Dotmatics
Protein Metrics

Byosphere® Protein Characterization Web Analysis Manual

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Protein Metrics LLC, Boston, Massachusetts, USA

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Overview

Byosphere® **Web Analysis** is a native web application that provides users with the ability to perform data analysis for protein characterization within the Byosphere Web Client. Web Analysis provides an interface embedded within the Byosphere enterprise server that improves customer experience by enabling interactive and iterative computations, providing platform independence, and avoiding upload and download issues that are inherent to the desktop client. Features include:

1. Protein characterization data analysis directly within the Byosphere Web Client
2. Adjustable processing flow to enable quicker, more targeted analysis
3. The ability to produce comprehensive reports
4. The ability to use and create Templates for Analysis that can be reused for multiple analyses

Web Analysis Modes

Currently, users are provided with two different analysis modes in Web Analysis: **Deconvolution** mode predominantly for Intact protein level analyses and **Feature Finder** mode for Peptide level analyses. Templates will be preset to the mode most relevant to the workflow.

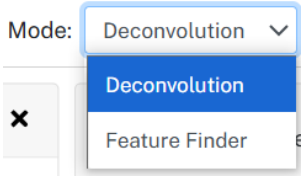


Figure 1: Web Analysis mode

User Privileges

A user must have the **Contributor** role entitlement to create or view a Web Analysis.

Multiple Users

Byosphere® **Web Analyses** can be accessed by multiple users at once if they have the appropriate access level, or by one user with multiple login points. As a result, it is possible that one user/browser might make a change that will not be apparent to one user until the other user refreshes their browser. This has the advantage that an analysis can be accessed simultaneously by different users in different locations.

Refreshing the browser will also reveal any additional changes that have been made by any other user that is accessing the analysis simultaneously. To ensure data fidelity on the underlying database, users can take advantage of the Publish functionality, which will create a “snapshot” of the current state of a Web Analysis at a given moment.

Introduction

The **Web Analysis** application is embedded directly within the Byosphere Web Client.

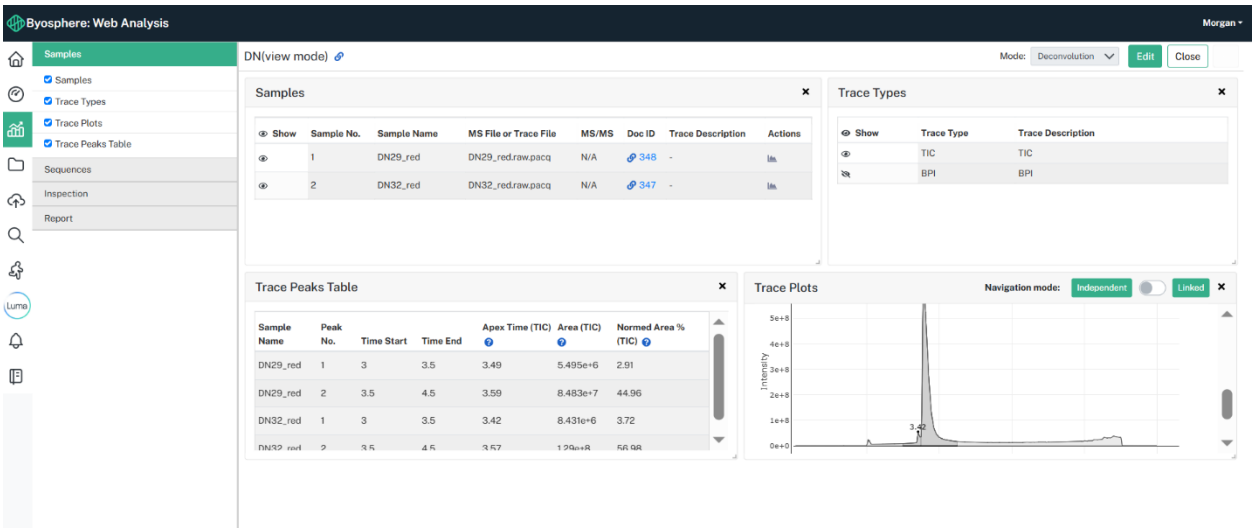


Figure 2: Analysis from Edit Mode in the Samples room view of Deconvolution mode

Users can access Web Analysis from the left navigation panel of Byosphere by clicking the icon.

Within Web Analysis, there are four **rooms**: **Samples**, **Sequences**, **Inspection**, and **Report**. Each room contains widgets relating to a specific part of the overall analysis which vary based upon the selected analysis mode.

Deconvolution mode

- **Samples**: Add samples/traces and integrate peaks using trace range rules, which are visualized within the Trace Plots.
- **Sequences**: Enter sequences and/or specify masses, add sequence combinations, add modifications, adjust Mass Matching parameters, and modify Deconvolution zoom plot settings.
- **Inspection**: Sample Status Review, Inspect Trace Peaks, Inspect Heatmaps (if generated), edit/set Deconvolution presets, as well as view masses and generated MS1 and Deconvolved Mass plots based upon trace peaks.
- **Report**: View analysis summaries, generated plots, and customize charts and tables visualizing analysis data.

Feature Finder mode

- **Samples**: Add samples/traces and integrate peaks using trace range rules, which are visualized within the Trace Plots, and view Features.
- **Sequences**: Enter sequences and/or specify masses, add sequence combinations, add modifications, and adjust Mass Matching parameters.
- **Inspection**: View peptides and associated features, a Sequence Coverage Map and Coverage Summary table, as well as XIC and Isotope plots for the selected feature.
- **Report**: View analysis summaries, parameters used, and generated XIC/Isotope plots.

Creating An Analysis with Templates

Users can create an analysis by clicking on a **Web Analysis System Analysis Template**, as shown below. Templates are prepopulated with modifications, custom presets, and preconfigured reports so that users can easily start their analysis. Web Analysis provides multiple standard templates (`*.wat` files) accessible from the home page:

- **Progressive Deconvolution:** Identification and relative quantification of intact protein species via Progressive Deconvolution
- **Intact**: Identification, characterization, and relative quantification of large molecules
- **Isotope Resolved**: Identification, characterization, and relative quantification of smaller molecules with individual isotopes resolved.
- **Mass Check**: Fast deconvolution to obtain a nominal mass.
- **Native**: Identification, characterization, and relative quantification of non-denatured molecule
- **PTM with MS2**: Identification and Quantification of post-translational modifications
- **Reduced**: Identification, characterization, and relative quantification of reduced large molecules
- **Optical Trace**: Optical Trace (e.g., UV trace) analysis and peak quantitation

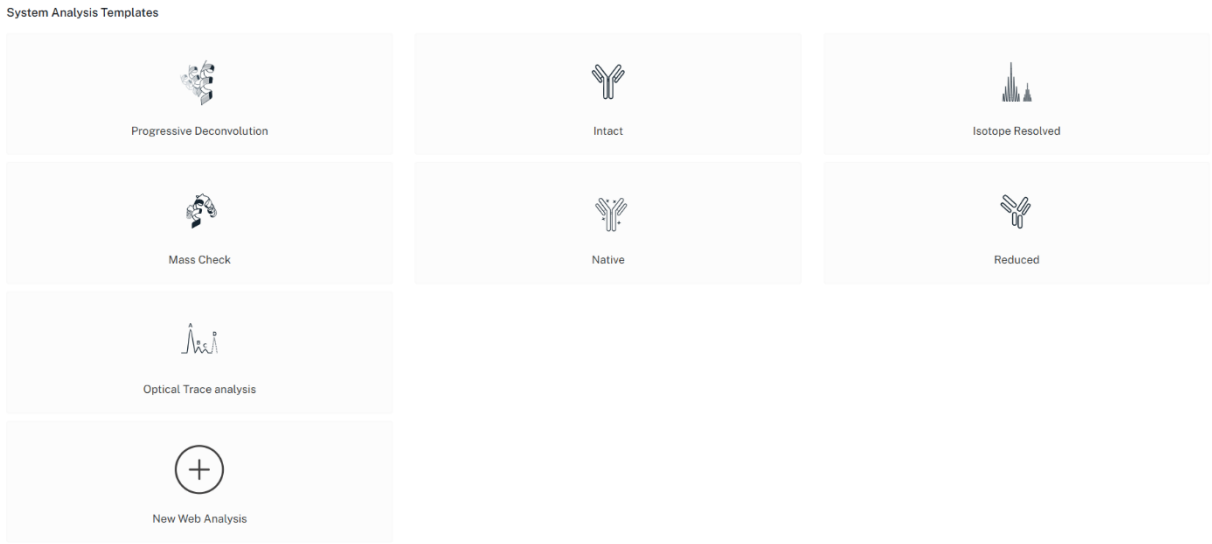


Figure 3: Project Templates

An empty analysis can also be created from the Web Analysis page by clicking “New Web Analysis”.

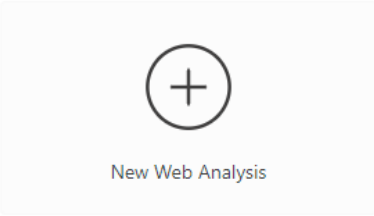


Figure 4: Create a new Web Analysis with no preconfigured settings

Opening an Analysis

When an Analysis is first opened, it is set to **View** Mode. To make any changes to the Analysis, the user must be in **Edit** mode. To enter Edit mode, click **Edit**.

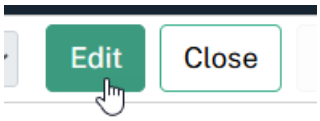


Figure 5: Analysis Header in View Mode

Note that when opening an analysis created in an older version of Byosphere, the user should recompute to ensure that all data is repopulated. For more information on computing, see Compute.

Deconvolution mode

When the Analysis is set to **Deconvolution mode**, room features are configured for the analysis of **intact proteins**.

Samples Room (Deconvolution mode)

The **Samples Room** consists of four widgets: **Samples**, **Trace Types**, **Trace Peaks Table**, and **Trace Plot**

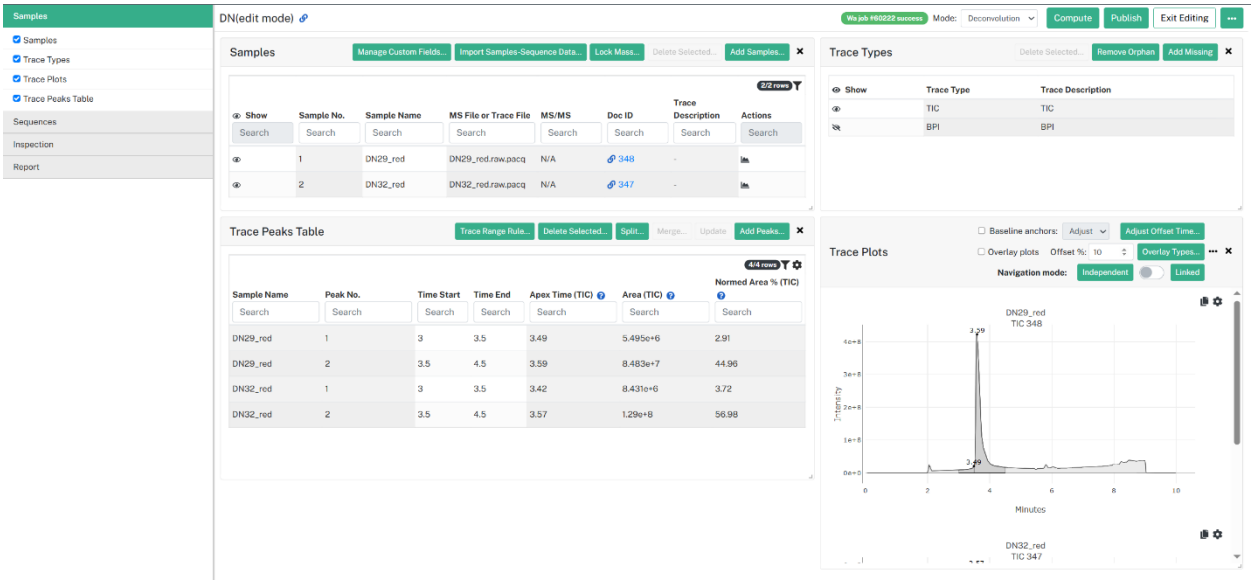


Figure 6: Samples Room (Edit mode)

Samples

The **Samples** view controls the addition, deletion, and visibility of sample files and trace files.

Sample(s) can be added within the **Samples** room by clicking **Add Samples.** The **Select sample to add** dialog list all folders to which the user has access. To add a sample, select a folder from the left pane and check the sample(s) of interest, then click **Add Sample**.

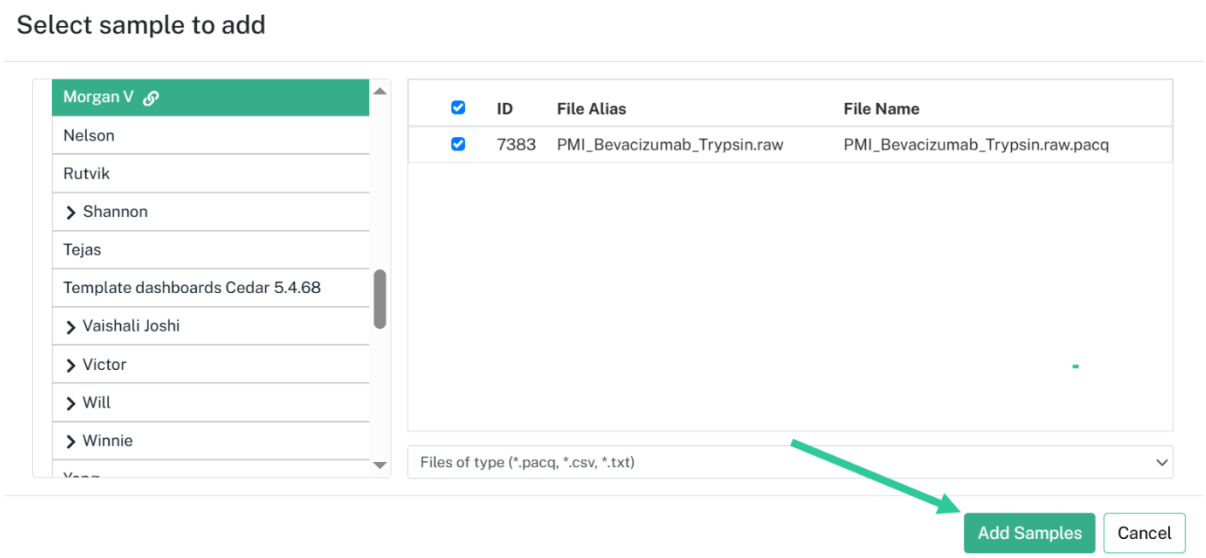


Figure 7: Add Samples dialog

MS files (`*.pacq`) and/or trace files (`*.csv` or `*.txt`) can be added as samples. A progress bar will show the progress of the sample upload. Once finished, the sample will show up in the Samples table and will be associated with a **Sample No.** Sample numbers (Sample No.) are added in sequential order and cannot be edited. **Sample Name** can be edited by clicking in the cell that holds the auto-generated name for each sample and typing a custom entry. The name is the full sample name without the associated MS and `*.pacq` extensions by default. Clicking out of a sample name will immediately save any changes made. For traces, the **MS File or Trace File** and **Trace Description** columns reflect those values from the Trace Types table for individual trace files associated with the sample. These *cannot* be edited within the Samples table. **Doc ID** provides a hyperlink to the associated file within the Byosphere search page.

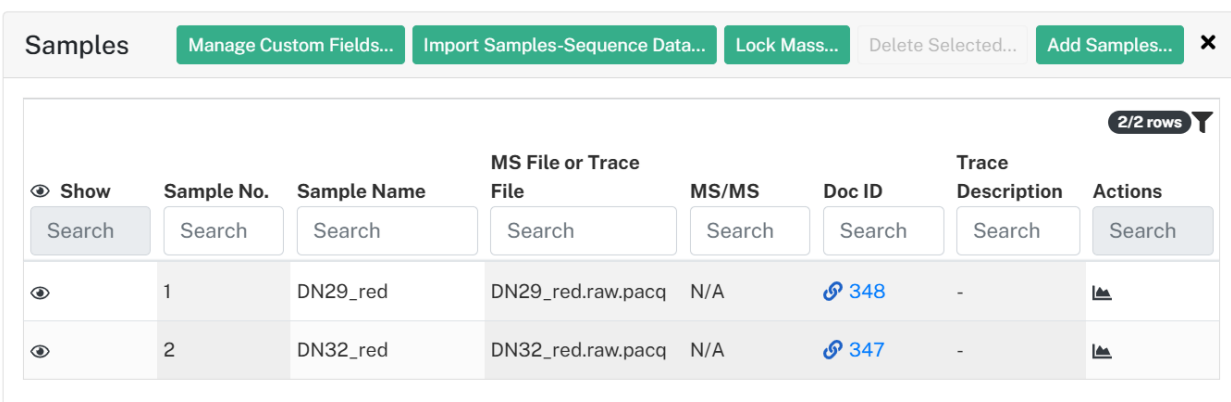


Figure 8: Samples Room

Clicking the Show icon enables/disables the visibility of a sample on the Trace plot, as well as the rows within the Trace Peaks table. When clicked, the icon changes to Hide to denote the visibility of a sample is disabled. The user has the option to disable visibility of a *single* sample or *all* samples, which is performed by clicking the icon in the *header* of the Samples view.

To select samples, click on the rows of the samples of interest. This highlights the samples in blue. Once the samples are selected, the user has the option to delete the samples by clicking **Delete Selected**

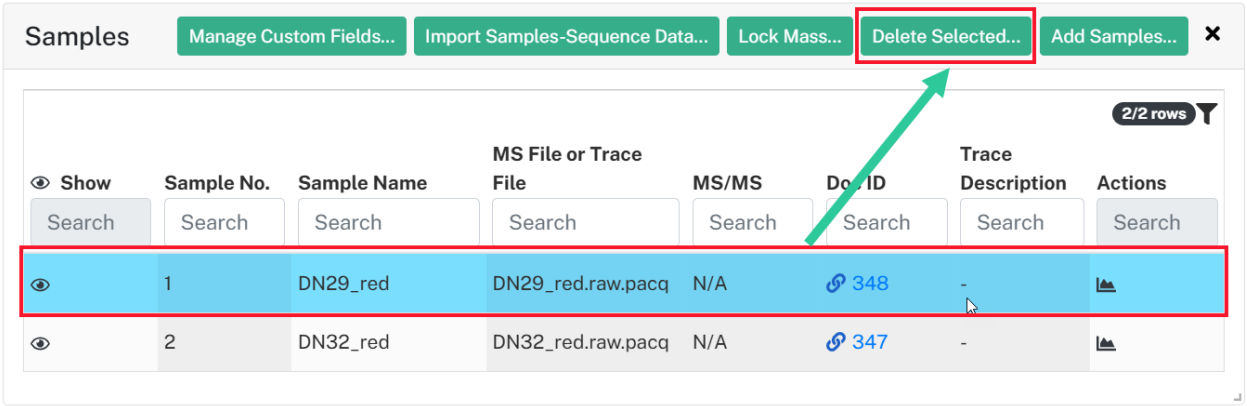


Figure 9: A selected Sample can be deleted by clicking "Delete Selected"

The **Actions** column (currently only available in Deconvolution mode) allows the user to add trace files to sample by clicking the Add Trace File icon for the sample of interest. This launches a file selector that allows the user to browse for and check a trace file to associate with a sample.

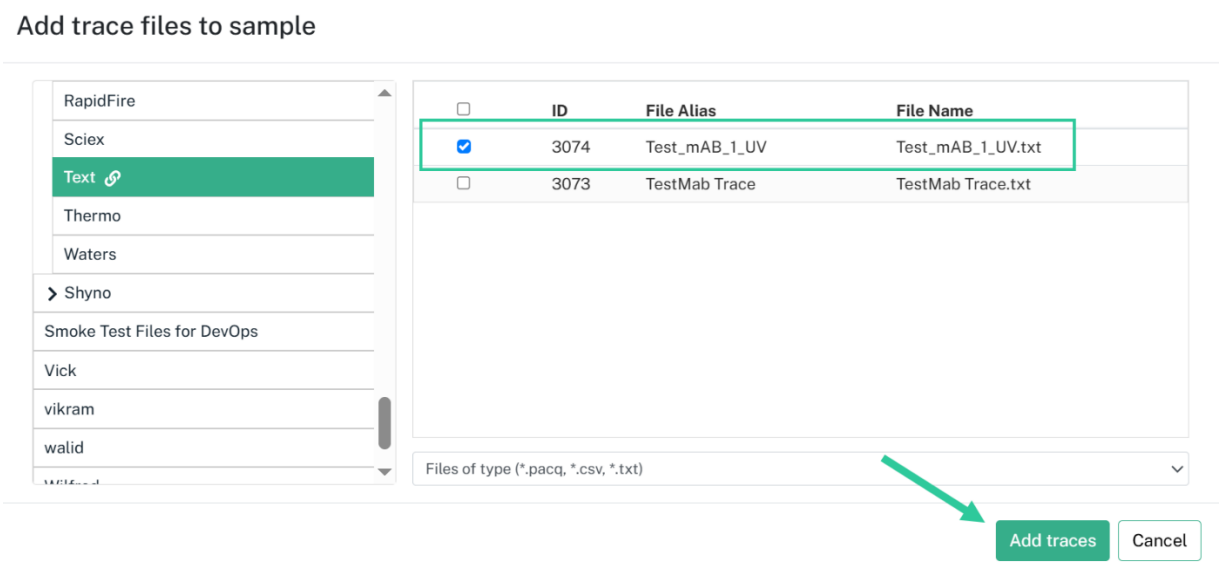


Figure 10: Adding Trace Files

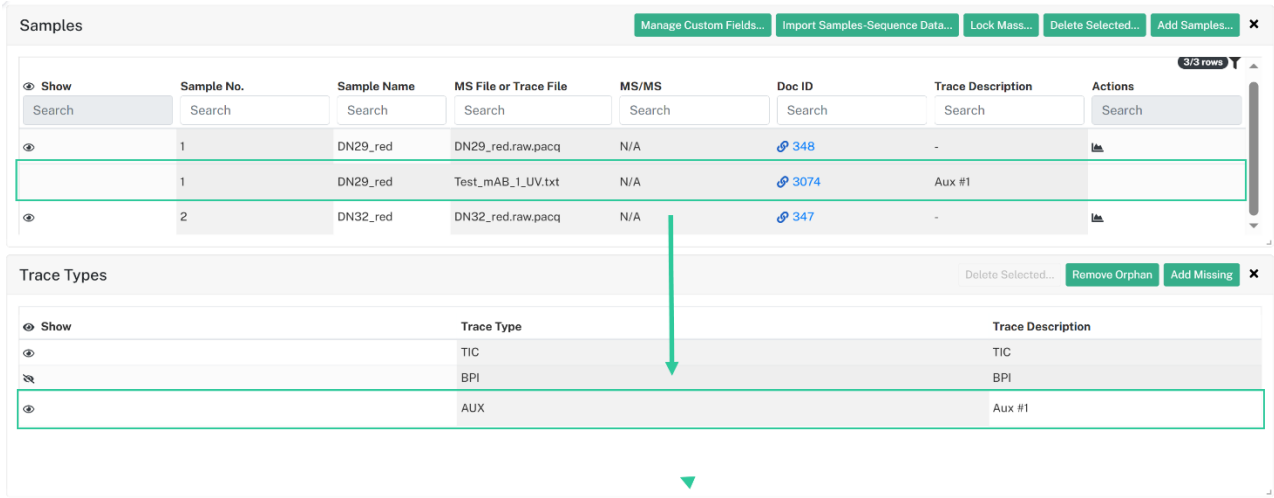


Figure 11: Custom trace added to Sample Name DN29_red

The associated trace files do not become new sample records; rather, they inherit the Sample No. and Sample Name values for the associated sample. The Trace Description values for the trace files (editable in the Trace Types table) are displayed in the Samples view. Under Trace Types, they have the type “AUX”.

If the analysis is saved as a template, the samples will be removed from the resulting template.

Clicking **Import Samples-Sequence Data** opens a dialog to import a CSV file to the analysis. Once it has been imported, the analysis will be populated with the relevant information contained within the file.

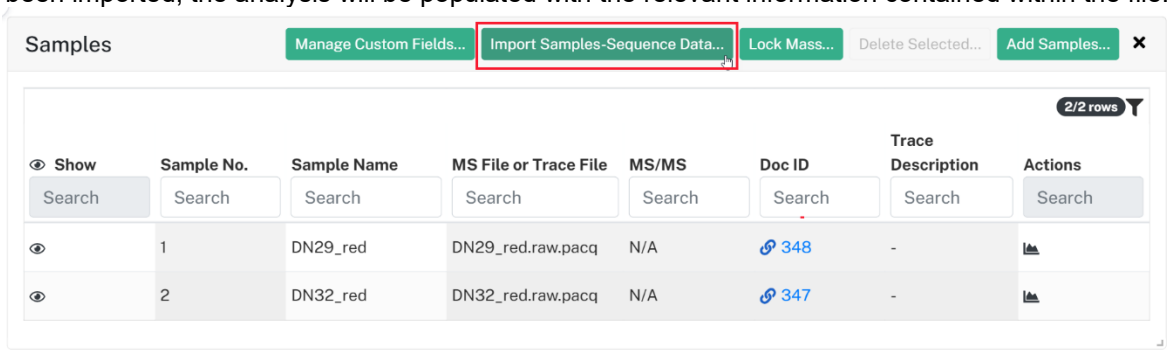


Figure 12: Import Samples-Sequence Data

Users must use the following format for their CSV files to be imported successfully into WA:

ColumnName	FilePath	Sample Name	Sequence or Protein Name 1	Sequence or Protein Name 2
Expectation			Desired or Undesired	Desired or Undesired
ColumnType		SampleName	Mass	Mass
	C:\ExamplePath	Example Sample Name	Sequence1	Sequence2

Table 1: Sample-Sequences Data import format for CSV

Sequences and Combinations tables will also populate if the CSV contains relevant information present in the CSV file. More information on Sequences and Combinations can be found in the Sequences room section.

Clicking on **Manage Custom Fields** opens a dialog that allows the user to add additional custom columns to their Samples table.

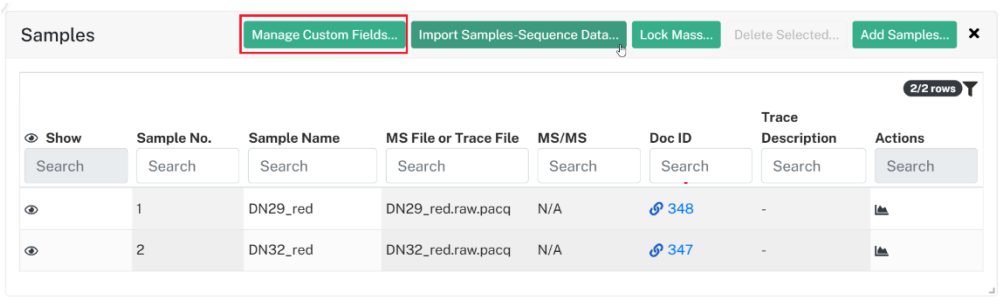


Figure 13: Custom Fields in the Samples table

There are two types of fields that users can introduce using Custom columns:

- **System metadata fields**: Fields that are associated with sample files (usually pacq) within Byosphere
- **Manual custom columns**: Freeform user editable columns within a single analysis, where a user can create a field with whatever name or value they want

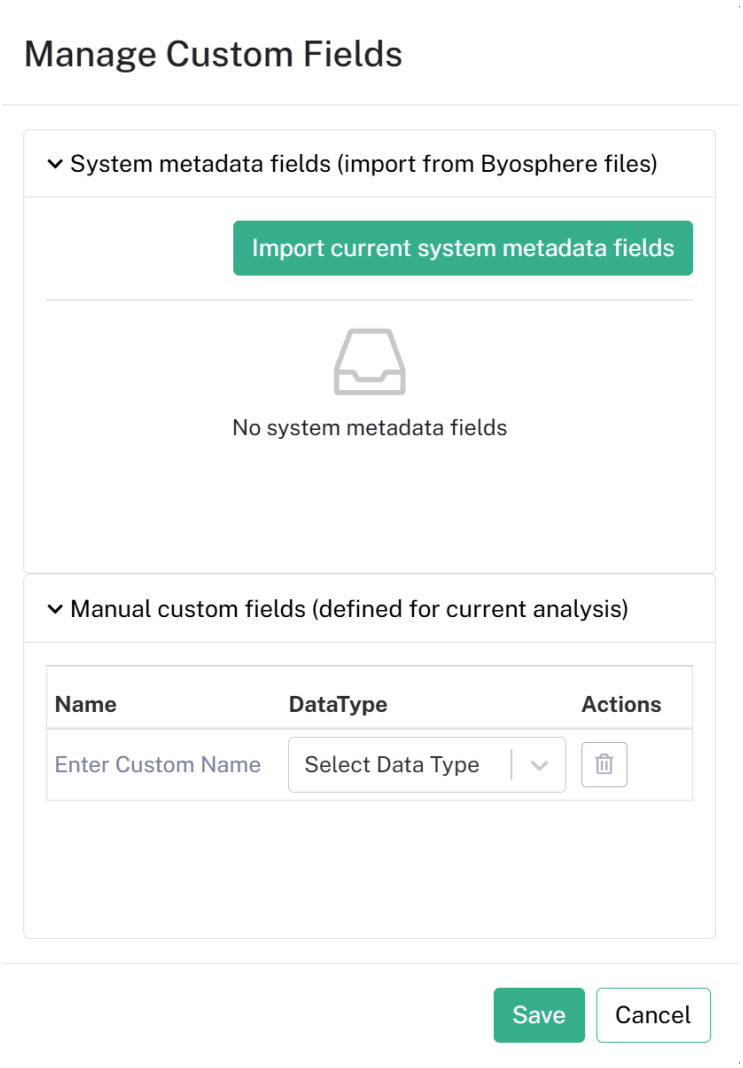


Figure 14: Manage Custom Fields

Clicking **Import Current System Metadata Fields** will provide the user with options to bring in columns for all available system metadata fields:

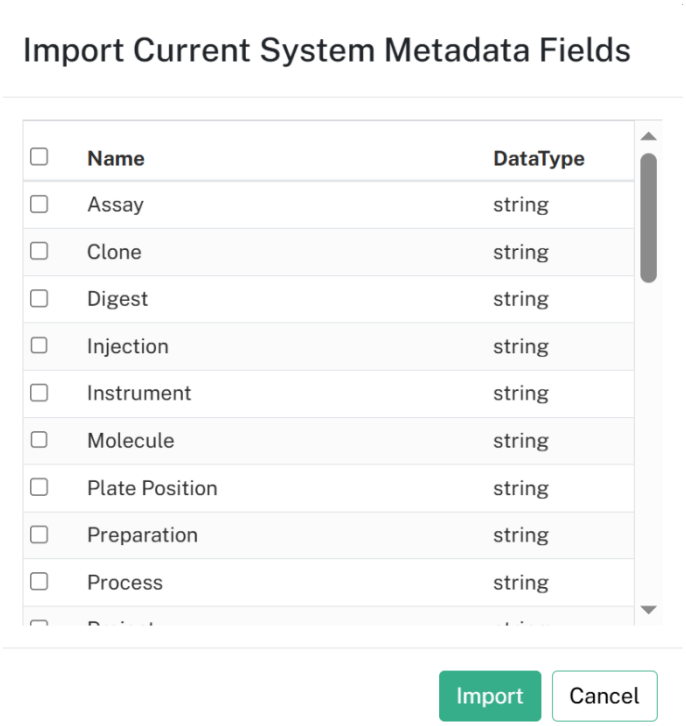


Figure 15: Import Current System Metadata Fields dialog

System metadata fields can be added or removed but not edited within Web Analysis.

In the **Manual custom fields** section, the user can define a string, integer, or real number field with a user-entered value for each row.

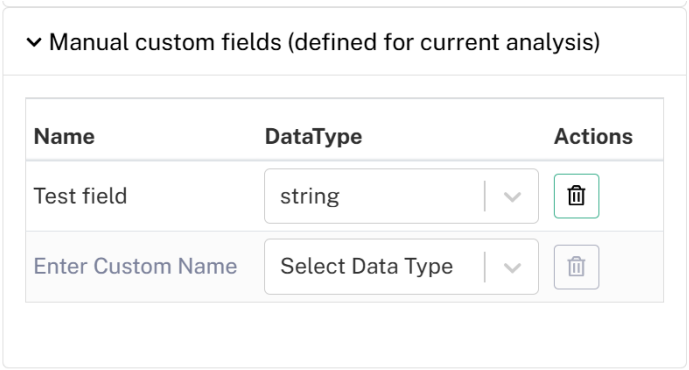


Figure 16: Manual custom fields dialog

Once the custom fields have been added, they will appear at the right side of the Samples table.

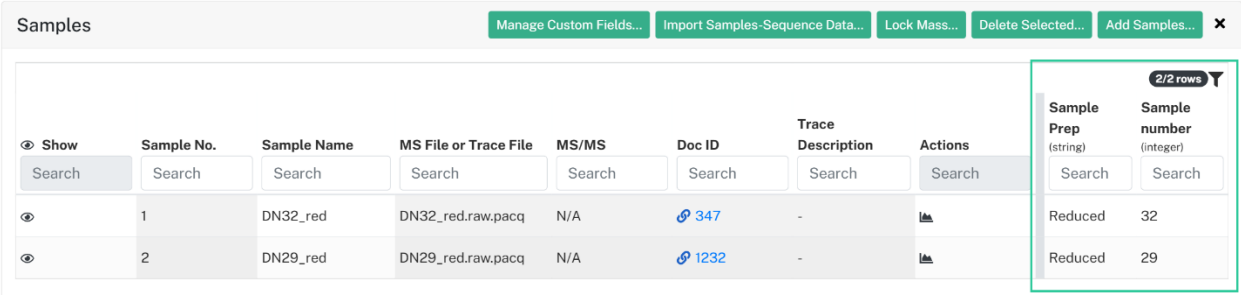


Figure 17: Samples table with custom fields

Trace Types

The **Trace Types** table displays trace types included with uploads of samples or traces.

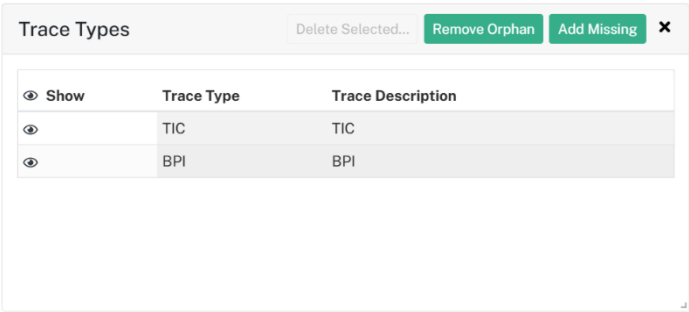


Figure 18: Trace Types Table

The **Show** column allows the user to select or deselect which traces are visualized in the **Trace Plots** and the **Area** columns of the **Trace Peaks Table**. This is performed by clicking the Show icon next to the row of interest. If the user wishes to enable/disable views for *all* trace plots, the user must click the Show icon in the header of the table.

The **Trace Type** column provides information on the type of trace that has been imported. This information cannot be edited, although the user can provide additional information by changing the **Trace Description**, which is editable by clicking within the cell. This field cannot be null and must be unique for each row; additionally, edits to an existing trace description entry with leading or trailing whitespace are not allowed, nor are edits that are duplicates with different casing.

Note that if `*.csv` or `*.txt` files are added, the Trace types are designated as “AUX”.

The Trace Types table buttons include **Delete Selected**, which deletes any selected rows. When rows are selected, they are highlighted blue. Multiple rows can be selected by holding down CTRL when clicking on each row. The Delete Selected button is disabled until at least one row has been selected. The **Remove Orphans** button is used to identify and remove traces added with samples that were later deleted (note: when samples are deleted from the Samples table, the associated Traces uploaded to the Trace Table are unaffected by default. These traces remain “orphaned” within the Trace Types table and must be removed manually using the Remove Orphans button). The **Add Missing** button is used to replace **Trace Types** imported with samples but later deleted manually.

If the analysis is saved as a template, the Trace Type records will be preserved in the resulting template.

Trace Peaks Table

The **Trace Peaks Table** shows the peak assignments for all visible samples after the **Trace Range Rule** is applied. Peak rows are generated by clicking the **Update** button within the Trace Range Rule view.

Sample name comes directly from the name of the sample as shown in the **Samples** table. Peak rows are sorted for each sample, first by **Time start** and second by **Time end** and then identified peaks are assigned sequential **Peak No.** counts (not IDs). **Area** (denoted with the trace type in parentheses) is calculated for each peak and a column is added and named for each **Trace Description** that is visible in the **Trace Types** table. Note that Trace Description may be long and require truncation. By default, rows are sorted first by Sample name and second by Peak No.

The Sample Name and Peak No. columns are not editable, as designated by their darker shading. **Time start** and **Time end** can be edited, although the row will be crossed out until the user clicks **Update**, which will update the associated values accordingly.

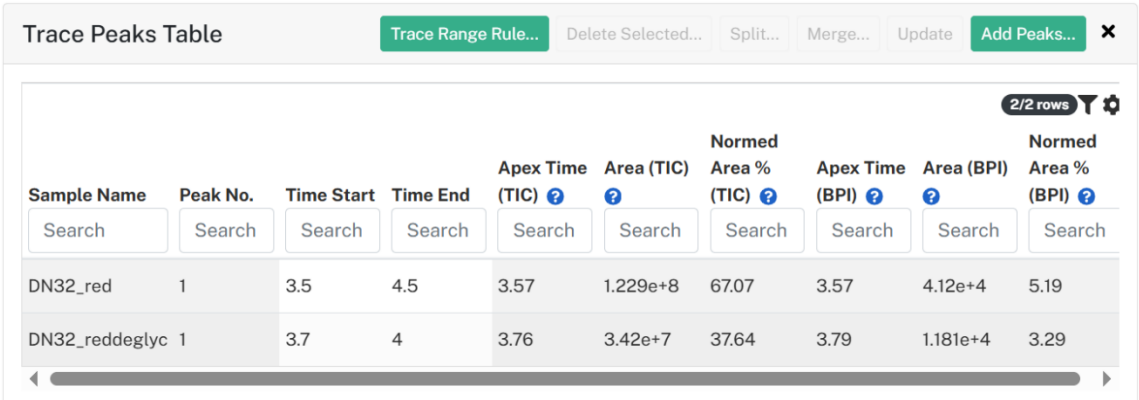


Figure 19: Trace Peaks Table

Specific Apex Time and Area are provided in the Trace Peaks Table for all *visible* trace types.

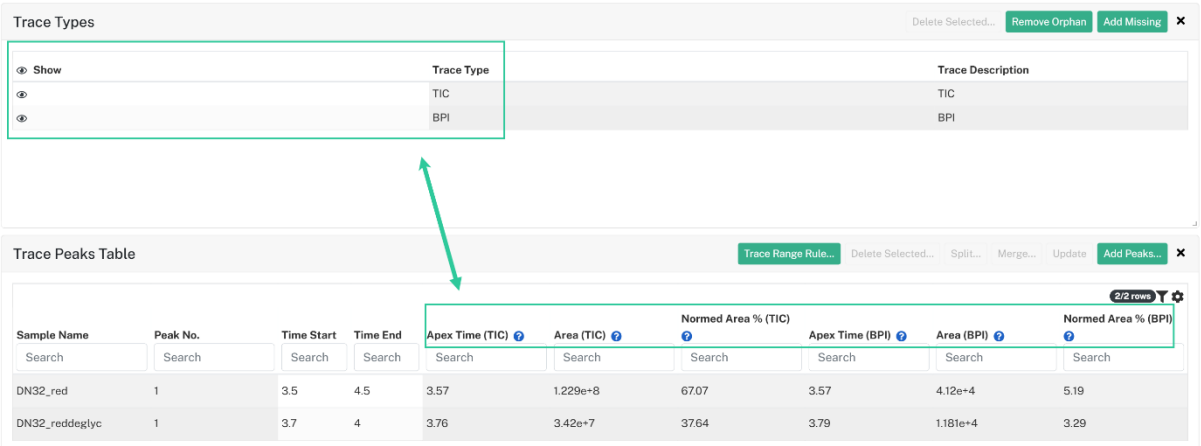


Figure 20: Apex Time and Area for TIC and BPI trace types, when both are set to visible

Before any trace peaks have been added, all buttons at the top are visible, but only **Add Peaks** can be selected. Once peak(s) are added, the other two become available. Time start/Time end values can be added manually to the Trace Peaks table by clicking **Add Peaks**.

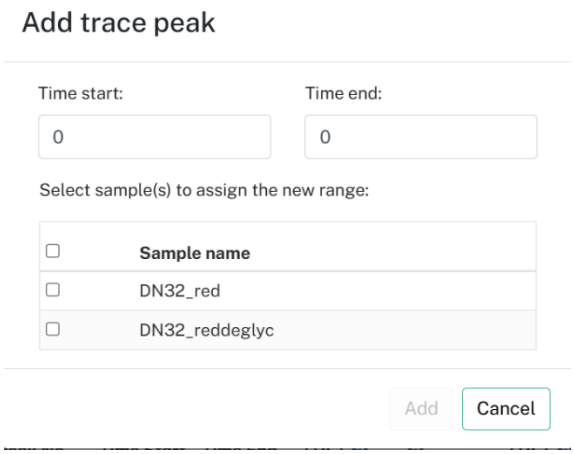


Figure 21: Add Trace Peak dialog

The **Trace Range Rule** button allows the user to configure the settings used to generate slices from the traces in the samples (See Trace Range Rule). Once a trace range rule is defined and the user clicks **Compute**, the Trace Peaks Table will be populated with trace peaks based upon the trace range rule set.

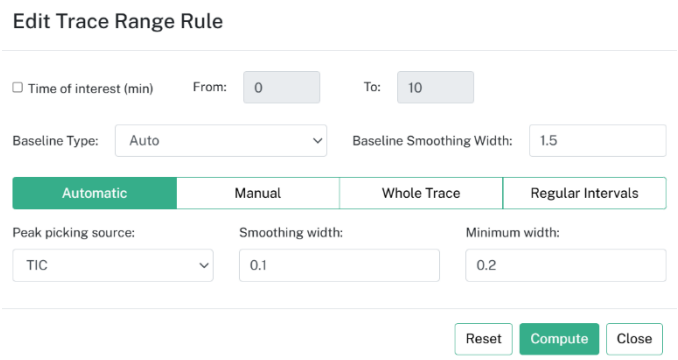


Figure 22: Trace Range Rule

The user must click **Compute** in the dialog to save any changes, then **Update** in the Trace Peaks Table to validate the new Area values and remove the strikethroughs.

The **Update** button in the Trace Peaks Table recalculates the Area column values when the Time start or Time end values are changed in the table or when the magenta integration bars in the trace plot are moved. Time settings invalidate the old Area values, which will be displayed with a strikethrough to signify that they are obsolete prior to being updated.

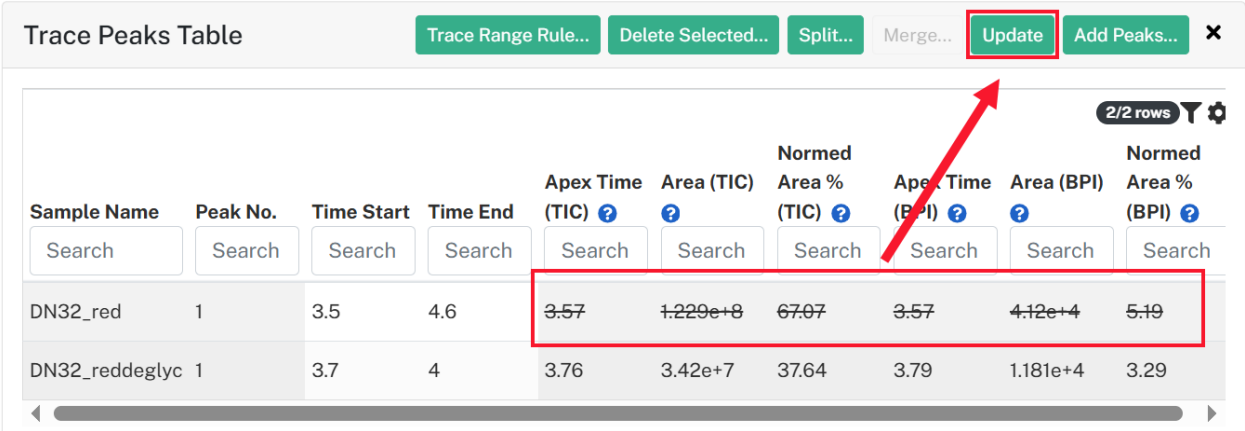


Figure 23: Invalidated area values after changing Time values

Edits to the Trace Peak Table Time start and end values also update the integration boundaries in the **Trace Plots** for that sample, as shown below.

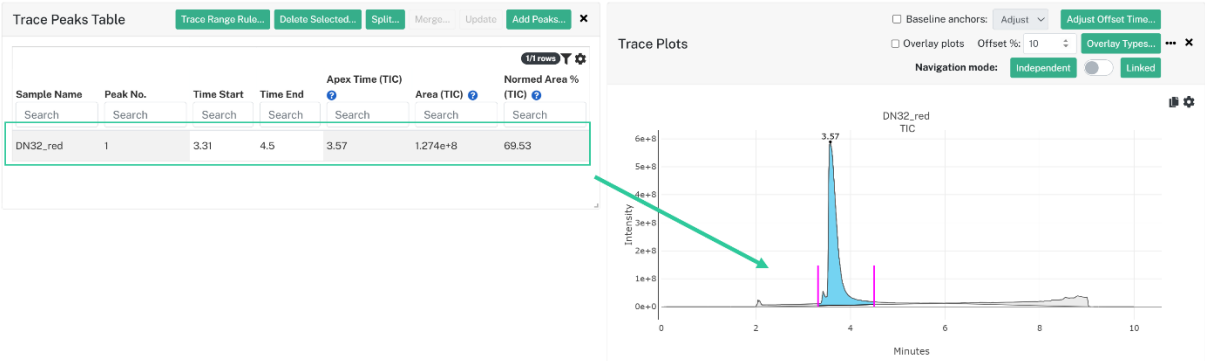


Figure 24: Trace plot integration limits and corresponding Trace Peak

To delete a trace peak, click on the row in a region that does not allow user entry (colored in gray versus white). The row should highlight blue and the **Delete selected** button can now be clicked. To multi-select rows to be deleted, hold down Shift or CTRL when clicking rows and click Delete selected when finished. If the Delete button is clicked, the highlighted trace peak row(s) are removed from the Trace Peaks Table as well as from the Trace Plots (any integration bars associated with the deleted row will be removed).

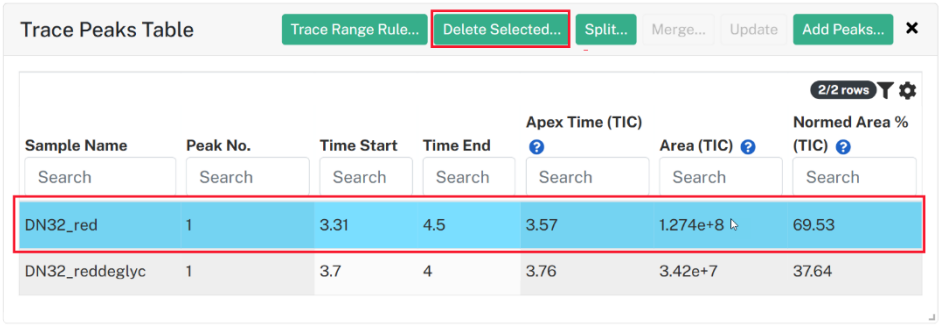


Figure 25: Delete Selected; multi-selected rows are highlighted in blue

If the analysis is saved as a template, the Trace Peak Table records will be removed, along with the samples from the resulting template.

Trace Range Rule

Trace Range Rule settings can be configured by clicking **Trace Range Rule** in the Trace Peaks Table view.

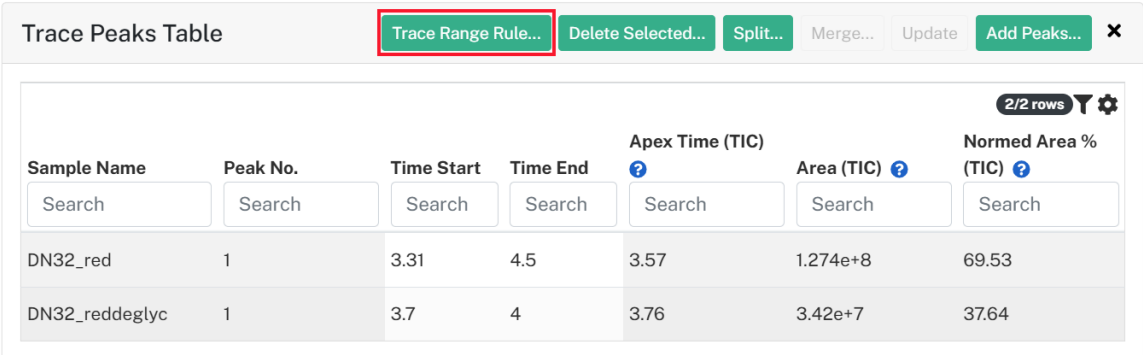


Figure 26: Trace Range Rule settings are modified from the Trace Peaks Table

This opens the **Edit Trace Range Rule** dialog, which allows the user to configure the computations used to generate slices from the traces in the samples.

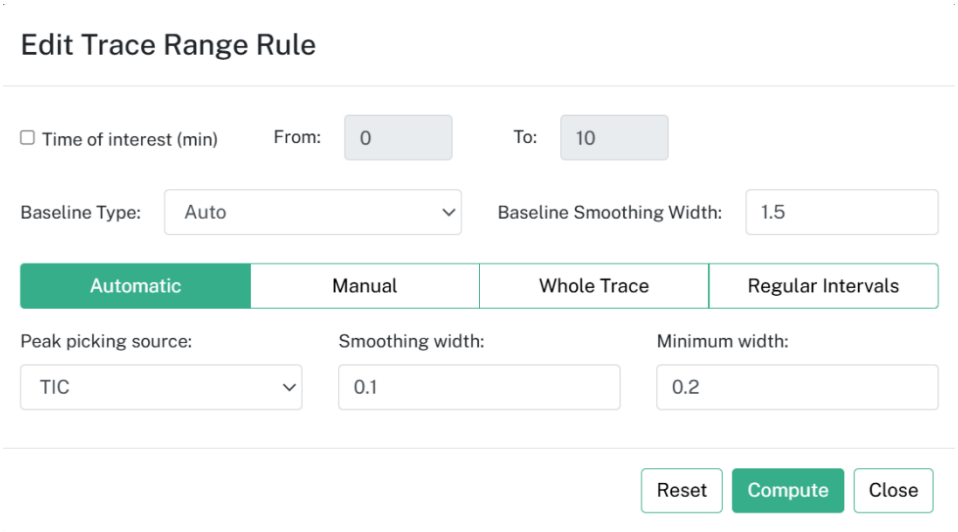


Figure 27: Trace Range Rule dialog

When **Time of Interest (min)** is checked (unchecked by default), calculated peaks are defined within trace **Time start** and **Time end** values. When applied with the Automatic trace range rule, any peak with an apex that falls within the user-defined Time of Interest range will be fully integrated and will not truncate the end of the peak.

Compute gives the user the option to select which samples the Trace Range rule should apply to.

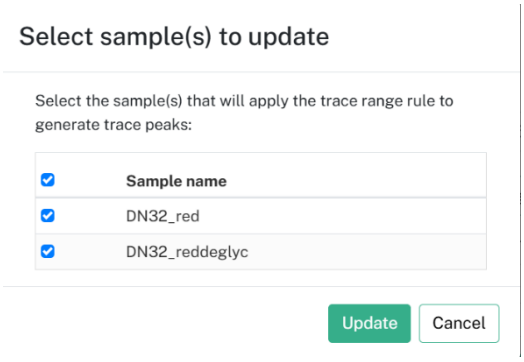


Figure 28: Compute dialog

Clicking **Update** in the dialog generates peaks in the **Trace Peaks Table** as defined by the Trace Range Rule and calculates areas for those peaks, replacing any existing rows. Hiding or deleting Trace Types hides the corresponding Area columns in the **Trace Peaks Table**. Similarly, showing (deselecting the Hide icon) or adding Trace Types will show the corresponding Area columns in the Trace Peaks Table, but will not populate them without clicking **Compute** within the Trace Range Rule widget or **Compute** at the top of the Analysis.

Trace Range Rule Computation options (Deconvolution mode only)

Time of interest (min)

Automatic: When applied with the Automatic trace range rule, any peak with an apex that falls within the user defined Time of Interest range will be fully integrated and will not truncate the end of the peak.

Manual: When applied with the Manual trace range rule, will constrain the min and max times for all generated peaks. Regardless of the manual peaks defined, if a peak is defined with a time that lies outside of the Time of interest, the peak start/end time will be moved to the edge of the Time of Interest.

Whole Trace: The only additional input that can be set for Whole Trace— if checked, the Whole Trace will have its start and end time defined by the Time of Interest

Regular Intervals: Required to be checked for generating regular intervals. The regular intervals will be generated between the start and end time of interest.

Automatic

The automatic option uses the specified parameters to automatically identify individual trace peak start and end times (and calculate the associated peak areas) for each sample:

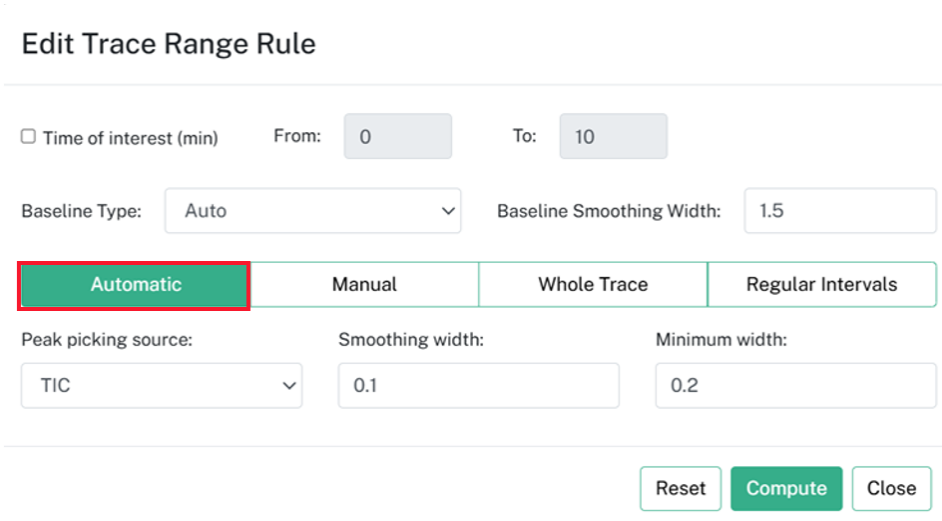


Figure 29: Trace Range Rule – Automatic

The user can select a **Peak picking source** by using the dropdown of available and unique *Trace Descriptions* available from the Trace Types table (including those that are hidden with the Hide icon). This Trace Description determines the trace used to calculate peak slices. If a trace type is deleted from the Trace Types table, it will not be available in the dropdown. The **Peak picking source** trace type is determined by the trace selected within Sample Selection when updating the trace range rule. TIC is selected by default when a TIC is an available trace. Other trace types may be selected from the drop down to be used to determine trace integration peaks.

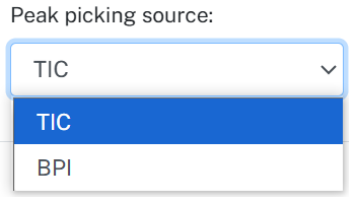


Figure 30: Peak Picking Source

Smoothing Width and **Minimum Peak Width** are **time settings.** The time entered for Smoothing Width relates to the amount of smoothing that is applied to the trace peak, whereas Minimum Peak Width defines the minimum width (baseline) that a peak must be to be picked as a peak and integrated. For narrow chromatographic peaks this value may need to be lowered; however, a too narrow setting may cause background to be integrated as a peak. The default value of 0.2 minutes for peak width should work well for most intact chromatography. To determine a suitable value, it is recommended to inspect the Trace Plot.

Manual

The **Manual** trace option uses a table containing peak start and end times for the user to define the peaks explicitly. The table is empty by default, and peaks are added by clicking **Add**. If an analysis with values in the Manual table is saved as a template, these values will persist in the template and appear when the template is used to generate a new analysis.

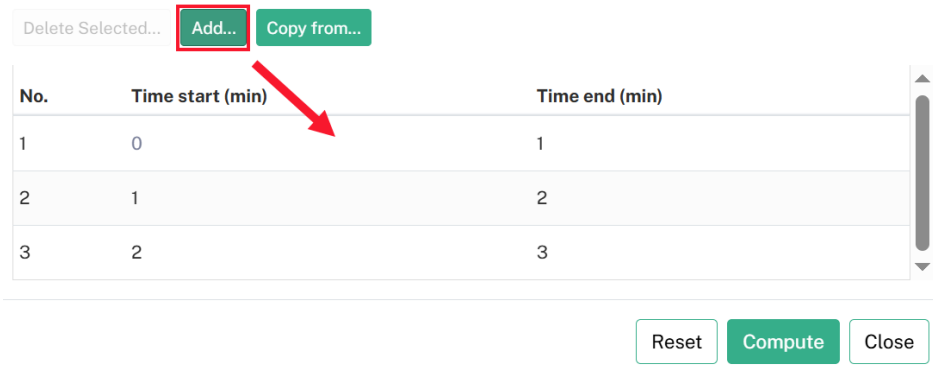


Figure 31: Trace Range Rule - Manual

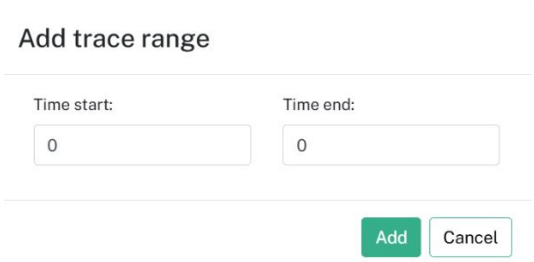


Figure 32: Add Trace Range dialog

Time start (min) and **Time end (min)** are editable by the user. The **Copy From** button populates the Manual table with Time start and end values of a single sample in the Trace Peaks Table (Note: this option is only enabled if the Trace Peaks Table is populated). The following dialog allows the user to select a sample from which the trace ranges present in the Trace Peaks Table are copied to populate the Manual trace ranges.

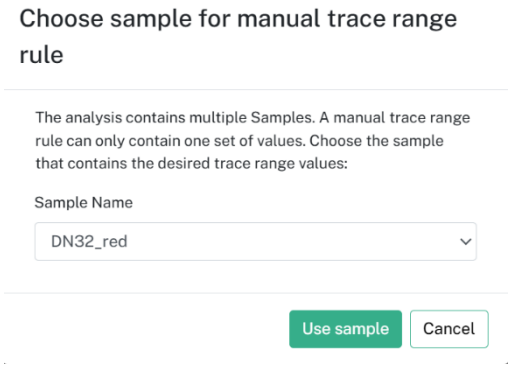


Figure 33: "Copy From" dialog

Note that the Copy From option will overwrite any table values already present with the values from the selected sample.

Whole Trace

The **Whole Trace** option treats the entire trace as a single trace range. The only input parameter is the option **Time of interest (min)** checkbox already present at the top of the dialog.

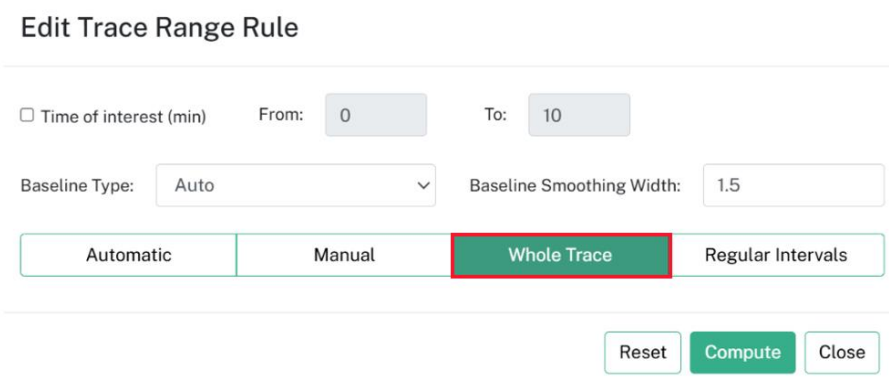


Figure 34: Trace Range Rule - Whole Trace

Regular Intervals

The **Regular Intervals** option generates equal width time slices.

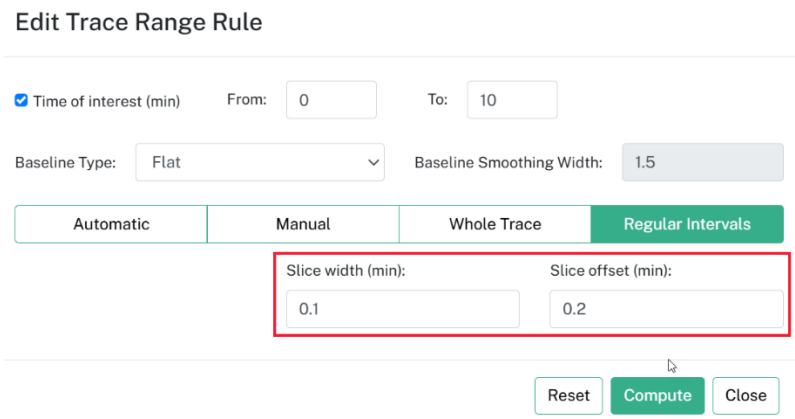


Figure 35: Trace Range Rule - Regular Intervals

The user can control:

- **Time of interest (min)**: Used to define regular intervals (defines the time range over which to apply the regular interval slices).
- **Slice width (min):** The time range for each slice (real number entry)
- **Slice offset:** The time after the Start time for each interval when the next interval is defined to start (real number entry)

For example, if a user wished to create consecutive slices of 0.1 minutes over a time range from 3-4 minutes, they would set the time of interest from 3 to 4 minutes. The slice width would be set to 0.1.

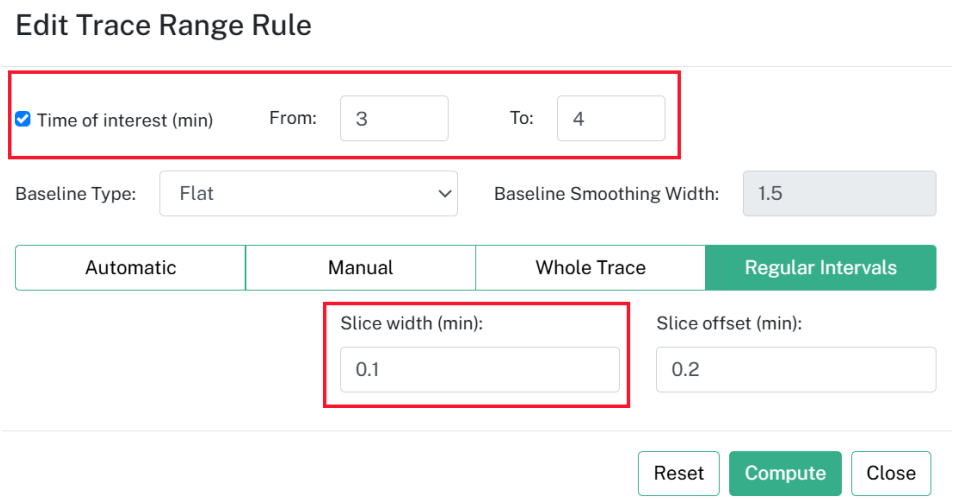


Figure 36: Regular Intervals Example

The first slice would start at 3 minutes, and end at 3.1 minutes.

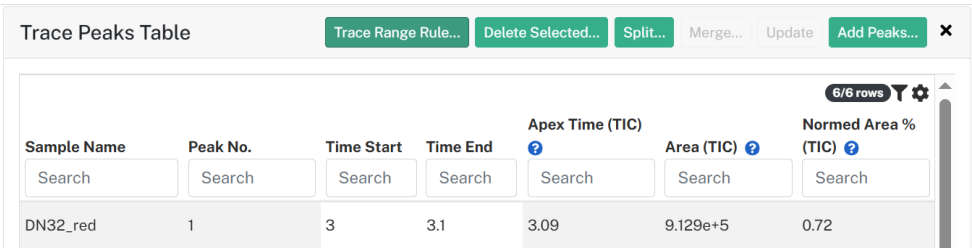


Figure 37: First slice from above

The slice offset will define the time taken from the start of the previous slice to the start of the next slice. In this example, a slice offset of 0.2 would mean that the next slice would begin at 3.2 minutes (offset 0.2 from the previous peak start).

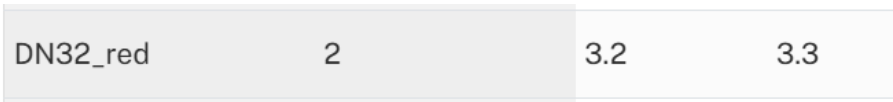


Figure 38: Second peak with Slice width of 0.1 and offset of 0.2

Changing the Trace Range Rule

- When a trace range rule option is changed for an analysis with one or more samples, the new rule is not applied automatically to those samples. The user must click **Update** to apply the new rule to existing samples. Once the new trace range rule is updated, the previous traces for the selected sample are removed and replaced with the new traces.
- When a trace range rule is changed, the default values for the new rule are displayed. Those values do not reflect the previous rule settings or Trace Peaks Table values. In the case of the Manual option, the default setting is to have no ranges defined.
- When samples or trace types are added to the analysis, the current Trace Range Rule is not applied automatically, and the values must be computed by clicking **Update**.

When **Update** is clicked, the **Select sample(s) to update** dialog is opened, which allows the user to choose which samples to apply the updated trace range rules to generate trace peaks from:

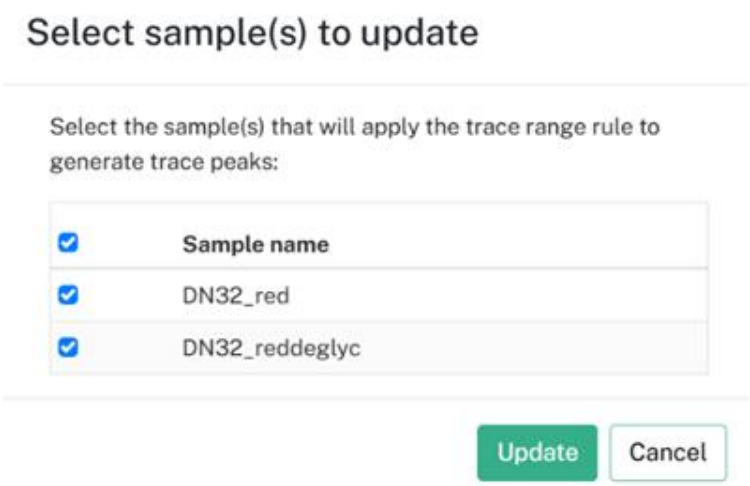


Figure 39: Select sample(s) to update dialog

If the analysis is saved as a template, the Trace Range Rule settings (including Manual peak times) will be preserved in the resulting template.

Flat vs Auto Baseline

There are two modes that the user can select for applying the baseline in any of the trace range rule computation options.

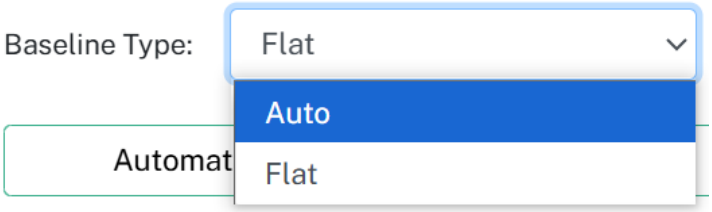


Figure 40: Baseline options

For an **Auto** baseline with **smoothing**, the baseline fits to the base of the peak, allowing for quantitation that excludes any dip in baseline (if there is a dip in baseline, that extra area may be integrated, and so adjust the peak area, changing the quantitation). For a **Flat** baseline, there is no fit to the base of the peak. **Auto** is the default option.

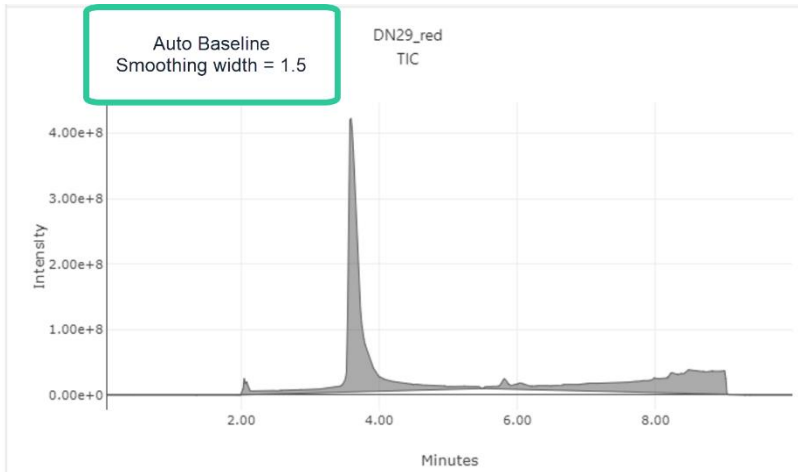


Figure 41: Auto Baseline

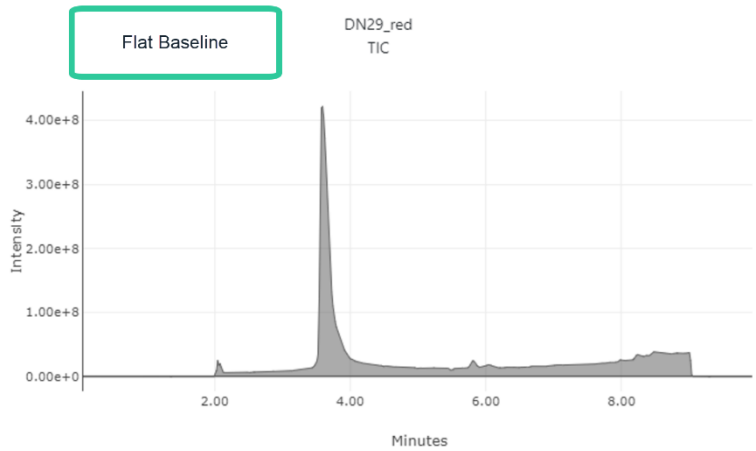


Figure 42: Flat Baseline

Splitting and Merging Peaks

Users can merge or split trace peaks using the **Split** and **Merge** buttons in the Trace Peaks Table.

When a user highlights a single peak and clicks Split, two peaks will result; the start time of the first new peak has the same start time as the original peak and the end time of the second new peak has the same end time as the original peak.

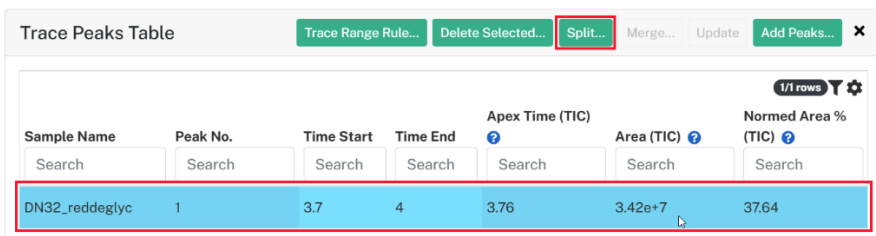


Figure 43: Split Trace Peak

DN32_reddeglyc	1	3.7	3.85	3.76	2.231e+7	24.55
DN32_reddeglyc	2	3.85	4	3.86	1.19e+7	13.09

Figure 44: Resulting two peaks from a Split

When a user highlights two or more adjacent peaks in the trace peaks table and clicks Merge, the start time of the single merged peak will equal the start time of the first highlighted peak and the end time of the single merged peak will equal the end time of the last highlighted peak.

The user must click Update for AUC values for these new peaks to populate.

Trace Plots

The **Trace Plots** widget shows all sample traces that are set to be visible. All visible trace plots can be shown by scrolling within the Trace Plot view.

When a peak is selected in the Trace Peaks Table, the integration boundaries (start and end times) are displayed within the Trace Plots as magenta vertical lines with the area under the curve (AUC) shaded blue.

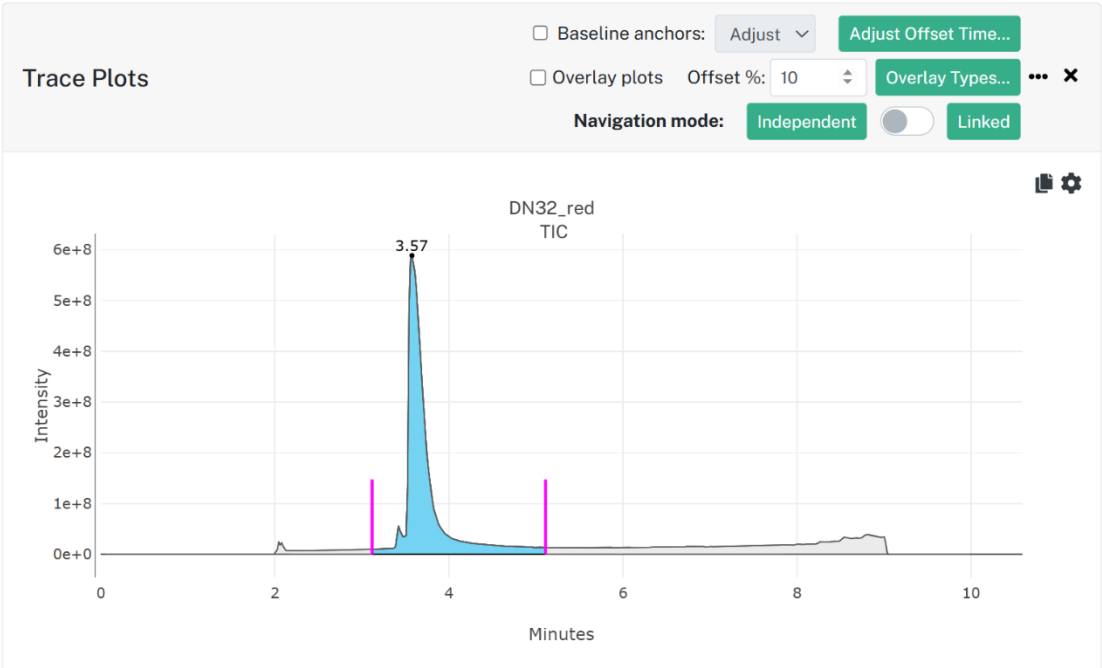


Figure 45: Trace Plot with integration boundaries

The user can adjust these integration bars by clicking and dragging them within the Trace plot. The integration lines move only along the x-axis. When the mouse is released, the line is fixed at that time. The corresponding peak row within the **Trace Peaks Table** is updated with the new start and/or end time and the areas can be recomputed by clicking **Update**.

Changes made to the start and end time in the Trace Peaks Table, in turn, will update the position of the integration lines.

Clicking **Adjust Offset Time** opens a dialog that gives the user the ability to adjust the offset time for each trace. Changes to the offset time will render a trace obsolete until it is updated, parallel to the behavior described above for changing Time start/Time end. **Offset Time** represents the amount of time in minutes that the respective trace in the Sample (a row in the table below) is offset from the original value obtained upon the initial trace extraction.

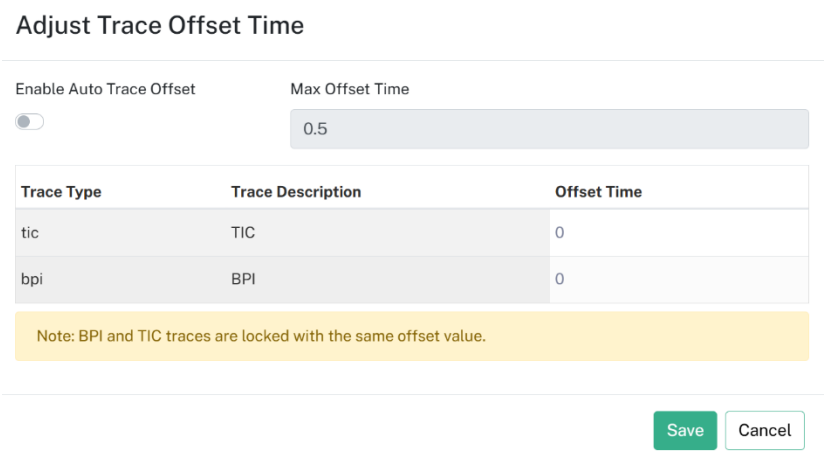


Figure 46: Adjust Offset Time

Users can set the **Max Offset Time**, as well as allow for **Auto Trace Offset**. **Auto Trace Offset** enables auto alignment among traces within a given sample, which is needed sometimes due to the MS instrument's attachment with tandem secondary sensors such as UV detectors. It is important to note that all the non-mass spectrometric traces (e.g., UV, FLR) are aligned with respect to the mass spectrometric trace (e.g., TIC) if present in the sample by automatically computing their respective offset times.

Note: When using Enable Auto Trace Offset *after* adding samples and/or traces or when using Adjust Offset Time, the user needs to apply a **Compute** step with **Extract Traces** only for that sample and check **Clear first**. If the Compute step is not applied, the Trace Alignment rules are simply set and not *applied* to the desired traces.

The user can zoom into the Trace Plot by clicking and dragging over the area of interest. A box will appear around the region over which the view will be zoomed when the mouse is released.

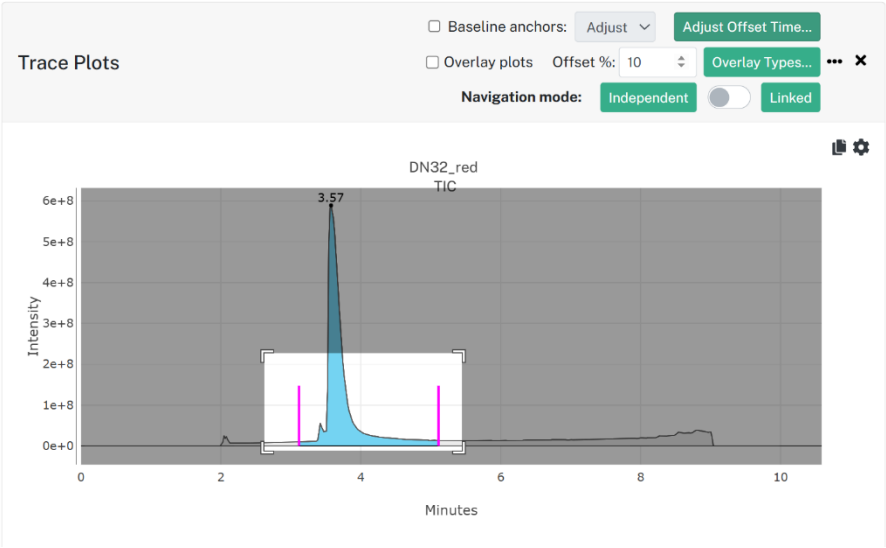


Figure 47: Before zoom

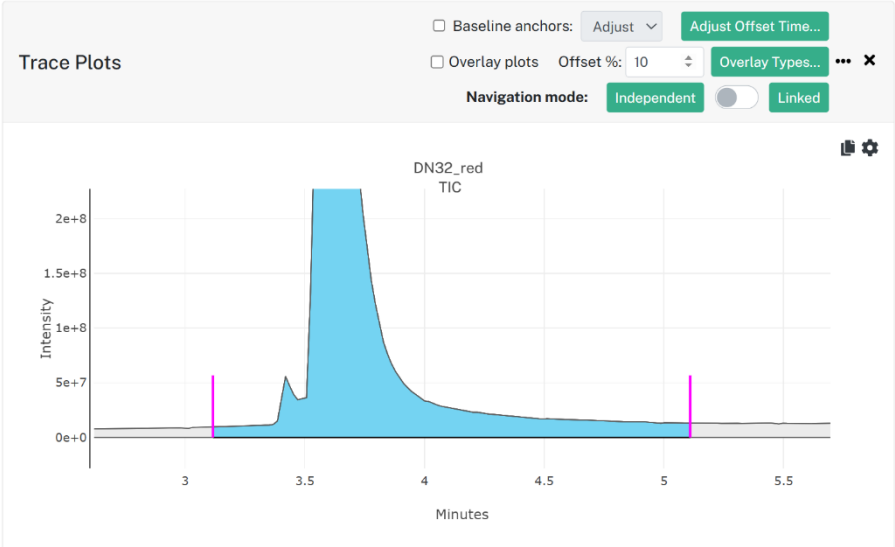


Figure 48: After zoom

To pan, hover over either the X or Y axis until the mouse turns into a double arrow ⇔ , then click and drag the axis left/right or up/down.

The vertical integration lines are not affected by zooming, panning, or resetting the view.

If the analysis is saved as a template, the Trace Plots will be removed, along with the samples from the resulting template.

Baseline Anchors

Users can manually adjust baseline anchors using Adjust, Insert, and Delete operations.

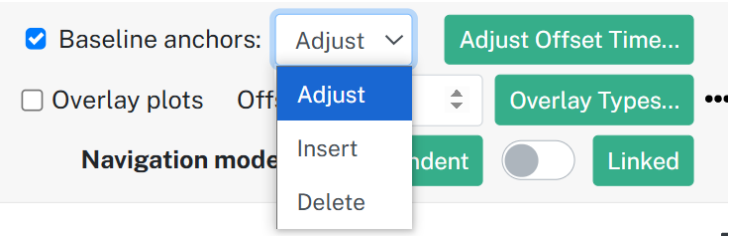


Figure 49: Baseline anchors options

When **Adjust** is selected, the user can click on and move the red anchors to any spot on the plot:

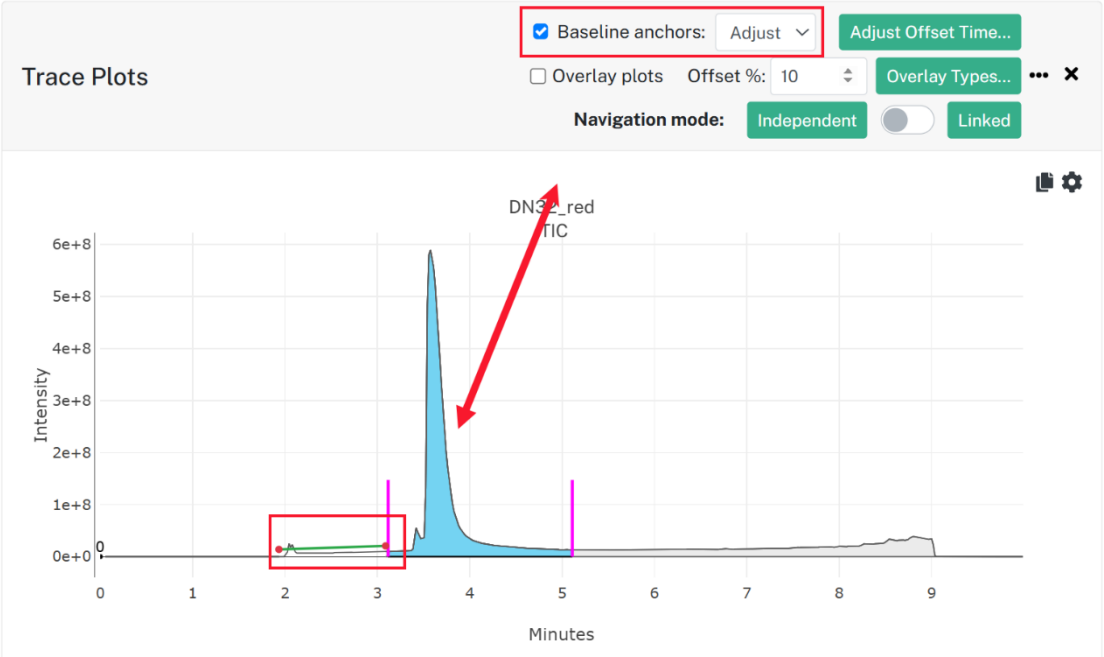


Figure 50: Adjust anchor

When **Insert** is selected, a red dot representing an anchor will be added wherever on the plot the user clicks.

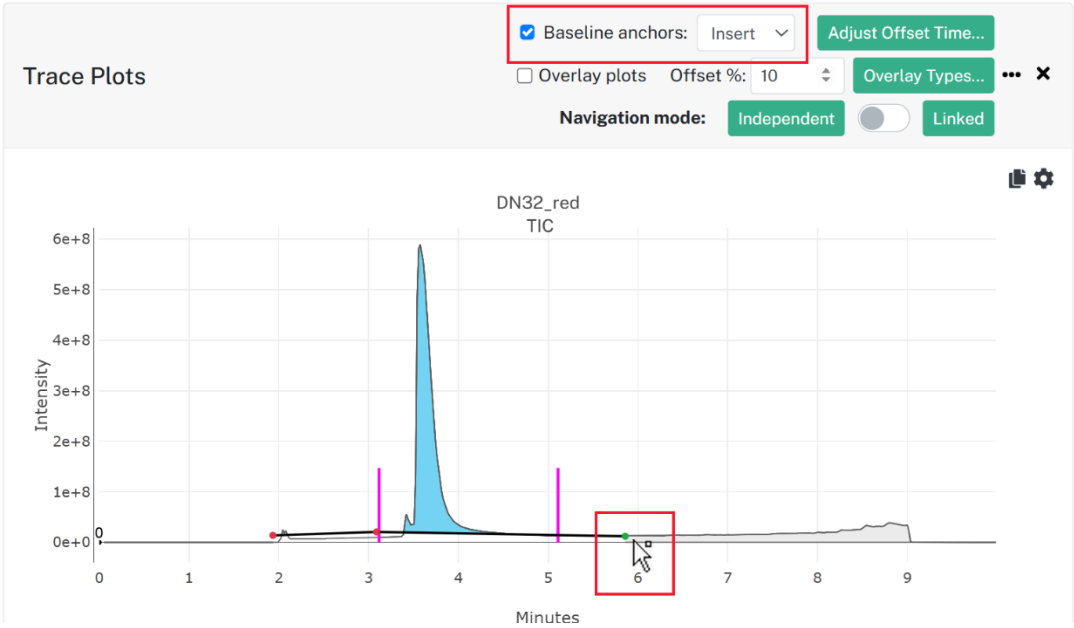


Figure 51: Insert anchor

When **Delete** is selected, hovering over and clicking any anchor will delete the anchor from the plot, as long as there are more than 2 anchors present. The user cannot delete an anchor if there are only two left.

Figure 52: Delete anchor

Trace Overlay

Traces of the same trace type (e.g., TIC, BPI) can now be overlayed within the same plot. Users can enable Trace Overlay by checking the **Overlay Plots** box on the Trace Plots widget.

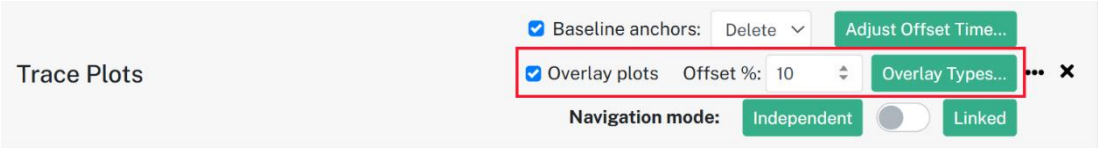


Figure 53: Overlay trace plots

Only trace types that are currently marked as visible with the eye icon will be enabled in the list of trace types to overlay. Users can select which traces to create overlays for by clicking **Overlay Types** in the Trace Plots widget. Note that only traces currently set to visible (with the eye icon) will be available to check. In the figure below, BPI is currently hidden.

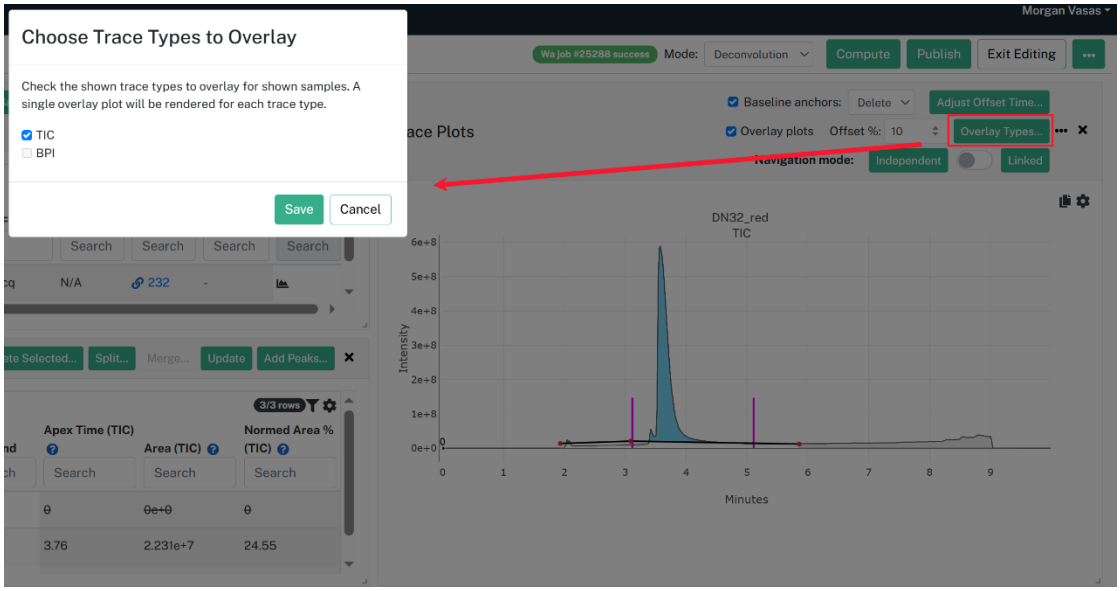


Figure 54: Overlay selection dialog

Once an overlay has been created, the offset between each plot can be configured to be anywhere based on percentage. A 0% overlay will result in all plots being positioned directly on top of one another, while the distance between each plot will increase with each successive percent value.

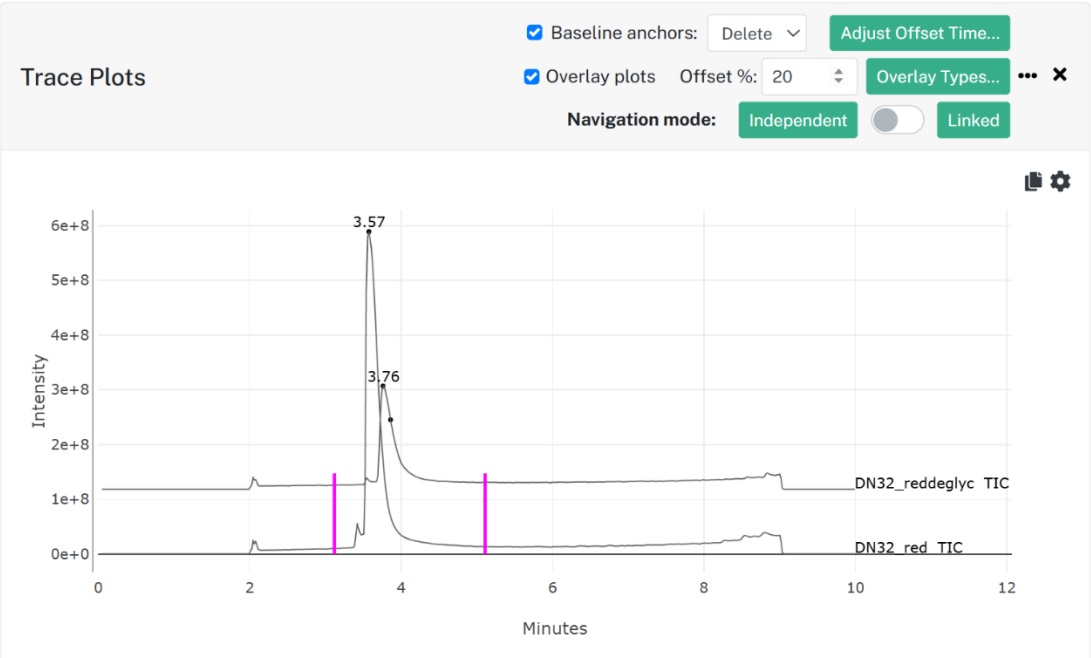


Figure 55: Overlay with 20% offset

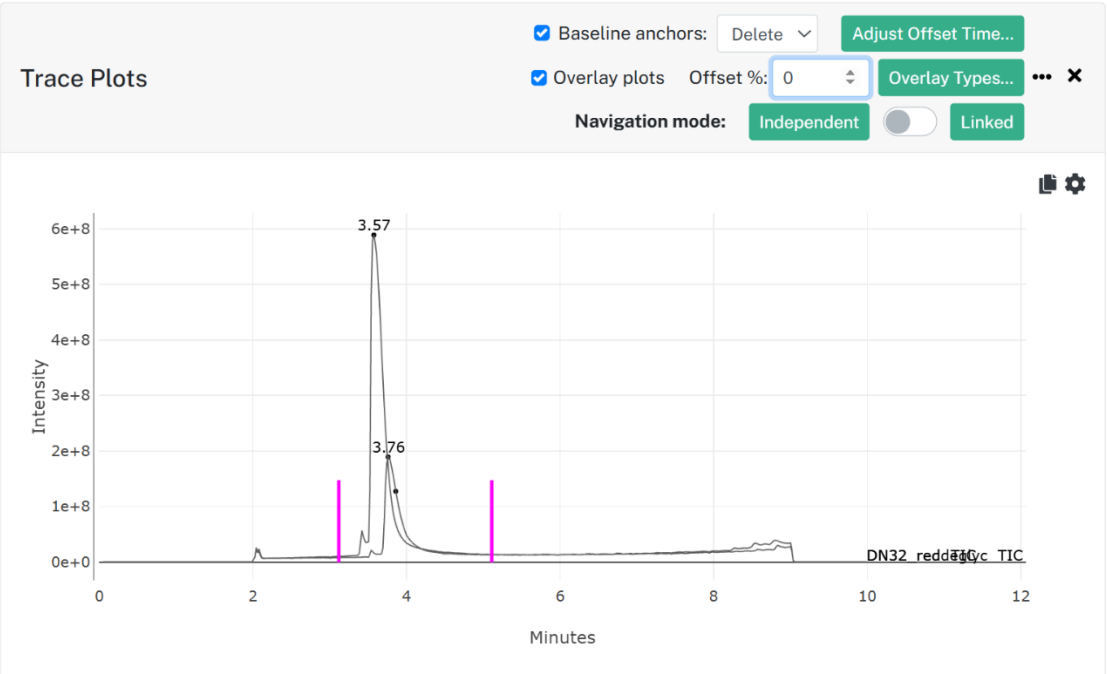


Figure 56: Overlay with 0% offset

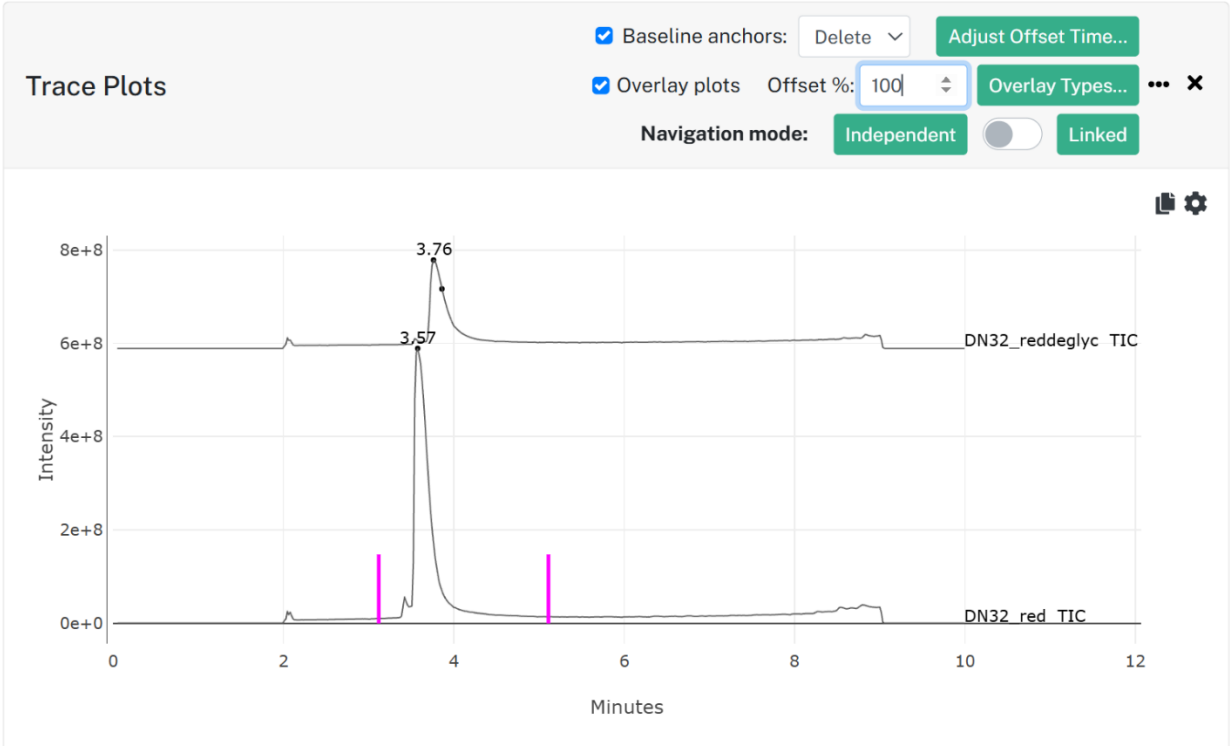


Figure 57: Overlay with 100% offset

Navigation mode

Navigation mode provides the user with two options for plot navigation for all selected plots stacked in the plots widget. **Linked** mode allows users to perform simultaneous adjustment of the zoom ratio for all plots within the same widget.

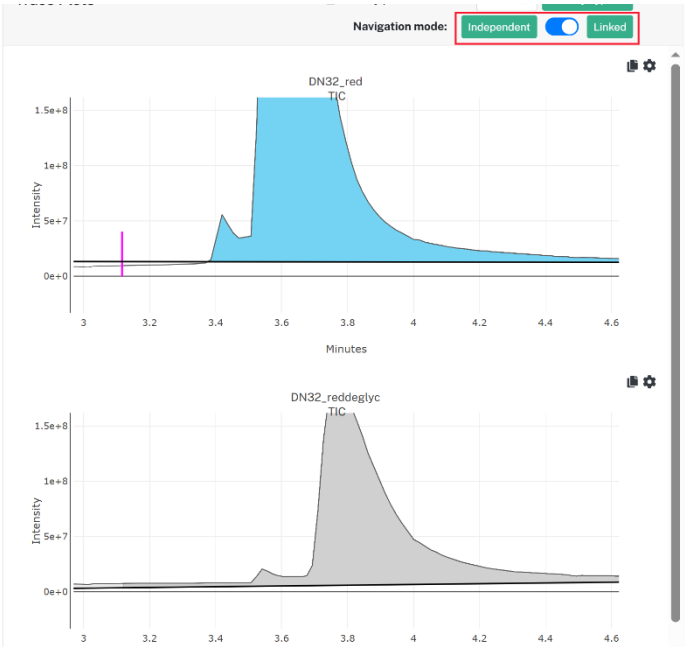
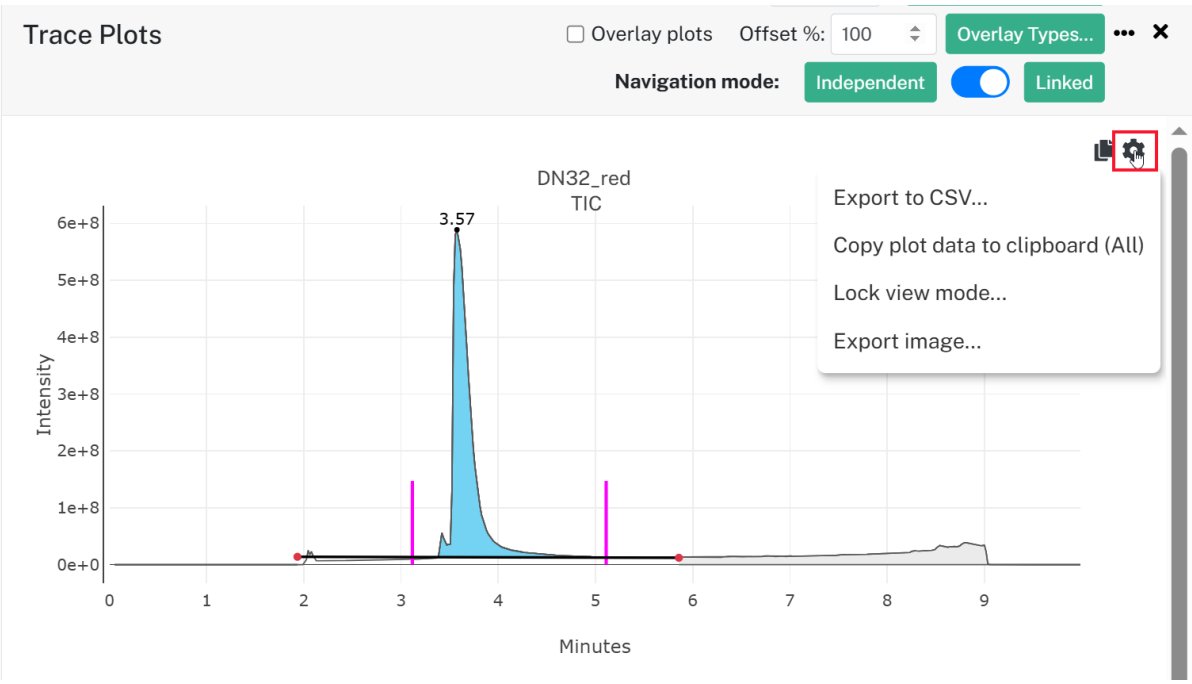


Figure 58: Two traces set to the same zoom level in linked navigation mode

Navigation mode is set to Independent by default, which represents default behavior (only a single plot will have adjusted zoom levels). If a user wants to re-adjust integration start and end times they can do that for individual traces in the Independent mode.

Individual Plot tools

Settings under the gear icon present for each *individual* plot within the Trace Plots widget apply only to the plot in question. Tools include **Export to CSV**, **Copy Plot Data to Clipboard (all**), **Lock View Mode**, and **Export Image.**



Copy plot image to Clipboard

Users can click on the icon to copy a plot (as an image) to the clipboard to paste in external document creating and editing applications. Copy will include overlaid plots as well provided they are in the same plot area.

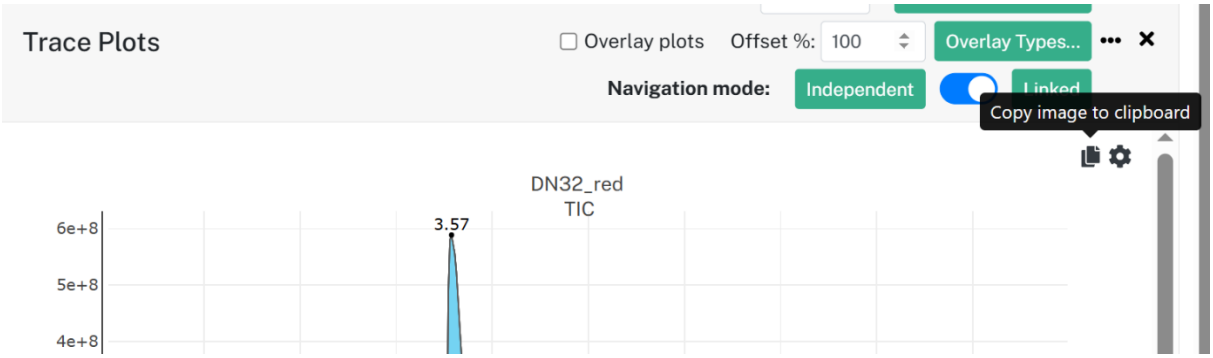


Figure 59: Copy plot image to clipboard

Export to CSV

Clicking on **Export to CSV** will automatically generate and download a CSV file containing numerical plot data to the user's browser.

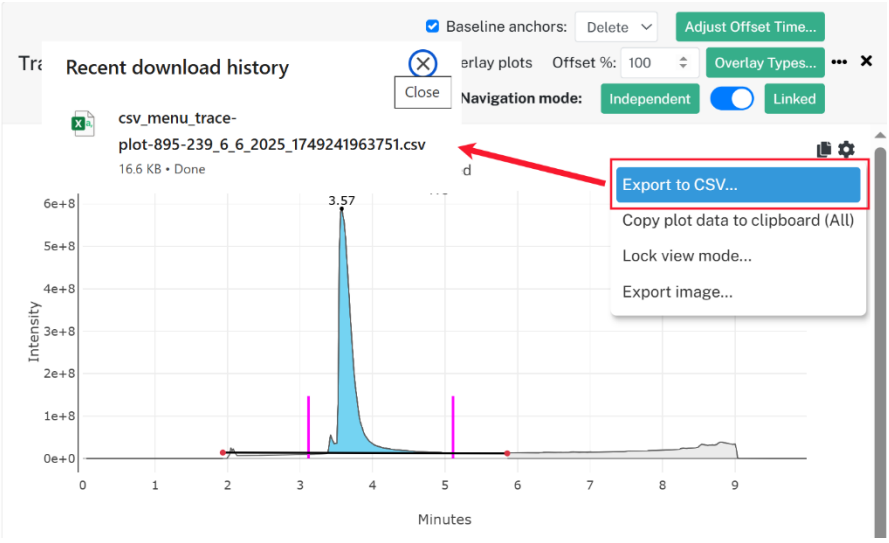


Figure 60: Export to CSV

Copy plot data to clipboard (All)

Clicking on **Copy plot data to clipboard (All)** copies all x,y plot data values to the user's clipboard. This action does not download any files to the user's browser.

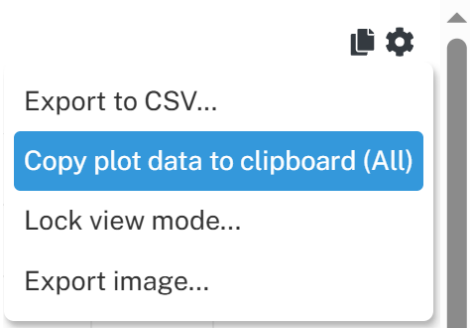


Figure 61: Copy plot data to clipboard (All)

Lock view mode

Enabling different lock view modes allow the user to maintain axis zoom levels when selecting different samples to be viewed in the Plots widgets:

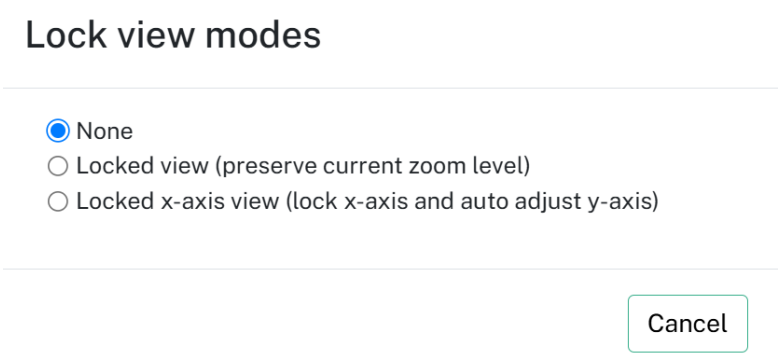


Figure 62: Lock view modes dialog

Users can choose to preserve the current zoom level on both axes or on just the x-axis with the y-axis auto adjusting for each successive plot.

Export Image

Users can export an image of their plots in Web Analysis as a PNG, JPEG, or SVG file. The dimensions of the image can also be adjusted prior to downloading, and dimensions can be adjusted with a fixed aspect ratio. The file will be automatically downloaded to the browser if the user clicks Download.

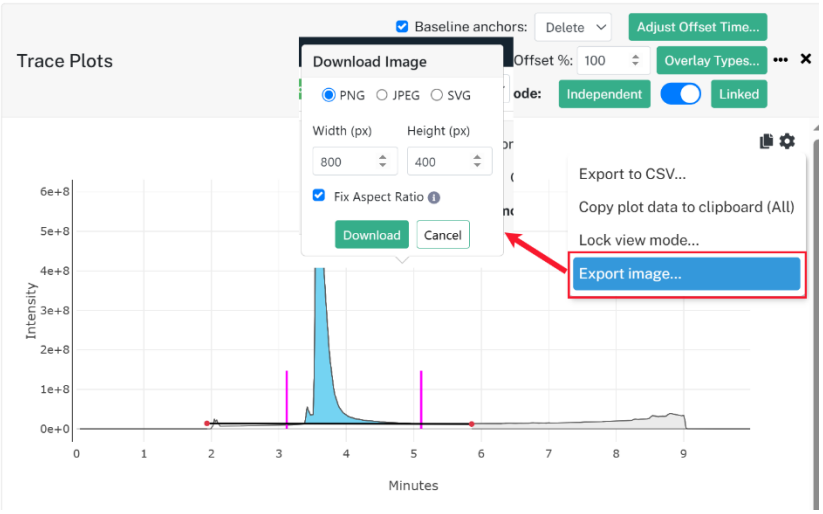


Figure 63: Export Plot Image

Lock Mass

Clicking **Lock Mass** in the Samples view opens the Lock Mass dialog.

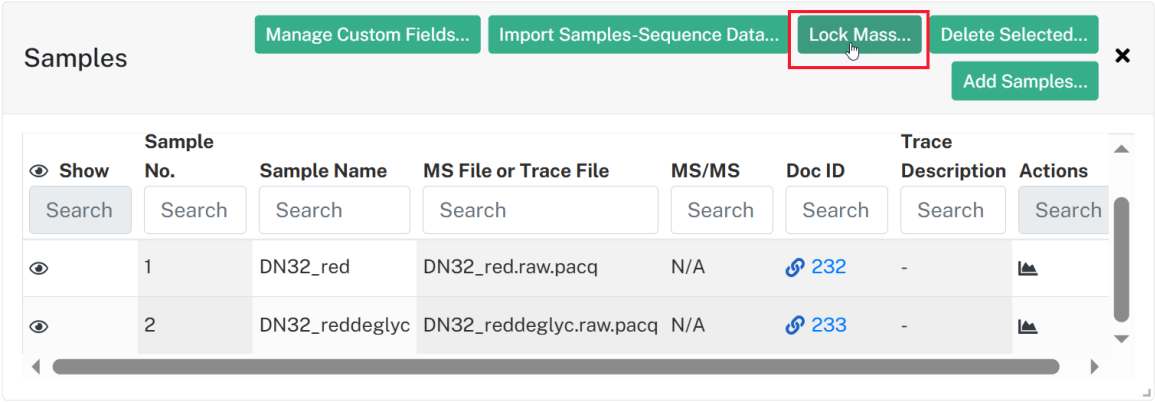


Figure 64: Lock Mass options can be found in the Samples view

Enable lock mass is unchecked by default. When checked, the other two entries become enabled for entry.

Enabling Lock Mass will allow the user to define a m/z of a known value used as an internal calibrant to improve mass accuracy (such as Glu-Fib or Agilent Tune mix). The dropdown for **Lock mass (m/z)** includes various common calibrant values.

Tolerance (ppm) defines the window in ppm for locating the uncalibrated lock mass m/z peak.

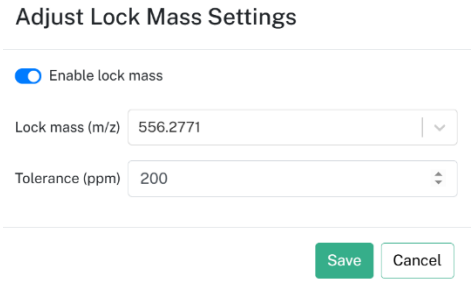


Figure 65: Lock Mass dialog

If the analysis is saved as a template, the Lock Mass settings will be preserved in the resulting template.

Compute

Once Samples and the Trace Range Rule are added, the user is provided options to **Compute**. The **Compute** button opens the following dialog:

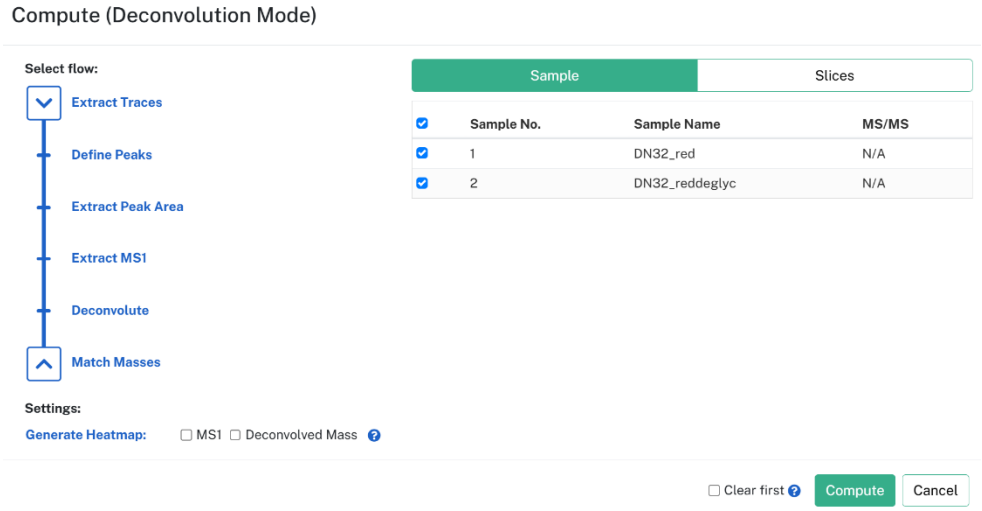


Figure 66: Compute dialog (Deconvolution mode)

If the user checks **Clear first**, *all* existing results will be removed and re-computed, including any existing matches and preset associations (e.g., Deconvolution options). This option is only recommended if the user wants to remove all previous work and re-compute, which may be useful when experimenting with different parameters.

Not all samples must be part of the computation. Only samples selected within the **Samples** table will undergo the selected computations.

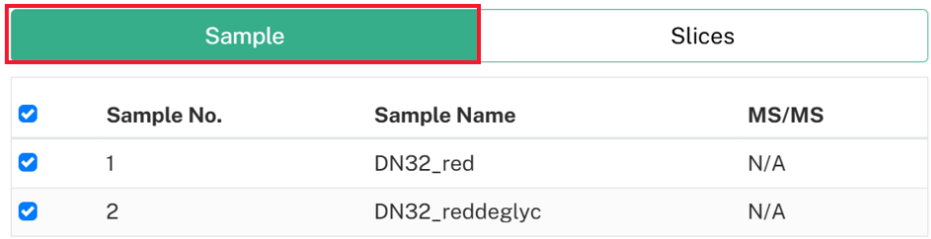


Figure 67: Sample tab in Computation

Alternatively, the **Slice** tab allows the user to select specific slices to undergo calculations.

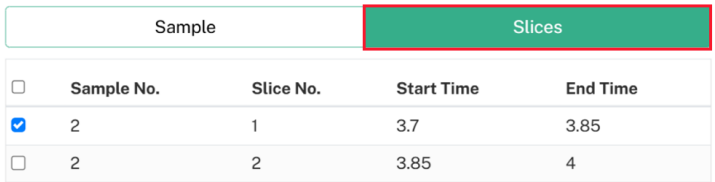


Figure 68: Slices tab in Computation

The **Flow** allows the user to select the range of operations that are performed when clicking **Compute**. This can save both time and computational power, since more intensive calculations can be omitted if not desired. The arrows on the flow chart can be dragged to encompass all calculations to be included in the computation. Note that the default Flow options for Compute are specific to each room. The full flow is as follows:

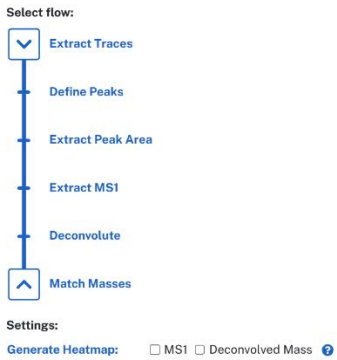


Figure 69: Computation Flow Chart

The Flow shown in the above figure includes all possible computations that can be performed on the analysis.

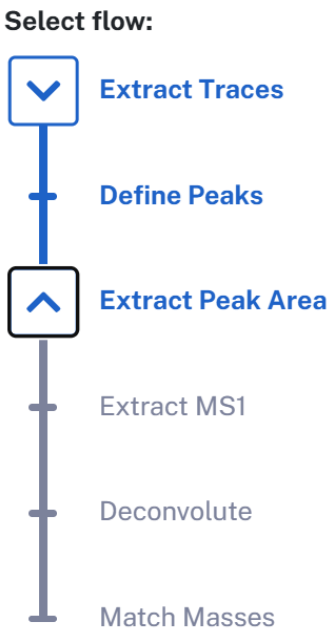


Figure 70: Truncated Flow Chart

The flow shown in the above image shows the steps used in the Samples room in computation.

Note that steps *cannot* be skipped between stages in the flow chart; rather, the flow chart can only be started or ended at a determined point e.g., the user cannot select to perform all operations except “Extract MS1”—if the user wishes to skip Extract MS1, they must either choose the flow Extract Traces > Define Peaks > Extract Peak Area *or* Deconvolute > Match.

One advantage that Web Analysis offers over traditional Byos Intact is the ability to fine-tune calculations such as Deconvolution: the user can make modifications and compute *only* Deconvolution multiple times without having to restart an entire analysis or perform computations on every sample each time.

Sequences (Deconvolution and Feature Finder modes)

The **Sequences** room contains four widgets: **Sequences**, **Combinations, Modifications**, **Deconvolution Plot Zoom Settings**¸ and **Deconvolved Mass Matching**.

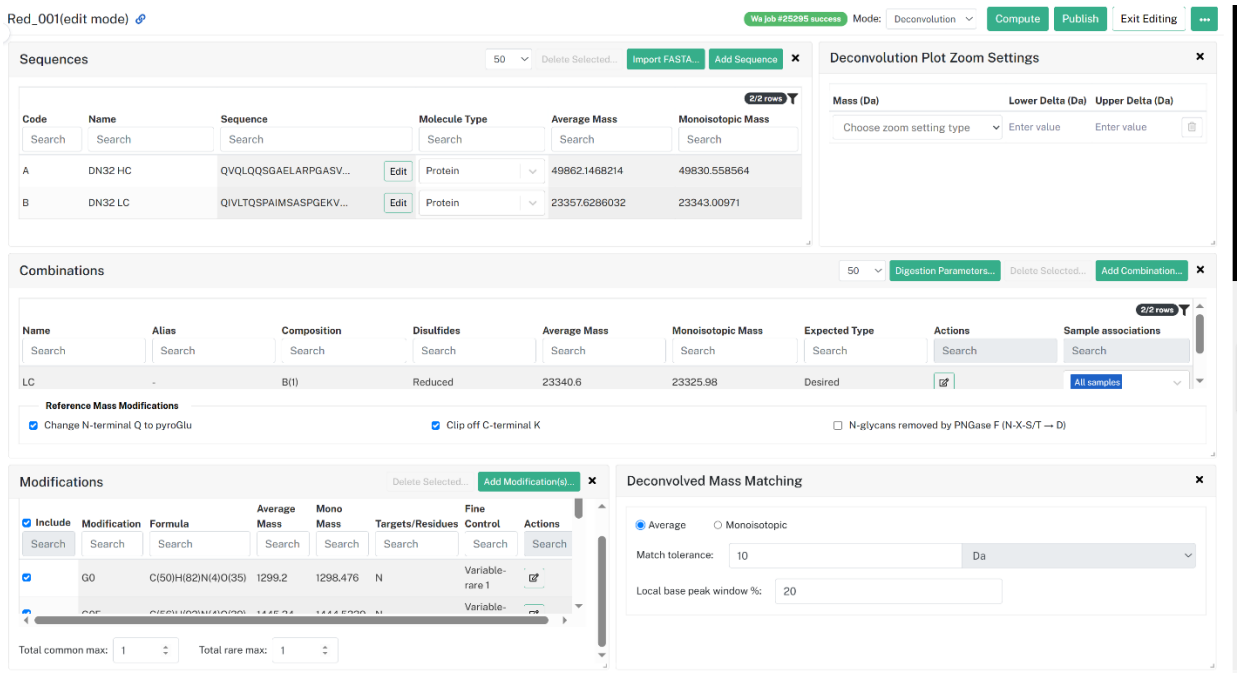


Figure 71: Sequences Room (Edit mode)

Sequences

The **Sequences** room opens with the **Sequences table**, where records are added manually or through imported `*.FASTA` or `*.fa` files. The **Import FASTA** button opens those files to display the names and sequences of all contained records. All checked sequences are imported with the **Import FASTA** button to the Sequences table as separate rows.

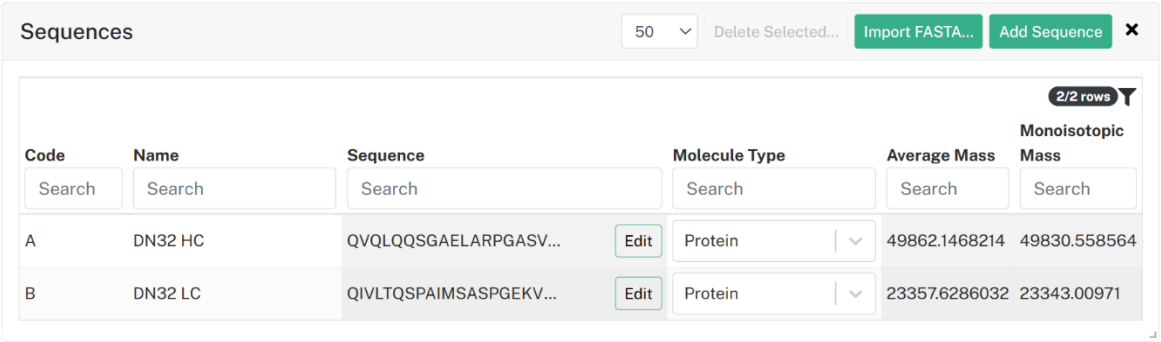


Figure 72: Sequences

Using **Import FASTA** allows the user to import FASTA sequences directly. Once they are added, the masses are updated automatically. **FASTA** files are imported from folders available to users.

Clicking **Add Sequence** adds an empty row populated with a **Code** letter (e.g., A, B, C which are added in alphabetical order). However, the user has the option to edit the Code by clicking into the cell and typing in an alphanumeric value. Clicking once on the **Name** cell allows the user to type in a name for each sequence. The sequence can be added (or updated) by clicking **Edit**. Unlike the Code and Name columns, this value cannot be changed outside of the Edit dialog. Average and Monoisotopic Mass *cannot* be edited if a sequence value is present, but if there is no entry in the Sequences column, a user can add these mass values manually; however, this disables the ability for the user to enter a Sequence value.

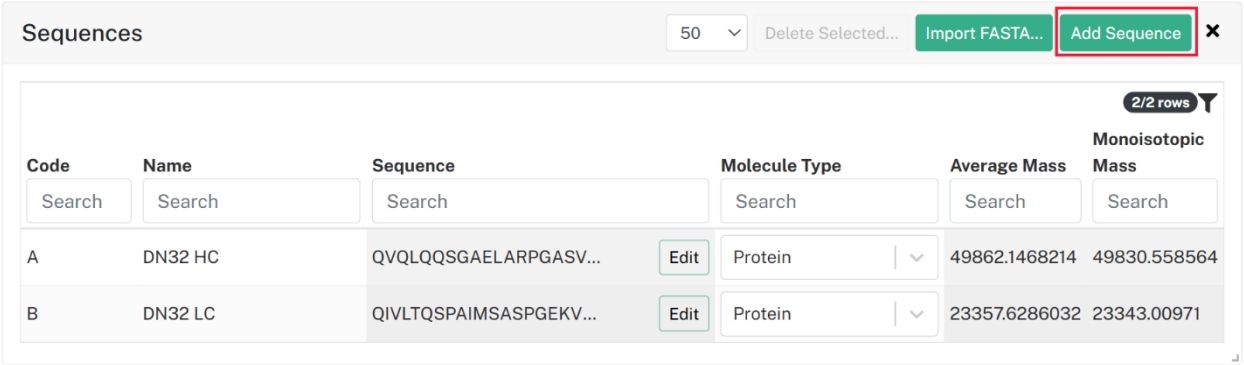


Figure 73: Add Sequences

To select a row, click on the row of interest. This highlights the row in blue. Once the row(s) are selected, the user has the option to delete them by clicking **Delete Selected**

The following confirmation dialog pops up to ensure the user wishes to delete the selected row(s).

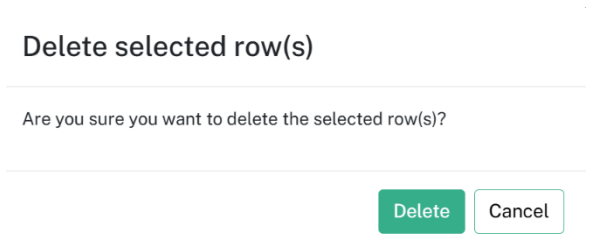


Figure 74: Delete selected row(s) confirmation dialog

Molecule Type allows the user to specify whether the molecule is a Protein or an oligonucleotide. This value defaults to **Protein**.

Combinations

The **Combinations** table allows for the addition of new combinations when entries in the Sequence table contain values for Sequence, Average Mass, or Monoisotopic Mass.

A Sequence Combination consists of a **Name**, an **Alias** (optional)**,** and a **Count** of each checked component sequence chain. The user checks the samples the combination will apply to. Finally, options are chosen for Disulfides (Non-Reduced, Reduced, and Disulfide Count) and Expected Types (Desired/Undesired).

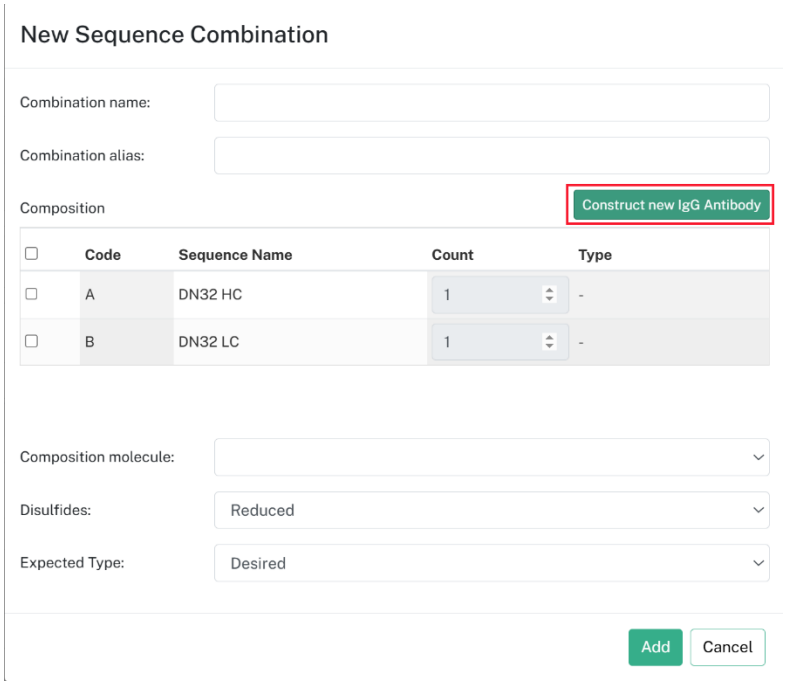


Figure 75: New Sequence Combination dialog

Clicking on **Construct new IgG Antibody** within the New Sequence Combination dialog will open dialog that can be used to construct an IgG antibody based upon the heavy chain and light chain sequences present in the Sequences table.

Figure 76: Construct new IgG Antibody

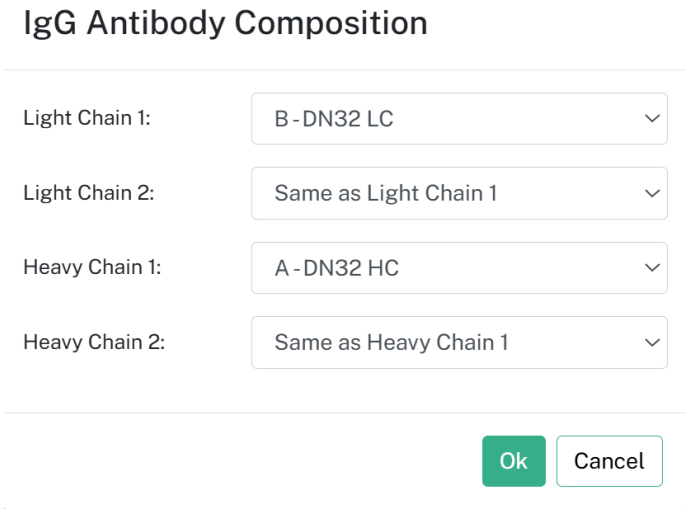


Figure 77: IgG Antibody Composition dialog

All IgG antibody structures constructed using this dialog will default to non-reduced for Disulfides.

Additional options are available at the bottom that allow the user to specify the Composition (will default to IgG Antibody if the above tool is used), the number of Disulfides (with options of reduced, non-reduced, and user defined), and the Expected Type (desired or undesired).

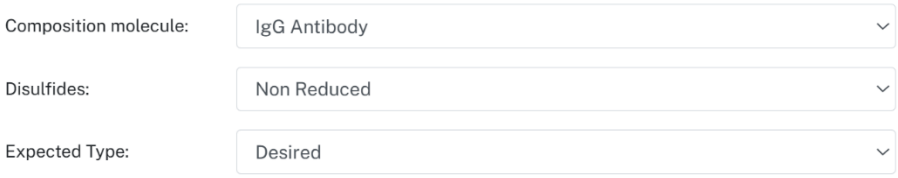


Figure 78: Additional combination options

Note that if the user selects “User Defined” for disulfides, a box will be added to add the custom number of disulfides.

Figure 79: User Defined Disulfides

Once the user has added a Combination, it is added as a row to the Combinations table and the default Sample associations are set to “All Samples”. If “All Samples” is provided as the sample association, combinations will be considered for matching all samples in the analysis, including those added after the associations are set.

Clicking on the **Sample associations** dropdown within a row opens the **Sample Associations** dialog, which lists all current associations and provides all available fields, including Samples, to form associations on.

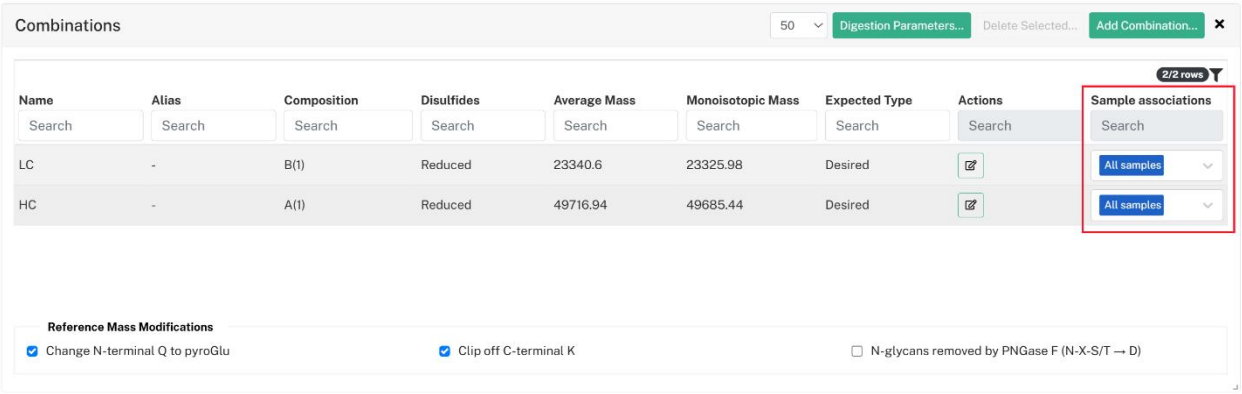


Figure 80: Sample associations column

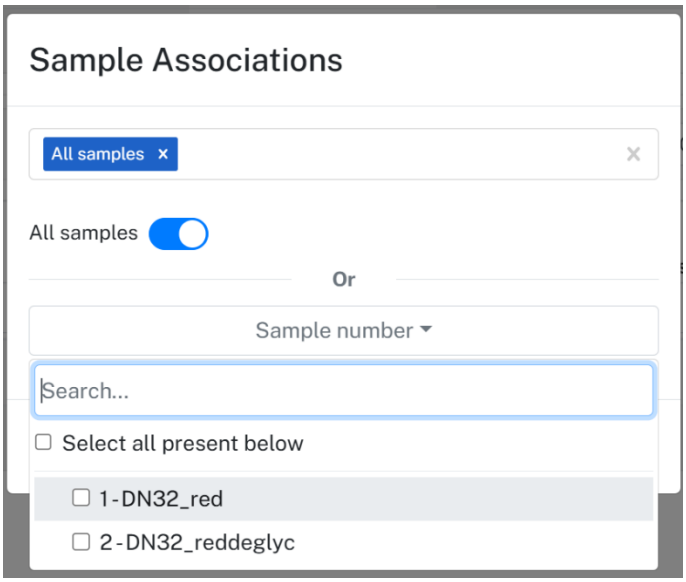


Figure 81: Sample Associations dialog

In addition to Samples, users can also associate combinations with custom or metadata fields defined in the Samples room. Shown below is an example where the field “Glycosylation” with values of “null” and “Degly” can be associated with a combination.

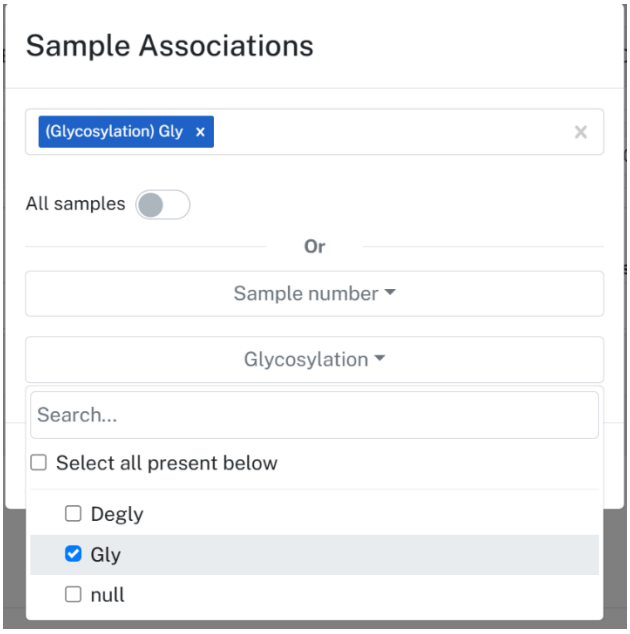


Figure 82: Sample Associations with Custom Fields

In the below combinations table, sample associations are made between DN29 HC and Sample 1 (DN29_red) and the Glycosylation field with the value = Degly. For the other combination, DN29 LC, all samples will be associated and considered for matching when processing the data, including any samples that are added in the future.

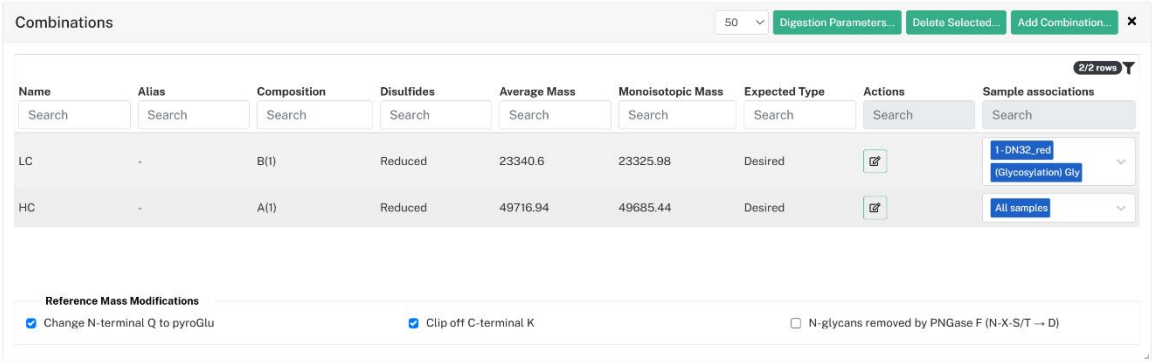


Figure 83: Combinations table with Sample associations populated

Digestion Parameters

Clicking on **Digestion Parameters** within the header of the Combinations table opens a dialog within which the user can specify the protease and alkylating agent used in sample preparation, as well as the number of potential missed cleavages

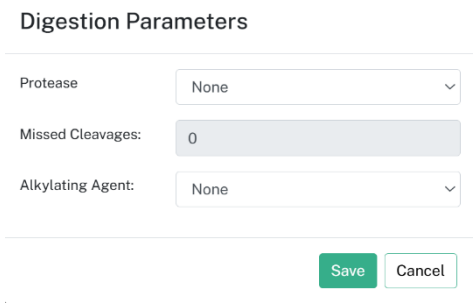


Figure 84: Digestion Parameters dialog

Protease options include Trypsin R, K C-termini, LysC K C-termini, AspN D N-termini, and GluC D, E C-termini. Default is set to none. As of v5.8, Web Analysis can now assign masses to sequence fragments based upon enzymatic digestion using IdeS (FabRICATOR), FabULUOUS and FabALACTICA enzymes for both reduced and non-reduced IgG structures. GlySERIAS digestion and digestion of more complex molecular structures have been also introduced as a **beta** version.

Alkylating agent options include Iodoacetamide, Iodo acetic acid, and NEM.

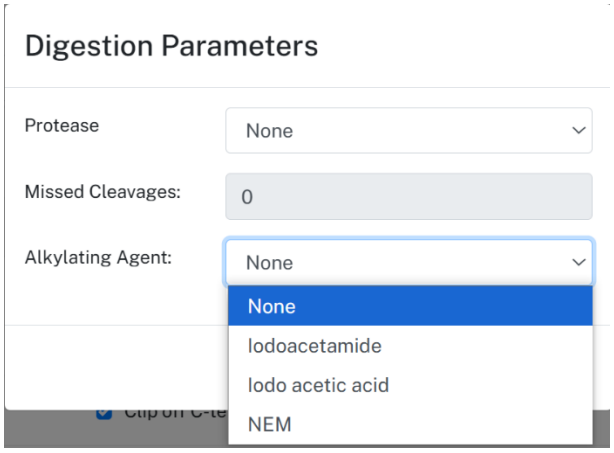


Figure 85: Alkylating Agents dropdown

The **Mass computation options (Deconvolution mode *only*)** enable the user to edit the reference mass with delta masses that are common on an unmodified protein. Users are granted the following options:

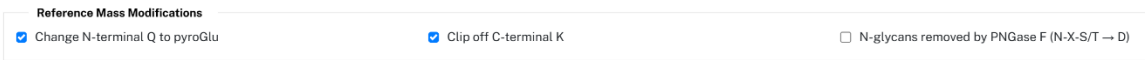
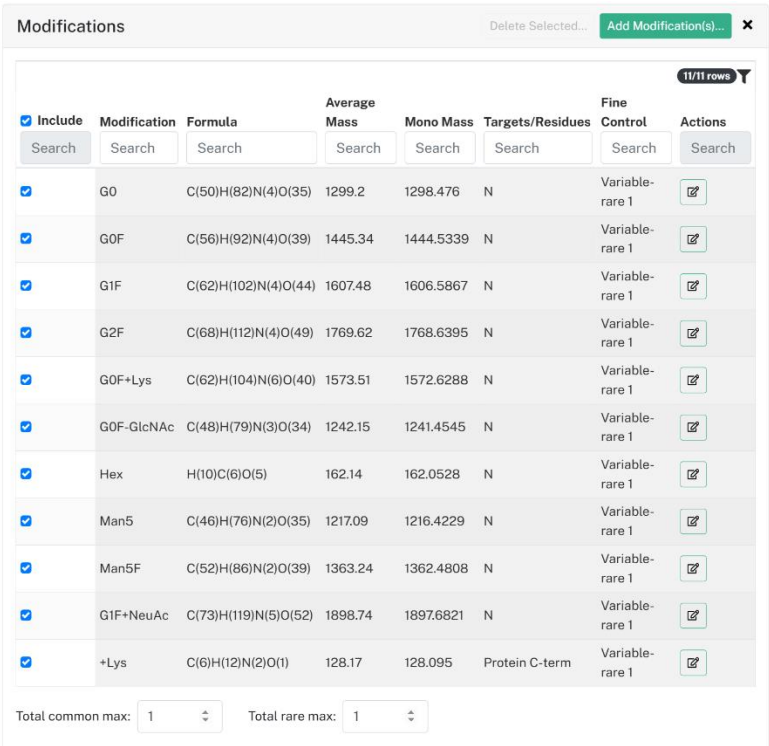


Figure 86: Mass Computation Options

Modifications

Users add Modifications in the **Modifications** widget.



Include	Modification	Formula	Average Mass	Mono Mass	Targets/Residues	Fine Control	Actions
G0	C(50)H(82)N(4)O(35)	1299.2	1298.476	N		Variable-rare 1	
G0F	C(56)H(92)N(4)O(39)	1445.34	1444.5339	N		Variable-rare 1	
G1F	C(62)H(102)N(4)O(44)	1607.48	1606.5867	N		Variable-rare 1	
G2F	C(68)H(112)N(4)O(49)	1769.62	1768.6395	N		Variable-rare 1	
G0F+Lys	C(62)H(104)N(6)O(40)	1573.51	1572.6288	N		Variable-rare 1	
G0F-GlcNAc	C(48)H(79)N(3)O(34)	1242.15	1241.4545	N		Variable-rare 1	
Hex	H(10)C(6)O(5)	162.14	162.0528	N		Variable-rare 1	
Man5	C(46)H(76)N(2)O(35)	1217.09	1216.4229	N		Variable-rare 1	
Man5F	C(52)H(86)N(2)O(39)	1363.24	1362.4808	N		Variable-rare 1	
G1F+NeuAc	C(73)H(119)N(5)O(52)	1898.74	1897.6821	N		Variable-rare 1	
+Lys	C(6)H(12)N(2)O(1)	128.17	128.095	Protein C-term		Variable-rare 1	

Figure 87: Modifications Table

New Modifications are populated in the list by clicking the **Add Modification** button, which is always enabled. This launches the dialog as shown below:

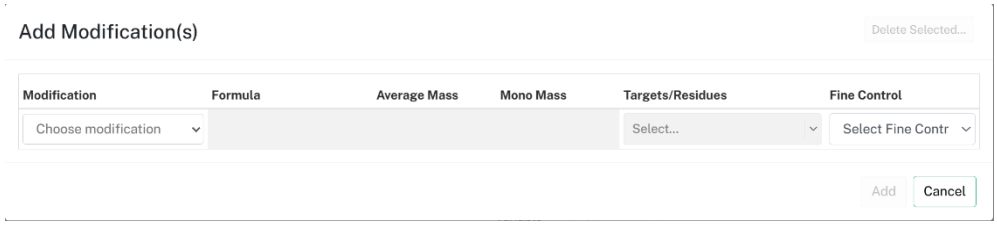


Figure 88: Add Modification

Modifications are selected from a dropdown which sources modification types from the UniProt database. When a modification is selected from the list, the Formula, Average Mass, and Monoisotopic Mass are

populated automatically. Users can add a custom option by typing in “Custom”; when this option is selected, a custom Modification name can be entered and Formula, Average Mass, and Monoisotopic Mass can be typed in manually by double-clicking the space in the column.

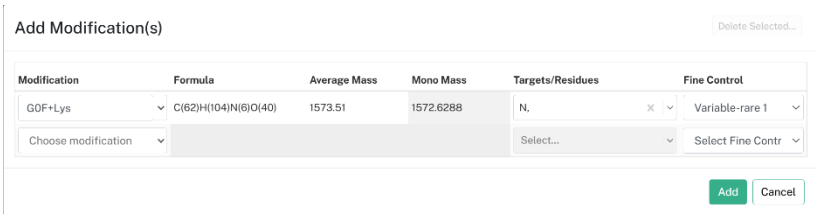


Figure 89: Adding a custom modification "G0F+Lys"

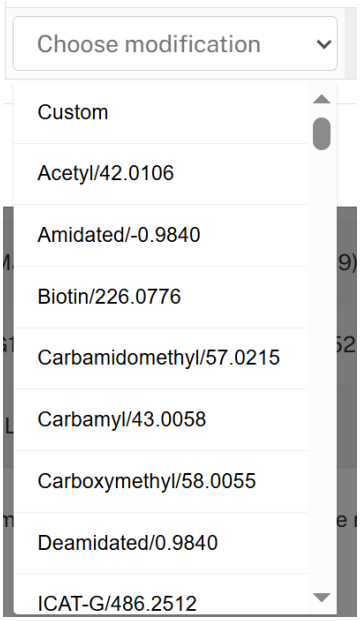


Figure 90: Choose Modification dropdown

Targets/Residues displays a list of possible target locations associated with the selected modification name in a dropdown. Targets form a comma-separated list.

Applying **Modification Fine Control™** when adding a Modification enables the user to search for 10s or even 100s of modification types at a time without a combinatorial explosion. Fine Control options allow a user to mark the modification as belonging to one of two categories. Each of those categories has a maximum count of that modification, so that inappropriate mathematical matches are not made. Note that for Intact/Deconvolution analysis, the UI is the same, but the location of exact amino acids cannot be located on the whole Intact chain in this case.

For example, in a peptide analysis, a user might allow up to three phosphoserines, S[+80],per peptide, but allow a maximum of one beta elimination, S[-18], and at most one deamidated asparagine, N[+1].To further reduce the search, the user might allow at most one of either S[-18] or N[+1],that is, disallowing peptides containing one of each. Modification Fine Control empowers the user to tailor the search to the sample, and thus avoid overly narrow searches that miss interesting peptides and overly broad searches that run for hours or days and produce “noisy” results with many false positives.

Modifications can be deleted by checking the box for the sample of interest and clicking **Delete Selected**.

Note that modifications are **not selected by default**. The user must manually select the modifications of interest once they are created.

Once a modification is created, it can be edited by clicking the icon under the **Actions** column. This will bring up the **Edit Modification** dialog, which is parallel to the dialog for adding modifications.

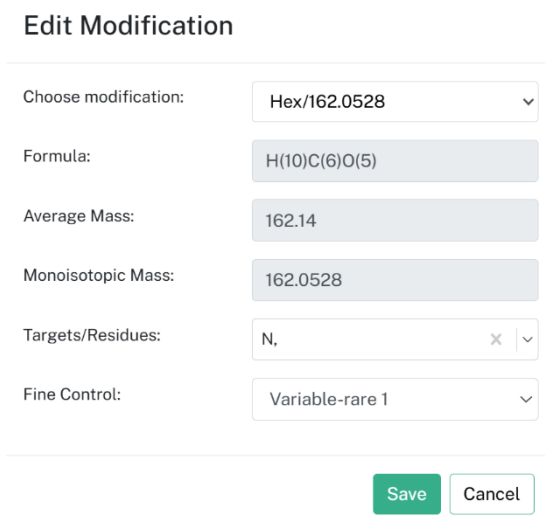


Figure 91: Edit Modifications Dialog

If the **Edit** icon for a Modification record is clicked, the Edit Modification dialog opens populated with the row's values. If the values are edited and saved, the Modification will update accordingly.

The **Include** checkboxes for the Set Modifications table indicate whether each modification is used to generate modification combinations. All are checked by default, but if a modification is unchecked, the modification combinations are recalculated without the unchecked modification.

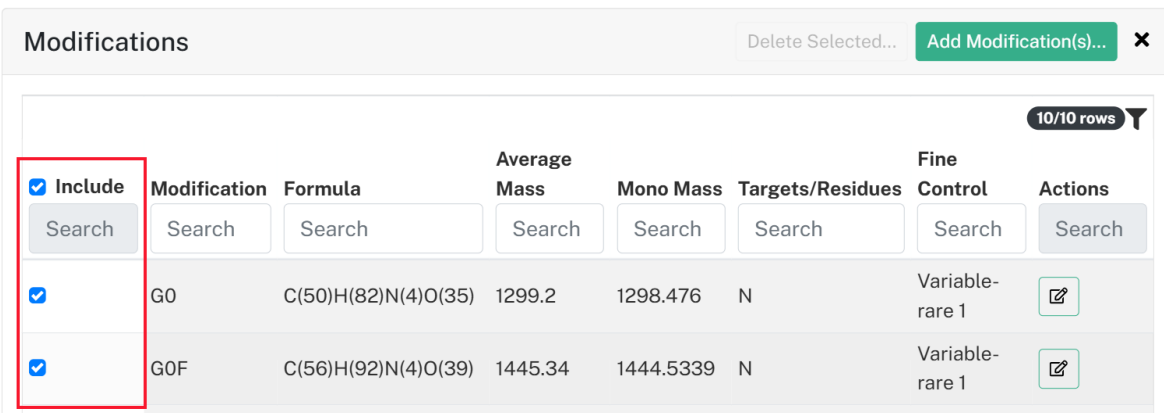


Figure 92: Include modifications

Note that modifications can be generated and saved to a template *without* Samples table or Sequence table entries.

Deconvolved Mass Matching

The **Deconvolved Mass Matching** view includes parameters that control automatic mass peak assignment.

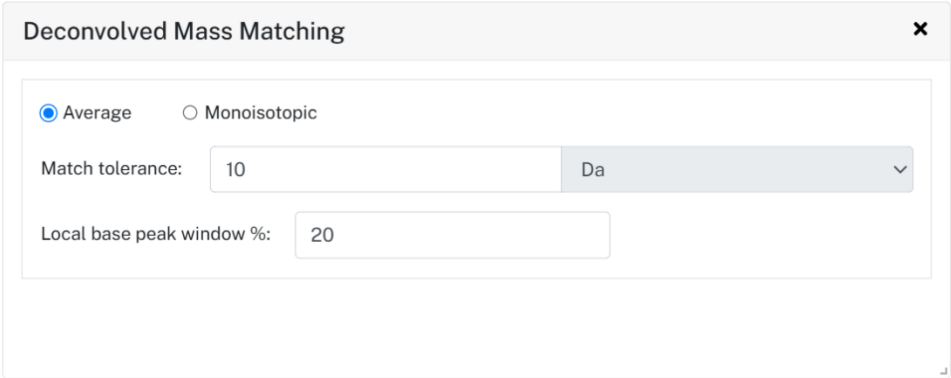


Figure 93: Mass Matching view

The user can choose between **Average** and **Monoisotopic** mass.

The **Match Tolerance** determines the value +/- a peak must match to a potential mass to be assigned. The default value is 10 Da, but the user has the option to enter in any number, as well as the units (Da or ppm).

The **Local base peak window%** sets the percentage range of the major mass peak that defines a local peak group. For example, a 20% window would compare 20-30 kDa forms to a locally tallest (base) peak at 25 kDa and 40-60 kDa forms to a base peak at 50 kDa.

If the analysis is saved as a template, the Sequence room views will be preserved in the resulting template.

Deconvolution Zoom Plot Settings

The **Deconvolution Zoom Plot Settings** view allows the user to set different methods of generating plots zoomed to regions of interest based upon user-defined criteria.

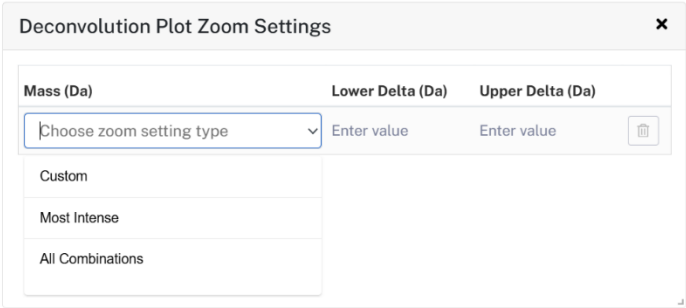


Figure 94: Deconvolution Zoom Plot Setting widget showing dropdown options

Users can establish zoom criteria based upon the most intense mass, all combinations, or a Custom within a delta range. Users have the option to generate multiple zoomed plot settings. These settings will apply to the plots generated (and shown in the Inspection room) when the user performs computation and zoom plot settings options will also be added to the list of available zoom segments as described in the **Select/Add Zoomed Segments** section.

Inspection room (Deconvolution mode)

The **Inspection** room contains six widgets: **Sample Status Review, Trace Peaks Table**, **Trace Plot**, **Masses Table**, **MS1 Plot**, and **Deconvolved Mass Plot**.

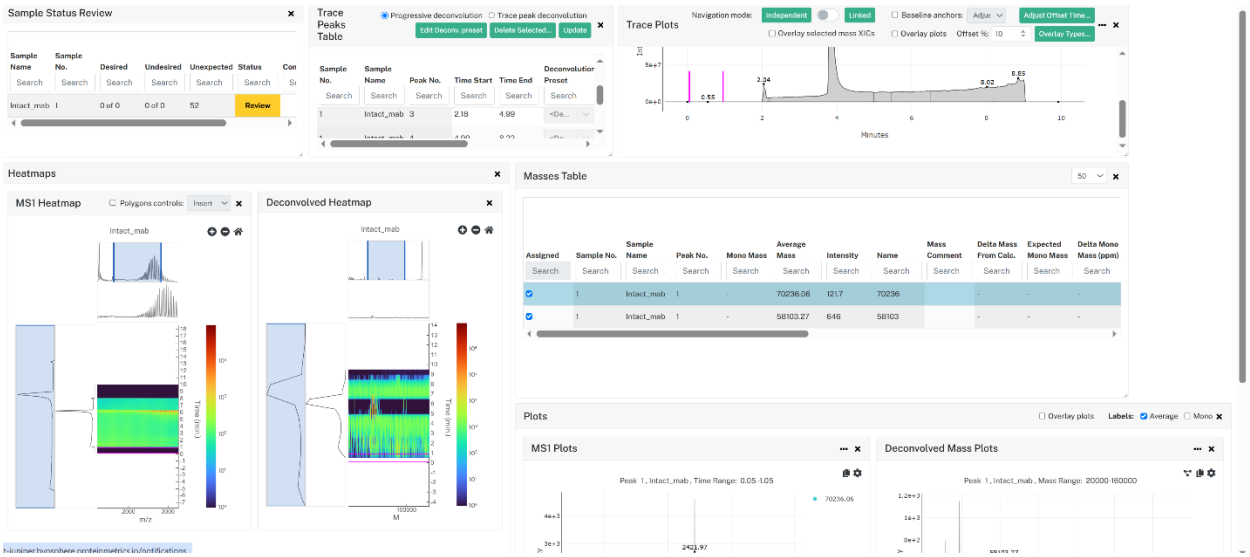


Figure 95: Inspection Room

Trace Peaks Table

The **Trace Peaks Table** allows the user to adjust peak integration settings and apply deconvolution presets (see Edit Deconvolution Presets). The following columns are editable: Time start, Time end, Deconvolution preset, and Peak comment columns. All other columns are grayed out and cannot be changed within this view.

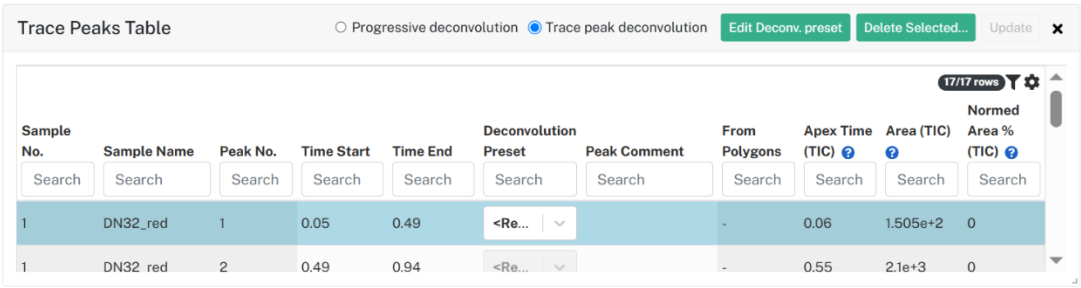


Figure 96: Trace Peaks Table

The Trace Peaks table will remain consistent with changes made within the same room as well as other rooms.

Click **Update** to recalculate the **Area** values and **Apex Time** after the Time Start or Time End is edited.

The user can select a trace by clicking it, which will highlight the row blue. To delete the highlighted peak rows, click **Delete** Selected. To deselect a trace peak, click on an uneditable part of the trace peak's row (such as the Sample name).

Deconvolution Modes

Users can specify the deconvolution mode they wish to use when performing deconvolution within the header of the Trace Peaks Table in the Inspection room. Users have the option to perform either **Progressive Deconvolution** or **Trace Peak Deconvolution**.

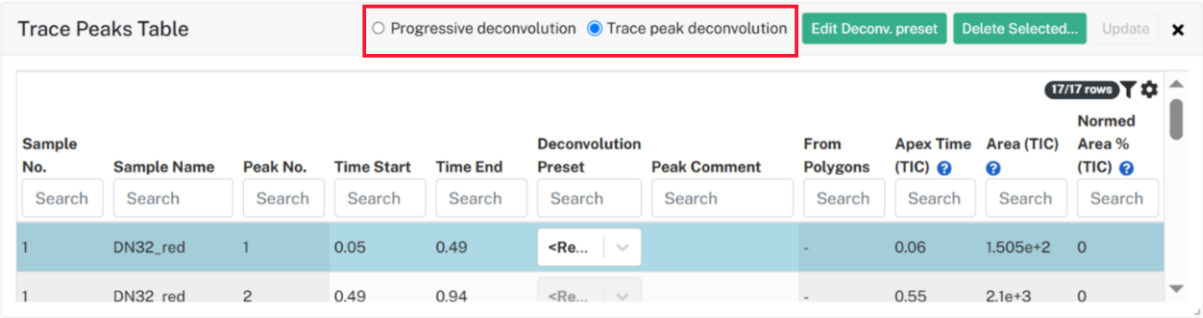


Figure 97: Deconvolution options

When a user selects **Trace Peak Deconvolution**, the range used for deconvolution is directly associated with the range from trace peak integration. The figure below shows the range for the MS1 plot corresponds to the highlighted peak, where the time range in this example is 1.5-2.07 minutes:

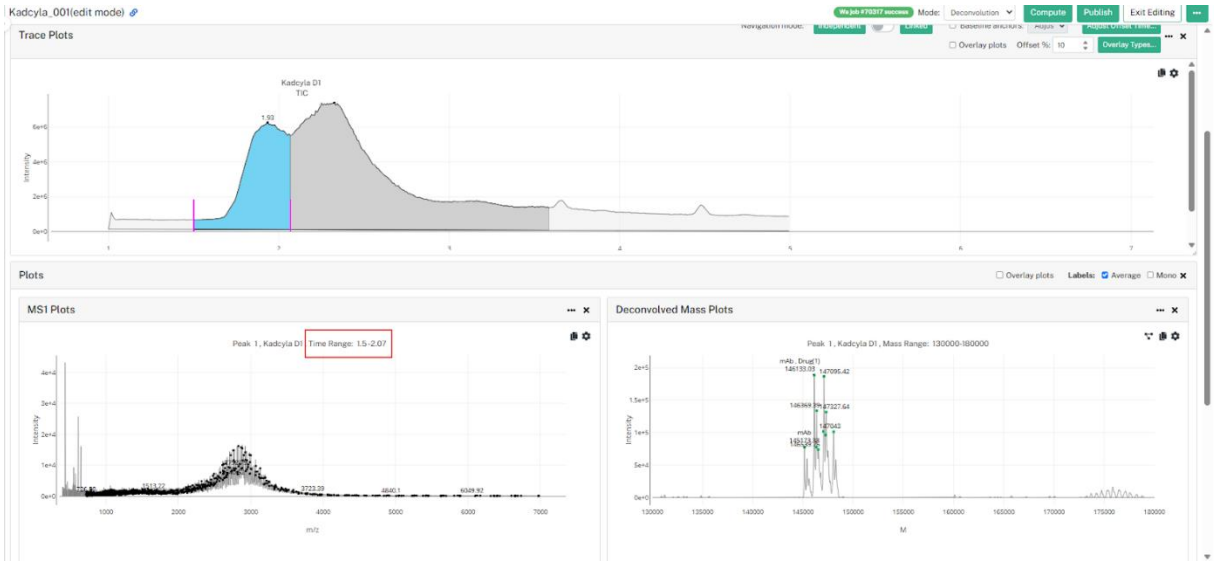


Figure 98: Example of Trace Peak Deconvolution

Progressive Deconvolution mode utilizes the **sliding window deconvolution** approach, which creates narrow sliding overlapping time windows and performs deconvolution iteratively on sequential time ranges. Masses observed across stretches of consecutive slices are grouped together to identify mass features. Isomers are distinguished based on the differential elution profiles and reported separately. Mass XICs (Extracted Ion Chromatograms) are available to display on the trace plot. Either the AUC (Area Under the Curve) or apex intensity for each Mass XIC may be for feature quantitation.

For example, when a user selects **Trace Peak Deconvolution**, the range used for deconvolution is directly associated with the range from trace peak integration. The figure below shows the range for the MS1 plot corresponds with the highlighted peak, where the time range is 1.5-2.07 minutes.

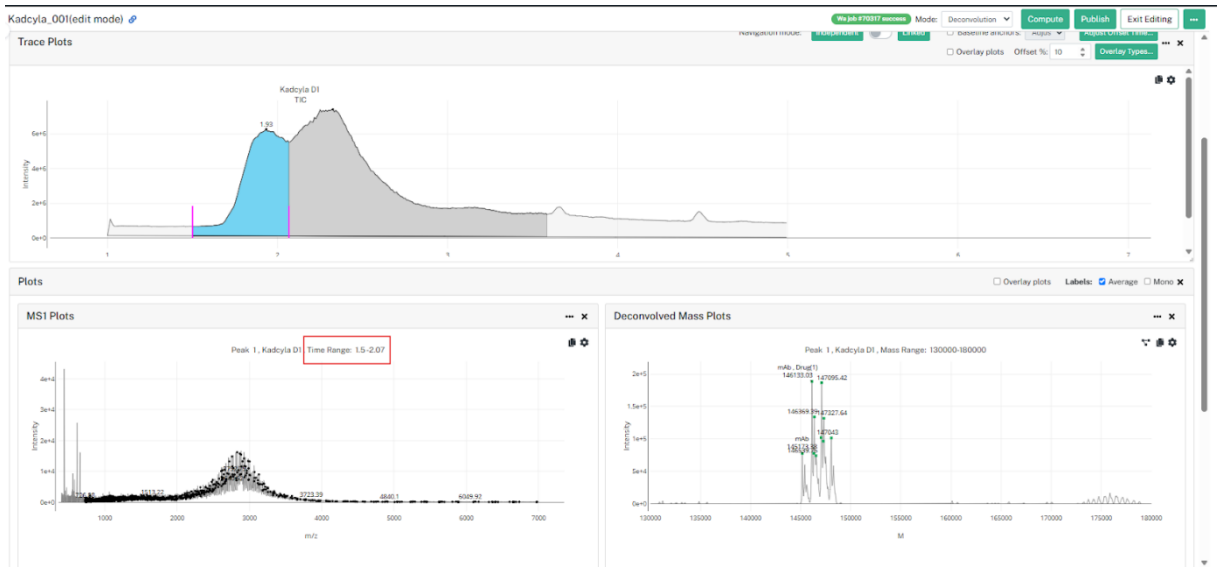


Figure 99: Example of Trace Peak deconvolution

When a user selects **Progressive Deconvolution**, deconvolution is no longer restricted to the integrated trace peaks. Instead, a user may specify windows to produce multiple slices for deconvolution. They may also specify an overlap between these windows. In this way, Deconvolution is no longer restricted to integrated trace peaks and may be applied across an entire trace. These settings may be accessed via the Deconvolution Presets in the inspection room, or by using the new Progressive Template available as a system resource default template in Web Analysis.

Settings to generate slices may applied over the entire time range or be restricted to a time of interest as set in the Trace Peak Integration Settings in the Samples Room.

Additional Progressive Deconvolution settings may be accessed as part of the **Deconvolution Parameters** if Progressive Deconvolution is selected as the method of Deconvolution (see Progressive Deconvolution).

The example below outlines the result of these settings being configured. In the example below. a time range of interest from 1.5-3.5 minutes was specified, and Trace Peak integration was performed, resulting in two integrated peaks from the TIC trace:

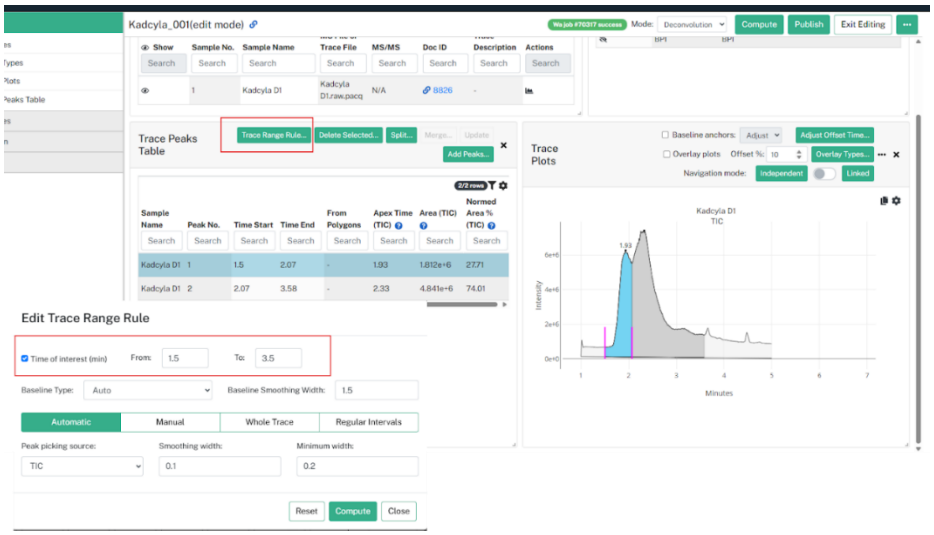


Figure 100: Two trace peaks generated

The next figure outlines how the first two slices are calculated and applied when using the default settings in the progressive deconvolution template:

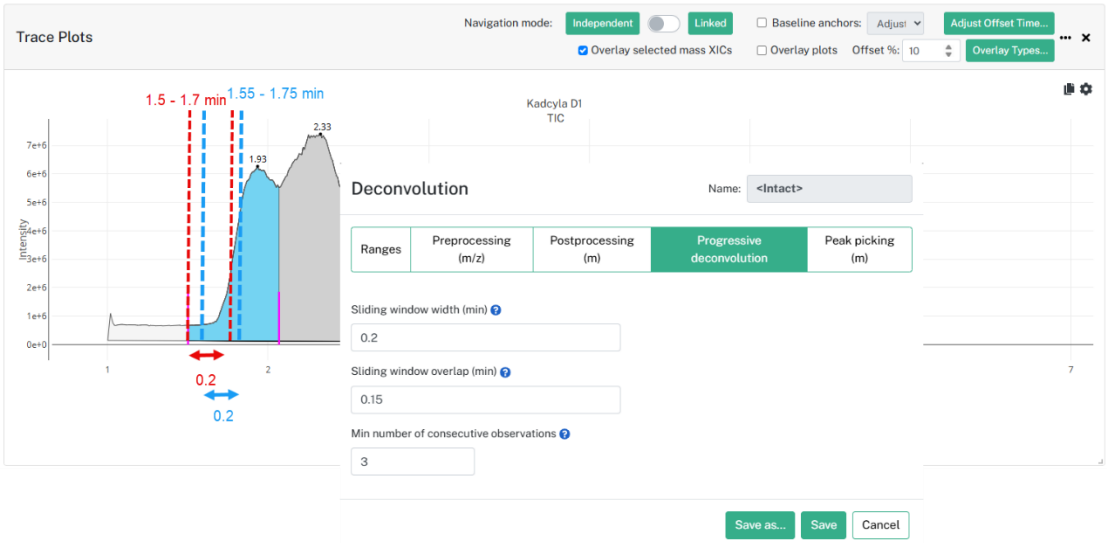


Figure 101: Progressive Deconvolution presets applied

Each slice window in this example is **0.2**. Since the Time Range of interest was set at **1.5-3 minutes**, the first slice will start at **1.50** and end at **1.70** (1.50 + 0.2 = 1.70). The overlap was set to **0.15**, so the second slice will overlap the previous slice by **0.15 minutes**, starting at **1.55 minutes** (this is calculated by subtracting the overlap value from the end time of the slice, in this case, 1.70 - 0.15 = 1.55 minutes).

Again, as the window is set to 0.2, the second slice will end at **1.75**, making the time range of the second slice **1.55 - 1.75 minutes** (1.55 + 0.2 = 1.75). Deconvolution will be performed on *each* slice using the deconvolution parameters specified in the preset tab. The table below shows all the slices that would be generated in this example:

Slice Start Time	Slice End Time
1.50	1.70
1.55	1.75
1.60	1.80
1.65	1.85
1.70	1.90
1.75	1.95
1.80	2.00
1.85	2.05
1.90	2.10
1.95	2.15
2.00	2.20
2.05	2.25
2.10	2.30
2.15	2.35
2.20	2.40
2.25	2.45
2.30	2.50
2.35	2.55
2.40	2.60
2.45	2.65
2.50	2.70
2.55	2.75
2.60	2.80
2.65	2.85
2.70	2.90
2.75	2.95
2.80	3.00

Figure 102: Resultant slices

As a rule, the default parameters in the Progressive Deconvolution Template are a good starting point for fully intact mAb data.

To view the masses identified and spectra associated with slices from performing Progressive Deconvolution, a trace peak(s) from the trace peaks table must be selected. This will populate the masses table with all masses that were identified and have generated a mass XIC (Extracted Ion Chromatogram) where the apex of that XIC is within the boundaries of that trace peak.

Users can view the overlay of selected mass XICs on the trace peaks by selecting the mass of interest and selecting the check box for overlay mass XICs, another new feature added in this release.

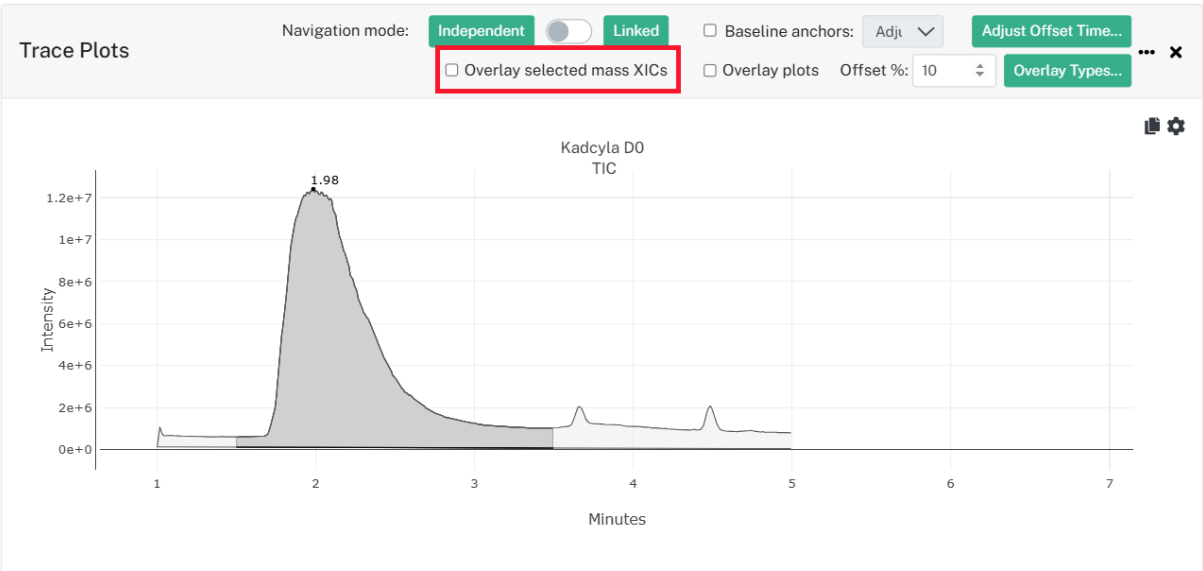


Figure 103: Overlay selected mass XICs

Users can select more than one trace peak at a time and the Masses table will populate with masses from all selected peaks. The mass value reported is an aggregation of masses for that Mass ID from all slices that fall within the time range of the trace peak.

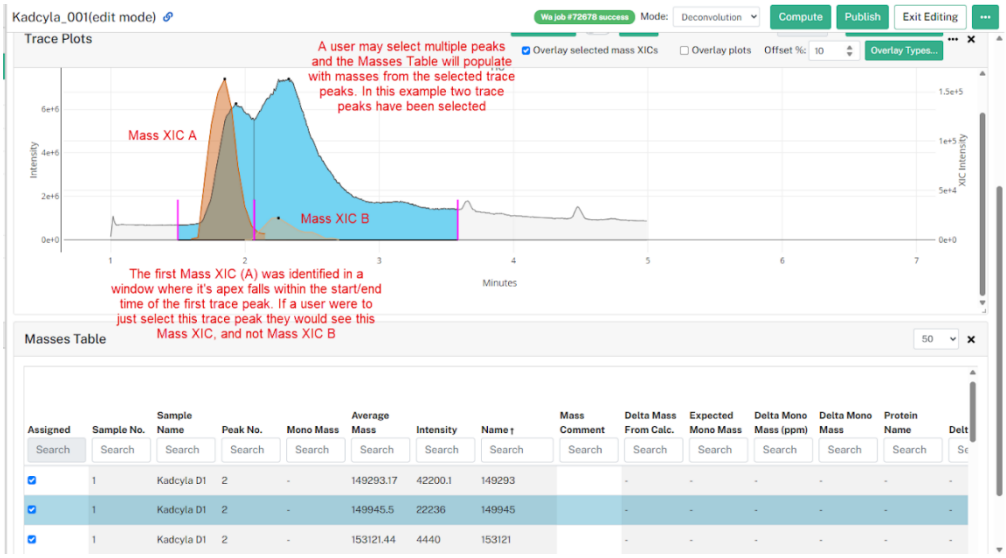


Figure 104: Peak selection

When a user selects a mass from the Masses Table, the MS1 and Deconvolution plots displayed are taken from this slice:

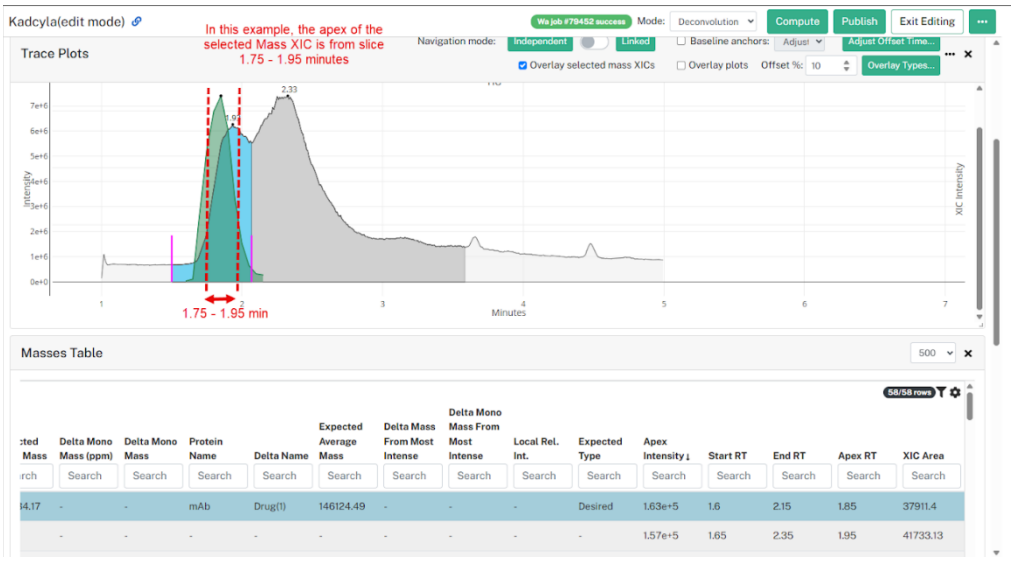


Figure 105: Peak overlay

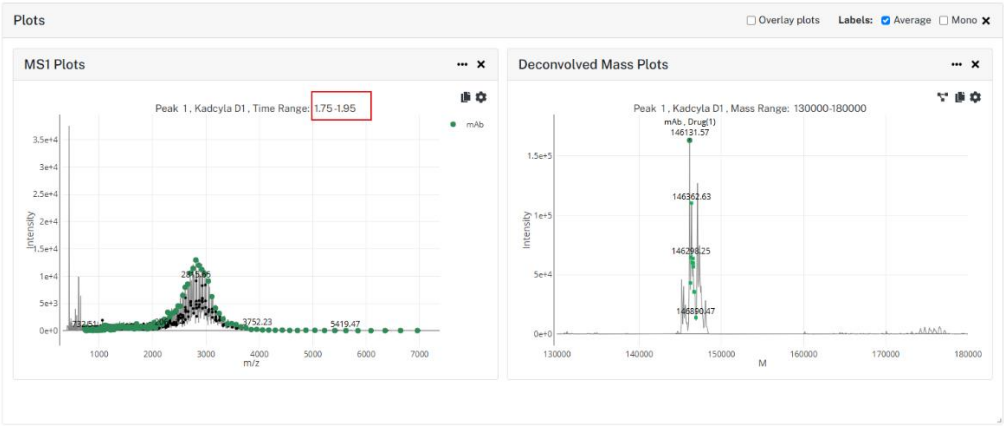


Figure 106: Corresponding MS1 plot with designated range

Deconvolution Presets

Intact Web Analysis includes **Deconvolution Presets**. The **Deconvolution Preset** column has a dropdown menu with a list of system-provided presets to allow for quick selection. Deconvolution presets contain all the parameters used to perform deconvolution for frequent analyses; system-provided default presets are configured for specific data types (Default, IgG Monoclonal Antibodies; Super Heavy Chain, Super HC; Heavy Chain, HC, Light Chain, LC; and Edit Current). These presets can be edited by clicking **Edit Deconv. Preset** or by clicking **Edit Current** under the Deconvolution Preset dropdown of a specific sample.

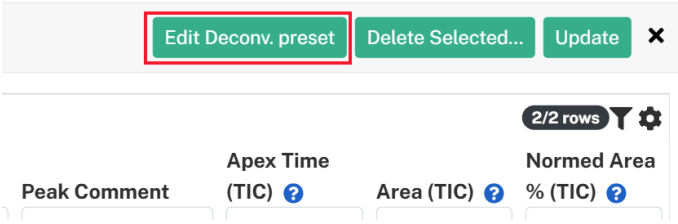


Figure 107: The two ways to modify deconvolution presets

Presets can be edited, renamed, or deleted. Different presets can be applied to individual separation peaks within a single trace.

The default Deconvolution set is named **<Default>**.

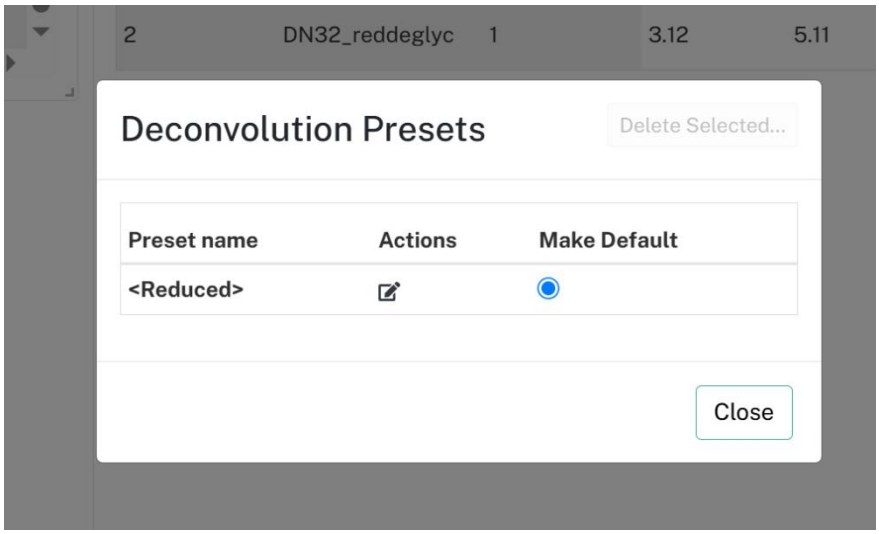


Figure 108: Renaming presets

The **Edit Deconv. preset** dialog includes four tabs: **Ranges, Preprocessing (m/z), Postprocessing (m),** and **Peak Picking.**

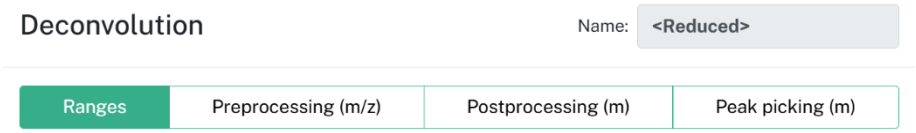


Figure 109: Deconvolution tabs

Each parameter provided within the Deconvolution presets has a question mark icon next to it that, when hovered over, provides the user with a tooltip describing its utility.

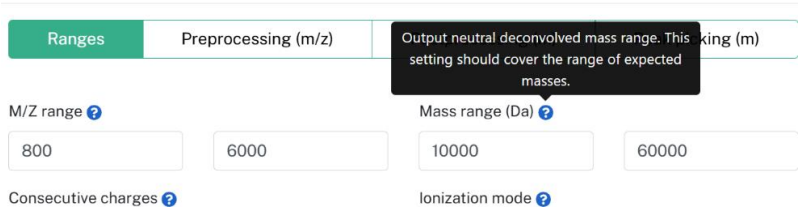


Figure 110: Deconvolution Tooltip

Ranges

The **Ranges** tab provides the user with the ability to define input and output parameters for deconvolution.

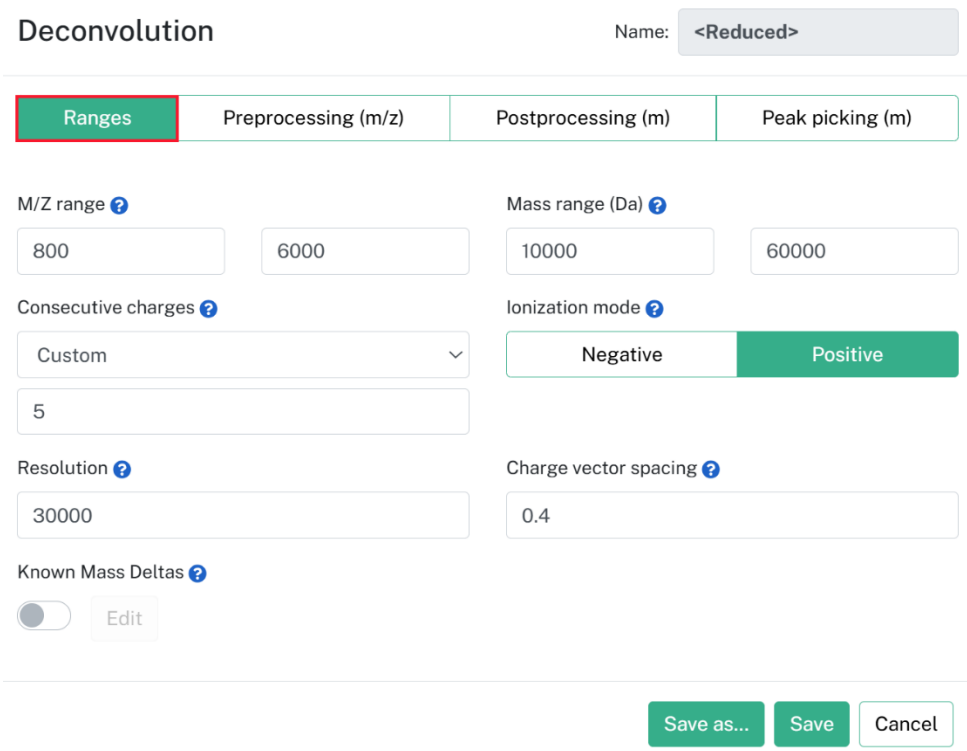


Figure 111: Deconvolution Ranges tab

Mass range and **m/z range** set ranges for the deconvolved *neutral* masses and *m/z*, respectively. Mass range defines the range of *expected* masses displayed in the Deconvolved mass spectrum. **m/z** range defines the segment of the MS1 spectrum used to perform deconvolution. The mass range should include the expected mass.

Consecutive Charges adjusts charge envelope weights. This is useful for heterogenous or high background MS1 spectra to determine which m/z peaks relate to a charge state envelope. Ions found in the charge state envelope in the MS1 spectra are expected to follow a consecutive number of charges. More charge states would be expected for **denatured data** (such as reduced and intact mAbs), so a higher value of 7 may be used or select denatured in the dropdown. In **native-MS** there are far fewer charge states, so a value of 3 (or select Native) may be more appropriate. For **lower mass** (such as <10 kDa) a value of 5 or less is often used. Note that odd numbers must be used.

Charge vectors spacing assigns charge probabilities for each small interval of *m/z* points. Deconvolutions of most MS1 spectra will be almost exactly the same with any charge vectors spacing from 0.2 to 1.0 *m/z* units (thomsons), but a narrow spacing of 0.1 may give better results on isotope-resolved MS1 spectra with interleaved signals, and a wide spacing of 2 may give better results for native MS with broad *m/z* peaks. A value of 0.6 will work well for most average mass data.

Ionization mode denotes the ionization charge used (positive or negative). For proteins, positive polarity is most used. Ensure that the Ionization mode matches the mode of the instrument.

Resolution denotes the observed mass resolution (instrument resolution). A setting that is higher than the mass resolution won't provide any additional improvements to deconvolution. However, it will increase processing time.

Known mass deltas is toggled **off** by default. When toggled **On**, an **Edit** box becomes available that enables the user to select from a list of known mass deltas, representing both monoisotopically resolved and average mass. This setting is useful in cases where there is a mass that is known to repeat in different species, such as glycoforms, where the mass shift between species is 162 Da. This will help to determine charge of m/z peaks.

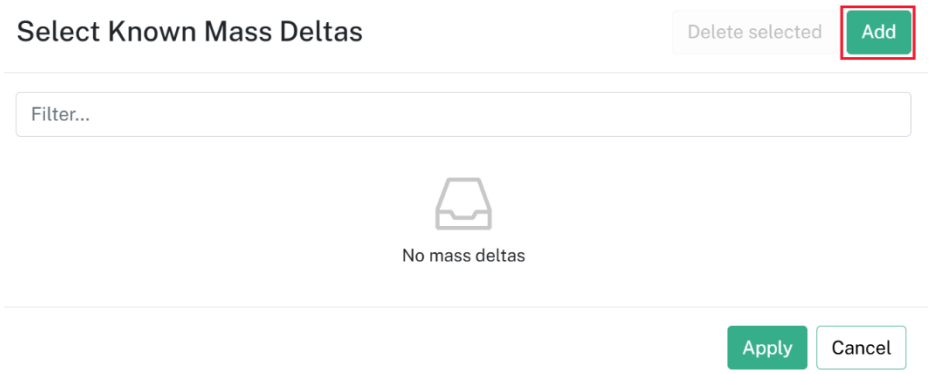


Figure 112: Select Known Mass Deltas—none selected

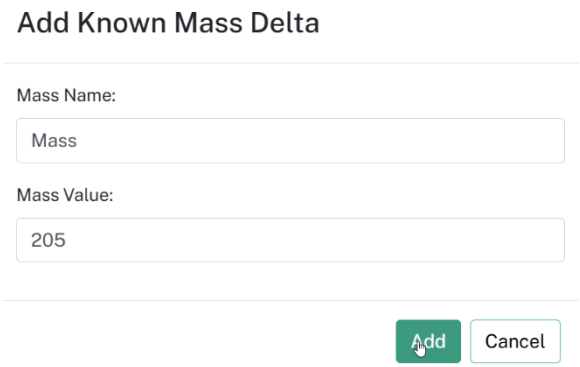


Figure 113: Add Known Mass Deltas selection dialog

The user can only add up to *three* known delta masses at a time. Once all desired mass deltas have been added, the user should click **Apply**.

Note that when a user adds a negative value, the application converts it to an absolute value.

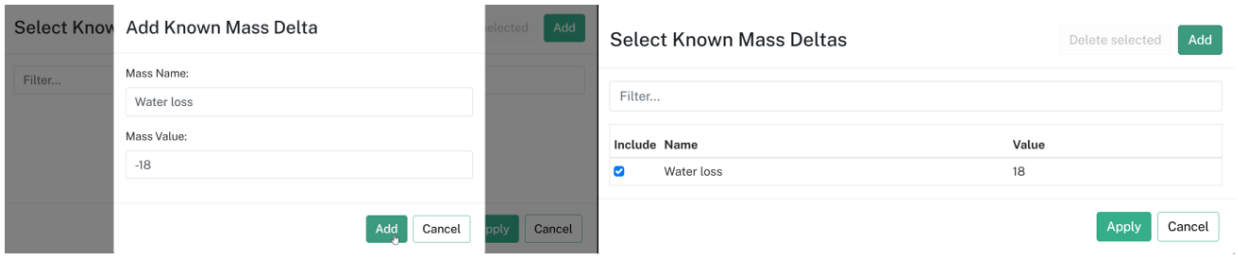


Figure 114: Negative Known mass delta values are converted to absolute value

Preprocessing (m/z)

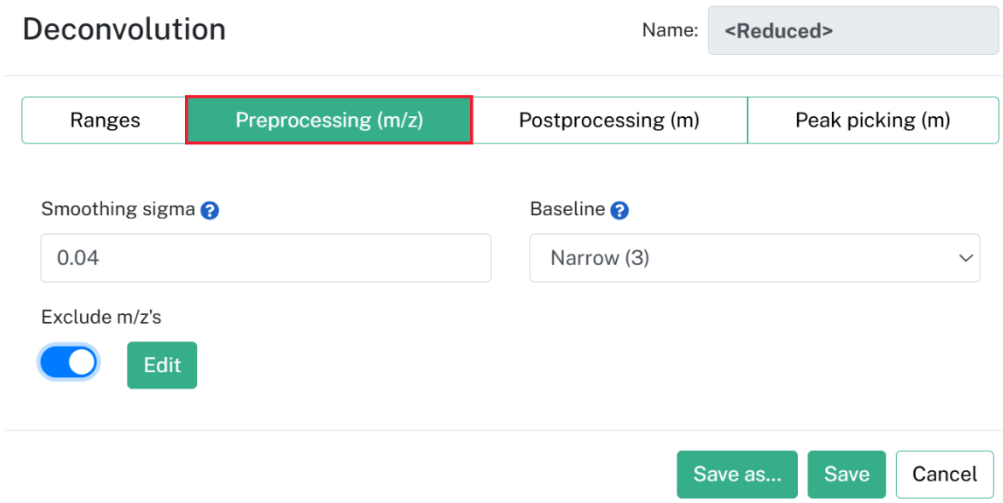


Figure 115: Preprocessing tab

In the **Preprocessing (m/z) tab**, users can apply preprocessing parameters to m/z spectrum for deconvolution.

Smoothing sigma (m/z) controls the level of smoothing in the MS1 spectra. A value of 0.005 may be used for isotopically resolved spectra. For most average mass, such as reduced and intact denatured mAbs, a value of 0.04 is recommended. For broader peaks associated with high mass and native-MS, a value of 0.4 may be more appropriate. Lower values are usually used for narrow m/z peaks, and higher values may be required for broader peaks.

Baseline radius: Controls the stiffness of the baseline on m/z peaks. Narrow (3) is useful for low mass (<60kDa) and isotopically resolved MS1 data. Medium (15) will work well for most average mass data (such as denatured mAbs), whereas a much higher setting of wide (100) may be required for broad peaks observed at high mass or native-MS. Narrow peaks usually use lower values, broader peaks will use higher values. Inappropriate settings may distort peak areas.

When **Exclude m/z** is toggled **on**, it allows the user to specify one or more m/z values so that deconvolution can occur without those ions being included in the processing or output. Clicking Edit opens the “Select excluded m/z values” dialog comes up. From here, the user can click **Add**, which opens a dialog where the user can provide a name, formula, and charge values for the m/z value to be excluded.

Once the value has been added, it will be added to a table and the Mono M and Mono m/z’s will be calculated automatically. Here, the user also has the option to exclude C13 isotopes.

Postprocessing

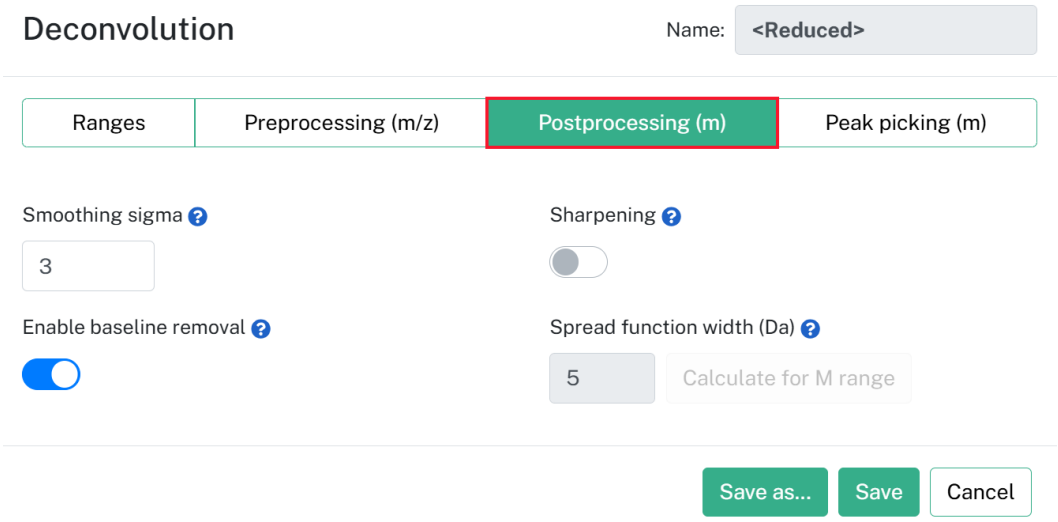


Figure 116: Deconvolution Postprocessing tab

Smoothing Sigma: Controls smoothing in the neutral deconvolved spectra. A value of 0.1 may be used for isotopically resolved spectra. For most average mass, such as reduced and intact denatured mAbs, a value of 3 is recommended. For broader peaks associated with high mass and native-MS, a value of 6 may be more appropriate. Lower values are usually used for narrow m/z peaks, and higher values may be required for broader peaks.

Sharpening: Toggle on to enable. Sharpening may help to resolve shoulder peaks on the neutral deconvolved spectra. Not recommended for use with isotopically resolved data.mo

Enable baseline removal: Toggle on to enable. Will remove baseline from deconvolved spectra, and is only recommended for noisy baseline.

Spread function width: Used for peak sharpening. To enable, **Sharpening** must also be enabled. This defines the halfwidth of the point spread function. It can be auto calculated by clicking calculate for M range, and is dependent on the deconvolution mass range set in the ranges tab. The calculation is based upon the square root of a neutral mass peak in kDa.

Progressive Deconvolution (Progressive Deconvolution mode only)

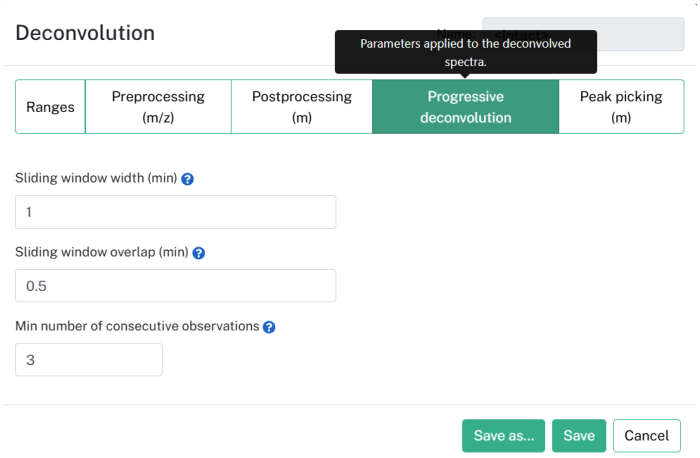


Figure 117: Progressive deconvolution tab

Sliding window width (min): Width of the individual deconvolution windows (slice) applied across the entire time range to perform progressive deconvolution. The sliding window width must be greater than the sliding window overlap

Sliding window overlap (min): Time overlaps between consecutive deconvolution windows (slices) for progressive deconvolution.

Min number of consecutive observations: Minimum number of same mass observations to generate deconvolved mass feature. For example, if this value is set to 3, then the same mass must be observed in 3 consecutive windows/slices. The aim of this is to reduce noise. It is suggested that the number is decreased if the sliding window overlap results in < 3 slices generated across the time range of trace peaks

Peak Picking

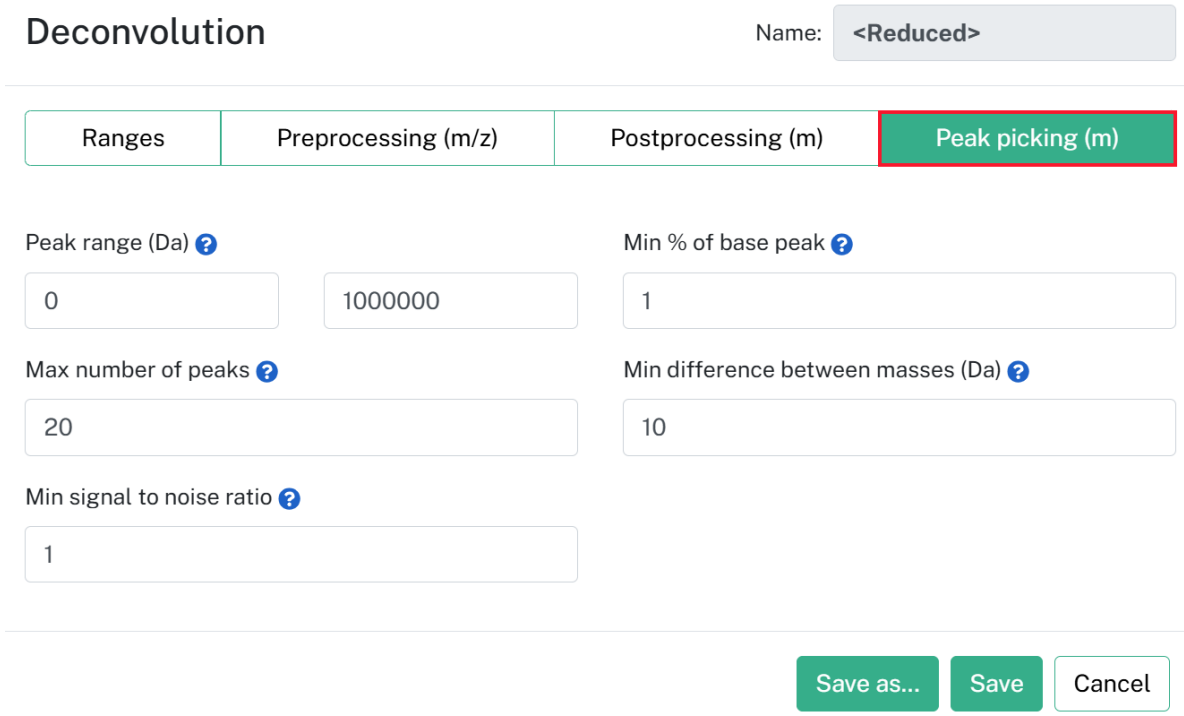


Figure 118: Deconvolution Peak picking tab

Peak Range: Defines the range to be used for peak picking for mass matching. Values cannot exceed the minimum and maximum values set for deconvolved mass range set in the ranges tab.

Min percent of base peak: Will only allow peaks above this threshold % of the base peak (highest intensity) to be picked and used for mass matching.

Max number of peaks: Sets a limit on the number of peaks used for mass matching. The top n peaks will be annotated (based upon intensity).

Min difference between masses (Da): This will define the minimum delta mass (Da) between deconvolved peaks that are separate peaks to be used for mass matching and is highly dependent on resolution. Isotopically resolved data will give greater resolution, hence a lower delta value of 7 Da between mass peaks may be useful. For higher mass, broader, less resolved peaks (such as intact mAbs and native data) a value of 15 may work well. For reduced mAbs a value of 10 may be more appropriate. Low values may be used with more resolved data, higher values will be needed for less resolved data.

Minimal signal to nose ratio: Will set the minimum signal-to-noise ratio (amplitude of mass peak relative to the amplitude of background noise peaks) for picking peaks to be used for mass matching. This setting is dependent on the level of noise observed in the deconvolution spectra. Low values (such as <2) will allow low level peaks close to background noise to be picked, whereas a high value such as 10 will only pick those peaks that are clearly above the background noise.

Masses Table

The **Masses** table reports the masses of the most intense peaks that are selected in the Trace Peaks table.

Masses will be shown based upon peaks selected in the Trace Peaks table. The only columns that are editable are Assigned, Name, and Mass comment.

Selecting multiple peaks in the Trace Peaks table displays all associated masses within the Masses table.

Masses Table

Assigned	Sample No.	Sample Name	Peak No.	Mono Mass	Average Mass	Intensity	Name	Mass Comment	Delta Mass From Calc.	Expected Mono Mass	Delta Mono Mass (ppm)	Delta Mono Mass	Protein Name	Delta Name	Expected Average Mass	Delta Mass From Most Intense	Delta M Mass F Most Intense
Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Sear
☑	1	DN32_red	1	-	50952.35	2391254.1	HC, G0F-GlcNAc		-6.74	50926.89	-	-	HC	G0F-GlcNAc	50959.09	-365.46	-
☑	1	DN32_red	1	-	50790.59	648508.6	50791		-	-	-	-	-	-	-	-527.22	-
☑	1	DN32_red	1	-	50748.92	364837.6	50749		-	-	-	-	-	-	-	-568.89	-
☑	1	DN32_red	1	-	23336.75	4914231.8	LC		-3.85	23325.98	-	-	LC	-	23340.6	-27981.06	-
☑	1	DN32_red	1	-	14653.22	1356775.3	14653		-	-	-	-	-	-	-	-36664.59	-

Figure 119: Masses table (truncated)

Within the Masses table, the user can sort columns in ascending/descending order by clicking the column of interest. An up arrow indicates that the column has been sorted by ascending order and a down arrow indicates that the column has been sorted by descending order.

Average Mass	Intensity
Search	Toggle SortBy
51479.92	2575196.6
51521.48	391104.7
51627.69	453688.5
51639.98	468827.2
51787.34	544965.4

Figure 120: Column with arrow indicating that values are sorted in ascending order

Note: User will see a minor difference in masses between WA and Byos due to the fact that WA sources values from IsoSpec. Source: https://pubs.acs.org/doi/10.1021/acs.analchem.0c00959

Sample Status Review

The **Sample Status Review** table allows the user to perform validation on their samples. The number of desired and undesired is listed and based upon the presence of desired/undesired properties for each combination specified in the Sequences room (Expected Type as added to sequence combinations). Each sample is marked as either Pass (green), Fail (Red), or Review (Yellow).

Sample Status Review

2/2 rows

Sample Name	Sample No.	Desired	Undesired	Unexpected	Status	Comment	Validate
Search	Search	Search	Search	Search	Search	Search	Search
DN32_red	1	2 of 2	0 of 0	15	Review		Pass
DN32_reddeglyc	2	1 of 1	0 of 0	14	Review		

Figure 121: Sample Status Review

The following rules apply to samples to determine their status:

Desired	Undesired/Unexpected	Status
100%	0%	PASS
<100%	Any	FAIL
100%	Any	REVIEW

Figure 122: Sample Status rules

In addition to the original Validation status determined by the application logic of the software, there is an opportunity for a user to define a validation status by using the **Validate** column input option.

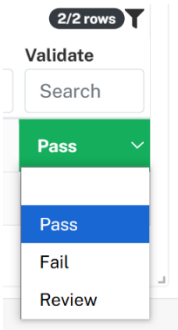


Figure 123: Validate column

The **Comment** column is user editable by clicking in the cell when a drop down appear and the same status options become available for the user to choose. Entry into this column is optional. If the user wants to take note of the outcome of their review or any changes they make, they can edit the content of the comment column.

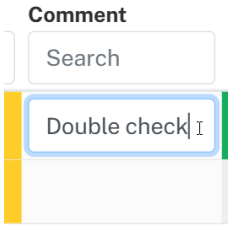


Figure 124: Comment column

Trace Plots

The behavior of the Trace Plots in the Inspection room is the same as the Trace Plots in the Samples room.

All sample traces that are set to be visible in the Samples room will be shown in the Inspection room. All visible trace plots can be shown by scrolling within the Trace Plot view.

When a peak is selected in the Trace Peaks Table, the integration boundaries (start and end times) are displayed within the Trace Plots as magenta vertical lines with the area under the curve (AUC) shaded blue.

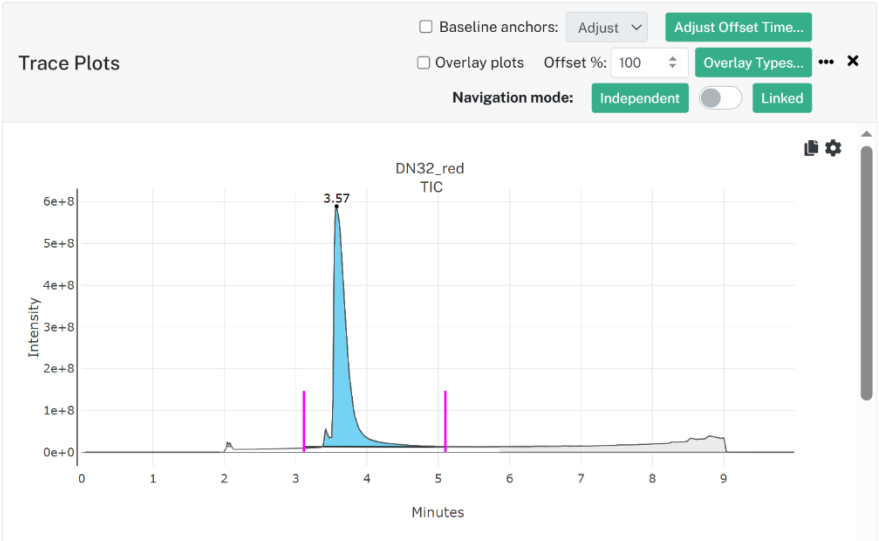


Figure 125: Trace Plot with integration windows for selected time ranges

The Baseline Anchor, Navigation mode, and Overlay plots settings are the same for Trace Plots within both the Samples and the Inspection room. See Baseline Anchors, Navigation Mode, and Trace Overlay.

Heatmaps

The **Heatmaps** widget provides an interactive way to visualize MS1 and Deconvolved Mass data.

Heatmaps to visualize data are present in the Inspection and Report rooms. This feature allows users to visualize data trends and patterns more effectively, facilitating better decision-making based on the analysis results. These interactive heatmaps are present in the Inspection and Report rooms. Note that the Deconvolved Heatmap is only generated if the user processes their data using **Progressive Deconvolution**.

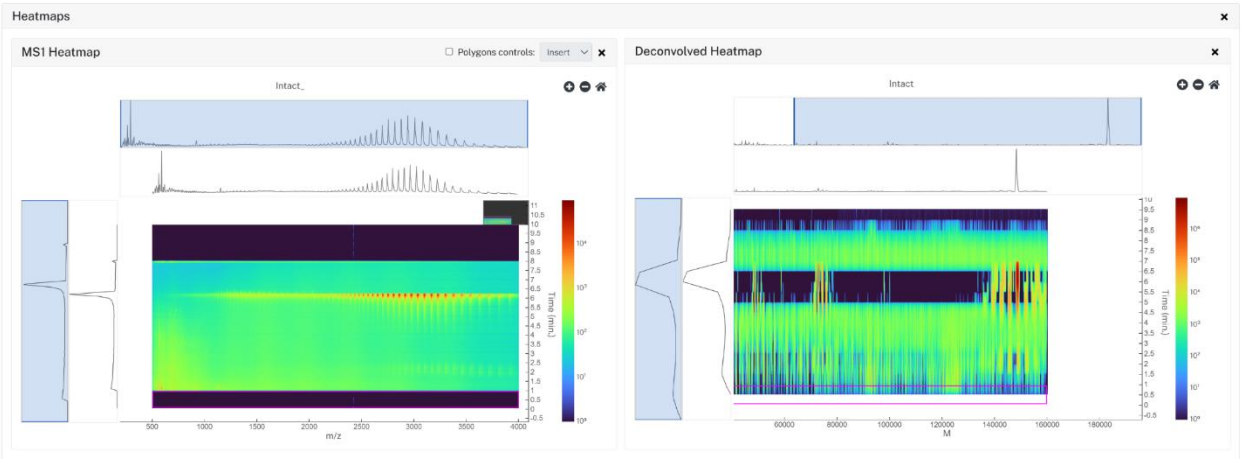


Figure 126: Heatmaps widget

Heatmaps visualize m/z vs time (MS1 Heatmap) and M vs time (Deconvolved Heatmap). When a row is selected, the corresponding area is highlighted with a red box on the heatmap.

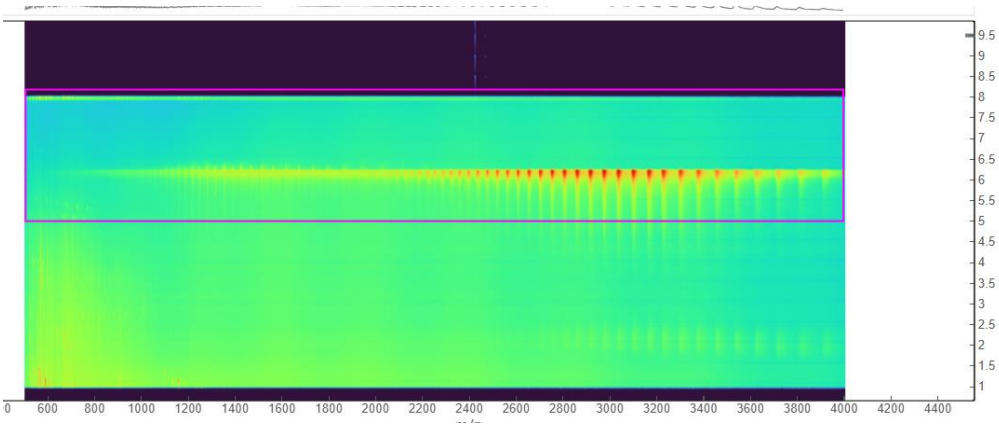


Figure 127: Red box around corresponding selection

If multiple rows are selected in the trace peaks table, multiple regions will be highlighted on the heatmap. Heatmaps are generated for each input sample. For a sample's heatmap to be displayed, a trace peak from that sample must be selected.

The heatmaps have the following dynamic **zoom controls**:

- Users can zoom in and out on the heatmap as well as return to home position (Home icon)
- Zoom in with + icon or left clicking the heatmap
- Mouse scroll to zoom in/out while hovering over the heatmap

When a user selects **Progressive Deconvolution** mode, deconvolution is no longer restricted to the integrated trace peaks. Instead, a user may specify windows to produce multiple slices for deconvolution. As a part of selecting Progressive Deconvolution, an additional Deconvolved heatmap is created which provides the same level of granularity as the progressive deconvolution.

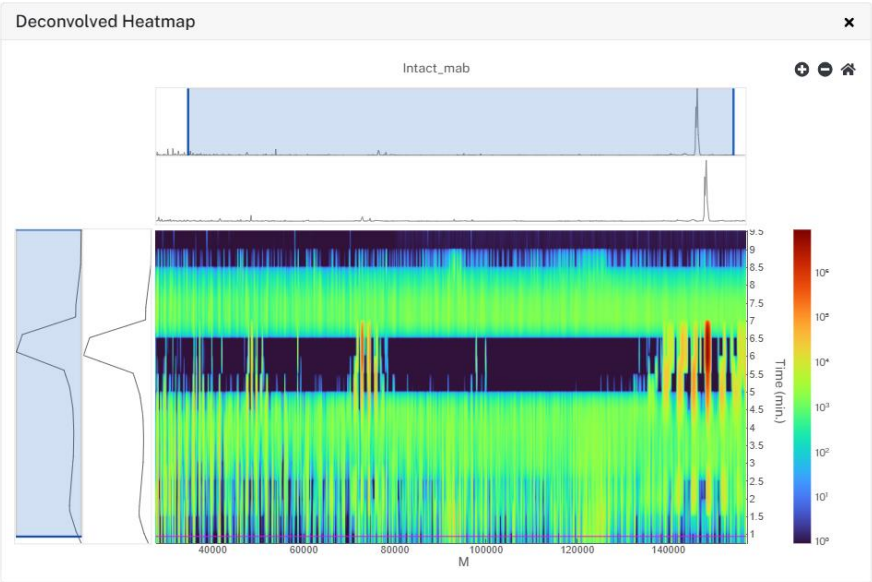


Figure 128: Deconvolved Heatmap from Progressive Deconvolution

Alongside each heatmap are **margin plots** which display the corresponding trace plot and the m/z or M spectra, respectively. Each margin plot contains two stacked instances of the same data – the bottom trace

will change boundaries as the user zooms into areas of the heatmap. The top trace will remain at full zoom and show vertical bars representing the zoom level of the trace below.

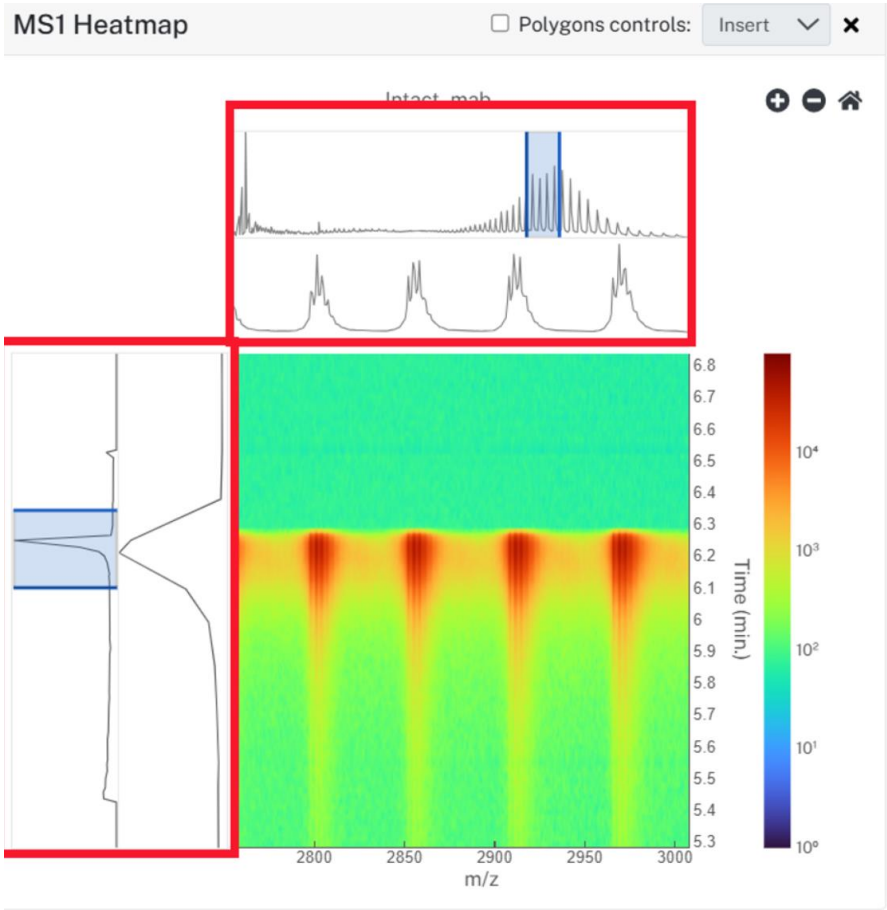


Figure 129: Margin plots

A **color scale** on the right side of the Heatmap denotes the relationship between pixel colors and signal intensity.

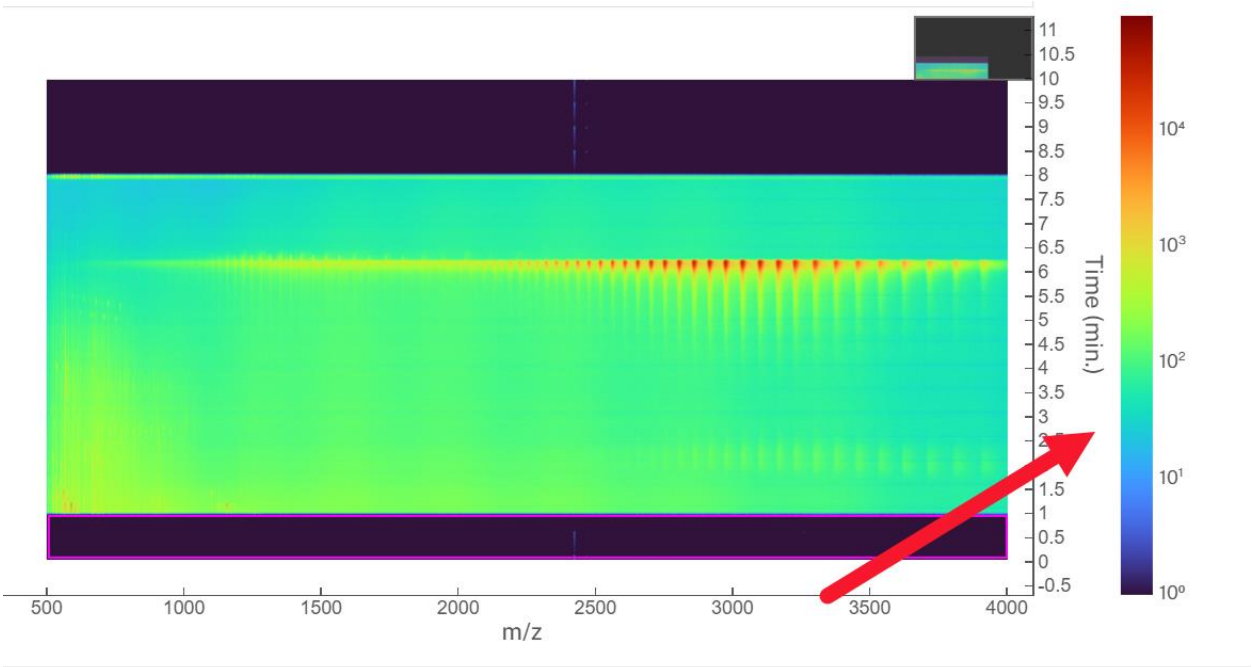


Figure 130: Heatmap color scale

An additional feature available in the MS1 Heatmap is **MS1 polygon filtering.** Using the new polygon drawing tools, users can draw a polygon around a region of interest within the heatmap and the polygon selection will be saved to the Trace Peaks table and a corresponding summed MS1 and Deconvolved spectrum will be generated in the existing spectrum plots.

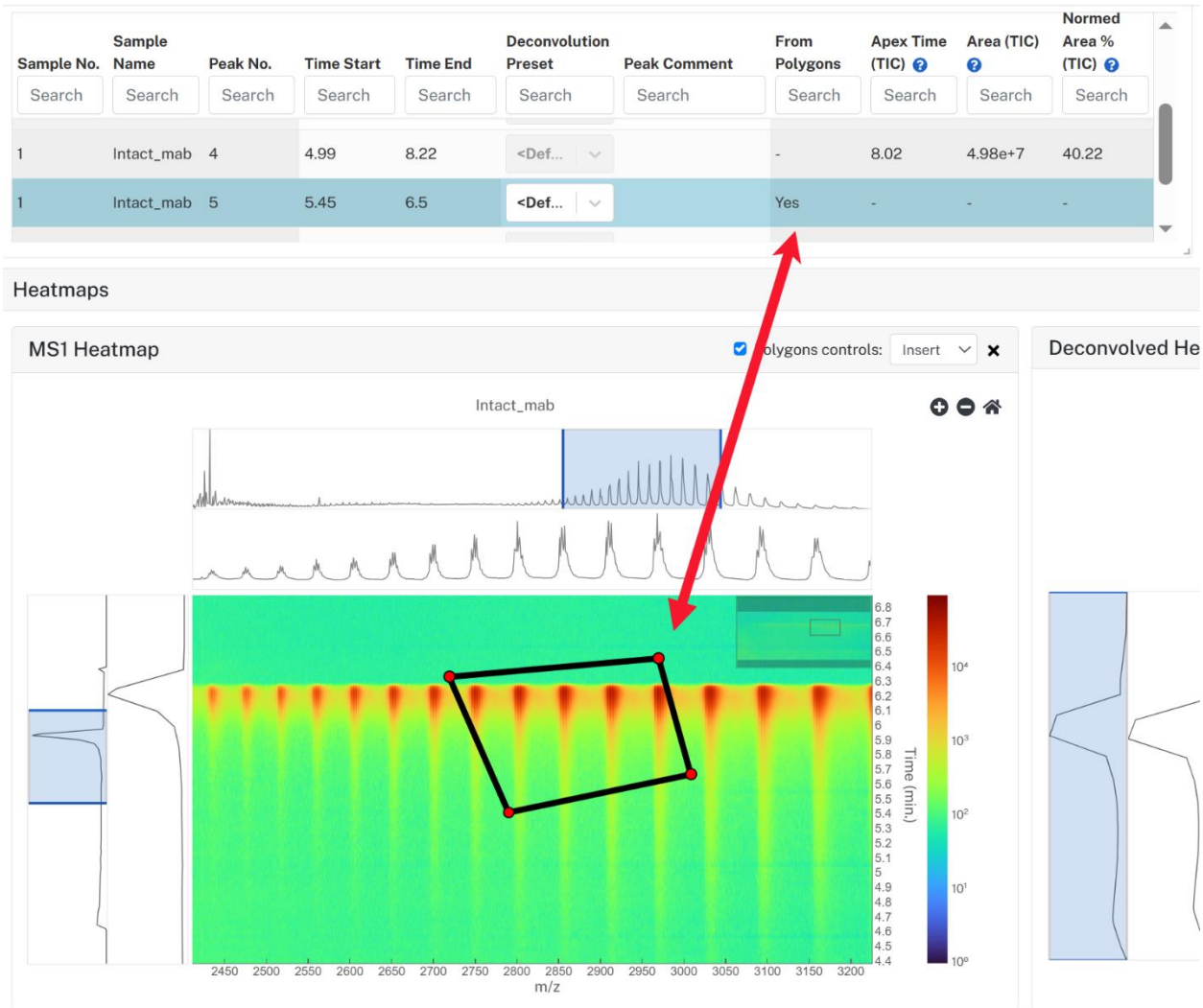


Figure 131: Heatmap polygon filtering

When Polygon controls are enabled, users can select to **Insert**, **Adjust,** or **Delete** anchor points in the Polygon. If the user wishes to delete the Polygon entirely, this can be done by deleting the resultant Trace Peak row created by the bounded polygon.

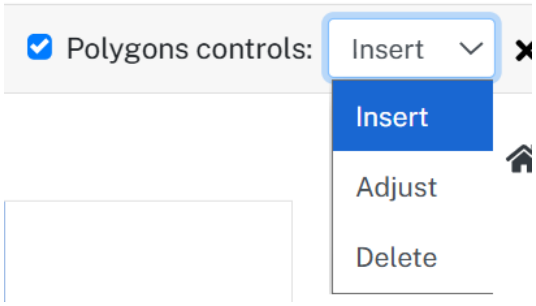


Figure 132: Polygon controls

MS1 and Deconvolved Mass Plots

MS1 and **Deconvolved Mass Plots** appear side-by-side for each peak.

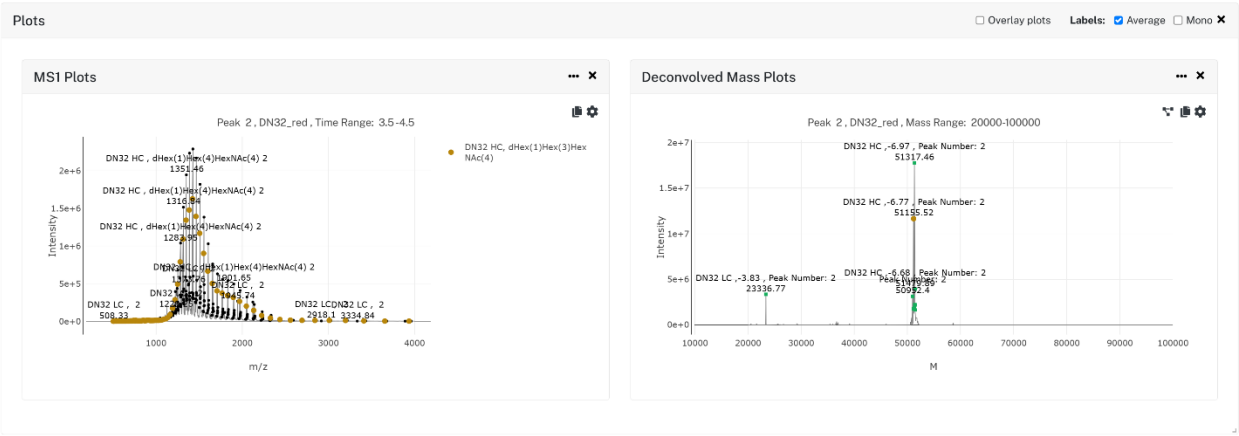


Figure 133: MS1 and Deconvolved Mass Plots in Inspection room

The **MS1** plot shows the summed m/z plot for the trace peak selected.

The **Deconvolved Mass spectrum** displays neutral masses plotted for the summed m/z plot associated with the trace peak selected. This spectrum is computed using the specialized Intact analysis algorithm. The neutral mass peaks within the deconvolved mass spectrum represent proteoforms within the sample.

Users can check Mono, Average, or both types to annotate their plot.

If the start/end time is updated within the Trace Peaks table within the Inspection Room, the MS1 and Deconvolved plots are hidden until they have been recomputed.

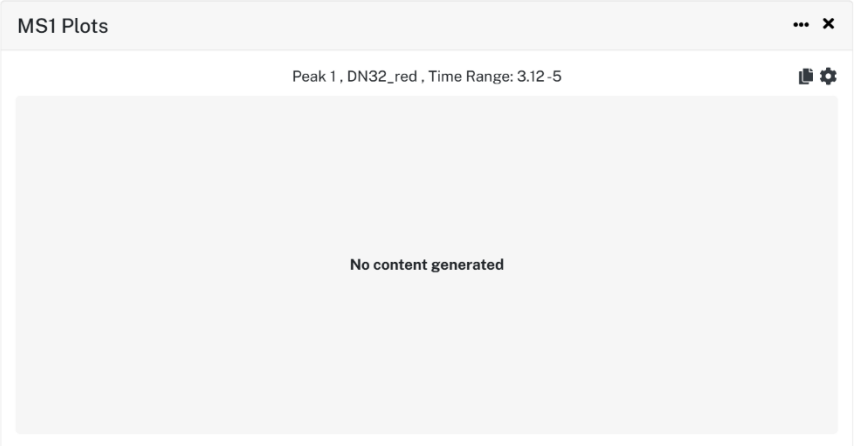


Figure 134: Plots show "No content generated" when changes are made until recomputed

The Deconvolved mass plot also contains a **selection marquee.** Clicking on the icon sets the cursor to selection mode. When a peak is selected in the plot, a colored dot appears over that peak and the same-colored dots appear over the corresponding peaks in the MS1 plot. Clicking on another deconvolved peak will assign a different colored dot to that peak and its MS1 peaks. The colored dots in the MS1 spectrum are the best way to tell true mass peaks from artifacts; the MS1 dots for true mass peaks have contiguous charges and tend to hit local maxima for m/z intensity.

Figure 135: Selection marquee

When the user selects a row in the masses table, colored dots representing the mass can been seen on the MS1 and deconvolved mass spectrum.

Figure 136: Color-coordinated dots represent masses

Multiple rows can be selected, leading to multiple colors being represented on the plot. As seen in the image below, the MS1 plot contains a legend that reflects the selected rows from the Masses table.

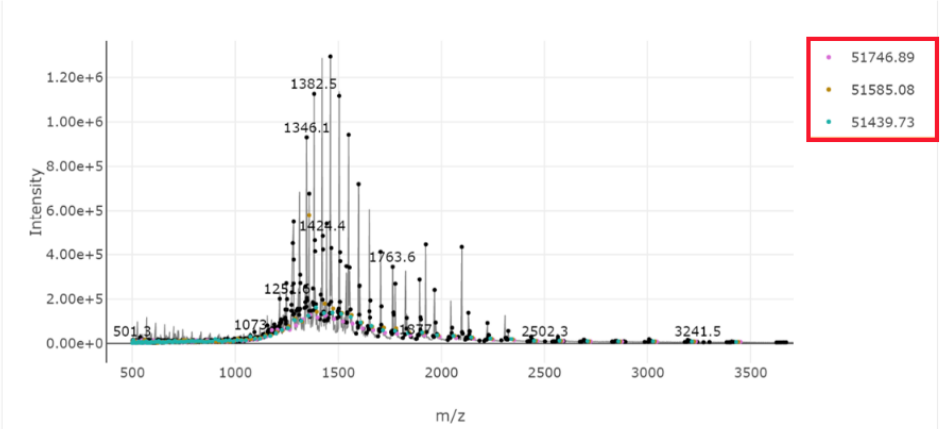


Figure 137: MS1 plot with legend

Checking **Overlay plots** enables the overlay of all multi-selected sample plots.

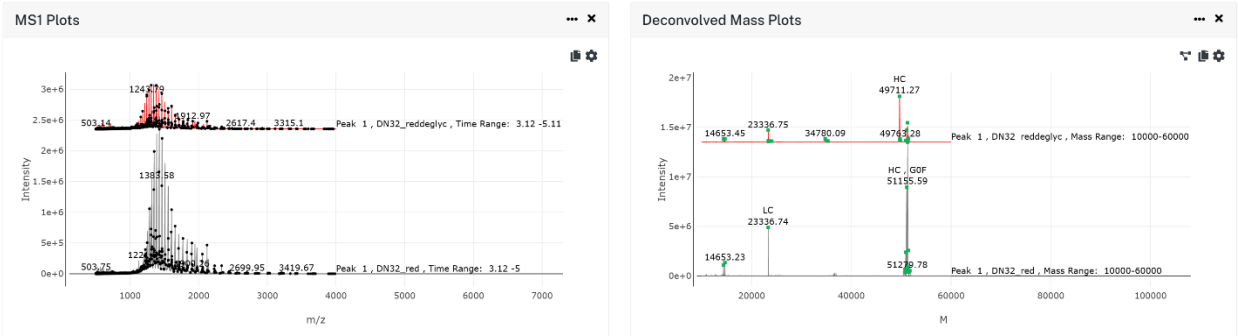


Figure 138: Overlay plots with 100% offset for both plot types

The **Offset control**, enabled when the user checks **Overlay plots**, is accessed by clicking the icon. The offset value can range from 0-500%. When the value is set to 0%, the plots are completely overlapped. When the value is set to 100%, the plots are separated completely.

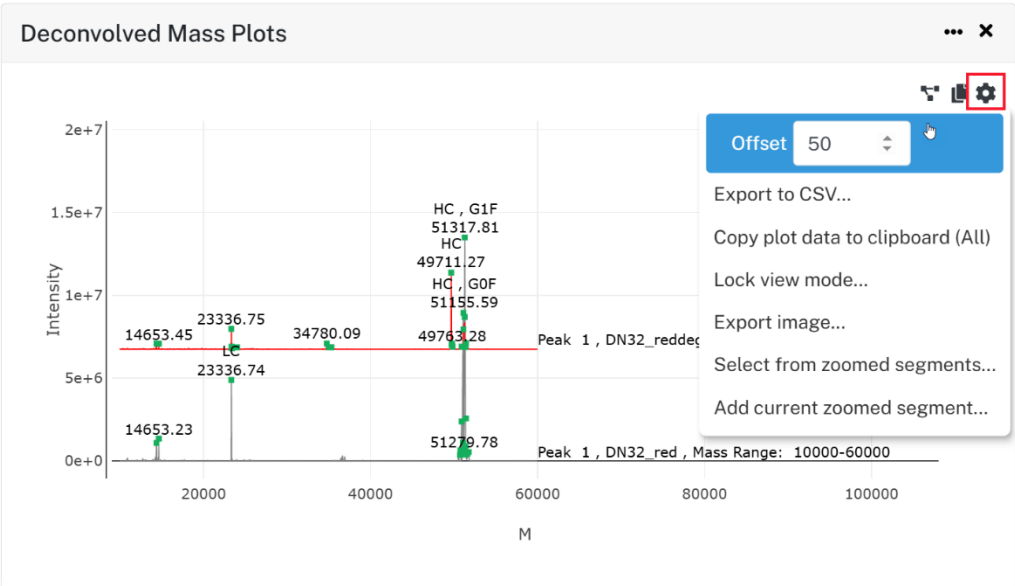


Figure 139: Overlay plots with 50% offset for DCM plots

Individual Plot Tools (Inspection room)

Additional settings can be accessed by clicking the icon. Information on the first four options can be found in the [Individual Plot Tools]() section for the Samples room section as the behavior of these tools is the same. Additional individual plot tools are detailed below.

Select/Add Zoomed Segments

Within each plot widget, users have the option to add a new zoom segment or select to view from the list of zoomed segments available.

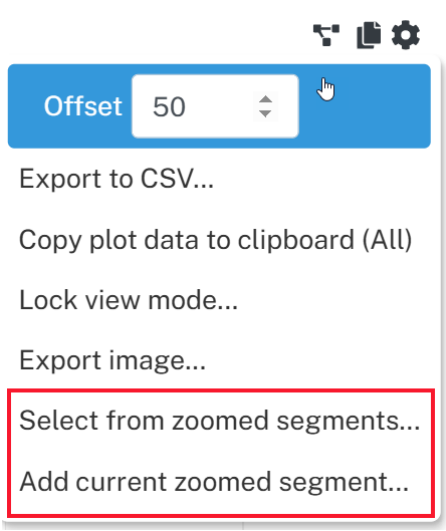


Figure 140: Zoomed segment options in plot settings

Zoomed segments can be associated with the current peak (default), all peaks in current sample, or all peaks in all samples. Users can specify min-max Mass as well as min-max intensity (defining the zoom parameters both on the X and Y-axes).

Clicking **Select from zoomed segments** opens a pop-up window that contains all currently available zoom segments. All fields are editable by the user except the Segment number field.

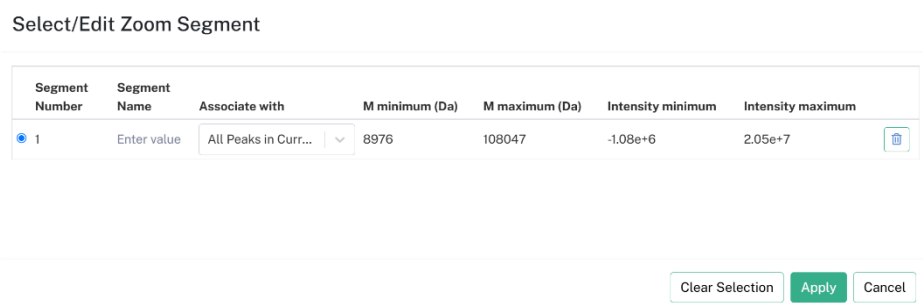


Figure 141:Select/Edit Segment

Clicking **Add current zoomed segment** allows the user to save the current zoom settings as a zoomed segment option that can be applied to the current selected peak, all peaks in the current sample, or all peaks in all samples.

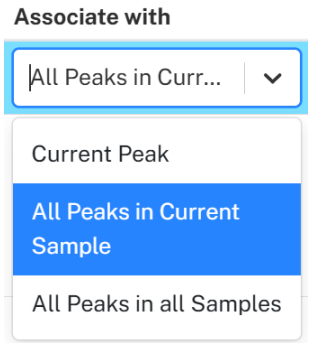
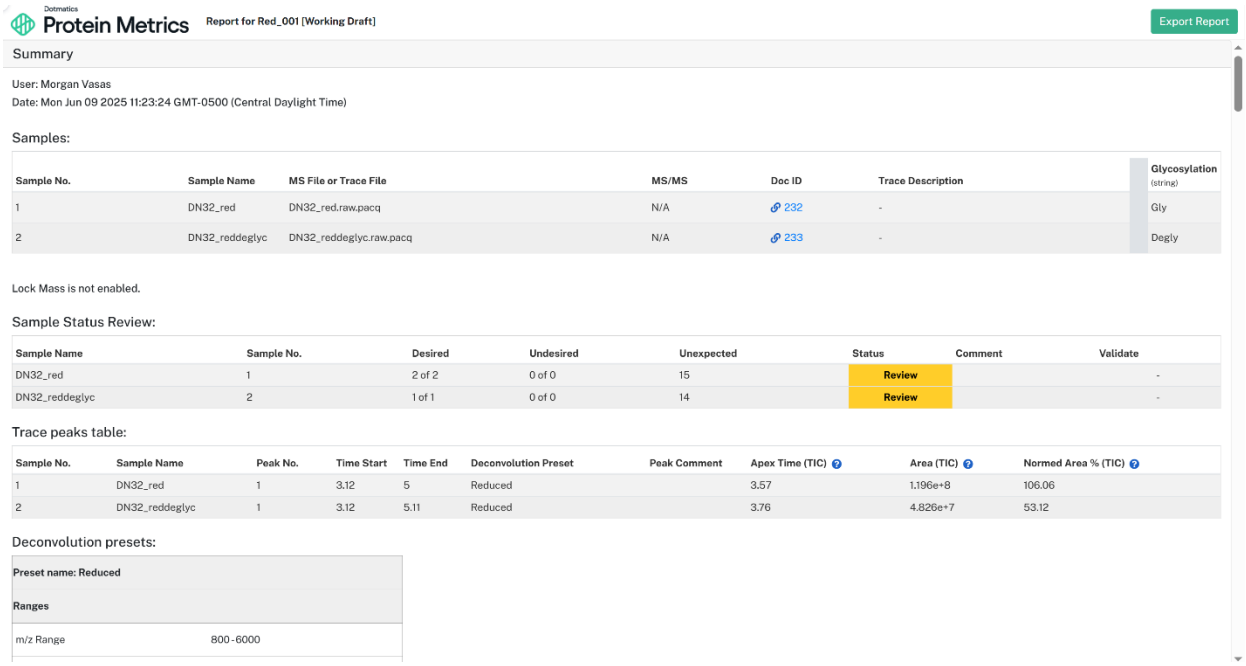


Figure 142: Add Current Zoom Segment

The values for Da and Intensity can be edited by the user by double clicking on the value in the table.

Report room (Deconvolution mode)

The **Report** room provides a summarization of the analysis, as well as Trace, MS1 and Deconvoluted Mass plots, and provides the user with the ability to create Visualizations to accompany their analysis.



Dotmatics Protein Metrics Report for Red_001 [Working Draft] Export Report

Summary

User: Morgan Vasas
Date: Mon Jun 09 2025 11:23:24 GMT-0500 (Central Daylight Time)

Samples:

Sample No.	Sample Name	MS File or Trace File	MS/MS	Doc ID	Trace Description	Glycosylation (string)
1	DN32_red	DN32_red.raw.pacq	N/A	232	-	Gly
2	DN32_reddeglyc	DN32_reddeglyc.raw.pacq	N/A	233	-	Degly

Lock Mass is not enabled.

Sample Status Review:

Sample Name	Sample No.	Desired	Undesired	Unexpected	Status	Comment	Validate
DN32_red	1	2 of 2	0 of 0	15	Review		-
DN32_reddeglyc	2	1 of 1	0 of 0	14	Review		-

Trace peaks table:

Sample No.	Sample Name	Peak No.	Time Start	Time End	Deconvolution Preset	Peak Comment	Apex Time (TIC)	Area (TIC)	Normed Area % (TIC)
1	DN32_red	1	3.12	5	Reduced		3.57	1.196e+8	106.06
2	DN32_reddeglyc	1	3.12	5.11	Reduced		3.76	4.826e+7	53.12

Deconvolution presets:

Preset name: Reduced	
Ranges	
m/z Range	800 - 6000

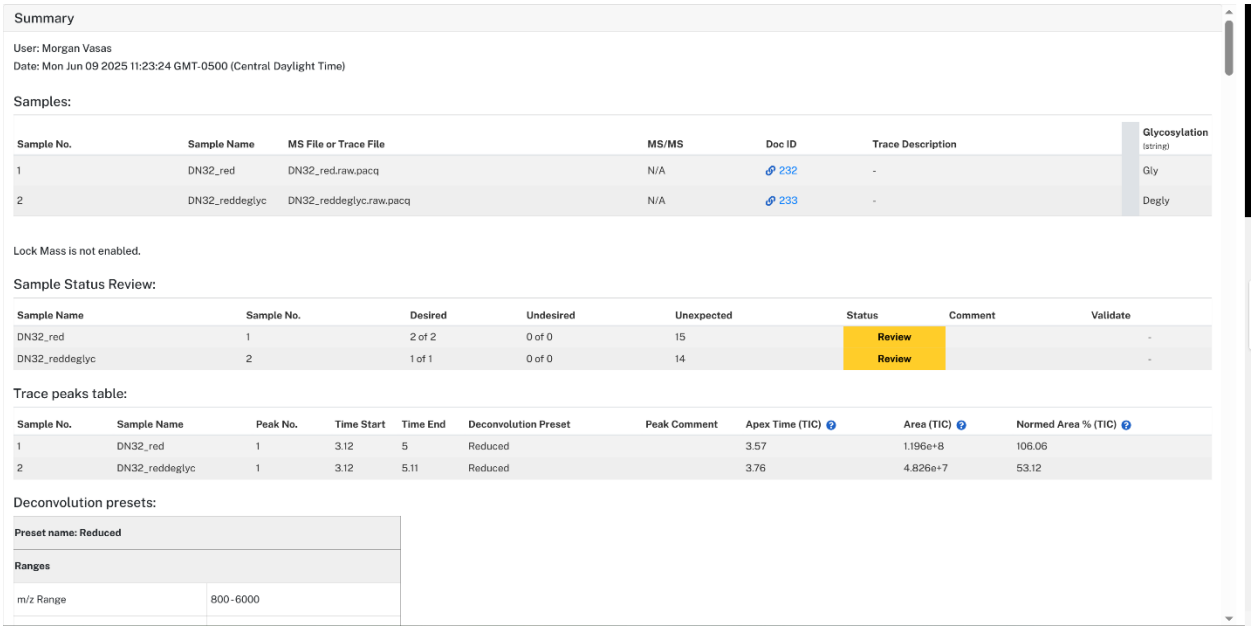
Figure 143: Snapshot of Report room

Note that in the Report room, the header only shows the following options when in Edit mode.

Figure 144: Header options for the Report room

Summary

The **Summary** view provides a summary of the settings used to create an analysis.



Summary

User: Morgan Vasas
Date: Mon Jun 09 2025 11:23:24 GMT-0500 (Central Daylight Time)

Samples:

Sample No.	Sample Name	MS File or Trace File	MS/MS	Doc ID	Trace Description	Glycosylation (string)
1	DN32_red	DN32_red.raw.pacq	N/A	232	-	Gly
2	DN32_reddeglyc	DN32_reddeglyc.raw.pacq	N/A	233	-	Degly

Lock Mass is not enabled.

Sample Status Review:

Sample Name	Sample No.	Desired	Undesired	Unexpected	Status	Comment	Validate
DN32_red	1	2 of 2	0 of 0	15	Review		-
DN32_reddeglyc	2	1 of 1	0 of 0	14	Review		-

Trace peaks table:

Sample No.	Sample Name	Peak No.	Time Start	Time End	Deconvolution Preset	Peak Comment	Apex Time (TIC)	Area (TIC)	Normed Area % (TIC)
1	DN32_red	1	3.12	5	Reduced		3.57	1.196e+8	106.06
2	DN32_reddeglyc	1	3.12	5.11	Reduced		3.76	4.826e+7	53.12

Deconvolution presets:

Preset name: Reduced	
Ranges	
m/z Range	800-6000

Figure 145: Snapshot of Summary view within the Report room

The **Summary** section of the Report room provides an overview of the key inputs and outputs for an analysis. Information includes:

- **Samples** used in the analysis
- **Sample Status Review** (from the Inspection Room)
- **Trace Peaks** (from the Inspection Room)
- **Deconvolution preset details**
- **Trace Types** table
- **Masses** table
- **Trace Range Rules** applied
- **Sequences** table
- **Combinations** table
- **Digestion Parameters**

This information will update within the Summary view as changes are made within the respective rooms.

Note: If you change the name of the analysis, the name at the top of the report also changes.

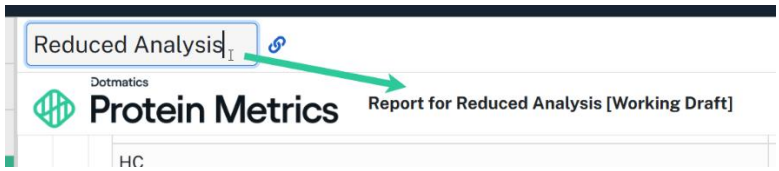


Figure 146: Change name of report

Charts & Tables

Users can create and view charts and tables within a Web Analysis report using similar mechanism that of Deep Query Dashboards for data visualization. For more information on Dashboards, see the **Deep Query Dashboards Manual**.

In a Web Analysis, data is pulled from the samples provided in the analysis, rather than a Data Source as seen in Deep Query. Upon opening a previous Analysis, the Dashboard and associated Visualizations are displayed.

Configuring the Visualization

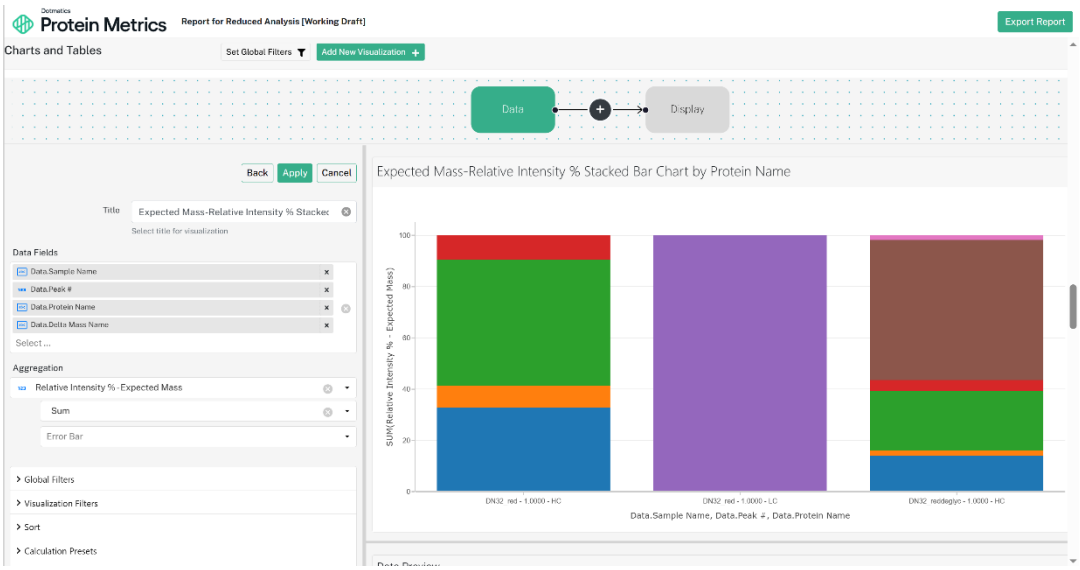


Figure 147: Building a Visualization for Report

To add a visualization to the Report, click **Add New Visualization**.

The **Reset** button resets any applied filters, graphical changes, or deleted visualizations that were made *prior* to clicking **Save Changes**. Thus, any changes the user made could be reversed to the last saved version.

Note: The Background Alert functionality does not work within Web Analysis.

Work is retained when switching pages without publishing. When the user has unsaved changes within Charts and Tables, the **Save Changes** and **Reset** button are enabled. When all changes have been saved, these buttons are disabled. Upon clicking **Save Changes**, all unsaved changes in Charts and Tables are saved to the working draft of the Analysis. Upon clicking **Reset**, the Charts and Tables section is reset to the last saved version of the working draft.

Plots (Deconvolution mode)

All the plots from the Analysis application (Trace, MS1, and Deconvolved Mass Spectrum plots) are available in the Report room to be added to a report. Controls under the **Report** tab enable the user to hide/show different components, such as the Summary, Chart & Tables, Trace Plot, MS1 Plot, and Deconvolved Mass Plot.

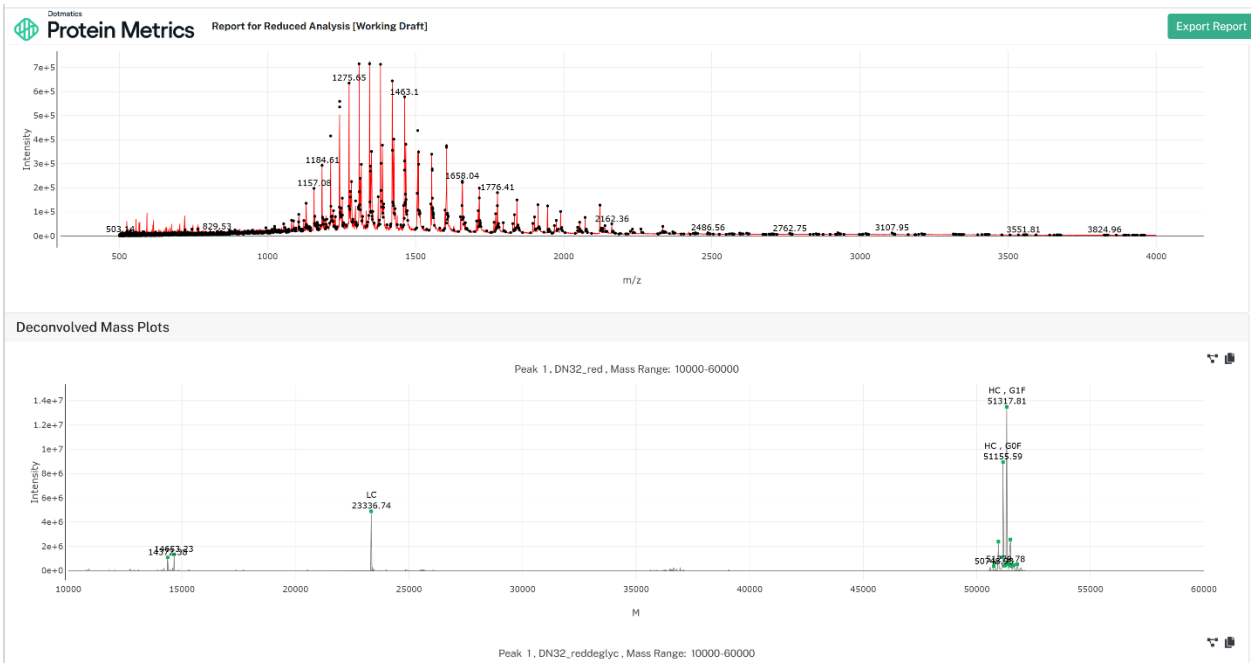


Figure 148: Plots within Report

The **Trace Plot**, **MS1**, and **Deconvolved Mass** are all included in the Report; however, the user has the option to deselect undesired plots in the side pane to remove them from the Report.

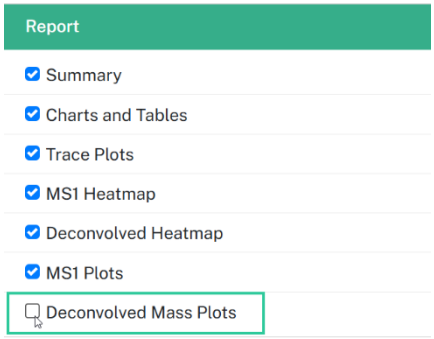


Figure 149: Deselected plot; in this case, the Deconvolved Mass plot will not be included in the Report room

Heatmaps (Deconvolution mode)

The Deconvolved and MS1 Heatmaps can also be included in the Report, and users have the ability to utilize zoom controls while in the Report room.

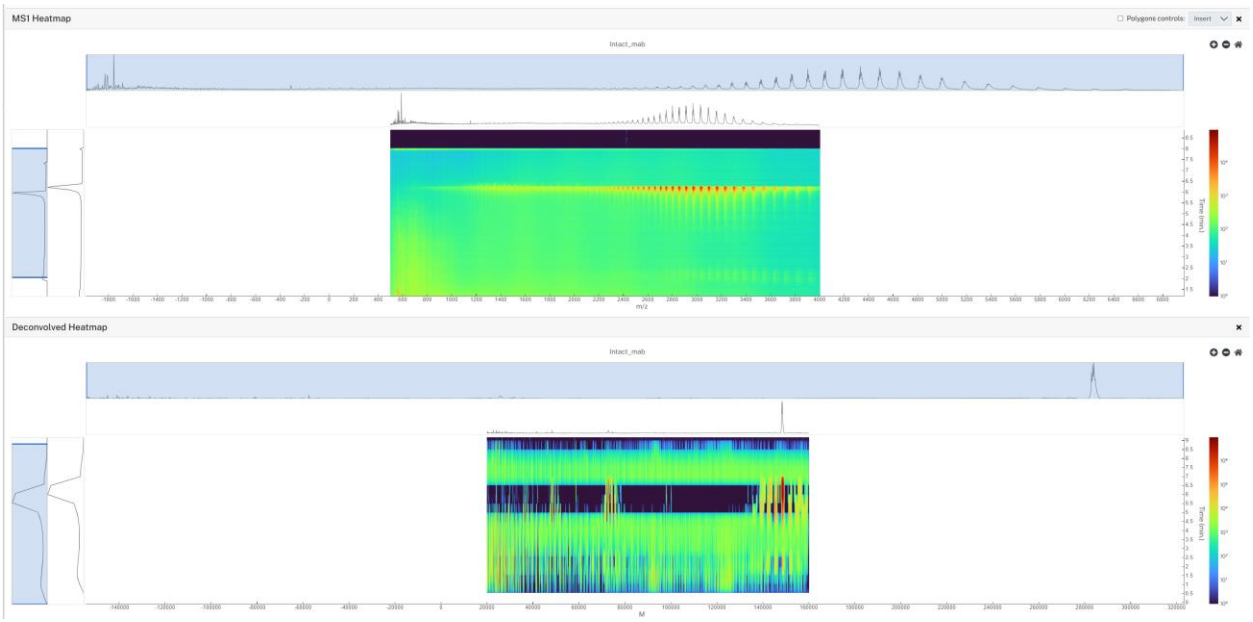


Figure 150: Heatmaps from within the Report room

Additional Tools

Numerical display settings

Users can control the numeric display settings on plots and tables. This widget can be found by clicking on the gear icon present in any of the tables or the three-dot icon ••• in the header for the plots within the Samples and Inspection rooms. For example, from the Masses table:

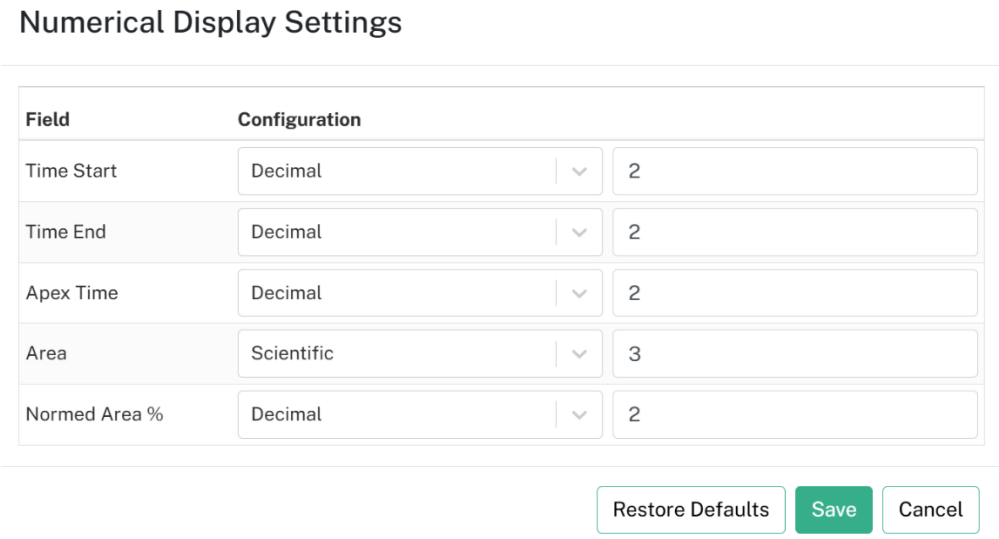


Figure 151: Numerical Display Settings dialog for the Masses table

Only numerical values will be listed in the configuration dialog.

For the plots, numerical display settings are included in a list of various tools. Clicking on “Numerical Display Settings” opens the following dialog:

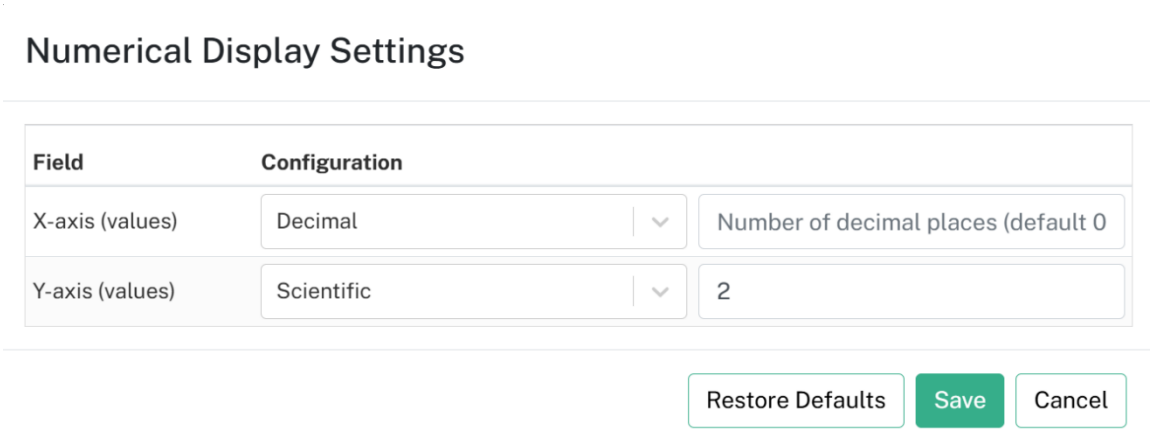


Figure 152: Numerical Display Settings dialog for plots

All values will have a default number of 2 decimal places prior to reconfiguration by the user.

Basic and Advanced Filters

Users can filter the results in their tables in two different ways. For **basic** filtering, there is a search bar on all columns within the tables in Web Analysis where users can type in a value to narrow down available values in the table to only those of interest. When a user enters a string, the rows in the table filter to only display those rows that contain the same user input string values:

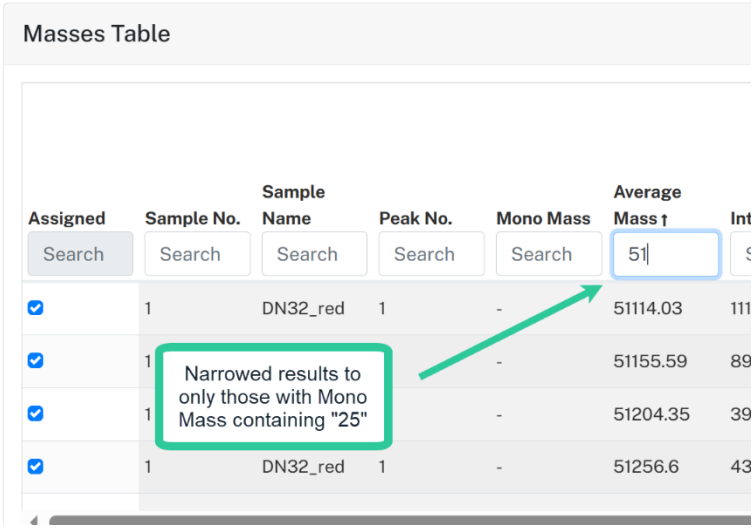


Figure 153: Basic Filtering

If the user enters string values in multiple rows, the individual text box entries act in conjunction with each other as an AND function.

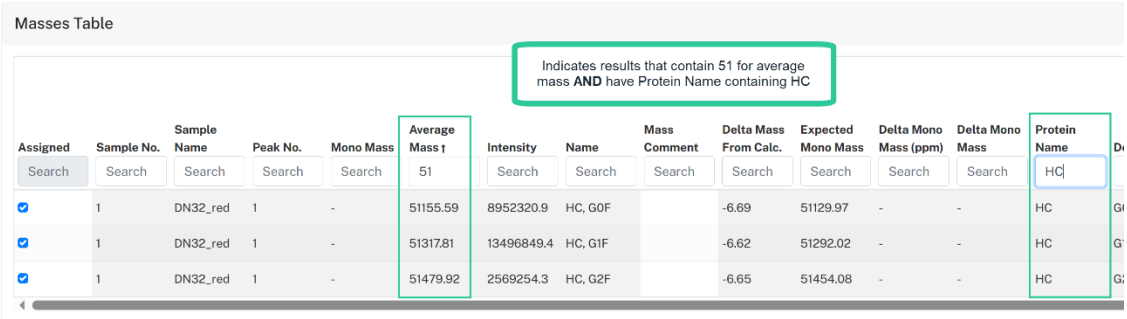


Figure 154: AND logic for multiple basic filters

Users can also apply more **advanced** filter functions on tables by clicking the icon. These advanced filters work in conjunction with the basic filters described above.

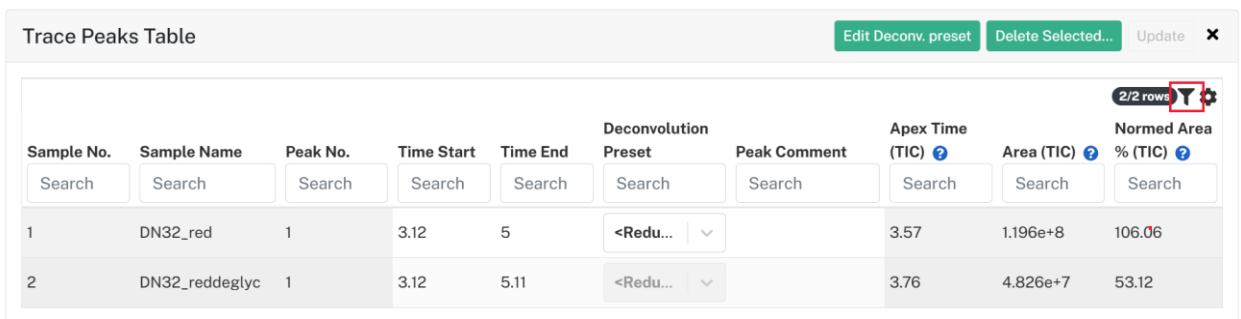


Figure 155: Advanced Filter icon

Conditions in the advanced filters can be applied as an ALL or ANY function to anything present in the text filters. For string entries, users can enter numbers, text, and symbol, and these entries are not case sensitive. For numeric entries only numbers can be entered apart from “e”. Users can add multiple filters.

Users have the option to select from multiple operators:

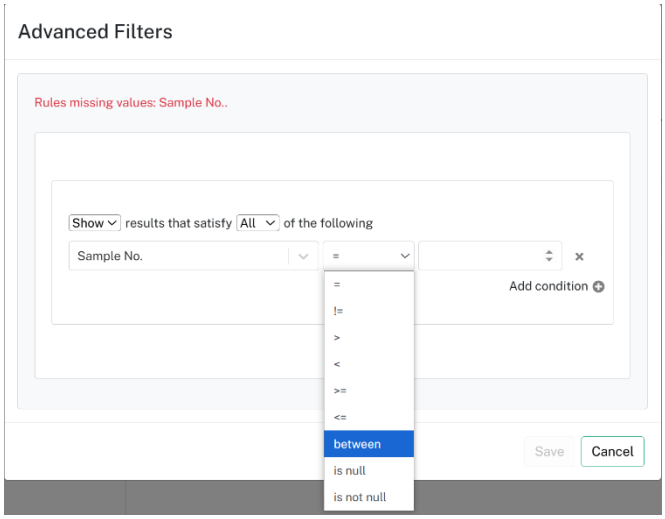


Figure 156: Advanced filters

It is viewable from the table how many rows are visible out of all total rows when filters are applied.

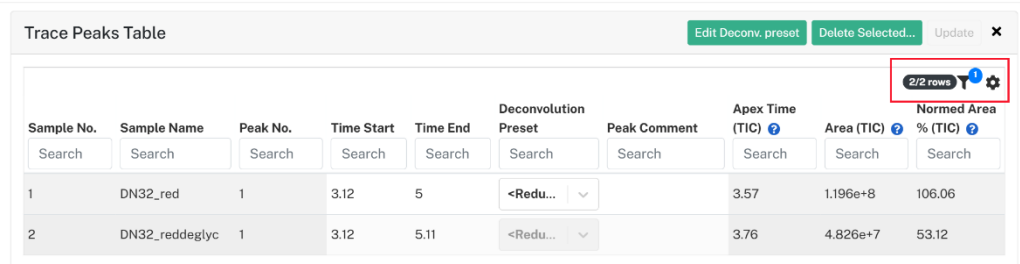


Figure 157: Number of advanced filters applied

Filtering is a *visual* tool only and will not impact the final reported data or calculations. Filters can only be applied in Edit mode.

Edit Plot Titles and Annotations

Users can customize the content of their plot titles and peak annotations to contain different fields as well as free text. Annotation tools can be accessed from the three-dot icon present in all plot widgets.

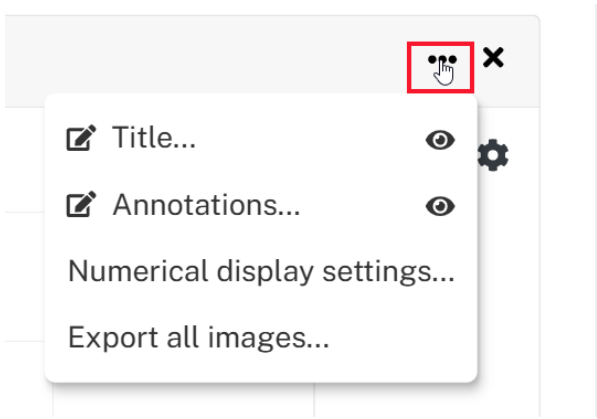


Figure 158: Title and Plot annotations tool access

Clicking **Title** opens the **Edit Title** dialog. Users can search for applicable fields to add to all plot titles in the Plots widget. Users also have the option to enter free text, including delimiters. Values are spaced out by default, but users can add a space or additional delimiters by simply pressing the key (e.g., space, comma, colon) within the text box.

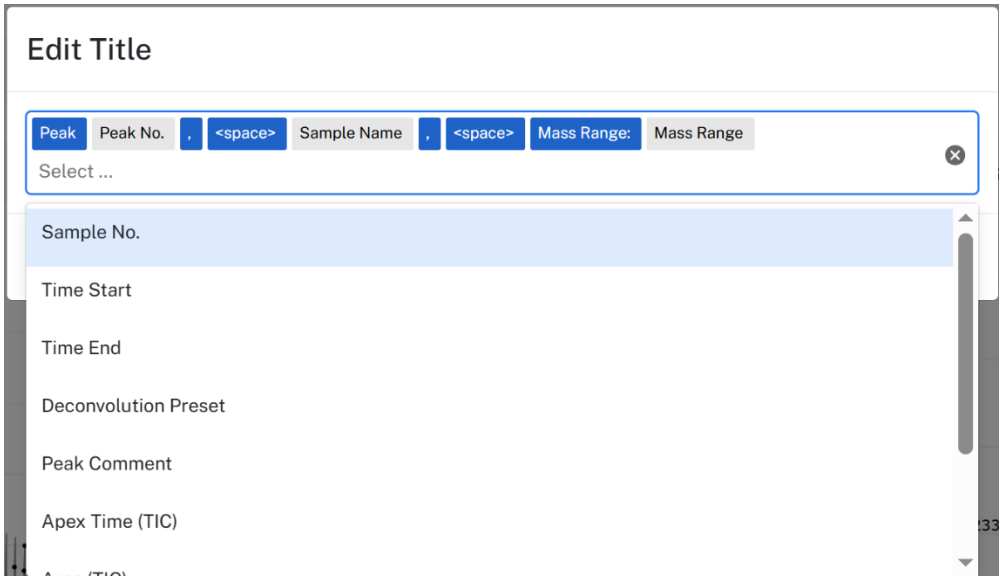


Figure 159: Edit Title dialog

Figure 160: Title resulting from the above settings

Clicking on **Annotations** opens the **Edit Annotations** dialog, which provides users with the same tools as described above but with fields relevant to the specific plot.

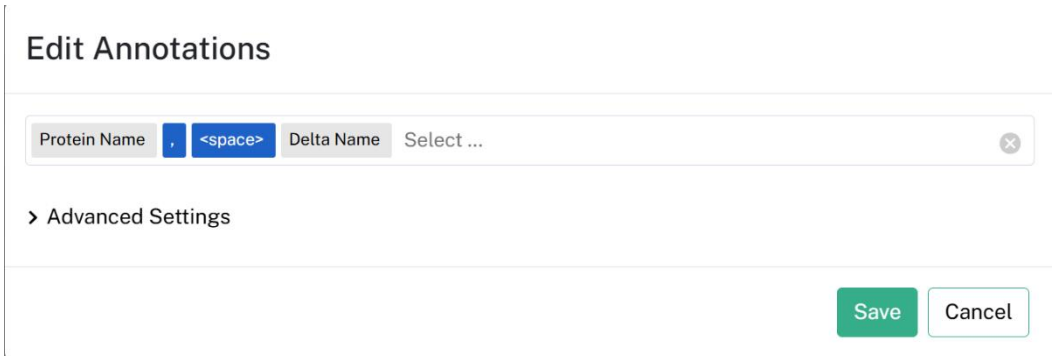


Figure 161: Edit Annotations dialog

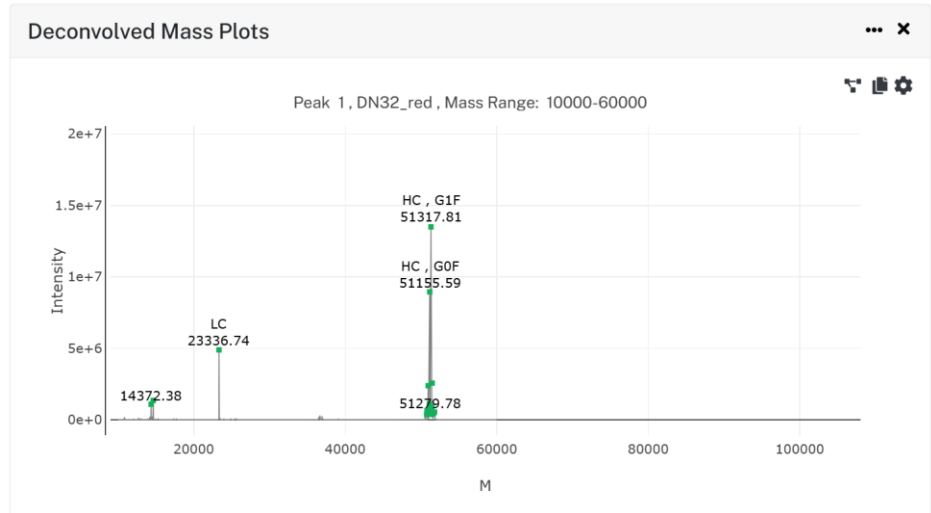


Figure 162: Plot with annotations resulting from the above settings

Under **Advanced Settings**, users can apply conditions that dictate which plot points receive annotations:

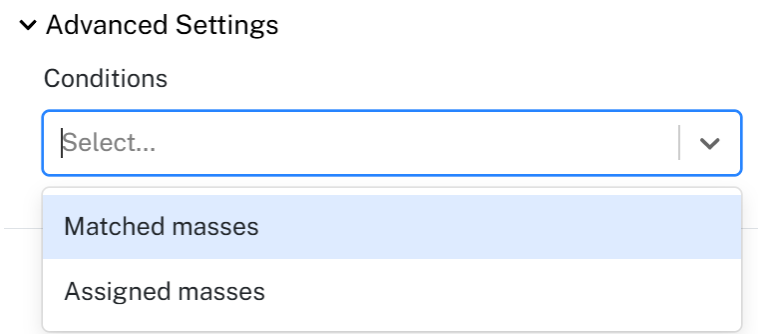


Figure 163: Annotations Advanced Settings

Export to Template

Export to Template can be accessed by clicking on the three-dot icon in the header of the analysis in Edit mode.

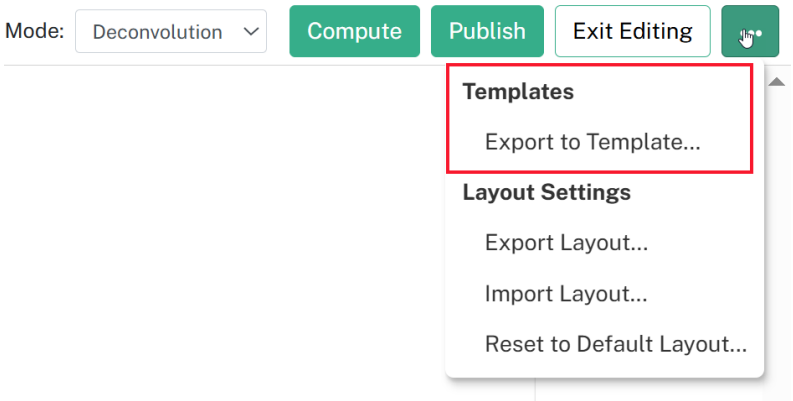


Figure 164: Export to Template

Clicking on Export to Template opens a dialog where the user can save the current project settings as a unique template. The current analysis can be saved in a Folder as a template record for future use with the appropriate extension (`*.wat`). User-saved templates are accessible through the Folder within which they are saved and will *not* be included among the system templates on the Home page. They will show in the **Recent Files** section, where they can be used to launch new analyses.

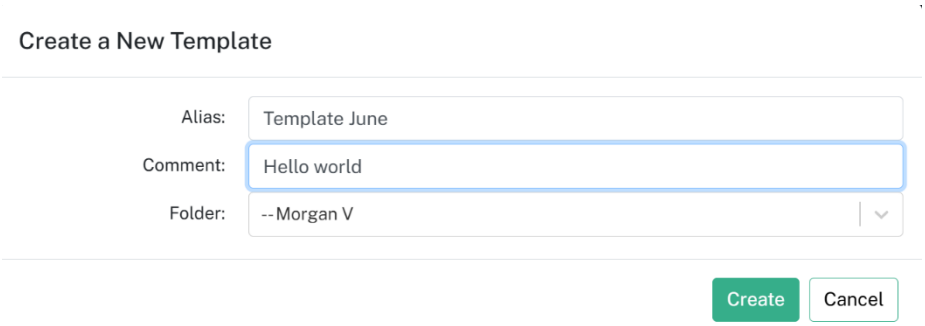


Figure 165: Create Template File

Folder	ID	File Alias	File Name
17Jun25 WA Templates	7769	Intact	Intact.wat

Figure 166: Templates have the extension ".wat"

To launch an analysis from a saved template, click the Web Analysis icon. The new analysis created from the template does not have any samples from the original analysis.

Users can copy a link to the Analysis Template document and share with others. The shared link will open the Byosphere search page with the desired template displayed.

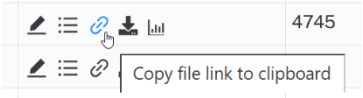


Figure 167: Users can copy link to share Analysis Template

Users can also download the Analysis template file and share it with users directly.

Layouts

Users can save the layout of their widgets within Web Analysis. This tool can be accessed by clicking on the three-dot icon in the header of the analysis in Edit mode.

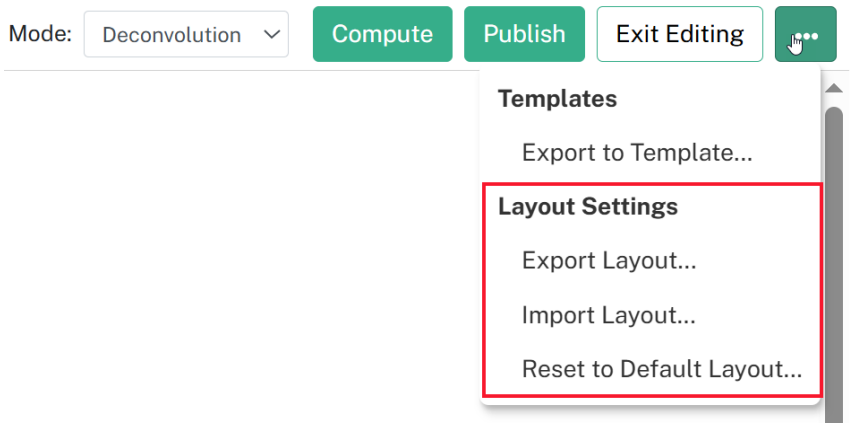


Figure 168: Layout Settings

Layouts can be saved (Export) and loaded from preexisting Layout files. Layout files are exported as `*.bdisp` files which will be saved on the Byosphere Server in a folder of the user's choice. The user can also restore the default layout if they wish to do so. These layout files may then be imported into another Web Analysis.

The location where users can save the current analysis as a template (`*.wat`) has been relocated under the same three-dot icon as the Layout settings.

Feature Finder mode

When the Analysis is set to **Feature Finder mode**, room features are configured for the analysis of **peptides**.

Samples Room (Feature Finder mode)

The **Samples Room** consists of five widgets: **Samples**, **Features Table, Trace Types**, **Trace Peaks Table**, and **Trace Plot.**

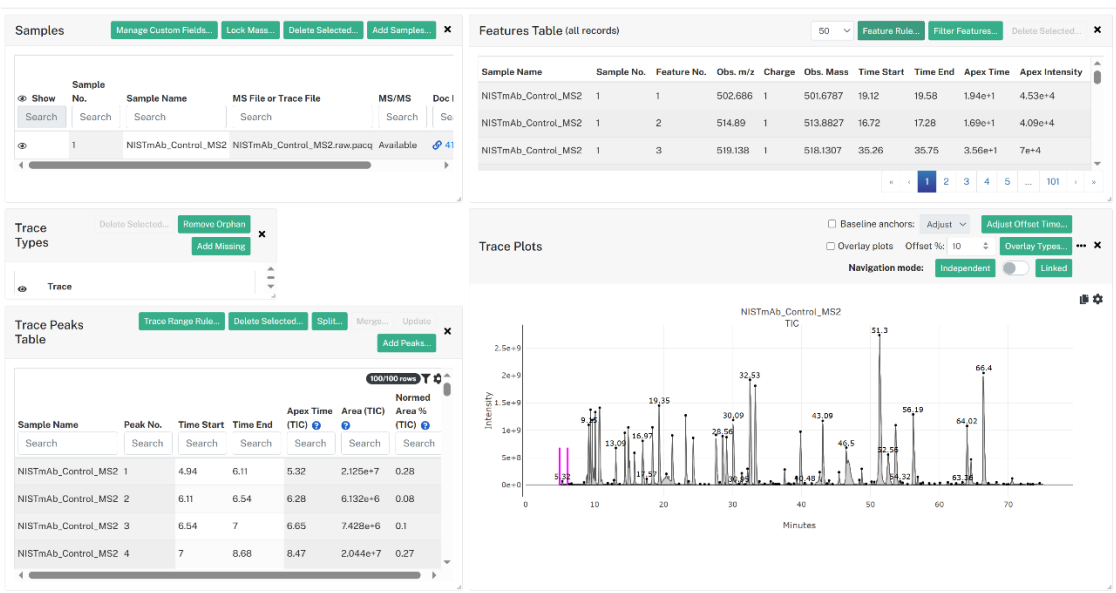


Figure 169: Samples Room (View Mode)

Samples

The **Samples** view controls the addition, deletion, and visibility of sample files and trace files. It also states which sample files contain fragment ion scans (MS/MS data).

Sample(s) can be added within the **Samples** room by clicking **Add Samples.** The **Select sample to add** dialog list all folders to which the user has access. To add a sample, select a folder from the left pane and check the sample(s) of interest, then click **Add Sample**.

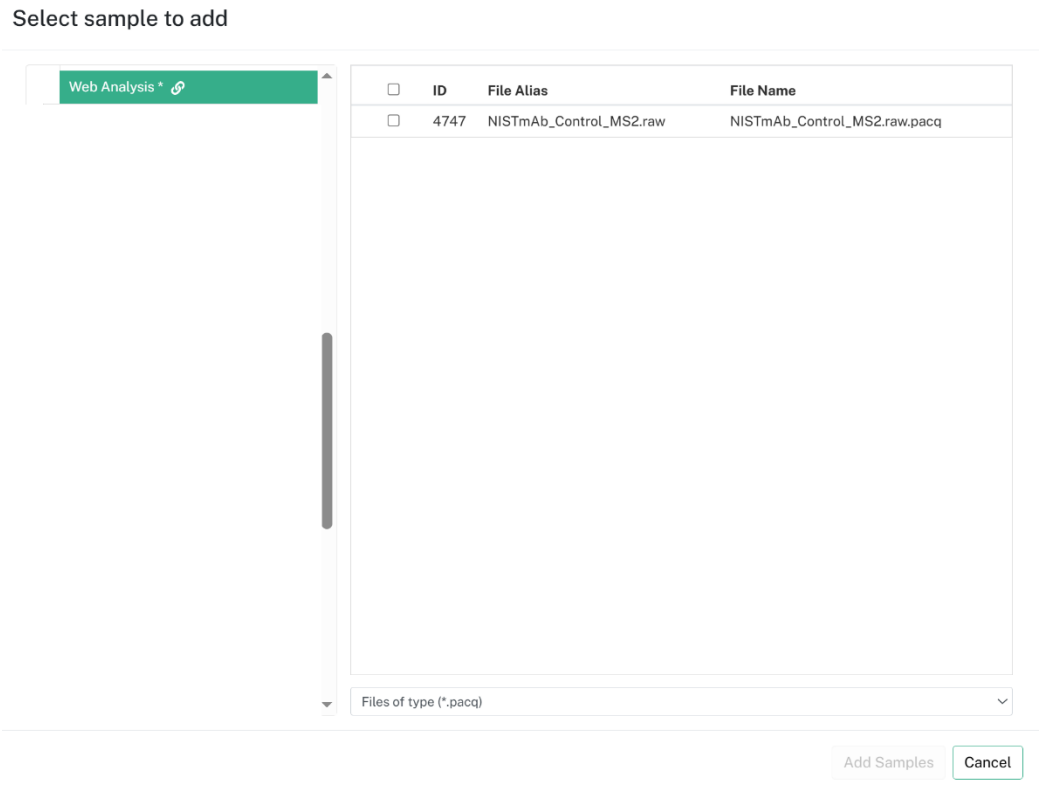


Figure 170: Add Samples dialog

MS files (`*.pacq`) and/or trace files (`*.csv` or `*.txt`) can be added as samples. A progress bar will show the progress of the sample upload. Once finished, the sample will show up in the Samples table and will be associated with a **Sample No.** Sample numbers (Sample No.) are added in sequential order and cannot be edited. **Sample Name** can be edited by clicking in the cell that holds the auto-generated name for each sample and typing a custom entry. The name is the full sample name without the associated MS and `*.pacq` extensions by default. Clicking out of a sample name will immediately save any changes made.

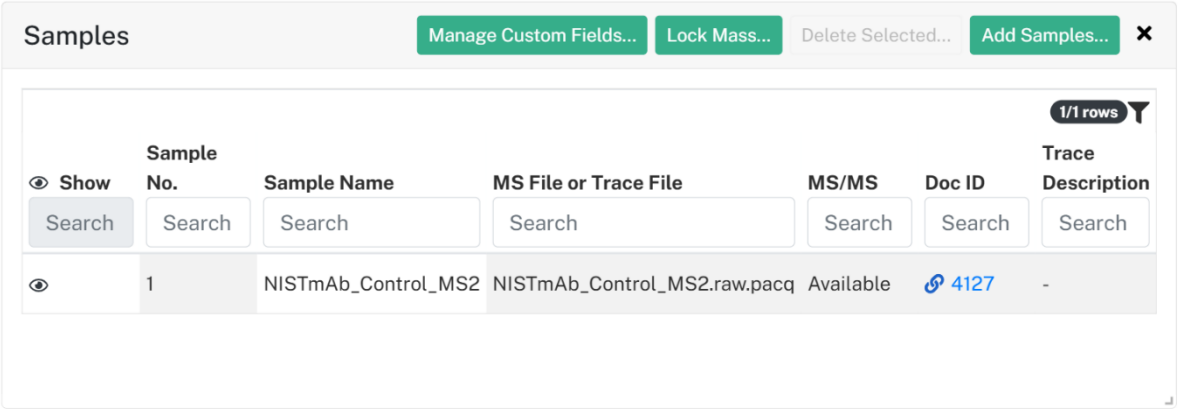


Figure 171: Samples

To select samples, click on the rows of the samples of interest. This highlights the samples in blue. Once the samples are selected, the user has the option to delete the samples by clicking **Delete Selected**

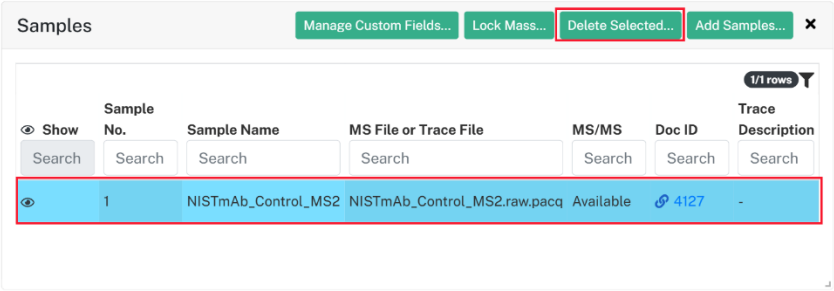


Figure 172: Delete the selected sample

Features table

The **Features** table provides automatically determined, putative peptide signals. Observed masses for the peptide features are used to match theoretical peptide masses defined in the Sequences room.

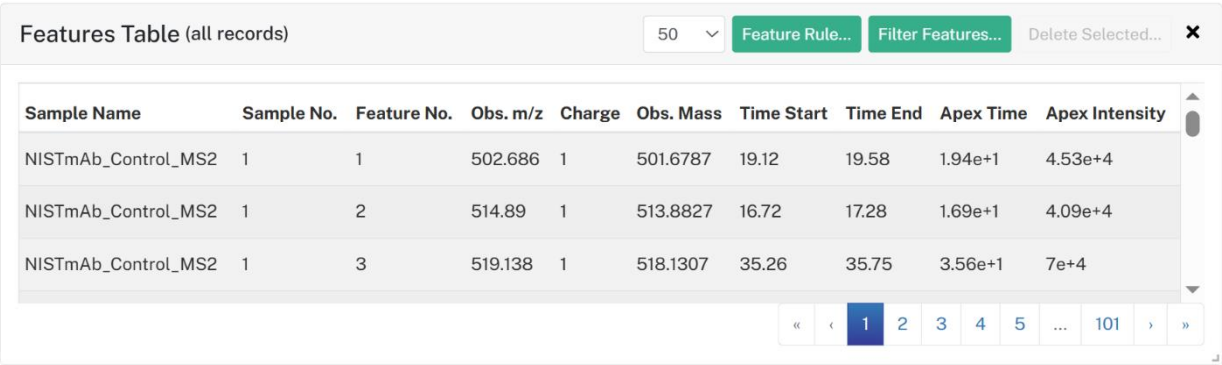


Figure 173:Features Table

Feature Rule

Clicking **Feature Rule** opens a dialog that allows the user to define the ppm window for feature finding and XIC extraction. This value can then be applied to all, or individual samples.

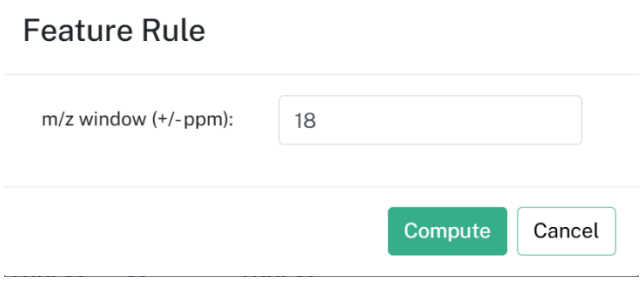


Figure 174: Feature Rule

When a user clicks on “Compute”, features will be extracted for the selected samples only, using the m/z window values provided.

Filter Features

The user can filter features based upon any of the columns within the table. If “Sample Name” is selected, the user can type in the name of the sample that they wish to see within the table. If any of the other options are selected, the user can provide the minimum and maximum numerical value within which the feature must fall for the selected column.

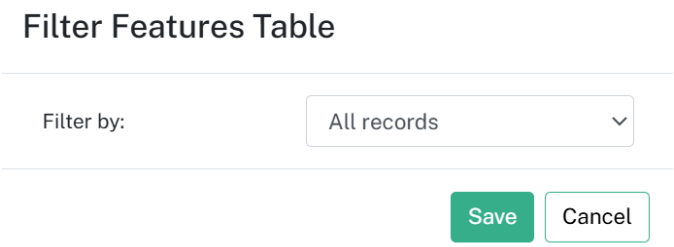


Figure 175: Filter Features dialog

Trace Types

See **Trace Types**.

Trace Plots

See **Trace Plots**.

Trace Range Rule

See Trace Range Rule.

Trace Peaks Table

See **Trace Peaks Table**.

Compute

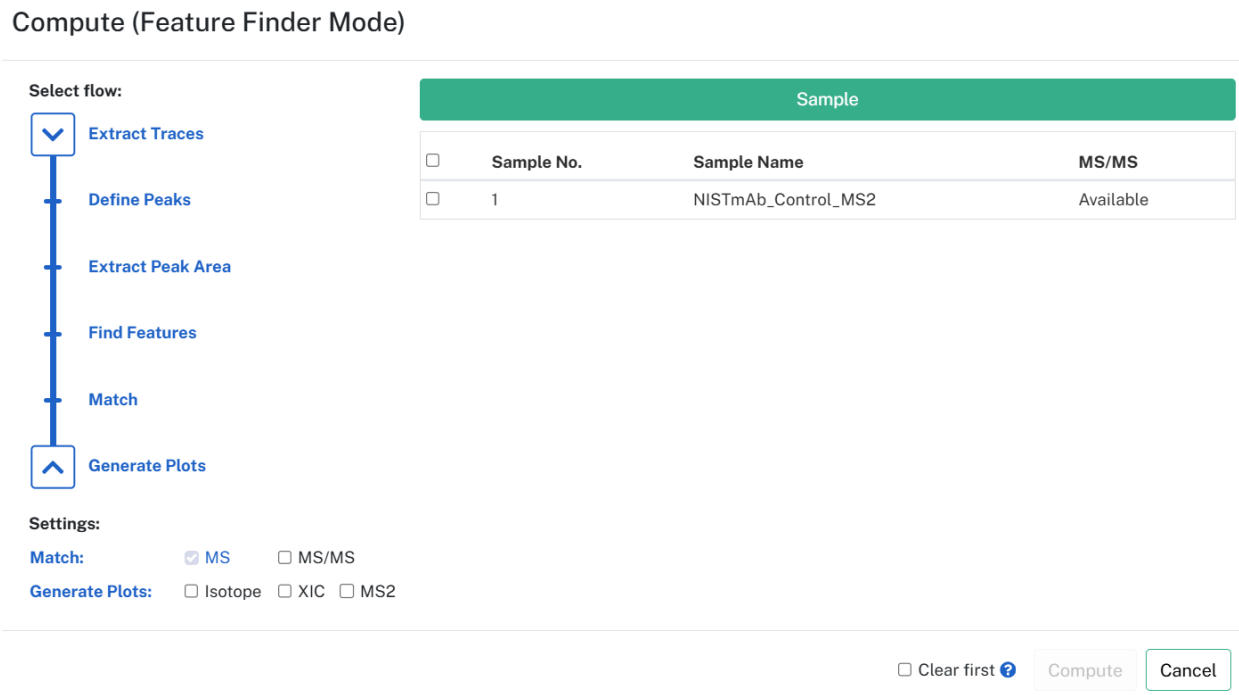


Figure 176: Compute dialog

If the user checks **Clear first**, *all* existing results will be removed and re-computed, including any existing matches and preset associations (e.g., Feature rule options). This option is only recommended if the user wants to remove all previous work and re-compute, which may be useful when experimenting with different parameters.

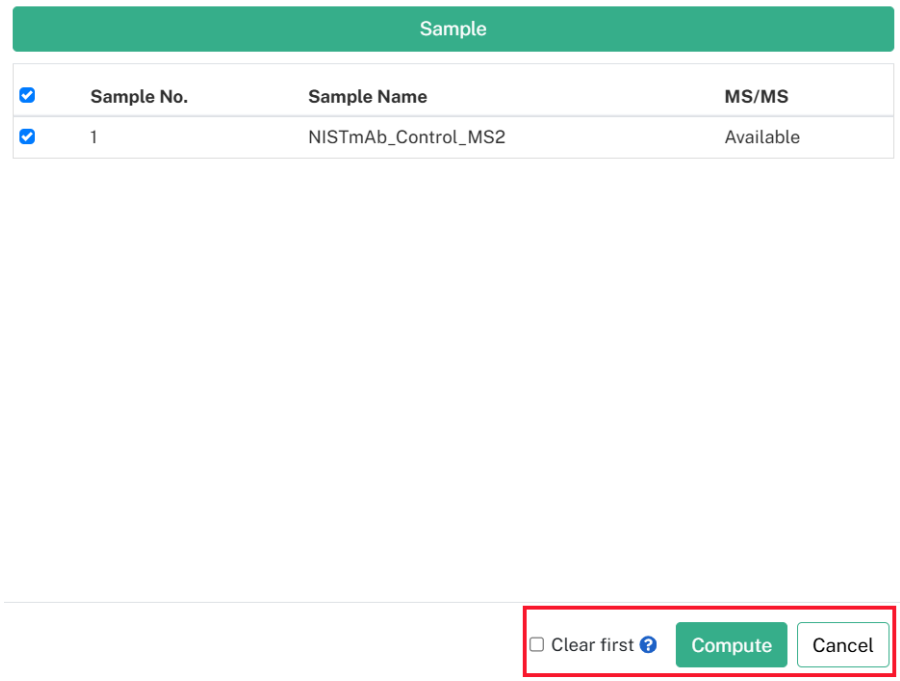


Figure 177: Clear first

Not all samples must be part of the computation. Only samples selected within the **Samples** table will undergo the selected computations.

Sample

	Sample No.	Sample Name	MS/MS
☐	1	NISTmAb_Control_MS2	Available

Figure 178: Sample tab in Computation with one sample selected

The **Flow** allows the user to select a range of operations that are performed when clicking **Compute**. This can save both time and computational power, since more intensive calculations can be omitted if not desired. The arrows on the flow chart can be dragged to encompass all calculations to be included in the computation. Note that the default Flow options for Compute are specific to each Room. The full flow is as follows:

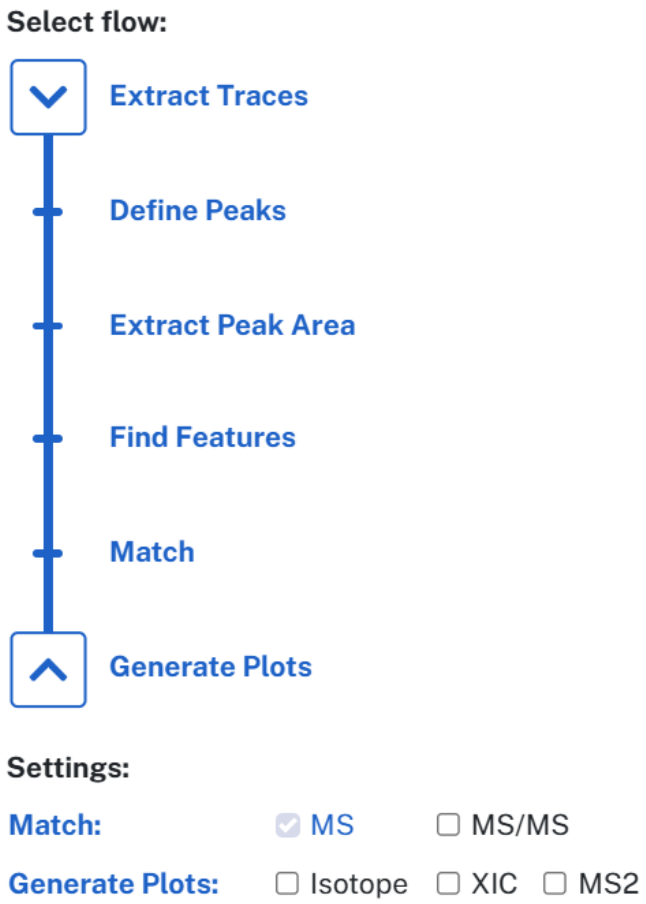


Figure 179: Flow Chart with Settings

In the **Samples** and **Sequences** rooms, the default selected compute flow range is:

1. Extract Traces

2. Define Peaks
3. Extract Peak Area
4. Find Features

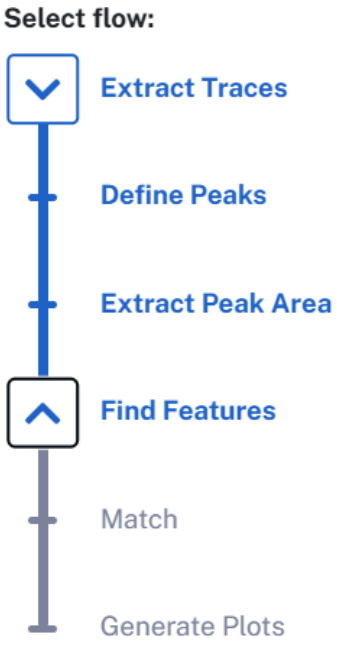


Figure 180: Samples room flow

The user must specify whether they wish to compute for MS data only or MS and MS/MS data, as well as which plots they wish to generate, by checking each under the Settings.

The settings will remain grayed out when the Match option is not included within the flow.

In the **Inspection** room, *all* Flow options are selected and the setting provide the user with the ability to Match MS alone or both MS and MS/MS data, as well as which plots to generate.

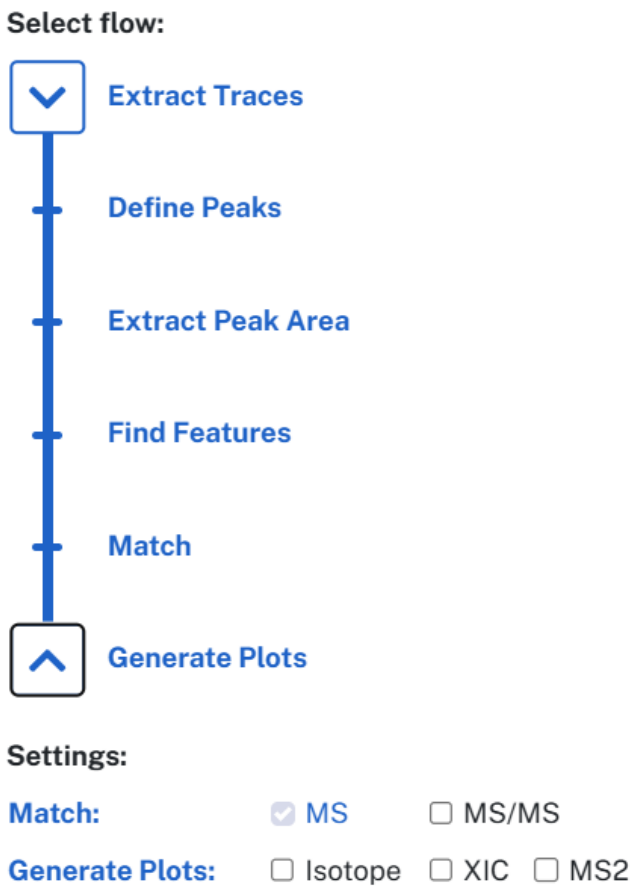


Figure 181: Inspection room flow

Note that steps *cannot* be skipped between stages in the flow chart; rather, the flow chart can only be started or ended at a determined point. Plot generation is not contingent on the generation of another plot.

Lock Mass

See Lock Mass. These settings are the same in both modes.

Sequences Room (Feature Finder mode)

The **Sequences** room in Feature Finder analysis mode contains the same four views as Deconvolution mode, with some key differences. For more information on the overarching schemata, see Sequences room. Only the differences will be detailed below.

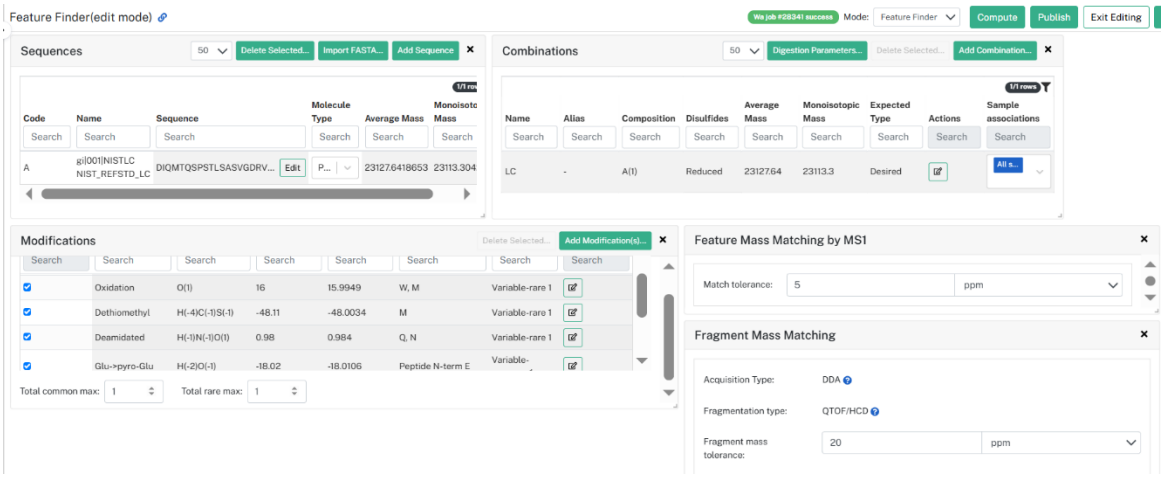


Figure 182: Sequences Room (Feature Finder mode, Edit mode)

Feature Mass Matching

The **Feature Mass Matching by MS1** options include parameters that control automatic mass peak assignment.

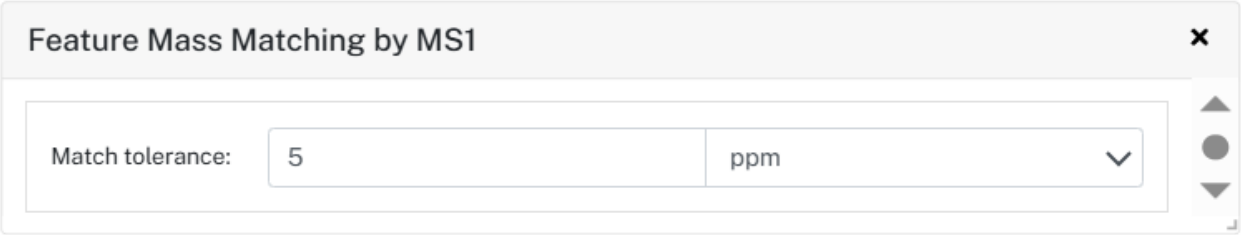


Figure 183: Feature Mass Matching by MS1

The **Match Tolerance** determines the value +/- a peak must match to a potential mass to be assigned. The default value is 10 Da, but the user has the option to enter in any number, as well as the units (Da or ppm).

If the analysis is saved as a template, the Sequence room views will be preserved in the resulting template.

Fragment Mass Matching

The **Fragment Mass Matching** options include parameters that control MS2-related assignments.

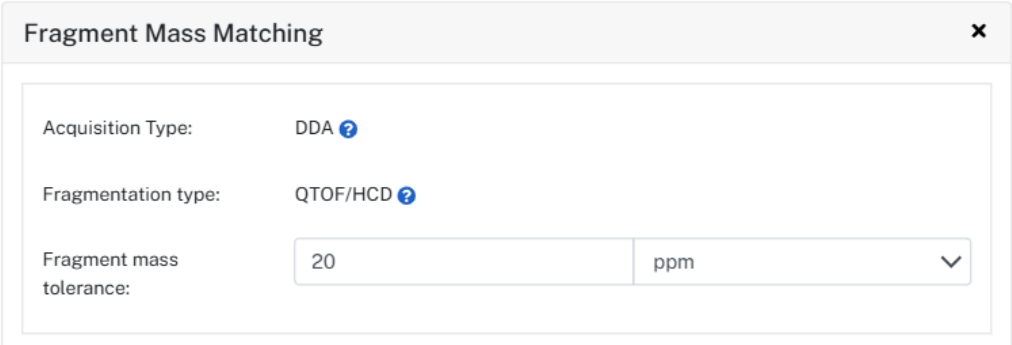


Figure 184: Fragment Mass Matching

As of v5.5, Peptide Web Analysis currently supports the DDA-Acquisition type. DIA will be available in future releases.

Peptide fragment annotation is currently available for QTOF/HCD and additional fragmentation types will become available in future releases.

Inspection room (Feature Finder mode)

The **Inspection** room in Feature Finder mode allows the user to view features associated with peptides and their associated XIC, Isotope, and MS2 plots.

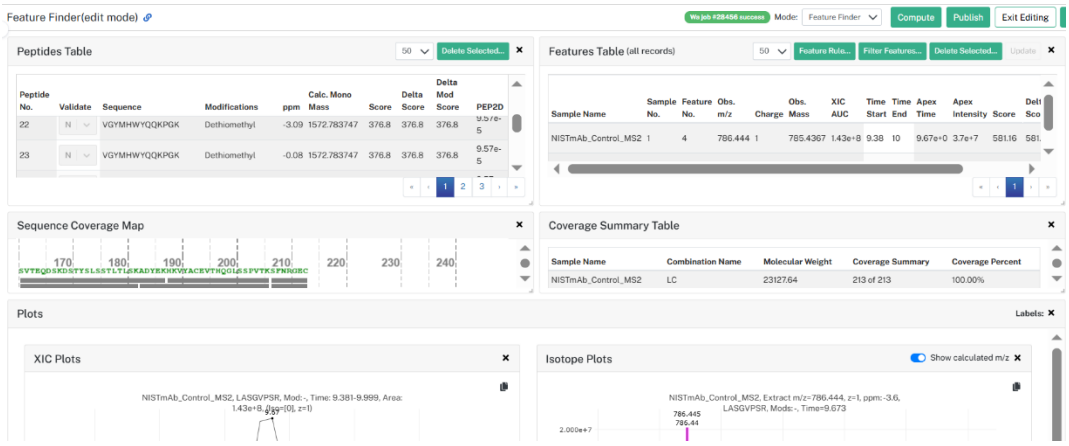


Figure 185: Feature Finder mode Inspection room

Peptides Table

The **Peptides Table** in the Inspection room provides a list of matched and identified peptides. Within the table, the user can review and validate peptide mapping PTMs and choose whether to assign 'True-positive', 'False-positive', 'Uncertain', or no entry to the peptides.

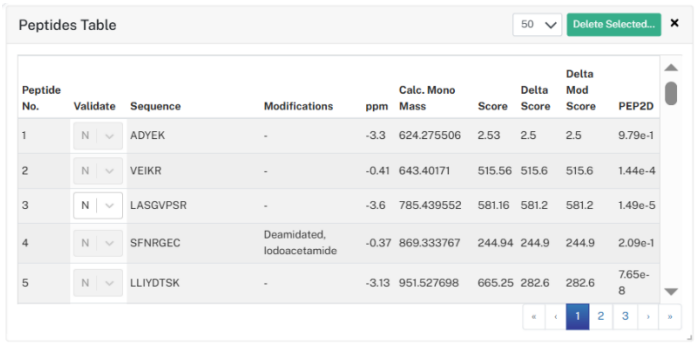
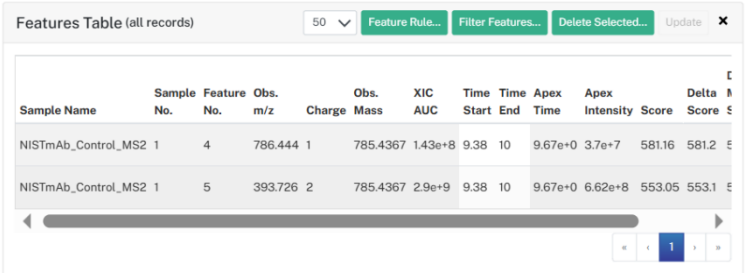


Figure 186: Peptides table

Features Table

The **Features Table** in the Inspection room displays a list of the corresponding features of an identified peptide.



Sample Name	Sample No.	Feature No.	Obs. m/z	Charge	Obs. Mass	XIC AUC	Time Start	Time End	Apex Time	Apex Intensity	Score	Delta Score
NISTmAb_Control_MS2	1	4	786.444	1	785.4367	1.43e+8	9.38	10	9.67e+0	3.7e+7	581.16	581.2
NISTmAb_Control_MS2	1	5	393.726	2	785.4367	2.9e+9	9.38	10	9.67e+0	6.62e+8	553.05	553.1

Figure 187: Features Table

Selecting a peptide in the Peptides table populates the Features Table with the features associated with the selected peptide. The highest scoring Feature will be automatically selected when a user selects a peptide.

See Feature Rule and Filter Features in the Samples room section for more information on these functionalities within the Features table.

Plots

XIC Plots

The **XIC plot** shows the extracted ion chromatogram for the selected feature. Within the XIC plot are magenta integration bars that can be dragged by the user to adjust the integration window. The following information is available in the header of the plot: Sample name, selected sequence, modifications (if present), start and end time, XIC area summed, isotope information, and charge state.

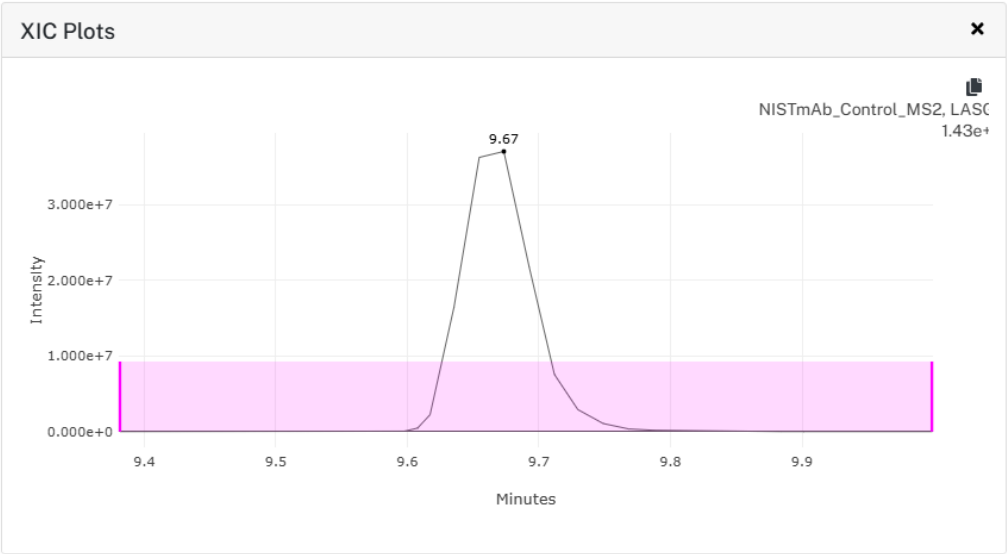


Figure 188: XIC plot

When the user moves the integration bars, the following cells in both the Samples and Inspection room are updated: XIC Area, Time, and Apex Intensity. These values will be marked dirty with a strikethrough until the user clicks **Update**.

Isotope Plots

The **Isotope plot** shows the MS1 isotope spread of the selected feature. The following information is available in the header of the plot: The sample name, extract m/z, charge (z=), sequence, any modifications present, and the retention time window.

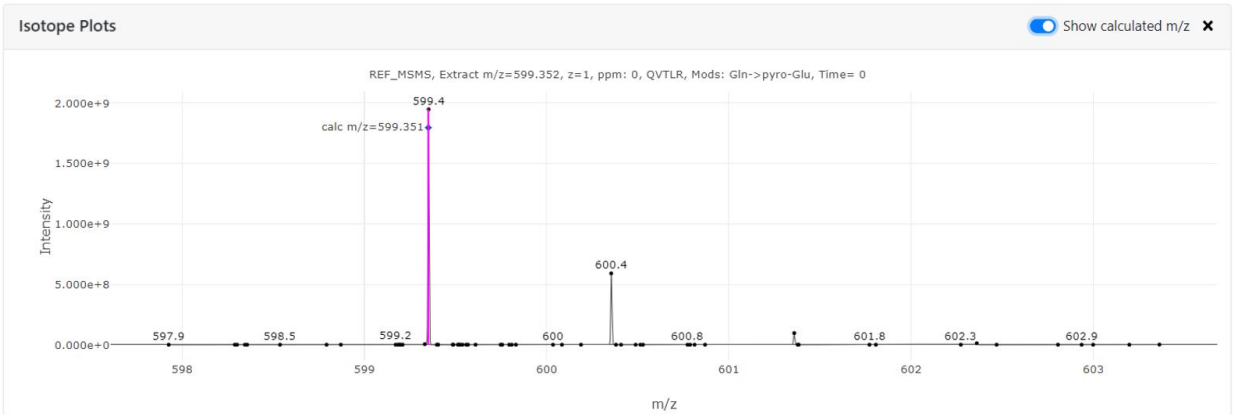


Figure 189: Isotope plot

Toggling on **Show calculated m/z** will add a blue diamond at the theoretical isotope apex that represents the calculated m/z, and shows the calculated m/z value itself on the plot. A pink bar indicates the isotope peak that is used to generate the XIC plot. This is based off of the calculated m/z that uses the ppm window as defined in the **Feature Rule** options in the Features table.

If the user hovers over the actual m/z value on the isotope peak, the label changes to the value of the mass shift relative to other peaks.

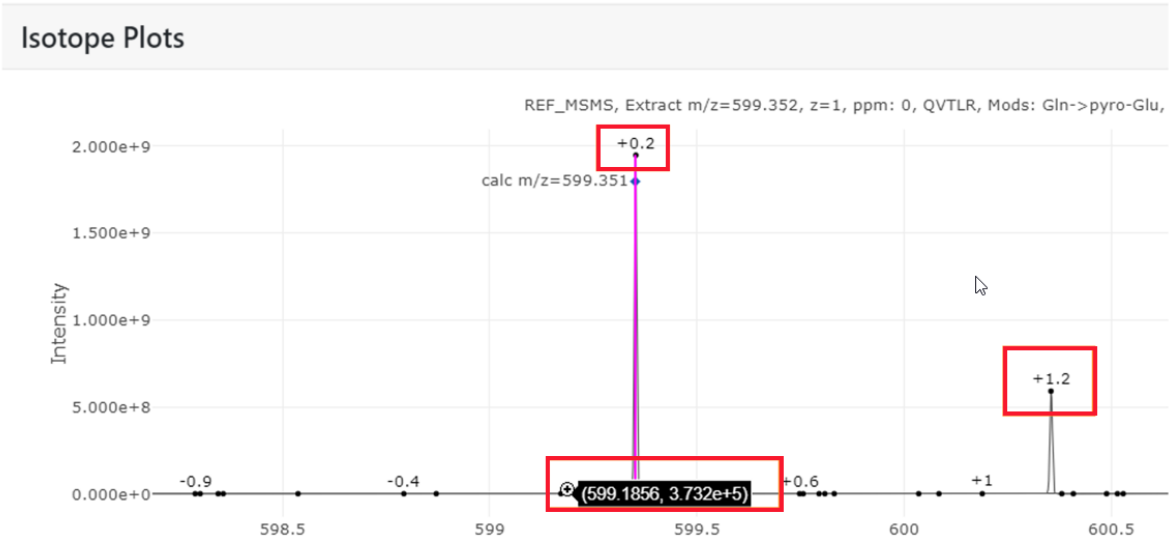


Figure 190: Mass shift relative to other peaks

MS2 Plots

The **MS2** plots display the plots of the MS2 fragments when available.

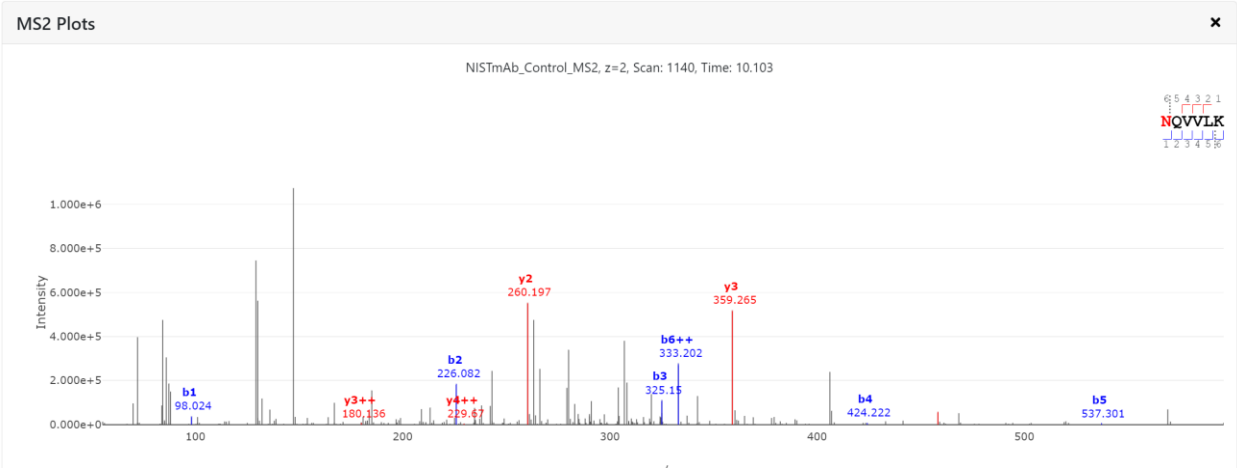
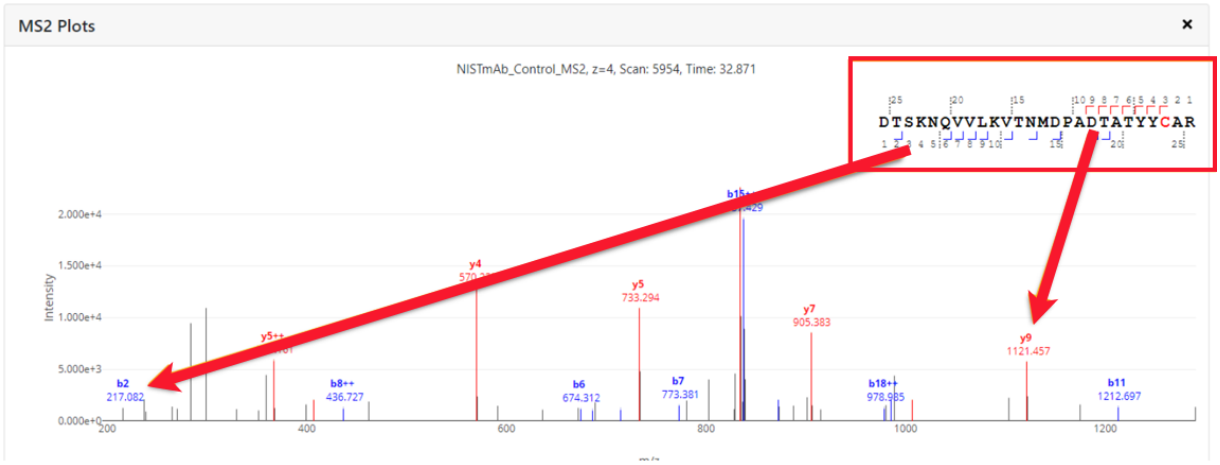


Figure 191: An MS2 plot with modification present

Modifications are labeled on the peptide fragment legend which consists of the sequence of the peptide that was identified (along with the potentially modified residues being highlighted in *red*. Additionally, the peptide sequence also contains an indication of where the peptide bond was broken during fragmentation which gave rise to the ion that Byonic used for an ID (with N-terminus b ions being blue, C-terminus y ions being red).



Each plot includes the sample name, scan number, charge state, and time.

Sequence Coverage Map

The **Sequence Coverage Map** shows the visual coverage of identified peptides (including different charge states) against the sequence of a protein for each sample. Each sample is represented by a different color and each line represents the sequence section of the peptide. The sequences come from the chains entered into the Combinations table under the sequences tab, defined for mass matching. There is a line for each feature that was mass matched to this given peptide sequence. This could be different charge states, different modified forms, and features from different retention times.

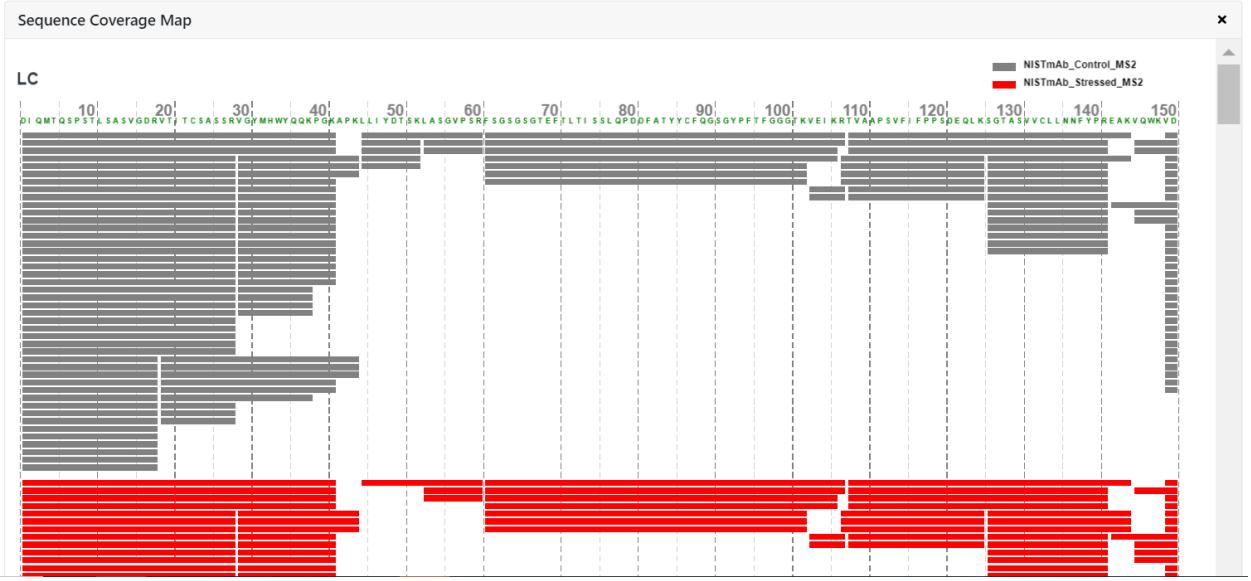


Figure 192: Sequence Coverage Map

Coverage Summary Table

The **Coverage Summary Table** provides the molecular weight, coverage summary (amino acids covered within the sequence), and coverage percentage for each protein chain combination per sample.

Coverage Summary Table

Sample Name	Combination Name	Molecular Weight	Coverage Summary	Coverage Percent
NISTmAb_Stressed_MS2	LC	23113.30	213 of 213	100.00%
NISTmAb_Control_MS2	LC	23113.30	213 of 213	100.00%
NISTmAb_Stressed_MS2	HC	49447.75	412 of 449	91.76%
NISTmAb_Control_MS2	HC	49447.75	400 of 449	89.09%

Figure 193: Coverage Summary Table

Report room (Feature Finder mode)

The **Report** room provides a summarization of the analysis as well as the plots generated in the analysis.

Note that in the Report room, the header only shows the following options when in Edit mode.

Figure 194: Header options for the Report room

Summary

The **Summary** view provides a summary of the settings used to create an analysis.

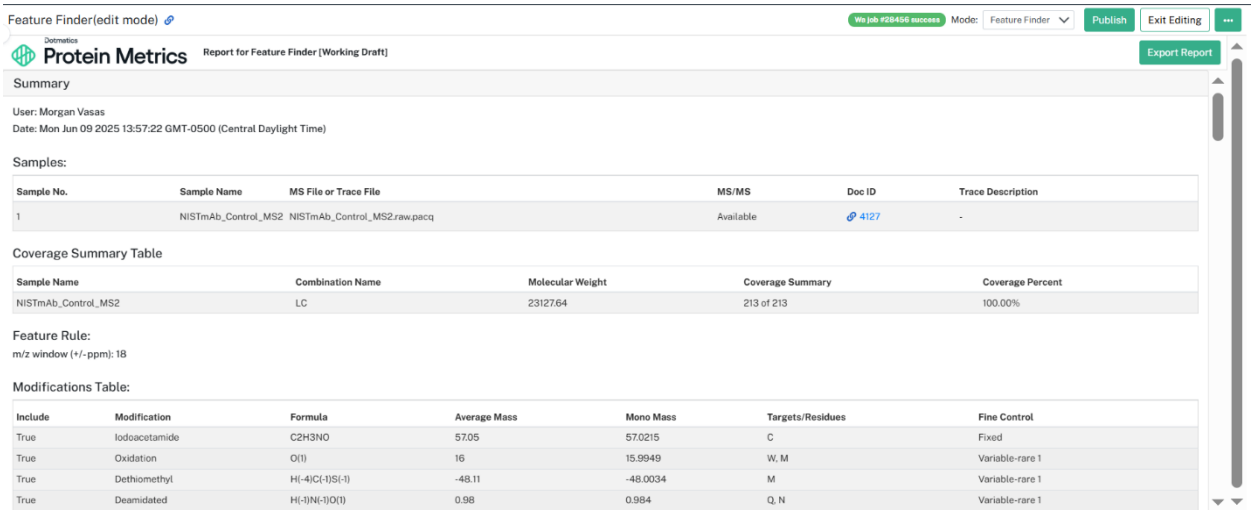


Figure 195: Snapshot of Summary view within the Report room

The **Summary** section of the Report room provides an overview of the key inputs and outputs for an analysis. Information includes:

- User
- The date the report was made (Day of the week in shorthand i.e., “Thurs”, Month (i.e., May), day, whole year, time HH:MM:SS in military time, differential from GMT (for example EST is GMT-0400) and timezone “i.e., Eastern Daylight Time).
- Samples Table (Brought in from the Samples Widget in the Samples Room)
- Feature Rule (From the Feature Rule pop up on the Features Table in the Samples Room)
- Digestion Parameters (From Combinations Widget in the Sequences Room)
- Modifications Table (From the Modifications Table in the Sequences Room)
- Feature Mass Matching (From the Feature Mass Matching Widget in the Sequences Room)
- Fragment Mass Matching (From the Fragment Mass Matching Widget in the Sequences Room)
- Sequences table (From the Sequences table in the Sequences Room)
- Combinations table (From the Sequences table in the Sequences Room)
- Peptides Table (From the Peptides Table in the Inspection Room)
- Coverage Summary Table (from the Inspection table)

The **Summary** section of the Report room provides an overview of the key inputs and outputs for an analysis.

This information will be updated within the Summary view as changes are made within the respective rooms.

Note: If you change the name of the analysis, the name at the top of the report also changes.

Plots

The **XIC** and **Isotope** plots are deselected in the in the Report by default and the **MS2** plots are selected by default. If they are selected, every plot associated with every feature will be generated and populated within the Report.

Note: Charts and Tables are not yet supported in Feature Finder analyses

Analysis (Edit Mode)

Once in **Edit** mode, the user can interact with any of the rooms within the Analysis. The header now consists of the following options:

Figure 196: Analysis Header in Edit Mode for both modes

Users can only have one Dashboard open in Edit mode at a time. If a user has a Dashboard currently open in Edit mode, they will not be able to create a new Dashboard until it has been closed.

Rearrange Views

Users can move individual tables/views around a room by clicking the header of the view and dragging it to any location within the room. When the view is dropped, the other views within the room will be moved aside to accommodate the change.

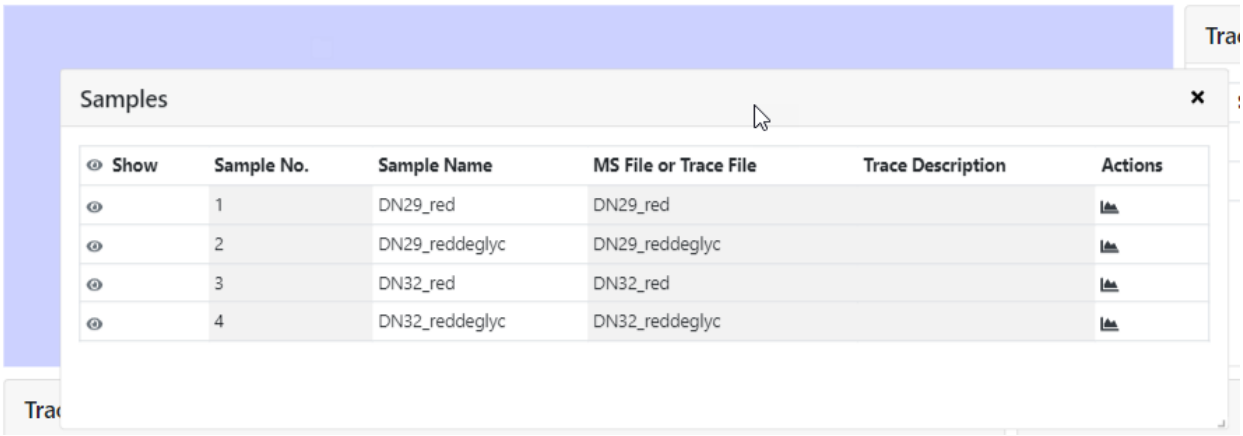


Figure 197: When moving a view, the space it previously inhabited is highlighted blue

To move the Trace Plot view back to its original position, the user can adjust the size of the Samples table by clicking on the lower right corner of the view window and dragging in to reduce its size. A icon will appear over the corner indicating that the user can adjust the window's size. When performing the adjustment, the other windows will move in real time and the new window size will be reflected by a blue highlighted box.

Compute

Information on the Compute tool can be found here.

Exit Editing

This takes the user out of **Edit** mode and back into **View** mode. When clicked, all unpublished changes made in the analysis are immediately saved, although any other users who open the analysis will only see the version of the analysis that has been *published* and not the saved (still unpublished) edits. When in View mode, click **Close** to close the analysis. Note that if an analysis is being edited, a second analysis cannot be opened until the first analysis is set to View mode.

Publish

Publish makes the current version available to be opened by other users as the newest published version of the analysis.

Users will be asked if they would like to generate a PDF report whenever a Web Analysis is published. Note that reports with many plots can take a long time to complete.

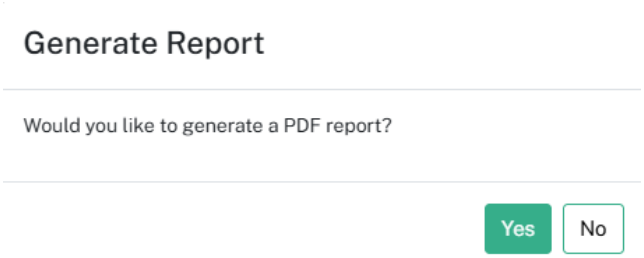


Figure 198: Generate PDF dialog

If the user chooses to generate a PDF of their published analysis, the report can be found under the Actions column of their project within the File Navigator. When this icon is clicked, the published PDF will automatically be saved within the browser.

Figure 199: View published report

The user also has the option to generate a PDF without publishing the analysis, by clicking on the export Report button in the top right corner of the report area. A watermark is added to every page when a PDF is generated from an analysis in **Edit** mode indicating that the PDF contents are from a "Working Draft" of Analysis report.

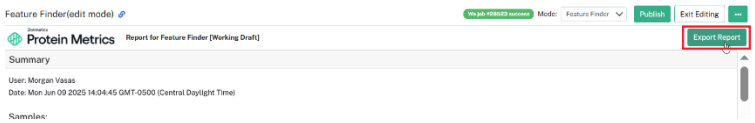


Figure 200: Export Report

Note that editing and then publishing a WA without generating another PDF leaves the last generated PDF in place.

Note: Export Report currently not supported for users on CentOS/RHEL 7.

Analysis Locked

When an active job is still occurring, the analysis will be temporarily locked for editing and the following graphic shown. This also enables users to **view active jobs**, which can be canceled by clicking **Cancel Job**.

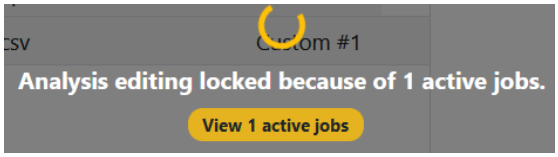


Figure 201: Analysis locked for Editing

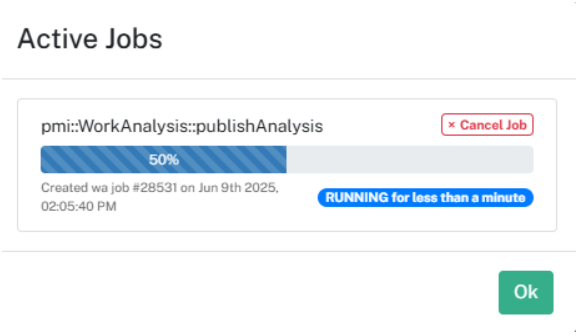


Figure 202: Active Job