Weighing in on research: mass spectrometry-based proteomics at MSKCC

Mara Monetti, PhD

Proteomics and Metabolomics Core
and Proteomics Innovation Laboratory Director at MSKCC

GSK Cancer Engineering Course

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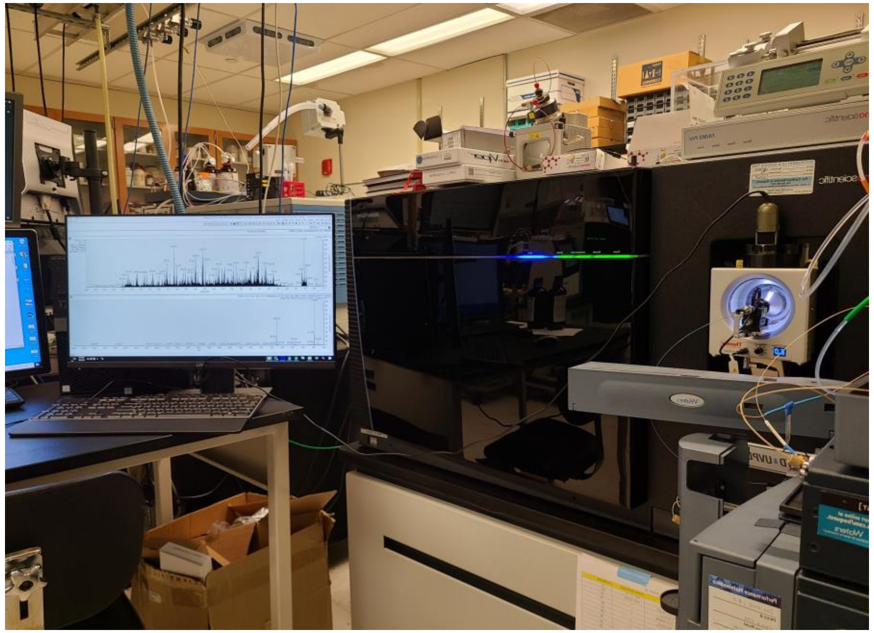
Memorial Sloan Kettering
Cancer Center

Outline

- Introduction to mass spectrometry-based proteomics
- Interpretation of mass spectra
- Instruments
- Quantification methods
- Applications
- Paper discussion

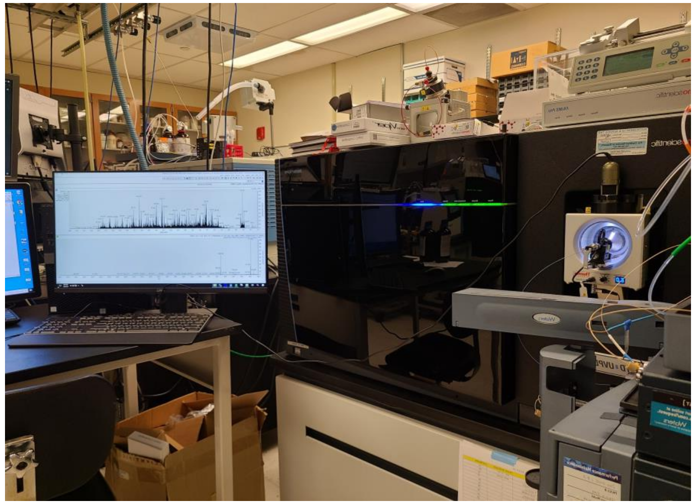


Proteomics at Memorial Sloan Kettering Cancer Center

- **What is the proteome?**
  - The **proteome** is the complete set of proteins in cells, tissues, biofluids.
  - Reflects the functional state of a biological system.

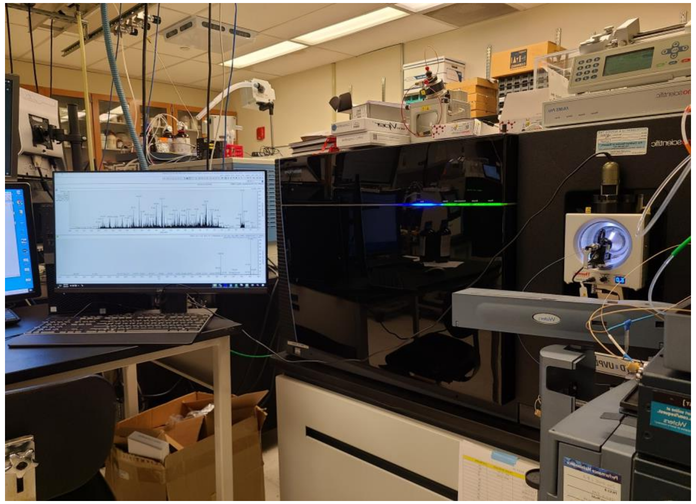
- **What is proteomics?**
  - Large-scale study of proteins.
  - Used to compare biological conditions or disease states.

- **Why study proteins?**
- Proteins are the ‘working bees’ of the cell, essential for:
  - Biochemical reactions
  - Signaling
  - Transport
  - Structural support



Why mass spectrometry?

- **Mass Spectrometry in proteomics:**
  - Identifies and quantifies proteins in complex samples,
  - Hight-throughput and sensitive
  - Detects post-translational modifications
- **Applications:**
  - biomarker discovery
  - drug development
  - system biology
  - among others

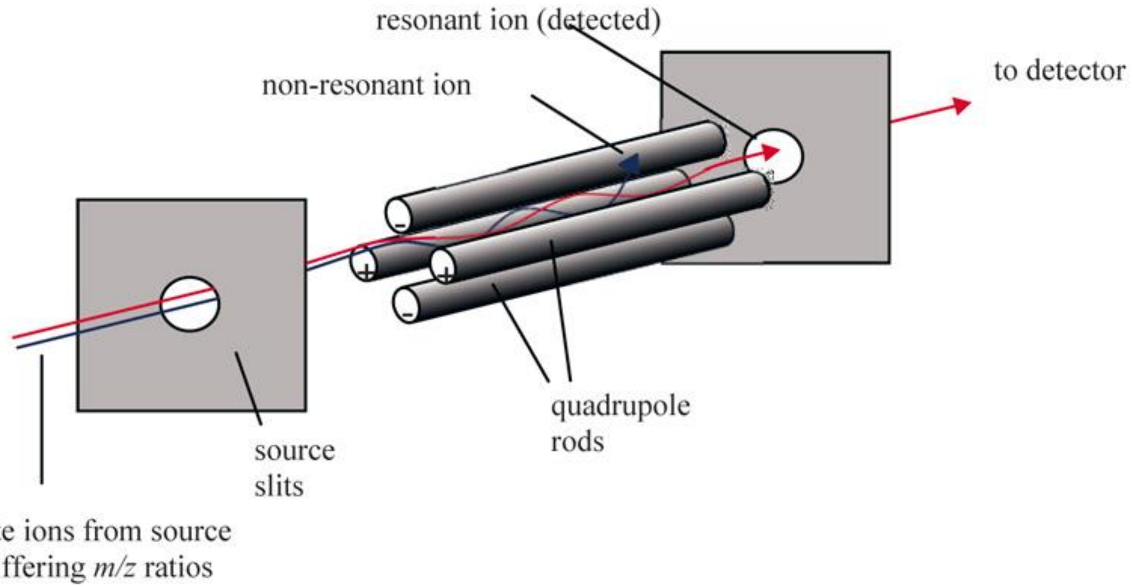


How do mass spectrometers work?

- Measures **mass-to-charge (m/z)** ratio of ions
  - m=mass of the ion (peptide or peptide fragments)
  - z=charge of the ion
- Ions with different m/z ratios are **separated by the mass analyzer** inside the mass spectrometer
- The instrument records the **signal intensity (ions abundance) vs m/z** to create a **mass spectrum**

How do mass spectrometers measure m/z?

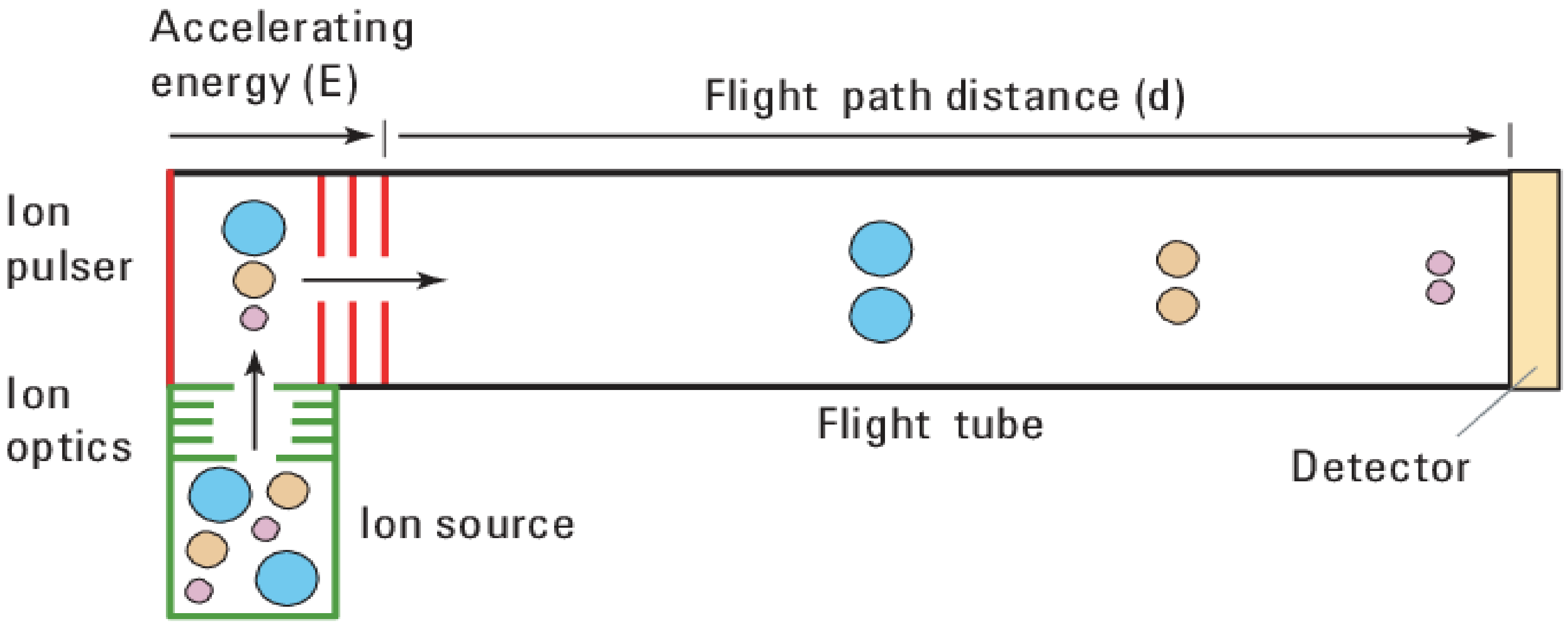
- Different MS instruments use different mass analyzer and principles to measure m/z ratio
  - **Quadrupole:** uses oscillating electric fields to filter ions based on m/z



How does the mass spectrometer measure m/z?

- Different MS instruments use different mass analyzer and principles to measure m/z ratio

  - **TOF (time-of-flight):** measure the time ions take to travel a fixed distance, after acceleration. Lighter ions (lower m/z) reach the detector faster



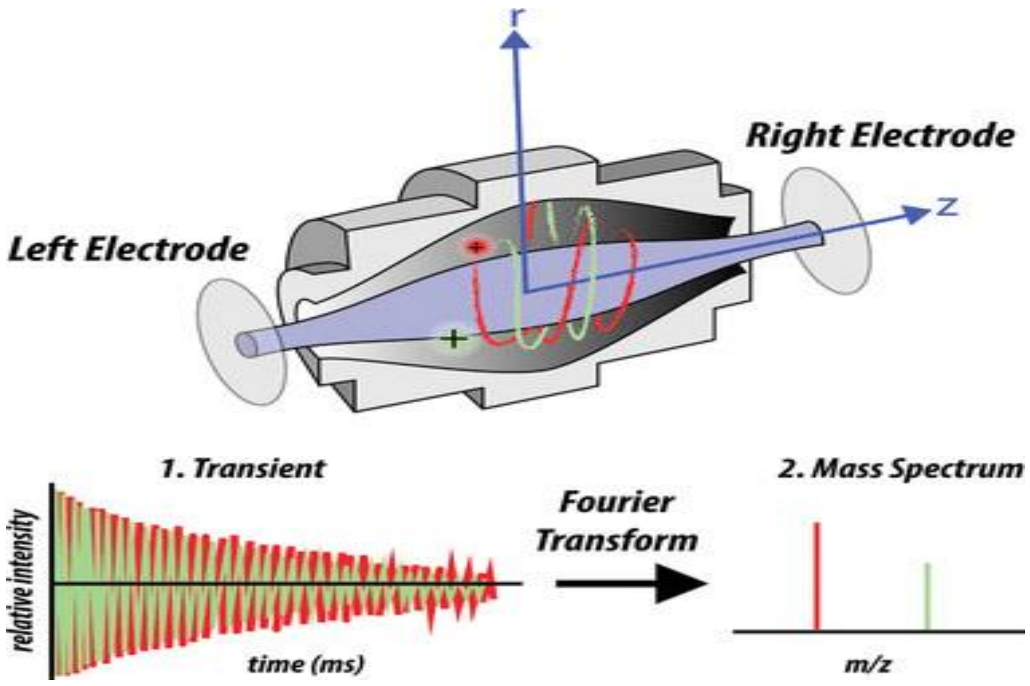
How does the mass spectrometer measure m/z?

- **Orbitrap:** traps ions in an electrostatic field and measure their oscillation frequency

  - The electric field cause ions:
    - to **orbit around** the central electrode
    - to **oscillate back and forth** along the z-axis

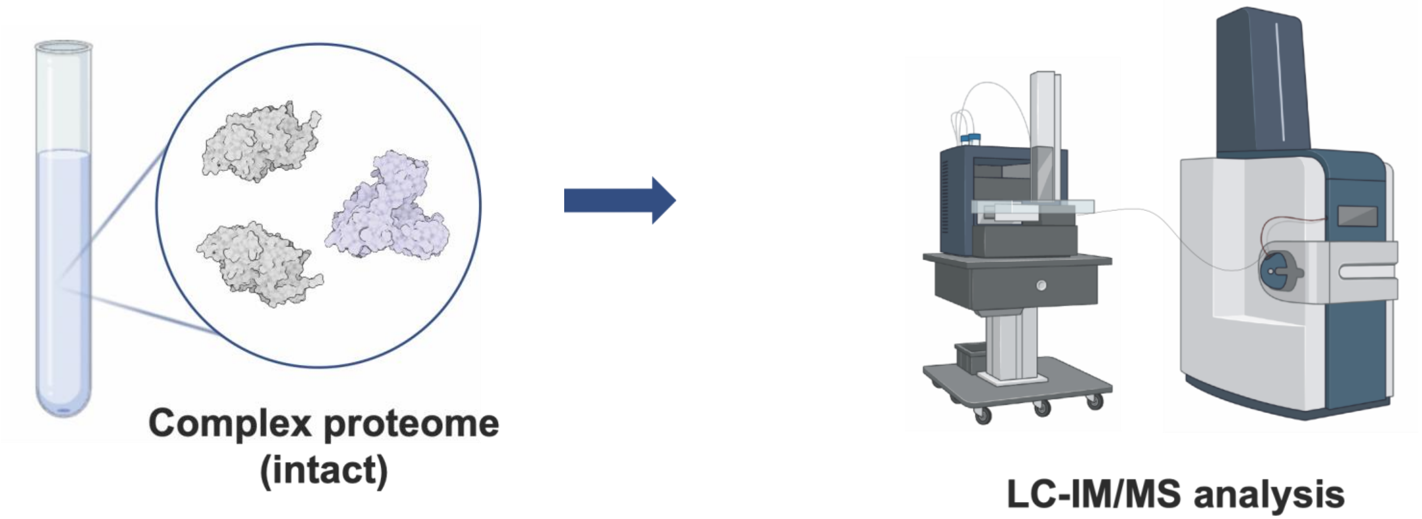
  - The **frequency of the axial oscillation depends on the ion m/z**

  - This frequency is used to determine m/z via **Fourier Transform**



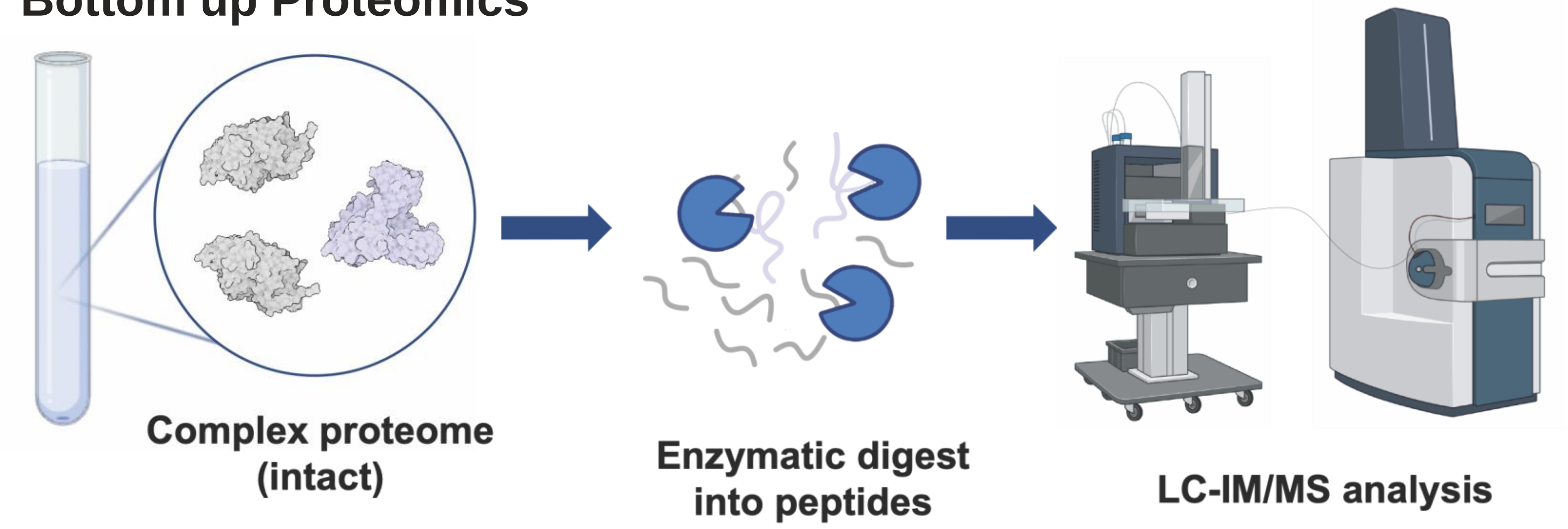
Proteins or peptides?

Top down Proteomics



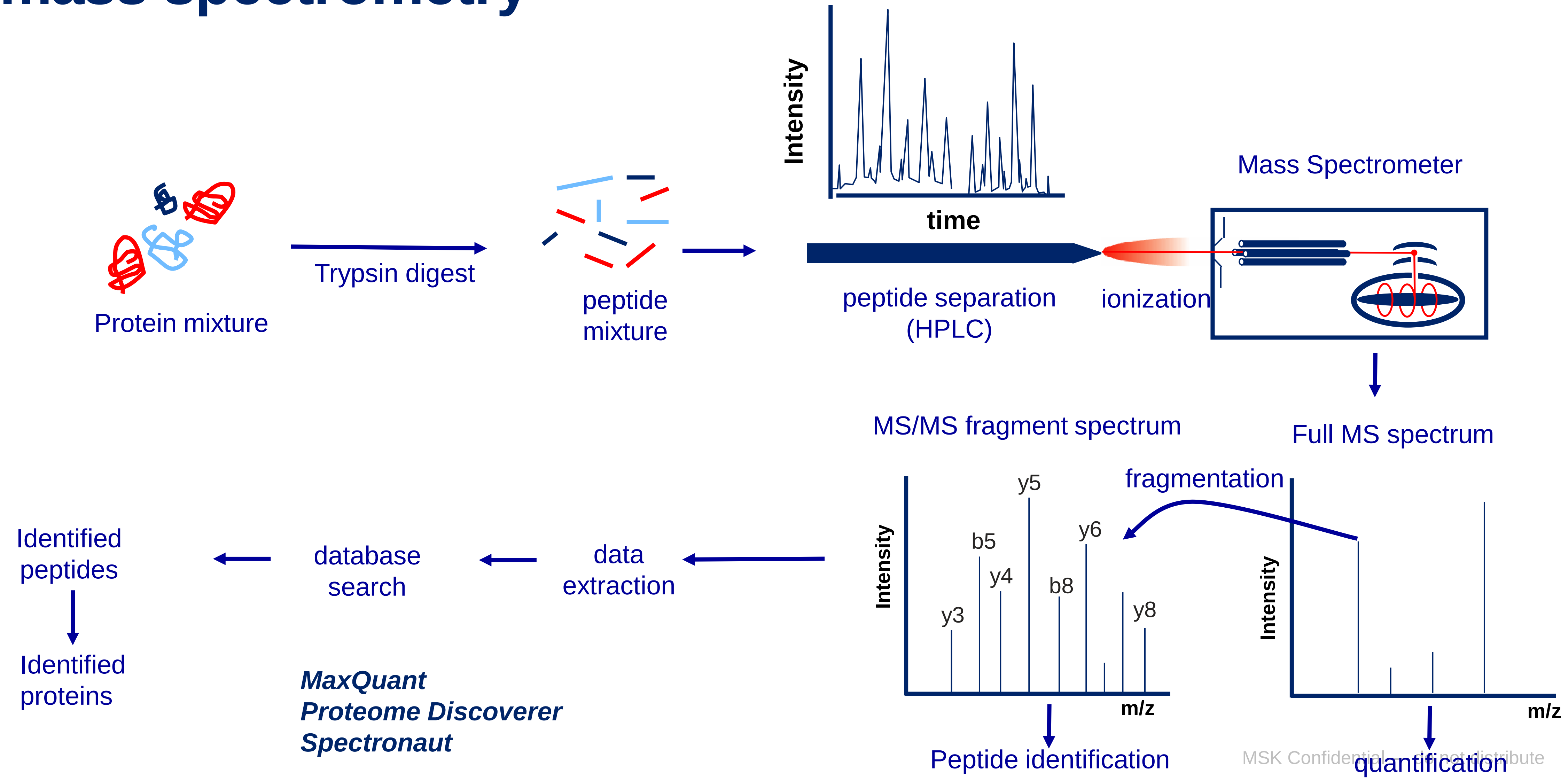
- Different solubilities
- Poor chromatographic separation
- Complex charge states
- Poor ionization
- PTM stoichiometry
- No inference (uniqueness)

Bottom up Proteomics



- Better ions
- Better chromatographic separation
- Site-resolved amino acid modification
- Increased complexity
- Protein inference (need unique peptides)

Shotgun proteomics by HPLC coupled to high resolution mass spectrometry



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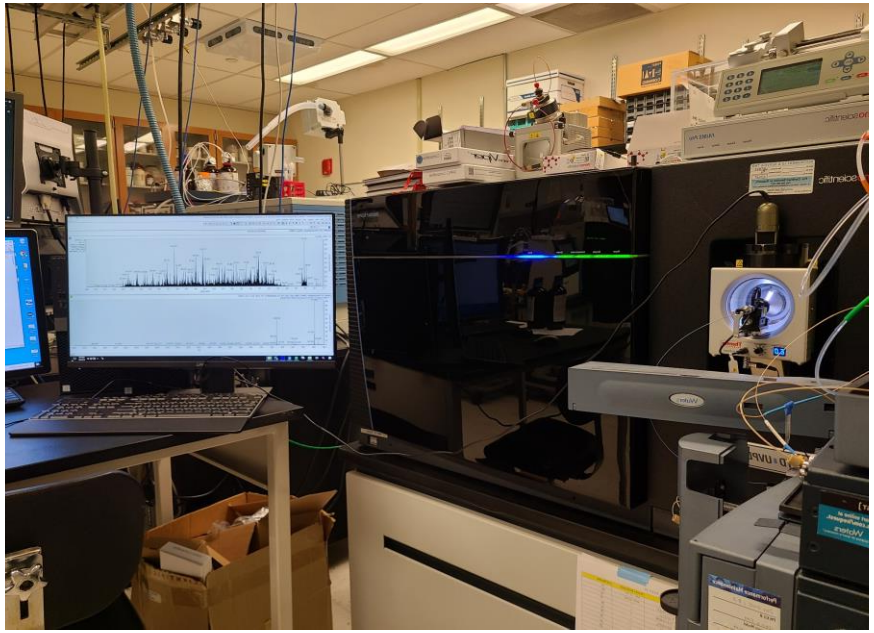
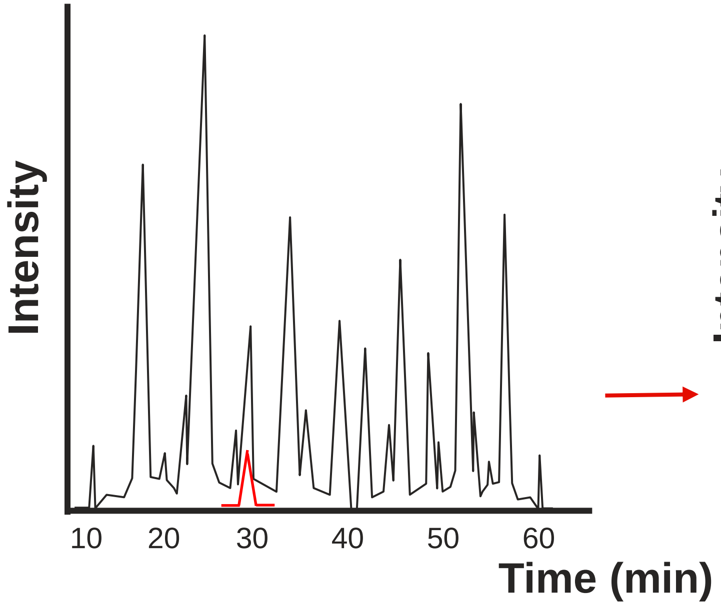


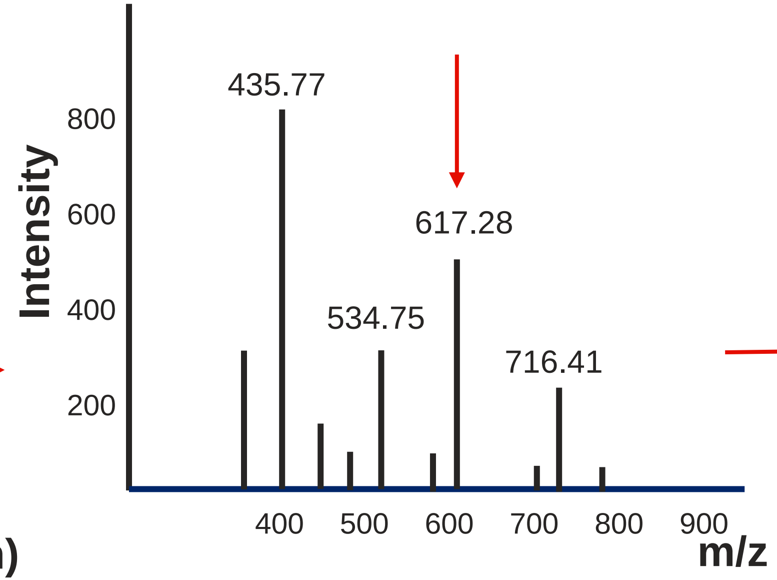
How we identify peptides (and proteins) by MS?

- **Chromatogram and Total Ion Current:** sum of all ion signals detected at a given time point in an MS run
- **MS spectrum:** spectrum of the precursor ion
- **MS/MS spectrum:** spectrum of the fragmented ions

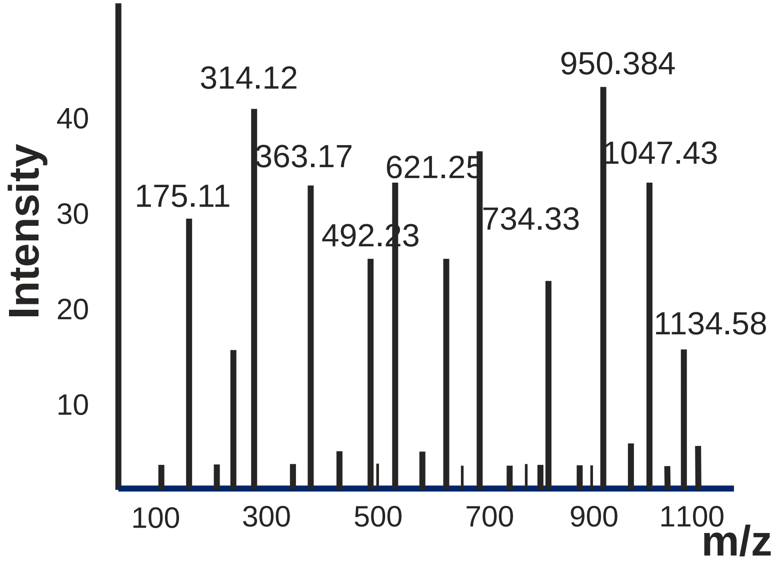
Total Ion Current



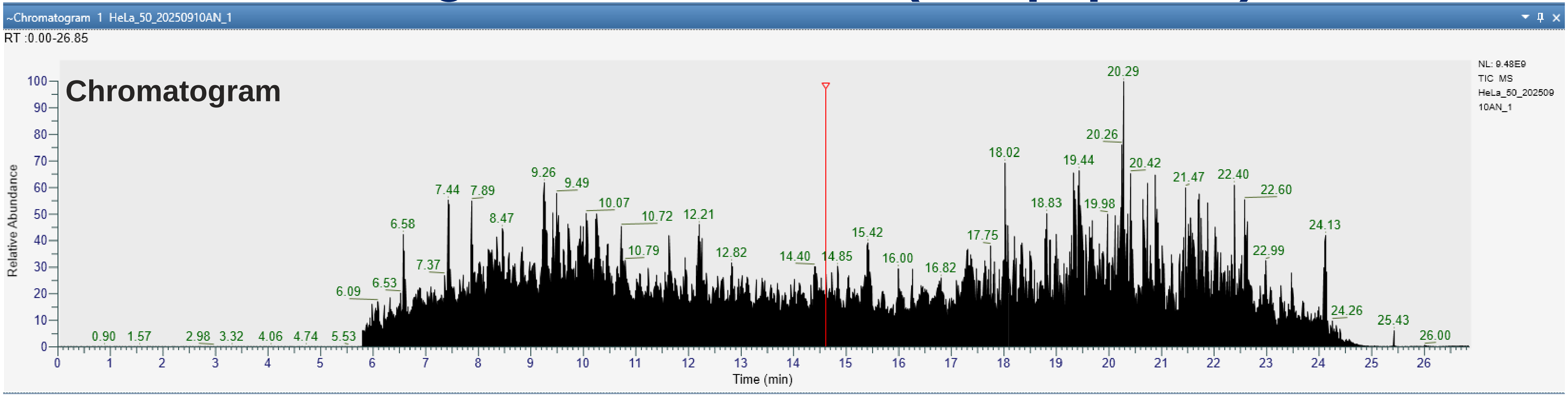
MS spectrum
MS1 spectrum
Parental Ions spectrum



MS/MS spectrum
MS2 spectrum
Fragmented ions spectrum



How do we assign z and m of ions (and peptides)?



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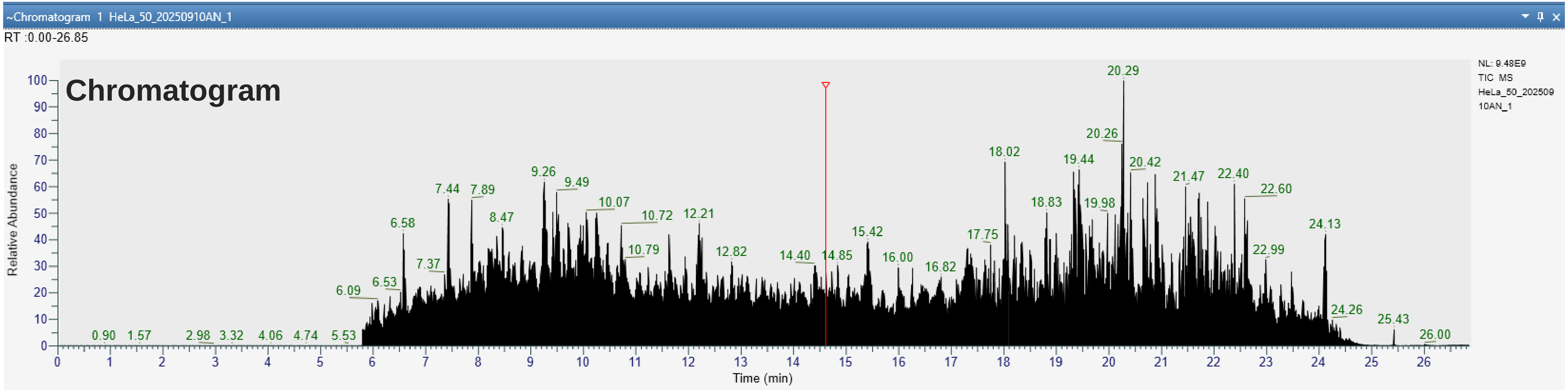

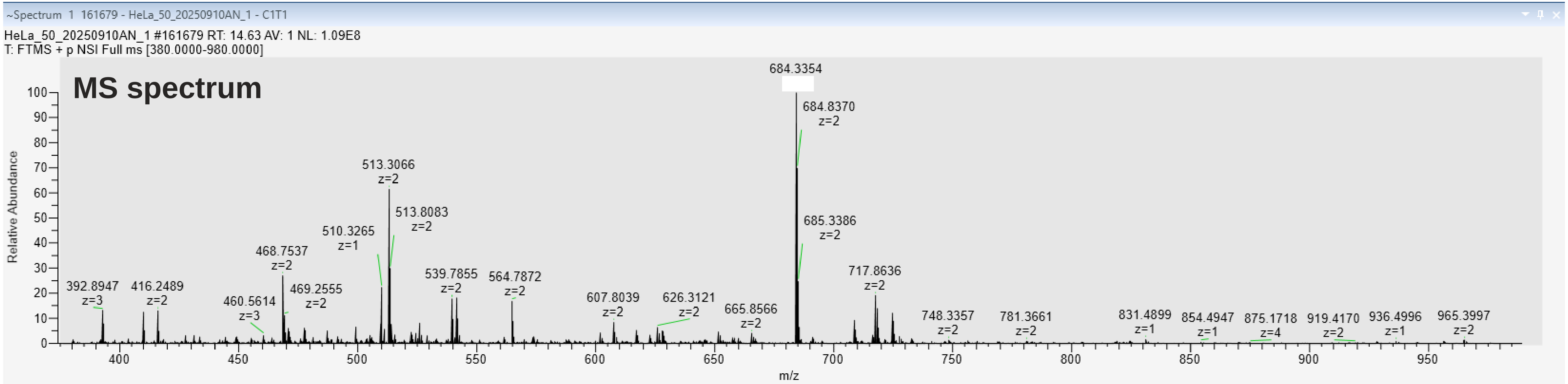

Assigning an ion z and m from a MS spectrum

- **Calculate the charge (z) of the ion:**

- Look for a **cluster of peaks** (isotopic pattern)
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- Identify the **monoisotopic peak**
  - usually the leftmost peak, the peptide with all C12
- Calculate the mass difference (Δm) between 2 adjacent peaks
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- Calculate the **peak spacing** Δ m/z
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- Calculate the **charge state (z):**

$$z=\Delta m/\Delta\ m/z$$

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- Calculate the **ion mass (m)**

$$m=(m/z)\times z$$

m =

- Calculate the **neutral mass (m-(z x 1.0073))**
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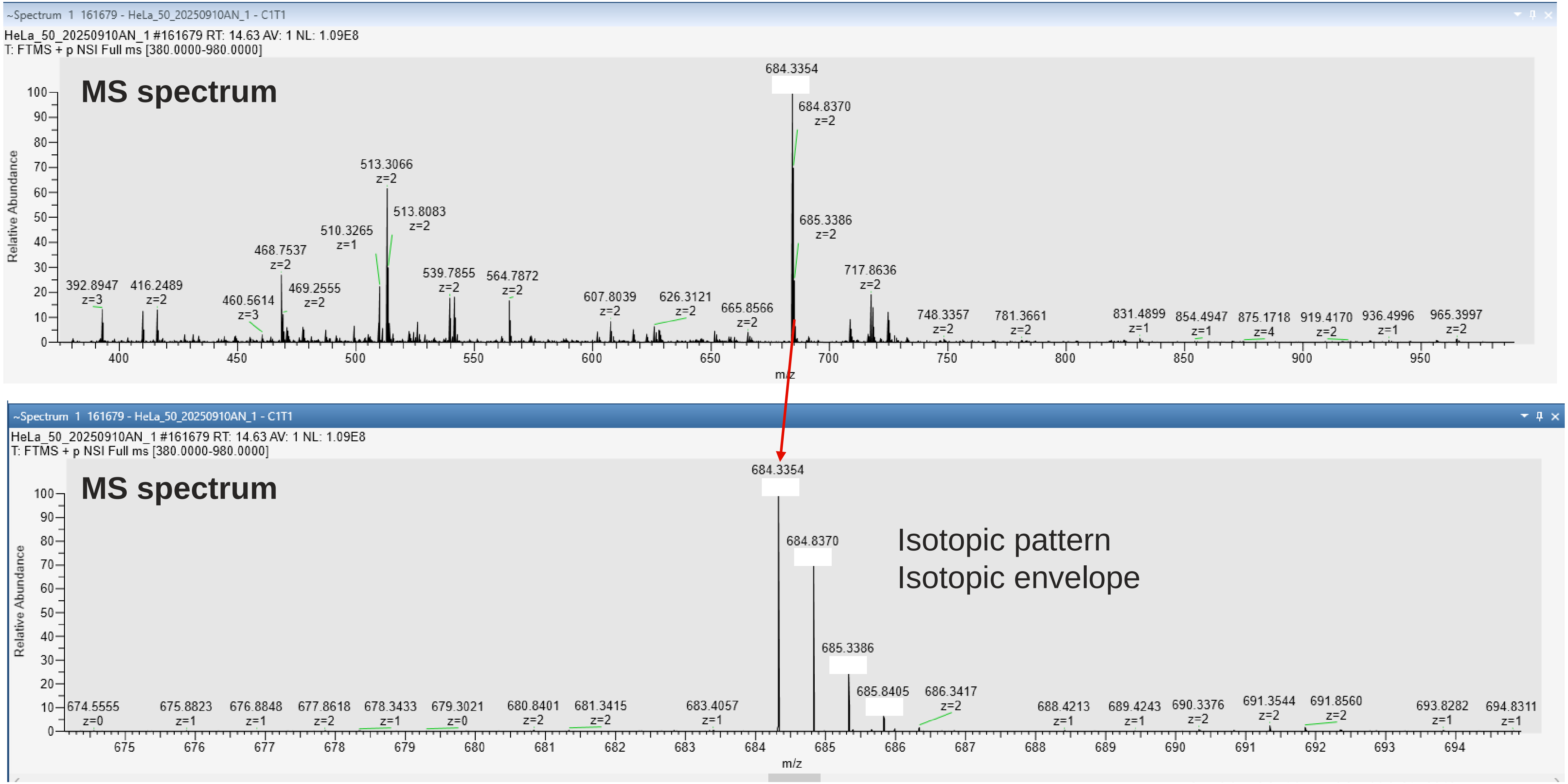

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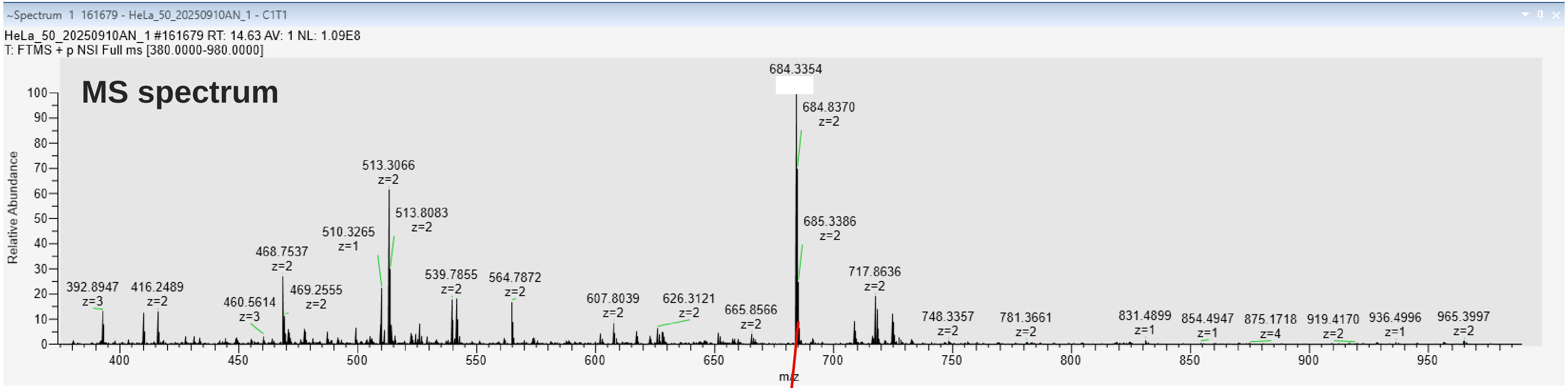

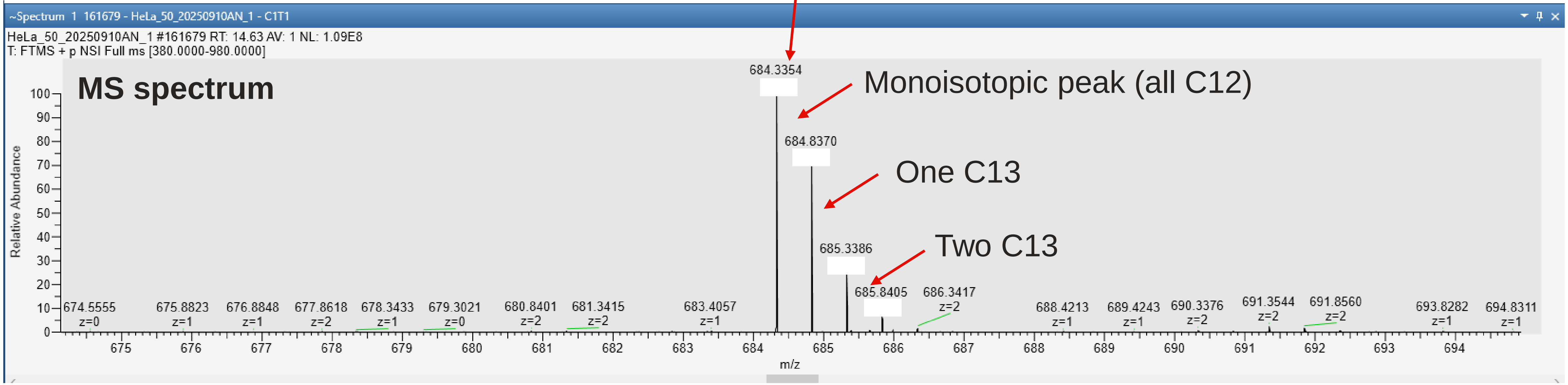

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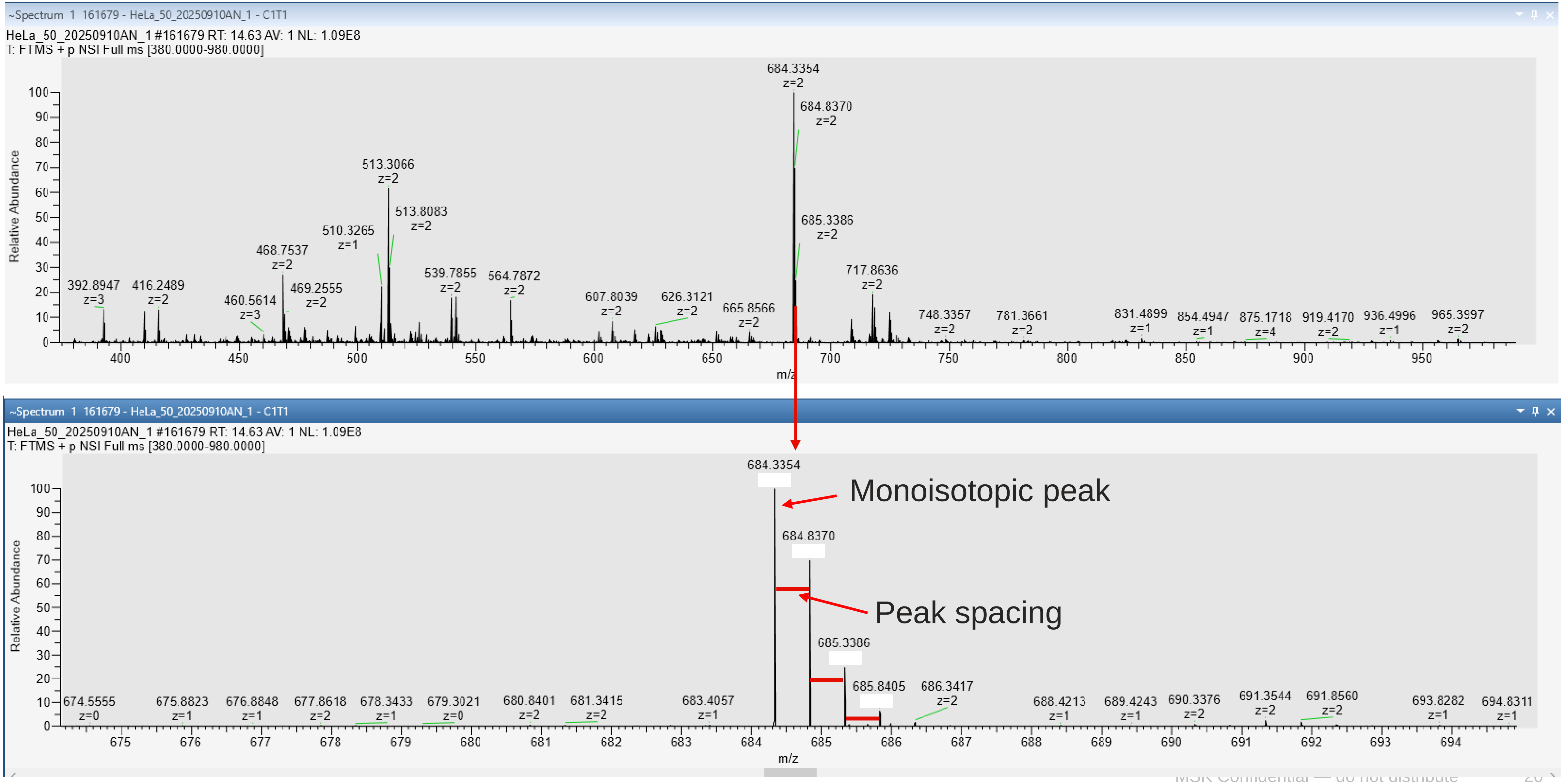

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- Calculate the **peak spacing** Δ m/z
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  - Δ m/z = 684.83 - 684.33 = **0.5**

- Calculate the **charge state (z):**

$$z=\Delta m/\Delta\ m/z$$

  - **z** = 1 / 0.5 = **2** the peptide ion has a charge of +2

- Calculate the **ion mass (m)**

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m = 684.33 x 2 = **1368.66 Da**

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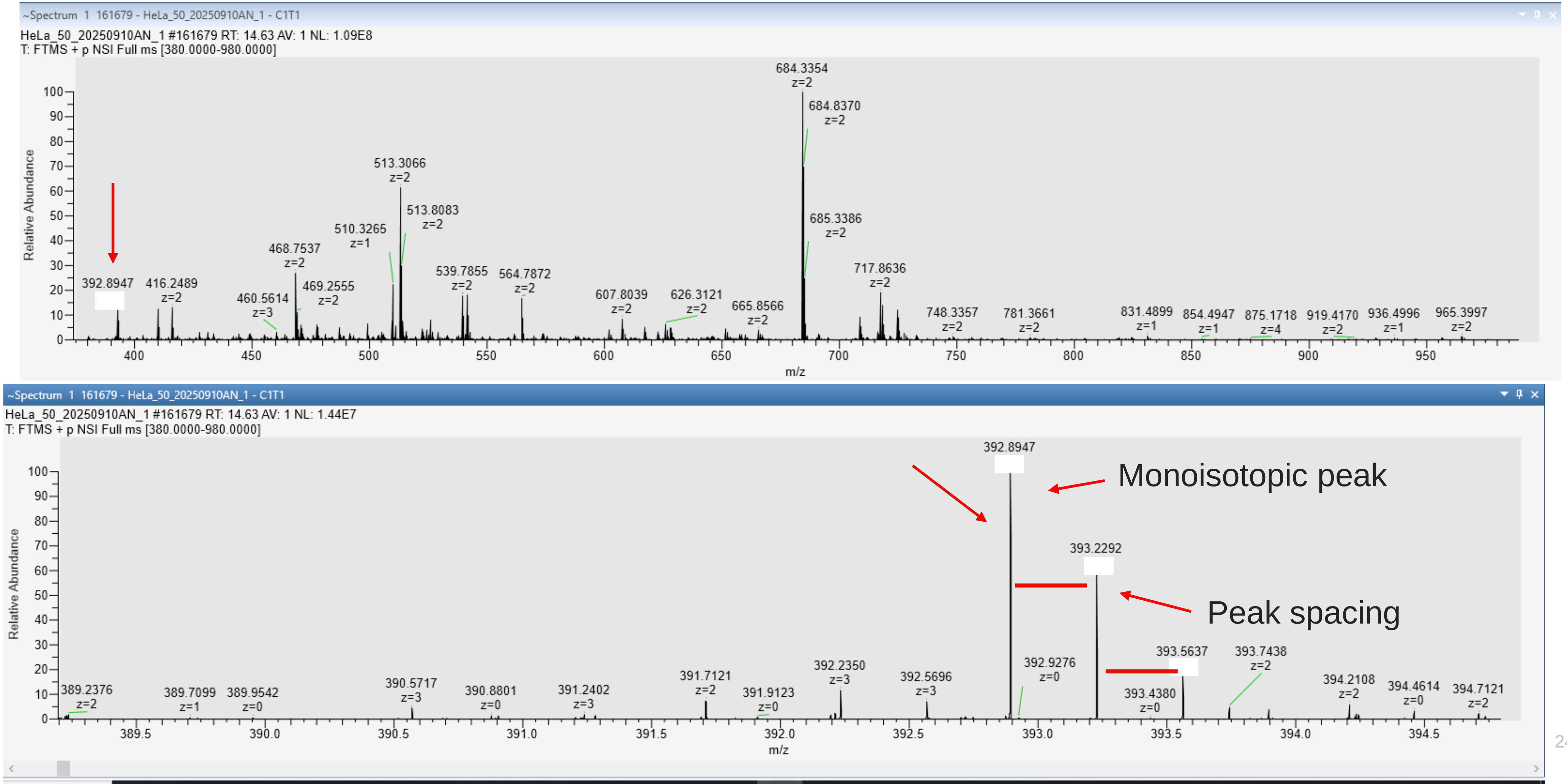
Find the mass and the charge of ion m/z 392.89



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- Calculate the **peak spacing** Δ m/z
  - Measure the difference in the m/z values between 2 adjacent isotopic peaks in the cluster
  - Δ m/z = 393.22 – 392.89 = **0.33**

- Calculate the **charge state (z):**

$$z=\Delta m/\Delta\ m/z$$

  - **z** =

- Calculate the **ion mass (m)**

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- Calculate the **charge state (z):**

$$z=\Delta m/\Delta\ m/z$$

  - **z** = 1 / 0.33 = **3 t**he peptide ion has a charge of +3

- Calculate the **ion mass (m)**

$$m=(m/z)\times z$$

m =

- Calculate the **neutral mass (m-(z x 1.0073))**
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- Calculate the **ion mass (m)**

$$m=(m/z)\times z$$

m = 393.22 x 3 = **1191.57 Da**

- Calculate the **neutral mass (m-(z x 1.0073))**
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  - **Neutral mass** = m − (z × 1.0073) =

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  - Δ m/z = 393.22 – 392.89 = **0.33**

- Calculate the **charge state (z):**

$$z=\Delta m/\Delta\ m/z$$

  - **z** = 1 / 0.33 = **2 t**he peptide ion has a charge of +3

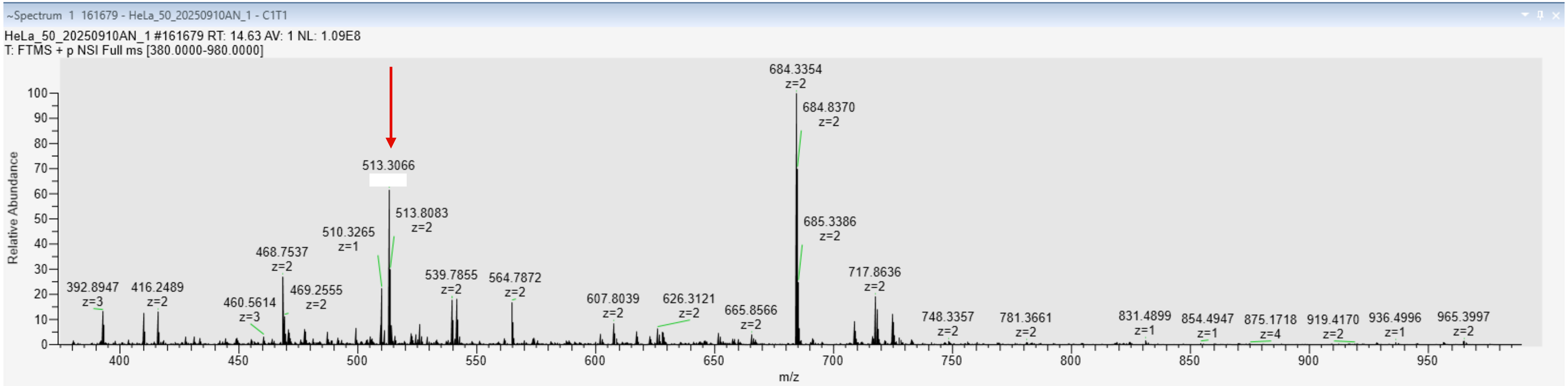
- Calculate the **ion mass (m)**

$$m=(m/z)\times z$$

m = 393.22 x 3 = **1191.57 Da**

- Calculate the **neutral mass (m-(z x 1.0073))**
  - Proton mass=1.0073
  - **Neutral mass** = m − (z × 1.0073) = 1191.57 – 3.0219 = **1188.5 Da**

Find the mass and the charge of ion m/z 513.30



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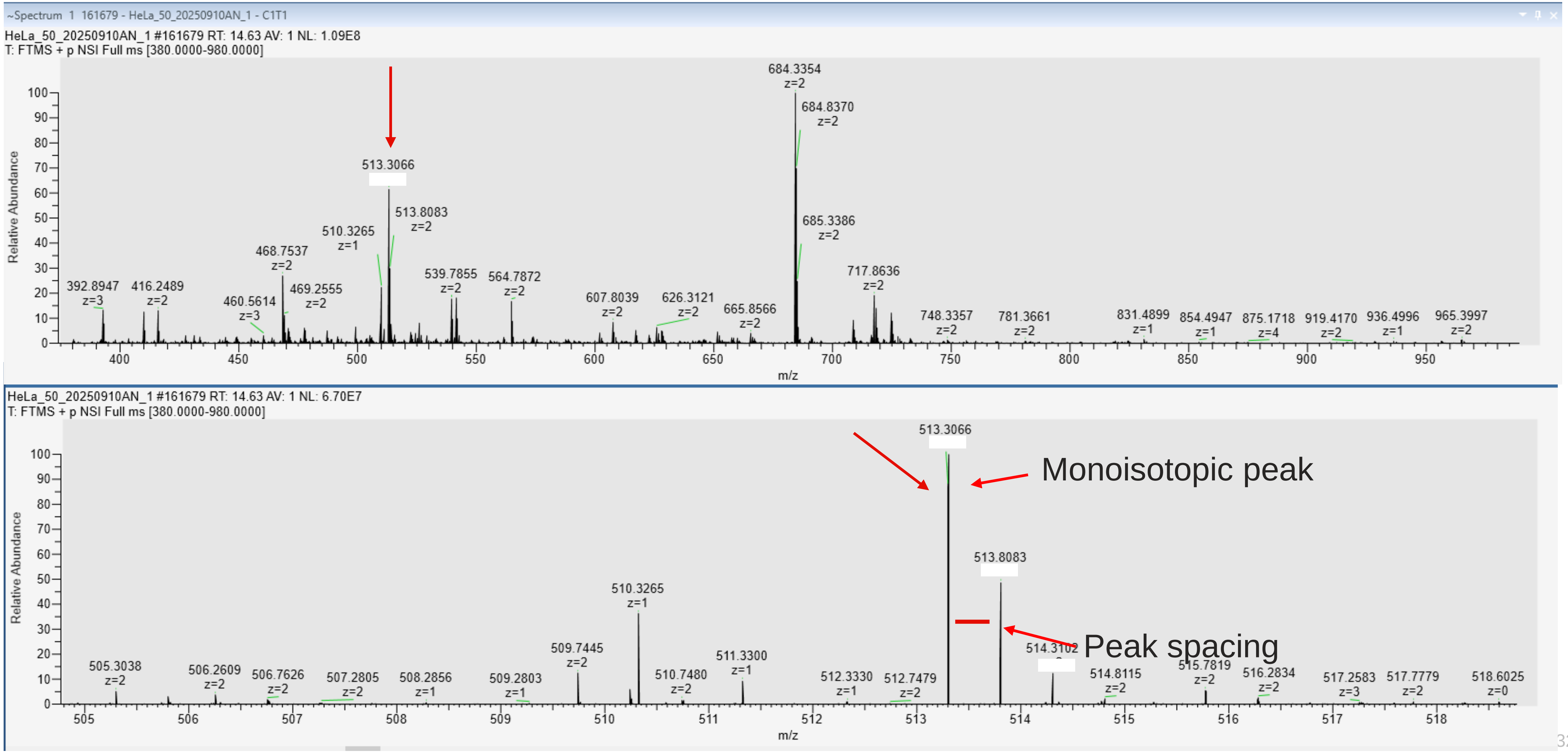

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- Calculate the mass difference (Δm) between 2 adjacent peaks
  - Δm=C13-C12=1 Dalton

- Calculate the **peak spacing Δ m/z**
  - Measure the difference in the m/z values between 2 adjacent isotopic peaks in the cluster
  - Δ m/z=513.30-513.80=0.5

- Calculate the **charge state (z):**

z=Δm/Δ m/z

  - Z=1/0.5=2
  - The peptide ion has a charge of +2

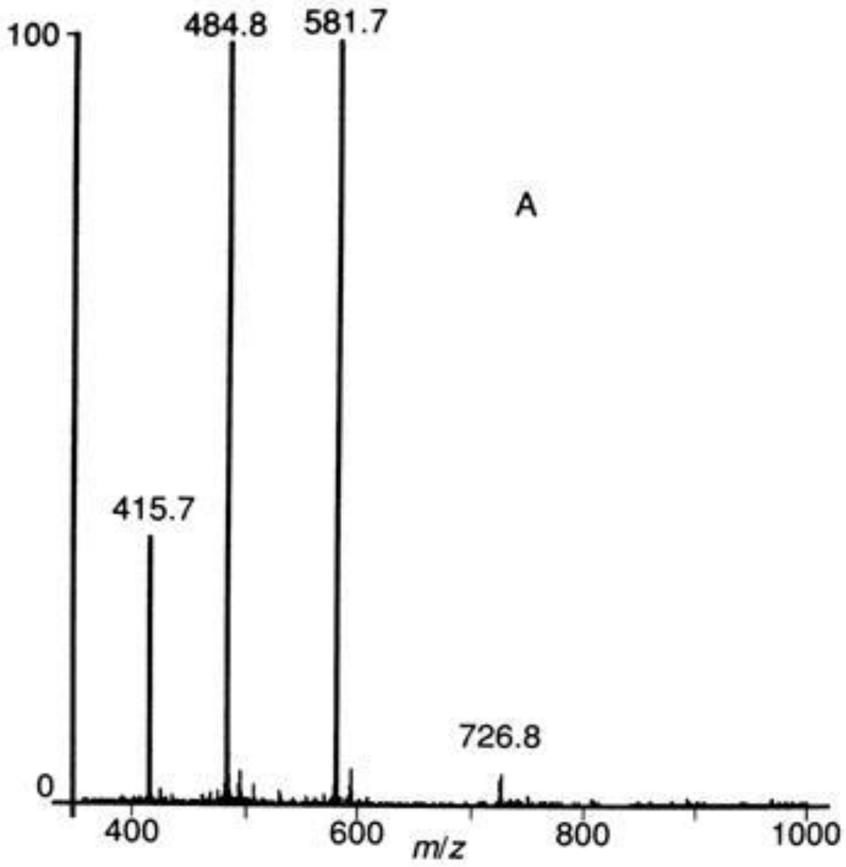
- Calculate the **ion mass (m)**

m=(m/z)×z

m=513.30 X 2 =1036.6 Da

- Calculate the **neutral mass (m-(z x 1.0073))**
  - Proton mass=1.0073
  - **Neutral mass**=m−(z×1.0073) = 1368.66-2.0146=1024.58 Da

How do we calculate the charge and mass of a protein?



- In an ESI spectrum, each observed peak corresponds to the same protein with a different charge

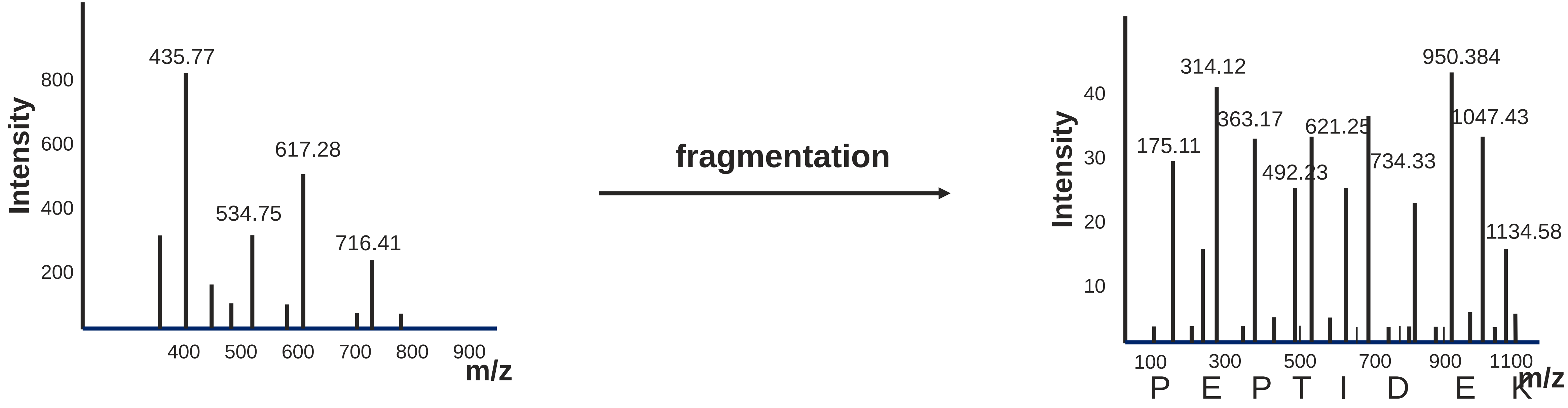
- f you have two neighboring peaks at m/z values m1 and m2 (where m2>m1), the lower m/z peak corresponds to charge z1, and the higher m/z peak corresponds to z1−1.

- Calculate the charge of the lower m/z peak
- **z1 = (m2 -1) / (m2 – m1)**

- Calculate the protein neutral mass M
  - M=(m1 x z1) – (z1 x 1.0073)

How do we identify a peptide sequence?

MS spectrum **MS/MS spectrum**



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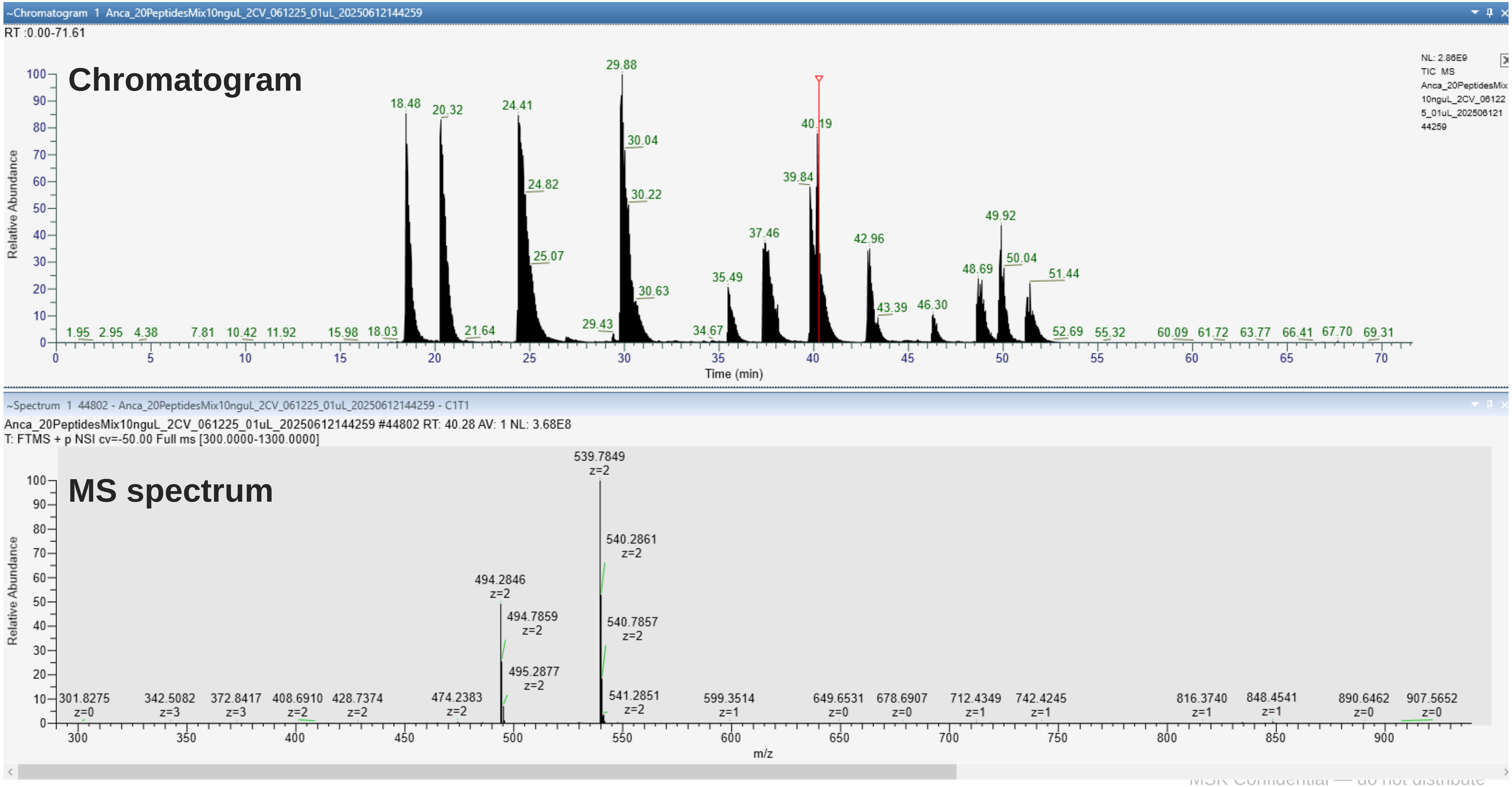

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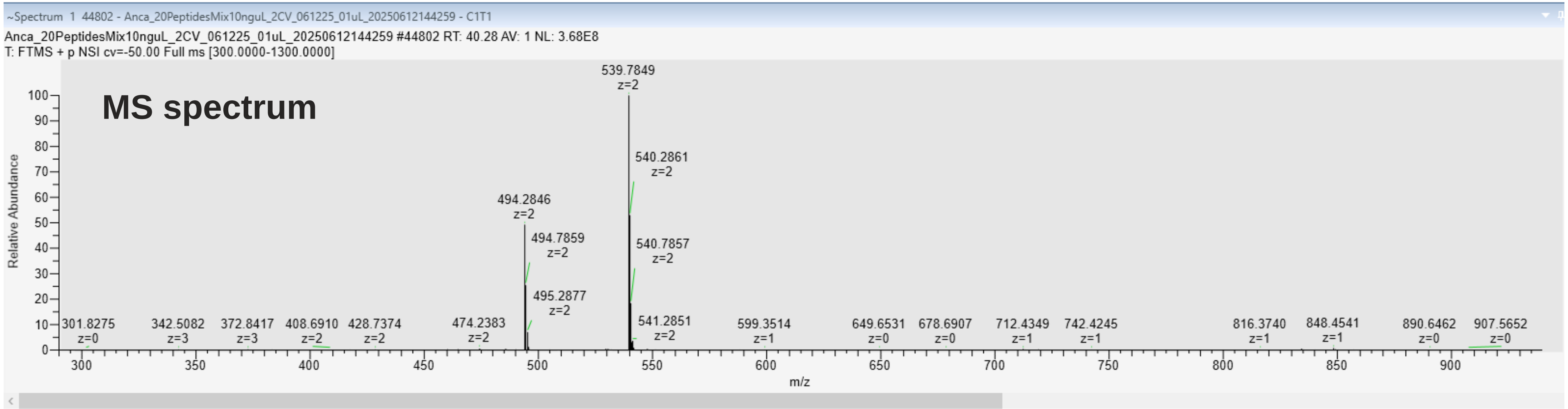

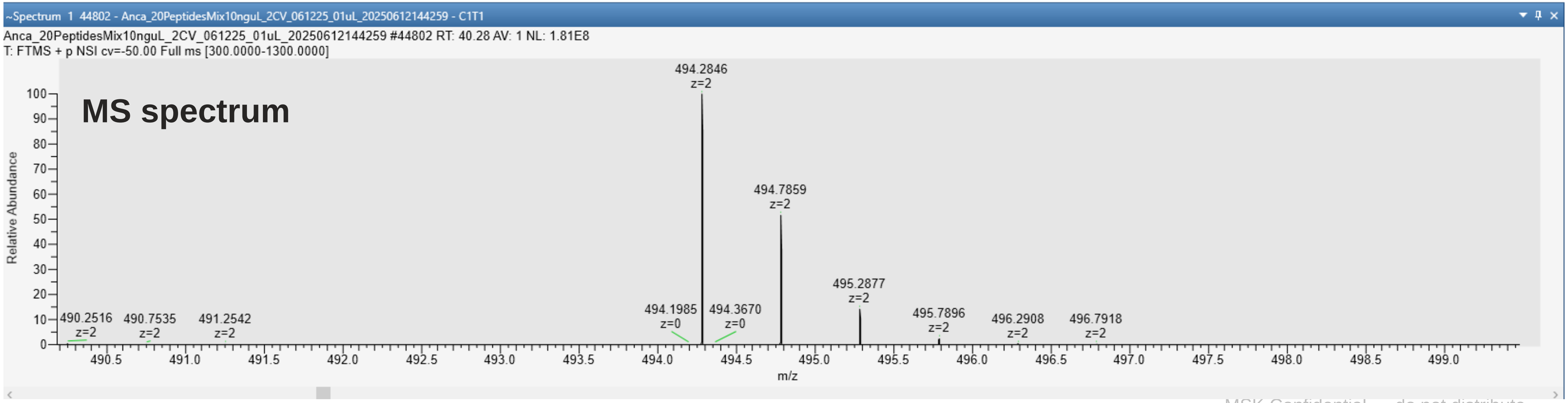

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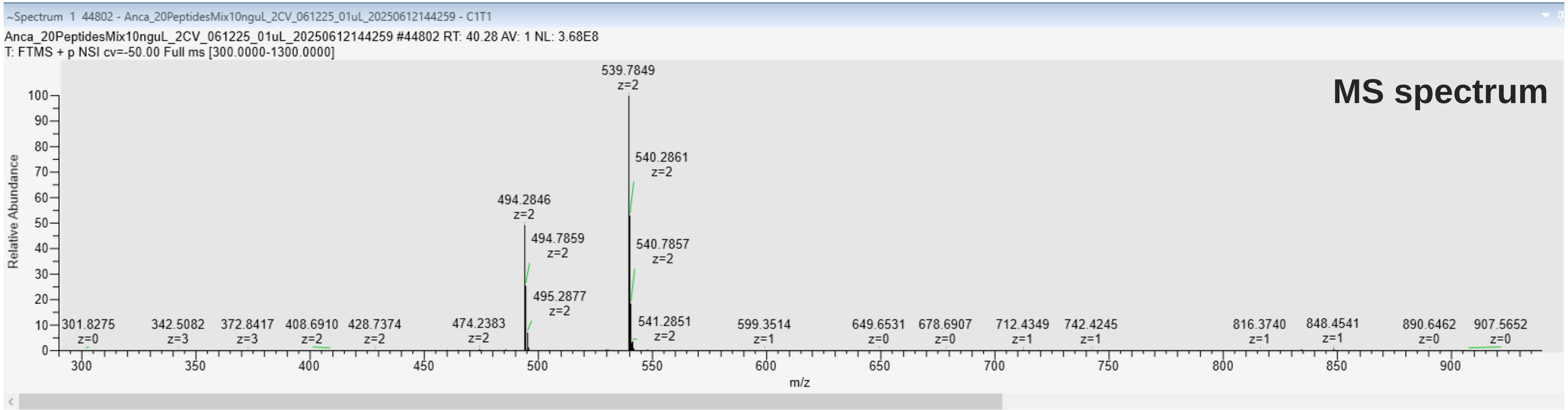
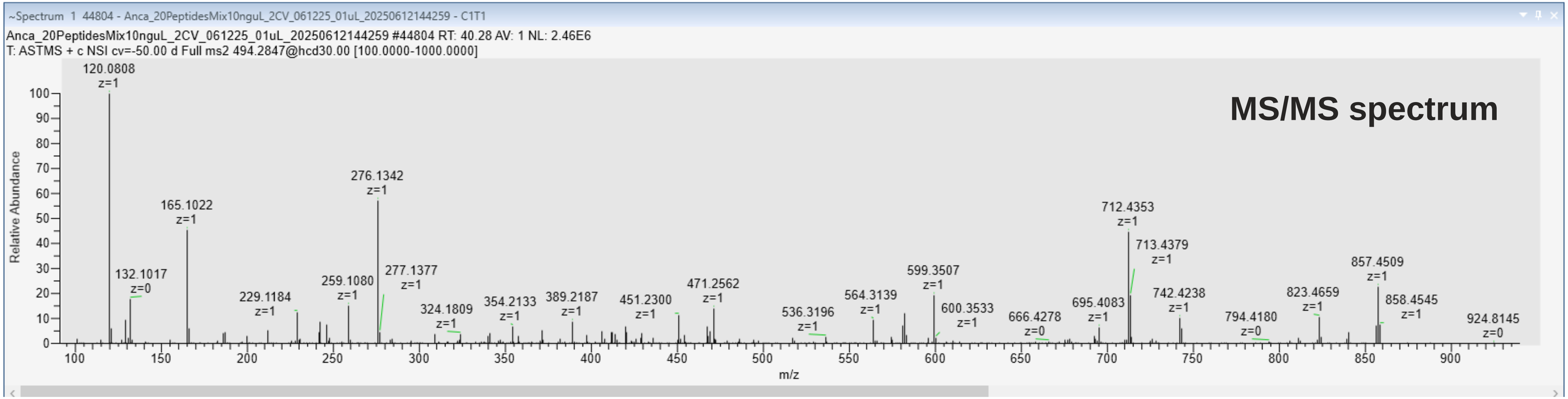


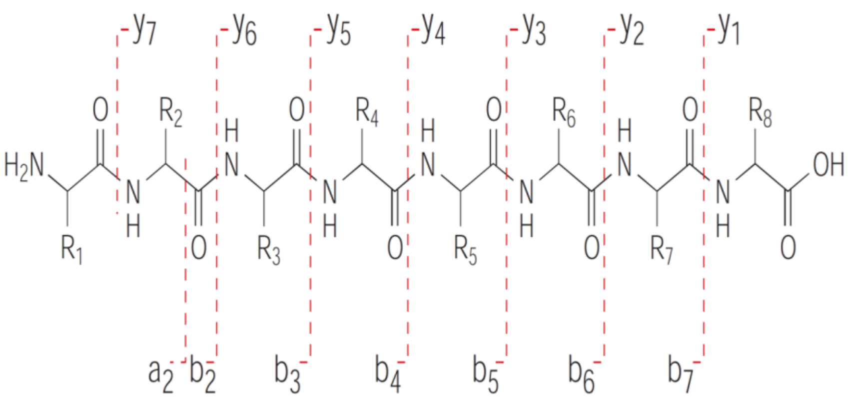


How do we identify a peptide sequence?

- This fragmentation generates MS/MS spectra, with incomplete ladders of peaks
- The spacing among peaks correspond to amino acid masses

Parent ion

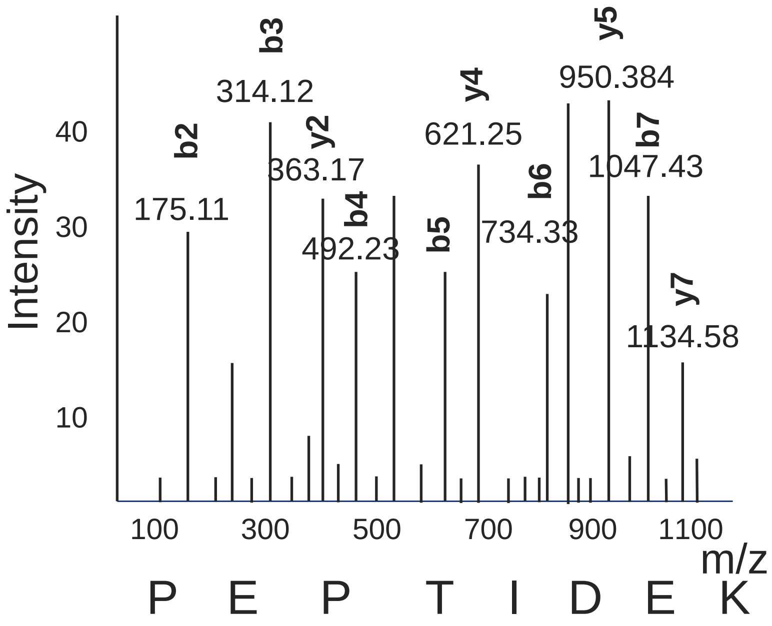
P E P T I D E K



Fragmented ion

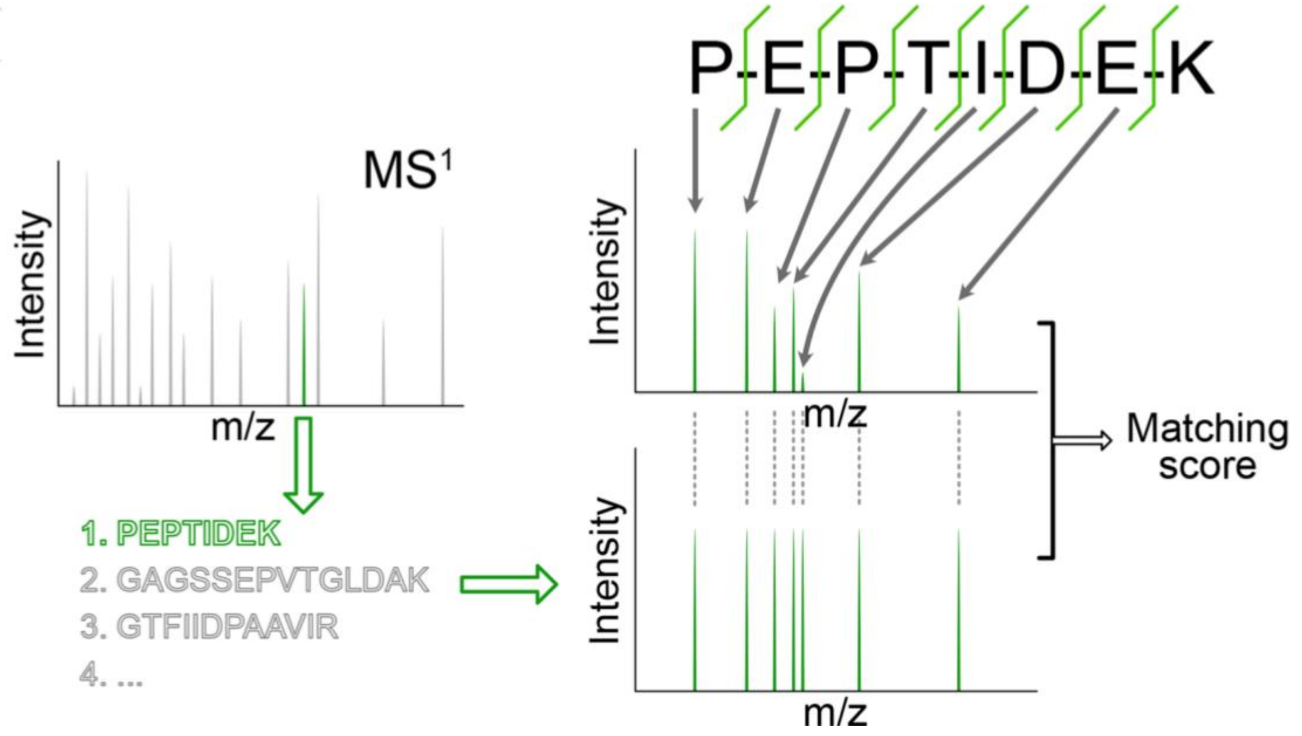
b1	P	EPTIDEK	y7
b2	PE	PTIDEK	y6
b3	PEP	TIDEK	y5
b4	PEPT	IDEK	y4
b5	PEPTI	DEK	y3
b6	PEPTID	EK	y2
b7	PEPTIDE	K	y1

MS/MS Spectrum



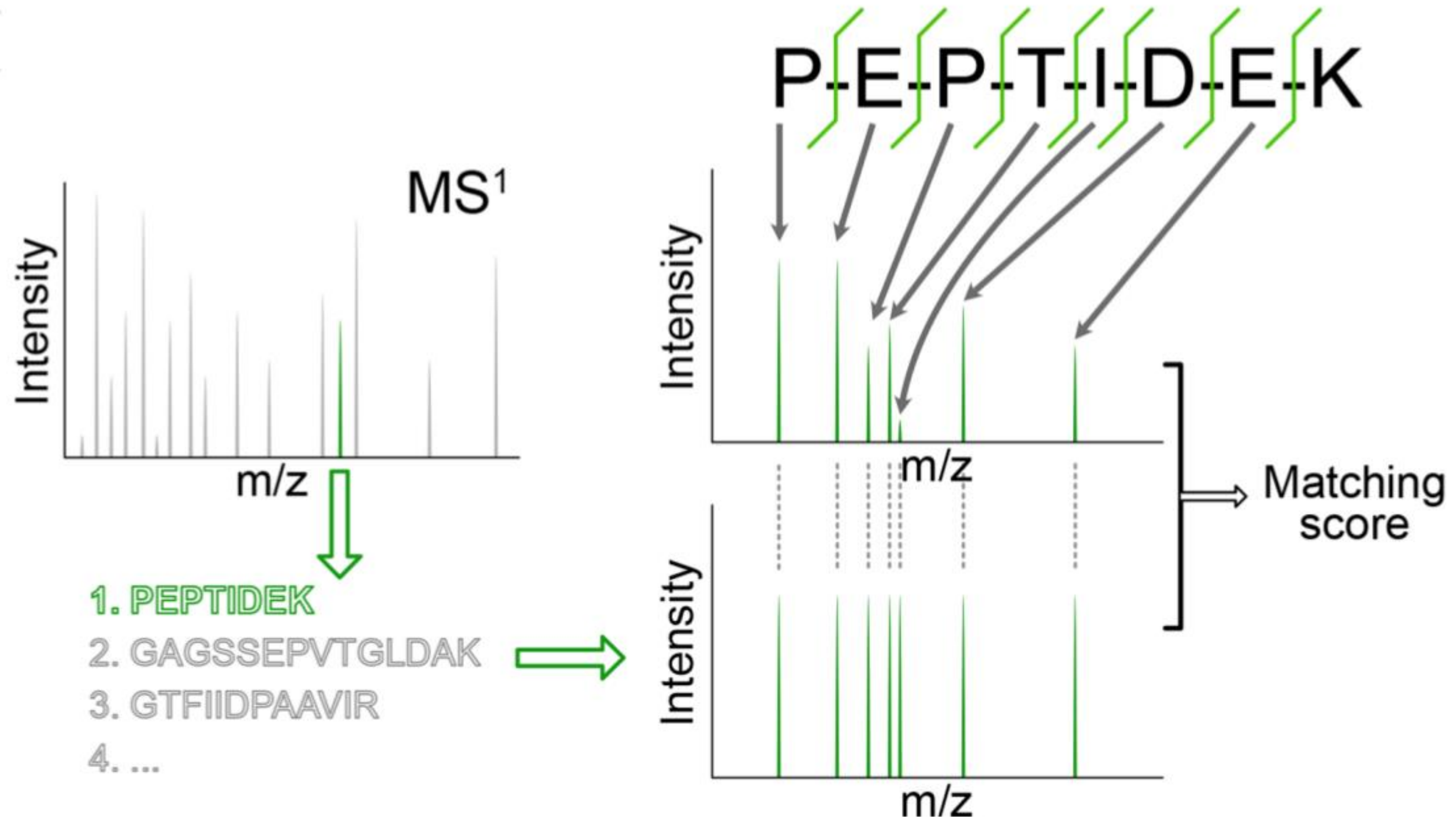
How we identify peptides from MS spectra?

- Database identification
- uses software to simulate enzymatic digestion of all proteins in the database to create a peptide list
- Each peptide is theoretically fragmented to create predicted MS/MS spectra
- Each experimental spectrum is compared to all candidate theoretical spectra
- The software score how well each theoretical spectrum matches the experimental one
- Each spectrum is assigned the best matching peptide
- Peptides matches across all spectra are grouped to identify proteins



How we identify peptides from MS spectra?

- To ensure matches are not due to random chances:
- Search is also run against a decoy database (reversed or scrambled sequence)
- FDR=false matches/total matches
- Typically set at 1% (high confidence identification)



How to identify peptides and proteins from spectra?

Raw MS/MS spectra

↓

Protein database (FASTA)

↓

In silico digestion (trypsin)

↓

Generates theoretical peptides and fragment ions

↓

Match experimental spectra to theoretical one

↓

Score matches-assigned peptides

↓

Group peptides-identify proteins

↓

Control FDR using decoy database

Outline

- Introduction to mass spectrometry-based proteomics
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- Instruments
- Quantification methods
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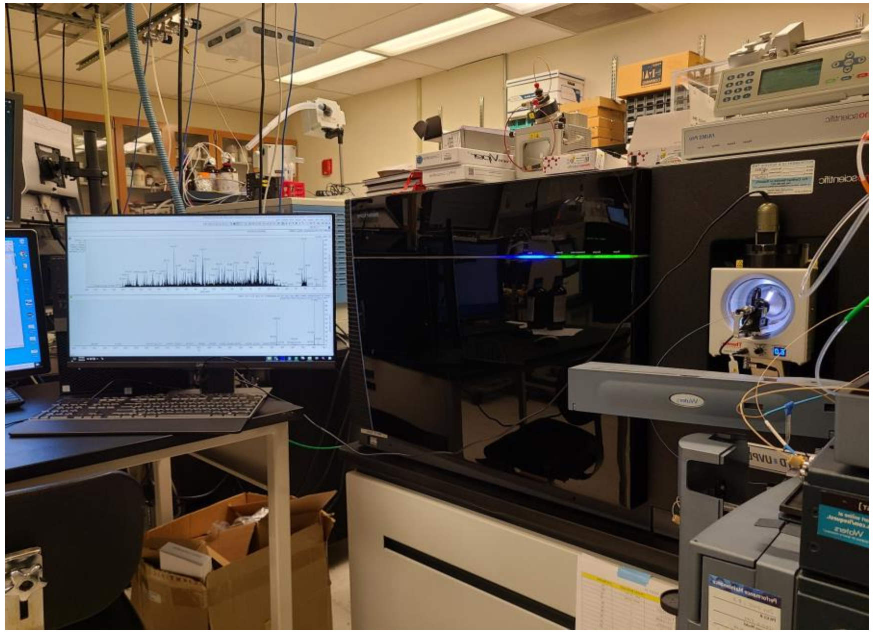
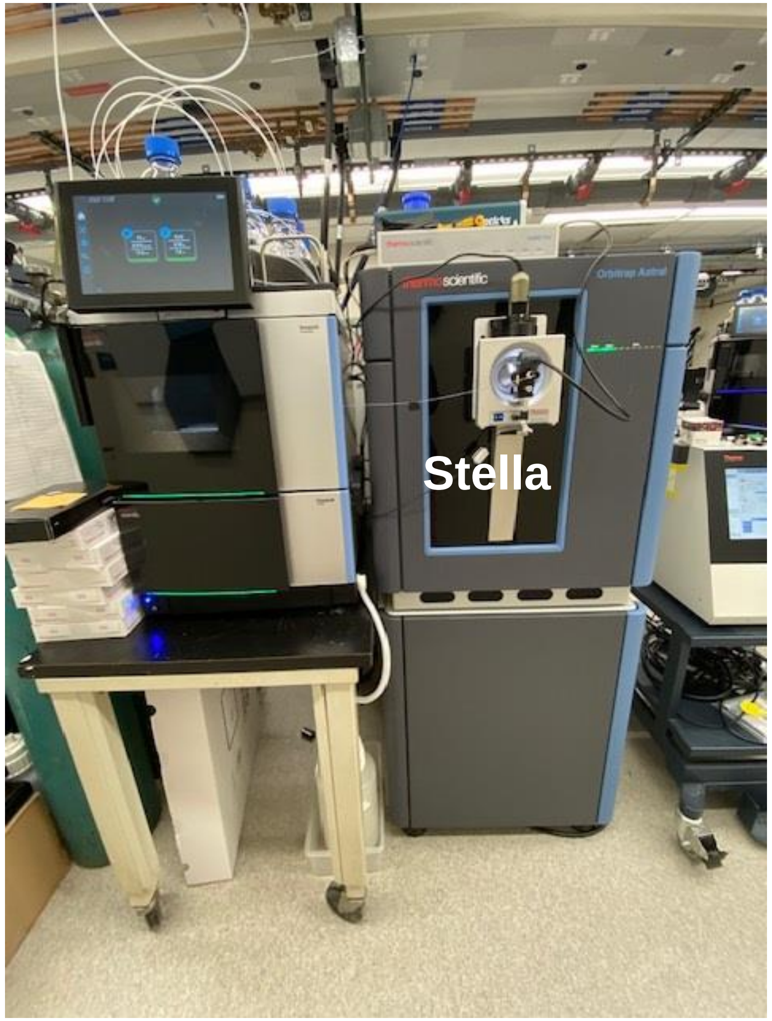
- Paper discussion



High resolution Mass Spectrometers at MSKCC

TimsTOF

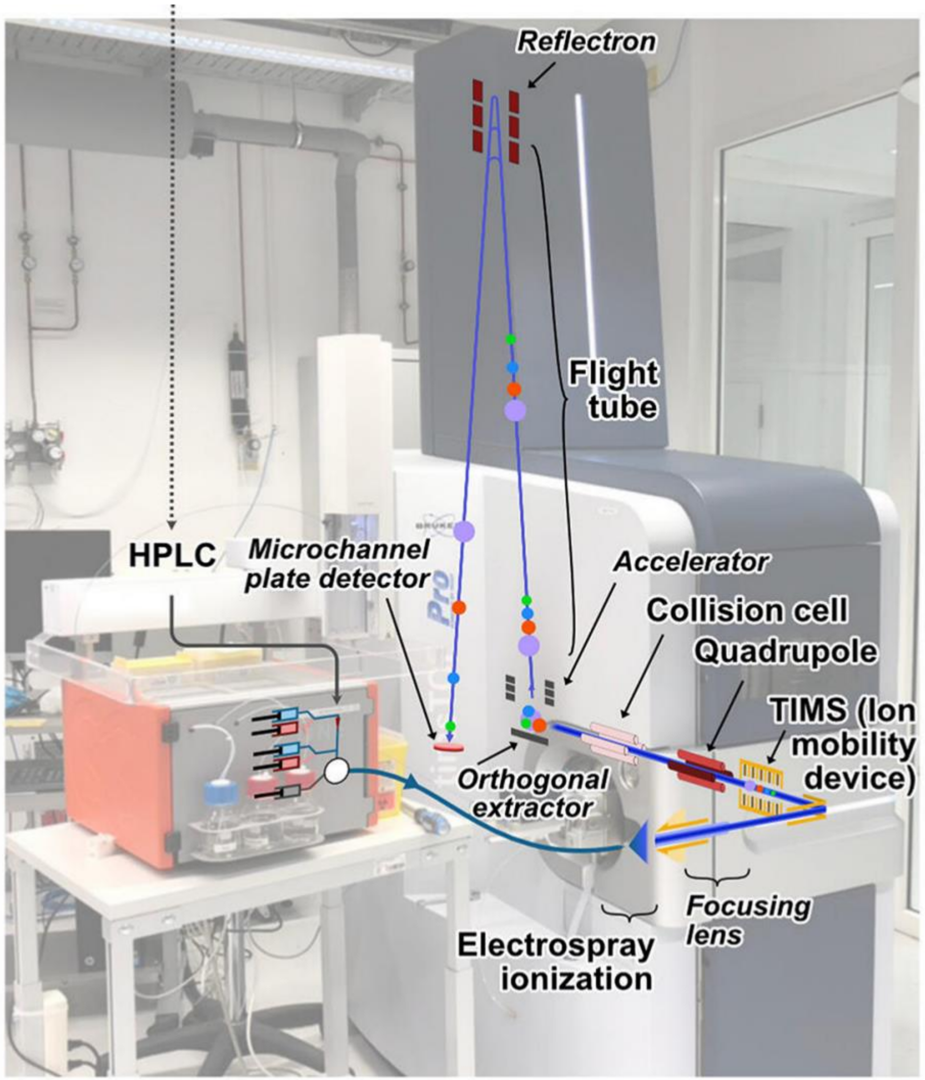
Astral



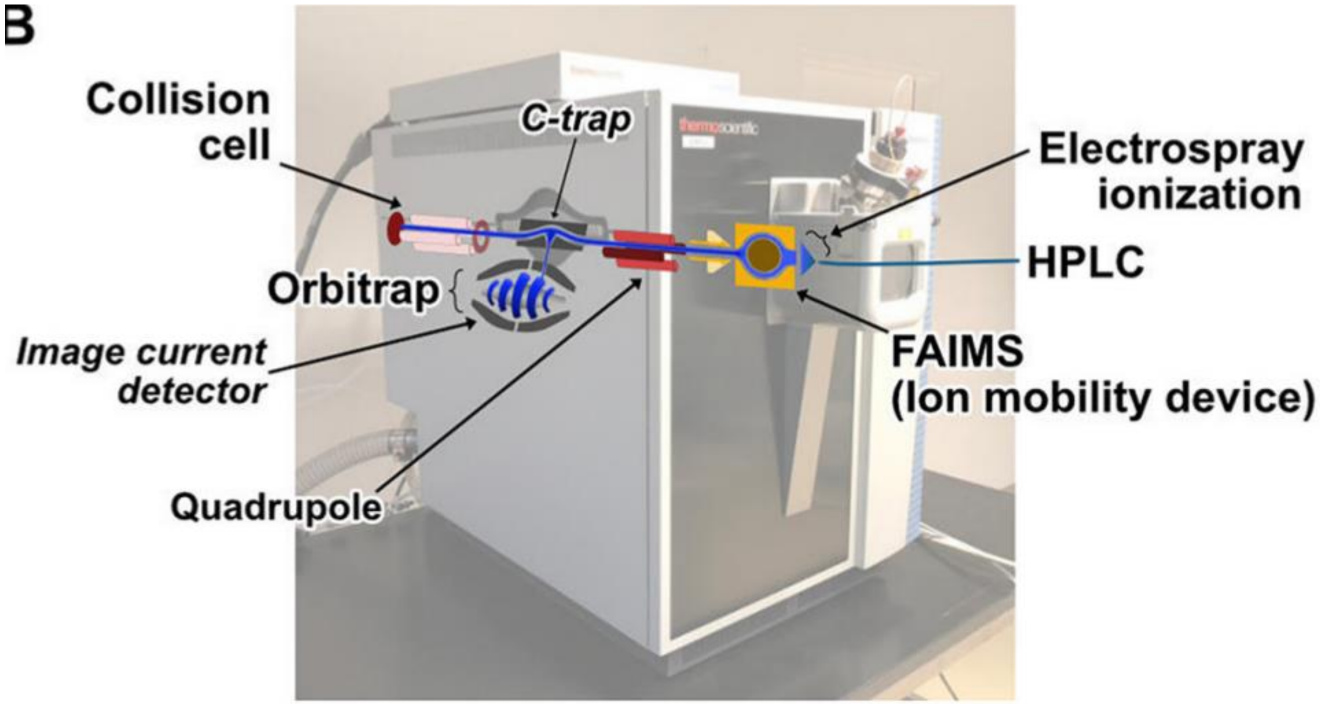
Lumos/Eclipse

Inside of a Mass Spectrometer

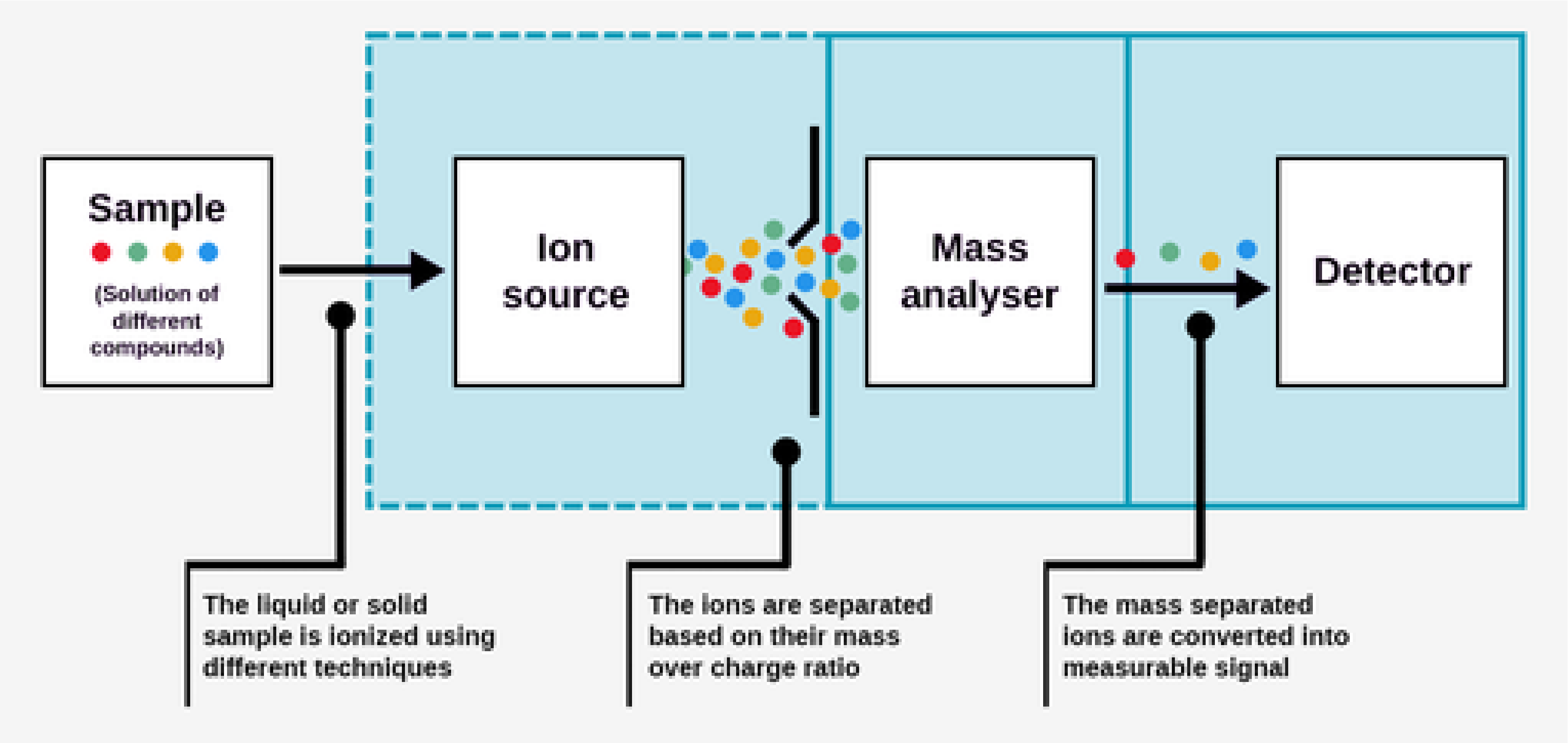
TOF



Orbitrap

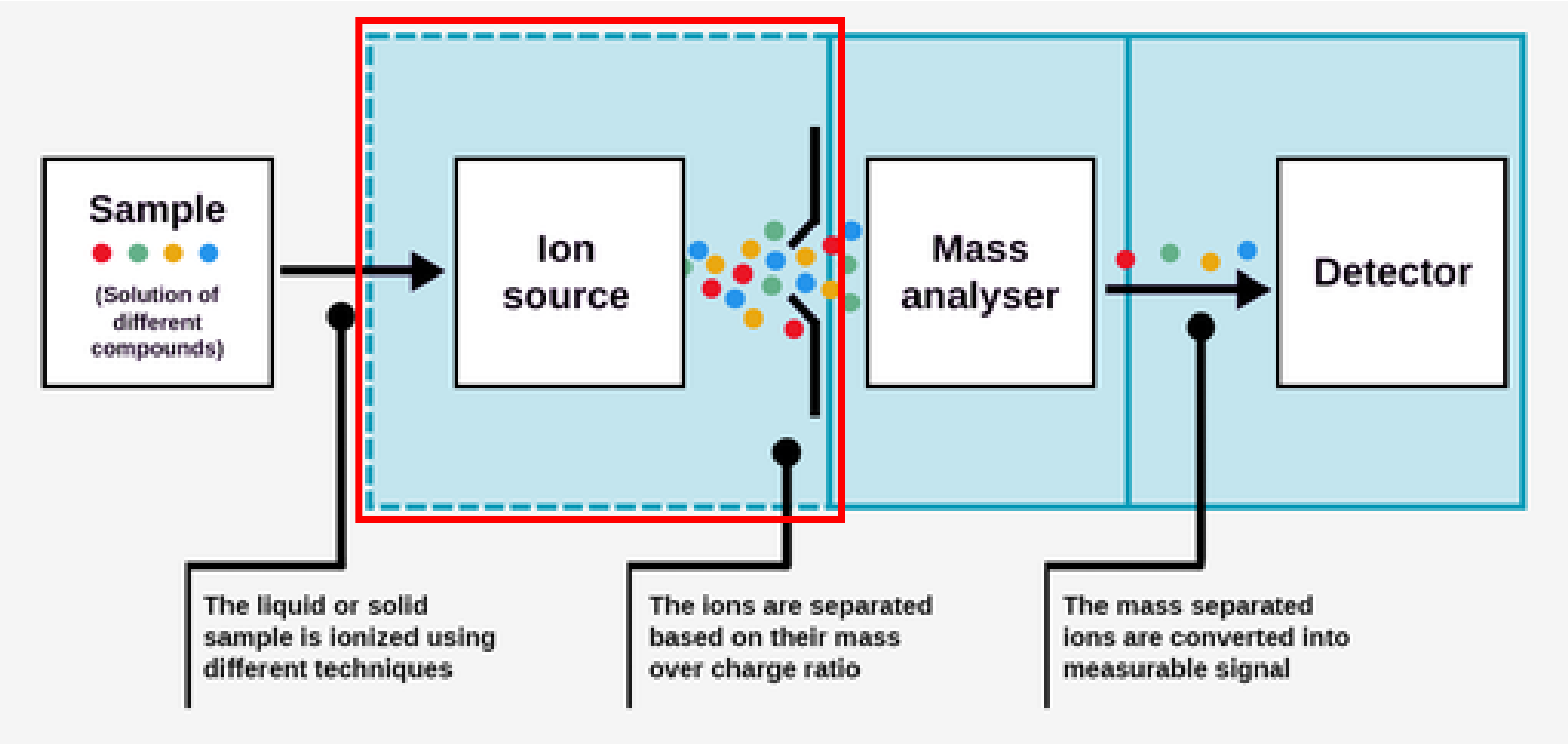


Liquid chromatography coupled to mass spectrometry



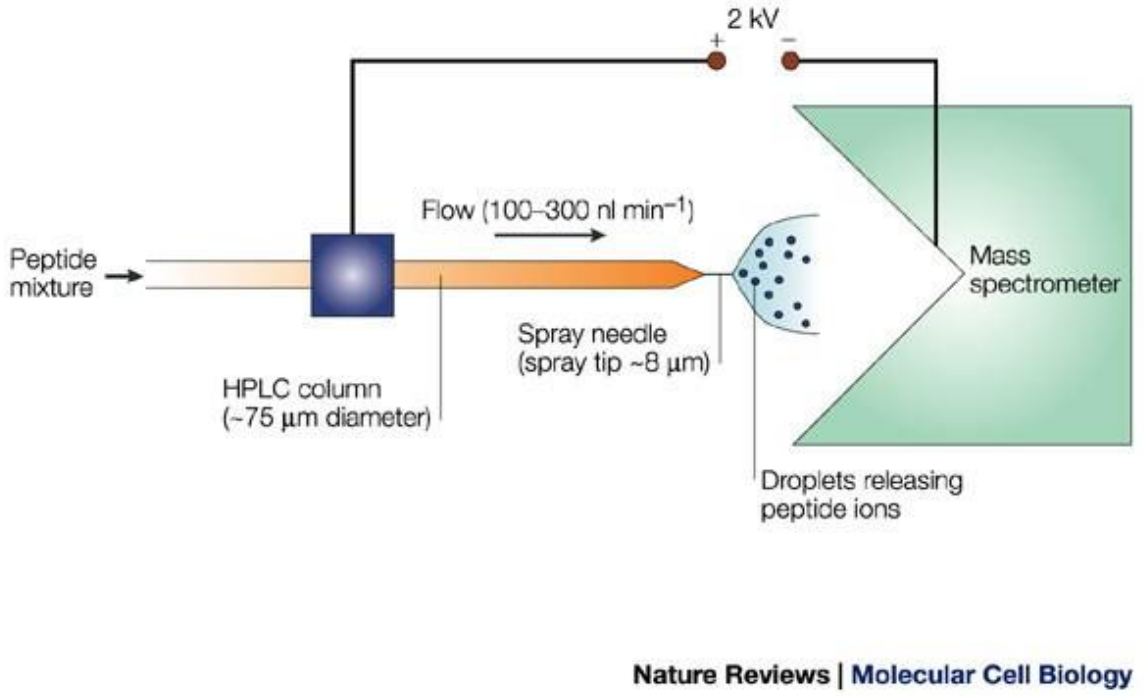
Ion source

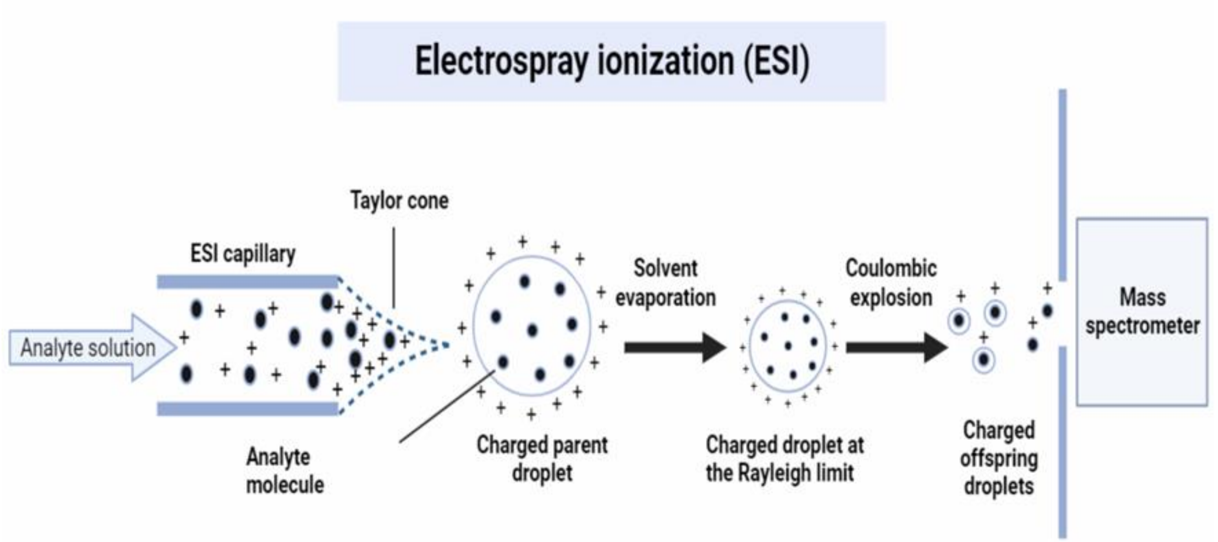
- Ionizes the liquid or solid samples using different techniques
- The sample is turned into a gas and charged with electric and magnetic fields



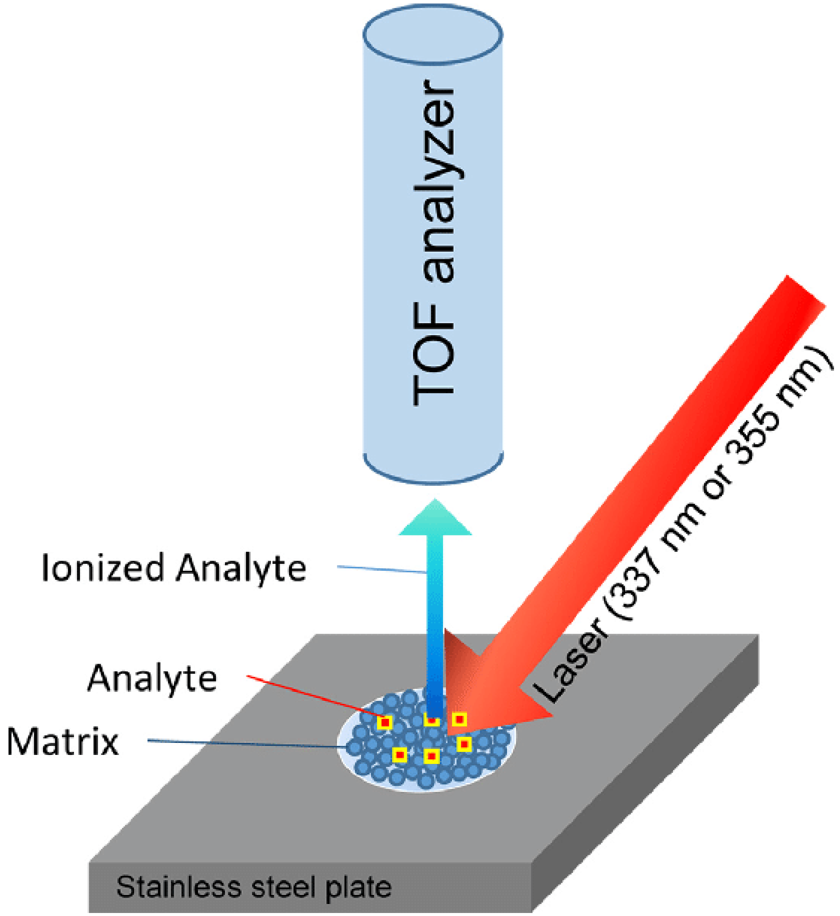
Electrospray Ionization (ESI)

•The liquid containing the peptides flow through a micrometer-sized needle held at a high voltage (2–4 kV).
•Upon reaching the needle, the liquid disintegrates into extremely small, highly charged and rapidly evaporating charged droplets, leaving peptide ions in the gas phase.
•John Fenn received the Nobel Prize for this discovery, the exact mechanisms are not completely understood.



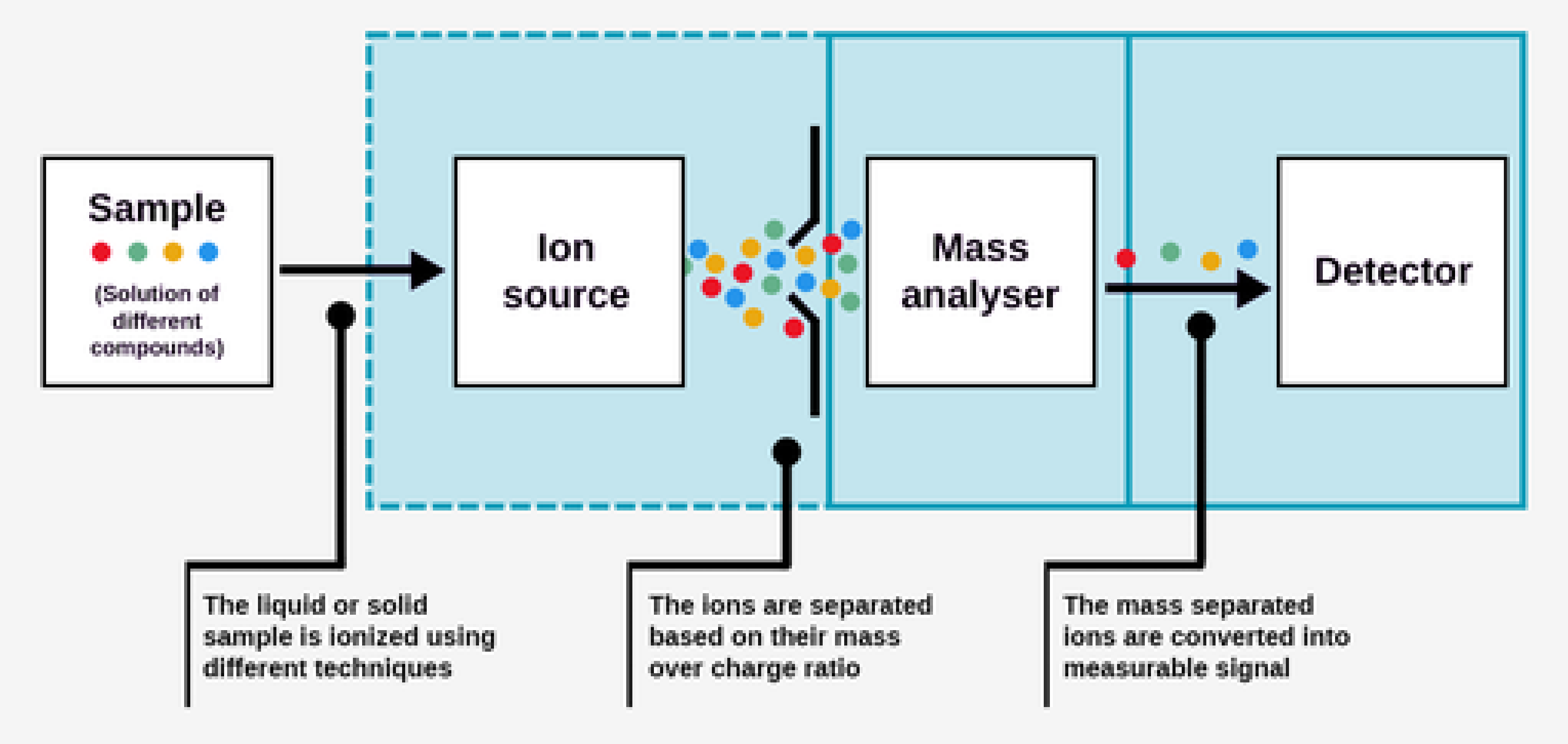


Matrix Assisted Laser Desorption Ionization



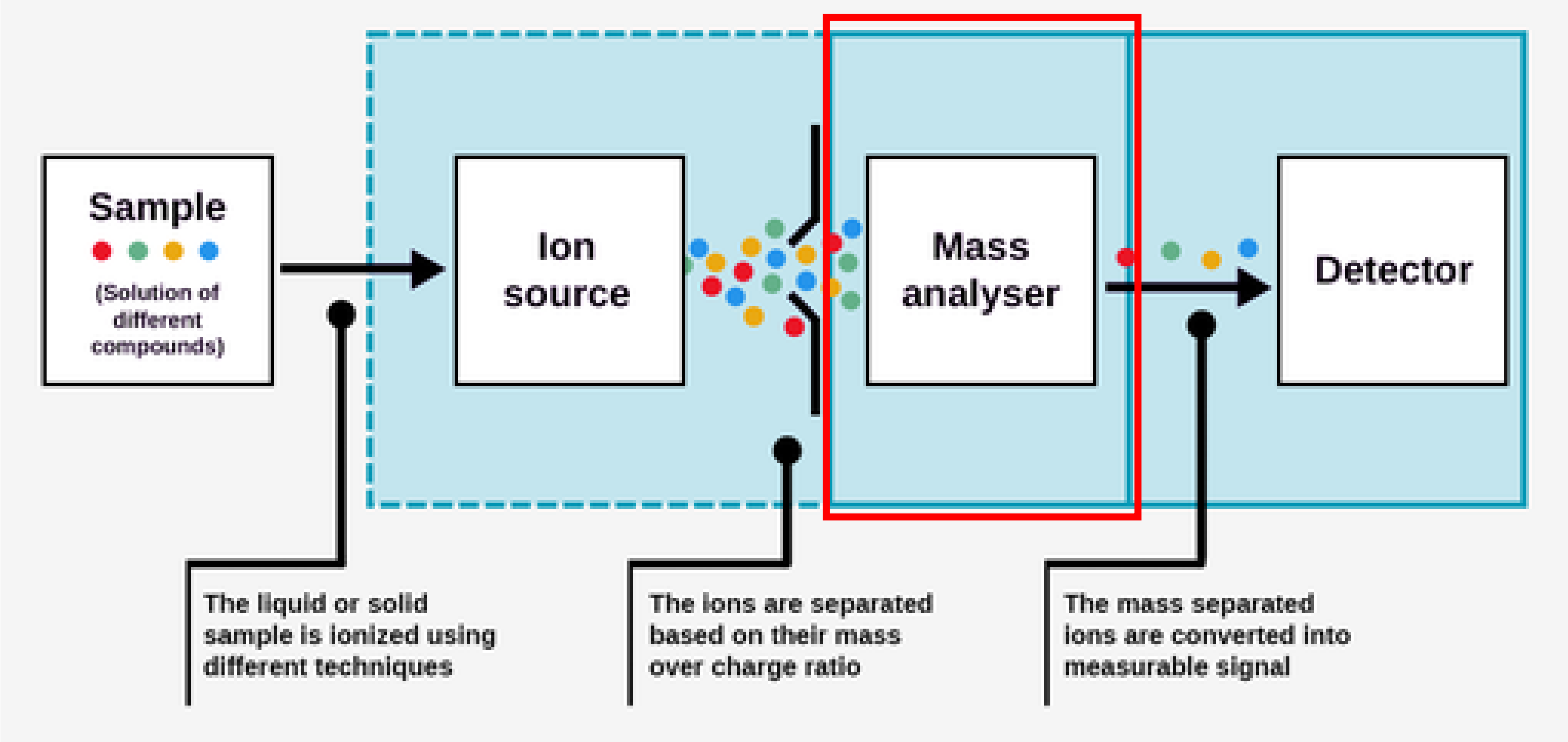
What are the tasks of a mass spectrometer in proteomics?

- Create ions from analyte molecules
- Separate the ions based on charge and mass
- Detect ions and determine their mass-to-charge (m/z) (**precursor ion**)
- Select and fragment ions of interest to provide structural information (MS/MS) (**fragment ion**)



Mass analyzers

- Separate ions based on their mass-to-charge ratios (m/z)
- Light and charged fragments will be accelerated by the fields and go through the analyzer faster
- Differ in the principle they use for separating ions
  - Quadrupole
  - Time of flight (TOF)
  - Orbitrap



Mass Spectrometer performance factors

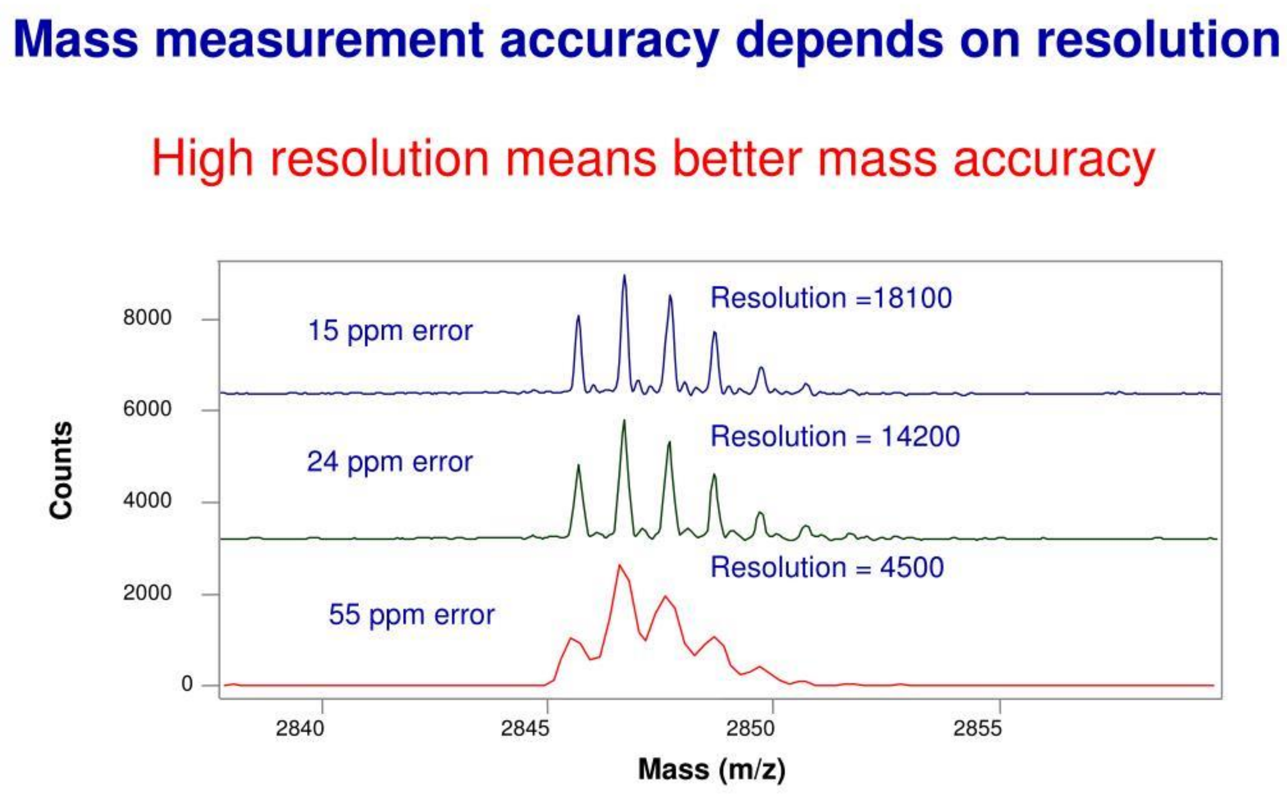
Mass accuracy: how accurate is the mass measurement

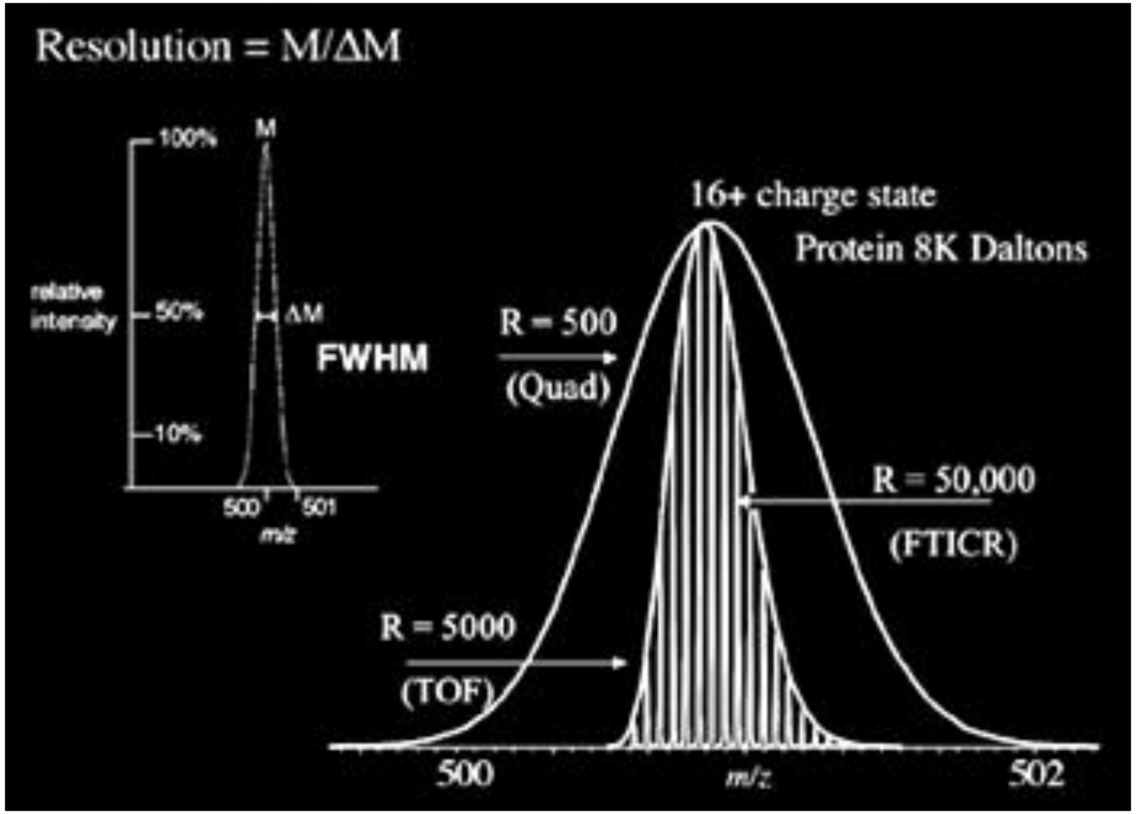
Resolution: how well separated are the peaks from each other

Sensitivity: how small an amount can be analyzed

Resolution and mass accuracy

- Resolution is the ability to separate spectra in MS
- The term resolving power is often used to describe the ability of a mass spectrometer to resolve adjacent peaks in a mass spectrum





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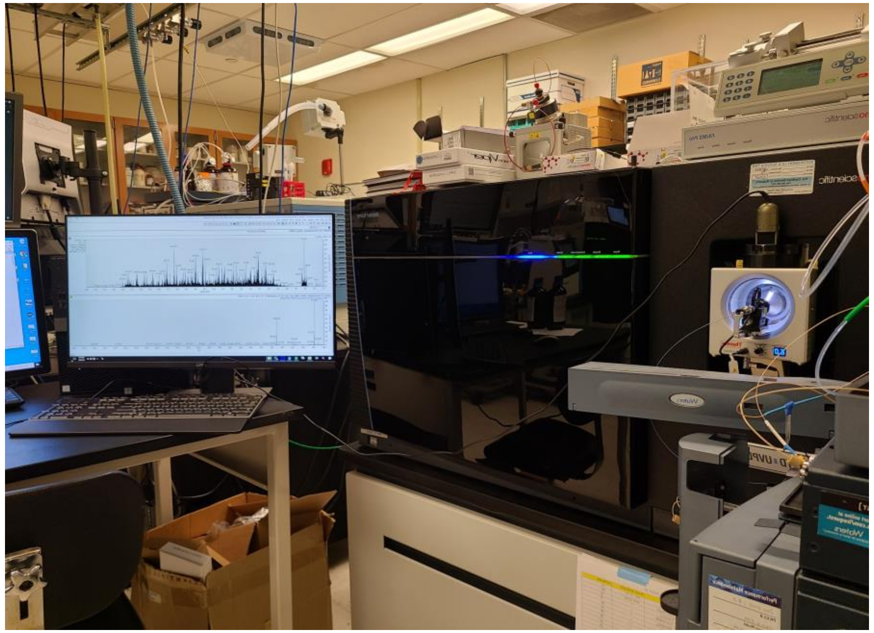


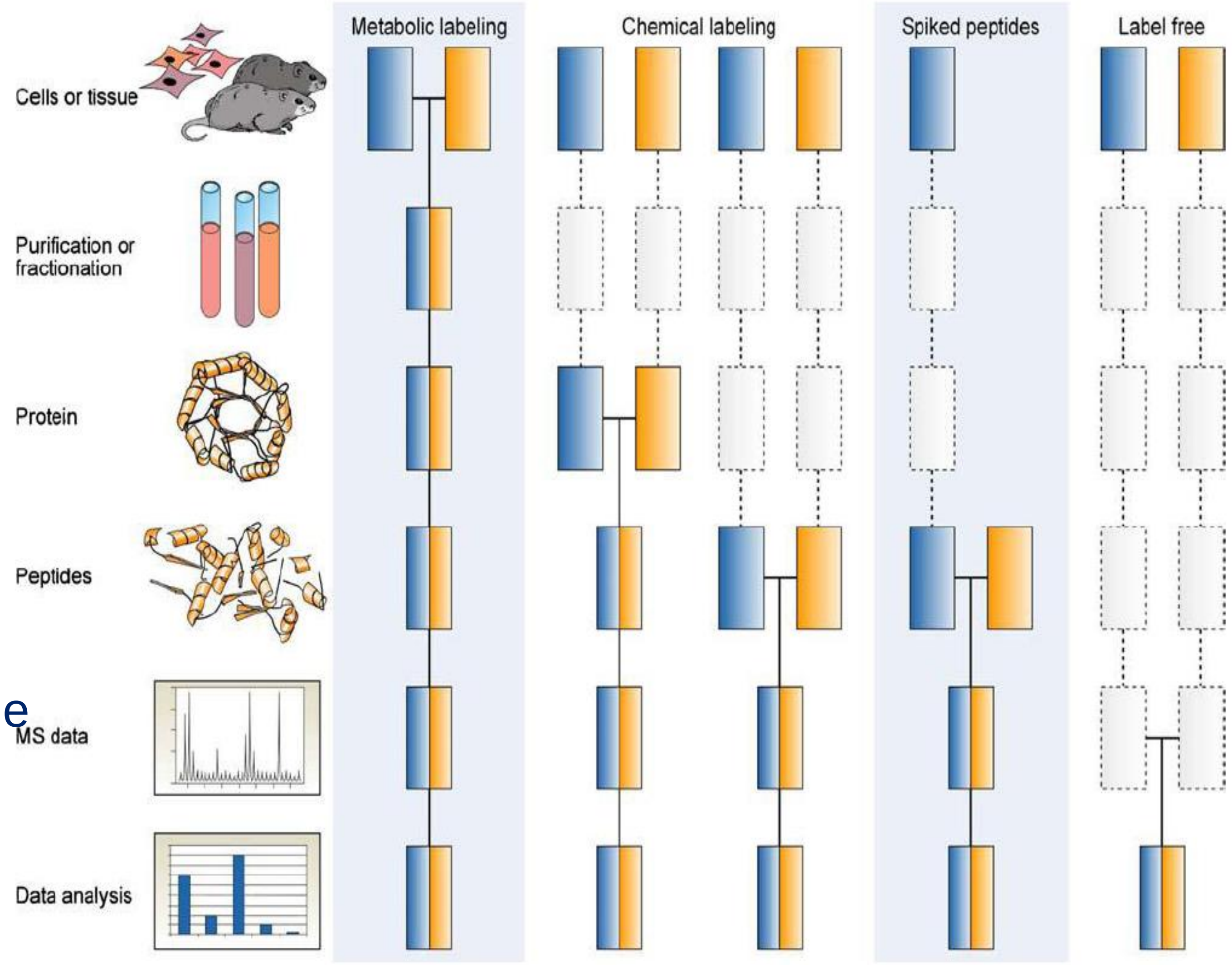
Methods to quantify proteins by mass spectrometry

Relative quantification:

- SILAC and superSILAC mix
- TMT (Tandem Mass Tag) labeling
- Label-free quantification (DDA, DIA)

Absolute quantification:

- Protein quantification relative to a peptide standard



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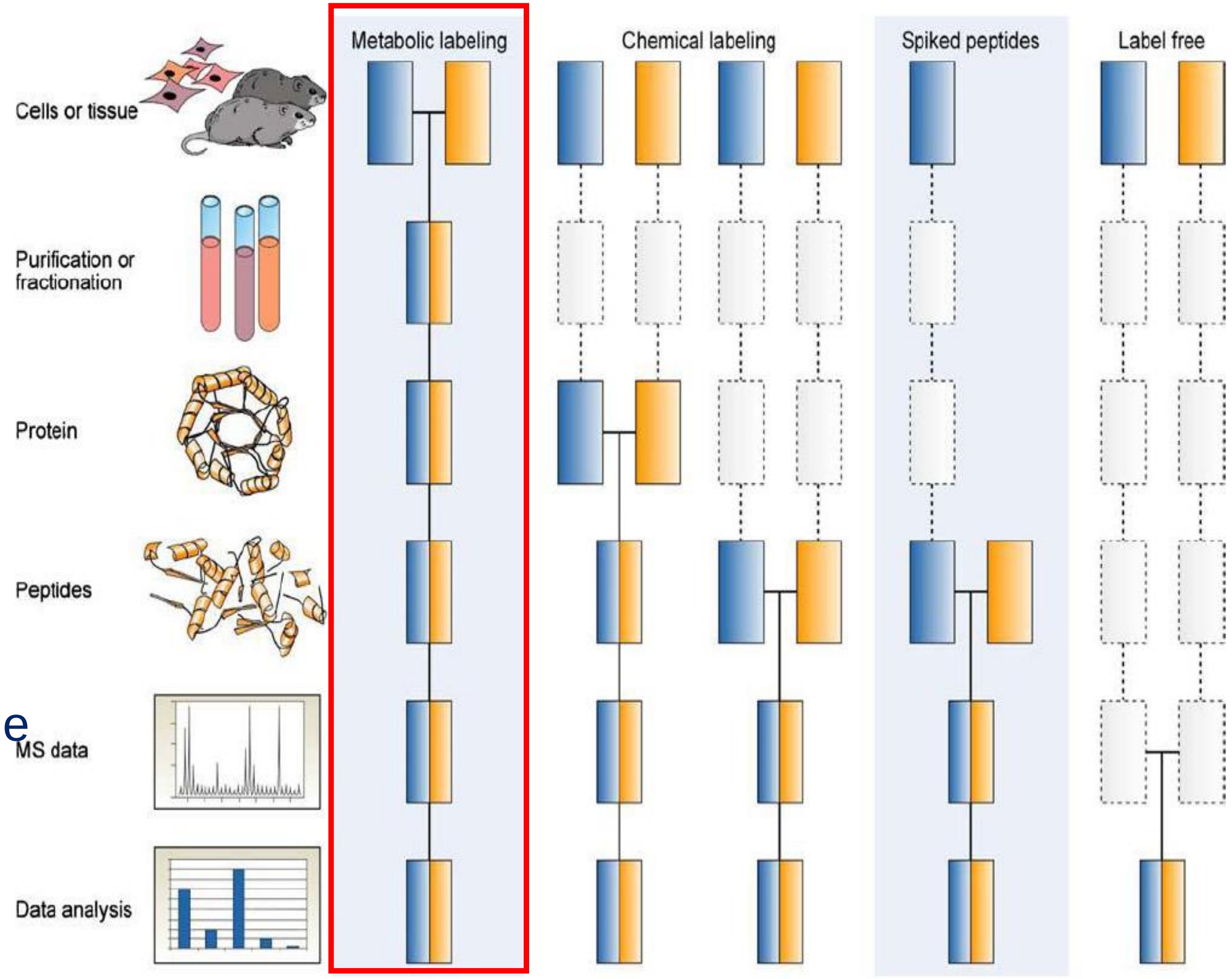
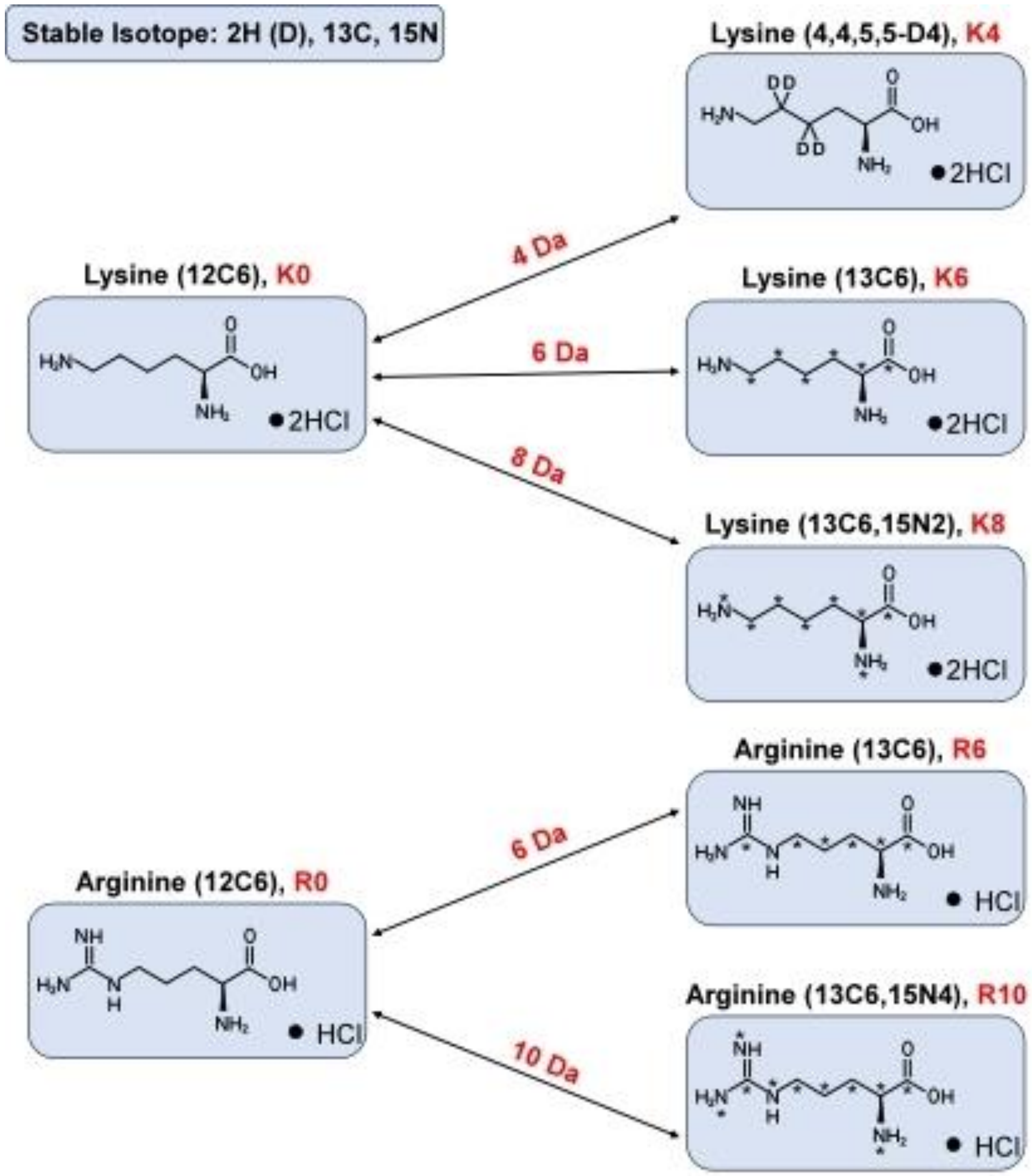


Stable Isotope Labeling of Amino acids in Culture



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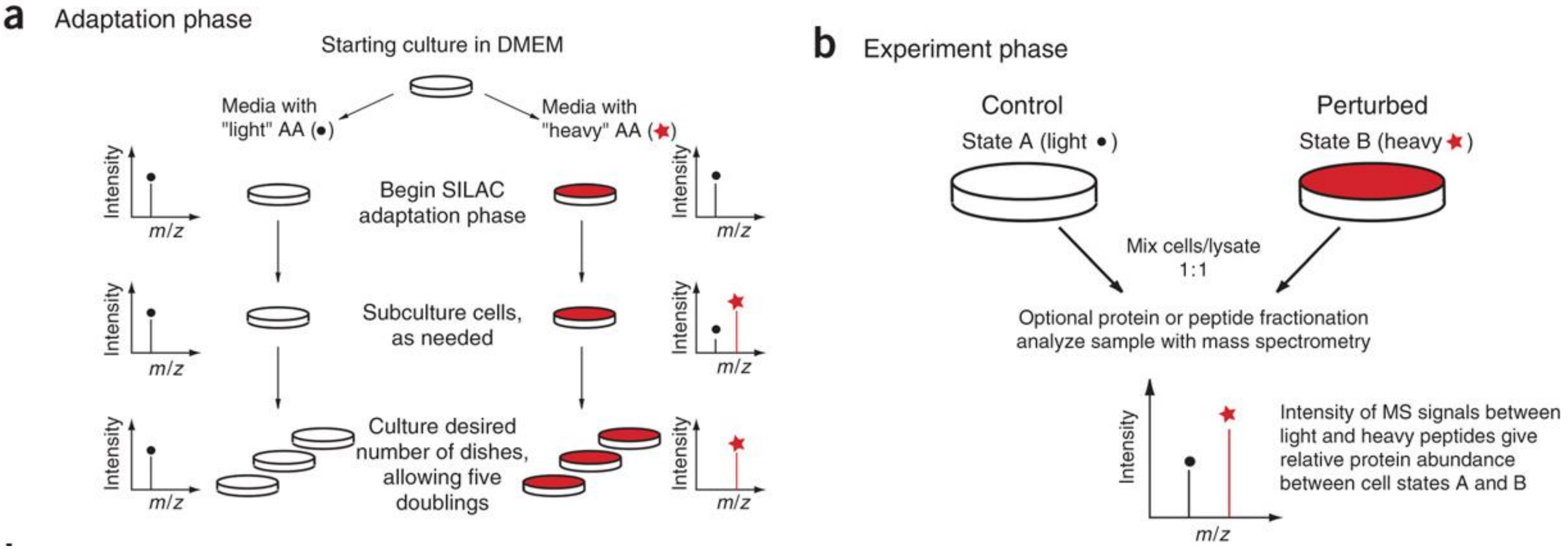

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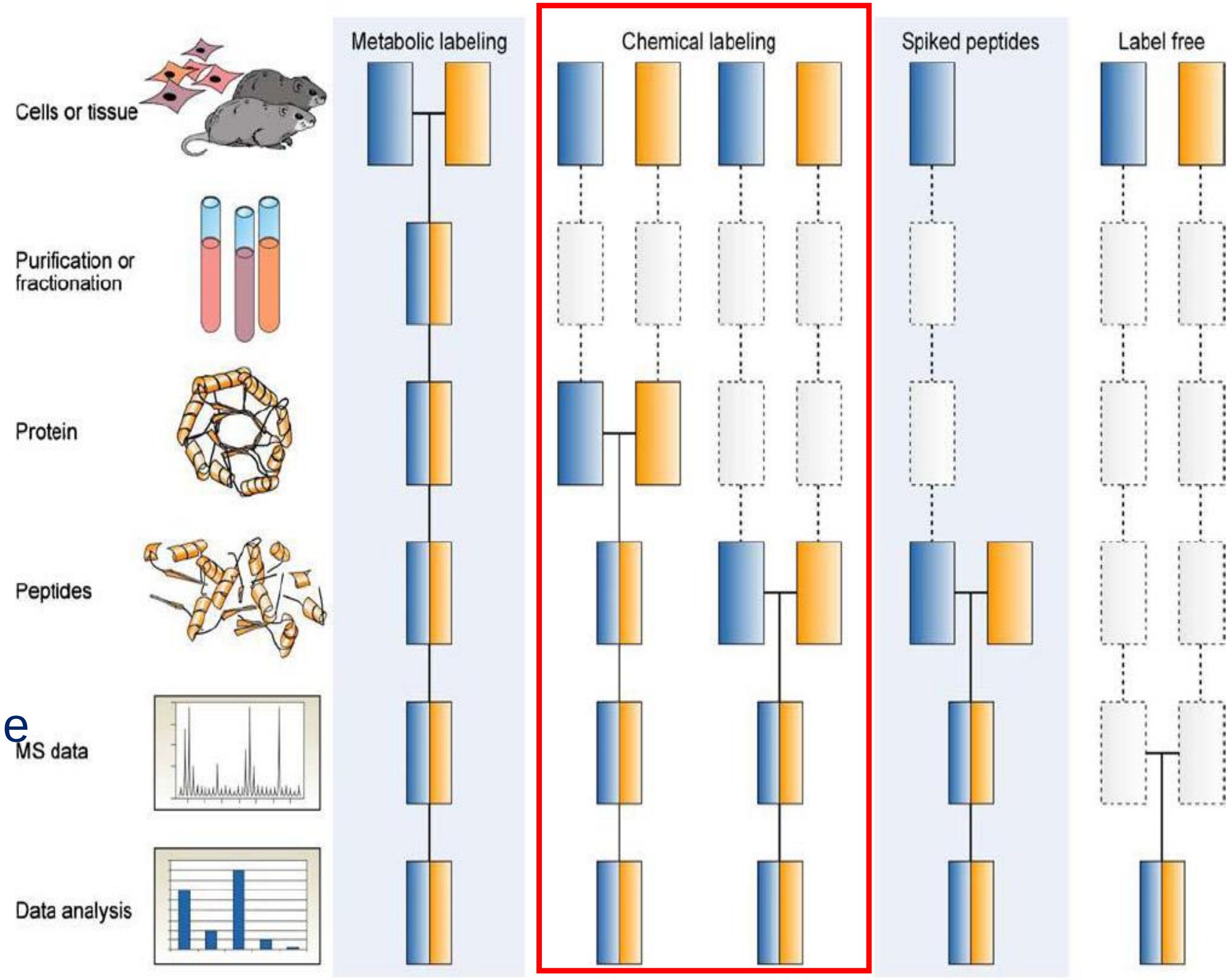
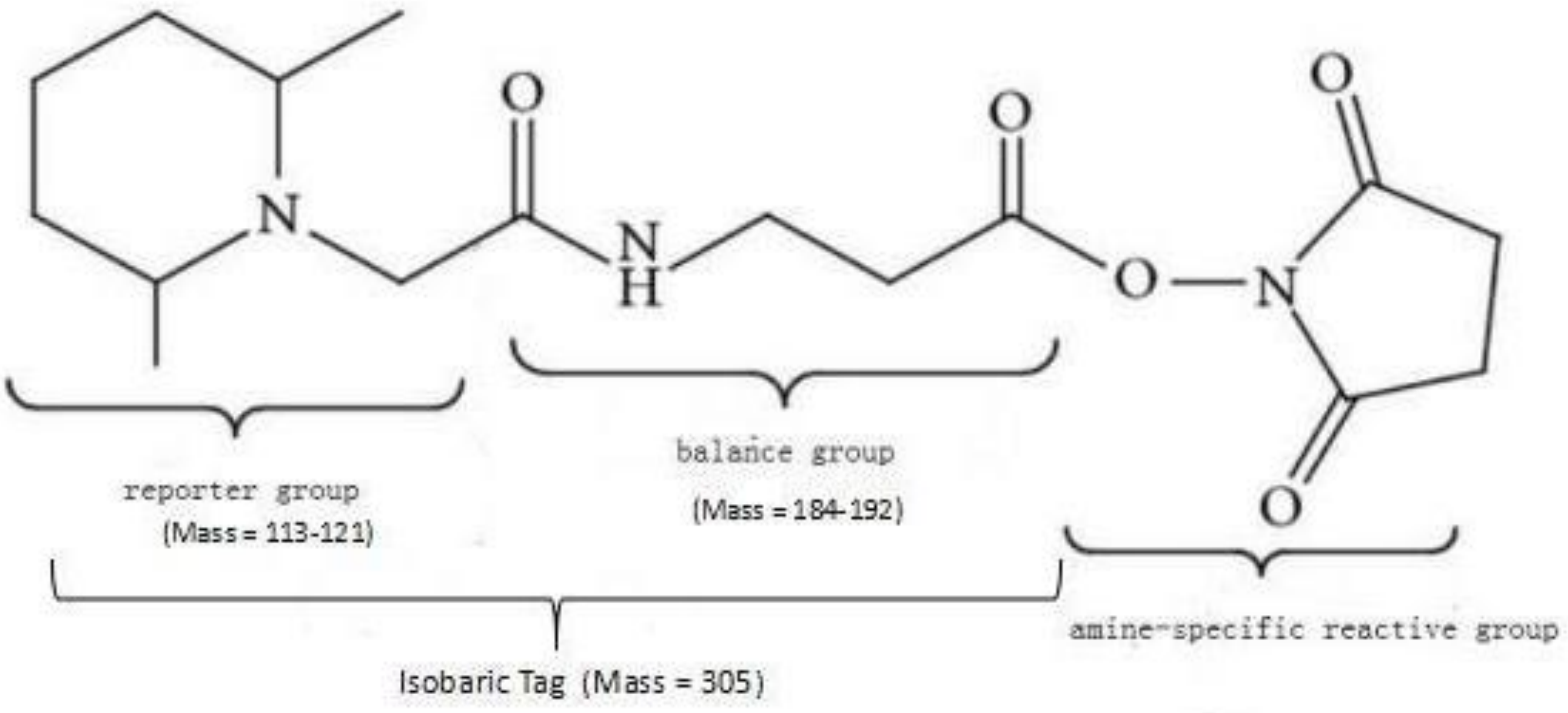
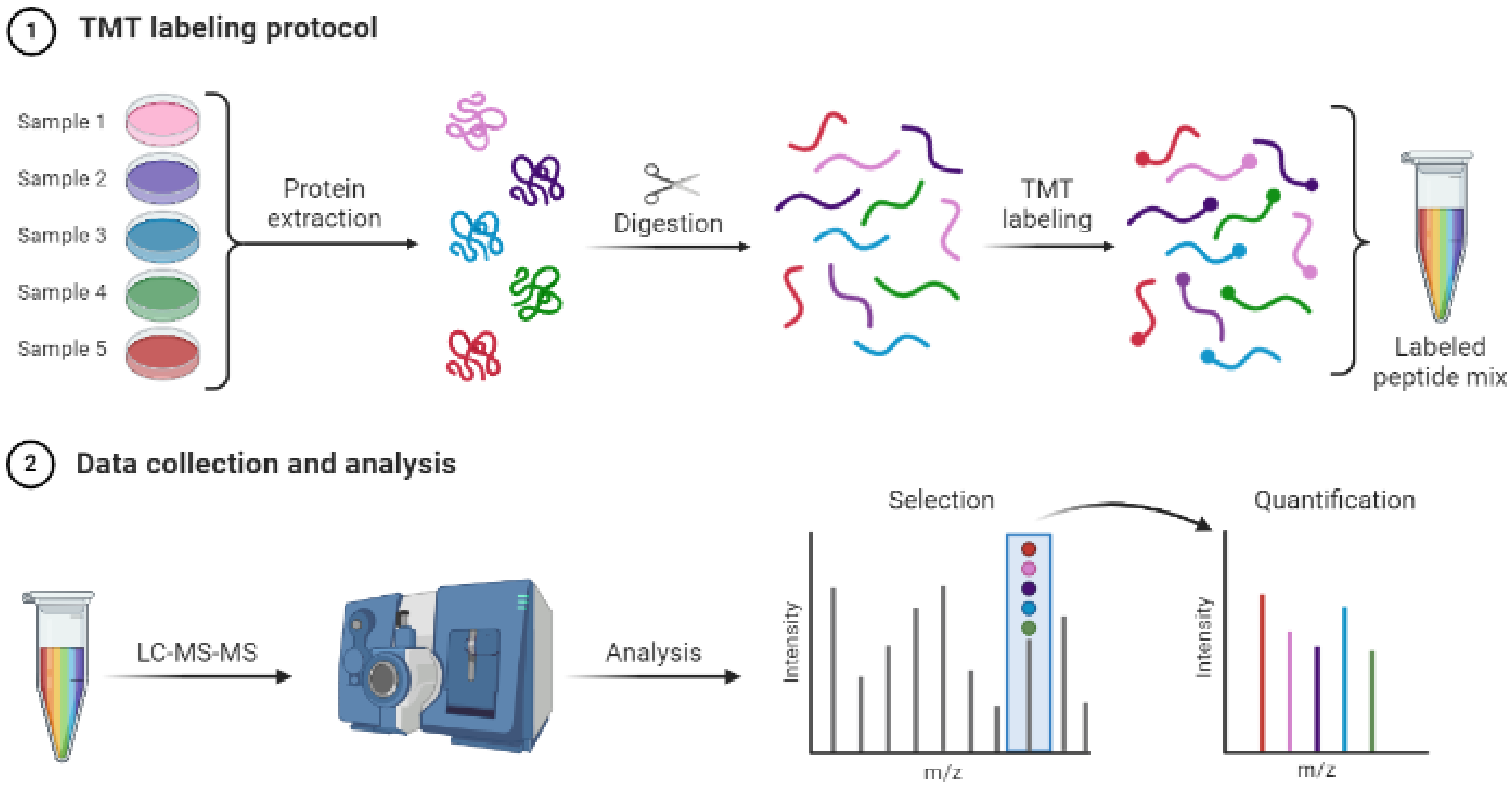


Chemical labeling-Tandem Mass Tag



TMT Tag

Chemical labeling-Tandem Mass Tag



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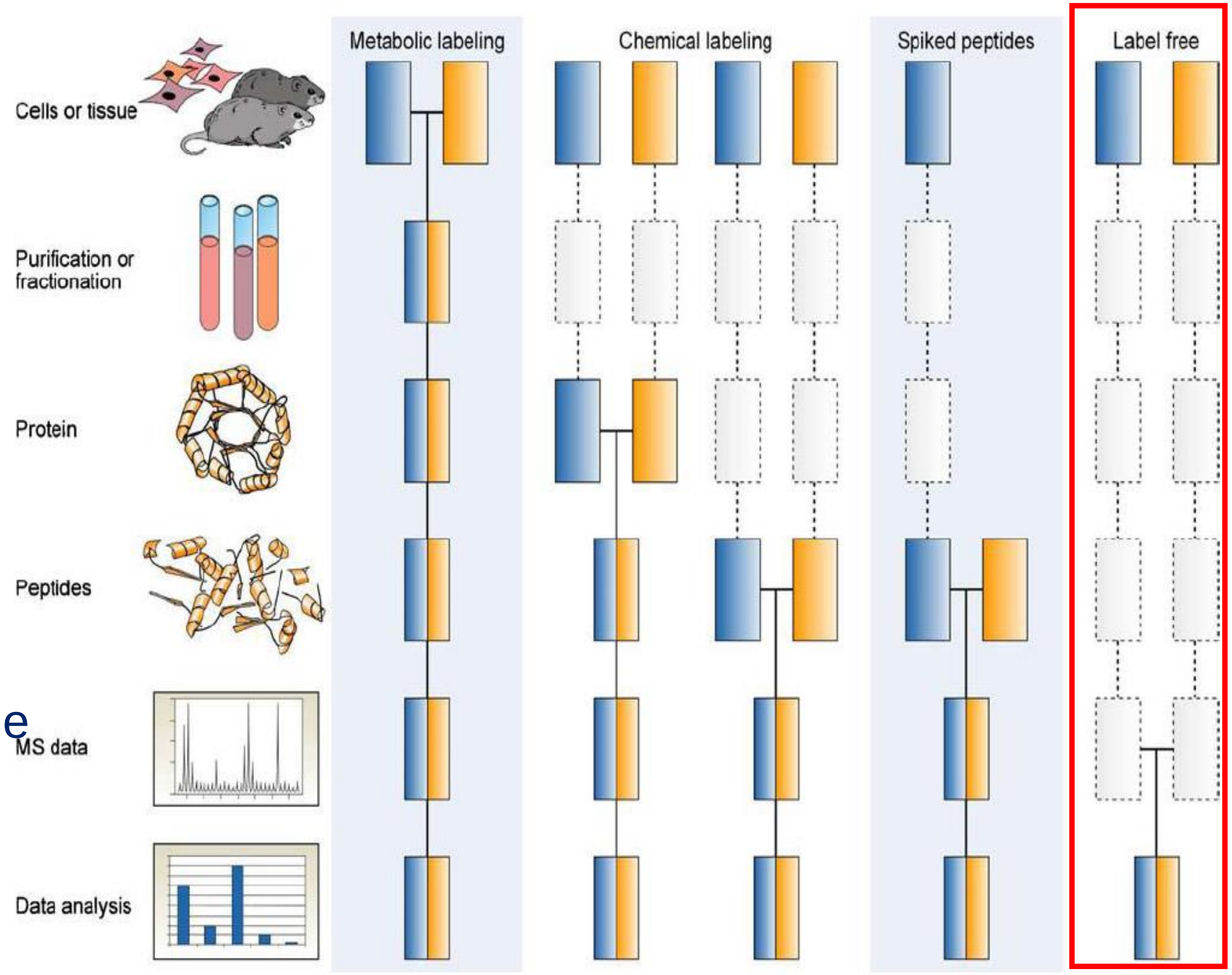
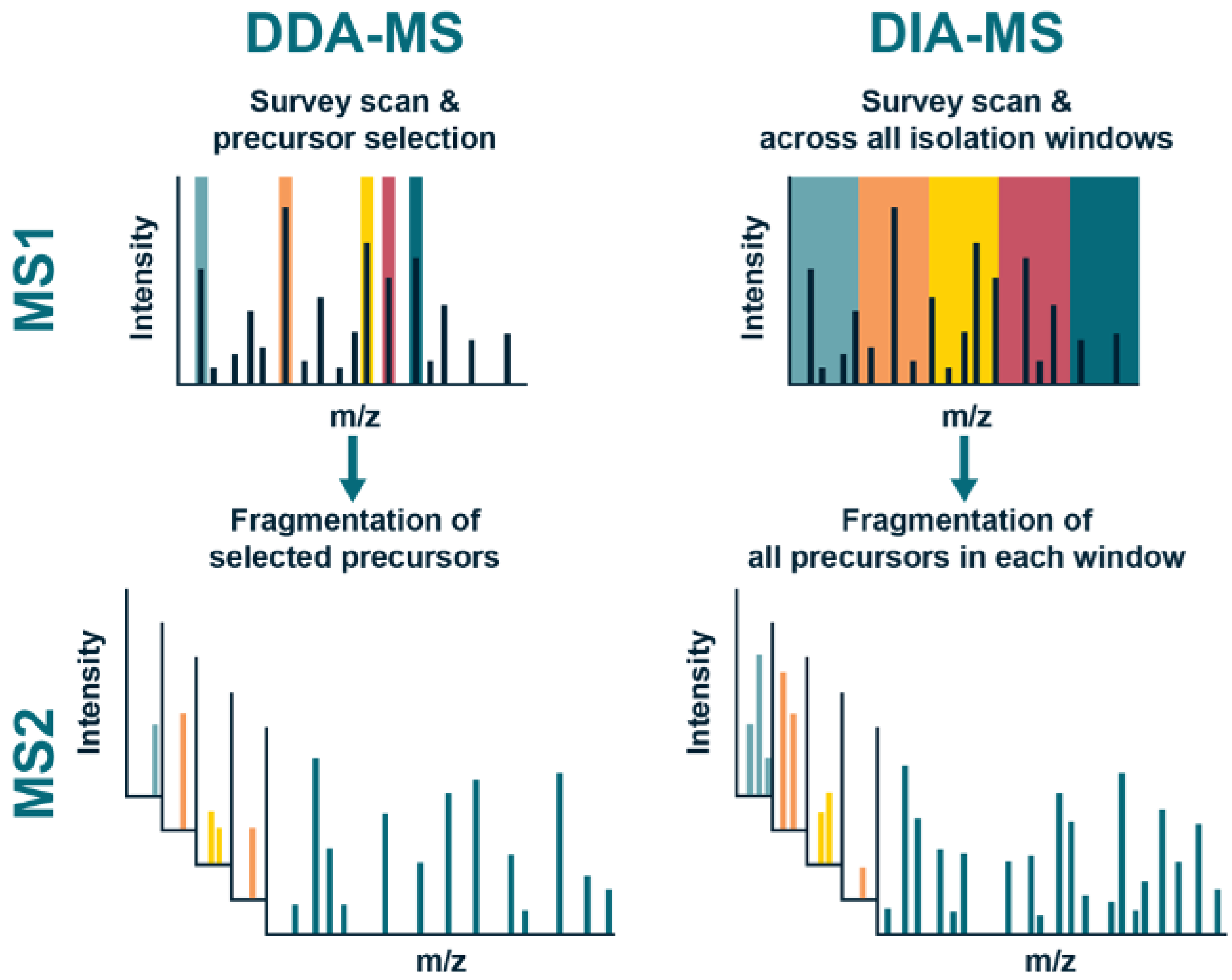


Data Dependent and Data Independent Acquisition



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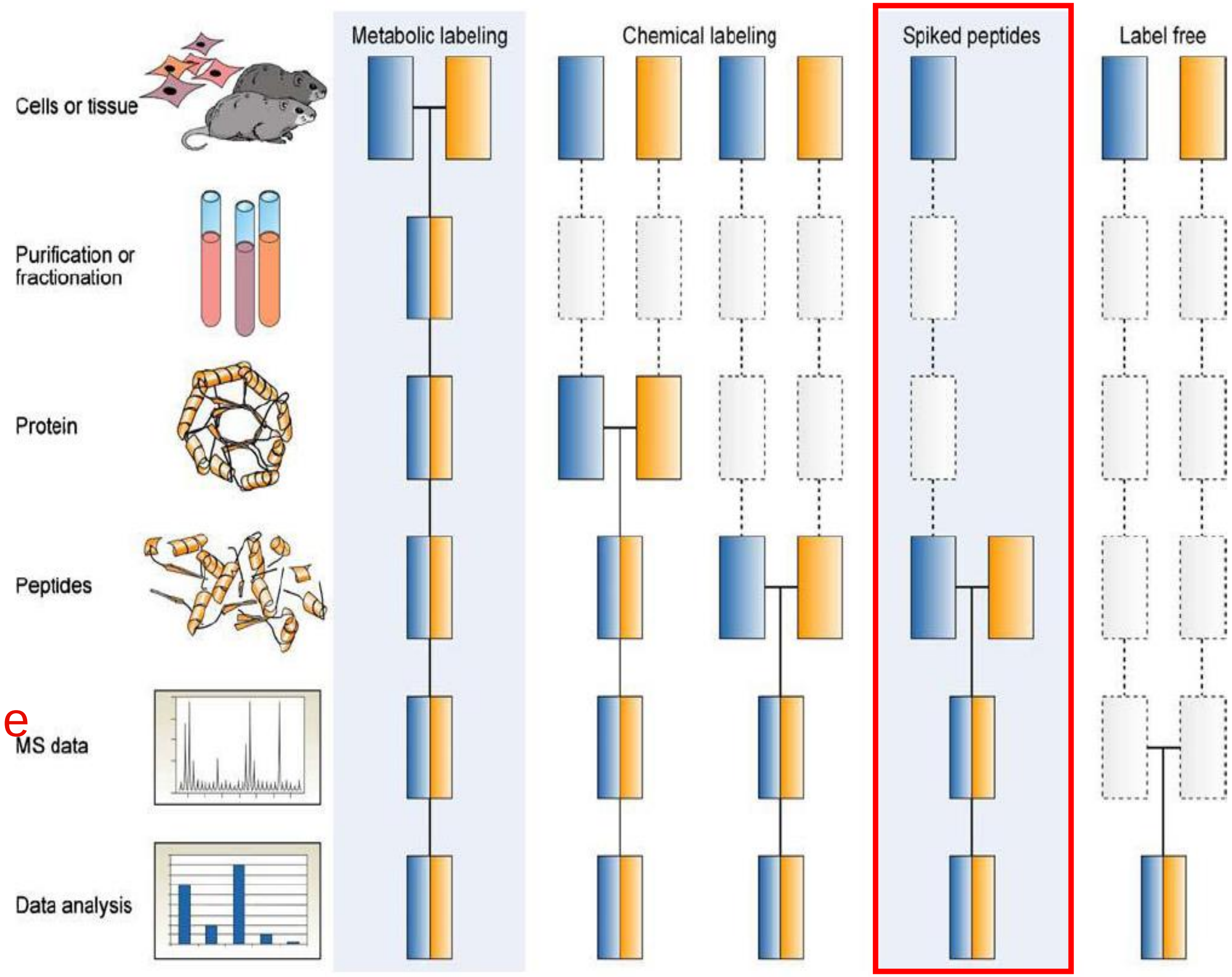

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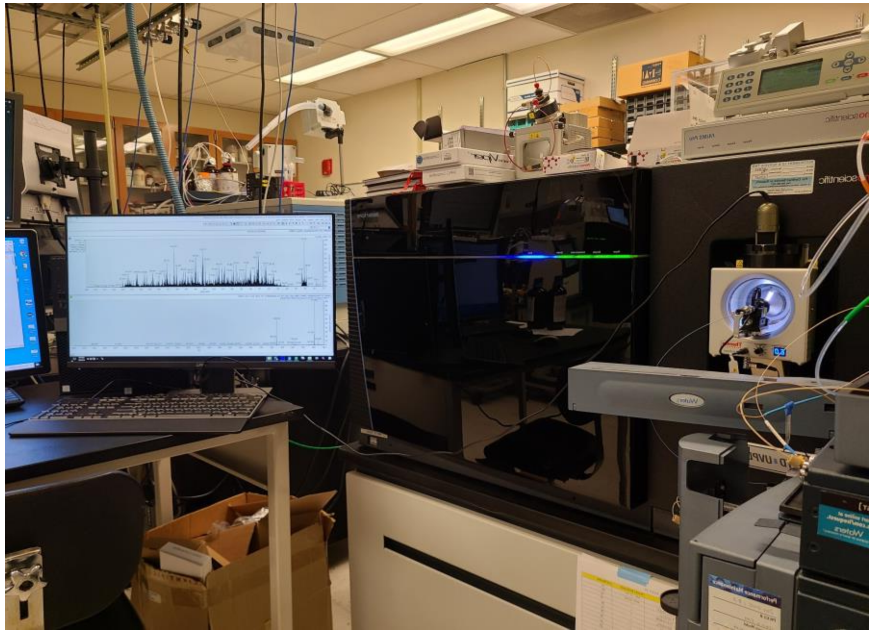
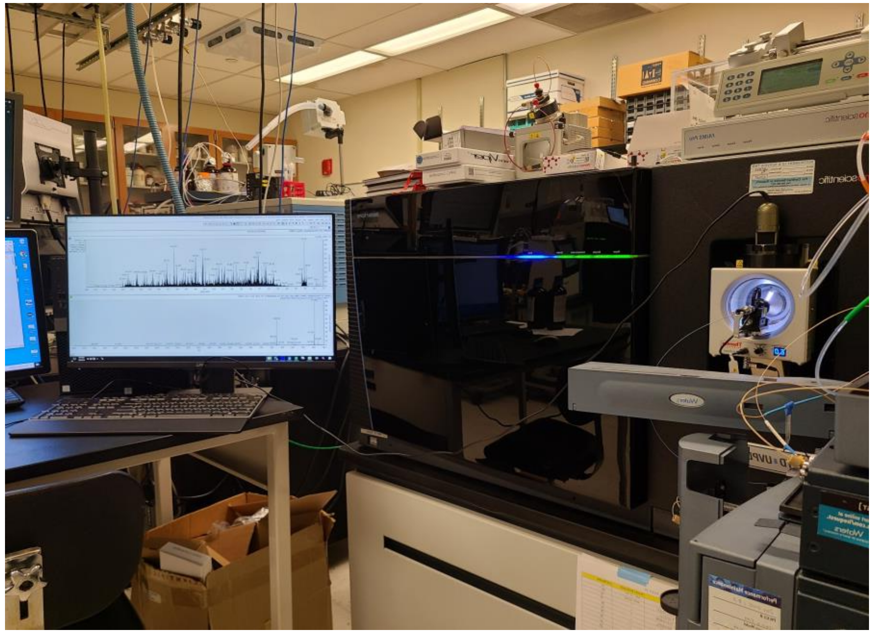


Questions to address with proteomics

- What is the protein composition of a sample?

- How are proteins modified?

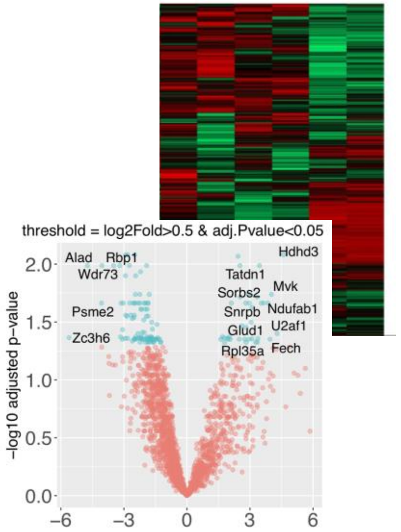
- What are the interaction partners of a protein? What is the structure of proteins and their complexes?



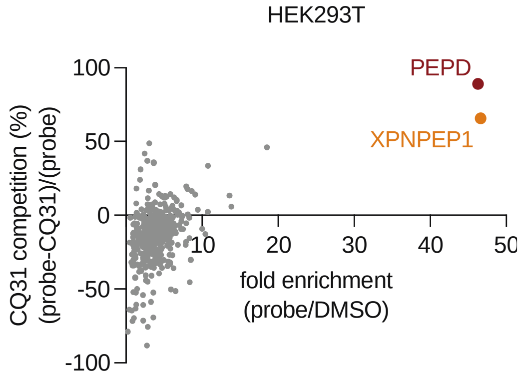
Applications of proteomics

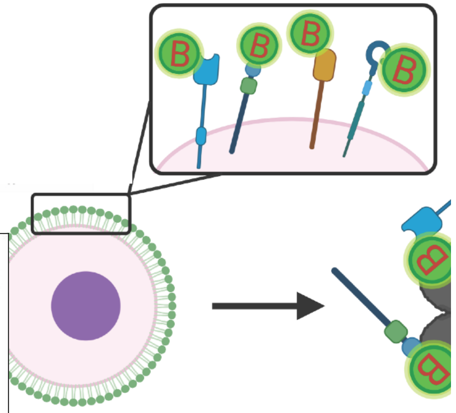
- Identity proteins and measure their abundance in a sample

- Measure post-translational modifications (PTM) of proteins (phosphorylation, ubiquitination…)

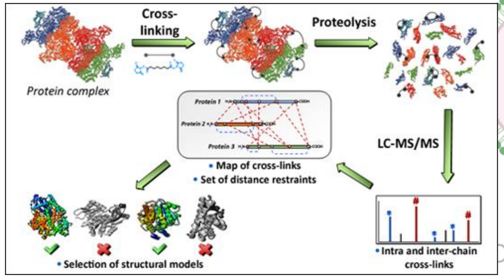


- Identify
  - Interaction partners of proteins
  - proteins in specific spatial locations
  - HLA associated peptides
  - Targets of compounds



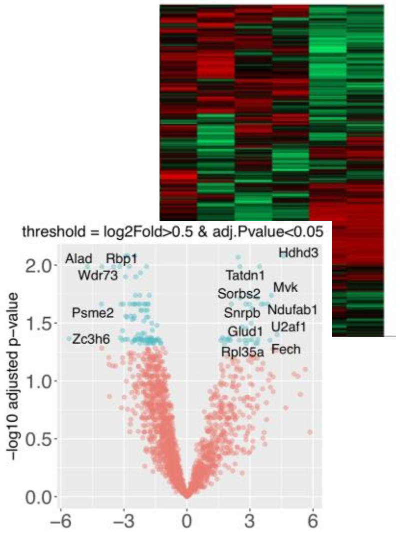


- Investigate protein structure by cross-linking mass spectrometry



Capabilities of the Proteomics Core

- Identity proteins and measure their abundance in a sample



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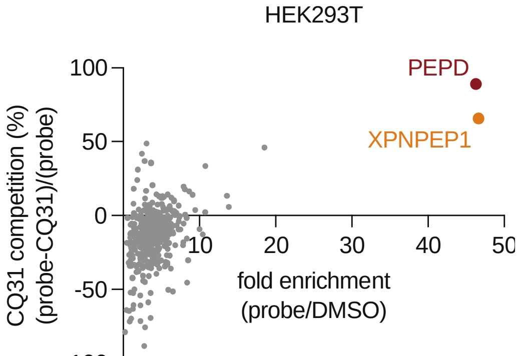

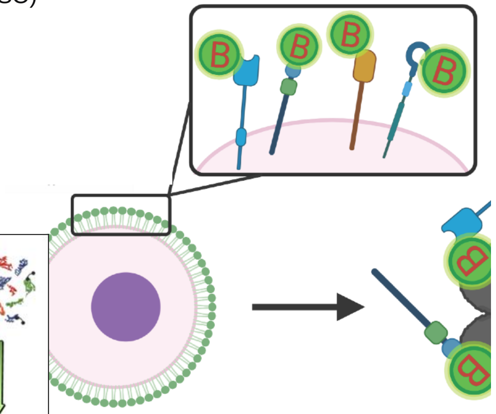

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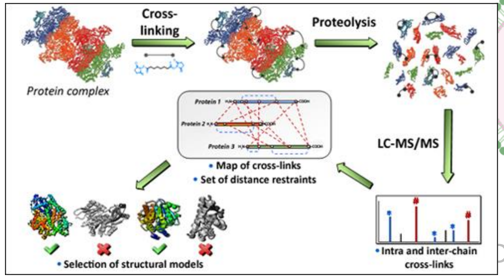
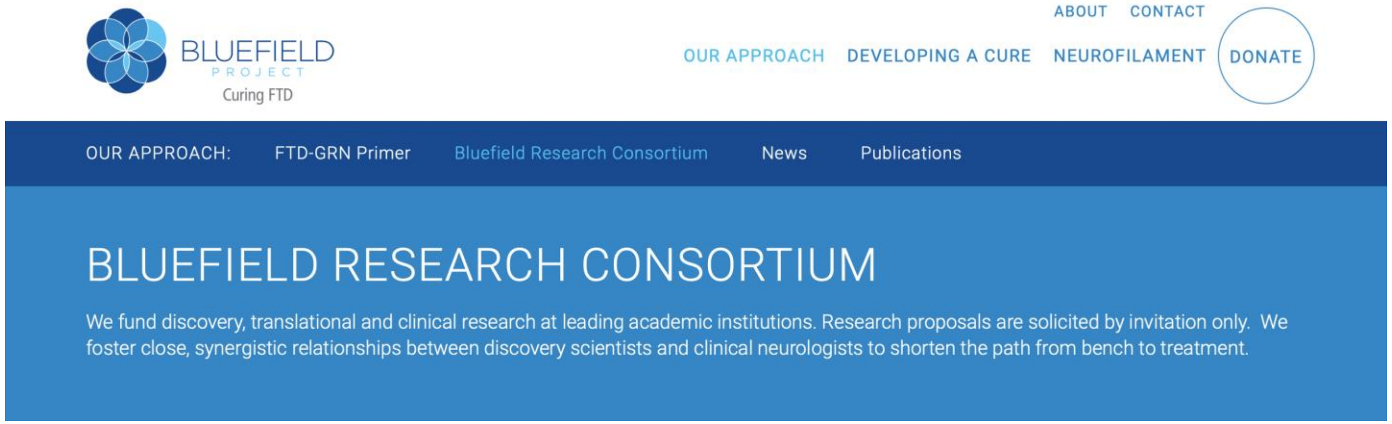
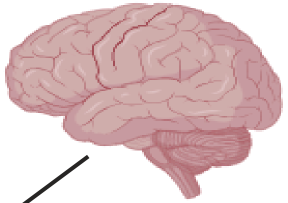


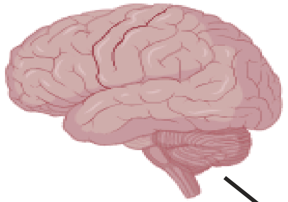
Proteomics and lipidomic to study Frontotemporal Dementia-GRN pathology



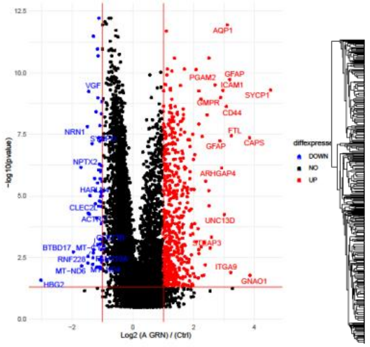
Control (n=6)

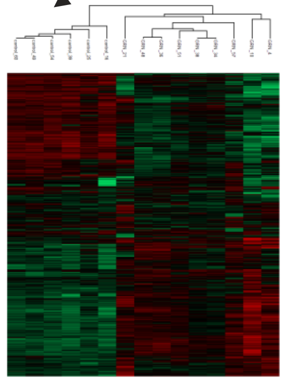


FTD-GRN (n=9)

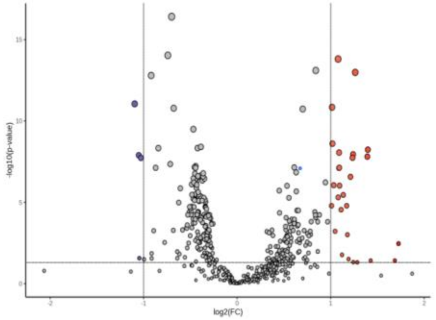


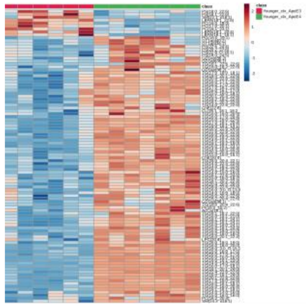
Proteomics





Lipidomics





Yohannes Ambaw, Bob Farese, Tobi Walther

Workflow to acquire brain proteomics data

Sample Collection

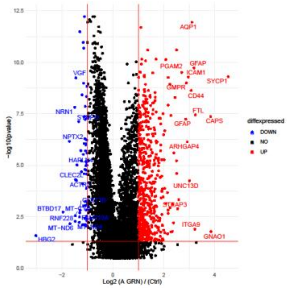
Sample processing

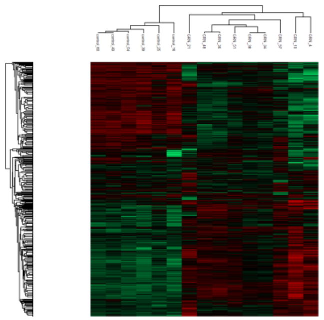
LC-MS/MS

Data processing and Visualizations

control

FTD-GRN





Human brains

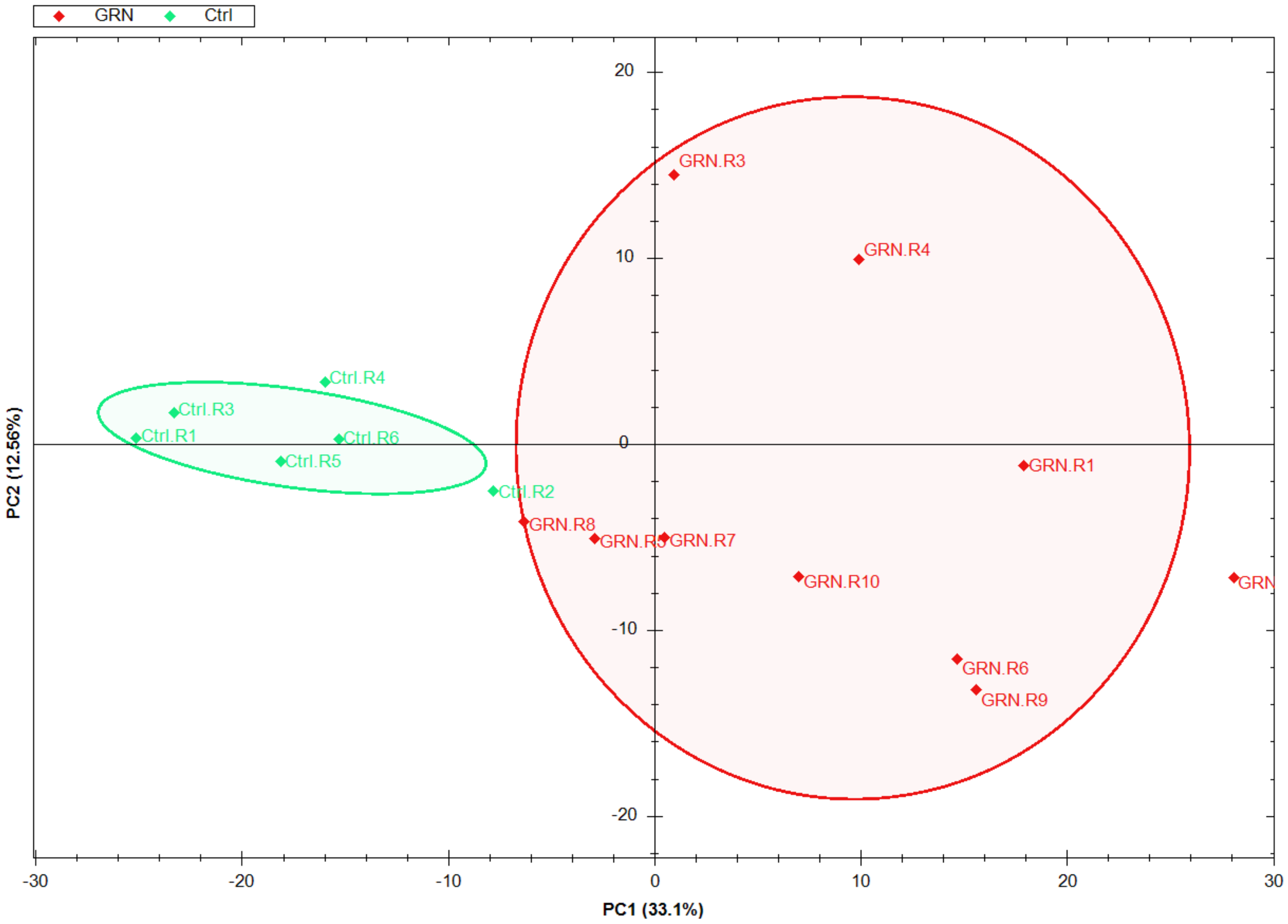
Homogenization
Digestion
Clean up

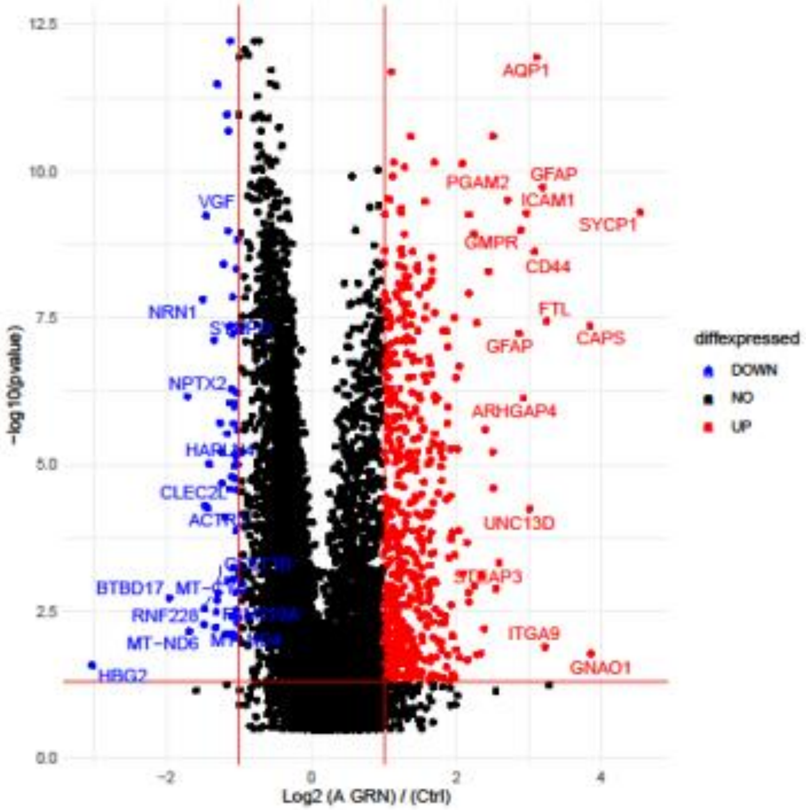
Orbitrap
Astral

Spectronaut
Perseus

Laura Tuffery, Zhuoning Li

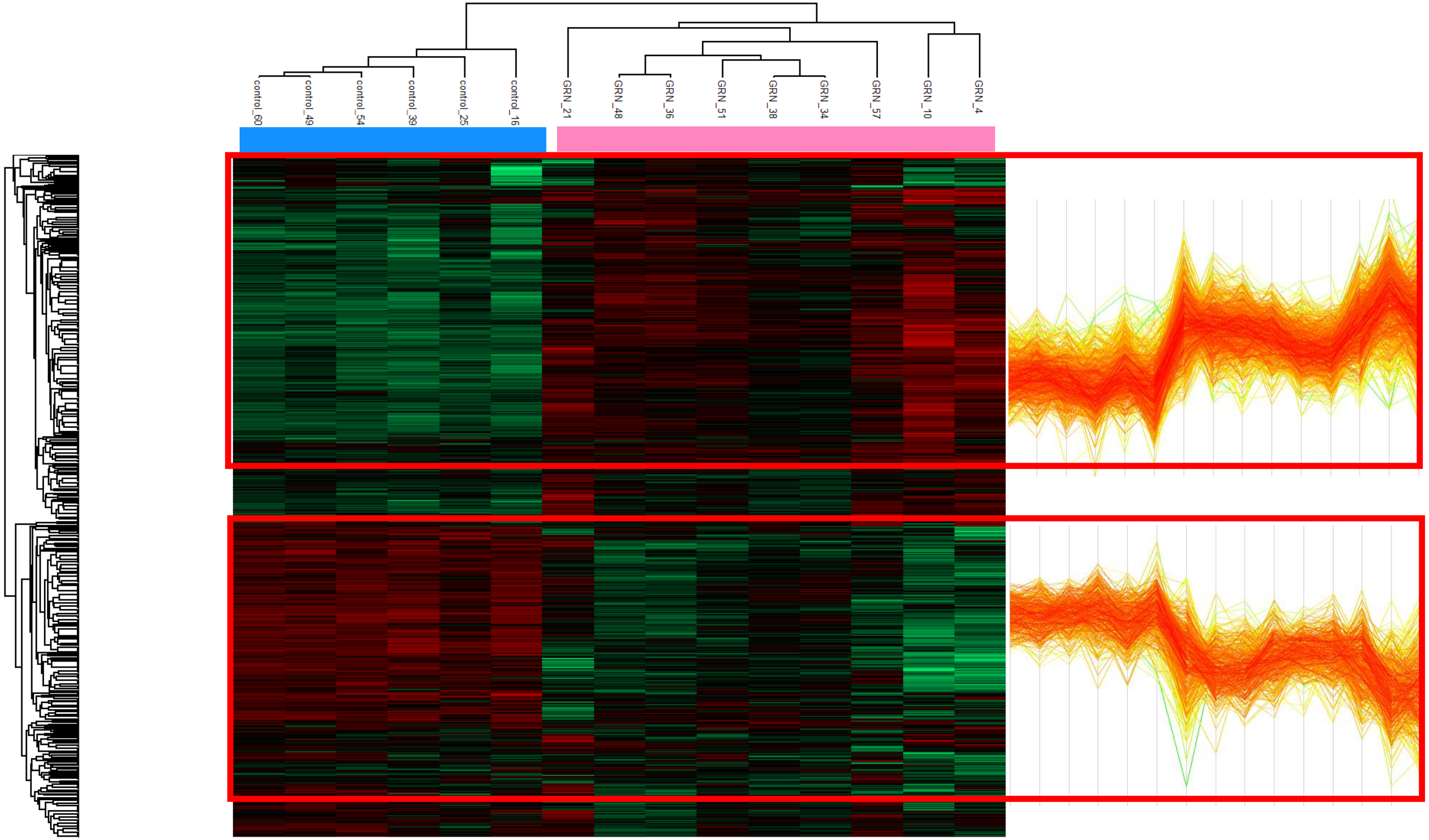
Distinct proteome of healthy versus FTD-GRN brains





Laura Tuffery, Zhuoning Li

Proteomics to study the etiology of frontotemporal dementia



control

FTD-GRN

Neurodegeneration
Inflammation

Lysosome, lytic vacuole
Lipid metabolism

Synaptic membrane and signaling
Neuron projection and neuronal cell body

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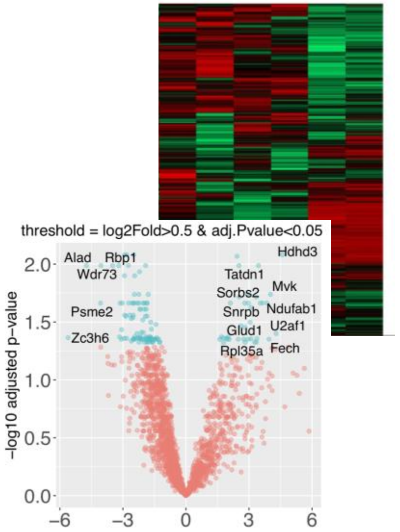
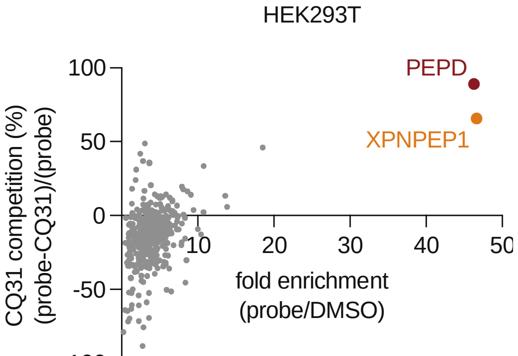
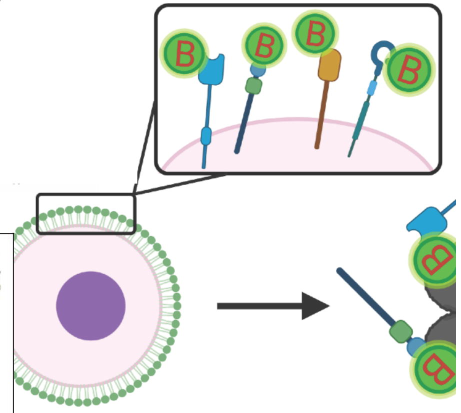
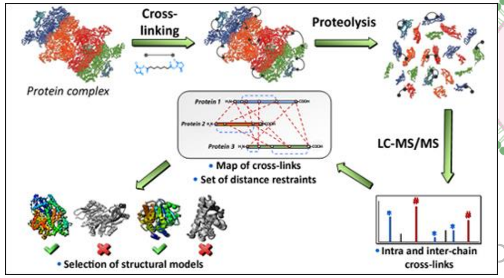






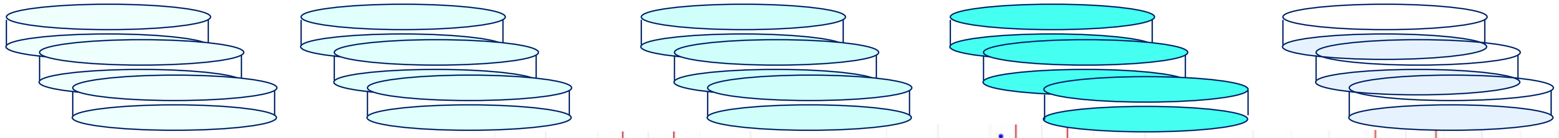


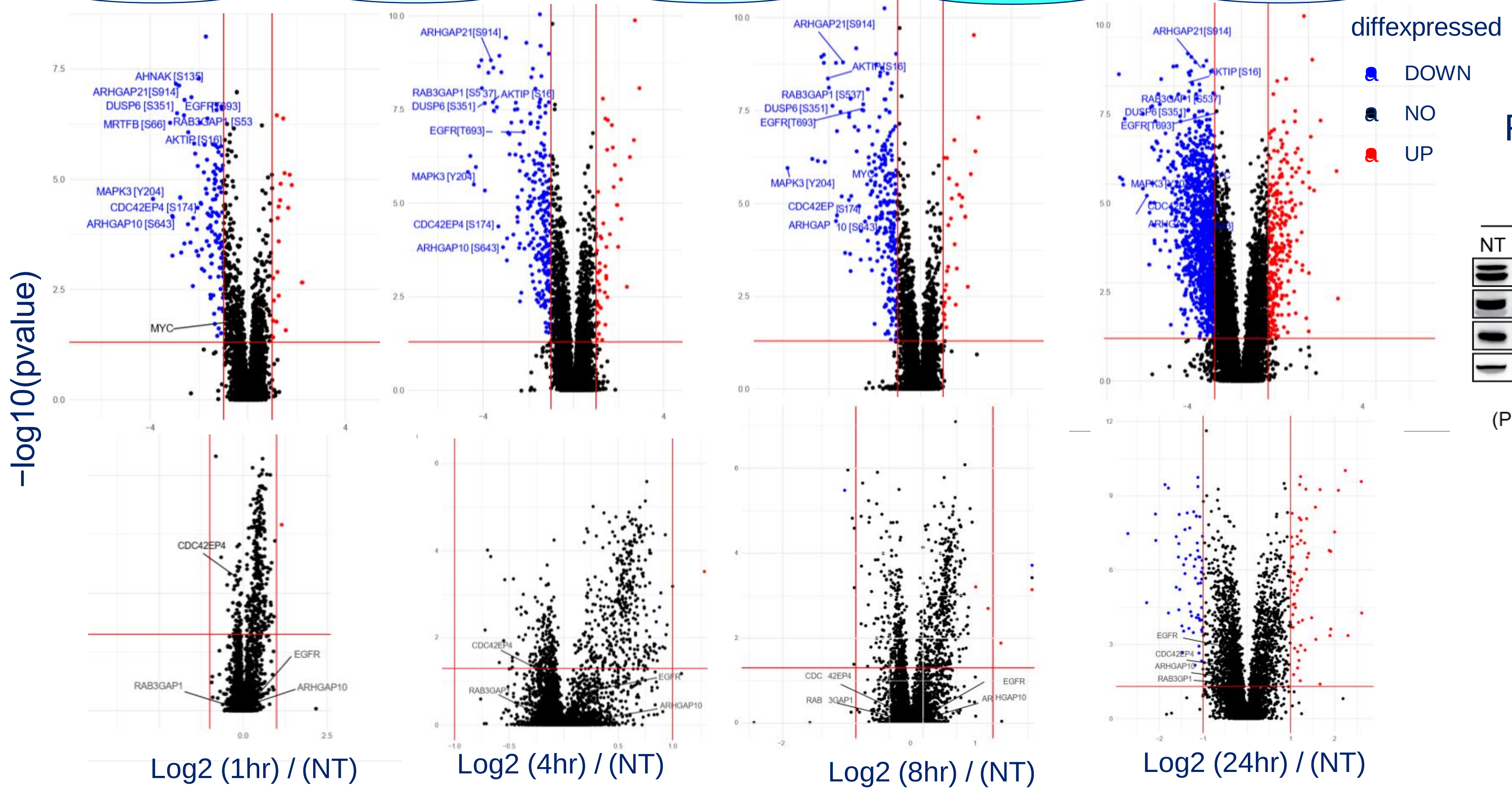
Determining the time-resolved phosphoproteomic signature of RAS inhibition in pancreatic cancer

AsPC-1 (PDAC, KRAS G12D)

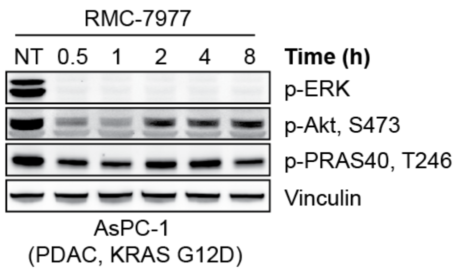
DMSO | RMC-79771 h | RMC-7977 4 h | RMC-7977 8 h | RMC-7977 24 h

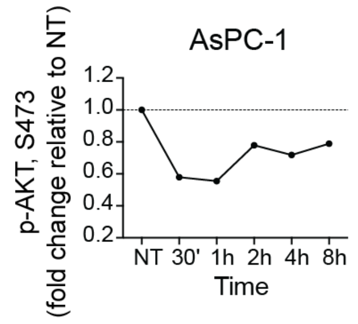
RMC-7977=RAS-GTP inhibitor





RAS inhibition impairs PI3K signaling





Michelangelo Marasco, Neal Rosen

Capabilities of the Proteomics Core

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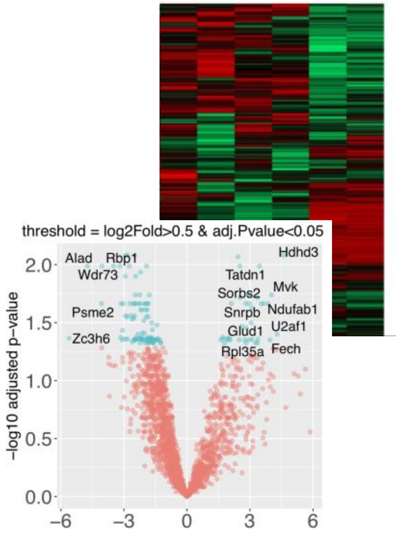
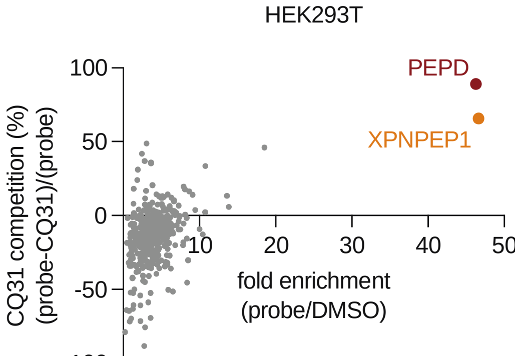
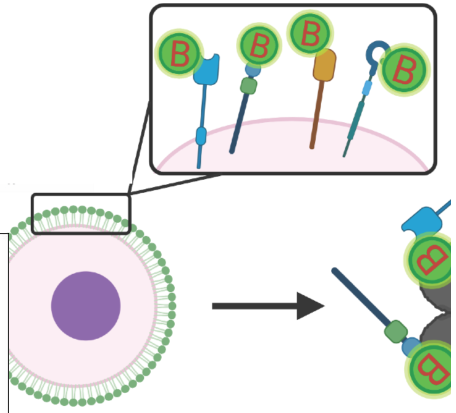
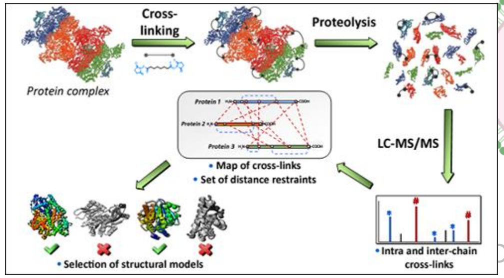
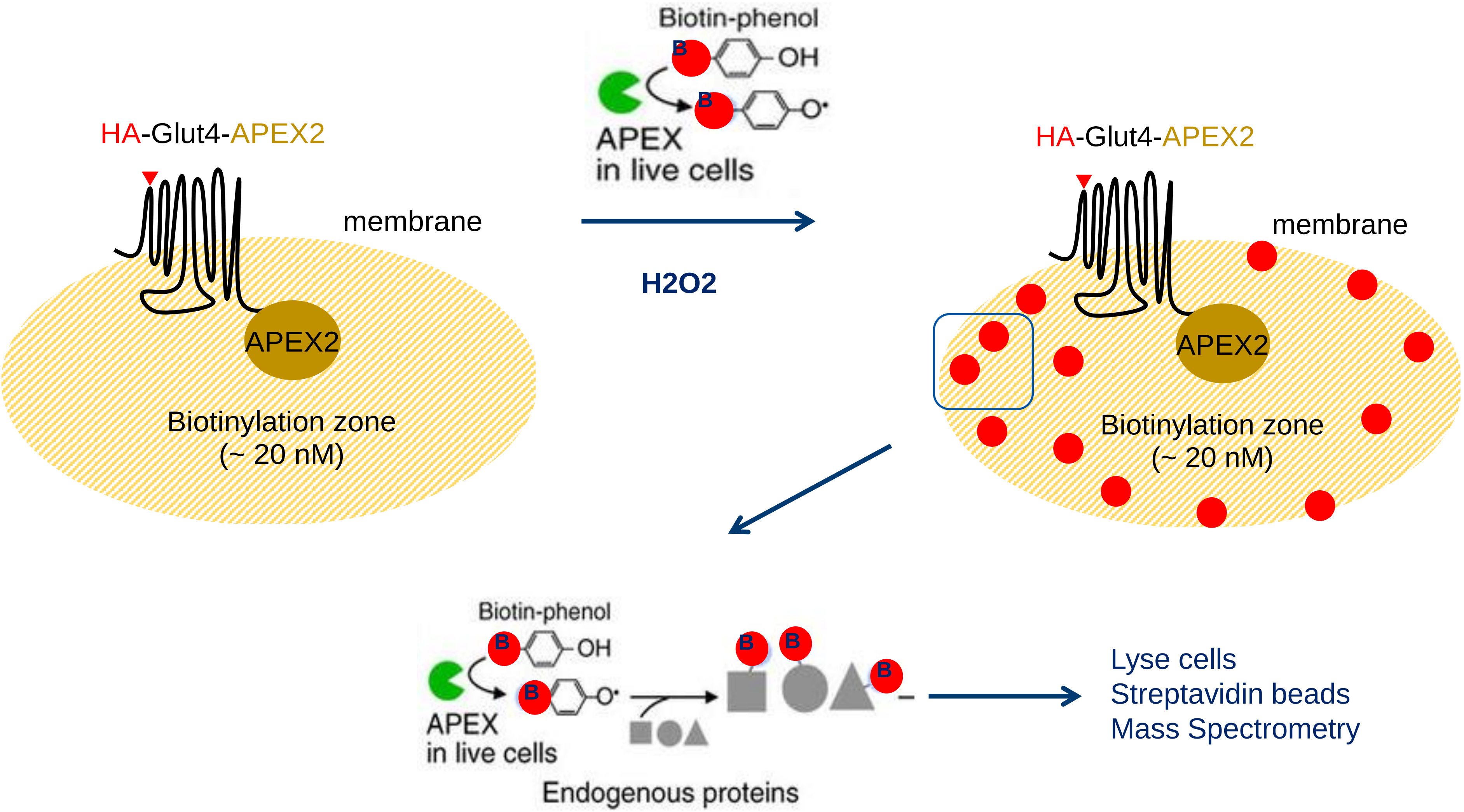








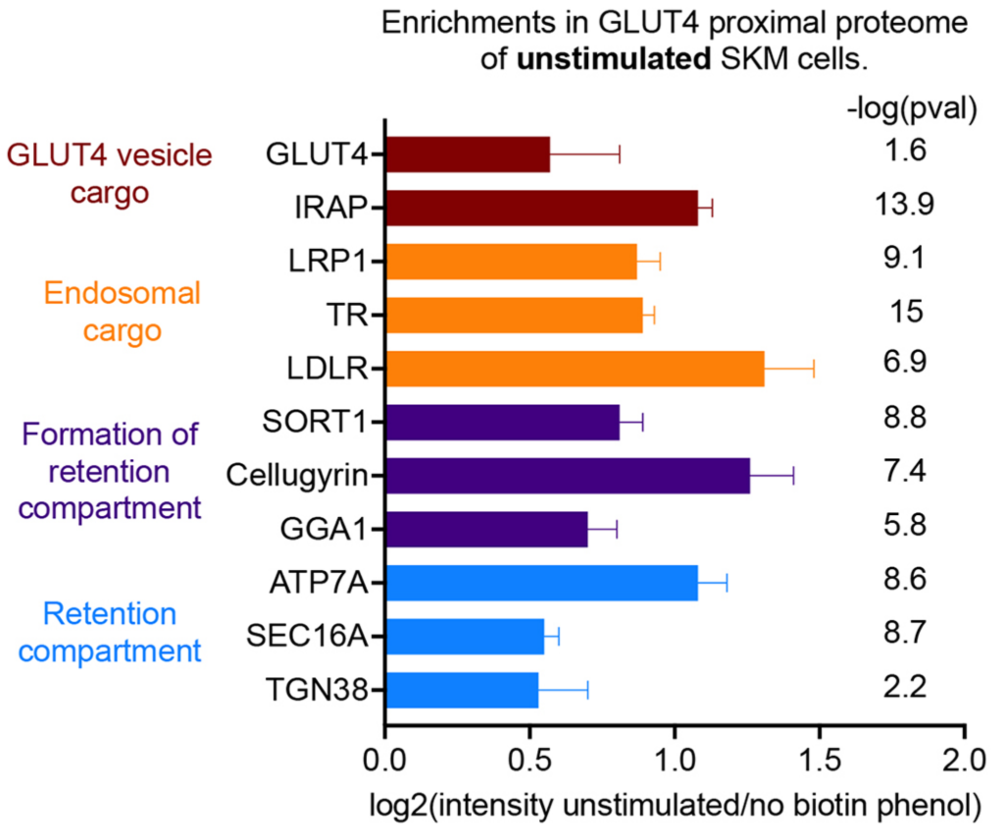
Defining the Glut4 vesicle composition by proximity biotinylation

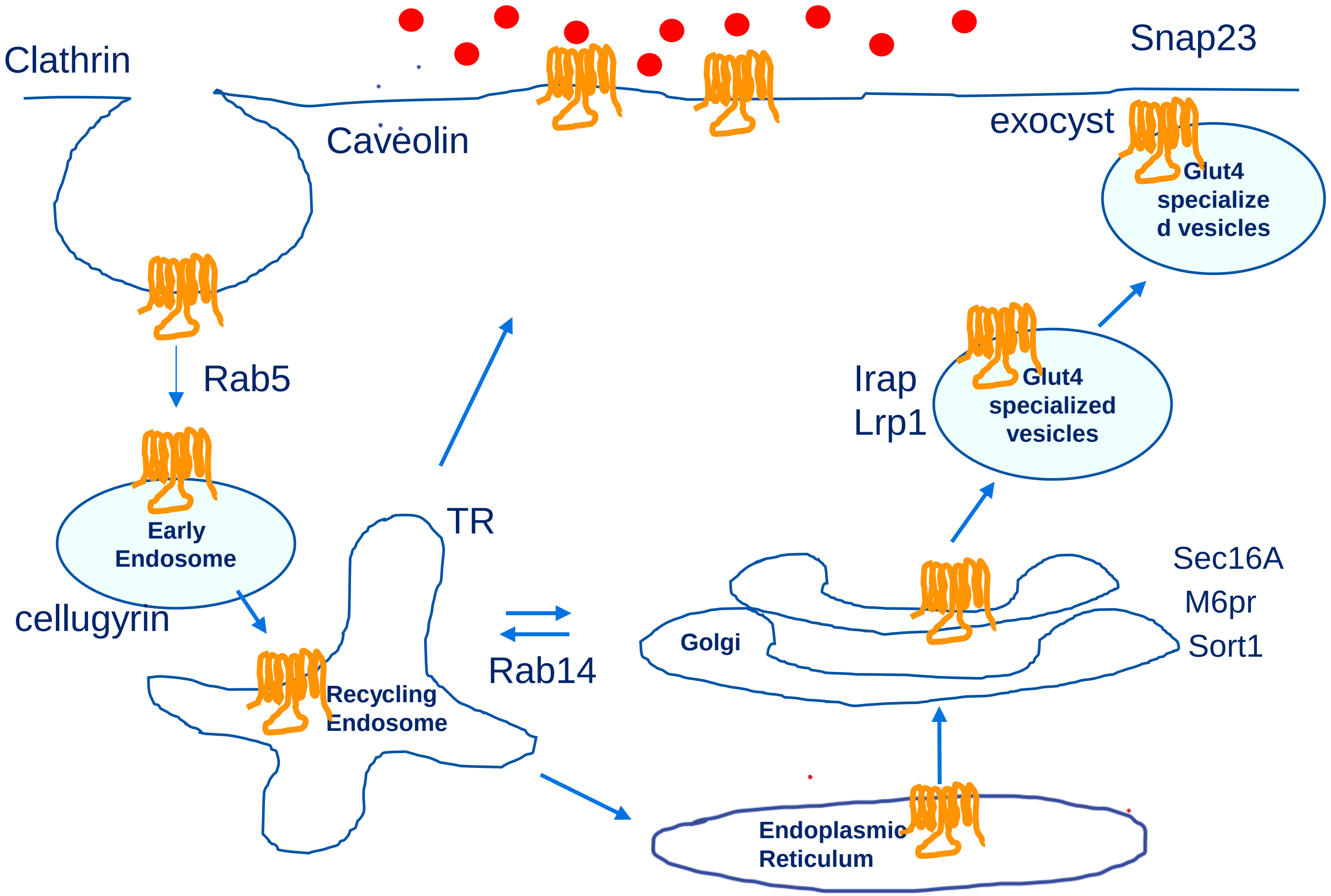


Adapted from Han S and all, Cell Chemical Biology, 2017

Anuttoma Ray, Tim McGraw

Proximity biotinylation identifies known mediators of Glut4 trafficking





Ray at all, J Cell Sci, 2023

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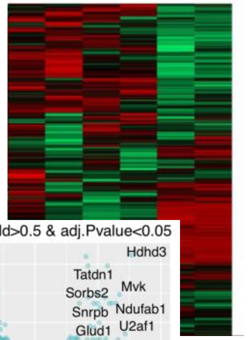
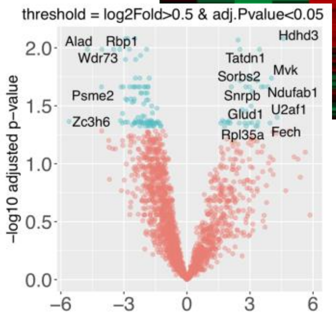
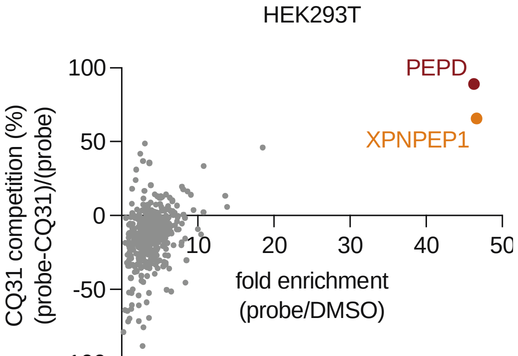
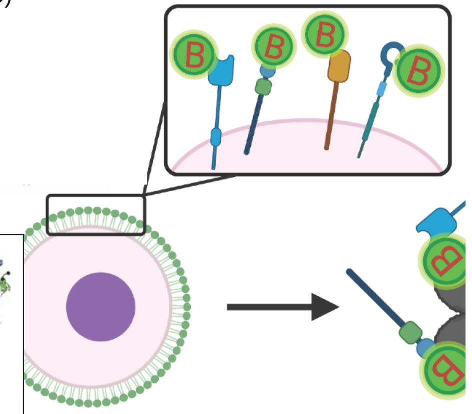
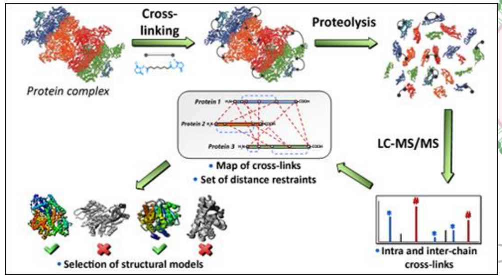


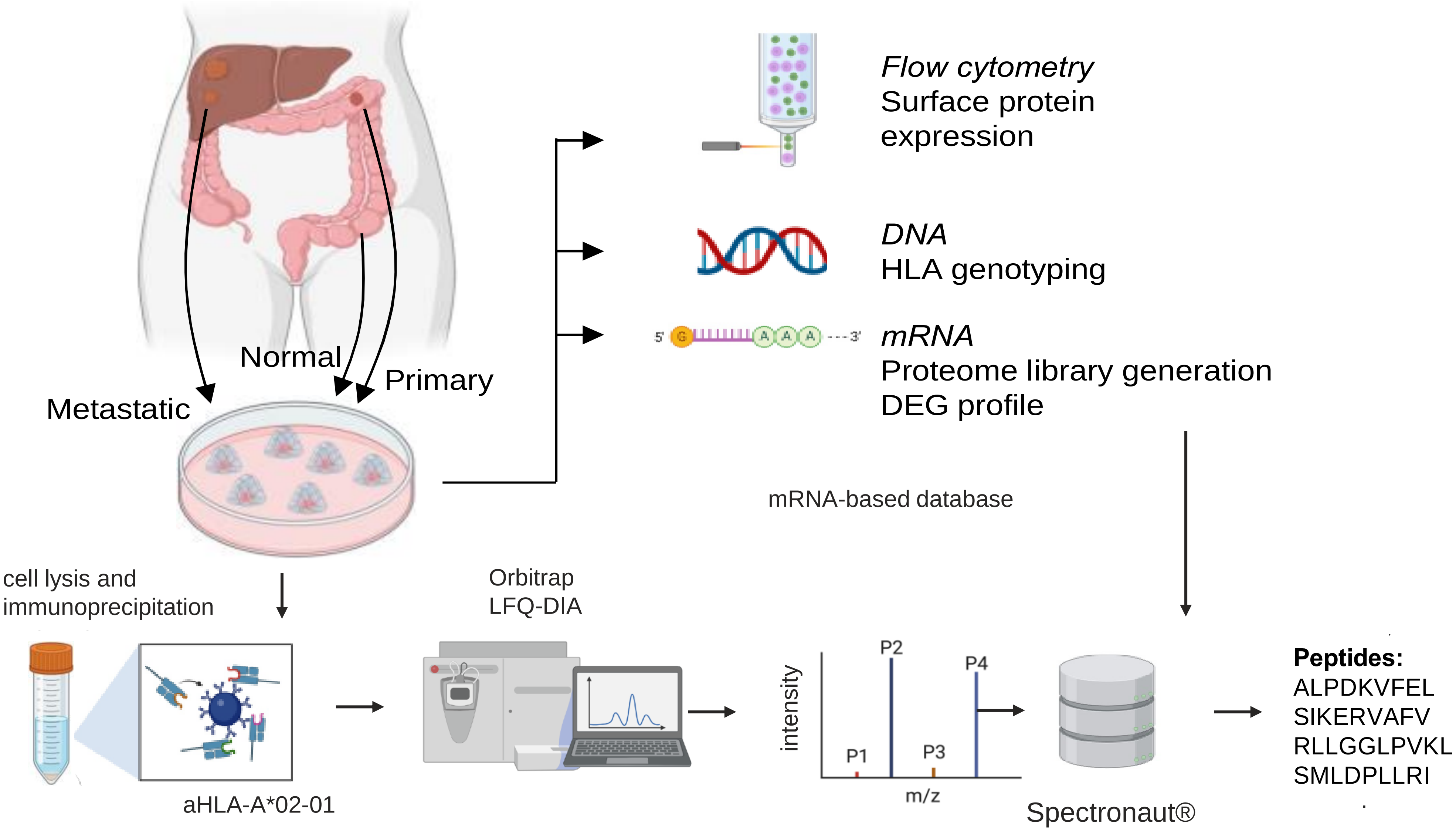








Identification of HLA associated peptides by immunopeptidomics



J. Lee and D. Sheinberg

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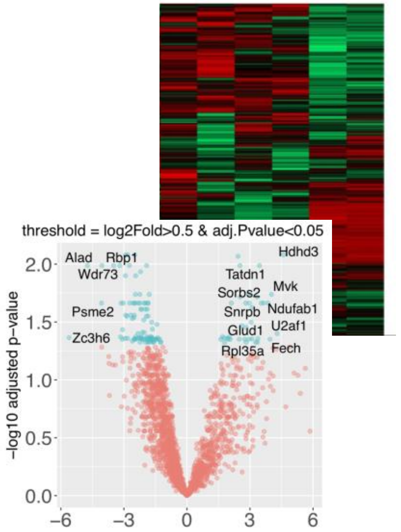
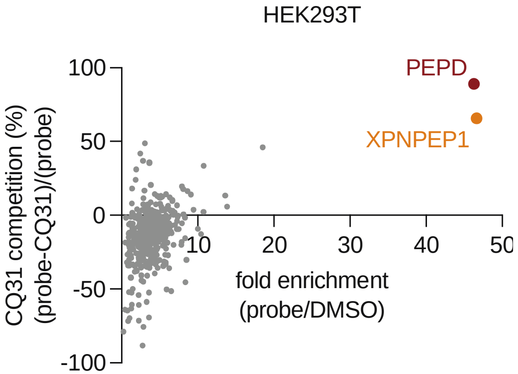
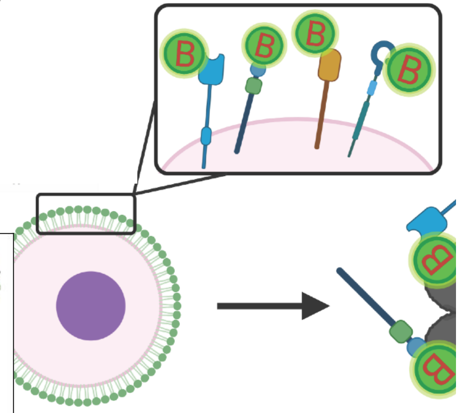
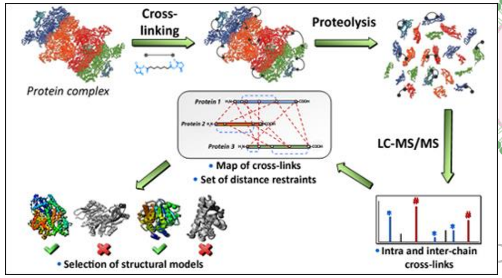
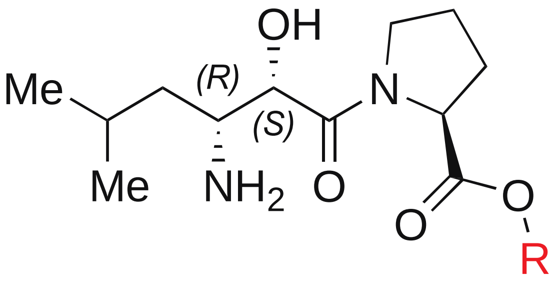




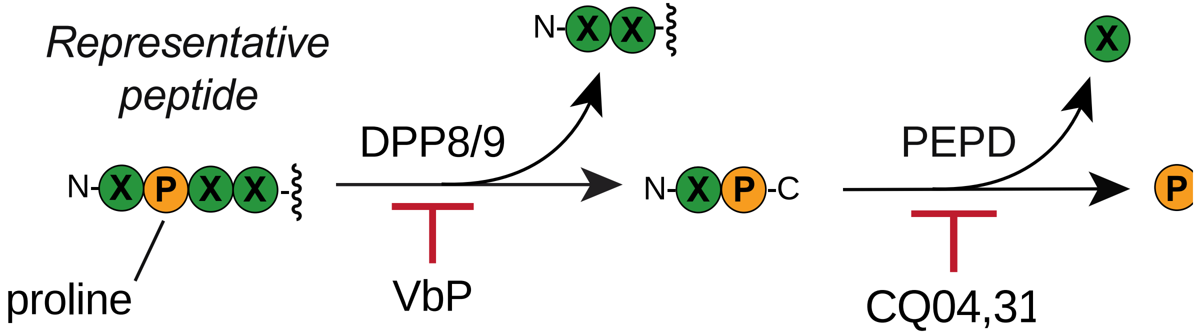




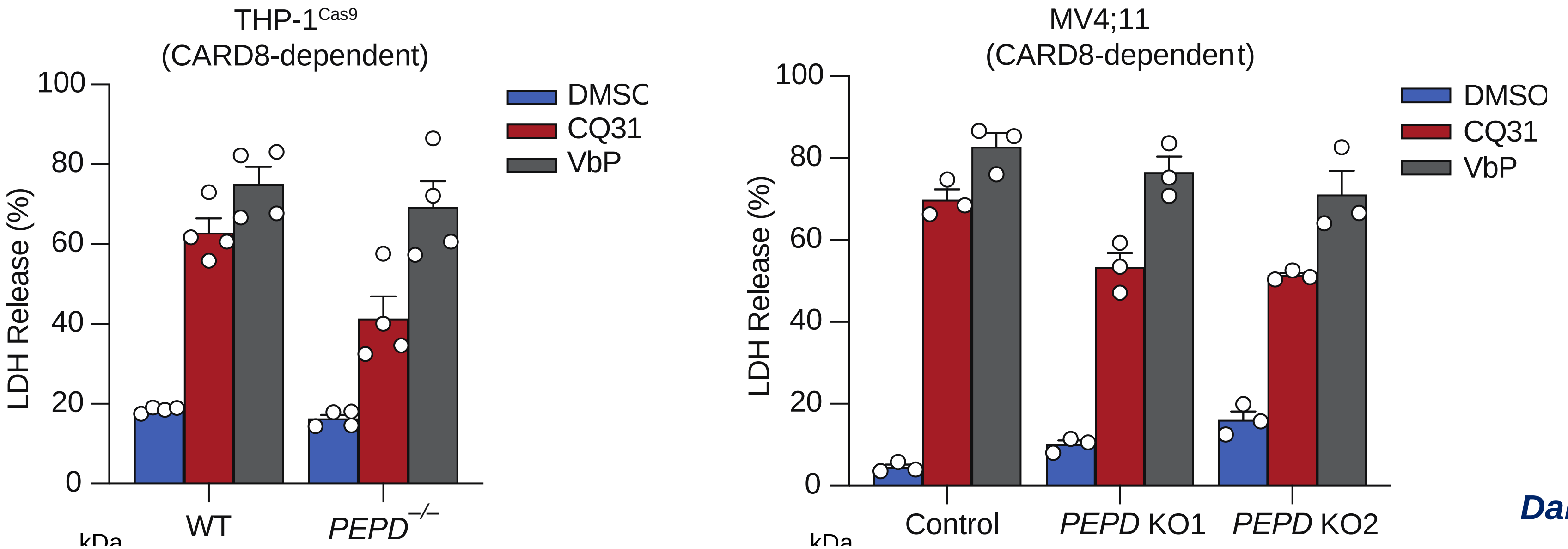
CQ31 is a potent prolidase (PEPD) inhibitor



CQ04: R = H; PEPD (IC_{50} = 160 nM)
CQ31: R = Me; PEPD (IC_{50} = 675 nM)

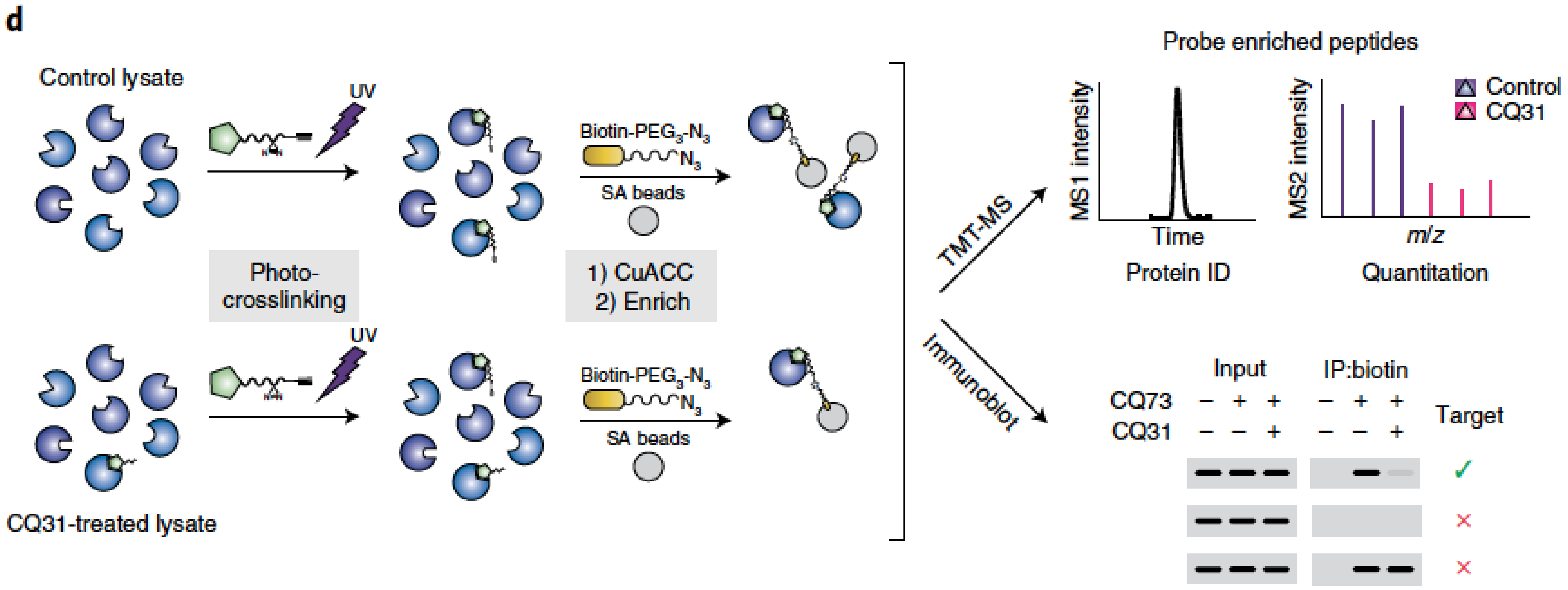


PEPD KO cells still respond to CQ31



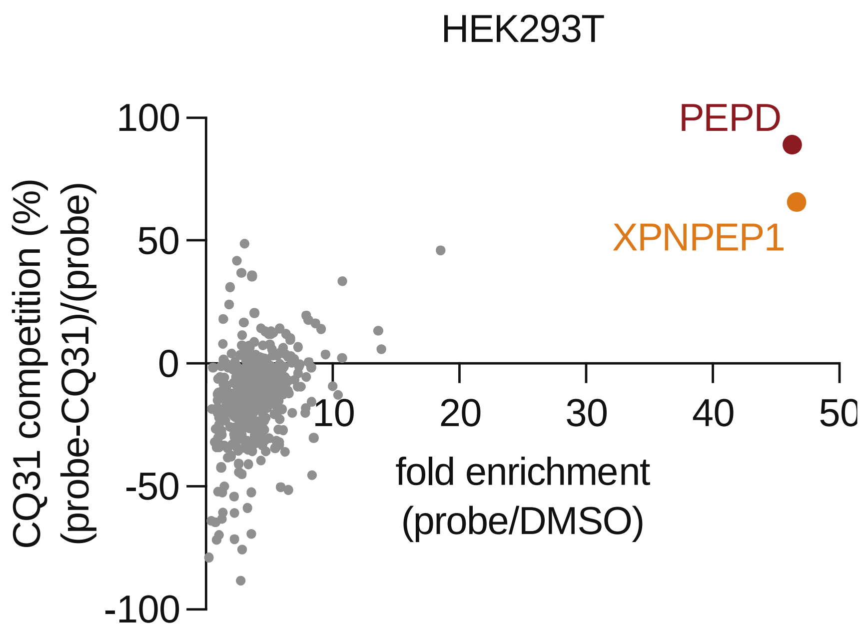
Dan Bachovchin

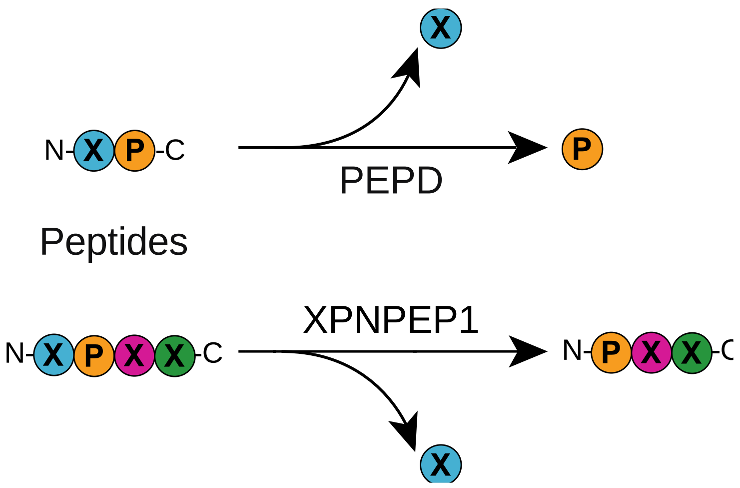
Approach to discover other CQ31 targets

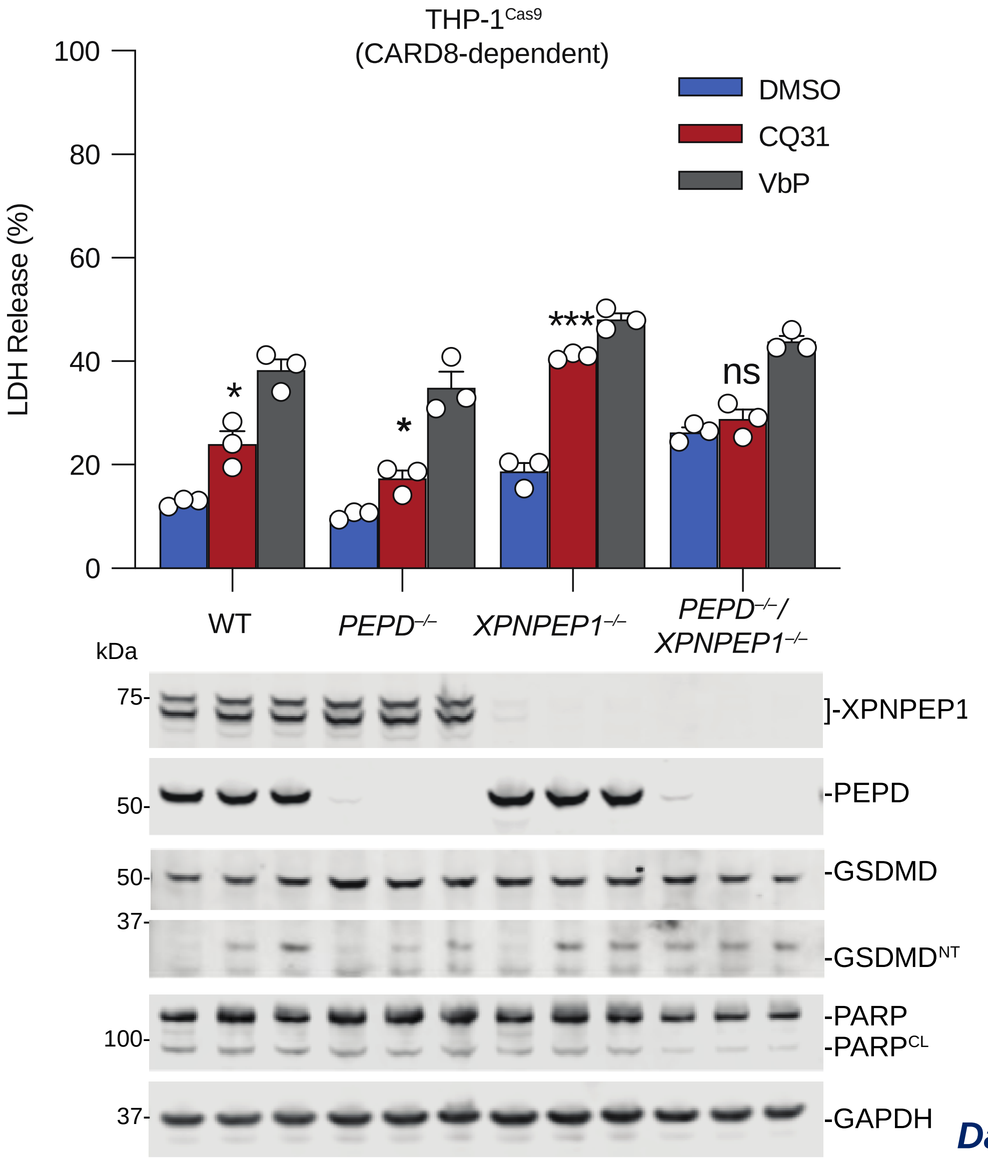


Dan Bachovchin

CQ31 inhibits PEPD and XPNPEP1







Dan Bachovchin, Matt Miele

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