Some Recent Enhancements to Protein Prospector

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Multi-Sample Data

The ABI 4700 TOFTOF instrument allows each spot on a sample plate to contain a single digest sample with both MS and MSMS data available. As the MSMS data isn’t acquired for every peak the MS data can be used to identify post-translation modifications, peptides formed by non-specific enzymatic cleavages or to distinguish proteins with similar sequences.

The MS data can either be searched against the proteins identified by MSMS or the whole database.

The MSMS hits can be used to calibrate the MS scan.
Display of Multi-Sample Results

The Batch-Tag and Search Compare programs can produce separate protein and peptide reports for each spot on a sample plate. Both the MS and MSMS matches for the proteins identified can be displayed.

Better Matches Using MS-Homology

**Swiss Prot Matches**

- **HUMAN Keratin, type II cytoskeletal 1**
- **DICDI 26S protease subunit 6B homolog**

**NCBI Ref Match identified by MS-Homology**

- **DROME 26S proteasome regulatory complex subunit p50**

Identified by using the MSMS hit sequences in an MS-Homology search.

Recalibration of Data Based on Results

A histogram of the MSMS parent errors of the correctly identified peptides shows a normal distribution with a systematic error of −27ppm. The data can be resubmitted with an error tolerance of 35 ppm rather than the original 100 ppm.

Recalibration of Multi-Fraction Data

Each fraction in a multi-fraction data set can have a separate calibration.

Distribution of Errors in a 6 Fraction Data Set

The calibration enabled the data to be re-searched with a tolerance of ±50 ppm rather than the original ±120 ppm.
LC MSMS Waterfall Plots

Visualization of Peaks Chosen for MSMS

Numbers in green represent the charge.
Support for LTQ Data

Discriminant score graph from data set with 116075 spectra from 82 fractions.

New ion types supported:
- $b^{+2}$, $b-\text{NH}_3^{+2}$, $b-\text{H}_2\text{O}^{+2}$, $b-\text{SOCH}_4^{+2}$, $b-\text{H}_3\text{PO}_4^{+2}$
- $y^{+2}$, $y-\text{NH}_3^{+2}$, $y-\text{H}_2\text{O}^{+2}$, $y-\text{SOCH}_4^{+2}$, $y-\text{H}_3\text{PO}_4^{+2}$

MS-Tag of Protein ECD

The ECD spectrum with 198 fragment ions contains two major modification states of Histone H3.

MS-Tag correctly identifies the modification sites of these two components.
### iTRAQ Quantitation

Link to raw quantitation data

![Graph showing iTRAQ quantitation data](image)

### Acknowledgements

- Funding: NIH NCRR grant 01614 and the Vincent Coates Foundation.

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