



Byosphere[®] Intact Web Analysis Quick Start Manual

December 2025

5.12

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Intact 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 such as Intact protein analysis within the Byosphere Web Client. The Web Analysis application is embedded directly within the Byosphere Web Client.

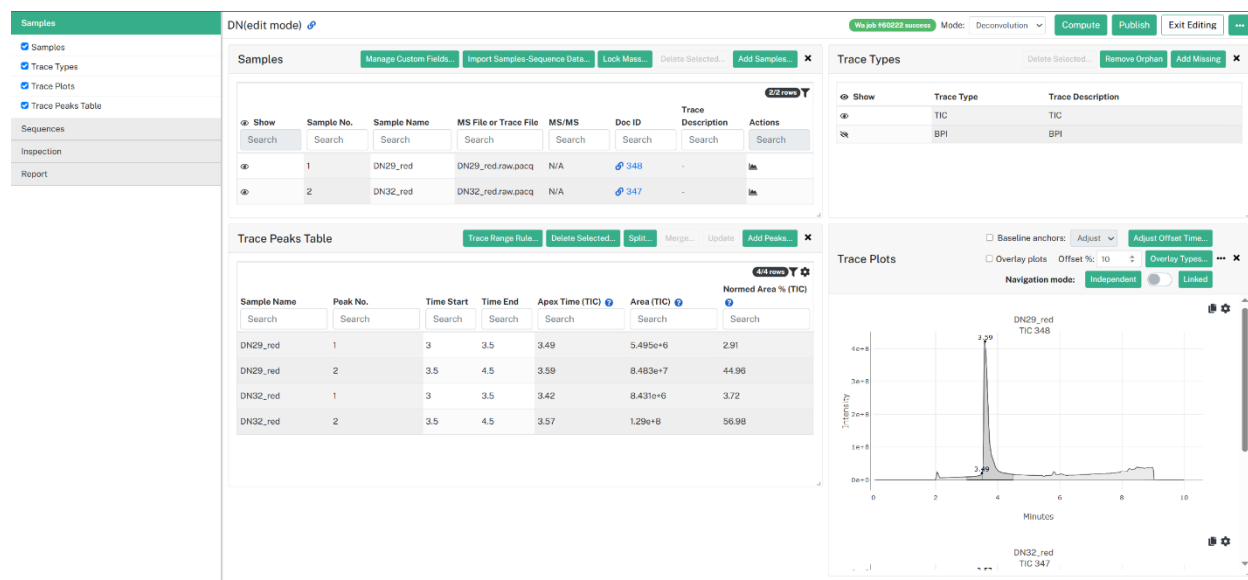


Figure 1: Analysis from Deconvolution Edit Mode , with the Samples Room selected

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 the **Deconvolution** mode in Web Analysis, which is used to analyze Intact projects.

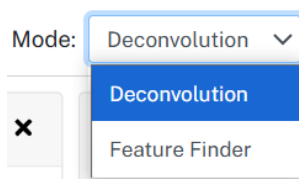


Figure 2: Web Analysis mode

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 (*.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
- **ADC-Intact:** Mass confirmation and DAR for ADCs with multi-sample reporting.

- **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

System Analysis Templates

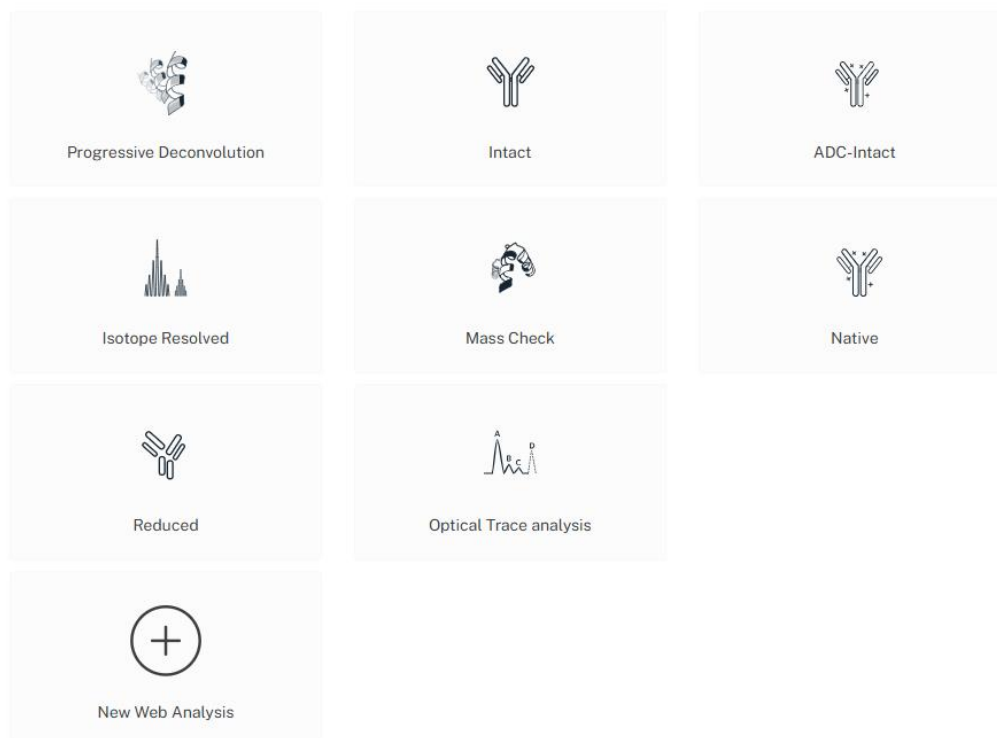


Figure 3: Project Templates

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.

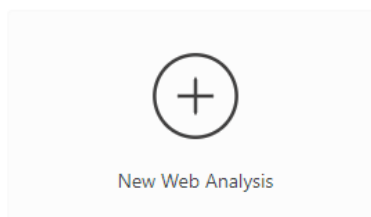


Figure 4: Create a new Web Analysis with no preconfigured settings

Samples Room

Add Samples

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, check the sample(s) of interest, and then click **Add Sample**.

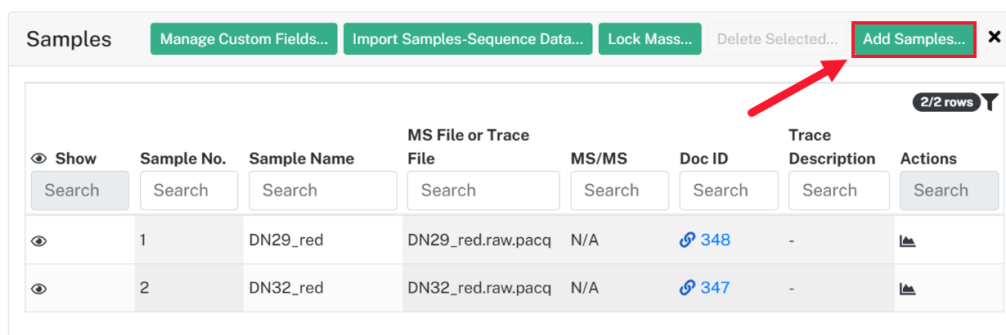


Figure 5: Add Samples in Samples Room

Select sample to add

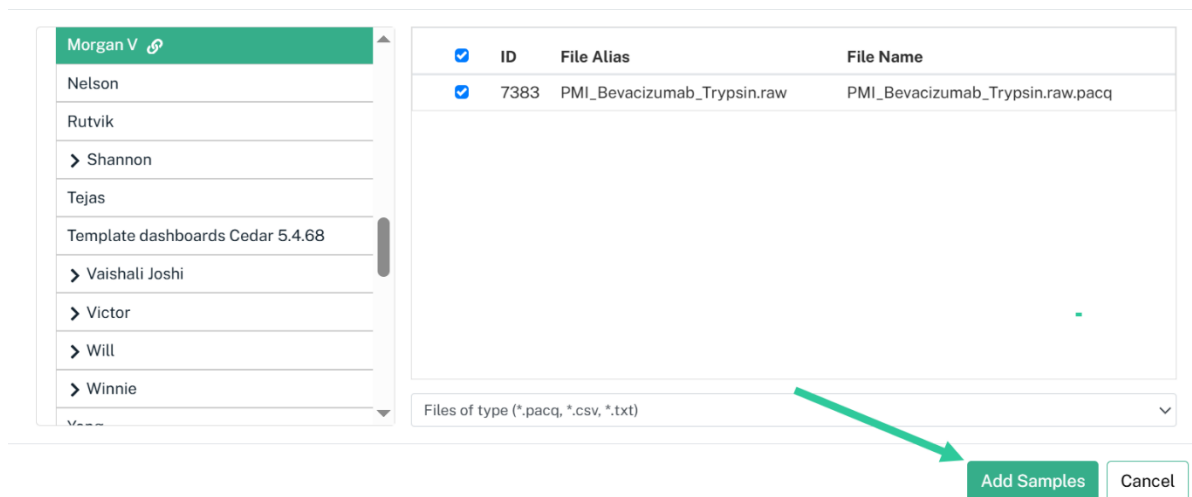




Figure 6: Add Samples Dialog

The **MS File or Trace File** and the **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.

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.

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**

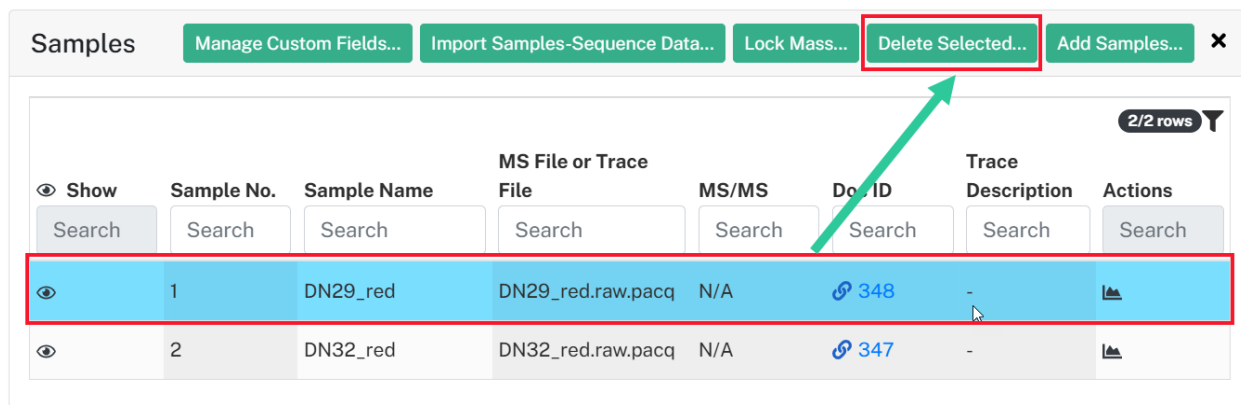



Figure 7: A selected Sample can be deleted by clicking "Delete Selected"

The user can double click a sample name within the row to rename the sample. The name will be immediately saved once the user clicks out of the sample box.

Adding a *.pacq file will create a corresponding sample with the same name (without the *.pacq extension). The user can add multiple *.pacq files at once to the Samples table.

Add Trace Files to Samples

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.

Add trace files to sample

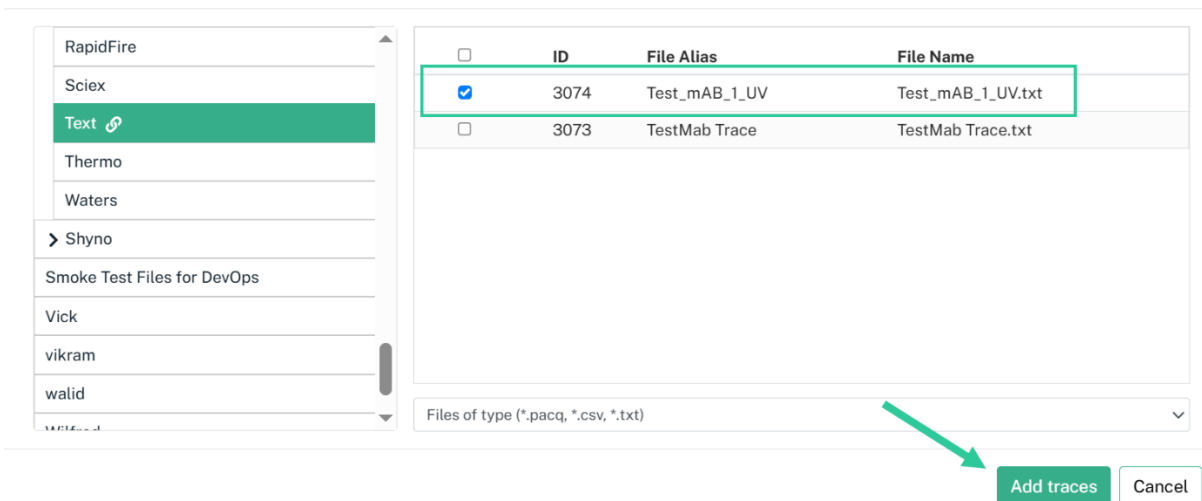
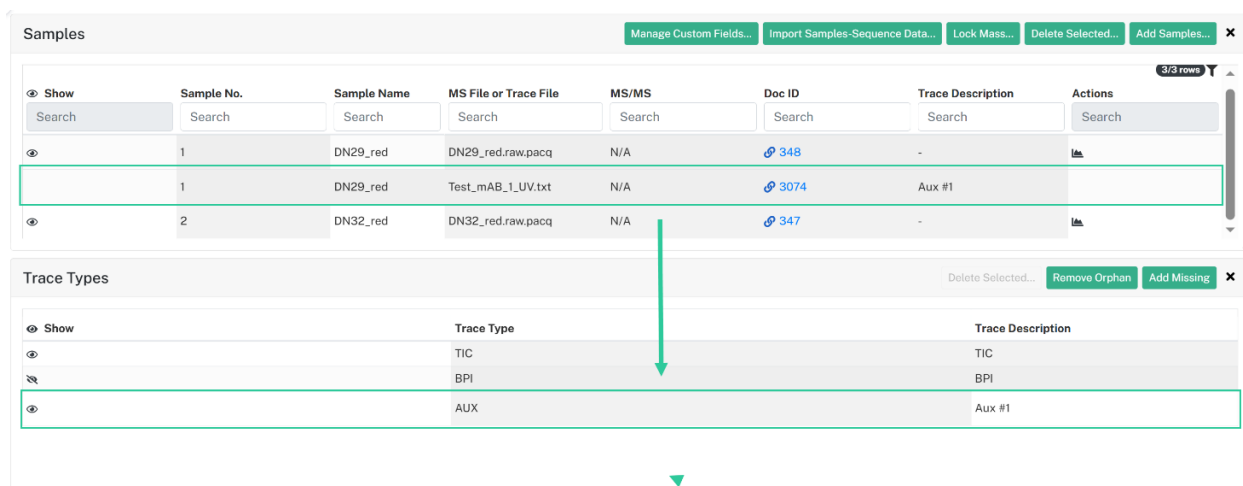


Figure 8: Adding Trace Files



Samples

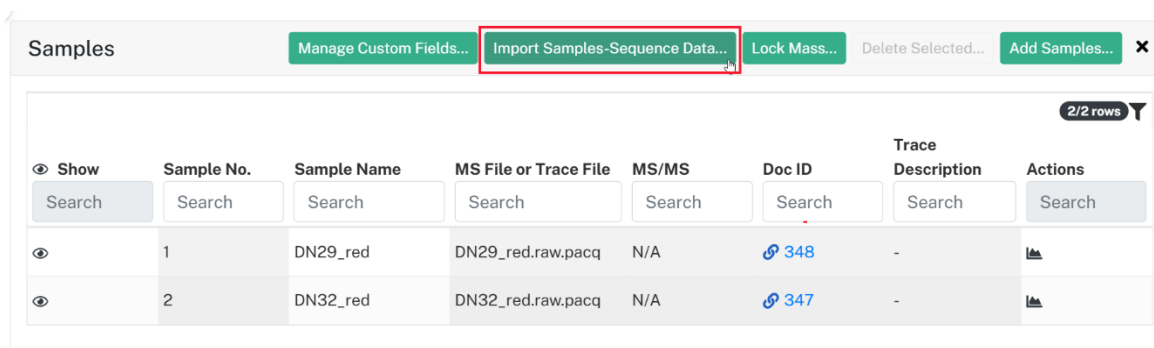
Show	Sample No.	Sample Name	MS File or Trace File	MS/MS	Doc ID	Trace Description	Actions
<input type="checkbox"/>	1	DN29_red	DN29_red.raw.pacq	N/A	348	-	
<input type="checkbox"/>	1	DN29_red	Test_mAB_1_UV.txt	N/A	3074	Aux #1	
<input type="checkbox"/>	2	DN32_red	DN32_red.raw.pacq	N/A	347	-	

Trace Types

Show	Trace Type	Trace Description
<input type="checkbox"/>	TIC	TIC
<input type="checkbox"/>	BPI	BPI
<input type="checkbox"/>	AUX	Aux #1

Figure 9: Custom trace added to Sample Name DN29_red

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.



Samples

Show	Sample No.	Sample Name	MS File or Trace File	MS/MS	Doc ID	Trace Description	Actions
<input type="checkbox"/>	1	DN29_red	DN29_red.raw.pacq	N/A	348	-	
<input type="checkbox"/>	2	DN32_red	DN32_red.raw.pacq	N/A	347	-	

Figure 10: Import Samples-Sequence Data

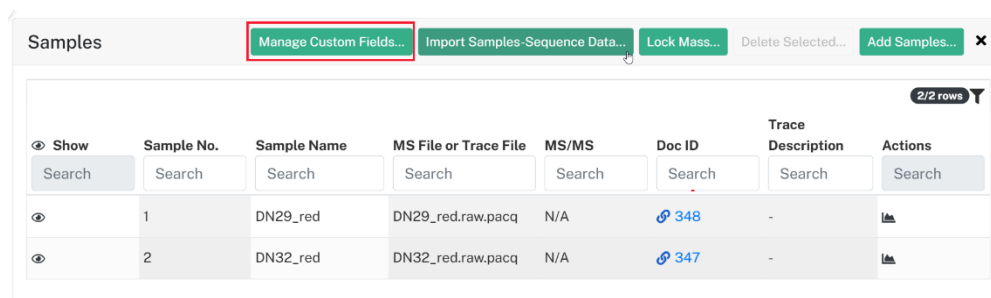
Users must use the following format for their CSV files to be imported successfully into WA:

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.



Sample No.	Sample Name	MS File or Trace File	MS/MS	Doc ID	Trace Description	Actions
1	DN29_red	DN29_red.raw.pacq	N/A	348	-	
2	DN32_red	DN32_red.raw.pacq	N/A	347	-	

Figure 11: 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

Import Current System Metadata Fields

<input type="checkbox"/>	Name	DataType
<input type="checkbox"/>	Assay	string
<input type="checkbox"/>	Clone	string
<input type="checkbox"/>	Digest	string
<input type="checkbox"/>	Injection	string
<input type="checkbox"/>	Instrument	string
<input type="checkbox"/>	Molecule	string
<input type="checkbox"/>	Plate Position	string
<input type="checkbox"/>	Preparation	string
<input type="checkbox"/>	Process	string

Figure 12: Metadata fields

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.

▼ Manual custom fields (defined for current analysis)

Name	DataType	Actions
Test field	string	
Enter Custom Name	Select Data Type	

Figure 13: Manual fields

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			<input type="button" value="Delete Selected..."/> <input type="button" value="Remove Orphan"/> <input type="button" value="Add Missing"/> <input type="button" value="X"/>
Show	Trace Type	Trace Description	
	TIC	TIC	
	BPI	BPI	

Figure 14: 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.

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 15: 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:

Reset
Compute
Close

Figure 16: 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:

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

Update Cancel

Figure 17: 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: 0 To: 10

Baseline Type: Auto Baseline Smoothing Width: 1.5


Automatic Manual Whole Trace Regular Intervals

Peak picking source: Smoothing width: Minimum width:

TIC 0.1 0.2

Reset Compute Close

Figure 18: 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:

TIC

TIC

BPI

Figure 19: 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.

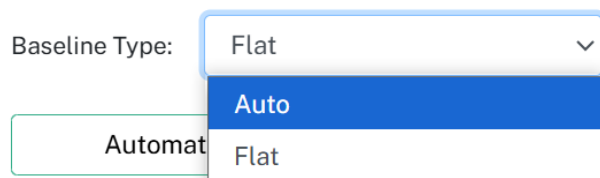


Figure 20: Baseline options

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.

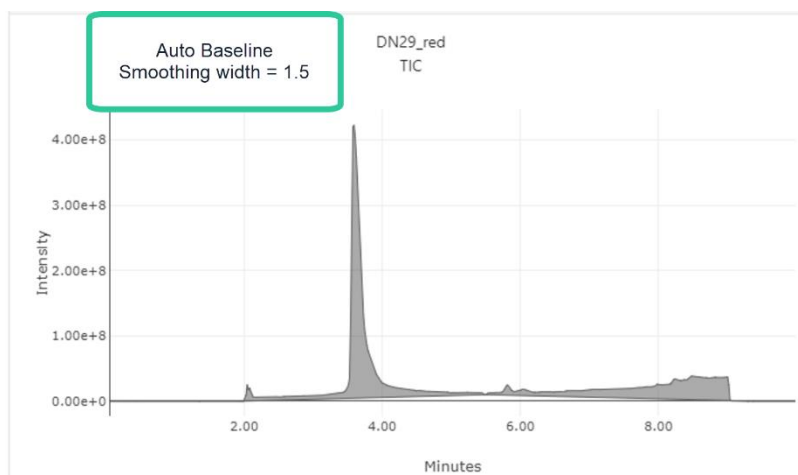


Figure 21: Auto Baseline

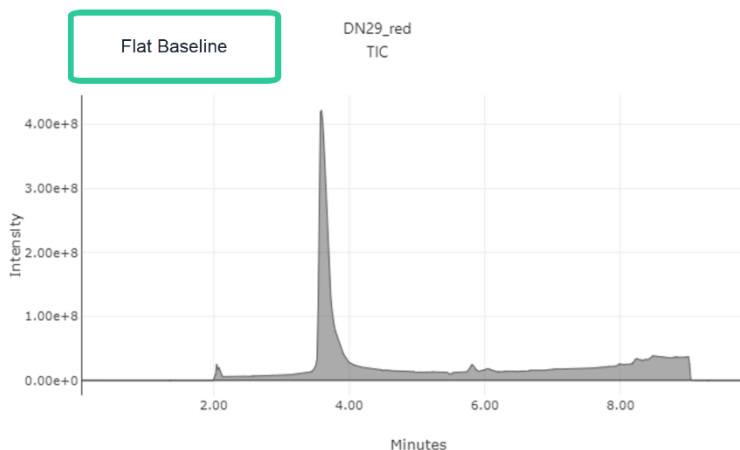


Figure 22: Flat Baseline

Lock Mass

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

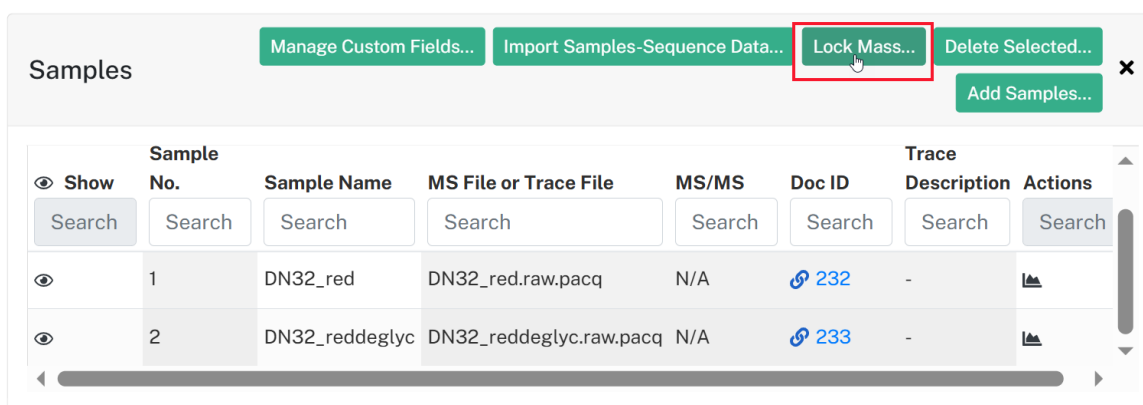


Figure 23: Lock mass dialog from the Samples view

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 24: Lock Mass dialog

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

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.

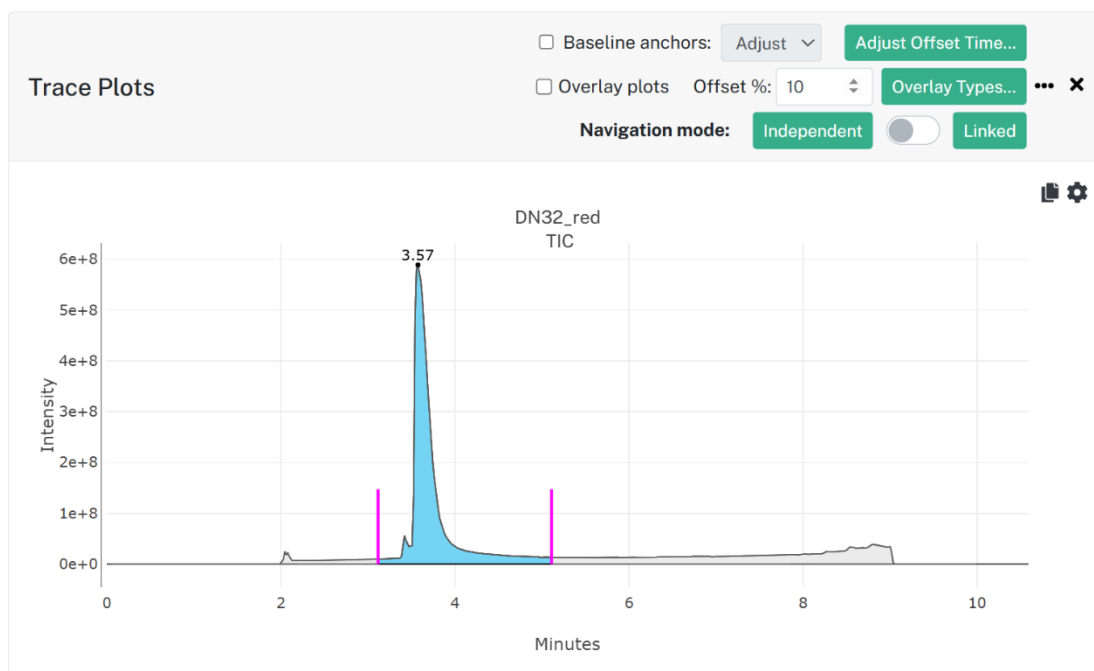


Figure 25: Trace Plot with integration boundaries

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 26: 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.

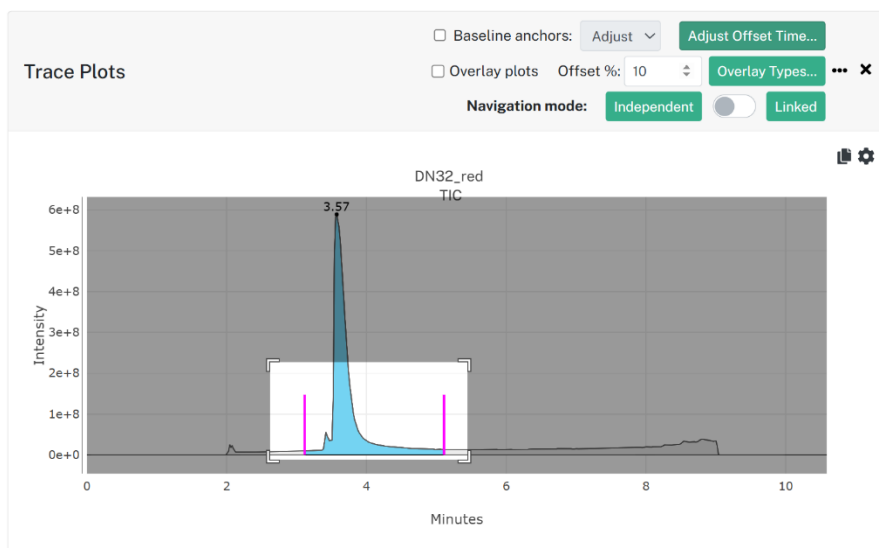


Figure 27: Before zoom

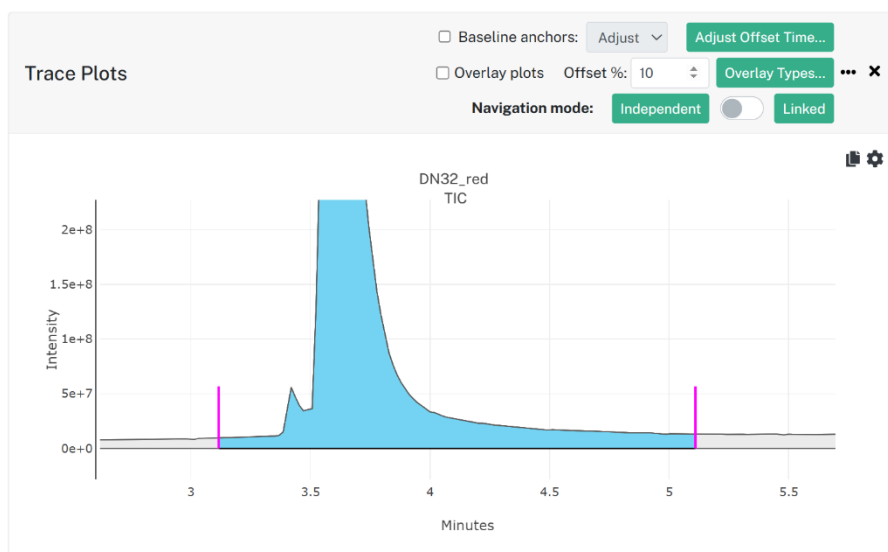



Figure 28: After zoom

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.

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						
Trace Range Rule...		Delete Selected...	Split...	Merge...	Update	Add Peaks...
1/1 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_reddeglyc	1	3.7	4	3.76	3.42e+7	37.64

Figure 29: 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 30: 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 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									
<div>Trace Range Rule... Delete Selected... Split... Merge... Update Add Peaks... X</div>									
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	4.5	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 31: Trace Peaks Table

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

Trace Types									
Delete Selected... Remove Orphan Add Missing X									
Show	Trace Type			Trace Description					
<input checked="" type="checkbox"/>	TIC			TIC					
<input checked="" type="checkbox"/>	BPI			BPI					

Trace Peaks Table									
Trace Range Rule... Delete Selected... Split... Merge... Update Add 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	4.5	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 32: Apex Time and Area for TIC and BPI trace types, when both are set to visible

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 33: 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 34: 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...

Update

Add Peaks...

✕

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) ?
<div>Search</div>	<div>Search</div>	<div>Search</div>	<div>Search</div>	<div>Search</div>	<div>Search</div>	<div>Search</div>	<div>Search</div>	<div>Search</div>	<div>Search</div>
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 35: 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.

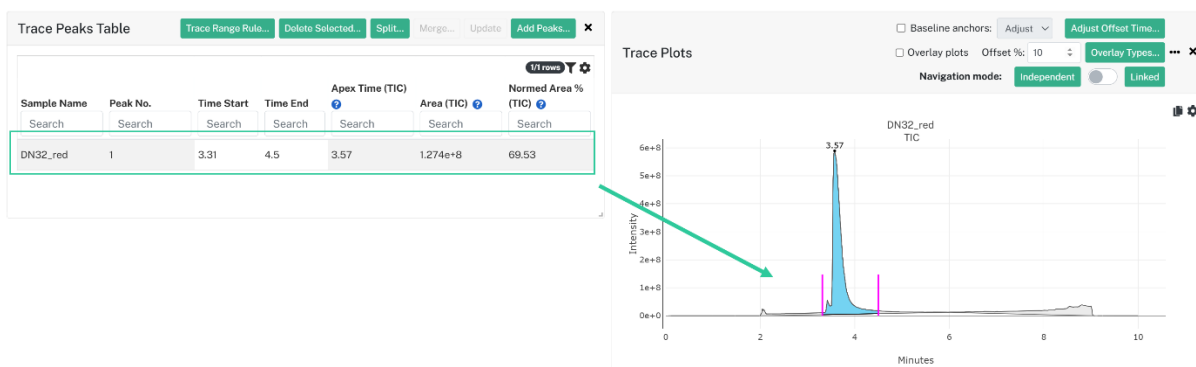


Figure 36: Trace plot integration limits and corresponding Trace Peak

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.

Baseline Anchors

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

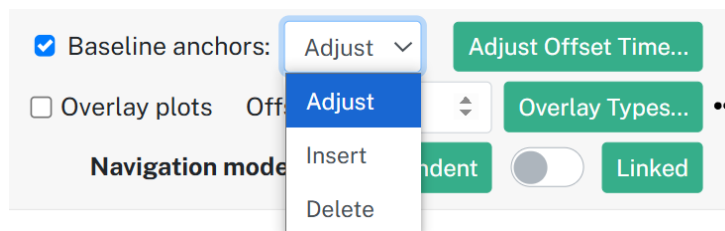


Figure 37: Baseline anchors options

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

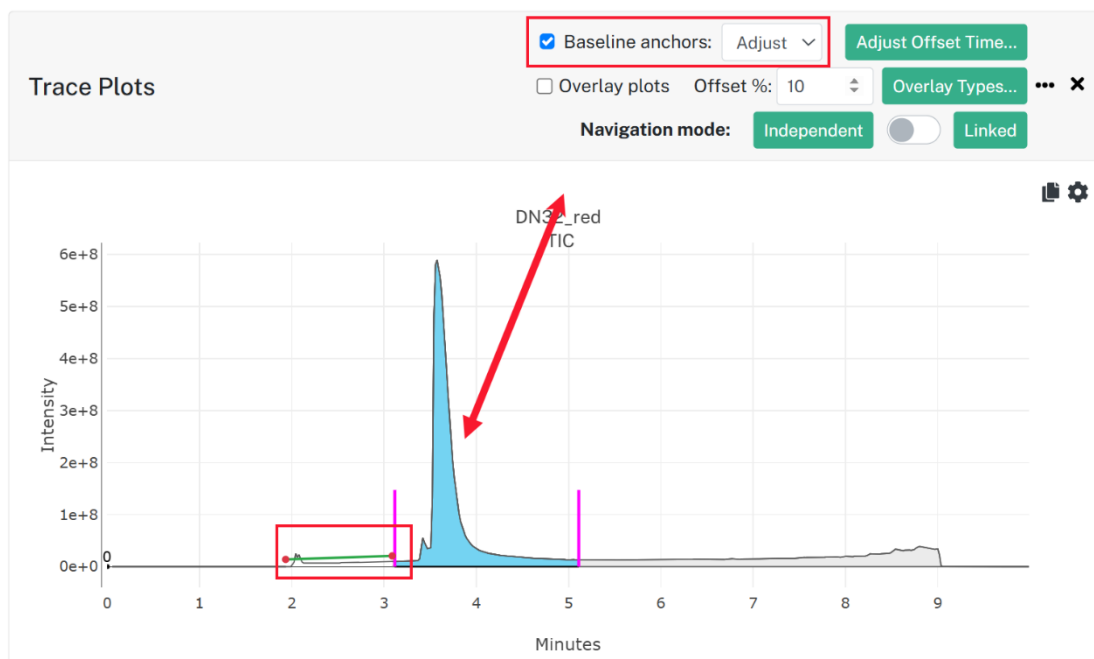


Figure 38: Adjust anchor

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

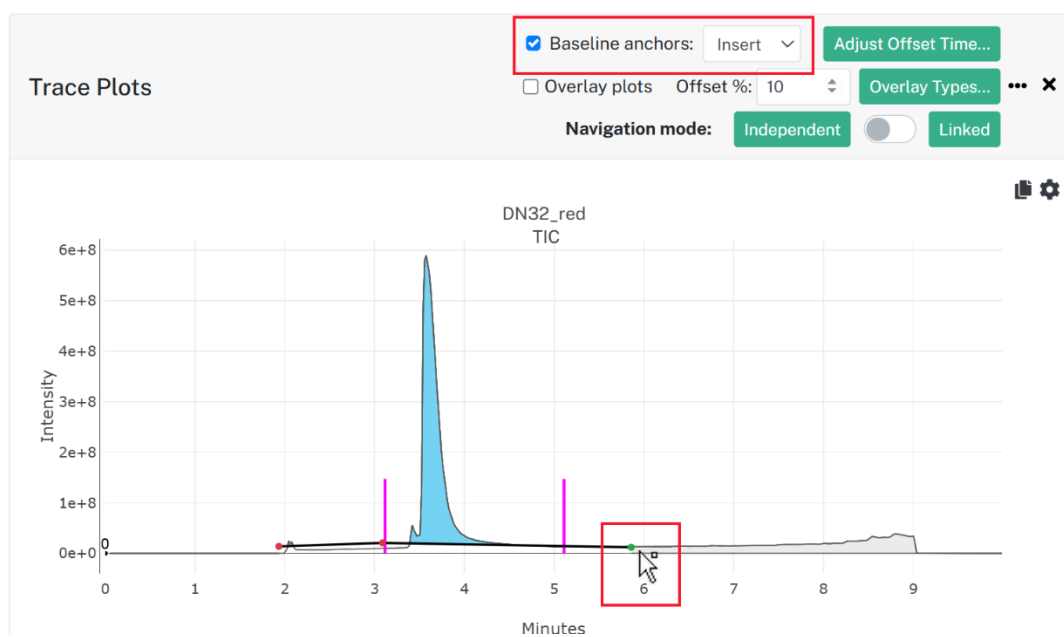


Figure 39: Insert anchor

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.

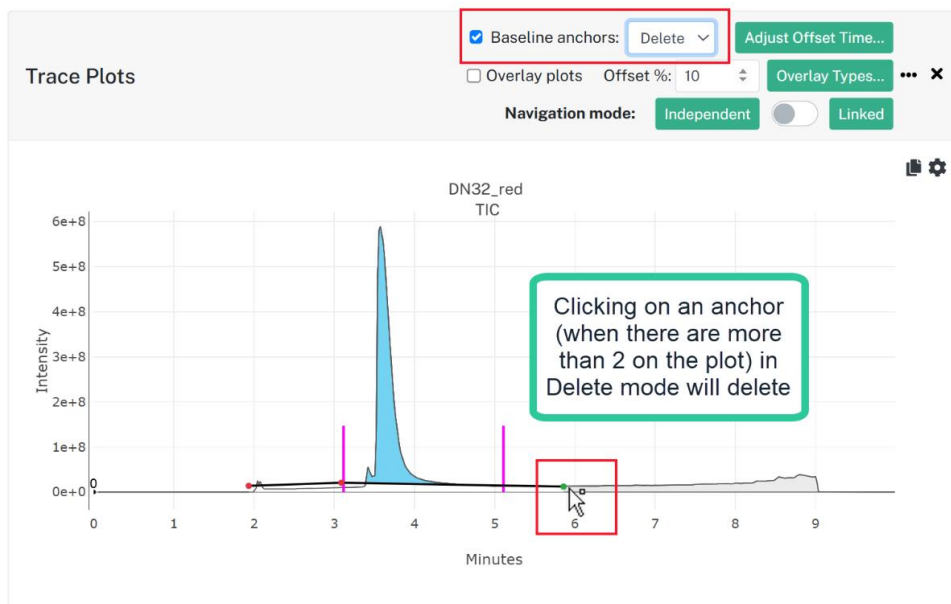


Figure 40: Delete anchor

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.

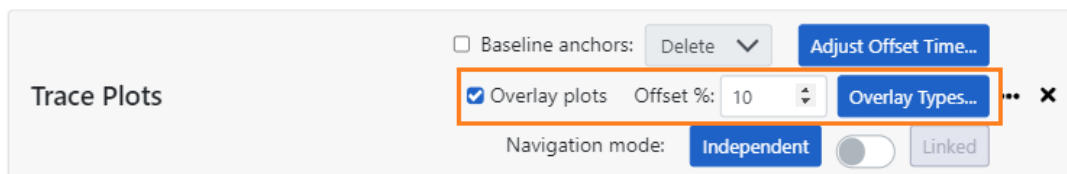


Figure 41: Overlay trace plots

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

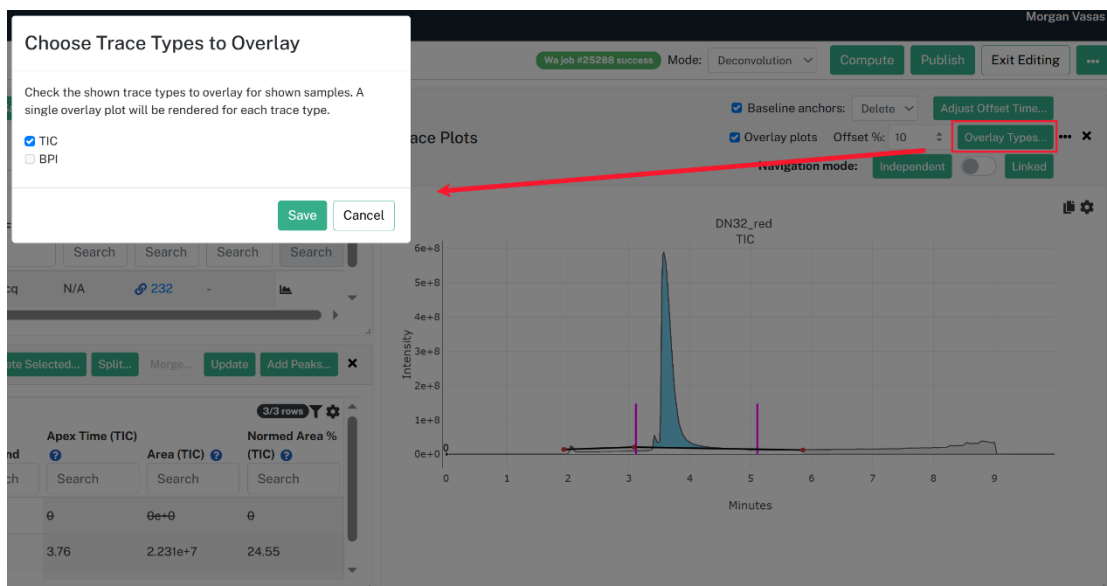


Figure 42: Overlay plots dialog

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.

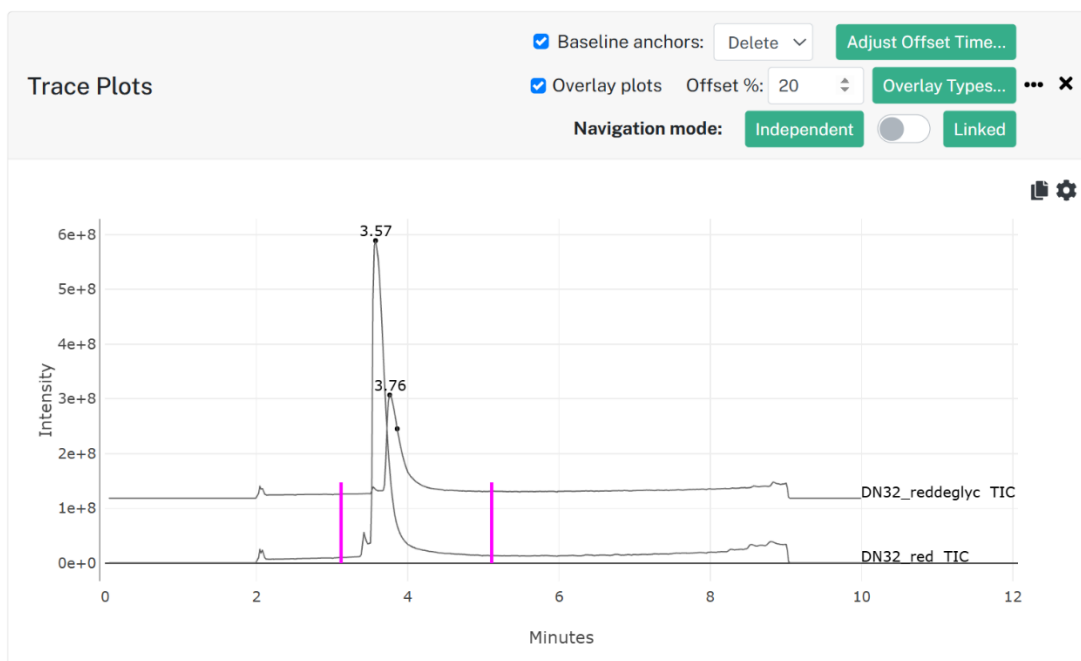


Figure 43: Overlay with 20% offset

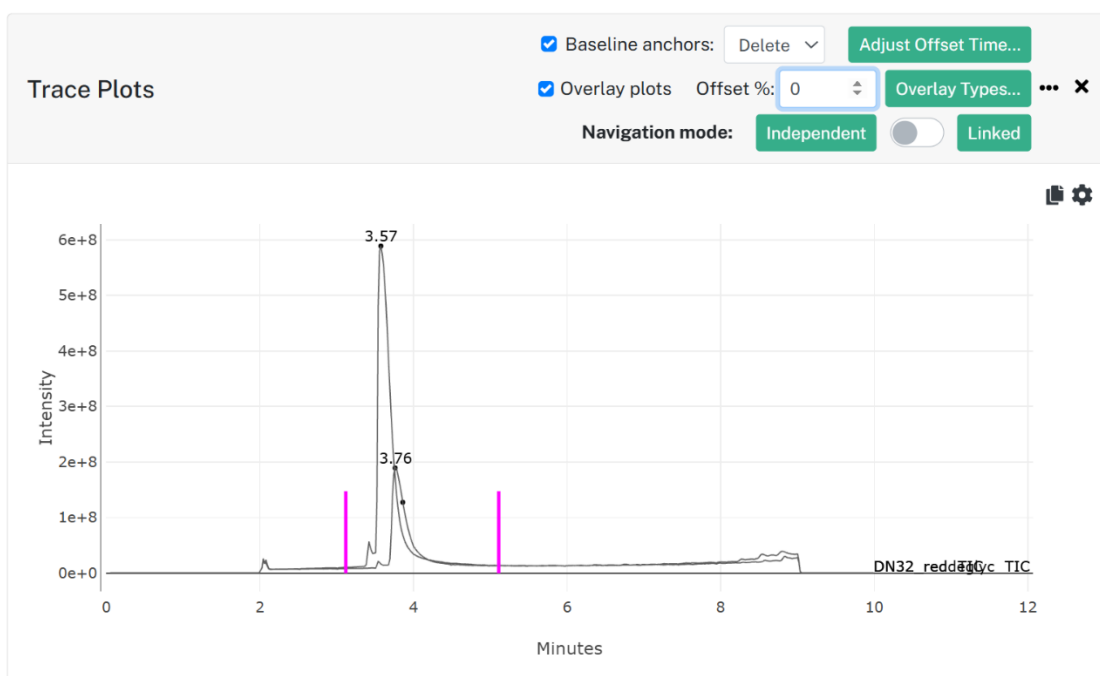


Figure 44: Overlay with 0% offset

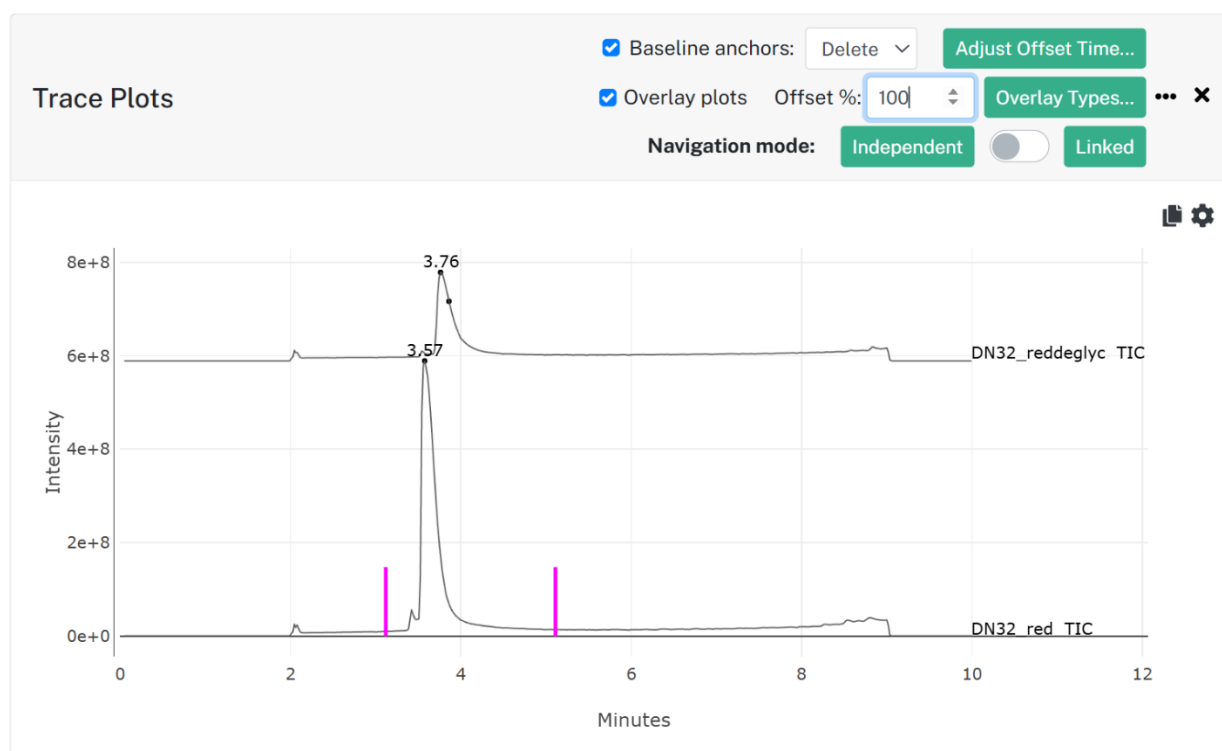


Figure 45: Overlay with 100% offset

Navigation mode

The user can enable linked **Navigation mode** to sync up plot zooming amongst all Trace Plots in the widget. This can be useful in cases where a user might be looking for a specific chromatographic peak in multiple samples.

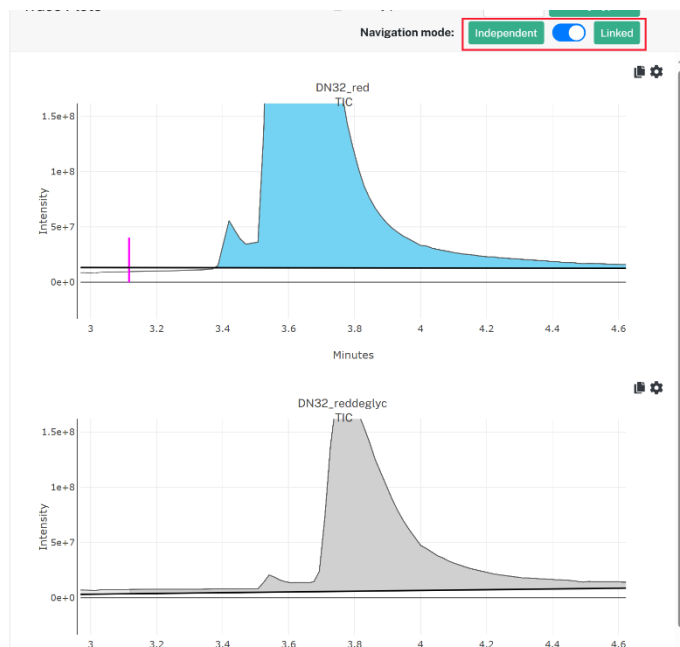


Figure 46: Two traces set to the same zoom level in linked navigation mode

Navigation mode is set to Independent by default, which represents default behavior (only a single plot will have adjusted zoom levels).

Compute

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

Compute (Deconvolution Mode)

Select flow:

- ☒ Extract Traces
- ☐ Define Peaks
- ☐ Extract Peak Area
- ☐ Extract MS1
- ☐ Deconvolute
- ☒ Match Masses

Settings:

Generate Heatmap: ☐ MS1 ☐ Deconvolved Mass [?](#)

Sample		Slices
<input checked="" type="checkbox"/>	Sample No.	Sample Name
<input checked="" type="checkbox"/>	1	DN32_red
<input checked="" type="checkbox"/>	2	DN32_reddeglyc

☐ Clear first [?](#)

Figure 47: 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., 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.

Sample		Slices	
<input checked="" type="checkbox"/>	Sample No.	Sample Name	MS/MS
<input checked="" type="checkbox"/>	1	DN32_red	N/A
<input checked="" type="checkbox"/>	2	DN32_reddeglyc	N/A

Figure 48: Sample tab in Computation

Alternatively, the **Slice** tab allows the user to select specific slices to undergo calculations. To select more than one slice, hold CTRL when checking each row of interest.

Sample		Slices		
<input type="checkbox"/>	Sample No.	Slice No.	Start Time	End Time
<input checked="" type="checkbox"/>	2	1	3.7	3.85
<input type="checkbox"/>	2	2	3.85	4

Figure 49: Slices 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:

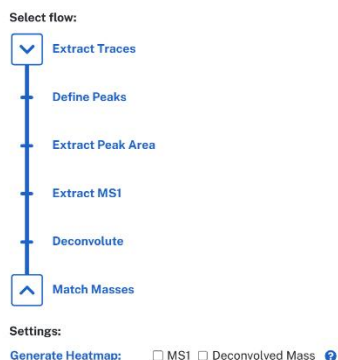


Figure 50: Computation Flow Chart

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

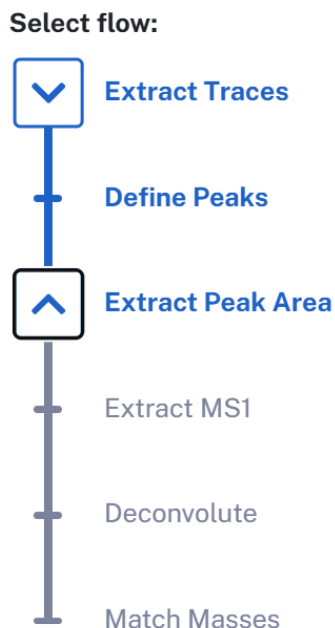


Figure 51: Truncated Flow Chart

The flow shown in the above image shows the steps used in the Sample 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 Masses).


Sequences Room



The **Sequences** room consists of three different molecule workflow options: **Library**, **Chains and Compositions**, and **Sequences and Combinations**. These options enhance the user experience by providing different methods of defining compositions of interest to be used in mass matching downstream. **Users are advised not to switch between molecule workflows once inputs have already been made as progress may be lost.**

Molecule Workflow: Sequences and Combinations



The different sections of the **Sequences and Combinations** workflow are outlined below.

Reduced(edit mode)  No job #1301 success Mode: Deconvolution  Compute Publish Exit Editing 



Molecule Workflow Option: Sequences and Combinations 


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





Code	Name	Sequence	Molecule Class	Average Mass	Monoisotopic Mass
A	DN29 HC	EVQLVESGGGLVQPKGSL...	Protein	49804.0709644	49772.5444916


Combinations 50  Digestion Parameters... Delete Selected... Add Combination... 


Name	Alias	Composition	Disulfides	Average Mass	Monoisotopic Mass	Expected Type	Actions	Sample associations
Reference Mass Modifications <input checked="" type="checkbox"/> Change N-terminal Q to pyroGlu <input checked="" type="checkbox"/> Clip off C-terminal K <input type="checkbox"/> N-glycans removed by PNGase F (N-X-S/T → D)								


Modifications 50  Delete Selected... Add Modification(s)... 

Include	Modification	Formula	Average Mass	Mono Mass	Targets/Residues	Fine Control	Actions
<input checked="" type="checkbox"/>	G0	C(50)H(82)N(4)O(35)	1299.2	1298.476	N	Variable-rare 1	


Total common max: 1   Total rare max: 1  

Deconvolution Plot Zoom Settings 

Mass (Da)	Lower Delta (Da)	Upper Delta (Da)
Choose zoom setting type 	Enter value	Enter value

Deconvolved Mass Matching 

☒ Average ☐ Monoisotopic



Match tolerance: 10  Da


Local base peak window %: 20

Figure 52: Sequences and Combinations molecule workflow

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. Once they are added, the masses are updated automatically.

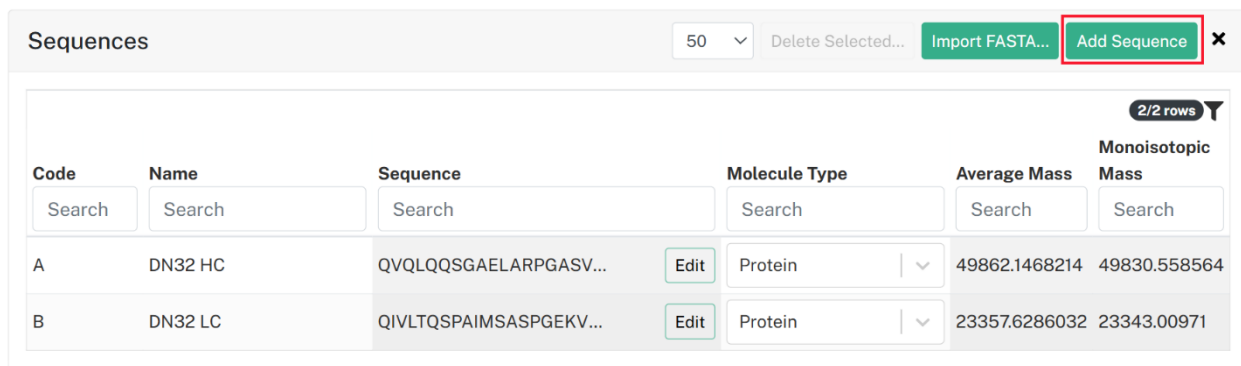
Sequences 50  Delete Selected... Import FASTA... Add Sequence 

2/2 rows 

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

Figure 53: Sequences

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.



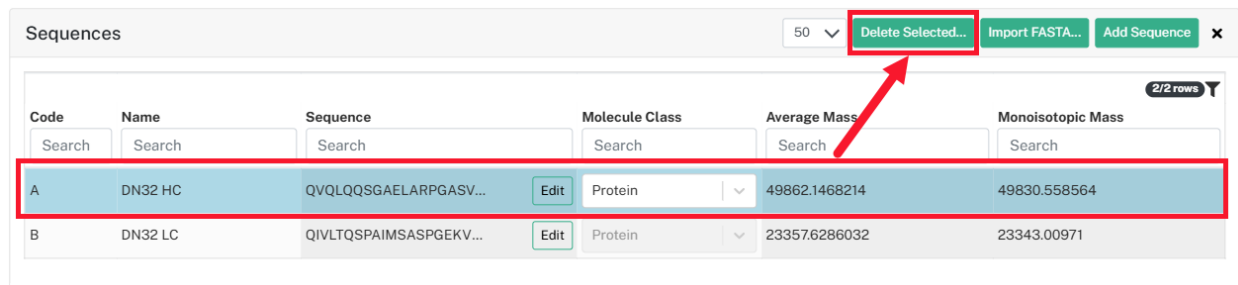
Sequences 50 ▾ Delete Selected... Import FASTA... **Add Sequence** ✕

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	QIVLTQSPAIMASASPGEKV...	Edit	Protein ▾	23357.6286032	23343.00971

Figure 54: 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**



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

2/2 rows

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

Figure 55: Selected row

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

Delete selected row(s)

Are you sure you want to delete the selected row(s)?

Delete Cancel

Figure 56: 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).

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:

Figure 57: 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 58: Construct new IgG Antibody

IgG Antibody Composition

Light Chain 1:

Light Chain 2:

Heavy Chain 1:

Heavy Chain 2:

Figure 59: 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).

Composition molecule:

Disulfides:

Expected Type:

Figure 60: 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.

Disulfides:

Figure 61: 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.

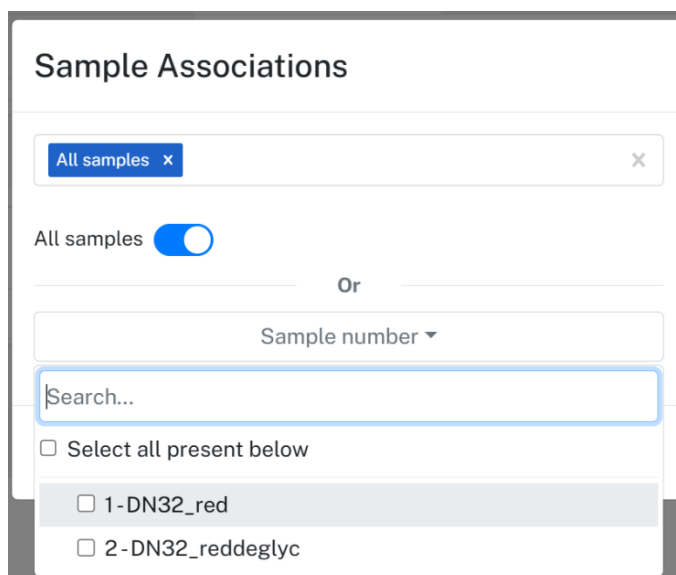
Combinations 50 Digestion Parameters... Delete Selected... Add Combination... ×

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		<div>2/2 rows</div> <input type="text" value="Search"/> <div>All samples</div> <div>All samples</div>
HC	-	A(l)	Reduced	49716.94	49685.44	Desired		<div>2/2 rows</div> <input type="text" value="Search"/> <div>All samples</div> <div>All samples</div>

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)

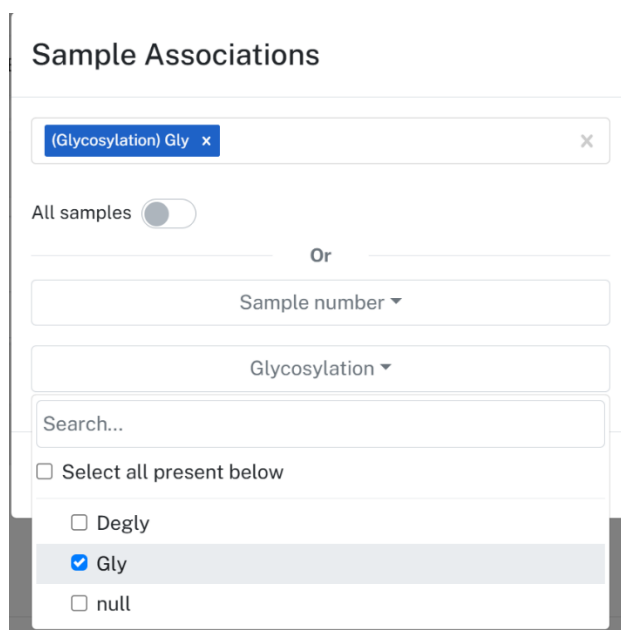
Figure 62: Sample associations column



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 63: 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 dropdowns. Underneath the search field is a checkbox labeled "Select all present below". A list of values for the "Glycosylation" field is shown below the checkbox, with the value "Gly" highlighted in light blue and marked with a blue checkmark. The other values in the list are "Degly" and "null".

Figure 64: 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.

Combinations								
					50	Digestion Parameters...	Delete Selected...	Add Combination...
Name	Alias	Composition	Disulfides	Average Mass	Monoisotopic Mass	Expected Type	Actions	Sample associations
Search	Search	Search	Search	Search	Search	Search	Search	Search
LC	-	B(1)	Reduced	23340.6	23325.98	Desired		1-DN32_red (Glycosylation) Gly
HC	-	A(1)	Reduced	49716.94	49685.44	Desired		All samples

2/2 rows

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 65: 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

Digestion Parameters

Protease

None

Missed Cleavages:

0

Alkylating Agent:

None

Save

Cancel

Figure 66: 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.

Digestion Parameters

Protease

None

Missed Cleavages:

0

Alkylating Agent:

None

None

Iodoacetamide

Iodo acetic acid

NEM

Figure 67: 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:

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 68: Mass Computation Options

Molecule Workflow: Library

Library

In the **Library** table users can provide a FASTA database reference. All sequences in the FASTA file are selected by default.

Clicking **Add Library** will launch the file navigator dialog where the user can point to a FASTA file. Each uploaded FASTA reference will have its own row within the Library table where Sample associations can be provided.

Library				
		50	Delete Selected...	Add Library x
1/1 rows				
Molecule Class	Name	Doc ID	Sample associations	Preview Contents
Search	Search	Search	Search	Search
Protein	DN29-32.fasta	2357	All samples	

Figure 69: Library table

Within the Library table is the **Preview Contents** column. Clicking on the eyeglass within this column per file will open the Preview Contents dialog which displays all sequences included within the FASTA file. This dialog is read-only and cannot be edited.

Preview Contents

Name	Sequence
DN29 HC	EVQLVESGGGLVQPKGSLKLSAASGFTFTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQLLYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGLTLTVSAAKTTTPSVYPLAPGSAATNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTPVPSSTWPSETVTCNVAHPASSTKVDDKIVPRDCGCKPCICTVPEVSSVFIFFPKPKDVLITLTPKVTCTVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPTPKQMAKDKVSLTMITNFFPEDITVEWQWNGQPAENYKNTQPIMDGGSYFVYSKLVNQKSNWEAGNTFTCSVLHGLHNHHTTEKSLSHSPGK
DN29 LC	QAVVTQESALTTSPGETVTLTCSRSTGAVTTSNYANWVQKPDHLFTGLIGGNTKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVLGQPKSSPSVTLFPPSSSELETNKATLVCTITDFYPGVTVVDWKVDGTPVTQGMETTPQSKSQNNKYMSSYLTLTARAWERHSSYSQVTHEGHTVEKLSLRADCS
DN32 HC	QVQLQQSGAELARPGASVKMSCKASGYFTTRYTMHWVKRPGQQLLEWIGYINPSRGVYTNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGLTLTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDDKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIDVLMISLSPITCVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMSQGEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVLTCTMVDFTFMDIYVEWNTNNGKTELNYKNTPEVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHGLHNHHTTKFSRTPGK
DN32 LC	QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEADAATYYCQQWSSNPFTFGSGTKLEINRADTAPTVSIFPPSSEQLTSGGASVCFLNFPYKIDINVKWKIDGSRQNGVLNSWTDQSKDSTYSMSSTLTLTKDEYERHNSYTCATHKSTSPIKVKSFRNEC

Ok

Figure 70: Preview Contents dialog

Molecule Workflow: Chains and Compositions

Chains and Compositions

The **Chains and Compositions** table includes the compositions of interest that are used in mass matching downstream.

Chains and compositions											
50					Delete Selected...		Import FASTA...		Add Sequence...		Add Composition...
Molecule Class	Code	Name	Alias	Sequence	Composition	X-Links	Average Mass	Monoisotopic Mass	Expected Type	Sample associations	Actions
Protein	A	DN29 HC	DN29 HC	EVQLVESGGGLVQPKGSL...	Edit	All possible	49663.8	49632.36	Desired	All...	
Protein	B	DN29 LC	DN29 LC	QAVVTQESALTTSPGETV...	Edit	All possible	23078.57	23064.28	Desired	All...	
Protein	C	DN32 HC	DN32 HC	QVQLQQSGAELARPGASV...	Edit	All possible	49704.85	49673.34	Desired	All...	
Protein	D	DN32 LC	DN32 LC	QIVLTQSPAIMSASPGEKV...	Edit	All possible	23336.57	23321.95	Desired	All...	

Reference Mass Modifications

☒ Change N-terminal Q to pyroGlu ☒ Clip off C-terminal K

Figure 71: Chains and Compositions table

To add a composition to the table, click **Add Composition**. This will launch the corresponding dialog:

Add Composition

Name:

Alias:

Composition type:

IgG antibody	Multispecifics	Custom
Light Chain 1:		
Light Chain 2:	Same as Light Chain 1	
Heavy Chain 1:		
Heavy Chain 2:	Same as Heavy Chain 1	

X-Links:

Basic	Advanced
Disulfides:	All possible

Expected type: Desired

Figure 72: Add Composition

There are three composition types the user can choose to build: **IgG antibody**, **Multispecifics**, and **Custom**.

The **IgG antibody** composition tab allows the user to select from a dropdown which sequences correspond with each light chain and heavy chain of the IgG construct.

Composition type:

IgG antibody	Multispecifics	Custom
Light Chain 1:		
Light Chain 2:	Same as Light Chain 1	
Heavy Chain 1:		
Heavy Chain 2:	Same as Heavy Chain 1	

Figure 73: IgG Antibody composition type

The **Multispecifics** tab allows the user to select the permutation count for all included sequences, as well as set a max number of chains per composition.

Composition type:

IgG antibody
Multispecifics
Custom

Set chains permutation count:

Molecule Class	Code	Name	Count
Protein	A	DN29 HC	0
Protein	B	DN29 LC	0
Protein	C	DN32 HC	0

Max chains per composition: 1

Figure 74: Multispecifics composition type

After the user sets the rules in the table and clicks **Add**, all possible chain permutations based upon the counts selected will be added to the Chains and Compositions table, from one chain to the number defined in the Count column chains up to the Max chains per composition number of chains in a single composition. All entries created via permutation will have the same Expected Type as specified in the dialog.

The **Custom** tab allows the user to provide a simple count of each sequence as well as its type.

Composition type:

IgG antibody
Multispecifics
Custom

<input type="checkbox"/>	Code	Name	Count	Type
<input checked="" type="checkbox"/>	A	DN29 HC	1	-
<input type="checkbox"/>	B	DN29 LC	1	-
<input type="checkbox"/>	C	DN32 HC	1	-

light_chain
heavy_chain

Figure 75: Custom composition type

The **X-links** section is available for all composition types, with the options of Non-Reduced, Reduced, and Disulfide Count (numerical user input).

X-Links:

Basic
Advanced

Disulfides: All possible

Figure 76: Crosslinks

The **Advanced** X-links tab allows users to define a fine grain setting of x-links between any two chains. It contains the columns for specifying the 'from' chain selection and corresponding Chain ID and 'from' residue number, and similar columns for 'to' chain. The user also specifies the Bond type to distinguish between peptide and disulfide bonds.

Composition type:

IgG antibody	Multispecifics	Custom
Light Chain 1:	B - DN29 LC	▼
Light Chain 2:	B - DN29 LC	▼
Heavy Chain 1:	C - DN32 HC	▼
Heavy Chain 2:	C - DN32 HC	▼

X-Links:

Basic						Advanced
						Delete selected
From Chain	From Chain Id	From residue	To Chain	To Chain Id	To residue	Bond Type
B ▼	2	1	C ▼	3	1	Peptide ▼
▼			▼			Disulfide
						Peptide

Figure 77: Advanced crosslinks tab

Note that the **Chain Id** equates to each row in the Composition type table in sequential order. In the example above, the crosslink is defined as going from residue 1 of Chain B of Chain Id 2, which is Light Chain 2 in the table above, to residue 1 of Chain C of Chain Id 3, which is Heavy Chain 1 in the table above.

The **Expected Type** dropdown is also available for all composition types with the options of Desired and Undesired.

Expected type:

Desired ▼

Desired

Undesired

Figure 78: Expected Type dropdown

Shared sections in the sequences room

The following sections are present in all molecule workflows within the Sequences room.

Modifications

Users can add Modifications in the **Modifications** widget. If the user builds their analysis from a System Template, it will be prepopulated with corresponding modifications commonly associated with the type of analysis the template is used for.

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="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input checked="" type="checkbox"/>	G0	C(50)H(82)N(4)O(35)	1299.2	1298.476	N	Variable-rare 1	
<input checked="" type="checkbox"/>	G0F	C(56)H(92)N(4)O(39)	1445.34	1444.5339	N	Variable-rare 1	
<input checked="" type="checkbox"/>	G1F	C(62)H(102)N(4)O(44)	1607.48	1606.5867	N	Variable-rare 1	
<input checked="" type="checkbox"/>	G2F	C(68)H(112)N(4)O(49)	1769.62	1768.6395	N	Variable-rare 1	
<input checked="" type="checkbox"/>	G0F+Lys	C(62)H(104)N(6)O(40)	1573.51	1572.6288	N	Variable-rare 1	
<input checked="" type="checkbox"/>	G0F-GlcNAc	C(48)H(79)N(3)O(34)	1242.15	1241.4545	N	Variable-rare 1	
<input checked="" type="checkbox"/>	Hex	H(10)C(6)O(5)	162.14	162.0528	N	Variable-rare 1	
<input checked="" type="checkbox"/>	Man5	C(46)H(76)N(2)O(35)	1217.09	1216.4229	N	Variable-rare 1	
<input checked="" type="checkbox"/>	Man5F	C(52)H(86)N(2)O(39)	1363.24	1362.4808	N	Variable-rare 1	
<input checked="" type="checkbox"/>	G1F+NeuAc	C(73)H(119)N(5)O(52)	1898.74	1897.6821	N	Variable-rare 1	
<input checked="" type="checkbox"/>	+Lys	C(6)H(12)N(2)O(1)	128.17	128.095	Protein C-term	Variable-rare 1	

Total common max: Total rare max:

Figure 79: 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:

Add Modification(s) Delete Selected...

Modification	Formula	Average Mass	Mono Mass	Targets/Residues	Fine Control
Choose modification				Select...	Select Fine Contr

Add Cancel

Figure 80: 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.

Add Modification(s) Delete Selected...

Modification	Formula	Average Mass	Mono Mass	Targets/Residues	Fine Control
G0F+Lys	C(62)H(104)N(6)O(40)	1573.51	1572.6288	N	Variable-rare 1
Choose modification				Select...	Select Fine Contr

Add Cancel

Figure 81: Adding a custom modification "G0F+Lys"

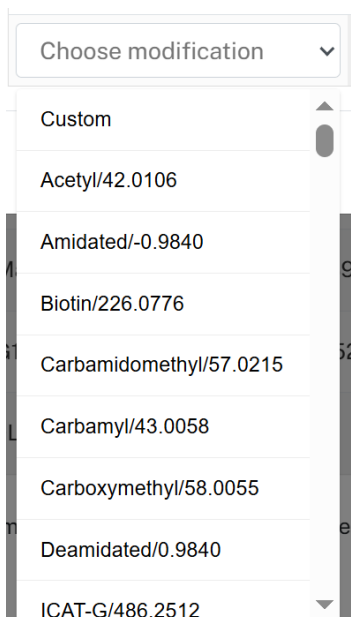


Figure 82: Choose Modification dropdown


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.

Edit Modification

Choose modification:	Hex/162.0528
Formula:	H(10)C(6)O(5)
Average Mass:	162.14
Monoisotopic Mass:	162.0528
Targets/Residues:	N, × ↓
Fine Control:	Variable-rare 1

Save
Cancel

Figure 83: 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.



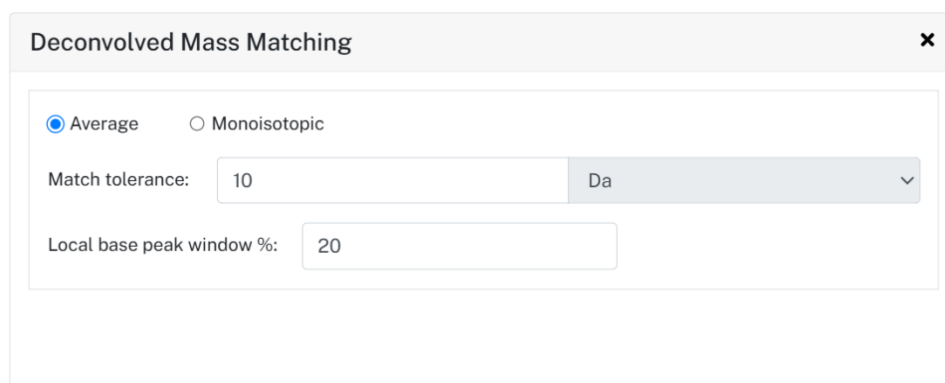
Modifications								Delete Selected... Add Modification(s)...
								10/10 rows
<input checked="" type="checkbox"/> Include Search	Modification	Formula	Average Mass	Mono Mass	Targets/Residues	Fine Control	Actions	
<input checked="" type="checkbox"/>	G0	C(50)H(82)N(4)O(35)	1299.2	1298.476	N	Variable-rare 1		
<input checked="" type="checkbox"/>	G0F	C(56)H(92)N(4)O(39)	1445.34	1444.5339	N	Variable-rare 1		

Figure 84: 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.



Deconvolved Mass Matching [X]

☒ Average ☐ Monoisotopic

Match tolerance: Da [v]

Local base peak window %:

Figure 85: Mass Matching view

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

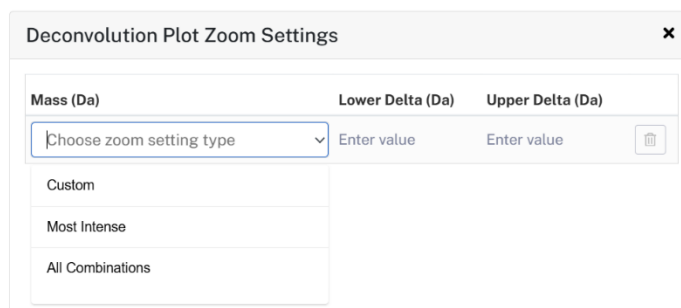
The **Match Tolerance** determines the value +/- a peak must match to a potential mass to be assigned. The default value is 10 Da, but the user has the option to enter in any number, as well as the units (Da or ppm).

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.



Deconvolution Plot Zoom Settings [X]

Mass (Da)	Lower Delta (Da)	Upper Delta (Da)
Choose zoom setting type [v]	Enter value	Enter value [X]
Custom		
Most Intense		
All Combinations		

Figure 86: Deconvolution Zoom Plot Setting widget showing dropdown options

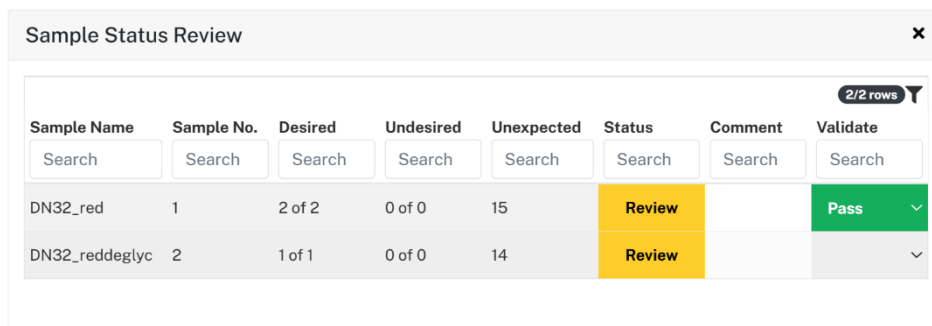
Users can establish zoom criteria based upon the most intense mass, all combinations, or a Custom within a delta range. Users have the option to generate multiple zoomed plot settings. These settings will apply to the plots generated (and shown in the Inspection room) when the user performs computation 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

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

Figure 87: Sample Status Review

The following rules apply to samples to determine their status:

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

Figure 88: Determination of Sample Status

The user can select a Validate option for each sample which provides the opportunity to override the original assignment from the software. The Comment column is user editable by clicking in the cell and allowing a user to enter text or number values. Entry into this column is optional.

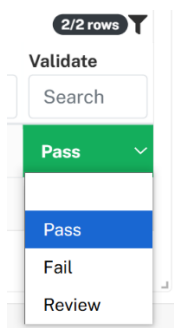


Figure 89: Validate column

Using the Trace Peaks Table

The **Trace Peaks Table** allows the user to adjust peak integration settings and apply **Deconvolution Presets**. The following columns are editable: Time start, Time end, Deconvolution preset, and Peak comment columns.

Trace Peaks Table										Edit Deconv. preset	Delete Selected...	Update	×
Sample No.	Sample Name	Peak No.	Time Start	Time End	Deconvolution Preset	Peak Comment	Apex Time (TIC)	Area (TIC)	Normed Area % (TIC)	2/2 rows			
Search	Search	Search	Search	Search	Search	Search	Search	Search	Search				
1	DN32_red	1	3.12	5.11	<Redu...		3.57	1.197e+8	106.15				
2	DN32_reddeglyc	1	3.12	5.11	<Redu...		3.76	4.826e+7	53.12				

Figure 90: Trace Peaks Table

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

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**.

Trace Peaks Table

☐ Progressive deconvolution

☒ Trace peak deconvolution

Edit Deconv. preset

Delete Selected...

Update

Sample No.	Sample Name	Peak No.	Time Start	Time End	Deconvolution Preset	Peak Comment	From Polygons	Apex Time (TIC)	Area (TIC)	Normed Area % (TIC)
Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search
1	DN32_red	1	0.05	0.49	<Re... <div></div>		-	0.06	1.505e+2	0
1	DN32 red	2	0.49	0.94	<Re... <div></div>		-	0.55	2.1e+3	0

Figure 91: 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:

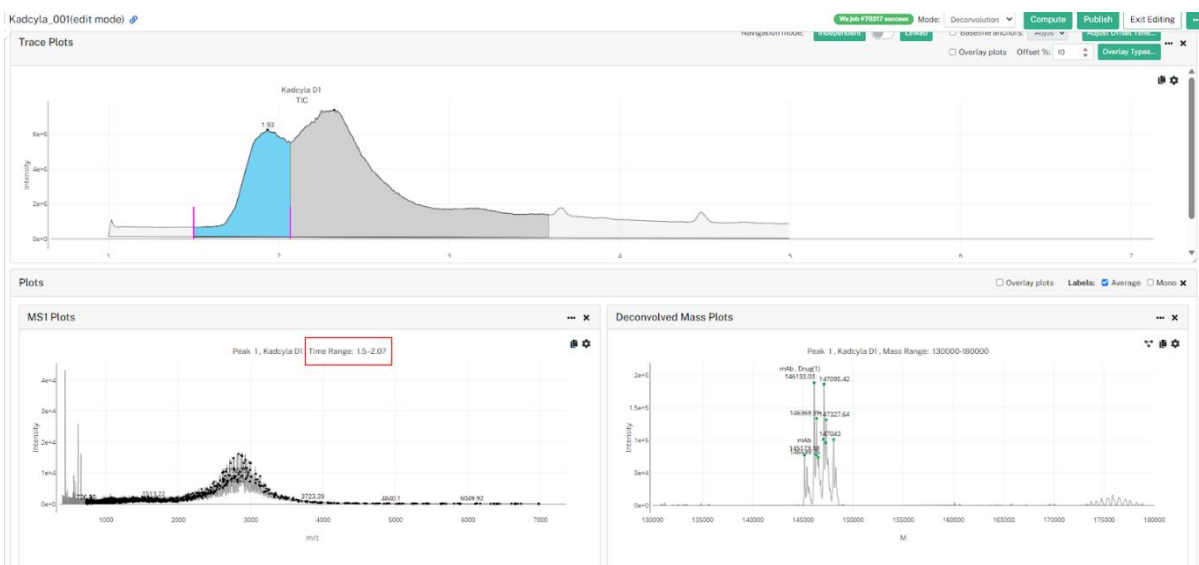


Figure 92: Example of Trace Peak Deconvolution

Progressive Deconvolution mode utilizes the **sliding window deconvolution** approach, which creates narrow sliding overlapping time windows and performs deconvolution iteratively on sequential time ranges. Mass XICs (Extracted Ion Chromatograms) may also be obtained, including the AUC (Area Under the Curve) for each of the Mass XICs and may be subsequently used for quantitation, rather than using deconvolved mass intensity.

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 be run 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.

A more detailed overview of Progressive Deconvolution can be found in the **Byosphere Web Analysis Manual**.

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. These presets can be edited by clicking **Edit deconv. Preset**.

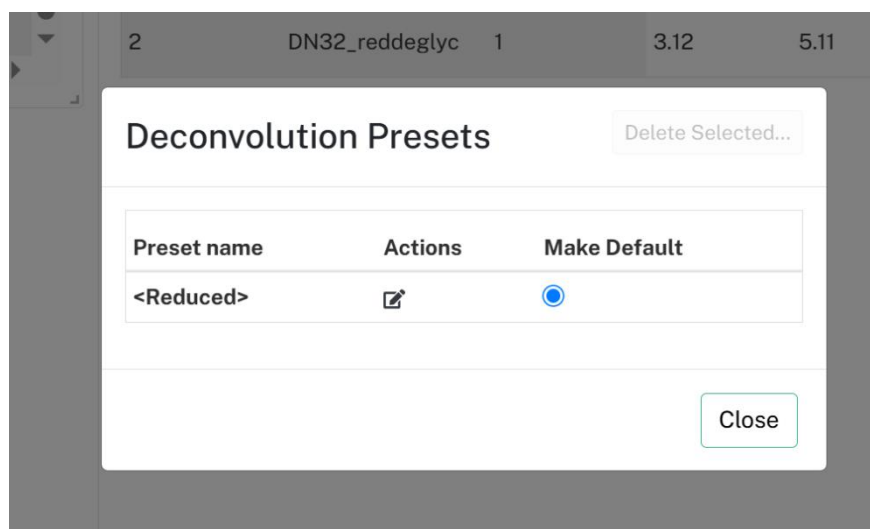


Figure 93: Edit Deconvolution Presets

Presets can be edited or deleted, except for the Default presets (or template presets) which cannot be overwritten or deleted (however, the user can use the Default preset as a base and Save as a new preset). Different presets can be applied to individual separation peaks within a single trace. To create a new preset, click **Save as**, which will prompt the user to name the new preset.

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

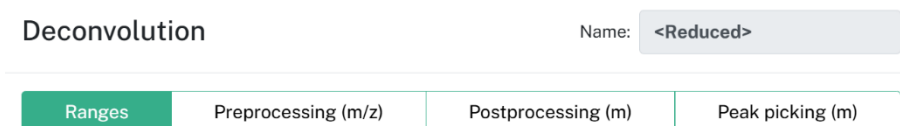


Figure 94: Deconvolution tabs

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.

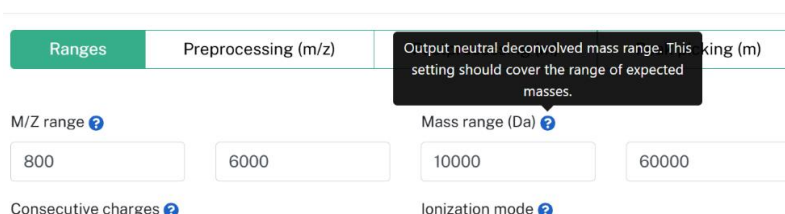


Figure 95: Deconvolution Tooltip

More information about Deconvolution Presets can be found in the **Byosphere Web Analysis Manual**.

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.

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 Mass F	Delta Mass F
Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search
<input checked="" type="checkbox"/>	1	DN32_red	1	-	50952.35	2391254.1	HC, G0F-GlcNAc		-6.74	50926.89	-	-	HC	G0F-GlcNAc	50959.09	-365.46	-	-
<input checked="" type="checkbox"/>	1	DN32_red	1	-	50790.59	648508.6	50791		-	-	-	-	-	-	-	-527.22	-	-
<input checked="" type="checkbox"/>	1	DN32_red	1	-	50748.92	364837.6	50749		-	-	-	-	-	-	-	-568.89	-	-
<input checked="" type="checkbox"/>	1	DN32_red	1	-	23336.75	4914231.8	LC		-3.85	23325.98	-	-	LC	-	23340.6	-27981.06	-	-
<input checked="" type="checkbox"/>	1	DN32_red	1	-	14653.22	1356775.3	14653		-	-	-	-	-	-	-	-36664.59	-	-

Figure 96: Masses table

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>

Trace Plots

There will be one **Trace Plot** per sample.

When the user selects a Trace Peak, the associated integration window will be shown on the Trace Plot.

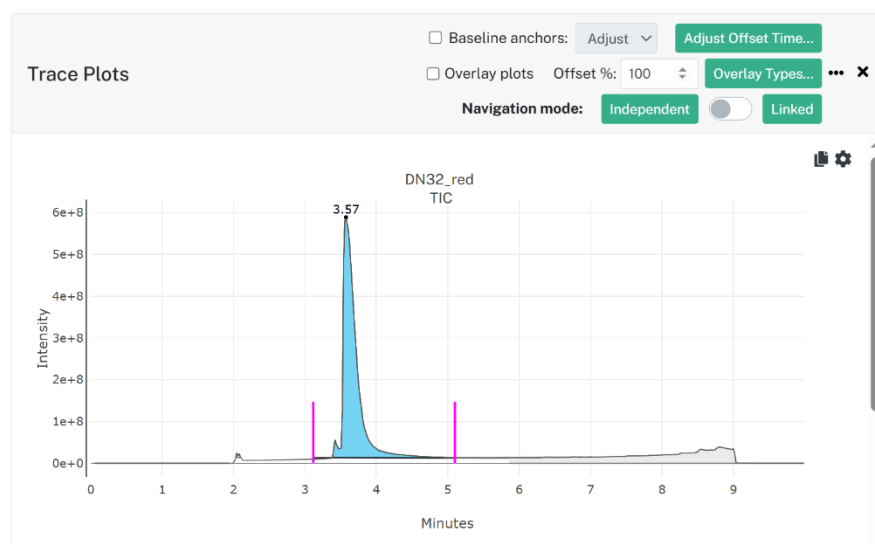


Figure 97: Trace Plot with integration windows for selected time ranges

The Baseline Anchor, Navigation mode, and Overlay plots settings are the same for Trace Plots within both the Samples and 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](#).

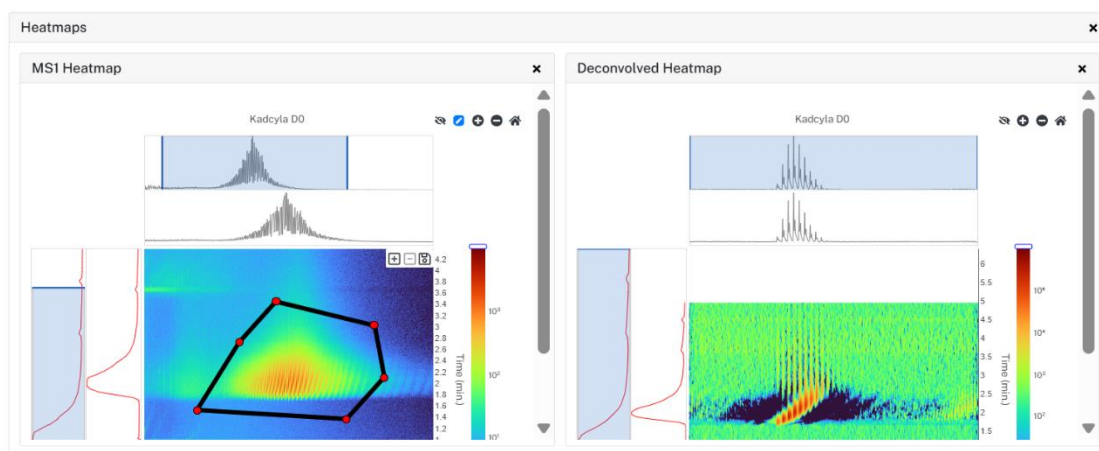


Figure 98: Heatmaps widget

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.

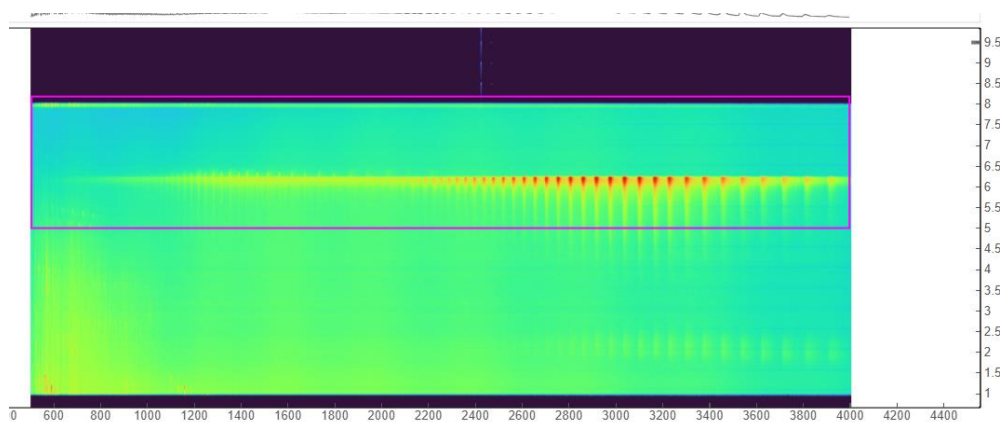


Figure 99: Red box around corresponding selection

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.

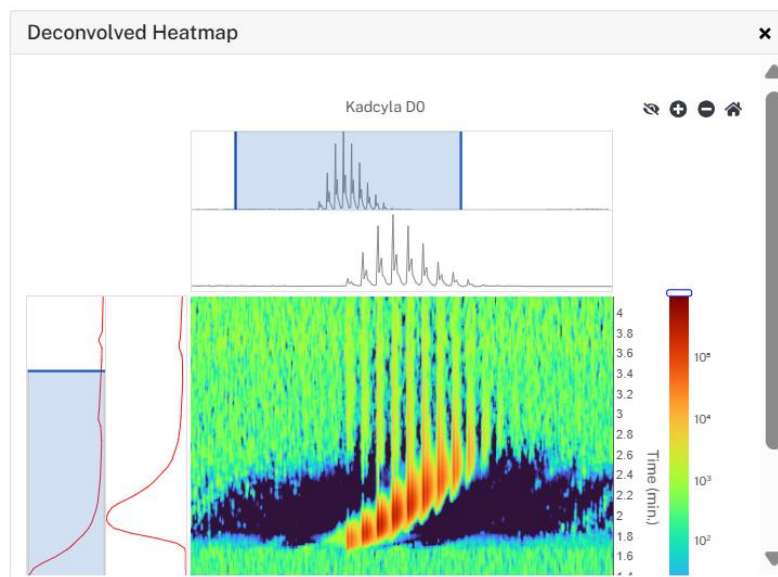


Figure 100: Deconvolved Heatmap from Progressive Deconvolution

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.

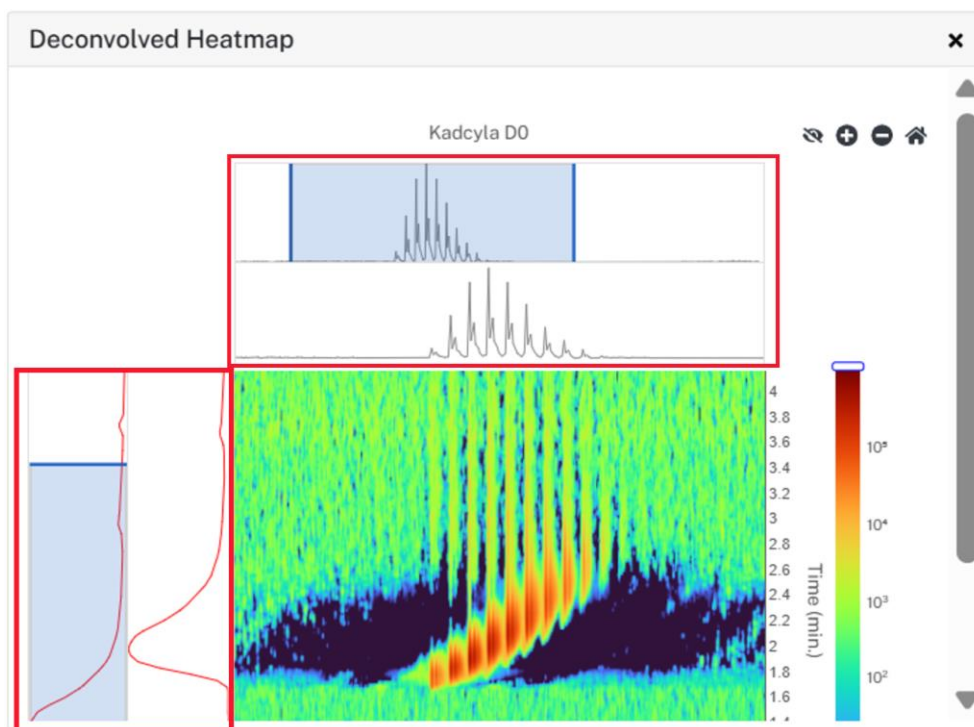


Figure 101: Margin plots

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

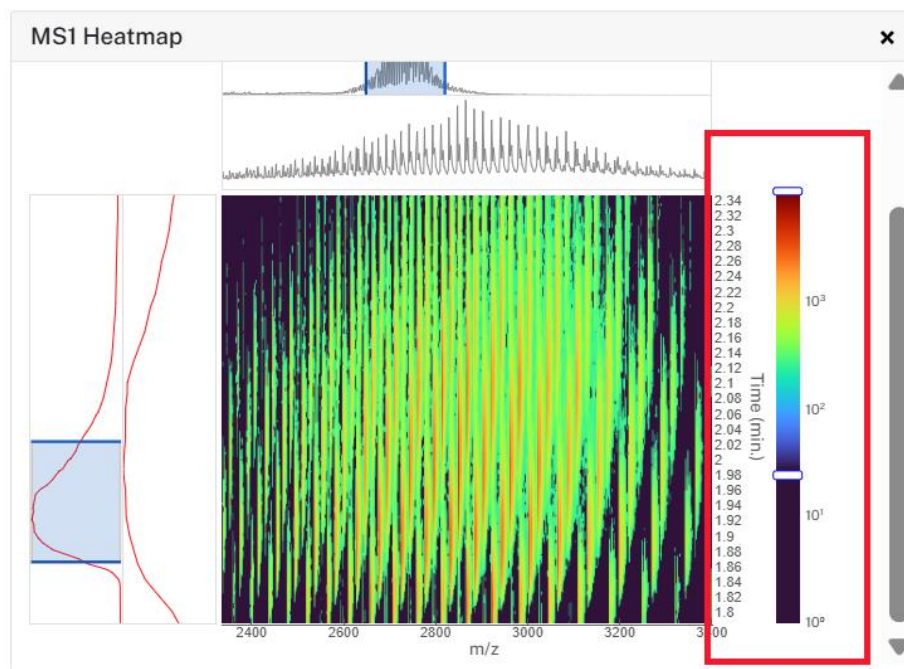


Figure 102: Heatmap color scale

Both margin plots and the color gradient can be shown or hidden by clicking on the eye icon on the heatmap:

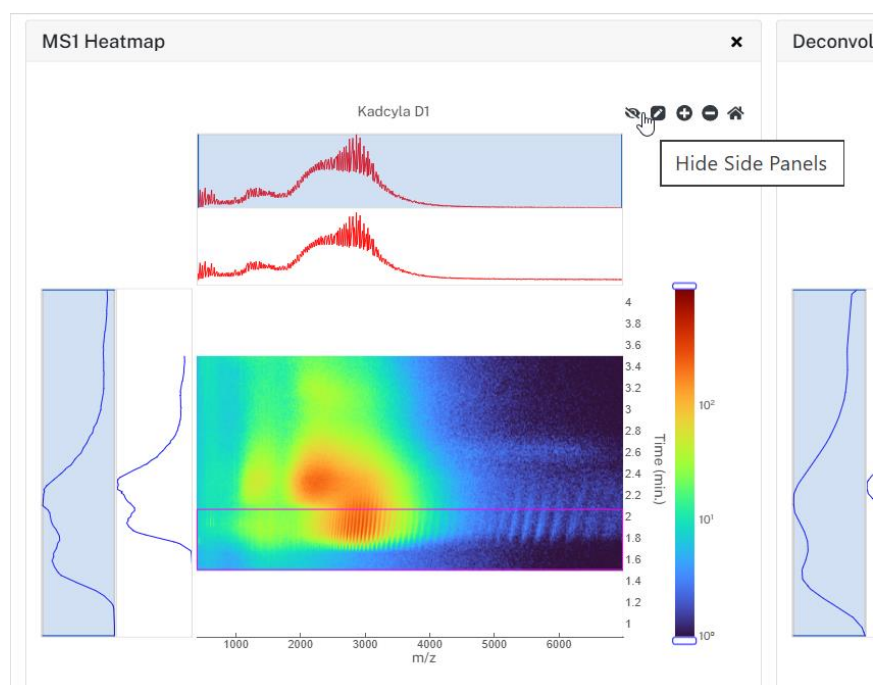



Figure 103: Hide side panels

Clicking the eye icon when crossed out will hide the side panels and clicking the eye icon when fully visible will reveal the side panels.


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.

Polygon creation controls have been removed from the header of the MS1 heatmap widget and are now located with the zoom controls. The controls available for each heatmap are as follows:






 controls side panel (gradient control, margin plot) visibility

 (new) enables polygon creation controls on the heatmap

 zooms heatmap in

 zooms heatmap out

 returns heatmap to home view

When polygon controls  are enabled, the edit icon turns blue . Clicking on the heatmap when polygon controls are enabled and the  icon is selected adds a single red node of a polygon to the heatmap. Clicking again will add another node which connects to the previous node.

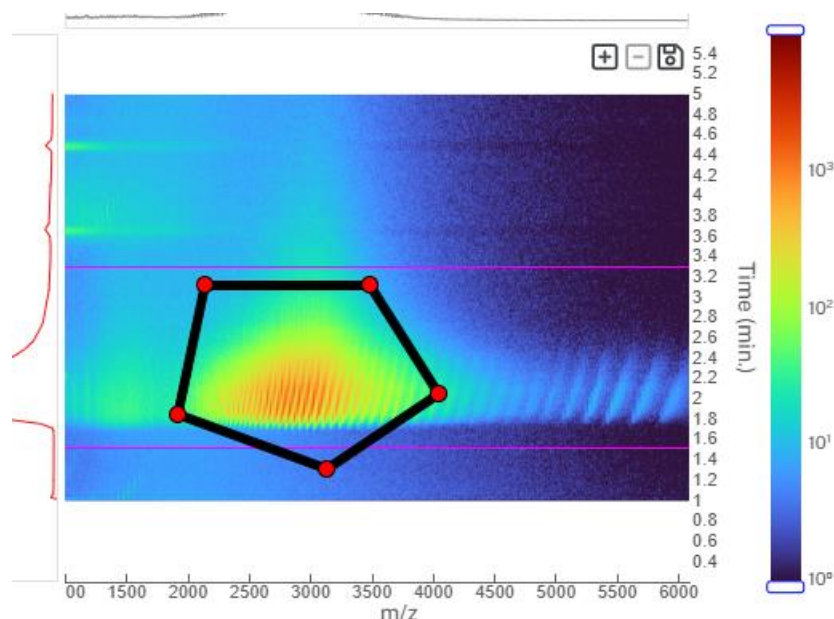




Figure 104: Drawn polygon in edit mode

Hovering over an existing node will illuminate it in magenta. Clicking on the node and dragging will allow the user to move it within the heatmap.



Figure 105: Polygon node in hover state

Clicking the  icon turns on deletion mode. When this icon is selected, clicking on a node will delete it from the heatmap and the polygon shape will adjust accordingly. Deleting all nodes will remove the polygon all together.

Clicking the  icon will save the parameters of the heatmap and add/update the Trace Peaks Table with a trace peak derived from the polygon.

Interactive sliders on the heatmap color scale allow users to control the dynamic range, remove background signal, and focus on areas of interest. The gradient will be applied between the user-set positions of the sliders.

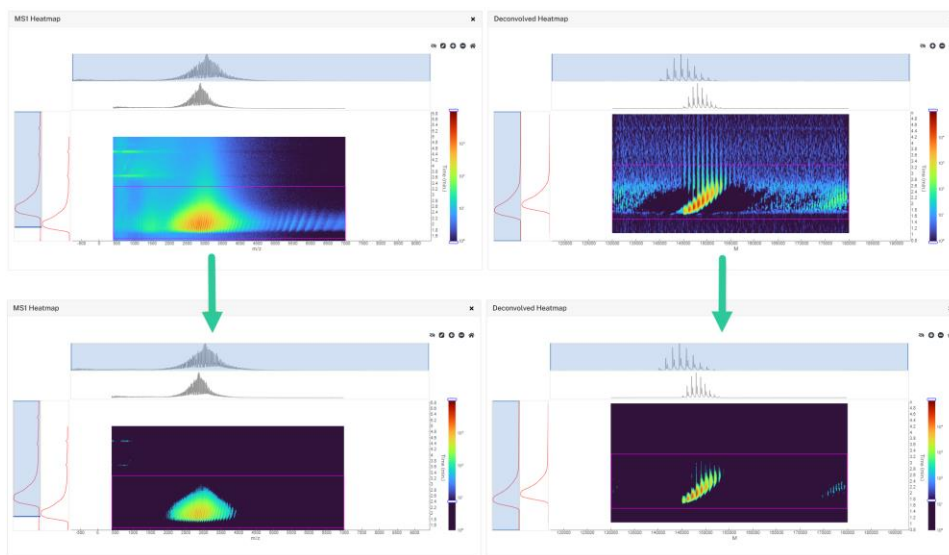


Figure 106: Heatmap gradient controls

Microtiter Plate (MTP)

A **Microtiter Plate** widget is available in Web Analysis in the Inspection room. This widget enables users to visualize their data in customizable plate layouts, with options for selecting associated metadata associated with each plate as well as connection to the [Sample Status Review](#) table.

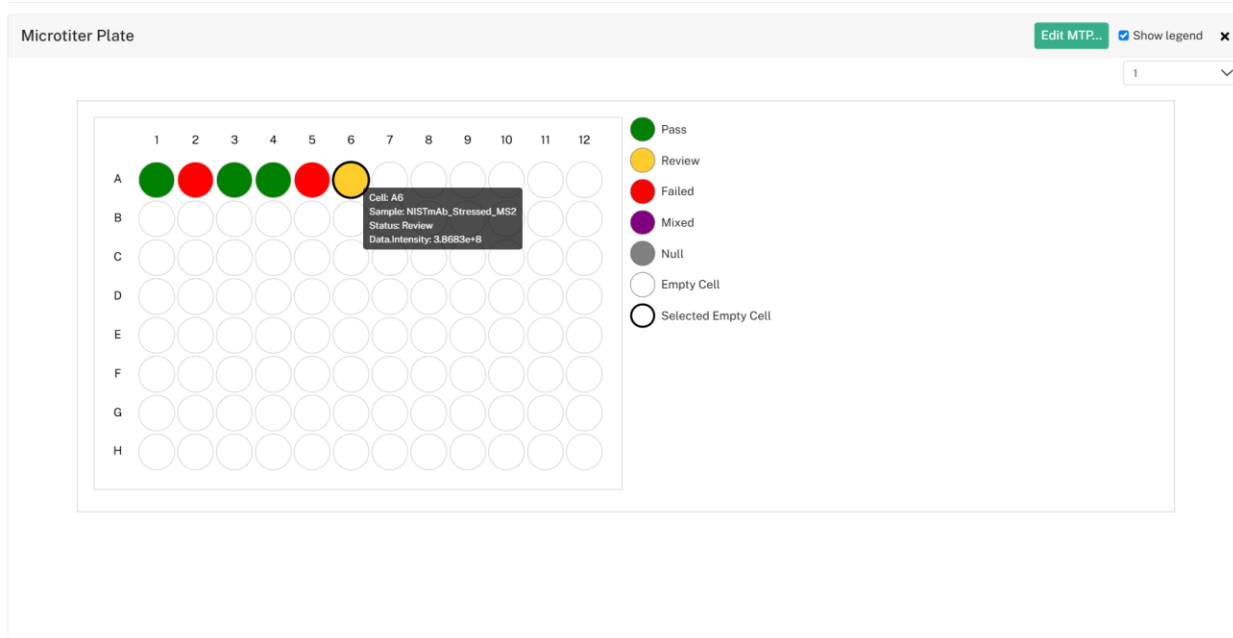


Figure 107: Microtiter Plate widget

If a user specifies a different value in the Validate column of the Sample Status Review table, this color and updated status will be reflected in the MTP widget for the corresponding sample.

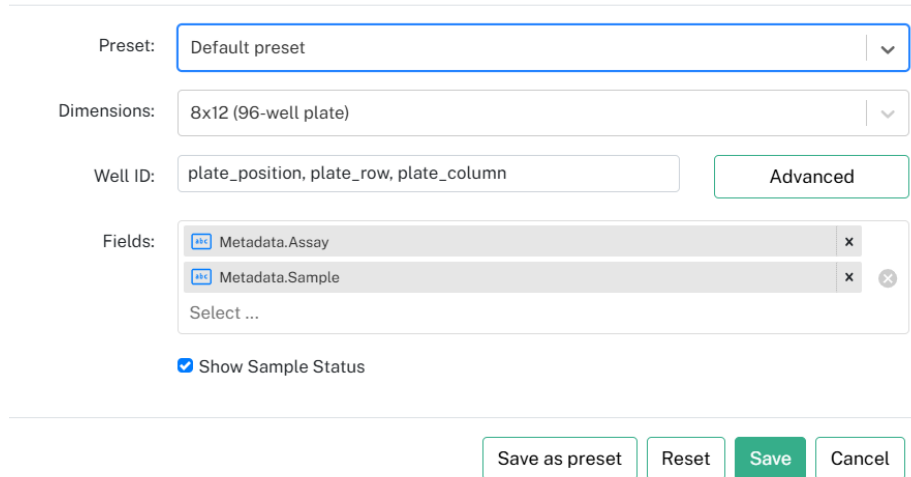
Cell states are represented by the following colors, which correspond with those in the Sample Status Review Table:

- Green for Pass.
- Yellow for Review.
- Red for Failed.
- Purple for Mixed (if a well contains multiple injections with conflicting status values)
- Gray for Null. Null refers to a cell that contains a sample, but the values of interest is neither measurable nor detectable.
- White for Empty. Empty refers to a cell that contains no sample.

Information surrounding each cell can be found in a tooltip available when hovering over the cell. Information includes well identifier (e.g., A1), sample name, and metadata fields defined by the Preset.

Clicking **Edit MTP** opens a dialog which allows the user to select or define a **preset** to use which dictates the information available in the tooltip.

Edit MTP



Preset: Default preset

Dimensions: 8x12 (96-well plate)

Well ID: plate_position, plate_row, plate_column

Fields: Metadata.Assay, Metadata.Sample

☒ Show Sample Status

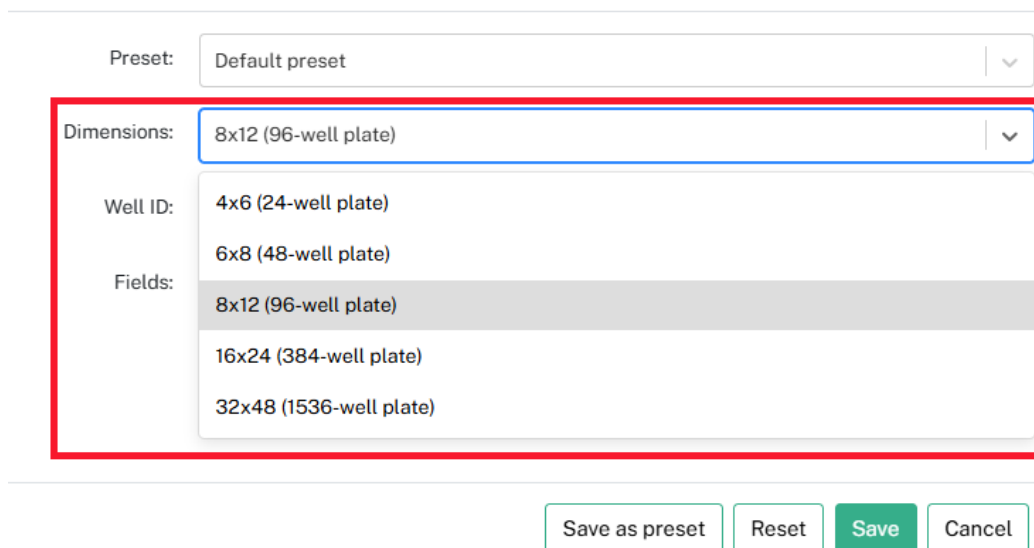
Save as preset Reset Save Cancel

Figure 108: Edit MTP dialog

Two system presets, **Intact** and **Default**, are available for selection in the dropdown. The Default preset is selected by default.

Parameters in the Edit MTP dialog include **Dimensions**, with available options in a dropdown:

Edit MTP



Preset: Default preset

Dimensions: 8x12 (96-well plate)

Well ID: 4x6 (24-well plate), 6x8 (48-well plate), 8x12 (96-well plate), 16x24 (384-well plate), 32x48 (1536-well plate)

Fields:

Save as preset Reset Save Cancel

Figure 109: Edit MTP dimensions

The **Well ID** refers to the metadata fields used to form the association between a sample and its corresponding well in the microtiter plate. These fields are currently **read-only**.

Edit MTP

The 'Edit MTP' form contains the following elements:

- Preset:** A dropdown menu with 'Default preset' selected.
- Dimensions:** A dropdown menu with '8x12 (96-well plate)' selected.
- Well ID:** A text input field containing 'plate_position, plate_row, plate_column'. This field is highlighted with a red rectangular box.
- Advanced:** A green button located to the right of the 'Well ID' field.
- Fields:** A list of selected fields: 'Metadata.Assay' and 'Metadata.Sample'. Each field has a small 'x' icon to its right. Below the list is a 'Select ...' button.
- Show Sample Status:** A checkbox that is checked.
- Buttons:** At the bottom right, there are four buttons: 'Save as preset', 'Reset', 'Save' (in green), and 'Cancel'.

Figure 110: Edit MTP Well ID

The **Fields** specify which fields (Data or Metadata) will be included in the tooltip for each cell associated with a sample.

Edit MTP

This screenshot shows the same 'Edit MTP' form as Figure 110, but with the 'Fields' list highlighted by a red rectangular box. The 'Well ID' field now contains 'plate_position, plate_row, plate_column'. All other elements, including the 'Preset', 'Dimensions', 'Advanced' button, 'Show Sample Status' checkbox, and bottom buttons, remain the same.

Figure 111: Edit MTP fields

For example, if the **Default** preset is selected, the Metadata.Assay and Metadata.Sample information associated with a sample will be viewable in the tooltip for that sample's cell:

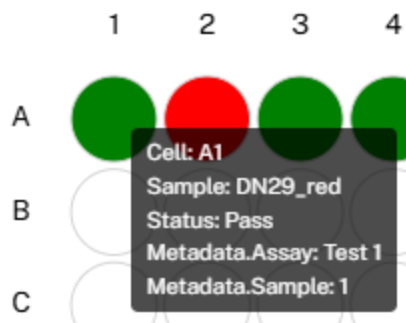


Figure 112: MTP cell tooltip

Users can create their own custom preset by specifying the parameters they wish to save in the Edit MTP box then clicking **Save as Preset** and providing a unique name.

Checking **Show Sample Status** enables the view of the **Sample Status Review** table, which provides corresponding information surrounding a sample's status.

The **Advanced Plate Configuration** is intended to shield end-users from the complexity of parsing plate, row, and column information such as A2, G:A2, G:A,2 (representing optional plate G, row A, column 2).

Advanced Plate Configuration

Sample:
Add new +

Mapping:

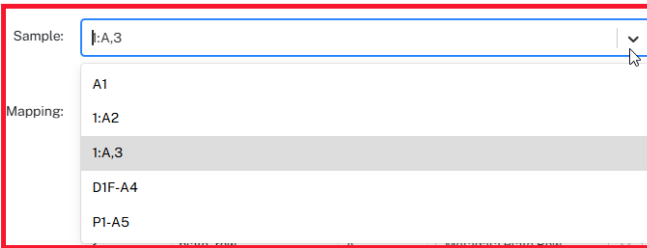
Factor	Group	Value	Metadata Field
1	plate_position		Metadata.Plate Position ▼
2	plate_row	A	Metadata.Plate Row ▼
3	plate_col	1	Metadata.Plate Column ▼

Apply
Cancel

Figure 113: Advanced Plate Configuration

The **Sample dropdown** provides predefined options and can also support user-defined custom samples.

Advanced Plate Configuration



Sample: 1:A,3

Mapping:

A1
1:A2
1:A,3
D1F-A4
P1-A5

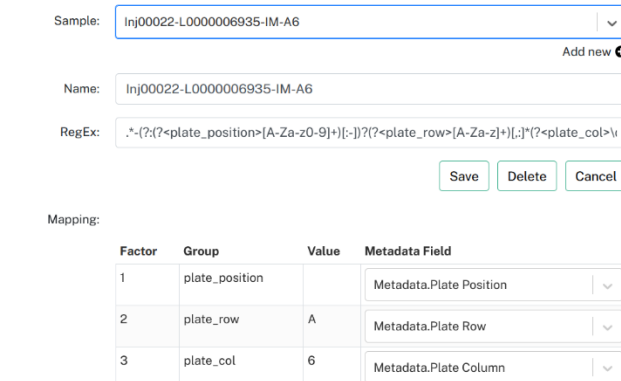
3	plate_col	3	Metadata.Plate Column
---	-----------	---	-----------------------

Apply Cancel

Figure 114: Advanced plate configuration Samples

If the user clicks **Add new**, the **RegEx** box will be populated with a preset value which supports multiple instrument sample vial configurations, but users can define custom RegEx as well.

Advanced Plate Configuration



Sample: Inj00022-L0000006935-IM-A6

Add new

Name: Inj00022-L0000006935-IM-A6

RegEx: .*-(?<plate_position>[A-Za-z0-9]+)[-]?(?<plate_row>[A-Za-z]+)[.]*(?<plate_col>[0-9]+)

Save Delete Cancel

Mapping:

Factor	Group	Value	Metadata Field
1	plate_position		Metadata.Plate Position
2	plate_row	A	Metadata.Plate Row
3	plate_col	6	Metadata.Plate Column

Apply Cancel

Figure 115: Regular Expression box

The **Mapping** table outlines the parsed values based upon the selected Sample or custom RegEx which the user can then provide field associations for under the **Metadata Field**. The screenshot below shows the resultant mapping table if the user selects 1:A,3:

Advanced Plate Configuration

Sample: 1:A,3 Add new

Mapping:

Factor	Group	Value	Metadata Field
1	plate_position	1	Metadata.Plate Position
2	plate_row	A	Metadata.Plate Row
3	plate_col	3	Metadata.Plate Column

Figure 116: Metadata mapping table

All Micro Titer plates can be displayed within the **Report** room.

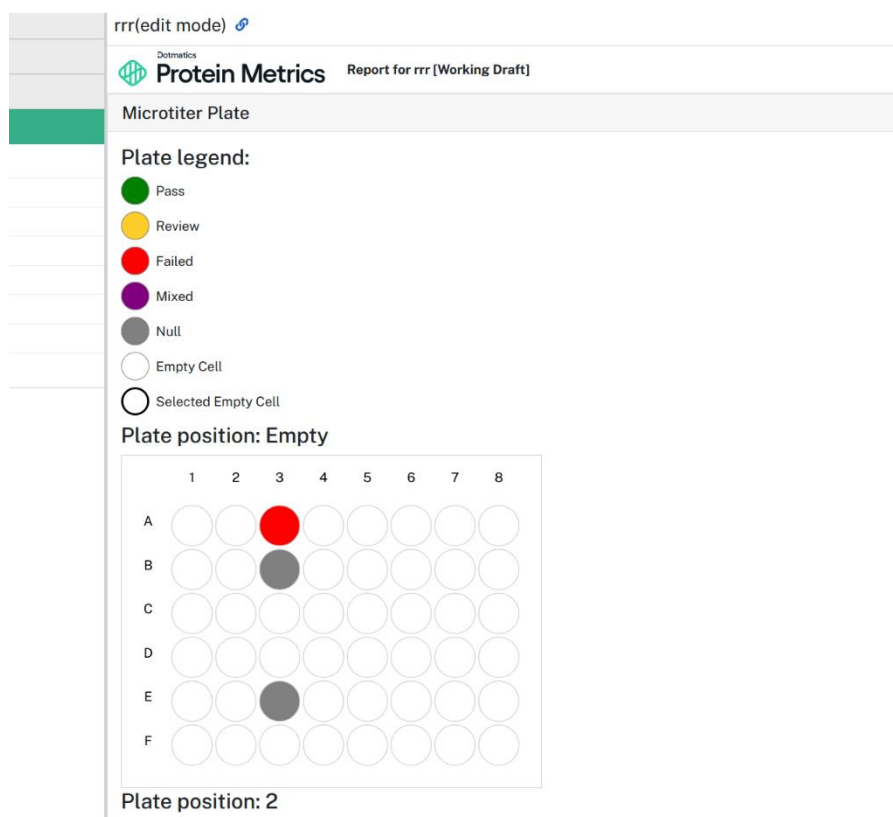


Figure 117: MTP in Reports

MS1 and Deconvolved Mass

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

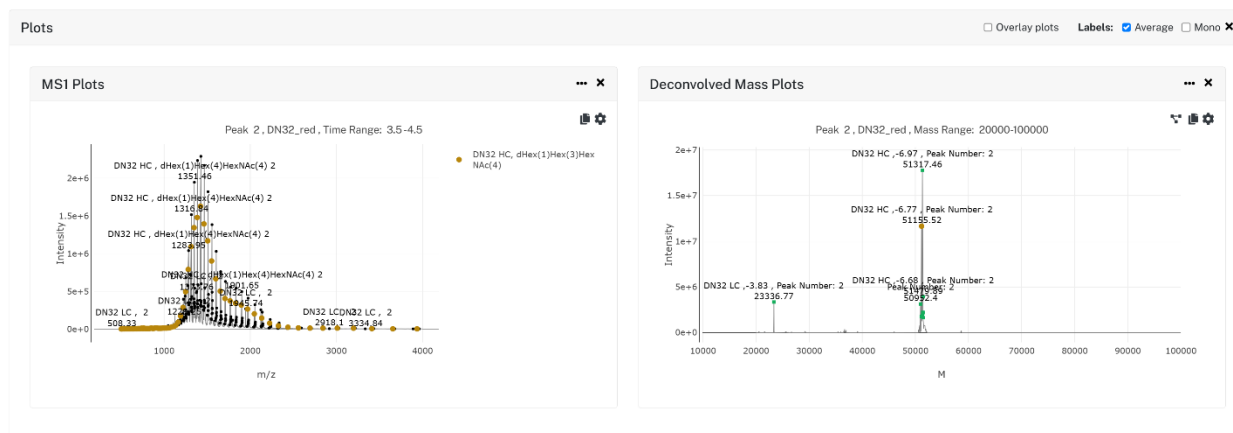


Figure 118: MS1 and Deconvolved Mass Plots in Inspection room

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.

When the user selects a row in the masses table, colored dots representing the mass can be seen on the MS1 and deconvolved mass spectrum. Multiple rows can be selected, leading to multiple colors being represented on the plot.

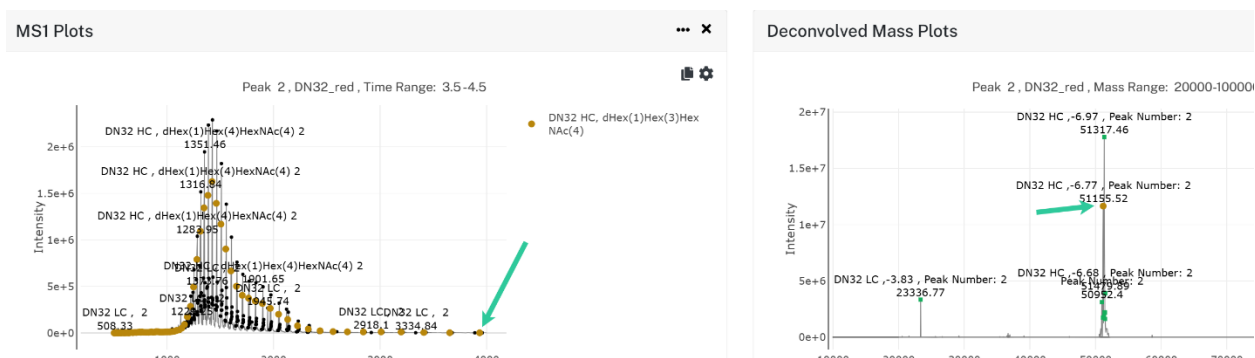


Figure 119: Colored dots represent masses on the plots


The Deconvolved 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 120: Selection marquee

As seen in the image above (and on Figure 53 below), the MS1 plot contains a legend that reflects the selected Names from the Masses table.

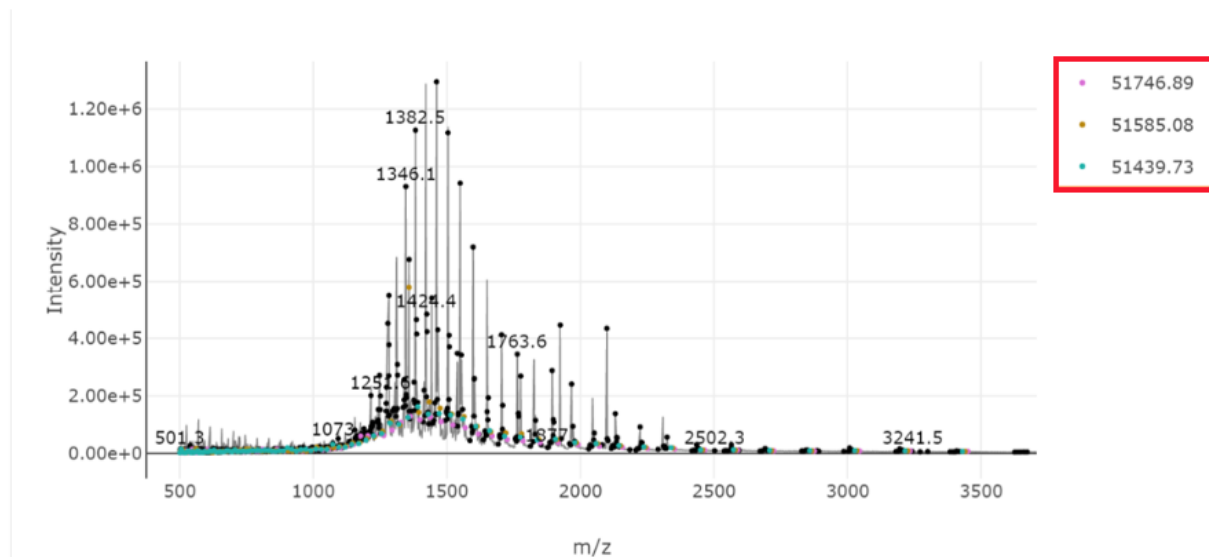


Figure 121: MS1 plot with legend

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

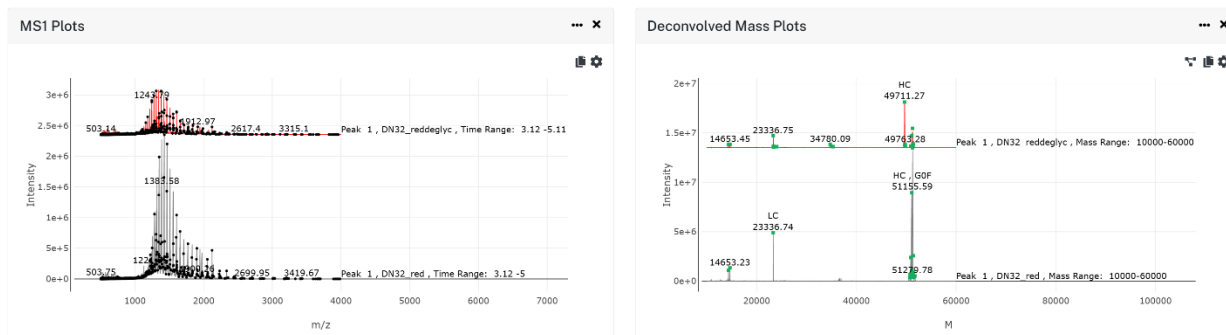



Figure 122: Overlay plots with 100% offset for both plot types

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.

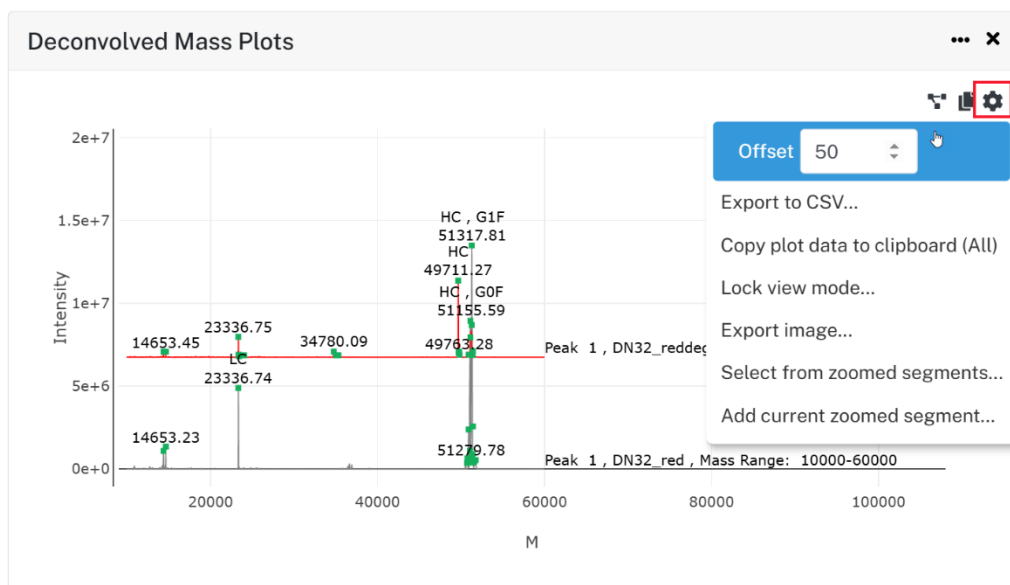



Figure 123: Overlay plots with 50% offset for DCM plots

Additional settings can be accessed by clicking the  icon. In the MS1 Plots, settings include **Show Charge Labels (Enable/Disable)**, **Copy to Clipboard (All)**, **Export to CSV**, and **Numerical Display Settings**. In the Deconvolved Mass Plots, settings include **Copy to Clipboard (All)**, **Export to CSV**, and **Numerical Display Settings**.

Report

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

Summary

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

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 (adding)
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 Range800 - 6000

Figure 124: 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.

Charts & Tables

Configuring the Visualization

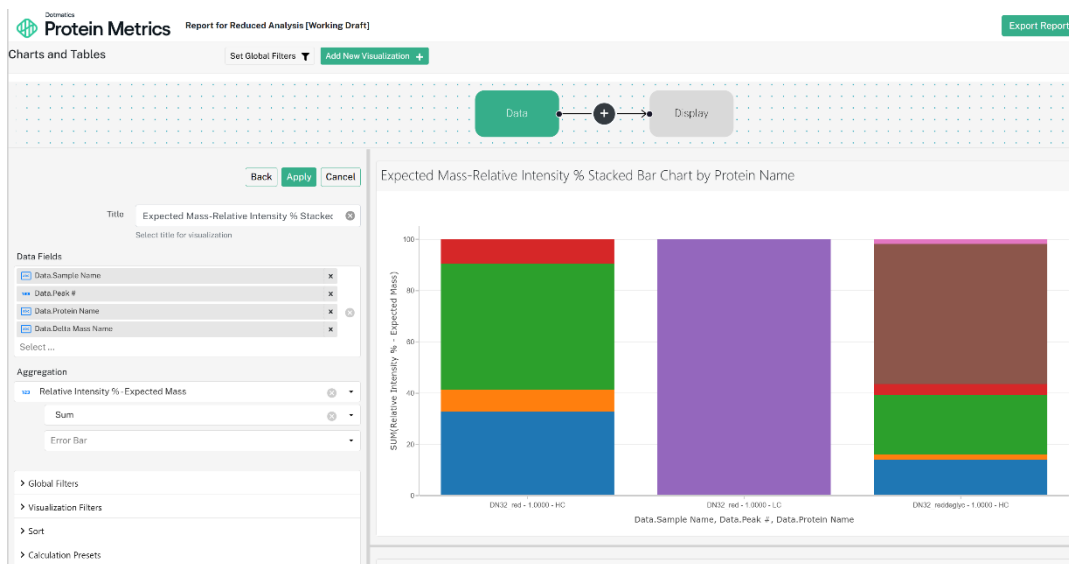


Figure 125: Building a Visualization for Report

To add a visualization to the Report, click **Add New Visualization**. The **Reset** button on the top right corner of the Charts and Tables widget 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.

For more information on using Dashboards, see the **Deep Query Dashboards Manual**.

Plots

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.

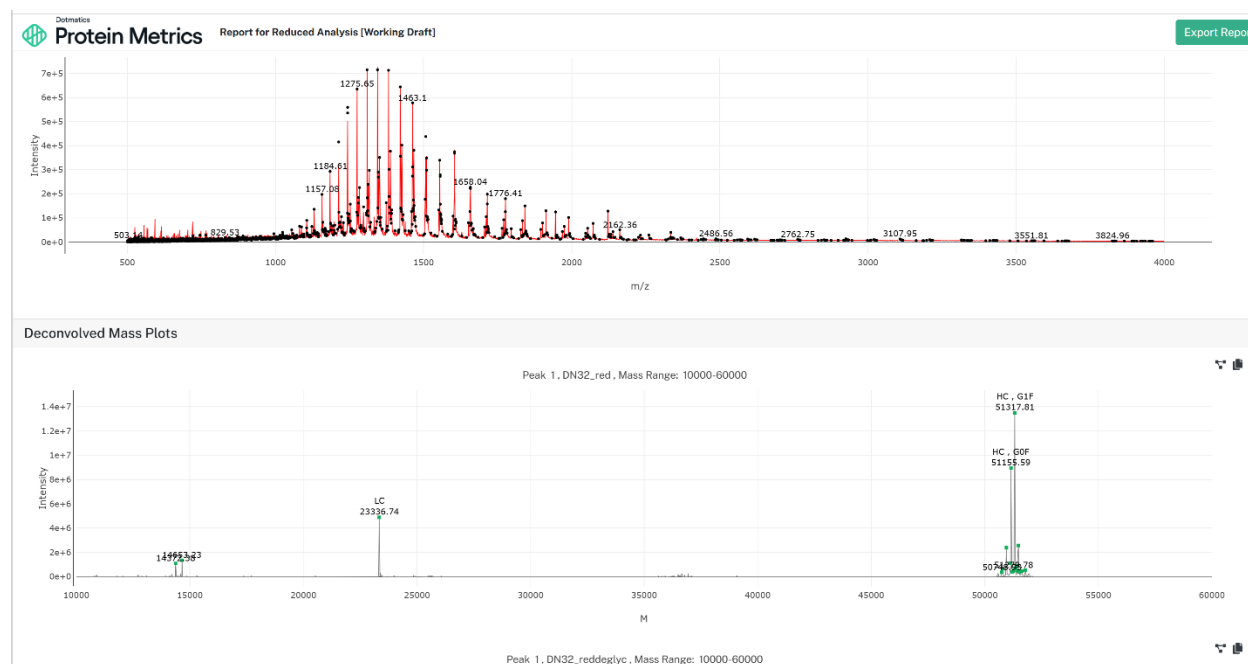


Figure 126: Plots within Report

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.

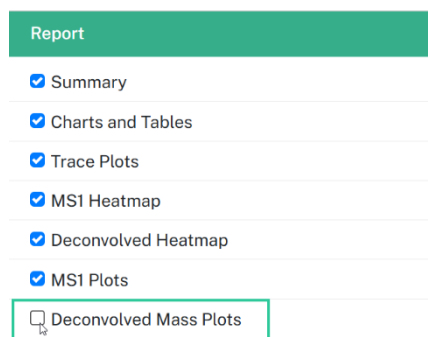




Figure 127: Deselected plot; in this case, the Deconvolved Mass plot will not be included in the Report room

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:

Numerical Display Settings

Field	Configuration
Time Start	Decimal 2
Time End	Decimal 2
Apex Time	Decimal 2
Area	Scientific 3
Normed Area %	Decimal 2

[Restore Defaults](#) [Save](#) [Cancel](#)

Figure 128: 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:

Numerical Display Settings

Field	Configuration
X-axis (values)	Decimal Number of decimal places (default 0)
Y-axis (values)	Scientific 2

[Restore Defaults](#) [Save](#) [Cancel](#)

Figure 129: 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:


Assigned	Sample No.	Sample Name	Peak No.	Mono Mass	Average Mass †	Int
<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="51"/>	<input type="text" value="Search"/>
<input checked="" type="checkbox"/>	1	DN32_red	1	-	51114.03	111
<input checked="" type="checkbox"/>	1			-	51155.59	89
<input checked="" type="checkbox"/>	1			-	51204.35	39
<input checked="" type="checkbox"/>	1	DN32_red	1	-	51256.6	43

Figure 130: 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.

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	D
<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="51"/>	<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="HC"/>	
<input checked="" type="checkbox"/>	1	DN32_red	1	-	51155.59	8952320.9	HC, G0F		-6.69	51129.97	-	-	HC	GI
<input checked="" type="checkbox"/>	1	DN32_red	1	-	51317.81	13496849.4	HC, G1F		-6.62	51292.02	-	-	HC	G
<input checked="" type="checkbox"/>	1	DN32_red	1	-	51479.92	2569254.3	HC, G2F		-6.65	51454.08	-	-	HC	G

Figure 131: AND logic for multiple basic filters

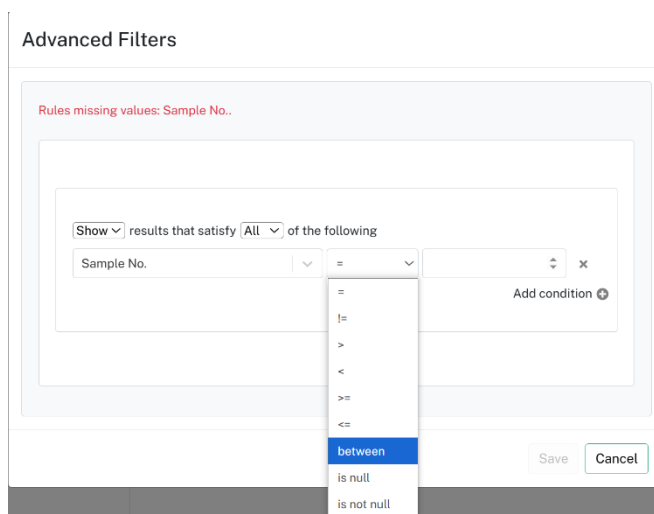
Users can also apply more **advanced** filter functions on tables by clicking the  icon. These advanced filters work in conjunction with the basic filters described above.

Sample No.	Sample Name	Peak No.	Time Start	Time End	Deconvolution Preset	Peak Comment	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"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>	<input type="text" value="Search"/>
1	DN32_red	1	3.12	5	<Redu...		3.57	1.196e+8	106.06
2	DN32_reddeglyc	1	3.12	5.11	<Redu...		3.76	4.826e+7	53.12

Figure 132: 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:



Advanced Filters

Rules missing values: Sample No..

Show ▾ results that satisfy All ▾ of the following

Sample No. ▾ = ▾ ▾ ×

▾

=

!=

>

<

>=

<=

between

is null

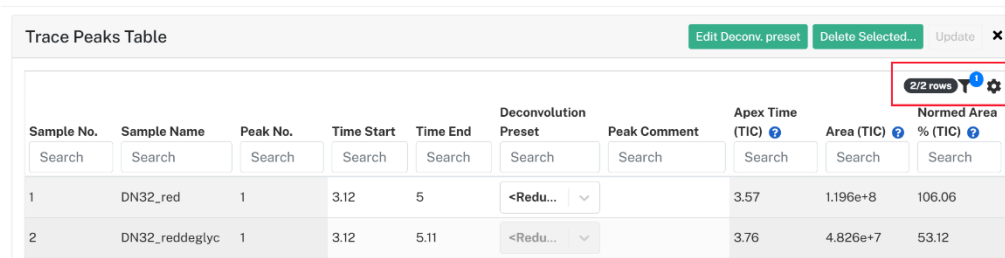
is not null

Add condition +

Save Cancel

Figure 133: Advanced filters

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



Trace Peaks Table

Edit Deconv. preset Delete Selected... Update ×

2/2 rows

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

Figure 134: 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.

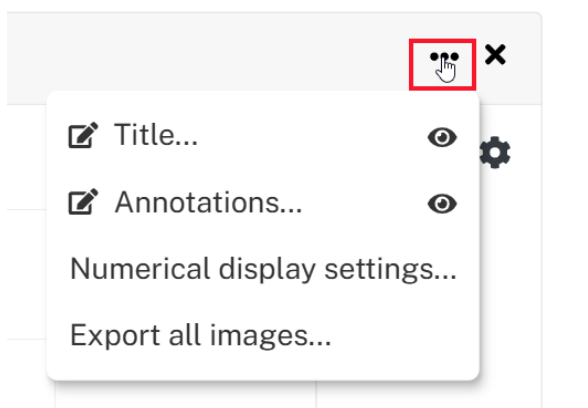


Figure 135: Title and Plot annotations tool access

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.

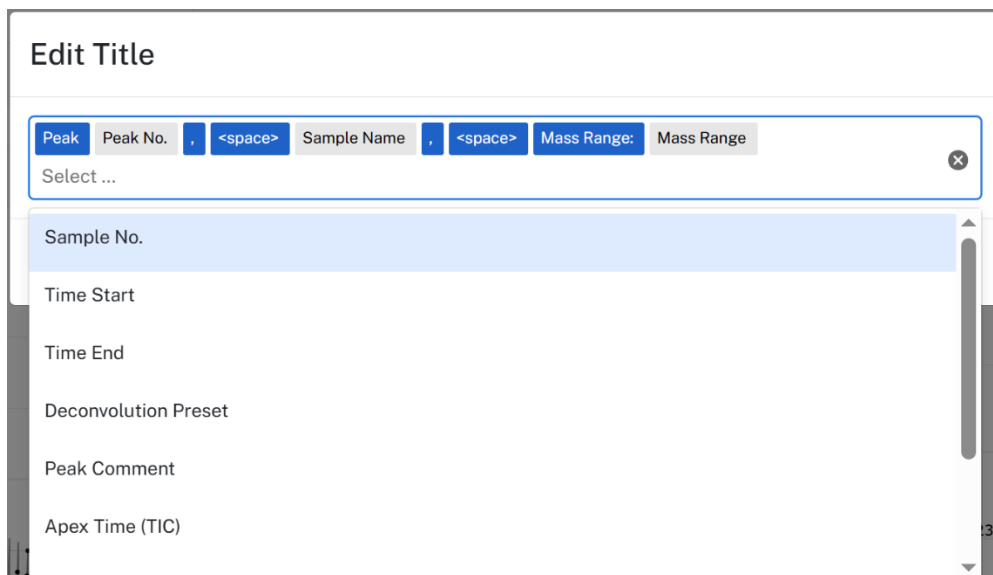


Figure 136: Edit Title dialog



Figure 137: 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.

Edit Annotations

Protein Name Delta Name

> Advanced Settings

Figure 138: Edit Annotations dialog

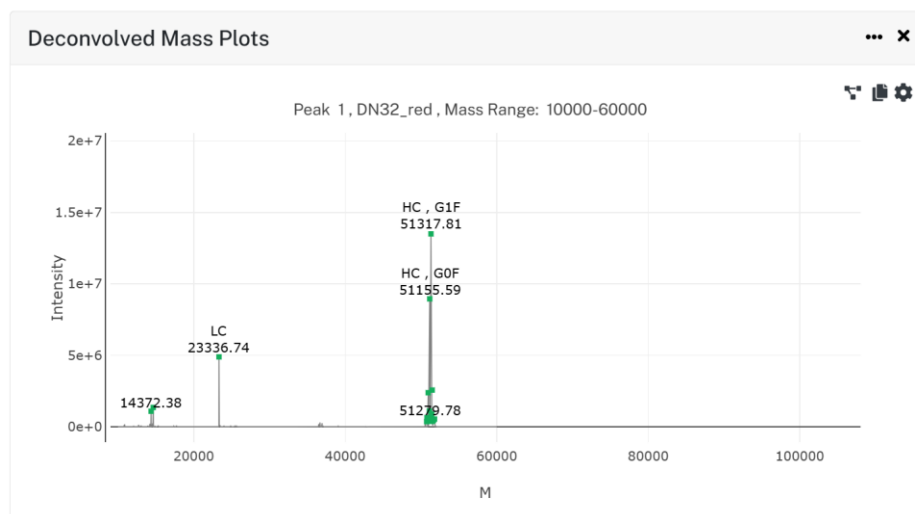
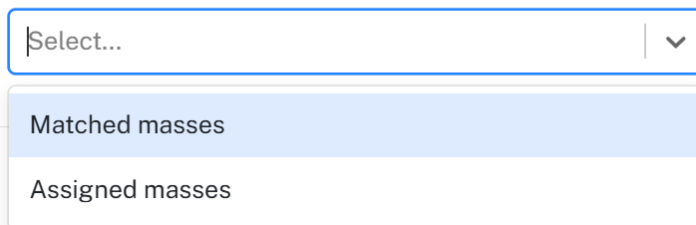


Figure 139: Plot with annotations resulting from the above settings

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

▼ Advanced Settings

Conditions



Select... ▼

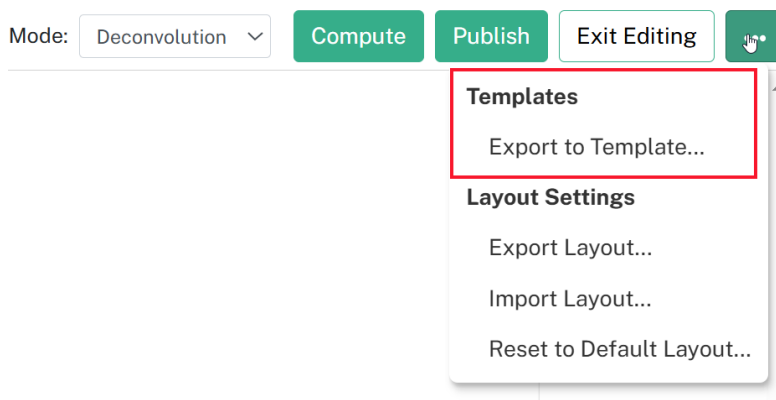
Matched masses

Assigned masses

Figure 140: 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.



Mode: Deconvolution ▼ Compute Publish Exit Editing ⋮

Templates

Export to Template...

Layout Settings

Export Layout...

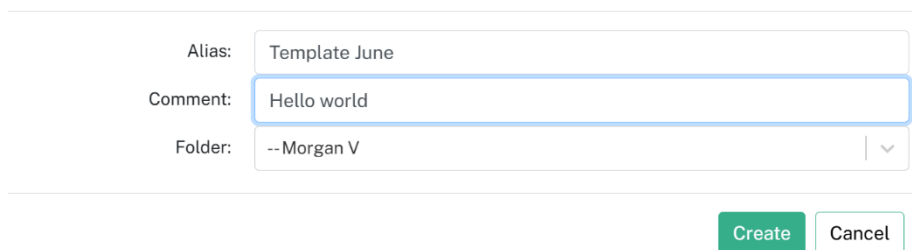
Import Layout...

Reset to Default Layout...

Figure 141: 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.

Create a New Template



Alias: Template June

Comment: Hello world


Folder: --Morgan V ▼

Create Cancel

Figure 142: Create Template File

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

Figure 143: 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.

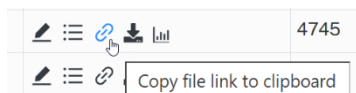


Figure 144: Users can copy link to share Analysis Template

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.

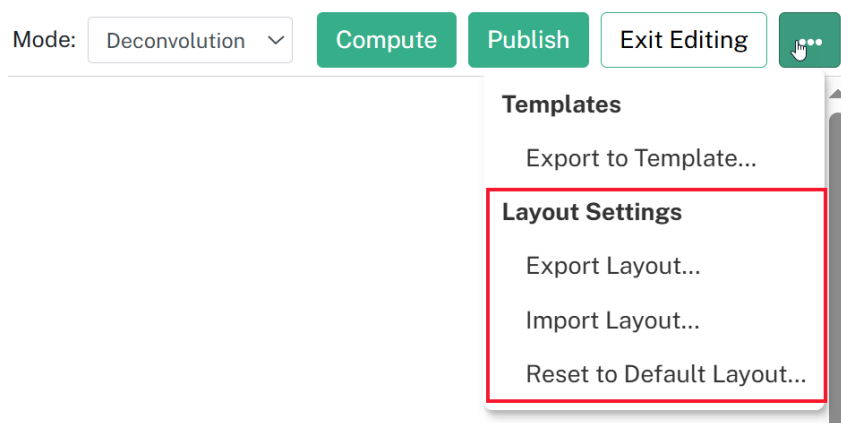


Figure 145: Layout Settings

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.