



PROTEIN TURNOVER CALCULATIONS FROM PARTIAL METABOLIC LABELING IN PLANTA EXPERIMENTS

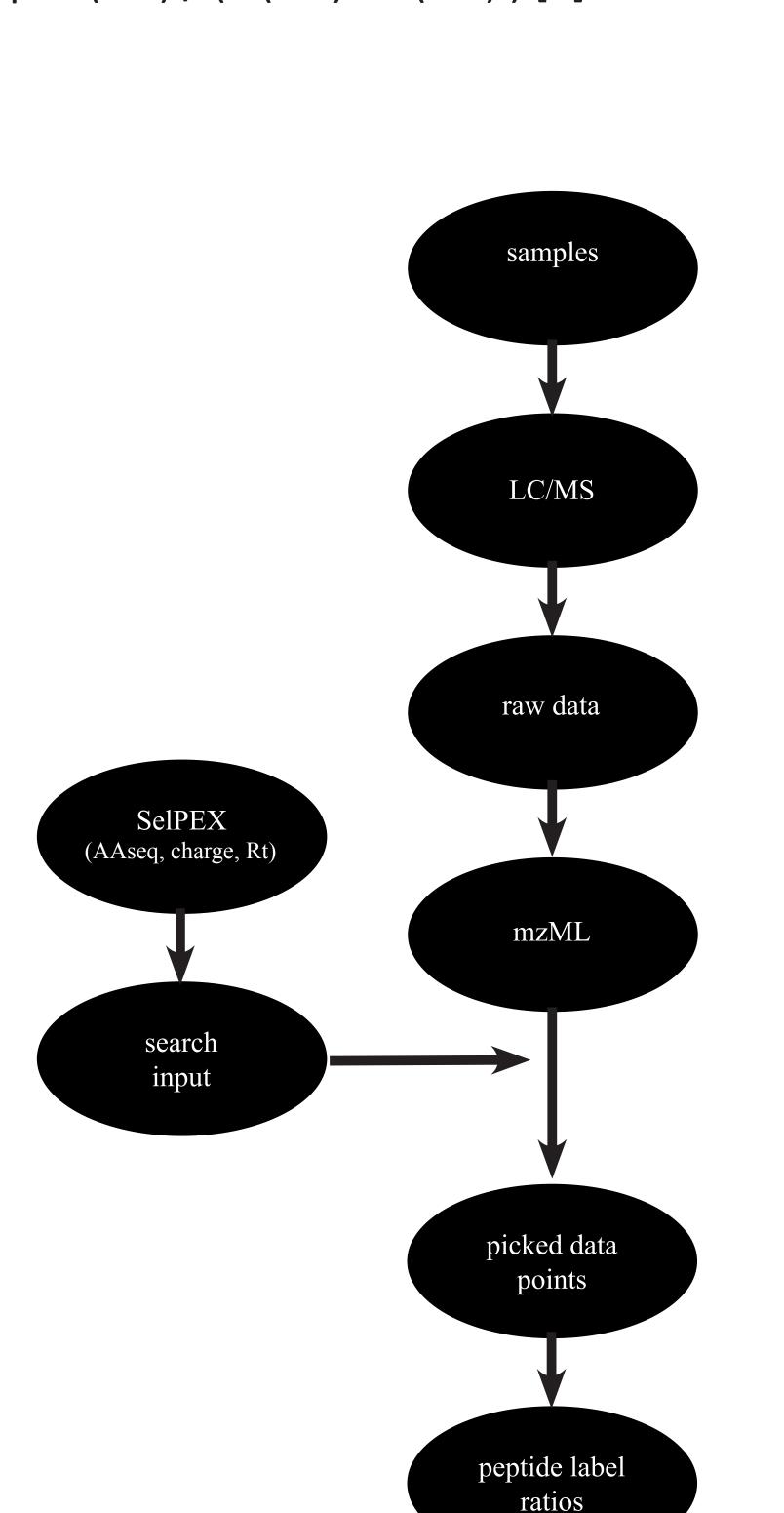
David Lyon, Ma Angeles Castillejo, Chritiana Staudinger, Vlora Mehmeti, Volker Egelhofer and Stefanie Wienkoop

Introduction

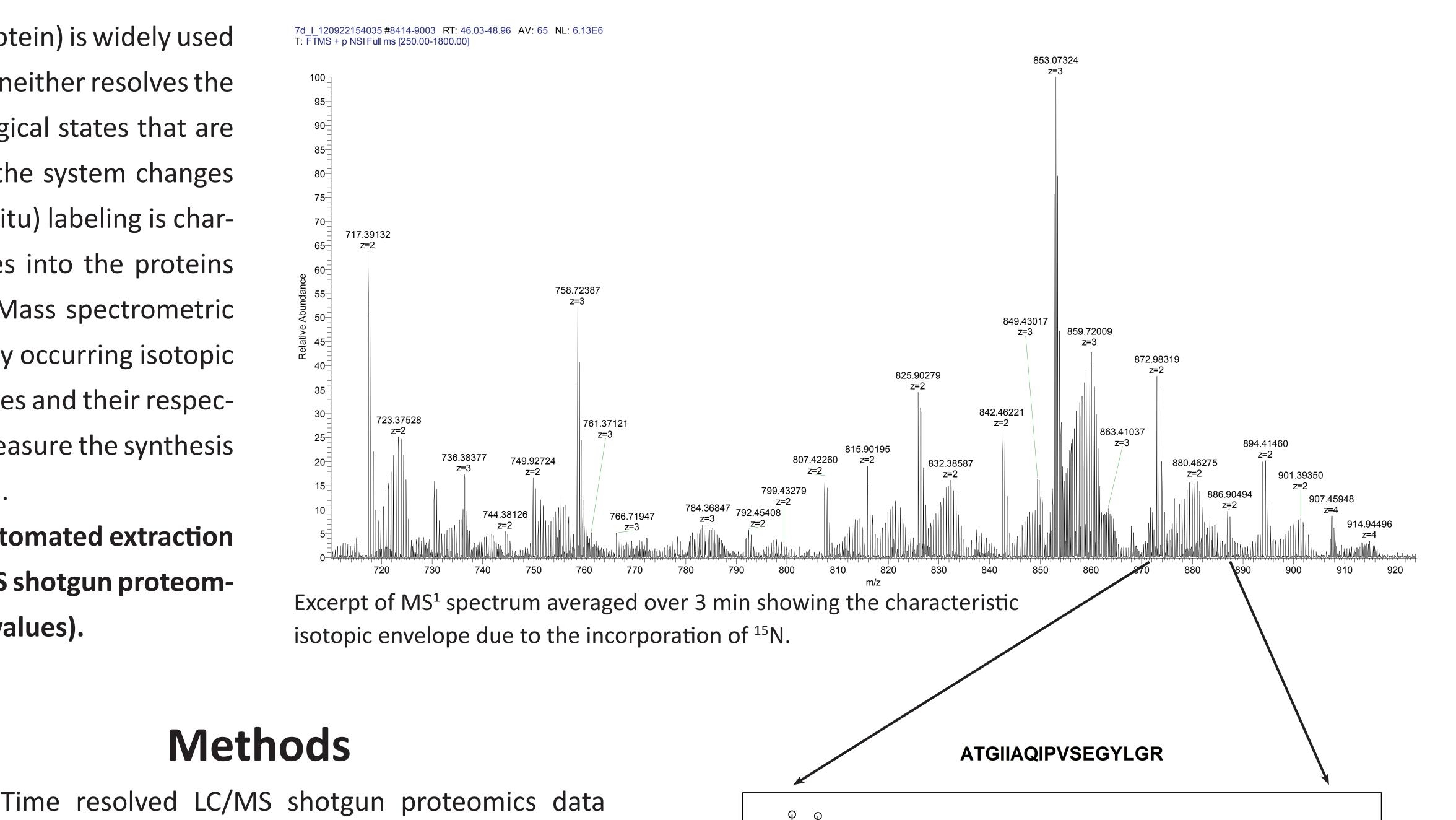
In shotgun proteomics the FCP (Fold Change in Protein) is widely used to compare protein levels of various samples, but neither resolves the dynamics of the proteome in the different biological states that are being compared nor the mechanisms whereby the system changes from one state to the other [1–3]. Metabolic (in situ) labeling is characterized by the incorporation of stable isotopes into the proteins of organisms via growth on media or food [4]. Mass spectrometric measurement of the ratio between light (naturally occurring isotopic distribution) and heavy (enriched with ¹⁵N) isotopes and their respective degrees of enrichment provide a means to measure the synthesis and degradation rates of individual proteins [1,5].

We hereby present an approach enabling the automated extraction and quantification of ¹⁵N partial metabolic LC/MS shotgun proteomics data, to calculate ¹⁵N incorporation rates (q-values).

 $q = I(^{15}N) / (I(^{14}N) + I(^{15}N)) [6]$



Overview of the workflow. Only the SelPEX list is organism specific, the workflow and the program are not. The program calculates the possible isotopic envelope of a given sequence and charge state and extracts the corresponding spectrum from the raw data. The ratio (q-value) of ¹⁵N (all ions of the isotopic envelope due to ¹⁵N enrichment) to ¹⁴N (all ions of the naturally occuring isotopic envelope) reflects the dynamics of protein synthesis.



from ¹⁵N partial metabolic labeling In Planta (SILIP [7]) of the model plant Medicago truncatula were analysed. The plants were grown for 12 weeks and then fertilized with enriched ¹⁵NH₄ ¹⁵NO₃ for five days. Samples were taken in regular intervals and subjected to LC/MS/MS shotgun proteomics using an LTQ-Orbitrap-XL and GC/MS/MS metabolomics. A Selective Peptide EXtraction (SelPEX* [8]) approach was applied, resulting from database dependent identification of peptides/proteins, to generate a list of 100 peptide sequences, their charge state and retention time. When inspecting the MS¹ spectra of the selected peptides, a time dependent

increase of the isotopic envelope could automati-

cally be extracted within minutes. GC/MS data also

showed a time dependent increase of ¹⁵N labeled

metabolites.

874 876 878 880 m/z Example of the isotopic envelope of the SelPEX peptide ATGIIAQIPVSE-GYLGR (ATPase). Six measurements of increasing incorporation of stable isotope labeling are plotted above each other (from bottom to top, maximally labeled on top). Each data point in a spectrum is normalized to the

Discussion

Our preliminary results demonstrate that an automated analysis of protein synthesis and degradation for previously identified SelPEX targets from complex samples is now possible. This approach is not restricted to any organism or tissue.

basepeak of the given spectrum.

168

120

12

We expect to find differentially regulated q-ratios between treatments (drought vs. control) alluding to physiological mechanisms characteristic for drought. By combining data from GC/MS metabolomics and physiological parameters with the presented LC/MS proteomics data, we aim for a holistic understanding of the underlying processes. To the best of the author's knowledge, this is the first SILIP gel-free shotgun proteomics study.

SelPEX is based on database dependent protein identification. Peptides with good ionisation properties (and ideally proteotypic), are selected for a robust ¹⁵N analysis. This approach allows for the best accuracy for quantification and identification.

Kline, K. G.; Sussman, M. R. Annual review of biophysics 2010, 39, 291–308.

Cargile, B. J.; Bundy, J. L.; Grunden, A. M.; Stephenson, J. L. Analytical chemistry 2004, 76, 86–97. Gustavsson, N.; Greber, B.; Kreitler, T.; Himmelbauer, H.; Lehrach, H.; Gobom, J. Proteomics 2005, 5, 3563–70.

(7) Schaff, J. E.; Mbeunkui, F.; Blackburn, K.; Bird, D. M.; Goshe, M. B. The Plant journal: for cell and molecular biology 2008, 56, 840–54.

(8) Castillejo, Ma. A. C.; Staudinger, C.; Egelhofer, V. and Wienkoop, S. Chapter 15 In: Plant Proteomics Methods and Protocols. Ed. J.V. Jorrin Novo, S. Komatsu, S. Wienkoop, W. Weckwerth: Springer New York. (in press)

FWF Project Number: P23441-B20

etty, J.; Riba-Garcia, I.; Robertson, D. H. L.; Gaskell, S. J.; Oliver, S. G.; Beynon, R. J. Molecular & Cellular Proteomics 2002, 1, 579–591.

W.; Wienkoop, S. Rapid Communications in Mass Spectrometry 2010, 3609–3614. ; Nelson, C. J.; Solheim, C.; Whelan, J.; Millar, a H. Molecular & cellular proteomics: MCP 2012, 11, M111.010025.