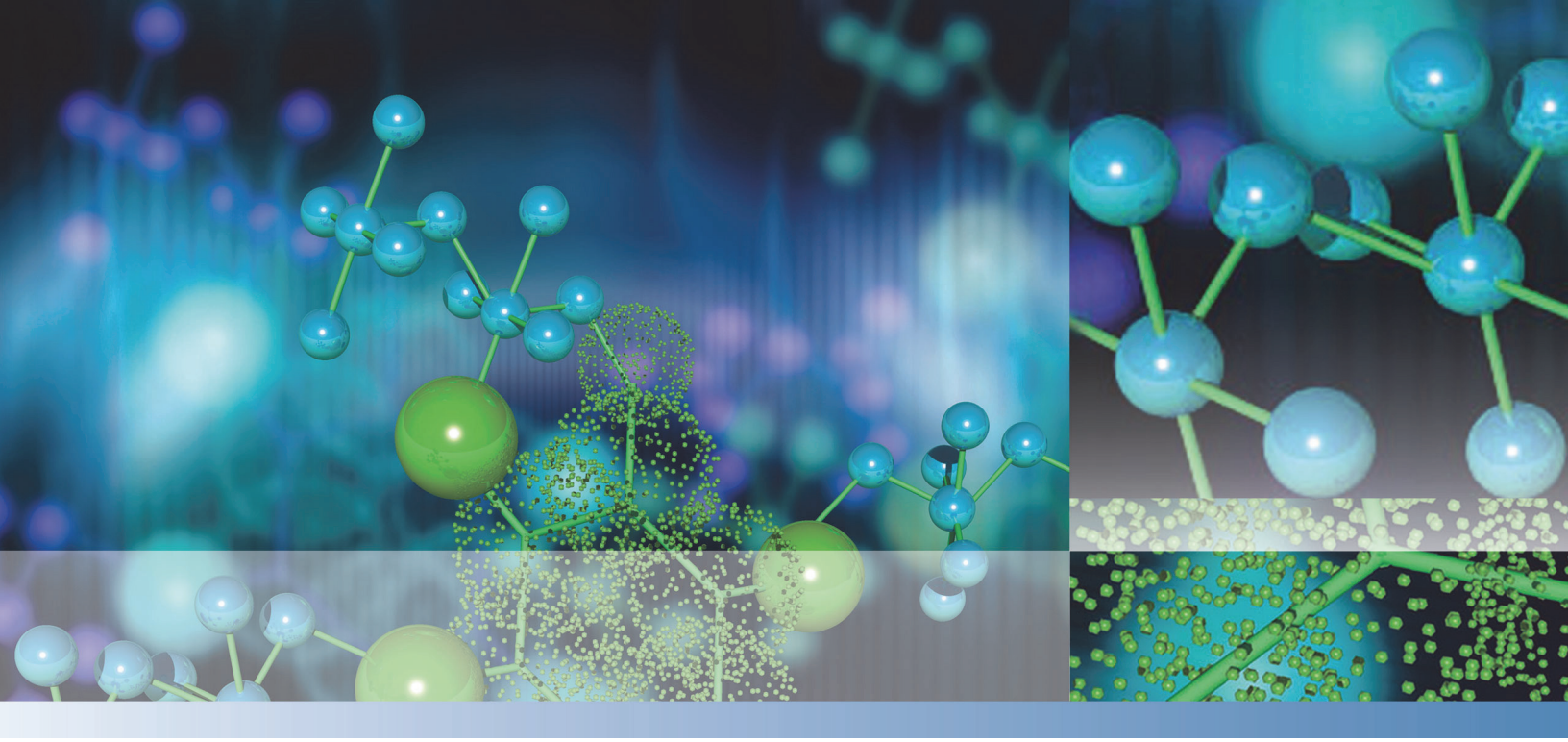

Thermo

PepFinder

User Guide

Software Version 1.0

XCALI-97661 Revision A February 2014

DOCUMENTATION SURVEY

Thermo SCIENTIFIC

Release history: Revision A, February 2014

Software version: PepFinder 1.0.x

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

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Contents

Preface

This guide describes how to use the PepFinder automated software solution for complete peptide mass fingerprinting (also known as peptide mapping) for proteolytic digestion of a recombinant (or other) protein and subsequent identification of the peptides. The application automatically finds all ions in the MS/MS or LC/MS run (excluding background ions), determines their charge states and masses, and then determines the identity of each ion in an MS/MS or LC/MS peptide mapping experiment.

Contents

Understanding the PepFinder Application

The PepFinder application does the following:

- Detects and quantifies peptide ions, distinguishing true LC components from the background signal.
- Determines the identity of a peptide ion by matching the experimental tandem spectrum to the predicted tandem spectra of peptides or modified peptides from the target protein sequence. The predicted spectra can also account for fragmentation patterns of glycosylated peptides.
- Identifies both user-specified and unexpected modifications using an error-tolerant search.
- Identifies disulfide-linked peptides, including situations where several peptides are bound by more than one dislufide bond.
- Produces peptide maps from matched MS and MS/MS peptides.
- Quantifies site occupancy of modifications.
- Compares ions in different LC/MS runs in spreadsheet format. You can import more than one LC/MS run into the application to be processed simultaneously. Then, you can export all ions found in these runs into a spreadsheet application with equivalent ions aligned in the same row. Finally, use the PepFinder application to compare these ions directly in the spreadsheet application to detect any differences among these runs.

The PepFinder application provides other output formats:

- Chromatograms with peaks that are labeled with mass values or peptide identities
- A retention time table with the *m/z* value, ion intensity, and area; the charge state, average mass, and monoisotopic mass; and the peptide identity of each ion

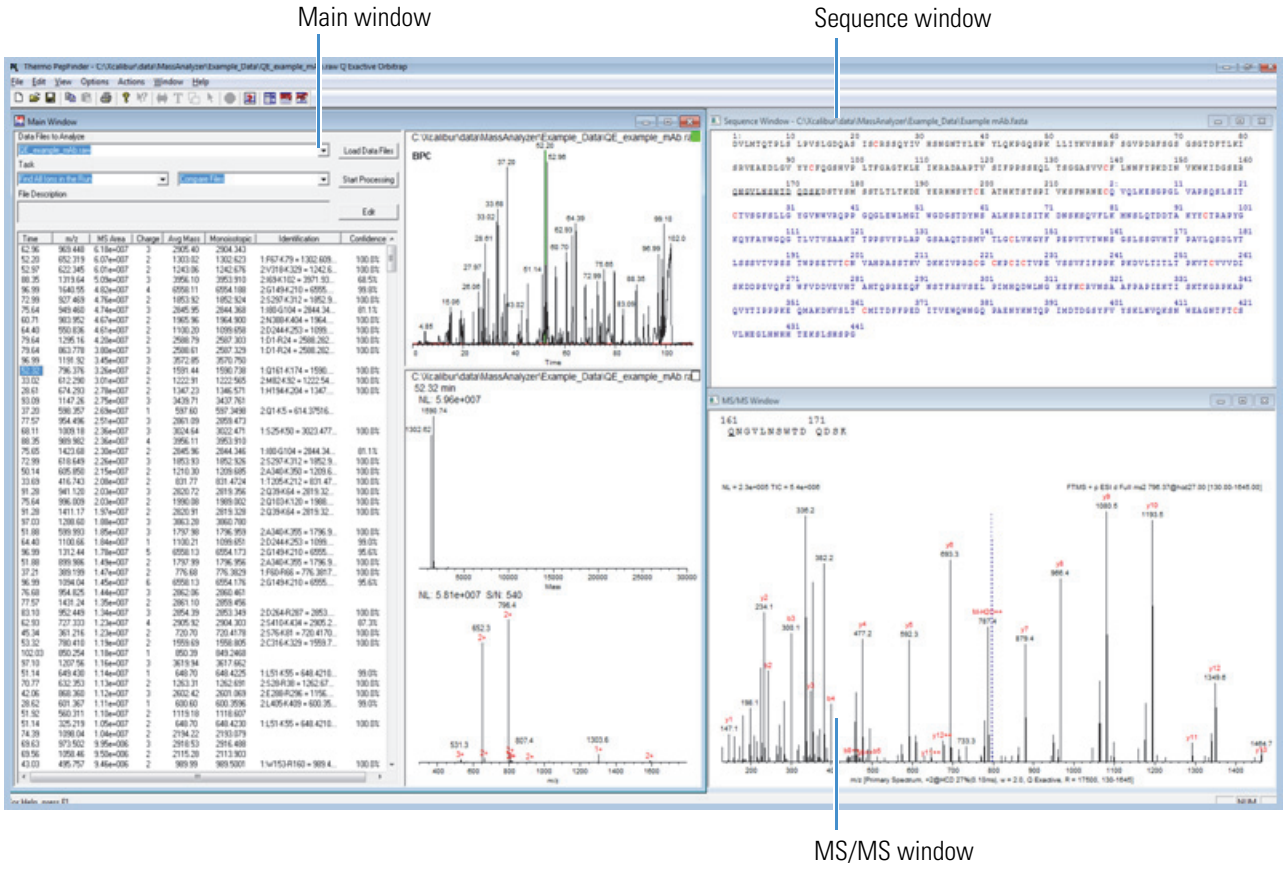
The PepFinder application reads only Thermo raw data files (acquired using Thermo Scientific™ instruments such as the Orbitrap™, Orbitrap Fusion™, Orbitrap Elite™, Q Exactive™, LCQ™, LTQ™, and LTQ FT™ mass spectrometers). For other types of Thermo instruments, you can perform limited analysis manually, including fragment ion assignments, peptide identification from a single MS/MS scan or spectrum (imported by using the Clipboard), and so forth.

The application uses three major windows: the main application window, the Sequence window, and the MS/MS window. You can access the Sequence window and MS/MS window from the View menu. The application performs top-down protein characterization in the MS/MS window.

The Main window contains three view areas: the ion list view, the chromatogram view, and the spectrum view. All three are interactive. For information about viewing and modifying results in these views, see Chapter 2, “Working with Results in the Main Window.”

The Sequence window displays the protein sequences used for identification. This window interacts with the Main window when you click an ion time in the ion list. For information about using the Sequence window, see Chapter 3, "Viewing Results in the Sequence Window."

The MS/MS window displays the experimental and predicted MS/MS spectrum, along with the predicted sequence. This window also interacts with the Main window when you click an ion time in the ion list. For information about using the MS/MS window, see Chapter 4, "Viewing Results in the MS/MS Window."



Related Documentation

In addition to this guide, the PepFinder application includes the *PepFinder Getting Started Guide* and Help.

For access to documents or the application Help, follow these procedures.

❖ **To view the product manuals**

From the Microsoft™ Windows™ taskbar, do the following:

- Choose **Start > All Programs > Thermo PepFinder > Manuals**.

–or–

- From the application, choose **Help > Manuals**.

❖ **To view application-specific Help**

- From the application window, choose **Help > PepFinder Help**.

For more information, visit www.thermoscientific.com.

System Requirements

The PepFinder application requires a license. In addition, your system must meet these minimum requirements.

System	Requirements
Hardware	• (Recommended) 32- or 64-bit computer with 16 GB RAM • 2 GHz processor with 2 GB RAM • 300 GB hard drive • CD/R-ROM drive • Video card and monitor capable of 1680 × 1050 resolution (SXGA) • NTFS format
Instruments (supported or required)	All ion trap and Orbitrap-based instruments
Software	• Microsoft™ Windows™ 7 Professional 32- or 64-bit operating system with Service Pack 1 • MSFileReader 3.0 • Microsoft .NET Framework 4.5

Activating and Deactivating the Software License

Use the Thermo Scientific Product Licensing wizard to activate or deactivate the PepFinder application. To activate the PepFinder application, you must have an activation code from Thermo Fisher Scientific. You must deactivate the PepFinder application before you transfer the license to another computer.

❖ To open the Product Licensing wizard

1. Open the PepFinder application.
2. Choose **File > About PepFinder**.
3. Click **Activate** (or **Deactivate**) to start the activation or deactivation process as applicable.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

CAUTION Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

For Thermo Scientific™ products	Access by phone, fax, email, or website
Technical Support	(U.S.) Phone: 1 (800) 532-4752 Fax: 1 (561) 688-8736 Email: us.techsupport.analyze@thermofisher.com Web—for product support, technical documentation, and knowledge bases: www.thermoscientific.com/support
Customer Service (Sales and service)	(U.S.) Phone: 1 (800) 532-4752 Fax: 1 (561) 688-8731 Email: us.customer-support.analyze@thermofisher.com Web—for product information: www.thermoscientific.com/lc-ms Web—for customizing your service request: 1. From any Products & Services Web page, click **Contact Us**. 2. In the Contact Us box, complete the information requested, scroll to the bottom, and click **Send**.
User Documentation	Web—for downloading documents: mssupport.thermo.com 1. On the Terms and Conditions Web page, click **I Agree**. 2. In the left pane, click **Customer Manuals**. 3. To locate the document, click **Search** and enter your search criteria. For Document Type, select **Manuals**. Email—to send feedback directly to Technical Publications: techpubs-lcms@thermofisher.com Web—to complete a survey about this Thermo Scientific document: www.surveymonkey.com/s/PQM6P62

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Processing Data

Use the PepFinder application to perform peptide mass fingerprinting of proteolytic digestion of a recombinant (or other) protein and subsequent identification of the peptides from LC/MS experiments. The application identifies peptides by matching the intact peptides masses using high mass accuracy and potential retention time, based on hydrophobicity or by LC/tandem mass spectrometry. You can compare the identified peptides across different LC/MS runs and compare results using the interactive tools within the application, including the modification summary report.

Contents

Acquiring Data

The PepFinder application can interpret any kind of MS/MS data from Thermo Scientific instruments, including CID (collision-induced dissociation), ETD (electron-transfer dissociation), and HCD (higher energy collision-induced). You can compare LC/MS/MS runs of similar proteins, using one of these methods:

- For an Orbitrap or LTQ FT instrument, acquire data using a high-resolution scan followed by several MS/MS scans, either in high resolution or low resolution, in centroid mode.
- For an LCQ/LTQ instrument, acquire the data in triple-play mode, for example, a full scan followed by a data-dependent zoom scan or ultra-zoom scan, followed by a data-dependent MS/MS scan in centroid mode.

Creating a Process

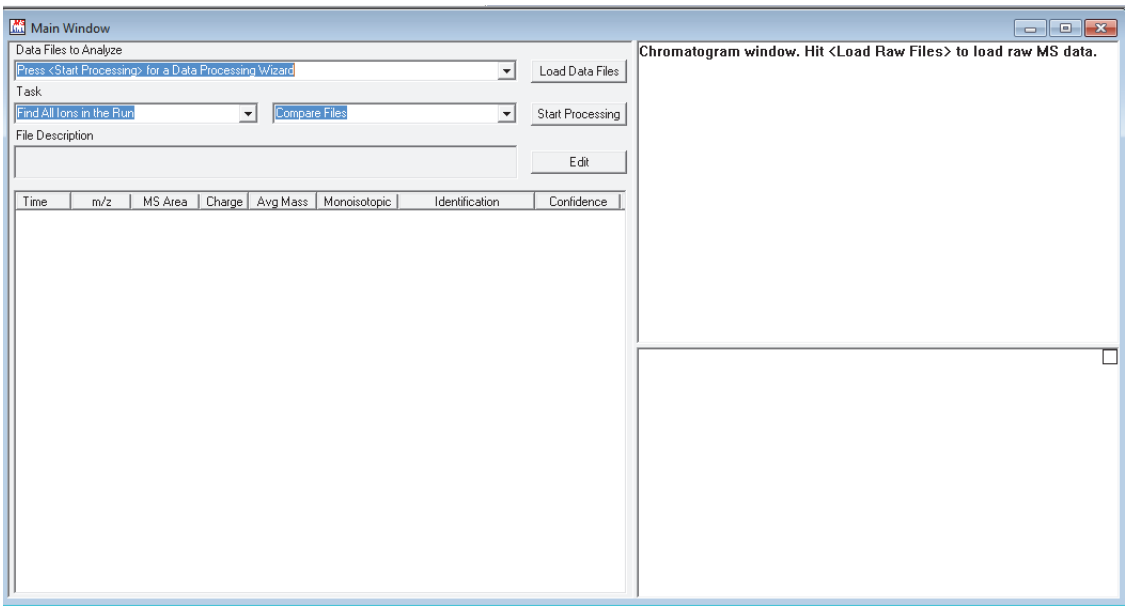
This section provides a detailed description for setting up an experiment. For guidance through the application, refer to the *PepFinder Quick Start Guide*.

Because the application repeatedly accesses the raw data files during data processing, your processing time is very slow if the application accesses the data files through a network. Copy the raw data files into your local computer for faster processing speed.

❖ To define a new experiment

Double-click the **PepFinder** icon ( ) to open the application.

The application displays a blank project in the Main window.



Adding and Modifying a Protein Sequence

The sequence establishes the target protein and helps the application match detected ions to potential identifications. Without a protein sequence, the PepFinder application still performs an analysis, but the ion list has no identification information.

Adding a Protein Sequence

❖ To add a protein sequence

1. Choose **View > Sequence Window**.
2. Choose **File > Add a Protein Sequence** to add a sequence to the sequence window.

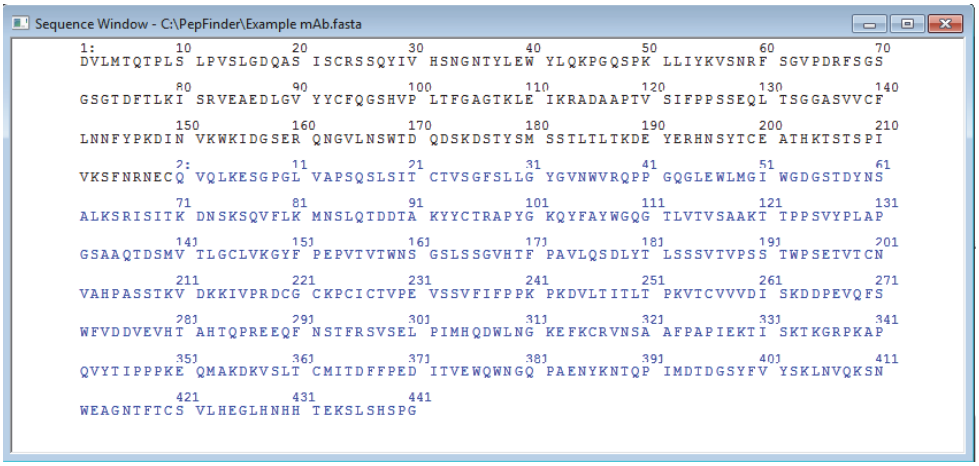
   The application displays a browse box where you can define a text file containing the target protein sequence. You can use a FASTA-formatted file to import more than one sequence at a time (IgG light and heavy chains).

   To replace a sequence in the Sequence window, choose **File > Import Protein Sequence**.

   To manually edit a sequence, choose **Sequence > Input/Edit**. The application displays a text editor window where you can make changes to the sequences that appear in the sequence window.

3. Select the protein sequence file (a text or FASTA file) and click **Open**.

   The application updates the Sequence window with the new or modified sequences.



Working with Protease and Modifications Definitions

Use the following processes to select and define the protease that is used to digest the target protein and to define expected variable modifications that are expected for the sample analysis. Oxidized methionine and deamidation are common examples of such modifications.

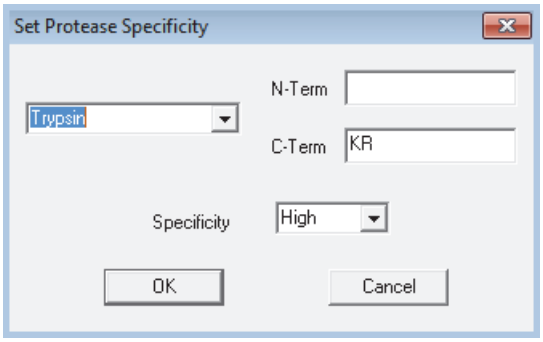
- Selecting a Protease
- Working with Modification Lists
- Defining Mass Calculations

Selecting a Protease

Use the Set Protease Specificity dialog box to select the protease used in the digestion of the target protein and to define the specificity of the protease that you used for the digestion.

❖ To define protease values

1. In the Sequence window, choose **Options > Select Protease** to open the Set Protease Specificity dialog Box.



   If your protease does not appear on the list, you can add custom proteases by opening the Sequence view and choosing Sequence > Edit Protease and Modification List. Thermo Fisher Scientific recommends this action if you expect to use the protease in subsequent analyses. You can also add a protease to the list by modifying the Mspep.ini file using a text editor.

   When you select the level of specificity (Strict, High, Medium, or Low), the application selects peptides that meet the selected specificity level. For the application to identify a peptide, at least one of the two cleavage sites must meet the user-defined specificity of the protease. If you set the level to Strict, both ends of the peptide must match the selected protease specificity.

   No limitation is applied to the maximum number of missed cleavages inside the peptide.

2. To set protease specificity, select an option from the Specificity list.

Working with Modification Lists

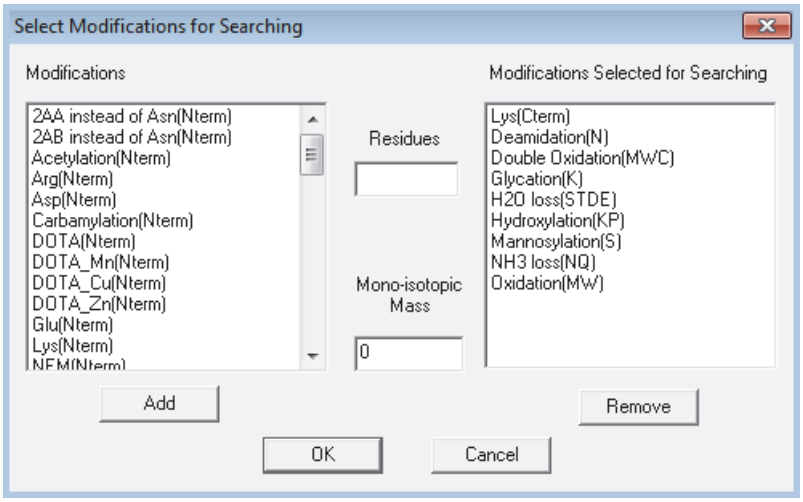
You can use the PepFinder application to specify a list of modifications to be used in the search. Select or modify the type of modifications for automated searching of modified peptides and modification sites using the Select Modifications for Search dialog box.

You can use post-translational modifications (such as phosphorylation) or modifications due to artifacts from sample handling or digestion (such as overalkylation, oxidation, or deamidation). Choose the settings for N-glycosylation, an error tolerant search, and amino acid substitutions in the Data Processing Options dialog box. For more information, see "Changing Data Processing Options" on page 7.

Editing the Modification List in PepFinder

❖ To select modifications

1. In the Sequence window, choose **Sequence > Select Modifications for Searching** to open the dialog box.



   The list on the left of the dialog box shows the available modifications and the list on the right shows the list of selected modifications to be used as variable modifications during the identification step of the process.

   If your modification does not appear on the list, you can add custom modifications by opening the Sequence view and choosing Sequence > Edit Protease and Modification List. Thermo Fisher Scientific recommends this action if you expect to use the modification in subsequent analyses. You can also add a modification to the list by modifying the Mspep.ini file using a text editor.

   To identify modification sites automatically, make sure the modification is selected for searching under Options > Select Modifications for Searching. Most unspecified modifications are detected if enough information is available.

2. To remove modifications from the Modifications Selected for Searching list, select the modification and click **Remove**.

3. To add modifications to the Modifications Selected for Searching list, select the modification and click **Add**.
4. Click **OK** to accept the changes.

   For more information about manually changing modification lists, see Manually Changing Modification Lists.

   The PepFinder application identifies proteolytic peptides by searching the mass of the ion against the known protein sequence by following the rules of the protease that you select. At least one of the two cleavage sites must match the protease specificity to be considered a peptide candidate. No limitation is applied to the maximum number of missed cleavages inside a peptide when the application is identifying peptide candidates.[1]

Manually Changing Modification Lists

❖ To edit the modification lists

1. Open the **C:\Program Data/Thermo/PepFinder** folder.

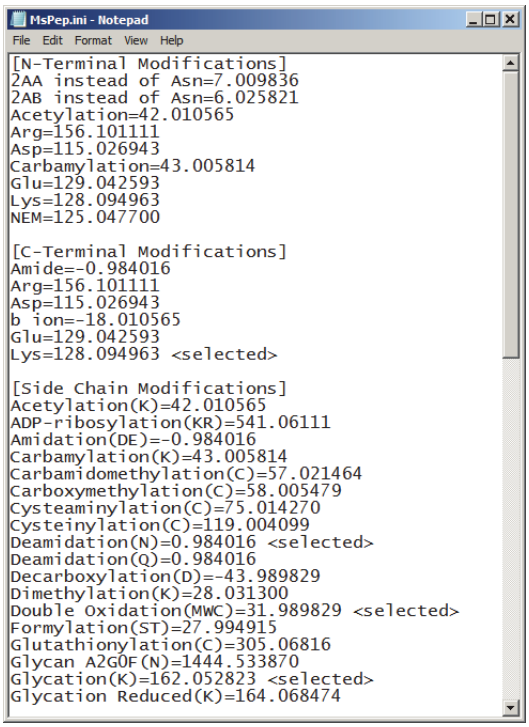
   The default list of available modifications is in the Mspep.ini file. You can edit the list of default modifications by adding or removing the <selected> terms on the end of the modifications. Thermo Fisher Scientific recommends that you add new modifications using the Edit Modification and Protease dialog box available from the Edit menu in the Sequence windows. Changes made in this dialog box are saved in the Mspep.ini file. For more information, see "Working with Protease and Modifications Definitions" on page 4.

2. Save a backup copy of the MSpep.ini file in a different location.

   IMPORTANT Make sure to edit the MSpep.ini file correctly, or the application cannot perform accurate peptide identification.

[1] Published in: Zhang, Z. Large-scale Identification and Quantification of Covalent Modifications in Therapeutic Proteins. *Anal. Chem*. **2009**, 81(20), 8354-8364.

3. Select the **MSpep.ini** file and right-click to open the file in a text editor, such as Notepad.



4. To change the default Modifications Selected for Searching list in the Select Modifications for Searching dialog box, remove the <selected> comment after items that should not appear on the list and add <selected> to items that should appear on the list.

5. Save the file to the same location.

Defining Mass Calculations

❖ **To specify a method for displaying a theoretical mass**

1. In the Sequence window, to display the monoisotopic mass in all displayed mass calculations, choose **Options > Mass > Monoisotopic**.

2. To display the average mass in all displayed mass calculations, choose **Options > Mass > Averaged**.

Changing Data Processing Options

Use the data processing options to control options for the peak detection, retention time alignment, monoisotopic and average mass determination, and peptide identification. The PepFinder application automatically determines some of the parameters, such as the absolute signal threshold and retention time shift, based on the raw data files, while you must manually select other parameters, such as those for peptide ID, that are appropriate for the sample analysis.

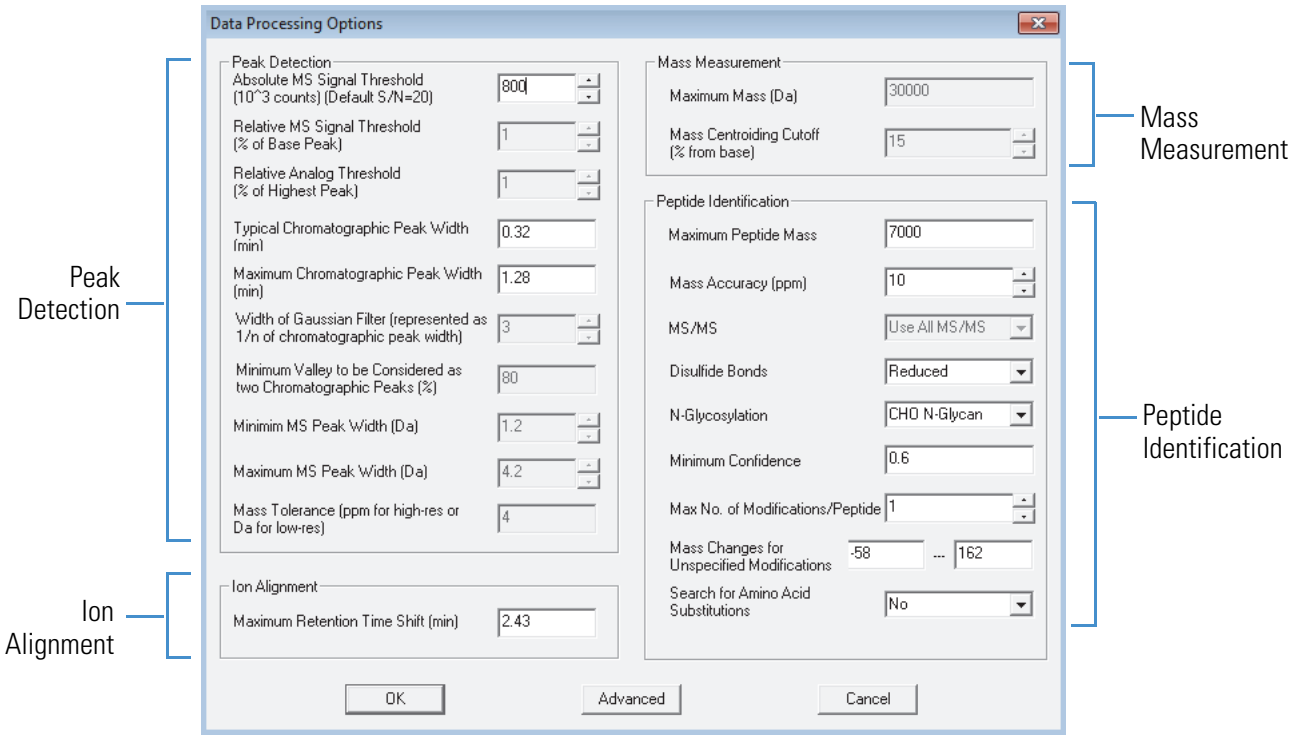
The Data Processing Options dialog box is divided into four different subprocesses: Peak Detection, Ion Alignment, Mass Measurement and Peptide Identification. For information about the processes and values for each parameter, see the following:

- Setting Peak Detection Values
- Aligning Ions
- Measuring Masses
- Identifying Peptides
- Saving Data Processing Options
- Selecting a Deconvolution Method

❖ **To open the Data Processing Options dialog box**

Click  or choose **Options > Process Options > Peak Detection/Mass Measurement/Peptide ID**.

The Data Processing Options dialog box opens. To access grayed out values, click **Advanced**.



Setting Peak Detection Values

Use the Data Processing Options dialog box to define peak detection options during processing. Click **Advanced** to modify options that are grayed out.

❖ To set peak detection values

1. Absolute MS Signal Threshold: To change the absolute MS signal threshold in counts for the application to detect an ion, type a value in the box.

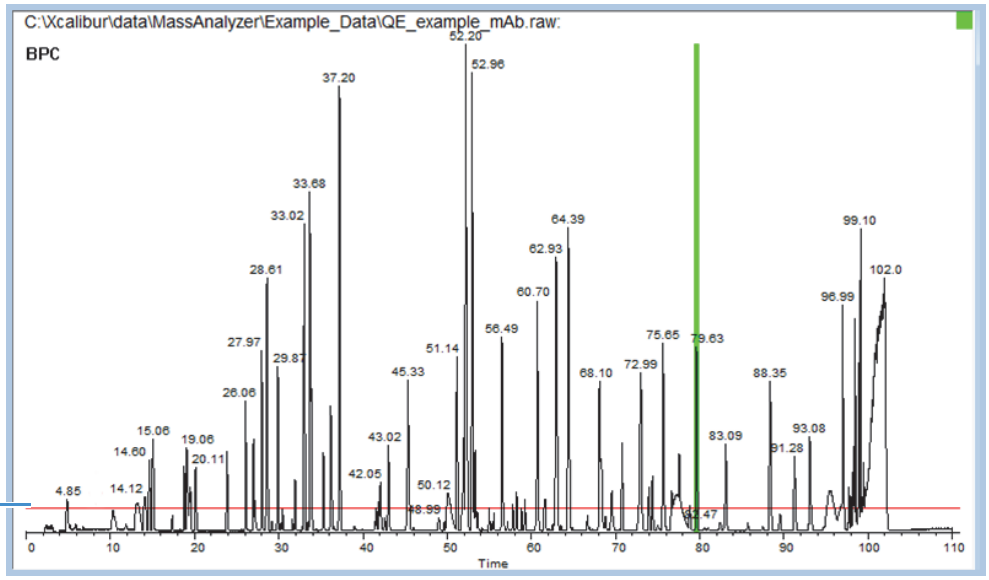
   The application automatically calculates the MS signal threshold (noise level) from the raw data file and sets this value automatically to reflect a signal-to-noise level of 20.

   The application provides a visual aid for this signal threshold by displaying a thin red horizontal line on the chromatograms displayed in the main window. As you change the absolute MS signal threshold value and click OK in the Data Processing Options dialog box, the red line reflects this change on the chromatogram. In general, processing takes much more time if this red line is well below the background noise level in the base peak chromatogram (BPC) or total ion chromatogram (TIC).

   To change the minimum MS signal in counts for the application to detect an ion, type a value in the box.

   Valid values: This value depends on the instrument. The minimum value is 0 and the maximum value is any number, but never larger than the maximum intensity component in the sample.

   Default: Calculated from the data file



2. Relative MS Signal Threshold: To set the relative MS signal threshold so that the application can detect an ion at a given point in the chromatogram, type a number in the box.

This MS signal threshold sets the minimal MS signal for an ion to be detected at a given point in the chromatogram. This parameter defines the relative threshold abundance, as a percentage, for the detection of a component that coelutes with a larger component in the same experiment.

Valid values: 0 to 100%

Default: 10%

3. Relative Analog Threshold: To define the relative analog threshold that the application detects in relation to the strongest signal in the chromatogram, type a value in the box.

   This relative analog threshold defines the minimal relative analog signal that the application detects in relation to the strongest signal in the chromatogram.

   Use this parameter only when setting up a process to find peaks using the Analog Chromatogram option. For options to set up a process, see "Creating a Process" on page 2.

   Valid values: 0 to 100%

   Default: 1%

   Valid values: 0 to 100%

   Default: 1%

4. Typical Chromatographic Peak Width (min): To set the typical chromatographic peak width (min) in the LC/MS run, type a value in minutes in the box.

   The application automatically determines the typical chromatographic peak width in minutes of the experimental LC/MS data file and sets the value to the width of the highest peak in the chromatogram.

   Valid values: 0.02 to 20 min

   Default: Calculated from the data file

5. Maximum Chromatographic Peak Width (min): To set the maximum chromatographic peak width (min) in the LC/MS run, type a value in minutes in the box.

   The maximum chromatographic peak width in the LC/MS run is automatically set by the application. This initial value is set to the geometric mean of the width of the highest peak and the range of the chromatogram. The application considers any peak wider than this value to be part of the background and does not include it in the results.

   Valid values: 0.05 to 50 min

   Default: Calculated from the data file

6. Width of Gaussian Filter: To set the width of the Gaussian filter to $1/n$ of the typical chromatographic peak width, type a value in this box.

The application uses a Gaussian filter when reading the LC/MS file and averages nearby full MS scans by applying a moving Gaussian function to improve the signal-to-noise ratio of each scan. Setting the width of the Gaussian filter is important to optimize the S/N of each scan.

For example, a value of 4 means the filter width is 40 percent of the chromatographic peak width. Lower the number to optimize sensitivity, and increase the number to optimize chromatographic resolution. A value of 1 represents a Gaussian filter for maximum sensitivity.

Valid values: 1 to 10

Default: 3

7. Minimum Valley To Be Considered as Two Chromatographic Peaks: To set the size for the minimum valley to be considered as two chromatographic peaks, type a value in the box.

   This value represents the minimum valley that the application uses to treat two chromatographic peaks as separate chromatograms.

   Valid values: 5 to 99.9%

   Default: 80%

8. Minimum MS Peak Width: To set the minimum ms peak width measured in daltons, type a value in this box.

   The application uses the minimum MS peak width in Dalton with the maximum MS peak width to generate a range for the isotope envelope of the ion.

   Valid values: 0.5 to 100 Da

   Default: 1.2 Da

9. Maximum MS Peak Width: To define the maximum ms peak width measured in daltons, type a value in this box.

   The application uses the maximum MS peak width in Dalton with the minimum MS peak width to generate a range for the isotope envelope of the ion.

   Valid values: 0.5 to 100 Da

   Default: 1.2 Da

10. Mass Tolerance: To set the maximum mass difference of the same ion in different scans, type a value in the box.

    Mass Tolerance value is the maximum mass difference of the same ion in different scans. The application measures this value in ppm for high resolution and Da for low resolution.

    Valid values: –0.01 to 50 ppm

    Default: 4 ppm

Aligning Ions

Use the Ion Alignment area of the Data Processing Options dialog box for setting the maximum retention time shift.

❖ **To specify the maximum retention shift time**

Maximum Retention Time Shift: To set the Maximum Retention Time Shift in minutes when comparing two or more LC/MS runs, type a value in the box.

Valid values: 2 to 200 min

Default: Calculated automatically from the raw data

Measuring Masses

Use the Mass Measurement area of the Data Processing Options dialog box to define options for measuring mass during processing. Click **Advanced** to modify options that are grayed out.

❖ **To define how to measure masses**

1. Maximum Mass: To change the calculation for the average mass of an ion, type a value in this box. You can set the maximum mass of the peptide or protein in the LC/MS run.

   Valid values: 1000 to 500000 Da

   Default: 7000 Da

2. Mass Centroiding Cutoff: To change the calculation for the average mass of an ion, type a value in this box.

   Valid values: 0 to 99%

   Default: 15%

Identifying Peptides

Use the Peptide Identification area of the Data Processing Options dialog box to define peptide options for processing.

❖ **To define peptide options**

1. Maximum Peptide Mass: To specify the maximum peptide mass to be identified, type a value in the Maximum Peptide Mass box. Increase this value when looking for disulfide bonds in non-reduced samples.

   Valid values: 500 to 20000 Da

   Default: 7000 Da

2. Mass Accuracy (ppm): To set the maximum mass deviation (ppm) for the comparison of the theoretical peptide mass to the calculated mass of a particular ion, type a value in the Mass Accuracy box.

   Valid values: 1 to 2000 ppm

   Default: 5 ppm

3. MS/MS: To turn on the MS/MS list, select a value to define the type of data to process.

   Valid values: Use All MS/MS, Use CID/HCD (energy collision-induced dissociation/higher energy collision-induced dissociation), Use ETD (electron-transfer dissociation)/ECD (electron-capture dissociation) Only, Ignore MS/MS

   Default: Use All MS/MS

4. Disulfide Bonds: Use this parameter to select how the application searches the disulfide bonds in the protein search. If the disulfide bonds are reduced during digestion, then select **Reduced**. If no reduction is performed and the disulfide bonds are intact, then select **Non-Reduced.**

   Valid values: Reduced, Non-Reduced

   Default: Reduced

5. N-Glycosylation: To search for N-Glycosylation, select a value from the N-Glycosylation list. Change the glycosylation parameter to match the expression system (Chinese Hamster Ovary (CHO) cell line, Human cell line, or none) used for the target protein.

   Valid values: CHO Glycan, Human N-Glycan, None

   Default: CHO Glycan

6. Minimum Confidence: Set the minimum confidence level to be reported for a peptide assignment on a 0-to-1 scale with 1 having the highest confidence.

   Valid values: 0 to 1

   Default: 0.8

7. Max No. of Modifications/Peptide: To specify the maximum number of modifications on each peptide, type a value in the Max No. of Modifications/Peptide box. Increasing this number might substantially increase processing time.

   Valid values: 0 to 8

   Default: 1

8. Mass Changes for Unspecified Modifications: To determine unspecified modifications is critical to full characterization of a target protein. To identify an unspecified modification, the PepFinder application searches for an identified peptide sequence that is similar to the unknown peptide by a user-defined mass change. If the exact modification site cannot be determined, the application places a tilde (~) symbol in front of the modification site to indicate the approximate location of the unspecified modification.

For example, an unspecific modification on a peptide, ~C310-57.0212 stands for the loss of 57.0212 u near Cys-310, indicating an incomplete alkylation. The loss of –57.0215 u was not specified in the search and without the unspecified search would not be identified.

Valid values: All positive and negative integers

Default: –58 to 162

9. Search for Amino Acid Substitutions: In the Search for Amino Acid Substitutions list, select an appropriate option:

   - To avoid searching for substitutions, select **No**.
   - Because amino acid substitutions caused by DNA mutations rarely have more than one base change in the codons of the two amino acids, to search for DNA mutations, select **Single Base Change**. With this option selected, the application displays amino acid substitutions involving only one base change in their codons.
   - To find all substitutions, select **All Substitutions**.

   Valid values: No, All Substitutions, Single Base Change

   Default: No

Saving Data Processing Options

❖ **To save data processing options**

After making changes, click **OK** to close the options dialog window.

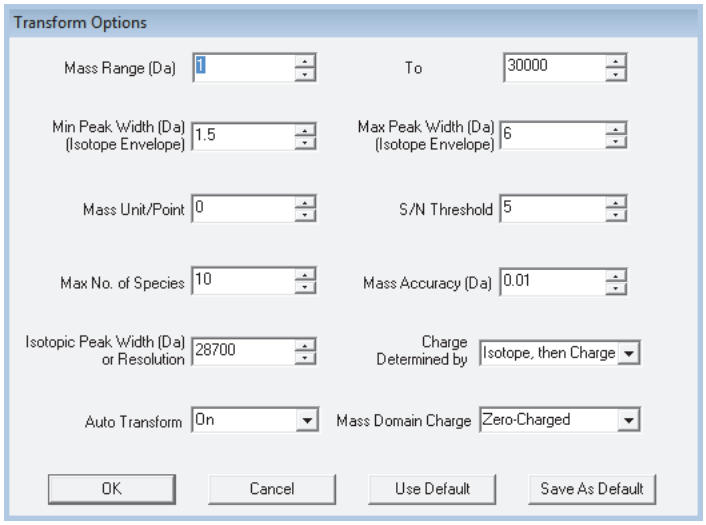
Selecting a Deconvolution Method

To perform charge state deconvolution using a deconvolution method other than a Gaussian deconvolution method or to define transform options for processing so that the application can determine the monoisotopic mass, follow this procedure.

❖ **To define transform options**

1. Activate the Spectrum view by clicking the upper right corner.
2. In the Main window, move the cursor to the Spectrum view.
3. Right-click a spectrum to open a shortcut menu, and choose **Transform Options**.

The Transform Options dialog box opens.



4. To define the minimum and maximum possible masses measured in daltons, type a value in the Mass Range (Da) box.

   Valid values: 0 to 400000 Da

   Default: 1 Da (low) 30000 Da (high)

5. To define the minimum width of a peak (at half height, including all isotopic peaks) measured in daltons, type a value in the Min Peak Width (Da) box.

   Valid values: 0.1 to 50 Da

   Default: Calculated from the data file in daltons

6. To define the maximum width of a peak (including all isotopic peaks) measured in daltons, type a value in the Max Peak Width (Da) box.

   Valid values: 0.2 to 100

   Default: Calculated from the data file

7. To define the interval (measured in daltons) between data points in the transformed mass domain (type **0** for automatic selection by the application), type a value in the Mass Unit/Point box.

   Valid values: 0 to 200 Da

   Default: 1 Da

8. To set the minimum signal-to-noise ratio for signals to be in the transformed spectrum, type a value in the S/N Threshold box.

   Valid values: 1.01 to 100

   Default: 5

9. To set the maximum number of possible output masses in the transformed spectrum, type a value in the Max No. of Species box.

   Valid values: 1 to 99

   Default: 10

10. To specify the accuracy of mass measurement in the original spectrum, type a value in the Mass Accuracy (Da) box.

    Valid values: 0.001 to 10 Da

    Default: 0.01 Da

11. To set the peak width (measured in daltons) at half the height of a single isotopic peak, or the resolution of the spectrum, whichever is constant, type a value in the Isotopic Peak Width or Resolution box.

    The application uses this parameter to determine the charge state by isotopic peaks.

    Valid values: 0.0001 to 1e+700

    Default: Read from the data file

12. To select the way charge states are determined, select an option in the Charge Determined By list.

    For low resolution data of intact proteins, select the **Charge Envelope Only** option. For transforming high resolution data of peptides or smaller proteins, Thermo Fisher Scientific recommends **Isotope, then Charge**.

    Valid values: Charge Envelope Only; Charge Isotope Only; Charge, then Isotope; Isotope, then Charge

    Default: Isotope, then Charge

13. To turn Auto Transform on if you want to start transforming automatically when a new spectrum is imported or options change, select an option from the Auto Transform list.

    Valid values: On, Off

    Default: On

14. To display the mass domain spectrum in a zero charged mass or MH+, select an option from the Mass Domain Charge list.

    Valid values: Zero-Charged, Protonated (MH+)

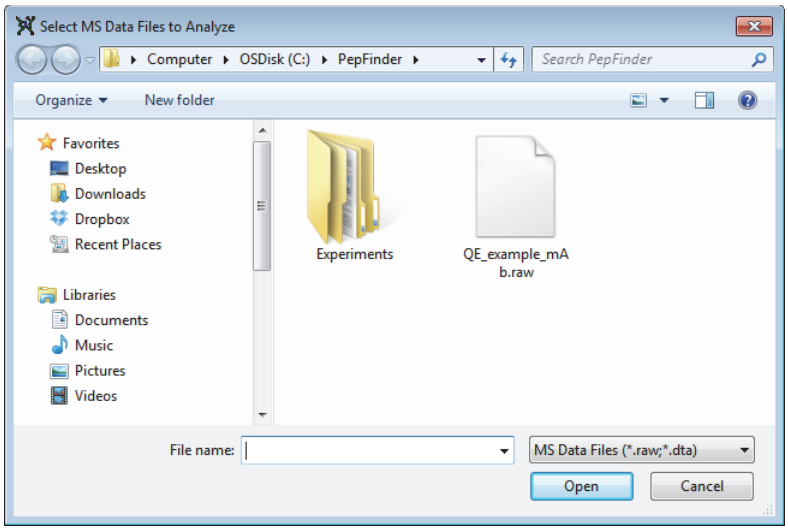
    Default: Zero-Charged

Loading Data Files

Before opening data files, you must import a protein sequence. For more information, see "Adding and Modifying a Protein Sequence" on page 3. Without the sequence, the PepFinder application performs ion detection, but the ion list has no identification information. If you performed the initial ion detection without importing a sequence, to identify ions, import a sequence and choose **Actions > Identify All Ions**.

❖ To select and load raw data files

1. In the Main window, click **Load Data Files** to open a browse window.

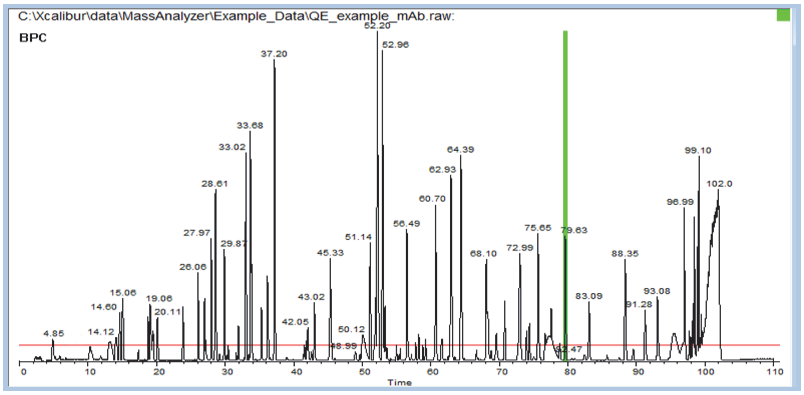


2. Browse to and select Thermo raw data files for processing.

   Load all the raw data files that you will analyze together by using the CTRL or SHIFT key.

3. Click **Open**.

4. After the application has opened the files, review the chromatogram displayed to the right of the Ion List view. If the red line is below the general level for noise, increase the peak detection value to a more appropriate value before processing.



Beginning Processing

❖ To begin processing the data

1. From the first Task list in the Main window, define the experiment task.

   The default task is Find All Ions in the Run. Information about each ion is displayed in an ion list, including retention time, *m/z*, MS peak area, charge, and so forth. You can customize the information in the ion list as described in "Selecting Ion Properties to Display" on page 31.

   If you set a project to a specific option that is not the default in the Task list and then close the application, you must reset the Main window Task options to the specified values (for example, Find All Masses in the Run) before you can reopen the experiment and see the data.

   You can specify these additional tasks:

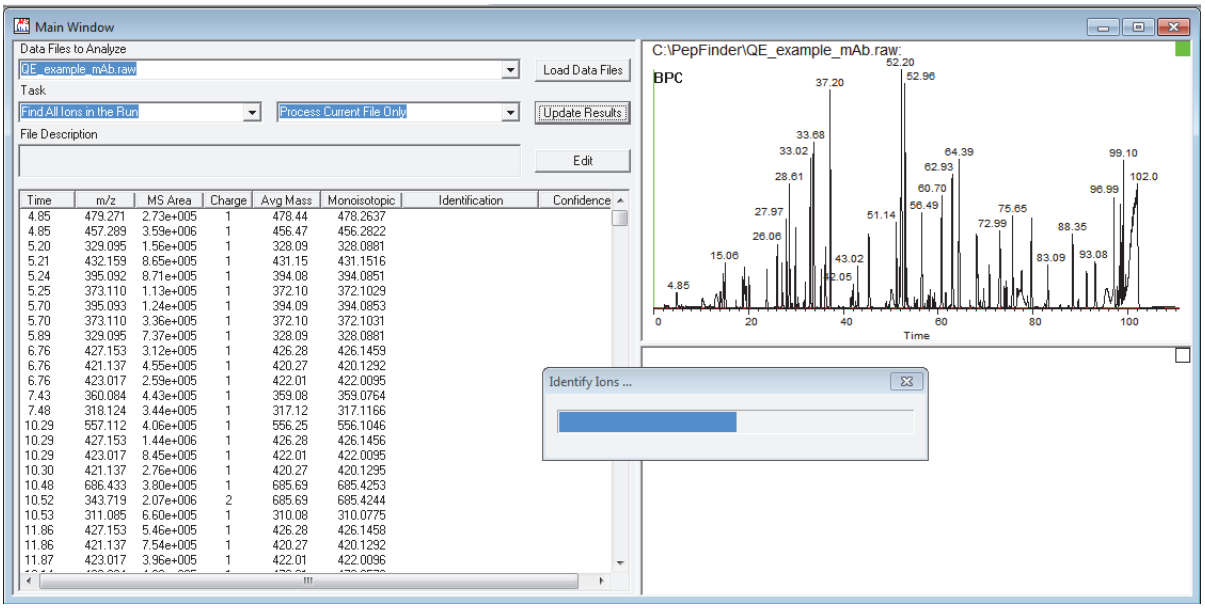
   - Find all Masses in the Run: Combines multiple charge states into a single entry.
   - Find Peaks in the Total Ion Chromatogram: Detects peaks in the Total Ion Chromatogram, deconvolves the spectra, and identifies MS/MS spectra associated with the major ions under these peaks.
   - Find Peaks in the Base Peak Chromatogram: Detects peaks in the Base Peak Chromatogram, deconvolves the spectra, and identifies MS/MS spectra associated with the major ions under these peaks.
   - Find Peaks in the Analog Chromatogram: Detects peaks in the analog chromatogram.
   - Find all Ions with MS/MS: Detects ions that have an associated MS/MS spectrum.

2. From the second Task list, limit your search as follows:

   - To compare ions in all selected files, select **Compare Files**.
   - To process files individually, select **Process Files Separately**.
   - To process the currently displayed file, select **Process Current File Only**.

3. To start processing the files, click **Start Processing** in the application Main window.

   To view the state of processing at any point, click **Update Results**. You can click this button as often as you want. The application updates the ion list to reflect newly detected ions and their identifications. You can click ions in the list to see the identified MS/MS spectra.



When you want to stop most time-consuming tasks, click the **Stop Processing** icon () on the toolbar. The application still displays the current state of processing in the ion list.

Interpreting Results

After the application finishes (approximately 5 to 10 minutes per file, depending on the processing computer), the application displays a list of ions with their identifications and confidences on the left side of the window and a Chromatogram view and a Spectrum view for the selected ion on the right side.

For information about viewing and modifying results, see Chapter 2, "Working with Results in the Main Window."

Saving Experiment Results

After processing is complete and you have made changes to the data, save your results using this procedure. The application saves the file as a PMF file.

❖ To save your experiment results

- Choose **File > Save** to overwrite a previous file for that experiment.

–or–

1. Choose **File > Save As** to save the file under a new name.
2. Click **OK**.

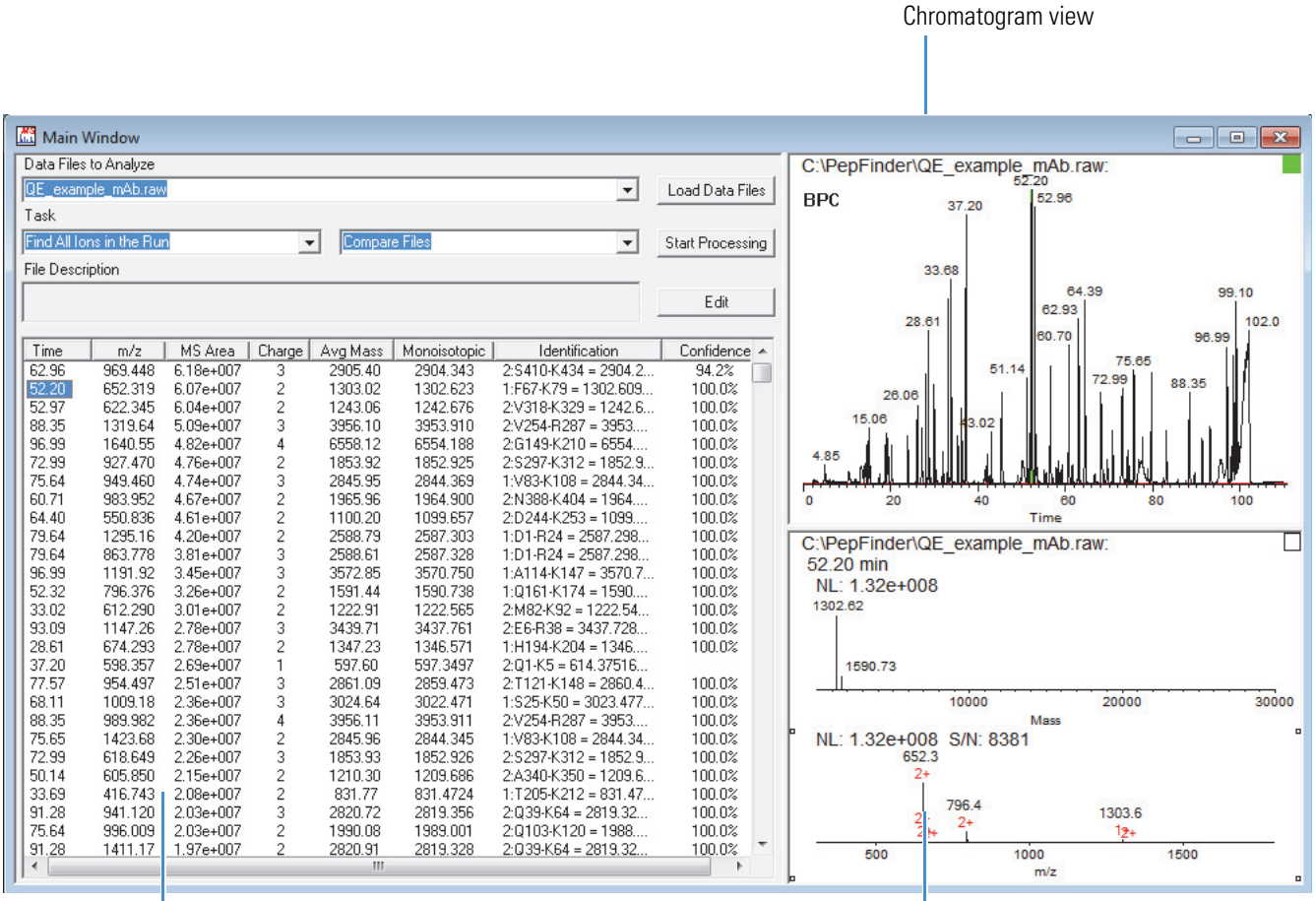
2

Working with Results in the Main Window

After the PepFinder application finishes processing data, it displays processing information in the Main window.

Contents

The Main window contains three view areas. The view on the left shows the ion list. The other two views show chromatograms and spectra. You can use the chromatograms and spectra views together to manually explore the data (similar to the Xcalibur™ Qual Browser application).



To access some of the commands in the PepFinder application menus, you must be in a specific area such as the Spectrum view of the Main window.

❖ To make an area active

Click the square in the upper right corner. The square turns green and the application changes the menu options accordingly.

–or–

Select an object or place your cursor in the area you want to make active.

Viewing Results in the Main Window

❖ To open the Sequence window

Choose **View > Sequence Window**, or click  .

❖ To display the peptide MS/MS Spectrum window

Choose **View > MS/MS Window**, or click  .

❖ To show or hide the toolbar

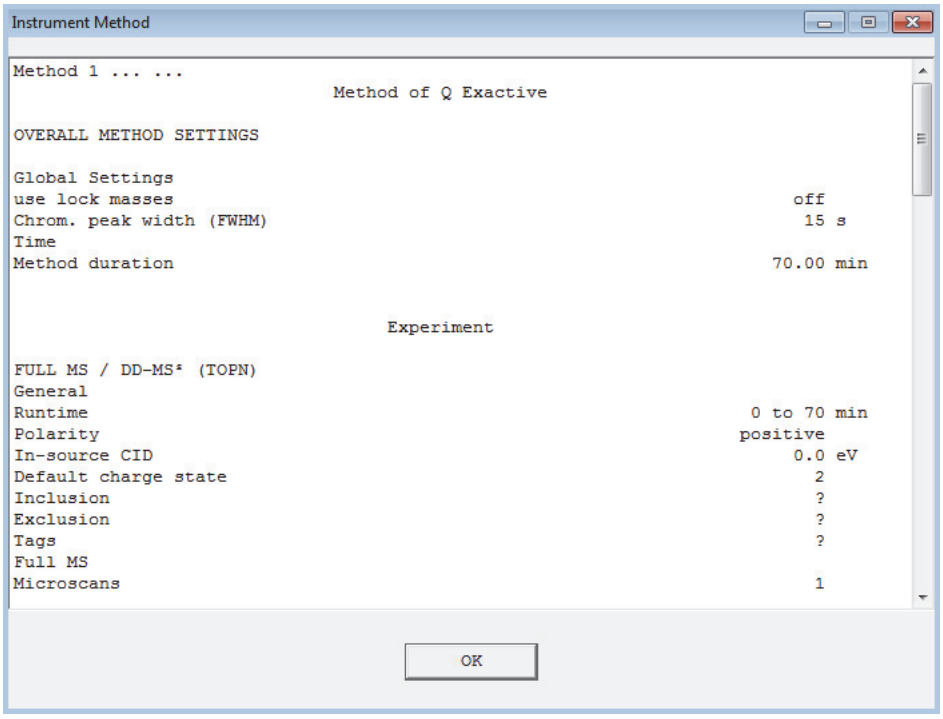
Choose **View > Toolbar**.

❖ To show or hide the status bar

Choose **View > Status Bar**.

❖ To display the instrument method

1. To view the instrument method, click the ion list view to make it active.
2. Choose **View > Instrument Method**.



Editing the Main Window Contents

To view results data in the Ion List view, the Chromatogram view and the Spectrum view, follow these procedures.

- Working with the Ion List View
- Working with the Chromatogram View
- Working with the Spectrum View

Working with the Ion List View

To search and make changes within the Ion List view, follow these procedures.

- Copying an Ion List
- Searching an Ion List
- Viewing and Modifying Specific Ions
- Setting Display Options for the Ion List

Copying an Ion List

❖ **To copy an ion list from a project to the Clipboard**

1. Open the project in the Main window and choose **Edit > Copy Ion List**.

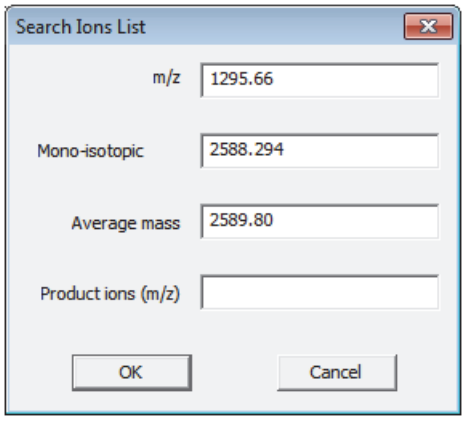
   The application copies the ion list to the Clipboard.

2. Open a file and paste the list into it.
3. Save the file.

Searching an Ion List

❖ To search the ion list

1. From the review display, choose **Actions > Search Ion List** to display the Search Ions List dialog box.



   Use this dialog box to search the ion list for ions with the specified properties.

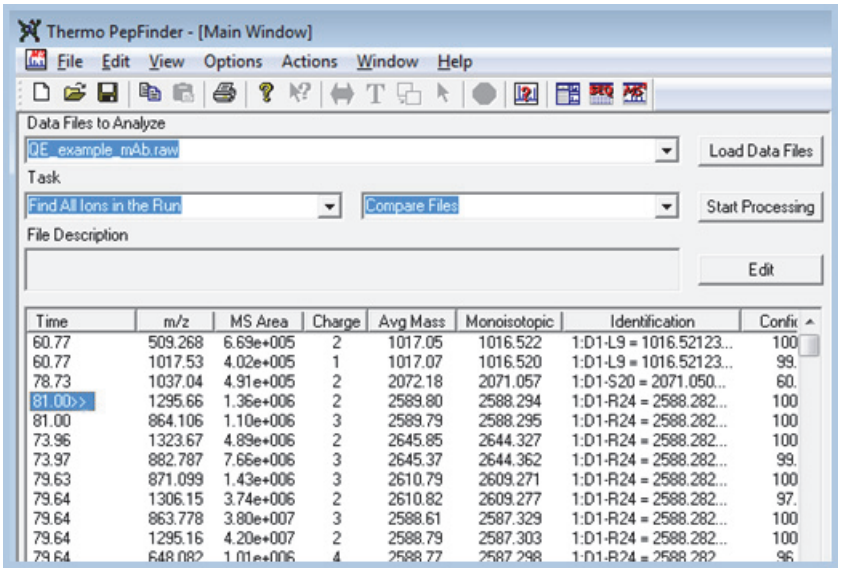
2. Type the *m/z*, monoisotopic mass, and average mass values as single numbers.

3. Type the *m/z* values of the product ions as numbers separated by commas (for example, **345.6,789.1,345.7**).

   The application labels identified ions after the retention time.

4. Click **OK** to start searching.

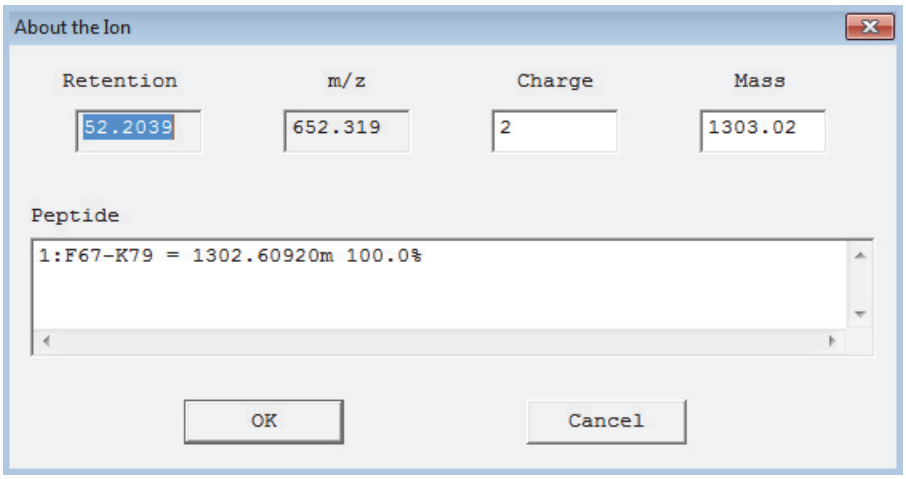
   The application places >> to the right of the time if it finds a match.



Viewing and Modifying Specific Ions

❖ To view information about a specific ion

1. In the ion list, double-click an ion retention time to display the About the Ion dialog box.



2. To change the charge and mass values, type new values in their corresponding boxes.

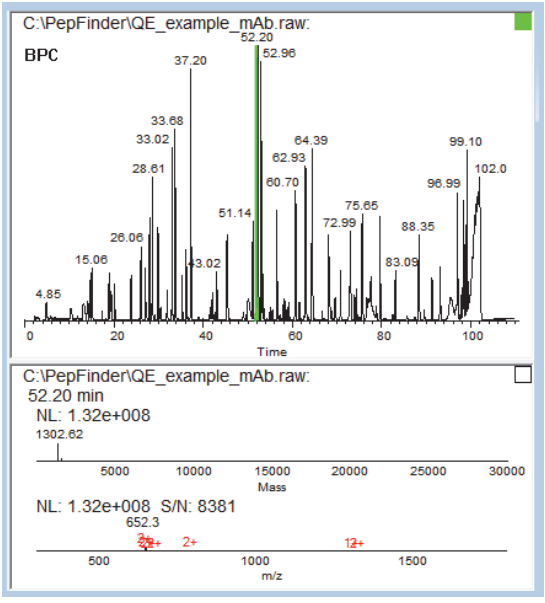
   You can also add, modify, or delete information in the Peptide area.

3. Click **OK** to save your changes and close the dialog box.

❖ To identify an ion

1. To manually process a single ion, right-click the retention time of an ion of interest to open a shortcut menu.

   The application highlights the location of the ion in the chromatogram with a green line.



2. Choose **Identify This Ion**.

   The application defines the formula in the Identification column of the ion list.

Removing Identification Information

❖ To remove data from the Identification field

1. Select the ion retention time.
2. Right-click and choose **Remove Identification**.

   The application removes the information.

Setting Display Options for the Ion List

To make changes to the display options within the ion list and to sort the ion list, follow these procedures.

- Reviewing an Ion List
- Sorting Ions
- Selecting Ion Properties to Display
- Setting the Mass Range
- Quantifying Deamidation
- Determining Isotopic Distribution

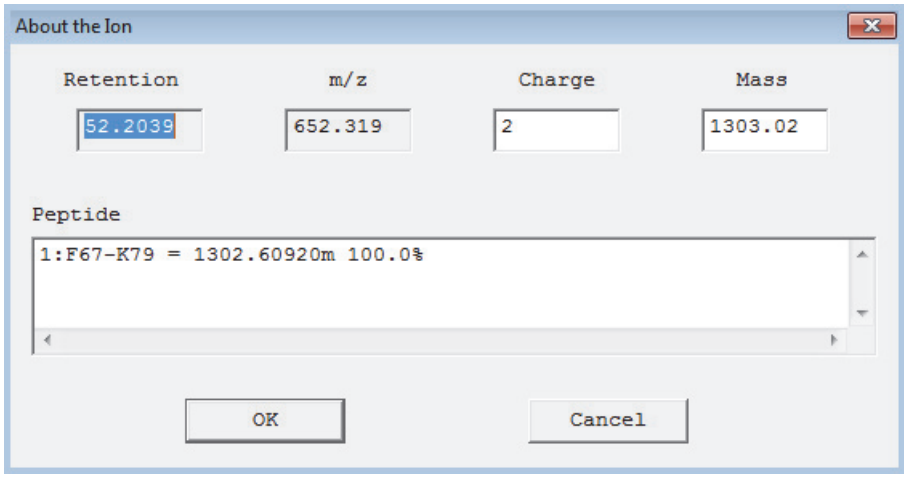
Reviewing an Ion List

❖ To review an ion list

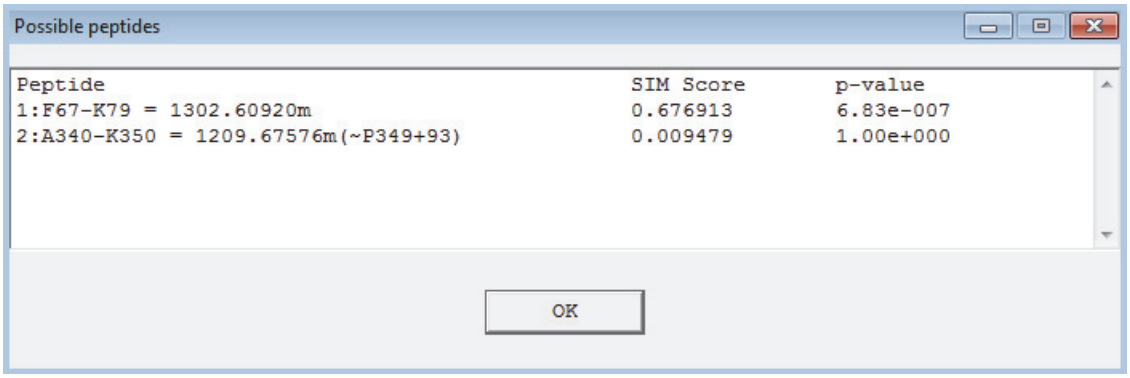
1. To update an ion in the Chromatogram view, Spectrum view, MS/MS window and Sequence window, click the retention time.

   The spectrum shown in the Spectrum view is optimized for the signal-to-noise ratio of the selected ion (matched filtering and background subtraction).

2. To show more information about the ion, double-click the retention time. Some fields are editable.



3. To perform a single ion assignment, right-click the retention time and choose from the shortcut menu.



Sorting Ions

❖ To sort ions in the ion list

1. To view options for sorting ions in the Main window, choose **Options > Display Options > Sort Ions By...**

   You can also sort the ion list according to these properties using the sort button at the top of each column. Click the retention time of any entry in the ion list to see the position of the ion in the chromatogram, the full scan spectrum of the ion, and its deconvoluted spectrum.

2. Choose from the listed options.

   - To sort the ion list by mass-to-charge ratio, choose ***m/z***.
   - To sort the ion list peak height, choose **MS Peak Height**.
   - To sort the ion list peak area, choose **MS Peak Area**.

- To sort the ion list weight, choose **Mass**.
- To sort the ion list ion location from N to C, choose **Identification**.
- To sort the ion list confidence from highest to lowest, choose **Confidence**.
- To list flagged ions, choose **Flag**.

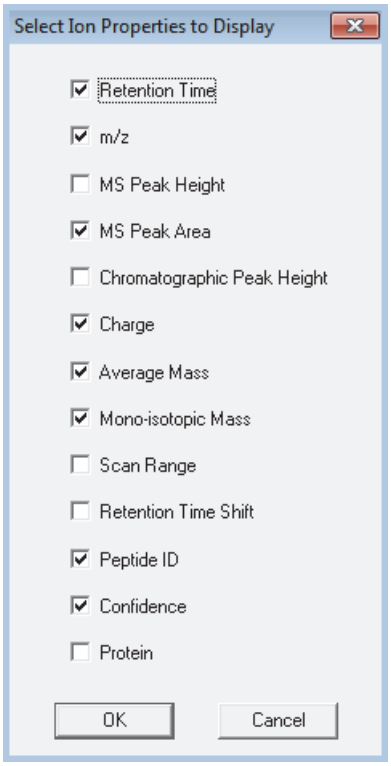
  The application flags identical ions from two separate files when the MS area is significantly different.

Selecting Ion Properties to Display

❖ To select ion properties

1. To view options for sorting ions in the Main window, choose **Options > Display Options > Select Ion Properties to Display.**

   The application displays the Select Ion Properties to Display dialog box. You can select the properties for the application to display.



2. Select features you want to display in the Ion List view and clear features you want to hide.
3. Click **OK**.

Setting the Mass Range

❖ To change the range display

1. To display the range dialog box, choose **Display Options > Mass Range**.

   The application shows the Mass Range Information dialog box.

2. Type the range start and stop values in the appropriate fields.

3. Click **OK**.

Quantifying Deamidation

❖ To quantify degradation in a specific ion

1. To quantify the amount of degradation in residues due to deamidation, right-click the ion retention time and choose **Quantify Deamidation**.

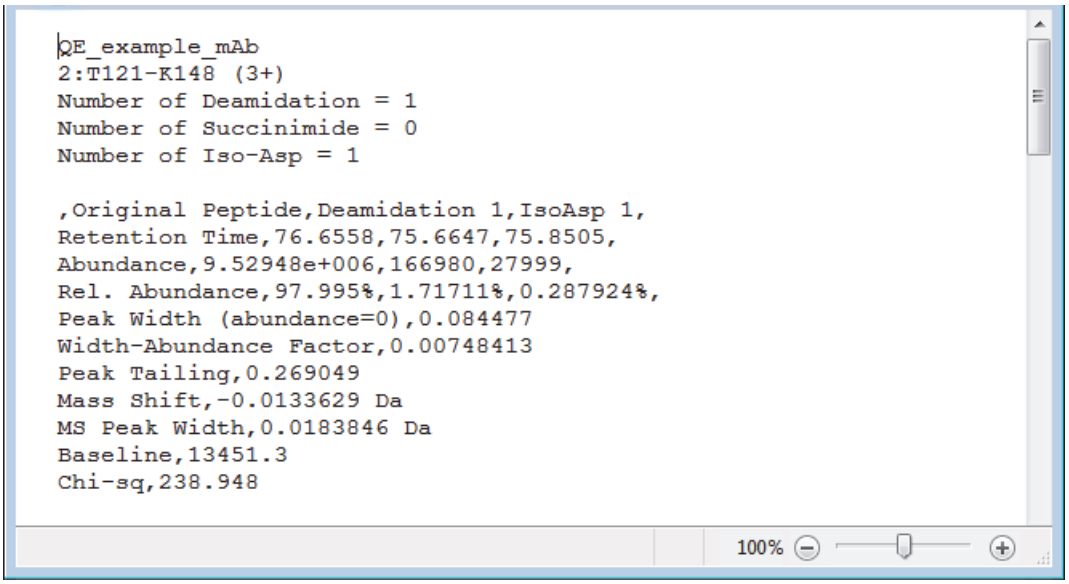
2. Select an unmodified peptide, one that has only the base sequence information (no attachments), with N or Q in it.

   The application writes the data to a text file and saves it in your standard file location for the PepFinder application. The file name contains the name of the raw data file, the peptide sequence, and the charge state.

❖ To quantify degradation in your results file

To quantify the amount of degradation in residues due to deamidation, choose **Actions > Quantify Deamidation/Isomerization**.

The application writes the data for each peptide to a text file and saves it in your standard file location for the PepFinder application. The file name contains the name of the raw data file, the peptide sequence, and the charge state.

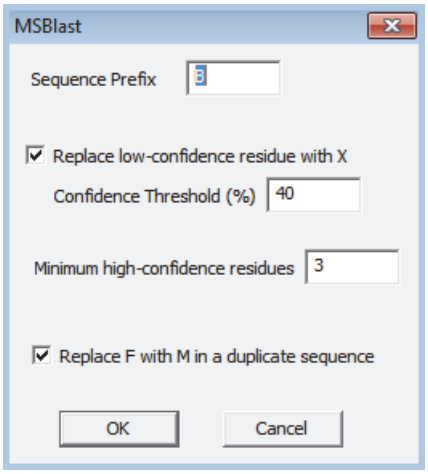


Determining Isotopic Distribution

❖ To determine isotopic distribution

1. Select the ion retention time.
2. Choose **Actions > Determine Isotopic Distribution**.

   The MSBlast dialog box opens.



3. To define the sequence prefix, type a value in the Sequence Prefix box.

   Valid values: Any sequence value

   Default: B

4. To replace low confidence residue with a specified threshold, type a value in the Confidence Threshold (%) box.

   Valid values: 1 to 100

   Default: 40

5. To specify a minimum level for high-confidence residues, type a value in the Minimum High-Confidence Residues box.

   Valid values: 1 to 10

   Default: 3

6. To replace F with an M in a duplicate sequence, select the **Replace F with M in a Duplicate Sequence** check box.
7. To save your changes, click **OK**.

Working with the Chromatogram View

To make changes within the Chromatogram view, follow these procedures.

- Truncating a Chromatogram
- Changing a Chromatogram View
- Resetting Chromatogram to Full Range
- Labeling Peaks
- Modifying Chromatogram Options
- Copying Chromatography Data

Truncating a Chromatogram

❖ To truncate a chromatogram

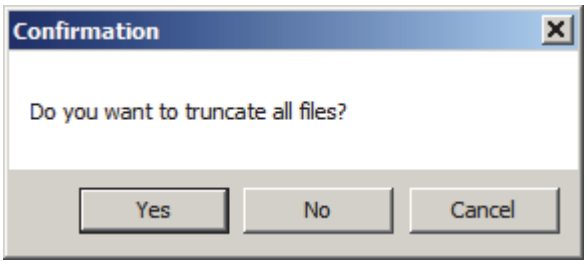
1. Select the ion retention time.

   To truncate the chromatogram, reduce the displayed range to an appropriate area. The application does not process the part outside the displayed range. When you truncate a chromatogram, the application does not process any information outside of the area you identify as valid.

2. Narrow the display window to the width you want to process.

3. Choose **Edit > Truncate Chromatogram**.

   The application shows a warning:



4. Click **Yes** to truncate chromatograms in all open files.

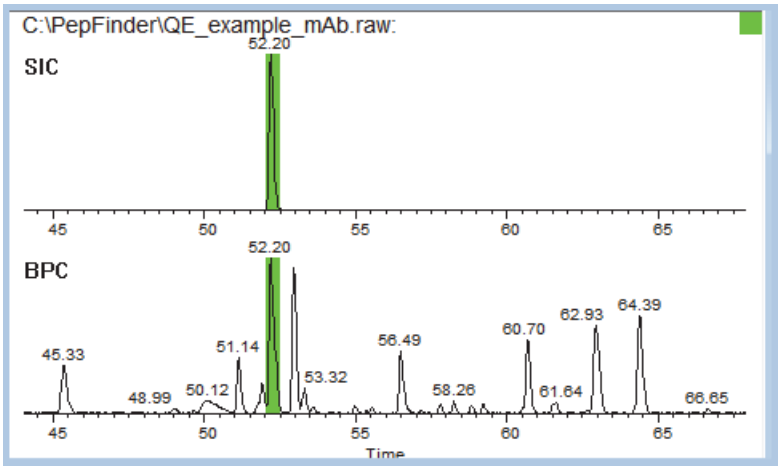
   To reset the chromatograms, see "To restore the full range of the chromatogram."

Changing a Chromatogram View

❖ To change a Chromatogram view

Click the top-right corner square to activate the area and select one of the following options to change the area.

- To zoom the chromatogram, drag the active area. Double-click to return the chromatogram to the original size.
- To show averaged spectrum of the selected time range, drag the cursor over the active Spectrum view. When you drag the cursor in the selected-ion chromatogram (SIC), a matched filter is applied for optimal signal-to-noise ratio of the ion. The time delay between analog signal and MS signal is automatically adjusted.
- Right-click to open a shortcut menu where you can choose the type of chromatogram to display, paste an external chromatogram from the Clipboard, and so forth.



Resetting Chromatogram to Full Range

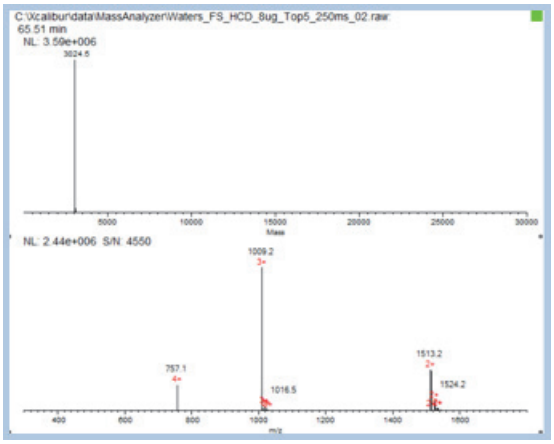
❖ To restore the full range of the chromatogram

1. Select the ion retention time.
2. Choose **Edit > Reset Chromatogram to Full Range**.

Labeling Peaks

❖ To label peaks

1. To select a method for peak labels in the Main window, click the green corner of the upper spectrum view to make the view active or click the marker in the lower spectrum to activate that view.



2. Choose **Options > Display Options > Peak Label**.
3. Select from **Peak Top** or **Centroid** and click **OK** to change the label.

   The application places the correct peak labels on the peaks.

Modifying Chromatogram Options

Use the procedures in this section to modify the chromatogram presentation or values.

- Selecting a Chromatogram
- Defining the Time Range
- Changing Fonts
- Adding or Changing Labels

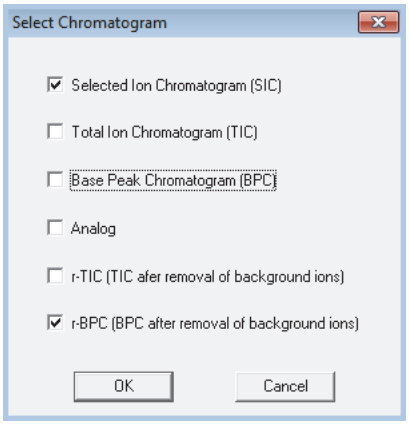
Selecting a Chromatogram

❖ To select the type of chromatogram to display

1. Select the ion retention time and right-click the Chromatogram window.

   You can choose one or several types of chromatograms to view in the results window.

2. Choose **Select Chromatogram** to display the chromatogram options.

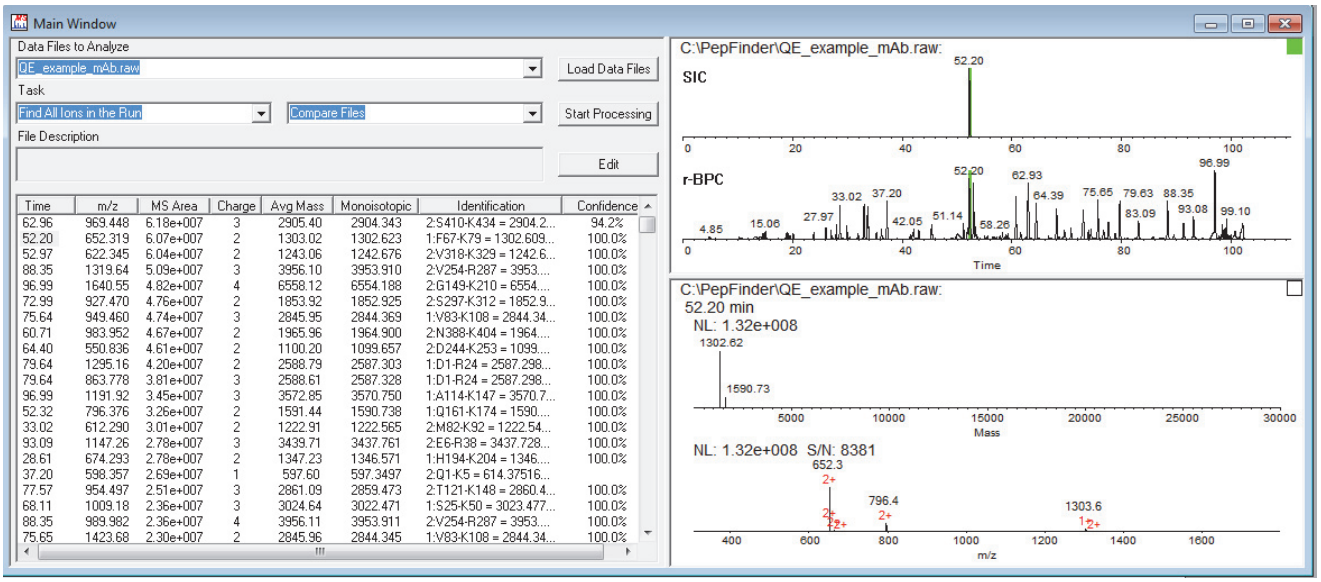


3. Select one or several chromatogram options.

   The chromatogram window displays up to 6 options at one time. You can view the selected ion chromatogram for a given component as well as view all the components that were detected. Do this immediately after processing for the most dependable information.

   - To choose a selected ion chromatogram (SIC), select its check box.
   - To choose a total ion chromatogram (TIC), select its check box.
   - To choose a base-peak chromatogram (BPC), select its check box.
   - To choose the analog signal received by the spectrometer, select the **Analog** check box.
   - To choose a TIC with background ions and weak ions (below threshold) removed, select the **r-TIC** check box (reduced TIC).
   - To choose a BPC with background ions and weak ions (below threshold) removed, select the **r-BPC** check box (reduced BPC).

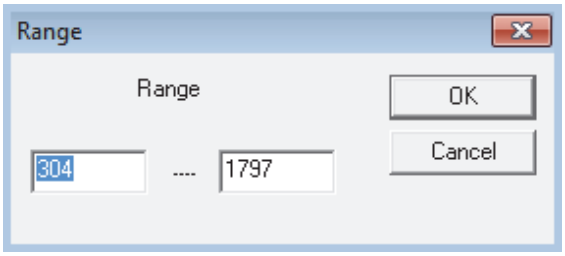
4. Click **OK** to display your selections.



Defining the Time Range

❖ To define the time range

1. Select the ion and right-click the chromatogram window.
2. Choose **Time Range** to display the Range dialog box.

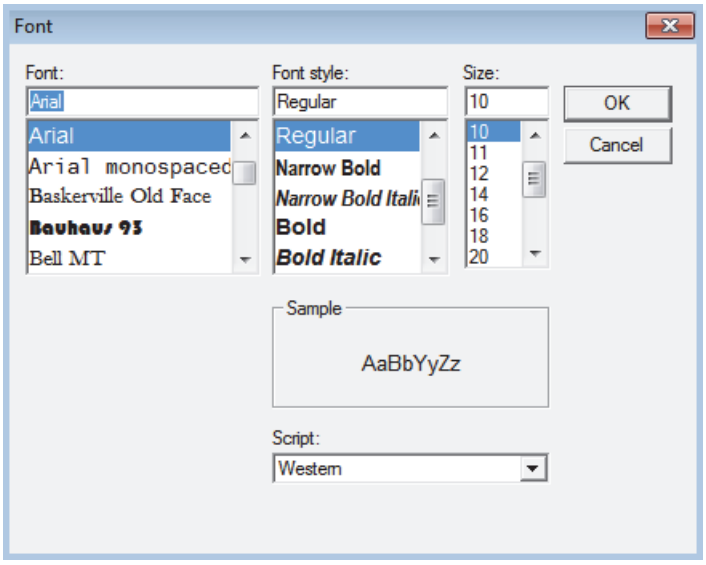


3. Type a new time range (starting or ending or both) and click **OK**.

Changing Fonts

❖ To change the font size or style on the labels

1. Select the ion retention time and right-click the chromatogram window.
2. Choose **Font** to display font options.
3. Select from the fonts, font styles, and sizes displayed.
4. Click **OK**.

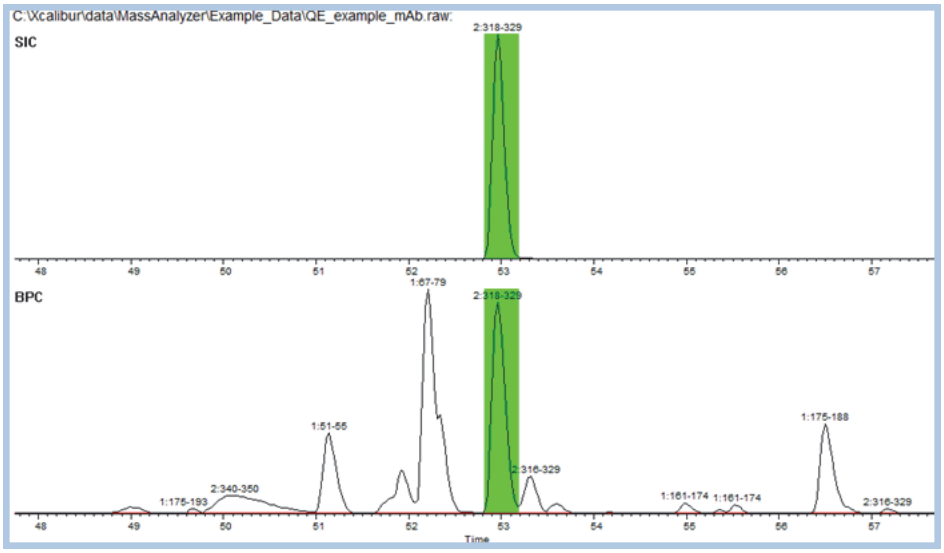


Adding or Changing Labels

❖ To add labels to a chromatogram

1. Select the ion retention time and right-click the chromatogram window.
2. Choose **Label** to display a list of label options.
3. Choose **Retention Time**, **Mass**, or **Peptide** to display labels on the chromatogram.

In this case, the graphic displays peptide labels.



Copying Chromatography Data

❖ **To copy chromatogram data for comparison or to replace another chromatogram**

1. Right-click the chromatogram window and choose **Copy Chromatogram Data**.
2. Select the retention time for the ion you want to change and choose **Paste External Chromatogram**.

   If the pasted chromatogram is the same type as the selected ion, the pasted chromatogram replaces the ion chromatogram. If it is a different type of chromatogram, the application displays both in the chromatogram window.

Working with the Spectrum View

The bottom spectrum in the Spectrum view of the PepFinder Main page shows the *m/z*-domain spectrum. The top spectrum shows the mass-domain spectrum. Both areas are movable (by using the move icon [ ] in the toolbar) and resizable by moving the small squares in the corners in or out.

When you load a number of spectra through the MS/MS Data menu in the Main window, view these spectra by pressing the UP and DOWN arrow keys.

The MS/MS window is automatically updated every time you click the retention time of an entry in the ion list of the Main window. The disulfide linkage that the application automatically displays in the window is just an example, not necessarily the correct linkage. You can also manually copy an intensity list from any MS data system to the Clipboard and paste the spectrum into the MS/MS window for analysis.

- Changing the Spectrum View
- Modifying Spectrum Information

Changing the Spectrum View

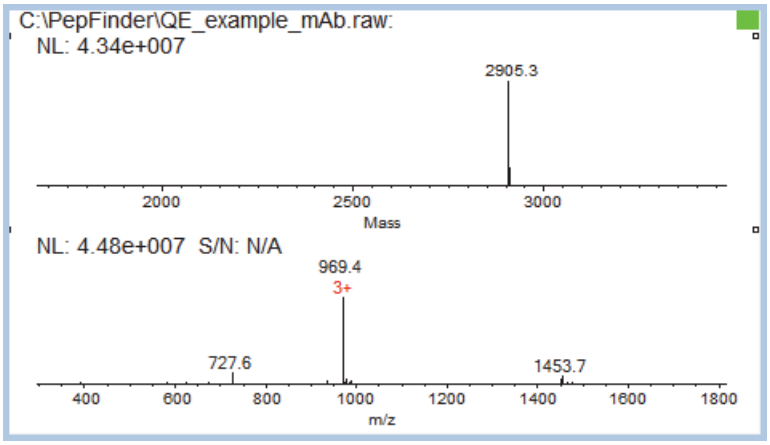
Use the procedures in this section to modify the Spectrum view.

- Increasing the Zoom Level in a Spectrum
- Displaying a Full-scan Spectrum
- Viewing a Peak List of Ions
- Displaying the Mass Range for a Spectrum
- Modifying Line Width on the Spectrum
- Copying a Spectrum
- Printing the Spectrum

Increasing the Zoom Level in a Spectrum

❖ To increase the zoom level in the spectrum

1. Click in the Spectrum view to make it active.
2. Drag the cursor over the area of interest.



Displaying a Full-scan Spectrum

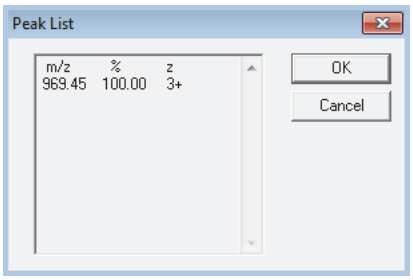
❖ To display the full-scan spectrum

1. To decrease the zoom level in the Spectrum view, make the Spectrum view active.
2. Choose **View > Full Scan**.

Viewing a Peak List of Ions

❖ To show a peak list of ions in the displayed spectrum

1. Make the Spectrum view active.
2. Choose **View > Peak List in the Spectrum**.



Displaying the Mass Range for a Spectrum

❖ To view a specific mass range for a spectrum

1. Select the ion retention time.
2. In the MS/MS window, right-click the spectrum and choose **Mass Range**.

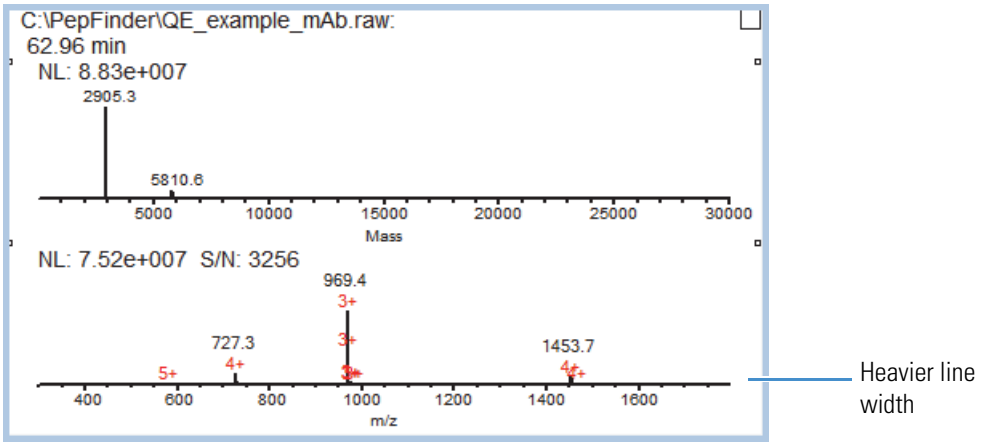
   A Range dialog box opens.
3. Type in values for the mass range.
4. Click **OK** to change the mass range.

Modifying Line Width on the Spectrum

❖ To change the line width of the spectrum

1. To change the line width on the spectrum, right-click and choose **Line Width**.
2. Type a value in the dialog box and click **OK**.

The application changes the spectrum display to the new line width.



Copying a Spectrum

❖ **To copy a spectrum for comparison**

Right-click the Spectrum view and choose **Copy Spectrum**.

Printing the Spectrum

❖ **To print the Spectrum view**

1. Right-click the spectrum.
2. Choose **Print**.

Modifying Spectrum Information

Use any of these procedures as appropriate.

- Changing a Spectrum View
- Adding Text to a Spectrum View
- Truncating Spectra
- Displaying the Residual Spectrum
- Identifying the Peptide Responsible for a Spectrum
- Selecting Transform Options
- Changing the Spectrum Header
- Creating a Mass List
- Exporting a Primary/Secondary Spectrum

Changing a Spectrum View

❖ To change a Spectrum view

Click the top-right corner square to activate the area and use one of the following options to change the Spectrum view.

- To zoom the view, drag across the spectrum.
- To return to the original spectrum, double-click the Spectrum view.
- To show the selected-ion chromatogram (SIC) of the selected range, drag across the active chromatogram area.
- Right-click to open a shortcut menu where you can change the transform options, show the zoom-scan, and so forth. See "Changing the Spectrum View in the MS/MS Window" on page 77.

Adding Text to a Spectrum View

❖ To add text to a Spectrum view

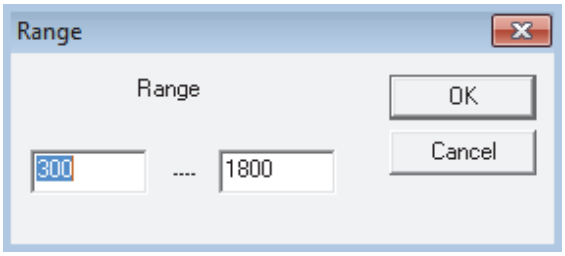
1. Click the **Insert Text** icon (T) in the toolbar.
2. Edit the text by double-clicking it or move the text by dragging it.
3. To change an area's font, click in the area, right-click and choose **Font**.
4. To hide the inserted text, right-click the text and choose **Hide**.

Truncating Spectra

❖ To truncate spectra

1. Choose **Edit > Truncate Spectra**.

   The Range dialog box opens.

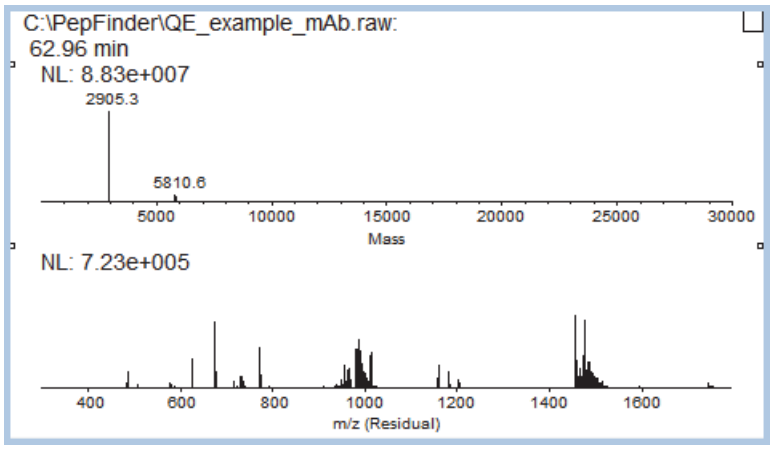


2. Change the range values to display the areas of interest.
3. To truncate all spectra, click **OK** in the confirmation dialog box.

Displaying the Residual Spectrum

❖ To display the residual spectrum

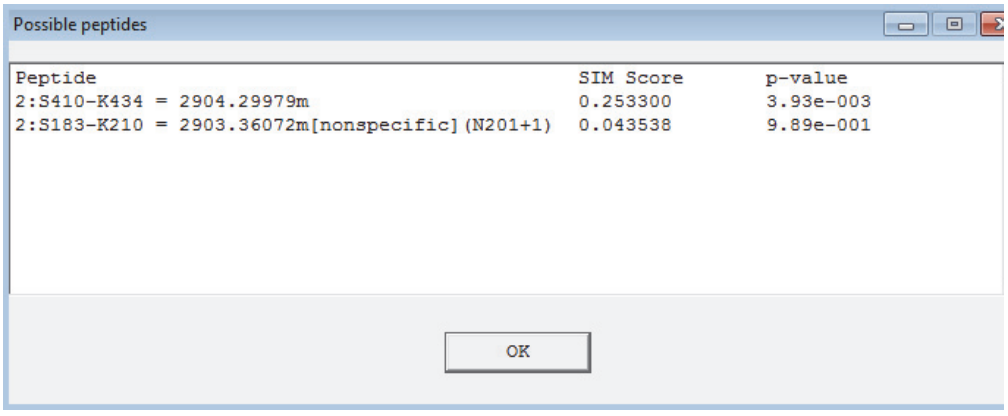
1. To display the residual spectrum after deconvolution in the Spectrum view, make the Spectrum view active.
2. Choose **View > Residual Spectrum** to display the residual spectrum at the bottom of the Spectrum view.



Identifying the Peptide Responsible for a Spectrum

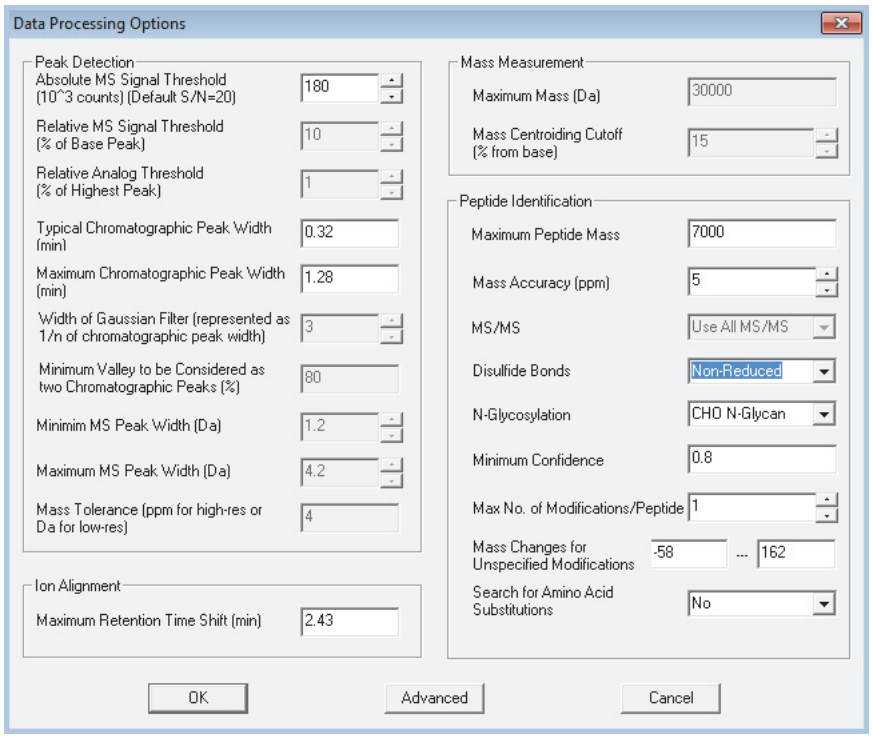
❖ To identify the peptide responsible for this spectrum

1. If the protein sequence is provided in the Sequence window, choose the **MS/MS** icon () to open the MS/MS window.
2. Choose **Actions > Identify the Peptide** to display the Possible Peptides dialog box.



3. To include peptides with disulfide bonds in your experiment results, set the Disulfide Bonds option to **Non-Reduced** in the Data Processing Options dialog box (for more

information about setting data processing options, see "Changing Data Processing Options" on page 7).



4. If the acquisition process has acquired ETD spectra in addition to CID spectra, to display the ETD spectrum, right-click the spectrum and choose **Select Fragmentation Type**.
5. To help visualize low-abundance ions and to display the spectra in square-root scale or log-scale, right-click the spectrum and choose **Select Intensity Scale**.

Selecting Transform Options

To display Transform options, see "Selecting a Deconvolution Method" on page 14.

Changing the Spectrum Header

❖ To modify the spectrum header

Click the Spectrum view to make it active.

- To change the header, click in the header area.
  - To hide the header, right-click and choose **Hide**.
  - To delete the header, right-click and choose **Delete**.
- To change the font, right-click and choose **Font**.

  After selecting font information, click **OK**.

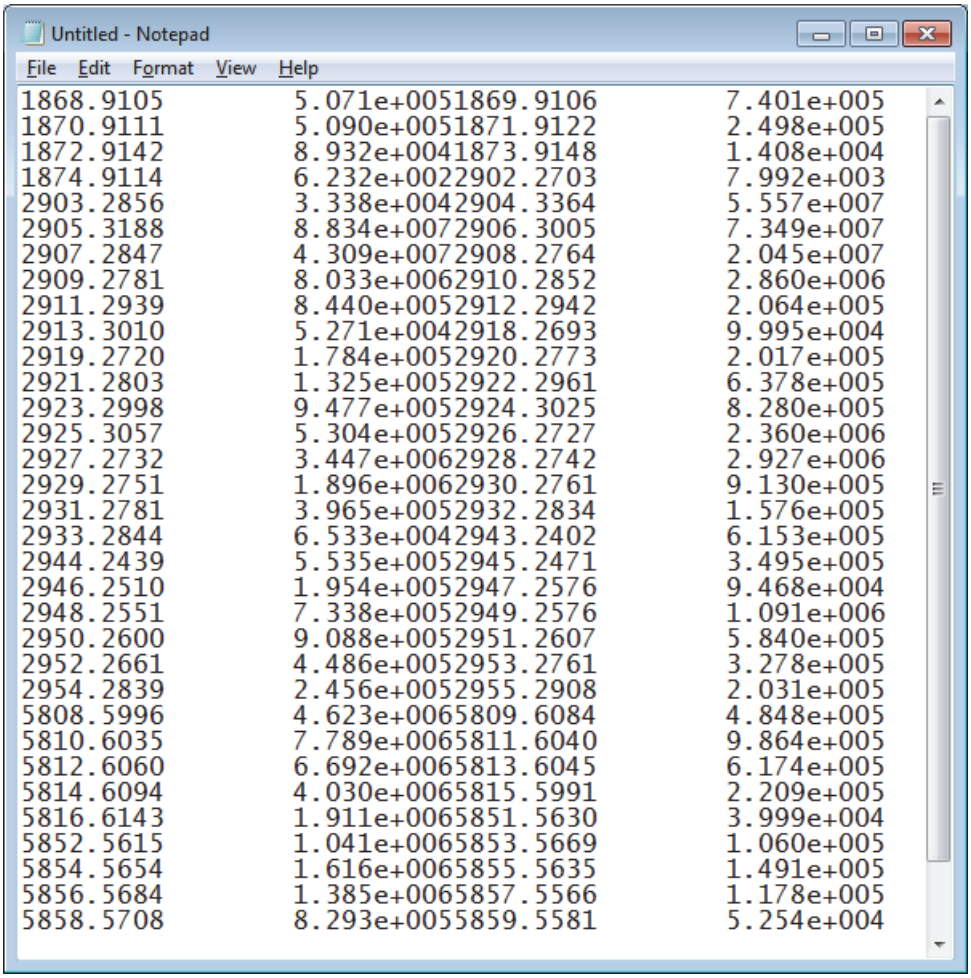
Creating a Mass List

❖ To create a list of masses from the spectrum

1. Right-click the Spectrum view and choose **Copy Mass List**.

   The application copies the mass list information, containing the *m/z* and intensity, to the Clipboard.

2. Open a text file and paste the list into it.



3. Save the mass list file.

Exporting a Primary/Secondary Spectrum

Export the intensity list of the primary or secondary spectrum into a text file for plotting using another application.

Working with Experiment Results

Use the procedures in this section to display and export experiment results.

- Exporting an Ion List
- Exporting Ion Lists for All Files
- Creating a Sequence Coverage Map
- Creating a Modification Summary

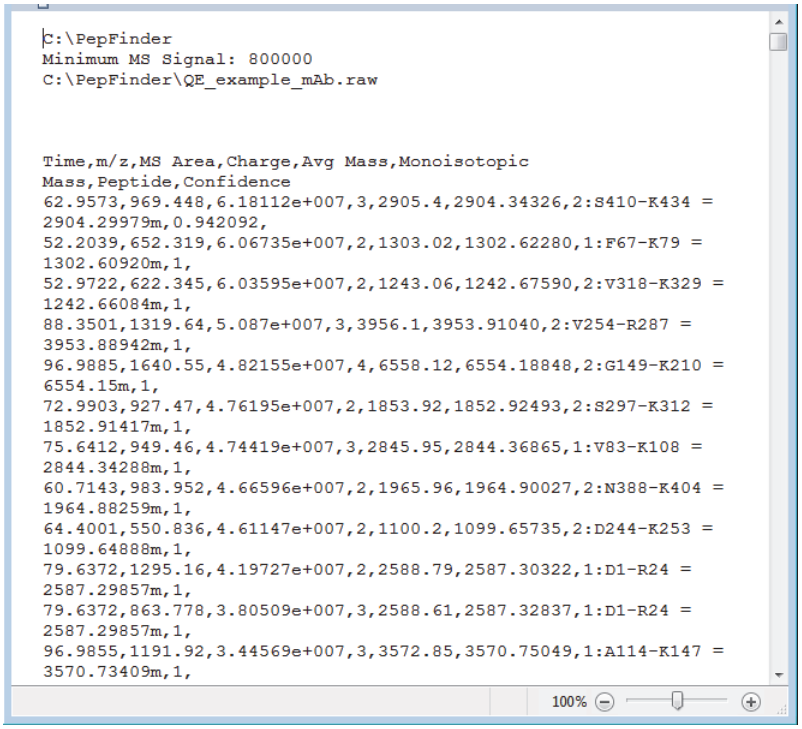
Exporting an Ion List

Export the ion list into a text file using the following procedure.

❖ To export the ion list

1. To export the ion list into a text file, choose **File > Export Ion List** to open a browse box.
2. Browse to a preferred location and type a name for the ion list file.
3. To display and save the file, click **Save**.

   The list that you created will look similar to this one.



```
C:\PepFinder
Minimum MS Signal: 800000
C:\PepFinder\QE_example_mAb.raw


Time,m/z,MS Area,Charge,Avg Mass,Monoisotopic
Mass,Peptide,Confidence
62.9573,969.448,6.18112e+007,3,2905.4,2904.34326,2:S410-K434 =
2904.29979m,0.942092,
52.2039,652.319,6.06735e+007,2,1303.02,1302.62280,1:F67-K79 =
1302.60920m,1,
52.9722,622.345,6.03595e+007,2,1243.06,1242.67590,2:V318-K329 =
1242.66084m,1,
88.3501,1319.64,5.087e+007,3,3956.1,3953.91040,2:V254-R287 =
3953.88942m,1,
96.9885,1640.55,4.82155e+007,4,6558.12,6554.18848,2:G149-K210 =
6554.15m,1,
72.9903,927.47,4.76195e+007,2,1853.92,1852.92493,2:S297-K312 =
1852.91417m,1,
75.6412,949.46,4.74419e+007,3,2845.95,2844.36865,1:V83-K108 =
2844.34288m,1,
60.7143,983.952,4.66596e+007,2,1965.96,1964.90027,2:N388-K404 =
1964.88259m,1,
64.4001,550.836,4.61147e+007,2,1100.2,1099.65735,2:D244-K253 =
1099.64888m,1,
79.6372,1295.16,4.19727e+007,2,2588.79,2587.30322,1:D1-R24 =
2587.29857m,1,
79.6372,863.778,3.80509e+007,3,2588.61,2587.32837,1:D1-R24 =
2587.29857m,1,
96.9855,1191.92,3.44569e+007,3,3572.85,3570.75049,1:A114-K147 =
3570.73409m,1,
```

4. To open a saved file, right-click the file and choose **Open With**.
5. Choose a text editor and click **Open**.

Exporting Ion Lists for All Files

To export ion lists for all files in the open project into a text file for comparison, use this procedure.

❖ **To export the ion lists for all files in an open project**

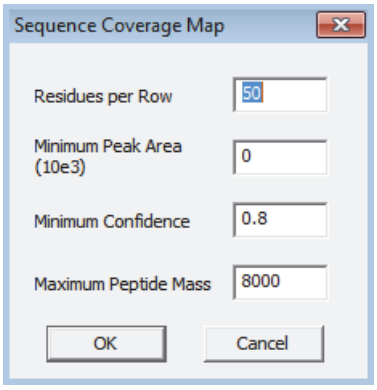
1. Choose **File > Export Ion List** to open a browse box.
2. Name the ion list and click **Save** to close the box.

Creating a Sequence Coverage Map

Use this procedure to create an HTML file showing the sequence coverage map report for the data in the active Main window showing the percent of protein sequence coverage. You can open the created HTML file in a Web browser or in an Excel™ spreadsheet. The PepFinder application labels each peptide with its retention time and color-codes the peptide by intensity (red, yellow, green and cyan), with red as most intense and cyan as least intense.

❖ **To create a sequence coverage map**

1. Choose **File > Create Fragment Coverage Map** to display the dialog box.



2. Specify the number of residues per row.

   Valid values: 2 to 500

   Default: 50

3. Define the minimum peak area.

   Valid values: 0 to 1e+006

   Default: 0

4. Specify a level for minimum confidence.

   Valid values: 0 to 1

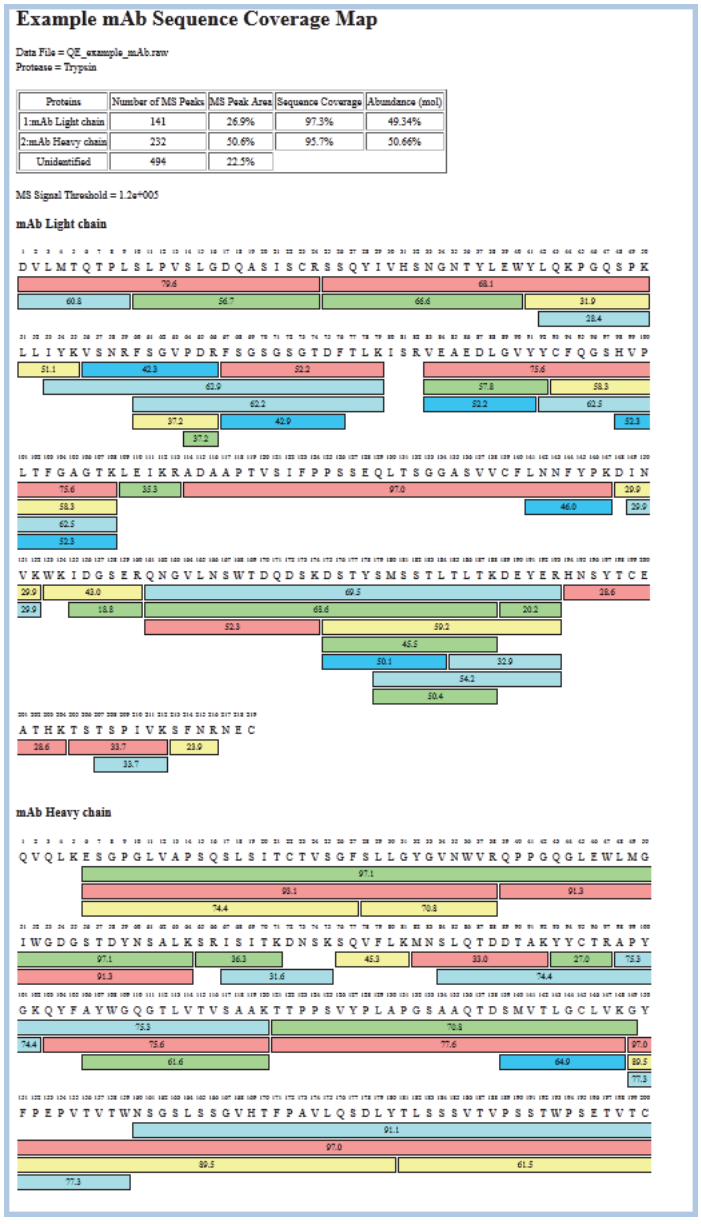
   Default: 0.8

5. Define the maximum peptide mass.

   Valid values: 100 to 100000

   Default: 8000

6. Click **OK** to display the sequence coverage map, as in the following example for the mAb experiment.



Example mAb Sequence Coverage Map

Data File = QE_example_mAb.raw
Protease = Trypsin

Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
1:mAb Light chain	141	26.9%	97.3%	49.34%
2:mAb Heavy chain	232	50.6%	95.7%	50.66%
Unidentified	494	22.5%		

MS Signal Threshold = 1.2e+005

mAb Light chain

D V L M T Q T P L S L P V S L G D Q A S I S C R S S Q Y I V H S N G N T Y L E W Y L Q K P G Q S P K

L L I Y K V S N R F S G V P D R F S G S G S G T D F T L K I S R V E A E D L G V Y Y C F Q G S H V P

L T F G A G T K L E I K R A D A A P T V S I F P P S S E Q L T S G G A S V V C F L N N F Y P K D I N

V K W K I D G S E R Q N G V L N S W T D Q D S K D S T Y S M S S T L T L T K D E Y E R H N S Y T C E

A T H K T S T S P I V K S F N R N E C

mAb Heavy chain

Q V Q L K E S G P G L V A P S Q S L S I T C T V S G F S L L G Y G V N W V R Q P P G Q G L E W L M G

I W G D G S T D Y N S A L K S R I S I T K D N S K S Q V F L K M N S L Q T D D T A K Y Y C T R A P Y

G K Q Y F A Y W G Q G T L V T V S A A K T T P P S V Y P L A P G S A A Q T D S M V T L G C L V K G Y

F P E P V T V T W N S G S L S S G V H T F P A V L Q S D L Y T L S S S V T V P S S T W P S E T V T C

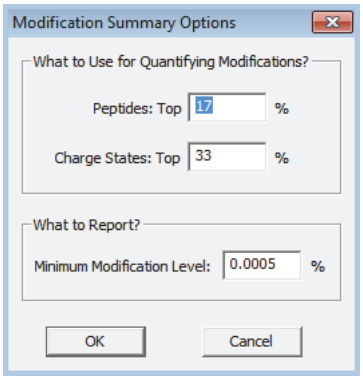
Creating a Modification Summary

To create an HTML modification summary report for the data in the active Main window showing the abundance of all detected modifications, create a modification summary.

❖ To create a modification summary

1. Choose **File > Create Modification Summary**.

   The Modification Summary Options dialog box opens.



2. To define the percentage of top modifications for peptides, type a value from **1** to **100** in the Peptides: Top box.
3. To define the percentage of top modifications for charge states, type a value from **1** to **100** in the Charge States: Top box.
4. To specify the minimum modification level to display, type a value from **1** to **100** in the Minimum Modification Level box.

   The application uses the following equation when calculating the relative area % of modification in the summary.

$$\text{Relative area \% of modification} = \frac{\text{Area of modified peptide}}{\text{Area sum of all related peptides (native + all modified forms)}} \times 100$$

5. Click **OK** to create a report similar to the one below.

Example mAb Modification Summary

Data File = QE_example_mAb.raw
Protease = Trypsin

Protein	Modification	Recovery	Abundance
mAb Light chain	~V2+56.9910	Good	8.7549%
mAb Light chain	~Q6+0.9840	Good	2.3020%
mAb Light chain	~N33+NH3 loss	Fair	15.3095%
mAb Light chain	~E39+H2O loss	Fair	15.8464%
mAb Light chain	~L51+57.0211	Good	2.4874%
mAb Light chain	~F60+57.0209	Good	3.2691%
mAb Light chain	S61+H2O loss	Good	2.0692%
mAb Light chain	~T74+21.9648	Good	4.0653%
mAb Light chain	~L78+57.0089	Good	2.0839%
mAb Light chain	~V83+57.0022	Good	1.5330%
mAb Light chain	~Y92+43.9333	Good	1.9759%
mAb Light chain	~Q95+0.9840	Good	1.6760%
mAb Light chain	~Q95+57.0066	Good	1.6246%
mAb Light chain	~W153+57.0157	Fair	4.4147%
mAb Light chain	~Q161+39.9904	Good	0.7809%
mAb Light chain	N162+Deamidation	Good	18.0914%
mAb Light chain	W168+Double Oxidation	Good	1.0037%
mAb Light chain	~S179+57.0238	Fair	76.7000%
mAb Light chain	~M180-48.0017	Fair	5.3324%
mAb Light chain	~L184+57.0112	Good	17.7936%
mAb Light chain	~H194+57.0215	Good	0.8429%
mAb Heavy chain	~T137+20.9966	Good	3.4217%
mAb Heavy chain	~D138-0.9890	Good	42.8132%
mAb Heavy chain	~S139+14.9956	Good	2.1580%
mAb Heavy chain	~W159+43.9746	Good	2.6066%
mAb Heavy chain	~H169+56.9521	Good	5.5933%
mAb Heavy chain	~H169-16.0557	Good	1.6631%
mAb Heavy chain	D244+57.0177	Good	1.5915%
mAb Heavy chain	~T251+H2O loss	Good	1.0687%
mAb Heavy chain	~V254+43.9385	Good	2.8949%
mAb Heavy chain	~V254+56.9824	Good	3.0492%
mAb Heavy chain	~F270+38.9265	Good	0.7478%
mAb Heavy chain	N292+A1G0F	Good	6.4236%
mAb Heavy chain	N292+A2G0F	Good	43.4050%
mAb Heavy chain	N292+A2G1F	Good	41.5864%
mAb Heavy chain	N292+A2G2F	Good	8.5525%
mAb Heavy chain	~L301+57.0264	Good	8.7837%
mAb Heavy chain	M304+Oxidation	Good	4.1258%
mAb Heavy chain	~H305+57.0121	Good	1.7691%
mAb Heavy chain	N310+Deamidation	Good	3.1520%
mAb Heavy chain	~N310+NH3 loss	Good	2.4734%
mAb Heavy chain	~K312+57.0222	Good	1.0023%
mAb Heavy chain	~V318+57.0123	Good	2.2719%
mAb Heavy chain	N319+Deamidation	Good	0.8170%
mAb Heavy chain	N319+NH3 loss	Good	11.0229%
mAb Heavy chain	~A322+52.8967	Good	0.5218%

3

Viewing Results in the Sequence Window

Use the Sequence window to type in or paste the protein sequence. You can use only uppercase single-letter codes to define the sequence. You can also use the sequence window to import a FASTA file or type or paste a protein sequence in FASTA format. For information on making changes to a protein sequence, see "Adding and Modifying a Protein Sequence" on page 3.

The Sequence window displays the sequence detail for peptides in the project.

Contents

Modifying Data in the Sequence Window

In the Sequence window, you can modify the sequence view and data in many ways and search for specific sequences.

Viewing Protein Sequences

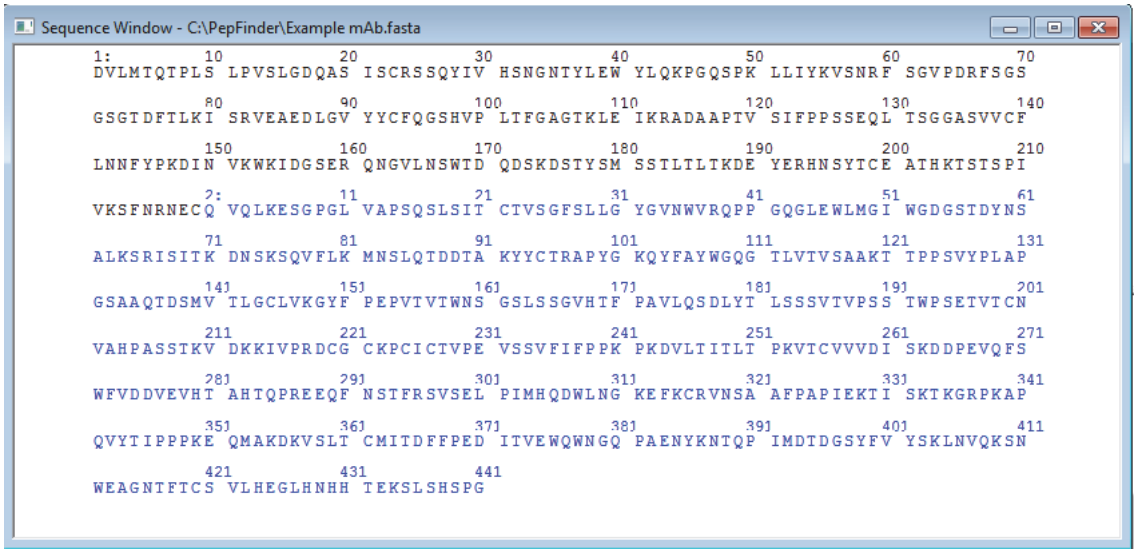
Use the PepFinder application to load one or more target protein sequences. You can view the sequences as complete units so that you can view the structure of the amino acid sequences of the target protein.

Opening the Sequence Window

To import or change a sequence, open the Sequence window.

❖ **To open the Sequence window**

Choose **View > Sequence Window**, or click  .



Displaying Mass Values

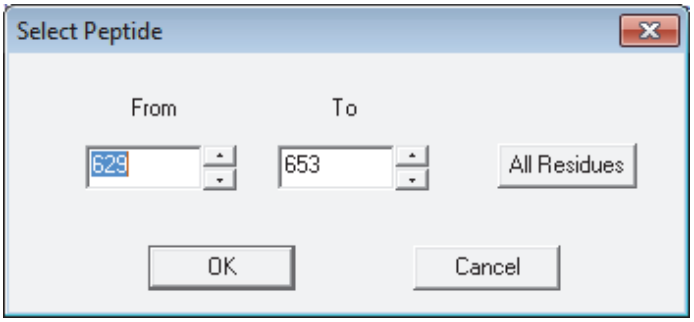
❖ **To select a method for displaying the value**

Choose **Option > Mass** > **Monoisotopic** or **Average**.

Selecting a Peptide

❖ To select a peptide

1. Choose **Sequence > Select Peptide**.
2. In the dialog box, type a range for the peptide and click **OK**.

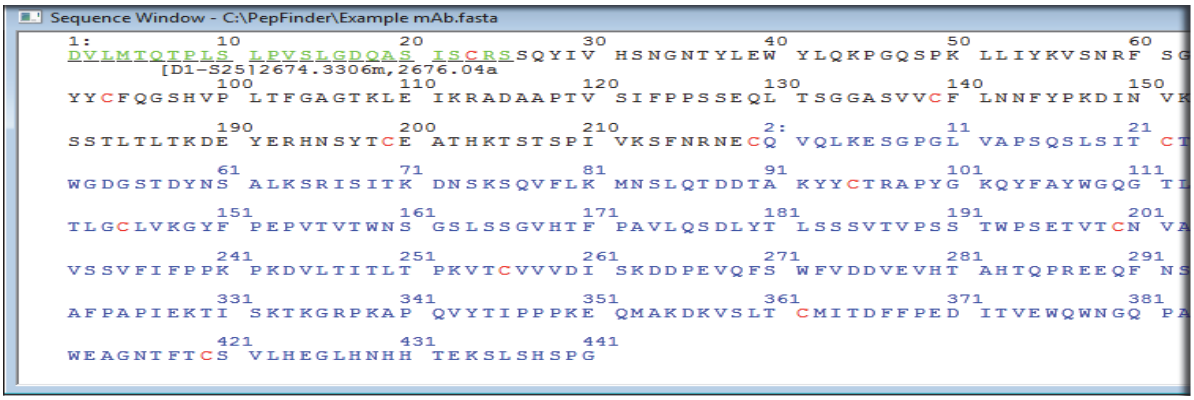


Displaying a Protein's Mass

❖ To see the mass of a specific protein

1. Click the Sequence window.
2. Drag across the complete protein.

   The application displays the mass under the protein.

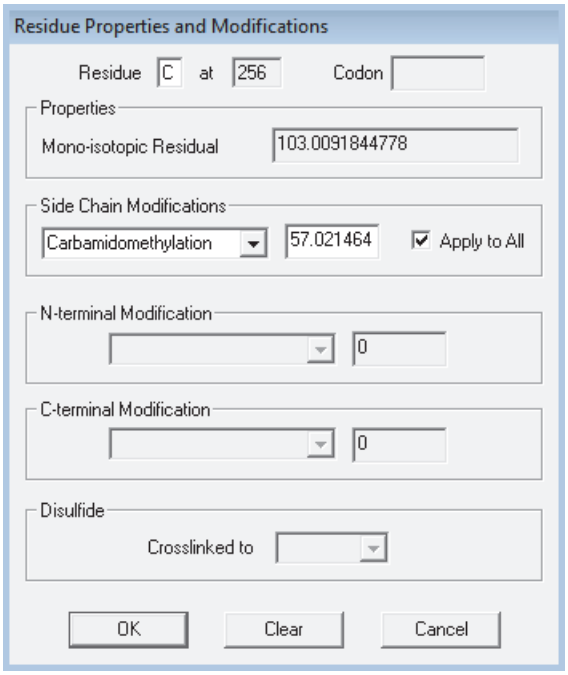


In this example, [D1-S25] 2674.3306m, 2676.04a, the m is the monoisotopic mass and the a is the average mass.

Finding Residue Information

❖ To find residue information

1. Choose **View > Sequence Window** to open the Sequence window.
2. To view or modify a protein, double-click any residue in the Sequence window to open the Residue Properties and Modifications dialog box.



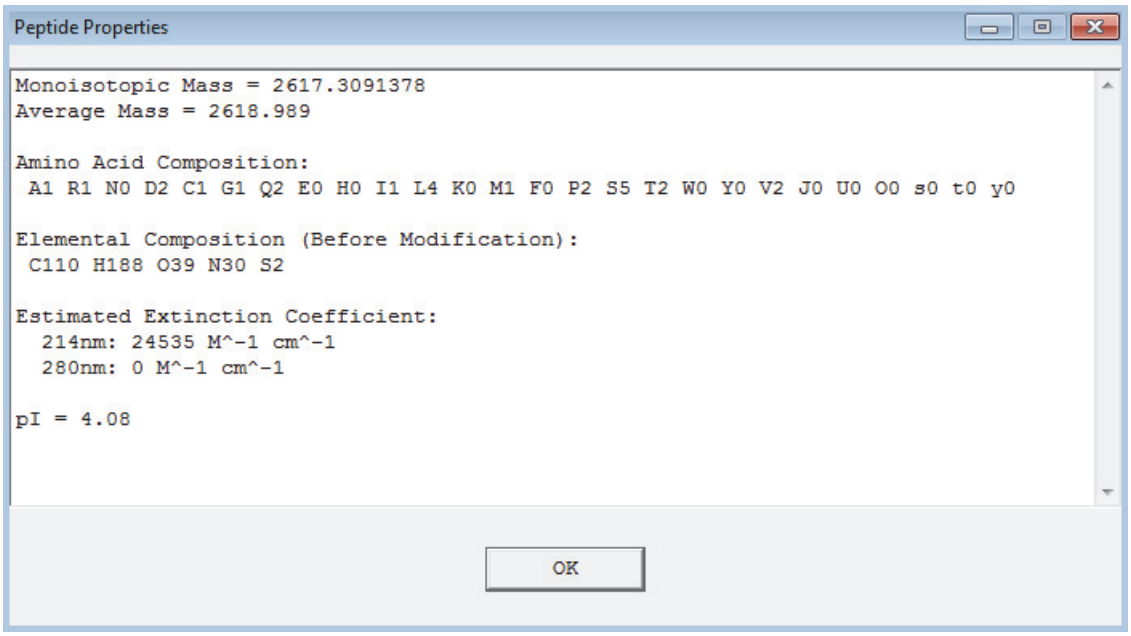
3. Click **OK**.

Viewing Peptide Information

To view information about a specific peptide, follow this procedure.

❖ To view peptide properties

1. To view peptide and display properties, right-click the peptide and choose **Select Peptide/Display Properties.**



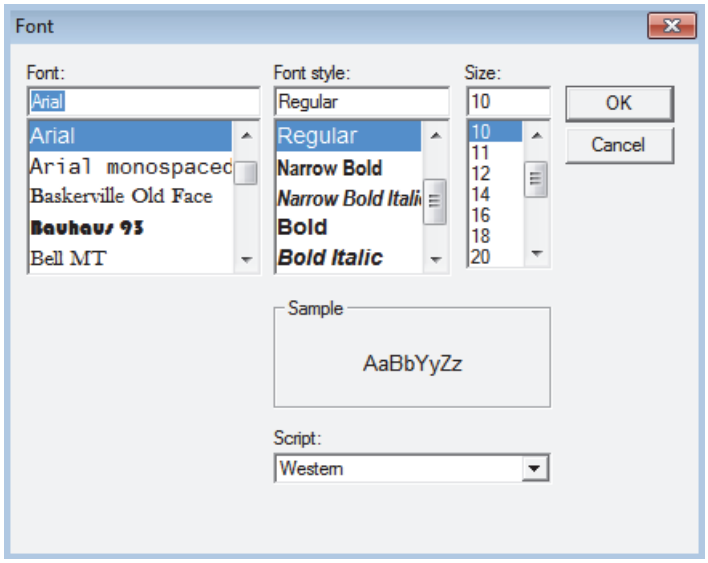
2. Click **OK**.

Changing Font Appearance

To make the screen results easier to read, change the font or the font size.

❖ To change the font type, style, or size in the Sequence window

1. Right-click an area of the Sequence window and choose **Font** to display the Font dialog box.



2. Choose font values and click **OK.**

Copying the Sequence Window Contents

❖ To copy the contents of the Sequence window

1. In the Sequence window, right-click and choose **Copy**.
2. Paste the contents of the Clipboard into a document.

Reviewing the Print Setup

❖ To review and make changes to the print setup

1. Choose **File > Print Setup**.
2. Make changes as needed.
3. Click **OK**.

Printing a Protein Sequence

❖ **To print a protein sequence**

1. Choose **File > Print** (or choose **File > Print Preview** to view the file before printing).
2. Click **OK**.

Modifying Sequences

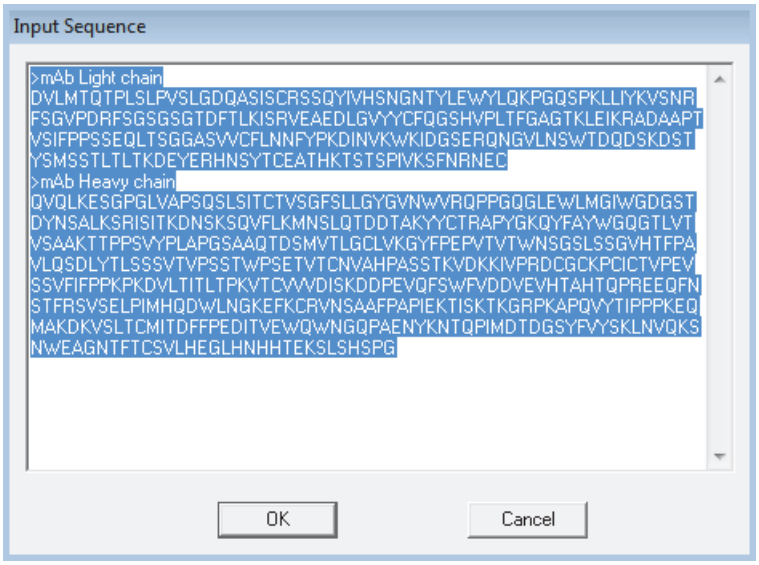
Follow these procedures to make changes to your protein sequence. In a FASTA file, the right arrow (>) indicates the description line for a new sequence.

- Adding or Editing a Sequence Manually
- Adding a Protein Sequence File
- Importing a Protein Sequence
- Importing a DNA Sequence File
- Saving a Protein Sequence
- Displaying Mass Values
- Identifying False Positives

Adding or Editing a Sequence Manually

❖ **To add or edit a sequence manually**

1. Choose **Sequence > Input/Edit** sequence to open the Input Sequence dialog box.
2. Paste a sequence into the dialog box, and click **OK**.



Adding a Protein Sequence File

❖ To add a protein sequence file

1. Choose **File > Add a Protein Sequence** to open a browse box.
2. Select a FASTA file and click **Open** to add it.

   If some residues are permanently modified, such as carboxymethylation of all cysteines, you can permanently modify the residue by double-clicking the residue, and then selecting an appropriate modification in the dialog box.

Importing a Protein Sequence

❖ To import a protein sequence

1. Choose **File > Import Protein Sequence**.
2. Select the protein sequence file (PMF) from the browse box and click **Open**.

   The protein sequence file can be in FASTA format, or it can be a text file containing nothing but one-letter codes (uppercase) with a ">" between chains. Importing a sequence replaces the existing sequence.

Importing a DNA Sequence File

❖ To import a DNA sequence file

1. Choose **File > Import DNA Sequence**.
2. Browse to the location of the DNA file and select the file.
3. Click **Open** to import it.

Saving a Protein Sequence

❖ To save a protein sequence

1. Choose **File > Save Sequence As...**
2. Type a name in the browse box and click **Save** to save the file.

Identifying False Positives

❖ **To identify false positives**

To identify false positives, do one or both of the following:

- To copy the sequence in reverse to the bottom of the sequence, choose **Sequence > Append Reverse Sequence**, paste a sequence into the dialog box, and click **OK**.
- To add a randomized version of the sequence to the end of the sequence, choose **Sequence > Append Randomized Sequence** and click **OK**.

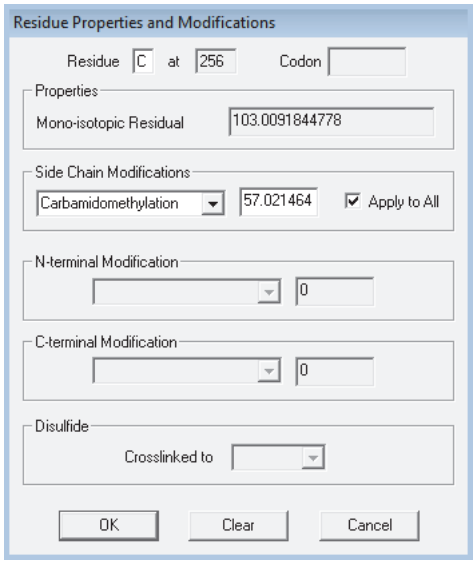
After processing the data, examine the confidence score for ions. If the confidence score drops below 50 percent, you are most likely looking at false positives.

Modifying Residue Properties

Use the Residue Properties and Modifications dialog box to add modifications to the residue. The application displays this dialog box when you double-click a residue. You can change the residue or add a modification to it and the residue changes color. If you do not find a needed modification in the list, add the mass changes (monoisotopic) directly to the mass change box.

❖ **To modify residue properties**

1. From the Sequence window, double-click a residue to display the Residue Properties and Modifications dialog box.



2. Select a side chain modification.
3. Click **OK** to make the changes.

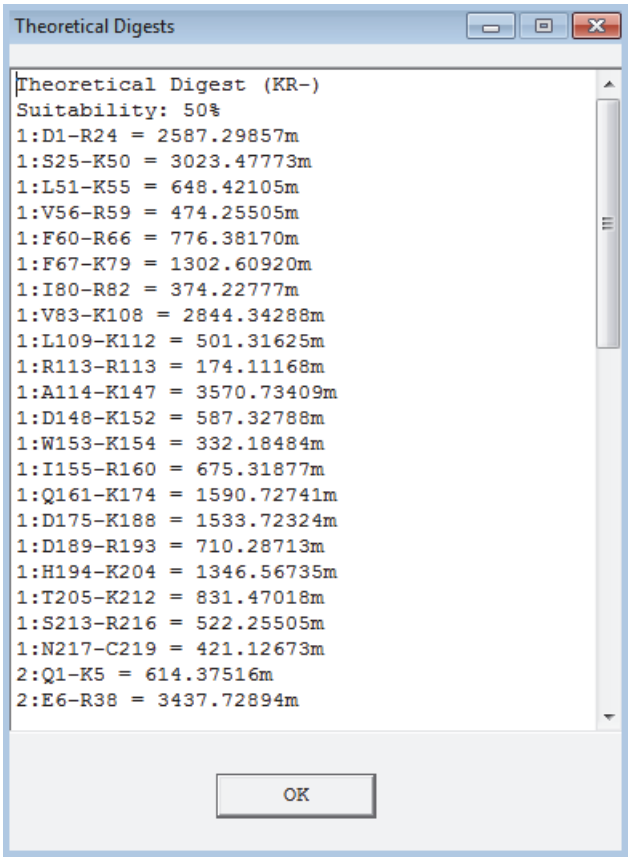
Performing a Theoretical Digest

Perform a theoretical digest to show where cleavages can happen because of the protease. The best results produce smaller fragments. Try a different enzyme if the fragments are too large.

❖ To perform a theoretical digest

1. In the Sequence window, choose **Actions > Theoretical Digest**.

   The Theoretical Digests window opens.

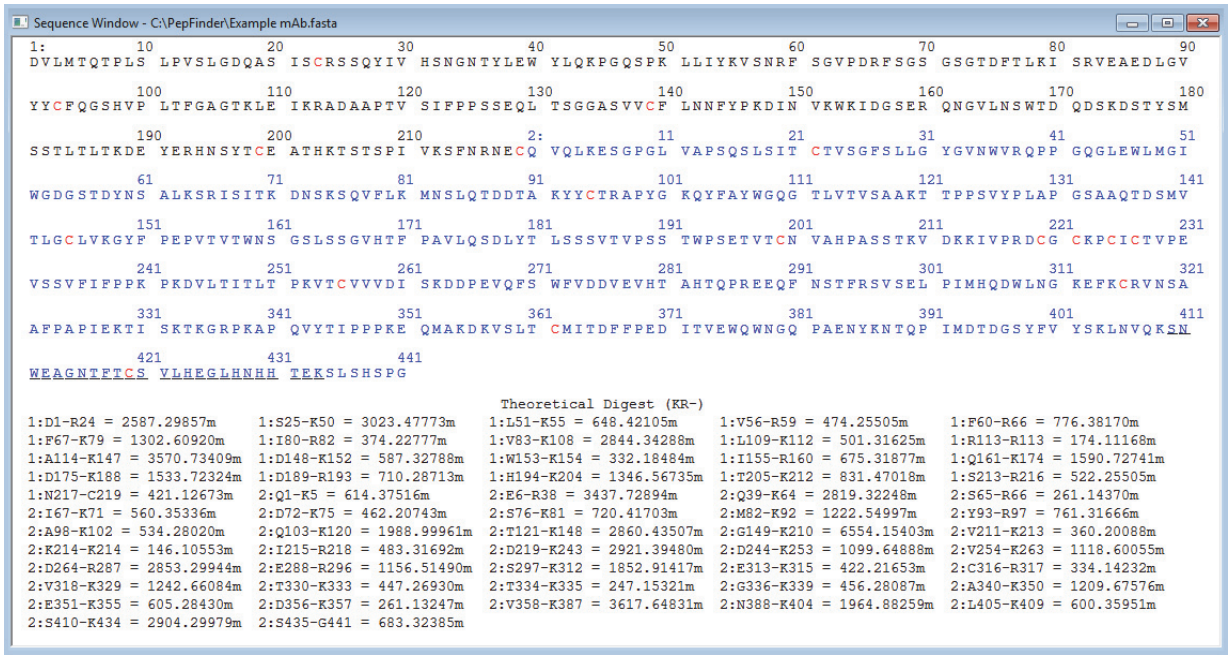


   The PepFinder application produces a list of potential cleavage locations when you perform a theoretical digest.

2. To make changes to the mass before performing the search, choose **Actions > Search Mass** to change the mass value or clear the **Average Mass** check box.

3. Click **OK**.

The application displays results in the Sequence window.



Searching in the Sequence Window

Use the Sequence window to detect a mass in the spectrum. You can see what matches the sequence and look for potential mismatches where two or more fragments are joined.

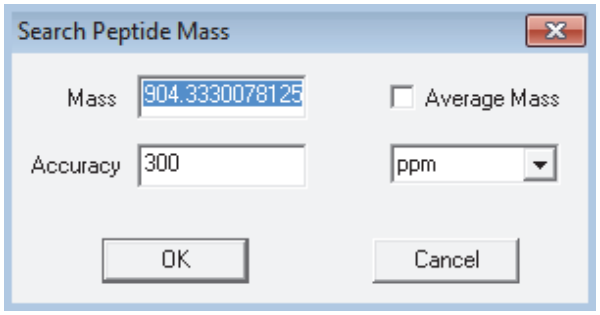
- Searching for a Specified Mass
- Searching Disulfide-linked Peptides for a Specified Mass
- Searching for Disulfide-Bond Assignments
- Searching for Proteolytic Fragments

Searching for a Specified Mass

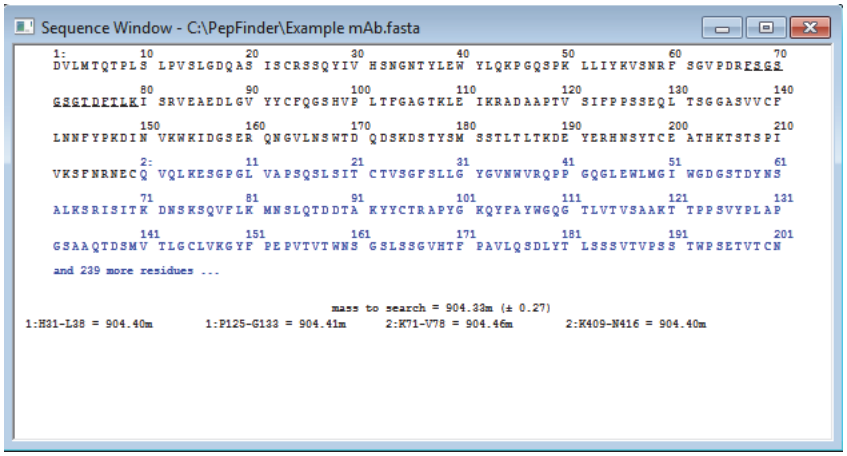
❖ **To search for a peptide fragment matching a specific mass in a sequence**

1. In the Sequence window, choose **Actions > Search Mass**.

   The application opens the Search Peptide Mass dialog box.



2. To make changes to the mass, change the mass parameter value.
3. To change how the peptide mass is calculated, clear the **Average Mass** check box.
4. Click **OK**.



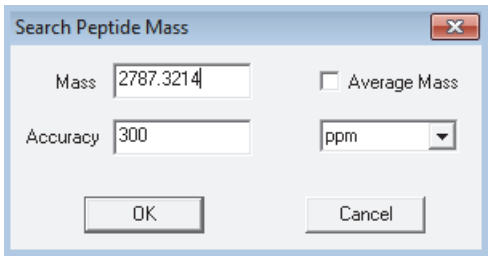
Searching Disulfide-linked Peptides for a Specified Mass

❖ **To search for a disulfide-linked peptide fragment matching a specific mass in a sequence**

1. To look for linked peptides, in the Sequence window, choose **Actions > Search Mass of Disulfide-Linked Fragments**.

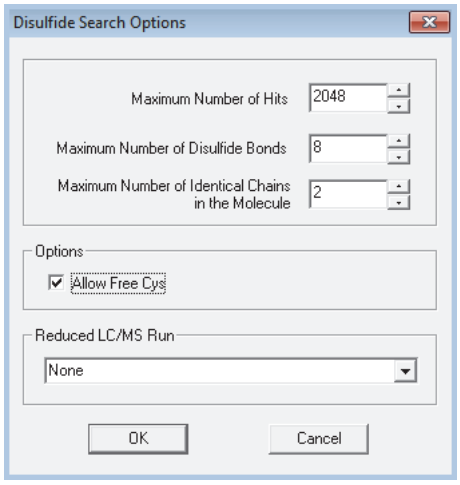
   The disulfide bond is the weak link in many molecules. The most important reaction of disulfide bonds is their cleavage, which occurs by reduction using a variety of reductants.

   The application opens the Search Peptide Mass dialog box.



2. To make changes to the mass, change the Mass parameter value or clear the **Average Mass** check box.
3. Define the accuracy of the search in ppm.
4. Click **OK**.

   The Disulfide Search Options dialog box opens.



5. Set the maximum number of search results before the application stops searching for more disulfide-linked peptides.

   Valid values: 2 to 4000

   Default: 2048

6. Specify the maximum number of disulfide bonds.

   Valid values: 1 to 16

   Default: The number of cysteines in the sequence divided by 2

7. Set the maximum number of identical chains in the molecule.

   For example, if the molecule is a disulfide-linked homodimer, then set this parameter to 2.

   Valid values: 1 to 8

   Default: 2

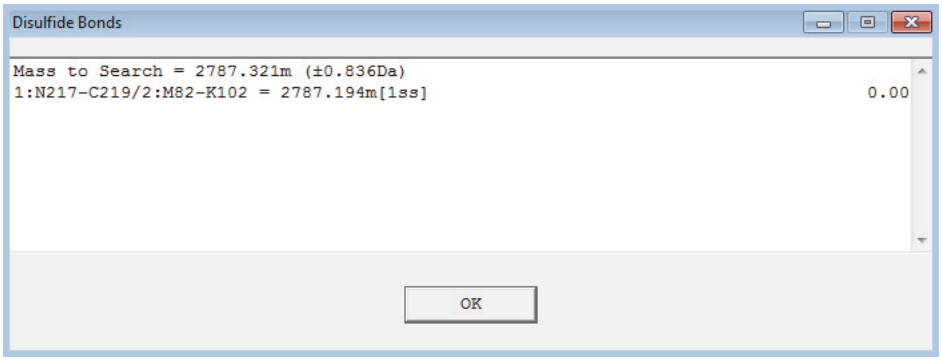
8. If you allow free cysteine residues in the molecule, select the check box.

9. Select a file containing a reduced LC/MS run.

   If you have both reduced and non-reduced data files, you can process both data files in the same experiment to generate more reliable disulfide assignments. When loading your data files (.raw), select both reduced and non-reduced at the same time by using the CTRL or SHIFT key to select multiple files. Then in the Disulfide Search Options box, select the reduced raw file name from the list under Reduced LC/MS Run.

10. Click **OK**.

11. The application opens the Disulfide Bonds dialog box.



12. Click **OK** to close the box.

Searching for Disulfide-Bond Assignments

❖ To show masses of fragments generated from CN-induced cleavages

1. To search for masses of fragments generated from CN-induced cleavages for disulfide bond assignments (cleavages by aqueous ammonia at the N-terminus of cyanylated cysteine residues), in the Sequence window choose **Actions > Masses from CN Induced Cleavages.**

   Immunoglobulin G (IgG) is the most abundant antibody isotope found in human blood and works to control infection. Use the application to calculate elemental composition and mass of different forms of IgG.

   If you do not have too many cysteines in the sample, the application opens the CN Induced Cleavages message box.

2. Click **OK**.

❖ To automatically calculate the elemental compositions and average masses of different chains and glycoforms of the IgG molecule

1. In the Sequence window choose **Actions > Masses from CN Induced Cleavages**.

   If the imported sequence is an IgG molecule, the application opens the Calculate IgG Properties information? box.

   The application uses atomic weights of elements from organic sources instead of IUPAC standard atomic weights.

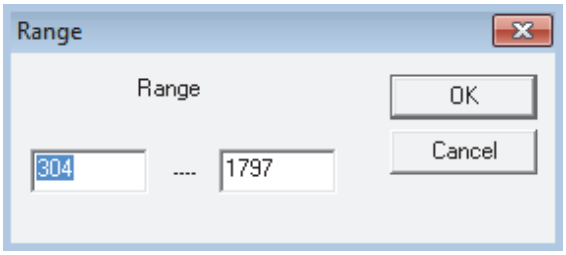
2. Click **OK**.

Proteolytic Fragments

❖ To show all proteolytic fragments within a specified mass range

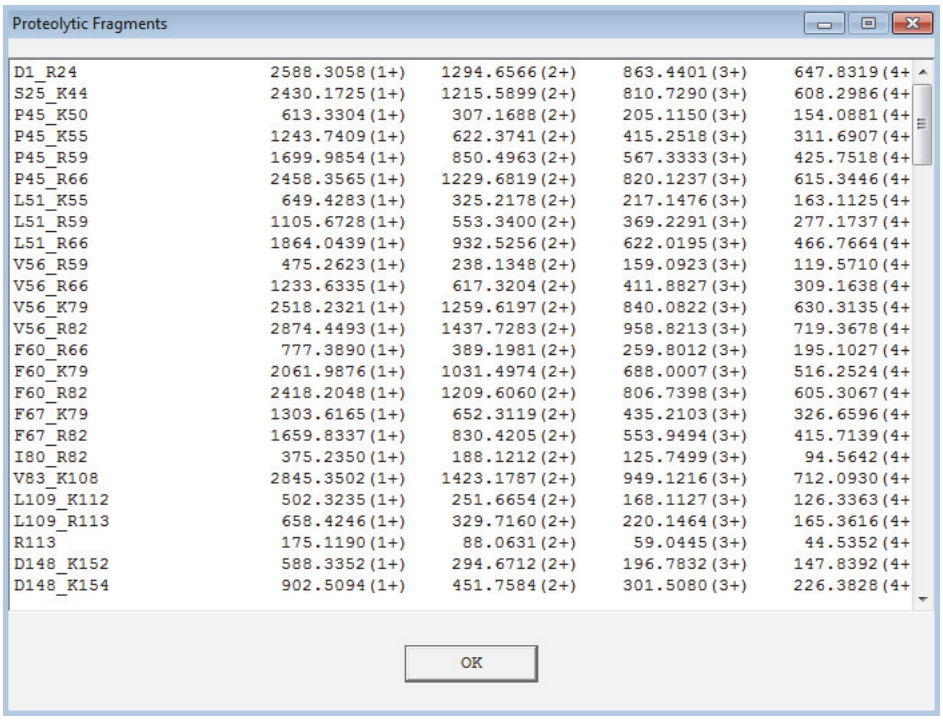
1. To show all peptide fragments within a mass range that have been created in response to enzymes, whether the digestion is complete or not, in the Sequence window, choose **Actions > Show all Proteolytic Fragments.**

   The application opens the Range dialog box.



2. Make changes to the range if needed.
3. Click **OK**.

   The application displays a list of proteolytic fragments, containing the sequence and possible values for the theoretical *m/z*.



Working with Amino Acid Substitutions

Due to limitations in the MS/MS data, the PepFinder application might interpret many artifacts produced during digestion and ionization as amino acid substitutions. To minimize this action, the application also automatically performs a search of common digestion/ionization artifacts before searching for amino acid substitutions.

However, you cannot eliminate all artifacts without eliminating potential amino acid substitutions. Table 1 lists some common artifacts that the application might assign as amino acid substitutions. Considering the inaccuracies in measurement (for example, the application determines that an oxidized peptide has a change of 15 Da instead of 16 Da), the list can be much larger.

Among these artifacts, the application most frequently assigns oxidations. Oxidation of nearby residues causes a large proportion of misassigned amino acid substitutions because a large proportion of misassigned amino acid substitutions results from oxidation of nearby residues.

After the application detects a potential amino acid substitution, you must confirm it. Use any of the methods below to confirm the amino acid substitution.

- High resolution spectrometry: Accurate measurement of the change on a high resolution instrument will determine the exact elemental composition causing the change.
- Electron-transfer dissociation (ETD): ETD usually generates much higher sequence coverage than CID, which helps determine the exact location of the change.
- Digestion with a different protease to generate a different peptide containing the substitution: MS/MS analysis of this peptide can provide complementary information.
- N-terminal sequencing: When enough purified sample is available, perform N-terminal sequencing for more definite confirmation when enough purified sample is available.
- Synthesis of the substituted peptides: You can compare their retention times and MS/MS results to the peptide in the digest for confirmation.
- DNA confirmation of mutation (as opposed to amino acid misincorporation) through sequencing a large number of library colonies.

Table 1. Common artifacts that can be assigned as amino acid substitutions (Sheet 1 of 2)

Assigned substitutions	Change	Possible modifications of nearby residues
D-G	–58	Incomplete carboxymethylation of Cys
E-A	–58	Incomplete carboxymethylation of Cys
F-V	–48	Loss of 64 from oxidized Met
Y-D	–48	Loss of 64 from oxidized Met
M-L	–18	Dehydration
D-N	–1	Amidation

Table 1. Common artifacts that can be assigned as amino acid substitutions (Sheet 2 of 2)

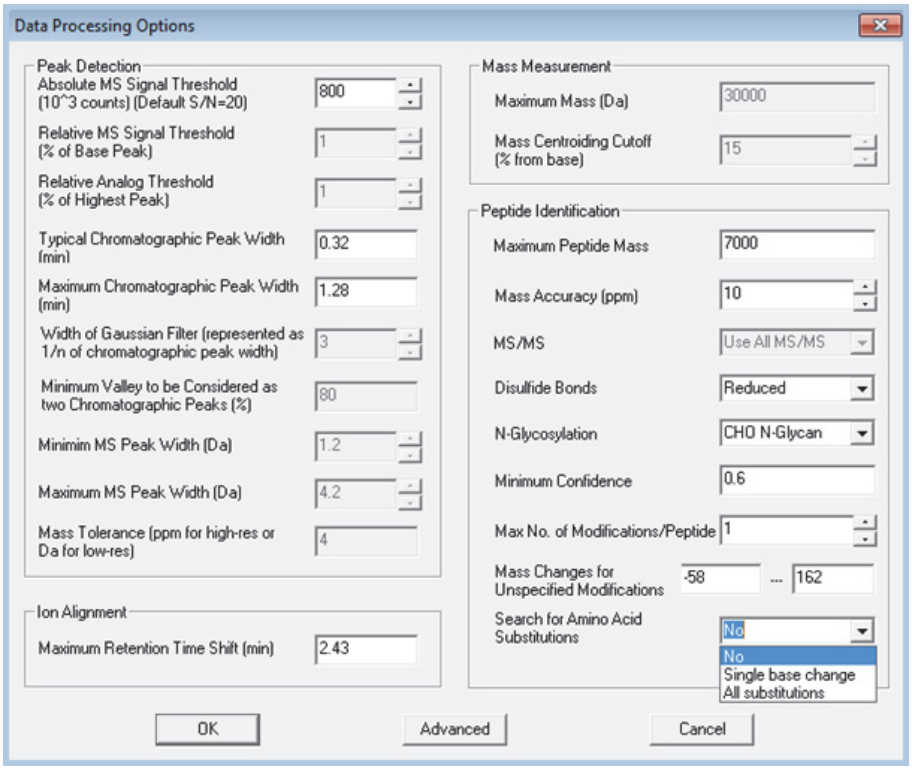
Assigned substitutions	Change	Possible modifications of nearby residues
E-K	–1	Amidation
E-Q	–1	Amidation
N-I	–1	Amidation
I-N	1	Deamidation of N or Q
K-E	1	Deamidation of N or Q
P-T	4	Trp oxidation
A-S	16	Oxidation
F-Y	16	Oxidation
P-L	16	Oxidation
S-C	16	Oxidation
V-D	16	Oxidation
D-H	22	Na+ adduct
V-M	32	Double Oxidation
G-V	42	Acetylation
I-R	43	Carbamylation of N-terminus or Lys
L-R	43	Carbamylation of N-terminus or Lys
A-D	44	Double Na+ adduct
C-F	44	Double Na+ adduct
D-Y	48	Triple oxidation
V-F	48	Triple oxidation
A-E	58	Carboxymethylation
G-D	58	Carboxymethylation

To evaluate the impact on your data results, consider these limitations:

- The original peptide must be present, and the acquisition process must provide good quality MS/MS data. This means the peptide map must have enough coverage to cover the amino acid in question.
- The substituted peptide ion must be present, and the acquisition process must provide good quality MS/MS data. To increase the possibility that the application can collect your MS/MS data, adjust the data-dependent settings in "Changing Data Processing Options" on page 7.
- Even if the application obtains good quality MS/MS data, detecting amino acid substitutions is not guaranteed due to potential artifacts previously mentioned.

❖ To turn on the amino acid substitution search

1. Click [?] to open the Data Processing Options dialog box.
2. In the Search for Amino Acid Substitutions list, select the appropriate substitutions. Select from No, All Substitutions, or Single Base Change:
   - To avoid searching for substitutions, select **No**.
   - Because amino acid substitutions caused by DNA mutations rarely have more than one base change in the codons of the two amino acids, to search for DNA mutations, select **Single Base Change**. With this option selected, the application displays amino acid substitutions involving only one base change in their codons.
   - To find all substitutions, select **All Substitutions**.



4

Viewing Results in the MS/MS Window

In the MS/MS window, you can perform any of the following calculations on the peptide and tandem spectrum.

- Predicting the theoretical tandem spectrum of the selected peptide sequencing
- Modifying a residue or making a disulfide bridge in the selected peptide by double-clicking the residue
- Predicting a theoretical tandem spectrum of a modified or disulfide-linked peptide

Read these sections for information about viewing and changing results in the MS/MS window.

Contents

Using the MS/MS Window to View Spectra

The MS/MS window provides a clear way to analyze and compare spectra from different peptides.

Opening the MS/MS Window

❖ To open the MS/MS window

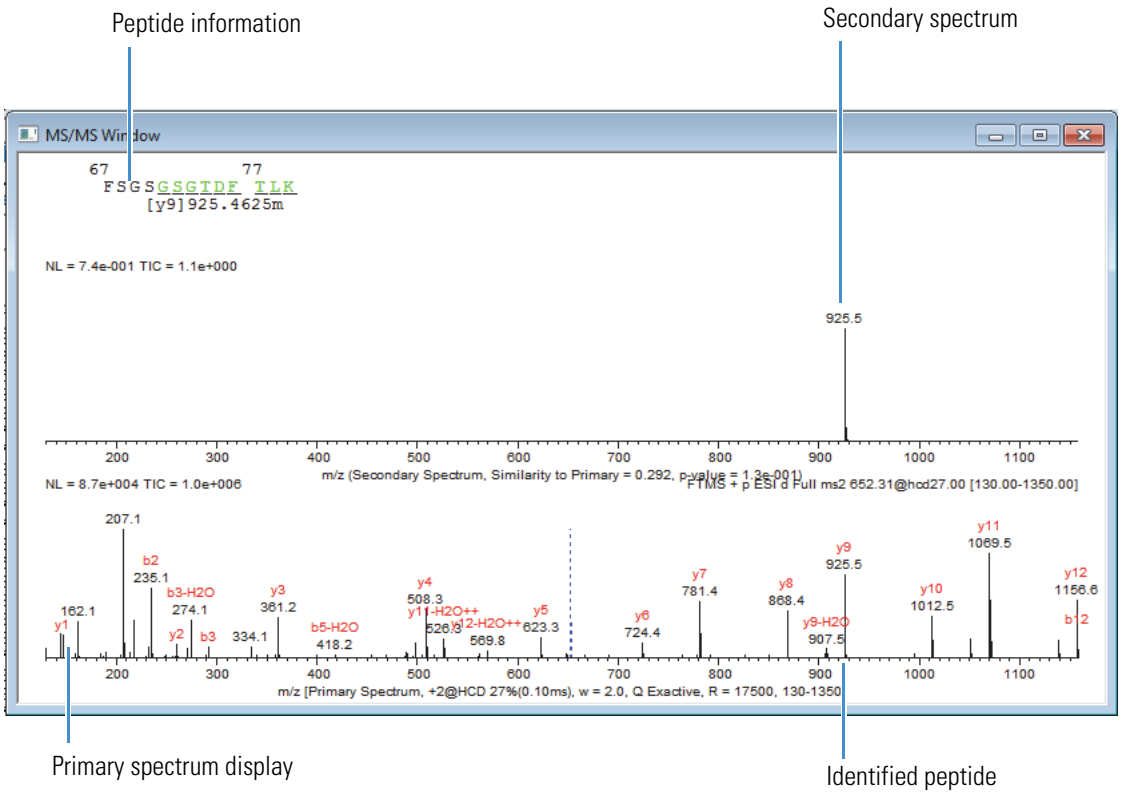
1. In the Main window, open a saved project.
2. Choose **View > MS/MS Window**, or click the **MS/MS** icon ( ).

   The MS/MS window can display two spectra at one time for comparison. The bottom spectrum is called the primary spectrum and the top spectrum is called the secondary spectrum. The application displays information about the selected peptide at the top of the window. The application displays fragment assignments in the primary spectrum.

Viewing Spectra in the MS/MS Window

Review these sections for information about viewing spectra in the MS/MS window.

- Viewing the Primary Spectrum
- Showing a Secondary Spectrum



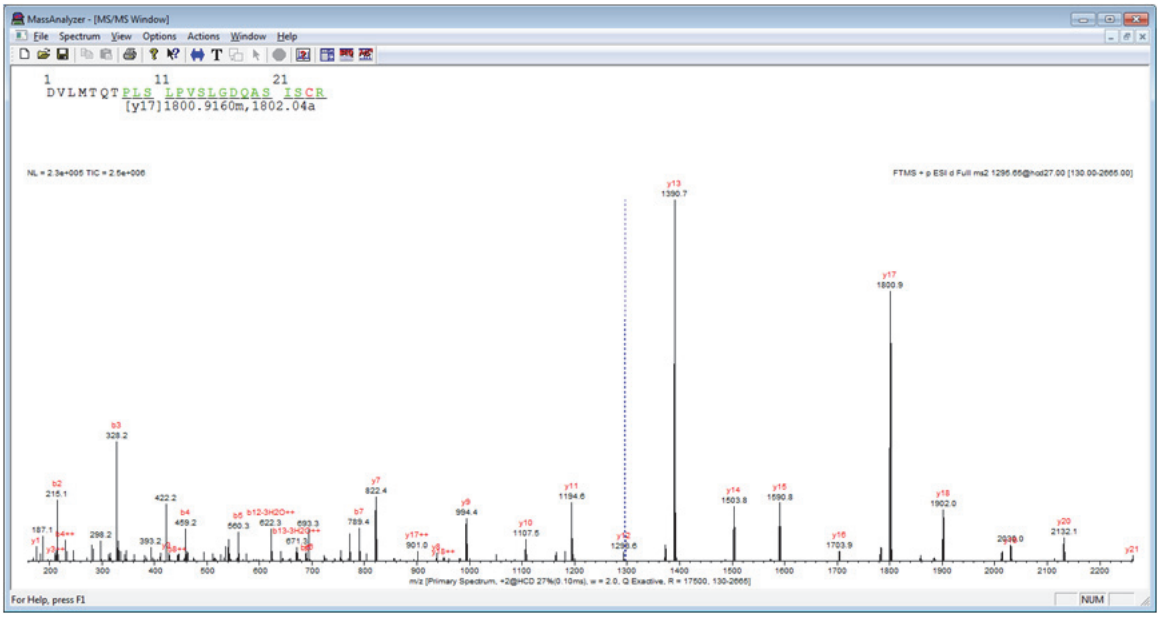
Viewing the Primary Spectrum

❖ To view the primary spectrum

1. From the ion list view, click an ion retention time.
2. Choose **View > MS/MS Window** or click  .

   The application displays the MS/MS window.

   The application can display two spectra in this window for comparison. You can move the primary spectrum (bottom) to the top (secondary). You can also perform spectrum prediction in this window. The application automatically updates the window whenever you click the retention time of another entry in the ion list.

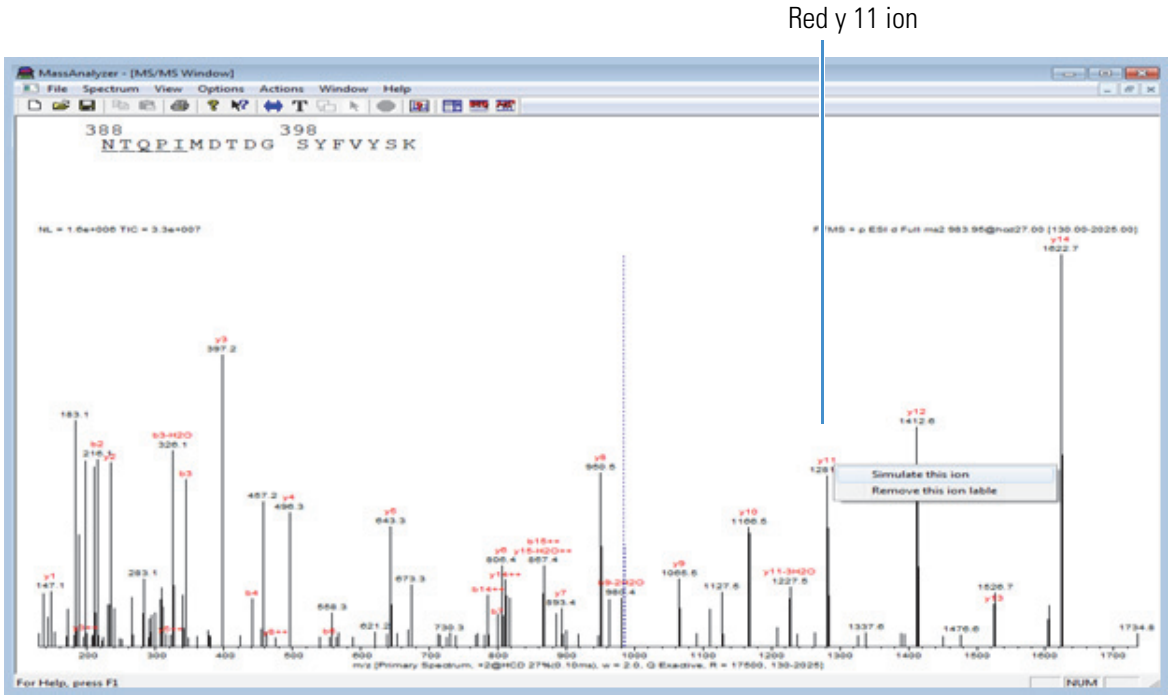


Showing a Secondary Spectrum

❖ To show the ion in the secondary spectrum

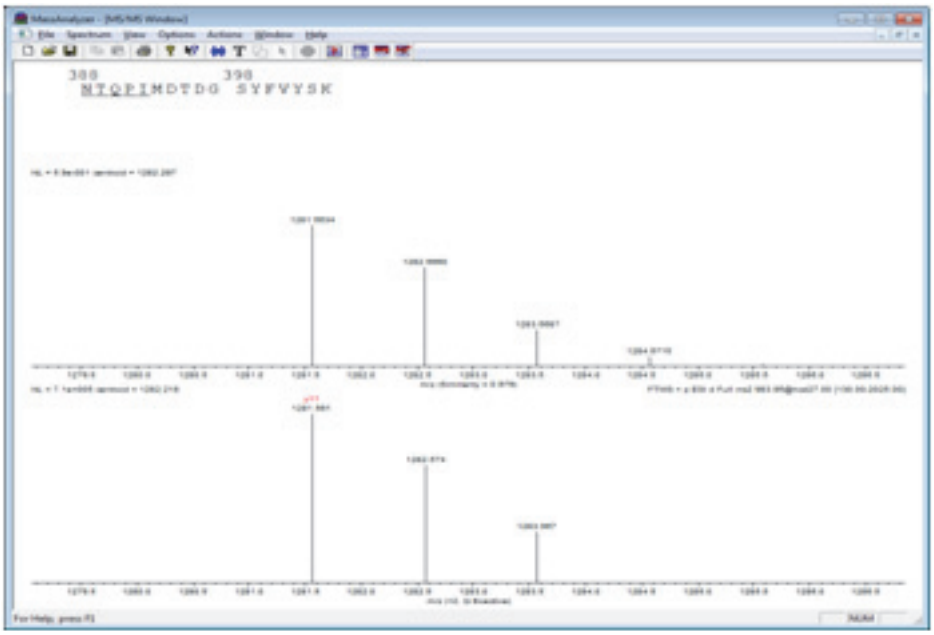
Right-click an ion assignment in the primary spectrum and choose **Simulate This Ion**.

In this example, the MS/MS window shows selection of the red y11 ion assignment in the primary spectrum.



In the secondary spectrum, the PepFinder application simulates what the ion for that peptide should look like for an HCD high resolution MS/MS scan, allowing for quick manual validation of the identification assignment.

In the graphic below, the application simulates what the y11 ion for peptide NTQPIMDTDGSYFVYSK should look like for an HCD high resolution MS/MS scan, allowing for quick manual validation of the identification assignment.



Changing the Spectrum View in the MS/MS Window

Follow these procedures to change the look of the Spectrum window or the spectrum itself.

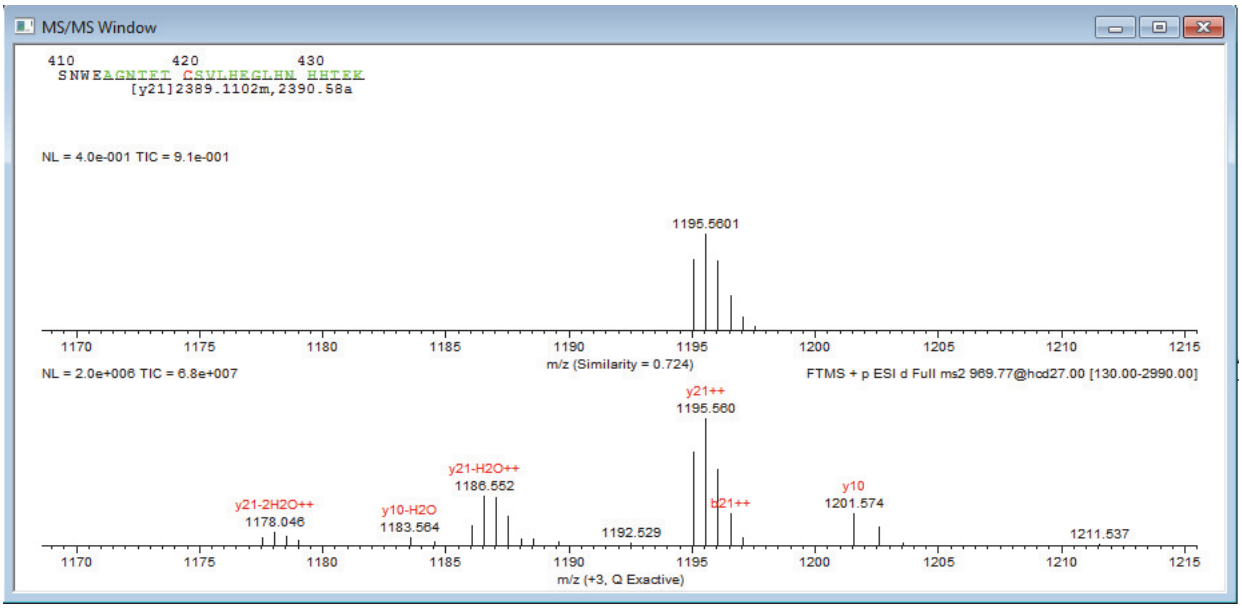
- Increasing the Zoom Level in the Spectrum Window
- Restoring the Full-scan Spectrum
- Changing the Spectrum Location
- Deleting the Secondary Spectrum
- Displaying a Specific Mass Range (Spectrum)
- Changing Line Width
- Changing Fonts
- Adding Text to an MS/MS Window

Increasing the Zoom Level in the Spectrum Window

❖ **To increase the zoom level in the MS/MS window**

1. Choose either the primary or secondary spectra.
2. Drag the cursor over the area of interest.

The application changes the zoom level of both spectra.



Restoring the Full-scan Spectrum

❖ **To display the full-scan spectrum after changing the zoom level**

In the Spectrum view, double-click the Spectrum window to restore the original version.

The application restores both spectra.

Changing the Spectrum Location

❖ **To change the primary spectrum location**

1. Select the primary spectrum.
2. Right-click and choose **Move to Secondary**.

   The application moves the primary spectrum into the secondary location.

Deleting the Secondary Spectrum

❖ **To delete the secondary spectrum**

1. Select the secondary spectrum.
2. Right-click and choose **Delete Spectrum**.

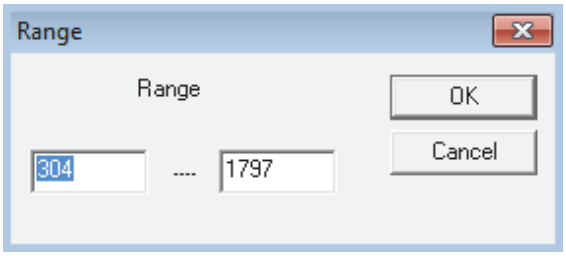
   The application deletes the secondary spectrum.

Displaying a Specific Mass Range (Spectrum)

❖ To display a specific mass range for a spectrum

1. Select the ion retention time.
2. In the MS/MS window, right-click an ion, and choose **Mass Range**, or select an ion and choose **Edit > Range**.

   A Range dialog box opens, displaying the current mass range.



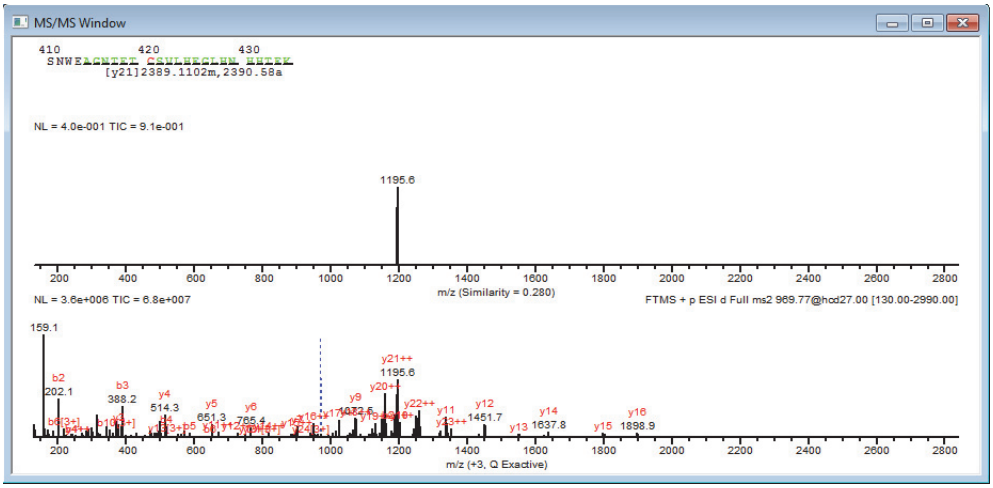
3. Type in values to display a new mass range.
4. Click **OK** to change the visible mass range.

Changing Line Width

❖ To change the line width of the primary spectrum

1. To change the line width on the primary spectrum, right-click the spectrum and choose **Line Width**.
2. Type a value in the dialog box and click **OK** to change the line width, measured in points.

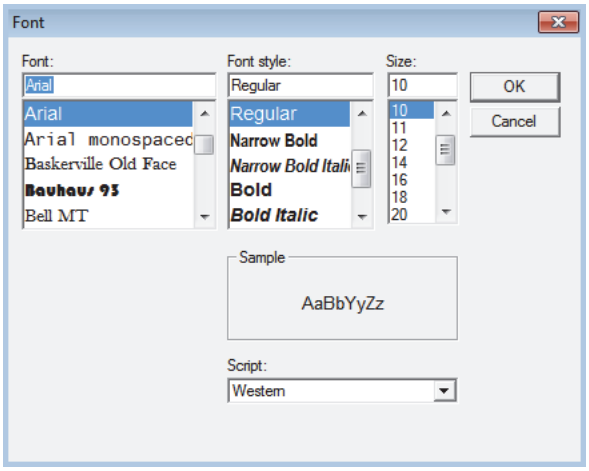
   The application changes the spectrum display to the new line width.



Changing Fonts

❖ To change the font size or style in the Spectrum window

1. Select an area in the window and right-click.
2. Choose **Font** to display font options.
3. Choose from among the fonts, font styles, and sizes displayed.
4. Click **OK**.



Adding Text to an MS/MS Window

❖ To add text to or modify text in an MS/MS window

1. Click the **Add Text** icon (T) in the toolbar to open the Text dialog box.
2. Type text into the box and click **OK** to add it to the Spectrum view.
3. To hide the inserted text, right-click the text and choose **Hide**
4. To move the text, drag it to a preferred location.
5. To edit the text, double-click the message and type in changes.
6. To change the font, right-click and choose **Font**, choose a font, and click **OK**.
7. To delete a text block, right-click the block and choose **Delete**.

Using the MS/MS Window to Modify Spectra

You can use the MS/MS window to define or change a spectrum.

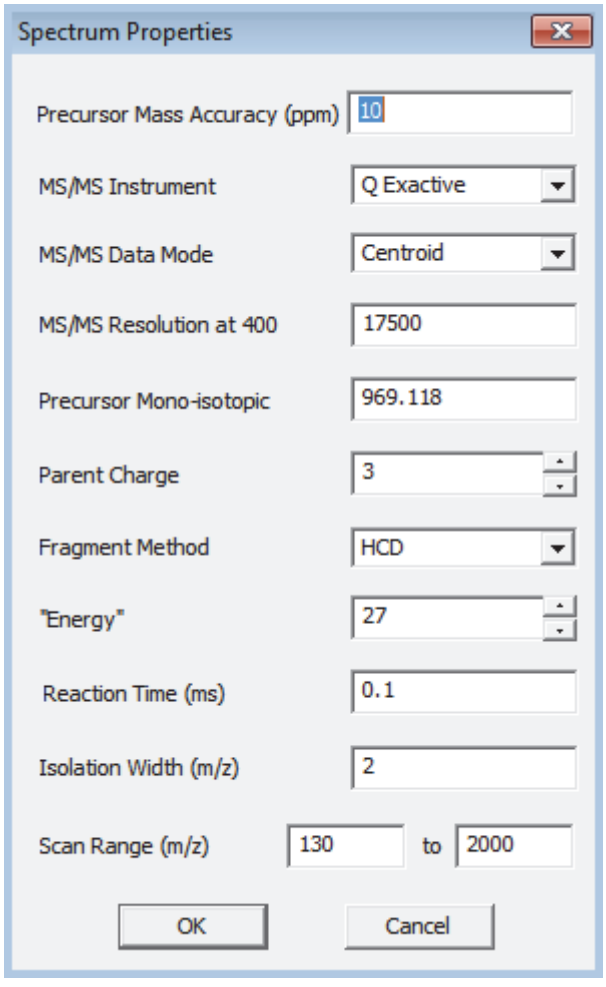
- Defining Spectrum Properties
- Recalibrating a Spectrum

Defining Spectrum Properties

❖ **To define spectrum properties**

1. In the MS/MS window, choose **Options > Spectrum Properties.**

   The Spectrum Properties dialog box opens.



2. To specify the precursor mass accuracy in ppm, type a value in the box.
3. To select an instrument type, select an instrument from the MS/MS Instrument list.

4. To choose a mode for the MS/MS data, select from these options:
   - Select **Centroid** to show a single line.
   - Select **Profile** to show the profile.

   For automated processing, the application loads the spectra automatically as centroid data, but when you perform manual spectral averaging in the displayed chromatogram, the application displays it as a profile dataset.
5. To specify the resolution, type a value in the box.
6. To specify the *m/z* of the precursor ion, type a value in the box.
7. To identify the parent charge, type a value in the box.
8. To select a fragment method, select one of these options:
   - ETD (electron-transfer dissociation)
   - ETD with sa (electron-transfer dissociation with supplemental activation)
   - HCD (higher energy collision-induced)
9. To specify a theoretical closing energy level, type a value in the box.
10. To set a reaction time, type a value in the box.
11. To specify the *m/z* of the isolation width, type a value in the box.
12. To define the scan range, type a start and end time in the boxes.
13. Click **OK** to change the spectrum.

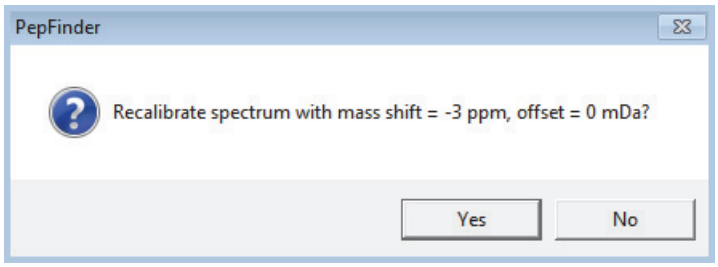
Recalibrating a Spectrum

To recalibrate the spectrum so that you can compare it to external libraries, use this procedure.

❖ To recalibrate a spectrum

1. Choose **Spectrum > Recalibrate Spectrum**.

   The application displays a message similar to this one:



2. Click **Yes**.

Working With Spectra Files

To view or export spectrum data, use any of these procedures.

Exporting the Primary Spectrum

❖ **To export the primary spectrum**

1. In the MS/MS window, choose **File > Export Primary Spectrum**.
2. In the browse box, name the file and click **Save**.

   The application exports a file containing the primary spectrum in the MS/MS window.

Exporting the Secondary Spectrum

❖ **To export the secondary spectrum**

1. In the MS/MS window, choose **File > Export Secondary Spectrum**.
2. In the browse box, name the file and click **Save**.

   The application exports a file containing the secondary spectrum in the MS/MS window.

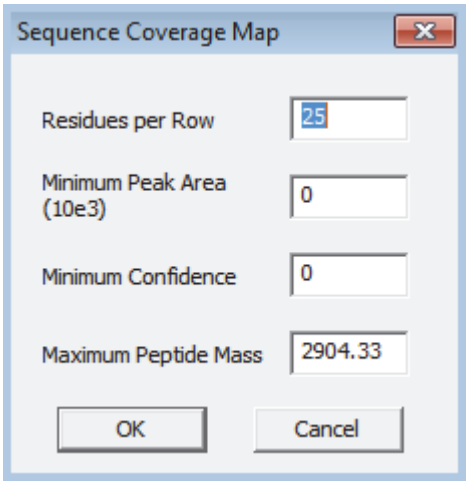
Creating a Fragment Coverage Map for the Spectrum

❖ **To create a sequence coverage map for a spectrum**

1. In the MS/MS window, choose **File > Create Fragment Coverage Map.**

   The Sequence Coverage Map dialog box opens.

   The application calculates the percent of coverage. Peptides are labeled with their retention time and color-coded with their intensity (red, yellow, green and cyan), with red as most intense and cyan as the least intense.



2. Specify the number of residues per row.

   Valid values: 2 to 500

   Default: 50

3. Define the minimum peak area.

   Valid values: 0 to 1e+006

   Default: 0

4. Specify a level for minimum confidence.

   Valid values: 0 to 1

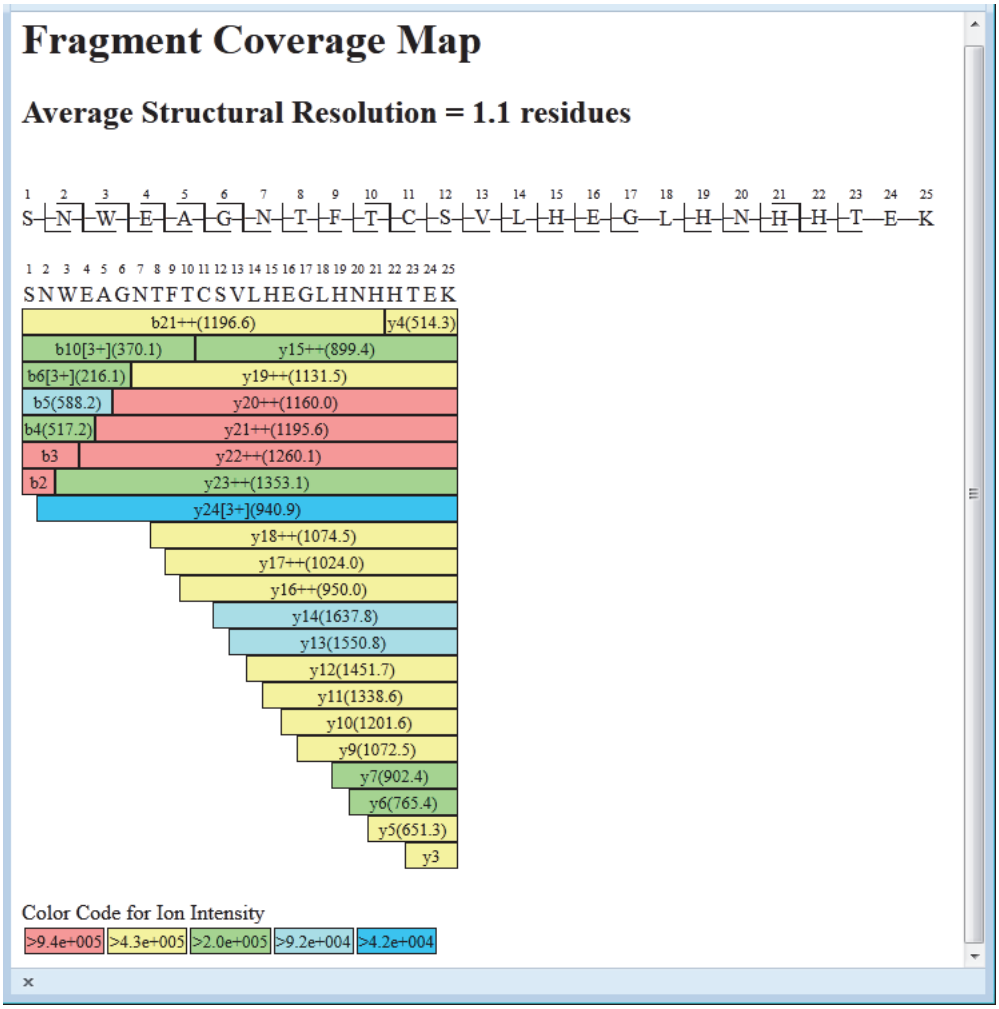
   Default: 0.8

5. Define the maximum peptide mass.

   Valid values: 100 to 100000

   Default: 8000

6. Click **OK** to open a browse box and save the fragment coverage map.



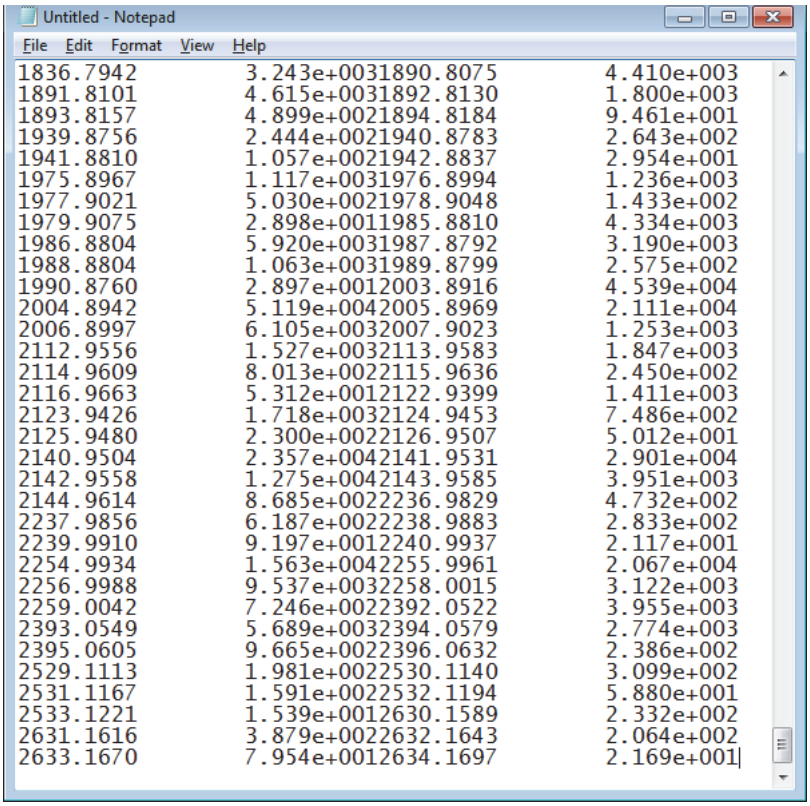
Copying a Spectrum List

❖ To copy a spectrum list

1. In the MS/MS window, display the spectrum.
2. Choose **Spectrum > Copy *type of spectrum* Spectrum List**, or right-click the Spectrum view and choose **Copy Spectrum List**.

   If you chose Primary, the application copies the primary spectrum list. If you chose Secondary, the application copies the secondary spectrum list.

   The application copies the list, containing the *m/z* and the intensity, to the Clipboard.
3. Paste the list into a text application.



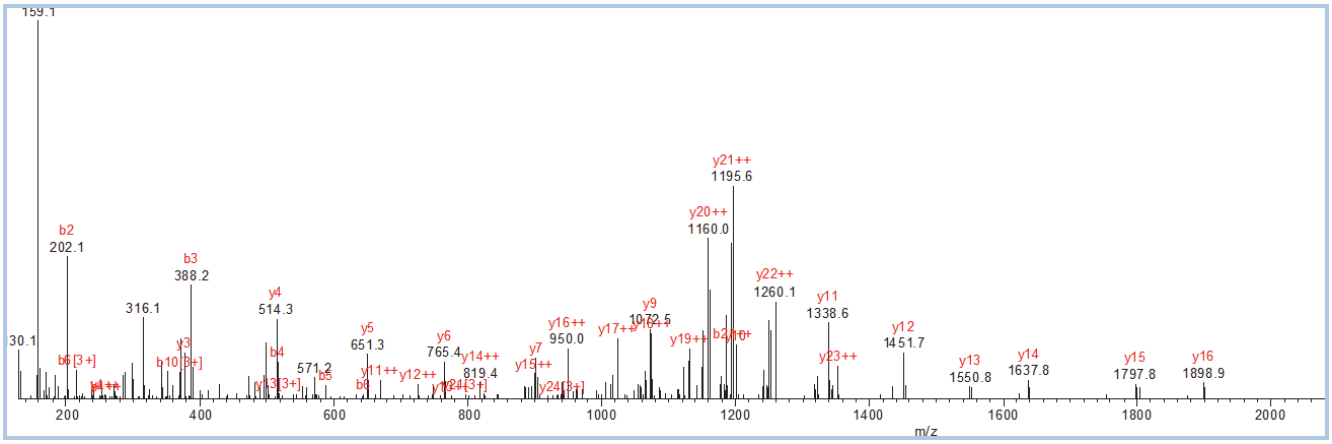
Copying a Spectrum

❖ To copy a spectrum

1. In the MS/MS window, display the spectrum.
2. Choose **Spectrum > Copy *type of spectrum* Spectrum** or right-click the Spectrum view and choose **Copy Spectrum**.

   If you chose Primary, the application copies the primary spectrum. If you chose Secondary, the application copies the secondary spectrum.

   The application copies the spectrum to the Clipboard.



Pasting a Spectrum

❖ To compare two spectra with different retention times

1. Select the new retention time.
2. Make the Spectrum view active by clicking the square in the corner of the area.
3. Copy the spectrum.
4. Select the retention time of the spectrum you want to use for comparison.
5. Choose **Spectrum > Paste Data as *type of spectrum* Spectrum** to paste the spectrum into the MS/MS window to compare the two spectra.

   If you chose Primary, the application pastes the spectrum as the primary spectrum. If you chose Secondary, the application pastes the spectrum as the secondary spectrum.

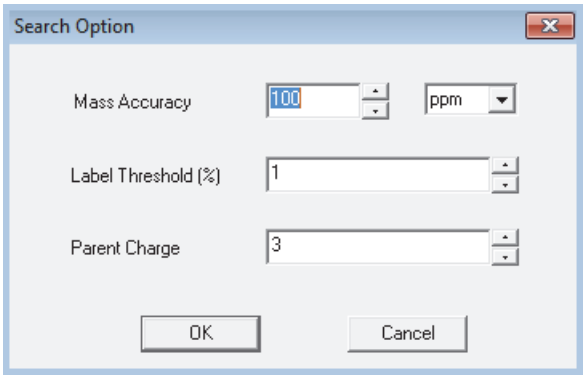
Setting Search Options

❖ To define fragment ion search options

1. In the Spectrum window, choose **Options > Search Criteria**.

Use the Search Criteria dialog box to set the criteria when searching fragment ions to assign ion types in the spectrum.

The Search Option dialog box opens.



2. To set a level for the mass accuracy, type a value in the Mass Accuracy box and select either **ppm** or **daltons** to specify the value type.

   Valid values: 0.0001 to 2000

   Default: 100

3. To specify the smallest ion (relative to the most intense ion) that is labeled, type a value in the Label Threshold (%) box.

   Valid values: 0 to 100

   Default: 1

4. To specify the charge state of the parent ion, type a charge level in the Parent Charge box.

   Valid values: All integers

   Default: 2

Working with Ions

You can analyze or simulate an ion (performing a theoretical calculation of the isotope pattern) or remove an ion label by right-clicking the label.

- Removing the Ion Label
- Analyzing an Ion
- Working with Fragment Ions

Removing the Ion Label

❖ **To remove the ion label**

1. Right-click the ion in the spectrum.
2. Choose **Remove This Ion Label**.

   The application removes the label.

Analyzing an Ion

You can create a theoretical calculation of the isotope pattern by simulating an ion.

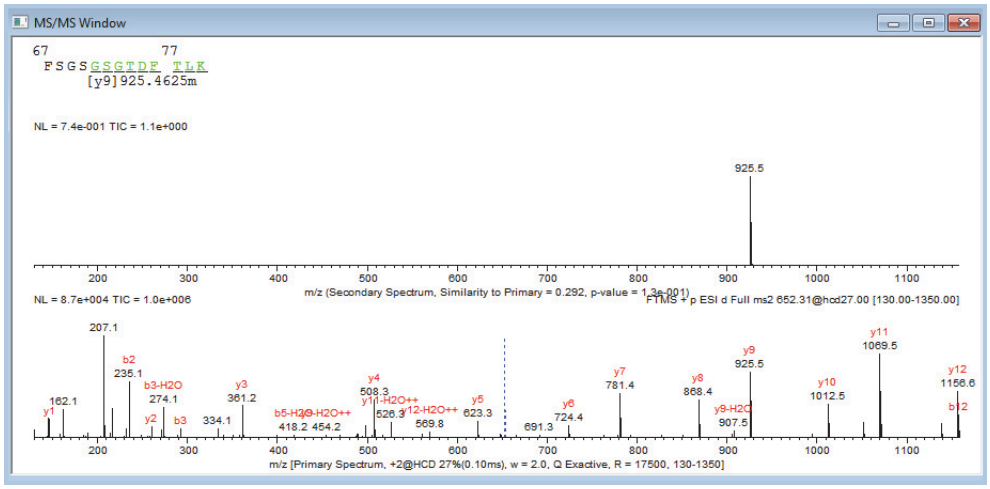
Performing a protein analysis

❖ **To simulate an ion and perform top-down protein analysis in the MS/MS window**

1. Open the MS/MS window by choosing **View > MS/MS Window** or by clicking .

1. Select the ion and right-click the ion label (in red).
2. Choose **Simulate This Ion** to calculate the theoretical isotope pattern of the ion and compare the results to the theoretical pattern to confirm the fragment assignments.

   The application displays the theoretical calculation of the isotope pattern in the upper spectrum.



   The application calculates theoretical isotope patterns based on the following:

   - Binomial expansion if the ion is less than 25 kDa
   - Simulation of 200000 molecules if the ion is above 25 kDa

3. To perform isotope simulation from the elemental composition (CHNOS only), choose **Action > Simulate Ion Isotope Pattern**.

After you are satisfied with the fragment assignments, you can create a sequence coverage map so that you can specify breakage points for your ion (for example, after K). For more information, see "To create a sequence coverage map for a spectrum" on page 84.

Working with Fragment Ions

Use these procedures to define and export fragment ions.

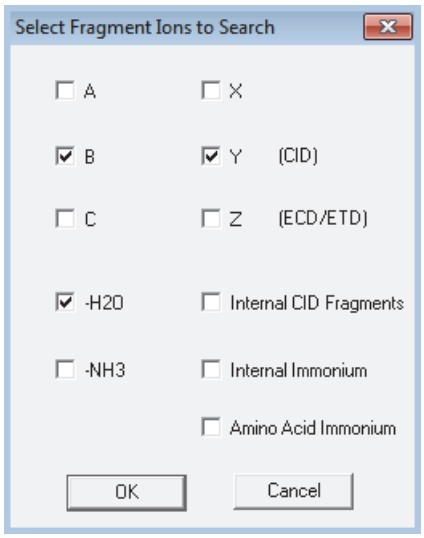
- Searching for Fragment Ion Types
- Exporting Fragment Ions

Searching for Fragment Ion Types

❖ **To select types of fragment ions**

1. In the MS/MS window, choose **Options > Select Fragment Ions to Search**.

   The Select Fragment Ions to Search dialog box opens.



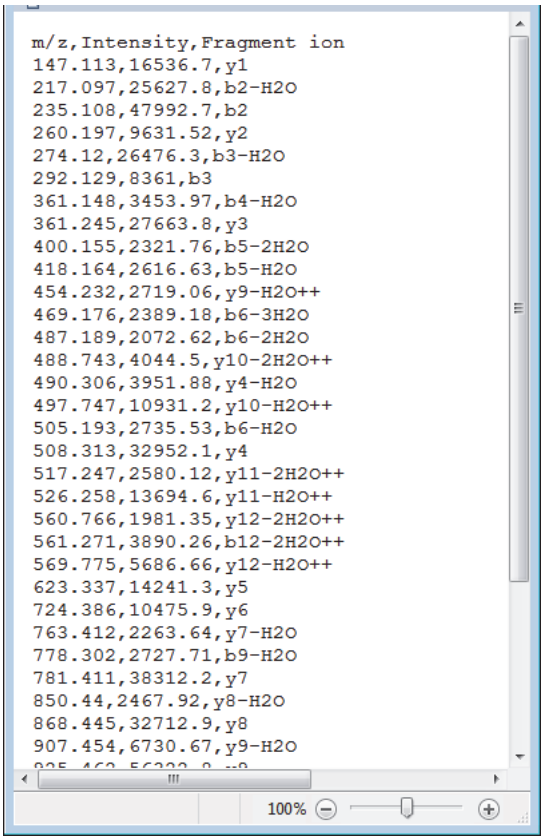
2. To set the criteria for searching fragment ions for assigning ion types in the spectrum, select options in this dialog box that you want identified as fragment types.
3. Click **OK** to start the search.

Exporting Fragment Ions

❖ **To export a list of fragment ions**

1. In the MS/MS window, choose **File > Export Fragment Ions** to export a list of fragment ions that match the assigned peptide sequence.
2. In the browse box, name the file and click **Save**.

   The application exports the primary spectrum in the MS/MS window.



```
m/z,Intensity,Fragment ion
147.113,16536.7,y1
217.097,25627.8,b2-H2O
235.108,47992.7,b2
260.197,9631.52,y2
274.12,26476.3,b3-H2O
292.129,8361,b3
361.148,3453.97,b4-H2O
361.245,27663.8,y3
400.155,2321.76,b5-2H2O
418.164,2616.63,b5-H2O
454.232,2719.06,y9-H2O++
469.176,2389.18,b6-3H2O
487.189,2072.62,b6-2H2O
488.743,4044.5,y10-2H2O++
490.306,3951.88,y4-H2O
497.747,10931.2,y10-H2O++
505.193,2735.53,b6-H2O
508.313,32952.1,y4
517.247,2580.12,y11-2H2O++
526.258,13694.6,y11-H2O++
560.766,1981.35,y12-2H2O++
561.271,3890.26,b12-2H2O++
569.775,5686.66,y12-H2O++
623.337,14241.3,y5
724.386,10475.9,y6
763.412,2263.64,y7-H2O
778.302,2727.71,b9-H2O
781.411,38312.2,y7
850.44,2467.92,y8-H2O
868.445,32712.9,y8
907.454,6730.67,y9-H2O
```

100%

Working with Peptides

Use these procedures to view peptide properties and label proteolytic peptides. You can also create a list of proteolytic fragments.

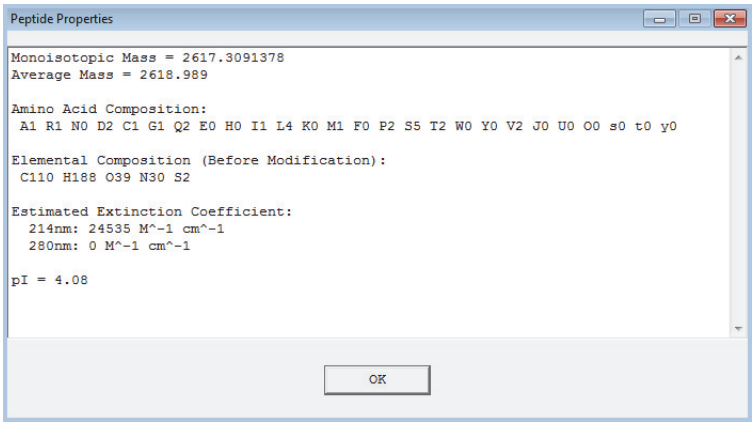
- Showing Peptide Properties
- Predicting MS/MS

Showing Peptide Properties

❖ To show peptide properties

1. In the MS/MS window, choose **Actions > Show Peptide Properties**.

   The Peptide Properties window opens.



2. Click **OK**.

Predicting MS/MS

The PepFinder application uses kinetic models to predict peptide MS/MS. Use uppercase one-letter code for peptide sequence. You can also use the following special codes.

Code	Definition
J	carboxymethylated cysteine
U	carboxyamidomethylated cysteine
O	oxidized methionine
s, t, y	phosphorylated serine, threonine, and tyrosine, respectively

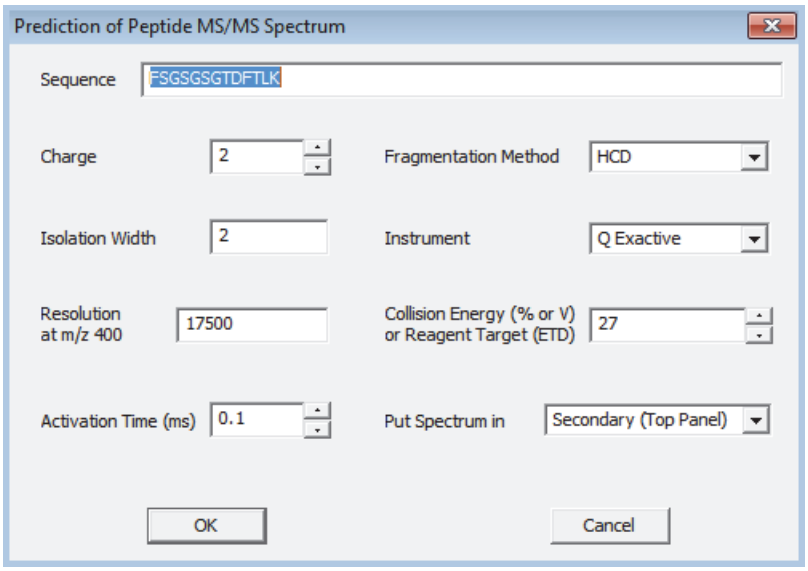
- For a modification, put the modification in parentheses after the sequence. For example, AADECFGHK(C5+250)(H8–9) means Cys at position 5 is modified by +250 u, and His at position 8 is modified by –9 u. AANASAA(N3+A2G0F) means Asn at position 3 is glycosylates with A2G0F.
- To define a disulfide bond, put the bond in parenthesis. For example, ADCAGHTYCHPEK(C3-C9) means Cys at position 3 and Cys at position 9 are linked by a disulfide bond.
- For collision energy, specify normalized collision energy (%) for LCQ/LTQ, volts for Q-TOF, and the reagent ion AGC target value for ETD.

❖ To perform batch prediction of a list of peptides

1. Save the peptide list to a text file.

   Each line in the file contains the peptide sequence followed by the charge state. Almost all deliminators work. If the charge is not given, the application estimates the number of charges based on the amino acid composition and peptide length.

2. In the MS/MS window, choose **Actions > Predict Peptide MS/MS (Kinetic Model)** to perform batch prediction for a peptide dataset.



3. In the Prediction of Peptide MS/MS Spectrum dialog box, set the appropriate collision energy, isolation width, and instrument model.

   a. To set charge values, type a value in the box.

      Valid values: 1–100

      Default: 0.8

   b. To define the Isolation Width Valid, type a value in the box.

      Valid values: 0.1 to 40

      Default: 1

   c. To define resolution at *m/z* 400, type a value in the box.

      Valid values: 100 to 5000000

      Default: 17500

   d. To specify activation time (ms), type a value in the box.

      Valid values: 0 to 1000

      Default: 1000

e. To define a fragmentation method, select from the following:

Valid values: CID, CID with WB activation, ETD, ETD with supplemental activation, ECD, HCD

Default: HCD

f. To choose an instrument, select an instrument name from the list.

g. To define collision energy (% or V) or reagent target (ETD), type a value in the box.

Valid values: 350 to 1e+007

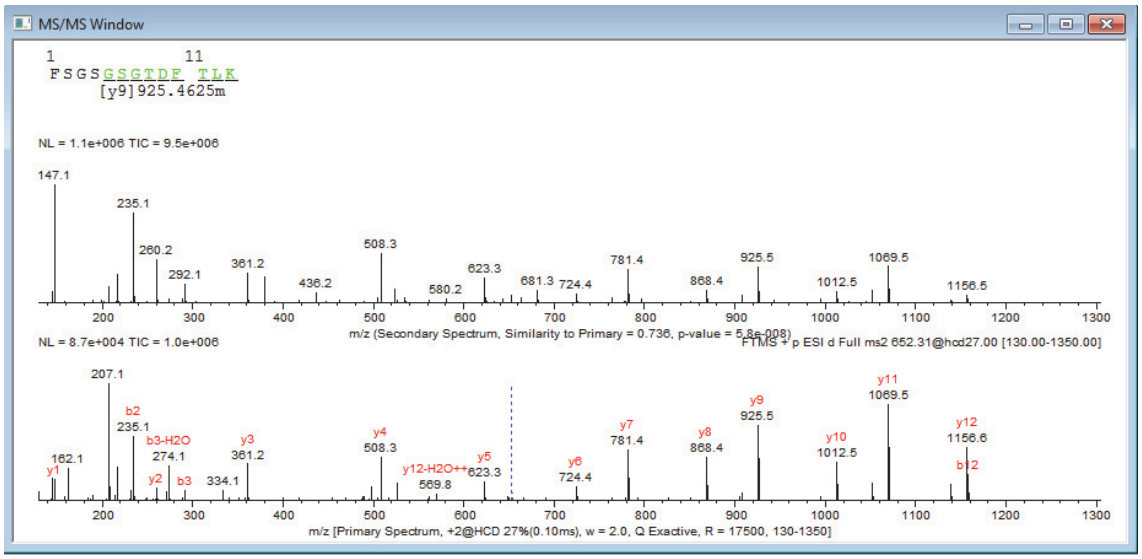
Default: Read from raw data file

h. To put the spectrum in a specific location, select from **Primary (Bottom Panel)** or **Secondary (Top Panel)**.

Default: Secondary (Top Panel)

4. Click **OK** if you want isotopic peaks to be predicted (otherwise, each ion is represented as a single ion at average).

The application displays the changes in the MS/MS window.



The application also saves the results in a file called MSMSPredict.txt. Rename it to a meaningful name.

Below is the format of MSMSPredict.txt:

Line 1: Total number of spectra in the file

Line 2: Sequence of peptide 1

Line 3: Description of peptide 1

Line 4: Charge of peptide 1

Line 5: Fragment method of peptide 1 (1 for CID, 2 for ETD, 3 for ETD with supplemental activation)

Line 6: Collision energy (or reagent ion target value for ETD) of peptide 1

Line 7: Reaction time of peptide 1 (in seconds)

Line 8: Isolation width of peptide 1

Line 9: Instrument model of peptide 1 (1 for LCQ, 2 for LTQ, 3 for Orbitrap, 4 for LTQFT, 5 for QTOF)

Line 10: Resolution of peptide 1 at *m/z* 400

Line 11: Scan range of peptide 1

Line 12: Total number of mass-intensity pairs of peptide 1

Lines 13 to n: Mass-intensity pairs of peptide 1

Line n+1: Sequence of peptide 2

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Index

L

M

P

R

S

T

V

Z