Measuring Protein Half Life In Vivo While Compensating For Tissue-Dependent Amino Acid Tracer Absorption Rates

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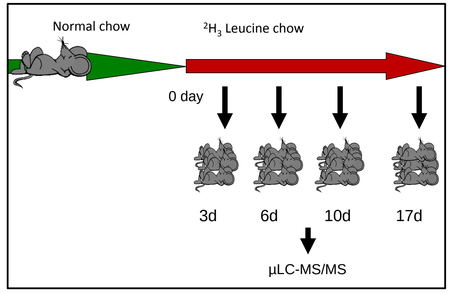
http://proteome.gs.washington.edu/software/topograph

Overview:

Protein kinetics can be observed by altering an organism's diet to include a heavy isotope labeled amino acid and then observing the appearance of that label in proteins. Converting the rate of label incorporation into an absolute rate of protein turnover is complicated by the fact that the amino acid precursor pool in the tissue of interest does not immediately change to reflect the new diet. By examining partial labeling of peptides containing multiple labeled amino acids, this time lag can be corrected for.
We have developed the open source computer program ***Topograph*** which analyzes data from shotgun protein turnover experiments.

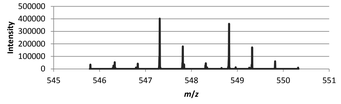
Methods:

48 mice were fed a diet consisting of $^2H_3$ labeled leucine. They were sacrificed at 3 days, 6 days, 10 days, and 17 days.
Liver, heart, and brain samples were taken.
Both whole cell samples and mitochondrial enriched samples were analyzed.

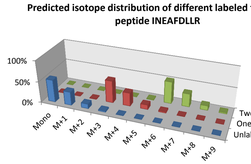


Peptide samples were separated chromatographically over a 40cm (75 µm ID) micro capillary column (Phenomenex Jupiter 4µm Proteo 90Å C-12 resin) using a 3 hour linear gradient from 9% acetonitrile, 0.1% formic acid to 36% acetonitrile, 0.1% formic acid. The HPLC used was a Waters nanoAcquity.
The mass spectrometer used was a LTQFT Ultra (Thermo Scientific). Spectra were acquired with a scan cycle of 1 MS full scan acquired in the FTICR (400 - 1,400 m/z, 50,000 resolution, profile mode) followed by 5 data dependent MS/MS scans in the LTQ ion trap.

Amount of label in one MS1 scan



The observed peak areas are arranged in a column vector **P**



The predicted abundances are arranged in a matrix **A** with a column for each of the labeled forms of the peptide, and a row for each mass.

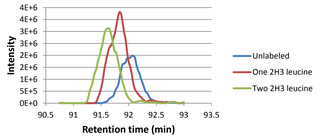
For the vector **X** representing the amount of each labeled form of the peptide present in the sample, the least squares solution to **X** can be efficiently computed by a matrix equation

$$\mathbf{X} = (\mathbf{A}^T\mathbf{A})^{-1}\mathbf{A}^T\mathbf{P}$$

That is, **X** is equal to the product of the *inverse* of **A** *transpose* **A**, times **A** *transpose*, times **P**.[1]

Amount of label in sample

Because deuterated leucine causes a retention time shift, the amount of label found in a single MS1 scan is not representative of the entire sample. The amount found in each scan can be plotted in a chromatogram, and the retention time shift can be seen.



Precursor pool enrichment

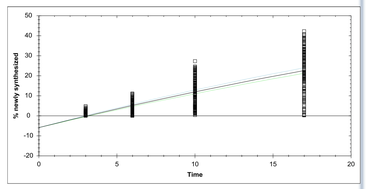
The peptide in the sample comes from a mixture of old and newly synthesized protein.
If we assume that the newly synthesized protein had a constant precursor pool enrichment then there are three unknowns: quantity of old protein, quantity of new protein, and precursor enrichment ε . This can be solved if the peptide contains two or more leucines.

For a peptide with *n* leucines synthesized from a precursor pool with enrichment ε, the probability that it will have *k* heavy leucines is:

$$P_{new}(k) = \varepsilon^k(1-\varepsilon)^{n-k}\binom{n}{k}$$

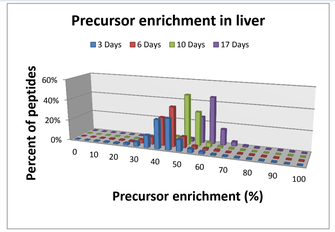
Protein half life

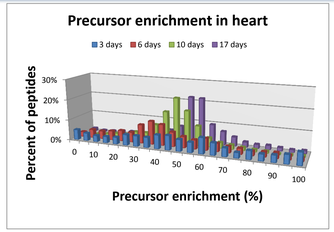
In the entire sample, the median value of the precursor enrichment across all peptides is used to compute the amount of newly synthesized peptide. These values are plotted for all peptides from a given protein at all time points, and an exponential curve is fit through the data.



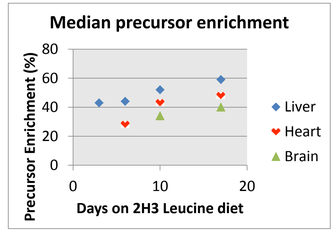
Half life of Aconitate hydratase (Aco2) in mouse heart is 37.6±2.1 days

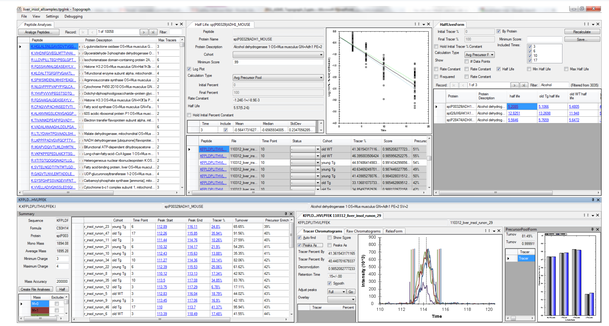
Results:



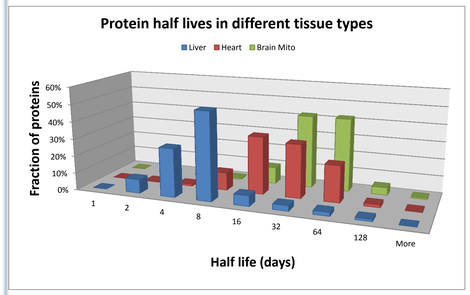


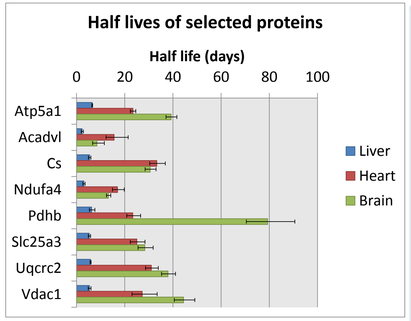
Three days after the mouse diet is altered to include deuterated leucine, there is enough newly synthesized protein incorporating the label to be able to estimate the precursor pool enrichment at 41%.
In the heart, however, the time lag for the label in the diet to be incorporated into new protein was too long, making it difficult at 3 days to distinguish newly synthesized from existing protein.
This problem was worse in the brain, where it was not possible to get an accurate estimate of the precursor enrichment until the tenth day.





The computer program ***Topograph*** provides a graphical user interface for analyzing data in protein turnover experiments. ***Topograph*** performs the calculations to determine the median precursor enrichment in each data file, and uses that value to compute the fraction of protein that was newly synthesized at each time point. ***Topograph*** performs a simple linear regression to calculate half lives for hundreds of proteins.





Half lives were measured for a large number of proteins in the mouse liver, heart, and brain. Proteins turn over fastest in the liver, and slowest in the brain.

Conclusions:

Leucine precursor enrichments can be estimated by analyzing multiple leucine containing peptides. Precursor enrichment values can be used to compute protein half lives.

References:
(1) Brauman, J.I. Anal Chem. 1966, 38(4): 607-610
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