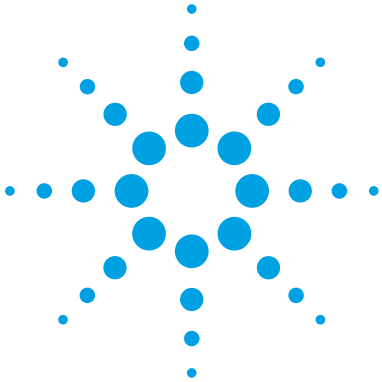


Agilent MassHunter BioConfirm Software

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*
- Agilent MassHunter BioConfirm eFamiliarization
- Agilent MassHunter BioConfirm Training Videos
- Online Help provides in-depth information and can be displayed in the following ways:
  - Click **Contents** 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 drive to your computer hard drive. We recommend copying the data files to the D:\MassHunter\Data folder.
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**, and 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, **NIST mAb1.d**, **Nist 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.09.00** 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.

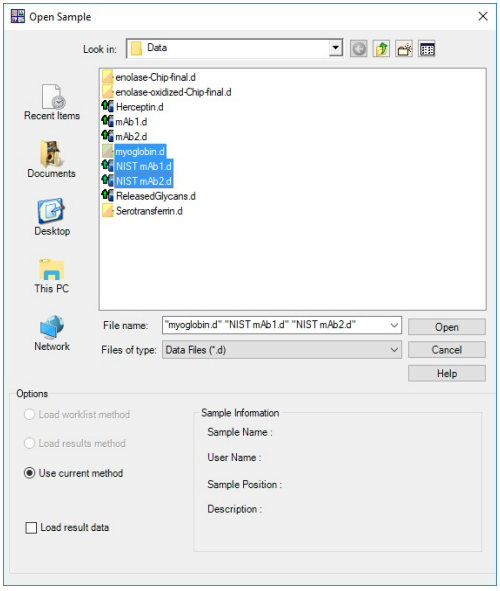


Figure 1 Open data files when opening software

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 **Nist mAb1.d**, **Nist mAb2.d** and **myoglobin.d**. **d** Clear the **Load result data** check box. **e** 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. **f** 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.	• Click **Configuration > Window Layouts > Restore Default Layout**.	• 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

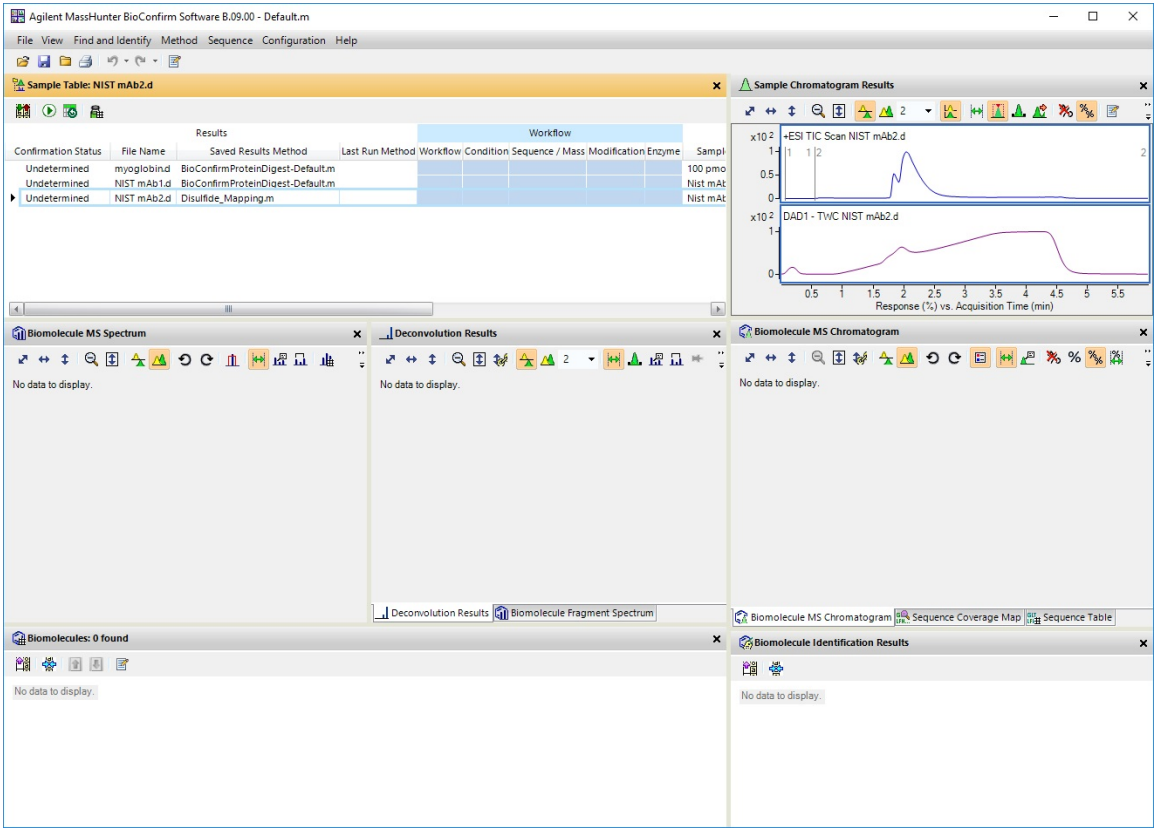


Figure 2 BioConfirm main window

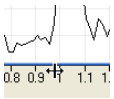
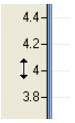
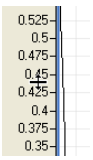
Task 2. Zoom in and out of the chromatogram

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.	**a** 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. **b** Repeat step b. **c** Click the **Autoscale Y-axis during Zoom** icon, , in the toolbar. **d** 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. **e** Click the **Unzoom** icon, , to undo the last zoom operation. You can undo the last fifteen zoom operations. **f** Click the **Autoscale X-axis and Y-axis** icon, , to zoom out completely.	• You can also use these zoom features 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. Default layouts are available for each workflow.

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 load a layout, click **Configuration > Windows Layouts > Load Layout**. • To save a window layout, click **Configuration > Window Layouts > Save Layout**. • To lock or 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. • The following layouts are shipped with the software: Default_IntactProtein.xml Deafult_Protein_Digest.xml Default_Released_Glycans.xml

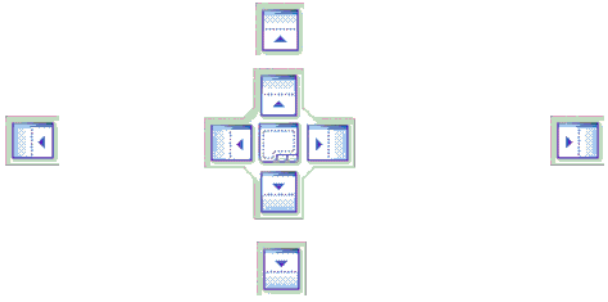


Figure 3 Window repositioning tools

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 default 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 16.

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 **Sequence > 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.

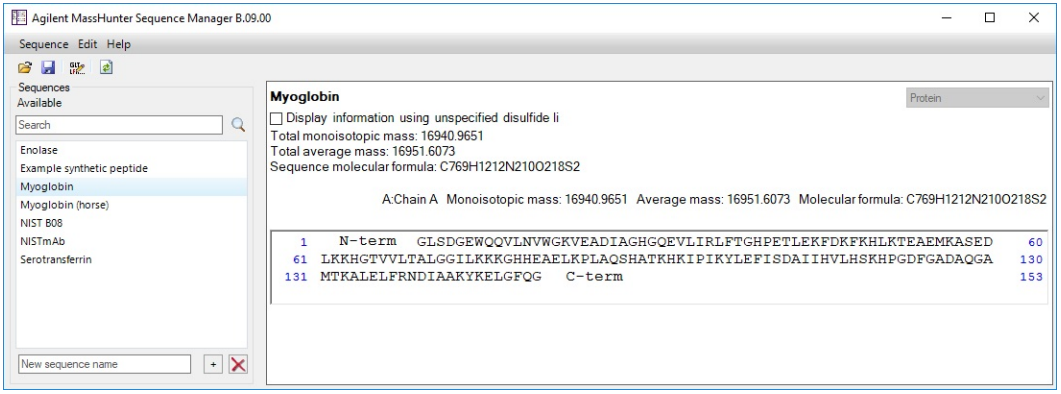


Figure 4 Creating a sequence file of myoglobin in the Sequence Manager program

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

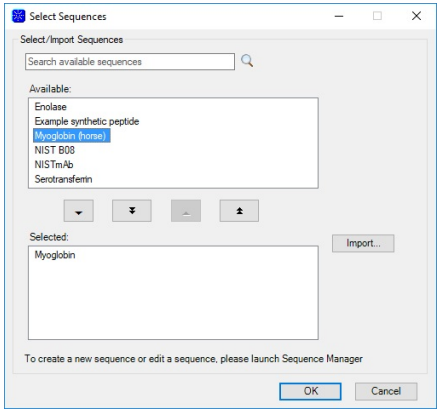
Step 9 - Print report.

Exercise 1. Interactive Intact Protein Workflow

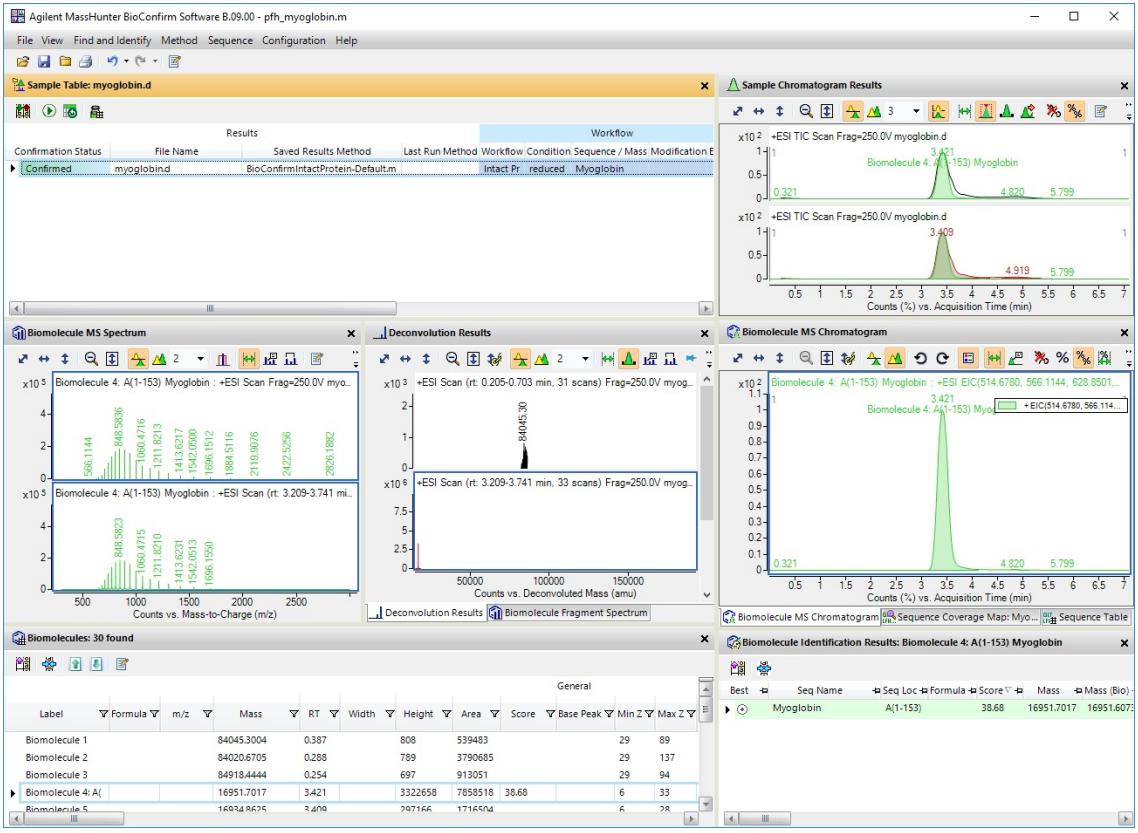
This exercise shows you how to set method parameters, match an intact protein sequence, and view the results. This exercise uses the *iii*_**myoglob.psq** sequence file created in Exercise 3 and the **myoglobin.d** data file copied before you started. See "Before you start" on page 2.

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*.

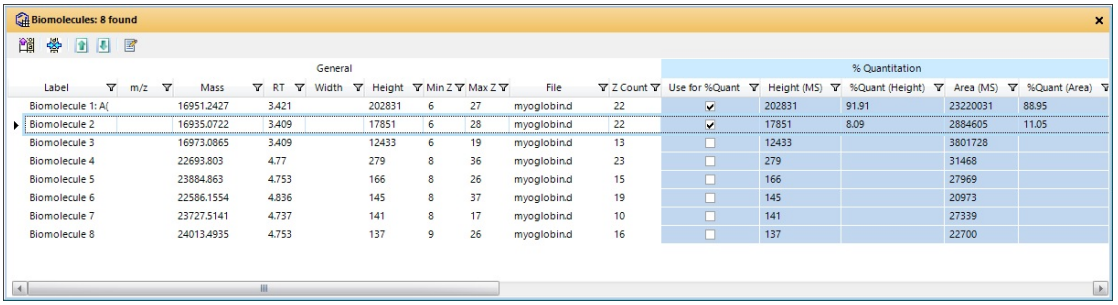
Steps	Detailed Instructions	Comments
		
7 Start the match search.	**a** Click **Intact Protein > Match Tolerances**. **b** Select the fourth biomolecule in the Biomolecules window. **c** Click  on the Method Editor toolbar. **d** Select the myoglobin.d data file and click **Match**.	Alternate methods: • 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 2.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_**myoglobin.m**, where *iii* represents your initials. **c** Click **Save**.	
10 Investigate the Relative Quantitation feature. You normally use this feature to quantitate proteoforms (PTMs on the protein). Myoglobin is used just for demonstrating this feature.	**a** In the Biomolecules window, clear all empty columns. **b** Mark the **Use for %Quant** column for Biomolecule 1. **c** Mark the **Use for %Quant** column for Biomolecule 2. **d** Review the values for the **%Quant (Height)** and **%Quant (Area)**.	• You can right-click the window and click **Add/Remove** columns to change the columns that are available.

Steps	Detailed Instructions	Comments

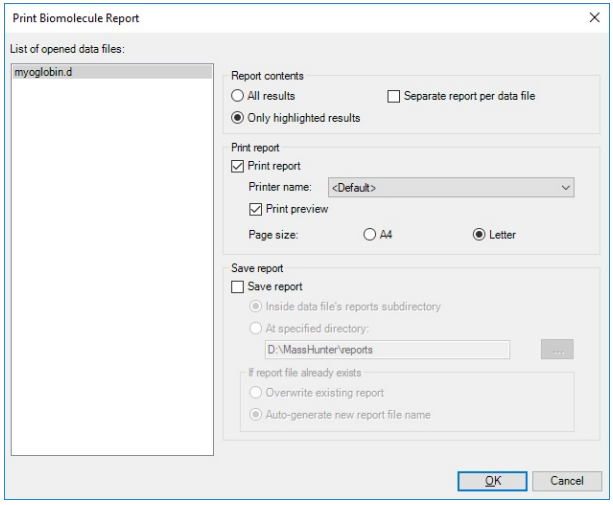


Exercise 2. Automated Intact Protein Workflow

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 Open the automation section in the Method Editor window.	• Click **Method Automation > Workflow and Sequences** in the Method Editor window.	
3 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*.
4 Import the myoglobin sequence.	**a** Select **reduced** for the **Condition**. **b** Click the ... button next to the **Sequences** parameter. The **Select Sequences** dialog box opens. **c** If myoglobin is not available, click **Import**. **d** Select *iii*_**myoglob.psq** and click **Open**. **e** Verify that the Myoglobin sequence is in the **Selected** list. **f** Click **OK**.	• The *iii*_**myoglob.psq** sequence file was created in "Task 4. Creating a Protein Sequence File" on page 9. • 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*.
5 Save the method.	• Click **Method > Save**.	
6 Run the method workflow or run method automation.	• Click **Method > Run Method Workflow**. • Click **Method > Run Method Automation (Workflow + Reports)**. • Click  on the Method Editor toolbar. • Reprocess the sample. See "Exercise 7. Reprocessing Samples" on page 29.	• Method Automation first runs the method workflow, and then extracts additional chromatograms and generates a biomolecule report and exports results.

Steps	Detailed Instructions	Comments
7 (optional) Review the printed Biomolecule reports.	• If you clicked **Run Method Automation**, then a report is generated automatically. • You can click **File > Print > Biomolecule Report** to generate a report for the current sample.	• You set report options in the Method Editor window in the Method Automation > Reports section. • If you are creating a report interactively, you specify some of these parameters in the **Print Biomolecule Report** dialog box.



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.

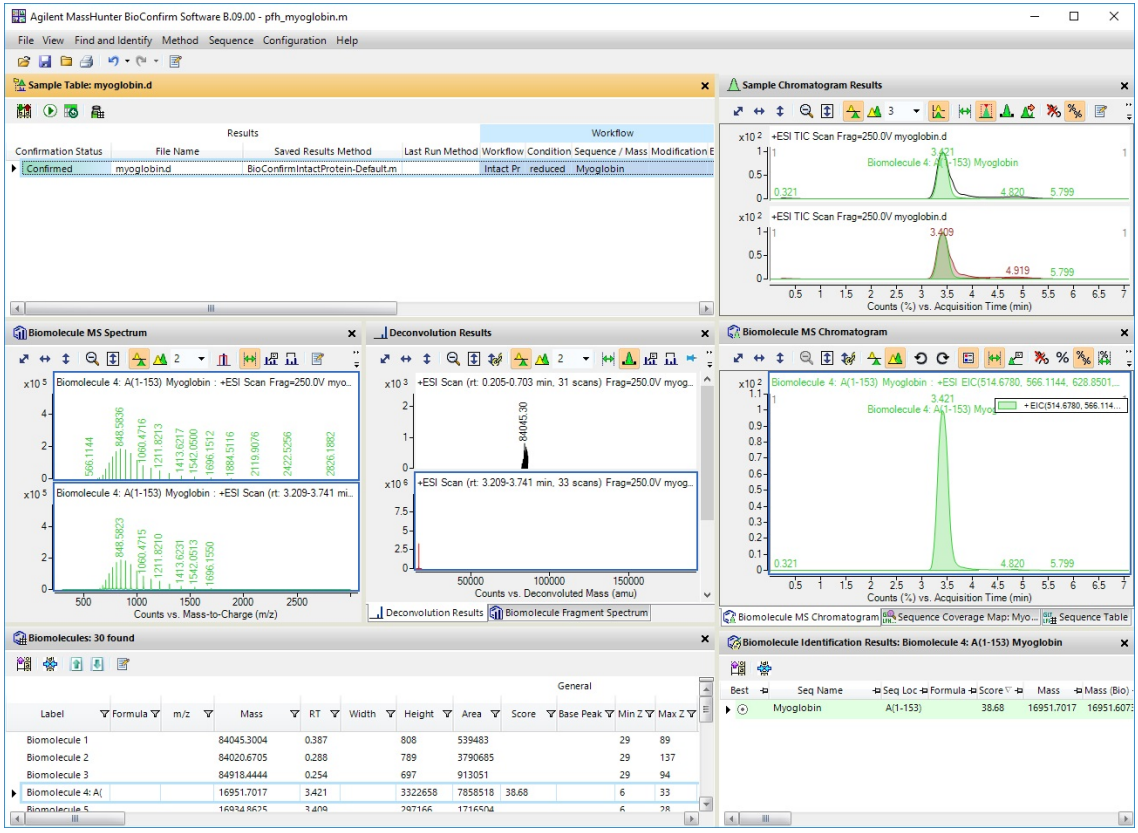
If you select the Protein Digest workflow, the Find Peptides algorithm runs and uses the enzyme selected in the Workflow and Peptides section and then runs the Protein Digest matching rules. See "Before you start" on page 2.

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** sample. **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 **MS-Only Extraction** tab. **d** Review the parameters. For the example file, you can restrict the mass range to 300 - 1700. **e** In the MS-Only Extraction tab, enter 500 for the **Use peaks with height >=** 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 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**.

Steps	Detailed Instructions	Comments
5 Import the sequence.	**a** Click **Method Automation > Workflow and Seqauences** 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/Masses** 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**.	• 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.
6 Review parameters on the Mass Matching tab.	**a** Click the **Mass Matching** tab in the Protein Digest > Match Tolerances section of the Method Editor window. **b** Review the parameters.	
7 Review the Matching Rules.	**a** Click the Matching Rules tab in the Protein Digest > 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**. **d** Review the other parameters.	
8 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**.	
9 Start the match search.	**a** Click **Find and Identify > Match Sequences**. **b** Select **enolase-Chip-final.d**. **c** Click **Match**.	*Alternate methods*: • Click  on the Method Editor toolbar. • Click **Match Sequences** on the Method Editor shortcut menu.

Steps	Detailed Instructions	Comments
10 Review the results.	**a** Highlight Biomolecule 3 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 biomolecule in the Biomolecules windows.	• If the biomolecule was identified, the **ID Techniques Applied** column contains **Sequence Match**.
11 View sequence coverage results.	**a** Click **View > Sequence Coverage Map**. **b** Select a different biomolecule in the Biomolecules table to view a different result.	
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** • **Specified Applied Links** • **View Digest List**	
12 Save the results	**a** Click **File > Save Results** to save your results to the data file folder.	• You can also click  to save results.

Steps	Detailed Instructions	Comments



13 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 biomolecules (step 4).
d Match sequences (step 9).
e Save the results to the second data file (step 12).

- Most of the processing parameters used for the first data file are the same for the second data file.

Exercise 4. Automated Protein Digest Workflow

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 3 (*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.	• You can instead click the Method Editor icon on the main toolbar.
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
4 Save the method.	• Click **Method > Save**.	
5 Run the method workflow or run method automation.	• Click **Method > Run Method Workflow**. • Click **Method > Run Method Automation (Workflow + Reports)**. • Click  on the Method Editor toolbar. • Reprocess the sample. See "Exercise 7. Reprocessing Samples" on page 29.	• Method Automation first runs the method workflow and then extracts additional chromatograms, generates a biomolecule report, and exports results.
6 (optional) Review the printed Biomolecule reports.	• If you clicked **Run Method Automation (Workflow + Reports)**, then a report is generated automatically. • You can click **File > Print > Biomolecule Report** to generate a report for the current sample.	• You set report options in the Method Editor window in the Method Automation > Reports section. • If you are creating a report interactively, you specify some of these parameters in the **Print Biomolecule Report** dialog box.

Released Glycans Workflow

The steps outlined below show the workflow for Released Glycans with Agilent MassHunter BioConfirm Software.

Step 1 - Open the data file of interest.

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

Step 3-Select the **Workflow** on the **Workflow and Sequences** tab.

Step 4 - Select the **Target glycan source.**

Step 5 - Select the tag which you used. 2-AB and InstantPC are listed, and you can create your own.

Step 6 - Run the Method Workflow.

Step 9 - Review the results which are shown in these windows:
Sample Chromatogram Results
Biomolecule MS Chromatogram
Biomolecules table
Biomolecule Identification Results
Biomolecule MS Spectrum
Biomolecule Fragment Spectrum
Glycan Structure Viewer

Step 9 - Print report.

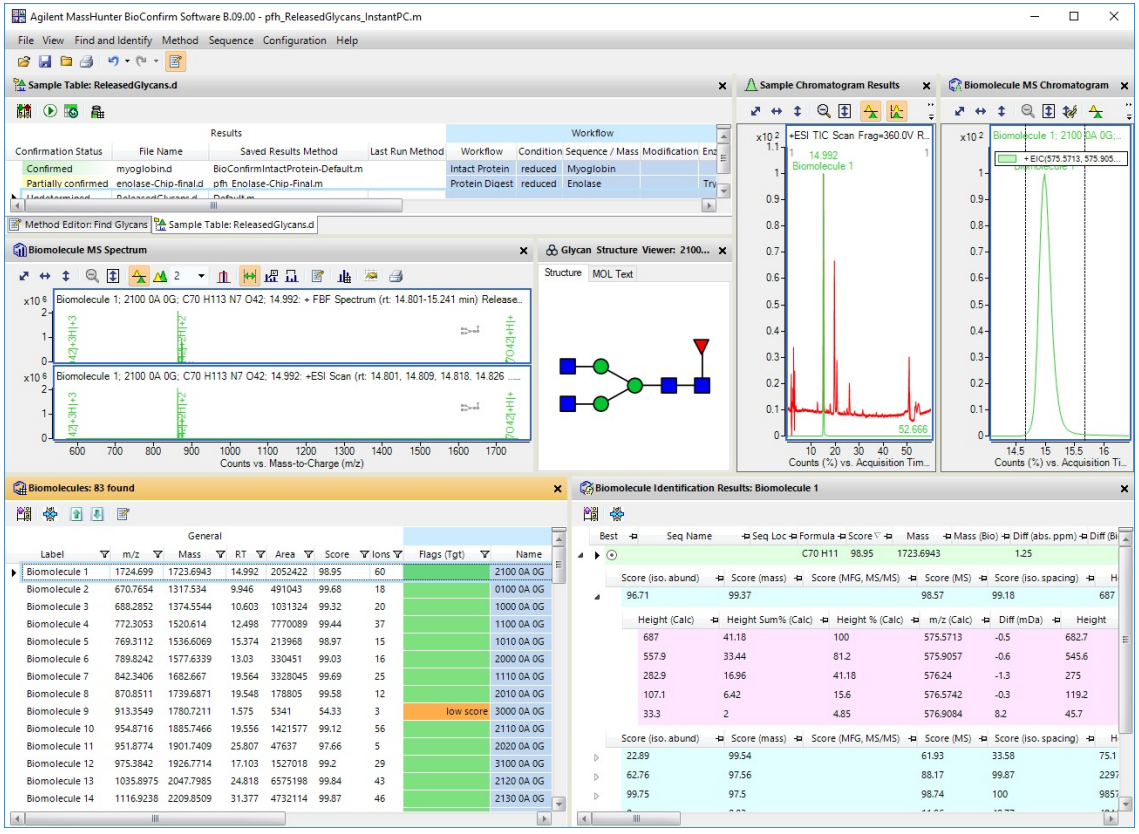
Exercise 5. Interactive Released Glycans

This exercise shows you how to find released glycans interactively.

If you select the Released Glycans workflow, the Find Glycans algorithm runs. 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 **BioConfirmReleasedGlycans-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 **Released Glycans > Find Glycans** in the Method Editor window. **b** Review the settings on the various tabs of the Find Glycans section. **c** Select `Glycans_Composition_AM_PCD.cdb` for the **Target glycan source**. **d** Click the **Tag** tab. **e** Click the option for the correct tag. For the example data file, click **InstantPC**.	• You can change the default parameters as described in the next steps. • 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 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. **c** Click **View > Glycan Structure Viewer** to show this window.	
5 Save the method for use in Exercise 6.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_ReleasedGlycans_InstantPC.m, where *iii* represents your initials. **c** Click **Save**.	

Steps	Detailed Instructions	Comments
6 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.	• Several changes were made to the default layout for the image below. The Glycan Structure Viewer window is visible, and the Biomolecule Identification Results window is docked next to the Biomolecules window. Also, the **Flags (Tgt)** column was moved.



Steps	Detailed Instructions	Comments
7 Save the results	**a** Click **File > Save Results** to save your results to the data file folder.	• You can also click  to save results.

Exercise 6. Automated Released Glycans Workflow

This exercise guides you through the setup of a worklist to automatically run the Released Glycans workflow.

If you select the Released Glycans workflow, the Find Glycans algorithm runs and uses the **Target glycan source** selected in the Workflow and Peptides section.

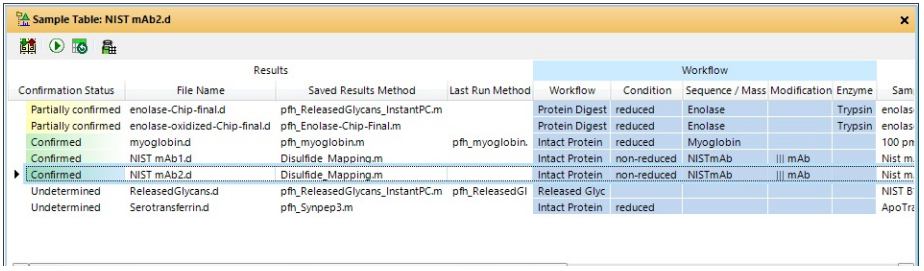
Steps	Detailed Instructions	Comments
1 Open the method.	**a** Click **Method > Open**. **b** Select the *iii*_**ReleasedGlycans_InstantPC.m** folder. **c** Click **Open**.	• This method was created in Exercise 5 (*iii* represents your initials).
2 Display the Method Automation > Workflow and Sequences 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.	• You can instead click the Method Editor icon on the main toolbar.
3 Select the appropriate workflow.	**a** Select **Released Glycans** for the Workflow. **b** Select the **Target glycan source**. **c** Clear the **Require RT match if database contains a RT for the target glycan** check box.	• The Released Glycans workflow automatically runs the Find Glycans algorithm.
4 Save the method.	• Click **Method > Save**.	
5 Run the method workflow or run method automation.	• Click **Method > Run Method Workflow**. • Click **Method > Run Method Automation (Workflow + Reports)**. • Click  on the Method Editor toolbar. • Reprocess the sample. See "Exercise 7. Reprocessing Samples" on page 29.	• Method Automation first runs the method workflow and then extracts additional chromatograms, generates a biomolecule report, and exports results.

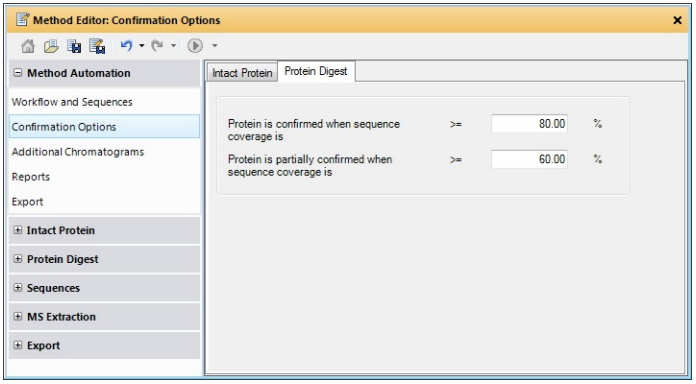
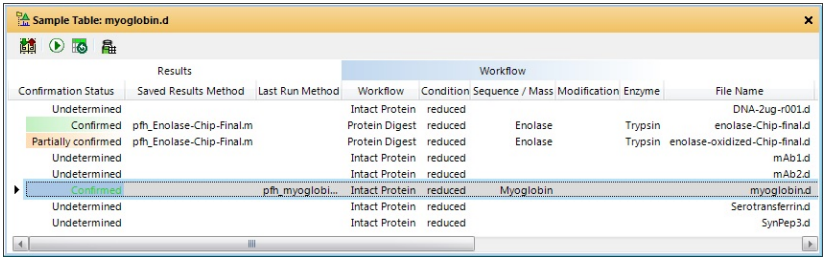
Steps	Detailed Instructions	Comments
6 (optional) Review the printed Biomolecule reports.	• If you clicked **Run Method Automation (Workflow + Reports)**, then a report is generated automatically. • You can click **File > Print > Biomolecule Report** to generate a report for the current sample.	• You set report options in the Method Editor window in the Method Automation > Reports section. • If you are creating a report interactively, you specify some of these parameters in the **Print Biomolecule Report** dialog box.

Review Results

Exercise 7. 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.

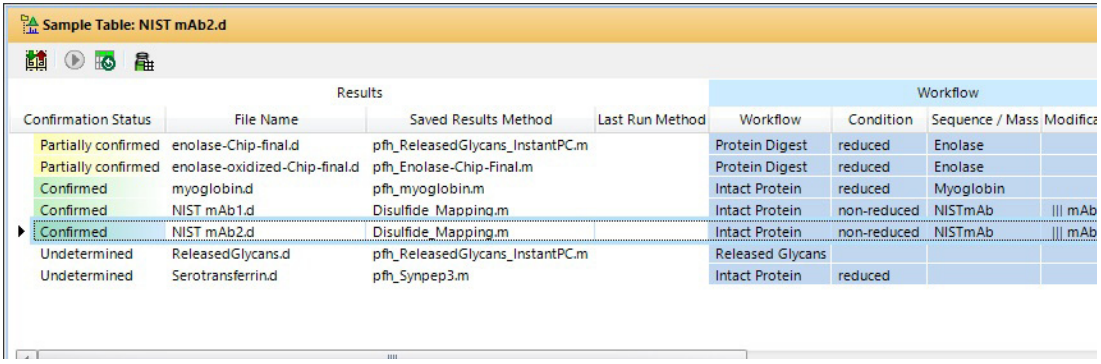
Steps	Detailed Instructions	Comments
1 Open several data files.	**a** Click **File > Open Sample Files**. **b** Select all of the example files. **c** Click **Open**.	• To select multiple files, click the first file. Then, press **Shift** and click the last file.
2 Review results in Sample Table window.	**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 selection for the **Protein is partially confirmed when sequence coverage is >=** option.	• 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 Save the results for the samples that you reprocessed.	**a** Click **File > Save Results**. **b** Click **Save**.	

Exercise 8. Using Result Review mode

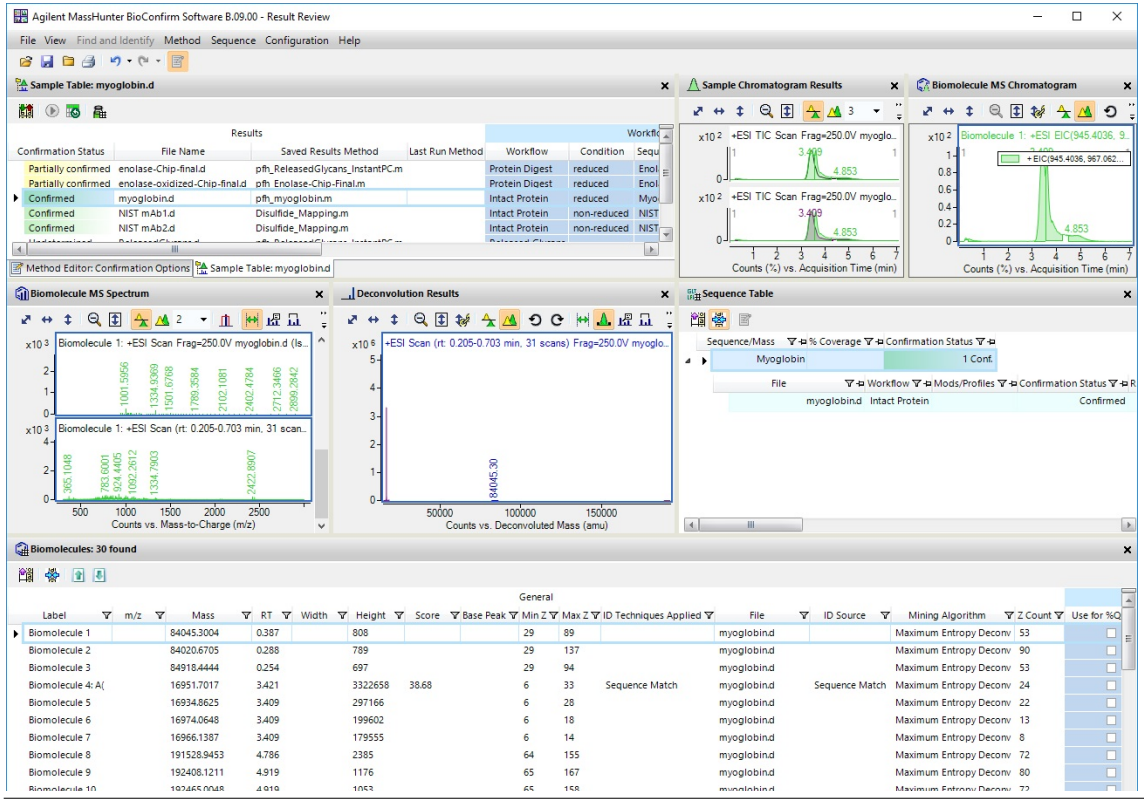
This exercise shows you how to use the Result Review mode. When this mode is enabled, you cannot edit a method. You also cannot run the algorithms in the Find and Identify menu.

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.	•



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.

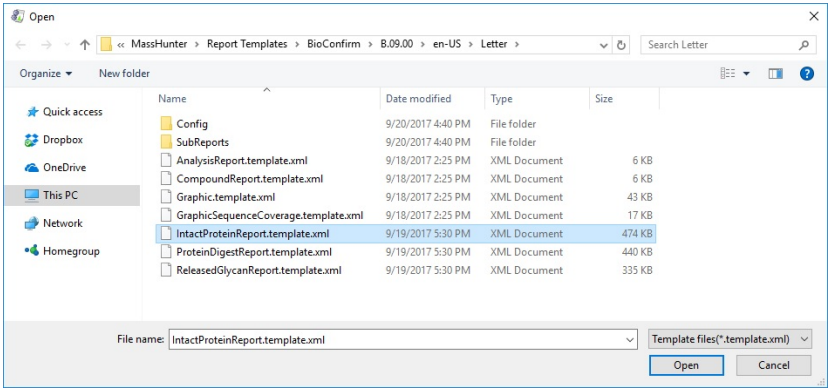
Steps	Detailed Instructions	Comments
4 Reprocess the myoglobin.d data file.	**a** In the Sample Table, click the row containing myoglobin.d. **b** Click **Method > Open**. **c** Select the *iii*_**myoglobin.m** folder. **d** Click **Open**. **e** Click the button to open the **Reprocess Sample** dialog 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.

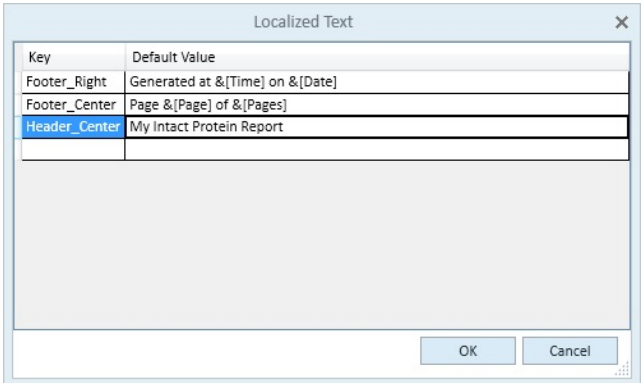


Steps	Detailed Instructions	Comments
5 Save the results for the samples that you reprocessed.	**a** Click **File > Save Results**. **b** Click **Save**.	

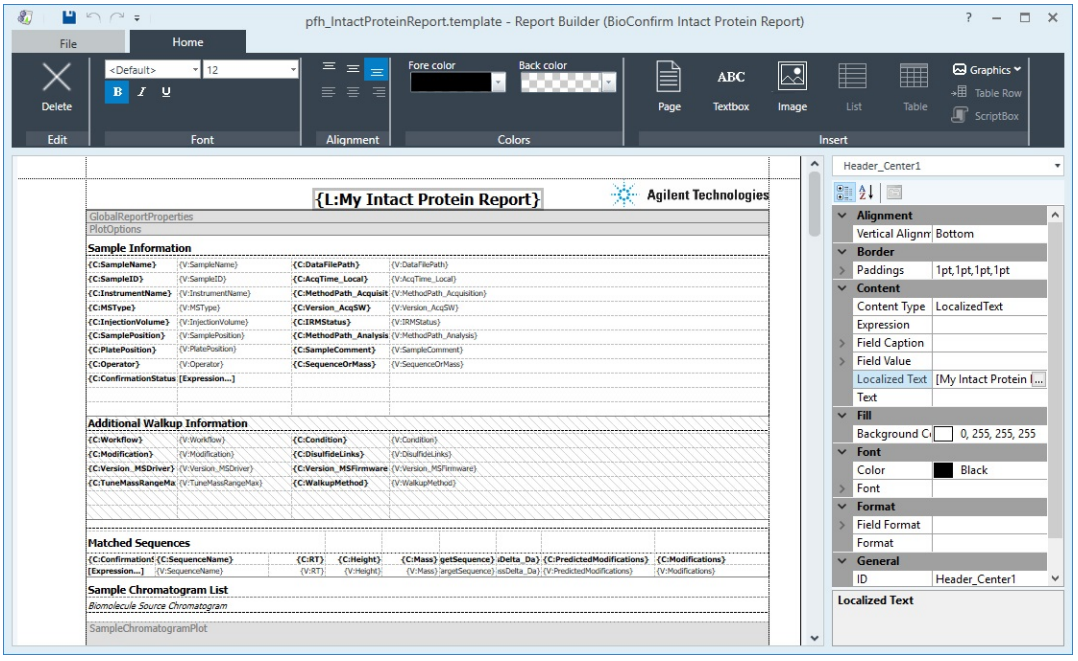
Exercise 9. Using Report Builder

This exercise shows you the program to modify PDF templates. If you click **Use PDF Report Builder** in the Method Automation > Reports > Templates tab, then you can use Report Builder to modify those templates.

Steps	Detailed Instructions	Comments
1 Open Report Builder program.	**a** Double-click **Report Builder** in the Tools for BioConfirm B.09.00 folder in the Agilent MassHunter Workstation program folder. **b** In Windows 10, click **Agilent MassHunter Report Builder > Report Builder B.09.00**.	• You can also start the Report Builder program when you click the Edit button next to the template in the Method Automation > Reports > Template tab. These buttons are only available if you click **Configuration > Show Advanced Settings**.
2 Open an existing template.	**a** Click **File > Open > Browse**. **b** Select a template and click **Open**.	• Report templates are installed in the *MassHunter**Report Templates**BioConfirm* folder.
		**Agilent recommends that you do not modify the default templates. Instead, make a copy of the template and modify the copy.**

Steps	Detailed Instructions	Comments
3 Review the template in Report Builder.	**a** Click an item in the template. Notice that the right pane changes. **b** Click the title of the report. **c** In the right pane, click **Localized Text** in the Content section. **d** Click the ... button. The Localized Text dialog box opens. **e** Click the Header_Center. **f** Enter **My Intact Protein Report**. **g** Click **OK**.	• The left pane shows the template. The right pane shows the parameters for the current selection.
		• You can make many different changes to the report. This exercise only shows you one possibility. Press F1 to access the online Help to learn more about customizing a report template.
4 Save the template.	**a** Click **File > Save As > Browse**. **b** Enter a file name and click **Save**. **c** Close the **Report Builder** program.	• You can instead click **File > Save**, and the file is saved to the current method. Agilent recommends that you do not modify the default templates.

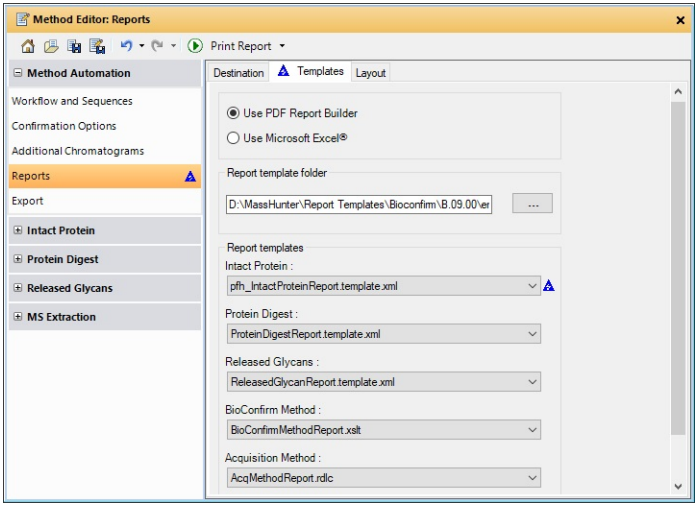
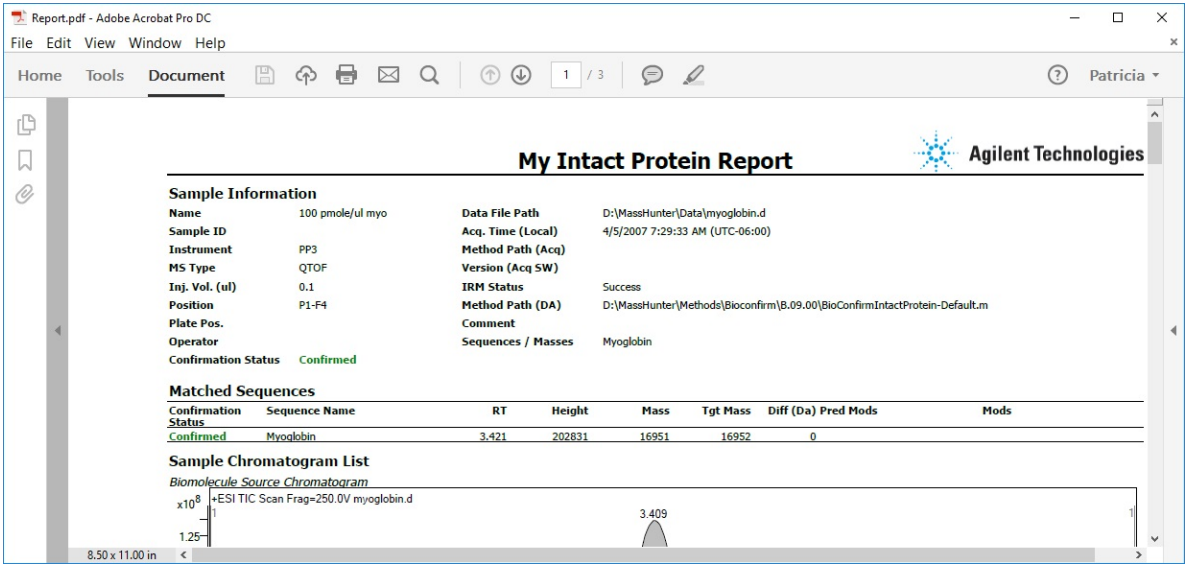
Steps	Detailed Instructions	Comments



5 Use this new template in a method.

a Open the Method Editor window. Click View > Method Editor if it is not visible.
b Select Method Automation > Reports.
c Click the **Templates** tab.
d Select the changed report for the corresponding report template type. In this example, the **Intact Protein** report template was modified.

- Different reports use different report templates. If you modified an Intact Protein report template, then you select the modified template for the **Intact Protein** report template.
- When you print a biomolecule report, the report template corresponding to the selected workflow is used.

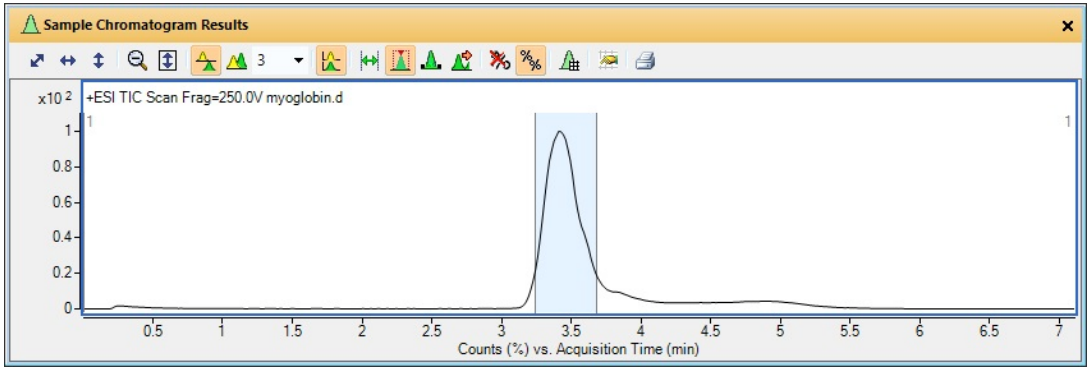
Steps	Detailed Instructions	Comments
		
6 Print a Biomolecule report.	**a** Click **File > Print > Biomolecule Report**. **b** Mark the **Print preview** check box. **c** Click **OK**.	• When you print a biomolecule report, the report template corresponding to the selected workflow is used.
		

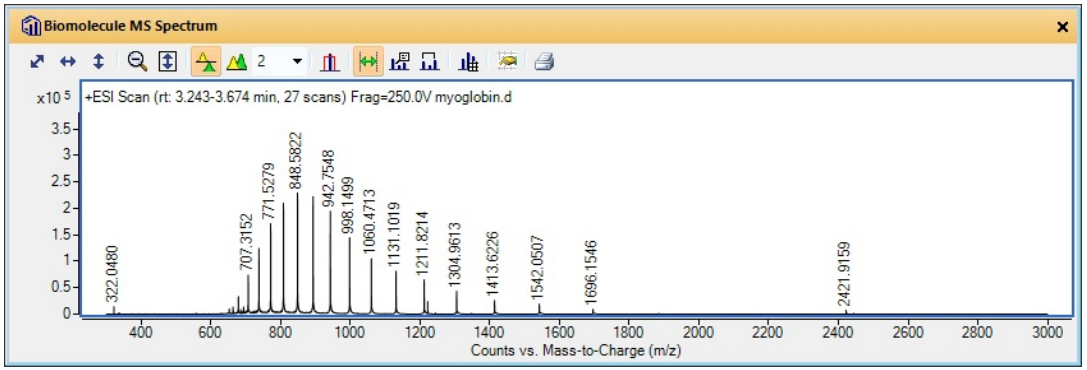
Deconvolution

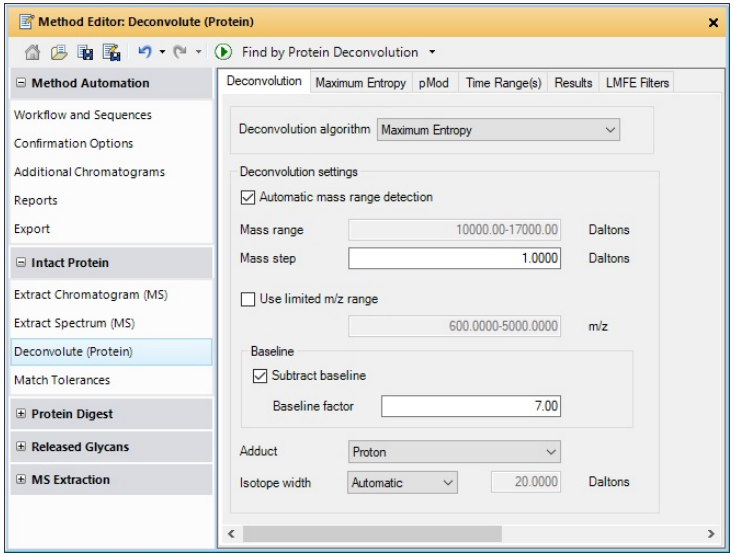
Exercise 10. Interactive Protein Molecular Weight Determination

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.

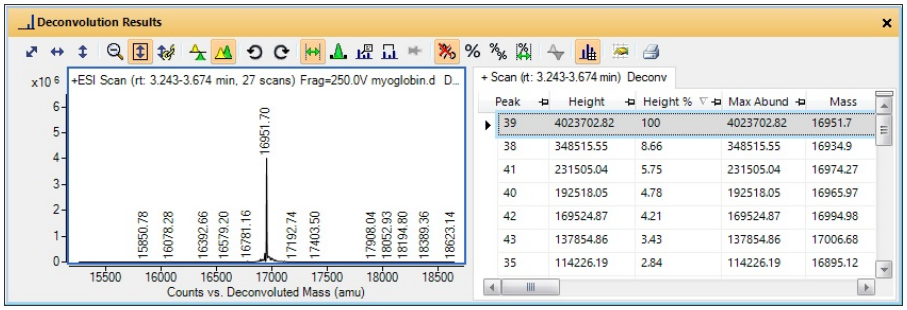
Steps	Detailed Instructions	Comments
1 Open the data file.	**a** Click **File > Open Data File**. **b** Locate the **myoglobin.d** folder. **c** Clear the **Load Result** check box. **d** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
2 Extract a peak 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 and the Biomolecules 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 11. Using the Mirror Plot window" on page 41 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.

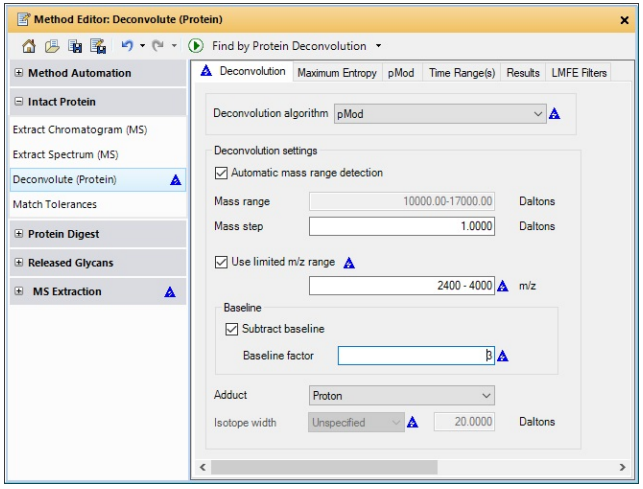


10 Save the method to iii_Deconvolution_MaxEnt.m where iii are your initials	**a** Click **Method > Save As**. **b** Enter ***iii*_Deconvolution_MaxEnt.m** for the method name. **c** Click **Save**.	

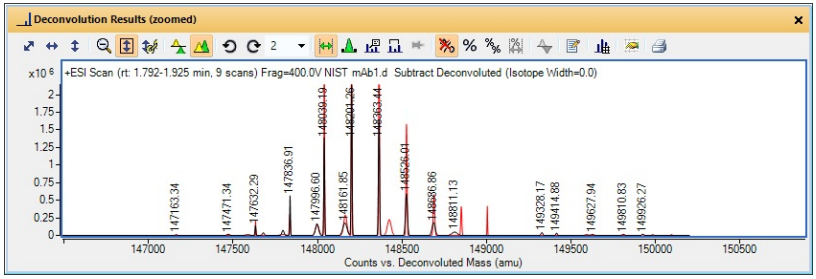
Exercise 11. Using the Mirror Plot window

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

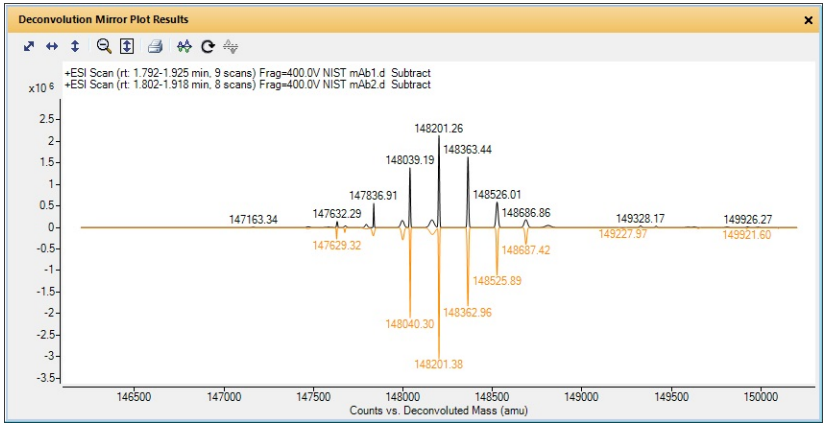
Steps	Detailed Instructions	Comments
1 Open the NIST mAb1.d data file.	**a** Click **File > Open Data File**. **b** Locate the **NIST mAb1.d** folder. **c** Click **Open**.	• The TIC is automatically displayed in the Chromatogram Results window.
2 Open the Deconvolute (Protein) Method Editor section.	• 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 NIST mAb2.d data file.	**a** Click **File > Open Data File**. **b** Locate the **NIST 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** Click the button to show the spectra in list mode. **b** Select a spectra from the Deconvoluton window. **c** Press the **Ctrl** button and select another spectra from the other data file. **d** Click the button to display the spectra in the Deconvolution Mirror Plot Results window.	

Steps	Detailed Instructions	Comments



Exercise 12. 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 10. Interactive Protein Molecular Weight Determination" on page 37.	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 45.
3 Select the biomolecule with mass around 16974.3.	• Click the row which has a mass around 16974.3 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 46. • 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 Biomolecule MS Spectrum Results window.	• Click  on the toolbar in the Biomolecule MS Spectrum Results window.	• See Figure 7 on page 46.

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 biomolecule 2 in the Biomolecules window.	• 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 PDF-based reporting or Microsoft Excel reporting. • When you print a Biomolecule Report, it uses the **Intact Protein**, the **Protein Digest**, or the **Released Glycans** 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.

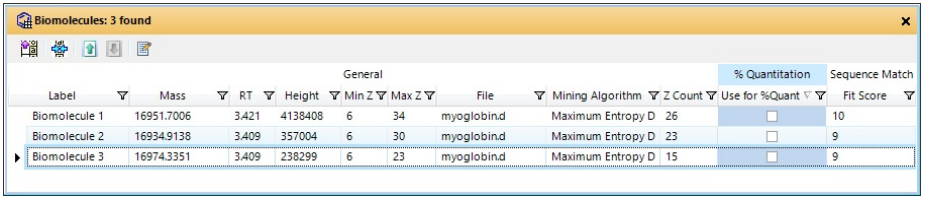
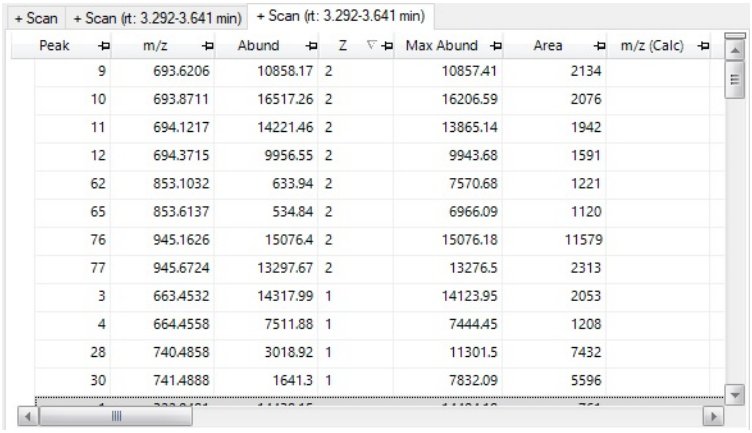


Figure 5 Biomolecules window for myoglobin.d



+ Scan | + Scan (rt: 3.292-3.641 min) | + Scan (rt: 3.292-3.641 min)

Peak	m/z	Abund	Z	Max Abund	Area	m/z (Calc)
9	693.6206	10858.17	2	10857.41	2134	
10	693.8711	16517.26	2	16206.59	2076	
11	694.1217	14221.46	2	13865.14	1942	
12	694.3715	9956.55	2	9943.68	1591	
62	853.1032	633.94	2	7570.68	1221	
65	853.6137	534.84	2	6966.09	1120	
76	945.1626	15076.4	2	15076.18	11579	
77	945.6724	13297.67	2	13276.5	2313	
3	663.4532	14317.99	1	14123.95	2053	
4	664.4558	7511.88	1	7444.45	1208	
28	740.4858	3018.92	1	11301.5	7432	
30	741.4888	1641.3	1	7832.09	5596	

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

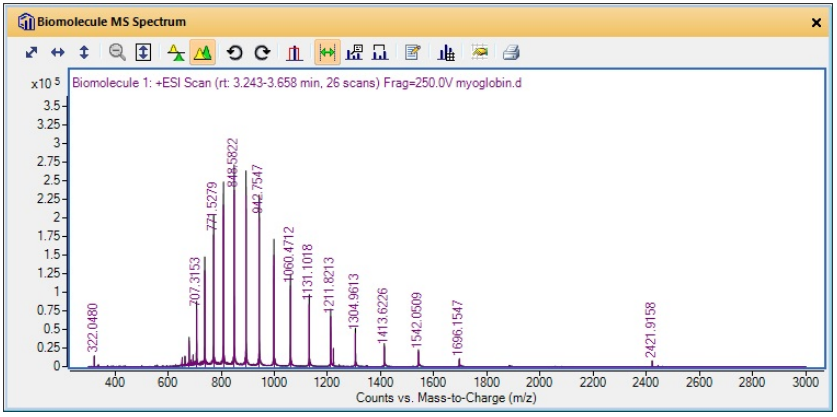


Figure 7 Biomolecule MS Spectrum Results window for myoglobin.d (Overlay Mode)

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