P05.09 Assessment of Differences Between Literature and Empirical N-Values for Kinetic Proteomics

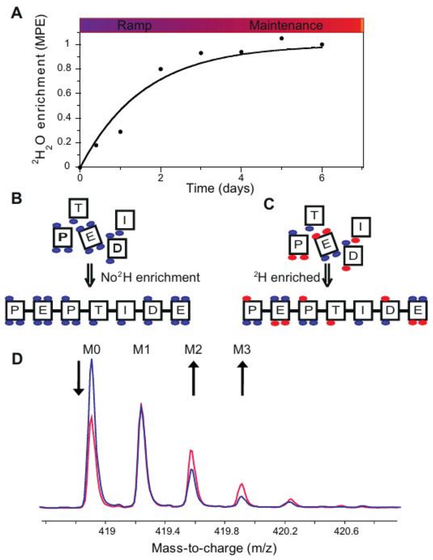
Benjamin Driggs, Coleman Nielsen, J.C. Price

Introduction

The Price Lab ran a mouse diet experiment to see if we could detect changes in amino acid metabolism according to diet. The diets used were low-protein ad libitum (LP AL), low-protein CR (LP CR), high protein ad libitum (HP AL), and high protein CR (HP CR). Multiple individuals were randomly assigned to each diet.The mice were given an initial shot of deuterated water (D2O or heavy water) and provided water with D2O to keep them at a consistent level of 5% D2O enrichment.

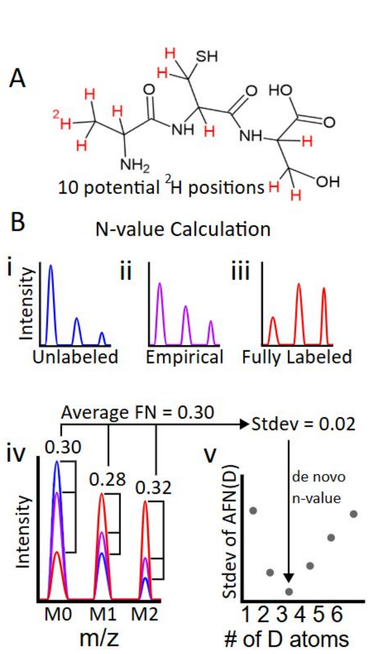
Extracting D2O labelled samples across several time points allows us to use mass spectrometry to measure both protein concentration and turnover (1). These are measured by analyzing the shift in isotopic peaks (2).

Figure 1



N-Value Calculations

Figure 2 - A molecule will have a number of locations where hydrogen can be replaced by deuterium, marked in red (A). Subtracting a theoretical fully labeled distribution (iii) from the unlabeled distribution (i) gives the maximum abundance of a molecule that can be new in the population. The percent of that maximum abundance that is empirically measured (ii) is called fraction new (FN). The theoretical distribution with the lowest standard deviation is selected to represent the n-value for that molecule (iv) (3).



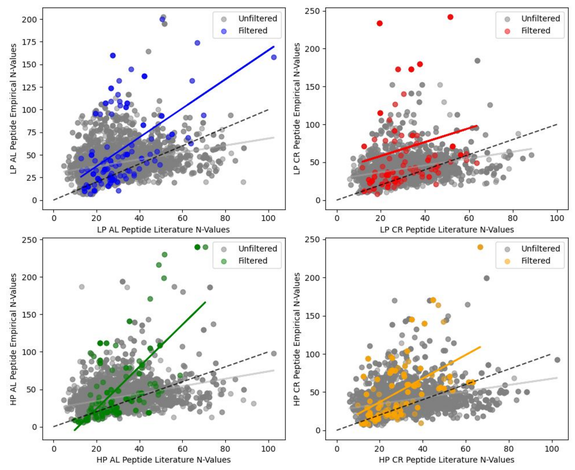
Empirical vs Literature N-Values

Amino Acid	LP AL N-Values	LP CR N-Values	HP AL N-Values	HP CR N-Values	Literature N-Values	Max Labeling Sites
Phenylalanine	8	8	8	8	0.47	8
Histidine	5	2.56	5	0	2.15	5
Isoleucine	6.51	0	4.18	0	0.8	10
Lysine	0	9	4.82	8.13	0.3	9
Leucine	3.38	10	8.23	10	0.7	10
Methionine	3.77	0	0	7.11	1.02	8
Threonine	0	5	0	1.22	0.21	5
Valine	7.83	8	0.74	2.5	0.79	8
Tryptophan	4.5	2.11	1.43	3.25	0.06	8
Average Essential AA Change	**3.61**	**4.24**	**2.88**	**3.75**		
Alanine	4	0	4	4	3.95	4
Cysteine	0	3	0	0	1.67	3
Aspartic Acid	3	3	0	0	2.5	3
Glutamic Acid	4.43	0	5	3.11	4.18	5
Glycine	2	2	0	0	1.84	2
Asparagine	0	3	1.47	0	1.15	3
Proline	0	7	0	0	1.57	7
Glutamine	4.68	0	5	5	3.62	5
Arginine	5.29	1.35	0	7	3.4	7
Serine	3	0	3	1.66	1.96	3
Tyrosine	7	7	7	6.19	0.5	7
Average Non-Essential AA Change	**0.64**	**0**	**-0.08**	**0.06**		
Combined Average AA Change	**1.98**	**1.91**	**1.25**	**1.72**		

Figure 3 - Estimated amino acid n-values using a least-squares solution for the rectangular, overdetermined matrix of peptides (rows) and amino acids (columns)(4).

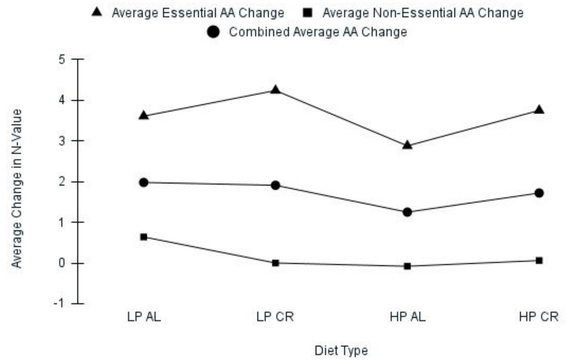
Noise Reduction and Quality Control

Figure 4 - Filtering out noise based on n-value standard deviations (<=0.05) and intensity (top 75%). Empirical n-values would ideally be similar to the literature n-values and would follow a y=x trend (dashed line). The grey points and linear fit show the peptide data before filtering. Colored points and linear fit represent the results of the filters and show mixed results in matching the y=x trend (4).



Results

- Filtering by n-value standard deviation and intensity moderately decreased noise.
- Figure 5 (below) In theory, we should see less variance in essential amino acid n-values since they will be obtained through diet. However, essential amino acid n-value changes were higher than non-essential amino acid changes.



- Better criteria for filtering out noisy peptide data would decrease confounding factors that affect the accuracy of the least-squares matrix solution and increase the confidence of our empirical amino acid n-values.
- Utilizing min/max bounds for matrix solution helped reduce noise. This prevented amino acid n-values from being negative or larger than the number of possible labeling sites.

References and Contact Information

1. DeuteRater: https://github.com/JC-Price/DeuteRater
2. Price, J. C., et al. (2012). "Measurement of human plasma proteome dynamics with (2)H(2)O and liquid chromatography tandem mass spectrometry." Anal Biochem 420(1): 73-83.
3. Carson, R. H., et al. (2017). "Imaging regiospecific lipid turnover in mouse brain with desorption electrospray ionization mass spectrometry." J Lipid Res 58(9): 1884-1892.
4. Amino Acid N-Value Calculations: https://github.com/StarLicker/Amino_Acid_N_Value_Calculator

LinkedIn:

Email: bendriggs99gmail.com

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