PEAKS® X PRO

COMPLETE & VENDOR NEUTRAL SOLUTION FOR DISCOVERY PROTEOMICS WITH DDA & DIA SUPPORT

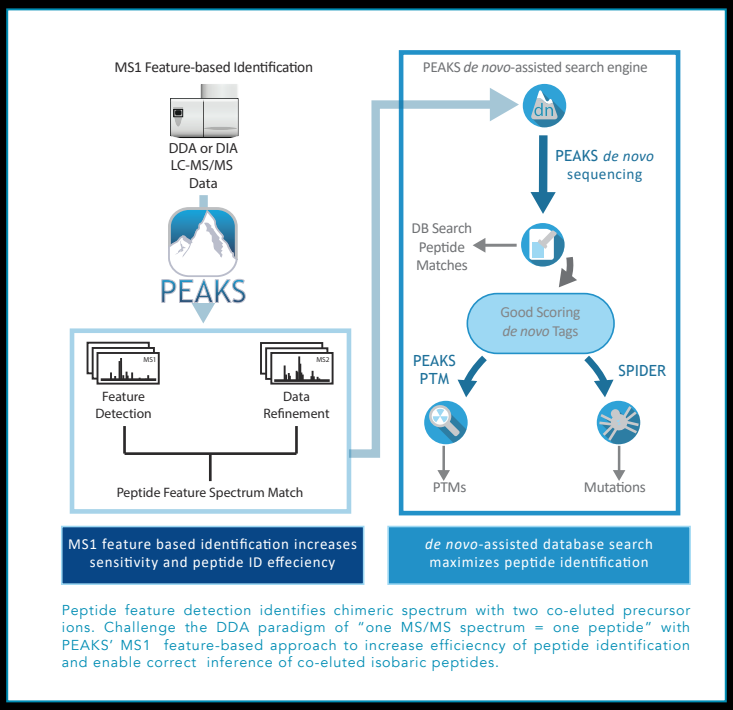
PEAKS: IDENTIFYING THE HIDDEN PROTEOME

Understanding fundamental biological processes requires a complete grasp of all entities within a proteome. This means going beyond what is already known. As mass spectrometry instrumentation achieve higher resolution and become more advanced, we aim to uncover additional information by generating more data with higher quality. However, many applications in proteomic mass spectrometry currently face limitations in the tools used to analyze this information rich data. Furthermore, researchers seek a vendor neutral solution to automate and facilitate the overall goal of their projects. Thus, to support the advancement of biological discoveries and characterize the unknown, we need to further exploit DDA & DIA data to uncover the hidden parts of the complex proteome.

PEAKS Feature-based Identification Workflow

Today's mass spectrometry data is acquired with higher resolution and mass accuracy and this can be exploited by increasing sensitivity of data analysis.

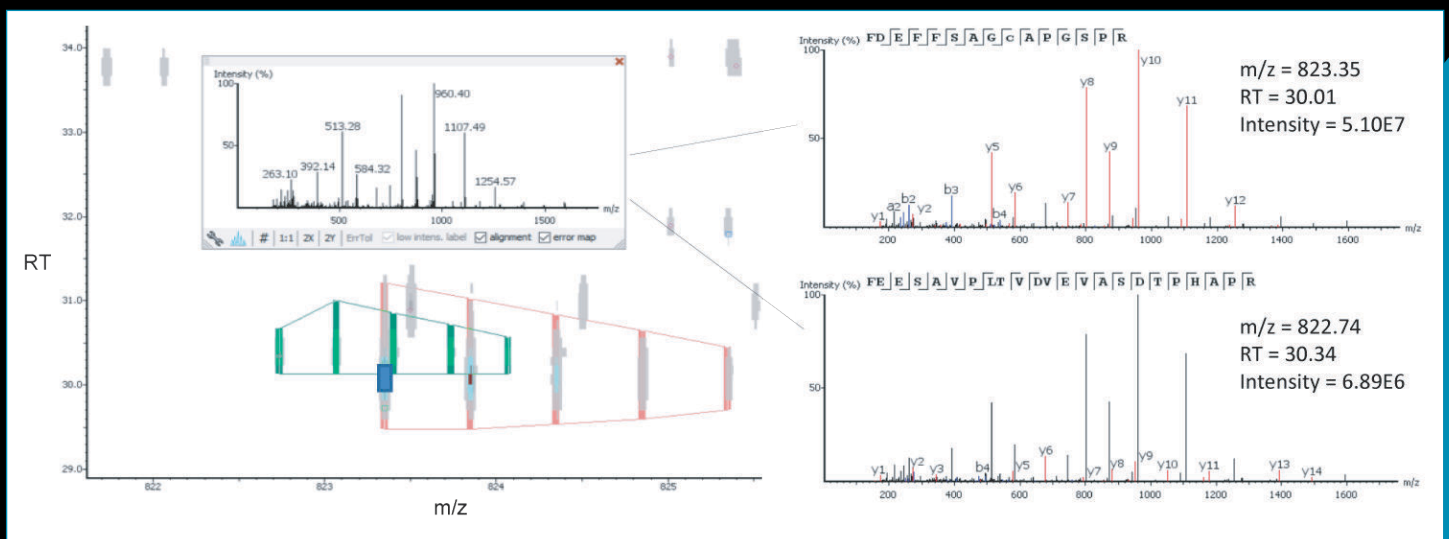
With this in mind, integration of a peptide feature-based identification algorithm to the unique PEAKS *de novo*-assisted workflow offers increased sensitivity and maximized peptide identification efficiency for in-depth shotgun analysis of complex proteomes.

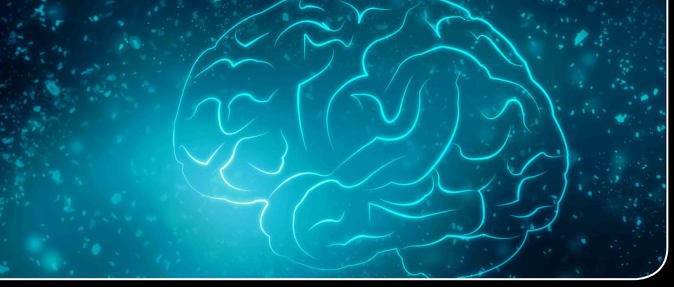


Peptide feature detection identifies chimeric spectrum with two co-eluted precursor ions. Challenge the DDA paradigm of "one MS/MS spectrum = one peptide" with PEAKS' MS1 feature-based approach to increase efficiecncy of peptide identification and enable correct inference of co-eluted isobaric peptides.

FEATURE-BASED PEPTIDE IDENTIFICATION OCCURS IN 3 MAIN STEPS:

1. PEPTIDE-FEATURE DETECTION 2. FEATURE-SPECTRUM ASSOCIATION 3. PEPTIDE FEATURE SPECTRUM MATCH





Generally speaking, there are three common ways to interpret tandem mass spectrometry data: database search, *de novo* sequencing and spectral library search:

DATABASE SEARCH:
Given a spectrum and a protein sequence database, find a peptide in the database that has the best match with the spectrum.

DE NOVO SEQUENCING:
Given a spectrum, find a peptide that has the best match with the spectrum.

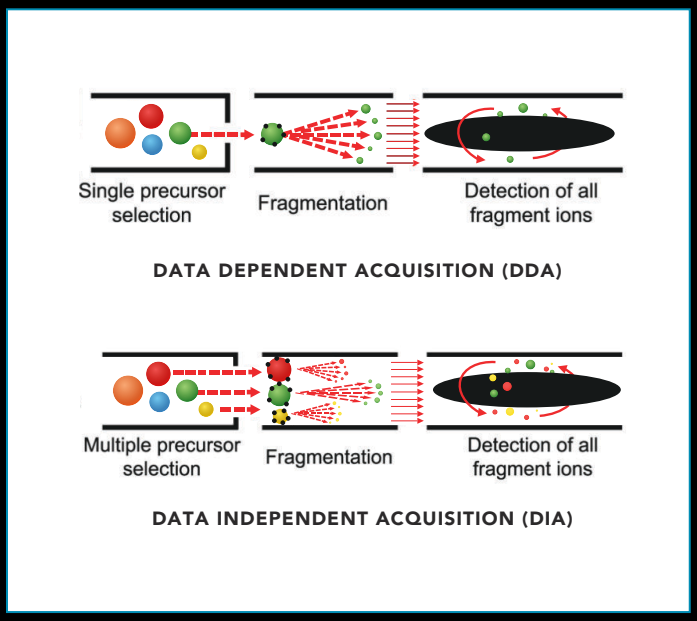
SPECTRAL LIBRARY SEARCH:
Given a spectrum and spectral library, find a peptide in the spectral library that has the best match with the spectrum.

1. When the peptides are believed to be in a protein sequence database, then a database search approach is preferred.
2. When studying a particular proteome, a peptide spectral library for the targetted biological system being studied can be used to focus your analysis.
3. However, when such a sequence database or spectral library is unavailable, de novo sequencing is needed to derive the peptide sequence directly from the spectrum.

Perform Everything in PEAKS: The vendor- and acquisition-neutral proteomics software solution

Both DDA and DIA technologies are rapidly advancing, and researchers need an analysis method that harmonizes the benefits of both acquisition methods. In recent years, DIA has become increasingly popular due to its parallel nature of acquiring all fragment ions for all precursors within a selected m/z range. This overcomes the limitations of sequential MS/MS acquisition in DDA.

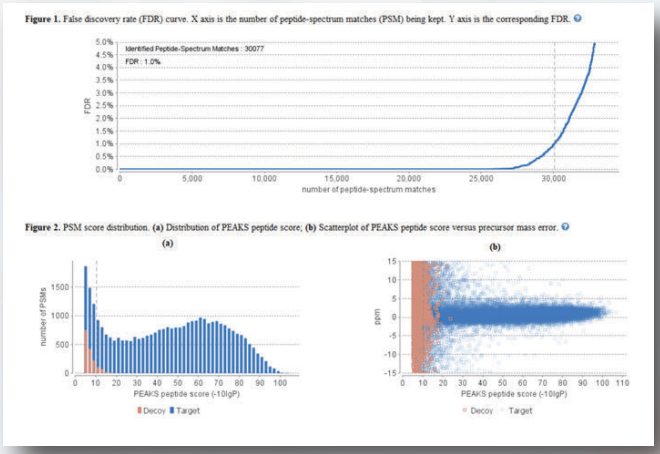
As a vendor neutral proteomics software developer, we strive to provide a comprehensive solution to facilitate proteomics research and support efficiency in mass spec labs. Avoid the need to use different software for various analytical and acquisition methods.



Spectral libraries provide a refined search space that matches the fragment ion pattern, expected retention time, and other unique features of the experimental data to previously identified spectra in the library

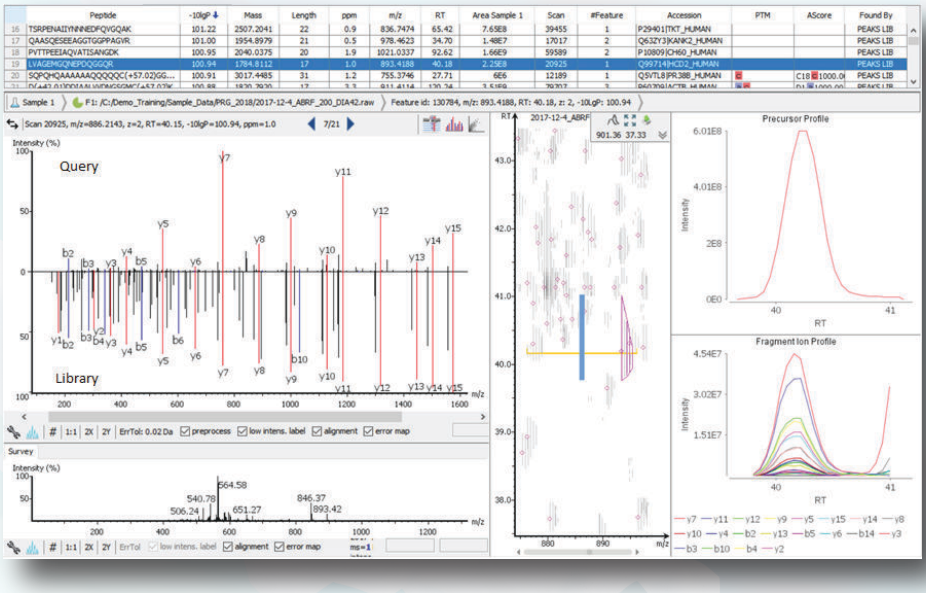
Generate reproducible, accurate identification of known peptides in DDA and DIA datasets with the spectral library searching in PEAKS Xpro.

The enhanced PEAKS Xpro spectral library search uses m/z, intensity values, normalised retention time plus ion-mobility spectrometry values (CCS/CV values with PEAKS IMS add-on module), and is designed to work with both DDA and DIA spectral library searches. With precise FDR estimation users can detect more peptides in each dataset without sacrificing accuracy for both, DDA and DIA analyses. The addition of the spectral library search to the current functions in PEAKS allows users to perform their whole pipeline in one software tool, from creating a spectral library to spectral library searching.



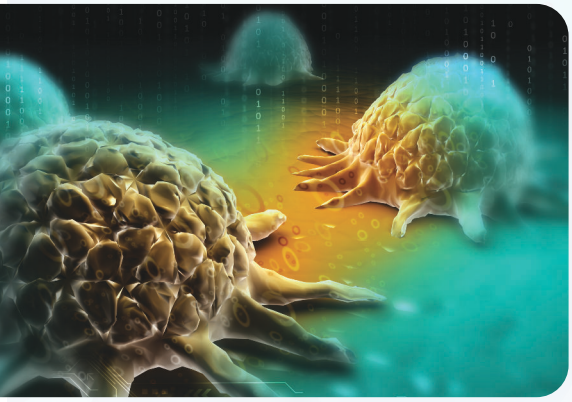
DID YOU KNOW:

PEAKS' spectral library searches use RT, CCS and AI-based spectral prediction to improve the separation of true and false hits



PEAKS Spectral Library Viewer & Editor for PEAKS-Generated and 3rd Party Libraries

Utilise the Spectral Library Viewer and Editor within PEAKS Xpro to assess core spectral library information and gain important insight into the attributes of the library you are utilizing for your search. With detailed tables and interactive view of the spectral library, you can validate the library and easily make the necessary changes to ensure a high-quality peptide spectral library is used for all of your searches.



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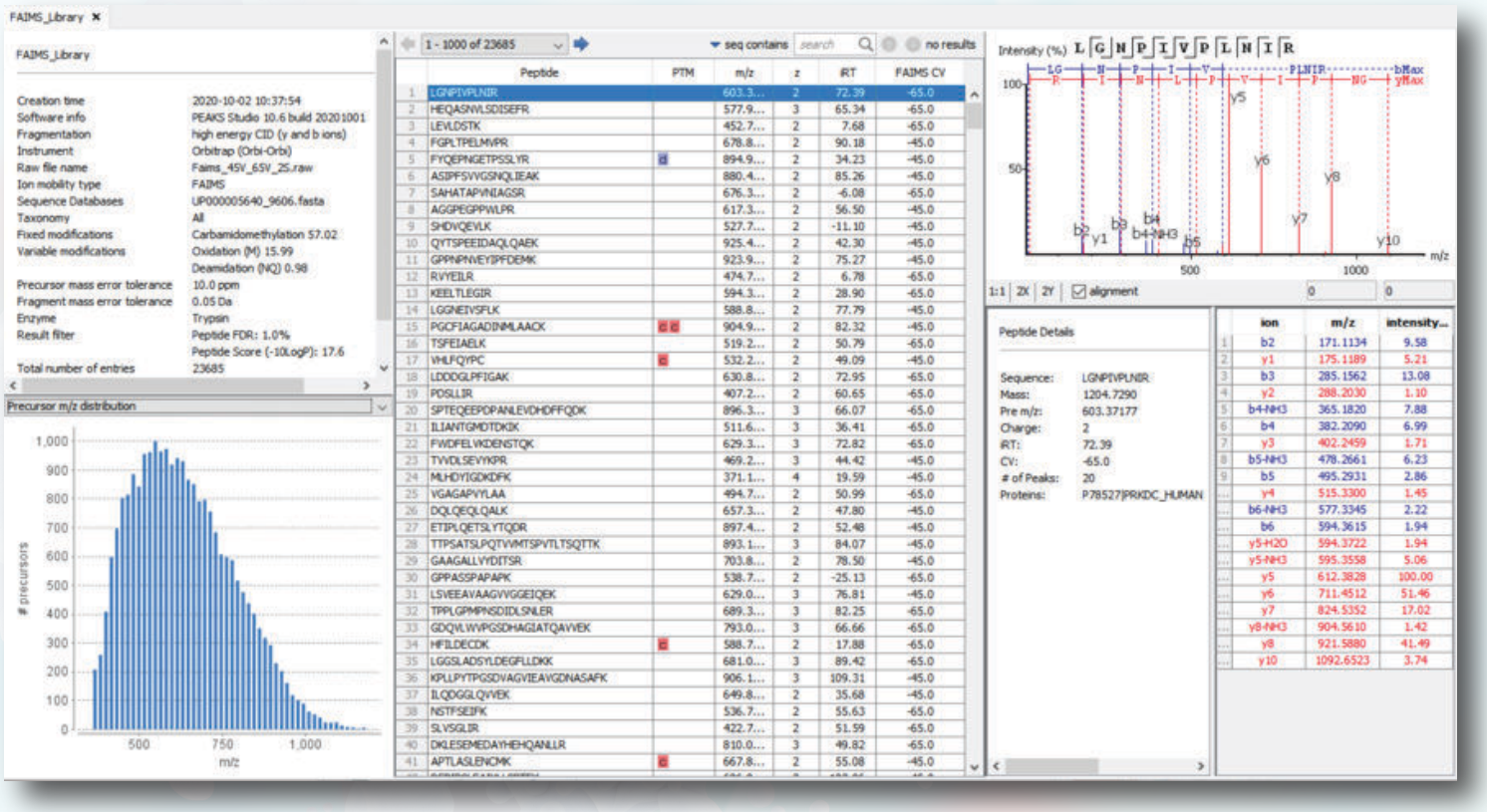
Spectral Library editor and viewer compatible with PEAKS_LB, OpenMS_LB, text file libraries, and more. Easily view third party libraries and utilise them in PEAKS with Spectral Library Viewer!



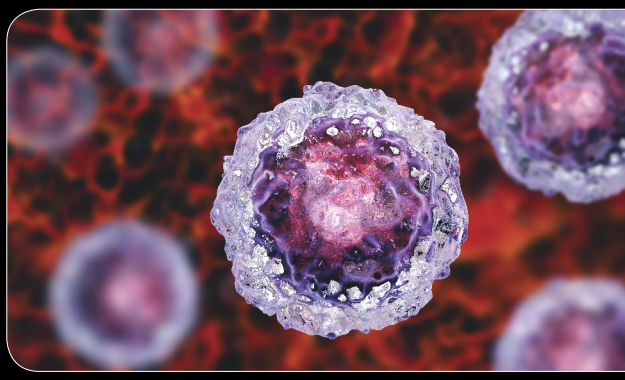
Using PEAKS Xpro spectral library search you can expect a fast, accurate and easy use solution to analyze your DIA, FAIMS DIA, SWATH, and diaPASEF. Spectral library searches are a great way to screen samples for known peptides/proteins and with PEAKS Xpro you expect; improved sensitivity for single cell proteomics; and other low abundant data.

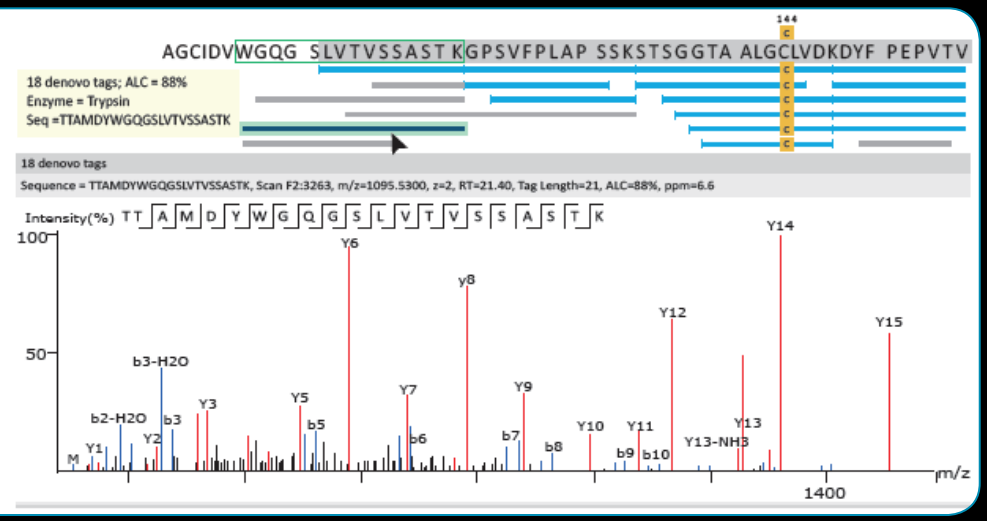
PEAKS' *de novo* sequencing is world renowned and the base of all PEAKS analyses.

In mass spectrometry, *de novo* sequencing derives an amino acid sequence from a mass spectrum without the need of a sequence database. In contrast to the popular 'database search' peptide identification approach, *de novo* sequencing is the only choice when the sequence database is not available. This makes PEAKS the preferred method for identifying novel peptides and proteins from unsequenced organisms.

Why not use both? The added power of *de novo*-assisted discovery

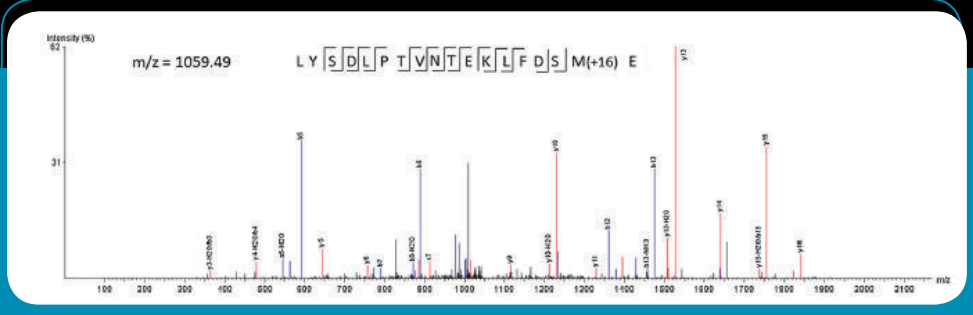
They say, two is better than one. Be the leader in your field of research; overcome the limits of a traditional database search, while minimizing *de novo* sequencing errors when you combine both de novo sequencing and database search identification methods together.





PEAKS offers a unique approach to its data analysis by combining the derived *de novo* sequence with the corresponding database search result. *De novo* peptide sequences are aligned with protein database entries to facilitate the identification of:

- PTMs
- Homologous Peptides
- Mutations
- Novel Peptides



DID YOU KNOW:

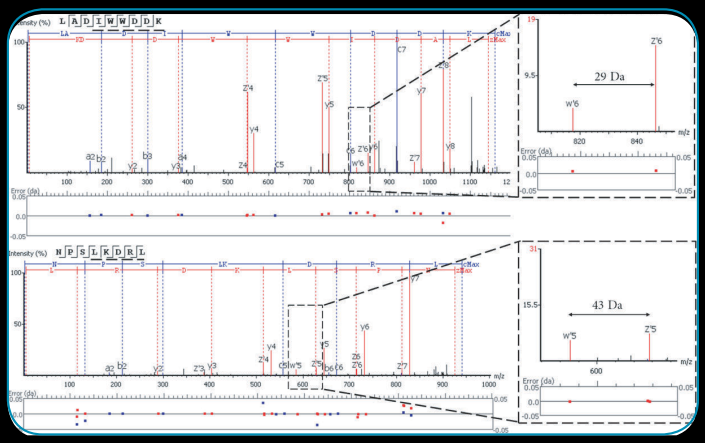
Camel milk and urine have also been used as medicines to treat various ailments including cancer. However, there is a lack of detail on the constituents of camel milk. Using PEAKS' de novo-assisted search, novel peptides in camel's milk can be identified.

With PEAKS, you can ensure that new instrument fragmentation methods are optimized for peptide sequence reconstruction.

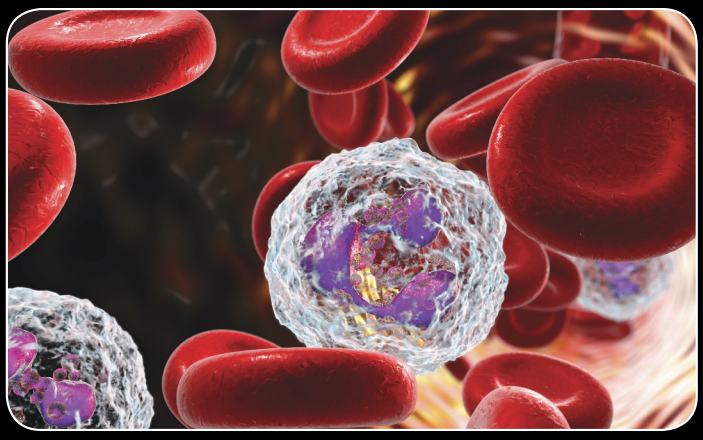
Before support within the software, PEAKS undergoes an extensive fragmentation-specific algorithm training to confidently analyze various fragmentation data. Supported Fragmentation: CID/CAD, HCD, ETD/ECD, EThcD, and mixed/complementary fragmentation.

PEAKS provides enhanced separation of true and false hits by incorporating *de novo* sequencing into a database search. This unique *de novo*-assisted approach will allow you to identify more peptides and proteins with greater confidence.

The detailed PEAKS Studio interface allows users to quickly define, filter and visualize results as desired. With a few clicks you can specify a false discovery rate, or draw project-wide comparisons between your samples.



With endogenous peptides, there are inherent challenges with the abundance and natural complexity that pose analytical road blocks. By utilizing EThcD, the MS/MS rich spectra provides a new technique to increase the accuracy and confidence of de novo sequencing and database search results. It further allows the direct discrimination between the isobaric amino acids, isoleucine and leucine, based on a signature w-ion generated by each amino acid.



Designed to discover hidden modifications

In PEAKS PTM and SPIDER, the highly confident spectra with a good *de novo* score are reanalyzed to assess any unknown PTMs or sequence variants.

PEAKS PTM

Specify the PTMs of interest or search all 313 naturally occurring biological modifications from the Unimod database in your PEAKS PTM search. Don't let your computational resources limit you.

SPIDER

Cross-species homology search with SPIDER

De novo tag homology search tolerates common *de novo* sequencing errors such as (AT/TA) and (N/GG). Find confident hits that do not exist in the database with our *de novo* based homology search.

SPIDER provides a specialized tool for:

- Resolving database errors
- Antibody sequence confirmation
- Potential biomarker discovery
- Mutated peptide identification

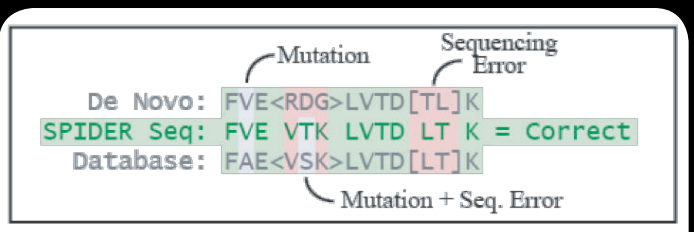
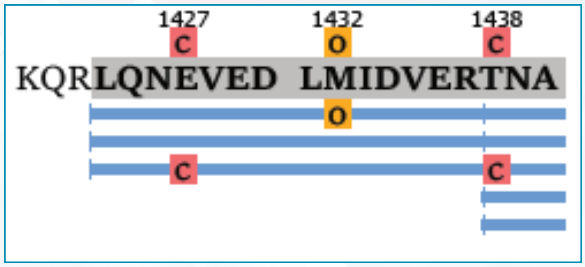


Figure 1: Illustrating how SPIDER reconstructs the correct sequence from a de novo sequence and a homologous peptide.

The analysis of post-translational modifications (PTMs) is essential for the understanding of biological pathways. With PEAKS, you can maximize ID efficiency and thoroughly characterize PTMs in a complex proteome.

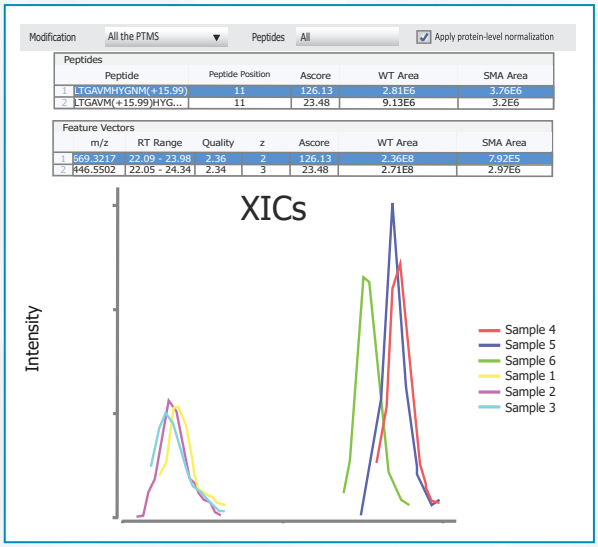
Site determination & validation

Identified modifications in PEAKS can be confidently assessed using A-Score, a positional confidence assessment score, or the minimal ion intensity. Use PEAKS to validate your results accurately before reporting them.



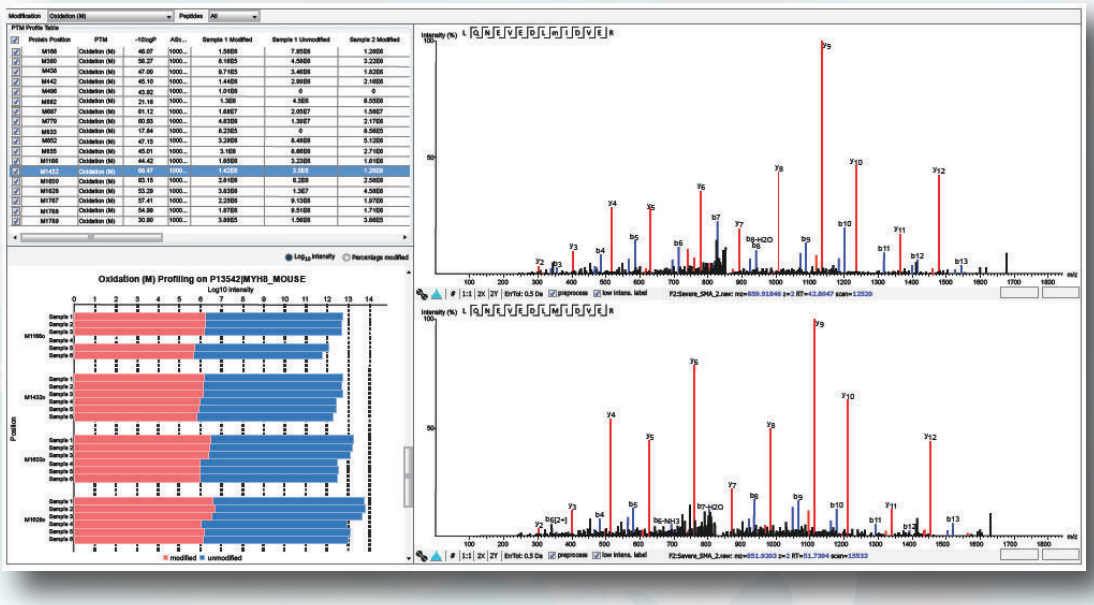
Resolve abundance variation to accurately quantify PTMs

Discover more with PEAKS Q and harness the information that can be extracted from experimental datasets. Report PTM changes confidently between samples by normalizing the quantified PTM with protein abundance.



Decipher differences between experimental groups with PTM profiling

With the PTM Profiling tool, you can profile the abundance of modified and unmodified forms at each identified PTM site. Researchers can analyze PTM abundance at the positional level across time, replicates and conditions.

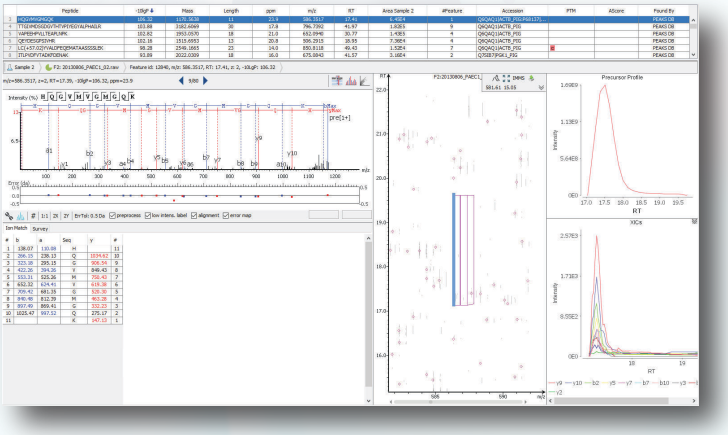


Ion Mobility Spectrometry - Mass Spectrometry (IMS-MS) provides a compelling analytical workflow for complex biological and chemical mixtures by adding an additional dimension of ion separation; a 4th-dimension.

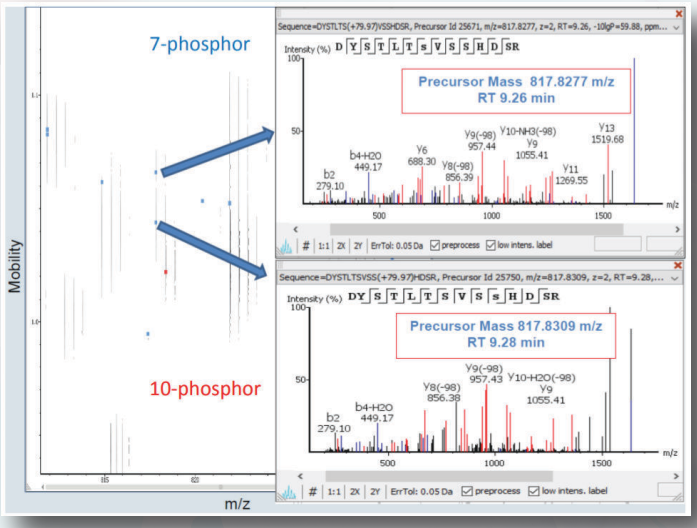
With IMS-MS, ions are separated based on their mobility through a buffer gas, which provides the capability to differentiate ions based on their size, shape, charge and mass mobilities. Thus, it is possible to resolve ions that may be indistinguishable by traditional mass spectrometry.

Using PEAKS, the ion mobility data can be viewed in the Mobility-LC-MS 4th-Dimension. The additional dimensionenables increased identification sensitivity with smaller sample amounts.

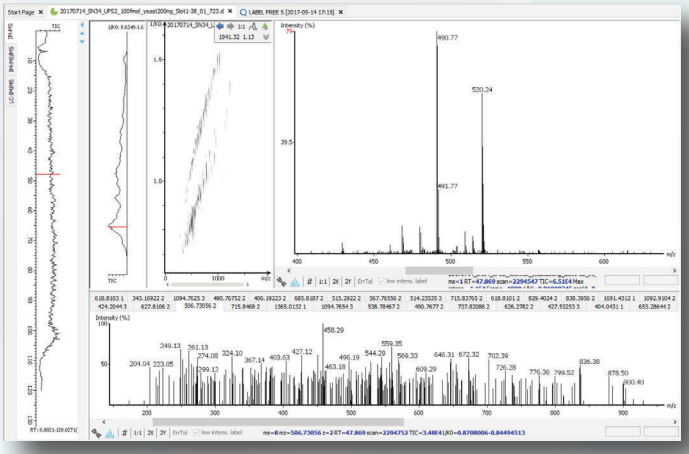
- Analyze IMS-MS data using PEAKS de novo, identification and quantification workflows
- **Interactive data visualization tools to view data projected on m/z-rt or m/z-1/k0 dimensions**
- Vendor neutral; PEAKS is able to support IMS data from any instrument
- **Enable accurate and sensitive quantification analyses for IMS-based proteomics studies (Ex. label-free, SILAC, TMT/iTRAQ)**



Take advantage of HDMSe data by extracting the accurate mass and CCS information obtained for all precursor and fragment ions to improve accuracy and reproducibility of peptide/protein identification and quantification.



PEAKS was used to analyze an extract of a HEK cell digest after a PASEF acquisition. The two co-eluting parent ions were separated in the ion mobility dimension, revealing two isobaric peptides differing only in the position of phosphorylation.

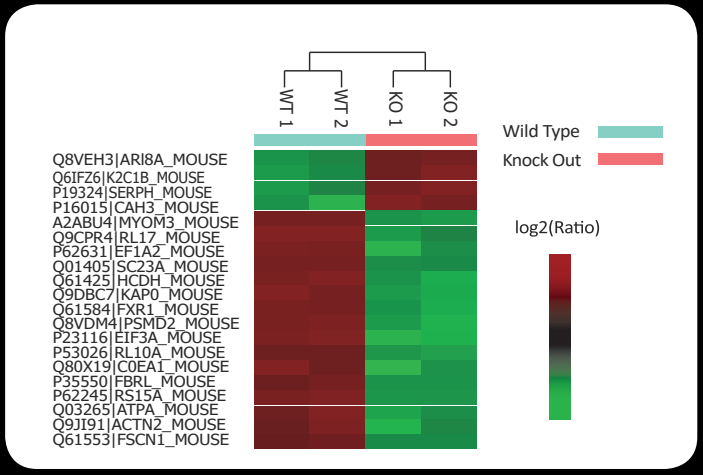


Easy-to-use PEAKS graphical user interface categorizes the raw data into IM-MS, IM-MS/MS, and LC-IM/MS. Researchers can easily view 4-D feature detection and feature separation based on ion-mobility.

Quantification provides greater insight into proteomic mysteries. Researchers need a software tool to support them as they press further in to the understanding of life sciences.

PEAKS is equipped with not only a powerful identification algorithm, but also embraces paralleled quantification capabilities to perform:

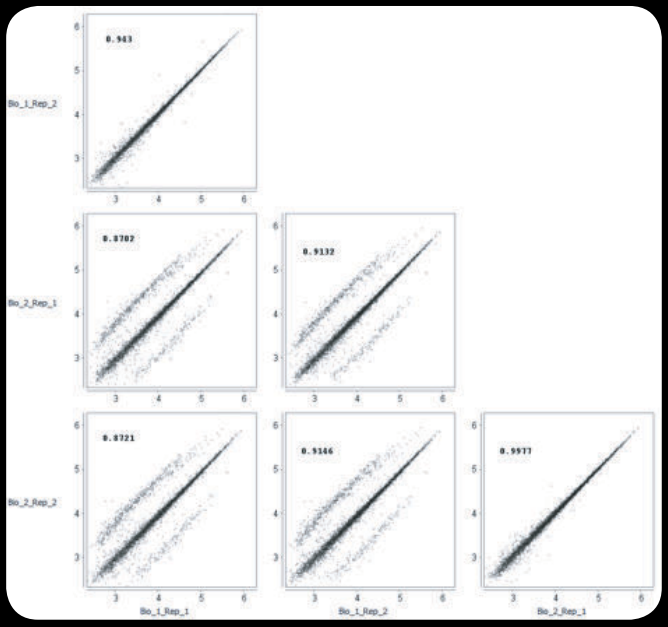
- Label-free Quantification (LFQ)
- TMT/iTRAQ
- SILAC/Dimethylation labelling
- Custom methods support



OBSERVE AND VALIDATE WITH EASE

Validate significant changes seen in the heat map by assessing individual significance and quality scores.

Cut hours out of analysis time and make filtering out false positives easy. These filters change dynamically and will automatically be reflected in the heat map and other PEAKS-based quantification results.



Label-free quantification

PEAKS Q's LFQ function provides researchers with the option to calculate protein abundance either by using the well-known Top-3 peptides method or by using all unique supporting peptides. Researchers can then thoroughly investigate differences in peptide/protein abundance between samples while confident in high-level, accurate results. The module provides detailed peptide-specific results, and researchers are given additional options to handle the following cases:

- Peptides with both modified and unmodified forms, and
- Redundant peptides
  Peptides without ID

PEAKS Q calculates based on the extracted ion chromatograms (XICs) of the whole isotopic envelope on the MS1 level to provide accurate and sensitive MS1-based quantification for low abundance proteins. PEAKS Q also extracts and uses the LC retention time and MS features to align different runs.

Precursor Ion Quantification

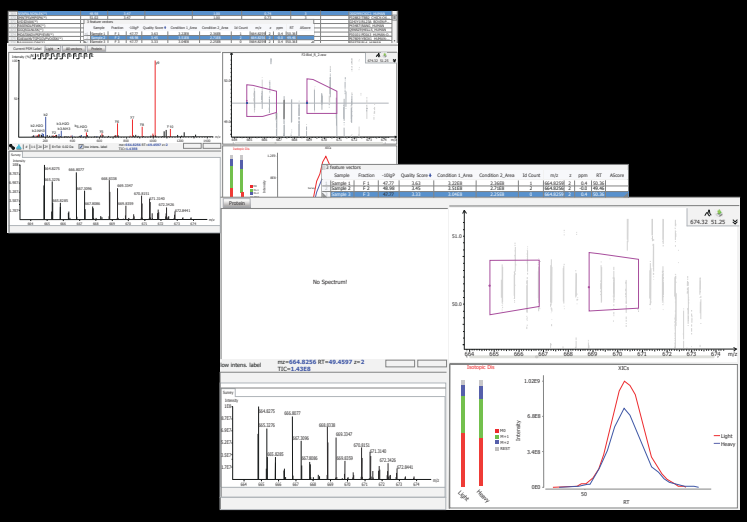
Stable Isotope Labelling by Amino Acids in Cell Culture (SILAC) is a powerful and popular approach for mass spectrometry (MS)-based quantitative proteomics. PEAKS Q's SILAC quantification enables unsurpassed sensitivity of peptide feature detection through a novel peptide feature detection algorithm to find peptide feature pairs.Researchers can take advantage of the intuitive interface showing paired features at first glance and minimize the biases from missing values.

ARGININE TO PROLINE CONVERSION CORRECTION

A limitation of SILAC quantification is that some cells convert high concentrations of Arg to Pro. As a result, the abundance of the heavy label could be incorrect. PEAKS Q, however, considers this common phenomenon and will also consider heavy labeled Pro to correct this innate matter.

COMPLEX SILAC DATASET ANALYSIS AND QUANTITATIVE PTM PROFILING

PEAKS supports complex SILAC dataset analysis and quantitative PTM profiling of individual protein and all identified proteins. In PEAKS Q, experimental design supports analyses of SILAC and super-SILAC types of data. This pool of spiked-in heavy proteins can be used as an internal standard for quantification.



Reporter Ion Quantification

Isobaric tags (ex. TMT/iTRAQ) have identical masses and chemical properties that allow heavy and light isotopologues to co-elute. The tags are then cleaved from the peptide by collision-induced dissociation during MS/MS, which is used for quantification.

COMBINING EXPERIMENTS WITH REFERENCE CHANNELS

For large-scale, protein quantification studies, researchers can use PEAKS Q to expand the sample size with reference channels to enhance the accuracy of quantification.

ACCURATE AND SENSITIVE QUANTIFICATION

To ensure a high confidence of accuracy, PEAKS Q provides users the ability to perform labeled quantification analysis using the latest methodology. Researchers can analyze both MS2- and MS3 synchronous precursor selection (SPS)-based reporter ion quantification. The MS3 approach removes ratio distortion of interfering signals from co-isolated species; thus, providing high-quality quantification results.

DID YOU KNOW: Retention times of different LC-MS runs in PEAKS Q are first aligned, then the MS/MS and ID can be matched from another run by aligning features within tight mass ranges and retention times, allowing quantification of SILAC pairs without any ID.

From Service to Software tool, PEAKS AB, for all your therapeutic protein characterization needs

Antibody Characterization Service

- Full sequence coverage and 100% accuracy guaranteed.
- Comprehensive sequence validation by our expert application scientists.
- Identification of PTMs and sequence variants.
- Quantitative analysis of PTMs and sequence variants.
- In less than two weeks, receive a comprehensive and PEAKS-based report.

Antibody Characterization Software

- Automated antibody protein sequencing with LC-MS/MS.
- Antibody protein sequence validation.
- Qualitative and quantitative analysis of PTMs and sequence variants.
- Assessment of impurities.
- Comprehensive and automated report generation.

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