Agilent MassHunter BioConfirm Software

Familiarization Guide

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

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:

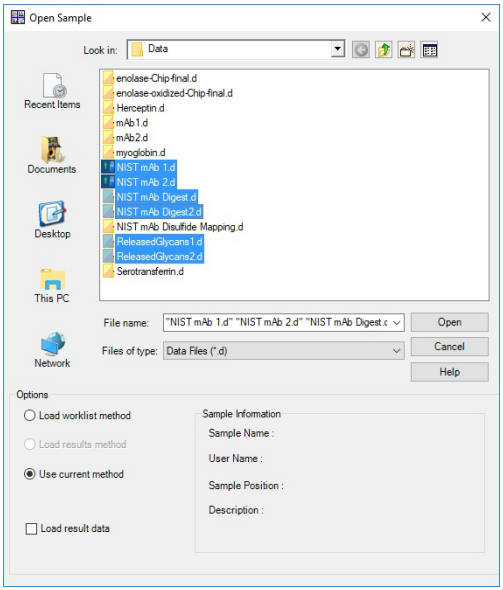
1. Copy all of the data files from the **Data** folder on the BioConfirm setup media to your computer hard drive. We recommend copying the data files to the **D:\MassHunter\Data** folder.
2. Make sure you have both read and write permissions for the folder you just created on your computer. This is required 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**.
3. Copy all of the sequences from the **ProteinSequences** folder on the BioConfirm setup media to your computer hard drive. We recommend copying the data files to the **D:\MassHunter\ProteinSequences** folder.

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 these data files NIST mAb 1.d Nist mAb 2.d NIST mAb Digest.d NIST mAb Digest2.d ReleasedGlycans1.d ReleasedGlycans2.d in the folder **D:\MassHunter\Data**, or in the folder where you copied them. • Make sure that the **Use current method** button is clicked. • Make sure that the **Load result data** check box is cleared.	**a** Double-click the **Agilent MassHunter BioConfirm 10.0** icon. The system displays the **Open Sample** dialog box. **b** Go to the folder **D:\MassHunter\Data** or to the folder where the example files are located.  **Figure 1.** Open data files when opening software	• You can get help for any window, dialog box, or tab by pressing the **F1** key when that window is active.

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

Steps	Detailed Instructions	Comments
	c Click the **NIST mAb 1.d** file. **d** Press and hold the **Shift** key while you click **NIST mAb Digest.d**. **e** Press and hold the **Ctrl** key while you click **ReleasedGlycans1.d** and **ReleasedGlycans2.d**. **f** Clear the **Load result data** check box. **g** Click Open. All the 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. **h** Click the **List Mode** button in the Sample Chromatogram Results toolbar. **i** Click the **NIST mAb 1.d** data file.	• If you press the **Shift** key, you can pick a group of files that are directly next to each other. • 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 button, the background of the button changes to orange.
2 Return the main window to the default Intact Protein layout.	• Click **Intact Protein Layout** in the main toolbar.	• You click the  button in the graphics window to change the display options. • You can switch between layouts for the different workflows when you click the buttons in the main toolbar. • 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

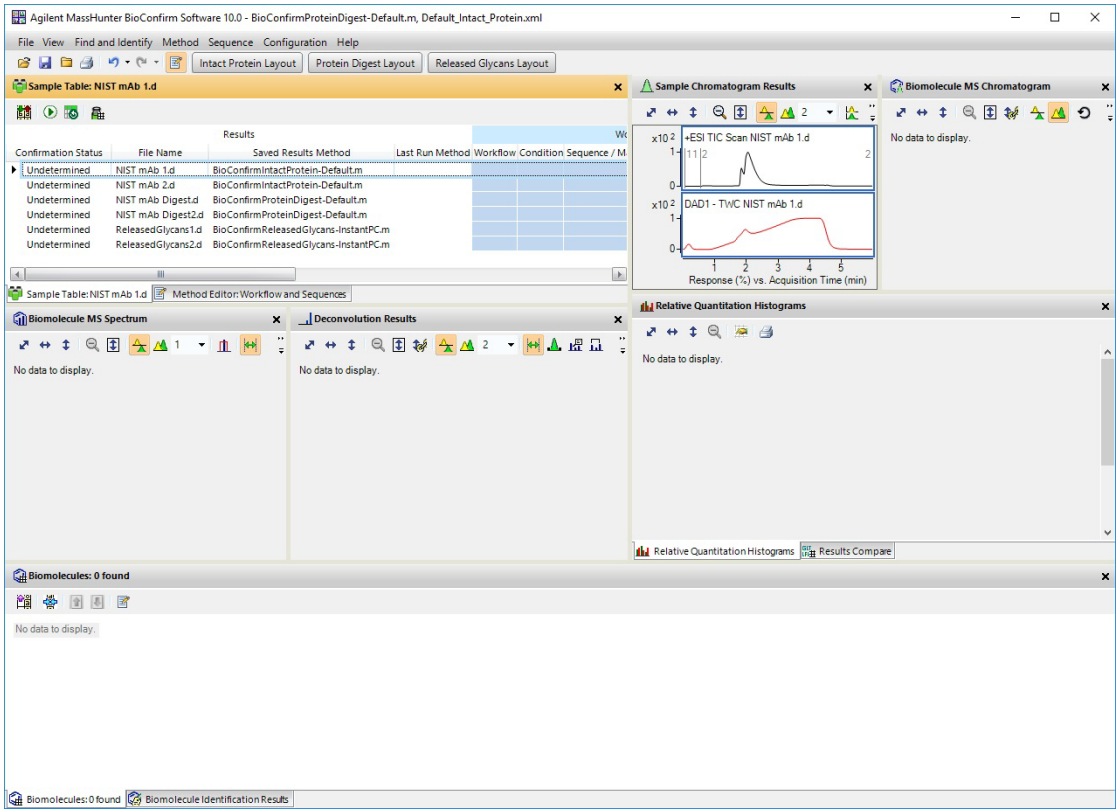


Figure 2. BioConfirm main window

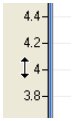
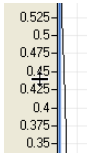
Task 2. Zoom in and out of the chromatogram

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 button,  , is not selected for this step. **b** Repeat step a. **c** Click the Autoscale Y-axis during Zoom button,  , 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 button,  , to undo the last zoom operation. You can undo the last fifteen zoom operations. **f** Click the Autoscale X-axis and Y-axis button,  , 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. • In addition to those windows, you can also zoom on the x-axis and y-axis and use the toolbar buttons in the Relative Quantitation Histograms window. You cannot drag over an area in the Relative Quantitation Histograms window. • A selected button 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 button, ↔, 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 button, ↕, 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 the default layout for a workflow, click one of the buttons in the main toolbar: **Intact Protein Layout**, **Protein Digest Layout**, and **Released Glycans Layout**. • 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

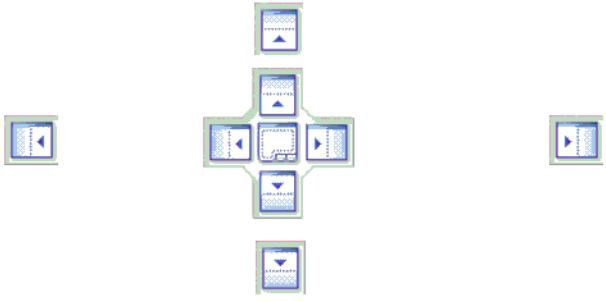


Figure 3. Window repositioning tools

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 **Intact Protein Layout** in the main toolbar.	• The cursor must be over one of the arrows in a box in order for repositioning to occur. • Clicking the **Configuration > Load Default Layout** command restores the default layout. The default layout is different than the default layout for each workflow.

Task 4. Creating a Protein Sequence File

This task guides you through the creation of a myoglobin sequence file.

Steps	Detailed Instructions	Comments
1 Start the Agilent MassHunter Sequence Manager.	• Click **Sequence > Sequence Manager**.	
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. GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEM KASEDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIH VLHSKHPGDFGADAQGAMTKALELFRNDIAAKYKELGFQG	• 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. • 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*.

Steps	Detailed Instructions	Comments
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.

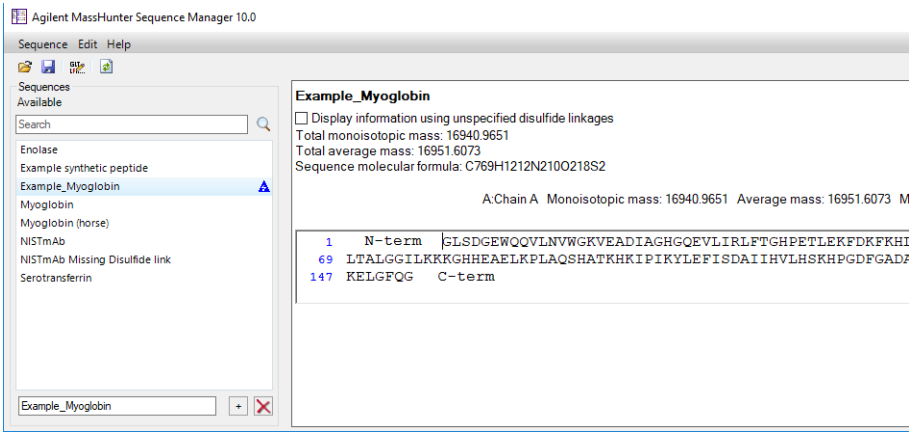


Figure 4. Creating a sequence file of myoglobin in the Sequence Manager program

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
Deconvolution Results
Biomolecule MS Chromatogram
Biomolecule MS Spectrum
Biomolecule Fragment Spectrum
Results Compare
Relative Quantitation Histograms

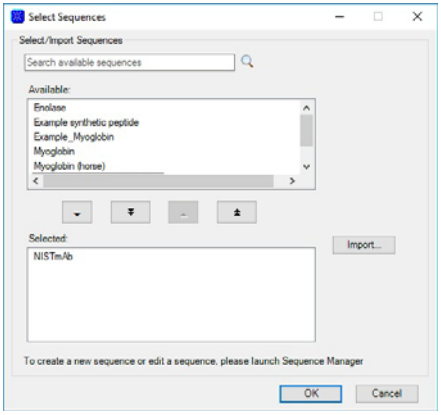
Step 9 - Print report.

Exercise 1. Interactive Intact Protein Workflow

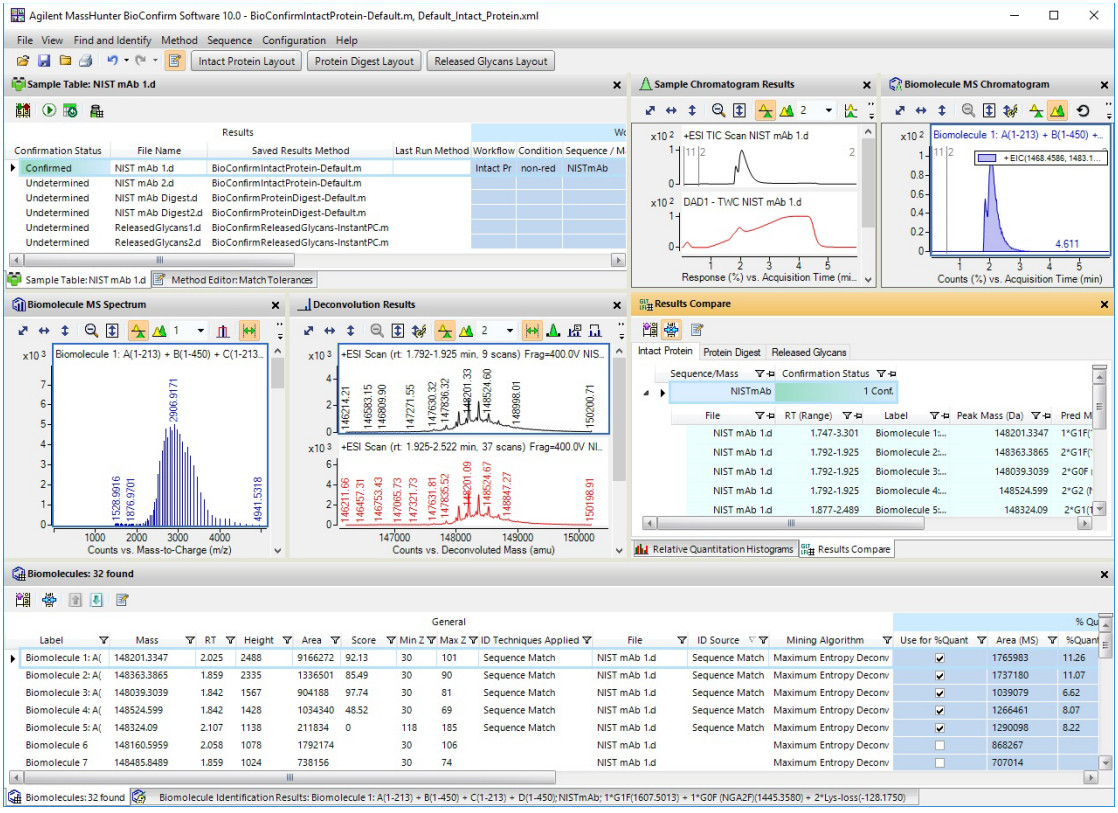
This exercise shows you how to set method parameters, match an intact protein sequence, and view the results. This exercise uses the **NISTmAb.seq** sequence file and the **NIST mAb1.d** data file copied before you started. See **"Before you start"** on page 2.

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 **NIST mAb1.d** data file is not already open, open it.	**a** Click **File > Open Data File**. **b** Locate the **NIST mAb1.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** If the Find Proteins dialog box opens, select NIST mAb 1.d and click **Find**. **d** 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*. • If you have more than one data file open, the Find Proteins dialog box opens.
5 Display the Workflow and Sequences section in the Method Editor window.	• Click **Method Automation > Workflow and Sequences** in the Method Editor window.	

Steps	Detailed Instructions	Comments
6 Import the NISTmAb sequence.	**a** Select **Intact Protein** for the Workflow. **b** Select **non-reduced** for the Condition. **c** Click the ... button next to the **Sequences** parameter. The **Select Sequences** dialog box opens. **d** Double-click **NISTmAb**. If **NISTmAb** is not available, click **Import**. **e** Select **NISTmAb.psq** and click **Open**. **f** Verify that the NISTmAb sequence is in the **Selected** list. **g** Click **OK**. 	• You will use the sequence as is. You can add or modify modifications and links to sequences as described in *online Help* and the *Quick Start Guide*.
7 Select the mAb modification.	**a** Click the ... button next to the **Modifications** parameter. The **Select Modifications and Profiles** dialog box opens. **b** Double-click **mAb** in the **Available** list in the Modifications and profiles section. **c** Click **OK**.	• The mAb sequence has modifications. You can learn how to add modifications in the *online Help* and the *Quick Start Guide*.
8 Start the match search.	**a** Click **Intact Protein > Match Tolerances**. **b** Click  on the Method Editor toolbar. **c** Select NIST mAb 1.d and click **Match**.	Alternate methods: • Click **Find and Identify > Match Sequences**.
9 Review the results.	• Select the **Biomolecule 1** row in the Biomolecules table.	• In the BioConfirmIntactProtein-default layout, the Biomolecule Identification Results window is tabbed with the Biomolecules window.

Steps	Detailed Instructions	Comments



10 Save the method for use in Exercise 2.

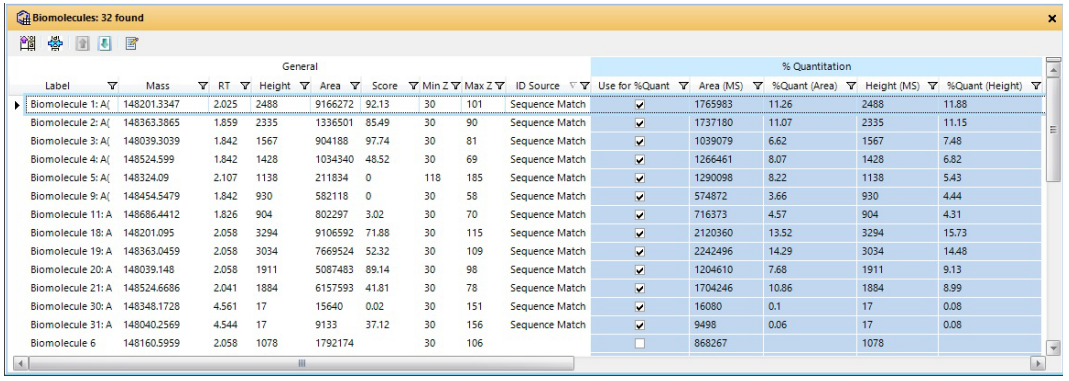
a Click **Method > Save As**.
b Type the **File name** *iii*_**NIST_mAb.m**, where *iii* represents your initials.
c Click **Save**.

11 Investigate the Relative Quantitation feature. You normally use this feature to quantitate proteoforms (PTMs on the protein).

a In the Biomolecules window, hide all empty columns. Click  in the Biomolecules toolbar.
b Review the values for **Use for %Quant** column for the biomolecules.
c Review the values for the **%Quant (Height)** and **%Quant (Area)**.

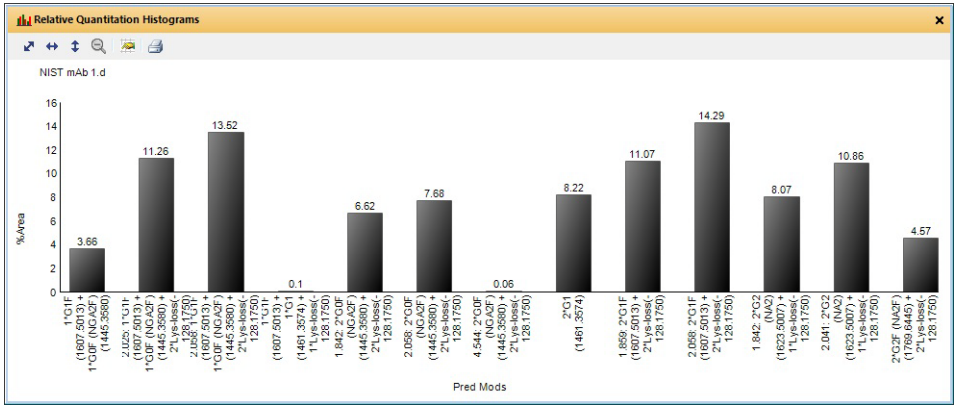
- You can right-click the window and click **Add/Remove** columns to change the columns that are available.

Steps	Detailed Instructions	Comments



Label	Mass	RT	Height	Area	Score	Min Z	Max Z	ID Source	Use for %Quant	Area (MS)	%Quant (Area)	Height (MS)	%Quant (Height)
Biomolecule 1: A(	148201.3347	2.025	2488	9166272	92.13	30	101	Sequence Match	☑	1765983	11.26	2488	11.88
Biomolecule 2: A(	148363.3865	1.859	2335	1336501	85.49	30	90	Sequence Match	☑	1737180	11.07	2335	11.15
Biomolecule 3: A(	148039.3039	1.842	1567	904188	97.74	30	81	Sequence Match	☑	1039079	6.62	1567	7.48
Biomolecule 4: A(	148524.599	1.842	1428	1034340	48.52	30	69	Sequence Match	☑	1266461	8.07	1428	6.82
Biomolecule 5: A(	148324.09	2.107	1138	211834	0	118	185	Sequence Match	☑	1290098	8.22	1138	5.43
Biomolecule 9: A(	148454.5479	1.842	930	582118	0	30	58	Sequence Match	☑	574872	3.66	930	4.44
Biomolecule 11: A	148686.4412	1.826	904	802297	3.02	30	70	Sequence Match	☑	716373	4.57	904	4.31
Biomolecule 18: A	148201.095	2.058	3294	9106592	71.88	30	115	Sequence Match	☑	2120360	13.52	3294	15.73
Biomolecule 19: A	148363.0459	2.058	3034	7669524	52.32	30	109	Sequence Match	☑	2242496	14.29	3034	14.48
Biomolecule 20: A	148039.148	2.058	1911	5087483	89.14	30	98	Sequence Match	☑	1204610	7.68	1911	9.13
Biomolecule 21: A	148524.6686	2.041	1884	6157593	41.81	30	78	Sequence Match	☑	1704246	10.86	1884	8.99
Biomolecule 30: A	148348.1728	4.561	17	15640	0.02	30	151	Sequence Match	☑	16080	0.1	17	0.08
Biomolecule 31: A	148040.2569	4.544	17	9133	37.12	30	156	Sequence Match	☑	9498	0.06	17	0.08
Biomolecule 6	148160.5959	2.058	1078	1792174		30	106		☐	868267		1078	

d Review the results in the Relative Quantitation Histograms window. For an Intact Protein workflow, this window only contains data if the **Use for %Quant** check box is marked in the Biomolecules window for one or more biomolecules and if the sequence has predicted modifications.

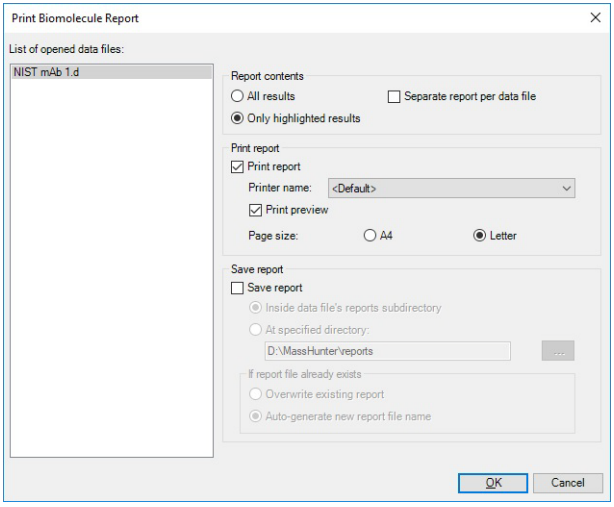


Exercise 2. Automated Intact Protein Workflow

This exercise guides you through the setup of a worklist to automatically confirm the presence of NISTmAb in a previously acquired sample. This exercise uses the **NIST mAb1.d** data file copied in Exercise 1.

Steps	Detailed Instructions	Comments
1 If not already open, open the method *iii*_**NIST_mAb_Intact.m**.	**a** Click **Method > Open**. **b** Select the *iii*_**NIST_mAb_Intact.m** folder. **c** Click **Open**.	This method was created in **"Exercise 1. Interactive Intact Protein Workflow"** on page 13.
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.	• Confirm that **Intact Protein** is selected 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 NISTmAb sequence.	**a** Select **non-reduced** for the Condition. **b** Click the ... button next to the **Sequences** parameter. The **Select Sequences** dialog box opens. **c** If NISTmAb is not available, click **Import**. **d** Select *NISTmAb*.**psq** and click **Open**. **e** Verify that the NISTmAb sequence is in the **Selected** list. **f** Click **OK**.	• The **NISTmAb.psq** sequence file is available on the BioConfirm setup media. • You can learn about modifications and links in the *online Help* and in 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 33.	• 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
Peptide Relative Quantitation Results
Results Compare
Relative Quantitation Histograms

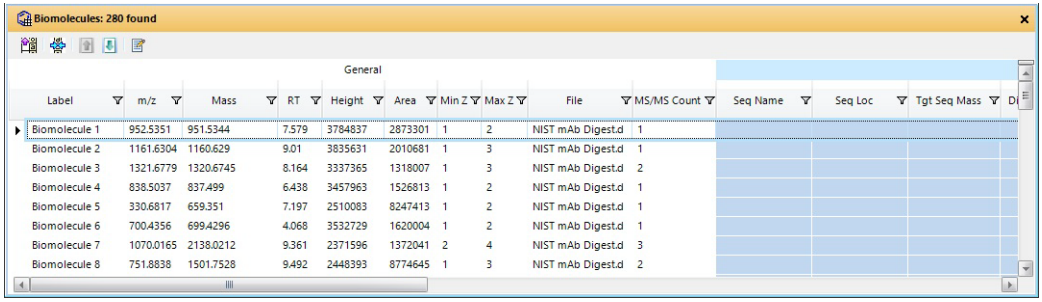
Step 9 - Print report.

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 Load the Protein Digest default layout.	• Click **Protein Digest Layout** on the main toolbar.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Select NIST mAb Digest.d. If necessary, open the example sample file.	• If available, select **NIST mAb Digest.d**. otherwise **a** Click **File > Open Data File**. **b** Locate the **NIST mAb Digest.d** sample. **c** Clear the **Load result data** check box. **d** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
4 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.
5 Find biomolecules.	**a** Click  on the Method Editor toolbar to start the biomolecule search. **b** If the **Find Peptides** dialog box opens, select **NIST mAb Digest.d** and click **Find**. **c** When processing is complete, review the results in the Biomolecules window.	• You can instead click **Find and Identify > Find Peptides**.

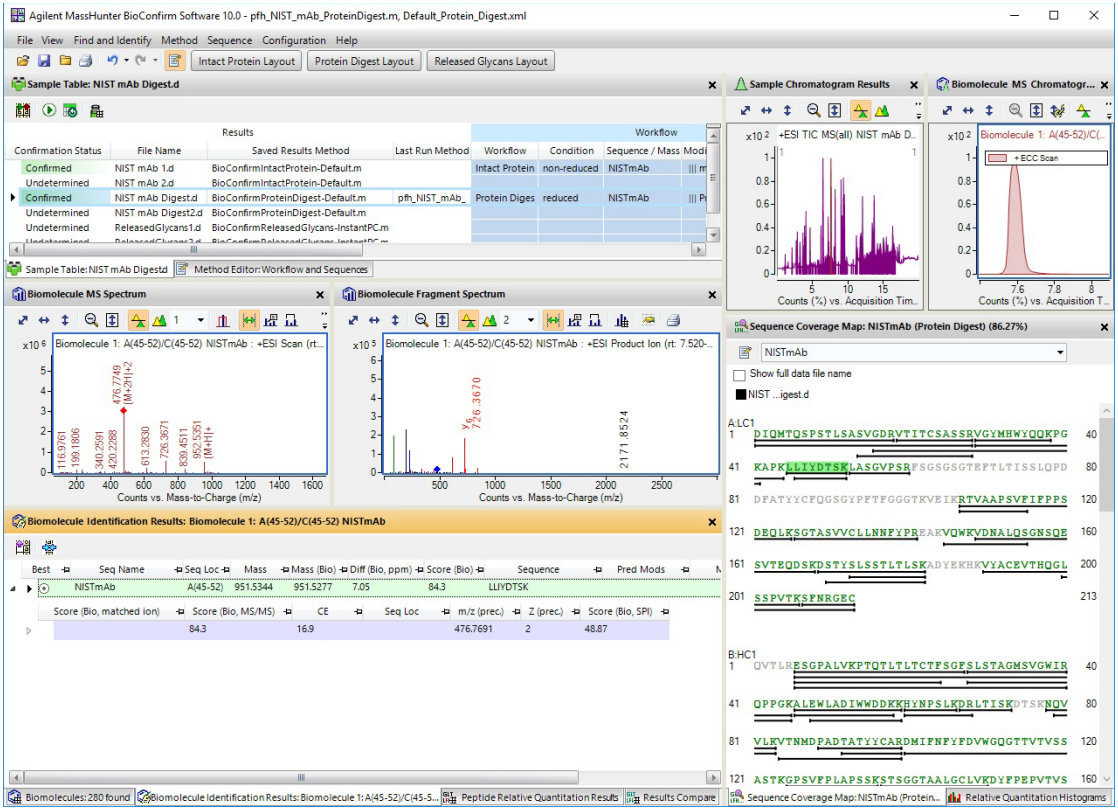


Label	m/z	Mass	RT	Height	Area	Min Z	Max Z	File	MS/MS Count	Seq Name	Seq Loc	Tgt Seq Mass
Biomolecule 1	952.5351	951.5344	7.579	3784837	2873301	1	2	NIST mAb Digest.d	1			
Biomolecule 2	1161.6304	1160.629	9.01	3835631	2010681	1	3	NIST mAb Digest.d	1			
Biomolecule 3	1321.6779	1320.6745	8.164	3337365	1318007	1	3	NIST mAb Digest.d	2			
Biomolecule 4	838.5037	837.499	6.438	3457963	1526813	1	2	NIST mAb Digest.d	1			
Biomolecule 5	330.6817	659.351	7.197	2510083	8247413	1	2	NIST mAb Digest.d	1			
Biomolecule 6	700.4356	699.4296	4.068	3532729	1620004	1	2	NIST mAb Digest.d	1			
Biomolecule 7	1070.0165	2138.0212	9.361	2371596	1372041	2	4	NIST mAb Digest.d	3			
Biomolecule 8	751.8838	1501.7528	9.492	2448393	8774645	1	3	NIST mAb Digest.d	2			

Steps	Detailed Instructions	Comments
6 Import the sequence.	**a** Click **Method Automation > Workflow and Sequences** 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** Double-click **NISTmAb**. **f** Click **OK** in the **Select Sequences** dialog box. **g** Click the [...] next to the **Mods and Profiles** parameter. **h** Double-click **Protein Digest (Reduced+Alkylated)**. **i** Click **OK** in the **Select Modifications and Profiles** dialog box. **j** 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.
7 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.	
8 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.	
9 Save the method for use in Exercise 7.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_NIST_mAb_ProteinDigest.m, where *iii* represents your initials. **c** Click **Save**.	

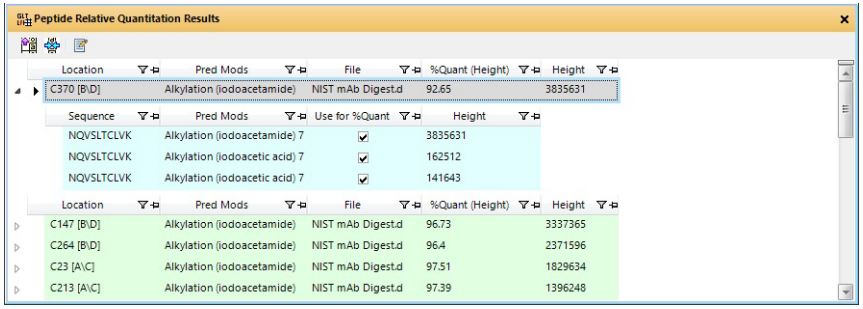
Steps	Detailed Instructions	Comments
10 Start the match search.	**a** Click **Find and Identify > Match Sequences**. **b** Select **NIST mAb Digest.d**. **c** Click **Match**.	*Alternate methods*: • Click  on the Method Editor toolbar. • Click **Match Sequences** on the Method Editor shortcut menu.
11 Review the results.	**a** Highlight Biomolecule 3 in the Biomolecules window. **b** Click the **Biomolecule Identification Results** tab which is tabbed with the Biomolecules window. **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 window.	• If the biomolecule was identified, the **ID Techniques Applied** column contains **Sequence Match**.
12 View sequence coverage results.	**a** If necessary, click **View > Sequence Coverage Map**. **b** Select a different biomolecule in the Biomolecules table to view a different result.	• Amino acids that are matched are either green (MS/MS) or black (MS-only) in a matched sequence. • Amino acids that are not matched are gray. • A line is added below the AA sequence to display where peptides have been identified. • See the online Help for more information.
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**	

Steps	Detailed Instructions	Comments
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 Review the results in the Peptide Relative Quantitation Results window.	**a** Click the **Peptide Relative Quantitation Results** tab. It is tabbed with the Biomolecules window. **b** Click the first triangle next to the first row. **c** Note that **Use for %Quant** is marked for both rows.	• For Protein Digest workflow, the software uses the **Use for %Quant** check box in the Peptide Relative Quantitation Results window.

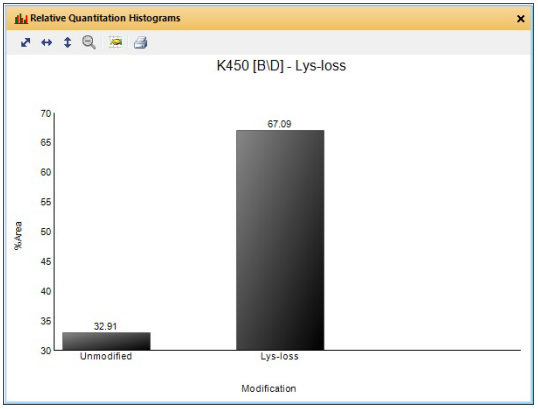
Steps	Detailed Instructions	Comments



15 Review the results in the Relative Quantitation Histograms window.

a Click the **Relative Quantitation Histograms** window.
b Click the first triangle next to the first row.
c Note that **Use for %Quant** is marked for both rows.

- For Protein Digest workflow, the software uses the **Use for %Quant** check box in the Peptide Relative Quantitation Results window.



16 Repeat the interactive processing with *NIST mAb Digest2.d*.

a Open the data file **NIST mAb Digest2.d** (see **step 3**).
b Select Find Peptides in the Method Editor and verify the parameters(**step 4**).
c Find biomolecules (**step 5**).
d Match sequences (**step 10**).
e Save the results to the NIST mAb Digest2.d file (**step 13**).

- Most of the processing parameters used for the first data file are the same for the second data file.
- These results are used in **"Exercise 8. Using Result Review mode"** on page 36.

Exercise 4. Automated Protein Digest Workflow

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

If you select the Protein Digest workflow, the Find Peptides algorithm runs and uses the enzymes 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*_**NIST_mAb_ProteinDigest.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 button,  , on the main toolbar.
3 Select the appropriate workflow.	**a** Select **Protein Digest** for the Workflow. **b** Select the **Condition**. **c** Verify that **NISTmAb** is the sequence. **d** Verify that **Protein Digest (Reduced+Alkylated)** is the Mods and Profiles. **e** 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 33.	• 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
Results Compare
Relative Quantitation Histograms

Step 9 - Print report.

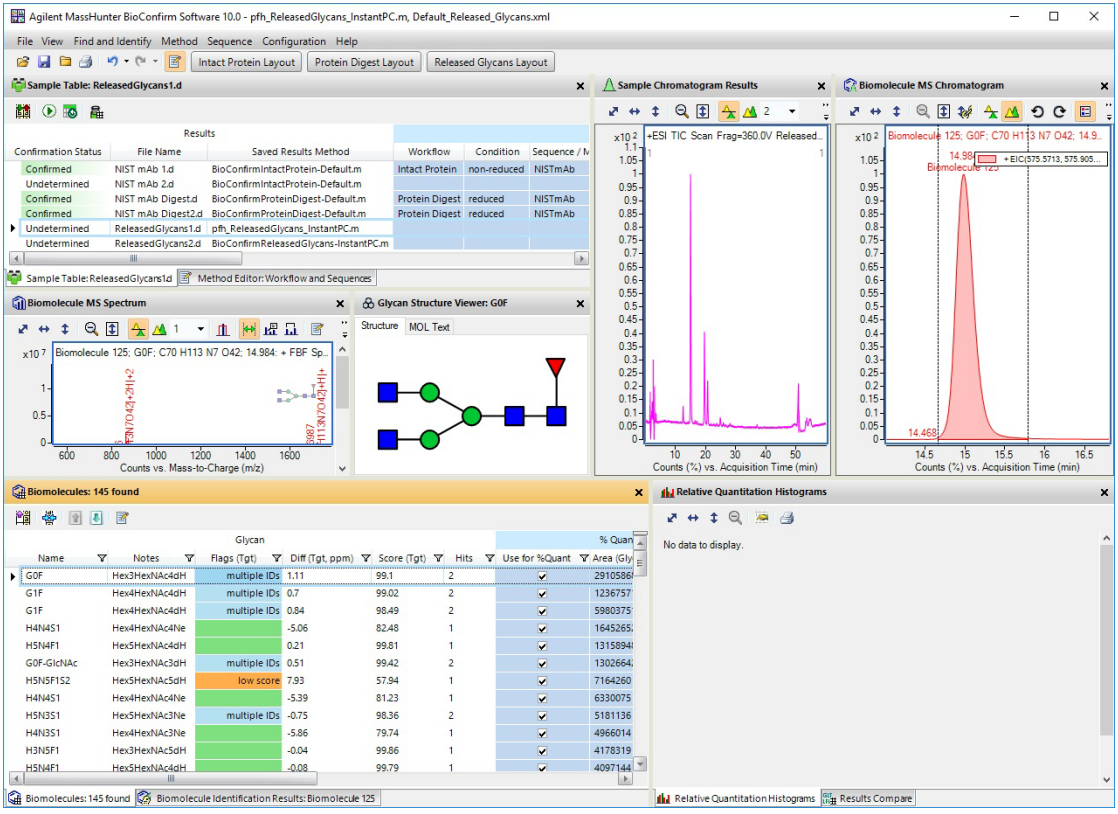
Exercise 5. Interactive Released Glycans

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 **ReleasedGlycans1.d** folder. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Load the Released Glycans layout.	• Click **Released Glycans Layout** in the main toolbar.	
4 Review the parameters in the Find Peptides section in the Method Editor window.	**a** Select **Method Automation > Workflow and Sequences** in the Method Editor window. **b** Enter `Example` in the Glycan group. **c** Clear **Require RT match if database contains a RT for the target glycan**. **d** Select **Released Glycans > Find Glycans** in the Method Editor window. **e** Select `Glycans_mAb_AM_PCD.cdb` for the **Target glycan source**. **f** Click the **Tag** tab. **g** 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.
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. **c** Click **View > Glycan Structure Viewer**.	
6 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
7 Review the results.	**a** In the Biomolecules window, click the header of the **Area (Glycan)** column to sort the table by this column. If necessary, click the header again so that the largest areas are at the top of the table. **b** Highlight **G0F** in the Biomolecules window. **c** Click the **Biomolecule Identification Results** tab. The Biomolecules Identification Results window is tabbed with the Biomolecules window. **d** 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. Also, the **Flags (Tgt)** column was moved. • The Relative Quantitation Histograms window only contains information when you run a workflow.



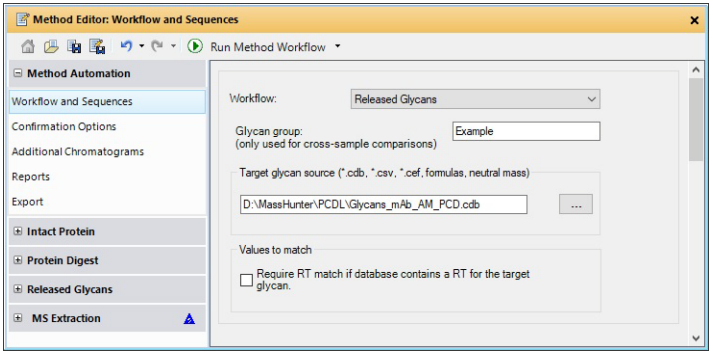
8 Save the results	**a** Click **File > Save Results** to save your results to the data file folder.	• You can also click to save results.

Exercise 6. Automated Released Glycans Workflow

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

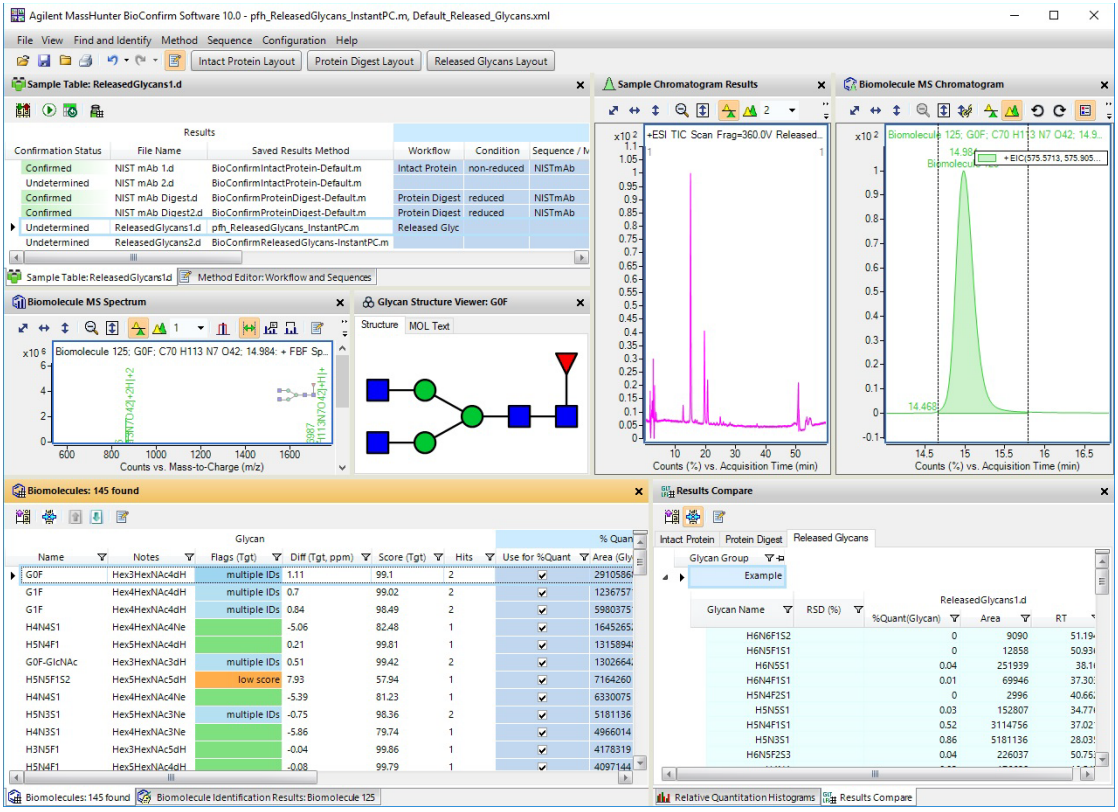
If you select the Released Glycans workflow, the Find Glycans algorithm runs and uses the **Target glycan source** selected in the Workflow and Peptides section.

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 button, , on the main toolbar.
3 Select the appropriate workflow.	**a** Select **Released Glycans** for the Workflow. **b** Enter `Example` in the **Glycan group**. **c** Select **Glycans_mAb_AM_PCD.cdb** as the **Target glycan source**. **d** 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. • The Glycan group is used to organize the results in the Results Compare window.
4 Save the method.	• Click **Method > Save**.	

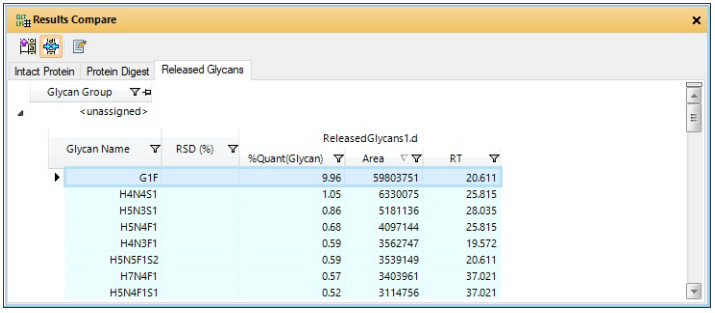


Steps	Detailed Instructions	Comments
5 Run the method workflow or run method automation.	• Click **Method > Run Method Workflow**. • Click **Method > Run Method Automation (Workflow + Reports)**. • Click  on the Method Editor toolbar when the Workflow and Sequences section is showing. • Reprocess the sample. See **"Exercise 7. Reprocessing Samples"** on page 33.	• Method Automation first runs the method workflow and then extracts additional chromatograms, generates a biomolecule report, and exports results. • The Workflow column is set to "Released Glycans". The Relative Quantitation Histograms window and the Results Compare window contain 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.

Steps	Detailed Instructions	Comments



Steps	Detailed Instructions	Comments
7 Review the results in the Results Compare window.	**a** Click the **Results Compare** window. **b** Click the **Released Glycans** tab. **c** Note that RSD (%) is empty because only one sample is selected. If you select two or more samples and they belong to the same Glycan Group, then the results are shown in the same table. If the same glycan is in multiple samples, then the RSD (%) is calculated.	• For Released Glycans workflow, the software uses the **Use for %Quant** check box in the Biomolecules window.
8 Save the results and close the data file.	**a** Click **File > Save Results**. **b** Click **File > Close All**. **c** Click Yes to save results.	

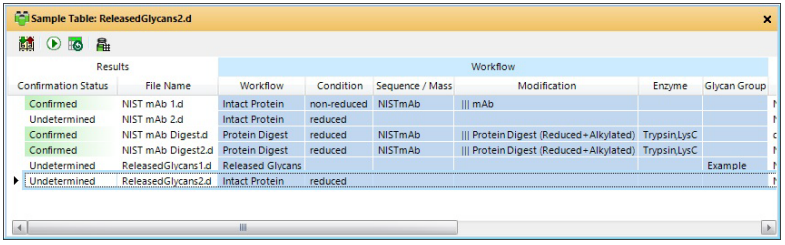


Review Results

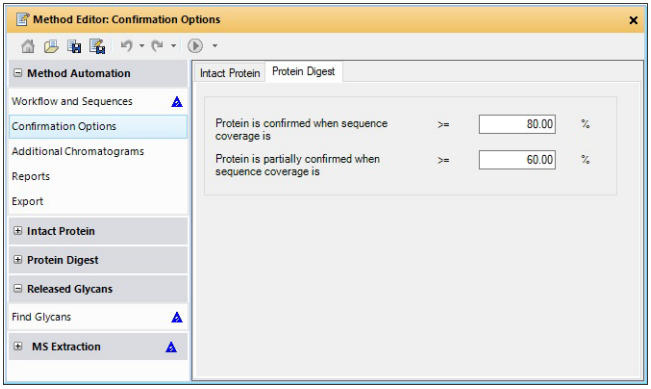
Exercise 7. Reprocessing Samples

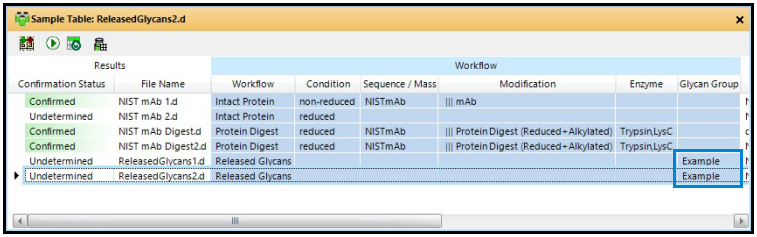
This exercise shows you how to reprocess samples in the Sample Table. You can quickly check the Confirmation Status of each sample and determine if you need to reprocess the sample.

Steps	Detailed Instructions	Comments
1 Open several data files.	**a** Click **File > Open Sample Files**. **b** Select these example files: **NIST mAb1.d**, **NIST mAb 2.d**, **NIST mAb Digest.d**, **NIST mAb Digest2.d**, **ReleasedGlycans1.d**, and **ReleasedGlycans2.d**. **c** Mark the **Load result data** check box. **d** Click **Open**.	• To select multiple files, click the first file. Then, press **Shift** and click the last file.
2 Review results in the Sample Table window.	**a** Look at the **Confirmation Status** column.	• If you saved results, the table contains information on confirmation.



Steps	Detailed Instructions	Comments
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**. • You are not changing these options. You are only seeing what the software checks to determine if the protein is confirmed.

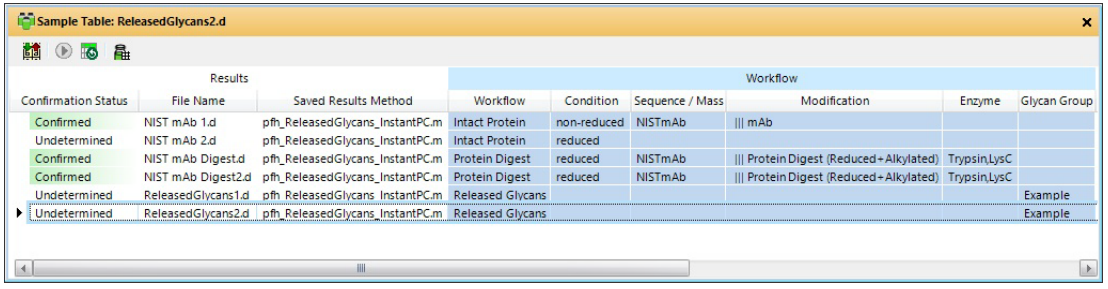


Steps	Detailed Instructions	Comments
4 Reprocess the ReleasedGlycans2.d data file.	**a** In the Sample Table, click the row containing ReleasedGlycans2.d. **b** Click **Method > Open**. **c** Select the *iii*_**ReleasedGlycans-InstantPC.m** folder. **d** Click **Open**. **e** Click the  button to open the **Reprocess Sample** dialog box. **f** Select **Released Glycans** for the workflow. **g** Enter `Example` for the **Glycan group**. **h** Select **Glycans_mAb_AM_PCD.cdb** for the **Target glycan source**. **i** Click **Reprocess**.	• To reprocess a sample, you need to first load the correct method and then complete the Reprocess Sample dialog box. • You can also double-click the Sample Table row to open 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.
Both data files that used the Released Glycans workflow have the same Glycan Group.		
5 Save the results for the samples that you reprocessed.	**a** Click **File > Save Results**. **b** Click **Save**.	

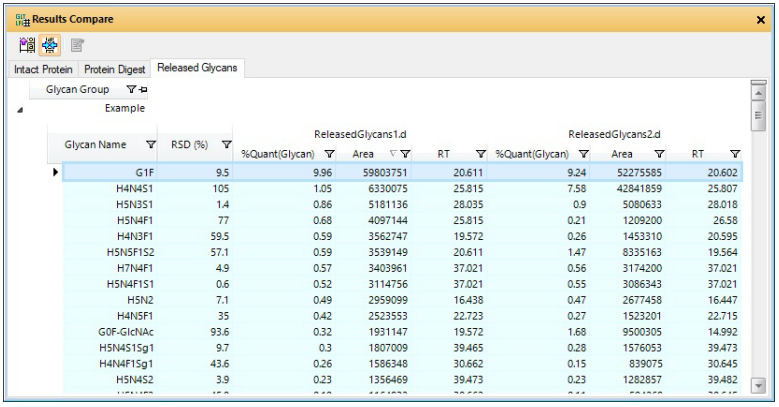
Exercise 8. Using Result Review mode

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 clicking this same comment 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.	

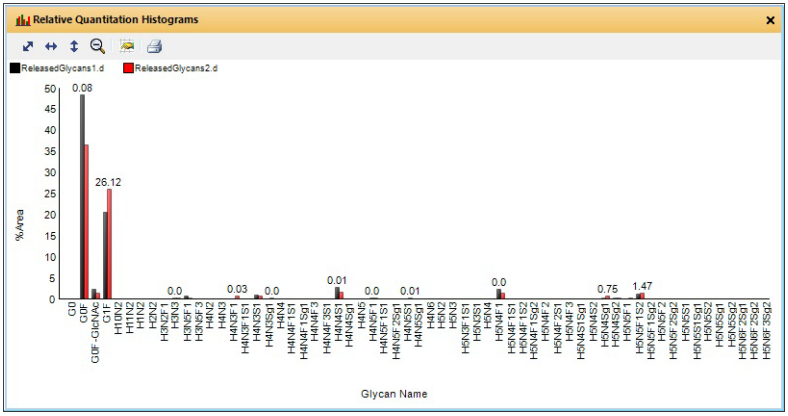


Steps	Detailed Instructions	Comments
3 Review and compare the *ReleasedGlycans1.d* and *ReleasedGlycans2.d* data files.	**a** In the Sample Table, click the row containing ReleasedGlycans1.d. **b** Press the **Shift** key and click the row containing ReleasedGlycans2.d. **c** Review the results in the Results Compare window. The RSD (%) column has been calculated for glycans that are in both samples.	

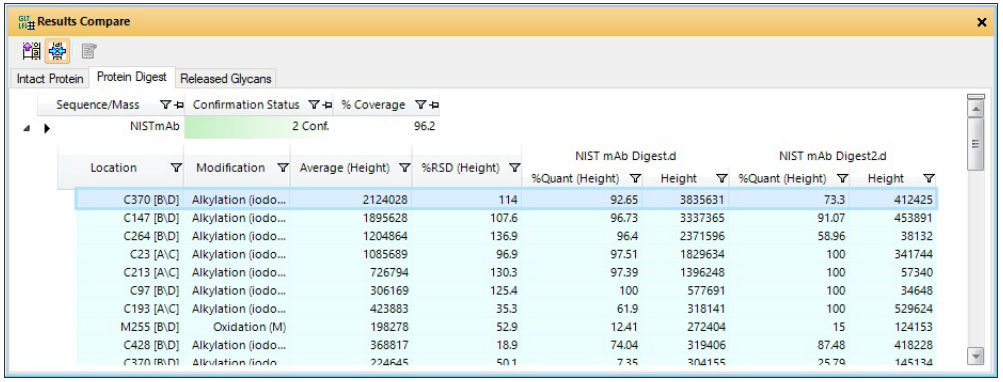


Glycan Name	RSD (%)	ReleasedGlycans1.d %Quant(Glycan)	ReleasedGlycans1.d Area	ReleasedGlycans1.d RT	ReleasedGlycans2.d %Quant(Glycan)	ReleasedGlycans2.d Area	ReleasedGlycans2.d RT
G1F	9.5	9.96	59803751	20.611	9.24	52275585	20.602
H4N4S1	105	1.05	6330075	25.815	7.58	42841859	25.807
H5N3S1	1.4	0.86	5181136	28.035	0.9	5080633	28.018
H5N4F1	77	0.68	4097144	25.815	0.21	1209200	26.58
H4N3F1	59.5	0.59	3562747	19.572	0.26	1453310	20.595
H5N5F1S2	57.1	0.59	3539149	20.611	1.47	8335163	19.564
H7N4F1	4.9	0.57	3403961	37.021	0.56	3174200	37.021
H5N4F1S1	0.6	0.52	3114756	37.021	0.55	3086343	37.021
H5N2	7.1	0.49	2959099	16.438	0.47	2677458	16.447
H4N5F1	35	0.42	2523553	22.723	0.27	1523201	22.715
G0F-GlcNAc	93.6	0.32	1931147	19.572	1.68	9500305	14.992
H5N4S1Sg1	9.7	0.3	1807009	39.465	0.28	1576053	39.473
H4N4F1Sg1	43.6	0.26	1586348	30.662	0.15	839075	30.645
H5N4S2	3.9	0.23	1356469	39.473	0.23	1282857	39.482

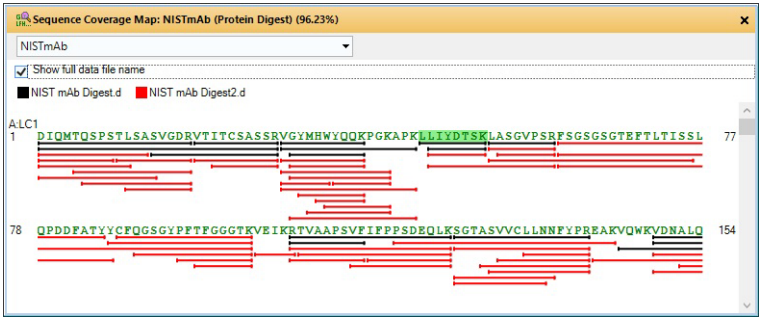
	d Review the Relative Quantitation Histograms window.	• You can visually compare the relative quantitation results for different glycans.



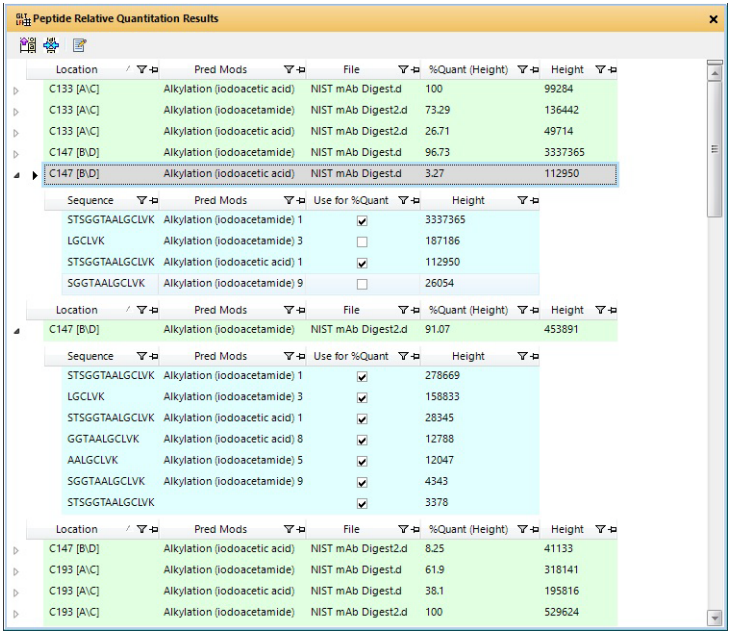
Steps	Detailed Instructions	Comments
4 Review and compare the *NIST mAb Digest.d* and *NIST mAb Digest2.d* data files.	**a** In the Sample Table, click the row containing NIST mAb Digest.d. **b** Press the **Shift** key and click the row containing NIST mAbDigest2.d. **c** Click the **Protein Digest Layout** button in the main toolbar. **d** Review the results in the Results Compare window. The RSD (%) column has been calculated for many modifications that are in both samples.	•



	e Review the results in the Sequence Coverage Map window. A legend is added to the top of the window and the lines under the sequence are color coded to show which sample is described.	•



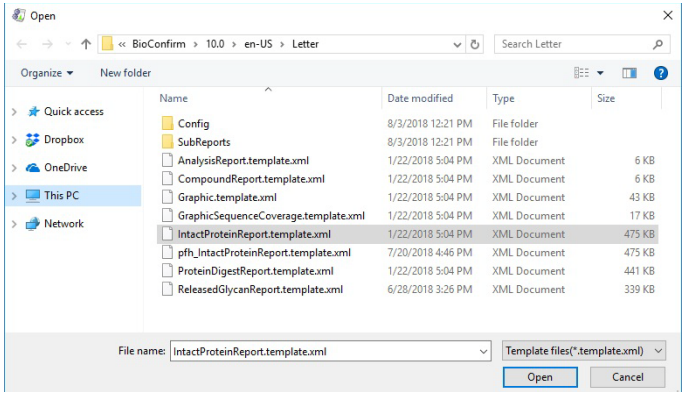
Steps	Detailed Instructions	Comments
	f Review the results in the Peptide Relative Quantitation Results window. The Location C147 [B/D] has two rows because this location is in both samples.	• For Protein Digest workflows, you mark the **Use for %Quant** check box in the Peptide Relative Quantitation Results window.
5 Save the results for the samples that you reprocessed.	**a** Click **File > Save Results**. **b** Click **Save**.	



Exercise 9. Using Report Builder

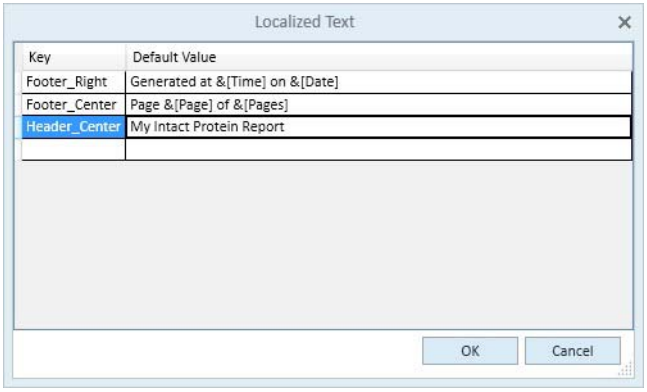
This exercise shows you the program to modify PDF templates. If you click **Use PDF Report Builder** in the Method Automation > Reports > Templates tab, then you can use Report Builder to modify those templates.

Steps	Detailed Instructions	Comments
1 Open Report Builder program.	**a** Double-click **Report Builder** in the Tools for BioConfirm 10.0 folder in the Agilent MassHunter Workstation program folder. **b** In Windows 10, click **Agilent MassHunter Report Builder > Report Builder 10.0**.	• You can also start the Report Builder program when you click one of the **Edit** button next to a template in the Method Automation > Reports > Template tab. These **Edit** 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 **D:\MassHunter\Report Templates\BioConfirm** folder.

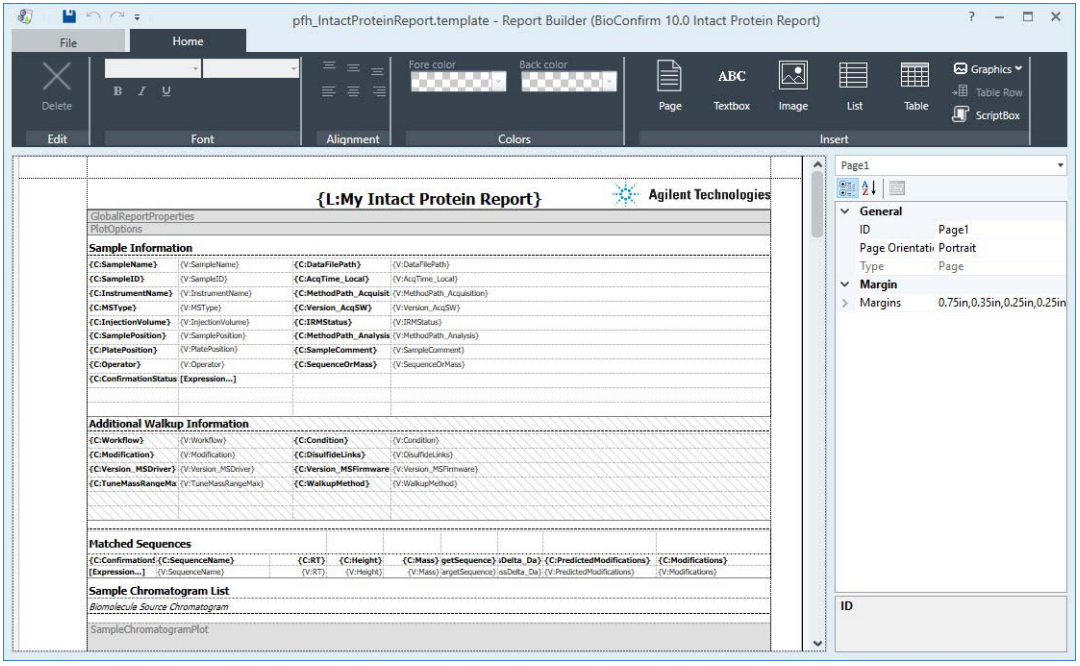


Agilent recommends that you do not modify the default templates. Instead, make a copy of the template and modify the copy.

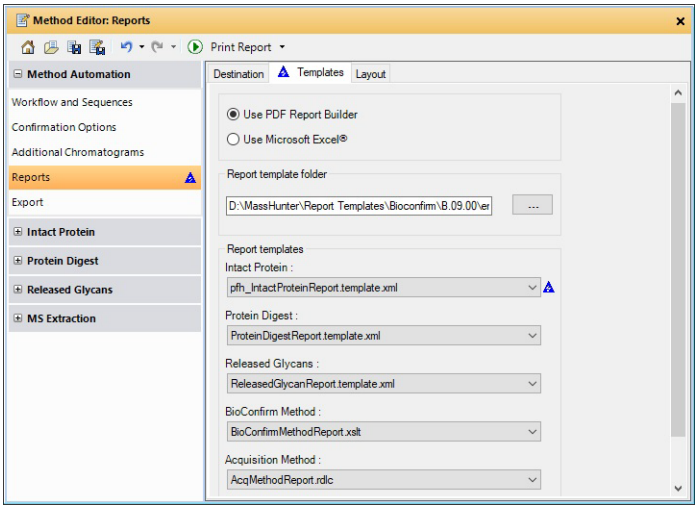
Steps	Detailed Instructions	Comments
3 Review the template in Report Builder.	**a** Click an item in the template. Notice that the right pane changes. **b** Click the title of the report. **c** In the right pane, click **Localized Text** in the Content section. **d** Click the ... button. The **Localized 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.



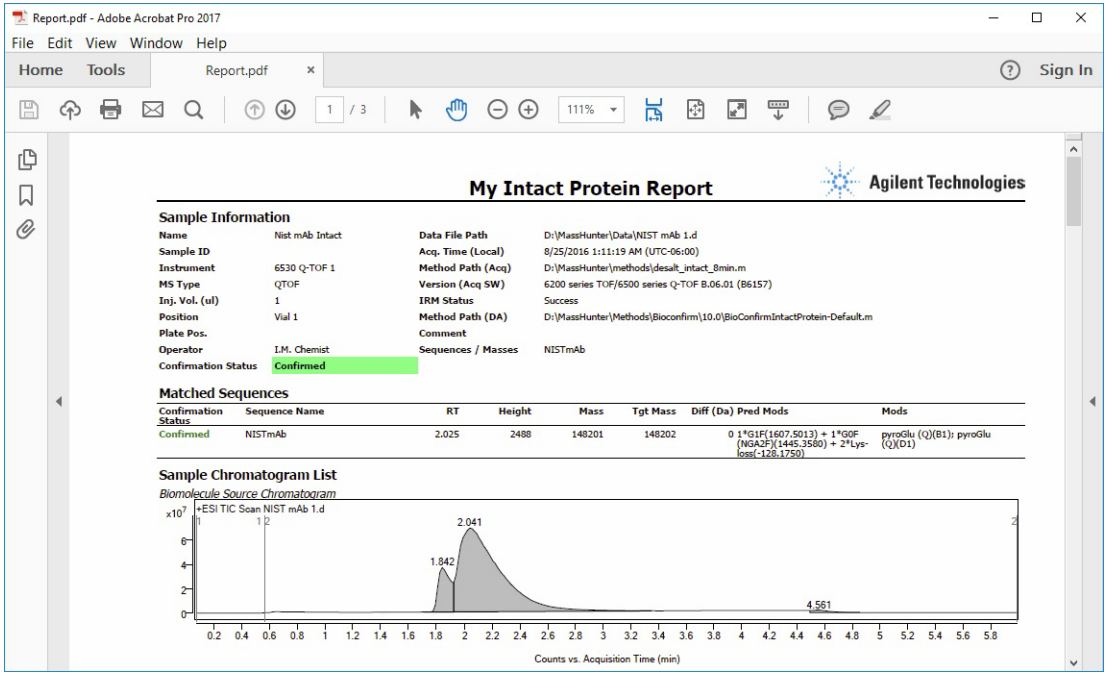
Steps	Detailed Instructions	Comments
4 Save the template.	**a** Click **File > Save As > Browse**. **b** Enter a file name and click **Save**. **c** Close the **Report Builder** program.	• You can instead click **File > Save**, and the file is saved to the current method. Agilent recommends that you 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.

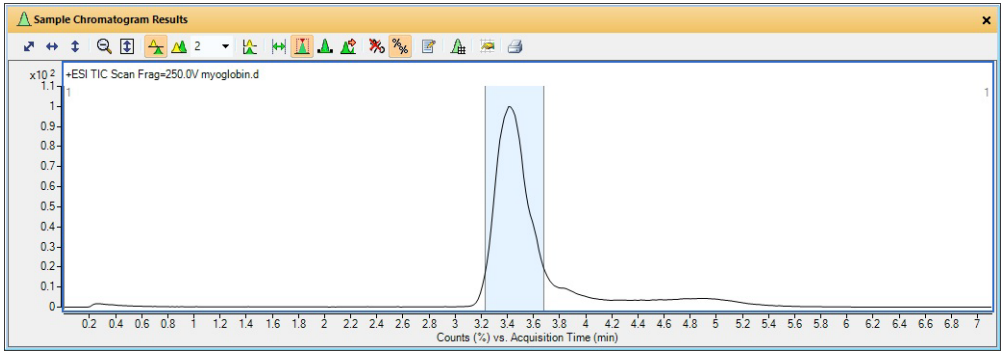


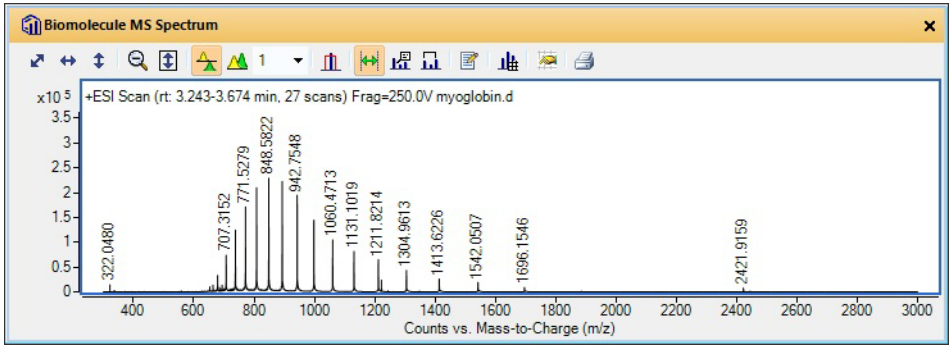
Deconvolution

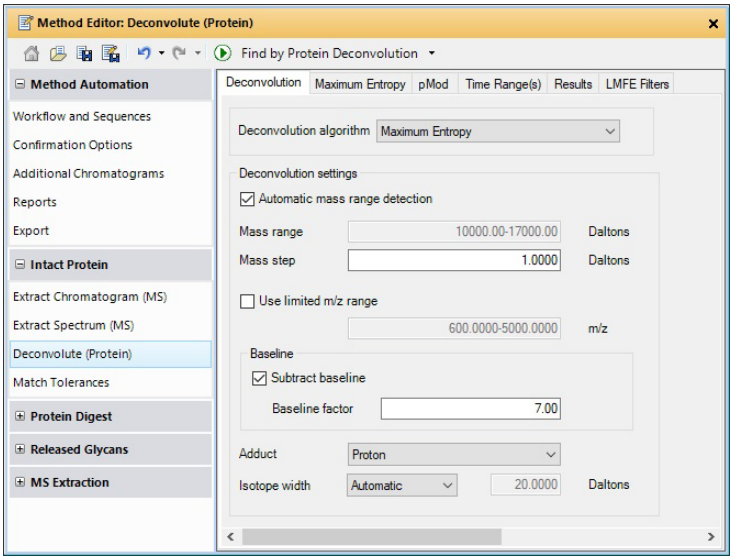
Exercise 10. Interactive Protein Molecular Weight Determination

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.

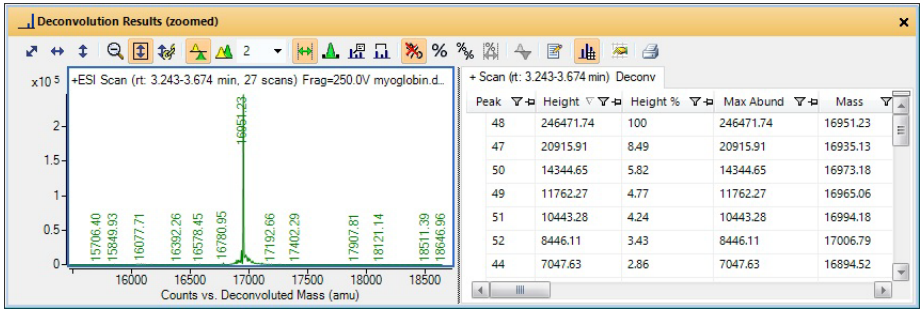
Steps	Detailed Instructions	Comments
1 Open the data file.	**a** Click **File > Open Data File**. **b** Locate the **myoglobin.d** folder. **c** Clear the **Load Result** check box. **d** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
2 Extract a peak 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 default Intact Protein method and open the Deconvolute (Protein) Method Editor section.	**a** Click **Method > Open**. **b** Select *BioConfirmIntactProtein-Default.m*. **c** Click **View > Method Editor**. **d** 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 button 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 Set the **Mass step** to 1.	• Enter 1 for the **Mass step**. 	
7 Select the extracted MS peak spectrum.	• Click the spectrum in the Biomolecule MS Spectrum window.	
8 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)**.
9 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 Create Mirror Plot 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 49 for more information.

Steps	Detailed Instructions	Comments
10 View peak information.	**a** Click the spectrum in the Deconvolution Results window to select it. **b** Click the Spectrum Peak List button ( ) . **c** Click the **Max Abund** column heading to sort results by abundance. **d** Click the Spectrum Peak List button ( ) 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.



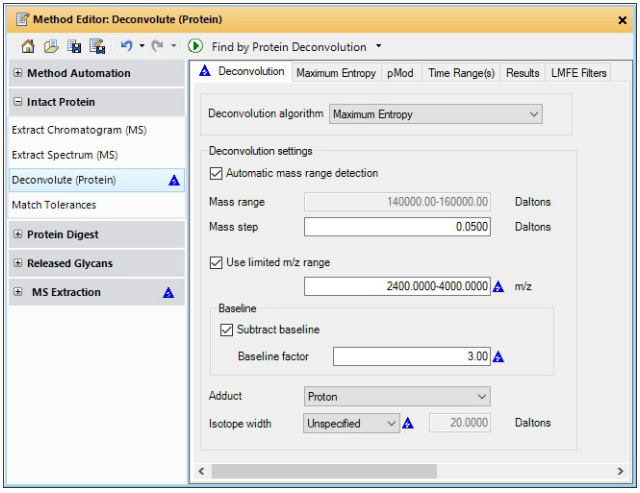
11 Save the method to iii_Deconvolution_MaxEnt.m where iii are your initials	**a** Click **Method > Save As**. **b** Enter ***iii*_Deconvolution_MaxEnt.m** for the method name. **c** Click Save.	

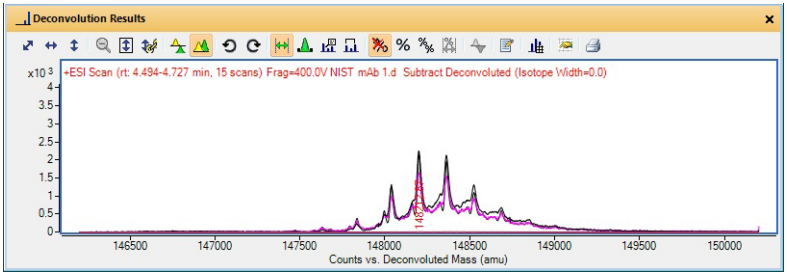
Exercise 11. Using the Mirror Plot window

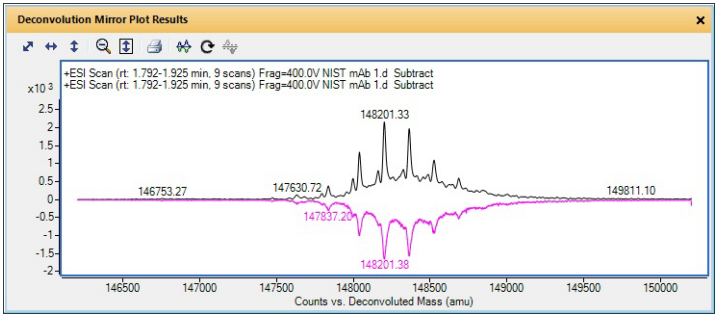
This section shows how to display a Mirror Plot of two deconvoluted biomolecules.

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** Clear **Load Result Data**. **d** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
2 Open the method to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select **BioConfirmIntactProtein-Default.m** **c** Click **Open**.	
3 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.

Steps	Detailed Instructions	Comments
4 Select the deconvolution algorithm. • Use Maximum Entropy. • 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 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**.
5 Use the default settings for Maximum Entropy deconvolution.	• Click the **Maximum Entropy** tab to review settings.	
6 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)**.



Steps	Detailed Instructions	Comments
7 Review deconvolution results.	• The results appear in the Deconvolution Results window. 	
8 Open the NIST mAb 2.d data file.	**a** Click **File > Open Data File**. **b** Locate the **NIST mAb 2.d** sample file. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
9 Run the Find by Protein Deconvolution algorithm on NIST mAb 2.d.	• Click **Find and Identify > Find by Protein Deconvolution**.	
10 Review deconvolution results.	• The results appear in the Deconvolution Results window.	
11 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.

Steps	Detailed Instructions	Comments
12 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 Deconvolution 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.	
		

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 45.	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 54.
3 Select the biomolecule with mass around 16973.1.	• Click the row which has a mass around 16973.1 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 3 spectrum in the Biomolecule MS Spectrum Results window.	• Click the graphics area for the spectrum for Biomolecule 3.	• 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 54. • 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 55.
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.

Steps	Detailed Instructions	Comments
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.

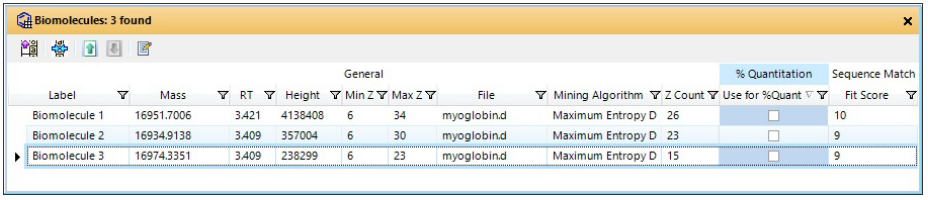


Figure 5. Biomolecules window for myoglobin.d

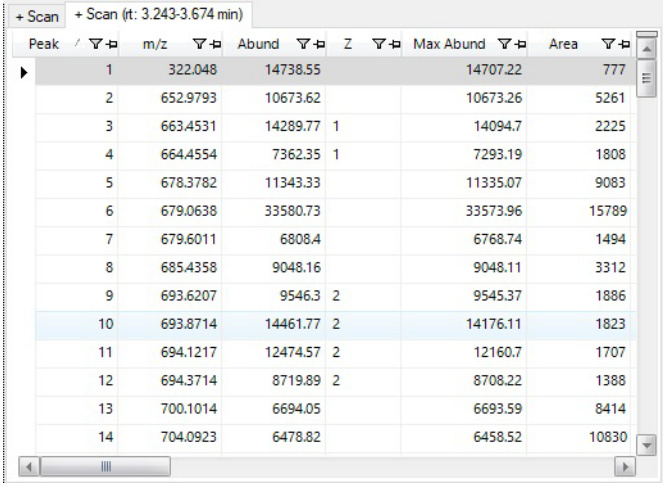


Figure 6. Peak information for myoglobin.d displayed in the Biomolecule MS Spectrum window

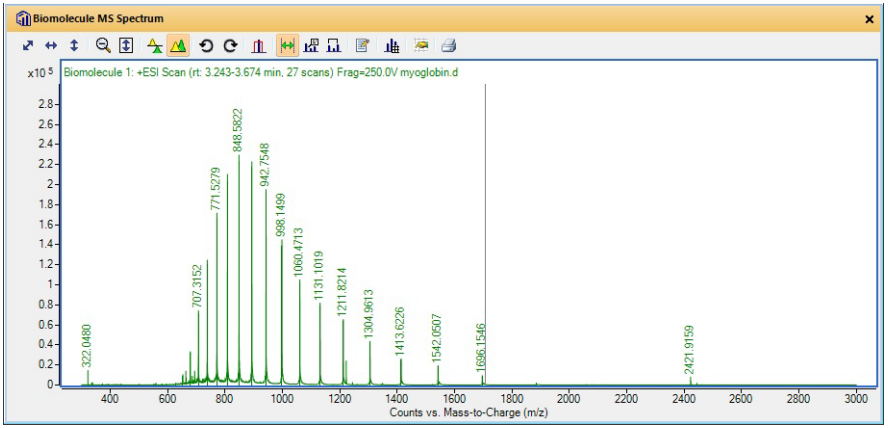


Figure 7. Biomolecule MS Spectrum Results window for myoglobin.d (Overlay Mode)

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