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Software and Algorithm for Differential Characterization of Post-Translational Modifications

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Novel Aspect

Developed a method for scoring post-translationally modified peptides corresponding to the strength of their differential observation using spectral counting

Introduction

Protein post-translational modifications (PTMs) play a critical role in normal cell function and the mechanisms of disease. Precisely characterizing these PTMs across experimental conditions is a significant priority in contemporary biomedical research. PTMs must be characterized by direct means, such as by liquid chromatography-mass spectrometry (LC-MS). Using the major database search engines, we obtain lists of peptide assignments, potential PTMs, and the associated proteins to which they map. Organizing these lists of putative PTMs in a manner amenable to interpreting their significance in a complex, large-scale differential proteomics experiment is a major challenge. To expedite the biological interpretation of PTM and proteomics data, we developed the Software Tool for the Rapid Annotation of Proteins: PTM edition (STRAP PTM).

Methods

STRAP PTM is software centered on graphically assisted interpretation of differentially observed PTMs. STRAP PTM is written in C# and runs on Microsoft Windows XP or higher with version 4.0 of the Microsoft.NET Framework. The software imports data from protXML files (obtained from the ISB's Trans-Proteomics Pipeline), and then ranks peptides based on differential post-translational modification. We used STRAP PTM to gain insight into several differential proteomics experiments, most notably PTMs associated with in vitro oxidation of CD40 ligand (CD40L) a key co-stimulatory molecule which has recently emerged as a key player in cardiovascular disease and thrombosis. We further tested and validated STRAP PTM algorithms through a comparative study against traditional label-free methods using Nonlinear Dynamics Progenesis LC-MS.

Preliminary Data

STRAP PTM is an easy-to-use, freely available application designed for the mapping and characterization of differentially observed PTMs. STRAP PTM allows for global analysis of proteins and their PTMs allowing for users to compare sample groups within large experiments. STRAP PTM performs comparisons between protXML files and/or sample groups comprised of protXML files. One of the products of this comparison is a PTM map that overlays PTMs on the primary sequence of their parent proteins. This visualization aids data interpretation by illustrating differences in PTMs between sample groups. The peptides containing PTMs are also assigned a score corresponding to the strength of their differential observation using a spectral counting approach. Several algorithms were tested to arrive at the one currently in use. STRAP PTM results can be exported to CSV format, and the PTM map may be saved in various image formats.

Preliminary data from an in vitro oxidation experiment on CD40L was used in STRAP PTM and compared with a label-free, peak intensity approach using Progenesis LC-MS. The scoring function used by STRAP PTM was able to highlight differentially modified peptides of interest by bringing them to the top of the list of peptides associated with individual proteins. In the CD40L study, STRAP PTM scored dinitrotyrosine and cysteine sulphonic acid modifications among the highest of all modified peptides assigned to CD40L. Non-specific oxidant modifications were ranked with low scores thus differentiating real from false positive results. The observed modifications were in strong agreement with the differential analyses conducted using label free methodology and literature search results of the identity and location of known and putative PTMs. Additional data from an on-going analysis of a cardiovascular proteomics model will be presented further highlighting the utility of our PTM counting approach.