Software for Differential Characterization of PTMs: Approaches in Data Acquisition and Processing

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Introduction
The identification of post-translational modifications (PTMs) across different states is critical for determining biomarkers and therapeutic targets in proteomics studies. In a typical shotgun proteomics experiment, data sets contain greater than 10,000 MS/MS spectra identifying thousands of peptides and hundreds of PTMs. Although software can identify peptides, and by inference proteins, identification of PTMs is more challenging. Once PTMs are identified, few techniques exist for analyzing significant trends. STRAP PTM (Software Tool for Rapid Annotation of Proteins: Post-Translational Modification edition) was developed to facilitate multi-sample comparison by collating and ranking PTMs. In this study, we investigate the power of STRAP PTM to characterize differential PTMs in increasingly complex data sets and determine the effects of data acquisition and processing parameters.

Methods
STRAP PTM uses spectral counting and a novel scoring algorithm to accelerate the identification of differential PTMs. Recent modifications extended the capability to read data file formats from protXML (Trans-Proteomic Pipeline, ISB) to handle those generated by select search engines and thus bypass TPP processing. STRAP PTM is freely available on the BUSM Cardiovascular Proteomics Center website:

www.bumc.bu.edu/cardiovascularproteomics/cpctools/

Model data were acquired by LC-MS/MS using a Q Exactive mass spectrometer (Thermo Fisher) coupled with a Waters NanoAcquity HPLC. MS/MS data were analyzed using Proteome Discoverer (Thermo Fisher) and Mascot (Matrix Science) software, searching custom protein databases using both variable-modification and error-tolerant search modes. Label-free quantification was conducted using both Scaffold (Proteome Software) and Progenesis LCMS (Nonlinear Dynamics).

Preliminary Results/Abstract
STRAP PTM was originally tested with model data, CD40L protein with *in vitro* oxidative stress-induced PTMs, and the results were independently validated with label-free quantification using Progenesis LCMS. Additional data sets representing a complex matrix were obtained using PTM-modified peptides (Protea Biosciences) spiked into a mixture of depleted mouse plasma and protein standards (Sigma). A study of EGFR phosphorylation in response to nucleotide and EGF stimulation provided further data sets that tracked differential phosphorylation. LC-MS/MS data were initially obtained using instrument data acquisition/processing parameters typical for shotgun proteomics. STRAP PTM analysis revealed PTM changes that were readily visualized in proteins and across sample groups and showed a strong correlation with label-free analysis. In an attempt to increase the sensitivity, accuracy, and precision of STRAP PTM results, different parameters for data acquisition/processing were explored. For example, the number of precursor MS/MS per duty cycle was varied, targeted inclusion lists were investigated, and multiplexing or MSX was explored, in which more than one precursor can be selected, isolated, and summed together, prior to MS/MS, per duty cycle. Data analysis parameters included customized UniMod PTM libraries, customized peptide and protein databases, and the use of spectral libraries. The identification and differential relative quantification of PTM-modified peptides in the representative data sets were optimized with increasing degrees of success. Lastly, different analytical parameters used in the database search and processing of data with the TPP were observed to affect the outcome of the analyses with STRAP PTM. Through selective optimization of each of the experimental and data analysis parameters, several scenarios were demonstrated where PTM counting with STRAP PTM offers a fast, efficient, and reliable means for elucidating differential PTMs across varied and complex proteomics data sets.

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Novel Aspect
STRAP PTM is a powerful software tool for differential comparison of PTMs in large proteomics data sets.