



Byosphere[®] Peptide Web Analysis Quick Start Manual

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Protein Metrics LLC, Boston, Massachusetts, USA

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Peptide Web Analysis Quick Start Manual

Byosphere® **Web Analysis** is a native web application that provides users with the ability to perform Protein Characterization data processing within the Byosphere Web Client.

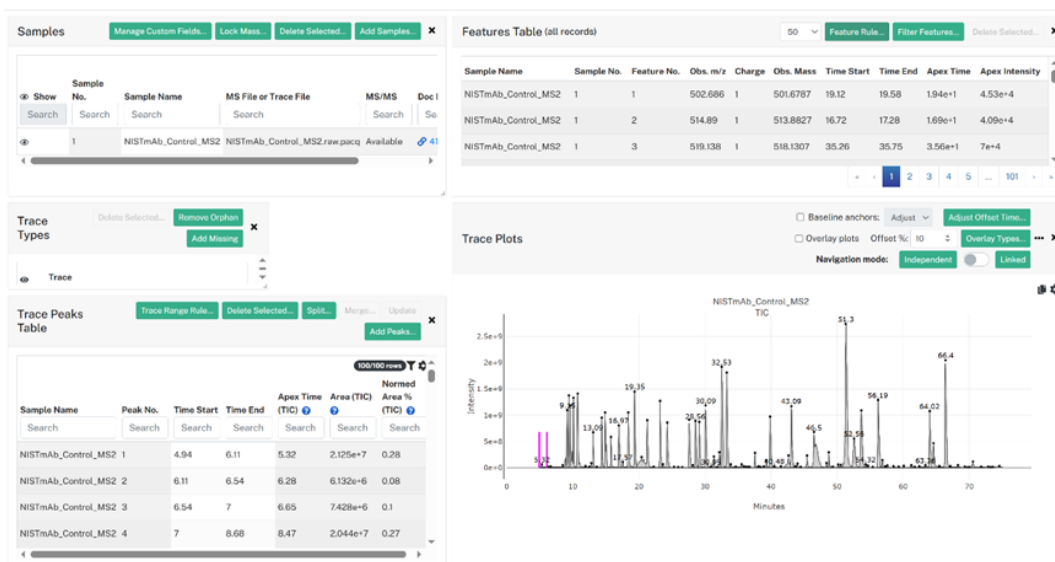


Figure 1: Analysis from Edit Mode, with the Samples Room selected

Web Analysis Modes

Currently, users are provided with two different analysis modes in Web Analysis: **Deconvolution** mode for Intact protein analyses and **Feature Finder** mode for Peptide Analyses. Templates will be preset to the mode most relevant to the workflow. This quick start manual covers building an analysis from scratch in **Feature Finder** mode in Web Analysis, which is used to analyze Peptide projects.

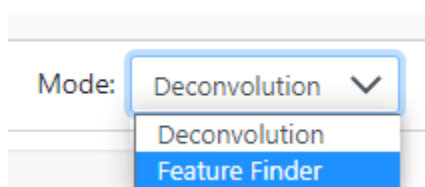


Figure 2: Web Analysis mode

Creating An Analysis with Templates

Users can create an analysis by clicking on a **Web Analysis Project 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 (.bproj files) accessible from the home page:

- 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 molecules.

- PeptidePTM: Identification and Quantification of post-translational modifications
- Reduced: Identification, characterization, and relative quantification of reduced large molecules.

System Analysis Templates

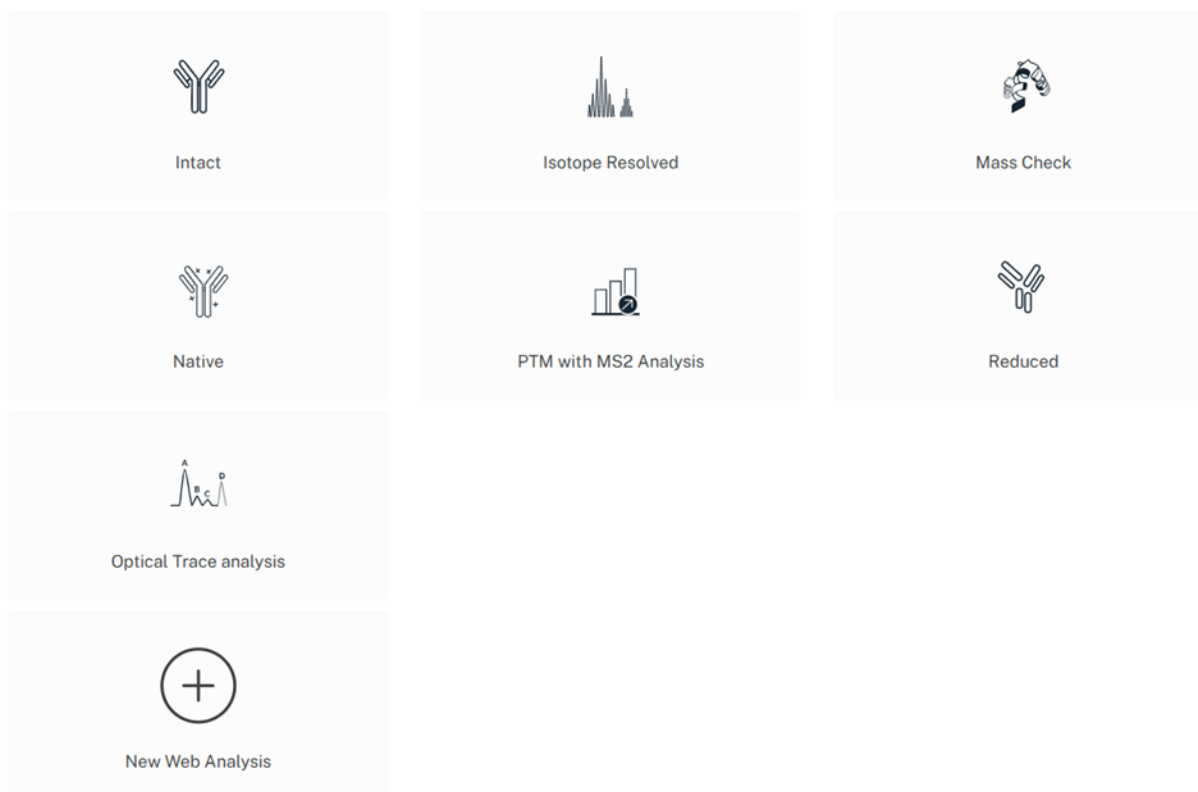


Figure 3: Project Templates

An empty analysis can also be created from the Web Analysis page by clicking “New Web Analysis”. This quick start manual will detail the process of creating a Web analysis from scratch.

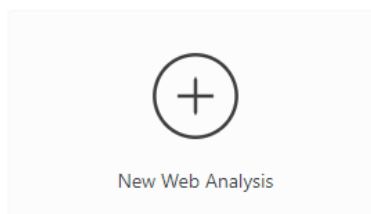


Figure 4: Create a new Web Analysis with no preconfigured settings

Samples room

Add Samples

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

Select sample to add

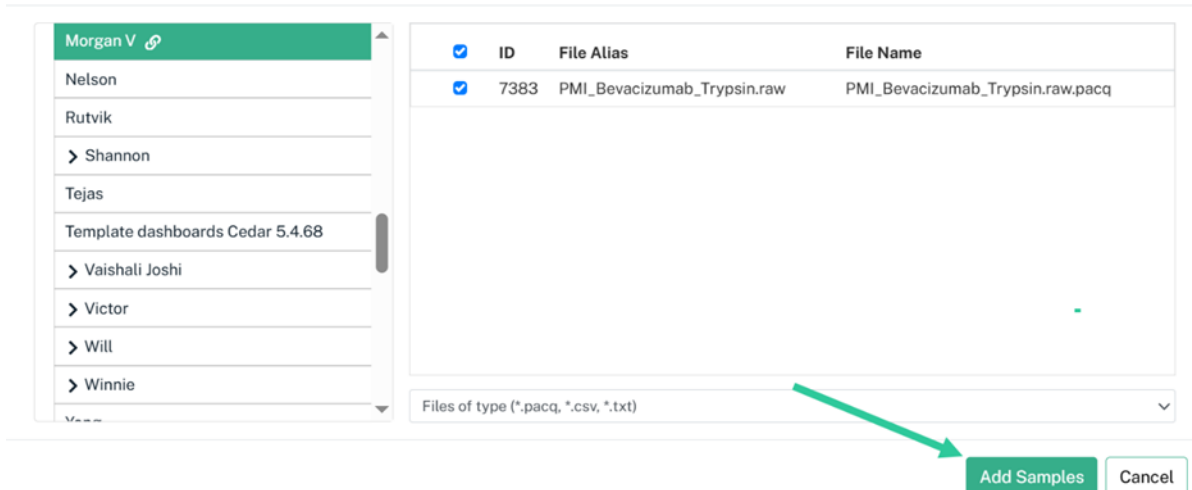


Figure 5: Add Samples dialog

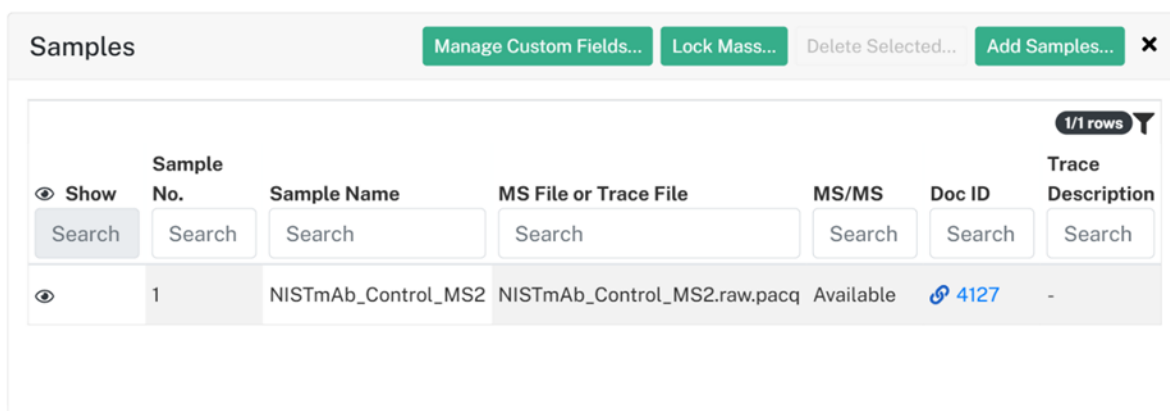


Figure 6: Samples Room

View Trace Types

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

Trace Types			Delete Selected...	Remove Orphan	Add Missing	×
👁 Show	Trace Type	Trace Description				
👁	TIC	TIC				
👁	BPI	BPI				

Figure 7: Trace Types Table

Users can select/ deselect which traces are visualized in the **Trace Plots** 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 Shown icon in the header of the table.

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. 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.

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 checked in the **Trace Types** table.

Only **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.

Trace Peaks Table										Trace Range Rule...	Delete Selected...	Split...	Merge...	Update	Add Peaks...	×
Sample Name	Peak No.	Time Start	Time End	Apex Time (TIC) ?	Area (TIC) ?	Normed Area % (TIC) ?	Apex Time (BPI) ?	Area (BPI) ?	Normed Area % (BPI) ?							
Search	Search	Search	Search	Search	Search	Search	Search	Search	Search							
DN32_red	1	3.5	4.5	3.57	1.229e+8	67.07	3.57	4.12e+4	5.19							
DN32_reddeglyc	1	3.7	4	3.76	3.42e+7	37.64	3.79	1.181e+4	3.29							

Figure 8: Trace Peaks Table

Additionally, Time start/Time end values can be added manually to the Trace Peaks table by clicking **Add Peaks**.

Add trace peak

Time start: Time end:

Select sample(s) to assign the new range:

<input type="checkbox"/>	Sample name
<input type="checkbox"/>	DN32_red
<input type="checkbox"/>	DN32_reddeglyc

Figure 9: 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. 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.

Edit Trace Range Rule

☐ Time of interest (min) From: To:

Baseline Type: Baseline Smoothing Width:

Peak picking source: Smoothing width: Minimum width:

Figure 10: Edit Trace Range Rule

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 in Trace Plots when the magenta integration bars 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.

Trace Peaks Table

Trace Range Rule...Delete Selected...Split...Merge...UpdateAdd Peaks...X

2/2 rows

Sample Name	Peak No.	Time Start	Time End	Apex Time (TIC) ?	Area (TIC) ?	Normed Area % (TIC) ?	Apex Time (BPI) ?	Area (BPI) ?	Normed Area % (BPI) ?
Search	Search	Search	Search	Search	Search	Search	Search	Search	Search
DN32_red	1	3.5	4.6	3.57	1.229e+8	67.07	3.57	4.12e+4	5.19
DN32_reddeglyc	1	3.7	4	3.76	3.42e+7	37.64	3.79	1.181e+4	3.29

Figure 11: Invalidated mass 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.

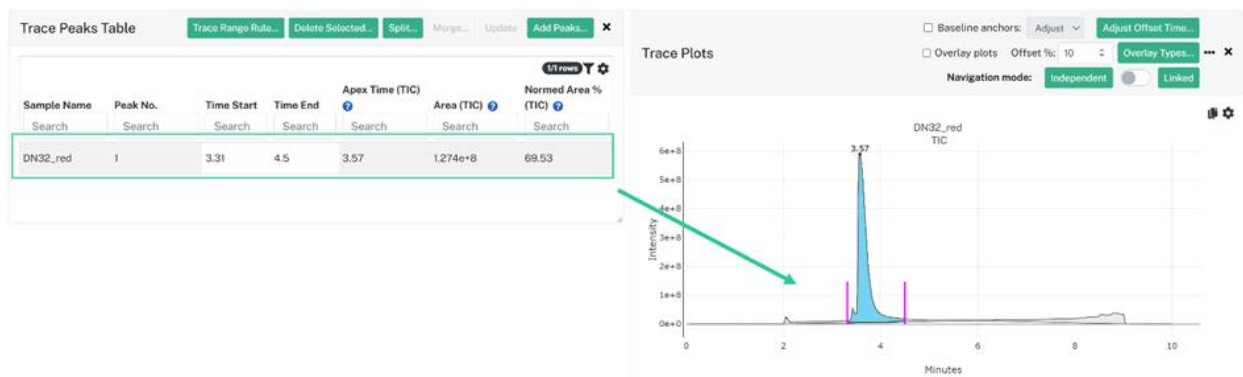


Figure 12: Trace plot integration limits and corresponding Trace Peak

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. 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 delete row alone will be removed).

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

Apply Trace Range Rule

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


Trace Peaks Table							Trace Range Rule...	Delete Selected...	Split...	Merge...	Update	Add Peaks...	✕
							2/2 rows 						
Sample Name	Peak No.	Time Start	Time End	Apex Time (TIC)	Area (TIC)	Normed Area % (TIC)							
<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>							
DN32_red	1	3.31	4.5	3.57	1.274e+8	69.53							
DN32_reddeglyc	1	3.7	4	3.76	3.42e+7	37.64							

Figure 13: Trace Range Rule button

The **Edit Trace Range Rule** dialog allows the user to configure the computations used to generate slices from the traces in the samples. There are four options: **Automatic**, **Manual**, **Whole Trace**, and **Regular Intervals**. More details on each rule can be found in the **Byosphere Intact Web Analysis Manual**. The following instructions detail the **Automatic** trace range rule.

Edit Trace Range Rule

☐ Time of interest (min) From: To:

Baseline Type: Baseline Smoothing Width:

☒ Automatic
 ☐ Manual
 ☐ Whole Trace
 ☐ Regular Intervals

Peak picking source:
 Smoothing width:
 Minimum width:

Figure 14: Trace Range Rule View

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. Additionally, Time start/Time end values can be added manually to the Trace Peaks table by clicking **Add**.


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

Select sample(s) to update

Select the sample(s) that will apply the trace range rule to generate trace peaks:

<input checked="" type="checkbox"/>	Sample name
<input checked="" type="checkbox"/>	DN32_red
<input checked="" type="checkbox"/>	DN32_reddeglyc

Figure 15: Update 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, displaying (deselecting the Hide ) or adding Trace Types will show the corresponding Area columns in the Trace Peaks Table, but will not populate them without clicking **Update** in the Trace Range Rule or **Compute** at the top of the Analysis.

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:

Edit Trace Range Rule


☐ Time of interest (min) From: To:

Baseline Type: Baseline Smoothing Width:

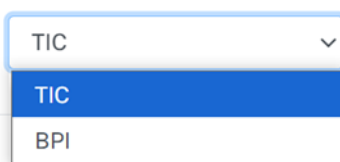
Automatic Manual Whole Trace Regular Intervals

Peak picking source: Smoothing width: Minimum width:

Figure 16: 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. If multiple traces are selected, the first trace will be shown by default.

Peak picking source:



A dropdown menu with a light blue border and a downward arrow on the right. The menu is open, showing three options: 'TIC' (highlighted in blue), 'TIC', and 'BPI'.

Figure 17: 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.

Details for other options (Manual, Whole Trace, and Regular Intervals) can be found in the **Byosphere Intact Web Analysis Manual**.

When samples or trace types are added to or deleted from the analysis, the current Trace Range Rule is not applied automatically, and the new samples/traces must be selected and updated within the trace range rule dialog.

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

Flat vs Auto Baseline

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

View 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 magenta shaded windows indicating the trace range.

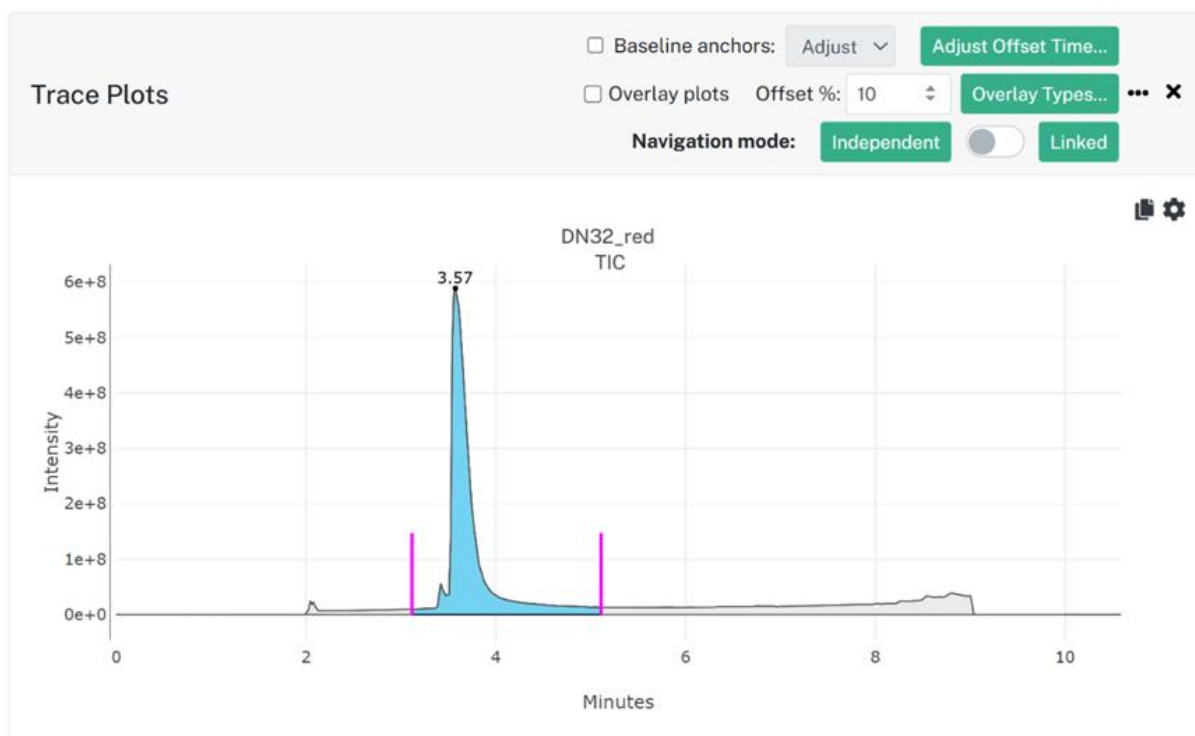


Figure 18: Trace Plot with integration boundaries

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 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.

Adjust Trace Offset Time

Enable Auto Trace Offset ☐ Max Offset Time

Trace Type	Trace Description	Offset Time
tic	TIC	0
bpi	BPI	0

Note: BPI and TIC traces are locked with the same offset value.

Figure 19: Adjust Offset Time

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.

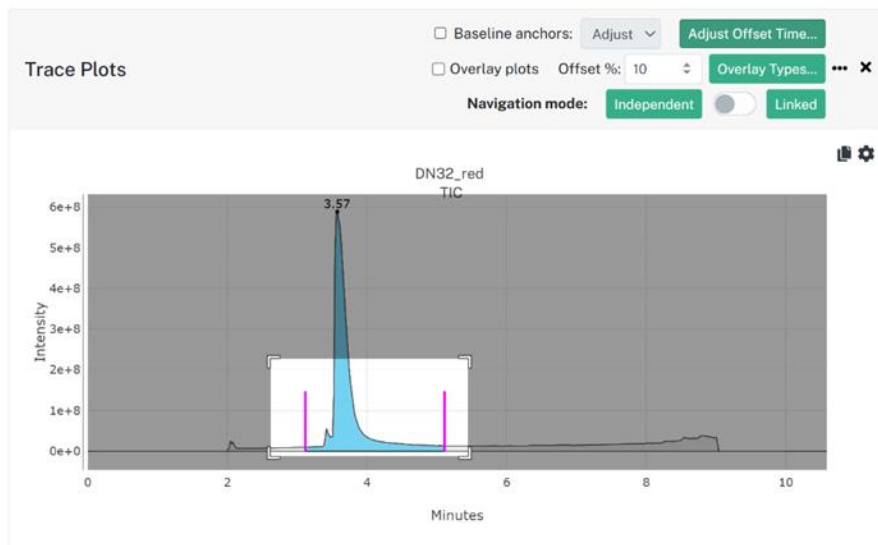


Figure 20: Before zoom

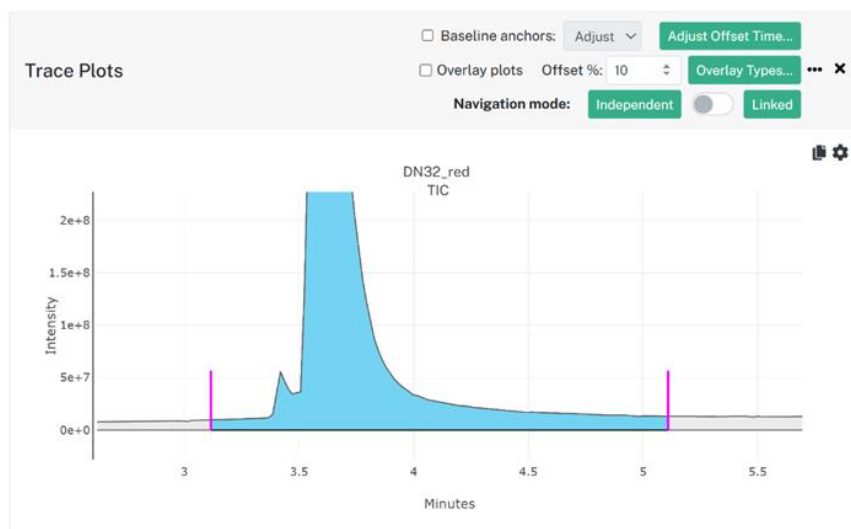


Figure 21: After zoom

To pan, hover over either the X or Y axis until the mouse turns into a double arrow \leftrightarrow , 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.

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.

Trace Peaks Table						
<div>Trace Range Rule... Delete Selected... Split... Merge... Update Add Peaks... X</div>						
1/1 rows						
Sample Name	Peak No.	Time Start	Time End	Apex Time (TIC)	Area (TIC)	Normed Area % (TIC)
Search	Search	Search	Search	Search	Search	Search
DN32_reddeglyc	1	3.7	4	3.76	3.42e+7	37.64

Figure 22: Split Trace Peak

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.

Baseline Anchors

Users can manually adjust baseline anchors using Add, Move, and Delete operations.

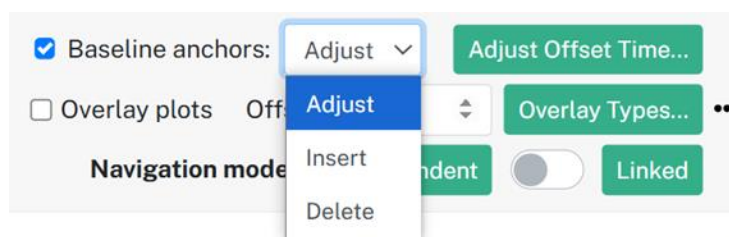


Figure 23: Baseline anchors options

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

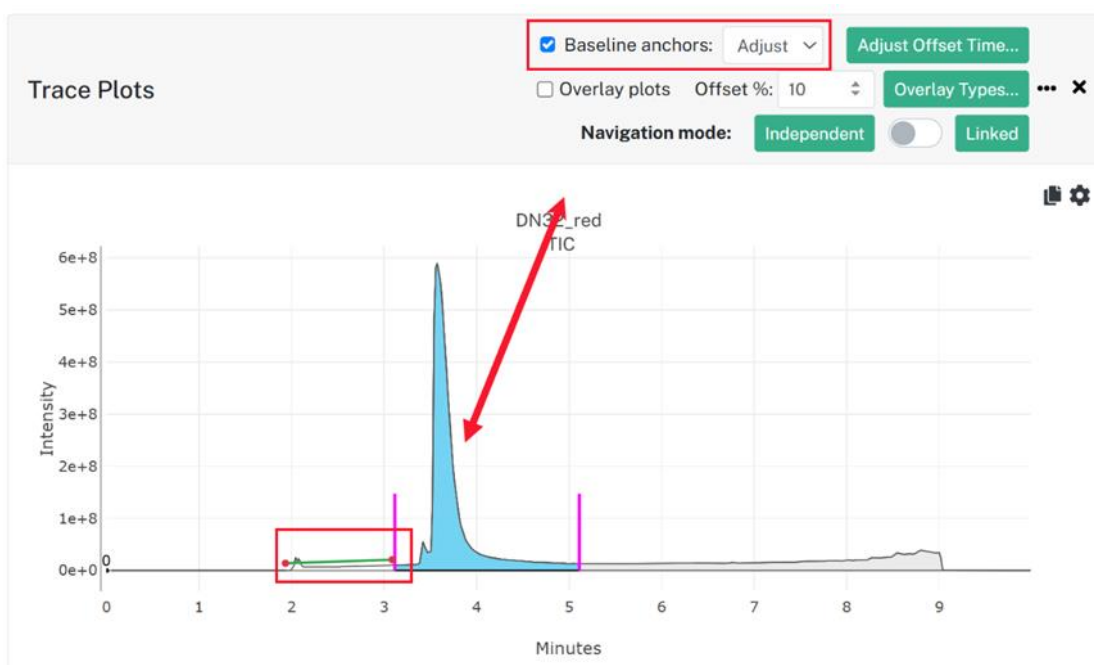


Figure 24: Adjust anchor

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

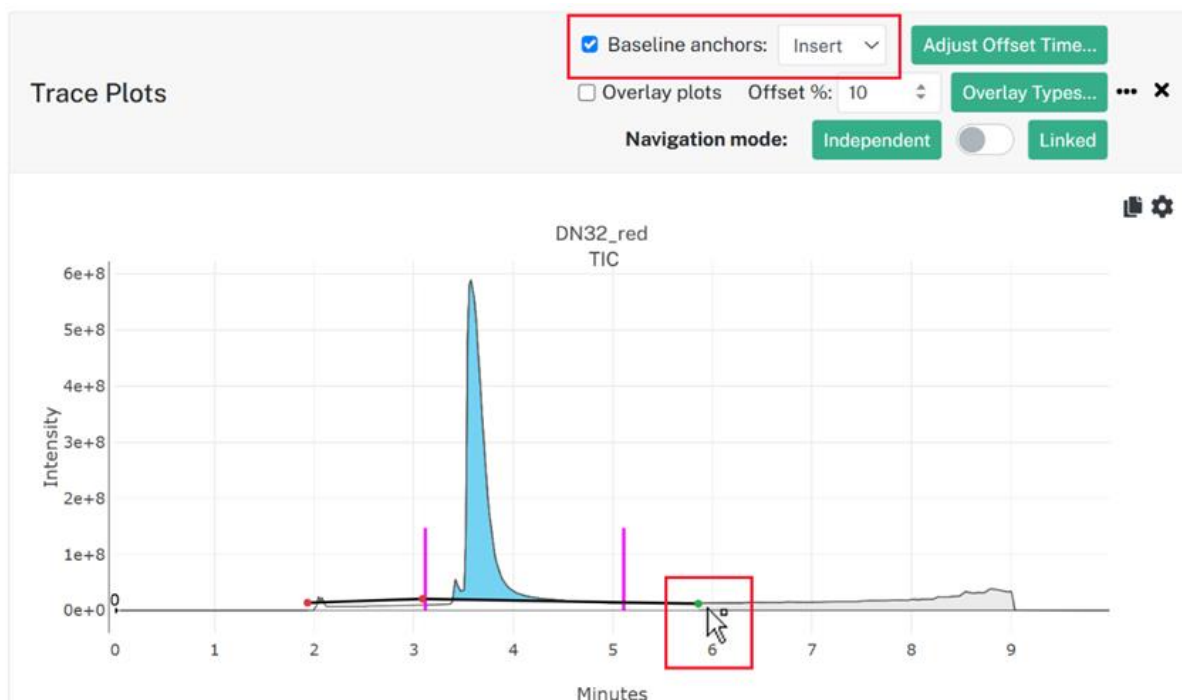


Figure 25: Insert anchor

When **Delete** is selected, hovering over and clicking any anchor will delete the anchor from the plot, as long as there are more than 3 anchors present. The user cannot delete an anchor if there are only two left.

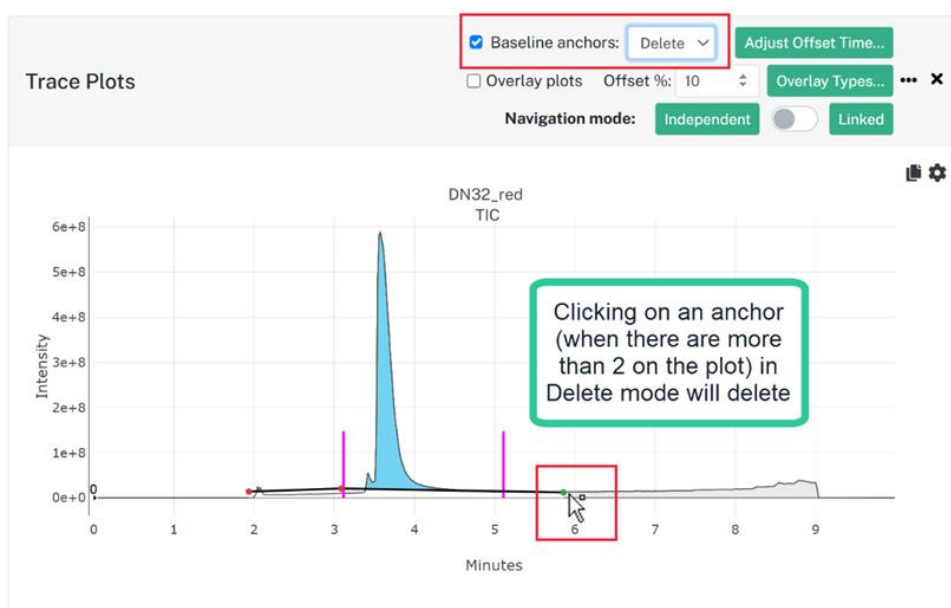


Figure 26: Delete anchor

Trace Overlay

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

Only trace types that are currently marked as visible with the eye icon will be included in the list of trace types of overlay. Users can select which traces to create overlays for by clicking **Overlay Types** in the Trace Plots widget.

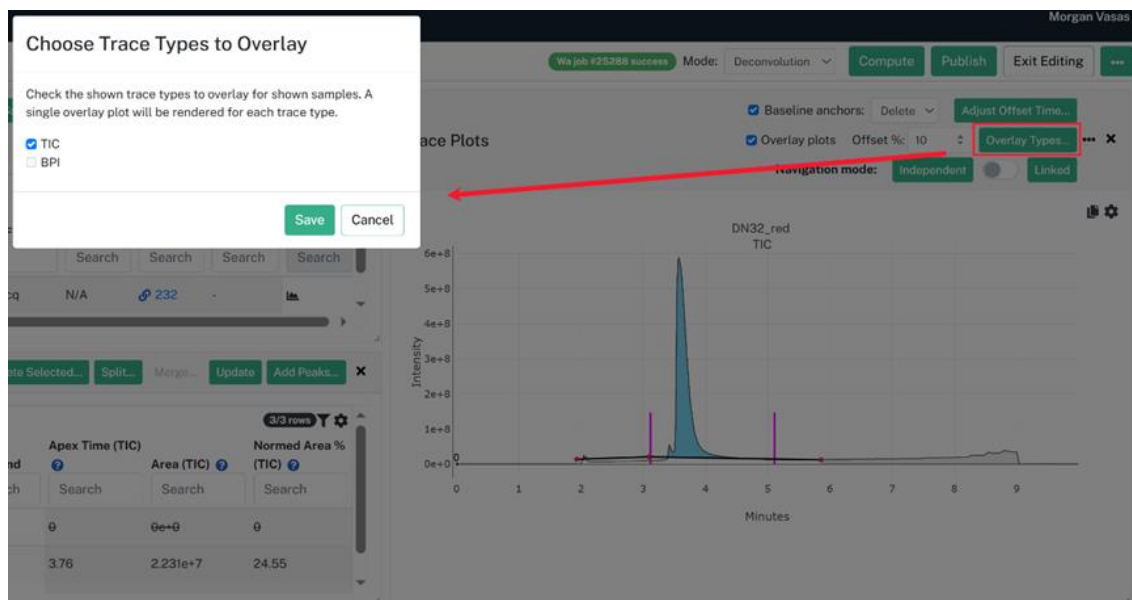


Figure 27: Overlay selection dialog

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.

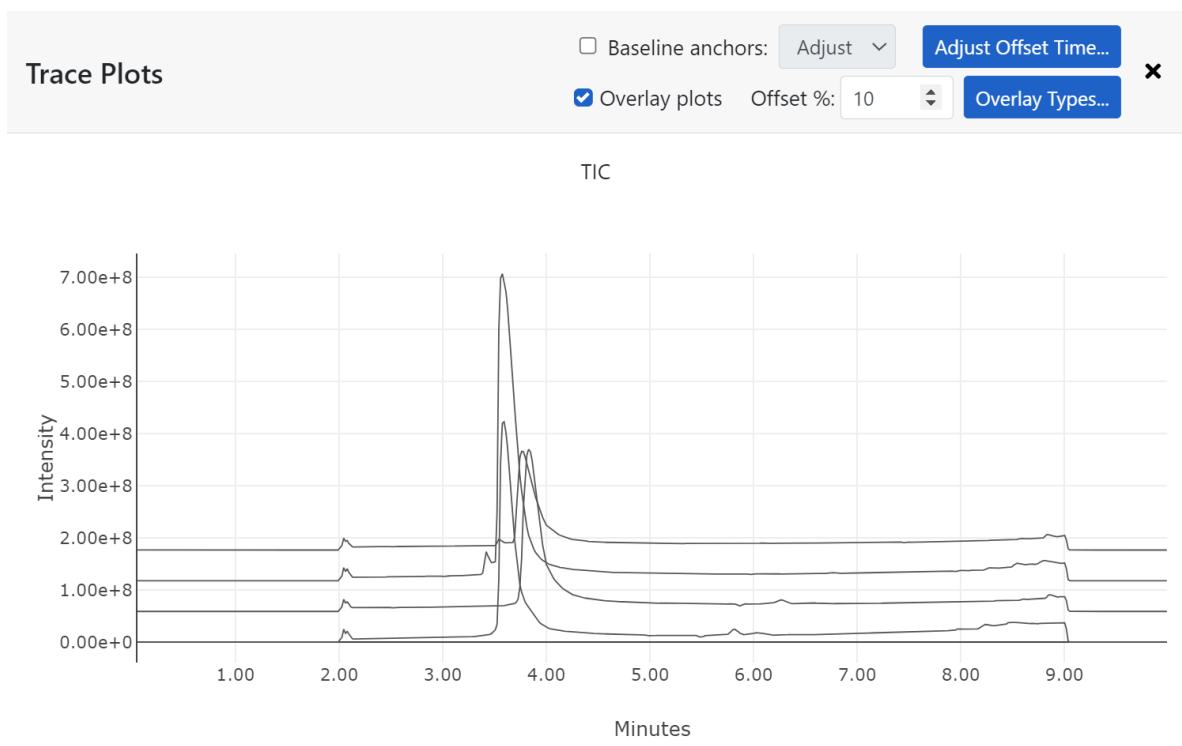


Figure 28: Overlay with 10% offset

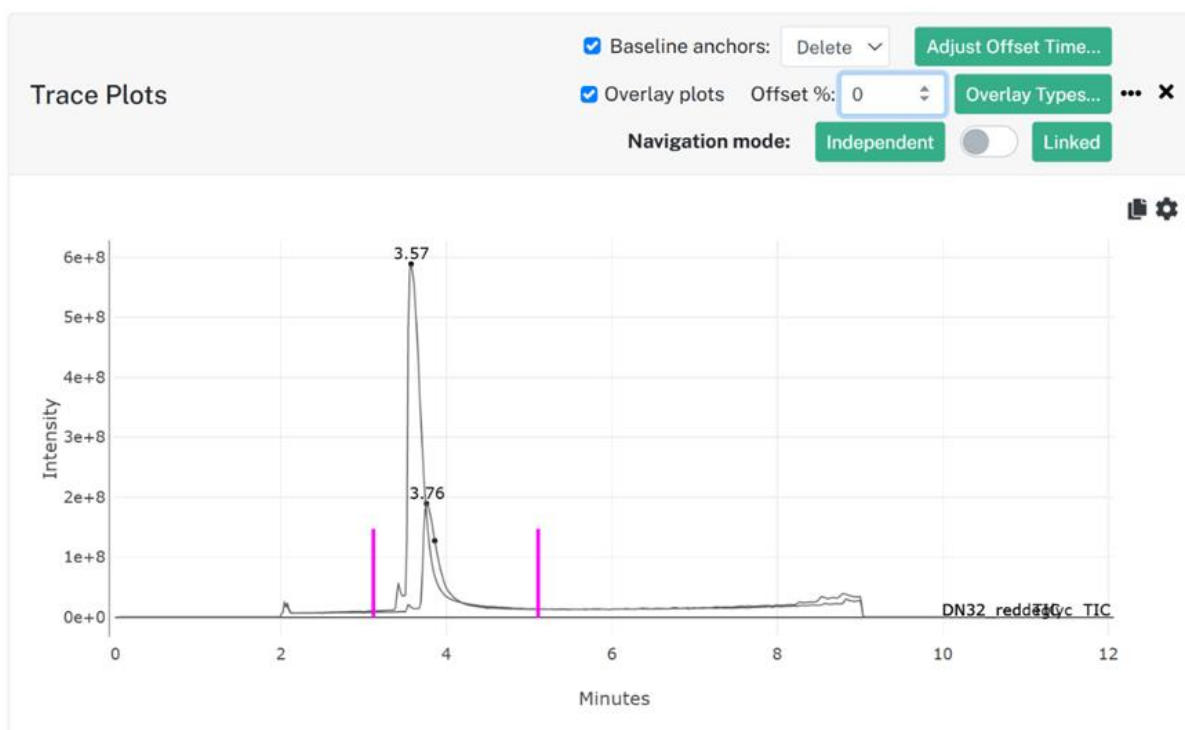


Figure 29: Overlay with 0% offset

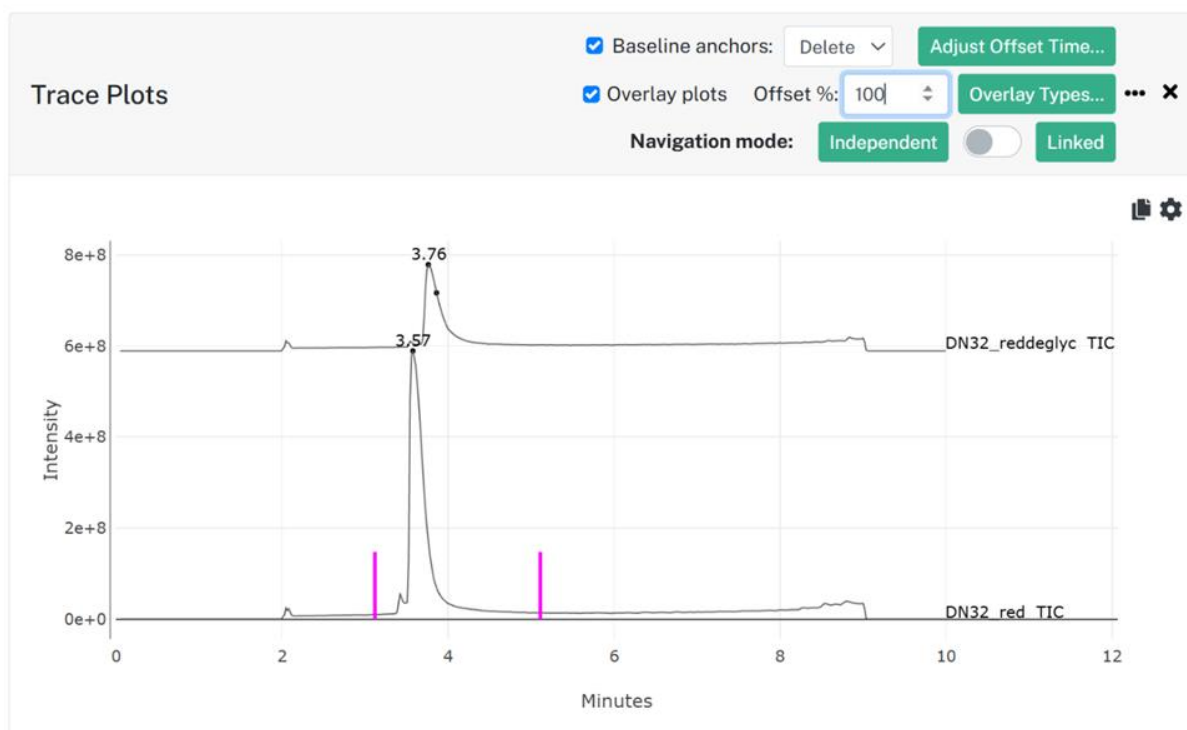


Figure 30: Overlay with 100% offset

Lock Mass

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

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.

Adjust Lock Mass Settings

☒ Enable lock mass

Lock mass (m/z)

Tolerance (ppm)

Figure 31: Lock Mass dialog

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

View Features

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 Sequence room.

Features Table (all records) 50 Feature Rule... Filter Features... Delete Selected... ✕

Sample Name	Sample No.	Feature No.	Obs. m/z	Charge	Obs. Mass	Time Start	Time End	Apex Time	Apex Intensity
NISTmAb_Control_MS2	1	1	502.686	1	501.6787	19.12	19.58	1.94e+1	4.53e+4
NISTmAb_Control_MS2	1	2	514.89	1	513.8827	16.72	17.28	1.69e+1	4.09e+4
NISTmAb_Control_MS2	1	3	519.138	1	518.1307	35.26	35.75	3.56e+1	7e+4

« ‹ 1 2 3 4 5 ... 101 › »

Figure 32: 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.

Feature Rule

m/z window (+/- ppm):

Compute Cancel

Figure 33: 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.

Feature Rule

m/z window (+/- ppm):

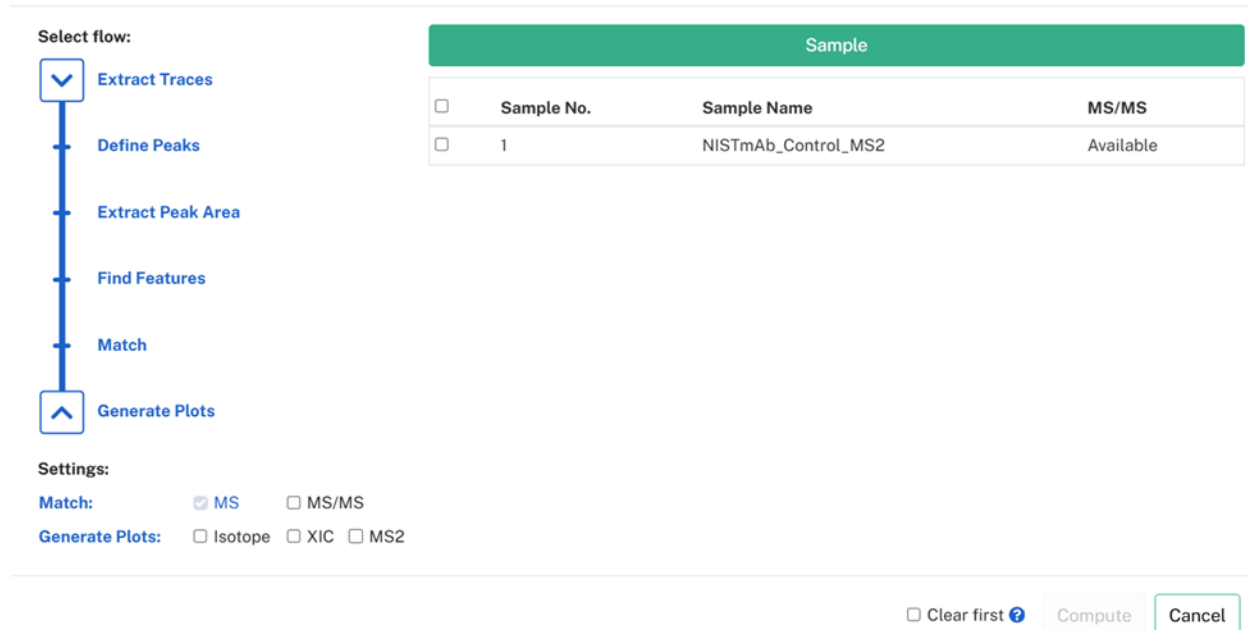
Compute Cancel

Figure 34: Filter Features dialog

Compute (Feature Finder Mode)

The **Compute** options in Feature Finder Mode differ from those found in Deconvolution mode. Users have the option to **Extract Traces**, **Define Peaks**, **Extract Peak Area**, **Find Features**, **Match (MS and MS/MS)**, and Generate Plots.

Compute (Feature Finder Mode)



Select flow:

☐ Extract Traces

☐ Define Peaks

☐ Extract Peak Area

☐ Find Features

☐ Match

☐ Generate Plots

Settings:

Match: ☒ MS ☐ MS/MS

Generate Plots: ☐ Isotope ☐ XIC ☐ MS2

Sample			
<input type="checkbox"/>	Sample No.	Sample Name	MS/MS
<input type="checkbox"/>	1	NISTmAb_Control_MS2	Available

☐ Clear first [?](#)

Figure 35: 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 rules 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.

Sample			
<input type="checkbox"/>	Sample No.	Sample Name	MS/MS
<input type="checkbox"/>	1	NISTmAb_Control_MS2	Available

Figure 36: Sample tab in Computation

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:

Select flow:**Settings:****Match:** ☒ MS ☐ MS/MS**Generate Plots:** ☐ Isotope ☐ XIC ☐ MS2

Figure 37: Computation Flow Chart

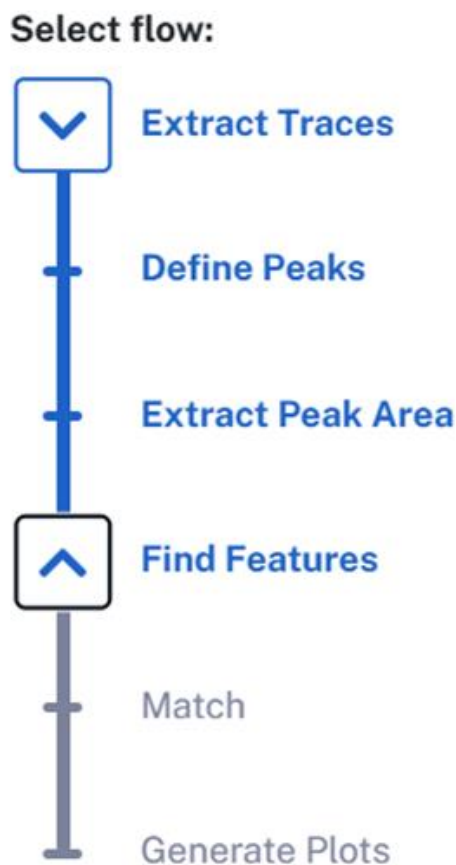


Figure 38: Truncated Flow Chart

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

In the **Inspection** room, all options are included in the compute flow range.

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.

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.

Sequences room

Add Sequences

Sequences can be added manually or through an 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 imported with the **Import FASTA** button to the Sequences table as separate rows.

Sequences 50 Delete Selected... Import FASTA... Add Sequence ✕

2/2 rows

Code	Name	Sequence		Molecule Type	Average Mass	Monoisotopic Mass
Search	Search	Search		Search	Search	Search
A	DN32 HC	QVQLQQSGAELARPGASV...	Edit	Protein	49862.1468214	49830.558564
B	DN32 LC	QIVLTQSPAIMSASPGEKV...	Edit	Protein	23357.6286032	23343.00971

Figure 39: Sequences

Using **Import FASTA** allows the user to import FASTA sequences directly. Once they're added, the masses are updated automatically. **FASTA** files are imported from folders available to users. FASTA sequences can also be entered manually.

Clicking **Add Sequence** adds an empty row populated with a **Code** letter (e.g., A, B, C which are added in alphabetical order); Clicking **Edit** in this row launches a dialog where a user can enter a sequence. Masses will be calculated automatically.

Sequences 50 Delete Selected... Import FASTA... Add Sequence ✕

2/2 rows

Code	Name	Sequence		Molecule Type	Average Mass	Monoisotopic Mass
Search	Search	Search		Search	Search	Search
A	DN32 HC	QVQLQQSGAELARPGASV...	Edit	Protein	49862.1468214	49830.558564
B	DN32 LC	QIVLTQSPAIMSASPGEKV...	Edit	Protein	23357.6286032	23343.00971

Figure 40: Add Sequences

Enter Sequence

Sequence

Enter sequence name

Save

Cancel

Figure 41: Manual sequence entry

Add Combinations

The **Add Combination** button becomes available once a sequence has been added.

When adding a new combination, mass values are updated automatically.

New Sequence Combination

Combination name:

Combination alias:

Composition Construct new IgG Antibody

<input type="checkbox"/>	Code	Sequence Name	Count	Type
<input type="checkbox"/>	A	DN32 HC	1	-
<input type="checkbox"/>	B	DN32 LC	1	-

Composition molecule:

Disulfides:

Expected Type:

Add Cancel

Figure 42: New Sequence Combination

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.

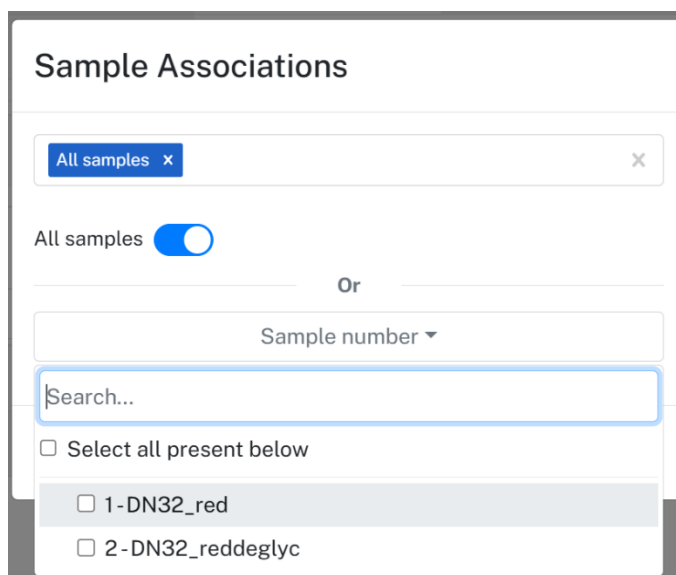
Clicking on the **Sample associations** dropdown within a row opens the **Sample Associations** dialog, which lists all current associations and provides all available fields, including Samples, to form associations on.

Combinations								2/2 rows	
Name	Alias	Composition	Disulfides	Average Mass	Monoisotopic Mass	Expected Type	Actions	Sample associations	
<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	
LC	-	B(l)	Reduced	23340.6	23325.98	Desired		<input type="text" value="All samples"/>	
HC	-	A(l)	Reduced	49716.94	49685.44	Desired		<input type="text" value="All samples"/>	

Reference Mass Modifications

☒ Change N-terminal Q to pyroGlu ☒ Clip off C-terminal K ☐ N-glycans removed by PNGase F (N-X-S/T → D)

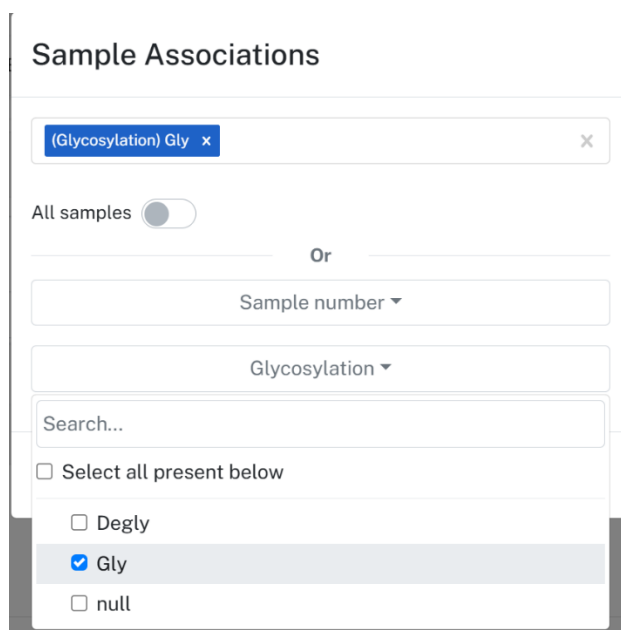
Figure 43: Sample associations column



The dialog box is titled "Sample Associations". It features a search bar at the top containing the text "All samples" with a close button (x). Below the search bar is a toggle switch labeled "All samples" which is currently turned on. A horizontal separator with the word "Or" in the center follows. Below this, there is a dropdown menu labeled "Sample number". A search input field with the placeholder text "Search..." is positioned below the dropdown. Underneath the search field is a checkbox labeled "Select all present below". A list of sample identifiers is shown below the checkbox, with the first item, "1 - DN32_red", highlighted in light blue. The second item is "2 - DN32_reddeglyc".

Figure 44: 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.



This dialog box is also titled "Sample Associations". The search bar at the top contains the text "(Glycosylation) Gly" with a close button (x). Below the search bar is a toggle switch labeled "All samples" which is currently turned off. A horizontal separator with the word "Or" in the center follows. Below this, there are two dropdown menus: the first is labeled "Sample number" and the second is labeled "Glycosylation". A search input field with the placeholder text "Search..." is positioned below the "Glycosylation" dropdown. Underneath the search field is a checkbox labeled "Select all present below". A list of glycosylation values is shown below the checkbox, with the second item, "Gly", highlighted in light blue and marked with a blue checkmark. The other items are "Degly" and "null".

Figure 45: 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 46: Combinations table with Sample associations populated

Digestion Parameters

Feature Finder analysis provides a dialog within which to specify the protease and alkylating agent used for sample preparation, as well as the number of potential missed cleavages. This dialog can be accessed within the **Combinations** header.

Figure 47: Digestion Parameters dialog

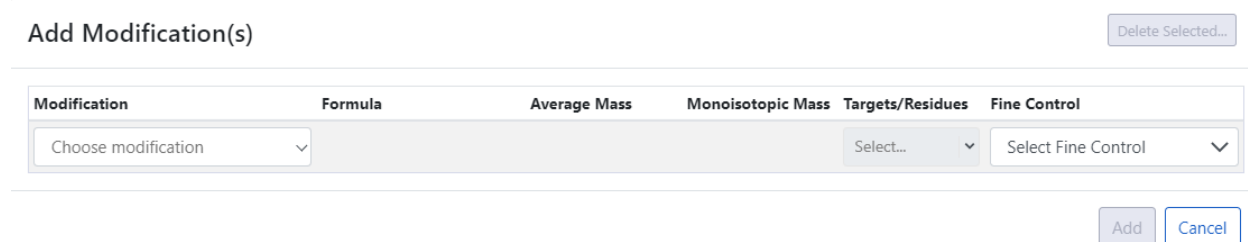
Protease options include Trypsin R, K C-termini, LysC K C-termini, AspN D N-termini, and GluC D, E C-termini.

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

Figure 48: Alkylating Agents dropdown

Modifications

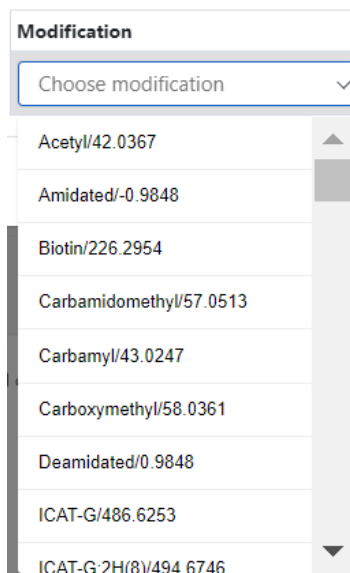
New Modifications are generated by clicking the **Add Modification** button, which is always enabled. This launches the dialog as shown below:



The dialog box is titled "Add Modification(s)" and includes a "Delete Selected..." button in the top right corner. It features a table with the following columns: Modification, Formula, Average Mass, Monoisotopic Mass, Targets/Residues, and Fine Control. The "Modification" column contains a dropdown menu with the text "Choose modification". The "Targets/Residues" column contains a dropdown menu with the text "Select...". The "Fine Control" column contains a dropdown menu with the text "Select Fine Control". Below the table are "Add" and "Cancel" buttons.

Figure 49: Add Modification

Modifications are selected from a dropdown which sources modification types and corresponding information 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. If a custom formula is entered, Average and Monoisotopic mass are automatically calculated.




The dropdown menu is titled "Modification" and shows a list of modification types with their corresponding average masses. The list includes: Acetyl/42.0367, Amidated/-0.9848, Biotin/226.2954, Carbamidomethyl/57.0513, Carbamyl/43.0247, Carboxymethyl/58.0361, Deamidated/0.9848, ICAT-G/486.6253, and ICAT-G-2H(8)/494.6746. The dropdown is currently open, showing the list of options.

Figure 50: 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. More information about Modification Fine Control™ can be found in the **Byonic Manual**.

Modifications can be deleted by selecting the row and clicking **Delete Selected**.

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

Edit Modification

Choose modification:

Formula:

Average Mass:

Monoisotopic Mass:

Residues: × ▼

Fine Control:

Figure 51: 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 Modifications table will update accordingly.

Modifications Delete Selected... Add Modification(s)... ×							
11/11 rows							
<input checked="" type="checkbox"/> Include	Modification	Formula	Average Mass	Mono Mass	Targets/Residues	Fine Control	Actions
<input type="button" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="button" value="Search"/>
<input checked="" type="checkbox"/>	G0	C(50)H(82)N(4)O(35)	1299.2	1298.476	N	Variable-rare 1	<input type="button" value="Edit"/>
<input checked="" type="checkbox"/>	G0F	C(56)H(92)N(4)O(39)	1445.34	1444.5339	N	Variable-rare 1	<input type="button" value="Edit"/>
<input checked="" type="checkbox"/>	G1F	C(62)H(102)N(4)O(44)	1607.48	1606.5867	N	Variable-rare 1	<input type="button" value="Edit"/>
<input checked="" type="checkbox"/>	G2F	C(68)H(112)N(4)O(49)	1769.62	1768.6395	N	Variable-rare 1	<input type="button" value="Edit"/>
<input checked="" type="checkbox"/>	G0F+Lys	C(62)H(104)N(6)O(40)	1573.51	1572.6288	N	Variable-rare 1	<input type="button" value="Edit"/>
<input checked="" type="checkbox"/>	G0F-GlcNAc	C(48)H(79)N(3)O(34)	1242.15	1241.4545	N	Variable-rare 1	<input type="button" value="Edit"/>
<input checked="" type="checkbox"/>	Hex	H(10)C(6)O(5)	162.14	162.0528	N	Variable-rare 1	<input type="button" value="Edit"/>
<input checked="" type="checkbox"/>	Man5	C(46)H(76)N(2)O(35)	1217.09	1216.4229	N	Variable-rare 1	<input type="button" value="Edit"/>
<input checked="" type="checkbox"/>	Man5F	C(52)H(86)N(2)O(39)	1363.24	1362.4808	N	Variable-rare 1	<input type="button" value="Edit"/>
<input checked="" type="checkbox"/>	G1F+NeuAc	C(73)H(119)N(5)O(52)	1898.74	1897.6821	N	Variable-rare 1	<input type="button" value="Edit"/>
<input checked="" type="checkbox"/>	+Lys	C(6)H(12)N(2)O(1)	128.17	128.095	Protein C-term	Variable-rare 1	<input type="button" value="Edit"/>
Total common max: <input type="text" value="1"/> Total rare max: <input type="text" value="1"/>							

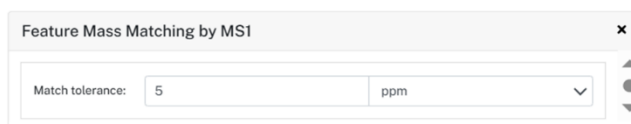
Figure 52: Modifications Table

Users have the option to include a modification by checking **Include** or include *all* modifications in the table by checking the **Include** box in the *header*.

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

Feature Mass Matching

The **Feature Mass Matching** view includes parameters that control automatic mass peak assignment of peptide sequences to detected features.



Feature Mass Matching by MS1

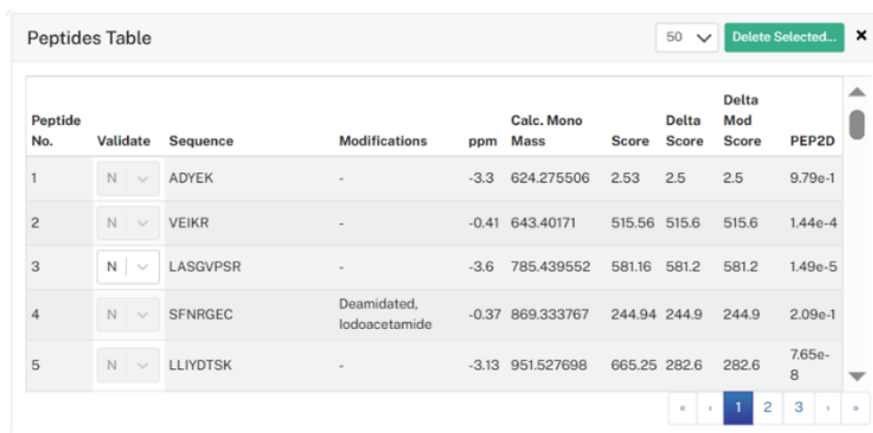
Match tolerance: ppm

Figure 53: Mass Matching

Inspection room

View Peptides

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.

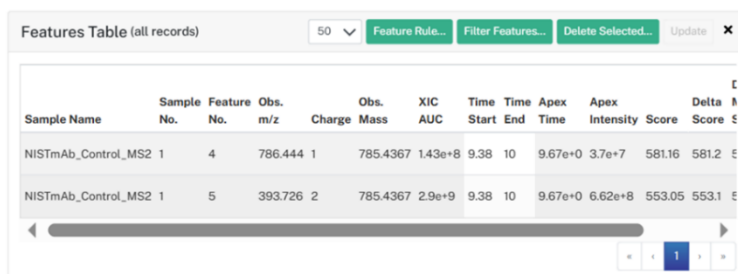


Peptide No.	Validate	Sequence	Modifications	ppm	Calc. Mono Mass	Score	Delta Score	Delta Mod Score	PEP2D
1	<input type="button" value="N"/>	ADYEK	-	-3.3	624.275506	2.53	2.5	2.5	9.79e-1
2	<input type="button" value="N"/>	VEIKR	-	-0.41	643.40171	515.56	515.6	515.6	1.44e-4
3	<input type="button" value="N"/>	LASGVPSR	-	-3.6	785.439552	581.16	581.2	581.2	1.49e-5
4	<input type="button" value="N"/>	SFNRGEC	Deamidated, Iodoacetamide	-0.37	869.333767	244.94	244.9	244.9	2.09e-1
5	<input type="button" value="N"/>	LLIYDTSK	-	-3.13	951.527698	665.25	282.6	282.6	7.65e-8

Figure 54: Peptides table

Features Corresponding with Peptides

The **Features Table** in the Inspection room displays a list of the corresponding Features when the user selects a peptide in the Peptides table. Also included in the table are any modifications included on the peptide, the ppm, and the calculated mono mass.



Sample Name	Sample No.	Feature No.	Obs. m/z	Obs. 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 55: Features Table

Clicking a peptide will populate the Features table with associated features.

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

Also note that if Feature rules are assigned in the Samples room, changing these settings in the Inspection room will reset them and the Mass Matching step of computation will have to be performed again.

Plots

XIC

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.

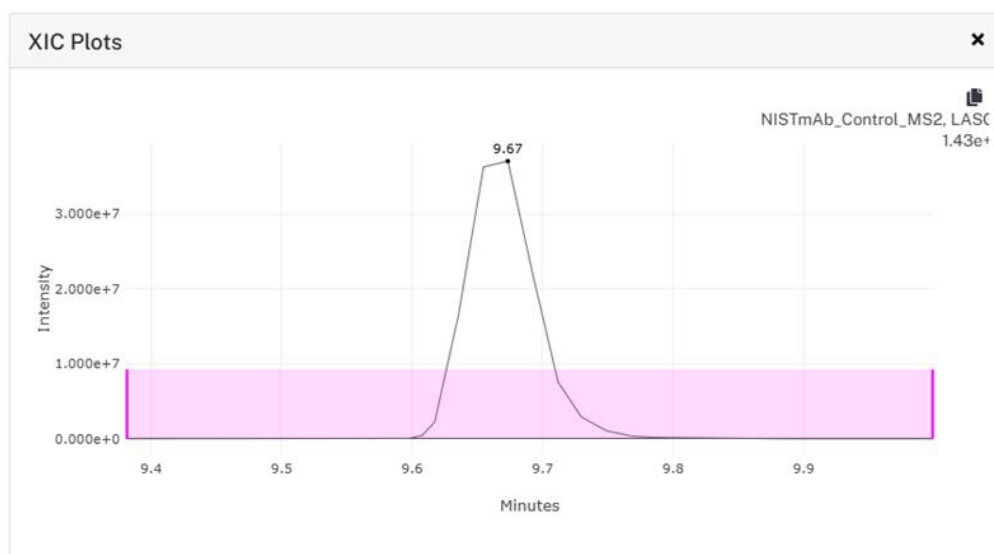


Figure 56: XIC plot

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

The **Isotope plot** shows the MS1 isotope spread of the selected feature, corresponding with the XIC plot. 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.

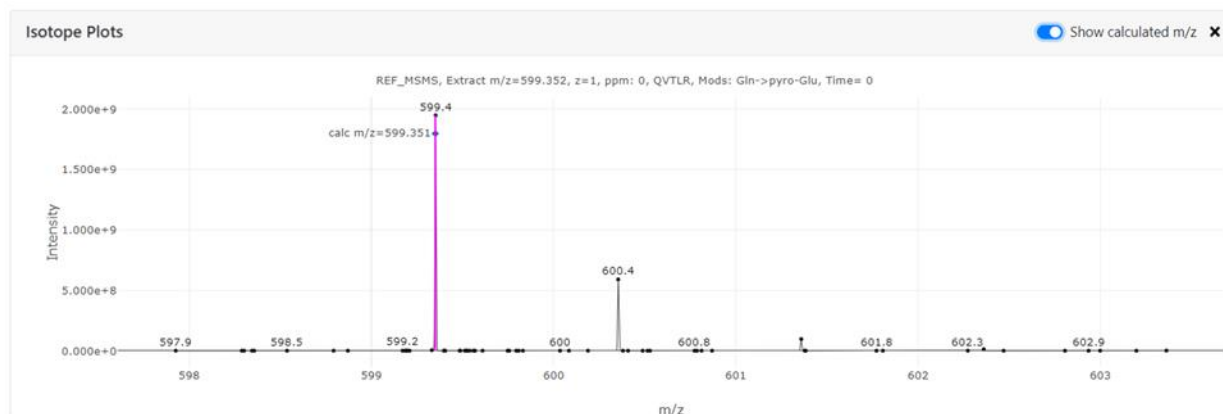


Figure 57: Isotope plot

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.

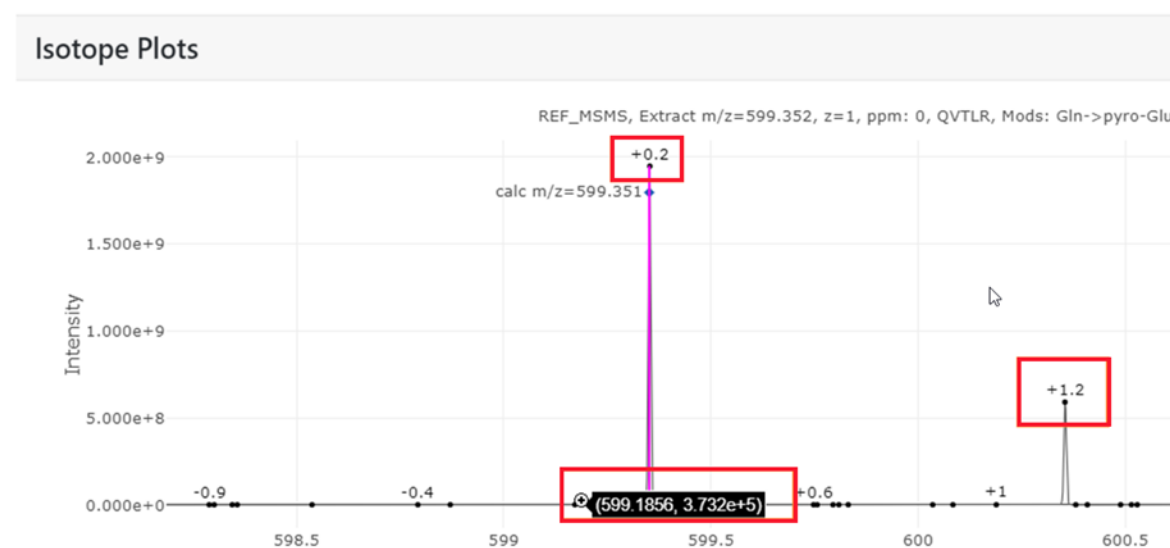


Figure 58: Mass shift relative to other peaks

MS2 Plots

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

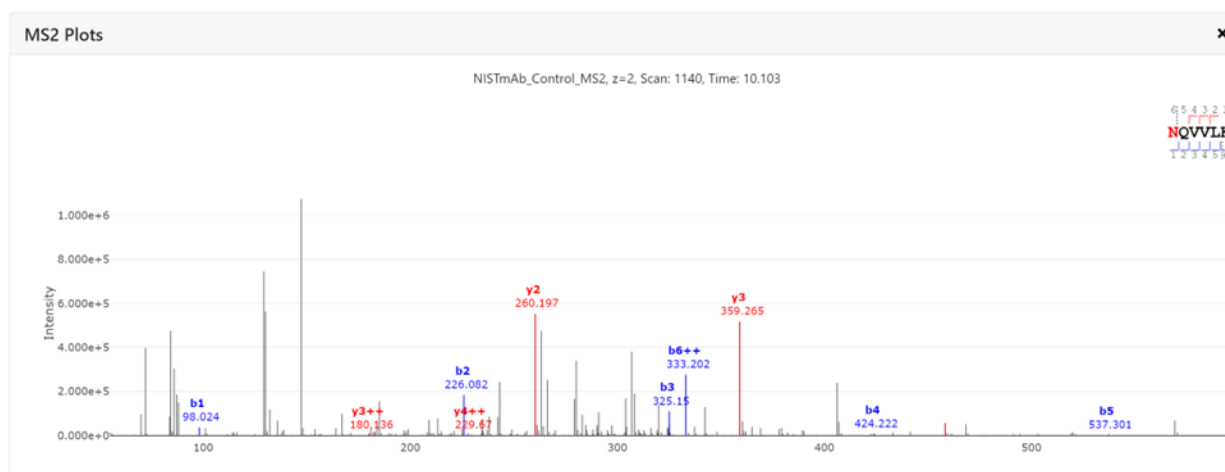
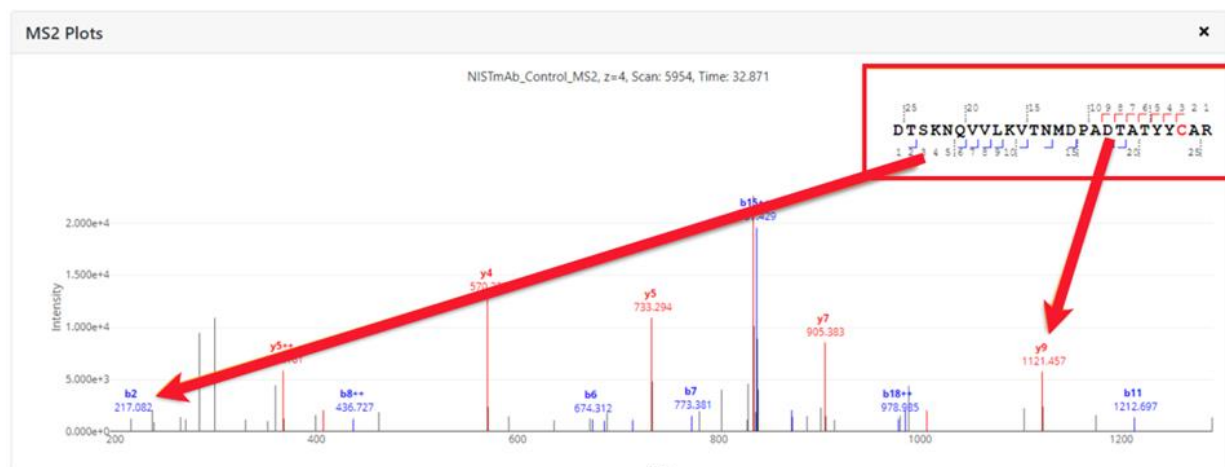


Figure 59: An MS2 plot with modification present

Modifications are labeled on the peptide fragment legend which consists of the sequence of the peptide that was ID'd (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.

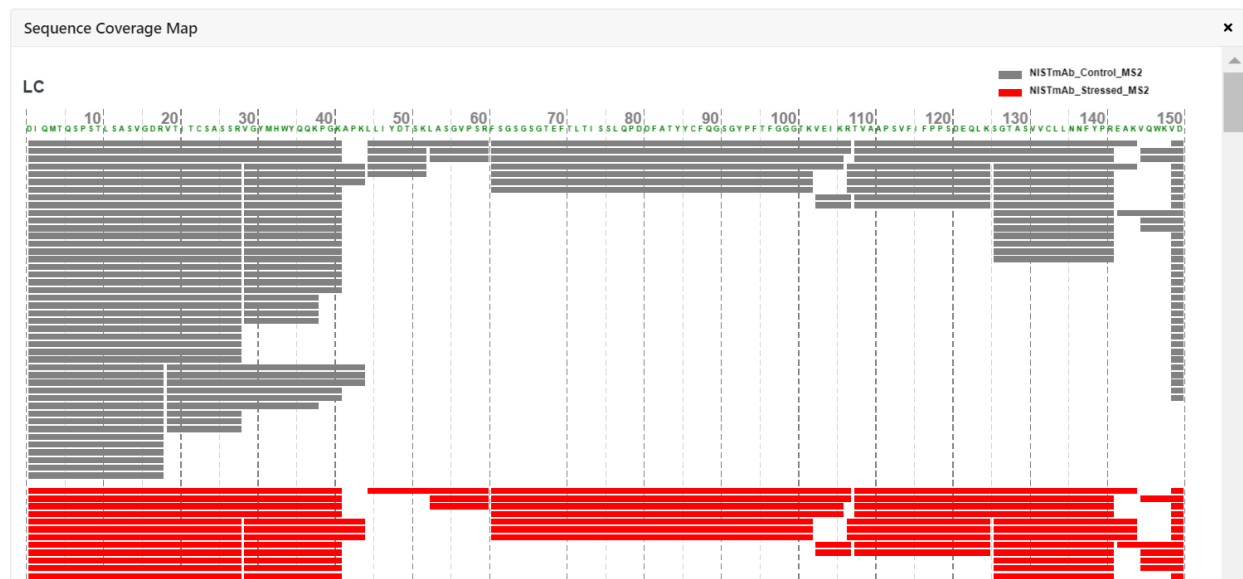


Figure 60: Sequence Coverage Map

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.

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 61: Coverage Summary Table

Report room

The **Report** room provides a summarization of the analysis, as well as XIC and Isotope plots generated (if selected).

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

Publish

Exit Editing

Figure 62: Header options for the Report room

Summary

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

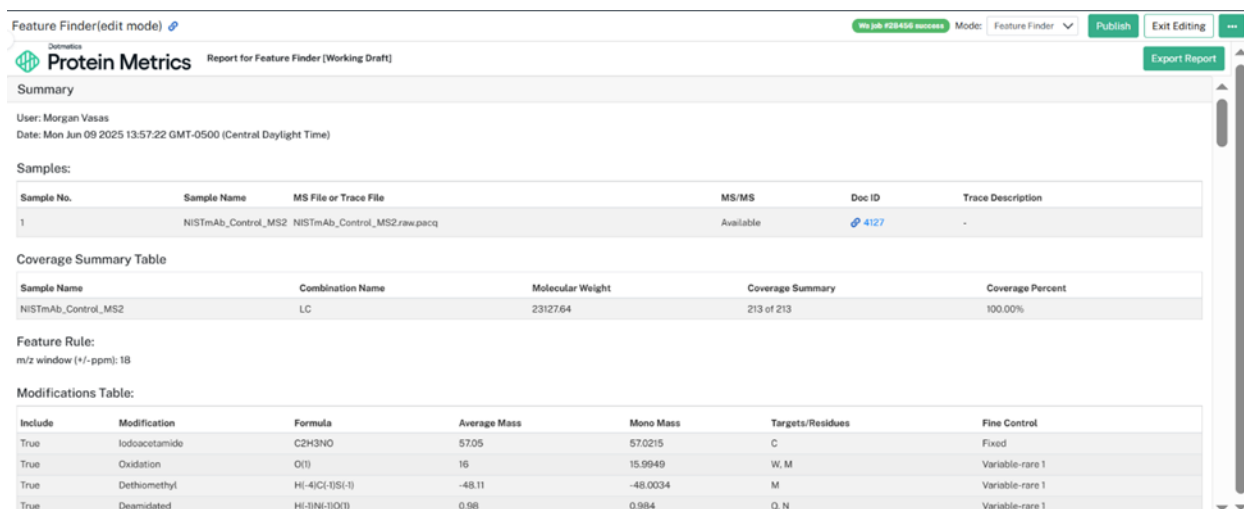


Figure 63: Snapshot of Summary view within the Report room

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

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. If they are selected, every XIC and Isotope 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 Settings in Edit mode

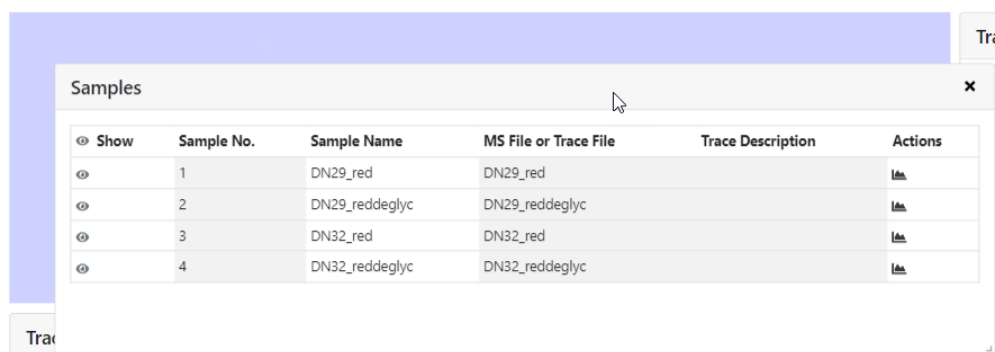
When in **Edit** mode, the user can interact with any of the rooms within the Analysis. The header now consists of the following options:



Figure 64: Analysis Header in Edit Mode

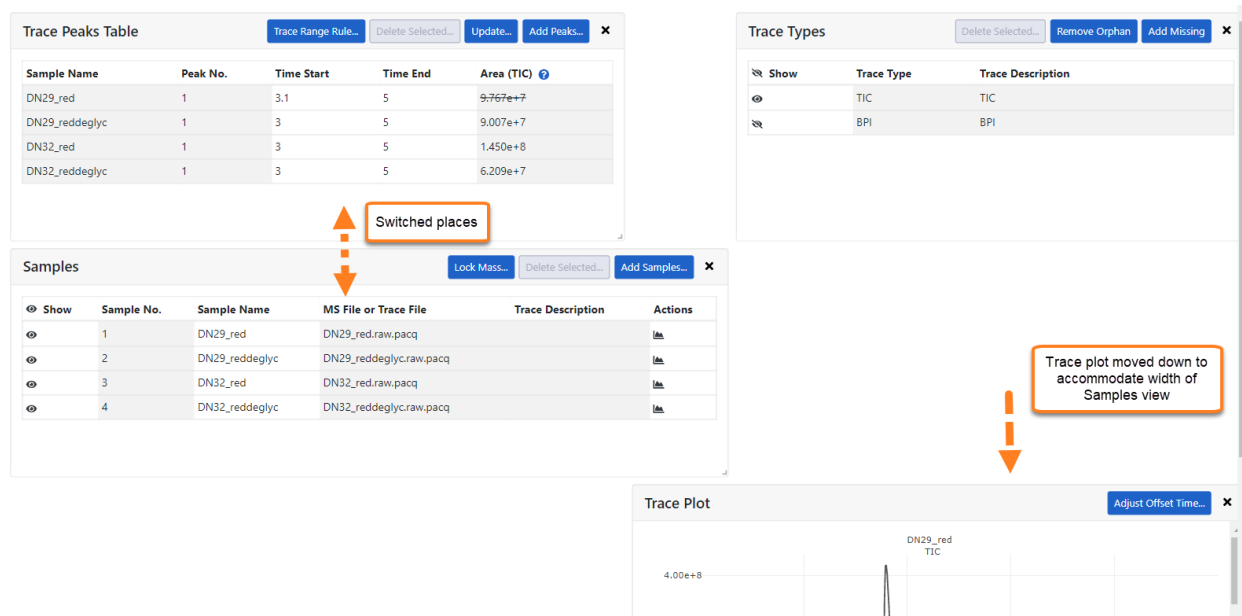
Rearrange Views

The user 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.



Show	Sample No.	Sample Name	MS File or Trace File	Trace Description	Actions
	1	DN29_red	DN29_red		
	2	DN29_reddeglyc	DN29_reddeglyc		
	3	DN32_red	DN32_red		
	4	DN32_reddeglyc	DN32_reddeglyc		

Figure 65: When moving a view, the space it previously inhabited is highlighted blue



Trace Peaks Table

Sample Name	Peak No.	Time Start	Time End	Area (TIC)
DN29_red	1	3.1	5	9.767e+7
DN29_reddeglyc	1	3	5	9.007e+7
DN32_red	1	3	5	1.450e+8
DN32_reddeglyc	1	3	5	6.209e+7

Switched places

Samples

Show	Sample No.	Sample Name	MS File or Trace File	Trace Description	Actions
	1	DN29_red	DN29_red.raw.pacq		
	2	DN29_reddeglyc	DN29_reddeglyc.raw.pacq		
	3	DN32_red	DN32_red.raw.pacq		
	4	DN32_reddeglyc	DN32_reddeglyc.raw.pacq		

Trace Types

Show	Trace Type	Trace Description
	TIC	TIC
	BPI	BPI

Trace plot moved down to accommodate width of Samples view

Trace Plot

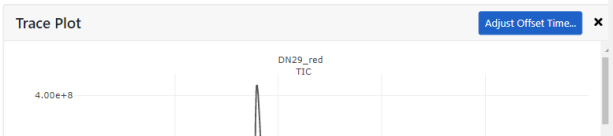



Figure 66: Rearranging Views

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 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.

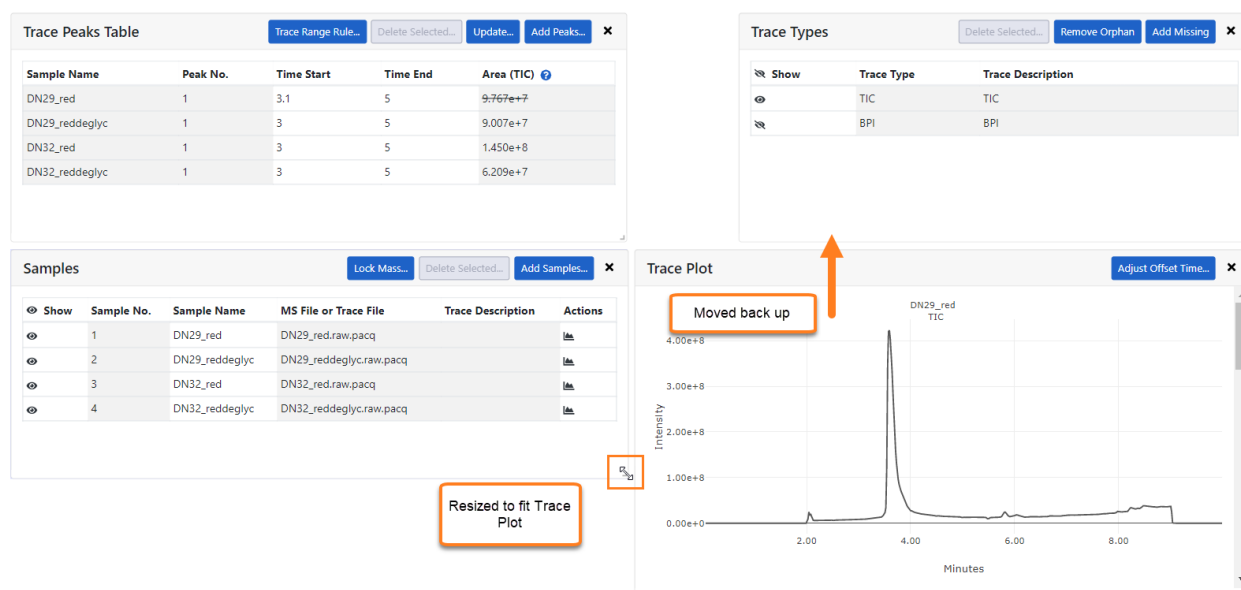


Figure 67: Rearranging Views part 2

Compute

Information on Compute 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.

Save as Template

The current analysis can be **Saved as Template** in a Folder as a template record for future use with the appropriate extension (.bproj). user-saved templates are accessible through the Folder within which they are saved and will *not* be including among the system templates on the Home page.

To launch an analysis from the 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.

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.

Generate Report

Would you like to generate a PDF report?

Yes

No

Figure 68: 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.

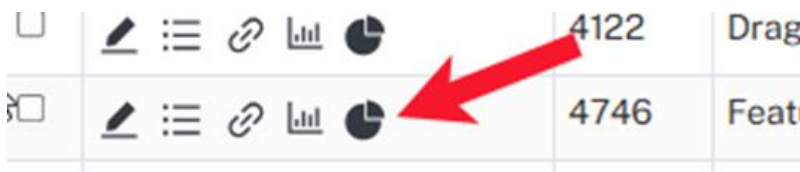


Figure 69: View published report

The user 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.

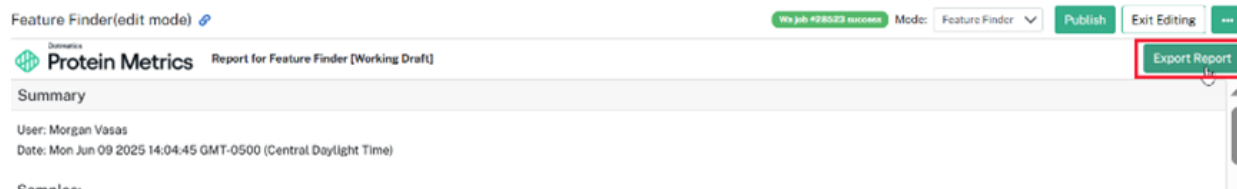


Figure 70: Export Report