A new software tool for analyzing mass spectrometry data in protein turnover experiments

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Overview:

Protein kinetics can be observed by altering an organism's diet to contain 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 software program Topograph which provides a graphical user interface and automates many of the calculations necessary to analyze protein turnover data.

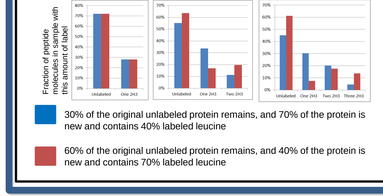
Introduction:

After an organism's diet is changed to include a heavy isotope labeled amino acid, newly synthesized protein incorporates that label. The fraction of labeled amino acid found in a particular protein at a later time is the product of the fraction of that protein that was newly synthesized and the fraction of amino acids that were labeled when that protein was synthesized.

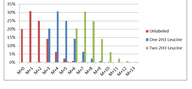
In a peptide containing multiple labeled amino acids, the amount of partial labeling observed can be used to infer the fraction of amino acids that were labeled.

$^2H_3$ leucine is a good amino acid to use in protein turnover experiments because it is an essential amino acid in animals, and, unlike lysine, will yield many partially labeled peptides when digested with trypsin.

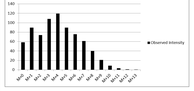
Peptides containing multiple leucine residues can be used to distinguish scenarios that are indistinguishable for a peptide containing only one leucine.



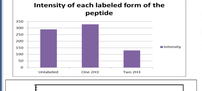
Steps to determine amount of each labeled form of a peptide from MS scans



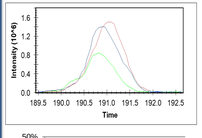
Step 1: Generate the theoretical intensities for each isotopomer of the peptide. For $^2H_3$ labeled leucine, the distributions are each offset by 3 Daltons.



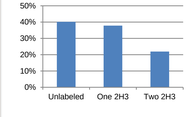
Step 2: Examine an MS scan and record the intensities at the *m/z* values that are part of the distribution of at least isotopomer of the peptide.



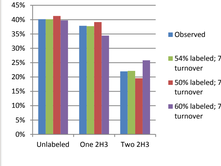
Step 3: Determine the linear scaling of the isotopomer distributions which will most closely match the observed intensities.



Step 4: For each MS scan, plot the amount of each isotopomer calculated in step 3.



Step 5: Integrate the areas under the chromatograms in step 4 to determine the relative amounts of each isotopomer in the entire sample.[1]

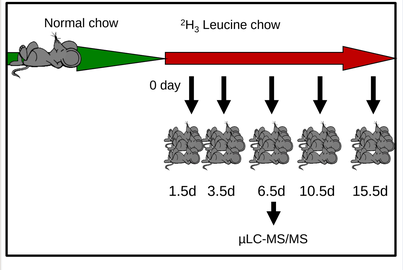


Step 6: Compare the observed distribution of isotopomers to those which one would predict for different values of the rate of turnover, and the fraction of labeled amino acid precursor. Find the combination of values for fraction of protein that is newly synthesized and the fraction of the amino acids in that new protein that is labeled that most closely matches the observed distribution of isotopomers.

Methods:

Fifteen mice were fed a diet consisting of $2H_3$ labeled leucine. They were sacrificed at 1.5 days, 3.5 days, 10.5 days, and 15.5 days.

- Dounce homogenization
- 800g centrifugation (collect supernatant)
- 4000g centrifugation (collect pellets)
- Resuspend pellets in 19% Percoll
- Percoll step-gradient (60%, 30% and 19%) centrifugation 10000g
- Collect mitochondrial fraction between 30% and 60% Percoll layer



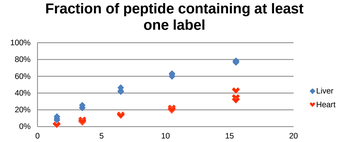
Peptide samples were separated chromatographically over a 40cm (75 µm ID) micro capillary column (Phenomenex Jupiter 4µm Proteo 90Å C-12 resin) using a 3 hour linear gradient from 9% acetonitrile, 0.1% formic acid to 36% acetonitrile, 0.1% formic acid. The HPLC used was a Waters nanoAcquity.

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.

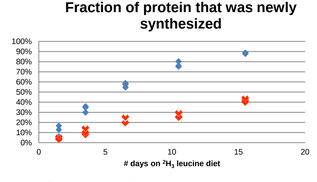
Results:

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

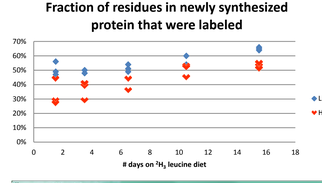
Over 7000 peptides were identified by LC MS/MS in the mouse heart and/or liver samples. Topograph was used to analyze all of the peptides which contained at least one leucine. The peptide K.IVAPELYIAVGISGAIQHLAGMK.D (Alpha-ETF: electron transfer flavoprotein subunit alpha, mitochondrial precursor) was abundant in both heart and liver samples, and its detailed analysis is presented here as an example of the results obtained using Topograph.

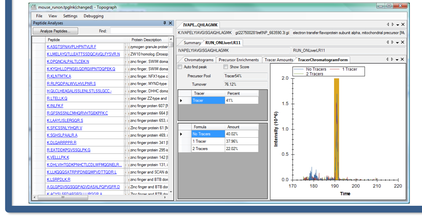


Graph of the amount of singly and doubly labeled peptide found in samples. Since the fraction of labeled amino acid within the tissue was not 100%, the fraction of protein that was newly synthesized will always be greater than the fraction of peptide with at least one label.



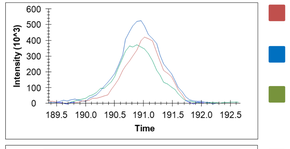
Estimate of the fraction of protein that was newly synthesized. By analyzing the fraction of peptides with 0, 1 and 2 labels, Topograph inferred the fraction of protein that was newly synthesized and the fraction of amino acids that were labeled at the time of protein synthesis. During this experiment, the amount of label in the cells was changing. A more complex mathematical model of the distribution of unlabeled, singly, and doubly labeled peptides may be necessary to calculate more accurate protein turnover values.





The computer program "Topograph" provides a graphical user interface to manage a workspace containing thousands of peptides across multiple MS data files. It performs proteome wide calculations of labeled peptide percentages, and enables the user to curate individual results by hand.

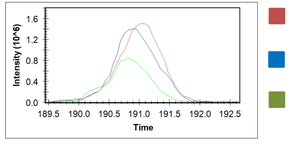
Chromatograms for specific *m/z* channels compared to deconvoluted chromatograms



Monoisotopic mass (M) of peptide (2351 Da)

M+3 (2354 Da)

M+6 (2357 Da)



Unlabeled peptide

One $^2H_3$ leucine

Two $^2H_3$ leucine

Topograph determines the amount of each labeled form of the peptide in each MS scan. In the upper graph, three chromatograms are shown for the peptide K.IVAPELYIAVGISGAIQHLAGMK.D.
The chromatogram for M+3 (3 Daltons above the monoisotopic mass of the unlabeled peptide) has contributions from both the unlabeled peptide (primarily containing $^{13}C_2$), and from one $^2H_3$ labeled peptides.
After deconvoluting the labeled forms of the peptide (lower graph), it is more apparent that the $^2H_3$ labeled peptides elute earlier.

Conclusions:

- Partially labeled peptides can be used to infer amino acid precursor enrichment levels
- Overlapping isotopomer distributions can be deconvoluted in individual MS scans, resulting in chromatograms that show the retention time shift of the $^2H_3$ leucine label

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

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