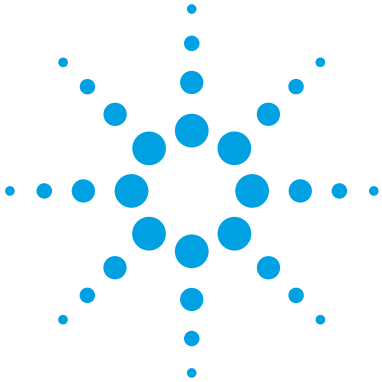


Agilent MassHunter BioConfirm Software

Familiarization Guide

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

Agilent Technologies

Where to find more information

- *Agilent MassHunter BioConfirm Software Quick Start Guide*
- Online Help provides in-depth information and can be displayed in the following ways:
  - Click **Contents**, **Index**, or **Search** from the BioConfirm software Help menu.
  - Press the **F1** key to get more information about a window or dialog box.

How to use this guide

Try to do these familiarization exercises initially using the steps listed in the first column. Then if you need more information, follow the detailed instructions in the second column.

Before you start

Copy the data files used for these tasks onto your hard disk as follows:

1. Copy all of the data files from the **Data** directory on the BioConfirm setup disk to your computer hard drive.
2. Make sure you have both read and write permissions for the folder you just created on your computer. This is required if you want to save results.
   a. In Windows Explorer right-click the folder where you copied the data files and click **Properties** from the shortcut menu.
   b. *Clear* the **Read-only Attributes** check box if it is marked.
   c. In the Confirm Attribute Changes dialog, click **Apply changes to this folder, subfolders, and files**, then click **OK**.

Basic Tasks

Task 1. Open the BioConfirm program

In this task you open multiple data files using the current method.

Task 1. Open the BioConfirm program with multiple data files

Steps	Detailed Instructions	Comments
1 Open the BioConfirm program. • Open the data files, **mAb1.d**, **mAb2.d** and **myoglobin.d** in the folder **\\MassHunter\Data**, or in the folder where you copied them. • Make sure that the **Use current method** button is clicked.	**a** Double-click the **Agilent MassHunter BioConfirm B.08.00** icon . The system displays the Open Sample dialog box. **b** Go to the folder **\\MassHunter\Data** or to the folder where the example files are located.	• You can get help for any window, dialog box, or tab by pressing the **F1** key when that window is active.

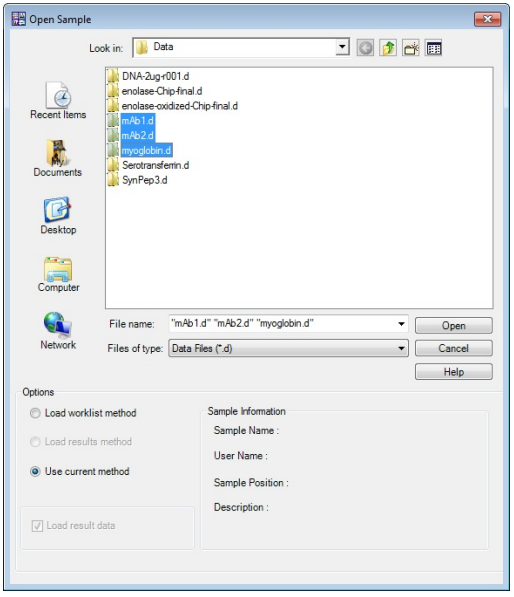


Figure 1 Open data files when opening software

Task 1. Open the BioConfirm program with multiple data files (continued)

Steps	Detailed Instructions	Comments
	c Press and hold the **Shift** key while you click **mAb1.d**, **mAb2.d** and **myoglobin.d**. **d** Click **Open**. All three data files are displayed in the Sample Table window. The selected sample in the Sample Table is also shown in the Sample Chromatogram Results window. **e** Click the **List Mode** icon in the Sample Chromatogram Results toolbar.	• If you press the **Ctrl** key, you can pick files which are not directly next to each other in the list. • What you see in the main window at this point depends on the method, layout, display and plot settings used before you opened these files. • When you click the List Mode icon, the background of the icon changes to orange.
2 Return the main window to the default layout.	**a** Click **Configuration > Window Layouts > Restore Default Layout**. **b** Click the down arrow next to the Maximum Number of List Panes icon in the Sample Chromatogram Results Toolbar, and select **3**.	• You click the  button in the graphics window to change the display options. • You can change the layout if you click **Configuration > Window Layouts > Load Layout**.

Task 1. Open the BioConfirm program with multiple data files (continued)

Steps	Detailed Instructions	Comments

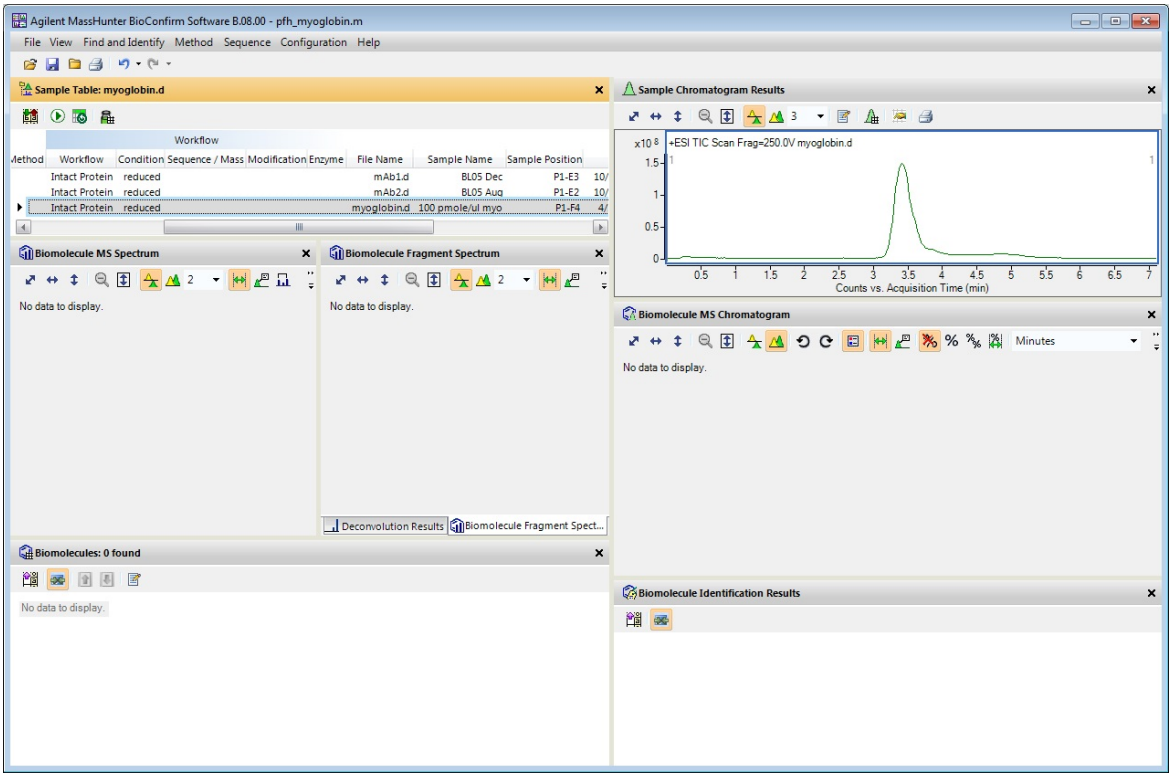


Figure 2 Qualitative Analysis main window in the Data Navigator view with the General Workflow selected.

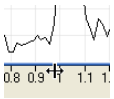
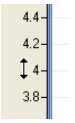
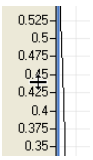
Task 2. Zoom in and out of the chromatogram

In this task, you become familiar with the zoom in and zoom out features of the BioConfirm program.

Task 2. Zoom in and out of the chromatogram

Steps	Detailed Instructions	Comments
1 Practice zooming in and out on the chromatogram in the Sample Chromatogram Results window. • Zoom in twice on the peak. • Zoom in one more time autoscaling the y-axis. • Zoom out once to the previous zoom position. • Completely zoom out to the original chromatogram.	**c** Click the right mouse button and drag over an area on the last peak. Make sure that the **Autoscale Y-axis during Zoom** icon, , is not selected for this step. **d** Repeat step b. **e** Click the **Autoscale Y-axis during Zoom** icon, , in the toolbar. **f** Click the right mouse button again and drag over an area of the peak for the third time. The BioConfirm program automatically scales the y-axis to the largest point in the range. **g** Click the **Unzoom** icon, , to undo the last zoom operation. You can undo the last fifteen zoom operations. **h** Click the **Autoscale X-axis and Y-axis** icon, , to zoom out completely.	• You can also use these zoom features on spectra in the Biomolecule MS Spectrum window, the Biomolecule Fragment Spectrum window, the Deconvolution Results window, the Deconvolution Mirror Plot window, and the Biomolecule MS Chromatogram window. • A selected icon has an orange background color.

Task 2. Zoom in and out of the chromatogram (continued)

Steps	Detailed Instructions	Comments
2 Practice zooming in and out on each axis separately. • Zoom in only along the x-axis. Hint: Right-click the x-axis values and move cursor from left to right. • Partially zoom out the x-axis. Hint: Move cursor in opposite direction. • Completely zoom out of the x-axis. • Repeat the previous steps for the y-axis.	**a** To zoom in on the x-axis, move the cursor to the x-axis values until a horizontal double arrow appears. **b** Click the right mouse button and drag the new cursor from left to right across the x-axis values. **c** To zoom out on the x-axis, click the right mouse button and drag from right to left on the x-axis values. **d** Click the **Autoscale X-axis** icon, ↔, to completely zoom out on the x-axis. **a** To zoom in on the y-axis, move the cursor to the y-axis values until a vertical double arrow appears. **b** Click the right mouse button and drag the new cursor from bottom to top across the y-axis values. **c** To zoom out on the y-axis, click the right mouse button and drag from the top towards the bottom of the y-axis values. **d** Click the **Autoscale Y-axis** icon, ↕, to completely zoom out on the y-axis.	 **Horizontal Double Arrow**  **New cursor appears when you right-click the x-axis value**  **Vertical Double Arrow**  **New cursor appears when you right-click the y-axis values.**

Task 3. Change window layouts

In this task, you move windows within the main view and create various window layouts.

Task 4. Change window layout

Steps	Detailed Instructions	Comments
1 Change the window layout: • Change the window size. • Save a window layout. • Unlock the layout. • Change the Chromatogram Results window to be floating. • Move the Chromatogram Results window. • Display the tools for repositioning the windows.	• To change the size of a window, drag the boundary between the windows. • To save a window layout, click **Configuration > Window Layouts > Save Layout**. • To unlock a layout, click **Configuration > Window Layouts > Lock Layout**. • To make a window float, right-click the title bar of the window, and click **Floating** from the shortcut menu. • To move a window, click the title bar of the window and drag the window to the desired location. • To display the repositioning tools, drag the window over one of the other windows. When one window is overlapped with another, the program displays several layout tools, as shown in Figure 3.	• If the layout is locked, the system displays a check mark next to the Lock Layout menu. • You can only use the repositioning tools when the layout is unlocked. • You can also make a window float by double-clicking the title bar of the window. • If the BioConfirm program is installed, it has several different workflows and layouts.

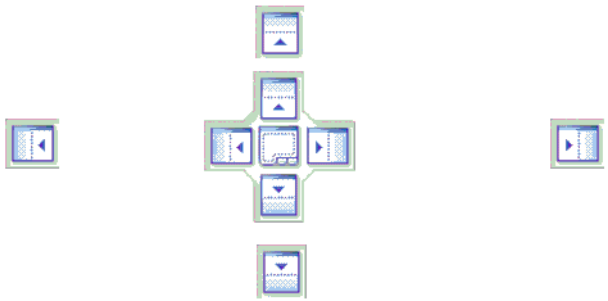


Figure 3 Window repositioning tools

Task 4. Change window layout (continued)

Steps	Detailed Instructions	Comments
2 Reposition the Sample Chromatogram Results window. • Move the window so that it is at the top, to the left, to the right and then at the bottom of the other windows. • Move two windows together so that they are on top of one another and available only through the tabs at the bottom. • Restore the default layout.	• If you drag the cursor over one of the smaller icons, the window you are dragging will be placed above, to the right, below, or to the left of all of the other windows. • Drag the cursor over the larger icon. The window can also be placed above, to the right, below, or to the left of the other window by dragging the cursor over the edges of the larger icon. • To tab two windows together, drag the cursor over the center of the larger icon. You will see a shadow version of the two windows tabbed together. Stop dragging the mouse. The two windows will be tabbed together. • Click **Configuration > Window Layouts > Restore Default Layout**.	• The cursor must be over one of the arrows in a box in order for repositioning to occur. • Clicking the **Restore Default Layout** command restores the layout.

Task 4. Creating a Protein Sequence File

This task guides you through the creation of a myoglobin sequence file that you will use in "Exercise 1. Interactive Intact Protein Workflow" on page 11 and "Exercise 2. Automated Intact Protein Workflow" on page 15.

Steps	Detailed Instructions	Comments
1 Start the Agilent MassHunter Sequence Manager.	Click **Sequence > Sequence Manager**.	

Steps	Detailed Instructions	Comments
2 Create a new sequence.	**a** Type Myoglobin for the name of the Sequence. **b** Click the + button. The Sequence Editor pane opens automatically with a new sequence displayed for editing.	• Protein is automatically selected for the sequence type.
3 Enter the amino acid sequence shown below into the Sequence Manager.	• Type in individual amino acids one at a time between the N-term and C-term symbols.	• Use the single-character (letter) amino acids abbreviations.
GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEK FDKFKHLKTEAEMKASEDLKKHGTVVLTALGGILKKKGHHEAE LKPLAQSHATKHKIPIKYLEFISDAIIHVLHSKHPGDFGADAQG AMTKALELFRNDIAAKYKELGFQG		• Tip: If you are reading this document as a PDF file on your computer, you can copy and paste the sequence into the Sequence Manager window.

Note: The myoglobin sequence does not have any links or modifications, but some sequences do. In that case, add links and modifications as described in the *Quick Start Guide* or *online Help*.

4 Save the sequence as the name *iii*_**myoglob.psq**, where *iii* represents your initials.	**a** Click **File > Export Sequences**. **b** Type *iii*_myoglob in the File name box. **c** Click **Save**.	• The sequence is saved as a **.psq** file that can be imported for use in other methods as described in Exercise 4 or referenced from worklists as described in Exercise 5.

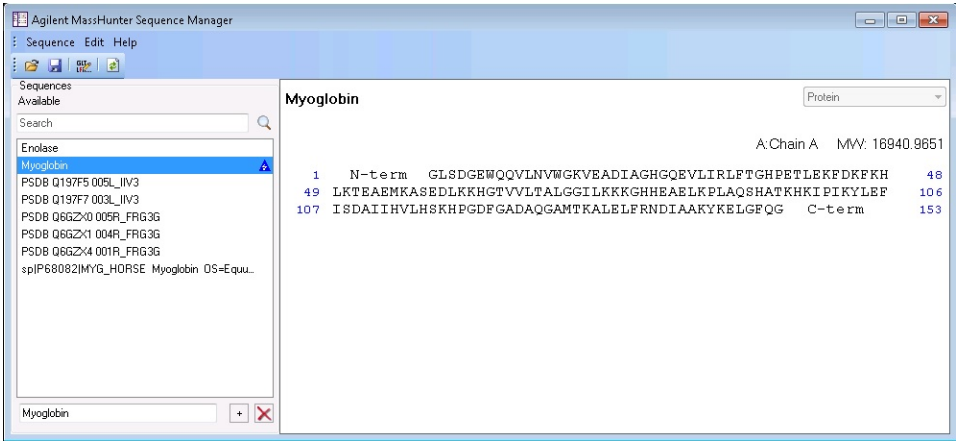


Figure 4 Creating a sequence file of myoglobin in the Sequence Manager program

Intact Protein Workflow

Step 1 - Open the data file of interest.

Step 2 - Open a BioConfirm Method or create a new one.

Step 3 - Edit sequences if necessary in the Sequence Manager program:

- Add or edit the sequence text.
- Apply or edit modifications
- Apply or edit links

Step 4 -Select **Intact Protein** for the **Workflow** on the **Workflow and Sequences** tab. Select the **Condition**.

Step 5 - Select the **Sequence/Masses** to match on the Workflow and Sequences tab. If the sequence you want to match is not in the method or Sample Table, then:

Import or create a sequence.

Step 6 - Select the **Mods and Profiles** on the Workflow and Sequences tab.

Step 7 - Run the Method Workflow.

Step 8 - Review the results which are shown in these windows:
Sample table
Biomolecules table
Biomolecule Identification Results
Sequence Coverage Map
Biomolecule MS Chromatogram
Biomolecule MS Spectrum
Biomolecule Fragment Spectrum

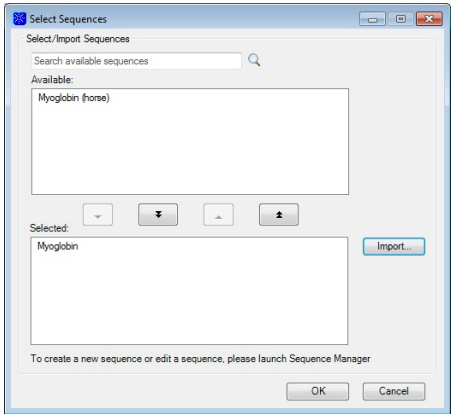
Step 9 - Print report.

Exercise 1. Interactive Intact Protein Workflow

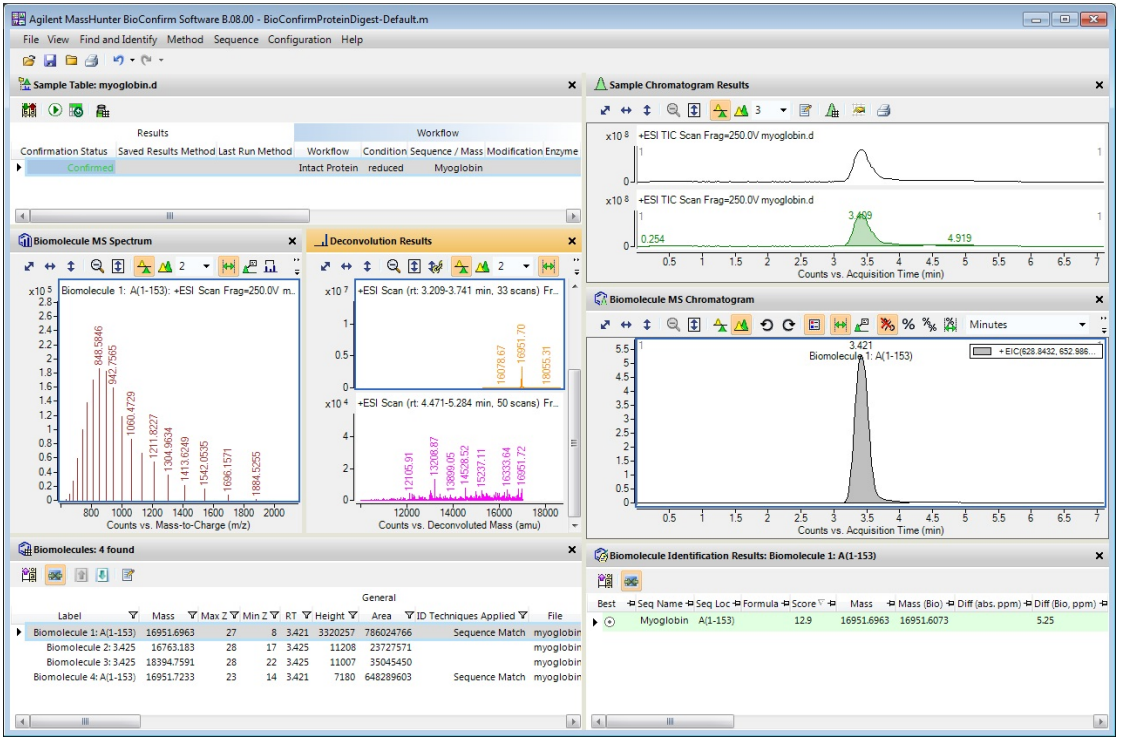
This exercise shows you how to set method parameters, match an intact protein sequence, and view the results. This exercise uses the *iii*_**myoglob.psq** sequence file created in Exercise 3 and the **myoglobin.d** data file copied before you started. See "Before you start" on page 2.

If you select the Intact Protein workflow, the Find by Protein Deconvolution algorithm runs and uses protein Matching Rules (Intact Protein, and Predicted Modifications). You can select whether or not Protein Truncation is done.

Steps	Detailed Instructions	Comments
1 Open the method to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select the **BioConfirmIntactProtein-Default.m** folder. **c** Click **Open**.	
2 If the **myoglobin.d** data file is not already open, open it.	**a** Click **File > Open Data File**. **b** Locate the **myoglobin.d** folder. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Display the Deconvolute (Protein) section in the Method Editor window.	**a** Click **View > Method Editor** if the method editor is not visible. **b** Select **Intact Protein > Deconvolute (Protein)** in the Method Editor window.	
4 Run the Find by Protein Deconvolution algorithm.	**a** Review the settings and modify them if necessary. **b** Click  on the Method Editor toolbar to start the Find by Protein Deconvolution algorithm. **c** Review the results in the Biomolecules window.	• In this case you are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*.
5 Display the Workflow and Sequences section in the Method Editor window.	• Click **Method Automation > Workflow and Sequences** in the Method Editor window.	
6 Import the myoglobin sequence.	**a** Select **Intact Protein** for the Workflow. **b** Select **reduced** for the Condition. **c** Click the  button next to the **Sequences** parameter. The **Select Sequences** dialog box opens. **d** If myoglobin is not available, click **Import**. **e** Select *iii*_**myoglob.psq** and click **Open**. **f** Verify that the Myoglobin sequence is in the **Selected** list. **g** Click **OK**.	• The *iii*_**myoglob.psq** sequence file was created in Exercise 3. • For this exercise, you will use the sequence as is, but you can add modifications and links to sequences as described in *online Help* and the *Quick Start Guide*.

Steps	Detailed Instructions	Comments
		
7 Start the match search.	**a** Click **Intact Protein > Match Tolerances**. **b** Select the first biomolecule in the Biomolecules window. **c** Click  on the Method Editor toolbar.	Alternate methods: • Click **Find and Identify > Match Sequences**.
8 Review the results.	**a** Display the Biomolecule Identification Results window. If it is not visible, click **View > Biomolecule Identification Results**. **b** Select the **Biomolecule 1** row in the Biomolecules table.	

Steps	Detailed Instructions	Comments



9 Save the method for use in Exercise 5.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_**myoglobin.m**, where *iii* represents your initials. **c** Click **Save**.	

Exercise 2. Automated Intact Protein Workflow

This exercise guides you through the setup of a worklist to automatically confirm the presence of myoglobin in a previously acquired sample. This exercise uses the *iii*_**myoglob.psq** sequence file created in Exercise 1 and the **myoglobin.d** data file copied in Exercise 1.

Steps	Detailed Instructions	Comments
1 If not already open, open the method *iii*_**myoglobin.m**.	**a** Click **Method > Open**. **b** Select the *iii*_**myoglobin.m** folder. **c** Click **Open**.	This method was created in "Exercise 1. Interactive Intact Protein Workflow" on page 11.
2 Select Intact Protein as the workflow.	**a** Click **Method Automation > Workflow and Sequences** in the Method Editor window. **b** Select **Intact Protein** as the **Workflow**.	
3 Display the Workflow and Sequences section in the Method Editor.	• Click **Method Automation > Workflow and Sequences** in the Method Editor window.	
4 Use the **Intact Protein** Workflow.	• Select **Intact Protein** for the Workflow.	• In this case you are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*. • Actions are executed in the order they appear in the list. You can reorder them using the Up and Down arrow buttons to the right of the list
5 Save the method.	• Click **Method > Save**.	
6 Create a worklist of one sample in the MassHunter Workstation Data Acquisition software.	**a** Display the Worklist pane. **b** Click **Worklist > Add Sample**. A new sample row is added to the Worklist table.	If you plan to do batch data analysis using the worklist, consider using the DA Reprocessor tool that is installed with Agilent MassHunter Workstation Software - Data Acquisition program.

Steps	Detailed Instructions	Comments
7 Specify the myoglobin sequence file, *iii*_**myoglob.psq**, in the worklist.	**a** Click **Worklist > Add Column**. **b** When the Add Column dialog box appears, select **Protein** as the Column Type. **c** Enter the **Column** name as **Protein.** **d** Select the *iii*_**myoglob.psq** file as the Value. **e** Click **OK**.	The *iii*_**myoglob.psq** file was created in Exercise 3.
8 Edit the acquisition method to link to the iii_myoglobin.m method.	**a** Open the Method Editor window in Data Acquisition. **b** Click the **DA** tab. **c** Click the **BioConfirm** tab. **d** Mark the **BioConfirm Automation** check box. **e** Click either the **Copy** or **Link** button. **f** Select the **iii_myoglobin.m** method. **g** Save the method to ***iii*_acqmyoglobin.m**.	The method iii_myoglobin.m was saved in Step 9 above. The *iii* represents your initials
9 Select parameters in the worklist. *iii*_**myoglobin.m** as the **Method** myoglobin.d as the Data File *iii*_**myoglob.psq** as the **Protein**.	**a** Select **iii_acqmyoglobin.m** in the Method in the Worklist. **b** Enter **myoglobin.d** in the data file. **c** Select ***iii*_myoglob.psq** in the **Protein** column.	The *iii* represents your initials
10 Set up to run the worklist for data analysis only.	**a** Click **Worklist > Worklist Run Parameters**. **b** Select **DA Only** as **Part of method to run**. **c** Select the paths for the method and data file, and then click **OK**.	
11 Run the worklist.	Click **Worklist > Run**.	
12 Review the printed Biomolecule reports.		

Protein Digest Workflow

The steps outlined below show the workflow for Protein Digest 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 necessary in the Sequence Manager program:

- Add or edit the sequence text.
- Apply or edit modifications
- Apply or edit links

Step 4 -Select the **Workflow** on the **Workflow and Sequences** tab. Select the **Condition**.

Step 5 - Select the **Sequence/Masses** to match on the Workflow and Sequences tab.
If the sequence you want to match is not in the method or Sample Table, then:

Import or create a sequence.

Step 6 - Select the **Mods and Profiles** on the Workflow and Sequences tab.

Step 7 - Mark the **Enzymes** on the Workflow and Sequences tab.

Step 8 - Run the Method Workflow.

Step 8 - Review the results which are shown in these windows:
Biomolecules table
Biomolecule Identification Results
Sequence Coverage Map
Biomolecule MS Spectrum
Biomolecule Fragment Spectrum

Step 9 - Print report.

Exercise 3. Interactive Protein Digest Sequence Matching

This exercise shows you how to confirm protein digests interactively.

If you select the Protein Digest workflow, the Find Peptides algorithm runs and uses the enzyme selected in the Workflow and Peptides section and then runs the Protein Digest matching rules. See "Before you start" on page 2.

Steps	Detailed Instructions	Comments
1 Open the method to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select the **BioConfirmProteinDigest-Default.m** folder. **c** Click **Open**.	• The parameters in the BioConfirmProteinDigest-Default.m method are a good starting point for Protein Digests.
2 Open the example sample file.	**a** Click **File > Open Data File**. **b** Locate the **Enolase-Chip-final.d** folder. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Review the parameters in the Find Peptides section in the Method Editor window.	**a** Select **Protein Digest > Find Peptides** in the Method Editor window. **b** Review the settings on the various tabs of the Find Peptides section. **c** Click the **Extraction** tab. **d** Review the parameters. For the example file, you can restrict the mass range to 300 - 1700. **e** In the Extraction tab, click **Use peaks with height >=** button and enter `500` counts.	• You can change the default parameters as described in the next steps. You can also use the method without any changes. • For some data files, you will need to use different parameters as described in the *online Help*. • A very low peak height filter can result in greater sequence coverage but requires much more time to process.
4 For MS/MS data, set parameters to extract MS/MS spectra.	**a** Click the **Results** tab of Find Peptides in the Method Editor. **b** Verify that the **Extract MS/MS Spectrum** check box is marked. **c** Verify that the **Deisotope MS/MS Spectrum** check box is marked.	

Steps	Detailed Instructions	Comments
5 Find biomolecules.	**a** Click  on the Method Editor toolbar to start the biomolecule search. **b** When processing is complete, review the results in the Biomolecules window.	• You can instead click **Find and Identify > Find Peptides**.
6 Import the sequence.	**a** Click **Sequences > Default Sample Prep** in the Method Editor window. **b** Select **Protein Digest** as the **Workflow**. **c** Select **reduced** as the **Condition**. **d** Click the next to the **Sequences** parameter. **e** Click **Import**. **f** Select **EnolaseDigest.psq**. **g** Click **Open** in the **Select Protein Sequence File(s)** dialog box. **h** Click **OK** in the **Select Sequences** dialog box. **i** Mark the **Trypsin** check box under **Enzymes**.	• You can choose either the Default Sample Prep section or the Method Automation > Workflow and Sequences section. They are the same. • For this exercise, you use the sequence as is, but you can add modifications and links to sequences as described in *online Help*. • You can customize the list of available reagents using the Chemical Data Dictionary; see *online Help* for more information.
7 Review the Matching Rules.	**a** Click the Matching Rules tab in the Match Tolerances section in the Method Editor. **b** Mark the **Allow free cysteines (non-reduced condition)** check box. **c** Enter 2 for the **Allow missed cleavages up to**.	
8 Review parameters on the Scoring tab.	**a** Click the **Scoring** tab in the Match Tolerances section of the Method Editor window. **b** Review the parameters.	For MS/MS data, you can adjust the following parameters on the **Scoring** tab: • **MS/MS score** to increase or decrease its contribution to the overall Score (Bio). • **MS/MS scored peak intensity** and **MS/MS matched ion score** contribute to Score (Bio MS/MS).
9 Save the method for use in Exercise 7.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_Enolase-Chip-Final.m, where *iii* represents your initials. **c** Click **Save**.	

Steps	Detailed Instructions	Comments
10 Start the match search.	• Click **Find and Identify > Match Sequences**.	*Alternate methods*: • Click  on the Method Editor toolbar. • Click **Match Sequences** on the Method Editor shortcut menu.
11 Review the results.	**a** Highlight Biomolecule 1 in the Biomolecules window. **b** If the Biomolecule Identification Results window is not visible, click **View > Biomolecule Identification Results**. **c** When you open the window, the window displays the results for the first biomolecule that is selected in the Biomolecules window. **d** Select another sequence match result to view by selecting a different compound in the Biomolecules windows.	• If the biomolecule was identified, the **ID Techniques Applied** column contains **Sequence Match**.
12 View sequence coverage results.	**a** Click **View > Sequence Coverage Map**. **b** Select a different biomolecule in the Biomolecules table to view a different result.	
13 Save the results	**a** Click File > Save Results to save your results to the data file folder.	• You can also click  to save results.
14 Repeat the interactive processing with *enolase-oxidized-chip-final.d*.	**a** Open the data file **enolase-oxidized-chip-final.d** (see step 2). **b** Select Find Peptides in the Method Editor and verify the parameters(step 3). **c** Find compounds (step 5). **d** Match sequences (step 10). **e** Save the results to the second data file (step 13).	• Most of the processing parameters used for the first data file are the same for the second data file.
To view more information.	Click the following items on the Sequence Coverage Map window shortcut menu to view more information about the sequence: • **Applied Modifications** • **Applied Links** • **Show Sequence Description**	

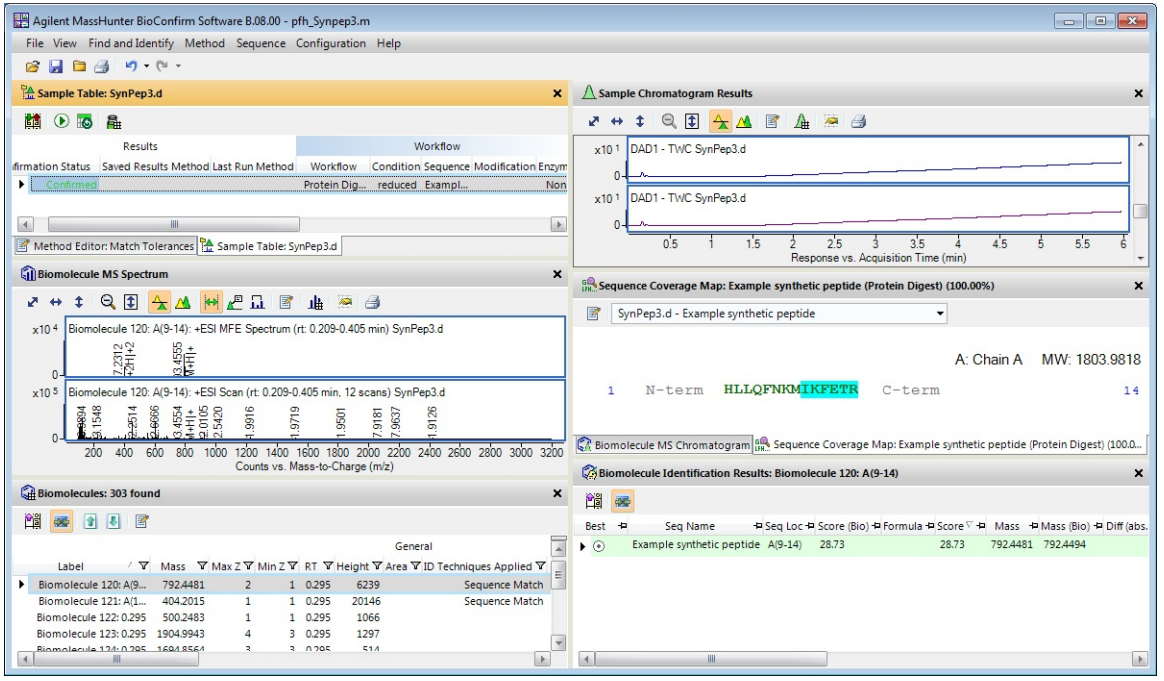
Exercise 4. Interactive Synthetic Peptide Sequence Matching

This exercise shows you how to set method parameters, import a sequence, match a synthetic peptide sequence, and view the results.

Steps	Detailed Instructions	Comments
1 Open the method **BioConfirmProteinDigest-Default** to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select the **BioConfirmProteinDigest-Default.m** folder. **c** Click **Open**.	
2 Open the SynPep3.d data file.	**a** Click **File > Open Data File**. **b** Locate the **SynPep3.d** folder. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window
3 Display the **Find Peptids** section in the Method Editor window.	• Select **Protein Digest > Find Peptides** in the Method Editor window.	
4 For MS/MS data, set parameters to extract MS/MS spectra.	**a** Click the **Results** tab of Find Peptides section in the Method Editor. **b** Verify that the **Extract MS/MS Spectrum** check box is marked. **c** Verify that **Deisotope MS/MS Spectrum** is marked.	
5 Find compounds.	**a** Review the settings and modify them if necessary. **b** Click  on the Method Editor toolbar to start the biomolecule search. **c** Review the results in the Biomolecules window.	In this case you are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*.

Steps	Detailed Instructions	Comments
6 Import the sequence.	**a** Click the **Method Automation > Workflow and Sequences** section of the Method Editor window. **b** Select **Protein Digest** as the **Workflow**. **c** Select **reduced** as the **Condition**. **d** Click the [...] next to the **Sequences** parameter. **e** Click **Import**. **f** Select **SynPep3.psq**. **g** Click **Open** in the **Select Protein Sequence File(s)** dialog box. **h** Click **OK** in the **Select Sequences** dialog box. **i** Clear the **Trypsin** check box under **Enzymes**. **j** Mark the **Nonspecific** check box under **Enzymes**.	For this exercise, you will use the sequence as is, but you can add modifications and links to sequences as described in *online Help* and the *Quick Start Guide*.
7 Display the Match Tolerances section in the Method Editor window and review the parameters.	**a** Click **Protein Digest > Match Tolerances** in the Method Editor window. **b** Review these parameters.	Make sure to select the Match Tolerances in the Protein Digest section.
8 Save the method for use in Exercise 9.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_SynPep3.m, where *iii* represents your initials. **c** Click **Save**.	
9 Start the match search.	Click **Find and Identify > Match Sequences**.	Alternate methods: • Click  on the Method Editor toolbar. • Click **Match Sequences** on the Method Editor shortcut menu.
10 Review the results.	**a** Click **View > Biomolecule Identification Results** window. **b** Select the first Biomolecule which has identification results. **c** Select another sequence match result to view by selecting a different biomolecule in the Biomolecules window.	• If Match Sequences found a result, then the **ID Techniques Applied** column contains **Sequence Match**.

Steps	Detailed Instructions	Comments



Exercise 5. Automated Protein Digest Sequence Matching

This exercise guides you through the setup of a worklist to automatically confirm the presence of serotransferrin in a previously acquired sample.

If you select the Protein Digest workflow, the Find Peptides algorithm runs and uses the enzyme selected in the Workflow and Peptides section and then runs the Protein Digest matching rules.

Steps	Detailed Instructions	Comments
1 Open the method.	**a** Click **Method > Open**. **b** Select the *iii*_**Enolase-Chip-Final.m** folder. **c** Click **Open**.	This method was created in Exercise 6 (*iii* represents your initials).
2 Display the Method Automation > Workflow and Sequences section in the Method Editor.	**a** If the Method Editor is not visible, click **View > Method Editor**. **b** Click **Method Automation > Workflow and Sequences** in the Method Editor window.	
3 Select the appropriate workflow.	**a** Select Protein Digest for the Workflow. **b** Select the **Condition**. **c** Verify that Enolase is the sequence. **d** Mark the **Trypsin** check box.	The Protein Digest workflow automatically runs the following actions: • Find Peptides • Match Sequences • Generate Biomolecule Report.
4 Save the method.	Click **Method > Save**.	
5 Create a worklist of one sample in the MassHunter Workstation Data Acquisition software and add a Protein column.	**a** Display the Worklist window. **b** Click **Worklist > Add Sample**. A new sample row is added to the Worklist table. **c** Click **Worklist > Add Column**. **d** When the Add Column dialog box appears, select **Protein** as the Column Type. **e** Enter the Column name as **EnolaseDigest.** **f** Select **EnolaseDigest.psq** as the Value. **g** Click **OK**.	If you plan to do batch data analysis using the worklist, consider using the DA Reprocessor tool that is installed with MassHunter Workstation Software - Data Acquisition program. In that case, skip Step 10 below.

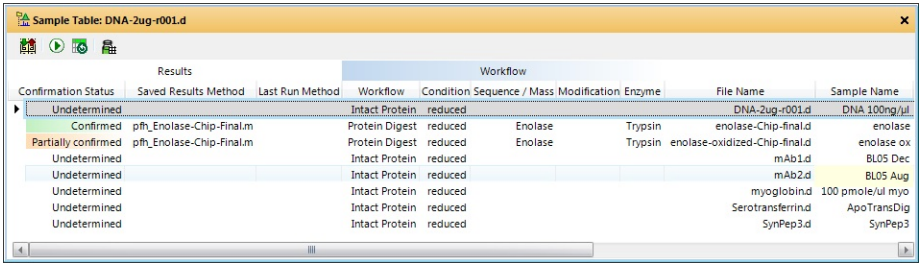
Steps	Detailed Instructions	Comments
6 Edit the acquisition method to link to the iii_myoglobin.m method.	**a** Open the Method Editor window in Data Acquisition. **b** Click the **DA** tab. **c** Click the **BioConfirm** tab. **d** Mark the **BioConfirm Automation** check box. **e** Click either the **Copy** or **Link** button. **f** Select the *iii*_**Enolase-Chip-Final.m** method. **g** Save the method to ***iii*_acqEnolase-Chip-Final.m**.	• The method iii_Enolase-Chip-Final.m was saved in "Exercise 3. Interactive Protein Digest Sequence Matching" on page 18. • The *iii* represents your initials
7 Enter parameters for the new sample.	**a** Select *iii*_**acqEnolase-Chip-Final.m** in the **Method** column of the worklist. **b** Enter **Enolase-Chip-Final.d** in the Data File column of the worklist.	
8 Set up to run the worklist for data analysis only.	**a** Click **Worklist > Worklist Run Parameters**. **b** Select **DA Only** as **Part of method to run**. **c** Select the paths for the method and data file, and then click **OK**.	
9 Run the worklist.	Click **Worklist > Run**.	
10 Review the printed Biomolecule reports.		

Review Results

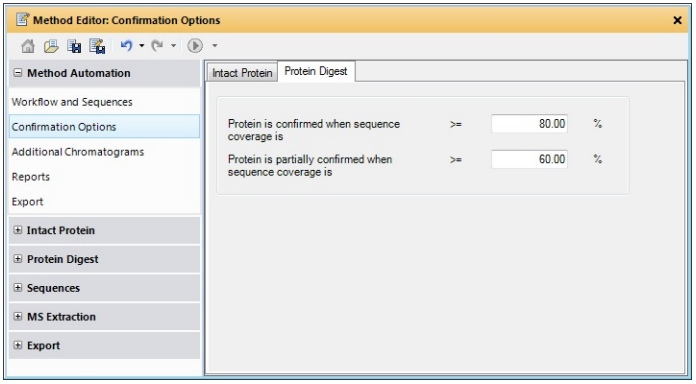
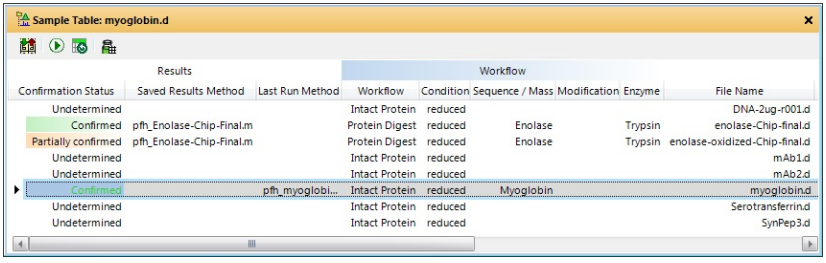
Exercise 6. Reprocessing Samples

This exercise shows you how to reprocess samples in the Sample Table. You can quickly check the Confirmation Status of each sample and determine if you need to reprocess the sample.

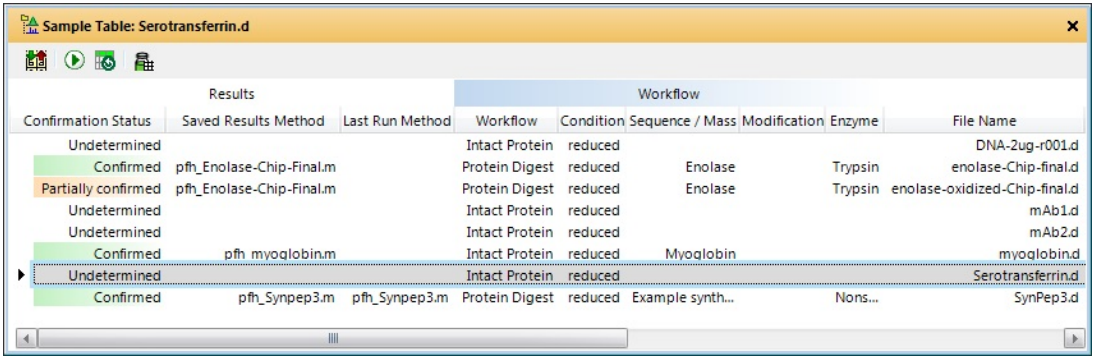
Steps	Detailed Instructions	Comments
1 Open several data files.	**a** Click **File > Open Sample Files**. **b** Select all of the example files. **c** Click **Open**.	• To select multiple files, click the first file. Then, press **Shift** and click the last file.
2 Review results in Sample Table window.	**a** Look at the **Confirmation Status** column.	• If you saved results, the table contains information on confirmation.



3 Review values in Method Automation > Confirmation Options.	**a** Click View > Method Editor, if necessary. **b** Select **Method Automation > Confirmation Options**. **c** Click the **Intact Protein** tab. **d** Review selection for the **Intact Protein match found but not for the most abundant peak** option. **e** Click the **Protein Digest** tab. **f** Review parameters on this tab.	• These tabs explain what it means to be **Confirmed** and **Partially confirmed**.

Steps	Detailed Instructions	Comments
		• You are not changing these options. You are only seeing what the software checks to determine if the protein is confirmed.
4 Reprocess the myoglobin.d data file.	**a** In the Sample Table, click the row containing myoglobin.d. **b** Click **Method > Open**. **c** Select the *iii*_**myoglobin.m** folder. **d** Click **Open**. **e** Click the  button to open the **Reprocess Sample** dialog box. **f** Select **Intact Protein** for the workflow. **g** Select **reduced** for the **Condition**. **h** Click the  button next to the **Sequences** parameter. The **Select Sequences** dialog box opens. **i** Move **Myoglobin** to the **Selected** list. **j** Click **OK**. **k** Click **Reprocess**.	• To reprocess a sample, you need to first load the correct method and then complete the Reprocess Sample dialog box. • You can either use the current method, or if you have previously saved results, you can use the sample result method.
		
5 Continue to reprocess samples.	• The following table shows how some of the samples were reprocessed.	

Steps	Detailed Instructions	Comments



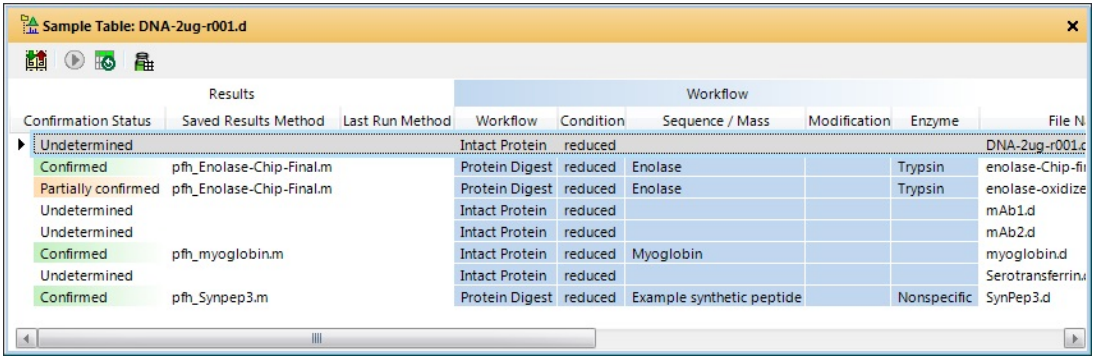
6 Save the results for the samples that you reprocessed.	**a** Click **File > Save Results**. **b** Click **Save**.	

Exercise 7. Using Result Review mode

This exercise shows you how to use the Result Review mode. When this mode is enabled, you cannot edit a method. You also cannot run the algorithms in the Find and Identify menu.

Steps	Detailed Instructions	Comments
1 Enable Result Review mode.	• Click **Configuration > Enable Result Review (Disables Method Editing)**.	• You can toggle this mode off by using this command again.
2 Review results in Sample Table window.	• All of the options in this window are available except for the **Run Method Workflow** button. You can still reprocess samples.	•

Steps	Detailed Instructions	Comments

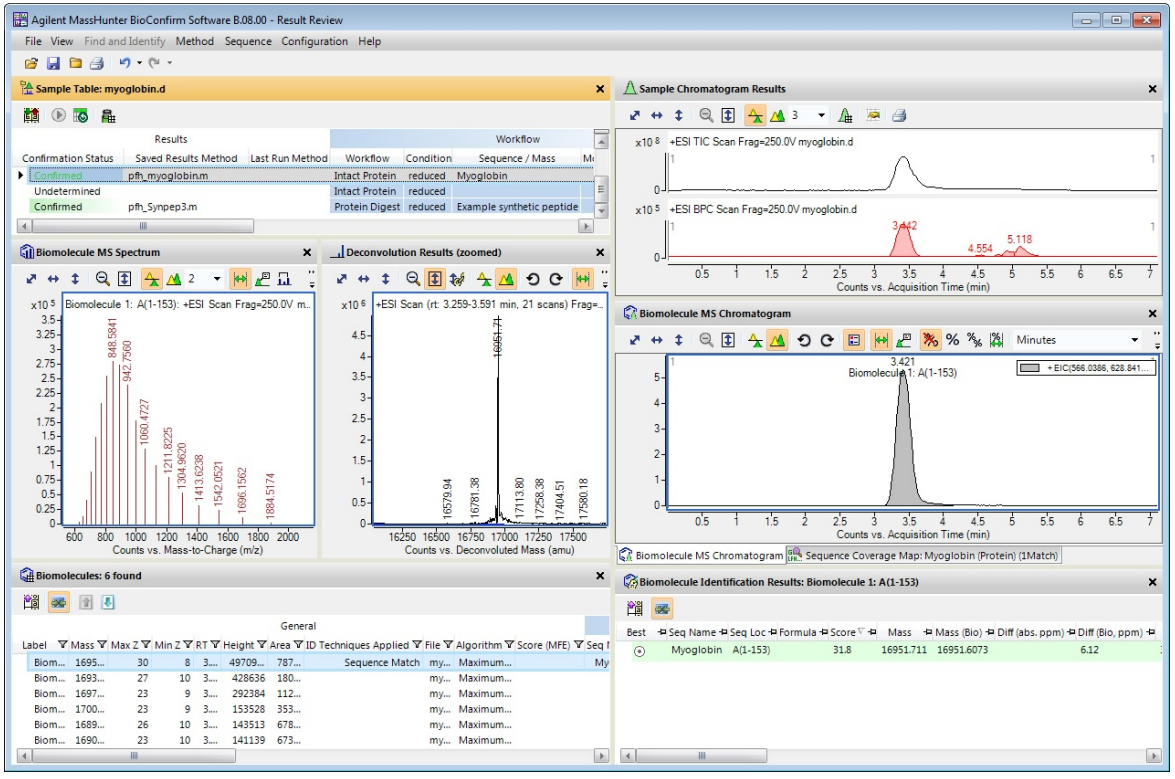


3 Reprocess the myoglobin.d data file.

a In the Sample Table, click the row containing myoglobin.d.
b Click **Method > Open**.
c Select the *iii*_**myoglobin.m** folder.
d Click **Open**.
e Click the button to open the **Reprocess Sample** dialog box.
f Select **Intact Protein** for the workflow.
g Select **reduced** for the **Condition**.
h Click the ... button next to the **Sequences** parameter. The **Select Sequences** dialog box opens.
i Move **Myoglobin** to the **Selected** list.
j Click **OK**.
k Click **Reprocess**.

- This step does not change when you enable **Result Review** mode.

Steps	Detailed Instructions	Comments
4 Reprocess the myoglobin.d data file.	**a** In the Sample Table, click the row containing myoglobin.d. **b** Click **Method > Open**. **c** Select the *iii*_**myoglobin.m** folder. **d** Click **Open**. **e** Click the  button to open the **Reprocess Sample** dialog box. **f** Select **Intact Protein** for the workflow. **g** Select **reduced** for the **Condition**. **h** Click the  button next to the **Sequences** parameter. The **Select Sequences** dialog box opens. **i** Move **Myoglobin** to the **Selected** list. **j** Click **OK**. **k** Click **Reprocess**.	• To reprocess a sample, you need to first load the correct method and then complete the Reprocess Sample dialog box. • You can either use the current method, or if you have previously saved results, you can use the sample result method.



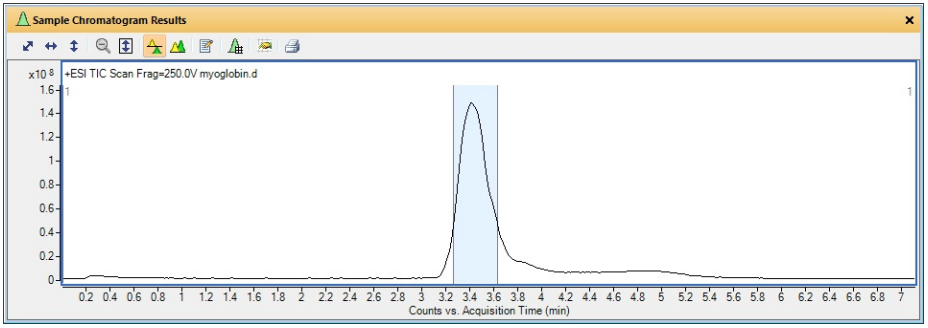
Steps	Detailed Instructions	Comments
5 Save the results for the samples that you reprocessed.	**a** Click **File > Save Results**. **b** Click **Save**.	

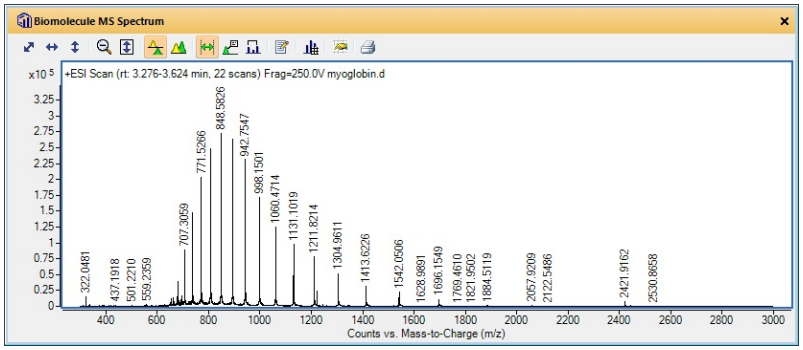
Deconvolution

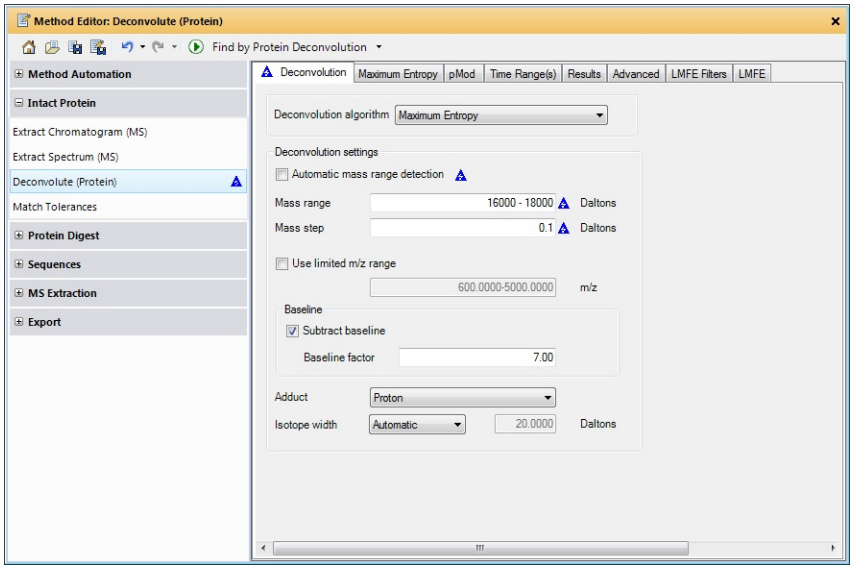
Exercise 7. Interactive Protein Molecular Weight Determination

This exercise shows you how to open a data file, extract spectra, deconvolute and view results. Deconvolution software does charge state deconvolution of mass spectra of large molecules with high charge states, such as proteins. See "Before you start" on page 2.

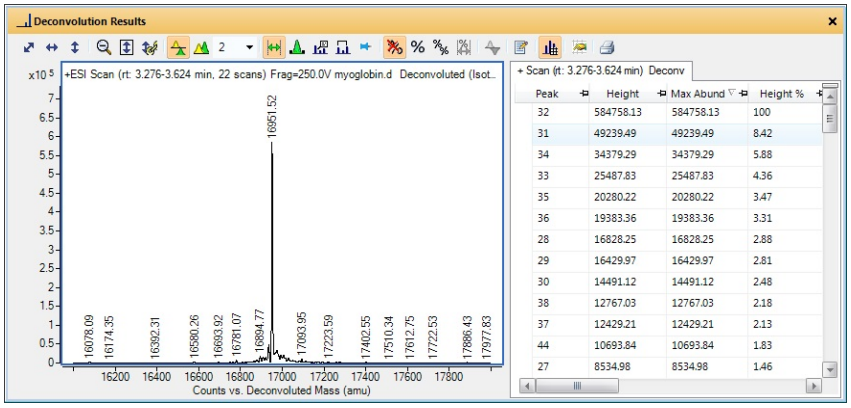
Steps	Detailed Instructions	Comments
1 Open the data file.	**a** Click **File > Open Data File**. **b** Locate the **myoglobin.d** folder. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
2 Extract a peak spectrum.	**a** Select a range around the peak at 3.5 minutes. **b** Double-click this range.	• To select a range, click one side of the peak and drag to the other side of the peak.



Steps	Detailed Instructions	Comments
		
3 Open the Deconvolute (Protein) Method Editor section.	**a** Click **View > Method Editor**. **b** Select **Intact Protein > Deconvolute (Protein)**.	• The commands in the View menu toggle whether or not a window is visible. If the command is shown in blue and the icon has an orange box around it, then the window is currently visible.
4 Select Maximum Entropy as the deconvolution algorithm.	• On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, verify that **Maximum Entropy** is selected for **Deconvolution algorithm**.	
5 Verify that the Mass range is automatically detected.	• Verify that the **Automatic mass range detection** check box is marked.	• If you clear this check box, then you need to manually enter the Mass range which can vary for different intact proteins.

Steps	Detailed Instructions	Comments
		
6 Select the extracted MS peak spectrum.	• Click the spectrum in the Biomolecule MS Spectrum window.	
7 Deconvolute the spectrum.	• Right-click the Biomolecule MS Spectrum window and click **Deconvolute** to start the deconvolution process.	You can also click the arrow next to the run button in the Method Editor toolbar and select **Deconvolute (Protein)**.
8 Review deconvolution results.	The results appear in the Deconvolution Results window. For information on changing the display of data in the Deconvolution Results window, see *online Help*.	• To compare two deconvoluted spectra, select the spectra of interest; then, click the button on the Deconvolution toolbar. If necessary, click **View > Deconvolution Mirror Plot**. The spectra are displayed in the Deconvolution Mirror Plot Results window. See "Exercise 8. Using the Mirror Plot window" on page 36 for more information.

Steps	Detailed Instructions	Comments
9 View peak information.	**a** Click the spectrum in the Deconvolution Results window to select it. **b** Click the  . **c** Click the **Max Abund** column heading to sort results by abundance. **d** Click  on the Deconvolution Results toolbar to close the peak list tab.	• Mass (*m/z*), Abundance, and Fit score are listed for each peak in the spectrum. • You can change the size of the graphics pane and the table pane in the Deconvolution Results window. Select the line between them and drag it to the right or left.

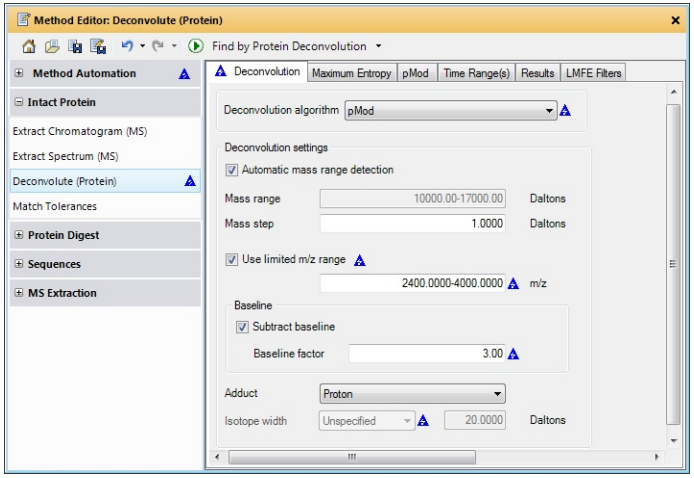


10 Save the method to iii_Deconvolution_MaxEnt.m where iii are your initials	**a** Click **Method > Save As**. **b** Enter ***iii*_Deconvolution_MaxEnt.m** for the method name. **c** Click **Save**.	

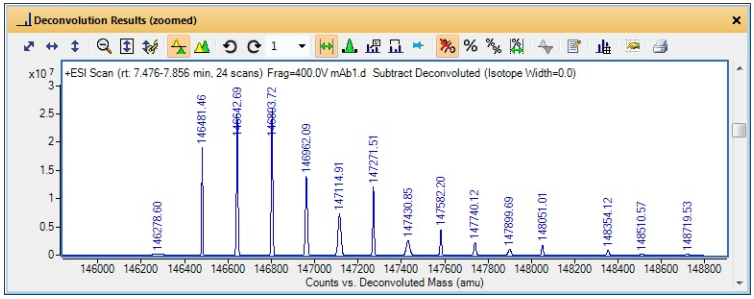
Exercise 8. Using the Mirror Plot window

This section shows how to display a Mirror Plot of two deconvoluted biomolecules.

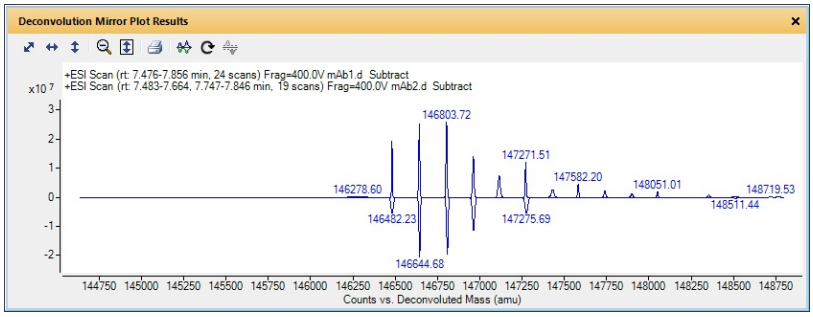
Steps	Detailed Instructions	Comments
1 Open the 1st data file.	**a** Click **File > Open Data File**. **b** Locate the **mAb1.d** folder. **c** Click **Open**.	• The TIC is automatically displayed in the Chromatogram Results window.
2 Open the Deconvolute (Protein) Method Editor section.	• Select **Deconvolute (Protein)** from the Intact Protein section of the Method Editor.	If the Method Editor window is not visible, click **View > Method Editor** to display it.
3 Select pMod as the deconvolution algorithm. • Use the automated mass range detection. • Use the limited m/z range of 2400 - 4000. • Use 3 for the baseline factor.	**a** On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select **pMod** for the **Deconvolution algorithm**. **b** Mark the **Automatic mass range detection** check box. **c** Mark the **Use limited m/z range** check box. **d** Enter 2400 - 4000 for the *m/z* range. **e** Enter 3 for the **Baseline factor**.	• For more information on these parameters, press **F1**.



4 Use the default settings for pMod deconvolution.	• Click the **pMod** tab to review settings.	

Steps	Detailed Instructions	Comments
5 Run the Find by Protein Deconvolution algorithm.	• Click **Find and Identify > Find by Protein Deconvolution**.	You can also click the arrow next to the run button in the Method Editor window, and select **Deconvolute (Protein)**.
6 Review deconvolution results.	The results appear in the Deconvolution Results window.	
		
7 Open the mAb2.d data file.	**a** Click **File > Open Data File**. **b** Locate the **mAb2.d** sample file. **c** Click **Open**.	The TIC is automatically displayed in the Sample Chromatogram Results window.
8 Run the Find by Protein Deconvolution algorithm on mAb2.d.	• Click **Find and Identify > Find by Protein Deconvolution**.	
9 Review deconvolution results.	The results appear in the Deconvolution Results window.	
10 Select both data files in the Sample Table window.	**a** Select one of the sample files in the Sample Table window. **b** Press the **Ctrl** button and click the other sample file.	• The results for the sample files selected in the Sample Table are shown in the Deconvolution window and other windows.
11 Use Mirror Plot to compare two deconvoluted spectra.	**a** Select a spectra from the Deconvoluton window. **b** Press the **Ctrl** button and select another spectra. **c** Click the button to display the spectra in the Deconvolution Mirror Plot Results window.	

Steps	Detailed Instructions	Comments



Exercise 9. Viewing Biomolecule Information

This exercise shows you how to view biomolecule information for deconvoluted spectra.

Steps	Detailed Instructions	Comments
1 Deconvolute **myoglobin.d** spectrum.	• See "Exercise 7. Interactive Protein Molecular Weight Determination" on page 32.	You do not need to repeat the deconvolution steps, if you have already done them in Exercise 1.
2 View the biomolecule list.	• Click **View > Biomolecules**	See Figure 5 on page 40.
3 Select the biomolecule with mass 16951.5.	• Click the row which has mass 16951.5 in the Biomolecules window.	• The Biomolecule MS Spectrum window and the Deconvolution Results window are both updated. • A biomolecule spectrum that displays all the charge states from the original *m/z* data for that specific protein mass is shown in the Biomolecule MS Spectrum Results window.
4 Select the Biomolecule 1 spectrum in the Biomolecule MS Spectrum Results window.	• Click the graphics area for the spectrum for Biomolecule 1.	• You can right-click the title of the window and click **Floating**. Then, you can make the window wider.
5 View the charge states found for the protein.	**a** Click  on the Biomolecule MS Spectrum toolbar to show the peak information. **b** Right-click the table and click **Add/Remove Columns**. **c** Select the columns in the Available Columns list which you want to see. **d** Click either **Add** or **Add All ->>**	• The following information is displayed for the ion set spectrum: ◦ m/z ◦ Abundance ◦ Charge state See Figure 6 on page 41. • If you cannot see the graphics when the table is displayed, move the cursor to between the graphics and the table until it looks like  . Then, click and drag to the right to increase the size of the graphics.
6 Switch from List mode to Overlay mode in the MS Spectrum Results window.	• Click  on the toolbar in the Biomolecule MS Spectrum Results window.	• See Figure 7 on page 41.

Steps	Detailed Instructions	Comments
7 Select biomolecule 1 in the biomolecule list.	• Click the first line of the Biomolecules table.	• Notice that the spectrum in the Biomolecule MS Spectrum window is updated.
8 Select compound 2 in the compound list.	• Click the second line of the Biomolecules table.	• Notice that a different spectrum is shown in the Biomolecule MS Spectrum window.
9 Print a biomolecule report.	**a** Display the Reports section in the Method Editor by selecting **Method Automation > Reports**. **b** Review the parameters in both the **Templates** and **Layout** tabs. **c** Click **Biomolecule Report** from the **File > Print** menu to print the report.	• You can use either Microsoft Excel for reporting or use PDF-based reporting. • When you print a Biomolecule Report, it uses either the **Intact Protein report template** or the **Protein Digest report template**, depending on the workflow selected in the Sample Table window. • If the workflow is **Custom**, then if you use the Find Peptides command, the **Peptide Digest report template** is used; otherwise, the **Intact Protein report template** is used.

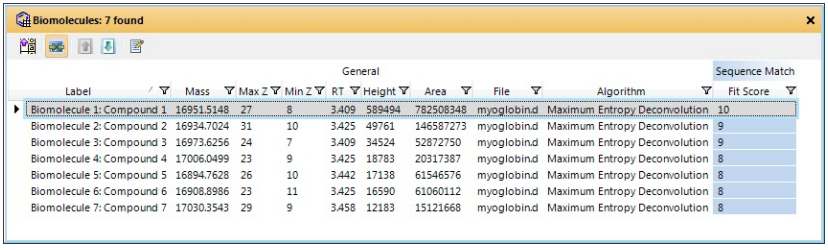
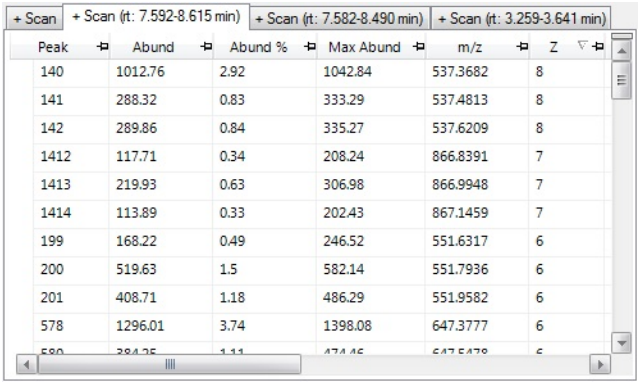


Figure 5 Biomolecules window for myoglobin.d



+ Scan | + Scan (rt: 7.592-8.615 min) | + Scan (rt: 7.582-8.490 min) | + Scan (rt: 3.259-3.641 min)

Peak	Abund	Abund %	Max Abund	m/z	Z
140	1012.76	2.92	1042.84	537.3682	8
141	288.32	0.83	333.29	537.4813	8
142	289.86	0.84	335.27	537.6209	8
1412	117.71	0.34	208.24	866.8391	7
1413	219.93	0.63	306.98	866.9948	7
1414	113.89	0.33	202.43	867.1459	7
199	168.22	0.49	246.52	551.6317	6
200	519.63	1.5	582.14	551.7936	6
201	408.71	1.18	486.29	551.9582	6
578	1296.01	3.74	1398.08	647.3777	6

Figure 6 Peak information for myoglobin.d displayed in the Biomolecule MS Spectrum window

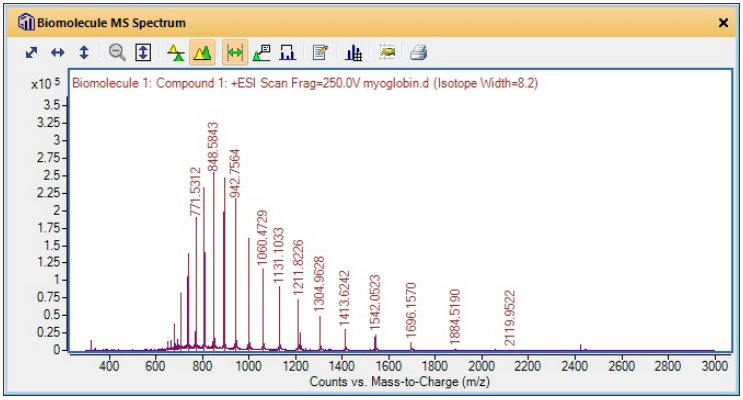


Figure 7 Biomolecule MS Spectrum Results window for myoglobin.d (Overlay Mode)

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