

# Agilent MassHunter BioConfirm Software

## Quick Start Guide

***For Research Use Only. Not for use in diagnostic procedures.***

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**Agilent Technologies**

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## Where to find more information

- *Agilent MassHunter BioConfirm Software Familiarization Guide*
- *Agilent MassHunter BioConfirm eFamiliarization Guide*
- *Agilent MassHunter BioConfirm Training Videos*
- *Online Help* provides more information and can be displayed in the following ways:
  - Click **Contents** or **Search** from the **Help** menu.
  - Press the **F1** key to get more information about a window or dialog box.

## What is Agilent MassHunter BioConfirm Software?

Agilent MassHunter BioConfirm Software provides automated and interactive protein confirmation for TOF and Q-TOF data, with the following features:

- Automated post acquisition data analysis and report generation.
- Biomolecule-centric navigation for peptides and proteins.
- Intact protein UI functionality, most notably the visualization of ion sets and showing deconvoluted spectra in a separate window.
- Protein sequence matching, including target protein and potential modifications for intact protein and protein digest sequence types.
- Finding glycans from the target glycan source.
- Lets you assign site specific variable modification for protein, protein digest and synthetic peptide sequence types.
- Protein Molecular Feature Extractor for finding protein compounds in complex LC/MS data.
- Relative protein level can be measured either by height from the deconvoluted spectrum or height/area of EIC using all ion set peaks.
- Protein biomolecule quality filters, which prevent “noise” peaks from the deconvoluted spectrum being considered a biomolecule, by requiring sufficient evidence in the  $m/z$  spectrum.
- Sequence editing/matching.
- Linked navigation between biomolecules with assigned protein digest matches and peptide sequence within the Sequence Coverage Map.
- Sequential-enzyme digestion, where a protein digest is first digested with a first reagent, and then the resulting digest is digested with the second reagent, and so on.

## What's New in B.09.00

### New Released Glycans workflow

- The Glycan structure is shown in the Biomolecule MS Spectrum for Released Glycans results.
- You can view the glycan structure in the Glycan Structure Viewer window.
- You can select to only report qualified biomolecules for the Released Glycans workflow.
- You can see the predicted isotope distribution in the Biomolecule MS Spectrum.
- You can specify the glycan tag information on the Tag tab in the Method Editor.
- You can select multiple glycan species, and they are mixed and matched for positive mode Find Glycans.
- You can select multiple glycan species, and they are mixed and matched for negative mode Find Glycans.
- A default Method is available for the Released Glycans workflow.
- A default layout is available for the Released Glycans workflow.

### Enhancements to Intact Protein workflow

- You can integrate a sample chromatogram.
- The software can calculate and display the results of relative quantitation of intact protein isoforms.
- Several columns are available in the Biomolecules window to support relative quantitation: **Use for %Quant**, **%Quant (Height)**, and **%Quant (Area)**.
- You can label deconvoluted peaks with the **%Quant (Area)** or **%Quant (Height)** values determined from relative quantitation.
- You can use manual integration on deconvoluted maximum entropy spectrum.
- Several columns for relative quantitation are available in the peak list in the Biomolecule MS Spectrum window.
- You can view the Sequence Table for Batch Comparison.

- The Sequence Table has nested levels for batch comparison of Intact Protein data.
- You can select Specified or Unspecified for Disulfide links when you use the Match Sequences algorithm with the Intact Protein workflow.
- You can create a peak from within a deconvoluted spectrum.
- You can label deconvolution peaks with found modifications (for example +1 phosphorylation, or N-acetylation).
- You can adjust the peak threshold in the Deconvolution Results window using the shortcut menu.
- A Disulfide links column is available for Walkup & Data Acquisition worklist.
- The default method for Intact Protein workflow is updated.
- The default window Layout for the Intact Protein workflow has been changed.

### **Enhancements to Protein Digest workflow**

- The Peptide Mapping algorithm has been enhanced.
- Several Peptide Mapping columns have been added to the Biomolecule table.
- You can filter the spectral abundance filter for the Find Peptides algorithm.
- You can see the sequence coverage for multiple sequences in the Sequence Coverage Map window. The lines in the Sequence Coverage map show the sequence coverage. If you select multiple samples, the display shows the sequence coverage in different colors in the Sequence Coverage Map.
- You can view the Sequence Coverage Map with MS/MS matches only.
- A legend is available in the Sequence Coverage Map.
- The default method for Protein Digest workflow is updated.
- The Find Peptides section in the Method Editor is changed for the new peptide mapping algorithm.
- The Find Peptides > Advanced tab and Find Peptides > Match Tolerances section have been updated.
- You can load a default window layout for the Protein Digest workflow.

## What is Agilent MassHunter BioConfirm Software?

### What's New in B.09.00

#### Enhancements to Reporting

- The MassHunter PDF Report Builder is integrated with BioConfirm. You use this program to modify templates which you use with the **Use PDF Report Builder** option.
- A default report template is available for each workflow.
- You can print graphics windows using the PDF Report Builder.

#### Enhancements to Sequences

- The new Sequence Coverage Map window is available.
- In the Sequence Manager program, the display of the "Total monoisotopic mass", "Total average mass" and "Sequence molecular formula" values has been changed.
- In the Sequence Manager program, you can create a duplicate copy of a protein using a command in the Sequence menu.

# Agilent MassHunter BioConfirm Software Installation

## To install the software

If the G6829AA Agilent MassHunter BioConfirm Software is not already installed on your system, install it as follows.

- 1 Insert the disk labeled **G6829AA** into the disk drive.
- 2 From the installation disk, right-click **Setup.exe**, and then select **Run as administrator**.
- 3 Follow the instructions on the screen to install the software.

For Excel based reporting to work properly in the BioConfirm program, after installing Excel 2016, you need to open Excel and click **Accept** on the “First things first” message.

## To remove the software

Use **Programs and Features** in Windows Control Panel to remove **Agilent MassHunter Workstation BioConfirm Software**.

# User Interface

## Main Functional Areas

The main BioConfirm window consists of three parts: (1) the Menu Bar, (2) the Toolbar, and (3) the Main Window. The main functional areas are shown in Figure 1, Figure 2 on page 9, and Figure 3 on page 10. Some windows are not shown because they are not in the default layout.

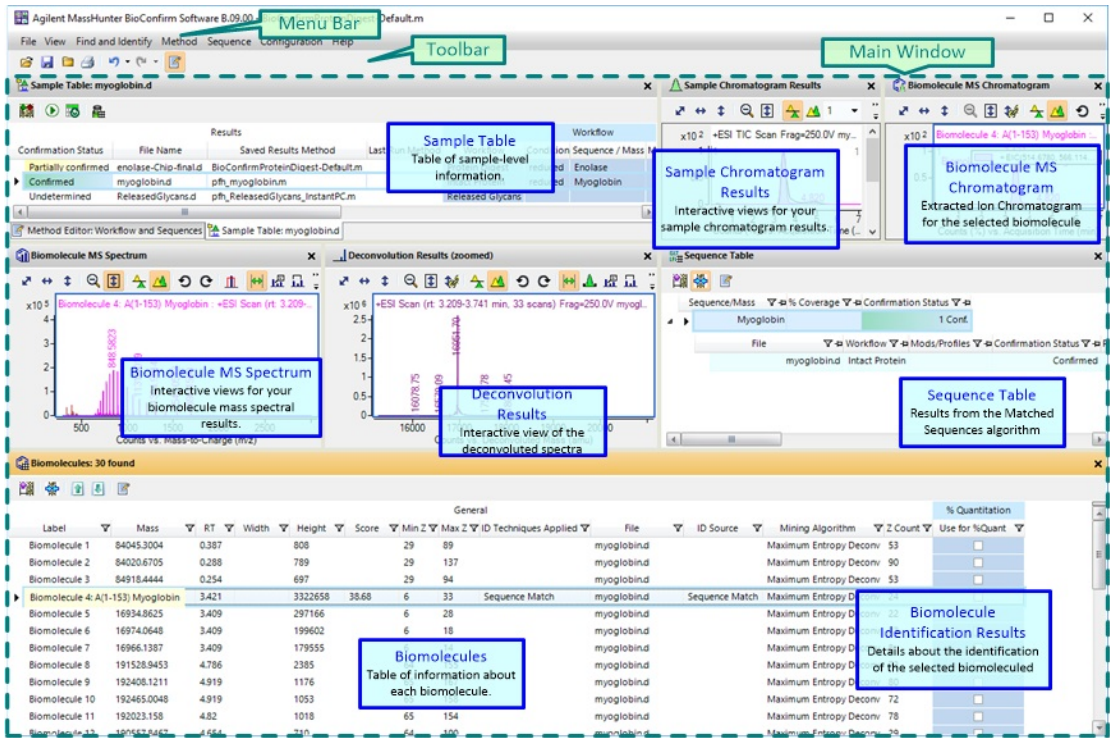
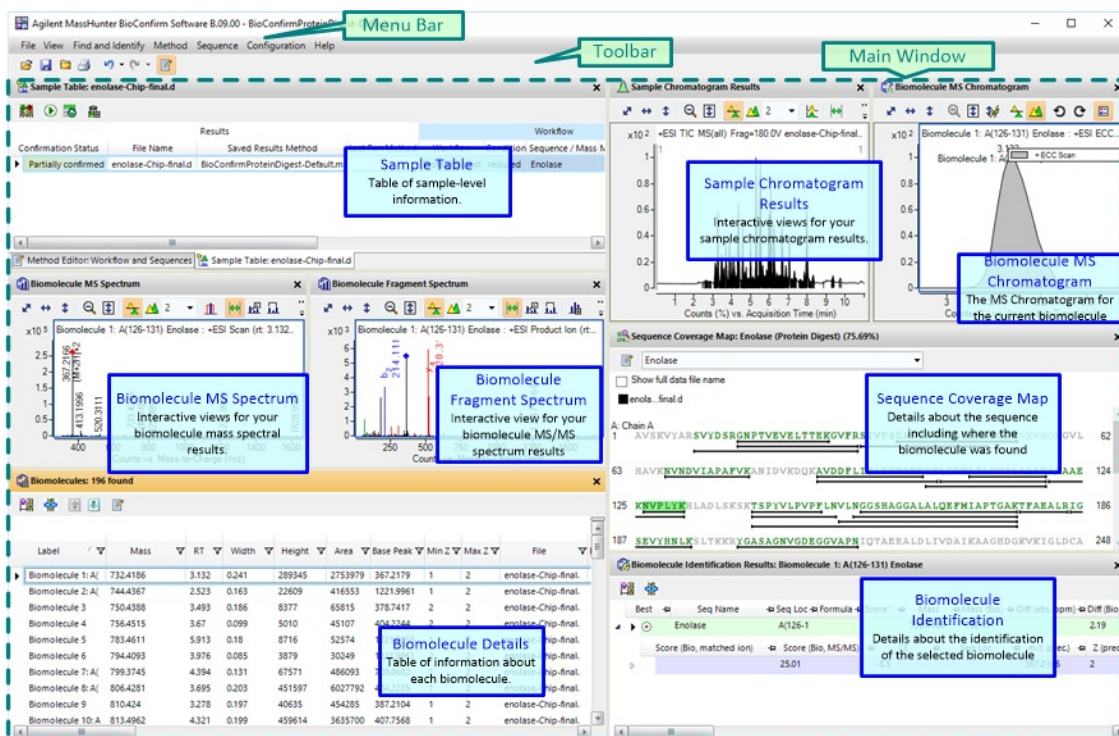


Figure 1 The main functional areas of BioConfirm for an Intact Protein

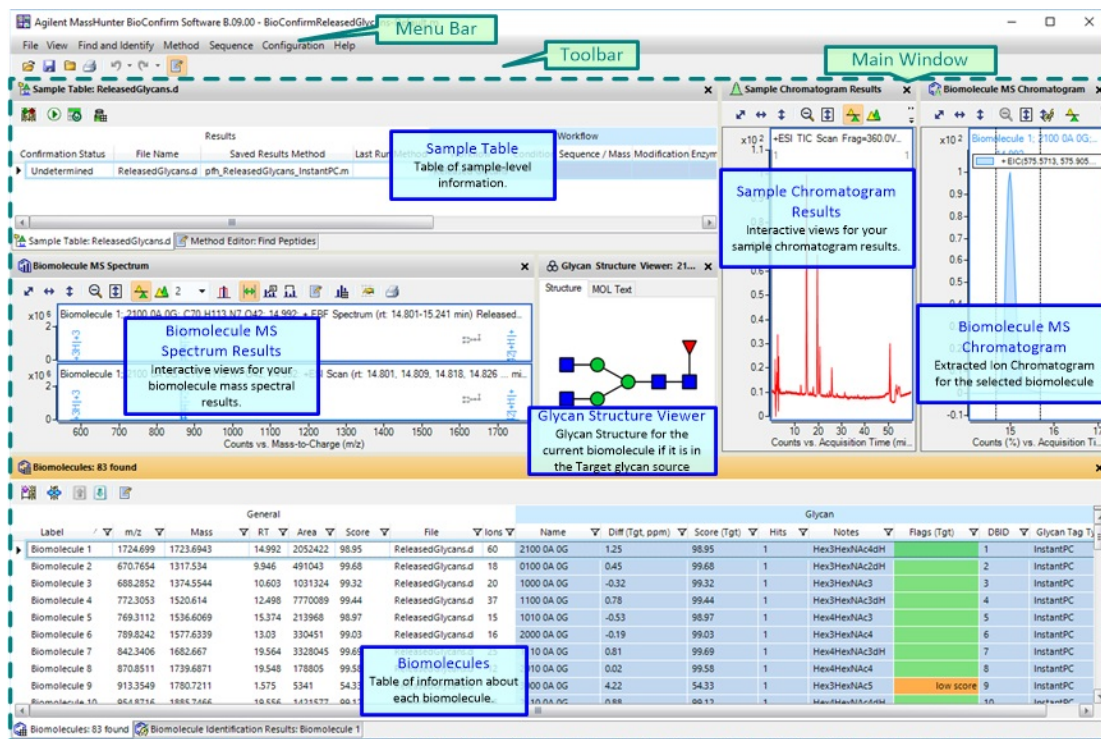




**Figure 2** The main functional areas of BioConfirm for a Protein Digest

## User Interface

### Main Functional Areas



**Figure 3** The main functional areas of BioConfirm for a Released Glycans

### 1. Menu Bar

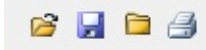


The menu bar (Figure 4 on page 10) provides actions that are used for finding and identifying biomolecules, printing and exporting reports, and editing sequences.

File View Find and Identify Method Sequence Configuration Help

**Figure 4** Menu bar

## 2. Toolbar

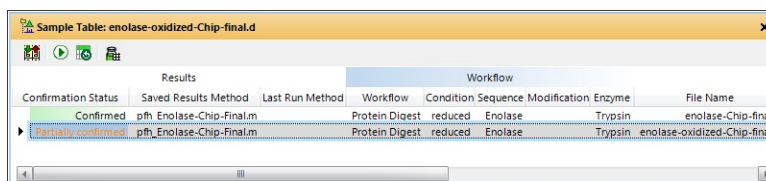
The toolbar provides actions that are used for opening data files and closing data files. You can also save results and print a Biomolecule report. Two icons allow you to undo or redo the last actions performed. The last icon toggles whether the Method Editor window is open.

Toolbar Icon	Action
	<ul style="list-style-type: none"> <li>• File &gt; Open Data File</li> <li>• File &gt; Save Results</li> <li>• File &gt; Close Data File</li> <li>• File &gt; Print &gt; Biomolecule Report</li> </ul>
	<ul style="list-style-type: none"> <li>• Undoes last action performed</li> <li>• Redoes last action undone.</li> </ul>
	<ul style="list-style-type: none"> <li>• Toggles whether the Method Editor window is open.</li> </ul>

## 3. Main window

The main window, see [Figure 2](#) on page 9, is further divided into up to thirteen areas: Sample Table, Method Editor, Sample Chromatogram Results, Biomolecule MS Chromatogram, Biomolecule MS Spectrum, Biomolecule Fragment Spectrum, Deconvolution Results, Deconvolution Mirror Plot, Biomolecules, Biomolecule Identification Results, Sequence Table, Sequence Coverage Map, and Glycan Structure Viewer. You toggle whether these windows are visible in the **View** menu.

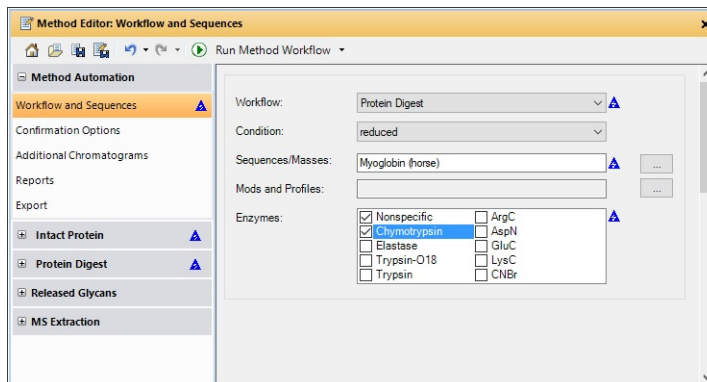
**Sample Table** The Sample Table shows information for each sample that is opened. The sample or samples which you select in this window are displayed in the other windows. You can reprocess the selected sample.



**Figure 5** Sample Table window

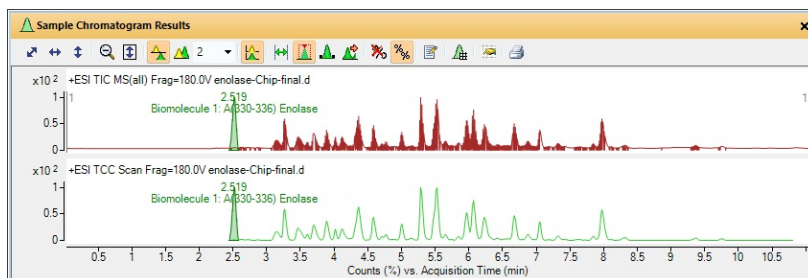
**Method Editor** A method is a set of parameters that are associated with the different algorithms that you can run. Methods containing these parameters can be saved using unique file names.

You select the section of the method to display in the left pane. The right pane contains either a single section or multiple tabs. You can get help for each tab or section in the Method Editor when you press **F1**.



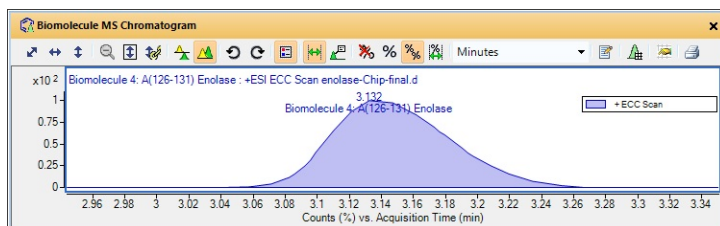
**Figure 6** Method Editor window

**Sample Chromatogram Results** This window shows the chromatograms for each sample that is selected in the Sample Table window. This chromatogram may be a Total Ion Chromatogram (TIC) or a Base Peak Chromatogram (BPC). You can overlay the chromatogram for the selected biomolecule.



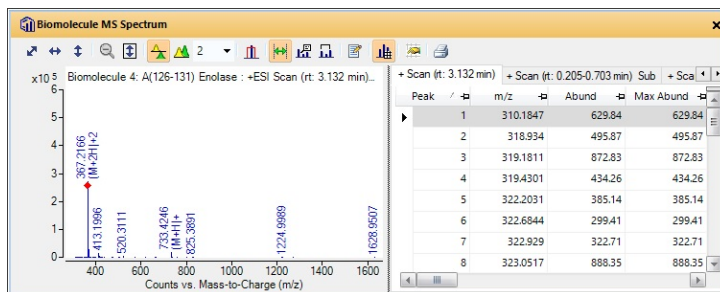
**Figure 7** Sample Chromatogram Results window

**Biomolecule MS Chromatogram** This window shows an Extracted Ion Chromatogram (EIC) for each of the biomolecule you selected in the Biomolecules window. You can display a legend in the upper right corner of the graphic if you select **Overlaid mode** for the chromatograms. You can add annotations to the graphic. You can also export or print the graphic.



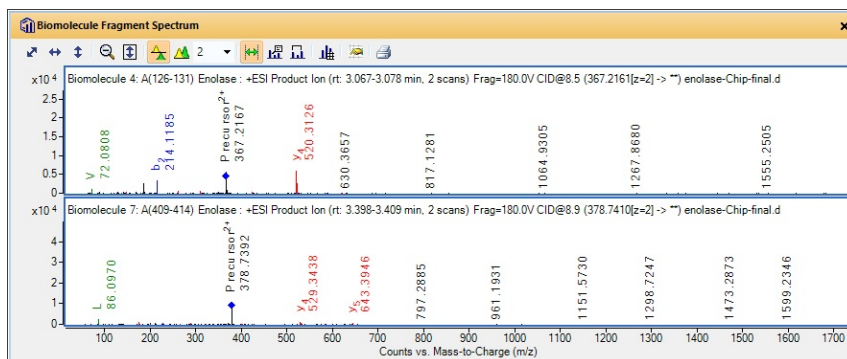
**Figure 8** Biomolecule MS Chromatogram window

**Biomolecule MS Spectrum** This window shows any MS spectrum. MS/MS spectra are displayed in the Biomolecule Fragment Spectrum window. You can add annotations and calipers to a spectrum in this window. You can also display the peak list which is displayed in a table on the right-side of this window. You can deconvolute, print, and export spectra in this window.



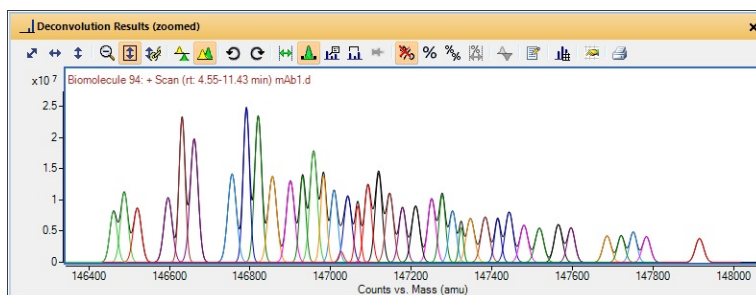
**Figure 9** Biomolecule MS Spectrum window

**Biomolecule Fragment Spectrum** This window shows any MS/MS spectrum. MS spectra are displayed in the Biomolecule MS Spectrum window. You can also annotate and add calipers to a Fragment Spectrum.



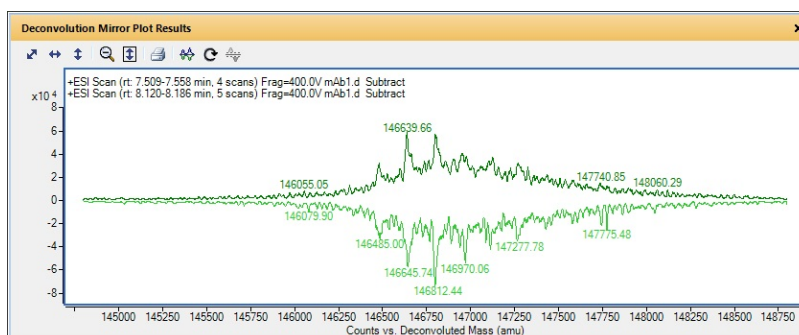
**Figure 10** Biomolecule Fragment Spectrum window

**Deconvolution Results** This window shows deconvoluted mass spectra. You can add annotations and calipers to a spectrum in this window. You can also display the peak list which is displayed in a table on the right-side of this window. You can deconvolute, print, and export spectra in this window.



**Figure 11** Deconvolution Results window in **Overlaid** mode

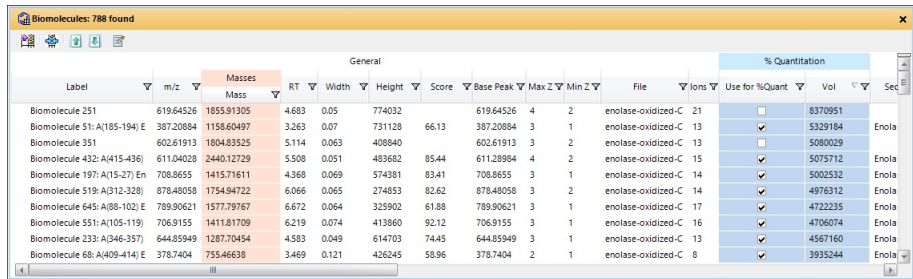
**Deconvolution Mirror Plot** This window displays two deconvoluted spectra selected from the Deconvolution Results window. The first spectra is displayed as the top plot, and the second spectra is displayed as the inverted or bottom plot.



**Figure 12** Deconvolution Mirror Plot

**Biomolecules** This window shows all of the biomolecules which were found for the selected sample files. You can add and remove columns from this table, and you can change the order of the columns.



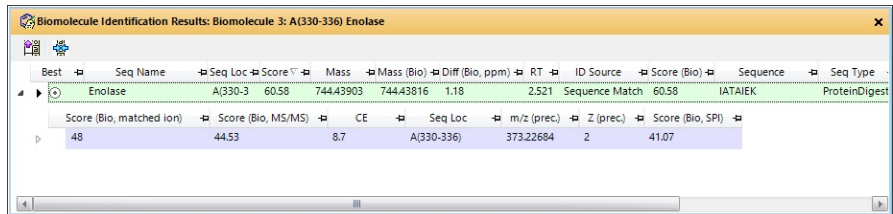


Biomolecules: 788 found

General											% Quantitation			
Label	m/z	Masses	RT	Width	Height	Score	Base Peak	Max Z	Min Z	File	Ions	Use for %Quant	Vol	Sec
		Mass												
Biomolecule 251	619.64526	1855.91305	4.683	0.05	774032		619.64526	4	2	enolase-oxidized-C	21	<input type="checkbox"/>	8370951	
Biomolecule 51: A(185-194) E	387.20884	1158.60497	3.263	0.07	731128	66.13	387.20884	3	1	enolase-oxidized-C	13	<input checked="" type="checkbox"/>	5329184	Enola
Biomolecule 351	602.61913	1804.83525	5.114	0.063	408840		602.61913	3	2	enolase-oxidized-C	13	<input type="checkbox"/>	5080029	
Biomolecule 432: A(415-436)	611.04028	2440.12729	5.508	0.051	483682	85.44	611.28984	4	2	enolase-oxidized-C	15	<input checked="" type="checkbox"/>	5075712	Enola
Biomolecule 197: A(15-27) En	708.8655	1415.71611	4.368	0.069	574381	83.41	708.8655	3	1	enolase-oxidized-C	14	<input checked="" type="checkbox"/>	5002532	Enola
Biomolecule 519: A(312-328)	878.48058	1754.94722	6.066	0.065	274853	82.62	878.48058	3	2	enolase-oxidized-C	14	<input checked="" type="checkbox"/>	4976312	Enola
Biomolecule 645: A(88-102) E	789.90621	1577.79767	6.672	0.064	325902	61.88	789.90621	3	1	enolase-oxidized-C	17	<input checked="" type="checkbox"/>	4722235	Enola
Biomolecule 551: A(105-119)	706.9155	1411.81709	6.219	0.074	413860	92.12	706.9155	3	1	enolase-oxidized-C	16	<input checked="" type="checkbox"/>	4706074	Enola
Biomolecule 233: A(346-357)	644.85949	1287.70454	4.583	0.049	614703	74.45	644.85949	3	1	enolase-oxidized-C	13	<input checked="" type="checkbox"/>	4567160	Enola
Biomolecule 68: A(409-414) E	378.7404	755.46638	3.469	0.121	426245	58.96	378.7404	2	1	enolase-oxidized-C	8	<input checked="" type="checkbox"/>	3935244	Enola

Figure 13 Biomolecules window

**Biomolecule Identification Results** This window shows the results of running the Match Sequences algorithm on the biomolecules in the Biomolecules table. If you see **Sequence Match** as the value for the **ID Techniques Applied** column, then you can see additional information about that match in this window.



Biomolecule Identification Results: Biomolecule 3: A(330-336) Enolase

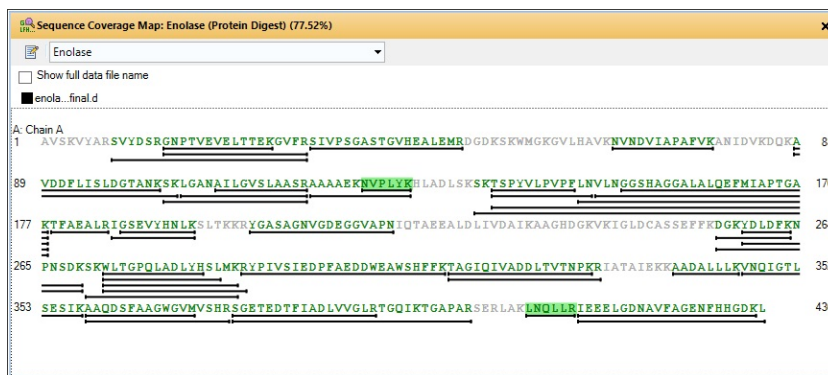
Best	Seq Name	Seq Loc	Score	Mass	Mass (Bio)	Diff (Bio, ppm)	RT	ID Source	Score (Bio)	Sequence	Seq Type
▶	Enolase	A(330-3)	60.58	744.43903	744.43816	1.18	2.521	Sequence Match	60.58	IATAIEK	ProteinDigest
▶	Score (Bio, matched ion)	Score (Bio, MS/MS)	CE	Seq Loc	m/z (prec.)	Z (prec.)	Score (Bio, SPI)				
▶	48	44.53	8.7	A(330-336)	373.22684	2	41.07				

Figure 14 Biomolecule Identification Results window

**Sequence Coverage Map** This window displays information for the protein digest sequence selected in the Workflow and Sequences section of the Method Editor window and the biomolecule selected in the Biomolecules window. The highlighted area in the sequence shows where the current biomolecule matches the current sequence. Different lines underneath parts of the sequence show where a biomolecule was matched in the sequence.

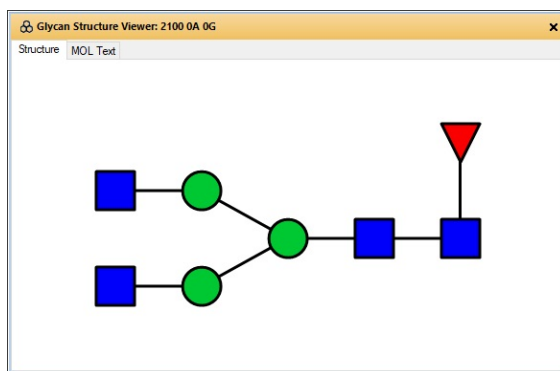
If you run Match Sequences on multiple sequences, then you can see multiple sequences in this window.





**Figure 15** Sequence Coverage Map window

**Glycan Structure Viewer** This window displays glycan structures. The structure is also shown in the Biomolecule MS Spectrum window.



**Figure 16** Glycan Structure Viewer window

## Sequence Dialog Boxes

The following dialog boxes are some of the dialog boxes available in Agilent MassHunter BioConfirm Software. See *online Help* for more information.

### Chemical Data Dictionary Dialog Box

Lets you customize the list of modifications, links, and reagents for use with MassHunter BioConfirm software. The factory-supplied default lists cannot be modified, but you can add to them.

**To open this dialog box** Click **Open Chemical Data Dictionary Editor** on the Sequence menu.

### Applied Description Dialog Box

Lets you review descriptive information that is stored with both method sequences and result sequences.

**To open this dialog box** Click **Show Sequence Description** on the Sequence menu. In the Sequence Manager program, you can use the **Edit Description** command on the Sequence menu or the Sequence Editor shortcut menu to change the description for a sequence.

### Applied Links

Lets you review the amino acids linked in the sequence.

**To open this dialog box** Click **Applied Links** on the Sequence menu or the Sequence Coverage Map shortcut menu. In the Sequence Manager program, you can use the **Edit Link** item on the Sequence menu or the Sequence Editor shortcut menu to add a link to a sequence. See “To apply or edit links” on page 55.

### Modifications

Lets you view modifications to the sequence.

**To open this dialog box** Click **Applied Modifications** on either the Sequence menu or the Sequence Editor shortcut menu. In the Sequence Manager program, you can use the **Edit Modifications** item on the Sequence menu or the Sequence Editor shortcut menu to add a modification to a sequence.

## Workflows and Sequences

In the Method Editor in the Method Automation > Workflow and Sequences section, you set several parameters including the Workflow and the Sequences.

### Workflows

You select a workflow in the Method Editor window in the **Method Automation > Workflow and Sequences** section. This workflow decides what operations are to be run and how the sequence match is to be done when you run the method. You select one of these four workflows: **Intact Protein**, **Protein Digest**, **Custom**, and **Released Glycans**.

#### Intact Protein

The workflow runs the Find by Protein Deconvolution algorithm, and then runs Intact Protein matching rules. A Biomolecule report is generated using the **Intact Protein report template** selected on the Method Automation > Reports > Templates tab.

#### Protein Digest

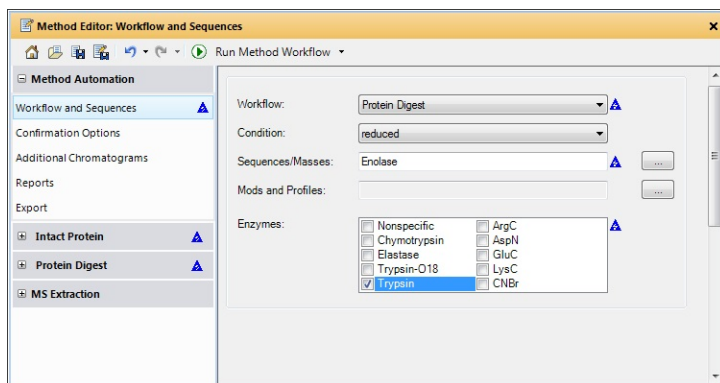
This workflow runs the Find Peptides algorithm, and uses protein matching rules (Protein Digest, Predicted Modifications). You can select whether or not to use Protein Truncation. The workflow also runs the Match Sequences algorithm with the Sequence or mass that you entered and digests the sequence using the enzyme selected. See [Figure 17](#) on page 20. A Biomolecule report is generated using the **Protein Digest report template** selected on the Method Automation > Reports > Templates tab.

#### Released Glycans

This workflow runs the Find Glycans algorithm and uses the **Target glycan source** that you entered. See [Figure 17](#) on page 20. A Biomolecule report is generated using the **Released Glycans report template** selected on the Method Automation > Reports > Templates tab.

## Custom

This workflow runs the actions which you select in the Workflow and Sequences section. You select the actions from the **Available actions** list and place them in the **Actions to be run** list. The order of the actions in the **Actions to be run** list is the order in which the actions are executed. If you generate a biomolecule report, it uses the **Protein Digest report template** if you used the **Find Peptides** algorithm; otherwise, it uses the **Intact Protein report template**.



**Figure 17** Method Automation > Workflow and Sequences section

## Sequences

You create and edit sequence files in the Agilent MassHunter Sequence Manager program. You can add a sequence manually, or you can import a sequence from a sequence file (psq), text file (txt), or FASTA file. You edit sequences in the Sequence Manager program.

You specify a sequence in the Method Editor window in the Method Automation > Workflow and Sequences section.

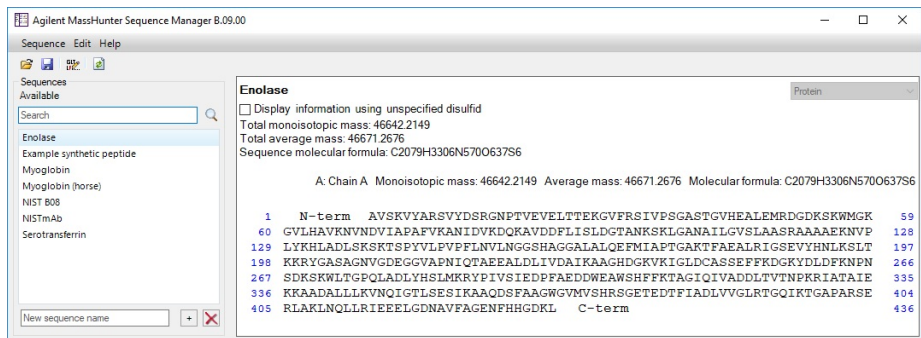


Figure 18 MassHunter Sequence Manager program

## Intact Protein Workflow

The topics in this section will help you get started using the Intact Protein workflow features of Agilent MassHunter BioConfirm Software.

- “To run the Intact Protein workflow” on page 23
- “To find biomolecules by Protein Deconvolution” on page 24
- “To match sequences for intact protein biomolecules” on page 26
- “To view protein deconvolution results” on page 27
- “To view deconvolution biomolecules” on page 31
- “To print a report with deconvolution results” on page 32
- “To automate protein confirmation” on page 33

### What is deconvolution?

Deconvolution software does charge state deconvolution of mass spectra of large molecules with high charge states. Singly-charged ions with  $m/z$  values greater than a few thousand Thomsons are beyond the mass range of the Agilent TOF instrument. However, multiply-charged ions can be observed if their mass-to-charge ratio ( $m/z$ ) falls within the instrument range. This applies to proteins which typically become multiply-charged.

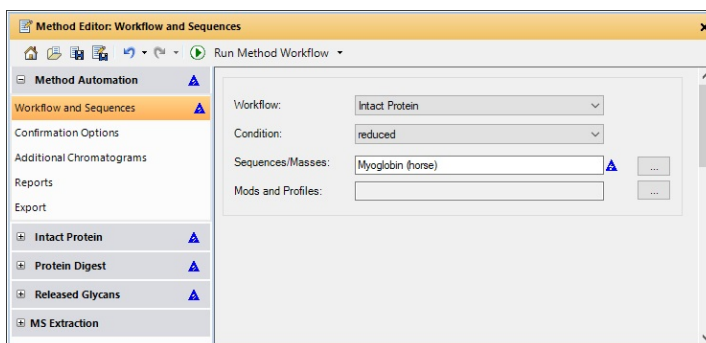
Based on the maximum entropy result, peak modeling (pMod) automatically generates peak models without manual intervention and applies these models through fitting and validating procedures to provide a highly resolved zero-charge spectrum and a set of mass error assessments for each peak. **pMod** can be selected as the **Deconvolution algorithm** on the Deconvolution tab.

## To run the Intact Protein workflow


When you run an **Intact Protein** workflow, the workflow automatically does these steps:

- Find by Protein Deconvolution
- Match Sequences

- 1 Open the data file that contains the biomolecules of interest.
- 2 Open the **BioConfirmProteinDigest-Default.m** method. Click **Method > Open**.
- 3 Select Method Automation > Workflow and Sequences.
- 4 Select **Intact Protein** for the **Workflow**.
- 5 Select either **reduced** or **non-reduced** for the **Condition**.
- 6 Click the  button next to the **Sequences/Masses** parameter to select a sequence. You can instead enter one or more masses.
- 7 Click the  button next to the **Mods and Profiles** parameter to select modifications and profiles.
- 8 Save the method. Click **Method > Save As** to save to a new name.




**Figure 19** Workflow and Sequences section for an Intact Protein workflow

- 9 Click the  button on the Method Editor toolbar to run the method workflow.
- 10 View biomolecules as described in [“To view deconvolution biomolecules”](#) on page 31.

## To find biomolecules by Protein Deconvolution


Use this procedure to deconvolute proteins and create a biomolecule list.

- 1 Open the data file that contains the spectra of interest as described in *online Help*.
- 2 In the Method Editor window, select **Intact Protein > Deconvolute (Protein)** in the left pane.
- 3 Set parameters in the Method Editor window in the Deconvolute (Protein) > Deconvolution section.
- 4 Click the  button on the Method Editor toolbar to start processing. You can instead click **Find and Identify > Find by Protein Deconvolution**.
- 5 Review results in the Deconvolution Results window. If this window is not currently displayed, click **View > Deconvolution Results**.
- 6 View deconvolution biomolecules as described in [“To view deconvolution biomolecules”](#) on page 31.






## To deconvolute selected spectra

Use this procedure to deconvolute selected  $m/z$  spectra and create a biomolecule list.

- 1 Open the data file that contains the spectra of interest. Click **File > Open Data File**. This process is described in *online Help*.
  - 2 Select a spectrum as described in *online Help*.
    - Double-click the Sample Chromatogram Results window. or
    - Select a range in the Sample Chromatogram Results window and double-click that range.
  - 3 In the Method Editor window, select **Intact Protein > Deconvolute (Protein)** in the left pane.
  - 4 Set parameters in the Method Editor window in the Deconvolute (Protein) > Deconvolution section.
  - 5 Click the arrow next to the  button on the Method Editor toolbar and select **Deconvolute (Protein)** to start processing.
- Tip** You can also initiate deconvolution when you right-click an MS spectrum in the Biomolecule MS Spectrum window and click **Deconvolute** from the shortcut menu.
- 6 Review results in the Deconvolution Results window. If this window is not currently displayed, click **View > Deconvolution Results**. You can manually integrate peaks in the Deconvolution Results window.
  - 7 View deconvolution biomolecules as described in [“To view deconvolution biomolecules”](#) on page 31.

## To match sequences for intact protein biomolecules

Use this procedure to match sequences for biomolecules that were found using deconvolution. You need to specify the sequence and modifications and profiles in the Method Automation > Workflow and Sequences section.

- 1 Create biomolecules through deconvolution. See [“To find biomolecules by Protein Deconvolution”](#) on page 24 or [“To deconvolute selected spectra”](#) on page 25.
- 2 Select Method Automation > Workflow and Sequences.
- 3 Select **Intact Protein** for the **Workflow**.
- 4 Select either **reduced** or **non-reduced** for the **Condition**.
- 5 Click the  button next to the **Sequences/Masses** parameter to select a sequence.
- 6 Click the  button next to the **Mods and Profiles** parameter to select modifications and profiles.
- 7 In the Method Editor window, select **Intact Protein > Match Tolerances** in the left pane.  
Make sure to choose the Intact Protein > Match Tolerances section.  
Do *not* select the Protein Digest > Match Tolerances section.
- 8 Review the parameters on the Mass Matching, Matching Rules, and Results tabs.
- 9 Click the  button on the Method Editor toolbar to run the Match Sequences algorithm.

**Tip** You can also start Match Sequences when you click **Find and Identify > Match Sequences**.

- 10 View deconvolution biomolecules as described in [“To view deconvolution biomolecules”](#) on page 31.

## To view protein deconvolution results















Use this procedure to review the results from the following deconvolution process:










- “To run the Intact Protein workflow” on page 23
  - “To find biomolecules by Protein Deconvolution” on page 24
  - “To deconvolute selected spectra” on page 25
- 1 If the Deconvolution Results window is not currently displayed, click **View > Deconvolution Results**.
  - 2 If the Biomolecules window is not currently displayed, click **View > Biomolecules**.
  - 3 Select a biomolecule in the Biomolecules window.
  - 4 Use the following mouse actions to change the display of data:
    - Click to select a single mass in the spectrum.
    - Drag to select a mass range in the spectrum.
    - **Ctrl+drag** to select another area and keeps the previous area/time selected.
    - Drag axes to scroll the axes in the direction you are moving the mouse.
    - Right-drag to expand the selected area. The area you define is shown as a rectangle outlined in black. The Y-scale of the zoomed in display is controlled by the Auto-Scale Y-axis mode.
    - Right-drag axes to scale the axis. Dragging to the right (x-axis) or to the top (y-axis) zooms in on that axis. Dragging to the left (x-axis) or to the bottom (y-axis) zooms out on that axis.



**Tip** To return to the previous display scale, click the **Unzoom** toolbar button




- 5 Use the following toolbar buttons in the Deconvolution Results window to change the display of data:

Toolbar button	Action/Meaning
	Scales the x-axis and y-axis automatically to fit the displayed data.
	Scales the x-axis automatically to fit the displayed data.
	Scales the y-axis automatically to fit the displayed data.
	Returns to the previous display scale (undoes last zoom operation).
	<ul style="list-style-type: none"> <li>When this mode is on, the vertical plot range is automatically scaled to fit the data in the selected horizontal range when you zoom in.</li> <li>When the <b>Autoscale y-axis during Zoom</b> mode is off, the vertical plot range is set to the limits you specify by dragging the mouse.</li> </ul>
	Plots each spectrum separately. The spectra share the same x-axis, but each spectrum has a separate y-axis.
	Overlays all spectra. The spectra are shown with the same x- and y-axes.
	Cycles to previous chromatogram. This toolbar button is only available when you have overlaid the chromatograms.
	Cycles to next chromatogram. This toolbar button is only available when you have overlaid the chromatograms.
	Turns on the Range Select tool.
	Turns on the Manual Peak Integration tool. When you select a peak, two black boxes are drawn at the beginning and end of that peak. You can drag those boxes to change the integration.
	Turns on the Annotate tool, which lets you add various annotations.
	Turns on the Caliper tool, which lets you measure Modifications, Amino Acids, or Delta Mass between peaks or points.
	Pins the selected plot to the window which is useful when you want to compare deconvoluted spectra from different data files in the Deconvolution Mirror plot.

Toolbar button	Action/Meaning
	Stops normalizing the deconvolution spectra.
	Normalizes all deconvoluted spectra. All deconvoluted spectra are normalized to the largest peak in any of the chromatograms.
	Normalizes all chromatograms. Each chromatogram is normalized to the largest peak in itself.
	Normalizes each chromatogram to the highest peak within the selected range.
	Displays the two, selected deconvoluted spectra in the Deconvolution Mirror Plot window. This button is only available if two deconvoluted spectra are selected.
	Displays the Method Editor window as a floating window. This button is not a toggle.
	Toggles the display of the peak list tabs in this window. If this option is on, then the right pane of this window contains a tab for each deconvoluted spectra. Each tab contains a table of information about a deconvoluted spectrum.
	Opens the <b>Deconvoluted Spectra Display Options</b> dialog box.
	Prints an image of the contents of the Deconvolution Results window and puts it in a PDF file. This PDF is opened automatically.


- 6 Right-click the graph to display the following shortcut menu commands:
  - **Copy Deconvolution Settings to Method** - Copies the settings that were used to create the Deconvolution Results the Deconvolute (MS): Maximum Entropy section of the Method Editor window.
  - **Include in %Quant** - If you select the Manual Integration icon and you are displaying the results in List mode, then you can specify to include the selected peak in the Relative Quantitation results. The **Use in %Quant** check box for this peak is marked in the Biomolecules table.
  - **Pin/Remove Pin**- toggles whether to pin the selected spectrum to its location.
  - **Delete** - Deletes the currently highlighted chromatograms and spectra.
  - **Copy to Clipboard** - Copies spectra that are currently visible in the Deconvolution Results window to the Clipboard for use with other applications.
  - **Print** - Lets you print the Deconvolution Results window.
  - **Export** - Lets you export data and graphics.
  - **Adjust Peak Threshold**- Lets you change the threshold for identifying peaks.
- 7 (optional) To change the number of significant digits in the deconvoluted spectrum, click  in the Deconvolution Results toolbar. Set the **Digits after the decimal value**, and click **OK**.
- 8 To compare two deconvoluted spectra:
  - a Highlight the two spectra of interest in the Deconvolution Results window.
  - b Click the **Deconvolution Mirror Plot Results** button .

The Deconvolution Mirror Plot Results window appears with the first spectra displayed as the top plot and the second spectra displayed as the inverted or bottom plot. See “[Deconvolution Mirror Plot](#)” on page 15.
  - c Click the  button to reverse the two plots.

See *online Help* for more display options in the Deconvolution Mirror Plot Results window.
- 9 (optional) To move the window, drag it to a new position on the screen. To return it to its default position, right-click the title bar and click the **Floating** command.


## To view deconvolution biomolecules

Biomolecules are created for proteins that are confidently found. You can view these compounds as follows:

- 1 If necessary, click **View > Biomolecules**.
- 2 If necessary, click **View > Biomolecule Identification Results**.
- 3 Click a biomolecule of interest. Associated data in the following windows is displayed:
  - Deconvolution Results window
  - Biomolecule MS Chromatogram window shows an EIC
  - Biomolecule MS Spectrum window displays all the different peaks in the raw  $m/z$  spectrum that indicate the presence of the deconvoluted protein mass in the Biomolecule MS Spectrum Results window
  - Biomolecule Identification Results if you also ran the Match Sequences algorithm.
  - Sequence Coverage Map, if you selected the Protein Digest workflow
- 4 Review the results in the Deconvolution Results window. See [“To view protein deconvolution results”](#) on page 27.
- 5 Click  on the Deconvolution Results toolbar.
- 6 View the following information for the deconvoluted spectrum in the Deconvolution Results window:

• Peak	• Charge	• Mass	• Height
• <i>Fit</i>	• Max Abund	• Expected $m/z$	• Area
- 7 Review the columns in the Biomolecule Identification Results window.
- 8 Right-click the table in the Biomolecule Identification Results window to see the shortcut menu. You can add and remove columns, copy to Clipboard, print, export and other features.

## To print a report with deconvolution results

- 1 Select **Method Automation > Reports** in the Method Editor window.
- 2 Review the options in the **Destination** section.
- 3 Review the parameters in both the **Templates** and **Layouts** tabs. Select the **Intact Protein**, the **Protein Digest**, and the **Released Glycans** report templates.
  - **IntactProteinReport.template.xml** (for intact proteins)
  - **ProteinDigestReport.template.xml** (for protein digests)
  - **ReleasedGlycanReport.template.xml** (for released glycans)
- 4 Click **Biomolecule Report** from the **File > Print** menu to print the report. You can instead click the  button on the main toolbar.






**Tip** To print deconvolution spectra, right-click the graph area of the Deconvolution Results window and click **Print** from the shortcut menu.

**Tip** A report is automatically created if you run Method Automation. Method Automation first runs the method workflow and then generates a biomolecule report. You can run Method Automation when you click **Method > Run Method Automation (Workflow + Reports)**.



## To automate protein confirmation

Use this procedure to do protein confirmation automatically for samples in a worklist. See the BioConfirm Familiarization Guide for more information.

- 1 In the Method Editor window, select Method Automation > Workflow and Sequences.
- 2 Select **Intact Protein** as the **Workflow**.
- 3 Select either **reduced** or **non-reduced** as the **Condition**.
- 4 Click the  button to select a sequence. The **Select Sequences** dialog box opens. Select the sequence or sequences, and then click **OK**.
- 5 Click the  button to select the Mods and Profiles. The **Select Modifications and Profiles** dialog box opens. Select the modifications and profiles, and then click **OK**.
- 6 Save the method in any of the following ways:
  - Click **Save** or **Save As** from the Method menu.
  - Click  or  in the Method Editor toolbar.
  - Click **Save Method** from Method Editor shortcut menu (right-click).
- 7 Assign this method to samples of interest when setting up a worklist as described in the online Help for your instrument.
- 8 To run this workflow interactively, do the following:
  - Click **Method > Run Method Workflow** *or*
  - Click the  button in the Sample Table.
- 9 To run this workflow and generate a report interactively, do the following:
  - Click **Method > Run Method Automation (Workflow + Reports)**.

# Protein Digest Workflow

The steps outlined below show the workflow for sequence matching with Agilent MassHunter BioConfirm Software.

**Step 1** - Open the data file of interest.

**Step 2** - Open a BioConfirm method or create a new one.

**Step 3** - Edit sequences if needed. You edit sequences in the Sequence Manager program.

- Add or edit the sequence text.
- Apply or edit modifications.
- Apply or edit links.
- Assign or edit digest reagents (Protein Digest sequences only).

**Step 4** - Select the **Protein Digest** workflow.

**Step 5** - Select the **Condition**.

**Step 6** - Select the **Sequences** to match.

If the sequence you want to match is not in the method, then:

- Import a sequence. You create and modify sequences in the Sequence Manager program.
- Select modifications and profiles

**Step 7** - Mark the **Enzymes** to include.

**Step 8** - Run the method workflow.

**Step 9** - Review the results.

**Step 10** - For protein digests only:

- View sequence coverage results in the Sequence Coverage Map window.

**Step 10** - Print report.

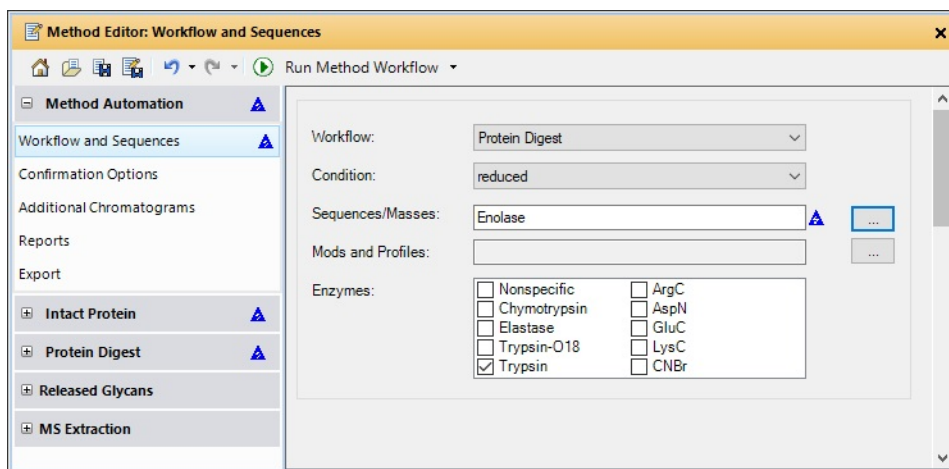
The topics in this section will help you get started using the Protein Digest workflow features of Agilent MassHunter BioConfirm Software.

- [“To run the Protein Digest workflow”](#) on page 36
- [“To find peptides”](#) on page 38
- [“To match sequences for protein digest biomolecules”](#) on page 39
- [“To view peptide biomolecules”](#) on page 41
- [“To view sequence coverage results for protein digests”](#) on page 42
- [“To print a report with peptide results”](#) on page 43


## To run the Protein Digest workflow

When you run a **Protein Digest** workflow, the workflow automatically does these steps:

- Find Peptides
  - Match Sequences
- 1 Open the data file that contains the spectra of interest as described in *online Help*.
  - 2 Open the *BioConfirmProteinDigest-Default.m* method. Click **Method > Open**.
  - 3 Select Method Automation > Workflow and Sequences.
  - 4 Select **Protein Digest** for the **Workflow**.
  - 5 Select either **reduced** or **non-reduced** for the **Condition**.
  - 6 Click the  button next to the **Sequences/Masses** parameter to select a sequence.
  - 7 Click the  button next to the **Mods and Profiles** parameter to select modifications and profiles.
  - 8 Mark the check box for the appropriate **Enzymes**. You cannot match sequences unless at least one Enzyme is marked.
  - 9 Save the method. Click **Method > Save As** to save to a new name.



**Figure 20** Workflow and Sequences section for a Protein Digest workflow

- 10 Click the  button on the Method Editor toolbar to run the method workflow. You could instead click **Method > Run Method Workflow**.
- 11 View biomolecules as described in “[To view peptide biomolecules](#)” on page 41.

## To find peptides

Use this procedure to run the Find Peptides algorithm to create a biomolecule list. This algorithm is part of the Protein Digest workflow.

The default method often does not need to be modified. The parameters for the Find Peptides algorithm are in the Protein Digest > Find Peptides section.

- 1 Open the data file.
- 2 Click **Method > Open** and open *BioConfirmProteinDigest-Default.m*.
- 3 Click **Find and Identify > Find Peptides**.
- 4 View biomolecules as described in [“To view peptide biomolecules”](#) on page 41.

## To match sequences for protein digest biomolecules

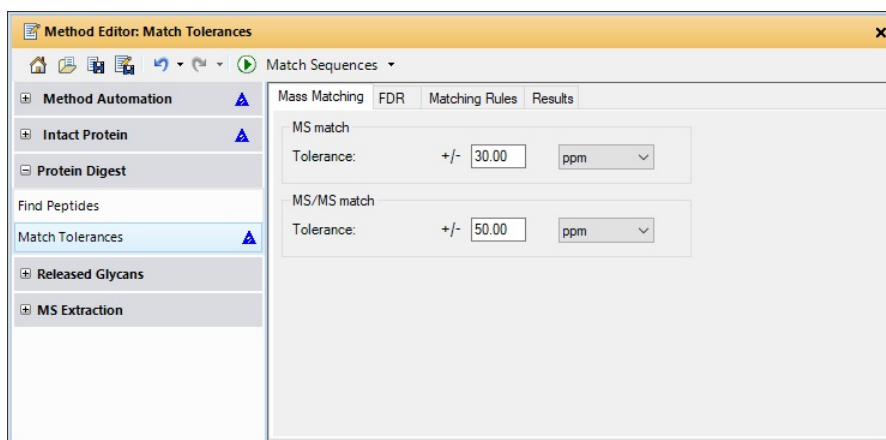
Use this procedure to match sequences for biomolecules that were found using Find Peptides. You need to specify the sequence and modifications and profiles in the Method Automation > Workflow and Sequences section.

- 1 Create biomolecules using Find Peptides. See “[To find peptides](#)” on page 38.
- 2 Select **Method Automation > Workflow and Sequences**.
- 3 Select **Protein Digest** for the **Workflow**.
- 4 Select either **reduced** or **non-reduced** for the **Condition**.
- 5 Click the  button next to the **Sequences/Masses** parameter to select a sequence.
- 6 Click the  button next to the **Mods and Profiles** parameter to select modifications and profiles.
- 7 Mark the check box for the appropriate **Enzymes**. You cannot match sequences unless at least one Enzyme is marked.
- 8 In the Method Editor window, select **Protein Digest > Match Tolerances** in the left pane.

Make sure to choose the Protein Digest > Match Tolerances section.

Do *not* select the Intact Protein > Match Tolerances section.


- 9 Review the parameters on the Mass Matching, FDR, Matching Rules, and Results tabs.



**Figure 21** Protein Digest > Match Tolerances section in the Method Editor

## Protein Digest Workflow

To match sequences for protein digest biomolecules

- 10 Click the  button on the Method Editor toolbar to run the Match Sequences algorithm.

**Tip** You can also start Match Sequences when you click **Find and Identify > Match Sequences**.

- 11 View peptide biomolecules as described in [“To view peptide biomolecules”](#) on page 41.



## To view peptide biomolecules

You can view these compounds as follows:


- 1 If necessary, click **View > Biomolecules**.
- 2 If necessary, click **View > Biomolecule Identification Results**.
- 3 Click a biomolecule of interest. The following windows are updated when you select a biomolecule:
  - Biomolecule MS Spectrum window
  - Biomolecule Fragment Spectrum window
  - Biomolecule Identification Results if you also ran the Match Sequences algorithm.
  - Sequence Coverage Map if you also ran the Match Sequences algorithm
- 4 To see other information for biomolecules in the list, right-click the table, and then click **Add/Remove Columns** from the shortcut menu.
- 5 See [“To view sequence coverage results for protein digests”](#) on page 42.
- 6 Review the columns in the Biomolecule Identification Results window.
- 7 Right-click the table in the Biomolecule Identification Results window to see the shortcut menu. You can add and remove columns, copy to Clipboard, print, export and other features.

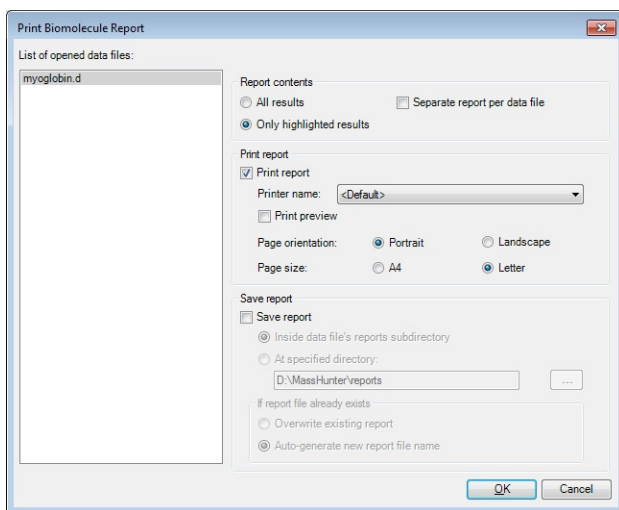
## To view sequence coverage results for protein digests

The location in the sequence which was matched is highlighted.

- 1 To display the Sequence Coverage Map window, click **View > Sequence Coverage Map**.
- 2 Click the following items on the Sequence Coverage Map shortcut menu to view more information about the current sequence:
  - **Applied Modifications**
  - **Applied Links**
  - **Show Sequence Description**
- 3 Select another sequence match result to view by selecting a different row in the Biomolecules window which has **Sequence Match** as the value in the **ID Techniques Applied** column.

## To print a report with peptide results

- 1 Select **Method Automation > Reports** in the Method Editor window.
- 2 Review the options in the **Destination** section.
- 3 Review the parameters in both the **Templates** and **Layouts** tabs. Select the **Protein Digest** report template.
  - **BioConfirmProteinDigestBiomoleculeReport.xltx** (for protein digests)
- 4 Click **Biomolecule Report** from the **File > Print** menu to print the report.  
You can instead click the  button on the main toolbar.



**Figure 22** Print Biomolecule Report Dialog Box

**Tip** To print deconvolution spectra, right-click the graph area of the Deconvolution Results window and click **Print** from the shortcut menu.

**Tip** A report is automatically created if you run Method Automation. Method Automation first runs the method workflow. It then extracts additional chromatograms, and then generates a biomolecule report. Finally, it exports the results. You can run Method Automation when you click **Method > Run Method Automation (Workflow + Reports)**.

# Released Glycans Workflow

The steps outlined below show the workflow for released glycans with Agilent MassHunter BioConfirm Software.

- Step 1** - Open the data file of interest.
- Step 2** - Open a BioConfirm method or create a new one.
- Step 3** - Select the **Released Glycans** workflow.
- Step 4** - Select the **Target glycan source**.
- Step 5** - Select the TAG in the Find Glycans section.
- Step 6** - Run the method workflow.
- Step 7** - Review the results.
- Step 8** - Print report.

The topics in this section will help you get started using the Released Glycans workflow features of Agilent MassHunter BioConfirm Software.

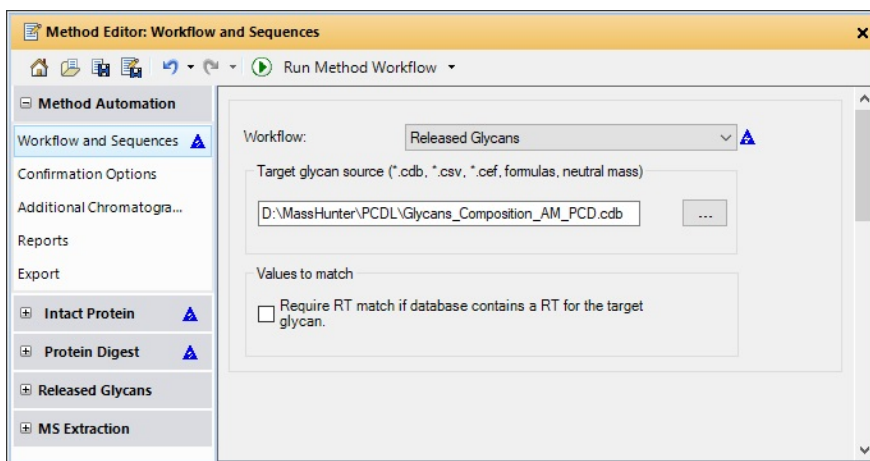
The topics in this section will help you get started using the Released Glycans workflow features of Agilent MassHunter BioConfirm Software.

- [“To run the Released Glycans workflow”](#) on page 45
- [“To find glycans”](#) on page 46
- [“To view released glycans biomolecules”](#) on page 47

## To run the Released Glycans workflow

When you run a **Released Glycans** workflow, the workflow automatically does these steps:

- Find Released Glycans
- 1 Open the data file that contains the data of interest.
- 2 Open the *BioConfirmReleasedGlycans-Default.m* method. Click **Method > Open**.
- 3 Select Method Automation > Workflow and Sequences in the Method Editor window.
- 4 Select **Released Glycans** for the **Workflow**.
- 5 Select the **Target glycan source**.
- 6 Mark or clear the **Require RT match if database contains an RT for the target glycan**.
- 7 Select **Released Glycans > Find Glycans**.
- 8 Click the **Tag** tab.
- 9 Select the tag that was used with this data file. 2-AB and InstantPC are defined in the software.
- 10 Save the method. Click **Method > Save As** to save to a new name.



**Figure 23** Workflow and Sequences section for a Released Glycans workflow

## Released Glycans Workflow

### To find glycans


**11** Click the  button on the Method Editor toolbar to run the method workflow. You could instead click **Method > Run Method Workflow**.

View biomolecules as described in [“To view released glycans biomolecules”](#) on page 47.

## To find glycans

Use this procedure to run the Find Glycans algorithm to create a biomolecule list. This algorithm is part of the Released Glycans workflow.

The parameters for the Find Glycans algorithm are in the Released Glycans > Find Glycans section. To find glycans, you need to specify the Target glycan source.

- 1** Open the data file.
- 2** Click **Method > Open** and open *BioConfirmReleasedGlycans-Default.m*.
- 3** Select **Released Glycans > Find Glycans** in the Method Editor window.
- 4** Enter the **Target glycan source**.
- 5** Click the **Tag** tab.
- 6** Select the tag that was used with this data file. 2-AB and InstantPC are defined in the software.
- 7** Click the  button on the Method Editor toolbar to run **Find Glycans**.

View biomolecules as described in [“To view released glycans biomolecules”](#) on page 47.

## To view released glycans biomolecules

You can view these compounds as follows:

- 1 Click Configuration > Windows Layouts > Load Layout.
- 2 Select *Default\_Released\_Glycans.xml* and click **Open**. The Biomolecules window and the Biomolecules Identification Results window are both open and tabbed in the lower left section of the main window.
- 3 Click a biomolecule of interest. The following windows are updated when you select a biomolecule:
  - Biomolecule MS Chromatogram
  - Biomolecule MS Spectrum window
  - Biomolecule Fragment Spectrum window
  - Biomolecule Identification Results window
  - Glycan Structure Viewer window (if a glycan structure is available)
- 4 To see other information for biomolecules in the list, right-click the table, and then click **Add/Remove Columns** from the shortcut menu.
- 5 Review the columns in the Biomolecule Identification Results window.

Right-click the table in the Biomolecule Identification Results window to see the shortcut menu. You can add and remove columns, copy to Clipboard, print, export and other features.




## Review Results

The topics in this section will help you get started using the Intact Protein workflow features of Agilent MassHunter BioConfirm Software.

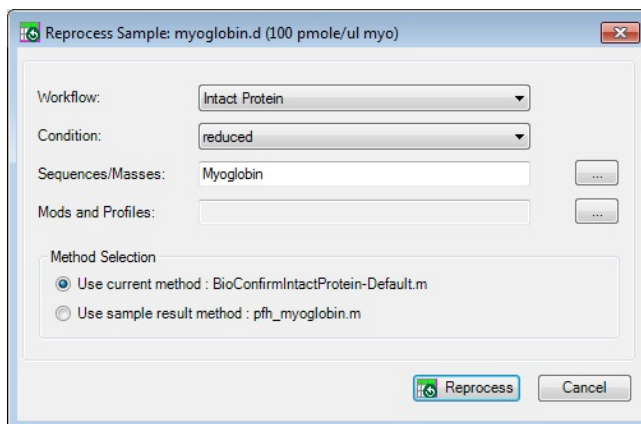
- “To reprocess samples” on page 48
- “To use Result Review mode” on page 50

### To reprocess samples

You can reprocess data files using the Sample Table toolbar, and you can review results in the usual windows.

- 1 Open multiple data files.
- 2 Examine the Confirmation Status column. The values can be **Confirmed**, **Partially Confirmed**, **Not Confirmed**, and **Undetermined**.
- 3 Select a row that is **Not Confirmed** or **Undetermined**.
- 4 (optional) Load a method to use with the sample you selected.
- 5 Click the  button in the Sample Table toolbar. The **Reprocess Sample** dialog box opens.
- 6 Select the **Workflow**.
- 7 If necessary, select the **Condition**.
- 8 Select the **Sequences/Masses**. Click the  button to select a different sequence.
- 9 If necessary, select the **Mods and Profiles**. Click the  button to select different modifications and profiles.
- 10 If the **Workflow** is **Protein Digest**, mark the **Enzymes** to use.
- 11 If you have a choice, click either **Use current method** or **Use sample result method**. You can reprocess using the current method in the BioConfirm program or you can reprocess using the same method that was used to create previous sample results.





**Figure 24** Reprocess Sample Dialog Box

**12** Click **Reprocess**.

**13** Review results. See “To view deconvolution biomolecules” on page 31 and “To view peptide biomolecules” on page 41 and “To view released glycans biomolecules” on page 47.

**14** Continue to reprocess other data files.

Sample Table: enolase-Chip-final.d						
Confirmation Status	Results		Workflow			
	Saved Results Method	Last Run Method	Workflow	Condition	Sequence / Mass	Modificat
Undetermined	pft_Synpep3.m		Intact Protein	reduced		
Confirmed	pft_Enolase-Chip-Final.m		Protein Digest	reduced	Enolase	
Partially confirmed	pft_Enolase-Chip-Final.m		Protein Digest	reduced	Enolase	
Undetermined	AJS-15min-MS-290-14-20-400-12.m		Intact Protein	reduced		
Undetermined	AJS-15min-MS-290-14-20-400-12.m		Intact Protein	reduced		
Confirmed	pft_myoglobin.m		Intact Protein	reduced	Myoglobin	
Undetermined	pft_Synpep3.m		Intact Protein	reduced		
Confirmed	pft_Synpep3.m		Protein Digest	reduced	Example synthetic peptide	

**Figure 25** Reprocess samples using the Sample Table

## To use Result Review mode

Result Review mode disables the Method Editor window and the **Find and Identify** menu. You can reprocess data files using the Sample Table toolbar, and you can review results in the usual windows.

- 1 Click the **Configuration > Enable Result Review (Disables Method Editing)**. It has a check mark next to the command when it is enabled.
- 2 Observe that the **Find and Identify** menu is grayed out.
- 3 The Method Editor window is closed if it was open.
- 4 Click the **Method** menu. Most commands are grayed out.
- 5 You can still reprocess samples. See [“To reprocess samples”](#) on page 48.
- 6 To end Result Review mode, click **Configuration > Enable Result Review (Disables Method Editing)**.

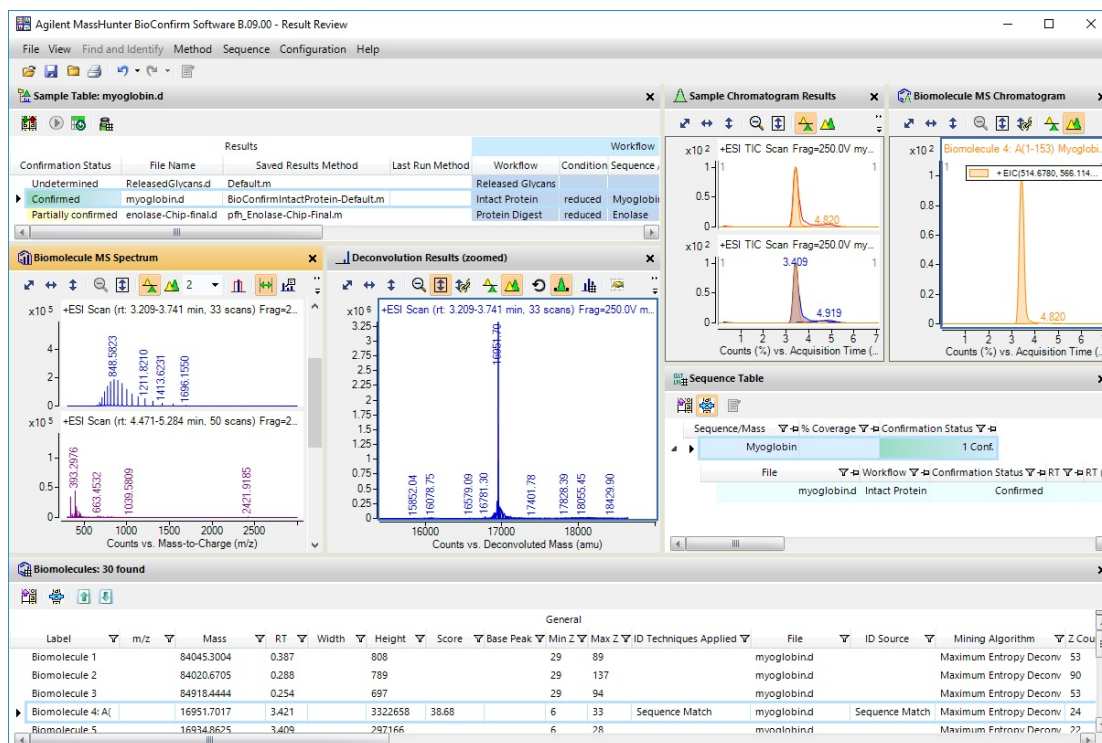


Figure 26 BioConfirm window in Result Review mode



## Setting Up Sequences

Topics in this section include:

- “To create or edit a sequence” on page 51
- “To add or edit the sequence text” on page 52
- “To apply or edit modifications” on page 53
- “To apply or edit links” on page 55

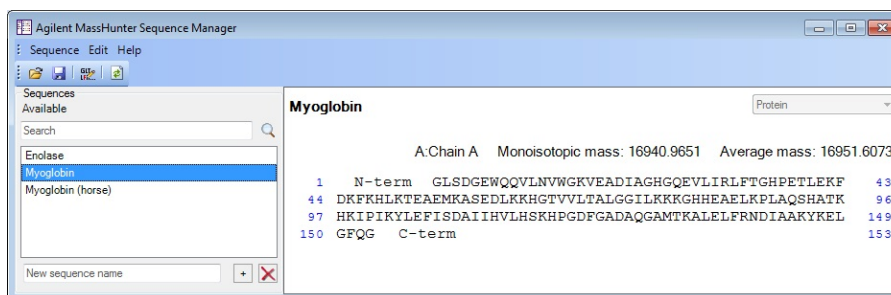
### To create or edit a sequence

You create and edit a sequence in the Sequence Manager program.

- 1 Open the Sequence Manager program. In the BioConfirm program, click **Sequence > Sequence Manager**. You can also click the Sequence Manager () shortcut or click **All Programs > Agilent > MassHunter Workstation > Sequence Manager B.09.00**. In Windows 10, you click the Windows icon and then click **Agilent MassHunter BioConfirm > Sequence Manager B.09.00**.
- 2 To add a sequence, type a sequence name and click the  button in the bottom left corner.
- 3 To edit a sequence, click the sequence in the left pane of the Sequence Manager.
- 4 In the Sequence Manager:
  - a Add or edit the sequence text as described in “To add or edit the sequence text” on page 52.
  - b Apply or edit modifications as described in “To apply or edit modifications” on page 53. *Note that modifications are not currently supported for Oligonucleotide sequences.*
  - c Apply or edit links as described in “To apply or edit links” on page 55. *Note that links are not currently supported for Oligonucleotide sequences.*
- 5 Click the **Sequence > Export Sequences** to save the sequence for use with a method.

## To add or edit the sequence text

- 1 In the Sequence Manager program, select the sequence of interest.
- 2 Select the correct chain to add or edit. Chain A is selected by default.
- 3 Click the sequence in the right pane.



**Figure 27** Sequence Manager program

- 4 Enter or edit the amino acids in the sequence text box in either of the following ways.
  - Type in individual amino acids one at a time between the N-term and C-term symbols.
  - Copy the sequence from a FASTA-formatted database or a text file. Right-click the sequence in the Sequence Manager and click **Paste** from the shortcut menu. The amino acid sequence will appear in the Sequence box, between the N-term and C-term symbols.

### NOTE

Only single-character (letter) amino acids are allowed for the protein sequence parameter. Three character amino acid symbols are not supported.

## To apply or edit modifications

- 1 In the Sequence Manager program, select the sequence of interest. *Note that modifications are not currently supported for Oligonucleotide sequences.*
- 2 Right-click that sequence, and click **Edit Modifications** from the shortcut menu to open the **Modifications** dialog box.
- 3 To select and apply global modifications:
  - a On the Global tab, select the desired modification from the list of **Available modifications**.
    - If desired, you can customize the list of available modifications using the Chemical Data Dictionary; *see the online Help for more information.*
    - Note that many N-linked glycans with numerical abbreviations, such as “2000 0A 0G”, are included in the Chemical Data Dictionary; *see online help for more information.*
  - b Select the amino acids to modify in the **Apply to all** list. Use **Shift+click** or **Ctrl+click** to select multiple amino acids, if desired.
  - c Click **Apply** to apply the specified modification to the selected amino acids throughout the sequence.
  - d Repeat [step a](#) - [step c](#) to select and apply other global modifications.
- 4 To select and apply local modifications:
  - a On the Local tab, select the location in the sequence to modify: **C-terminus**, **N-terminus**, or the position in the sequence. Use the blue index numbers shown for each row of the sequence in the Sequence Manager program to determine the proper index number for the selected position.
  - b Select the desired modification from the **Applicable modifications** list.
  - c Mark the **Variable modification** check box if you want to create a variable modification for sequence matching. When marked, sequences that contain that amino acid site are matched both *with and without* this modification.
  - d Click **Apply** to apply the selected modification to the specified location (amino acid) in the sequence.
  - e Repeat [step a](#) - [step d](#) to select and apply other local modifications.
- 5 Click **OK** to close the **Modifications** dialog box. Note that the molecular weight and formula have been updated in the Sequence Manager.

#### To remove modifications

- 1 Click **Edit Modifications** on either the Edit menu or the Sequence shortcut menu to open the **Modifications** dialog box.
- 2 Click the **Applied** tab and review the list of modifications.
- 3 Click to select the modifications you want to delete.
- 4 Click **Delete** to remove the selected modifications.
- 5 Click **OK**.

#### To migrate method from B.07.00 to B.08.00

It is recommended that you open an old method in B.08.00 and save it before using it through the Data Acquisition worklist or Walkup program.

- 1 In BioConfirm B.08.00, load a method saved from BioConfirm B.07.00.
- 2 Load the data file of interest.
- 3 Run **Find by Protein Deconvolution**.
- 4 Click **Sequence > Open Chemical Data Dictionary Editor**.
- 5 Create a custom modification profile, with name such as B07Mods.
- 6 Search for custom modification(s).

Once found, the modifications appear in the list of Available modifications.

- 7 Add the custom modification(s) found in the previous step to the custom modification profile created earlier (with a name such as B07Mods). You can add preset modifications to the custom profile as well.
- 8 Click **OK** to close the Chemical Data Dictionary and save the changes.

#### To use the migrated modification profile

- 1 In the Sequence Manager program, import the sequence of interest.
- 2 Click **Sequence > Edit Matching Rules** to open the Rules dialog box.
- 3 Move the Modification profile created above from the **Available modifications** list to the **Selected modifications** list, and then click OK.
- 4 Save the sequence.

## To apply or edit links

- 1 In the Sequence Manager program, select the sequence of interest in the left pane. *Note that links are not currently supported for Oligonucleotide sequences.*
- 2 Right-click the sequence in the pane on the right side, and click **Edit Links** from the shortcut menu to open the **Links** dialog box. You can also click **Edit > Edit Links**.
- 3 Enter the index number for one end of the link in the **From index** box and select the chain (if other than the default Chain A).
- 4 Enter the index number for the other end of the link in the **To index** box and select the chain (if other than the default Chain A).
- 5 Select a link from the **Link types** list.

**Tip** You can customize the list of links types using the Chemical Data Dictionary; *see online help for more information.*

- 6 Click **Apply** to link the selected amino acids in the sequence.
- 7 Repeat Steps 3 -6 to create additional links.
- 8 Click **OK** to close the Links dialog box. Note that the molecular weight and formula have been updated in the Sequence Editor.

## To remove links

- 1 Click **Edit Links** on either the Sequence menu or the Sequence Manager shortcut menu to open the **Links** dialog box.
- 2 Click the **Applied** tab and review the list of links.
- 3 Click the links you want to delete.
- 4 Click **Delete** to remove the selected links.

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## **In This Guide**

This guide has instructions for installing and using the Agilent MassHunter BioConfirm Software.

This guide is valid for the B.09.00 revision or higher of the G6829AA Agilent MassHunter BioConfirm Software, until superseded.

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