

Software Release Notes

June 2020

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 2020-06 (v3.9)

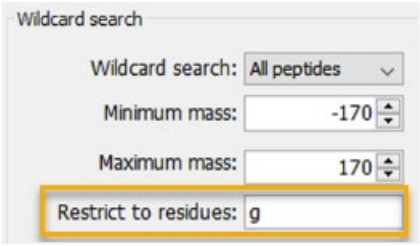
Byonic, Byos

- **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

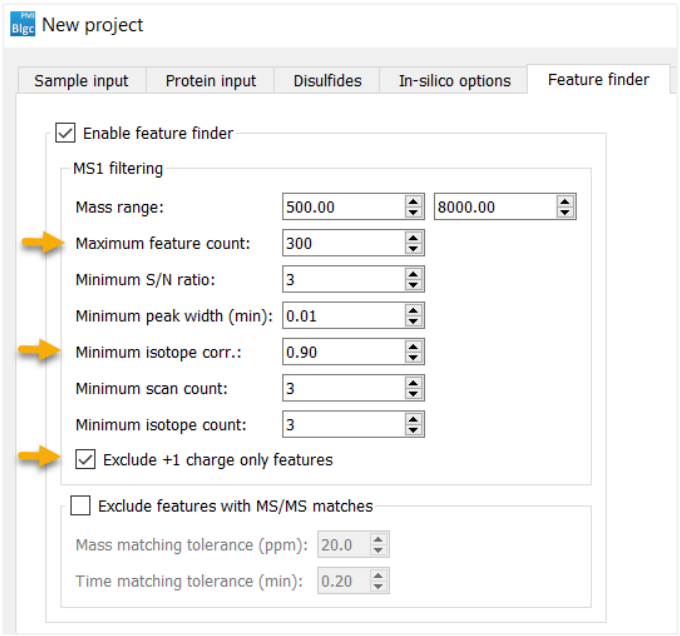


Peptide Analysis, Byos

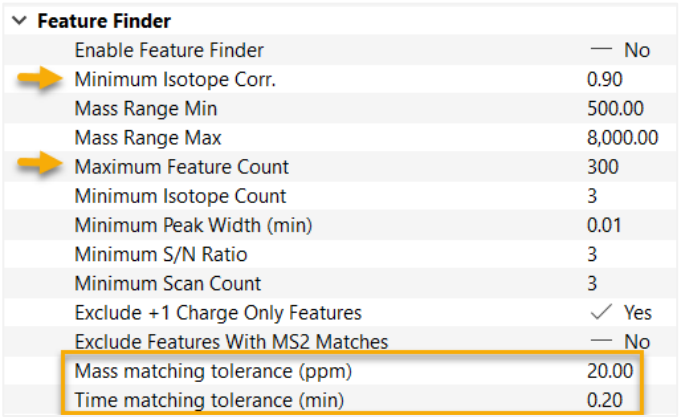
- **New parameters in Feature Finder**

  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.

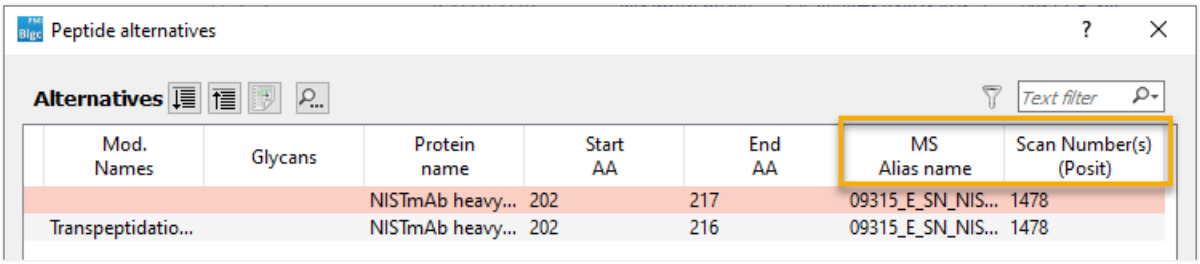
Feature Finder default values were also optimized in both Peptide Analysis and Byos:

- **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**

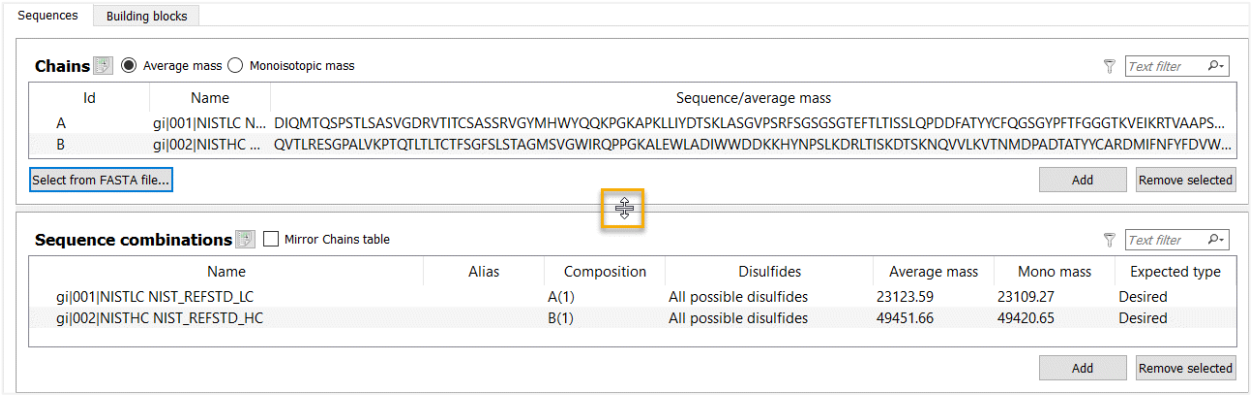
The columns **MS Alias name** and **Scan Number(s)** of origin were added to the **Peptide alternatives** dialog:



Intact Analysis, Byos

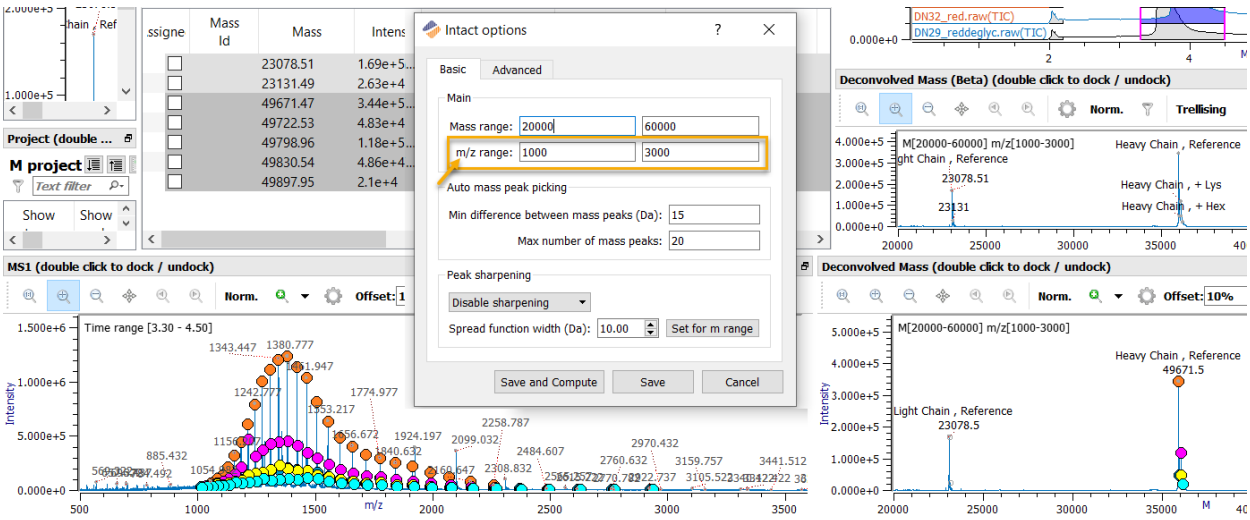
- **Resize capabilities in protein sections of Intact workflows**

  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.



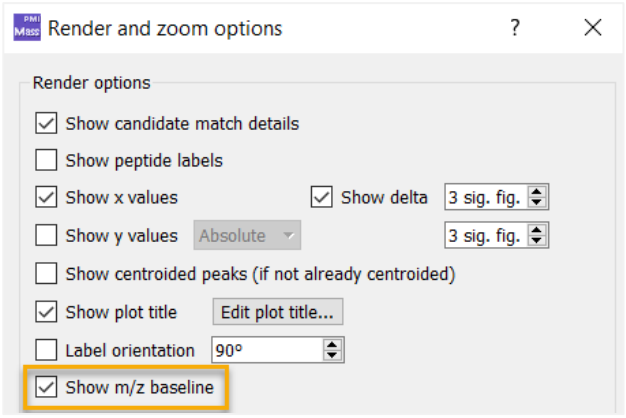
- **Display Colored Dots – Specify Range**

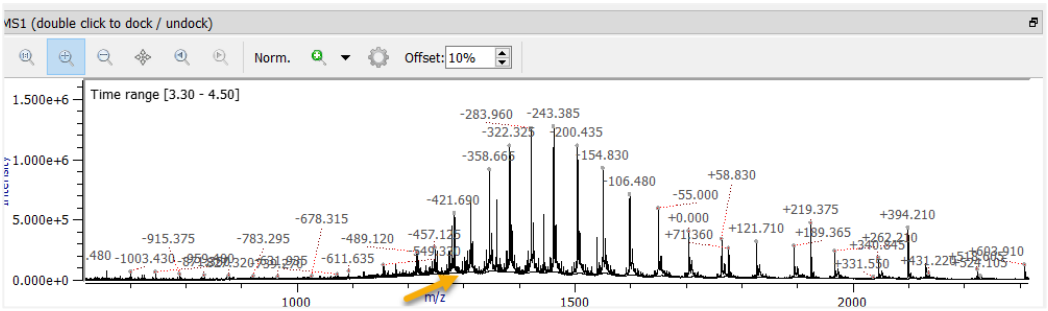
  Colored dots will now only be displayed within the specified m/z or charge state range.



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

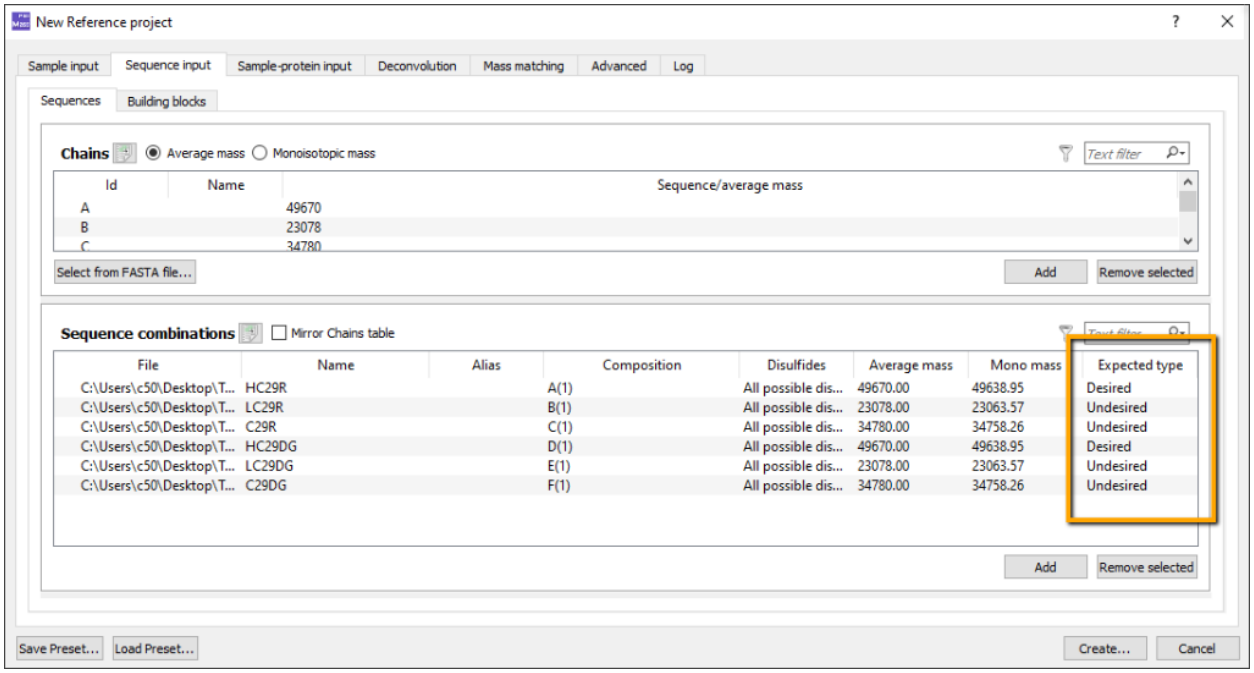
  The **Render and zoom options** dialog includes the option **Show m/z baseline** in **MS1** plots:





- **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:

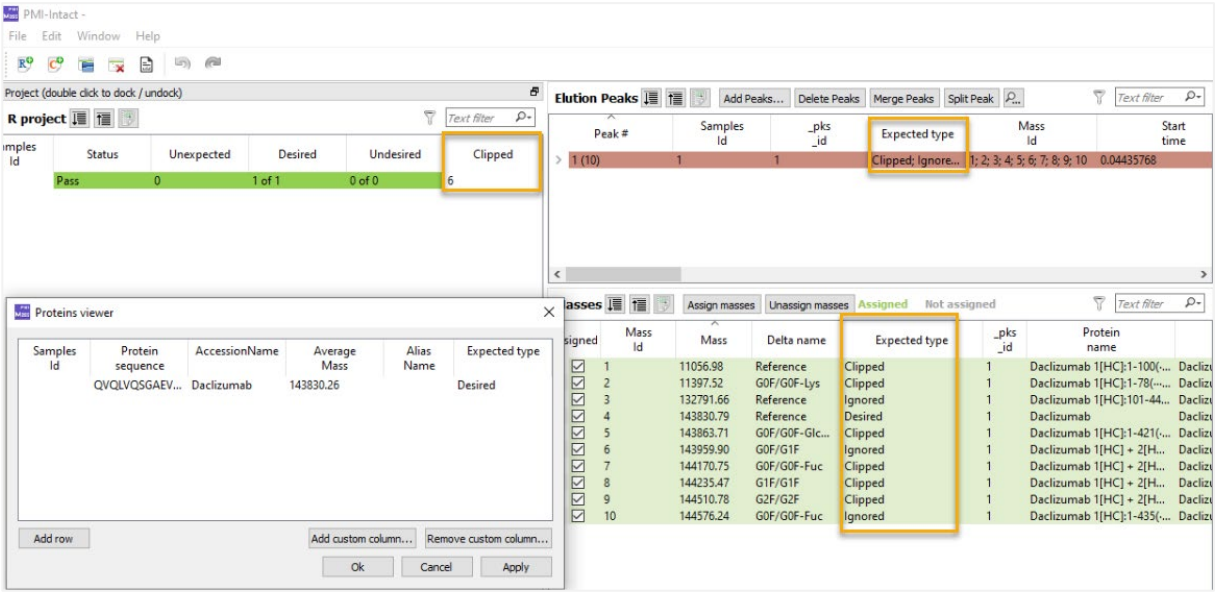


- **New Expected Type – Clipped**

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

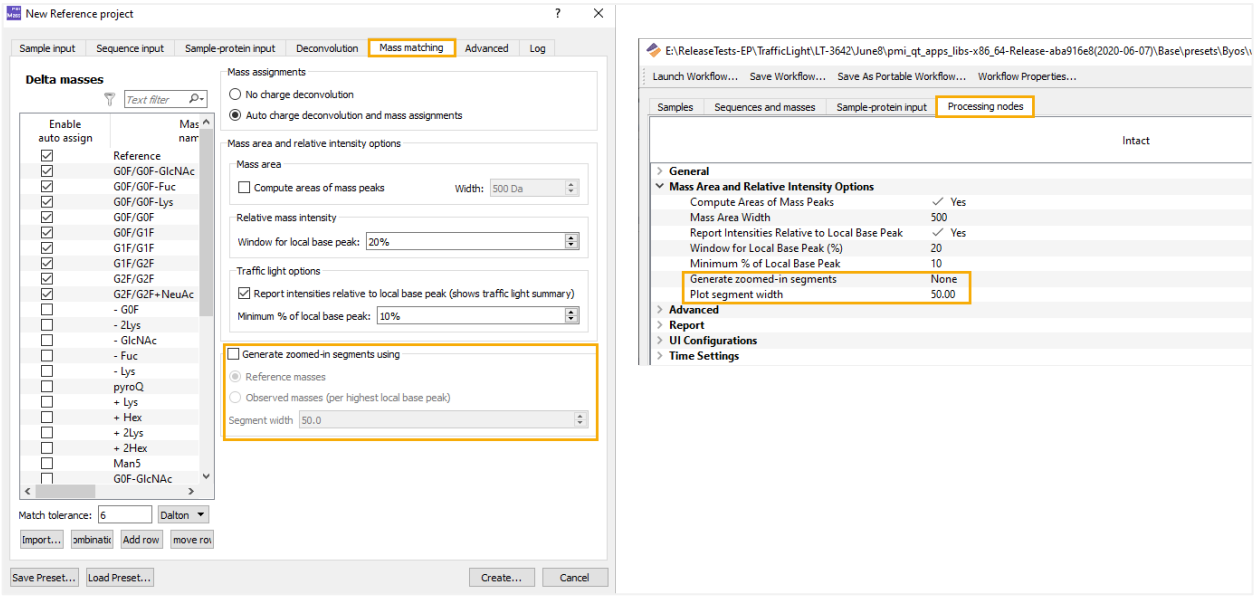


  Clipped is included as a new **Expected type** and counted the same: all occurrences of Expected type = Clipped, except for duplicates, that is, masses with the same name. Clipped is also displayed in the **Elution Peaks** and **Masses** tables:

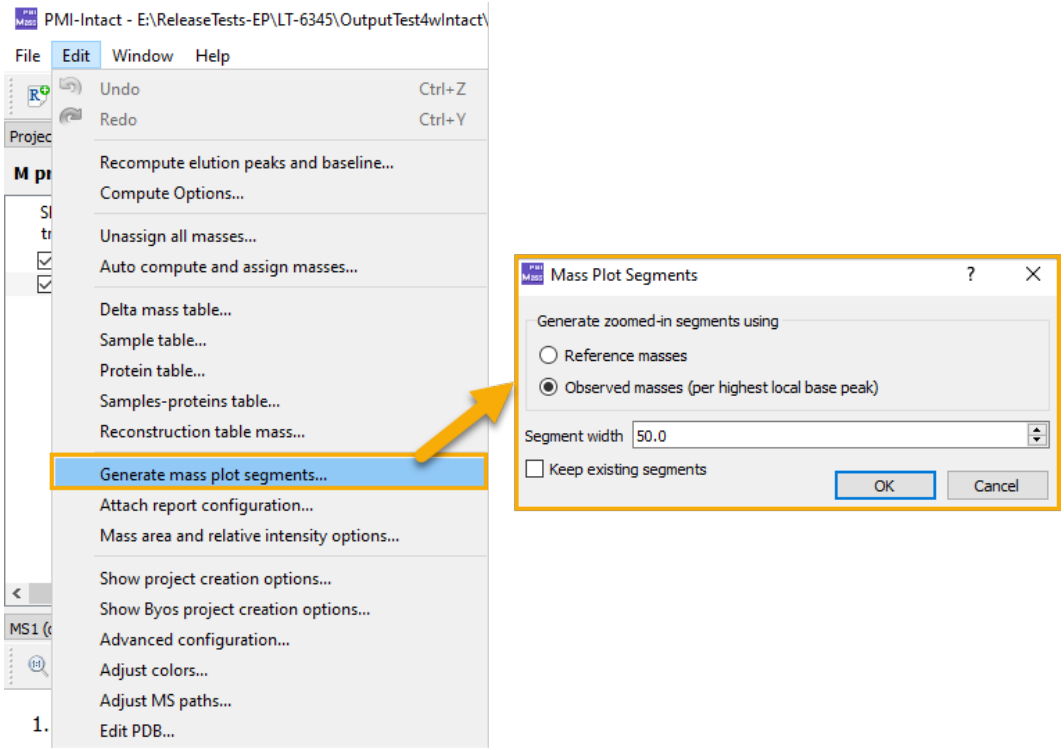


- **Traffic Lights – Logic Improvements**
  - The logic used in counting **Desired** and **Undesired** has been improved. All unique occurrences of a specific type for a given protein are counted. The uniqueness of the protein is defined by the **Protein name** and **Expected type** columns.
  - Editing 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**:



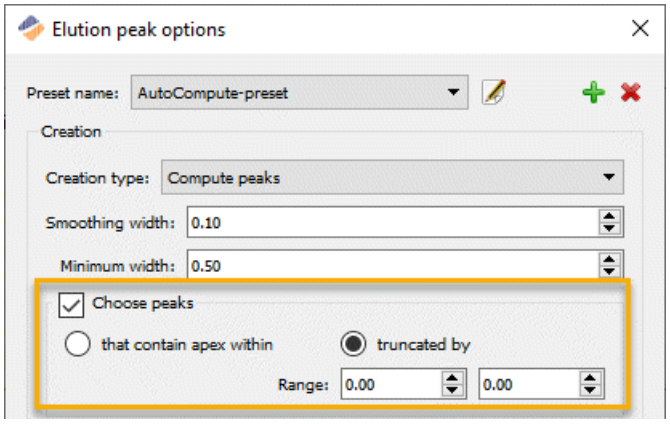
Chromatogram Analysis, Intact Analysis, Byos

- **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**

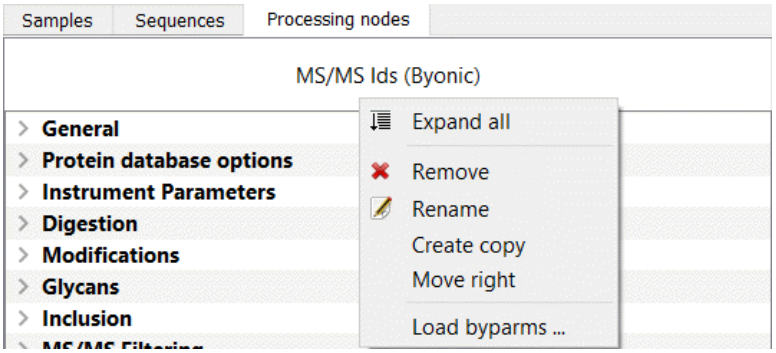
Users can choose have peaks truncated by a time range in the **Compute peaks** option of the **Elution peak options** dialog.



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.

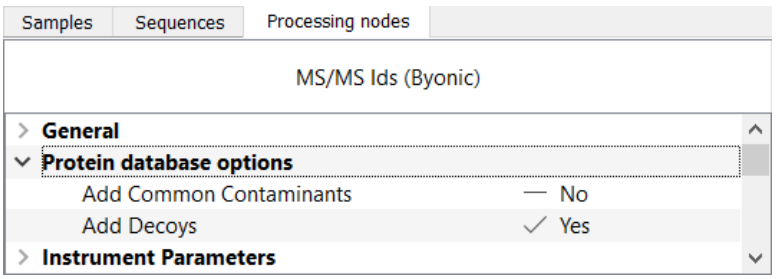
Processing Nodes Enhancements in Byos Workflows

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



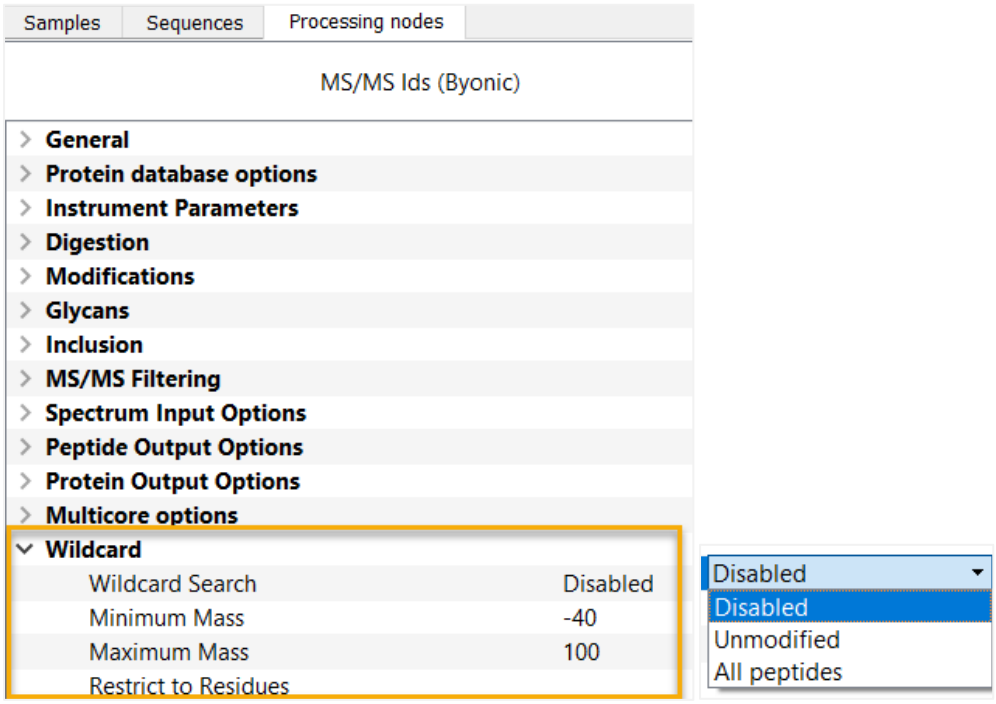
  - **Remove** – the user can remove the Byonic Processing node
  - **Rename** – the user can rename the Processing node
  - **Create copy** – the user can create a copy of the Byonic Processing node. For example, a copy can be used to support an analysis with more than one digestion
  - **Move right** – 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**



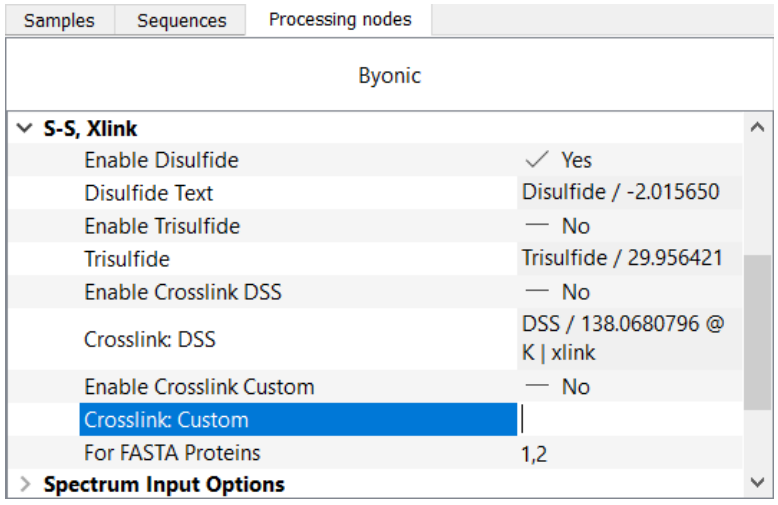
The protein database options **Add Common Contaminants** (default setting = **No**) and **Add Decoys** (default setting = **Yes**) are now part of the Byonic Processing node.

- **Wildcard parameters in Byonic Processing nodes**



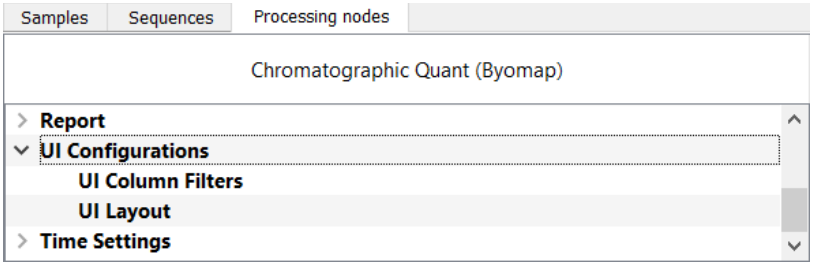
Users can now enable Wildcard modification search. This capability was previously only available in Byonic. The options Disabled, Unmodified, and All peptides are available through a drop-down menu.

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



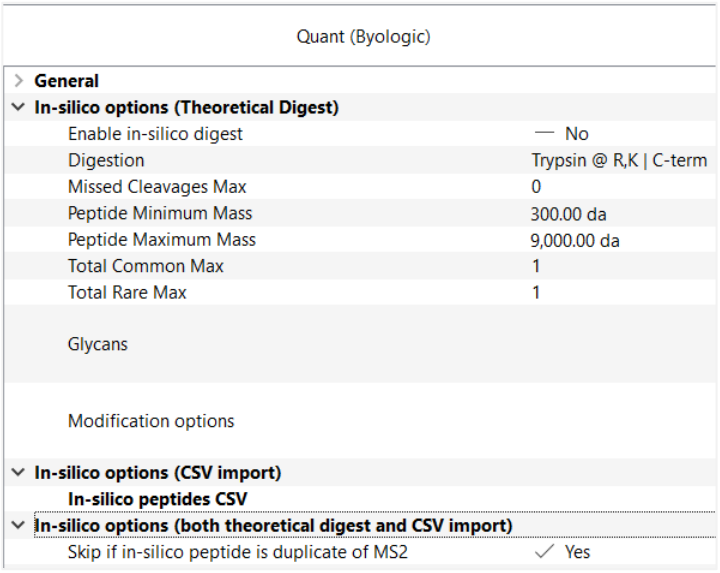
Users can now specify the Modification Fine Control used in a custom crosslink search. This capability was previously only available in Byonic.

- **UI Configurations in Byomap Processing nodes**



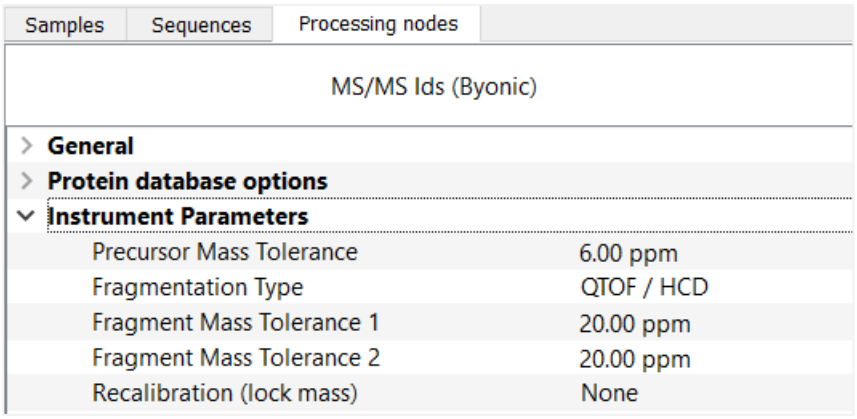
Users can now include UI Configurations in Byos workflows containing Byomap Processing nodes. This was previously unavailable in Byos.

- **In-silico options improvements**

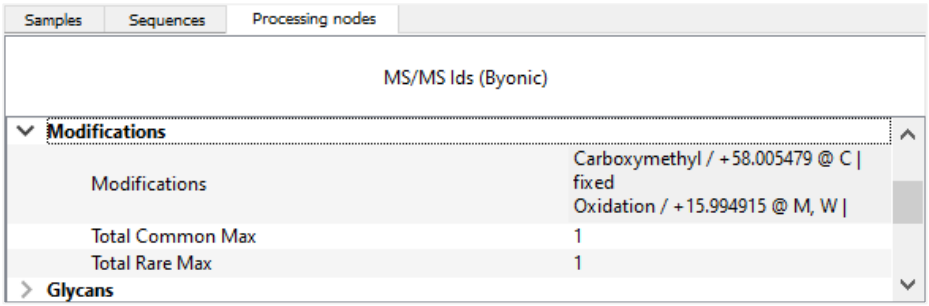


Parameters are separated into three sections to clarify which pertain to a **Theoretical Digest**, **CSV import**, and **both theoretical digest and CSV import**

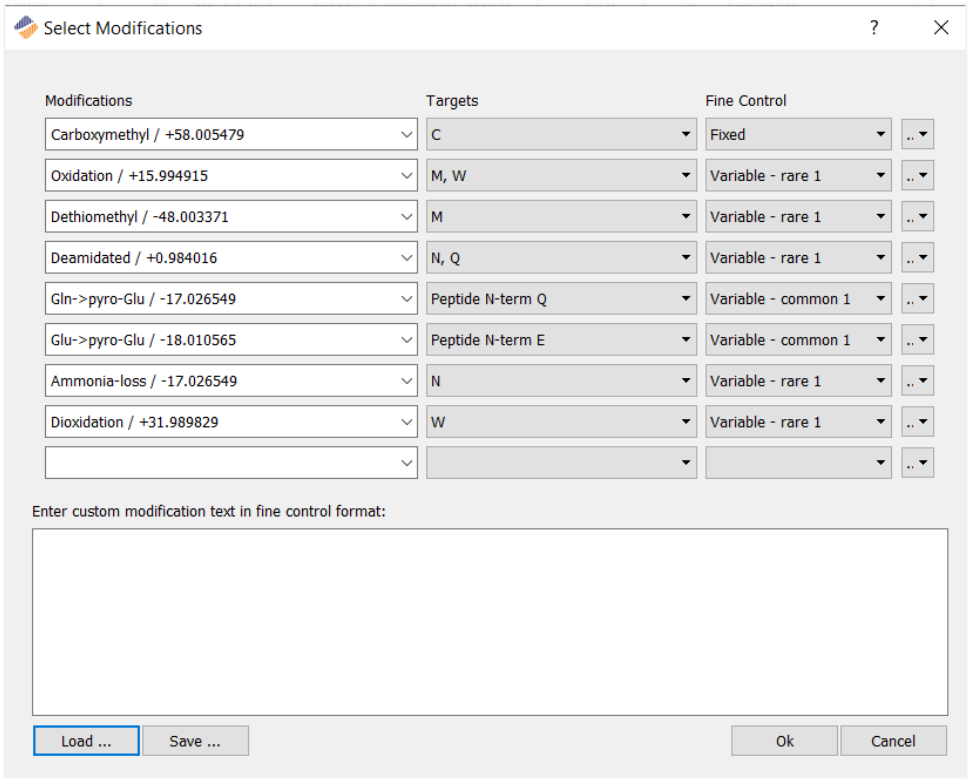
- **PTM.wflw parameter changes**
    - Byonic Processing nodes - Instrument Parameters - Fragment Mass Tolerance value Changed from 0.6 Da to 20.00 ppm



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

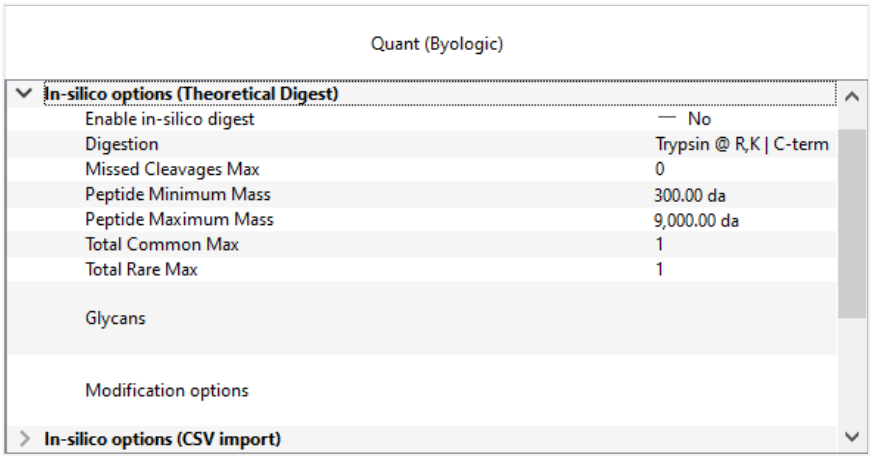


- Byonic Processing nodes - Modifications – Modifications List and Fine Control



  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

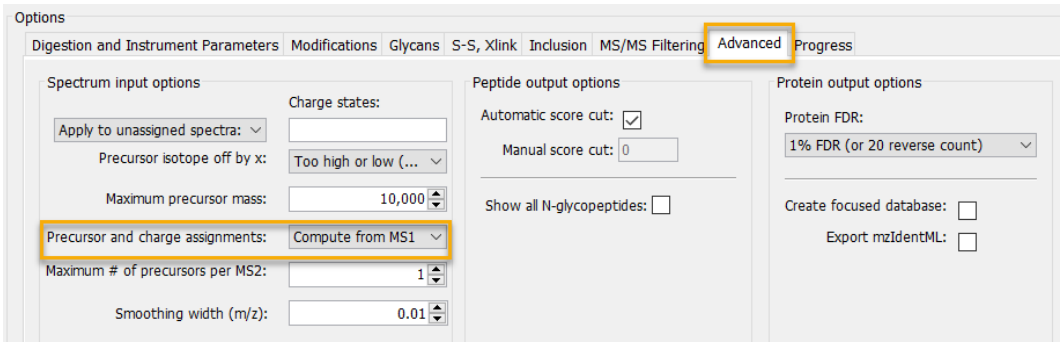
- Byologic Processing nodes - For PTM.wflw and PTM (pure in-silico).wflw, **In-silico options (Theoretical Digest)** – **Total Common Max** was changed from 2 to 1



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:



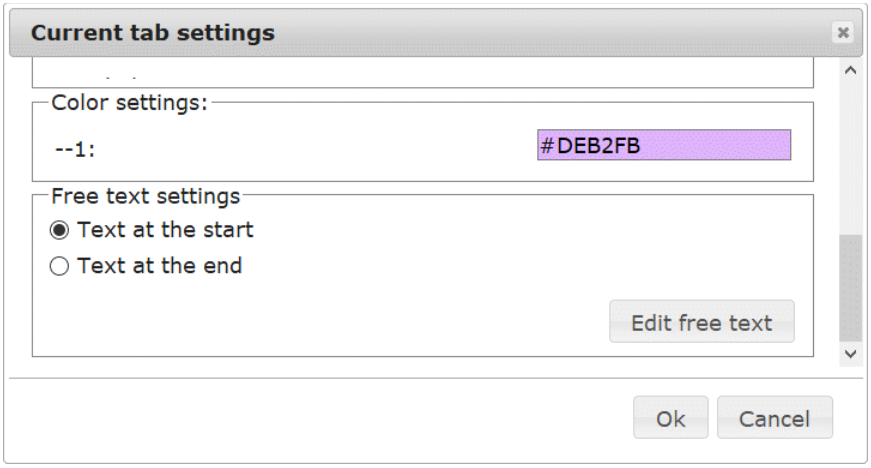
- **Bruker Data – File reader**

  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

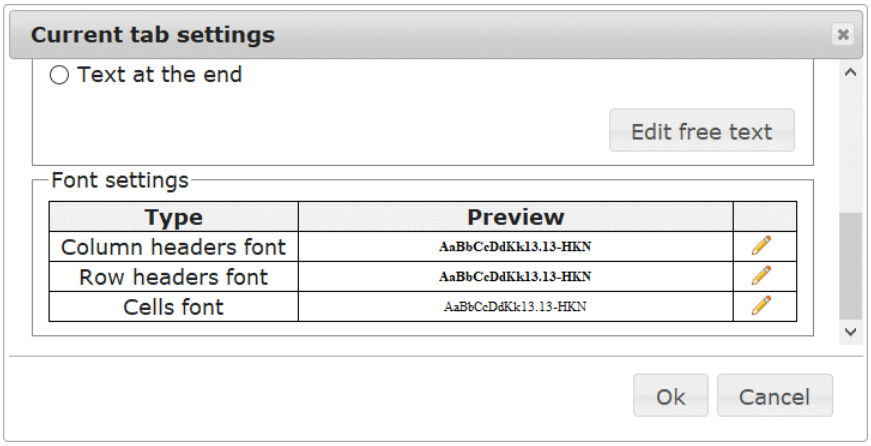
- **Free text entry in Pivot tabs**

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

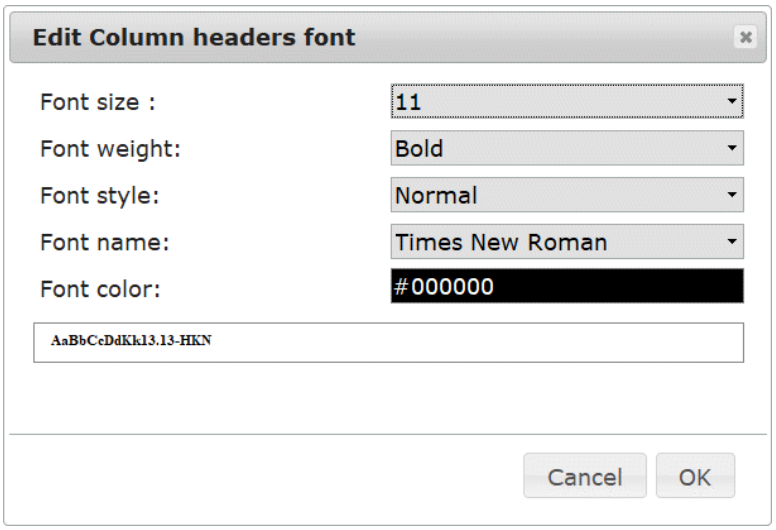


- **Edit Fonts in Pivot tabs**

  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):

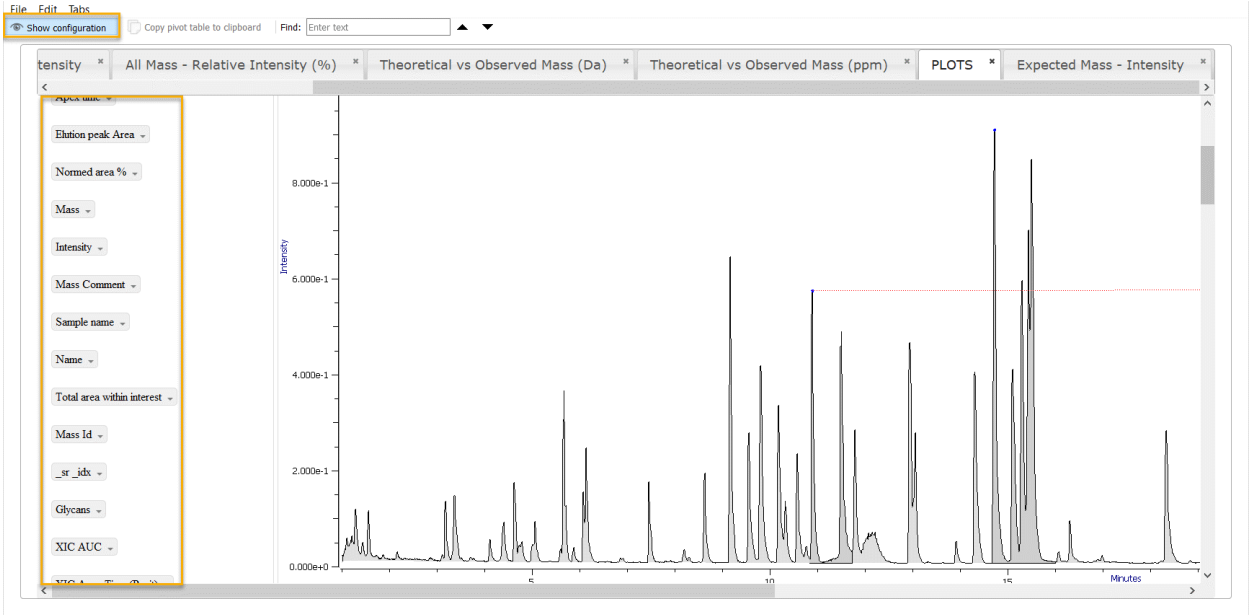


  Click the icon to edit the fonts for each text type:



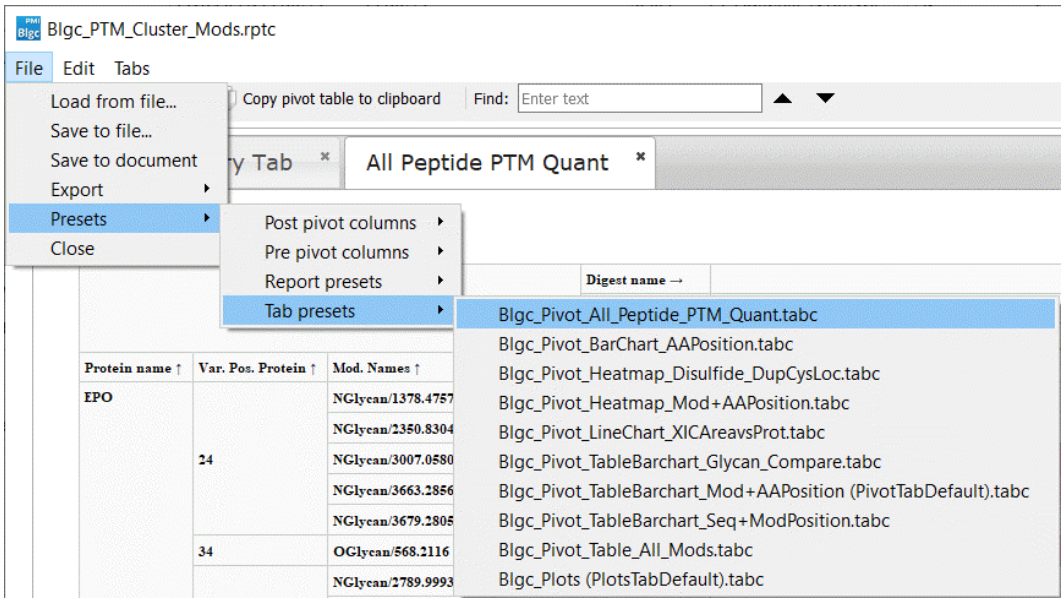
- **Filtering in the Plots tab**

  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**



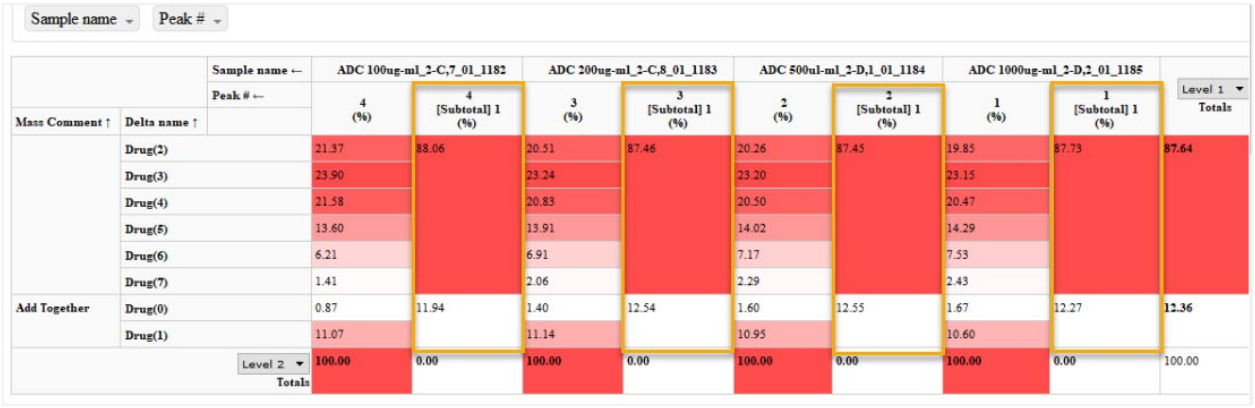
- **Feature Finder tab**

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

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

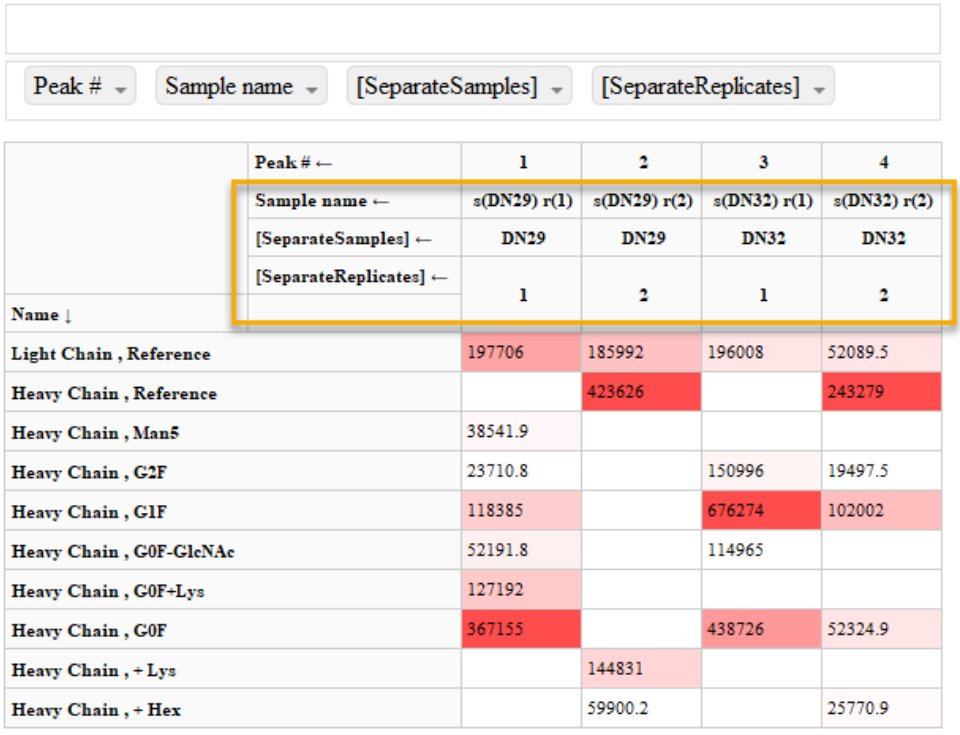
  Please reach out to the Customer Success team for additional details on the new enhancements.

  - **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.



- **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:



Release 2020-03 (v3.8)

Supported Hardware

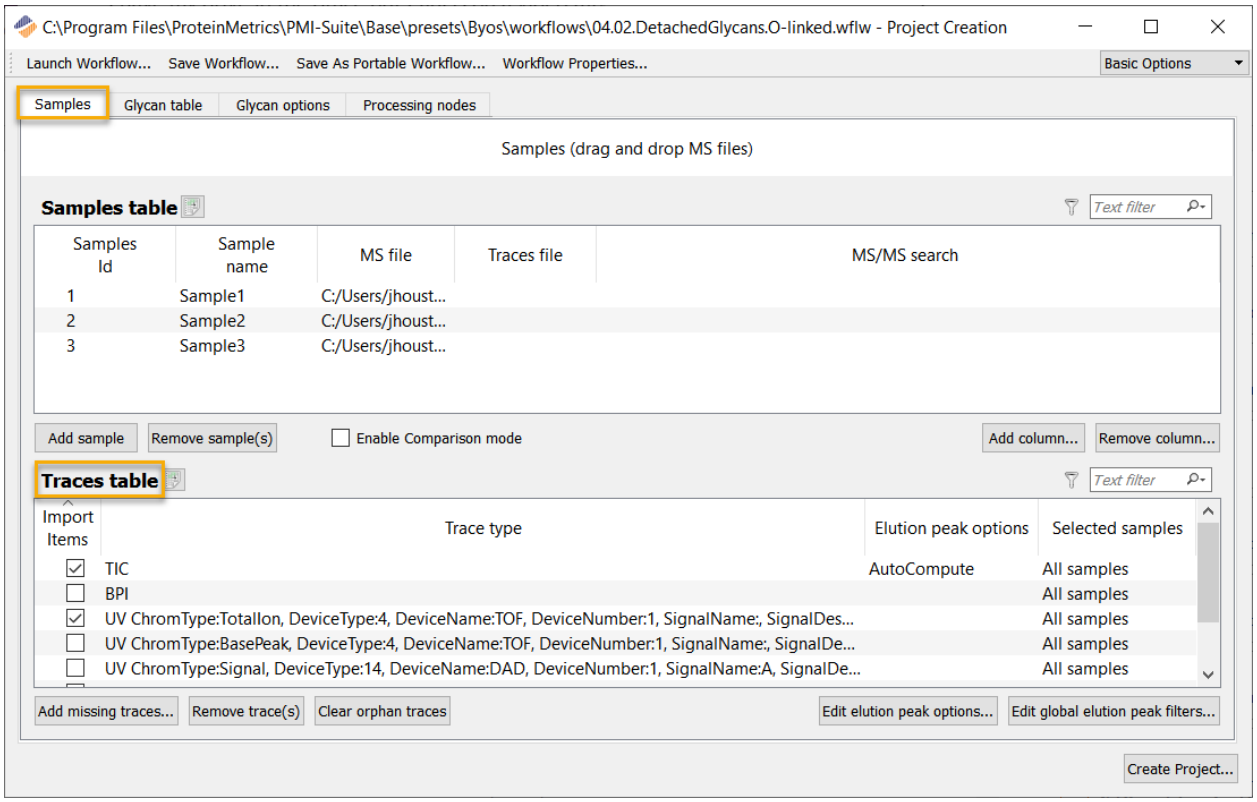
Beginning in March 2021, PMI software will not be supported on 32-bit operating systems. It is strongly recommended to install this and future releases on 64-bit operating systems.

Elution Peak and Traces Table – Intact Analysis and Chromatogram Analysis

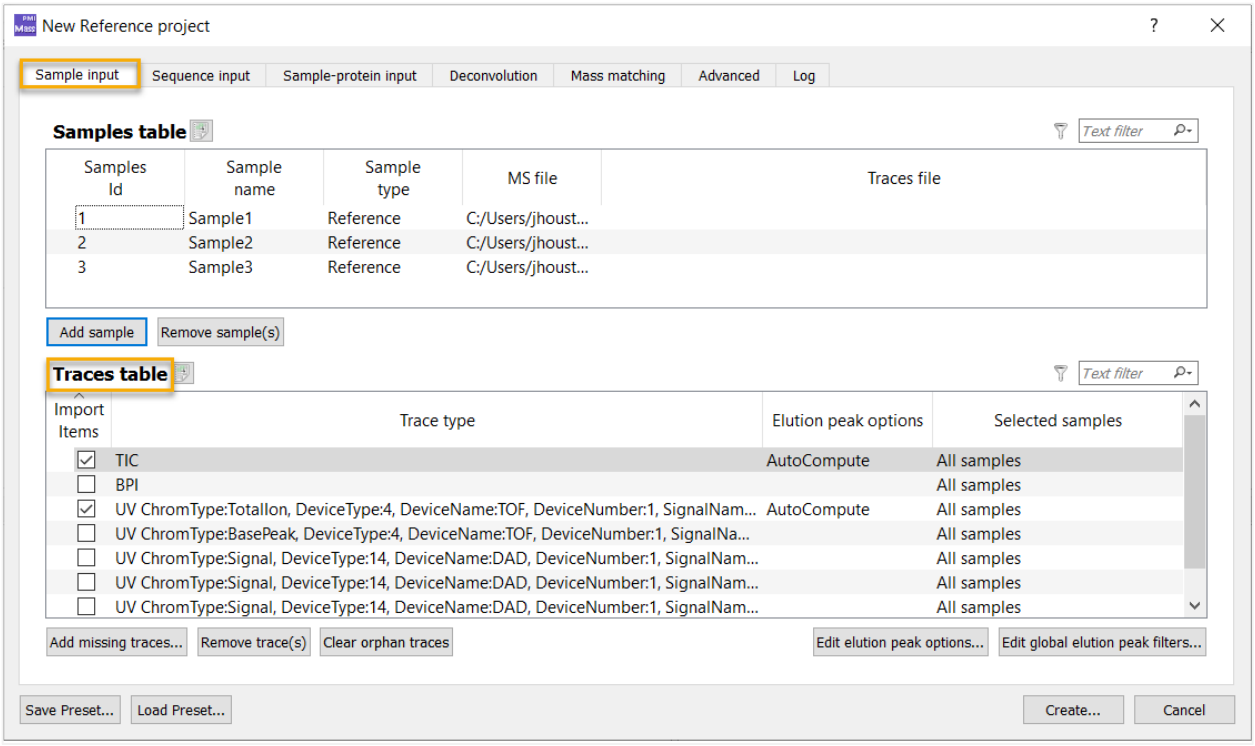
During project creation for Intact Analysis, Chromatogram Analysis, and associated Byos workflows, the elution peak calculation parameters have been moved to the Samples and Sample input tabs. Traces are no longer sub-records of samples; instead, they are organized in a Traces table within the Samples/Sample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

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

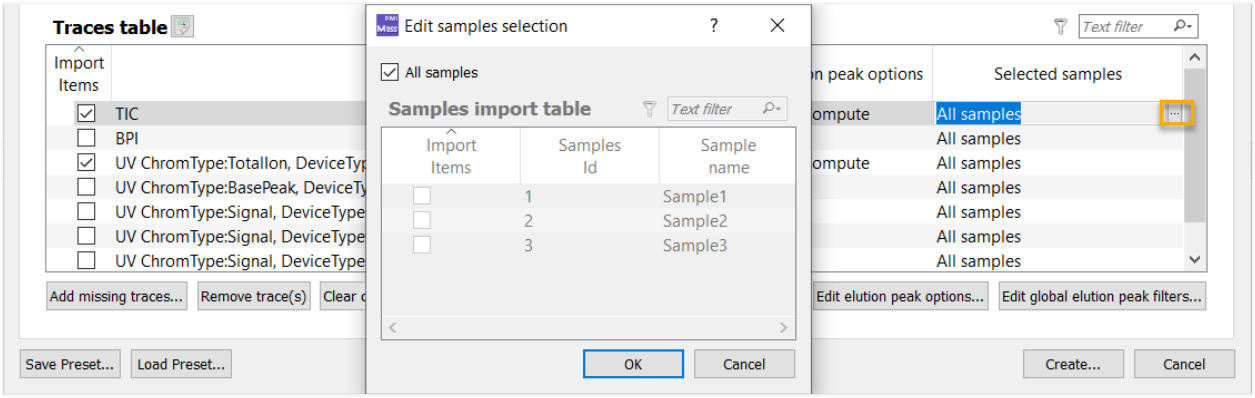
An example from Byos is shown below:



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

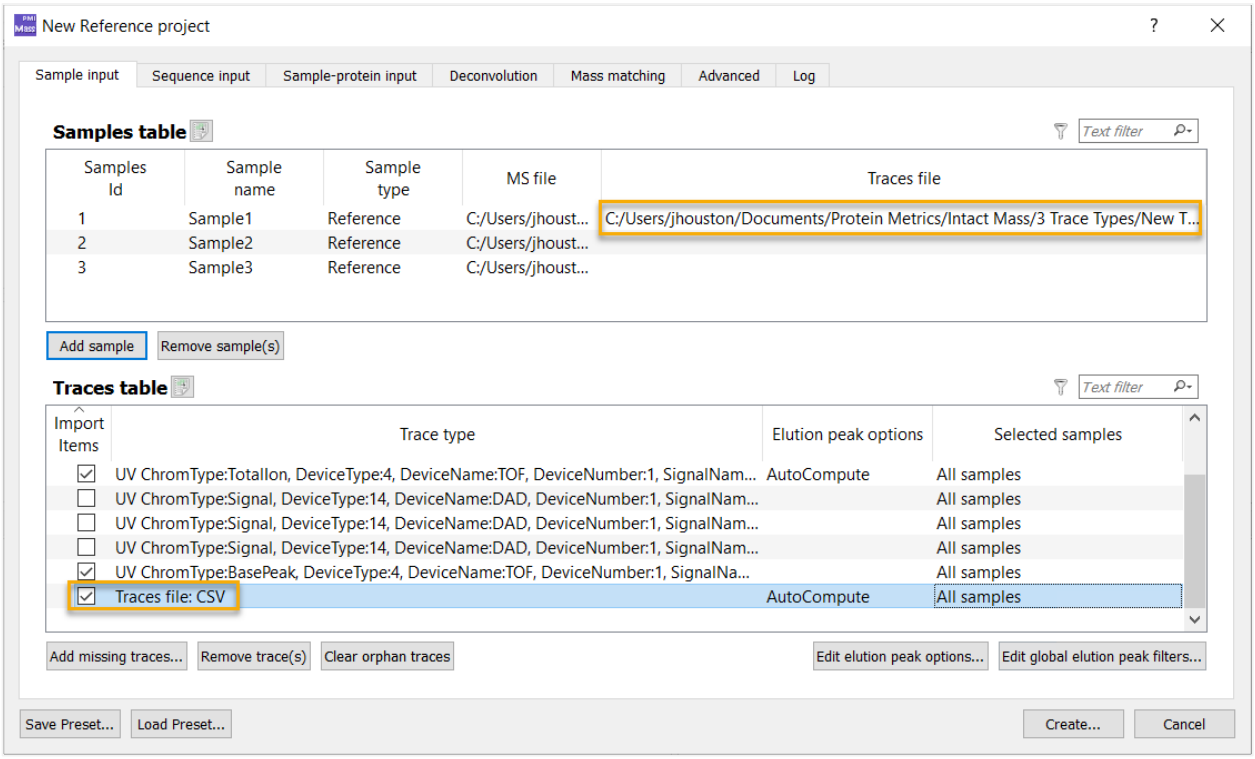


Selected traces can be removed with the Remove trace(s) button. Removed traces can be restored with the Add missing traces button. To associate specific traces to the samples, double-click the text in the Selected samples column, then click the ... button to open the Edit samples selection dialog:

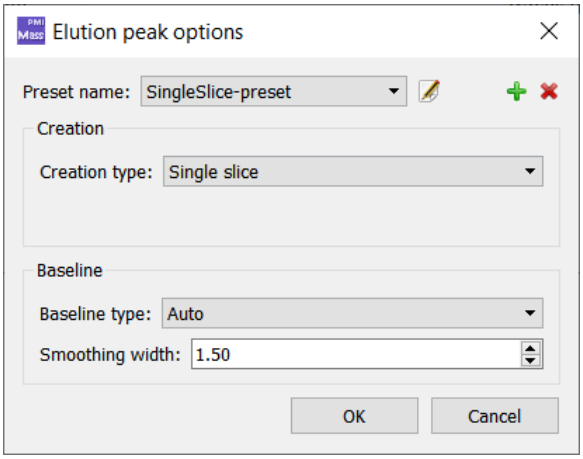


To associate the selected trace with individual samples, uncheck All samples and check those samples and click OK.

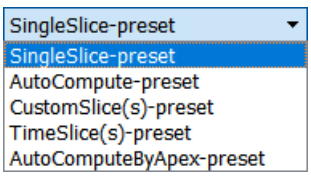
To import a trace from a *.csv file, drag the file into the Traces files column in the Samples table. The trace is added to the Traces table:



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

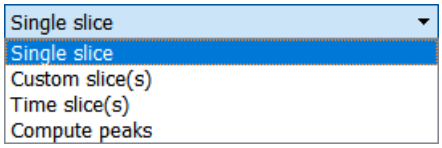


The Elution peak options dialog contains five default peak processing presets:



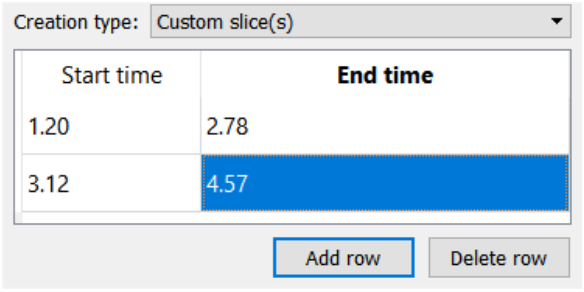
Select a processing preset and edit the peak parameters. To create a custom preset, click the ✚ button, add a preset name, and click **OK**. Next, select a creation type and edit those peak parameters. The new preset is added to the list. Click the ✎ button to edit a selected preset name. Click the ✖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

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

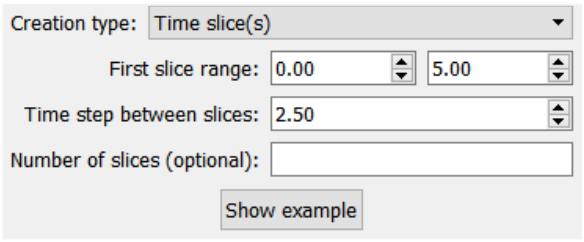


These Creation types use the following peak processing methods:

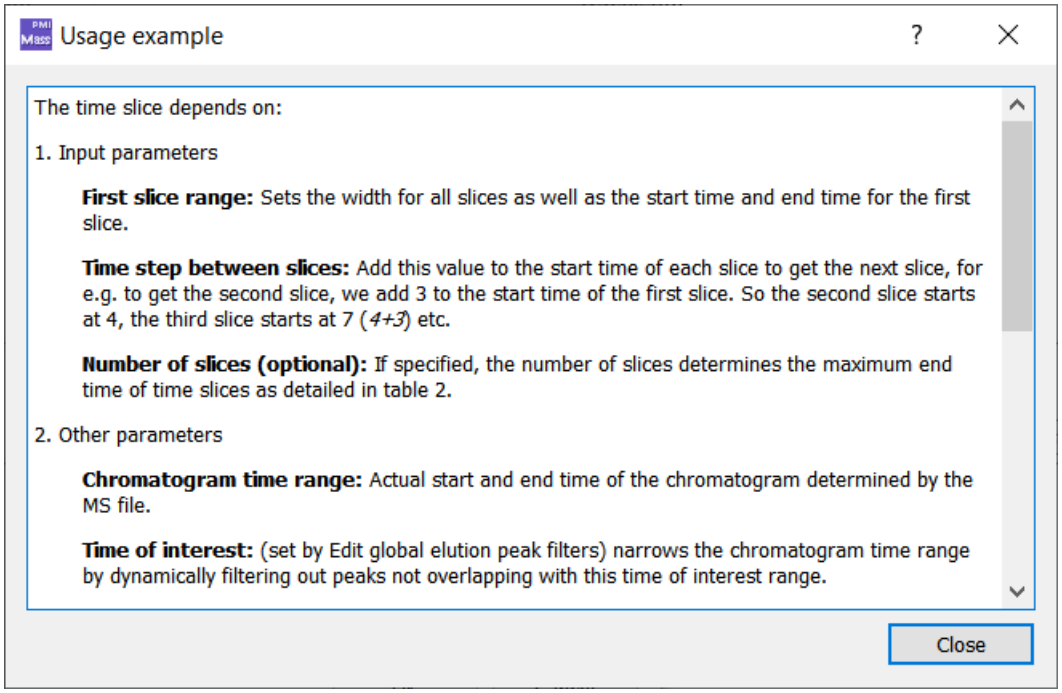
- **Single Slice** processes the entire chromatogram as single elution peak.
- **Custom slice(s)** divides elution peaks by user defined start and end times:

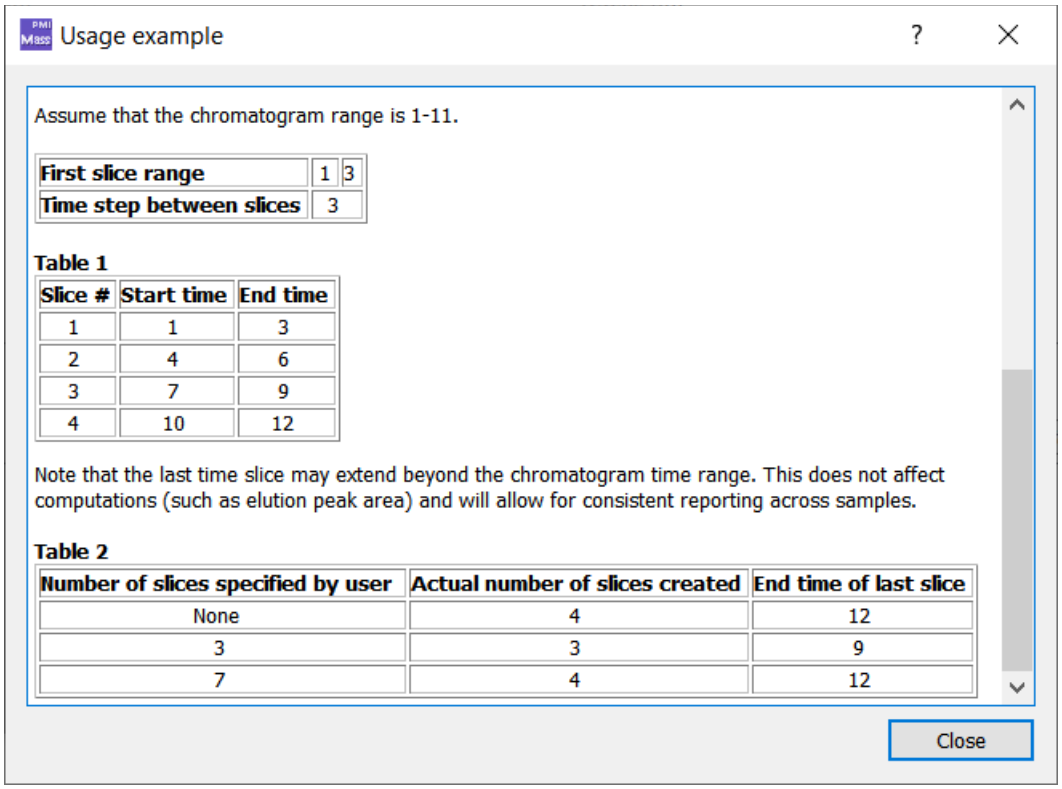


- Click **Add row** and enter start and end times for each slice. To remove a slice, select it and click **Delete row**.
- **Time slice(s)** divides elution peaks by regular time intervals defined by the user:

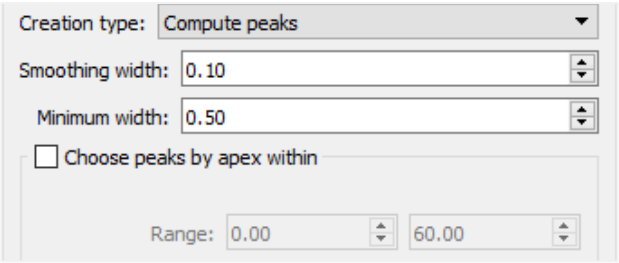


  - **First slice range** sets the start and end times for the first slice. This also sets the width for all time slices.
  - **Step** is the time delta that sets the start of subsequent time slices by adding the step value to the start of the preceding time slices. For example, the values above define 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:



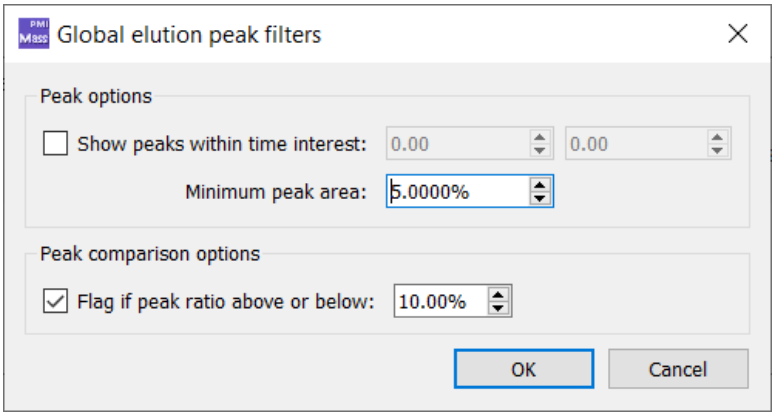


- **Compute peaks** automatically determines the elution peaks based on their minimum width, after smoothing:



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 %.

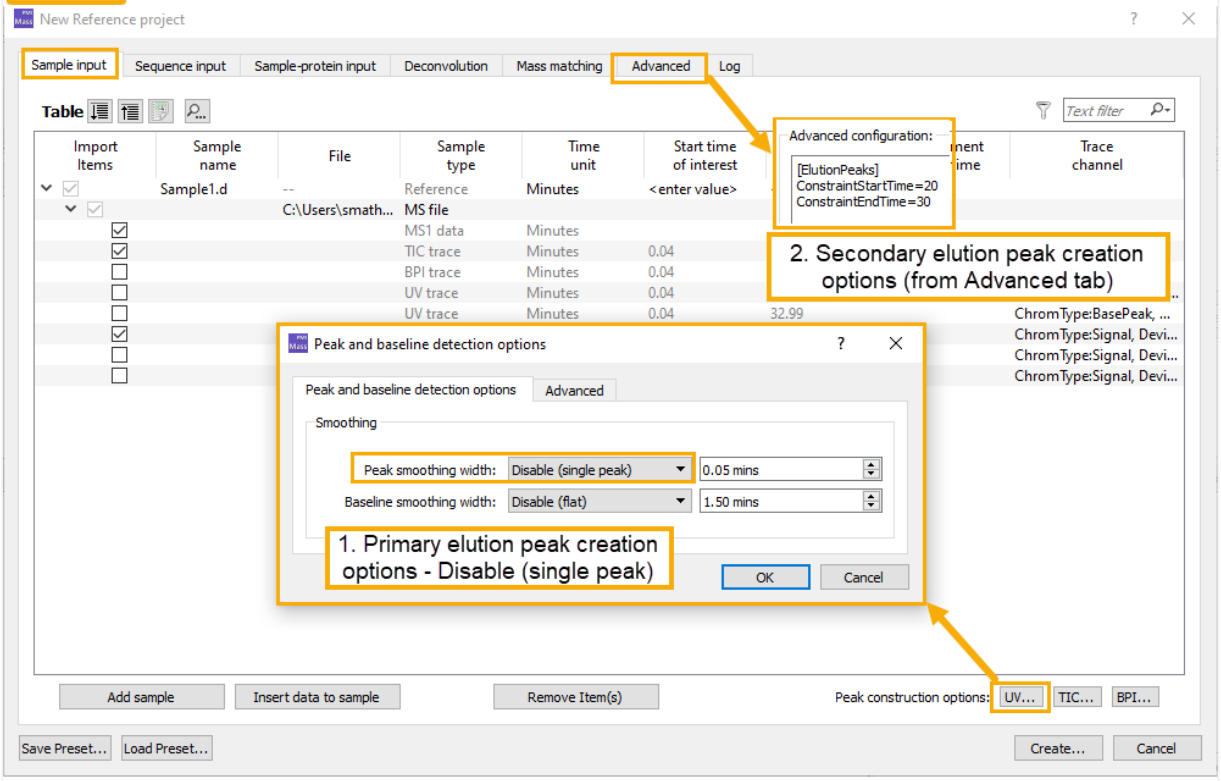
The new Traces table and elution peak settings are backward-compatible. Traces associated with samples in projects from software versions before 3.8 will correctly populate the Traces table in the current version. Elution peak settings and presets from versions before 3.8 will correctly migrate into 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

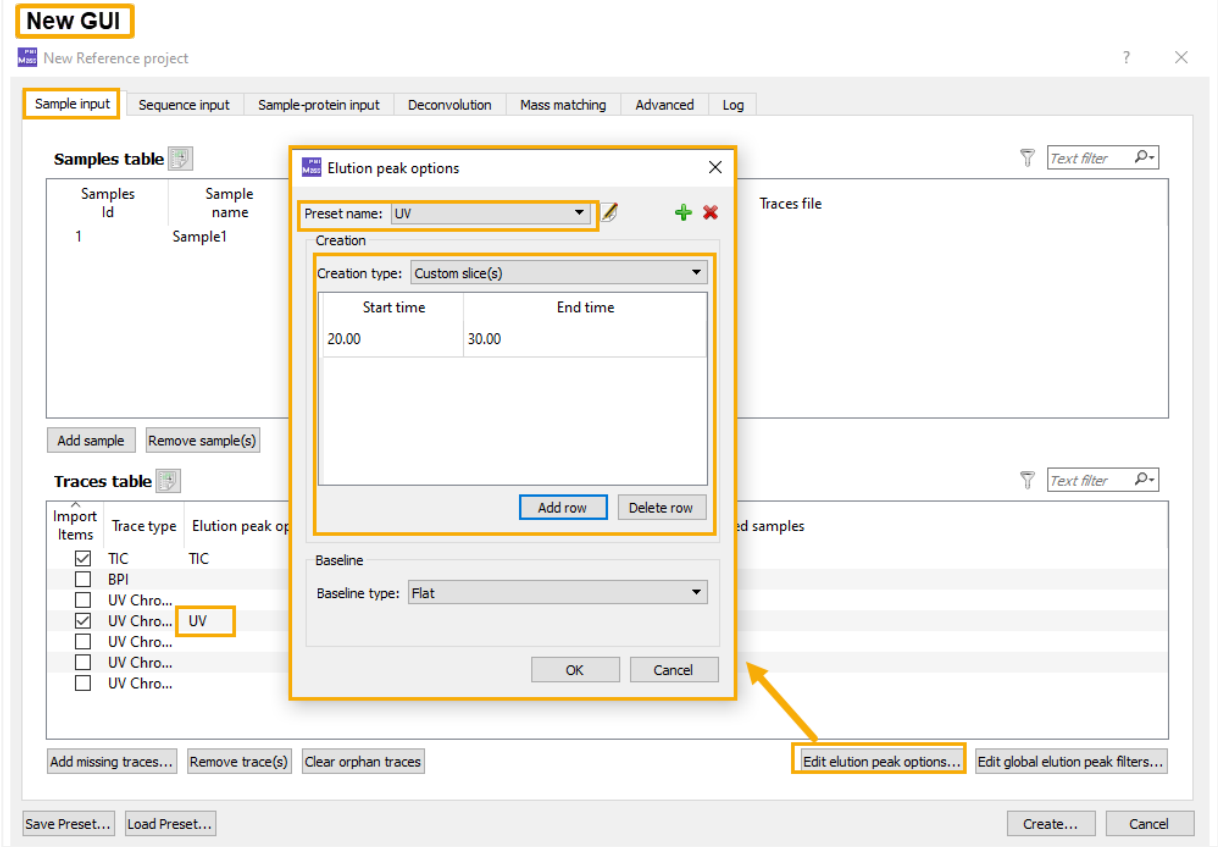
Several of the elution peak text commands have been migrated from the Advanced tab to Sample input tab. The following images illustrate how certain presets translate between previous versions and the current release. One to one matching was provided whenever possible.

Case I

Old GUI

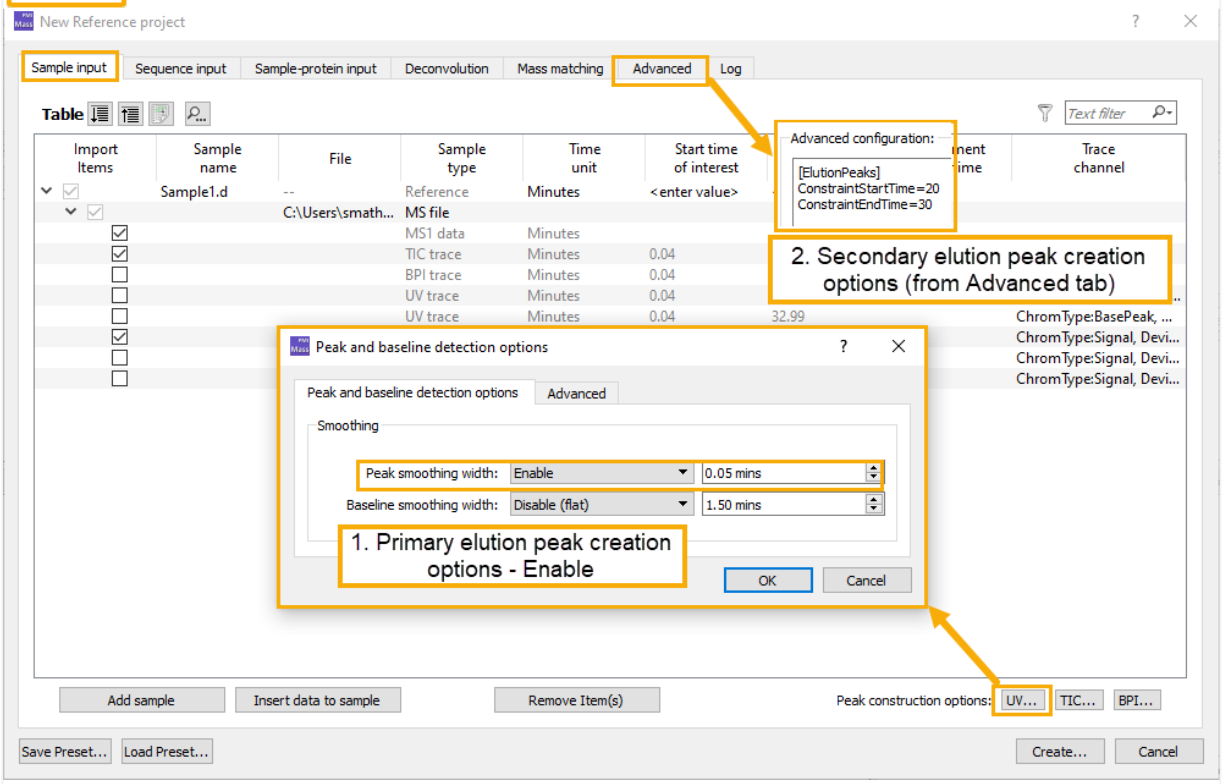


New GUI

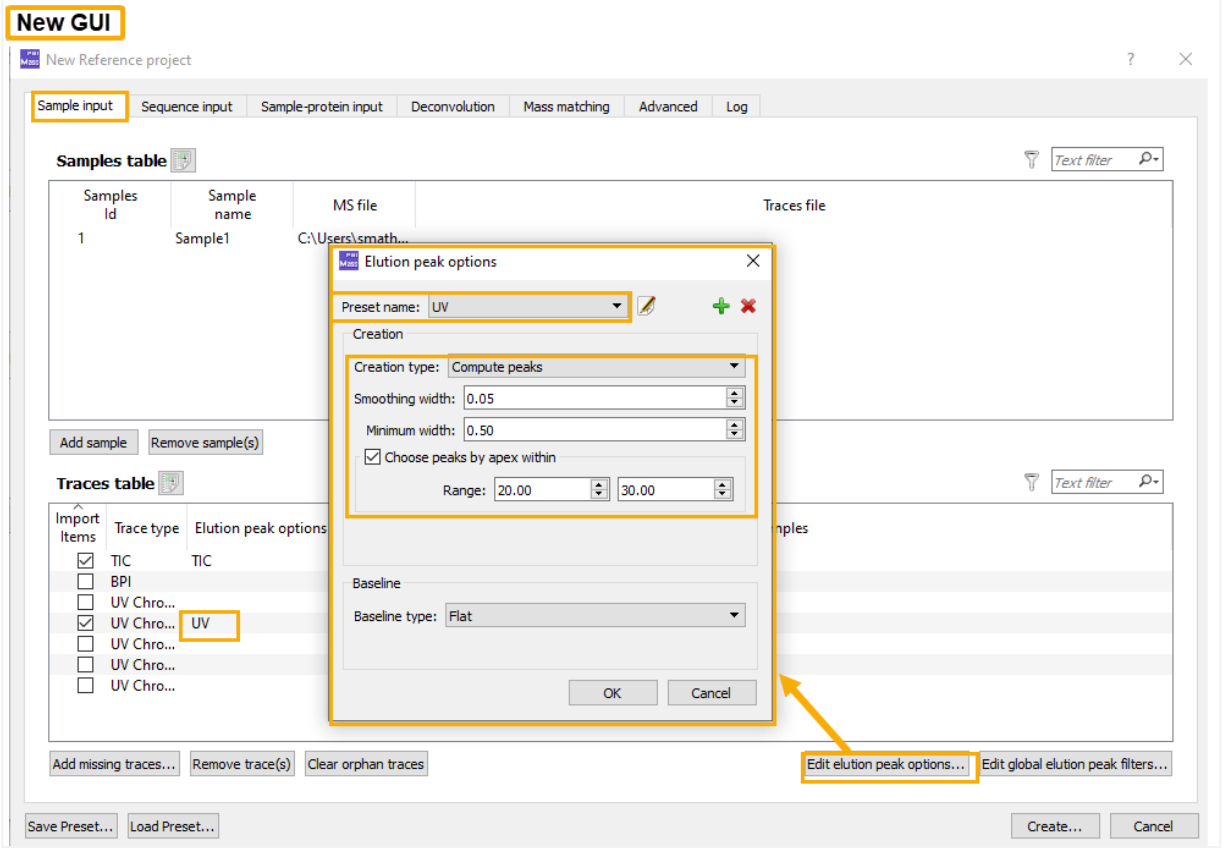


Case II

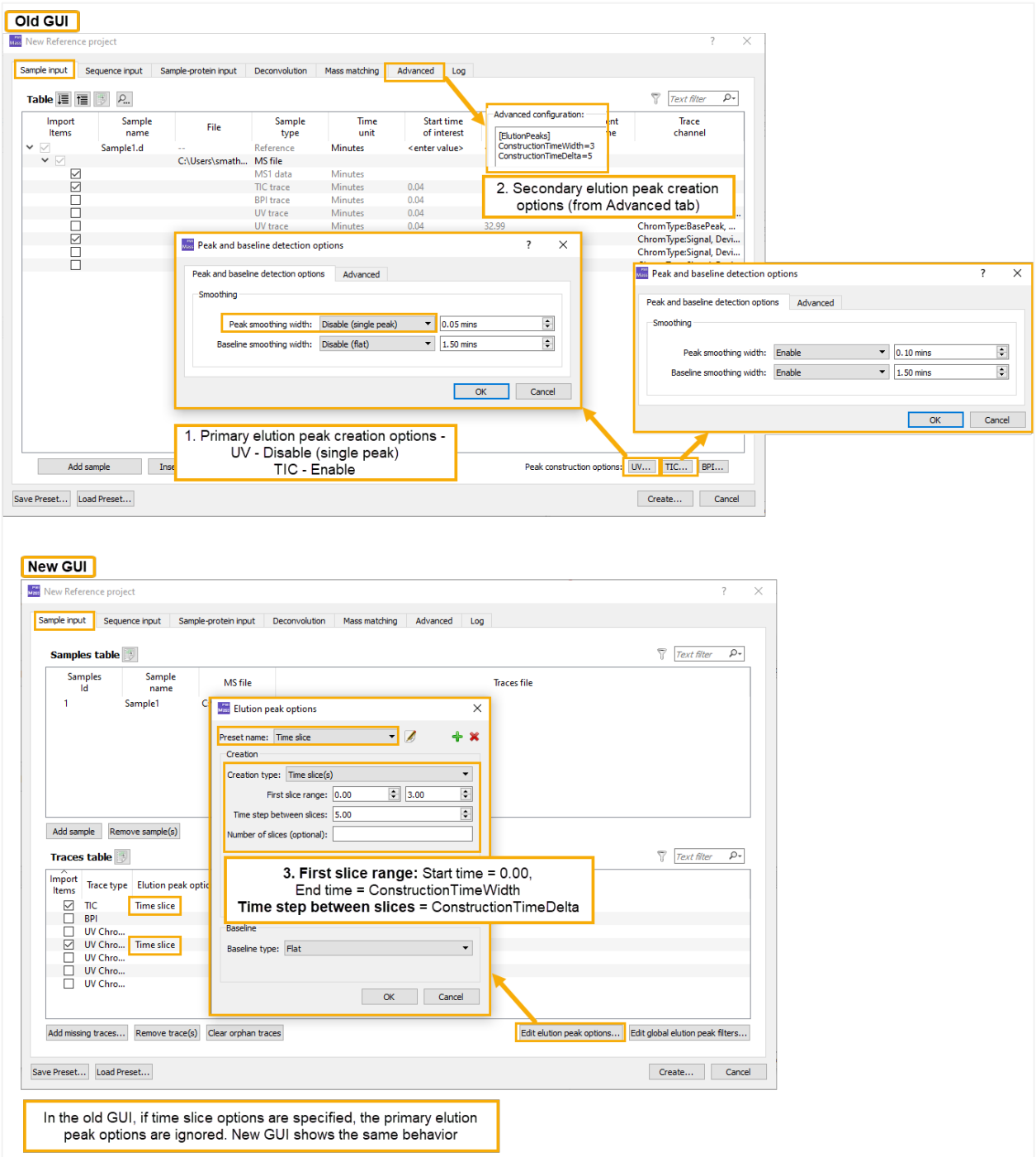
Old GUI



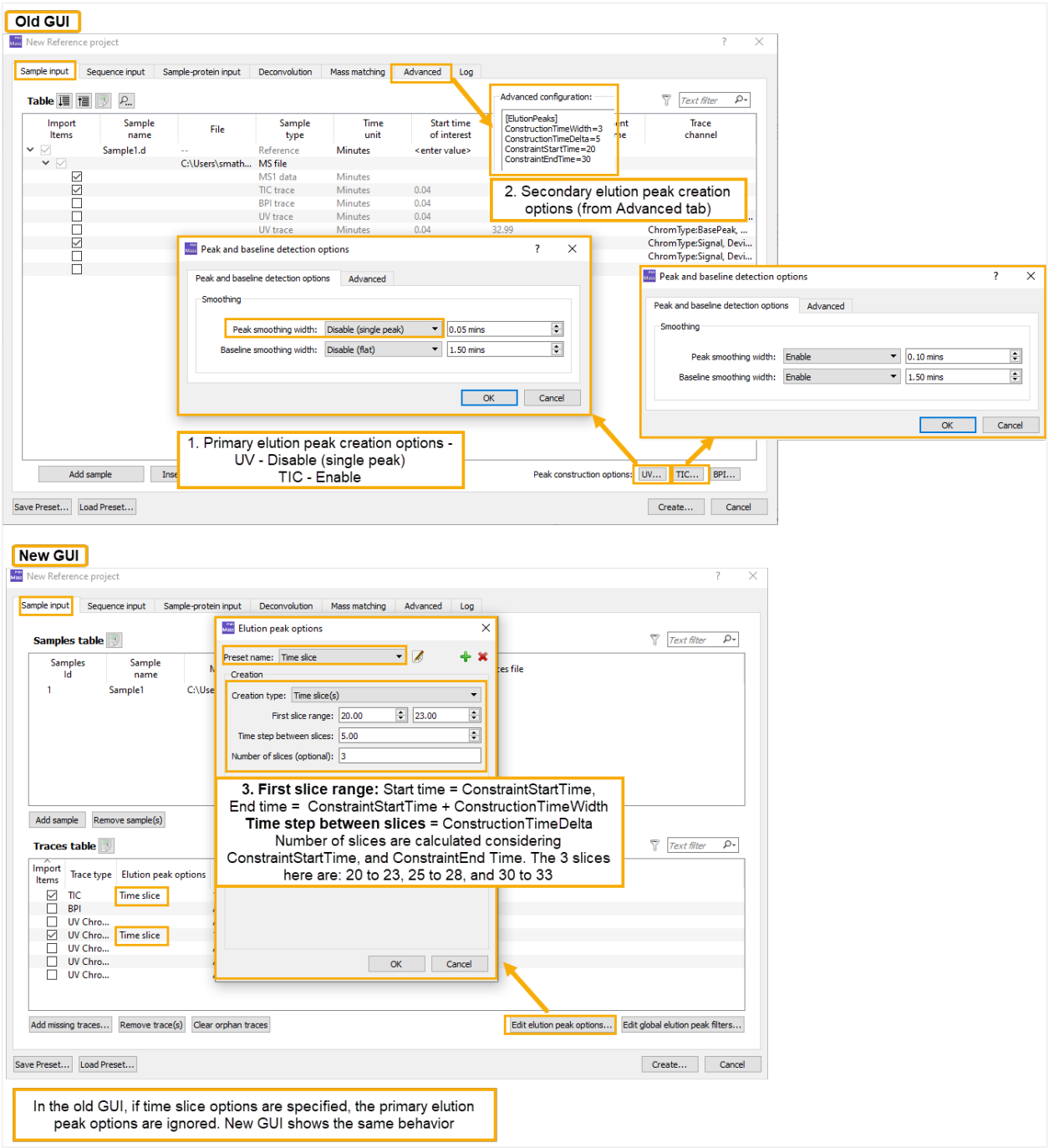
New GUI



Case III


Old GUI
Sample input
Sequence input
Sample-protein input
Deconvolution
Mass matching
Advanced
Log
Advanced configuration:
[ElutionPeaks]
ConstructionTimeWidth=3
ConstructionTimeDelta=5
2. Secondary elution peak creation options (from Advanced tab)
Peak and baseline detection options
Smoothing
Peak smoothing width: Disable (single peak) 0.05 mins
Baseline smoothing width: Disable (flat) 1.50 mins
OK
Cancel
Peak smoothing width: Enable 0.10 mins
Baseline smoothing width: Enable 1.50 mins
1. Primary elution peak creation options - UV - Disable (single peak) TIC - Enable
Peak construction options:
UV...
TIC...
BPI...
Add sample
Save Preset...
Load Preset...
Create...
Cancel
New GUI
Samples table
Traces table
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
Edit elution peak options...
Edit global elution peak filters...
Add missing traces...
Remove trace(s)
Clear orphan traces
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
Smoothing
Peak smoothing width: Disable (single peak) 0.05 mins
Baseline smoothing width: Disable (flat) 1.50 mins
OK
Cancel
Peak smoothing width: Enable 0.10 mins
Baseline smoothing width: Enable 1.50 mins
1. Primary elution peak creation options -
UV - Disable (single peak)
TIC - Enable
Peak construction options:
UV...
TIC...
BPI...
Add sample
Save Preset...
Load Preset...
Create...
Cancel
New GUI
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
Traces table
Trace type
Elution peak options
Time slice
Add missing traces...
Remove trace(s)
Clear orphan traces
Edit elution peak options...
Edit global elution peak filters...
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

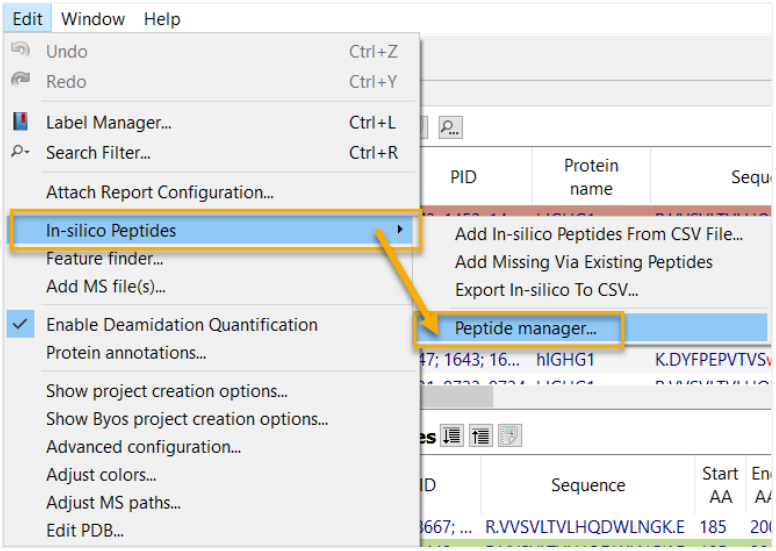
[Byomap]
ProjectCreateAutoAssign=true

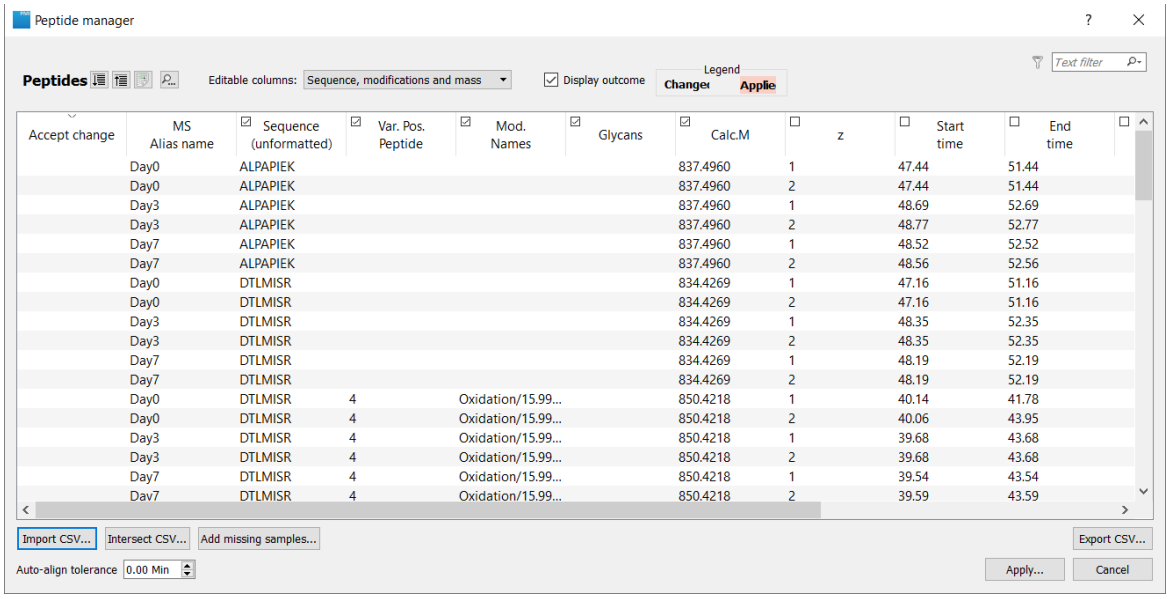

[Trace]
DefaultSelection=UV

[Trace]
DefaultUVTrace=2
```

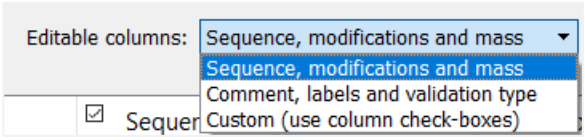
Peptide Manager - Peptide Analysis

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.

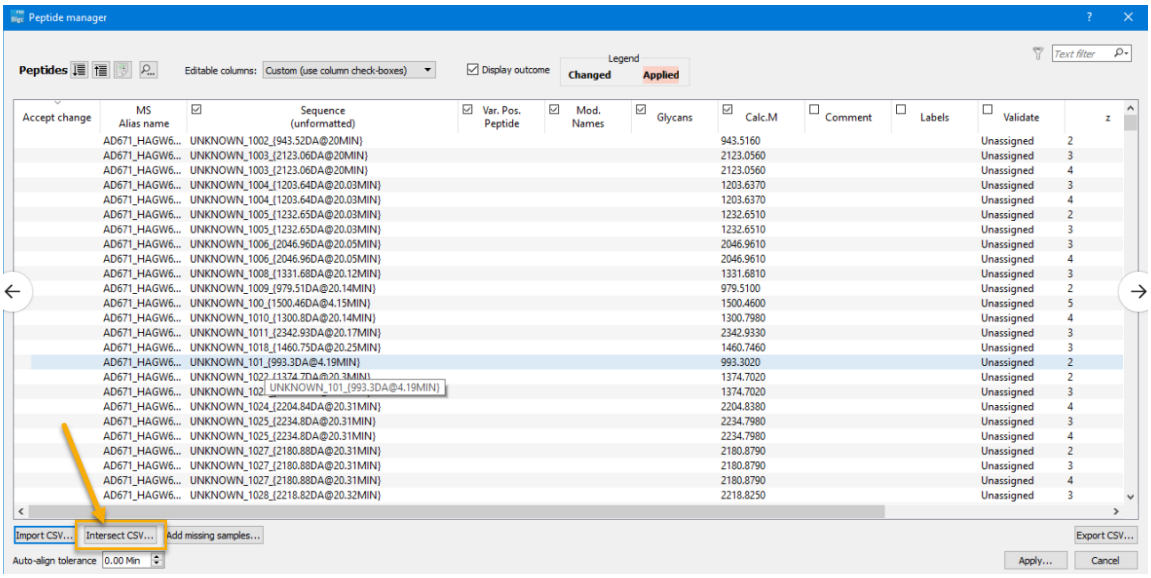




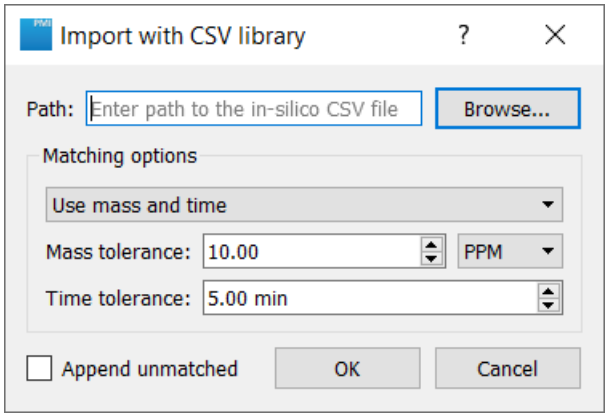
The user can set which columns are editable using the drop-down menu. Please note “Comment, labels and validation type” enables annotation transfer.



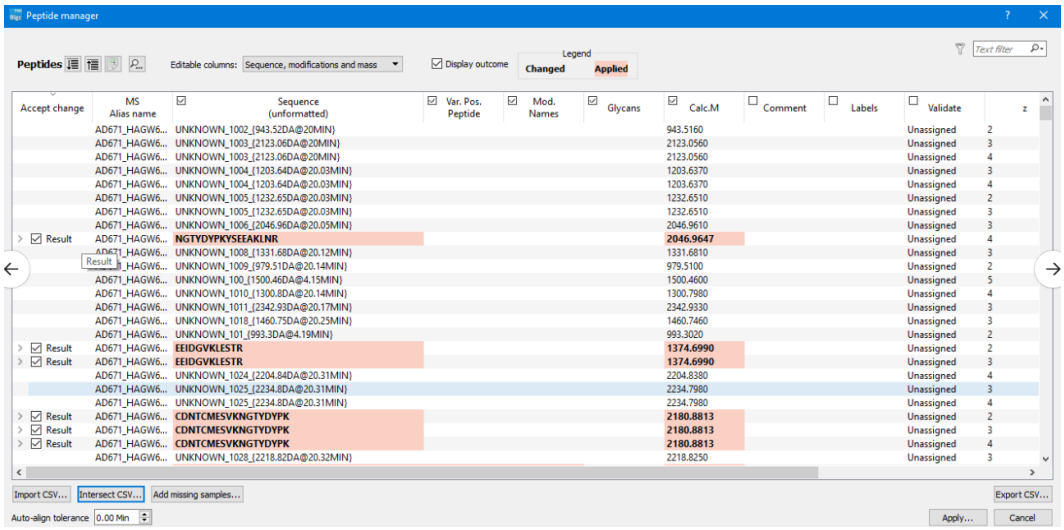
- Click **Export CSV** is the same as Edit > In-Silico > Export CSV.
    - Start and End times may differ from the previous CSV export. This is because Peptide Manager exports the most centered XIC start/end, while the old method exported the first available start/end range. The difference is considered 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.



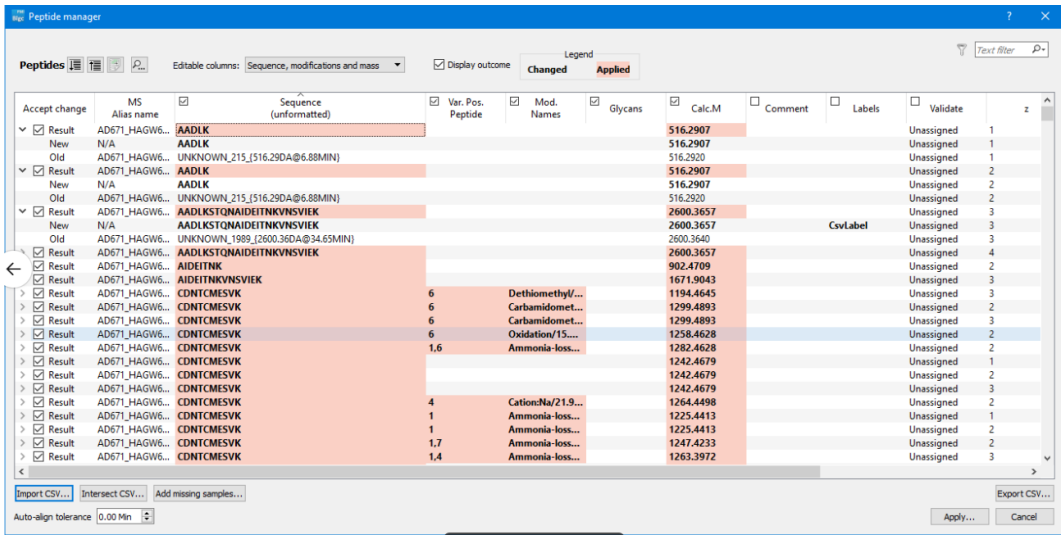
- Click Intersect CSV and select a CSV file:



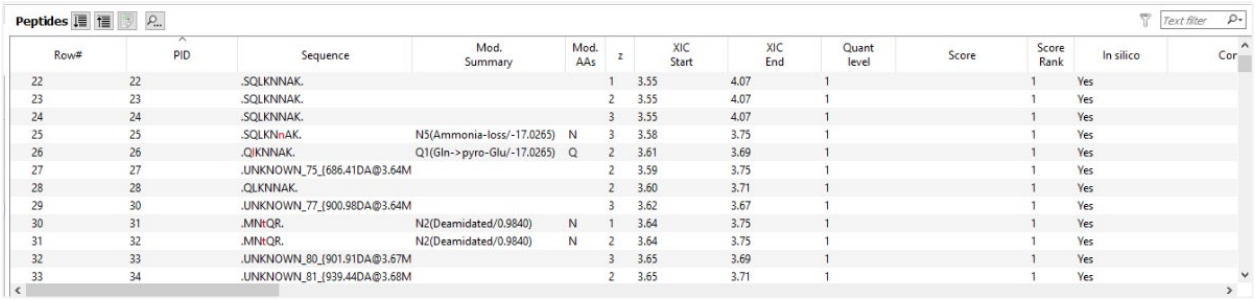
- Select a CSV file using the Browse button.
- Select between the Matching options “Use mass and time” and “Use sequence and modifications”
- Click OK to return to Peptide Manager and select columns to review. New values will be show in red.



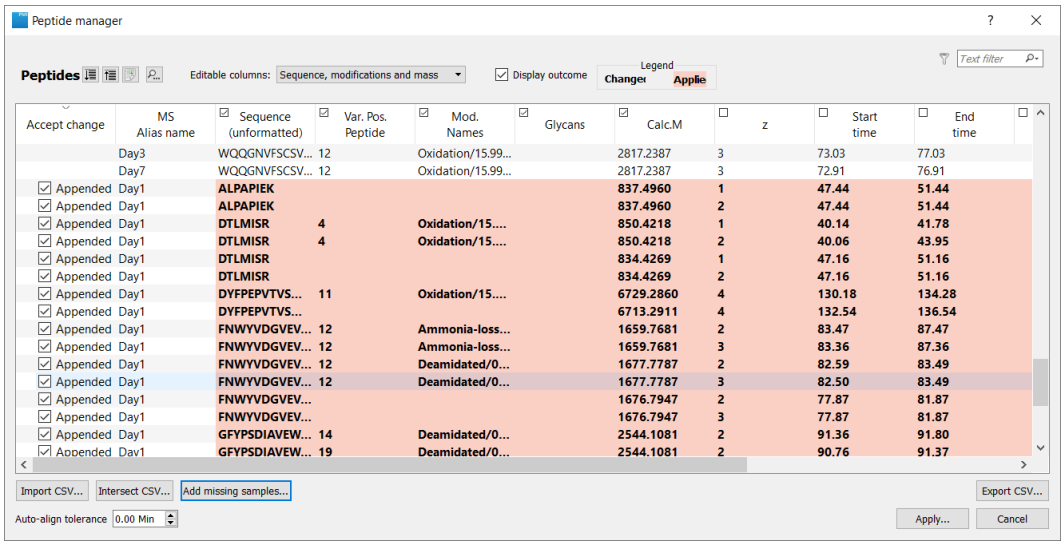
The below image displays expanded rows to showcase additional content



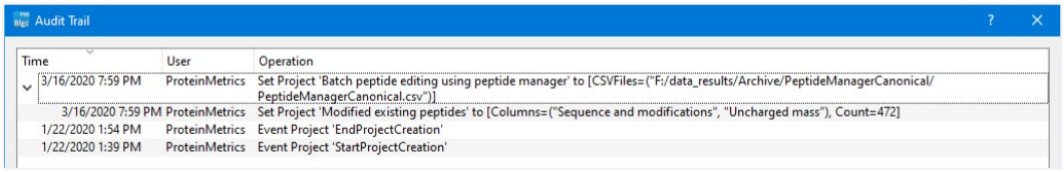
Click Apply to update the project. The below image displays a common use case – Feature Finder unknowns matched with in-silico content.



- **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

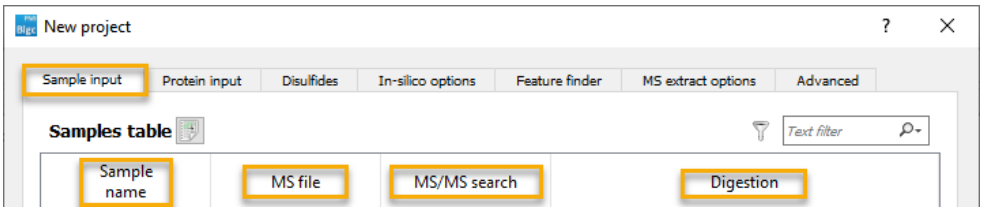


  - The CSV intersected
  - The columns altered
  - How many peptides were affected

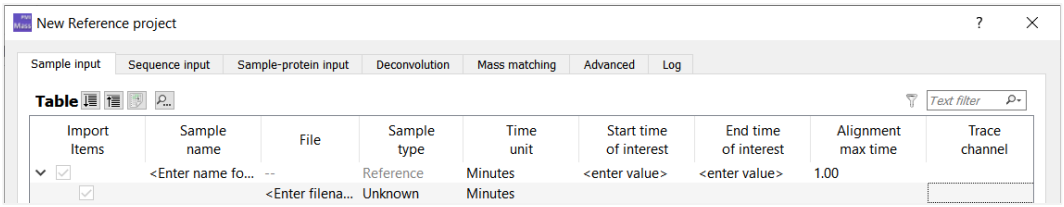
Sample Input Tab Redesign – Peptide Analysis, Intact Analysis, and Chromatogram Analysis

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

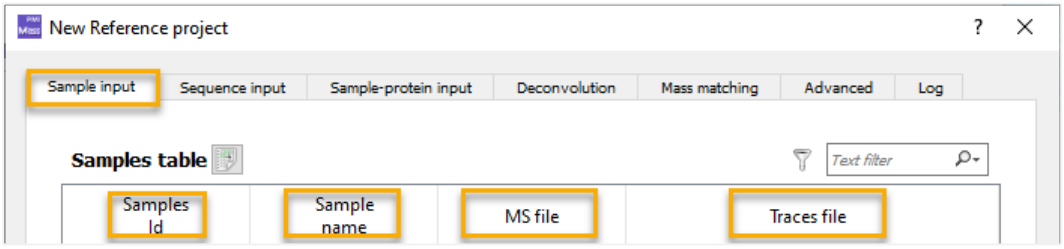
- In Peptide Analysis and the related Byos workflows, the **Sample input tab** header “Alias name” is now “Sample name”, “File” is now “MS file” and “Ms2 search” is now “MS/MS search”. **MS/MS search** is now opened directly in the **MS file** row, rather than as a sub-row of the MS file.



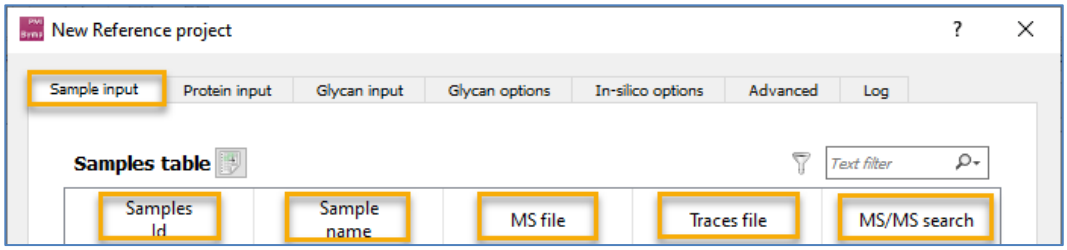
- In Intact Analysis, Chromatogram Analysis, and the related Byos workflows, the **Sample input tab** has been completely redesigned. The previous tab contents are shown below:



The new Sample tab contents for Intact Analysis are shown below



The new Sample tab contents for Chromatogram Analysis are shown below

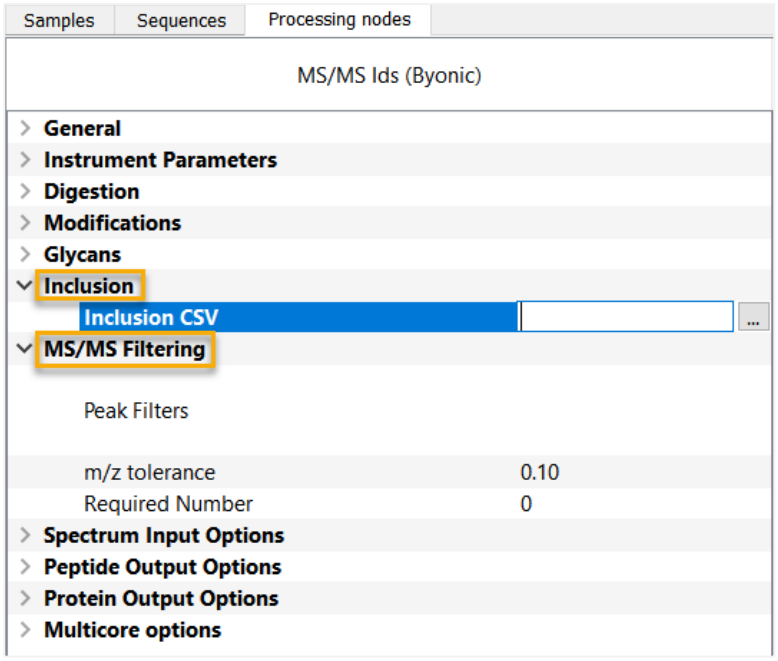


The **Start time** and **End time** columns have been moved to the **Global elution peak filters** dialog. The **Time unit** and **Alignment max time** columns have been moved to the **Advanced** tab. See the **Elution Peak Calculations and the Traces Table** section for more details about the new Sample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

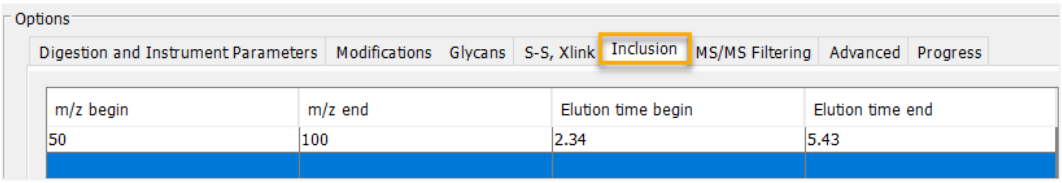
Byonic and Byos

- Inclusion and MS/MS Filtering tabs

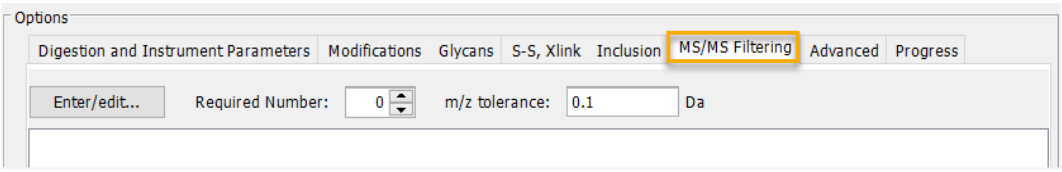
  In Byos, two new sections appear in the **Processing nodes** tab for workflows that include Byonic parameters – Inclusion and MS/MS Filtering. The Inclusion feature, added to Byonic in the 2019-12 release, is now part of Byonic workflows in Byos. MS/MS Filtering is new to both Byonic and Byonic workflows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



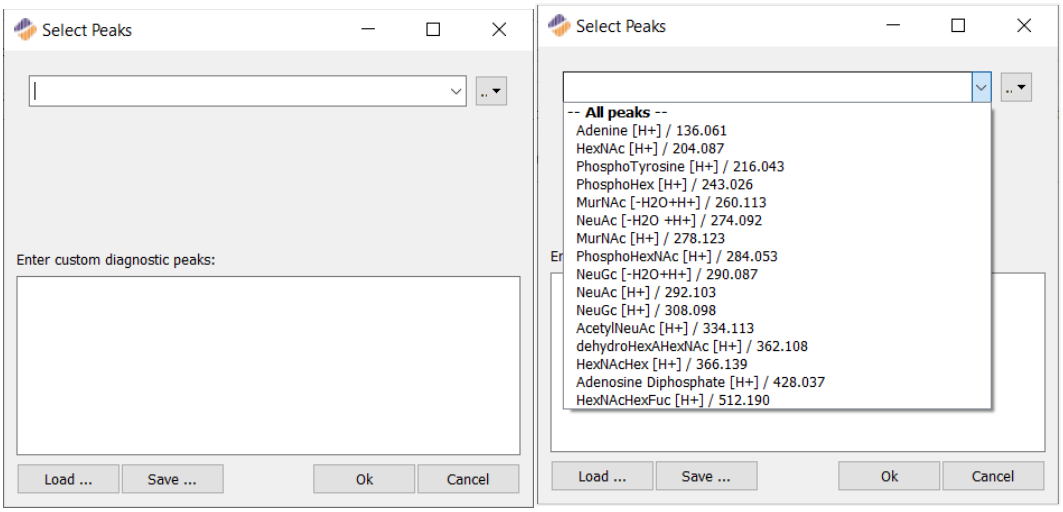
The **Inclusion** section allows the import of *.csv files which define m/z ratio ranges and/or elution time range segments to include in the search. In Byonic, **Inclusion** is represented as a tab:



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



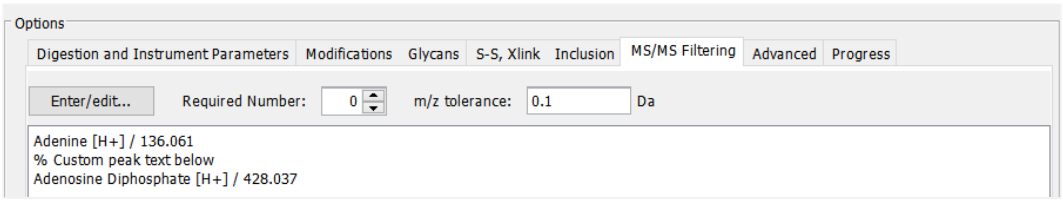
The **Enter/edit** button in Byonic and the **Peak Filters** ... button in Byos each open the **Select Peaks** dialog:



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

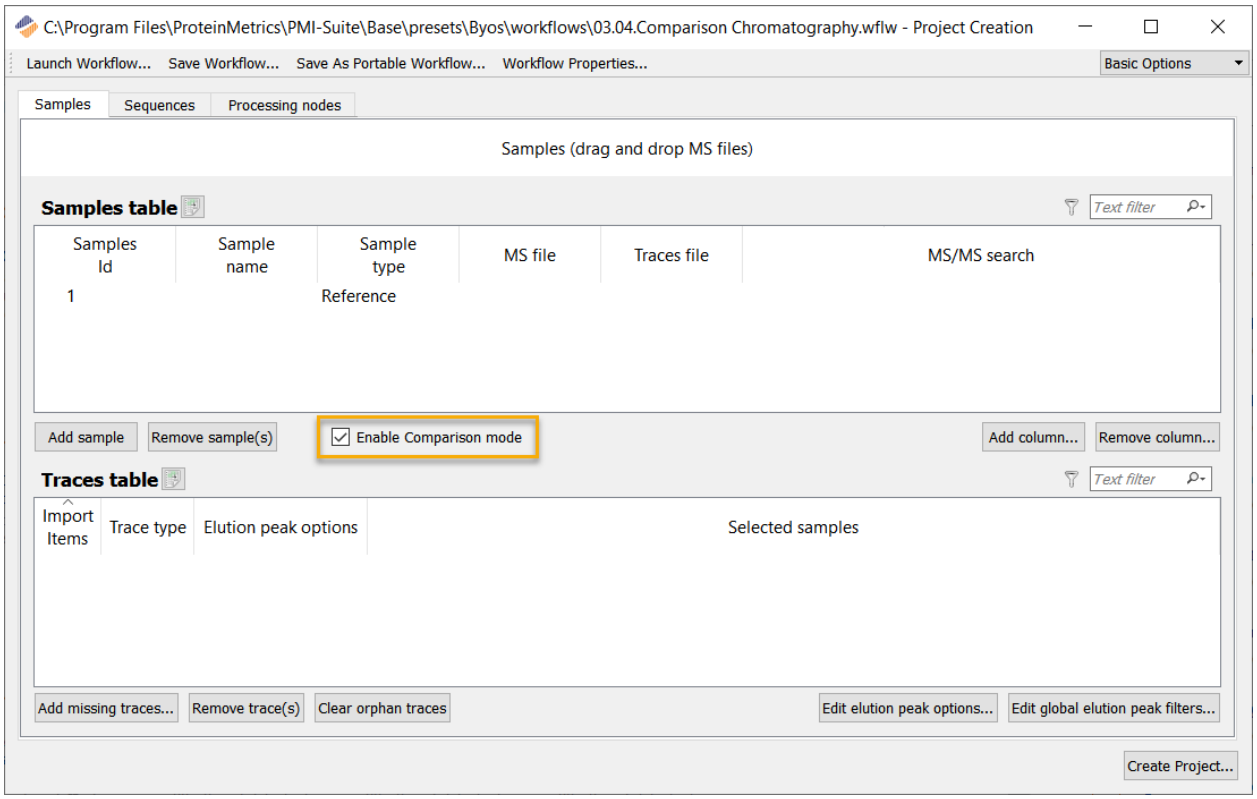
Click **OK** and the diagnostic peaks strings are now listed in the MS/MS Filtering tab:



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

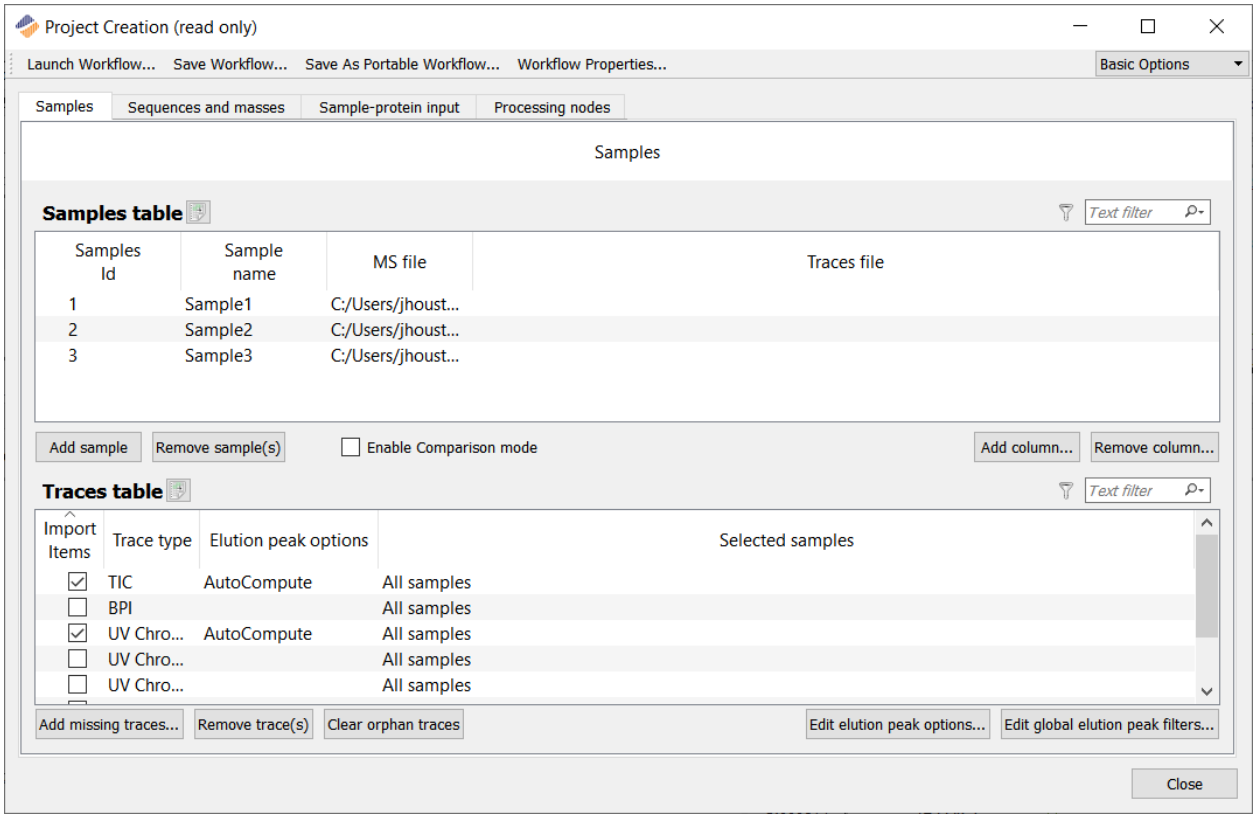
For Byos Intact Analysis and Chromatography Analysis workflows, new comparison projects are created by checking the **Enable Comparison mode** checkbox in the **Samples** tab:



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.

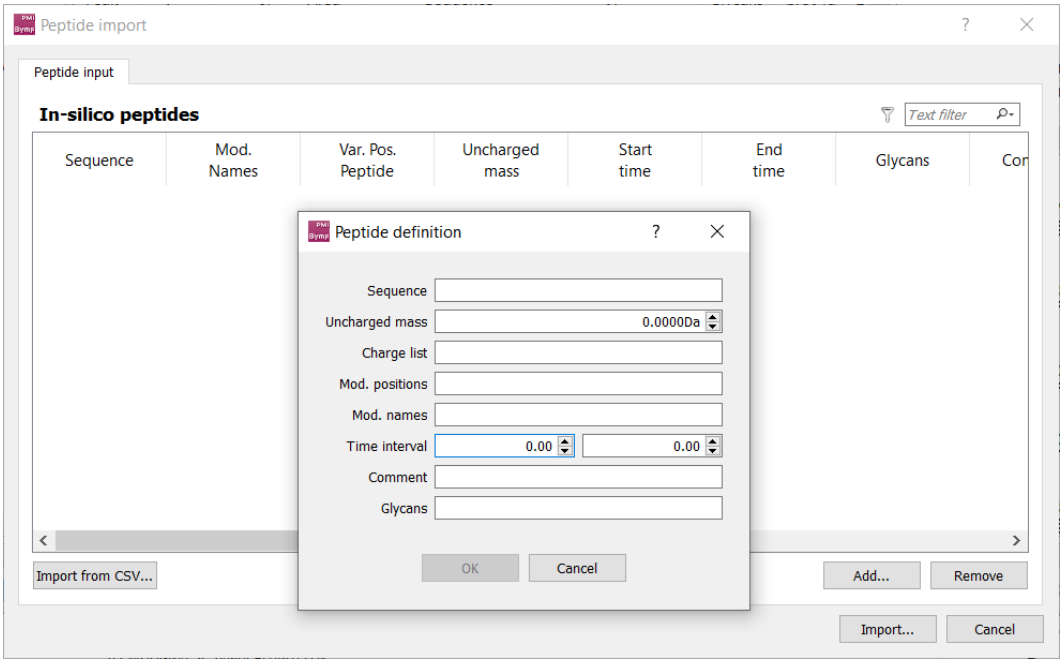
Show Byos project creation options

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:



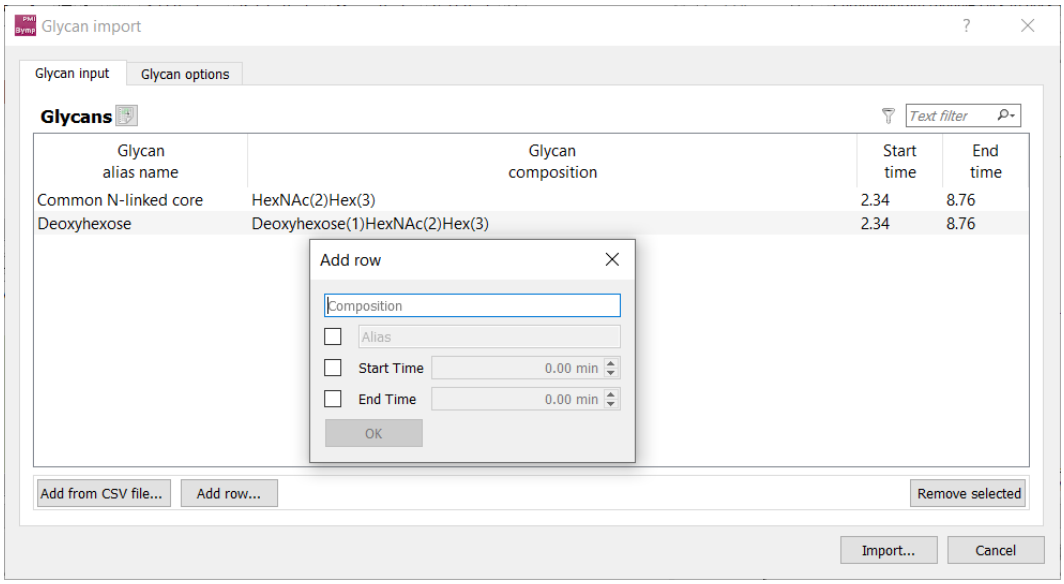
Glycan import – Chromatogram Analysis

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.

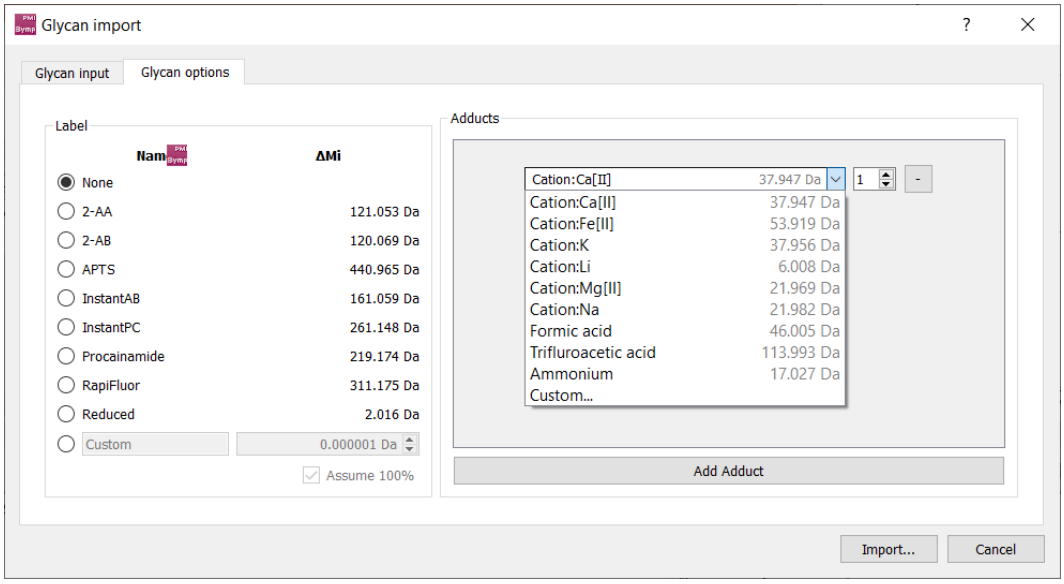
Edit > Import detached glycan candidates opens a dialog to import or manually enter glycans:



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:

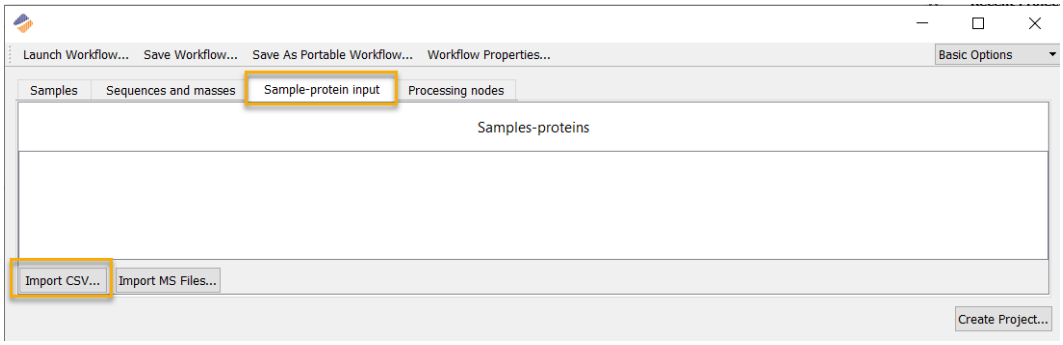


Hide Protein Coverage

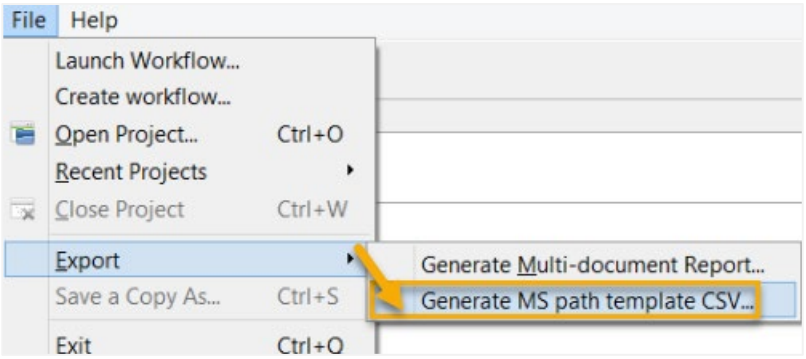
For Peptide and Chromatogram Analysis projects with a very large number of proteins, the Protein Coverage graphic can be turned off to speed loading of the project. The **Enable coverage rendering** checkbox can be accessed by the ⚙ icon or from right-click **Rendering options.**

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**

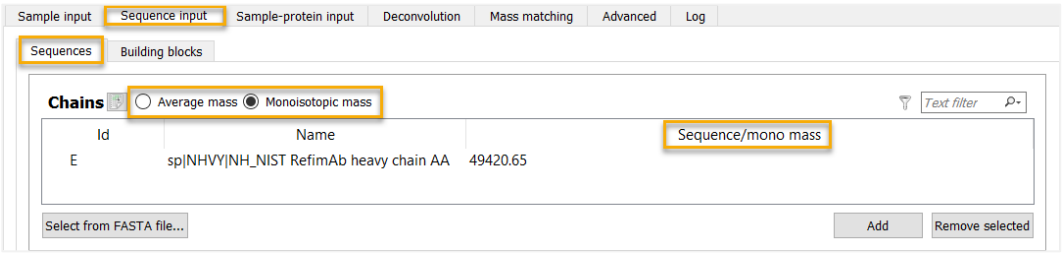
  In the new project creating Sequence input tab, fluorine (F) has been added to the Chemical Elements table in the Building blocks sub-tab:

Chemical Elements

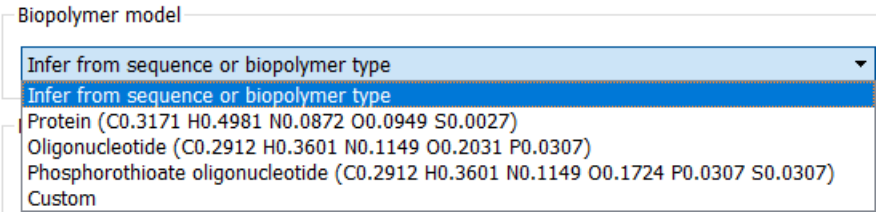
Element	Average mass
C	12.01079
H	1.007968
N	14.00669
O	15.99937
S	32.0639
P	30.973762
Se	78.971
F	18.998403163

- **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.

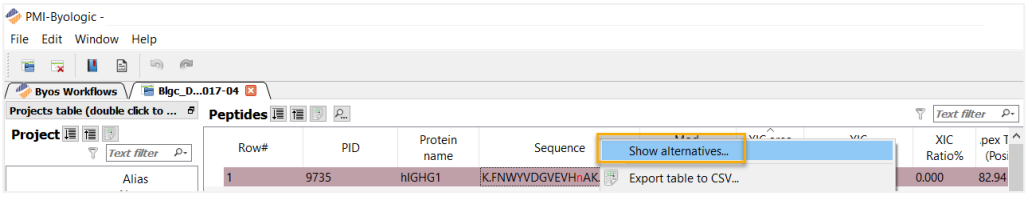
Peptide Analysis

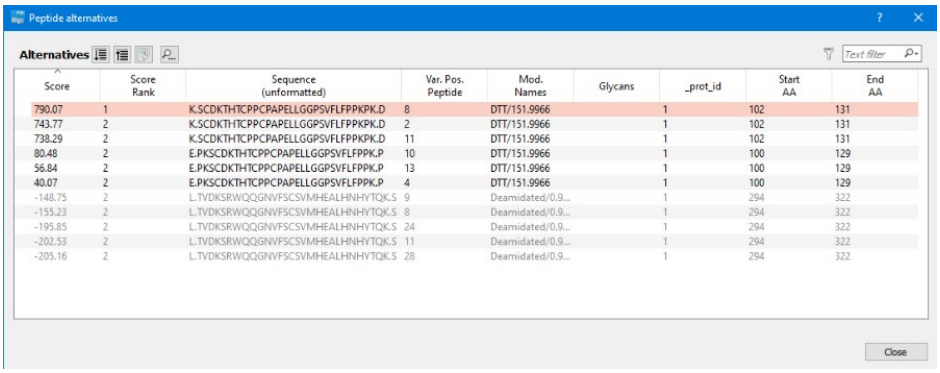
- **Isoelectric Point (pI value) Column**

  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.



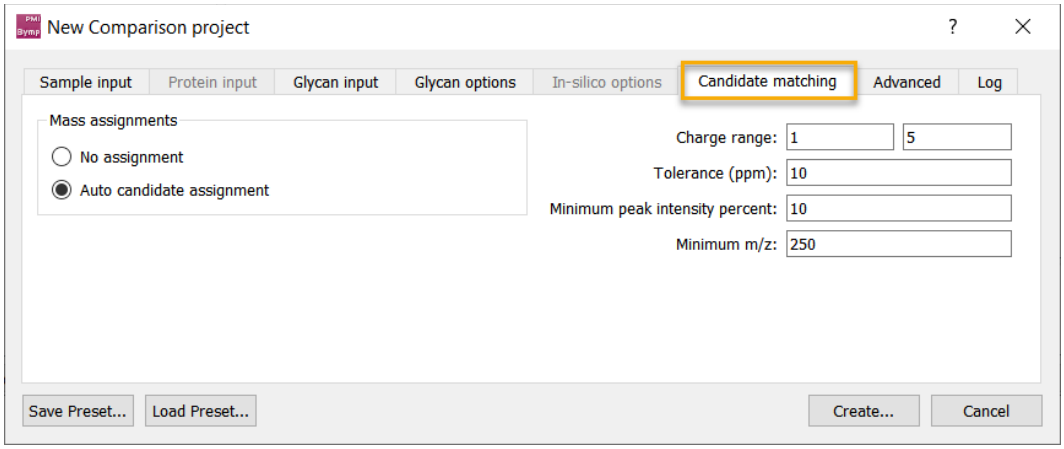


- The best candidate is highlighted in red
- The candidates scoring above the threshold are visible in black

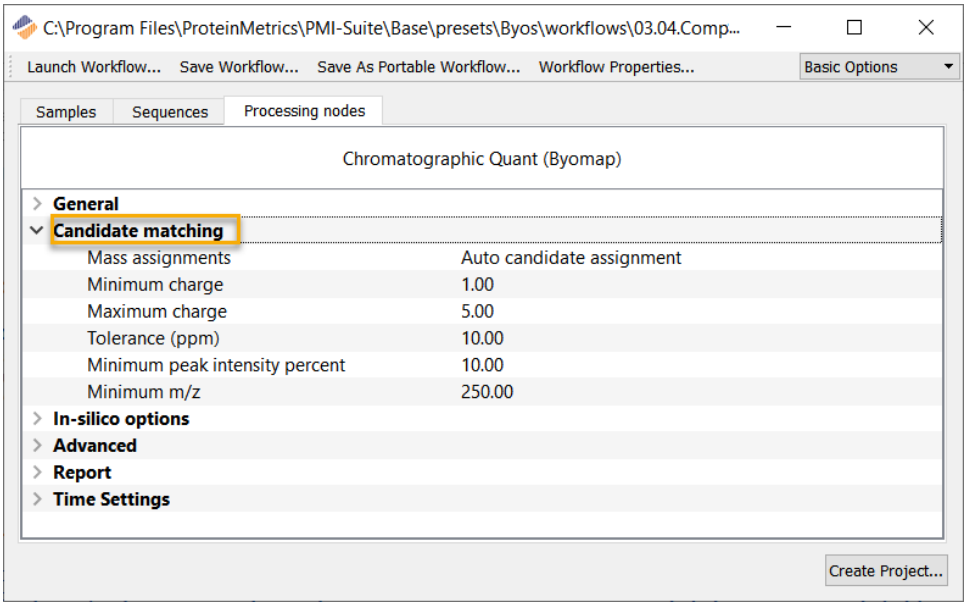
Chromatogram Analysis

- **Candidate Matching Tab**

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

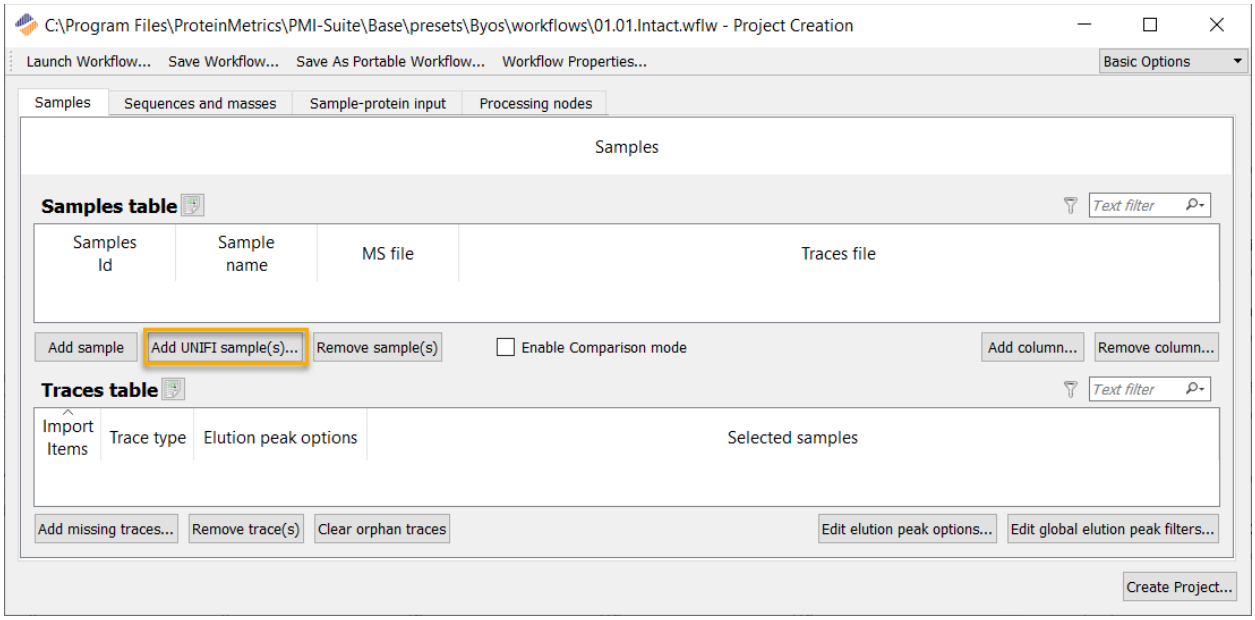


Candidate matching filters can now be modified before project creation. In previous versions, users could modify candidate matching filters only after project creation. In Byos, Candidate matching is found in the Processing node tab:

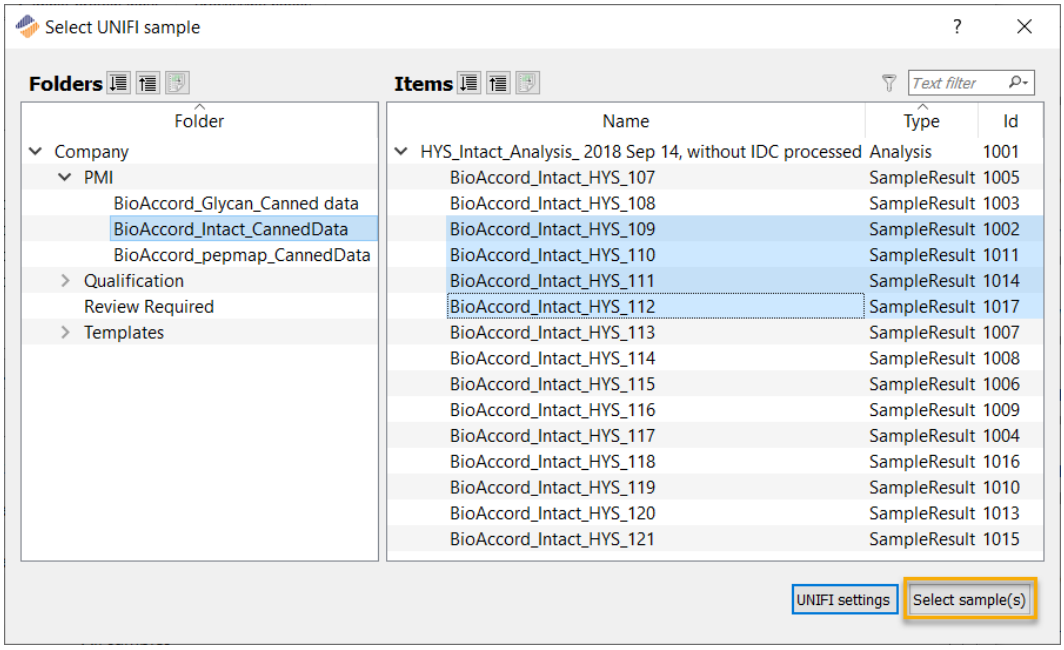


Byos UNIFI API Integration

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:



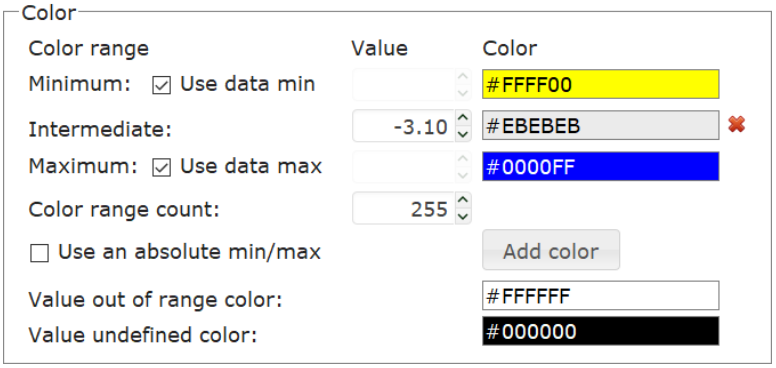
This opens the **Select UNIFI sample** dialog where MS data stored in UNIFI can be browsed and selected for download to Byos:



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**.

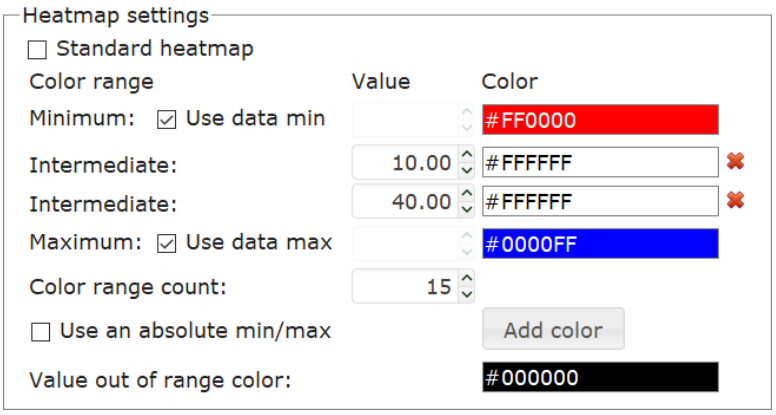
Reports

- **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.

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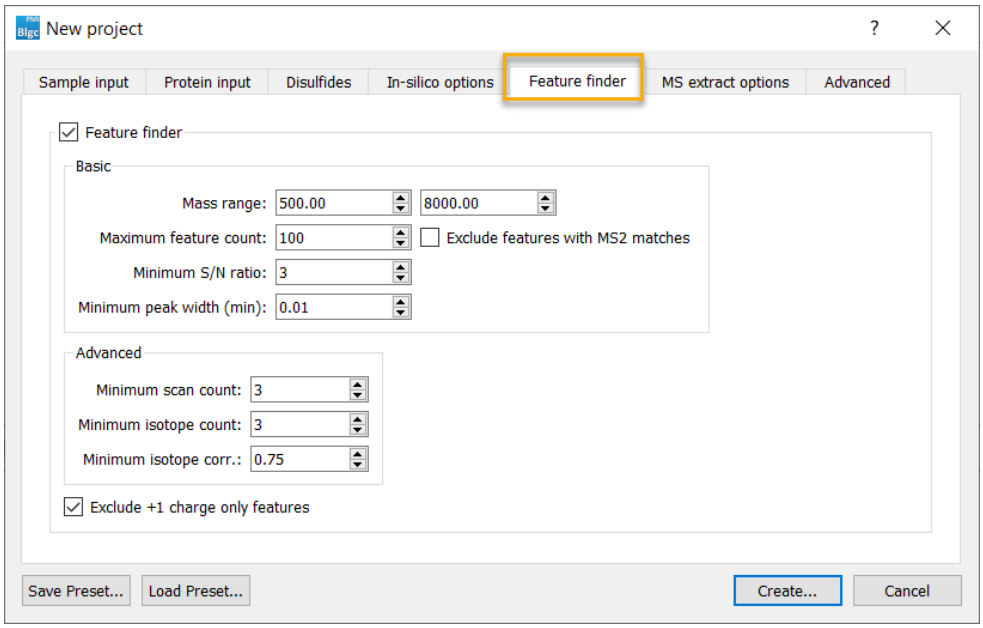
Feature Finder – Byos and Byologic

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.

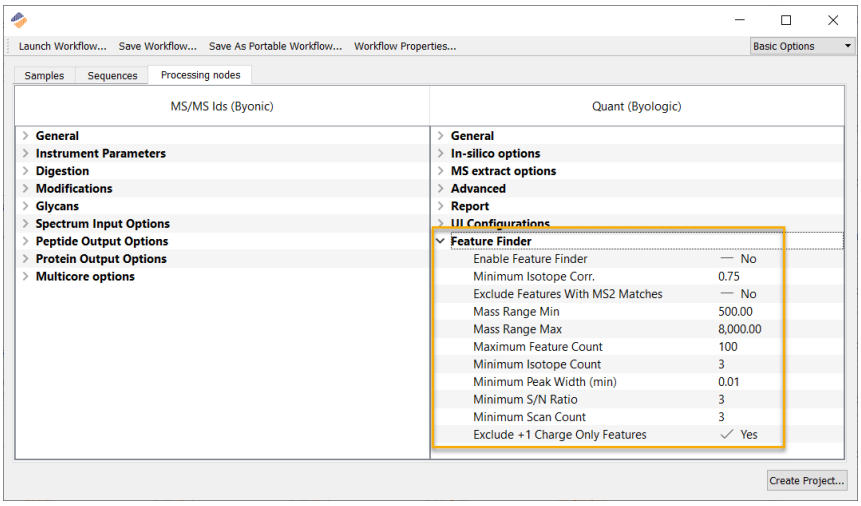
Feature Finder is an algorithm that allows the user to scan the MS1 domain for all existent peptides in a sample. In a typical data dependent acquisition assay, peptides are identified only if an MS2 scan is triggered from a precursor ion in an MS1 scan. This means that if a peptide ion does not trigger 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



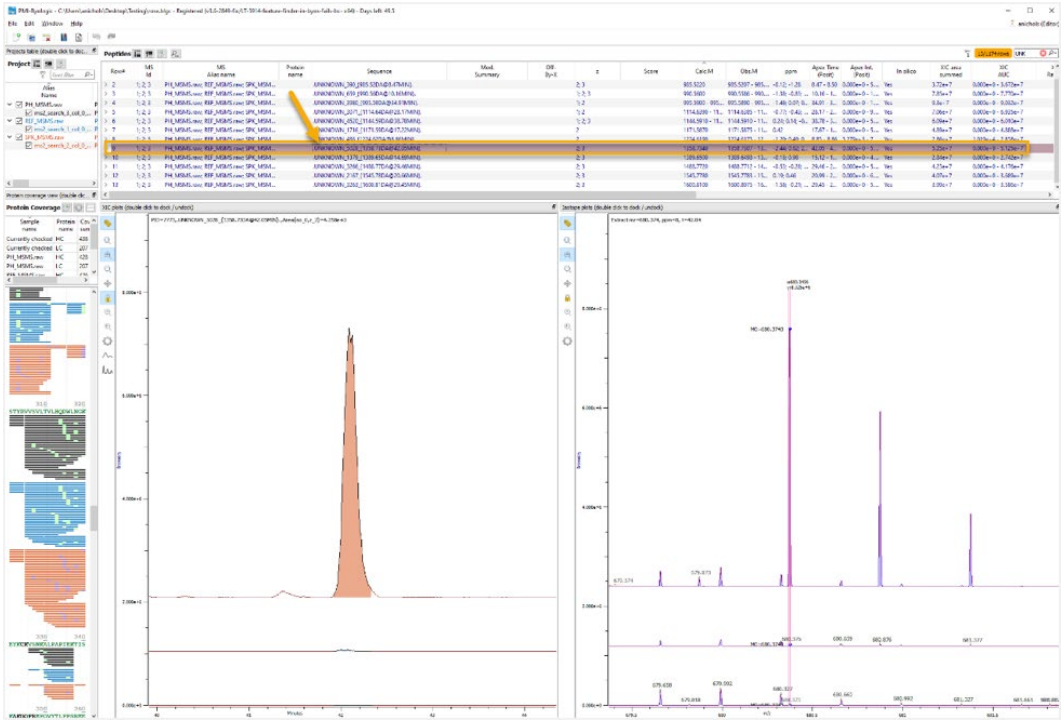
Byos – new “Feature Finder” section of Processing nodes



- 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

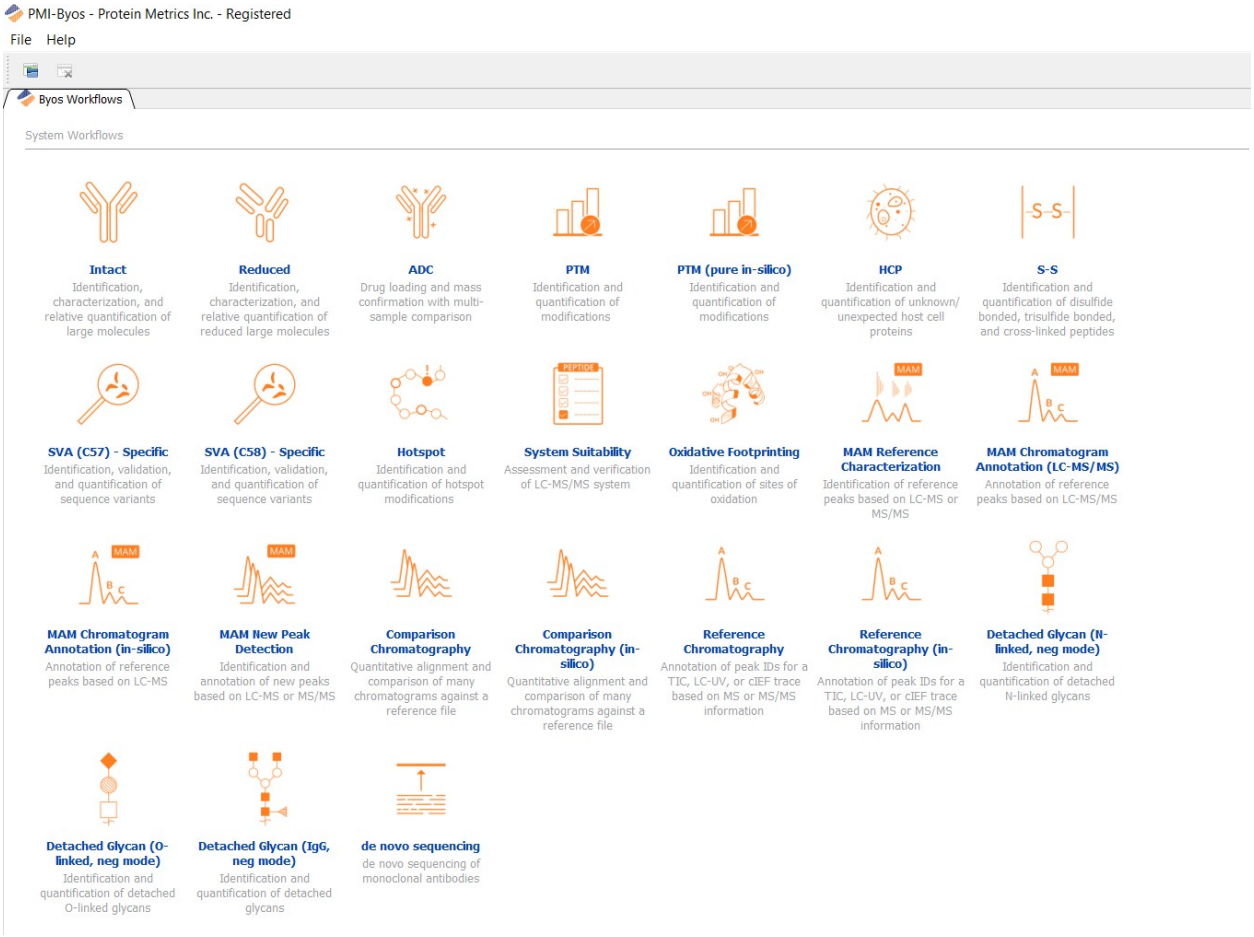
An example of the Feature Finder display:



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).



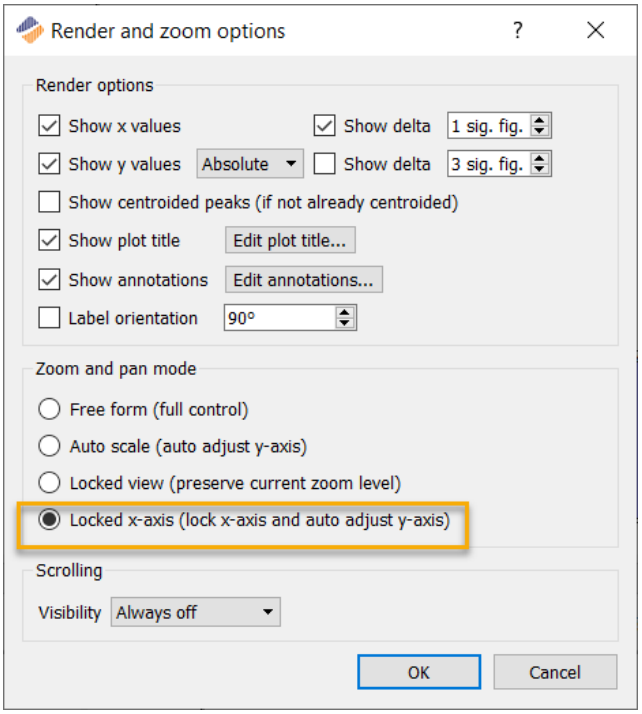
- **Projects to Open by Default in Byos**

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

Usability – Plots

- **Lock x-axis range**

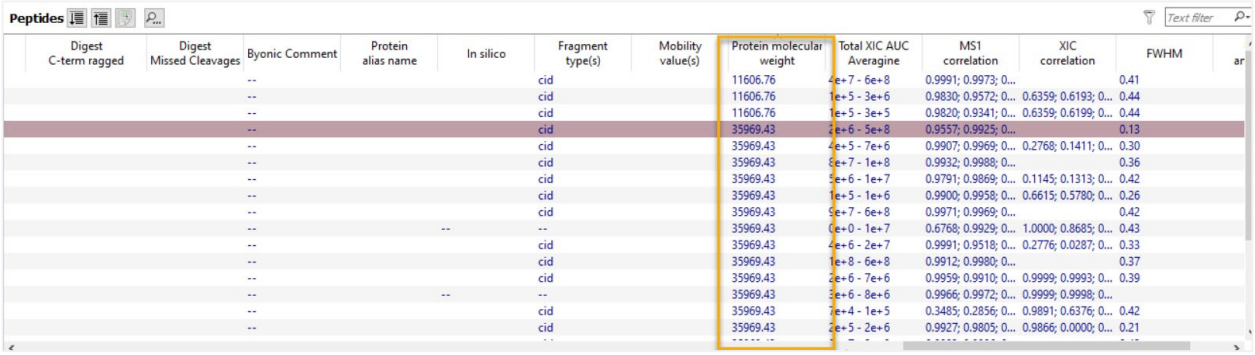
  New capability to selectively lock the x-axis range. This is enabled through the Render and zoom options, as shown below.



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):

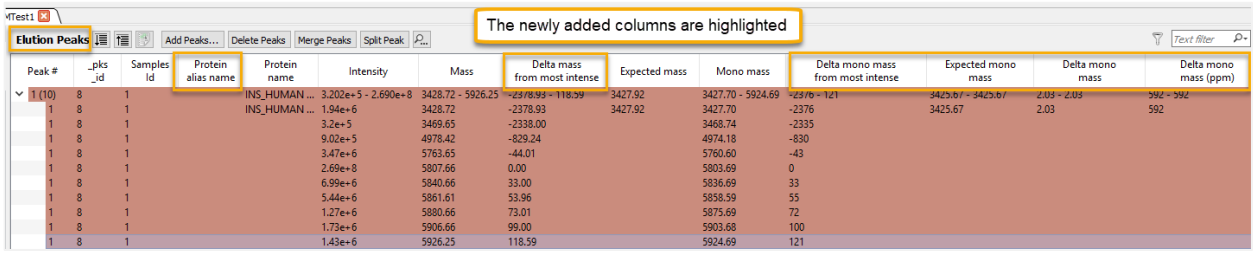
```
[Intact]

MaxMonoisotope=25000
```

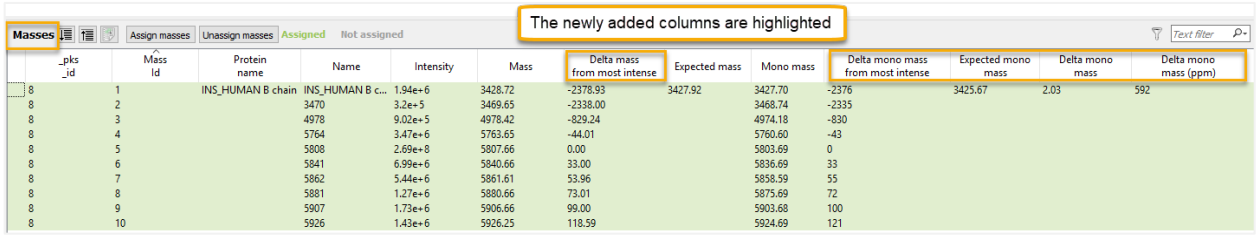
  MaxMonoisotope activates the calculation of monoisotopic masses. A value of 25000 will cause mass peaks below 25000 to have monoisotopic masses computed by matching the deconvolved mass peak against an averagine model with isotope-resolved peaks. Mass peaks above 25000

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

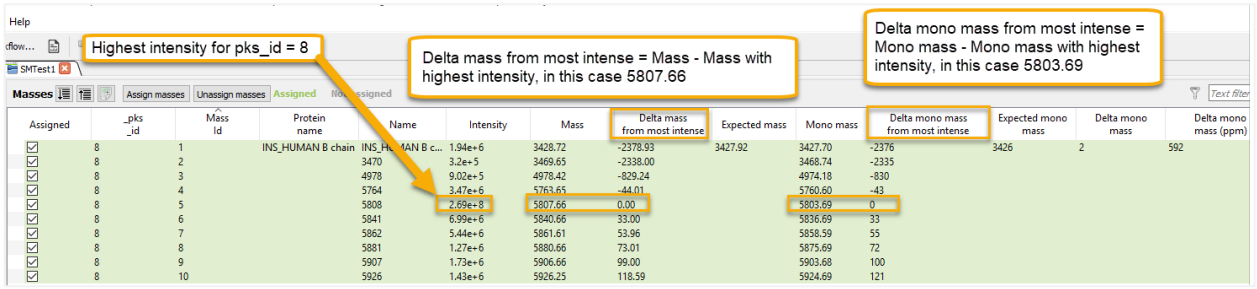


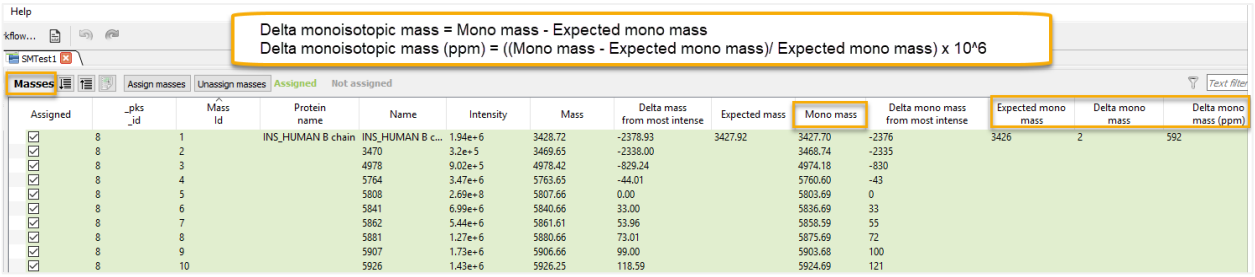
  - Protein alias name
  - Delta mass from most intense
  - Delta mono mass from most intense
  - Expected mono mass
  - Delta mono mass
  - Delta mono mass (ppm)



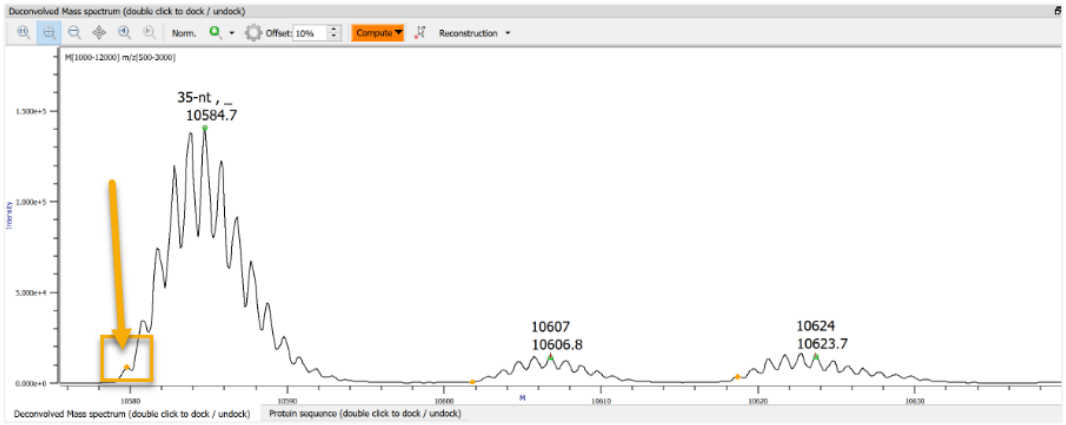
  - Delta mass from most intense
  - Delta mono mass from most intense
  - Expected mono mass
  - Delta mono mass
  - Delta mono mass (ppm)

- **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:

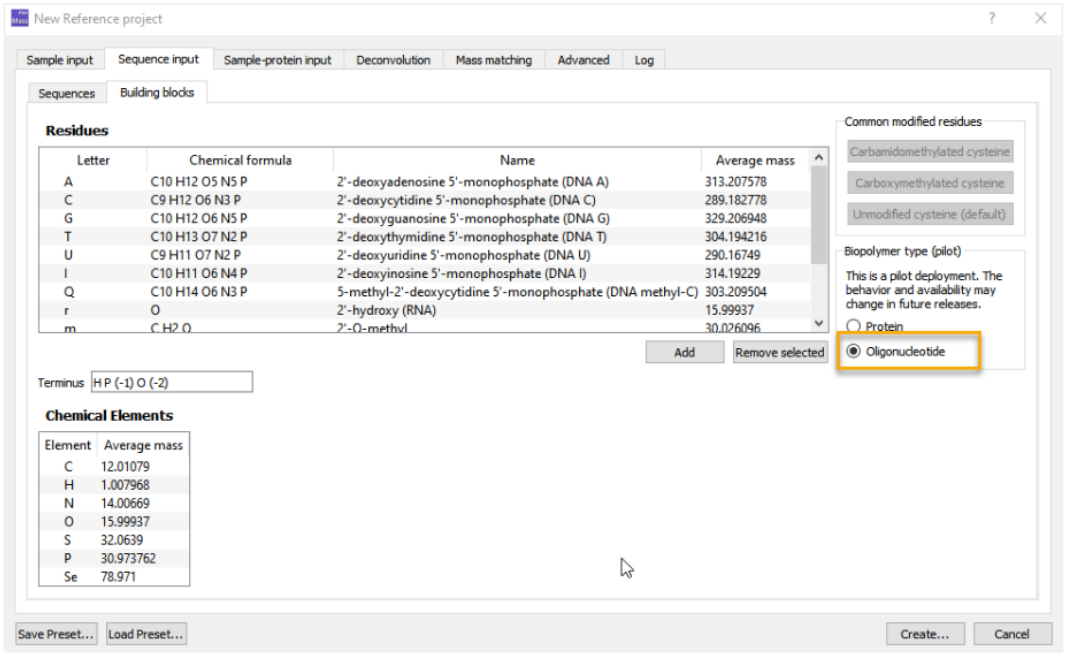
```
[Intact]

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

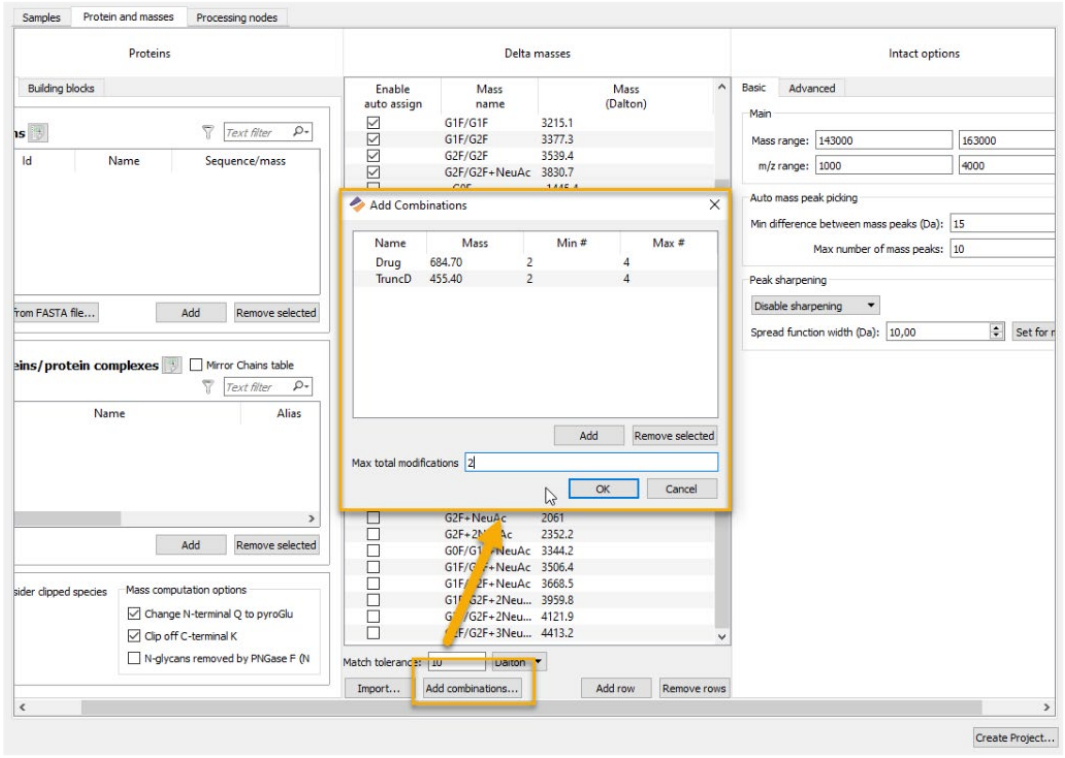
The Oligonucleotides setting alters the Sequences sub-tab in the following ways:

- 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

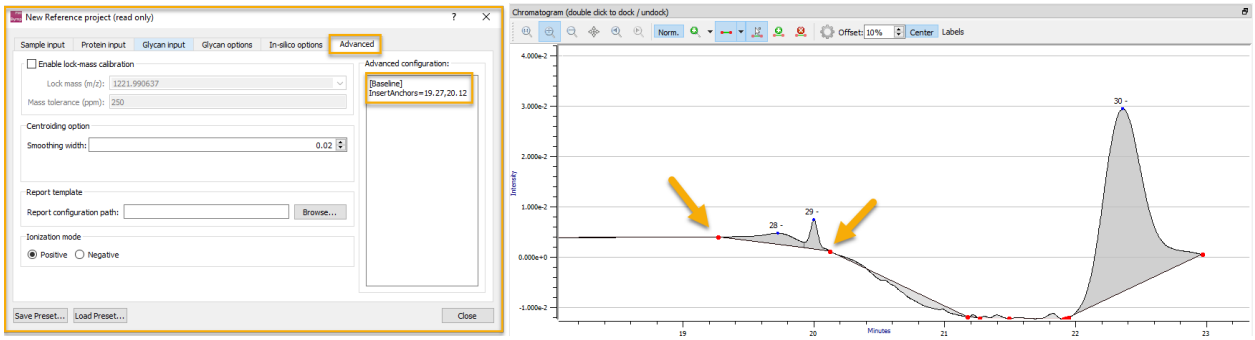
- **New advanced commands to inject manual baseline points**.

  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.

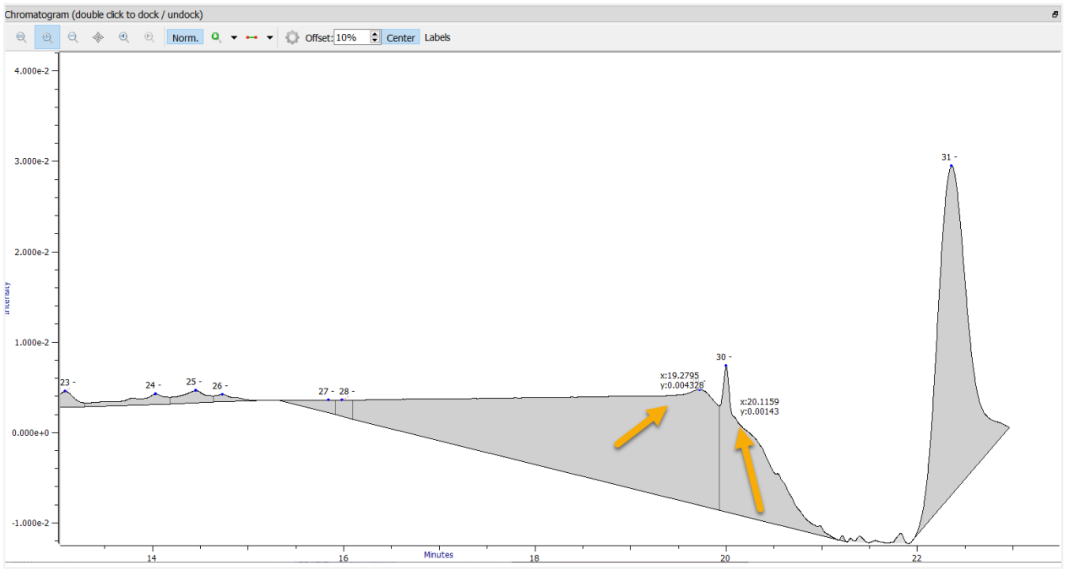
  Please note this will require the user to have knowledge of where to place the baseline points.

```
[Baseline]

InsertAnchors=19.27,20.12
```



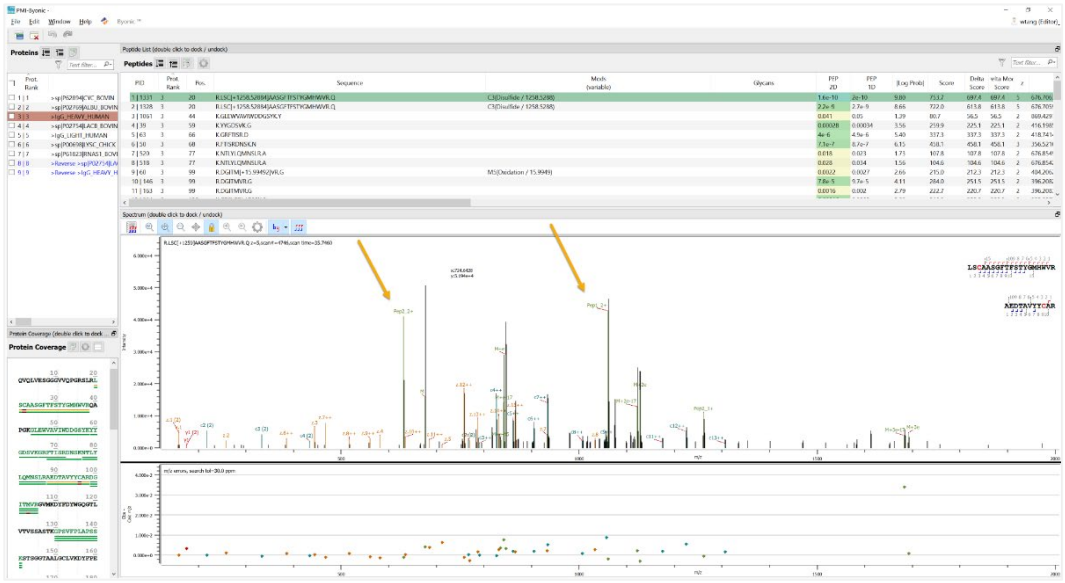
The values 19.27 and 20.12 were observed from the Chromatogram (the below image is before the advanced command was applied).



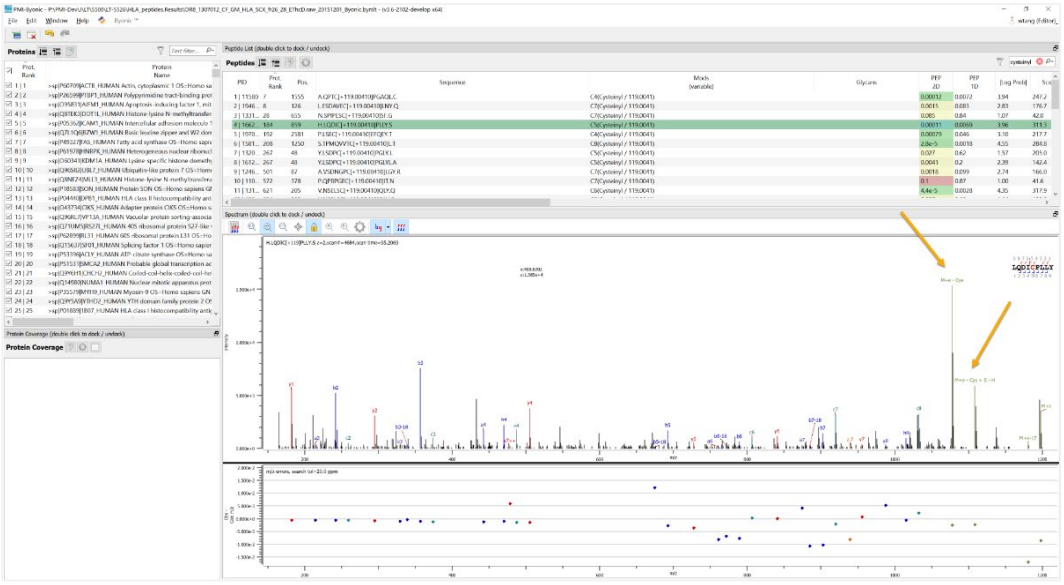
Byonic Viewer

- **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:



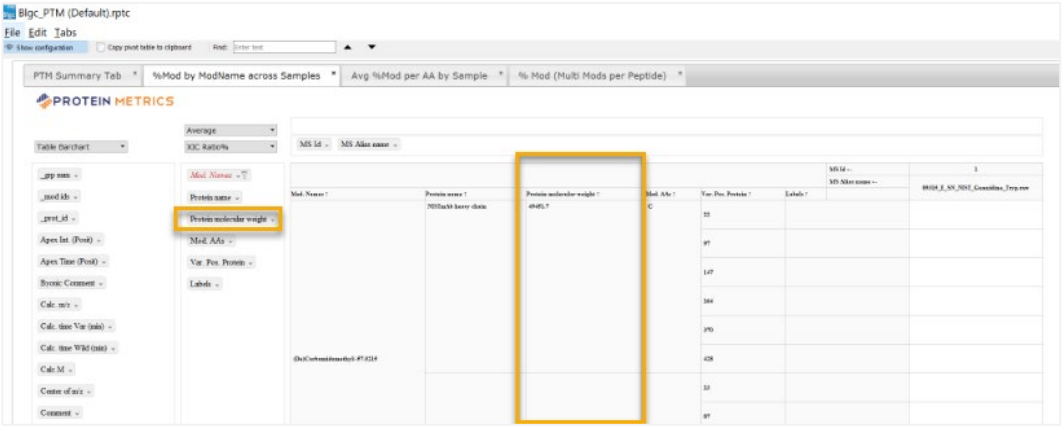
Neutral mass losses for cysteinylation (-119, -88, etc.), as shown below.



Reporting

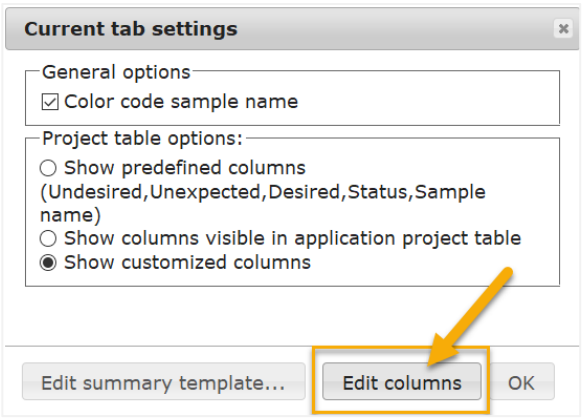
- **Protein Molecular Weight Column**

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

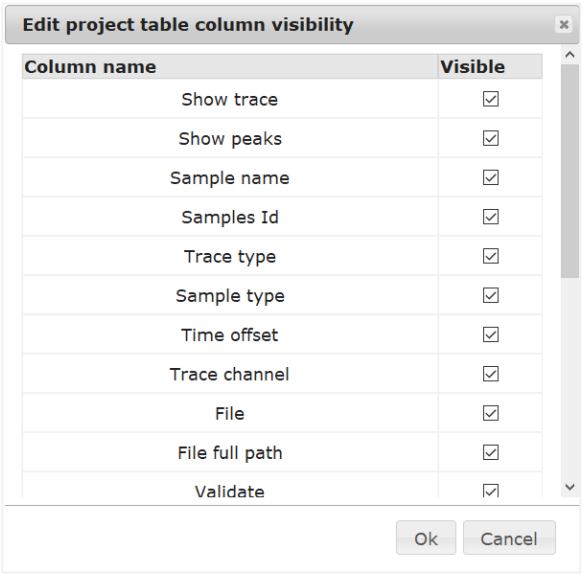


- **Define Order of Custom Columns – Project Table in Summary tab**

  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**.

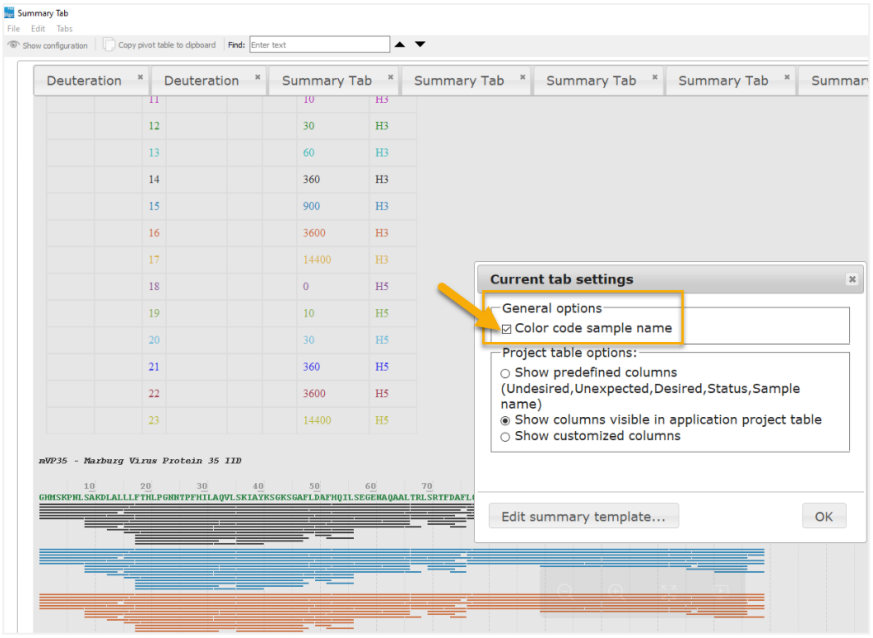


The user can then drag and drop the columns as desired.

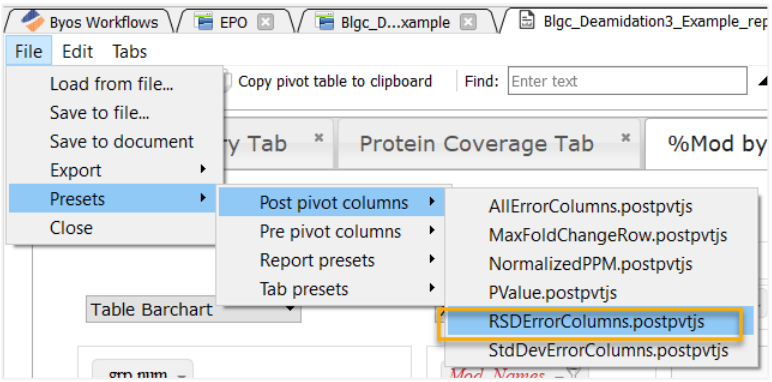


- **Color coding Samples in Summary tab**

  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.



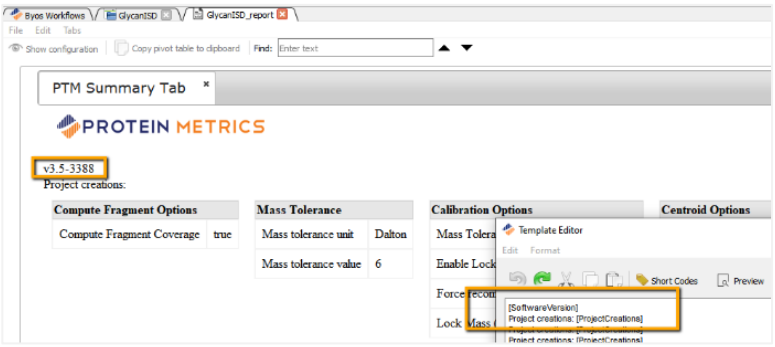
- **Post-pivot column preset**



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

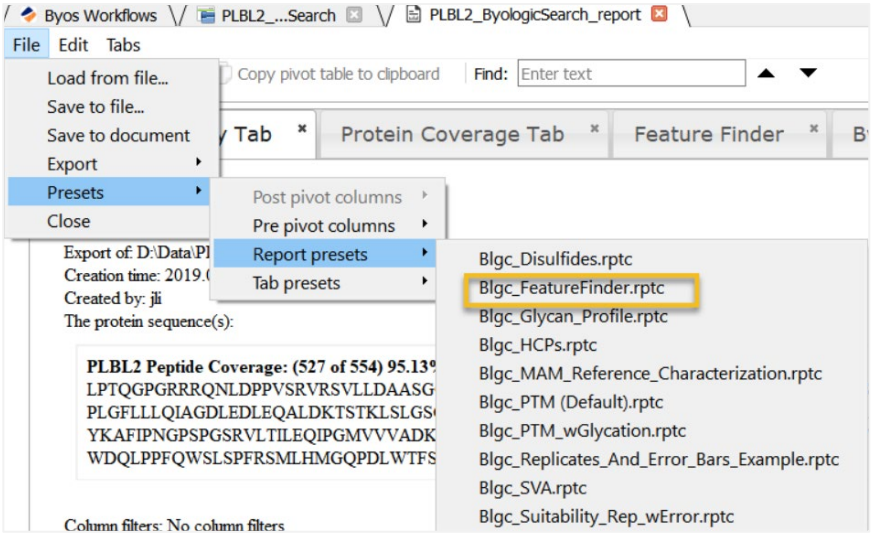
- **Software Version**

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.



Release 2019-09 (v3.6)

Usability - All Modules

- **Zoom Short-cuts**

  Double-left click: complete zoom out (including Chromatogram, MS1, Deconvolved Mass spectrum, MS2, XIC, and Isotope plots).

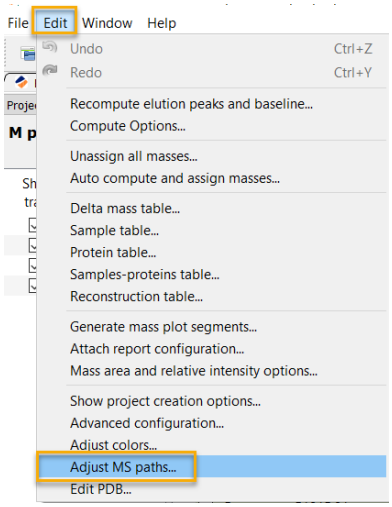
  Shift-Left key: undo zoom

  Shift-Right key: redo zoom

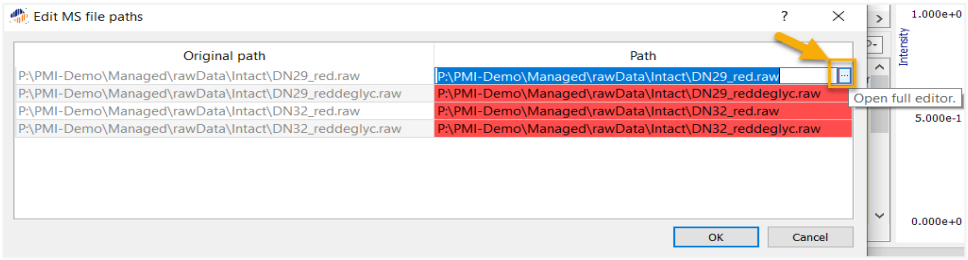
  Note: Alt-left/right key shortcut is reserved by Isotope plot to select next/previous scan time.

- **Update File Paths**

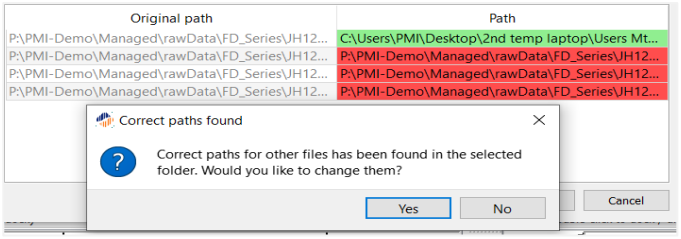
  The user can now update file path locations through *Edit -> Adjust MS paths…* This is useful when the raw files are moved in the file system, or the project file is moved to a different computer.



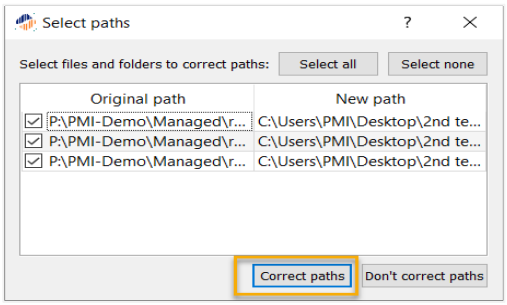
A table will be shown and the user can update the file paths using the "…" box, as shown below:



If there are several files, and they are saved within the same location, the rest will be populated after the first has been updated if **Yes** is clicked, as shown below.

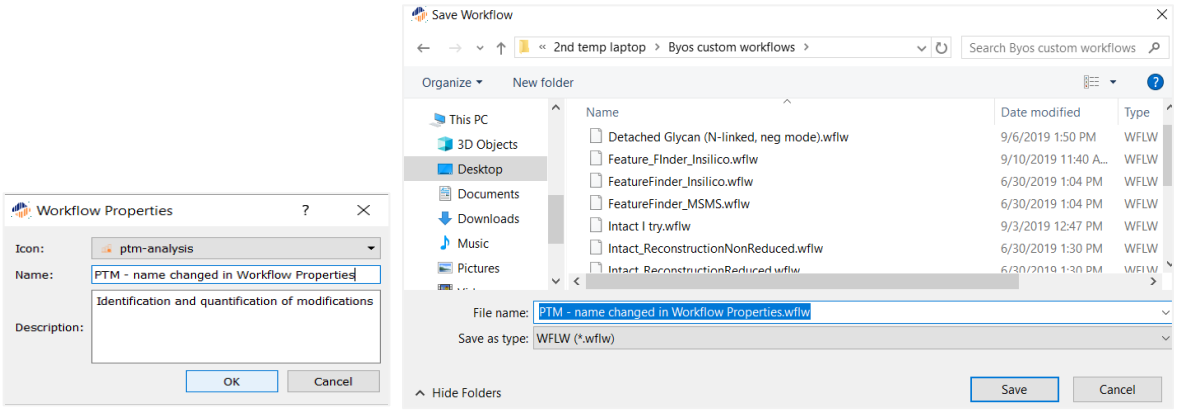


And then clicking "Correct paths", as shown below:



- **Byos – File Saving and Naming**

When saving a workflow, the file name entered by the user is now used to also set the workflow name. This removes the requirement to name the workflow as a separate step through Workflow Properties. In addition, if the user first changes the workflow name through Workflow Properties, that name is now automatically entered into the filename text input. The user will just have to set where the workflow should be saved.



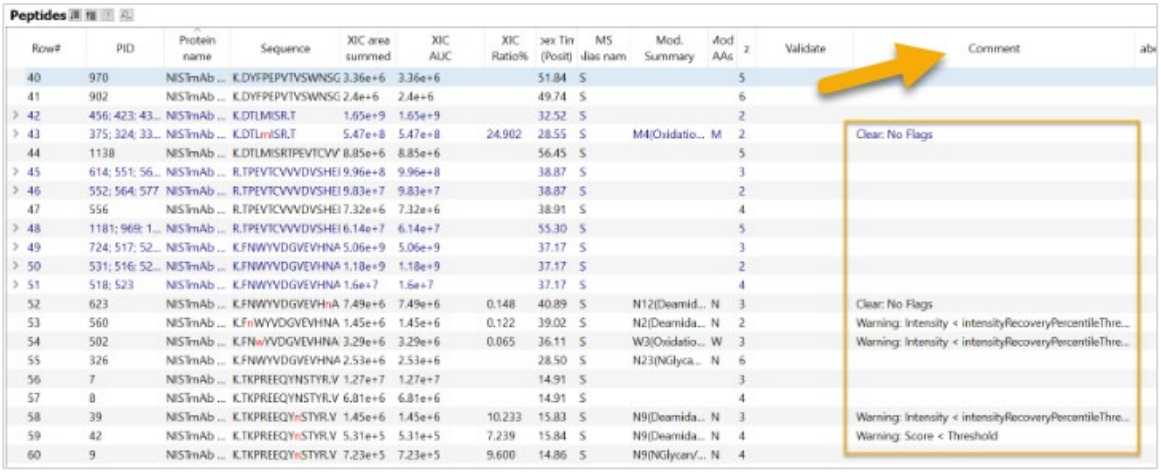
Byonic and Byonic Viewer

- **Glycopeptide Annotation Enhancements**
  - Byonic now applies score penalties for matches of EThcD and HCD spectra to glycopeptides containing NeuAc without a peak at 274, and similarly for matches to glycopeptides containing NeuGc without a peak at 290.
  - Byonic now includes dGLcA for deoxy-glucuronic acid to support proteoglycan linker peptides (O-glycopeptides on proteoglycan).
  - Byonic now supports the following: Pep + 2HexNAc, Pep + 2HexNAc+Hex, Pep + 2HexNAc+2Hex for O-glycopeptides.
  - Byonic assigns a mass of 819 for Hex-HexNAc-Hex-NeuAc.

Byologic

- **Peak Evaluation Logic – Mod Validator and SVA Validator**

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



- **Mod Validator**

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

  The feature can be activated in the advanced commands in Byologic / Byos as follows:

```
[Byologic]

ModValEnabled=true;
```

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

```
[ModValidatorOptions]

DeltaModScoreThreshold=10.0;
ScoreThreshold =225.0;
```

Mod Validator Option Parameter	Description
DeltaModScoreThreshold	The acceptable threshold of the delta mod score. If a degradation score falls below the set threshold, a warning is added to the Comments field to alert the user.
ScoreThreshold	Same as deltaModScoreThreshold except it will alert if a Score is below the threshold.
FwhmThreshold	The FWHM is used as the minimum retention time difference that categorizes a modification as not co-eluting with its wildtype. A setting of -1 determines the average fwhm of the input files and then automatically calculates the threshold. To set this manually, use any real number greater than 0. For example, if this is set to 0.05, this means that if a degradation has a retention time difference from its wildtype of more than 0.05 minutes, the user will be alerted in the Comments field.

IntensityRecoveryPercentileThreshold	This will alert the user if the apex intensity of the degradation lies within the bottom x percentile. For example, if this is set to 0.05, then whenever the intensity of a peak is in the bottom 5%, a warning will be added to the Comments field.

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

- **SVA Validator**

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

  The feature can be activated in the advanced commands in Byologic / Byos as follows:

```
[Byologic]

SvaEnabled = true;
```

```
[SvaOptions]

DeltaModScoreThreshold = 10.0;
PercentXICRatioThreshold = 1.0;
ScoreThreshold = 225.0;
FwhmThreshold = -1;
PredictedTimeDiffThreshold = 5.0;
```

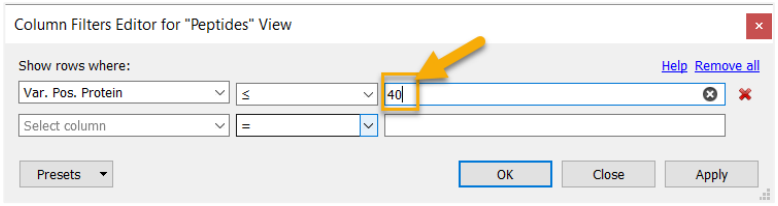
SVA Validator Option Parameter	**Description**
DeltaModScoreThreshold	See above
PercentXICRatioThreshold	The acceptable threshold of the XIC Percentage Ratio. If the ratio falls below the set threshold, a warning is added to the Comments field. Depending on how the *.rptc file is configured, the XIC Ratio % value may not match the quantification in the reports since the reports allow multiple kinds of quantifications (one charge state, multiple charge states, etc.).
ScoreThreshold	See above
FwhmThreshold	The FWHM is used as the minimum retention time difference that categorizes a modification as not co-eluting with its wildtype. A setting of -1 determines the average fwhm * 2 of the input files and then automatically calculates the threshold. This is the recommended setting for this parameter (feedback is welcomed). However, this can be manually set to any positive value. This is not recommended.
PredictedTimeDiffThreshold	This threshold alerts the user if the predicted retention

	time in minutes for the sequence variant exceeds the set threshold. A “Critical” label is displayed in the Comments field.
CommonModLookBothWaysFromModResid	uTehe count of residues on either side of the putative site of a sequence variance to search across for a more common modification that better explains the results. For example, consider the wildtype peptide AAVLAMK, when this parameter is set to 3. Then, a putative sequence variant of AAdLAMK (V->D) is proposed with a delta modification of ~16 Daltons. The program searches the 3 amino acid residues to the left and 3 to the right of this site for a more common modification (such as methionine oxidation) with the +16 Da shift. In this case, there is a methionine residue 3 residues to the right of the putative sequence variant, so a “Warning” label is displayed in the Comments field.

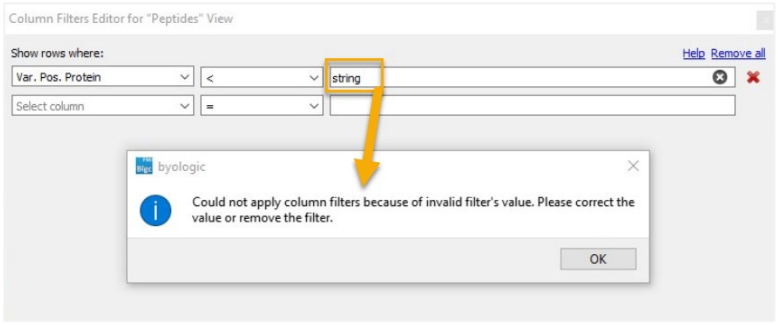
Now the Comments Field in the Peptide window will be filled out for various rows. Please note that this field sorts with no comments first, so the user may have to scroll to the end of the peptide window to find the labels.

- **Filtering – “Var. Pos. Protein” and “Var. Pos. Peptide”**

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



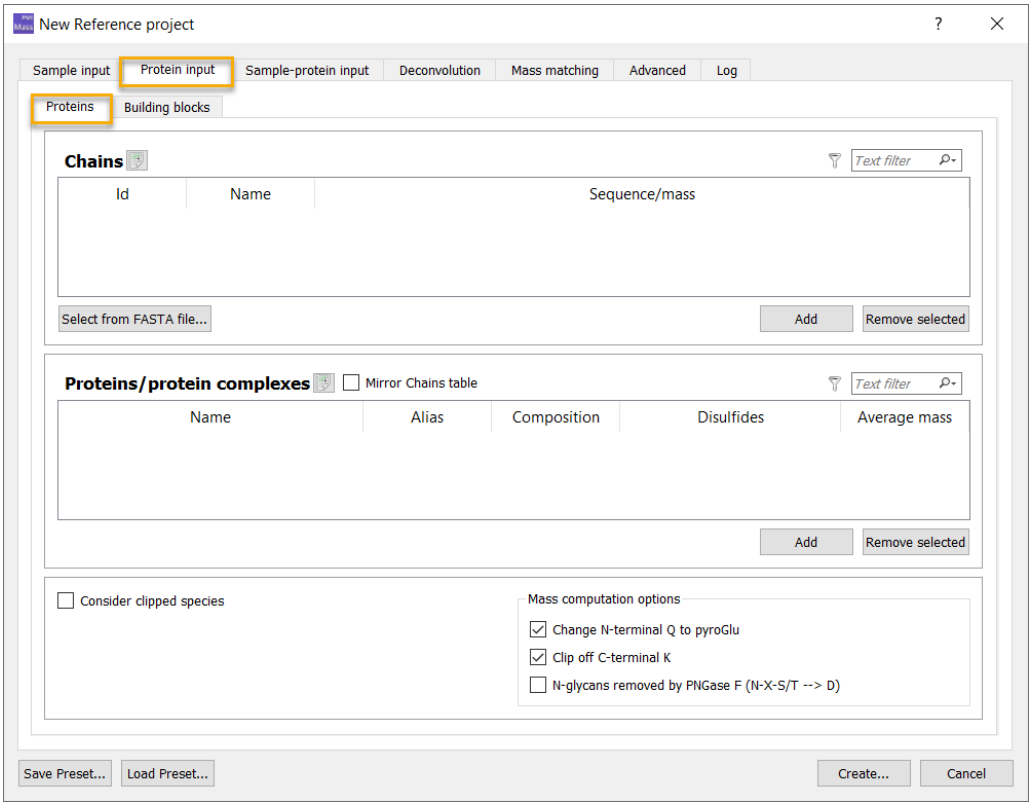
If a non-integer value is entered, the user will receive an error message to correct the value entered, as shown below.



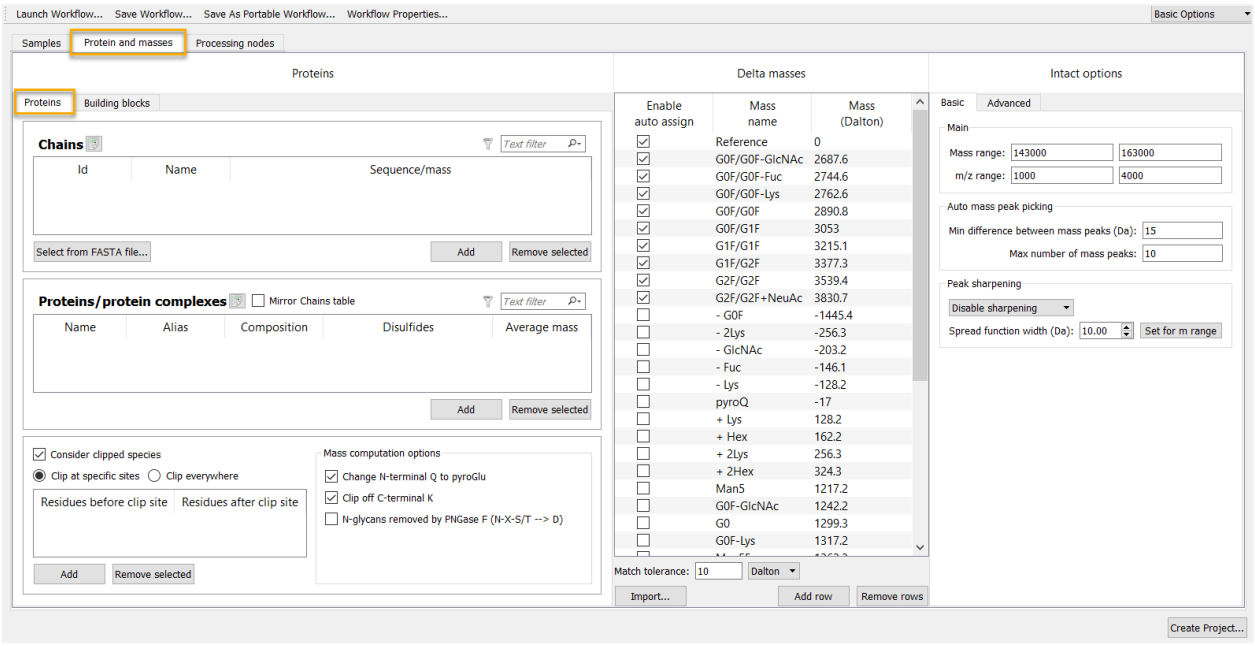
Intact

- **New UI for Protein Input UI**

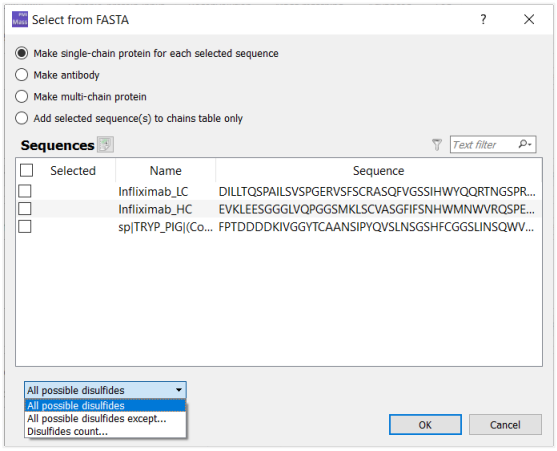
  The Intact Protein Input tab:



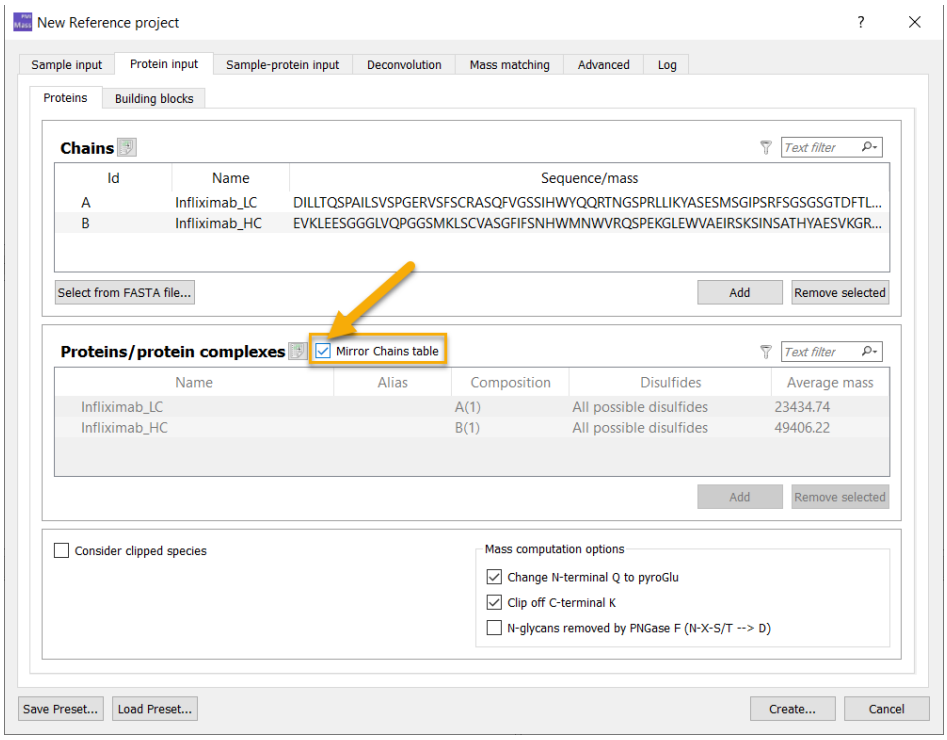
  The Byos Protein and masses tab:



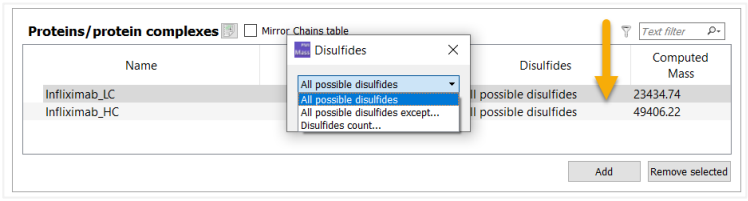
- The user can now add chains to the Chains table and based on these chains add rows to the Proteins table.
- The Chains table can be filled in manually or using the FASTA file. When using the FASTA file, a dialog appears that allows the user to add data to the Proteins table:



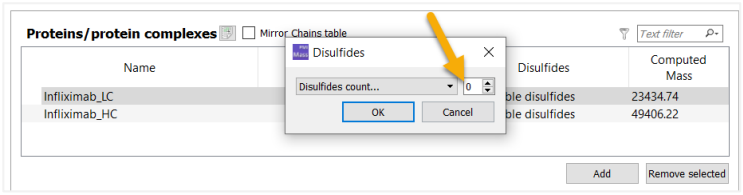
- The Mirror Mode allows the user to populate the Proteins table based on the Chains table automatically. For each row in the “Chains” table, a corresponding row is added to the “Proteins/protein complexes” table (with single-chain composition and all possible disulfides):



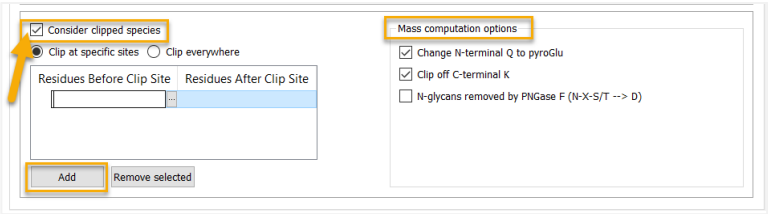
- The user can also set the number of disulfides within the Proteins/protein complexes table. The “Disulfides” window is activated by double clicking within the box within the table, as shown below:



To set a specific number, select “Disulfides count…” and enter a value, as shown below:

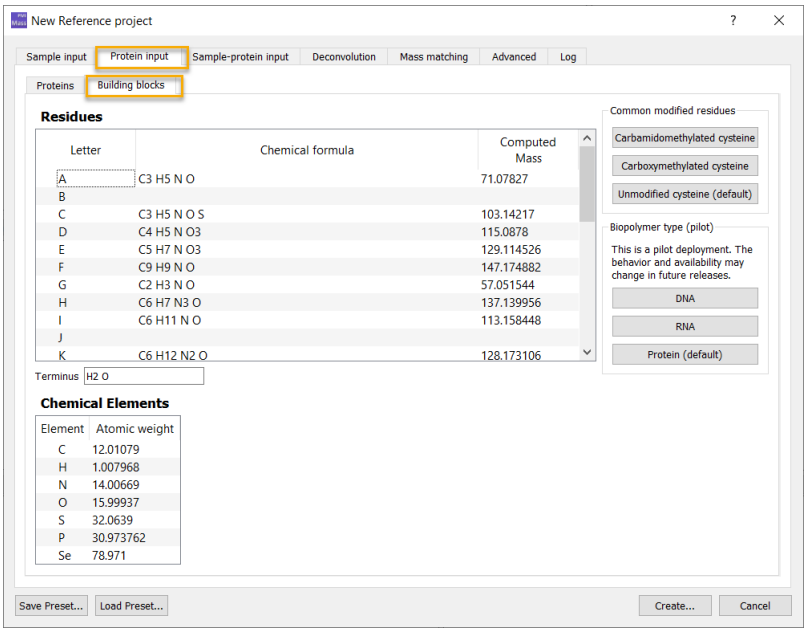


- Several items have been added to the Protein tab UI, removing the need to set advanced parameters:
  - Clipping - Select the box to activate – there are the options: 1. “Clip at specific sites” or 2. “Clip everywhere”
  - Click “Add” to enter specific residues, if that radio button is selected
  - Mass computation options - Select the box(es) to activate options

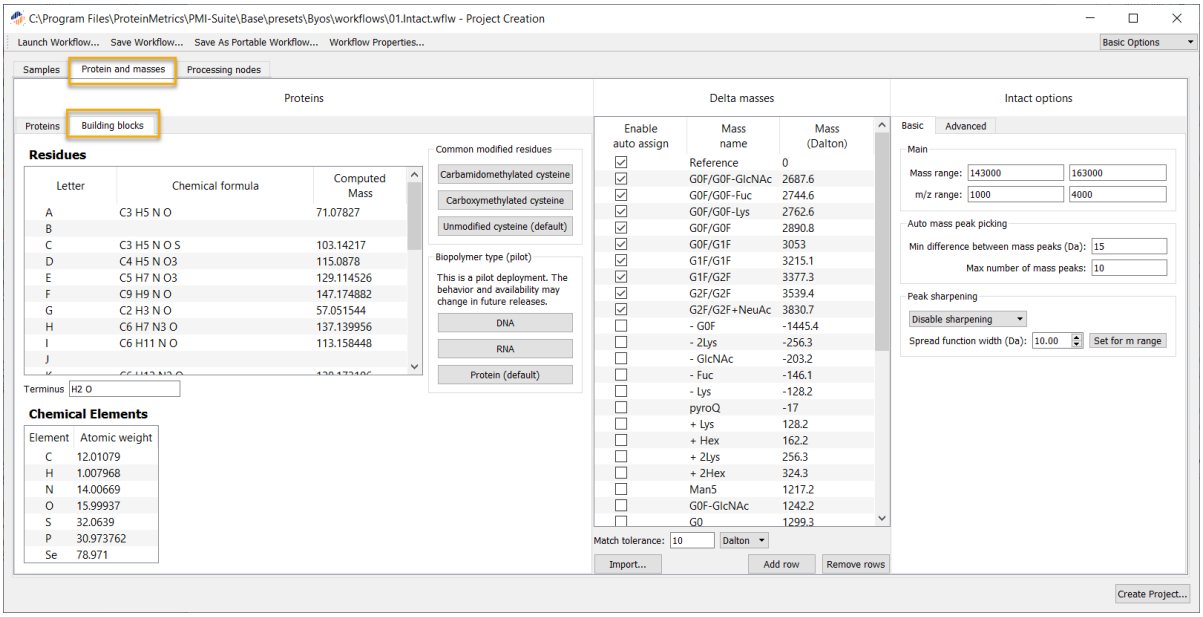


- Building blocks tab

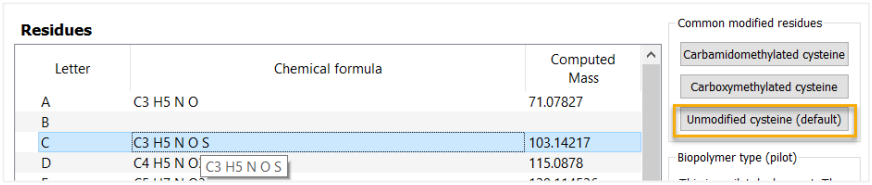
  The Intact Protein Input:

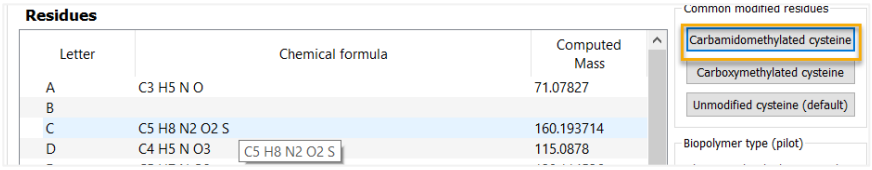


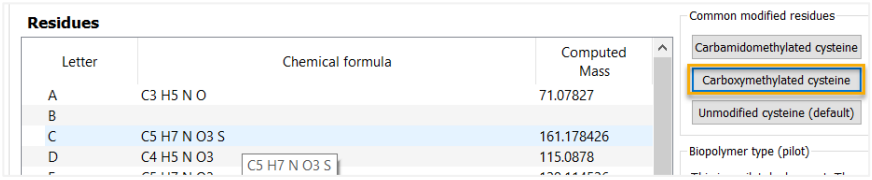
The Byos Protein and masses tab:



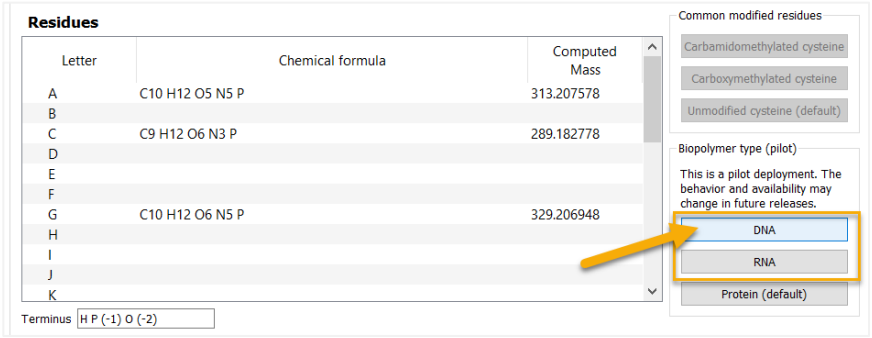
- The user can now set commonly modified residues that will affect the content of the Residues table:



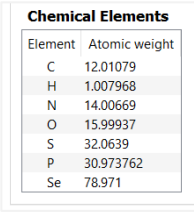




- The user can also select the Biopolymer type (default Protein, DNA, and RNA; please note this is a pilot deployment). The content of the table will be updated accordingly:



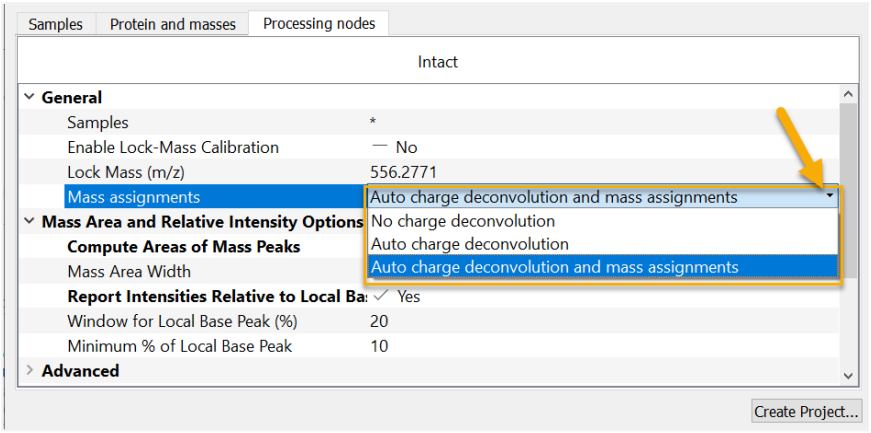
- The user can also specify the atomic weights:



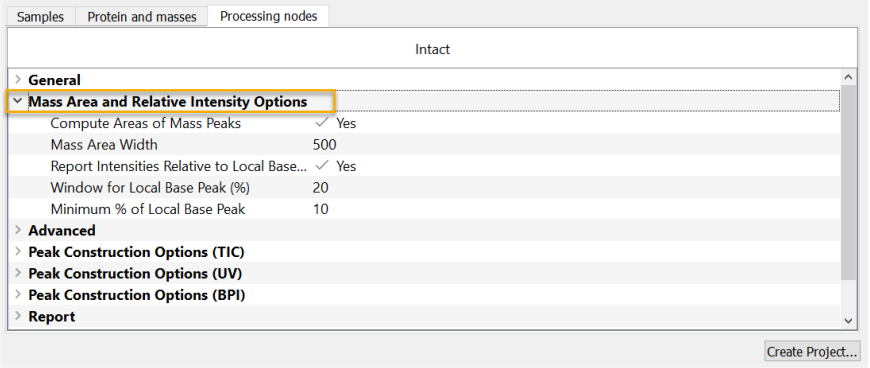
- **Mass Matching Parameters**

  Mass matching parameters are added to Intact-based workflows in Byos (Intact, Reduced). These were already available in the ADC workflow in Byos.

  - General → Mass assignments
    3 Mass assignments options are available in the Processing nodes tab through a drop-down menu, as shown in the image below



- Mass Area and Relative Intensity Options



  - Yes/No entry for Compute Areas of Mass Peaks
  - A numerical value for Mass Area Width, which defaults to 500 Da
  - Yes/No entry for Report Intensities Relative to Local Base Peak
  - 2 lines below with box to add numerical values. The default values are 20% and 10

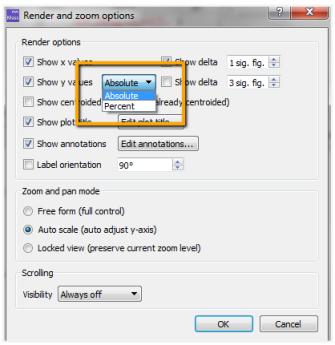
- **Stacked Plots – Reference, Comparison, and Multi-sample Projects**

  The user can now stack plots within the Chromatogram, Deconvolved Mass Spectrum, and MS1 plots in any Intact project (Reference, Comparison, and Multi-sample). To select more than one plot to stack, press Ctrl and select all the desired elution peaks in the Elution Peaks table. Similar to the stacked plots for the chromatogram, the user will then see stacked plots for the Deconvolved Mass Spectrum and MS1 plots.

  For each selected elution peak, the color of the deconvolved mass spectrum and MS1 plots match the sample colors from the Project table Labels column will appear only on the top layer in the Deconvolved Mass Spectrum.

- **Removal of "Fraction" from Y-axis Rendering Options**

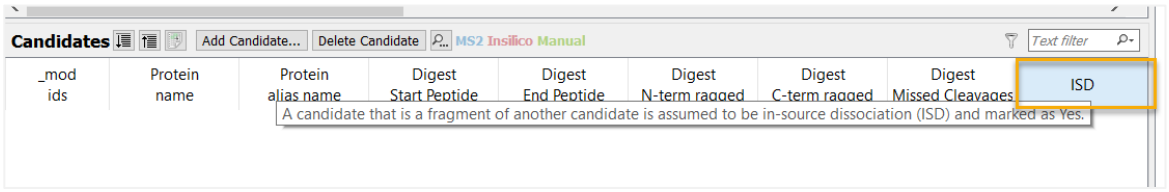
  The ability to set "Fraction" notation for y-axis labels has been removed. It was considered too similar to the percentage notation. The options are now Absolute and Percent.



Byomap

- **Support for In-source Dissociation (ISD)**

  A new ISD column was added to the “Candidates” table – the entries will be True, False, or none. If the candidate is a fragment of another candidate, it will be marked as “yes”.



  - True - the glycan is a subcomposition of another candidate glycan for the same peak
  - False - the glycan is either the same composition or a supercomposition of the other candidate glycans for the same peak.
  - (Blank) - the candidate does not represent a glycan.

  The below advanced commands are required for the auto assign options in Byomap:

```
[AutoAssign]

RemoveDuplicates = 0
```

  - If enabled (set to 1), candidates will only be assigned to the most abundant elution peak during auto-assignment.
  - If disabled (set to 0), candidates may be assigned to more than one elution peak.

```
[AutoAssign]

RemoveISD = 1
```

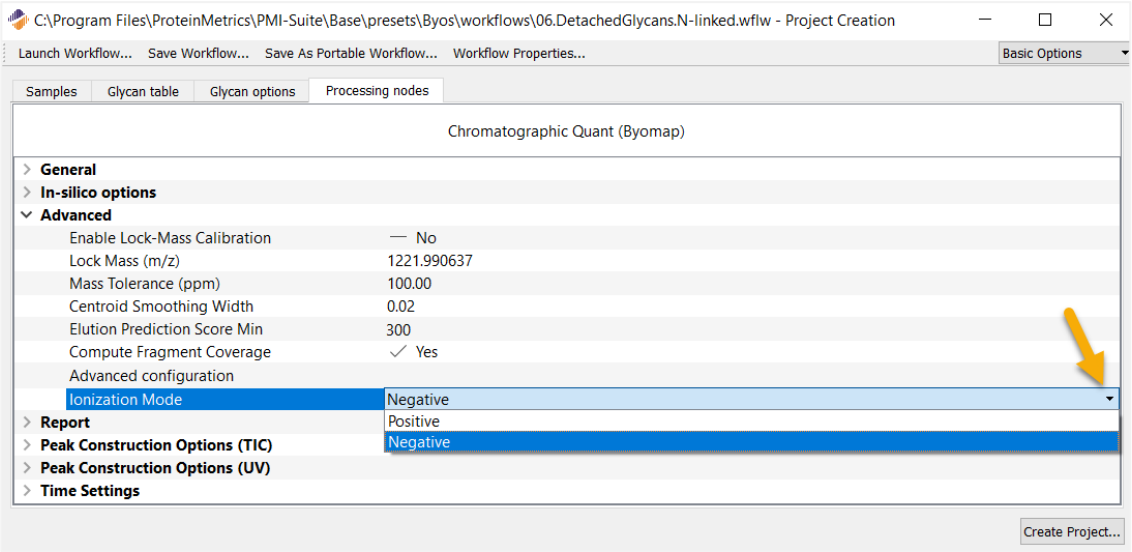
  - If enabled (set to 1), in-source dissociation candidates will be excluded from auto-assignment.
  - If disabled (set to 0), in-source dissociation candidates will be treated no differently from other candidates during auto-assignment.

    Please note:

- Candidates with ISD set to True will not be auto-assigned during Edit → Auto assign candidates….
- Auto-assignment will only refrain from assigning ISD candidates; it will not unassign them. If auto-assignment has already been run for the project in a build without this feature, the user should first run Edit → Remove all candidate assignments… to unassign possible ISD candidates.

- **Clarification – Negative Mode Processing for Detached Glycan Workflows**

  "Neg mode" has been added to the title of the three detached glycan workflows to clarify that the "Negative" ionization mode has been selected by default to complete processing in negative mode. If the user wishes to process instead in the positive ionization mode, this change can be made using the Advanced → Ionization Mode drop-down menu, as shown below:

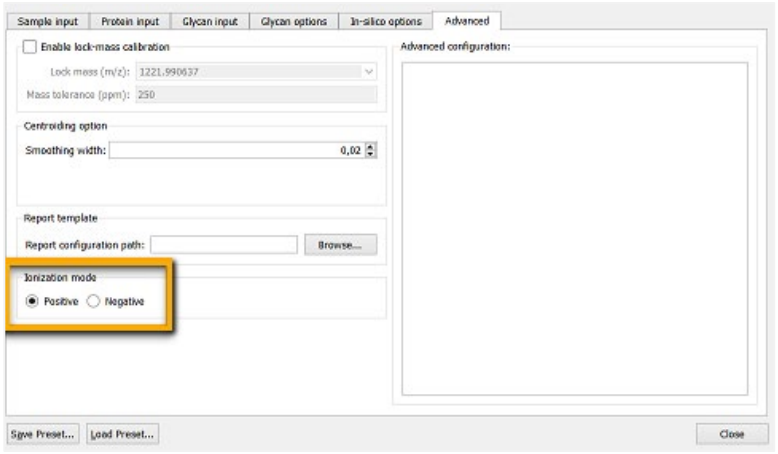


Intact/Byomap

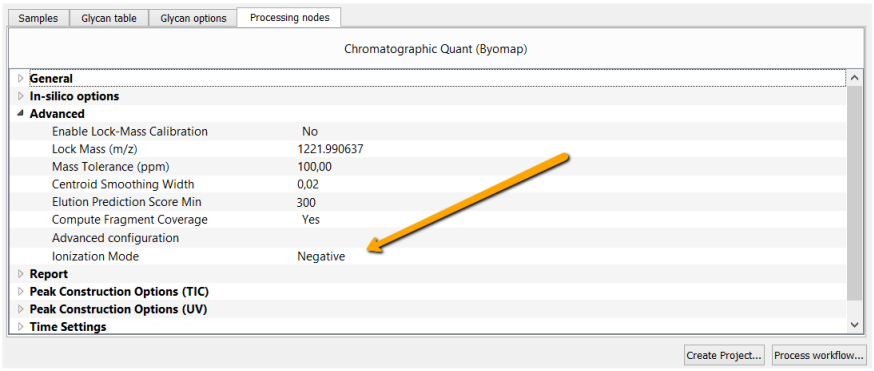
- **Ion mode (Positive and Negative) Moved into User Interface**

  The user can now select between positive or negative ion mode within the user interface. This replaces the need to enter an advanced command to have data processed in negative mode.

  In Intact and Byomap, this selection is made through a radio button in the Advanced tab, as shown below:



In Byos, this selection is made by toggling between Positive and Negative in the Processing nodes tab, as shown below:



Reporting

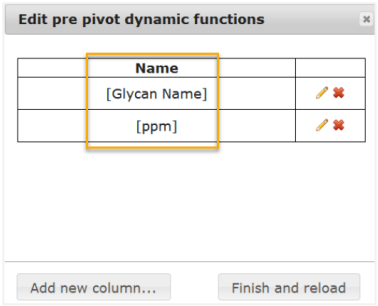
- **Update to Default PTM report template – IsoD**

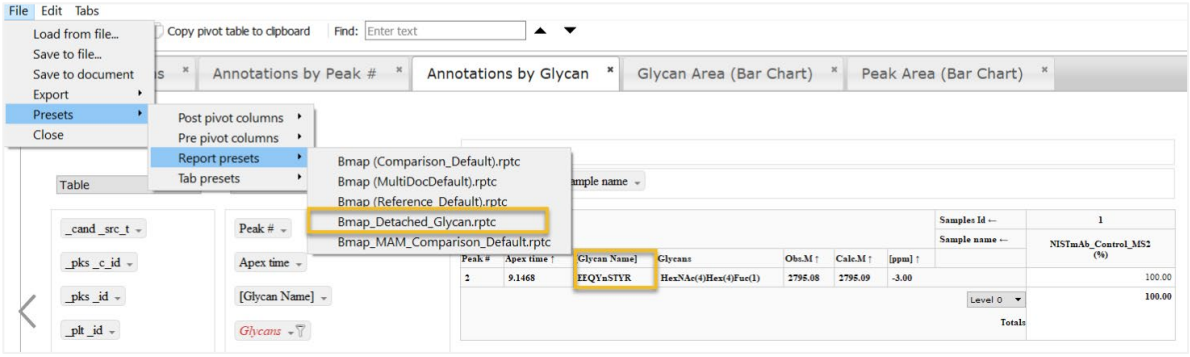
  The last tab ("% Mod (Multi Mods per Peptide)") of the default PTM workflow report template in Byos is modified to include "isoD/0.0000" in the Mod. Names column, if a peptide has "isoD" or "isoAsp" as a Label.

						MS Id ←	1	2	3	4
						MS Alias name ←	Day 0 (%)	Day 1 (%)	Day 3 (%)	Day 7 (%)
Sequence (unformatted) ↑	Mod. Names ↑	Protein name ↑	Mod. AAs ↑	Var. Pos. Protein ↑	Labels ↑					
DTLMISR	Oxidation/15.9949	hIGHG1	M	135			5.65	7.06	6.99	10.9
DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTK	Oxidation/15.9949	hIGHG1	W	41			1.69	1.68	1.6	1.08
FNWYVDGVEVHNAK	Ammonia-loss/-17.0265	hIGHG1	N	169			0.216	0.232	0.252	0.218
	Deamidated/0.9840	hIGHG1	N	169				0.131	0.681	1.78
	isoD/0.0000	hIGHG1	D		isoD		0.105	0.185	0.346	0.586
GFYPSDIAVEWESNGQPENNYK	Deamidated/0.9840	hIGHG1	N	267	N->D		0.422	1.28	3.09	4.39
					N->isoD,isoD		2.13	6.42	12.4	19.4
				272			1.32	8.11	17.5	29.5
SGTASVVCLLNNFYPR	Deamidated/0.9840	hIGKC	N	29			0.328	0.439	0.413	0.492
				30			0.0313	0.0426	0.0458	0.0554

- **Update to Default Detached Glycan report templates – Glycan Name**

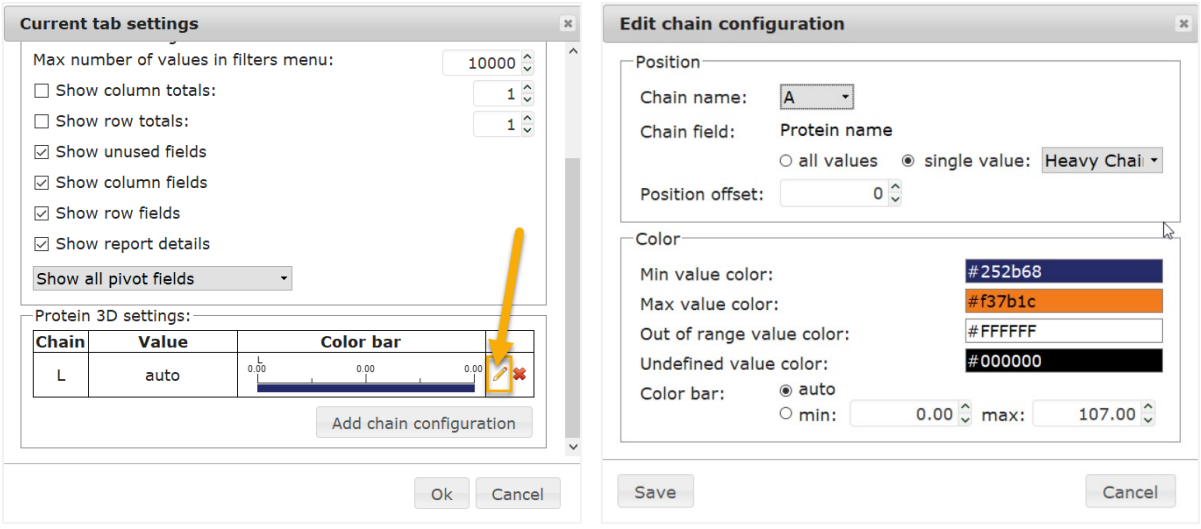
A new column has been added to the default detached glycan workflows report template in Byos. This is “Glycan Name”, which creates a column of glycan names without the tag:



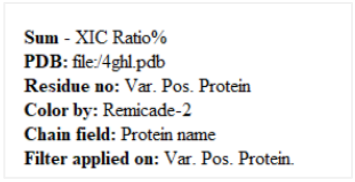


- **Protein 3D – UI Improvements**

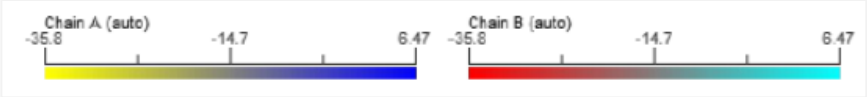
  The “Edit chain configuration” dialog has been simplified for enhanced user experience. This is accessed through Edit → Current tab settings and then clicking on the Protein 3D settings “pencil” icon to access “Edit chain configuration”, as shown below:



  - When the configuration is hidden, several items are added to the footer: Calculation, PDB, Residue no., Color by, Chain field, and Filter applied on

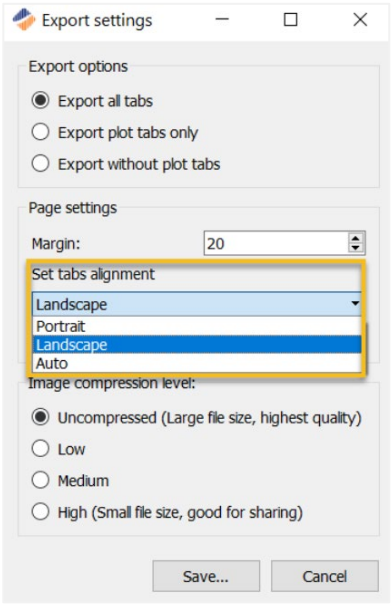


- The footer information included in an exported image no longer includes position or color by details.
- Color range values are now included in the color bar.



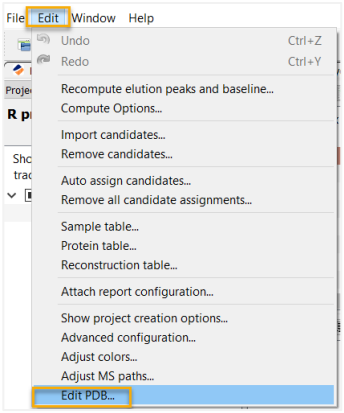
- **Orientation Mode for content exported to PDF**

  The user can now select the orientation mode during export to PDF for all tabs within the report module.



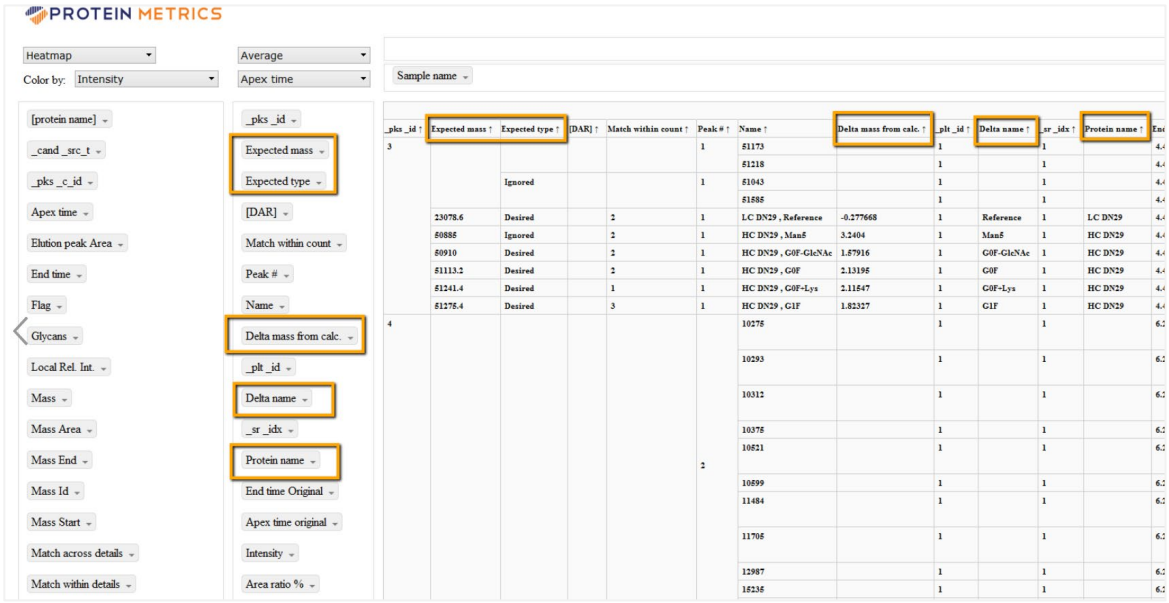
- **PDB Files**

  The user can now add their own PDB files in 3-D reporting. This capability is accessed through Edit -> Edit PDB… , as shown below:



- **Additional Pivot Fields**

  Additional items from the Intact Project table have been made available as pivot fields in the reports. These include Expected mass, Expected type, Protein name, Delta name, and Delta mass from calc.



Updates to Beta offering from 2019.09 Release Notes

Feature Finder – Beta

Update: additional advanced command to Remove Features that have only +1 charge state.

This will not find features that only have a +1 charge state. If feature X across all samples only has +1 charge, it will be removed because it is assumed to be undesirable. However, if feature X is found in multiple samples, but in one of them it has charge states of +1 and +2, it will not be removed. This exclusion is included within the advanced commands in the updated "FeatureFinder_MSMS.wflw" available as detailed below:

```
[FeatureFinderOptions]
```

```
removeChargeOneOnlyFeatures = true
```

From the 2019.06 Release Notes document:

> 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.

Please download the Beta Feature Finder Demo package available in the Current Release section at http://www.proteinmetrics.com/biopharma-download/.

Intact Reconstruction – Beta

Updates:

- Column header names – Protein Name, Protein Position, Modification Name, Modification Percent.
- Assured backward comparability with former names.
- Added descriptive tool tips.
- Added ability to mix the order of csv columns.
- Compliance and Audit trail – users without adequate permissions will not be able to access this function; intact reconstruction has been added to the Audit Trail.

From the 2019.06 Release Notes document:

> One challenge in analytical chemistry is the ability to compare orthogonal methods in a systematic fashion. Here we present a way to reconstruct Intact mass spectra of proteins from peptide mapping data. Protein degradations at the amino acid level quantified by peptide mapping methods are convolved against each other to reconstruct a theoretical intact mass spectrum. Comparing the theoretically reconstructed intact mass spectrum against the experimental spectrum provides a way to compare peptide mapping data against intact mass spectrometric analysis. The differences between the two spectra can then be used to compare how well each method correlates with the other. This can then be used to adjust acquisition or data analysis settings accordingly.

Please download the Beta Intact Reconstruction Demo package available in the Current Release section at http://www.proteinmetrics.com/biopharma-download/.