Agilent MassHunter BioConfirm Software

Quick Start Guide

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

Agilent Technologies

Where to find more information

- *Agilent MassHunter BioConfirm Software Familiarization Guide*
- *Online Help* provides in-depth information and can be displayed in the following ways:
  - Click **Contents**, **Index**, 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.
- 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.08.00

Major changes and enhancements

- BioConfirm is a separate program. It can be installed and uninstalled separately from the MassHunter Qualitative Analysis program.
- BioConfirm user interface has been highly streamlined and tightly focused for the Intact Protein and Protein Digest workflows.
- Result review mode allows batch review with ability to change simple parameters and then reprocess the sample for biologists.
- BioConfirm can automatically detect an appropriate mass range for deconvolution for your sample to allow detection of characterized proteins and variants.
- BioConfirm can automatically choose between the TIC or BPC MS chromatogram for intact protein deconvolution based on the signal to noise ratio for a given sample.
- The Method Automation section of the Method Editor allows you to run a workflow with a single click.
- BioConfirm considers disulfide bonds when calculating intact protein match so that you can analyze your native intact protein.
- BioConfirm can perform disulfide bond scrambling calculations for protein digest.
- Excel 2016 is supported.
- BioConfirm automation is available in the Data Acquisition program and in the Walkup program.
- The Agilent Sequence Manager program allows you to add, edit or remove sequences. You can also import and export sequences and manage chains and modifications for a sequence.

Sequence improvements

- The Sequence Coverage Map window has been simplified.
- When a sample with the same sequence, modifications, enzymes, and condition (non-reduced) is matched more than one time, the second time and onward the result generation for sequence matching is significantly faster.

- You can specify a default sequence in the Method Editor which is used if you don't specify one in the worklist.
- Multiple chains are shown in the same list in the Sequence Coverage Map window.
- You can limit the number of modifications per biomolecule in the Chemical Data Dictionary Editor.

Navigation improvements

- You can extract an EIC when you double-click a Biomolecule MS or MS/MS spectrum. This EIC is automatically integrated.
- The new Sample Table provides quick and easy navigation between results of different samples.
- You can reprocess a sample in the Sample Table and change the Workflow, the Condition, the Sequence, the Mods and Profiles, and the Enzymes.
- The Method Editor window contains a navigation pane to switch between sections in the method.
- You can set confirmation options for Intact Protein and Protein Digest workflows and then see the confirmation status in the Sample Table window.
- The Biomolecule Table has column super headers for better data interpretation: General, Sequence Match, and Molecular Feature.

Workflow improvements

- You can run a workflow on the selected sample in the Sample Table window with a single click.
- You can automatically extract additional chromatograms, specify the report to run, and specify how to export the results during method automation.
- You can interactively see how your method will behave when run through method automation.
- For Intact Protein workflow, you can set the Matching Rule for whether or not to allow protein truncation.
- For Protein Digest workflow, you can set the Matching Rule for whether or not to allow free cysteines (non-reduced condition).
- You can specify a different report template for Intact Protein and Protein Digest workflows.

- You can create a custom workflow in Method Automation. You specify the actions to run for this custom workflow.

- You can extract a TWC and use it as the starting chromatogram from the Intact Protein workflow.

- You can specify the maximum number of missed cleavages when you are using the Peptide Digest workflow.

Result windows improvements

- Comparison of Biomolecule Chromatograms from multiple files is available within Sample Chromatogram Results window.

- Comparison of Biomolecule Spectra from multiple files is available within Biomolecule MS Spectrum Results window.

- You can pin a deconvoluted spectra to the Deconvolution Results window.

- You can see the spectrum peak list for the deconvoluted spectrum in the Deconvolution Results window.

- You can see the integration peak list for the sample chromatograms in the Sample Chromatogram Results window.

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 2013 or 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 2 and Figure 2 on page 9.
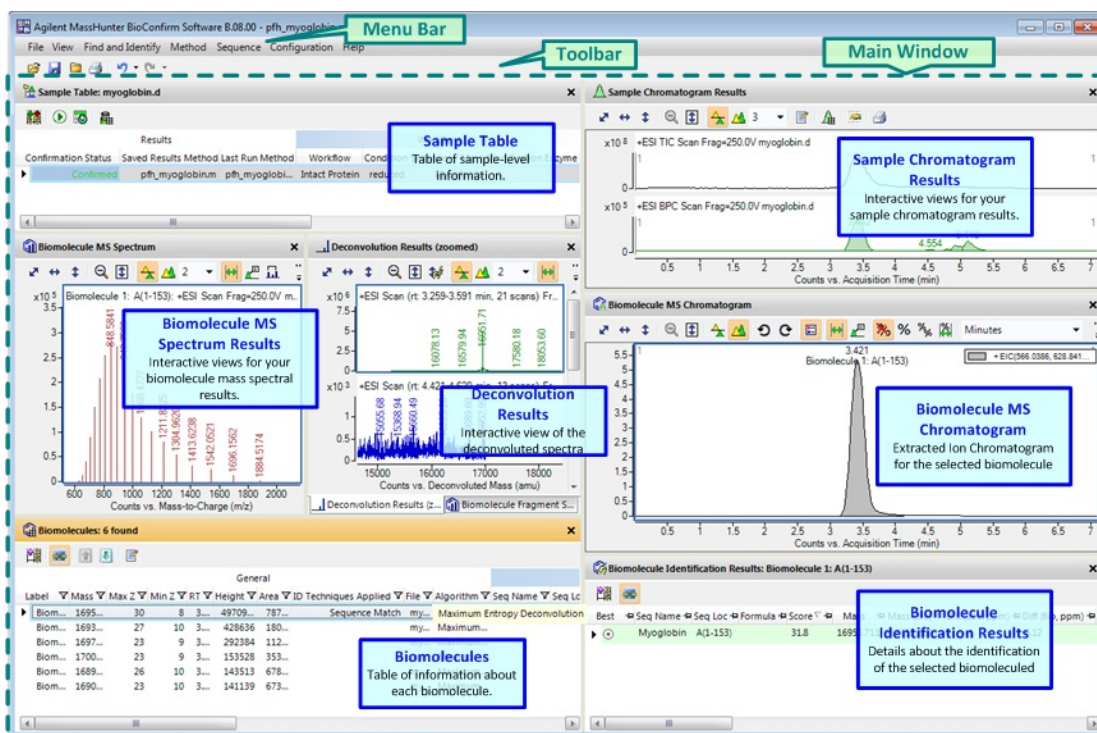

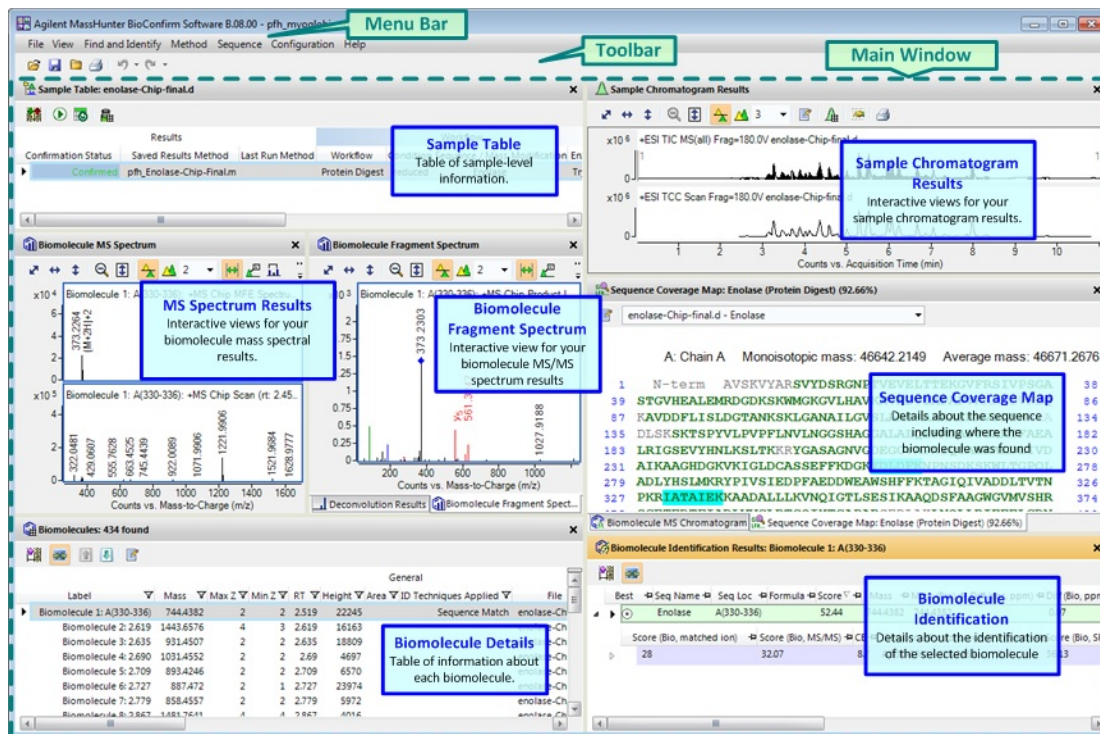**Figure 1**   The main functional areas of BioConfirm for an Intact Protein

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

1. Menu Bar

The menu bar (Figure 3 on page 9) provides actions that are used for finding and identifying biomolecules, printing and exporting reports, and editing sequences.
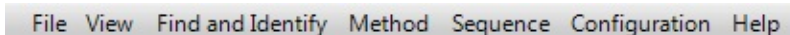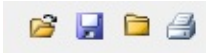

Figure 3   Menu bar

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. The last two icons allow you to undo or redo the last actions performed.

Toolbar Icon	Action
	• File > Open Data File • File > Save Results • File > Close Data File • File > Print > Biomolecule Report
	• Undoes last action performed • Redoes last action undone.

3. Main window

The main window, see Figure 2 on page 9, is further divided into up to eleven 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, and Sequence Coverage Map. 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.
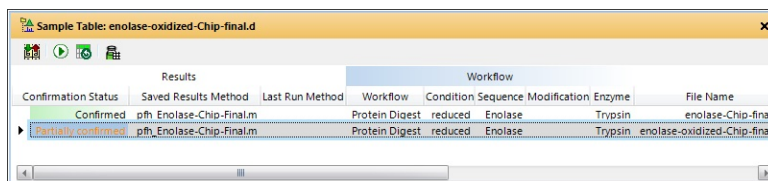

Figure 4   Sample Table window

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**.
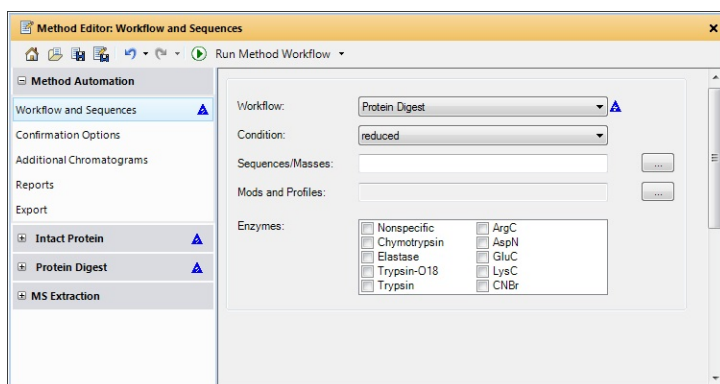
Figure 5   Method Editor window

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).
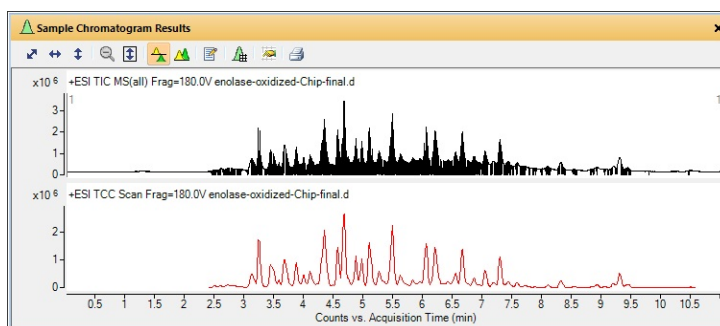

Figure 6   Sample Chromatogram Results window

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.
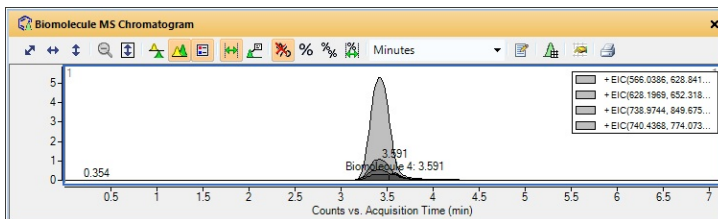
Figure 7 Biomolecule MS Chromatogram window

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.
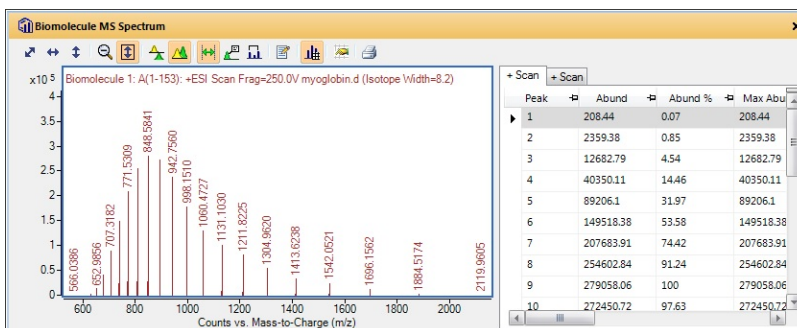

Figure 8 Biomolecule MS Spectrum window

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.
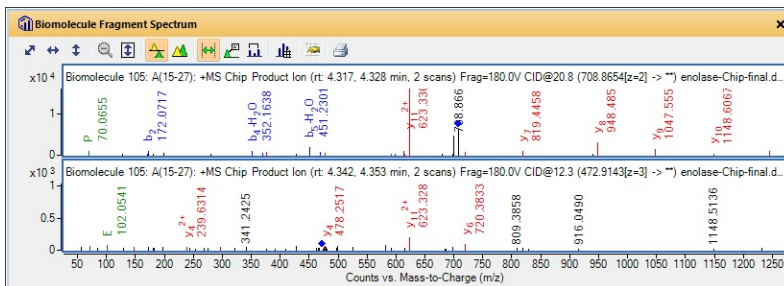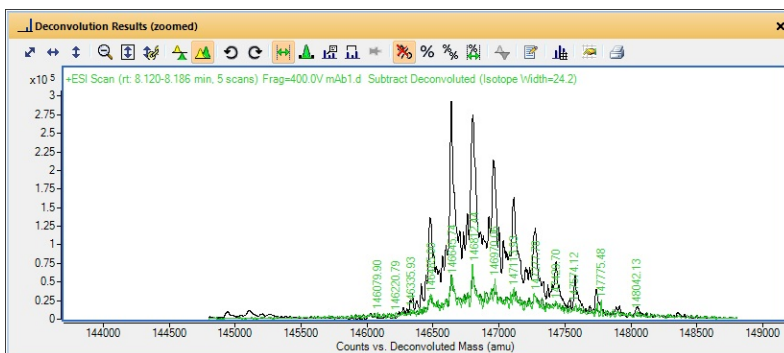
Figure 9   Biomolecule Fragment Spectrum window

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.
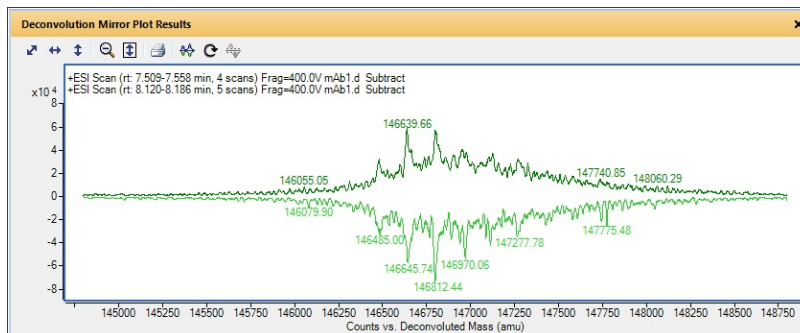

Figure 10   Deconvolution Results window in **Overlaid** mode

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 11   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.

Figure 12   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.

Figure 13   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.

Figure 14   Sequence Coverage Map 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 50.

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 three workflows: **Intact Protein**, **Protein Digest**, and **Custom**.

Intact Protein

The workflow runs the Find Peptides algorithm, digest sequence using the enzyme selected, and then runs Protein Digest 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 by Protein Deconvolution algorithm and uses protein matching rules (Intact Protein, Predicted Modifications). You can select whether or not to use Protein Truncation. The workflow also runs the Match Sequence algorithm with the Sequence you entered. See Figure 15 on page 18. A Biomolecule report is generated using the **Protein Digest 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**.
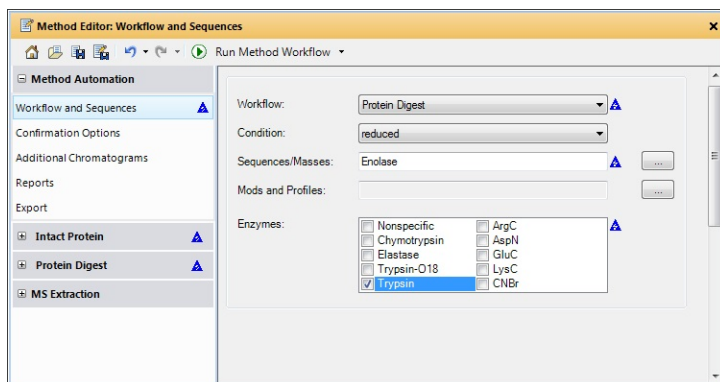
Figure 15   Method Automation > Workflow and Sequences section

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 Fast-A file (fasta). 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. You can view a sequence in
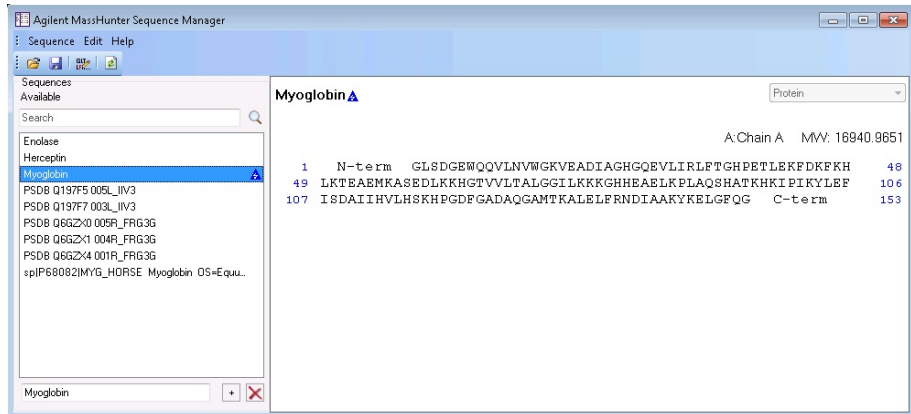the BioConfirm program.

Figure 16   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.

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.

Guidelines

- 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.

- For these BioConfirm analysis, use Find by Protein Deconvolution.

- Use **Find Peptides** for synthetic peptides, and peptides from protein digests with the **Target data type** on the **Find Peptides > Extraction** tab set to **Small molecules (chromatographic)**.

- For other BioConfirm analyses, use **Find Peptides**. You can set the **Target data type** to **Small Molecules (infusion)** or **Large molecules (proteins, oligos)**.

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 **native** for the **Condition**.

6 Click the [...] button next to the **Sequence/Masses** parameter to select a sequence.

7 Click the [...] button next to the **Mods and Profiles** parameter to select modifications and profiles.

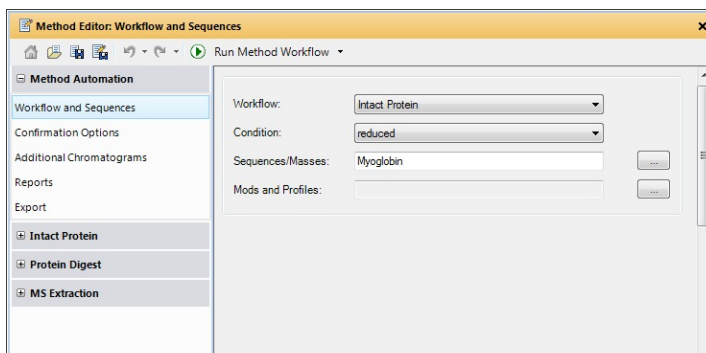**8** Save the method. Click **Method > Save As** to save to a new name.

Figure 17 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 30.

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 30.

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**.

7 View deconvolution biomolecules as described in "To view deconvolution biomolecules" on page 30.

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 23 or "To deconvolute selected spectra" on page 24.

2 Select Method Automation > Workflow and Sequences.

3 Select **Intact Protein** for the **Workflow**.

4 Select either **reduced** or **native** for the **Condition**.

5 Click the ⬚ button next to the **Sequence/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 30.

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 22
- "To find biomolecules by Protein Deconvolution" on page 23
- "To deconvolute selected spectra" on page 24

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. Click **Unzoom** from the shortcut menu to return to the previous display scale.

Tip   To return to the previous display scale, click the **Unzoom** toolbar button or click **Unzoom** from the shortcut menu.

5 Use the following toolbar buttons 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).
	• 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. • When the **Autoscale y-axis during Zoom** mode is off, the vertical plot range is set to the limits you specify by dragging the mouse.
	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.
	Stops normalizing the deconvolution spectra.

Toolbar button	Action/Meaning
%	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.
�ⱶⱶ	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 Deconvoluted Spectra Display Options 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:

- **Create Biomolecule from Highlighted Range on Deconvoluted Spectrum > Add Highest Peak** - Creates a new biomolecule using the highest peak in the selected range.

- **Create Compound from Highlighted Range on Deconvoluted Spectrum > Add Average Mass** - Creates a new biomolecule using the average mass in the selected range.

- **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.

- **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.

7 (*optional*) To change the number of significant digits in the deconvoluted spectrum, click **Configuration > Deconvoluted Spectra Display Options**. 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 13.

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 are displayed:

- Deconvolution Results window
- Biomolecule MS Chromatogram Results window shows an EIC
- Biomolecule MS spectrum 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 26.

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
- *Fit*
- 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 options in the Contents section. Verify that the sections that you want included in the report are marked.

4 Review the parameters in both the **Templates** and **Layouts** tabs. Select both the **Intact Protein report template** and the **Protein Digest report template**.

   • **BioConfirmProteinBiomoleculeReport.xslt** (for intact proteins)

   • **BioConfirmProteinDigestBiomoleculeReport.xslt** (for protein digests)

5 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**.

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 the **Save** button 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.

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 35
- "To find peptides" on page 37
- "To match sequences for protein digest biomolecules" on page 38
- "To view peptide biomolecules" on page 40
- "To view sequence coverage results for protein digests" on page 41
- "To print a report with peptide results" on page 42

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 **Sequence/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.
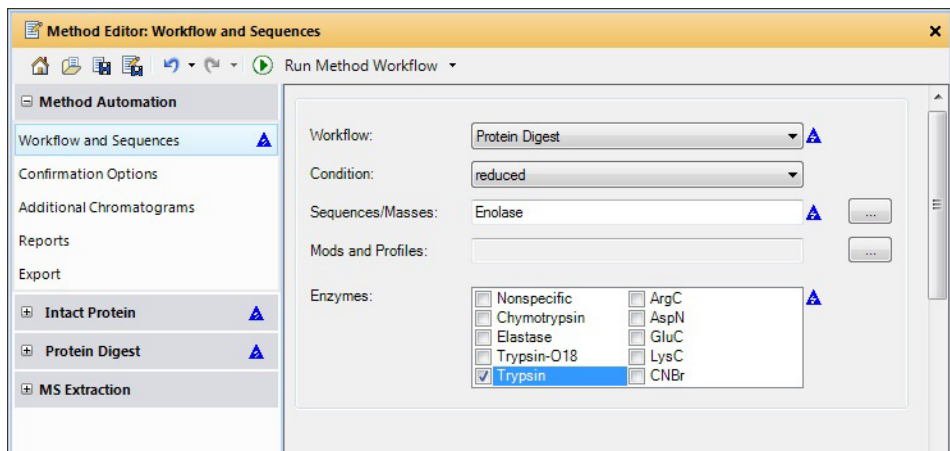
Figure 18   Workflow and Sequences section for a Protein Digest workflow

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 40.

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 40.

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 37.

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 **Sequence/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, Matching Rules, and Results tabs.
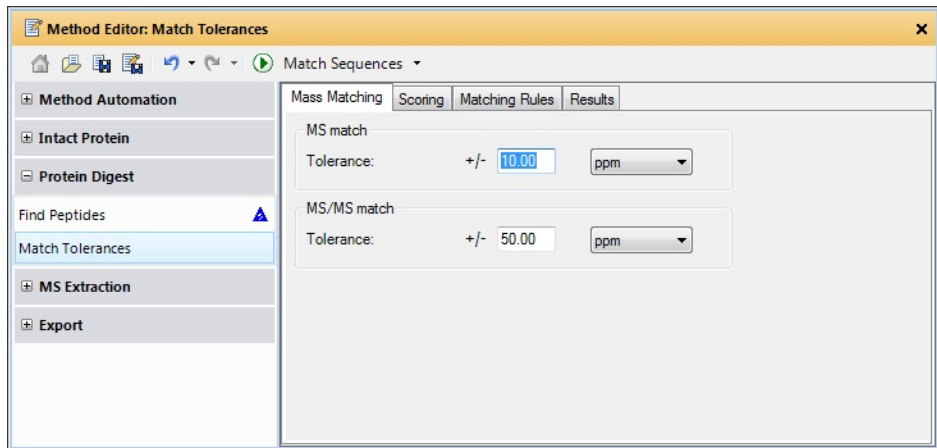
Figure 19   Protein Digest > Match Tolerances section in the Method Editor

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 40.

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 41.

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.
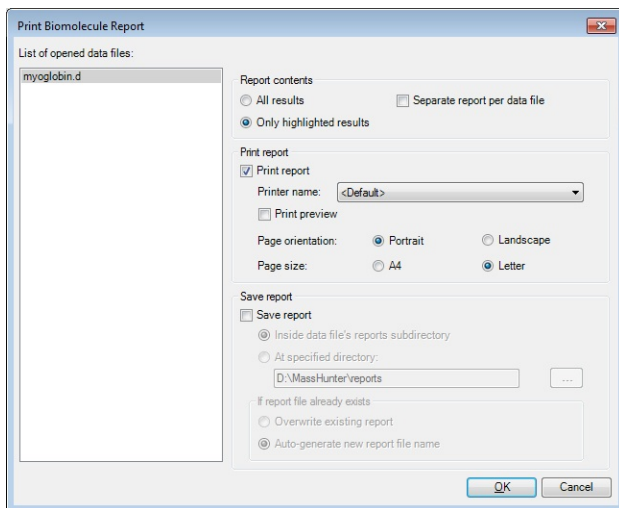
Figure 20 Print Biomolecule Report Dialog Box

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 and then generates a biomolecule report. You can run Method Automation when you click **Method > Run Method Automation**.

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 43
- "To use Result Review mode" on page 45

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 Select the **Condition**.

8 Select the **Sequences/Masses**. Click the [...] button to select a different sequence.

9 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.
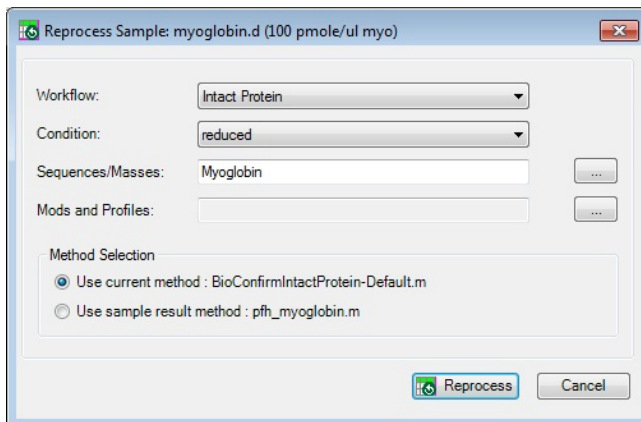
Figure 21   Reprocess Sample Dialog Box

12 Click **Reprocess**.

13 Review results. See "To view deconvolution biomolecules" on page 30 and "To view peptide biomolecules" on page 40.

14 Continue to reprocess other data files.

Figure 22   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. The **Method Editor** command is grayed out.

5 You can still reprocess samples. See "To reprocess samples" on page 43.

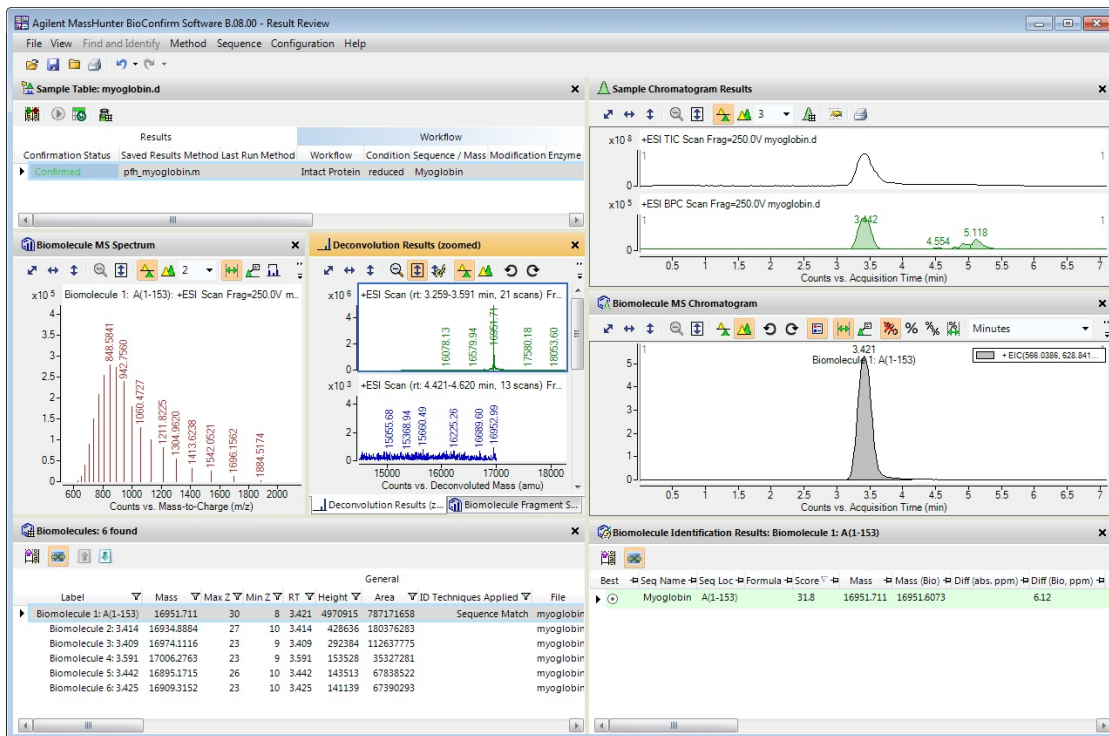6 To end Result Review mode, click **Configuration > Enable Result Review (Disables Method Editing)**.

Figure 23 BioConfirm window in Result Review mode

Setting Up Sequences

Topics in this section include:

- "To create or edit a sequence" on page 46
- "To add or edit the sequence text" on page 47
- "To apply or edit modifications" on page 48
- "To apply or edit links" on page 50

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 icon]) shortcut or click **All Programs > Agilent > MassHunter Workstation > Sequence Manager B.08.00**.

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 47.

   b Apply or edit modifications as described in "To apply or edit modifications" on page 48. *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 50. *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.
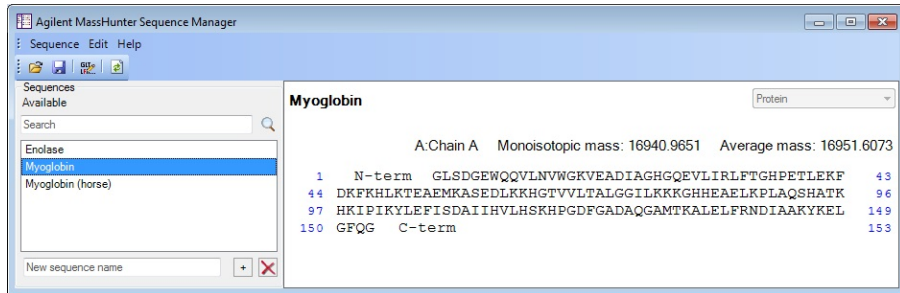
Figure 24   Sequence Manager program

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 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.08.00 revision or higher of the G6829AA Agilent MassHunter BioConfirm Software, until superseded.

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