Protein Metrics Software: A vendor-neutral solution.

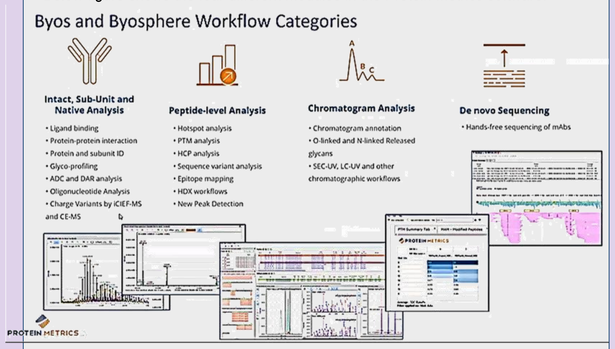
ACRNS Analytical Technologies is an authorized distributor of Protein Metrics software in India.

About Protein Metrics Software

Protein Metrics (PMI) software

PMI software offers the most accurate analysis of your data, quantifying species down to trace amounts. PMI software is powered by unique algorithms that help researchers minimize ambiguity, achieve reproducibility, and eliminate lab-to-lab variability.

Below figure shows the four different modules in the Protein Metrics software:



The user may select any default workflow from the System Workflows section or customized workflow from the My Workflows section. Default parameters for each workflow, including a report template, have been set in the Sequence and masses and Processing nodes tabs.
To create a project, the user will click on a workflow icon, drag and drop their raw data file(s) and a FASTA file, and click Create Project. A completed analysis and optimized report will then be available. This data flow is represented in Figure below.

Simplified diagram of the Byos data flow

Intact Module

Intact Analysis (Intact Mass™) software by Protein Metrics analyzes mass spectra of intact (undigested) proteins. All Byos Intact mass workflow relies on parsimonies charge deconvolution algorithm. This algorithm makes no assumption & requires separate evidences for each mass peak thereby, reducing harmonics & artifacts which result from assigning a number of charges to the *m/z* peak.

Features include:
1. Support for all major mass spectrometry instruments and vendors
2. Automatic or manual chromatographic time windows
3. Deconvolution of charge states to transform m/z spectra to neutral mass spectra
4. Automatic mass peak picking and intensity calculations
5. Side-by-side comparison of multiple samples
6. Reporting of summary data and figures

Intact module allows you to do Intact, Reduced, Subunit, Antibody-drug conjugate, Intabio iCIEF-MS analysis. These workflows all have the Samples, Proteins and masses, Sample-protein input and Processing nodes tabs. The default parameters to review are included within the Sequences and masses and Processing nodes tabs .The user can create Reference project, Comparison projects, Multi projects of the analysis.

Each workflow has a default report associated to it. The user can customize as per the requirement and save it for future datasets. All calculations can be done within the software (DAR, intensity, etc.) and data can be represented in various ways such as heatmaps, bar chart, line plots, pie-diagram, plots, etc. The reports can then be exported in pdfs, excel, csv, image formats.

Peptide Module

Peptide Analysis software considers LC-MS and LC-MS/MS results and enables label-free quantification, easy inspection of results, and reporting on summary and detail information for a variety of workflows that rely on peptide-level analysis. The raw data (MS1 recorded in profile or centroid mode) can originate from any major mass spectrometry vendor.

Peptide Analysis is a general tool that can be applied to a wide range of degradants, variants, and post-translation modifications. Thus, it can be used in workflows such as PTM analysis, peptide mapping, hotspot analysis, stress studies, MAM profiling, HCP analysis, glyco analysis, disulfide bond profiling, and more.

It offers a powerful platform for comparing and reporting across samples. More than one type of digestion enzyme may be examined at once, and each may include multiple LC-MS data acquisitions and multiple MS2 searches from any data acquisition. The fragmentation method may be any combination of low-energy CID, HCD/Q-TOF, and/or ETD. The quantification of a modification or variant relative to wildtype is performed by label-free quantification with extracted ion chromatograms (XICs), which have editable limits of integration.

Steps in Peptide module

Preview™ Protein Metrics Inc.
Preview is a fast searching program to guide parameter setting for subsequent full searches.
- Quickly samples MS data to measure:
  - mass errors, digestion specificity, & modifications in preparation for more thorough searches.
- Generates optimized parameters files for use in Byonic™ searches.
- Runs fast initial searches to recalibrate precursor & fragment masses based on confident identifications.

Byonic™ Protein Metrics Inc.
A software package for identifying peptides & proteins by tandem MS.
- Modification Fine Control
- Wildcard Search
- Glycopeptide search
- Disulfide bonds, trisulfide bonds, and general crosslinking

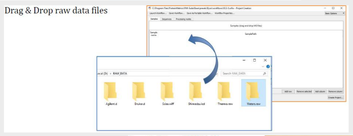
Byologic® Protein Metrics Inc.
Used for inspection, validation & reporting of the peptides.
- Simultaneous inspection of MS-2, MS-1 isotopic plots & XIC for individual modified peptide and its corresponding wildtype peptide.
- 30 different parameters can be optionally viewed for each individual peptide.
- Multiple features for interpretation, customization & automation (eg: filters)
- Report Generation: Customized reports can be generated within minutes

Steps to create Project in PMI - Byos

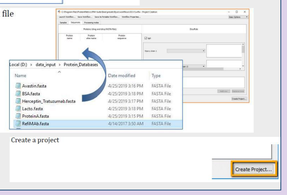
- Click on the workflow as per your analysis
- Drag & drop your raw data files
- Drag & drop your Fasta file
- In the processing tab, you can import Byonic parameters suggested by Preview and/or edit the parameters as per your instrument & analysis.
- Check Byologic parameters
- Click create project and give name to your file & save. The project creation starts.

Click S-S to launch the workflow

Drag & Drop raw data files

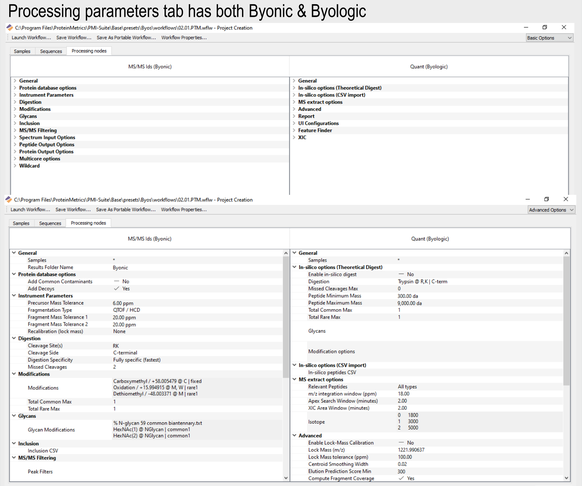


Drag & Drop a FASTA file



Processing parameters

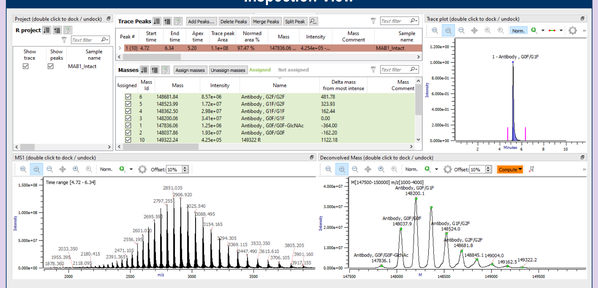
Processing parameters tab has both Byonic & Byologic



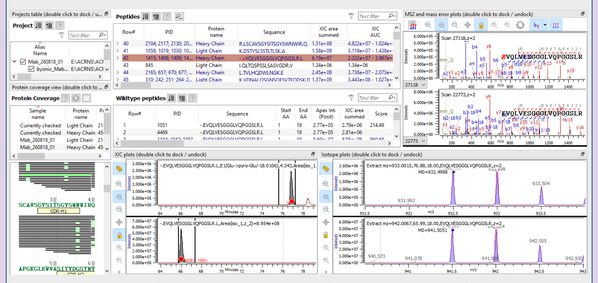
Byonic™ is a software package for identifying peptides and proteins by tandem mass spectrometry. Byonic plays the same role as Mascot, SEQUEST, and X!Tandem, but offers greater accuracy, sensitivity, and flexibility. Byonic provides several major features not found in the other search engines:
Modification Fine Control, Wildcard Search, Glycopeptide search, Top-down, middle-down, and bottom-up proteomics, Disulfide bonds, trisulfide bonds, and general crosslinking (For more details explanation check Byonic manual).

Byologic is for MS1 quantitation & insilico identifications. One of the parameter is **Feature Finder -** an algorithm that allows the user to scan the MS1 domain for all existent peptides in a sample. In a typical DDA assay, peptides are identified only if an MS2 scan is triggered from a precursor ion in an MS1 scan. Therefore, if a peptide ion does not trigger an MS2 scan, or its score excludes it from identification, it will not be detected. Feature Finder ensures that a peak will not go undetected by identifying all possible isotopic distributions in a sample that could potentially be a peptide.

Inspection View



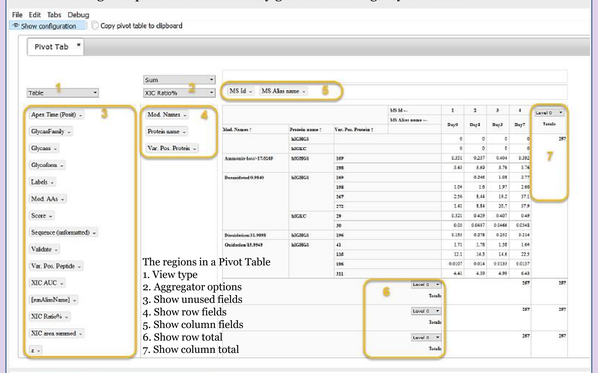
Intact module inspection view includes – Project view, Trace peak view, trace plot view, Masses table view, MS1 plot view (raw data), Deconvoluted mass plot view (neutral mass).

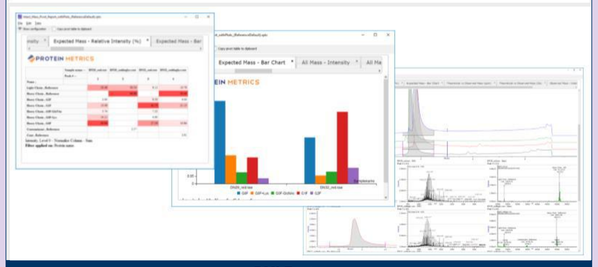


Peptide module inspection view includes – Project view, Protein Coverage view, Peptides table view, MS2 plot view, XIC plot view, Isotopic plot view.

Reporting View

Protein Metrics applications have common Reporting features:
- Review all plots on the same page through the Plot Tab.
- Export the report into PDF, Microsoft Excel workbook, or CSV format.
- Perform further data analysis through the Pivot Table.
- Organize project information into a Summary.
- Reuse the report design saved as a template on future data sets.
- Time-saving – Reports are automatically generated through Byos workflows.





PMI Features

- Multiple Workflow templates available as per your analysis: (PTM, HCP, S-S, MAM, SST etc.)
- 360° Comprehensive view: Inspection view has Project view, Coverage view, MS2 view, XIC plot view, Isotopic plot view.
- Peptide manager to identify unknowns based on previous experimental/in-silico data.
- Feature finder identifies low scoring unknown peptides by matching isotopic distribution, RT, S/N, peak width, intensity etc.
- Multiple features for interpretation, customization & automation: Many ways in which filters can be applied to interpret data of interest in short duration.
- Error free regulatory submissions: Report templates for complex calculations for comparative quantitation, statistical & graphical representations of data.

Protein Metrics Software	Other Vendors Software	Impact
Preview Module: Essential to select processing parameters (Precursor & Fragment mass accuracy), to ascertain health of sample preparation (digestion specificity) & chromatography /MS performance, common modifications, etc.	NOT AVAILABLE	Users can use parameters suggested by Preview (search parameter Byonic file) to confidently and comprehensively characterize modifications and sequence variants.
Identification of peptides (low abundance) based on MS1 data: Peptide manager & Feature finder	NOT AVAILABLE	Users benefit from this feature in automating identification of low & high abundant peptides
Reporting formats: Customizable and workflow specific formats for error free quantitation of impurities & modifications.	NOT AVAILABLE	Users can avoid complex calculations manually performed in excel, thus, ensures hustle-free & error-free reporting. Customizable reports for multiple in-process stages enable better interpretation of data
Characterization specific workflows: Captures workflow specific inputs with capability to cater specific needs such as number of shuffles v/s expected disulfides, fold change in case of MAM, duplicate peptides in case of HCP, sequence variants modification library in case of SV workflow, etc.	NOT AVAILABLE	Even fresher's with little experience on MS, can learn interpretation of data using optimized workflows & user friendly interface.

Contact ACRNS for more information

If you have PMI software, go to Help > Open User Guides to gain access to all the manuals.

You can have access to resources by clicking Help > Online resources. This will take you to the online resources on Protein Metrics website & you have access to all application notes & video tutorials.

For more information mail to info@acrnstech.com

To book your training session call +91 8097104991