

Software Release Notes

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Protein Metrics Inc., Cupertino, California, 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™, and Preview™.

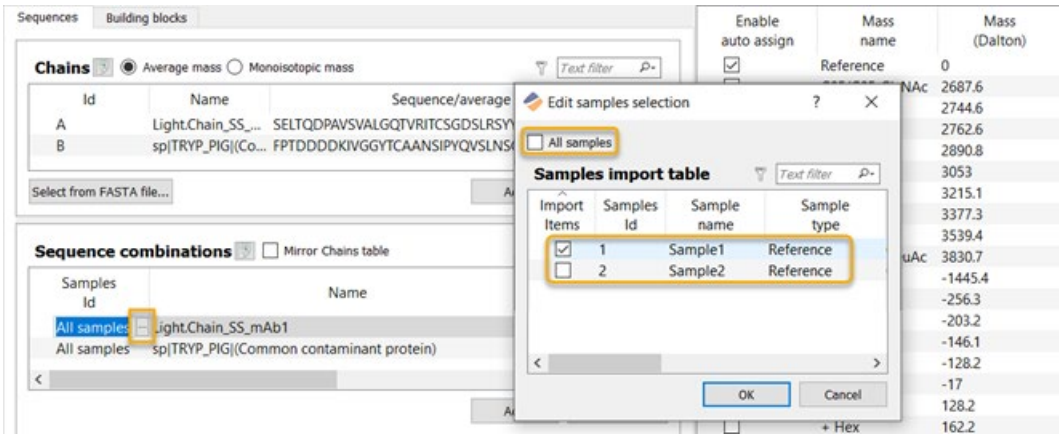
All enhancements are available within Byos.

Release 2020-09 (v3.10)

Sequence Combinations

- **Sequence combinations can be associated with individual samples**

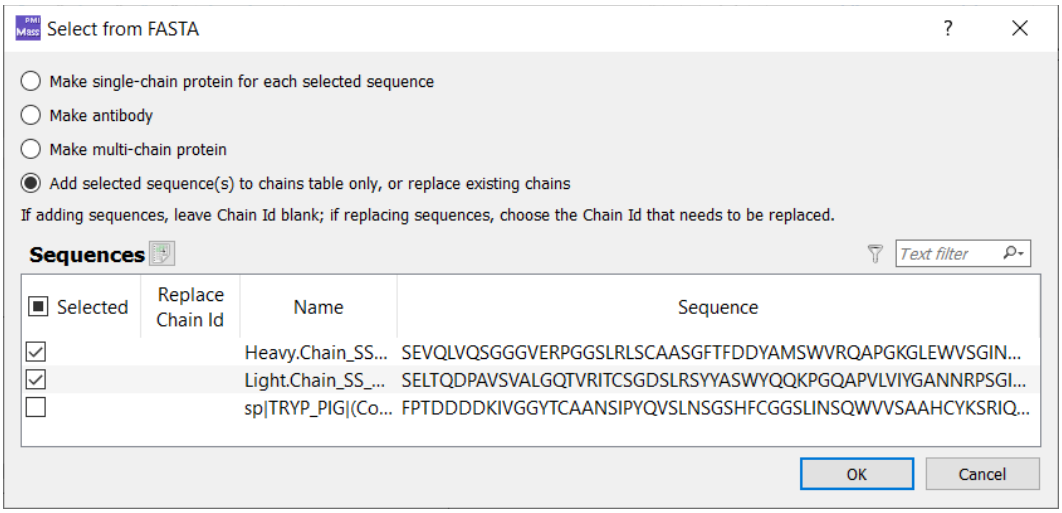
  Previously, Intact Analysis sequence combinations generated from sequences in FASTA files were always associated with all the samples. Now, a sequence combination can be associated with an individual sample:



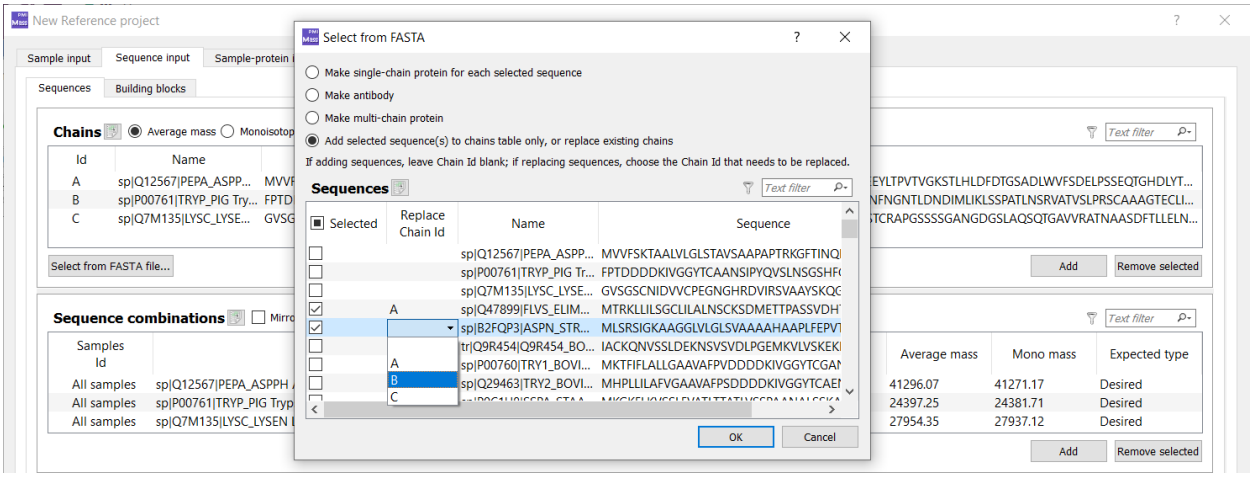
  Note that either **All samples** or a single sample ID can be checked. Multiple Sample Ids are not permitted.

- **Existing sequences can be replaced in the Chains and Sequence combinations tables**

  The **Select from FASTA** dialog radio button **Add selected sequence(s) to chains table only** has been extended to enable replacing existing sequences:



This option can be used to replace existing sequences added using any of the four sequence entry options. To replace existing sequences, click **Select from FASTA file** again and select the original or a new FASTA file. Select the **Add selected sequence(s) to chains table only, or replace existing chains** radio button. Choose the rows containing the new sequences by checking the boxes in the **Selected** column. Use the dropdowns in the **Replace Chain Id** column to select the Ids of the sequences to replace (by default, capital letters):

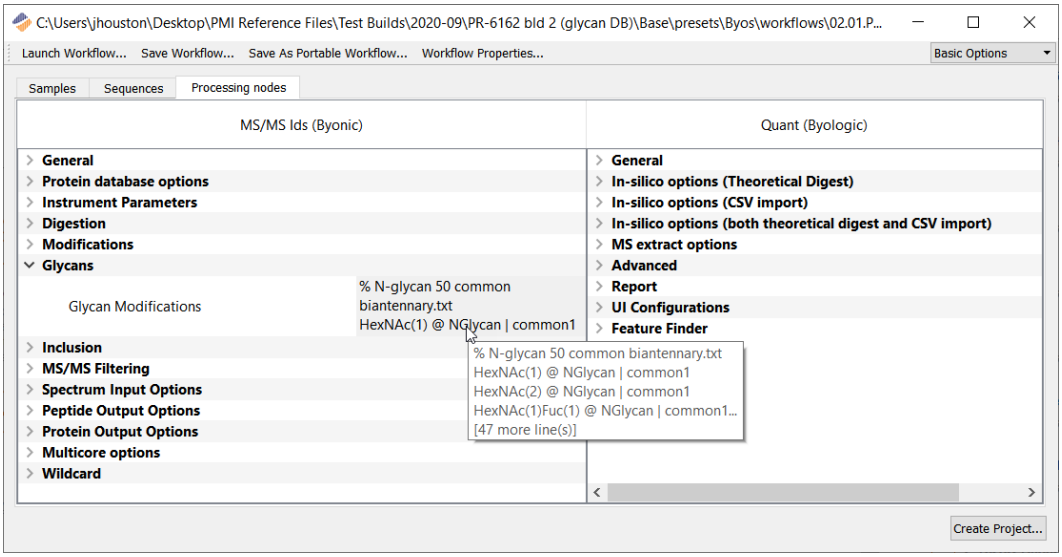


Click **OK** and the **Name** and **Sequence** entries in the Chains table are updated. Likewise, the Sequence combinations table records containing those updated sequences are also updated.

Glycans

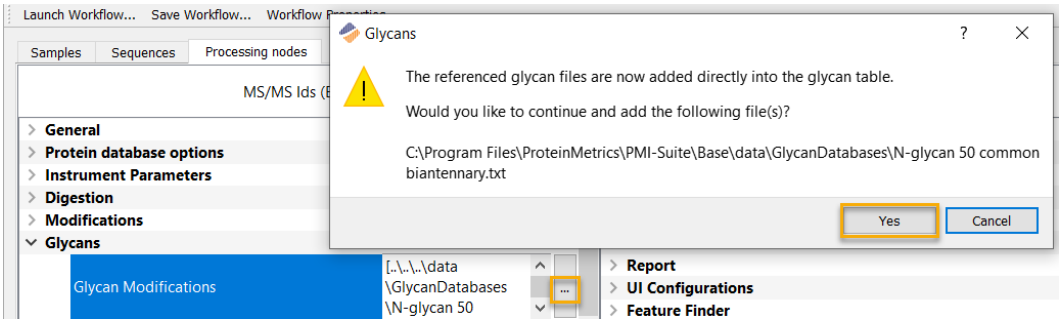
- **Detached Glycan Byos workflows use custom glycans in place of glycan DB file references**

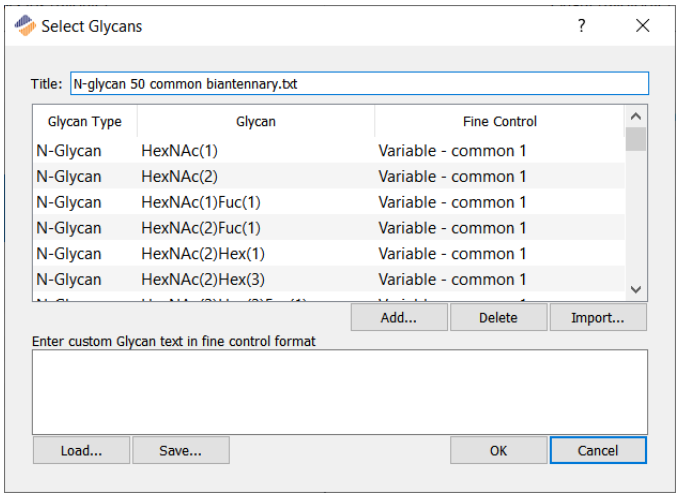
  In the standard Byos workflows, references to glycan DB files contain the content of those files as custom glycans:



- **Glycan DB file references in custom Byos workflows can be converted to custom glycans**

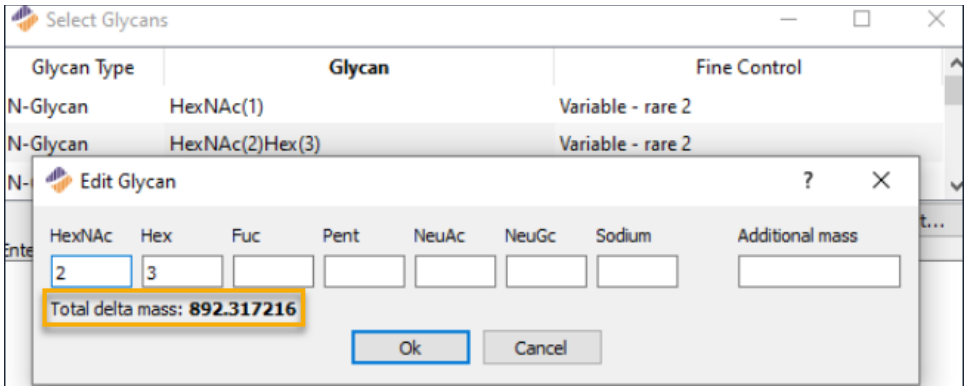
  For custom Byos workflows that contain references to glycan DB files, opening the glycan reference gives the option to convert them to custom glycans:





- **Glycan Total delta mass is displayed to 6 decimal places**

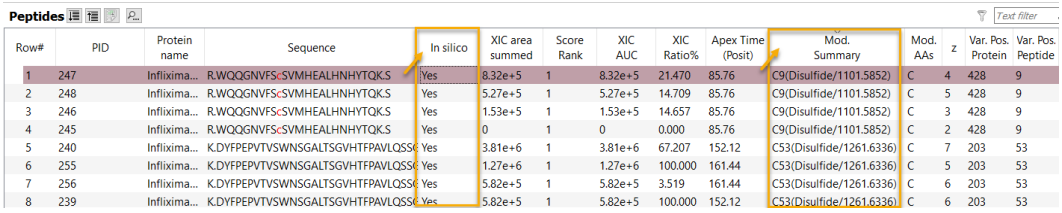
  When editing from the **Glycans** tab in Byonic, the **Edit Glycan** dialog displays **Total delta mass** to 6 decimal places:



Peptide Analysis, Byos

- **In-silico Disulfide mode**

In-silico has been generalized to handle disulfides. This is controlled by the new parameter **Disulfide mode** (on the **Processing nodes** tab in the Byos S-S workflow, or on the **In-silico options** tab in Byologic) which can be set to **Free peptides only**, **Disulfide complexes only**, or **Both**. For the latter two options, make sure to set the expected disulfide links (on the **Sequences** tab in the Byos S-S workflow, or on the **Disulfides** tab in Byologic). In the generated project, the disulfide data will populate the **In silico**, **Mod. Summary,** and **Xlink** columns in the **Peptides** table:



- **Mod Validator updates**

  The Mod Validator no longer treats pyroGlu (pyrolization of N-terminal glu/gln) as a sequence variant. Also, N-terminal pyrolization no longer triggers a wildtype error.

- **Wildtype Validator**

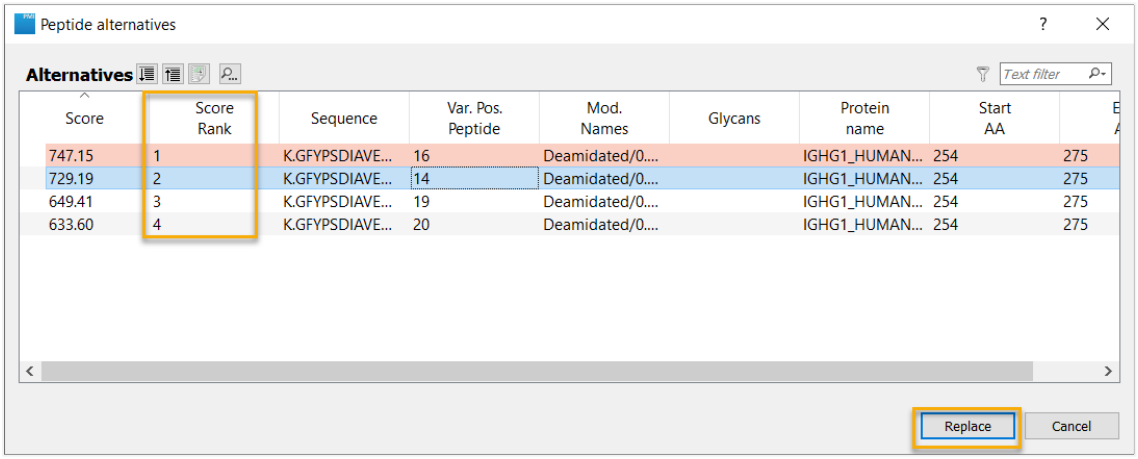
  The Wildtype Validator quickly scans wildtype PQMs for scoring violation. To use the Wildtype Validator, enter the following code in Advanced Commands:

```
[Byologic]
WildTypeValEnabled = true;


//defaults

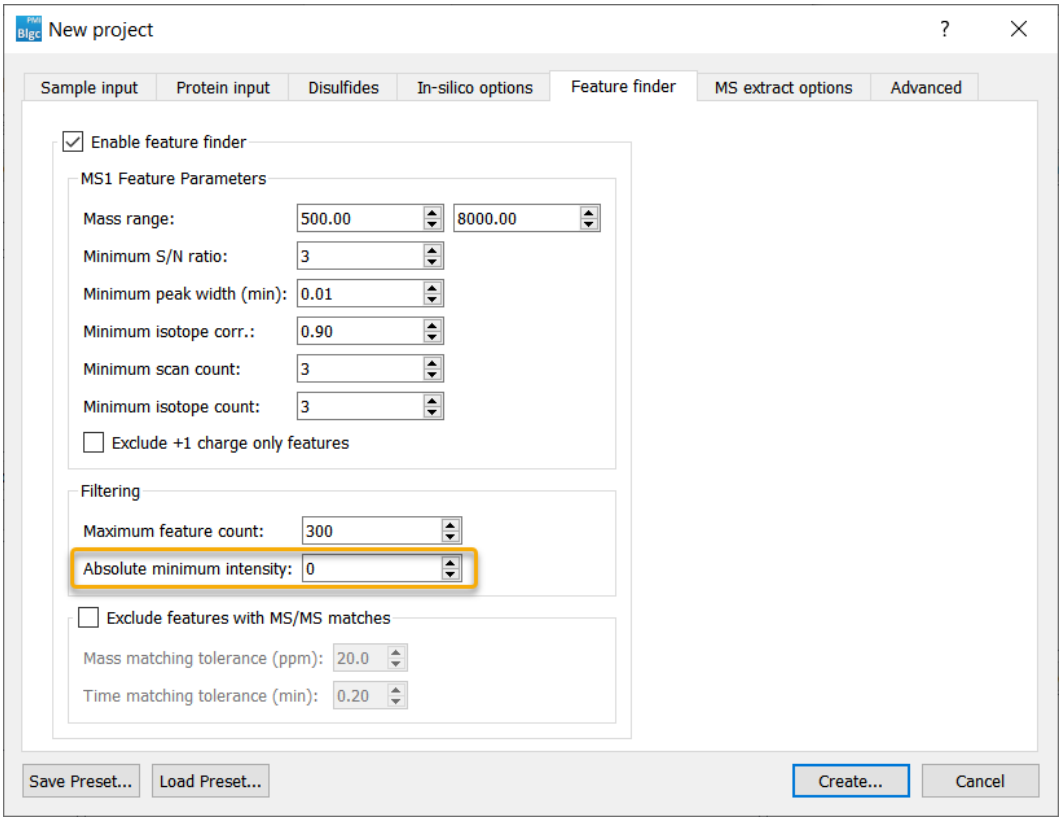

[WildTypeValidatorOptions]
ScoreThreshold = 225.0;
IntensityRecoveryPercentileThreshold = 0.05;
PPMStandardDeviations = 3;
```

- **Peptide Alternatives**
  It is now possible to select an alternative peptide assignment through the **Replace** button in the **Peptide Alternatives** dialog. Project values and features are updated when alternative proteins are selected:
    - Protein coverage updates
    - Calculated masses are updated for the fields Obs.M, Calc.M, Obs. m/z, Calc. m/z, and Off-By-X



- **New Feature Finder filter**

  A new filter in Feature Finder allows the user to narrow the list of features displayed in the project by **Absolute minimum intensity**. When filtering for projects that contain multiple samples, this setting will include a feature if the feature intensity from any sample passes above the threshold. The filter is applied on the summed intensities across all observed charge states and the user is encouraged to select **Combine charge states** under **Peptide grouping method** in the **Search filters** when viewing the data.



In the Peptide Analysis workflows in Byos, the new filter is in the Feature Finder section of the **Processing nodes** tab:

Quant (Byologic)

Advanced	
Report	
UI Configurations	
Feature Finder	
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

- **Protein summary list column**

  In the past, if a peptide was duplicate in more than one protein (i.e. in multi-specifics and HCPs) visibility into the other proteins was limited. In the new release, information about other proteins

can now be viewed in a new column **Protein summary list** which reports protein ID and other relevant information of all proteins containing the peptide sequence.

The new feature is enabled by adding the following advanced command:

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

- **Correcting primary proteins**

  In the past, if HCP searches against multiple samples yielded different primary proteins for a given sequence, there was no way to synchronize the samples. Now, inconsistent protein assignments can be corrected to assign the peptide in all samples to the highest-ranking protein. The new feature is enabled by adding the following advanced command:

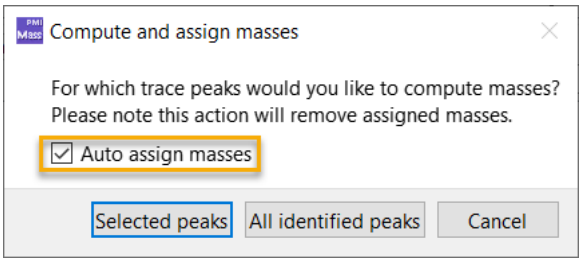
```
[Byologic]
AdjustPrimaryProtein = true;
```

Intact Analysis, Byos

- **Masses no longer become unassigned after deconvolution**

  When doing deconvolution after project creation, all found masses are assigned. Any previously present assigned masses are no longer protected, hence unassigned before the deconvolution and replaced with new masses in the new deconvolution.
  This corrected behavior applies to:

  - Clicking the orange **Compute** button in the **Deconvolved Mass** spectrum
  - Choosing **Edit > Compute Options**
  - Choosing **Edit > Auto compute and assign masses**. In addition, the **Auto assign masses** checkbox works correctly.

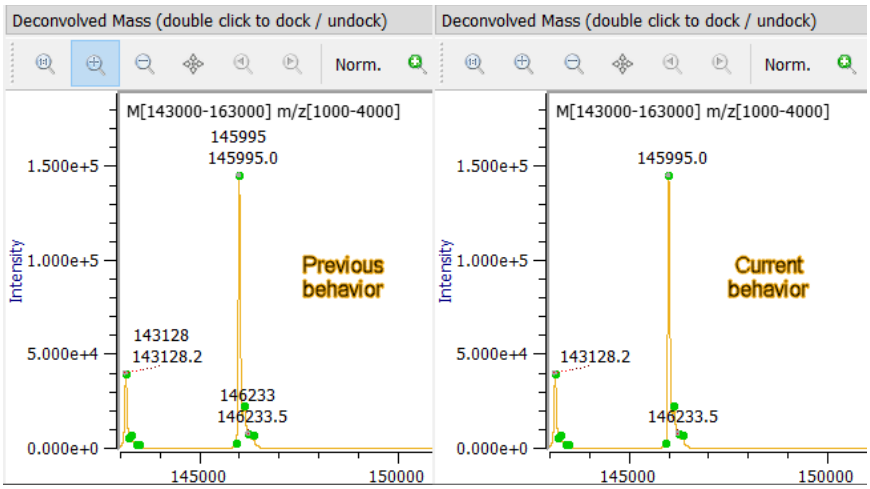


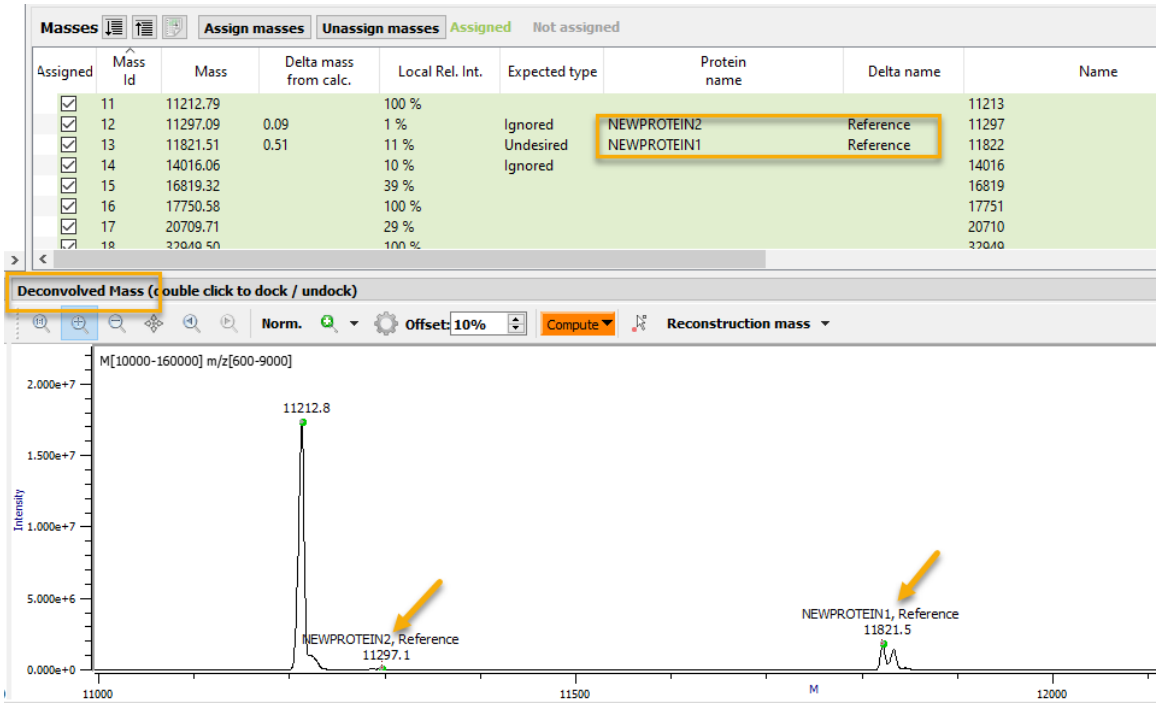
- **The Name column is updated after deconvolution**

  The **Name** column in the **Masses** table is updated when newly added delta masses or proteins have been matched after project creation. The new value in the Name column is most often created using the values in the **Protein name** and **Delta name** columns. The report is updated automatically with the new Name values.

- **Default candidate annotations**

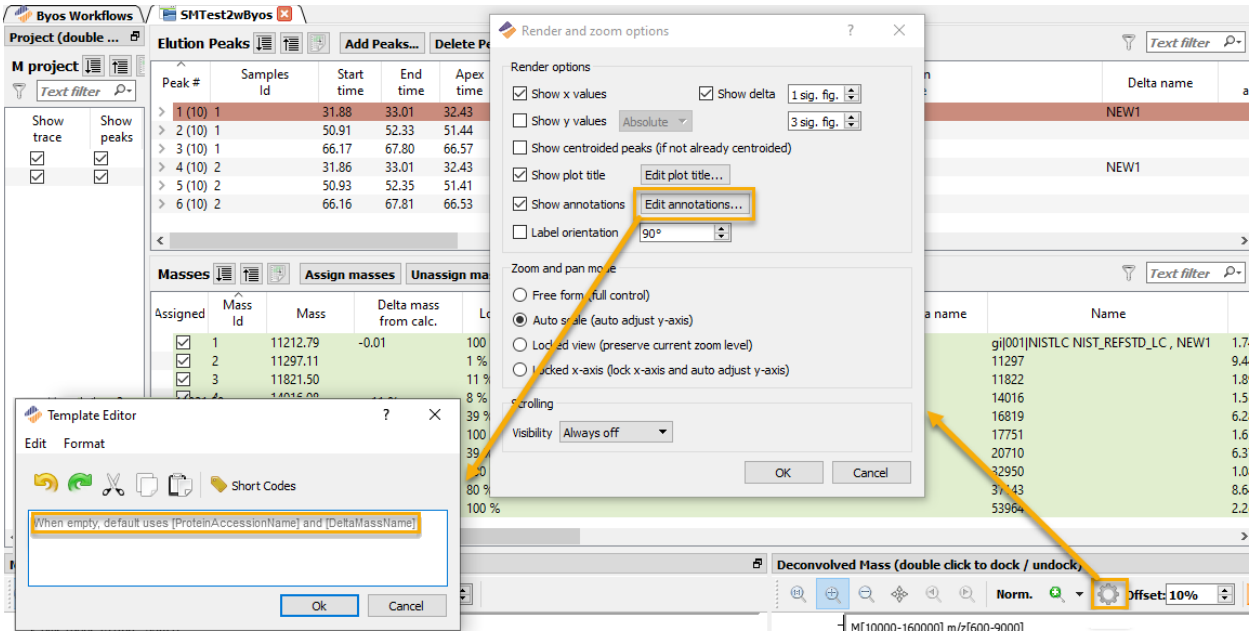
  Candidate annotations default to values from **Protein name** and **Delta name** instead of **Name**. This avoids duplication where Name values were derived from x-axis values:





- **Show annotations default**

  When the Template Editor for **Show annotations** is not assigned for a Deconvolved Mass plot, the editor shows the default annotations as **ProteinAccessionName** and **DeltaMassName**. To open the **Template Editor**, click the gear icon for the Deconvolved Mass plot, check **Show annotations**, and click **Edit annotations**:

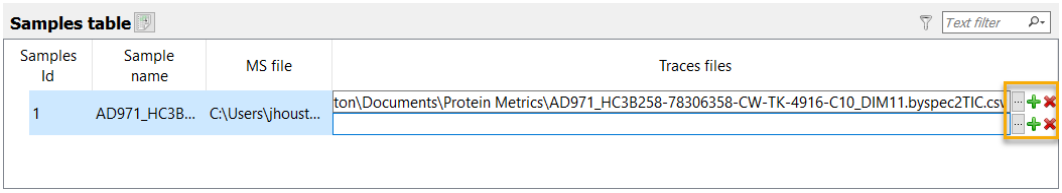


- **New Mass smoothing sigma default value**

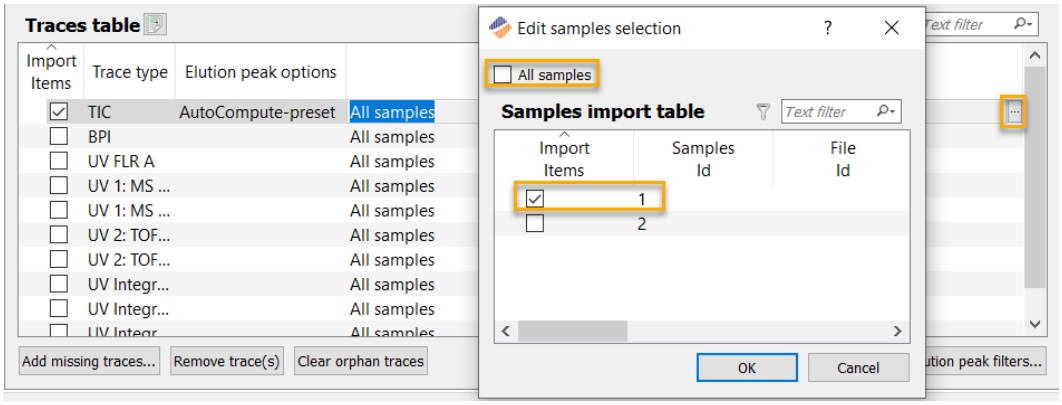
  In the Reduced workflow in Byos, the default value for **Mass smoothing sigma** is changed from **1** to **3**.

Chromatogram Analysis, Intact Analysis, Byos

- **Multiple traces can be added to a sample**



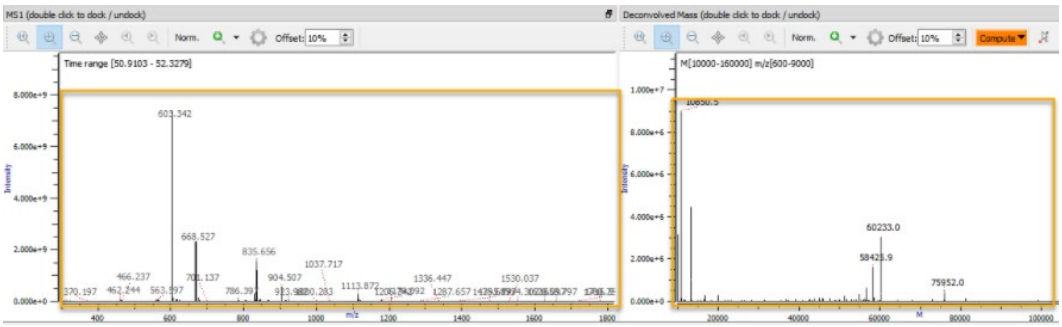
- **Either All samples or a single sample can be associated with a trace**



When associating samples to traces, only **All samples** or a single sample Id can be checked.

- **The dots displayed on plots can be turned off**

Advanced commands can be used to make the dots appear/disappear in Chromatogram, MS1 and deconvolved mass plots. Dots are visible by default.



To hide the dots, enter the following commands in the advanced configuration, and then click on another trace peak in the Trace peaks table:

```
[Rendering]
ShowMSLabelDots=false
ShowChromLabelDots=false
```

To make dots appear again, change false to true in the Advanced configuration, as shown below:

```
[Rendering]
ShowMSLabelDots=true
ShowChromLabelDots=true
```

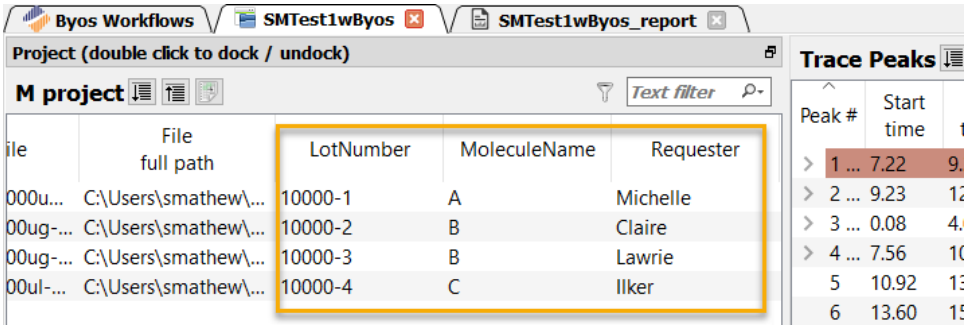
ShowChromLabelDots controls visibility of dots for Chromatogram plot, and ShowMSLabelDots controls visibility of dots for MS1 and Deconvolved Mass plots.

- **Time offset values default to 3 decimal places**

Byos

- **Sample-protein input imports into custom columns**

  CSV imports into the **Sample-protein input** tab now correctly populate custom columns in Intact Analysis workflows in Byos. CSV imports formerly only imported correctly into custom columns in Intact.



- **Relative file paths are updated to absolute files paths when saving workflows**

Relative file paths in legacy workflows (for example, `\...\Byomap\report_config\Bmap (Reference_Default).rptc`) are assigned their absolute paths when workflows are saved (for example, `C:\Program Files\ProteinMetrics\PMI-Suite\Base\presets\Byomap\report_config\Bmap (Reference_Default).rptc`). This applies to all reference file names, including report template files, glycan DB files, trace files, and UI column filter and layout files. This ensures that a workflow will continue to work if it is moved.

Byonic

- **Peak annotations for sulfated glycans**

  The Byonic Viewer now annotates peaks at 284 and 446 for sulfated HexNAc and sulfated HexNAc-Hex. These are small peaks and they do not always show clearly.

- **Custom peak scoring and annotation support**

  Byonic now supports user-specified custom peaks scoring and annotation. The theoretical peaks for the peptide fragments will contain these custom peaks. If the spectrum matches the custom peaks, they will be included in the Peptide Score.

  The custom peaks are constrained to belong to a particular modification rule in the **Modifications** tab in Byonic. An example of how to specify the custom peaks:

  ```
  [Woo2/+346.14575 @S,T | common2 | CustomPeaks{IsoTaG:347.1531, IsoTaG-36: 311.1320, IsoTaG-90: 257.11017}
  ```

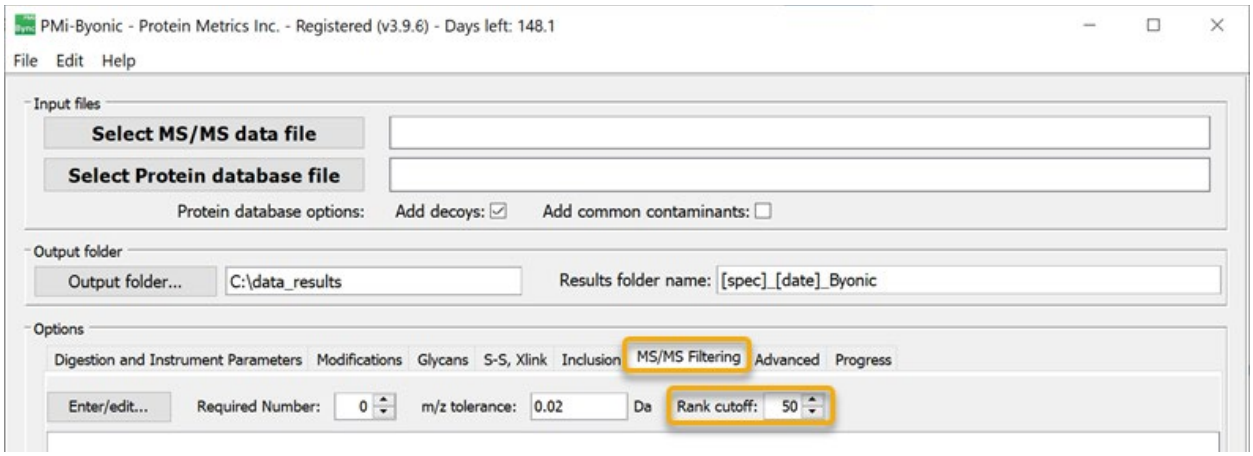
  - **Woo2/+346.145** - name/composition of the modification
  - **@ S, T** - target amino acid for modification
  - **common2** - type of modification (common, rare etc)
  - **CustomPeaks{peak_label: peak_mz} -** comma separated list of peak_label: peak_mz strings where you specify the strings of peak_label (ex IsoTaG-36) and peak_mz (ex 311.132)

- **Byonic Viewer displays the Thermo scan header**

  The Byonic Viewer captures the values in Thermo scan header and displays it in Byologic in the **Scan info** column.

- **Rank cutoff is set in the UI**

  The rank cutoff for filtering peaks can now be set in the **MS/MS Filtering** tab in Byonic. The default value is **50**. This means that a peak must be among the 50 most intense MS/MS peaks to count as a match.



Processing – File Reading

- **The Shimadzu vendor libraries have been updated**
- **Consistent chromatogram type naming**

  Chromatogram types and the naming convention of trace channels are consistent across all vendors (non-MS types are no longer all "UV").

- **Enhanced processing speeds**

  XIC processing can be accelerated by the following Advanced Command:

```
[MSReader]
XicMode=fast
```

The following optional settings are available for this command:

```
[MSReader]
FastXicGranularity=100000
FastXicMaxMz=3010
FastXicMaxIonCount=2000
```

Parameter	Description
FastXicGranularity	Integer value. 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 10000.
FastXicMaxMz	Integer value. The maximum m/z value during data acquisition. To acquire data in the range 200-2000, set MaxMz = 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. MaxIonCount = 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 2500is recommended. Default value is 1000.

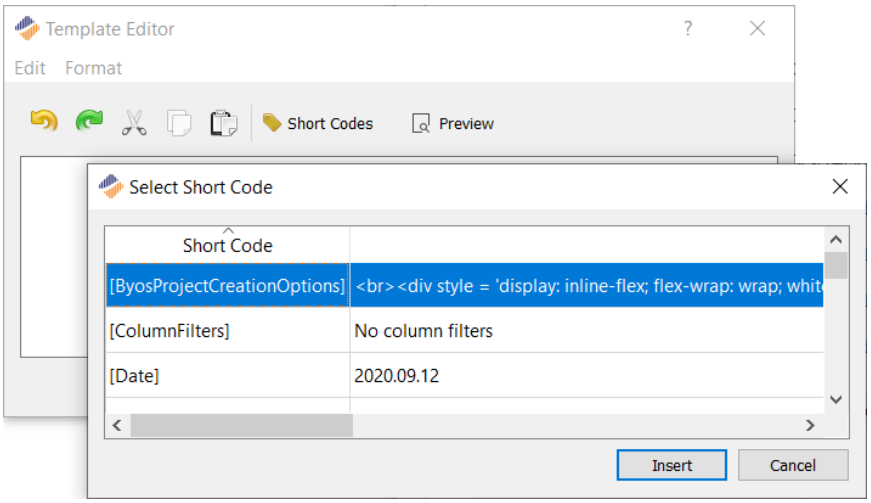
Reporting

- **Protein Summary List Column**
  The new column **Protein summary list** (see PMI_Suite_Software_Release_Notes) is available in a new rptc. Please reach out to support@proteinmetrics.com to receive the new file.

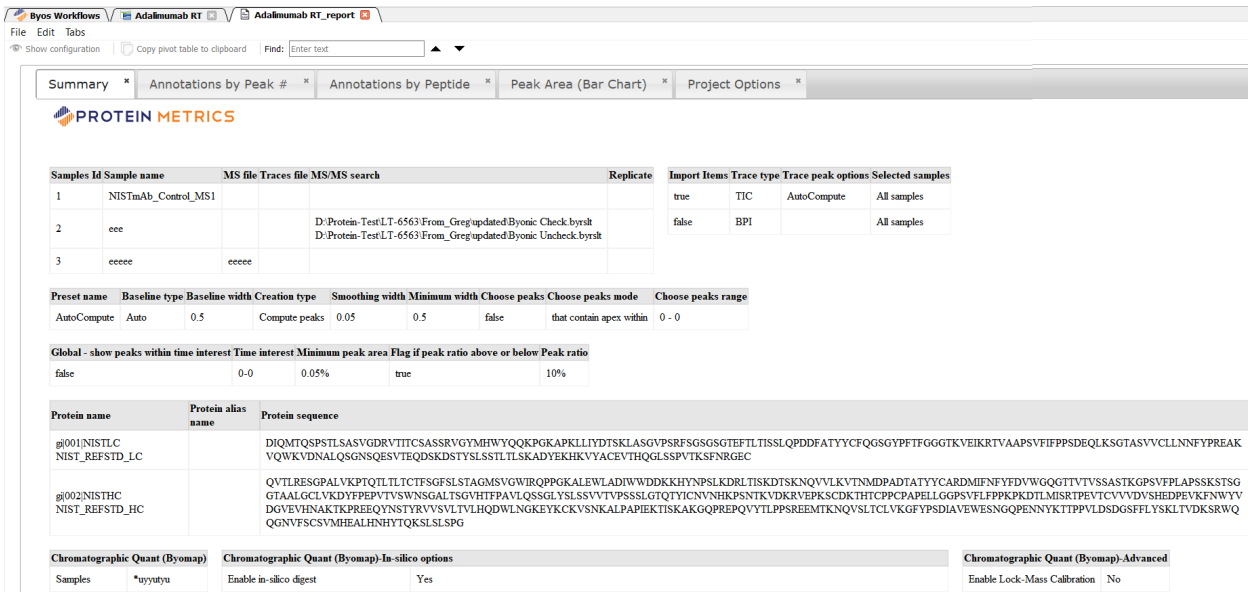
- **Byonic search parameters can be added to Chromatogram Analysis reports**

  The Byonic search parameters used to create Chromatogram Analysis projects in Byos can now be displayed in the Summary tab for reports. To display the search parameters, do the following:

  1. Open a new or existing **Summary** tab in the report
  2. Click **Edit > Current tab settings**
  3. Click **Edit summary template**
  4. Remove existing short code, if desired
  5. Click **Short codes**, select **[ByosProjectCreationOptions]** and click **Insert**

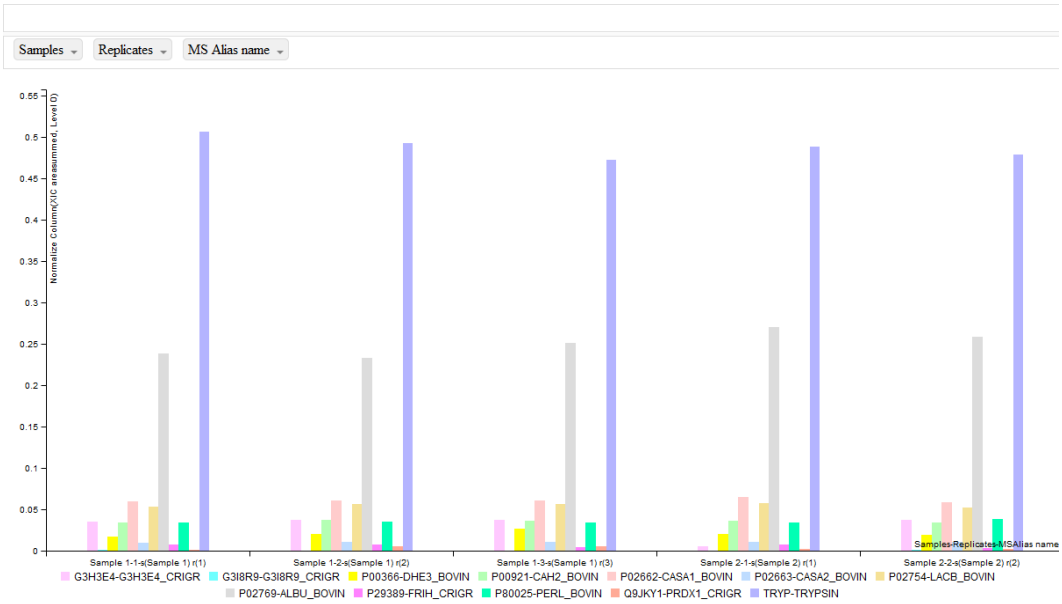


  6. Click **Ok** and the Summary tab is updated with the Byonic search parameters:



- **Default chart colors are pastels**

  The default colors used for report charts are now pastels:



  The default colors assigned by **Edit > Current tab setting** are also pastels:

Color settings:

G3H3E4-G3H3E4_CRIGR:	#FFC8FF
G3I8R9-G3I8R9_CRIGR:	#6EFFFF
P00366-DHE3_BOVIN:	#FFFF00
P00921-CAH2_BOVIN:	#B4FFB4
P02662-CASA1_BOVIN:	#FFCCCC
P02663-CASA2_BOVIN:	#BEDCFF
P02754-LACB_BOVIN:	#F5E196
P02769-ALBU_BOVIN:	#DCDCDC
P29389-FRIH_CRIGR:	#FF82FF
P80025-PERL_BOVIN:	#00FFB4
Q9JKY1-PRDX1_CRIGR:	#FFAA96
TRYP-TRYPSIN:	#B4B4FF

  Note that the colors used in reports created in previous versions are not replaced by the pastel palette.

- **Blgc_Glycan_Profile.rtpc updated with new glycan grouping options**

  The **Glycan profile by sample (table)** tab of the **Blgc_Glycan_Profile.rtpc** contains the new fields, **[GlyGroup1]**, **[GlyGroup2]**, and **[GlyGroup3]**:

  GlyGroup1:
  - Sialylated (contains NeuAc or NeuGc)
  - Fucosylated (contains Fuc)
  - M9, M8, M7, M6, M5 (HexNAc(2)Hex(9−3))
  - Hybrid (HexNAc(3)Hex(5−6))

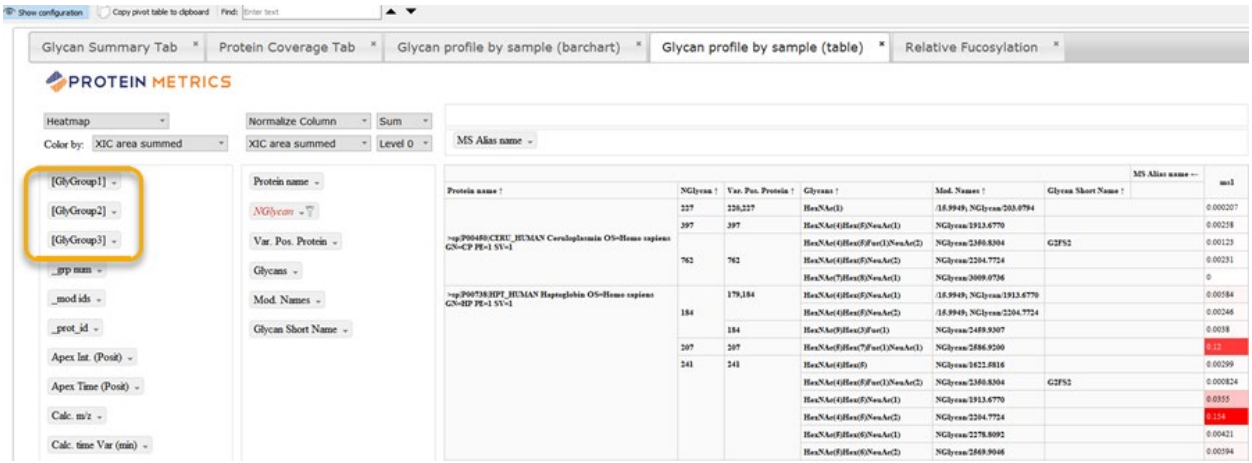
- Complex
- Unoccupied
- Multiple when a peptide has multiple glycans of different types.

GlyGroup2:

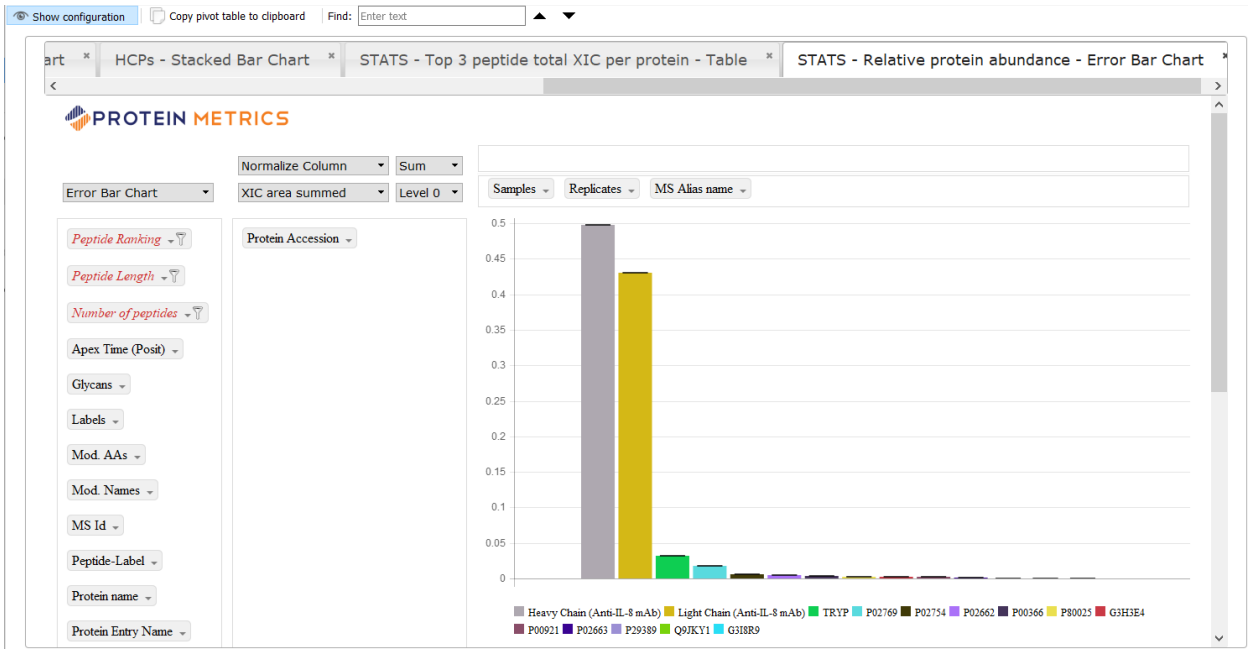
- High Mannose (HexNAc(2)Hex(9−3))
- Hybrid (HexNAc(3)Hex(5−6))
- Complex
- Unoccupied
- Multiple when a peptide has multiple glycans of different types.

GlyGroup3:

- Charged (contains NeuAc or NeuGc) (with number of charges0
- Fucose (contains Fuc)
- High Manose (contains HexNAc(2))
- Neutral a-fucosylated
- Mixed (when a peptide has multiple mods of different types)
- a-glycosylated

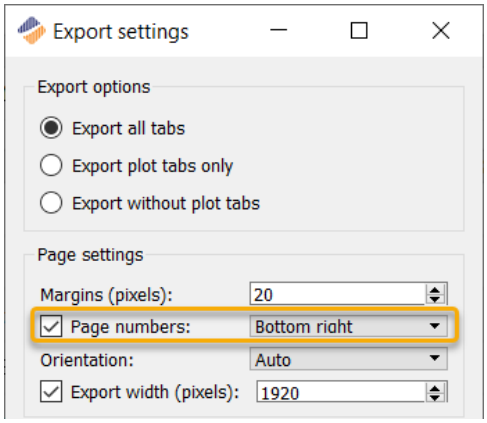


- **BIgc_HCPs.rptc updated to sort bar charts**



- **Exported PDF files from reports can contain page numbers**

  For reports, **File > Export > Export to PDF** allows configuration for page numbers:

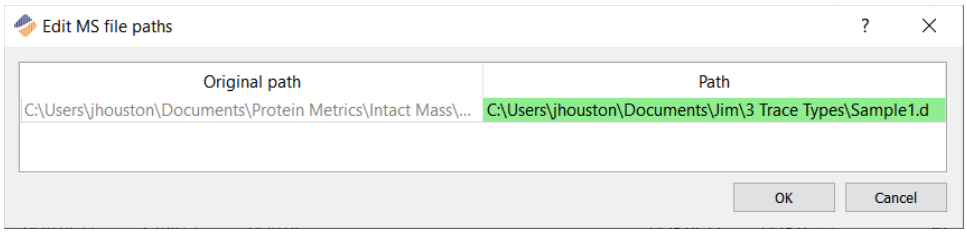


- **SeparateSamples and SeparateReplicates columns**

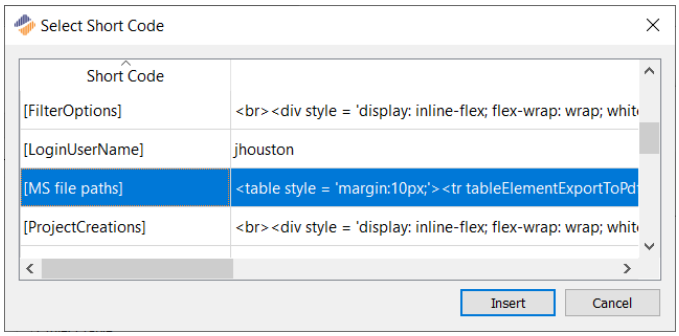
  Columns for [SeparateSamples] and [SeparateReplicates] were added to the report template Intact_Sample_Name_And_Error_Bars_Example.rptc.

- **Original and edited MS file paths can display in Summary tab**

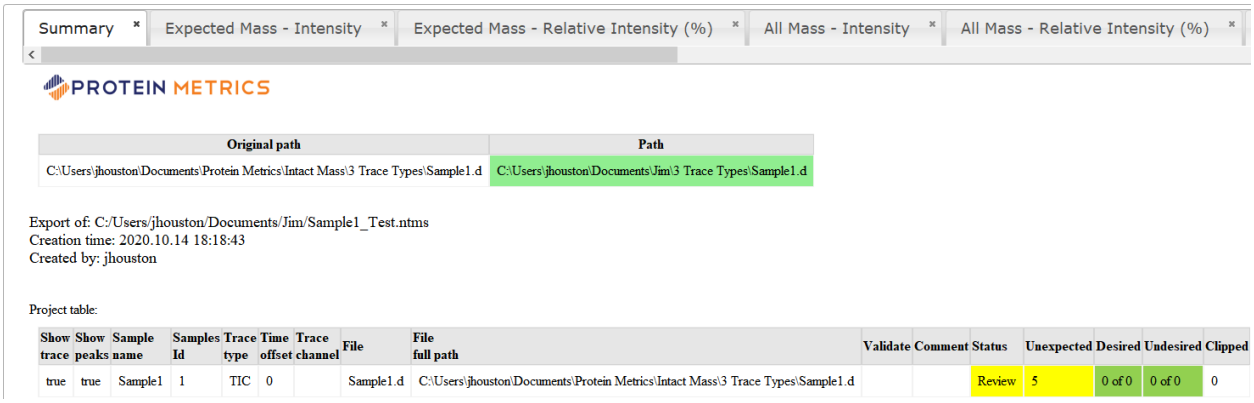
  The short code [MS file paths] was added for the report Summary tab. This will display the values shown in the **Edit MS file paths** dialog opened from **Edit > Adjust MS paths** for original and edited MS file paths:



To enable MS file paths, choose the report **Summary** tab, choose **Edit > Current tab setting**, click **Edit summary template**, and click **Short Codes**:

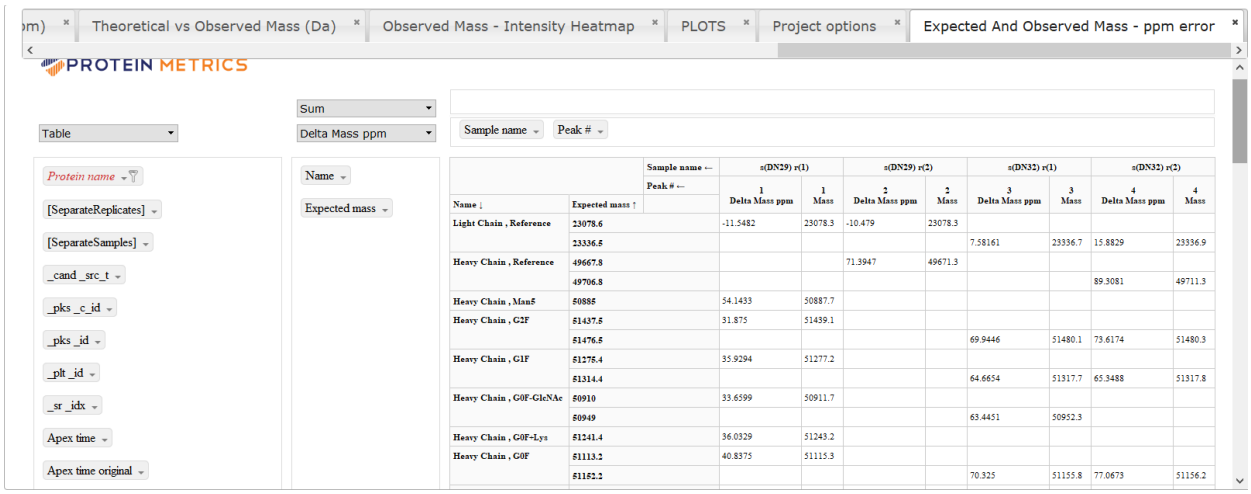


Select **[MS file paths]** and click **Insert**. Click **Ok** to close the **Template Editor**. The MS file paths are added to the Summary tab:



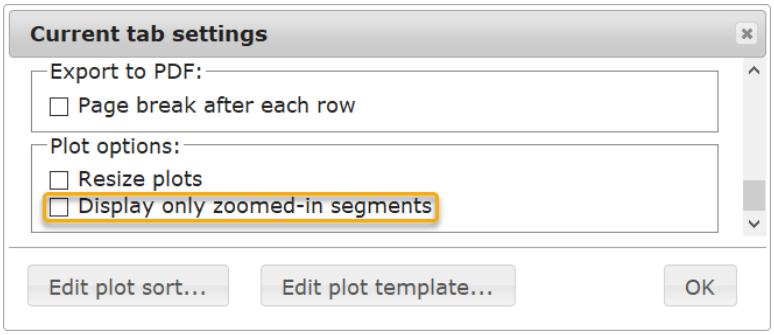
- **Intact_Expected_And_Observed_Mass_With_Ppm_Error.tabc**

  Introduced a new Intact report tab displaying expected and observed mass with ppm error. This uses a new function that allows multiple pivot columns based on the same aggregator. Contact the CS team at support@proteinmetrics.com for help with customization. This is added from **File > Presets > Tab presets** for Intact Analysis:



- **Display only zoomed-in segments**

  An option was added to display zoomed in segments in the Plots tab. Choose **Edit > Current tab setting** and check **Display only zoomed-in segments**:

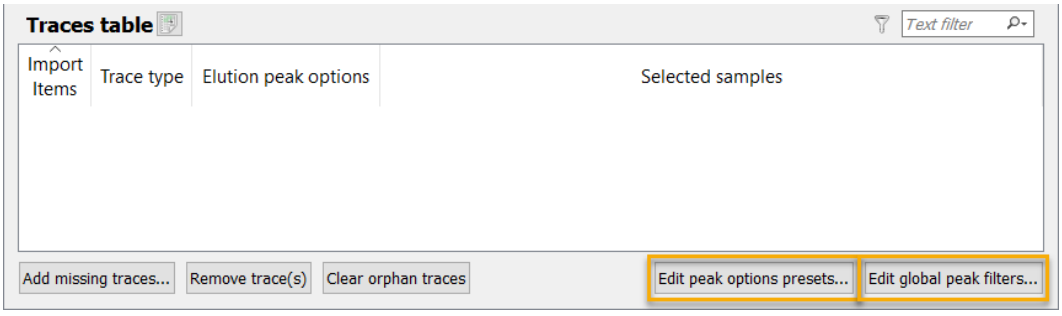


Elution Peaks

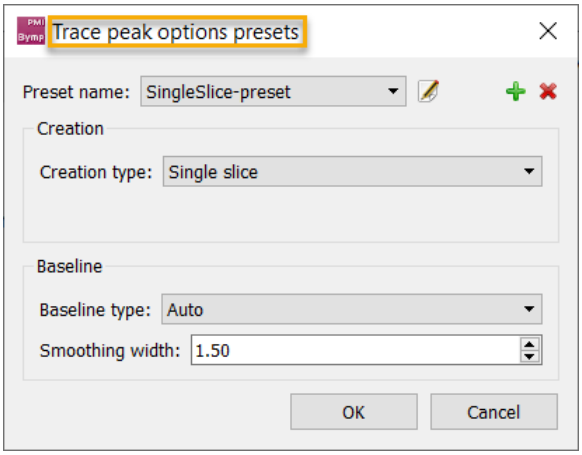
- **Elution peaks generalized to trace peaks**

  Trace peaks now accommodate technologies beyond elutions and x-axes beyond time units. The terminology used in the products has been generalized to support additional workflows.

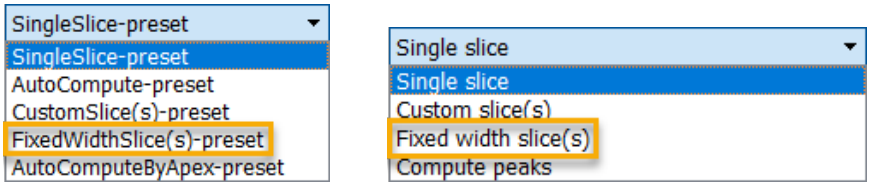
  - The **Sample input** tab buttons **Edit elution peak** options and **Edit global elution peak filters** have been renamed **Edit peak options presets** and **Edit global peak filters**:



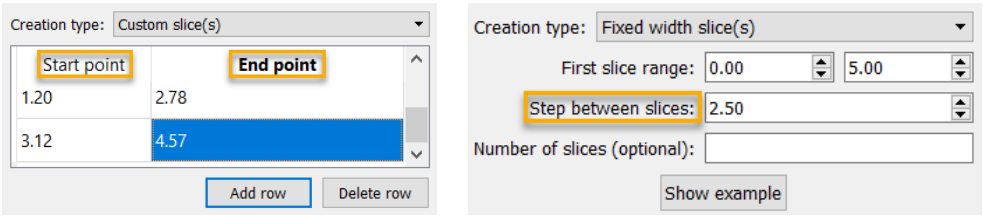
  - The **Elution peak options** dialog has been retitled **Trace peak options presets**:



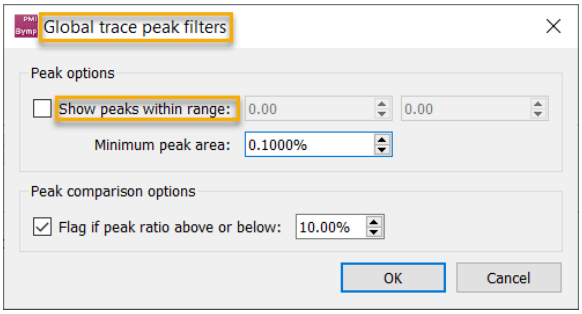
- The Trace peak options **TimeSlice(s)-preset** has been renamed **FixedWidthSlice(s)-preset**. The preset Creation type **Time slice(s)** has been renamed **Fixed width slice(s)**:



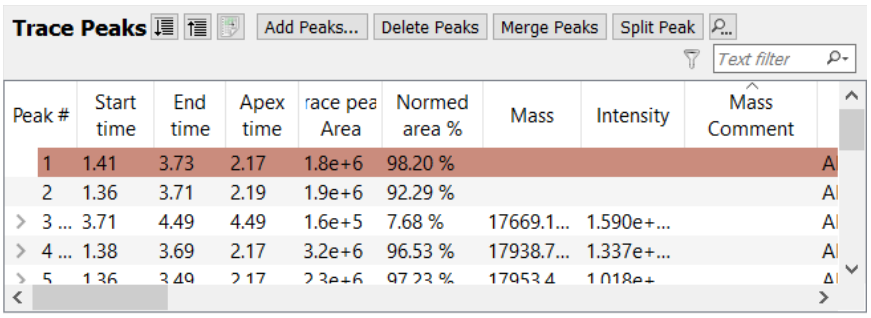
- Trace peak options references to time have been generalized:



- The **Global elution peak filters** dialog been retitled **Global trace peak filters** and the time value has been generalized:

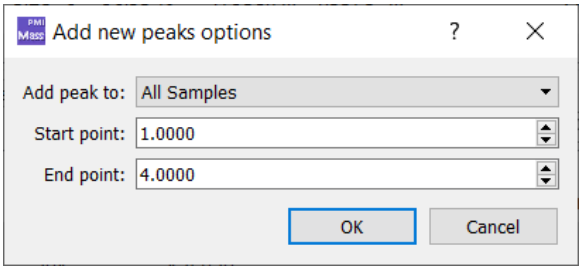


- The Intact Analysis **Elution Peaks** table has been renamed **Trace Peaks**:

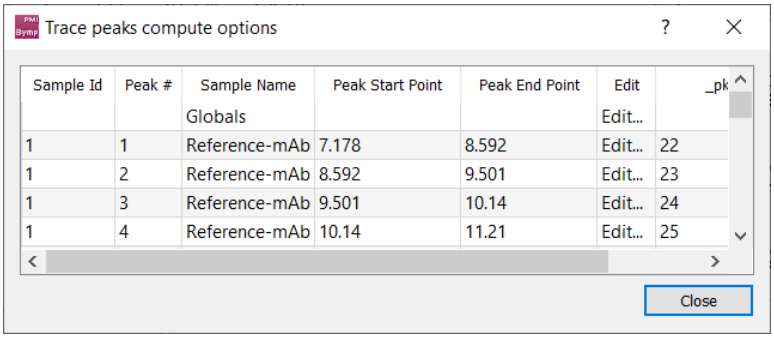


In addition, the column header **Elution peak Area** has been renamed **Trace peak Area**.

- In the **Add new peaks options** dialog opened from **Add peaks** in the Intact Analysis **Trace Peaks** table and Chromatogram Analysis **Peaks** table, **Start time** and **End time** have been renamed **Start point** and **End point**:



- The **Elution peak compute options** dialog, opened with **Edit > Compute Options**, has been renamed **Trace peak compute options**:

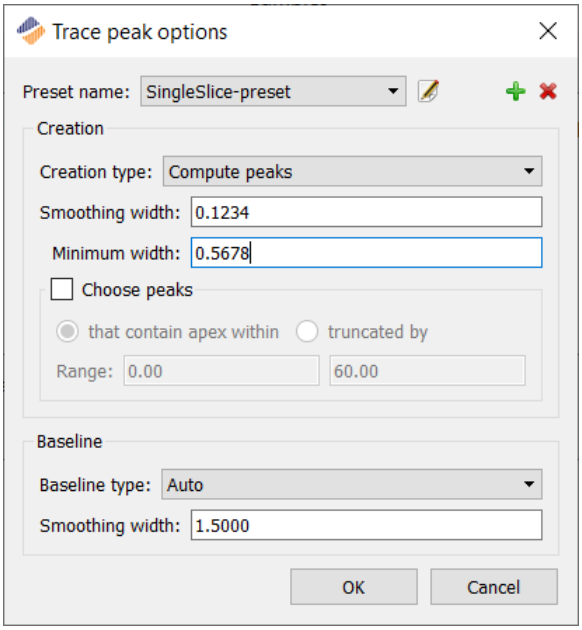


In addition, the column headers **Peak Start Time** and **Peak End Time** have been renamed **Peak Start Point** and **Peak End Point**.

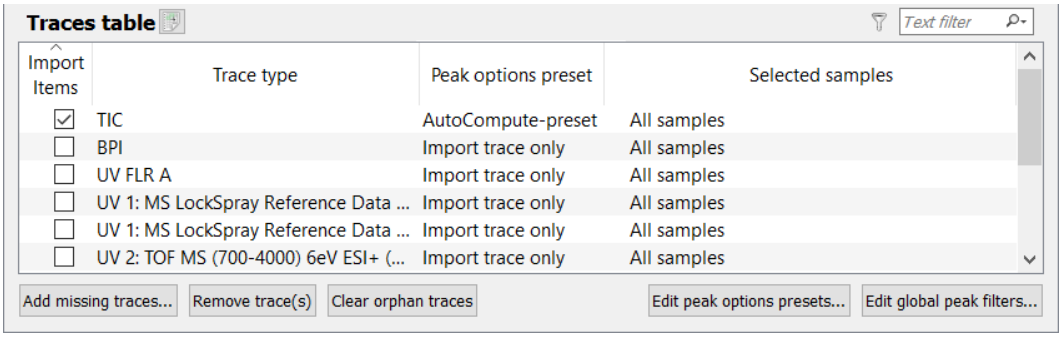
- In the new project **Advanced** tab, the **Time** section has been renamed **Trace alignment**:



- **Elution/Trace Peaks inputs allow more than two significant figures**



- **Traces table displays "Import trace only" for traces without peak option presets**:



Release 2020-06 (v3.9)

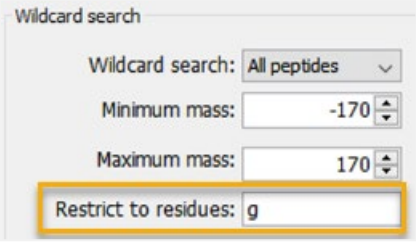
Byonic, Byos

- **Wildcard Search - Expanded to Glycopeptides**

  The wildcard search has been expanded to amino acid positions with an already present glycan mass modification in Byonic and Byonic Processing nodes in Byos. N-linked, O-linked, and other glycan motifs are supported.

  The user must use the below parameters:

  - **Wildcard search**: All peptides
  - **Minimum** and **Maximum mass:** Set values
  - **Restrict to residues**: set to "g" to search for wildcard modifications on top of glycan modifications

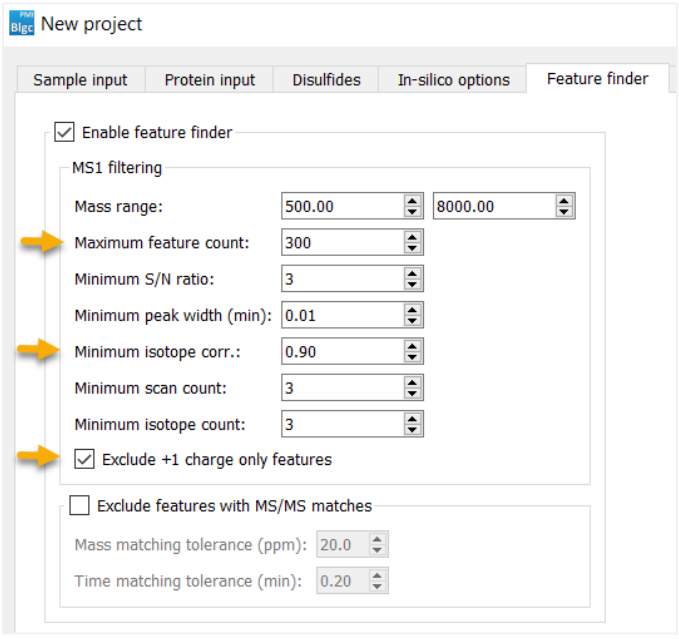


Peptide Analysis, Byos

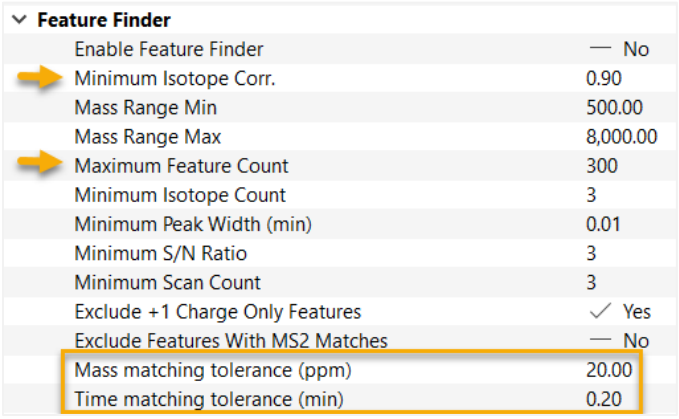
- **New parameters in Feature Finder**

  Two parameters formerly used in Advanced Settings have been added to Feature Finder in Peptide Analysis and Byos: **MassMatchingTolerance** and **ApexTimeMatchingTolerance**.

  In Peptide Analysis, the settings are added to the Feature Finder tab:



In Byos, the settings are added to the Feature Finder section of the Processing nodes tab:



These parameters are used to match and remove features that have already been identified by MS2 (when **Exclude Features With MS2 Matches** is checked/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.

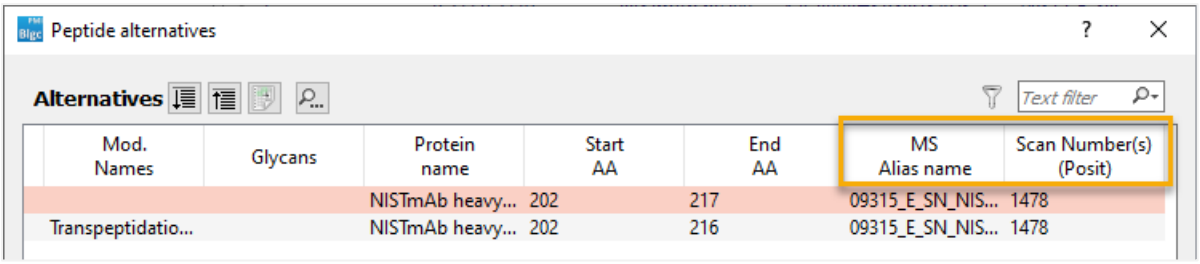
Feature Finder default values were also optimized in both Peptide Analysis and Byos:

- **Maximum feature count** changed from 100 to 300
- **Minimum isotope corr** changed from 0.75 to 0.90
- **Maximum feature count** changed from 100 in Peptide Analysis and 500 in Byos to 300
- **Exclude +1 Charge Only Features** is now checked/set to Yes in Peptide Analysis and Byos, respectively

The Feature Finder parameters were also regrouped in Byos. **Exclude Features With MS2 Matches** was moved down, directly above the two new parameters.

- **New columns in Peptide alternatives**

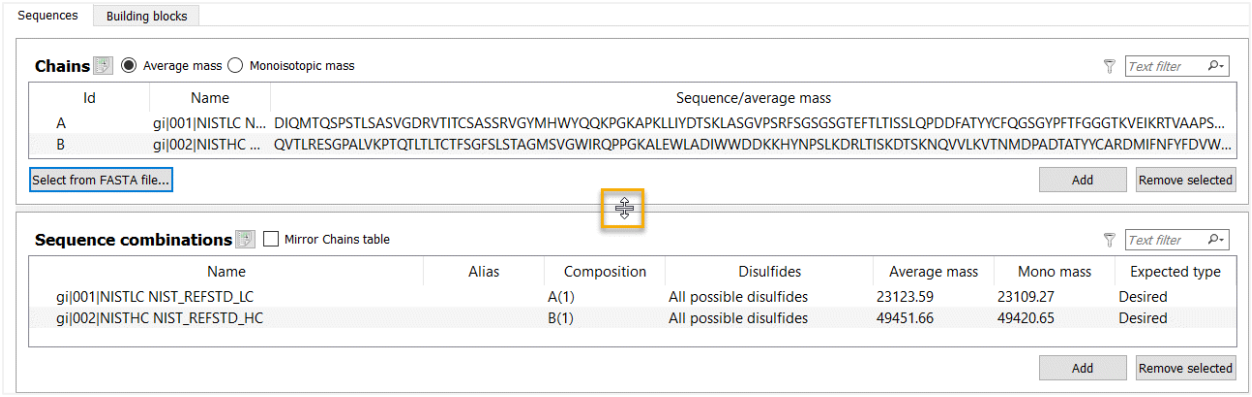
  The columns **MS Alias name** and **Scan Number(s)** of origin were added to the **Peptide alternatives** dialog:



Intact Analysis, Byos

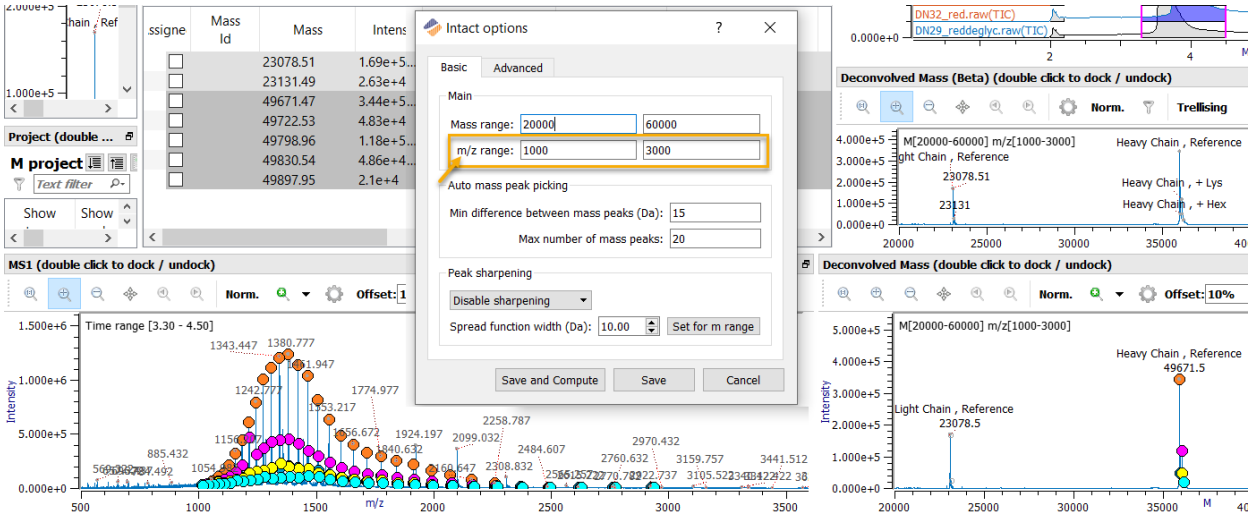
- **Resize capabilities in protein sections of Intact workflows**

  The protein sections in the **Sequences** tab of Intact Analysis and corresponding workflows in Byos are resizable. Splitter bars appear when hovering below the **Chains** and **Sequence combinations** sections. Click and drag to lengthen or shrink those sections.



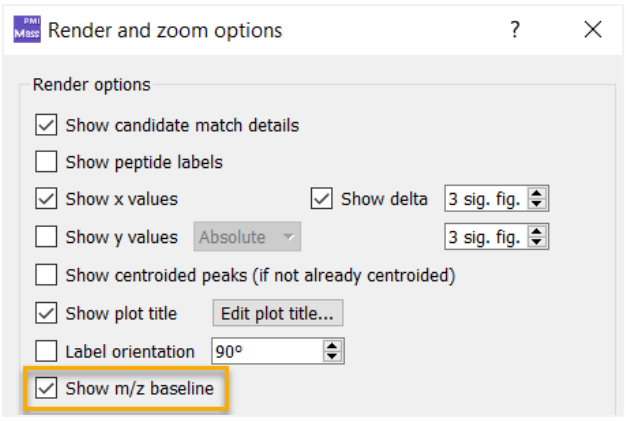
- **Display Colored Dots – Specify Range**

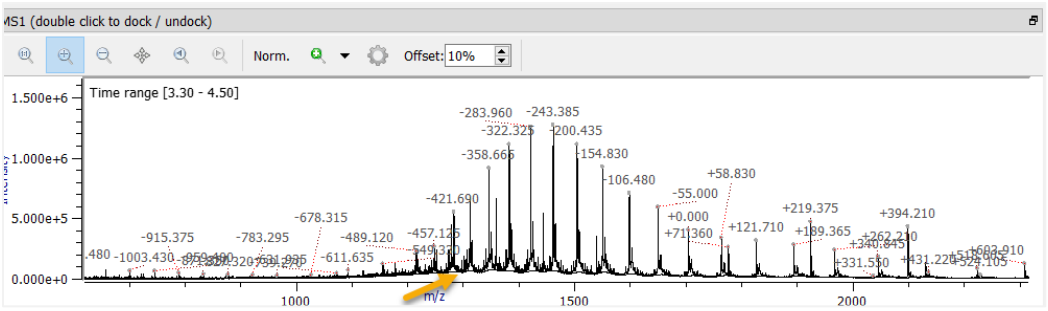
  Colored dots will now only be displayed within the specified m/z or charge state range.



- **Show m/z baseline in MS1 plots**

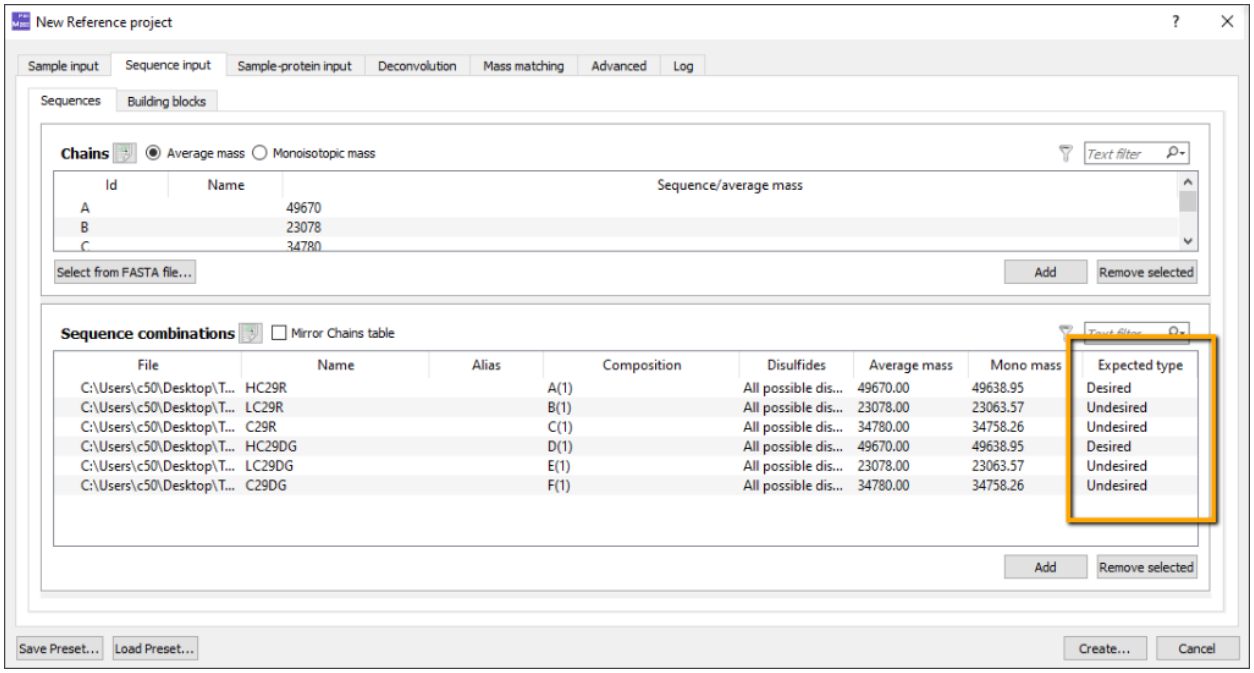
  The **Render and zoom options** dialog includes the option **Show m/z baseline** in **MS1** plots:





- **Expected type added to Sequence combinations table**

  The **Expected type** column was added to the **Sequence combinations** table in **Sequences and masses - Sequences** sub-tab in Intact Analysis and corresponding workflows in Byos:

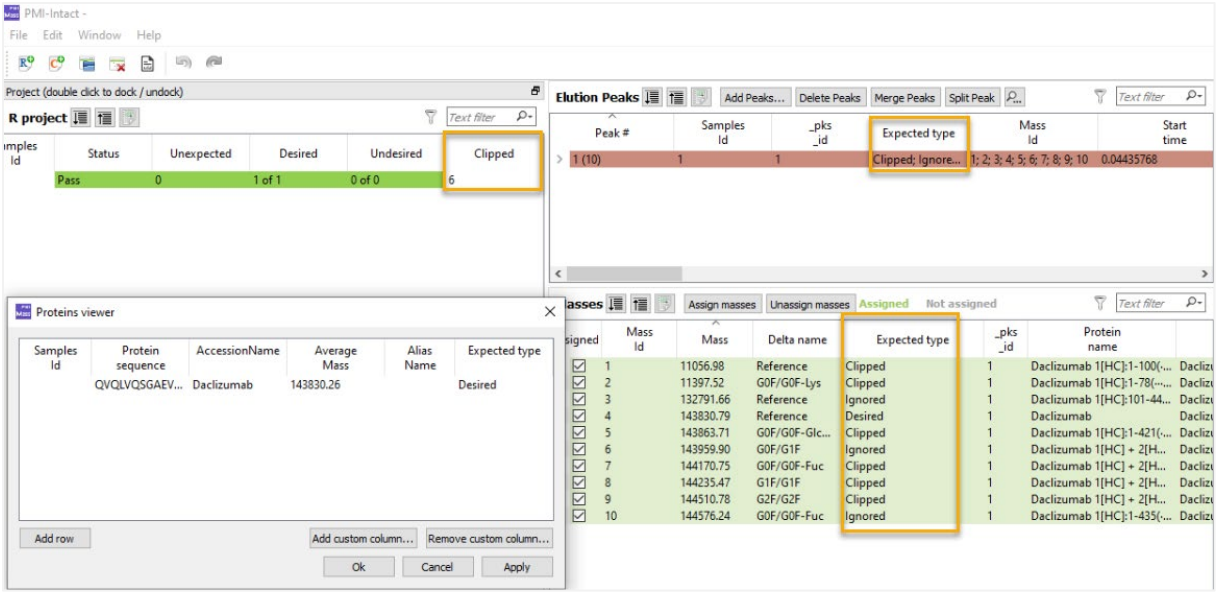


- **New Expected Type – Clipped**

  Clipped is counted in a separate column in the project table:

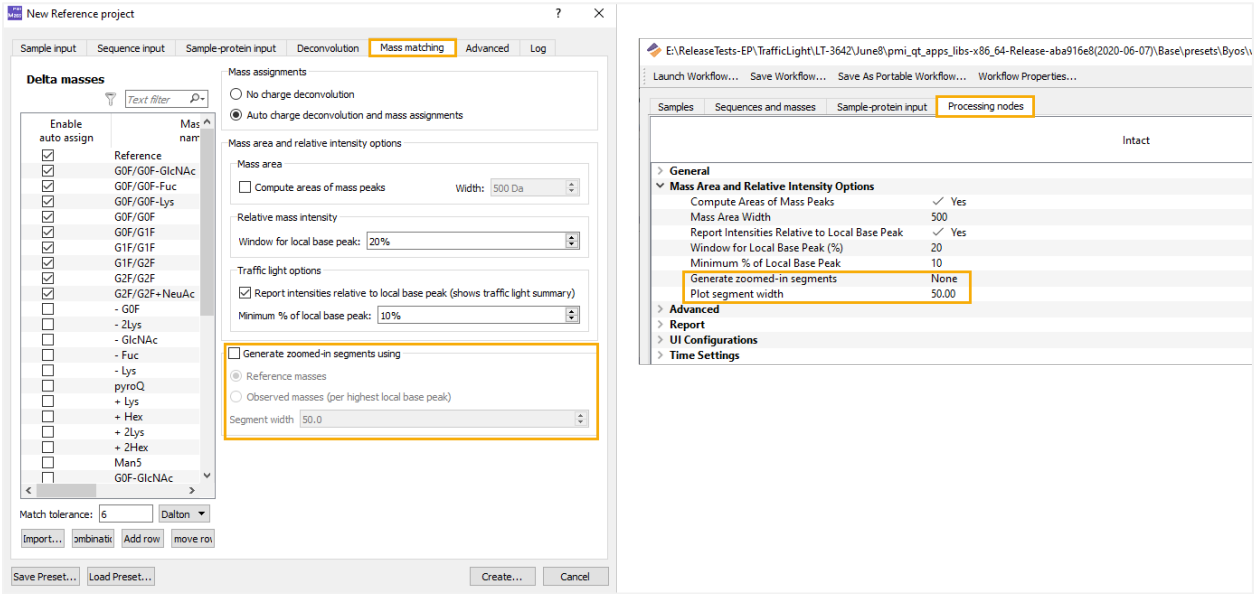


  Clipped is included as a new **Expected type** and counted the same: all occurrences of Expected type = Clipped, except for duplicates, that is, masses with the same name. Clipped is also displayed in the **Elution Peaks** and **Masses** tables:

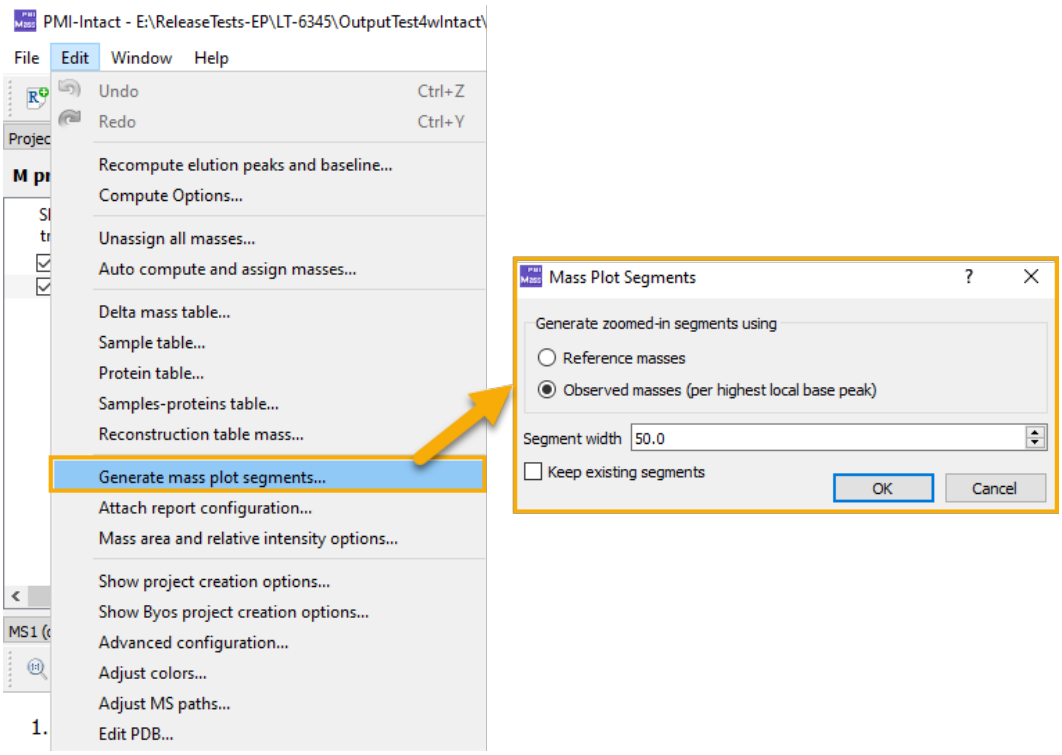


- **Traffic Lights – Logic Improvements**
  - The logic used in counting **Desired** and **Undesired** has been improved. All unique occurrences of a specific type for a given protein are counted. The uniqueness of the protein is defined by the **Protein name** and **Expected type** columns.
  - Editing the **Name** and **Expected type** columns in the **Sequence combinations** table is disabled if the data is loaded from a CSV file to help maintain consistency between tabs.
- **Generate zoomed-in segments for observed masses during project creation**

  Segments can be set to be automatically generated around reference/observed masses for MS1 or Deconvolved Mass plots during project creation. This option is available for both Intact Chromatogram and associated workflows in Byos.



After project creation, zoomed-in segments can still be generated around the reference/observed masses (per highest local base peak) by choosing **Edit > Generate mass plot segments**:



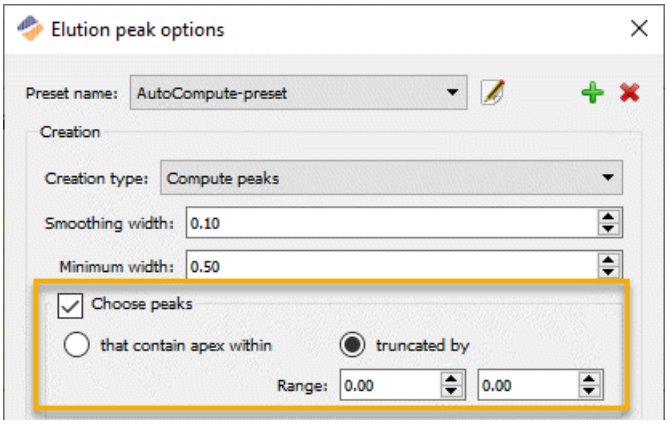
Chromatogram Analysis, Intact Analysis, Byos

- **Cancelling plot segment edits**

  When cancelling edits entered in the **Plot segments** dialog, the plot returns to its previous state.

- **Elution peak Auto truncated by time range**

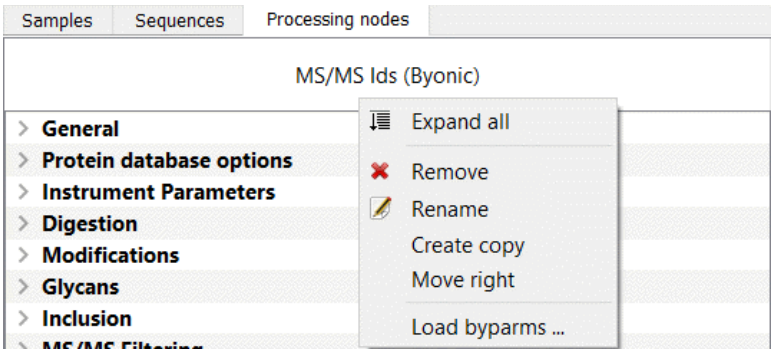
  Users can choose have peaks truncated by a time range in the **Compute peaks** option of the **Elution peak options** dialog.



Check **Choose peaks** and select **truncated by**. All peaks within the specified time range will be chosen. Additionally, the first and last peak will be trimmed to fit within the specified time range.

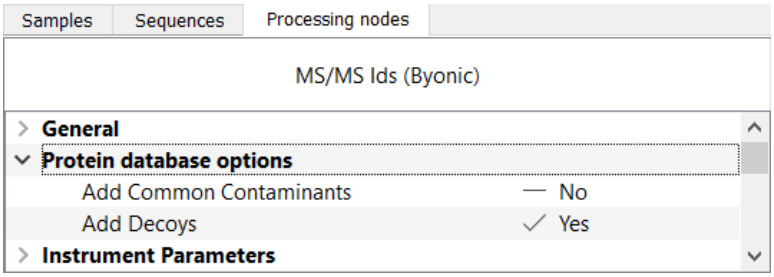
Processing Nodes Enhancements in Byos Workflows

- **Enhanced capabilities – new menu items in Byonic Processing nodes**



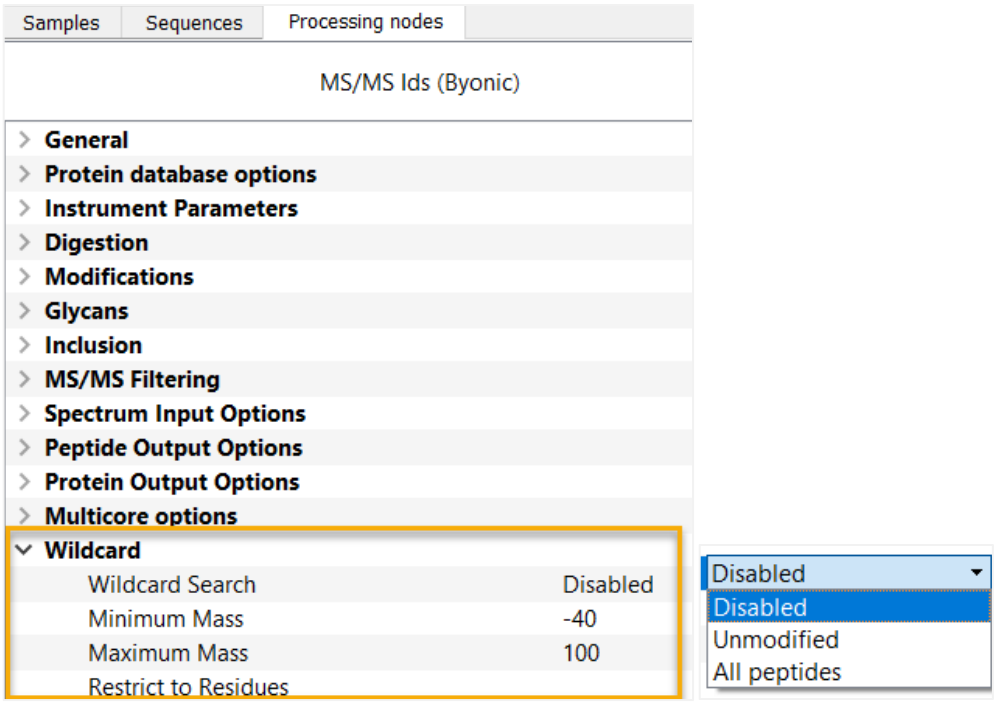
- **Remove** – the user can remove the Byonic Processing node
- **Rename** – the user can rename the Processing node
- **Create copy** – the user can create a copy of the Byonic Processing node. For example, a copy can be used to support an analysis with more than one digestion
- **Move right** – the Byonic Processing node will appear at the far right. The user can then move it back to the left or center with Move left.

- **Protein database options in Byonic Processing nodes**



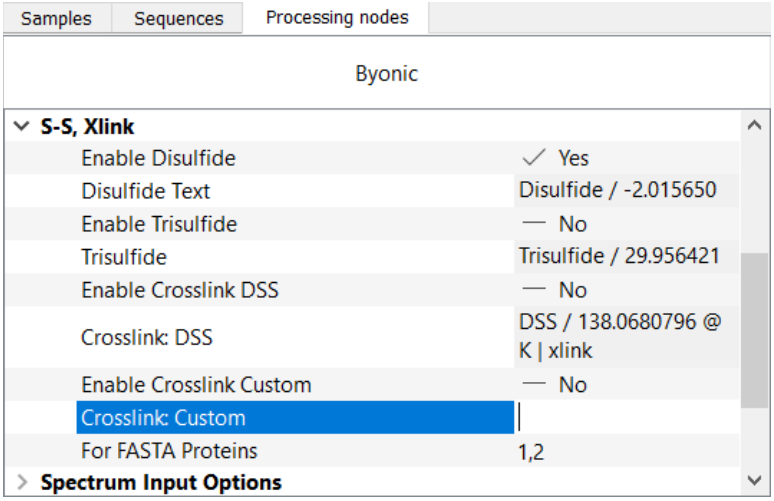
The protein database options **Add Common Contaminants** (default setting = **No**) and **Add Decoys** (default setting = **Yes**) are now part of the Byonic Processing node.

- **Wildcard parameters in Byonic Processing nodes**



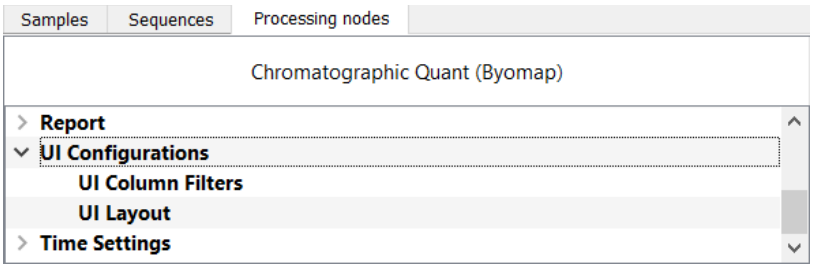
Users can now enable Wildcard modification search. This capability was previously only available in Byonic. The options Disabled, Unmodified, and All peptides are available through a drop-down menu.

- **S-S.wflw – Crosslink: Custom added to SS, Xlink parameter in Byonic Processing nodes**



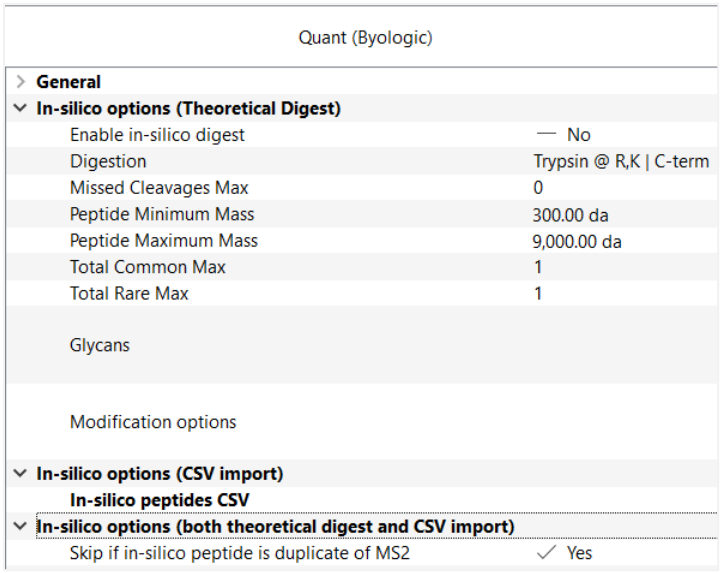
Users can now specify the Modification Fine Control used in a custom crosslink search. This capability was previously only available in Byonic.

- **UI Configurations in Byomap Processing nodes**



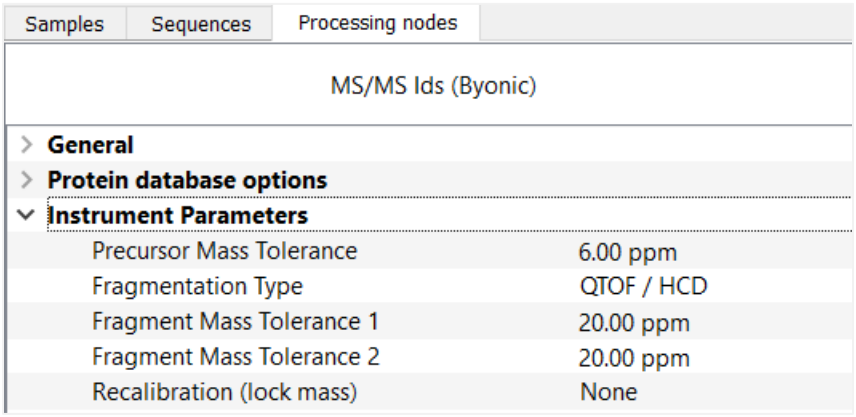
Users can now include UI Configurations in Byos workflows containing Byomap Processing nodes. This was previously unavailable in Byos.

- **In-silico options improvements**

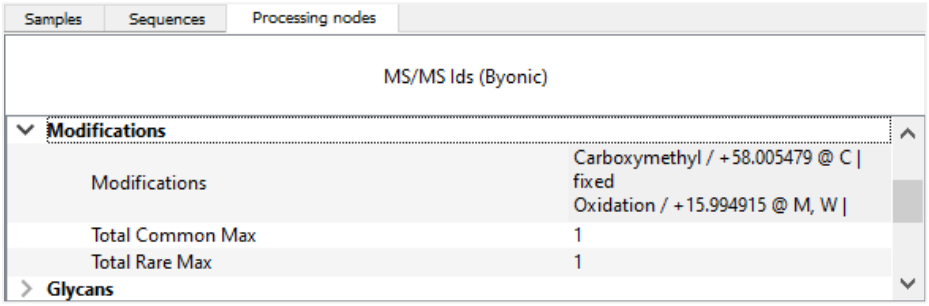


Parameters are separated into three sections to clarify which pertain to a **Theoretical Digest**, **CSV import**, and **both theoretical digest and CSV import**

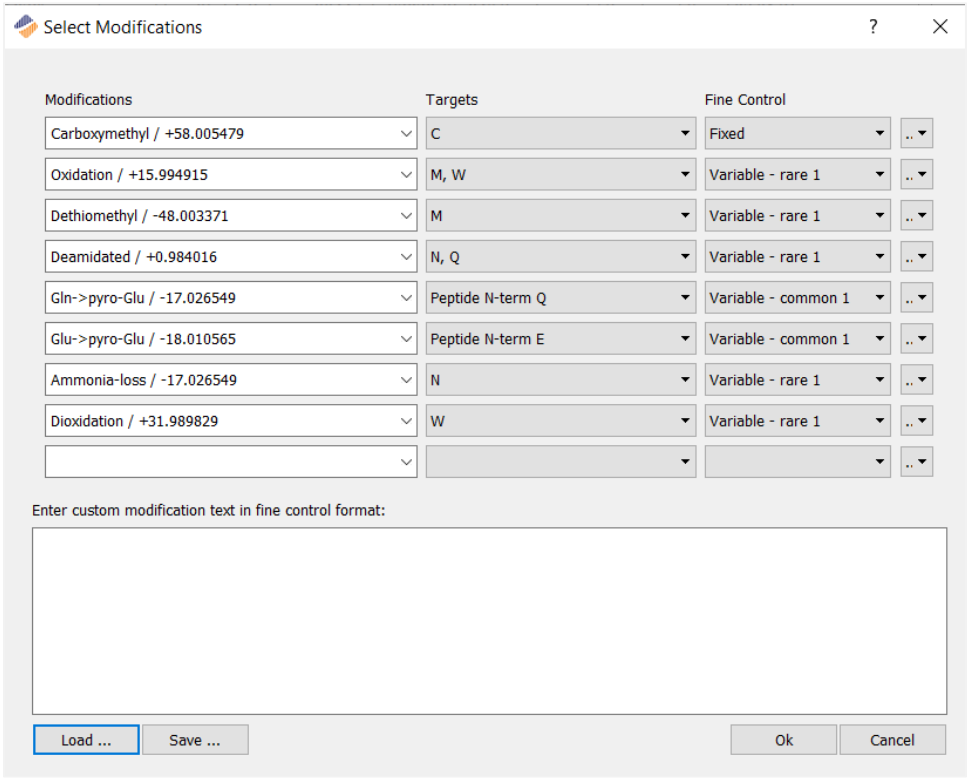
- **PTM.wflw parameter changes**
    - Byonic Processing nodes - Instrument Parameters - Fragment Mass Tolerance value Changed from 0.6 Da to 20.00 ppm



- Byonic Processing nodes - Modifications - Total Common Max Changed from 2 to 1

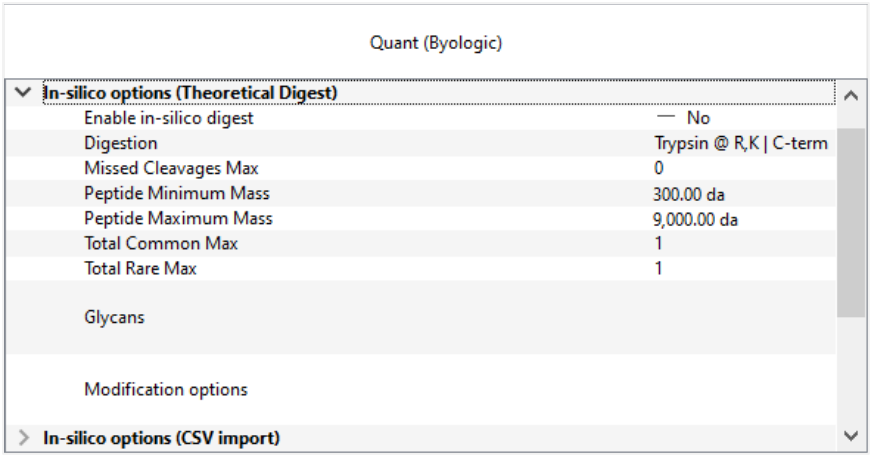


- Byonic Processing nodes - Modifications – Modifications List and Fine Control



  i. DTT / +151.996571 removed
  ii. (De) Carboxymethyl / -58.005479 removed
  iii. Carbamyl / +43.005814 removed
  iv. Acetyl / +42.010565 removed
  v. Deamidated / +0.984016 changed from Variable – common 2 to Variable – rare 1

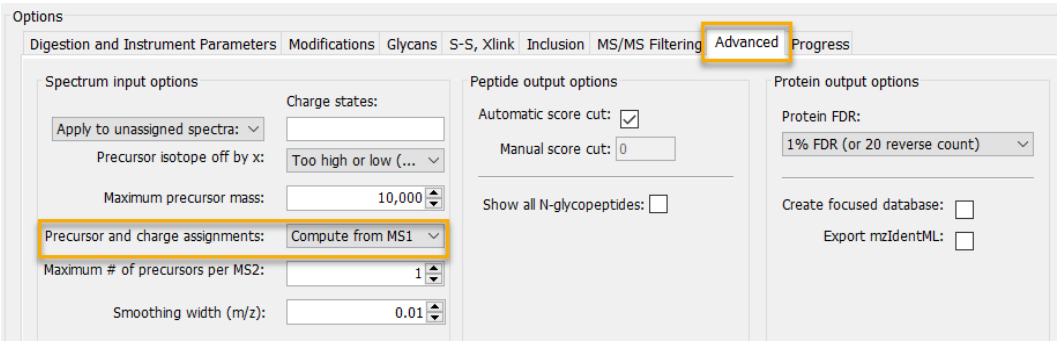
- Byologic Processing nodes - For PTM.wflw and PTM (pure in-silico).wflw, **In-silico options (Theoretical Digest)** – **Total Common Max** was changed from 2 to 1



Processing – Vendor Updates

- **Bruker Data – Byonic Processing Parameters**

  When processing recalibrated Bruker data in Byonic, it is recommended select Compute from MS1 for Precursor and charge assignments in the Options Advanced tab:



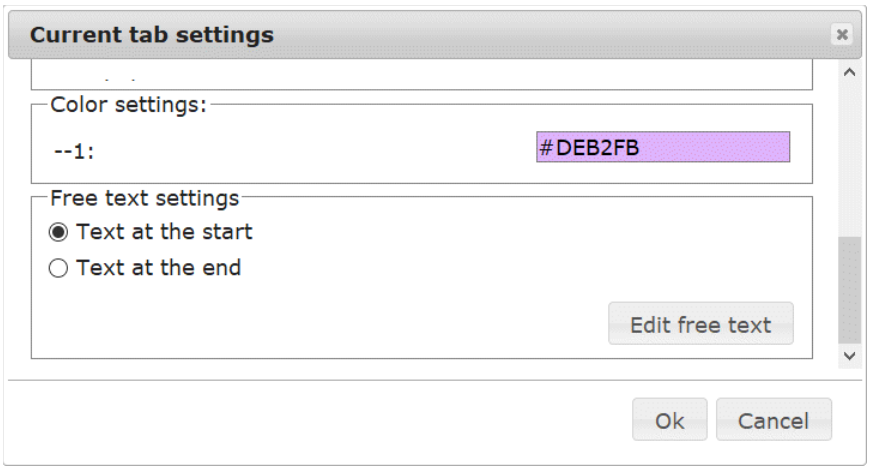
- **Bruker Data – File reader**

  The file reader has been updated for all Bruker data to the latest available from the vendor. There is no user action required, just an update that this has been implemented.

Reporting

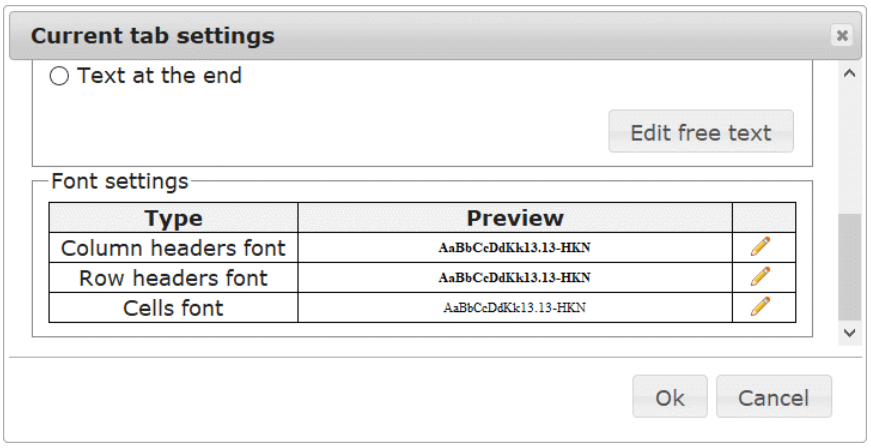
- **Free text entry in Pivot tabs**

  Custom text can be entered before or after Pivot tab displays from **Edit > Current tab settings**:

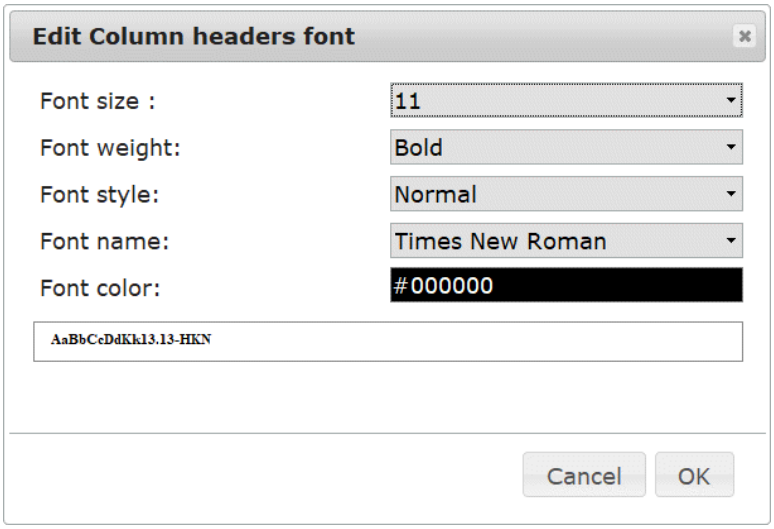


- **Edit Fonts in Pivot tabs**

  The Column headers, Row headers, and cells fonts in Pivot tabs can be edited from **Edit > Current tab settings** (font settings not available for PCA and Charts rendering):

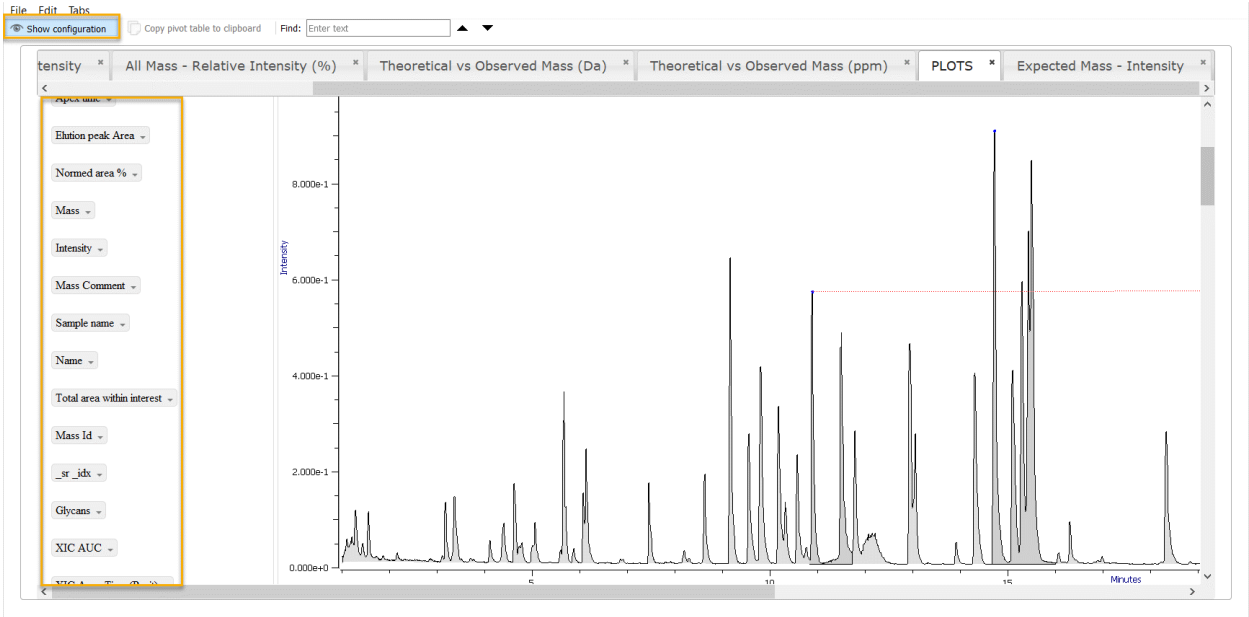


  Click the icon to edit the fonts for each text type:



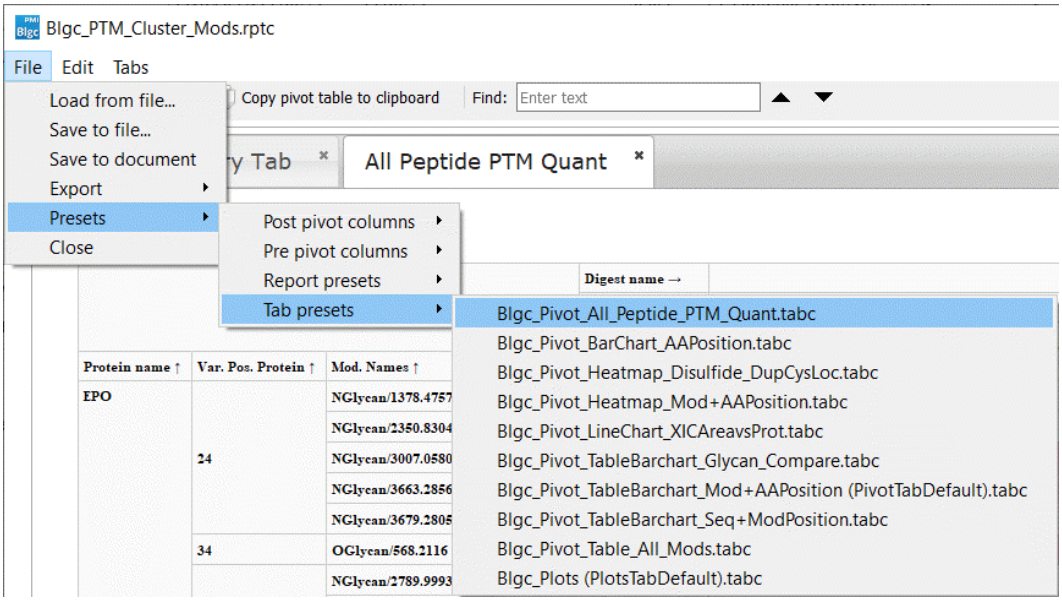
- **Filtering in the Plots tab**

  The **Show / Hide configuration** button is enabled for the Plots tab, allowing users to filter plots by available field values in same manner as in Pivot tabs:



- **PTM Cluster Mods tab**

  A new reporting option (tab) that groups all forms of peptides that share the same residue with a modified peptide is now available. The report tab uses duplicated wildtype rows as part of the processing to assure that all forms are included for each modification. This is available through **File > Presets > Bigc_Pivot_All_Peptide_PTM_Quant.tabc**



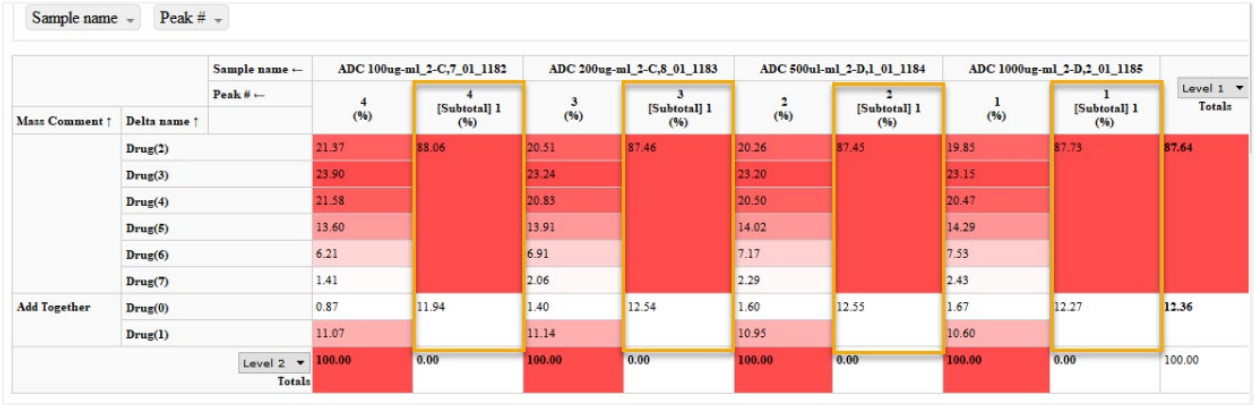
- **Feature Finder tab**

  The Feature Finder tab has been improved and properly named in the report template.

- **New pre-pivot and post-pivot scripts**

  Please reach out to the Customer Success team for additional details on the new enhancements.

  - **RenameCols.prepvtjs** was added to easily rename a column header in the Report.
  - **TotalGroup.postpvtjs** was added to support subtotal columns based on a pivot table row field. Choose **File > Presets > Postpivot columns > TotalGroup.postpvtjs** and assign a name to the script.

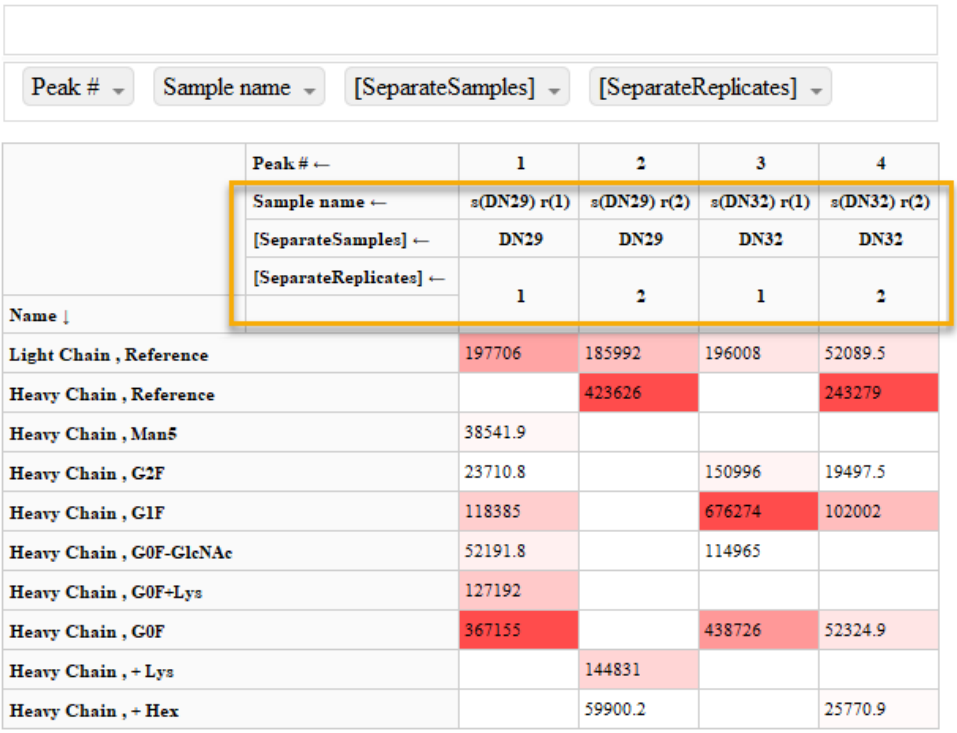


Sample name | Peak #

		Sample name ←	ADC 100ug-ml_2-C,7_01_1182		ADC 200ug-ml_2-C,8_01_1183		ADC 500ul-ml_2-D,1_01_1184		ADC 1000ug-ml_2-D,2_01_1185		
		Peak # ←	4 (%)	4 [Subtotal] 1 (%)	3 (%)	3 [Subtotal] 1 (%)	2 (%)	2 [Subtotal] 1 (%)	1 (%)	1 [Subtotal] 1 (%)	Level 1 Totals
Mass Comment ↑	Delta name ↑										
	Drug(2)		21.37	88.06	20.51	87.46	20.26	87.45	19.85	87.73	87.64
	Drug(3)		23.90		23.24		23.20		23.15		
	Drug(4)		21.58		20.83		20.50		20.47		
	Drug(5)		13.60		13.91		14.02		14.29		
	Drug(6)		6.21		6.91		7.17		7.53		
	Drug(7)		1.41		2.06		2.29		2.43		
Add Together	Drug(0)		0.87	11.94	1.40	12.54	1.60	12.55	1.67	12.27	12.36
	Drug(1)		11.07		11.14		10.95		10.60		
		Level 2 Totals	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00

- **Pre-pivot columns added to separate samples and replicates – now available in Intact**

  SeparateReplicates.prepvtjs and **SeparateSamples.prepvtjs** have been added to **File > Presets > Pre-pivot columns** to enable separation of samples and replicates in Pivot tabs:



Peak # | Sample name | [SeparateSamples] | [SeparateReplicates]

	Peak # ←	1	2	3	4
	Sample name ←	s(DN29) r(1)	s(DN29) r(2)	s(DN32) r(1)	s(DN32) r(2)
	[SeparateSamples] ←	DN29	DN29	DN32	DN32
	[SeparateReplicates] ←	1	2	1	2
Name ↓					
Light Chain , Reference		197706	185992	196008	52089.5
Heavy Chain , Reference			423626		243279
Heavy Chain , Man5		38541.9			
Heavy Chain , G2F		23710.8		150996	19497.5
Heavy Chain , G1F		118385		676274	102002
Heavy Chain , G0F-GlcNAc		52191.8		114965	
Heavy Chain , G0F+Lys		127192			
Heavy Chain , G0F		367155		438726	52324.9
Heavy Chain , + Lys			144831		
Heavy Chain , + Hex			59900.2		25770.9

Release 2020-03 (v3.8)

Supported Hardware

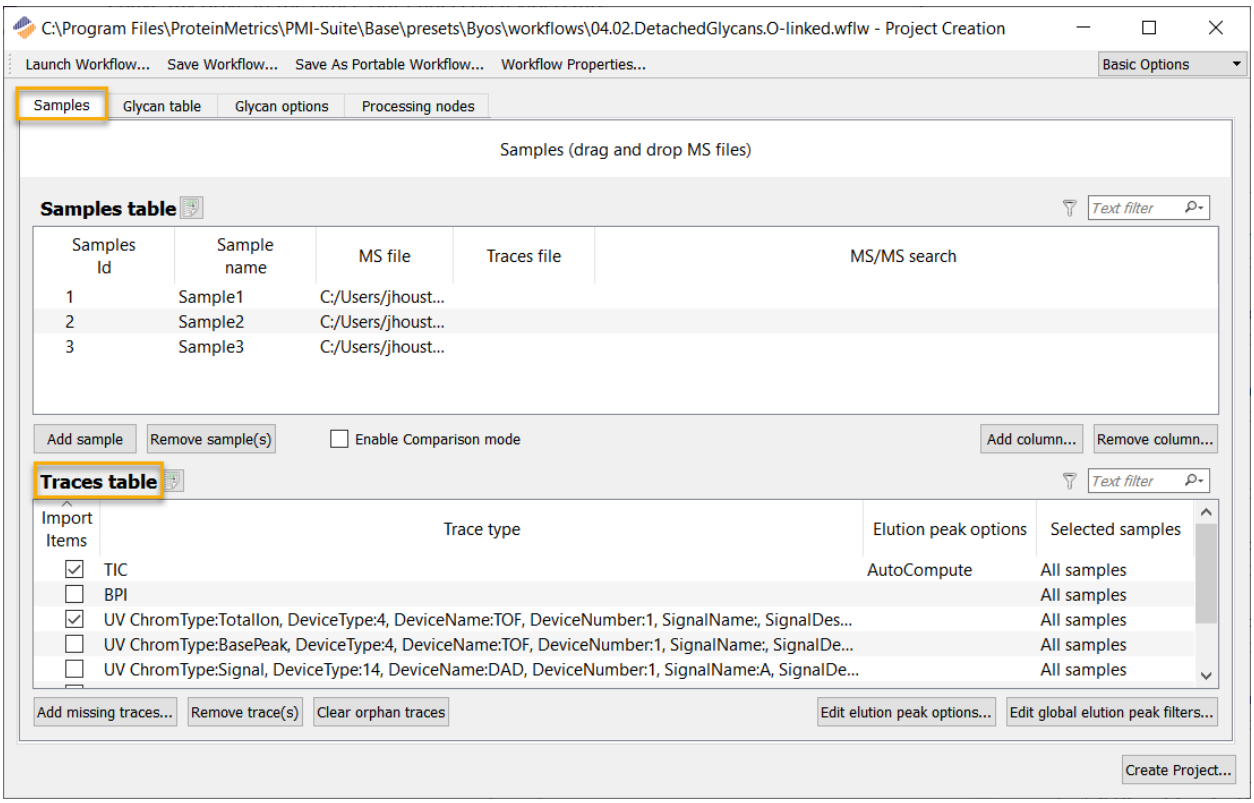
Beginning in March 2021, PMI software will not be supported on 32-bit operating systems. It is strongly recommended to install this and future releases on 64-bit operating systems.

Elution Peak and Traces Table – Intact Analysis and Chromatogram Analysis

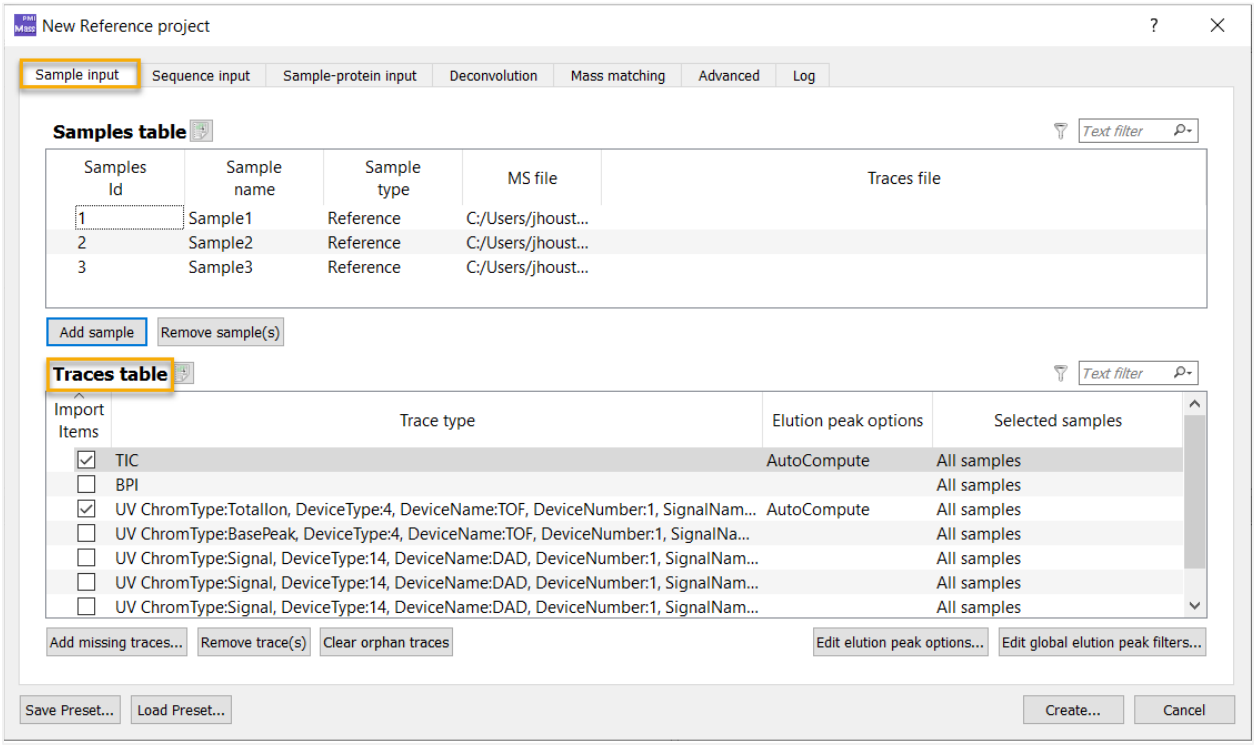
During project creation for Intact Analysis, Chromatogram Analysis, and associated Byos workflows, the elution peak calculation parameters have been moved to the Samples and Sample input tabs. Traces are no longer sub-records of samples; instead, they are organized in a Traces table within the Samples/Sample input tabs.

Please note the Samples table in Chromatogram Analysis and the associated Byos workflows has the additional column MS/MS search.

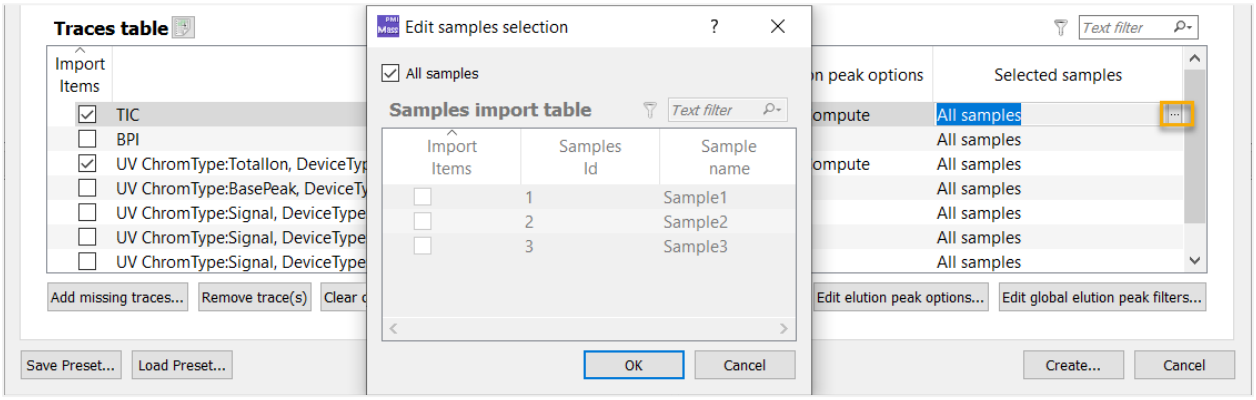
An example from Byos is shown below:



The Sample input tab in Intact Analysis and Chromatogram Analysis is very similar:

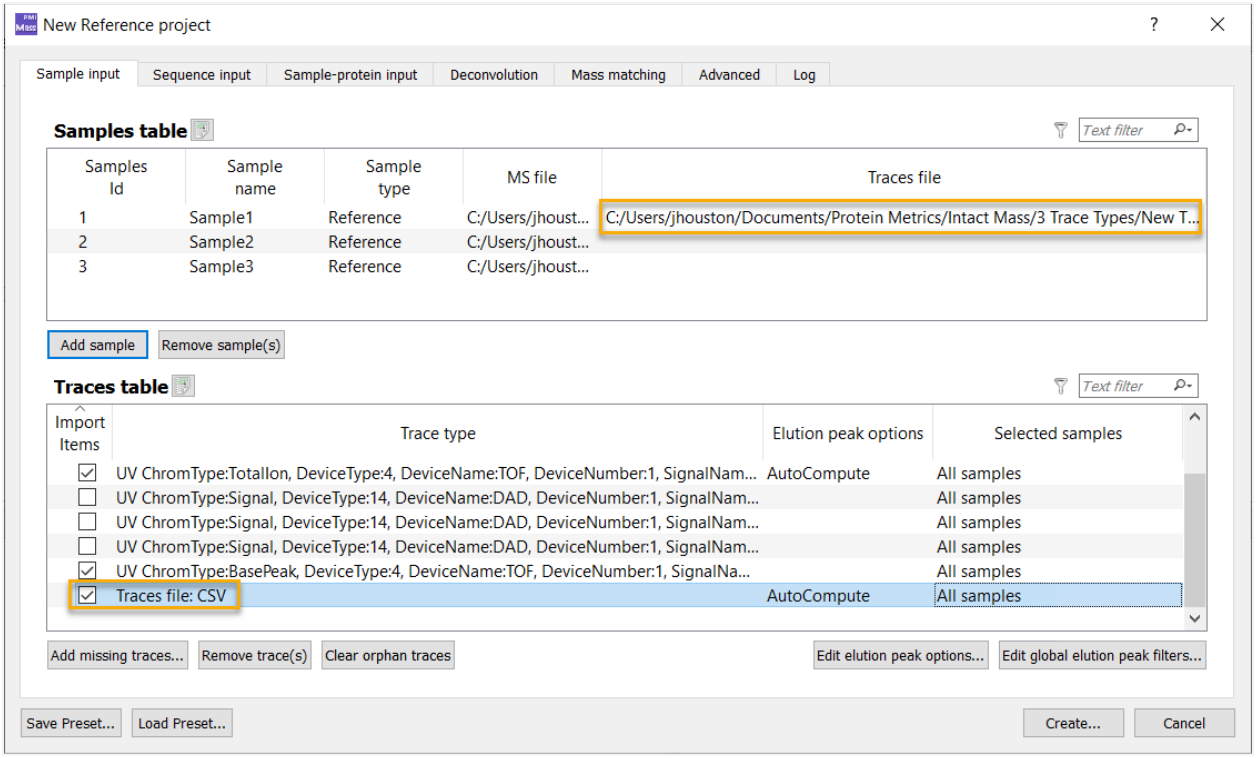


Selected traces can be removed with the Remove trace(s) button. Removed traces can be restored with the Add missing traces button. To associate specific traces to the samples, double-click the text in the Selected samples column, then click the ··· button to open the Edit samples selection dialog:

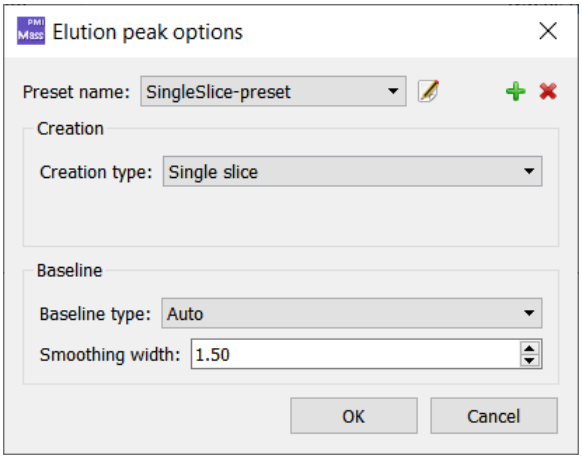


To associate the selected trace with individual samples, uncheck All samples and check those samples and click OK.

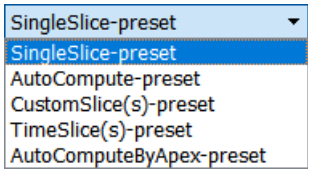
To import a trace from a *.csv file, drag the file into the Traces files column in the Samples table. The trace is added to the Traces table:



To control how the elution peaks are processed, click the Edit elution peak options button.

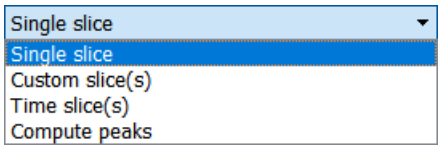


The Elution peak options dialog contains five default peak processing presets:



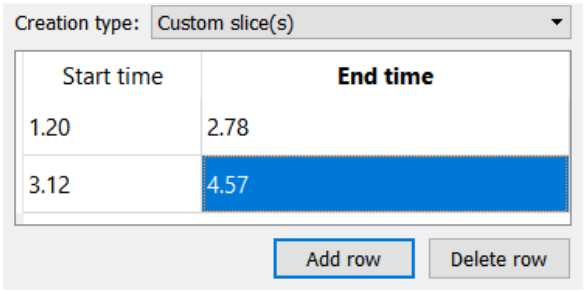
Select a processing preset and edit the peak parameters. To create a custom preset, click the ✚ button, add a preset name, and click **OK**. Next, select a creation type and edit those peak parameters. The new preset is added to the list. Click the ✎ button to edit a selected preset name. Click the ✖ button to delete a selected preset.

The default and custom peak processing presets can use one of four **Creation types**:

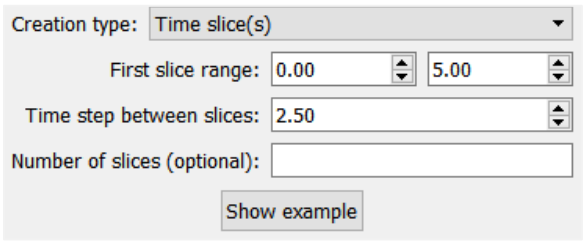


These Creation types use the following peak processing methods:

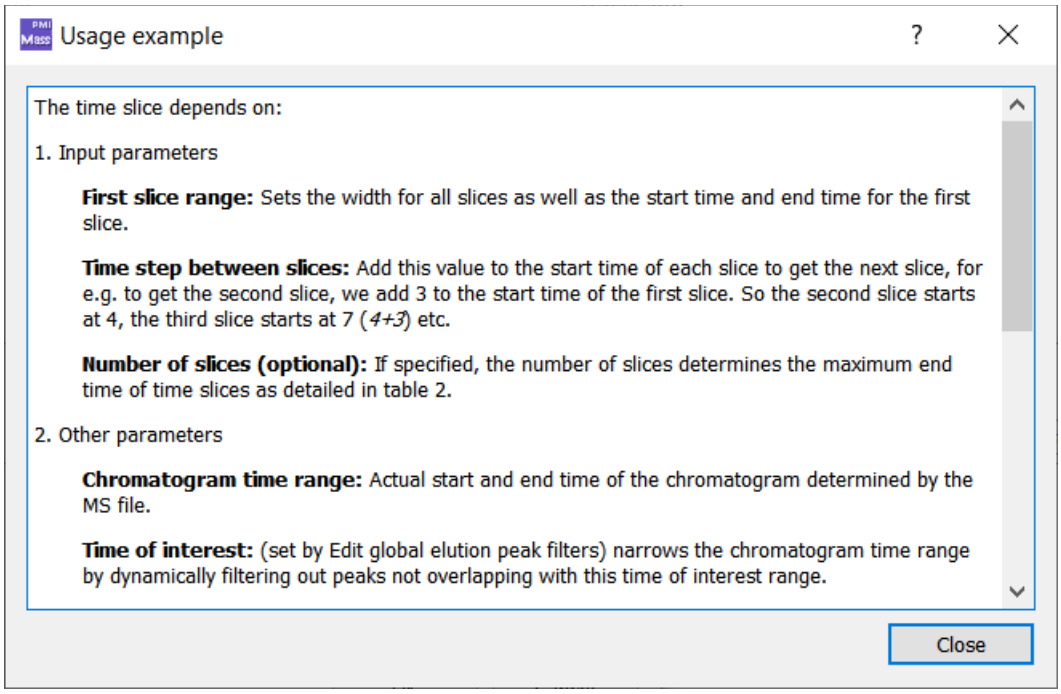
- **Single Slice** processes the entire chromatogram as single elution peak.
- **Custom slice(s)** divides elution peaks by user defined start and end times:

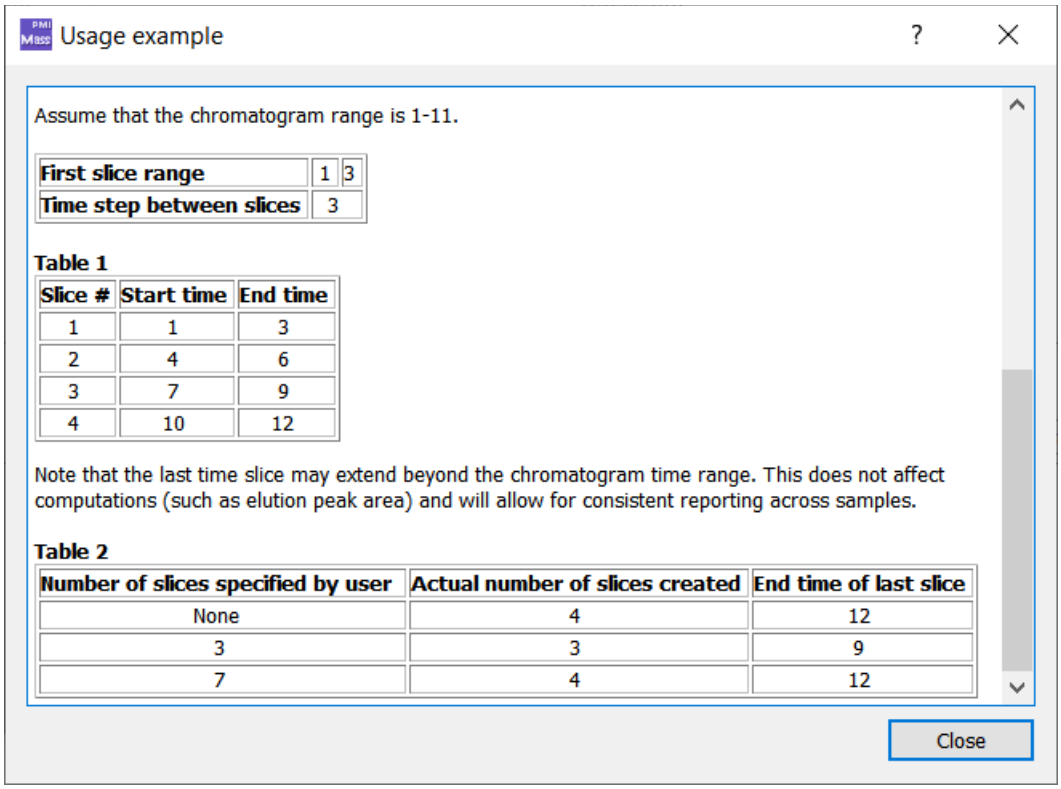


- Click **Add row** and enter start and end times for each slice. To remove a slice, select it and click **Delete row**.
- **Time slice(s)** divides elution peaks by regular time intervals defined by the user:

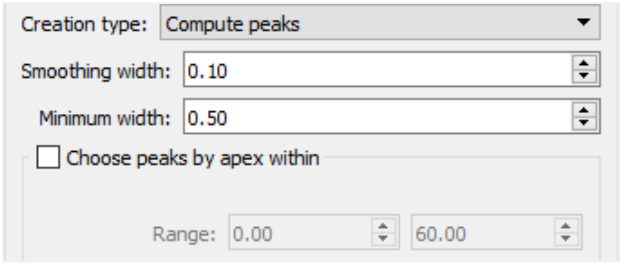


  - **First slice range** sets the start and end times for the first slice. This also sets the width for all time slices.
  - **Step** is the time delta that sets the start of subsequent time slices by adding the step value to the start of the preceding time slices. For example, the values above define the first slice as 0 - 5 min, the second slice as 2.5 – 5 min, etc., through the end of the chromatogram.
  - **Number of slices** sets the maximum number of defined slices. If blank, the last slice is determined by the end of the chromatogram time range.
  - **Show Example** displays an example time slice configuration with definitions and explanations:



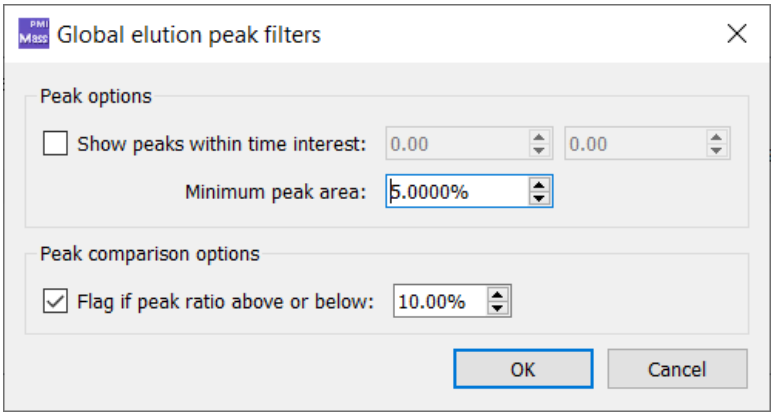


- **Compute peaks** automatically determines the elution peaks based on their minimum width, after smoothing:



Check **Choose peaks by apex within** to limit detected peaks to those with apex times within the specified time range. At the bottom of the Elution peak options dialog are the Baseline parameters. The baseline type can be set to **Auto** or **Flat**. Baseline smoothing width is also set here.

To set peak property filters across all the traces, click the **Edit Global elution peak filters** button:



Check **Show peaks within time interest** to manually set minimum and maximum times. **Minimum peak area** and a peak ratio threshold flag can be set in this dialog. The user now has the flexibility to specify minimum peak area as well as to flag if peak ratio above or below certain %.

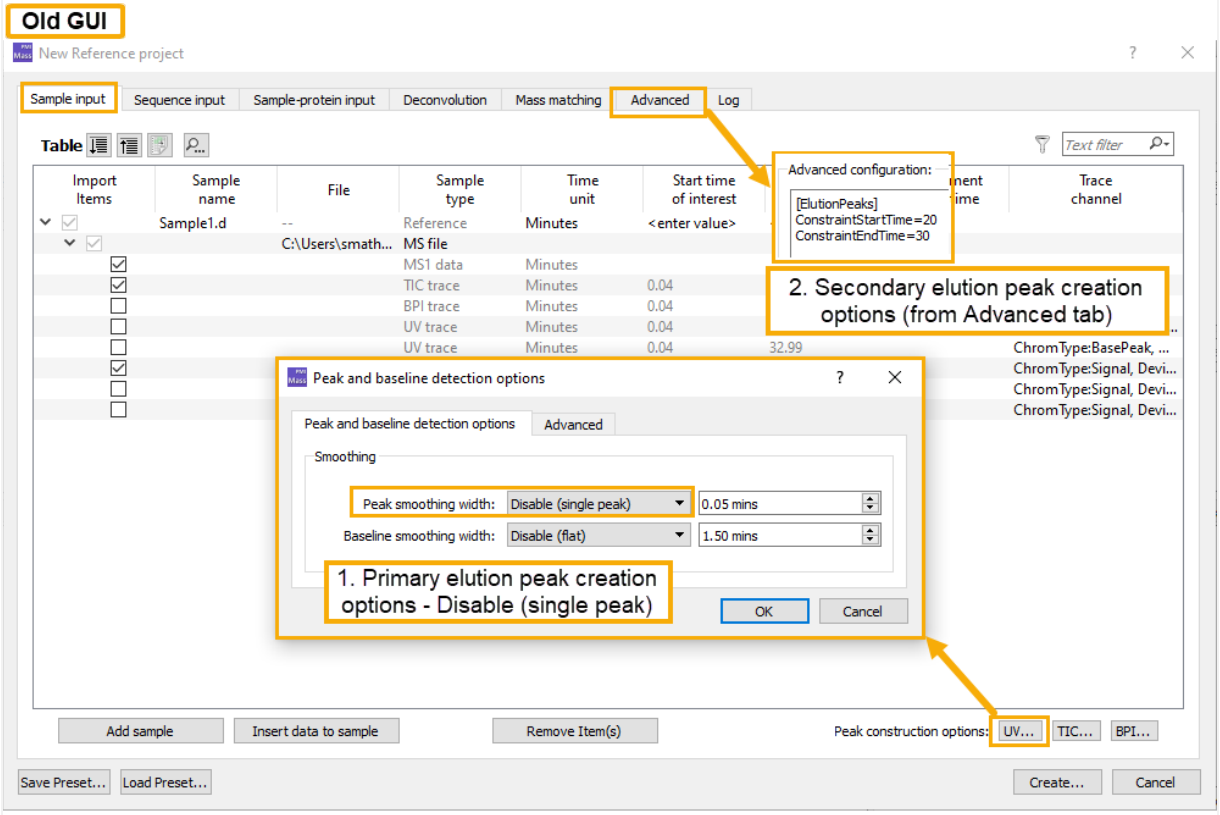
The new Traces table and elution peak settings are backward-compatible. Traces associated with samples in projects from software versions before 3.8 will correctly populate the Traces table in the current version. Elution peak settings and presets from versions before 3.8 will correctly migrate into the current version. However, Intact Analysis and Chromatogram Analysis projects created in versions 3.8 and above will not correctly open in older versions.

Elution Peak Backwards Compatibility

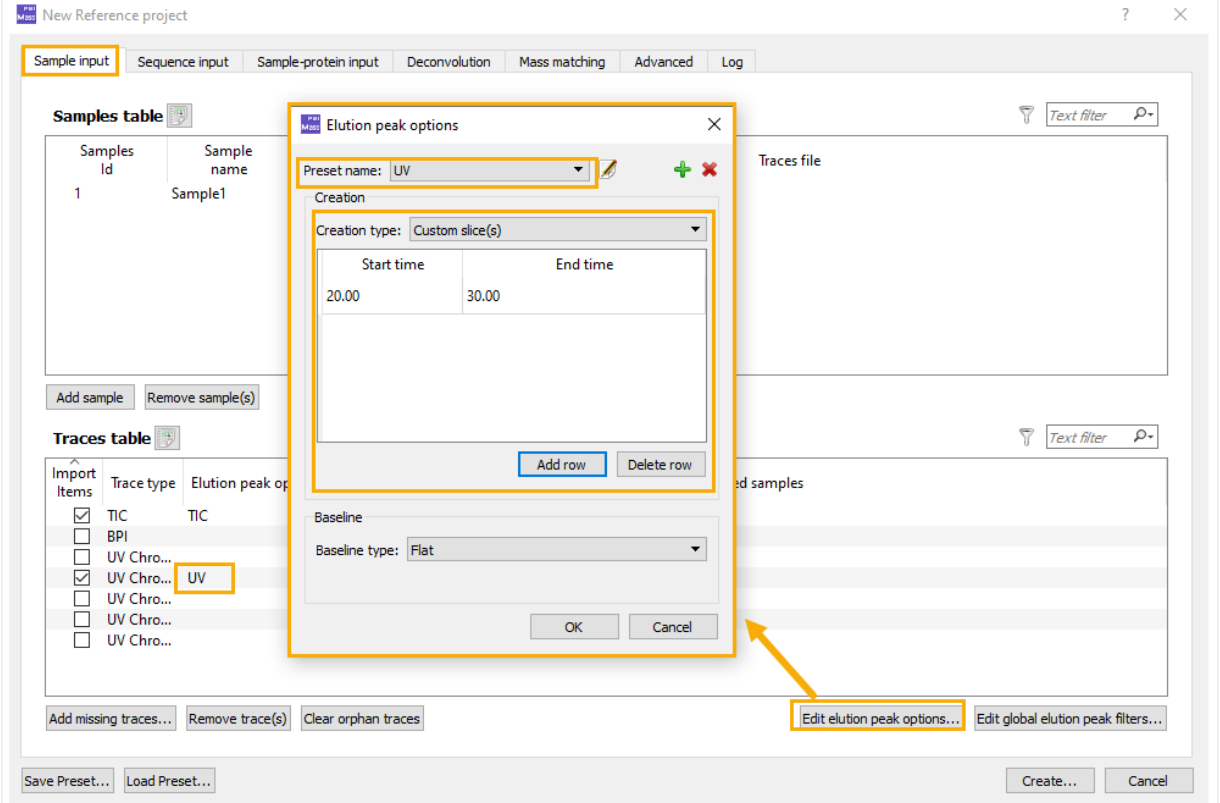
Several of the elution peak text commands have been migrated from the Advanced tab to Sample input tab. The following images illustrate how certain presets translate between previous versions and the current release. One to one matching was provided whenever possible.

Case I

Old GUI

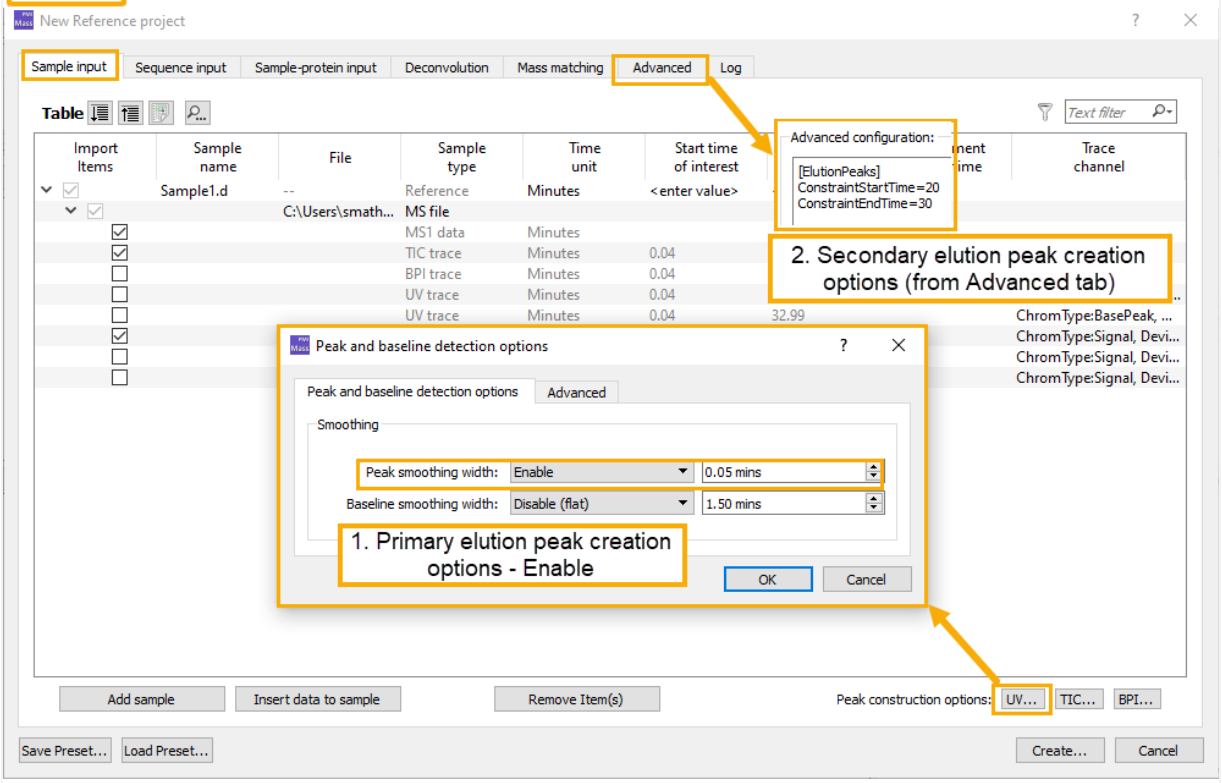


New GUI

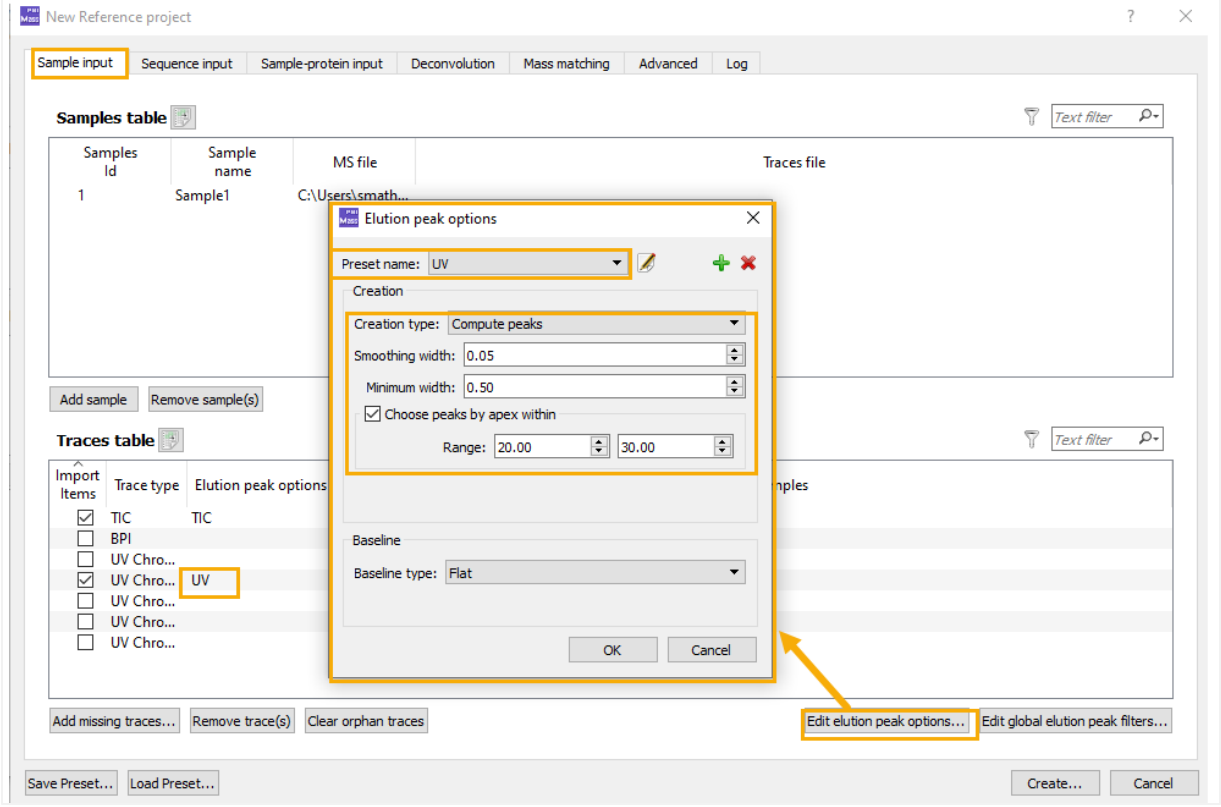


Case II

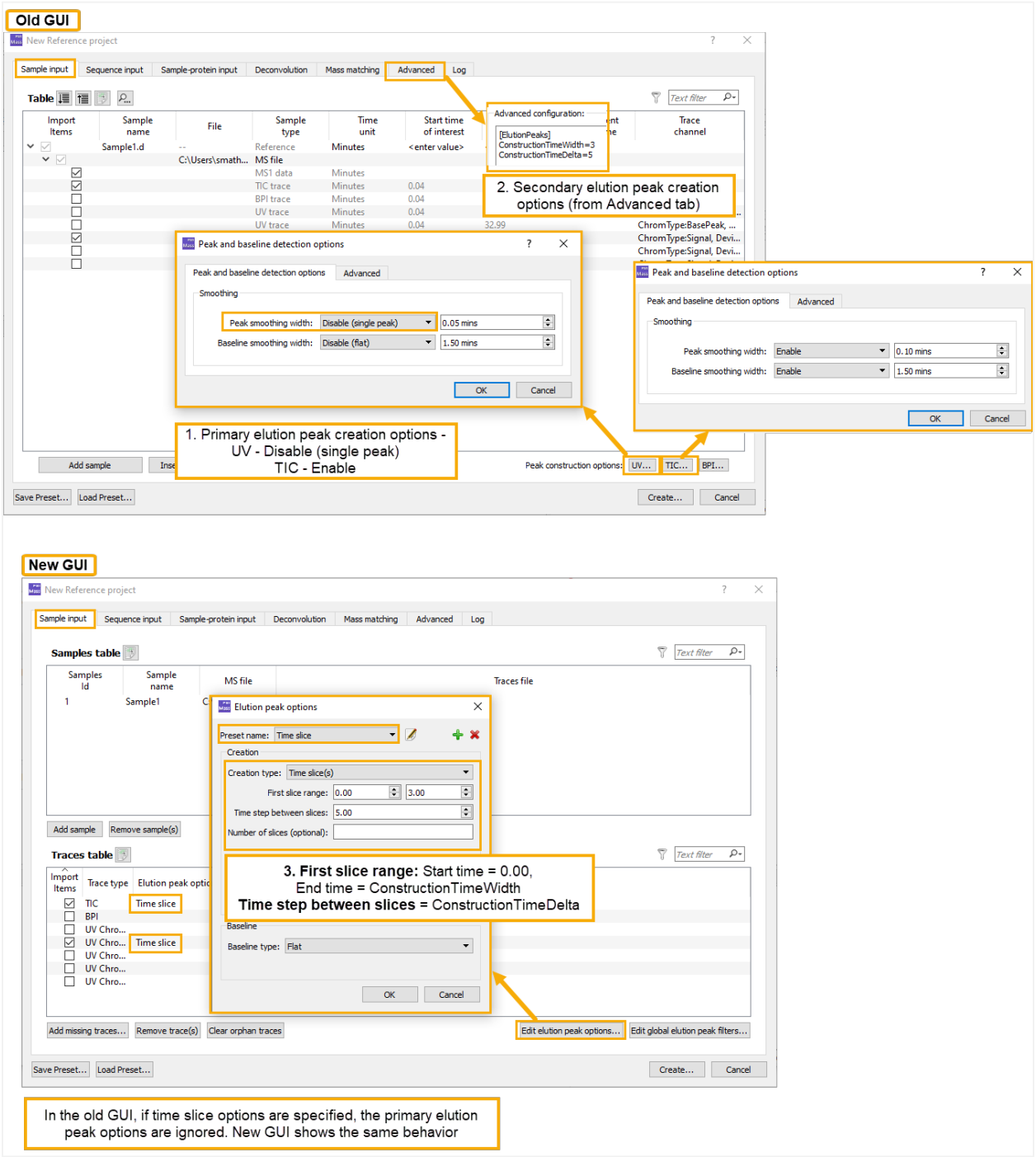
Old GUI



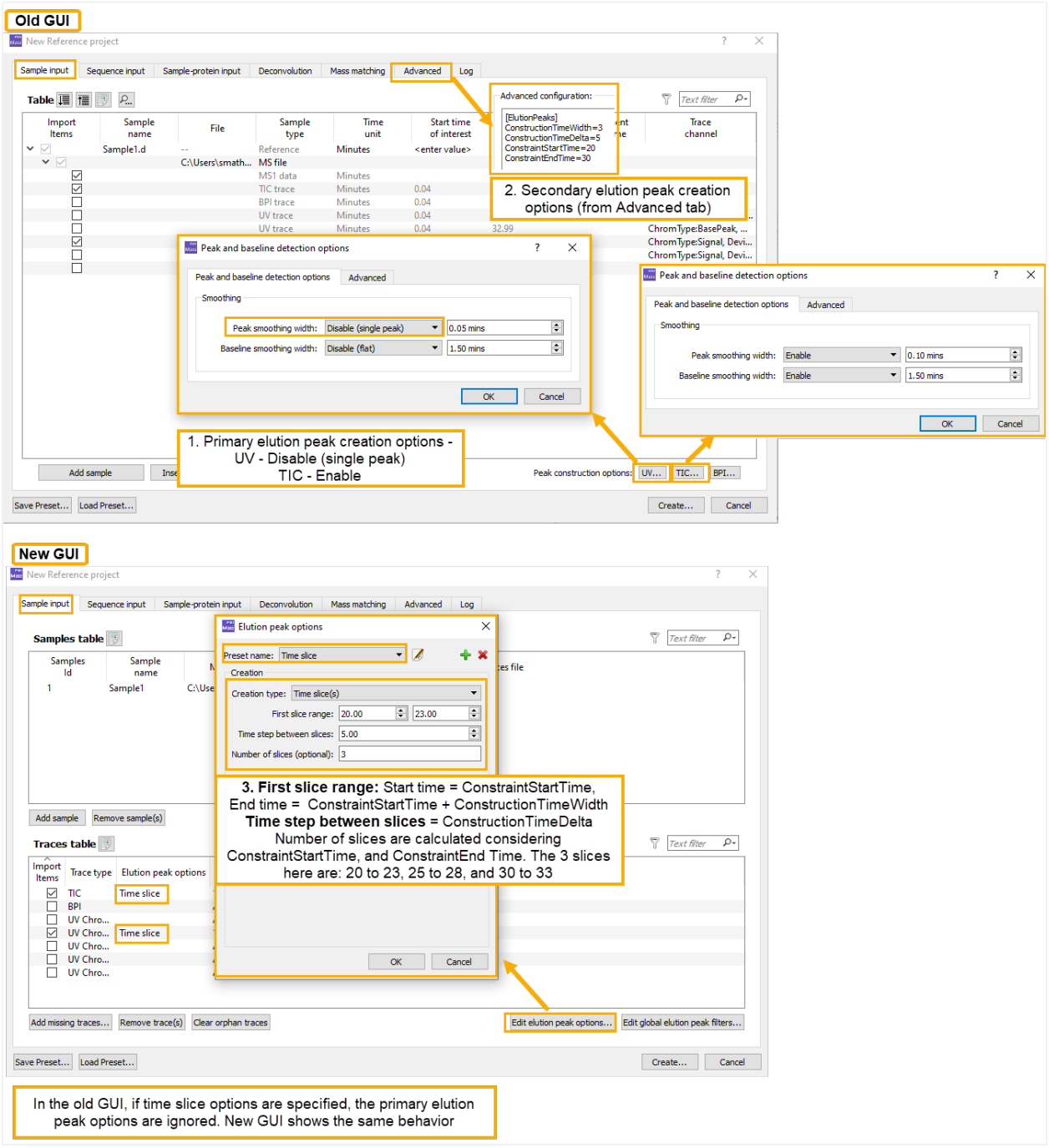
New GUI



Case III


Old GUI
New Reference project
Sample input
Sequence input
Sample-protein input
Deconvolution
Mass matching
Advanced
Log
Table
Text filter
Import Items
Sample name
File
Sample type
Time unit
Start time of interest
Trace channel
Sample1.d
Reference
Minutes
<enter value>
C:\Users\smath...
MS file
MS1 data
TIC trace
BPI trace
UV trace
Advanced configuration:
[ElutionPeaks]
ConstructionTimeWidth=3
ConstructionTimeDelta=5
2. Secondary elution peak creation options (from Advanced tab)
Peak and baseline detection options
Peak and baseline detection options
Advanced
Smoothing
Peak smoothing width:
Disable (single peak)
0.05 mins
Baseline smoothing width:
Disable (flat)
1.50 mins
OK
Cancel
Peak and baseline detection options
Peak and baseline detection options
Advanced
Smoothing
Peak smoothing width:
Enable
0.10 mins
Baseline smoothing width:
Enable
1.50 mins
OK
Cancel
1. Primary elution peak creation options -
UV - Disable (single peak)
TIC - Enable
Add sample
Peak construction options:
UV...
TIC...
BPI...
Save Preset...
Load Preset...
Create...
Cancel
New GUI
New Reference project
Sample input
Sequence input
Sample-protein input
Deconvolution
Mass matching
Advanced
Log
Samples table
Text filter
Samples Id
Sample name
MS file
Traces file
Elution peak options
Preset name:
Time slice
Creation
Creation type:
Time slice(s)
First slice range:
0.00
3.00
Time step between slices:
5.00
Number of slices (optional):
3. First slice range: Start time = 0.00,
End time = ConstructionTimeWidth
Time step between slices = ConstructionTimeDelta
Baseline
Baseline type:
Flat
OK
Cancel
Add sample
Remove sample(s)
Traces table
Import Items
Trace type
Elution peak optic
TIC
BPI
UV Chro...
Time slice
Add missing traces...
Remove trace(s)
Clear orphan traces
Edit elution peak options...
Edit global elution peak filters...
Save Preset...
Load Preset...
Create...
Cancel
In the old GUI, if time slice options are specified, the primary elution peak options are ignored. New GUI shows the same behavior

Case IV


Old GUI
New Reference project
Sample input
Sequence input
Sample-protein input
Deconvolution
Mass matching
Advanced
Log
Table
Advanced configuration:
[ElutionPeaks]
ConstructionTimeWidth=3
ConstructionTimeDelta=5
ConstraintStartTime=20
ConstraintEndTime=30
2. Secondary elution peak creation options (from Advanced tab)
Peak and baseline detection options
Peak and baseline detection options
Advanced
Smoothing
Peak smoothing width:
Disable (single peak)
0.05 mins
Baseline smoothing width:
Disable (flat)
1.50 mins
OK
Cancel
Peak and baseline detection options
Peak and baseline detection options
Advanced
Smoothing
Peak smoothing width:
Enable
0.10 mins
Baseline smoothing width:
Enable
1.50 mins
OK
Cancel
1. Primary elution peak creation options -
UV - Disable (single peak)
TIC - Enable
Add sample
Peak construction options:
UV...
TIC...
BPI...
Save Preset...
Load Preset...
Create...
Cancel
New GUI
New Reference project
Sample input
Sequence input
Sample-protein input
Deconvolution
Mass matching
Advanced
Log
Samples table
Elution peak options
Preset name:
Time slice
Creation
Creation type:
Time slice(s)
First slice range:
20.00
23.00
Time step between slices:
5.00
Number of slices (optional):
3
3. First slice range: Start time = ConstraintStartTime,
End time = ConstraintStartTime + ConstructionTimeWidth
Time step between slices = ConstructionTimeDelta
Number of slices are calculated considering
ConstraintStartTime, and ConstraintEnd Time. The 3 slices
here are: 20 to 23, 25 to 28, and 30 to 33
OK
Cancel
Add sample
Remove sample(s)
Traces table
Import Items
Trace type
Elution peak options
TIC
Time slice
BPI
UV Chro...
UV Chro...
Time slice
Add missing traces...
Remove trace(s)
Clear orphan traces
Edit elution peak options...
Edit global elution peak filters...
Save Preset...
Load Preset...
Create...
Cancel
In the old GUI, if time slice options are specified, the primary elution peak options are ignored. New GUI shows the same behavior

Migrated Commands from Advanced Tab

```
[ElutionPeaks]
ConstraintStartTime=0
ConstraintEndTime=0

[ElutionPeaks]
ConstructionTimeWidth=5
ConstructionTimeDelta=5


[ElutionPeak]
AutoCompute=UV
```

```
[ElutionPeaks]
MinPeakAreaPercent=0

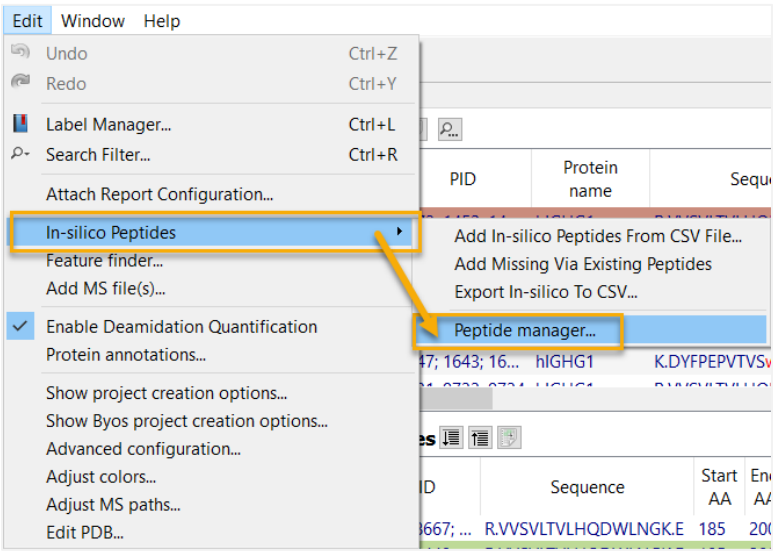
[Byomap]
ProjectCreateAutoAssign=true

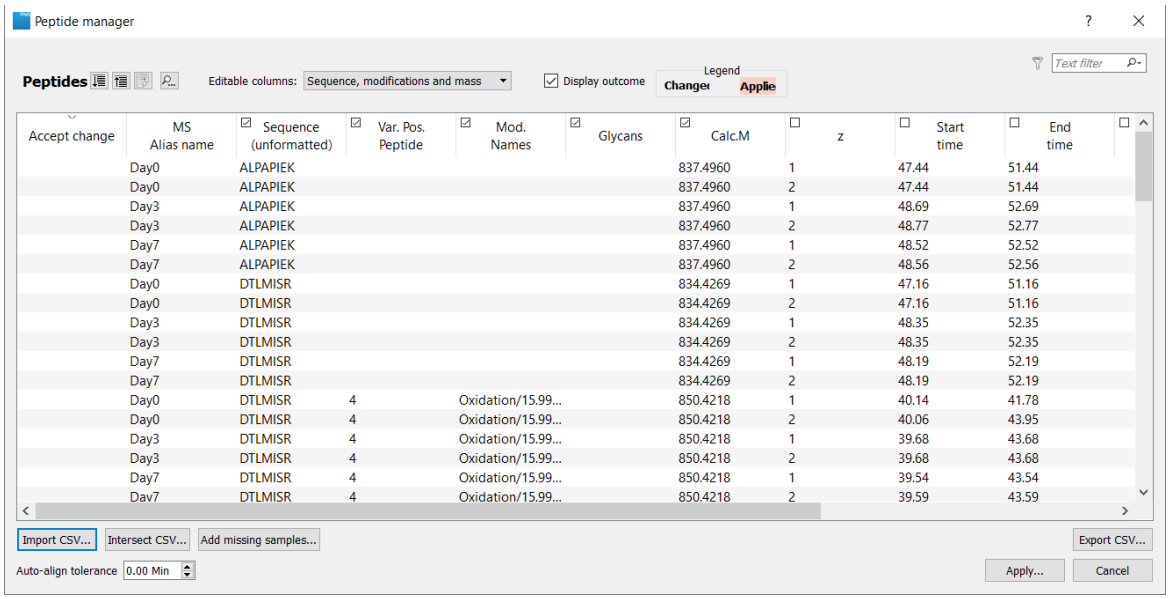

[Trace]
DefaultSelection=UV

[Trace]
DefaultUVTrace=2
```

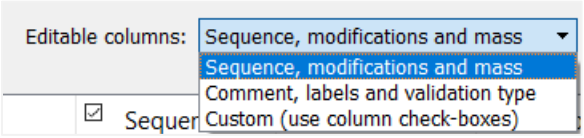
Peptide Manager - Peptide Analysis

This new tool allows the user to fine-tune in-silico processing. It combines all In-silico functionalities previously found in the Peptide Analysis **Edit > In-silico** menu with a color-coded preview of the project changes and enhanced control over which rows and columns are affected.

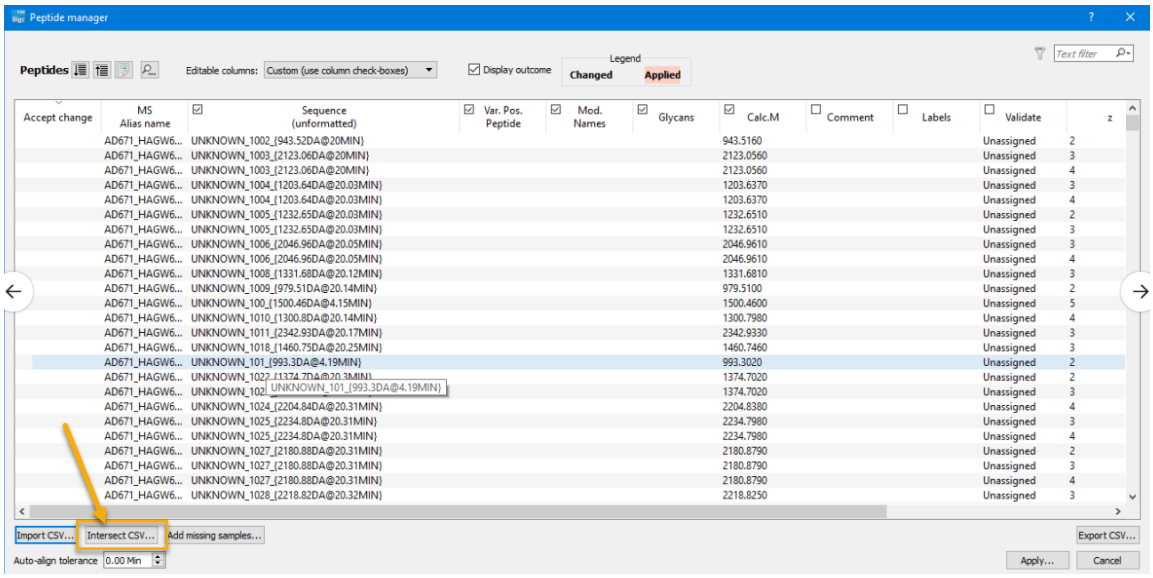




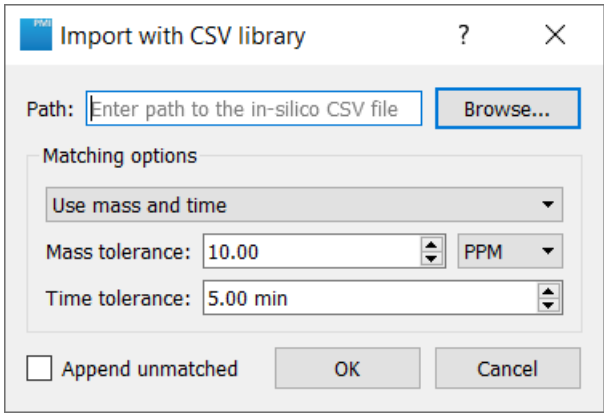
The user can set which columns are editable using the drop-down menu. Please note “Comment, labels and validation type” enables annotation transfer.



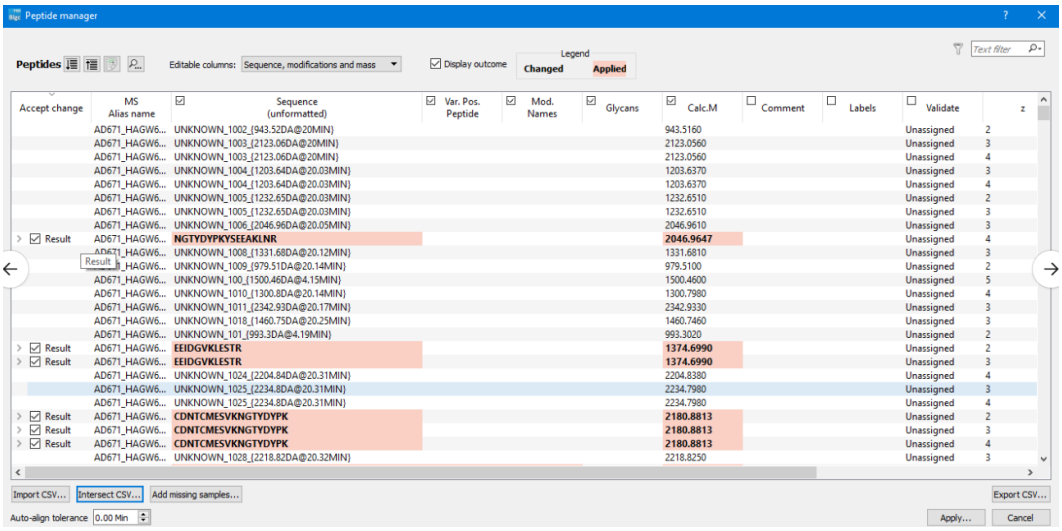
- Click **Export CSV** is the same as Edit > In-Silico > Export CSV.
    - Start and End times may differ from the previous CSV export. This is because Peptide Manager exports the most centered XIC start/end, while the old method exported the first available start/end range. The difference is considered an improvement.
- **Import CSV** is the same as in Edit > Import in silico from CSV
- **Intersect CSV** is the same as in previous menu item Edit > In-Silico > Intersect with CSV library. This menu item has been removed and can only be accessed from Peptide Manager.



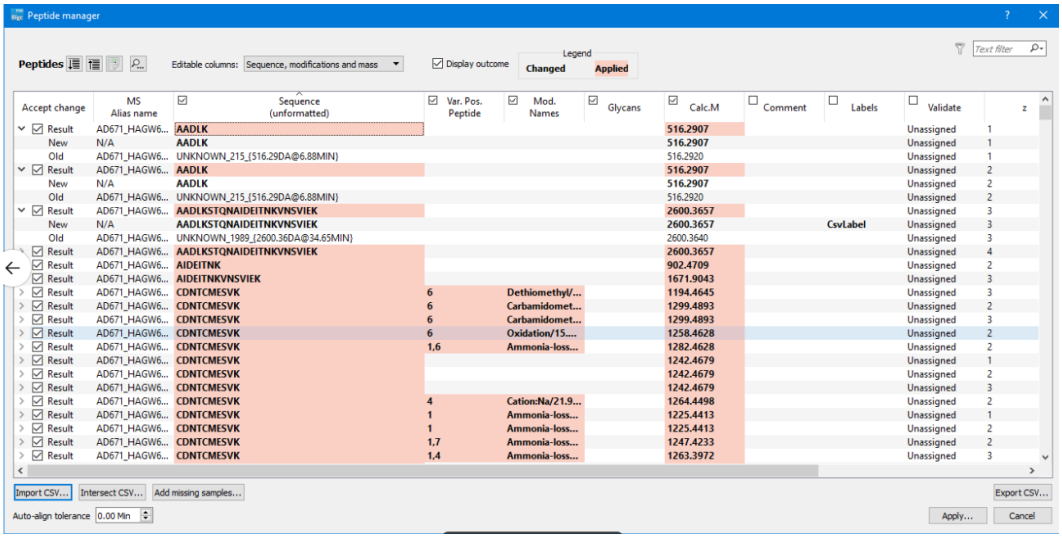
- Click Intersect CSV and select a CSV file:



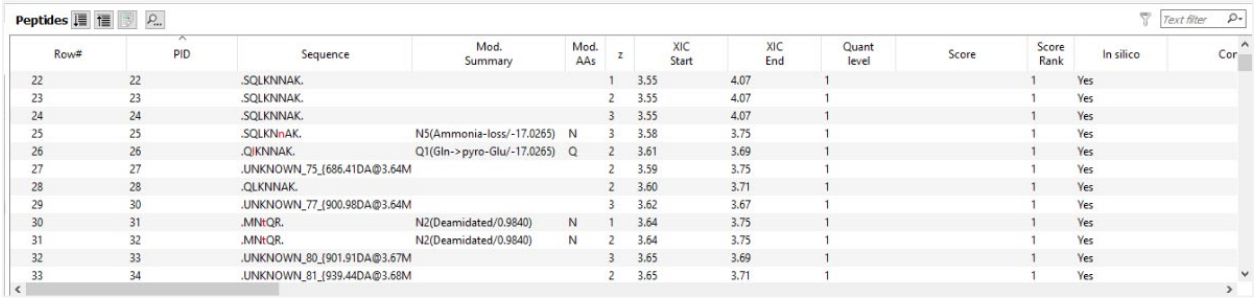
- Select a CSV file using the Browse button.
- Select between the Matching options “Use mass and time” and “Use sequence and modifications”
- Click OK to return to Peptide Manager and select columns to review. New values will be show in red.



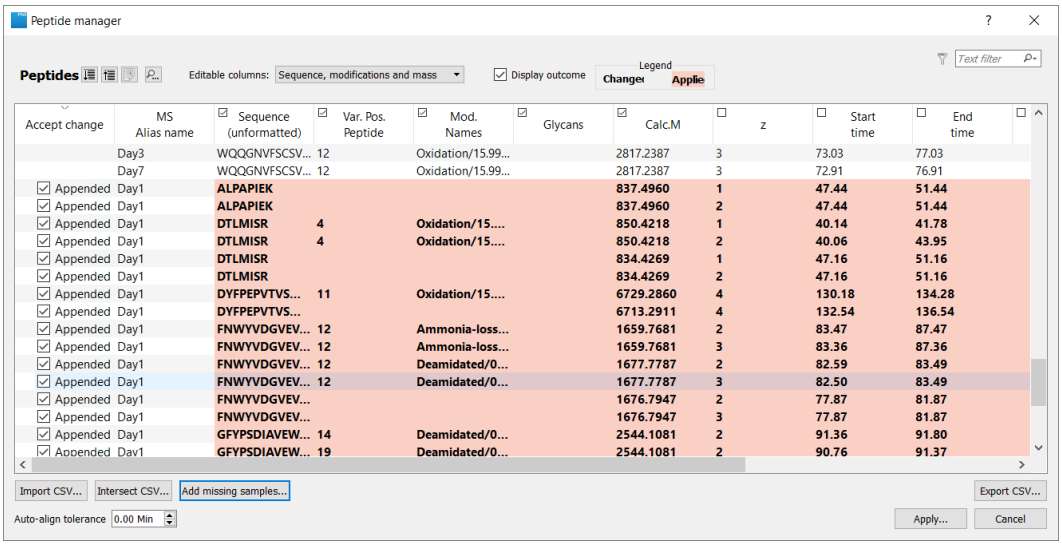
The below image displays expanded rows to showcase additional content



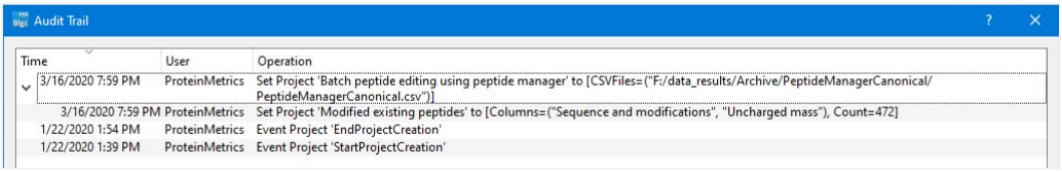
Click Apply to update the project. The below image displays a common use case – Feature Finder unknowns matched with in-silico content.



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



- Please note the Audit Trail documents all change made through the Peptide Manager

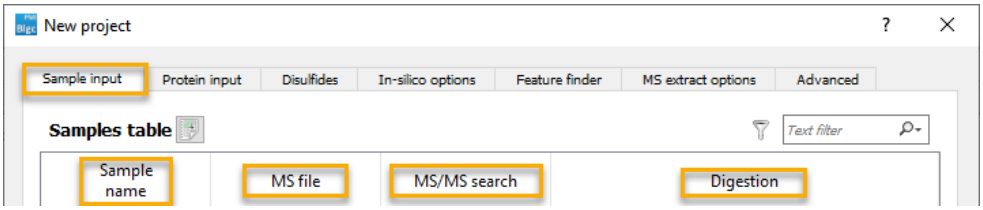


  - The CSV intersected
  - The columns altered
  - How many peptides were affected

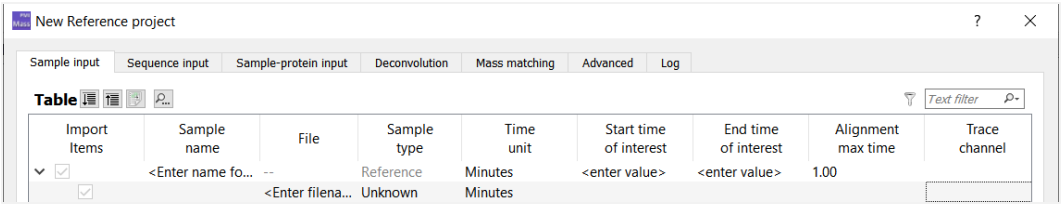
Sample Input Tab Redesign – Peptide Analysis, Intact Analysis, and Chromatogram Analysis

The headers in the new project creation Samples input tabs have been redesigned and renamed.

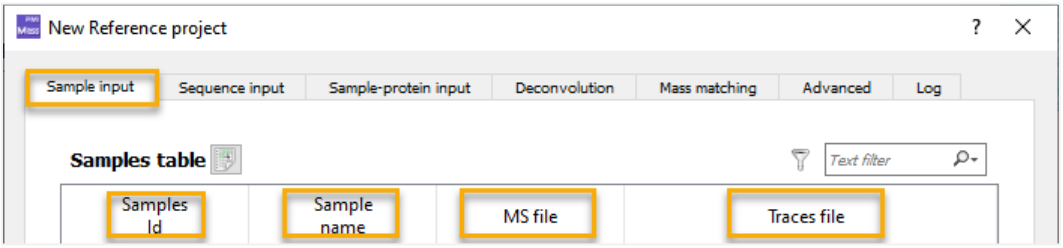
- In Peptide Analysis and the related Byos workflows, the **Sample input tab** header “Alias name” is now “Sample name”, “File” is now “MS file” and “Ms2 search” is now “MS/MS search”. **MS/MS search** is now opened directly in the **MS file** row, rather than as a sub-row of the MS file.



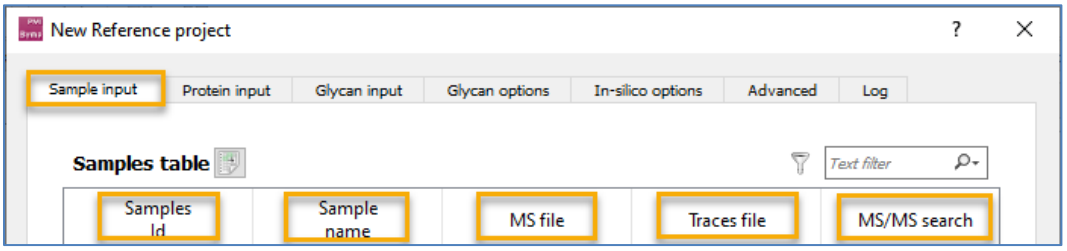
- In Intact Analysis, Chromatogram Analysis, and the related Byos workflows, the **Sample input tab** has been completely redesigned. The previous tab contents are shown below:



The new Sample tab contents for Intact Analysis are shown below



The new Sample tab contents for Chromatogram Analysis are shown below

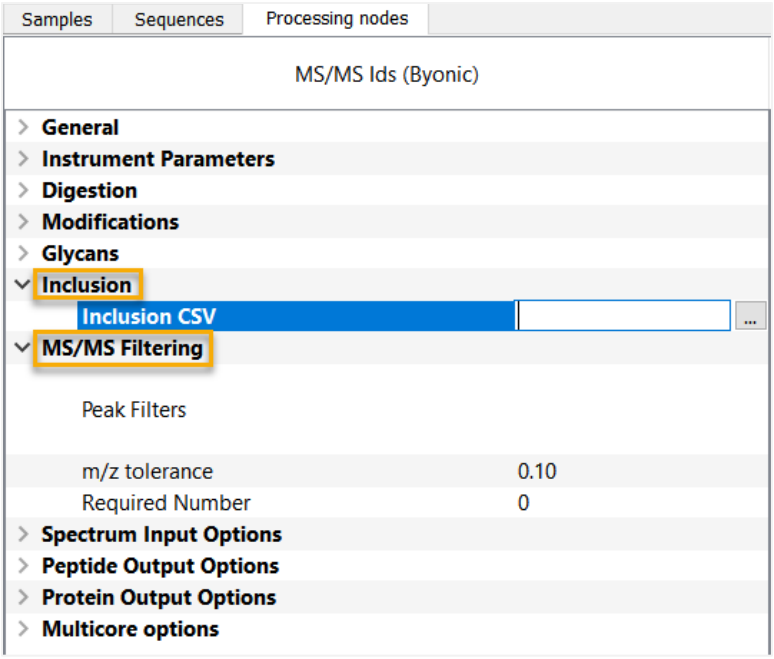


The **Start time** and **End time** columns have been moved to the **Global elution peak filters** dialog. The **Time unit** and **Alignment max time** columns have been moved to the **Advanced** tab. See the **Elution Peak Calculations and the Traces Table** section for more details about the new Sample input tab design.

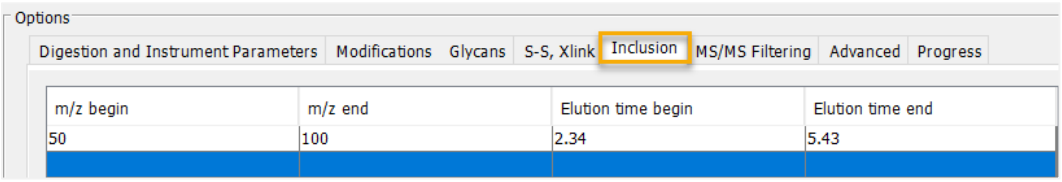
Byonic and Byos

- Inclusion and MS/MS Filtering tabs

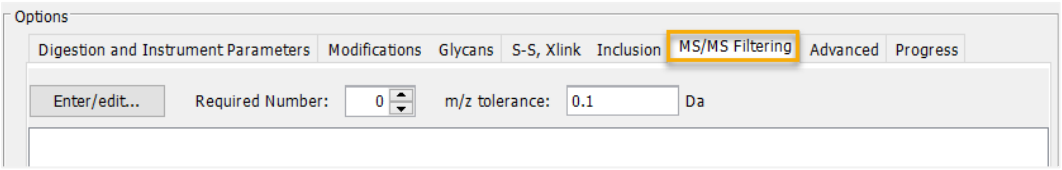
  In Byos, two new sections appear in the **Processing nodes** tab for workflows that include Byonic parameters – Inclusion and MS/MS Filtering. The Inclusion feature, added to Byonic in the 2019-12 release, is now part of Byonic workflows in Byos. MS/MS Filtering is new to both Byonic and Byonic workflows in Byos. In Byos, the features appear in the MS/MS Ids section of the **Processing nodes** tab:



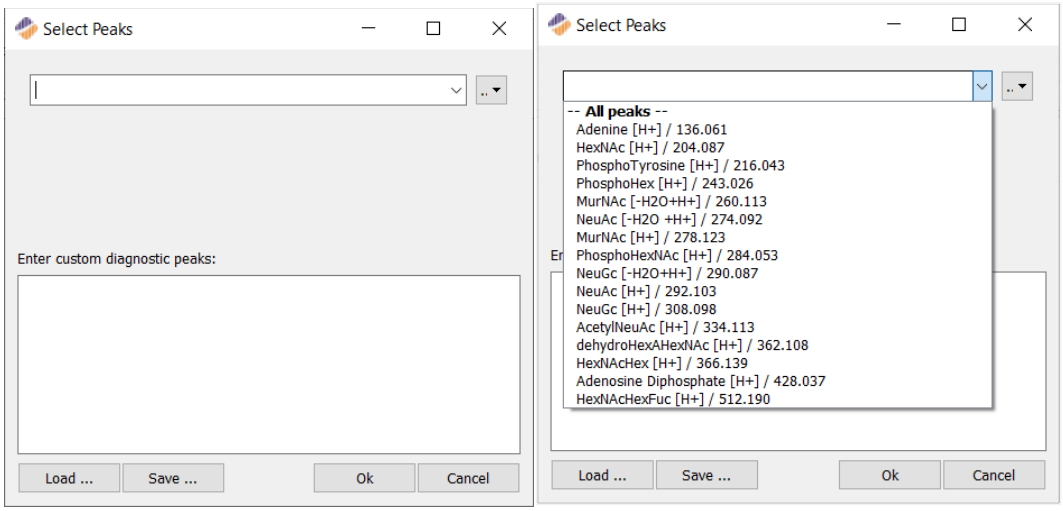
The **Inclusion** section allows the import of *.csv files which define m/z ratio ranges and/or elution time range segments to include in the search. In Byonic, **Inclusion** is represented as a tab:



The **MS/MS Filtering** tab/section allows the user to create MS/MS diagnostic peak filters:



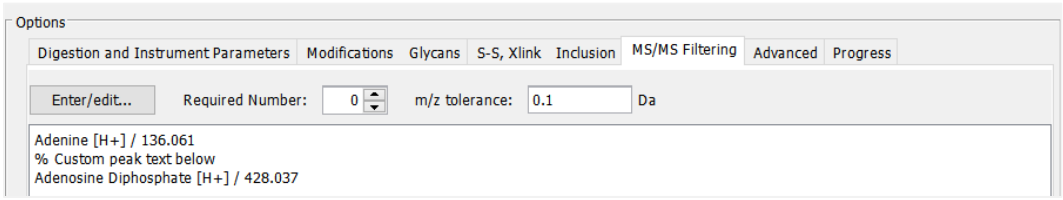
The **Enter/edit** button in Byonic and the **Peak Filters** ... button in Byos each open the **Select Peaks** dialog:



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

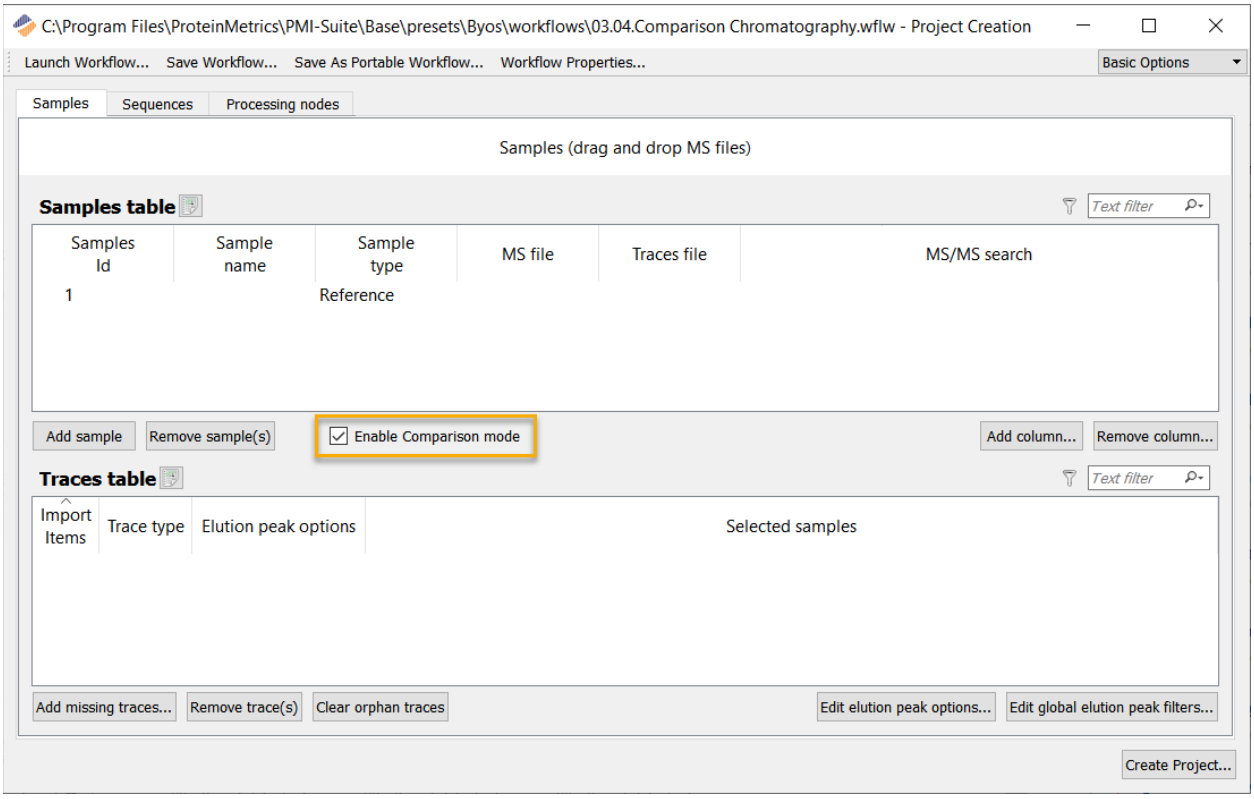
Click **OK** and the diagnostic peaks strings are now listed in the MS/MS Filtering tab:



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. A maximum m/z tolerance for applying the diagnostic peaks can also be set.

Byos – Enable Comparison Mode

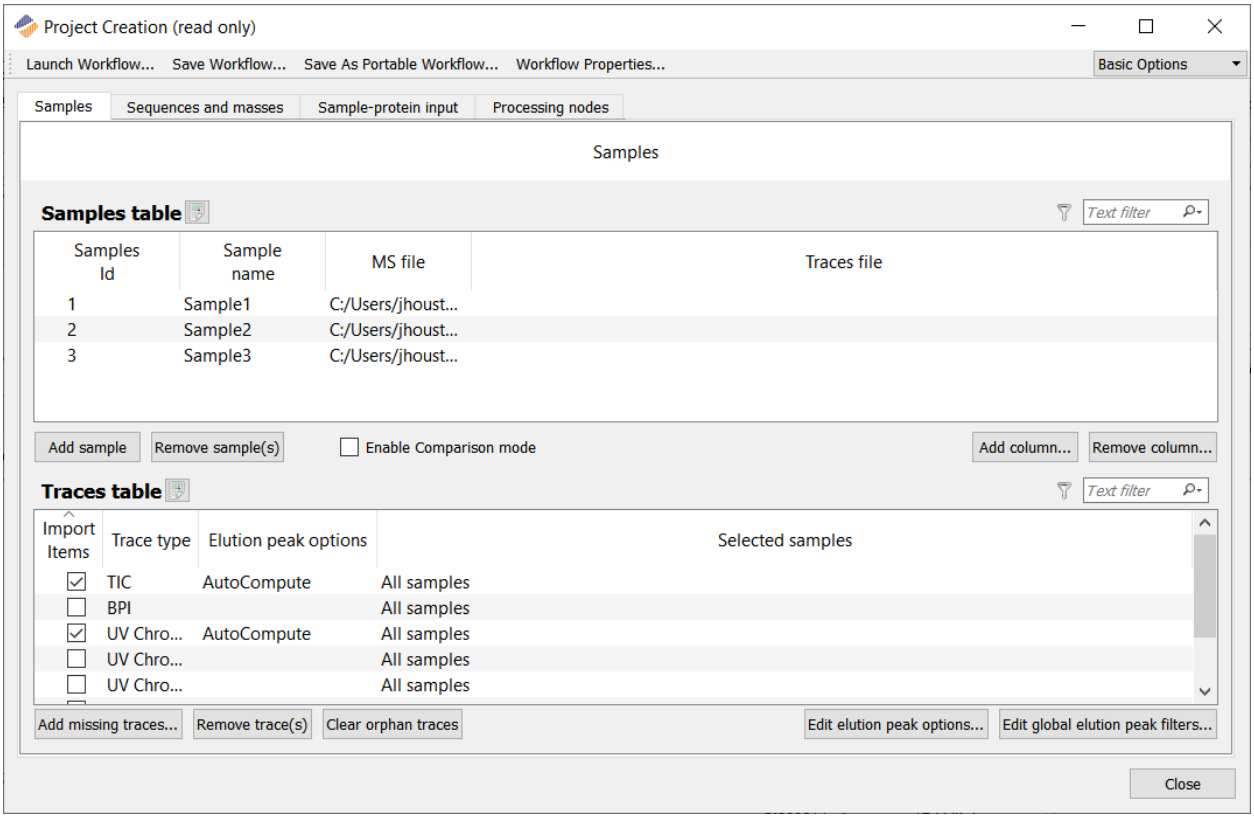
For Byos Intact Analysis and Chromatography Analysis workflows, new comparison projects are created by checking the **Enable Comparison mode** checkbox in the **Samples** tab:



Enable Comparison mode is unchecked by default except for the Comparison Chromatography, Comparison Chromatography (in-silico), and MAM New Peak Detection workflows. The column **Sample type** is displayed for comparison projects only.

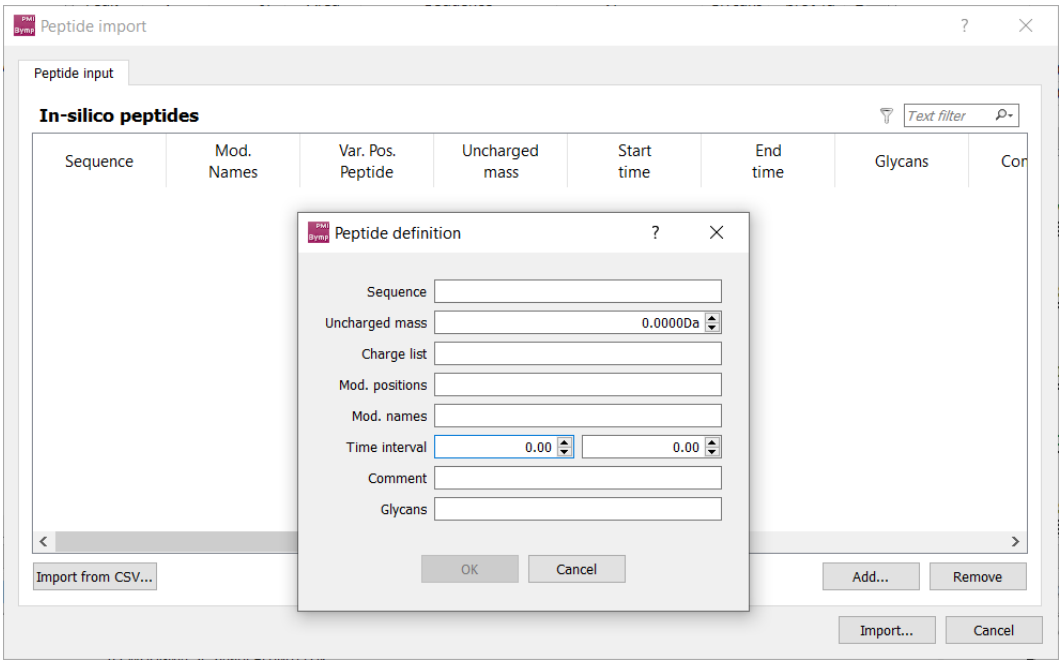
Show Byos project creation options

The Byos workflow project creation options used to generate a project can now be viewed when that project is opened. From Byos, choose **Edit > Show Byos project creation options**. Although this menu item is also available within Peptide Analysis. Intact Analysis and Chromatogram Analysis, this feature is only available for projects created through Byos. If the project were created in one of the legacy applications, **Edit > Show projects creation options** remains available. A read-only dialog similar to the Byos Project Creation dialog opens:



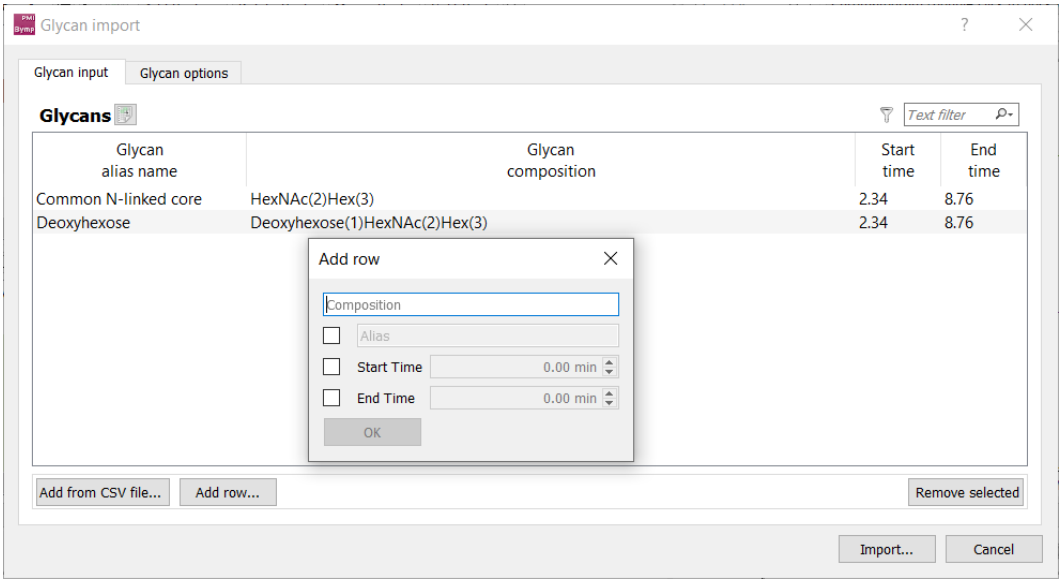
Glycan import – Chromatogram Analysis

The Chromatogram Analysis menu **Edit > Import candidates** has been divided into two Edit actions: **Import peptide candidates** opens a dialog to import or manually enter in-silico peptides:



Add from CSV file imports glycans from a *.csv file. **Add** opens the sub-dialog shown above, where the peptide sequence, mass and other properties can be added manually.

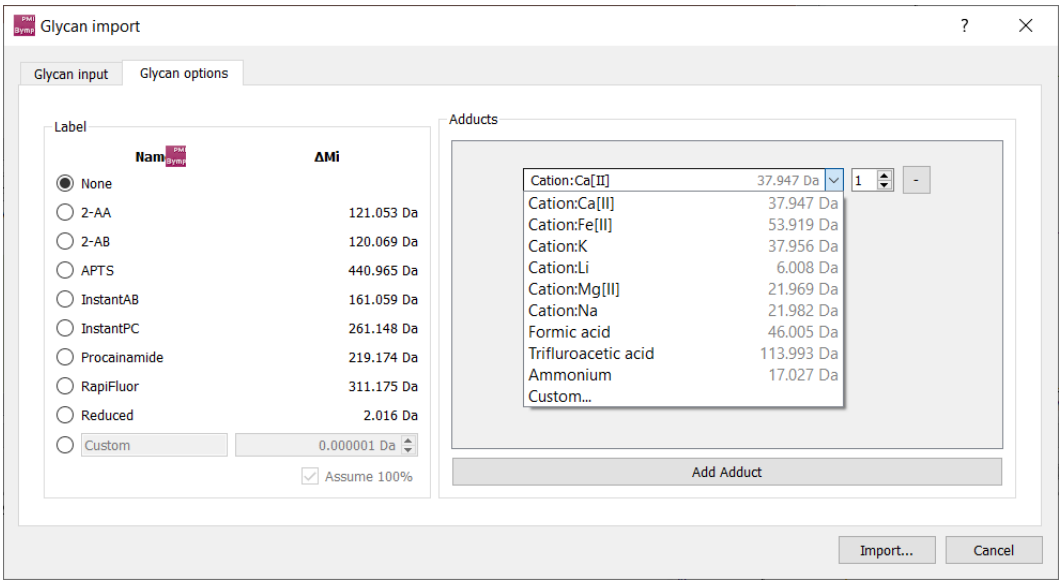
Edit > Import detached glycan candidates opens a dialog to import or manually enter glycans:



Add from CSV file imports glycans from a *.csv file. The file has the format:

```
GlycanAliasName,GlycanComposition,StartTime,EndTime
Common N-linked core,HexNAc(2)Hex(3),0,160
```

Add row opens the sub-dialog shown above, where a glycan composition can be added manually, along with optional entries for Alias, Start Time and End Time. The **Glycan options** tab defines labels and addicts for the glycans:

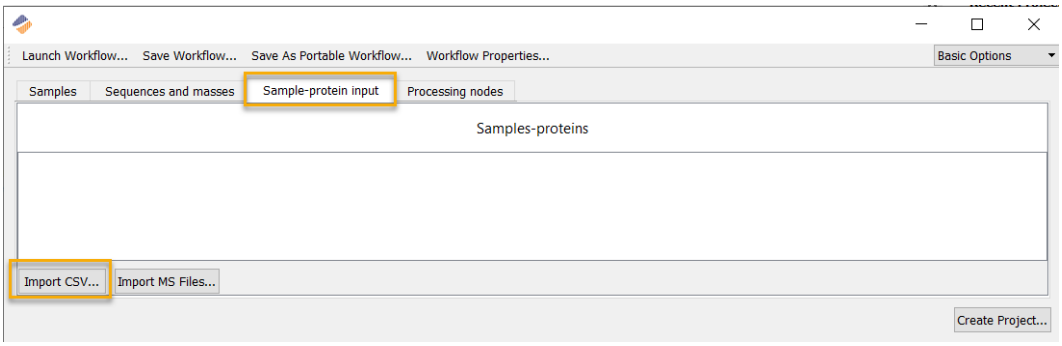


Hide Protein Coverage

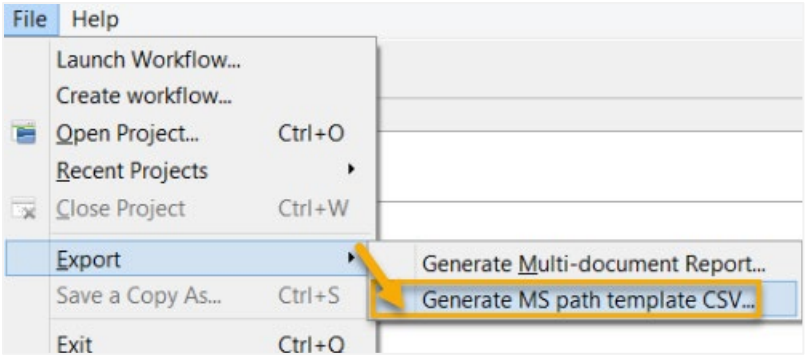
For Peptide and Chromatogram Analysis projects with a very large number of proteins, the Protein Coverage graphic can be turned off to speed loading of the project. The **Enable coverage rendering** checkbox can be accessed by the icon or from right-click **Rendering options.**

Intact Analysis

- **The Sample-protein input tab is now available in both Intact Analysis and Byos:**



The user can use the Import CSV button to import the sample information. The CSV is created through File > Export > Generate MS path template CSV.



- **Additional Chemical Element – Fluorine**

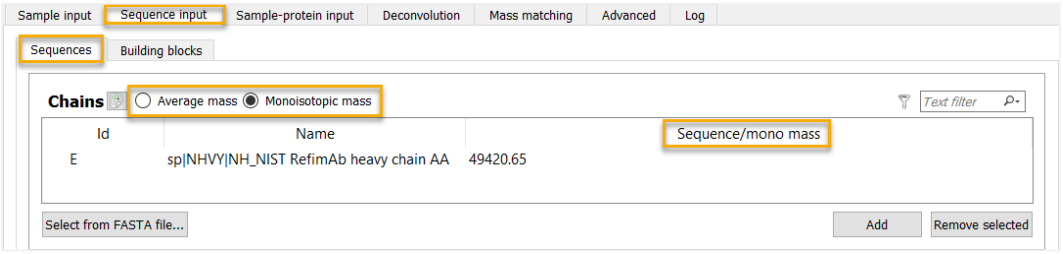
  In the new project creating Sequence input tab, fluorine (F) has been added to the Chemical Elements table in the Building blocks sub-tab:

Chemical Elements

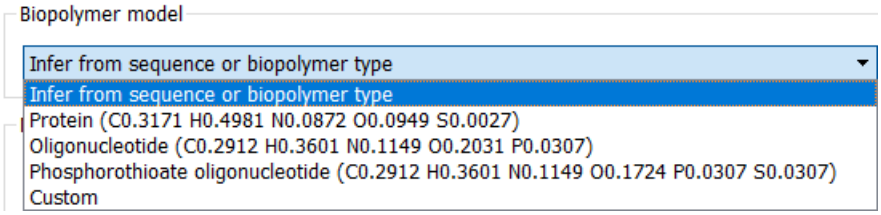
Element	Average mass
C	12.01079
H	1.007968
N	14.00669
O	15.99937
S	32.0639
P	30.973762
Se	78.971
F	18.998403163

- **Average Mass, Monoisotopic Mass and Biopolymer Models**

Users can now designate peptide chain masses without sequences as average mass or monoisotopic mass in Intact Analysis and the related workflows in Byos. In the **Chains** section of the **Sequences** sub-tab of the **Sequence input** tab, a radio button selects between the two types of masses:



- Two radio buttons **Average mass** and **Monoisotopic mass** have been added to the Sequences tab to specify the mass type that is entered in the sequence/average mass column in the Chains table. The selected mass type is only relevant if the mass (not sequence) has been entered in the Sequence/mass column in the Chains table. Selecting the **Monoisotopic mass** radio button will change the column to be **Sequence/mono mass**.
- Assignments will remain based upon the average mass.
- Users can select the kind of biopolymer model used to convert average mass to monoisotopic mass and monoisotopic mass to average mass:



Model options include:

- **Infer from sequence or biopolymer type** is the default selection, which uses the standard model that Intact Analysis has used to calculate mass.
- **Protein** (C0.3171 H0.4981 N0.0872 O0.0949 S0.0027) uses the displayed averagine formula to calculate mass.
- **Oligonucleotide** (C0.2912 H0.3601 N0.1149 O0.2031 P0.0307) uses the displayed formula to calculate mass.
- **Phosphorothioate oligonucleotide** (C0.2912 H0.3601 N0.1149 O0.1724 P0.0307 S0.0307) uses the displayed formula to calculate mass.
- **Custom** opens a cell to the right of the selection to add a custom formula to calculate mass.

- **Validation added to Reconstruction dialog**

  Additional validation has been added for the Protein Targets table in the Reconstruction dialog:

  - For each protein in Protein Targets table, there should be matching protein name in the Reconstruction table.
  - The protein name must be populated.

- The protein count value must be greater than 0.
- The gauss width value must be greater than 0.

The default values for the protein count and he gauss width has been added. By default:

- The protein count value is set to 1.
- The gauss width value is set to 5.

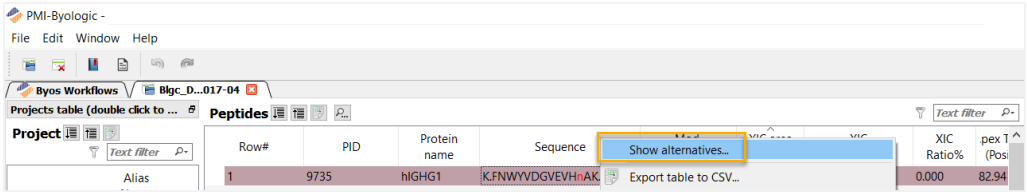
Peptide Analysis

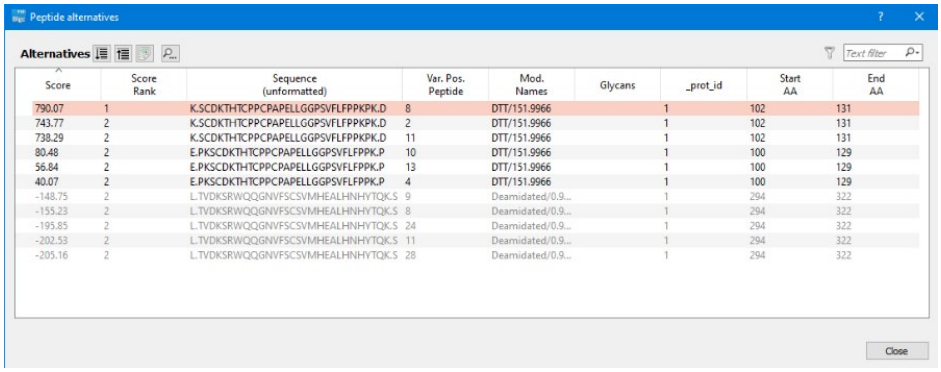
- **Isoelectric Point (pI value) Column**

  This information is now included in a new column in the Peptides table. This value is also included in the report.

- **Alternative MS2 Identifications**

  New ability to display the alternative MS2 identifications (of different sequence and/or modifications) for each peptide. This information is available through a right-click and selecting Show alternatives. A table will become available displaying the alternatives for each peptide along with their score. This feature is only available in for projects created in the 3.8 release.



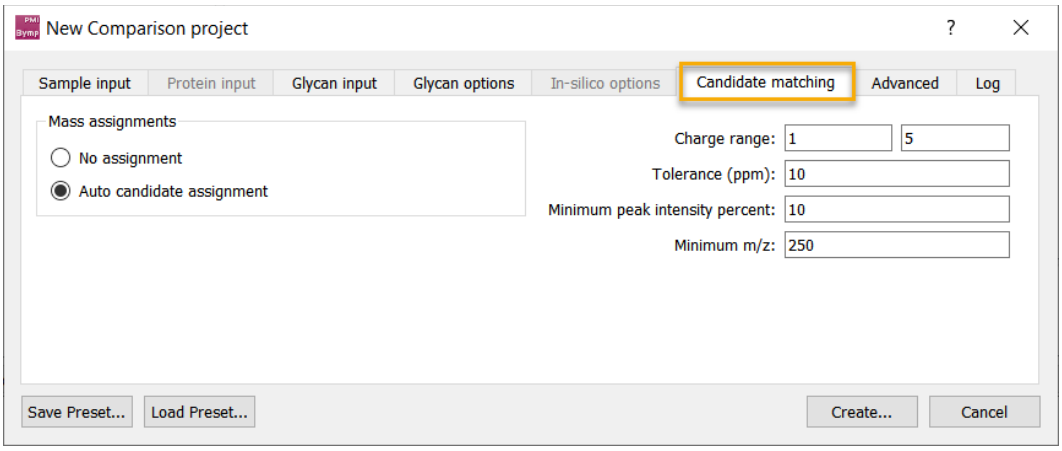


- The best candidate is highlighted in red
- The candidates scoring above the threshold are visible in black

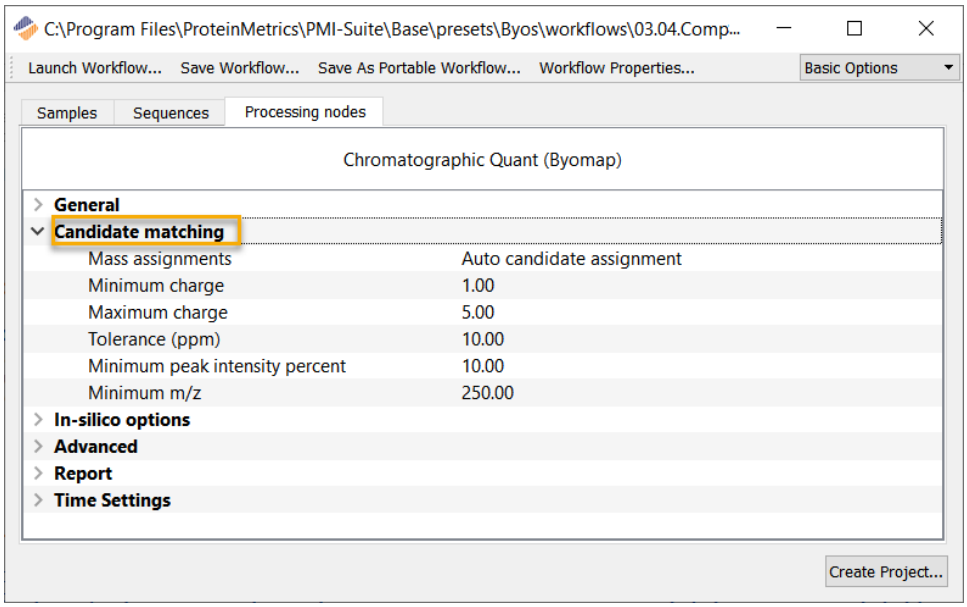
Chromatogram Analysis

- **Candidate Matching Tab**

  The **Candidate matching** tab was added to the project creation dialog for reference projects and comparison projects:

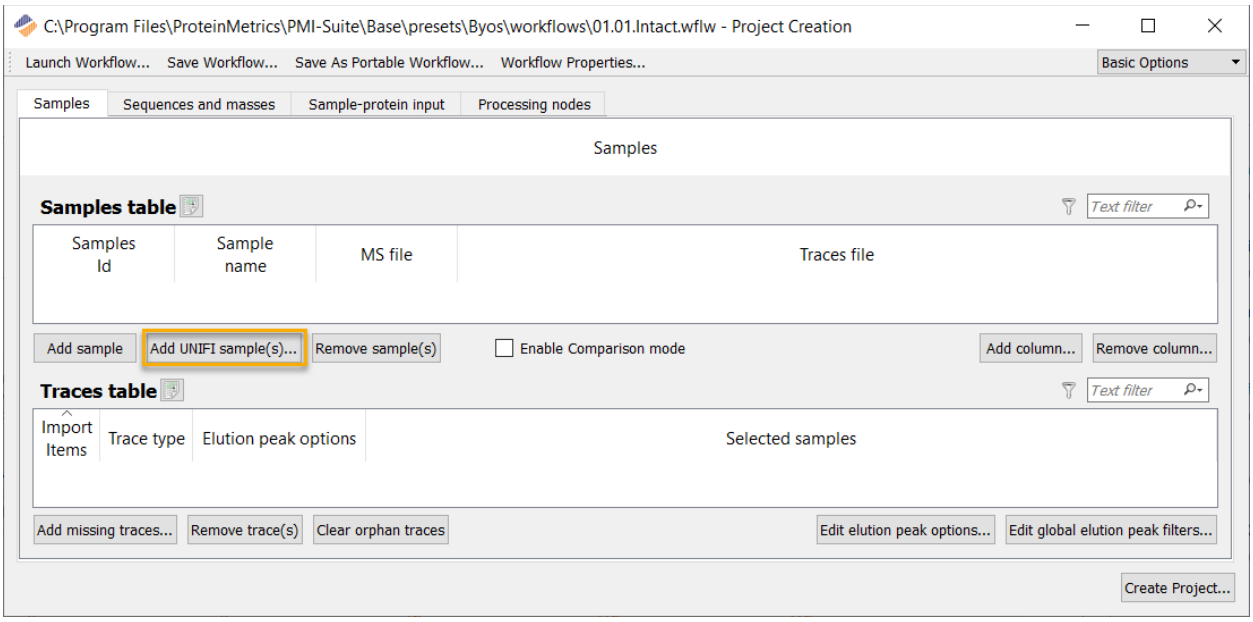


Candidate matching filters can now be modified before project creation. In previous versions, users could modify candidate matching filters only after project creation. In Byos, Candidate matching is found in the Processing node tab:

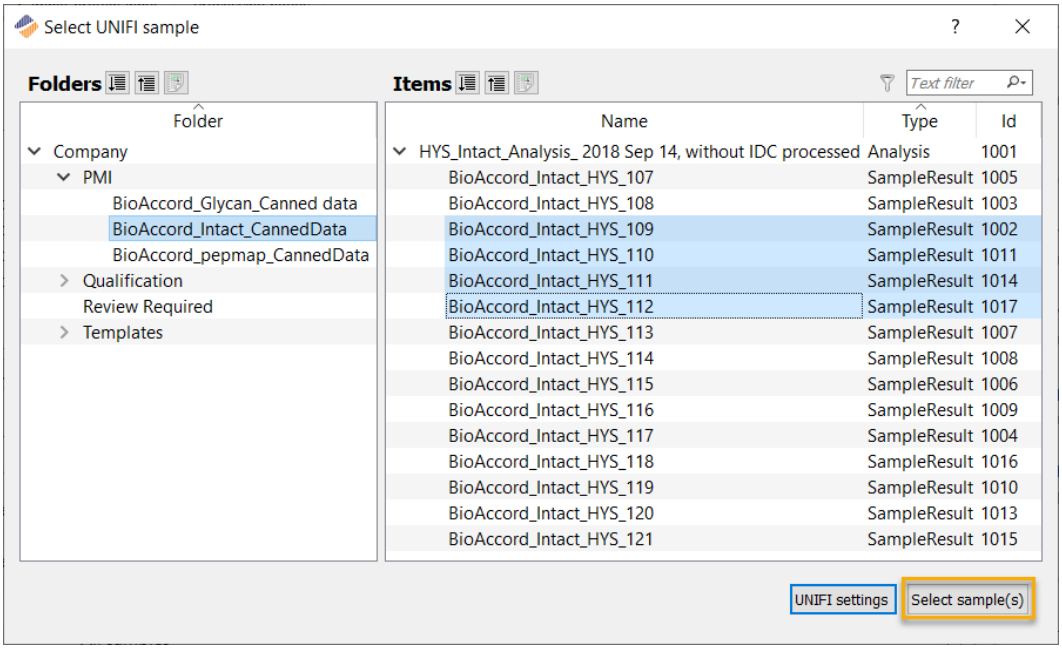


Byos UNIFI API Integration

Sample results stored in the Waters UNIFI Application Programming Interface (API) can now be loaded directly into Byos. The integration requires installation of the Waters UNIFI API and configuration of both UNIFI and Byos. The **Add UNIFI sample(s)** button appears in the **Samples** tab in Byos:



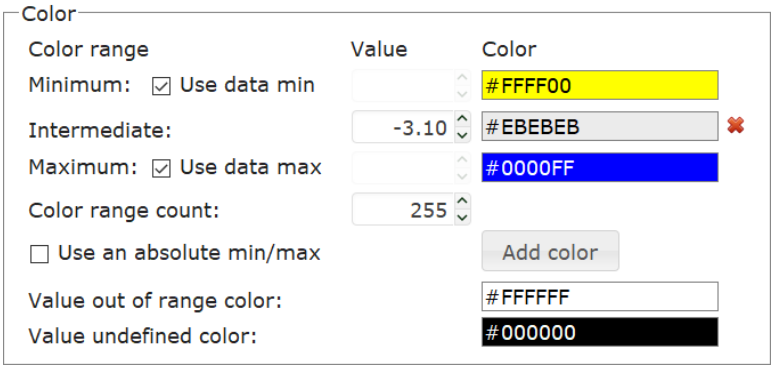
This opens the **Select UNIFI sample** dialog where MS data stored in UNIFI can be browsed and selected for download to Byos:



To learn how to access UNIFI from Byos, please refer to **PMI UNIFI Integration User Guide.docx**. Please start with the **PMI Byos UNIFI Integration Prerequisites Guide.docx**.

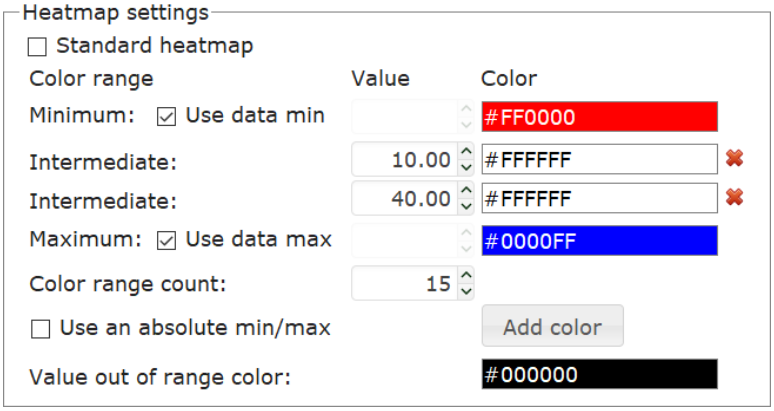
Reports

- **Protein 3D reports have advanced color-by features.**



- Specific minimum or maximum values can be assigned by unchecking **Use data min** or **max** and entering those values.
- One or more intermediate colors are inserted by the **Add color** button to create multiple color ranges. The default intermediate value is midway between the minimum and maximum values. This value and the intermediate color are editable. Additional intermediate colors can be inserted with the Add color button.
- The **Color range count** sets the number of color gradations or bins between the two color ranges. The maximum value, 255, creates a gradual shift in color across the range. A much smaller count creates distinct colors for a set of like ranges of values.
- **Use an absolute min/max** applies the larger of the absolute value of a negative minimum and the maximum value as the range to keep zero at its center.

- **Heatmap reports also support multiple color assignments**



- In addition to **Standard heatmap** (which behaves as before), any color can be assigned to the minimum value.
- **Use data min** or **max**, **Color range count** and **Use an absolute min/max** all function the same way as with Protein 3D reports.
- One or more intermediate colors are inserted by the **Add color** button, as with Protein 3D reports.

Release 2019-12 (v3.7)

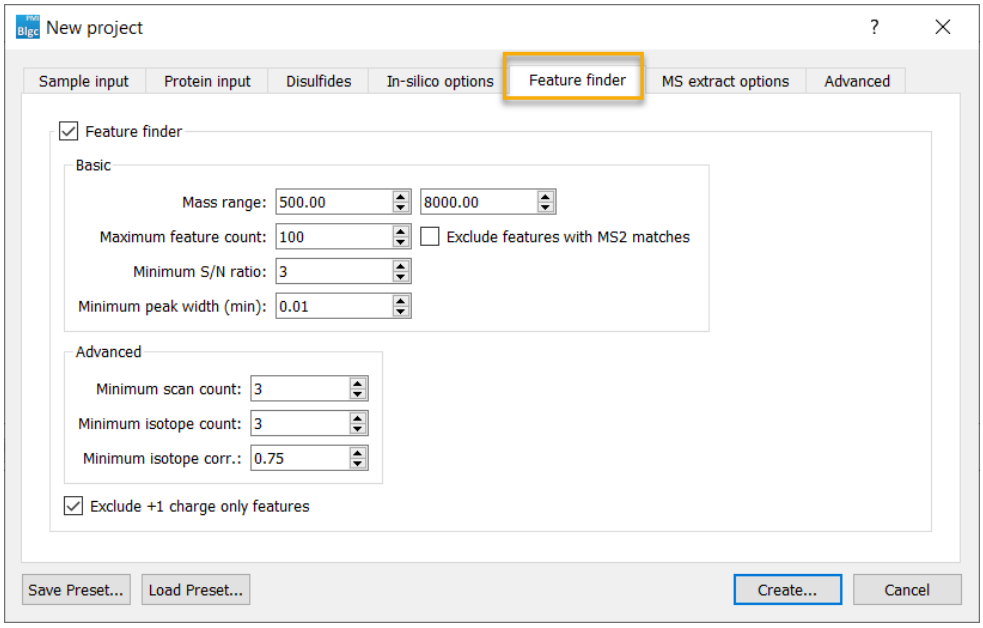
Feature Finder – Byos and Byologic

Feature Finder was first released as beta feature in 2019.06 Release. The advanced commands have now been added to the user interface. A new "Blgc_FeatureFinder.rptc" has also been added to the default set of Byos and Byologic report templates.

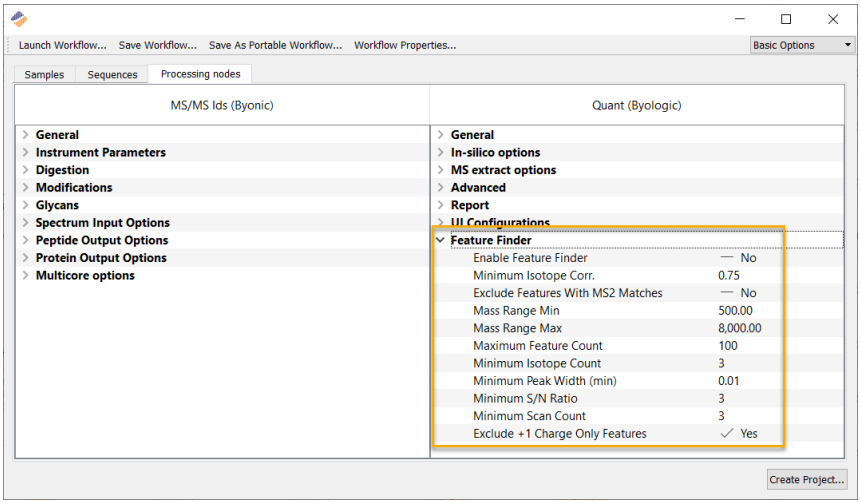
Feature Finder is an algorithm that allows the user to scan the MS1 domain for all existent peptides in a sample. In a typical data dependent acquisition assay, peptides are identified only if an MS2 scan is triggered from a precursor ion in an MS1 scan. This means that if a peptide ion does not trigger an MS2 scan, or its score excludes it from identification, its existence will go unknown. 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. Coupled with our proprietary time warping algorithms, differential expression and new peak detection become a trivial task when comparing samples.

This new option also allows the user to match these features to the current identifications and 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.

Byologic – new "Feature finder" tab



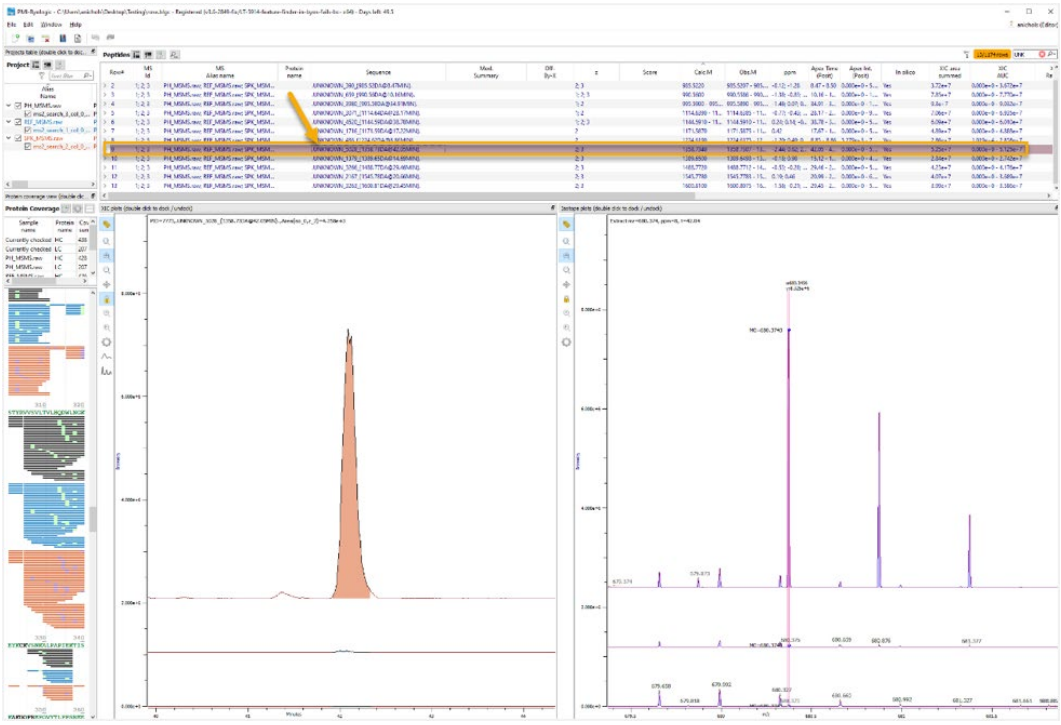
Byos – new "Feature Finder" section of Processing nodes



- Either check the box or toggle between No / Yes to activate Feature Finder
- Either check the box or toggle between No / 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.
- Either check the box or toggle between No / Yes to exclude singly charged features

Parameter	Description
Minimum Isotope Corr.	Minimum cutoff as to how close the found isotopic distribution matches the theoretical 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	Minimum mass to be considered as a potential feature
Mass Range Max	Maximum mass to be considered as a potential feature
Maximum Features Count	Only the most intense features will be included (in the above example, this is set to 100)
Minimum Isotope Count	Minimum number of isotope peaks required for something to be considered a feature if its charge is greater than 2 (does not apply to charge states less than 2).
Minimum Peak Width (min)	Minimum peak width (in minutes)
Minimum S/N Ratio	Requirement for S/N for a feature to be considered
Minimum Scan Count	Minimum scans across the peak for a feature to be detected

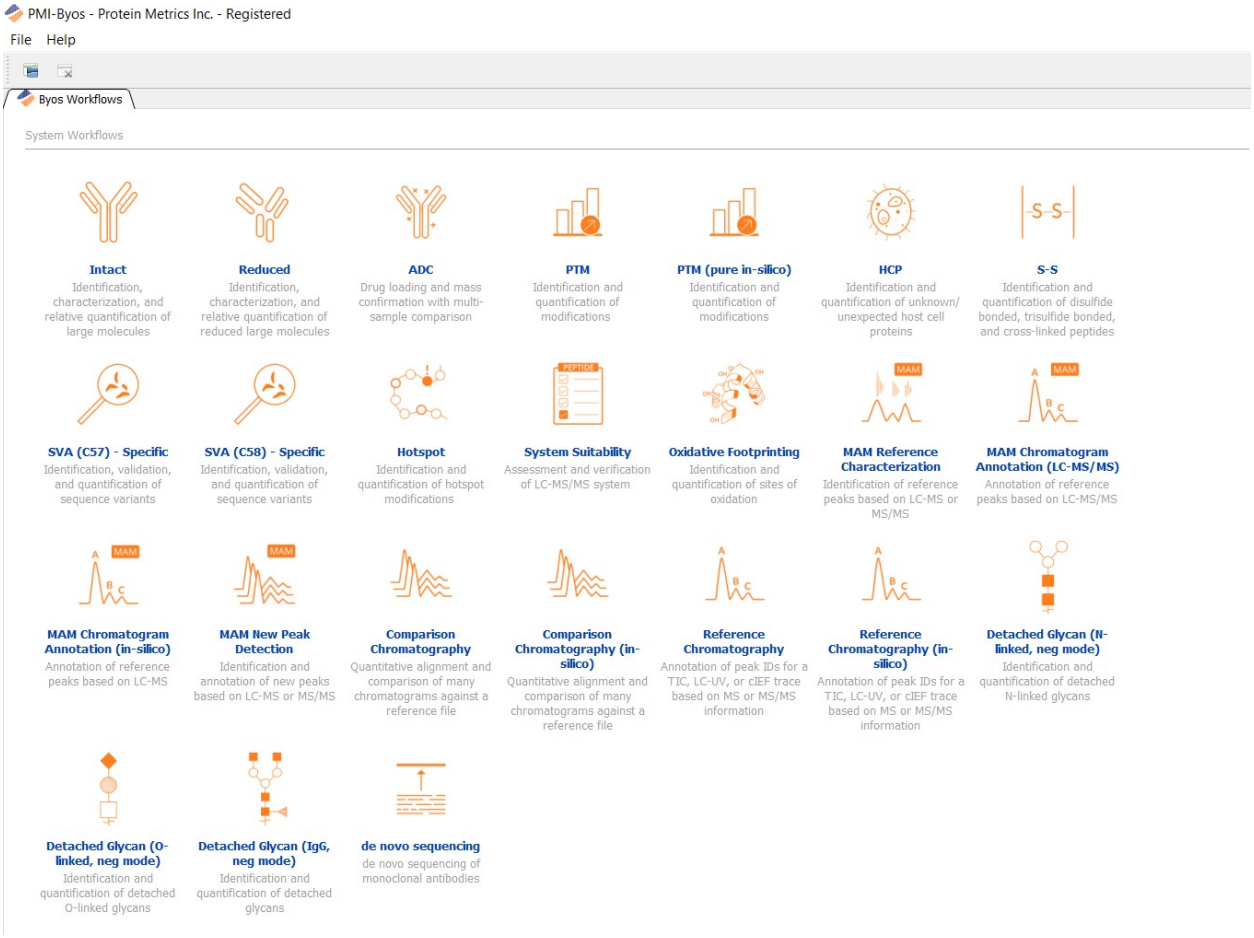
An example of the Feature Finder display:



Usability – Byos

- **System Workflows**

  The Byos default System Workflows have been reorganized to better represent the five sets (Intact, Peptide, Chromatogram, Detached Glycan, and De Novo Sequencing).



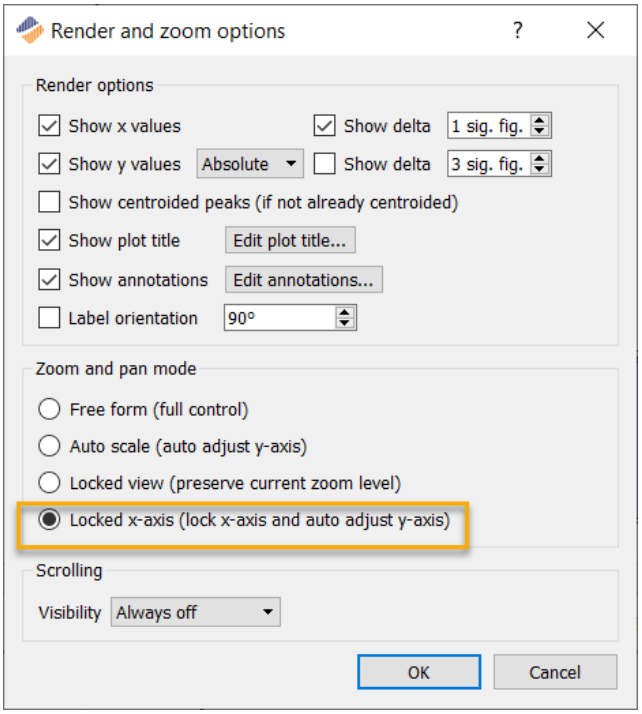
- **Projects to Open by Default in Byos**

  Byos is now the default application for **.byrslt*, **.blgc*, **.ntms*, and **.bmap* files to open.

Usability – Plots

- **Lock x-axis range**

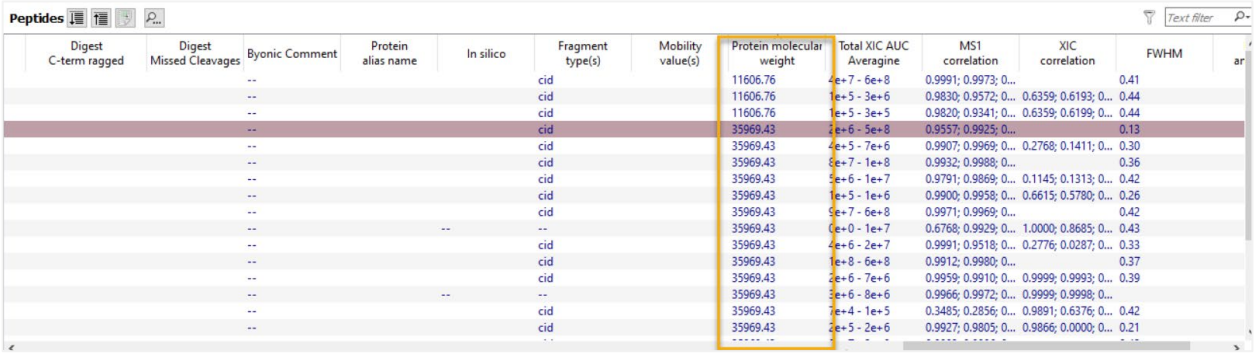
  New capability to selectively lock the x-axis range. This is enabled through the Render and zoom options, as shown below.



Byologic

- **Protein Molecular Weight Column**

  The Protein molecular weight column has been added to the Peptides table in Byologic.



Intact

- **Advanced Command to Calculate Monoisotopic Mass**

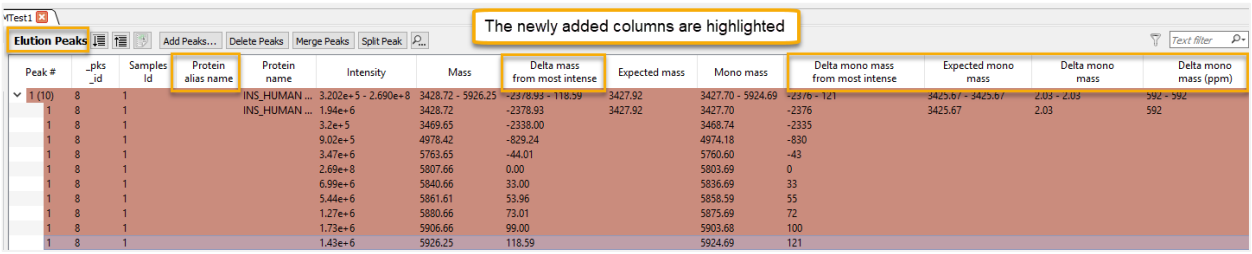
  As a reminder, the monoisotopic mass is calculated using the following advanced command (2018.09 release):

```
[Intact]

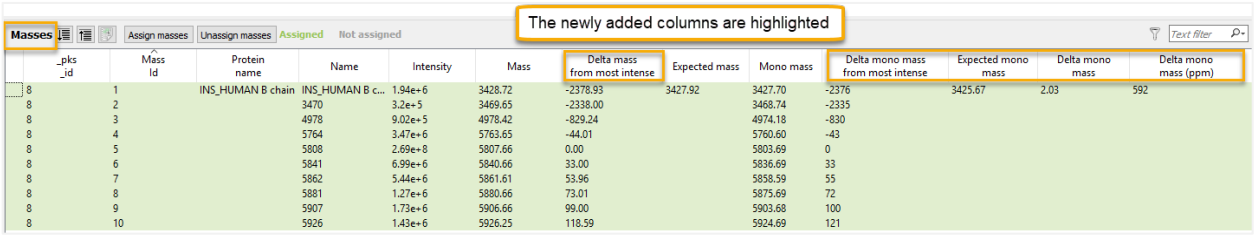
MaxMonoisotope=25000
```

MaxMonoisotope activates the calculation of monoisotopic masses. A value of 25000 will cause mass peaks below 25000 to have monoisotopic masses computed by matching the deconvolved mass peak against an averagine model with isotope-resolved peaks. Mass peaks above 25000 will have monoisotopic masses computed by simply subtracting an average-isotope-to-monoisotope correction.

- **New columns in the Elution Peaks table** – please note mono refers to monoisotopic

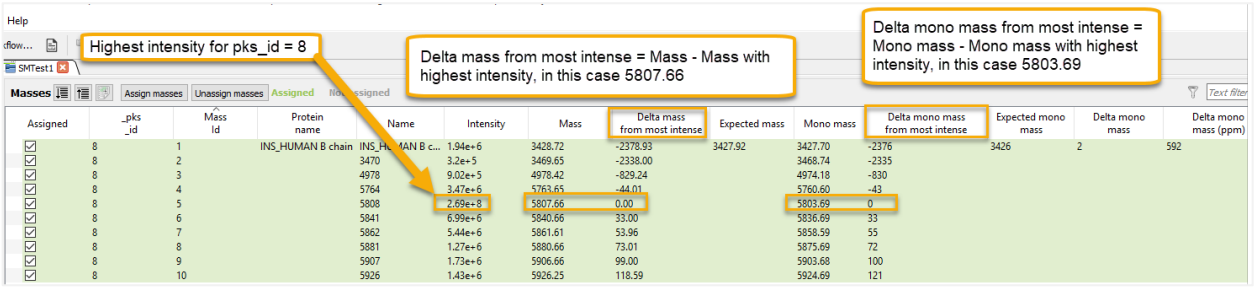


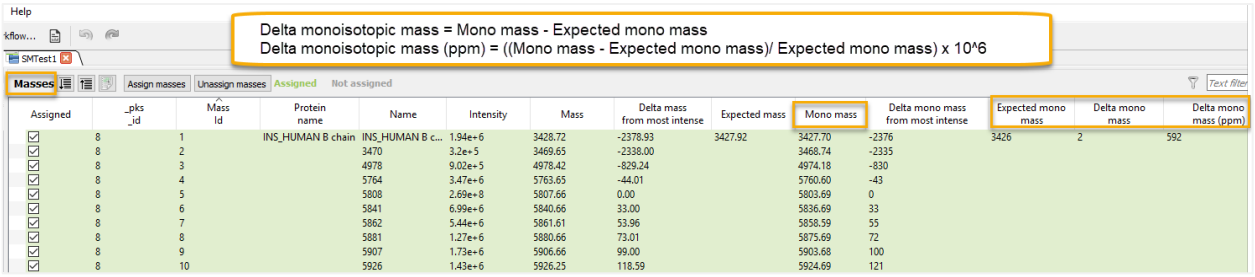
  - Protein alias name
  - Delta mass from most intense
  - Delta mono mass from most intense
  - Expected mono mass
  - Delta mono mass
  - Delta mono mass (ppm)



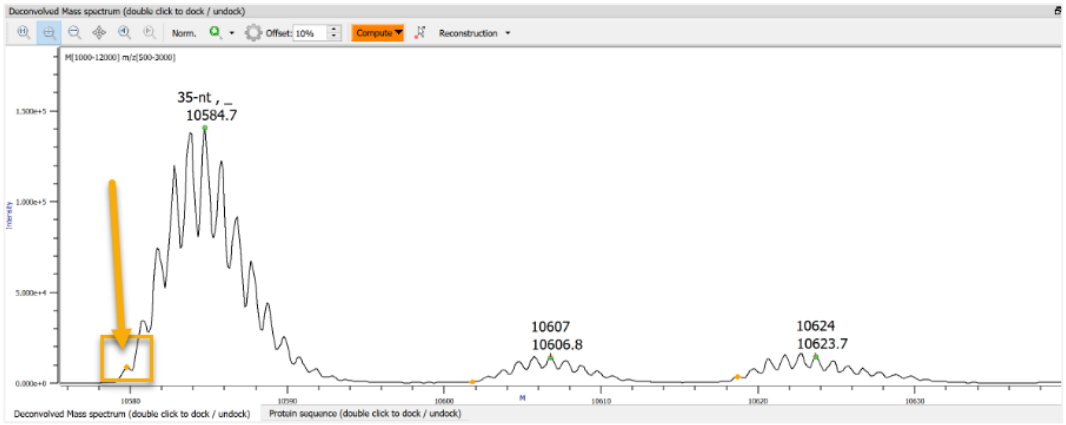
  - Delta mass from most intense
  - Delta mono mass from most intense
  - Expected mono mass
  - Delta mono mass
  - Delta mono mass (ppm)

- **Additional details on Delta mass from most intense and Delta mono mass from most intense columns**





- **Monoisotopic mass is also shown in the Deconvolved Mass Spectrum with an orange diamond**



The following advanced commands are required:

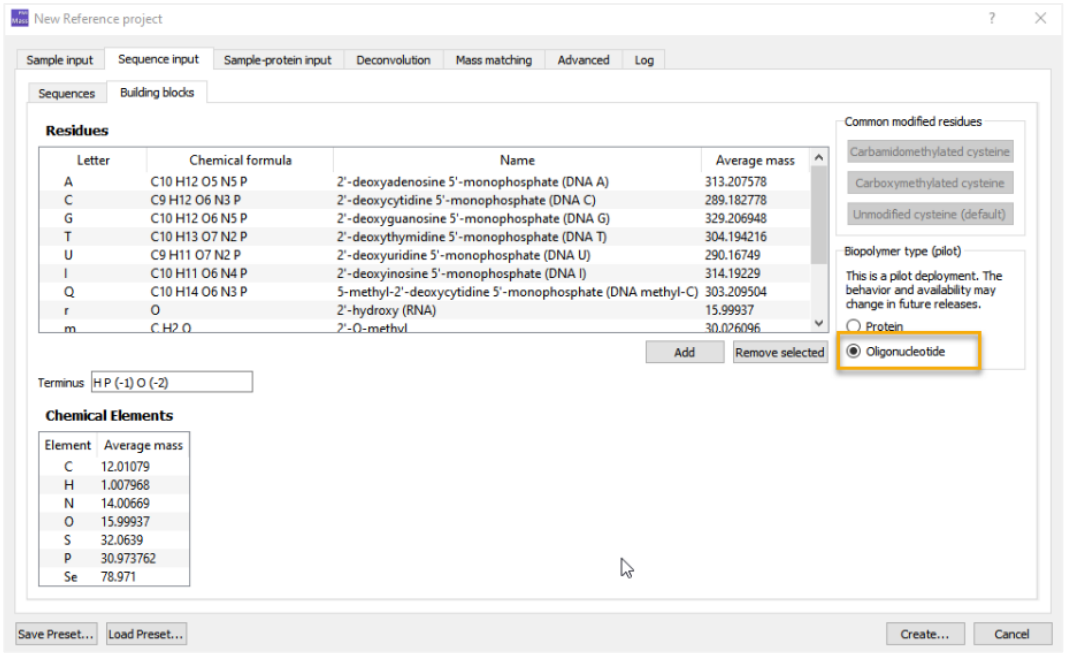
```
[Intact]

ShowObservedMono=true

MaxMonoisotope=25000
```

ShowObservedMono activates the display of the orange diamond. MaxMonoisotope activates the calculation of the monoisotopic mass (mentioned at the beginning of this section).

- **Support for Oligonucleotide sequences has been added through an “Oligonucleotide” radio button in the Building blocks sub-tab (legacy Intact and Byos)**



- The Letter column in the Residues table is editable
- When editing the Letter table, the string in the Letter column must have exactly 1 character and must be unique. The following characters are forbidden for the "Letter" column: 0 1 2 3 4 5 6 7 8 9 - ( )
- **Add** and **Remove selected** buttons appear below the Residues table
- The Common modified residues buttons are disabled

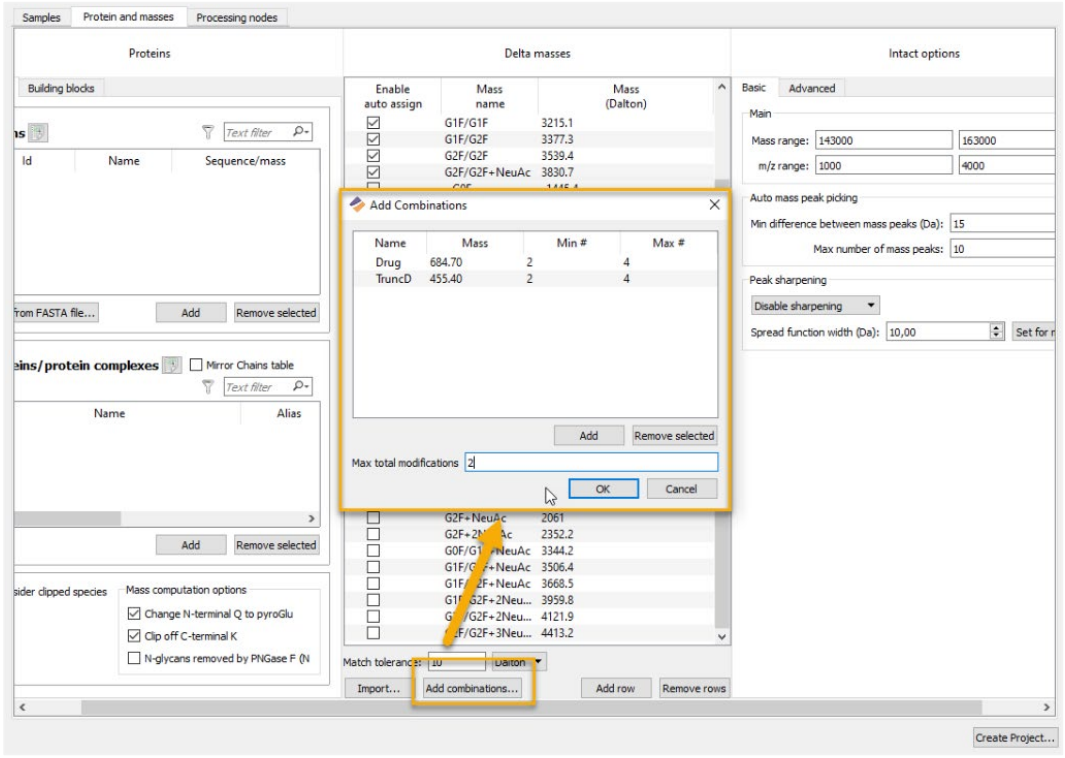
The Oligonucleotides setting alters the Sequences sub-tab in the following ways:

- The Mirror Chains table mode is enabled by default
- The Mass computation options are hidden
- The Disulfides column in the Sequence combinations table is hidden
- In the Chains table, added sequences are not converted to capital letters
- Terminus field is set to "H P (-1) O (-2)" by default

Sequences can be entered using the following rules (please note “X” identifies a base location):

- Standard and Mixed Bases must be expressed in UPPERCASE
- RNA must be expressed as “rX” (for example rA, rU)
- 2' O-methyl RNA bases are entered as “mX”
- Locked nucleic acid bases (LNA) are entered as “+X”
- Phosphorothioated DNA bases are entered as “X*”
- Phosphorothioated RNA bases are entered as “rX*”
- Phosphorothioated 2'-O-methyl RNA bases are entered as “mX*”
- Phosphorothioated LNA bases are entered as “+X*”
- The user can redefine letters and symbols as desired, as long as they are defined within the Building blocks tab. For example, see Q defined as 5-methyl-Cytosine in the above screen capture.

- The **Add Combinations** button was added to Delta Masses in the Mass-matching tab in Intact and the Proteins and masses tab in Byos. The Add Combinations button opens a dialog that allows the user to add modifications:



Byomap and Intact – Baseline Anchors

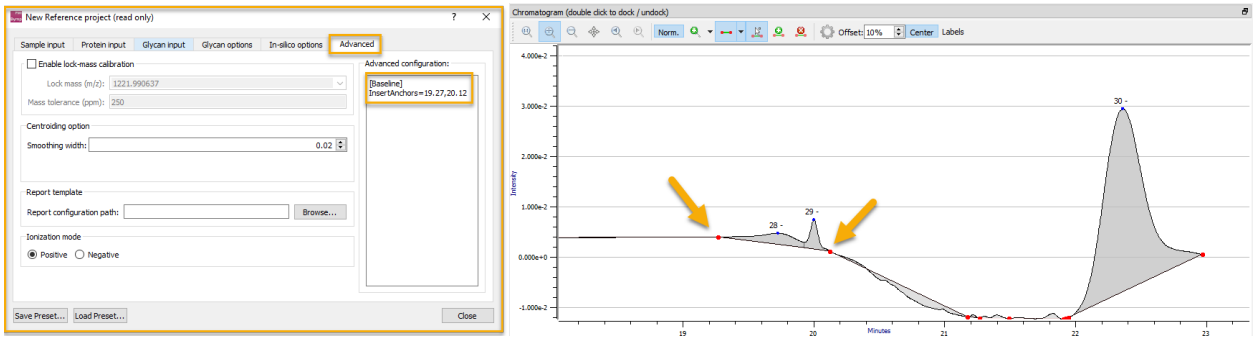
- **New advanced commands to inject manual baseline points**.

  This will result in baseline improvements. As depicted in the below before and after images, this will help to keep big dips out of the calculated area if points just outside the time range are included.

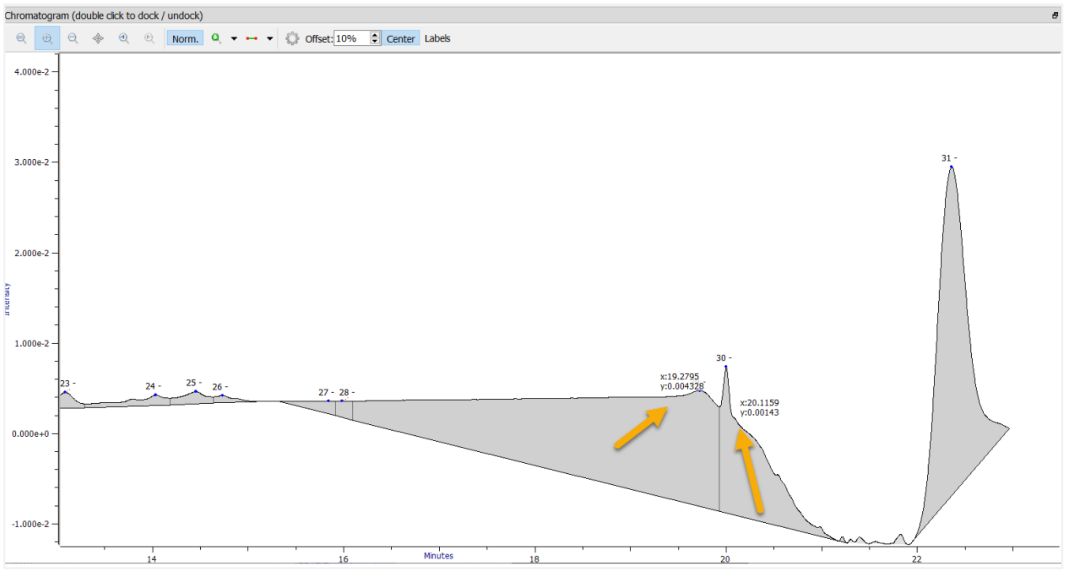
  Please note this will require the user to have knowledge of where to place the baseline points.

```
[Baseline]

InsertAnchors=19.27,20.12
```



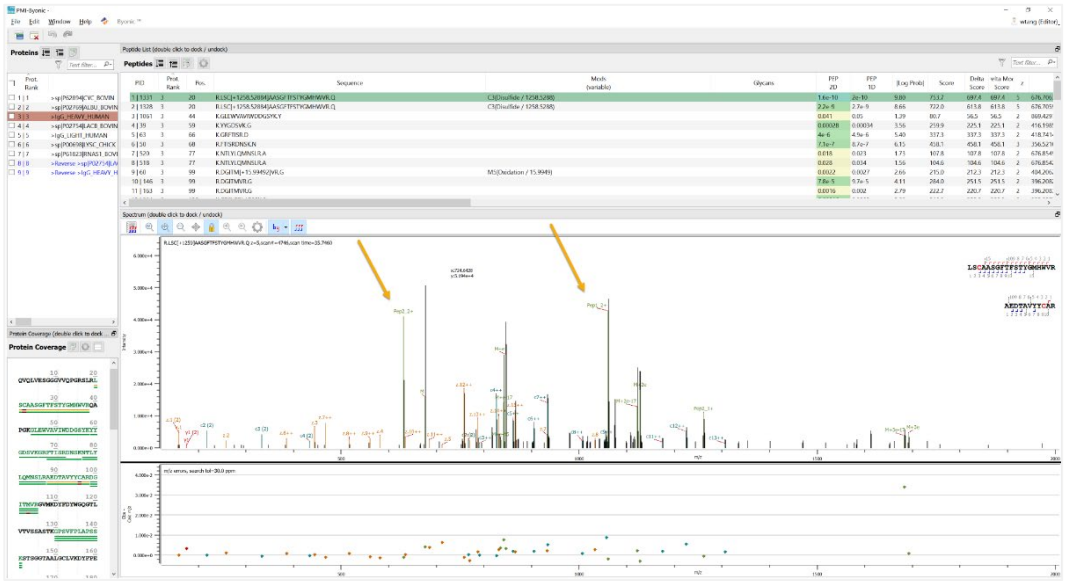
The values 19.27 and 20.12 were observed from the Chromatogram (the below image is before the advanced command was applied).



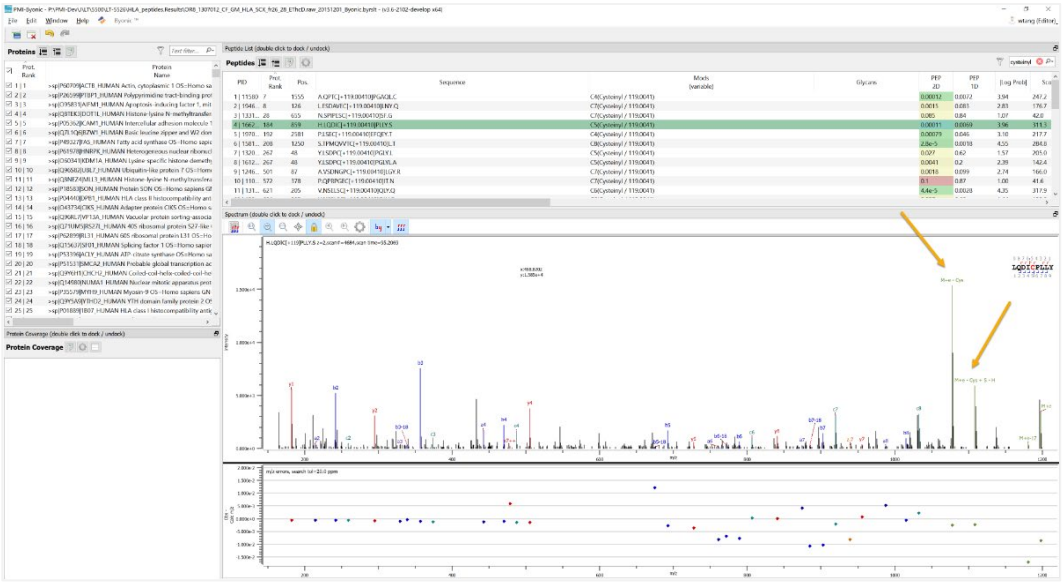
Byonic Viewer

- **Spectrum Annotations – split by ETD, EThcD, or UVPD fragmentation**

  The S-S bond in disulfide-bonded peptides is often broken by ETD/EThcD/UVPD, leading to individual peptides. These individual peptides in the MS2 spectrum are now annotated as Pep1_2+ and Pep2_2+, for example, as shown below:



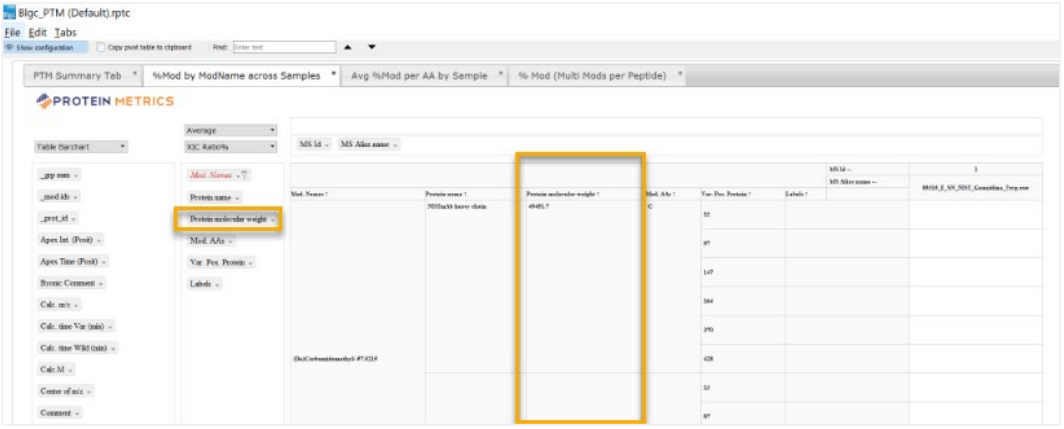
Neutral mass losses for cysteinylation (-119, -88, etc.), as shown below.



Reporting

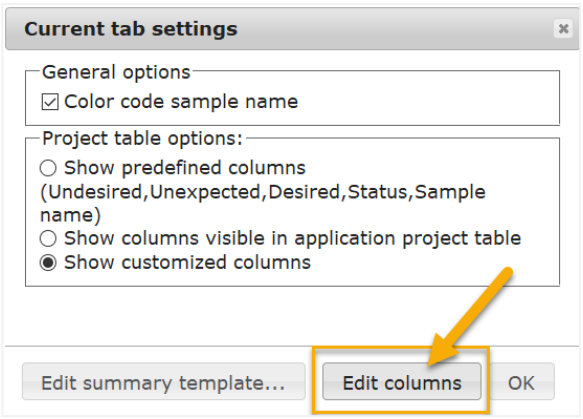
- **Protein Molecular Weight Column**

  The Protein molecular weight column new to the Peptides table in Byologic is also available in the Report.

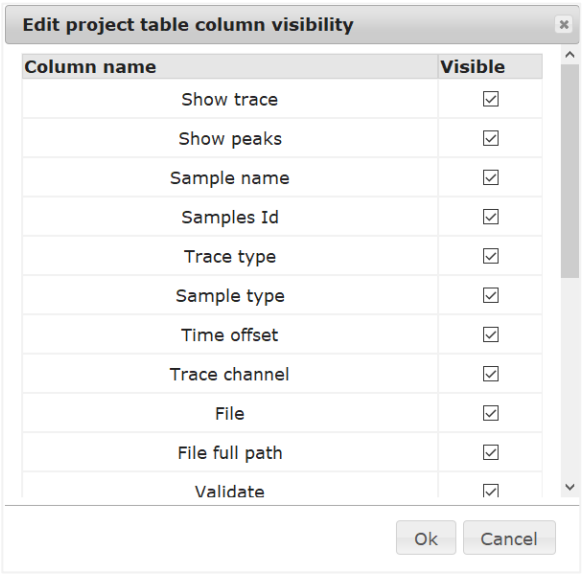


- **Define Order of Custom Columns – Project Table in Summary tab**

  New capability to define the order and visibility of custom columns for the project table in the Summary tab. This is activated through **Edit > Current tab settings > Edit columns**.

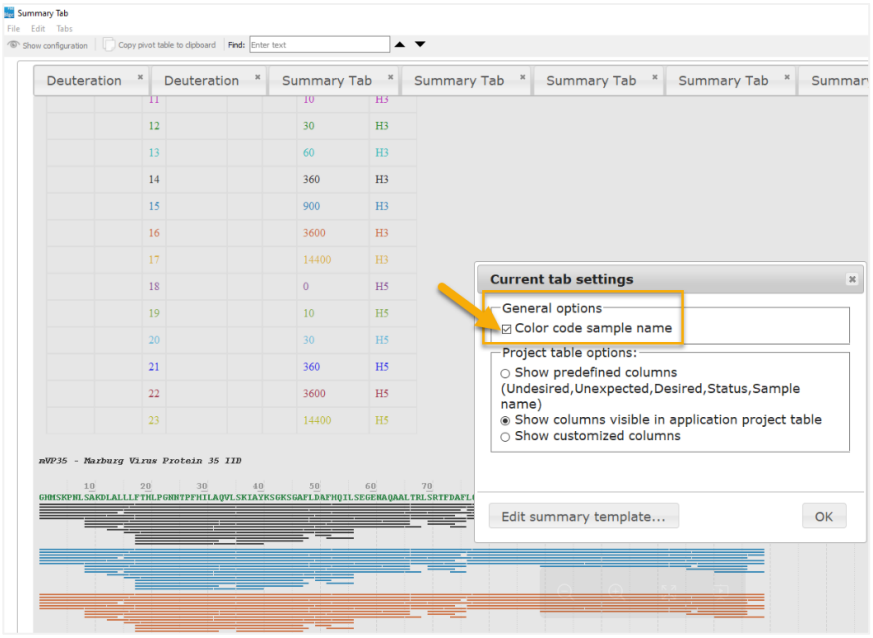


The user can then drag and drop the columns as desired.

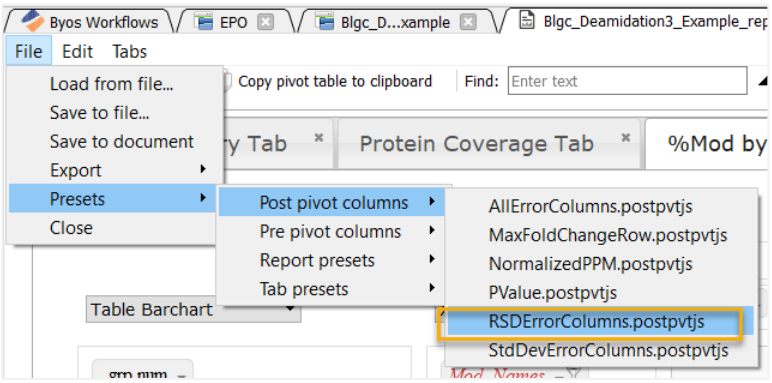


- **Color coding Samples in Summary tab**

  In the Summary Tab, the Project Table can be color coded according to the colors used in the Protein Coverage image in the main application. This is activated through *Edit -> Current tab settings…* and checking the “Color code sample name” box.



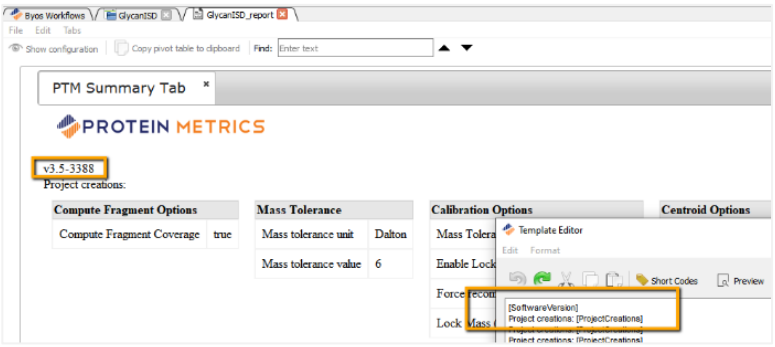
- **Post-pivot column preset**



RSDErrorColumns.postpvtjs" creates a relative standard deviation column. This is also included by default in the updated HCP report template.

- **Software Version**

Additional short code to show the software version has been added to Summary tab.



- **Feature Finder Report Template**

  A new Feature Finder-specific report template has been added to the default set for Byologic.

