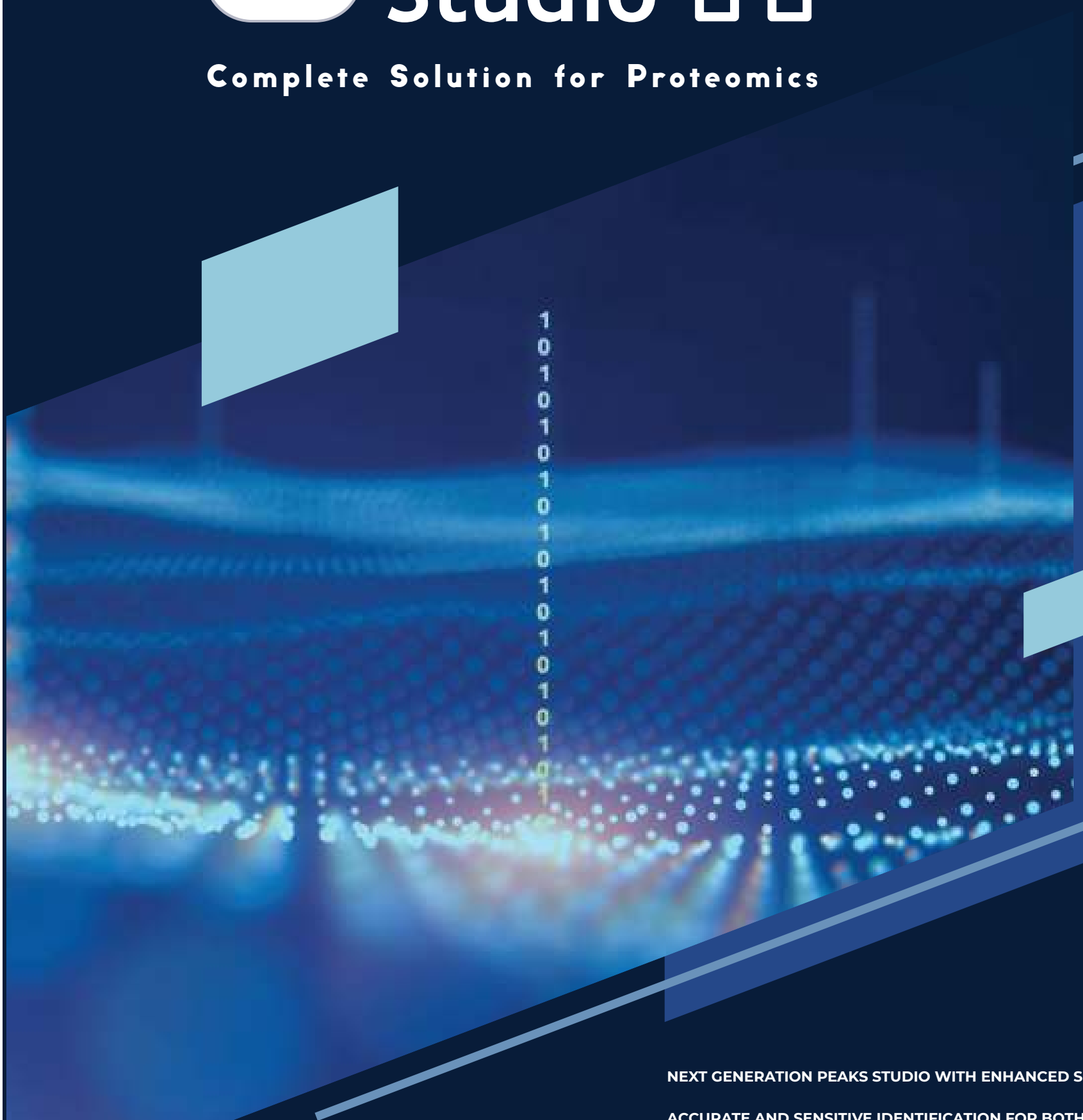
PEAKS® Studio 11

Complete Solution for Proteomics



NEXT GENERATION PEAKS STUDIO WITH ENHANCED SPEED AND STABILITY

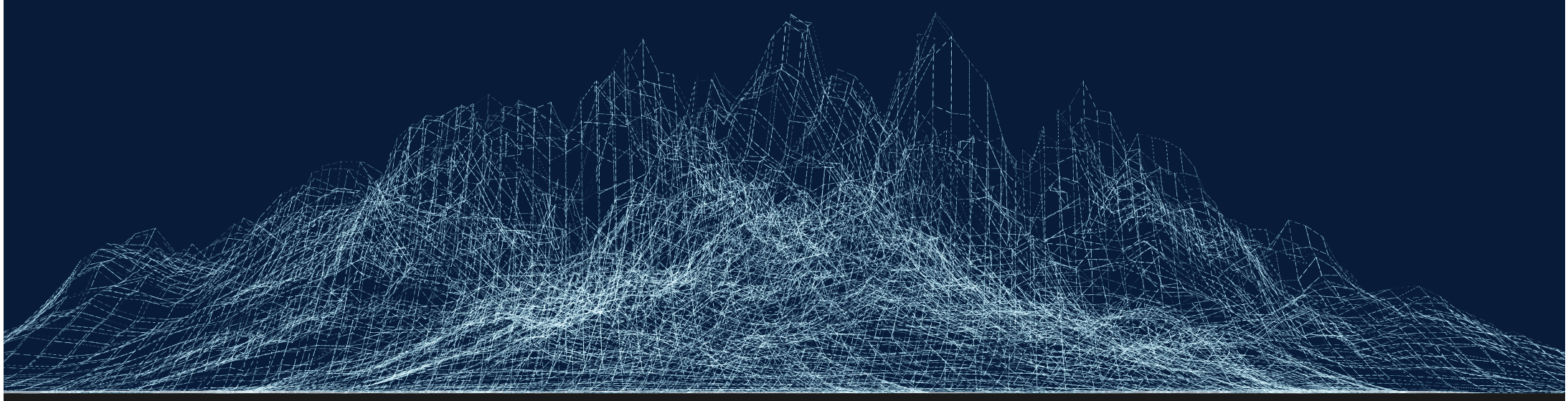
ACCURATE AND SENSITIVE IDENTIFICATION FOR BOTH DDA AND DIA ANALYSIS

ROBUST LABEL-FREE AND LABELLED QUANTIFICATION SUPPORT

NEW DEEP LEARNING ENABLED DIA WORKFLOW AND DEEPNOVO PEPTIDOME ANALYSIS

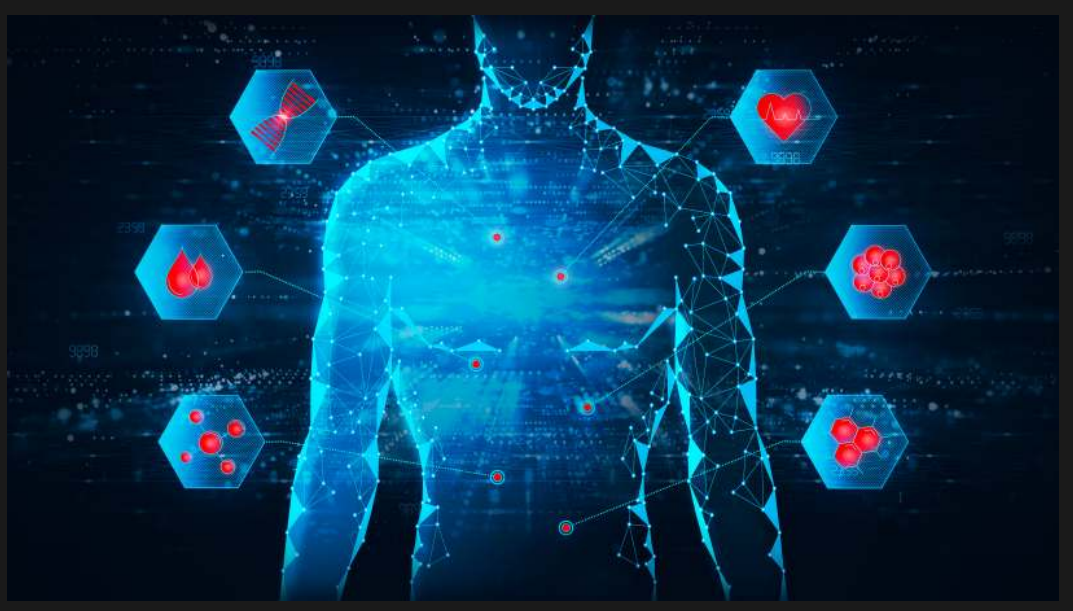
BIOINFORMATICS SOLUTIONS INC.

NEXT GEN PEAKS®



Mass spectrometers and other related analytical techniques have continued to improve at a rapid pace over the past few decades. It is important for researchers to have software that is up to date and can handle the continuously improving data outputs.

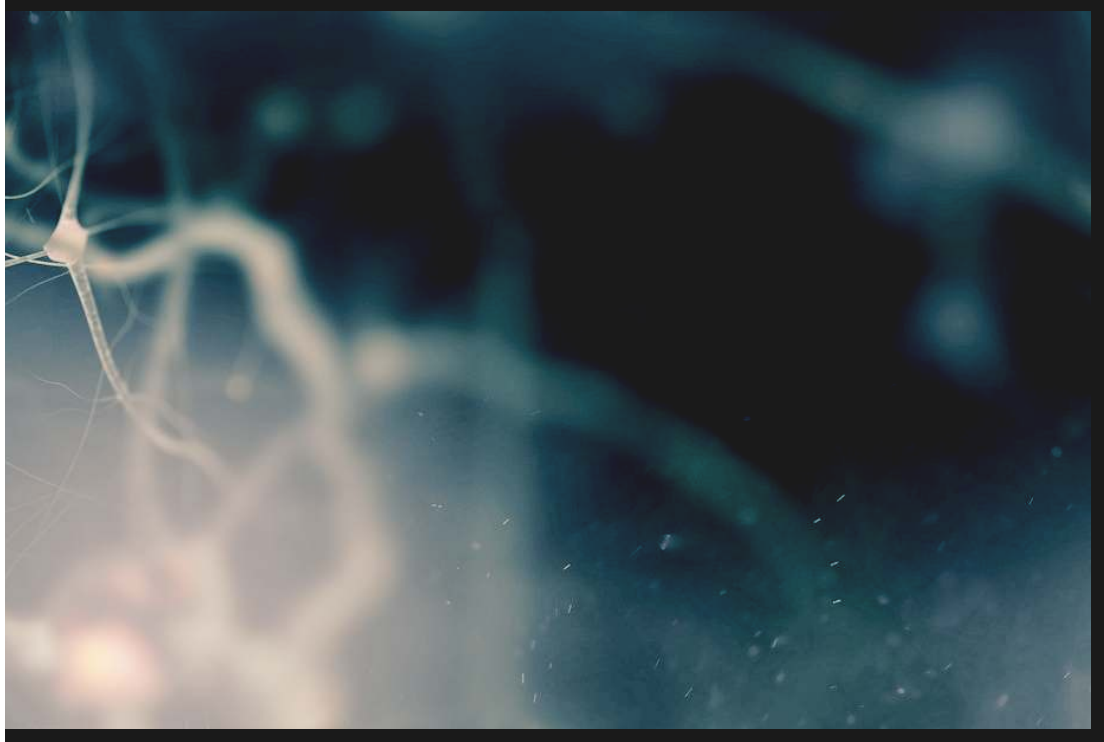
Developing innovative software and applying AI-driven technology to proteomics data analysis is vital to advancing research by providing faster, more accurate and sensitive identification and quantification. Together the latest mass spectromery technology and PEAKS® Studio will advance the frontier of biological research and facilitate drug discovery.



PEAKS® Studio 11 is the next generation of the studio platform and features a completely redesigned architecture to provide increased speed and stability. With the updated Graphical User Interface, users still get the intuitive data visualization that PEAKS® is known for but with a new look and optimized workflows to streamline your data analysis. From DDA to DIA data support, PEAKS® Studio 11 provides a complete solution to bring your research to new heights!

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 spectrometry labs. Avoid the need to use different software for various analytical and acquisition methods.



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 targeted 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.

PEAKS® provides a complete solution for all LC-MS/MS search methods!

DE NOVO SEQUENCING:

Given a spectrum, find a peptide that has the best match with the spectrum.

DATABASE SEARCH:

Given a spectrum and a protein sequence database, find a peptide in the database 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.

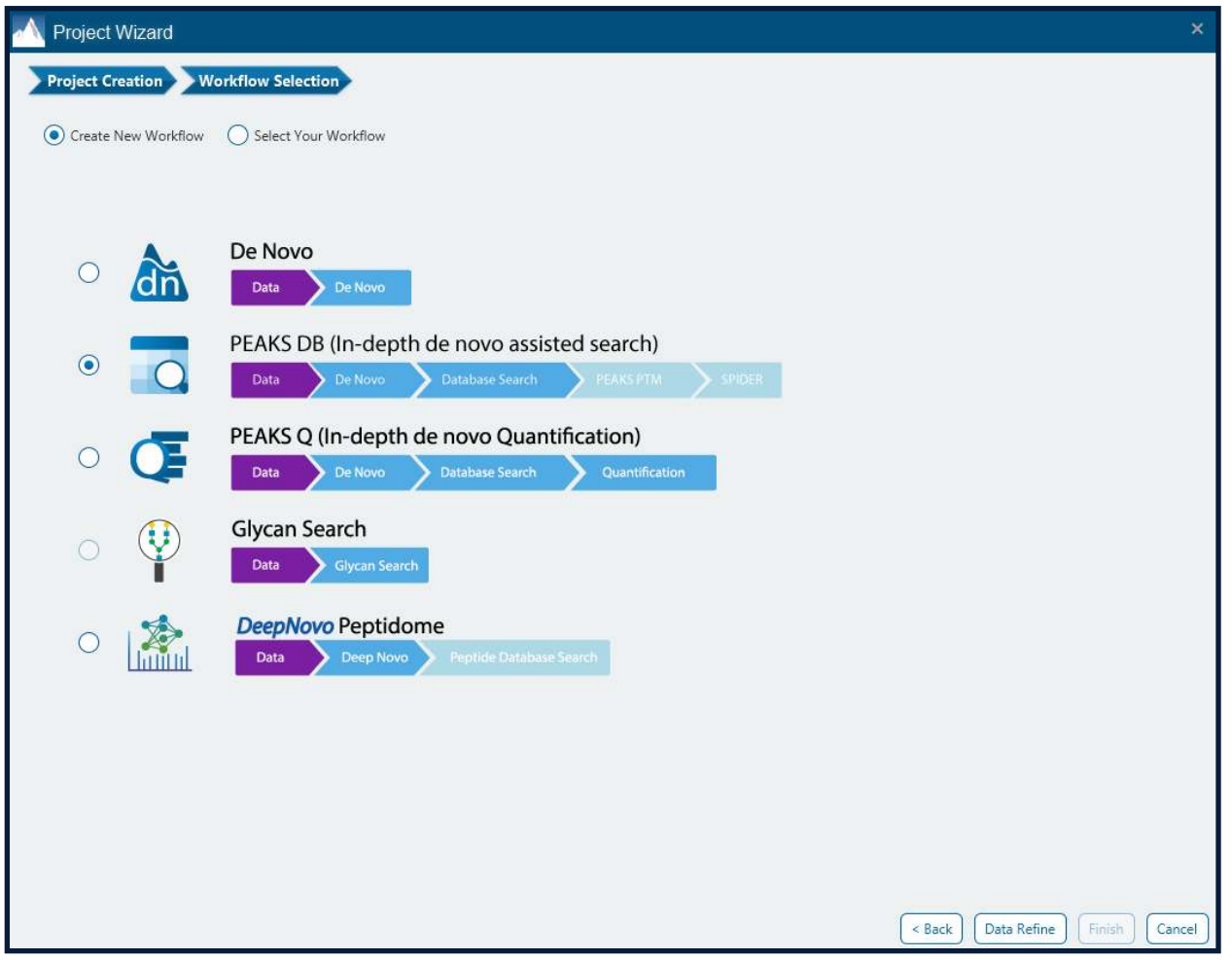
DIRECT DATABASE SEARCH:

Given a spectrum and protein sequence database, construct an in-silico spectral library using spectrum and iRT prediction models to find a peptide in the spectral library that has the best match with the spectrum.

Streamline your LC-MS/MS data analysis with

NEW WORKFLOWS

Seamlessly progress from raw file to report for all your bottom-up mass-spectrometry-based proteomics projects. PEAKS® Studio is a vendor-neutral software that significantly enhances laboratory efficiency, reproducibility, and reporting.

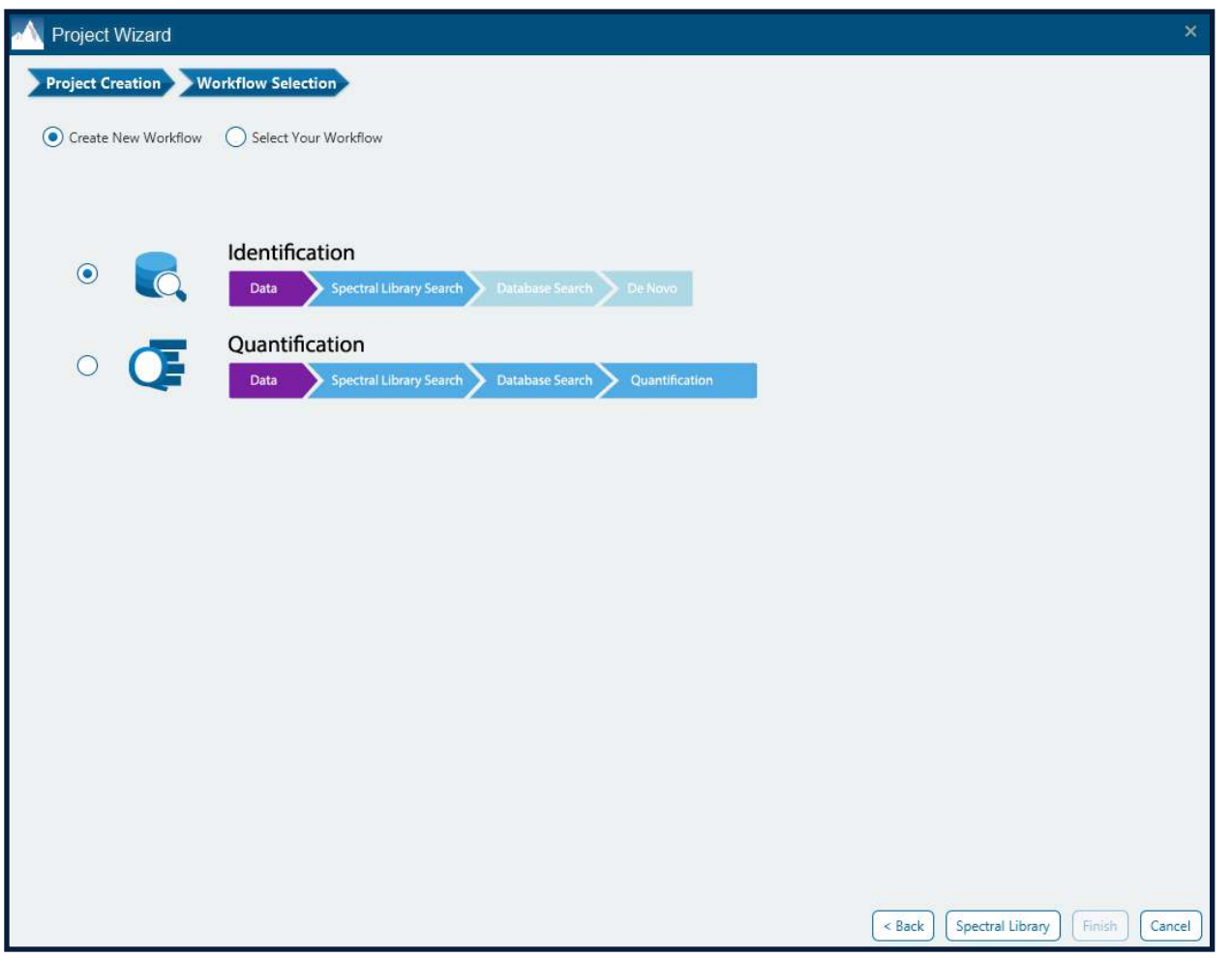


From *de novo* sequencing and database search to quantification, take advantage of the proven PEAKS® search algorithms effeciently as established workflows.

New to PEAKS® Studio 11:
- PEAKS® Glycan module add-on to enable glycoproteomicms analysis

- PEAKS® DeepNovo Peptidome, a unique workflow that integrates innovative deep learning technology for accurate and sensitive *de novo* peptide sequencing and database identification.

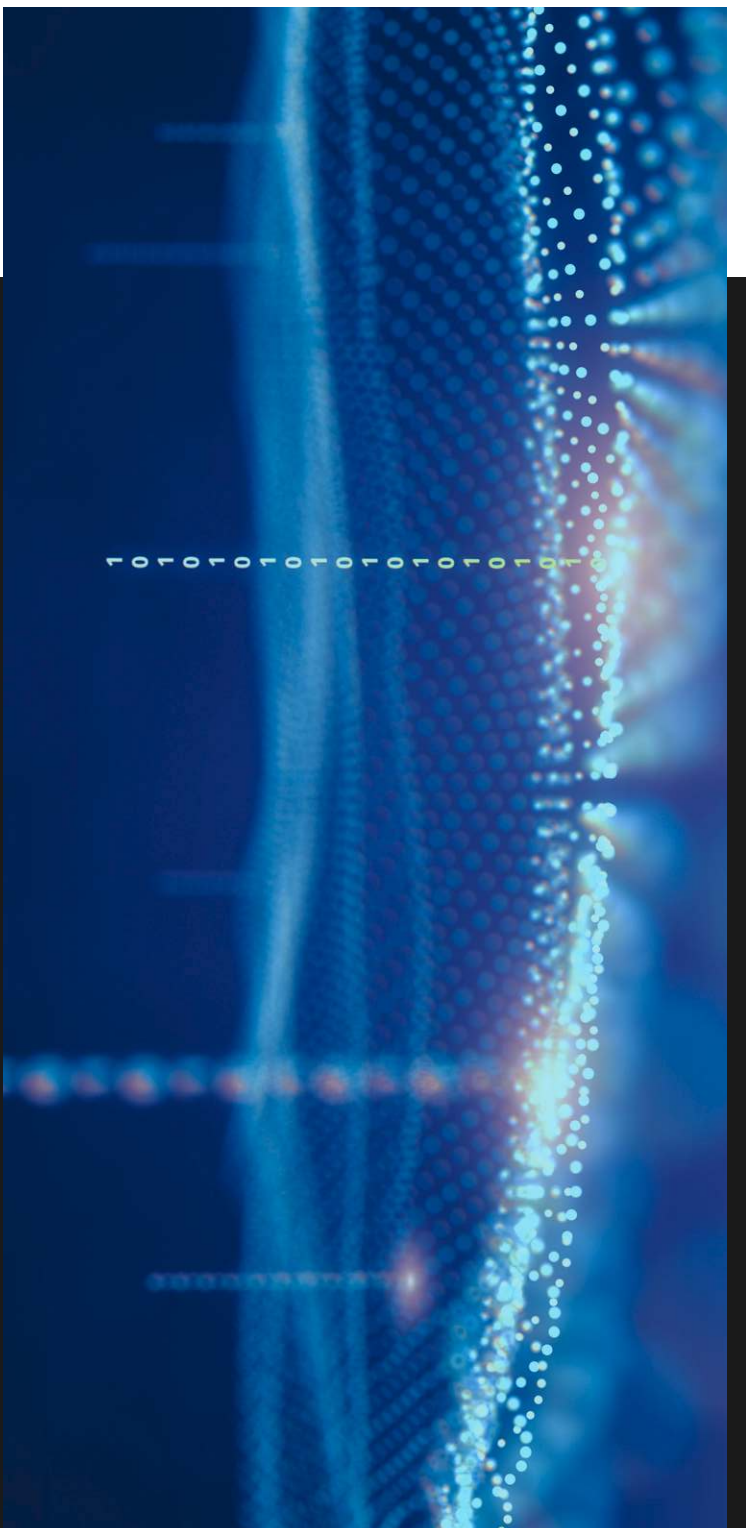
PEAKS® Studio 11 features a new DIA workflow that incorporates three methods of peptide identification: spectral library search, direct database search, and *de novo* sequencing. For quantification, the label-free method is available as an add-on module.



Updated Software Architecture and Graphical User Interface (GUI)

The updated software architecture provides increased speed and stability for identification and quantification. Support all your discovery proteomics research with higher throughput and robust data analysis for both, DDA and DIA support

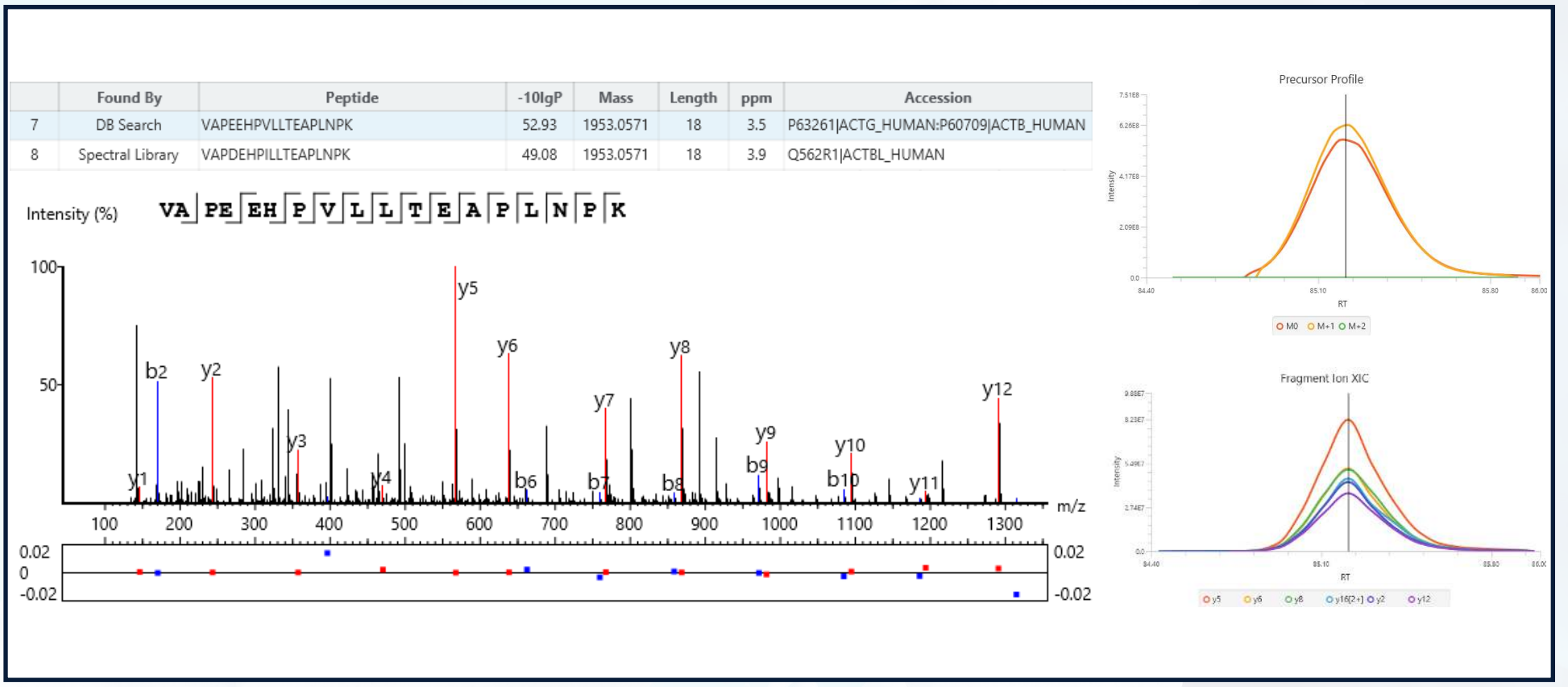
Users still get the intuitive data visualisation that PEAKS is known for but with a new look and optimised workflows to streamline your data analysis.



Deep Learning enabled Software

PEAKS® Studio 11 harnesses deep learning technology to improve identification accuracy and sensitivity by using algorithms to predict retention time, fragment ion intensity, and mass-to-charge ratios.

Deep learning is utilized in DDA workflows in PEAKS DeepNovo peptidome for immunopeptidomics and PEAKS+ to boost PEAKS DB search results. While in DIA workflows PEAKS database search for DIA searches the dataset with an in silico generated spectral library directly from the protein sequence database. In silico peptide details including the fragment ion pattern, indexed retention time, and ion mobility are predicted using deep learning.

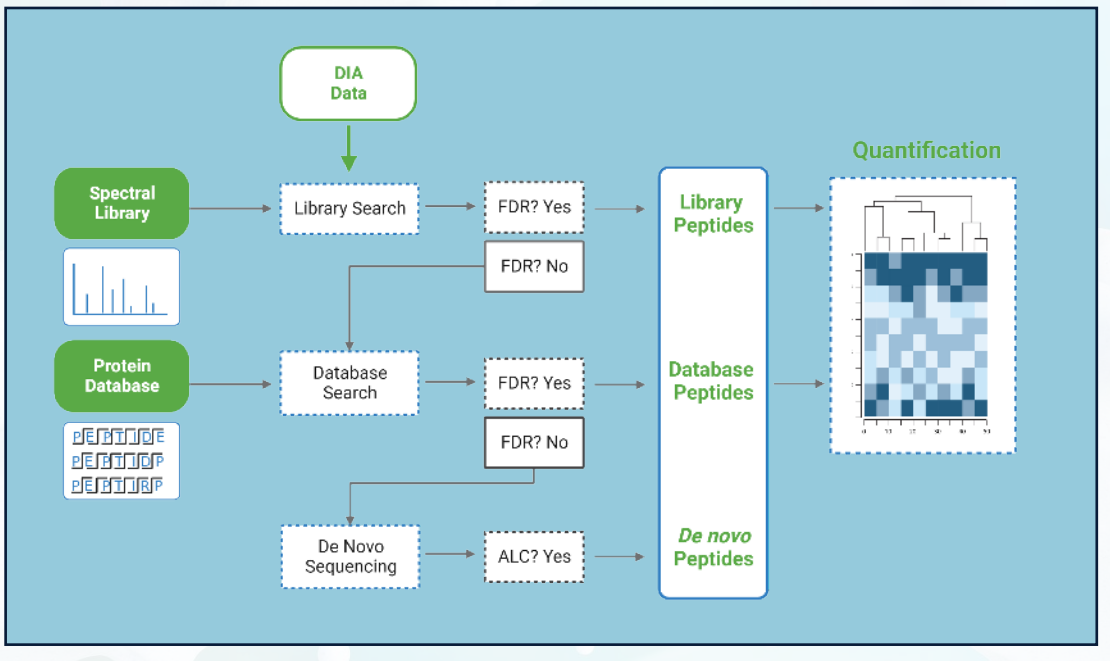


Advantages of a DIA Workflow

- Decrease bias by including all peptides in analysis
- Reproducibility of peptide detection and quantification across MS runs (3)
- Quantify proteins in complex mixtures over a dynamic range
- Eliminate under sampling
- Increased sensitivity and depth of proteome coverage
- Increased precision and reproducibility when compared to DDA
- Eliminate the cost and time with label free quantification

PEAKS® new DIA Workflow

PEAKS® offers a robust solution for DIA data analysis. It incorporates three methods of peptide identification: spectral library search, direct database search, and *de novo* sequencing. The search is performed using an expanding search space. First, a library search is performed against a library of previously identified spectra. By predicting the false discovery rate, peptides that pass the filter are saved. MS/MS spectra that don't match a peptide within the false discovery rate threshold are brought forward to a direct database search. Confident database matches are added to the result. Then, using the same FDR approach, unmatched spectra from the database search are analysed using *de novo* sequencing.



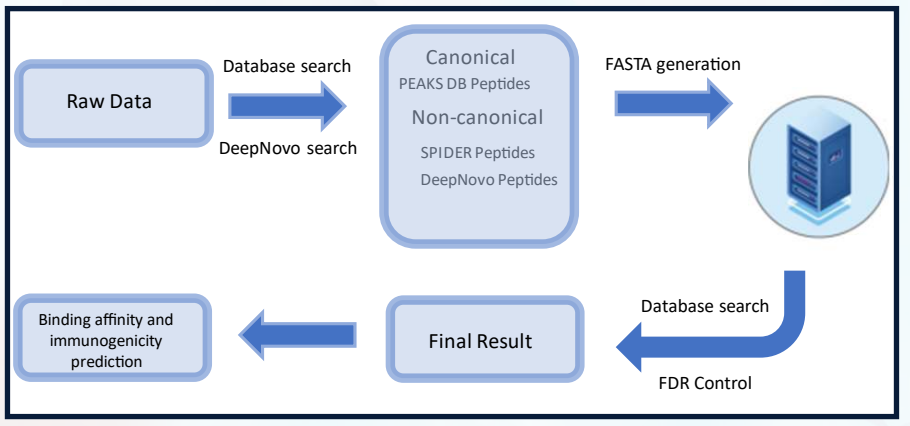
DID YOU KNOW:
Both new DIA workflow and PEAKS® DeepNovo Peptidome enable GPU accerlation

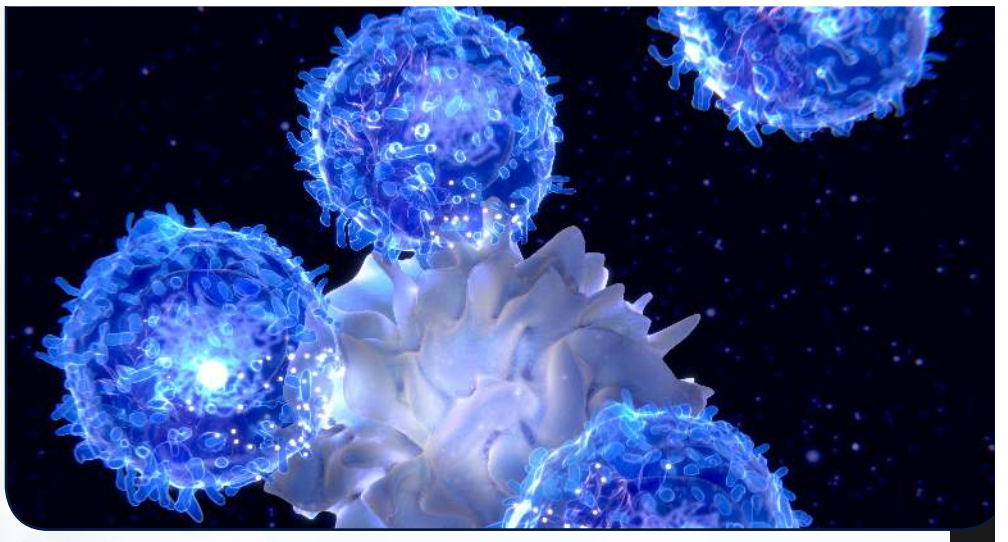
PEAKS® DeepNovo Peptidome

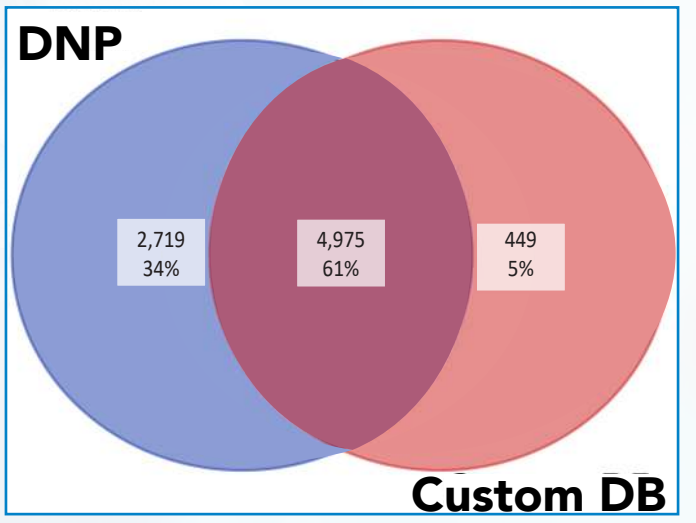
WORKFLOW

This newly developed solution is a specialized workflow for peptidomics data that combines database searching, *de novo* sequencing, and identification of mutated peptides. By training DeepNovo deep learning model using peptidomics datasets the sensitivity and accuracy of peptide identification can significantly be improved. Furthermore, *de novo* peptides (non canonical) are combined with database peptide (canonical) for more accurate estimation of false discovery rate.

The final output of peptides are categorized as Database, DeepNovo or Homologs (mutated peptides) and can be directly exported for binding affinity and immunogenicity predictions.

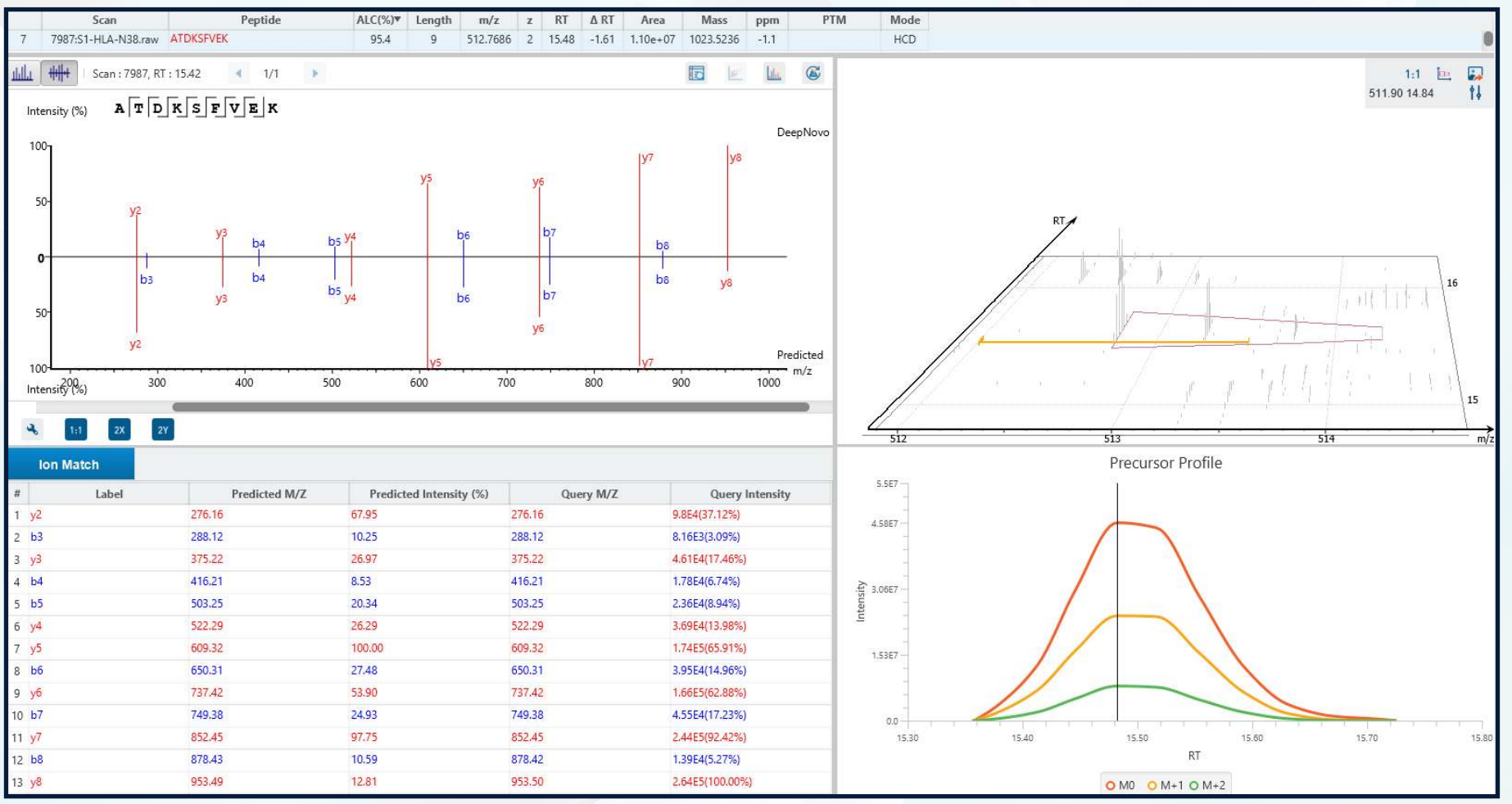






Advanced solution for Immopeptidomics

The peptides can be derived from non coding regions of the genome, aberrantly expressed transcripts, splicing of proteasomal products, or may contain mutations. DeepNovo Peptidome workflow uncovers the majority of peptides (>90%) without the requirement for custom database generation from RNA seq data.



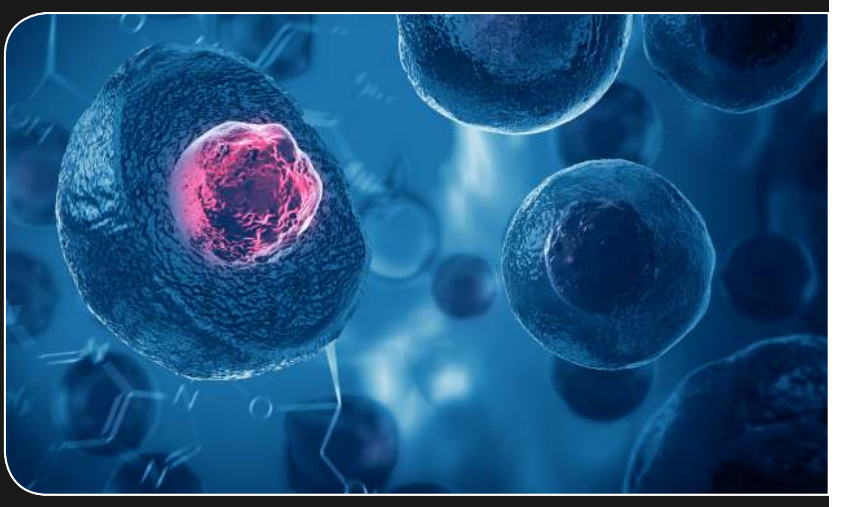
Scan	Peptide	ALC(%)	Length	m/z	z	RT	Δ RT	Area	Mass	ppm	PTM	Mode
7	7987:S1-HLA-N38.raw	ATDKSFVEK	95.4	9	512.7686	2	15.48	-1.61	1.10e+07	1023.5236	-1.1	HCD

Ion Match

#	Label	Predicted M/Z	Predicted Intensity (%)	Query M/Z	Query Intensity
1	y2	276.16	67.95	276.16	9.8E4(37.12%)
2	b3	288.12	10.25	288.12	8.16E3(3.09%)
3	y3	375.22	26.97	375.22	4.61E4(17.46%)
4	b4	416.21	8.53	416.21	1.78E4(6.74%)
5	b5	503.25	20.34	503.25	2.36E4(8.94%)
6	y4	522.29	26.29	522.29	3.69E4(13.98%)
7	y5	609.32	100.00	609.32	1.74E5(65.91%)
8	b6	650.31	27.48	650.31	3.95E4(14.96%)
9	y6	737.42	53.90	737.42	1.66E5(62.88%)
10	b7	749.38	24.93	749.38	4.55E4(17.23%)
11	y7	852.45	97.75	852.45	2.44E5(92.42%)
12	b8	878.43	10.59	878.42	1.39E4(5.27%)
13	y8	953.49	12.81	953.50	2.64E5(100.00%)

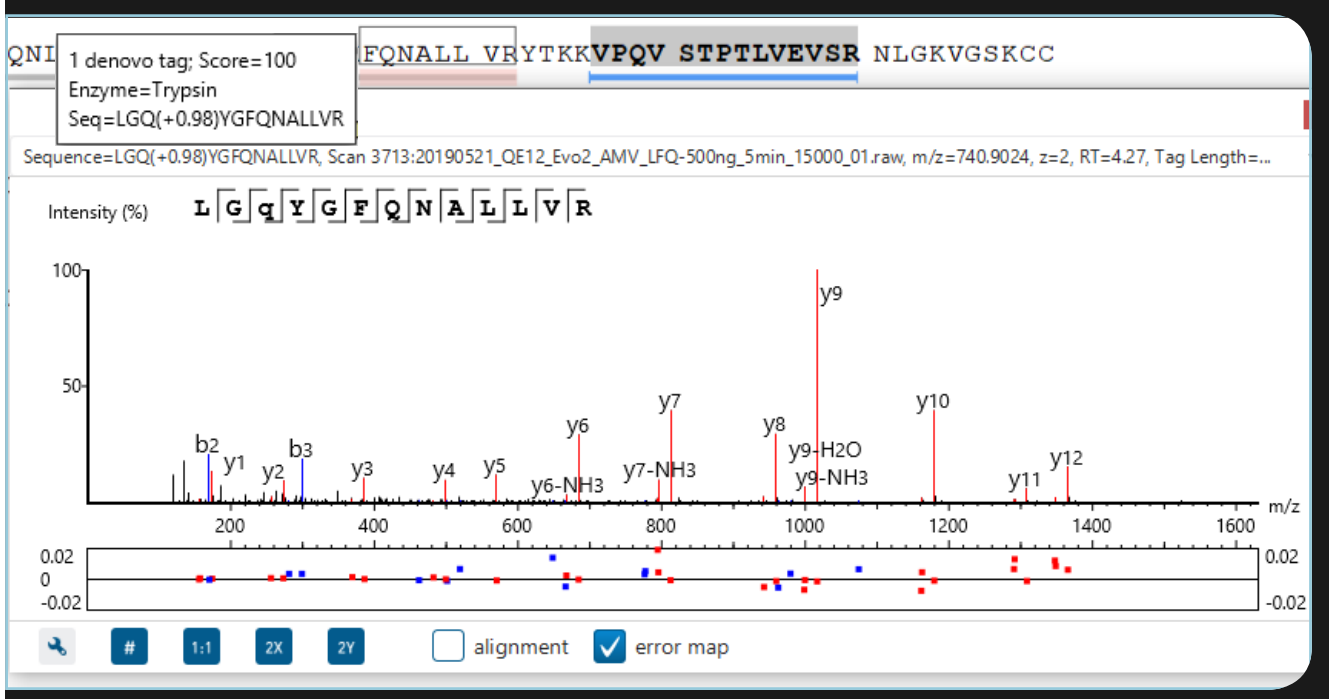
PEAKS® de novo sequencing is world renowned and the base of all PEAKS analyses.

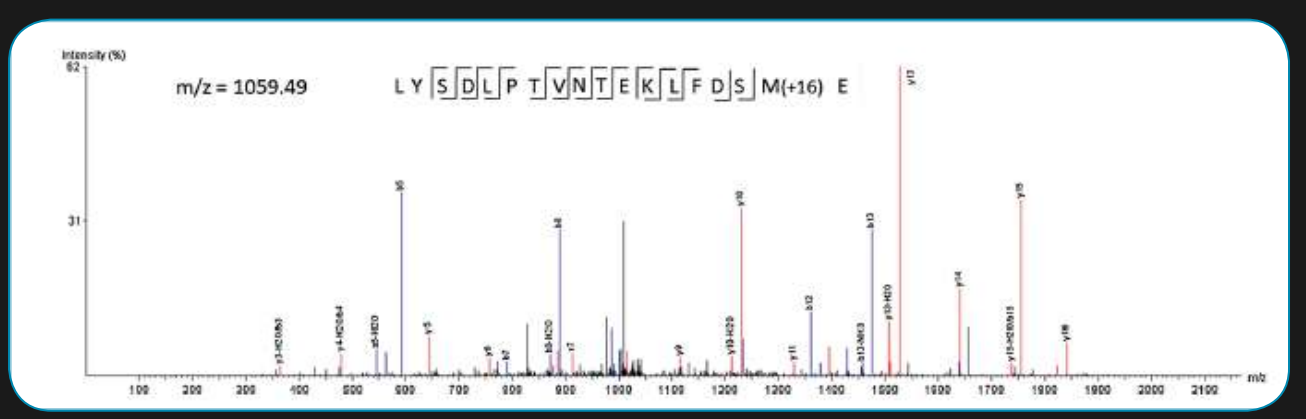
In mass spectrometry, *de novo* sequencing derives an amino acid sequence from a mass spectrum without the need of a sequence database. In contrast to the popular 'database search' peptide identification approach, *de novo* sequencing is the only choice when the sequence database is not available. This 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

Be the leader in your field of research; overcome the limits of a traditional database search, while minimizing *de novo* sequencing errors.





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' unique de novo-assisted search algorithm, novel peptides in camel's milk can be identified.

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:
Generate Spectral Libraries from DDA including PEAKS DB and de novo results and then perform DIA data analysis with spectral library

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

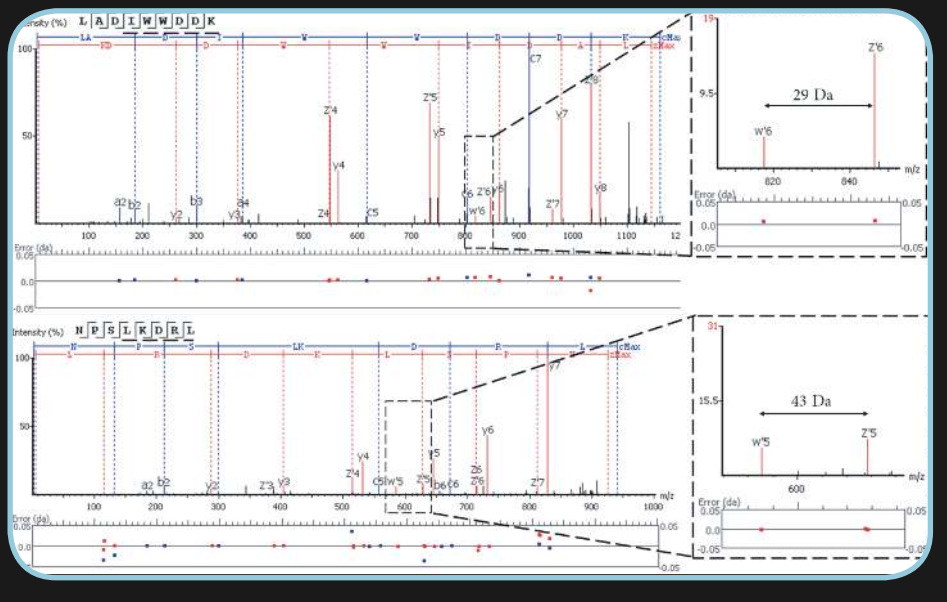
Before support within the software, PEAKS® undergoes an extensive fragmentation-specific algorithm training to confidently analyze various fragmentation data. Supported Fragmentation: CID/CAD, HCD, ETD/ECD, EThcD, and mixed/complementary fragmentation.

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.

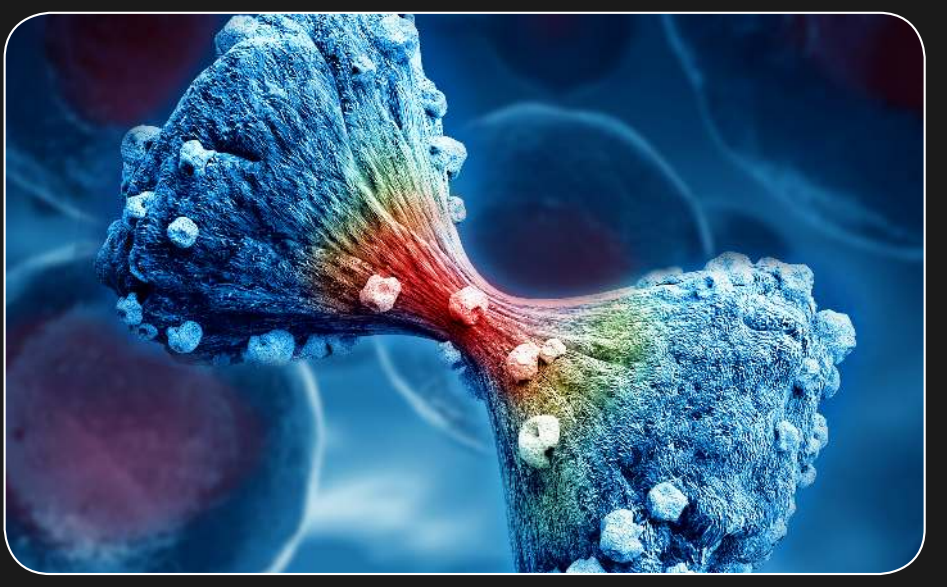
The detailed PEAKS® Studio interface allows users to quickly define, filter and visualize results as desired. With a few clicks you can specify a false discovery rate, or draw project-wide comparisons between your samples.

UNIFIED SCORING FOR EASY INTERPRETATION

PEAKS DB, PEAKS PTM, and SPIDER results are all scored using -10lgP. So, results from the three algorithms can be displayed together on the same scale.



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 aa.



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

De novo tag homology search tolerates amino acid mismatches between de novo sequence and database sequences. 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

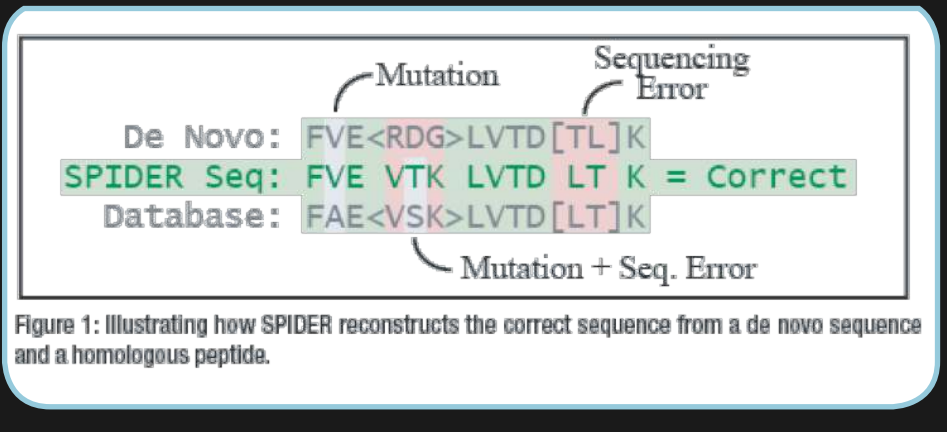
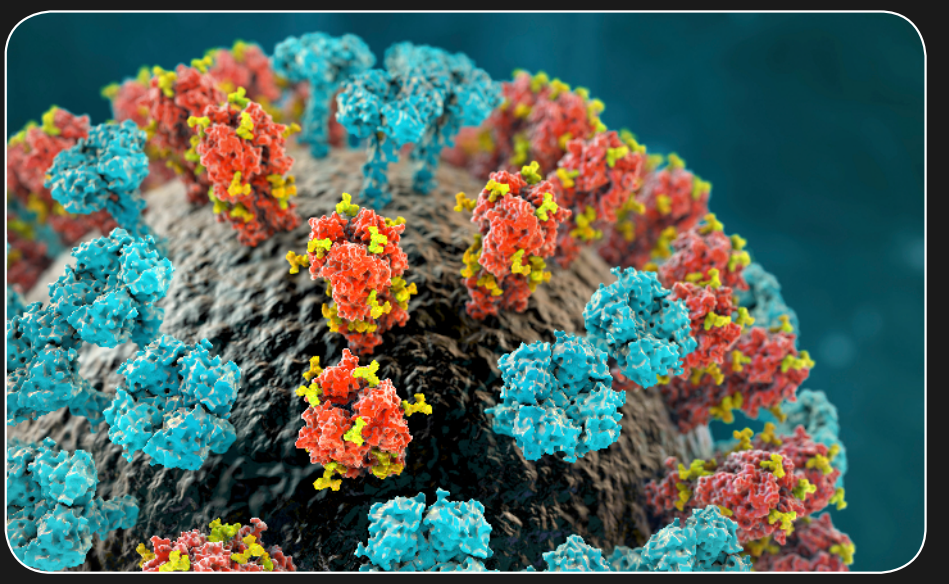


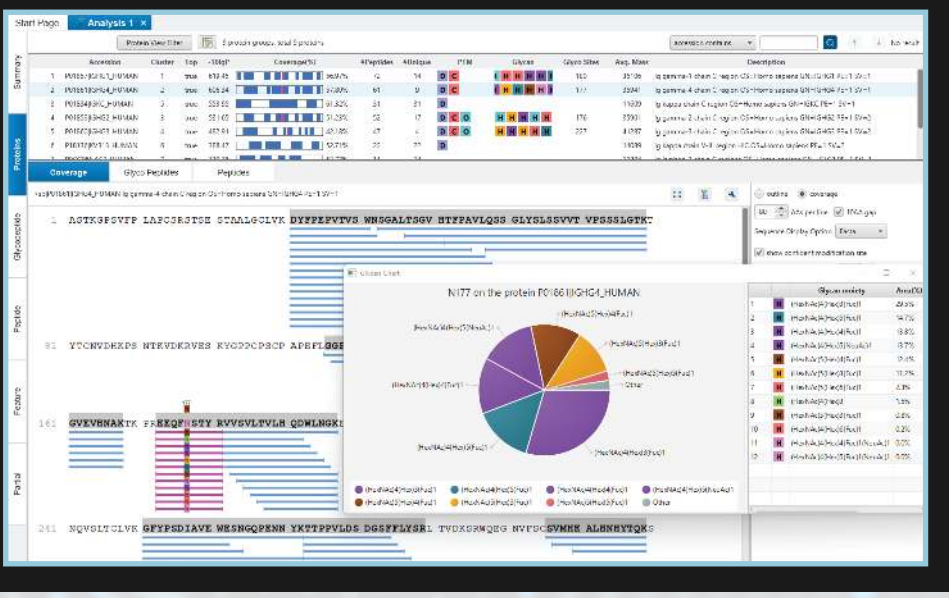
Figure 1: Illustrating how SPIDER reconstructs the correct sequence from a de novo sequence and a homologous peptide.

Add the PEAKS® Glycan Module to your PEAKS® Studio license to enable in-depth glycoproteomic analysis



Protein glycosylation is one of the most common post-translational modifications and plays a crucial role in important biological processes but is drastically understudied and deserves a specialized tool for both, N- and O-linked glycan analysis!

PEAKS® Glycan module is a comprehensive data analysis tool that provides a highly sensitive and accurate glycoproteomics software solution to advance our understanding of the glycoproteome. As an optional add-on module, PEAKS® Glycan Module add-on enables scientists to determine glycan site localization and glycan structures.

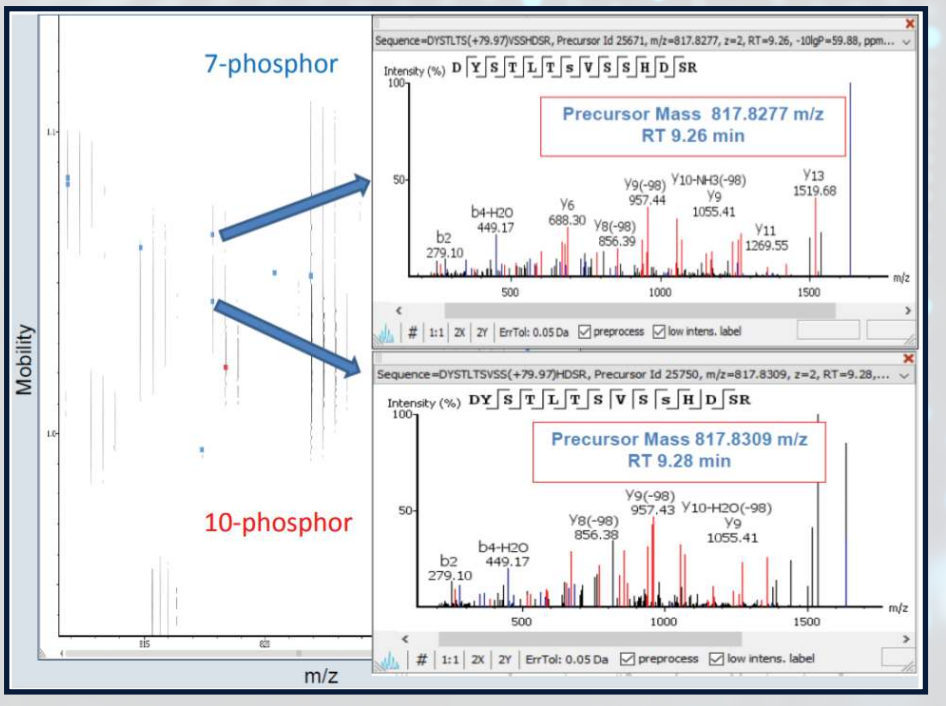


Ion Mobility Spectrometry - Mass Spectrometry (IMS-MS)

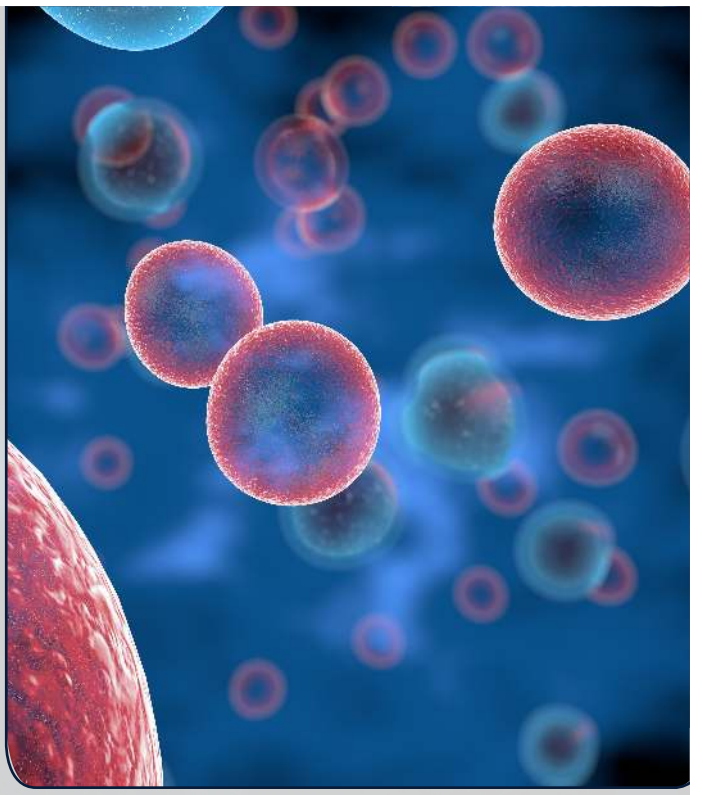
provides a compelling analytical workflow for complex biological and chemical mixtures by adding an additional dimension of ion separation; a 4th-dimension. With IMS-MS, ions are separated based on their mobility through a buffer gas, which provides the capability to differentiate ions based on their 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 dimension enables 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)



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.



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:

The ability to quantify the levels of proteins present in the samples by label-free quantification (LFQ) offers an efficient, cost-effective workflow to further understand the biological significance. 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 with confident and accurate results.

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.

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. 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.

Next generation PEAKS® Studio

Deep learning technology

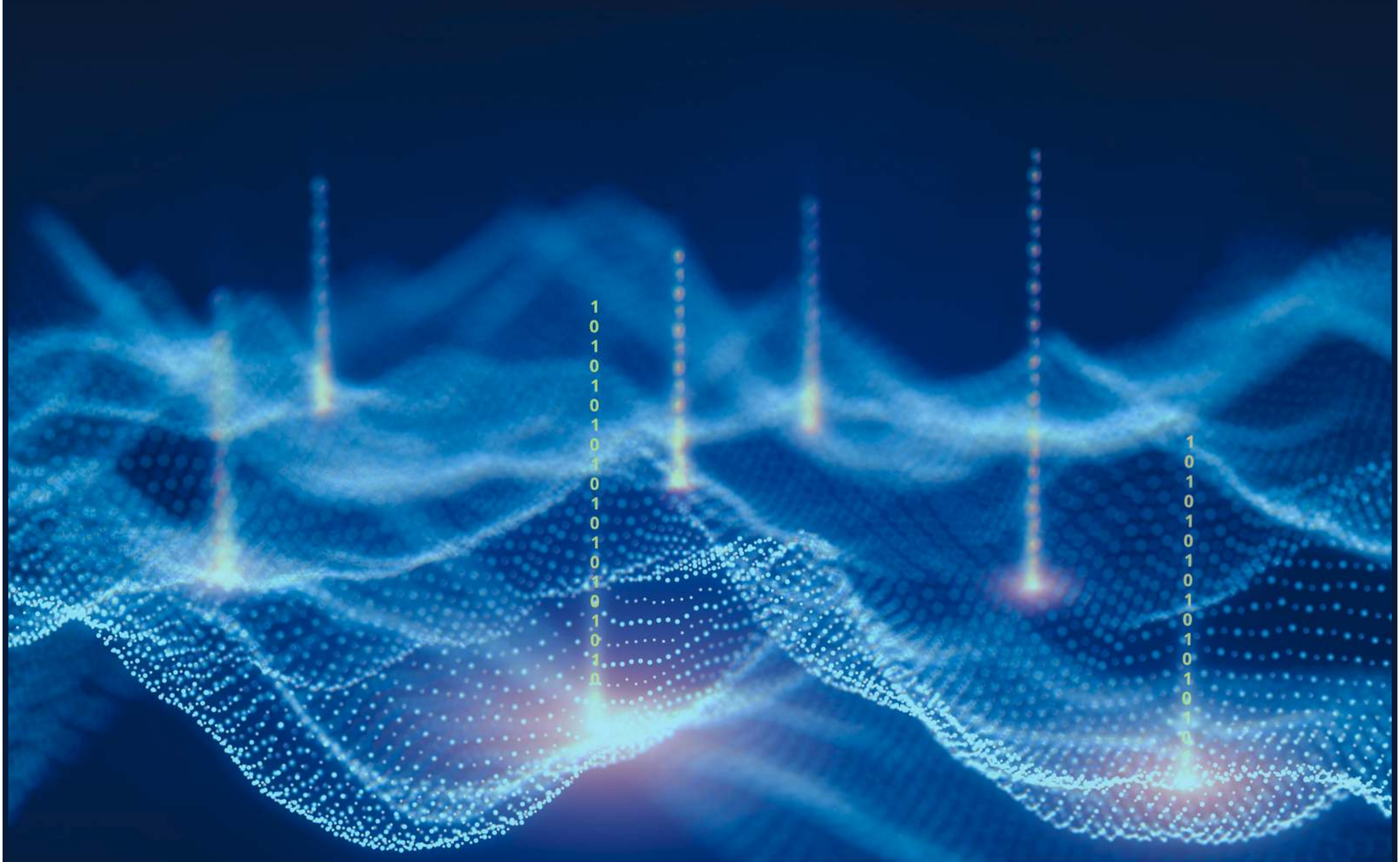
The brand new DIA workflow and DeepNovo Peptidome Analysis use deep learning technology to improve identification accuracy and sensitivity by using algorithms to predict retention time, fragment ion intensity, mass-to-charge ratios and ion mobility.

Updated intuitive GUI

With the updated Graphical User Interface (GUI), users still get the intuitive data visualization that PEAKS is known for but with a new look and optimized workflows to streamline your data analysis.

Increased speed & stability

The updated software architecture provides increased speed and stability for identification and quantification. Support all your discovery proteomics research with higher throughput and robust data analysis for both, DDA and DIA support.



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