



Byos Release Notes

December 2025

5.12

Protein Metrics LLC, Boston, Massachusetts, USA

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Protein Metrics Suite Software Release Notes

Byos®, MS/MS Analysis (Byonic™), Peptide Analysis (Byologic®), Chromatogram Analysis (Byomap™), Intact Analysis (Intact Mass™), Supernovo™, Footprint™, Oligonucleotide Analysis, HRIM Analysis, and Preview™.

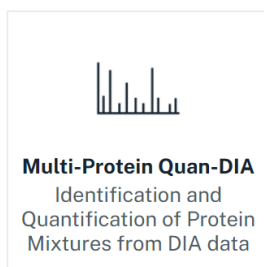
All enhancements are available within Byos.

Release 2025-12 (v5.12)

Multi-Protein Quantitation, Byos

- **New Multi-Protein Quantitation-DIA workflow in Byos**

The **Multi-Protein Quantitation-DIA** workflow, available with Multi-Protein Quantitation workflow license, enables the processing of data-independent acquisition (DIA) mass spec data via the MPQ pathway.



Data-independent acquisition (DIA) is sometimes preferred over data-dependent acquisition (DDA) for its improved quantitative accuracy, reproducibility, and deeper proteome coverage. DIA captures fragmentation data for all ions within specific mass ranges, making it more comprehensive and less prone to missing low-abundance peptides that DDA might skip, while also being more reproducible across multiple samples.

With this release, the new Multi-Protein Quan-DIA workflow is automatically enabled for users that already have the Multi-Protein Quantitation Workflow license!

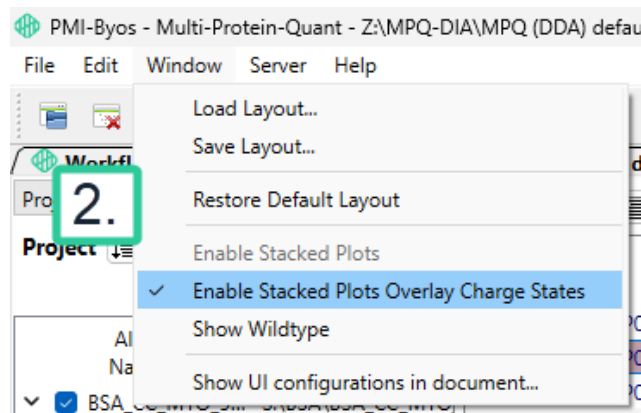
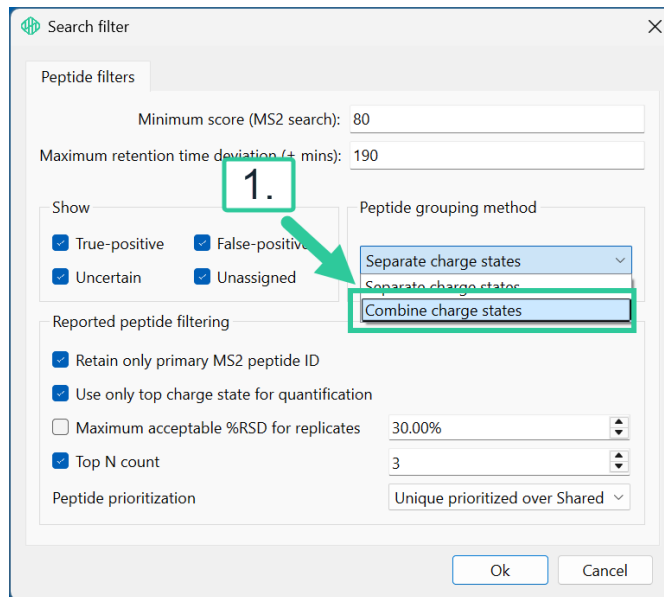
Note that this workflow is not yet available in Byosphere Desktop Client.

Peptide, Byos

- **Added ability to overlay charge states and stack samples in XIC plots**

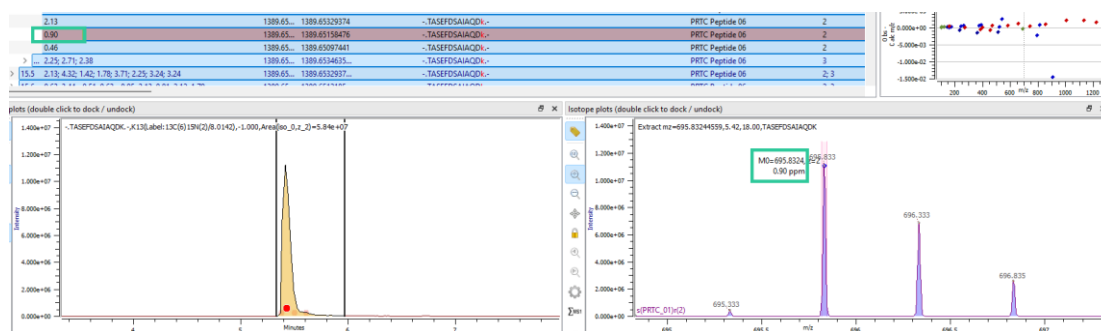
A new feature has been added to Byos Peptide workflows under **Window > Enable Stacked Plots - Overlay Charge States**.

This option is the same as Window > Enable Stacked Plots, except for the XIC plot widget where XICs with different charge states will be overlayed within the same sample. Users *must* ensure that charge states are combined prior to selecting this option.



- **Mass error is now included within the isotope plot**

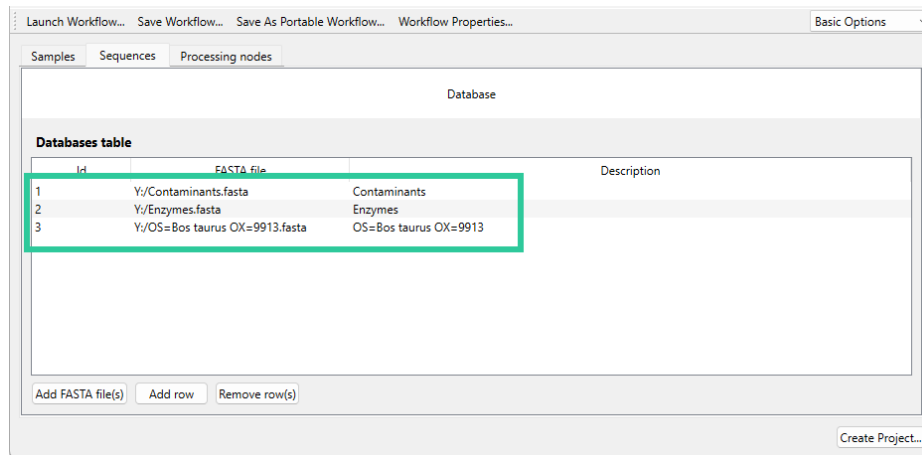
Mass error is now reported in PPM under the M0 label in isotope plots:



HCP, Byonic, MPQ, Byos

- **Users can now attach multiple FASTA files in various workflows**

Added the ability to provide multiple FASTA files in HCP, Byonic, and MPQ workflows.



MOBILion, Byos

- The MOBILion Reader has been updated to v1.12.0

Proteome Discoverer, Byos

- The Byonic and Preview Nodes have been updated to support Proteome Discoverer 3.3. These nodes can be downloaded from: <https://www.proteinmetrics.com/download/proteome-discoverer-nodes>

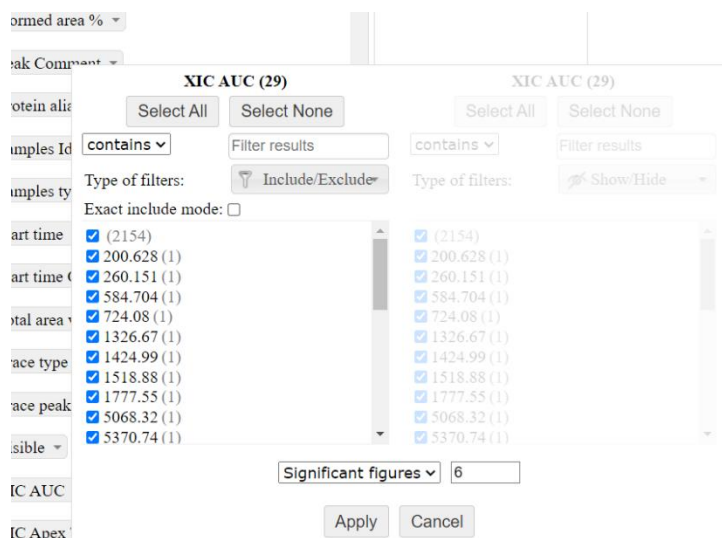
Progressive Deconvolution, Intact, Byos

- New Progressive Deconvolution workflow is now available

A **Progressive Deconvolution** workflow is now available as one of the default workflows in Byos. This workflow is useful for the identification and relative quantification of intact protein species via Progressive Deconvolution.



- **Mass XIC value has been added to the Progressive Deconvolution report.**
The XIC AUC value will now be populated in the Byos report for the Progressive Deconvolution.



- **Added the ability for a user to set the minimum number of consecutive Mass observations in Progressive Deconvolution workflow in Byos**

The following advanced command is now available as an option to filter progressive deconvolution results:

```
[Intact]
MinConsecutiveObservations = 3
```










































with 3 being the default value.

Release 2025-10 (v5.11)

Multi-protein Quantitation, Byos

- **Byos now displays -log probabilities (protein scores) for MPQ data**

Protein Log Probabilities values are now calculated for Multi-Protein Quantitation workflows.

Proteins                                         				Reset	
<input type="checkbox"/>	_prot_id	Protein name	Sequence (unformatted)	Log Prob	
<input checked="" type="checkbox"/>	1	nm NHEAVY NHVY_NIST NISTmAb heavy chain	QVTLRESGPALVKPTQLTLCTFSGFSLTAG...	432.76	
<input type="checkbox"/>	2	nm NLIGHT NLGT_NIST NISTmAb light chain	DIQMTQSPSTLSASVGDRVTITCSASSRVGYM..	201.62	
<input type="checkbox"/>	3	sp P00761 TRYP_PIG Trypsin	FPTDDDDKIVGGYTCAANSIPYQVSLNSGSHF..	53.65	
<input type="checkbox"/>	4	tr G3I4H6 G3I4H6_CRIGR Fructose-bisphosphate aldolase O...	MPYPYPALTPPEQKELSDIAHRIVAPGKGILAA...	47.12	
<input type="checkbox"/>	5	tr A0A8C2MCQ6 A0A8C2MCQ6_CRIGR Protein disulfide-iso...	MRLLGMARLGFLVSTCTFFLAASGLYSSDDV...	11.39	

- **Optimized persistence of Isotope plot data**

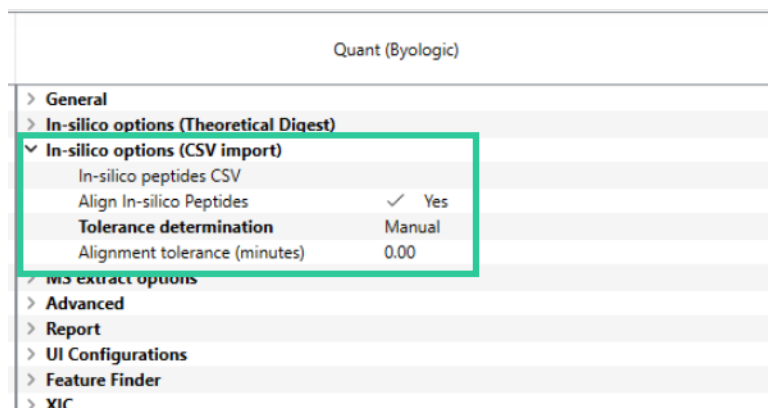
Rather than saving the entire scan, isotope plot persistence has been optimized in Byos v5.11 by only saving a small part of MS1 scans to the database for in-silico peptides. This significantly reduces the *.bgc and *.mpq file size while still providing ample context around the isotope masses in the mz domain.

- **New parameters have been added to the Quant (Byologic) processing node**

The following parameters have been added to the Quant (Byologic) processing node:

Under **In-silico options**:

- a. **Align In-silico Peptides** (True/False; Default = True)
- b. **Tolerance determination** (Auto/Manual; Default = Manual for Hotspot and MAM New Peak Detection workflows, Auto for all other workflows possessing this node)
- c. If **Tolerance determination** is = **Manual**, an additional **Alignment tolerance (minutes)** variable becomes available with the following default values:
 - i. **Hotspot:** 1.0
 - ii. **MAM New Peak Detection:** 0.25
 - iii. **All other workflows:** 0.0



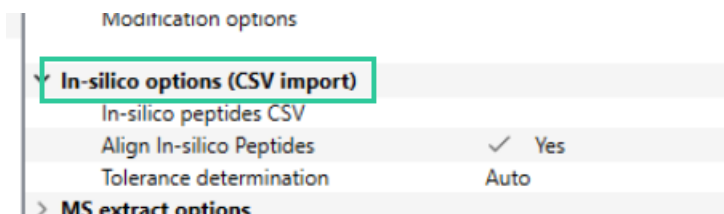
- **Users can now group multiple proteins by class or user-defined criterion**

Proteins can now be grouped by class or user-defined category.

This editing action is audit-logged. Undo/redo is not supported.

- **Updates to parameters within the Quant (Byologic) processing node**

The “In-silico peptides (CSV)” parameter has been renamed to “In-silico options (CSV import)” within the **Charge Variant Analysis with Reconstruction** workflow.



Digested Oligonucleotide, Byos

- **Enhanced inspection for Digested Oligo Mapping**

mRNA Oligo Mapping often results in digest products with sequences that map to multiple locations on the mRNA, as well as isomeric digest products (which have the same nucleotide composition but differ in order). The ability to monitor and filter unique masses/sequences from non-unique ones is significantly more critical for oligo mapping compared to peptide mapping.

To assess uniqueness, we utilize two existing metrics: Sequence occurrence(s) and the Delta score. The first metric, **Sequence occurrence(s)**, indicates how many times a digest sequence appears within and across intact oligo sequences. The **Delta** score, on the other hand, helps us determine if different sequences of the same mass could explain the MS2 spectrum differently. A

Delta score of 0.0 signifies that there is insufficient fragment information to differentiate between isomers.

We categorize oligo uniqueness into three distinct levels, which are color-coded as follows:

- **Green:** The most unique category, where Protein occurrence(s) = 1 in 1 and Delta score > 0.0.
- **Yellow:** This category includes rows where Protein occurrence(s) != 1 in 1 and Delta score > 0.0. Here, we are confident in the sequence assignment but cannot pinpoint the sequence to a specific location.
- **Red:** This category consists of rows with Delta score = 0.0, indicating that we cannot confidently distinguish the reported sequence from other isobaric sequences.

MS2 spectra are nested under parent rows in the Oligonucleotides table. To quickly view the colored rows, users may utilize the 'Expand rows' button at the top of the table.

Expand rows button

Color legend added to banner

Yellow = Delta Score > 0.0 & Protein Occurrences != '1 in 1'

Blue = New row selection color

Green = Delta Score > 0.0 & ProteinOccurrences = '1 in 1'

Red = Delta Score = 0.0

Oligonucleotides Unique Repeat sequence Non-unique isoform

Row#	Protein occurrence(s)	Delta Score	Sequence format	Validate	ppm	z	XIC area summed	AUC	m/z	Obs. m/z	Obs. m/z
1	3 in 1	41.4 - 66.1	ACC	1.99; 1.99; ...	-2	7.43e+06	1.57e+06 - 3.39e+06	621.5729	621.5742; 62...	gijml	
1.1	3 in 1	44.1 - 66.1	ACC	2.28; 2.48; ...	-2	3.39e+06	3.39e+06	621.5729	621.5743; 62...	gijml	
1.1.1	3 in 1	44.1 - 66.1	ACC	2.28; 2.48; ...	-2	3.39e+06	3.39e+06	621.5729	621.5743; 62...	gijml	
1.1.1.1	3 in 1	44.1	ACC	2.28	-2	3.39e+06	3.39e+06	621.5729	621.5743; 62...	gijml	
1.1.1.2	3 in 1	45.0	ACC	2.48	-2	3.39e+06	3.39e+06	621.5729	621.5743; 62...	gijml	
1.1.1.3	3 in 1	66.1	ACC	2.48	-2	3.39e+06	3.39e+06	621.5729	621.5743; 62...	gijml	
1.2	3 in 1	41.4 - 45.3	ACC	1.99; 1.99	-2	2.48e+06	2.48e+06	621.5729	621.5742; 62...	gijml	
1.3	3 in 1	71.3	ACC	2.18	-2	1.57e+06	1.57e+06	621.5729	621.5743	gijml	
2	8 in 1	71.3 - 71.3	GAGC	1.71; 2.18; ...	-2	1.37e+06	301 - 9.46e+05	661.0871	661.0882; 66...	gijml	
2.1	8 in 1	71.3	GAGC	1.71	-2	9.46e+05	9.46e+05	661.0871	661.0882	gijml	
2.2	8 in 1	71.3	GAGC	2.18	-2	4.24e+05	4.24e+05	661.0871	661.0882	gijml	
67	1 in 1	0.0 - 45.9	GGGG...	2.98; 2.98; ...	-3; -2	3.38e+07	3.38e+07	661.0871	661.0882	gijml	
67.1	1 in 1	0.0 - 45.2	GGGG...	2.58; 3.23; ...	-3; -2	2.14e+07	2.14e+07	661.0871	661.0882	gijml	
67.1.1	1 in 1	43.5 - 45.2	GGGG...	2.58; 3.23; ...	-3	6.16e+06	6.16e+06	751.4491	751.4510	gijml	
67.1.1.1	1 in 1	45.2	GGGG...	2.58	-3	6.16e+06	6.16e+06	751.4491	751.4510	gijml	
67.1.1.2	1 in 1	43.7	GGGG...	3.23	-3	6.16e+06	6.16e+06	751.4491	751.4510	gijml	
67.1.1.3	1 in 1	43.5	GGGG...	2.74	-3	6.16e+06	6.16e+06	751.4491	751.4510	gijml	
67.1.1.4	1 in 1	44.7	GGGG...	2.98	-3	6.16e+06	6.16e+06	751.4491	751.4510	gijml	
67.1.2	1 in 1	0.0 - 31.2	GGGG...	3.36; 3.82; ...	-2	1.52e+07	1.52e+07	1127.6773	1126.6777	gijml	
67.1.2.1	1 in 1	29.5	GGGG...	3.36	-2	1.52e+07	1.52e+07	1127.6773	1126.6777	gijml	
67.1.2.2	1 in 1	31.2	GGGG...	3.82	-2	1.52e+07	1.52e+07	1127.6773	1127.6816	gijml	
67.1.2.3	1 in 1	24.4	GGGG...	2.63	-2	1.52e+07	1.52e+07	1127.6773	1127.6802	gijml	
67.1.2.4	1 in 1	0.0	GGGG...	2.41	-2	1.52e+07	1.52e+07	1127.6773	1127.6802	gijml	
67.1.2.5	1 in 1	0.0	GGGG...	3.49	-2	1.52e+07	1.52e+07	1127.6773	1127.6802	gijml	
67.1.2.6	1 in 1	0.0	GGGG...	4.00	-2	1.52e+07	1.52e+07	1127.6773	1127.6802	gijml	
67.1.2.7	1 in 1	0.0	GGGG...	3.36	-2	1.52e+07	1.52e+07	1127.6773	1127.6802	gijml	

Release 2025-08 (v5.10)

Reports, Byos

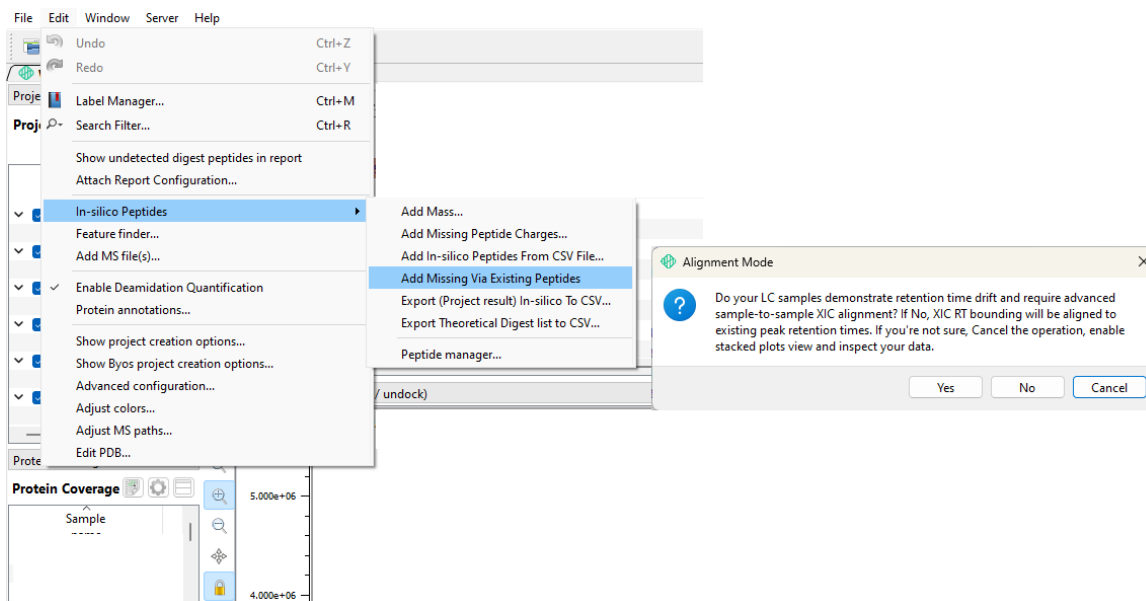
- **New template and tab preset available that support export to GraphPad Prism**

The new template, **MPQ_Prism_Ready_Export.rptc**, and tab preset, **MPQ_Prism_Ready_Export.tabc**, are now available. The report/tab preset contain a single tab titled "Export_Prism" comprising of a Pivot Table. This tab shows the report data in a format which facilitates exports from Byos to GraphPad Prism.

Peptide, Byos

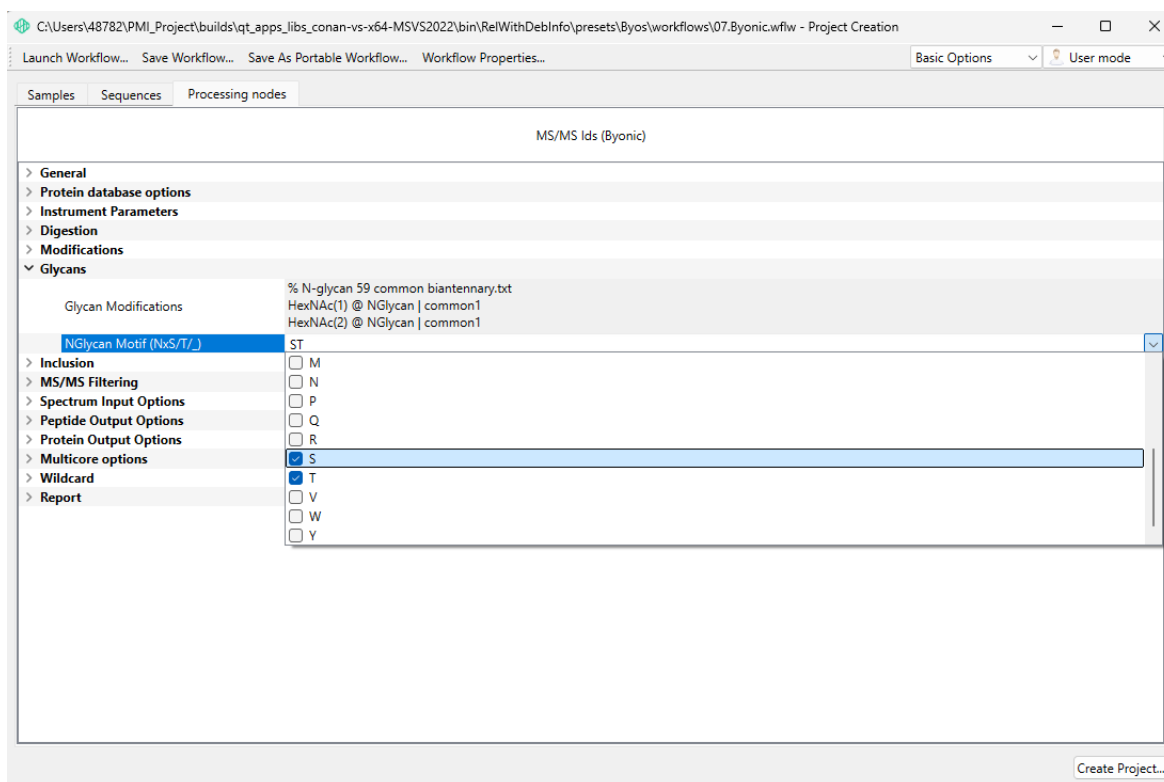
- **Users can now apply advanced sample-to-sample XIC alignment and peak detection to newly added peptides**

A dialog has been added to the Edit->Insilico Peptides->Add missing via existing peptides selection, allowing users the option to apply advanced sample-to-sample XIC alignment and peak detection to newly added peptides.



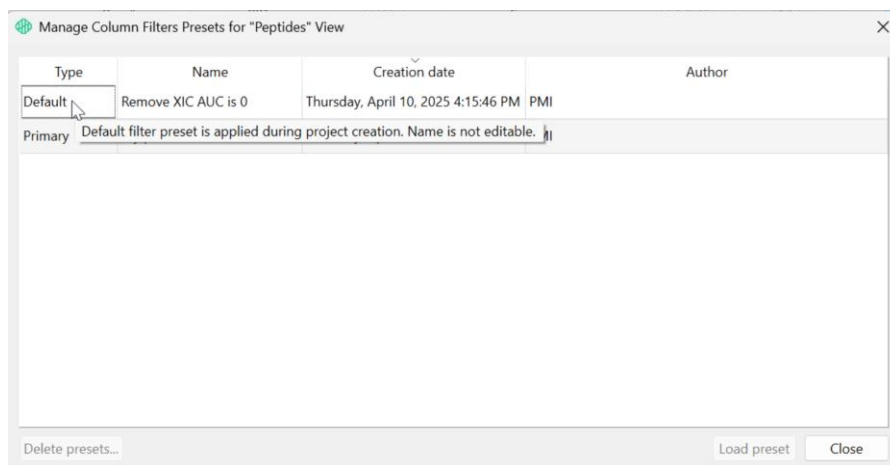
- **Added option to define third residues for Glycan motifs.**

Users now have the option to define the third residues for Glycan motifs. The default motif in default workflows is NXS/T, but users can now add or replace the third residue with other residues. This is performed through **Glycans>NGlycan Motif (NxS/T/_)** in all workflows containing the Byonic node excluding the Digested Oligonucleotides workflow.



- **Improvements to the Column Filter presets**

1. The preset name embedded in a given Workflow will not be overwritten; the original name of the preset will be maintained as it may indicate the purpose of the filter e.g. "Remove XIC AUC is 0".
2. The Default preset has been made read-only making its name not editable.
3. Custom tooltips have been added for both Default and Primary presets to help distinguish them from each other.



MOBILion, Byos

- Upgraded MOBILion Reader to v1.11.1

Multiprotein Quantitation, Byos

- A new "Inclusion List" field has been added to the MS/MS Identification node in Multi-protein workflows

Added a new field called **Inclusion list** to Multi-Protein Identification and Multi-Protein Quantitation workflows under MS/MS **Identification>Inclusion**. The field can be modified in a new Inclusion dialog where the user can enter inclusion values in a dedicated table:

Inclusion list

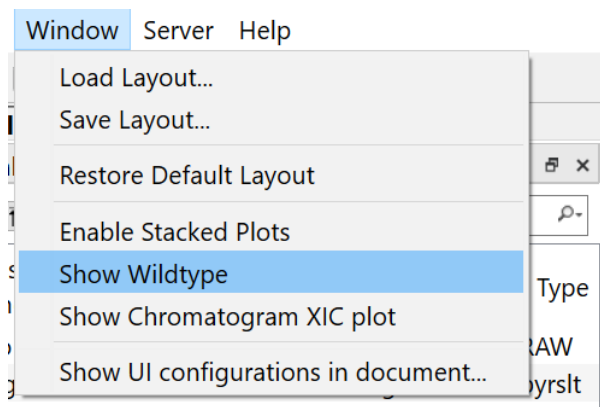
m/z begin	m/z end	Elution time begin	Elution time end
200.0000	500.0000	0.0000	25.0000
800.0000	1000.0000	45.0000	55.0000

Import CSV Export to CSV Add row Delete row Clear OK Cancel

Oligo, Byos

- **Updated Wildtype behavior for Digested Oligonucleotide projects**

The first time a Digested Oligo project is created the **Wildtype** widget will be turned **off** by default. From that point on, their state is controlled by the user and does not affect other projects.




Each subsequent Digested Oligo project will inherit the setting from the previous session; if Wildtype was *disabled* in the previous session, it will remain off when the next Digested Oligo project is opened or created. If Wildtype was *enabled* in the previous session, it will remain enabled for the next Digested Oligo document that is opened or created.

- **Byos will now use the Oligo mapping report template for multi doc reports generated from Digested Oligonucleotide projects**

The Oligo mapping template, **Blgc_Oligo_Mapping.rptc**, will now be used when generating multi-doc reports from Digested Oligonucleotide projects.

- **Collision Energy (CE) scan information for MS2 is now available in the MS2 data table**

MS2 Data 

Selected	Time	File	Precursor	z	CE	Mass
<input type="checkbox"/>	6.43	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.44	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.49	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.50	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.52	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.57	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.58	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.60	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.65	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.66	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.68	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.73	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.74	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.76	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	6.81	...es/Oligo/40-mer MSMS 17CS 8CE.d	722.06	17-	8	12286.12
<input type="checkbox"/>	4.59	...es/Oligo/40-mer MSMS 18CS 4CE.d	681.89	18-	4	12286.12
<input type="checkbox"/>	4.61	...es/Oligo/40-mer MSMS 18CS 4CE.d	681.89	18-	4	12286.12
<input type="checkbox"/>	4.65	...es/Oligo/40-mer MSMS 18CS 4CE.d	681.89	18-	4	12286.12
<input type="checkbox"/>	4.67	...es/Oligo/40-mer MSMS 18CS 4CE.d	681.89	18-	4	12286.12
<input type="checkbox"/>	4.69	...es/Oligo/40-mer MSMS 18CS 4CE.d	681.89	18-	4	12286.12

Byonic, Byos

- **Byonic can now search FASTA databases with more than 15 million sequences**

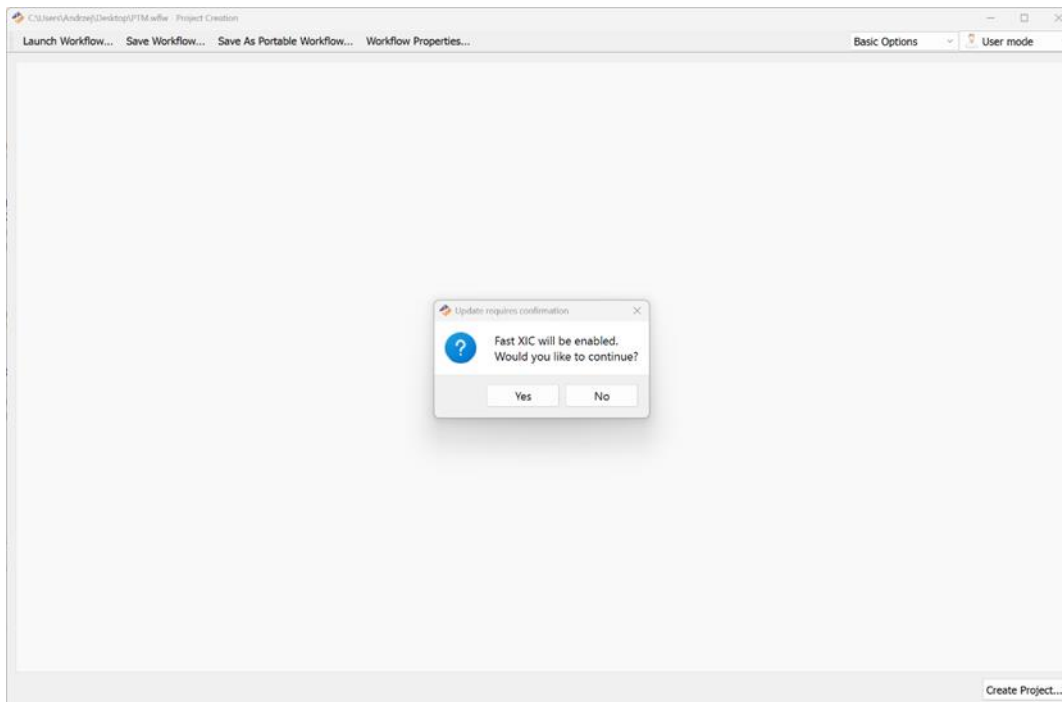
Byonic now can search FASTA databases with more than 15 million sequences. When searching large FASTA databases, 64 GB of RAM is the minimum amount of memory needed (128 GB RAM recommended). **Note: Normal and Heavy multicore options are not recommended for extremely large fasta database searches.**

Release 2025-04 (v5.9)

Peptide, Byos

- **Fast XIC will now be enabled during workflow update**

Fast XIC will now be enabled during workflow updates if the feature is not yet enabled. Users will be prompted with a dialog asking if they wish to proceed with this step.



- **Multiple annotations per peak are now allowed in the annotated MS2 spectrum plot**

Multiple annotations can now exist on a single peak within the annotated MS2 spectrum plot.

- **Asymmetric cleavage products have been extended to cleavable crosslinkers (DSBU) and MS2 spectra will now be automatically annotated**

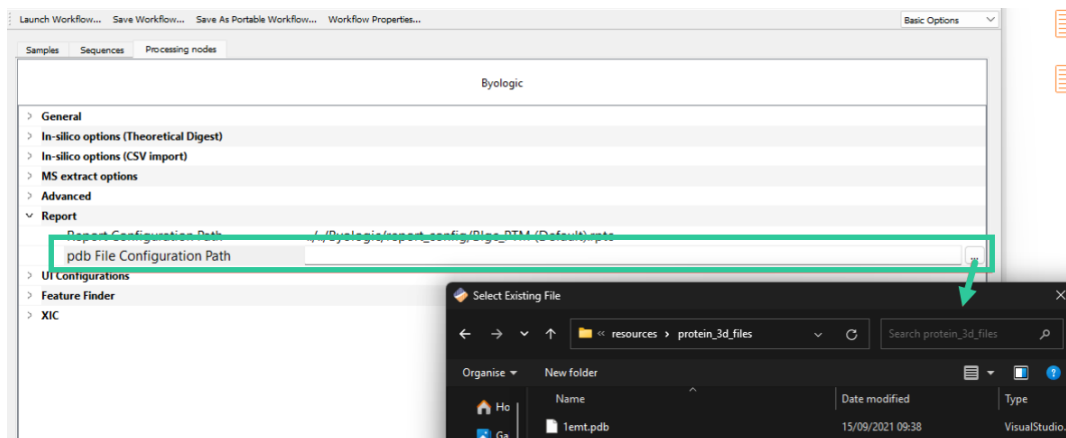
Asymmetric cleavage products, Pep+85 and Pep+111, are now made for DSBU-Xlinked peptide spectra. These annotations will only be made when **DSBU / 196.0848 @ K | xlink** is specified in the Project Creation parameters.

- **Improved fragmentation labels for disulfide neutral losses**

Annotation of neutral loss fragment ions, including Pep-NH3, Pep-SH2, and Pep-SCH2, from dissociated peptides following ExD fragmentation of crosslinked precursors are now available in MS2 plots.

- **Added ability to add pdb file into report during workflow setup**

Users can now add a pdb file into their report during Project Creation for Peptide-level workflows.



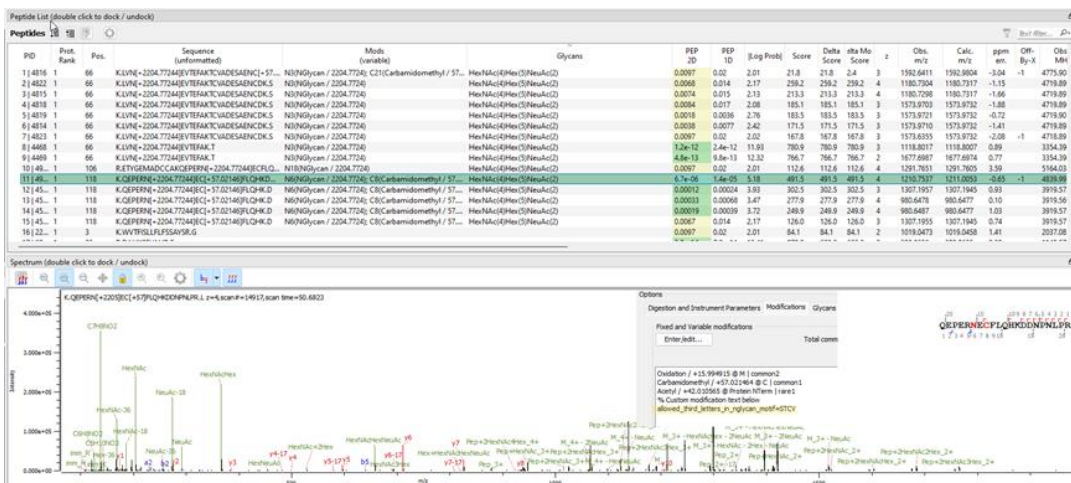
Glycans, Byos

- Improvements in glycan fragmentation annotation**

Sources of error in peak annotation are generally higher in glycan spectra compared to other biomolecule types since possible fragments are more likely to differ by just 1 or 2 Daltons. The glycan fragment annotation tool has been updated to prevent against annotation of +1, +2, etc. isotope peaks in glycan MS2 spectra.

- Enhanced customization of the N-glycan motif to consider non-canonical N-glycosites is now available**

Customization of the N-glycan motif to consider non-canonical N-glycosites is now available. This is done by entering `allowed_third_letters_in_nglycan_motif=[x]` into the Byonic modifications field, where [x] is a string of amino acid letters that are allowed in the +2 position. For example, using `allowed_third_letters_in_nglycan_motif=STC`, Byonic will search for that N-glycan mods only in cases where there is the motif **NX{S/T/C}**.



(Data is from doi.org/10.1186/s12967-024-05000-5)

General, Byos

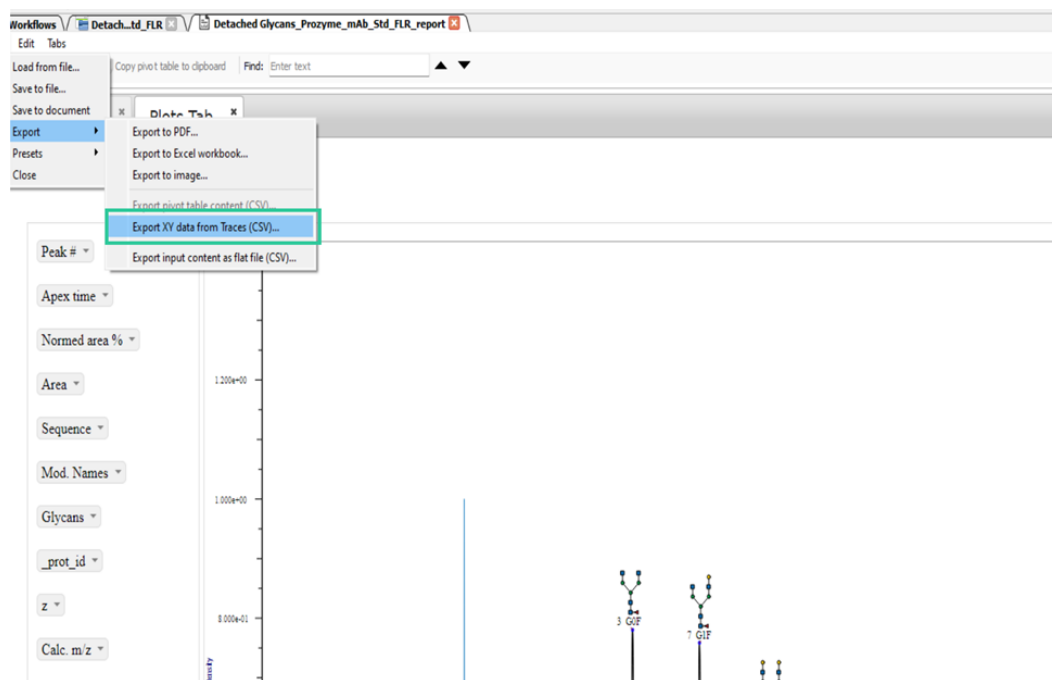
- Shimadzu reader has been upgraded to IoModule.4.2.0.7552**

Note: Readers supplied to Protein Metrics by mass spectrometry manufacturers are used as-is, without alteration. Any issues can be reported directly to the manufacturer with Protein Metrics support in cc.

Reports, Byos

- **Users can now export XY data from traces within Byos Reports**

Users can now export the underlying XY data from Trace plots within their Byos report. Doing so will provide the X and Y values needed to create the trace plot shown within a CSV file. Note that this export will not contain any integration information – users can export XY data for specific fragments directly from the Project by right-clicking on the plot of interest, clicking Export to CSV, and selecting Yes when asked to use current plot segment.



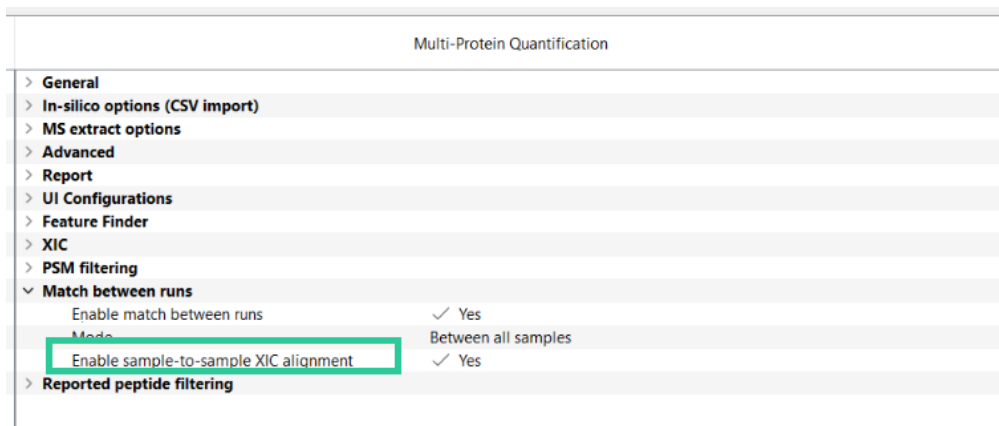
Multi-Protein Quantitation, Byos

- **Multi-Protein Identification reports will not be generated by default**

The Multi-Protein Identification report will not be generated upon project creation by default—users can now generate the report manually once the project has been loaded.

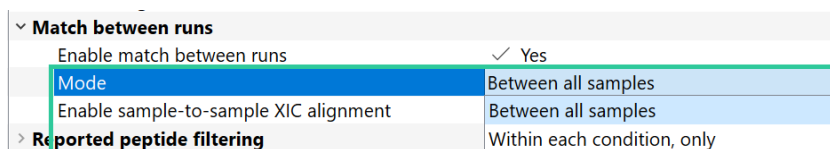
- **Enable sample-to-sample XIC alignment added and set to default within Multi-Protein Quantitation processing nodes**

A new **sample-to-sample XIC alignment** parameter is now enabled by default to improve XIC RT bounding for in-silico peaks added by the Match Between Runs parameter. Disabling this feature could reduce overall processing time by 10% or more, but the XIC RT bounding improvements will not be applied.



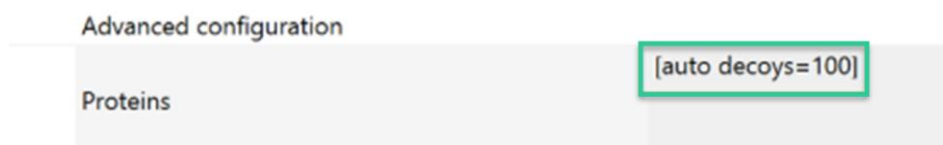
- **New option to restrict Match Between Runs within condition**

Users can now apply Match Between Runs between *all* samples (default) or within each condition. This mode is available under the Match between runs tab in the Processing Nodes for the Multi-Protein Quantitation workflow.



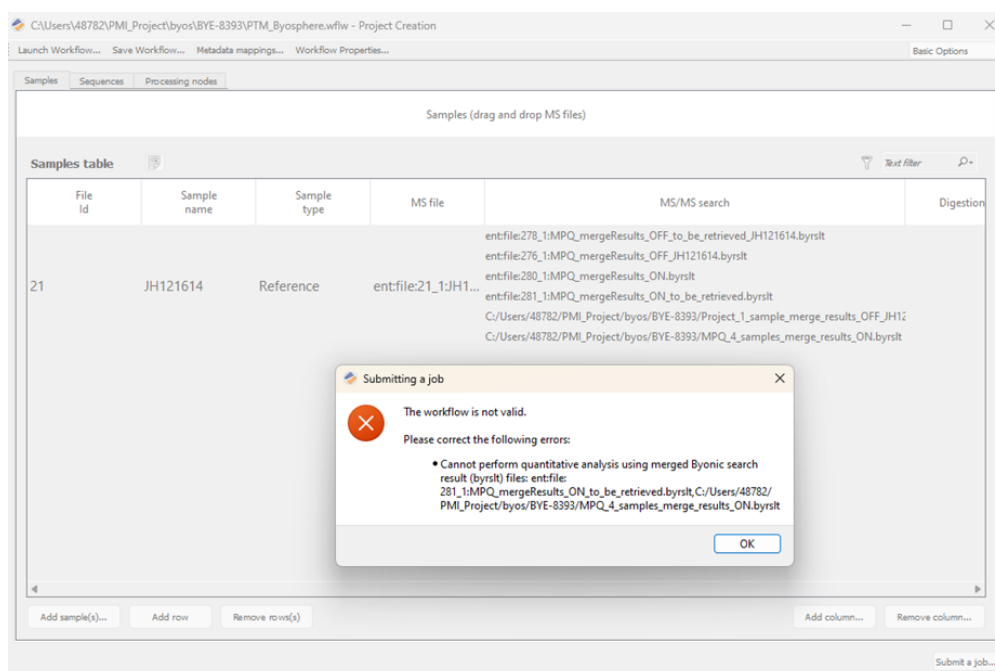
- **Updated Multi-Protein Quantitation workflows to have a default autodecoy value of 100**

The default value for 'auto decoys' has been updated to 100 in the MPQ workflow.



- **Users will now be informed if trying to start an MPQ analysis from a merged ByrsIt file**

Added a pre-flight check for all workflows containing "MS/MS search" column that prevents using .byrsIt file created using a merged byspec2 file.



- **Multi-Protein Preview analyses now create Byos temp and preset files within the target output folder**

Temporary folders are now created in the output directory for Preview-like workflows.

- **The Multi-Protein Quantitation workflow will now preferentially use unique peptides for quantitation when there are proteins that share the same peptides(s) as part of their ID**

In the **Multi-Protein Quantitation** workflow, peptide prioritization for TopN will now proceed using the following logic:

- Prioritize distinct peptide sequences (preferring a peptide ranked lower by AUC over a duplicate sequence with different modification).
- Prioritize peptides that are more abundant *per charge*. Thus, abundance of a peptide with regards to TopN is not the maximum per-charge average abundance (previously, average abundance across all charges).

Preview, Byos

- **Preview has been added to the Byos homepage as a standalone workflow**

Preview is a standalone desktop application that performs a first-pass, prospecting style search to identify key parameters that can subsequently be used for Byonic peptide search. Mass errors, digestion specificity, and common modifications are among the parameters that Preview will suggest. This update allows users to access the tools of Preview within a Byos workflow.



Preview

Preview for Byonic
Identification of Peptides
and PTMs

- **Preview now supports Waters raw files**

The standalone Preview application can now accept Waters raw data as an input.

icIEF-MS, Byos

- **Labels are now automatically applied to Deconvolved icIEF-MS plots**

Labels on the Deconvolved Mass plot are now rendered for icIEF-MS projects, with the functionality now the same as in Intact (.ntms; .olms) workflows.