Accurate Calculation of Protein Half-Lives with the TurnoveR External Tool in Skyline

National Institute on Aging

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Translational Geroproteomics Unit
Skyline User Group Meeting
June 5th, 2022

Translational Geroproteomics Unit (TGU)

Research Directions

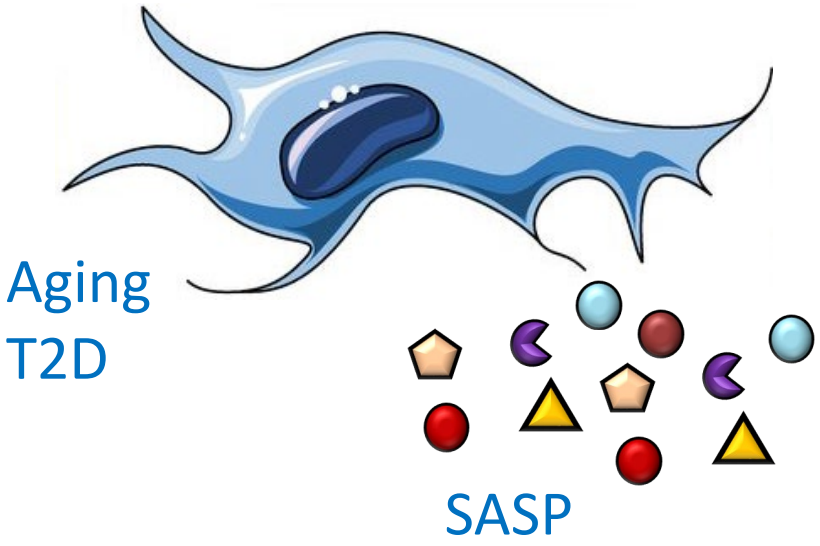
1. Development of a new generation of aging biomarkers

A. Senescence-based
B. PTMs and Isoforms

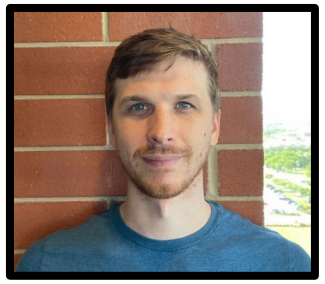
2. Protein & proteoform turnover

3. Proteomics-Driven Approaches to Quantify, Target and Isolate Senescent Cells in Humans/Tissues

A. Development and validation of senescence biomarkers
B. How 'senotype' drives aging phenotypes
C. Therapeutic targets



Matthew Payea

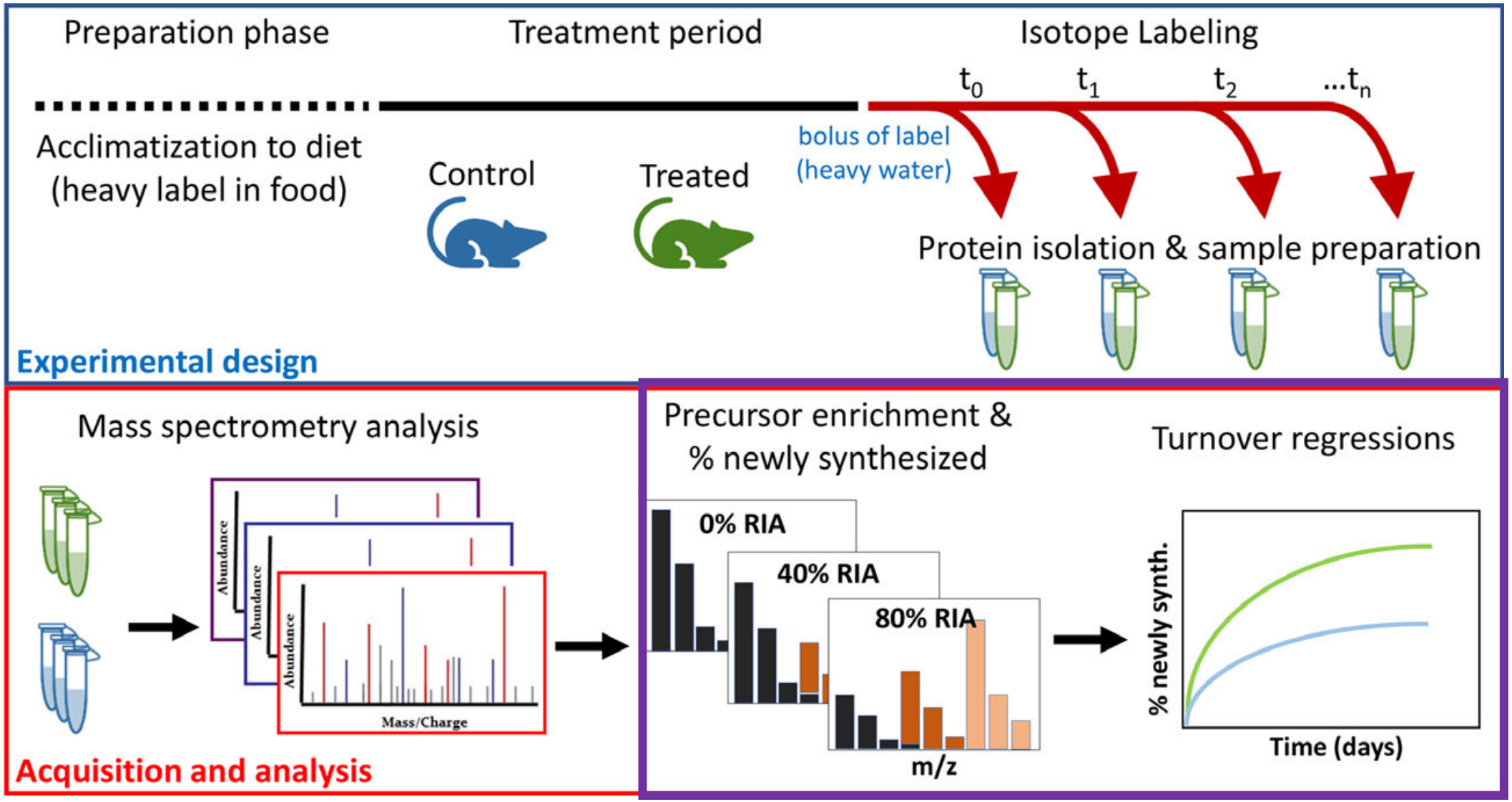


➢ ThP 466 - Quantitative Proteomic Analysis of Protein Half-Lives in Senescent Cells Using Pulsed SILAC

ASMS

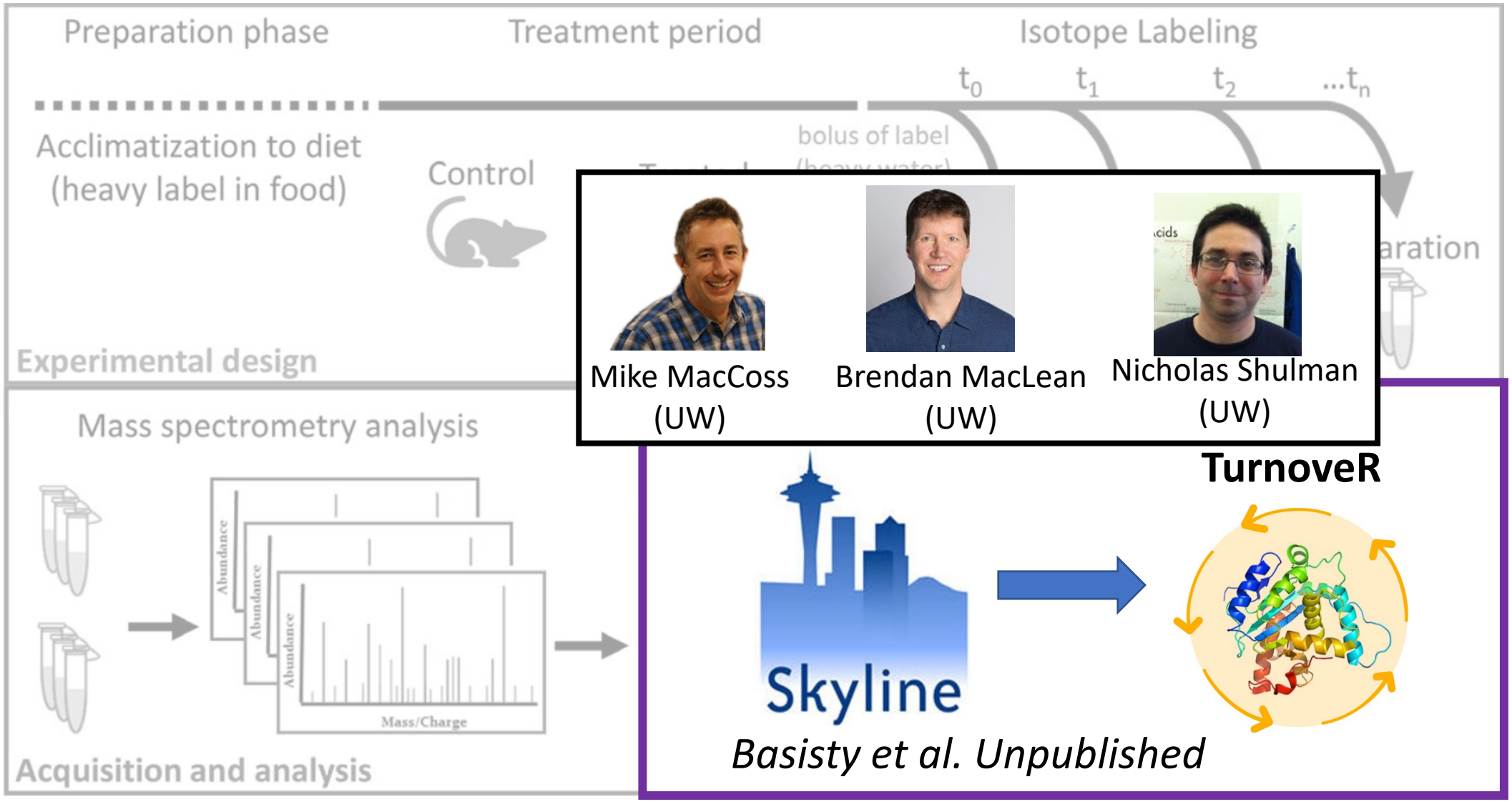
NIH National Institute on Aging

Metabolic Labeling and Mass Spectrometry Enable Comprehensive Measurement of Protein Turnover Rates



Basisty et al. Proteomics. 2018

Metabolic Labeling and Mass Spectrometry Enable Comprehensive Measurement of Protein Turnover Rates



Workflow for TurnoveR: A Skyline External Tool for the Analysis of Protein Turnover from Metabolic Labeling Studies

Labeling experiment and MS

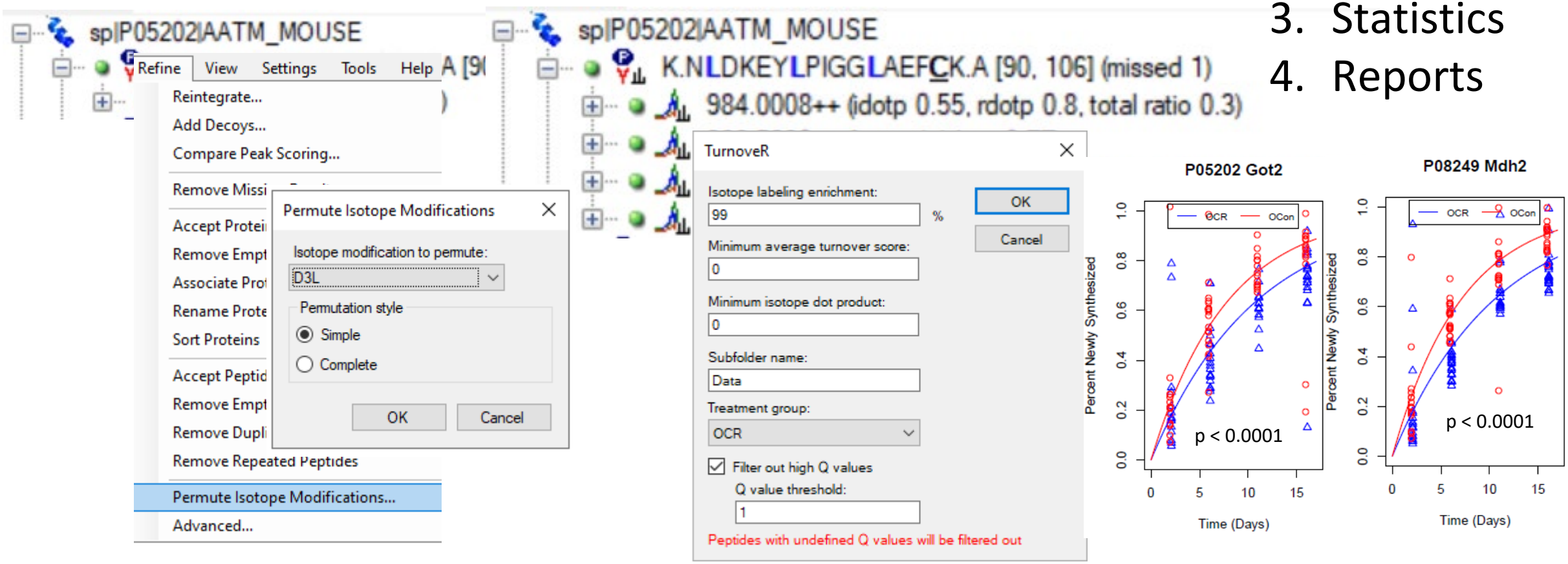
Skyline

Skyline Document

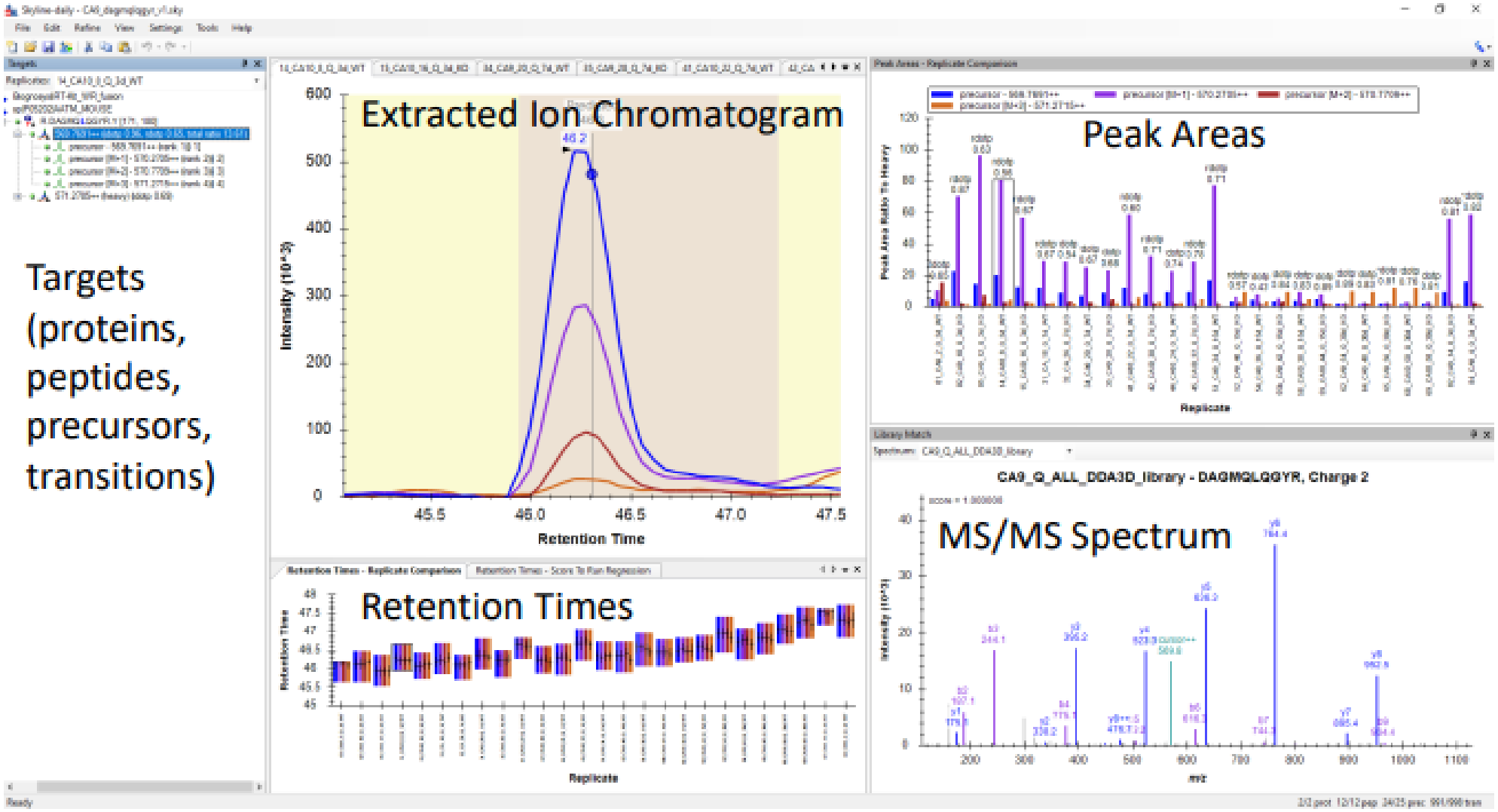
Populate Skyline w/ all isotope permutations

TurnoveR

1. Isotope analysis
2. Regressions
3. Statistics
4. Reports



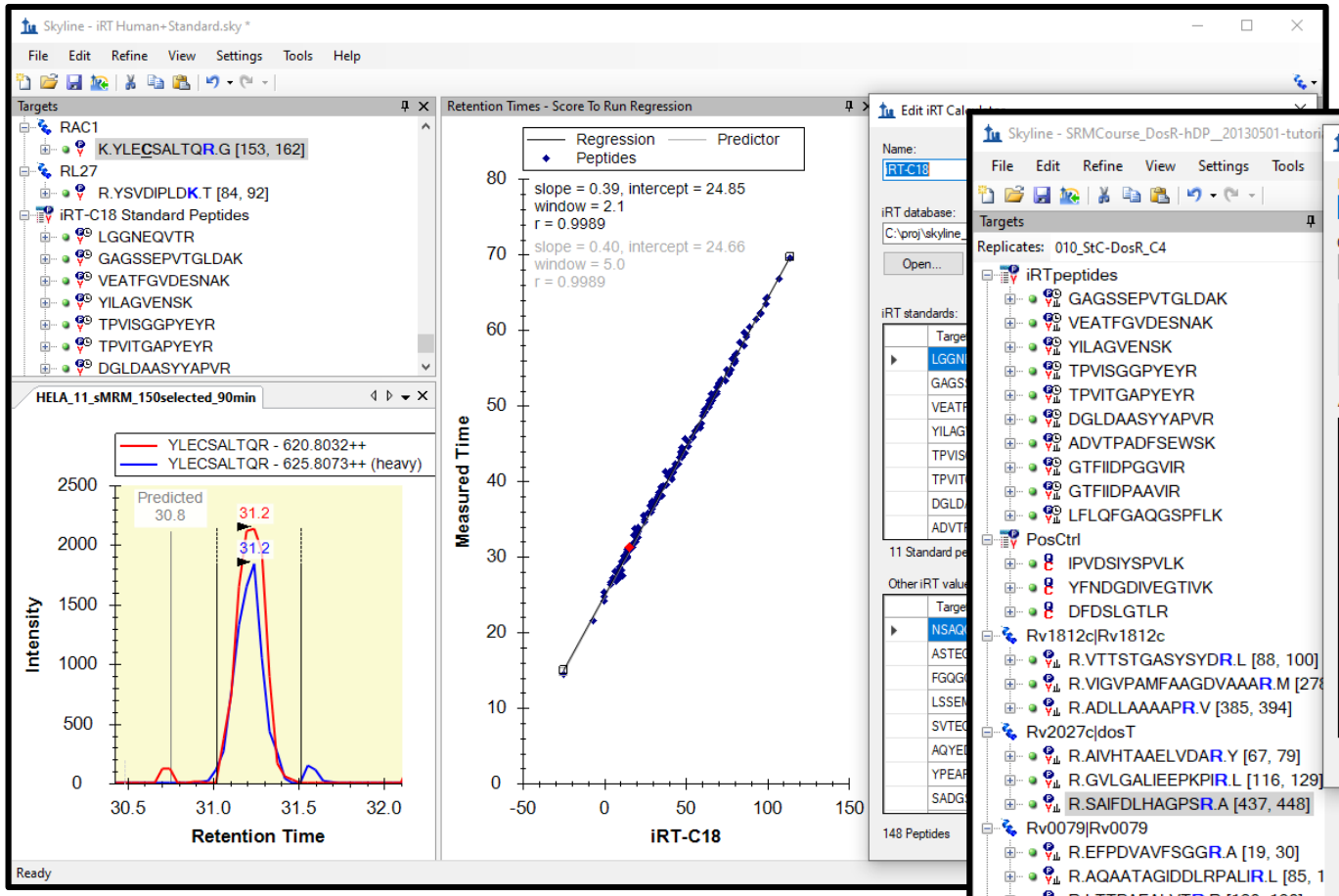
The Interactive Visual Graphical User Interface of Skyline



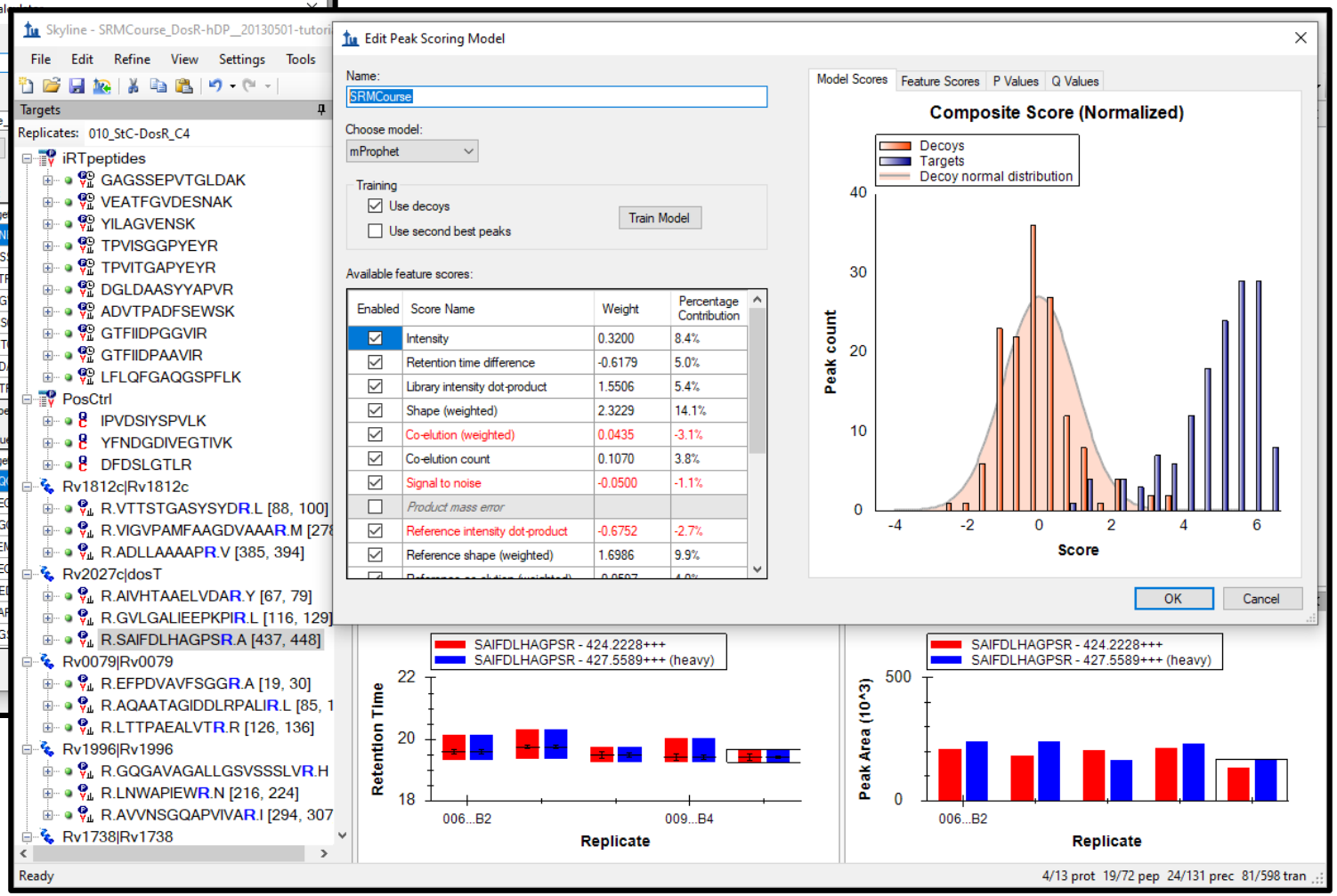
Targets (proteins, peptides, precursors, transitions)

Recommendations for Using the TurnoveR Tool in Skyline

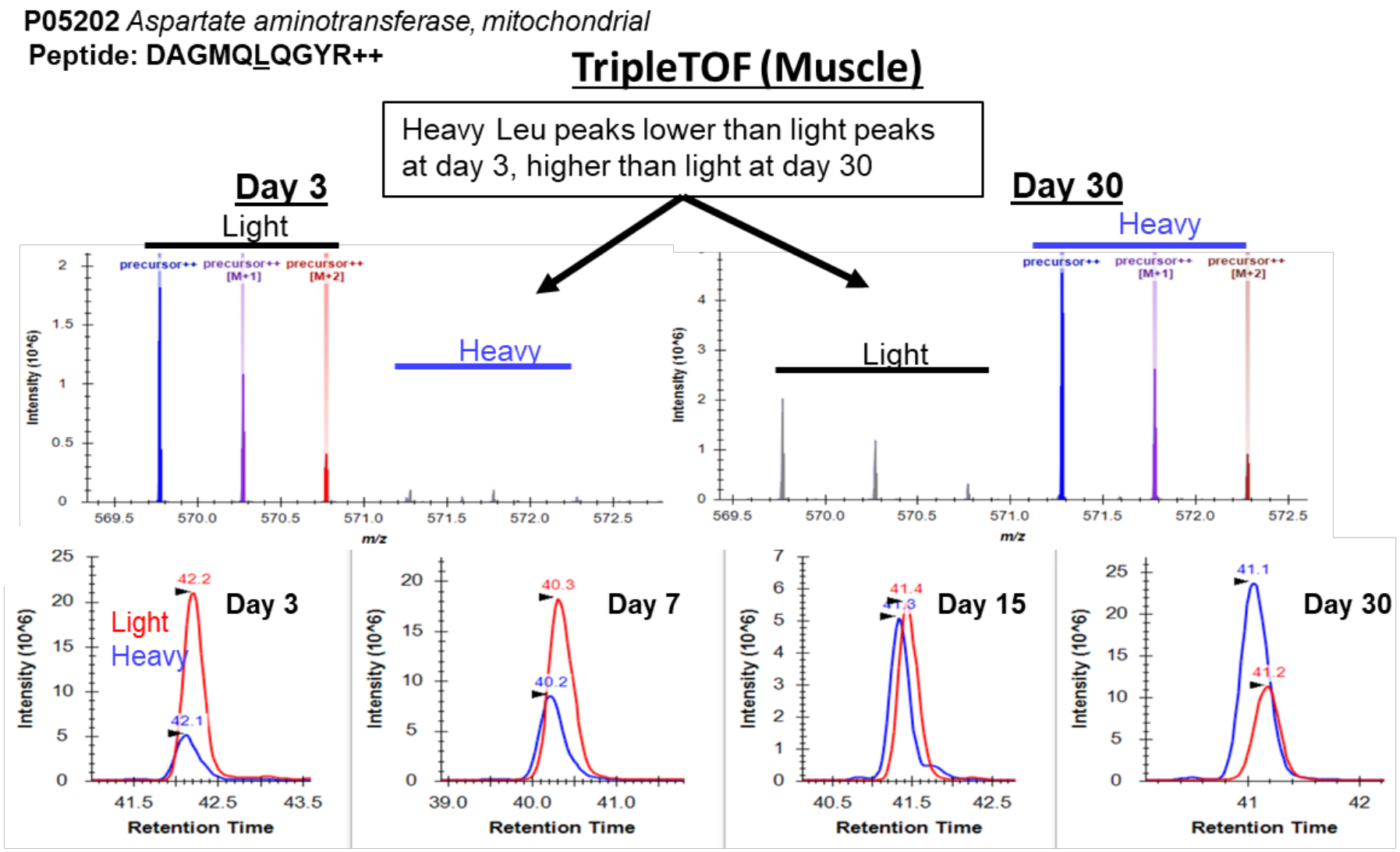
iRT alignment



mProphet peak scoring



Heavy leu incorporation is observable in Skyline software



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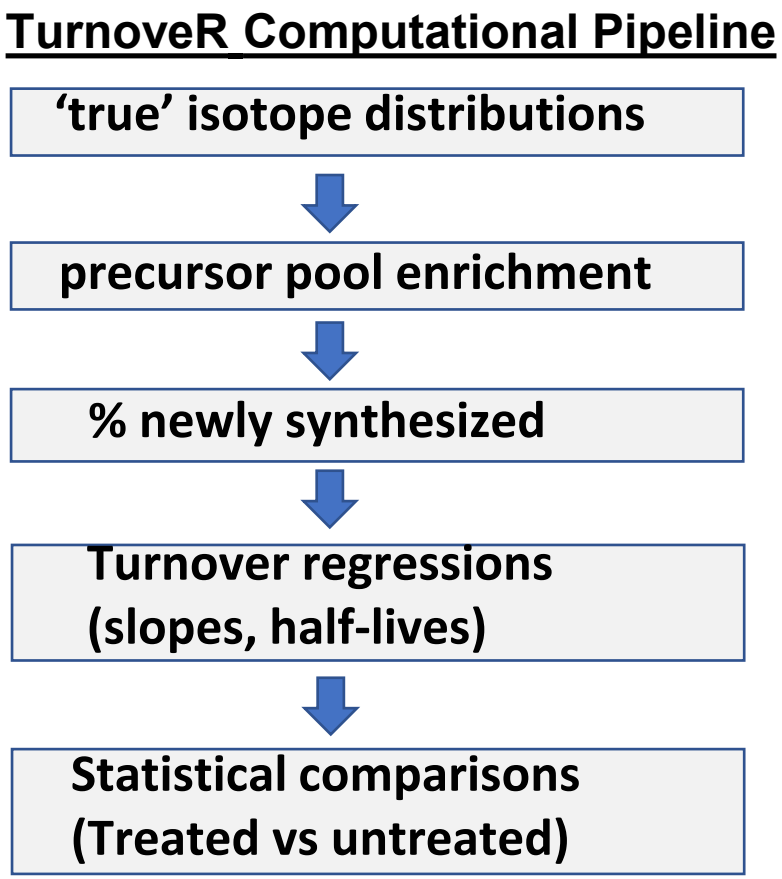
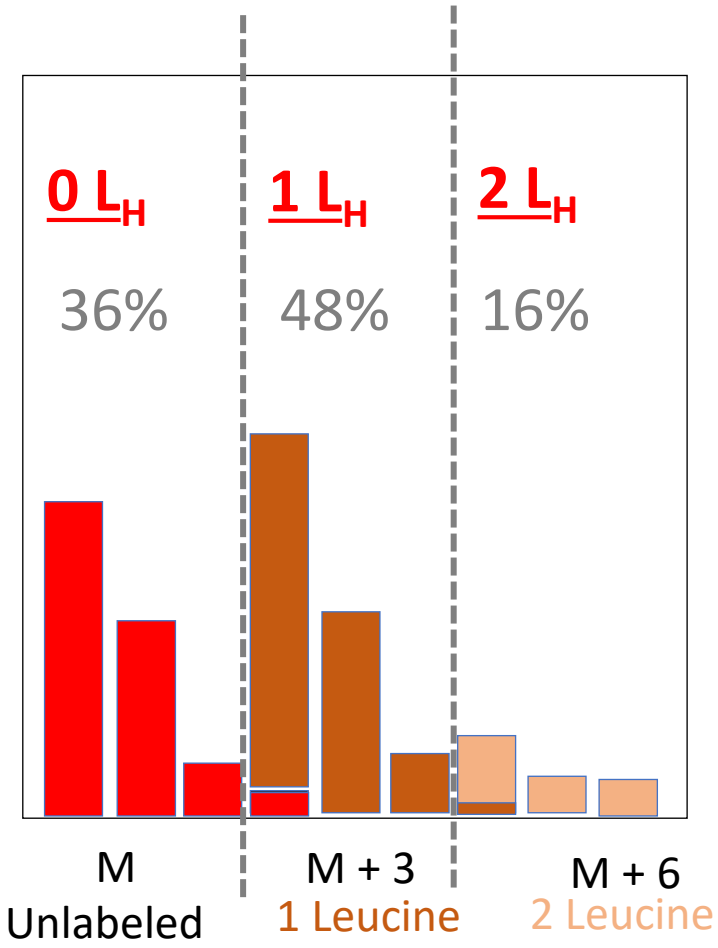


TurnoveR Calculates “True Isotope Distributions”

TurnoveR Computational Pipeline

- ‘true’ isotope distributions
- precursor pool enrichment
- % newly synthesized
- Turnover regressions (slopes, half-lives)
- Statistical comparisons (Treated vs untreated)



Isotope	% nat. abundance	atomic mass
^{1}H	99.985	1.007825
^{2}H	0.015	2.0140
^{12}C	98.89	12 (formerly by definition)
^{13}C	1.11	13.00335
^{14}N	99.64	14.00307
^{15}N	0.36	15.00011
^{16}O	99.76	15.99491
^{17}O	0.04	16.99913
^{18}O	0.2	17.99916

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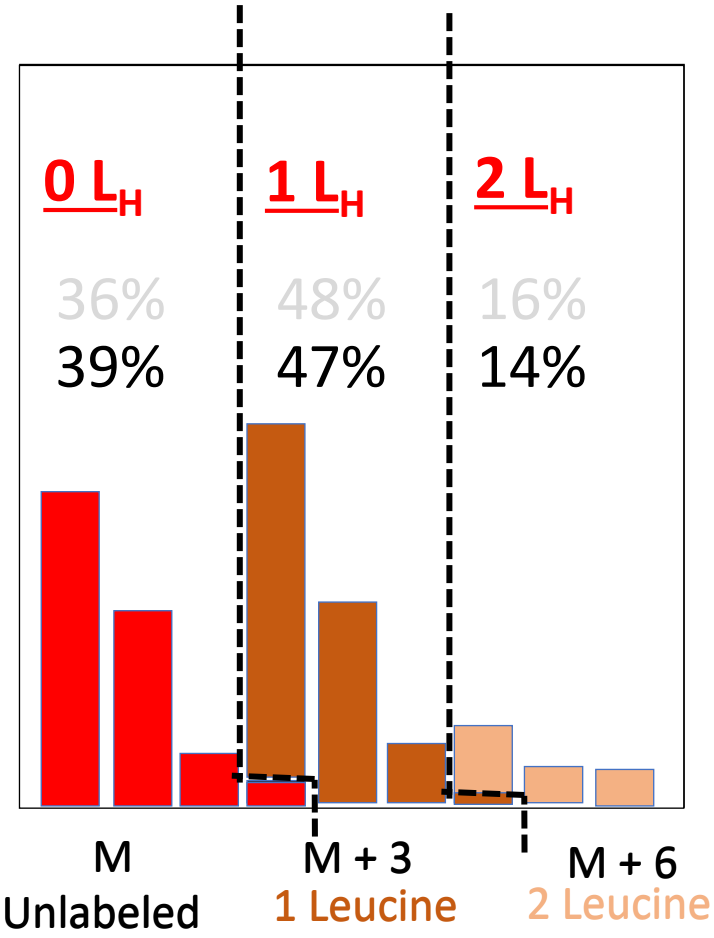

Natural isotope correction prevents systematic overestimation of heavy label enrichment

Brauman et al. 1966. Least Squares Analysis and Simplification of Multi-Isotope Mass Spectra

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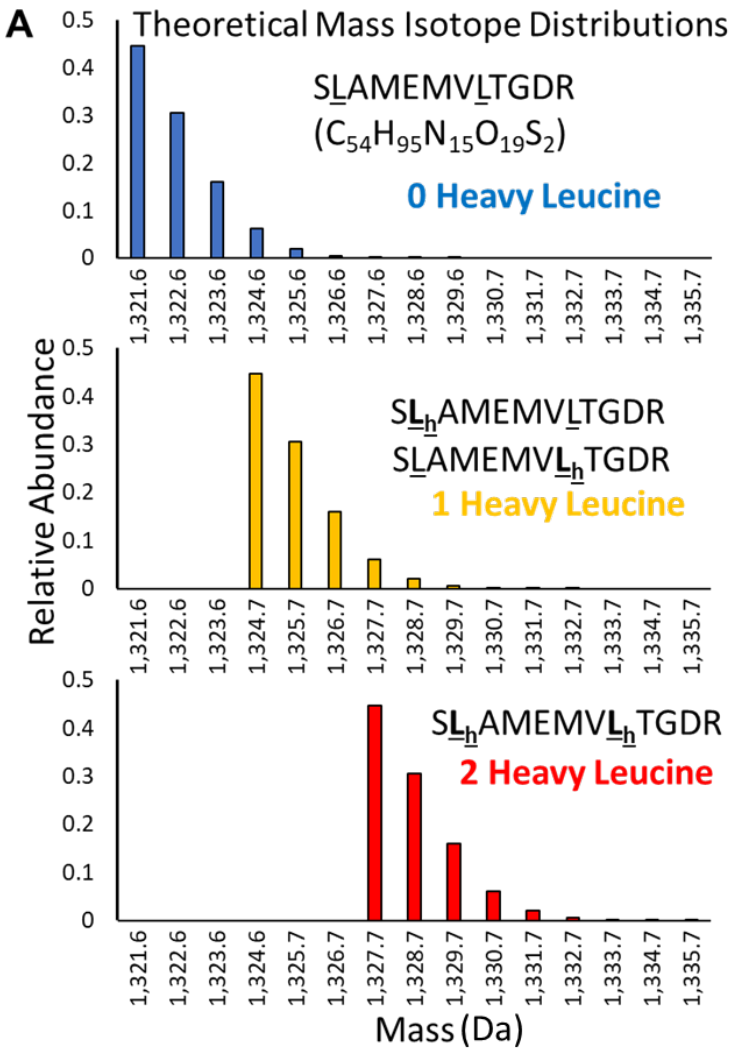

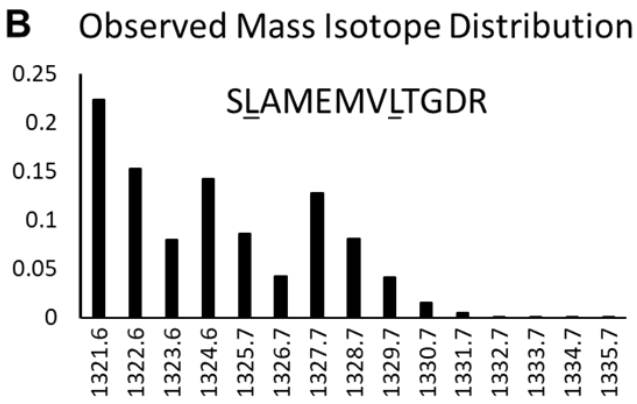

C

$$X = (A^tA)^{-1}A^tP \rightarrow X = \begin{bmatrix} 0.5 \\ 0.25 \\ 0.25 \end{bmatrix}$$

$$A = \begin{bmatrix} 0.446758 & 0 & 0 \\ 0.305383 & 0 & 0 \\ 0.159942 & 0 & 0 \\ 0.061458 & 0.446951 & 0 \\ 0.019516 & 0.30532 & 0 \\ 0.005246 & 0.159878 & 0 \\ 0.001223 & 0.061415 & 0.447144 \\ 0.00025 & 0.019498 & 0.305257 \\ 4.56E\text{-}05 & 0.005239 & 0.159815 \\ 0 & 0.001221 & 0.061372 \\ 0 & 0.000249 & 0.01948 \\ 0 & 4.55E\text{-}05 & 0.005233 \\ 0 & 0 & 0.00122 \\ 0 & 0 & 0.000249 \\ 0 & 0 & 4.54E\text{-}05 \end{bmatrix} \quad P = \begin{bmatrix} 0.223379 \\ 0.152691 \\ 0.079971 \\ 0.142467 \\ 0.086088 \\ 0.042592 \\ 0.127751 \\ 0.081314 \\ 0.041286 \\ 0.015648 \\ 0.004932 \\ 0.00132 \\ 0.000305 \\ 6.22E\text{-}05 \\ 1.14E\text{-}05 \end{bmatrix}$$

(0 D3L, 1 D3L, 2 D3L; Observed)

Brauman et al. 1966. Least Squares Analysis and Simplification of Multi-Isotope Mass Spectra

Precursor pool correction is required for accurate calculation of turnover

TurnoveR Computational Pipeline

- 'true' isotope distributions
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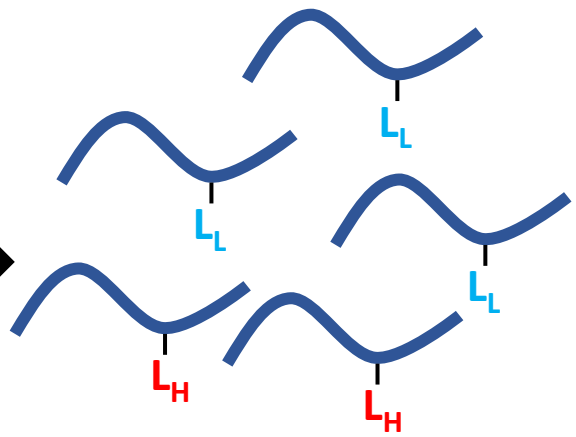
Protein synthesis



L_L = light leucine

L_H = heavy leucine

40% heavy labeled

40% newly synthesized?

Precursor pool correction is required for accurate calculation of turnover

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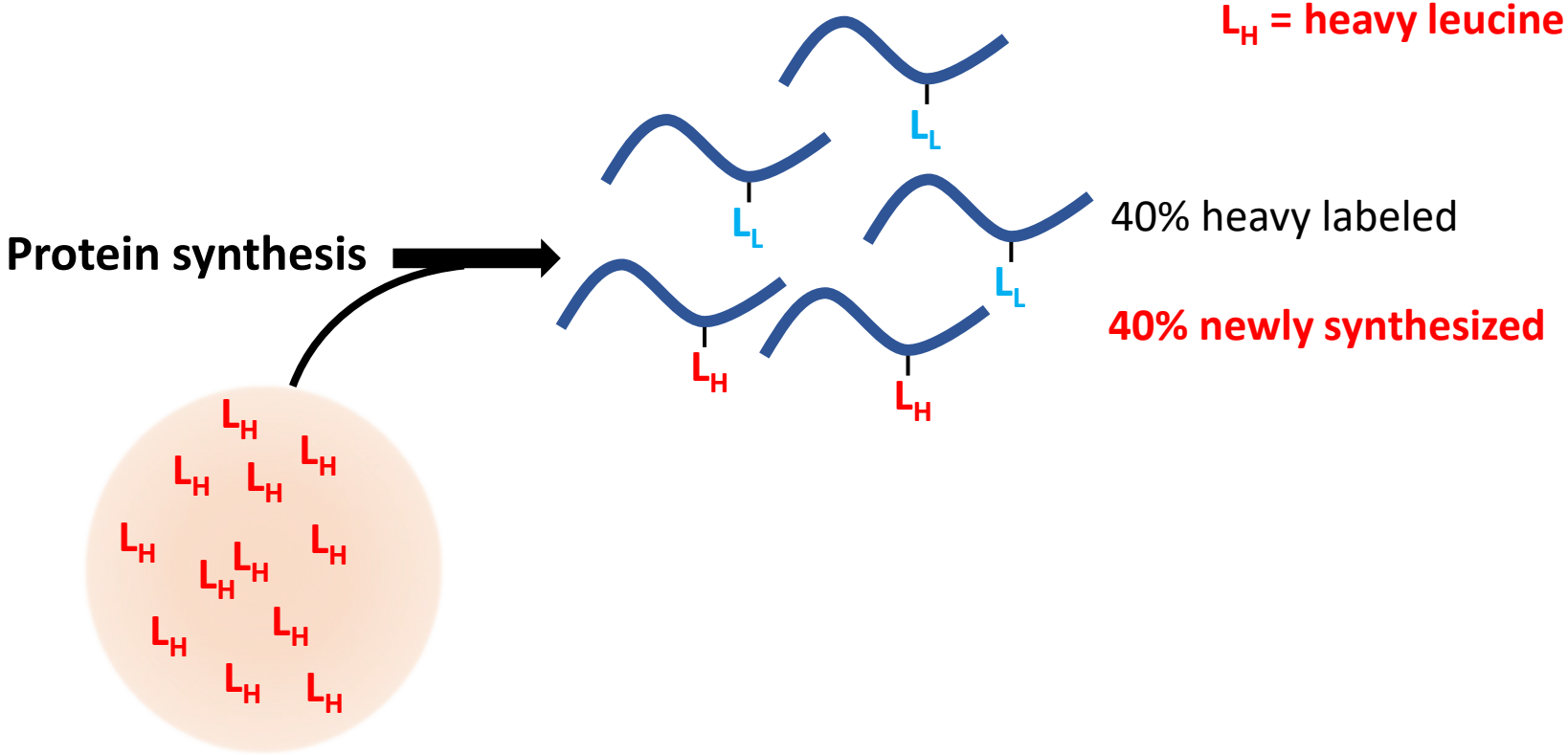

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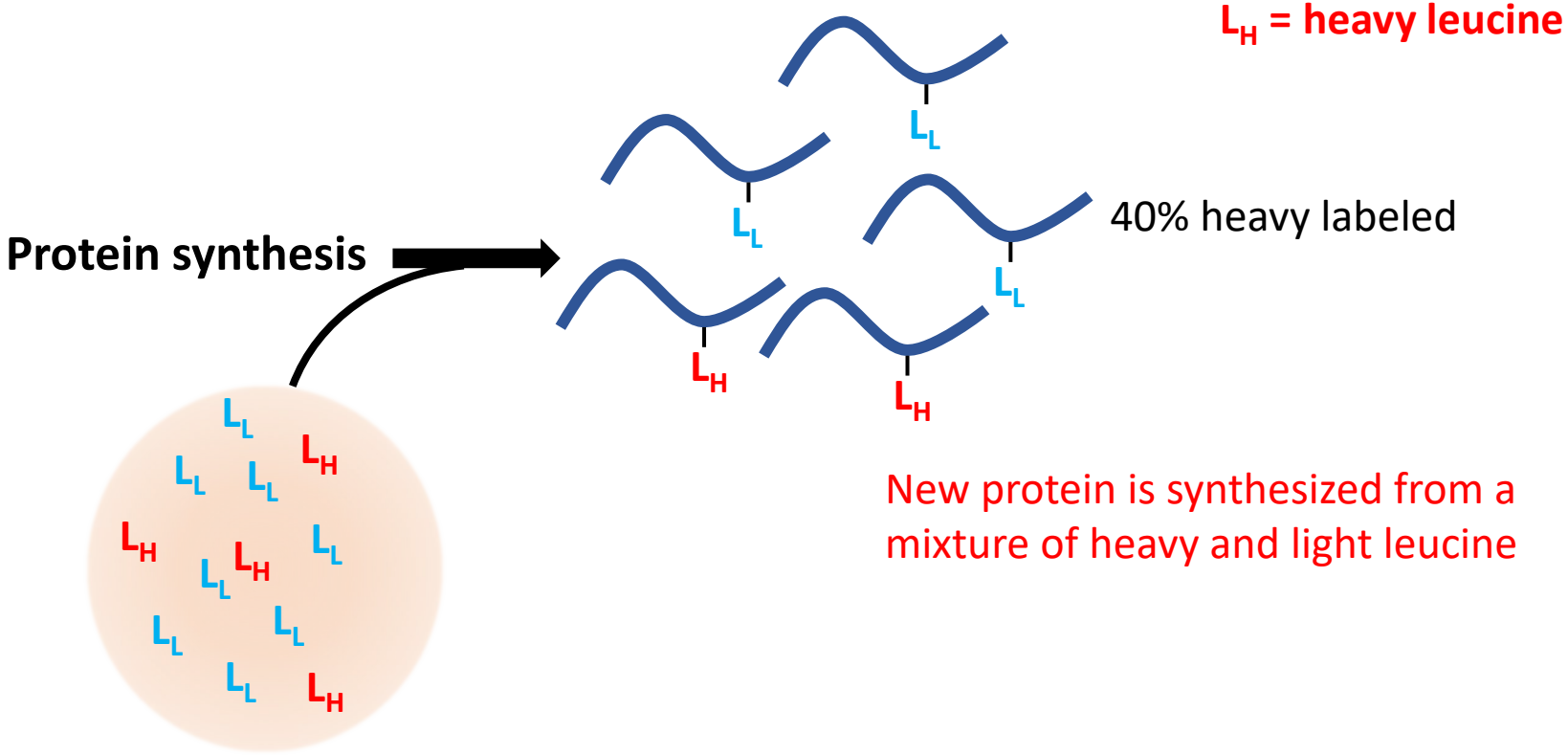

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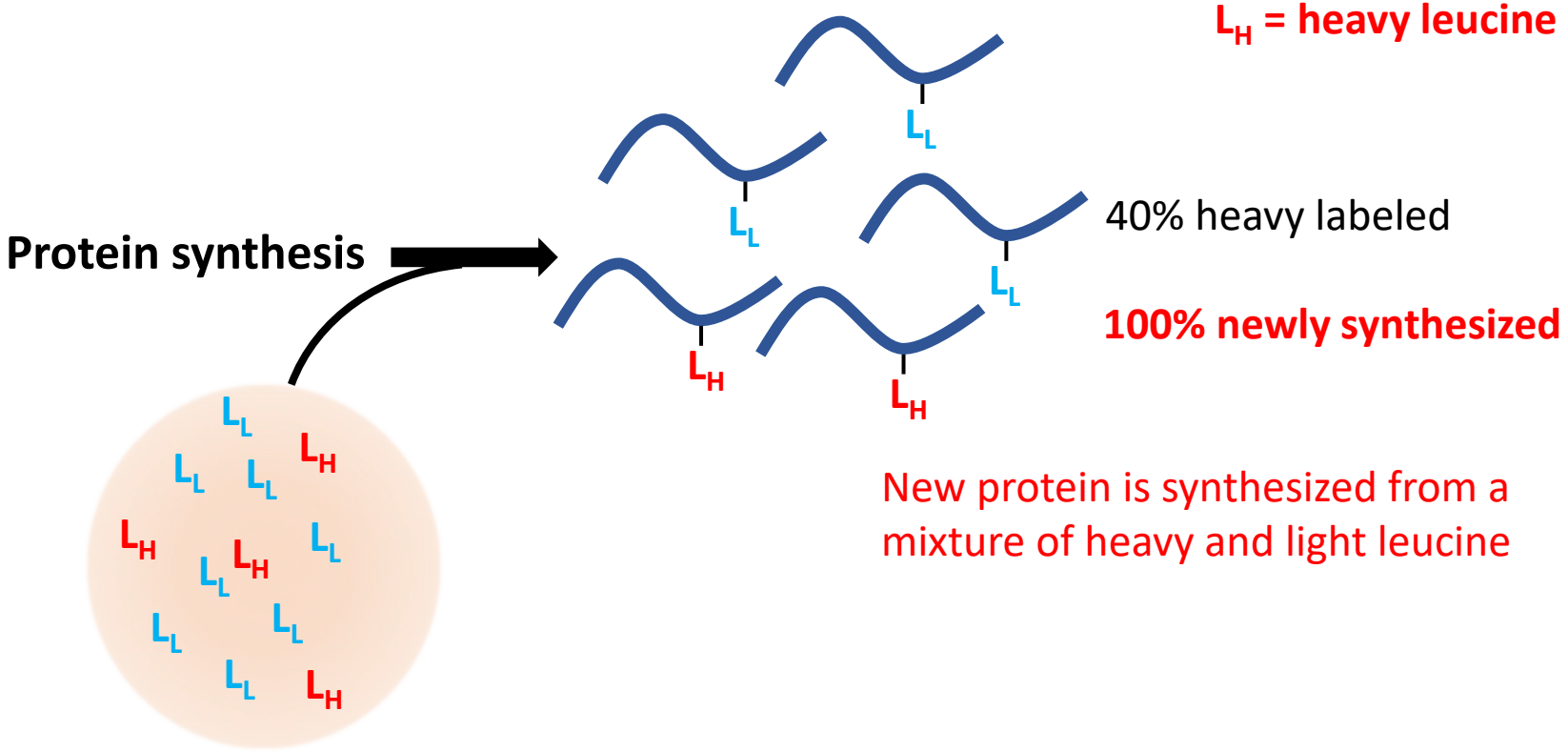

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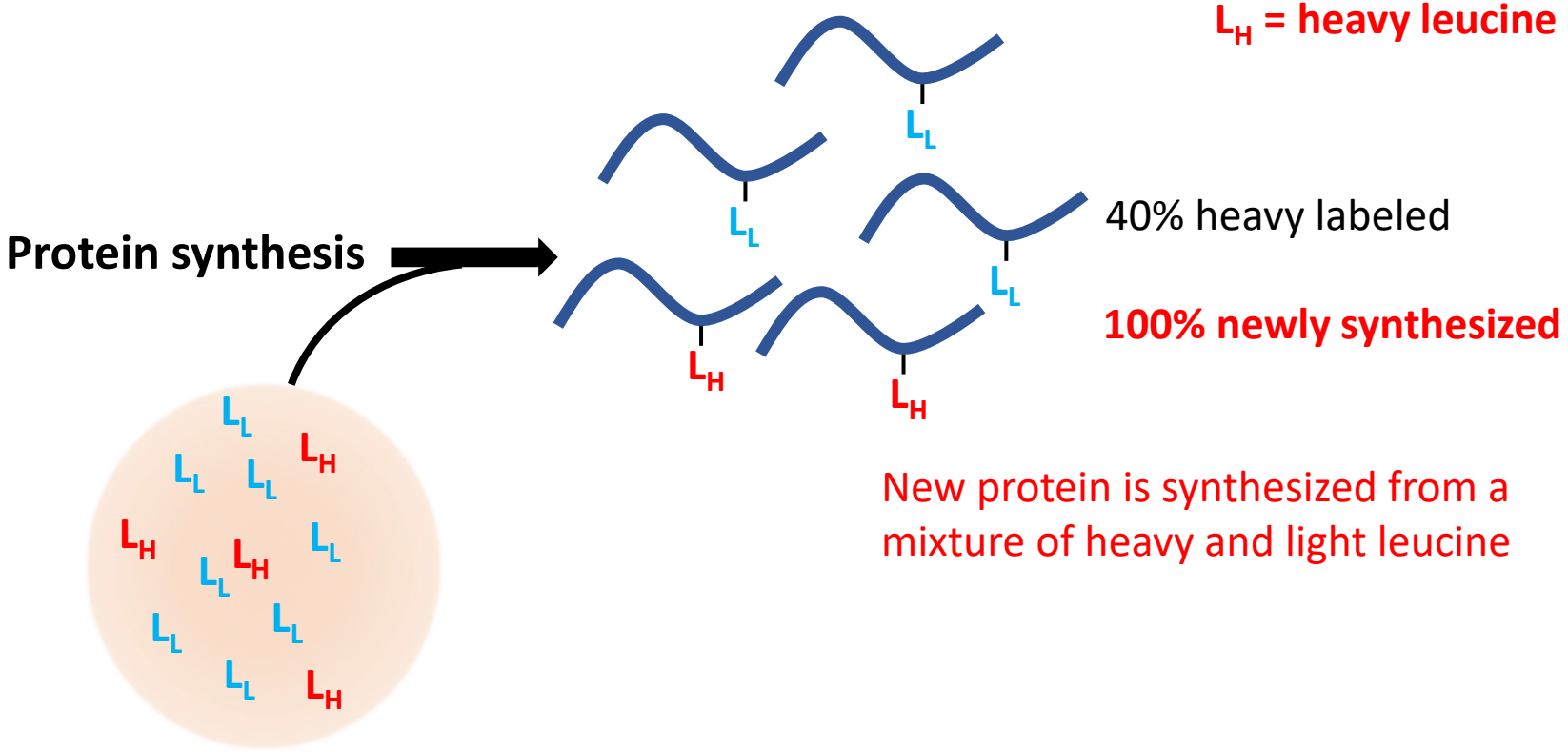

Precursor pool correction is required for accurate calculation of turnover

TurnoveR Computational Pipeline

- 'true' isotope distributions
- **precursor pool enrichment**
- % newly synthesized
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2 leucine peptide, 40% precursor pool label

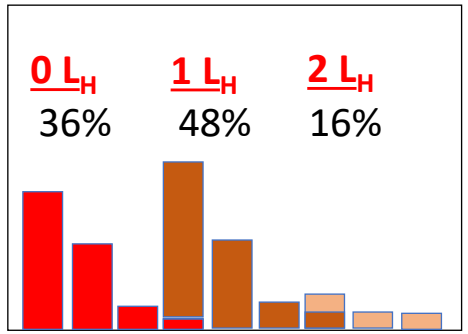
-60% of new leucines will be light (L_L)

-40% of new leucines will be heavy (L_H)

FTHL_LYTL_LIVK	FTHL_LYTL_HIVK	FTHL_HYTL_LIVK	FTHL_HYTL_HIVK
60% x 60%	60% x 40%	40% x 60%	40% x 40%
36%	24%	24%	16%

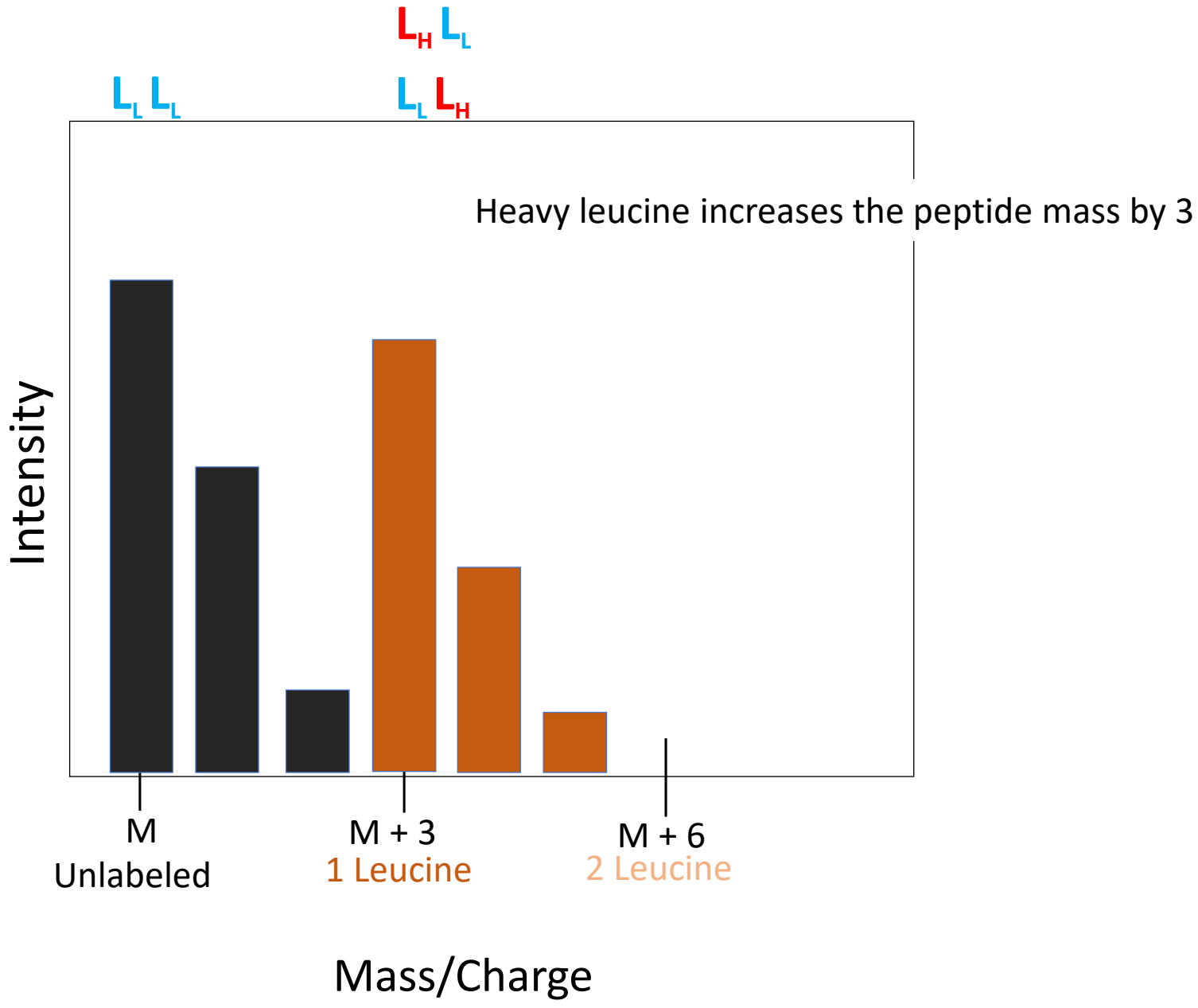
No heavy L	1 heavy L	2 heavy L
36%	48%	16%

40% Label



Binomial distribution calculation

Precursor Pool Estimation



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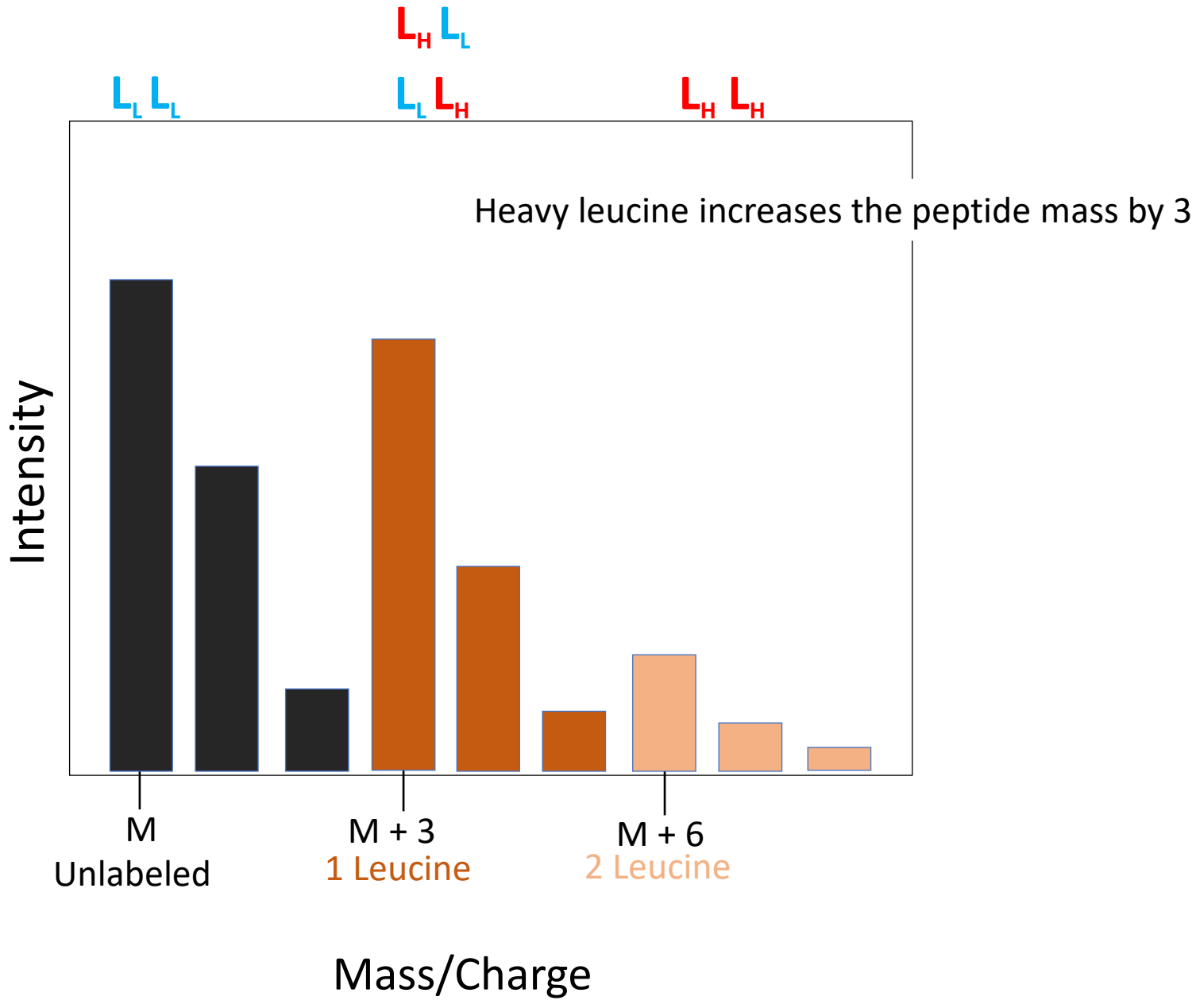
Precursor Pool Estimation



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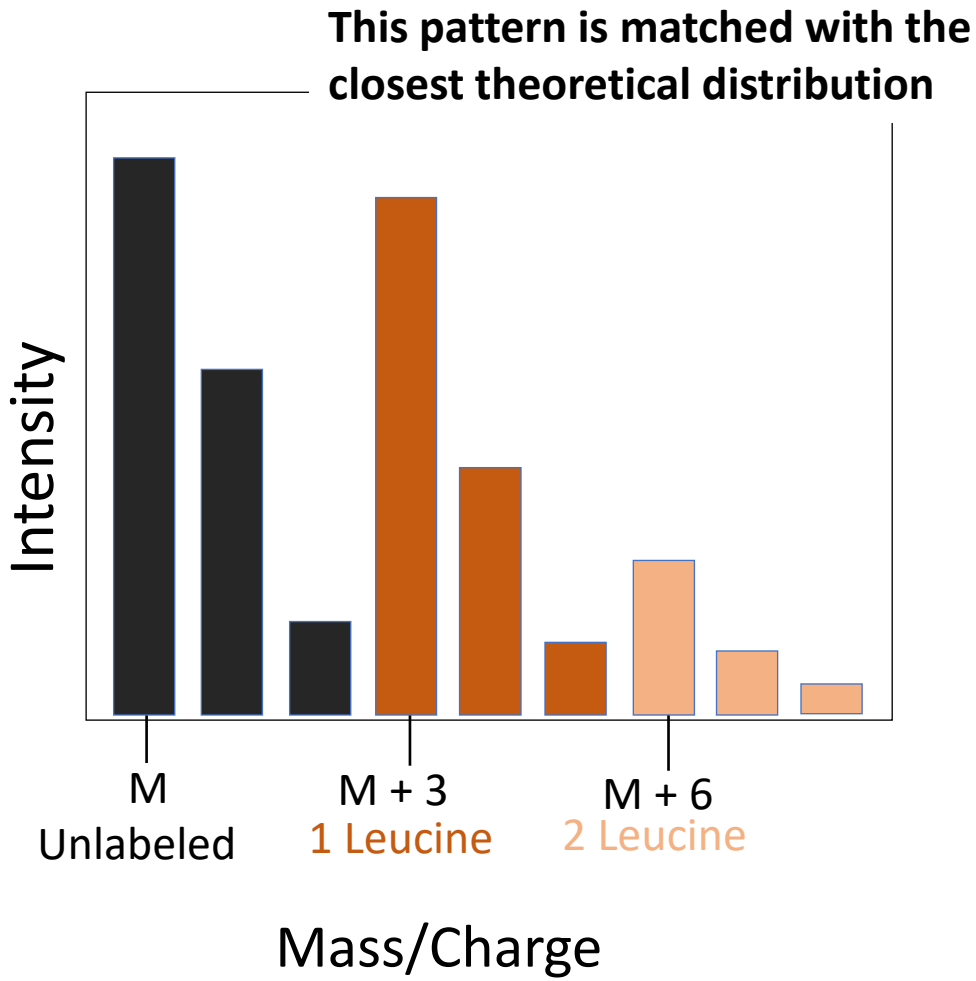
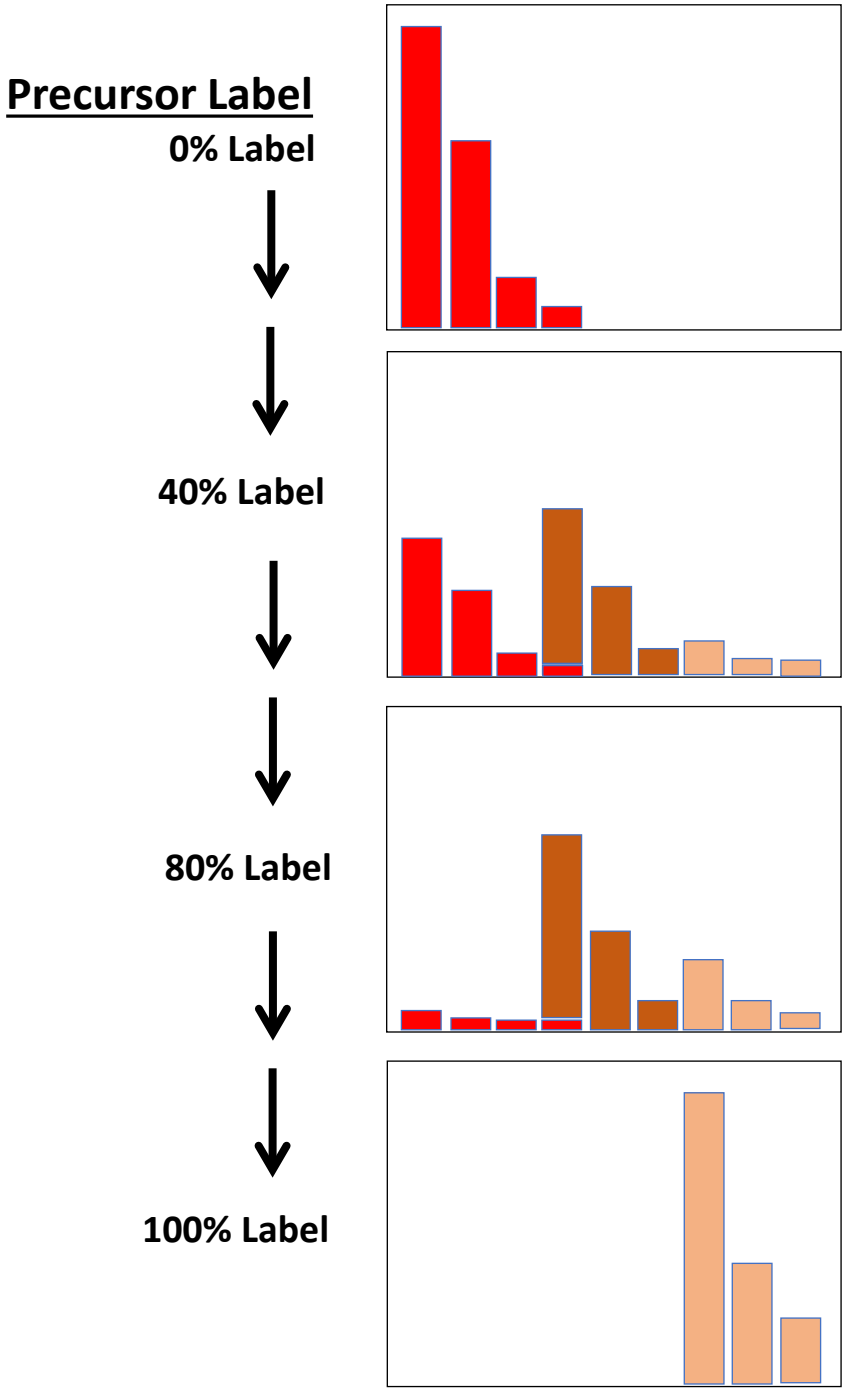
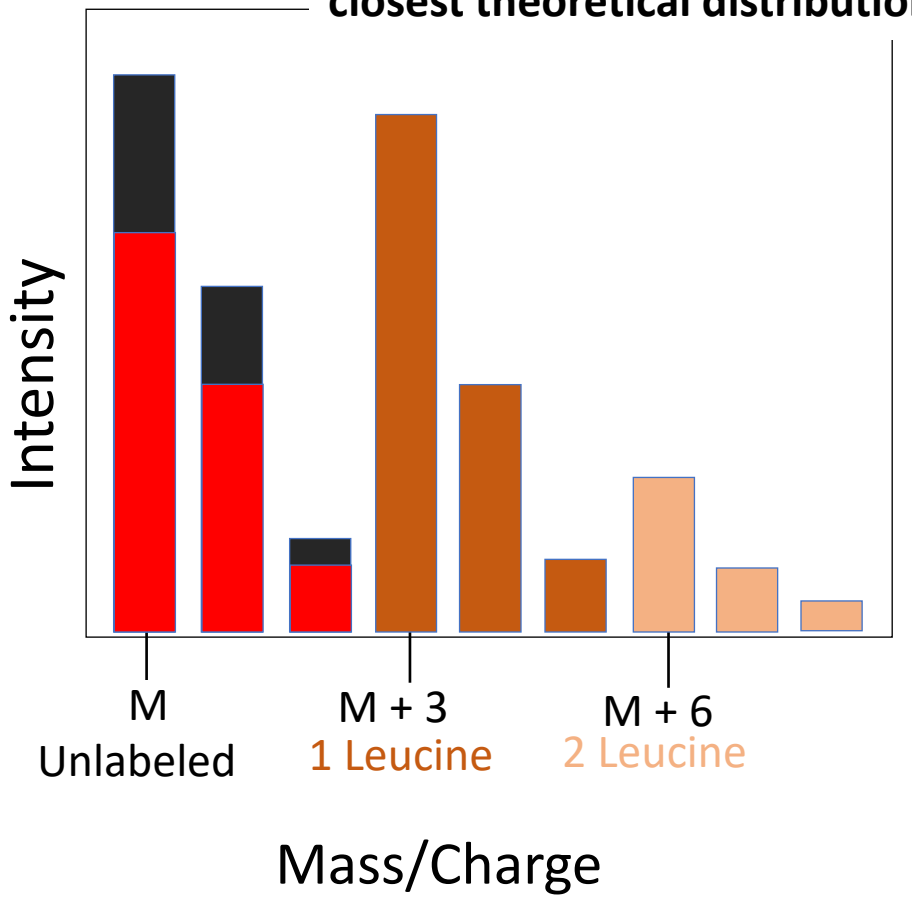

This pattern is matched with the closest theoretical distribution
Intensity
M
Unlabeled
M + 3
1 Leucine
M + 6
2 Leucine
Mass/Charge


Precursor Label
0% Label
40% Label
80% Label
100% Label

This peptide has:

40% Precursor Pool Label

This pattern is matched with the closest theoretical distribution



Precursor Label

0% Label

40% Label

$0\ L_H$ 36%

$1\ L_H$ 48%

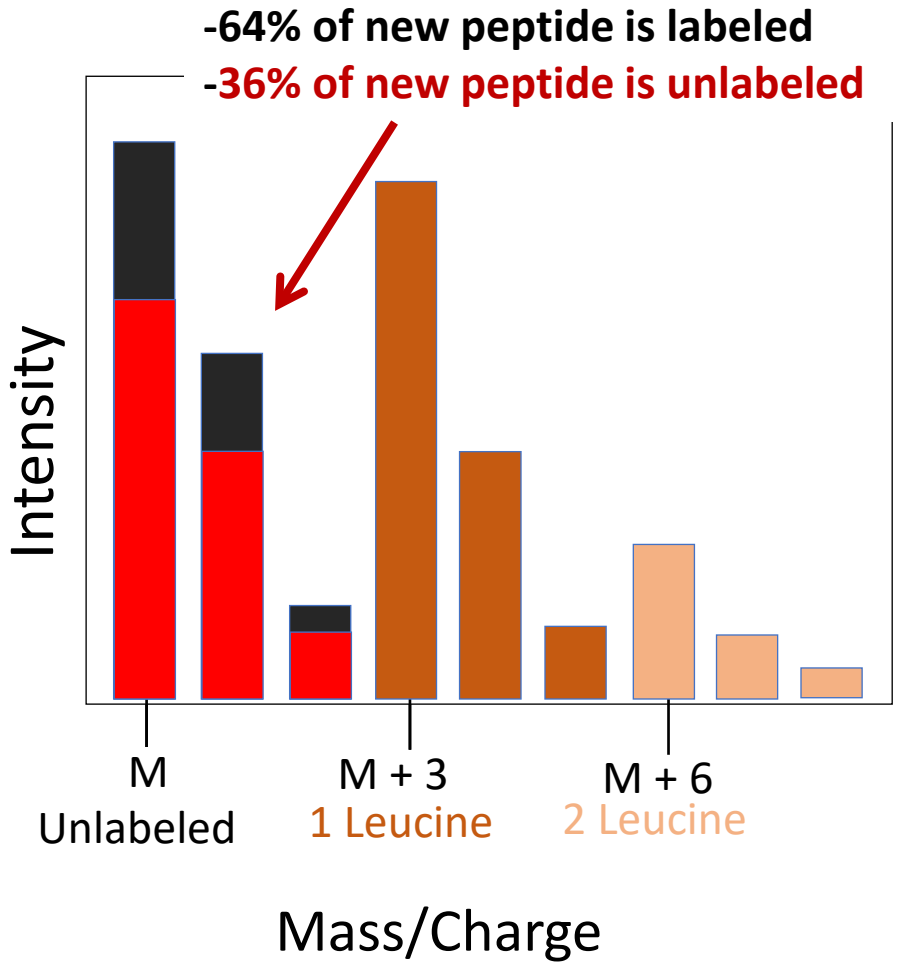
$2\ L_H$ 16%

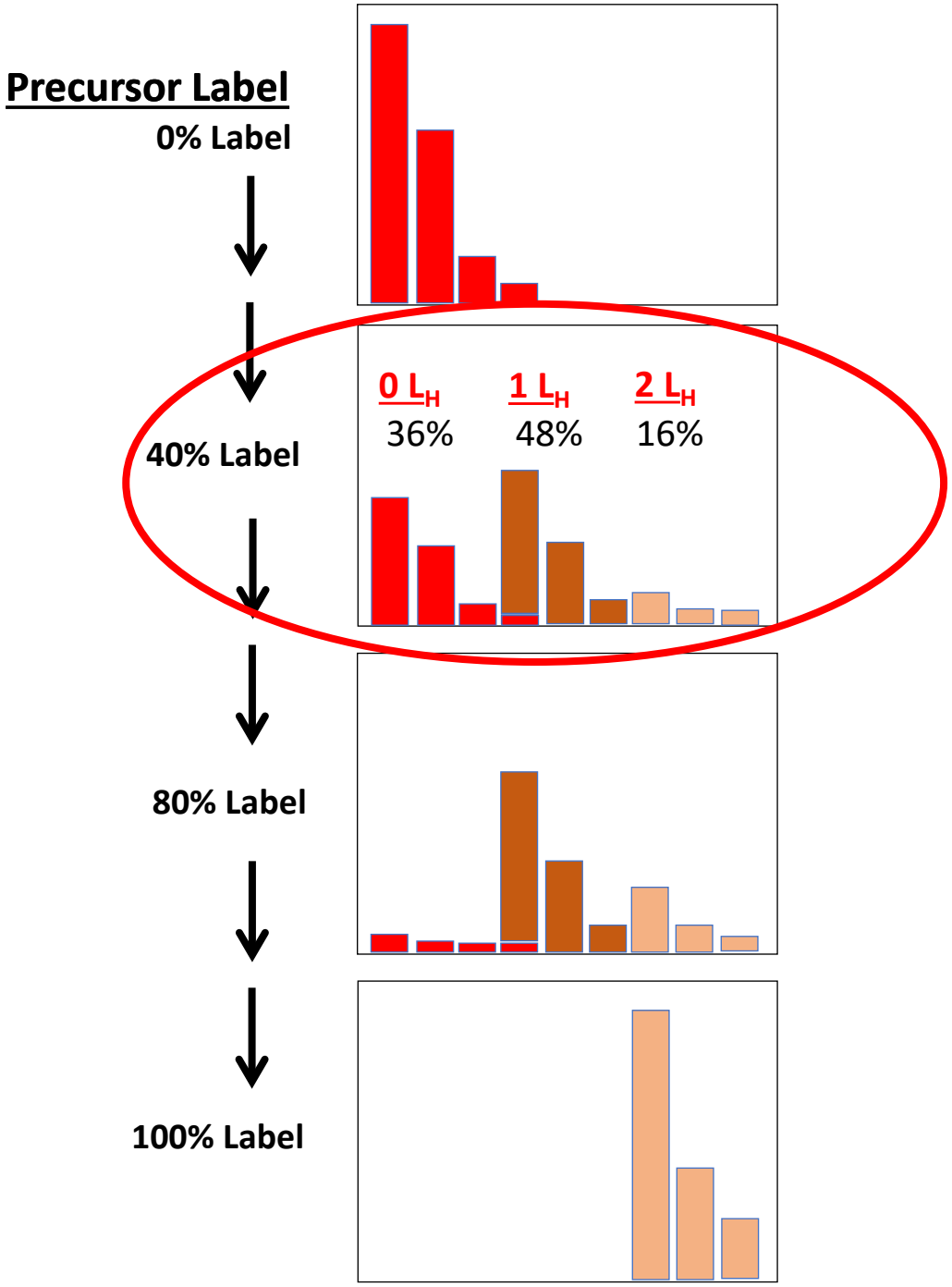
80% Label

100% Label

This peptide has:

40% Precursor Pool Label





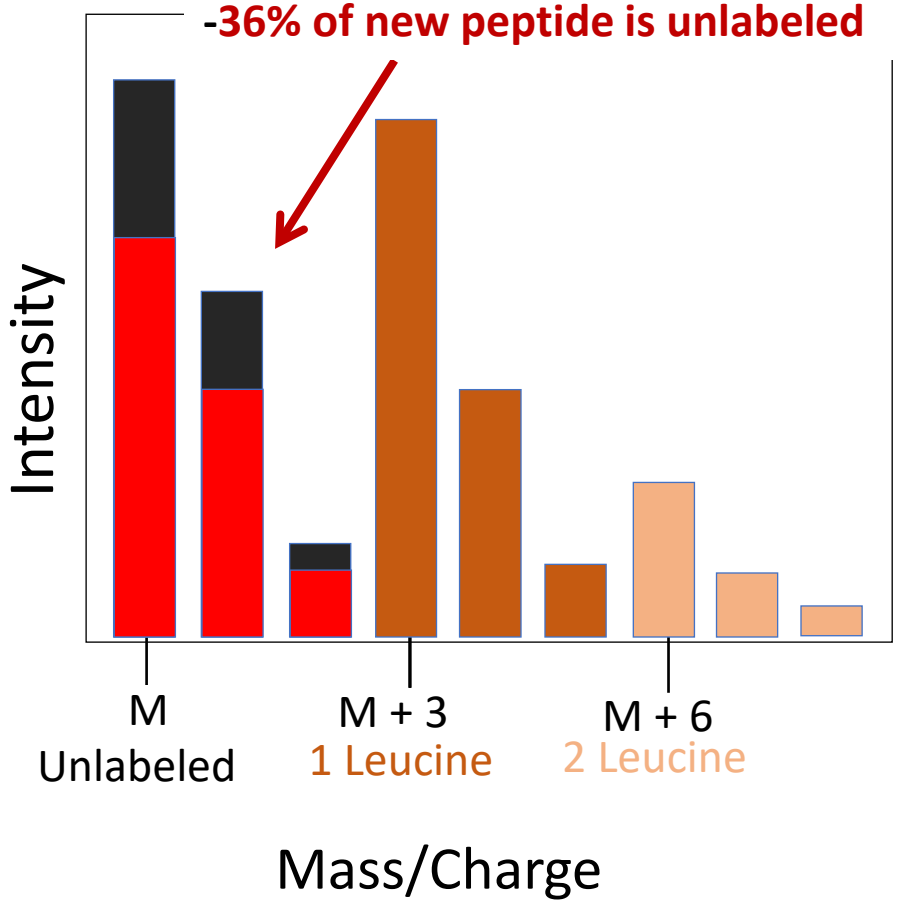
This peptide has:

40% Precursor Pool Label

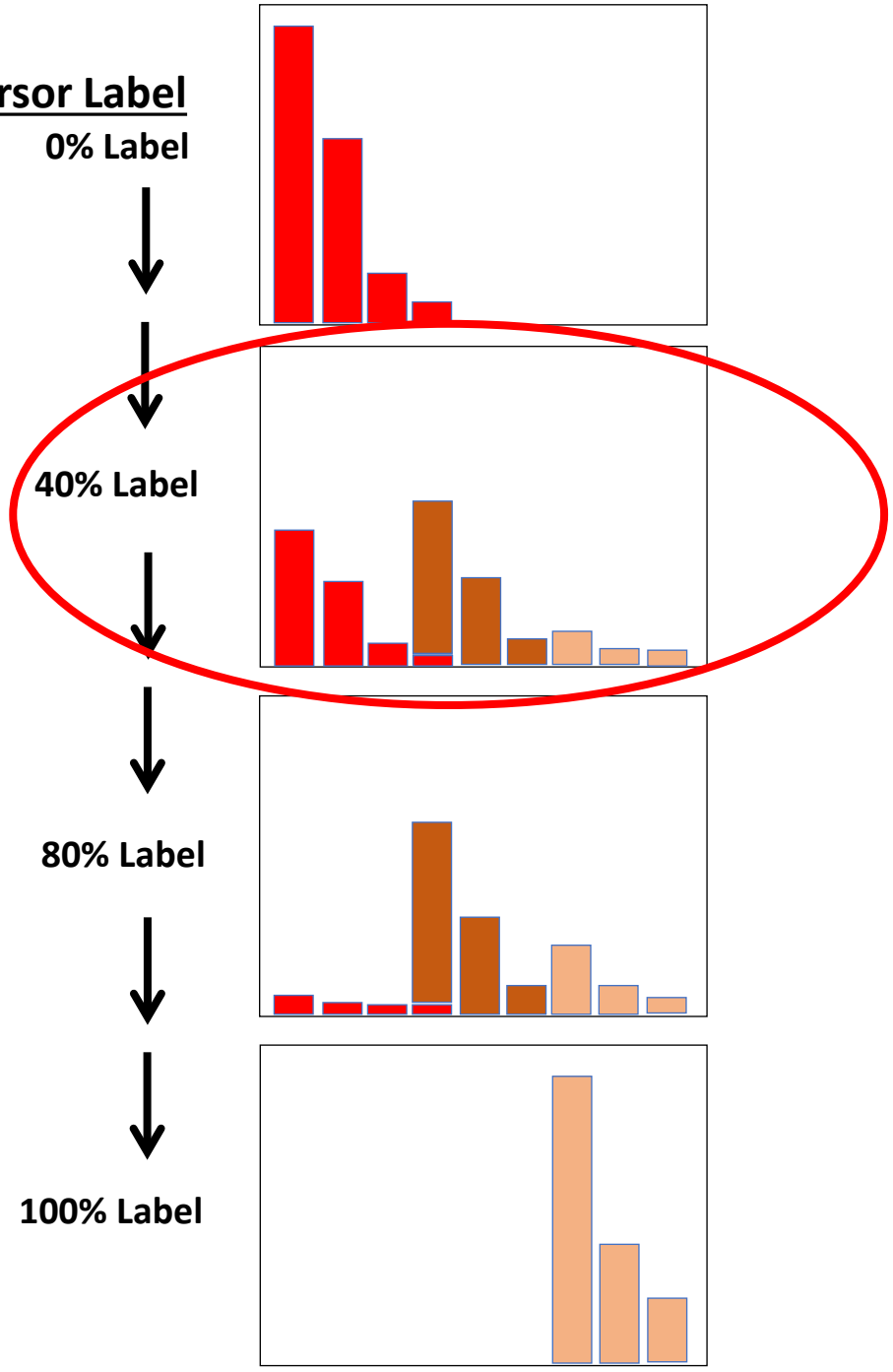
Total % New peptide = $\frac{\text{Unlabeled} + 1\text{ label} + 2\text{ label}}{\text{Total}}$

-64% of new peptide is labeled

-36% of new peptide is unlabeled



Precursor Label



This peptide has:

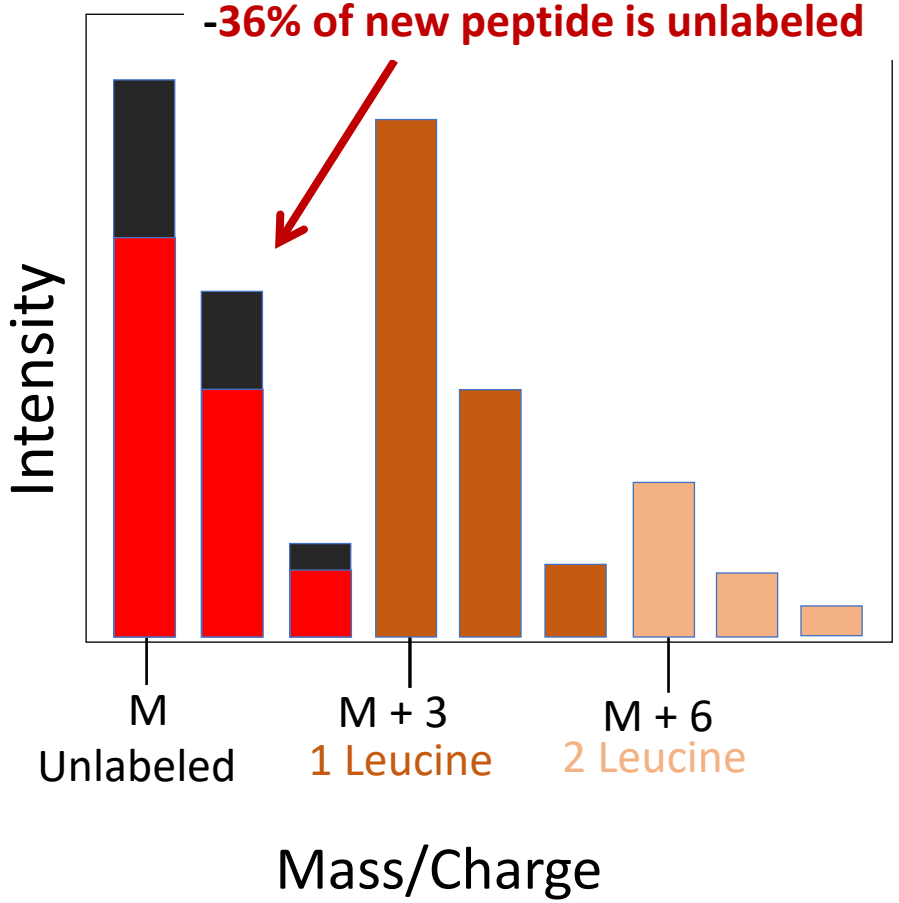
40% Precursor Pool Label

84% of Total Peptide is newly synthesized

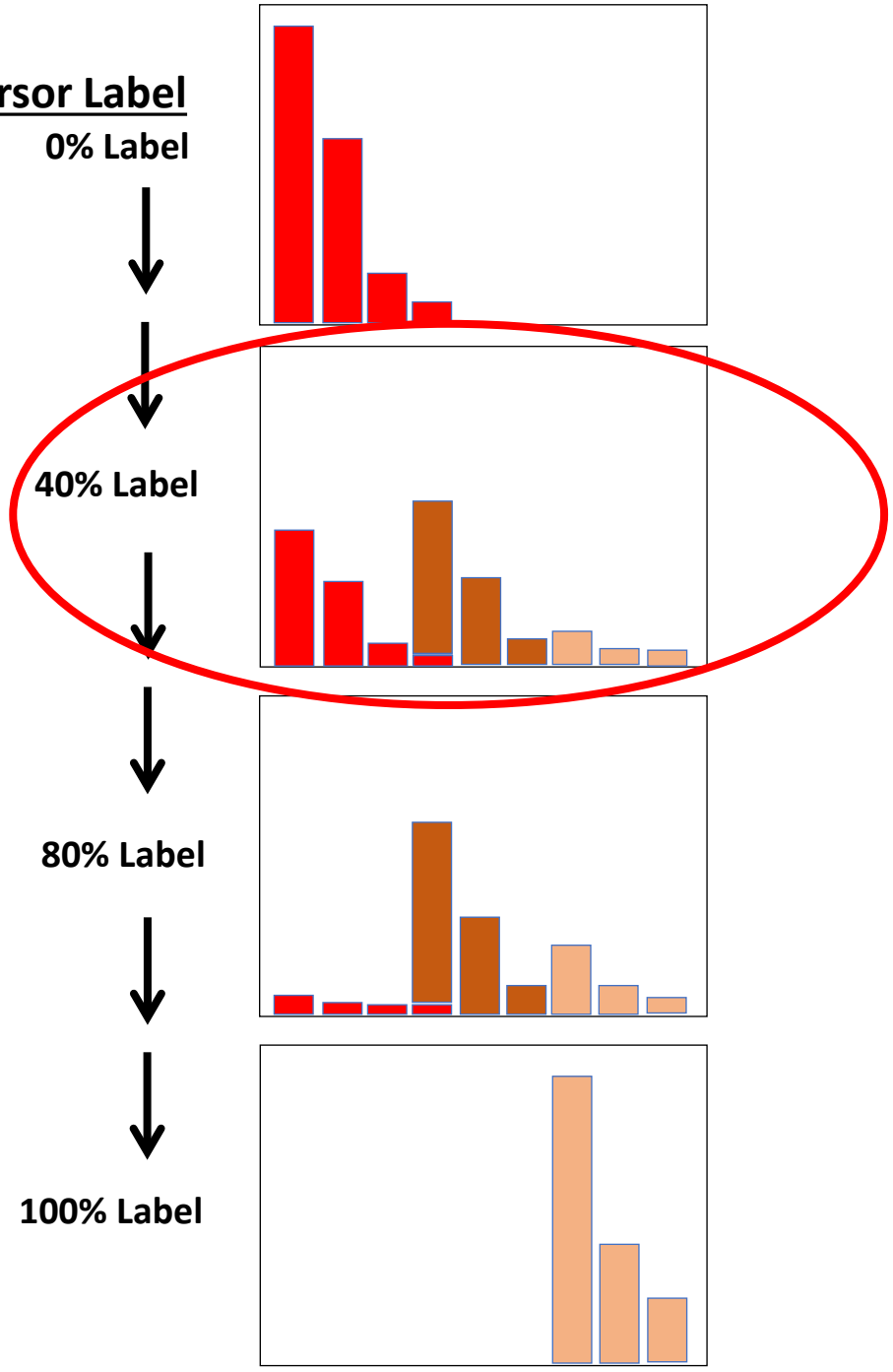
Total % New peptide = Unlabeled + 1 label + 2 label / Total

-64% of new peptide is labeled

-36% of new peptide is unlabeled



Precursor Label



Precursor pool correction is required for accurate calculation of turnover

TurnoveR Computational Pipeline

- 'true' isotope distributions
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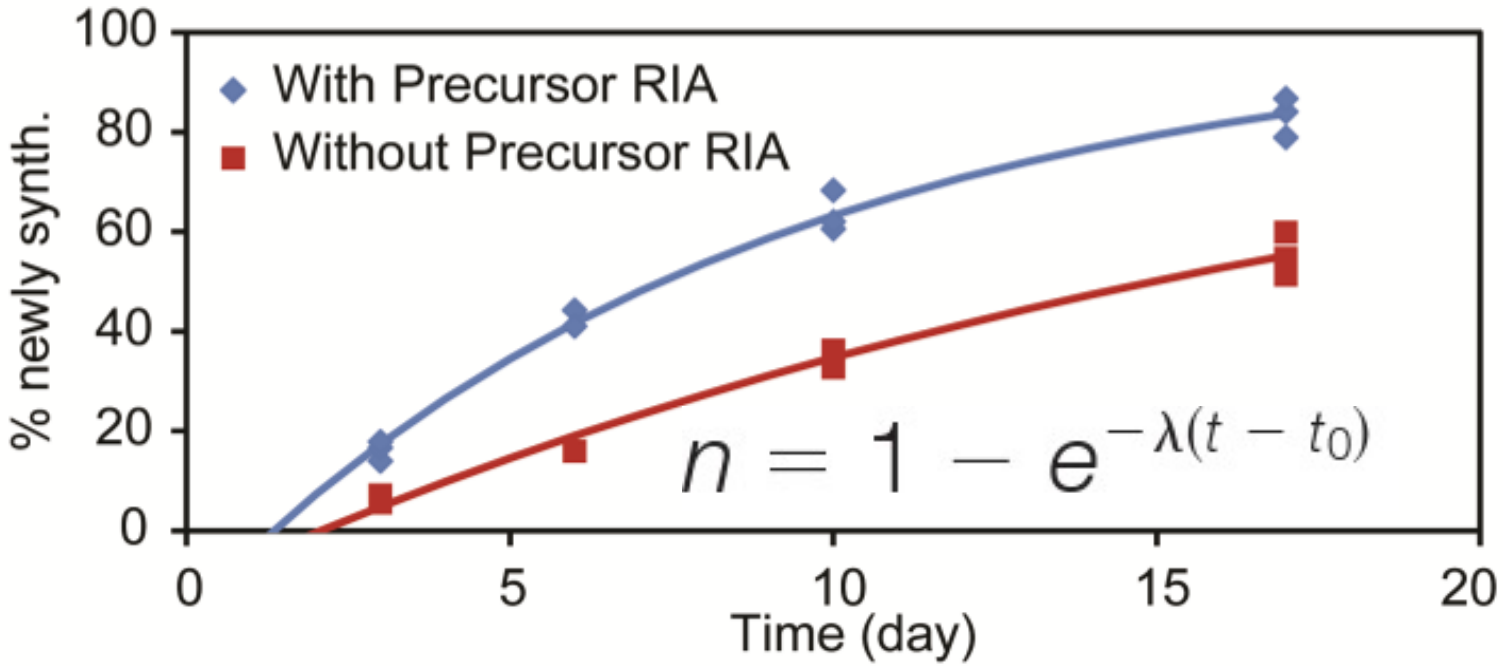
Always overestimated

Half-life without precursor RIA = 12.9 days

Half-life with precursor RIA = 6 days



Hsieh E, Shulman N … MacCoss. 2012. Molecular & Cellular Proteomics

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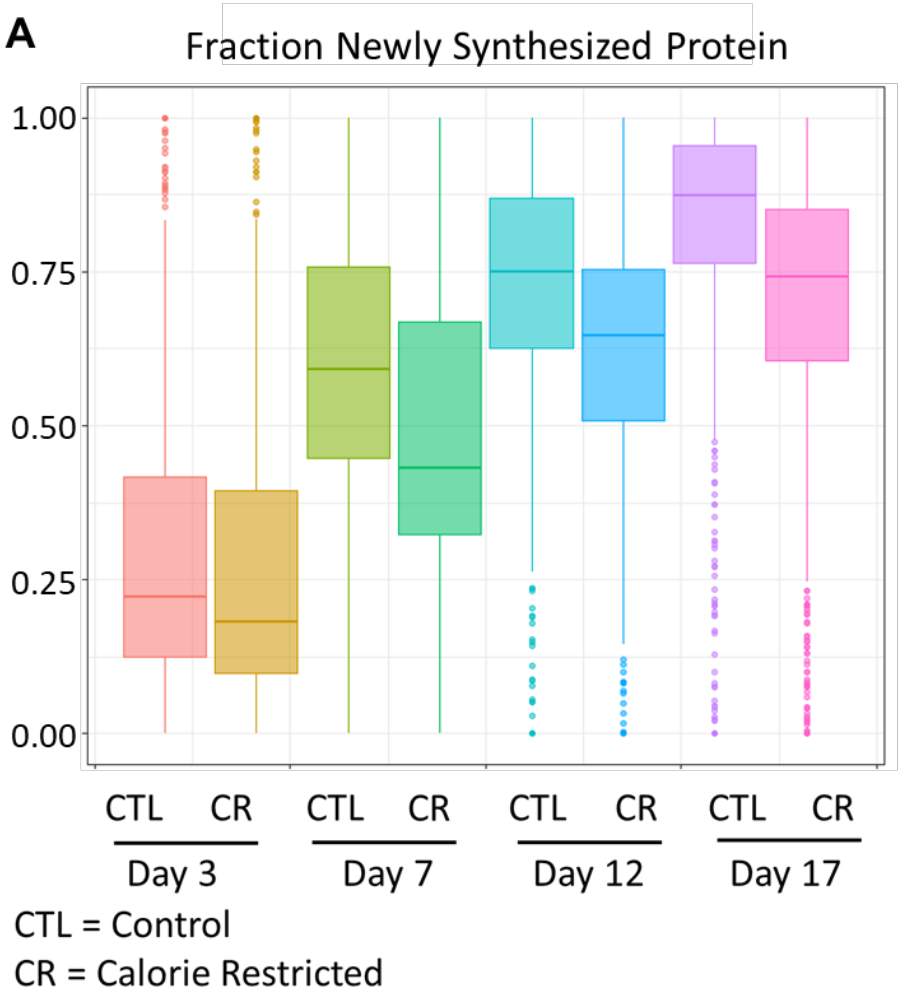
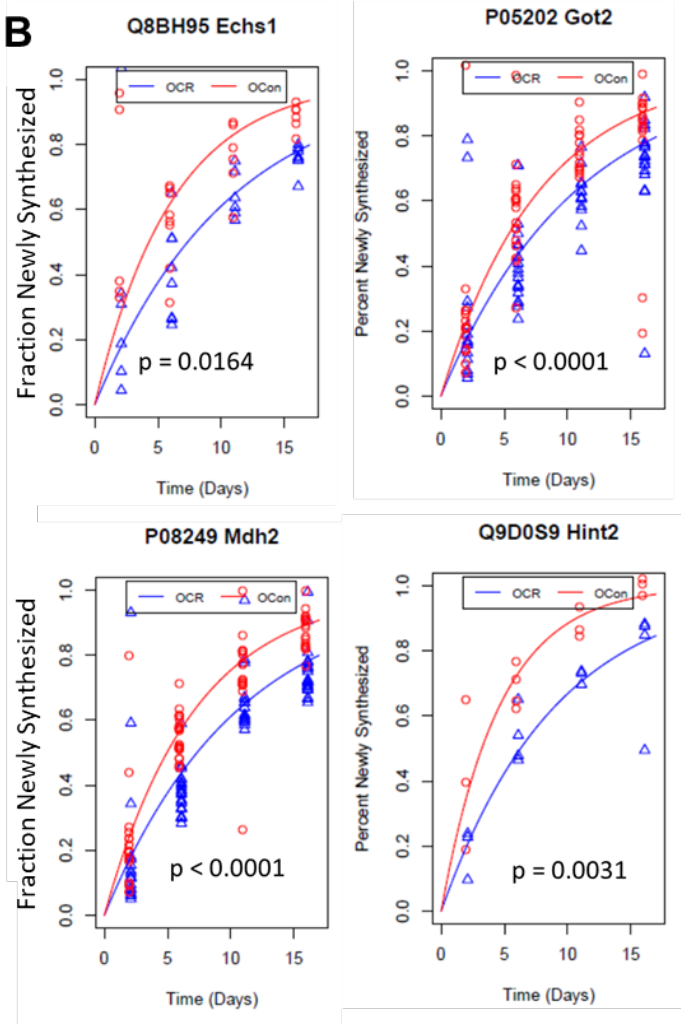
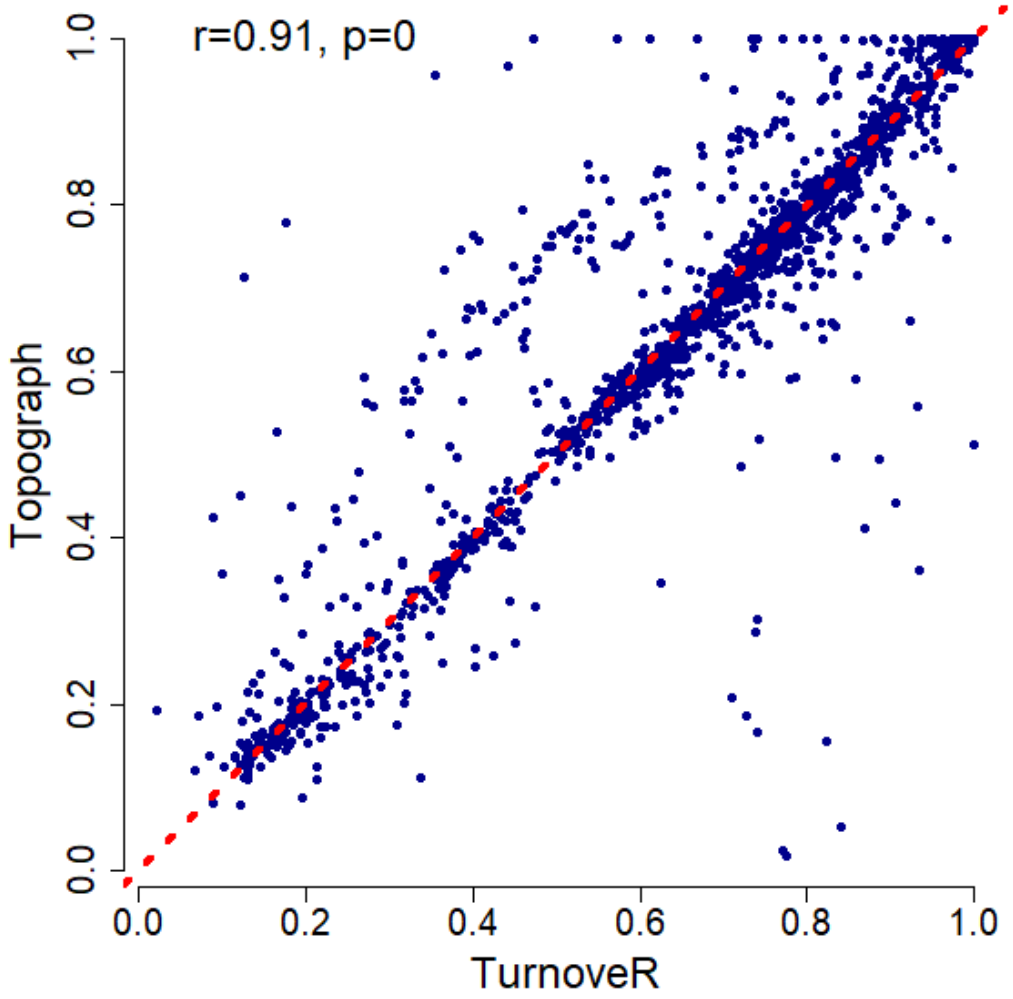




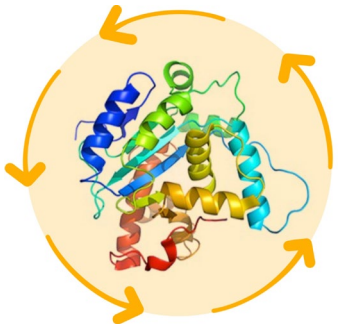
CTL = Control
CR = Calorie Restricted

TurnoveR Recapitulates Results Calculated by Topograph Software.

Topograph



TurnoveR



Next Steps in Method Development

- Compatibility with various metabolic labels (Heavy water)
- SILAC workflows

Outstanding Questions about Protein Turnover in Aging

- The turnover paradox of senescent cells
- How do PTMs (acylation, etc) affect protein turnover?
- Is longer half-life a biomarker for interventions that extend lifespan?

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Quinn Strassheim
Thedoe Nyunt

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U01AG060906,
Glenn Foundation

Skyline

NIH National Institute on Aging

Buck
Live better longer.

Visit our posters at ASMS:

- **Matthew Payea -** ThP 466
- **Nathan Basisty** - WP 028

Hiring Postdocs!

Translational Geroproteomics Unit (TGU)
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Nathan.basisty@nih.gov