



# Peptide Analysis User Manual

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## Introduction

Byos hosts a number of workflows incorporating peptide analysis that can be used for peptide-centric analysis of protein samples. From late discovery through clinical development, Peptide workflows in Byos are used for Protein Characterization and Multi-Attribute Methods (MAM).

Using the Peptide workflows available in Byos, researchers can confidently characterize their product down to trace components and reduce turn-around time by weeks. Dynamic, Editable reports for non-mass spectrometry stakeholders for internal review or for application filings are automatically linked to projects..

The 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 (Thermo Fisher Scientific™, Waters™, SCIEX™, Bruker™, Shimadzu™, or Agilent Technologies™).

Byos supports the analysis of a wide range of degradants, variants, and post-translational modifications (PTM). Workflows for PTM analysis, peptide mapping, hotspot analysis, stress studies, MAM profiling, host cell protein analysis, glyco analysis, disulfide bond profiling, comparability, oxidative footprinting, and more are available, providing 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.

Using Byos for peptide analysis enables the analyst to determine if a putative identification is true or false (validation) by making available all relevant information interactively in a single screen. This empowers the user to make this decision and add their own comments, rather than a black-box software algorithm. The application also makes room for one or more reviewers to enter their responses and comments. Comprehensive reports of results (tables and figures) may be exported for report generation to share with colleagues.

Analyses may also be deployed into a fully automated workflow to process large numbers of samples with consistent analysis and reporting.

## Windows Support

Byos Desktop is currently supported on Windows 10 and Windows 11. **Note that for Windows 10, you must have version 1809 or later.**

## System Specifications

- **Recommended PC:**
  - Windows 10/11 64-bit
  - 32 GB RAM
  - 1TB disk space (Solid State SSD)
  - Recent version of Intel Core i7 or i9 / AMD Ryzen 7 or 9 (with AVX support)
  - Oracle JRE or OpenJDK
  - C++ compiler version 16 or higher
- **Recommend PC for *high performance computing* (e.g. 32+ cores)**
  - Windows Server 2022 or Windows 10/11
  - 64 GB RAM
  - 2 TB disk space (Solid State SSD)

- Xeon CPU(s) (at least 16 physical cores) (with AVX support)
- Oracle JRE or OpenJDK
- C++ compiler version 16 or higher

Note that standalone applications are no longer supported outside of Byos and that Peptide Analysis is performed using Byos workflows.

Raw mass spec data are accepted from these vendors: Thermo Fisher, Waters, Sciex, Bruker, or Agilent. Optical data may be embedded in these files or imported via a \*.csv file of time-intensity data pairs

## Launching a Preset Workflow in Byos

To create a new analysis, go to the workflows tab and select a PTM workflow if you are performing a peptide map of a purified biotherapeutic protein with an MSMS search. Byos will open the **workflow**.

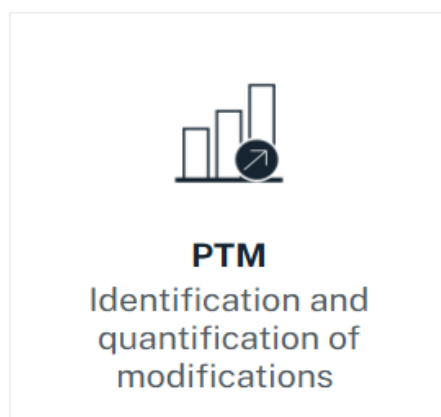


Figure 1: Default PTM workflow

This multi-tab window allows the analyst to select multiple mass spectrometer files to be analyzed, each with one or more set of MS/MS search results, as well as a corresponding set of protein sequences.

## Tour of Peptide Analysis Dashboard Views

The Peptide Analysis user interface (UI), or dashboard, shown in the figure below has seven highly interactive plot and table views:

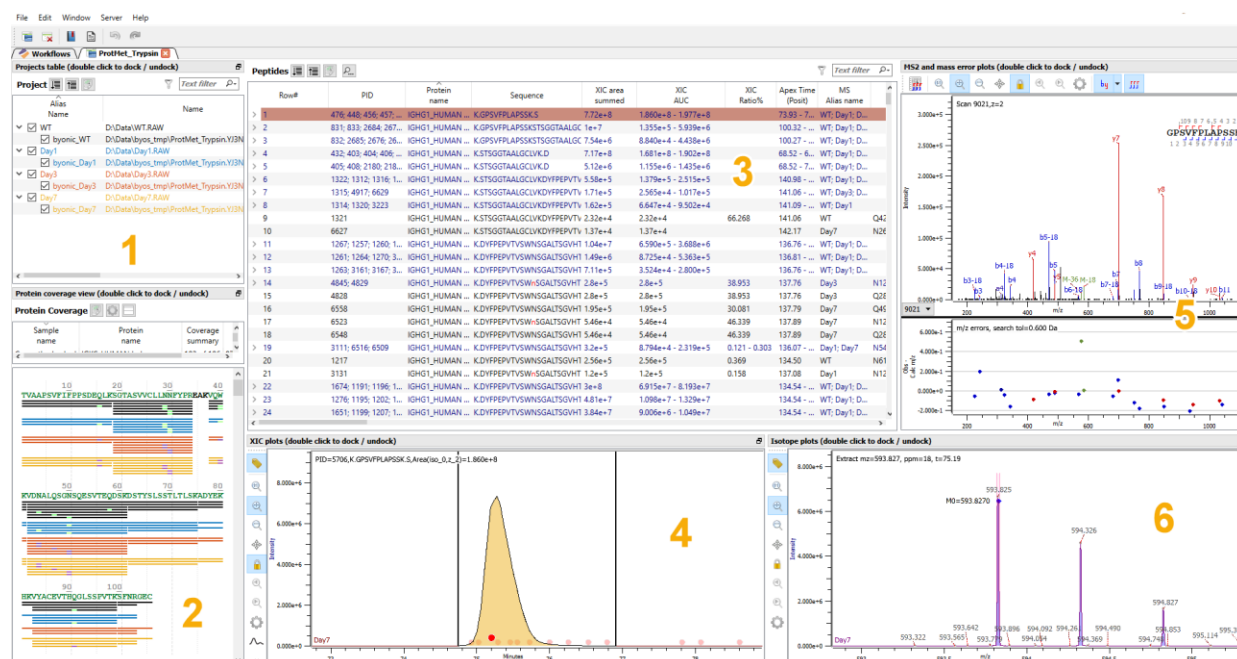


Figure 2: Peptide Analysis Dashboard

**Six Peptide Analysis views:** (1) **Project** table, (2) **Protein** coverage table, (3) **Peptides** table, (4) Ion chromatogram (XIC) plots, (5) MS2 and mass error plots, and (6) Isotope plots. Views can be rearranged, resized, docked, and undocked. Customized layouts can be saved and shared.

1. The **Project** view lists the data files in use. The paired check boxes for MS files and result files can be used to turn on or off the records displayed in the other views. The colors of the files correspond to colors used in the other views.
2. The **Protein Coverage** views display table data and identify sequences for peptides selected and filtered in the Peptides table in View 3. Bars below the sequences represent MS/MS scans. The bars and sequences share the colors of the corresponding MS files in the Project table in View 1.
3. The **Peptides** table lists all identified and filtered peptides. Selecting a peptide row in the table displays the corresponding wildtype peptides, XIC chromatograms, MS2 and mass error plots, and isotope plots in Views 4 through 7.
4. The **XIC plots** show the ion chromatograms for the peptide and corresponding wildtype peptides, if present, selected in the Peptides table in View 3. The plots are shaded with the colors of the source MS files in the Project table in View 1.
5. The **MS2 and mass error plots** show the MS2 spectrum of the peptide and corresponding wildtype peptides, if present, selected in the Peptides table in View 3. The peptide and wildtype peptide spectra share the same m/z scaling for easy comparison.
6. The **Isotopes plots** show the MS1 isotope spread of the peptide and corresponding wildtype peptides, if present, selected in the Peptides table in View 3.

The **Wildtype peptides** table lists wildtype peptides corresponding to the modified peptides selected in the **Peptides** table in View 3. Records are shaded with the colors of the source MS files in the **Project** table in View 1. This table is displayed when **Windows > Show Wildtype** is checked.


In the default mode, the **Chromatogram XIC Plot** displays the Total Ion Current (TIC) and Extracted Ion Chromatogram (XIC) of a specific peptide. With advanced settings as described in [Chromatogram XIC Plot and Menu](#) section, Summed Extracted Ion Chromatograms (XIC summed) of one or multiple peptides

eluting at a given time, and the Total Ion Current difference (TIC difference) can also be overlayed on the XIC plot. To open the Chromatogram XIC plot, choose **Window > Show Chromatogram XIC Plot**.

## Project Creation options

The following section details parameters in Project Creation for Peptide projects. These parameters are therefore relevant to the **HCP**, **HotSpot**, **PTM**, **PTM (in-silico)**, **SVA (C57) – Specific**, **SVA (C58) – Specific**, **S-S**, **System Suitability**, **Oxidative Footprinting**, and **MAM New Peak** Detection workflows.

## Samples

The **Samples** tab allows the user to load raw LC-MS sample files into the project. Byos accepts a variety of sample file types (Bruker: \*.d, Agilent: \*.d, Thermo: \*.raw, Waters: \*.raw, Sciex: \*.wiff, \*.wiff2, Shimadzu \*.lcd). Alternatively, a project can be opened from a \*.byrsIt file generated from a previous Byonic™ search. To load a file, drag and drop one or more MS files into the project window. Alternatively, click the **Add sample** button, double-click on the new row under the **MS file** header, click  and browse to the file. Click **Open**.

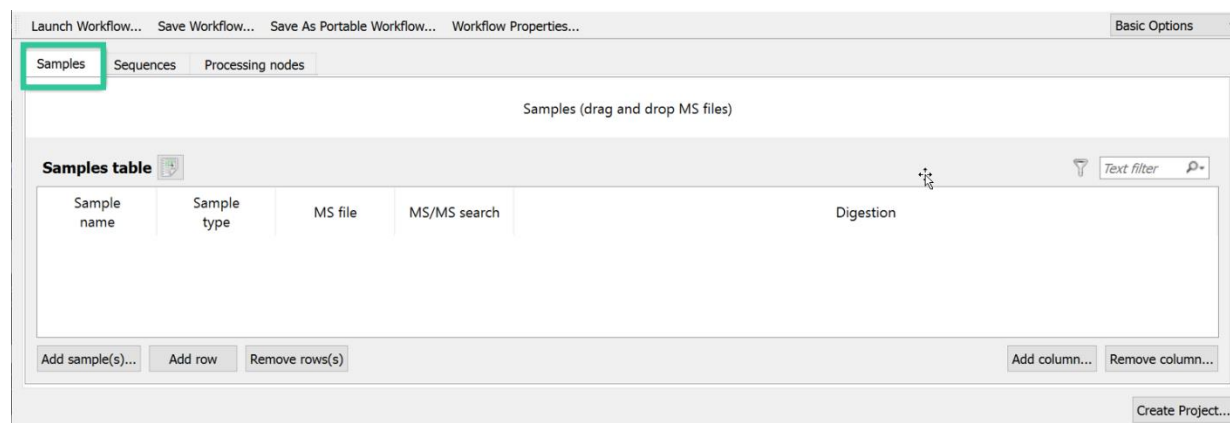


Figure 3: Sample input tab

**Sample name** defaults to the file name. To change the Sample name, double-click the name and edit the text. The name can also be updated later in the **Project** table.

To designate the enzyme used by a sample, double-click the sample row under **Digestion** and select the enzyme name. This enables Byos to determine the expected cleavage sites.

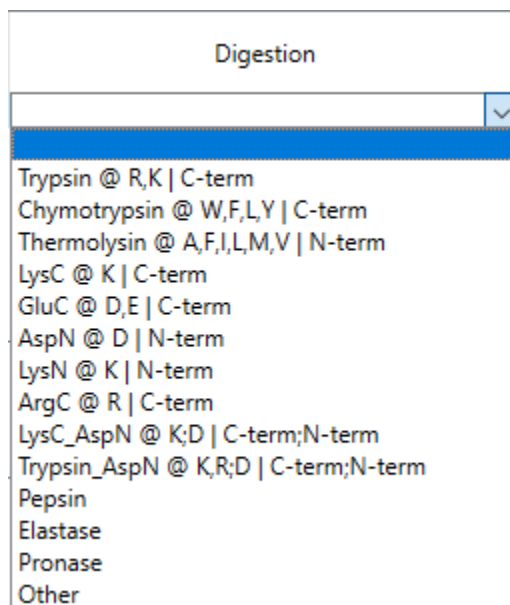




Figure 4: Digestion enzyme selection

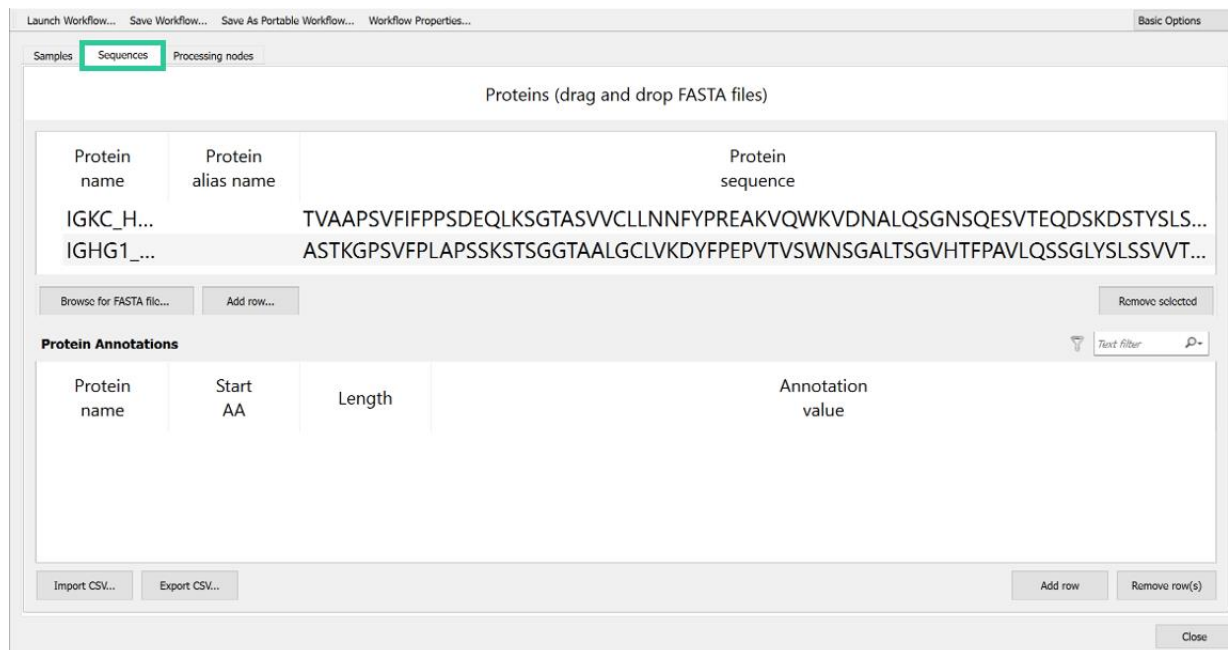
To apply the Digestion term to multiple Sample rows, select the group of Digestion cells, right-click **Edit selected rows**, select or enter the Digestion term, and click **OK**. Alternatively, copy the term from one cell, click each Digestion cell in turn, and past in the copied Digestion term.

The user has an option to provide a combination of C-Term and N-term proteases as well, e.g., AspN\_Trypsin @ D;R,K | N-term; C-term.

To add an incremental MS/MS search result to a sample, double-click in that sample row under the **MS/MS Search** header, click  and browse to the MS/MS search result ( \*.byrslt or \*.dat) file. Click **Open**. To attach additional MS/MS search result to that sample, click  to add a new MS/MS Search row and repeat the addition steps.

To remove an MS/MS search file, double-click the file name and click .

## Sequences



Launch Workflow... Save Workflow... Save As Portable Workflow... Workflow Properties... Basic Options

Samples **Sequences** Processing nodes

Proteins (drag and drop FASTA files)

Protein name	Protein alias name	Protein sequence
IGKC_H...		TVAAPSVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS...
IGHG1_...		ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVT...

Browse for FASTA file... Add row... Remove selected

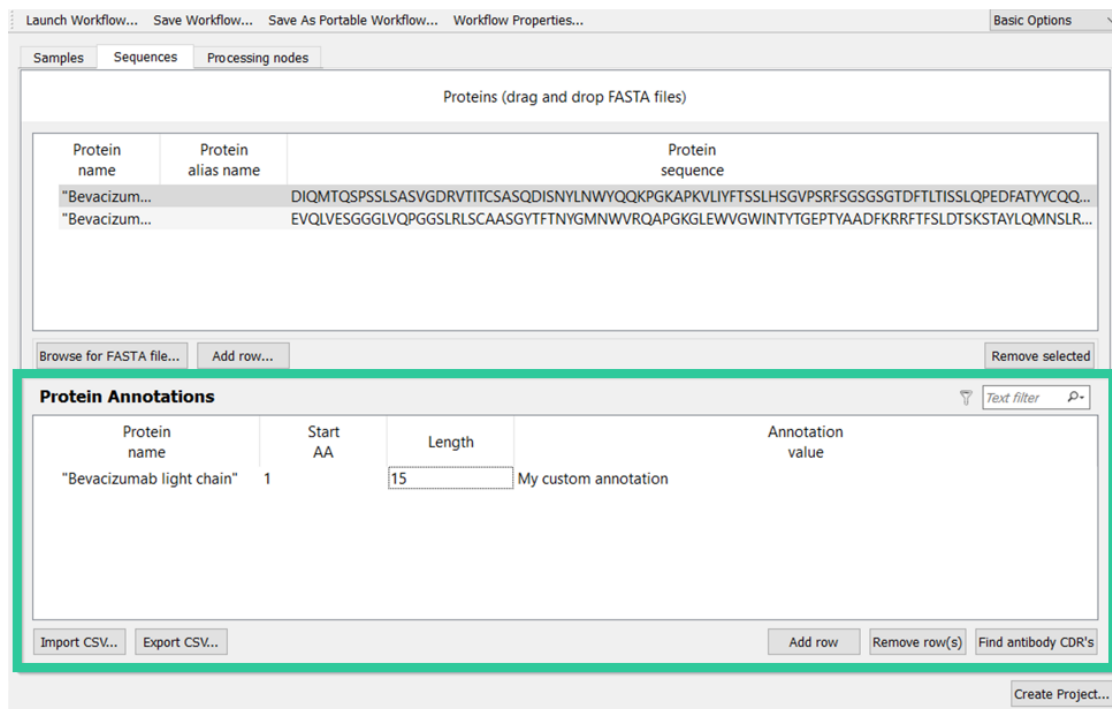
**Protein Annotations** Text filter

Protein name	Start AA	Length	Annotation value
--------------	----------	--------	------------------

Import CSV... Export CSV... Add row Remove row(s) Close

Figure 5: Sequences tab

It is also possible to define or import custom protein annotations during Project Creation within the Sequences tab.



Launch Workflow... Save Workflow... Save As Portable Workflow... Workflow Properties... Basic Options

Samples Sequences Processing nodes

Proteins (drag and drop FASTA files)

Protein name	Protein alias name	Protein sequence
"Bevacizumab..."		DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQ...
"Bevacizumab..."		EVQLVESGGGLVQPGGSLRLSCAASGYFTFTNYGMNWRVQAPGKLEWVGWINTYTGPTYAADFRRFTSLDTSKSTAYLQMNSLR...

Browse for FASTA file... Add row... Remove selected

**Protein Annotations** Text filter

Protein name	Start AA	Length	Annotation value
"Bevacizumab light chain"	1	15	My custom annotation

Import CSV... Export CSV... Add row Remove row(s) Find antibody CDR's Create Project...

Figure 6: Protein Annotations



There is also an option to import or export protein annotations after Project Creation in the **Protein Annotations** table. This feature has been implemented in a backward compatible fashion so that users can import annotations from CSV file created in previous versions.

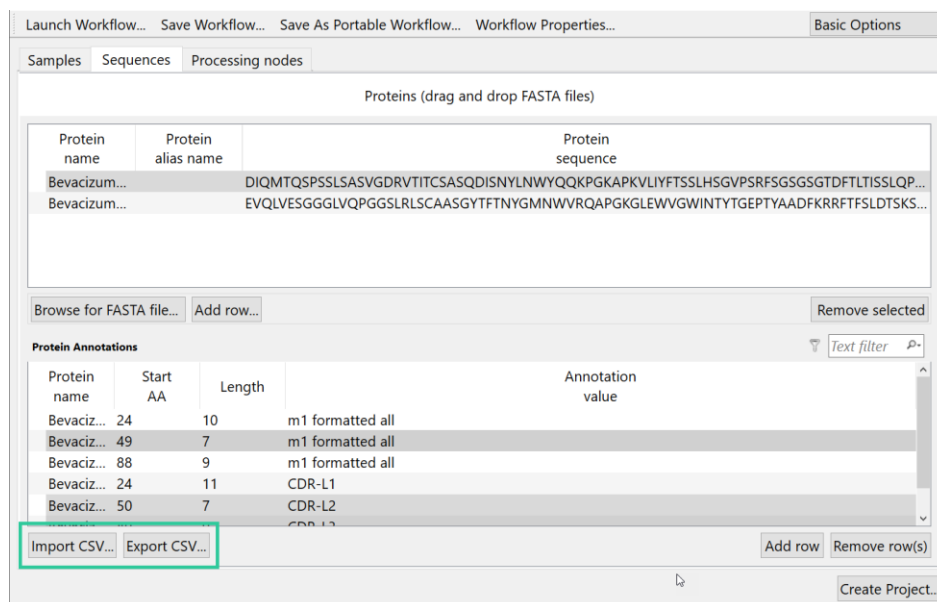


Figure 7: CSV options for Protein Annotations

## Processing nodes

The **Processing nodes** tab is where the user specifies the processing parameters to be applied during project creation and report generation. Each workflow is populated with default values, designed for the user to review once and save for future projects. This empowers every member of a team, scientist within a lab, or colleagues across a project to complete the exact same analysis and generate identical reports regardless of Byos user-skill level, scientific expertise or experience, or location. Please note the next section will only have to be completed once for each analysis type and the workflow used for perpetuity.

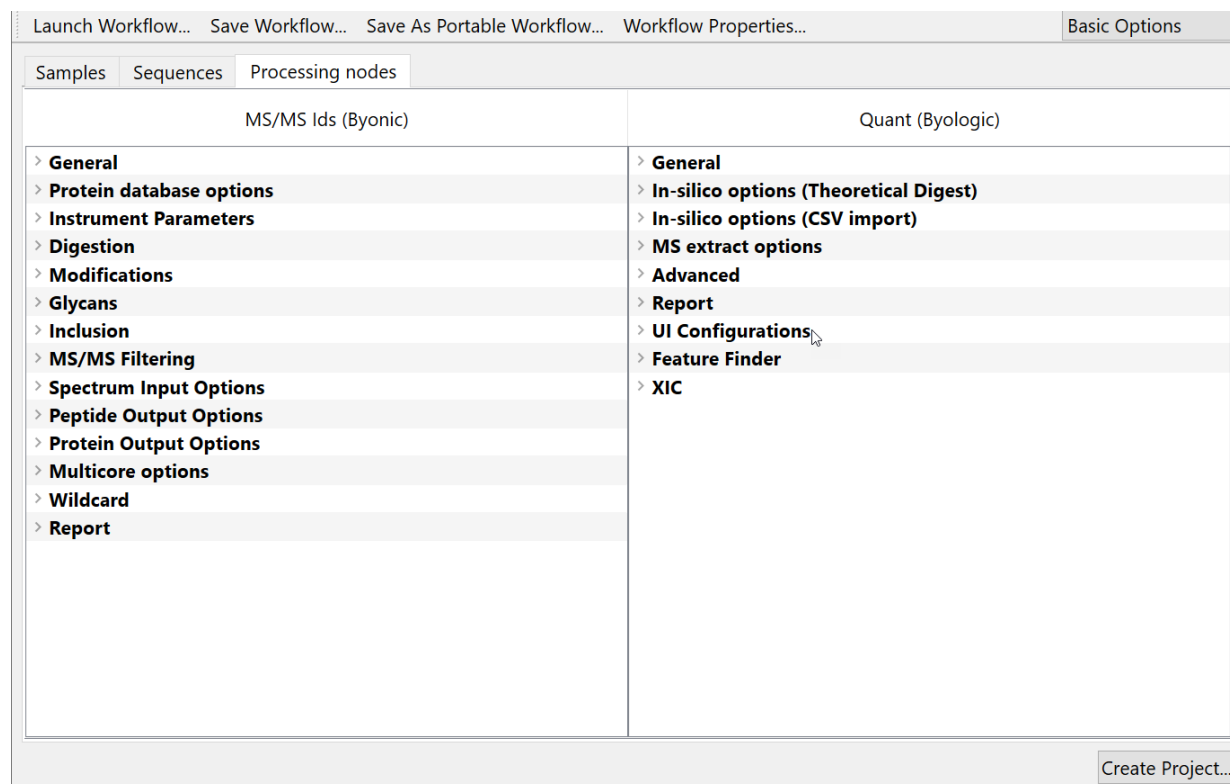


Figure 8: Processing nodes for the PTM workflow

NOTE: These parameters are not part of the PTM (in-silico) workflow. Please refer directly to the below Quant section.

- **General**

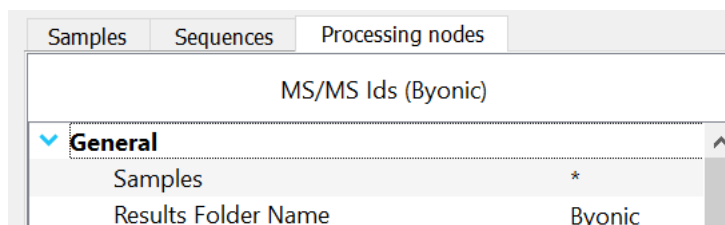


Figure 9: General.

- **Samples** - The "\*" will apply all parameters to all samples dragged and dropped into the Samples tab.
- **Results Folder Name** creates a folder of that name to save results, set to "Byonic" by default.

- **Protein Database options**

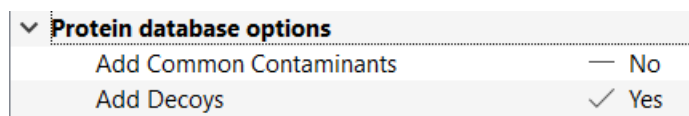


Figure 10: Protein database options.

The protein database options **Add Common Contaminants** (default setting = No) and **Add Decoys** (default setting = Yes) were added to the Byonic Processing node in v. 3.9. The protein database should contain both targets and decoys (recognized by protein names beginning >Reverse or >Decoy) for false discovery rate (FDR) estimation. Byonic will automatically add decoys if the **Add decoys** box is checked and contaminant proteins (e.g., trypsin, bovine serum albumin, and human keratins) if the **Add common contaminants** box is checked. Typical folders to store input files are: C:\data\_input\Mass\_Spectra and C:\data\_input\Protein\_Databases.

- **Instrument Parameters**

Instrument Parameters	
Precursor Mass Tolerance	6.00 ppm
Fragmentation Type	QTOF / HCD
Fragment Mass Tolerance 1	20.00 ppm
Fragment Mass Tolerance 2	20.00 ppm
Recalibration (lock mass)	None

Figure 11: Instrument Parameters. Optimize for the experiment completed.

In the figure above, the user set 6.0 ppm **Precursor Mass Tolerance**, 20.00 ppm **Fragment Mass Tolerance**, and QTOF/HCD as the **Fragmentation Type**. Both Dalton and ppm mass tolerances for precursors and fragments are supported, along with several fragmentation types. The Dalton tolerance applies to measured mass for precursors but measured m/z for fragments. The way scoring is completed changes at fragment tolerances of 0.1 Da or 100 ppm or less: high-resolution MS/MS is assumed, meaning resolution sufficient to distinguish charge states of fragment ions. For this reason, fragment tolerances larger than 0.1 Da should be used with low-resolution (ion trap) MS/MS analysis.

Internal models for most fragmentation types are included – CID low energy (ion trap), QTOF / HCD (beam-type CID), and ETD / ECD (electron transfer and electron capture dissociation), as well as a number of combinations of types. These internal models determine which fragment peak types will be scored and annotated. For example, prominent c- and z-ions and small y-ions are expected for ETD. Prominent oxonium ions are expected from glycopeptides with QTOF / HCD fragmentation, but small or missing oxonium ions from CID low energy.

- **Precursor Mass Tolerance**

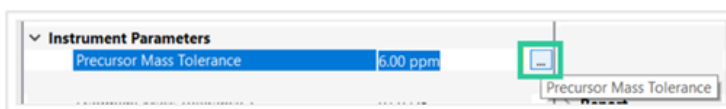


Figure 12: Precursor Mass Tolerance.

The user can change this value by clicking within the text box and then clicking on the activated blue “...” square. The user can then modify the text value and mass accuracy, as required. .

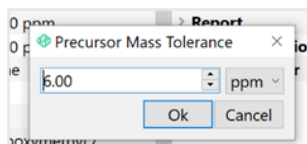


Figure 13: Modify the text value and mass accuracy.

- **Fragmentation Type**

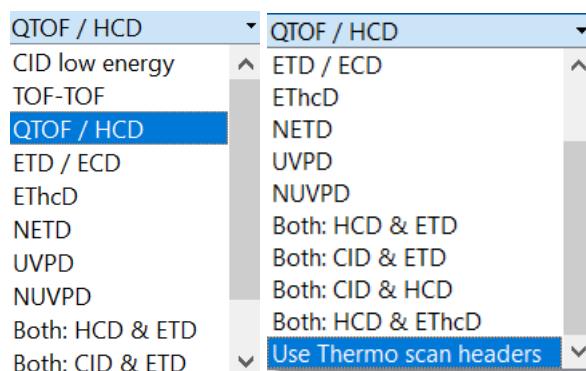


Figure 14: Fragmentation type.

The user can select from the available options using the drop-down menu. Additional options are visible using the scroll bar, including “Both:” for spectrum file(s) containing more than one fragmentation type and “Use Thermo scan headers” to read directly from Thermo raw data files.

- **Fragment Mass Tolerance 1** - The user can set the value used to acquire data in either ppm or Da. This is changed by editing **Fragment Mass Tolerance 1** shown in Figure 11.
- **Fragment Mass Tolerance 2** - If necessary, the user can set the value used to acquire data in either ppm or Da. This is only required for the “Both:” Fragmentation Types (selected as shown in Figure 11 above – the final entries). The user can set the value used to acquire data in either ppm or Da. No value will be applied if only a value for “Fragmentation Mass Tolerance 1” was entered.
- **Recalibration (lock mass)**

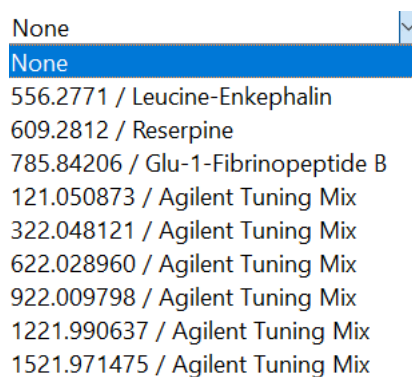


Figure 15: Lock mass calibration options.

The user can select from the available options using the drop-down menu.

- **Digestion**

▼ Digestion	
Cleavage Site(s)	RK
Cleavage Side	C-terminal
Digestion Specificity	Fully specific (fastest)
Missed Cleavages	2

Figure 16: Digestion options.

The Digestion settings allow the user to set the residues recognized by the digestion enzyme. In this example, the enzyme is trypsin, so the user entered RK for arginine and lysine for the Cleavage Site(s) and chose C-terminal for the Cleavage Side.

- **Cleavage Site(s)** - The user can change this value by entering text. If the user leaves the Cleavage Site(s) box empty, the only specific cleavage sites are protein termini.
- **Cleavage Side**

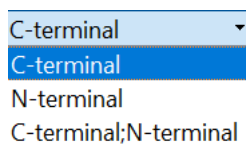


Figure 17: Cleavage Side options.

The user can change this by selecting another option from a drop-down menu. Click on the current selection to activate the drop-down menu to view the available options.

- **Digestion Specificity**

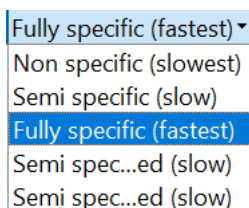


Figure 18: Digestion Specificity

In the figure above, the user chose a “Fully specific (fastest)” search, meaning that both the N- and C-terminal cleavages must be C-terminal to R or K. Nonspecific cleavage at either or both endpoints is supported. A nonspecific search with RK in the Cleavage Site(s) box searches all peptides but favors tryptic peptides; the user must leave the Cleavage Site(s) box empty for a true no-enzyme search. Digestion Specificity can be changed by selecting another option from a drop-down menu. The user can click on the current selection to activate the drop-down menu to view the available options.

- **Missed Cleavages** - The user selected 2 Missed Cleavages, as shown in Figure 16. This limits the maximum number of internal Rs and Ks not followed by P to 2; leaving Missed Cleavages at its default value of -1, which means any number of internal Rs and Ks. Missed Cleavages can be changed by entering text.
- **Modifications**

▼ <b>Modifications</b>	
Modifications	Carboxymethyl / +58.005479 @ C   fixed Oxidation / +15.994915 @
Total Common Max	1
Total Rare Max	1

Figure 19: Modifications options

Two categories of modifications are defined: fixed and variable. A fixed modification is assumed to occur on all the residues of that type, but a variable modification is optional, so that each site for a variable modification is considered with and without the modification.

- **Modifications**



Figure 20: Click within the text box to activate the light blue “...”button

To view the list of modifications included with the default workflow, the user can click within the text box to activate the light blue “...”button..

The user can specify any number of modification rules via a pull-down menu containing all the modifications listed in [www.unimod.org](http://www.unimod.org). For convenience, frequently used modifications are listed twice, at the top and again in the complete list. The three pull-down menus in each row select modification type, target residues, and fine control. There is a fourth pull-down, which lets the user delete, invert (as in (De)Carbamidomethyl), or add “attributes” to modifications. Attributes allow the user to define protein-specific modifications.

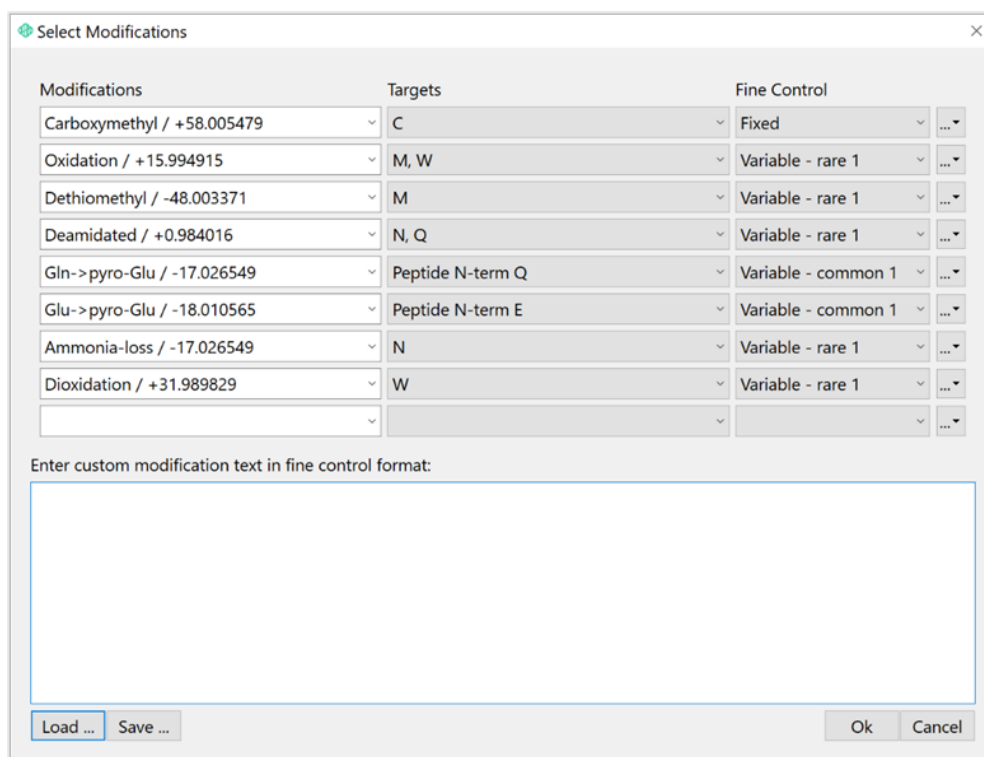


Figure 21: Select Modifications window

- **Total Common Max** and **Total Rare Max** - Protein Metrics uniquely provides a feature that dramatically increases search efficiency: the user designates each variable modification as either “common” or “rare”, with the names suggesting their use. The user can define separate limits on the number of occurrences of each variable modification, so that “common 2” means at most two occurrences per peptides. Separate limits can also be set for the total number of common and rare modifications per peptide. A typical search allows a total of at most two common modifications and a total of at most one rare modification per peptide. To search for, say, three phosphoserines per peptide, the user can change Total common modification max to 3 or split phosphorylated serine between two rules: common2 and rare1. Depending upon the other modification rules, the latter approach may give a

faster search. Please review the “Modification Fine Control” Application Note available at <https://www.proteinmetrics.com/resources/>.

NOTE: The single most important factor in search time is **Total Common Max** (shown in Figure 19). Roughly speaking, the search time grows as  $C \cdot T$  where C is the number of common modifications enabled and T is Total Common Max.

Conceptually, the search engine has one modification “slot” for each residue, along with slots for the peptide’s N- and C-termini. A variable modification such as +0.984016 @ N uses up the residue slot; a nonspecific terminal modification such as +57.021464 @ NTerm uses up the terminal slot; but residue-specific N-terminal modifications, such as -17.026549 @ NTerm Q, use up both the residue and the N-terminal slots.

The big open box (shown in the figure above) is a space for the user to type in custom modifications not listed in Unimod. The manual fine control format has the form:

Modification\_Name / Mass\_Delta @ Targets | Fine\_Control

Modification\_Name / is optional. The Targets field allows the 20 one-letter amino acid abbreviations, as well as four special locations: NTerm, CTerm, Protein NTerm, and Protein CTerm. NTerm, CTerm, Protein NTerm, and Protein CTerm can also be used as modifiers of amino acid residues. Targets form a comma-separated list.

Here is an example of a real modification not (yet) in Unimod:

DehydroFormyl / +9.98435 @ NTerm S, NTerm T | rare1

A limited number of nonstandard amino acid residues can be supported by redefining one-letter amino acid abbreviations using fixed modifications. B, Z, U, O, J, and X are accepted within FASTA protein databases, with masses, respectively, of 114.042927 (same as N), 128.058578 (same as Q), 150.95363 (selenocysteine), 237.052645 (pyrrolysine), 100.0, and 110.05 (close to averagine). By placing, for example, a fixed modification of +13.04768 on J, the user can make J in a FASTA database have mass 113.04768, correct for hydroxyproline. However, the amino acid sequence is used to predict peak intensity, so this fixed modification on J will not give the same scores as a +15.9949 variable modification on P.

For comprehensive sequence variant searches, or other searches with large numbers of modifications, it is more convenient to paste in a list of modifications in the custom modification box than to add all the modifications via the drop-down menus. Sequence variant lists are available from Protein Metrics by contacting [support@proteinmetrics.com](mailto:support@proteinmetrics.com).

- **Glycans**

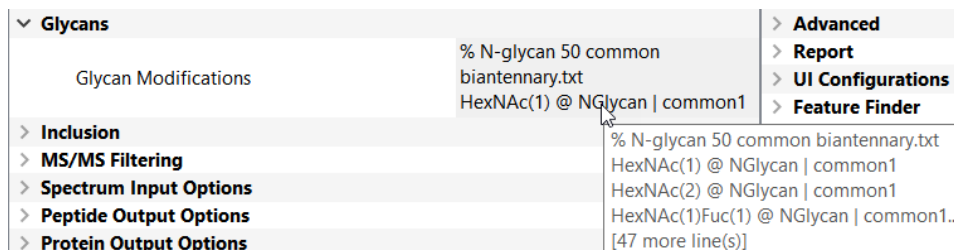


Figure 22: Glycans are now loaded as a list of records

Three ways to define glycan modifications are offered: internal preset tables, external glycan databases, and user-defined glycans. Click the activated light blue “...” button to pop up a window labeled **Select Glycans**:

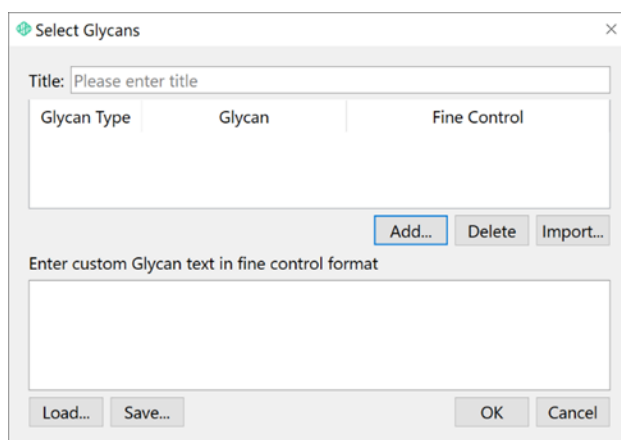


Figure 23: The Select Glycans

**Import** populates the dialog with a list of glycans from a glycan database text file:

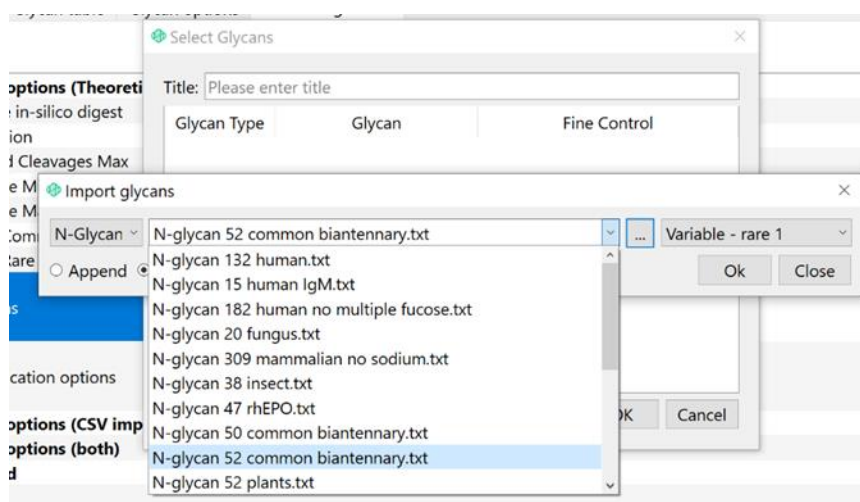


Figure 24: Importing glycan databases included with installation

Click the dropdown arrow to choose a glycan DB file. The dropdown displays a list of glycan database text files found in C:\Program Files\ProteinMetrics\PMI-Suite\Base\data\GlycanDatabases. These text files can be edited, and new glycan database text files can be added to the directory, where they become available in the dropdown (after closing and reopening Byos). This set of glycan databases is continually updated based on customer feedback. Please reach out to [support@proteinmetrics.com](mailto:support@proteinmetrics.com) to request additional content.

Alternatively, click the “...” button to open a custom glycan DB file from a different directory. The user can choose the Glycan type (N- or O-linked) and then set the Fine Control (rare1, common2, etc.).

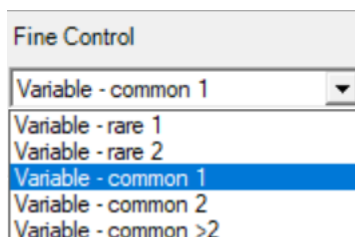


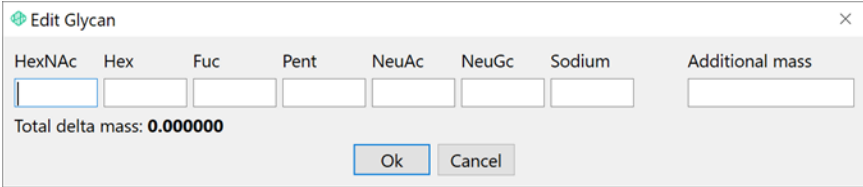


Figure 25: Glycan Fine Control options

The text files include one glycan composition per line; for example, the following gives five of the most common human O-glycans. Spaces between monosaccharides are optional, and unused monosaccharides can be left out or included with zero (0) occurrences.

```
HexNAc(1) Hex(0)
HexNAc(1) Hex(1) Fuc(0) NeuAc(0)
HexNAc(1)Hex(1)Fuc(0)NeuAc(1)NeuGc(0)Na(0)
HexNAc(1)Hex(1)Fuc(0)NeuAc(2)NeuGc(0)Na(0)
HexNAc(1)Hex(1)Fuc(1)NeuAc(0)NeuGc(0)Na(0)
```

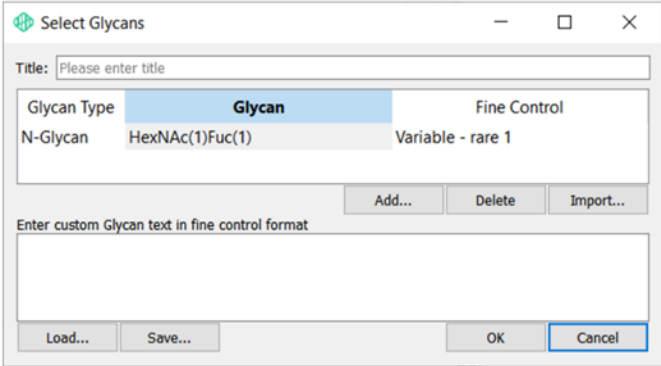
**Add** opens the **Edit Glycan** dialog to create glycans from internal preset tables: .



The **Edit Glycan** dialog box contains input fields for the following components: HexNAc, Hex, Fuc, Pent, NeuAc, NeuGc, Sodium, and Additional mass. Below these fields, the **Total delta mass** is displayed as 0.000000. At the bottom are **Ok** and **Cancel** buttons.

Figure 26: Edit glycans.

Enter the count of each used monosaccharide. Six monosaccharide residues are allowed: HexNAc, Hexose, Fucose, Pentose (common in plants), NeuAc, and NeuGc (common in non-humans). There is also a box for Sodium because it is a common adduct on sialic acids. Unused monosaccharides can be left blank or included with zero (0) occurrences. Other glycan masses and modifications such as sulfation and acetylation can be defined with the Additional mass box; this mass is added to the mass of the monosaccharides. The total delta mass will automatically populate to six decimal places. Click **Ok** to load the glycan to the Select Glycans dialog. Edit the Glycan Type and Fine Control, as needed:.



The **Select Glycans** dialog box features a **Title** field at the top. Below it is a table with three columns: **Glycan Type**, **Glycan**, and **Fine Control**. The table contains one entry: N-Glycan, HexNAc(1)Fuc(1), and Variable - rare 1. To the right of the table are **Add...**, **Delete**, and **Import...** buttons. Below the table is a text area labeled **Enter custom Glycan text in fine control format**. At the bottom are **Load...**, **Save...**, **OK**, and **Cancel** buttons.

Figure 27: Glycans assembled from monosaccharides

A third way to enter glycans is to enter or paste glycan text in the **Enter custom glycan text in fine control format** box at the bottom. These glycans are entered using the same format as for individually added glycans: Monosaccharide(count) @ OGlycan or NGlycan | fine control option:

Enter custom glycan text in fine control format:

```
HexNAc(1)Hex(1)Fuc(1) @ OGlycan | rare1
```

Figure 28: Custom glycan entry format

Legacy Glycan DB file references can be converted into a list of custom glycans. When a glycan DB file and path are shown in the Glycan Processing node, click the “...” button to convert the reference to custom glycans:

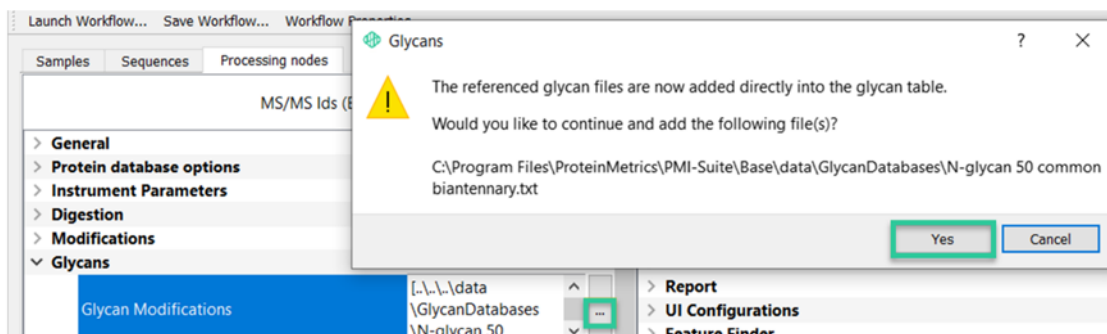


Figure 29: Converting glycan DB file reference to a glycan set

Click **Yes**, and the contents of the glycan DB file are converted into custom glycans. If the glycan DB file is no longer in the path specified, an error message gives the option to clear the obsolete file path reference:

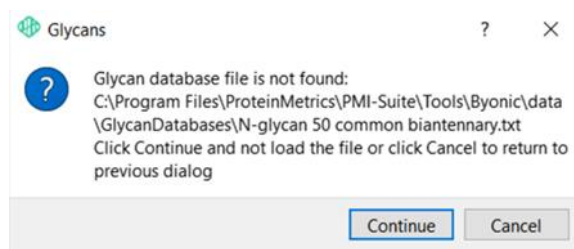


Figure 30: Click **Continue** to clear an obsolete glycan DB file reference

Click **Continue** and the **Select Glycans** dialog opens, cleared of the glycan DB file reference. Glycans can now be added using one of the methods described above.

For some helpful examples and best practices for conducting N-linked and O-linked glycan searches, see our Application Notes at <https://www.proteinmetrics.com/resources/>.

- **Inclusion**

**Inclusion** is used to import a \*.csv file which defines m/z ratio ranges and/or elution time range segments

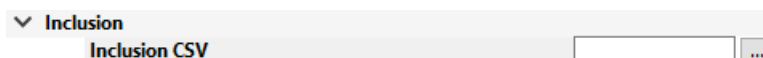


Figure 31: Inclusion option

- **MS/MS Filtering**

**MS/MS Filtering** creates MS/MS diagnostic peak filters.

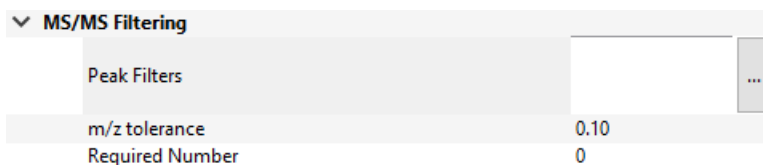


Figure 32: MS/MS diagnostic peak filtering

- The **Peak Filters**  button in Byos opens the **Select Peaks** dialog:

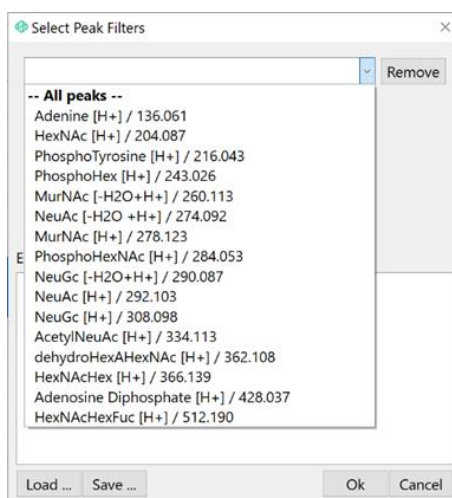


Figure 33: Select Peaks dialog for MS/MS Filtering, with dropdown

Click the drop-down arrow and select the diagnostic peak to filter. Alternatively, custom modifications can be entered manually in the box labeled **Enter custom diagnostic peaks**. The following is an example format of a diagnostic peak: Kdn / 251.076

- **m/z tolerance**  
Set a maximum **m/z tolerance** value. **Required Number** sets a required count of diagnostic peaks to be applied from within the list. To set no minimum requirement, leave the value at zero.
- **S-S, Xlink** (NOTE: This set of parameters is unique to the S-S disulfide analysis workflow.)

▼ <b>S-S, Xlink</b>	
Enable Disulfide	✓ Yes
Disulfide Text	Disulfide / -2.015650 @ C
Enable Trisulfide	— No
Trisulfide	Trisulfide / 29.956421 @ C
Enable Crosslink DSS	— No
Crosslink: DSS	DSS / 138.0680796 @ K   xlink
Enable Crosslink Custom	— No
Crosslink: Custom	
For FASTA Proteins	1,2

Figure 34: S-S and Xlink parameters.

This workflow allows the user to search for disulfide-bonded peptide pairs, trisulfide-bonded (also called persulfide-bonded) pairs, and more general cross-linking. This set of parameters also provides options to allow a user to search for expected and unexpected disulfide bonds. Enable S-S, Xlink analysis by setting **Enable Disulfide** parameter to “Yes”. Numerically designating which protein sequences from the FASTA database to consider in the **For FASTA**

**Proteins** parameter, an in-silico digestion will be completed based on the digestion parameters selected. The Xlink analysis considers every peptide that contains a cysteine and look to pair with other cysteine-containing peptides. The separation of numbers in the “For FASTA proteins” field below indicates how the potential pairings should be considered. For example, “1” searches for crosslinks in the first protein only. “4,5; 7” searches for all potential crosslinks on the 4th, 5th, and 7th protein, #4 and #5 may crosslink to each other, but not with #7.

The **Trisulfide** option allows a user to search for trisulfides within a single peptide and linking 2 peptides. Similarly, the Crosslink: DSS and Crosslink: Custom allow a user to search for crosslinks within a single peptide and linking 2 peptides.

The user can specify the modification fine control used in a custom crosslink search in the “Crosslink: Custom” text box.

- **Spectrum Input Options**

Spectrum Input Options help Byos cope with imperfect inputs. For example, on many MS instruments, precursor ion charges are uncertain for some or all spectra.

▼ Spectrum Input Options	
Apply Charges To	unassigned spectra
Charge States	
Precursor Isotope Off by X	No error check
Maximum Precursor Mass	12,000
Precursor and Charge Assignments	Compute from MS1
Maximum Number of Precursors per Scan	1
Smoothing Width (m/z)	0.01

Figure 35: Spectrum Input Options.

- **Apply Charges To** - By default, the assigned charge will be used for all spectra with assigned charges and +1, +2, +3 will be used for all CID spectra and +2, +3, +4 for all ETD spectra without assigned charges. The **Apply Charges To** parameter allows the user to override this default setting by instead selecting unassigned spectra.
- **Charge States** - All comma-separated charges detailed will be applied to each spectrum (based on the values entered into the Charge States box).
- **Precursor Isotope Off By X**

Precursor Isotope Off by X	No error check ▼
Maximum Precursor Mass	No error check
Precursor and Charge Assignments	Too high (narrow)
Maximum Number of Precursors per Scan	Too high (wide)
Smoothing Width (m/z)	Too high or low (narrow)
Side Output Options	Too high or low (wide)

Figure 36: Precursor Isotope Off By X.

Similarly, on many instruments the nominal precursor mass may actually be the mass of a <sup>13</sup>C isotope peak rather than of the base (all <sup>12</sup>C monoisotopic) peak, so the true precursor mass will within 10 ppm of 2350.120 Da or within 10 ppm of 2351.123 Da. Precursor Isotope Off By X is a pulldown menu with several options.

**No error check** will use only the assigned precursor; **Too high (narrow)** will allow the assigned precursor to be up to 2 Da too high; **Too high (wide)** will allow the assigned precursor to be up to n Da too high for a precursor of mass at least 1000n Da; **Too high or low (narrow)** will allow the assigned precursor to be up to 2 Da too high or 2 Da too

low; **Too high or low (wide)** will allow the assigned precursor to be up to n Da too high or 2 Da too low for a precursor of mass at least 1000n Da.

- **Maximum Precursor Mass** sets the Maximum Precursor Mass to be considered.
- **Precursor and Charge Assignments**

Precursor and Charge Assignments	Compute from MS1 ▾
Maximum Number of Precursors per Scan	Originally assigned
Smoothing Width (m/z)	Compute from MS1

Figure 37: Precursor and Charge Assignments.

The precursor and charge assignments will be calculated directly from the MS1 data or the originally assigned values will be applied. The user can set this using a drop-down menu.

- **Maximum Number of Precursors per Scan** - Multiple precursors per scan can also be considered – it is recommended for the user to set this to 2 for complex samples and 5-10 if processing MS<sup>E</sup> or DIA data.
  - **Smoothing Width** - The user can enter a sigma value in Thomsons for Gaussian smoothing and centroiding of Waters or Sciex data. Half-width at peak half maximum (~0.01 m/z) works well and is the default value already entered for the user.
- **Peptide Output Options**

▼ Peptide Output Options	
Automatic Score Cut	✓ Yes
Manual Score Cut	0
Show All N-glycopeptides	— No

Figure 38: Peptide Output Options.

The **Peptide Output Options** parameters offer options for filtering the peptide-spectrum matches (PSMs) by score. By default, PSM filtering is deferred until after protein ranking, and then filters to control PSM FDR on the “true” proteins—those ranked above the top-ranking decoy protein. This method gains sensitivity while simultaneously reducing both protein and PSM FDRs. See:

Two-dimensional target decoy strategy for shotgun proteomics, Journal of Proteome Research 10 (12), 5296-5301, 2011.

- **Automatic Score Cut** - To filter PSMs before protein ranking, the user can click **Yes** to activate Automatic score cut and type in a minimum score.
  - **Manual Score Cut** - The user can activate Automatic Score Cut and enter a minimum score. For example, a score threshold of 200 will remove weak matches and a threshold of 400 will remove all but the best matches. Filtering by score may be helpful in special cases, for example to eliminate from consideration all but the best wildcard PSMs.
  - **Show All N-Glycopeptides** - The user also has the option to Show All N-Glycopeptides. This will show N-Glycopeptide matches regardless of score or FDR. This is recommended for simple samples only. This can be especially useful for low energy CID data.
- **Protein Output Options**

▼ Protein Output Options	
Protein FDR	1% FDR (or 20 reverse count)
Create Focused Database	— No
Export mzIdentML	— No

Figure 39: Protein Output Options.

- **Protein FDR**

1% FDR (or 20 reverse count) ▼
1% FDR (or 20 reverse count)
2% FDR (or 50 reverse count)
No cuts

Figure 40: Protein FDR options

This gives the user control of the protein list cut-off. By default, the protein list is cut at 1% protein FDR or 20 decoy proteins, whichever comes last, but the user can ask for 2% protein FDR or a completely unfiltered (but still ranked) protein list, or No Cuts.

- **Create a Focused Database** - If the user clicks this to select Yes, the software is directed to output a new FASTA file (labeled focused and appearing in the output objs directory) containing only the proteins found in the search, along with suitable decoys (>Reverse) for unbiased FDR estimation. The focused database can then be used for subsequent wide searches, including more modifications and/or a wildcard. Of course, the user can also create focused databases outside of the software by editing existing FASTA files.
- **Export mzIdentML** - The user can also select to export this file as a mzIdentML file.

- **Multicore Options**

Multicore options	
Multicore options	Normal ▼
	Light
	Normal
	Heavy

Figure 41: Multicore processing options.

The user can control the number of computer cores of the CPU used through the drop-menu. The Light searches uses one core, Normal search uses all available cores minus two, and Heavy search uses all available cores.

- **Wildcard**

▼ Wildcard	
Wildcard Search	Disabled
Minimum Mass	-40
Maximum Mass	100
Restrict to Residues	

Figure 42: Wildcard parameters.

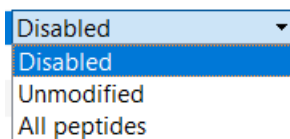


Figure 43: Wildcard Search drop-down menu options.

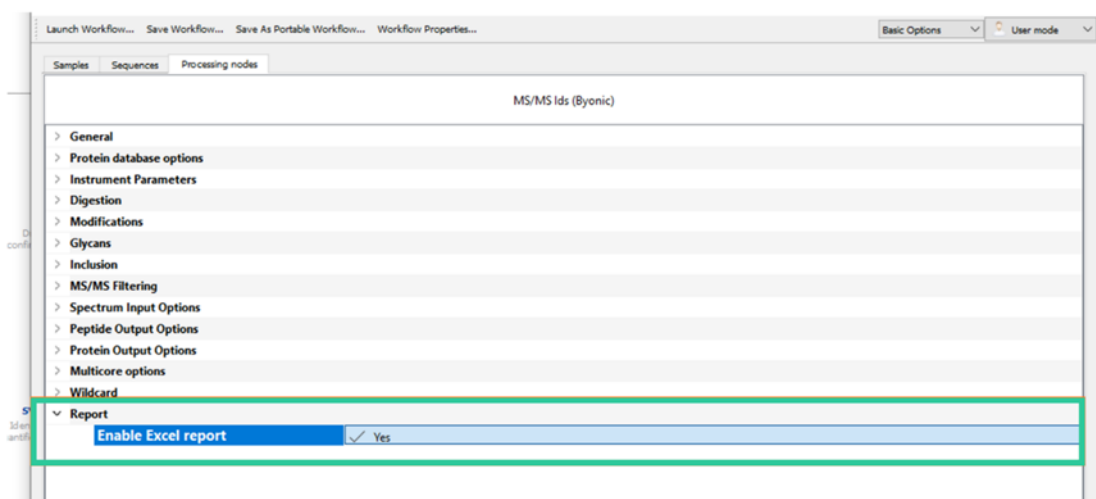
Wildcard lets the user turn on wildcard searches, set the range for the wildcard mass, and restrict the wildcard to certain residues if desired. The options Disabled, Unmodified, and All peptides are available through a drop-down menu.

The **Restrict to residues** box uses the common 20 single-letter residue abbreviations, and (lower case) n denotes peptide N-terminus and (lower case) c denotes peptide C-terminus (e.g. Kn searches lysine and N-terminus). Leaving the field blank searches all residues. A wildcard, even one with a mass range of only 50 or 60 Da, greatly increases the size of the search. It is best used with a focused database (see the Advanced tab section below) and used either alone or with only a few other modifications enabled. Most wildcard mass shifts will be recognizable by an expert; hence, a wildcard can be used to discover which known modifications should be enabled in a subsequent search. For more details about the wildcard search, see the application note “Byonic™: Wildcard Search™” at <https://www.proteinmetrics.com/resources/#application-notes>.

By specifying most modifications as rare, it is quite feasible to search for 10 – 20 modification types at once with Byonic. Even larger searches are possible with focused protein databases, for example with therapeutic proteins. Such a focused database easily allows efficient mutation searches with 200+ possible substitutions, or oxidative footprinting searches with 50+ types of oxidations. Glycans and wildcards can easily enlarge the search space by 2 to 3 orders of magnitude, so these options should be used with care, and in conjunction with only the most common variable modifications (such as oxidized methionine or pyro-Glu N-terminus). **NOTE:** The single most important factor in search time is Total common max. Roughly speaking, the search time grows as  $\underline{C}^{\underline{T}}$  where  $\underline{C}$  is the number of common modifications enabled and  $\underline{T}$  is Total common max.

The **Appendix** of the Byonic Manual provides examples of frequently found modifications and appropriate syntax for including those modifications in a Byonic search.

- **Report**



An Excel workbook can now be generated from the Byonic workflows in Byos. To enable Excel report, the user should go to **Processing nodes > Byonic column > Report** and check the **Enable Excel Report** option.

## Quant

NOTE: As mentioned in the above MS/MS Ids section, these parameters apply alone to the PTM (in-silico) Workflow.

- **General**



Figure 44: Samples to include in the project.

- **Samples** - The "\*" will apply all parameters to all samples dragged and dropped into the Samples tab.
- **In-Silico Options**

<div>&gt; General</div> <div> <div>▼ In-silico options (Theoretical Digest)</div> <div> <div>Enable in-silico digest</div> <div>— No</div> </div> <div> <div>Digestion</div> <div>Trypsin @ R,K   C-term</div> </div> <div> <div>Missed Cleavages Max</div> <div>0</div> </div> <div> <div>Peptide Minimum Mass</div> <div>300.00 da</div> </div> <div> <div>Peptide Maximum Mass</div> <div>9,000.00 da</div> </div> <div> <div>Total Common Max</div> <div>1</div> </div> <div> <div>Total Rare Max</div> <div>1</div> </div> </div>	
<div>Glycans</div>	
<div>Modification options</div>	
<div> <div>▼ In-silico options (CSV import)</div> <div>In-silico peptides CSV</div> </div>	
<div> <div>▼ In-silico options (both)</div> <div> <div>Skip if in-silico peptide is duplicate of MS2</div> <div>✓ Yes</div> </div> </div>	

Figure 45: In-silico options processing parameters.

Parameters are separated into three sections to clarify which pertain to a **Theoretical Digest**, **CSV import** and **both**.

- **Enable In-Silico** - The user has the option to click **Yes** to enable this parameter to generate an in-silico list of peptides. The below additional parameters must then be set.

NOTE: The option to add a list of in-silico peptides from a CSV file is offered through In-Silico Peptides CSV.

- **Disulfide mode (S-S workflow only)** – restricts disulfide bonds to **Free peptides only**, **Disulfide complexes only** and **Both**:

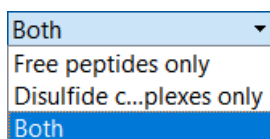


Figure 46: S-S Disulfide mode options

- **Digestion**



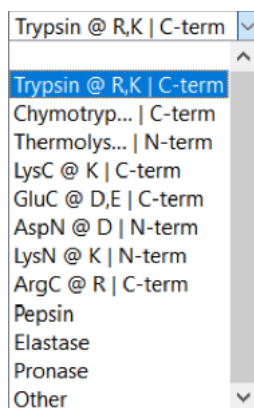


Figure 47: Digestion options.

The user can view several options through the drop-down menu. These are detailed as one letter residue codes adjacent to cleavage points. The user may enter a customized digestion by using the following syntax: Name @ Amino acid letters | C-term or N-term.

- **Missed Cleavages Max**

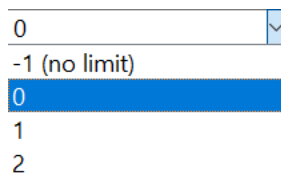
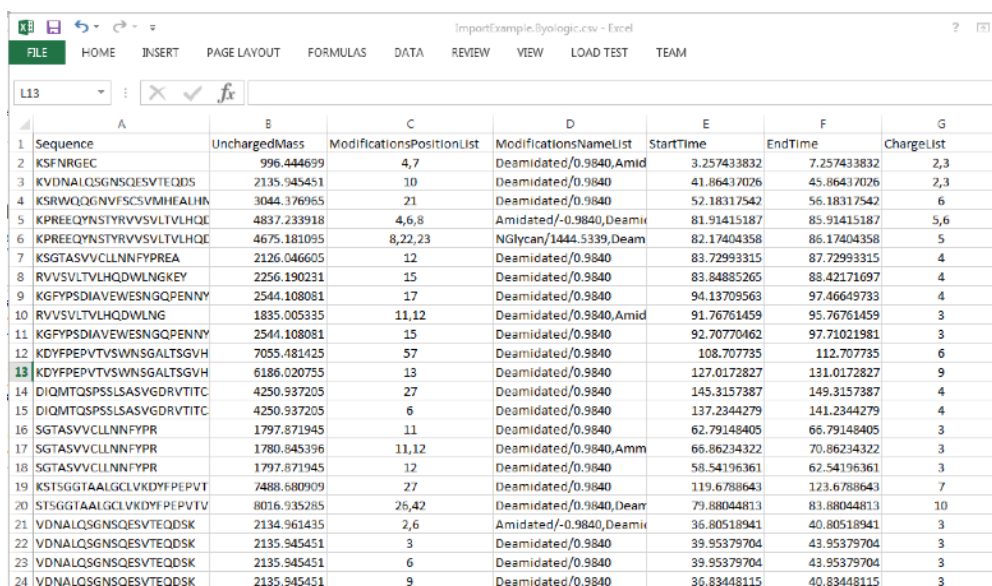


Figure 48: Missed Cleavages Max options.

The user can set the maximum number of missed cleavages per peptide by selecting a value using the drop-down menu. A value of -1 allows any number of cleavages.

- **Peptide Minimum Mass** - The user can set the minimum value of the peptide mass range.
- **Peptide Maximum Mass** - The user can set the maximum value of the peptide mass range.
- **Total Common Max** - The user can set the maximum number of common modifications per peptide. The search size grows by an order of magnitude with each increase in Total Common Max.
- **Total Rare Max** - The user can set the maximum number of rare modifications per peptide. The search size grows by an order of magnitude with each increase in Total Rare Max.
- **Glycans** - The user can specify glycan databases or custom glycans to include in the in-silico generation. This is done the same as detailed in the MS/MS Ids section.
- **Modifications Options** - The user can specify modifications to include in the in-silico generation. This is done the same as detailed in the MS/MS Ids section.
- **In-Silico Peptides CSV** - This option allows the user to add a list of in-silico peptides from an imported CSV file, thus there is no need to run an MS2 search if there are known modifications with known masses and retention times. The format for the CSV is shown below.



	A	B	C	D	E	F	G
1	Sequence	UnchargedMass	ModificationsPositionList	ModificationsNameList	StartTime	EndTime	ChargeList
2	KSFNRGEC	996.444699	4,7	Deamidated/0.9840,Amid	3.257433832	7.257433832	2,3
3	KVDNALQSGNSQESVTEQDS	2135.945451	10	Deamidated/0.9840	41.86437026	45.86437026	2,3
4	KSRVQQGNVFSCSVMHEALHN	3044.376965	21	Deamidated/0.9840	52.18317542	56.18317542	6
5	KPREEQYNSTYRVVSVLTVLHQE	4837.233918	4,6,8	Amidated/-0.9840,Deamid	81.91415187	85.91415187	5,6
6	KPREEQYNSTYRVVSVLTVLHQE	4675.181095	8,22,23	NGlycan/1444.5339,Deamid	82.17404358	86.17404358	5
7	KSGTASVVCLLNNFYPREA	2126.046605	12	Deamidated/0.9840	83.72993315	87.72993315	4
8	RVVSVLTVLHQDWLNGKEY	2256.190231	15	Deamidated/0.9840	83.84885265	88.42171697	4
9	KGFYPSDIAVEWESNGQPENNY	2544.108081	17	Deamidated/0.9840	94.13709563	97.46649733	4
10	RVVSVLTVLHQDWLNG	1835.005335	11,12	Deamidated/0.9840,Amid	91.76761459	95.76761459	3
11	KGFYPSDIAVEWESNGQPENNY	2544.108081	15	Deamidated/0.9840	92.70770462	97.71021981	3
12	KDYFPEPVTWSWNSGALTSVGH	7055.481425	57	Deamidated/0.9840	108.707735	112.707735	6
13	KDYFPEPVTWSWNSGALTSVGH	6186.020755	13	Deamidated/0.9840	127.0172827	131.0172827	9
14	DIQMTQSPSSLSASVGDRVTITC	4250.937205	27	Deamidated/0.9840	145.3157387	149.3157387	4
15	DIQMTQSPSSLSASVGDRVTITC	4250.937205	6	Deamidated/0.9840	137.2344279	141.2344279	4
16	SGTASVVCCLNNFYPR	1797.871945	11	Deamidated/0.9840	62.79148405	66.79148405	3
17	SGTASVVCCLNNFYPR	1780.845396	11,12	Deamidated/0.9840,Amid	66.86234322	70.86234322	3
18	SGTASVVCCLNNFYPR	1797.871945	12	Deamidated/0.9840	58.54196361	62.54196361	3
19	KSTSGGTAALGCLVKDYFPEPVIT	7488.680909	27	Deamidated/0.9840	119.6788643	123.6788643	7
20	STSGGTAALGCLVKDYFPEPVIT	8016.935285	26,42	Deamidated/0.9840,Deamid	79.88044813	83.88044813	10
21	VDNALQSGNSQESVTEQDSK	2134.961435	2,6	Amidated/-0.9840,Deamid	36.80518941	40.80518941	3
22	VDNALQSGNSQESVTEQDSK	2135.945451	3	Deamidated/0.9840	39.95379704	43.95379704	3
23	VDNALQSGNSQESVTEQDSK	2135.945451	6	Deamidated/0.9840	39.95379704	43.95379704	3
24	VDNALQSGNSQESVTEQDSK	2135.945451	9	Deamidated/0.9840	36.83448115	40.83448115	3

Figure 49: Format for an In-Silico Peptides CSV.

**Skip if in-silico peptide is duplicate of MS2** - The user can click to select **Yes**. This will skip any generated in-silico peptide if one already exists from the MS2 data. It is recommended to make this selection when generating in-silico peptides.

- MS extract options

MS extract options		
Relevant Peptides	All types	
m/z integration window (ppm)	18.00	
Apex Search Window (minutes)	2.00	
XIC Area Window (minutes)	2.00	
Isotope	0	1800
	1	3000
	2	5000

Figure 50: MS extract options.

There are various aspects to the XIC of a peptide which can help in distinguishing a true from false identification, or whether the peptide hit is relevant based on intensity.

- Relevant Peptides

All types
Seq.var., wildtypes
Seq.var., wildtypes, oxidations, deamidations
All types

Figure 51: Relevant Peptide options.

The user can select what types of peptides to consider relevant using the drop-down menu. Only peptides of interest will be considered for extracting additional information, such as XIC, isotope profile, and MS2 fragments. This pulldown menu option may affect the file size significantly. For SVA, the user may only need to choose Sequence variants and wildtypes. For peptide mapping, the user should choose All types.

- **m/z integration window (ppm)** - The user can define the maximum m/z error tolerance to be applied to the search.
- **Apex Search Window (minutes)** - The user can automatically define the search window for the peak apex.
- **XIC Area Window (minutes)** - The user can automatically set the time window for integration. The defined time limits will be visible as two vertical lines for each XIC in the project. These lines can be dragged by the user's mouse to adjust the integration time if needed.

- **Isotope**

XIC Area Window (minutes)	2.00
Isotope	0 1800
	1 3000
	2 5000

Figure 52: Isotope settings.

The user can set the mass ranges for the offset from the monoisotopic mass. Click within the box to activate the light blue “...” button and view the default values. These values can be modified by typing in values.

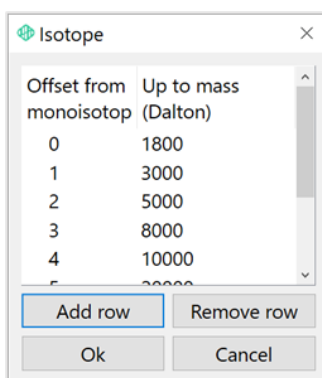


Figure 53: Isotope settings

- **Advanced**

▼ <b>Advanced</b>	
Enable Lock-Mass Calibration	— No
Lock Mass (m/z)	1221.990637
Lock Mass tolerance (ppm)	100.00
Centroid Smoothing Width	0.02
Elution Prediction Score Min	300
Compute Fragment Coverage	✓ Yes
Advanced configuration	

Figure 54: Advanced options

- **Enable Lock-Mass Calibration** - The user can click to select between **No** and **Yes**.
- **Lock Mass (m/z)**

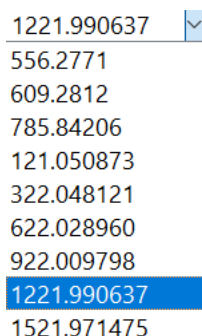


Figure 55: Lock mass m/z value options

The user can enter the calibrant m/z value. Several are also available using the drop-down menu or the user can type in a numerical value. If empty, no calibration will be applied.

- **Lock Mass tolerance (ppm)** - The user can enter the calibrant m/z mass tolerance value in ppm. The user can type in a numerical value or use the up and down arrow keys to increase or decrease the value. If empty, no calibration will be applied.
- **Centroid Smoothing Width** - The user can enter a sigma value in Thomsons for Gaussian smoothing and centroiding of Waters or Sciex data. Half-width at peak half maximum (~0.01 m/z) works well and is the default value already entered for the user.
- **Elution Prediction Score Min** - The user can set a value for this filter to find wildtype peptides with scores above this value to adjust the elution time.
- **Compute Fragment Coverage** - The user can click between **Yes** and **No** to compute fragmentation coverage at project creation.
- **Advanced Configuration** - The user can enter text commands to complete advanced processing. These are detailed in the Release Notes included with each quarterly release. Please reach out to [support@proteinmetrics.com](mailto:support@proteinmetrics.com) for additional details.

## • Report



Figure 56: Report Configuration Path

Each Byos default workflow includes a report template created by our Customer Success team that is optimized for the specific type of analysis. If the user prefers a customized report template, they can direct Byos to this file using the light blue “...” button. They will be prompted to select a file.

## • UI Configurations



Figure 57: UI Configurations – UI Column Filters and UI Layout

The user can import column filters as well as layout files. This is designed to standardize analyses across all users, labs, and sites. The user can direct Byos to the preferred file for each using the light blue “...” button. The user will be prompted to select a file.

## • Feature Finder

▼ <b>Feature Finder</b>	
Enable Feature Finder	— No
Minimum Isotope Corr.	0.90
Mass Range Min	500.00
Mass Range Max	8,000.00
Maximum Feature Count	300
Absolute minimum intensity	0.00
Minimum Isotope Count	3
Minimum Peak Width (min)	0.01
Minimum S/N Ratio	3
Minimum Scan Count	3
Exclude +1 Charge Only Features	— No
Exclude Features With MS2 Matches	— No
Mass matching tolerance (ppm)	20.00
Time matching tolerance (min)	0.20

Figure 58: Feature Finder

Feature Finder is an algorithm that allows the user to scan the MS1 domain for all existent peptides in a sample. In a typical data dependent acquisition 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.

Feature Finder also allows the user to match these features to the current identifications and to add a specified number of the unidentified features as unknowns to the project. Doing so will generate XICs for the unknowns and allow reporting on features such as retention time, mass, intensity, and fold change across samples.

- **Enable Feature Finder** - The user can click to select between **No** and **Yes** to enable Feature Finder.
- **Minimum Isotope Corr.** - The user can set the minimum cutoff to match the found isotopic distribution to the theoretical distribution using Pearson's correlation. If a feature has an isotopic distribution that is vastly different from its theoretical, this can be used to exclude it.
- **Mass Range Min** - The user can set the minimum mass for a feature to be considered.
- **Mass Range Max** - The user can set the maximum mass for a feature to be considered.
- **Maximum Features Count** - The user can set the maximum feature count to include the most intense features.
- **Absolute minimum intensity** - The user can set the minimum intensity for a feature to be considered.
- **Minimum Isotope Count** - The user can set the minimum number of isotope peaks required for a feature to be considered. This applies only if its charge is greater than 2.
- **Minimum Peak Width (min)** - The user can set the minimum peak width in minutes for a feature to be considered.
- **Minimum S/N Ratio** - The user can set the minimum S/N ratio for a feature to be considered.
- **Minimum Scan Count** - The user can set the minimum scans required across the peak for a feature to be considered.
- **Exclude +1 Charge Only Features** - The user can click to select between **Yes** and **No** to exclude singly charged features.
- **Exclude Features With MS2 Matches** - The user can click to select between **No** and **Yes** to exclude features with MS2 matches. Exclude will remove the feature if it coincides within the time and mass tolerances set – it will not be included in the top X features to avoid duplicates. It is recommended to check this box for projects containing MS2 data and Byonic searches.

- **Mass matching tolerance (ppm)** and **Time matching tolerance (min)** - These parameters are used to match and remove features that have already been identified by MS2 (when **Exclude Features With MS2 Matches** is set to Yes) so that they do not contribute to the maximum feature count. They help to match the retention time and mass of an unknown feature to a MS2 feature so that it may be excluded from the Peptides table in Peptide Analysis.
- **XIC**

**XIC** refers to the “Extracted Ion Chromatogram”, which represents the signal intensity of an ion or ions of interest over time extracted from the full mass spectrometry dataset. Fast XIC accelerates XIC processing. As of Byos v5.9, Fast XIC will be enabled during workflow updates if the feature is not yet enabled.



Enable Fast XIC	No
Granularity	100,000
Max Mz	3,010
Max Ion Count	3,000

Figure 59: Fast XIC parameters

If Enable Fast XIC is set to Yes, the below options are available:

Parameter	Description
Granularity	Integer value, relating to the precision. 10000 is 4 places past the decimal. For mass 1000.1236, a granularity of 1000 sets the value to 1000.124, while a granularity of 10000 keeps the value at 1000.1236. Default value is 100000.
Max Mz	Integer value. The maximum m/z value during data acquisition. To acquire data in the range 200-2000, set Max Mz = 2000, so that no ions are acquired past that value. Default value is 3010
Max Ion Count	Integer value. The maximum number of ions to be considered, sorted by intensity. Some files may contain ~2500 ions per scan. Max Ion Count = 1000 uses only the top 1000 ions by intensity. This is especially important for TOFs with ~25K ions per scan, where a higher value of 2500 is recommended. Default value is 3000.

## Main Menu Bar

The topmost menu bar includes four items: **File**, **Edit**, **Window**, **Server**, and **Help**.

## File Menu

The **File** pull-down menus manage project files and export reports and data:

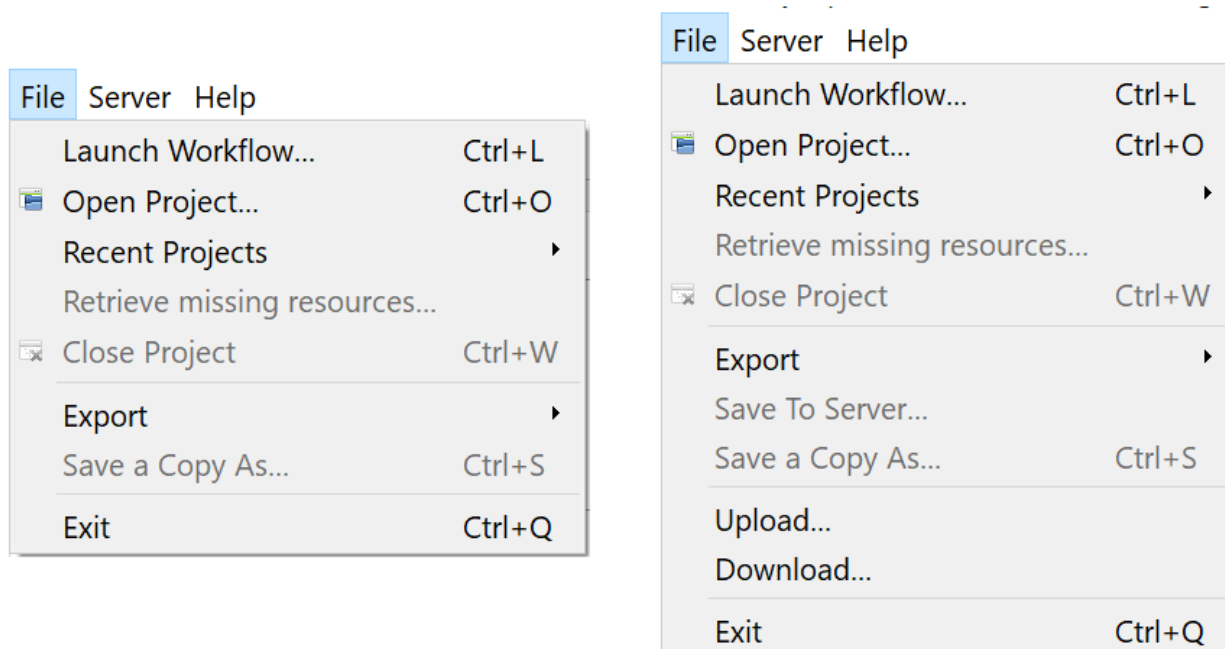


Figure 60: File menu (Standalone and Byosphere Client)

The File menus include items to create new projects, open previously created projects from saved files, save a copy of an open project, close a project (yet leave the application open), and exit Byos. Mouse over the **Recent Projects** menu to open a side window with a list of recently visited projects that can be reopened.

The File menu functions **New Project**, **Open Project**, **Retrieve missing** resources, **Close Project**, **Label Manager** and **Report**, respectively, are also available as icon buttons below the topmost menu bar:

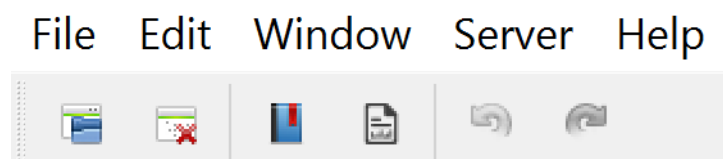


Figure 61: File menu icons

## File > Export Menu

There are also three File sub-menu items to export reports.

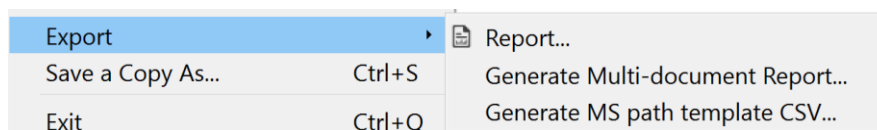


Figure 62: File &gt; Export menu

**File > Export > Report** generates a report that includes a summary of the project files, one or more pivot tables (that can be visualized as bar charts, etc.), along with plot images. There are other valuable tools for making customizable reports and regulatory filing documents. When a report is perfected, a copy of the report configuration can be saved to use with other reports. If no report configuration is attached to the project, a default report configuration is used to generate the report.

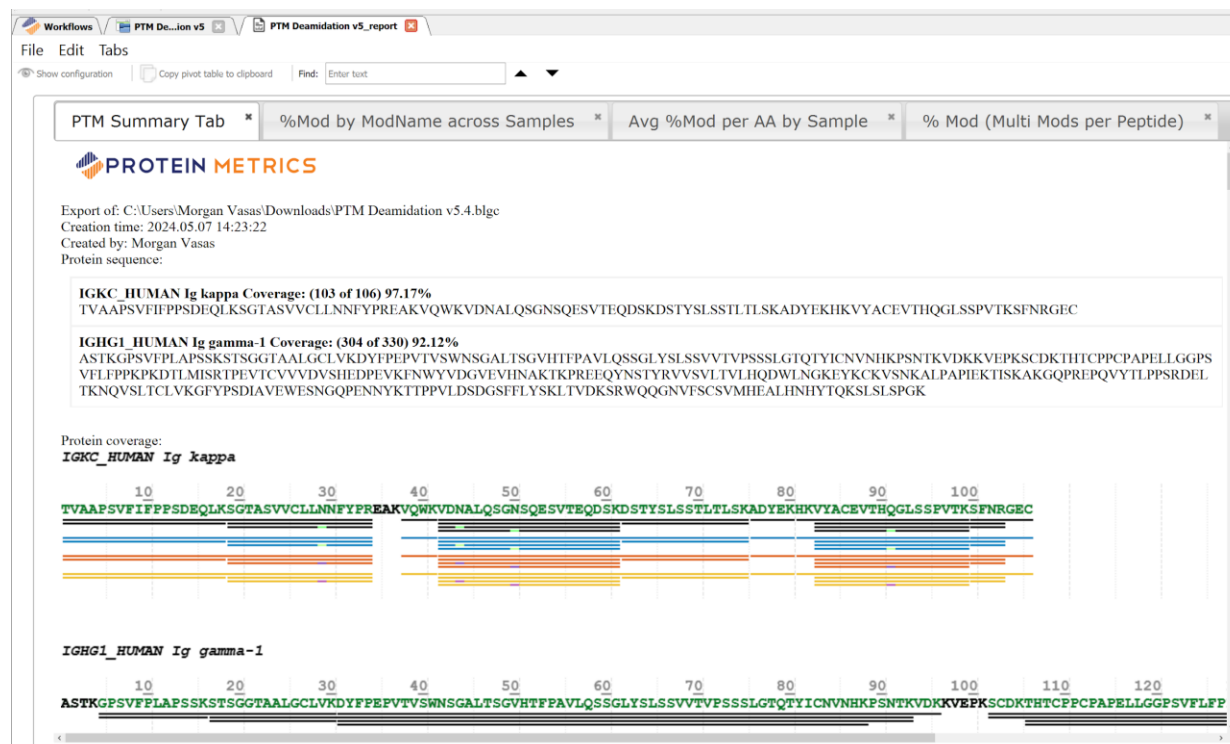


Figure 63: Default report format

To replace the report with another report template, choose in the report window **File > Presets > Report presets > <template name>**:

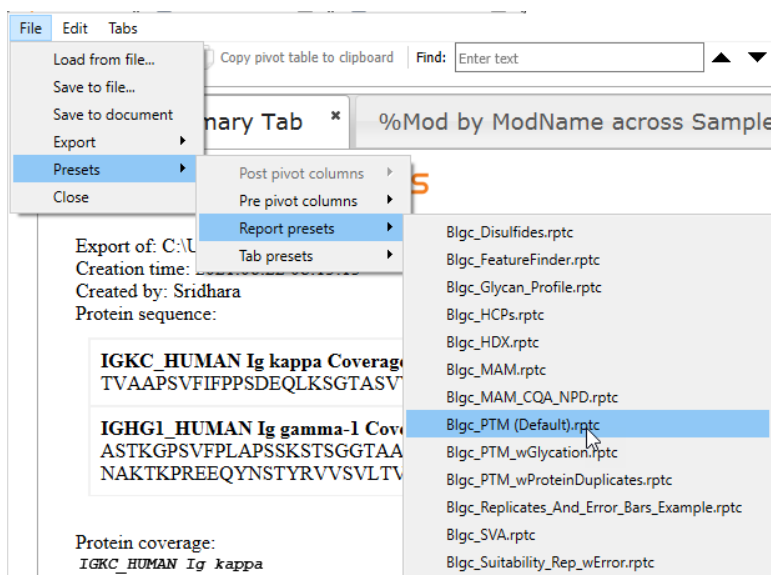


Figure 64: Changing the report template in a created report

**File > Export > Generate Multi-document report** opens a dialog into which the user drags and drops multiple Byos Peptide project \*.blgc files:



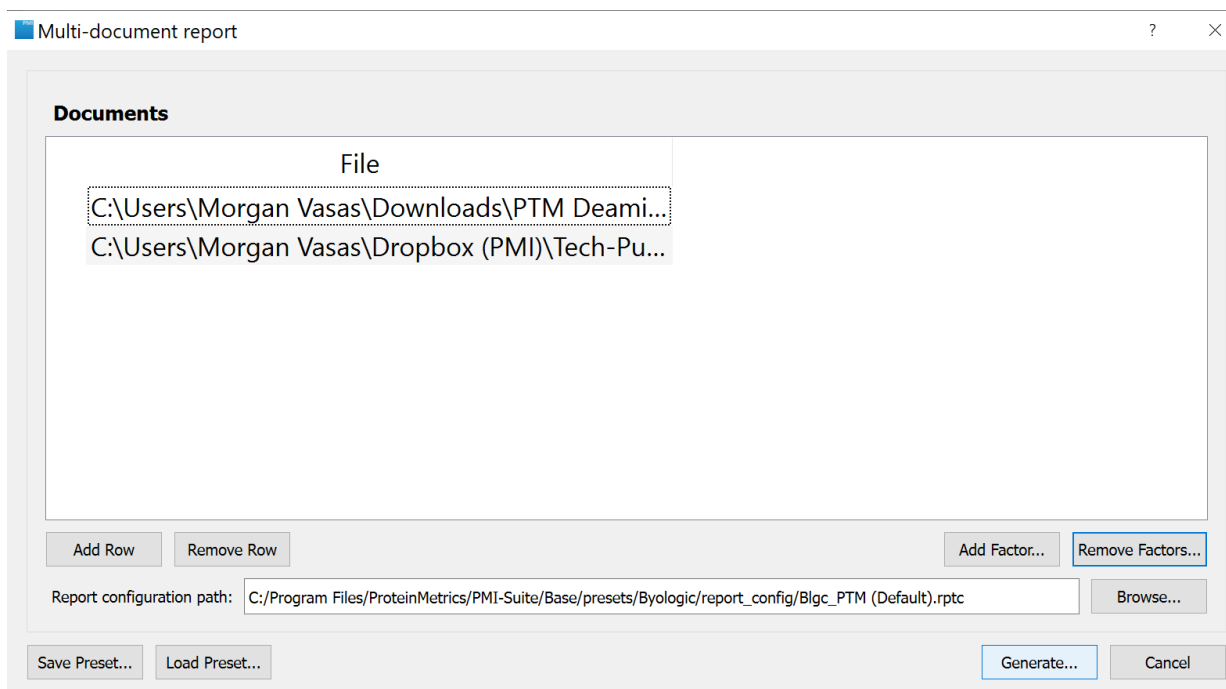


Figure 65: Multi-document report export

**File > Export > Generate MS path template CSV ...**

To learn about reports in detail see the document **PMI Reporting Manual.pdf**. Contact [support@proteinmetrics.com](mailto:support@proteinmetrics.com) for information on a variety of existing and new report templates.

**Edit Menu**

The **Edit** pull-down menu manages program settings.

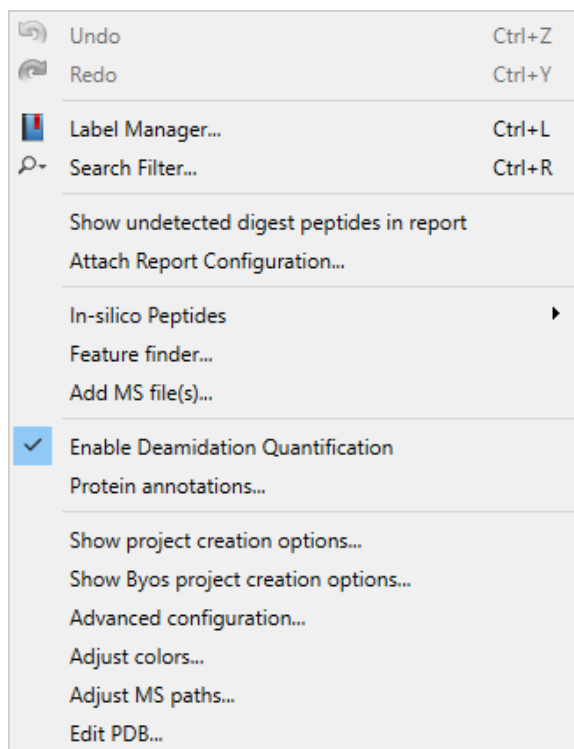
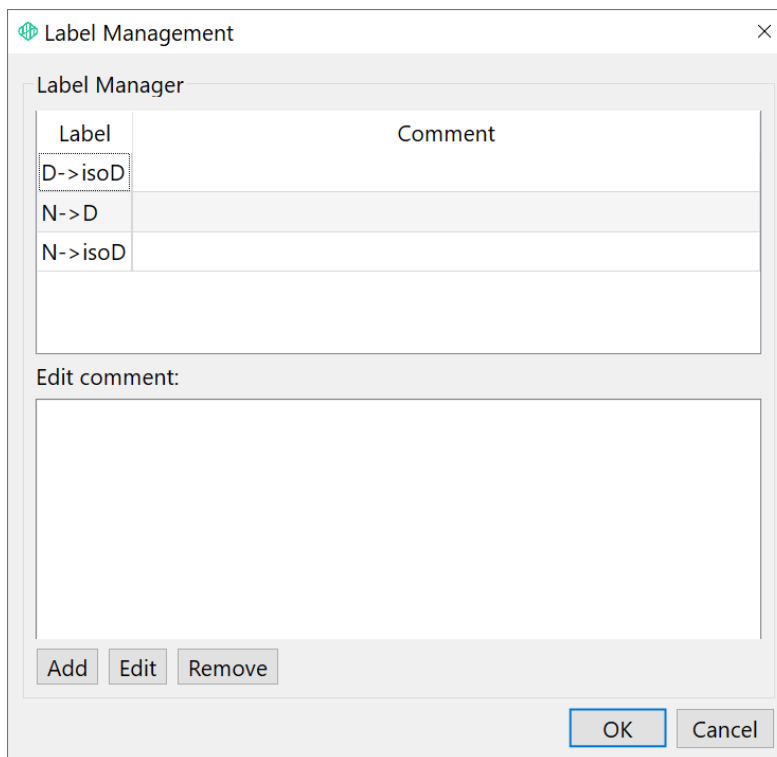


Figure 66: Edit menu

**Undo** and **Redo** allow the user to undo or redo changes. These **Edit** menu functions are also available as the arrow toolbar buttons below the menu bar to the right of the **Help** menu.

The **Label Manager** is used to add, remove and edit label values. These labels can then be assigned to records in the **Peptides** table. To add a new label, click the **Add** button, enter the label text, and click **OK**. To add or edit a comment to a new or existing label, select the label in the **Label Manager** view, and edit the comment in the **Edit comment** view. To edit a label, select it, click **Edit**, edit the label text, and click **OK**. To remove a label, select it, click **Remove**. Click **OK** in the main window to save all changes.



**Label Management**

Label Manager

Label	Comment
D->isoD	
N->D	
N->isoD	

Edit comment:

Add Edit Remove

OK Cancel

Figure 67: Label manager

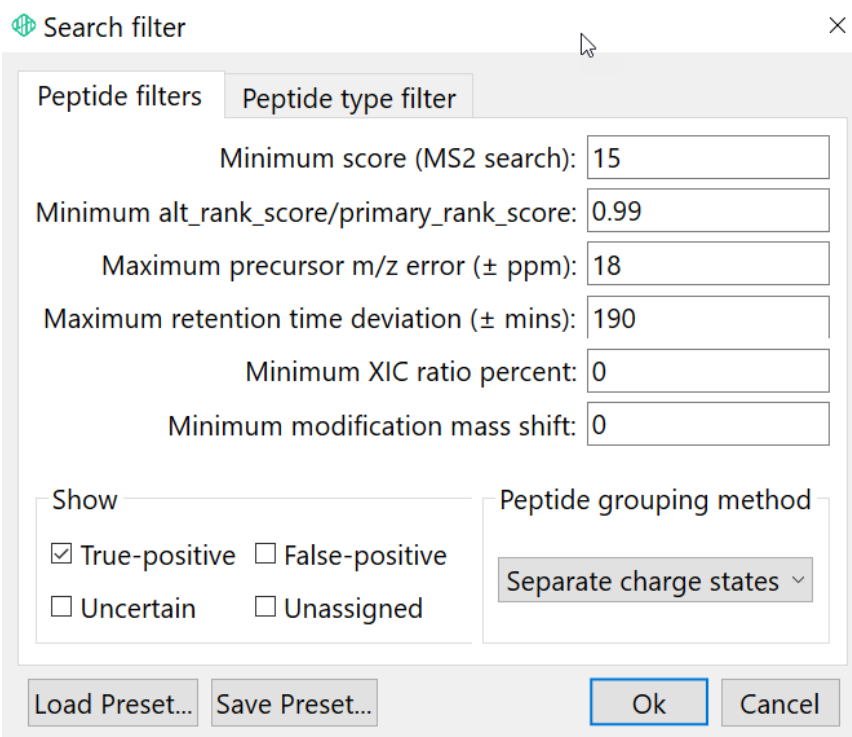
To add or remove labels to a record in the **Peptides** table, click the peptide row, scroll to the Labels column, click the cell in this column to open a dialog. Check or uncheck desired labels and click **OK**. The **Label Manager** can be opened from this dialog by a click on the **Manager** button.

Mod. Summary	Mod. AAs	z	Validate	ommer	Labels	
N14(Deamidated/0...	N	2	True-p...			<input type="checkbox"/> N->D
N14(Deamidated/0...	N	2	True-p...		N->isoD	<input checked="" type="checkbox"/> N->isoD
W12(Oxidation/15.9...	W	2	True-p...			
W12(Dioxidation/31...	W	2	True-p...			
		1	True-p...			
		2	True-p...			

Ok  
Manager

Figure 68: Adding labels in the Peptides table

The **Search Filter** filters records displayed in the **Peptides** table according to a variety of properties and categories. Edit values for properties to tighten or loosen restrictions. Check additional **Show** categories to include peptides that are not true positives. Click the **Peptide grouping method** drop-down arrow to combine peptides that differ only by their charge states.



Search filter

Peptide filters Peptide type filter

Minimum score (MS2 search): 15

Minimum alt\_rank\_score/primary\_rank\_score: 0.99

Maximum precursor m/z error (± ppm): 18

Maximum retention time deviation (± mins): 190

Minimum XIC ratio percent: 0

Minimum modification mass shift: 0

Show

☒ True-positive ☐ False-positive

☐ Uncertain ☐ Unassigned

Peptide grouping method

Separate charge states ▾

Load Preset... Save Preset... Ok Cancel

Figure 69: Peptide search filter

The **Peptide type filter** tab allows the inclusion or exclusion of peptide modification types. Check modifications to include and uncheck modifications to exclude. Byos defaults to all peptide types:

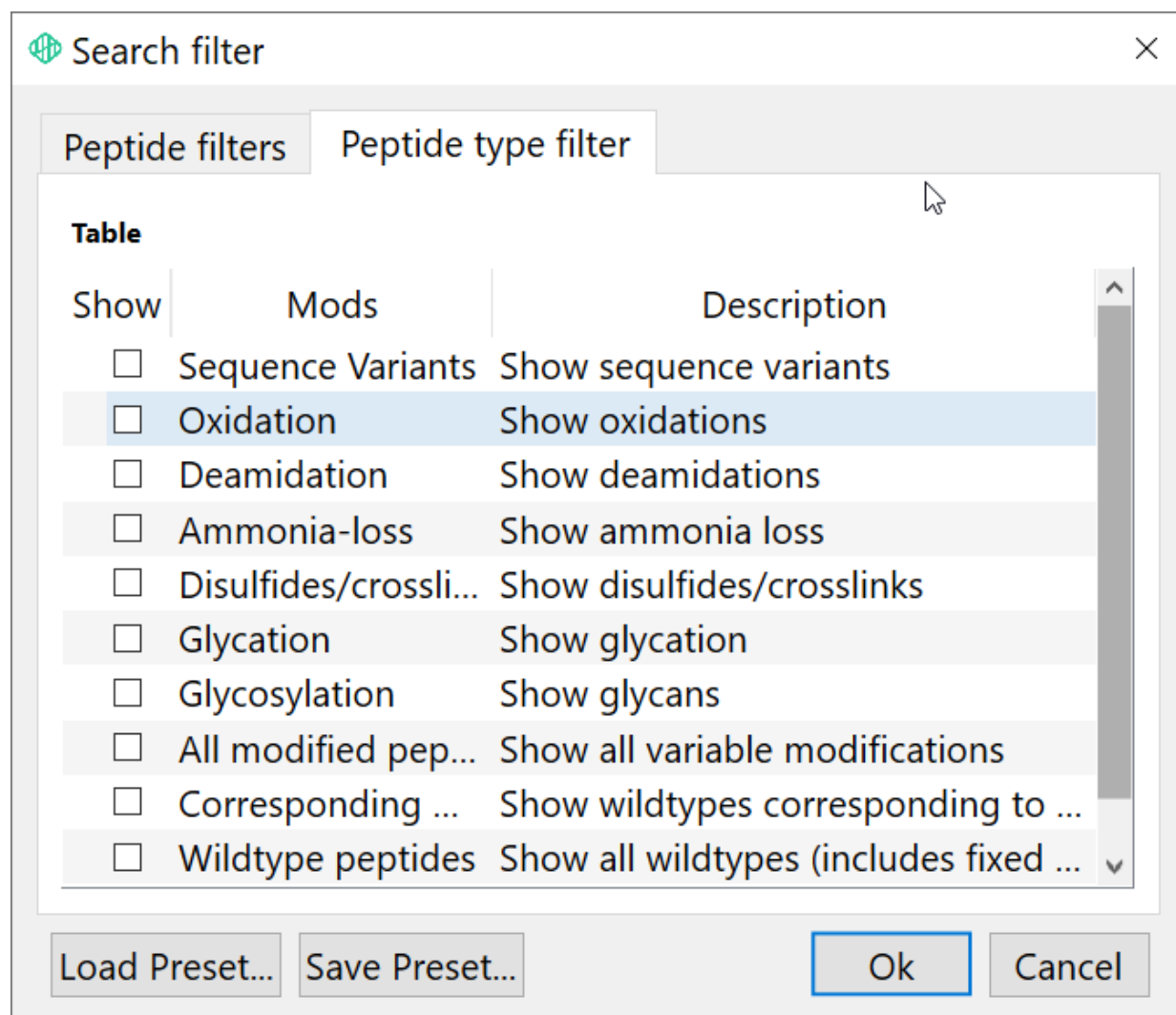


Figure 70: Filtering by peptide type

To save the edited settings from the two tabs to a configuration file, click the **Save Preset** button, enter a file name, navigate to a directory, and click **Save**. To load settings from a previously-saved configuration file, click the **Load Preset** button, navigate to the desired directory, select a \*.blgcsfson or \*.blgcsf file, and click **Open**.

**Show undetected digest peptides in report** allows for either including or excluding the undetected peptides for reporting. Enabling this setting will result in a new label "ND" in the **Comment** column in reporting tabs, denoting that the specific peptide is not detected in the analyses.

**Attach report configuration** attaches a report configuration file to the project, to be used instead of the default report configuration. Report configuration files (graphical layouts) are generated from **File > Export > Report** and have the extension \*.rptc. This menu can also be used to remove an attached report configuration file.

**Attach report configuration** attaches a report configuration file to the project to be used in place of the default report configuration. Report configuration files (graphical layouts) are generated from the project report opened using **File > Export > Report**, and then saved using **File > Save to file**. Configuration have the extensions \*.rptc, \*.tabc, \*.lvcfg, or \*.pvtcfg. This menu can also be used to remove an attached report configuration file.

**In-silico Peptides** contains five sub-menus to import or export in-silico peptide candidates before or after project creation:

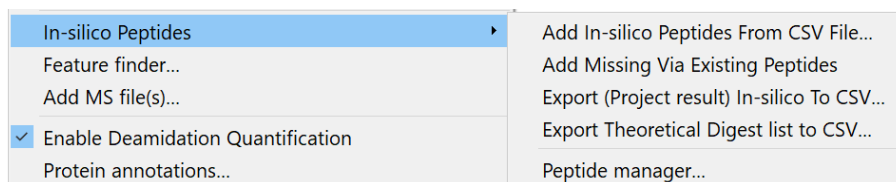


Figure 71: Edit > In-silico Peptides menu

- **Add In-silico Peptides From CSV File** opens a browser to load a \*.csv file of in-silico candidates. Use of the correct format for the \*.csv file is required. For an example of the correct \*.csv format, see the file **ImportExample.Byologic.csv** in Program Files\ProteinMetrics\PMI-Suite\ExampleData.
- **Add Missing Via Existing Peptides** identifies missing peptide sequences and fills in those sequences from equivalent peptides in the sample files. Support has been added in v5.10 of Byos allowing users the option to apply advanced sample-to-sample XIC alignment and peak detection to newly added peptides or not.
- **Export In-silico to CSV** exports the in-silico peptides to a \*.csv file. This peptide file is then available for import into a different project. Enter a file name, navigate to a directory, and click **Save**.
- **Export Theoretical Digest list to CSV** exports the theoretical digest information directly to a \*.csv file. This file is then available for import into a different project. Enter a file name, navigate to a directory, and click **Save**. Note that to be able to export theoretical digest in-silico, it must be enabled during Project Creation.
- **Peptide Manager** combines all of the In-silico functionalities from **Edit > In-silico peptides** with a color-coded preview of the project changes and enhanced control over which rows and columns are affected.

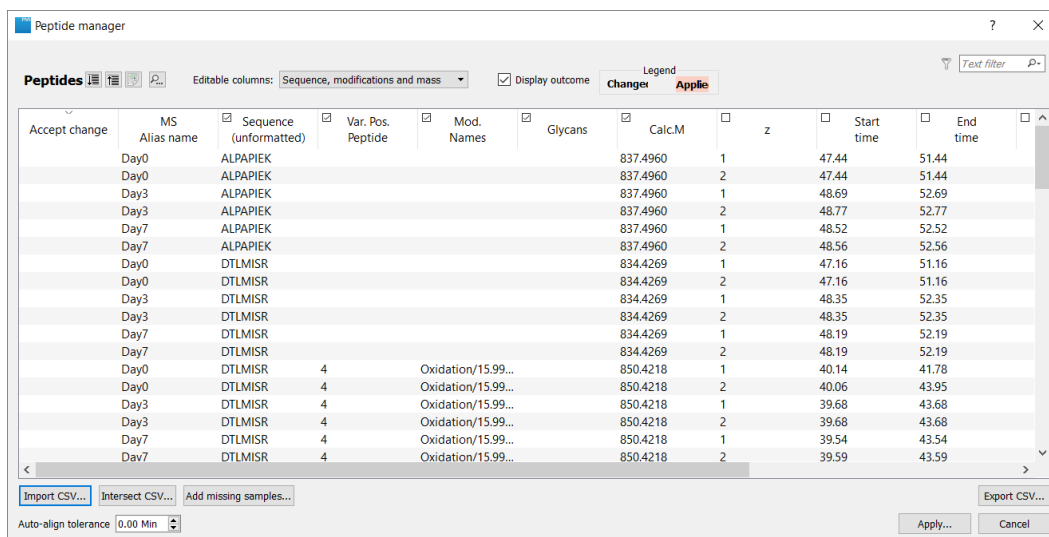


Figure 72: Peptide manager

The user can set which columns are editable using the drop-down menu.

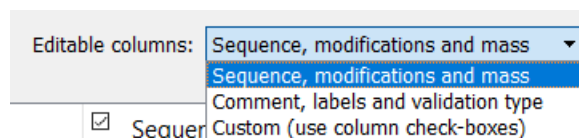


Figure 73: Editable columns in Peptide manager

- **Export CSV** is the same as **Edit > In-Silico > Export CSV**.

Start and End times may differ from the previous CSV export. This is because Peptide Manager exports the most centered XIC start/end, while the old method exported the first available start/end range. The difference is considered an improvement.

- **Import CSV** is the same as in **Edit > Import in silico from CSV**
- **Intersect CSV** replaces the old menu item **Edit > In-Silico > Intersect with CSV library**. This menu item has been removed and can only be accessed from Peptide Manager.

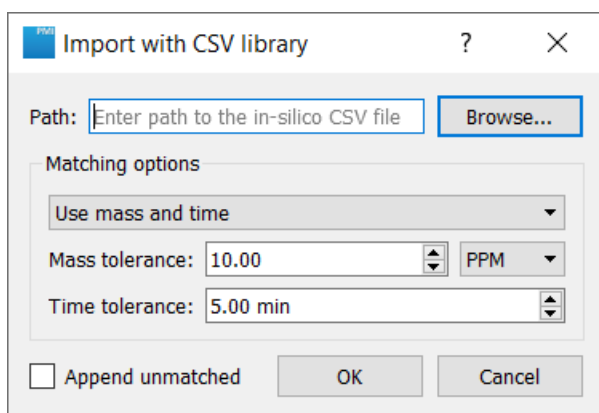


Figure 74: Intersect CSV: import with CSV library dialog

Select a CSV file using the **Browse** button. **Matching options** include **Use mass and time** and **Use sequence and modifications**. Set mass and time tolerances. Check **Append unmatched** to include non-intersected peptides. Click **OK** to return to Peptide Manager and select columns to review. New values will be show in red.

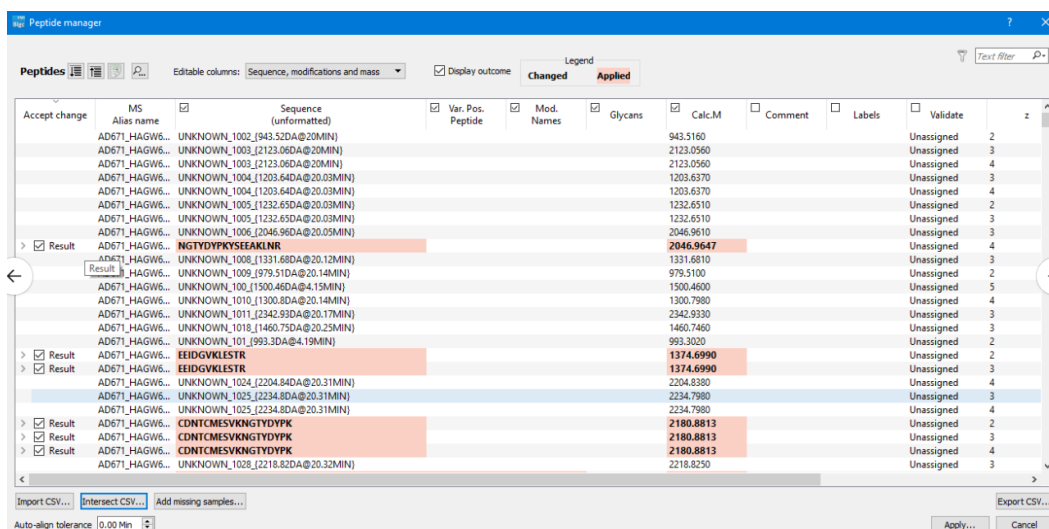
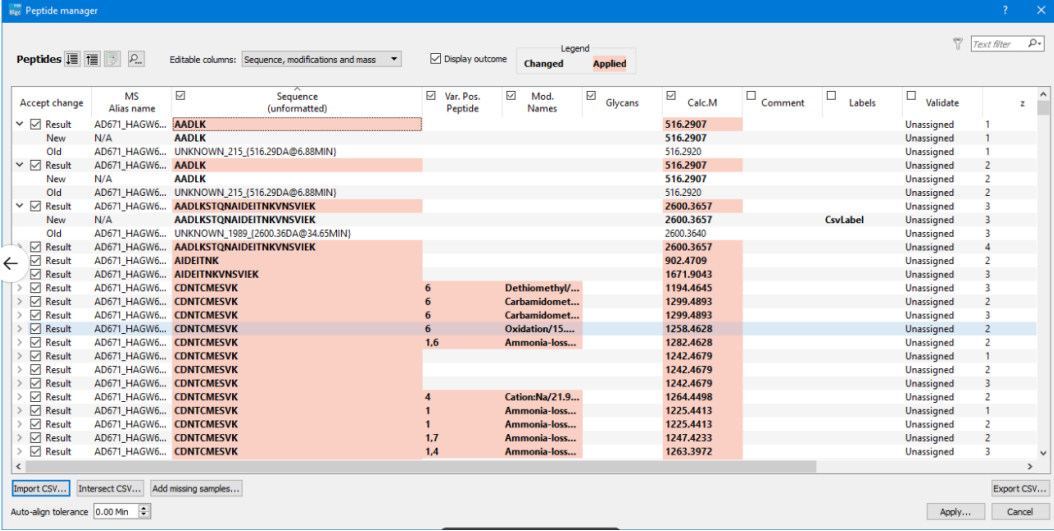


Figure 75: Peptides imported from CSV library are marked in red

Expanded rows to showcase additional content:

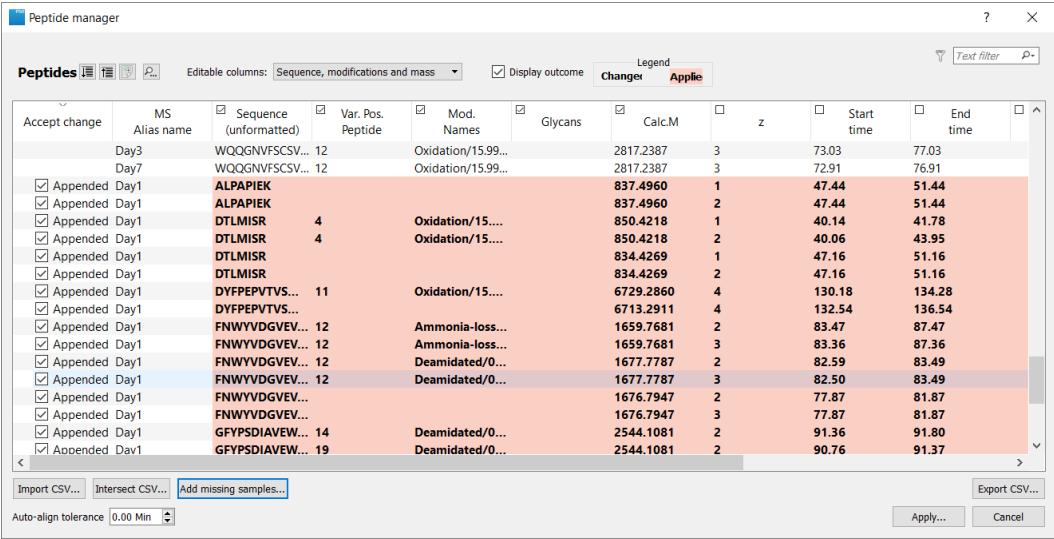


Accept change	MS	Alias name	Sequence (unformatted)	Var. Pos. Peptide	Mod. Names	Glycans	Calc.M	Comment	Labels	Validate	z
✓ Result	AD671_HAGW6...	AADLK					516.2907			Unassigned	1
✓ New	N/A	AADLK					516.2907			Unassigned	1
✓ Old	AD671_HAGW6...	UNKNOWN_215 [516.290A@6.88MIN]					516.2907			Unassigned	1
✓ Result	AD671_HAGW6...	AADLK					516.2907			Unassigned	2
✓ New	N/A	AADLK					516.2907			Unassigned	2
✓ Old	AD671_HAGW6...	UNKNOWN_215 [516.290A@6.88MIN]					516.2907			Unassigned	2
✓ Result	AD671_HAGW6...	AADLKSTQNAIDEITNKVNSVIEK					2600.3657			Unassigned	3
✓ New	N/A	AADLKSTQNAIDEITNKVNSVIEK					2600.3657		CsvLabel	Unassigned	3
✓ Old	AD671_HAGW6...	UNKNOWN_1999 [2600.365A@34.65MIN]					2600.3640			Unassigned	3
✓ Result	AD671_HAGW6...	AADLKSTQNAIDEITNKVNSVIEK					2600.3657			Unassigned	4
✓ Result	AD671_HAGW6...	AIDEITNKVNSVIEK					902.4709			Unassigned	2
✓ Result	AD671_HAGW6...	AIDEITNKVNSVIEK					1671.9043			Unassigned	3
✓ Result	AD671_HAGW6...	CDNTCMESVK		6	Dethiomethyl...		1194.4645			Unassigned	3
✓ Result	AD671_HAGW6...	CDNTCMESVK		6	Carbamidomet...		1299.4893			Unassigned	2
✓ Result	AD671_HAGW6...	CDNTCMESVK		6	Carbamidomet...		1299.4893			Unassigned	2
✓ Result	AD671_HAGW6...	CDNTCMESVK		6	Oxidation/15...		1258.4628			Unassigned	2
✓ Result	AD671_HAGW6...	CDNTCMESVK		1,6	Ammonia-loss...		1282.4628			Unassigned	2
✓ Result	AD671_HAGW6...	CDNTCMESVK					1242.4679			Unassigned	1
✓ Result	AD671_HAGW6...	CDNTCMESVK					1242.4679			Unassigned	2
✓ Result	AD671_HAGW6...	CDNTCMESVK					1242.4679			Unassigned	3
✓ Result	AD671_HAGW6...	CDNTCMESVK		4	Cation/Na/21.9...		1264.4498			Unassigned	2
✓ Result	AD671_HAGW6...	CDNTCMESVK		1	Ammonia-loss...		1225.4413			Unassigned	1
✓ Result	AD671_HAGW6...	CDNTCMESVK		1	Ammonia-loss...		1225.4413			Unassigned	2
✓ Result	AD671_HAGW6...	CDNTCMESVK		1,7	Ammonia-loss...		1247.4233			Unassigned	2
✓ Result	AD671_HAGW6...	CDNTCMESVK		1,4	Ammonia-loss...		1263.3972			Unassigned	3

Figure 76: Imported peptides, expanded

- **Add missing samples** is the same as in **Edit > In-Silico > Add missing**.

Click the button and then Apply to update the project with new in-silico peptides. These will be visible at the end of the list.



Accept change	MS	Alias name	Sequence (unformatted)	Var. Pos. Peptide	Mod. Names	Glycans	Calc.M	z	Start time	End time
	Day3		WQGGNVFSCSV...	12	Oxidation/15.99...		2817.2387	3	73.03	77.03
	Day7		WQGGNVFSCSV...	12	Oxidation/15.99...		2817.2387	3	72.91	76.91
✓ Appended	Day1		ALPAPIEK				837.4960	1	47.44	51.44
✓ Appended	Day1		ALPAPIEK				837.4960	2	47.44	51.44
✓ Appended	Day1		DTLMISR	4	Oxidation/15....		850.4218	1	40.14	41.78
✓ Appended	Day1		DTLMISR	4	Oxidation/15....		850.4218	2	40.06	43.95
✓ Appended	Day1		DTLMISR				834.4269	1	47.16	51.16
✓ Appended	Day1		DTLMISR				834.4269	2	47.16	51.16
✓ Appended	Day1		DYFPEPVTVS...	11	Oxidation/15....		6729.2860	4	130.18	134.28
✓ Appended	Day1		DYFPEPVTVS...				6713.2911	4	132.54	136.54
✓ Appended	Day1		FNWYVDGVEV...	12	Ammonia-loss...		1659.7681	2	83.47	87.47
✓ Appended	Day1		FNWYVDGVEV...	12	Ammonia-loss...		1659.7681	3	83.36	87.36
✓ Appended	Day1		FNWYVDGVEV...	12	Deamidated/0...		1677.7787	2	82.59	83.49
✓ Appended	Day1		FNWYVDGVEV...	12	Deamidated/0...		1677.7787	3	82.50	83.49
✓ Appended	Day1		FNWYVDGVEV...				1676.7947	2	77.87	81.87
✓ Appended	Day1		FNWYVDGVEV...				1676.7947	3	77.87	81.87
✓ Appended	Day1		GFYPSDIAVEW...	14	Deamidated/0...		2544.1081	2	91.36	91.80
✓ Appended	Day1		GFYPSDIAVEW...	19	Deamidated/0...		2544.1081	2	90.76	91.37

Figure 77: Peptide manager after using Add missing samples

Click **Apply** to update the project. The figure below displays a common use case: Feature Finder unknowns matched with in-silico content.



Peptides													Text filter
Row#	PID	Sequence	Mod. Summary	Mod. AAs	z	XIC Start	XIC End	Quant level	Score	Score Rank	In silico	Cor	
22	22	.SQLKNNAK.			1	3.55	4.07	1		1	Yes		
23	23	.SQLKNNAK.			2	3.55	4.07	1		1	Yes		
24	24	.SQLKNNAK.			3	3.55	4.07	1		1	Yes		
25	25	.SQLKNNAK.	N5(Ammonia-loss/-17.0265)	N	3	3.58	3.75	1		1	Yes		
26	26	.QIKNNNAK.	Q1(Gln->pyro-Glu/-17.0265)	Q	2	3.61	3.69	1		1	Yes		
27	27	.UNKNOWN_75_1686.41DA@3.64M			2	3.59	3.75	1		1	Yes		
28	28	.QLKNNAK.			2	3.60	3.71	1		1	Yes		
29	30	.UNKNOWN_77_1900.98DA@3.64M			3	3.62	3.67	1		1	Yes		
30	31	.MNNQRL	N2(Deamidated/0.9840)	N	1	3.64	3.75	1		1	Yes		
31	32	.MNNQRL	N2(Deamidated/0.9840)	N	2	3.64	3.75	1		1	Yes		
32	33	.UNKNOWN_80_1901.91DA@3.67M			3	3.65	3.69	1		1	Yes		
33	34	.UNKNOWN_81_1939.44DA@3.68M			2	3.65	3.71	1		1	Yes		

Figure 78: Peptides added with Add missing samples

- The **Audit Trail** documents all change made through the Peptide Manager, including
  - The CSV intersected
  - The columns altered
  - How many peptides were affected


Audit Trail 			?	×
Time	User	Operation		
3/16/2020 7:59 PM	ProteinMetrics	Set Project 'Batch peptide editing using peptide manager' to [CSVFiles=("/F:/data_results/Archive/PeptideManagerCanonical/PeptideManagerCanonical.csv")]		
3/16/2020 7:59 PM	ProteinMetrics	Set Project 'Modified existing peptides' to [Columns=("/Sequence and modifications", "Uncharged mass"), Count=472]		
1/22/2020 1:54 PM	ProteinMetrics	Event Project 'EndProjectCreation'		
1/22/2020 1:39 PM	ProteinMetrics	Event Project 'StartProjectCreation'		

Figure 79: Audit Trail after using Peptide manager

**Feature finder** opens a dialog which includes the settings available in the **Feature Finder** tab during project creation:

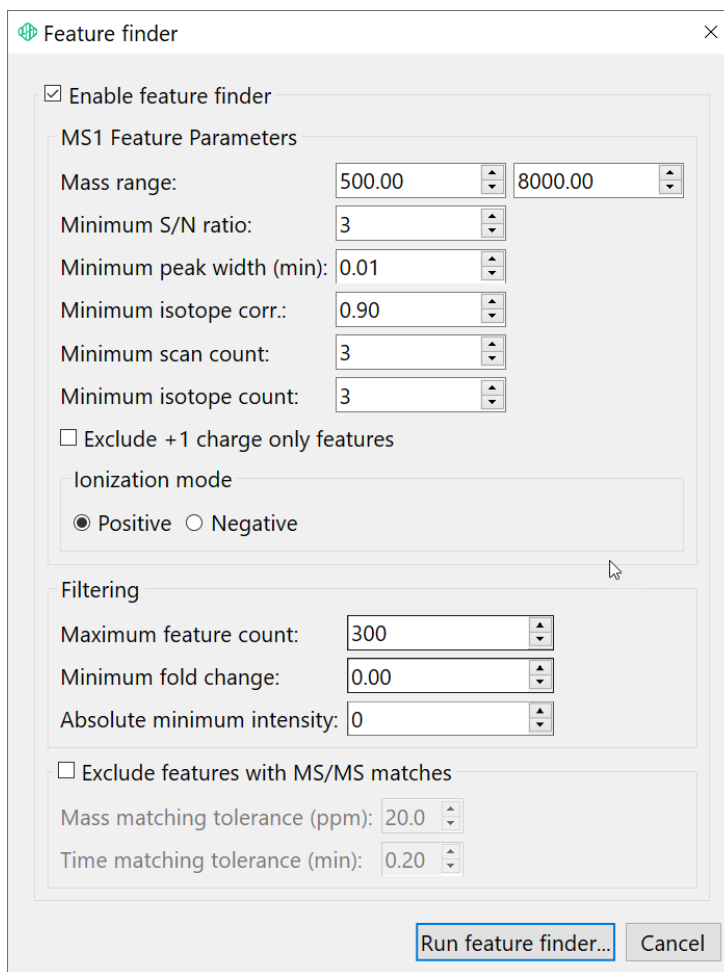


Figure 80: Feature finder dialog

**Add MS File(s)** imports additional MS files after project creation. The MS files have extensions \*.d, \*.raw, \*.wiff, \*.lcd, and \*.byspec2. Navigate to the desired file and click **Open**.

**Enable Deamidation Quantification** recalculates XIC integrations to account for the interference between a deamidated peptide and a wildtype peptide with a similar isotope value. The wildtype integration is subtracted from the total to yield a “pure deamidated” XIC plot.

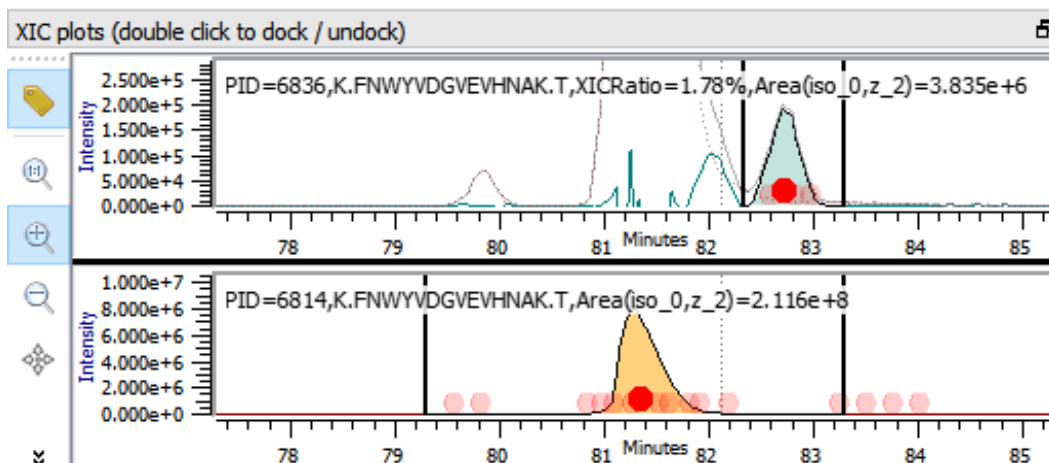
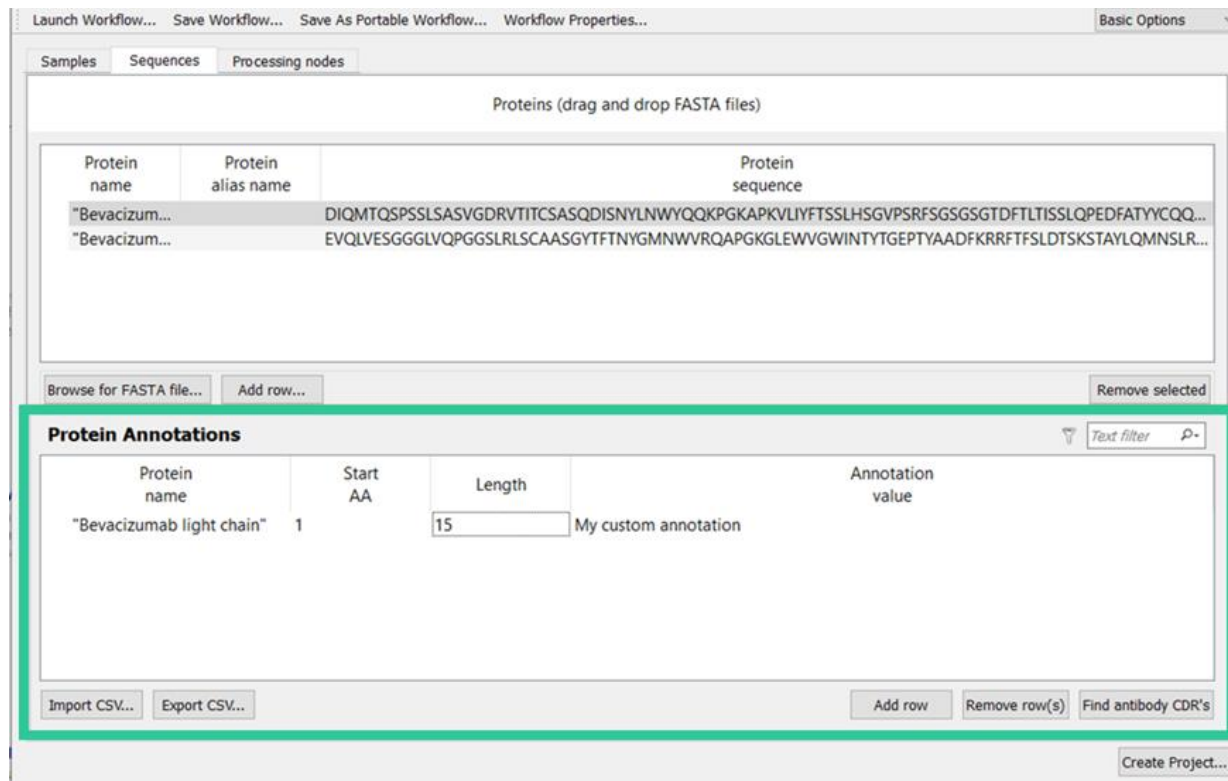


Figure 81: Deamidation quantification subtracts the wildtype integration from the total

**Protein Annotations** is used to annotate individual proteins for display in the **Peptides** table:



Launch Workflow... Save Workflow... Save As Portable Workflow... Workflow Properties... Basic Options

Samples Sequences Processing nodes

Proteins (drag and drop FASTA files)

Protein name	Protein alias name	Protein sequence
"Bevacizumab light chain"		DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFGSGSGTDFLTITSLQPEDFATYYCQQ...
"Bevacizumab heavy chain"		EVQLVESGGGLVQPGGSLRLSCAASGYTFITNYGMNWVRQAPGKLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLR...

Browse for FASTA file... Add row... Remove selected

**Protein Annotations** Text filter

Protein name	Start AA	Length	Annotation value
"Bevacizumab light chain"	1	15	My custom annotation

Import CSV... Export CSV... Add row Remove row(s) Find antibody CDR's

Create Project...

Figure 82: Protein Annotations

To annotate a protein, select it in the Proteins window, click **Add row**, specify the starting amino acid and sequence length, and then enter text under **Annotation value** in the Protein Annotations window. The **Find antibody CDR's** button finds antibody complementarity determining regions and displays them in the Protein Annotation column of the **Peptides** table. Click **Close** when finished.

**Show Byos project creation options** displays the workflow project creation options used to generate the project.

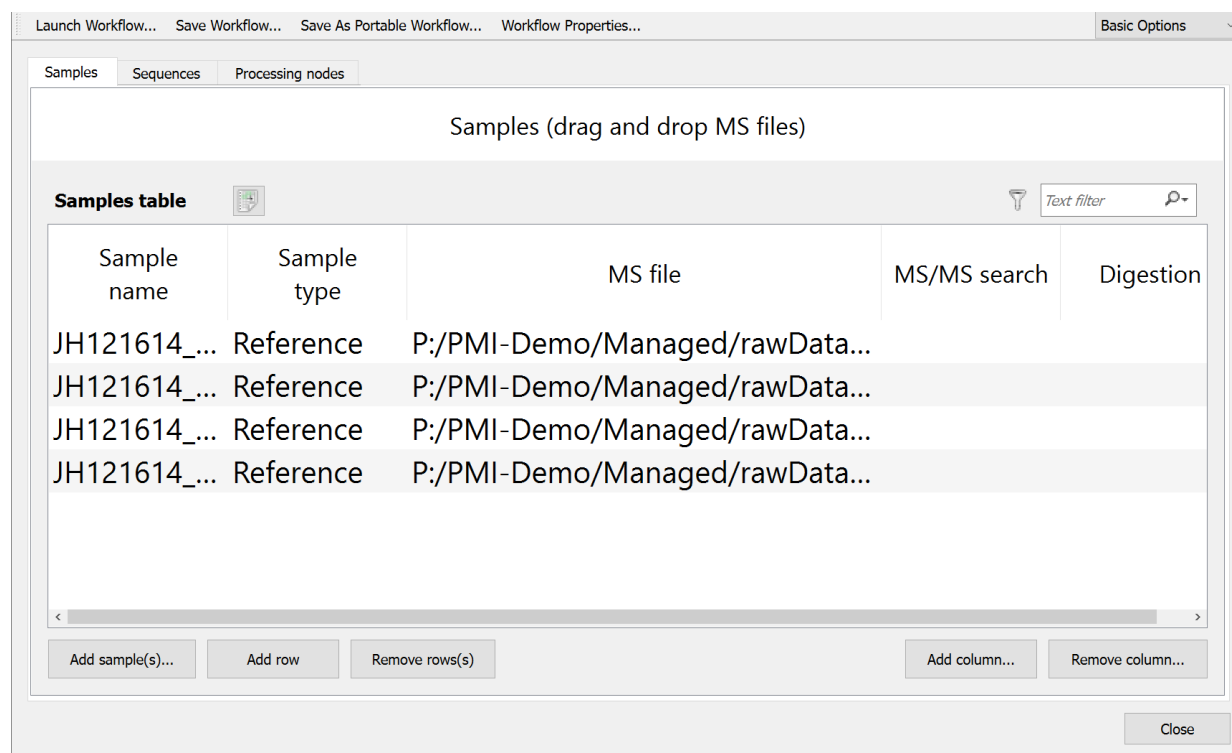


Figure 83: Show Byos project creation options

The **Sequences** and **Processing** nodes tabs open to show all the entries used in those tabs.

**Show project creation options** is obsolete and can be ignored.

The **Advanced configuration** dialog is used to enter text commands, which are often new features still in beta testing. A list of available advanced commands can be found [here](#).

**Adjust colors** is used to modify the default colors associated with MS samples and corresponding plots.

**Adjust MS paths** is used to modify the path to the source MS file, should the file be moved. Enter or navigate to the new directory containing the MS file.

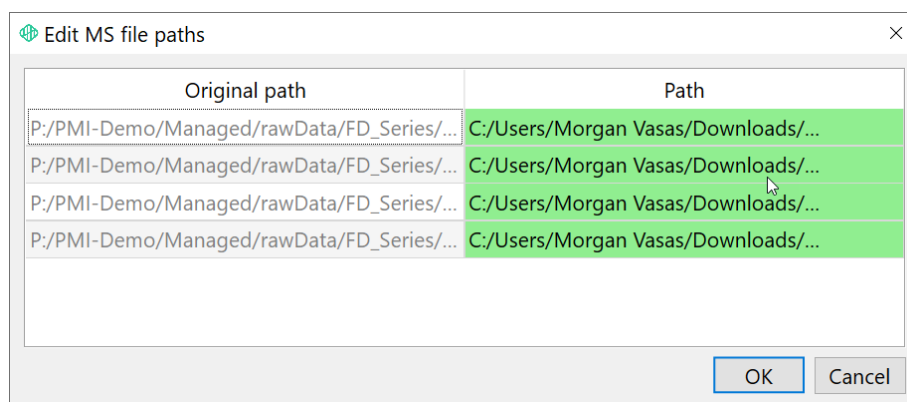


Figure 84: Editing the MS file path

**Edit PDB** loads protein database files for use by Protein 3D reports.

## Window Menu

The **Window** pull-down menu manages the visual layout of the user interface, and the arrangement and sizes of the various table and plot views:

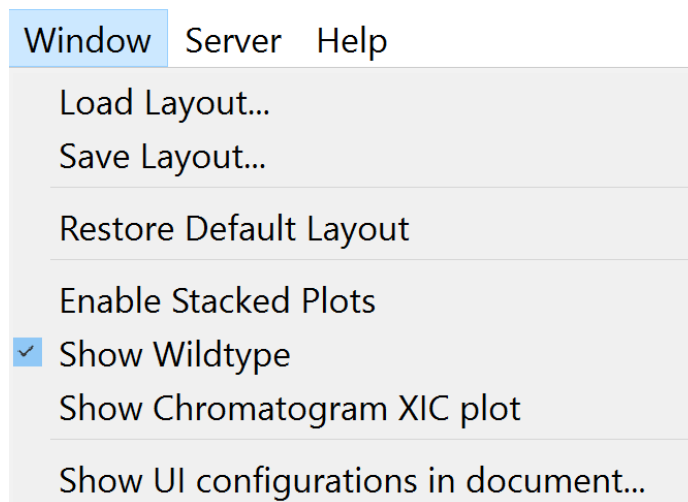
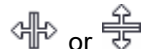


Figure 85: Window menu

**Load Layout** opens a saved layout stored to files with extension \*.ini. **Save Layout** saves the current layout to an \*.ini file, which can be used in other projects or shared with other users. **Restore Default Layout** applies the layout used when the application is first opened.

The current layout can be customized by moving, resizing, or editing the view panes. Many of the views (Project, Protein coverage, XIC, MS2 and mass error, and Isotope plots) can be undocked by double-clicking on the tops of that view to create standalone windows that can be moved to another monitor. These views can be click-dragged to new locations in the layout. The view can also be resized by hovering over the vertical or horizontal edges between table and plot views so that the cursor changes to arrows:



. Left click and drag the edges up or down or right or left to change the sizes of the views. Columns in the table views can be made larger or smaller in same manner. To reorder columns in a table, left click the name of the column, and drag it to the new location. To do a simple sort, left click the header once to sort ascending and twice to sort descending. Mouse over a column header to see a description of that field.

Column headers can be modified through the **Column Header Editor** dialog, opened by a right-click anywhere on a table column header.

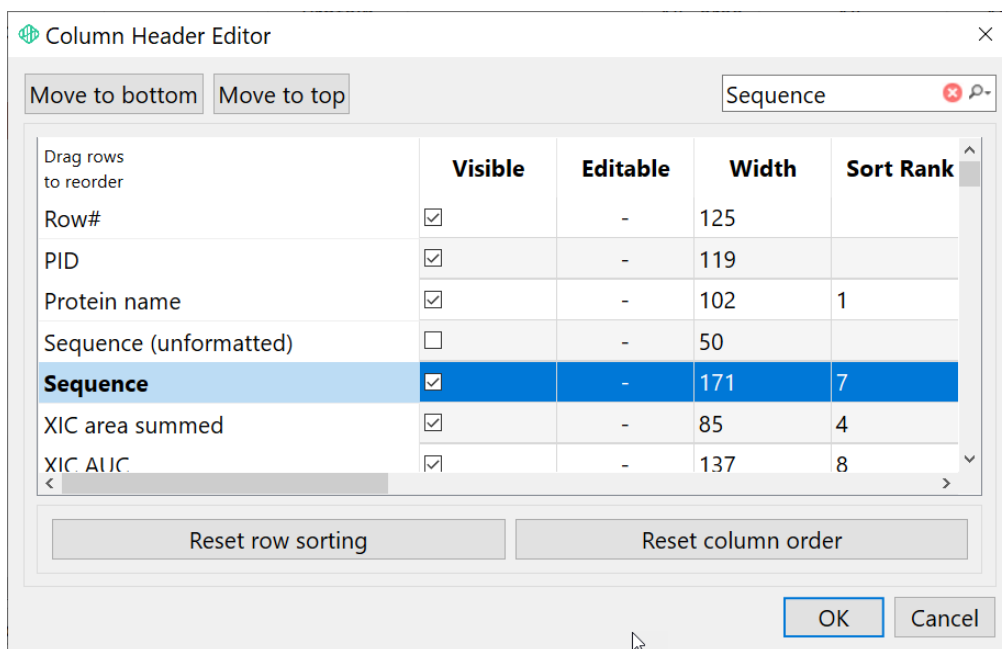


Figure 86: Column Header Editor

Columns can be rearranged by dragging the header names up or down. To hide a column, uncheck the **Visible** box for that column. This dialog can also be used to specify number of significant figures and ranked sorting. The **Window > Save Layout** menu preserves these edits for future use and sharing. The **Reset row sorting** and **Reset column order** buttons restore the default row and column properties.

The **Search Box** can be used to search for specific values or variables, with options to filter for only whole values, with case-sensitivity, and to only search in vertical header columns.

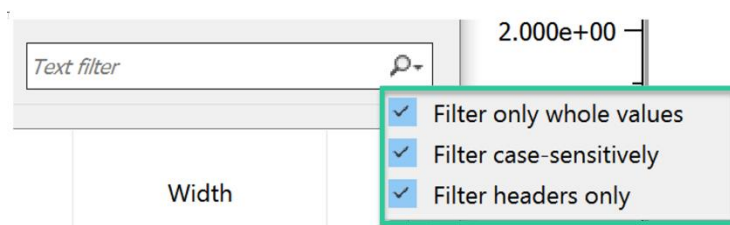


Figure 87: Search box filters

A selected row can be moved to the top or the bottom by clicking **Move to bottom** or **Move to top**; alternatively, the same actions can be performed by pressing Alt + t or Alt + b, respectively.

**Enable Stacked Plots** can be turned on to separate the plot view into a series of normalized traces of all the sample data:

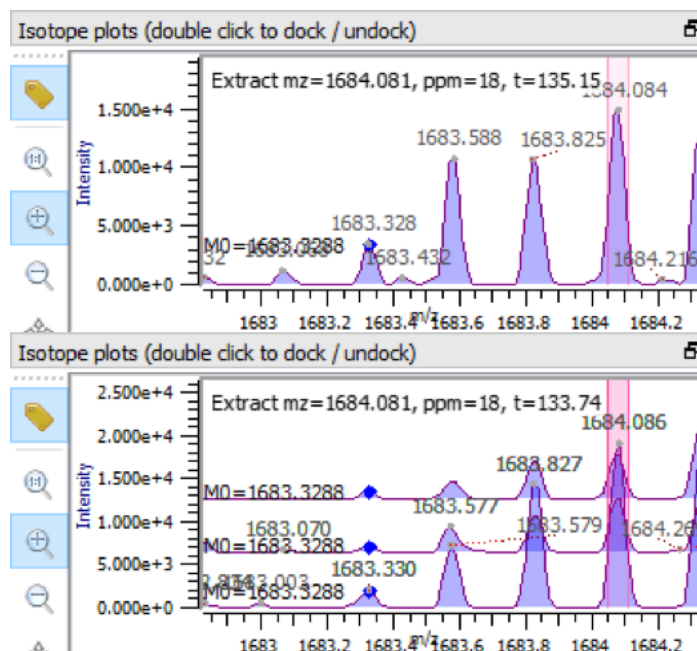


Figure 88: Example of stacked isotope plots

Turning on **Enable Stacked Plots - Overlay Charge States** functions the same as Window > Enable Stacked Plots, except for in the XIC plot widget where XICs with different charge states will be overlaid within the same sample. Users must ensure that charge states are combined prior to selecting this option.

**Hide Wildtype** removes the wildtype peptide records in the **Wildtype peptides** table and the corresponding wildtype peptide plots in the **XIC plots**, **MS2** and **mass error plots** and **Isotope plots**:

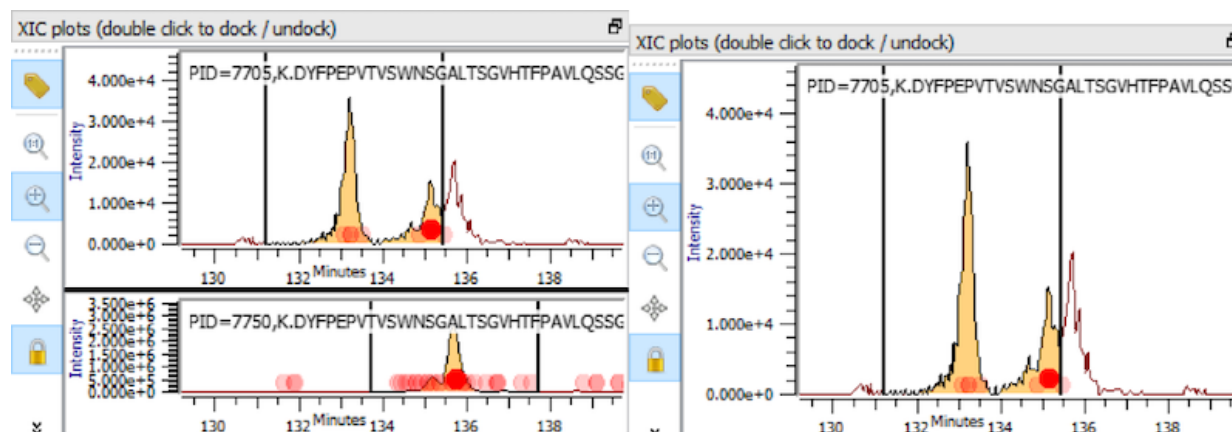


Figure 89: Example of hidden wildtype plot

The **Show Wildtype** menu displays the **Wildtype peptide** table records and wildtype plots again.

**Show Chromatogram XIC Plot** opens an undocked window displaying chromatograms for the projects. The traces are displayed in the colors of the samples they represent:



Figure 90: Undocked Chromatogram XIC plot

## Server Menu

Currently **Server > Configure** is supported for the Waters UNIFI server. See the **PMI UNIFI Integration User Guide** for instructions on how to configure UNIFI integration.

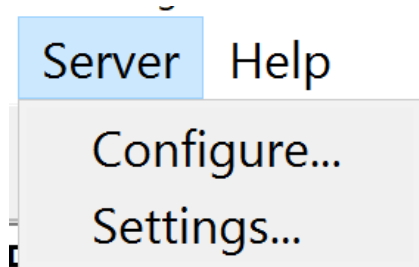


Figure 91: Server menu

## Help Menu

The **Help** pull-down menu provides information about the software:

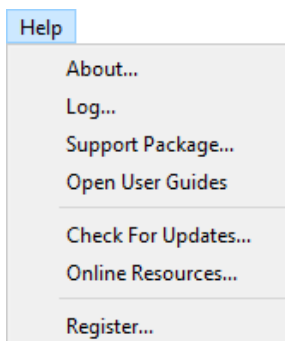






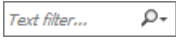

Figure 92: Help menu



The **About** menu shows the software version number, which is needed when reporting issues. The **Log** menu opens a log containing recent activity; this is helpful for troubleshooting problems together with Protein Metrics, Inc. staff via [support@proteinmetrics.com](mailto:support@proteinmetrics.com). **Support Package** opens a dialog to generate a comprehensive set of files to better enable Protein Metrics to troubleshoot problems. Click **Review & edit the package** to generate a directory of individual files as directed. Click **Compress** to generate a zip file of this support directory. An information box indicates where the zip file can be found and gives a link for [support@proteinmetrics.com](mailto:support@proteinmetrics.com). **Open User Guides** opens the installed folder with the manuals and quick start guides for all Byos workflows. **Check For Updates** will report if an online update is available for installation. **Online Resources** opens the Protein Metrics, Inc. Secure Resources webpage containing a variety of technical pages and tutorials related to Protein Metrics, Inc. software. **Register** is used to activate the software upon first use.

## Table Menus

The menu bars at the top of the three table views (Project, Peaks, and Candidates) share icons that manage hierarchical lists and filter on data.

- The  icon expands rows to show “sub-rows”.
- The  icon collapses rows to hide sub-rows. The use of sub-row depends on the table. For example, in the **Peptides** table, a row is a peptide record and a sub-row is the data resulting from an individual sample that contained the peptide.
- The  icon exports the table to a \*.csv file for opening with Excel.
- The  icon opens a dialog to create custom filters for that data table. (A single column filter can also be set by right-clicking on an entry in the column.) Select a column in the first cell, select an operator in the second cell, and enter text in the third cell. A second filter row becomes available for further entry. Click the red X after a filter row to delete it. Custom filters allow masses to be filtered by mass range, annotation, intensity, and so forth. Custom filters can be stored with the project document or exported and imported using the **Presets** dropdown.
- The  cell filters the entered text across the content of all columns in the protein and peptide tables. The records are filtered automatically as text is entered. Click the  icon to search the string as a whole word or as case sensitive.
- To sort the contents of a column by ascending value, click the column header. Click the column header again to sort by descending value.

## Table Right-Click Menus

The Project, Protein Coverage, Peptides, and Wildtype peptides tables have context menu items revealed by a right-click on the rows within the tables. (Recall that a right-click on the header of these tables opens the Column Header Editor dialog.)

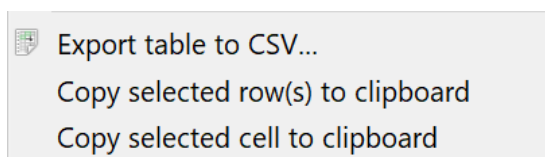


Figure 93: Table right-click menus

- **Export table to CSV** saves the table content and headers to a \*.csv file using parameters chosen in the **Export data** dialog.

- **Copy selected row(s) to clipboard** copies selected table rows, with their headers to be available for pasting into another application. (This menu is not available in the Protein Coverage table.)
- **Copy selected cell to clipboard** allows the user to copy the contents of the selected cell. It works just like CTRL+C.
- Table context menus contain a set of query options for searching the clicked text value using any of the available search operators. This is an easy way to filter a table based on a specific field value.

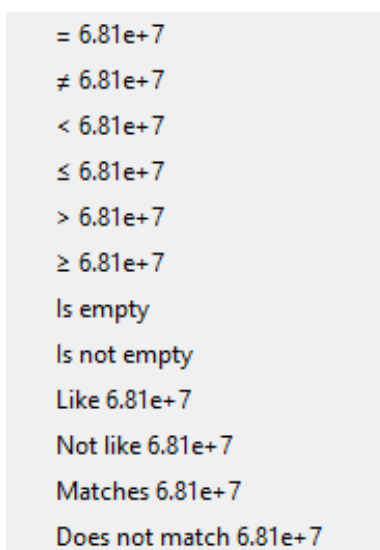


Figure 94: Search table values through a right-click menu

## Project Table

The **Project** table contains information about the MS samples and results files:

Projects table (double click to dock / undock)

Project	Alias Name	Name
▼ <input type="checkbox"/>	Day0	P:\PMI-Demo\Managed\rawData\FD_Series\
<input type="checkbox"/>	JH121614_Pr...	P:\PMI-Demo\Managed\rawData\FD_Series\
▼ <input checked="" type="checkbox"/>	Day1	P:\PMI-Demo\Managed\rawData\FD_Series\
<input checked="" type="checkbox"/>	JH121614_Pr...	P:\PMI-Demo\Managed\rawData\FD_Series\

Figure 95: Project table

To display or remove the MS sample and result combination, check or uncheck the box at left of either a sample or result record. To edit a MS sample or result alias name, right-click the entry to edit under the **Alias Name** header, click **Edit selected row(s)**, edit the name, and then click **OK**.

## Protein Coverage Table and Menu

The **Protein Coverage** table displays peptide sequences and corresponding protein coverage. The accompanying display maps the protein sequences of detected peptides to colored lines below each sequence representing colors assigned to different MS samples:

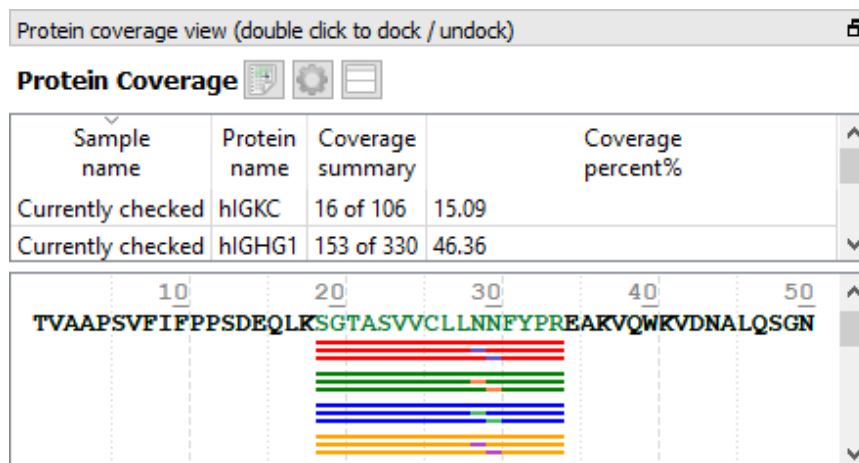



Figure 96: Protein Coverage table

The different colors within the colored bars indicate the presence of peptide modifications at those positions. (The colors used are chosen for contrast and do not reflect the colors assigned to the MS sample files.)

The Protein Coverage table also includes the following specialized menu icon buttons:

- The  icon opens the Protein coverage rendering options dialog:

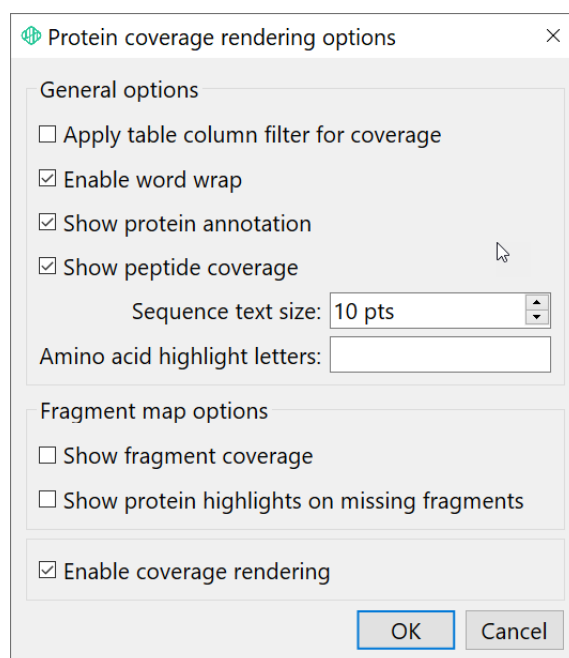


Figure 97: Protein coverage rendering options

- The  icon turns on and off the display of the tabular protein coverage data.

The Protein Coverage view has context menu items revealed by a right-click on the graphical portion of the display:

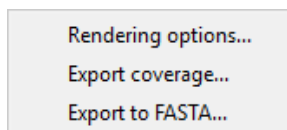
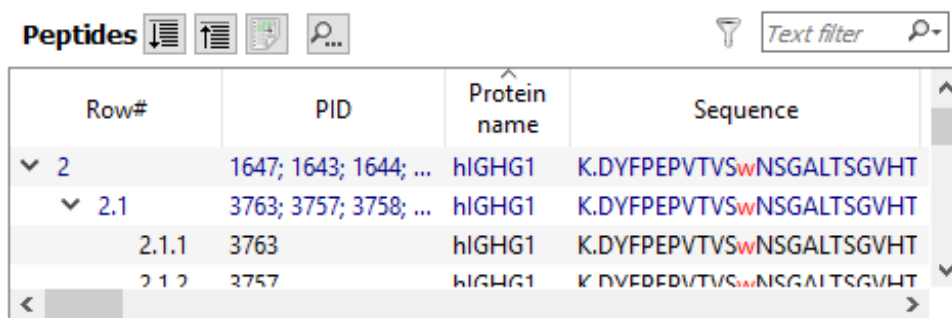


Figure 98: Protein Coverage right-click menu

- **Rendering options** also opens the Protein coverage rendering options dialog shown above.
- **Export coverage** creates a \*.png file of the graphical protein coverage display.
- **Export to FASTA** creates a \*.fasta file of the proteins found in the Protein Coverage view. The \*.fasta file is then available to be imported into new projects.

## Peptides Table and Menu

The **Peptides** table contains detailed information about all identified peptides:




Row#	PID	Protein name	Sequence
2	1647; 1643; 1644; ...	HIGHG1	K.DYFPEPVTVS <sup>sw</sup> NSGALTSGVHT
2.1	3763; 3757; 3758; ...	HIGHG1	K.DYFPEPVTVS <sup>sw</sup> NSGALTSGVHT
2.1.1	3763	HIGHG1	K.DYFPEPVTVS <sup>sw</sup> NSGALTSGVHT
2.1.2	3757	HIGHG1	K.DYFPEPVTVS <sup>sw</sup> NSGALTSGVHT

Figure 99: Peptides table

The **Peptide** table organizes records in the following hierarchy: Peptide > Sample > Charge state (if combined) > MS2 scan. Records in the **Peptide** table can be modified directly by the user for three fields: Validate, Comment, and Labels. In the Validate field, the user selects between “True-positive”, “False-positive”, and “Uncertain”. Comments are input directly. Values for Labels are selected from a list. To create new labels to use in the Labels field, see **Edit > Label Manager**.

Note: The MS1 correlation score compares the measured MS1 isotope distribution to the theoretical distribution calculated using cosine similarity. A score of 1 is a perfect match.

In addition to the usual table menu buttons, the **Peptides** table contains the  icon button which opens the same Search Filter dialog that is opened by the **Edit > Search Filter** menu. The *text filter* entry box allows the records to be filtered based on the occurrence of the given text string searched across all fields.

The **Peptides** table has context menu items applicable to specific peptides. The menu is revealed by a right-click on a peptide cell in the table:

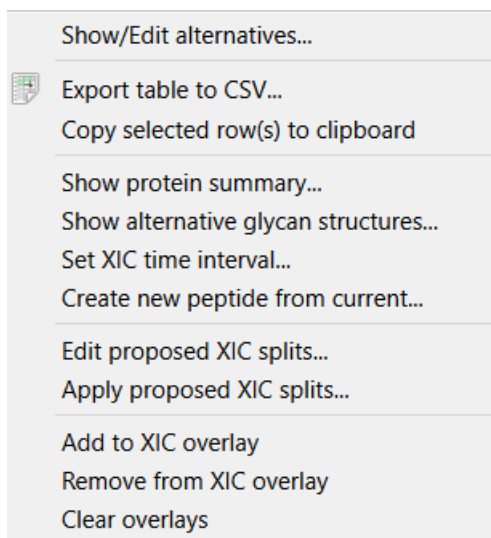
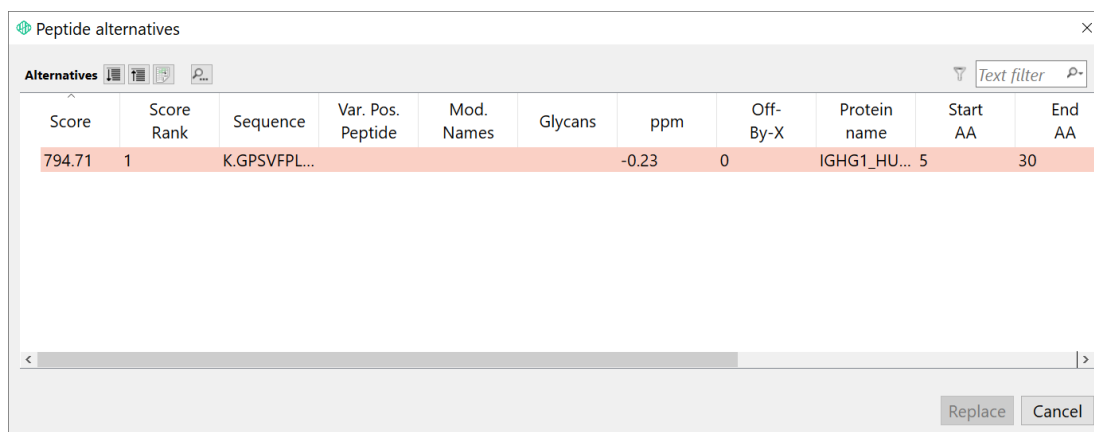


Figure 100: Peptides table right-click menus

- **Show/Edit alternatives** displays a table of alternative MS2 identifications (with different sequences and/or modifications) for the selected peptide, along with their scores:



Peptide alternatives

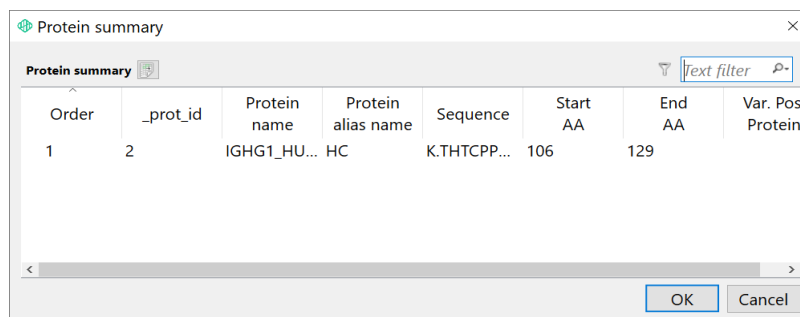
Score	Score Rank	Sequence	Var. Pos. Peptide	Mod. Names	Glycans	ppm	Off-By-X	Protein name	Start AA	End AA
794.71	1	K.GPSVFPL...				-0.23	0	IGHG1_HU...	5	30

Buttons: Replace, Cancel

Figure 101: Peptide alternatives table

Select an alternative peptide and click **Replace** to replace the selected peptide in the **Peptides** table. Protein coverage, observed and calculated masses and  $m/z$  ratios all are updated.

- **Show protein summary** opens a table with protein data for that peptide:



Protein summary

Order	_prot_id	Protein name	Protein alias name	Sequence	Start AA	End AA	Var. Pos Protein
1	2	IGHG1_HU...	HC	K.THTCPP...	106	129	




Buttons: OK, Cancel

Figure 102: Protein summary

The values in the Protein summary can be captured in a report using the template

**Blgc\_PRM\_wProteinDuplicates.rptc.**


- **Show alternative glycan structures...** allows the user to access alternative glycan structures rendered for glycopeptide modifications in generated reports featuring glycan cartoons. The table provides associated structures currently in the backend library. Users can also select **Add new structure from IUPAC...** to enter a new glycan structure to add to the library. See the **PMI Chromatogram Analysis Manual** for more information about building alternative glycan structures from IUPAC.


**Peptides**   


Row#	MS Alias name	Sequence	Mod.	Glycans
> 1	Day 0; Day 1; Day 3; Day 7	R.EEQYnSTYR.V		HexNAc(4)Hex(3)Fuc(1)
> 2	Day 0; Day 1; Day 3; Day 7	R.EEQYnSTYR.V		HexNAc(4)Hex(4)Fuc(1)
> 3	Day 0; Day 3; Day 7	R.EEQYnSTYR.V		HexNAc(2)Hex(5)
> 4	Day 0; Day 1; Day 3; Day 7	R.EEQYnSTYR.V		HexNAc(3)Hex(3)Fuc(1)
> 5	Day 0; Day 3; Day 7	R.EEQYnSTYR.V		HexNAc(5)Hex(4)Fuc(1)
> 6	Day 0; Day 1; Day 3; Day 7	R.EEQYnSTYR.V		HexNAc(3)Hex(4)Fuc(1)
> 7	Day 0; Day 1; Day 3; Day 7	R.EEQYnSTYR.V		HexNAc(4)Hex(5)Fuc(1)

Show/Edit alternatives...  
 Export table to CSV...  
 Copy selected row(s) to clipboard  
 Show protein summary...  
**Show alternative glycan structures...**  
 Set XIC time interval...  
 Create new peptide from current...

Figure 103: Show alternative glycan structures

 **Glycan Structure Selection** ✕

**Table** 

Selected	Composition	IUPAC	Glycan structure
<input checked="" type="radio"/>	HexNAc(3)...	GlcNAc(M)...	



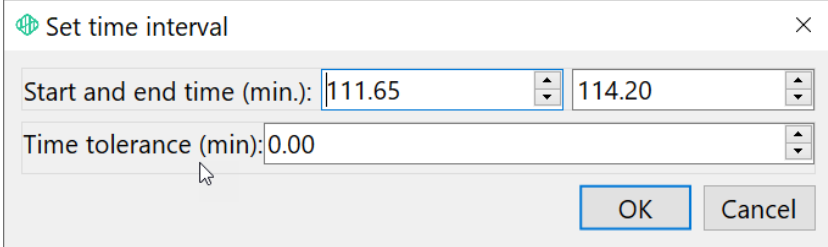
  

Figure 103: Glycan Structure Selection

- **Set XIC time interval** allows the user to edit the XIC start and end times for that peptide and to set the time tolerance (time warp) to improve performance:



Set time interval

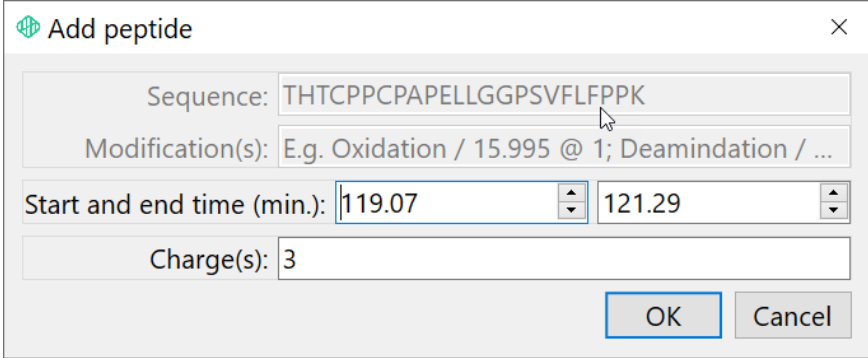
Start and end time (min.): 111.65 114.20

Time tolerance (min): 0.00

OK Cancel

Figure 104: Set XIC time interval

- **Create new peptide from current** makes a copy of that peptide. This allows XIC plots to be split according to separate integrations. The user edits the start and end times and the charge of the peptide before the copy is created:



Add peptide

Sequence: THTCPPCPAPELLGGPSVFLFPPK

Modification(s): E.g. Oxidation / 15.995 @ 1; Deamidation / ...

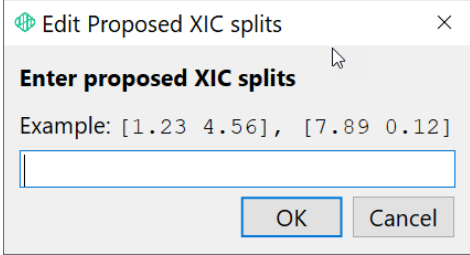
Start and end time (min.): 119.07 121.29

Charge(s): 3

OK Cancel

Figure 105: Create new peptide from current

- **Edit proposed XIC splits** opens a dialog to enter pairs of time ranges to split XIC plots for that peptide record into two or more chromatograms:



Edit Proposed XIC splits

**Enter proposed XIC splits**

Example: [1.23 4.56], [7.89 0.12]

OK Cancel

Figure 106: Edit proposed XIC splits

After a click on the **OK** button, the **Peptides** table record field **XIC proposals** is updated with the time range entries. The split must be applied to take effect.

- **Apply proposed XIC splits** separates a record in the **Peptides** table into two or more records using the time ranges entered using the Edit proposed XIC splits function.
- **Add to XIC overlay** adds the selected **Peptides** table record (subject to the right-click) or collection of records as an overlay to the XIC plots view mapped to the same time axis:

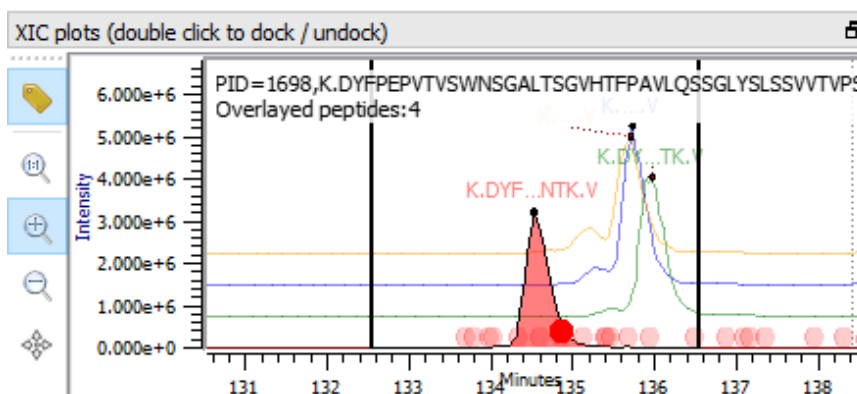





Figure 107: Add to XIC overlay

When this function is applied to a collection of records in the table, all of the records below it are added to the XIC plots view.

- **Remove from XIC overlay** removes the overlay from the selected Peptides table record (subject to the right-click) from the XIC plots view.
- **Clear overlays** removes all sample overlays from the XIC plots view.

## Wildtype Peptides Table and Menu

The **Wildtype peptides** table contains detailed information about naturally occurring or wildtype peptides associated with the peptide selected in the Peptides table:

**Wildtype peptides**   

Row#	PID	Sequence	Start AA	E
> 1	8176; 813; 9050...	K.FNWWYVDGVEVHNAK.T	158	17
> 2	822; 783; 797; 79...	K.FNWWYVDGVEVHNAK.T	158	17
> 3	828; 798; 807; 81...	K.FNWWYVDGVEVHNAK.T	158	17
> 4	8177; 8614; 9051...	K.FNWWYVDGVEVHNAK.T	158	17

Figure 108: Wildtype Peptides table

The Wildtype peptides table has one context menu item specific to wildtype peptides. The menu is revealed by a right-click on a wildtype peptide cell in the table. **Set primary wildtype** marks that record as the primary wildtype peptide for the selected record in the Peptides table.

## Validators

Additional validation is an essential step of degradant and sequence variants analysis. The SVA report, provided as a preset and used as the default report in the SVA workflow, contains a column for auto-validation called "SV auto-comment." These are now also available in the inspection view. The following two sets of advanced commands enable the user to complete validation through comments automatically populated in the **Peptides** table, as shown below.



Peptides 選 匯 圖

Row#	PID	Protein name	Sequence	XIC area summed	XIC AUC	XIC Ratio%	ex Tin (Posit)	MS alias nam	Mod. Summary	Mod AAs	z	Validate	Comment	ab
40	970	NIS1mAb ...	K.DYFPEPVTWNSG	3.36e+6	3.36e+6		51.84	S			5			
41	902	NIS1mAb ...	K.DYFPEPVTWNSG	2.4e+6	2.4e+6		49.74	S			6			
> 42	456; 423; 43...	NIS1mAb ...	K.DTLMSRLT	1.65e+9	1.65e+9		32.52	S			2			
> 43	375; 324; 33...	NIS1mAb ...	K.DTLMSRLT	5.47e+8	5.47e+8	24.902	28.55	S	M4(Oxidatio...	M	2			
44	1138	NIS1mAb ...	K.DTLMSRLT	8.85e+6	8.85e+6		56.45	S			5			
> 45	614; 551; 56...	NIS1mAb ...	R.TPEVTCVVDVSHEI	9.96e+8	9.96e+8		38.87	S			3			
> 46	552; 564; 577	NIS1mAb ...	R.TPEVTCVVDVSHEI	9.83e+7	9.83e+7		38.87	S			2			
47	556	NIS1mAb ...	R.TPEVTCVVDVSHEI	7.32e+6	7.32e+6		38.91	S			4			
> 48	1181; 969; 1...	NIS1mAb ...	R.TPEVTCVVDVSHEI	6.14e+7	6.14e+7		55.30	S			5			
> 49	724; 517; 52...	NIS1mAb ...	K.FNWWYVDGVEVHNA	5.06e+9	5.06e+9		37.17	S			3			
> 50	531; 516; 52...	NIS1mAb ...	K.FNWWYVDGVEVHNA	1.18e+9	1.18e+9		37.17	S			2			
> 51	518; 523	NIS1mAb ...	K.FNWWYVDGVEVHNA	1.6e+7	1.6e+7		37.17	S			4			
52	623	NIS1mAb ...	K.FNWWYVDGVEVHNA	7.49e+6	7.49e+6	0.148	40.89	S	N12(Deamid...	N	3		Clear: No Flags	
53	560	NIS1mAb ...	K.FNWWYVDGVEVHNA	1.45e+6	1.45e+6	0.122	39.02	S	N2(Deamid...	N	2		Warning: Intensity < intensityRecoveryPercentileThre...	
54	502	NIS1mAb ...	K.FNWWYVDGVEVHNA	3.29e+6	3.29e+6	0.065	36.11	S	W3(Oxidatio...	W	3		Warning: Intensity < intensityRecoveryPercentileThre...	
55	326	NIS1mAb ...	K.FNWWYVDGVEVHNA	2.53e+6	2.53e+6		28.50	S	N23(NGlyca...	N	6			
56	7	NIS1mAb ...	K.TKPREEQYNSTYRLV	1.27e+7	1.27e+7		14.91	S			3			
57	8	NIS1mAb ...	K.TKPREEQYNSTYRLV	6.81e+6	6.81e+6		14.91	S			4			
58	39	NIS1mAb ...	K.TKPREEQYNSTYRLV	1.45e+6	1.45e+6	10.233	15.83	S	N9(Deamida...	N	3		Warning: Intensity < intensityRecoveryPercentileThre...	
59	42	NIS1mAb ...	K.TKPREEQYNSTYRLV	5.31e+5	5.31e+5	7.239	15.84	S	N9(Deamida...	N	4		Warning: Score < Threshold	
60	9	NIS1mAb ...	K.TKPREEQYNSTYRLV	7.23e+5	7.23e+5	9.600	14.86	S	N9(NGlycar...	N	4			

Figure 109: Peptide table Comments apply the Mod and SVA Validators

## Mod Validator

Allows a user to assess the validity of a degradation analysis automatically. When this feature is activated, the algorithm will iterate row by row and assess whether the modification meets or fails certain thresholds (as explained below). Please note that this algorithm does not assess sequence variants. The SVA Validator algorithm is specifically designed for sequence variants (detailed directly after this).

The feature can be activated in the Advanced configuration section in Peptide Analysis/Byos with the following advanced commands:

```
[Byologic]
ModValEnabled = true;
```

Threshold parameters that can be modulated are as follows (with default settings):

```
[ModValidatorOptions]
DeltaModScoreThreshold = 10.0;
ScoreThreshold = 225.0;
FwhmThreshold = -1;
IntensityRecoveryPercentileThreshold = 0.05;
```

Mod Validator Option Parameter	Description
DeltaModScoreThreshold	The acceptable threshold of the delta mod score. If a degradation score falls below the set threshold, a warning is added to the Comments field to alert the user.
ScoreThreshold	Same as deltaModScoreThreshold except it will alert if a Score is below the threshold.
FwhmThreshold	The FWHM is used as the minimum retention time difference that categorizes a modification as coeluting with its wildtype. A setting of -1 determines the average FWHM of the input files and then automatically calculates the threshold. To set this manually, use any real number greater than 0. For example, if this is set to 0.05, this

	means that if a degradation has a retention time difference from its wildtype of less than 0.05 minutes, the user will be alerted in the Comments field.
IntensityRecoveryPercentileThreshold	This will alert the user if the apex intensity of the degradation lies within the bottom x percentile. For example, if this is set to 0.05, then whenever the intensity of a peak is in the bottom 5%, a warning will be added to the Comments field.

The Comments field in the Peptide window will be filled out for various rows. Please note that this field sorts with no comments first, so the user may have to scroll to the end of the peptide window to find the labels.

Starting with v. 3.10, the Mod Validator no longer treats pyroGlu ([pyrolization of N-terminal glu/gln](#)) as a sequence variant.

## SVA Validator

Allows a user to assess the validity of sequence variants automatically. When this feature is activated, the algorithm will iterate row by row and assess whether the sequence variant meets or fails certain thresholds as explained below.

[Reference: Zhongqi Zhang, et al, \*Biochemistry\*, 2013, 52, 45, 8165-8176.](#)

The feature can be activated in the Advanced configuration section in Peptide Analysis/Byos with the following advanced commands:

```
[Byologic]
SvaEnabled = true;
```

Threshold parameters that can be modulated are as follows (with default settings):

```
[SvaOptions]
DeltaModScoreThreshold = 10.0;
PercentXICRatioThreshold = 1.0;
ScoreThreshold = 225.0;
FwhmThreshold = -1;
PredictedTimeDiffThreshold = 5.0;
CommonModLookBothWaysFromModResidue = 3
```

SVA Validator Option Parameter	Description
DeltaModScoreThreshold	See Mod Validator above
PercentXICRatioThreshold	The acceptable threshold of the XIC Percentage Ratio. If the ratio falls below the set threshold, a warning is added to the Comments field. Depending on how the *.rptc file is configured, the XIC Ratio % value may not match the quantification in the reports since the reports allow multiple kinds of quantifications (one charge state, multiple charge states, etc.).
ScoreThreshold	See Mod Validator above
FwhmThreshold	The FWHM is used as the minimum retention time difference that categorizes a modification as not co-eluting with its wildtype.

	<p>A setting of -1 determines the average FWHM * 2 of the input files and then automatically calculates the threshold. This is the recommended setting for this parameter (feedback is welcomed).</p> <p>However, this can be manually set to any positive value (this is not recommended).</p>
PredictedTimeDiffThreshold	<p>This threshold alerts the user if the predicted retention time in minutes for the sequence variant exceeds the set threshold. A "Critical" label is displayed in the Comments field.</p>
CommonModLookBothWaysFromModResidue	<p>The count of residues on either side of the putative site of a sequence variance to search across for a more common modification that better explains the results.</p> <p>For example, consider the wildtype peptide AAVLAMK, when this parameter is set to 3.</p> <p>Then, a putative sequence variant of AAdLAMK (V-&gt;D) is proposed with a delta modification of ~16 Daltons.</p> <p>The program searches the 3 amino acid residues to the left and 3 to the right of this site for a more common modification (such as methionine oxidation) with the +16 Da shift.</p> <p>In this case, there is a methionine residue 3 residues to the right of the putative sequence variant, so a "Warning" label is displayed in the Comments field.</p>

Now the Comments field in the Peptide window will be filled out for various rows. Please note that this field sorts with no comments first, so the user may have to scroll to the end of the peptide window to find the labels.

- Filtering – "Var. Pos. Protein" and "Var. Pos. Peptide"

The values in "Var. Pos. Protein" and "Var. Pos. Peptide" columns during filtering are treated as a list of integers. For these columns only the list of integers (integers separated by a comma) is acceptable when entering filter conditions in the dialog.

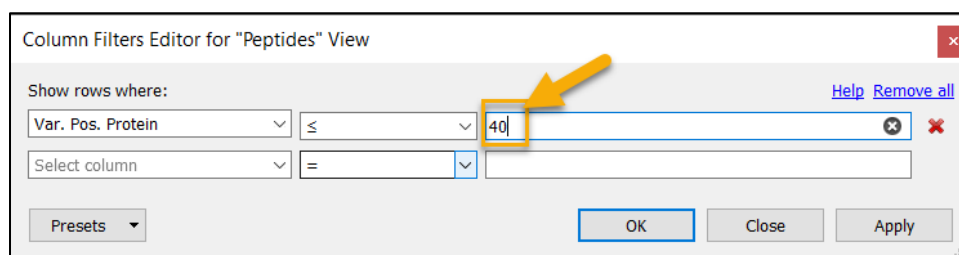


Figure 110: Filter by Var. Pos. Protein and Peptide

If a non-integer value is entered, the user will receive an error message to correct the value entered, as shown below.

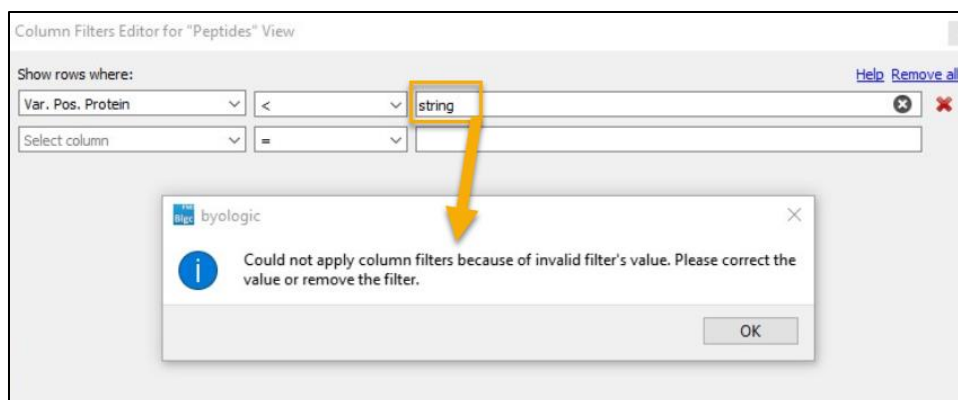


Figure 111: Var. Pos filtering requires integers

## Wildtype Validator

The Wildtype Validator quickly scans wildtype PQMs for scoring violation. To use the Wildtype Validator, enter the following code in Advanced Commands:

```
[Byologic]
WildTypeValEnabled = true;
```

Threshold parameters that can be modulated are as follows (with default settings):

```
[WildTypeValidatorOptions]
ScoreThreshold = 225.0;
IntensityRecoveryPercentileThreshold = 0.05;
PPMStandardDeviations = 3
```

Wildtype Validator Option Parameter	Description
ScoreThreshold	See Mod Validator above
IntensityRecoveryPercentileThreshold	This will alert the user if the apex intensity of the degradation lies within the bottom x percentile. For example, if this is set to 0.05, then whenever the intensity of a peak is in the bottom 5%, a warning will be added to the <b>Comments</b> field.
PPMStandardDeviations	The number of standard deviations (square-root of the variance) in ppm for a result to be considered

## Disulfide Validator

The Disulfide Validator analyzes disulfides similar to how the SVA Validator analyzes sequence variants and the Mod Validator analyzes degradations. Peptide analysis by the Disulfide Validator populates the **Comments** column. The following two sets of advanced commands enable the user to complete validation through comments automatically populated in the **Peptides** table, as shown below:

```
[Byologic]
DisulfideValEnabled=true;
```

Threshold parameters that can be modulated are as follows (with default settings):

```
[DisulfideValidatorOptions]
DeltaModScoreThreshold=10.0;
ScoreThreshold = 150.0;
FwhmThreshold = -1;
MS1Correlation = 0.8;
IntensityRecoveryPercentileThreshold = 0.05;
PPMStandardDeviations = 3
HingeDeltaModScoreThreshold = 10.0;
HingeScoreThreshold = 150.0
```

Disulfide Validator Option Parameter	Description
DeltaModScoreThreshold	See Mod Validator above
ScoreThreshold	See Mod Validator above
FwhmThreshold	The minimum retention time difference that categorizes a modification as not co-eluting with its wildtype. A setting of -1 determines the average FWHM of the input files and then automatically calculates the threshold. To set this manually, use any real number greater than 0. For example, if this is set to 0.05, then a degradation has a retention time difference from its wildtype of more than 0.05 minutes and the user will be alerted in the <b>Comments</b> field.
MS1Correlation	The minimum isotope distribution match needed for a result to be considered.
IntensityRecoveryPercentileThreshold	This will alert the user if the apex intensity of the degradation lies within the bottom x percentile. For example, if this is set to 0.05, then whenever the intensity of a peak is in the bottom 5%, a warning will be added to the <b>Comments</b> field.
PPMStandardDeviations	The number of standard deviations (square-root of the variance) in ppm for a result to be considered
HingeDeltaModScoreThreshold	The acceptable threshold of the delta mod score for a monoclonal antibody. If a degradation score falls below the set threshold, a warning is added to the <b>Comments</b> field to alert the user.
HingeScoreThreshold	Same as HingedeltaModScoreThreshold, except it will alert the user if a Score is below the threshold.

- The Disulfide Validator will operate on disulfide and trisulfide peptides, but the Mod Validator can also operate on disulfides and trisulfide peptides as well. Sequence variants will not operate w/ Mod Validator and vice versa.
- Users do not activate MS1 Correlation in the advanced settings. The code will apply this if it is set but will bypass it if it is not.
- PPM errors are normally distributed. This allows for usage of standard deviations from mean as a generalized and robust metric that is empirically derived from the data analyzed rather than ostensible vendor specifications.
- The validator also checks for ambiguous assignments, i.e., if a peptide sequence in the disulfide is found in multiple places within the protein, a flag will be added in the Comments field.

## Source Dissociation Validator










A validator was implemented to identify PSMs that are products of in-source dissociation. An identification is labelled as such in the comments column if it shares the same elution profile with another identification or feature with higher mass. The comment provides the PID and mass difference of the precursor from which the source dissociation product originates. To enable the validator in the **Advanced** tab, add the following in the **Advanced Configuration** section:

```
[Byologic]
SourceDissociationValEnabled =true;
```

As of Byos v5.4, this advanced command will also add a comment to any feature that coelutes with its wildtype peptide. This varies from the previous behavior, which requires the source artifact to have lesser mass than the attributed parent ID.

## Plot Menus

The menu bars at the top or sides of the three plot views, XIC, Isotope, and MS2 and mass error, share icons that support generic operations on plots. These icons appear in almost all Protein Metrics, Inc. software products.

- The  icon resets the plot to default zoom level. Shortcut = double left-click.
- The  icon enables zooming in. The cursor changes to this icon. Drag across the range of the plot to display to zoom to that x-range. By default, the plot's y-range scales according to the maximum y-value within the x-range, but the software also supports freeform zooming as enabled under the  icon.
- The  icon enables zooming out. Click anywhere in the plot to zoom out.
- The  icon enables moving (panning) across the plot. The cursor changes to this icon. Click the plot and drag up or down, right or left to view a part of the plot that is off-screen.
- The  icon locks the x-axis for stacked plots in a plot view to use the same ranges. Unselecting this allows stacked plots to apply independent x-axis ranges.
- The  icon performs an undo of the last zoom step. Shortcut = Shift-left arrow key.
- The  icon performs a redo of the last zoom step. Shortcut = Shift-right arrow key.
- The  icon manages how plots are displayed (render options), as well as zoom modes. The Render and zoom options include:

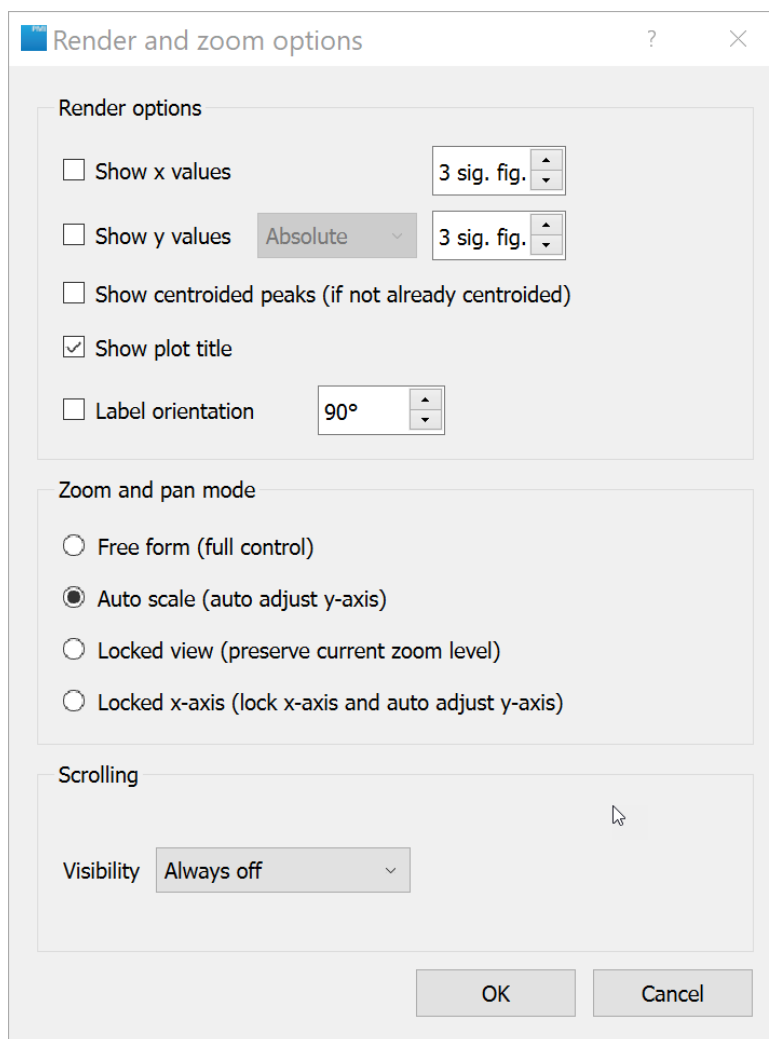


Figure 112: Render and zoom options

- **Show x values** and **Show y values** display the x- or y-coordinates beside plot peaks to the chosen number of significant figures.
- **Show delta**: when checked, displays the x or y differences from a reference peak when the cursor hovers over a peak tip.
- **Show centroided peaks** darkens the peak positions that are determined to be centroided.
- **Show plot title** displays the title of the plot, as generated from field values.
- **Label orientation**, when checked, orients labels to some angle other than horizontal.
- **Show XIC proposals** (XIC only) displays the proposed XIC plot splits generated by the **Edit proposed XIC splits** context menu in the Peptides table. The plot splitting is represented graphically as gray rectangles showing the edited integration limits:

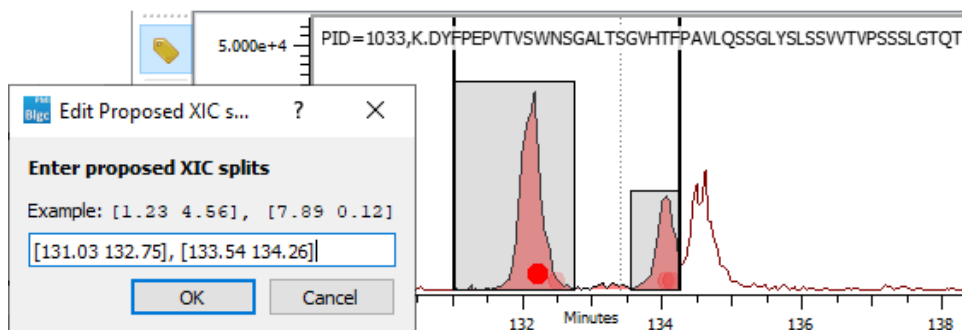



Figure 113: Edit proposed XIC splits

The **Zoom and pan mode** options include:

- **Match x-range**, when checked and followed by reset zoom (click on the ) the peptide and wildtype peptide plots are redrawn using the same x-axis values.
- **Free form** mode to manually select the desired y range as well as x range.
- **Auto scale** mode to select only the x range (the y range is then adjusted to the value of the highest peak).
- **Locked view** mode to keep the current x range (for either  $m/z$  and/or  $m$ ) when moving between elution peaks.
- **Locked x-axis** mode turns off autoscaling for the x-axis (but not the y-axis) and applies the current x-axis scale across all Peptide table selections.

Under **Scrolling**, the **Visibility** setting controls display of the scroll bars:

- **Always on** displays scroll bars even when the full plot is shown.
- **Always off** turns off display of the scroll bars even when a partial plot is shown.
- **Show as needed** displays scroll bars only when a partial plot is shown.

## Plot Right-Click Menus

The XIC, MS2 and mass error, and Isotope plots have a variety of context menus for plot styling and exporting revealed by a right-click on the plots:

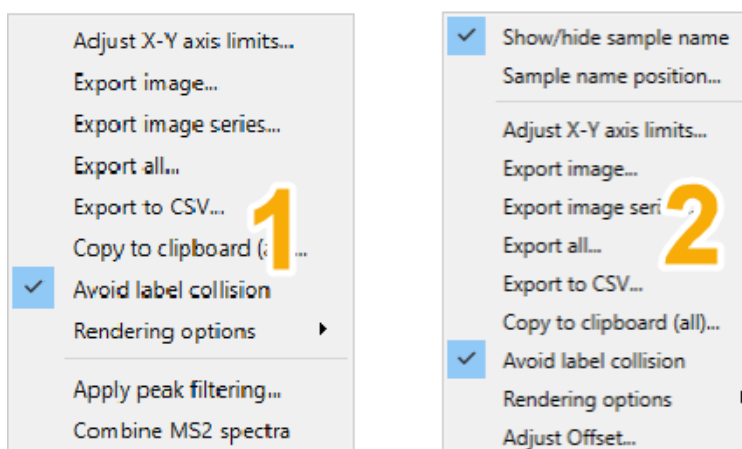




Figure 114: Plot right-click menus, [1] for MS2 and mass-error plots and [2] for XIC/Isotope plots



Figures exported can be useful for reports, publications, regulatory filings, or internal communications. There are many options for rendering and exporting so that the user has much freedom to prepare a plot or figure style as needed.

- **Adjust X-Y axis limits** opens a dialog for the user to manually edit the plots x and y maxima and minima. This is a less convenient but more precise (and reproducible) alternative to the  and  icons.
- **Export image** allows the user to save the plot as a \*.pdf file or as a \*.png, \*.wmf or \*.svg image file. It opens the **Plot Exporting Settings** dialog, which controls image size, file name and folder, and x and y minima and maxima:

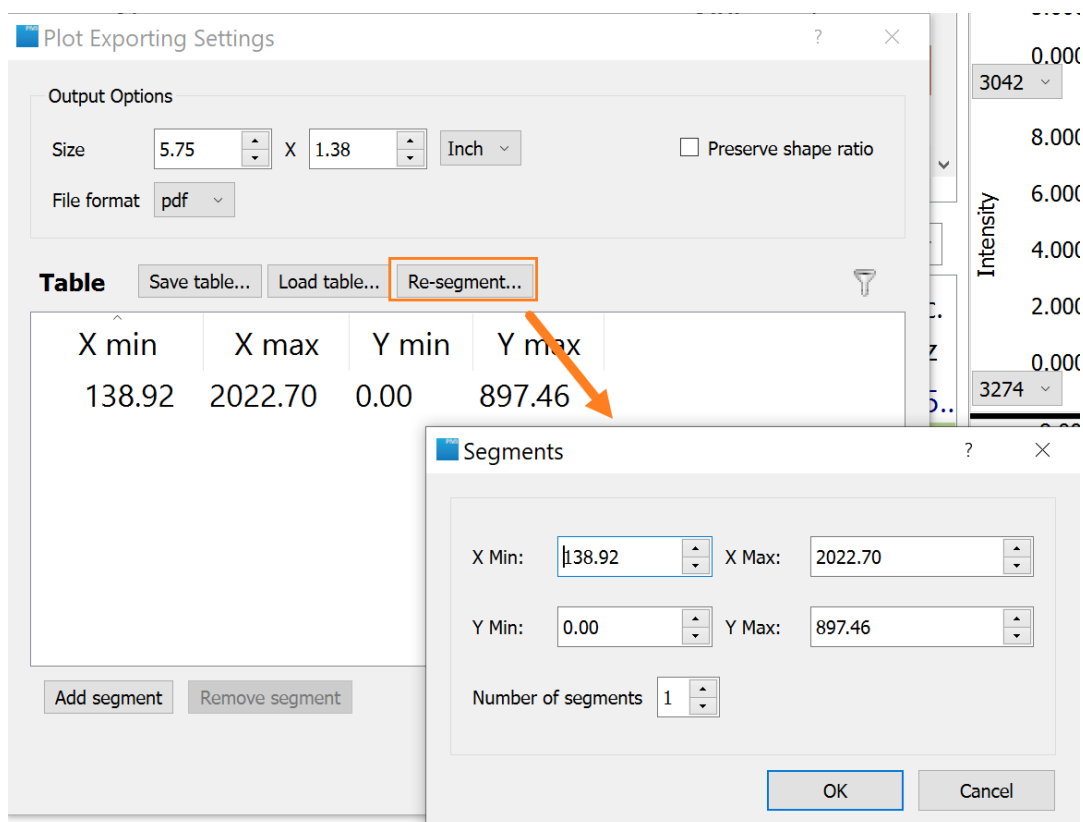


Figure 115: Plot export settings

Sometimes a user wishes to display a wide range across the x-axis and yet retain significant detail. This can be done by breaking up the plot into a series of panels. The **Add segment** button creates segments with user-defined x and y values. A series of these segments eliminates unneeded portions of the plot and increases the effective detail in the image. This is also a useful function for automated reporting. The **Re-segment** button allows edits to the reported x and y minima and maxima for existing segments.

**Note:** Image exports also support \*.svg image formats. In the file name cell, change the selected extension to **.svg** and click **Save**:

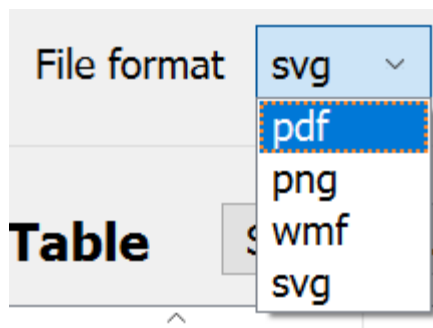


Figure 116: Plot export file options

- **Export image series** also opens the Plot Exporting Settings dialog, except that the segments are prepopulated with six equal sized segments. This simplifies the edits of the segments. The **Add segment** and **Remove segment** buttons control the number of segments that divide up the plot.
- **Export all** also opens the Plot Exporting Settings dialog, except that the wildtype peptide plots are exported along with the peptide plots. Segment editing functions are disabled for this option.
- **Export to CSV** exports the plot trace x-y points to a \*.csv file.
- **Copy to clipboard (all)** enables pasting the plot image into another application.
- **Avoid label collision** staggers the label text to reduce overlap.
- **Rendering options** controls how a plot is displayed in the application. It includes several sub-menus:

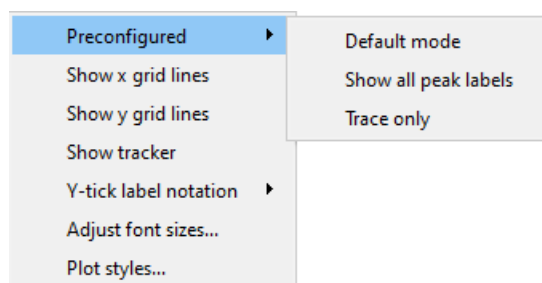


Figure 117: Rendering options menu

- **Preconfigured** sub-menus control what is displayed in the plots. **Default mode** displays the trace, peak labels and plot title. **Show all peak labels** does exactly that. **Trace only** turns off the peak labels and plot title.
- **Show x grid lines** turns on and off the x grid lines.
- **Show y grid lines** turns on and off the y grid lines.
- **Show tracker** displays a vertical dotted line that follows the cursor when moved in the plot. This allows a more precise determination of the X and Y positions of the cursor.
- **Y-tick label notation** toggles between y-axis notation options of absolute amounts or as percentages of the highest peak.
- **Adjust font sizes** controls the font size for each type of text on the plot.
- **Plot styles** allows changes to the graphic styling of the plot, including trace width, axis width, and total *m/z* dot colors to be used.

- **First Layer** (XIC and Isotope plots only) reveals sub-menus to turn on and off the display of traces, integrations, and labels in the plots. The show/hide all sub-menu turns all on or off.

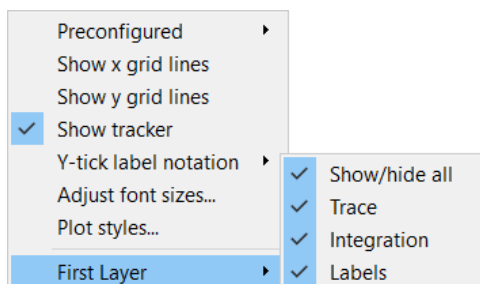


Figure 118: Rendering options &gt; First Layer menu

- **Adjust Offset** (XIC plots only) sets the x-axis offset between multiple plots as percentage of the highest peak when the top-level menu **Window > Enable Stacked Plots** is checked.
- **Show/hide sample name (XIC and Isotope plots only)** controls whether to display the sample names or not for both individual and stacked plots.
- **Sample name position (XIC and Isotope plots only)** reveals sub-menus to position the sample name with respect to the graphs.

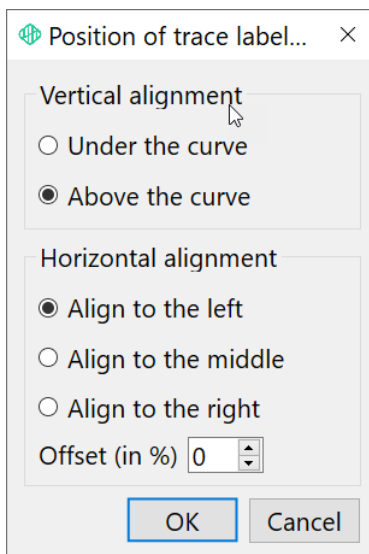


Figure 119: Sample name position sub-menu

## XIC Plots and Menu

The **XIC plots** view displays chromatograms for specific regions of the peptides and wildtype peptides associated with the record selected in the Peptides table:

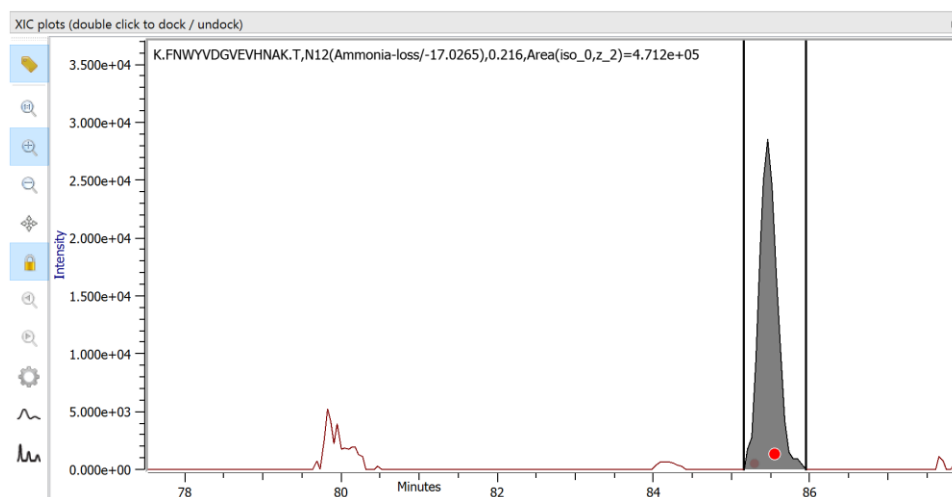







Figure 120: XIC Plots

In addition to the menu icon functions shared by all the plot views, there are some functions specific to XIC plots:

- The  icon turns plot annotations on or off. In the XIC plots, annotations are displayed as circles at the base of the chromatograms. Dark red circles represent the position of currently displayed MS2 scans. Light red circles represent the position of other MS2 scans with the same identification. Light gray circles represent the position of MS2 scans with the same precursor mass as the selected peptide, but with different attribution. Hover over the circles and the associated sequence ID and value are displayed. Note that the cursor changes to a hand. Click the circle and the corresponding Peptides table row is selected and the views are updated to display for that record.
- The  icon is used to compute the XIC plot integration for a specified time range. A dialog opens to enter start and end times.
- The  icon enables MS scan extraction. This displays isotope values derived from the XIC plot. A blue vertical line appears in the XIC plots view. As the line is dragged across the XIC trace, the Isotope plots view is updated to show the MS scan for that XIC time value. Two new icons appear on the Isotope plots menu bar in this mode: The  icon clears the extracted MS scan and restores the original isotope plots. The  icon saves the extracted MS scan to the project document.

If **Window > Enabled Stacked Plots** is selected, XIC plots for multiple samples can be overlaid and their explicit XIC integration limits defined.

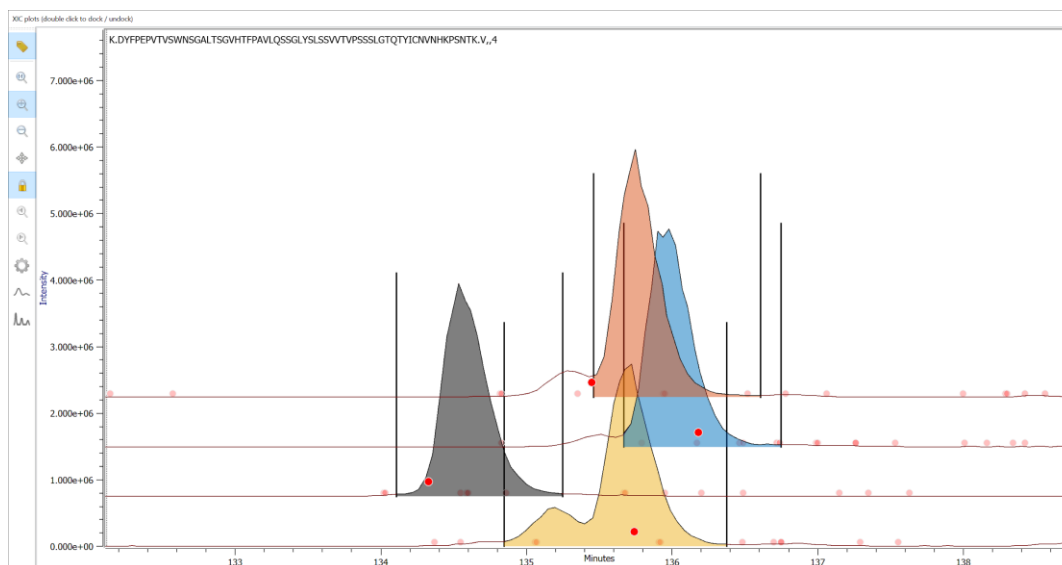


Figure 121: Stacked XIC plots

With stacked plots enabled (via **Window> Enable Stacked Plots**), users can click and drag integration boundaries within XIC plots to simultaneously update start and end times for all samples and charges of a peptide. Subsequent changes will be reflected in the Peptides table.

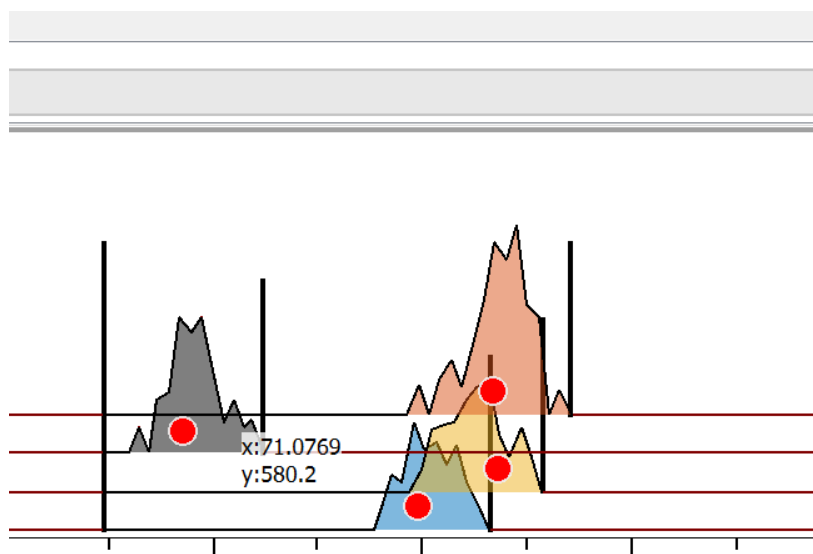


Figure 122: Selection of multiple plots

The option to edit multiple integration boundaries displayed in a set of stacked XICs at once is available with 'Shift+Click' and 'Ctrl+Click' actions. To move all start or end boundaries to the same retention time, hold down shift when clicking and dragging a boundary marker.

Alternatively, holding ctrl when clicking and dragging will result in boundary markers moving by the same amount.

If the user selects **Combine Charge States** under **Edit>Search Filter>Peptide Grouping Method** the application will display XIC traces for **multiple charge states** of the same molecule form in a single plot. This may provide additional evidence for the identity of the molecule.

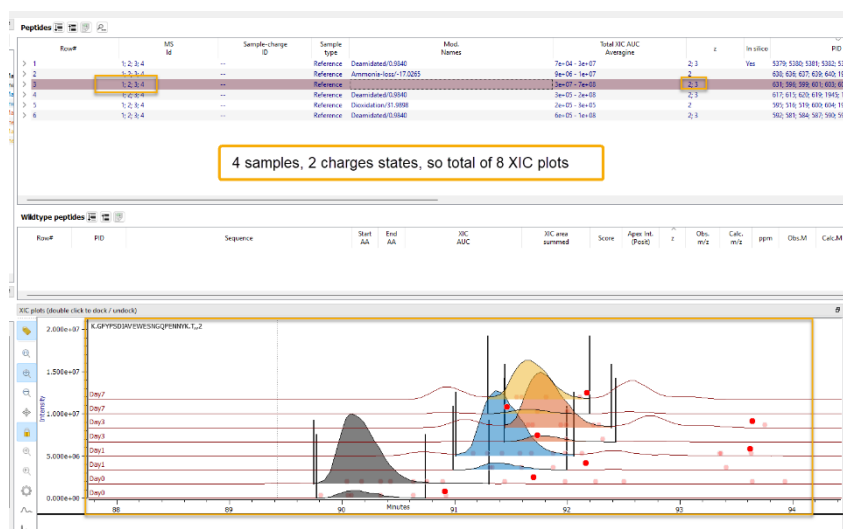


Figure 123: XIC traces for multiple charge states on one plot

## Isotope Plots and Menu

The **Isotopes** plots display the peptide isotope ( $m/z$  scan) for the selected Peptides table record:

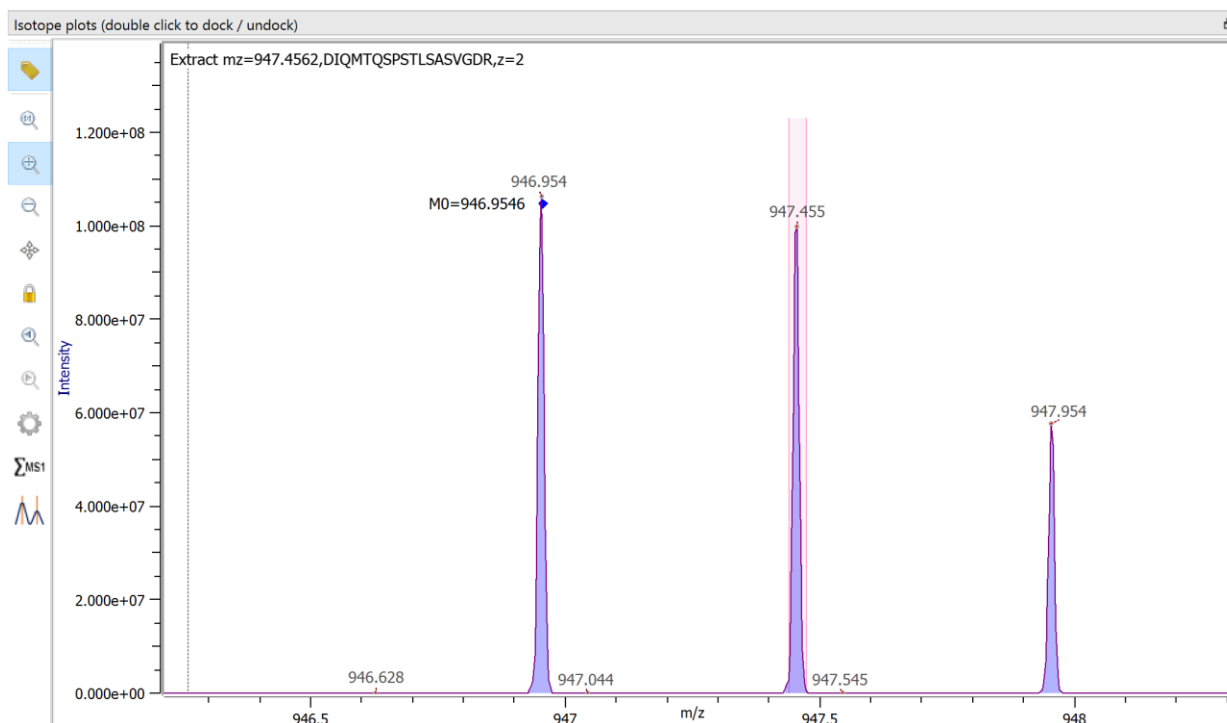





Figure 124: Isotope plots

In addition to the menu icon functions shared by all the plot views, there are some functions specific to Isotope plots:

- The  Show/hide annotations icon turns on and off the display of the molecular weight for the primary peptide beside a blue dot that identifies the peak with that calculated value.

- The  Sum MS1 icon is a local per-peptide summing option that sums MS1 scans for the selected peptide. If the XIC interval is adjusted by the user, the isotope plot will update accordingly. **Note:** Scan summing only works on projects created using v4.6 or later of Byos.
- The  Theoretical isotope distribution icon renders the theoretical average distribution. The theoretical isotope distribution is calculated using the average model rather than the exact chemical composition. Theoretical isotope distribution is rendered using green sticks surrounded by green shaded transparent rectangular boxes.

## MS2 and Mass Error Plots and Menu

The **MS2 and mass error plots** display plots of the MS2 peptide fragments with the corresponding m/z mass error table:

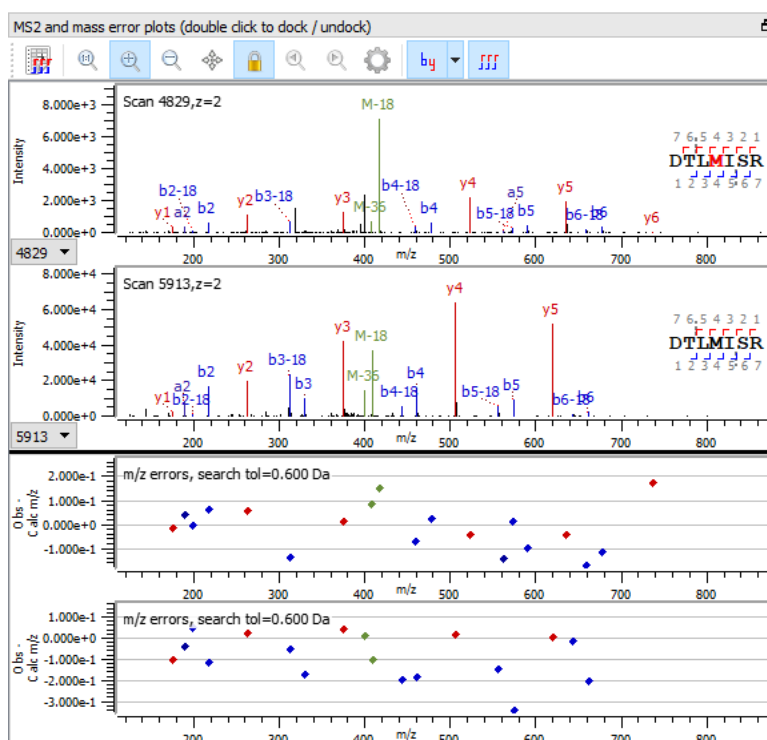





Figure 125: MS2 and mass error plots

The MS2 plots are identified by Scan ID. The plots are colored blue for N-terminus b ions and red for C-terminus y ions. Other fragments, including c and z ions are colored green

In addition to the menu icon functions shared by all the plot views, there are some functions specific to MS2 and mass error plots:

- The  icon switches between the default plot view and the mass table view:



MS2 and mass error plots (double click to dock / undock)




☒ Calculated ☐ Observed ☐ Delta

PID=239: DTLMISR    PID=337: DTLMISR

#	a calc.	b calc.	b-18 calc.	b-HexN calc.	Seq.	y calc.	y++ calc.	#
1	88.0393	116.0342	98.0237		D			7
2	<b>189.0870</b>	<b>217.0819</b>	<b>199.0713</b>		T	<b>736.4022</b>	368.7047	6
3	302.1710	330.1660	<b>312.1554</b>		L	<b>635.3545</b>	318.1809	5
4	449.2064	<b>477.2014</b>	<b>459.1908</b>		M	<b>522.2704</b>	261.6389	4
5	<b>562.2905</b>	<b>590.2854</b>	<b>572.2748</b>		I	<b>375.2350</b>	188.1212	3
6	649.3225	<b>677.3174</b>	<b>659.3069</b>		S	<b>262.1510</b>	131.5791	2
7					R	<b>175.1190</b>	88.0631	1

Figure 126: MS2 and mass error plots: mass table view

The reported fragment data that was confirmed in the analysis is displayed in red bold font. Unconfirmed fragment data is displayed in black font. In this view, the  icon switches the view back to MS2 plots. The  icon copies the content of this table to the clipboard.

- The  icon switches between showing and hiding the b and y ion labels. The icon is accompanied by a drop-down arrow that reveals three sub-menus:
  - **Open annotation options** opens a dialog that allows the user to set options for determining the assignment of the MS2 annotations:

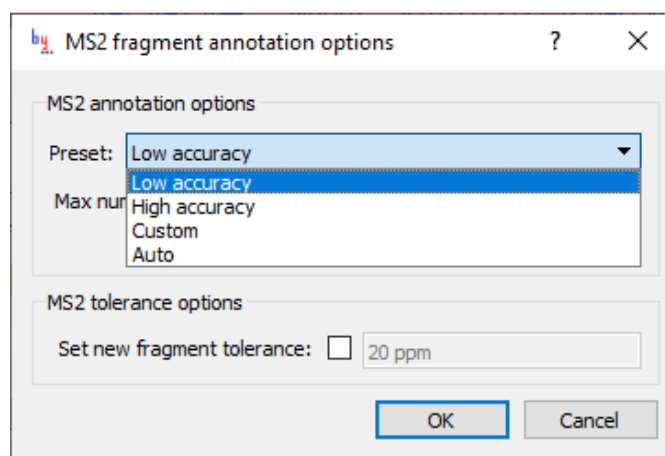



Figure 127: MS2 fragment annotation options

The maximum number of peaks per 100 m/z and MS2 mass-accuracy tolerance options can use presets for Low accuracy, High accuracy, or Custom, in which the user sets these values. The dialog defaults to the Auto preset, in which the software determines the MS2 fragment annotation settings.

- **Remove fragment coverage cache** clears the existing cache of detected fragments used for the protein fragment coverage rendering.



- **Export fragment to CSV**, exports the fragment sequence to a \*.csv file.
- The  icon turns on or off the fragment sequences displayed to the right of the plots.

The MS2 and mass error plots view has two specific context menu items revealed by a right-click in the plot:

- **Apply peak filtering** opens a dialog that allows the filtering of peaks by an entered signal-to-noise ratio value.

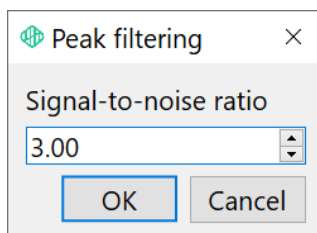
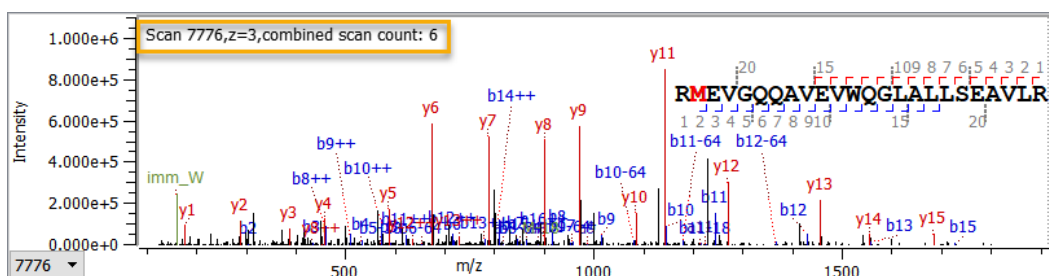


Figure 128: Peak filtering by signal-to-noise ratio

- **Combine MS2 spectra** (MS2 and mass error plots only) sums the MS2 Plots for all the peptides for a selected row in the Peptides table. The title of the plot updates to show the counts of the peptide scans in the combined MS2 plot:



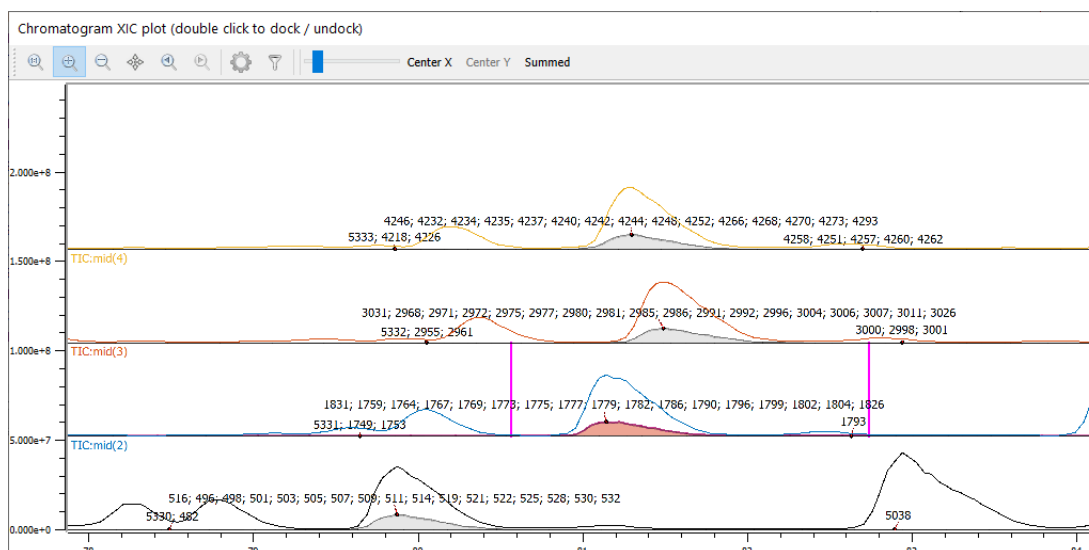



Figure 130: Default view of Chromatogram XIC plot

In addition, **XIC Summed** and **TIC Difference** traces can also be overlaid. **XIC summed** is calculated by summing the **XICs** of isotopes of peptides, including different charge states. **TIC difference** can then be calculated using the formula, **TIC – XIC summed**.

In addition to the menu icon functions shared by the other Peptide Analysis plot views, there are some functions specific to the Chromatogram XIC plot:

- The  **Settings** icon opens the **Plot Settings** dialog. The dialog includes navigation to four areas [a] General, [b] Labels, [c] Summed mode, [d] Zoom options.
  - **General** – contains options to show and edit the plot title and limit the number of displayed peptides:

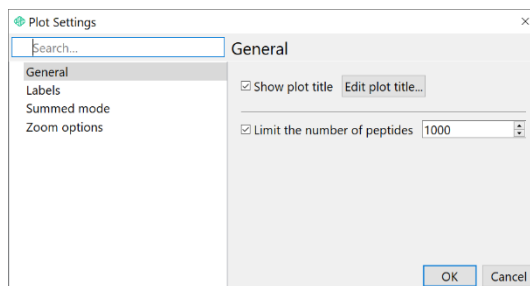


Figure 131: General tab - Chromatogram XIC plot menu item

Edit plot title opens the **Template Editor** dialog:

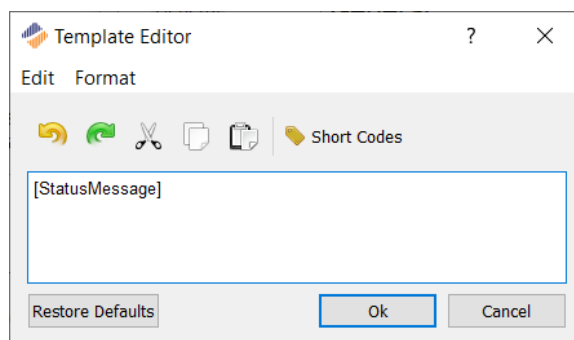


Figure 132: Template Editor within General tab

The Template Editor allows free entry of a plot title or the use of Short Codes.

- **Labels** – contains options to show and edit annotations, limit the total number of labels and orient (rotate) labels:

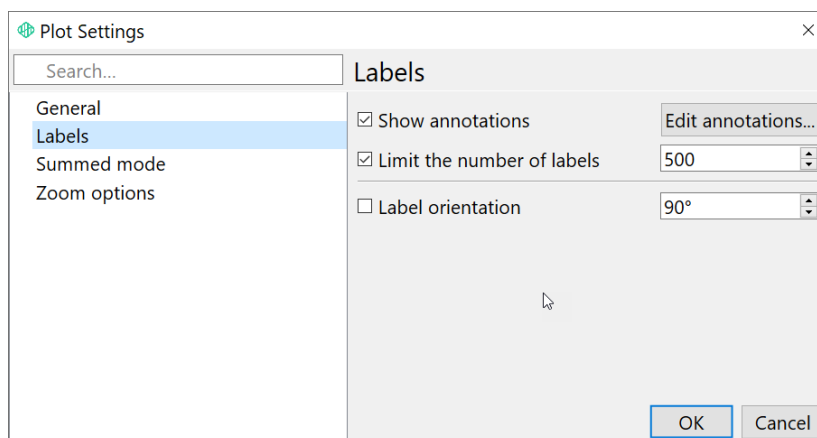


Figure 133: Labels tab - Chromatogram XIC plot menu item

**Edit annotations** also opens the **Template Editor** dialog. The default [PeptidesID] can be replaced with other annotations available in the “Short Codes” tab on the Template Editor menu (**Note:** a Chromatogram Analysis license is required to see the Labels section for Chromatogram XIC plots).

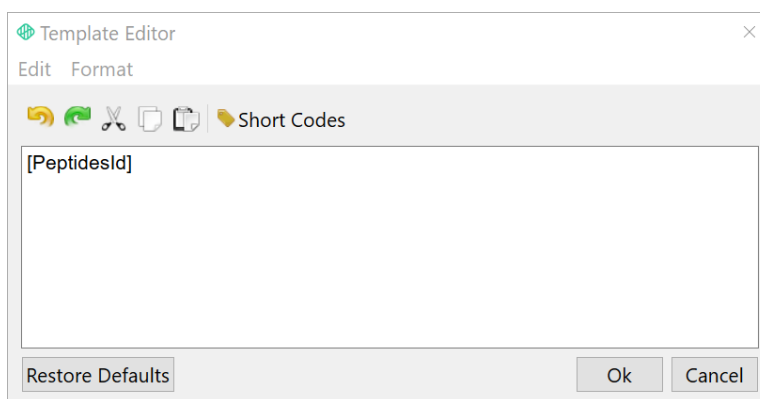


Figure 134: Edit annotations within Labels tab

Below are some of the options for the **Short Code** (1<sup>st</sup> column).

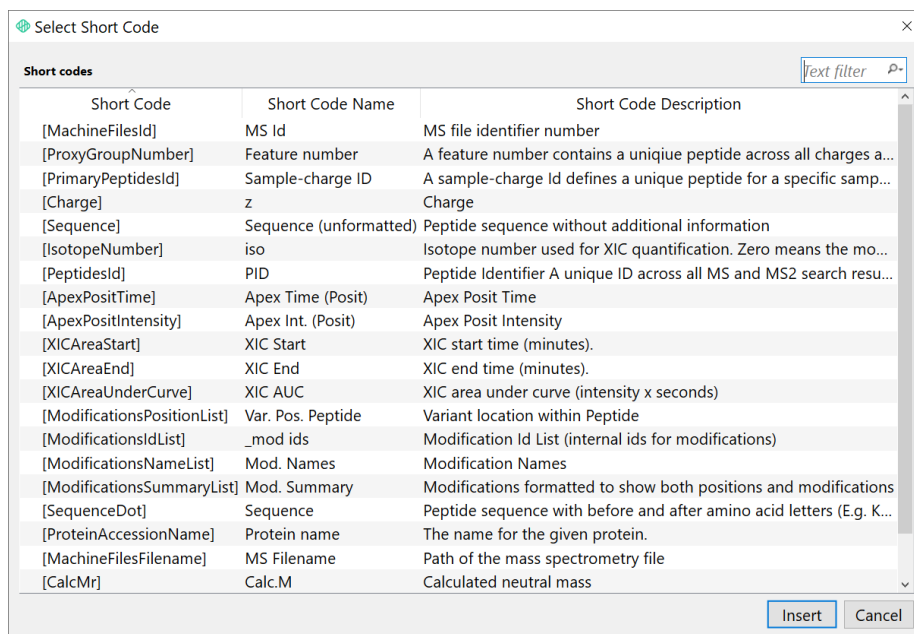


Figure 135: List of options for Short Code

Selecting the **[Sequence]** Short Code would result in the below annotation of the peaks.

Figure 136: Selecting **[Sequence]** Short Code to show peptide sequences as annotations

- **Summed mode** – contains the option to select TIC difference calculation, along with XIC summed:

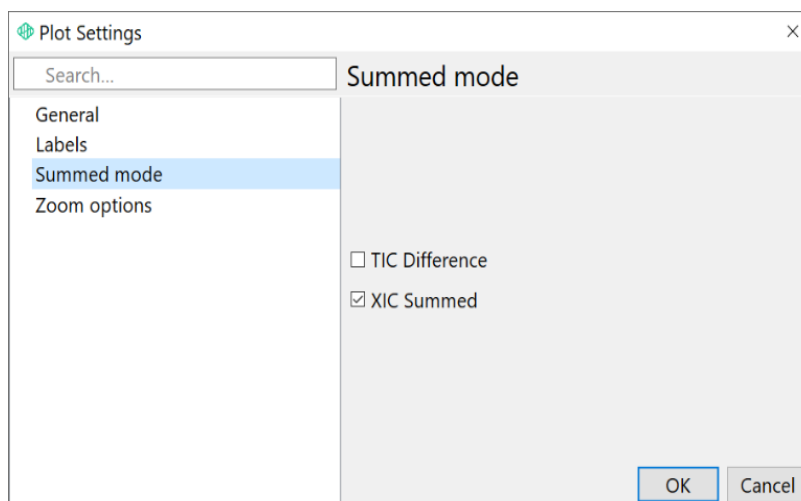


Figure 137: Summed mode tab - Chromatogram XIC plot menu item

- **Zoom options** – contains the standard zoom options, along with scrolling visibility:

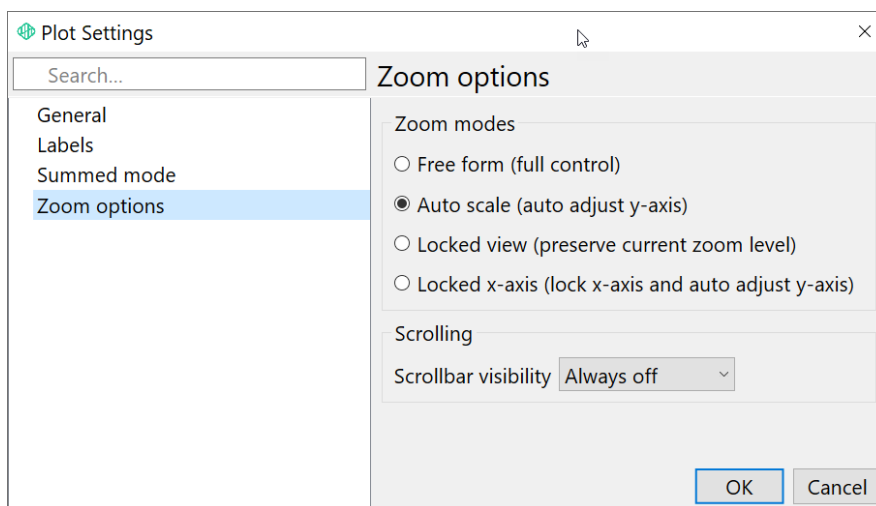


Figure 138: Zoom options tab - Chromatogram XIC plot menu item

- The **filter**  icon opens the Column Filters Editor dialog. The dialog enables filtering based on combinations of column values:

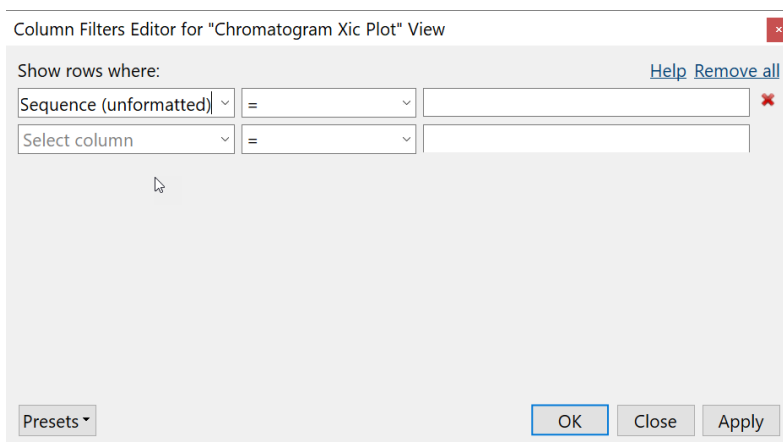


Figure 139: Column Filters Editor for filtering data to plot

- **Center X** zooms the segment and centers it in the frame:

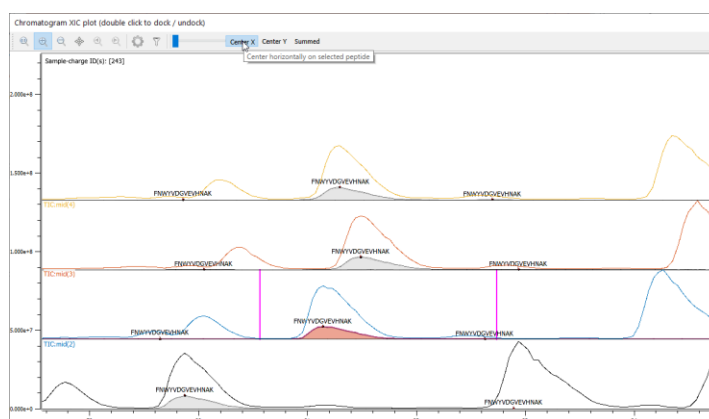


Figure 140: Center X allows to center the plot on X-axis

- **Center Y**, available when Center X is selected, zooms the segment and centers the height of the designated peak in the segment in the frame:

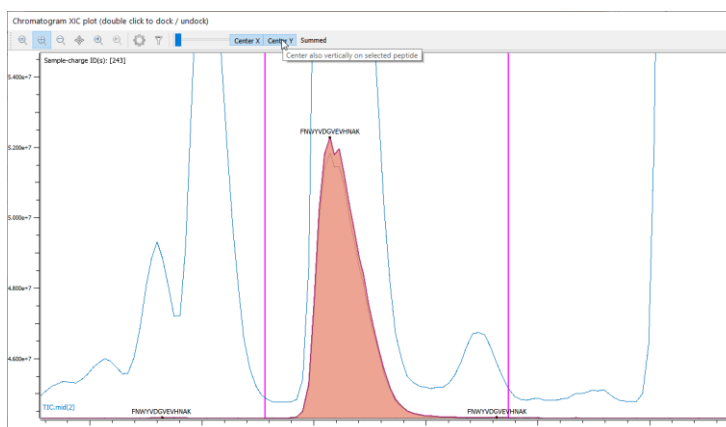


Figure 141: Center Y allows to center the plot vertically (i.e., on Y-axis)

- **Summed** button features the core step of the chromatogram XIC plot. This feature helps the user visualize how much of the total signal (represented by the TIC) is accounted for by the

collection of XICs. In addition to the default TIC and XIC layers, additional layer **XIC summed** is generated when **Summed** icon is clicked. **XIC Summed** is the sum of all XICs of all the isotopes and at different charge states of the peptide.

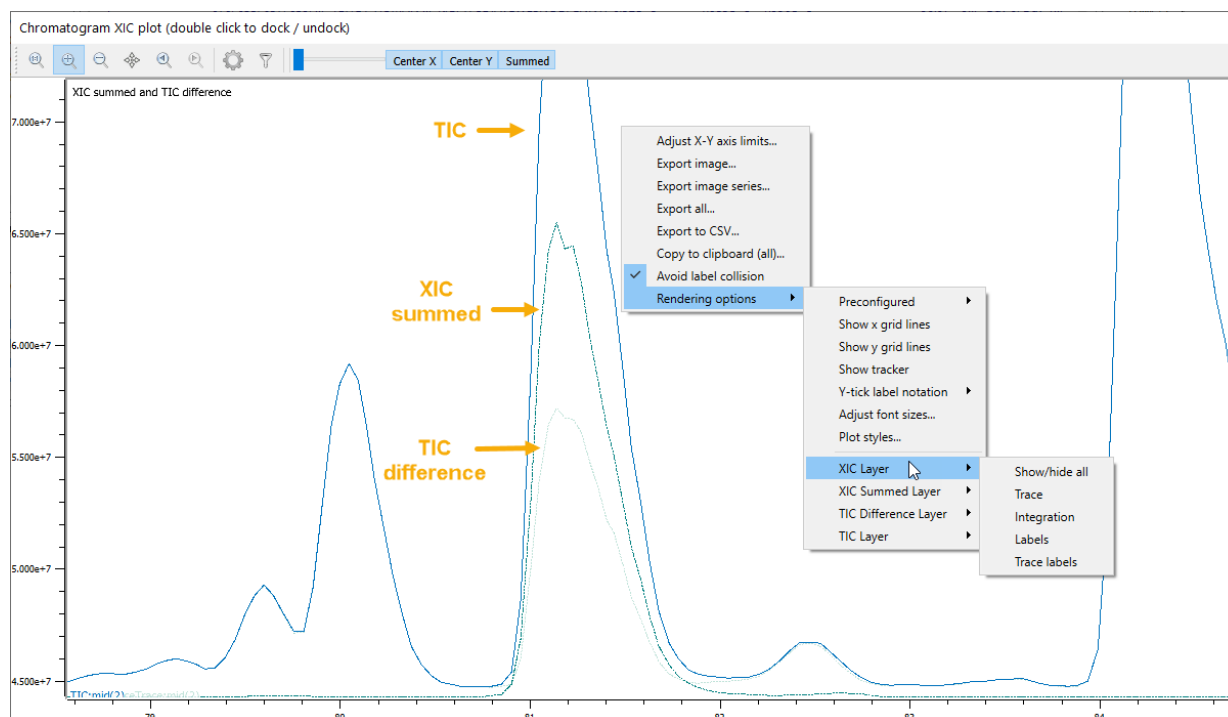


Figure 142: XIC Summed, TIC Difference and TIC curves

Note that the calculation of the Summed XIC (and TIC difference, if checkbox is enabled in the **Settings icon > Summed mode > TIC Difference** shown in Figure 137) might take a while depending on the size of the raw MS files and the RAM of the desktop computer.

The traces are plotted as layers (shown in the figure above) and user has an option to pick the set of traces to visualize. In the above figure, XIC layer rendering is hidden. This means, except the XIC layer, the rest of all of the layers can be seen.

## Appendix

### Advanced Commands

Peptide Analysis includes many ways to customize the functionality to fit specific needs. Protein Metrics uses Advanced Commands to test new ideas, beta-test new features, and enable specialized options, without adding complexity to the graphical user interface. This section describes several text-format Advanced Commands that will enable finer control over processing. Advanced commands may be entered during project creation in the **Advanced configuration** panel of the **Advanced** tab or after project creation by choosing **Edit -> Advanced configuration**.

1. **In-silico Align** allows shifts in the start and end times to better match the signal. FeatureCenterTolerance specifies how much a feature (start and end time pair) may shift in minutes. FeatureDurationTolerance specifies how much a feature may grow or shrink in minute.

```
[InSilicoAlign]
FeatureCenterTolerance=1
FeatureDurationTolerance=0.5
```

Signal processing has been updated in **Byos 5.3** to improve the XIC boundary for deamidated peptides that closely elute with the wildtype. The processing is automatically used for processing deamidated peptides when the following advanced command is applied:

```
[InSilicoAlign]
MultisotopeMethod=2
```

```
[InSilicoAlign]
FeatureCenterTolerance=1
FeatureDurationTolerance=0.5
```

As of Byos v5.4, users can enter the following command to enable multiple improvements in the In-silico Align workflow.

```
[InSilicoAlign]
Enabled=true
```

Note: If the in-silico align advanced command is being used, the start and end time of the added mass XIC may be different than what was entered by the user, in order to align across samples. If a user wants the XIC start and end to exactly match their input, then Add Mass must be done without using the in-silico align advanced commands.

If individual parameters are set in addition to this command, the set value(s) will take precedence.

2. **KeepIsoformOrder** improves logic for in-silico isoform assignments.

```
[InSilicoAlign]
KeepIsoformOrder=true
```

In the case of closely eluting isobaric candidates specified in the input CSV (e.g, the various deamidated forms of a peptide), the existing In-silico align logic will result in the same bounds for each candidate. Using this advanced command, the refined logic will attempt to assign input CSV candidates to correct peaks based on the elution order of the LC peaks found in the XIC. In some cases, it may also be necessary to include the following command to prevent the need for 'score' filtering:

```
[MSFeatureFinderSeg]
ScoreNegligibleRatio=-1.0
```

3. **AlwaysAddLabeledPeptide** allows correct grouping of in-silico peptides from CSV. These peptides will be grouped together with existing peptides unless they differ in retention times, in which case they form separate groups. Adding the below advanced command to the project will override the default isoD/isoAsp with whatever is in the command line.

```
[InSilico]
AlwaysAddLabeledPeptide=isoAsp|isoD
```

4. **Autosplit** automatically splits peptides during project creation if the peptide is wildtype, the sequence contains amino acid pairs DG, DS, or DD, and there are XIC proposals. The XIC proposals will be consistent across all charged states of the same peptide. New peptides will be marked "In-Silico" and a user-defined label will be visible in the Peptides table. In the example below, the label will be "IsoAsp".



```
[Byologic]
AutoSplitPeptides=DG|DS|DD
AutoSplitPeptidesLabels=IsoAsp
; Leave AutoSplitPeptides empty to disable
```

Note: If AutoSplitPeptides is set to <blank>, the function is disabled.

5. **MS feature align** performs a time alignment of all the samples and produces an ensemble average of the XIC signal. This reduces some noise and prepares multiple samples to search for XIC start and end points in an "ensemble" plot, as opposed to searching each plot separately). In some cases, especially with Iso-D, the input sample degrades and peaks may not be found in all samples. If MSFeatureAlign is disabled, the Iso-D start and end point proposal in one MS2 file sample may not be found in another. In such cases Iso-D splitting will not work.

MSFeatureAlign is enabled by setting it to one of two modes. For Mode=Full, all peptides will be aligned. For Mode=DifferentTopologyOnly, the ensemble average is used if different number of peaks are found in the various samples. After the peaks are found in the ensemble XIC, they get distributed back to the MS samples. This enables identification of peaks with zero signal between the XIC start and end points (e.g. where no IsoD is found).

```
[MSFeatureAlign]
Mode=DifferentTopologyOnly
OR
Mode=Full
```

6. **Negligible Ratio** settings can help identify very small peaks, (e.g. Iso-D peaks). The values assigned are ratios of the main peak; 0.001 is a peak sensitivity of 0.1% of the main peak.

```
[MSFeatureFinderSeg]
AUCNegligibleRatio=0.001
ScoreNegligibleRatio=0.0005
```

7. **Feature Finder Segments** speeds up peak finding and helps identify narrow XIC peaks. SmoothingStDev is used to tweak the peak finding algorithm to find narrow peaks. The default of 0.04 is good for identifying common peaks. A smaller value is better for narrow peaks. SamplesPerMinute can be reduced to increase processing speeds. The default rate is 100 samples per minute, which is generally considered a safe rate. A smaller value will speed up processing for large projects with many peptides and samples.

```
[MSFeatureFinderSeg]
SmoothingStDev=0.005
SamplesPerMinute=50.0
```

8. **Combined MS/MS Spectra** configures the number of summed spectra (averaged spectra to be annotated with the MS/MS fragments). Changes to this count can be made after processing to modify the results.

```
[Byologic]
MS2PeptideCombinedCount=5
```

9. **Auto-Annotate CDRs** enables/disables automatic annotation of CDR regions during project creation. The CDR regions in antibodies are automatically annotated (set to 1) during project creation by default.

```
[Byologic]
```

```
AutoAnnotateCDRs=0
```

10. **Enable XIC** Correlation computes XIC cosine correlation between variant and its wildtype peptide. This is disabled (set to 0) by default.

```
[Byologic]
EnableXICCorrelation=1
```

11. **Enable MS1 Correlation** checks the MS1 isotope distribution. This is disabled (set to 0) by default.

The **MS1 isotope envelope confidence** column will be added to the Peptides table when this advanced command is applied. The MS1 isotope envelope confidence column displays the values calculated using the KL metric that is currently used for MS2 fragment isotope confidence, normalized to 0-1 scale.

This metric facilitates better True Positive discrimination, fast calculation, lesser sensitivity of occasional interferences on minor isotopes, and better sensitivity to a shifted isotope pattern. Additionally, a single threshold can be applied across a wide range of data.

```
[Byologic]
EnableMS1Correlation=1
```

12. **Correcting primary proteins** corrects inconsistent protein assignments by assigning the peptide in all samples to the highest-ranking protein. If HCP searches against multiple samples yield different primary proteins for a given sequence, this can be used to synchronize the samples.

```
[Byologic]
AdjustPrimaryProtein = true;
```

13. **Enable Summary Protein List** creates the column **Protein summary list** in the **Peptides** table to display all protein IDs containing the peptide sequence. Thus, all multiple duplicates of a peptide (e.g. in multi-specifics and HCPs) are visible.

```
[Byologic]
EnableSummaryProteinList = true;
```

14. **PeakFindingRange** affects in-silico align and these advanced commands should only be used when the entire tolerance range specified will only contain the target peptide. In the default behavior, the original in-silico start/end time must at least touch a peak to find it. This range can then be moved by the user as much as the user-defined tolerance allows. Additional commands are as follows:

For PeakFindingRange=Extended, the most intense peak in the entire range specified by the user-provided tolerance is found.

When PeakFindingRange=Soft, distant peaks are gradually de-valued, and a good peak is identified as a compromise between time shift and intensity.

```
[Byologic]
PeakFindingRange = Soft;
OR
PeakFindingRange = Extended;
```

15. **PeptideMatchPolicy** allows the user to assign MS/MS identifications to in-silico peptides within the relevant XIC time interval. For example, if a peptide is split, this policy will re-assign MS/MS

identifications based on the new XIC limits of the original and the created in-silico peptide. This also helps the user to choose the appropriate MS1 for each peptide. The numbering is in binary, with the value of 1 denoting **on**.

```
[Byologic]
PeptideMatchPolicy=1
```

16. **AddDuplicates** controls how homologous peptide sequences are annotated in the **Protein coverage** table. Two options allow Protein coverage to be digestion-aware.

```
[ProteinCoverage]
AddDuplicates = 1
```

The options for AddDuplicates are:

- 0 - no duplication
- 1 - duplication within the same protein
- 2 - duplication across all proteins
- 3 - duplication within the same protein applying protease specificity
- 4 - duplication across all proteins applying protease specificity

**Note:** if the advanced command is not used, the default is option 4. The protease is specified in the **Digestion** column in the **Sample Input** tab during project creation. If no protease is specified (i.e, the **Digestion** column is blank in the **Samples** table), the project will be created with **AddDuplicates = 2**.

17. **FastXIC** accelerates XIC processing.

```
[MSReaderDev]
XicMode = fast
The following optional settings are available for this command:
[MSReaderDev]
FastXicGranularity = 100000
FastXicMaxMz = 3010
FastXicMaxIonCount = 2000
```

Parameter	Description
FastXicGranularity	Integer value, relates to the precision. 10000 is 4 places past the decimal. For mass 1000.1236, a granularity of 1000 sets the value to 1000.124, while a granularity of 10000 keeps the value at 1000.1236. Default value is 100000.
FastXicMaxMz	Integer value. The maximum m/z value during data acquisition. To acquire data in the range 200-2000, set FastXicMaxMz = 2000, so that no ions are acquired past that value. Default value is 3010
FastXicMaxIonCount	Integer value. The maximum number of ions to be considered, sorted by intensity. Some files may contain ~2500 ions per scan. FastXicMaxIonCount = 1000 uses only the top 1000 ions by intensity. This is especially important for TOFs with ~25K ions per scan, where a higher value of 2500 is recommended. Default value is 1000.

18. **AutoSplitWildTypeCoelution** captures in-source artifacts and process related modified peptides in separate peaks. Additionally, a previous advanced command will now flag in-source artifacts.

```
[Byologic]
AutoSplitWildTypeCoelution=true
```

19. **ReduceInSourceArtifacts** eliminates in-source modification artifacts from the XIC boundary windows. Potential in-source artifacts are specified by a comma separated list of chemical formulae.

For example, to account for in-source oxidation and dehydration one would use:

```
[InsilicoAlign]
ReduceInSourceArtifacts=O,H(-2)O(-1)
```

20. **ExportBottomUpCsv** enables the proper formatting of the 2nd tab in Byologic into a CSV for import into either Intact or Byomap within Intact and Charge Variant reconstruction workflows.

```
[Report]
ExportBottomUpCsv=1
```

21. **Improvements in XIC trace interval limits of components generated by Feature Finder**

```
[FeaturesFinderOptions]
PeakSegmentationMethod=2
RunInSilicoAlign = false
```

22. **Theoretical fragmentation for HCD data and isotopic distribution plots** can be activated using the above code in either the project's advanced configuration or pmi\_advanced.ini

```
[Byologic]
FragmentPrediction=true
IsotopeDistributionPrognosticator=true
```

23. **Balance peptide charges by generating missing XICs** using the following advanced command. When using this advanced command, in silico peptides will be added to the project so that any version of a peptide (unmodified and modified species) will all contain XICs for the same charge states, in order to allow for fair relative quantitation calculations across different species of the same peptide.

```
Byologic]
FillMissingChargeStates=true
```