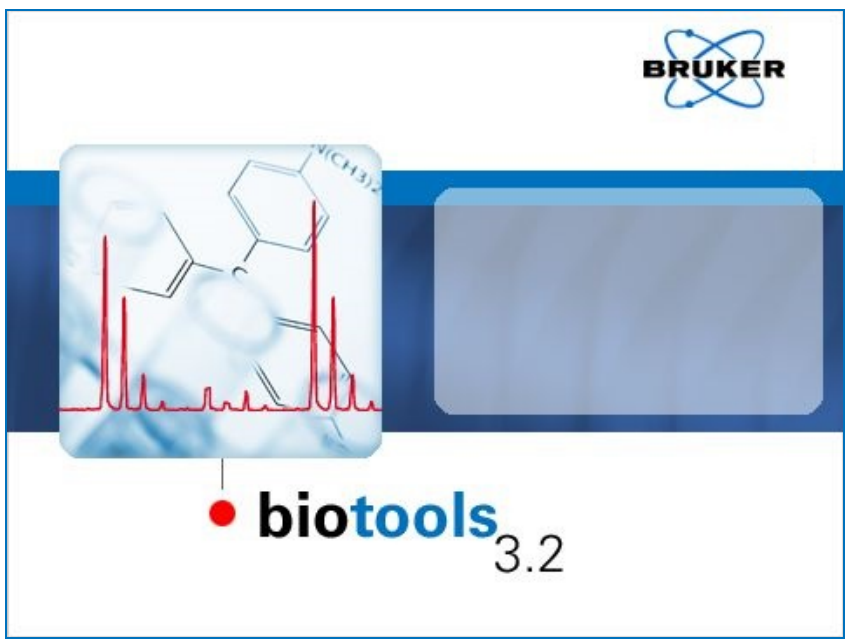
biotools 3.2

User Manual



Revision F (August 2015)

Legal and Regulatory Notices

Document History

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Table of Changes

Version	*Date*	*Changes*	*Remarks*
2.0	2000-10-14	Software versions: BioTools, Version 2.0 SequenceEditor, Version 1.0	
2.1	2002-01-18	Software versions: BioTools, Version 2.1 SequenceEditor, Version 2.1	
2.2	2002-08-01	Software versions: BioTools, Version 2.2 SequenceEditor, Version 2.1	
3.0	2005-05-12	Software versions: BioTools, Version 3.0 SequenceEditor, Version 3.0	
3.1	2007-01-26	Software versions: BioTools, Version 3.1 SequenceEditor, Version 3.1	
3.2	2008-10-28	Software versions: BioTools, Version 3.2 SequenceEditor, Version 3.2	
3.2 SR1	2010-03-15	Software versions: BioTools, Version 3.2 SR1 SequenceEditor, Version 3.2 SR1	
3.2 SR2	2011-05-15	Software versions: BioTools, Version 3.2 SR2 SequenceEditor, Version 3.2 SR2	
3.2 SR3	2011-11-15	Software versions: BioTools, Version 3.2 SR3 SequenceEditor, Version 3.2 SR3	
3.2 SR4	2012-12-10	Software versions: BioTools, Version 3.2 SR4 SequenceEditor, Version 3.2 SR4	
3.2 SR5	2015-08-31	Software versions: BioTools, Version 3.2 SR5 SequenceEditor, Version 3.2 SR5	

Table of Contents

1 INSTALLATION

1.1 System Requirements

- CPU: Intel Pentium III processor or better.
- Clock: 1 GHz processor for satisfying data handling.
- Main Memory: Minimum 512 Mbytes RAM or better.
- Operating systems supported:
  - Microsoft Windows Vista Business Edition, Service Pack 1, 32 bit
  - Microsoft Windows 7, Business Edition, 32 and 64 bit
- Internet Explorer 8.0 or better
- Graphic Resolution: 1024 x 768 pixel, 256 colors or better
- CD-ROM drive (4x or better)
- Ethernet connection
- Hard disk: at least 1 Gigabyte of free disk space.

1.2 Backup Methods

a. If no backup folder exists on the BioTools computer, create one. We recommend a location like D:\Bruker_Service

b. Create a subfolder for the actual backup. We recommend coding your current date, e.g. \backup_ddmmyy (dd=day, …)

c. If you're upgrading from a 2.x version of BioTools, browse to the BioTools installation directory, (usually this is C:\Bruker\BioTools) and copy the file **batch.ptr** to the newly created directory (necessary for old method import after the de-installation). When upgrading from BioTools 3.0 this step is not necessary, 3.0 methods can be read by BioTools 3.2.

d. Save the URL of your local Mascot Server. It can be found on the MS () or MSMS Mascot Search dialog () or the Batch Mode window (Query parameter tab).

e. You may backup these directories from the method folder: D:\Methods\BiotoolsParamFiles and D:\Methods\SEDigestMethods if you have defined building blocks, crosslinks or enzymes using a previous version of BioTools.

1.3 Uninstall old BioTools version

a. Make sure that you are logged on as Administrator or with administrator privileges, uninstall and install will not work with restricted user rights.

b. Go to the Windows **Start** menu → Settings → Control Panel and choose "Add/Remove Programs".

c. Choose BioTools and remove it from the system.

d. If there is a BioTools icon left on the desktop, remove it.

1.4 Install BioTools

a. Put the BioTools installation CD in the CD-ROM drive, wait for the launcher interface and choose "Install".

If the launcher interface does not open automatically, choose "CD_Start.exe" from the BioTools CD.

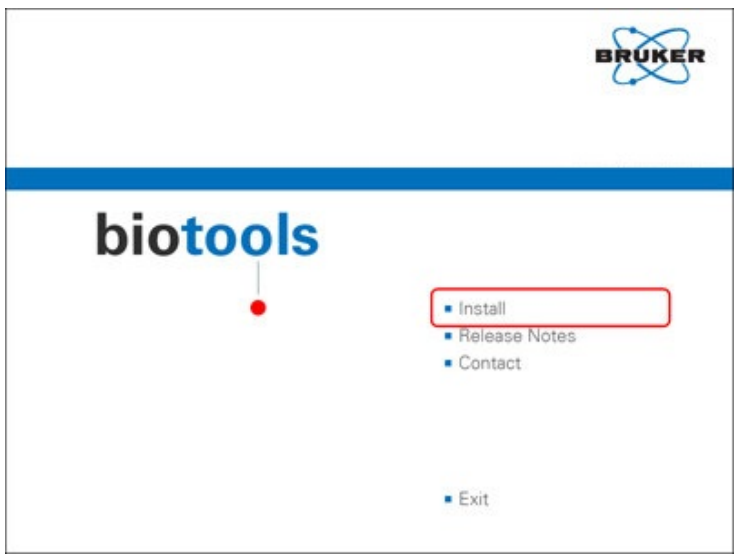


Figure 1-1 Choosing "Install"

b. To view the installation instructions click on "Installation Instructions".

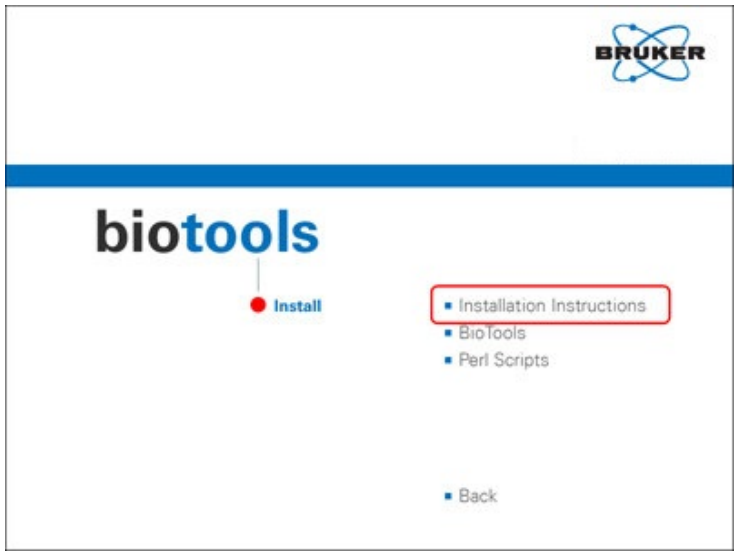


Figure 1-2 Choosing "Installation Instructions"

Follow the instructions on the screen to install the BioTools and the Perl Scripts (optionally).

c. Start the installation with a click on "BioTools" (or run the "CD_Start.exe" from the BioTools CD).

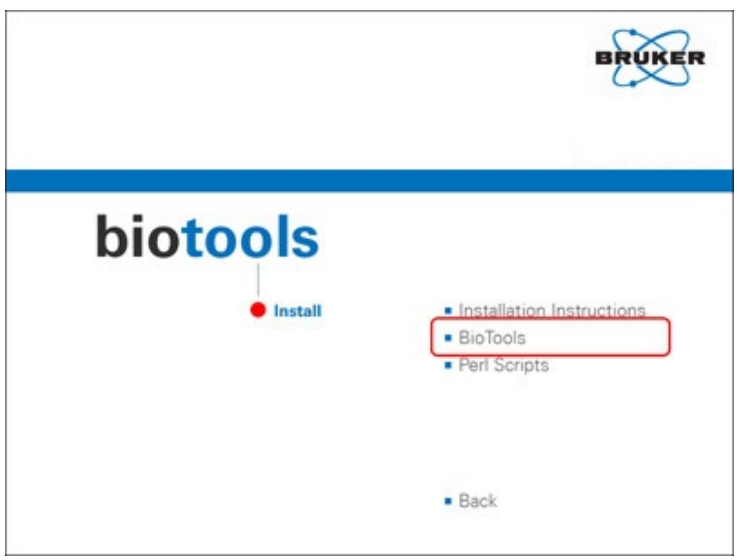


Figure 1-3 Choosing "BioTools"

d. If the following message appears, an old version of BioTools is still installed on the system.

Figure 1-4 Message about old BioTools version found

Click "OK" and remove the old version with the Windows "Add/Remove Programs" feature.

e. Go back to the BioTools launcher interface and start the installation again. Nearly always choose the preset options and confirm the following dialogs.

Click "Next" to start the installation

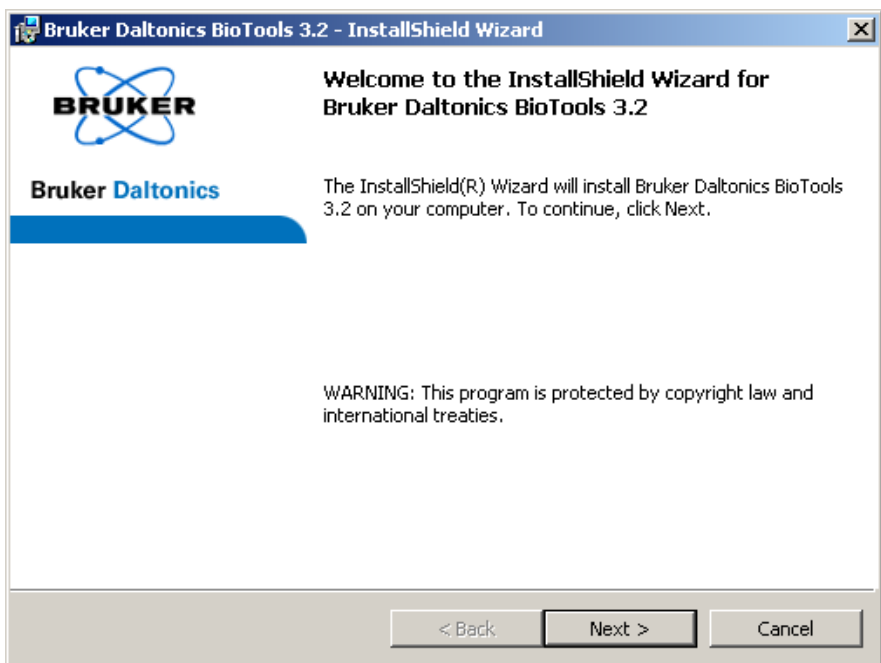


Figure 1-5 Installation started

f. Accept the License Agreement.

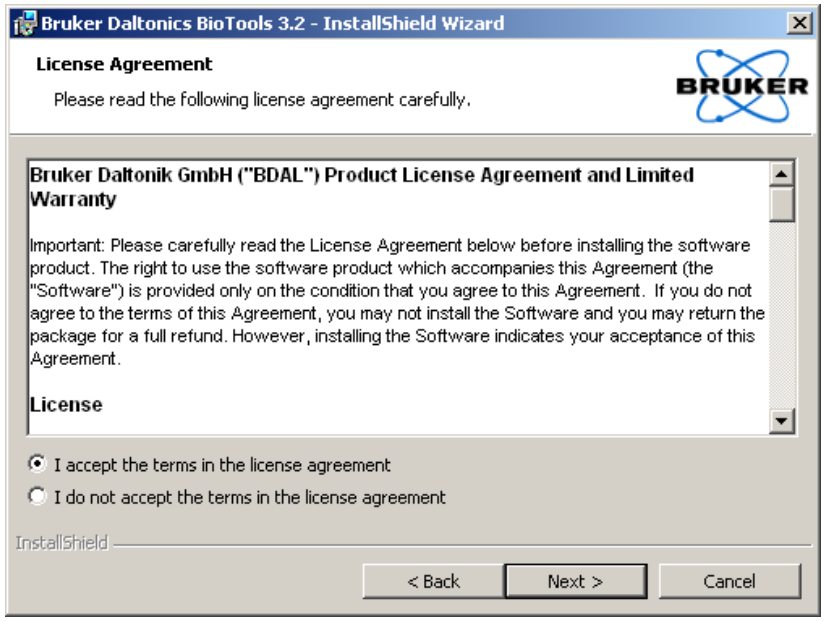


Figure 1-6 Accepting License Agreement

g. Choose the installation directory.

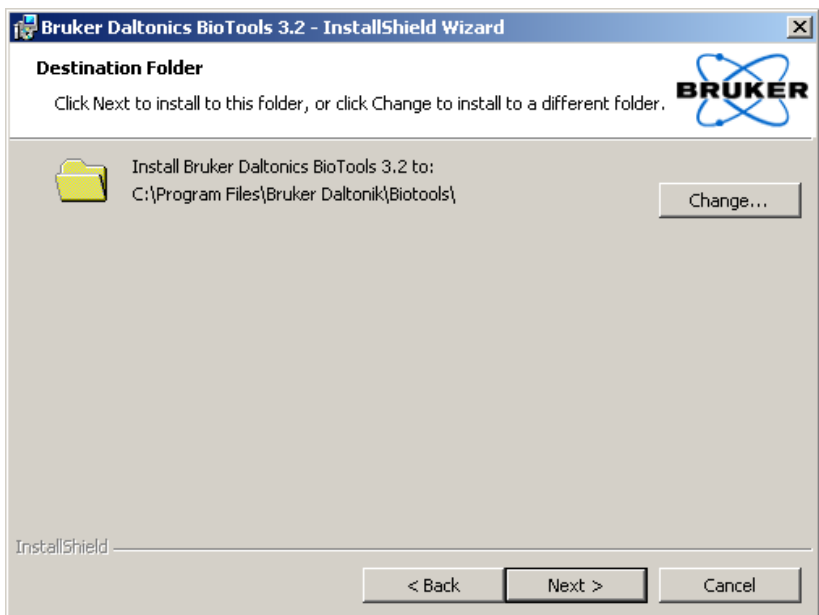


Figure 1-7 Choosing installation directory

h. You may be prompted to select a directory for the tutorial data that come with the installation. The selected folder will be used by other Bruker programs installed subsequently on that machine. A similar dialog prompting for the method directory may also appear.

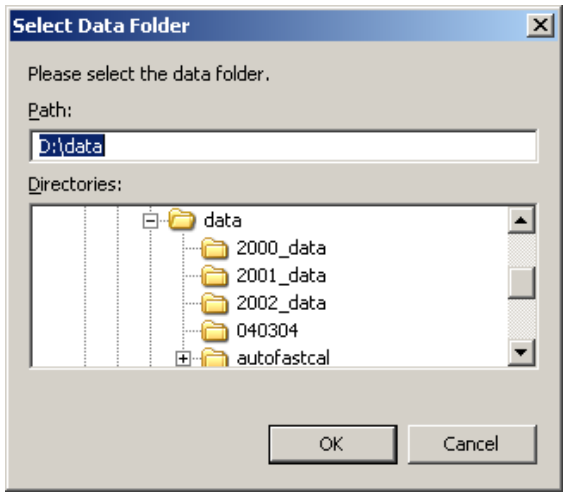


Figure 1-8 Selecting data folder

i. Select the check box to add desktop icons.

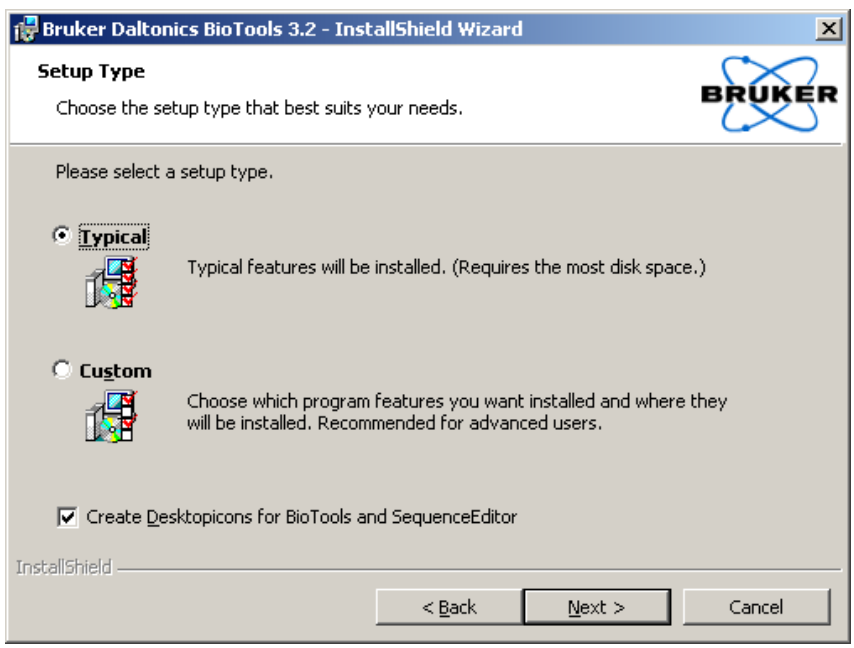


Figure 1-9 Choosing setup type and creation of desktop icons

j. Install the software by clicking on "Install".

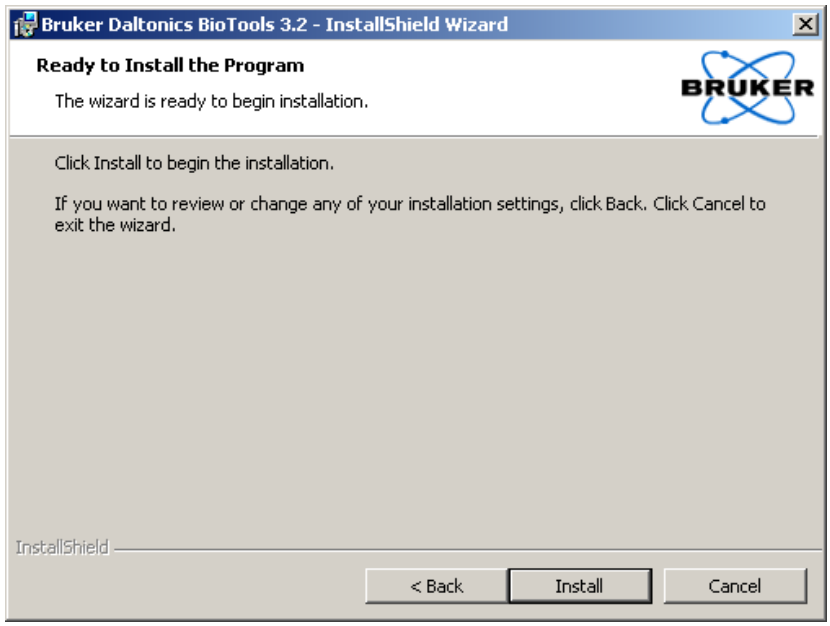


Figure 1-10 Starting installation by clicking "Install"

k. A progress indicator shows the status of the installation.

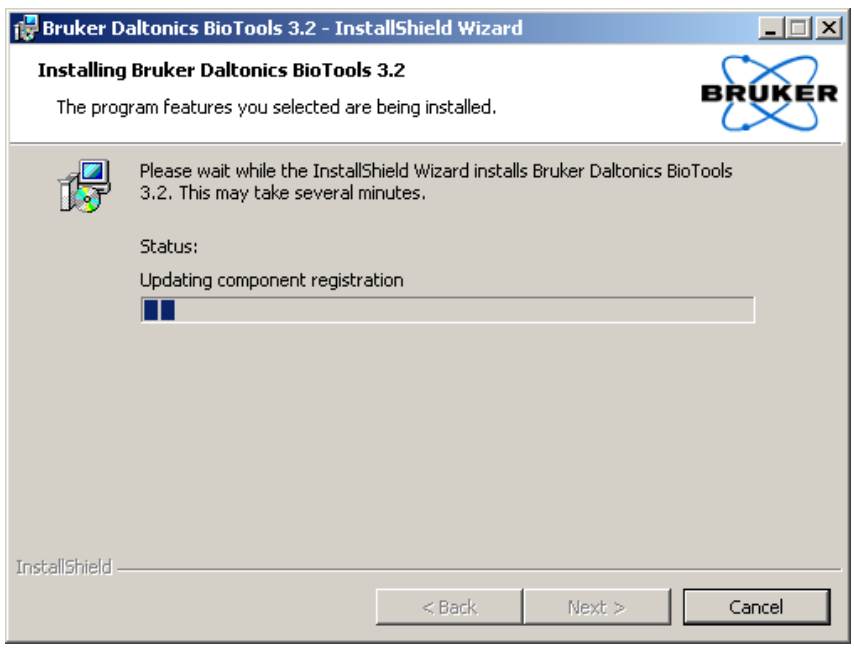


Figure 1-11 Installation running

l. If BioTools 3.2 is installed the first time on this computer, a 60-days license is created:

Figure 1-12 Temporary licenses created

Do not forget to enter your permanent license after the installation has been finished (menu **Compass** → **License**).

m. Click "Finish" to complete the installation.

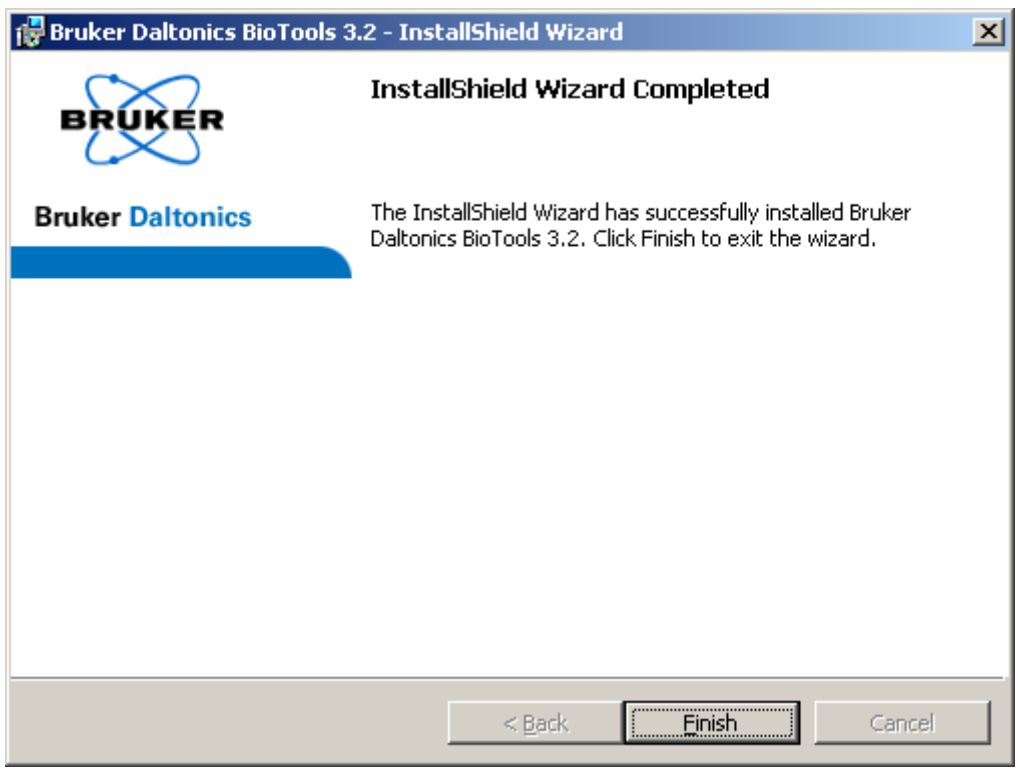


Figure 1-13 Installation finished

n. If an outdated version of Mascot is used as in-house server it may be necessary to install the Perl scripts by clicking on "Perl Scripts".
Follow the instructions on the screen to install them.

BioTools 3.2 can be run without installing Perl scripts from the CD when it is used with Mascot 2.1 or later. With earlier Mascot versions, make sure that the new **Perl scripts**, coming with the BioTools 3.2 installation CD, are installed **on the server (!)** before a batch mode search is started the first time. Otherwise there is no guarantee for correct communication between BioTools and the Mascot server (see chapter 1.6 Update Perl Scripts).

o. To leave the installation screen click on "Back".

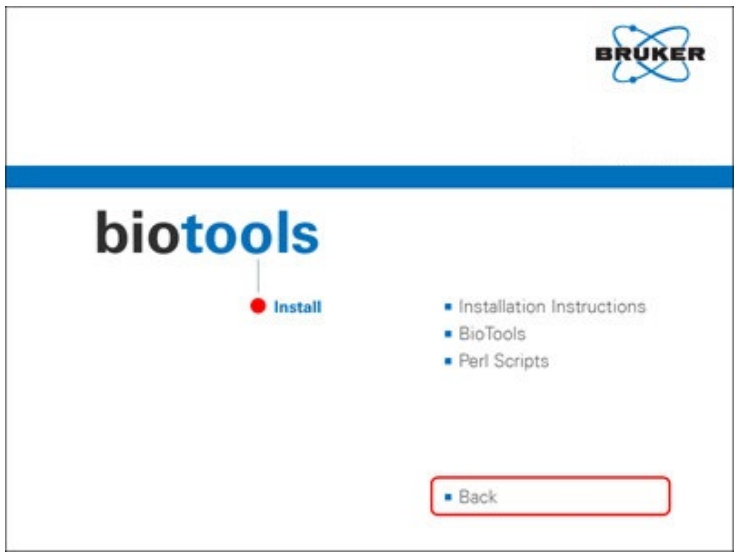


Figure 1-14 Choosing "Back"

p. To view the release notes click on "Release Notes". A new window with the release notes will be opened.

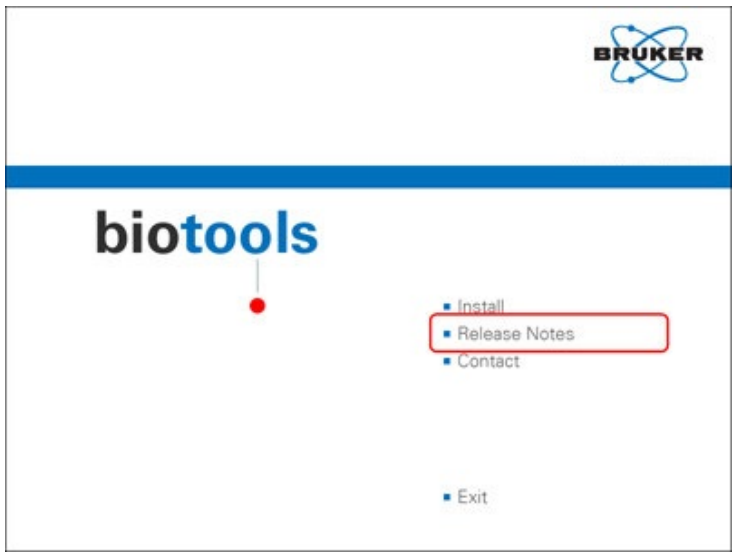


Figure 1-15 Choosing "Release Notes"

q. To view the contact information click on "Contact".

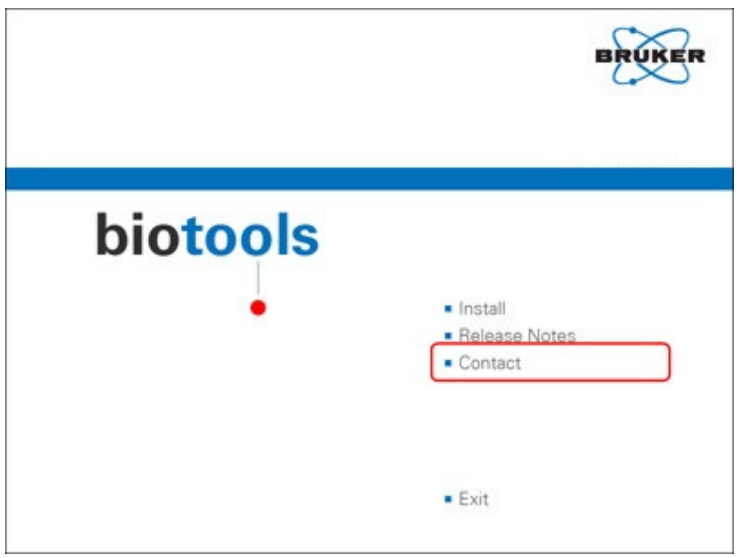


Figure 1-16 Choosing "Contact"

r. To close the contact information click on "Back".

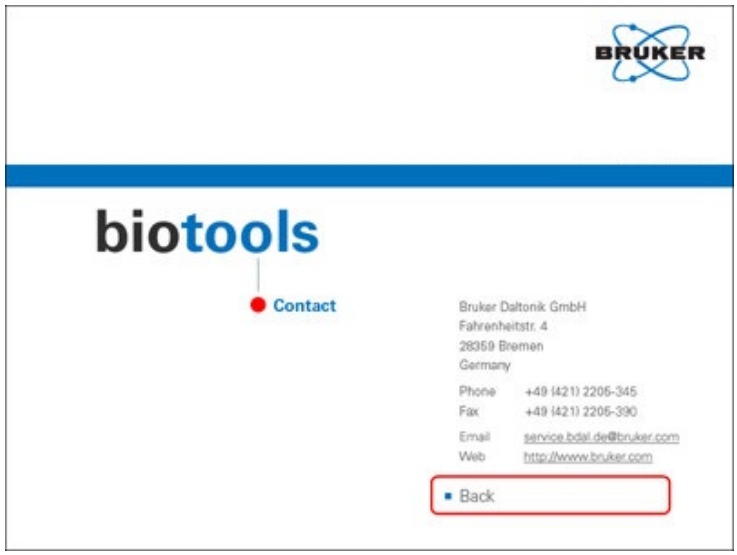


Figure 1-17 Choosing "Back"

s. To leave the launcher click on "Exit".

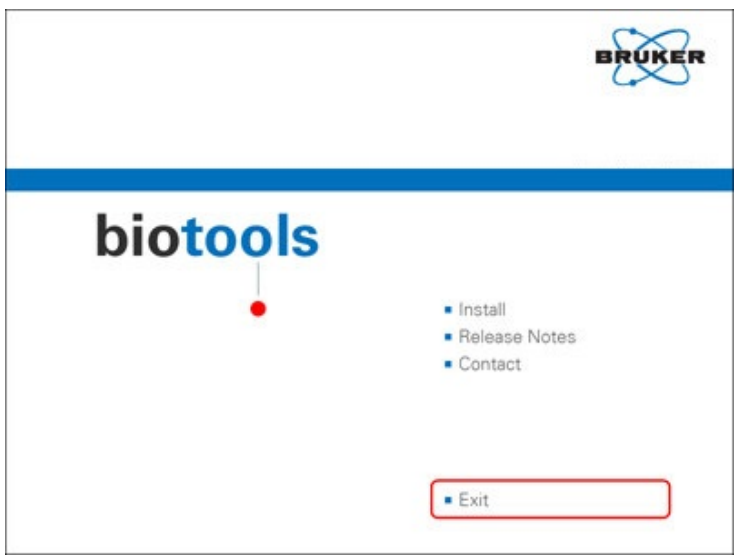


Figure 1-18 Choosing "Exit"

1.5 Getting Started

a. Open BioTools 3.2 with a double-click on the desktop icon  .

b. If the Bruker Daltonics Compass Security Pack (CSP) is not installed the following dialog appears and asks for an operator name (can be chosen arbitrarily).

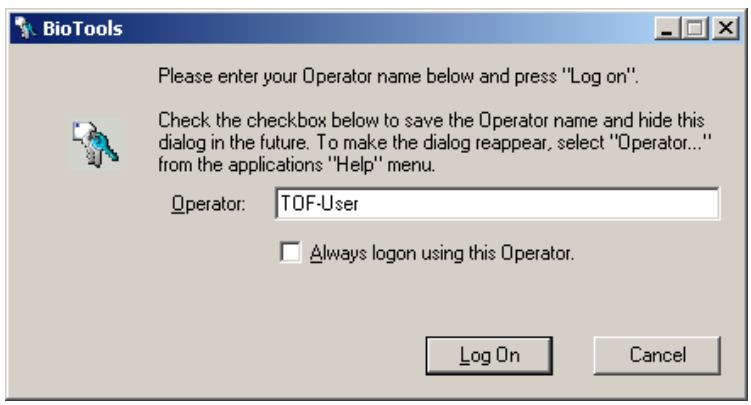


Figure 1-19 Login dialog when CSP is not installed

Otherwise, if the CSP is installed, the following dialog appears. Please ask your Bruker Daltonics CSP administrator for your login and password. Note that BioTools 3.2 SR5 needs a license for CSP 1.2 to use CSP 2.0, i.e. BioTools will ignore an installed CSP 2.0 if no CSP 1.2 license is available (so both licenses need to be added using menu Compass/License..).

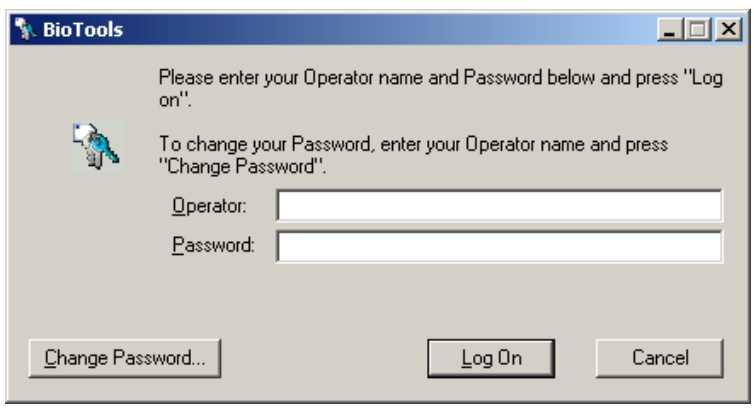


Figure 1-20 Login dialog when CSP is installed

c. After BioTools is started, at first the following dialog appears and asks for the URL of a possibly available local Mascot server. Click "Now" to enter the URL. This is strongly recommended if you want to run Mascot Batch Searches.

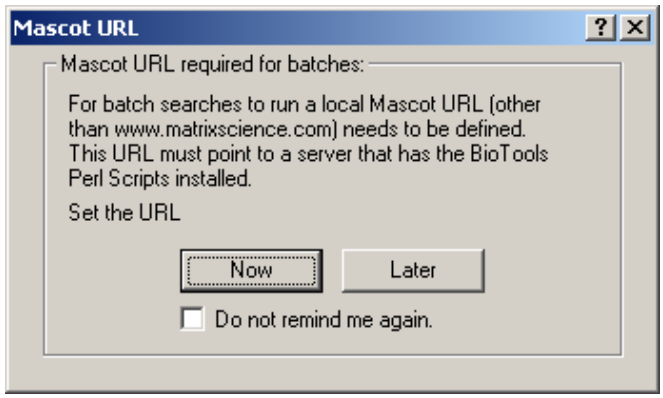


Figure 1-21 Setting URL of local Mascot server

d. In the next dialog click "Edit" and then **add** the Mascot server address (make sure the URL is entered correctly, with a standard Mascot installation only the server name needs to be entered in place of 'Enter Your Server Here'):

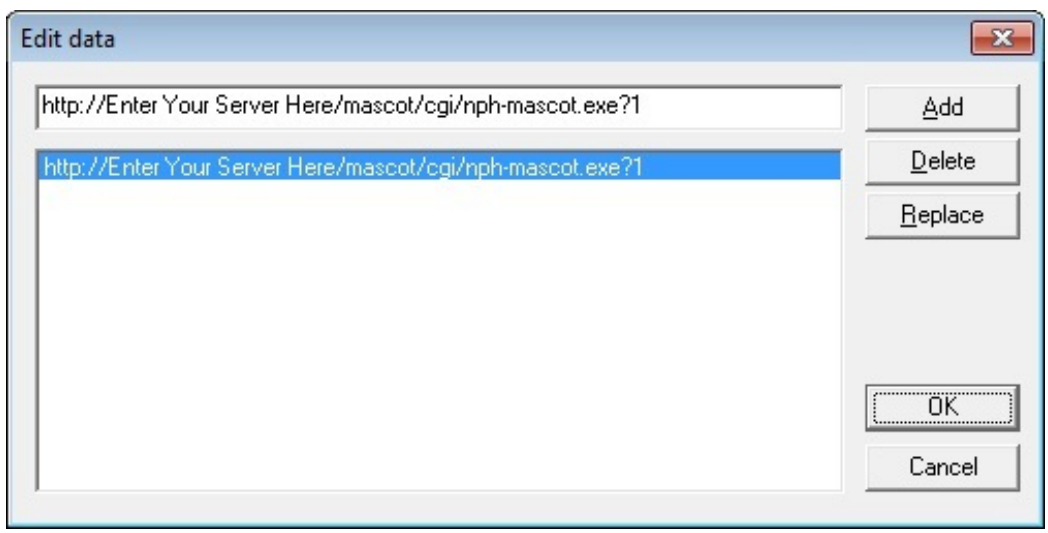


Figure 1-22 Adding Mascot server address

e. To import your old methods from BioTools 2.x open the Batch Mode dialog with the button  , switch to the "Method/Mail" tab and click the button "Import old method".

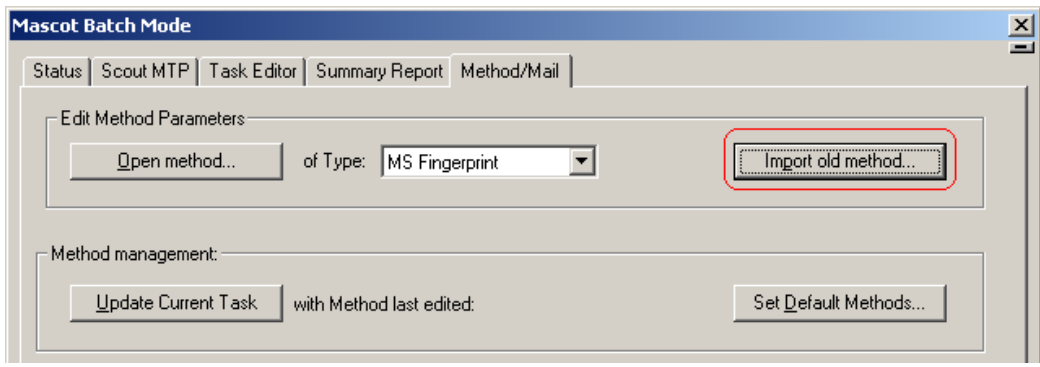


Figure 1-23 Choosing "Import old method"

f. Browse to the file **batch.ptr** that you saved in the beginning, probably to the directory D:\Bruker_Service and load it.

g. The methods you worked with in BioTools 2.x are offered in the drop down list.

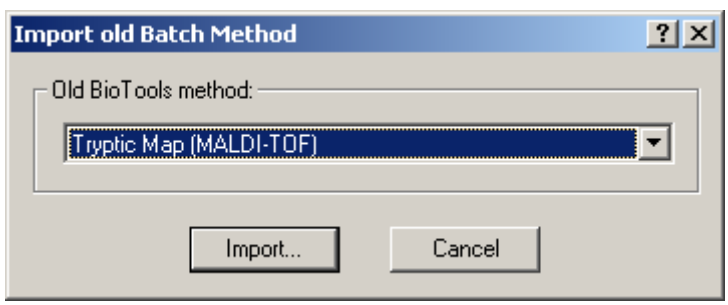


Figure 1-24 Selecting old method for import

Select them, respectively, and choose "Import" to open them in the new method editor where they can be saved as BioTools 3.2 methods.

1.6 Update Perl Scripts

BioTools 3.2 can be run without installing Perl scripts from the CD when it is used with current versions of Mascot (2.1 or later). With earlier Mascot versions, make sure that the new **Perl scripts**, coming with the BioTools 3.2 installation CD, are installed **on the server (!)** before a batch mode search is started the first time. Otherwise, there is no guarantee for correct communication between BioTools and the Mascot server.

a. Put the BioTools installation CD in the CD-ROM drive, wait for the launcher interface and choose "Install".

   If the launcher interface does not open automatically, choose "CD_Start.exe" from the BioTools CD

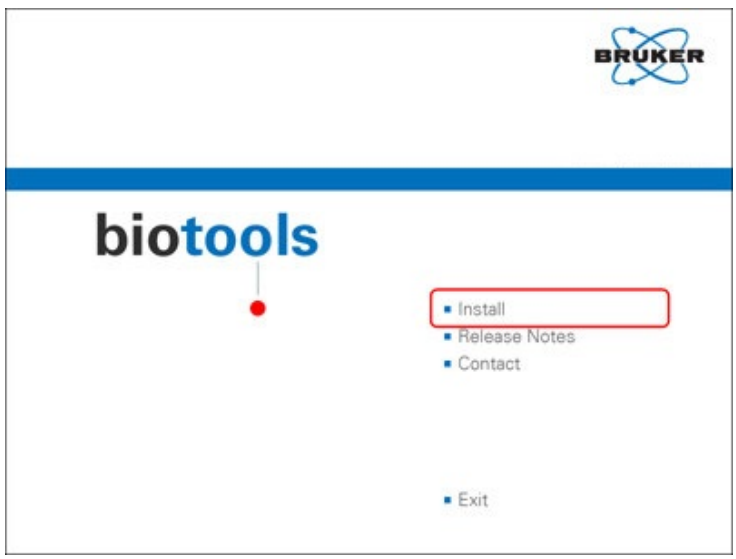


Figure 1-25 Choosing "Install"

b. Choose "Perl Scripts" and perform the installation as known, fulfill all necessary settings.

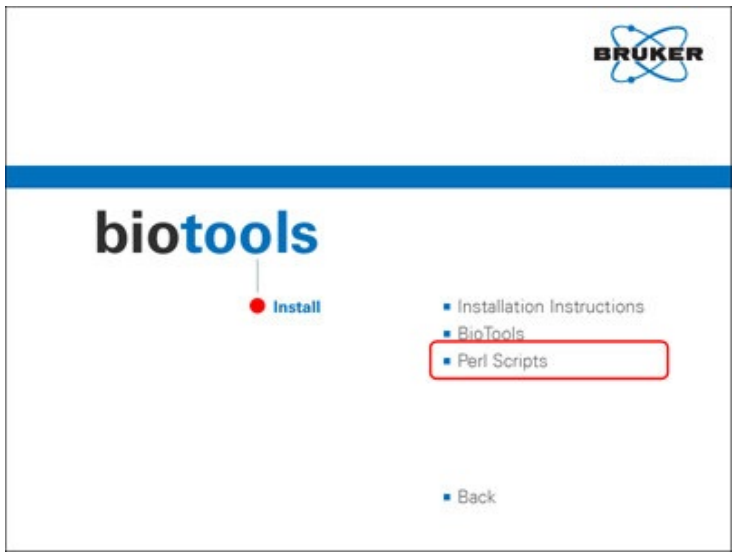


Figure 1-26 Choosing "Perl Scripts"

c. Select **Mascot Perl Scripts** and change the installation path.

d. After the necessary settings have been done click "Next" and "Install" to finish the installation.

Note: ***If the old Mascot server is installed on a Unix or Linux system, choose 'Perl Scripts' from the launcher interface and copy the offered scripts to the directory where the mascot-exe file is already available (probably /usr/local/mascot/cgi). Please keep in mind that we do not offer support for this.***

1.7 Starting the Program

Use the BioTools Icon from the program group you specified during program installation. If you receive the error message "A procedure entry point httpsendrequestExA could not be located in the dynamic linked library" during program start, the Microsoft Internet Explorer 6.0 or better is not installed on your system. The installation of the Microsoft Internet Explorer 6.0 or better will replace an existing WININET.DLL by a newer one.

BioTools will be installed with a temporary 60-days license key.

To avoid the popup, enter your appreciate license key for BioTools delivered be Bruker Daltonik GmbH. You will reach the LicenseManager via the menu **Compass** → **License**.

1.8 LicenseManager

After the 60-days temporary license key has expired you must enter a license key for BioTools and optionally for RapiDeNovo. The key(s) comes together with the BioTools documentation. Use the license manager to add or remove license keys (menu **Compass** → **License**).

If an invalid license key is entered, the program cannot be used.

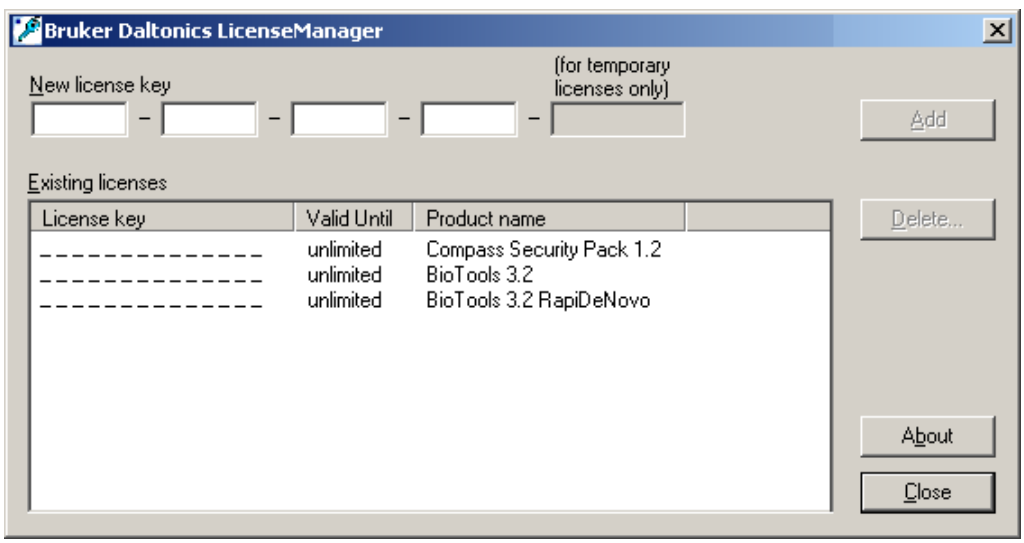


Figure 1-27 LicenseManager with permanent BioTools 3.2 licenses

1.9 Service Information

We will help you in the Support as precisely as possible. Therefore it is helpful to have the following information ready when contacting us:

- the full version number of the software that causes problems, i.e. version and build number (menu **Help** → **About**)
- try to describe exactly what you tried/want to do, or
- try to describe what happened and/or what you did before the error occurred
- also screenshots of error messages are highly appreciated

For questions, please contact:

service.bdal.de@bruker.com

2 QUICK GUIDE TO BIOTOOLS 3.2

2.1 Introduction

BioTools is a protein analysis package for Bruker Daltonics MALDI and ESI MS instruments.

BioTools work is applied to data (MS- and MS/MS-spectra and peak lists), which were previously processed using either flexAnalysis or DataAnalysis.

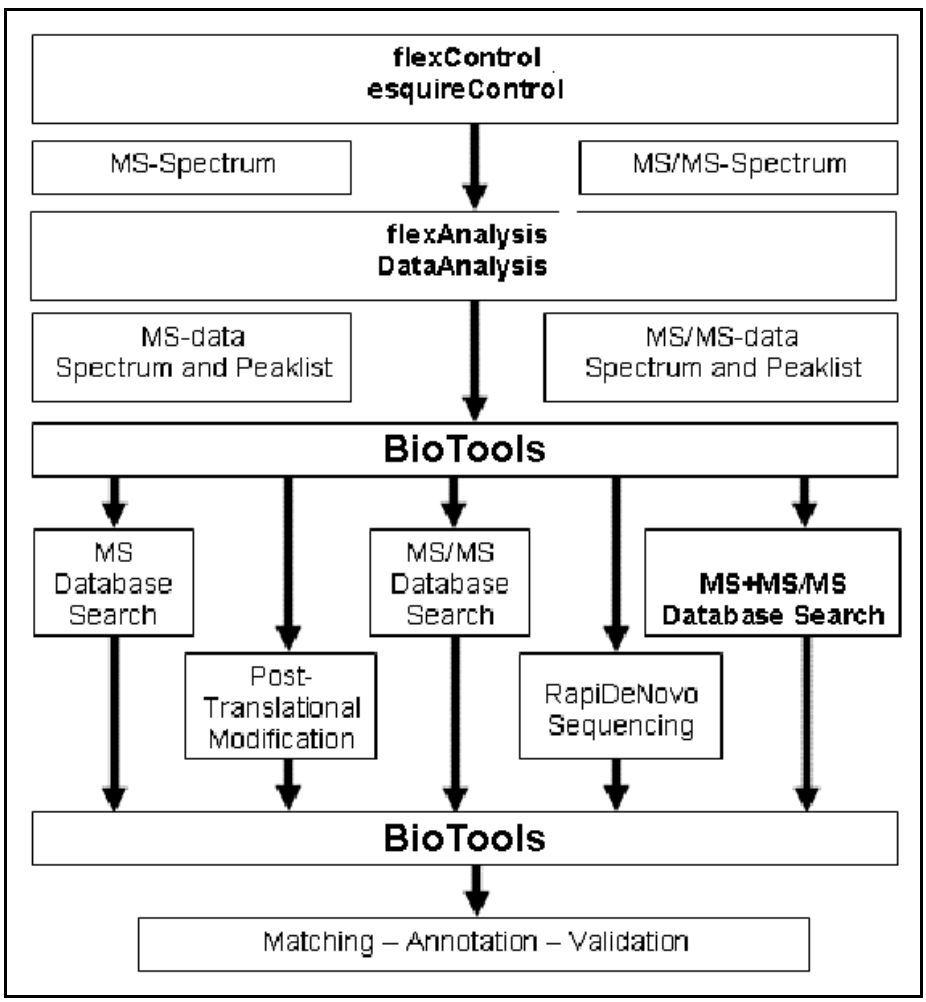


Figure 2-1 Protein Digests analysis flow

Initial sequence analysis can either occur through protein database searching using external search software such as Mascot or through SequenceEditor that allows entering the covalent protein structure including modifications and crosslinks in BioTools. A sequence assignment can be further enhanced: Additional information like creak peaks or modifications can be identified in this process.

2.1.1 What is New in BioTools 3.2 and 3.2 SR1-5?

Get updated on the new features of BioTools 3.2 and BioTools 3.2 SR1-5 in a breathtaking PowerPoint presentation "What´s new in BioTools 3.2" that you can access via menu **Help** → **What´s new in BioTools 3.2**.

2.1.2 Application Tutorials for BioTools and SequenceEditor

Application tutorials teach you various applications on various Bruker Daltonics mass spectrometers. They allow you to practice applications using tutorial data that is supplied with the BioTools 3.2 package.

2.2 Getting Help

1. Use the context sensitive help while you work: Press the F1 function key to access the software manual at exactly the relevant location for your problem.
2. Access the various application tutorials through this document that guide you through a number of applications from the sample preparation to final data analysis.
3. Get updated on the new features of BioTools 3.2 and BioTools 3.2 SR1-5 via menu **Help → What´s new in BioTools 3.2**.
4. Call or email the software support:

Bruker Daltonik GmbH
Fahrenheitstr. 4
D-28359 Bremen
Germany

Phone: +49 (4 21) 22 05-345
Fax: +49 (4 21) 22 05-390
E-Mail: service.bdal.de@bruker.com
Internet: www.bruker.com

2.3 Loading Processed Data

First the spectra must be processed in the Bruker software flexAnalysis 2.4 (or higher) or DataAnalysis 3.4 (or higher). Additional peak picking can be done in BioTools. It is also possible to export the processed data directly from flexAnalysis or DataAnalysis into BioTools (see flexAnalysis and DataAnalysis tutorials).

Note: ***When DataAnalysis 4.1 or higher was used to process the data set to be opened in BioTools the DataAnalysis 4.* software needs to be installed locally (license key is not necessary) for BioTools to read profile spectra for (xml/baf/yep) files.***

There are three ways to open processed data sets in menu **File**.

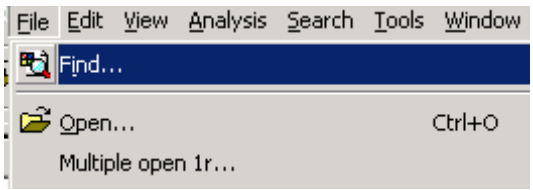


Figure 2-2 Load data: File menu

Open… will open one selected data set of the type specified in the drop down box on the File Open dialog.

And in **Find…** it is possible to search for types of data and to open one and more data.

Multiple open 1r… is a good option to open all 1r data of one folder included or excluded its subfolders.

When **Open Spectrum…** and **Find…** will be used a second time, the first time will be recollected. The path to the data or respectively the list of files that were found before are displayed again.

In this example go to menu **File/Find…**.

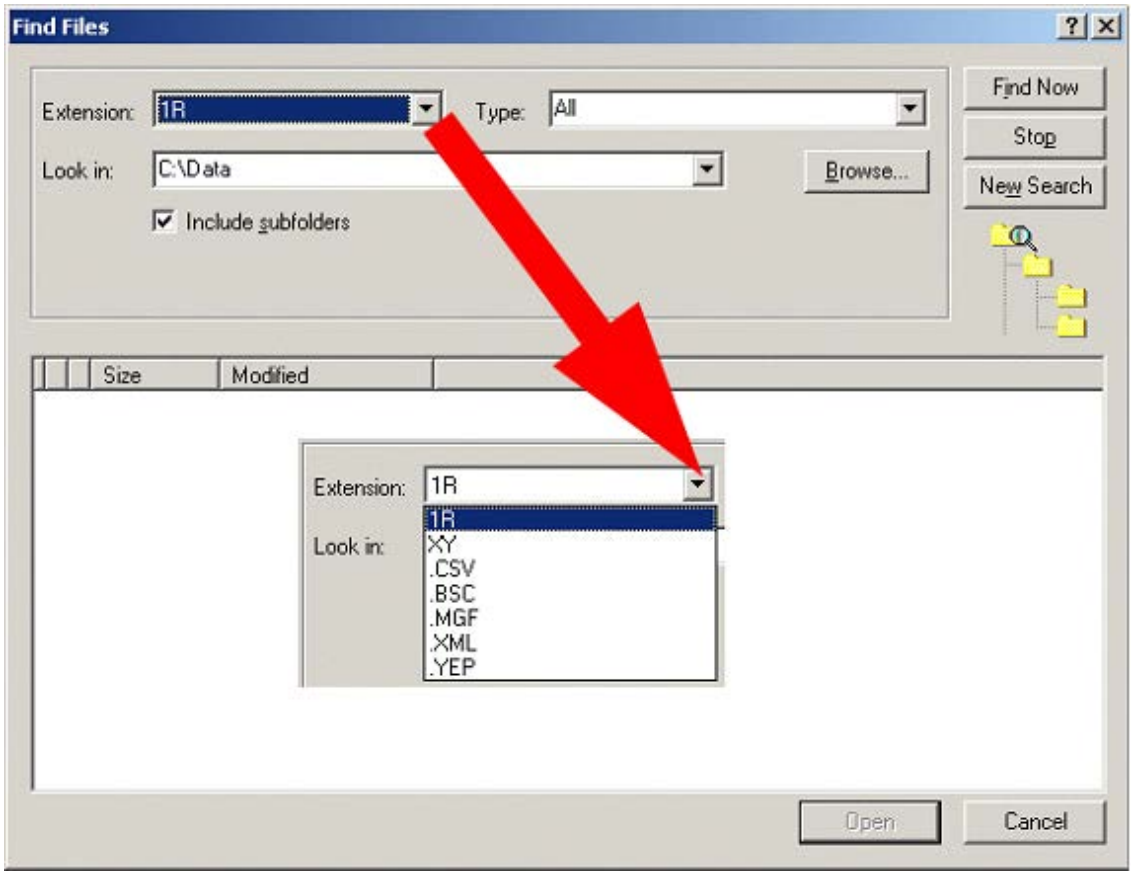


Figure 2-3 Load data: Find files with all possible file names

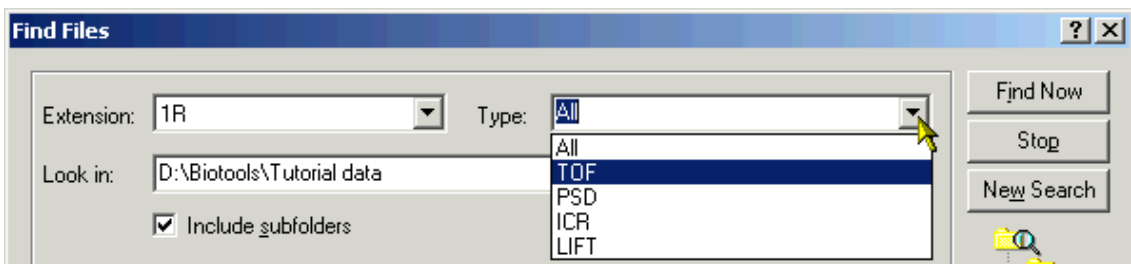


Figure 2-4 Load data: File types for 1R

Different files are possible to load for different instruments:

MALDI-TOF

PMF	1R	All, TOF
MS/MS	1R	PSD, LIFT
combined	.XML	TOF + nLIFT combined peaklist

esquire/HCT

YEP/.BAF	(Necessary to save results in DA before.)
.XML	DataAnalysis-processed LC MS(n)-Compounds (profile spectra will be shown with DataAnalysis 3.4 and higher)
.MGF	(No raw data will be shown.)
.BSC	(BioTools 3.2 also supports this older file format.)

micrOTOF, micrOTOF-Q, maXis

YEP/.BAF	(Necessary to save results in DataAnalysis before.)
.XML	DataAnalysis -processed LC MS(n)-Compounds (profile spectra will be shown with DataAnalysis 3.4 and higher)
.MGF	(No raw data will be shown.)
.BSC	(BioTools 3.2 also supports this older file format.)

FTMS

.YEP/.BAF	(Necessary to save results in DataAnalysis before.)
.XML	DataAnalysis -processed LC MS(n)-Compounds (profile spectra will be shown with DataAnalysis 3.4 and higher.)
.MGF	(No raw data will be shown.)
.BSC	(BioTools 3.2 also supports this older file format.)

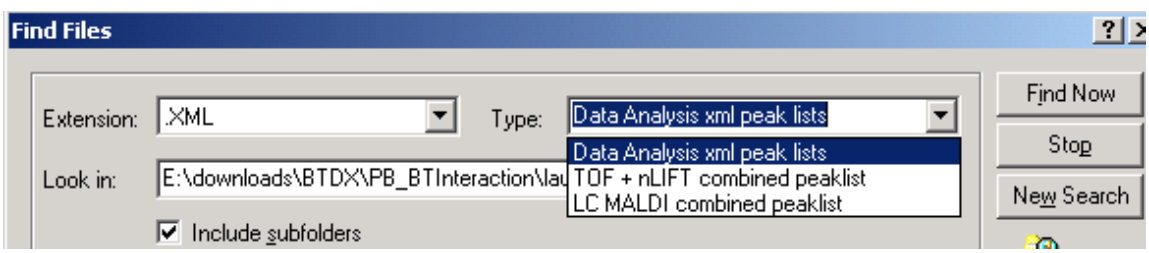


Figure 2-5 Load data: File types for .XML

Browse to the shown path after "Look in". This path is the folder where BioTools was saved during program installation. Subfolders will be included for the search also because a tick is set for "Include subfolders". Click on the button Find Now to start the search. All combined MALDI-TOF data sets of this folder are shown in the table underneath.

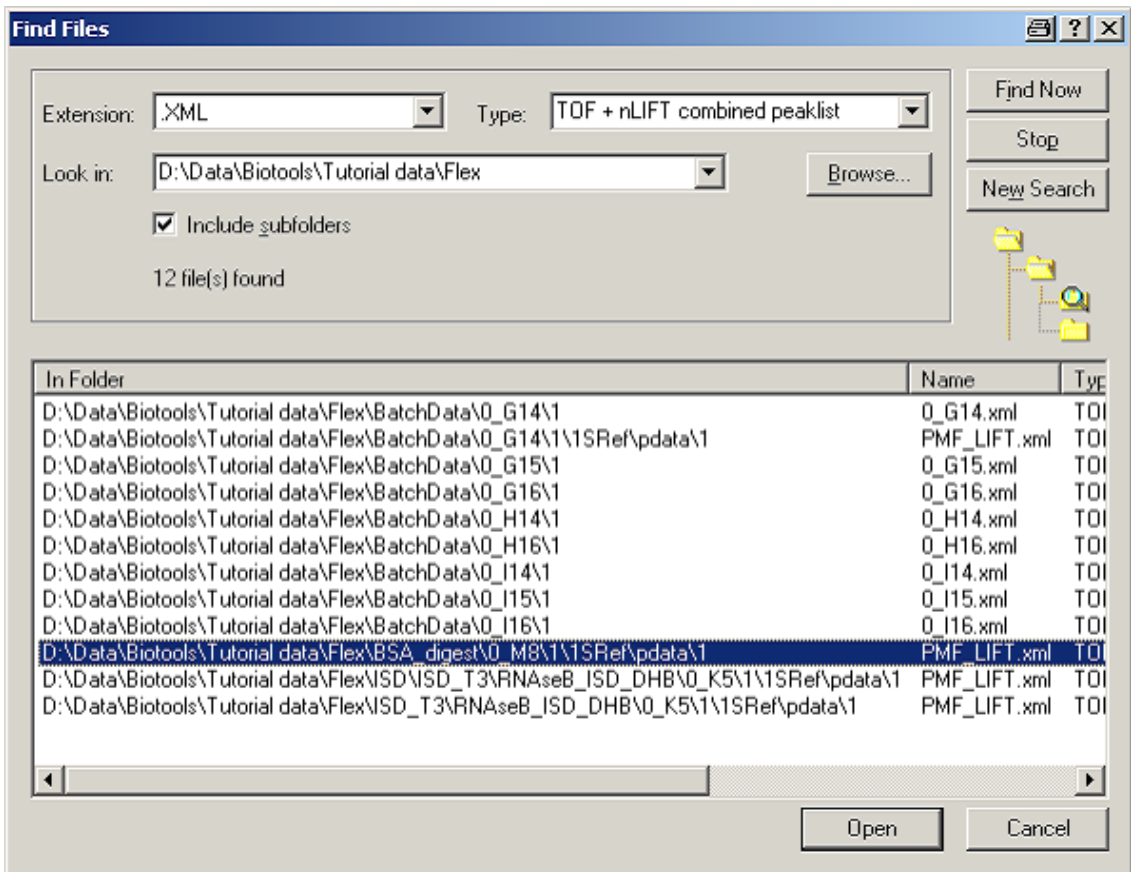


Figure 2-6 Load data: Select one or more of the found files

From the list of found files select the entry as shown in Figure 2-6 and load it using the button Open.

Load a second file for the navigating operations in BioTools. Go to menu/ Find Files, choose 1R and TOF as shown in Figure 2-4, select "BSA digest LysC" and open this or another MS-spectrum.

2.4 Navigating in BioTools

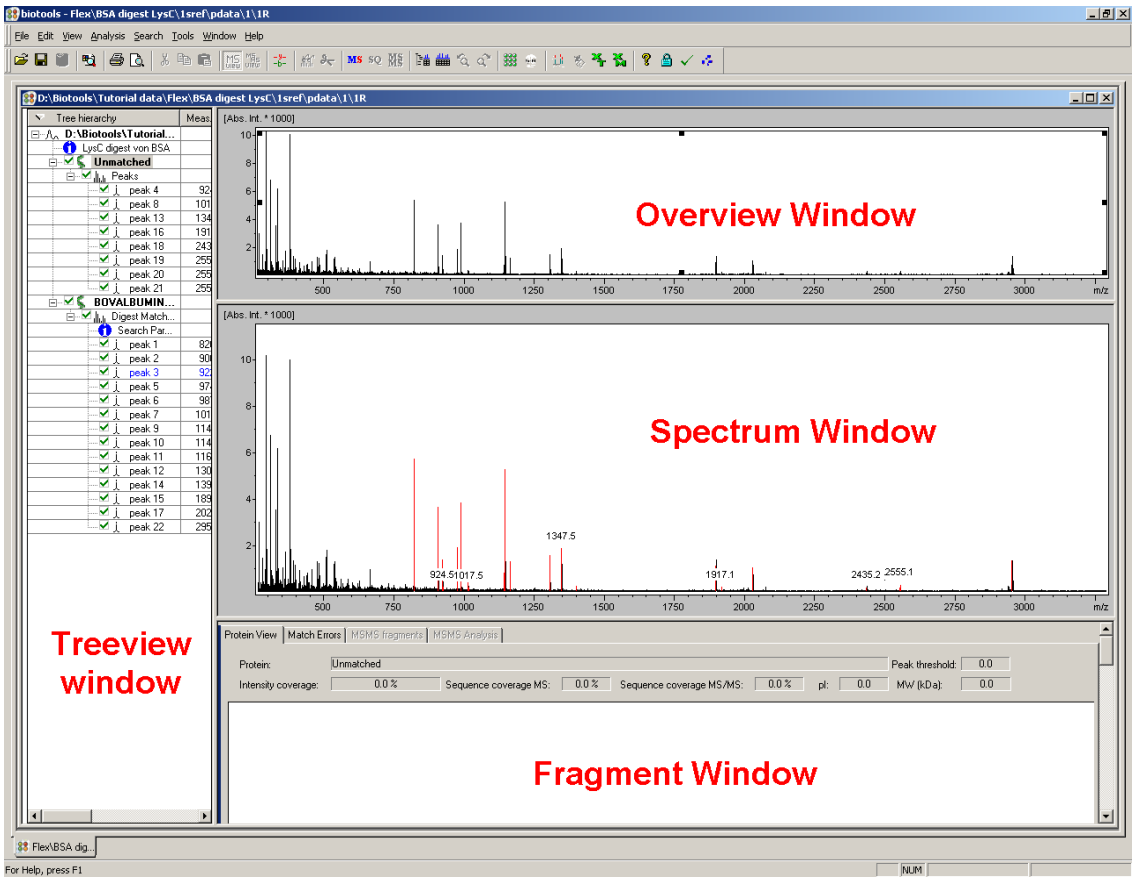


Figure 2-7 BioTools main display

BioTools shows the processed data in four windows. Some windows can be hidden using the toolbar buttons Show/Hide Treeview and Show/Hide Fragments. Each window can be changed in size. Move the mouse between two windows, the shape of the cursor will change to press the left mouse button (LMB) and drag the boundary to the desired position.

For more details on the toolbar buttons see chapter 3.3 Toolbar Reference and Shortcut List for BioTools.

The Treeview window on the left displays the peak list and already all matches to protein sequences. Before using the database searches in BioTools all masses of the peaks are listed under "Unmatched" peaks.

The upper window is the Overview window that always displays the complete spectrum. The lower window is the Spectrum window and shows the selected range of the spectrum. At the bottom the Sequence or Fragments window is displayed (for details see the chapter 2.7 Overview on BioTools Window).

All peaks of the mass list are signed with a vertical red line in the spectrum and the masses are annotated in black.

2.4.1 Zooming and Moving of Spectrum Views

In this chapter following descriptions will be used in a short form:

LMB Left mouse button

RMB Right mouse button

Drag Move the mouse cursor to a starting point, click LMB or RMB, go to the desired point and release the mouse button.

Note: After loading the spectrum, the Spectrum window shows the whole spectrum as in the Overview window. First, you have to zoom in the spectrum before you can zoom in the overview spectrum.

There are several ways to move and zoom within the spectrum.

2.4.2 Zoom Operation in the Spectrum Window

Drag LMB from the upper left corner to the bottom right of a rectangle like it is shown in Figure 2-8. The Overview window will frame the detail of the spectrum with a black rectangle (Figure 2-9).

Note: A single LMB click will reverse the last operation.
A double LMB click within the Spectrum window will reset the display to the whole spectrum.

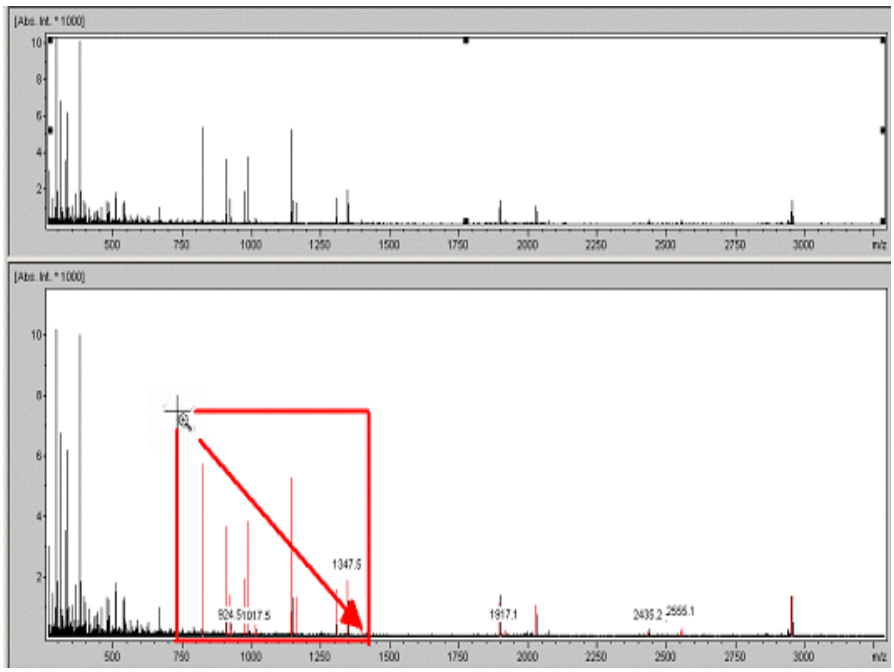


Figure 2-8 Zooming in spectrum

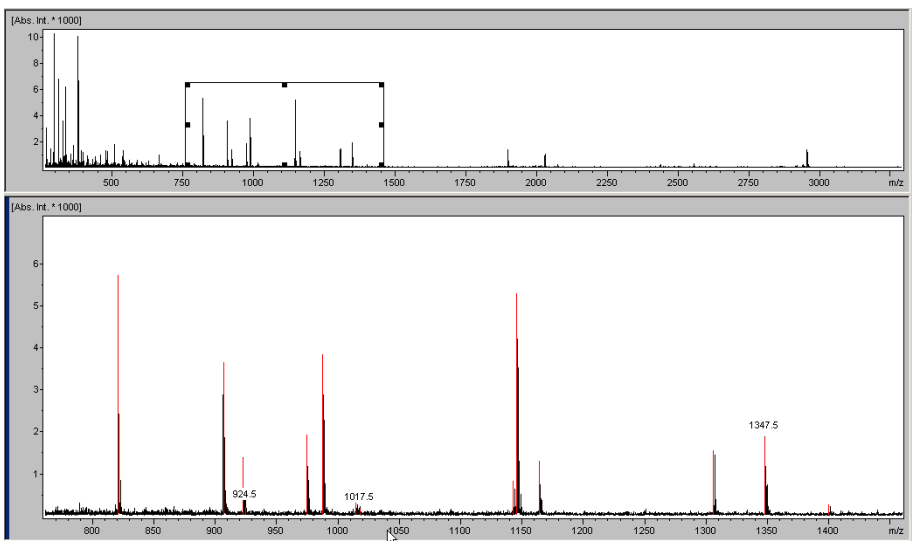


Figure 2-9 Zooming in spectrum, finished

The next figure and the table show all the possibilities to move and zoom in the spectrum. Navigate in the zoomed spectrum of Figure 2-9. Any operation in either the Overview window or the Spectrum window will set the context to the particular window.

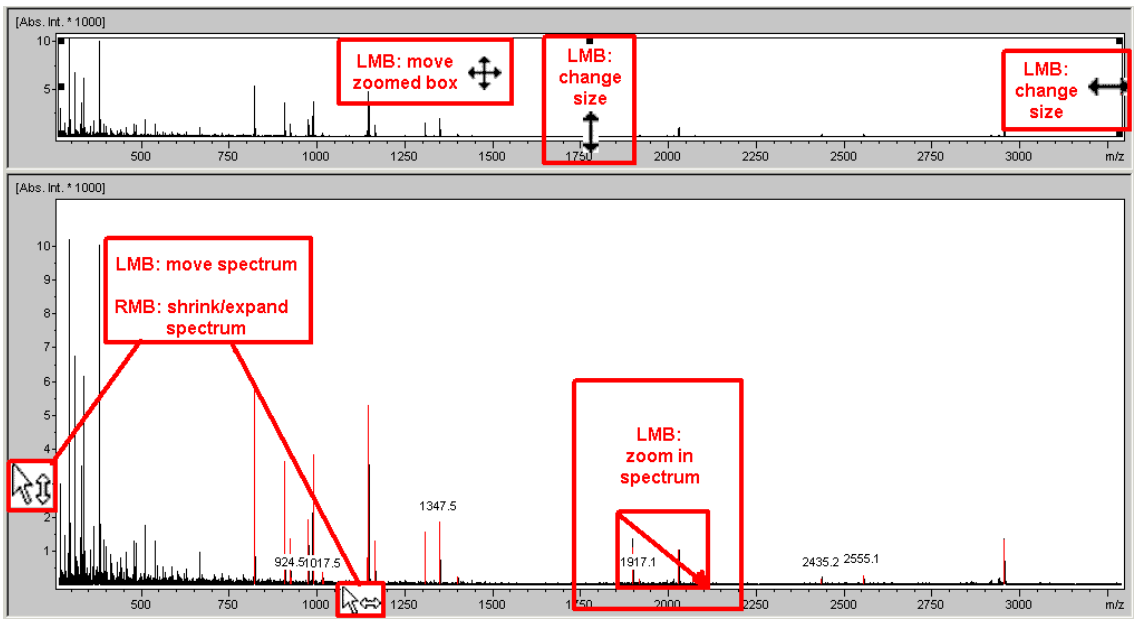


Figure 2-10 Operations for moving and zooming

2.4.2.1 Use Cursor Keys to Move the Zoom Range

Additional navigations are possible only by using following keyboard buttons, **provided the context is on the Overview window resp. the Spectrum window** (blue vertical bar on the left side, Figure 2-9 shows the context being on the Spectrum window):

	← → ↓ ↑	move	in 90% box size step
Shift	← → ↓ ↑	move	in 10% box size step
CTRL	← → ↓ ↑	shrink/expand	in 90% box size step
CTRL+ Shift	← → ↓ ↑	shrink/expand	in 10% box size step

2.4.2.2 Zoom onto Predicted MS/MS Fragments

A LMB click on a fragment mass in the tab MSMS fragments will zoom on the fragment ion in the spectrum.

For example load a combined PMF_LIFT spectrum.

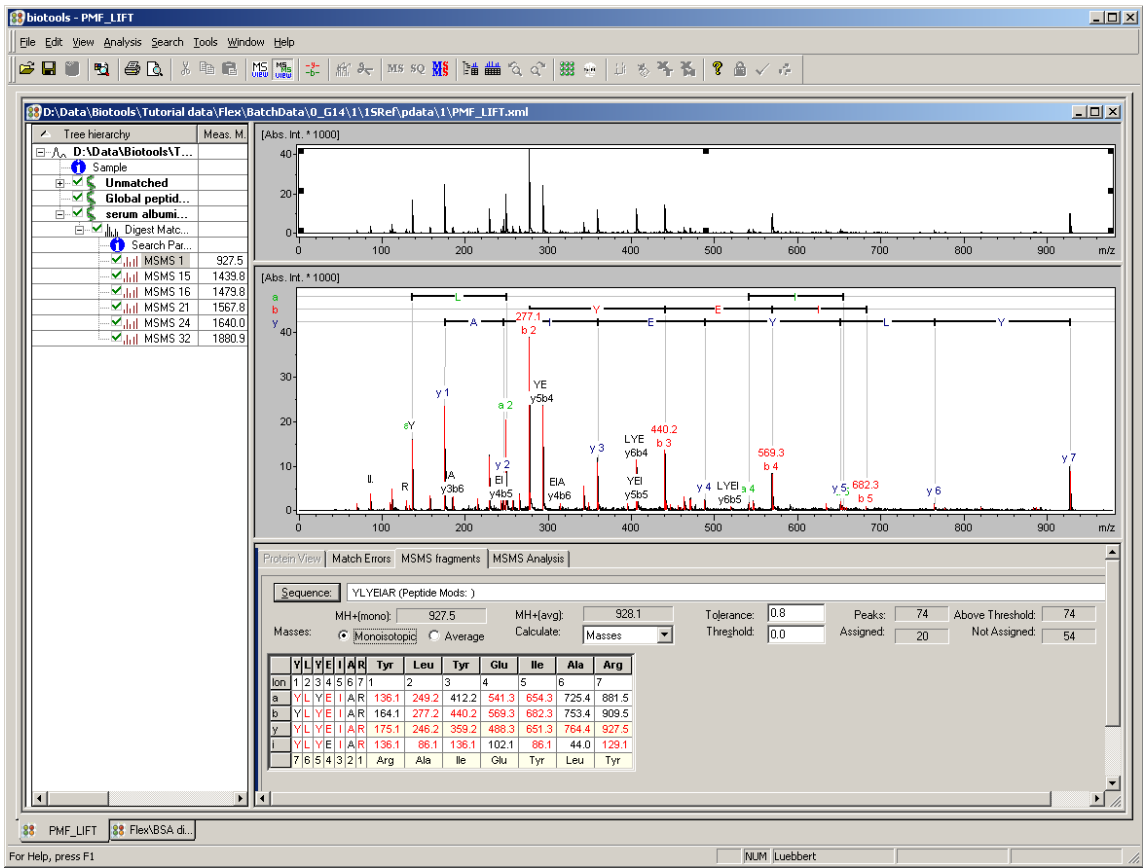


Figure 2-11 Zooming in on a fragment ion

Select MSMS1 in the treeview to get the fragments of this MS/MS spectrum. If the Protein view is still displayed click on the button . The MSMS fragments tab will be displayed Figure 2-11).

Click on the y1 fragment in the table (see mouse cursor) and the spectrum zooms in on this fragment ion Figure 2-12.

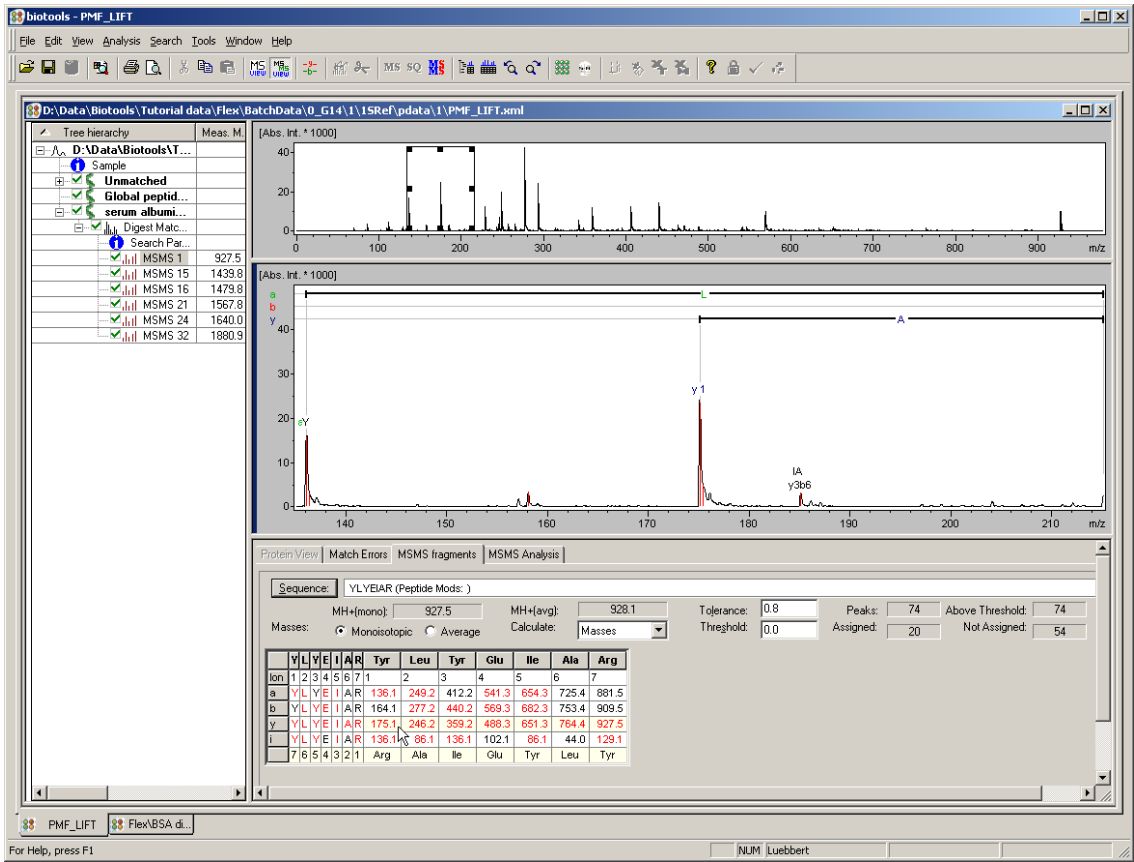


Figure 2-12 Zooming in on a fragment ion, finished

2.4.3 Edit Peak List

Picked peaks of the peak list are displayed with a red vertical line in the spectrum. To edit peaks in BioTools there are two features.

Manual peak picking:

Peaks can be manually picked or deleted by pressing the Ctrl key and dragging the mouse with LMB pressed across a mass range (Figure 2-15). The maximum intensity peak will be affected in the drag range. Therefore in crowded parts of the spectrum, better zoom in on the peaks of interest first. Alternatively, the context menu entry "Edit Peaks" can be used to enter the manual peak editing mode until the RMB is clicked in the spectrum.

With BioTools 3.2, the algorithm used for adding peaks in the manual peak editing can also be specified by using the menu entry "Configure Edit Peaks..". The Maximum algorithm is useful for arbitrary additions of peaks while the Sum PeakFinder should be used for micrOTOF-Q/maXis spectra (BioTools will attempt to use the same parameters as were previously used in DataAnalysis 3.4).

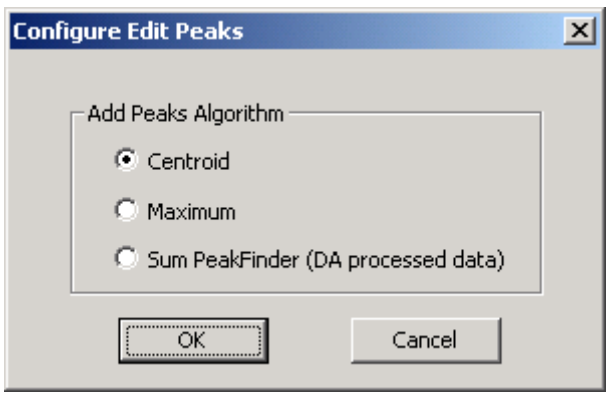


Figure 2-13 Zooming in on a fragment ion, finished

Note: ***If there are any problems in editing very narrow peaks, just click the peak directly (Ctrl + LMB). The "minimal mass difference" between peaks could be edited in the "Peak Finder" dialog, see Figure 2-16.***

Peak picking is often useful after a database search to align fragments which were not labeled.

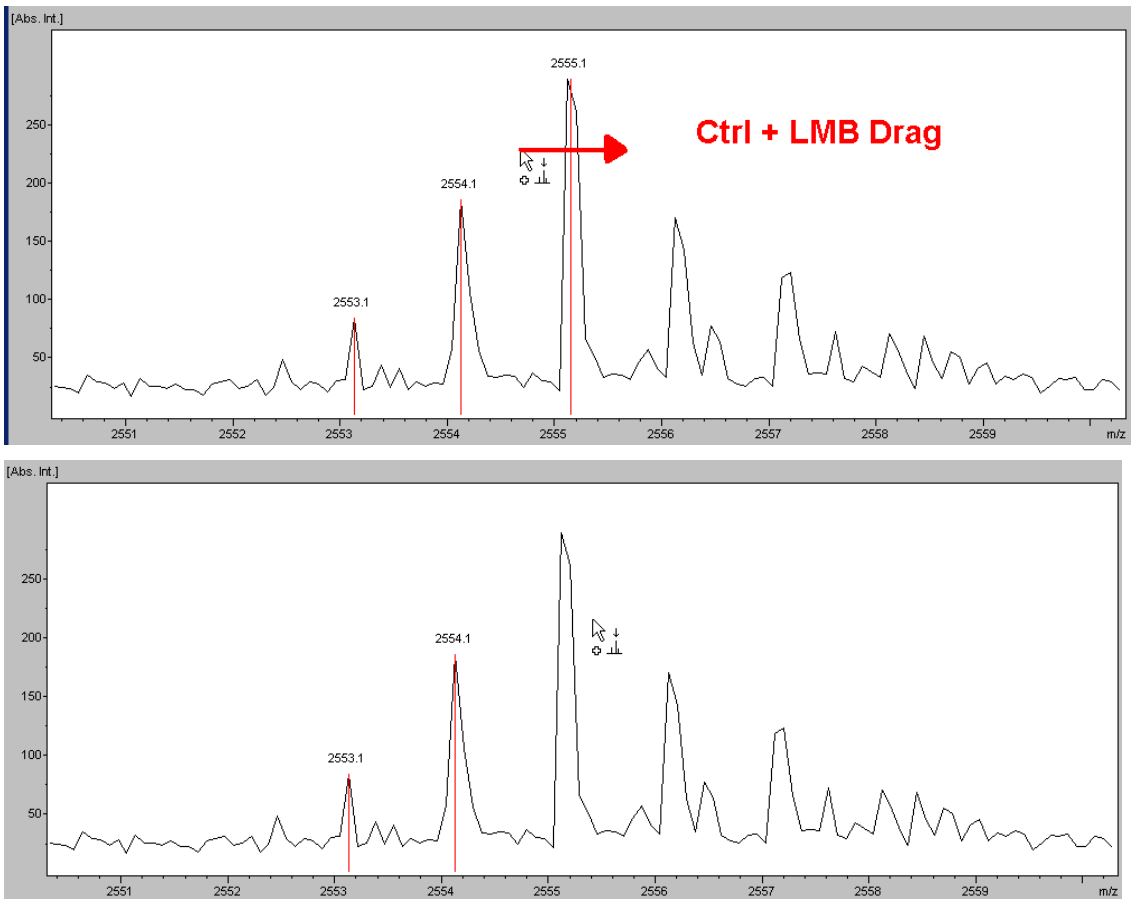


Figure 2-15 Manual peak picking/deleting

Automatic peak picking of a region or the entire spectrum:

Zoom in the region where the peaks should be picked again. Click on RMB and the context menu select "Peak Picking". The "Peak Finder" dialog (Figure 2-16) shows the parameters which can be changed for the automatic picking. Already picked peaks will be obtained. But it is possible to delete all peaks. The region for changing is the visible spectrum or the range which can be typed in.

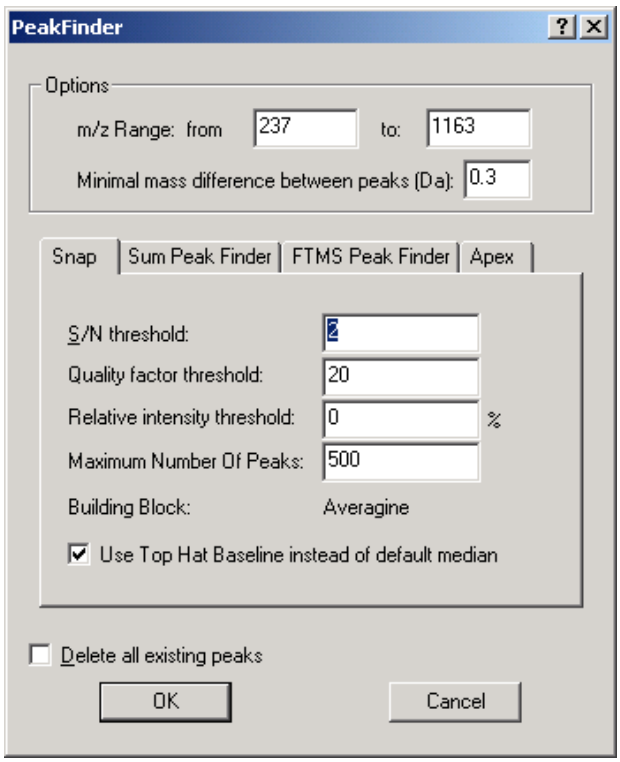


Figure 2-16 Automatic Peak Picking

The **PeakFinder** settings are identical to those in the flexAnalysis or DataAnalysis programs, except that some options are not available here.

2.5 Working with Combined MS + nMS/MS Data Sets

This chapter will give a short representation on how to work with BioTools especially for combined data sets. Detailed information about special topics you will find in the tutorials.

2.5.1 Combine Peaklist

An example is the data set of a PMF of BSA-digest and a number of LIFT-TOF/TOF MS/MS spectra from this sample. Load it as shown in Figure 2-6 or generate your own combined data set as described below.

Load exactly 1 MS spectrum and n MS/MS spectra as shown in Figure 2-17.

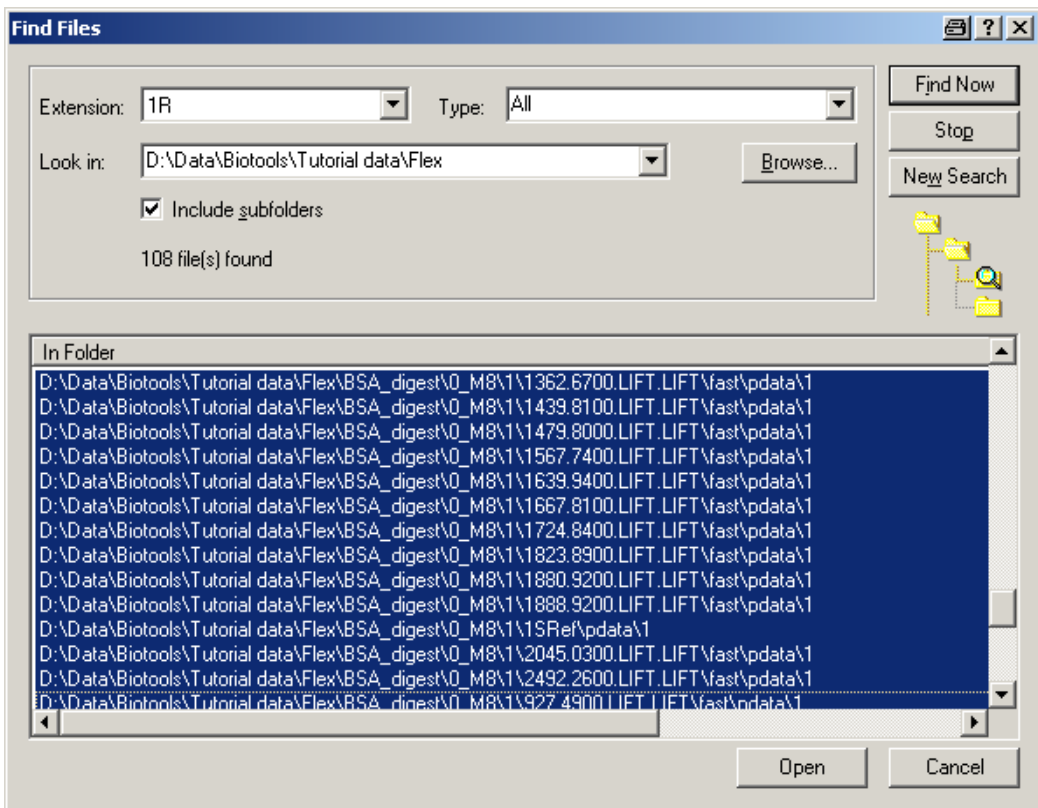


Figure 2-17 Load 1 MS and n MS/MS files into BioTools to manually generate a combined PMF+nLIFT data set

They can be analyzed now as individual data sets that are not related. In order to deal with them as a single combined data set that contains 1 PMF and n LIFT spectra, they need to be merged.

Combine the data: menu: **File/ Combine TOF + multiple LIFT spectra** (Figure 2-18).

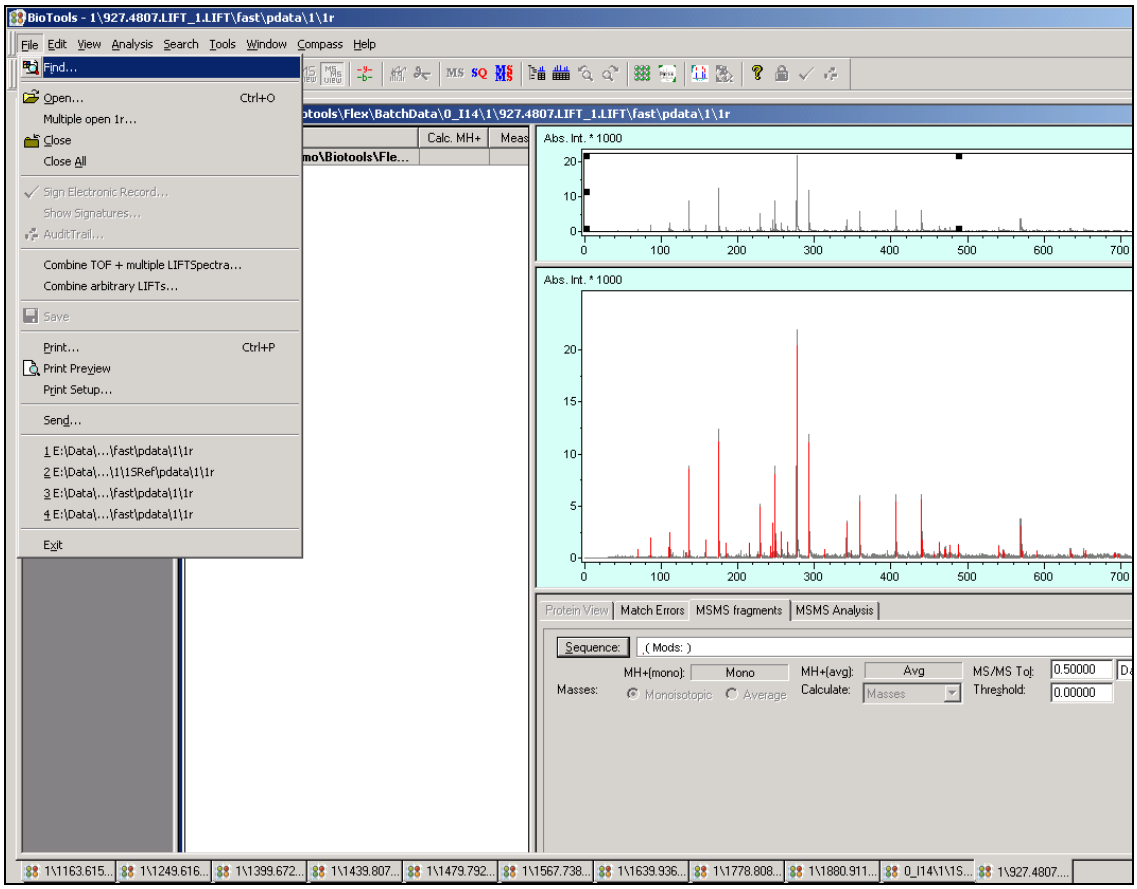


Figure 2-18 Combine multiple LIFT spectra

A dialog opens that lists all files currently open in BioTools (PMF_LIFT spectra need to be closed before you enter this dialog!).

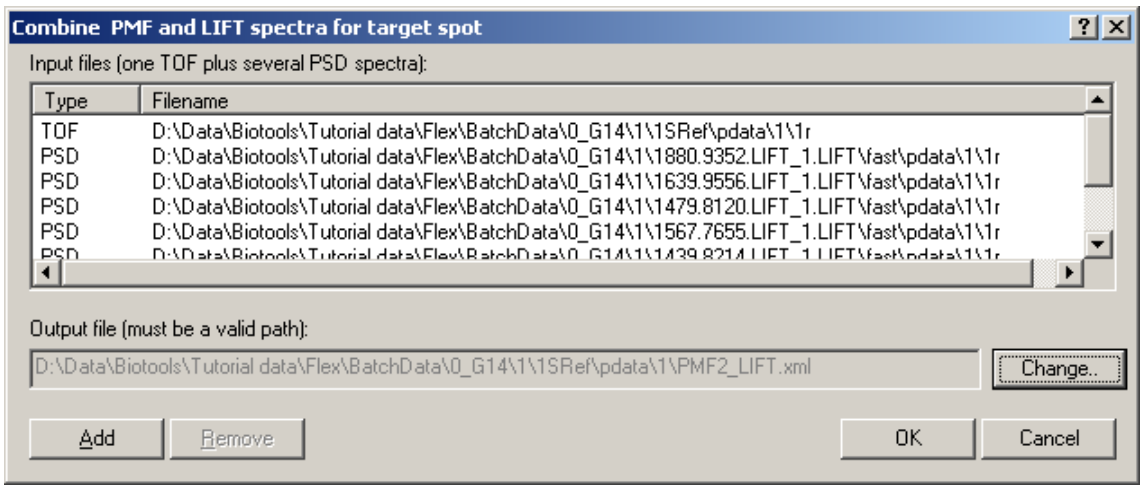


Figure 2-19 Select spectra

Remove open files of other samples currently open in BioTools by selecting them in the table and clicking on Remove. Change the "Output file" name if you want to save a second combined data set of the data from same sample position. Combine the listed files into that data set with OK

Note: Single MS and multiple MS/MS spectra can be combined. Even later, processed data can be added for improved results.

A new window PMF2_LIFT will show now the combined data set. Peaks of the MS spectrum and the done MS/MS spectrum of a peptide peak are listed in the treeview under "Unmatched" peaks.

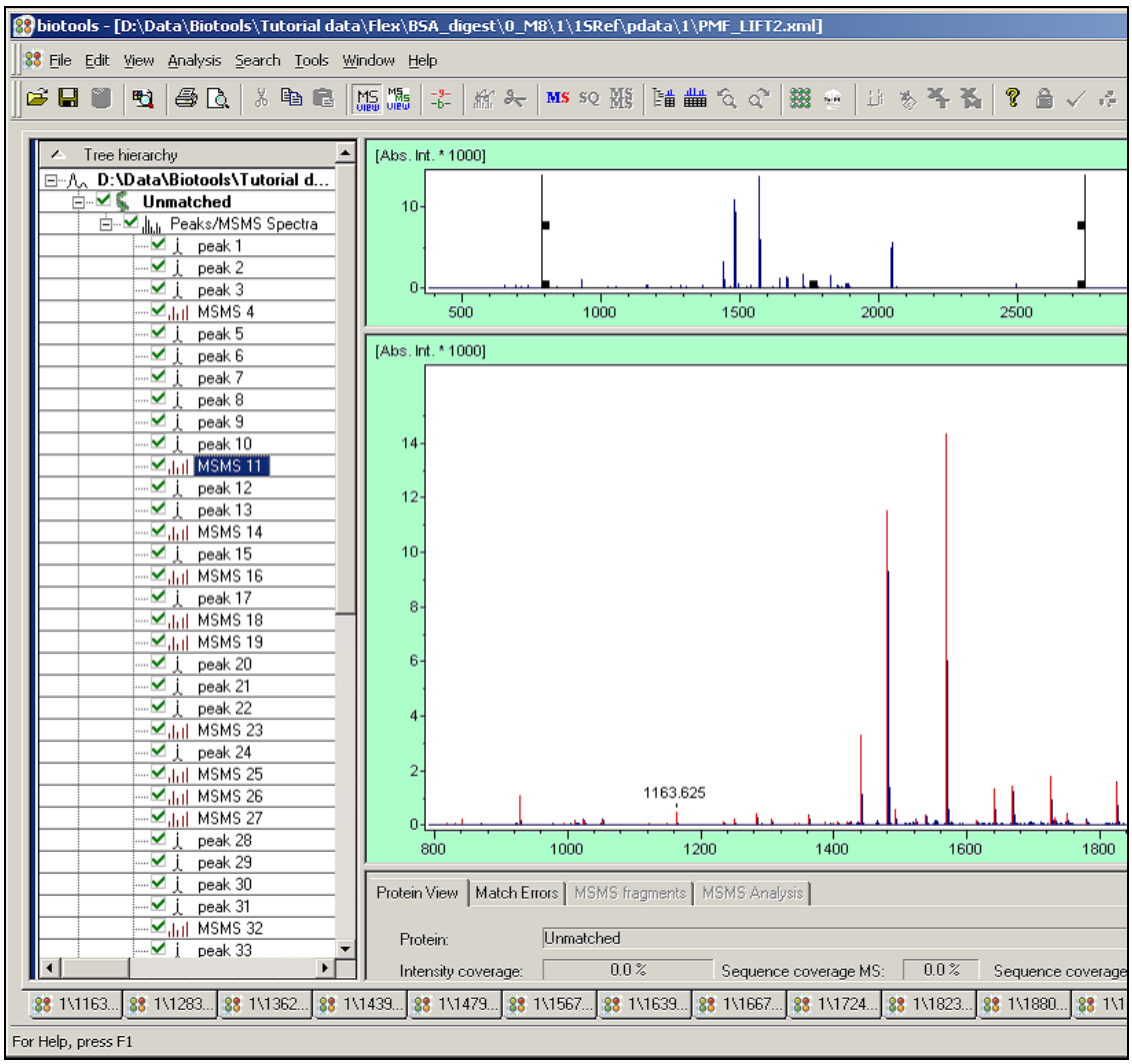


Figure 2-20 Combined data set: PMF_LIFT

2.5.2 MS and MS/MS View

With the toolbar buttons  and  you can switch between the display of the PMF type information/Protein Sequence Viewer (MS) and the Fragment Ion Spectrum of a selected treeview entry (MS/MS).

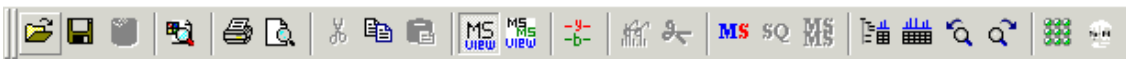


Figure 2-21 Toolbar detail

If the MS View was selected, a Mascot MS search can be done by clicking on  and after result import the whole protein sequence is displayed. If the MS/MS View was selected, a Mascot MS/MS search can be done by clicking on  . After result import MS/MS–fragment ions are assigned for each individual selected parent. For many samples and automated analysis use the batch mode that can be accessed through the  button (see also **Tutorial - Automated Processing of Multiple PMF Samples: WARP II, Batch Mode**).

2.5.3 Database Search in MS View (PMF Data)

In the MS view  click the  button to open the search dialog shown in Figure 2-22.

For details of Mascot query parameters use the F1-key context sensitive help or see the www.matrixscience.com online help.

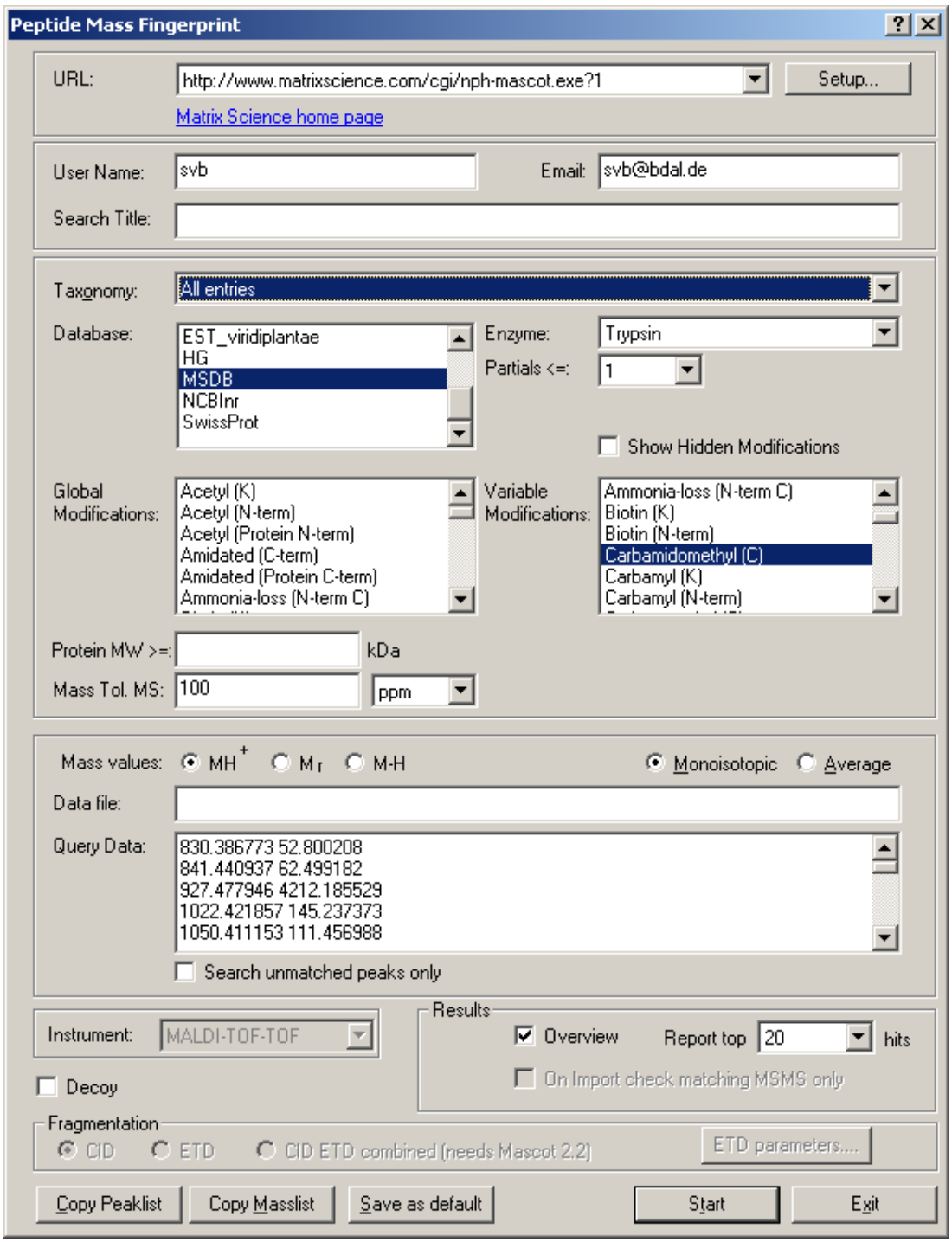


Figure 2-22 PMF search parameters

Adjust the search parameters and press Start. After some time the results of the query will be displayed in Query Results window, see Figure 2-23.

There will be an overview graphic of the significance, a list of the hits with links to the protein for more information and the search parameters at the end.

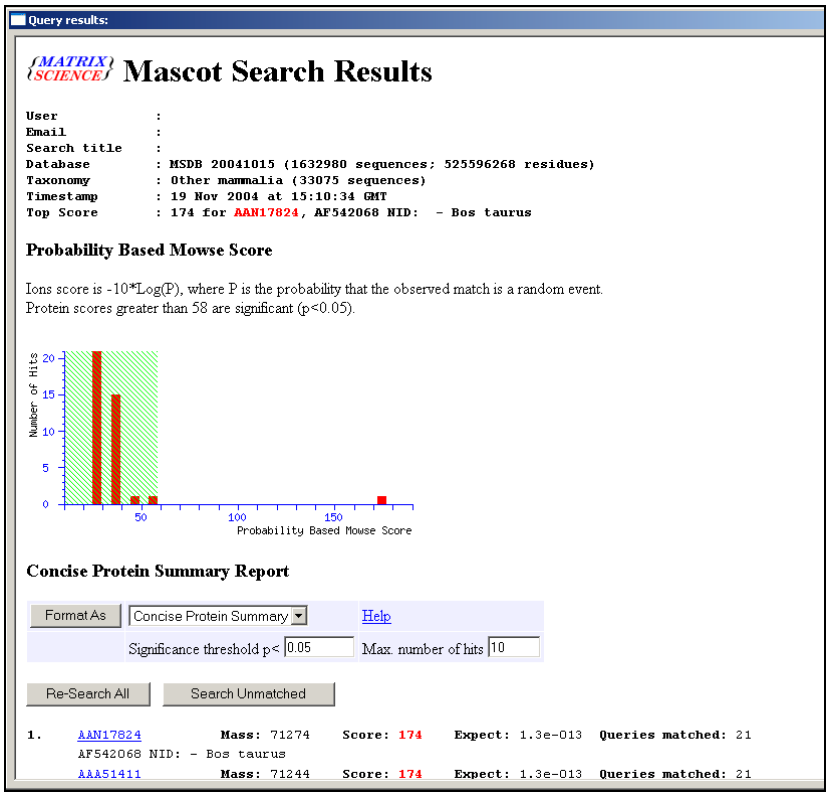


Figure 2-23 Results of MS search

Use the button Get Hit(s) to transfer the query results into BioTools. Number "1" means: get the top hit only. "1-3, 10" means: get hits 1-3 and 10.

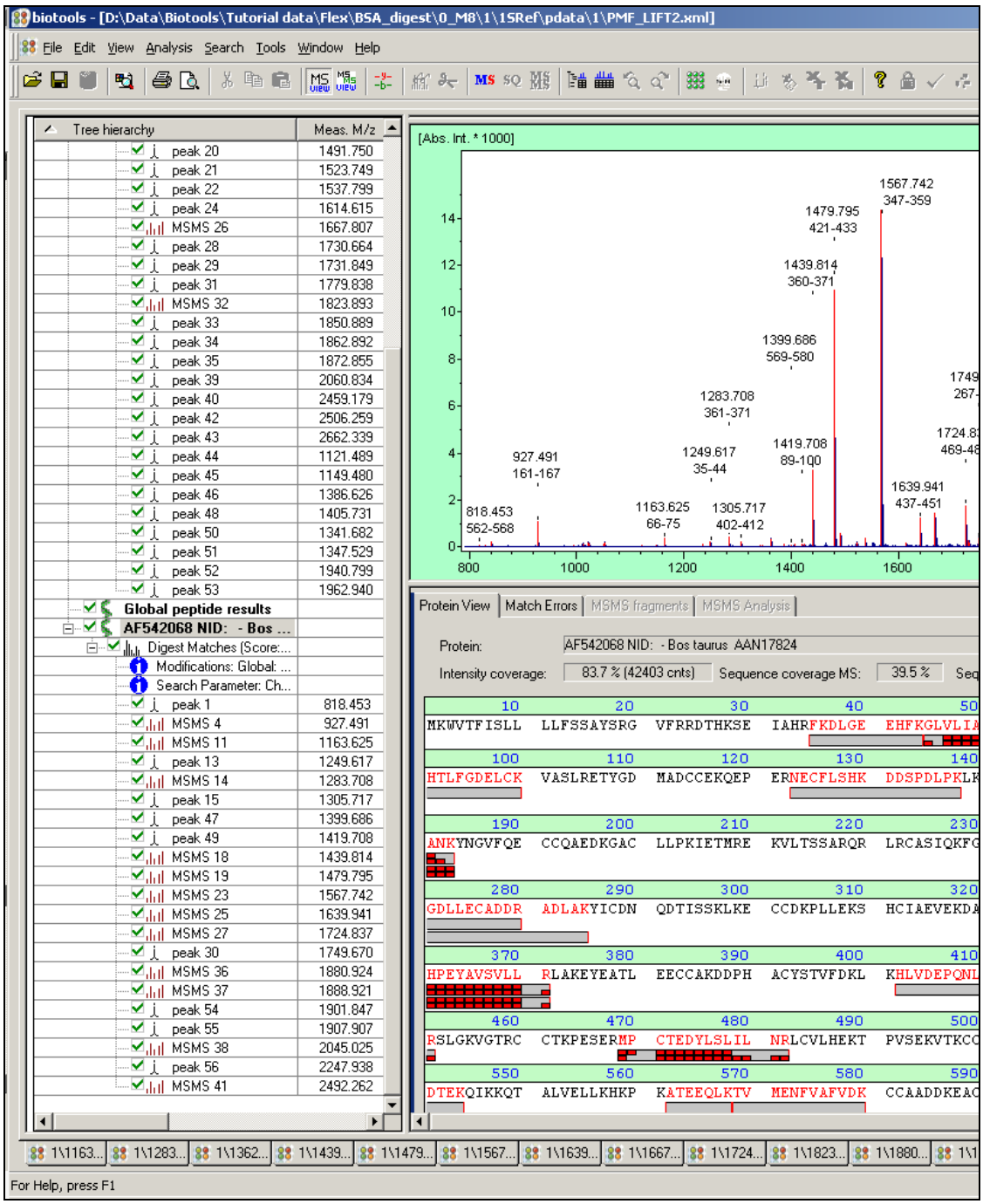


Figure 2-24 Database results of MS search in BioTools

The peptide masses are listed in the treeview on the left side of the BioTools window. If the protein sequence node is selected in the treeview, the PMF is properly annotated with the sequence positions and the entire protein sequence is shown at the bottom. Matching peptides (grey bars) are displayed and even the available matching MS/MS information (though not used for this search) is available from this display as series of red bricks (upper row: b-ions, lower row: y-ions). Peptides can be selected either in the sequence or in the treeview.

Alternatively to the Protein View tab for MS-spectra (Figure 2-24) also the differences between the calculated and the measured matched masses are displayed as a graph under the Match Errors tab.

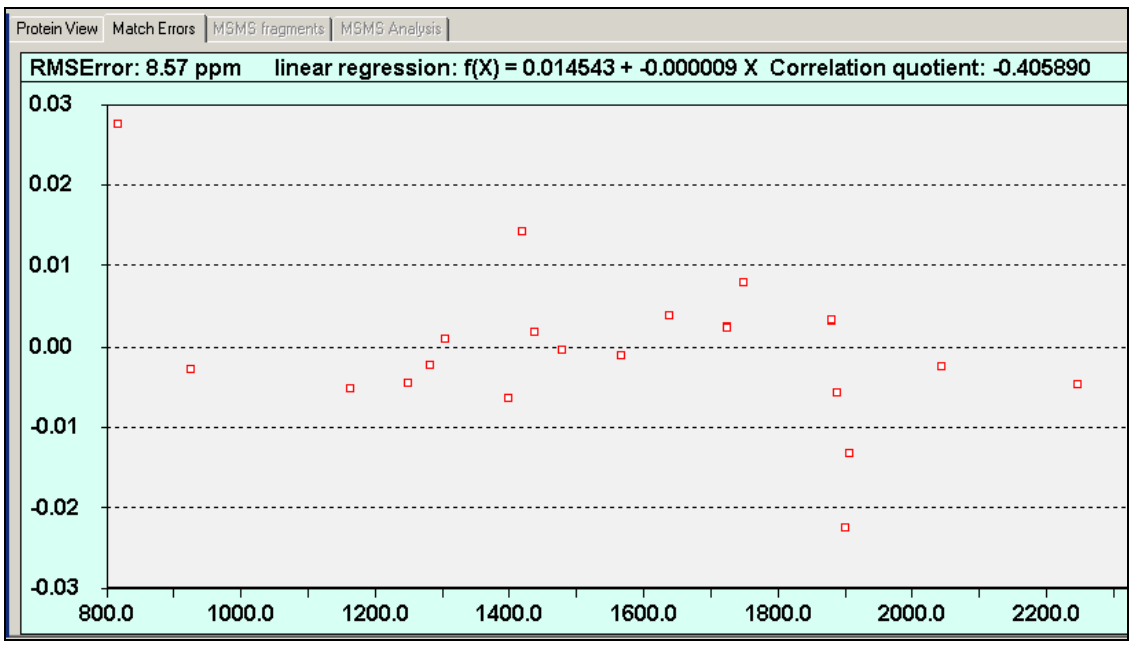


Figure 2-25 Error plot of the PMF: errors of the identified peptides vs. their molecular weight

2.5.4 Database Search in MS/MS View

Click the MS/MS button in the toolbar and MS button to do the database search for MS/MS ions.

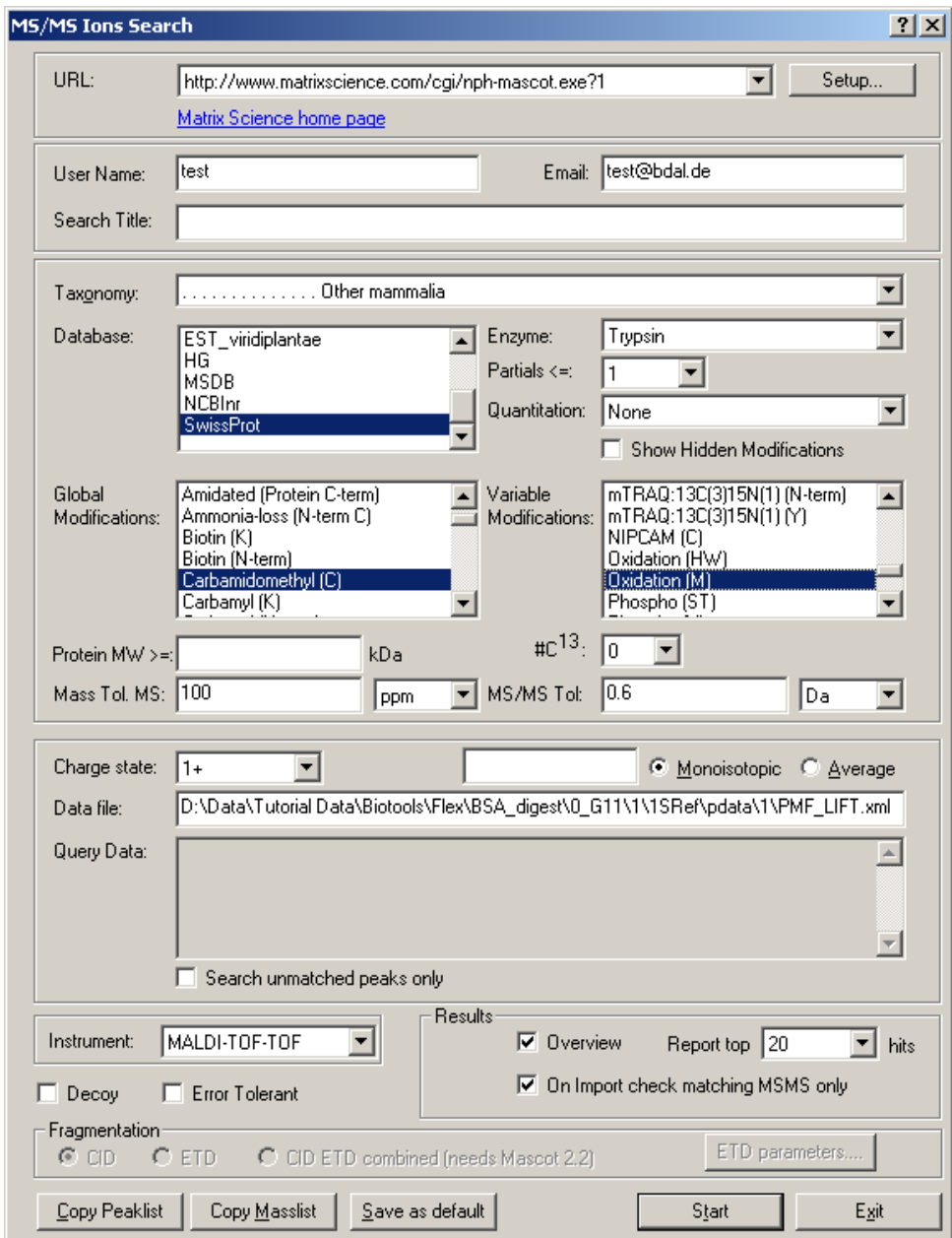


Figure 2-26 MS/MS Ions Search parameters

Click Start and after some time the results of the Mascot query will be displayed.

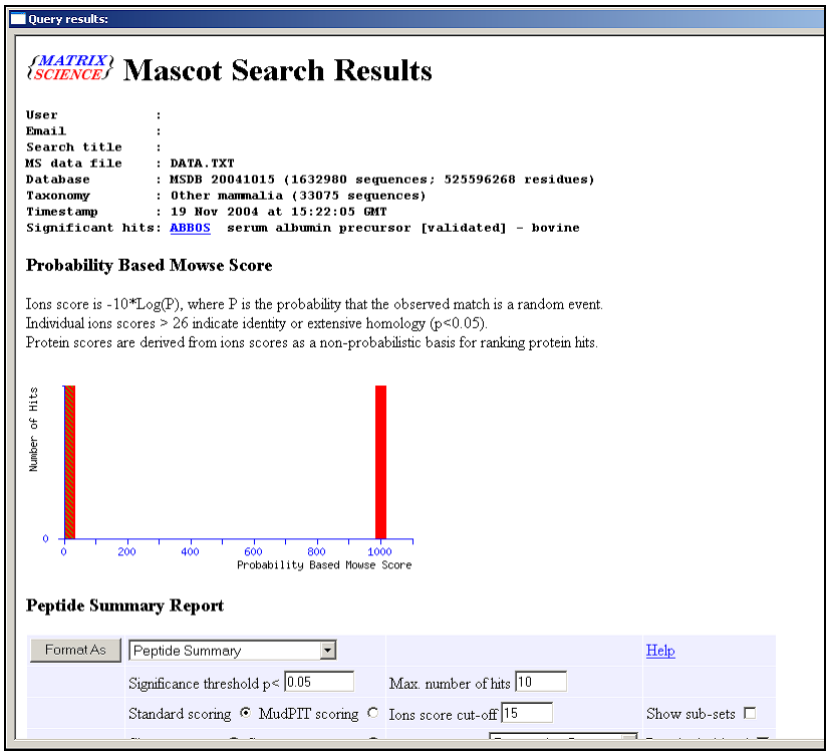


Figure 2-27 Results of MS/MS search

The result of this search is more specific as all MS/MS scores are accumulated (Mascot Score 999 instead of 174). Use the button Get Hit(s) to transfer the query results into BioTools.

After clicking in the treeview on a peptide entry the annotated fragment ion spectrum is displayed (Figure 2-28). The ion series have different colors in the spectrum for a better clarity. Click onto a theoretical mass in the table to zoom in on that peak in the spectrum. This targeted verification of the presence of small undetected peaks is a powerful **validation tool** to confirm sequence assignments, see also Edit Peak List.

More detailed descriptions and further analytical workflows are described in the Tutorials and in the Online Help.

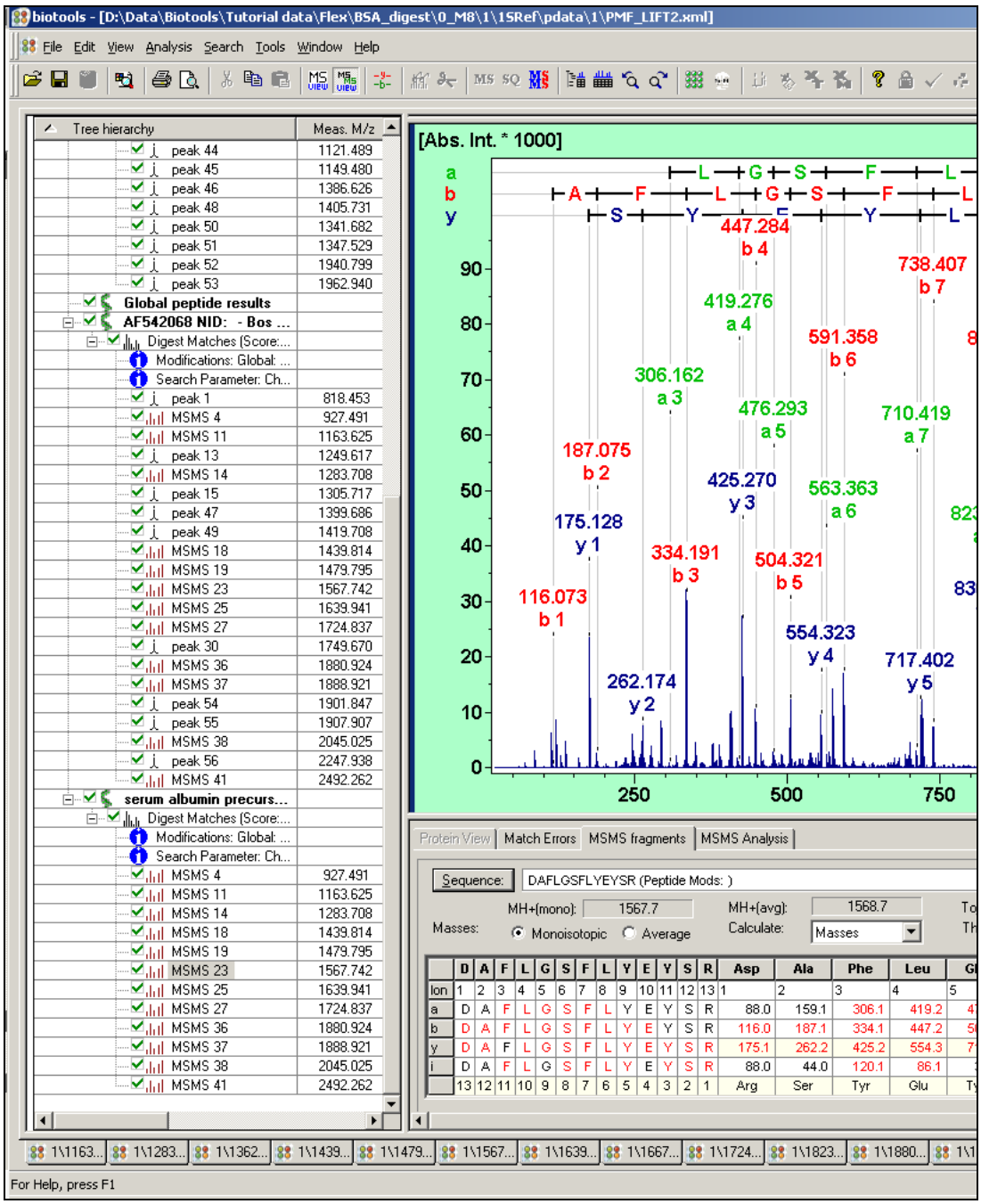


Figure 2-28 Database results of MS/MS search in BioTools

2.6 Useful Hints

2.6.1 How to Use this Manual and the Help Functionality within BioTools

The Operator Manuals are meant to be online help documents, which can be accessed using the F1 key at any point of entry of the program. In addition, they can be read like a book, which is not recommended whatsoever.

The two tutorial blocks with focus on either the SequenceEditor or BioTools contain procedure descriptions for specific analytical problems. Work your way through those applications, which you want to learn about and use the F1 access to online help only where needed.

2.6.2 Send Mascot Search Result Pages via Email to Somebody Who Does Not Have BioTools

- Perform the search under BioTools. Next to the data file opened (e g 1r) BioTools generates a copy of the HTML results page named *.html)
- On the same computer open this file in Internet Explorer (the first page opens up and connects to the Mascot server to display the full result page)
- This file can be sent to and opened by anybody.

2.7 Overview on BioTools Window

This chapter will give an explanation of all BioTools window areas and how to handle data and sequences.

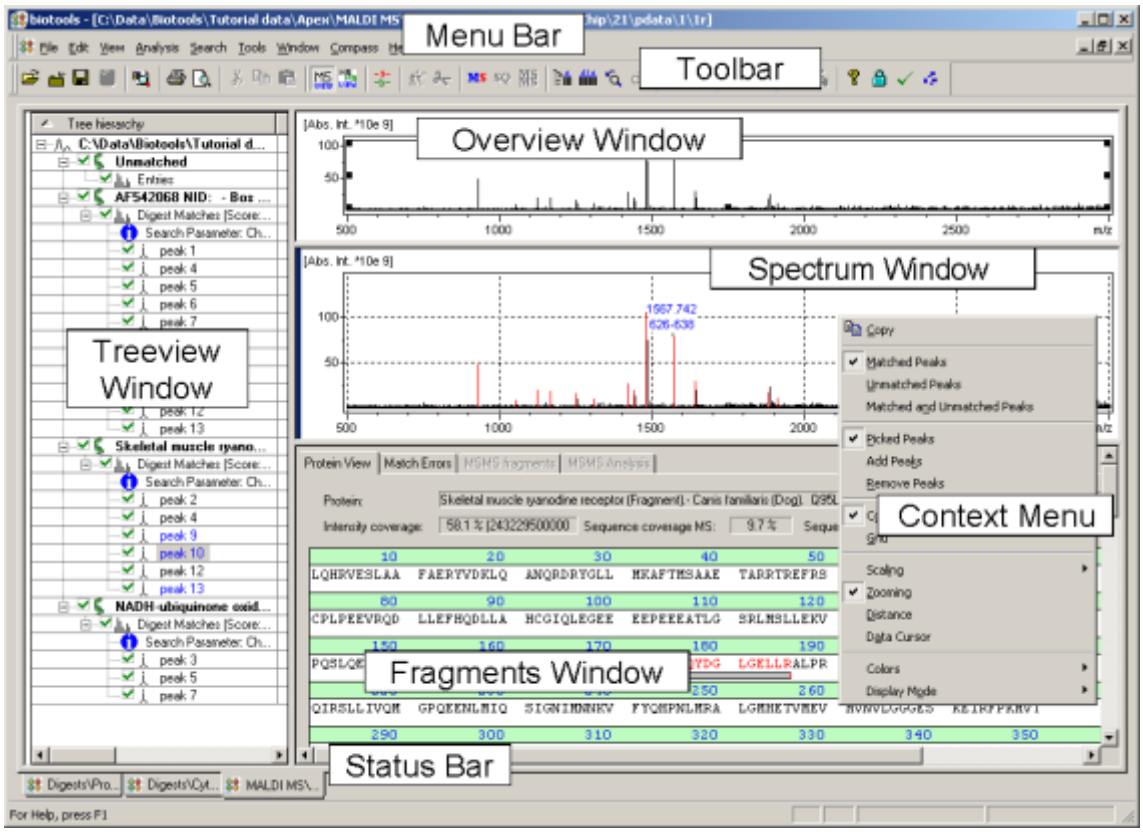


Figure 2-29 BioTools window

The active window is marked with a blue bar on the left.

The windows typically provide context menus that are accessed through a click with the right mouse button, while the cursor points to the window.

The working area within BioTools is separated into four windows and three bars:

Treeview window	Menu Bar
Overview window	Toolbar
Spectrum window	Status Bar
Fragments window	

2.7.1 Menu Bar

The menu bar can be moved with the mouse. Click with the left mouse button on the background of the menu bar and move the menu bar with the mouse button pressed to the desired position.

2.7.2 Toolbar

The toolbar can be dragged using the mouse. Click with the left mouse button on the background of the toolbar and move the toolbar with the mouse button pressed to the desired position. For details on the toolbar buttons see the chapter 3.3 Toolbar Reference and Shortcut List for BioTools.

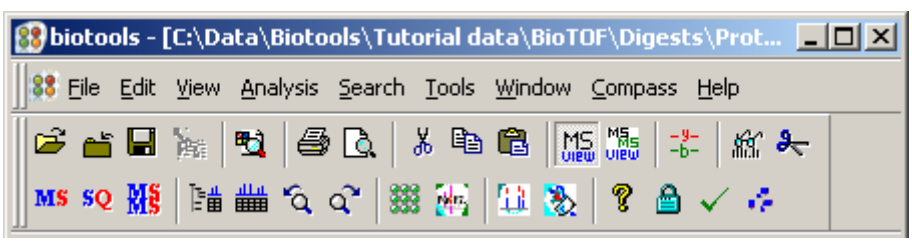
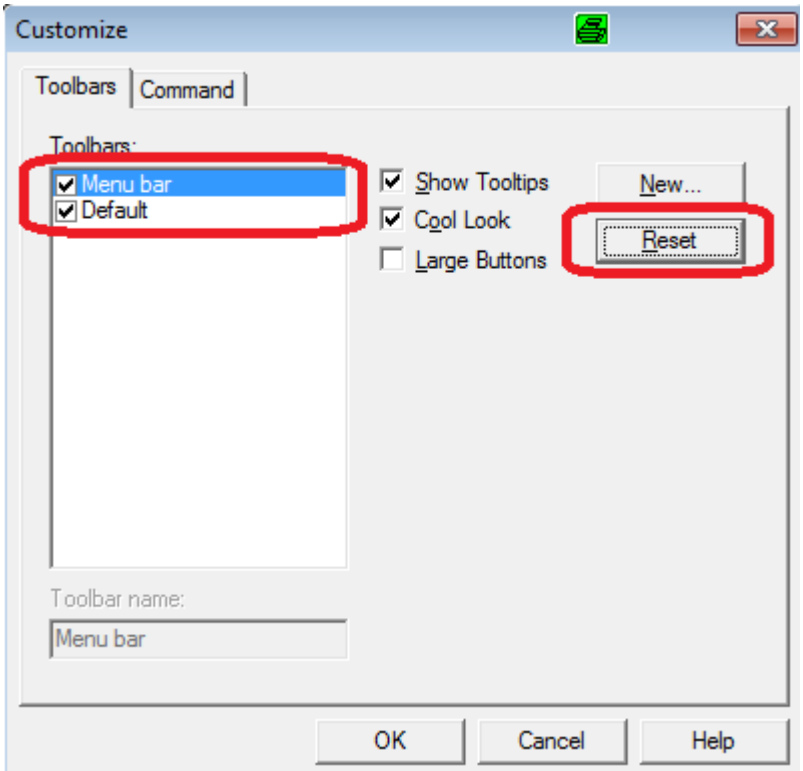


Figure 2-30 Toolbar

The toolbar can be updated to show all available buttons using menu Tools/Customize..: On the dialog select ‘Menu Bar’ and ‘Default’ toolbars and press the Reset button for each of those, this will add a TDS button to the Mascot search options:



2.7.3 Status Bar

By activating this option in the View menu the lower status bar is shown (standard) or hidden.

In the left corner is given a short help text corresponding to the cursor position and actions.

The next boxes show the activated caps lock and the numeric function of the numeric block, followed be the currently logged user.

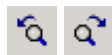
In the right corner is given the x-position and y-position (or x-distance) of the cursor (if not deactivated).

Figure 2-31 Status bar

2.7.4 Spectrum Window

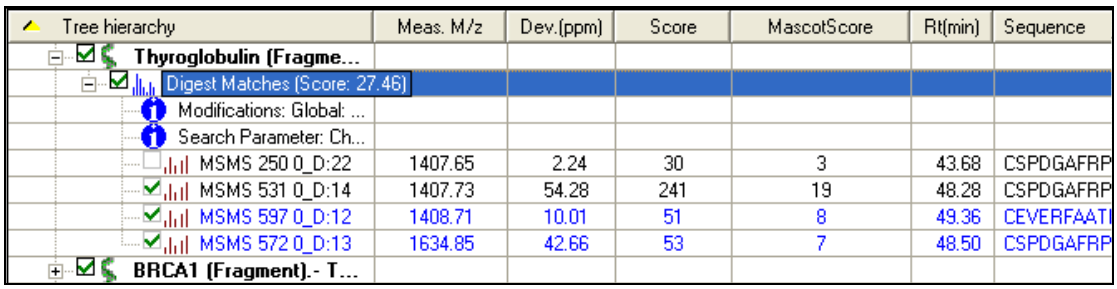
Displays one spectrum with sequence annotations and allows editing of the peak list. The Spectrum window can display either MS spectra (e.g. MALDI-PMF) or MS/MS spectra, depending on the selection of the display mode 

in the tool bar and the data type loaded. (F4) allows toggling between MS and MS/MS display mode.

- **zoom in**: drag cursor with left mouse button pressed (LMB) across the zoom area
- **zoom out to last view**: click LMB in the Spectrum window (4 repeats maximum)
- **undo and redo zooming** buttons  allow to toggle between views
- **zoom out to full spectrum**: double-click LMB in the Spectrum window
- **add or delete peaks** by CTRL-drag LMB across peak.
- **move axis:** drag LMB across scale bar of the axis of interest
- **expand/shrink axis** drag RMB (right mouse button) the scale bar of the axis of interest
- **format axis and spectrum labels:** RMB click on axis opens context menu that allows to
- Hide axis scale bar
- Format axis and peak labeling
- change axis background color
- Short keys for further x-axis operations:
  F9 zoom in x-axis.
  F10 zoomed out x-axis.
  F11 zoom box moves right.
  F12 zoom box moves left.
- The zoom view can be controlled using
  2.4.2 Zoom Operation in the Spectrum Window
  2.4.2.1 Use Cursor Keys to Move the Zoom Range
  2.4.2.2 Zoom onto Predicted MS/MS Fragments

2.7.5 Treeview Window

All protein sequence candidates with all matching peptides are listed in the treeview, a single protein or peptide can be selected for the spectrum display. The treeview provides a sorted, optionally filtered, view onto a data set in a tabulated form. The data set can be a single PMF with several sequence candidates associated, a single MS/MS spectrum or an entire LC-MS/MS data set.



Tree hierarchy	Meas. M/z	Dev.(ppm)	Score	MascotScore	Rt(min)	Sequence
Thyroglobulin (Fragme...						
Digest Matches (Score: 27.46)						
Modifications: Global: ...						
Search Parameter: Ch...						
MSMS 250 0_D:22	1407.65	2.24	30	3	43.68	CSPDGAFRP
MSMS 531 0_D:14	1407.73	54.28	241	19	48.28	CSPDGAFRP
MSMS 597 0_D:12	1408.71	10.01	51	8	49.36	CEVERFAAT
MSMS 572 0_D:13	1634.85	42.66	53	7	48.50	CSPDGAFRP
BRCA1 (Fragment).- T...						

Figure 2-32 Treeview appearance

Important Note: ***The Perform Digest and Mass Search function buttons***  ***are only accessible if the "Digest Matches" node underneath the protein name is selected***

- Send sequences to SequenceEditor
  - by double-click on the sequence
  - Click on Digest matches (see Fig.)
- Move through the treeview with arrow keys  and  .
- Unfold/fold sublevels with arrow keys  and  .
- Move through the treeview on one hierarchy levels using TAB and SHIFT TAB.
- Select a peptide sequence in the treeview
  - MS tab: to highlight it (green) in the Protein View and annotate its MS signal is in the Spectrum window.
  - MS/MS tab: to display the sequence with matching MS/MS fragments in the Fragments window and the annotations in the Spectrum window
- To hide a peak, uncheck it in the treeview. It will remove it from all calculations (e.g., sequence coverage) and from spectrum annotation, but it will not delete

it. This state will be saved with other data set processing, which is useful as it allows keeping rejected assignments for later reevaluation.

2.7.6 Overview Window

The Overview window always displays the entire spectrum. The spectrum view may show only a fraction of it after zooming in. A zoom box framing that detailed view is displayed in the spectrum overview. This zoom box can be arranged with the mouse by left-clicking on the borders. It can be moved by left-clicking in the rectangle and drawing.

- The zoom view can be controlled using
  2.4.2 Zoom Operation in the Spectrum Window
  2.4.2.1 Use Cursor Keys to Move the Zoom Range
  2.4.2.2 Zoom onto Predicted MS/MS Fragments

The arrangement of the rectangle can also be set with the arrow keys and/or **Ctrl** and/or S**hift** key.

Arrow	**-**	**Shift**	**Ctrl**	**Ctrl + Shift**
	moving with a little overlap	moving in short steps	scaling the rectangle roughly	scaling the rectangle in short steps
right 	right	right	increase width, center fix	increase width, center fix
left 	left	left	decrease width, center fix	decrease width, center fix
up 	up	up	increase height, baseline fix	increase height, baseline fix
down 	down	down	decrease height, baseline fix	decrease height, baseline fix

2.7.7 Fragments Window

The sequence view has different appearance and functionality depending on the displayed MS or MS/MS data type (see chapter 2.7.4 Spectrum Window).

MS View 

: the Protein View and the Match Errors tabs are active.

MS/MS View 

: the Match Errors, the MS/MS Fragments and the MS/MS Analysis is shown. In the MS/MS Analysis tab either the fingerprint info or the DeNovo Sequencing info is shown.

F4 allows toggling between MS and MS/MS display mode.

2.7.7.1 Protein View

A protein sequence selected in the treeview on the left is displayed and the detected peaks are annotated in the Spectrum window, the treeview and the Sequence window corresponding to this sequence.

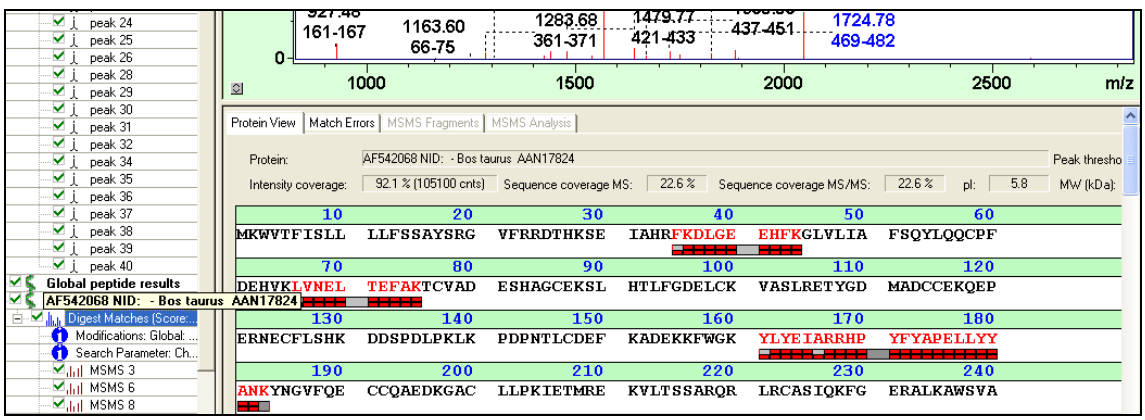


Figure 2-33 Protein View appearance

Interesting information available directly from the display:

Protein: name according to information imported from Mascot or Sequence Editor

Intensity coverage (%): Fraction of peak intensities that are assigned to the protein divided by the total intensities of all MS peaks in the data set. 100 % means all peaks in the spectrum are assigned to the protein. Very relevant diagnostic value that estimates to what degree a data set is accounted to identified proteins/peptide.

Sequence coverage MS: Fraction of amino acid residues that are assigned to the protein sequence by MS data vs. all residues in the protein sequence. Diagnostic parameter that expresses the degree of knowledge about a protein available from MS information in a data set (e.g. from MALDI-PMFs).

Sequence coverage MS/MS: Same as above, except only those peptides are accounted for that were IDed by MS/MS. Diagnostic parameter that expresses the degree of knowledge about a protein available from MS/MS information in a data set (e.g. from LC-MS/MS).

pI: isoelectric point calculated from entire protein sequence.

MW [kDa]: Molecular weight calculated from entire protein sequence.

Peak threshold: only peaks above this threshold are annotated. Threshold can be defined under Analysis > Set Threshold.

Visualization and interaction inside the Protein View tab:

- Individual peptides can be selected in the sequence view or in the treeview (background color changes to green).
- If MS/MS spectrum is available for the selected peptide – indicated by the red bricks inside the grey peptide bar – F4 key allows direct **access** to the sequence annotated **MS/MS spectrum** of a selected peptide (green background color).
- Red bricks indicate matching N-terminal fragment ions (upper row) and C-terminal fragment ions (lower row); can be defined via the Protein View context menu.
- N-Glycane consensus motif (NXS/NXT) is marked in yellow
- The appearance of the sequence view can be controlled through the Protein View context menu.

2.7.7.2 Match Errors

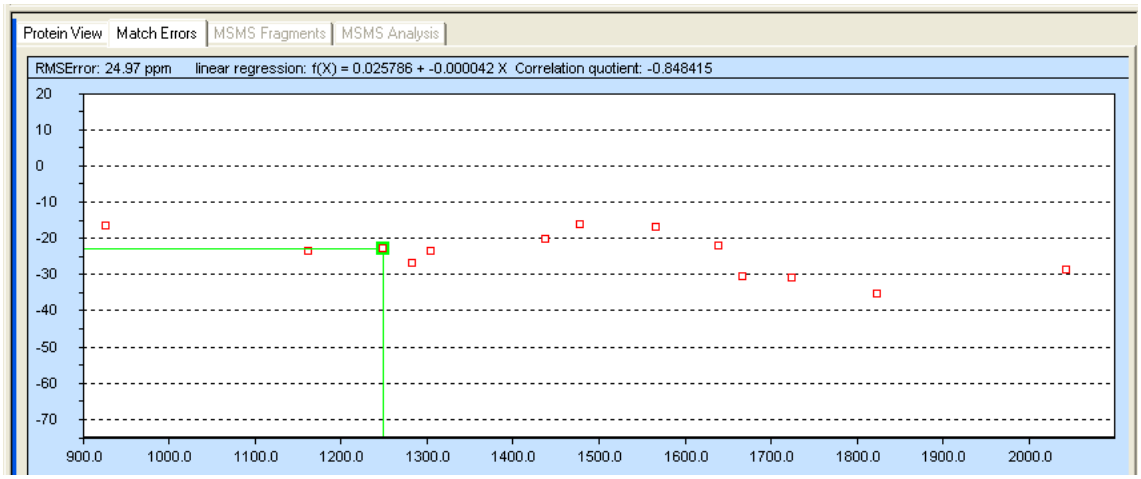


Figure 2-34 Match Errors

Displays the mass errors (Da or ppm) of the peptide peaks matching the protein sequence selected in the treeview. A selected peptide is highlighted in green as shown in the figure.

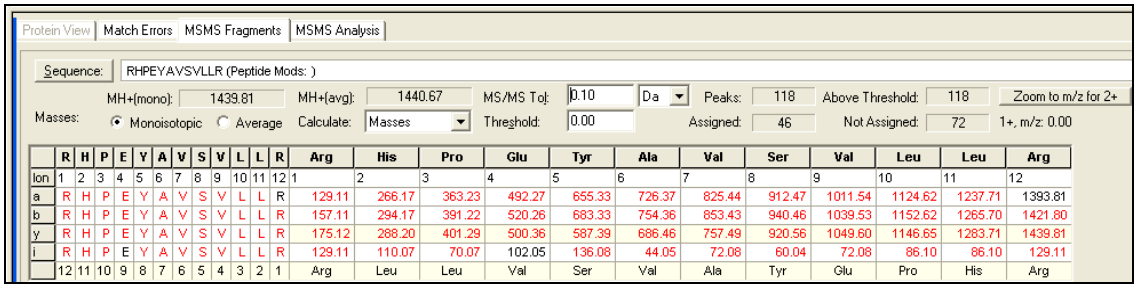
The RMS Error across all matching peptides is displayed on top in the selected unit.

Powerful diagnostic tool to estimate calibration drift of data sets, confidence range for improved database search settings etc.

The graphic can be copied as an image via the Match Errors context menu.

2.7.7.3 MS/MS Fragments

If an identified peptide sequence is selected in the treeview and the MS/MS display mode is active (), the annotated spectrum is displayed. Under the MS/MS Fragments tab, interaction with peaklist and spectrum are easily possible.



	R	H	P	E	Y	A	V	S	V	L	L	R	Arg	His	Pro	Glu	Tyr	Ala	Val	Ser	Val	Leu	Leu	Arg
Ion	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
a	R	H	P	E	Y	A	V	S	V	L	L	R	129.11	266.17	363.23	492.27	655.33	726.37	825.44	912.47	1011.54	1124.62	1237.71	1393.81
b	R	H	P	E	Y	A	V	S	V	L	L	R	157.11	294.17	391.22	520.26	683.33	754.36	853.43	940.46	1039.53	1152.62	1265.70	1421.80
y	R	H	P	E	Y	A	V	S	V	L	L	R	175.12	288.20	401.29	500.36	587.39	686.46	757.49	920.56	1049.60	1146.65	1283.71	1439.81
i	R	H	P	E	Y	A	V	S	V	L	L	R	129.11	110.07	70.07	102.05	136.08	44.05	72.08	60.04	72.08	86.10	86.10	129.11
	12	11	10	9	8	7	6	5	4	3	2	1	Arg	Leu	Leu	Val	Ser	Val	Ala	Tyr	Glu	Pro	His	Arg

Figure 2-35 Fragments window with parameters and annotation information

Most importantly, the matches between theoretically calculated fragment ion masses (as shown) and the peaks in the MS/MS spectrum of the IDed peptide are highlighted in red, theoretical fragments that do not match to an experimental peak are shown in black.

Note: ***The fragment ion series to be displayed and calculated can be selected or defined from the toolbar with the button -y- -b- (see chapter 2.7.2 Toolbar)! This is mandatory for proper display when switching between different fragmentation modes such as ETD/CID or ESI/MALDI.***

This provides a direct overview to what extent a peptide sequence is accounted for by the experimental data and an entry point for further evaluation.

Typical tasks:

Peaklist OK? Check if a fragment ion actually does not match or was, perhaps, not properly detected during peaklist generation:

- click on the black coded mismatch mass in the fragment table – the display will zoom onto that mass
- if a peak is actually present in the raw data add that peak to the list (keep Ctrl and LMB pressed and drag the cursor across the peak). Use the same procedure to remove peaks from the list.

Sequence + PTMs correct? Check which of 2 or more sequence candidates –or e.g. phosphorylation sites – better account for the experimental data:

- define the sequence or PTM variants in SequenceEditor and send them all to BioTools with the spectrum in question selected
- In the treeview context menu, use the **Match all entries** function to sort al variants according to their quality of match
- for each structure candidate check for matching and mismatching fragment ions to ensure sloppy peak picking does not account for preference of one sequence candidate over the other as described under **Peaklist OK?**.

Contents of the Fragments window:

Sequence:	Sends the displayed sequence to the SequenceEditor. The sequence, modifications and its title (in brackets) is shown in the field to the right. If there is a sequence available but not shown in the display: press the sequence button, type in/paste the sequence into SequenceEditor and send it back to BioTools for display at this point.
Symbol	If there is an irregular letter contained in the sequence (X, Z, B, etc), a message pops up and a symbol is placed in the title.
MH^+ (mono)	Monoisotopic mass of the single protonated sequence is shown.
MH^+ (avg)	Average mass of the single protonated sequence is shown. Masses Select the type of masses (Mono./Avg.) for calculation of the fragments below.
Calculate	Select the display format of the table below: **None** shows sequence only **Masses** calculates fragment ion masses (standard!) **Errors [Da]** calculates exp. fragment mass errors [Da] **Errors [ppm]** calculates exp. fragment mass errors [ppm]
MS/MS Tol.	Enter the tolerance (Da or ppm) for matching theoretical with experimental fragment ions. The drop-down box to the right allows the unit to be specified. Matches are indicated in red in the table below, mismatches in black.
Threshold	Define a threshold peak intensity according to the y-scale for the display of annotations.
Zoom to m/z for 4+ 2+	Zoom the displayed mass range to the one possibly displaying the next highest or lower charge state to check its presence – relevant for ESI-MS/$MS^{(n)}$ spectra. E.g., click on fragment 102.05 in the table, then Zoom to m/z for 2+, to zoom in on 56 Da.
Zoom +/-	Sets the Zoom range for the zoom to mass functionality in the spectrum (clicking on a fragment mass in the fragment table or error point in the error plot.)
Peaks	Number of picked peaks in the MS/MS peaklist
Above Threshold	Number of peaks above defined threshold
Assigned	Number of peaks matching the theoretical fragments calculated from sequence according to ion series selection under Toolbar button -y- -b-.

Not Assigned	Sum of **Assigned** and **Not Assigned** peaks equals the number of all peaks **Above Threshold**.

To view an already existing sequence via the Treeview window, select the desired sequence by clicking on it with the left mouse button.

2.7.7.4 MS/MS Analysis

This tab provides *DeNovo* Sequencing Tools for Bottom-Up and Top-Down work to extract sequence information from MS/MS data and to use it for database searching. Depending on data quality and type, these may constitute complete sequence suggestions or only "seed" sequences or sequence tags, which require further interactive work (via MS/MS Analysis context menu).

Procedure:

1. Select the Analysis Mode using a radio button on the top left to enable only those features that are useful for either Bottom-Up or Top-Down work.

Bottom-Up: protein digests, typically analyzed with CID type MS/MS methods on all instruments, ETD analysis of digests on the trap included.

Top-Down: Intact protein work using MALDI-TDS (ISD), ETD or ECD of peptides and proteins (see application tutorial on top-down sequencing in chapter 2.1.2 Application Tutorials for BioTools and SequenceEditor).

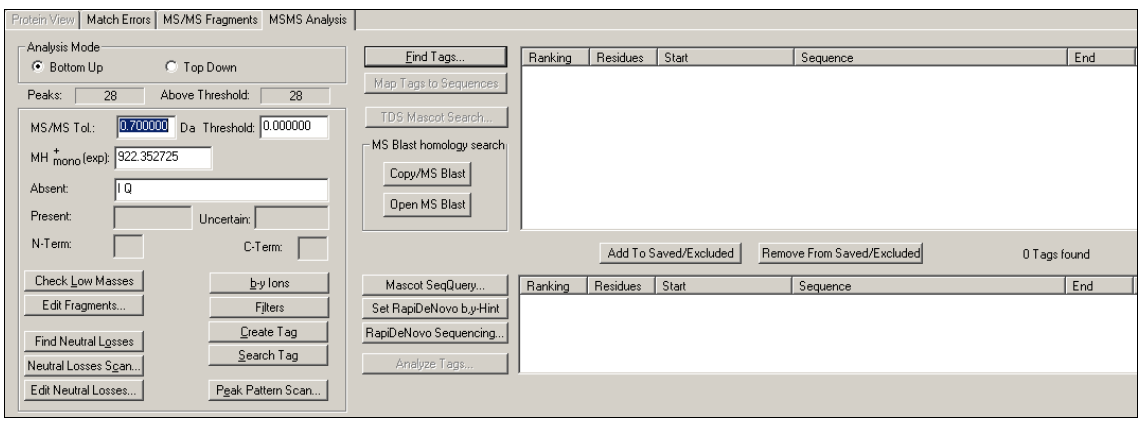


Figure 2-36 Fragments window with *DeNovo* sequencing parameters for Bottom-Up analysis

2. Enter proper MS/MS tolerance, threshold intensity and – if automatically available– the monoisotopic parent MH+.

3. Using MS/MS data providing immonium ions (i-type, e.g. from MALDI-TOF/TOF) click Check Low Masses to get information about existing amino acid residues, the absent residues obtained should only be left in the field if the information appears reliable! I and Q should be generally included as absent to avoid redundant sequence assignments. Absent masses are defined in a blank-separated list.

Edit Fragments	Allows modifying the fragment ion properties relevant for *Check Low Masses*. (Use only for development purposes)
Find Neutral Losses	Will screen for defined Neutral Loss groups such as phosphorylation, Met-Ox etc.
Edit Neutral Losses	Allows the list of Neutral Losses to be extended.

Contents of the sequence view tab MS/MS Analysis:

Peaks	Number of peaks in the peaklist
Above Threshold	Number of peaks above the entered threshold intensity
MS/MS Tol.	Enter the MS/MS mass tolerance (in m/z), for calculating a match
Threshold	Excludes peaks below the threshold intensity for all calculations and display
MH^+_{mono}(exp)	The monoisotopic mass of peptide MH^+ derived from the spectrum
Absent	Excludes listed amino acid residue types (space-separated) from any calculation. Typically at least I and Q are defined, I (Ile) only if MS/MS Tol. set to < 0.05
Uncertain	Shows the amino acid types suggested being uncertain by the *Low Masses* button operation
Present	Shows the amino acid residues types suggested to be present by the *Low Masses* button operation
b-y Ions	Annotates pairs of b and y ions in the data file, which result from the dissociation of the same peptide bond; the results are shown in the Spectrum window. This may assist in largely manual *DeNovo* sequencing studies but are typically not needed if RapiDeNovo is used.

Important: The mass error must be set 2x as high as the actual mass error for a single fragment ion.

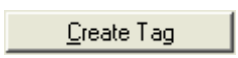
The following options are based on matching peak distances to particular theoretical masses.

Searches for all defined neutral losses in the currently open MSMS spectrum.

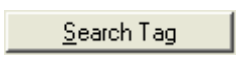
Opens an editor for the (user-defined) neutral losses that are stored in the method folder.

Opens a dialog that can be used to search for all MSMS spectra containing neutral losses that were defined using the button "Edit Neutral Losses..". The list of found MSMS spectra can be copied to the clipboard.

Opens a dialog that can be used to search for all MSMS spectra that contain an MSMS peak that is surrounded by a user-defined pattern, this can be used e g to find all MSMS spectra that contain the pattern -32/+34 Da that is characteristic of disulfide-linked peptides.



Creates sequence tags based on matched b-y ions; the results are shown in the spectrum.



Starts the sequence tag search on PeptideSearch at EMBL (this is deprecated).

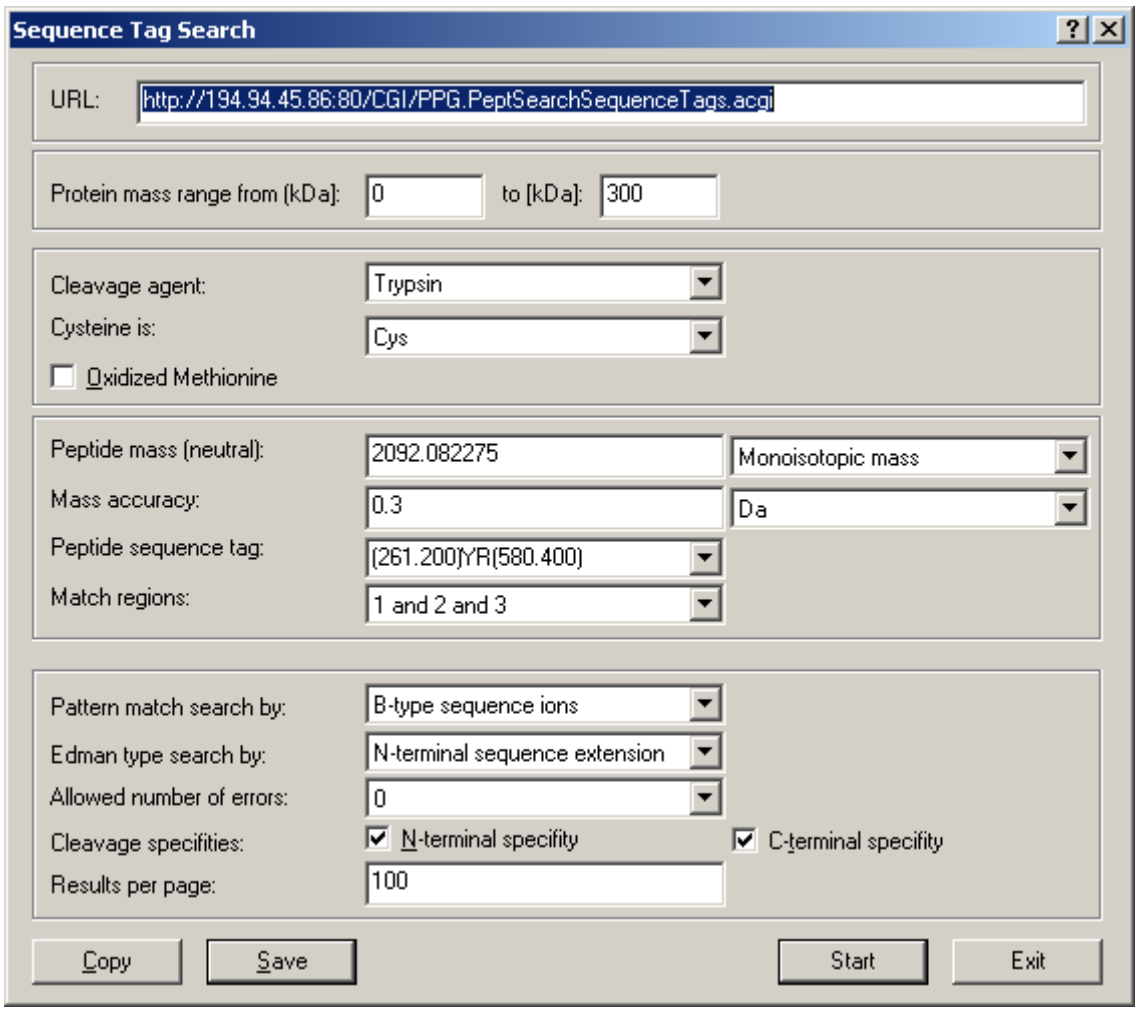


Figure 2-37 Sequence Tag Search dialog

Find Tags...

Opens the Find Tags dialog for sequencing workflows, Top-Down or Bottom-up. respectively.

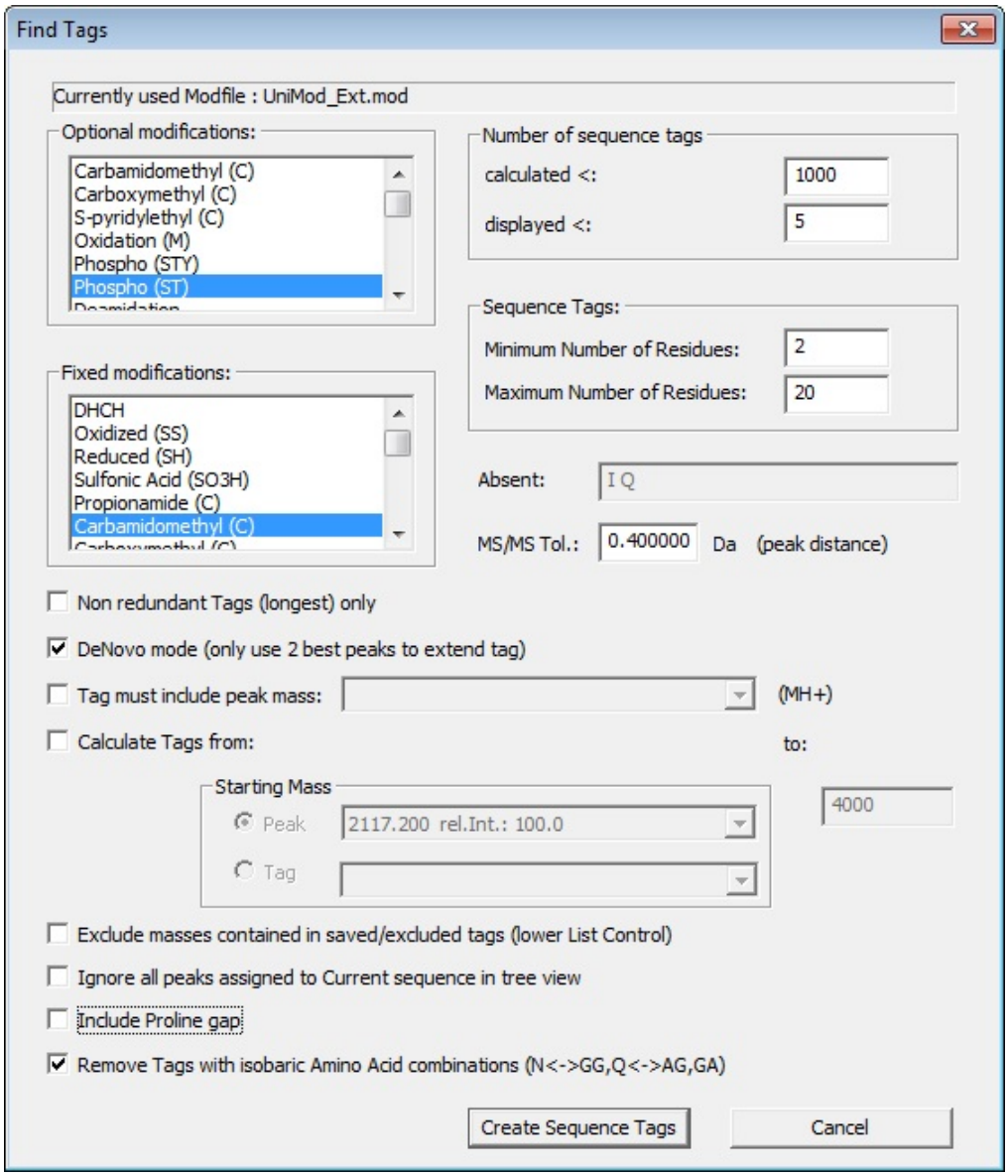


Figure 2-38 Find Tags dialog

Optional and Fixed modifications can be defined depending on suspected modifications and the protein chemistry employed.

The **number of sequence tags** created and annotated controls calculation time and display clarity and their **minimum** and **maximum number of residues** can be limited. MALDI-TDS (reISD) data may require a minimum setting of, e.g., 10 residues for c-ions, whereas typical Bottom-Up data require 2-5.

The **absent** amino acid residues were previously defined under the MS/MS Analysis tab.

The **MS/MS tolerance** is defined another time here, allowing for stricter accuracy requirements for sequencing (tag generation) than for annotation (as defined under the MS/MS Analysis tab.

The check boxes allow a better controlling particularly of Top-Down sequencing:

Non redundant Tags ..	Excludes shorter tags that are entirely covered by longer ones
DeNovo mode (only use 2 best peaks)	When this option is selected only the 2 highest intensity or closest-matching peaks are used to extend a tag; other potential matches are ignored. This option is useful if the most intense peaks in the spectrum correspond to the fragments of one series with MALDI ISD spectra.
Tag must include peak mass	Only returns tags that match the peak specified in the drop-down box. Powerful tool to direct the automated calculation to obvious peaks.
Calculate Tags from .. to:	Allows confining tag calculation to a mass range or to extend previously obtained tags contained in the list box.
Exclude masses…	Excludes any peaks from calculations that are matched by tags already and that are contained in the Saved/Excluded list of tags in the MS/MS Analysis tab (e.g. to sequence low intensity y-ion tags after completing high intensity c-ion tags in MALDI-TDS).
Ignore all peaks…	Excludes any peaks that are matched by the currently selected sequence in the treeview (e.g. to sequence tags belonging to another protein present in the sample).
Include Proline gap	Use for c-ion series based sequencing particularly in MALDI-TDS. This option accounts for a property of c-ion series obtained by ISD, ETD and ECD: c-ions (dominant in MALDI-TDS) are not produced N-terminal to Pro as this would involve ring cleavage, therefore X-Pro mass differences are included into the calculation

See also the application tutorial on the analysis of ISD data (see chapter 2.1.2 Application Tutorials for BioTools and SequenceEditor).

Filters

Opens the filters dialog to set the valence of the ions and the minimum and maximum values of the search. The number of hits depends to the previously selected value and order above the list window.

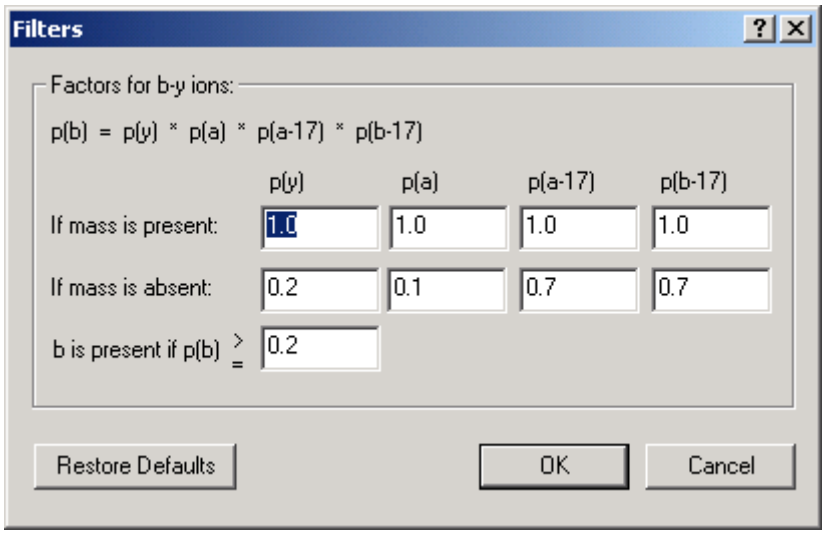


Figure 2-39 Filters dialog with settings for PSD. Ion trap settings should be 1.0 for "If mass is absent:" p(a), p(a-17) and p(b-17)

Map tags to Sequences

Top-Down only with a reference sequence loaded to the fragment ion spectrum: selects those tags in the upper list box matching the selected protein sequence in the BioTools treeview. In this way **Signal Peptides can be discovered**.

Mascot SeqQuery...

Opens the Mascot Sequence Query dialog containing the formatted data from the selected tags in the lower list box (for Top-Down Analysis as MassOfTag seq(*-TAG). For Bottom-Up Searches this should be reformatted as ParentMass tag(startmass, TAG, endmass). See Mascot Help for further details on the formatting of this query.

RapiDeNovo Sequencing...

Requires a RapiDeNovo license: for advanced ***DeNovo* sequencing**. For further information see the RapiDeNovo Tutorial.

TDS Mascot Search...

Top Down only: this starts the Mascot TDS search procedure, at first a dialog for selection of virtual parent masses for the MSMS peak list is shown.

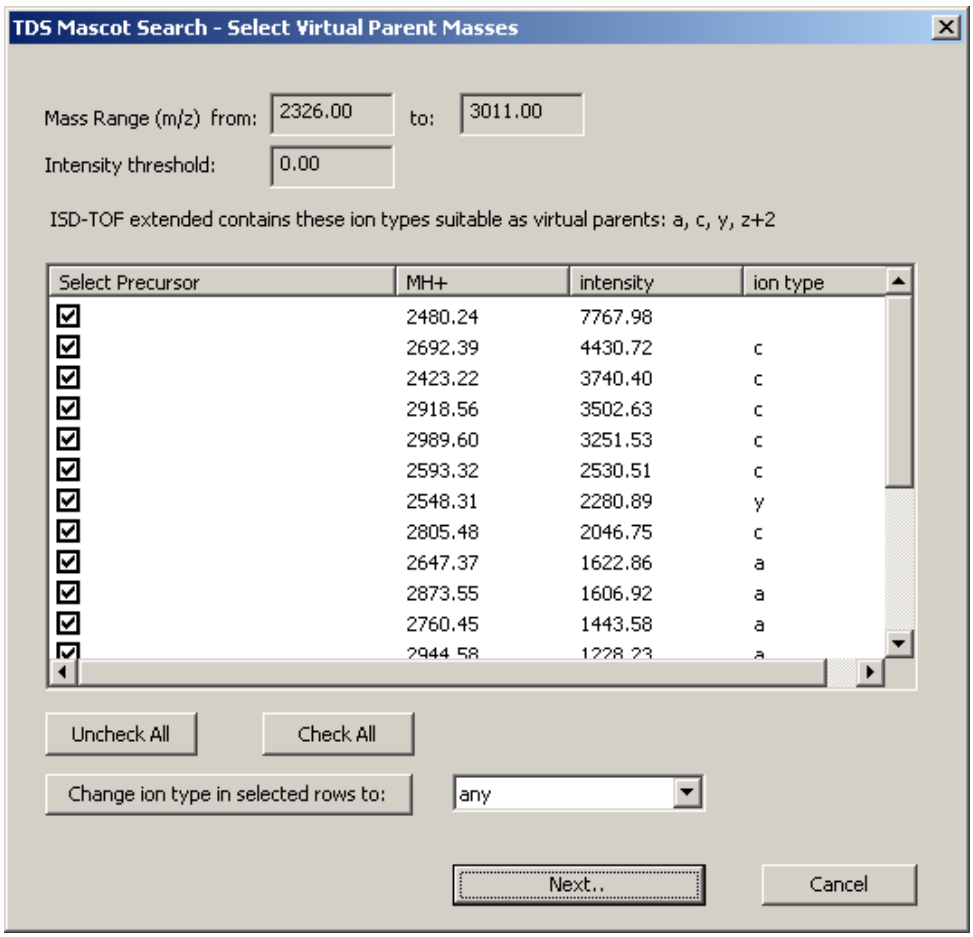


Figure 2-40 TDS Mascot Search – Select Virtual Parent Masses dialog

The mass range that was set previously by zooming in the spectrum to a mass range containing fragment ions is displayed as well as the intensity threshold set. This dialog calculates the masses of peptides based on an assumed ion type for the MSMS peak, if a peak of MH+ 1000.0 is assumed to be a c-ion the corresponding peptide from unspecific cleavage would be 1015. For other ion types a different mass offset is applied. The Virtual mass calculation recognizes 5 different types of ions: a, c, y, z+1, z+2. The subset of these contained in the currently selected ion series for annotation is used for virtual parent calculation and displayed on the dialog box above the list. The button "Change ion type in selected rows to:" can be used to change the ion type that is derived from c/a or z+2/y pairs in MALDI-ISD spectra.

All checkmarked peaks in the list will be used for virtual parent calculation. Clicking on "Next.." will open the Mascot MSMS search dialog with the virtual spectra already entered.

Please refer to the tutorial on top-down sequencing for details on the TDS search feature (see chapter 2.1.2 Application Tutorials for BioTools and SequenceEditor).

Analyze Tags...

This will search for a mass offset from a defined list of fragment mass offsets between start mass of the experimentally found tag and the one calculated from the protein sequences contained in the BioTools treeview). (Here, a c-ion match was found with the sequence of Serum Albumin precursor within 0.04 Da. In the native sequence (BSA) the signal peptide was correctly identified based on the start masses of the tags)!).

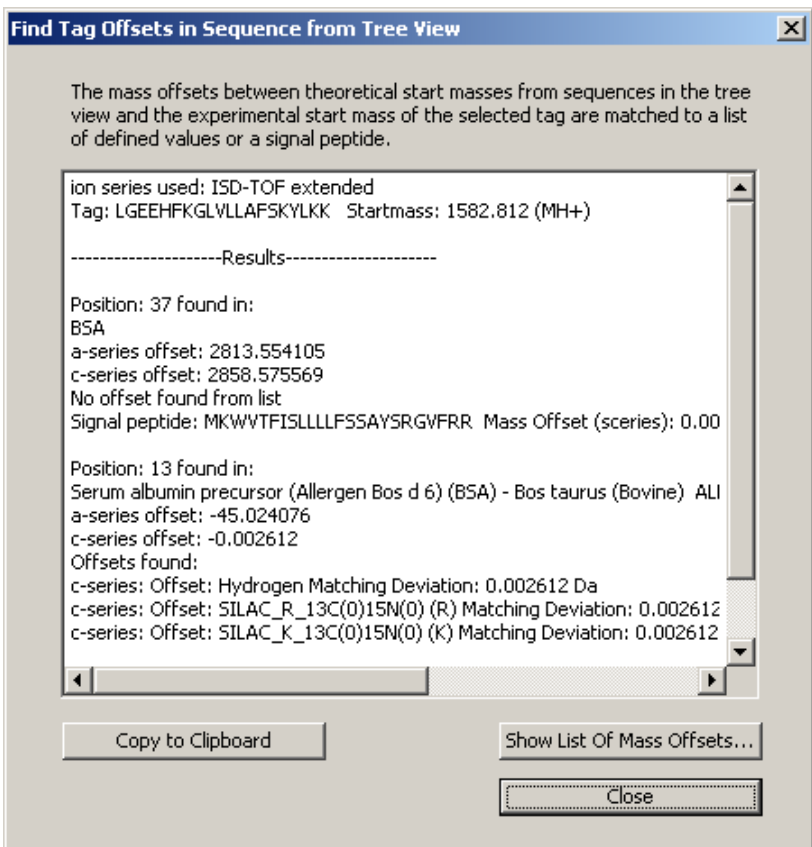


Figure 2-41 Find Tag Offsets dialog

Show List Of Mass Offsets...

Displays the current theoretical offsets as defined in the default modification file for the building blocks: Amino Acids. The list can be edited using the Sequence Editor (menu Edit → Modification-Types) by selecting a different .mod file.

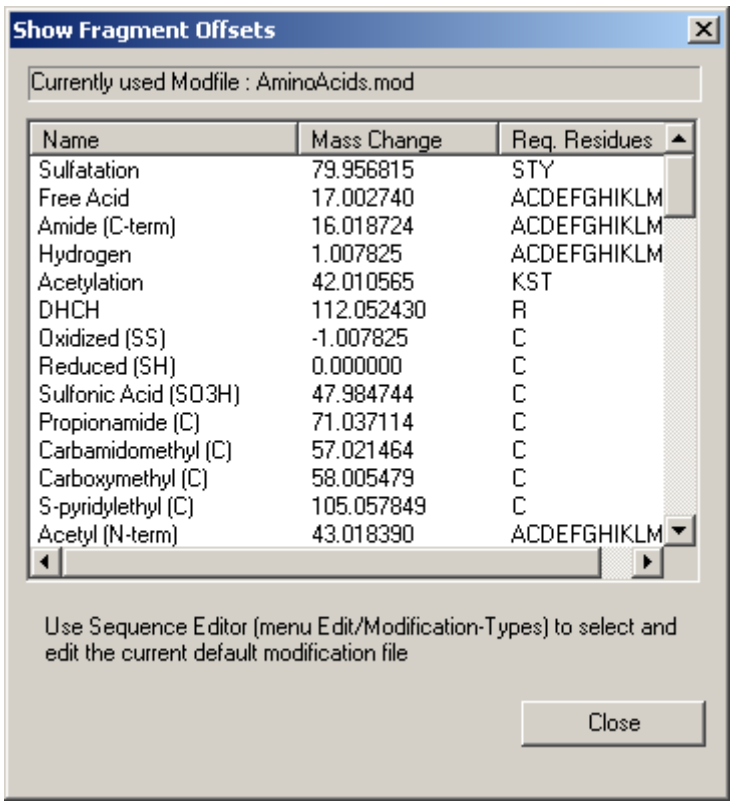


Figure 2-42 Show Fragments Offsets dialog

The following 2 buttons permit using the calculated sequence tags (preferably only one!) for error tolerant protein identification, seeding the more detailed characterization at a later stage using the *AnalyzeTags..* function.

Copy/MS Blast

Copies all selected sequence tags without any MS info (from both list boxes) into the clipboard formatted backwards and forwards for search with MS-BLAST (EMBL/Harvard).

Open MS Blast

Opens the Harvard web page for the MS-BLAST homology search via internet. **Please paste (Ctrl-V)** the previously copied sequences into the entry field on the website and submit the query.

The sequence tags in the lower *Saved/Excluded* list are secured while not interfering with further interactive work using *Find Tags*. Highlighted tags in that list are used for *Mascot Sequence Queries* and the *Analyze Tags* function.

Add To Saved/Excluded — Moves selected entries from the upper to the lower list control.

Remove From Saved/Excluded — Moves selected entries from the lower to the upper list control.

2.7.7.5 Sequence View

If an identified peptide sequence is selected in the treeview and the MS/MS display mode is active the Sequence View tab shows an overview of the fragment coverage of the peptide sequence.

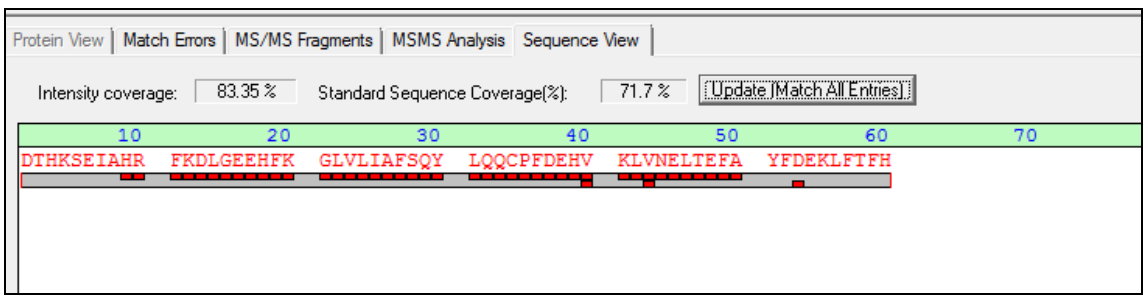


Figure 2-43 Sequence View tab of Fragments window

The display is calculated in the same way as for the Protein View (based on the selected ion series for annotation and the matching tolerance). Its main purpose is to provide a quick overview of the fragment and intensity coverages of Top Down data sets such as those generated by MALDI ISD.

Red bricks indicate matching N-terminal fragment ions (upper row) and C-terminal fragment ions (lower row).

Intensity coverage (%): Fraction of peak intensities that are assigned to the MSMS fragments divided by the total intensities of all MSMS peaks in the data set. Relevant diagnostic value that estimates to what degree a spectrum is explained by a peptide sequence.

Sequence coverage MS(%): Fraction of amino acid residues that were IDed by MS/MS. Diagnostic parameter that expresses the degree of knowledge about a protein available from MS/MS information in a data set.

There are 3 different ways of calculating the sequence coverage that can be selected using the Sequence View – Context Menu or Protein View – Context Menu.

Update (Match all Entries): This button updates the matches between MSMS peaks and fragments of the currently displayed protein sequence. The button can be used to ensure that the displayed fragment matches reflect the current state of the analysis.

3 APPENDIX

3.1 Amino Acid Residues

3.1.1 Single Letter Code

Most of the analysis programs use the single letter code - learn it by heart from: http://alpha2.bmc.uu.se/~kenth/bioinfo/singleletter.html

Name	Three Letter Code	Single Letter Code	Mnemonic
Alanine	Ala	A	(Alanine)
Cysteine	Cys	C	(Cysteine)
Aspartic Acid	Asp	D	(aciD)
Glutamic Acid	Glu	E	(E comes after D)
Phenylalanine	Phe	F	(Ph=F)
Glycine	Gly	G	(Glycine)
Histidine	His	H	(Histidine)
Isoleucine	Ile	I	(Isoleucine)
Lysine	Lys	K	(L follows K)
Leucine	Leu	L	(Leucine)
Methionine	Met	M	(Methionine)
Asparagine	Asn	N	(AsparagiNe)
Proline	Pro	P	(Proline)
Glutamine	Gln	Q	(Qlutamine)
Arginine	Arg	R	(aRginine)
Serine	Ser	S	(Serine)
Threonine	Thr	T	(Threonine)
Valine	Val	V	(Valine)
Tryptophan	Trp	W	(Double ring - W)
Tyrosine	Tyr	Y	(tYrosine)

Single Letter Code	Three Letter Code	Mnemonic
A	Ala	**A**lanine
C	Cys	**C**ysteine
D	Asp	aspar**D**ic acid
E	Glu	glu**E**tamic acid
F	Phe	**F**enylalanine
G	Gly	**G**lycine
H	His	**H**istidine
I	Ile	**I**soleucine
K	Lys	before **L**
L	Leu	**L**eucine
M	Met	**M**ethionine
N	Asn	Asparagi**N**e
P	Pro	**P**roline
Q	Gln	**Q**-tamine
R	Arg	a**R**ginine
S	Ser	**S**erine
T	Thr	**T**hreonine
V	Val	**V**aline
W	Trp	t**W**o rings
Y	Tyr	t**Y**rosine

3.1.2 Genetic Code

1.	T		C		2. A		G		3.
T	TTT	Phe [F]	TCT	Ser [S]	TAT	Tyr [Y]	TGT	Cys [C]	T
	TTC	Phe [F]	TCC	Ser [S]	TAC	Tyr [Y]	TGC	Cys [C]	C
	TTA	Leu [L]	TCA	Ser [S]	TAA	Ter [end]	TGA	Ter [end]	A
	TTG	Leu [L]	TCG	Ser [S]	TAG	Ter [end]	TGG	Trp [W]	G
C	CTT	Leu [L]	CCT	Pro [P]	CAT	His [H]	CGT	Arg [R]	T
	CTC	Leu [L]	CCC	Pro [P]	CAC	His [H]	CGC	Arg [R]	C
	CTA	Leu [L]	CCA	Pro [P]	CAA	Gln [Q]	CGA	Arg [R]	A
	CTG	Leu [L]	CCG	Pro [P]	CAG	Gln [Q]	CGG	Arg [R]	G
A	ATT	Ile [I]	ACT	Thr [T]	AAT	Asn [N]	AGT	Ser [S]	T
	ATC	Ile [I]	ACC	Thr [T]	AAC	Asn [N]	AGC	Ser [S]	C
	ATA	Ile [I]	ACA	Thr [T]	AAA	Lys [K]	AGA	Arg [R]	A
	ATG	Met [M]	ACG	Thr [T]	AAG	Lys [K]	AGG	Arg [R]	G
G	GTT	Val [V]	GCT	Ala [A]	GAT	Asp [D]	GGT	Gly [G]	T
	GTC	Val [V]	GCC	Ala [A]	GAC	Asp [D]	GGC	Gly [G]	C
	GTA	Val [V]	GCA	Ala [A]	GAA	Glu [E]	GGA	Gly [G]	A
	GTG	Val [V]	GCG	Ala [A]	GAG	Glu [E]	GGG	Gly [G]	G

3.1.3 Formulas and Molecular Weights

Amino acid residue masses (mono-isotopic and average) together with 3- and 1-letter code and elemental composition:

Name	Symbol	C	H	N	O	S	Monoisotopic Mass	Averaged Mass
Alanine	Ala (A)	3	5	1	1	0	71.03712	71.079
Cysteine	Cys (C)	3	5	1	1	1	103.00919	103.145
Aspartic acid	Asp (D)	4	5	1	3	0	115.02695	115.089
Glutamic acid	Glu (E)	5	7	1	3	0	129.0426	129.116
Phenylanaline	Phe (F)	9	9	1	1	0	147.06842	147.177
Glycine	Gly (G)	2	3	1	1	0	57.02146	57.052
Histidine	His (H)	6	7	3	1	0	137.05891	137.141
Isoleucine	Ile (I)	6	11	1	1	0	113.08407	113.159
Lysine	Lys (K)	6	12	2	1	0	128.09497	128.174
Leucine	Leu (L)	6	11	1	1	0	113.08407	113.159
Methionine	Met (M)	5	9	1	1	1	131.04049	131.199
Asparagine	Asn (N)	4	6	2	2	0	114.04293	114.104
Proline	Pro (P)	5	7	1	1	0	97.05277	97.117
Glutamine	Gln (Q)	5	8	2	2	0	128.05858	128.131
Arginine	Arg (R)	6	12	4	1	0	156.10112	156.188
Serine	Ser (S)	3	5	1	2	0	87.03203	87.078
Threonine	Thr (T)	4	7	1	2	0	101.04768	101.105
Valine	Val (V)	5	9	1	1	0	99.06842	99.133
Tryptophan	Trp (W)	11	10	2	1	0	186.07932	186.213
Tyrosine	Tyr (Y)	9	9	1	2	0	163.06333	163.176

3.1.4 Chemical Structures

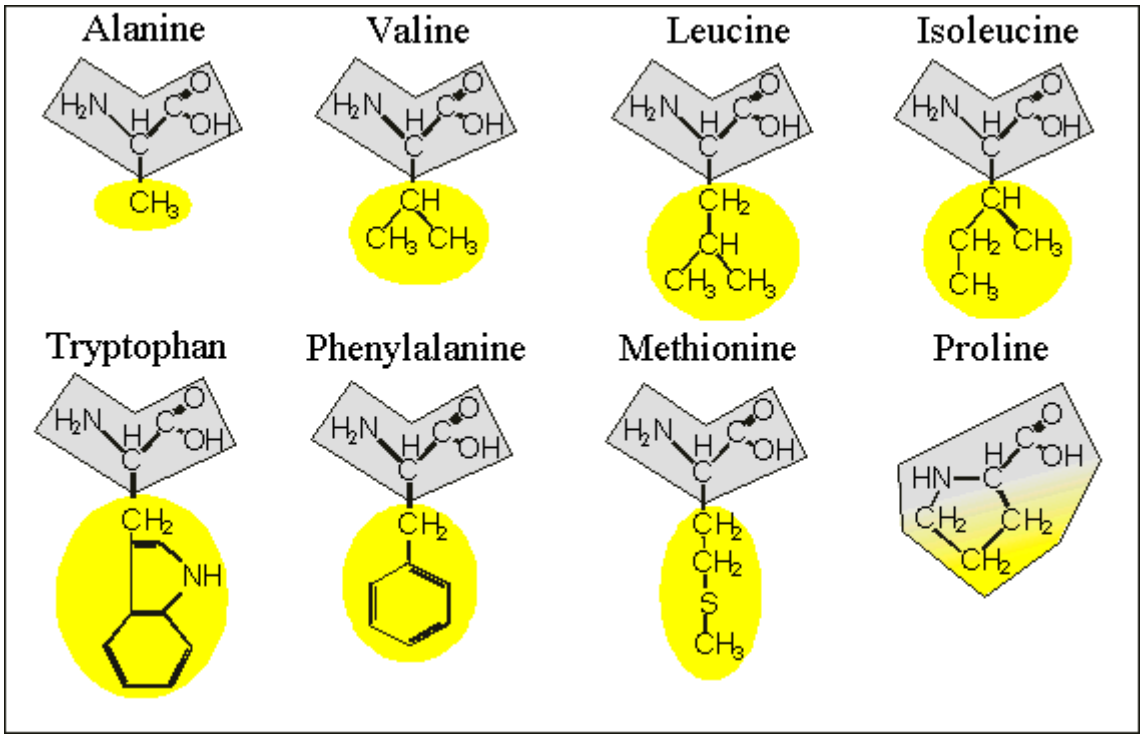


Figure 3-1 Neutral hydrophobic amino acids

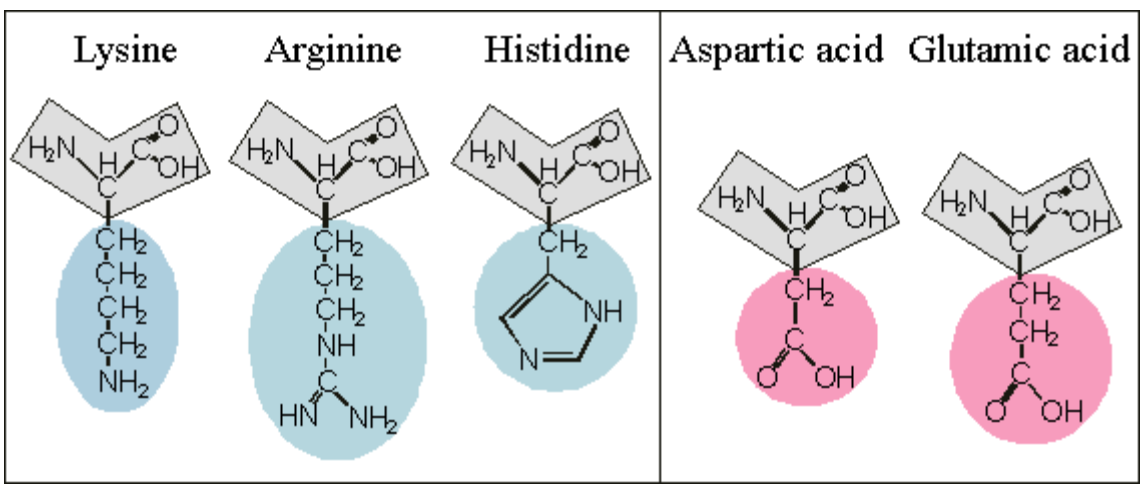


Figure 3-2 Basic and acidic amino acids

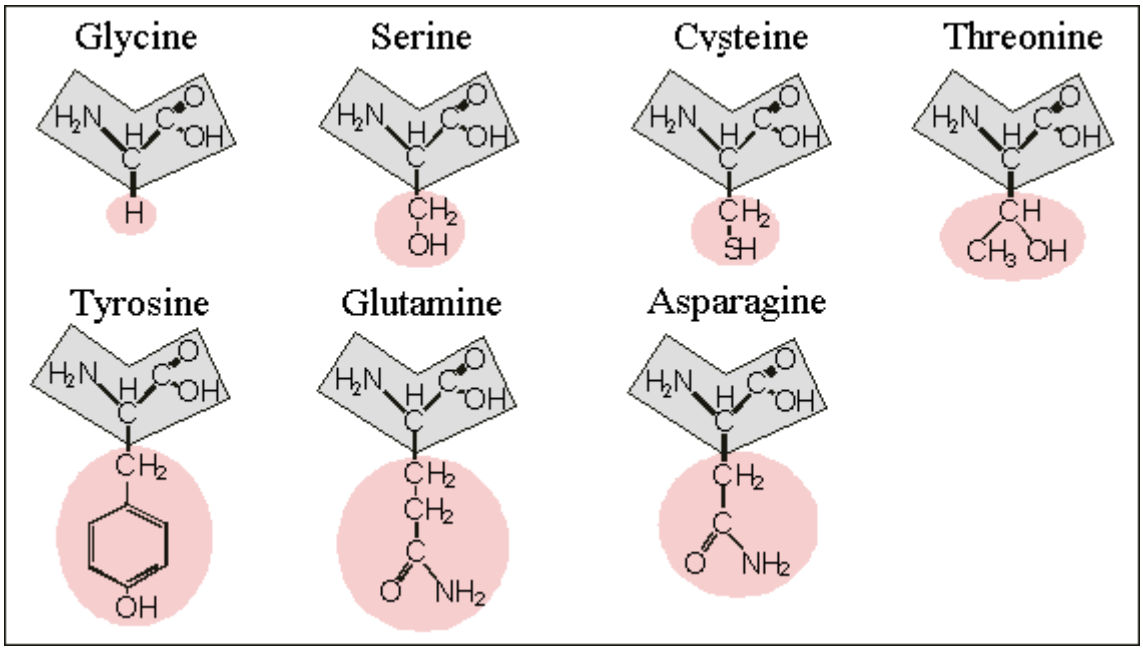


Figure 3-3 Neutral polar amino acids

3.2 Peptide Fragmentation

The types of fragment ions observed in an MS/MS spectrum depend on many factors including primary sequence, the amount of internal energy, how the energy was introduced, charge state, etc. The nomenclature used for fragment ions is the Biemann nomenclature (R. S. Johnson, S. A. Martin & K. Biemann (1988), Int. J. Mass Spec. Ion Procs. 86, 137-154).

Fragments will only be detected if they carry at least one charge. If this charge is retained on the N terminal fragment, the ion is classed as either *a*, *b* or *c*. If the charge is retained on the C terminal, the ion type is either *x*, *y* or *z*. An index indicates the number of residues in the fragment. In addition to the proton(s) carrying the charge, c ions and y ions abstract an additional proton from the precursor peptide. Note that these structures include a single charge carrying proton. In electrospray ionization, tryptic peptides generally carry two or more charges, so those fragment ions may carry more than one proton.

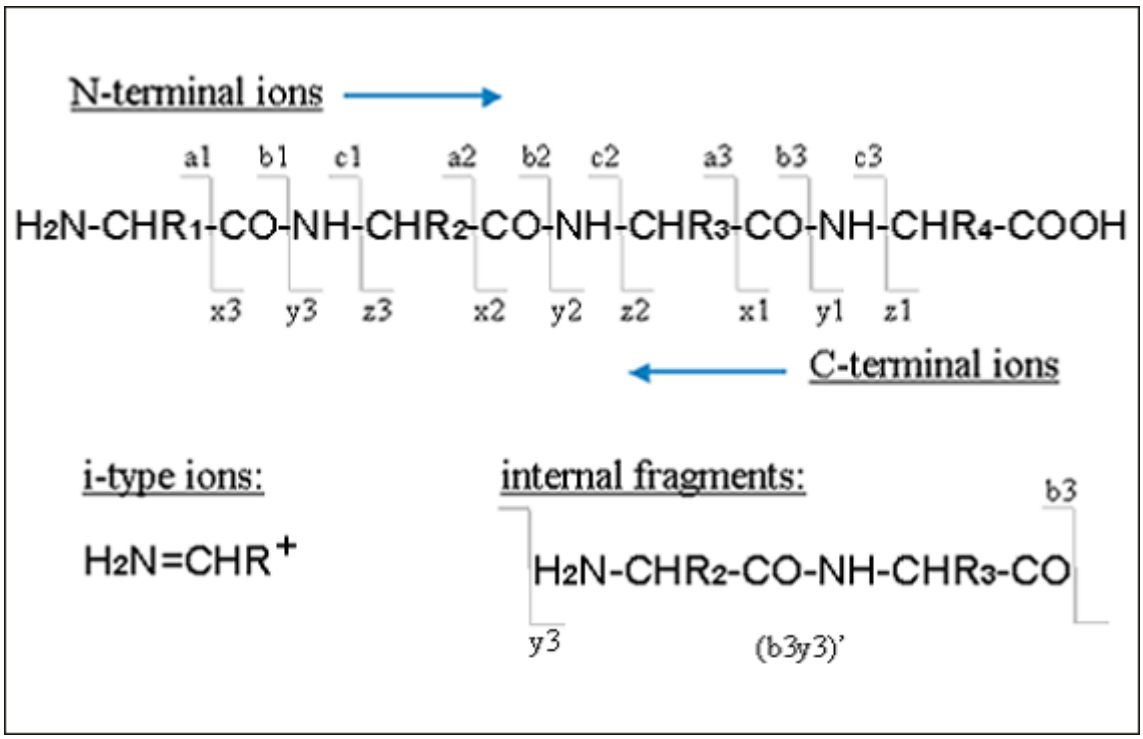


Figure 3-4 Peptide Fragmentation

Typical fragment ions observed are:

- Low energy CID: b and y
- PSD: a, b, y and i, including neutral losses of NH_3 from a and b
- ISD: c and y
- ECD-FTICR: c and z

Fragmentation of the backbone at two sites gives rise to internal fragments. Usually, these are formed by a combination of *b*-type and *y*-type cleavage to produce the illustrated structure, amino-acylium ion. Sometimes, internal ions can be formed by a combination of *a*-type and *y*-type digest, an amino-immonium ion.

An internal fragment with just a single side chain formed by a combination of *a* type and *y* type digest is called an immonium ion. The immonium ions can be used for *DeNovo* sequencing. The values from the following table are used to find these ions.

Immonium and related ion masses:

Residue	3-letter code	1-letter code	Immonium ion	Related ions
Alanine	Ala	A	44	
Cysteine	Cys	C	76	
Aspartic acid	Asp	D	88	
Glutamic acid	Glu	E	102	
Phenylalanine	Phe	F	120	148
Glycine	Gly	G	30	
Histidine	His	H	110	82 155
Isoleucine	Ile	I	86	44 72
Lysine	Lys	K	101	84
Leucine	Leu	L	86	44 72
Methionine	Met	M	104	60
Asparagine	Asn	N	87	
Proline	Pro	P	70	98
Glutamine	Gln	Q	76	
Arginine	Arg	R	112	100 87 70 60
Serine	Ser	S	60	
Threonine	Thr	T	74	
Valine	Val	V	72	
Tryptophan	Trp	W	159	
Tyrosine	Tyr	Y	136	

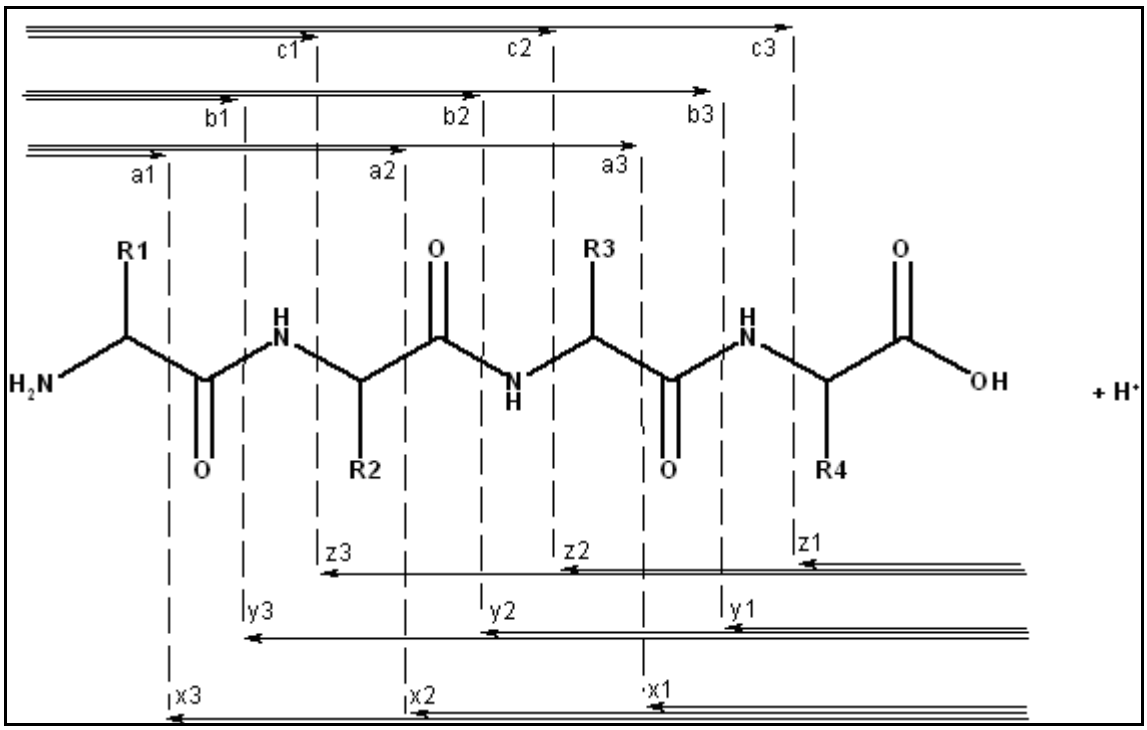


Figure 3-5 Fragmentation pattern for peptides

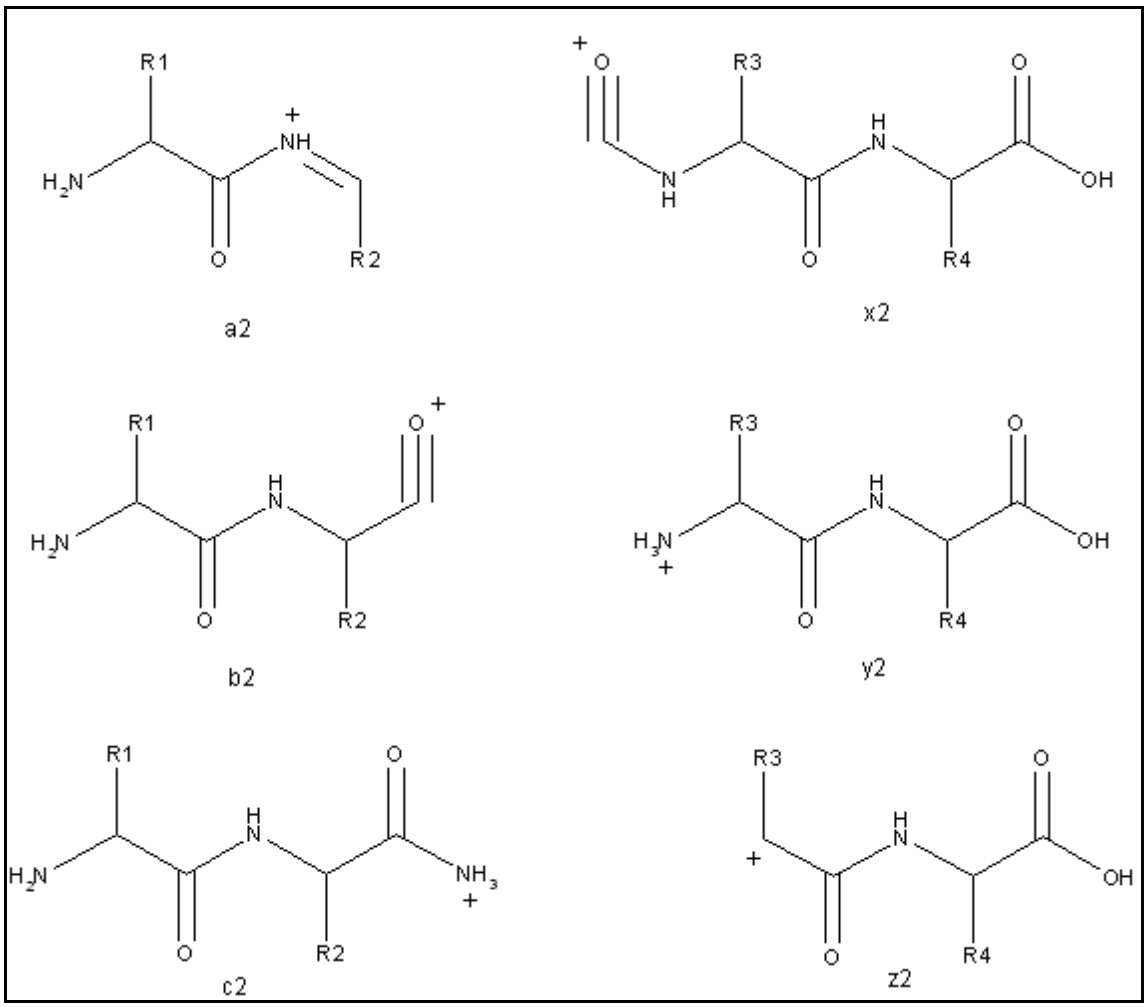


Figure 3-6 Structures of the fragments

3.3 Toolbar Reference and Shortcut List for BioTools

To hide or display the toolbar, choose menu *View - Toolbar*.

The toolbar can be dragged using the mouse. Click with the left mouse button on the background of the toolbar and move the toolbar with the mouse button pressed to the desired position.

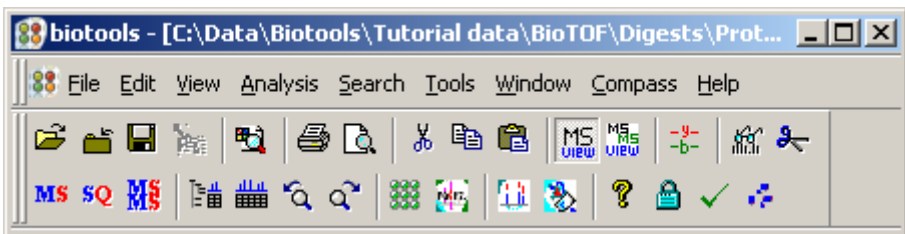


Figure 3-7 Toolbar of BioTools

Toolbar button	Menu option	Shortcut / Function key	Description
	File – Open Spectrum	*Ctrl + O*	Opens a file open dialog.
	File – Close All		Closes all opened data sets.
	File – Save	*Ctrl + S*	Saves the state of the active data file.
	File – Send		Sends the data file back to ProteinScape.
	File – Find		Opens a file manager for easier data handling and finding.
	File – Print		Prints the active data file immediately in accordance to the Print Setup.
	File – Print Preview		Shows a preview for controlling further printing.
	Edit – Cut	*Ctrl + X*	Deletes and copies into clipboard.
	Edit – Copy	*Ctrl + C*	Copies from clipboard to cursor position.
	Edit – Paste	*Ctrl + V*	Pastes from clipboard to cursor position.

Toolbar button	Menu option	Shortcut / Function key	Description
	*View – Activate MS view*	*F4 toggle*	Changes the view of the sequence in the Fragments window to MS view.
	*View – Activate MS/MS view*	*F4 toggle*	Changes the view of the sequence in the Fragments window to MS/MS view.
	*Analysis – Annotation Parameter*	-	Opens the annotation options dialog box.
	*Search – Search Mass One Protein SequenceEditor*	-	Opens the Search for mass dialog box in SequenceEditor (Search – Mass Search).
	*Search –Digest SequenceEditor*	-	Opens the Perform Digest dialog box in the SequenceEditor (Search – Perform Digest).
	*Search – Mascot Peptide Mass Fingerprint*	-	Opens the internet search via Peptide Mass Fingerprint.
	*Search – Mascot Sequence Query*	-	Opens the internet search via Sequence Query.
	*Search – Mascot MS/MS Ion Search*	-	Opens the internet search via MS/MS Ion Search.
	*Window – Show/Hide Treeview*	-	Show or hide the Treeview window.
	*Window – Show/Hide Fragments*	-	Show or hide the Fragments window.
	*View – Undo zooming*	-	The previous zoom action is undon.e
	*View – Redo zooming*	-	The previous zoom action is redone.
	*Search – Mascot Batch*	-	Opens the Mascot Batch Mode window.
	*Window – Start SequenceEditor*	-	Loads a sequence into the Sequence Editor and starts this program
	*Tools – Open Analysis in flexAnalysis*		This menu option starts the program flexAnalysis for TOF data.
	*Tools – Open Analysis in DataAnalysis*		This menu option starts the program DataAnalysis for ion trap data.
	*Help – About BioTools*	-	Opens About BioTools window.
	*Compass – Lock All Applications*	*Ctrl + Alt + K*	All applications will be locked and can only be unlocked be a certified user.

Toolbar button	Menu option	Shortcut / Function key	Description
	*File – Sign Electronic Record*		The active data file can be sign by a certified operator to the electronic record.
	*File – Audit Trail*		The file of the audit trail can be reviewed.
	File – Print	*Ctrl + P*	Opens the printer dialog.
	File – Exit	*Alt + F4*	Terminates the program.
	Analysis – Edit Peaks	*Ctrl*	The editing of peaks is permanent switched on. A click with RMB in the Spectrum window ends this function.
	Help – Topics	*F1*	Opens the BioTools Online Help.
	Special short keys for handling the zoomed area in the Spectrum window:	*F9*	The extracted area is zoomed in.
		F10	The extracted area is zoomed out.
		F11	The extracted area is moved right.
		F12	The extracted area is moved left.

3.4 Toolbar Reference and Shortcut List for SequenceEditor

To hide or display the Toolbar, choose menu *View - Toolbar*.

Figure 3-8 Toolbar of SequenceEditor

If at least one sequence is opened, this feature is focused to the top toolbar of the sequence window.

Toolbar button	Menu option	Shortcut/ Function key	Description
	File – New Sequence	Ctrl + N	Creates a new sequence, also from the web.
	File – Save	Ctrl + S	Saves the active sequence with its current name. If you have not named the sequence, your SequenceEditor displays the Save As dialog box.
	File – Append To Document		This appends the currently active sequence to the .sqs file it is based on. This button is the inverse of the "Delete Sequence" context menu available in the treeview and can be used to manage the contents of .sqs files flexibly.
	Edit – Cut	Ctrl + X	Removes selected data from the sequence and stores it on the clipboard.
	Edit – Copy	Ctrl + C	Copies the selection to the clipboard.
	Edit – Paste	Ctrl + V	Inserts the contents of the clipboard at the insertion point.
	-		The modified sequence or a marked range of it will be sent to BioTools for further processing.
	-		Prints the active data file immediately.
	Help – About SequenceEditor…		Opens About SequenceEditor window.
	-		Activates the context sensitive help.

Toolbar button	Menu option	Shortcut/ Function key	Description
	Compass – Lock All Applications	Ctrl + Alt + K	All applications will be locked and can only be unlocked be a certified user.
	File – Sign Electronic Record		The active data file can be sign by a certified operator to the electronic record
	File – View Signatures		Opens the View Signatures dialog.
	File – Audit Trail		The file of the audit trail can be reviewed.
	File – Print	Ctrl + P	Opens the printer dialog.
	File – Exit	Alt + F4	Terminates the program.

3.5 Part Numbers

1838877 Software-Package BioTools 3.2 SR5

8256590 License BioTools 3.2

8256591 License RapiDeNovo Sequencing

8256589 BioTools 3.2 User Manual

4 INDEX