Top-down proteomics has emerged as a technique that preserves labile post-translational modifications and offers full protein sequence coverage.

One of the major problems facing top-down MS/MS is the assignment of peaks due to the possibility of a large number of fragments from an intact protein.

Now in development is a web-based version of BUPID-Top-Down (Boston University Protein Identifier Top-Down) used to assign ions in top-down MS/MS spectra.

Theoretical and experimental ion masses are compared and scored using a log-likelihood ratio driven algorithm to find matches.

This program will facilitate the penetration of top-down techniques into a greater number of mass spectrometry laboratories.

Introduction to Top Down MS/MS

Top down proteomics involves introducing intact proteins into the mass spectrometer and fragmenting them using methods such as CID, ECD, ETD, etc. This has the potential for complete protein sequence and PTM identification without having to spend time digesting the protein. Making use of top-down data is very computationally taxing and the availability of software that can do this effectively is limited.

There are few tools available for top-down proteomics data analysis; these include:

- Mascot Top-Down (http://www.matrixscience.com): commercial, license required;

Here, we describe the development of a web-based continuation of BUPID-Top-Down redesigned for enhanced performance and with improved features which take advantage of the client-server approach.

Challenges

The original version of BUPID-Top-Down was developed as a desktop application to be run on laboratory PCs. This model is not ideal for the laboratory environment for several reasons:

- The speed of the search is limited by the resources available on the desktop minus the resources tied up by the other processes running on the system.
- In order to provide a familiar interface for the users, the original program was written in C# using the .NET framework. This, in part, caused the core code to become entangled with the user interface, preventing it from expanding to support new requirements.
- The PC platform restricted users to PC architecture and necessitated frequent updates to code, protein databases and PTM databases.

Algorithm for Pattern Matching

- BUPID-Top-Down begins by generating a list of theoretical ions that can be derived from the original sequence according to the properties associated with each fragmentation method. Primary fragmentation, internal fragments and post-translational modifications are included in the search.
- The score of the match is defined as the log-likelihood that the peak is generated by this ion over that the peak is generated by random background. If an ion matches with more than one peak, only the match with the highest score is kept for that ion.
- PTM searching is accomplished by shifting the original mass of each theoretical ion that meets the modification requirements before matching it with peaks. If a PTM is known to be on a specific residue, a fixed modification can be added to the search. Adding fixed modifications to a residue is equivalent to using a different mass for that residue in the subsequent search.

Inclusion of a database search to identify unknown proteins.

Loss of labile PTMs due to introduction of internal energy.

Improved ion matching accuracy.

Outstanding protein sequence coverage.

We designed a web interface to attach to the core program. Our interface was written in C# using the .NET framework. This, in part, caused the core code to be run on laboratory PCs. This model is not ideal for the laboratory environment for several reasons:

- The speed of the search is limited by the resources available on the desktop minus the resources tied up by the other processes running on the system.
- In order to provide a familiar interface for the users, the original program was written in C# using the .NET framework. This, in part, caused the core code to become entangled with the user interface, preventing it from expanding to support new requirements.
- The PC platform restricted users to PC architecture and necessitated frequent updates to code, protein databases and PTM databases.

Web Interface of BUPID-Top-Down

- BUPID-Top-Down analysis of MS/MS of Hb β.
- Example of Peak Assignment Using BUPID-Top-Down
- Comparison with ProSightPTM
- Comparison of BUPID-Top-Down analysis of MS/MS of Hb β with ProSightPTM
- Example of Peak Assignment Using BUPID-Top-Down

Results

- Example of Peak Assignment Using BUPID-Top-Down
- Comparison with ProSightPTM

Performance Improvements

We compared the data processing speed of the original BUPID-Top-Down with our improved program. We found that the improved version is able to process more complex datasets in linear time.

Discussion

- By designing the program with a client-server model in mind, we were able to alleviate many of the problems facing the desktop version. At the core is a standalone program that will accept the user data in the form of standard text files and command line options. This allows us to easily deploy the program with a variety of front ends without having to alter the underlying code.
- By writing the code in C and designing it from the ground up with a multithreaded ideology, we were able to achieve greater performance than was possible in the previous design.
- We designed a web interface to attach to the core program. Our interface allows users to easily submit searches as well as review the results in a variety of formats independent of platform.
- The PTM searching capabilities were extended to include all possible combinations of modifications rather than limiting the search to one modification for each ion, thus improving search results.

Conclusions

- Faster processing than BUPID-Top-Down Desktop version.
- Comparable results to ProSightPTM for unmodified searches.
- Assignment of PTMs and internal ions.
- Improved usability of search results.
- Use of BUPID-Top-Down will enable facile and accurate data analysis thus expanding this approach to many users in the field.

Future Work

- Inclusion of a database search to identify unknown proteins.
- Improved ion matching accuracy.

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