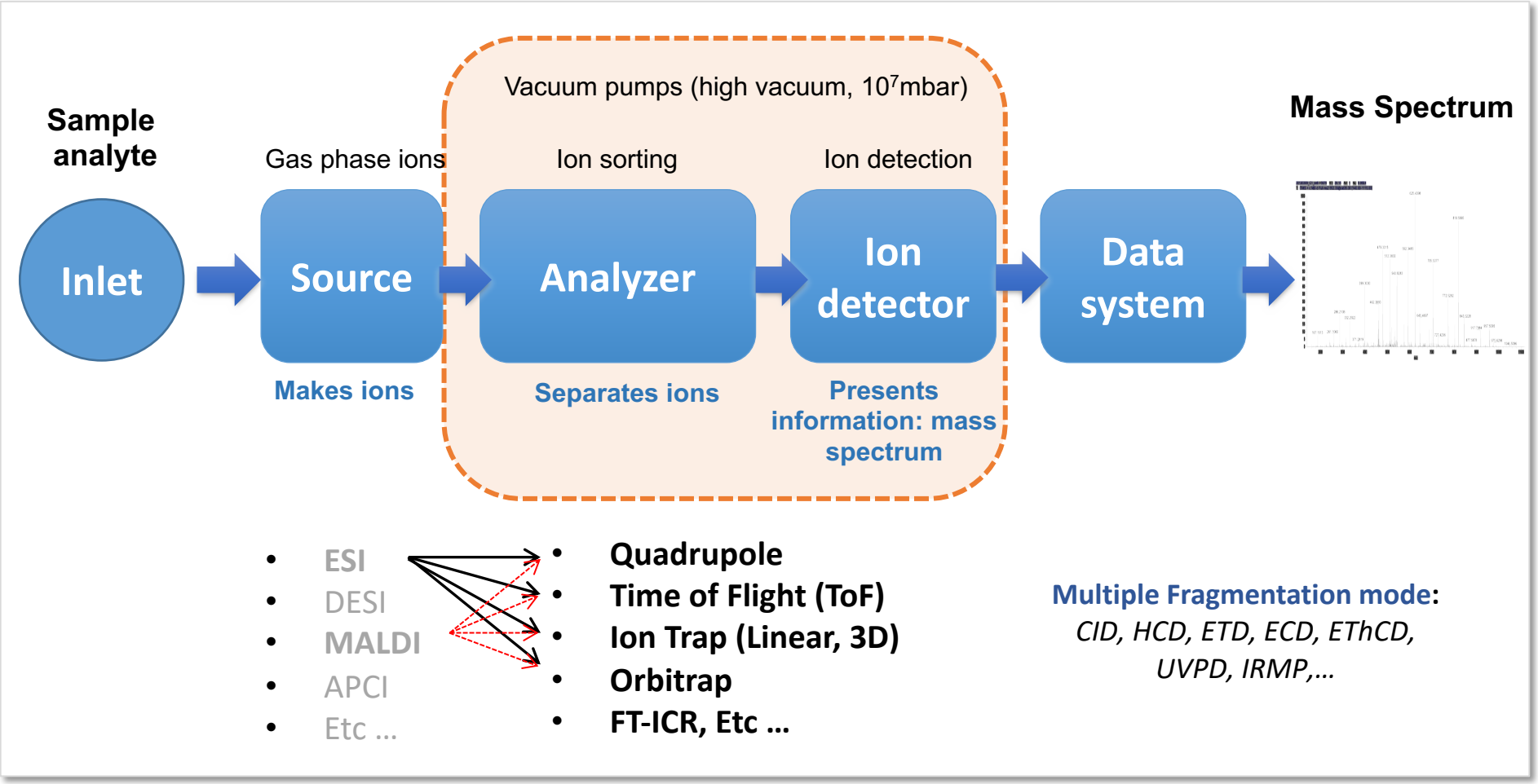
Electrospray

Koichi TANAKA

MALDI

Mass Spectrometer

- A **mass spectrometer** is a device for measuring the **mass-to-charge ratio** of ionized molecules.

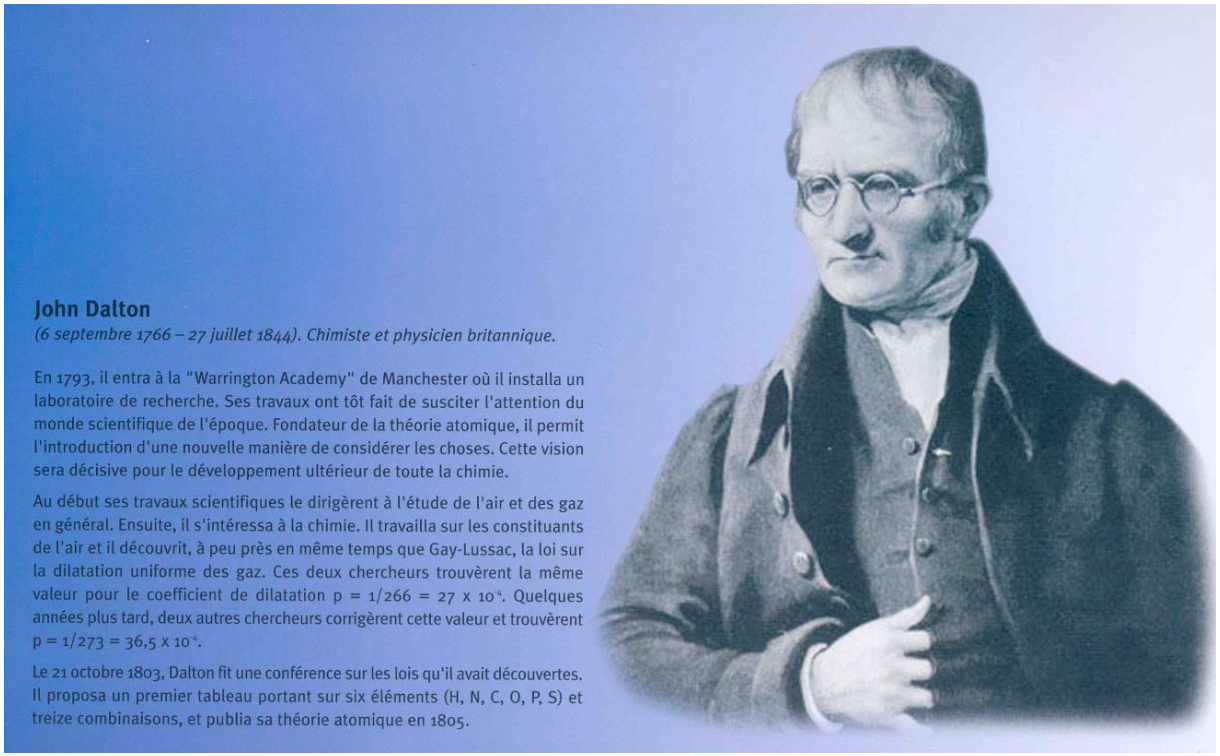


What happens in the mass analyzer ?

- It operates under **high vacuum** to keep ions from bumping into gas molecules
- **Ions are separated** according to their **mass-over-charge (m/z) ratio**

Measured in **Dalton (Da)**

1Da = one twelfth of the mass of a neutral atom of carbon-12



John Dalton

(6 septembre 1766 – 27 juillet 1844). Chimiste et physicien britannique.

En 1793, il entra à la "Warrington Academy" de Manchester où il installa un laboratoire de recherche. Ses travaux ont tôt fait de susciter l'attention du monde scientifique de l'époque. Fondateur de la théorie atomique, il permit l'introduction d'une nouvelle manière de considérer les choses. Cette vision sera décisive pour le développement ultérieur de toute la chimie.

Au début ses travaux scientifiques le dirigèrent à l'étude de l'air et des gaz en général. Ensuite, il s'intéressa à la chimie. Il travailla sur les constituants de l'air et il découvrit, à peu près en même temps que Gay-Lussac, la loi sur la dilatation uniforme des gaz. Ces deux chercheurs trouvèrent la même valeur pour le coefficient de dilatation $p = 1/266 = 27 \times 10^{-4}$. Quelques années plus tard, deux autres chercheurs corrigèrent cette valeur et trouvèrent $p = 1/273 = 36{,}5 \times 10^{-4}$.

Le 21 octobre 1803, Dalton fit une conférence sur les lois qu'il avait découvertes. Il proposa un premier tableau portant sur six éléments (H, N, C, O, P, S) et treize combinaisons, et publia sa théorie atomique en 1805.

What happens in the mass analyzer ?

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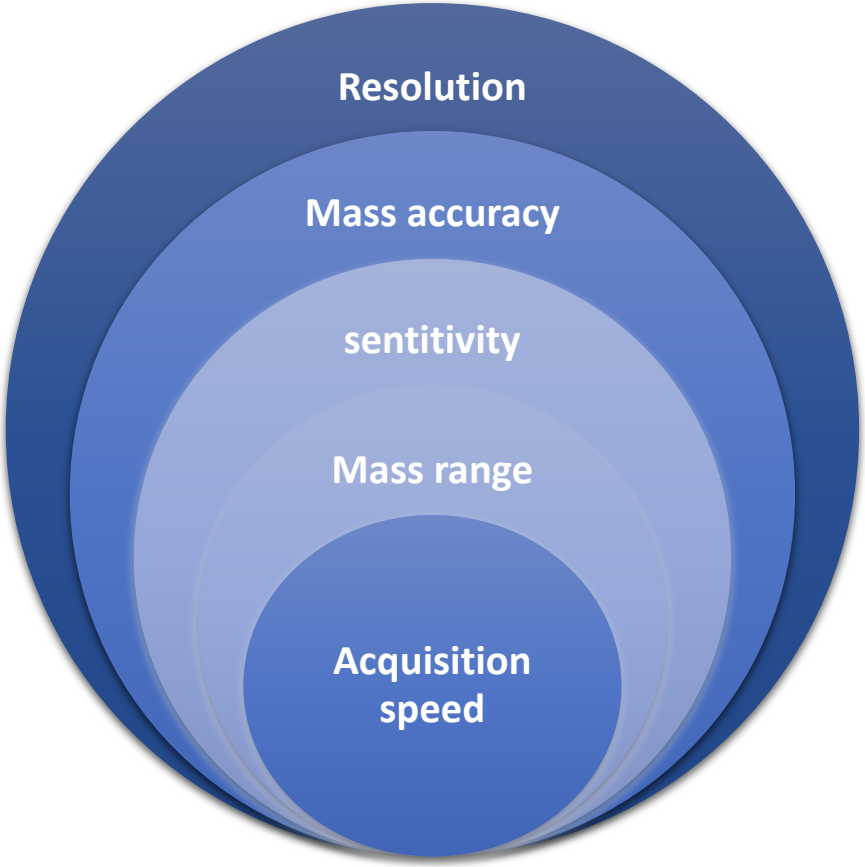
Key specifications of the analyzer are :

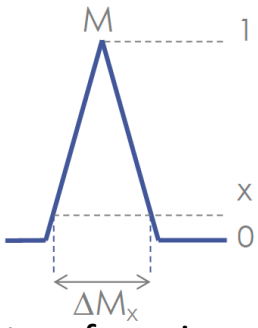


What happens in the mass analyzer ?

Key specifications of the analyzer are :

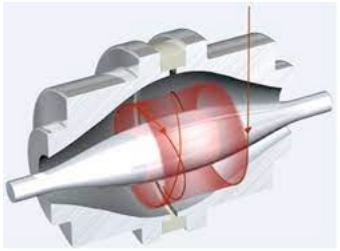
Resolution

$$R = \frac{M}{\Delta M_x}$$



ΔM_x : width of the peak measured at a specific fraction of the peak height

As important as for microscopy or structural biology, describes the ability of the instrument to resolve neighboring peaks



Example:
Orbitrap analyzer can have up to 1,000,000 FWHM **ultra-high resolution**

Mass accuracy

- Accuracy (ppm) = $10^6 \times \frac{M_{meas} - M_{theo}}{M_{theo}}$

Difference between **measured mass** and **calculated exact mass**, linked to resolution. Generally given as relative mass accuracy in parts per million (**ppm)**

Example:
Measured mass (M_{meas}) : 1000,001
Calculated exact mass (M_{theo}) : 1000,000
Difference: 0,001
Relative mass accuracy/mass error:
0,001/1000 = 1.10-6= 1 ppm

What happens in the mass analyzer ?

Institut Pasteur

Key specifications of the analyzer are :

sensitivity

Amount needed to see signal

1 mole	6.10^{23} molécules
1 millimole	6.10^{20} molécules
1 micromole	6.10^{17} molécules
1 nanomole	6.10^{14} molécules
1 picomole	6.10^{11} molécules
1 femtomole	6.10^{8} molécules
1 attomole	6.10^{5} molécules
1 zeptomole	6.10^{2} molécules

Avogadro number

$N = 6,022045 . 10^{23}$

amol(10^{-18}) sensitivity, 1 attomole equals ≈ 600 000 molecules

Performance of the analyser

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- A broad range of analyzers with different performances and cost
- Trend: higher resolution (R) and accuracy at higher speed

	Analyzer	Resolution	Accuracy	Mass Range
	Quadrupole	10^2 - 10^3	100 ppm	10^3
	Ion Trap	10^2 - 10^3	50 - 100 ppm	10^3
	ToF	10^3 - 10^4	5 - 50 ppm	> 10^5
FT-MS*	Orbitrap	10^5 - 5.10^5	2 - 5 ppm	4 000 (can be extended)
FT-MS*	FT-ICR	10^4 - 10^6	0.2 – 5 ppm	10^4

* FT-MS: Fourier Transform Mass Spectrometry

Importance of Isotopes

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- Most of the elements exist in nature as a mixture of isotopes
- Two atoms are called **isotopes** if they have the same number **of protons** but different number of **neutrons**

An element is defined by :

$$^{A}E_{Z}$$

– Atomic number Z (number of protons)
–Mass number A (number of protons + neutrons)

Isotopes: atoms of same atomic number (Z) but with different mass numbers (A)

Example: $^{12}C_6$ and $^{13}C_6$

$^{35}Cl_{17}$ and $^{37}Cl_{17}$

$^{79}Br_{35}$ and $^{81}Br_{35}$

Importance of Isotopes

Institut Pasteur

Element	isotope	%	Isotopic mass	isotope	%	Isotopic mass	isotope	%	Isotopic mass	Average mass
C	^{12}C	98.89	12.0000	^{13}C	1.1	13.0033				12.011
H	^{1}H	99.97	1.0078	^{2}H	0.015	2.0140				1.0079
N	^{14}N	99.64	14.0031	^{15}N	0.37	15.0001				14.0067
O	^{16}O	99.76	15.9949	^{17}O	0.04	16.9991	^{18}O	0.20	17.9992	15.9994
S	^{32}S	94.76	31.9721	^{33}S	0.789	32.9715	^{34}S	4.44	33.9679	32.066
F	^{19}F	100	18.9984							
Cl	^{35}Cl	75.76	34.9688				^{37}Cl	24.24	36.9659	35.453
Br	^{79}Br	50.69	78.9183				^{81}Br	49.31	80.9163	79.904

Importance of Isotopes

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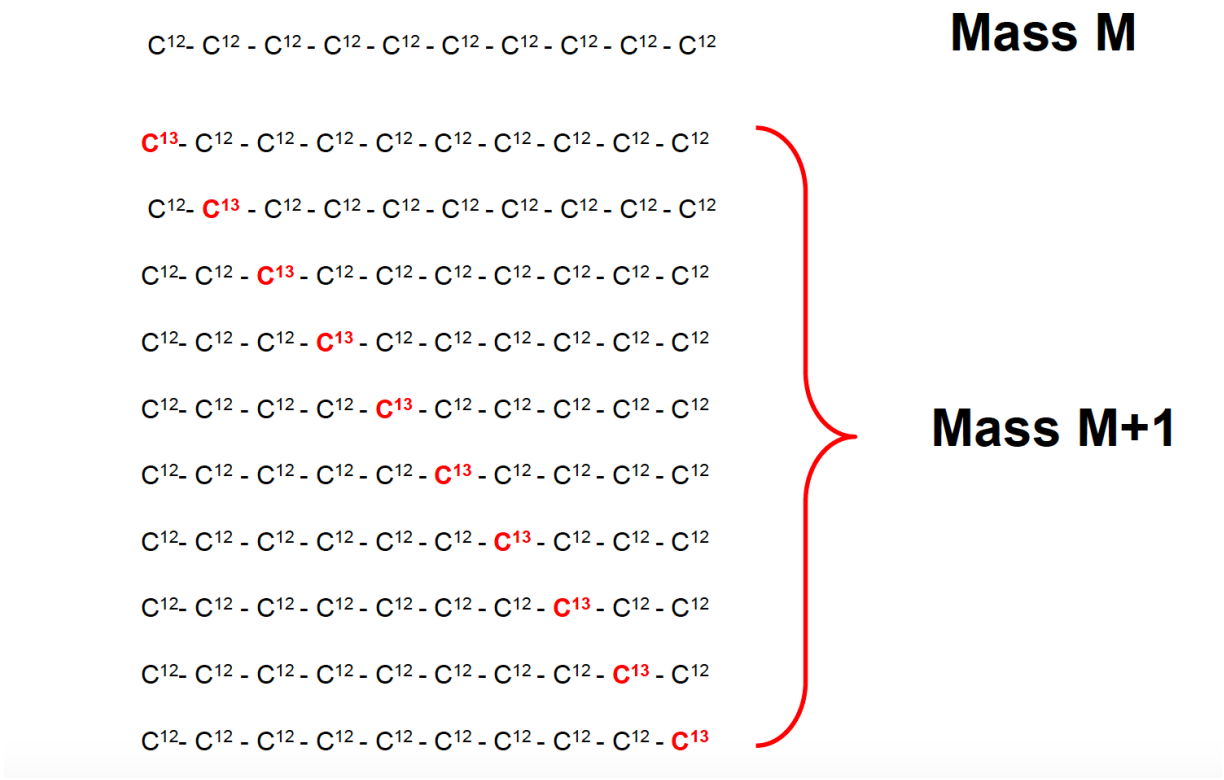
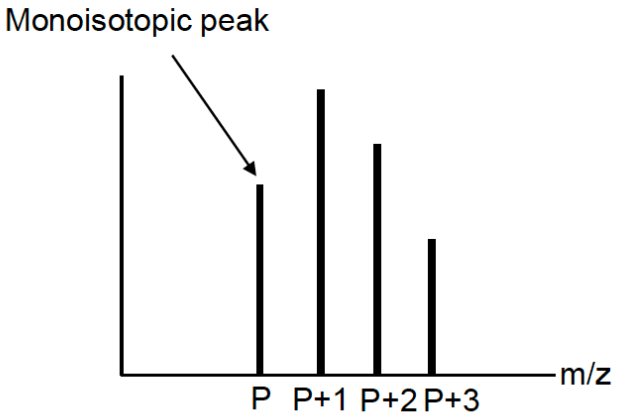
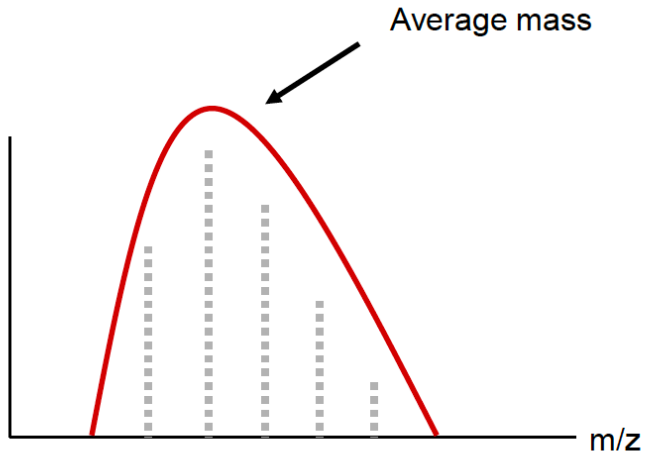


Effect of the presence of isotopes on MS detection





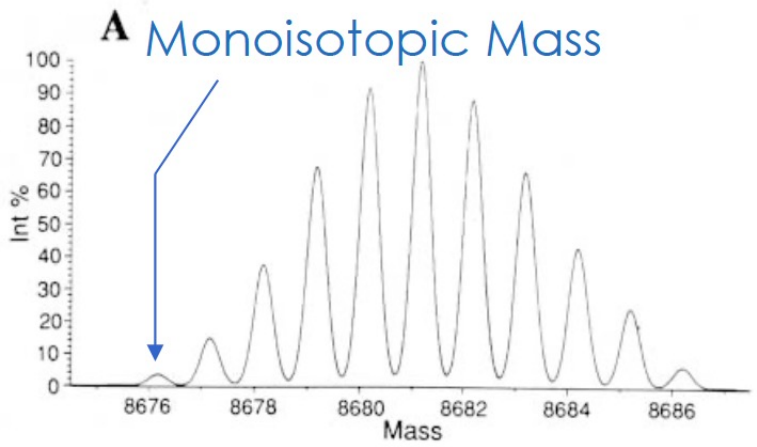
High resolution instruments

Low resolution instruments

Example of spectrum

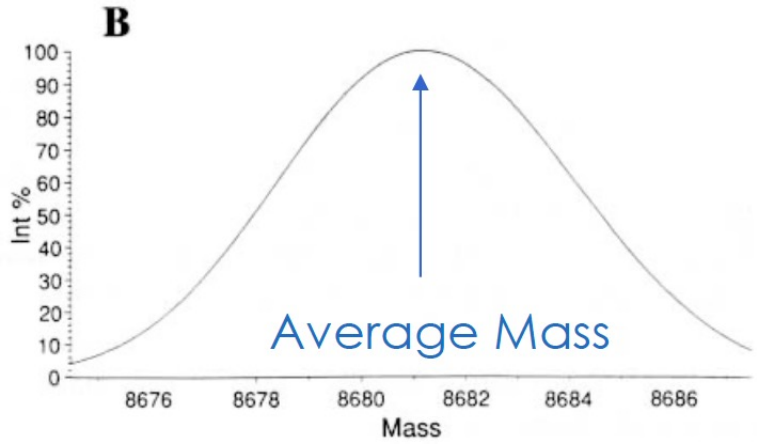
- Example: Bovin Proinsulin ($C_{381}H_{586}N_{107}O_{114}S_6$)

R=10 000



Monoisotopic mass:
Sum of the masses of the atoms in a molecule using the most abundant isotope for each element.

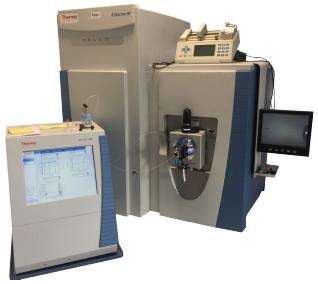
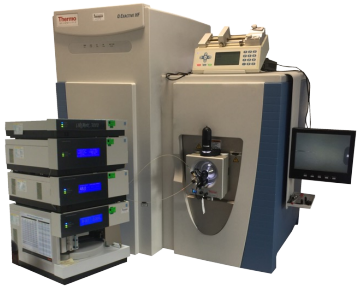
R=1 000



Average mass:
Sum of the average atomic masses of the constituent elements.

Mass Spectrometers available at the Institut Pasteur

Institut Pasteur

Instruments	 Velos OrbiTrap pro	 2 Q-exactive plus	 Q-exactive HF	 2 Orbitrap Lumos/ Eclipse	 Synapt G2-Si HDMS
Sources	ESI	ESI *High resolution, high sensitivity, high precision*		ESI *High resolution, high sensitivity, high precision*	
Geometry	Trap Orbitrap	Hybrid Quadrupole-Orbitrap	Hybrid Quadrupole-Orbitrap	Quadrupole-Trap Orbitrap	Quadrupole -IMS-ToF
Applications	• Protein quantification (label-free, TMT, SILAC etc…) • PTMs characterization • Protein identification • Interactomics • X-link etc …			• Native MS • IMS • HDX-MS } ***Structural proteomics*** • Top-down proteomics • Single cell proteomics	

Adapted from S. Brier

Mass Spectrometers available at the Institut Pasteur

Instrument	Source	Geometry	Applications
 Bruker Ultraflex	MALDI	ToF -ToF	• QC proteins • QC peptides • Lipids • Oligonucleotides • Etc …

MALDI-TOF/TOF

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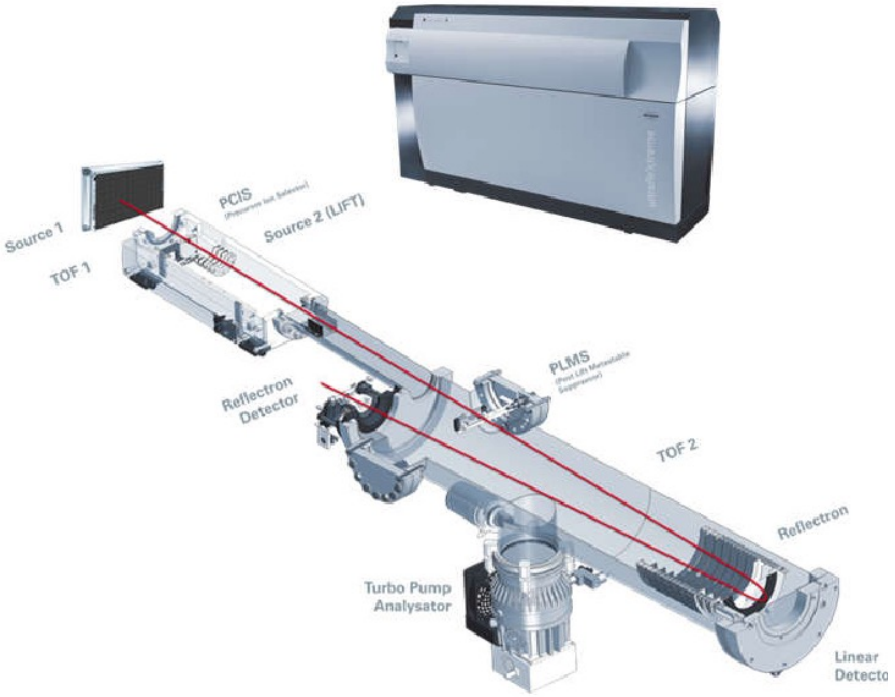
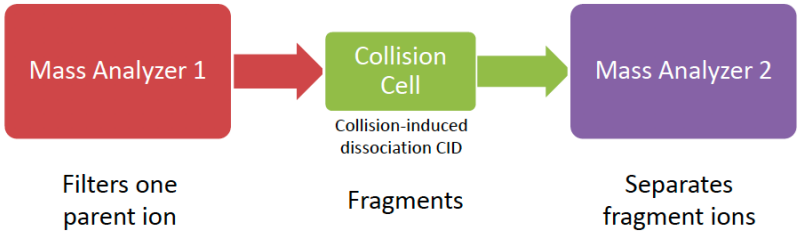
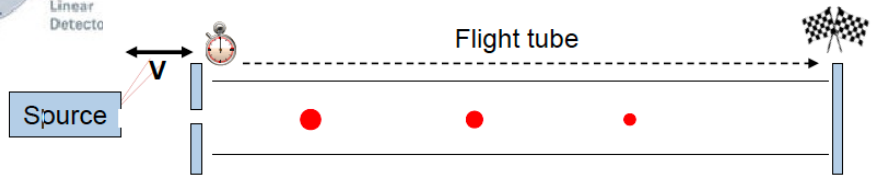
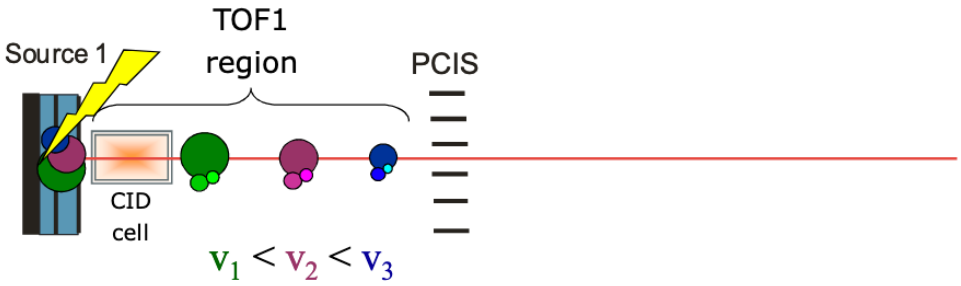




TOF principle

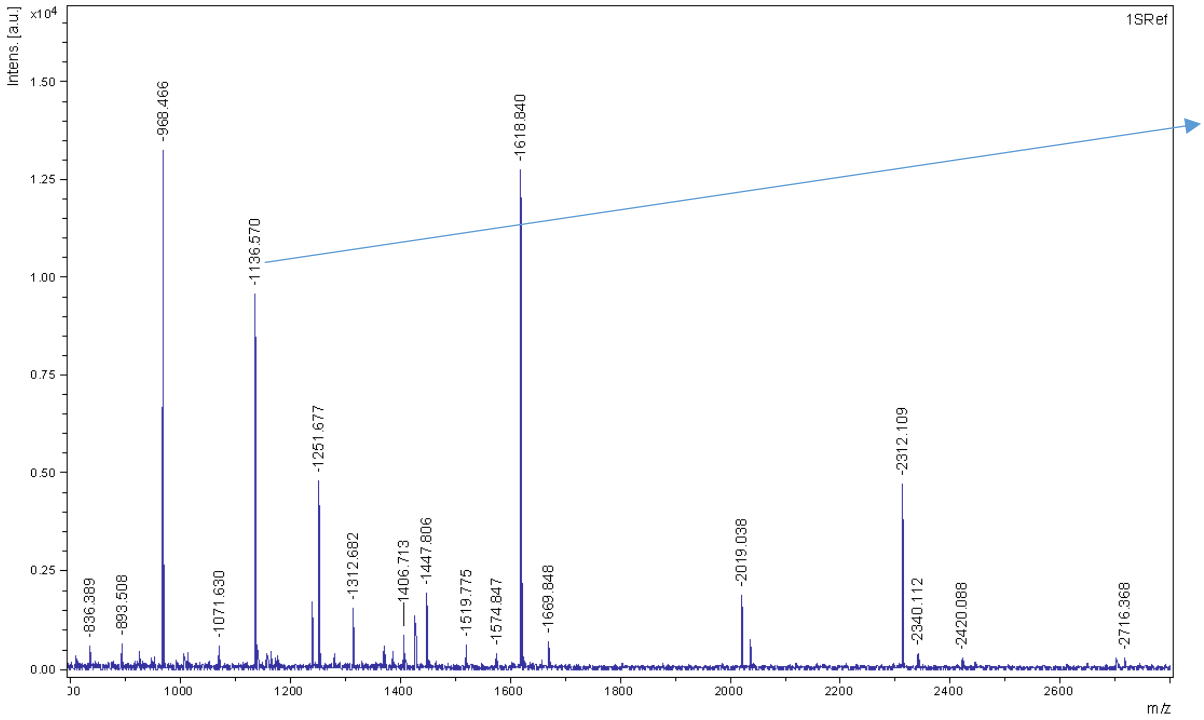


Time-of-Flight (TOF) analyzer distinguishes the molecules by their arrival times at the detector

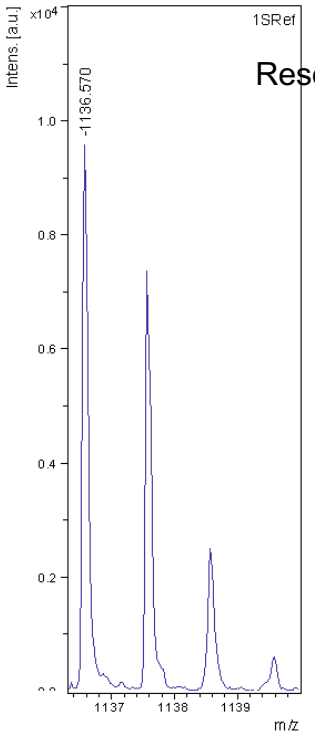


MALDI-TOF/TOF

MALDI-TOF MS spectrum of 100 fmol yeast ADH digested with trypsin



Isotopic resolution



Resolution : 12739

MALDI-TOF/TOF spectrum

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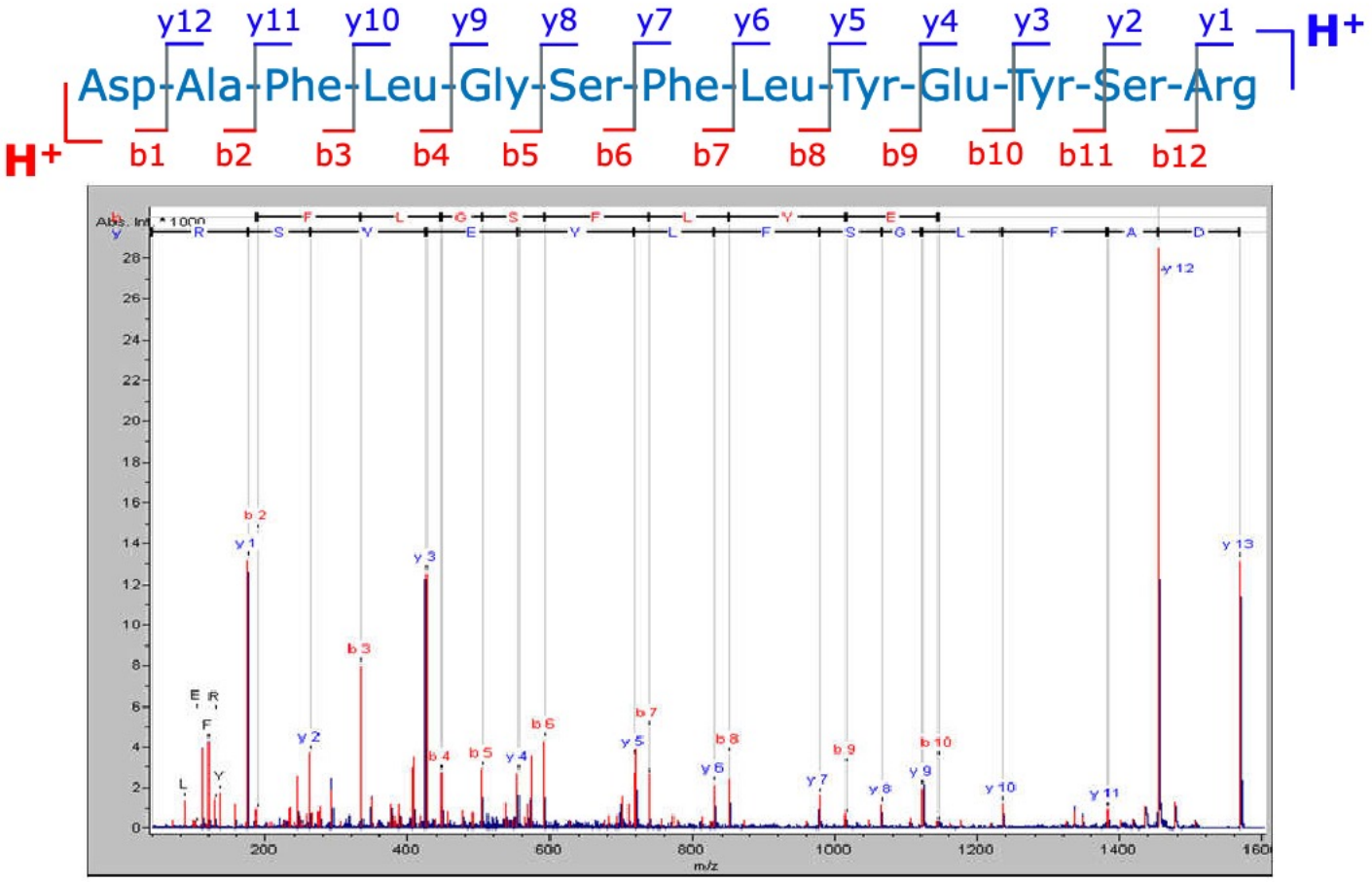
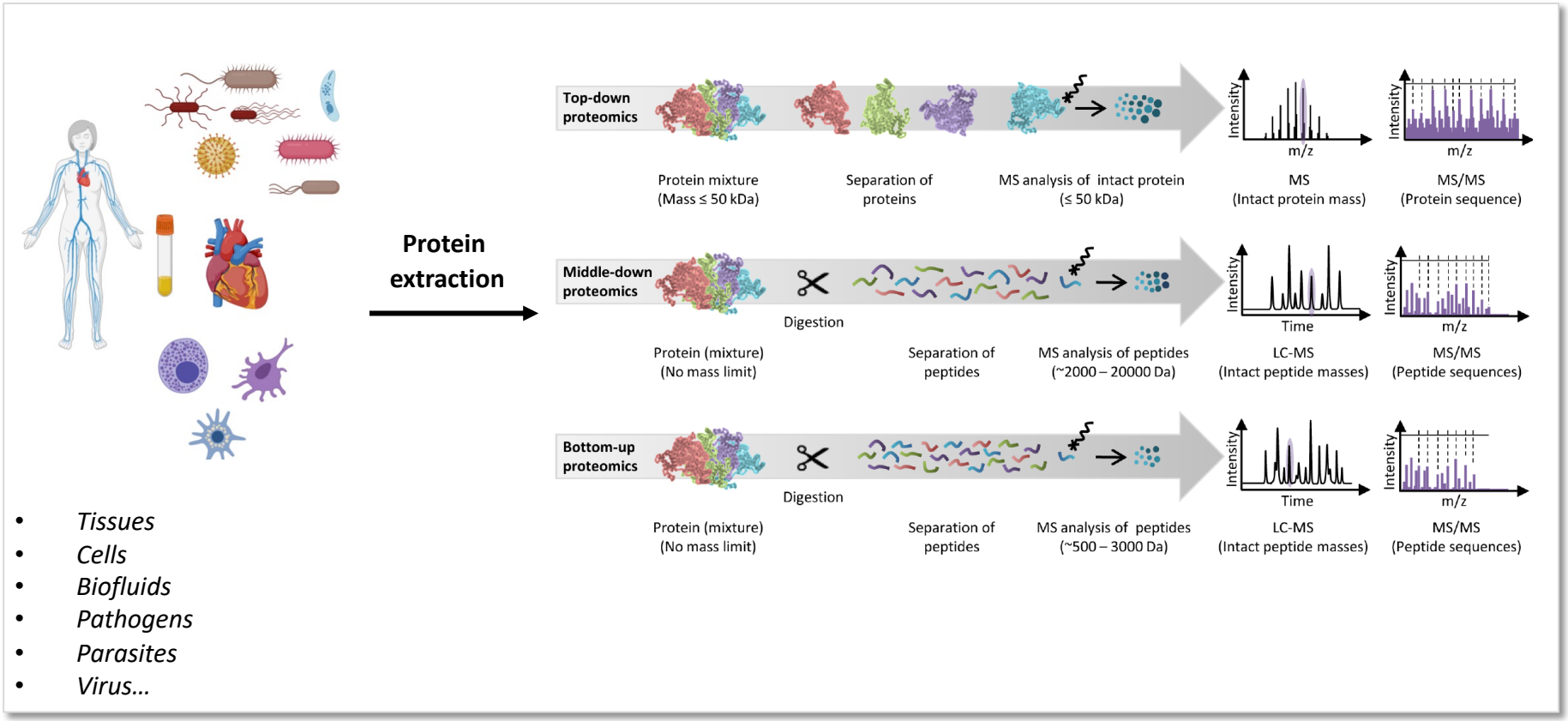


MS-based proteomics

General workflows and strategies

Overview of MS-based Proteomics Workflows

- The aim of the MS-based proteomics is **to identify and quantify all proteins** and their **post translational modifications** (PTMs)



- *Tissues*
- *Cells*
- *Biofluids*
- *Pathogens*
- *Parasites*
- *Virus…*

Linda Switzar & *al.*, *Journal of Proteome Research 2013*

Top-down proteomics

Top-down

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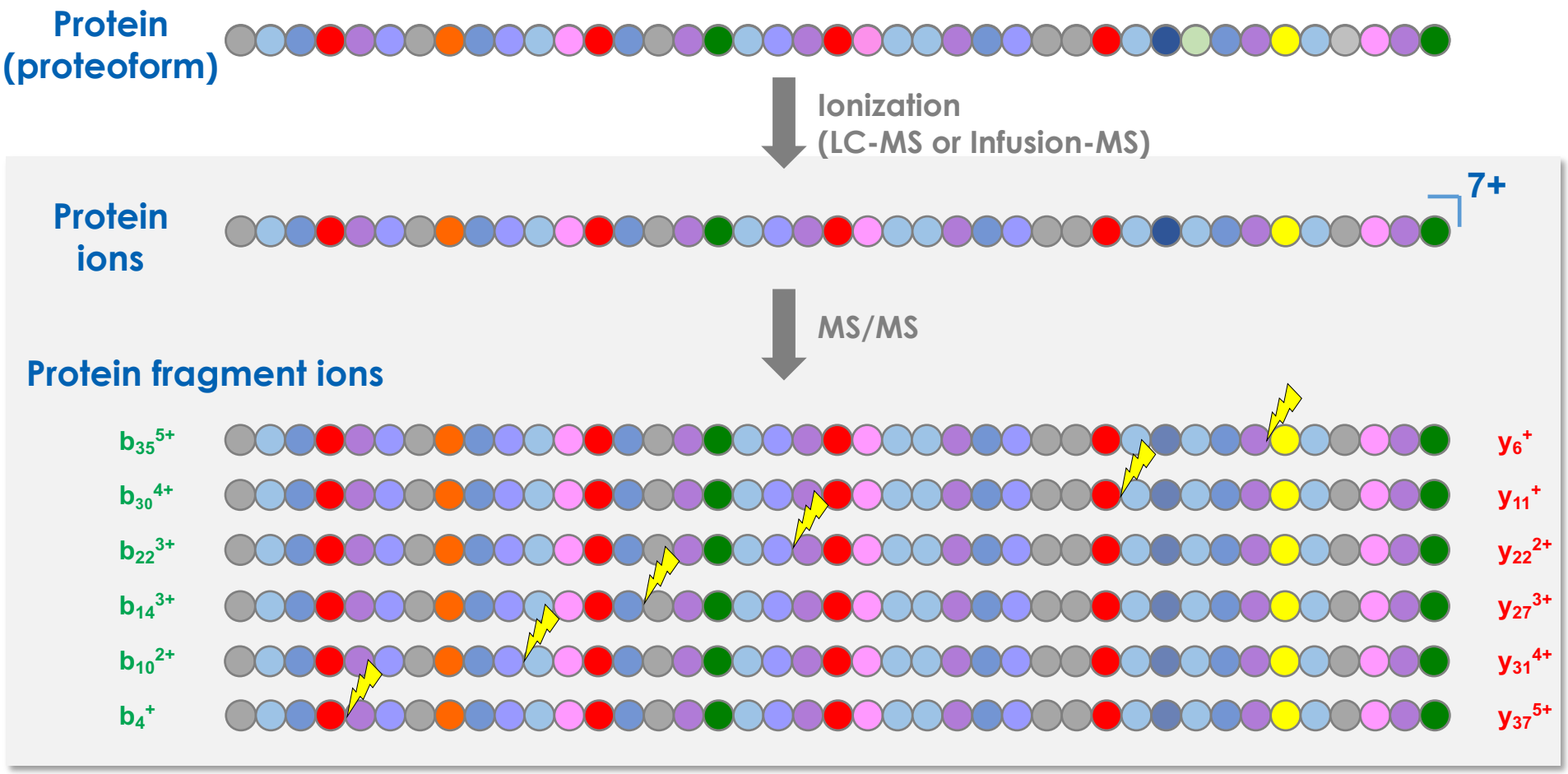


Proteoform Database

Proteoform characterization (potentially 100% sequence coverage)

Top-down proteomics challenges

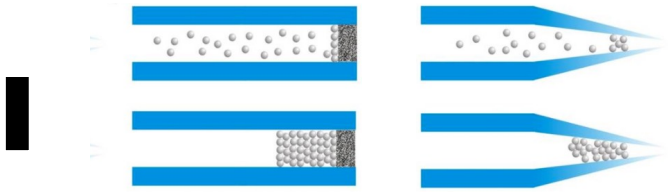
Institut Pasteur

- Poor protein solubility (SDS frequently used)
- Greater sample diversity at protein level (charge, hydrophobicity)
- Lack of « proteotypic » protein
- Decreased chromatographic resolution
- Lower throughput and reproducibility concerns

A. Doucette et al., *Exp. Rev. Proteomics* 8(6) 787-800 (2011)

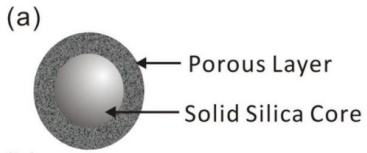
Online LC separation of intact proteins

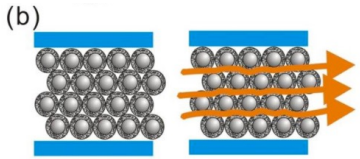
- Reverse Phase columns
  - C_4, C_5, C_8 porous particles



Home-made tip-packed nanoLC column (75 um, 50 cm)

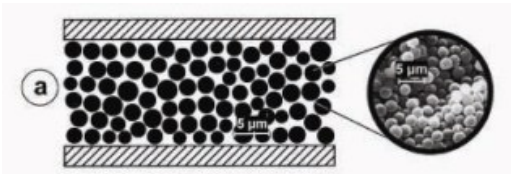
  - C_4, C_5, C_8 core-shell



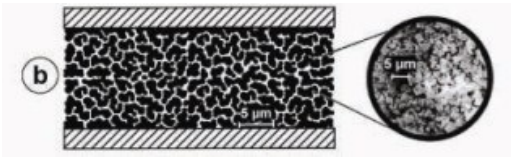


Y. Shen et al. *J. Chrom. A* In press

- Monolithic columns: pepswift PS-DVB, proswift RP-4H, proswift C4 RP-5H (Thermo Fisher)



Classical column



Monolithic column (PS-DVB)

Online LC separation (E. coli) – Different phases

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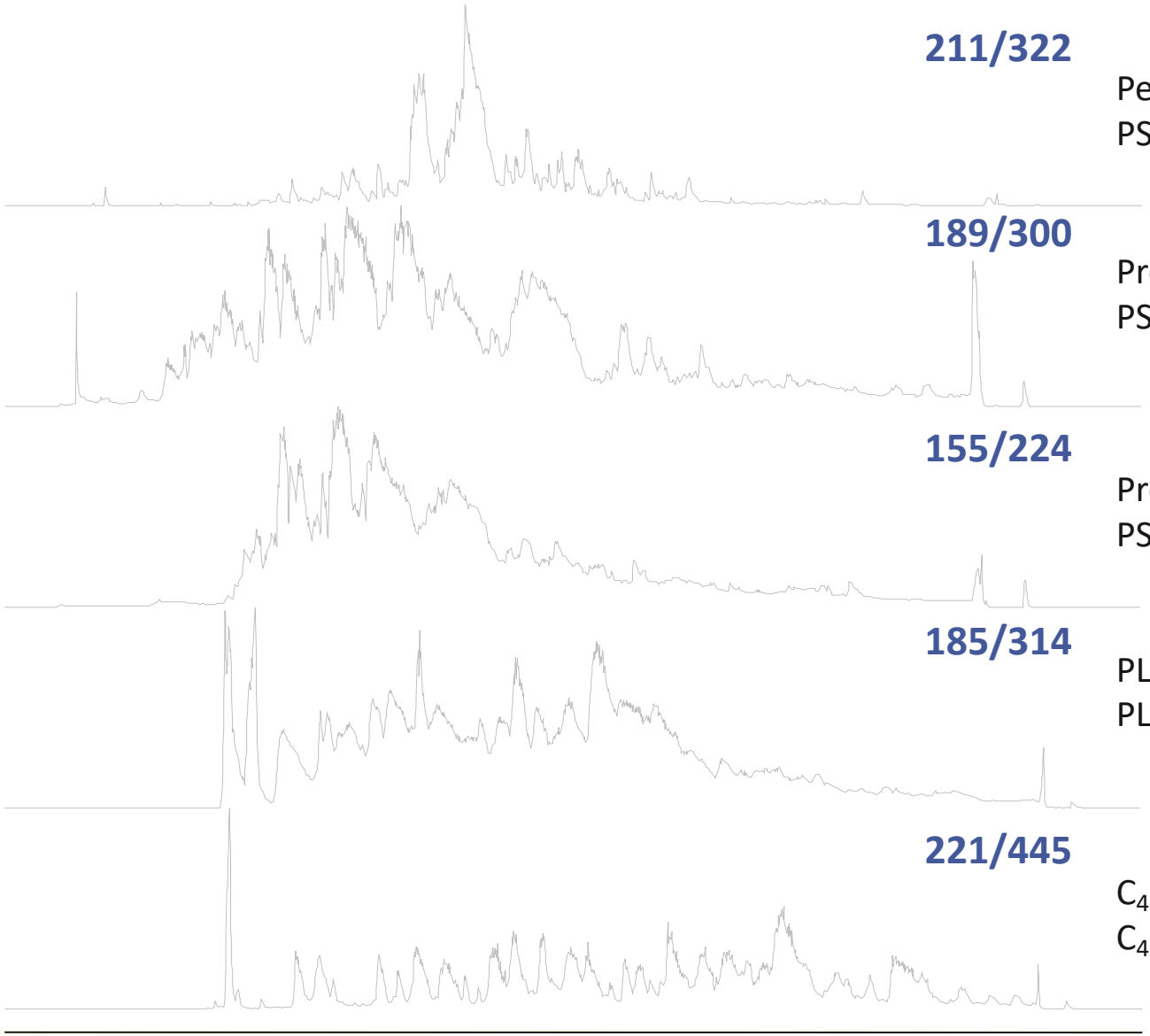

Pepswift PS-DVB 25cm,
PS-DVB 200um 5 mm

Proswift C4 RP-5H 100um 50cm,
PS-DVB 200um 5 mm

Proswift RP-4H 100um 50cm,
PS-DVB 200um 5 mm

PLRP-S, 1000A, 75um 50cm,
PLRP-S, 1000A 2cm

C_4 5um, 75um 60cm,
C_4 5um, 150 um, 3cm

Bottom-up proteomics

Bottom-up

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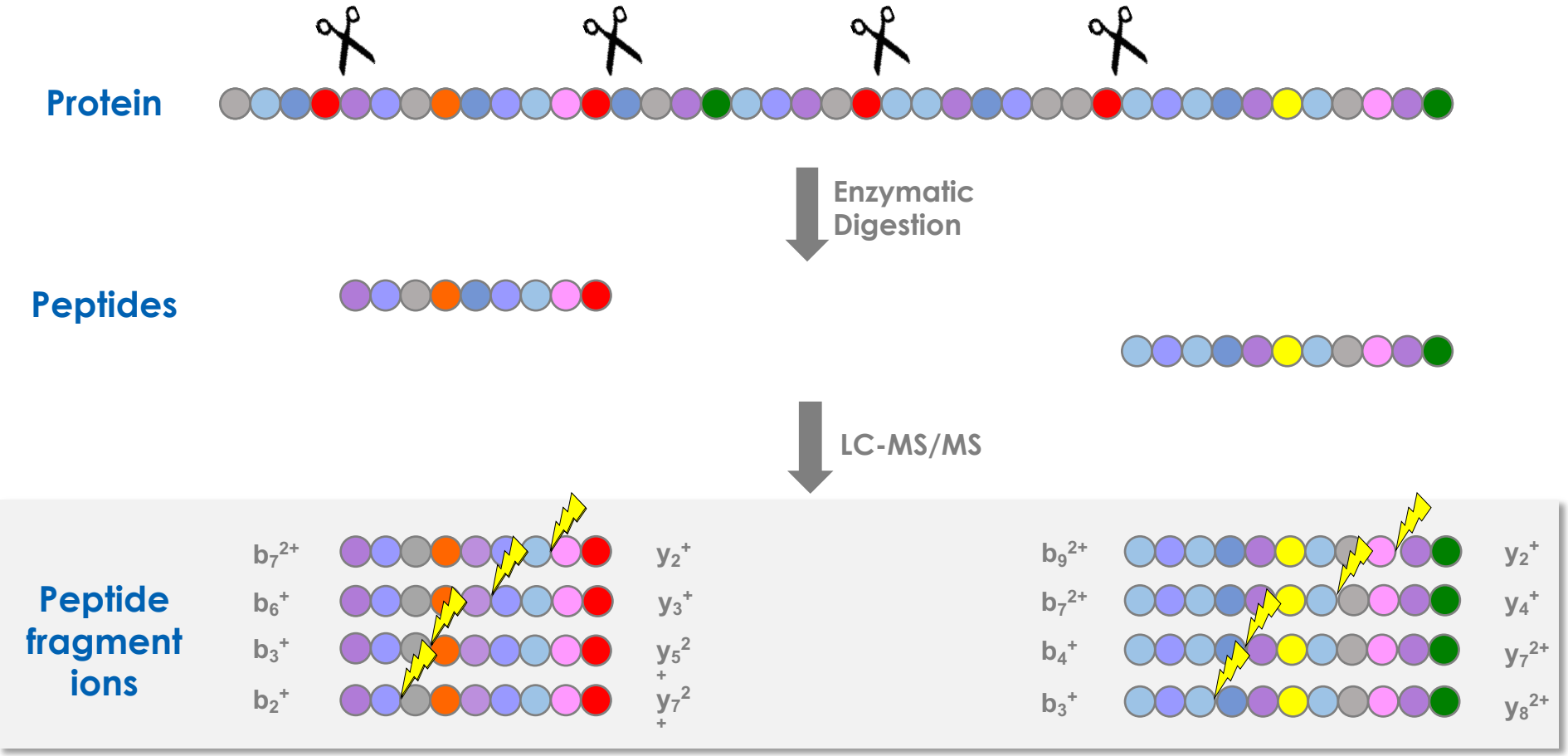
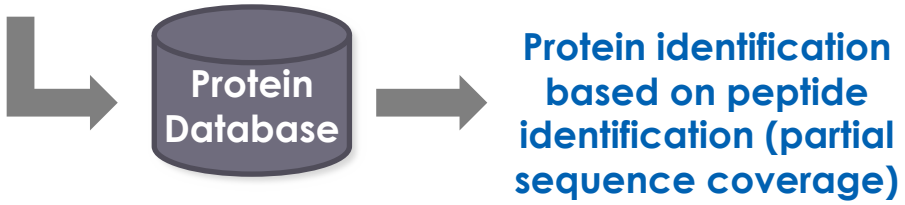
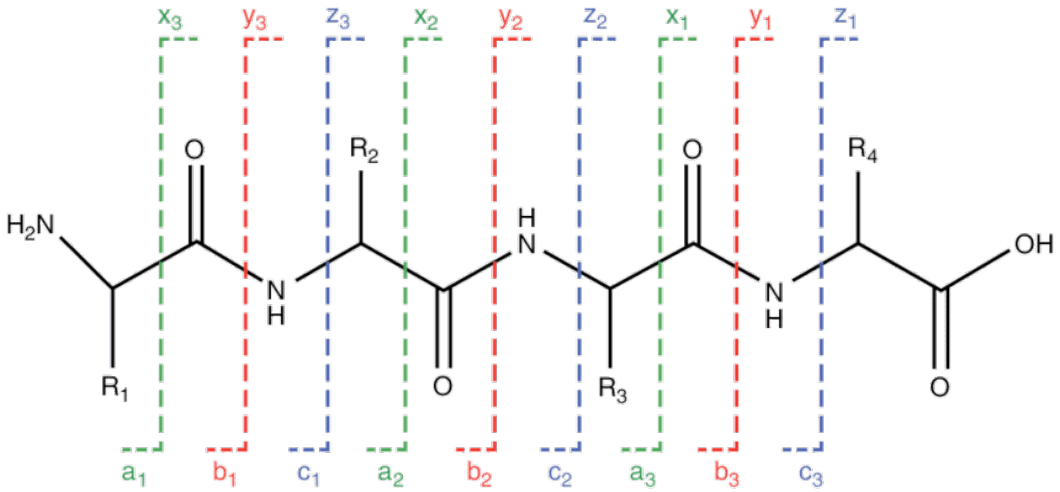
- Most used approach





Peptide fragmentation



Fragmentation mode

ETD c/z
CID/HCD b/y
etc…

Peptide bond is the weakest bond
Preferential fragmentation, tryptic peptides appropriate
Mass difference between two intense peaks corresponds to an amino acid

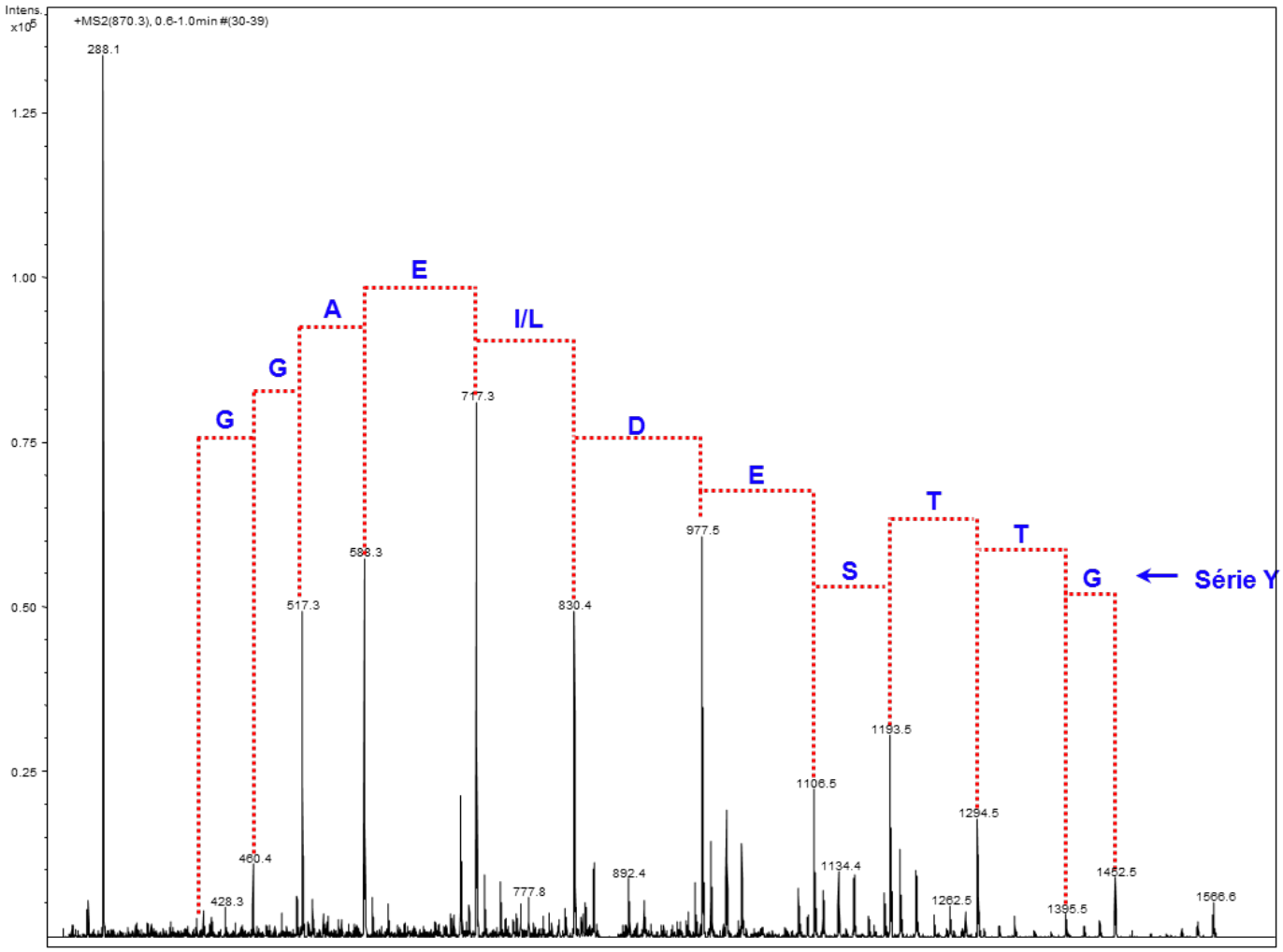
+ALLLFSDGR+
+ALLLFSDGR+
+ALLLFSDGR+
+ALLLFSDGR+
+ALLLFSDGR+
+ALLLFSDGR+
+ALLLFSDGR+

Fragmentation in the collision cell

b- type fragments	y- type fragments
+A	LLLFSDGR+
+AL	LLFSDGR+
+ALL	LFSDGR+
+ALLL	FSDGR+
+ALLLF	SDGR+
+ALLLFS	DGR+
+ALLLFSD	GR+
+ALLLFSDG	R+

Example of MS/MS spectrum

- MS/MS fragmentation Spectrum of a peptide



Bottom-up Proteomics workflows

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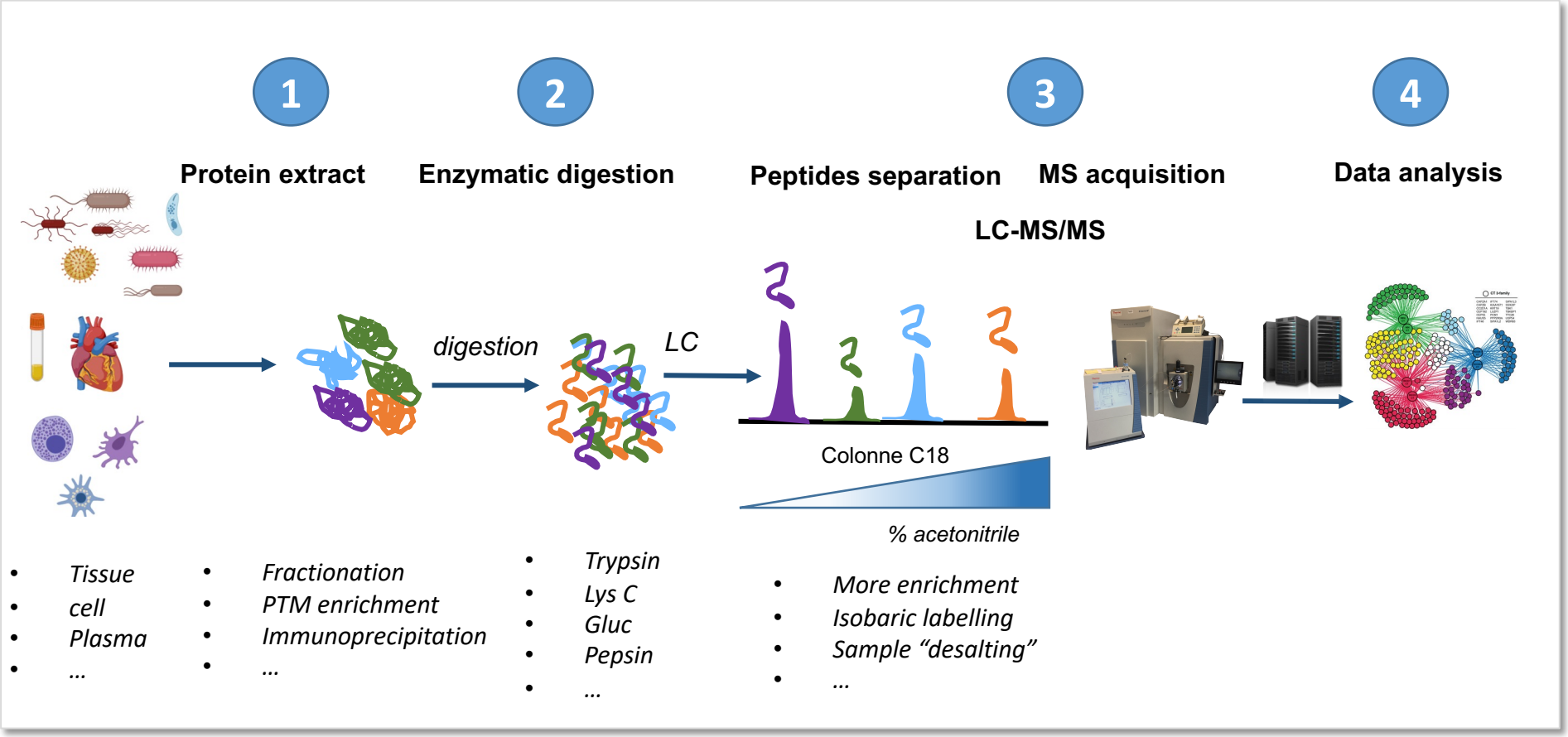
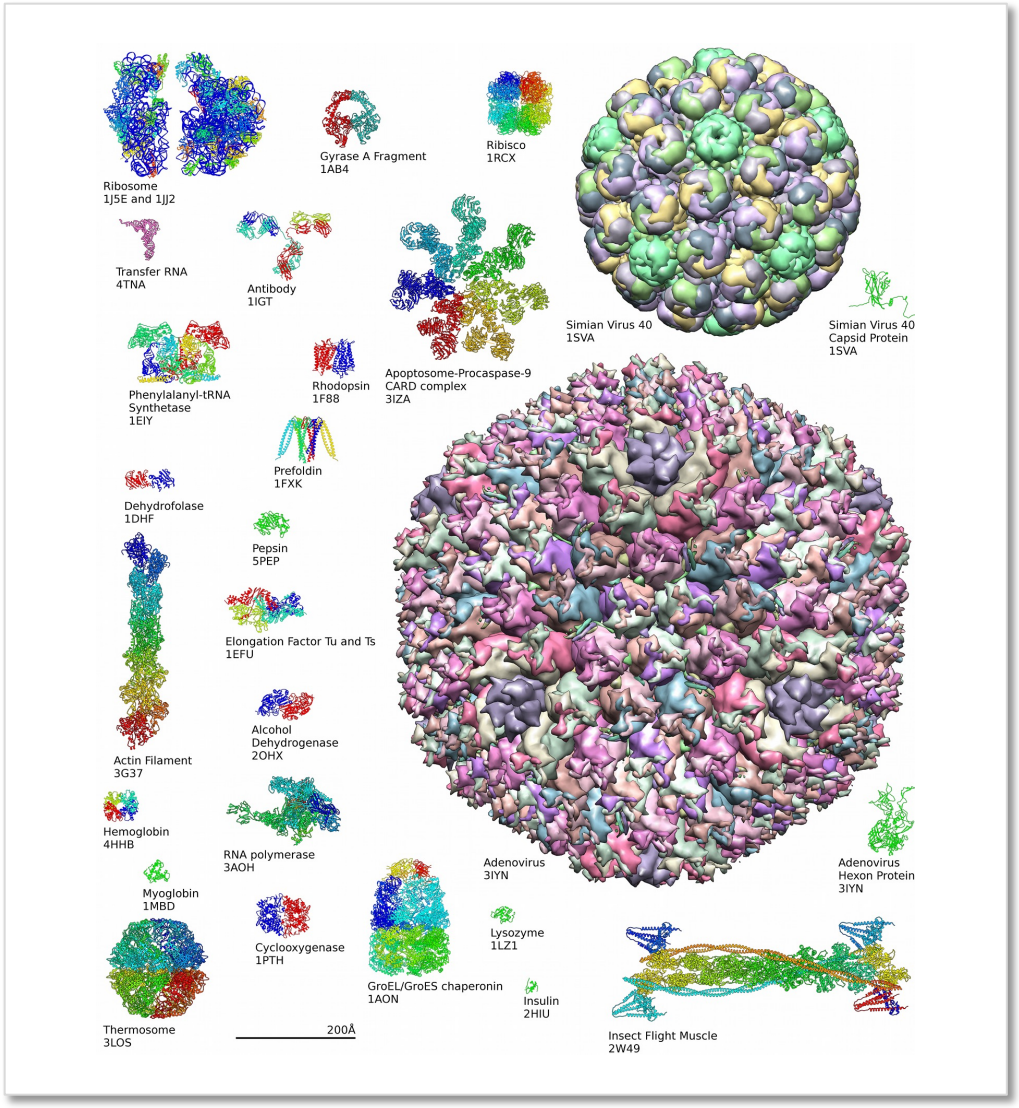
- Bottom-up proteomics is the most used and mature workflow

- Common method to identify proteins and characterize their amino acid sequences and post-translational modifications (PTMS) by **proteolytic digestion** of proteins prior to the LC-MS/MS analysis



Bottom-up approach: Protein diversity

- Proteins are heterogenuous chemical entities



Stability
Hydrophobicity
Chemical
Quantity
structure
PTM
Mass
Molecular

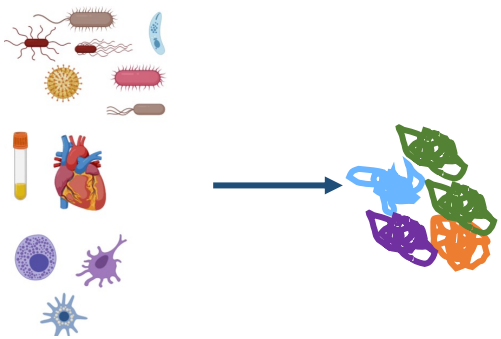
Bottom-up Proteomics workflows

Institut Pasteur

- Protein extraction: main issue in global protein analysis

1

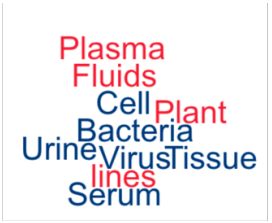
Protein extract



- *Tissue*
- *cell*
- *Plasma*
- *…*

- *Fractionation*
- *PTM enrichment*
- *Immunoprecipitation*
- *…*

For which sample type?



How to extract proteins?

Strongly dependent on the sample type and the biological question

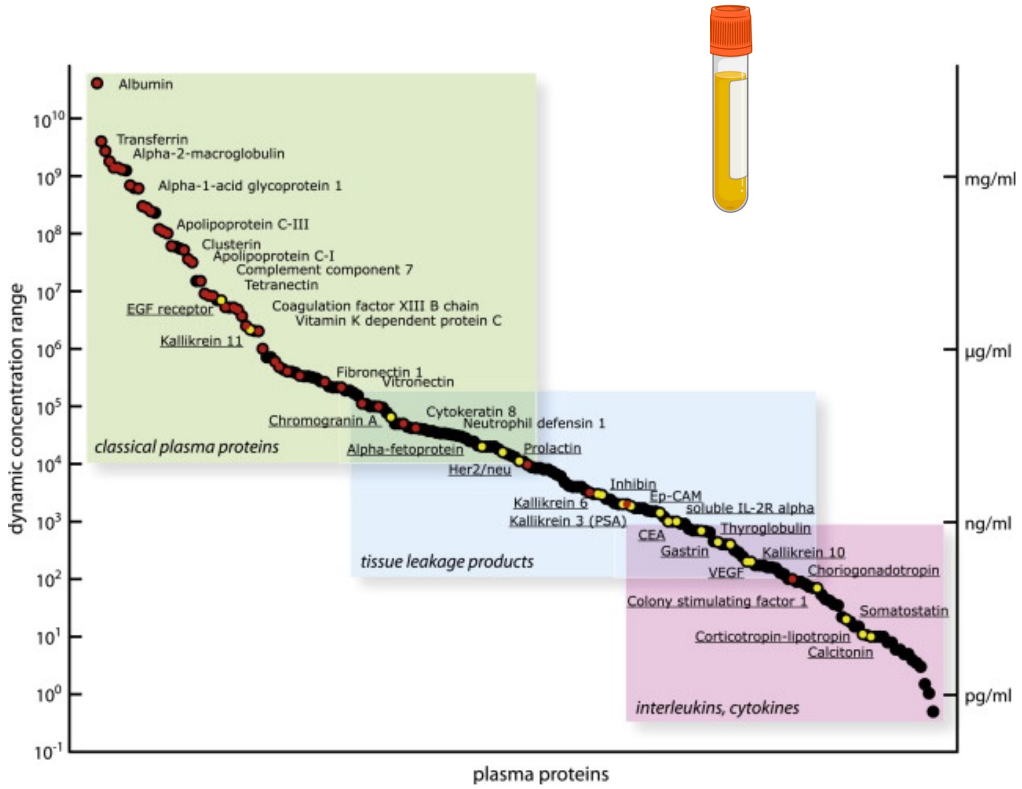
- **Mechanical cell disruption of cells**, e.g. by sonication or by freeze-thawing
- **Non-mechanical cell disruption**, e.g. by detergents (SDS, Chaps, Triton X100, NP40,…) or by chaotropes (Urea, Guanidinium Chloride, …)
- Prevent protein degradation: protease inhibitor cocktails, samples are kept on ice…

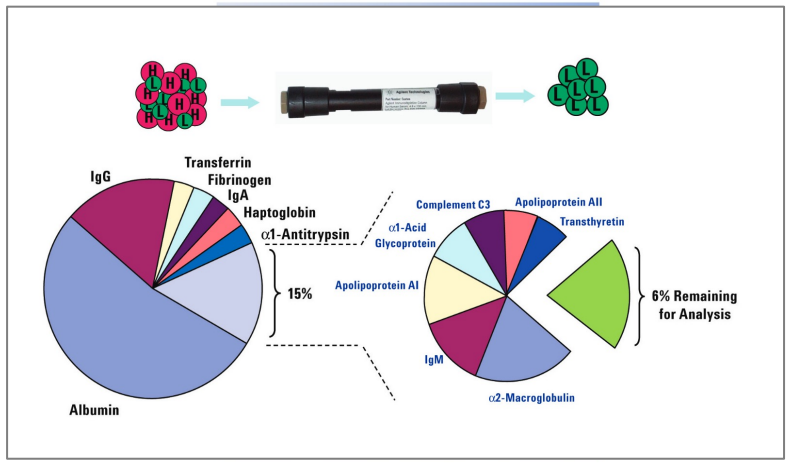
Main issue?

Complete extraction is difficult/impossible

Bottom-up approach: Sample preparation

- Impact of the dynamic range: Plasma, Serum



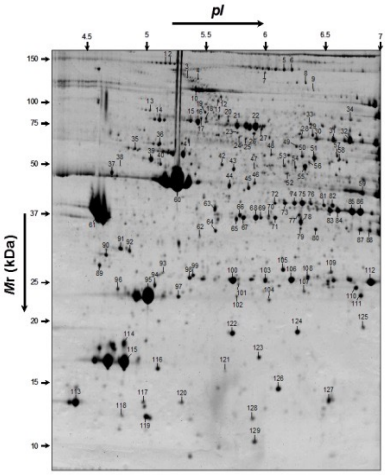


Plasma protein concentration as described by Anderson and Anderson (2002)

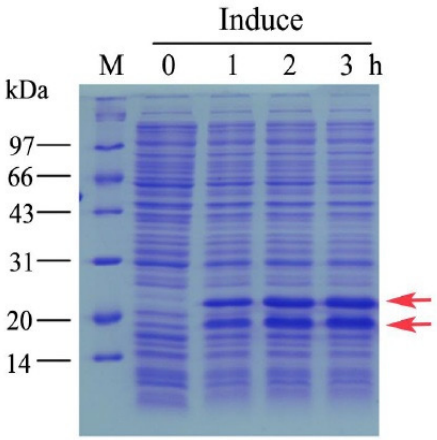
Bottom-up approach: Sample preparation

- Fractionation at protein level

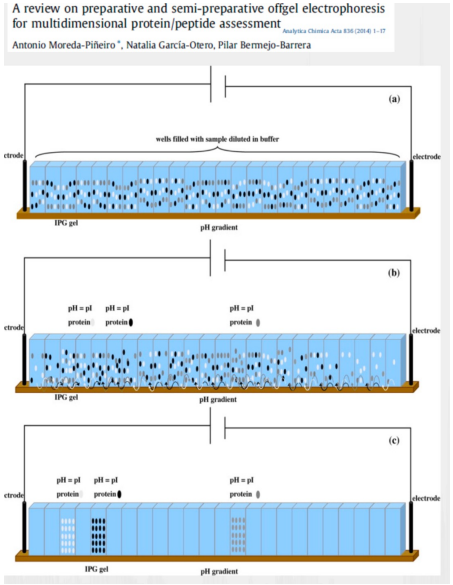
2D Gels



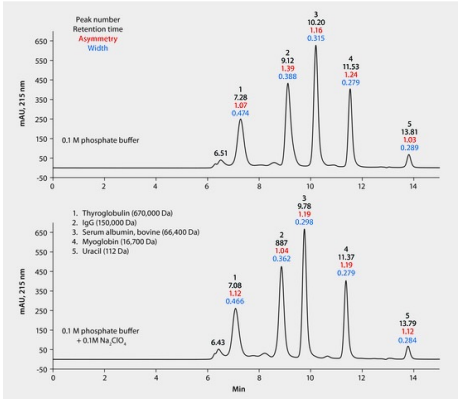
SDS-PAGE



OFFGEL



HPLC



Bottom-up Proteomics workflows

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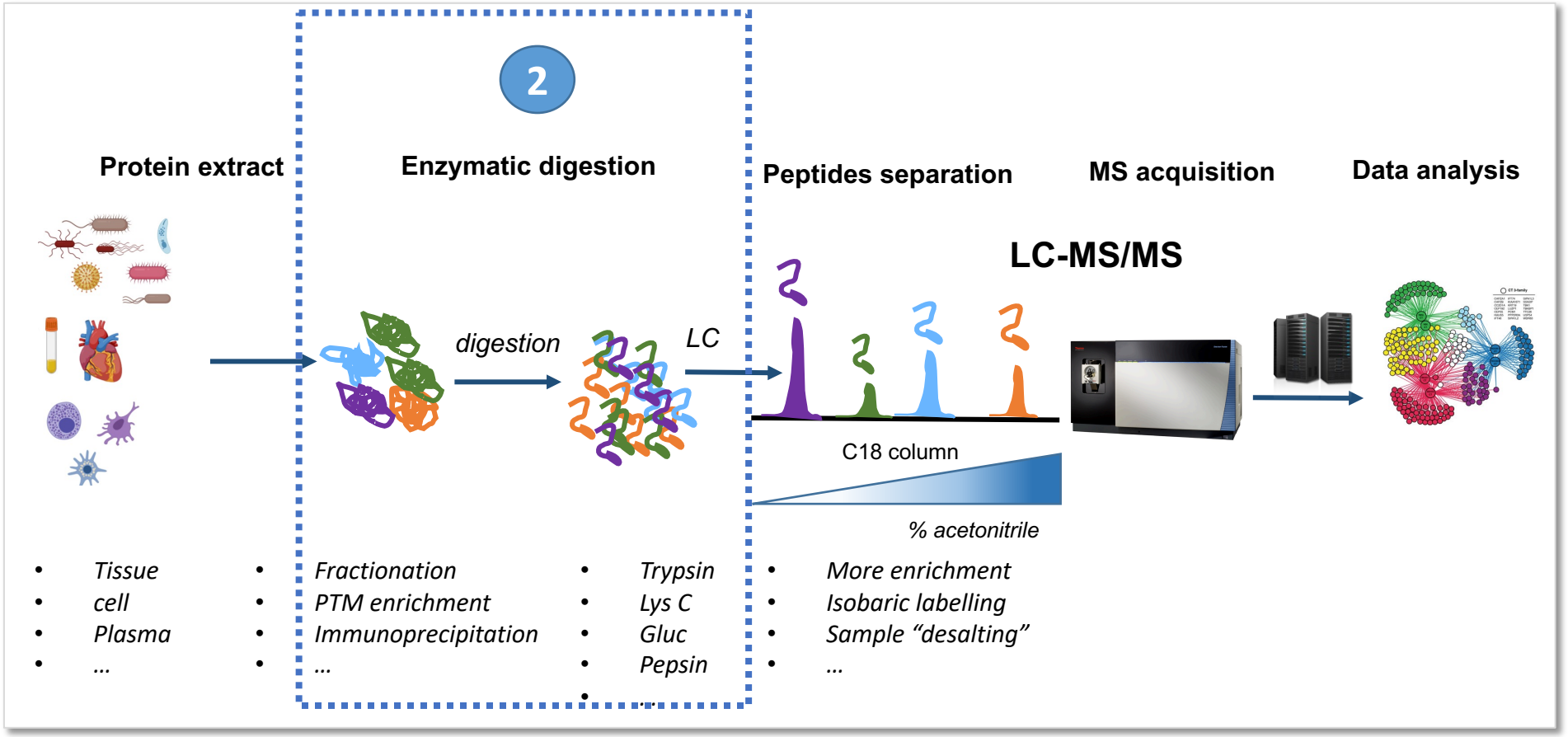
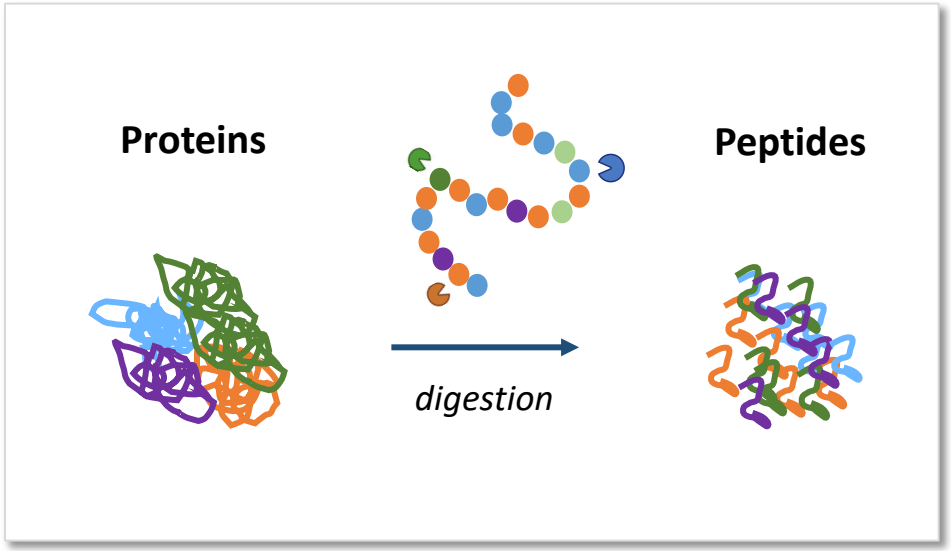
- Protein digestion



Bottom-up approach: Protein digestion

- Protein digestion: *from proteins to peptides*

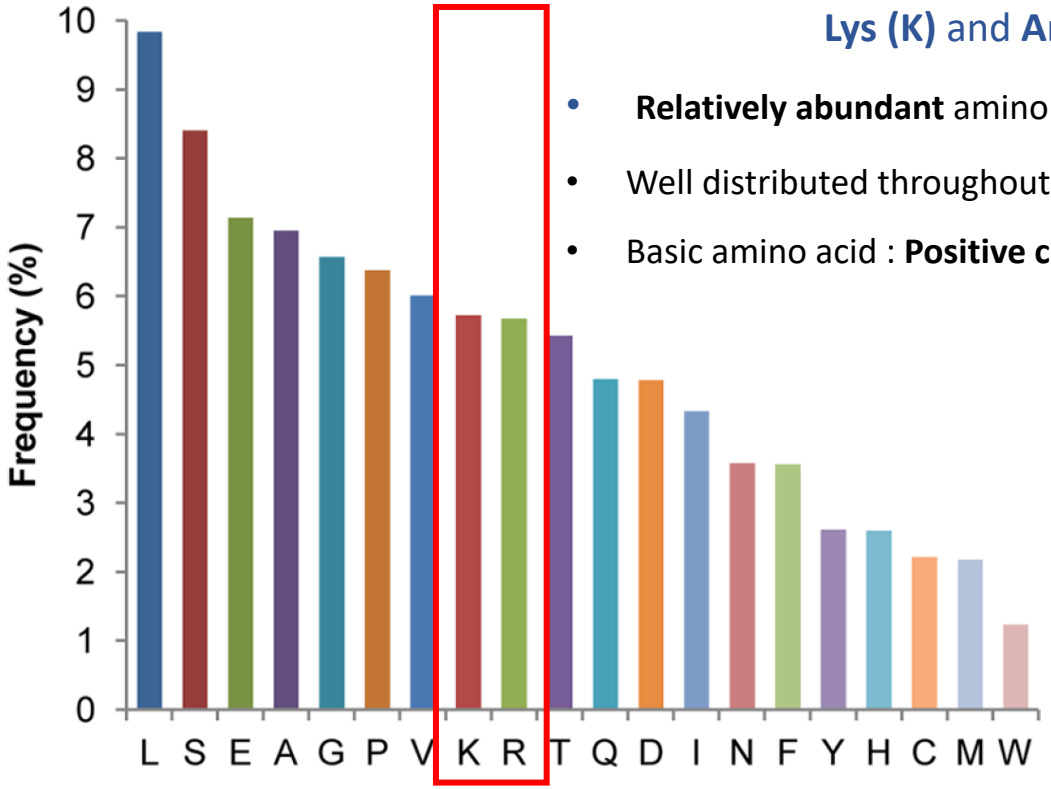


protease	organism	specificity	pH range
Arg-C	Clostridium histolyticum	R′	7.2–8.0[b]
Asp-N	*Pseudomonas fragi*	‘D	7.0–8.0[b]
Glu-C	*Staphylococcus aureus*	E′[b]	4.0–7.8[b]
Lys-C	Lysobacter enzymogenes	K′	8.5–8.8[b]
Lys-N	Lysobacter enzymogenes	‘K[c]	8.0[c]
Trypsin	*Bos taurus*	K,R′	8.0[b]
Chymotrypsin	*Bos taurus*	F,W,Y′	7.0–9.0[b]
Pepsin	Sus scrofa	‘F,L,W,Y′	1.3
		‘F,L′	2.0
Thermolysin	Bacillus thermoproteolyticus	‘A,F,I,L,M,V	8.0[h]
Papain	Carica papaya	R,K,D,H,G,Y[b]	6.0–7.0[b]
Pronase	*Streptomyces griseus*	A,E,F,I,L,T,V,W,Y′	6.0–7.5[b]

chemical	specificity	pH range
CNBr	M′	acidic
HAc	‘D′[d]	acidic
FA	D′	acidic
HCl	D′[e]	2.0[e]
NTCB	‘C[e]	9 –10[f]
Hydroxylamine	N–G	9.0[g]

Bottom-up approach: Protein digestion

- Sample Preparation : Protein digestion



Amino acid composition of the human proteome

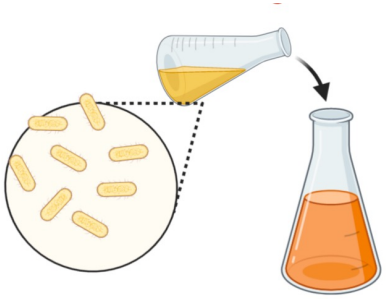
Lys (K) and **Arg (R)** are:

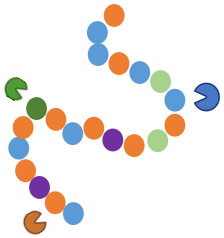
- **Relatively abundant** amino acids in the human
- Well distributed throughout a protein.
- Basic amino acid : **Positive charge, ionization**

Bottom-up approach: Protein digestion

- Importance of the combined enzymes for the digestion:

E. coli

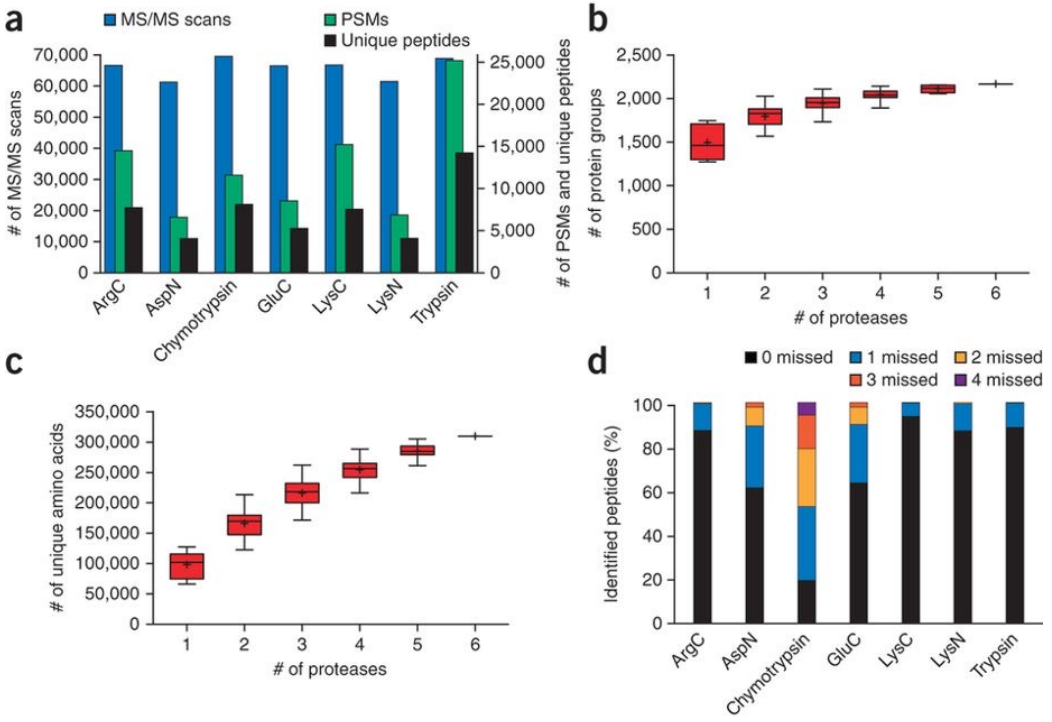




Digestion
With combined
enzymes

Peptides

LC-MS analysis of E. coli lysate digests



Piero Giansanti & al., Nature Protocols volume 11, pages 993–1006 (2016)

Bottom-up approach: Protein digestion

Institut Pasteur

- Protein digestion: **take home message**

  - Trypsin is most widely applied in bottom-up proteomics (gold standard)
  - Tryptic peptides lead to high quality MS/MS fragmentation spectra and confident peptide identification in protein database searches.
  - Arg-C and Lys-C : retain two basic amines in the peptide, N-terminal and Lys or Arg side chain leading to doubly protonated peptides.
  - Double digestion Trypsin / LysC for complex proteome

Linda Switzar & *al.*, *Journal of Proteome Research 2013*

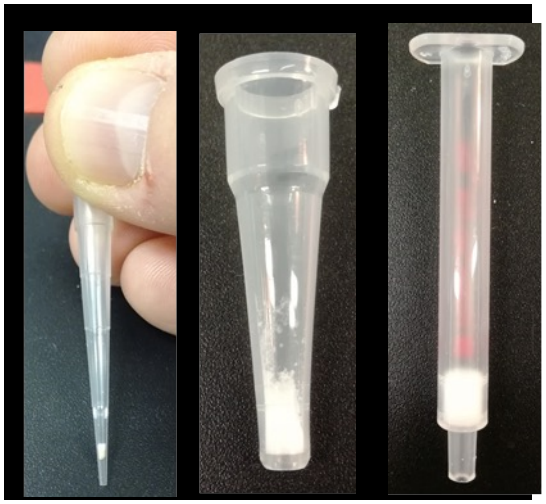
Bottom-up approach: Sample clean-up

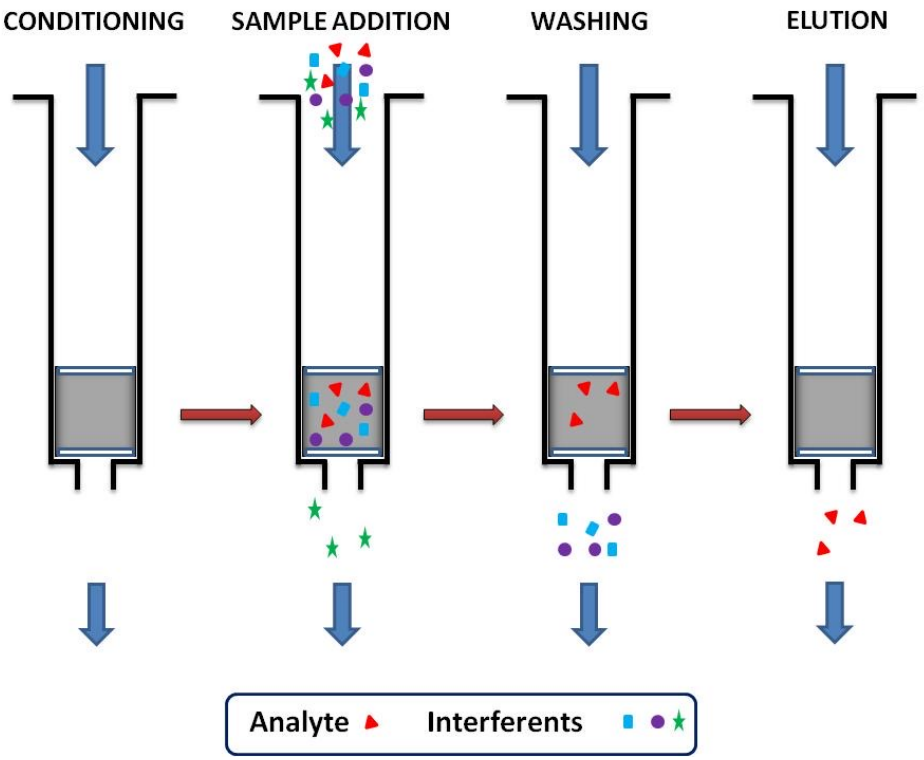
- Peptide Clean-UP: desalting

  Use of C18 materials in tips or columns to eliminate impurities/salt

Spin Column

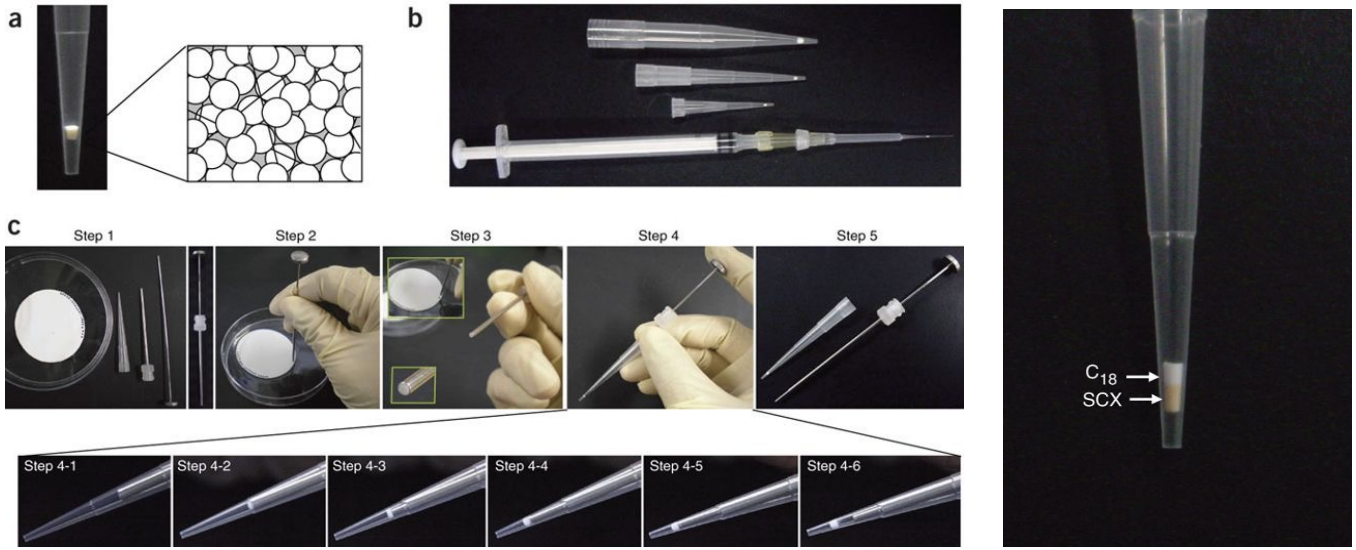
Stage Tip Sep-Pak





Bottom-up approach: sample clean-up

- Stage Tips



Empore material	Application in StageTips
C_{18}-silica	Desalting of peptides; fractionation of peptides at acidic and neutral pH
C_8-silica	Desalting of large peptides and proteins; usage as frit to retain beads in a tip
Activated carbon	No application described so far
Poly(styrene-divinylbenzene) copolymer (SDB)	Fractionation of peptides at basic pH
Anion exchange (SAX)	Fractionation of peptides by salt or pH steps
Cation exchange (SCX)	Fractionation of peptides by salt or pH steps
Chelating beads	No application described so far
Combinations by stacking of disks	
C_{18}-SCX	Desalting combined with fractionation of peptides by salt or pH steps
C_{18}-SCX-C_{18}	Desalting combined with fractionation of peptides by salt or pH steps followed by desalting again
SDB-SAX	Desalting combined with fractionation of peptides by salt or pH steps
Combination of beads and disks	
Metal oxide-C_8	Enrichment of phosphopeptides

Bottom-up approach: Peptide mixture simplification

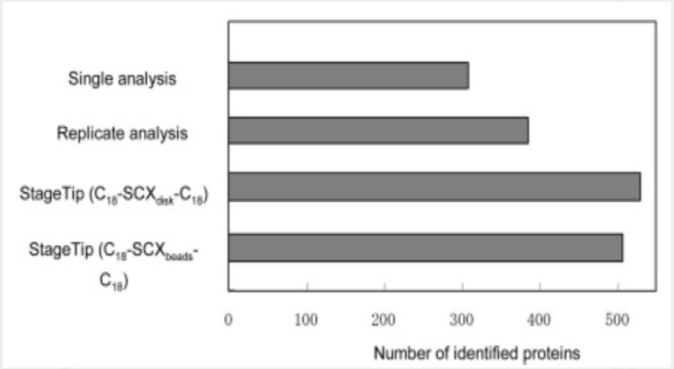
- Peptides fractionation

technical notes Journal of proteome research

Modular Stop and Go Extraction Tips with Stacked Disks for Parallel and Multidimensional Peptide Fractionation in Proteomics

Yasushi Ishihama,*,†,‖ Juri Rappsilber,‡,‖ and Matthias Mann*,§

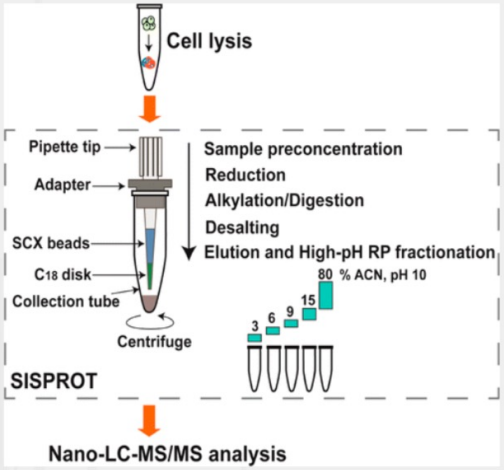
Journal of Proteome Research *2006, 5, 988−994*

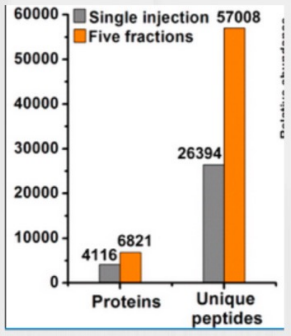


Simple and Integrated Spintip-Based Technology Applied for Deep Proteome Profiling

Wendong Chen†‡, Shuai Wang§, Subash Adhikari†, Zuhui Deng§, Lingjue Wang†, Lan Chen†, Mi Ke†, Pengyuan Yang‡, and Ruijun Tian*†∎

Anal. Chem., **2016**, *88* (9), pp 4864–4871

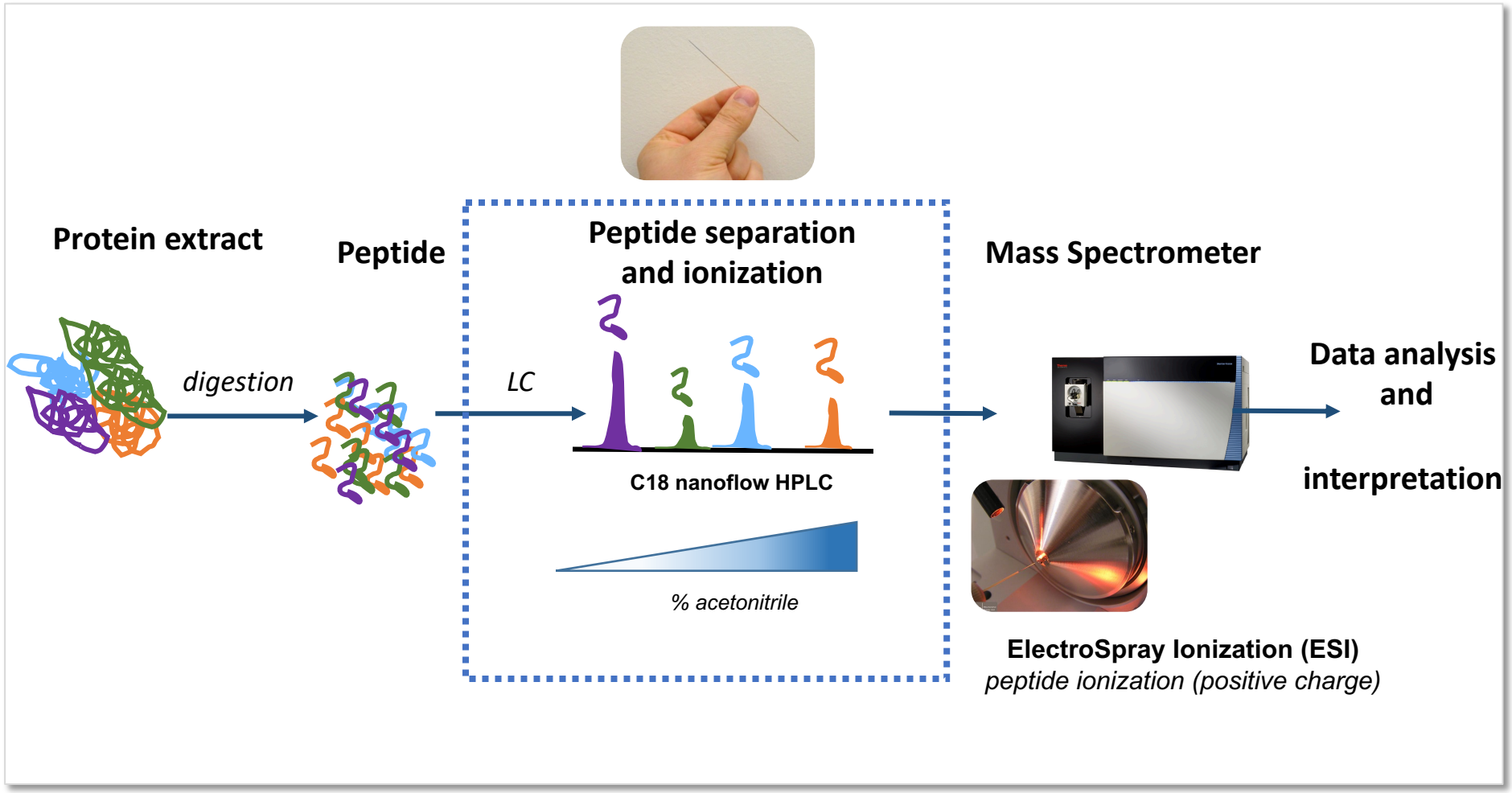




Bottom-up approach: Liquid Chromatography

Institut Pasteur

- Peptide separation and ionization



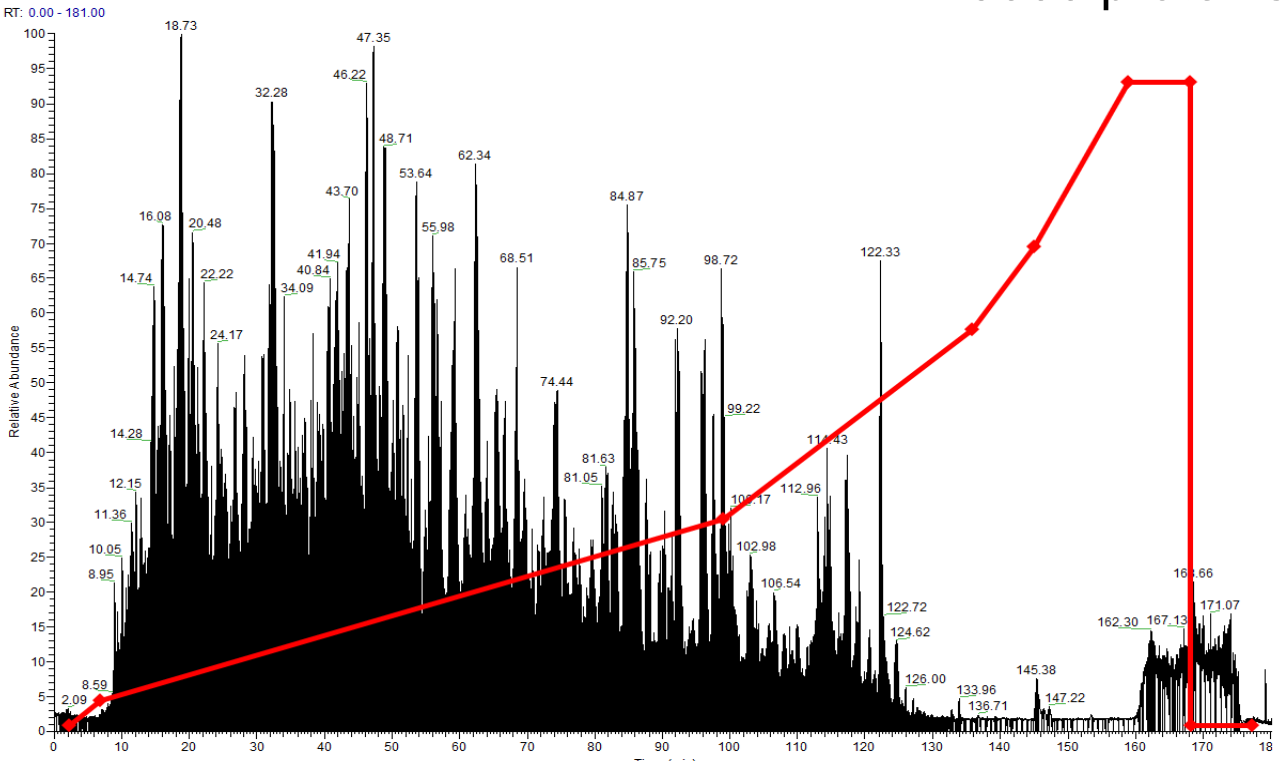
MS-based Proteomics : Bottom-up Proteomics

Institut Pasteur

- Separation of peptides using reverse phase nanoLC (C18) coupled to the mass spectrometer

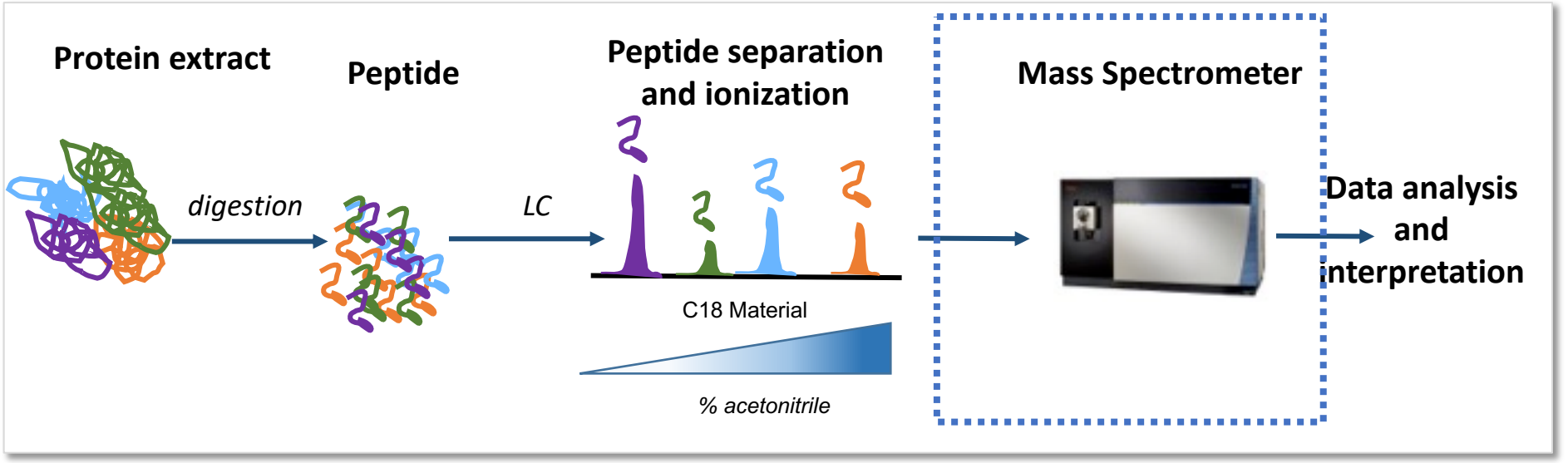
LC MS profile, 3h gradient
Proxeon, C18, 1.9µm, 100A 35 cm long, 75µm int Diam

> 5000 proteins identified



Bottom-up approach: Mass spectrometer acquisition

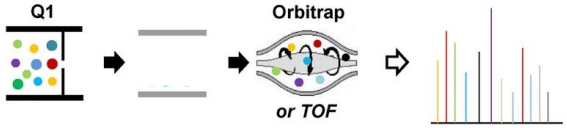
- Sample preparation :Protein digestion



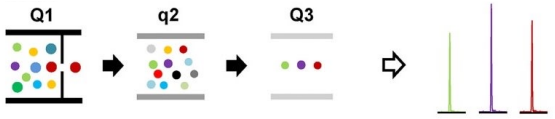
Bottom-up approach: Proteomics Workflows

- Three main MS acquisition methods

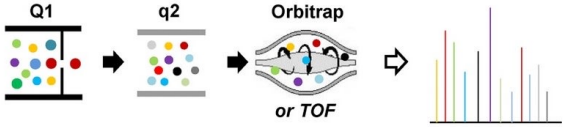
Data Dependant Acquisition (DDA)



Targeted

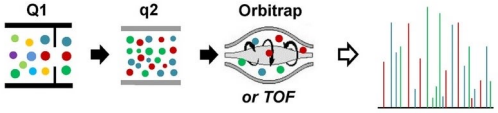


SRM



PRM

Data Independant Acquisition (DIA)



Bottom-up Proteomics

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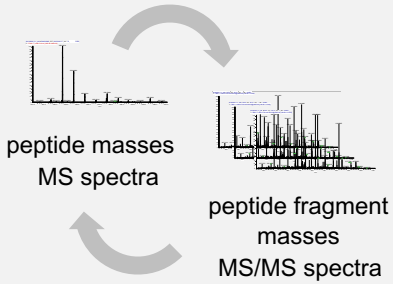
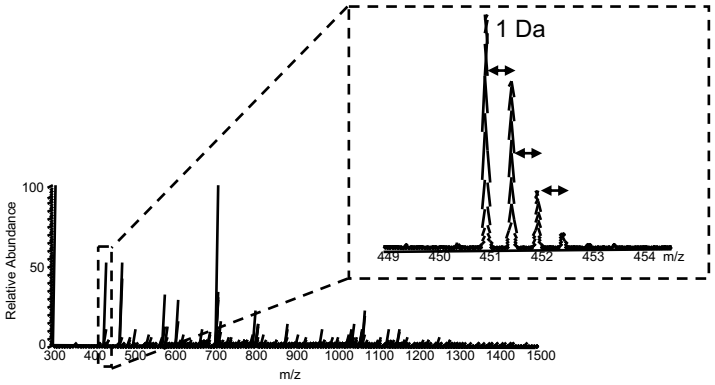
- Data Dependent Acquisition (DDA)- Tandem Mass Spectrometry (LC-MS/MS)

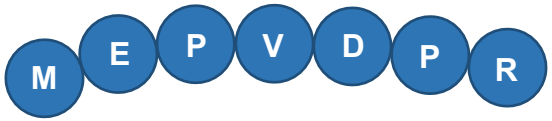
Tandem Mass Spectrometry

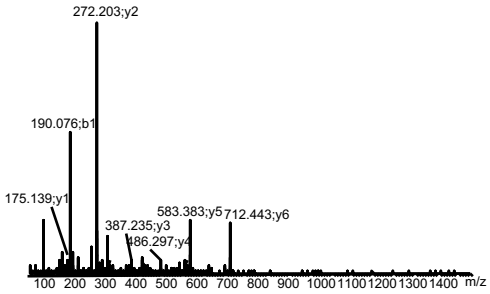


cycles of MS and
MS/MS spectra

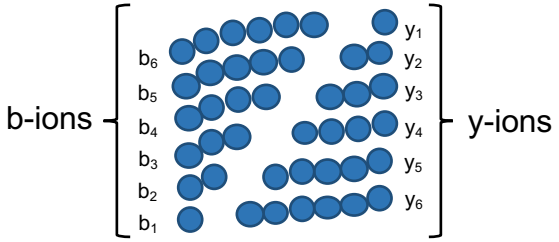


MS spectrum
=
mass of the intact peptides



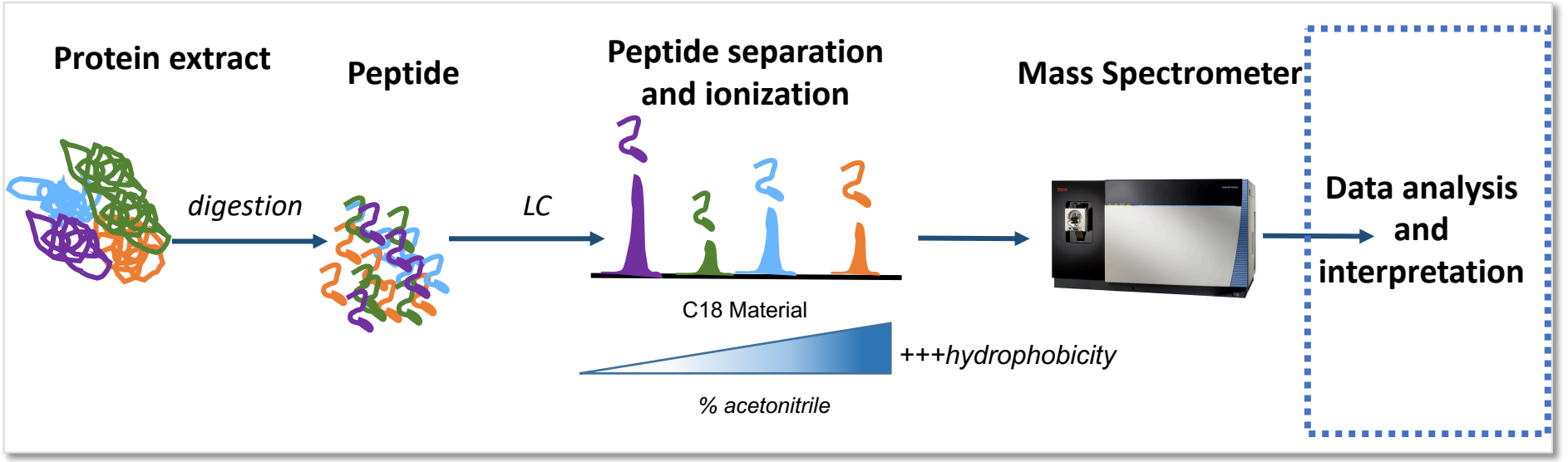


MS/MS spectrum
=
mass of the peptide fragments



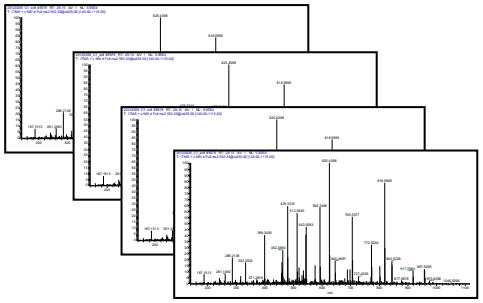
Bottom-up approach: data analysis and Interpretation

- Data analysis and interpretation



Bottom-up approach: data analysis and Interpretation

- **Data analysis**



DDA mode

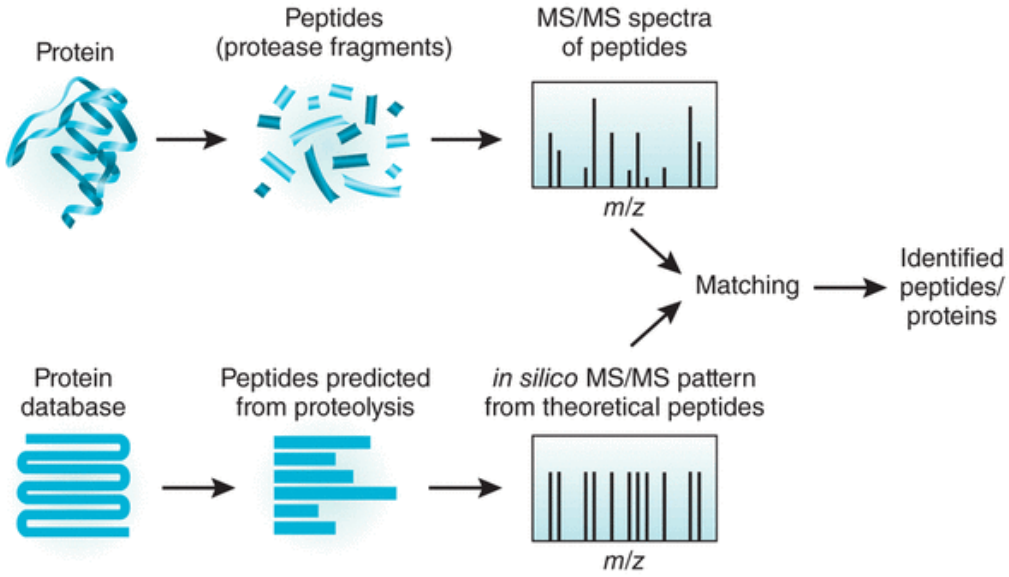
Spectra

Protein database

In silico digestion
Theoretical spectra
Mass list
Comparison

Algorithm to identify peptide & protein

(Mascot, Andromeda, Sequest…)



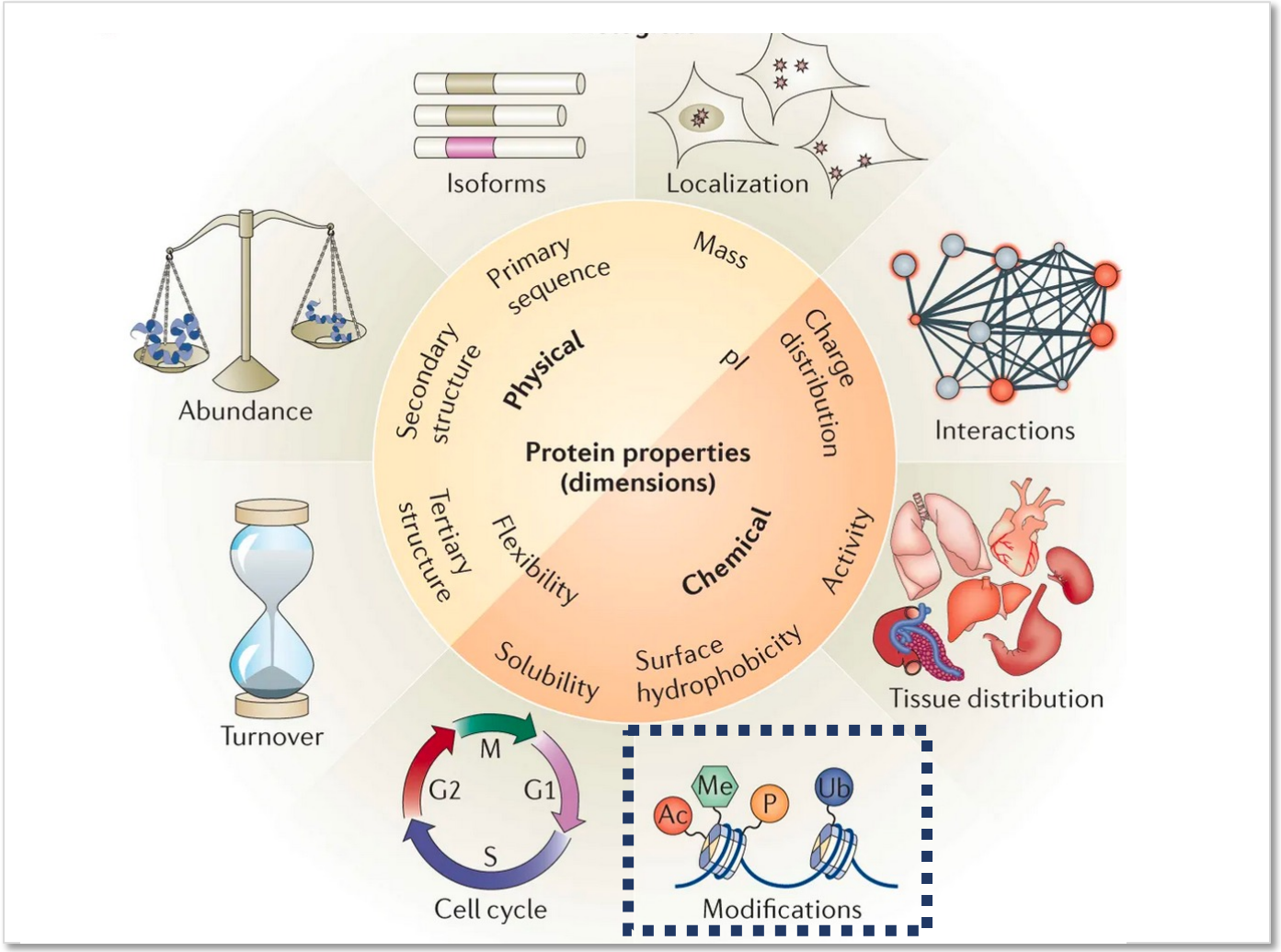
See Christine Carapito Part

Quantitative Proteomics

Mass-spectrometric exploration of the proteome

Quantitative Proteomics

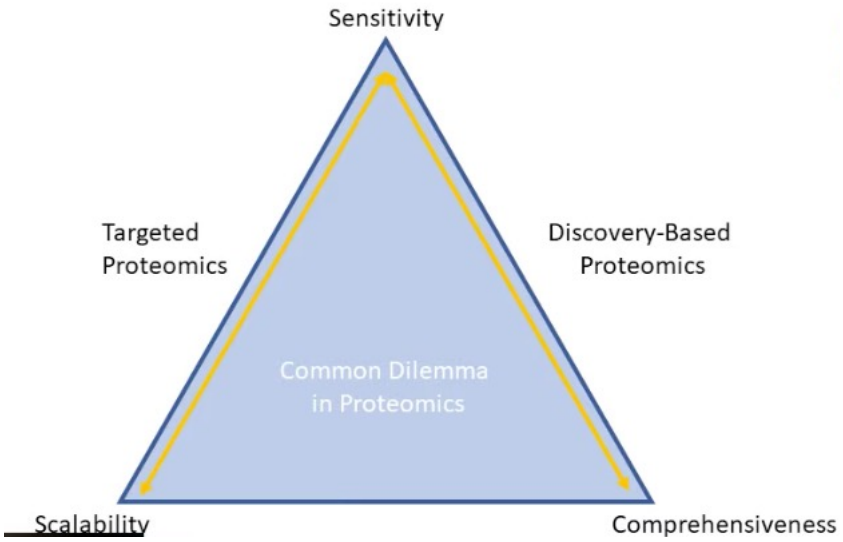
- Aim: Quantifying individual proteins and their modifications in biological systems



Mark Larance and Angus I. Lamond Mol Cell Bio 2015

Bottom-up approach: Proteomics Workflows

- Strategies for quantitative proteomics



Challenges of MS based Proteomics:

Quantitation of complex samples using MS is challenging due to:

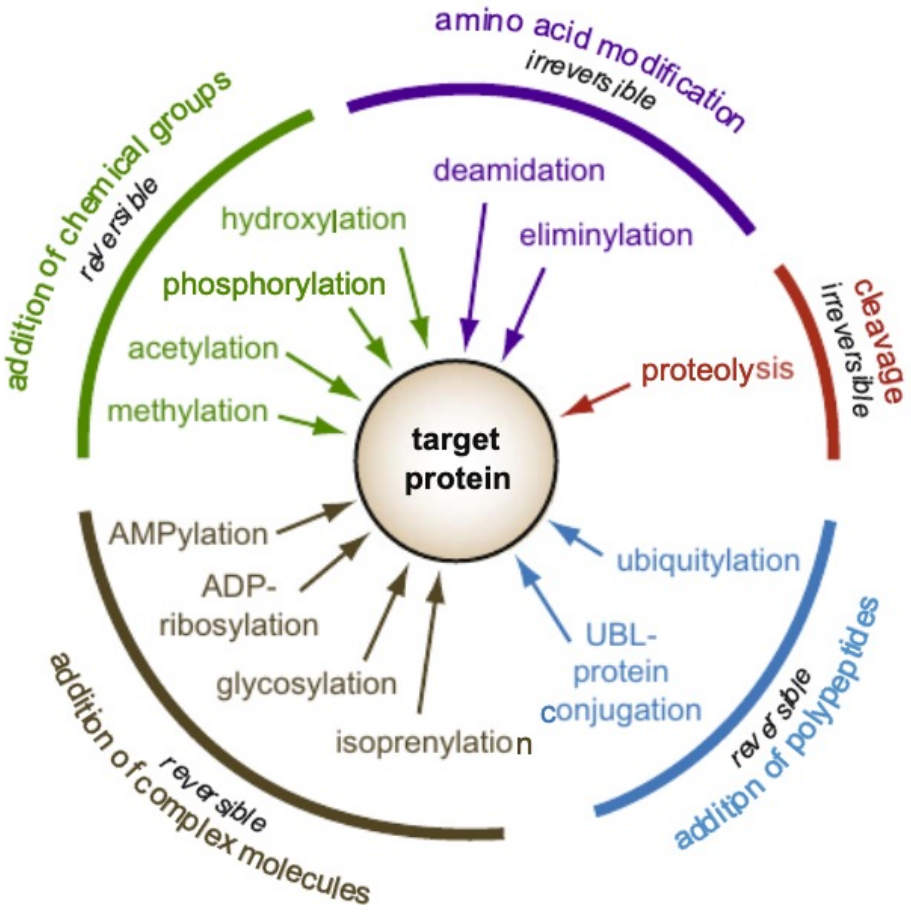
- Wide dynamic range of abundance
- Sensitivity limits of instrumentation
- Ionization suppression
- Missing information for quantitation

Solutions:

- Reduce sample complexity through enrichment (immunoprecipitation)
- Introduce isotopically labeled internal standards

Bottom-up Proteomics workflows

- Strategies to enrich for post-translational modification (PTM)



The most common PTMs in eucaryotic cells:

- Phosphorylation (S, T and Y)
- Ubiquitinylation (K)
- Glycosylation (N and O mainly)
- Acetylation (K, S)

Bottom-up Proteomics workflows

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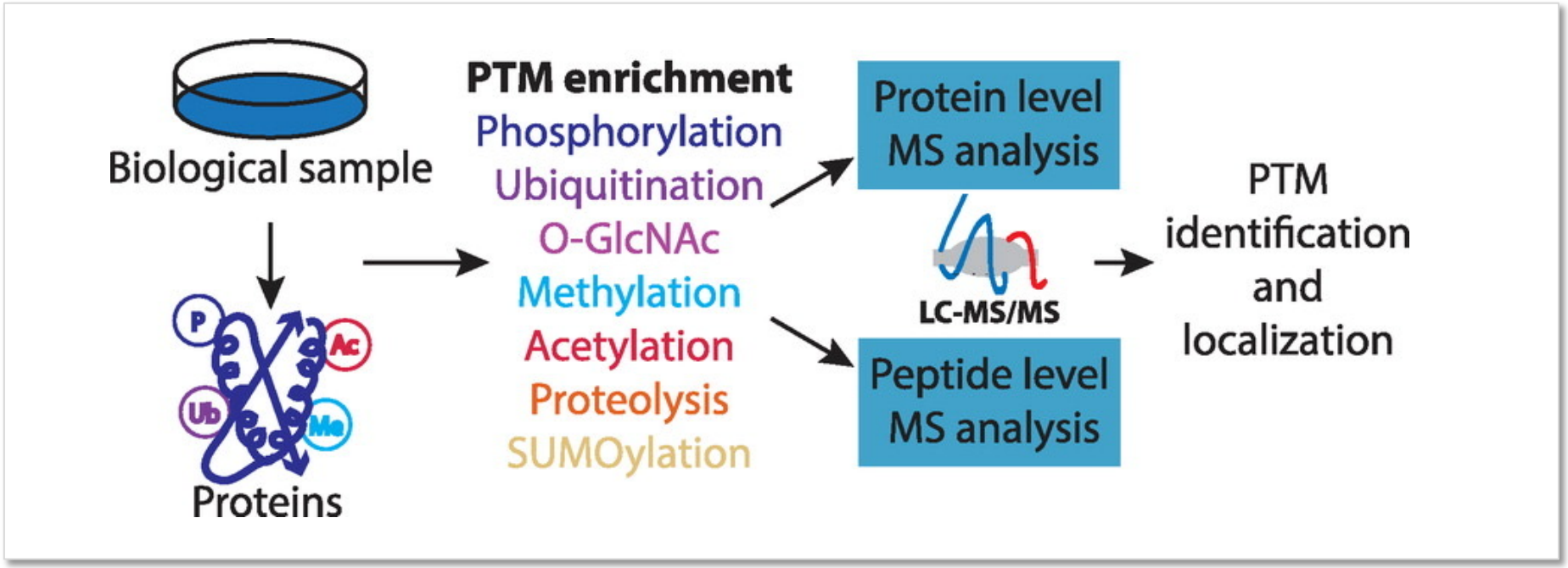
- How to identified PTMs by mass spectrometry ?



Bottom-up Proteomics workflows

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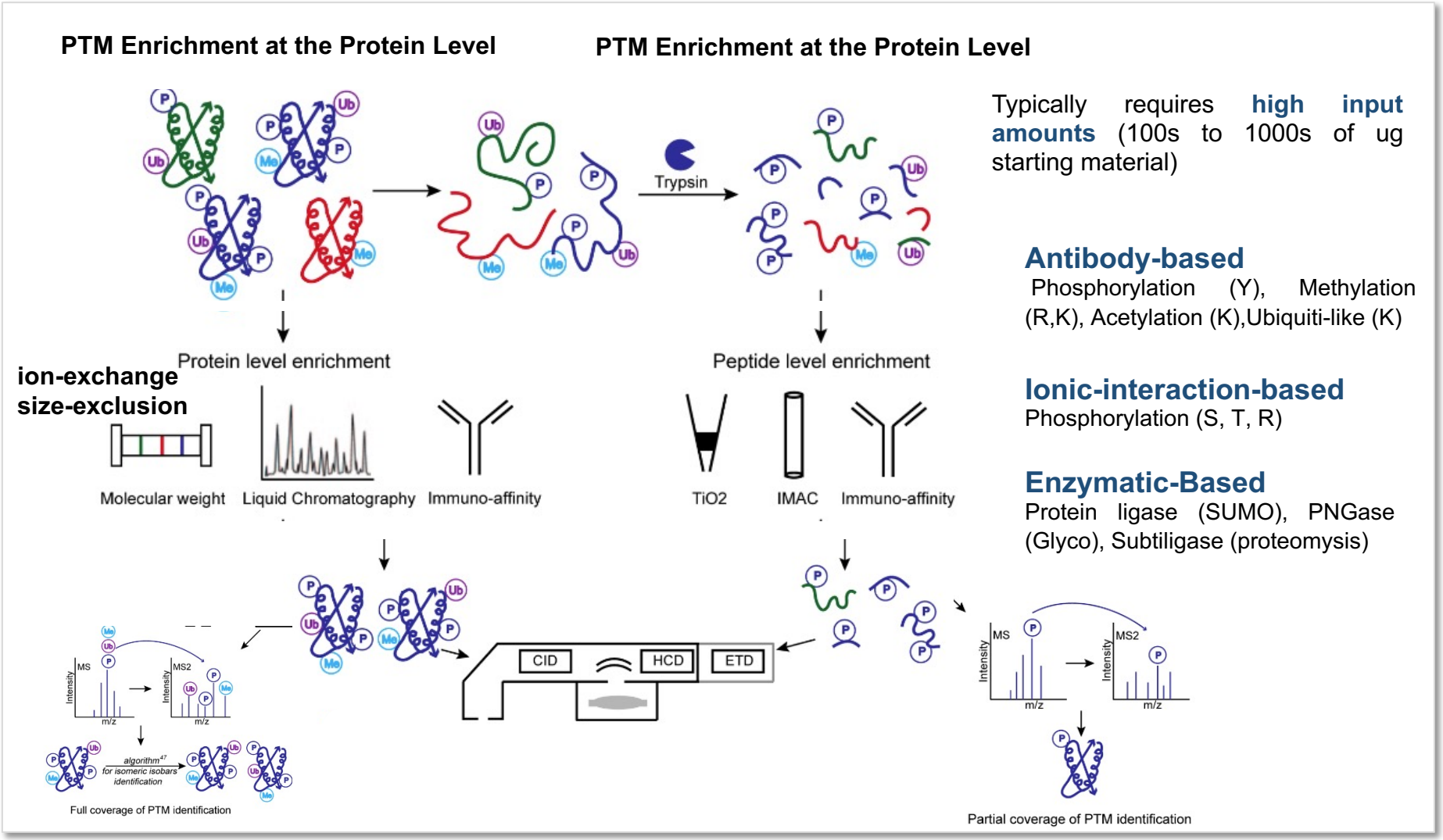
- PTM Enrichment Strategies



Typically requires **high input amounts** (100s to 1000s of ug starting material)

Antibody-based
Phosphorylation (Y), Methylation (R,K), Acetylation (K),Ubiquiti-like (K)

Ionic-interaction-based
Phosphorylation (S, T, R)

Enzymatic-Based
Protein ligase (SUMO), PNGase (Glyco), Subtiligase (proteomysis)

Doll & Burlingame, ACS Chem Biol, 2015

Discussion

Quantitative Proteomics

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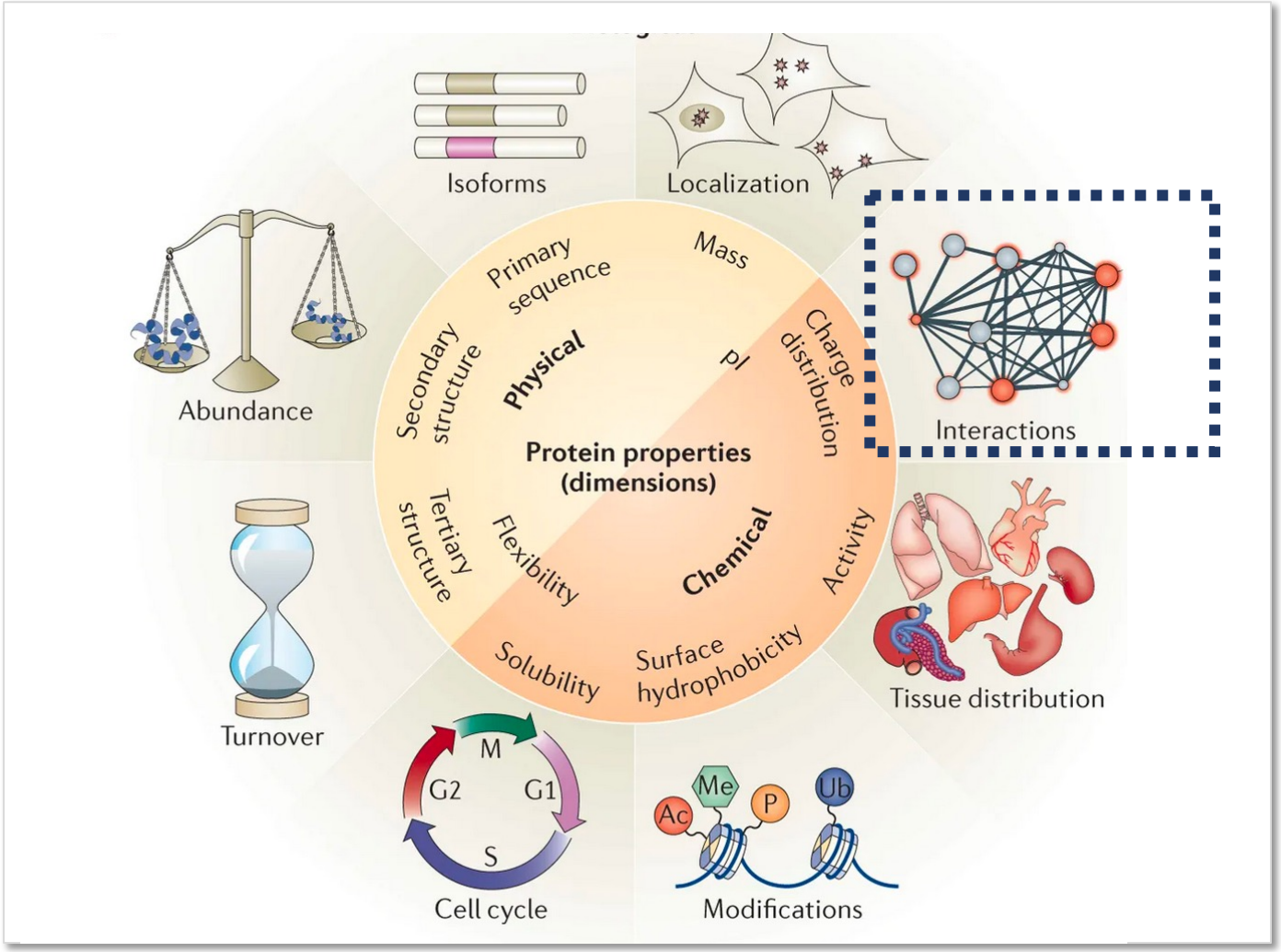
- Aim: Quantifying individual proteins and their modifications in biological systems



Mark Larance and Angus I. Lamond Mol Cell Bio 2015

Integrative structural biology

- Combined techniques to solve the structure of large dynamics complexes