



De novo Sequencing Analysis (SupernovoTM)

User's Manual

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Introduction

Supernovo is an application that makes end-to-end de novo sequence identification of monoclonal antibodies (mAbs) easier (i.e., drag and drop the raw files and start processing), generating a detailed project and report file.

Note: It is highly recommended to use Supernovo on a good 64-bit multi-core computer with a solid state drive. A solid state drive improves performance greatly. All Supernovo results (.blgc files) should be saved to the solid state drive.

Tour of De novo Sequencing Analysis Dashboard Views

The De novo Sequencing Analysis user interface (UI), or dashboard, shown in the figure below has six highly interactive plot and table views:

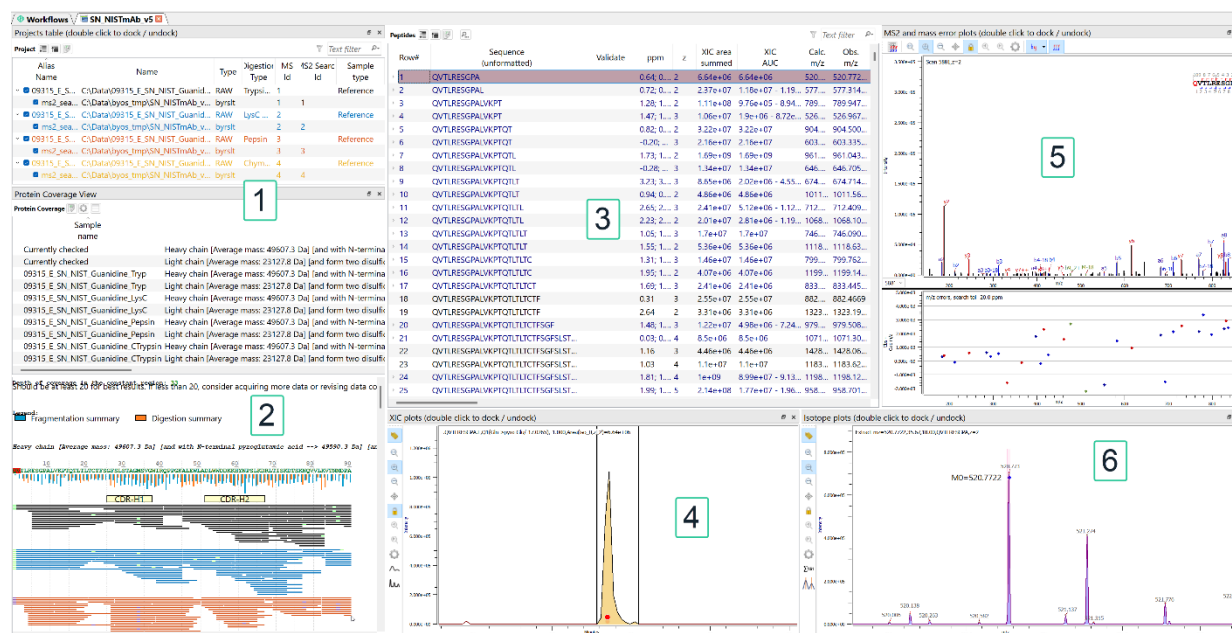


Figure 1: Six De novo Sequencing Analysis views: (1) Project table, (2) Protein coverage table, (3) Peptides table, (4) Ion chromatogram (XIC) plots, (5) MS2 and mass error plots, and (6) Isotope plots. Views can be rearranged, resized, docked, and undocked. Customized layouts can be saved and shared.

1. The **Project** view lists the data files in use. The paired check boxes for MS files and result files can be used to turn on or off the records displayed in the other views. The colors of the files correspond to colors used in the other views.
2. The **Protein Coverage** views display table data and identify sequences for peptides selected and filtered in the Peptides table in View 3. Bars below the sequences represent MS/MS scans. The bars and sequences share the colors of the corresponding MS files in the Project table in View 1. For a successful de novo sequence identification, understanding the **Protein Coverage** view is critical (see the [Summarizing De Novo Sequencing Analysis Results](#) section below for additional details).
3. The **Peptides** table lists all identified and filtered peptides. Selecting a peptide row in the table displays the corresponding wildtype peptides, XIC chromatograms, MS2 and mass error plots, and isotope plots in Views 4 through 7.

4. The **XIC plots** show the ion chromatograms for the peptide and corresponding wildtype peptides, if present, selected in the Peptides table in View 3. The plots are shaded with the colors of the source MS files in the Project table in View 1.
5. The **MS2 and mass error plots** show the MS2 spectrum of the peptide and corresponding wildtype peptides, if present, selected in the Peptides table in View 3. The peptide and wildtype peptide spectra share the same m/z scaling for easy comparison.
6. The **Isotopes plots** show the MS1 isotope spread of the peptide and corresponding wildtype peptides, if present, selected in the Peptides table in View 3.

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

Summarizing De Novo Sequencing Analysis Results

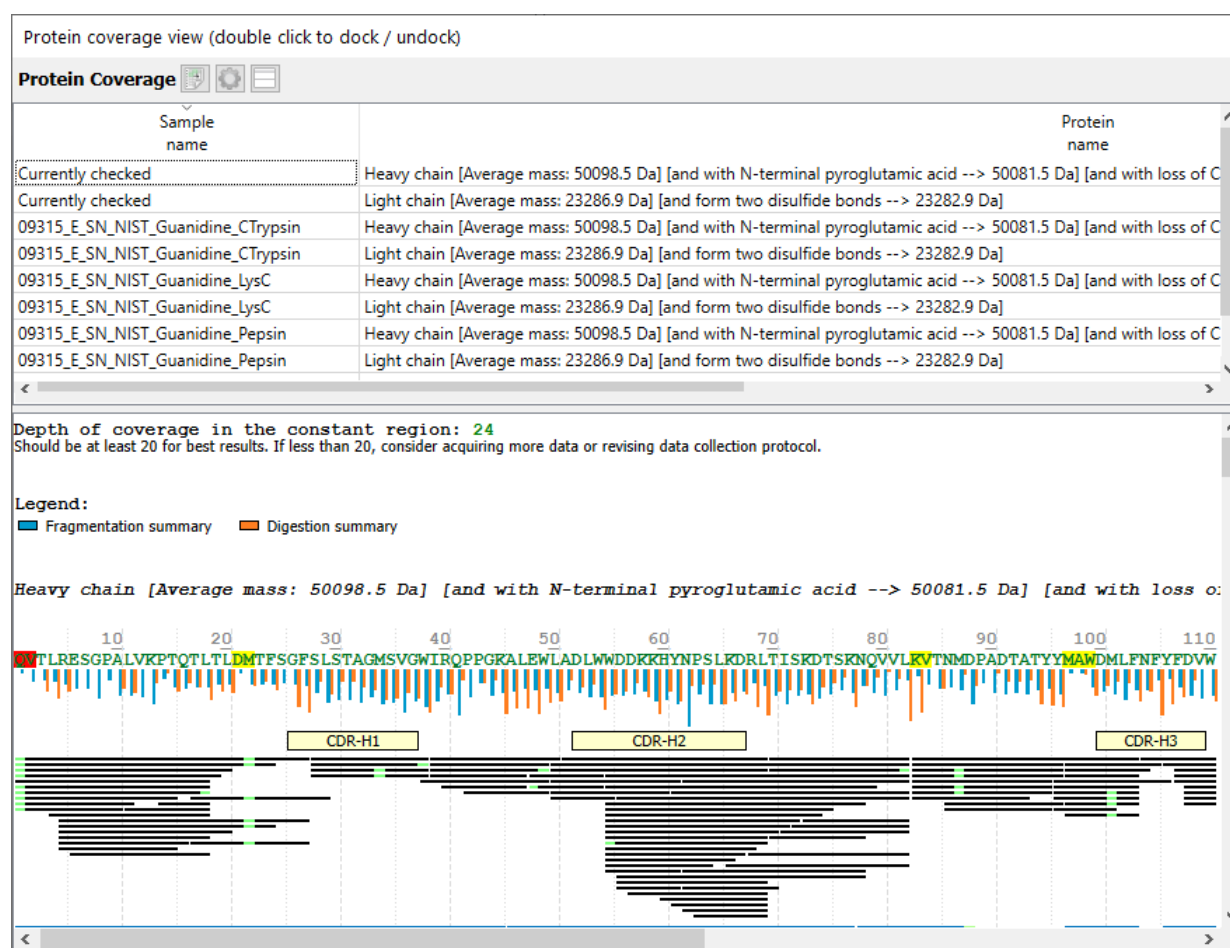


Figure 2: Protein Coverage view of Supernovo project

Protein Coverage view summarizes the de novo sequence analyses results. Right below the **Depth of coverage** info, there is de novo sequence identified and peptide coverage information. Segments of the sequence that are annotated in red and yellow colors need to be inspected. Right below this annotated sequence are the vertical bars that represent the confidence of the predicted amino acids.

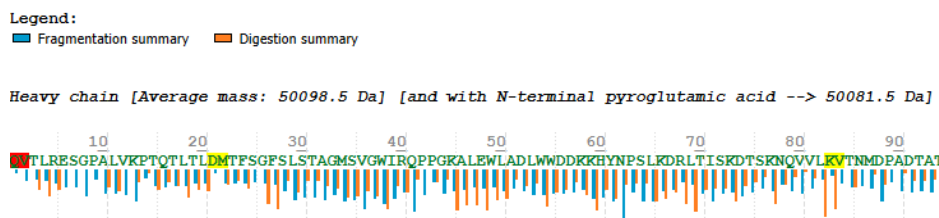


Figure 3: Confidence in amino acid identifications


Different colors of amino acids represent different error rates of de novo sequence analyses:

- **Red** – approximately 10% error rate
- **Yellow** – approximately 2% error rate

The vertical bars for each amino acid summarize the between-residue evidence accumulated over all peptides from all files

- **Blue** – accumulated fragmentation (MS^2) evidence
- **Orange** – accumulated digestion (MS^1) evidence

Project Creation

To create a new project, go to **File > New Project**, or click on the  icon directly below **File** on the toolbar. Supernovo will open the **New project** dialog:

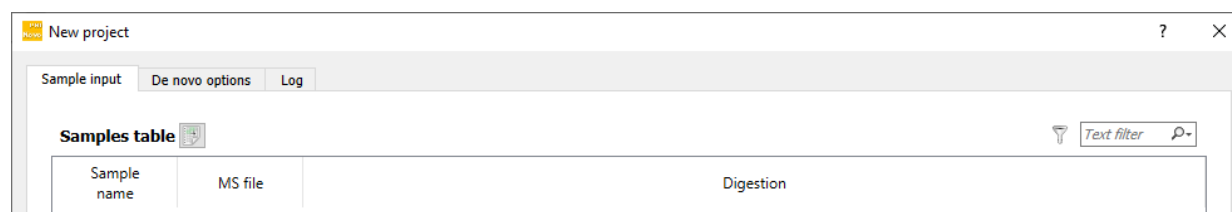


Figure 4: New project dialog headers

This multi-tab window allows the analyst to designate one or multiple mass spectrometer files to be analyzed.

Sample input Tab

The **Sample input** tab allows the user to load raw LC-MS sample files into the project. Supernovo accepts a variety of sample file types (Bruker: *.d, Agilent: *.d, Thermo: *.raw, Waters: *.raw, Sciex: *.wiff, Shimadzu *.lcd). To load a file, drag and drop one or more MS files into the project window.

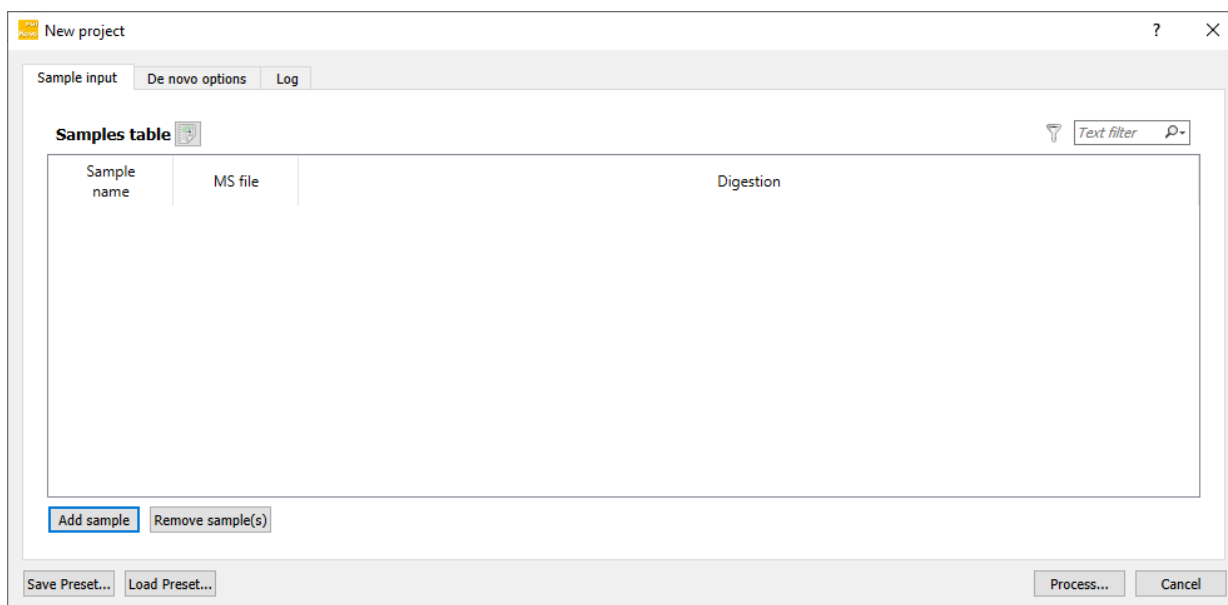


Figure 5: Sample input tab

Alternatively, click the **Add sample** button, double-click on the new row under the **MS file** header, click  and browse to the file. Click **Open**.

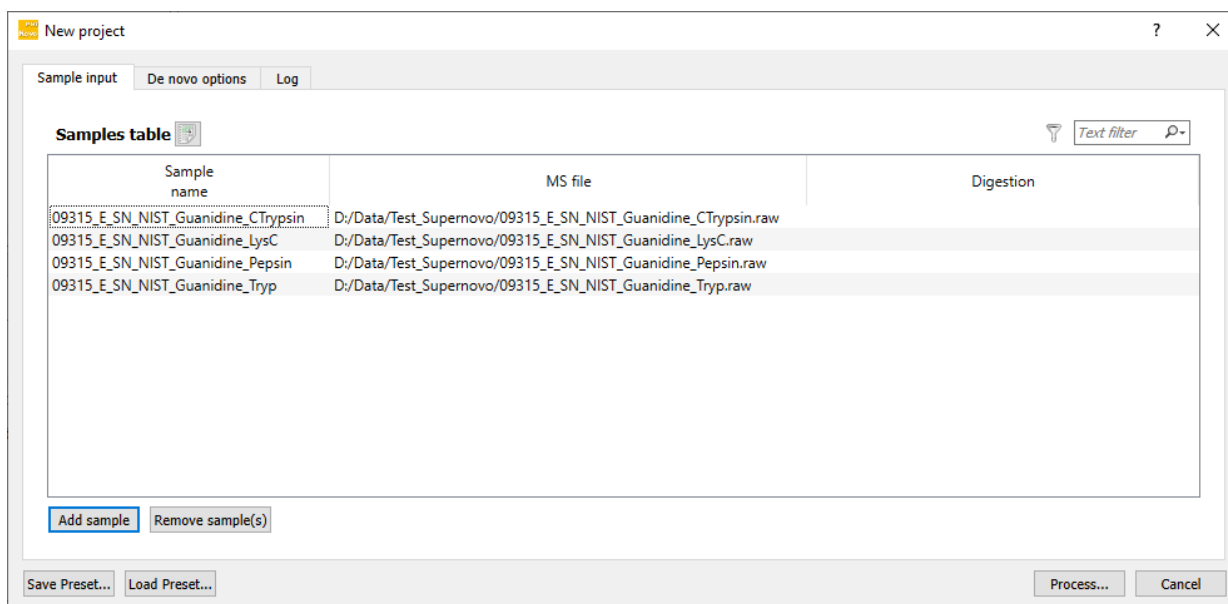



Figure 6: Loading MS files in the Samples table

The sample name defaults to the file name. To change the **Sample name**, double-click the name and edit the text or click  to edit in the text editor. The sample can be removed by selecting the row and clicking **Remove sample(s)**. **Digestion** column is ignored for Supernovo analysis but different digestion assignment of samples will result in different colors in the **Project** and **Protein Coverage** tables.

De novo options Tab

The **De novo options** allows the users to input parameters for the de novo sequencing analysis. There are two analyses modes available in Supernovo:

- **Full analysis mode** i.e., a pure de novo sequencing analysis – It is recommended to use a good 64-bit multi-core computer with a solid state drive for the full analysis.
- **Inspect project mode** i.e., quick way to test how well the MS/MS data matches to the predicted sequence (using a known sequence that is closely related to the sample of interest i.e., 80% match or more).




Figure 7: De novo options tab

- **Alternate Byonic parameter file**

By default, Supernovo uses a Byonic parameter file with

- [1] **Precursor mass tolerance** set to 10ppm,
- [2] **Fragment mass tolerance** set to 20ppm,
- [3] **Carbamidomethyl modification** set as fixed modification on **Cysteine (Carbamidomethyl / +57.021464 @ C | fixed)** and
- [4] **Fragmentation type** set to QTOF/HCD.

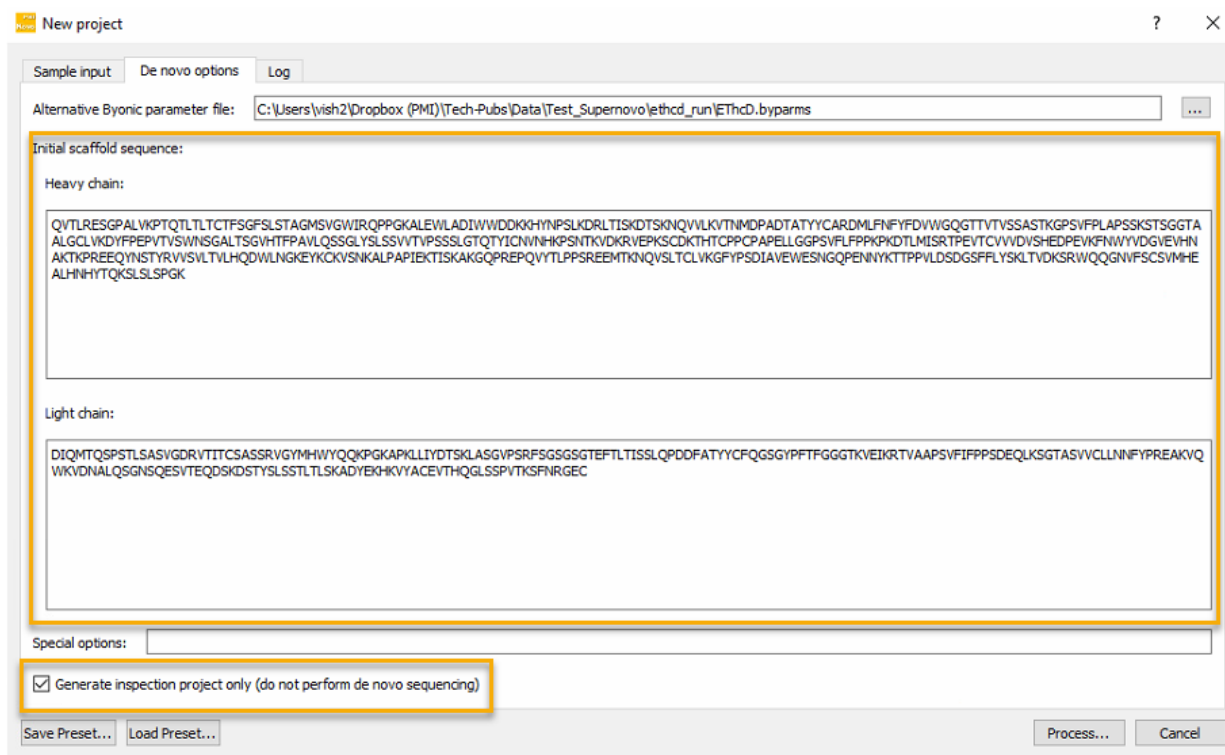
For a detailed list of the default Byonic parameters, open the file located at `C:\Program Files\ProteinMetrics\PMI-Suite\Tools\SupernovoCommandLine\Default.byparms`.

Another byparms file i.e., **Cysteine58.byparms** is also available at the same location. The **Cysteine58.byparms** file has the same mass tolerances, fragmentation type and other settings compared to the **Default.byparms** file, except the **Carboxymethyl modification** is set as fixed modification on **Cysteine (Carboxymethyl / +58.005479 @ C | fixed)**. In addition to the above 2 files, the user also has an option to input a new file by clicking  and uploading a ***.byparms** file. It is recommended to use the default Byonic parameters file as a template to make a new file.

- **Initial scaffold sequence**

For human, mouse, or rat mAbs, there is no need to input an initial scaffold sequence **in full analysis mode**. For other species, it is recommended to provide an initial scaffold sequence that matches closely (~80% or more) with the species of interest (i.e., sample). This applies to both **Heavy chain** and **Light chain**:

- **Heavy chain** – Text representing initial scaffold for the Heavy chain should be provided in the Heavy chain box.
- **Light chain** – Text representing initial scaffold for the Light chain should be provided in the Light chain box.
- **Special options**
This field can be ignored.
- **Generate Inspection project only (do not perform de novo sequencing)**



New project

Sample input De novo options Log

Alternative Byonic parameter file: C:\Users\vish2\Dropbox (PMI)\Tech-Pubs\Data\Test_Supernovo\ethcd_run\ETHcd.byparms

Initial scaffold sequence:

Heavy chain:

QVTLRESGPALVKPTQTLTLCTFSGFSLTAGMSVGVIRPPGKALEWLADIWWDKGGHYNPSLKDRLTISKDTSKNQVLLKVTNMDPADTATYYCARDMLFNIFYFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLWKDYFPEPVTYVWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHNKPSNTKVDKRVKPKCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDLMISRTPETVTCVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVISLVTLHQDWLNGKEYKKGVSNKALPAPIETISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGFFLYSKLTVDKSRWQQGNVIFSCSVHME
ALHNHYTQKSLSLSPGK

Light chain:

DIQMTQSPSTLSASVGRVTITCSASSRVGYMHWYQQKPKAPKLLIYDTSLKASGVPSRFSGSGSGTEFTLTISLQPDFAFYCYFGSGYPTFTGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSYLSSTLTLSKADYERHKVYACEVTHQGLSSPVTKSFNRGEC

Special options:

☒ Generate inspection project only (do not perform de novo sequencing)

Save Preset... Load Preset... Process... Cancel

Figure 8: Generate Inspection project for quick turnaround times for known sequences

If the **Generate Inspection project only** checkbox is checked, the analysis will be done faster and is not really a pure de novo sequencing analysis. However, this option allows the user to inspect the raw data quickly and see if the data matches well to predicted sequences of interest. The mandatory field(s) to input in this mode are initial scaffold sequences of **Heavy chain** and **Light chain**. It is recommended to provide these sequences as close as possible to the expected sequence. The **Generate Inspection project only** option is useful for the users who run multiple experiments with the same purified protein. The users will have an idea about what to expect for the sequence and providing that input in the **Heavy chain** and **Light chain** fields will give an estimate of the **depth of coverage in the constant region** quickly (e.g., 5 min compared to several hours in full analysis mode on a 16GB system with 8 cores)

Additional options

To save the edited settings from the **Sample input** and **De novo options** tabs to a configuration file, click the **Save Preset** button, enter a file name, and click **Save**. To load settings from a previously saved configuration file, click the **Load Preset** button, navigate to the file of interest, and click **Open**.

Finally, click **Process** to start the *de novo sequencing analysis*. A **Save File** dialog will open for the user to enter a file name to save the results as a **.blgc** file. To cancel the project creation click **Cancel**.

Log Tab

The **Log** tab reports progress on the analysis:



Figure 9: Log tab

The **Supernovo** program does multiple iterations of refining the *de novo* peptide identifications and assembling the refined peptides into scaffolds. A detailed project file with results and summary is then generated. The **full analysis** usually takes several hours, but the user has an option to do a quick inspection to make sure that the data acquired matches well to the predicted sequence (see **Generate Inspection project only** in the section [De novo options Tab](#) above).

During the initial phase of the **full analysis**, depth of the constant regions (of the antibody) is estimated. If the **Depth of coverage in the constant region** is greater than 20, the accuracy of the *de novo* sequence identified is high. Between 10 and 20, the accuracy is moderate. If the depth of coverage is less than 10, then it is advised to double-check the Byonic parameters, and if this still does not resolve the **Depth of coverage**, it is recommended to gather more data. The figure below shows the progress of Supernovo analysis in the **Log tab**.

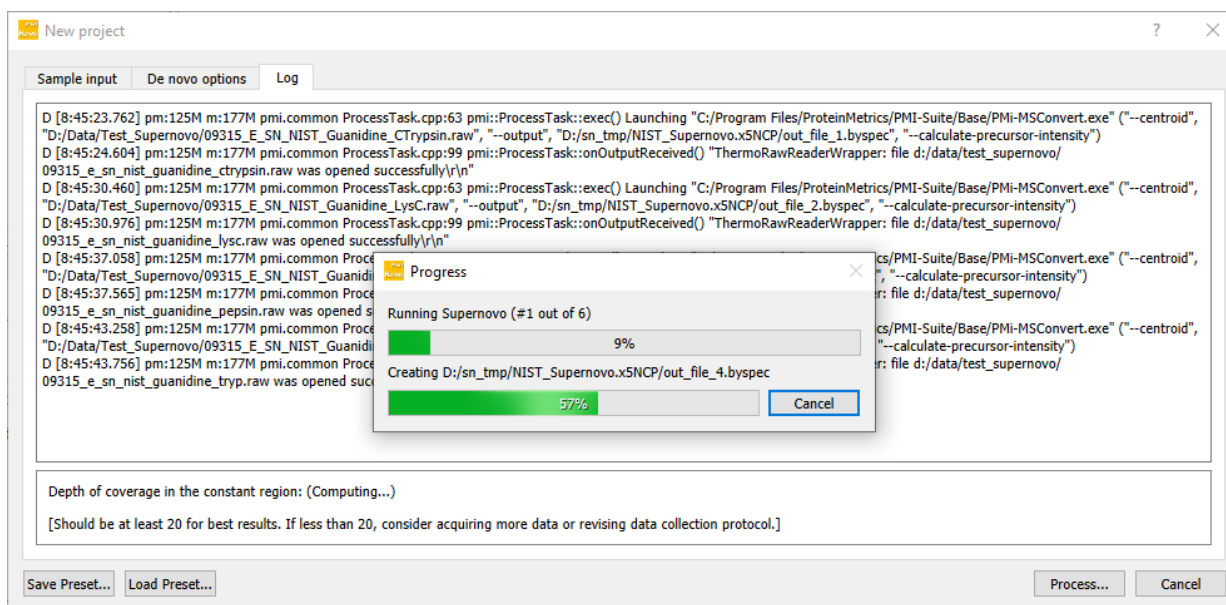


Figure 10: Progress of Supernovo analysis

Main Menu Bar

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

File Menu

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

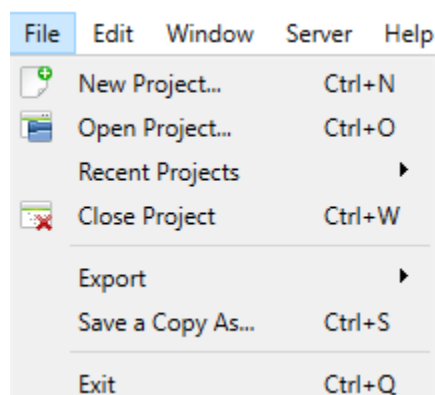


Figure 11: File menu

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

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

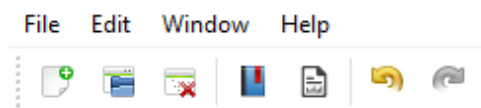


Figure 12: File menu icons

File > Export Menu

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

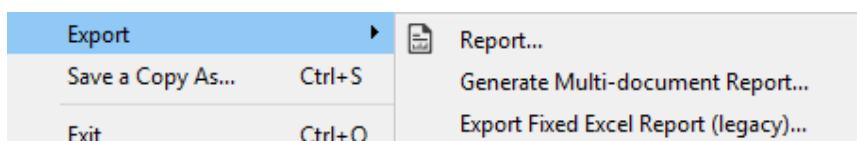


Figure 13: File > Export menu

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

Edit Menu

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

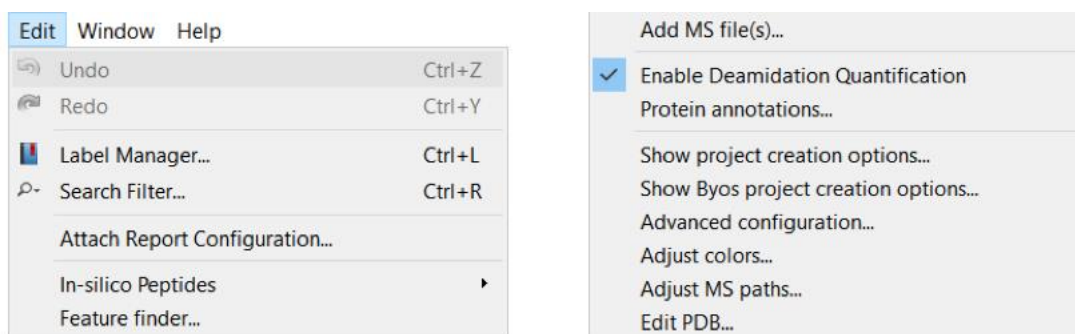


Figure 14: Edit menu

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

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

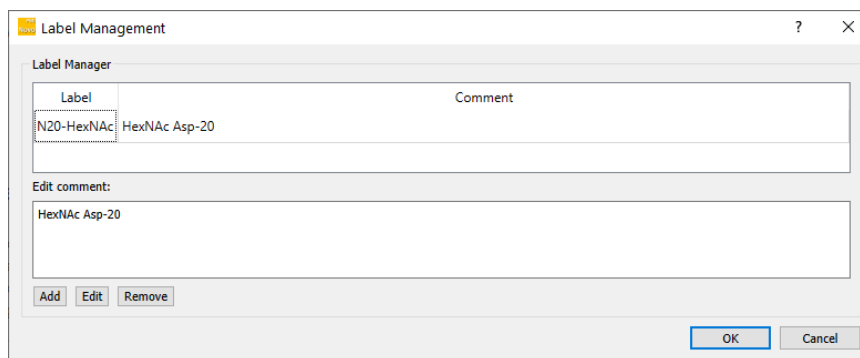


Figure 15: Label manager

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

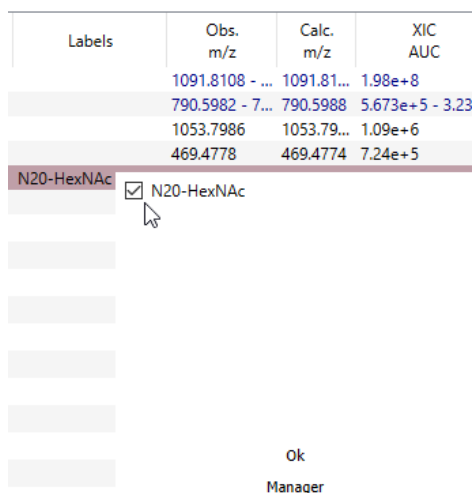
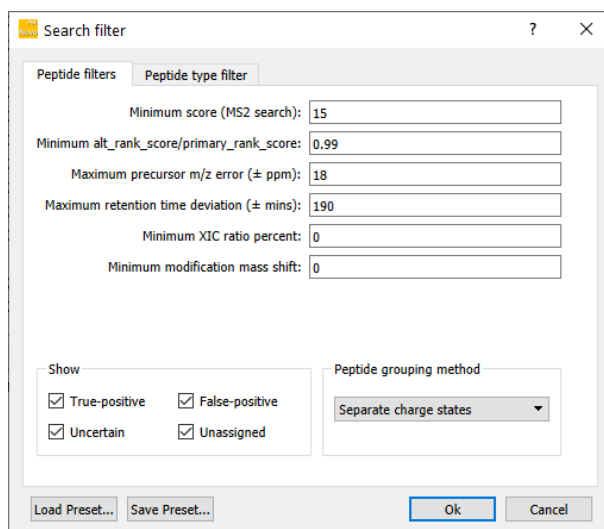


Figure 16: Adding labels in the Peptides table

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



Search filter

Peptide filters **Peptide type filter**

Minimum score (MS2 search): 15

Minimum alt_rank_score/primary_rank_score: 0.99

Maximum precursor m/z error (\pm ppm): 18

Maximum retention time deviation (\pm mins): 190

Minimum XIC ratio percent: 0

Minimum modification mass shift: 0

Show

☒ True-positive ☒ False-positive

☒ Uncertain ☒ Unassigned

Peptide grouping method

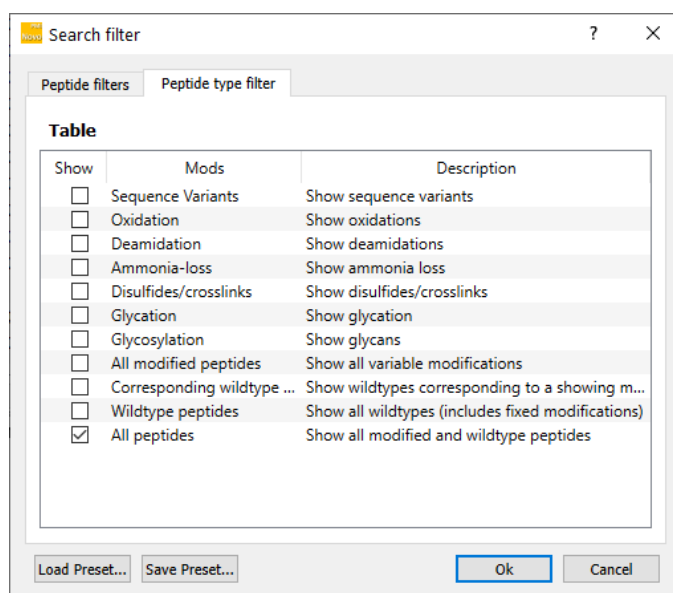
Separate charge states

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

Figure 17: Peptide search filter

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

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



Search filter

Peptide filters **Peptide type filter**

Table

| Show | Mods | Description |
|-------------------------------------|----------------------------|---|
| <input type="checkbox"/> | Sequence Variants | Show sequence variants |
| <input type="checkbox"/> | Oxidation | Show oxidations |
| <input type="checkbox"/> | Deamidation | Show deamidations |
| <input type="checkbox"/> | Ammonia-loss | Show ammonia loss |
| <input type="checkbox"/> | Disulfides/crosslinks | Show disulfides/crosslinks |
| <input type="checkbox"/> | Glycation | Show glycation |
| <input type="checkbox"/> | Glycosylation | Show glycans |
| <input type="checkbox"/> | All modified peptides | Show all variable modifications |
| <input type="checkbox"/> | Corresponding wildtype ... | Show wildtypes corresponding to a showing m... |
| <input type="checkbox"/> | Wildtype peptides | Show all wildtypes (includes fixed modifications) |
| <input checked="" type="checkbox"/> | All peptides | Show all modified and wildtype peptides |

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

Figure 18: Filtering by peptide type

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

Attach report configuration attaches a report configuration file to the project, to be used in place of the default report configuration. Report configuration files (graphical layouts) are generated from the project

report opened using **File > Export > Report**, and then saved using **File > Save to file**. Configuration have the extensions *.rptc, *.tabc, *.lvcfg, or *.pvtcfg. This menu can also be used to remove an attached report configuration file.

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

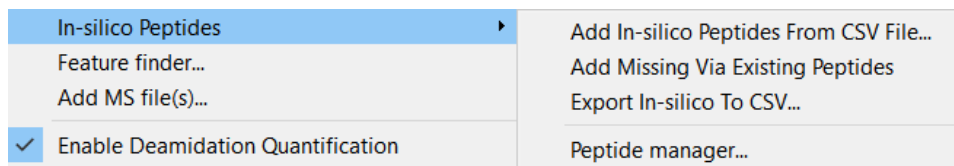


Figure 19: Edit > In-silico Peptides menu

- **Add In -silico Peptides From CSV File** opens a browser to load a *.csv file of in-silico candidates. Use of the correct format for the *.csv file is required. For an example of the correct *.csv format, see the file **ImportExample.Byologic.csv** in Program Files\ProteinMetrics\PMI-Suite\ExampleData.
- **Add Missing Via Existing Peptides** identifies missing peptide sequences and fills in those sequences from equivalent peptides in the sample files.
- **Export In -silico to CSV** exports the in-silico peptides to a *.csv file. This peptide file is then available for import into a different project. Enter a file name, navigate to a directory, and click **Save**.
- **Peptide Manager** combines all of the In-silico functionalities from **Edit > In-silico peptides** with a color-coded preview of the project changes and enhanced control over which rows and columns are affected.
 - **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** replaces the old menu item **Edit > In-Silico > Intersect with CSV library**. This menu item has been removed and can only be accessed from Peptide Manager.
Select a CSV file using the **Browse** button. **Matching options** include **Use mass and time** and **Use sequence and modifications**. Set mass and time tolerances. Check **Append unmatched** to include non-intersected peptides. Click **OK** to return to Peptide Manager and select columns to review. New values will be show in red.
 - **Add missing samples** is the same as in **Edit > In-Silico > Add missing**.
Click the button and then Apply to update the project with new in-silico peptides. These will be visible at the end of the list.
Click **Apply** to update the project. The figure below displays a common use case: Feature Finder unknowns matched with in-silico content.

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

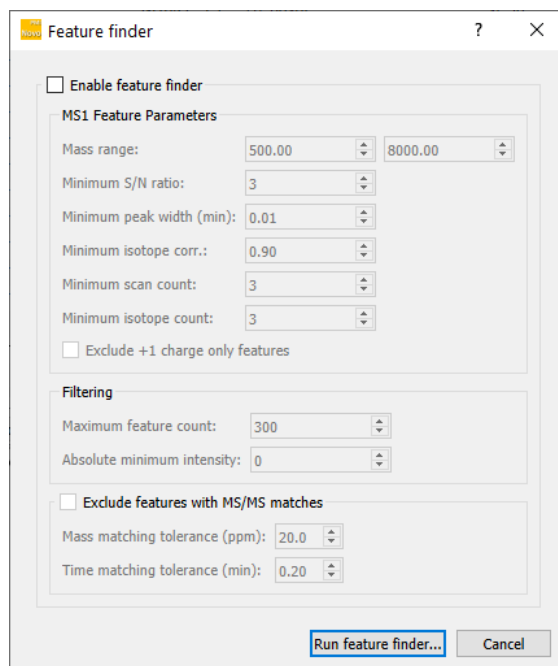


Figure 20: Feature finder dialog

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

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

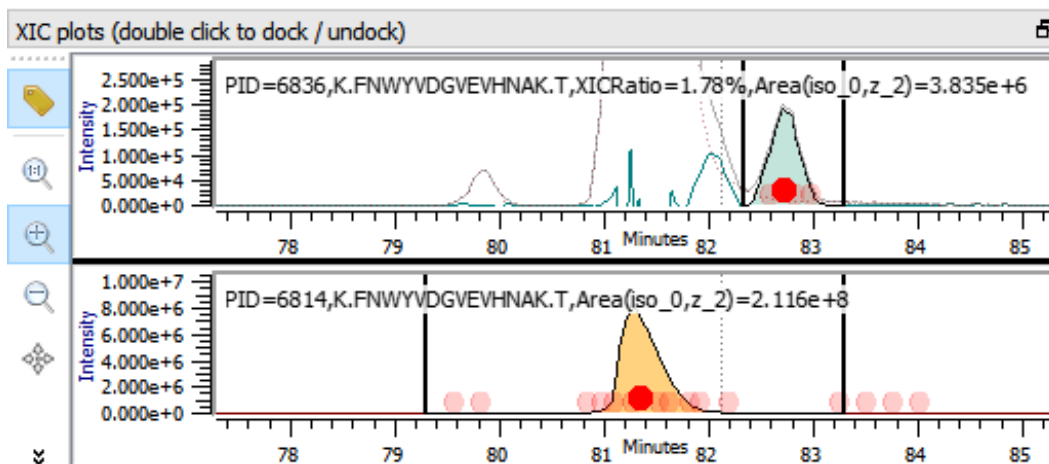
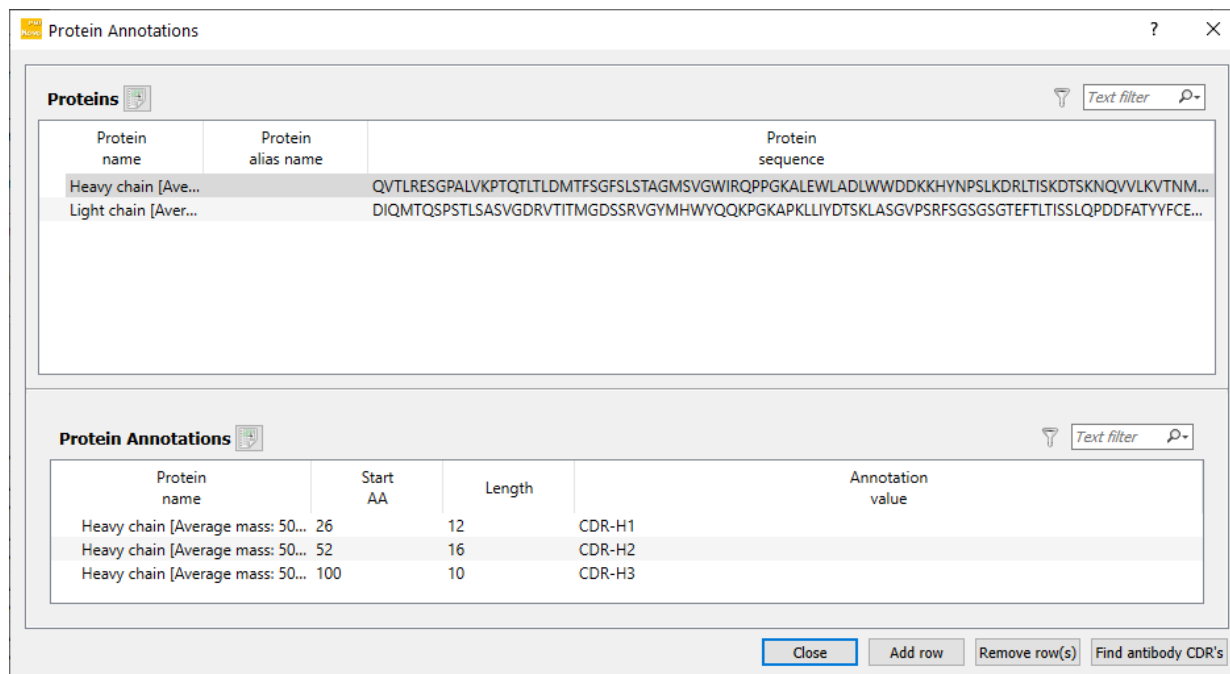


Figure 21: Deamidation quantification subtracts the wildtype integration from the total

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



Protein Annotations

Proteins

| Protein name | Protein alias name | Protein sequence |
|----------------------|--------------------|--|
| Heavy chain [Ave... | | QVTLRESGPALVKPTQLTLDMTFSGFSLTAGMSVGVIRQPPGKALEWLADLWDDKKHYNPSLKDRLTISKDTSKNQVVLKVTNM... |
| Light chain [Aver... | | DIQMTQSPSTLSASVGDRTITMGDSSRVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTEFTLTISLQPDFFATYYFCE... |

Protein Annotations

| Protein name | Start AA | Length | Annotation value |
|----------------------------------|----------|--------|------------------|
| Heavy chain [Average mass: 50... | 26 | 12 | CDR-H1 |
| Heavy chain [Average mass: 50... | 52 | 16 | CDR-H2 |
| Heavy chain [Average mass: 50... | 100 | 10 | CDR-H3 |

Close Add row Remove row(s) Find antibody CDR's

Figure 22: Protein Annotations

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

Show Byos project creation options displays the Byos workflow project creation options used to generate the project. This feature is only available for projects created through Byos.

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

Show project creation options displays in a single window the values and inputs used to create the current project. Apart from some import options, the data is generally read-only in these tabs. To edit specific functions and values, use the corresponding Edit menu items described in this section. This feature allows the user to save the current settings used in the project with the **Save Preset** button or load new ones with the **Load Preset** button.

Advanced configuration is used to enter text commands, which are often new features still in beta testing. This feature is generally not used.

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

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

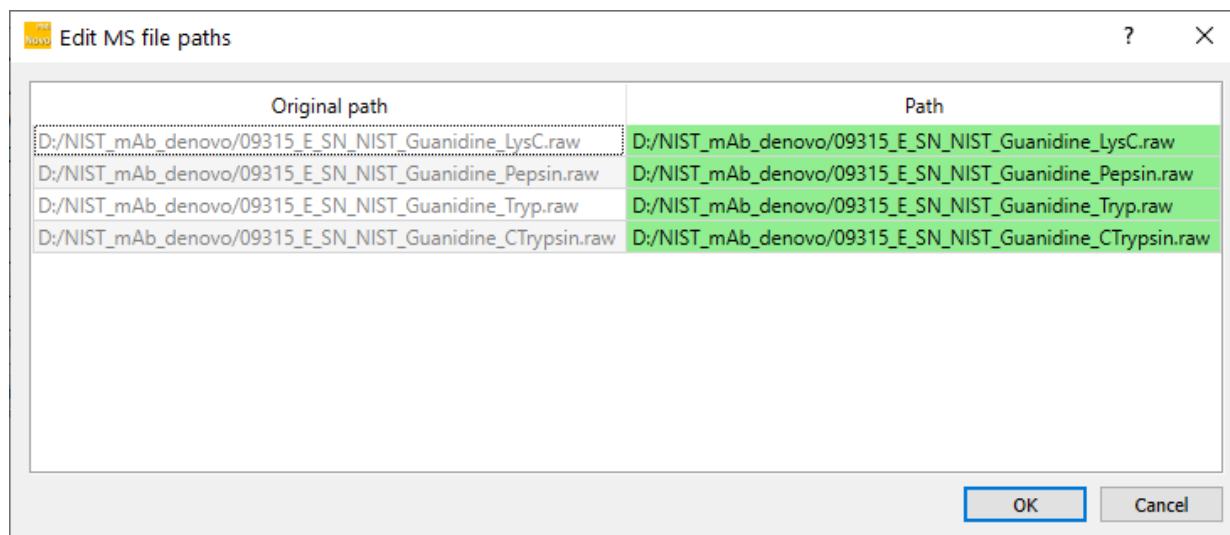


Figure 23: Editing the MS file path

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

Window Menu

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

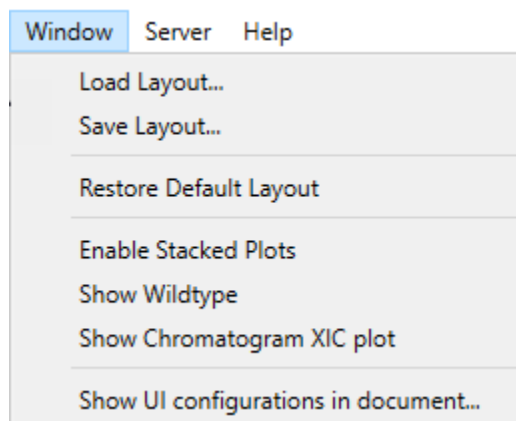
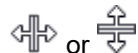


Figure 24: Window menu

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

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



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

once to sort ascending and twice to sort descending. Mouse over a column header to see a description of that field.

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

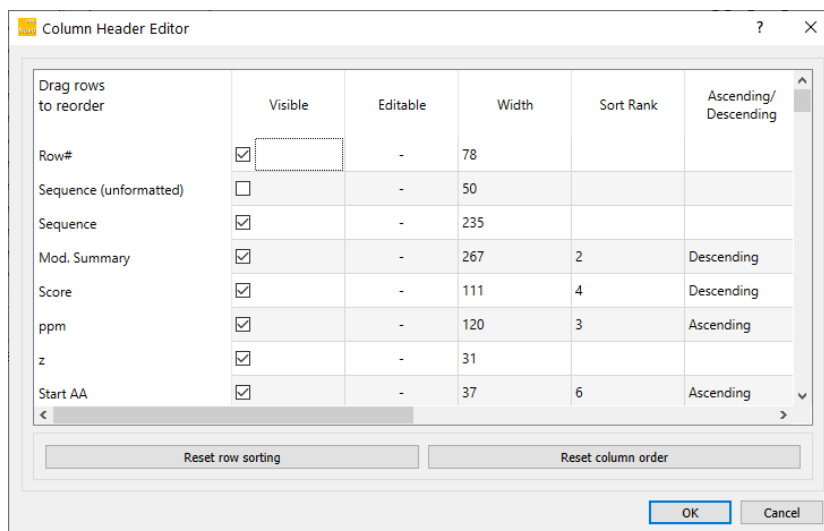


Figure 25: Column Header Editor

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

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

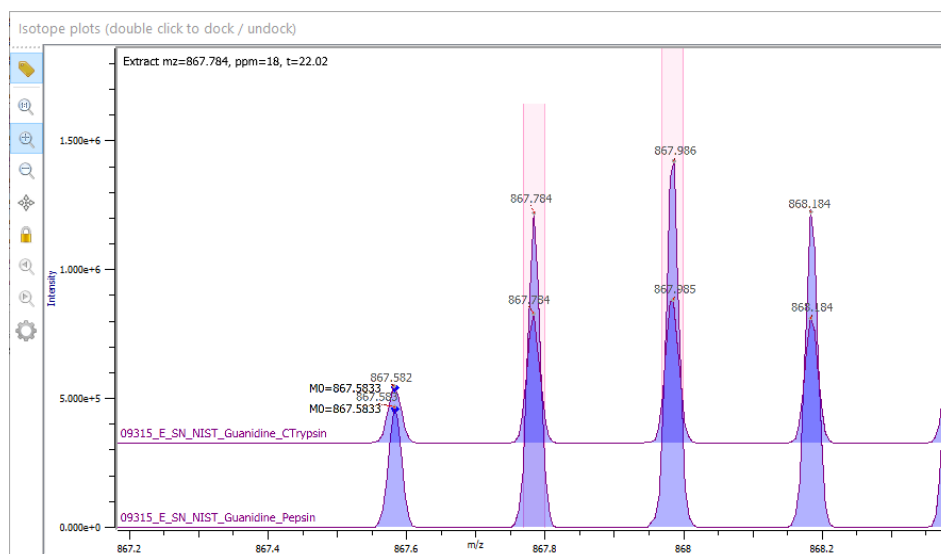


Figure 26: Example of stacked isotope plots

The **Show Wildtype** menu displays the Wildtype peptide table records and wildtype plots (**XIC plots**, **MS2 and mass error plots** and **Isotope plots**) again.

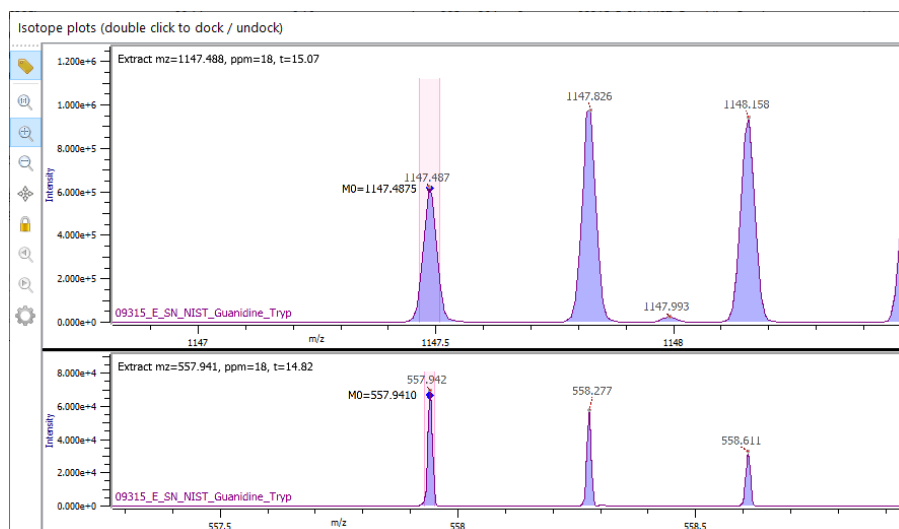


Figure 27: Example of peptide and its wildtype isotope plots

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

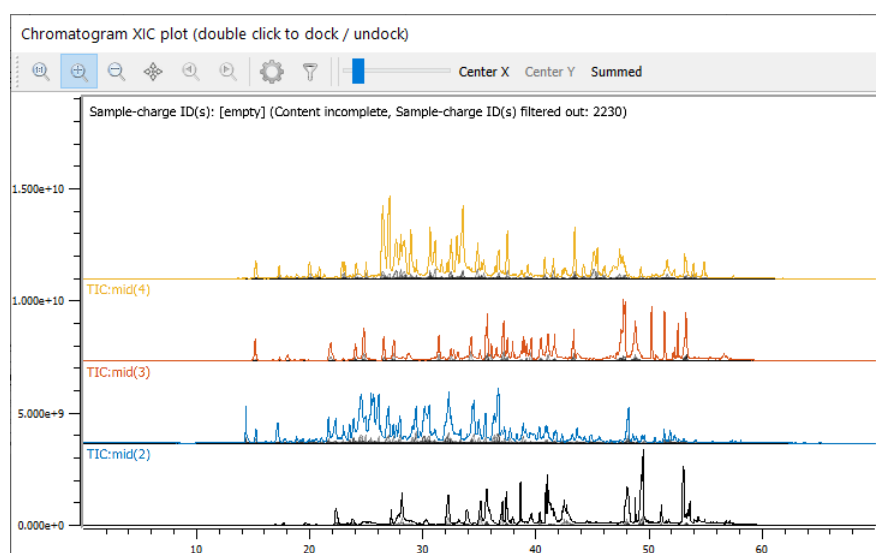


Figure 28: Undocked Chromatogram XIC plot

Server

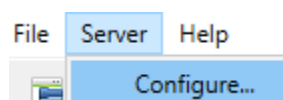


Figure 29: Server menu

| Menu | Sub Menu | Action and Notes |
|--------|-----------|---|
| Server | | |
| | Configure | Configure allows Byos integration with third party servers. |

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

Help Menu

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

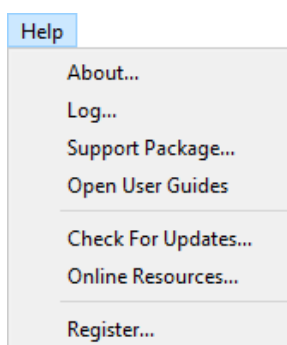






Figure 30: Help menu

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

Table Menus

The menu bars at the top of the three table views, Project, Peptides, and Wildtype peptides, share icons that manage hierarchical lists and filter on data.

- The  icon expands rows to show “sub-rows”.
- The  icon collapses rows to hide sub-rows. The use of sub-row depends on the table. For example, in the Peptides table, a row is a peptide record and a sub-row is the data resulting from an individual sample that contained the peptide.
- The  icon exports the table to a .csv file for opening with Excel.
- The  icon opens a dialog to create custom filters for that data table. (A single column filter can also be set by right-clicking on an entry in the column.) Select a column in the first cell, select an operator in the second cell, and enter text in the third cell. A second filter row becomes available for further entry. Click the red X after a filter row to delete it. Custom filters allow masses to be

filtered by mass range, annotation, intensity, and so forth. Custom filters can be stored with the project document or exported and imported using the **Presets** dropdown.


- The cell filters the entered text across the content of all columns in the protein and peptide tables. The records are filtered automatically as text is entered. Click the  icon to search the string as a whole word or as case sensitive.
- To sort the contents of a column by ascending value, click the column header. Click the column header again to sort by descending value.

Table Right-Click Menus

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

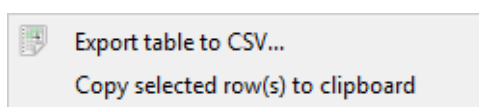


Figure 31: Table right-click menus

- **Export table to CSV** saves the table content and headers to a .csv file using parameters chosen in the **Export data** dialog.
- **Copy selected row(s) to clipboard** copies selected table rows, with their headers to be available for pasting into another application. (This menu is not available in the Protein Coverage table.)

Table context menus contain a set of query options for searching the clicked text value using any of the available search operators. This is an easy way to filter a table based on a specific field value.

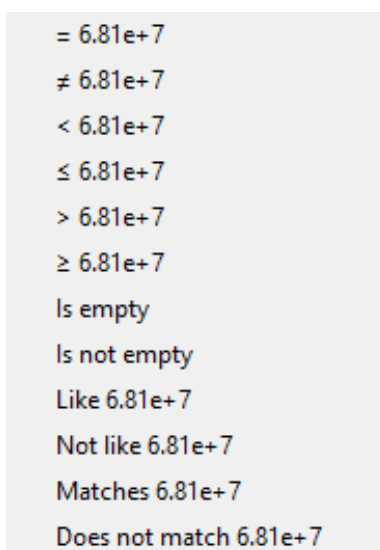







Figure 32: Search table values through a right-click menu

Project Table

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

Projects table (double click to dock / undock)

Project     

| Alias Name | Digestion Type | Name |
|--|----------------|---|
| ▼ <input checked="" type="checkbox"/> 09315_E_SN_NIST... | | D:\NIST_mAb_denovo\09315_E_SN_NIST_Guanidine_LysC.raw |
| <input checked="" type="checkbox"/> ms2_search_0... | | D:\NIST_mAb_denovo\sn_tmp\NIST_mAb_multiple_digests.mPfdP\ms2_s |
| ▼ <input checked="" type="checkbox"/> 09315_E_SN_NIST... | | D:\NIST_mAb_denovo\09315_E_SN_NIST_Guanidine_Pepsin.raw |
| <input checked="" type="checkbox"/> ms2_search_1... | | D:\NIST_mAb_denovo\sn_tmp\NIST_mAb_multiple_digests.mPfdP\ms2_s |
| ▼ <input checked="" type="checkbox"/> 09315_E_SN_NIST... | | D:\NIST_mAb_denovo\09315_E_SN_NIST_Guanidine_Tryp.raw |
| <input checked="" type="checkbox"/> ms2_search_2... | | D:\NIST_mAb_denovo\sn_tmp\NIST_mAb_multiple_digests.mPfdP\ms2_s |
| ▼ <input checked="" type="checkbox"/> 09315_E_SN_NIST... | | D:\NIST_mAb_denovo\09315_E_SN_NIST_Guanidine_CTrypsin.raw |
| <input checked="" type="checkbox"/> ms2_search_3... | | D:\NIST_mAb_denovo\sn_tmp\NIST_mAb_multiple_digests.mPfdP\ms2_s |

Figure 33: Project table

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

Protein Coverage Table and Menu

The **Protein Coverage** table displays peptide sequences and corresponding protein coverage. Different samples are assigned different colors:

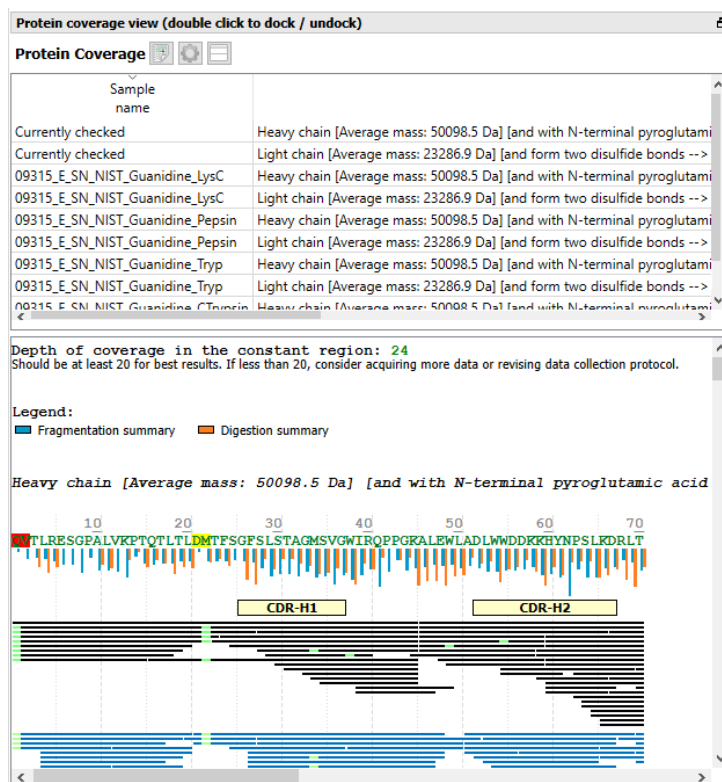



Figure 34: Protein Coverage table

The different colors within the colored bars indicate the presence of peptide modifications at those positions. (The colors used are chosen for contrast and do not reflect the colors assigned to the MS sample files.) The bar charts summarize between-residue evidence accumulated over all peptides from all samples.

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

- The  icon opens the Protein coverage rendering options dialog:

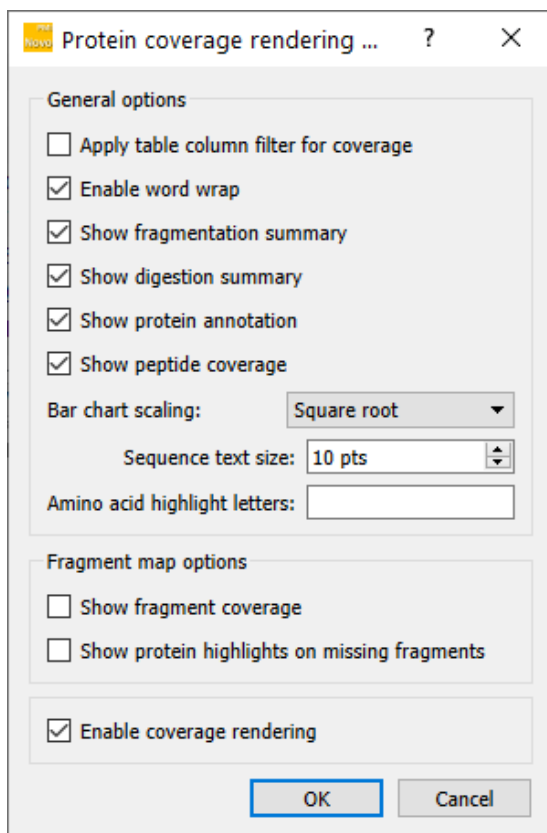


Figure 35: Protein coverage rendering options

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

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

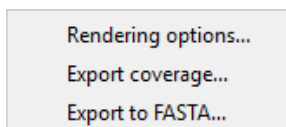


Figure 36: Protein Coverage right-click menu







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

Amino acids that should be inspected are shaded

- **Red** – approximately 10% error rate
- **Yellow** – approximately 2% error rate

Peptides Table and Menu

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


Peptides      Text filter 

| Row# | Sequence | Mod. Summary | Score | ppm | z | Start AA |
|------|--|-----------------------|-----------------|--------------------------|---|----------|
| 1 | -qVTLRESGPAL | Q1(Gln->pyro-Glu/-... | 728.91 | 0.64 | 2 | 1 |
| > 2 | -qVTLRESGPALV | Q1(Gln->pyro-Glu/-... | 658.83 - 660.41 | 0.72; -0.44 | 2 | 1 |
| > 3 | -qVTLRESGPALVKPT.Q | Q1(Gln->pyro-Glu/-... | 227.55 - 571.85 | 1.21; 1.28; 0.28 | 2 | 1 |
| > 4 | -qVTLRESGPALVKPT.Q | Q1(Gln->pyro-Glu/-... | 277.10 - 562.59 | 1.47; 1.59 | 3 | 1 |
| 5 | -qVTLRESGPALVKPTQTL | Q1(Gln->pyro-Glu/-... | 505.06 | 0.82 | 2 | 1 |
| 6 | -qVTLRESGPALVKPTQTL | Q1(Gln->pyro-Glu/-... | 492.41 | -0.20 | 3 | 1 |
| 7 | -QVTLRESGPALVKPTQTLT | | 469.88 | -0.28 | 3 | 1 |
| > 8 | -qVTLRESGPALVKPTQTLT | Q1(Gln->pyro-Glu/-... | 367.75 - 440.19 | 1.73; 1.60; -0.50; -0... | 2 | 1 |
| > 9 | -qVTLRESGPALVKPTQTLT | Q1(Gln->pyro-Glu/-... | 345.20 - 505.79 | 2.17; 1.60; 0.36; -1.27 | 3 | 1 |
| 10 | -qVTLRESGPALVKPTQTLT | Q1(Gln->pyro-Glu/-... | 604.35 | 1.32 | 3 | 1 |
| 11 | -qVTLRESGPALVKPTQTLT.L | Q1(Gln->pyro-Glu/-... | 392.83 | 0.94 | 2 | 1 |
| > 12 | -qVTLRESGPALVKPTQTLT.L | Q1(Gln->pyro-Glu/-... | 224.80 - 278.59 | 3.23; 0.42; 1.06 | 3 | 1 |
| > 13 | -qVTLRESGPALVKPTQTLT.D | Q1(Gln->pyro-Glu/-... | 333.20 - 400.50 | 2.23; 1.43; -0.29 | 2 | 1 |
| > 14 | -qVTLRESGPALVKPTQTLT.D | Q1(Gln->pyro-Glu/-... | 255.41 - 351.53 | 2.65; 1.62; 0.08 | 3 | 1 |
| 15 | -qVTLRESGPALVKPTQTLT.Dm.T | Q1(Gln->pyro-Glu/-... | 439.48 | 1.95 | 2 | 1 |
| 16 | -qVTLRESGPALVKPTQTLT.Dm.T | Q1(Gln->pyro-Glu/-... | 260.68 | 1.31 | 3 | 1 |
| 17 | -qVTLRESGPALVKPTQTLT.Dm.T.F | Q1(Gln->pyro-Glu/-... | 156.06 | 1.69 | 3 | 1 |
| 18 | -qVTLRESGPALVKPTQTLT.Dm.T.F.S | Q1(Gln->pyro-Glu/-... | 224.46 | 2.64 | 2 | 1 |
| 19 | -qVTLRESGPALVKPTQTLT.Dm.T.F.S | Q1(Gln->pyro-Glu/-... | 87.08 | 0.31 | 3 | 1 |
| > 20 | -qVTLRESGPALVKPTQTLT.Dm.T.F.S.F.S | Q1(Gln->pyro-Glu/-... | 158.31 - 348.80 | 1.47; 0.79 | 3 | 1 |
| 21 | -qVTLRESGPALVKPTQTLT.Dm.T.F.S.F.SLSTAG | Q1(Gln->pyro-Glu/-... | 488.62 | 1.16 | 3 | 1 |
| 22 | -qVTLRESGPALVKPTQTLT.Dm.T.F.S.F.SLSTAG | Q1(Gln->pyro-Glu/-... | 444.68 | 0.03 | 4 | 1 |
| 23 | -QVTLRESGPALVKPTQTLT.Dm.T.F.S.F.SLSTAG | | 343.41 | -4.41 | 5 | 1 |

Figure 37: Peptides table

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

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

In addition to the usual table menu buttons, the Peptides table contains the  icon button which opens the same Search Filter dialog that is opened by the **Edit > Search Filter** menu. Within the **Search Filter**, two other tabs to filter out the peptides and peptide types are present. The user has an option to filter out the false positives by clicking **Peptide filters** tab and unchecking **False-positive** box within the **Show** tab and clicking **OK**.

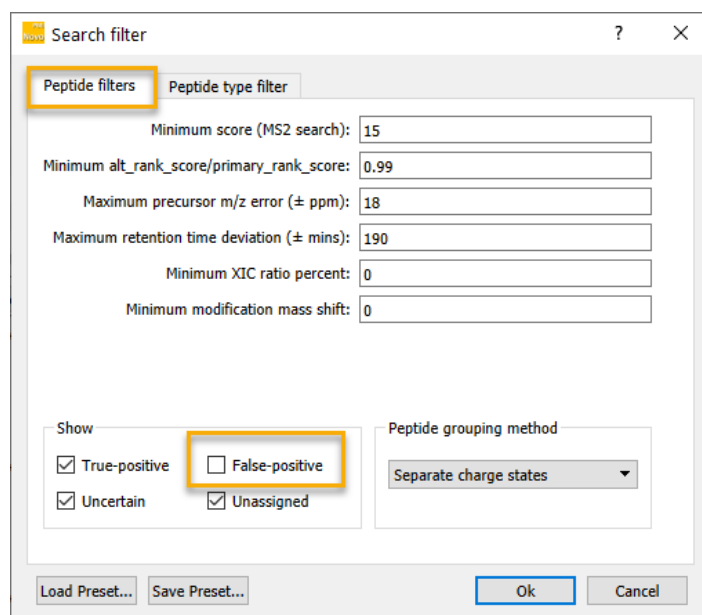


Figure 38: Peptides table - Search filter menu

The **text filter** entry box allows the records to be filtered based on the occurrence of the given text string searched across all fields.

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

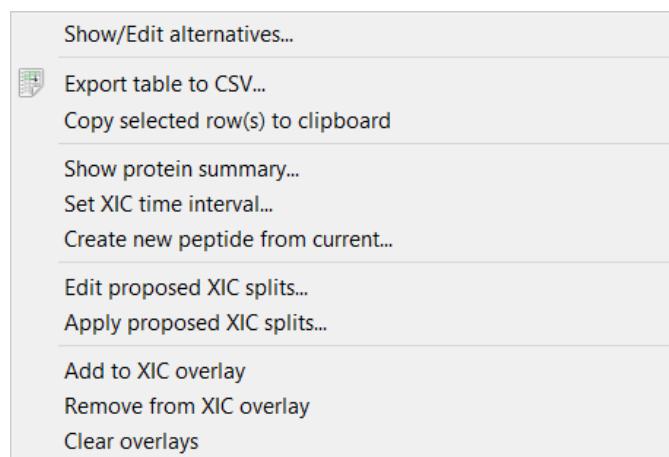
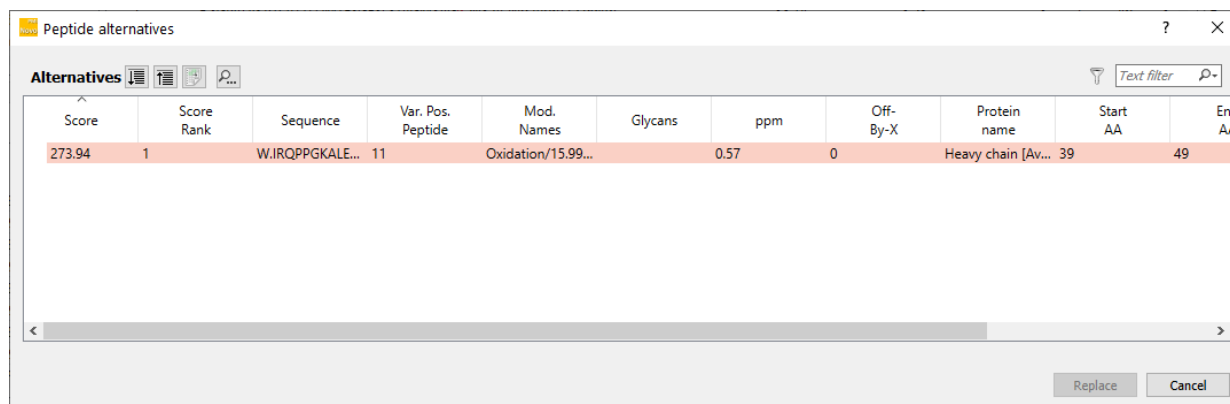


Figure 39: Peptides table right-click menus

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



Peptide alternatives

Alternatives

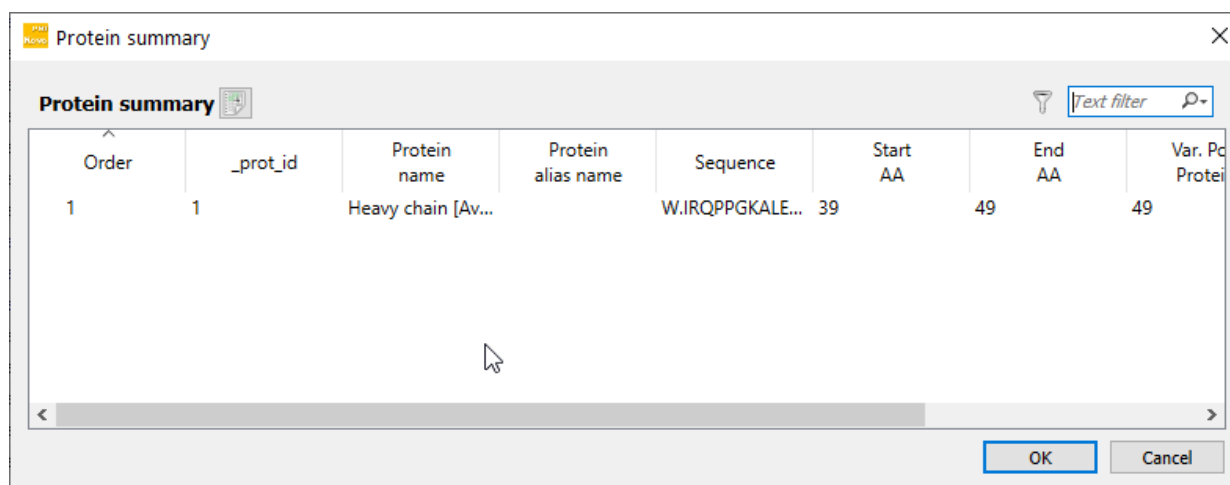
| Score | Score Rank | Sequence | Var. Pos. Peptide | Mod. Names | Glycans | ppm | Off-By-X | Protein name | Start AA | End AA |
|--------|------------|-----------------|-------------------|--------------------|---------|------|----------|--------------------|----------|--------|
| 273.94 | 1 | W.IRQPPGKALE... | 11 | Oxidation/15.99... | | 0.57 | 0 | Heavy chain [Av... | 39 | 49 |

Replace Cancel

Figure 40: Peptide alternatives table

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

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



Protein summary

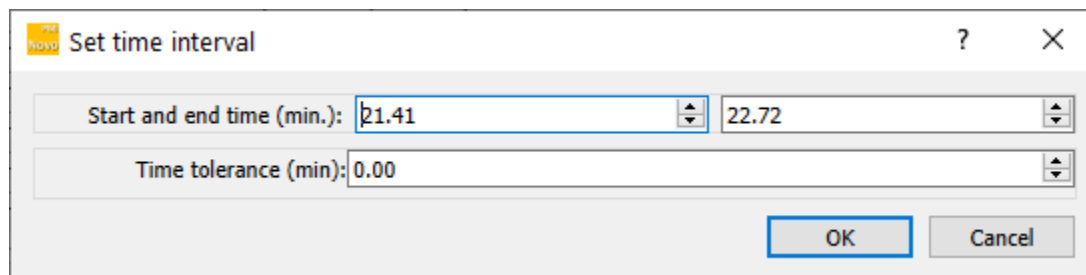
Protein summary

| Order | _prot_id | Protein name | Protein alias name | Sequence | Start AA | End AA | Var. Pos. Peptide |
|-------|----------|--------------------|--------------------|-----------------|----------|--------|-------------------|
| 1 | 1 | Heavy chain [Av... | | W.IRQPPGKALE... | 39 | 49 | 49 |

OK Cancel

Figure 41: Protein summary

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



Set time interval

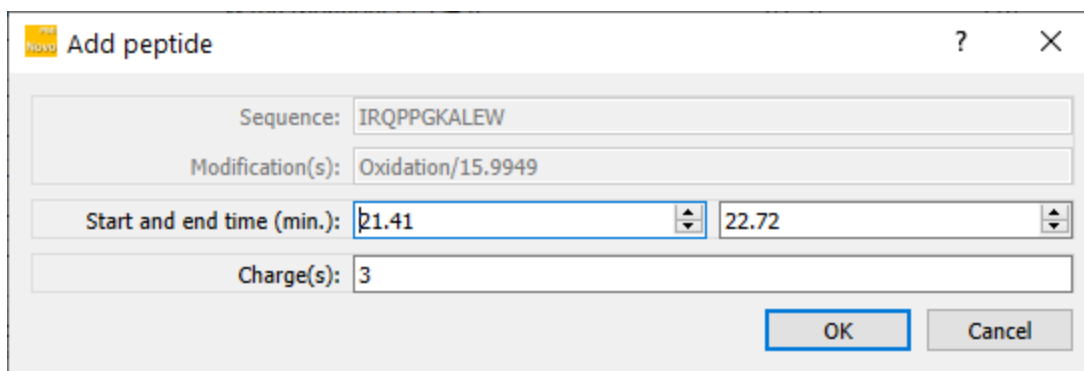
Start and end time (min.): 21.41 22.72

Time tolerance (min): 0.00

OK Cancel

Figure 42: Set XIC time interval

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

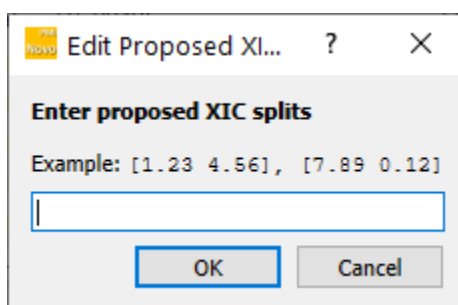


The 'Add peptide' dialog box contains the following fields and controls:

- Sequence:** Text field containing 'IRQPPGKALEW'.
- Modification(s):** Text field containing 'Oxidation/15.9949'.
- Start and end time (min.):** Two spinners. The first is set to 21.41 and the second to 22.72.
- Charge(s):** Text field containing '3'.
- Buttons:** 'OK' and 'Cancel' buttons at the bottom right.

Figure 43: Create new peptide from current

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



The 'Edit Proposed XIC splits' dialog box contains the following elements:

- Title:** 'Edit Proposed XIC splits'.
- Instruction:** 'Enter proposed XIC splits'.
- Example:** '[1.23 4.56], [7.89 0.12]'.
- Input:** A text field for entering the proposed splits.
- Buttons:** 'OK' and 'Cancel' buttons at the bottom.




Figure 44: Edit proposed XIC splits



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

- **Apply proposed XIC splits** separates a record in the Peptides table into two or more records using the time ranges entered using the Edit proposed XIC splits function.
- **Add to XIC overlay** adds the selected Peptides table record (subject to the right-click) or collection of records as an overlay to the XIC plots view mapped to the same time axis. When this function is applied to a collection of records in the table, all the records below it are added to the XIC plots view.
- **Remove from XIC overlay** removes the overlay from the selected Peptides table record (subject to the right-click) from the XIC plots view.
- **Clear overlays** removes all sample overlays from the XIC plots view.

Wildtype Peptides Table and Menu

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

Wildtype peptides   

 Text filter 










| Row# | PID | Sequence | Start AA | End AA | Apex Int. (Posit) | XIC area summed | Score | z | Obs. m/z |
|------|---------------------|--------------|----------|--------|-------------------|-----------------|-------------|---|----------|
| > 1 | 575; 433; 445; 4... | K.ALPAPIEK.T | 332 | 339 | 3.076e+8 ... | 7.24e+9 | 270.12 ~... | 2 | 419.754 |

Figure 45: Wildtype Peptides table

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

Plot Menus

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

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

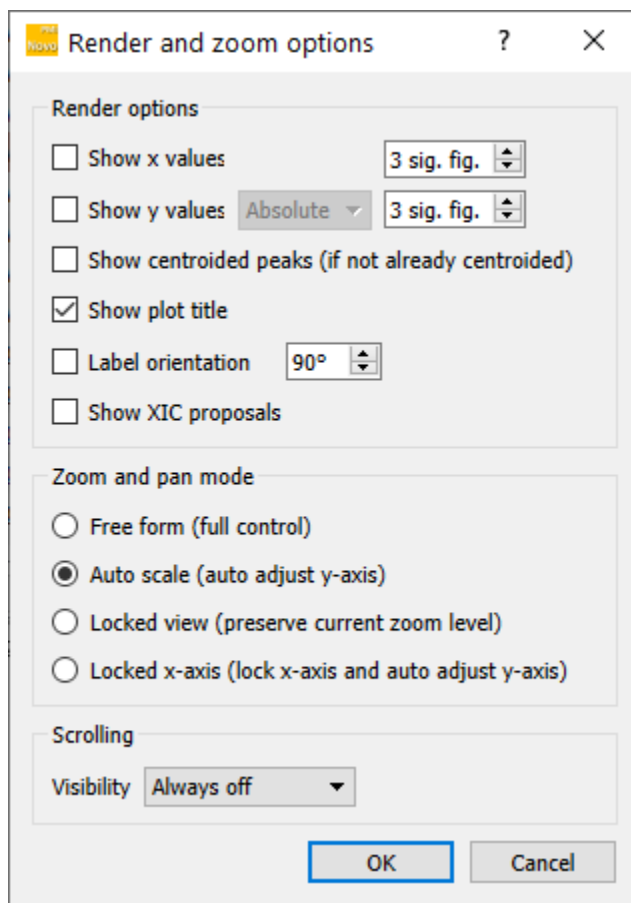



Figure 46: Render and zoom options

- **Show x values** and **Show y values** display the x- or y-coordinates beside plot peaks to the chosen number of significant figures.
- **Show delta**: when checked, displays the x or y differences from a reference peak when the cursor hovers over a peak tip.
- **Show centroided peaks** darkens the peak positions that are determined to be centroided.
- **Show plot title** displays the title of the plot, as generated from field values.
- **Label orientation**, when checked, orients labels to some angle other than horizontal.

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

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

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

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

Plot Right-Click Menus

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

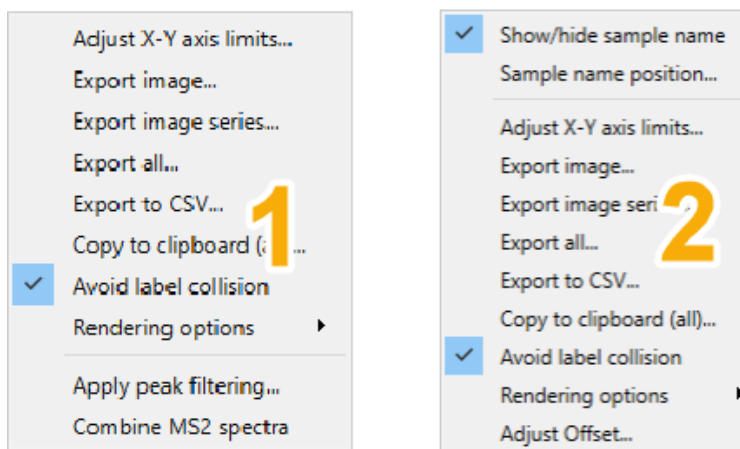




Figure 47: Plot right-click menus, [1] for MS2 and mass-error plots and [2] for XIC/Isotope plots

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

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

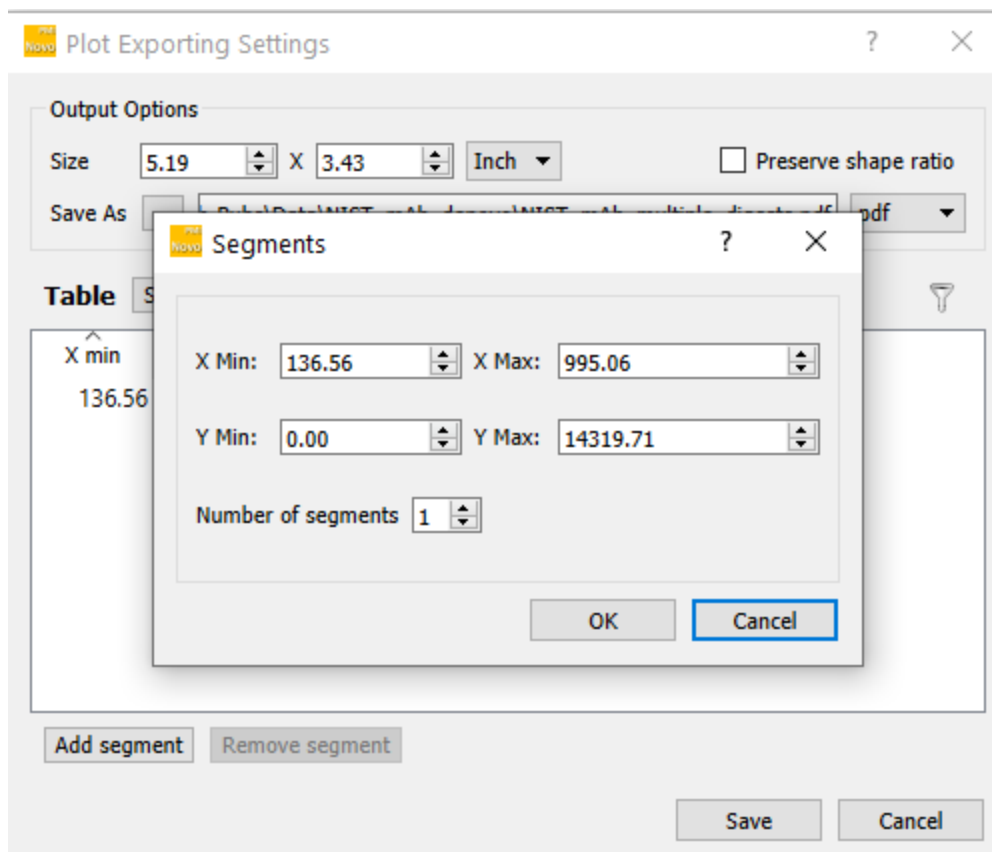


Figure 48: Plot export settings

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

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

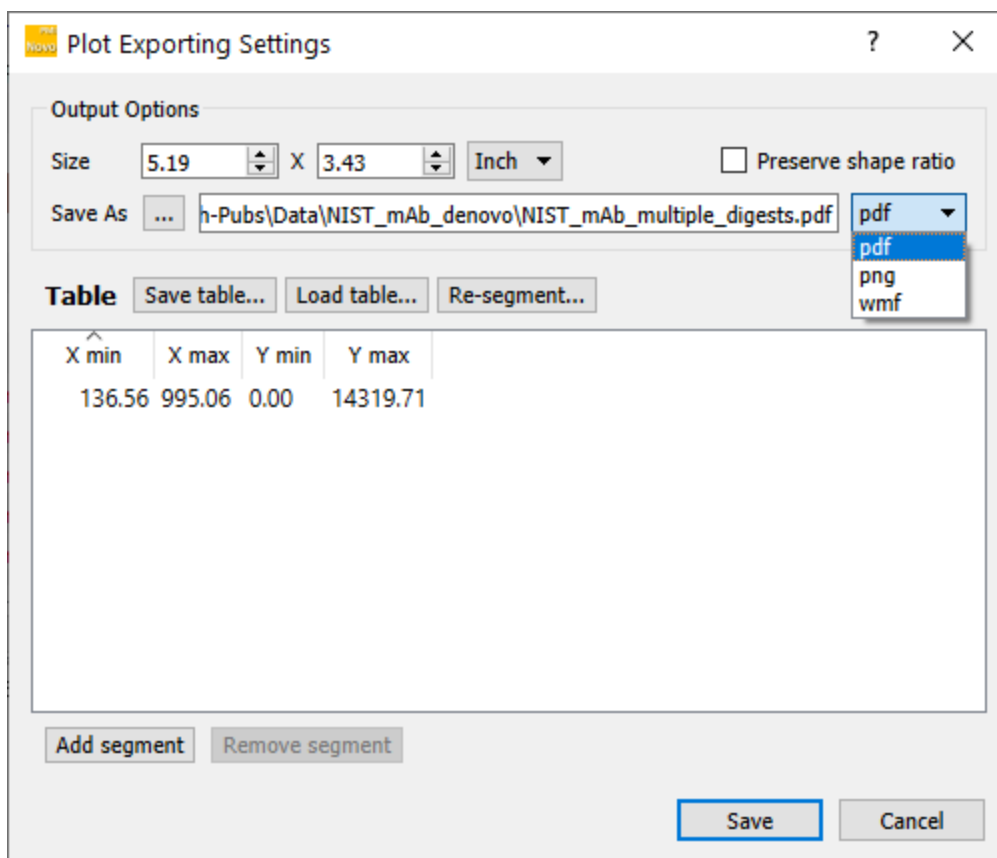


Figure 49: Plot export as a *.png file or a *.wmf file

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

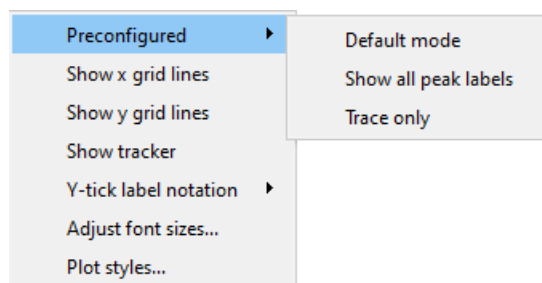


Figure 50: Rendering options menu

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

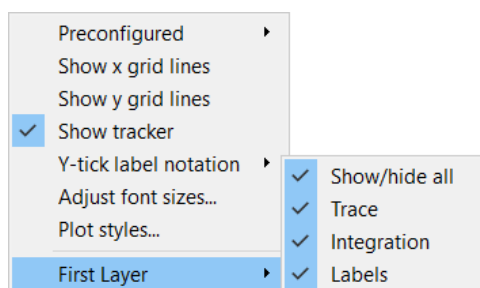


Figure 51: Rendering options > First Layer menu

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

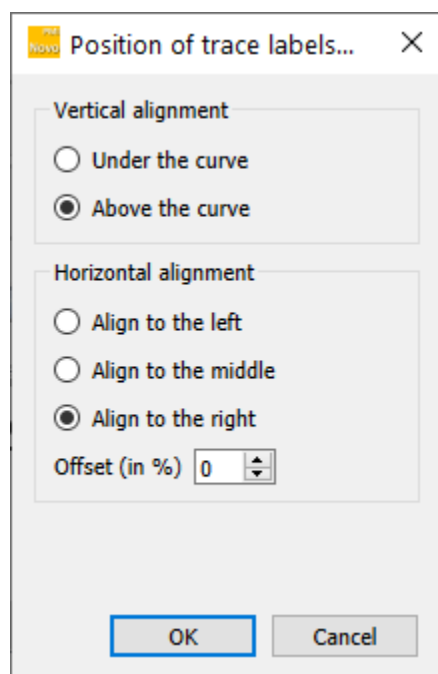


Figure 52: Sample name position sub-menu

XIC Plots and Menu

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

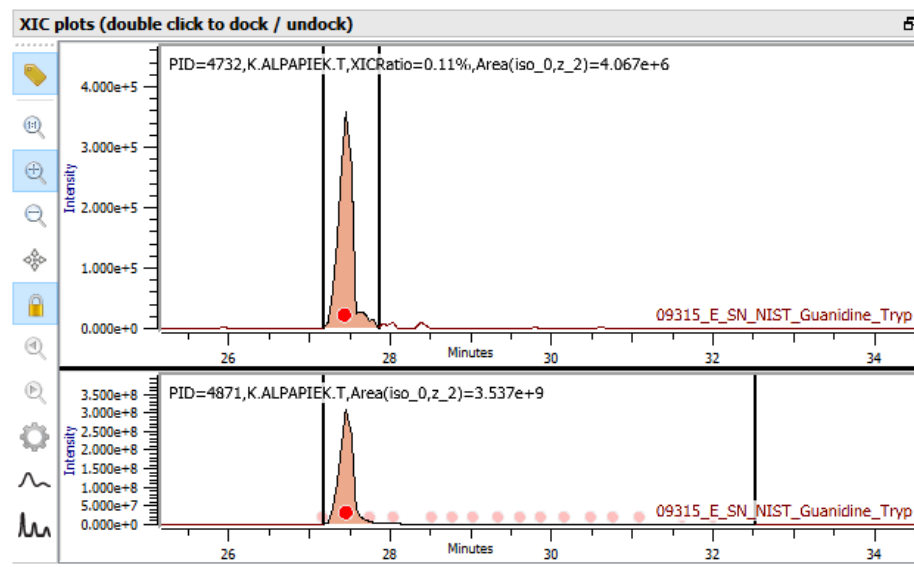







Figure 53: XIC Plots including wildtype peptides

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

- The  icon turns plot annotations on or off. In the XIC plots, annotations are displayed as circles at the base of the chromatograms. Dark red circles represent the position of currently displayed MS2 scans. Light red circles represent the position of other MS2 scans with the same

identification. Light gray circles represent the position of MS2 scans with the same precursor mass as the selected peptide, but with different attribution. Hover over the circles and the associated sequence ID and value are displayed. Note that the cursor changes to a hand. Click the circle and the corresponding Peptides table row is selected and the views are updated to display for that record.

- The  icon is used to compute the XIC plot integration for a specified time range. A dialog opens to enter start and end times.
- The  icon enables MS scan extraction. This displays isotope values derived from the XIC plot. A blue vertical line appears in the XIC plots view. As the line is dragged across the XIC trace, the Isotope plots view is updated to show the MS scan for that XIC time value. Two new icons appear on the Isotope plots menu bar in this mode: The  icon clears the extracted MS scan and restores the original isotope plots. The  icon saves the extracted MS scan to the project document.

Isotope Plots and Menu

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

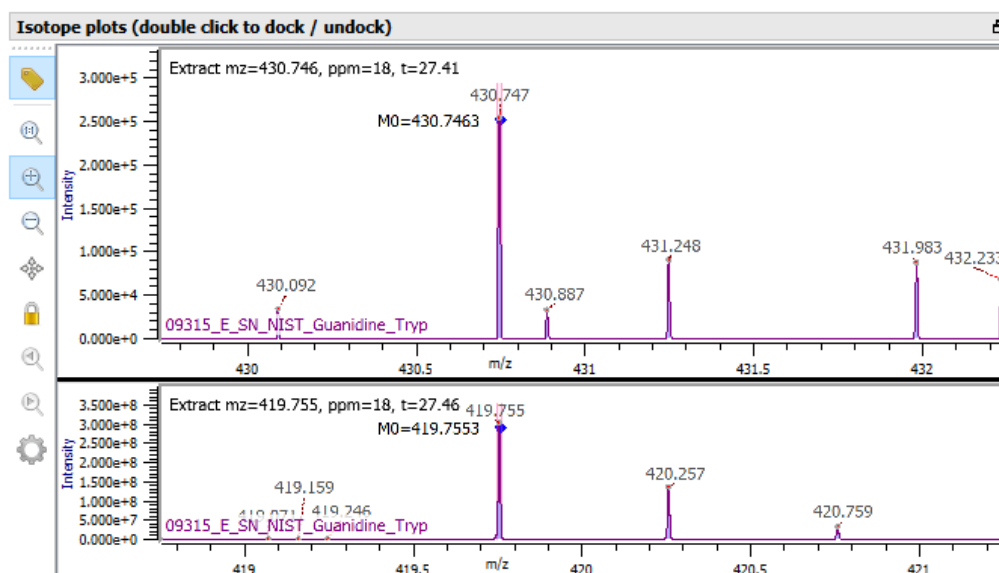



Figure 54: Isotope plots including wildtype peptides

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

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

MS2 and Mass Error Plots and Menu

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

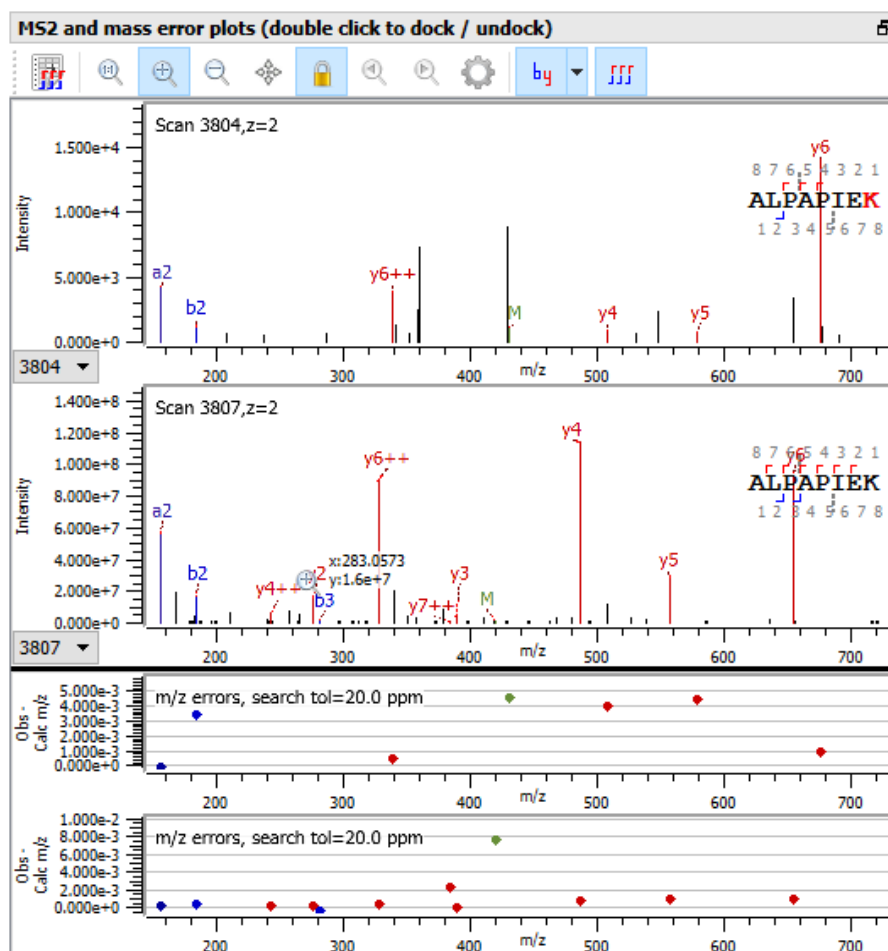





Figure 55: MS2 and mass error plots including wildtype peptides

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

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

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



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




☒ Calculated ☐ Observed ☐ Delta


PID=5252: DIQMTQSPSTLSASVGDRVTITMGDSSRVGYMHWYQQKPGKAPK

| # | a calc. | b calc. | b-18 calc. | b++ calc. | b++ -HexNAc calc. | b++ -HexN calc. | b-HexN calc. | b_3+ calc. |
|----|-----------------|-----------------|-----------------|--------------|----------------------|--------------------|-----------------|---------------|
| 1 | 88.0393 | 116.0342 | 98.0237 | 58.5207 | | | | 39.3496 |
| 2 | 201.1234 | 229.1183 | 211.1077 | 115.0628 | | | | 77.0443 |
| 3 | 329.1819 | 357.1769 | 339.1663 | 179.0921 | | | | 119.730 |
| 4 | 460.2224 | 488.2173 | 470.2068 | 244.6123 | | | | 163.410 |
| 5 | 561.2701 | 589.2650 | 571.2545 | 295.1361 | | | | 197.093 |
| 6 | 689.3287 | 717.3236 | 699.3130 | 359.1654 | | | | 239.779 |
| 7 | 776.3607 | 804.3556 | 786.3451 | 402.6815 | | | | 268.790 |
| 8 | 873.4135 | 901.4084 | 883.3978 | 451.2078 | | | | 301.141 |
| 9 | 960.4455 | 988.4404 | 970.4299 | 494.7238 | | | | 330.151 |
| 10 | 1061.4932 | 1089.4881 | 1071.4775 | 545.2477 | | | | 363.834 |

Figure 56: MS2 and mass error plots: mass table view

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

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

 MS2 fragment annotation options

MS2 annotation options

Preset: **Low accuracy**

Max num: **Low accuracy**

High accuracy

Custom

Auto


MS2 tolerance options

Set new fragment tolerance: ☐ 20 ppm

OK Cancel

Figure 57: MS2 fragment annotation options

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

- **Remove fragment coverage cache** clears the existing cache of detected fragments used for the protein fragment coverage rendering.
- **Export fragment to CSV**, exports the fragment sequence to a *.csv file.
- The  icon turns on or off the fragment sequences displayed to the right of the plots.

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

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

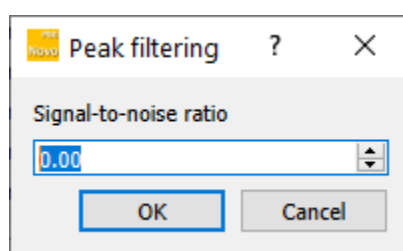
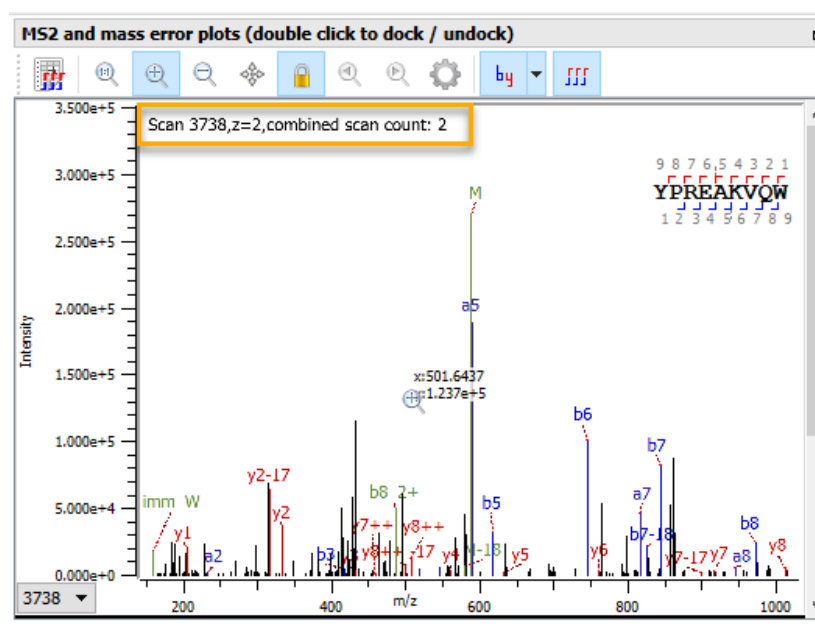


Figure 58: Peak filtering by signal-to-noise ratio

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



Appendix

Leucine/Isoleucine differentiation with EThcD data

The accuracy in differentiating leucine (L) and isoleucine (I) in mass-spectrometry based **de novo sequencing** analysis is difficult as the side chains of these amino acids are isomers and have the same mass of 113.08406 Da. However, using the samples from multiple proteases and running analysis on instrument that can generate EThcD data, the amino acids I/L can be differentiated with sophisticated analysis. Supernovo uses the **characteristic EThcD fragment ion peaks** and the **digestion or lack of digestion of C-terminal of these amino acids (I/L)** to distinguish Isoleucine (I) from Leucine (L).

Besides the .blgc file (Supernovo project file), there is an .html file (with the same base name as the project) that contains some additional information. For the EThcD analysis, this .html file (at the bottom section) summarizes the confidence of the I/L differentiation and other metrics and graphics. In the below figure, the actual sequence (highlighted in grey), the de novo predicted sequence (highlighted in black) and an additional row showing the confidence of I/L is shown. Different colors of I/L represent different confidences. **Green** is high confidence; **Orange** is medium confidence; and **Red** is low confidence. Regions highlighted in **Yellow** are **complementarity determining regions** (CDRs) of the antibody.

Heavy chain

```

QVTLRESGPALVKPTQTTLTCTFSGFSLSTSGMCVWIRQPPGKALEWLALIDWDDDKYYSTSLKTRLTISKDTSKNQV
QVTLRESGPALVKPTQTTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWDDKKHYNPSLKDRLTISKDTSKNQV
      L      L      L L      L      I      L L I      L      L I

VLTMTNMDPVDTATYYCARI      YYYYYGMDVWGQGTTTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
VLKVTNMDPADTATYYCARDMLFNFYF-----DVWGQGTTTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
      L                        L                        L      L L

PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAP
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAP
      L      L      L L      L      I

ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
LL      L      L I      L L

WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
      L      L I I      L      L L      I

TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
      L      L L      L      L L

```

Figure 60: NIST mAb heavy chain - de novo identification from Supernovo analysis

Light chain

DIQMTQSPSTLSASVGDRVTITCRASQISSWLAWYQQKPGKAPKLLIYKASSLESGVPSRFSGSGSGTEFTLTISLQP
DIQMTQSPSTLSASVGDRVTITCSAS-SRVGYMHYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTEFTLTISLQP
I L I LLI L L I L

DDFATYYCQQYNYSLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
DDFATYYCFQSGYPFTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
I I L LL L

ESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
ESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
L L L L

Figure 61: NIST mAb light chain - de novo identification from Supernovo analysis