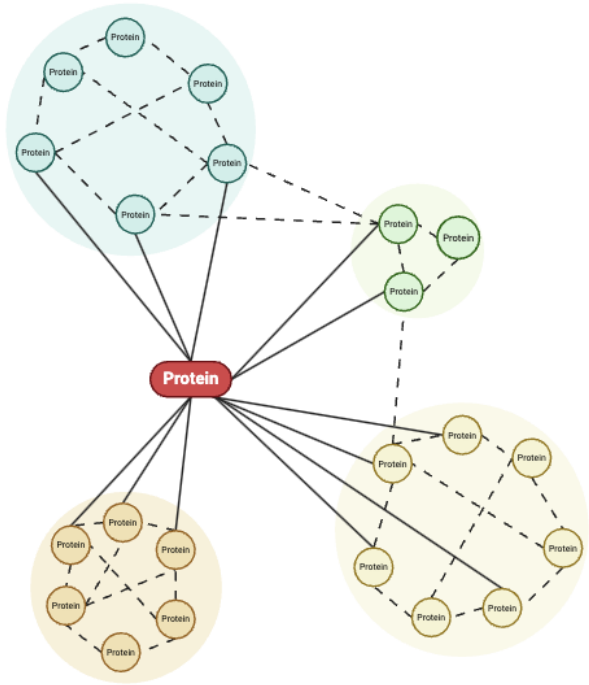
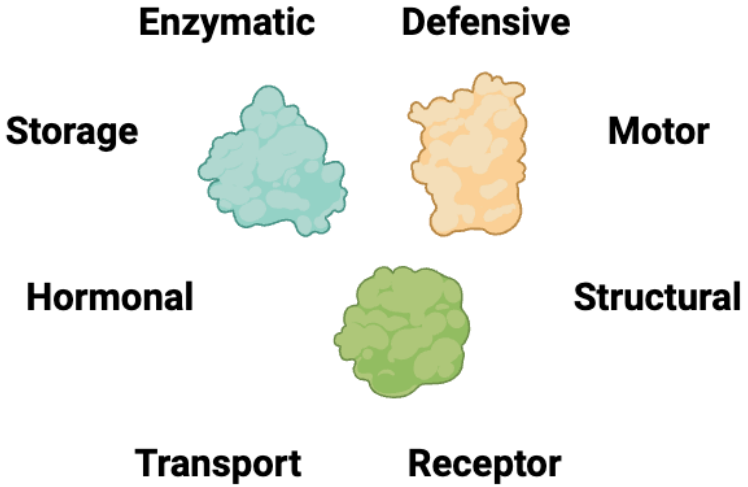
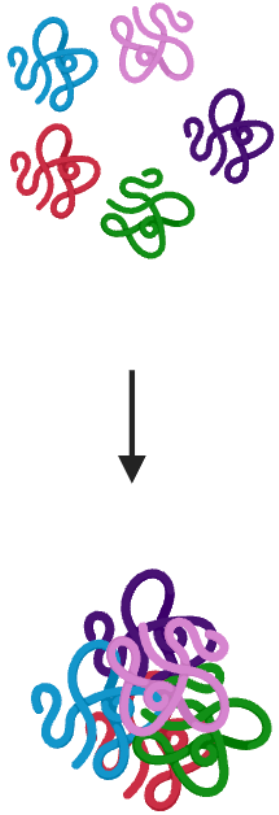
SILAC for Quantitative Proteomic Analysis

Madeline Blum and Samuel Kivi 4/4/2024

Proteomics: the study of all proteins, their interactions, and activities







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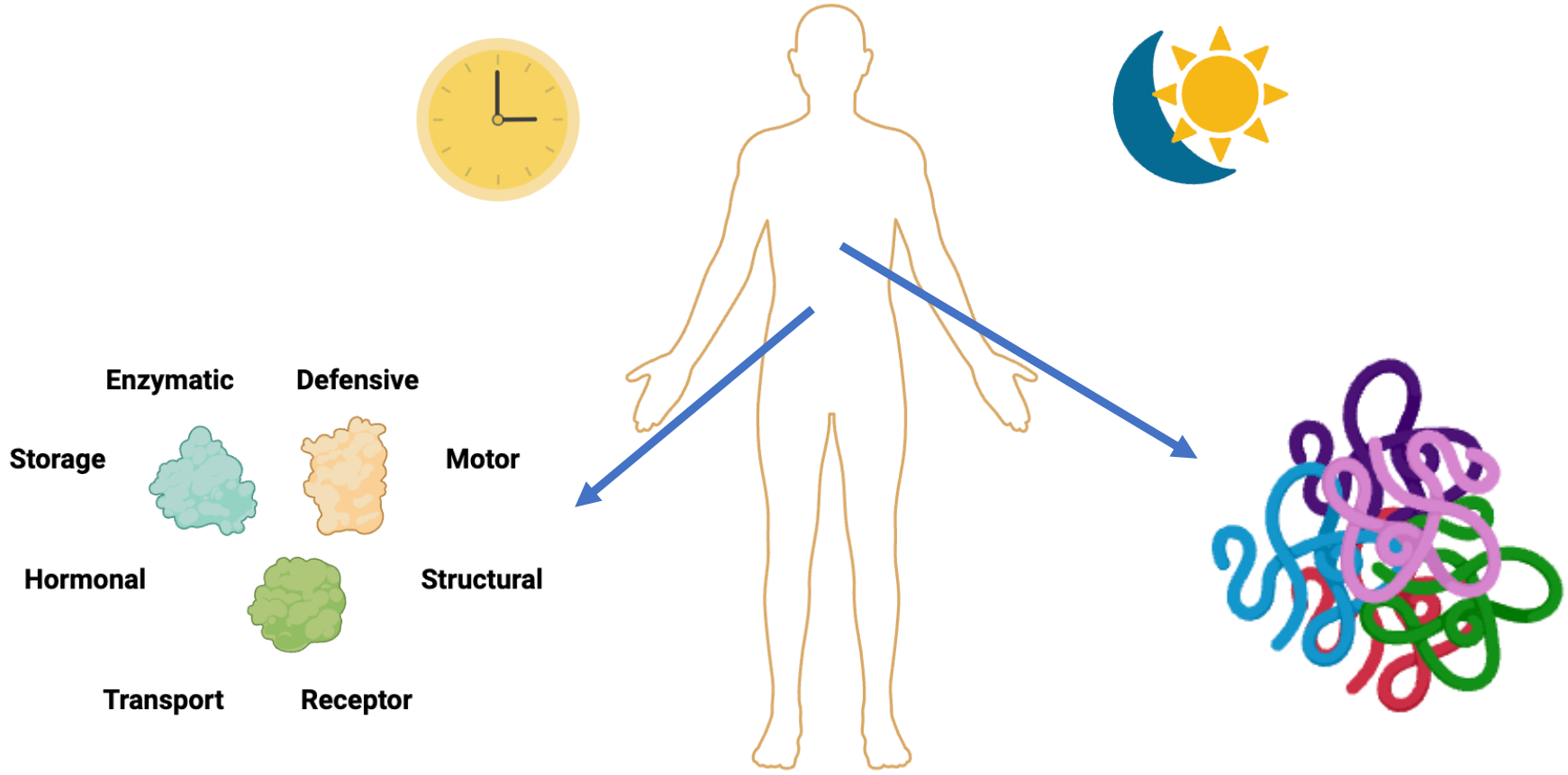
Proteomics considers the state of the system



This allows accurate reflection of structure and function

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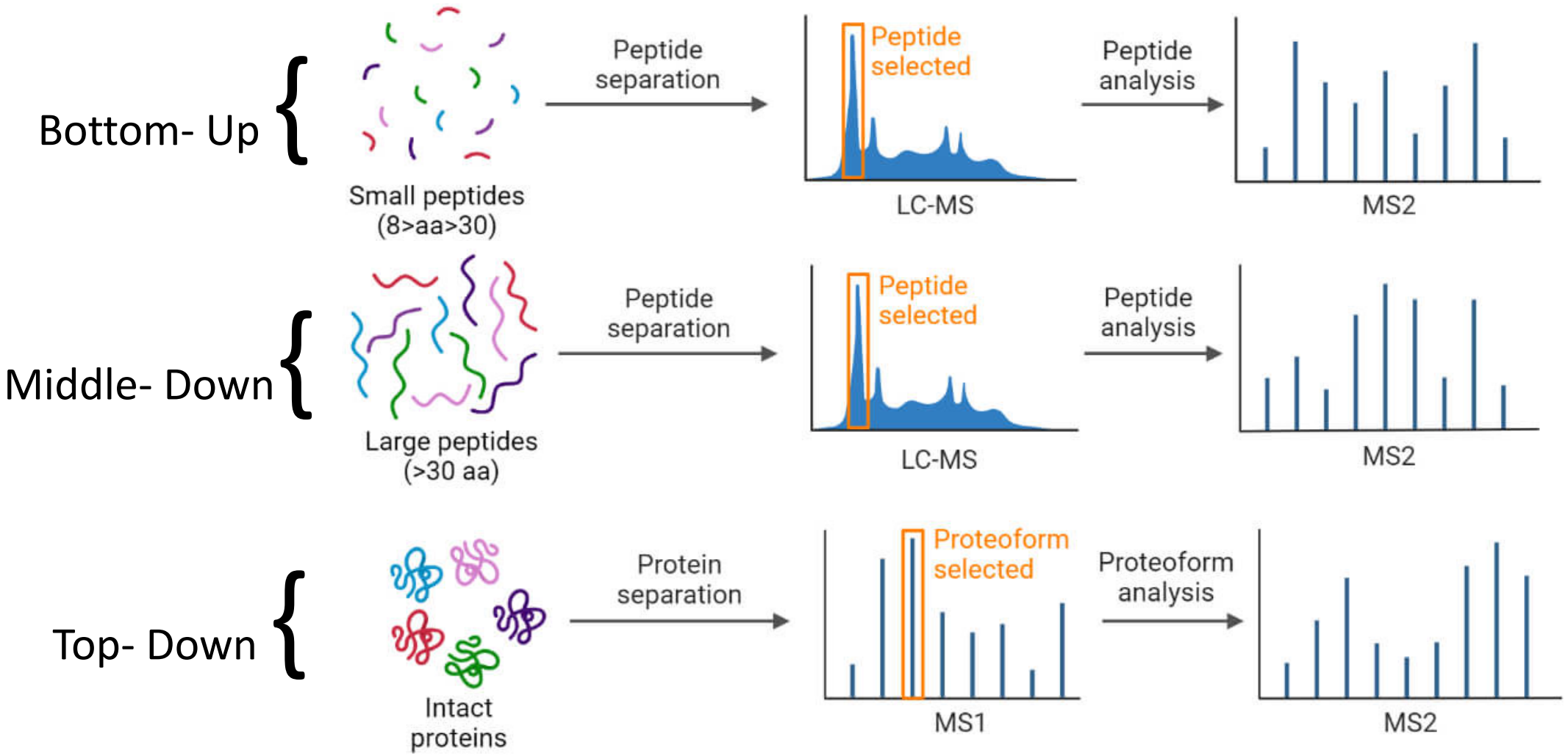
Proteomics assays can achieve multiple goals

1 Purification

- Chromatography-based techniques

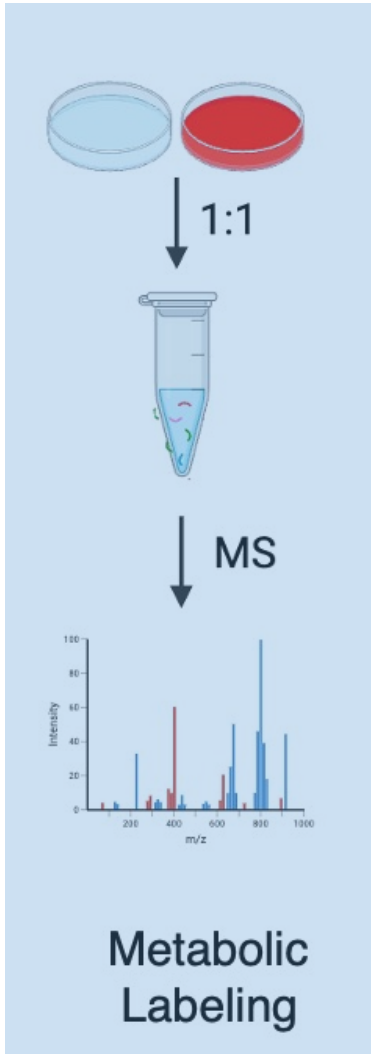
May be best to combine, depending on what your research question is.

Proteomics has three overarching approaches



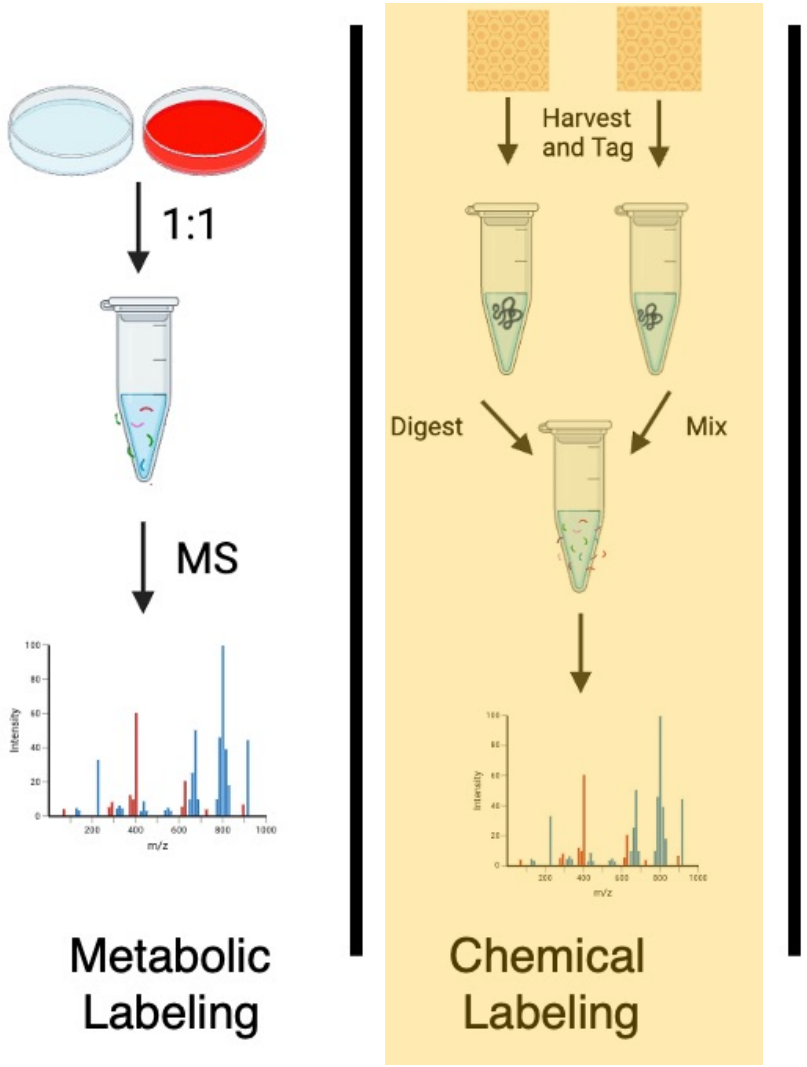
Bottom- up proteomics starts with proteolysis, and it works to analyze peptides. Top- down focuses on MS of full proteins, and this is used for PTM analysis

Quantitative approaches



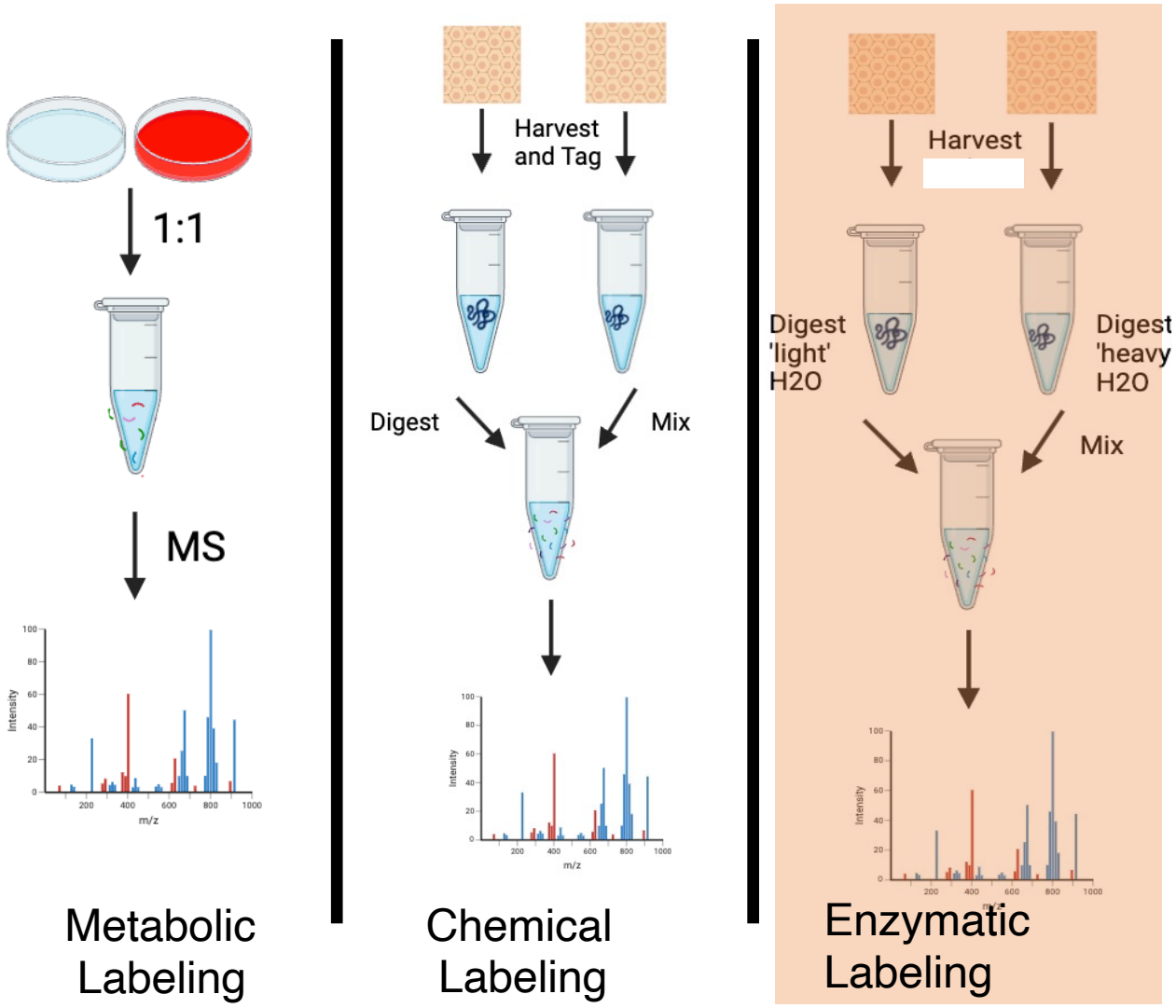
Use Metabolic Labeling by introducing different isotopes by mediums

Quantitative approaches



Chemical Labeling by introducing isotope tags in vitro

Quantitative approaches



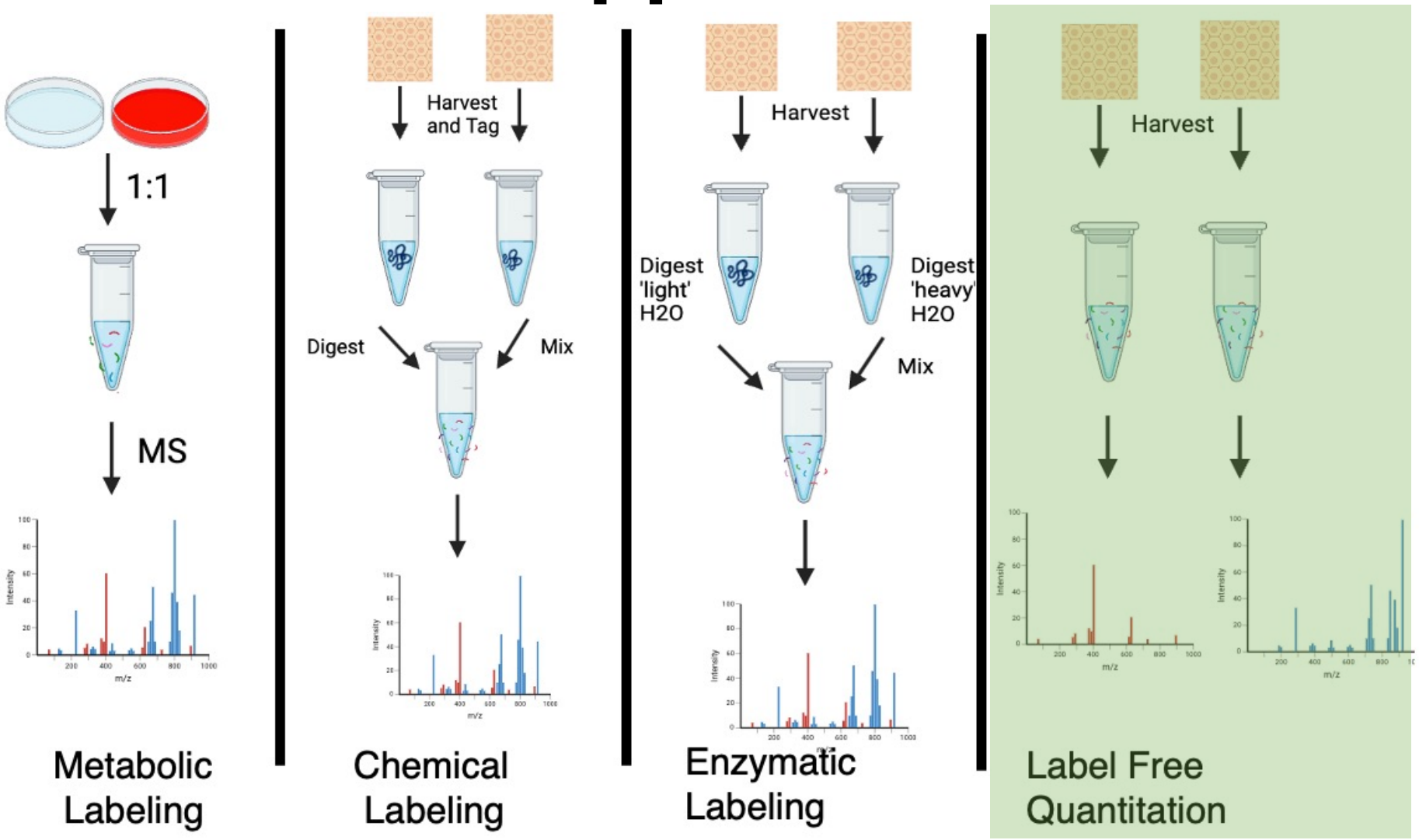
Metabolic Labeling

Chemical Labeling

Enzymatic Labeling

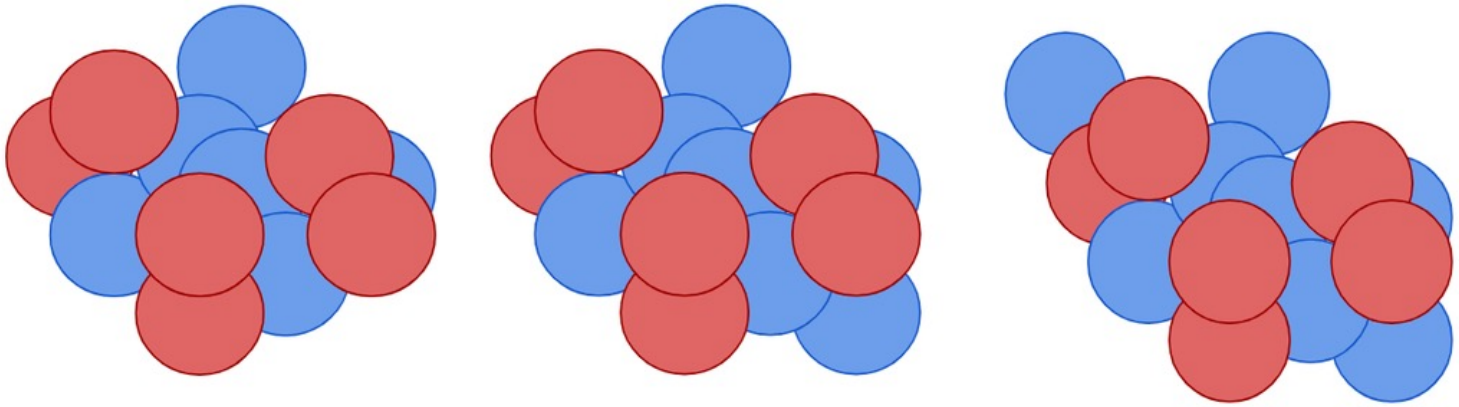
Enzymatic Labeling by proteolytic catalysis

Quantitative approaches



Label free strategies compares samples independent in MS analysis.

Isotopes have the **same charge** despite **different masses**

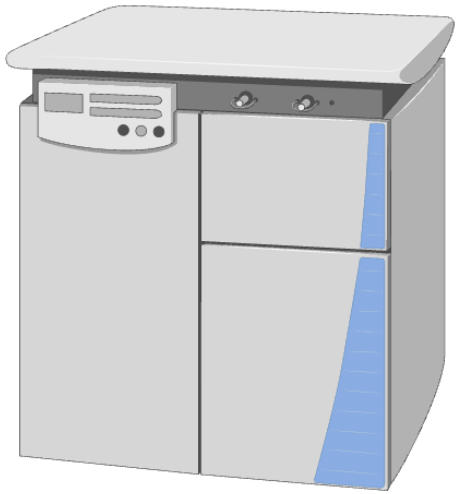


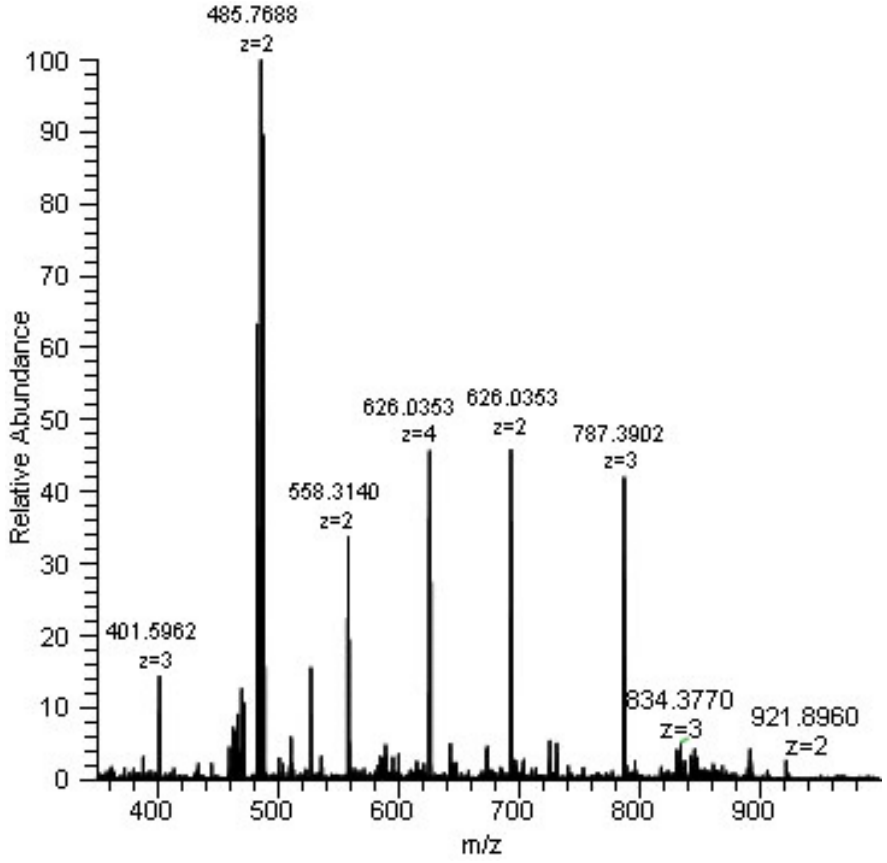
Carbon-12 Nucleus

Carbon-13 Nucleus

Carbon-14 Nucleus

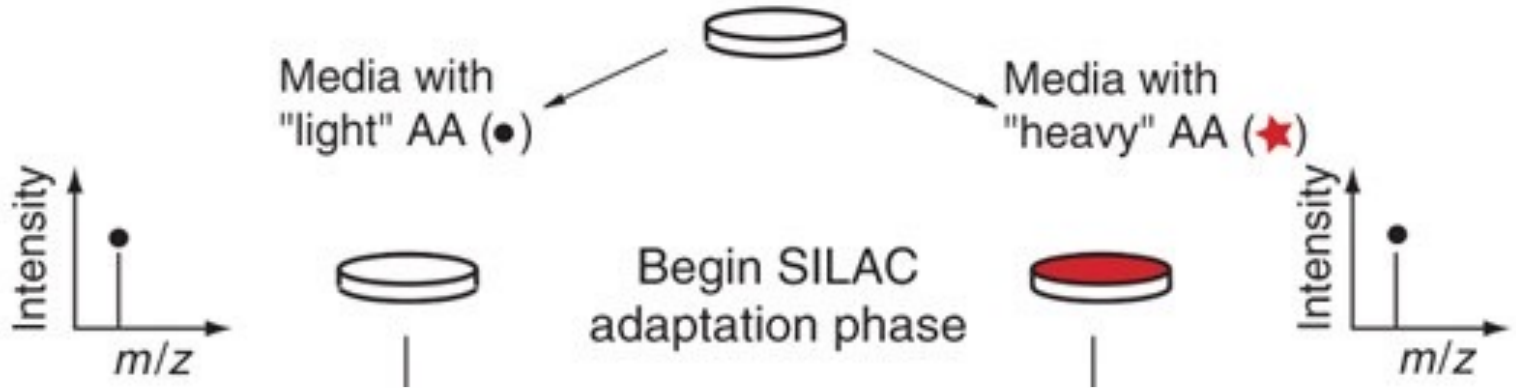
Mass Spectrometry represents the relative number of ions corresponding to a mass- to- charge ratio





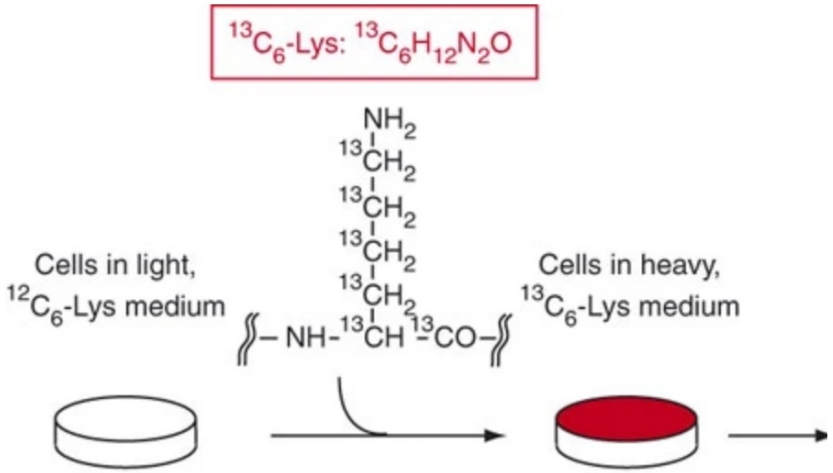
This ratio directly correlates to molecular weight.

Phase one of simple SILAC



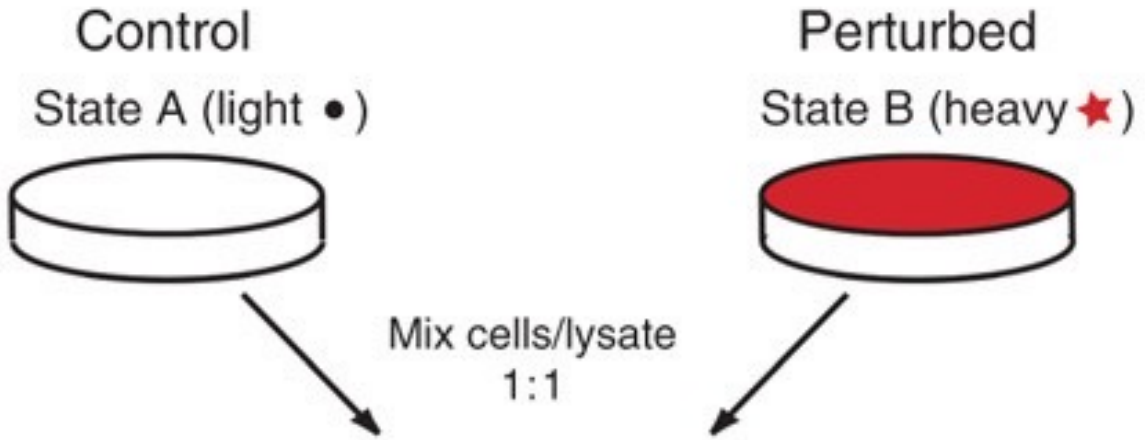
Two cultures are grown in mediums with light or heavy amino acids to allow for MS to discern the difference

Isotopes incorporation



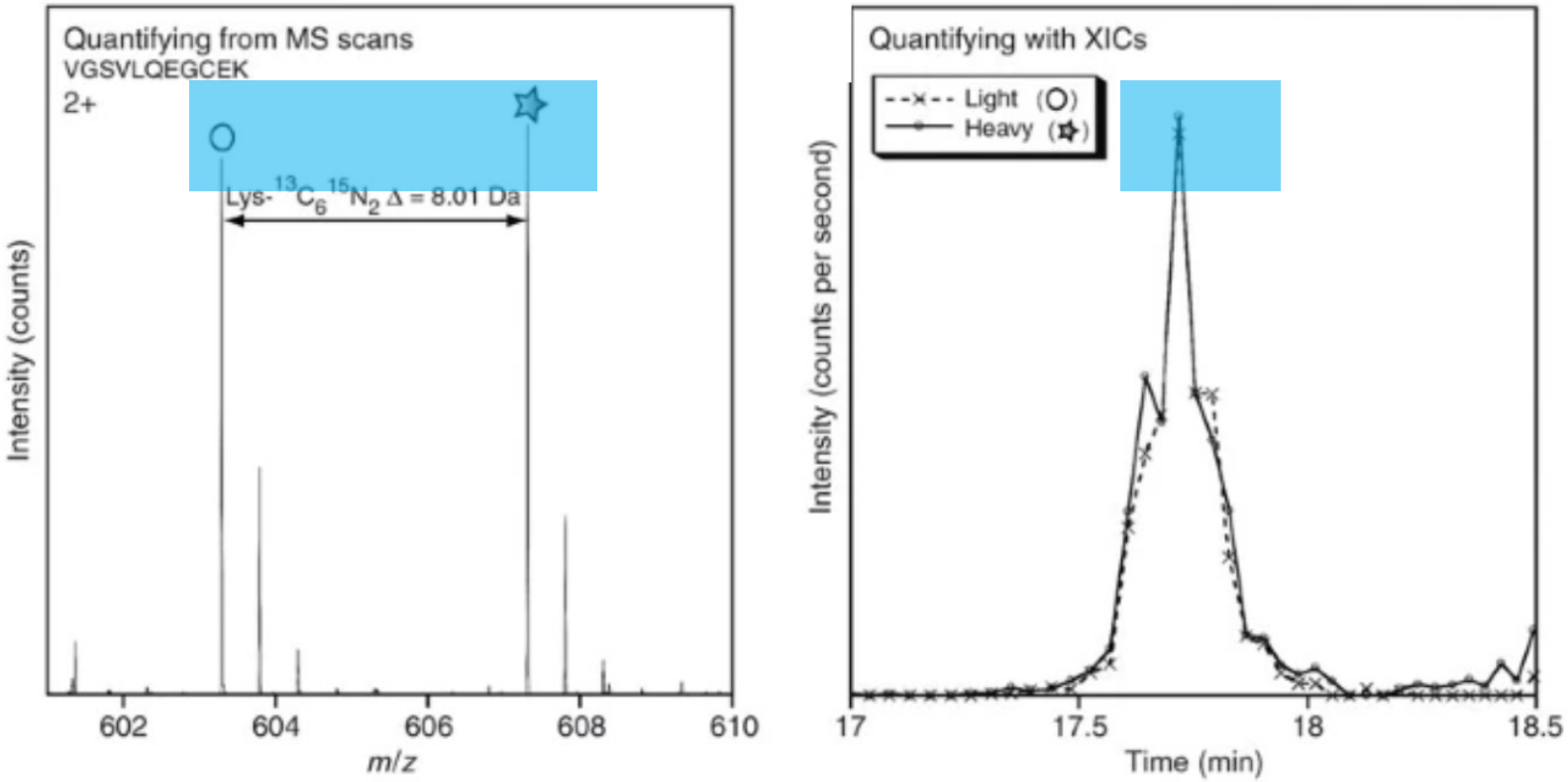
It would be wise to choose an amino acid essential to culture survival as well as have a large enough mass differential between control and experimental mediums

Phase two of simple SILAC



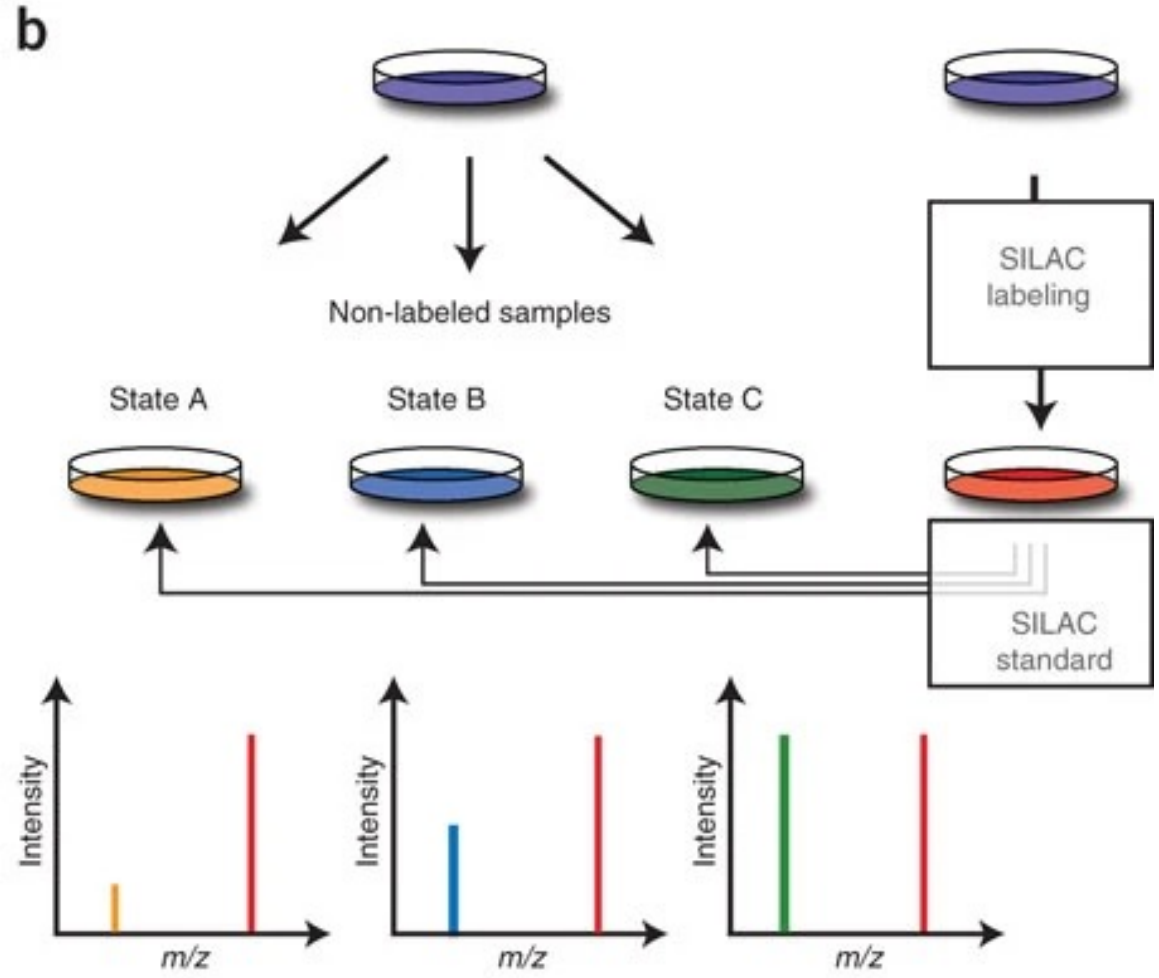
Perturbing in the heavy medium allows analysis of proteome in environment of your choice

MS Scans and XICs for analysis



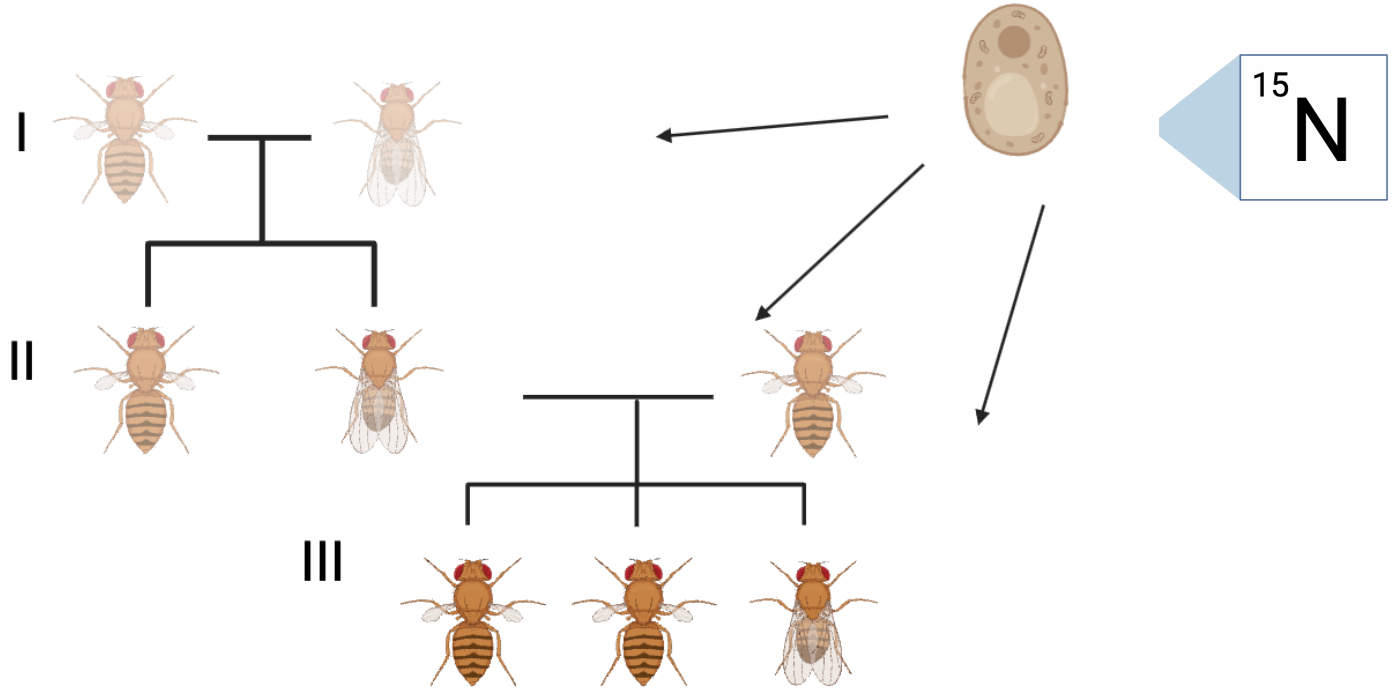
Mass difference between heavy and light peptides corresponds with respective amino acid isotopes.

Spike- in SILAC



“Spike” the experimental samples with a separate, SILAC labeled sample

Use of model organisms



The entire proteome of a model organism must be entirely turned over to be used as SILAC standard. This can be achieved within a couple of generations.

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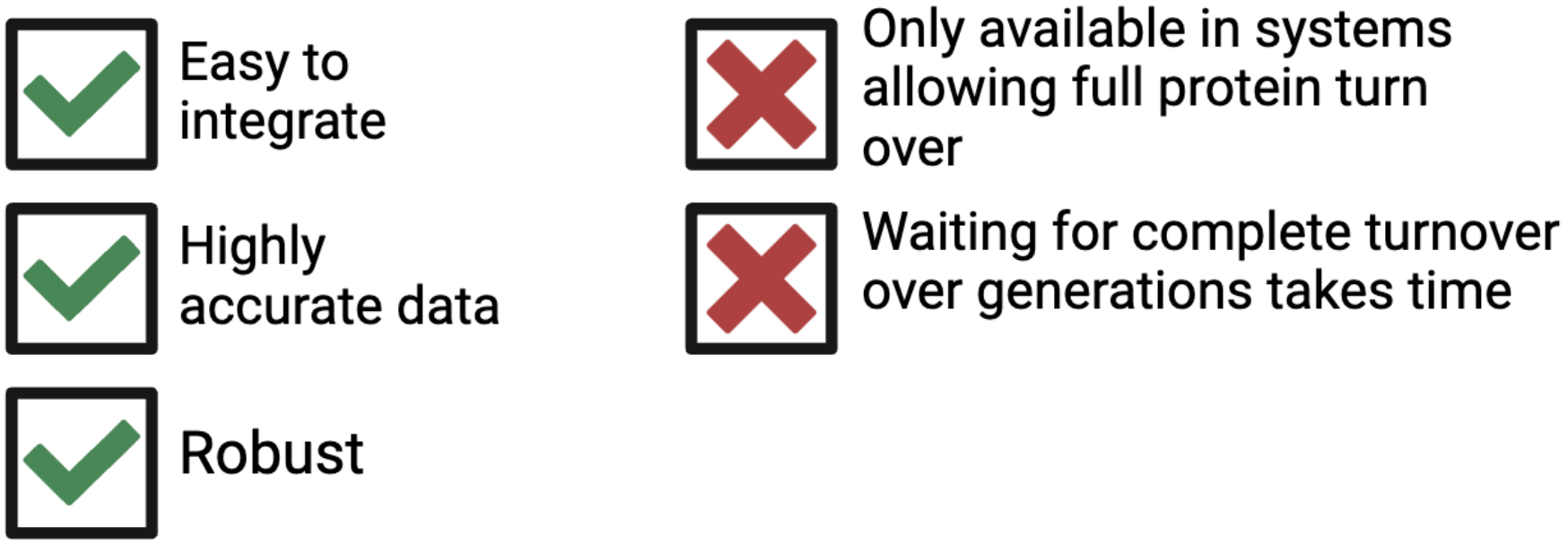
Advantages and disadvantages of SILAC



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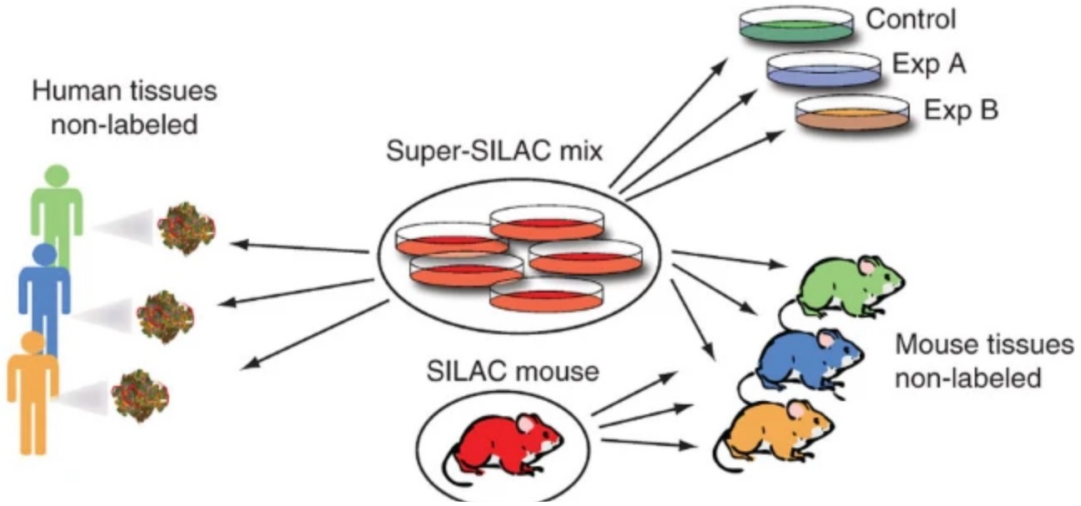
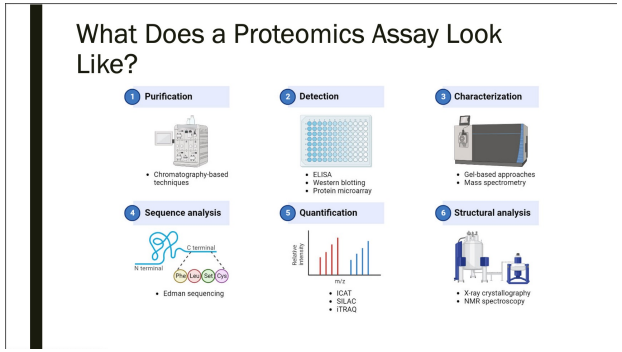
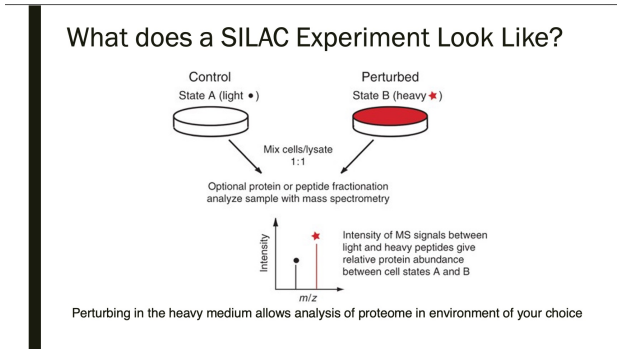
How can YOU apply SILAC to YOUR Aims?

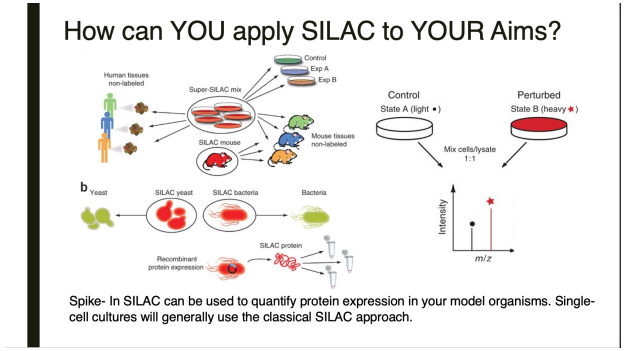


Spike- In SILAC can be used to quantify protein expression in your model organisms. Single-cell cultures will generally use the classical SILAC approach.

Summary







- Today, we reviewed what proteomics is and how we can assay the proteome
- We focused on the SILAC labeling method and how it uses isotopes to label protein interactions. Possible experimental design with this methodology was explored.
- We explored SILAC and its applications for your research projects, considering the possibilities of examining individual cultures and up to more complex systems like organisms

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Proteomics
Proteomics and Systems Biology

RESEARCH ARTICLE

Proteomic profiling of metformin effects in 3T3-L1 adipocytes by SILAC-based quantification

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Scientist profiles

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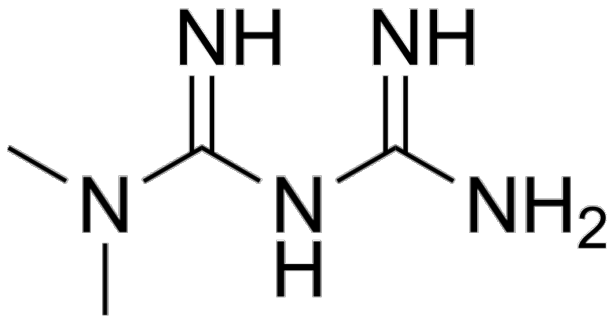
Postdoctoral Associate

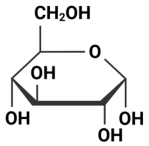
Rudolf Engelke
Instrumentation Supervisor

Neha Goswami
Lab Supervisor

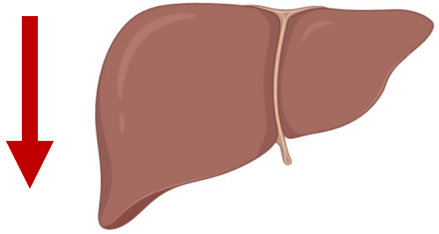
Dr. Frank Schmidt
Director of Proteomics Core

What is Metformin?

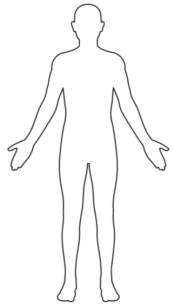




Glucose production



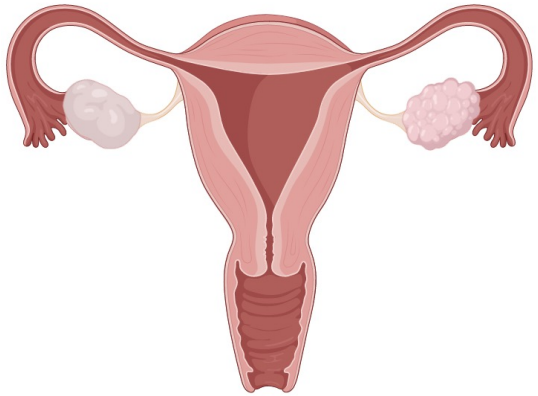
Insulin sensitivity



Metformin uses

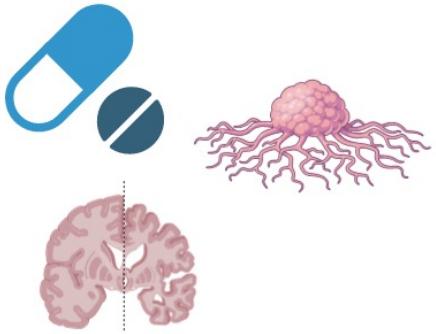
Type II Diabetes

Polycystic ovarian syndrome

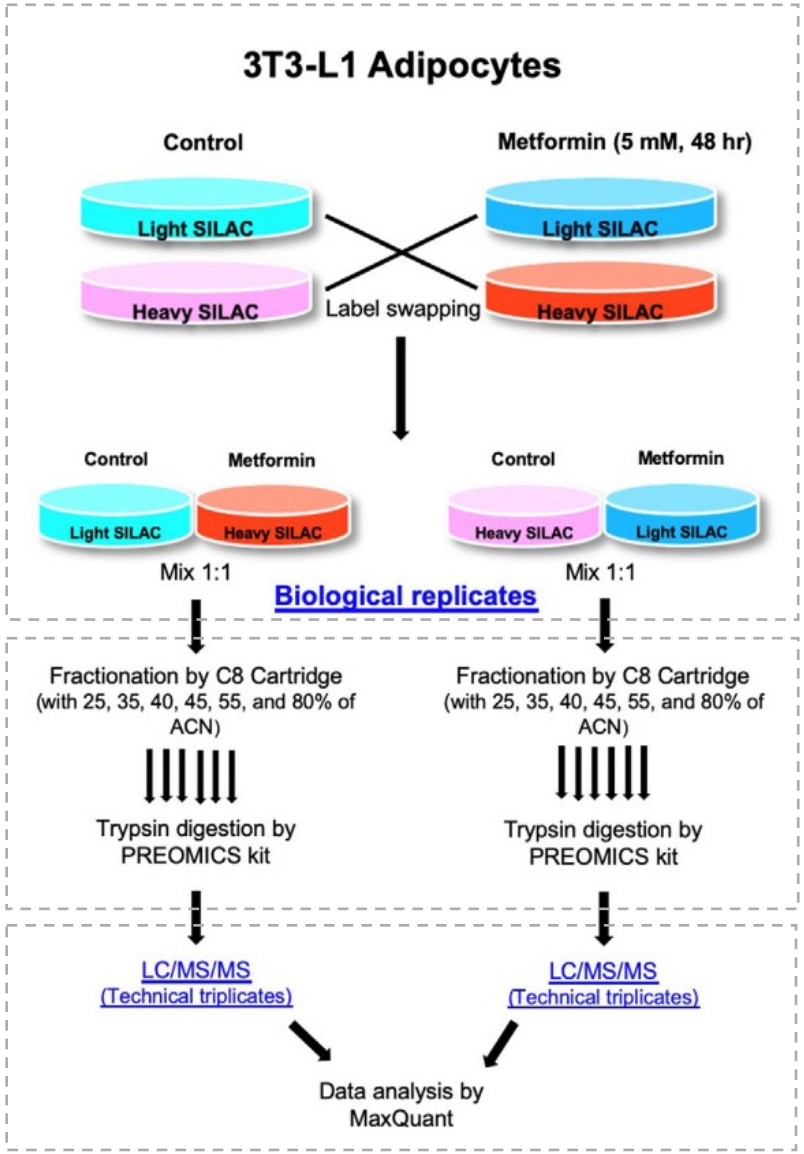


Weight Loss

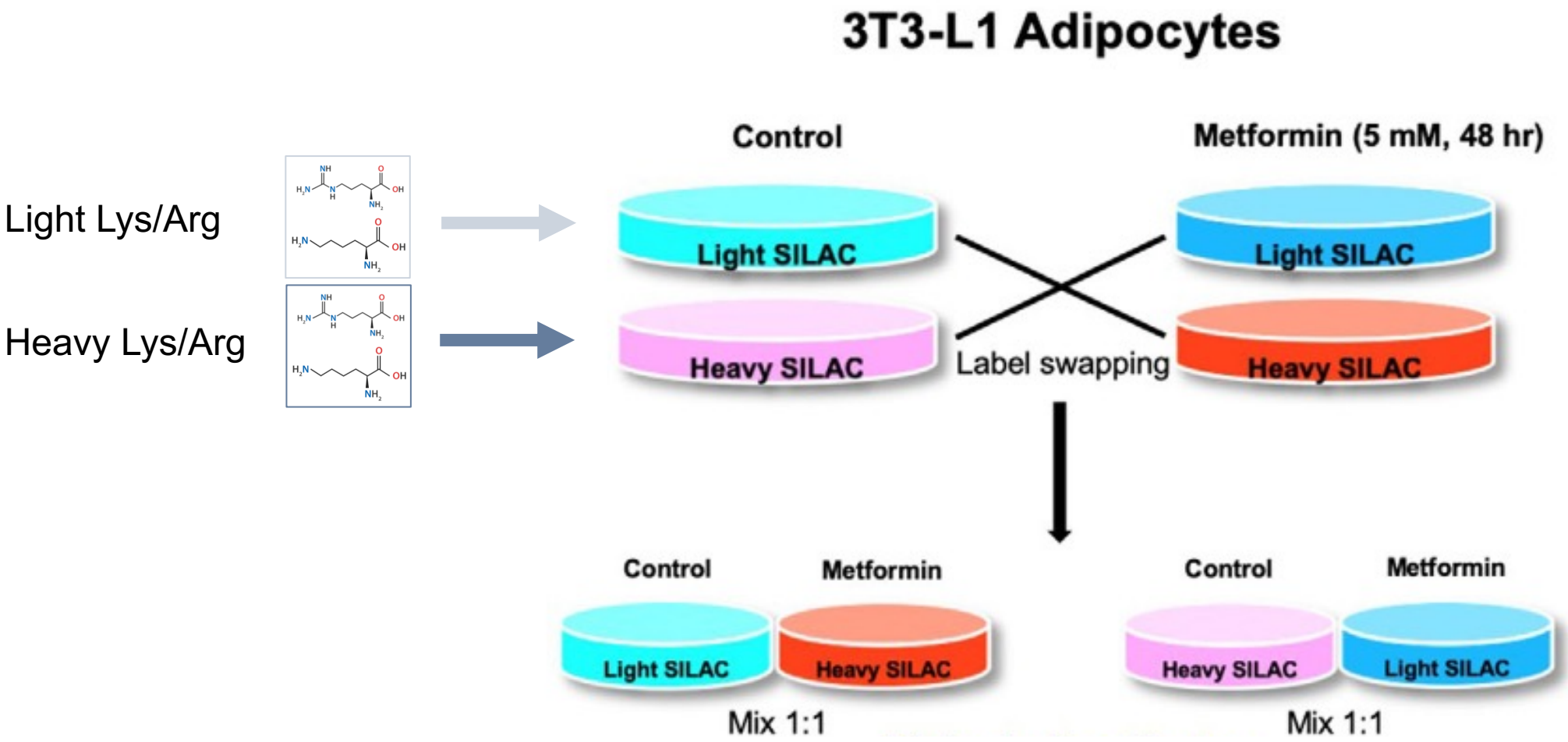
Other Possibilities



How did the authors profile metformin-treated 3T3-LI adipocytes?



Adipocyte labeling and culutring

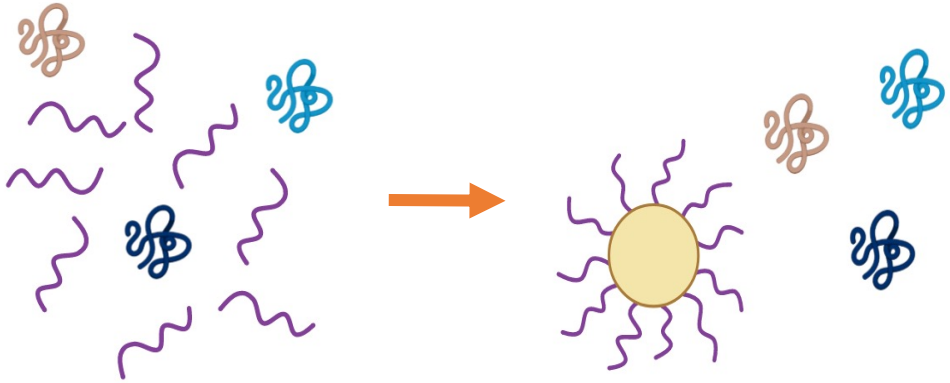


Protein fractionation and digestion

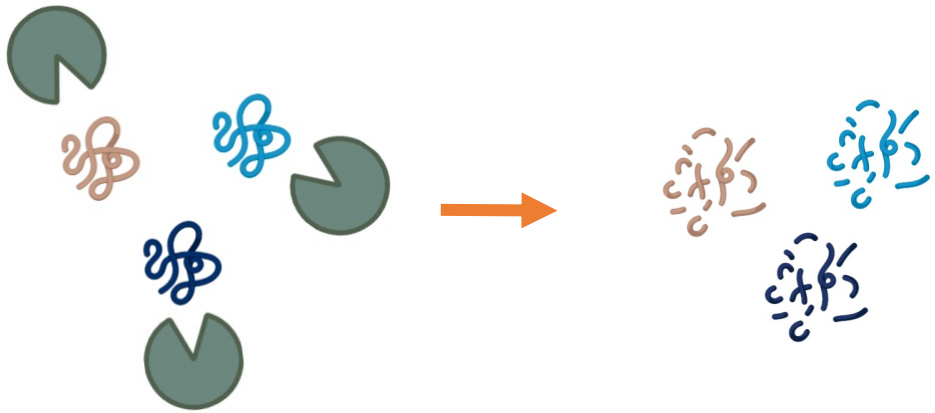
Fractionation by C8 Cartridge
(with 25, 35, 40, 45, 55, and 80% of ACN)

Trypsin digestion by PREOMICS kit

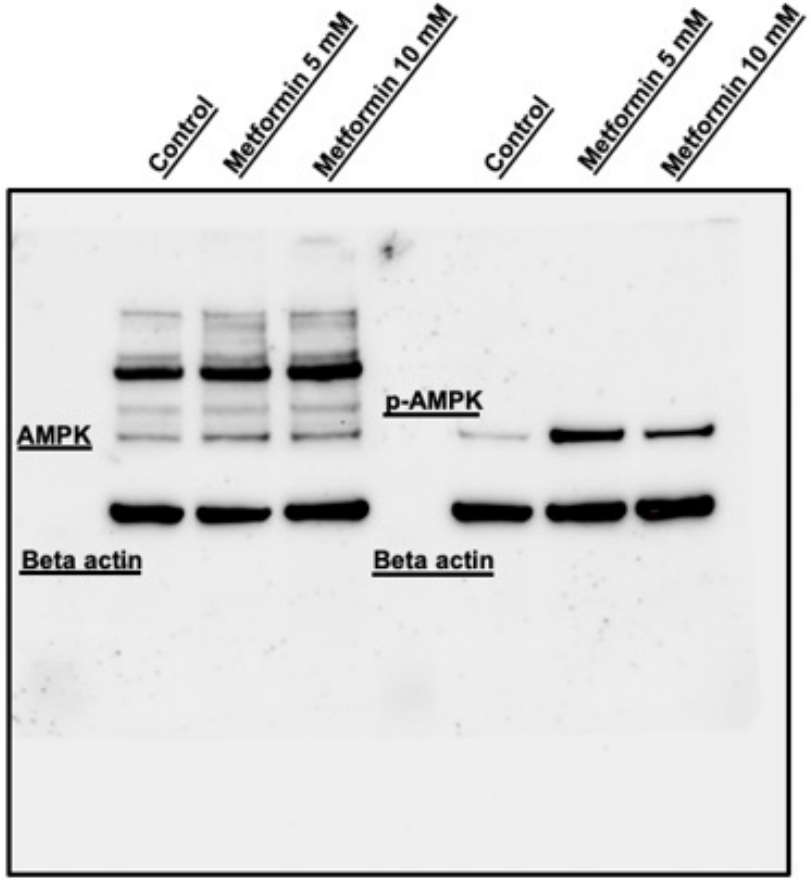
Fractionation



Digestion



Western blot confirming AMPK phosphorylation



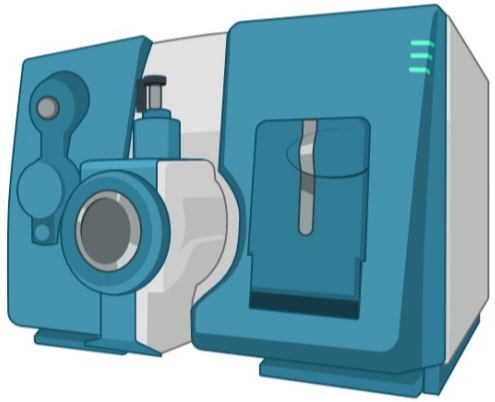
Western blotting

LC/MS/MS and analysis

LC/MS/MS
(Technical triplicates)

LC/MS/MS
(Technical triplicates)

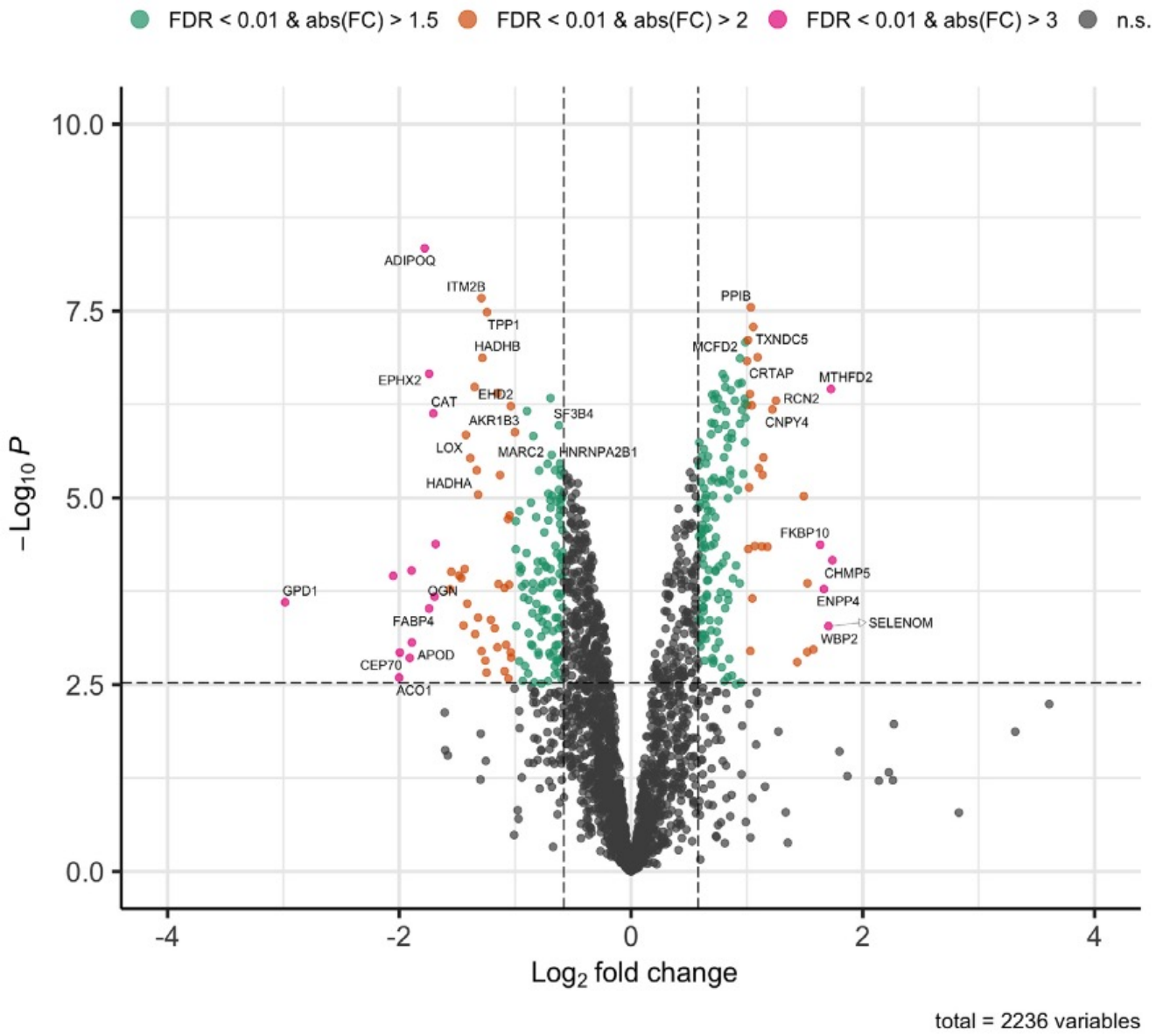
Data analysis by
MaxQuant



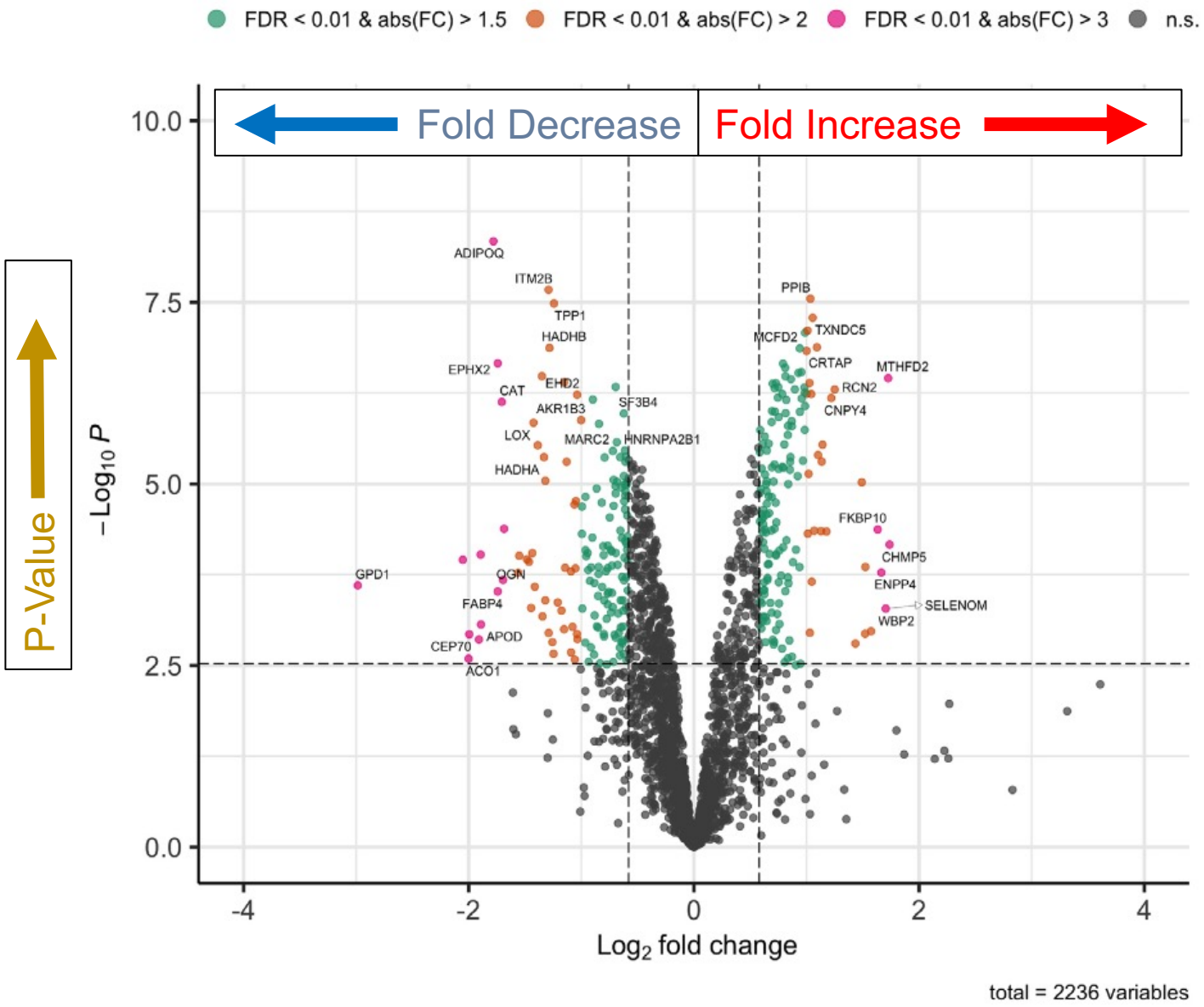
Liquid chromatography–tandem mass spectrometry

MaxQuant

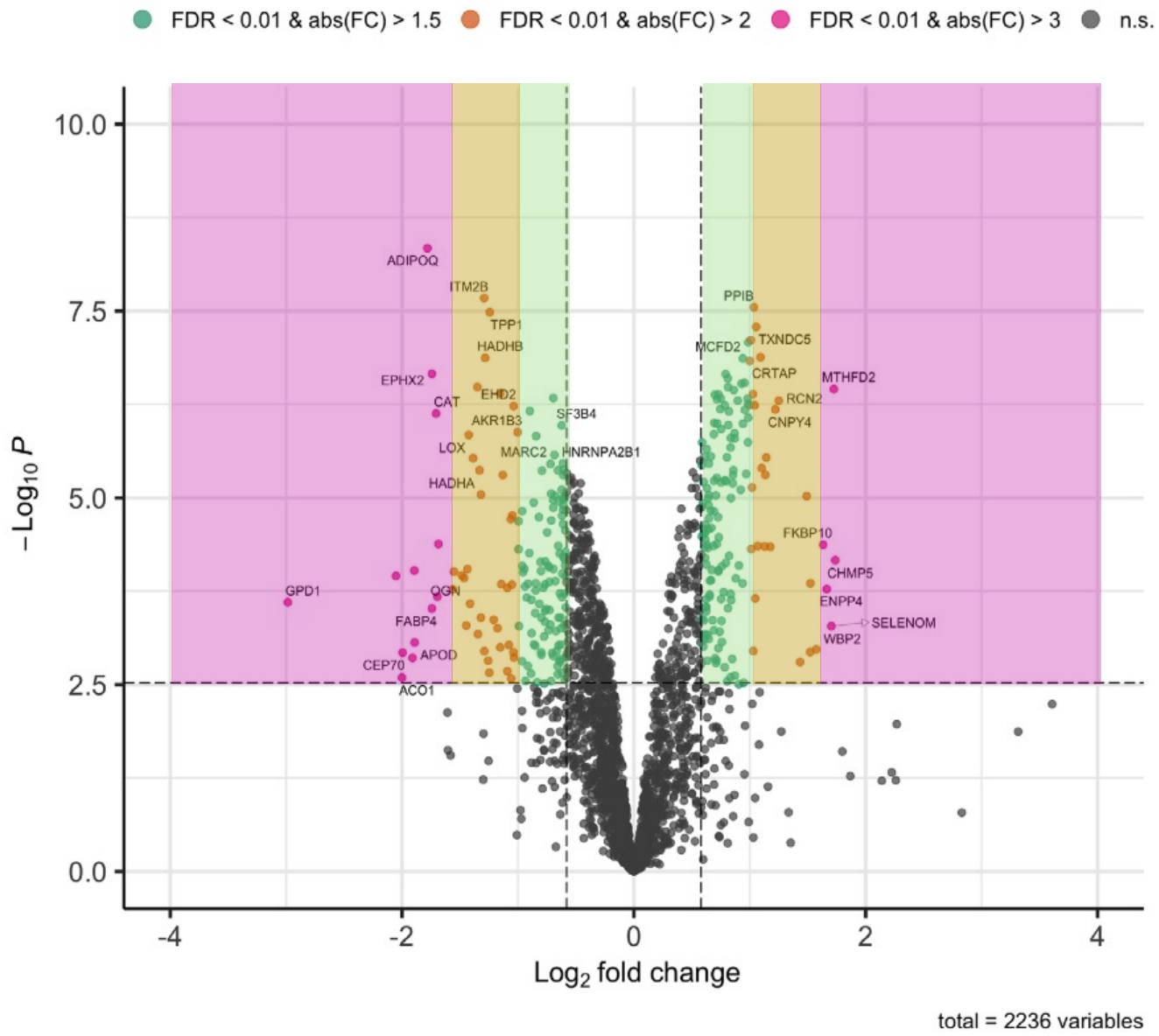
Volcano plots reveal proteins enriched after Metformin treatment



Volcano plots reveal proteins enriched after Metformin treatment



Volcano plots reveal proteins enriched after Metformin treatment

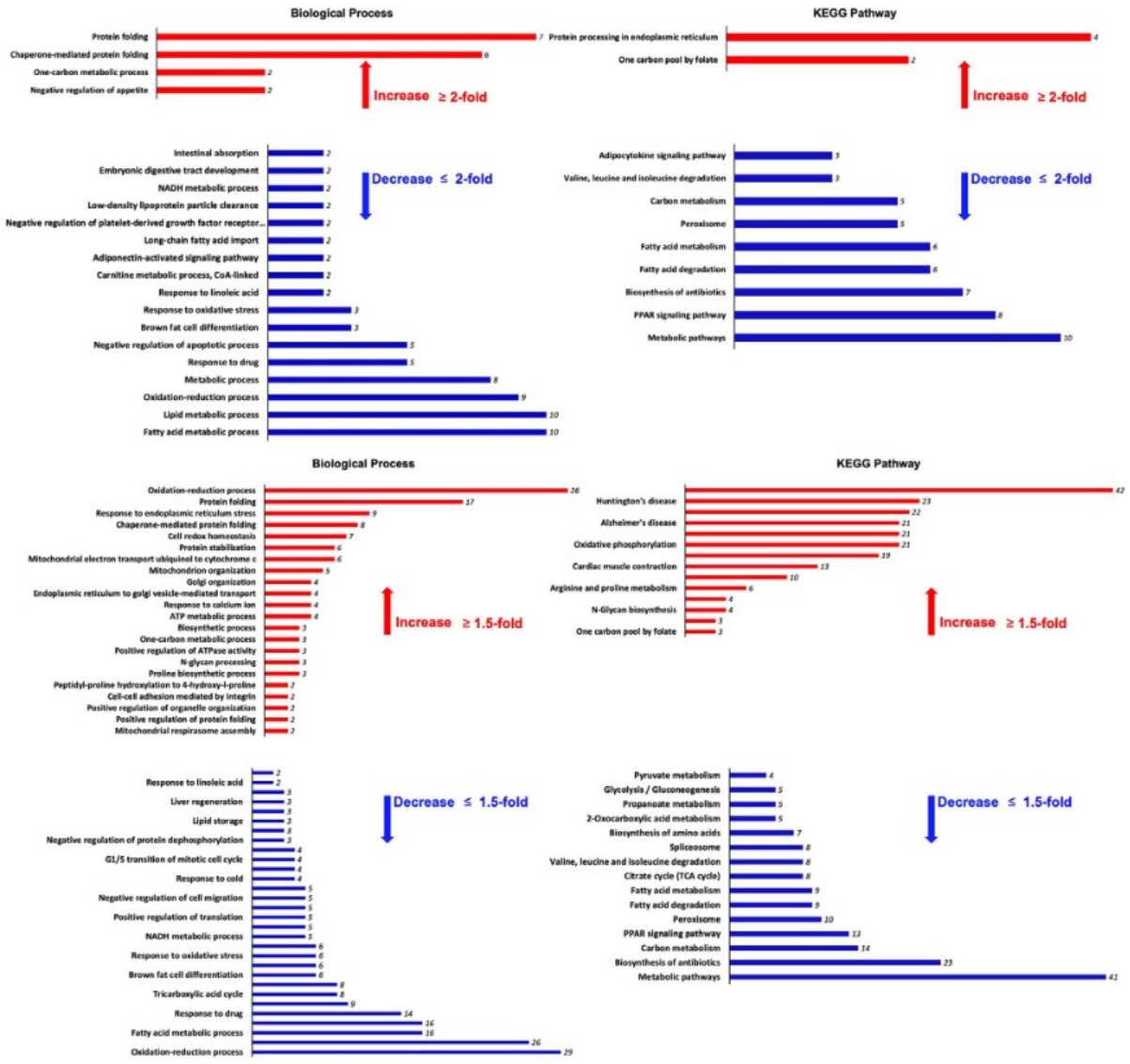


Fold Change > 1.5

Fold Change > 2

Fold Change > 3

Which types of proteins are increased or decreased after Metformin treatment?



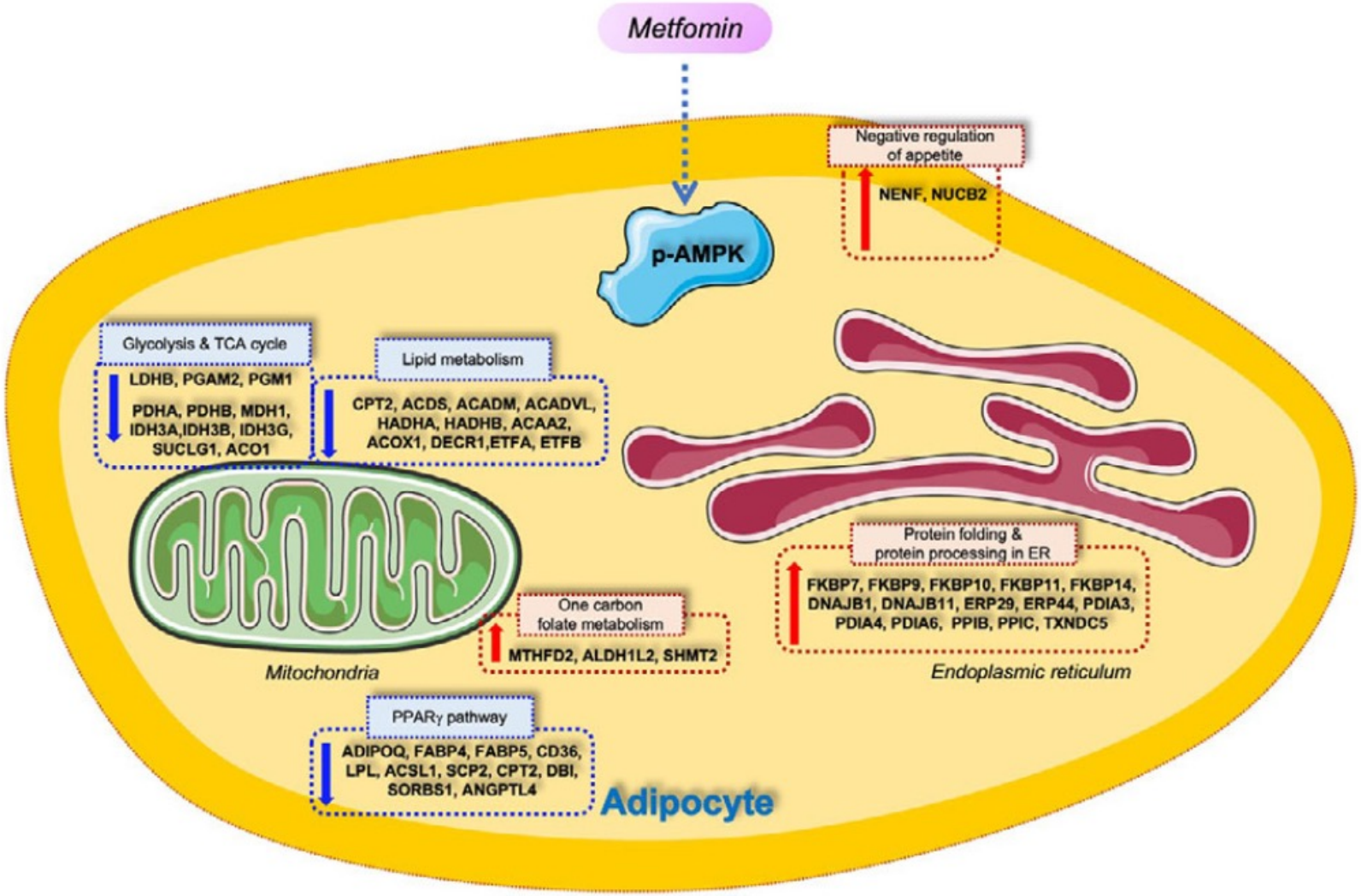
Increased protein levels

- Negative regulation of appetite
- One carbon folate metabolism
- Protein folding and protein processing in ER

Decreased protein levels

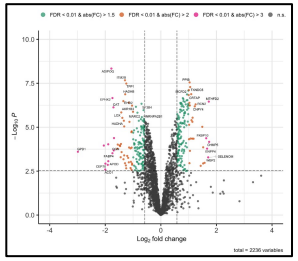
- Glycolysis/TCA cycle
- Lipid metabolism
- PPAR gamma pathway

Biological processes altered by Metformin

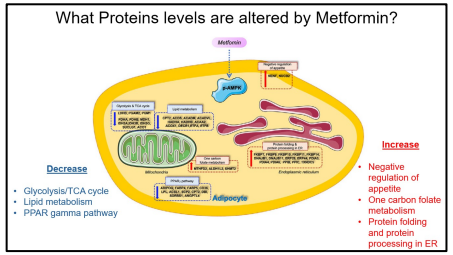


Conclusion

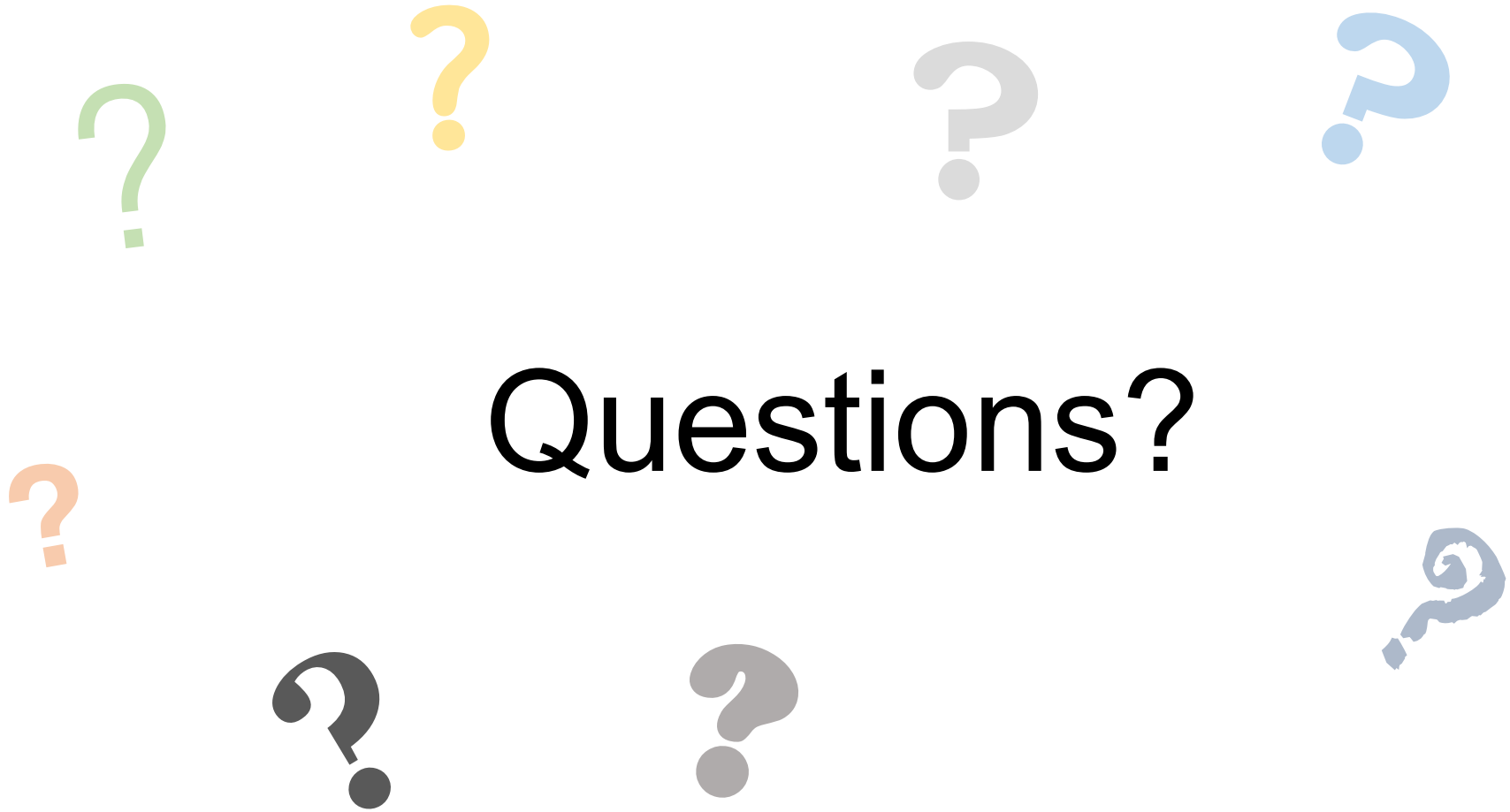
- Metformin is an important and widely used drug for Type II Diabetes and other diseases



- SILAC can be used to analyze the proteomic effects of Metformin



- Metformin is shown to increase and decrease many protein levels and affects numerous biological pathways


Questions?

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