Counting Strategies for Differential Characterization of Post-Translational Modifications

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Novel Aspects (max 20): Counting approach for ranking of post-translational modifications observed in differential proteomics experiments

Introduction (max 120):

There is an increasing interest in the identification and characterization of protein post-translational modifications (PTMs) as the field of mass spectrometry base proteomics begins to mature. Unfortunately, the vast amount of information obtained within a typical differential proteomics makes the facile measure of PTMs quite challenging. In addition to label-free approaches, we have begun to explore counting methods for differential analysis of PTM changes in proteomes. We have developed a software program, the Software Tool for Rapid Annotation of Proteins: Post-Translation Modification edition (STRAP PTM) to aid in our approach. Here we explore improved algorithms and demonstrate the utility of STRAP-PTM across different PTM/proteomics experiments.

Methods (max 120):

Multiples of existing data sets acquired obtained within the laboratory on a variety of LC-MS/MS platforms were used as models to establish algorithm parameters for PTM counting. We use a modified version of STRAP-PTM which afforded use the ability to rank individual factors which contributed to differential analyses. STRAP PTM was written in C# and runs on Microsoft OS with version 4.0 of the Microsoft.NET Framework. C# was chosen due to its object-oriented nature and productive programming environment. The application's code is written to import parsed prot.XML file data into an object model, and then rank peptides based on differential PTMs. Secondary analyses was with statistical packages within Microsoft Excel. Label-free analysis was with Progenesis LCMS (NonLinear Dynamics).

Preliminary Results (max 300):

While there is a great interest in PTMs due to their significant roles in disease, there are few tools for actually performing facile characterization of global PTM changes in large proteomics data sets and differential comparison of PTMs across different sample groups. In our quest to expand beyond these limitations we developed STRAP-PTM; a convenient Windows based graphical user interface shell for advanced differential analysis of PTMs in mass spectrometry based proteomics studies. Using simple and complex model data sets we explored different parameters affecting PTM counting. Simple data sets of singular PTM studies indicated that with appropriate MS/MS parameters and database search constraints we achieved a high degree of correlation between results obtained with counting approaches and label-free approaches. Expanding to more complex data sets with multiples of PTMs yielded similar results and afforded a more direct means to identify and rank unique and biologically relevant PTMs within a large data space. We observed that proper MS/MS experimental design was crucial to obtain sufficient counting information to obtain valid results. We also observed that database search parameters could be adjusted to influence and direct the results output to yield a limited yet significant analyses. Finally, differential comparison of large data sets with large search space

could be readily analyzed and the resulting PTM changes readily mapped to proteins and sample groups. An overview of our approaches will be presented with supporting data sets representing a wide range of proteomics experiments.

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