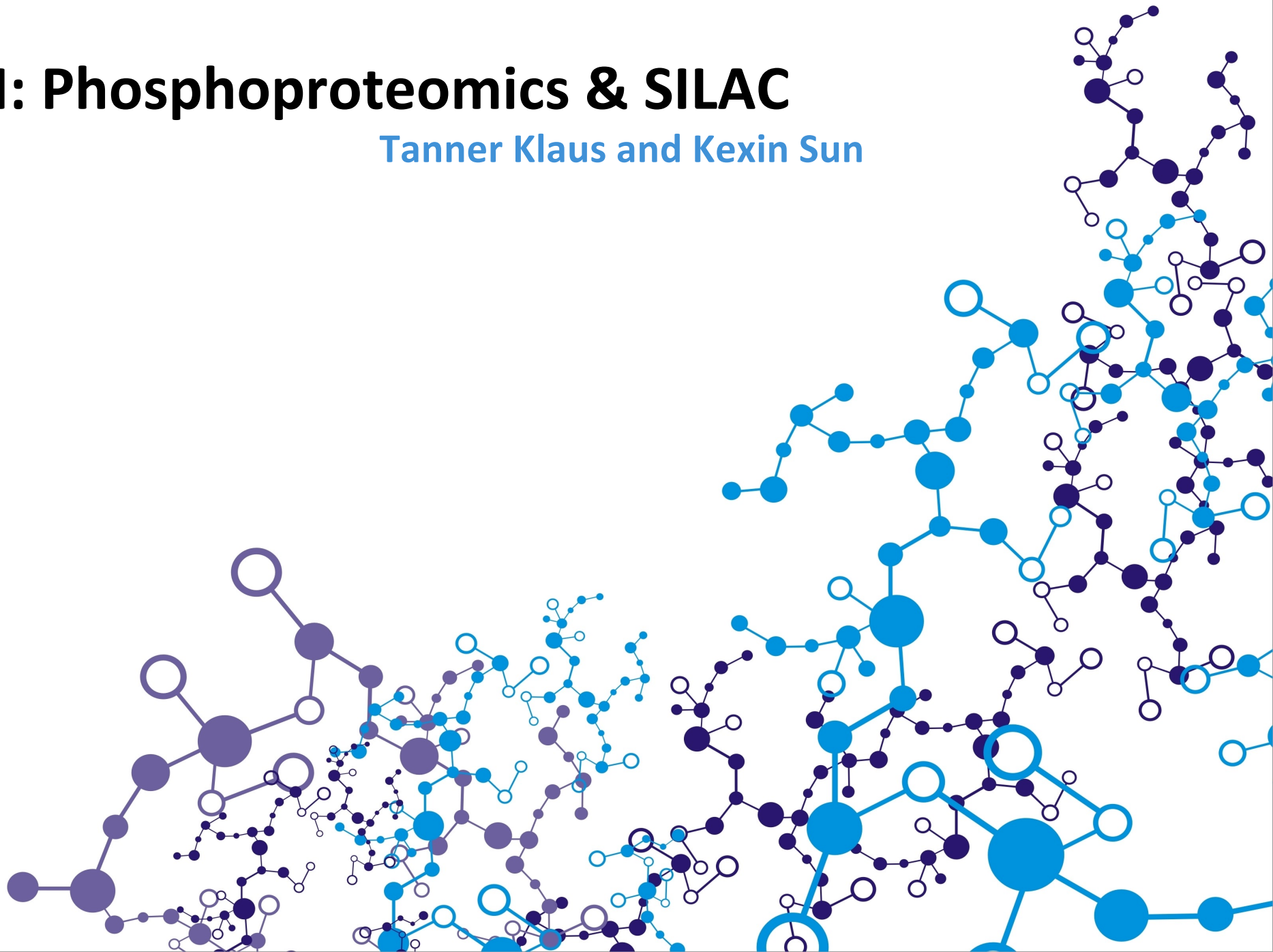
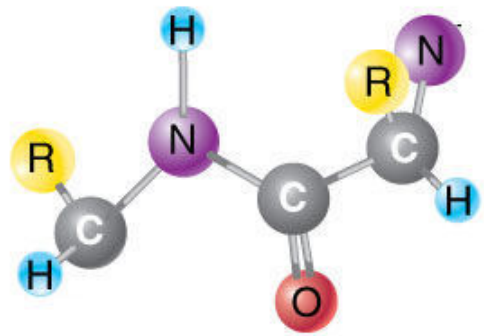
Proteomics II: Phosphoproteomics & SILAC

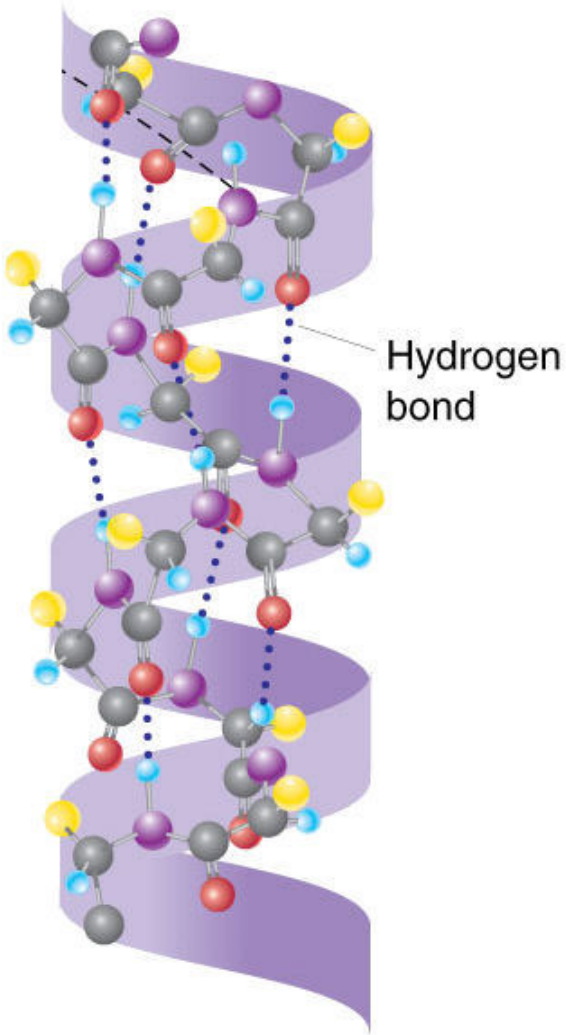
Tanner Klaus and Kexin Sun



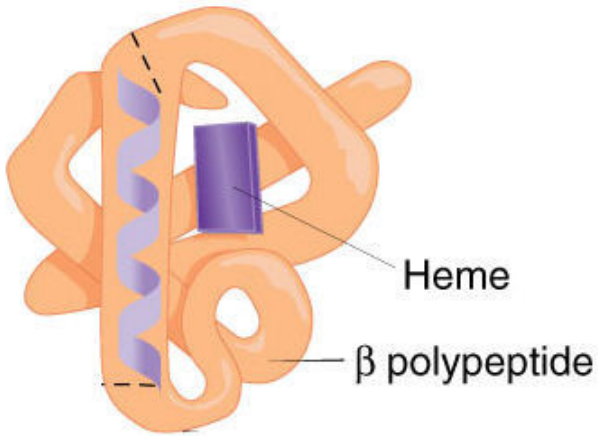
What are proteins?



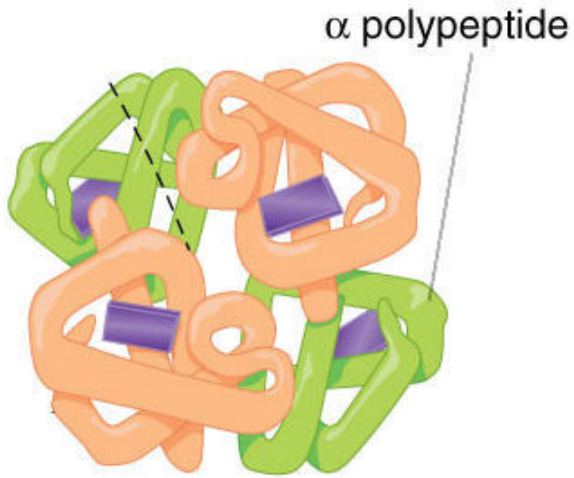
(a) Primary structure



(b) Secondary structure

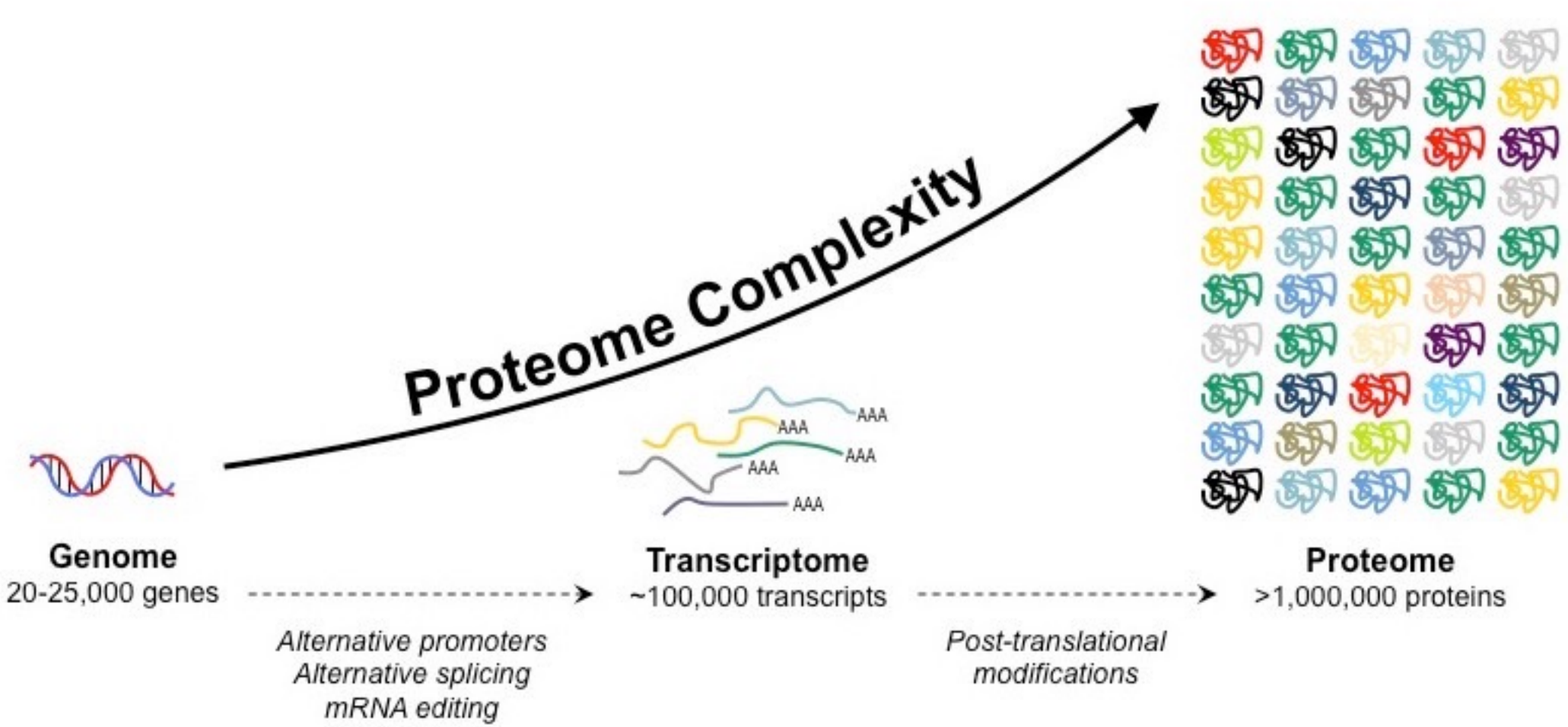


c) Tertiary structure

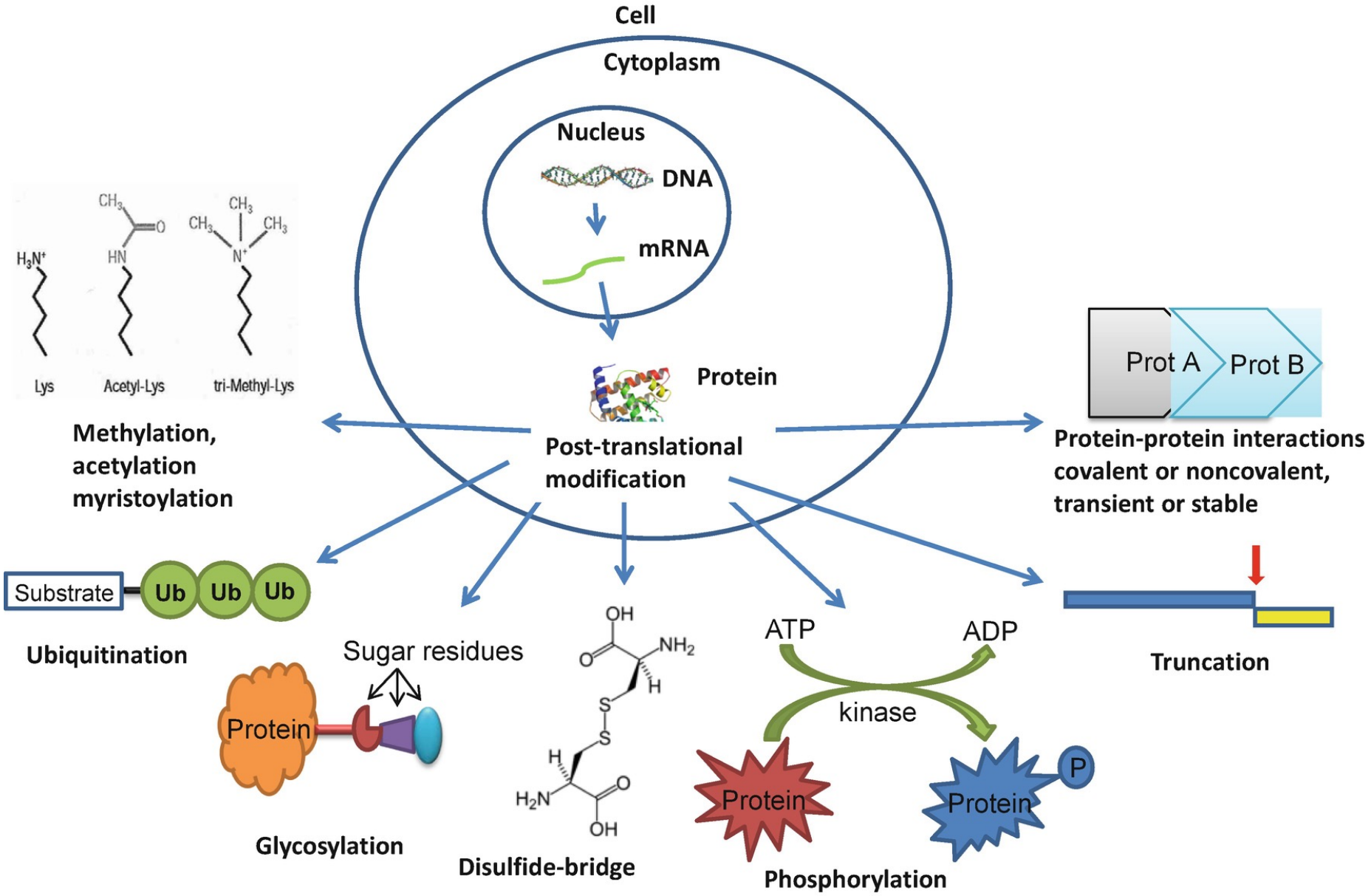


(d) Quaternary structure–

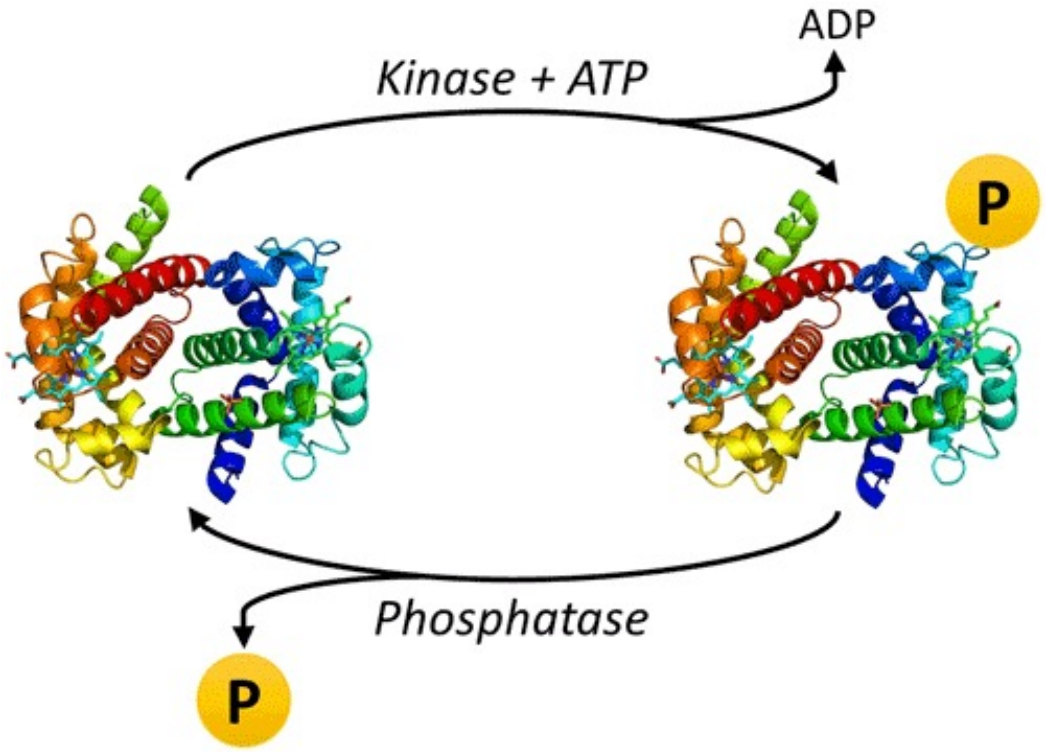
What is proteomics?

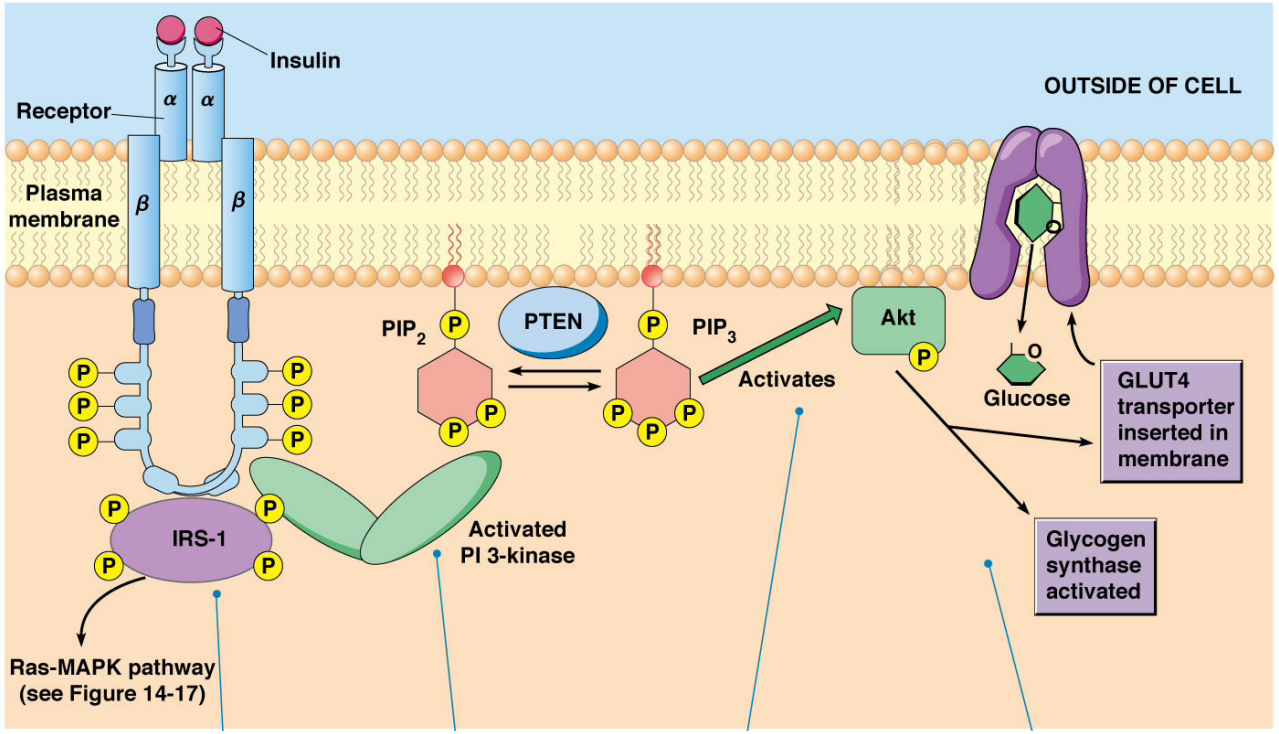


What are post translational modifications?



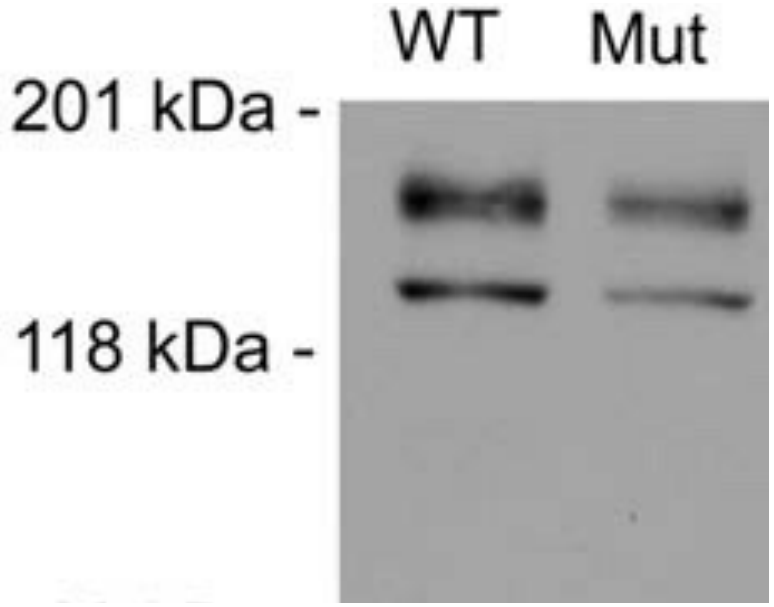
Why is phosphorylation important?

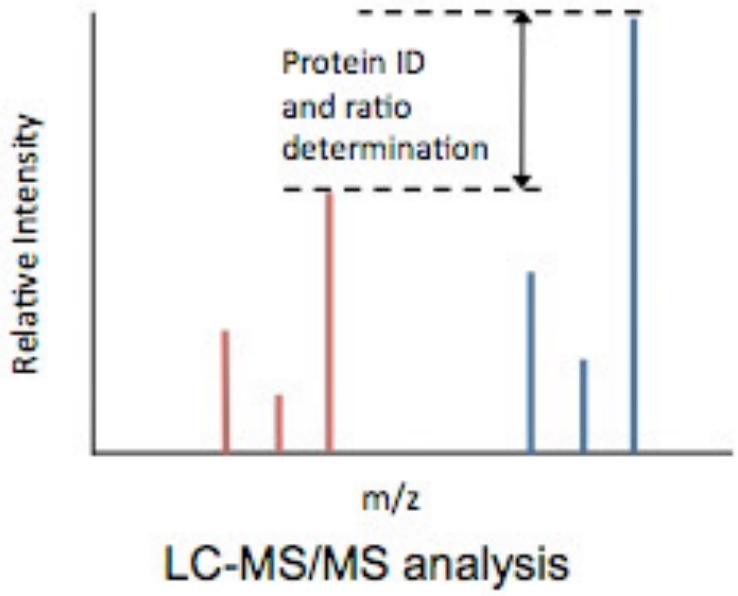




Phosphorylation regulates protein activity and sends signals throughout the cell via phosphorylation cascades

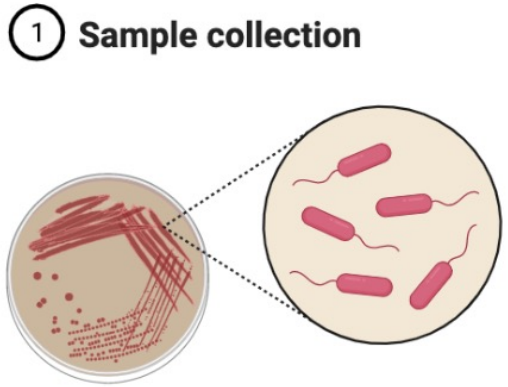
Why is it important to quantify protein levels?



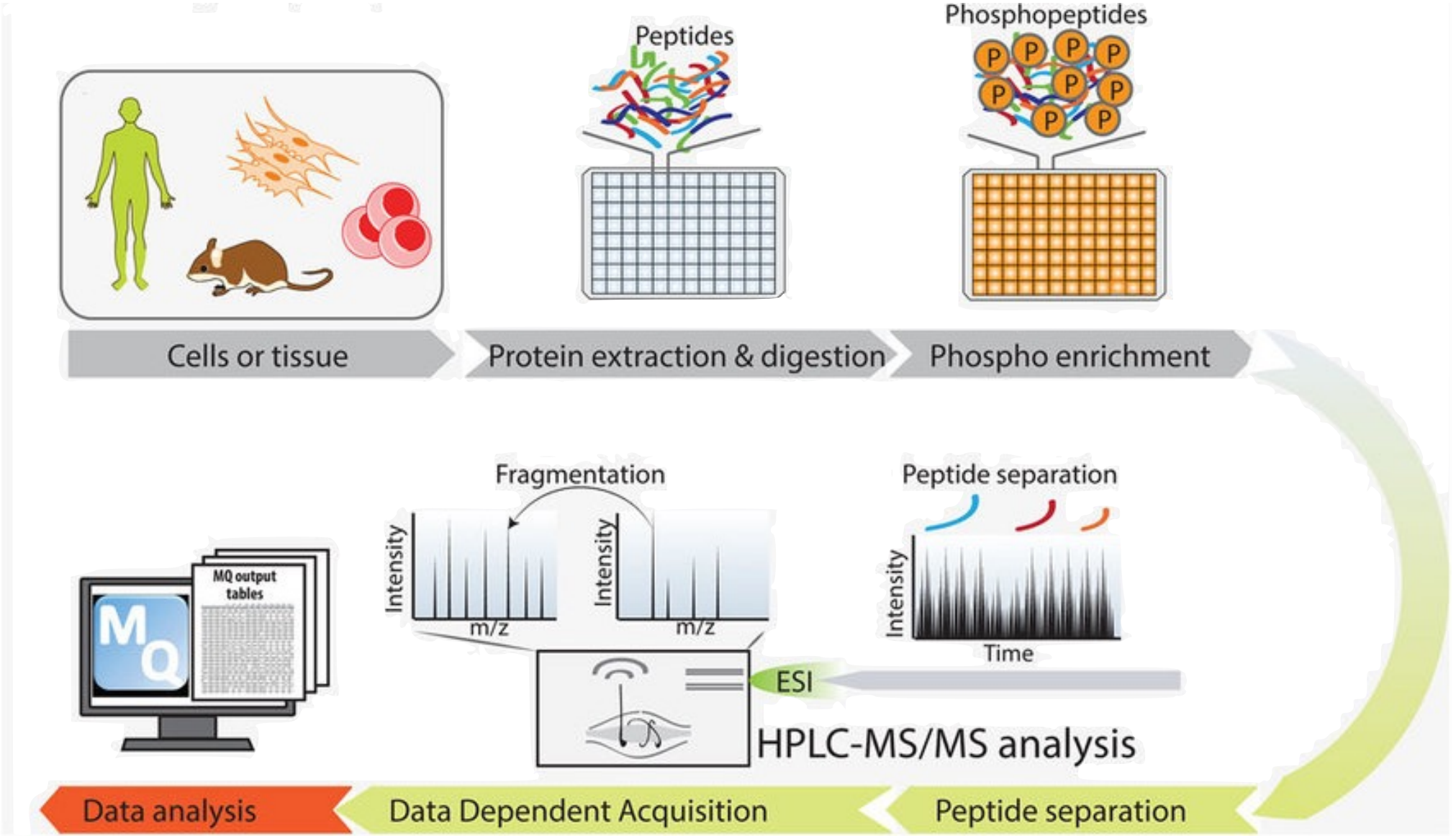


Quantifying protein levels allows us to compare protein expression and provide information about the physiological differences between two samples.

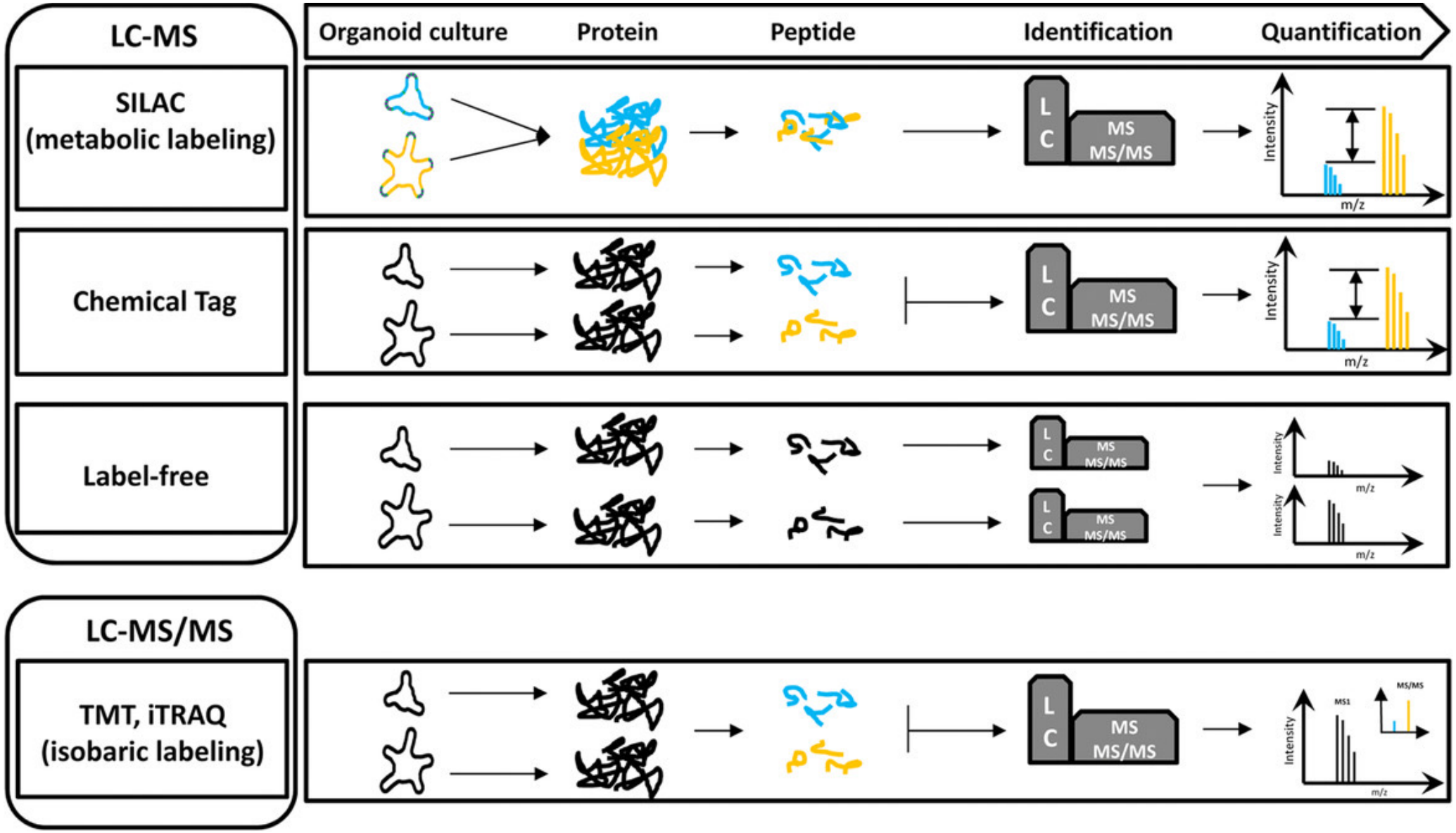
REVIEW: How does a basic proteomic analysis work?



What is phosphoproteomics?

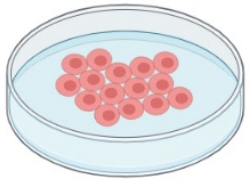


What are the different approaches to quantitative proteomics?

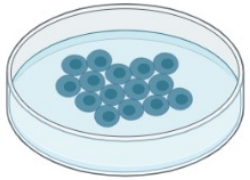


How does a typical SILAC experiment work?

Cells grown in heavy medium

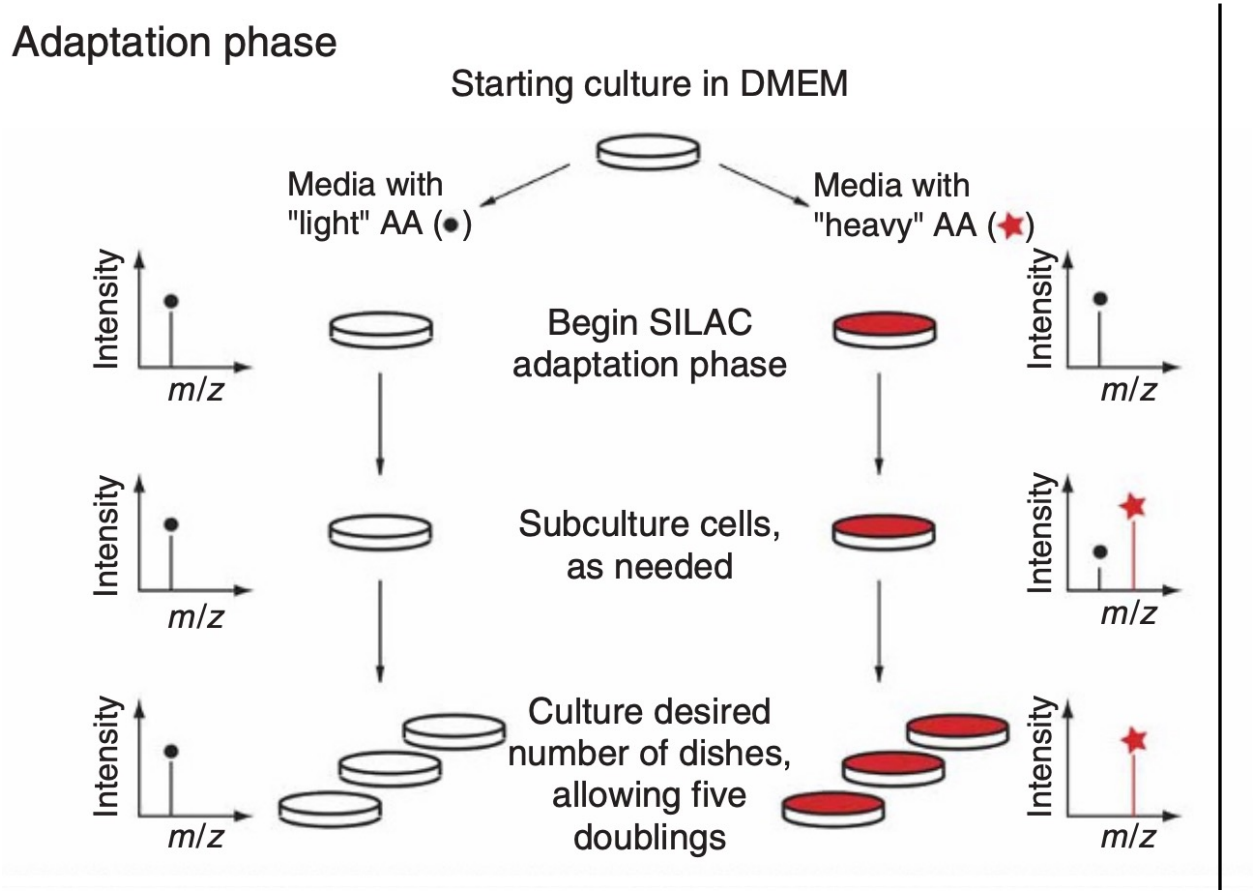


Cells grown in light medium

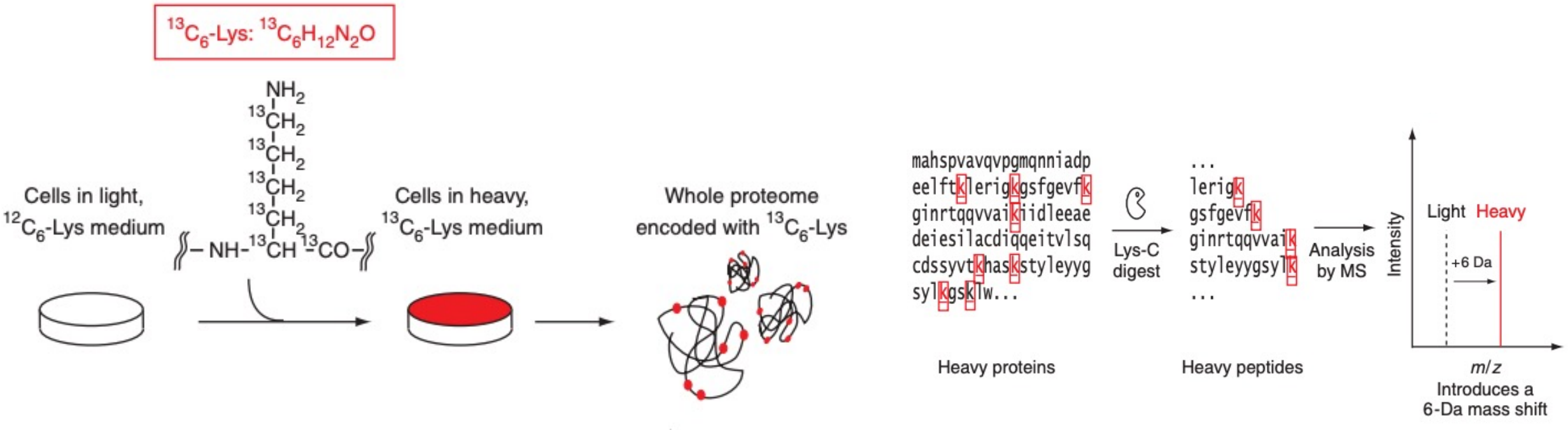


Stable Isotope Labeling by Amino acids in Cell culture

What are the two phases of SILAC?



How and what heavy isotopes incorporated?



What are the advantages and disadvantages of SILAC?

Advantages:

High quality of qualitative data

Easy to implement

Works well with existing experimental workflows

Can run multiple samples at once

Disadvantages:

Relatively expensive ($686 for a kit from Thermo Fischer)

Process takes a while (~8 days)

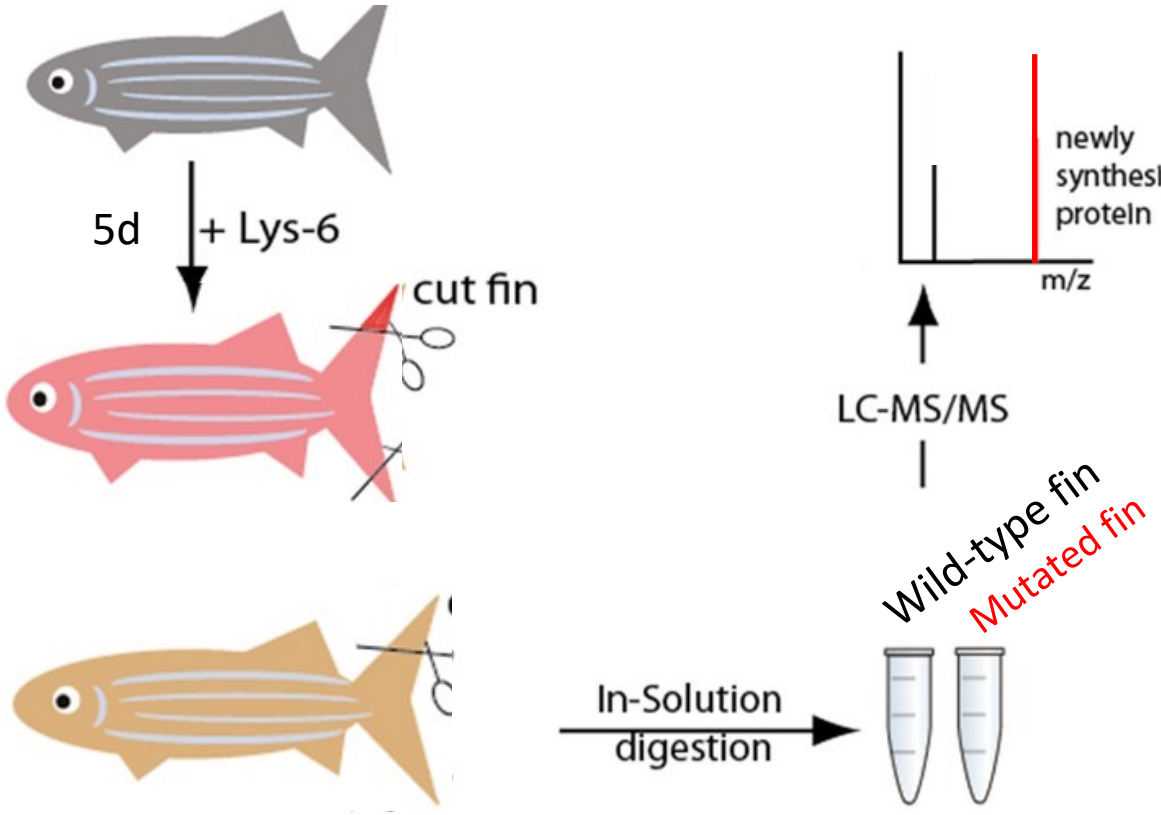
Conversion of arginine to proline must be accounted for

Cannot perform if metabolic labeling is not possible

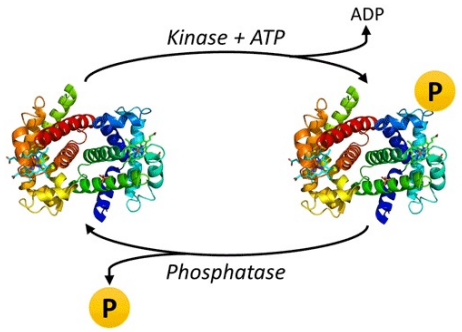
How can SILAC be applied to your AIMS?

Mutated version grown in heavy medium

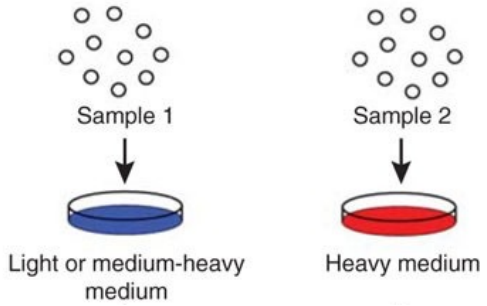
Wild-type grown in light medium



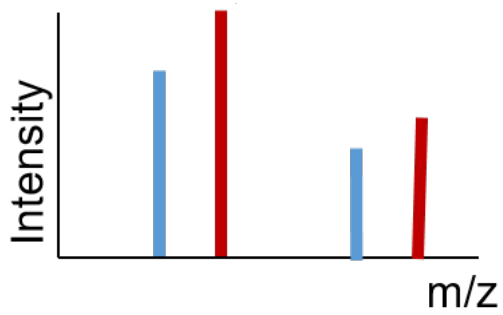
Summary



Phosphoproteomics allows us to identify and quantify proteins that are phosphorylated and determine their relative state of activity.



SILAC is used to differentiate two separate cell populations by growing one population in a light medium and another in a heavy medium.



Relative protein abundance is determined by obtaining a MS and calculating the difference in intensities between the two populations

Hetzer Lab at the Salk

Martin W. Hetzer, Ph.D.

His lab focuses on developmental and pathological changes in the organization and functions of the cell nucleus.

Bushwalter Lab at UCSF

Abigail Bushwalter, Ph.D.

Her lab focuses on the organization of the nucleus and how it is maintained over time to influence cellular identity.

References

Ong SE1, Mann M. A practical recipe for stable isotope labeling by amino acids in cell culture (SILAC). Nat Protoc. 2006;1(6):2650-60.

Gouw, Joost & Krijgsveld, Jeroen & Heck, Albert. Quantitative Proteomics by Metabolic Labeling of Model Organisms. Molecular & cellular proteomics : MCP. 2009; 10.1074/mcp.R900001-MCP200.

Chen X, Wei S, Ji Y, et al. Quantitative proteomics using SILAC: principles, applications, and developments. Proteomics, 2015; 15(18): 3175-3192.

Questions?