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# Proteomics Section of the Systems Technologies Core

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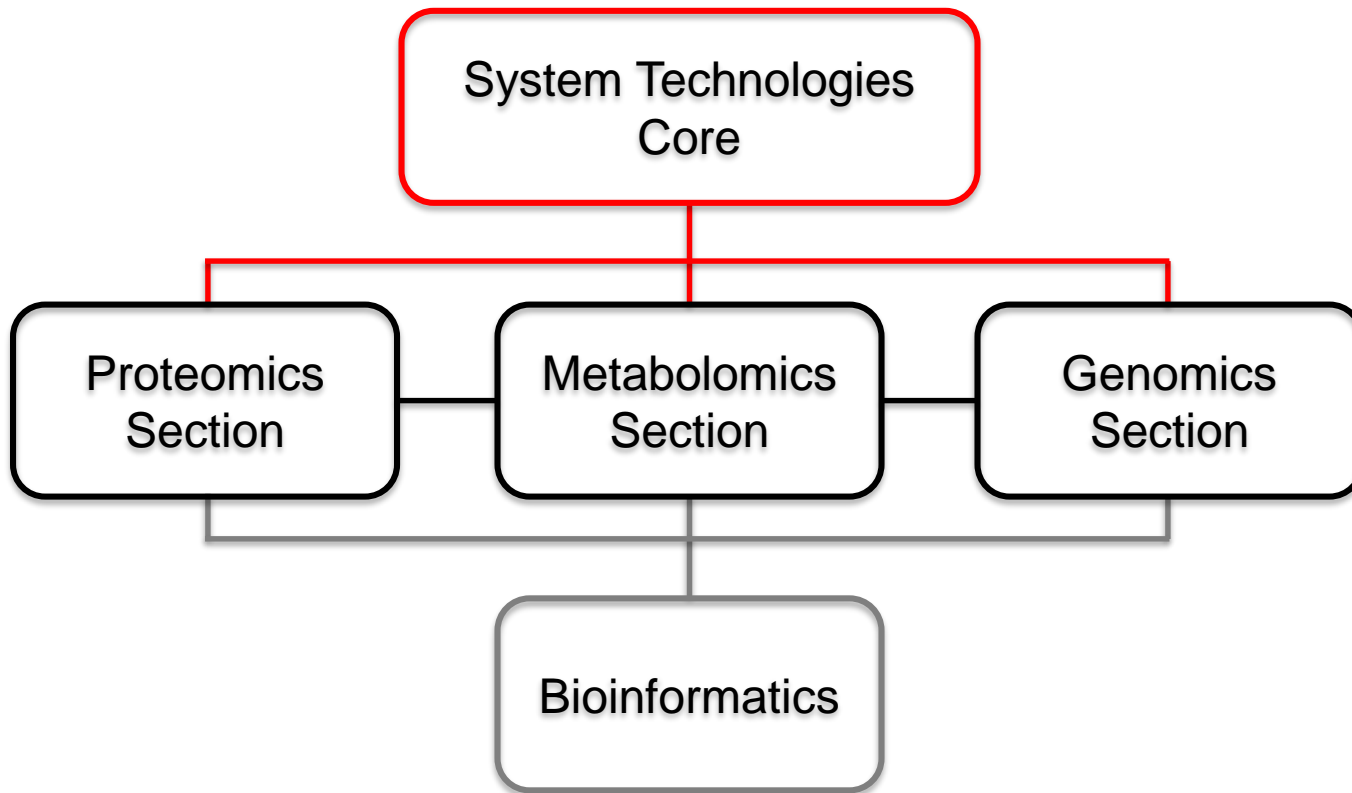
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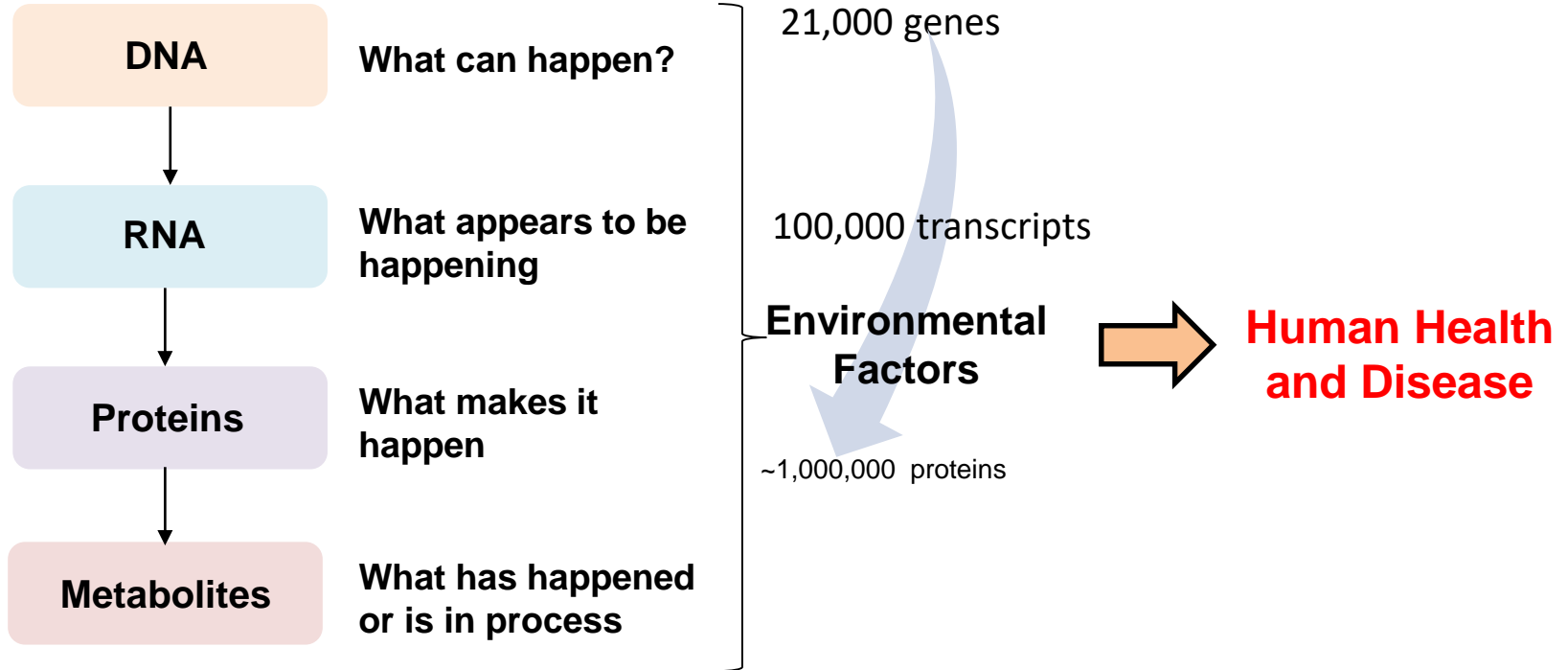


# Systems Technology Core

Molecular Services/Expertise with Integrated Bioinformatic Support

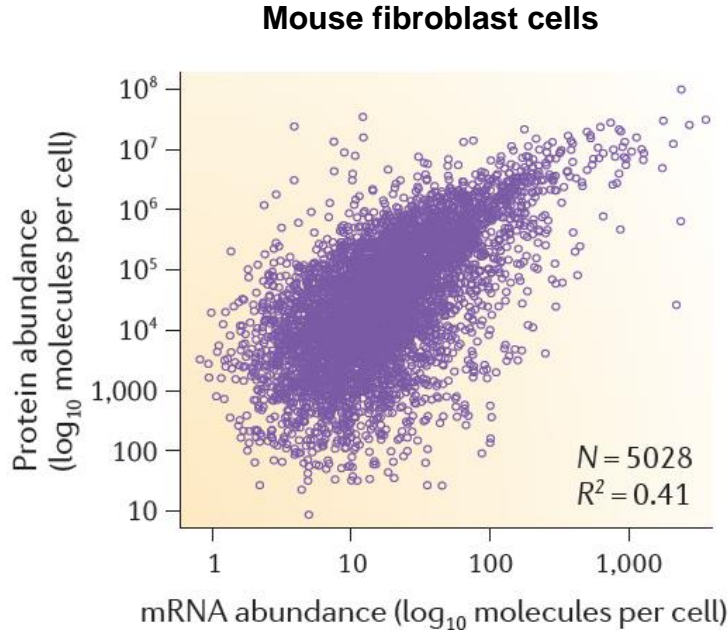


# Central Dogma of Biology



# Why Measure Proteins? – Molecules That Do Work

Christine Vogel and Edward M. Marcotte, *Nature Review Genetics*, 2012



- Intimately tied to cell state
- mRNA measurements are often not a true proxy

## Other information

Protein complexes  
PTMs  
Protein structure

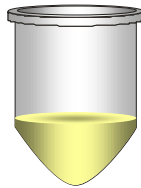
# Proteomic Experiments

“Proteomics is the **identities, quantities, structures, and biochemical and cellular functions** of all proteins in an organism, organ, or organelle, and how they vary in **space, time, and physiological state**”

Mol. Cell Proteomics 1:763:780 2002

## Discovery Proteomics

What Proteins are in my sample?

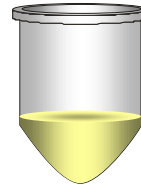


Shotgun proteomics  
GeLC MS/MS

“**Exploratory**”  
investigations

## Targeted Proteomics

Are Proteins A, B, C in my sample?



Relative or absolute  
quantitation

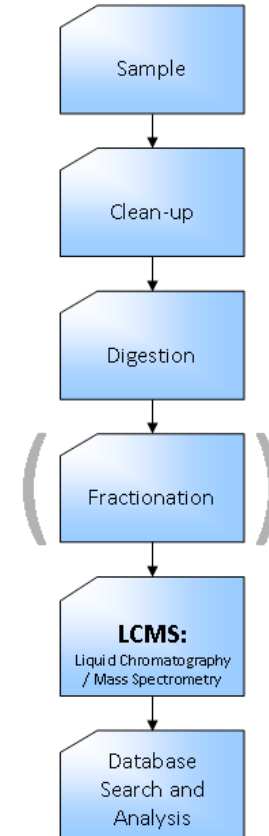
“**Hypothesis**” driven  
investigations

## What to expect

- **Routinely identify 2000-3000 proteins in a single injection – sample dependent**
- **Sensitive and precise relative quantification of a select group of proteins**
  - Targeted proteomics
- **ID of post-translational modifications upon enrichment**
  - Phosphorylation
  - Ubiquitination
- **Identification of protein interactions (Co-IP)**
  - Background
- **Limitations**
  - Protein abundance
  - Available databases
  - Reliable quantitation
  - Data mining

## CHHE Proteomics advises

- **Experimental design**
  - Number of replicates
  - Buffers
  - Extraction/Detergents
- **Sample Preparation**
  - Clean up/ Digestion
- **Data Acquisition**
  - LC MS/MS
- **Data Processing and Analysis**
  - Database search, results, pathway analysis



## CHHE Proteomics Center : Conclusions

- **Proteomics via LC MS/MS is a powerful technology used to measure proteins in complex mixtures**
- **Proteomics Section Mission is to provide expertise in experimental design, sample preparation, and analyses to members of CHHE**
- **Funding available for Proteomics through CHHE Seed Funds**  
<https://chhe.research.ncsu.edu/about/contact/seed-projects/>
- **Make an appointment to discuss your project today!**



# **INSTRUMENTATION**

# Thermo Fisher Scientific TSQ Quantiva™ Triple Quadrupole Mass Spectrometer

- Low-resolution instrument capable of quantitative analyses of peptides and small molecules
- Example studies:
  - Quantitative proteomic assay to determine absolute concentrations of a particular protein in a given matrix
  - Quantitation of pesticides in water/blood/urine
  - Measuring clearance of a chemical from culture media
  - Quantitating drug and metabolites in biological fluid



# Thermo Fisher Scientific Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ Mass Spectrometer



- High resolution instrument capable of discovery based analyses (e.g., discovery proteomics, untargeted metabolomics, etc)
- Example studies:
  - Determining protein expression profile changes as a result of chemical insult in order to elicit a toxicological mechanism of action
  - Co-immunoprecipitation workflow to determine associated proteins/complexes

# Thermo Fisher Scientific iCAP™ RQ ICP-MS

- Inductively couple plasma instrument capable of elemental analysis (e.g., metals)
- Example studies:
  - Tracking total cadmium in various organs of model organism
  - Total elemental analysis of food samples

