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

Jean L. Spencer, Vivek N. Bhatia, Amanuel Kehasse, Stephen A. Whelan, Christian F. Heckendorf, Catherine E. Costello and Mark E. McComb

Cardiovascular Proteomics Center, Boston University School of Medicine, Boston, MA 02118

Overview
- **Purpose:** Investigate software that characterizes differential PTMs in complex data sets and determine the effects of data acquisition/processing parameters on its capability.
- **Methods:** In-house software (STRAP PTM) uses spectral counting and a novel scoring algorithm to collapse and rank differential PTMs.
- **Results:** Trends in differential PTMs readily displayed and substantiated by quantitative analysis with results easily optimized by variation of acquisition/processing parameters.

Introduction
The identification of post-translational modifications (PTMs) across different states is critical for determining biomarkers and therapeutic targets in proteomics studies. Although software can identify peptides and infer proteins, identification of PTMs is more challenging. Once PTMs are identified, few methods exist to analyze significant trends. We created STRAP PTM (Software Tool for Rapid Annotation of Proteins: Post-Translational Modification edition) to facilitate multi-sample comparison by collating and ranking PTMs. Here we explore the utility of STRAP PTM and the effects of data acquisition and processing parameters on its capability.

Materials
- **PTM Peptide Standards in Plasma**
- **Label-Free Quantitative Analysis**
- **LC-MS/MS Analysis**
- **Methods**
  - **LC-MS/MS Analysis**
  - **Label-Free Quantitative Analysis**
  - **STRAP PTM Analysis**

Results
- **Effect of STRAP PTM Settings**
- **Effect of Instrument Settings**

Conclusions
- **STRAP PTM** is a powerful counting approach that:
  - Collates and ranks differential PTMs in complex data sets.
  - Generates results with trends substantiated by label-free, quantitative analysis.
  - Allows removal of poorer quality data for improved analysis.
  - Provides a rapid means to optimize instrument settings for best results.

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