

# Quantitative Analysis of the C2C12 and Mouse Skeletal Muscle Proteomes Using a Multiplexing Strategy



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## Overview

### Purpose:

To develop a multiplexing proteomic approach utilizing SILAC and iTRAQ labeling and apply this approach to a model of aging.

### Methods:

- SILAC labeling of C2C12 cells and myotubes
- iTRAQ labeling of cells, myotubes, and tissue
- In-gel digestion & MS analysis - Orbitrap Velos
- Application of MaxQuant and iQuant software through a proteomics pipeline in Galaxy P

### Results:

- Quantitation of dual-labeled standard sample validates methodology
- Application to a complex sample of aging mouse skeletal muscle, C2C12 cells, and myotubes

## Introduction

### 1) Quantitative Multiplexing Technologies:

- Methodologies currently being developed to analyze many conditions/analytes in a single MS sample
  - iTRAQ 8-plex<sup>1</sup>
  - TMT 6-plex + SILAC 3-plex in yeast<sup>2</sup>
- Need for a software and bioinformatics pipeline to efficiently analyze and quantify data (automation)

### 2) Proteome Comparisons:

- Super-SILAC: use of several cell lines increases number of labeled peptides in standard<sup>3</sup>
- Proteome dynamics during C2C12 myotube differentiation studied – complex process<sup>4-6</sup>
- Need a study to assess proteome coverage of myotubes with skeletal muscle—is Super-SILAC necessary?

### 3) Autophagy in an Aging Model:

- Autophagy:** intracellular digestion enabling cells to degrade overabundant or damaged proteins and organelles<sup>7</sup>
  - Impaired with aging<sup>8</sup>
- Caloric Restriction (CR), limiting caloric intake while obtaining adequate essential nutrients, induces autophagy in aging systems<sup>9</sup>

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## Methods

### Justification:

Creation of a simplified sample utilizing the multiplexing strategy serves as a standard for preparation and the data analysis workflow in Galaxy P.

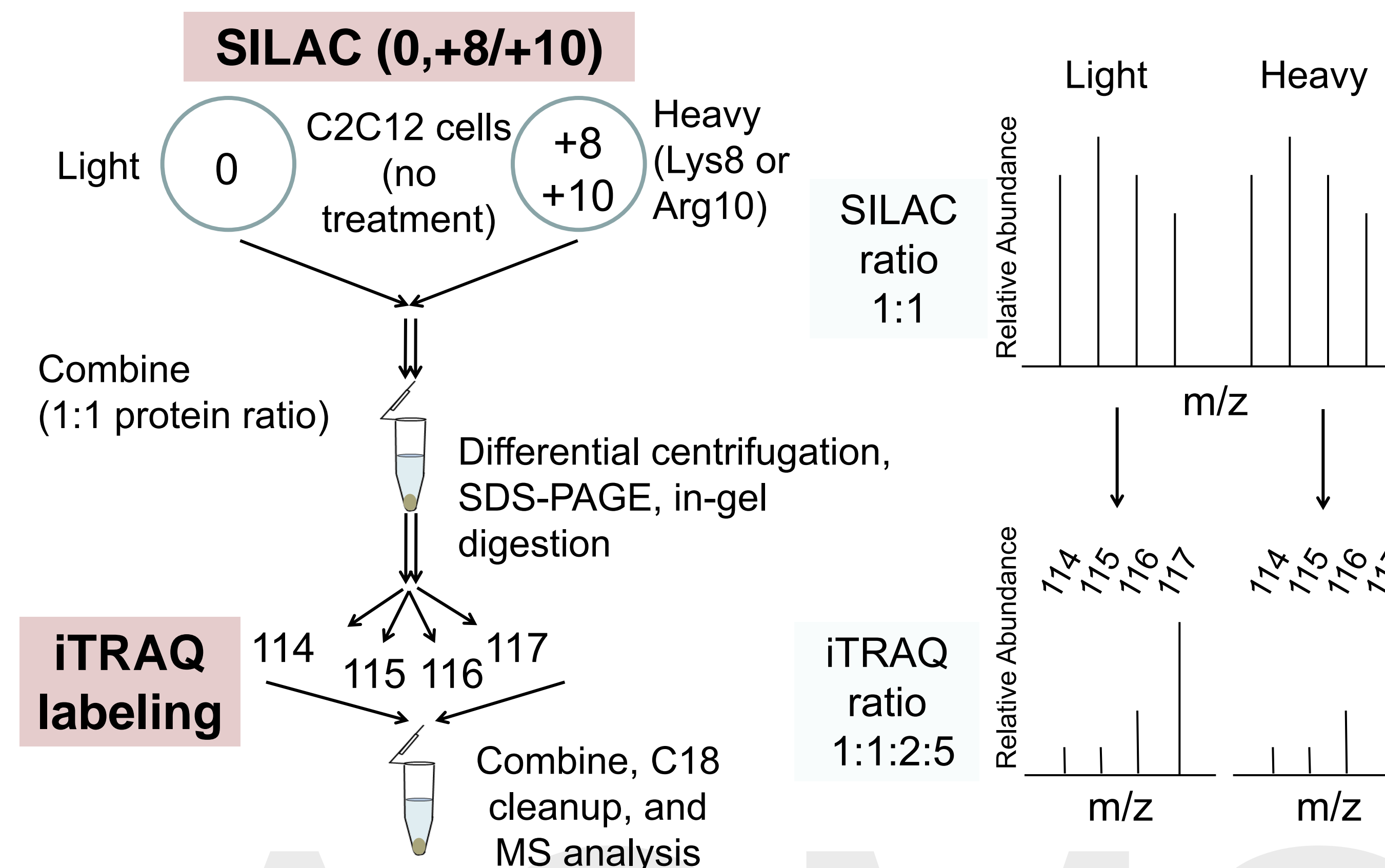
### Approach:

- Establish a dual-labeling procedure
- Ensure analysis software, MaxQuant and iQuant, can identify and quantify SILAC and iTRAQ labels, respectively.

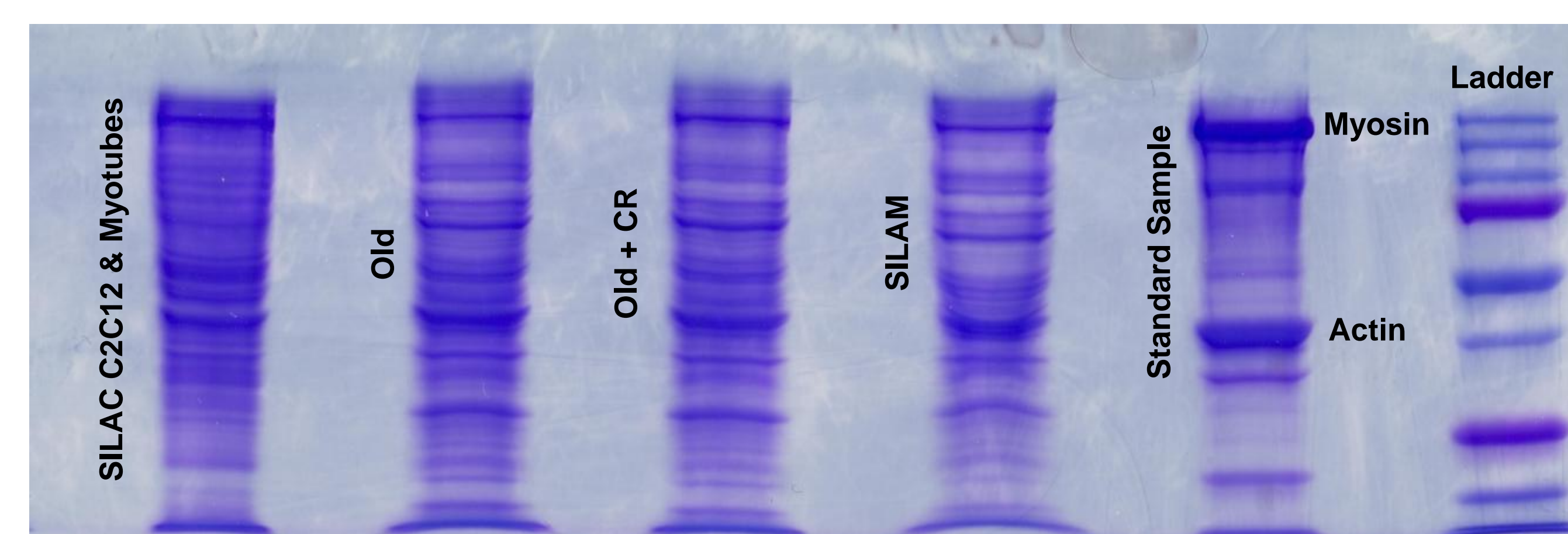
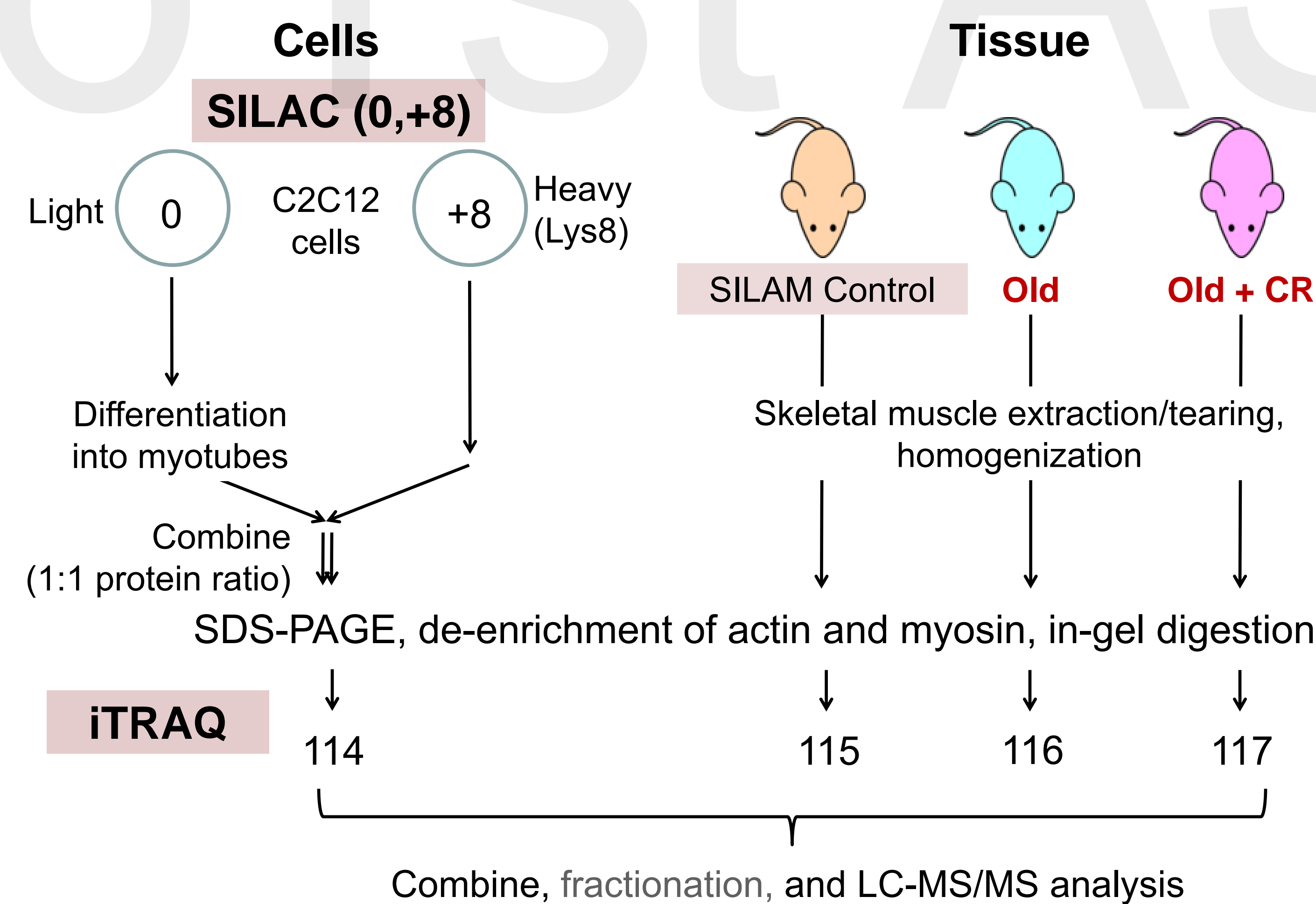
### Standard Sample Design:

- Simplified sample—untreated organelle fraction of C2C12 cells
- Controlled ratios
  - SILAC → 1:1
  - iTRAQ → 1:1:2:5

### Validation by Analysis of a Standard Sample



### C2C12 and Mouse Skeletal Muscle Sample



### Justification:

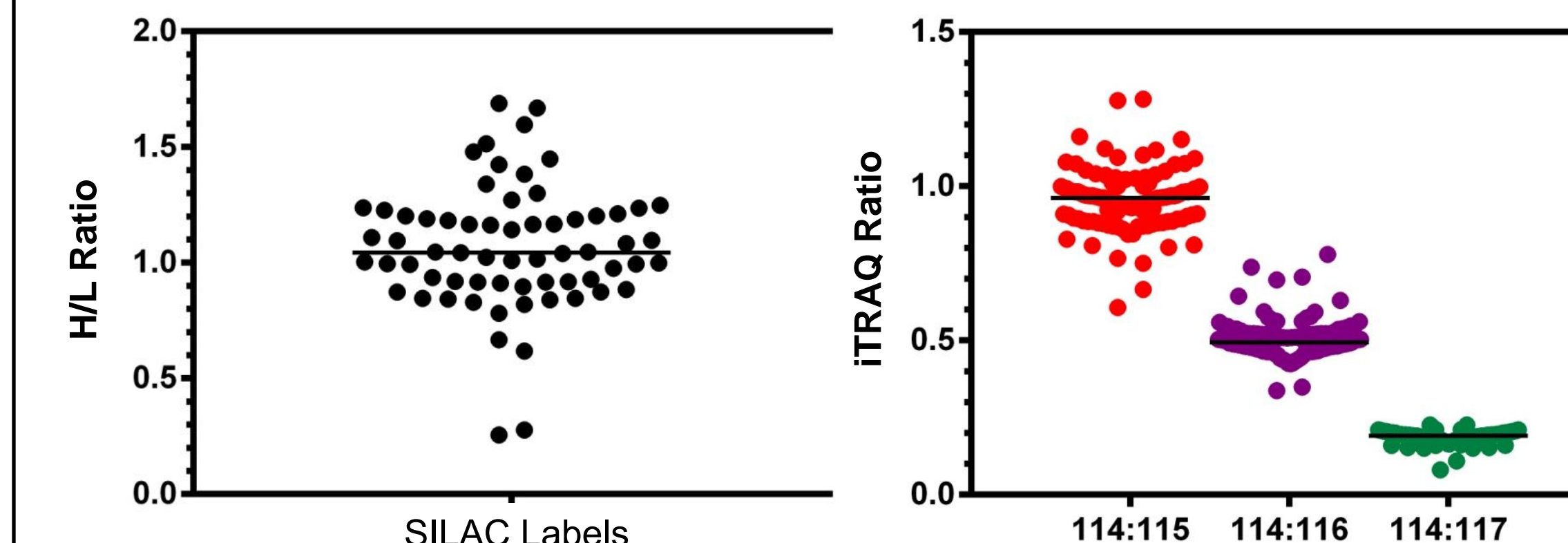
Application of techniques in a complex and biologically relevant sample to demonstrate utility of multiplexing approach and accessibility of Galaxy P data analysis workflow.

### Approach:

- Create a single sample containing light SILAC heavy C2C12 cells and light myotubes, and soleus tissue from a SILAM control mouse, old mouse, and calorically-restricted old mouse peptides.
- MS analysis
  - Eksigent nanoHPLC coupled to an Orbitrap Velos (Thermo)
  - C18 packed fused silica capillary
  - 140-min linear gradient
  - PicoTip™ emitter – electrospray ionization
  - HCD fragmentation
- Data analysis using the automated dual-label workflow in Galaxy P—SILAC and iTRAQ ratios
- Assess proteome overlap between C2C12 cells, myotubes, and tissue
- Biological/pathway analysis to assess effect of CR on soleus proteome of the aging mouse.

## Results & Conclusions

### SILAC and iTRAQ Quantification of Standard Sample



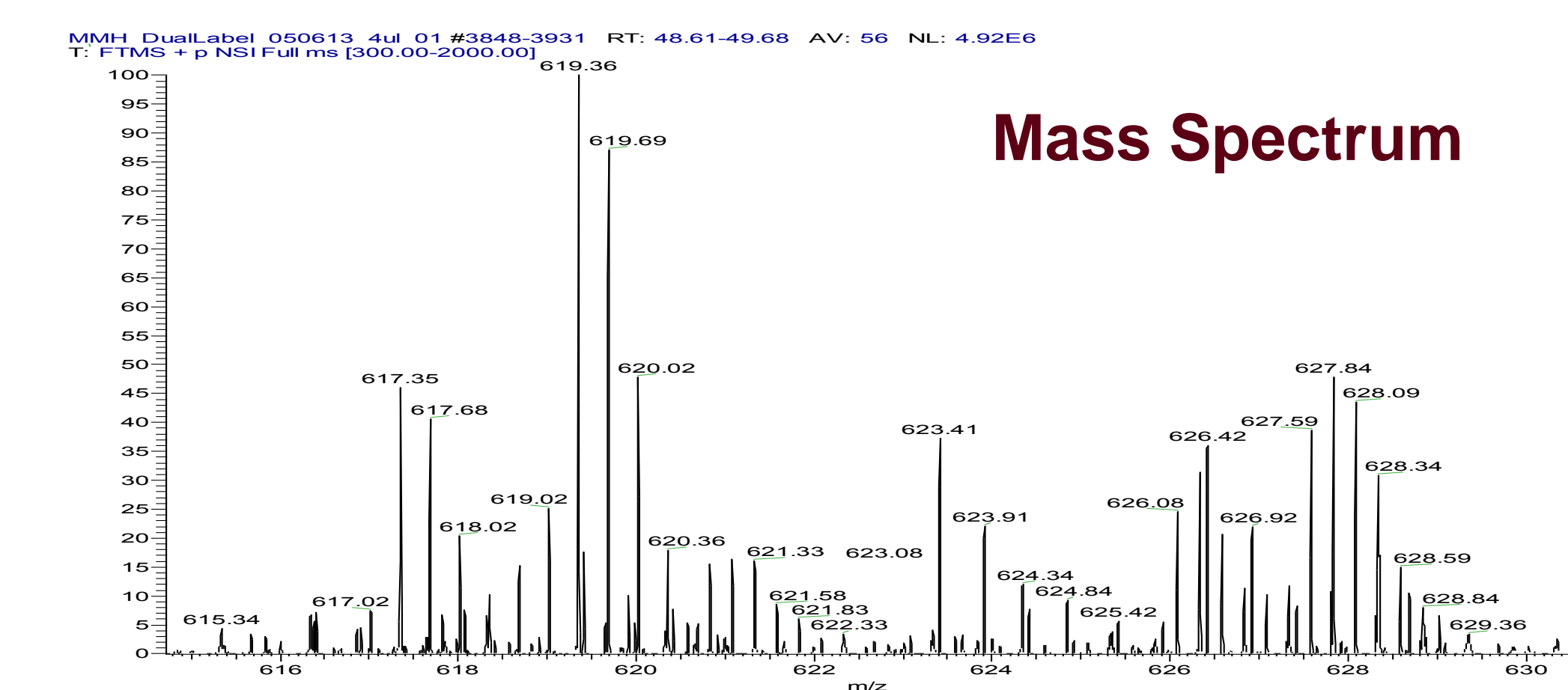
	H/L
Mean	1.07
Std. Deviation	0.27
Std. Error of Mean	0.033

	114:115	114:116	114:117
Mean	0.955	0.505	0.189
Std. Deviation	0.095	0.062	0.020
Std. Error of Mean	0.0087	0.0057	0.0078

- 65 proteins – heavy and light SILAC labels
- 120 proteins – all 4 iTRAQ labels

### Possible Issues with Applied Sample:

- Incomplete SILAC labeling
- Incomplete SILAM labeling in soleus tissue
- Lack of fractionation prior to MS analysis—co-eluting peptides (see mass spectrum below)
- Sample complexity still too high—only light SILAC peptides selected for MS/MS



### Future Work:

- Inhibit autophagy in a C2C12 cell/myotube system—compare proteome with old and calorically-restricted old mouse skeletal muscle using multiplexing approach.
- Enrich for different post-translational modifications after SILAC labeling and treatment—iTRAQ labeling for identification and quantification of a PTM on a specific protein.

## Acknowledgments

Dr. LeeAnn Higgins, Todd W. Markowski – Center for Mass Spectrometry and Proteomics  
 Dr. Peter Villalta – Masonic Cancer Center  
 Dr. K. Sreekumaran Nair, Dr. Ian R. Lanza – SILAM tissue