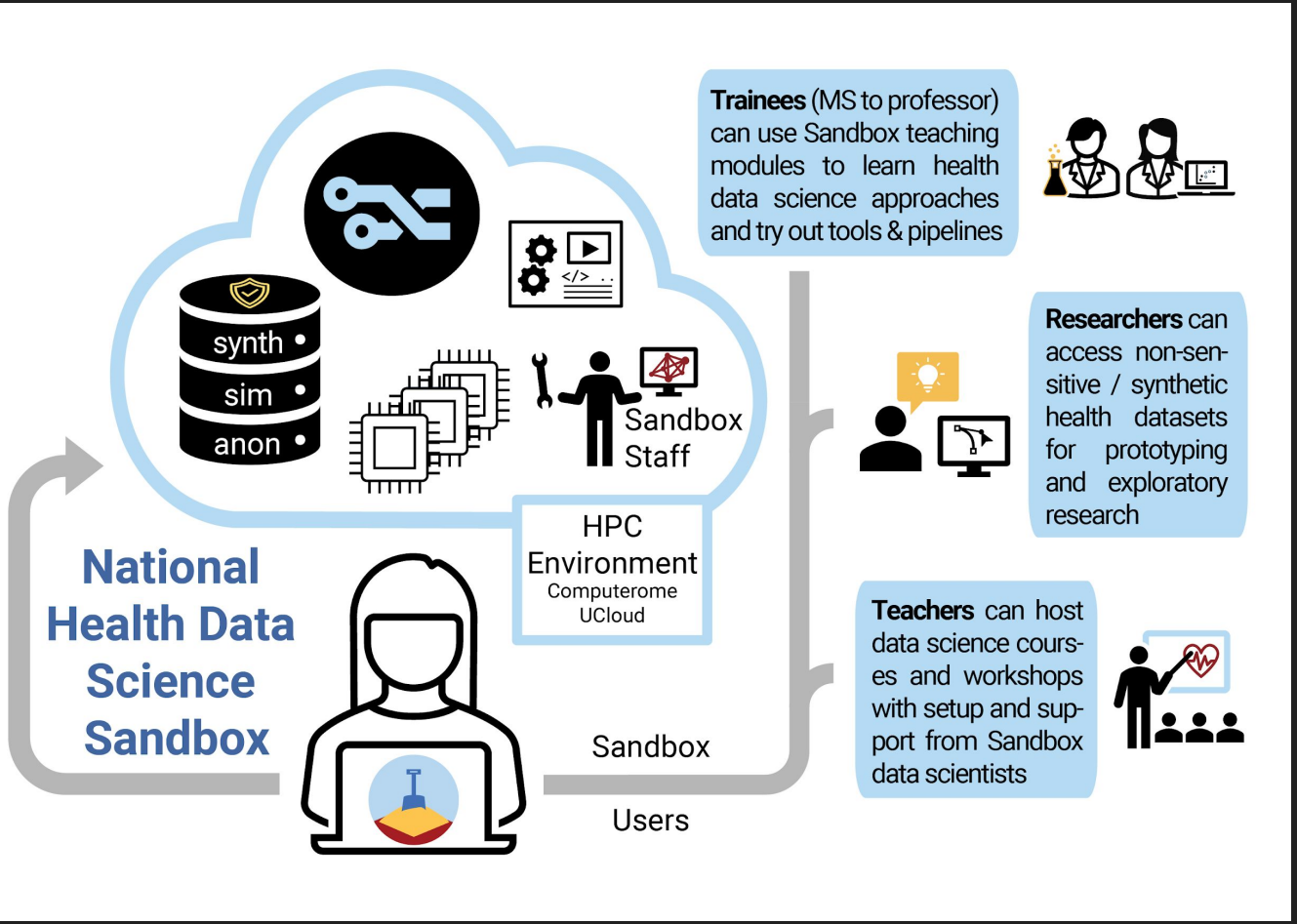
Proteomics

OMICS Workshop 31.august.2023

Samuele Soraggi
Health Data Science Sandbox

Health data science sandbox

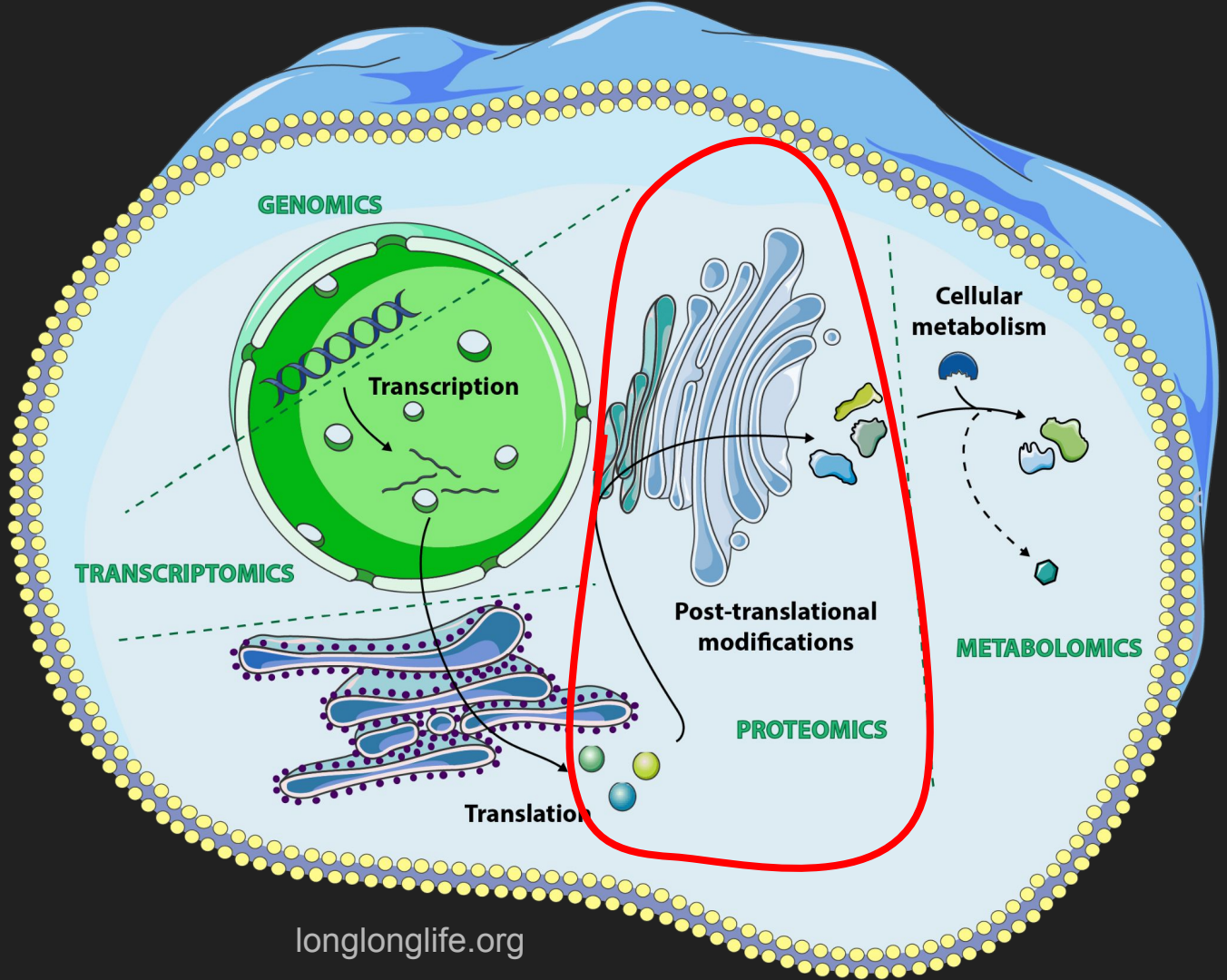


Home Page:

hds-sandbox.github.io

Contact:

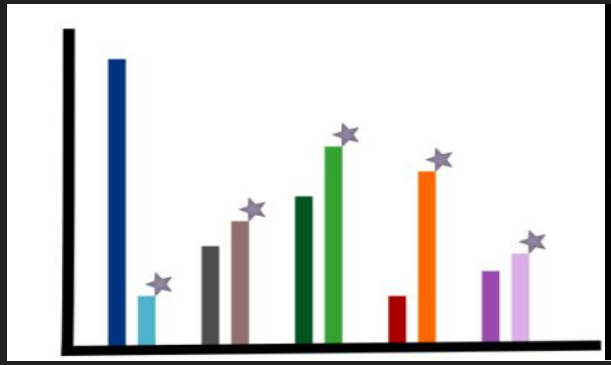
samuele@birc.au.dk



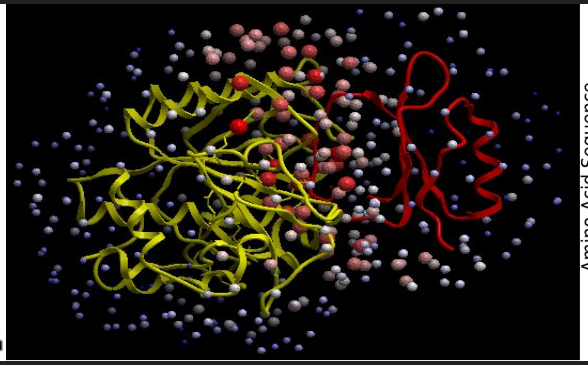
Program

9-9.30	Introduction to proteomics and tutorial format
9.30-9.45	Questions/Small break
9.45 - X	Log into uCloud, start the tutorial
X - X+15	Discussion and questions
X+15 - 13.00	Continue with the tutorial (If X small enough, we use the final time for discussion again)

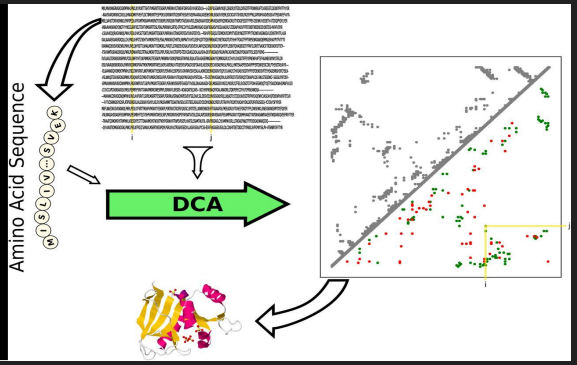
Proteomics - applications



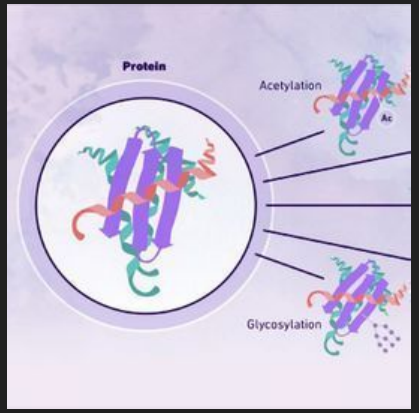
Peptide Identification and quantification



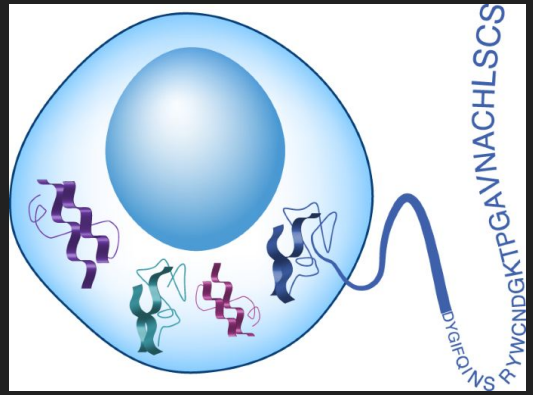
Protein-Protein Interactions



Structural prediction from aminoacids sequences

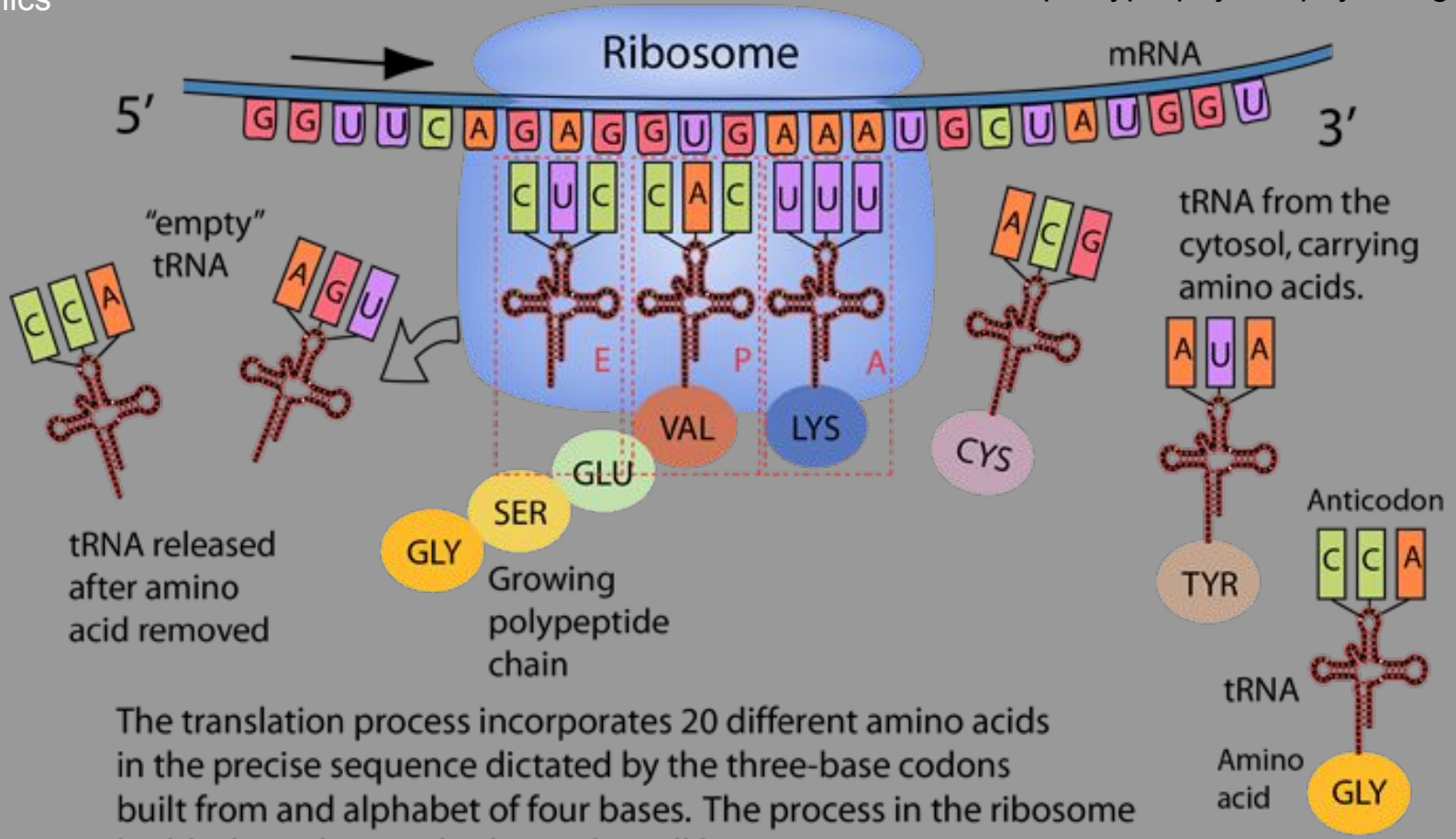


Post translational modification



Single cell proteomics

http://hyperphysics.phy-astr.gsu.edu/



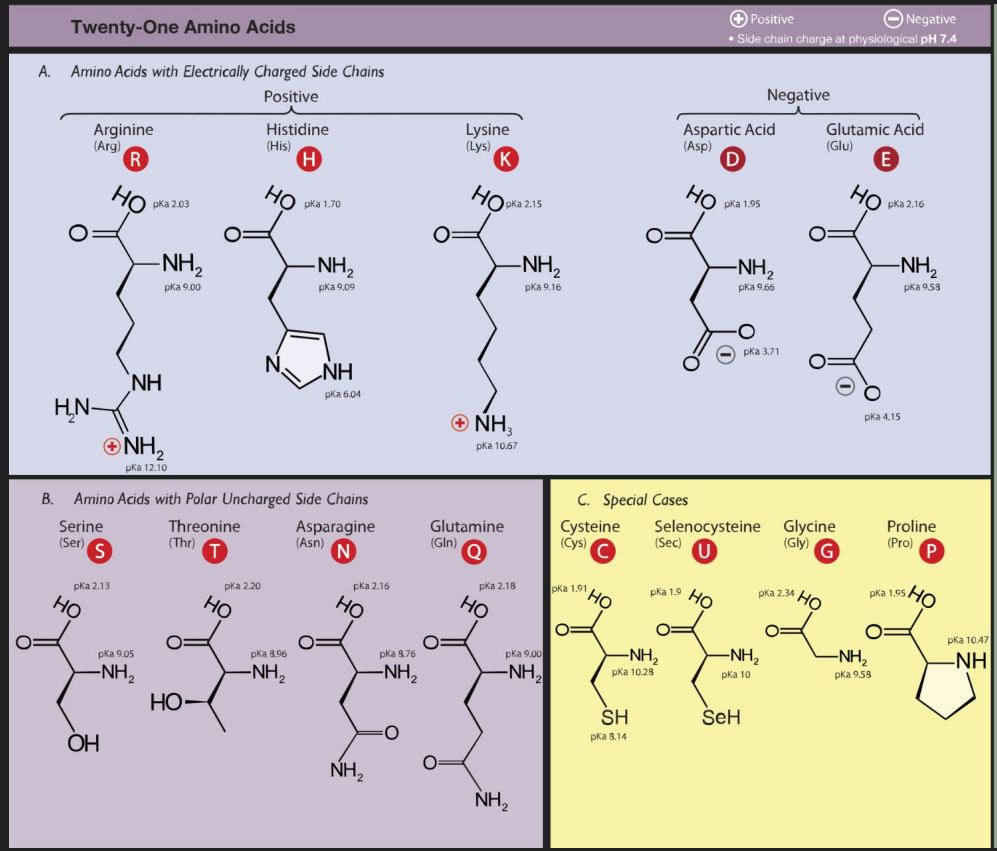
The translation process incorporates 20 different amino acids in the precise sequence dictated by the three-base codons built from and alphabet of four bases. The process in the ribosome builds the polypeptide chains tha will become proteins.

Wikipedia

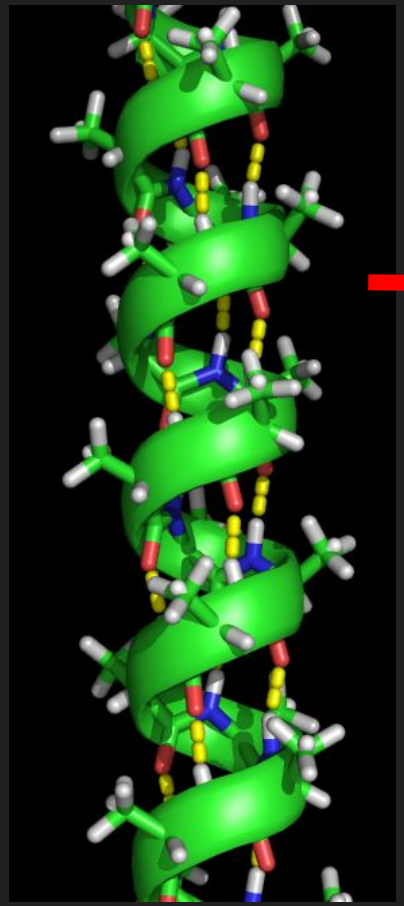
Aminoacids have specific properties such as

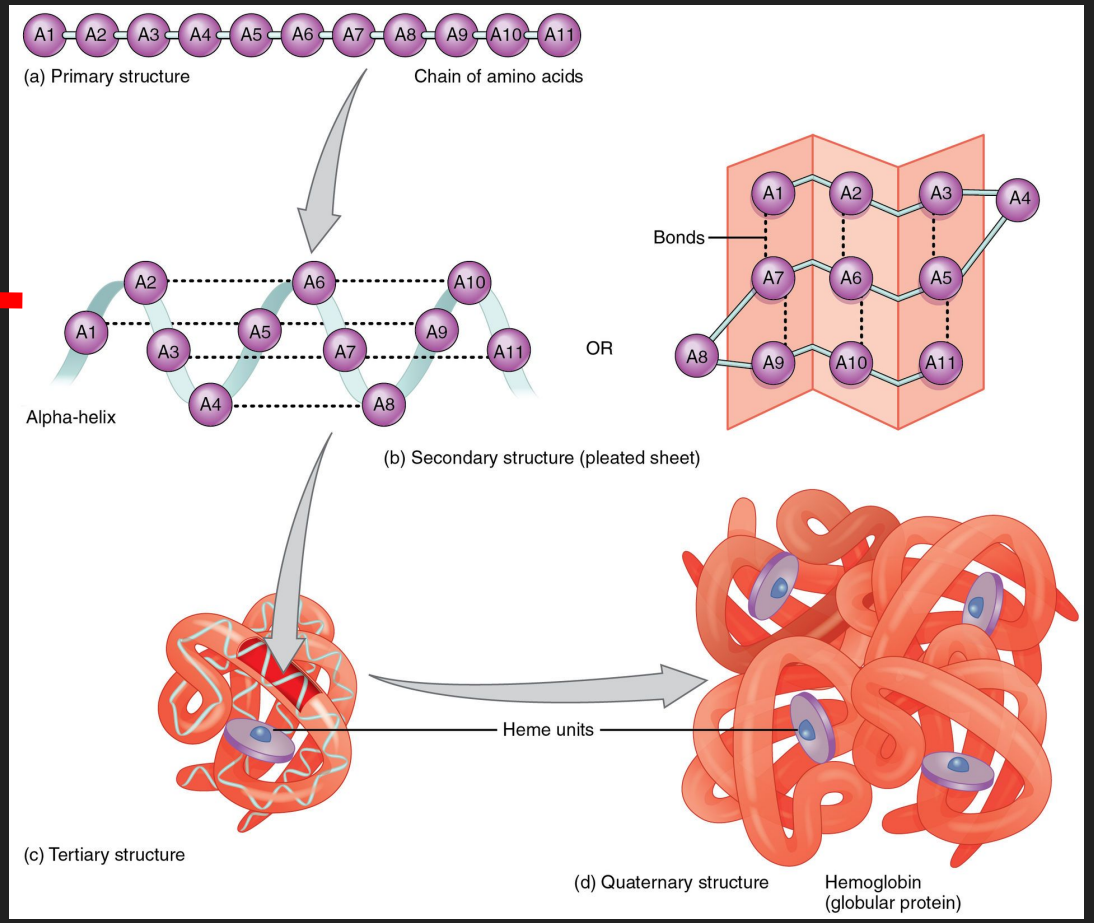
- charged
- uncharged
- hydrophobic

Those can create bonds between peptides



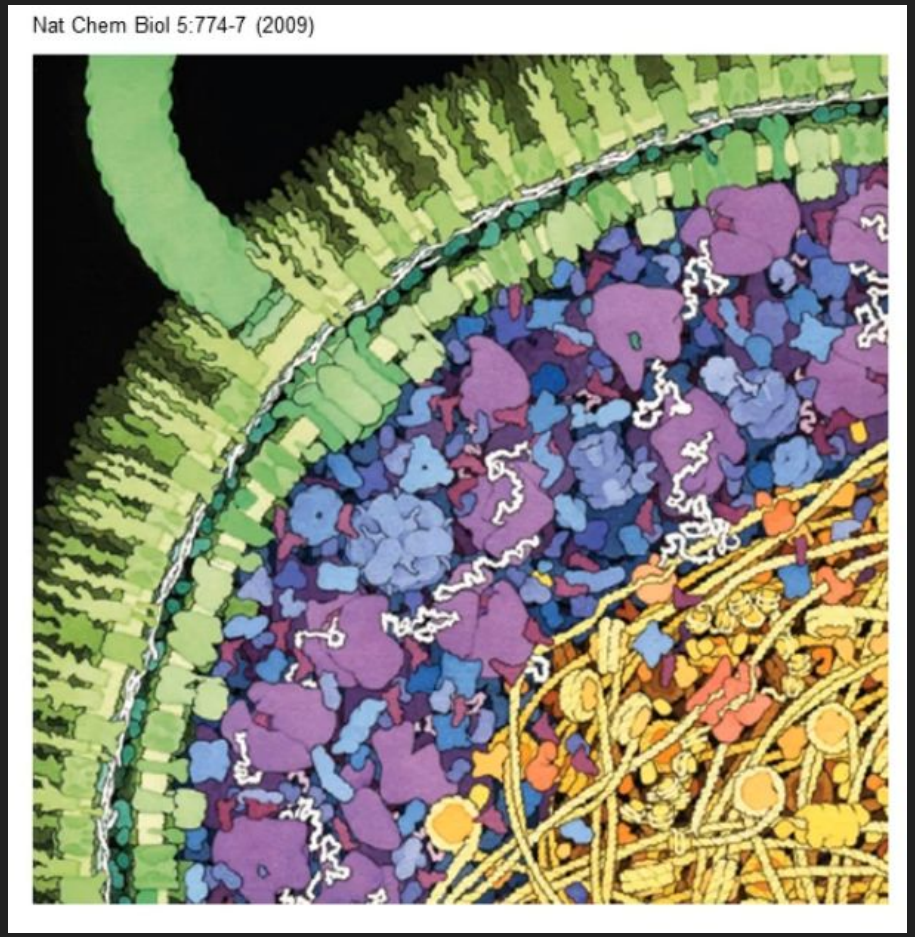
Proteomics - Protein geometry





Wikipedia

Proteomics - Protein/peptide isolation

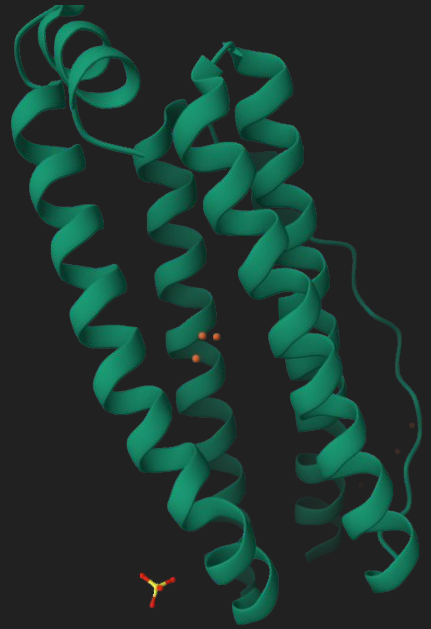


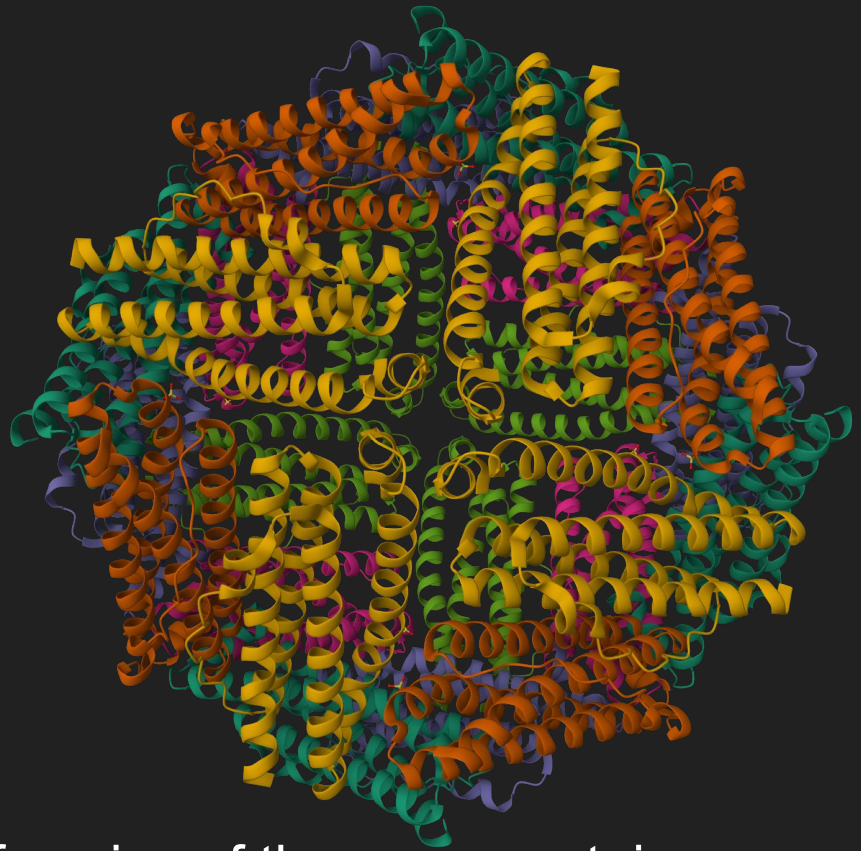
Challenge:

isolate proteins to detect the distinct chemical structures

Note: there is no PCR here

Proteomics - Protein/peptide isolation



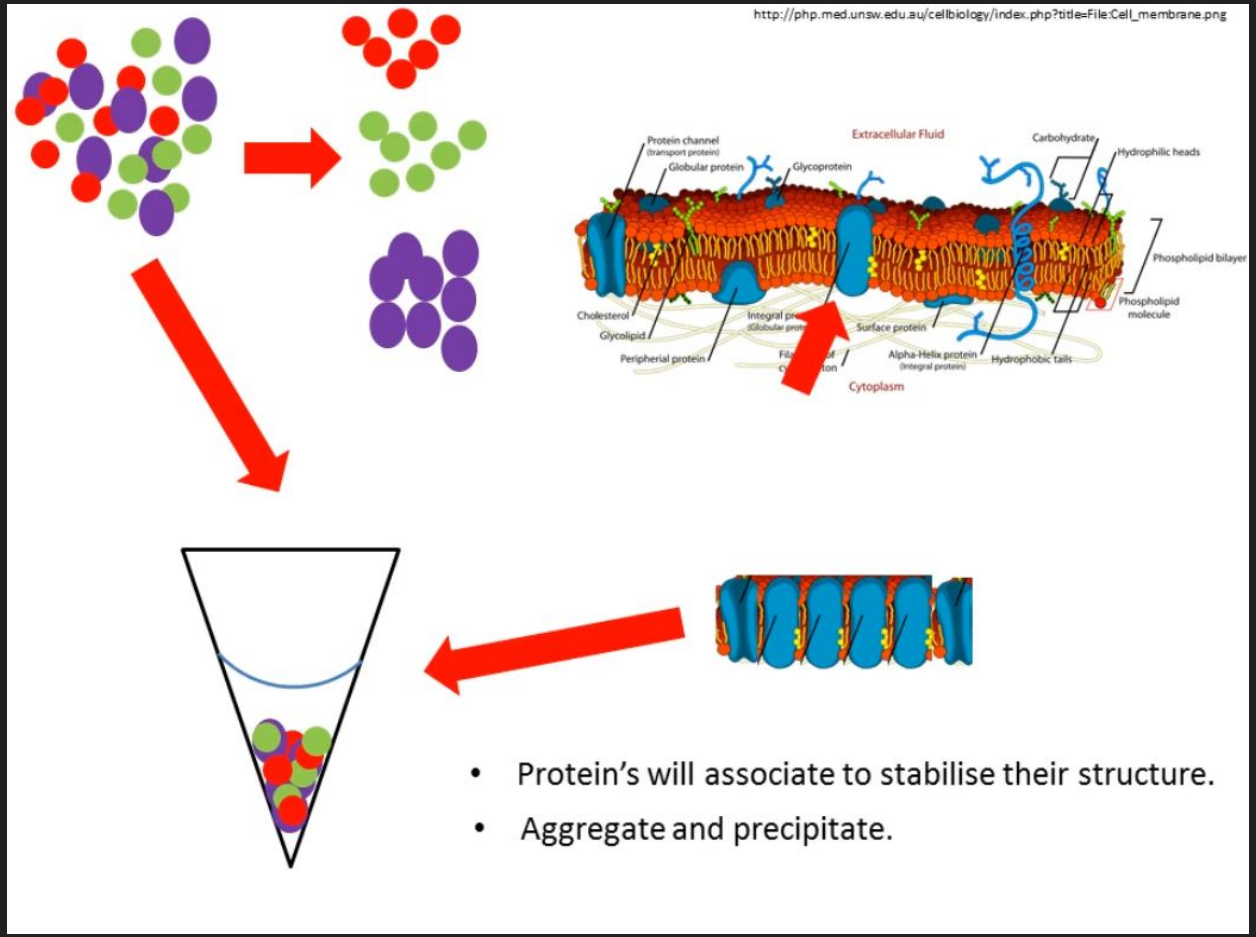


Not 1, but100s or 1000s of copies of the same protein

A Marchetti *et al. Nature* **000**, 1-4 (2008)
doi:10.1038/nature07539

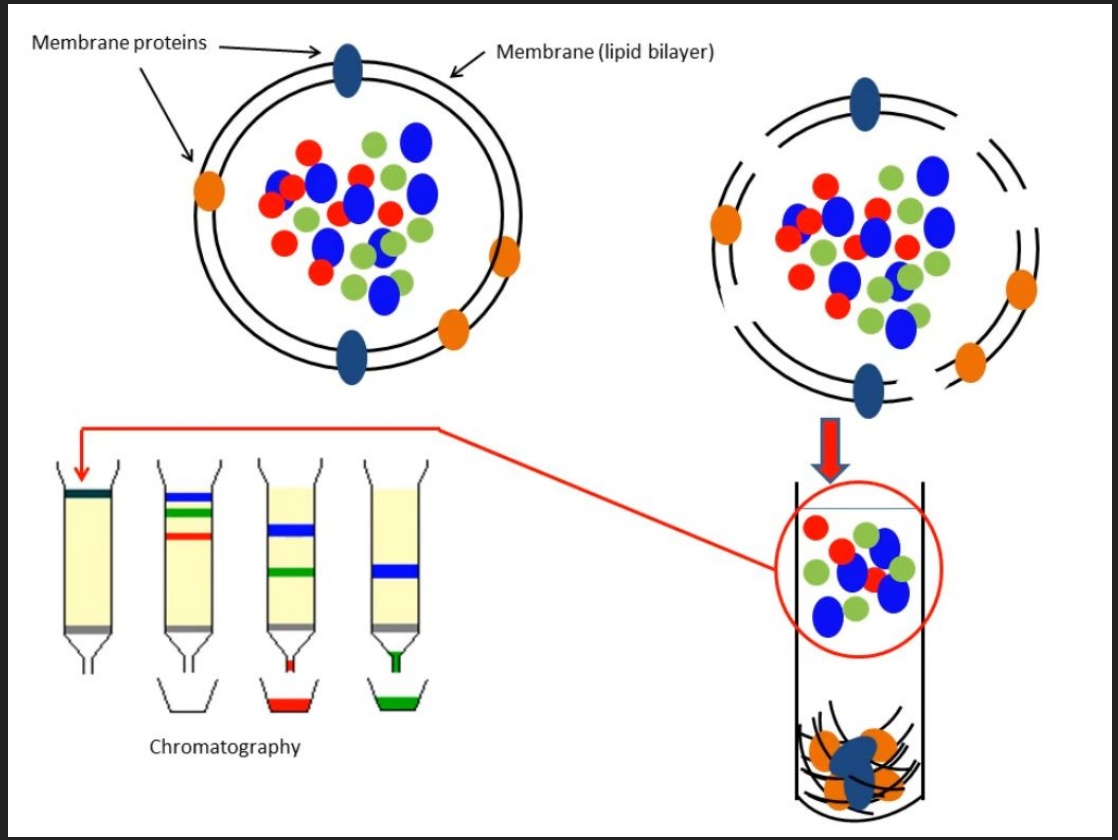
Fluidic isolation

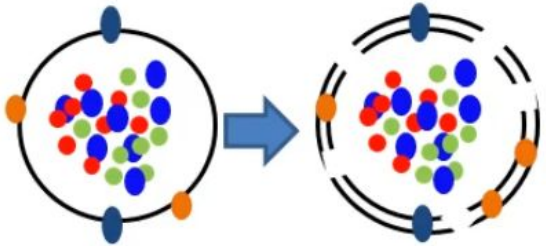
- recovers some peptides
- new associations to stabilize peptides (e.g. hydrophobic bonds)
- heavy bonds will be lost by precipitation

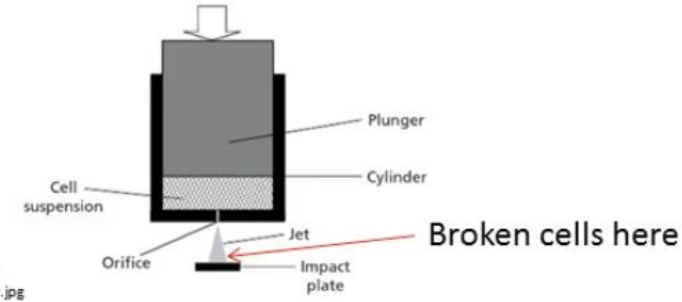


Fluidic isolation

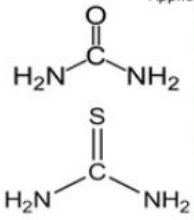
- centrifugation recovers more peptides
- a chromatograph can separate them
- specific liquid solutions reduce the precipitation loss further by avoiding bonds (e.g. hydrophobic)

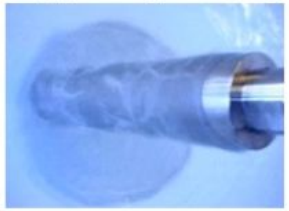


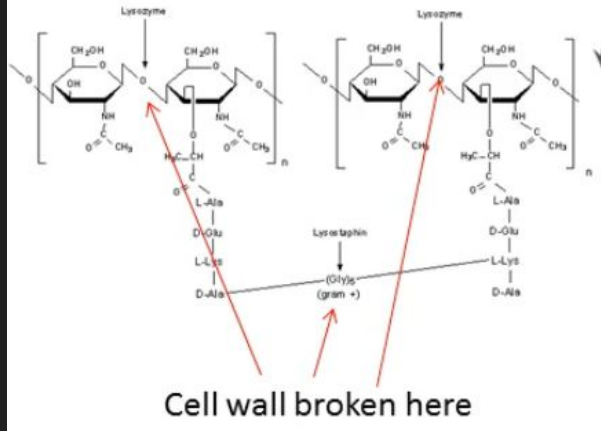


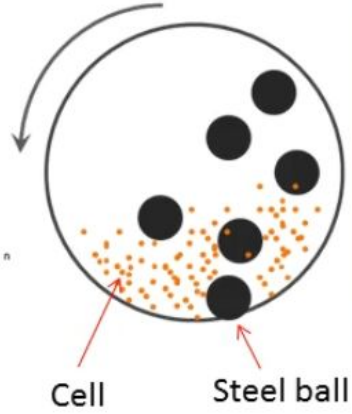


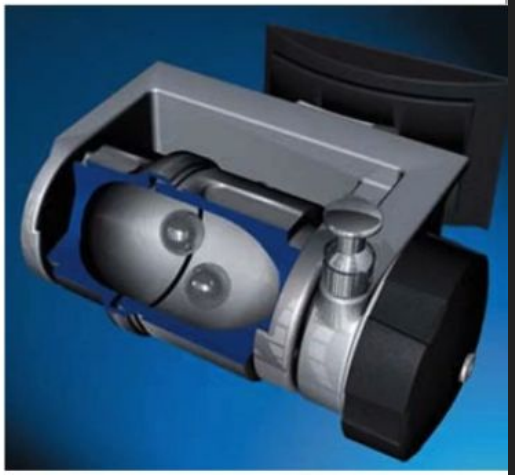
http://www.activeultrasonic.com/applications/files/images/Applications/LiquidProcessing/Sonocator/FW_Cavitation2aw.jpg







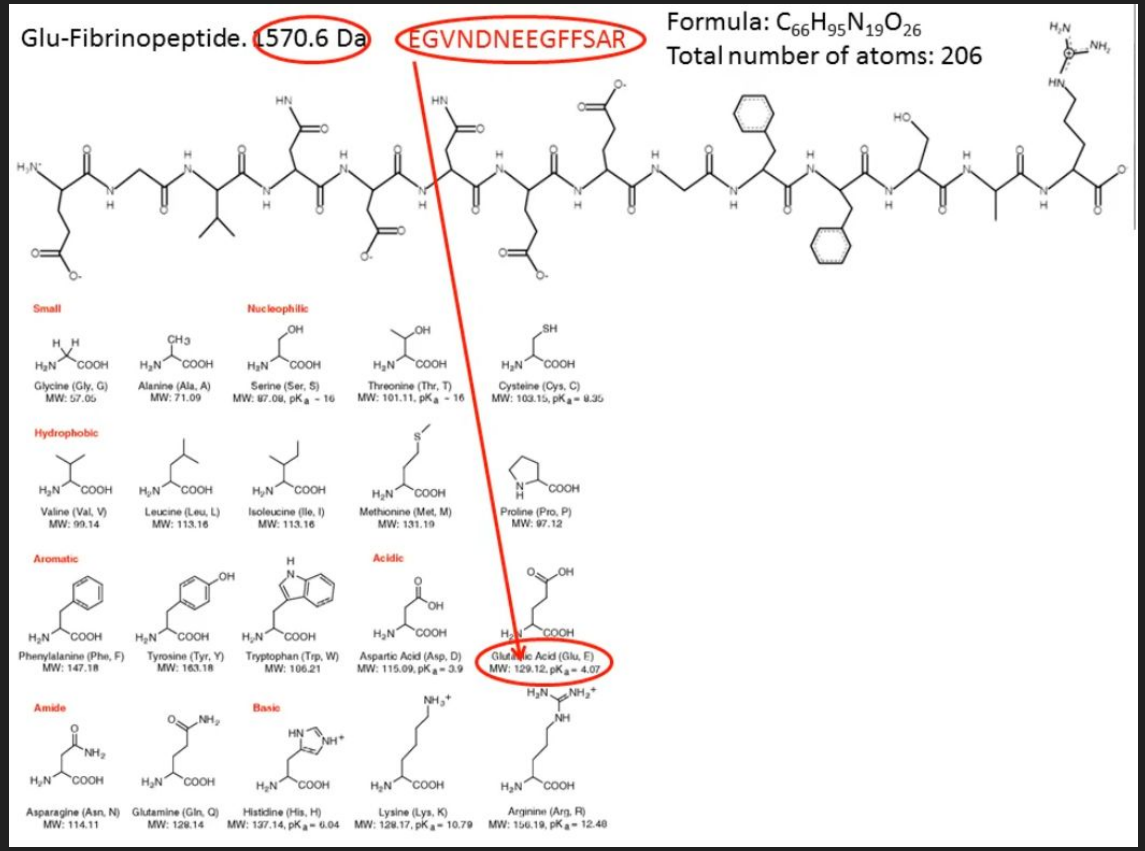




http://www.retsch.com/products/milling/ball-mills/mixer-mill-mm-400/function-features/

Mass is important

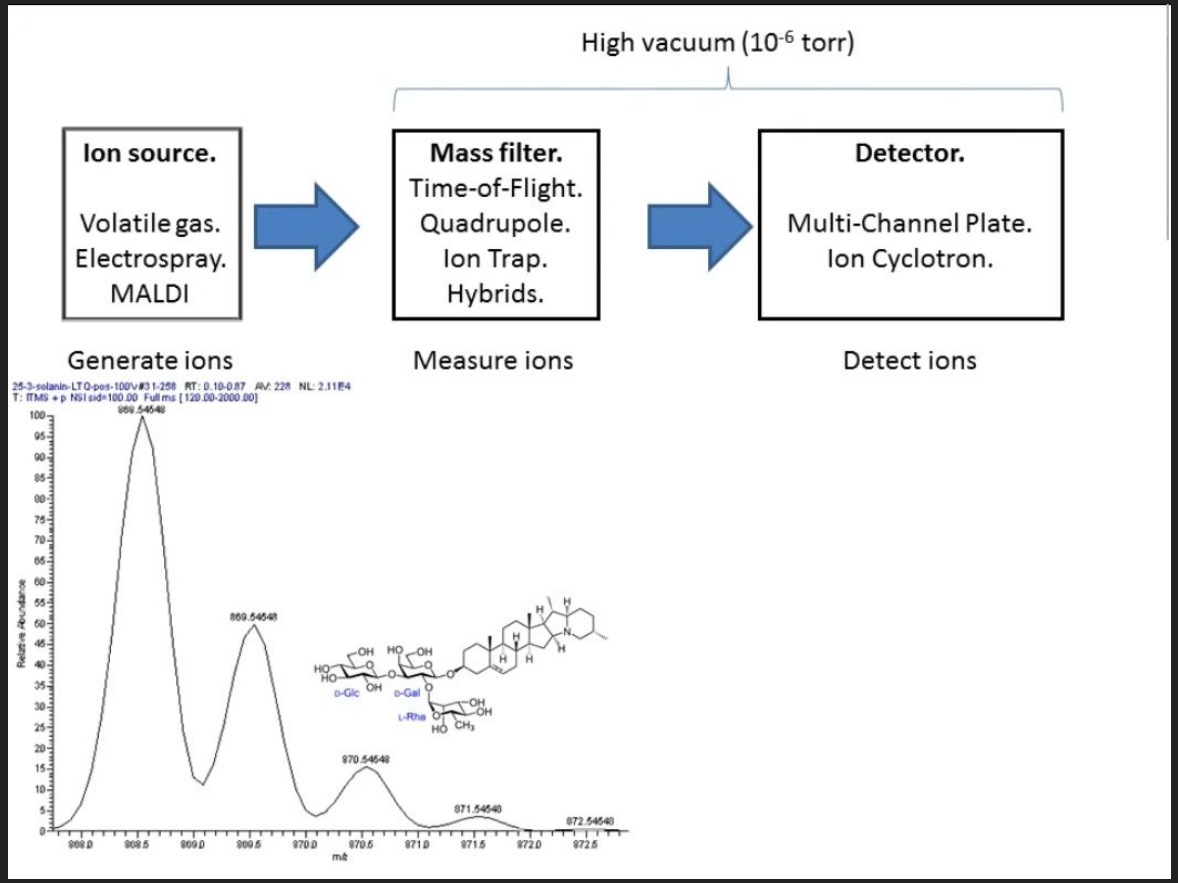
- elements in the periodic table have unique mass
- their compounds also do, including peptides
- We cannot calculate the mass of peptides when we do not know the sequence



Matt Padula

Mass spectrometry

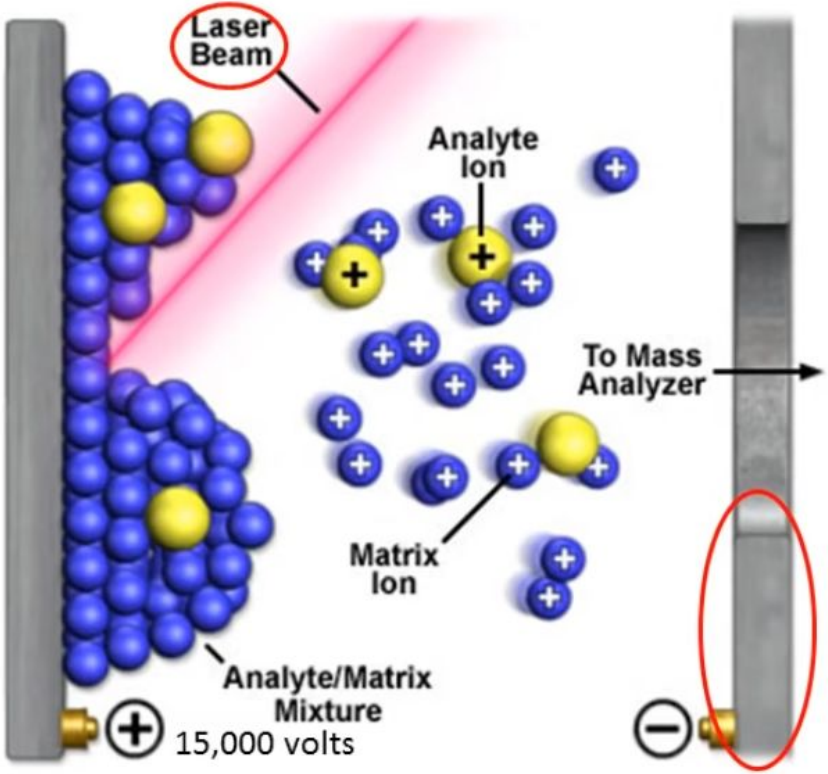
- We charge instead our particles
- We make them go through a dedicated device
  (e.g. a **T**ime **O**f **F**light device)
- We measure their mass

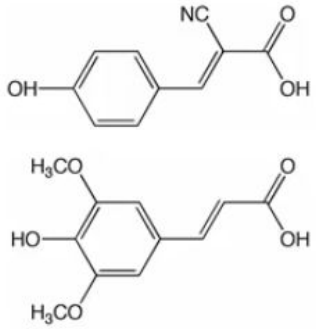


Matt Padula

Matrix Assisted Laser Desorption/Ionisation

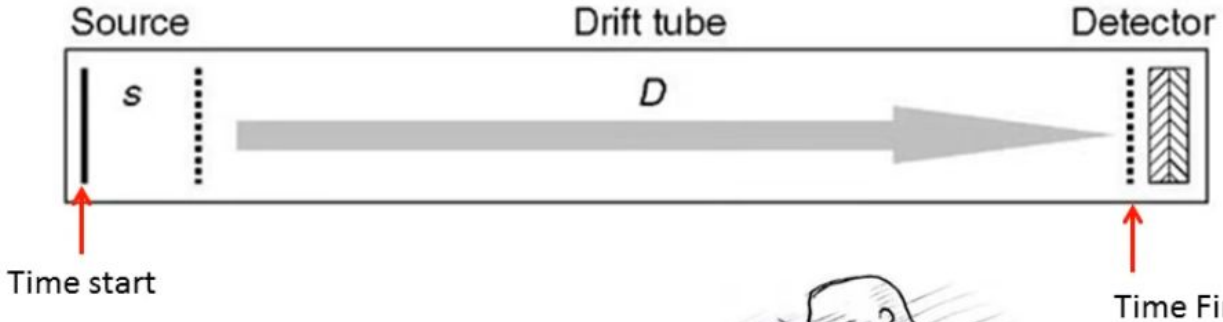
- Hillenkamp and Karas (1985) ionised amino acids and small peptides.

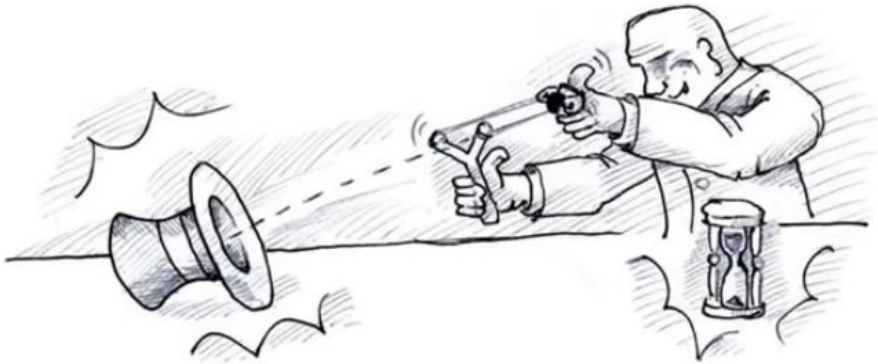




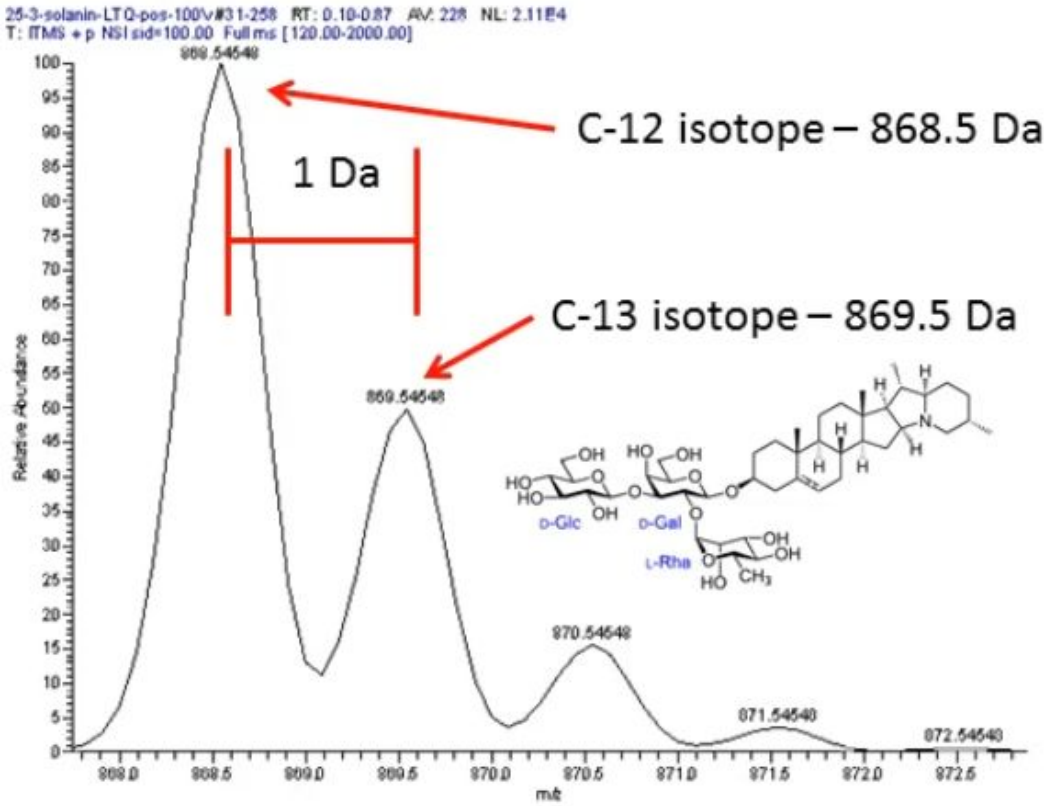
http://www.ms-textbook.com/2nd/downloads/maldi_anchor.jpg

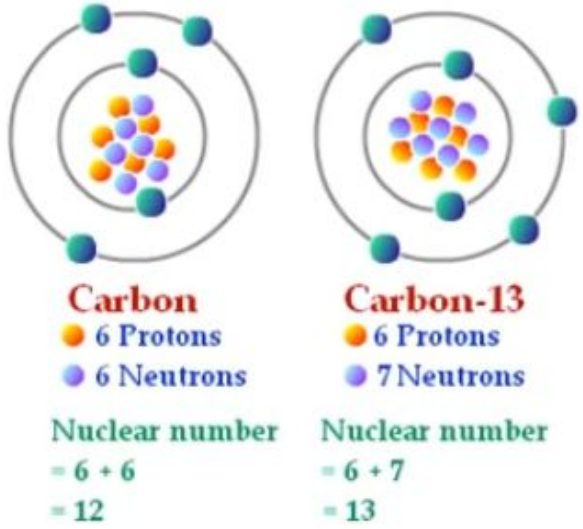
- Time of Flight: The time it takes an ion to go from ion source to detector is directly related to it's mass.
  - Heavier particles reach lower speeds.





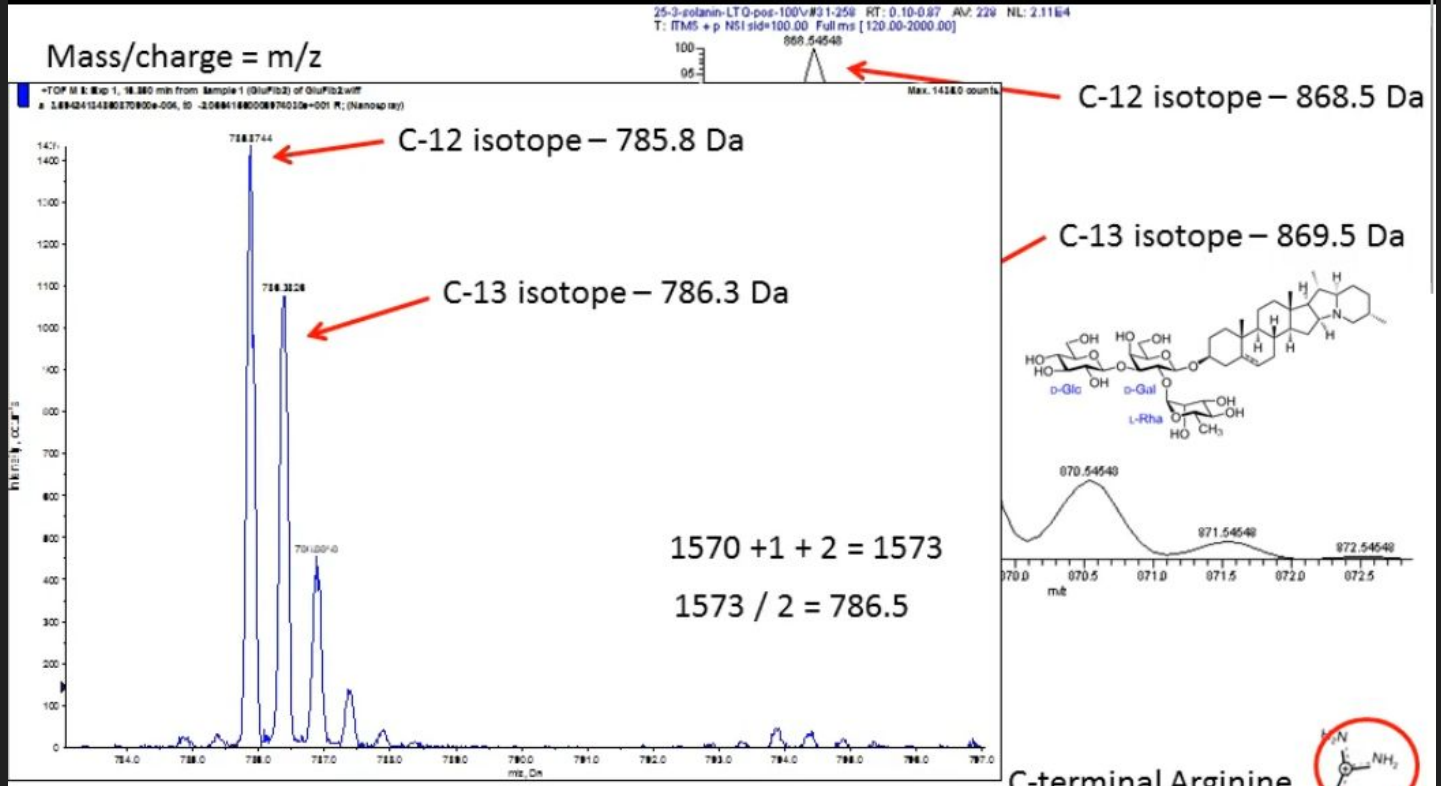
$$velocity = \sqrt{\frac{2 \times energy}{mass}} \qquad Flight\_time = \frac{drift\_length}{velocity} = drift\_length \times \sqrt{\frac{mass}{2 \times energy}}$$


25-3-solanin-LTQ-pos-100V#31-258 RT: 0.10-0.87 AV: 228 NL: 2.11E4
T: ITMS + p NSI sid=100.00 Full ms [120.00-2000.00]
868.54548
C-12 isotope – 868.5 Da
1 Da
C-13 isotope – 869.5 Da
869.54548
870.54548
871.54540
872.54540
D-Glc
D-Gal
L-Rha
Relative Abundance
m/z

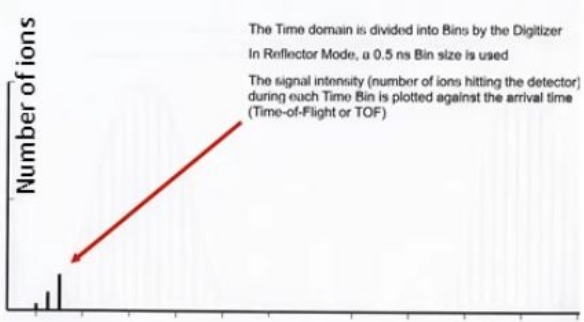

Carbon
6 Protons
6 Neutrons
Nuclear number
= 6 + 6
= 12
Carbon-13
6 Protons
7 Neutrons
Nuclear number
= 6 + 7
= 13

Matt Padula

Mass/charge = m/z

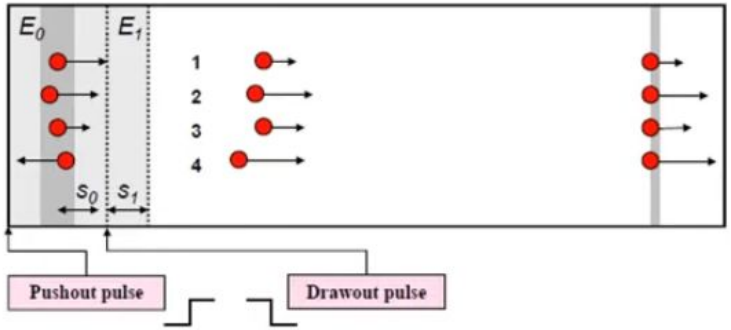


Matt Padula



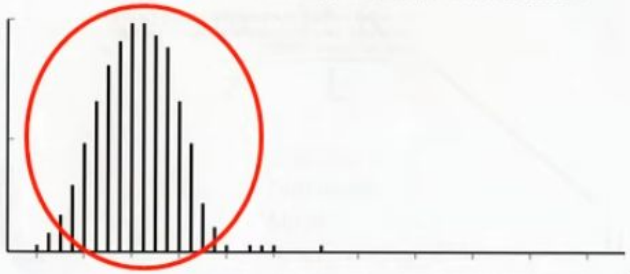
Arrival time of ions striking the detector (TOF)

Wiley, W.C.; McLaren, I.H., **Rev. Sci. Instrument. 26** (1955) 1150-1157.



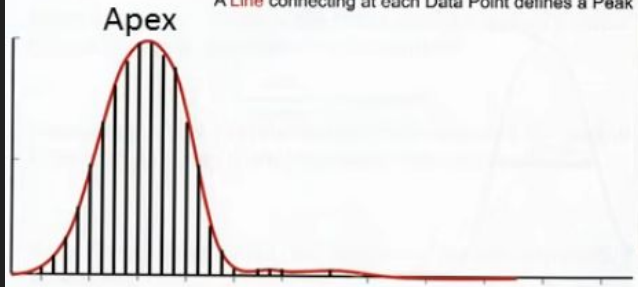
Display here is a Vertical Bar at each data point (Time

These data points define a peak in the mass spectrum

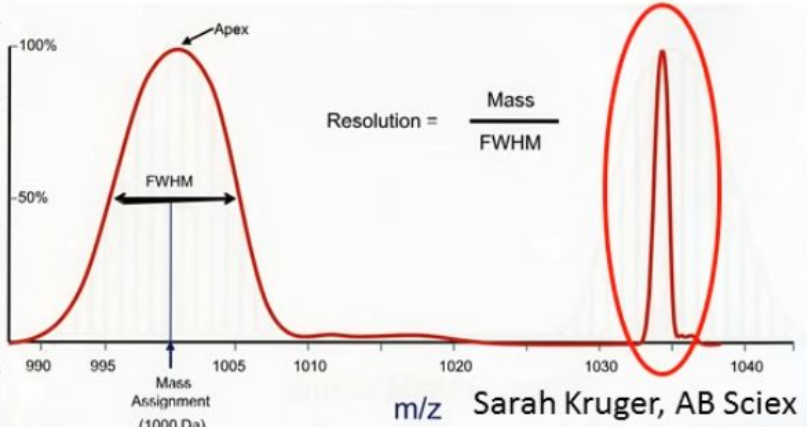


Time-of Flight → m/z

A Line connecting at each Data Point defines a Peak

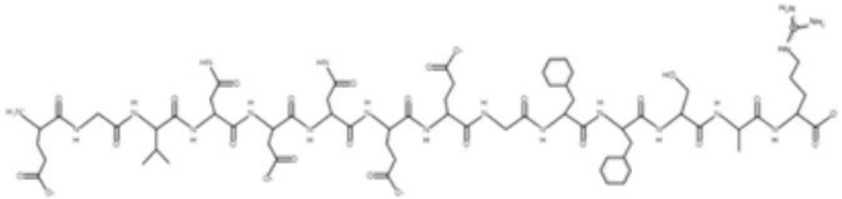


Time-of Flight → m/z



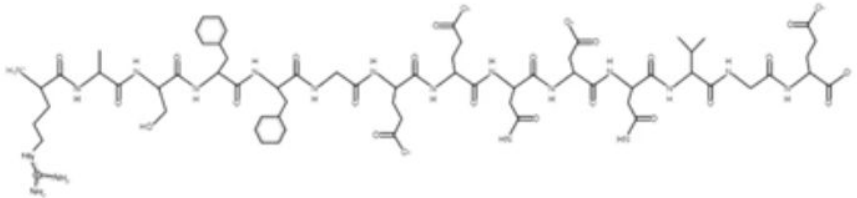
Sarah Kruger, AB Sciex

- But, in a more complex mixture of peptides from different protein isoforms, isobaric peptides exist.



EGVNDNEEGFFSAR

1570 Da



RASFFGEENDNVGE

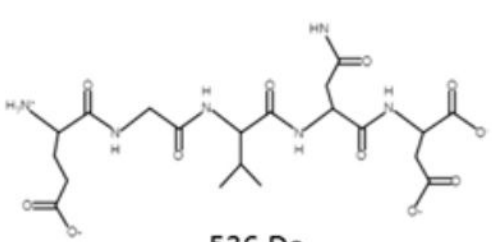
- Same mass, but different structure.
- Measuring intact mass doesn't reveal isomers.

http://www.tulane.edu/~biochem/WW/PepDraw/index.html

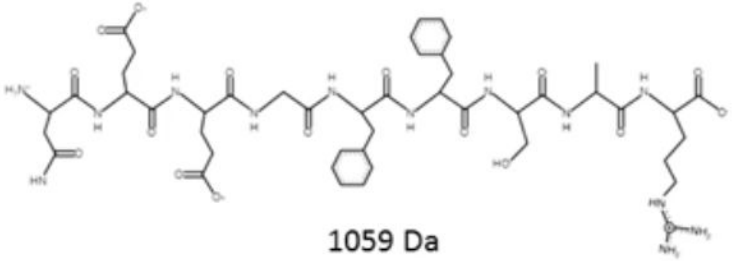
Matt Padula

- Break molecule into smaller pieces and measure their masses.

EGVNDNEEGFFSAR

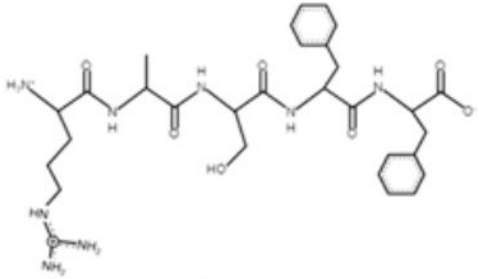


536 Da

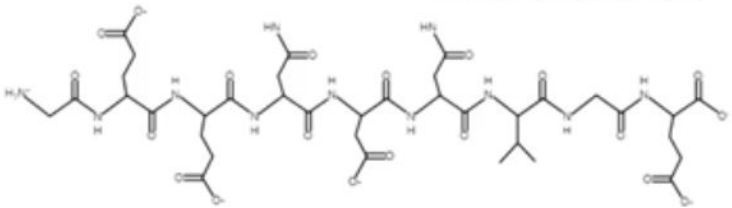


1059 Da

RASFFGEENDNVGE

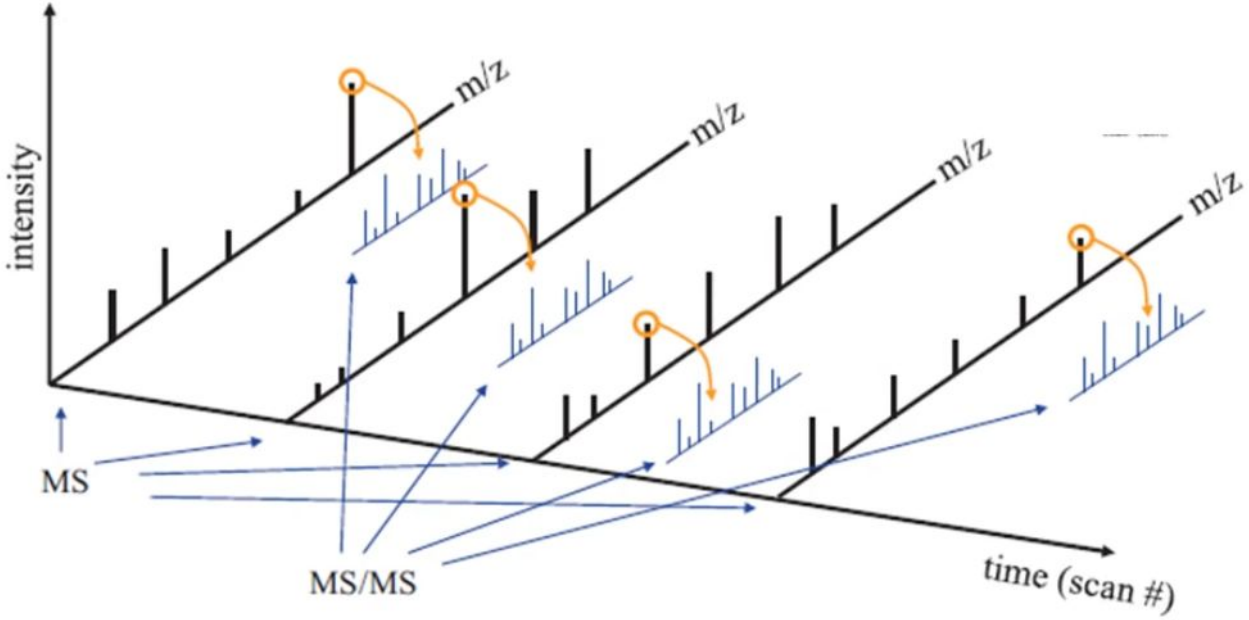


626 Da



969 Da

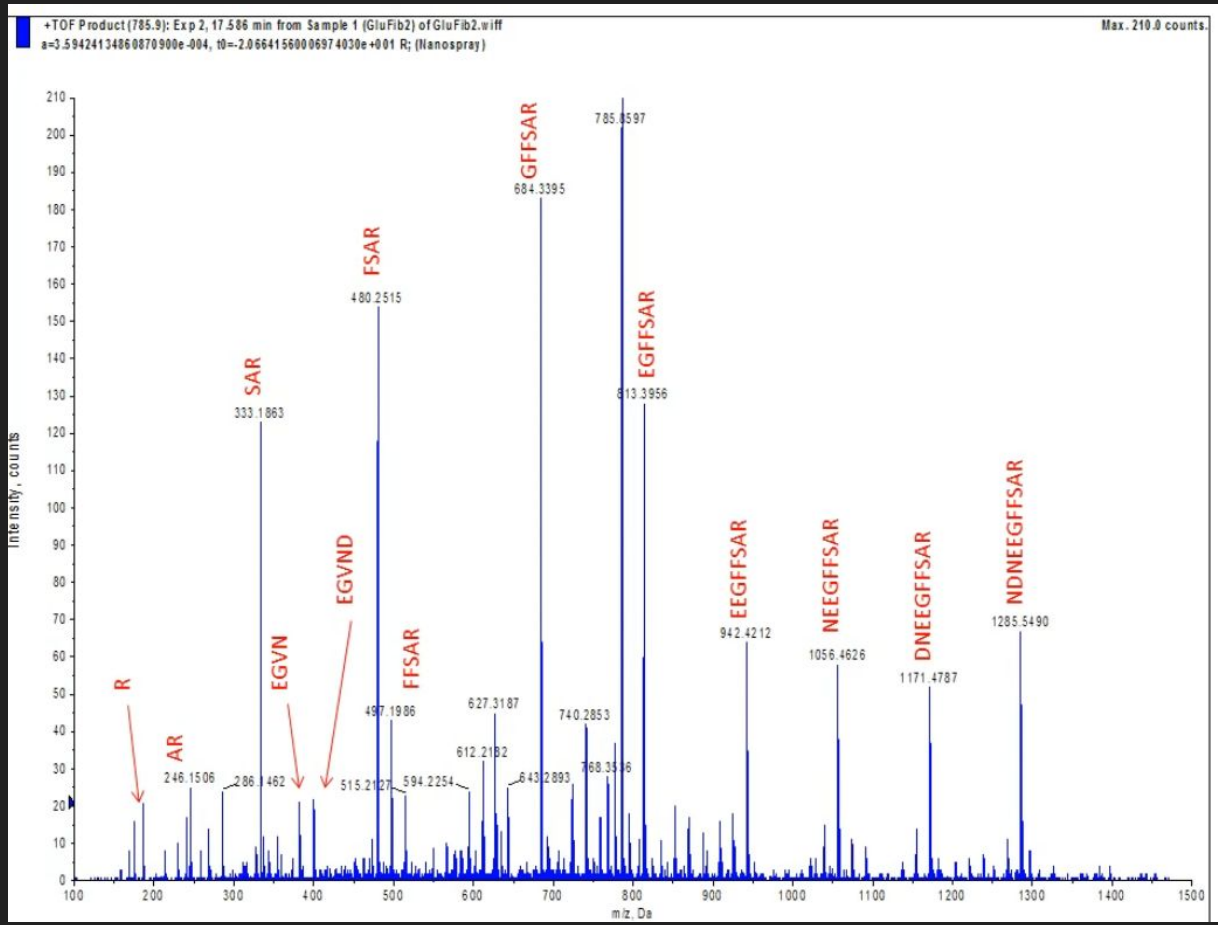
The MS scans continuously for intact peptide ions, selecting one of those ions, fragmenting it, and measuring the mass of each fragment



Luis Mendoza, Institute for Systems Biology

Mass spectrometry

The compound sequence is not known, but can be built back by putting together peaks which difference is the mass of an aminoacid.



Matt Padula

Acknowledgements:

Most slides are taken from or inspired by the course in Proteomics by Matthew Padula. All his proteomics lectures are stored on the youtube channel https://www.youtube.com/@MatthewPadula/videos and are highly recommended for a more thorough introduction than my own.

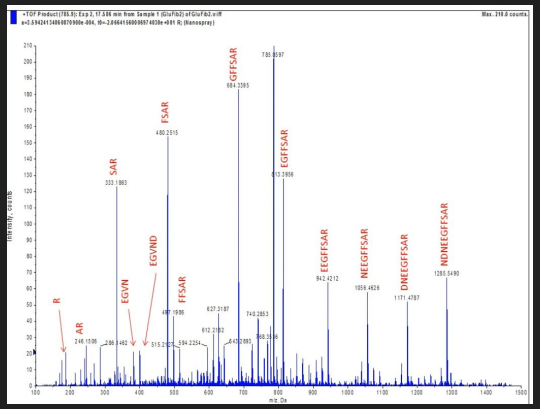
Tutorial

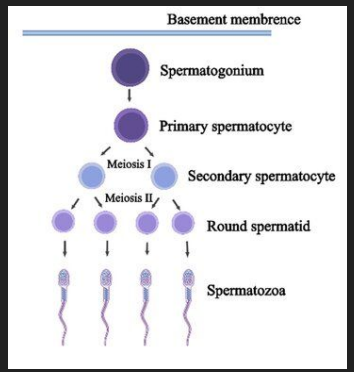
- Raw data from 6 samples: 3 fertile and 3 infertile men using Liquid Chromatography Mass Spectrometry (LC-MS)

- Pipeline to identify peptides and infer protein occurrences in the spectra

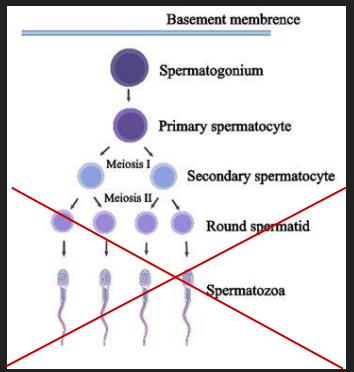
- Export-Import data and find significant proteins between the conditions

- Examples of biologically relevant information from the protein/gene list





VS



- Go to https://hds-sandbox.github.io/OMICS-workshop/
- Follow the "**uCloud access**" instructions in the menu if this is your first access on uCloud
- Go on "**Day 3 - Proteomics**" on the webpage for instructions to work on the tutorial