

Harnessing Quantitative Proteomics to Study Disease

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What Is Quantitative Proteomics?

Proteomics: “study of the entire complement of proteins expressed by a genome” – Wilkens, M.; coined in 1994.

Quantitative Proteomics: the measurement of changes in protein levels between experimental conditions

Goal = determine effects of disease or condition on:

- 1. the expression of individual proteins (up or down)**
- 2. post-translational modifications**

Overview

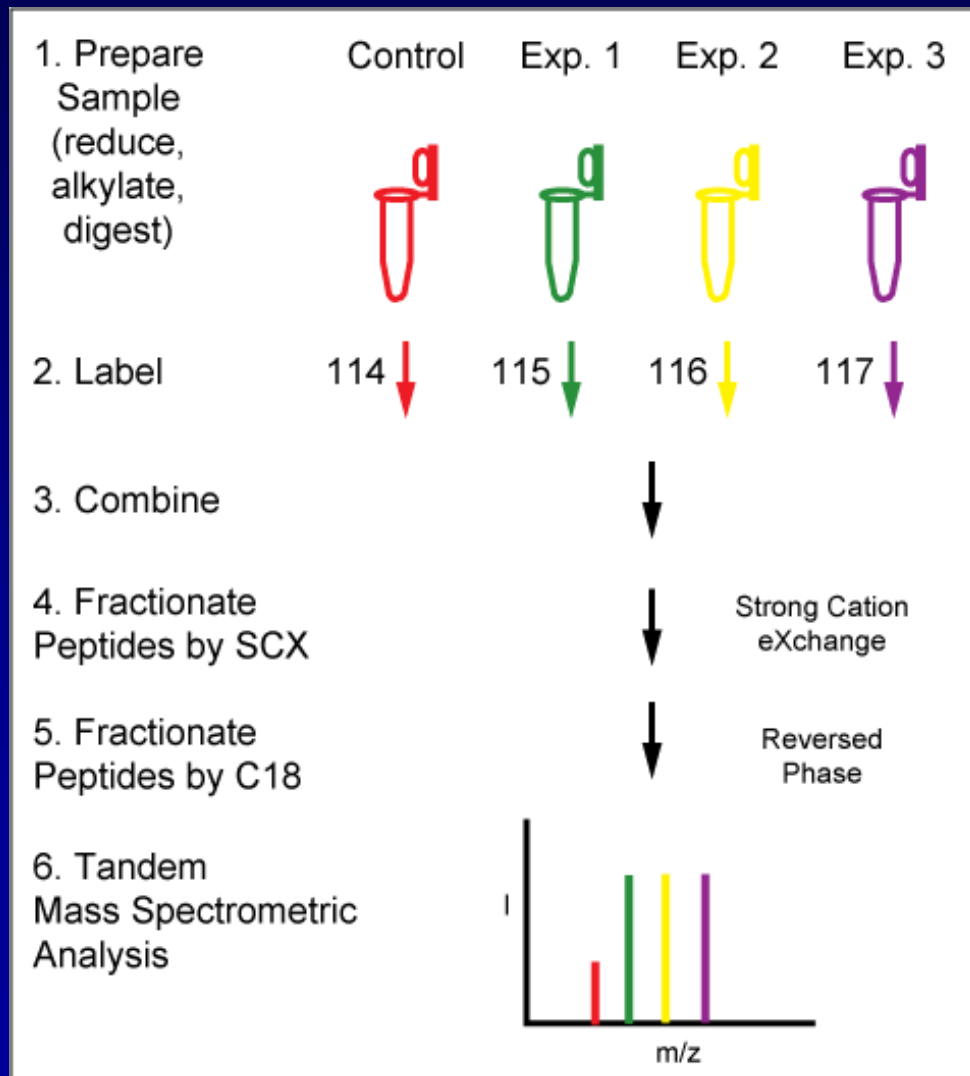
1. Introduction

2. The iTRAQ Method

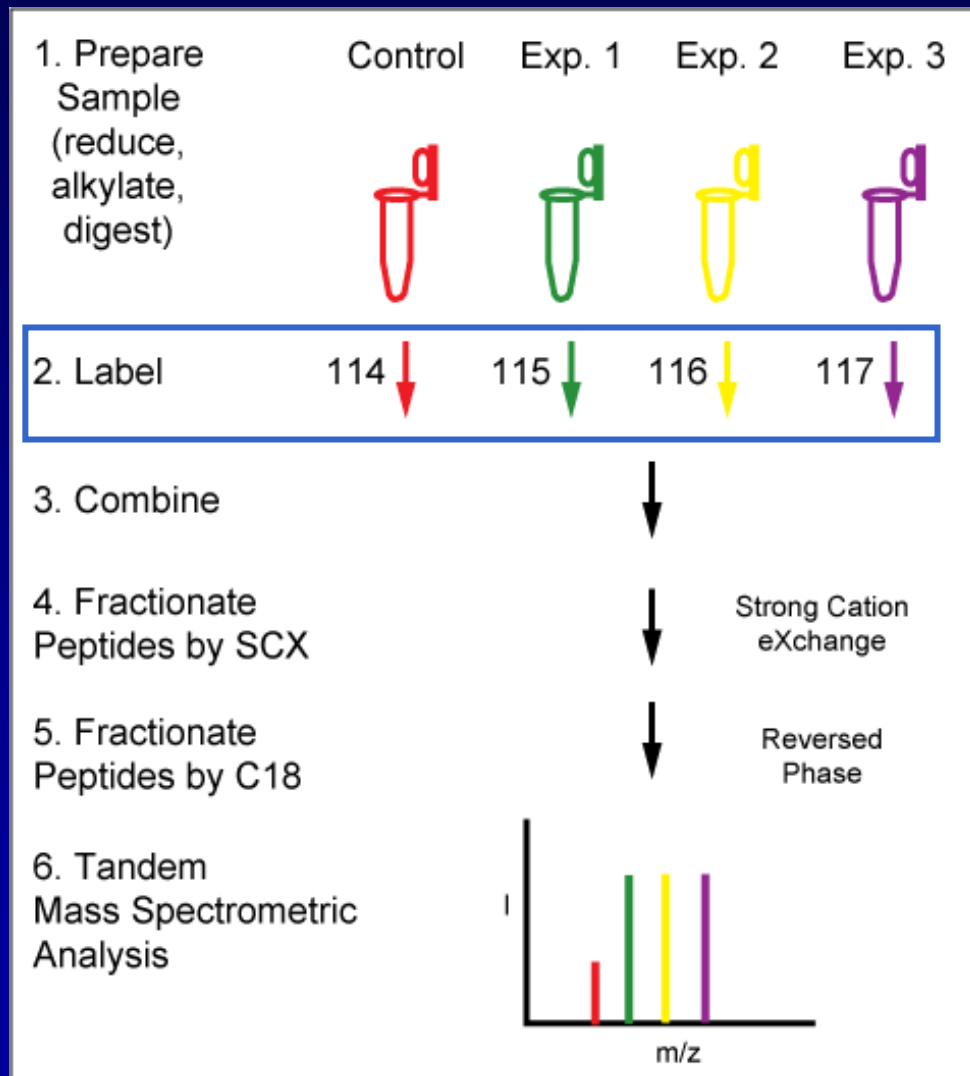
3. Analyzing the effect of age on differential protein expression

4. Targeting extracellular proteins for iTRAQ analysis

Anatomy of an iTRAQ experiment (isobaric Tagging for Relative and Absolute Quantitation)



Anatomy of an iTRAQ experiment (isobaric Tagging for Relative and Absolute Quantitation)



The unique isotopic composition of the four covalent labels allows quantitation by tandem mass spectrometry

Scope

- **71,296 tandem mass spectra acquired over 2 iTRAQ runs.**
- **4,085 unique peptides identified.**
- **Yielding 1,040 unique protein identifications.**



Overview

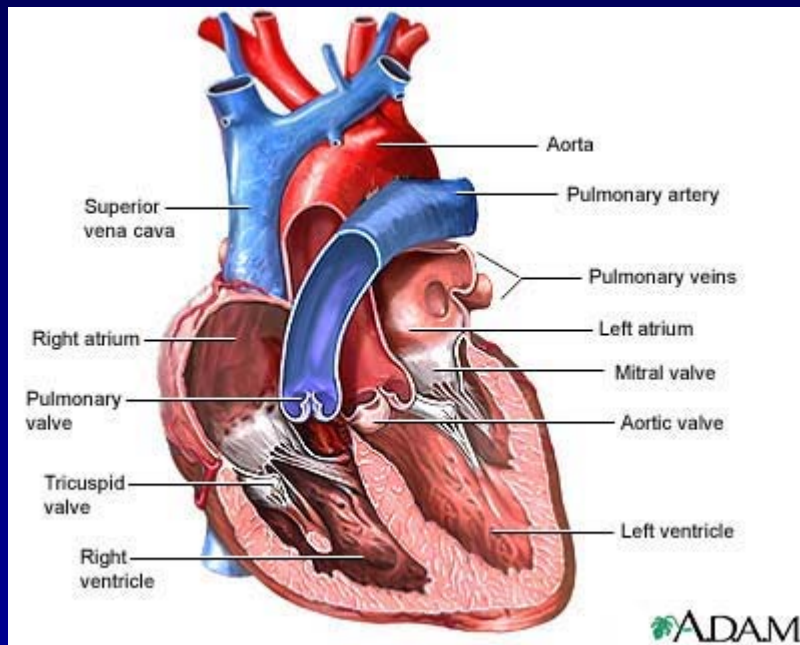
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Left Ventricular Hypertrophy (LVH)

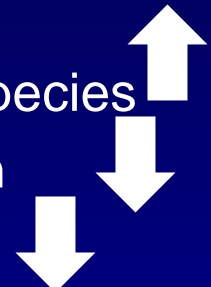


left ventricle

-Site of greatest hypertrophic response

-LV hypertrophy is an important predictor of cardiovascular risk

Age-Associated Hypertrophy

- On average 20% hypertrophic growth of left ventricle over lifetime
 - Reactive oxygen species
 - Fatty acid oxidation
 - ATP production
 - Increased Cardiac Fibrosis
- 
- ```
graph TD; ROS[Reactive oxygen species] --> FAO[Fatty acid oxidation]; FAO --> ATP[ATP production]; ATP --> CF[Increased Cardiac Fibrosis];
```

Can quantitative proteomics determine a fundamental protein signature describing age-related hypertrophy?

# Objective

- I. To determine the effects of aging on the proteome of the left ventricle using quantitative proteomics

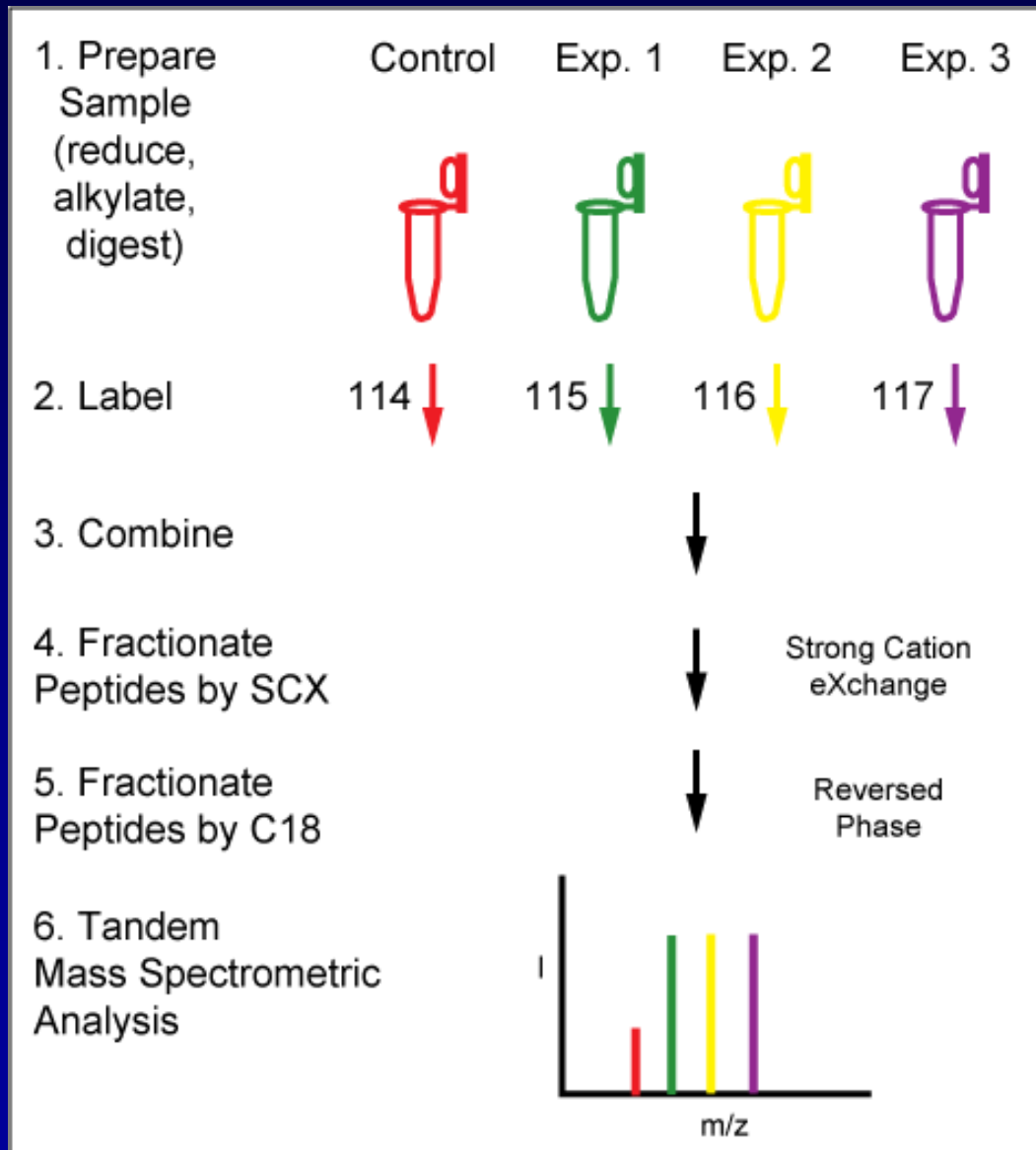
# An Aged Rat Model of Cardiac Hypertrophy

A comparison of old (24 months) vs. young (4 months) hearts

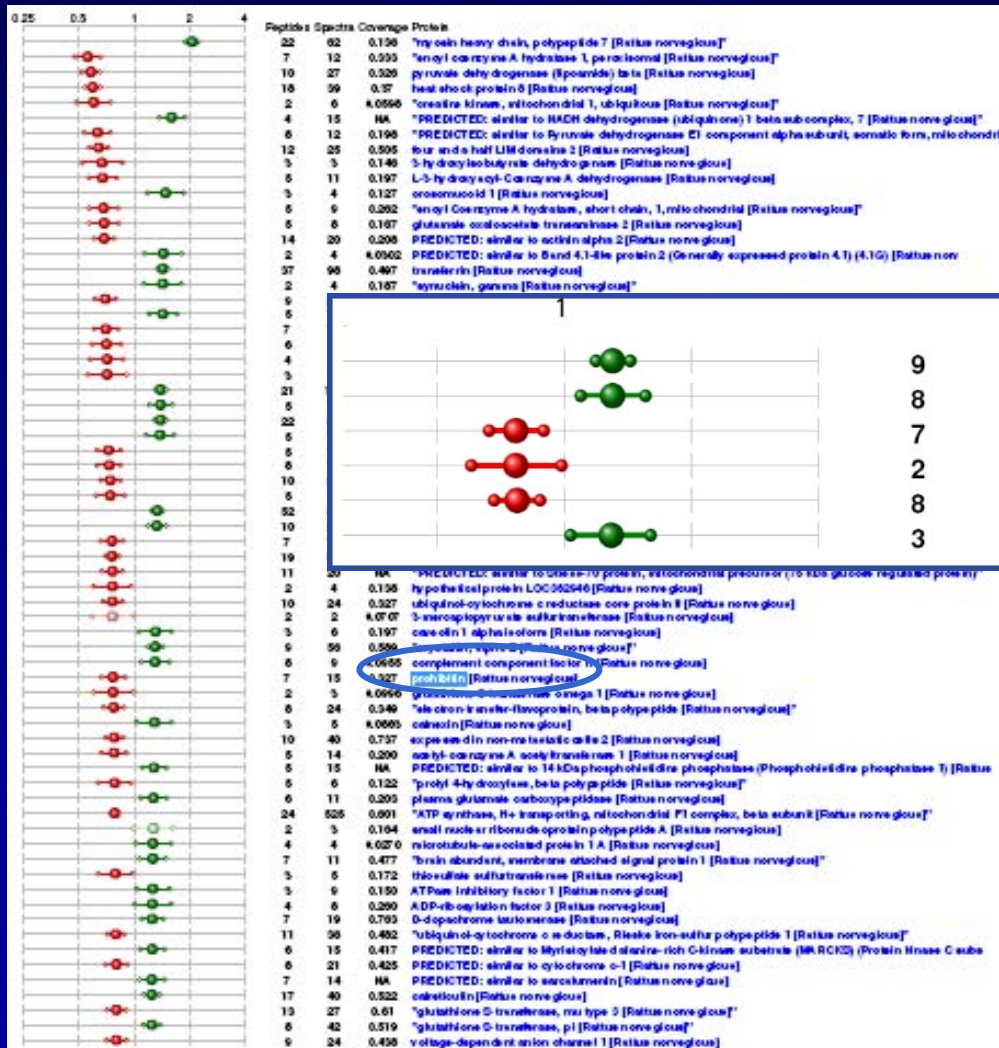


- whole extract from the left ventricles of Fisher 344 rats
- Isobaric Tagging for Relative and Absolute Quantitation (iTRAQ)

# Anatomy of an iTRAQ experiment



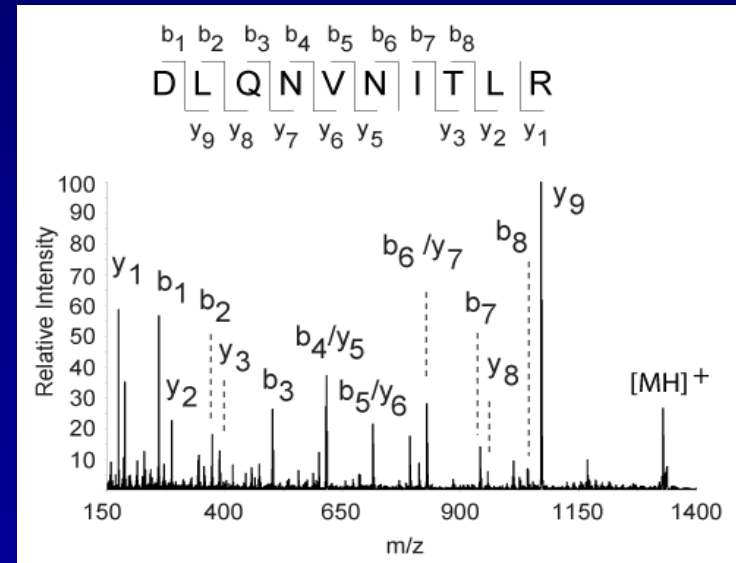
# A Customized Bayesian Statistical Software Package for Defining Proteins Significantly Up- or Down- Regulated



John Schwacke, Ph.D.

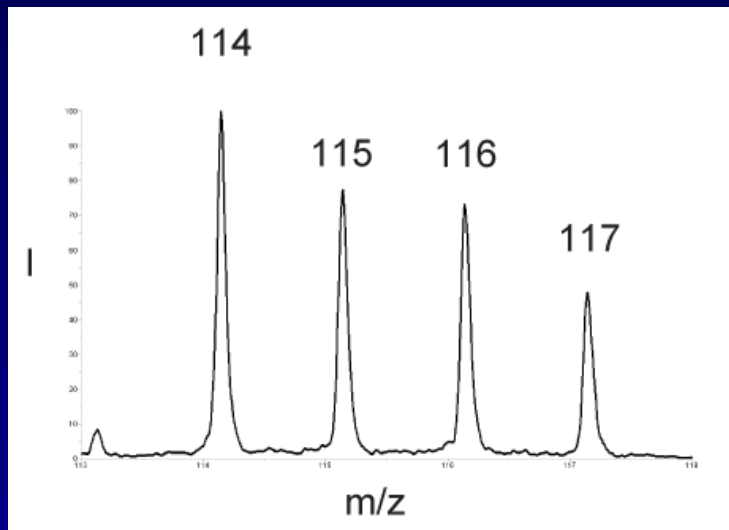
# MS/MS Data for a Prohibitin Peptide

## Peptide Sequence



# MS/MS Data for a Prohibitin Peptide

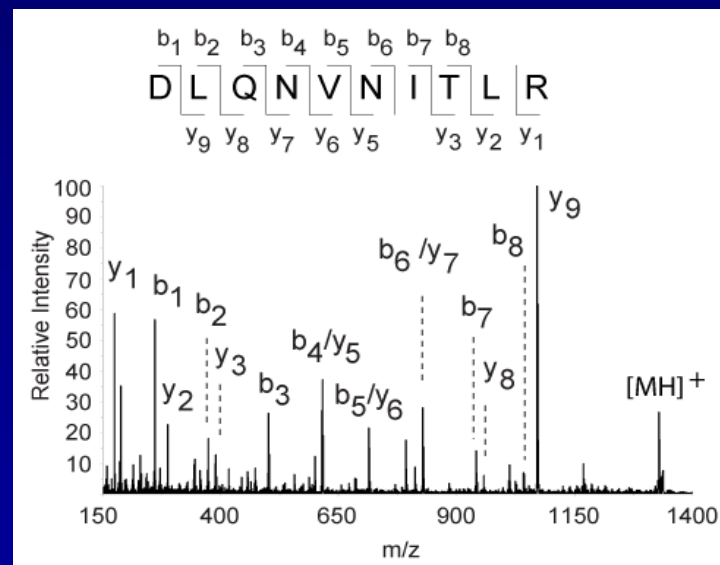
## iTRAQ Reporter Region



young      aged

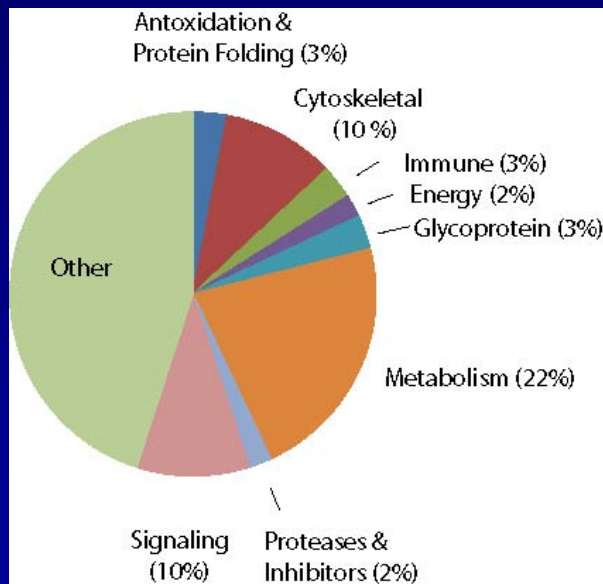
iTRAQ ratio ~ 0.72

## Peptide Sequence



# Summary: iTRAQ Analysis of Aged Rat Whole Cell Extract

295 proteins shared between two iTRAQ experiments



88 proteins differentially expressed  
in hypertrophy

10 glycoproteins and/or ECM proteins

In whole cell extract, glycoproteins are found at a low abundance

## iTRAQ analysis provides a global view of differential protein expression

### Challenges:

1. Experimental Design (good controls, etc.)
2. Complexity of biological specimens (i.e. tissue)
3. Sensitivity of detection

The greatest challenge to quantitative proteomics is to **enrich samples intelligently** prior to analysis.

# Overview

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4. Targeting extracellular proteins for iTRAQ analysis

\* Integrating biochemistry with the iTRAQ method

# Motivations and Opportunities

1. Cardiac fibrosis is characterized by increased production of extracellular matrix (ECM), which reduces cardiac efficiency and performance.
2. The ECM remains largely undefined by protein mass spectrometric methods.

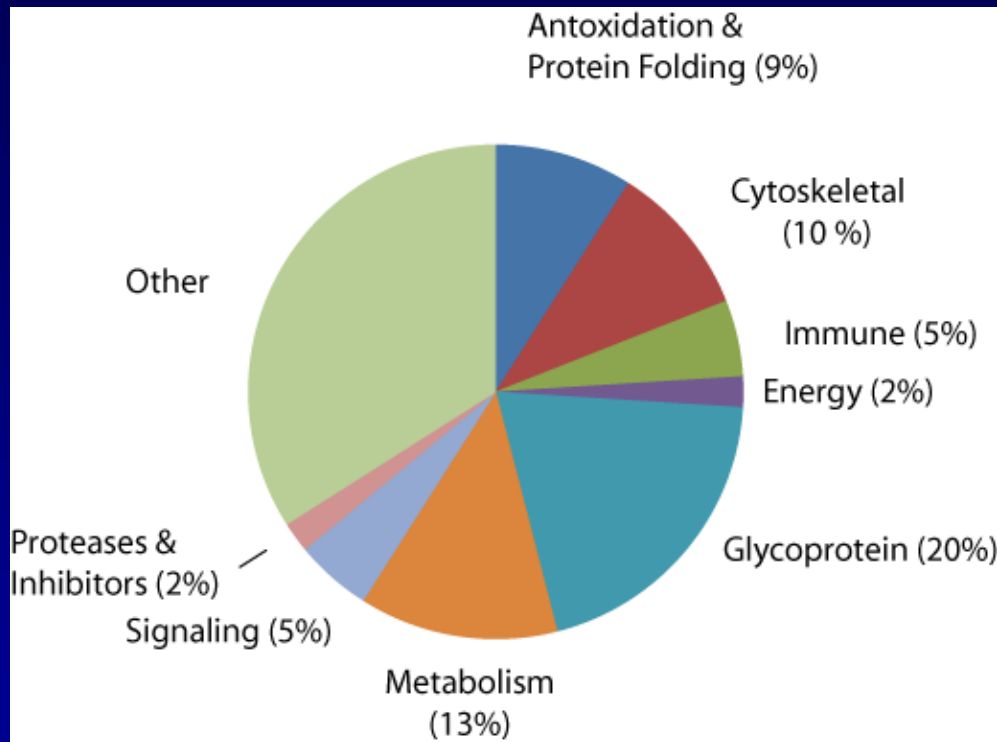
# Enrichment of ECM Proteins Prior To iTRAQ Analysis

Wheat-Germ Agglutinin Affinity  
Chromatography



iTRAQ analysis

# Test Labeling of a post-Wheat Germ Agglutinin (WGA) eluate (iTRAQ 114/117)



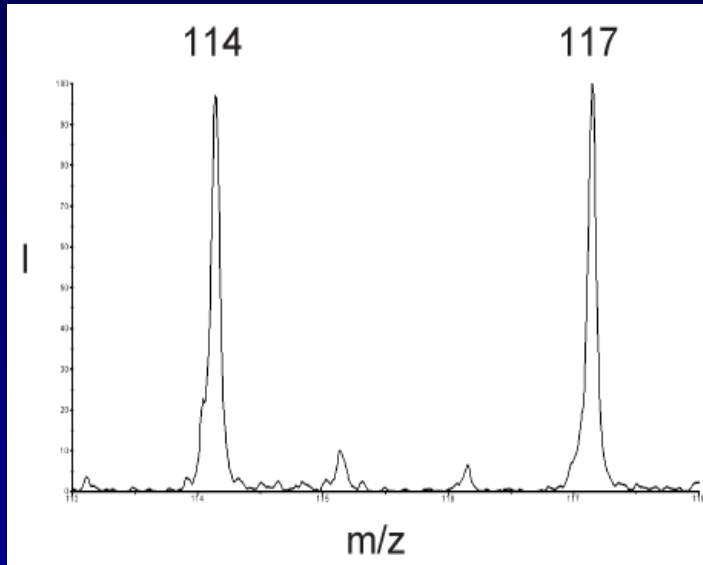
79 proteins found

16 glycoproteins (20%)

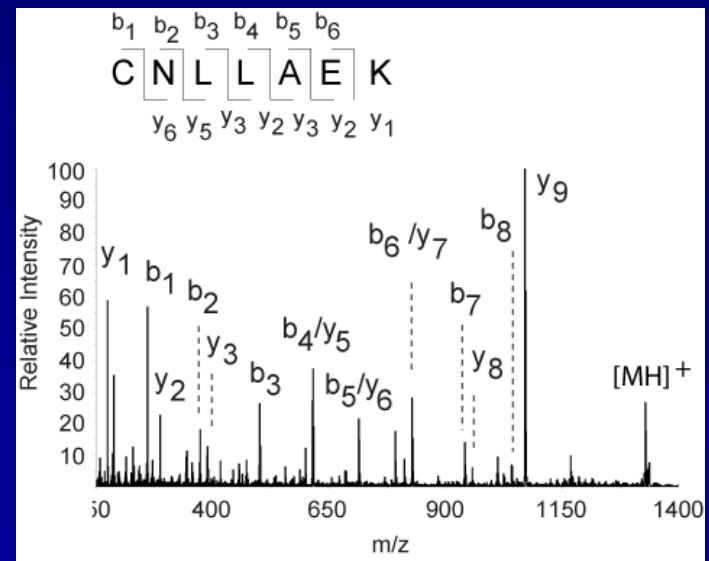
WGA chromatography enriched ECM protein content  
To 20 % (vs. 3% in whole cell extracts)

# $\alpha 2$ -HS Glycoprotein: an example

## iTRAQ reporter region



## Peptide Sequencing



## Summary for WGA Experiments

WGA columns isolated sufficient protein quantities for iTRAQ analysis, and this material was enriched in ECM proteins.

# Summary

iTRAQ is a useful quantitative tool  
for analyzing proteomes and sub-proteomes

# Future Directions

**iTRAQ analysis of WGA-purified  
ECM proteins from control vs.  
aged rats**

**Comparison of Aged-Related Hypertrophy  
to experimentally induced models of  
Hypertrophy and Heart Failure**

# Acknowledgements

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# Posters

## **MUSC IRACDA Scholars:**

Andrew Hunter, Ph.D.

Heather Trentham-Davis, Ph.D.

# A Customized Bayesian Statistical Software Package for Defining Proteins Significantly Up- or Down- Regulated

## 5.50 prohibitin [Rattus norvegicus]

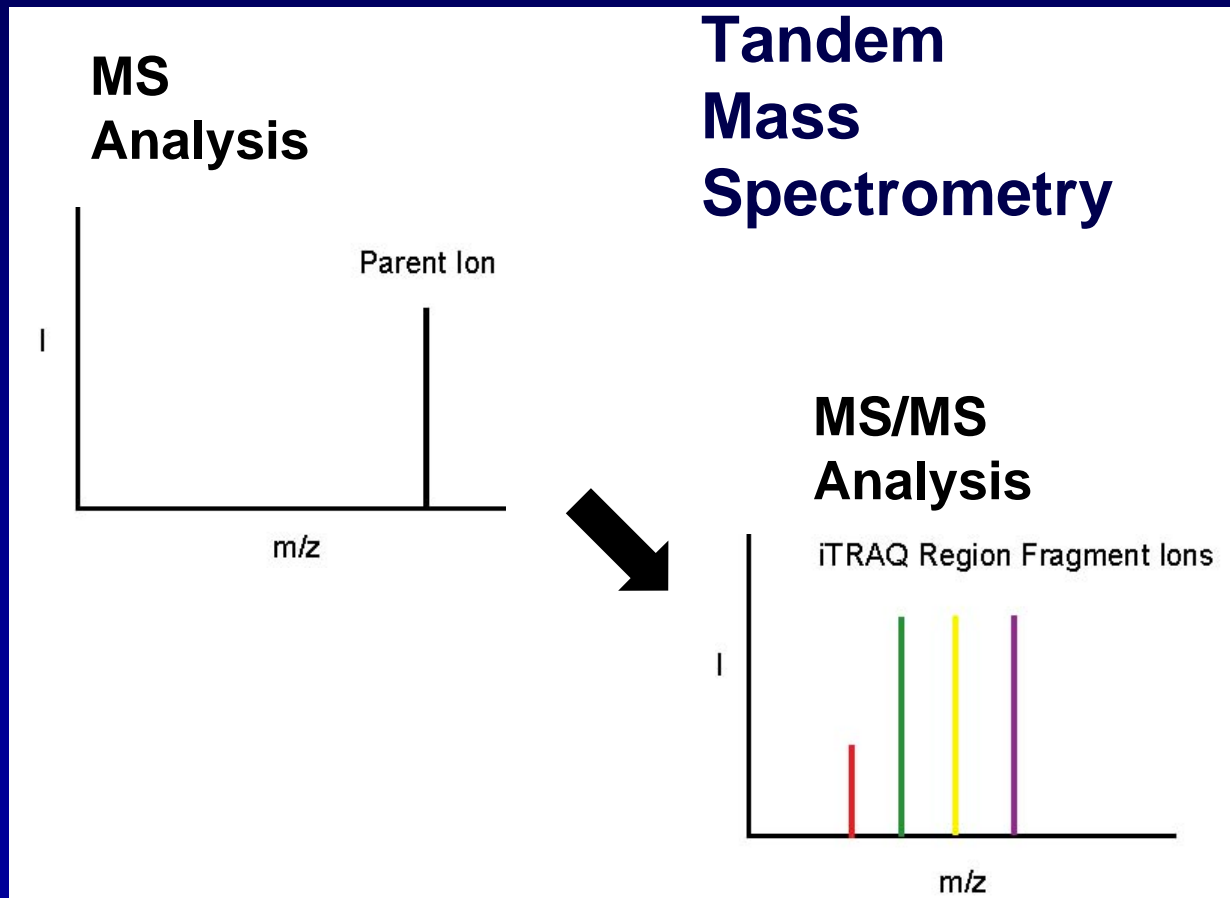
|                              |                |       |
|------------------------------|----------------|-------|
| Protein Accession            | gi 13937353    |       |
| Mean Expression Ratio        | 0.769          |       |
| Median Expression Ratio      | 0.768          |       |
| Credible Interval            | (0.663, 0.892) |       |
| Associated Peptides          | 7              |       |
| Associated Spectra           | 15             |       |
| Coverage                     | 0.327          |       |
| Experiment                   | A              | B     |
| Avg..iTRAQ.Ratio....115.114. | 0.785          | 0.563 |
| Avg..iTRAQ.Ratio....116.114. | 0.827          | 0.589 |
| Avg..iTRAQ.Ratio....117.114. | 0.718          | 0.607 |

```

1 MAARVFESIG KFGELAVAG GVNNSALYNV DAGHRAVIFD RFRGVQDIVV GEGTHFLIPW VQKPIIFDCR SRPRNVPVIT
81 SKDLQNVNI TLRILRPVA SQLPRIYTSI GEDYDERVLP SITTEILKSV VARFDAGELI TQRELVSRQV SDDLTERAAT
161 FGLILDDVSL THLTFGKEFT EAVEAKQVAQ QEAERARFVV EKAEQQKAA IISAEGDSKA AELIANSLAT AGDGLIELRK
241 LEAAEDIAYQ LSRSRNITYL PAGQSVLLQL PQ

```

# Readout provided in an iTRAQ experiment



# ECM-Related Proteins Revealed in iTRAQ Test Experiment

## Examples:

- alpha-1-B glycoprotein
- PREDICTED: similar to laminin B1 subunit 1
- histidine-rich glycoprotein
- PREDICTED: similar to Laminin alpha-2 chain precursor
- basigin
- PREDICTED: similar to peptidoglycan recognition protein 2
- laminin, beta 2
- phospholamban
- basal cell adhesion molecule