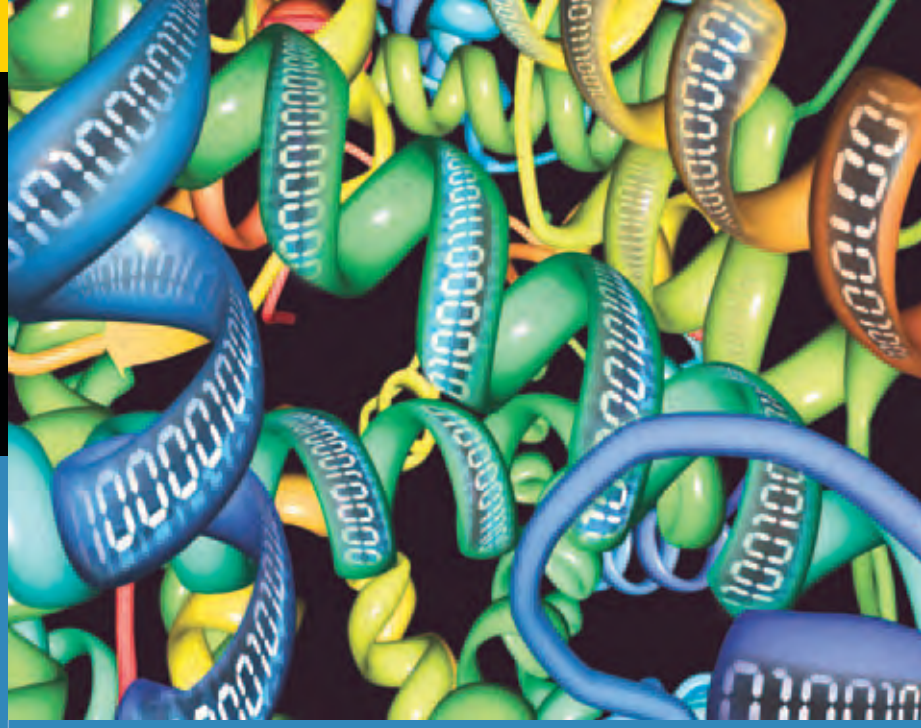
MASS SPECTROMETRY

Thermo Scientific Proteome Discoverer



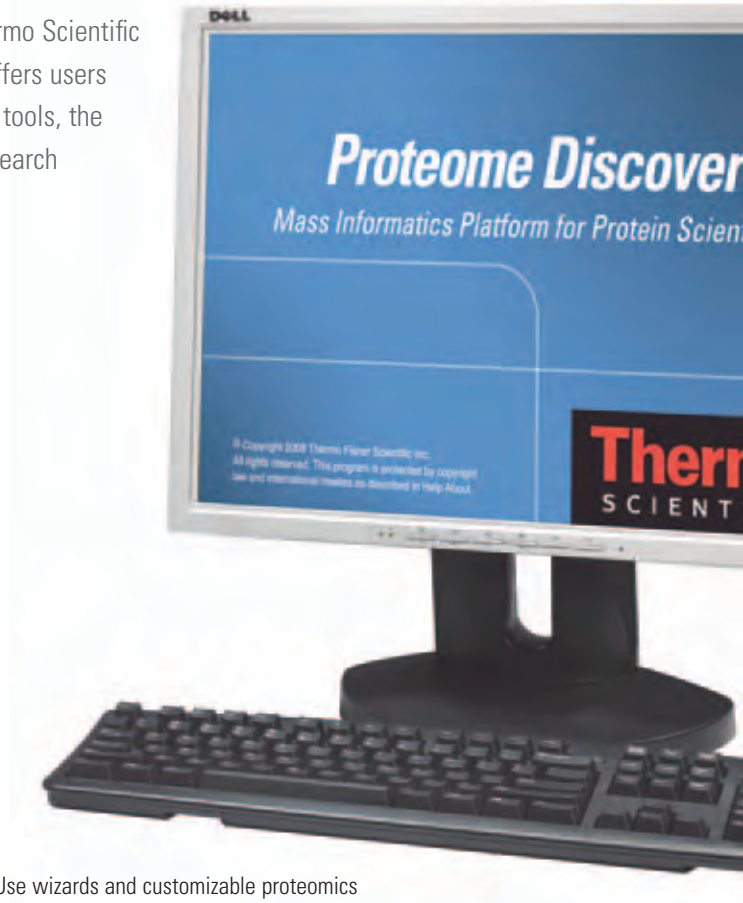
Mass Informatics Platform for Protein Scientists

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

A Comprehensive and Expandable Software Platform for the Analysis of Qualitative and Quantitative Proteomics Data

Outstanding sensitivity and the rapid growth of public databases have made mass spectrometry the primary method of identifying proteins in complex biological samples. Widely available database search algorithms such as SEQUEST® and Mascot™ facilitate the identification of proteins from mass spectral data. Proteomics research is rapidly expanding beyond simple identification, creating the need for more advanced and flexible tools. Thermo Scientific Proteome Discoverer software offers users an expansive range of analytical tools, the flexibility to address multiple research workflows, and an easy-to-use, wizard-driven interface.



With Proteome Discoverer:

- Choose the optimum database search algorithm (SEQUEST, Z-Core, Mascot, etc.,) or combine output from multiple algorithms to maximize and cross-validate results
- Merge results from multiple dissociation techniques such as ETD, HCD, and CID to identify more PTMs and increase confidence in those identifications
- Measure and report the relative expression levels of isotopically labeled peptides
- Leverage high-mass-accuracy MALDI data with a Mascot-based peptide mass fingerprinting workflow
- Validate protein IDs using False Discovery Rate (FDR) determination
- Integrate custom tools into the robust, expandable Proteome Discoverer software platform
- Use wizards and customizable proteomics workflows to analyze MS^n data from raw spectra through protein annotation
- Apply GO annotation to illuminate the biological context of the proteins identified
- Take advantage of data standards developed by the HUPO Proteomics Standards Initiative
- Use the SRF file import wizard for a seamless transition from the Thermo Scientific BioWorks platform

Proteomics Data

Maximum Protein Sequence Coverage and Confidence – Integrating Search Engines and Dissociation Techniques

Proteome Discoverer includes multiple database search engines to complement the breadth of dissociation techniques such as CID, ETD, and HCD available with LTQ™ linear ion trap-based mass spectrometers. Different dissociation techniques provide complementary results and often increase protein coverage and the number of identified proteins.

- **Integrate database search results from industry-leading search engines such as SEQUEST, Z-Core, and Mascot**
- **Combine information from multiple dissociation techniques**
- **Utilize advanced tools for database search results – from validation through automated false discovery rate calculations**
- **Exploit the benefits of high-mass-accuracy, high-resolution data**

Use the Z-Core search algorithm to exploit the power of ETD

The novel Z-Core database search algorithm included in Proteome Discoverer specifically takes into account the unique characteristics of spectra generated by electron transfer dissociation (ETD). It includes a data pre-processing step that assigns a charge state to precursor ions.

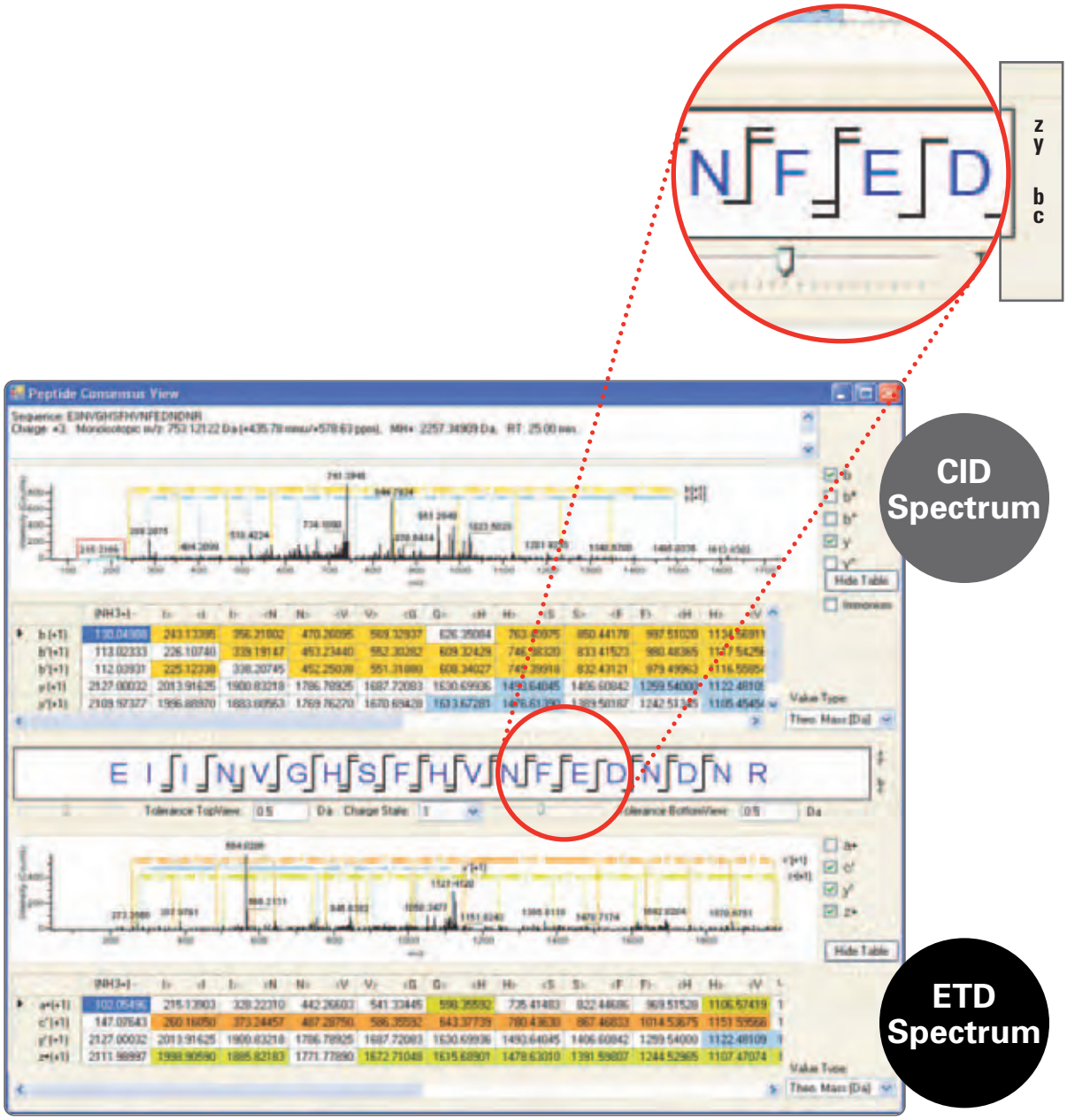
Using Z-Core to search ETD spectra:

- Increases throughput – An ETD spectrum need only be searched once to account for all precursor charge states
- Maximizes search efficiency – The number of data files searched is reduced
- Increases confidence in protein ID results – The number of false positive identifications generated from multiple searches of each ETD spectrum is decreased
- Reduces time spent on data interrogation – CID and ETD fragment ion information can be consolidated into one informative report

Combine CID and ETD data for greater sequence coverage and confidence

Proteome Discoverer includes many interactive results views that facilitate the interpretation of database search results. The Peptide Consensus View highlights the fragment ions in a peptide MS/MS spectrum that match predicted fragment masses. It color-codes the amino acid table to show the portion of the corresponding peptide sequence that was identified.

Multiple ion types (series) can be displayed, accommodating the differences in the predominant ions produced by different dissociation techniques.



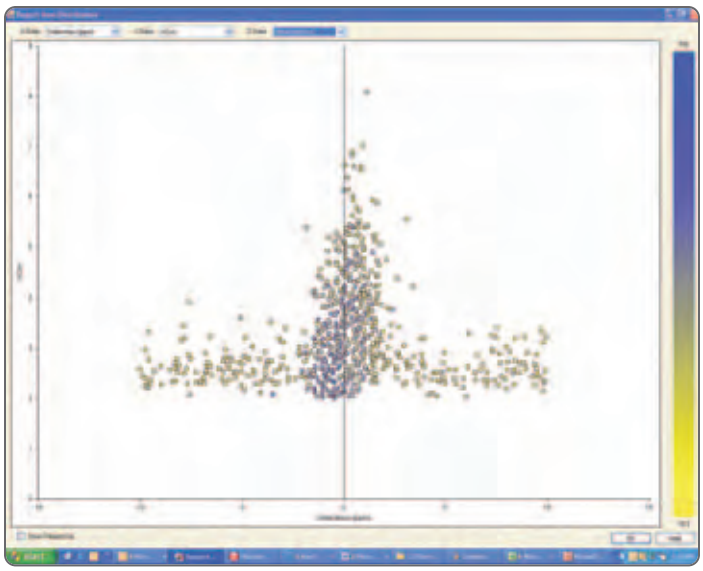
In this Peptide Consensus View, the combination of ETD and CID fragment ion matches increases the sequence coverage of a large triply charged peptide.

Effortless Results Interpretation – Quickly Identifying Peptide Sequences and Locating Modifications

Proteome Discoverer provides a wealth of tools that make it easy to identify sequences, investigate post-translational and other modifications, and validate search results.

Advanced visualization tools

- **Merge peptide hits into a comprehensive protein sequence coverage report using the Protein Results View**
- **Expand and cross-validate PTM information via direct access to the Unimod database**
- **Organize multiple complex data sets with an easy-to-use results table**
- **Export graphics for publication using a simple clipboard and common file formats**



New plotting capabilities

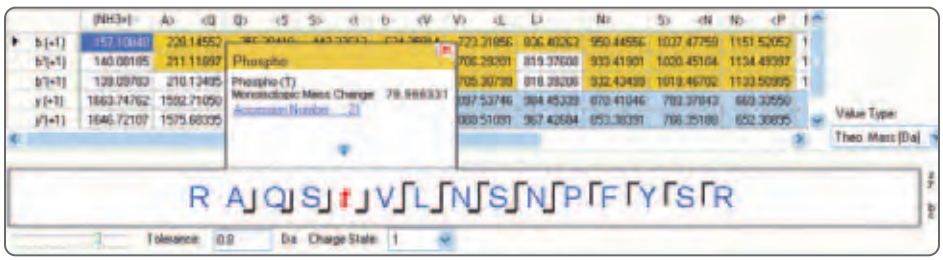
The Distribution Chart shows how closely fragment ion masses identified from a database search match theoretical expected masses, and how well the results correspond to XCorr scores and percentage of matched fragment ions.

Validation of MS/MS database searches

The False Discovery Rate (false positive rate) is a statistical value that estimates the number of false positive identifications amongst all identifications found in a database search, and thus is a measure of the certainty of the identification. Inclusion of False Discovery Rates (FDR) is frequently demanded by peer-reviewed publications. Proteome Discoverer includes a Decoy Database Search for determining FDR.

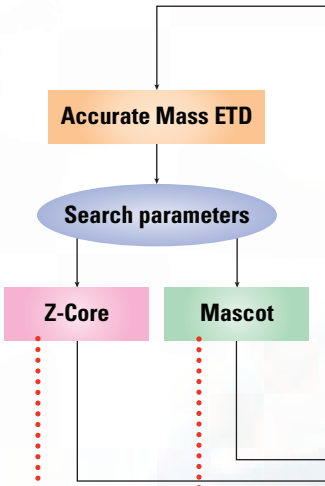
More information about PTMs

The Peptide Consensus View clearly identifies modified amino acids with lower case letters. Clicking on the letter displays a pop-up with a description of the modification. Clicking the hyperlink connects to the Unimod website for even more information.



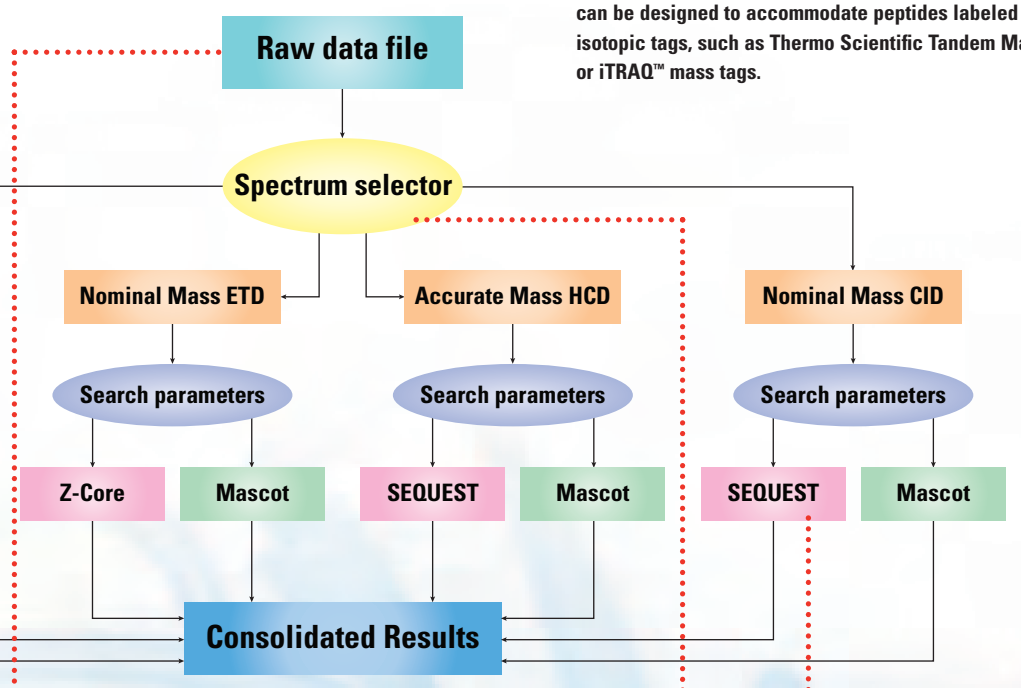
From Wizards to Workflows – Customizing the Process of MS and MS^n Data Mining

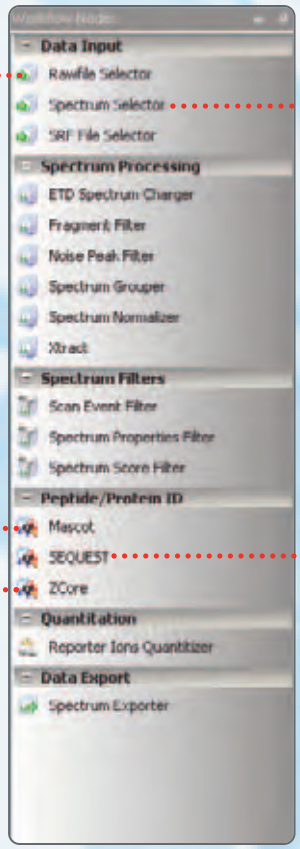
The task of analyzing proteomics data from LC/MS^n experiments can be daunting, in part because of the numerous and diverse ways to acquire and subsequently interrogate the data. For example, fragment ion spectra from peptides dissociated by ETD have different characteristics than spectra from CID and must be searched differently. Accurate-mass data require different search conditions than nominal-mass data, and both benefit from being searched by complementary algorithms to increase the confidence in the proteins identified. The Proteome Discoverer workflow editor allows users to create a fully customized data analysis workflow from a comprehensive menu of search algorithms, dissociation methods, and results filters. Results can be consolidated into a single, easy-to-read report.



- **Generate workflows for customized data analysis strategies in minutes**
- **Utilize comprehensive tools for sophisticated raw-data manipulation**
- **Import data from a variety of sources and output results in several data formats**
- **Integrate customized tools through the software developer's kit (SDK)**

This example workflow includes eight separate searches performed on the same raw file and incorporated into a single qualitative report. Similarly, quantitative workflows can be designed to accommodate peptides labeled with isotopic tags, such as Thermo Scientific Tandem Mass Tags or iTRAQ™ mass tags.



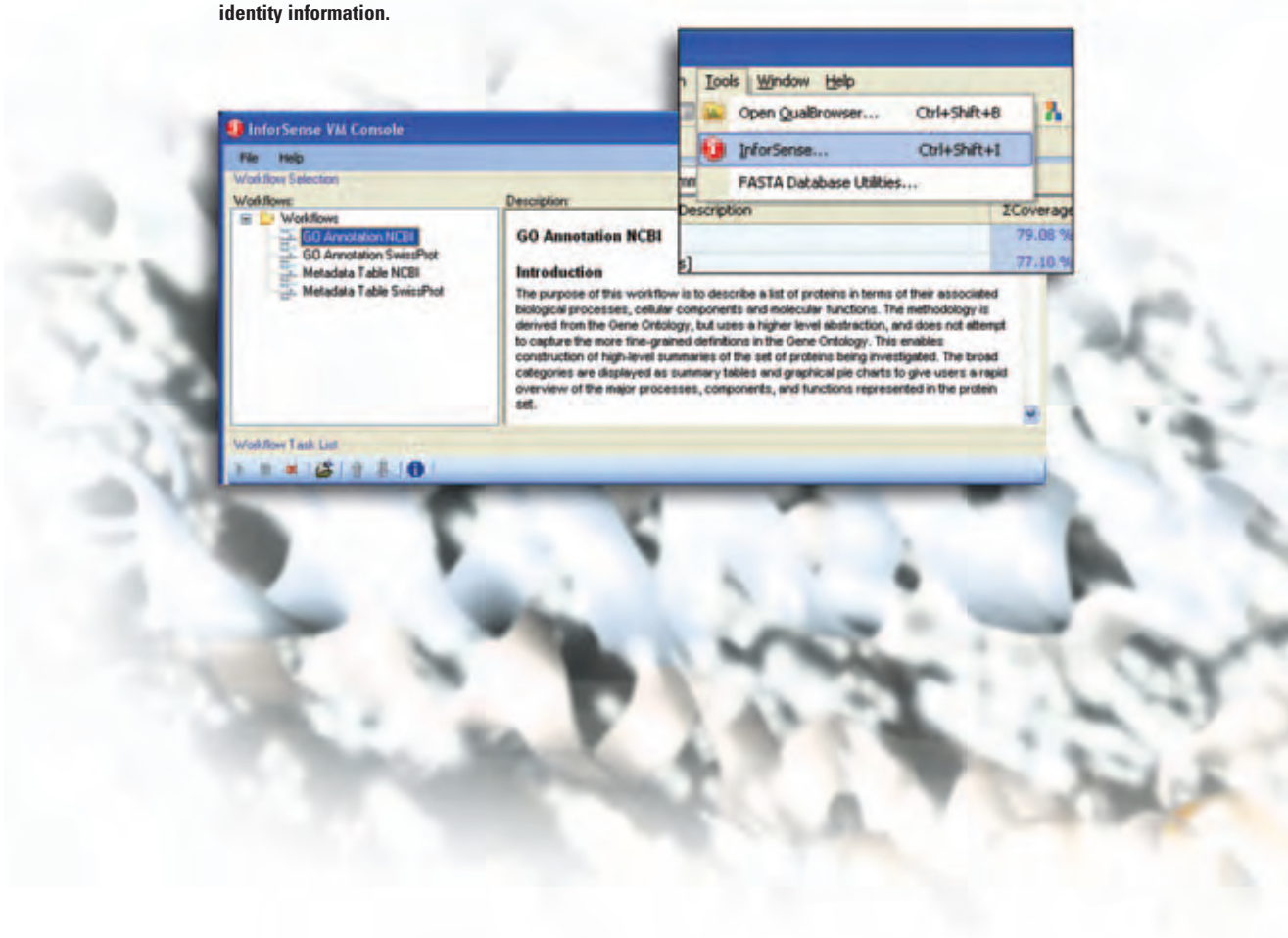


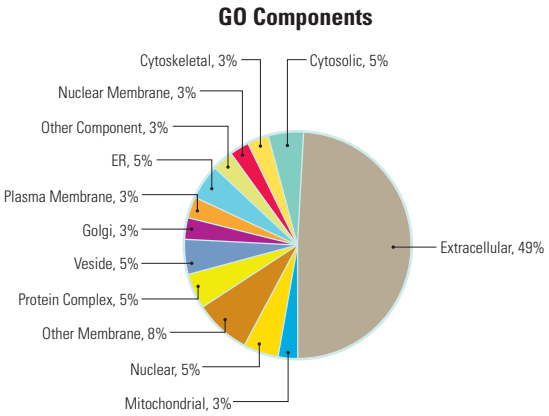
From Protein Identification to Biological Context – Annotating Results with Information from Public Databases

LC/MS analysis of complex protein mixtures such as whole cell digests or digests of biological fluids generally produces high numbers of protein identifications. Insight into biological meaning, however, requires extensive and specific information about each identified protein. Public databases such as NCBI Protein and UniprotKB/Swiss-Prot provide comprehensive descriptions of proteins, but without additional tools, researchers have had no alternative except querying these databases one protein at a time – a laborious and time-consuming process.

Using its integrated InforSense virtual machine (VM), Proteome Discoverer can automatically retrieve pertinent information about each identified protein – including gene ontology (GO) classifications, sites of post-translational modifications, and literature references – from the relevant public databases. Proteome Discoverer users have complete control over the choice of databases to be searched. Annotated results are easily exported to Excel®.

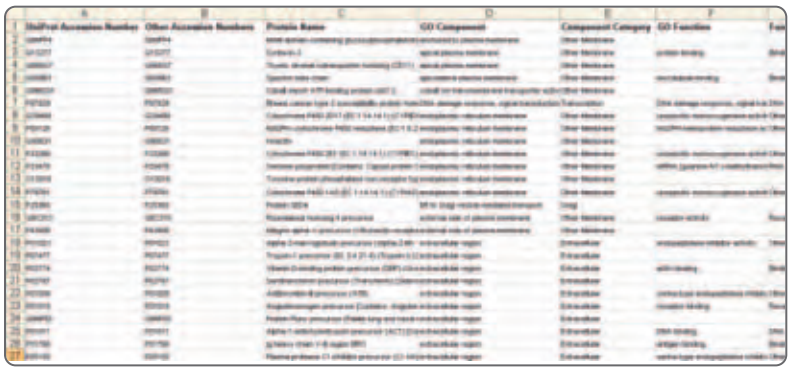
The InforSense virtual machine included in Proteome Discoverer can automatically extract useful metadata from public databases and integrate it with protein identity information.

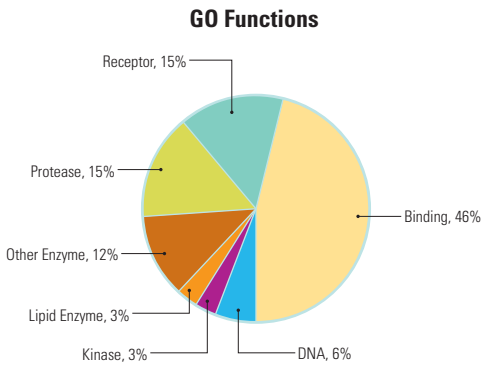


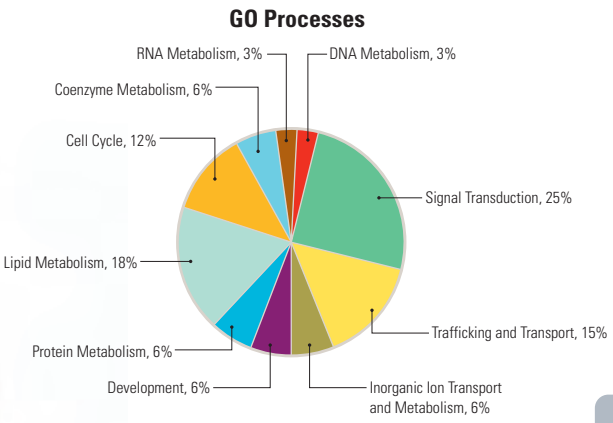


Automated GO annotation

- **Provides specific biological context about complex protein mixtures**
- **Provides critical information for additional data mining steps and targeted follow-up experiments**
- **Produces annotations in both pie chart and tabular form**







The pie charts here represent only GO-annotated proteins, but the GO annotation wizard in Proteome Discoverer can be selected to create charts for all identified proteins from a particular database search.

Automated Metadata Extraction

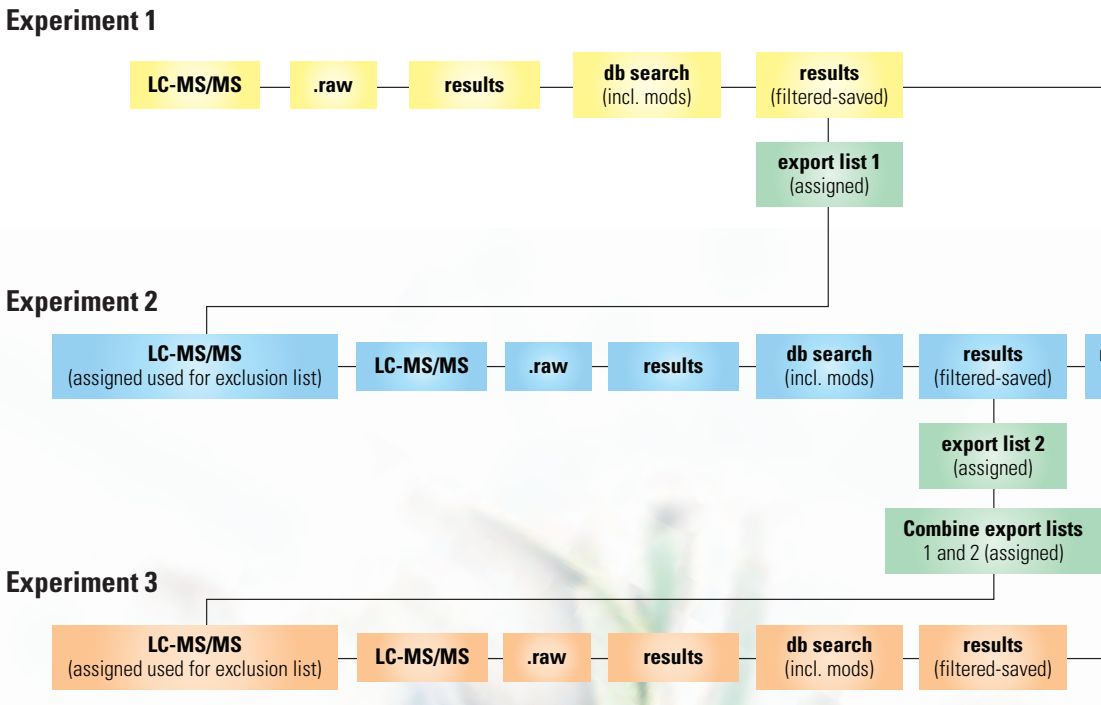
- **Provides sequence positions for known and predicted PTMs**
- **Provides descriptive information such as function, subcellular location, and tissue specificity**
- **Provides descriptions in an easy-to-use tabular format**

ACCESSION	GI	Protein_Name	COMMENT	MW	PUBMED	Sequence	Amino_	active	binding	glycosy	metal_b	methyla	modifie	phosph	acetylat	mut
NP_001054	4557871	transferrin [Homo	REVIEWED	76919	[17976429, 1	MRLAVGAL	698									
P06732	125305	Creatine kinase f	On Mar 15, 2		[3778498, 29	MPFGNTH	381	283	335,292							

Digging Deeper into Each Sample – Combining Data Acquisition with Creative Data Interrogation Strategies

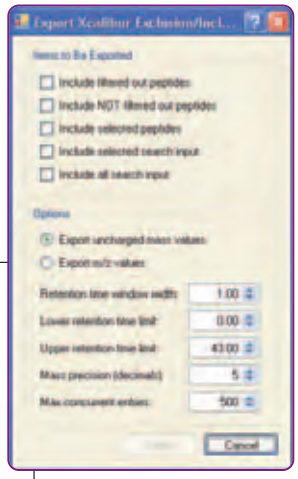
Iterative analysis strategies are a powerful tool for getting the most possible information from each sample. The combination of Proteome Discoverer and Thermo Scientific Xcalibur data acquisition software facilitates these sophisticated analyses.

- **Create inclusion and exclusion lists for direct import into Xcalibur™ instrument methods**
- **Design iterative analysis strategies to detect low-level sample components**
- **Export results using standard open file formats**



Quantitation – Using Proteome Discoverer to Analyze Isotopically Labeled Peptides

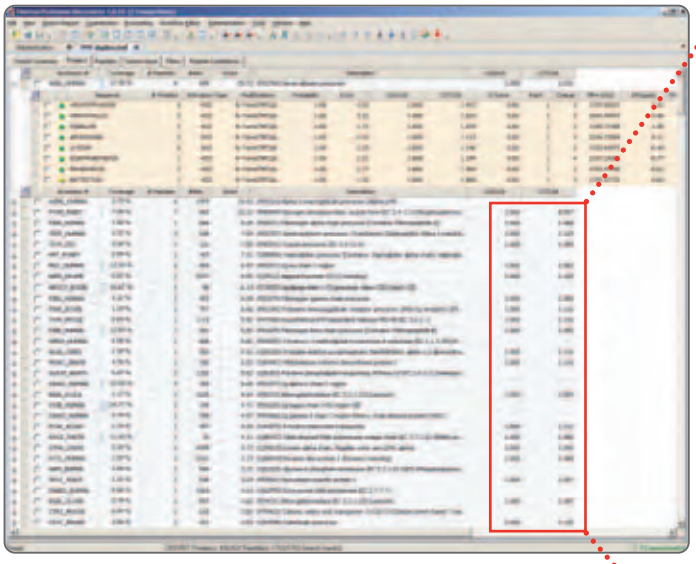
Changes in protein expression levels are often indicative of biological processes such as disease. Isobaric mass tagging has become a common technique for measuring these relative changes. Proteome Discoverer can accommodate several relative quantitation methods, including Tandem Mass Tags™ (TMT) which allow as many as six different samples to be multiplexed for comparison in a single experiment. The peptides identified and their relative quantitation ratios can be presented in a simple, graphical manner.



merge results (filtered-saved)

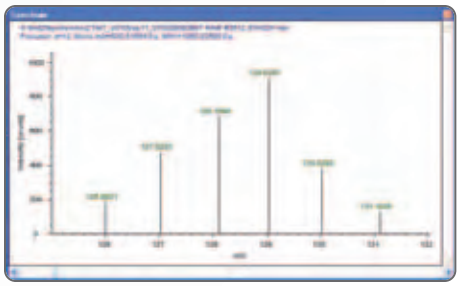
report (filtered-saved)

- **Easy and accurate quantitation with common isobaric labels such as TMT and iTRAQ**
- **Rigorous statistical data treatment for multiplexed assays**
- **Customizable filters – use only unique peptides to calculate accurate relative protein quantitation**
- **Normalization of peptide concentration corrects for experimental errors**

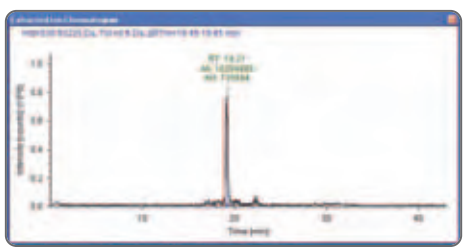


Report ratios of relative change between identified peptides.

126/126	127/126
1.000	0.597
1.000	1.060
1.000	1.129
1.000	1.050
1.000	1.062
1.000	1.128
1.000	1.000
1.000	1.116
1.000	1.116
1.000	1.095
1.000	1.116
1.000	1.116
1.000	1.059
1.000	1.112
1.000	1.000
1.000	1.092
1.000	1.080
1.000	1.057
1.000	1.087
1.000	1.125



Zoom in to view all diagnostic ions measured in a 6-plex TMT isobaric tag experiment.



Examine the calculated height and area for each extracted ion chromatogram.

Laboratory Solutions Backed by Worldwide Service and Support

Tap our expertise throughout the life of your instrument. Thermo Scientific Services extends its support throughout our worldwide network of highly trained and certified engineers who are experts in laboratory technologies and applications. Put our team of experts to work for you in a range of disciplines – from system installation, training and technical support, to complete asset management and regulatory compliance consulting. Improve your productivity and lower the cost of instrument ownership through our product support services. Maximize uptime while eliminating the uncontrollable cost of unplanned maintenance and repairs. When it's time to enhance your system, we also offer certified parts and a range of accessories and consumables suited to your application.

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