SV PTECH PTP-PCF

Physics of Life (PHYS-468)

Mass Spectrometry of Protein Samples

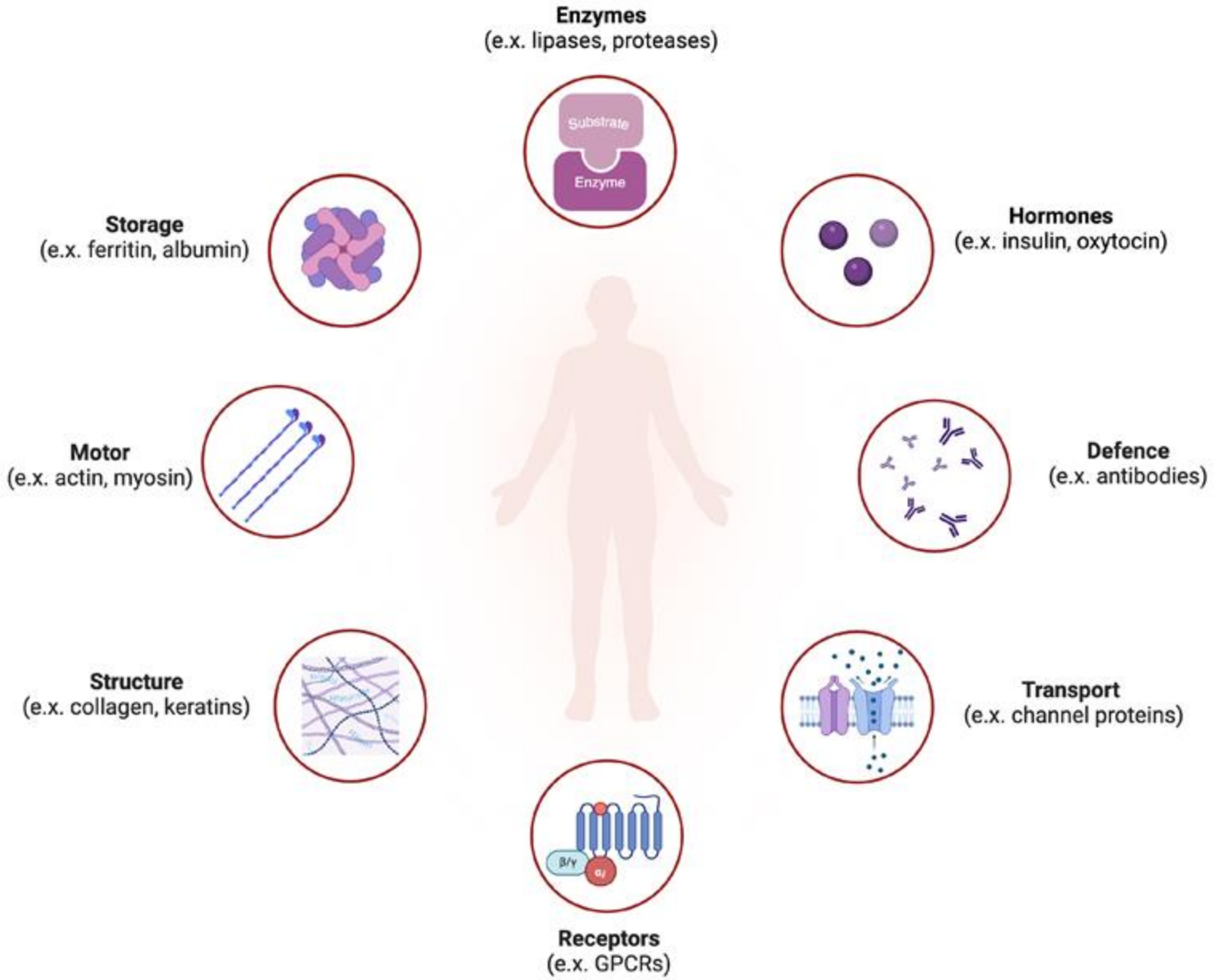
Maria Pavlou, PhD

Head of Proteomics Core Facility

Name your favorite protein

Maria Pavlou

Meet the most famous ones



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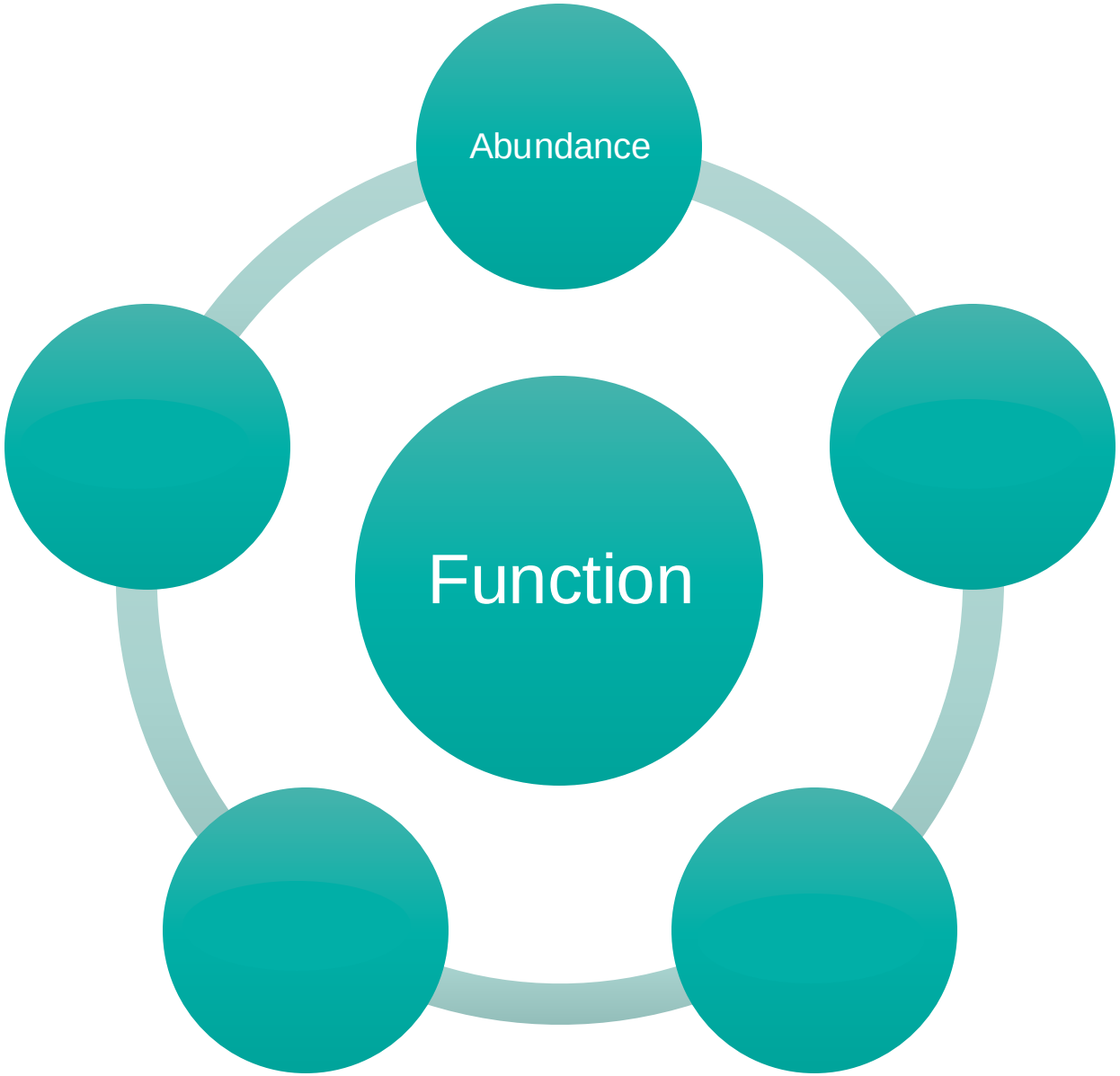
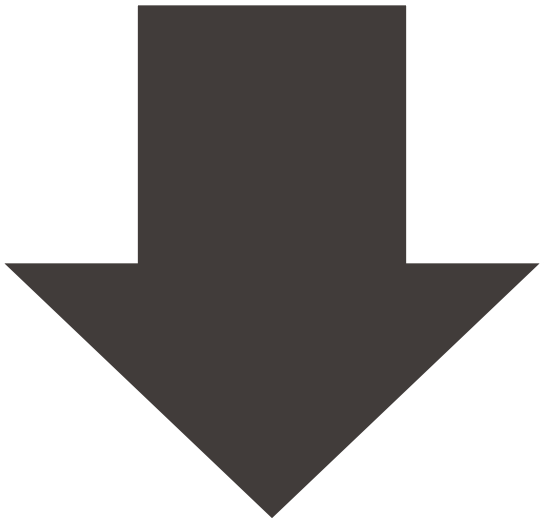
Proteins are the building blocks of biology



The good, the bad and the ugly of proteins (compared to mRNA)

5x more stable

2800x more abundant



10^4 x higher dynamic range

5x more complex

No PCR

Munro V et al. Proteomics 2024 24:2200220

Proteomes and Proteomics

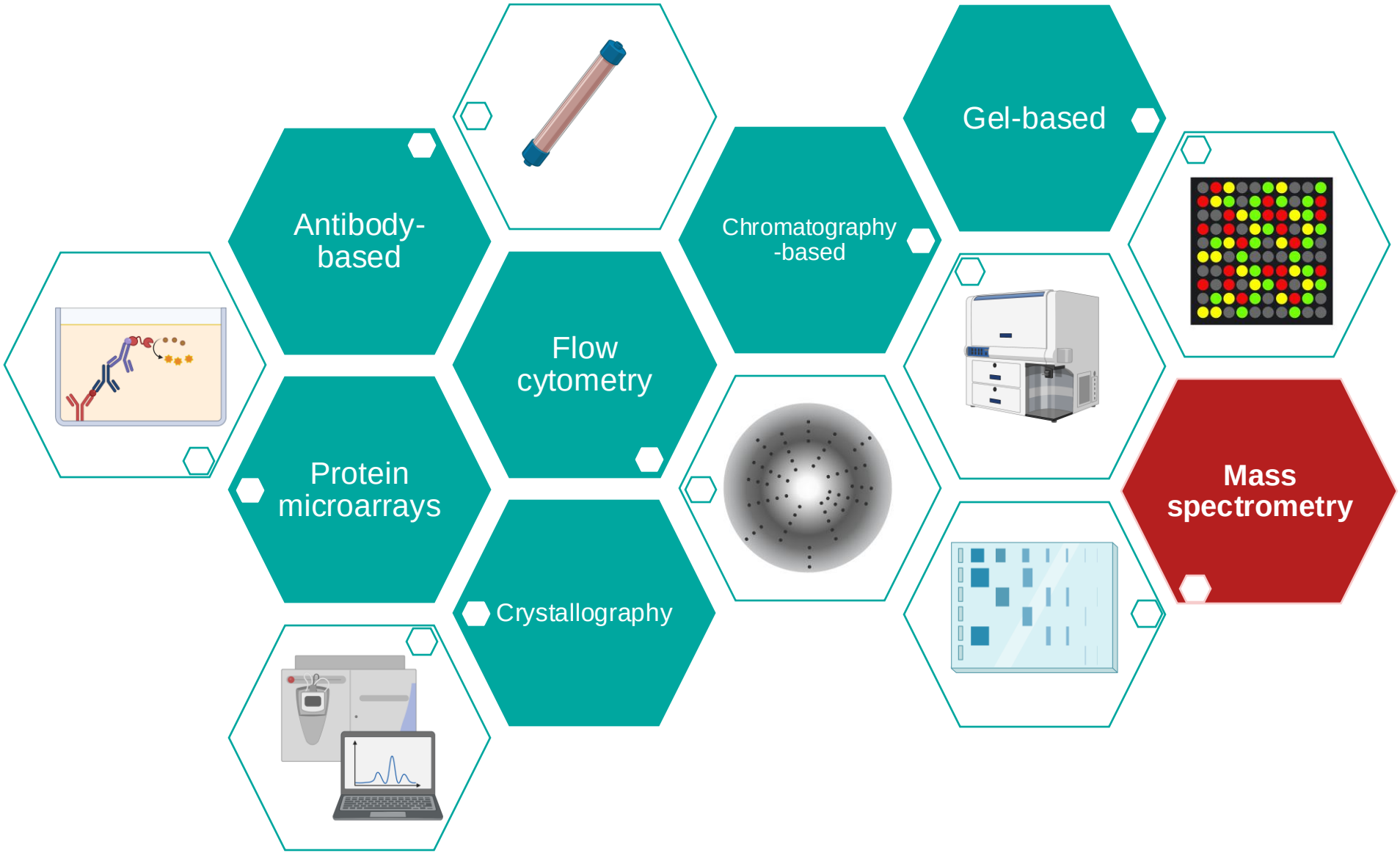
"**Proteome**": **PROTE**ins expressed by a gen**OME**

"**Proteomics**": methods (-omics) dedicated to the analysis of **proteomes**

Represents the effort to establish the *identities*, *quantities*, *structures*, and *biochemical and cellular functions* of all proteins in an organism, organ, or organelle, and how these properties vary in space, time, or physiological state.

MCP 1.10 pg 675 National Research Council Steering committee

Proteomic tools



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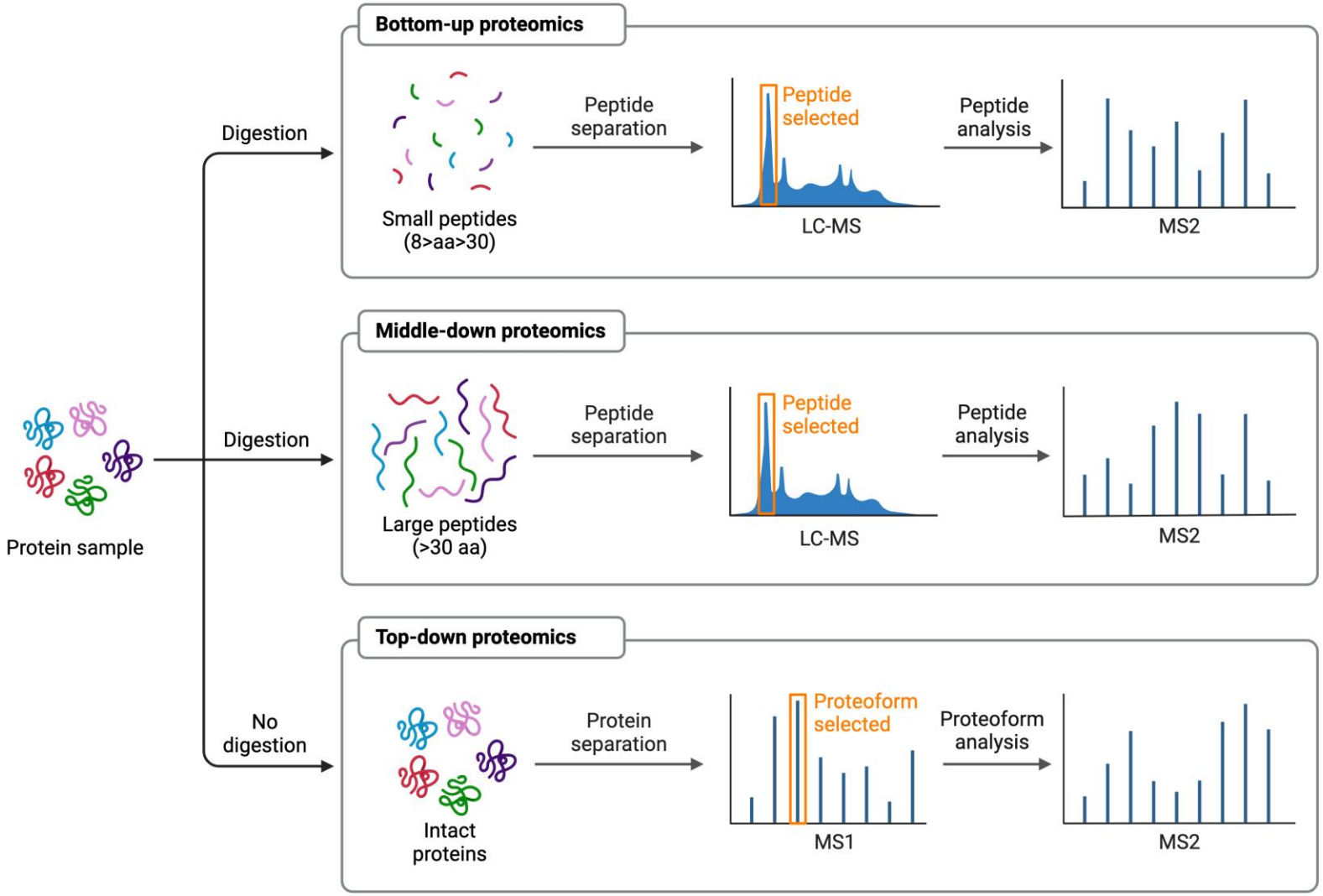
MS-based proteomics

MS-based proteomics workflows
Top or bottom? Up or down?



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PCF

SB
MSEAP

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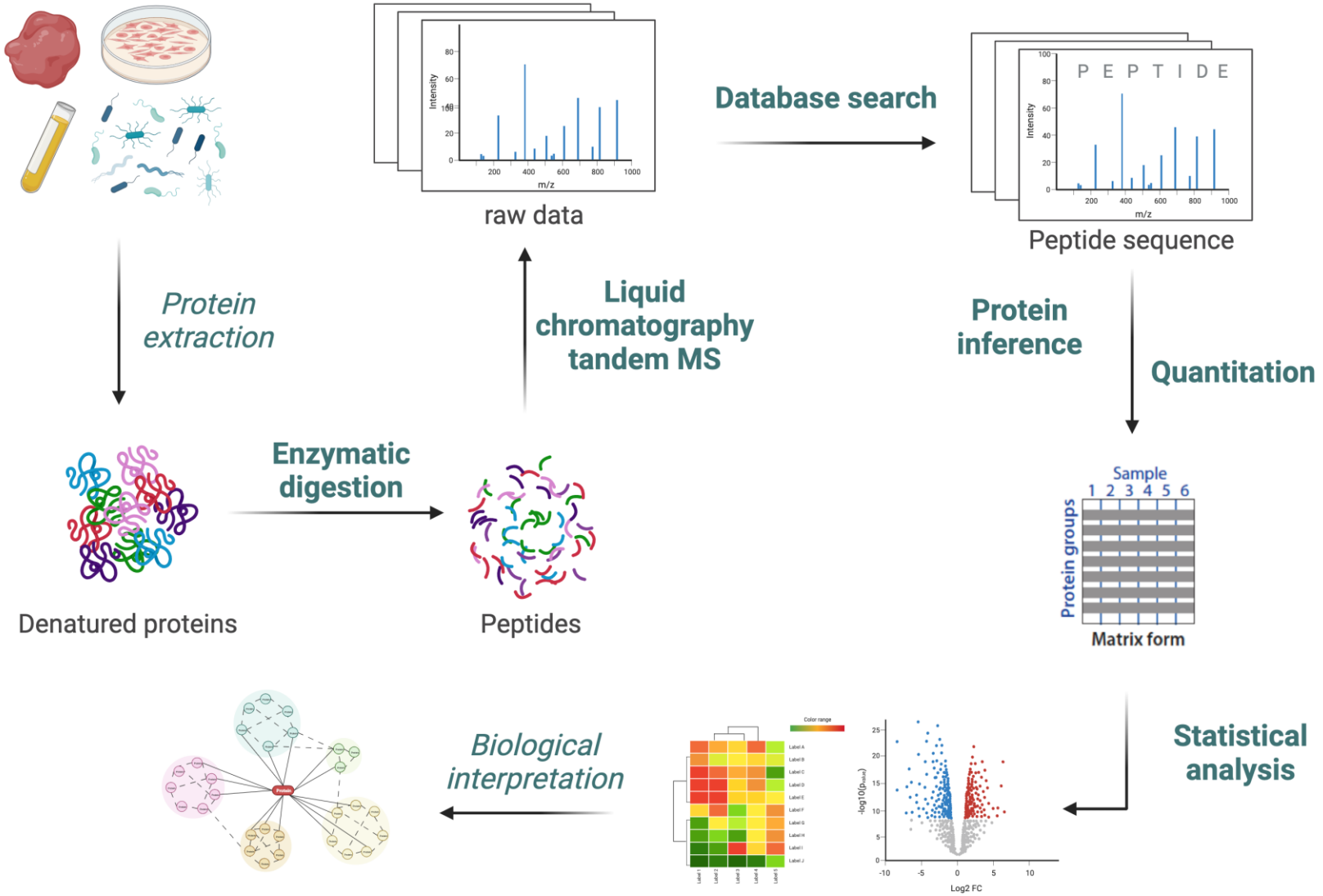
Typical bottom-up workflow



Protein extraction

Physical disruption

- Sonication
- Bead-beating
- Freeze-thaw
- Grinding

Detergents and chaotropic substances

- Protein extraction
- Protein solubilisation

Detergents and MS

Common detergents are incompatible with

1. Reverse phase liquid chromatography (compromise fractionation)
2. Mass spectrometry (ion suppression)

Detergent removal

✓Dialysis

✓Filtration

✓Electrophoresis (eg. SDS)

✓Protein precipitation

✓Dilution (eg. Urea)

MS-compatible detergents

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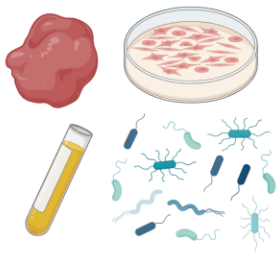
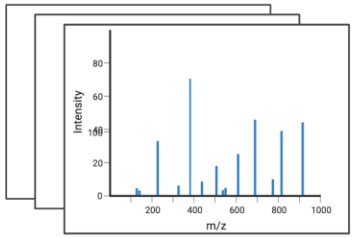
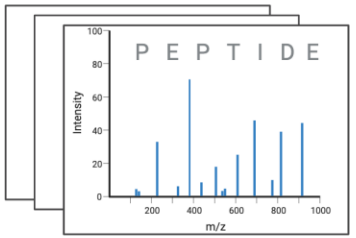
Typical bottom-up workflow





Database search



Protein extraction

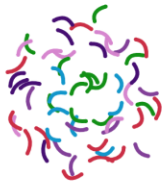
Liquid chromatography tandem MS

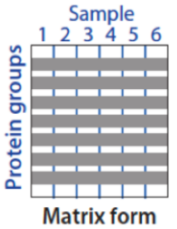
Protein inference

Quantitation

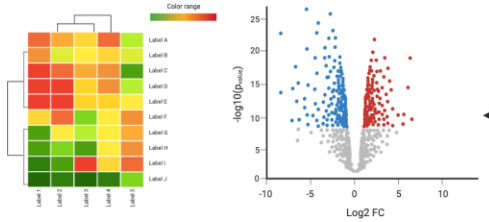

Denatured proteins

Enzymatic digestion


Peptides



Biological interpretation



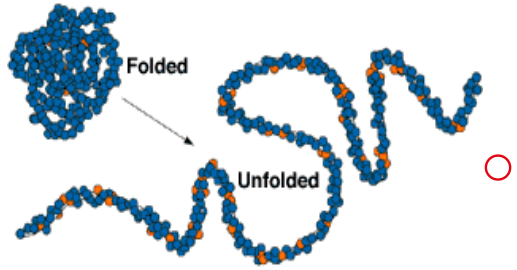
Statistical analysis

Trypsin- the star of proteases

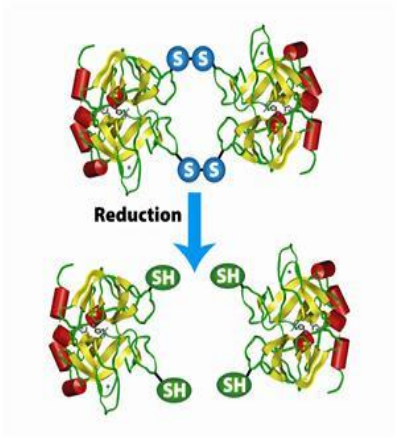
- ✓Highly specific and efficient; C-term of the basic residues Lysine and Arginine (except when followed by Proline)
- ✓Lys and Arg are relatively abundant and usually well distributed throughout a protein; many peptides of MS-reasonable size
- ✓Relatively cheap
- ✓Produces peptides with at least two charges (important for ionization)

Digestion of proteins

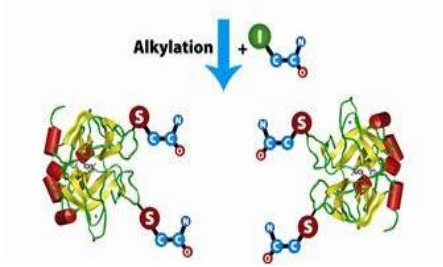
1. Denaturation



2. Reduction



3. Alkylation



- Denaturing agents
  - Urea, guanidinium chloride, SDS, Rapigest...
  - ✧Think about the way how to remove the detergent afterwards!
  - ✧Don’t forget to dilute denaturing agents before adding the digestion enzyme (***why?***)
- Buffers
  - Tris, HEPES, Ammonium bicarbonate
  - ✧ Be aware of the optimal pH of your digestion enzyme

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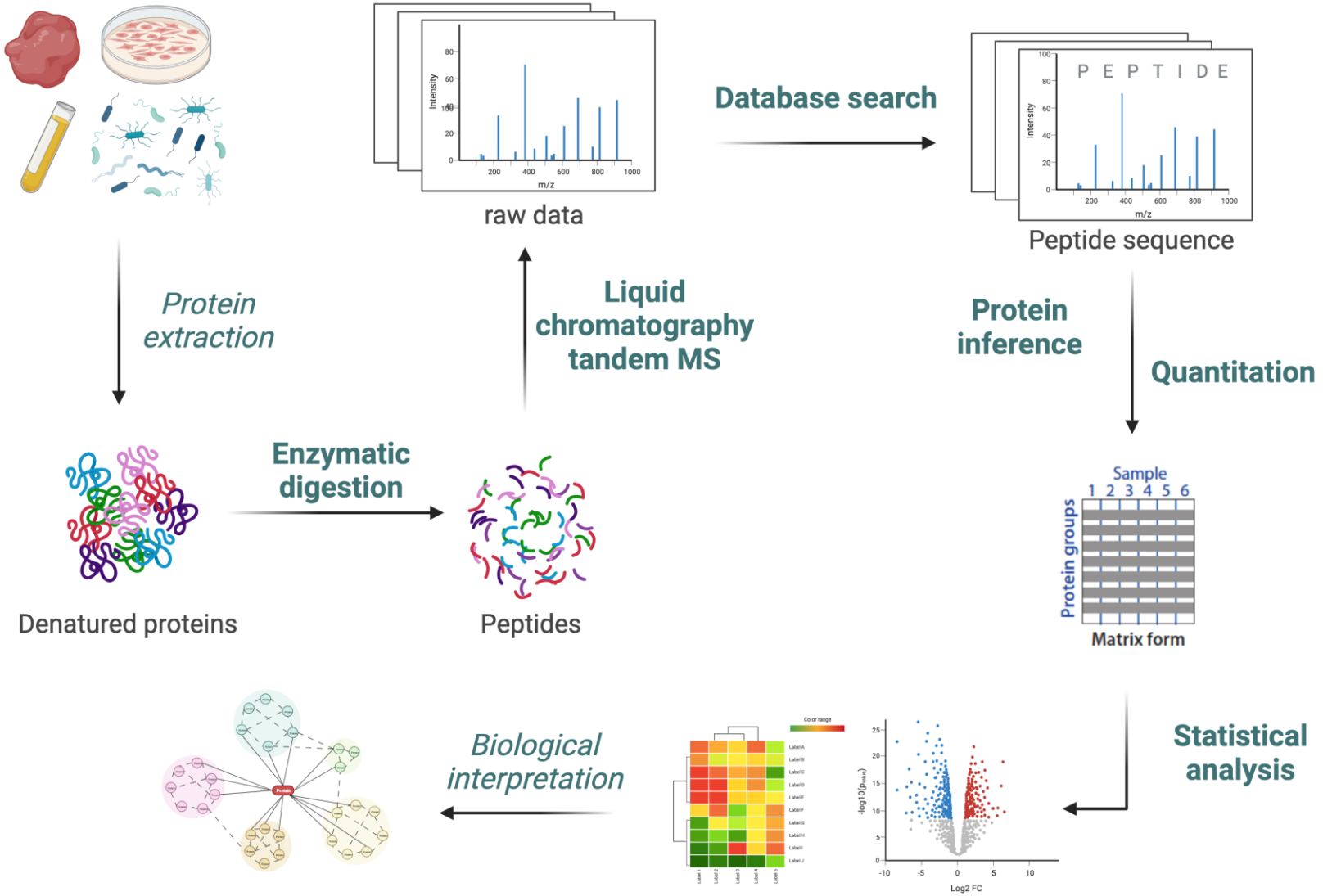
Typical bottom-up workflow



Reverse Phase (RP) chromatography

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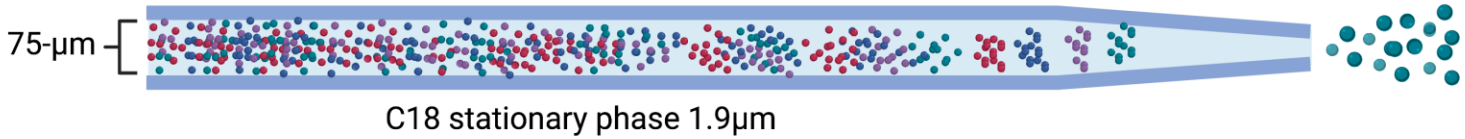
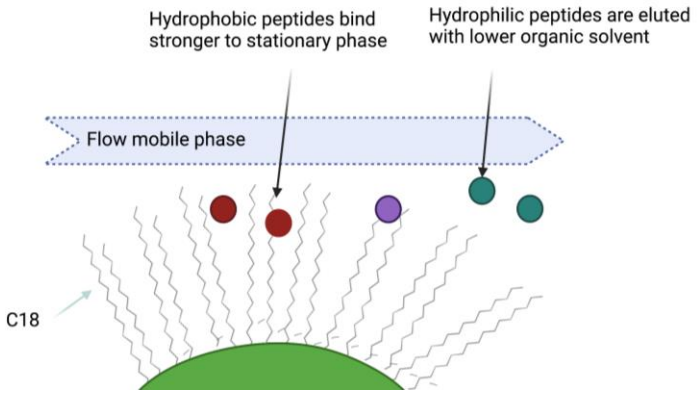
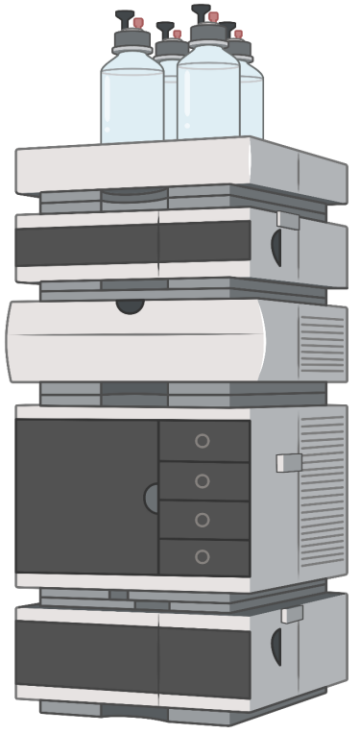
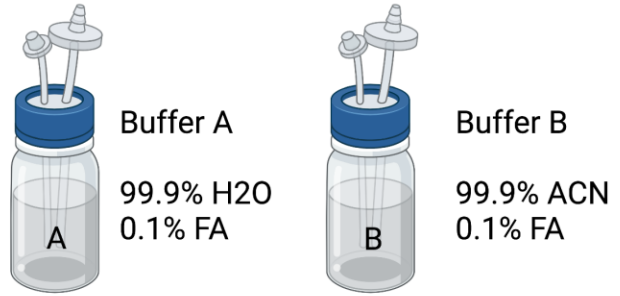


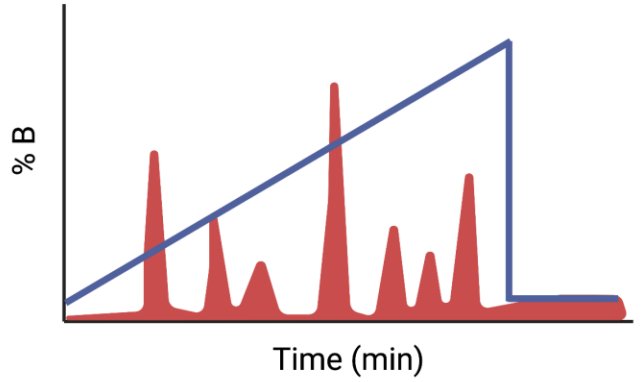


Hydrophobic stationary phase

Gradient: 3-90% organic solvent







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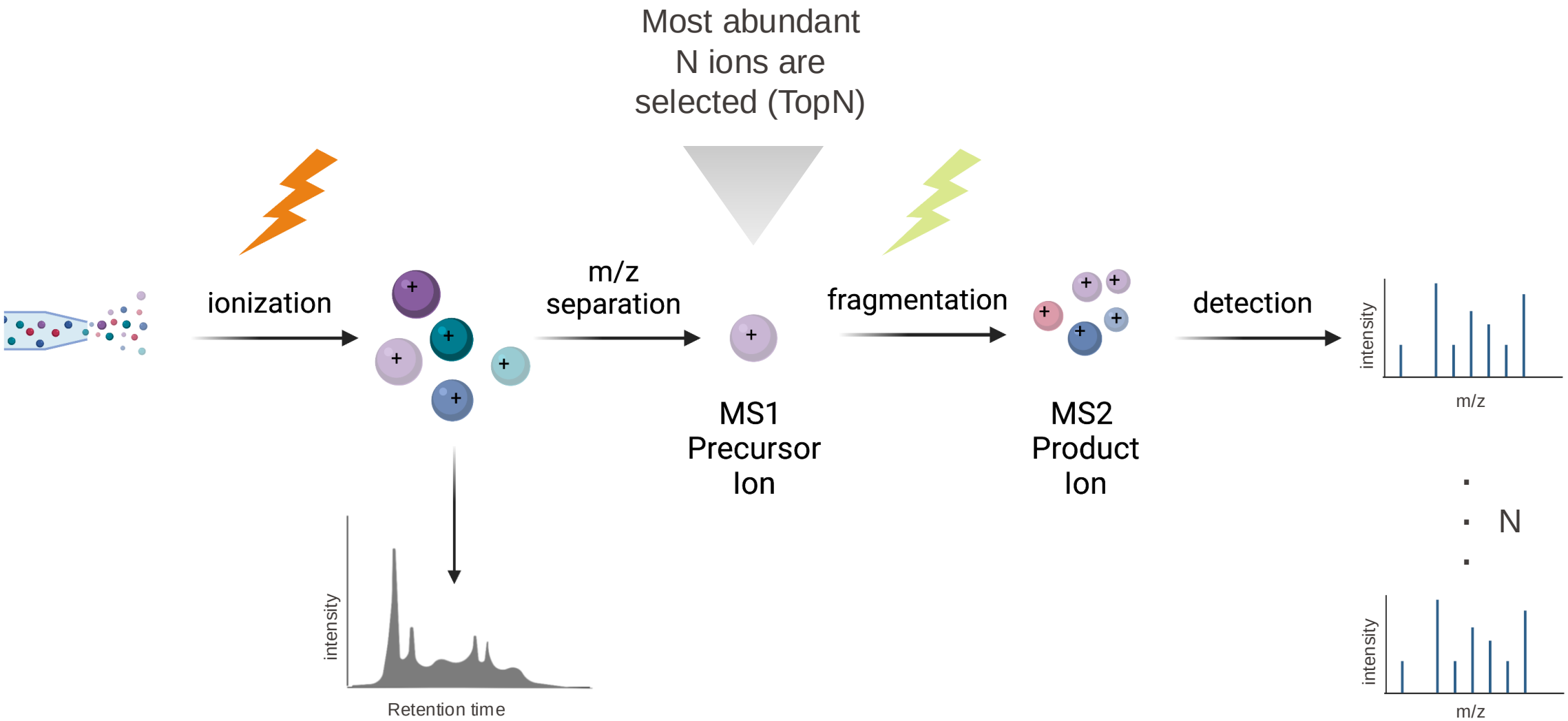
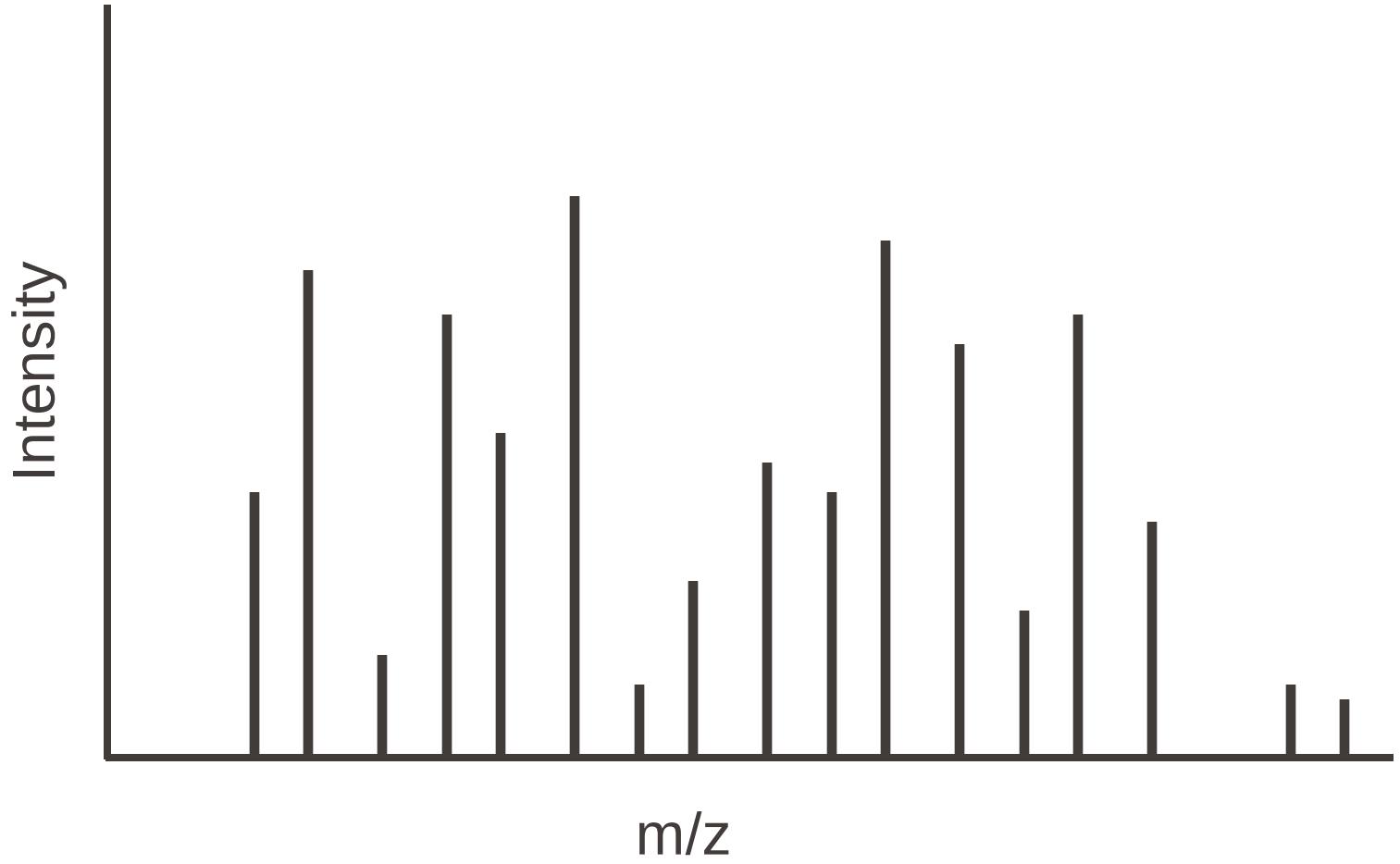
Tandem MS (MS/MS)
Data Dependent Acquisition (DDA)



MS2 spectrum in 2D



Peptide sequence identification

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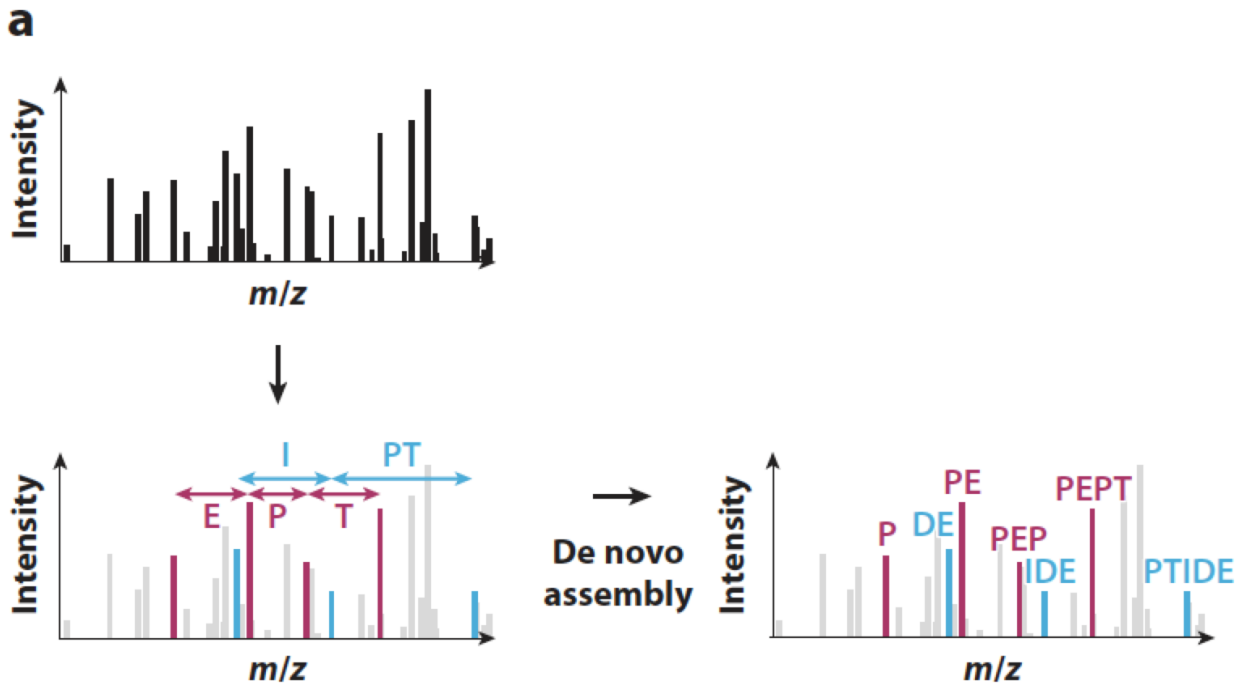

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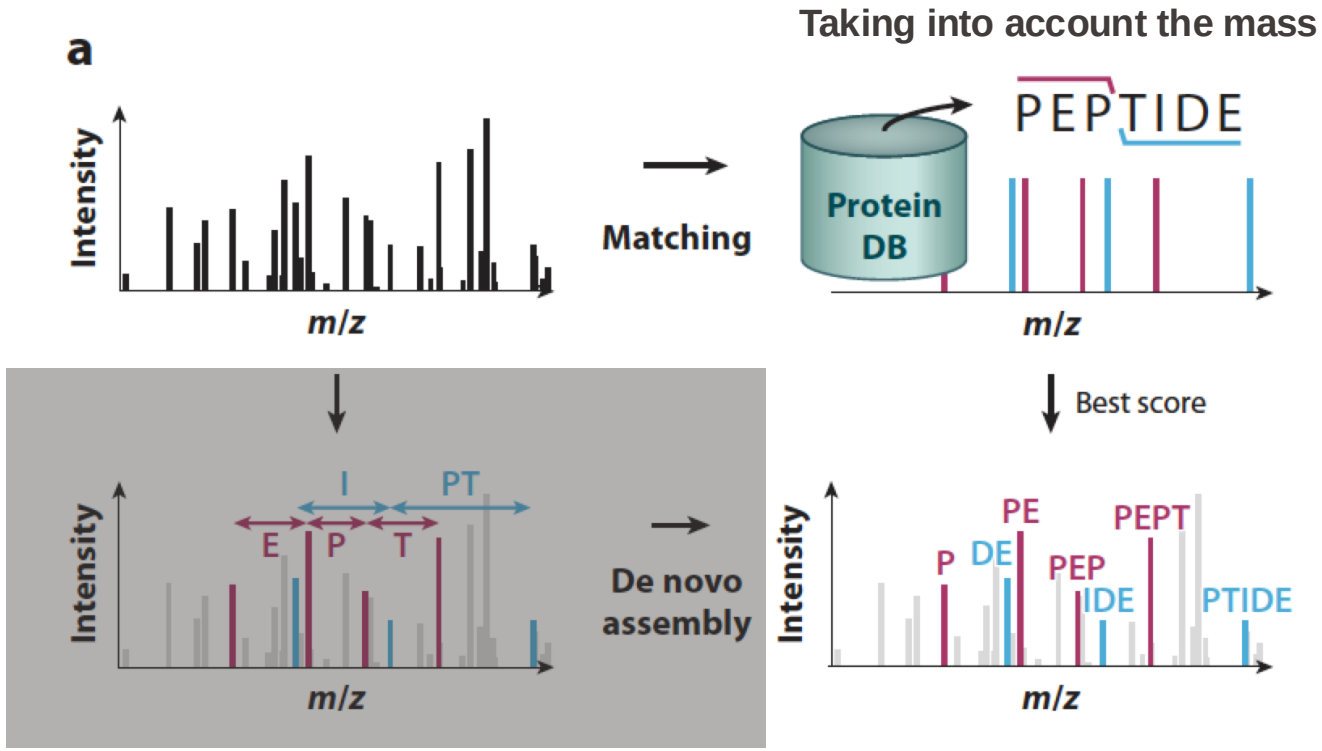

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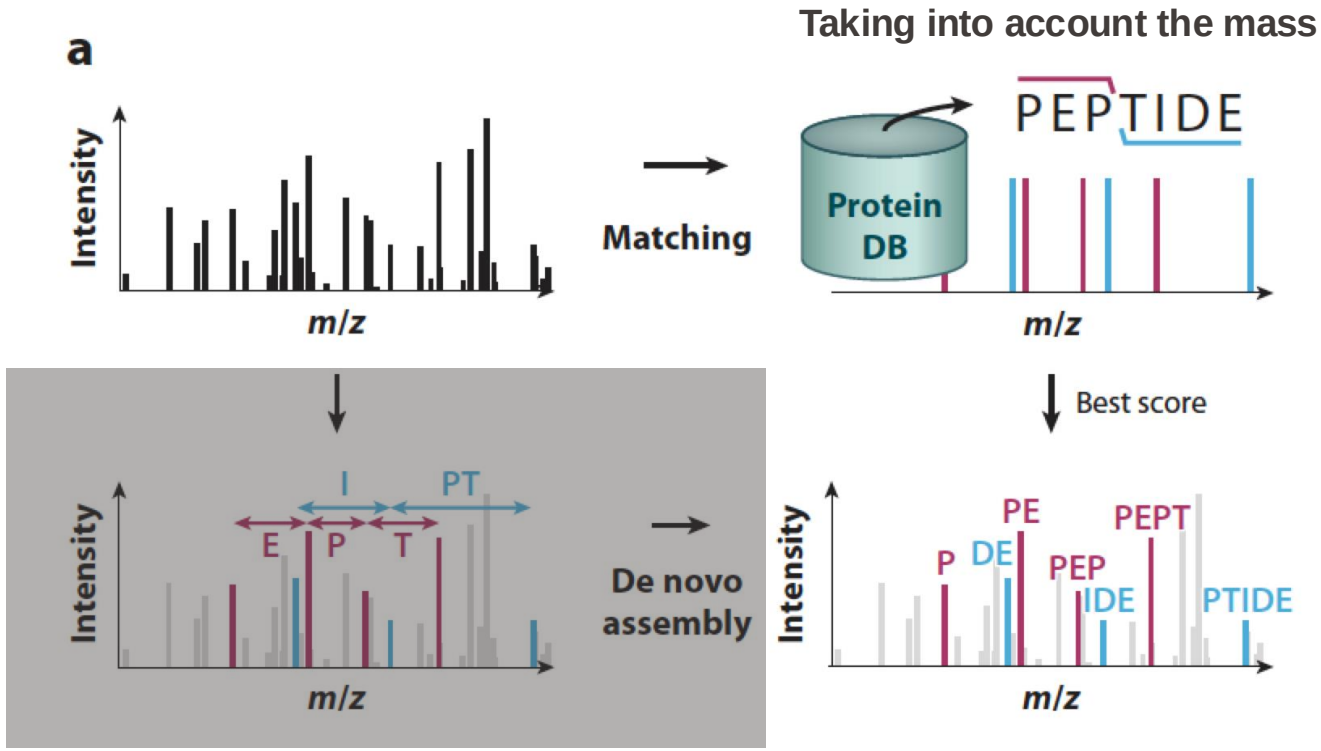
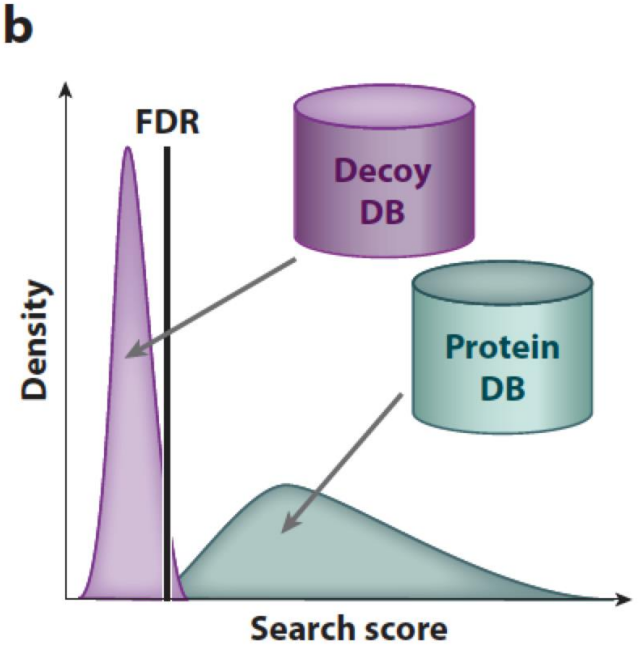
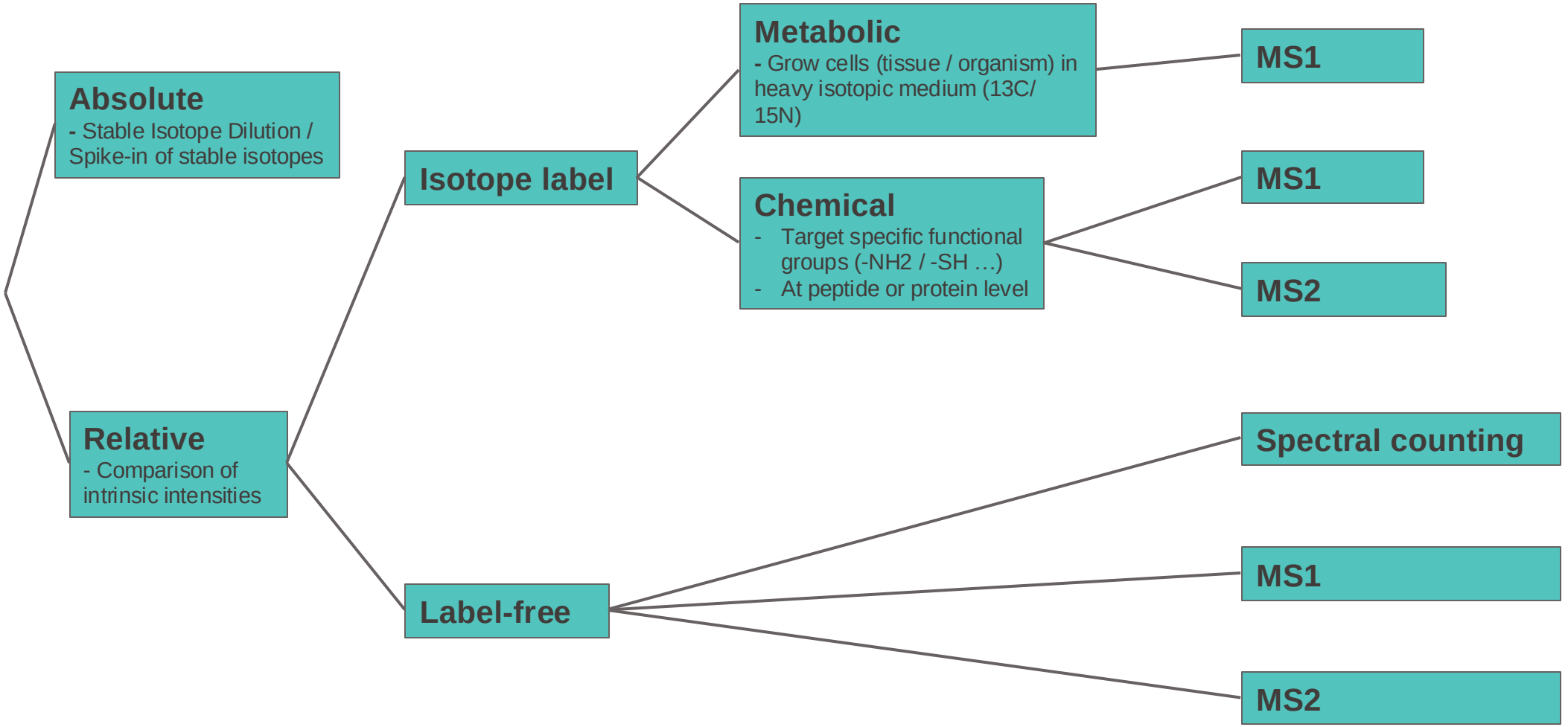




Quantitative proteomics strategies



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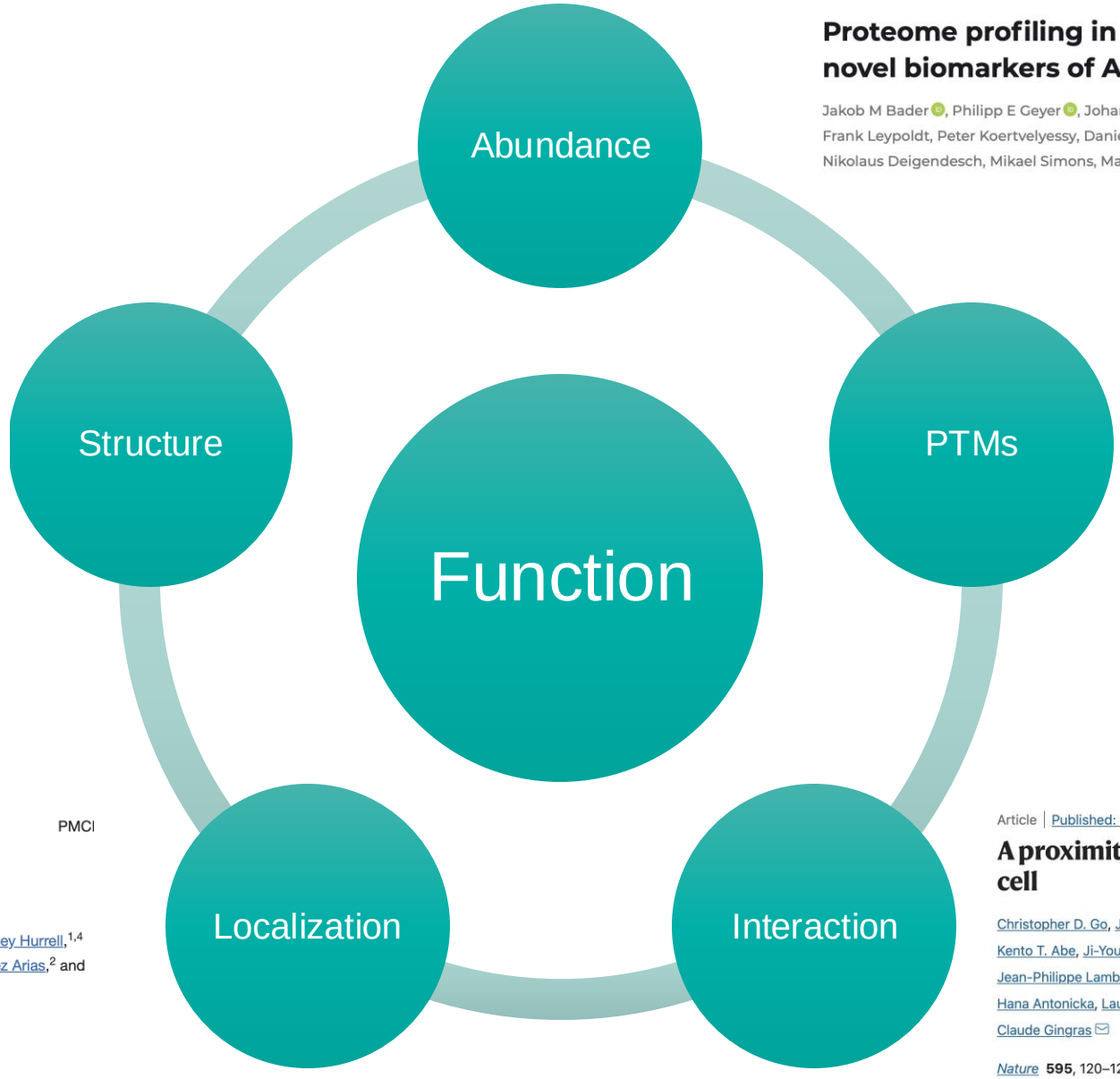


Article | 2 June 2020 | OPEN ACCESS | TRANSPARENT PROCESS

Proteome profiling in cerebrospinal fluid reveals novel biomarkers of Alzheimer's disease

Jakob M Bader, Philipp E Geyer, Johannes B Müller, Maximilian T Strauss, Manja Koch, Frank Leypoldt, Peter Koertvelyessy, Daniel Bittner, Carola G Schipke, Enise I Incesoy, Oliver Peters, Nikolaus Deigendesch, Mikael Simons, Majken K Jensen, Henrik Zetterberg, Matthias Mann

Measuring protein structural changes on a proteome-wide scale using limited proteolysis-coupled mass spectrometry

Simone Schopper, Abdullah Kahraman, Pascal Leuenberger, Yuehan Feng, Ilaria Piazza, Oliver Müller, Paul J Boersema & Paola Picotti

Nature Protocols **12**, 2391–2410 (2017) | Cite this article

Article | Open access | Published: 07 December 2022

Phosphoproteomic analysis of neoadjuvant breast cancer suggests that increased sensitivity to paclitaxel is driven by CDK4 and filamin A

S. Mouron, M. J. Bueno, A. Lluch, L. Manso, I. Calvo, J. Cortes, J. A. Garcia-Saenz, M. Gil-Gil, N. Martinez-Janez, J. V. Apala, E. Caleiras, Pilar Ximénez-Embún, J. Muñoz, L. Gonzalez-Cortijo, R. Murillo, R. Sánchez-Bayona, J. M. Cejalvo, G. Gómez-López, C. Fustero-Torre, S. Sabroso-Lasa, N. Malats, M. Martinez, A. Moreno, D. Megias, ... M. Quintela-Fandino + Show authors

Nature Communications **13**, Article number: 7529 (2022) | Cite this article

Nat Commun. 2016; 7: 9992. PMCI
Published online 2016 Jan 12. doi: 10.1038/ncomms9992

A draft map of the mouse pluripotent stem cell spatial proteome

Andy Christoforou,[1,2] Claire M. Mulvey,[1,2] Lisa M. Breckels,[1,3] Aikaterini Geladaki,[1,2] Tracey Hurrell,[1,4] Penelope C. Hayward,[2] Thomas Naake,[1,3] Laurent Gatto,[1,3] Rosa Viner,[5] Alfonso Martinez Arias,[2] and Kathryn S. Lilley[a,1]

Article | Published: 02 June 2021

A proximity-dependent biotinylation map of a human cell

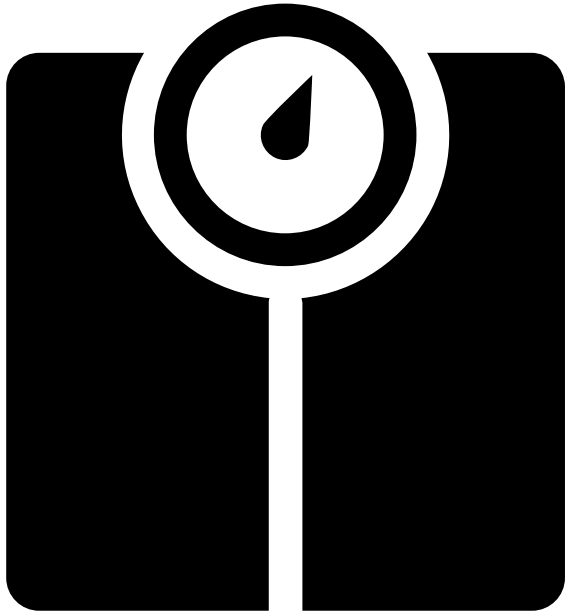
Christopher D. Go, James D. R. Knight, Archita Rajasekharan, Bhavisha Rathod, Geoffrey G. Hesketh, Kento T. Abe, Ji-Young Youn, Payman Samavarchi-Tehrani, Hui Zhang, Lucie Y. Zhu, Evelyn Popiel, Jean-Philippe Lambert, Étienne Coyaud, Sally W. T. Cheung, Dushyandi Rajendran, Cassandra J. Wong, Hana Antonicka, Laurence Pelletier, Alexander F. Palazzo, Eric A. Shoubridge, Brian Raught & Anne-Claude Gingras

Nature **595**, 120–124 (2021) | Cite this article

Mass Spectrometry

What is a mass spectrometer?

A Mass Spectrometer (MS) measures the mass-to-charge ratio (m/z) of ions

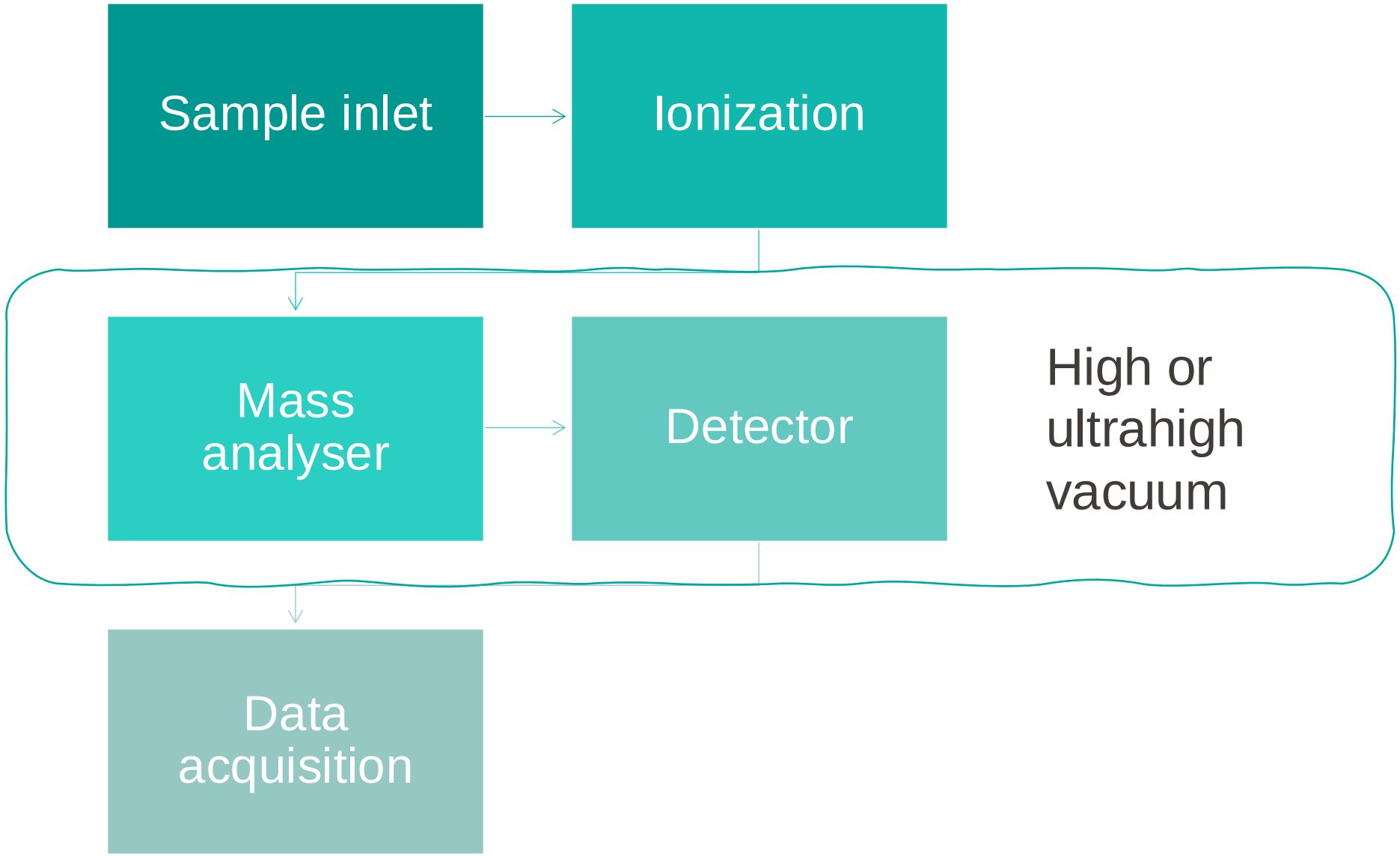


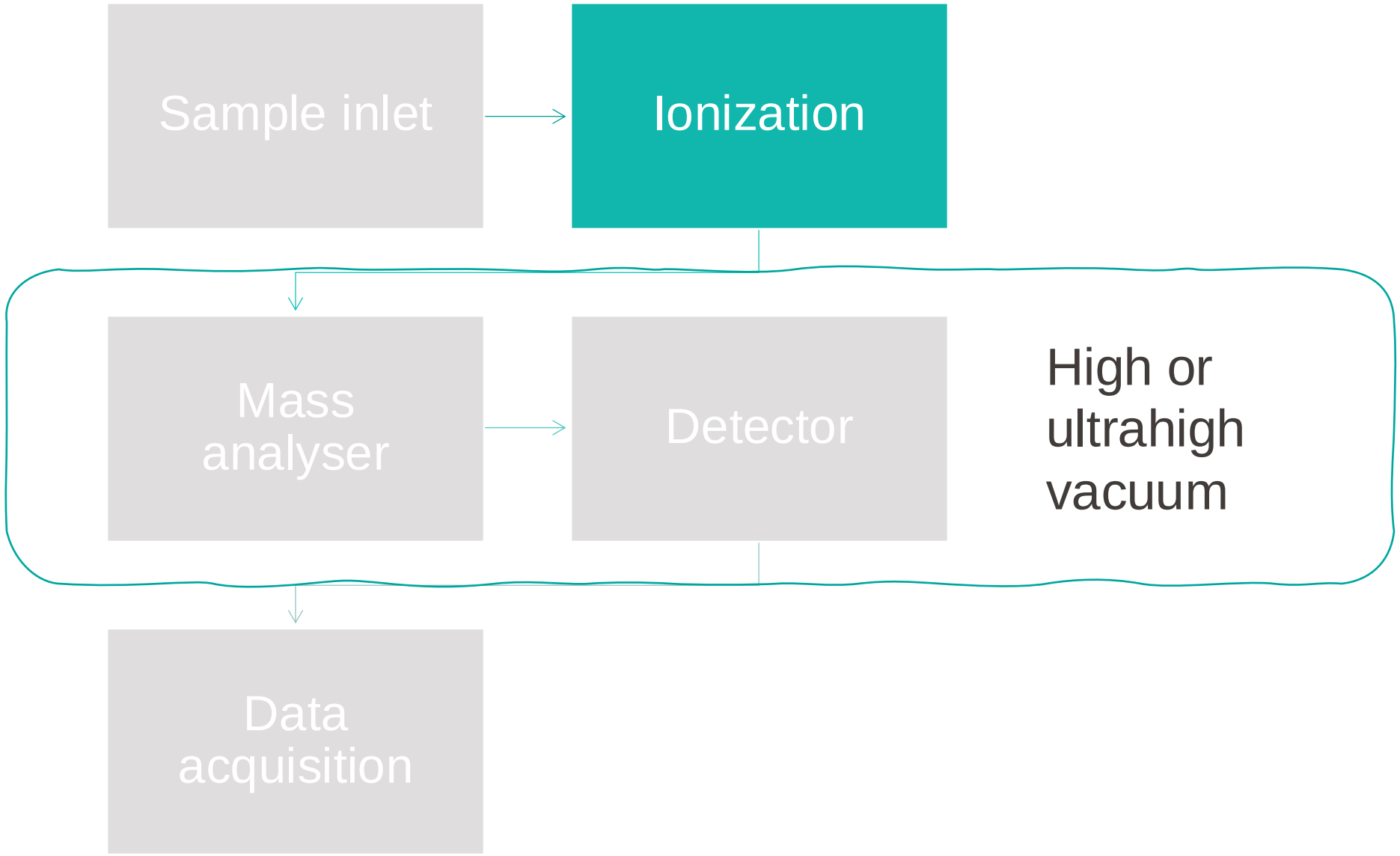
Molecular Scale

A mass-spec has 4 tasks…

1. Create ions from analyte molecules
2. Separate the ions based on charge and mass
3. Detect ions and determine their mass-to-charge (m/z)
4. Select and fragment ions of interest to provide structural information (MS/MS)

...and 5 parts


Sample inlet
Ionization
Mass analyser
Detector
High or ultrahigh vacuum
Data acquisition


Sample inlet
Ionization
Mass analyser
Detector
High or ultrahigh vacuum
Data acquisition

Ionization techniques

- Mass spectrometers measure the mass-to-charge (m/z) ratios of gas phase ions
  - Molecules (in solution or solid state) have to be turned into gas phase ions before they enter the mass spectrometer
  - Proteins are polar, non-volatile and thermally labile: Can an elephant fly?

- Most common in Proteomics
  - Electrospray ionization (ESI)
  - Matrix-assisted laser desorption ionization (MALDI)

- Advantages
  - Atmospheric pressure
  - No unwanted fragmentation (soft ionization)

The Nobel Prize in Chemistry 2002

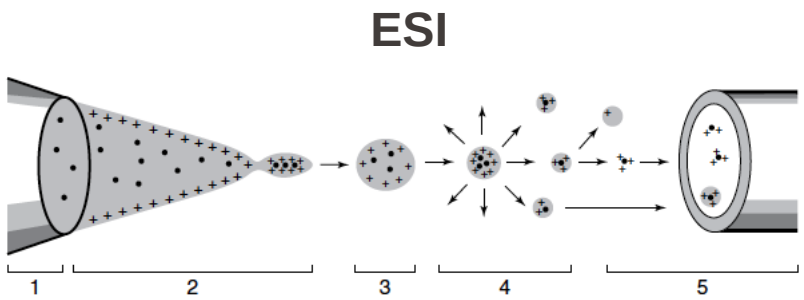
John B. Fenn

Prize share: 1/4

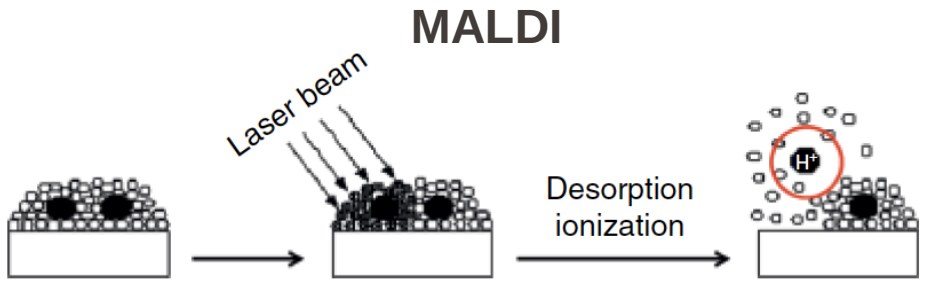
Koichi Tanaka

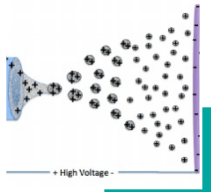
Prize share: 1/4

ESI versus MALDI



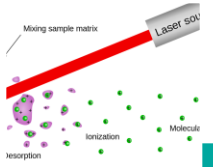
VS





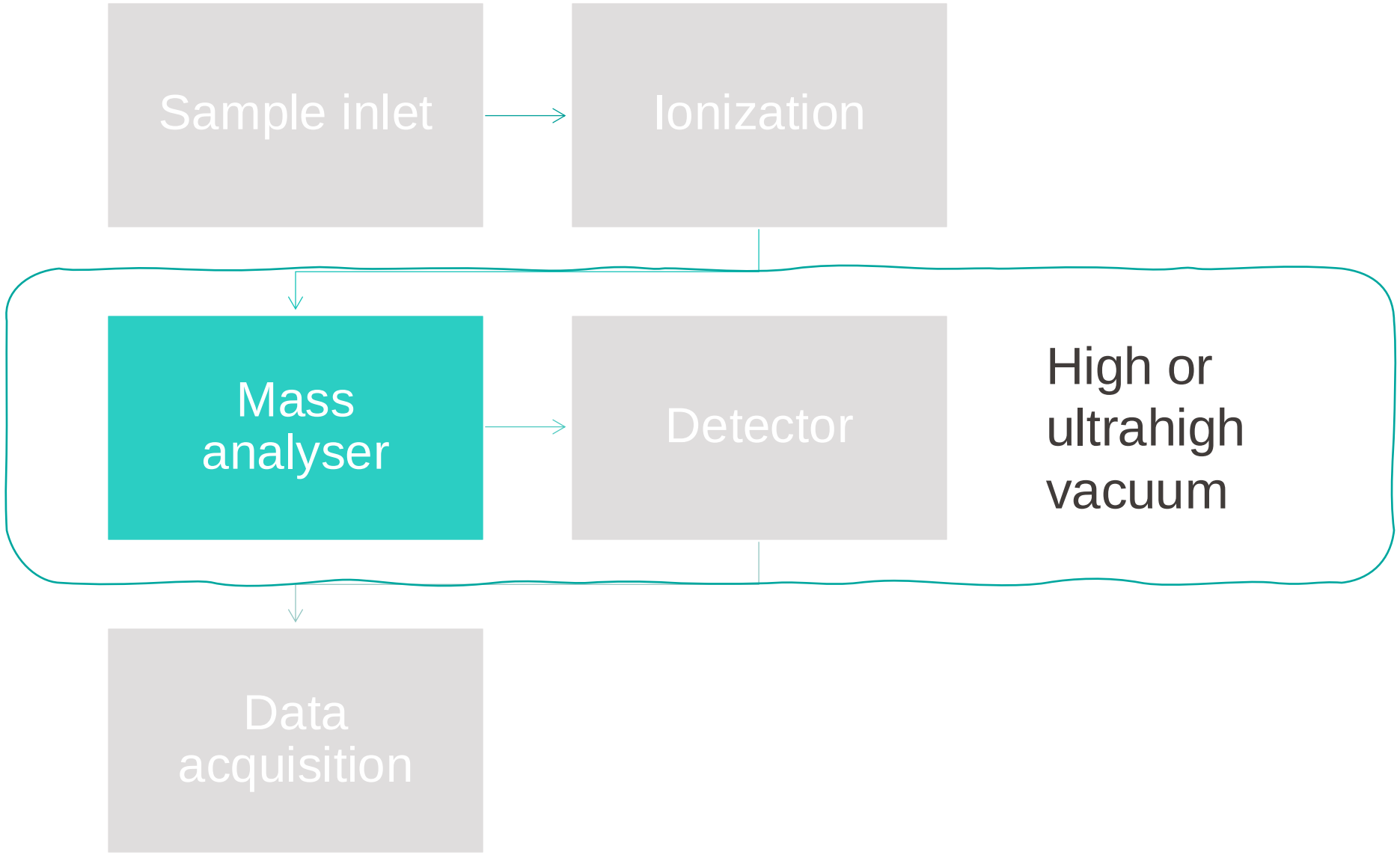
ESI

- Molecules in solution
- Continuous ion flow
- Multiple charge ions
- Sensitive to salts and detergents

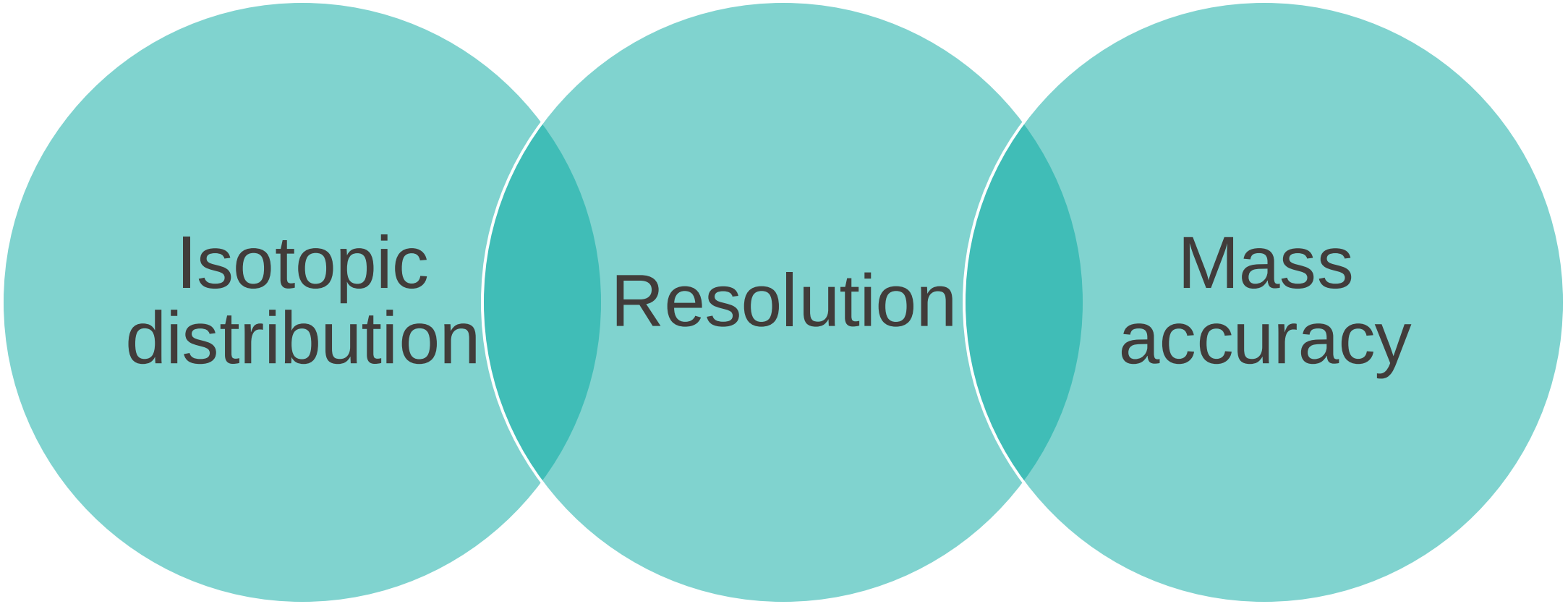


MALDI

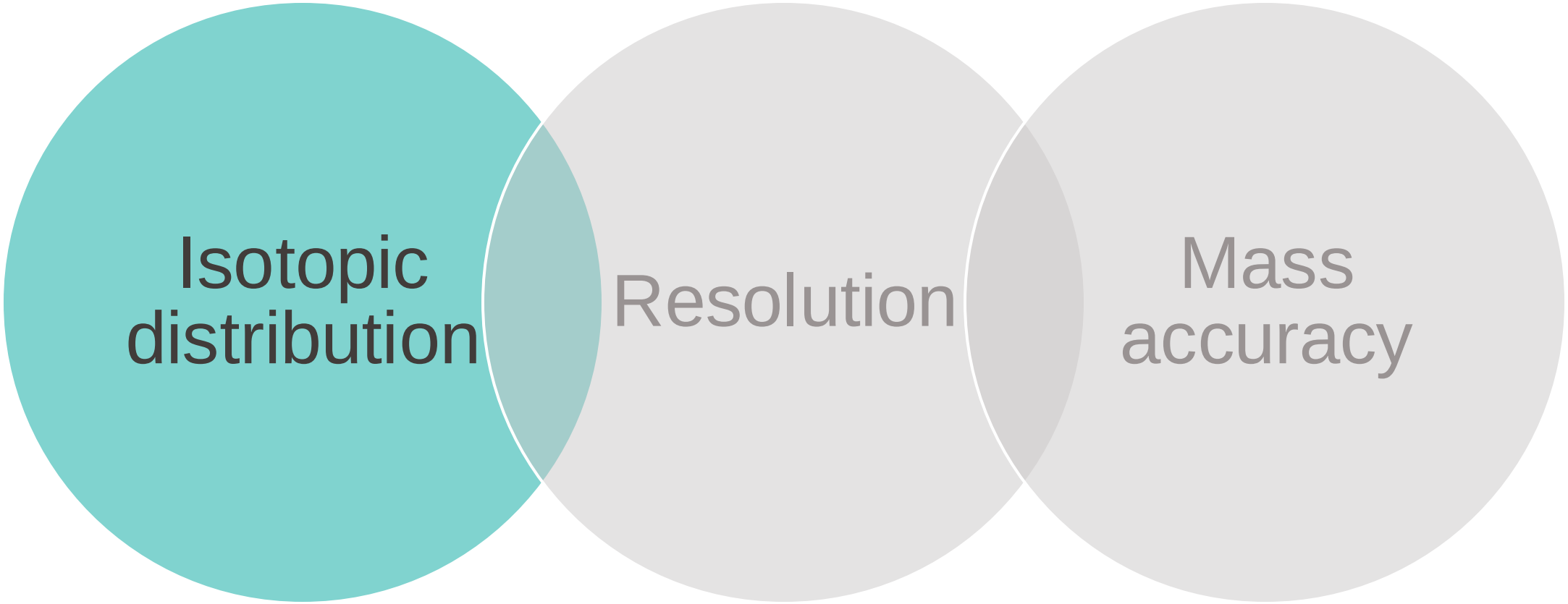
- Molecules in solid matrix
- Ion packages
- Single charge ions
- More tolerant in salts and detergents


Sample inlet
Ionization
Mass analyser
Detector
High or ultrahigh vacuum
Data acquisition

The basics of mass meassurements



The basics of mass meassurements



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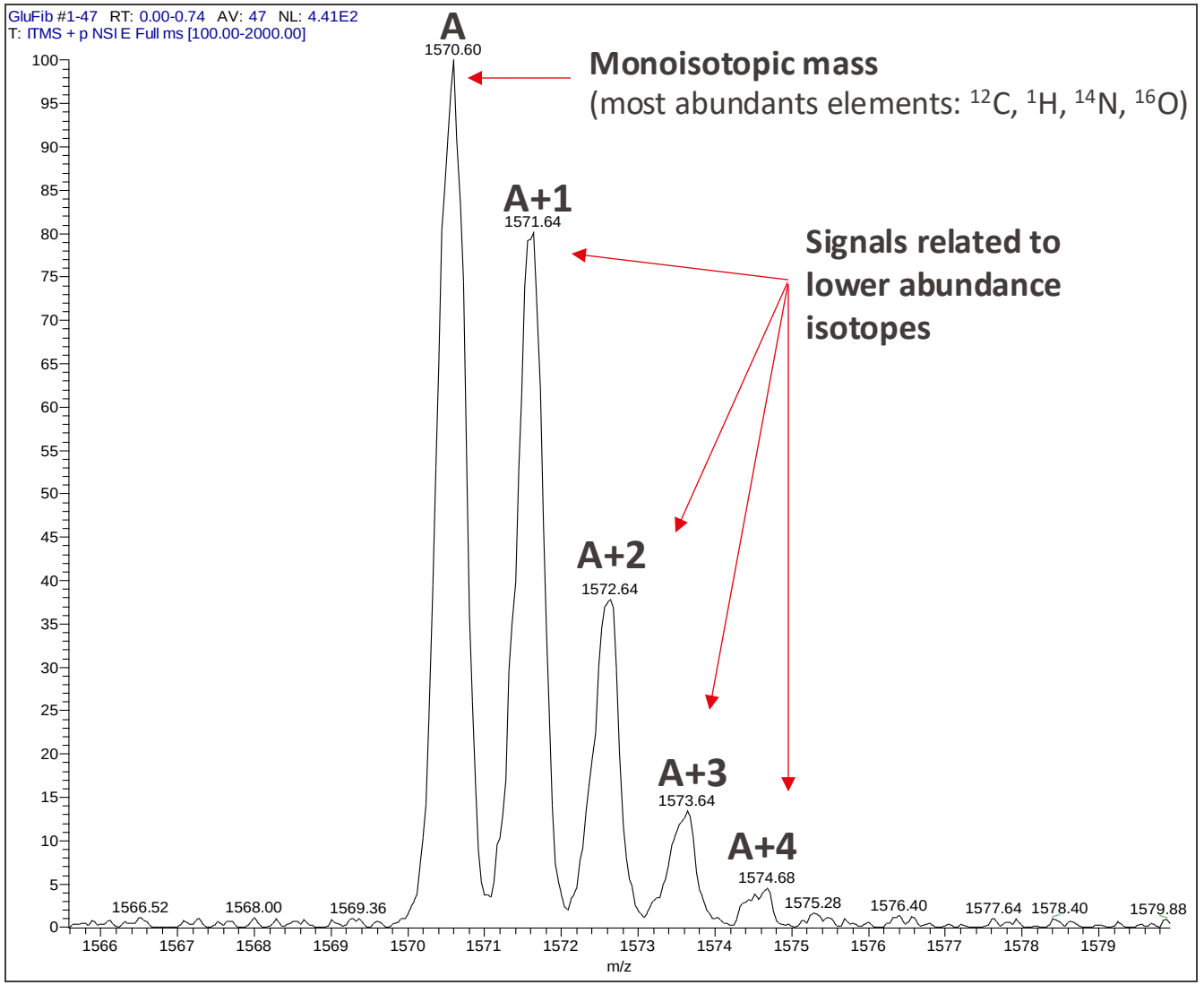
Natural isotopic distribution: relative abundance of isotopes

- Most elements occur in nature as a mixture of isotopes
- Isotopes are atom species of the same chemical element that have different masses
- They have the same number of protons and electrons, but a different number of neutrons (1Da)
- The main elements occurring in proteins are CHNOPS

element (symbol)	isotope	abundance %
hydrogen (H)	^{1}H	99.988 %
	^{2}H	0.012 %
carbon (C)	^{12}C	98.93 %
	^{13}C	1.07 %
nitrogen (N)	^{14}N	99.636 %
	^{15}N	0.364 %
oxygen (O)	^{16}O	99.757 %
	^{17}O	0.038 %
	^{18}O	0.205 %
phosphor (P)	^{31}P	100 %
sulfur (S)	^{32}S	94.99 %
	^{33}S	0.75 %
	^{34}S	4.25 %
	^{36}S	0.01 %

Mass meassurements

- Average mass: equivalent to taking the centroid of the complete isotopic envelope
- Monoisotopic mass: the mass of the first peak of the isotope distribution.



GluFib: ***EGVNDNEEGFFSAR***
MW: 1569.6696 Da

Chemical Formula:

- $C_{66}H_{95}N_{19}O_{26}$

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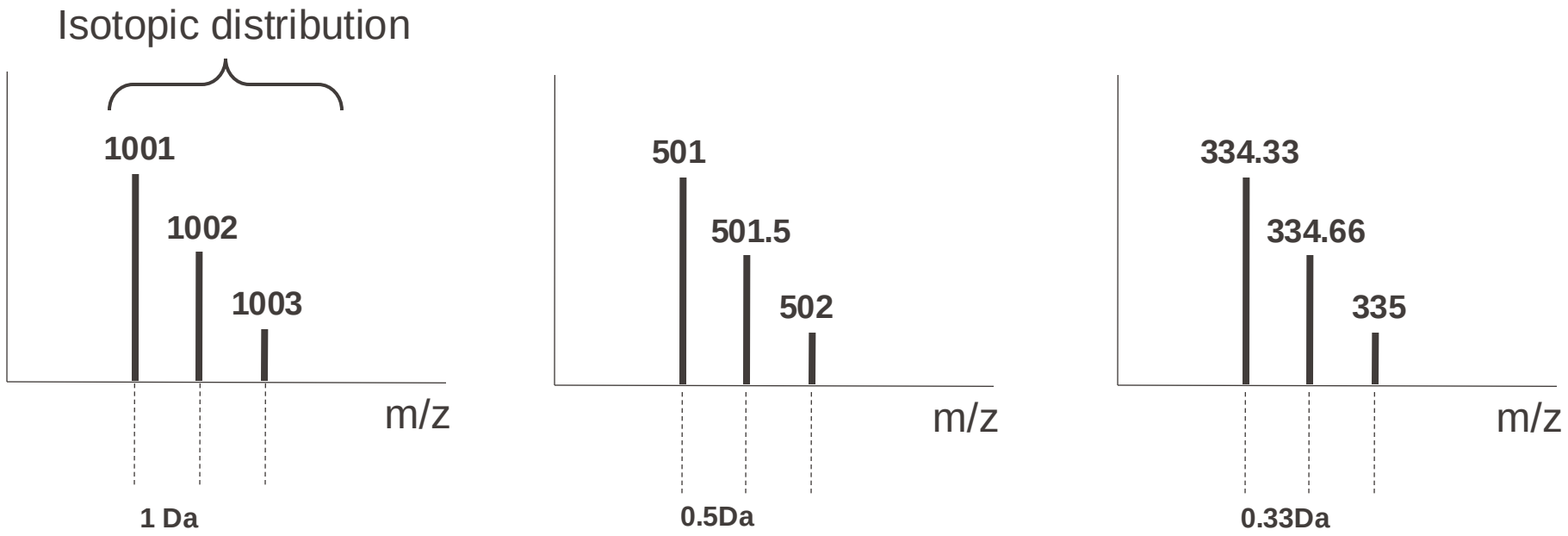
The distances between isotopic peaks reveal charge state

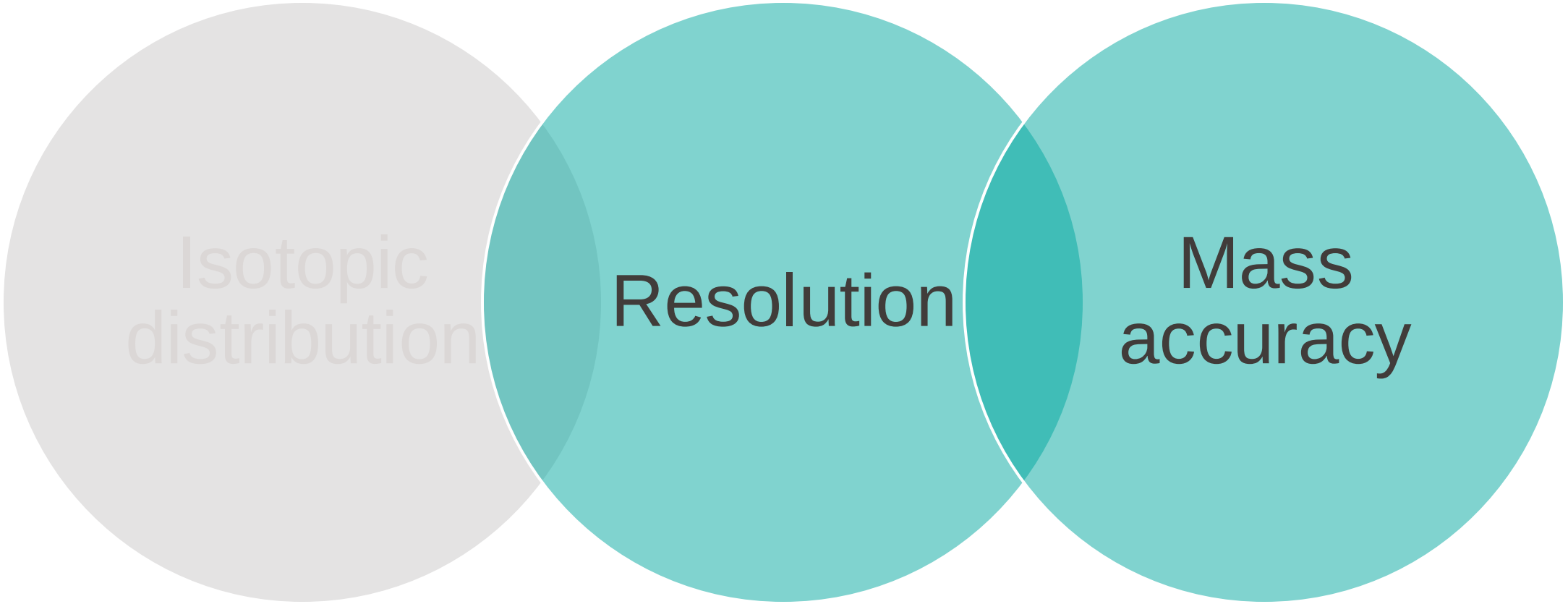
- M = 1000 Da (not charged)
- $[M+1H]^{1+} \longrightarrow$ z = 1
- m/z = 1001 Da (Monoiso.)

- M = 1000 Da
- $[M+2H]^{2+} \longrightarrow$ z = 2
- m/z = 501 Da

- M = 1000 Da
- $[M+3H]^{3+} \longrightarrow$ z = 3
- m/z = 334.33 Da



The basics of mass meassurements



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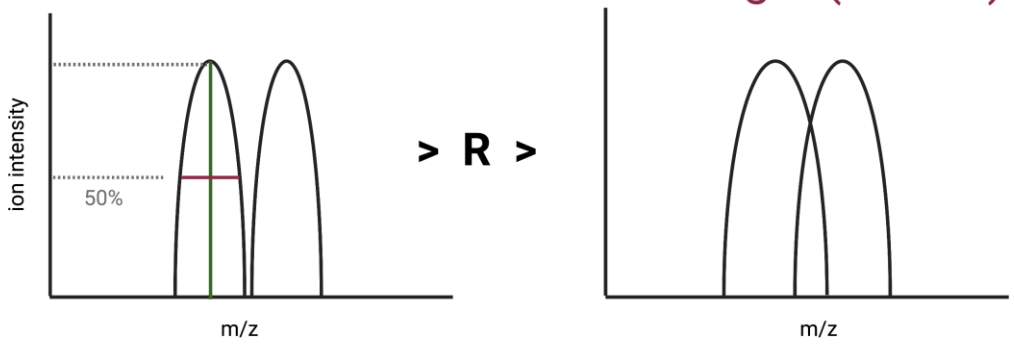
Resolution and mass accuracy

Mass resolution (R)=

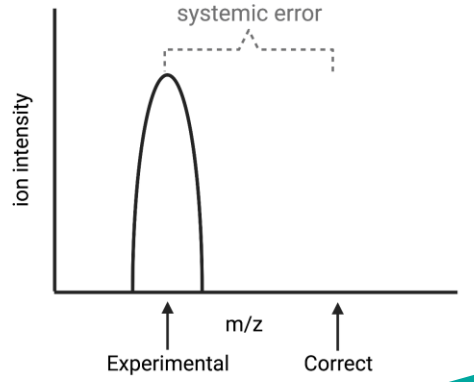
Peak mass/ Peak Width at Half Height (FWHM)



Resolution: The ability to discriminate molecules of similar mass

Mass accuracy (parts per million, ppm) =

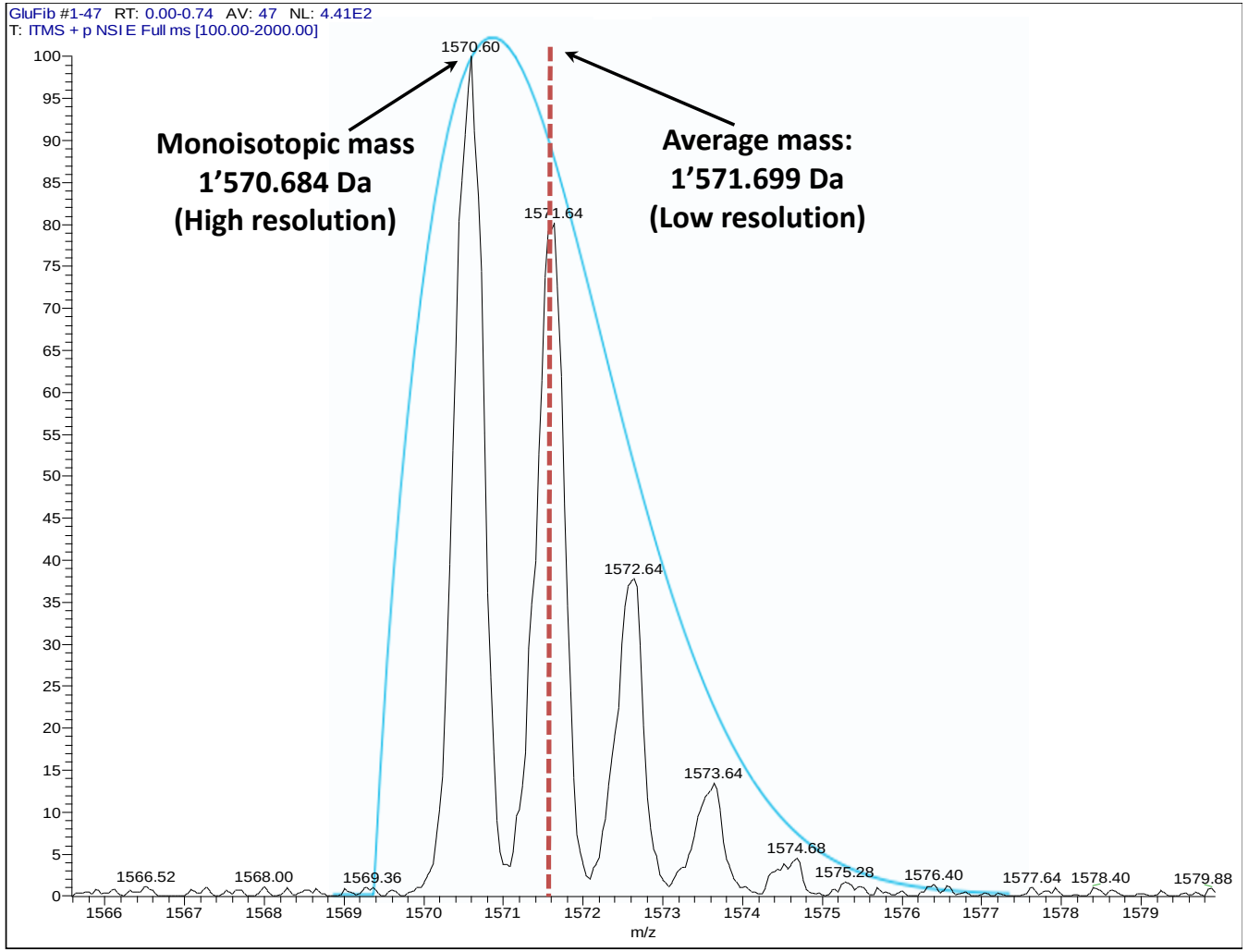
$$\frac{\text{Meassured mass - Calculated mass}}{\text{Calculated mass}} \times 10^6$$



Mass accuracy: the ability to accurately measure the mass of a molecule

Low vs high resolution

- Average mass: equivalent to taking the centroid of the complete isotopic envelope
- Monoisotopic mass: the mass of the first peak of the isotope distribution.

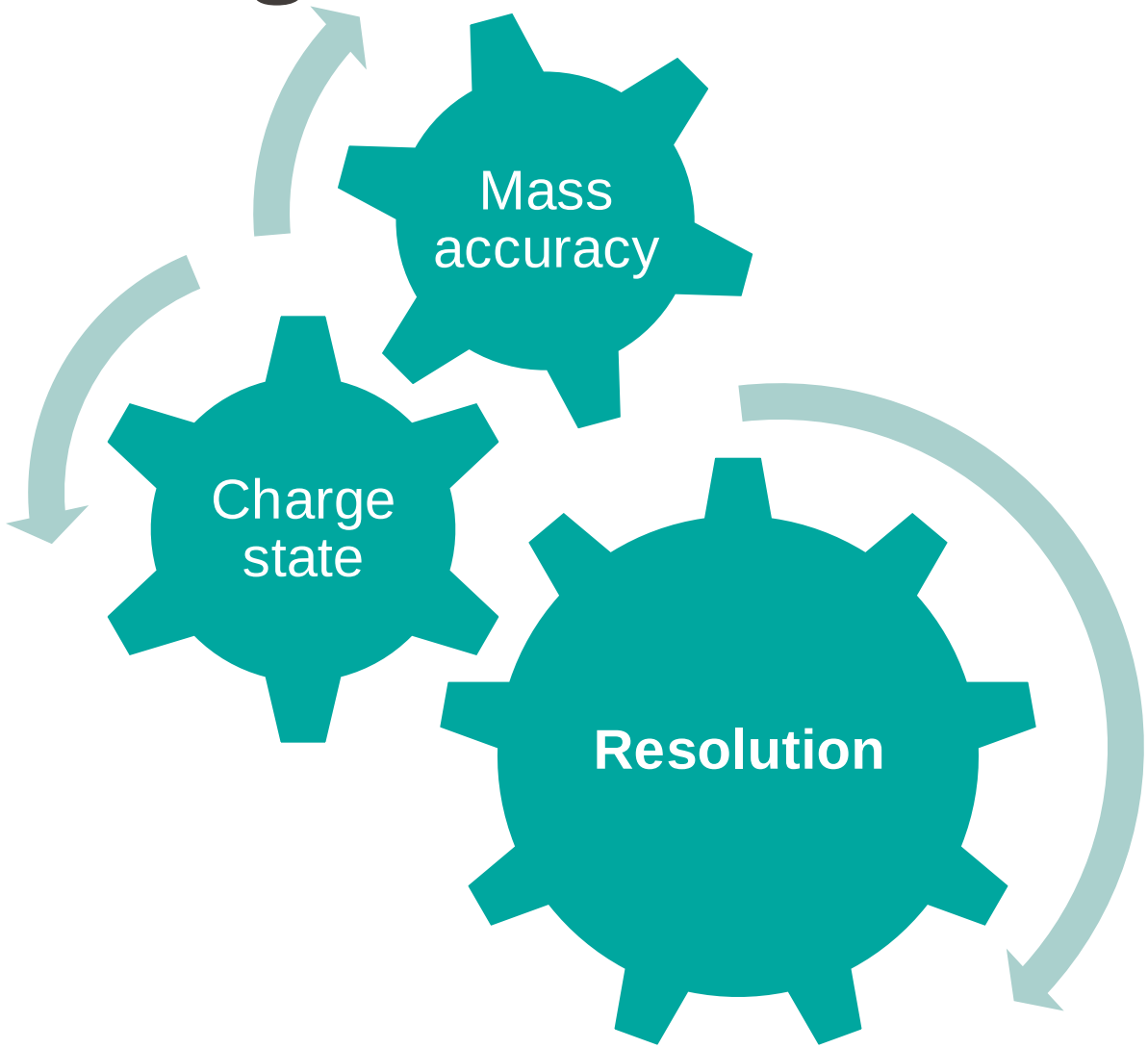


GluFib: ***EGVNDNEEGFFSAR***
MW: 1569.6696 Da

Chemical Formula:

- $C_{66}H_{95}N_{19}O_{26}$

The resolution drives mass accuracy and allows charge deconvolution



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The effect of electromagnetic fields on ions

- The force applied to an ion in a field is governed by 2 laws:

Lorentz force law and Newton's second law

$$\mathbf{F} = q(\mathbf{E} + \mathbf{v} \times \mathbf{B}) \qquad F = m \cdot a$$

$\mathbf{F}$ = force

q = electric charge

$\mathbf{E}$ = external electric field

$\mathbf{v}$ = velocity

$\mathbf{B}$ = magnetic field

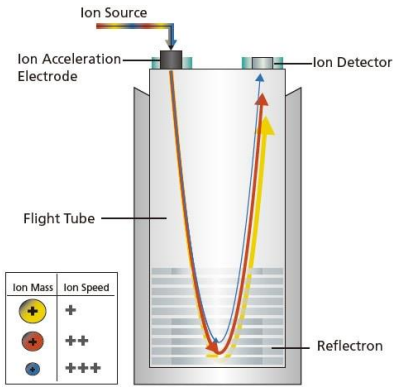
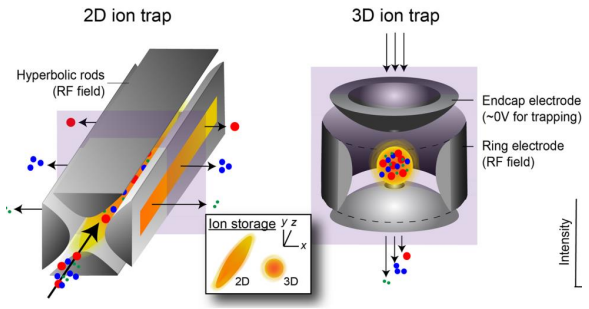
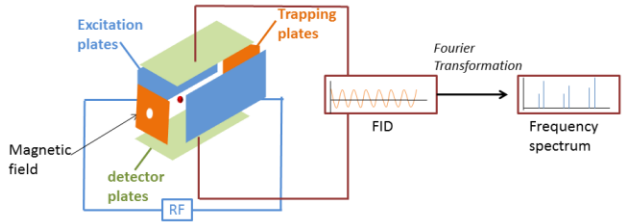
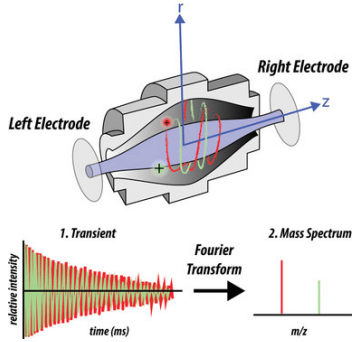
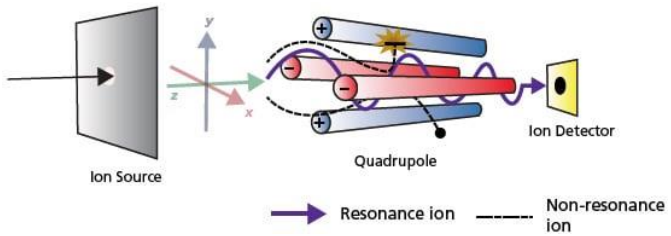
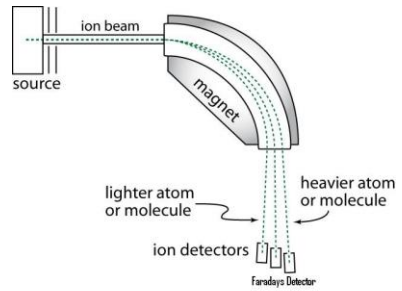
m = mass of an object

a = acceleration

Maria Pavlou

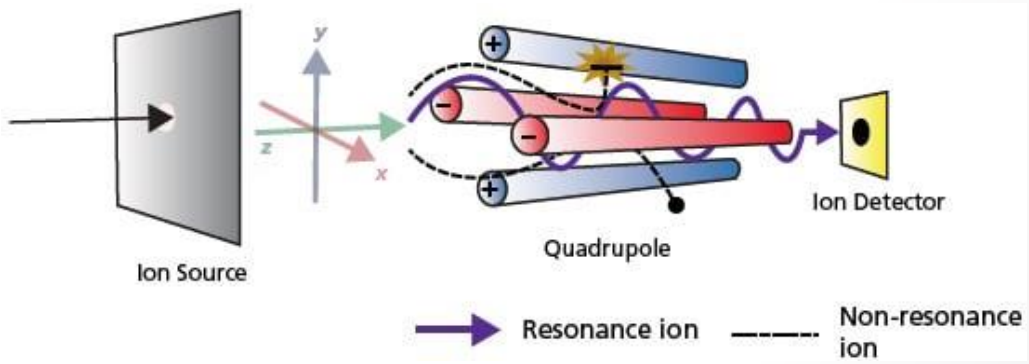
Mass analysers

- Separate ions based on mass-to-charge (m/z) ratio
- 6 main types
- Different analyser ☞different principle ☞ different characteristics ☞ different application

Field-free	Electric Field	Magnetic Field
 **Time-of-Flight (TOF)**	 **Ion Traps (IT)**	 **Ion cyclotron resonance (ICR)**
 **Orbitrap**	 **Quadrupole**	 **Magnetic sector**

Quadrupole Theory

- 4 parallel metal rods of opposite polarity
- RF voltage applied between one pair of rods and the other
- Direct Current (DC) voltage superimposed on the RF voltage
- Ions can travel between the rods at given RF and DC voltages

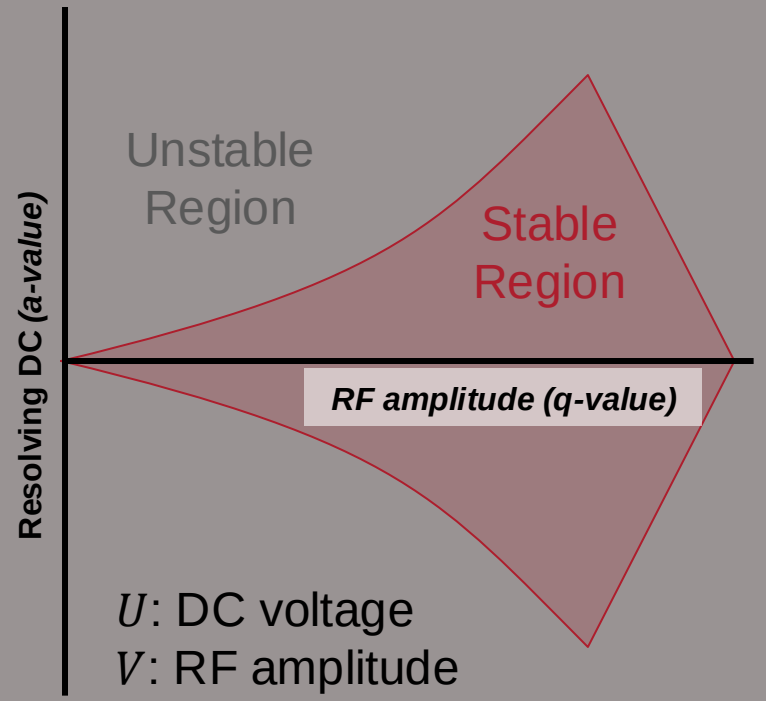


$$a_x = -a_y = \frac{4qU}{m_i {r_0}^2 \omega^2}$$

DC Component

$$q_x = -q_y = \frac{2qV}{m_i {r_0}^2 \omega^2}$$

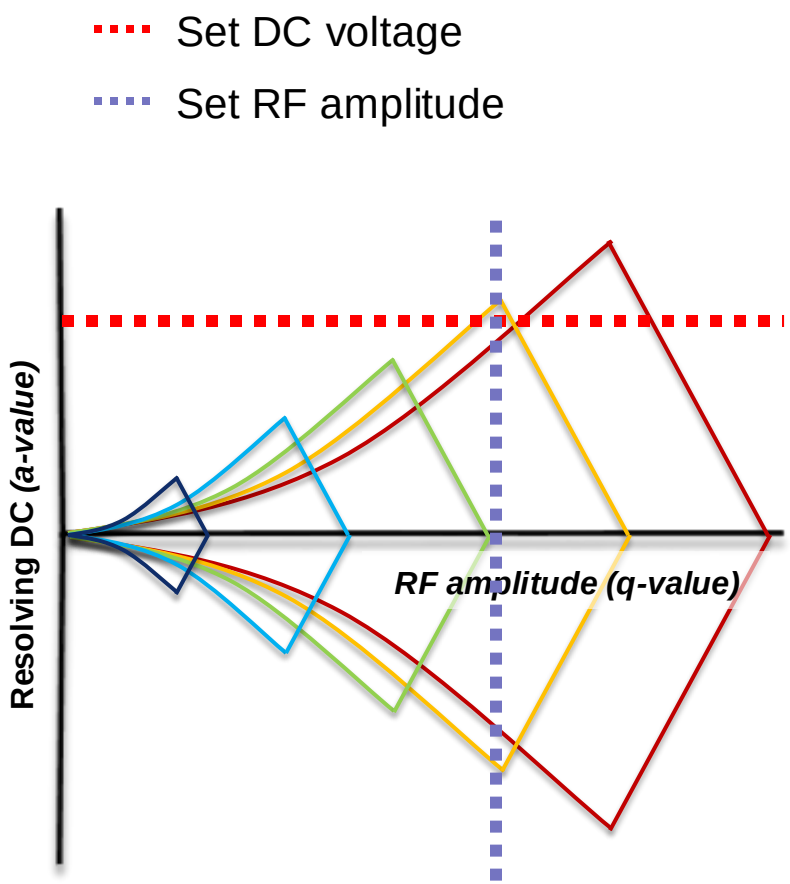
RF Component

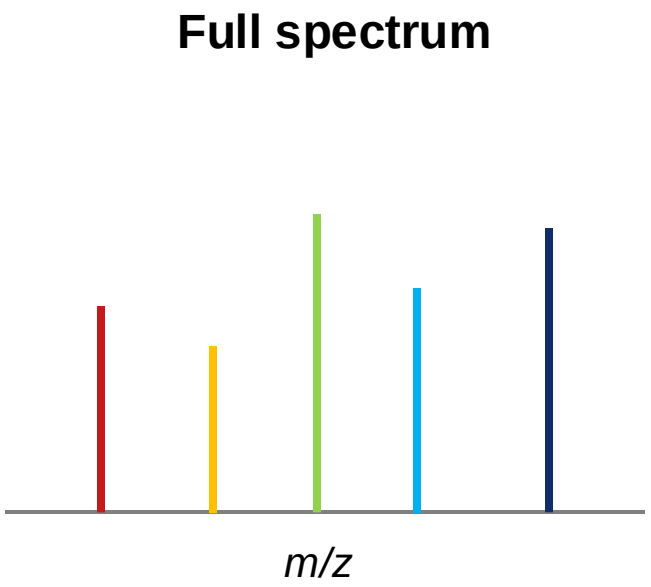


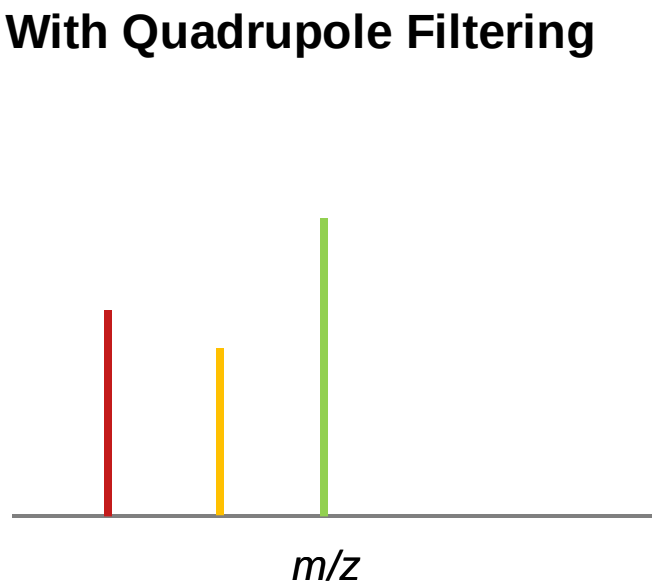
U: DC voltage
V: RF amplitude
q: ion charge
m_i: ion mass
r_0: quadrupole radius
ω: RF frequency

Isolation Using Quadrupoles

- By varying the DC voltages and RF amplitudes, it is possible to select ions with different *m/z* values and to vary isolation width.





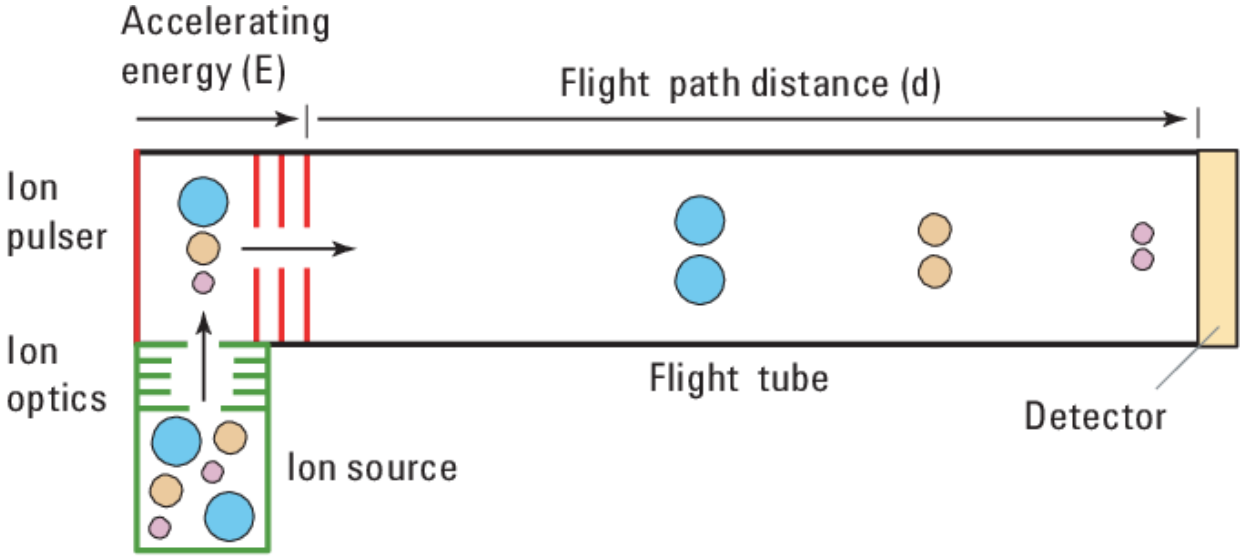


TOF

$$T = eV = \frac{mv^2}{2} \qquad v = \frac{L}{t}$$

$$\frac{m}{e} = \frac{2Vt^2}{L^2} \rightarrow t = L\sqrt{\frac{m}{e}\frac{1}{2V}}$$

- Consists of an acceleration region with a homogeneous electric field and a field-free drift region
- Different m/z -> different velocities -> different time to reach the detector
- Requires a pulsed ion source (ex. MALDI) or pulsing ion packages out of a continuous beam (pulser for ESI)

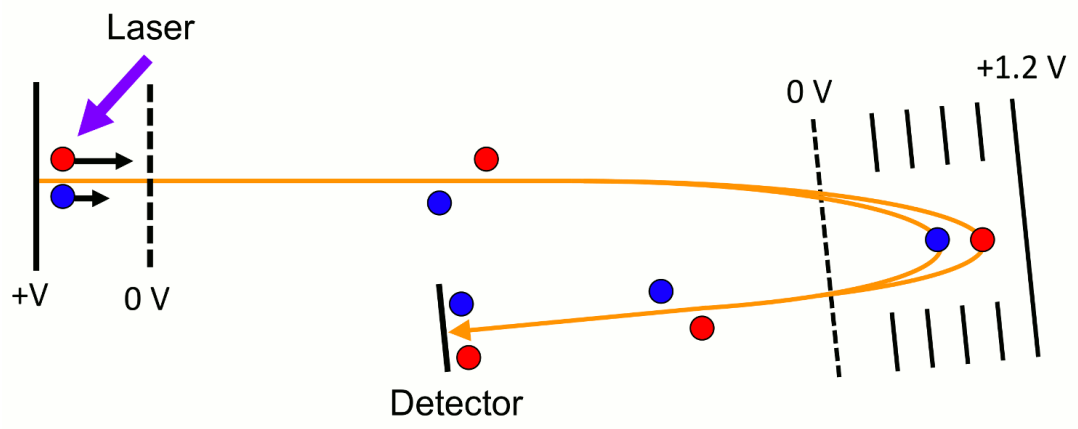


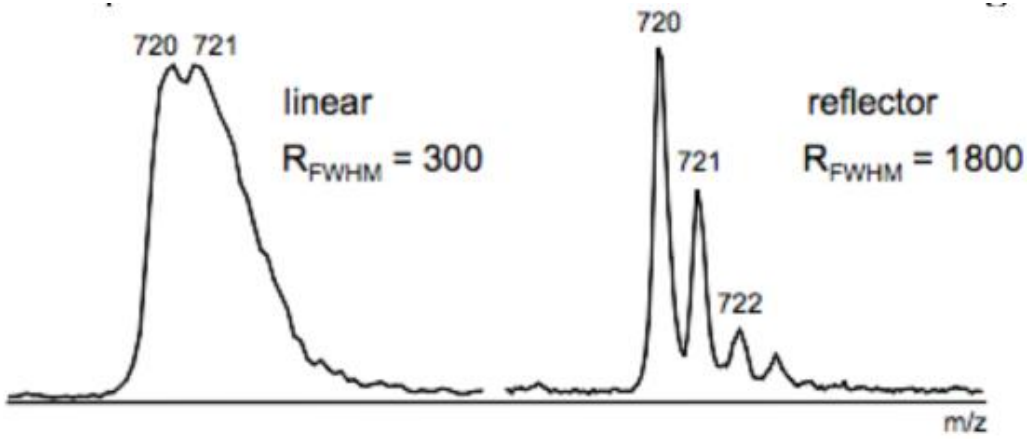
https://doi.org/10.1002/mas.21520

TOF: reflector

- Reasons for poor resolution

I. Different starting times
II. Different initial kinetic energies
III. Different starting locations
IV. Different initial directions of motion





Ion Traps (IT) 3D and 2D

- Operate on the same plysical principles as quadrupoles
- Filled with He

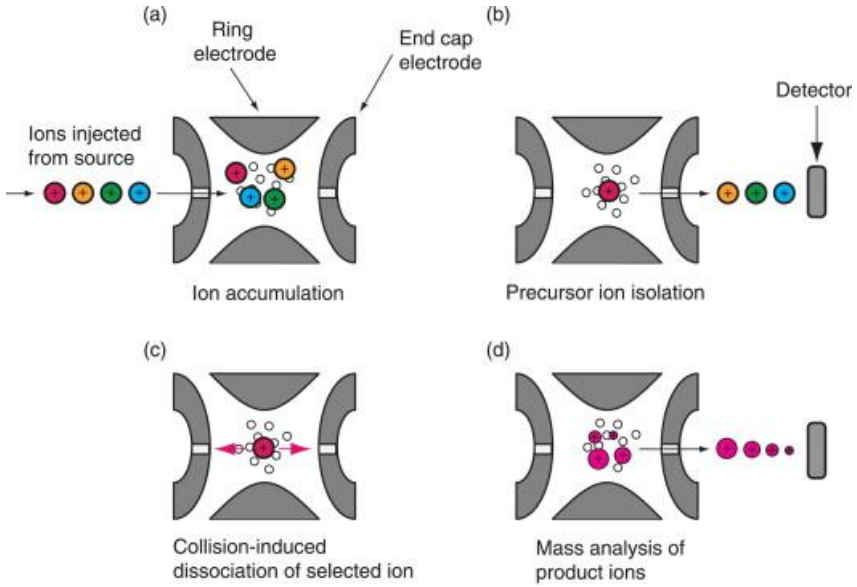
1. Trap
2. Isolate
3. Fragment (Collision Induced Dissociation, CID)

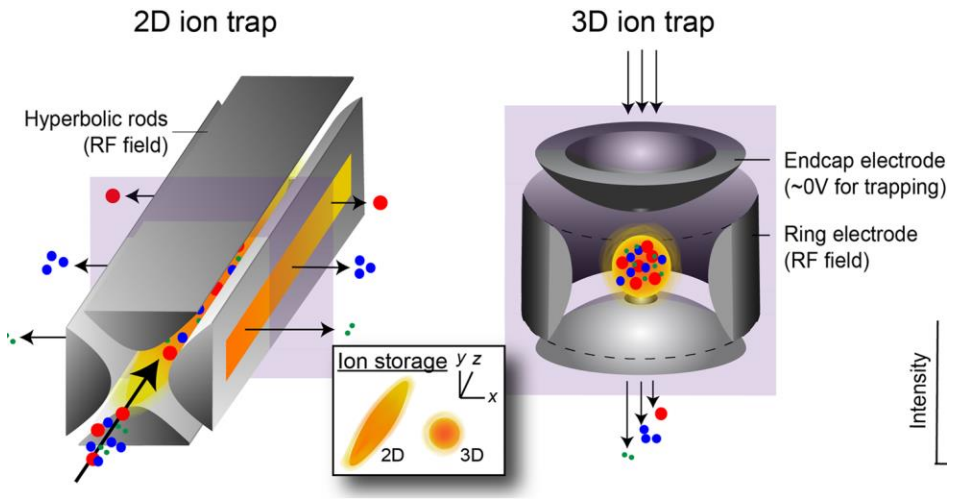
- Quadrupole IT (aka 3D): Ions trapped in 3 dimensions, space charging effect

- Advantages of Linear IT (aka 2D)

1. 50x ion storage capacity
2. 20x higher injection efficiency
3. simplicity of construction

- http://dx.doi.org/10.1016/j.cplett.2016.11.011





Orbitrap

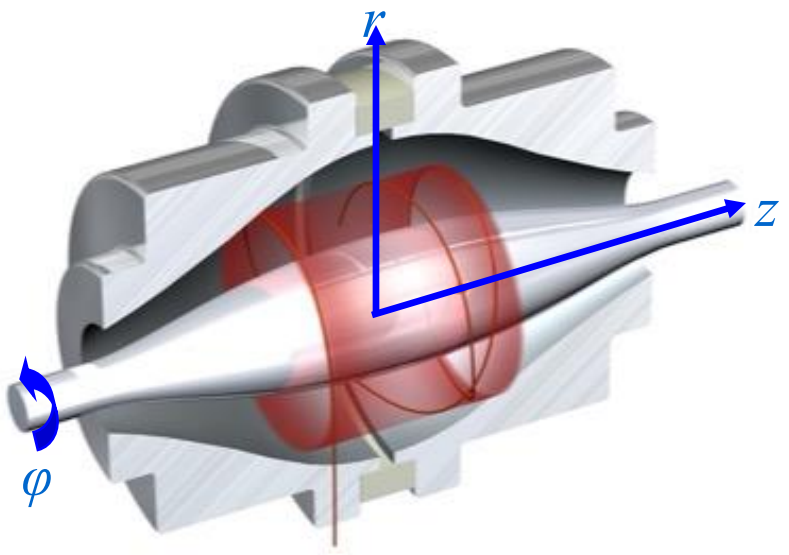
$$\omega_\varphi = \frac{\omega_z}{\sqrt{2}}\sqrt{\left(\frac{R_m}{R}\right)^2 - 1}$$

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

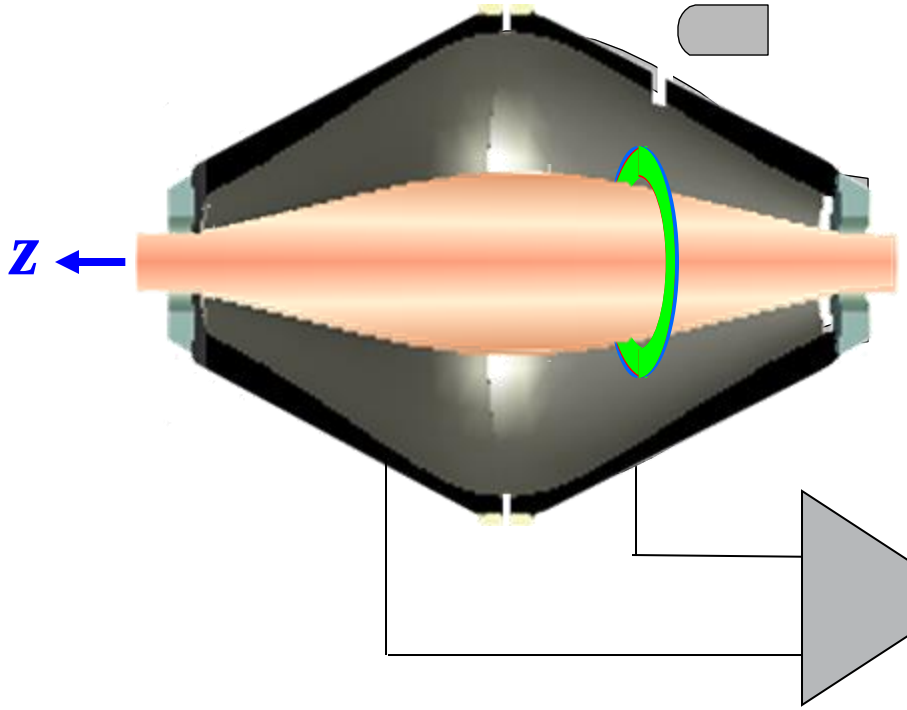
$$\omega_z = \sqrt{\frac{k}{m/z}}$$

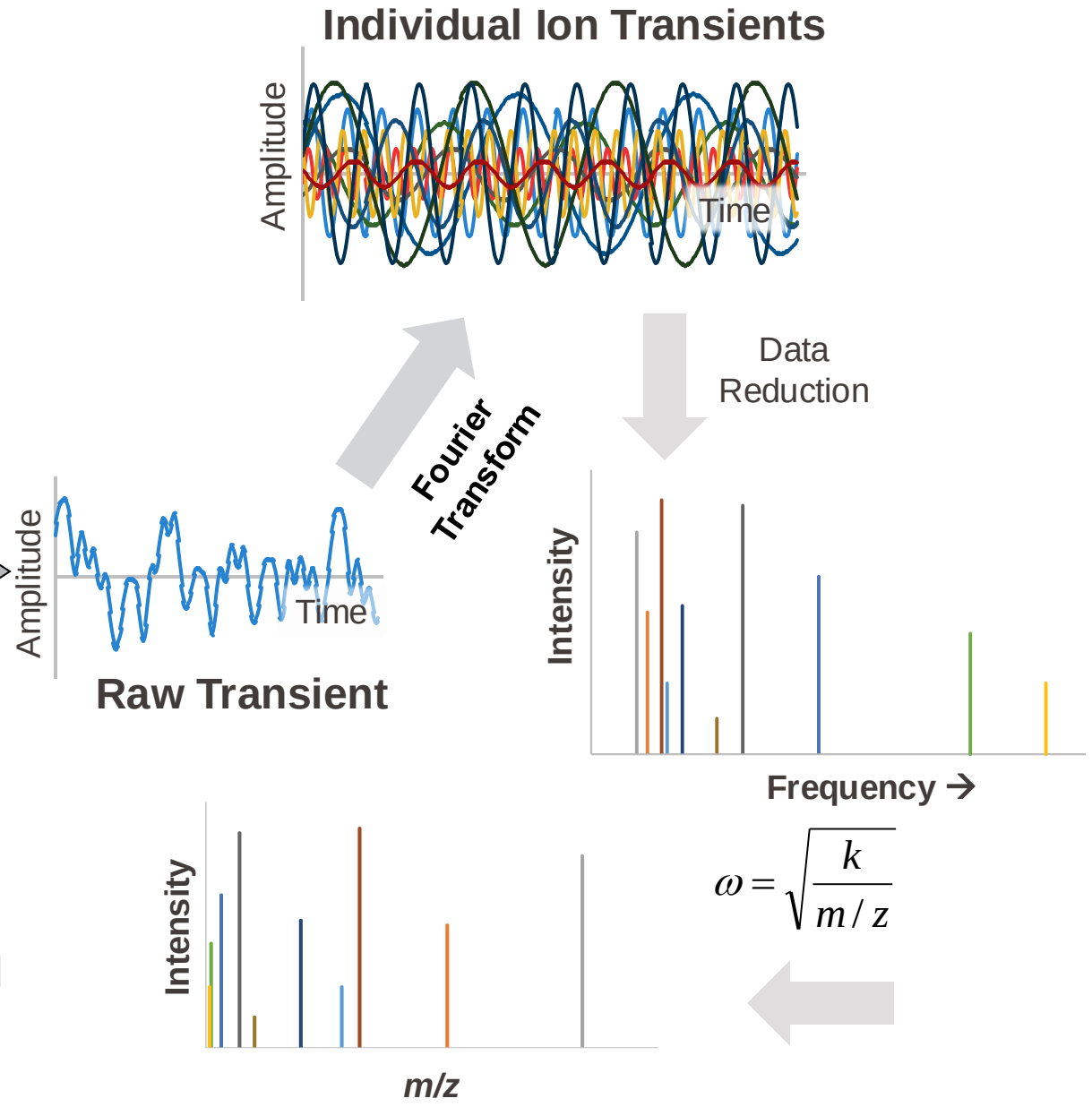
- Electrostatic field applied between 1 central spindle electrode and 2 halves of outer shell electrode

- A short ion packet of one *m/z* enters the field tangentially, off-equator
- Ions are squeezed towards the central electrode by increasing voltage on the central electrode
- In the axial direction, ions are forced to move away from the narrow gap towards the wider gap near the equator
- This initiates axial oscillations without the need for any further excitation
- ("excitation by injection")
- After the voltage increase stops, ion trajectories become a stable spiral



https://doi.org/10.1021/ac4001223



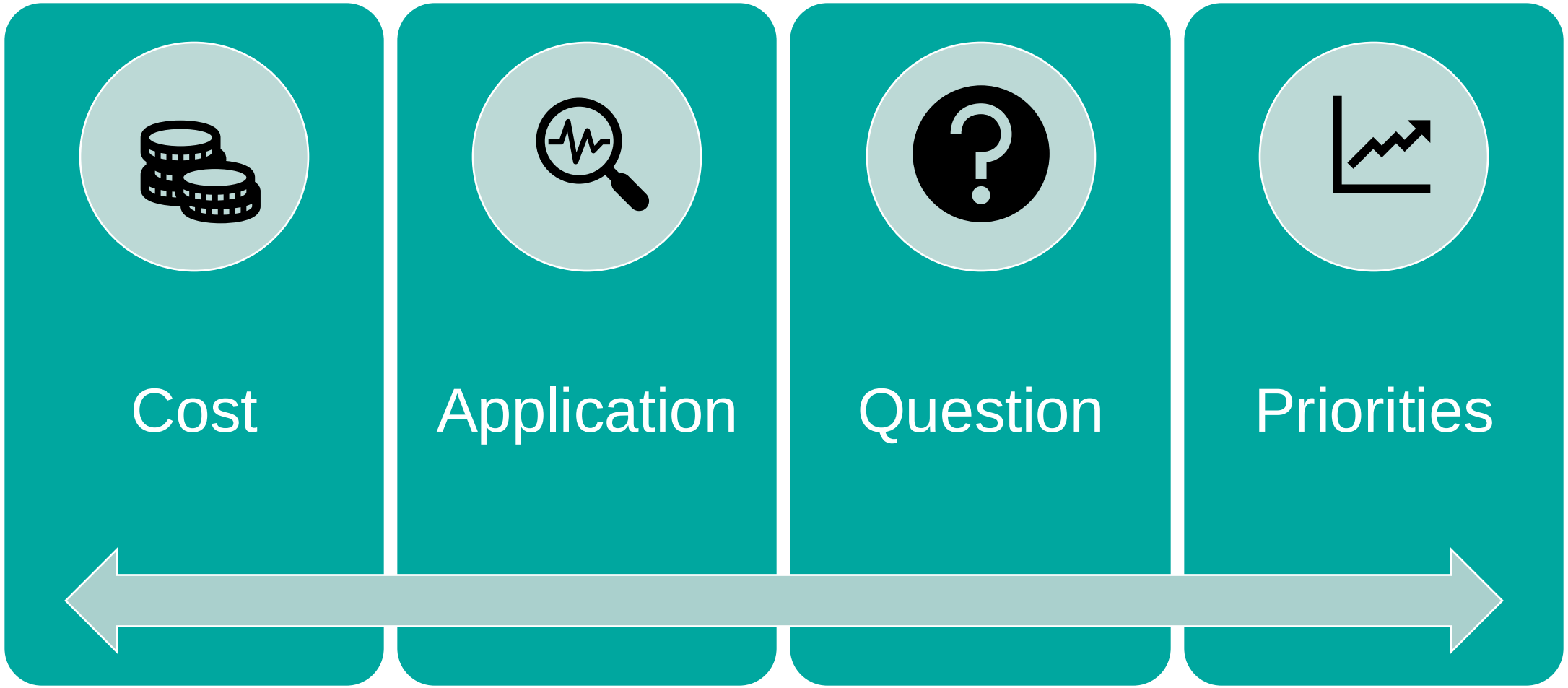


- The two outer electrodes are used to detect the image current produced by ions oscillating along the z-axis.
- Many ions in the Orbitrap generate a complex signal whose individual frequencies are determined using a Fourier Transformation

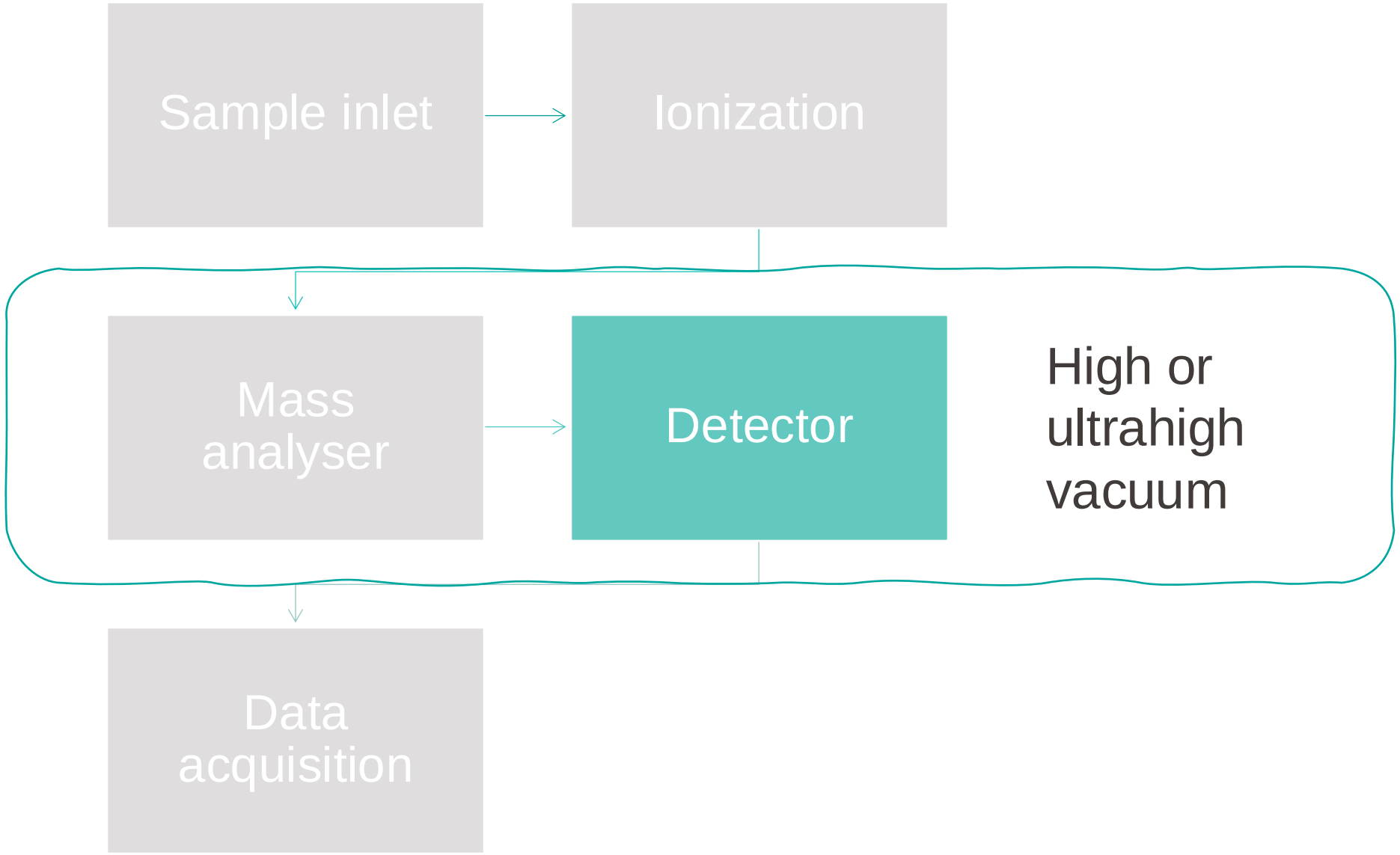
Characteristics of different mass analyzers

Mass Analyzer	Mass Accuracy	Resolution (FWHM)	Scan Rate	m/z Range	Cost
TOF	Very good 5 ppm	High 20,000	Very fast µs	Very wide	$$
Ion trap	Good 100 ppm	Low Unit mass	Fast ms	100 – 4000	$
Quadrupole	Good 100 ppm	Low Unit mass	Normal s	100 – 4000	$
Orbitrap	Excellent < 2 ppm	Very high >100,000	Normal s	100 - 4000	$$$

Disclaimer: No size fits all

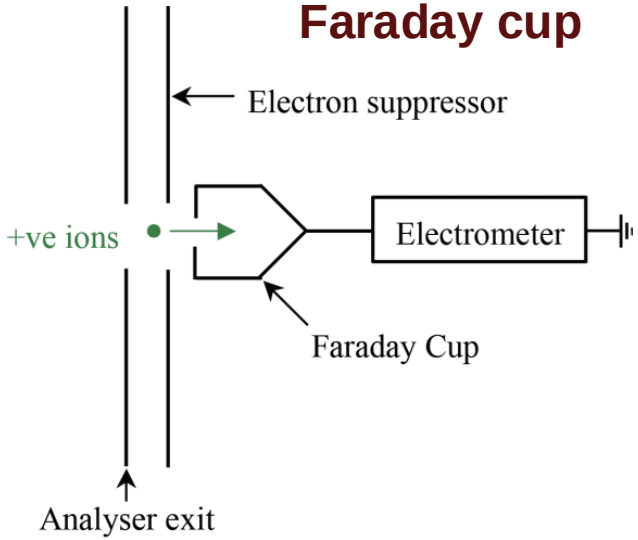


...and 5 parts

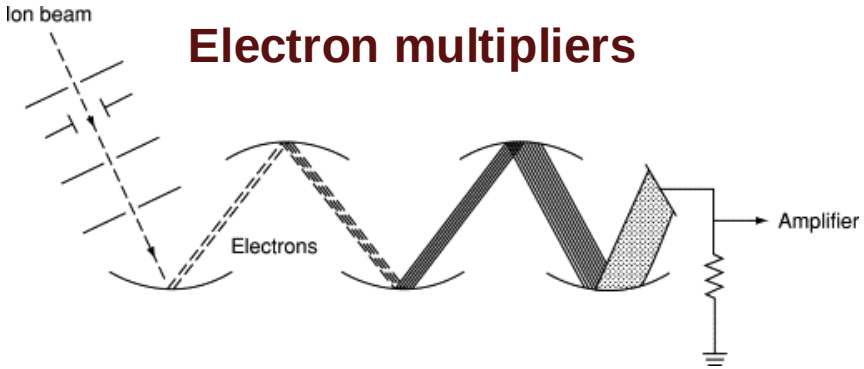

Sample inlet
Ionization
Mass analyser
Detector
High or ultrahigh vacuum
Data acquisition

Detectors

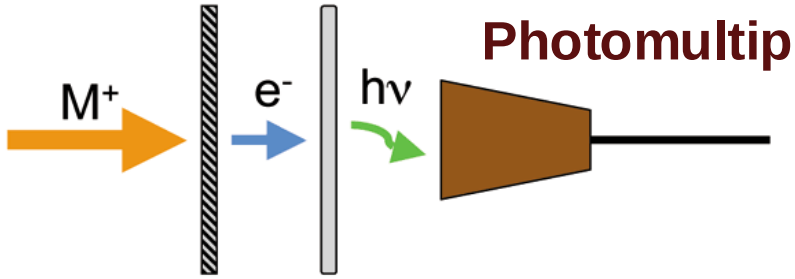
Faraday cup



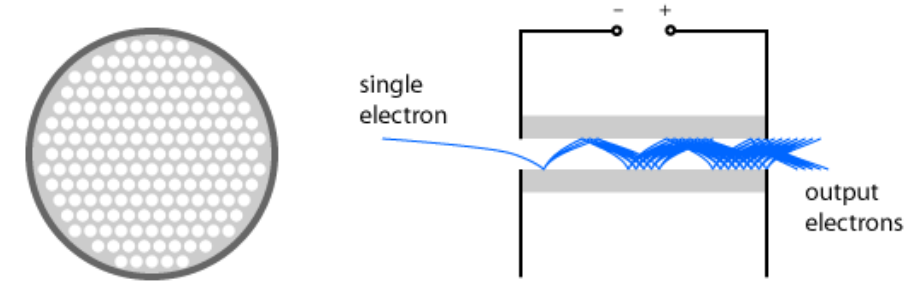
Electron multipliers



Photomultipliers



Multichannel plates



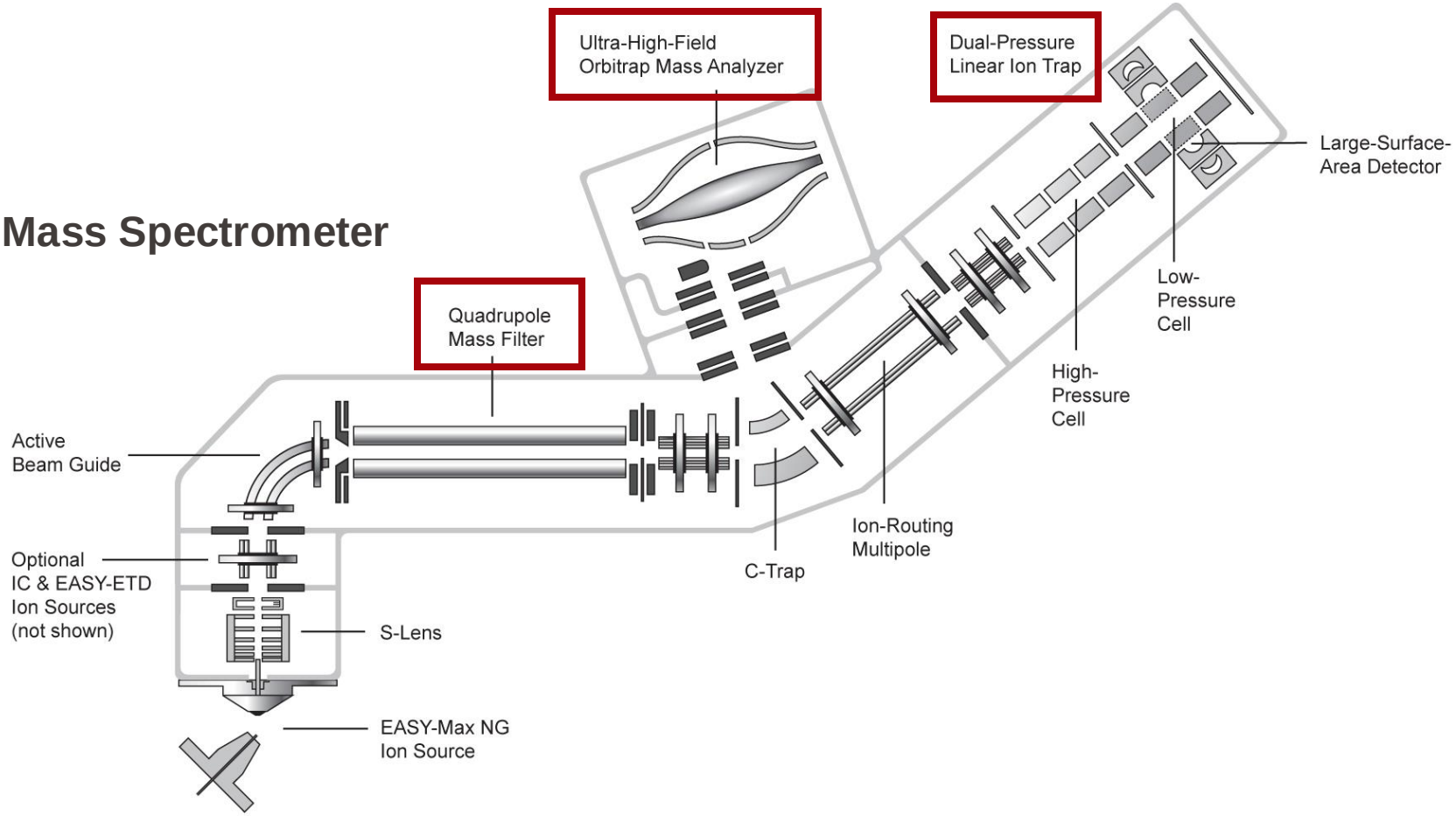
a) front view of microchannel plate

b) electron avalanche in a single channel

Maria Pavlou

Hybrid MS: why having one when you can have (almost) all?

Tribrid Mass Spectrometer



https://youtu.be/zJagpUbnv-Y

MS-based proteomics to elucidate protein structure

Limited proteolysis (LiP) MS

Measuring protein structural changes on a proteome-wide scale using limited proteolysis-coupled mass spectrometry

Simone Schopper, Abdullah Kahraman, Pascal Leuenberger, Yuehan Feng, Ilaria Piazza, Oliver Müller, Paul J Boersema & Paola Picotti

Nature Protocols **12**, 2391–2410 (2017) | Cite this article

- https://biognosys.com/resources/lip-ms-a-novel-target-deconvolution-approach/