Characterization of Post-Translational Modifications Using Counting Approaches

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Abstract

There is an increasing interest in the identification and characterization of protein post-translational modifications (PTMs). The vast amount of information obtained within a typical differential proteomics makes the measure of PTMs challenging. 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. Existing data sets were used to model our counting approaches. 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. Comparative label-free analysis was with Progenesis LCMS (NonLinear Dynamics). Using model data sets we explored different parameters affecting PTM counting. Singular PTM studies indicated that with appropriate MS/MS parameters and database constraints we achieved a high correlation between 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 direct the results output to yield limited yet significant analyses. Finally, differential comparison of large data sets with large search space could be readily analyzed and the resulting PTM changes mapped to proteins and sample groups. This project was funded by NIH-NCRR grants P41 RR010888/ GM104603, S10 RR015942, S10 RR020946, S10 RR025082 and NIH-NHLBI contract N01 HV00239.