

PEAKS 4.2

PEAKS Studio ▲ PEAKS Client ▲ PEAKS Viewer

User's Manual

superior
de novo sequencing and protein ID
software



BIOINFORMATICS SOLUTIONS INC

PEAKS 4.2 User's Manual

PEAKS Studio (S)

PEAKS Client (C)

PEAKS Viewer (V)

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INTRODUCTION	4
INTRODUCTION TO PEAKS 4.2	4
HOW TO USE THIS MANUAL.....	4
Scope.....	5
Terminology and Abbreviations Glossary.....	5
Files and Format Glossary.....	7
GETTING STARTED WITH PEAKS 4.2.....	8
SCV WHAT WE WILL NEED	8
Package contents.....	8
System requirements	8
Instrumentation	8
SCV INSTALLATION	9
SC CONNECTING TO A PEAKS ONLINE SERVER	10
SC REGISTERING PEAKS.....	10
SC DATABASE CONFIGURATION.....	11
FEATURES WALKTHROUGH	17
SC BEGIN THE WALKTHROUGH.....	17
SVC VIEWING RESULTS	19
SVC MANIPULATING RESULTS.....	21
S INCHORUS SEARCHING.....	22
GRAPHICAL USER INTERFACE.....	25
SVC WINDOWS, DIALOGUES, FRAMES AND REPORTS.....	26
SVC PEAKS 4.2 <i>main window</i>	26
SC <i>Auto de Novo Parameters Dialogue</i>	27
SC <i>Protein Identification Parameters Dialogue</i>	29
SCV PEAKS <i>Properties Dialogue</i>	31
SCV <i>Enzyme Editor Dialogue</i>	32
SC <i>PTM Selector Dialogue</i>	33
SCV <i>PTM Editing Dialogue</i>	34
SCV <i>Environment Preferences Window</i>	34
SCV <i>Protein Identification Result Window</i>	35
De Novo view.....	35
Peptide View.....	35
Protein View	36
Search parameters.....	37
Filter pane	37
SCV <i>Main Processing Window</i>	40
SCV <i>Ion Editor</i>	42
SCV <i>Export Image Dialogue</i>	43
SCV <i>Print Image Dialogue</i>	44
SVC TOOLBARS	44
SCV <i>Main window toolbar</i>	44

SCV	Main Processing Window Toolbar	46
PEAKS STUDIO CONFIGURATION		47
SCV	PEAKS PROPERTIES CONFIGURATION.....	48
SV	Creating and defining PTM	48
	Creating a New PTM.....	49
	Editing a PTM.....	50
	Removing a PTM.....	50
S	Database Manager	50
	Load/Configure a new database	51
	Remove a Database.....	51
	Edit a Database.....	51
	Moving/Updating a Database	51
SV	IMPORTING AND EXPORTING PEAKS PROPERTIES	52
SVC	PEAKS ENVIRONMENT PREFERENCE CONFIGURATION.....	53
S	Removing Saved Parameters	54
SCV	Configuring the ion table	55
SCV	Environment – Changing default file handling settings	56
SC	Environment – client to PEAKS Online.....	56
SCV	Changing Colours.....	56
SCV	Display – Changing typefaces and peak width	57
SCV	Changing Manual de novo defaults.....	58
LOADING AND PREPARING DATA		61
SCV	LOADING DATA INTO PEAKS 4.2.....	62
SCV	Opening data files	62
SCV	Loading a directory full of DTA files	63
SCV	Loading Thermo RAW data	64
SCV	Importing Applied Biosystems WIFF data	64
SCV	Importing Masslynx RAW data	64
SCV	Importing Data from the ABI 4700 or ABI 4800	65
	System Requirements	65
	Configuration:	65
	Data extraction procedure:	66
SC	REFINING DATA BEFORE ANALYSIS.....	66
SCV	MANUALLY MANIPULATING DATA FILES	69
SCV	Editing Precursor information	69
SCV	Manually merging MS/MS scans of the same peptide	69
SCV	Cutting and Copying Spectrum Data	70
SCV	Pasting Spectrum Data.....	70
ANALYSING DATA WITH PEAKS 4.2		71
SCV	USING PEAKS STUDIO WITH MODIFICATIONS (PTM)	72
SC	AUTO DE NOVO SEQUENCING.....	73
SCV	VIEWING AUTO DE NOVO RESULTS	77
SCV	EDITING SEQUENCING RESULTS (PREPARATION)	78

SCV	MANUAL DE NOVO SEQUENCING.....	80
SCV	<i>Creating a fresh spectrum for sequencing</i>	80
SCV	Manual De novo Operations	80
	Selecting a peak	80
	Measuring distance along the m/z scale	80
	Measure the m/z difference between two PEAKS.....	81
	Deselect a peak	81
	Zoom in on part of the spectrum	81
	Add (remove) ions to (from) a peak	81
	Using sequence tags.....	82
	Undoing an edit	82
	Redoing an edit	82
SCV	SUGGESTING A SEQUENCE TO SEE HOW IT FITS THE DATA	83
SC	PROTEIN IDENTIFICATION.....	85
SCV	PEAKS protein identification	85
S	<i>inChorus protein identification</i>	88
SC	CREATING A HIGH-THROUGHPUT WORKFLOW	89
SCV	SELECTING UNMATCHED DE NOVO RESULTS	90
SC	RUNNING PROTEIN IDENTIFICATION ON SELECT SPECTRA	91
SCV	USING THE MASS CALCULATOR.....	92
	WORKING WITH RESULTS.....	94
SCV	VIEWING PROTEIN IDENTIFICATION RESULTS.....	95
SCV	COMPARING PROTEINS – MULTIPLE SEQUENCE ALIGNMENT.....	99
SCV	FILTERING RESULTS.....	100
SCV	<i>How filters act on de novo sequences</i>	102
SCV	Filter examples.....	103
	Publication	103
	Digging for a protein by name.....	103
	Setting a protein mass range	103
SCV	<i>Saving/Loading Filter sets</i>	103
SCV	Toggling columns	104
SCV	EXPORTING RESULTS	104
SCV	WYSIWYG reports.....	104
SCV	Exporting peak lists	105
SCV	Exporting Sequences by spectrum.....	105
SCV	Exporting Exclusion Lists	105
SCV	Exporting high-resolution spectral images.....	105
SCV	SAVING RESULTS.....	107
	ABOUT BIOINFORMATICS SOLUTIONS INC.	108
	PEAKS SOFTWARE LICENSE	109
	REFERENCE: PEAKS PAPER.....	111

Introduction

Introduction to PEAKS 4.2

PEAKS makes the interpretation of MS/MS data easier and faster.

PEAKS is an innovative software system designed to derive amino acid sequences and identify proteins from tandem mass spectrometry data. After running MS/MS on a protein sample, PEAKS performs *de novo* sequencing and database search identification of the protein(s) and peptides using raw experimental data.

PEAKS 4.2 provides peptide sequence and protein identification results via an intuitive interface, allowing for rapid visual interpretation. PEAKS provides both auto and manual *de novo* sequencing tools for detailed examination of MS/MS spectra, providing the flexibility to manually modify auto *de novo* results when searching for additional sequence possibilities.

How to use this manual

This user's manual is intended to help us get started using PEAKS 4.2, acquaint us with its functionality, show us how to customize PEAKS to our application, allow us to work efficiently with the interface, provide a task based reference, and help us with troubleshooting. As such, this manual is organized into chapters based on these categories. Use the table of contents at the front of this manual to access the relevant section. If searching for the definition of a particular term (or abbreviation), please consult the glossary – found in this section. The glossary will tell us what a particular term means, but it will not tell us how it applies to PEAKS usage.

Since PEAKS Studio, PEAKS Viewer and PEAKS Client share the same user's interface, this manual covers all three software programs. **Where a section is**

applicable to **PEAKS Studio**, an '**S**' is noted, '**C**' denotes **PEAKS Client**, and '**V**' denotes **PEAKS Viewer**. Some sections apply to all three, so 'SCV' is noted.

Scope

PEAKS users are assumed to be familiar with computer usage, and the operating system environment. As such, it is beyond the scope of this manual to instruct the user on the use of windows, dialogue boxes, menus, file storage etc. Please refer to the operating system's manual, or computer help books for such information. Similarly, PEAKS users are expected to be familiar with mass spectrometry, standard operating practices and data.

Terminology and Abbreviations Glossary

a-ions: an N-terminal fragment holding at least one charge; similar to b-ions and c-ions. This is a prefix fragment of the peptide. The a-ion's mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues, subtract the mass of Carbon Monoxide.

b-ions: an N-terminal fragment holding at least one charge; similar to a-ions and c-ions. This is a prefix fragment of the peptide. The b-ion's mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues.

BSI (Bioinformatics Solutions Inc.): The makers of PEAKS and other fine bioinformatics software.

c-ions: an N-terminal fragment holding at least one charge; similar to a-ions and b-ions. This is a prefix fragment of the peptide. The c-ion's mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues, plus the mass of ammonia.

Deconvolution: rearrangement of the spectrum to show each monoisotopic peak as if it were singly charged. Thus, to reposition them on the scale, PEAKS multiplies the m/z of ion's that were doubly charged by two. Note that the deconvolved scale PEAKS shows is 'at +1.'

Fixed modification: selecting a post-translational modification as a fixed modification tells PEAKS that this modification is applied to all occurrences of the residue(s) that the PTM can act on.

Enzyme: The residues PEAKS can find in different positions in the sequence. This is based on information about the enzyme used to digest our protein sample.

ESI (Electrospray Ionization): A method for ionizing a sample into the mass spectrometer.

m/z : mass to charge ratio.

MALDI (Matrix-Assisted Laser Desorption/Ionization): A method for ionizing a sample into the mass spectrometer. This has a characteristic effect of only producing singly charged ions.

Mass accuracy: this refers to the accuracy of the spectrometer and its resulting data. On a spectrum, this is reflected by how close the peaks are to the actual masses of the ions they represent.

PTM (Post Translational Modification): A protein, just translated and hence newly formed, may differ from its final form as a result of interaction with the cellular environment (or the experimental environment). As they interact chemically with the environment, residues may gain or lose molecules. This change is referred to as a post-translational modification. Since PTM changes the mass of residues, it must be accounted for when sequencing peptides by mass spectrometry.

- **Built-in PTM:** PEAKS comes equipped with a library of possible post-translational modifications. These can be incorporated into a *de novo* analysis at the click of a button.
- **Customized PTM:** If the post-translational modification we are looking for is not in the PEAKS PTM set, we may create our own entry, or modify an existing one. This will appear as a customized PTM in the set.
- **PTM library:** A listing of all possible (built-in and custom entered) post-translational modifications that PEAKS can use as a part of its analysis.

Residue: as used in this manual, a residue refers to what remains of an amino acid once it has become part of a peptide, or peptide fragment. In this manual, residues are referred to by their original amino acid names.

Resolution: refers to the precision of an instrument. On a spectrum, this is reflected by how close together can two peaks be and still be told apart.

Variable modification: selecting a post-translational modification as a variable modification tells PEAKS that this modification may or may not be applied to any given occurrence of the residue(s) that the PTM can act on.

x-ions: a C terminal fragment holding at least one charge; similar to y-ions and z-ions. This is a suffix fragment of the peptide. The x-ion's mass will be the sum of the masses of the C terminal group, plus the intervening neutral amino acid residues, plus the mass of Carbon Monoxide.

y-ions: a C terminal fragment holding at least one charge; similar to x-ions and z-ions. This is a prefix fragment of the peptide. The y-ion's mass will be the sum of

the masses of the C terminal group, plus the intervening neutral amino acid residues, plus the mass of H₂.

z-ions: a C terminal fragment holding at least one charge; similar to x-ions and y-ions. This is a suffix fragment of the peptide. The z-ion's mass will be the sum of the masses of the N terminal group, plus the intervening neutral amino acid residues, subtract the mass of ammonia.

Files and Format Glossary

ANN data file: within the ANZ file, a folder contains ANN data files that store the MS/MS information and peptide information of one spectrum.

ANN index file: within the ANZ file, is one compressed file used to organize the data; the ANN index file links to a directory containing multiple ANN data files.

ANZ file: a PEAKS 'zip-compressed' XML-based Annotated spectrum file format. ANZ files preserve all the information from the PEAKS session.

DTA: The file format associated with SEQUEST software.

FASTA (Fast-All): A standard sequence database file format used for protein identification. PEAKS can identify proteins from any FASTA-format database of proteins.

MGF: The file format associated with Mascot software.

PKL: The file format associated with Micromass instruments.

Getting started with PEAKS 4.2

Everything we need to know from the beginning and step by step.

This section of the manual will guide us through the process of installation and configuration of PEAKS 4.2. If problems persist, contact technical support.

SCV

What we will need

Package contents

The PEAKS 4.2 package should contain:

- This manual
- PEAKS 4.2

System requirements

PEAKS 4.2 will run on most platforms with the following requirements:

- Equivalent or superior processing power to a Pentium at 500 MHz.
- At least 1 GB of memory (RAM). 1.5GB is recommended. For PEAKS Studio / PEAKS Client. PEAKS Viewer requires only 500MB.
- JAVA Virtual Machine 1.5 or better (provided on installation).

Instrumentation

PEAKS 4.2 will work with data from any type of tandem mass spectrometer designed for proteomics work.

PEAKS will accept data in the following formats:

mzXML is a standard data format from the Sashimi Project. It is an XML based format.

mzData is a standard data format from the Human Proteome Organization

Applied Biosystems instrument's data in .wiff format provided that PEAKS, the Infocromics converter plug-in and Analyst are installed on the same computer. PEAKS has the ability to read directly from the 4700/4800 Oracle database.

Bruker instrument's data in .yep, .baf and .fid formats.

Thermo Electron instrument's data in RAW format provided that PEAKS and XCalibur are installed on the same computer, or dta format (and concatenated dta formats), with the ability to load an entire folder full of dta's.

Waters/Micromass instrument's data in .RAW format, provided that PEAKS and MassLynx are installed on the same computer, or pkl files.

All instrument's data as can be converted into mzXML, mzData, .pkl, dta, or .mgf.

SCV

Installation

To make sure we only use the latest information available to PEAKS, if we already have PEAKS installed on our system, we must uninstall it before proceeding.

1. Close all programs that are currently running and end all non-system tasks.
2. Insert the PEAKS 4.2 disc into the CD-ROM drive.
3. Auto-run should automatically load the installation software. If it does not, find the CD-ROM drive and open it to access the disc. Click on the exe file.
4. A menu screen will appear. Select the top item "Install".
5. The installation utility will begin the install. Wait while it does so. Choose 'English' as the language for installation instructions. When the "PEAKS 4.2" installation dialogue appears, click the "Next" button.
6. Read the license agreement. If we agree to it, we change the radio button at the bottom to select "I accept the terms of the License Agreement" and click "Next".
7. Next we choose the folder/directory in which we'd like to install PEAKS 4.2. Press the "Choose..." button to browse our system and make a selection, or type a folder name in the textbox. Click "Next".
8. Choose where we'd like to place icons for PEAKS 4.2. The default will put these icons in the programs section of our start menu. Click "Next".

9. Review the choices we have made. We can click “Previous” if we’d like to make any changes or click “Next” if those choices are correct.
10. PEAKS 4.2 will now install on our system. We may cancel at any time by pressing the “Cancel” button in the lower left corner.
11. When installation is complete, click “Done”. The “PEAKS 4.2” menu screen should still be open. One may view movies and materials from here. To access this menu again, we simply insert the disc in our CD-ROM drive.

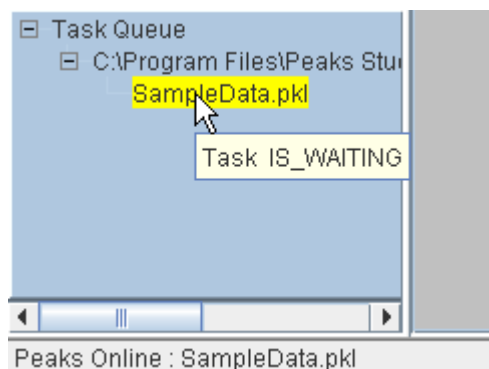
SC

Connecting to a PEAKS Online Server

PEAKS Client sends all its intensive computation jobs, like PEAKS auto de novo, and PEAKS Protein ID, to a PEAKS Online server, installed somewhere in the lab or computing facility. PEAKS Studio can optionally do this too.

In the **Environment** tab of the Environment preferences dialogue, change the “Default PEAKS Invocation” to “PEAKS Online. Then specify the IP address or URL of the PEAKS Online server, the port number to use, and a username and email address. Press ok. The email is used to notify us that a job has been completed, in the event that we close PEAKS 4.2 before finishing.

It’s important to monitor the task queue when running jobs on the PEAKS Online server. Hold the mouse over a job that’s in the queue to see it’s status.



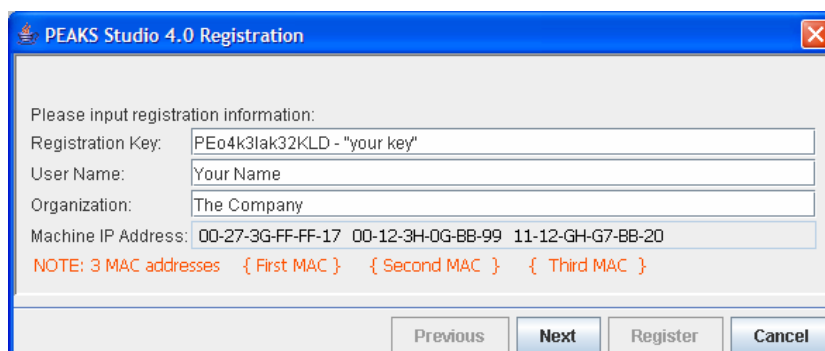
SC

Registering PEAKS

The first time we run PEAKS, we will be told that the product is not registered. Press the “Ok” button and a dialogue will appear. Enter the registration key that came with the product – whether it be a key for the full version or time limited trial version. We must also enter our name, the name of our organization, and the MAC (Media Access Control) address of the machine we are going to use PEAKS on. If we are connected

to the internet, registration will be completed automatically. If all is well, a dialogue will show “Registration Successful” and PEAKS will load.

The software uses your computer’s MAC address as a unique identifier for the computer. As such, if you have more than one MAC address for your computer, you may experience problems in registering PEAKS. The display will be something like this:



If this occurs, try disabling your wireless network card, restarting windows and plug into an Ethernet cable only. Then register with PEAKS. If problems still occur, please contact BSI technical support.

Re-registering PEAKS may be necessary if our license has expired or if we wish to update the license. We will need to obtain a new registration key from BSI. Once we have obtained this new key, select “Register Peaks” from the Help menu. The “License Upgrade” dialogue box will appear, cautioning us that we are about to update the license. Press the “Ok” button to continue. Follow the on-screen instructions.

SC

Database Configuration

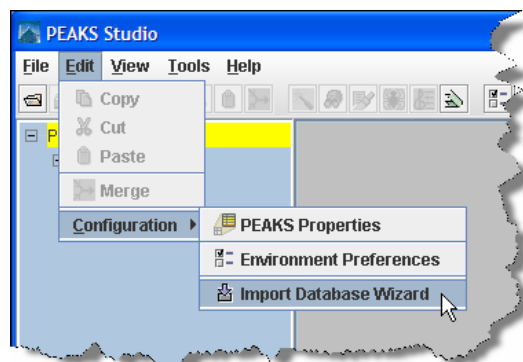
In addition to *de novo* sequencing of peptides, PEAKS 4.2 also has the ability to search through a database search to identify proteins. But in order to use this function, PEAKS must have access to a protein or EST database in FASTA format or an EST database of DNA sequences. We can point PEAKS to an existing database on our system, or download one. Additionally, we can associate taxonomy with certain databases. This is database configuration.

We can use PEAKS without the database search; PEAKS can perform *de novo* sequencing.

WARNING: Downloading a database can take a long time (8+ hours), depending on connection speed. Most only take 20 - 30 minutes.

To configure a database:

1. Load PEAKS 4.2. If we have not yet configured a database, the wizard will appear automatically. Otherwise...
2. In the 'edit' menu, select "Configuration", then "Import Database Wizard"
3. The Import Database Wizard will load and ask us to select a database to download – from the dropdown list. **If we already have a database we wish to use, we can select "Other database" from the dropdown list and skip to step6.** Click "Next"
4. Having selected a database, the Import Database Wizard will provide us with some information about that database. If this is, in fact, the database we wish to use, click the provided link to begin downloading. A dialogue box will appear with instructions on downloading using file transfer protocol (FTP). It does not matter where we put the download file, but we must remember where it is.



Microsoft Internet Explorer, for example, is an FTP client. We may use Internet explorer and the provided URL to download a database.

The next section provides a walkthrough of PEAKS 4.2's main functionality using the NCBI nr database.

A note on downloading databases

The links in the Wizard may be outdated because the owners of those download locations may change their URL periodically. If this is the case, remove all but the domain name and browse from there.

`ftp://ftp.ebi.ac.uk/pub/databases/MassSpecDB/msdb.fasta.z`
becomes:

`ftp://ftp.ebi.ac.uk/`

Best practices: configuring databases for use with X!Tandem

At the time of this writing, X!Tandem had trouble searching through large databases, and would crash. It is therefore suggested that X!Tandem only be used with small databases; or if used with a large database, a taxon should be specified. The NCBI nr and Swiss Prot databases are ideal for this purpose.

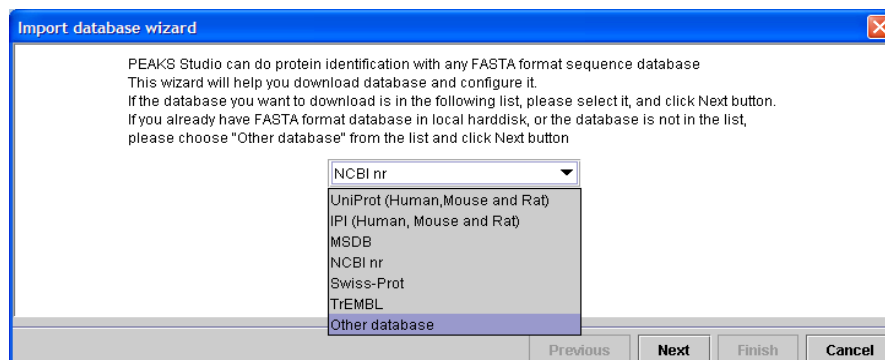
Best practices: configuring databases for use with OMSSA

At the time of this writing, we could not use OMSSA with databases that were not in NCBI format, or Swiss-Prot format, and have those results available to inChorus.

Also, a bug in OMSSA prevents us from easily using databases with OMSSA when they are stored in a folder that contains a space in its path. This creates problems when PEAKS creates temporary databases on our behalf. To avoid this, best practices suggest we put all our databases in a folder “c:\peaksdatabases”. The folder “c:\my documents\databases” wouldn’t work because it contains a space between ‘my’ and ‘documents’. Using spaces in the database file name causes the same problem. So after we download and extract our database we should call the database file “ncbinr.fas”, or “ncbi_nr.fas” rather than “ncbi nr.fas”.

5. The database we downloaded may be in a compressed file, perhaps a .zip or a .gz file. We must find the file and use a decompression utility, such as WinZip, or WinRar to extract its contents. The file inside the compressed file will be a FASTA format text file (a .fas or a .fasta file).
6. Return to the Import Database Wizard and click the “Next” button. This screen will allow us to configure the database. Click on the hyperlink next to

The taxonomy options are only available if the NCBI nr or Swiss-Prot database is selected and the Apply button has been pressed, or either database was selected on a previous screen.



each field for more information.

Import database wizard

Database Nickname:

Path:

☐ EST database

Advanced Options - Fasta header format set up

Use built-in database format: UniProt

Rule to parse accession/id string from FASTA title:

Rule to parse description string from FASTA title:

URL to get detailed information with accession/id:

Delimiter:

Taxonomy Options

gi_taxid

taxdmp

- PEAKS will ask us to enter the database nickname. This is a nickname that we chose to represent the database we are configuring. It doesn't matter what name we enter, but we must enter at least one character.
- The Path textbox shows where the database is located. It will be blank, so we must tell PEAKS where the database is. Type the location of the file into the textbox, or we can browse to find the file on our system. We must be sure to select the FASTA database, **not the compressed file of the same name (see step 5)**.
- If we chose one of the public standard databases (in step: 3) its format style will be displayed in the advanced options box. The selected database format is shown in the dropdown list. Accession number information and the way PEAKS parses the database headers – i.e. the parsing rules – are shown in the textboxes below.
- If our database is an EST database containing DNA sequences, check the 'EST database' checkbox.

Database header format is important for protein ID result reports. If parsed correctly, accession numbers and protein names will be shown in full.

- If we chose an “other database” (in step: 3) we must enter parsing parameters ourselves by typing in the textboxes. Alternatively, if our database format is the same as one of the public databases, we can choose to apply that database’s format when PEAKS reads our database. Select the database that is similar to ours from the dropdown list and **press the apply button** to fill the textboxes with the appropriate parsing rules.

The delimiter is the character used to separate multiple headers.

- If we are configuring the NCBI nr database or the Swiss-Prot database, we may choose to point PEAKS 4.2 to the location of the taxonomy files associated with that database. Under “Taxonomy Options” we must type the location of the taxonomy files, or click browse to find the file on our system. If we do not specify these taxonomy files, we will not be able to limit our database search to a specific taxon. We can use the compressed (.zip or .gz) files; **no decompression is required for the taxon files**.

A note on choosing the taxonomy files for NCBI nr

At the time of printing, the gi_taxid file was called: *gi_taxid_prot.dmp.gz* and the taxdmp file was called: *taxdmp.zip*. Select these files.

A note on choosing the taxonomy files for Swiss Prot

At the time of printing, the gi_taxid file was called: *speclist.txt* and the taxdmp file was called: *taxdmp.zip* – the same one as used by NCBI.

7. Press the “Finish” button to complete the database configuration.

We can repeat this process to configure a number of other databases. Once configured, a database need not be configured again unless we update the database itself.

Trouble shooting

Some problems with a database may not appear until we run a search. While PEAKS is quite tolerant of format errors in databases, other search engines called from the inChorus tool may not be. If there is an error in the search, it will be reported in a summary screen after the work has finished. If there is a problem, check the best practices outlined in this section. If the problem persists, it is possible that the database download was corrupted; try downloading again. Please contact technical support for help.

Features Walkthrough


Let's familiarize ourselves with PEAKS

This section of the manual will walk us through most of the basic functionality of PEAKS 4.2. After completing this section we will have seen how easy it is to load and view a data file, perform *de novo* sequencing, and database search protein identification.

SC

Begin the walkthrough

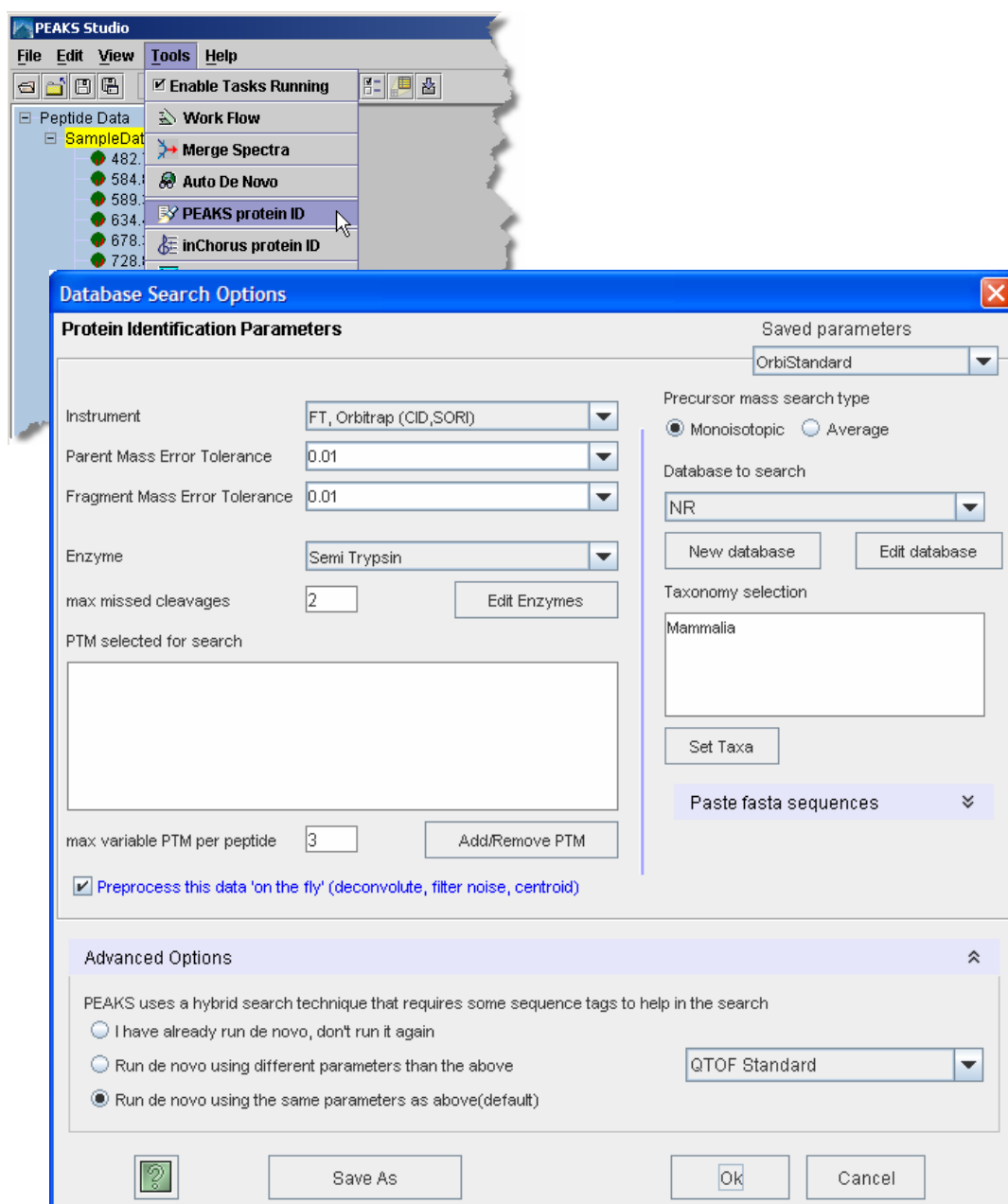
Run PEAKS 4.2, then download and configure the NCBI nr database. The procedures for doing so are outlined in the previous section.

The demo sample data should load automatically on startup under the heading OrbiOrbi.pkl. If it is not loaded, open the data file by clicking the  icon on the toolbar in the upper left corner of the PEAKS window, or selecting “Open” from the “File” menu. Sample data is located in the “C:\Program Files\PEAKS Studio\data\” folder. Load the file “OrbiOrbi.pkl” by clicking on it, then clicking “Open.”

The data file will appear in the left-hand frame. Make sure “OrbiOrbi.pkl” (i.e. the data file) is selected.

In the “Tools” menu, select “PEAKS Protein ID”. The protein identification options dialogue will appear.

Enter the settings as shown. Settings can be changed by clicking on the drop down list and selecting one of the options.



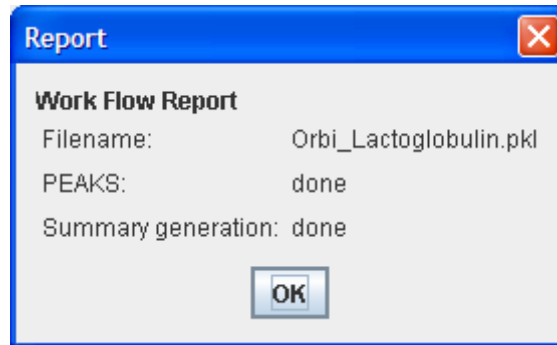
After entering the settings, as shown, click the “Save As” button to save these parameters for future use (PEAKS Studio only). When prompted, type OrbiStandard and press enter. Click “OK” to commence analysis. Analysis will be initialized (most of this time is spent creating a partial database, which only has to be done once) this takes one or two minutes.

After this, *de novo* sequencing will commence. For this sample it takes just over a minute, after which PEAKS database search will proceed. In total, the process takes less than two minutes for this sample (depending on the system’s processing speed and

memory). The PEAKS auto *de novo* algorithm derives sequence candidates for each of the 22 spectra in our example data file. These sequence candidate results for all eleven spectra in the example are then used for the database search component of PEAKS 4.2. PEAKS uses a unique sequence tag plus peptide fragment fingerprinting approach to protein identification.

SVC

Viewing Results



After the search is finished, the protein identification results will appear on screen. The 'Peptide View' is displayed by default. The display shows each spectrum for which PEAKS found a matching peptide. The spectra are grouped/sorted by index number. Since a spectrum may match to more than one peptide, there may be more than one entry

per spectrum. The list is sort-able; click the heading on each column to experiment with sorting by score, by mass, etc.

Click the 'Protein View' tab. PEAKS 4.2 presents a list of proteins that it believes to be the best match for the sample. The top section is an index, listing them by accession number, ranked in descending order from highest score on downward.

De Novo View Peptide View Protein View Search parameters Filter Pane									
Accession	Mass	Display	Score (%)	Coverage...	Query matc...	Marked	Description		
PEAKS DB Sea						<input type="checkbox"/>			
gi 229460	18,367		99	57.41%	18	<input type="checkbox"/>	lactoglobulin beta		
gi 1094913	64,669		29	0.83%	1	<input type="checkbox"/>	PREDICTED: similar to 82-kD FMRP Interacting P...		

The numbers under the "Mass" heading represent the mass of the protein displayed. The "Coverage" numbers represent the percentage of the proteins sequence covered by the matching peptides.

The correct protein, Lactoglobulin beta, is shown at the top of the list and with high score. Since one cannot distinguish between the different forms of Lactoglobulin Beta, PEAKS 4.2 groups them all together, thus avoiding cluttering the report. Click the plus sign next to gi|229460 for a listing of other possible lactoglobulin. The peptides matching these homologues will be the same set or a subset of Lactoglobulin beta matches. Collapse this list of homologues by clicking the minus sign next to gi|229460.

The listing, as shown above, is simply an index. We will find this useful, in the future, when dealing with complex mixtures. Clicking any protein's gi number will display the peptides matched to that protein in the bottom pane.

NCBI BLAST search of [gi|229460](#)

Links to retrieve entries containing this sequence

Accession/ID **Description**
[gi|229460](#) lactoglobulin beta

Peptides List

Index	m/z	z	Peptide
1	452.28497	2	TKIPAVFH
2	458.73856	2	LDAINEN
3	467.2729	2	LIVTQTMK
4	533.2925	2	VLVLDTD
5	545.92804	3	TPEVDD
7	597.3386	2	VLVLDTD
8	623.2947	2	TPEVDD
9	673.38513	1	GLDIQK
10	674.4233	1	IPAVFK
12	697.7167	3	LDAINEN
13	771.75635	3	VYVEELK
14	818.3876	2	TPEVDD
16	903.1304	3	VAGTWYS
17	903.5675	1	TKIPAVFH
18	933.5426	1	LIVTQTMK
20	1065.586	1	VLVLDTD
21	1157.1289	2	VYVEELK
22	1354.1898	2	VAGTWYSLAMAASDISLLDAQSAPLR

Matched peptides shown in Red

1 LIVTQTMKGL DIQKVAGTWY SLAMAASDIS LLDAQSAPLR VYVEELKPTP
 51 EGDLEILLQK WENDECAQKK IIAEKTKIPA VFKLDAINEN KVLVLDTDYK
 101 KYLLFCMENS AEPEQSLVCQ CLVRTPEVDD EALEKFDKAL KALPMHIRLS
 151 FMPTLQEEQC HI

467.2729 2 [Main processing window]

Above, 18 of the original 22 spectra indicated a peptide sequence matching with Lactoglobulin Beta. Most peptide matches shows a high confidence – strong evidence for having found the correct protein. We can also see exactly where the peptide fits into the protein sequence; with the matching sequences highlighted in red at the bottom.

As mentioned above, the peptide sequence results are based on a database search guided by an initial *de novo* sequencing analysis. Let's see how the *de novo* sequencing was able to help. Click on the entry for spectrum #3, **467.2729**. This will bring up the main processing window for spectrum 467.2729 2. Look in the top left frame to see the *de novo* and database results.

Color coding shows positional confidence scores. By the letters coded in red we can see that the PEAKS auto *de novo* analysis returned, with >90% confidence, the partial peptide sequence LLVXXTMK, but was not as sure of the middle two residues. The PEAKS DB Search was able to confirm this result, returning the peptide: LLVTQTMK.

Selecting another spectrum from the Peptide Data frame (left), e.g. 545.928 3, will allow us to view the results from that spectrum without having to return to the protein identification result. Click on the time and date stamp beneath the filename to return to the report.

Manipulating Results

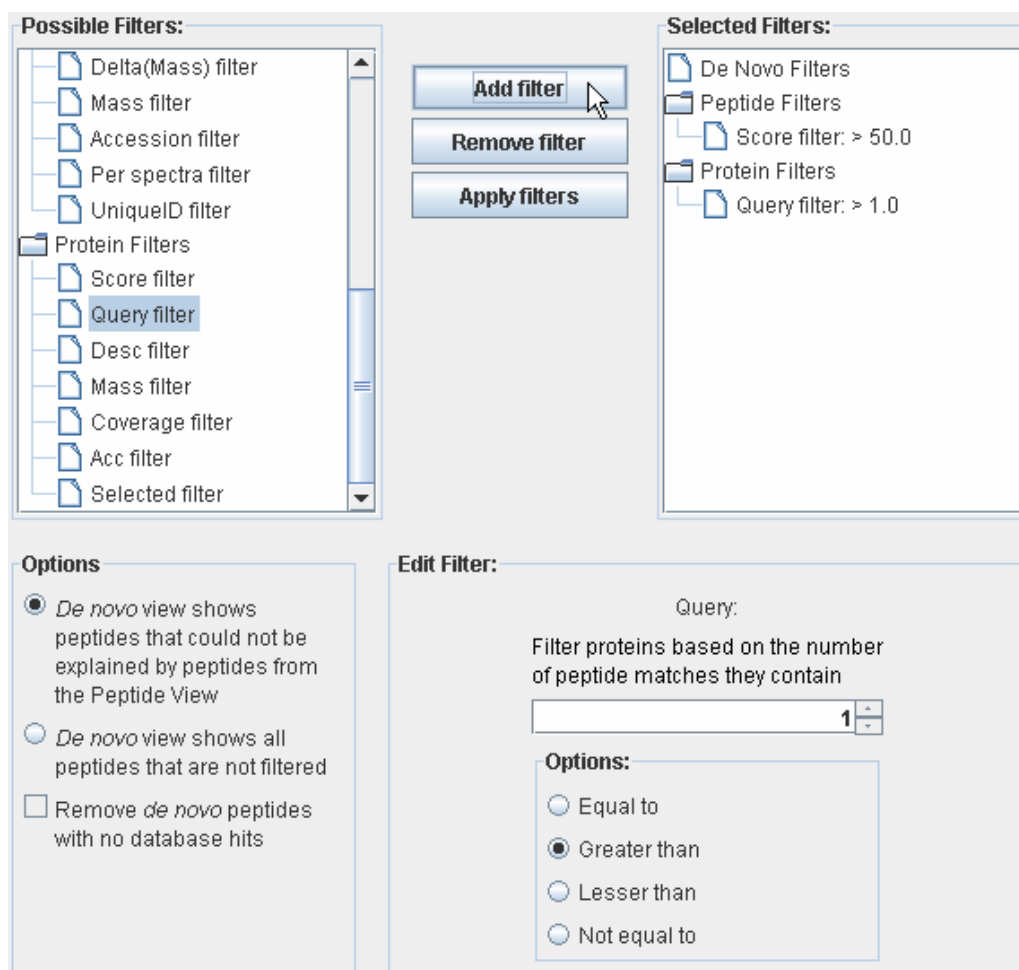
We already know that the sample was a simple digest of Lactoglobulin Beta; let's see if we can filter out the other spurious hits from the report. Click on the Filter Pane tab. Here we can add to the filter pane and add a filter that shows only database peptides with scores greater than 50% or any other value we choose. Scroll down the Possible Filters list and select the *Peptide Filter* called Score Filter. In the [Edit Filter] frame, remember to select "greater than" and type 50, then press the Add Filter button. The filter now appears in the list of Selected Filters. *The filter can be edited while in either listing and can be added multiple times (in case, for some reason, you only wanted peptides with a score greater than 50% but less than 90%).* Hit the Apply Filters button and let's examine the results. Click the 'Protein View' tab.

De Novo View	Peptide View	Protein View	Search parameters	Filter Pane				
Accession	Mass	Display	Score (...)	Coverage(...)	Query mat...	Marked	Description	
PEAKS DB Search								
gij229460	18,367		99	50.00%	12	<input type="checkbox"/>	lactoglobulin beta	

Ok, so we removed the other protein, and we're left with Lactoglobulin beta. The other protein only had one hit IGDLLQK, with score 50%. That peptide was removed from the report. Since the other protein no longer had any supporting peptides, it was also removed from the report. Now we're left with our lactoglobulin beta group. Click the plus sign here to expand this group further.

gij229460	18,367		99	50.00%	12	<input type="checkbox"/>	lactoglobulin beta	
gij2194	18,309		99	50.00%	12	<input type="checkbox"/>	Chain B, Bovine Beta-Lactoglobulin, Latti...	
gij4925	18,351		99	50.00%	12	<input type="checkbox"/>	Chain X, The Cys121ser Mutant Of Beta-L...	
gij7207	18,267		99	50.00%	12	<input type="checkbox"/>	beta-lactoglobulin - water buffalo	
gij7576	18,367		99	50.00%	12	<input type="checkbox"/>	Chain D, The Structure Of Bovine B-Lacto...	
gij2173	18,281		99	50.00%	12	<input type="checkbox"/>	Chain A, Bovine Beta-Lactoglobulin Com...	
gij1627	17,167		99	53.64%	12	<input type="checkbox"/>	beta-lactoglobulin	
gij7245	18,375		99	50.00%	12	<input type="checkbox"/>	Chain A, Structural Changes Accompanyi...	
gij1259	19,883		99	45.51%	12	<input type="checkbox"/>	Beta-lactoglobulin precursor (Beta-LG) (A...	
gij2780	19,921		99	45.51%	12	<input type="checkbox"/>	lactoglobulin, beta [Bos taurus]	
gij3808	20,023		99	45.00%	12	<input type="checkbox"/>	beta-lactoglobulin [Bubalus bubalis]	
gij2237	18,177		99	30.25%	7	<input type="checkbox"/>	lactoglobulin beta	
gij4949	19,976		99	27.22%	7	<input type="checkbox"/>	beta-lactoglobulin [Capra hircus]	
gij1658	19,947		99	27.22%	7	<input type="checkbox"/>	beta-lactoglobulin	
gij1279	17,678		99	15.00%	5	<input type="checkbox"/>	beta-lacto-globulin [Ovis aries]	
gij5403	18,151		99	14.81%	5	<input type="checkbox"/>	Beta-lactoglobulin (Beta-LG)	
gij1314	19,921		99	13.33%	5	<input type="checkbox"/>	beta-lactoglobulin [Ovis aries]	
gij5308	6,501		99	22.81%	3	<input type="checkbox"/>	beta-lactoglobulin	
gij5579	4,090		96	25.71%	2	<input type="checkbox"/>	beta-lactoglobulin [Bubalus bubalis]	
gij3703	5,189		81	53.19%	2	<input type="checkbox"/>	beta-lactoglobulin variant D [Bos taurus]	
gij1151	17,864		55	3.12%	1	<input type="checkbox"/>	Chain A, Structure Of Porcine Beta-Lacto...	
gij4067	19,720		55	2.81%	1	<input type="checkbox"/>	beta-lactoglobulin [Sus scrofa]	
gij3455	19,734		55	2.81%	1	<input type="checkbox"/>	beta-lactoglobulin [Sus scrofa]	
gij6347	29,091		55	1.92%	1	<input type="checkbox"/>	PREDICTED: similar to 60S ribosomal pr...	
gij5564	31,223		55	1.80%	1	<input type="checkbox"/>	PREDICTED: similar to 60S ribosomal pr...	
gij7400	33,147		55	1.69%	1	<input type="checkbox"/>	PREDICTED: similar to 60S ribosomal pr...	

Remember, all proteins in this group contain the same set or a subset of the same peptides. We'll see that there are still some hits down there that aren't very relevant. Yes, they contain a good peptide, but only one. Let's see if we can remove these one hit wonders. Go back to the Filter Pane tab. The Selected Filters list still shows our Peptide Score Filter > 50%. Let's add one to it. Scroll down the list of Possible filters and select the *protein filter* called Query filter. In [Edit Filter], select the 'greater than' option and type '1' in the box, then press the Add Filter button.

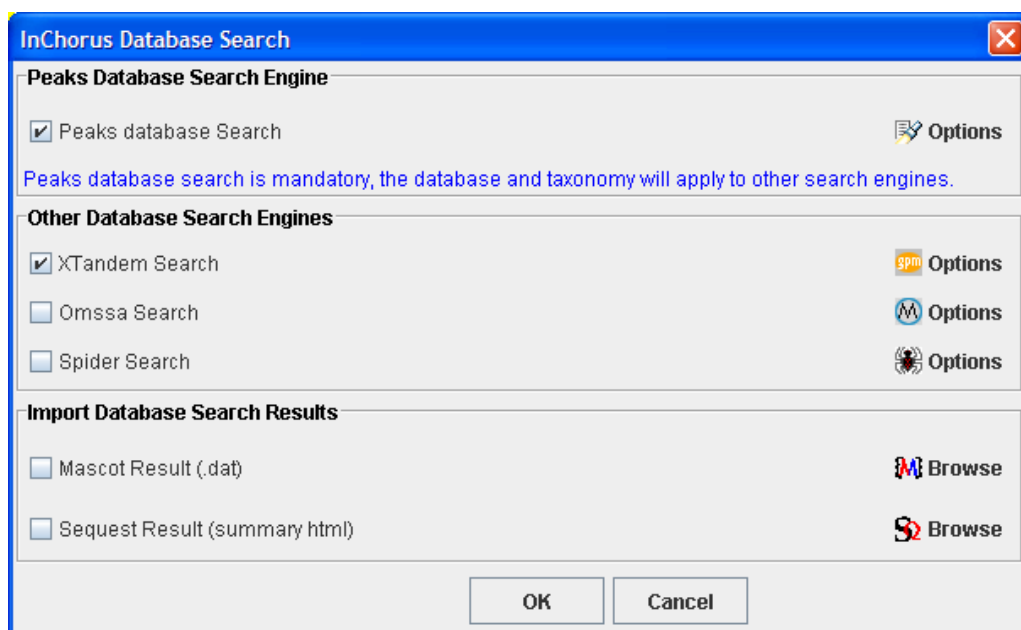


Now both filters appear in the list. Press the Apply Filters button to use them. Going back to the Protein View tab, and expanding the plus sign reveals that we are only left with variants of lactoglobulin.

S inChorus Searching

Let's try another kind of search. This time we'll use inChorus database searching – this technology, unique to PEAKS, allows us to launch other search engines that will help improve the results. The best confirmation of results comes from using two or more methods to confirm the peptide matches.

Select 'OrbiOrbi.pkl' from the Peptide Data frame (left) and choose 'inChorus protein ID' from the Tools menu. The inChorus Database search dialogue appears:



Make sure that “Peaks database Search” and “X!Tandem Search” are selected. Notice that there are three ‘Options’ icons on the right. They correspond to each search engine.

Click the Peaks database Search options button. The options pane is similar to the one we’ve seen already. The settings that we used before should still be there. If not, select “OrbiStandard” from the drop down list in the top right corner. Before pressing the “OK” button, we can make one change. Since we already have *de novo* sequencing results, we don’t need to do *de novo* sequencing again. Click the option ‘I have already run de novo, don’t do it again’, then press the OK button.

Click the X!Tandem search options button (top). This window allows us to set options for the X!Tandem search tool. This window is set up to behave almost exactly the same as the X!Tandem interface, so it may look familiar. Leave the fragment error at 0.1, and make sure there are no modifications turned on. Under “7. Predefined methods” choose FTICR. To learn more about X!Tandem settings, double-click any of the question marks. Press the OK button.

Now that we’ve set everything up for the inChorus search, press the OK button on the inChorus Database search dialogue. inChorus will call on each search engine, wait until they are finished, then compile their results together, ensuring the integrity of the data-results relationship.

Watch the task queue (bottom-left of PEAKS Studio). After everything is finished, new search results will appear in the Peptide Data frame (left), stamped with the date and time. The task queue will be empty, and the results will display. There’s also a nice little report to tell us if there were any errors.

05-Jun-06 14:47:33_NR [inChorus protein ID results]

Peptide View

Protein View

Search parameters

Index	m/z	z	mass	Peptide	Mr(Calc)	Delta(Mass)	Score (%)	Start	End	Accession No.	Peaks	X!Tandem
1	452.2...	2	902.55...	TKIPAVFK	902.55...	-0.0035	68	76	83	gil229460	<input checked="" type="checkbox"/>	<input type="checkbox"/>
2	458.7...	2	915.46...	LDAINENK	915.46...	-0.0035	71	84	91	gil229460	<input checked="" type="checkbox"/>	<input type="checkbox"/>
2	458.7...	2	915.46...	IDALNENK	915.46...	-0.0035	71	84	91	gil2194089	<input checked="" type="checkbox"/>	<input type="checkbox"/>
3	467.2...	2	932.53...	LIVTQTMK	932.53...	-0.0053	35	1	8	gil229460	<input checked="" type="checkbox"/>	<input type="checkbox"/>
3	467.2...	2	932.53...	IIVTQTMK	932.53...	-0.0053	35	1	8	gil72079	<input checked="" type="checkbox"/>	<input type="checkbox"/>
4	533.2...	2	1,064...	VLVLDTDYK	1,064...	-0.0048	85	92	100	gil229460	<input checked="" type="checkbox"/>	<input type="checkbox"/>
5	545.9...	3	1,634...	TPEVDDEALEKFDK	1,634...	-0.0050	100	125	138	gil229460	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
7	597.3...	2	1,192...	VLVLDTDYKK	1,192...	-0.0074	100	92	101	gil229460	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
8	623.2...	2	1,244...	TPEVDDEALEK	1,244...	-0.0023	100	125	135	gil229460	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
9	673.3...	1	672.37...	IGDLQK	672.38...	-0.0028	50	619	624	gil28972720	<input checked="" type="checkbox"/>	<input type="checkbox"/>
9	673.3...	1	672.37...	LGDLQK	672.38...	-0.0028	50	136	141	gil52782259	<input checked="" type="checkbox"/>	<input type="checkbox"/>
9	673.3...	1	672.37...	LGDIQK	672.38...	-0.0028	50	736	741	gil63586345	<input checked="" type="checkbox"/>	<input type="checkbox"/>
9	673.3...	1	672.37...	GLDIQK	672.38...	-0.0028	19	9	14	gil229460	<input checked="" type="checkbox"/>	<input type="checkbox"/>
10	674.4...	1	673.41...	IPAVFK	673.41...	-0.0002	76	78	83	gil229460	<input checked="" type="checkbox"/>	<input type="checkbox"/>
10	674.4...	1	673.41...	LPAVFK	673.41...	-0.0002	76	24	29	gil62659199	<input checked="" type="checkbox"/>	<input type="checkbox"/>
12	697.7...	3	2,090...	LDAINENKVLVLDTDYKK	2,090...	0.0022	100	84	101	gil229460	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
12	697.7...	3	2,090...	IDALNENKVLVLDTDYKK	2,090...	0.0022	100	84	101	gil2194089	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
13	771.7...	3	2,312...	VYVEELKPTPEGDLLEILLQK	2,312...	-0.0044	100	41	60	gil229460	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
14	818.3...	2	1,634...	TPEVDDEALEKFDK	1,634...	-0.0067	100	125	138	gil229460	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
16	903.1...	3	2,706...	VAGTWYSLAMAASDISLLDAQSAPLR	2,706...	0.0007	99	15	40	gil229460	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
17	903.5...	1	902.56...	TKIPAVFK	902.55...	0.0013	3	76	83	gil229460	<input checked="" type="checkbox"/>	<input type="checkbox"/>
18	933.5...	1	932.53...	LIVTQTMK	932.53...	-0.0012	34	1	8	gil229460	<input checked="" type="checkbox"/>	<input type="checkbox"/>
18	933.5...	1	932.53...	IIVTQTMK	932.53...	-0.0012	34	1	8	gil72079	<input checked="" type="checkbox"/>	<input type="checkbox"/>
20	1065...	1	1,064...	VLVLDTDYK	1,064...	0.0035	5	92	100	gil229460	<input checked="" type="checkbox"/>	<input type="checkbox"/>
21	1157...	2	2,312...	VYVEELKPTPEGDLLEILLQK	2,312...	-0.0085	100	41	60	gil229460	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
22	1354...	2	2,706...	VAGTWYSLAMAASDISLLDAQSAPLR	2,706...	-0.0037	90	15	40	gil229460	<input checked="" type="checkbox"/>	<input type="checkbox"/>

PEAKS displays the same 'Peptide View' as before. Now, X!Tandem search results are considered as well. Where X!Tandem agrees with a PEAKS assignment, there's a checkmark in the Peaks column and a checkmark in the X!Tandem column. Also, the score on this peptide is increased – in this way we increase confidence in the assignment. Since the two tools take different approaches, we may discover that PEAKS finds some peptides that X!Tandem misses, and vice-versa. Where this is the case, only one checkmark will be displayed and the score is penalized slightly in some cases. Sometimes we can find a good hit that the other search engine would have missed. In this way we increase coverage.

Click the 'Protein View' tab to see a summary of PEAKS' and X!Tandem's results at the protein level.

Thus concludes our walkthrough of PEAKS 4.2's basic features.

Graphical User Interface

A reference section to help us find our way around.

This chapter deals with interface elements. It is meant to be used as a reference so we can look up certain interface elements when we get stuck. For instructions on how to use PEAKS Studio to perform certain tasks, the section entitled “Using PEAKS Studio” will be more instructive.

The first part of this chapter describes **windows, dialogues, frames and reports**. This tells us what certain dialogue boxes, windows and frames do and how to read them.

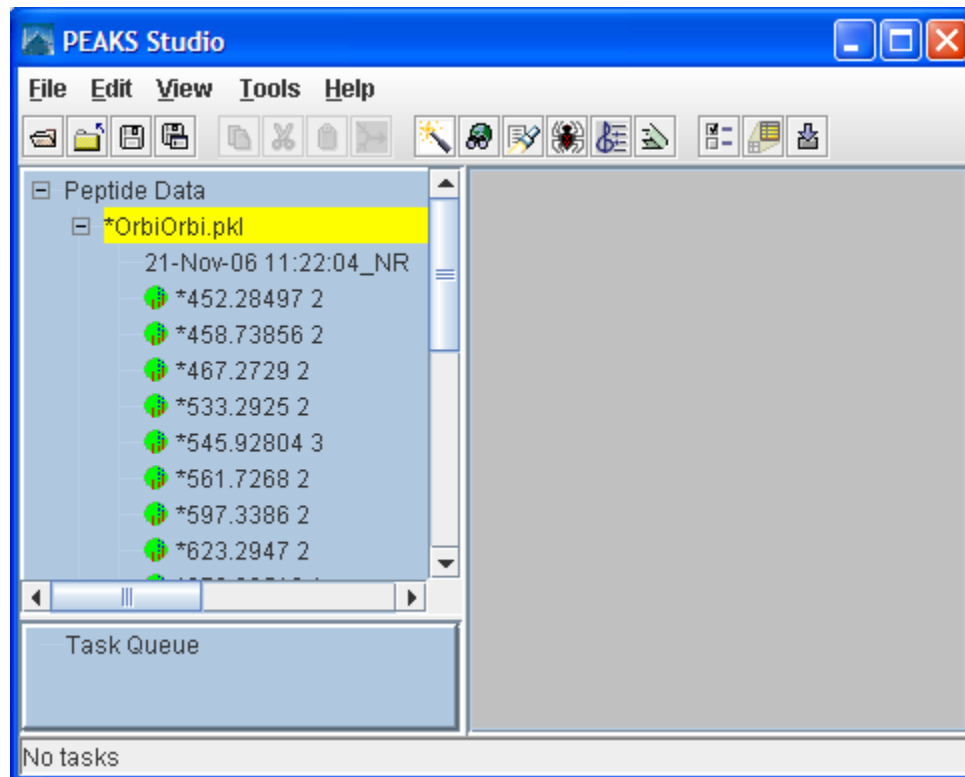
The second part of this chapter deals with **toolbars**. Toolbars are a very useful way to quickly get at the functions we use most.

SVC

SVC

Windows, Dialogues, Frames and Reports

PEAKS 4.2 main window



Comprises:

- **Peptide data frame (left):** This displays a listing of parent ions by m/z and charge. Clicking on one will bring up its MS/MS spectrum. The colored dot by each spectrum appears dark green for unprocessed, or light green for sequenced (or partially sequenced). An asterisk (*) next to a spectrum shows that it contains unsaved information.

Spectra are grouped by data files, or by nodes (which act like data files). Select a data file or node by clicking on its name (i.e. click on CytC-ESI.anz in the above example), or a spectrum within a data file by clicking on it. Use the '+' and '-' boxes to expand and collapse the view.

- **Task Queue frame (bottom left):** Shows running tasks, sorted by priority.

- **Working area (right):** This is where the Protein Identification Result Window and the Main Processing windows appear.
- **Menu bar:** access file, edit, view, tools, windows, and help commands.
- **Main window toolbar:** quick access to many commands. See “Toolbars” section below

SC

Auto de Novo Parameters Dialogue

De Novo Options

De Novo Sequencing Parameters Saved parameters

SampleData

Instrument: Quadrupole-TOF

Parent Mass Error Tolerance: 0.1

Fragment Mass Error Tolerance: 0.1

Enzyme: Semi Trypsin

Report up to (# peptides): 5 Edit Enzymes

PTM selected for search

max variable PTM per peptide: 3 Add/Remove PTM

☒ Deconvolute the data for analysis (use for non-deconvoluted data)

Save As Ok Cancel

- **Instrument:** choose the type of spectrometer that produced our data. Choose from a dropdown list.
- **Parent mass error tolerance:** determines how much random and systematic experimental error on the parent/precursor ion PEAKS will account for in its analysis. Select a tolerance from the dropdown list. Daltons are the units here.

- **Fragment mass error tolerance:** determines how much random and systematic experimental error on the fragment/daughter ion PEAKS will account for in its analysis. Select a tolerance from the dropdown list. Daltons are the units here.
- **Enzyme:** choose from a dropdown list of enzymes that we used to digest our protein sample. Click the “Edit Enzymes” button to edit the enzymes defined in this list, or to add to it.
- **Report up to:** set how many de novo sequence candidates PEAKS will report. Choose from a dropdown list.
- **PTM selected for search:** this box displays the modifications currently selected for analysis, these will be considered during auto de novo sequencing. To change this, click the Add/Remove PTM button.
- **Max variable PTM per peptide:** this allows us to restrict the number of variable PTM that will appear on any given peptide.

Protein Identification Parameters Dialogue

Database Search Options

Protein Identification Parameters

Instrument: FT, Orbitrap (CID,SORI)

Parent Mass Error Tolerance: 0.01

Fragment Mass Error Tolerance: 0.01

Enzyme: Semi Trypsin

max missed cleavages: 2

PTM selected for search:

max variable PTM per peptide: 3

☒ Preprocess this data 'on the fly' (deconvolute, filter noise, centroid)

Precursor mass search type: ☒ Monoisotopic ☐ Average

Database to search: NR

Taxonomy selection: Mammalia

Paste fasta sequences

Advanced Options

PEAKS uses a hybrid search technique that requires some sequence tags to help in the search

☐ I have already run de novo, don't run it again

☐ Run de novo using different parameters than the above

☒ Run de novo using the same parameters as above(default)

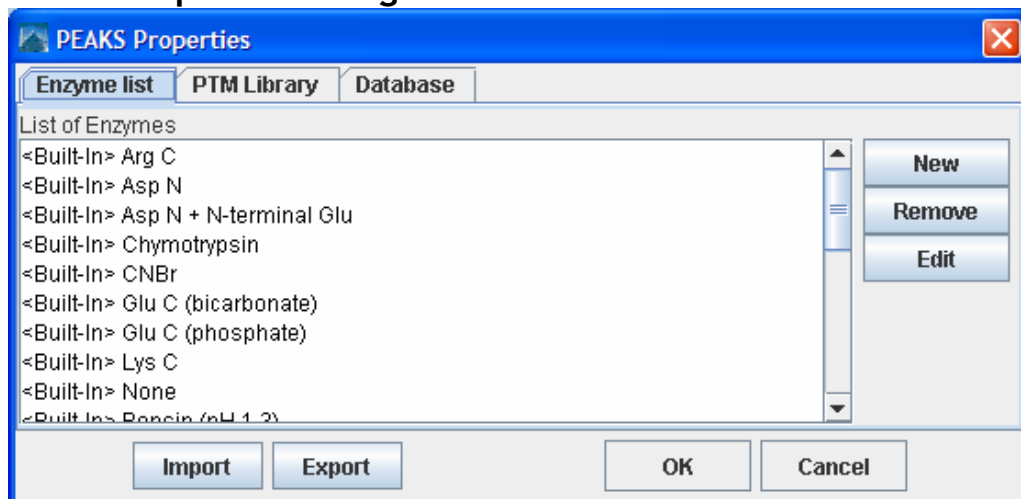
QTOF Standard

Buttons: ? Save As Ok Cancel

- **Instrument:** choose the type of spectrometer that produced our data. Choose from a dropdown list.
- **Parent mass error tolerance:** determines how much random and systematic experimental error on the parent/precursor ion PEAKS will account for in its analysis. Select a tolerance from the dropdown list. Daltons are the units here.
- **Fragment mass error tolerance:** determines how much random and systematic experimental error on the fragment/daughter ion PEAKS will account for in its analysis. Select a tolerance from the dropdown list. Daltons are the units here.

- **Enzyme:** choose from a dropdown list of enzymes that we used to digest our protein sample. Click the “Edit Enzymes” button to edit the enzymes defined in this list, or to add to it.
- **PTM selected for search:** this box displays the modifications currently selected for analysis, these will be considered during database searching. To change this, click the Add/Remove PTM button.
- **Database to search:** This dropdown allows us to choose which FASTA format database to search
- **Taxonomy selection:** if the ‘Database to search’ has been configured for taxon based searching, this list will allow us to select limit which taxa are searched.
- **Paste fasta sequences:** Paste a few sequences in this box and PEAKS 4.2 will search through those sequences as opposed to the database selected.
- **Preprocess this data ‘on the fly’:** PEAKS Studio has its own built-in preprocessor for removing noise, centroiding, and peak charge recognition from MS/MS data. Check this box to turn preprocessing on.
- **Advanced options (de novo sequencing):** The PEAKS approach to protein identification uses de novo sequences to help out in the search. This section allows you to decide how to obtain the de novo sequences required for the search.

PEAKS Properties Dialogue



- **Enzyme list tab:** Displays a list of built-in and user-defined enzymes. We may edit and create enzymes from here.
- **PTM library tab:** Displays a list of built-in and user-defined PTMs. We may edit and create PTM from here.
- **Database tab:** Displays a list of databases available to PEAKS. We may make new databases available to PEAKS from here.

Enzyme Editor Dialogue

Enzyme

Enzyme Definition

Saved enzymes: Chymotrypsin

Digestion Rules:

...residues at the end of a peptide	cleavage site	start of a new peptide...
FLMWY	{P}	start of a new peptide...
		start of a new peptide...
		start of a new peptide...
		start of a new peptide...

use set brackets {} around a residue to denote "any amino acid except the ones enclosed in these bracket". Use X to denote "any residue".

Specificity Parameters:

☒ Find peptides that satisfy the above at both ends

☐ Find peptides that satisfy the above only at C-terminus and/or peptides that satisfy the above only at N-terminus

example of what peptides can be returned

[KR]	[P]	[KR]	[P]
		[KR]	[P]
[KR]	[P]		

Shorthand notation for the above

-digest "([FLMWY])(P)" -semi 0

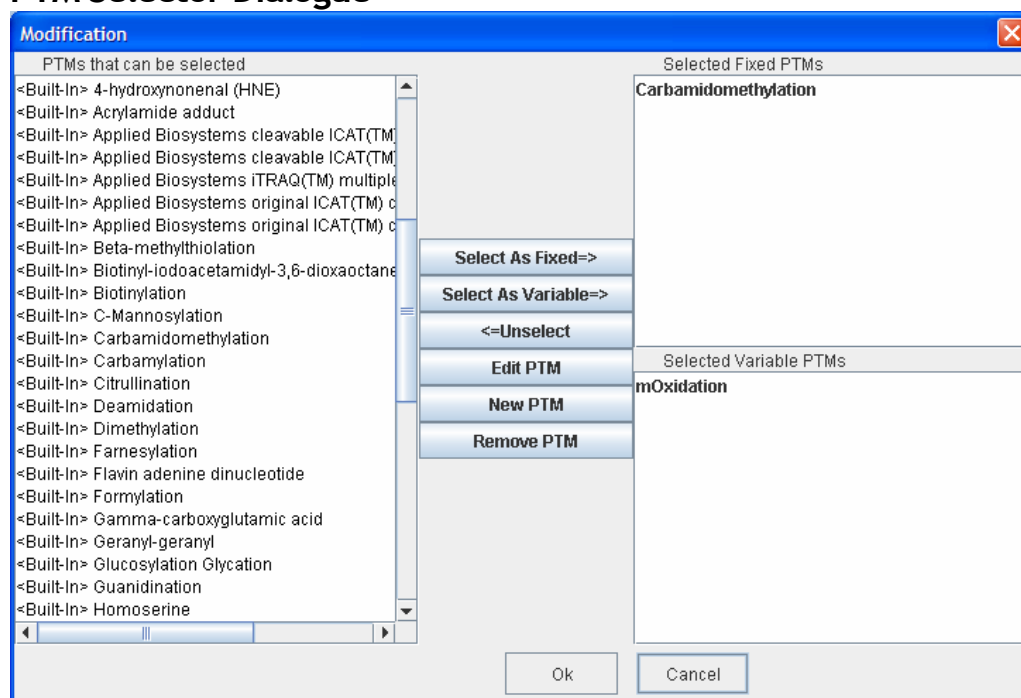
Save Enzyme Ok Cancel

- **Digestion Rules:** This is how we specify where our enzyme will cleave the protein between two amino acids to create peptides. Use set brackets {} around a residue to denote “any amino acid except the ones enclosed in these brackets”. Use X to denote “any residue”. Listing several amino acids in one box means “any one of these residues”.
- **Specificity Parameters:** Peptides can break down such that only one end is a cleavage site. Check the boxes to tell PEAKS to search for only for peptides that have proper cleavage sites on both ends, or to require that only one end be a proper cleavage site.
- **Shorthand notation:** Advanced users may specify their enzyme cleavage in shorthand notation, but it is not required.
- **Saving/Loading Enzymes:** After setting up an enzyme, we can save it for future use. Click the "Save Parameters" button, and choose a name for future reference if prompted. Don't worry, we can't accidentally overwrite the defaults. Any enzyme we save will be available in the drop-down list at the top of the window. To see what's inside, just select one, and the enzymes digest rules boxes will be populated.

SC

This dialogue allows us to create or edit a PTM

PTM Selector Dialogue



Here we can form a list of available post-translational modifications. We can choose any PTM as Fixed PTM or Varied PTM (to tell PEAKS that it may or may not occur). To make this selection, click on a PTM in the list at left, and then click the “Select As Fixed=>” or the “Select as Varied=>” button. If a PTM is already selected as a fixed PTM, it cannot be selected as varied PTM, and vice versa.

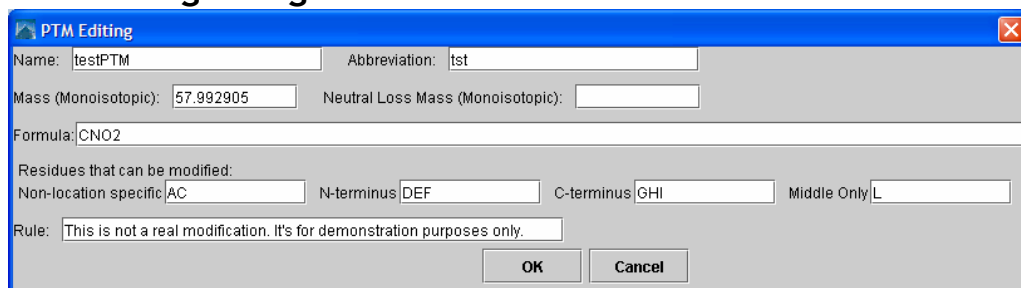
If we change our mind about a PTM after having selected it, it is still possible to unselect it. Click the erroneous PTM from the list of ‘Selected Fixed PTM’ or ‘Selected Varied PTM’, and then click the “<=Unselect” button to remove it from either list of Selected PTM.

PEAKS software ships with some pre-defined PTMs. These are listed as <Built-In>. If we want to create a new PTM, we can click ‘New PTM’ to create a new one. The **Editing a PTM** and **Creating a New PTM** sections below describes how this is done.

SCV

This dialogue allows us to create or edit a PTM

PTM Editing Dialogue



- **Name:** This will appear in the PTM list
- **Abbreviation:** This will appear in the auto *de novo* results, if it is found.
- **Mass (monoisotopic):** The mass that the residue gains or loses as a result of the PTM. Enter this numerically here, or enter the chemical formula below.
- **Neutral Loss Mass:** The mass that the modified residue loses as a result of fragmentation. E.g. 28 would signify a loss of 28 Daltons.
- **Formula:** The chemical formula of the PTM. This will automatically enter the mass.
- **Residues that can be modified:** Enter residues that can be modified anywhere, residues that can only be modified if they are at the N-terminus and residues that can only be modified at the C-terminus, and residues that can only be modified if they are not on either terminus.
- **Rule:** user entered, a comment for our reference.

SCV

The 'Basic' and 'Advanced' Ion tables differ only in the number of ions they can display. The 'Basic' table displays up to six ions.

Environment Preferences Window

This window controls the basic, user configurable environment. Colours, displays, file handling and default settings can all be changed from here. The window contains 7 tabs:

Parameters tab: Our saved parameters for de novo sequencing and protein ID are shown and can be deleted from here.

Basic Ion Table Editor tab: allows configuration of the basic ion table

Advanced Ion Table Editor tab: allows configuration of the advanced ion table

Environment tab: Allows us to set default file locations, default file handling functions, and to specify local processing or remote PEAKS Online processing

Colour tab: from here we can set the colours to use for various spectrum annotations.

Display tab: from here we can set the type-face to use across the PEAKS 4.2 user interface. The width of spectrum peaks can also be set from here.

Manual De Novo tab: default options for manual de novo sequencing can be set from here.

SCV

Protein Identification Result Window

The protein identification result window contains the results from one protein identification run on one data set. It is organized into three tabs: **peptide view**, **protein view** and **search parameters**.

De Novo view

The De Novo view summarizes the *de novo* sequencing results for each spectrum in the data file. The exact contents of the list are determined by filters set on the **Filter Pane**. By default the peptides are grouped by spectrum, but the list is sort-able by any of the columns in the table. Selecting one or more (click and drag, or use shift+click) items in this list brings those spectra in the peptide data tree (found on the left hand side).

Peptide View

The peptide view summarizes the results for each MS/MS spectrum. All peptides that match to each spectrum are displayed. The exact contents of the list are determined by filters set on the **Filter Pane**. By default the peptides are grouped by spectrum, but the list is sort-able by any of the columns in the table. Selecting one or more (click and drag, or use shift+click) items in this list selects those spectra in the peptide data tree (found on the left hand side). The bottom panel of this view shows more details about the peptide identification that is highlighted in the top section. In this view, we can see the protein that the peptide came from, and a simple alignment between the original de novo sequence for this spectrum (if available) and the peptide found in the database.

Peptide View showing peptides grouped by spectrum

21-Nov-06 11:22:04_NR [inChorus protein ID results]

De Novo View

Peptide View

Protein View

Search parameters

Filter Pane

Index	m/z	z	Mass	Peptide	Mr(Calc)	Score(%)	Start	End	Accession ...	UniqueID	Peaks
1	452.28497	2	902.5554	TKIPAVFK	902.5589	69	76	83	gi 229460	0	✓
2	458.73856	2	915.4626	LDAINENK	915.4661	43	84	91	gi 229460	1	✓
2	458.73856	2	915.4626	IDALNENK	915.4661	43	84	91	gi 4388846	2	✓
3	467.2729	2	932.5312	LIVTQTMK	932.5366	42	1	8	gi 229460	3	✓
3	467.2729	2	932.5312	IIVTQTMK	932.5366	42	1	8	gi 72079	4	✓
4	533.2925	2	1,064.5704	VLVLDTDYK	1,064.5752	87	92	100	gi 229460	5	✓
5	545.92804	3	1,634.7623	TPEVDDALEKFDK	1,634.7673	99	125	138	gi 229460	6	✓
7	597.3386	2	1,192.6627	VLVLDTDYKK	1,192.6702	99	92	101	gi 229460	7	✓
8	623.2947	2	1,244.5748	TPEVDDALEK	1,244.5771	99	125	135	gi 229460	8	✓
9	673.38513	1	672.3779	IGDLQK	672.3807	50	504	509	gi 1094913...	9	✓
9	673.38513	1	672.3779	GLDIQK	672.3807	19	9	14	gi 229460	10	✓
9	673.38513	1	672.3779	LGDLQK	672.3807	50	136	141	gi 62510779	11	✓
10	674.4233	1	673.4160	IPAVFK	673.4163	85	78	83	gi 229460	12	✓
10	674.4233	1	673.4160	LPAVFK	673.4163	85	24	29	gi 1145653...	13	✓

Peptide Details

Links to retrieve spectrum information:

458.73856 2

De novo LDALNENK

Database LDAINENK

>gi|229460|prf|732164A lactoglobulin beta

Matched peptides shown in Red

1 LIVTQTMKGL DIQKVAGTMY SLAMAASDIS LLDAQSAFLR VYVEELKPTP

51 EGDLEHLLQK WENDECAQKK IIAEKTKIPA VFKLDAINEN KVLVLDTDYK

101 KYLLFCMENS AEPEQSLVCQ CLVRTPEVDD EALEKFDKAL KALPMHIRLS

151 FNPTLQEEQC HI

NCBI BLAST search of de novo sequence LDALNENK

NCBI BLAST search of database search sequence LDAINENK

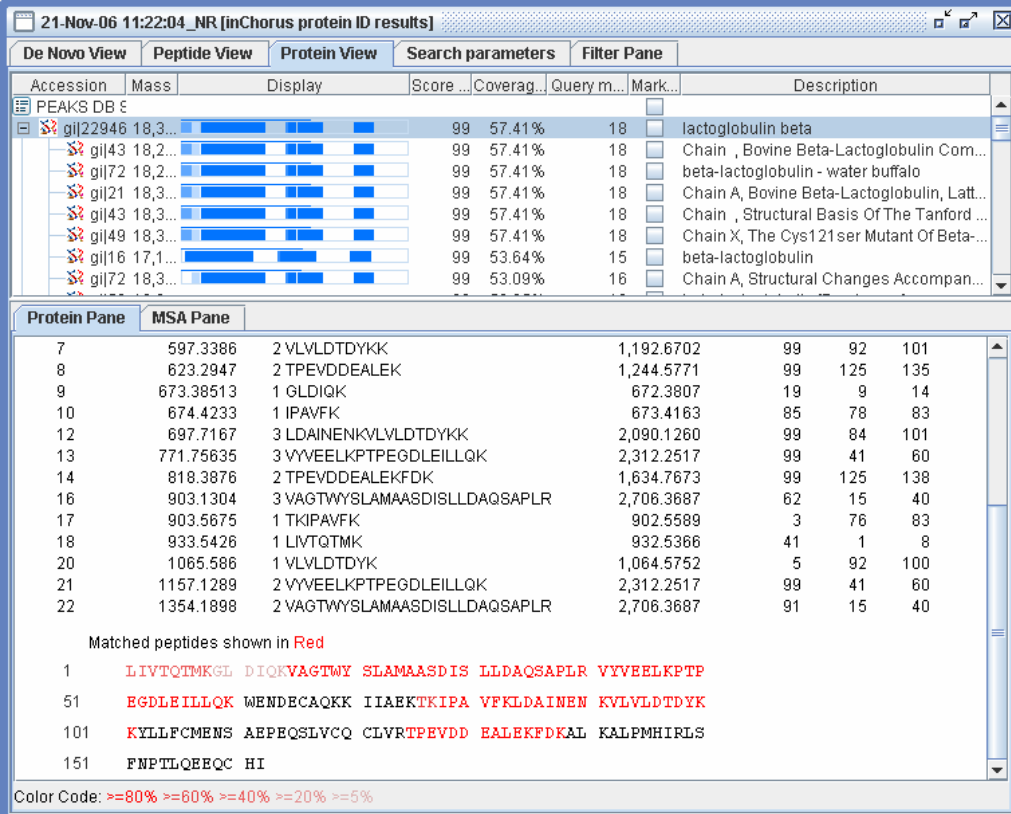
Protein View

The protein view is most useful as a summary of what proteins were present in a sample, and the peptides matched to them. It has two sections:

- **Index (top section):** PEAKS presents a list of proteins that it believes to be the best match for the sample. This index lists them by accession number, ranked in descending order by score. *Very similar proteins (i.e. ones that contain most of or all of the sequences identified by PEAKS) are grouped together – only the first entry in this group is shown here. Show the whole group by clicking the + sign.* In the example below, lactoglobulin beta is the top ranked protein candidate.
- **Peptide Match Reports (bottom section):** PEAKS presents each protein candidate with a peptide match list beneath it. Each peptide that matched the protein sequence is shown, in order, by spectrum. The confidence that the correct peptide sequence was found is displayed next to each peptide sequence. At the bottom of this list, the complete protein sequence is shown with matching peptides highlighted in red.
- **MSA Pane (bottom section):** This panel displays a multiple sequence alignment between any protein that is ‘marked’ in the

Protein View showing two proteins in the index, and beginning the full report

Index (top section). The MSA is only displayed when the button is pressed.



Accession	Mass	Display	Score	Coverag	Query m	Mark	Description
gi 22946 18,3...			99	57.41%	18		lactoglobulin beta
gi 43 18,2...			99	57.41%	18		Chain , Bovine Beta-Lactoglobulin Com...
gi 72 18,2...			99	57.41%	18		beta-lactoglobulin - water buffalo
gi 21 18,3...			99	57.41%	18		Chain A, Bovine Beta-Lactoglobulin, Latt...
gi 43 18,3...			99	57.41%	18		Chain , Structural Basis Of The Tanford ...
gi 49 18,3...			99	57.41%	18		Chain X, The Cys121 ser Mutant Of Beta-...
gi 16 17,1...			99	53.64%	15		beta-lactoglobulin
gi 72 18,3...			99	53.09%	16		Chain A, Structural Changes Accompan...

Protein Pane	MSA Pane
7	597.3386 2 VLVLDTDYKK 1,192.6702 99 92 101
8	623.2947 2 TPEVDDEALEK 1,244.5771 99 125 135
9	673.38513 1 GLDIQK 672.3807 19 9 14
10	674.4233 1 IPAVFK 673.4163 85 78 83
12	697.7167 3 LDAINENKVLVLDTDYKK 2,090.1260 99 84 101
13	771.75635 3 VYVEELKPTPEGDLLEILLQK 2,312.2517 99 41 60
14	818.3876 2 TPEVDDEALEKFDK 1,634.7673 99 125 138
16	903.1304 3 VAGTWYSLAMAASDISLLDAQSAPLR 2,706.3687 62 15 40
17	903.5675 1 TKIPAVFK 902.5589 3 76 83
18	933.5426 1 LVTQTMK 932.5366 41 1 8
20	1065.586 1 VLVLDTDYK 1,064.5752 5 92 100
21	1157.1289 2 VYVEELKPTPEGDLLEILLQK 2,312.2517 99 41 60
22	1354.1898 2 VAGTWYSLAMAASDISLLDAQSAPLR 2,706.3687 91 15 40

Matched peptides shown in Red

1 LIVTQTMKGL DIQKVAGTWY SLAMAASDIS LLDAQSAPLR VYVEELKPTP

51 EGDLEILLQK WENDECAQKK IIAEKTKIPA VFKLDAINEN KVLVLDTDYK

101 KYLLFCMENS AEPEQSLVCQ CLVRTPEVDD EALEKFDKAL KALPMHIRLS

151 FMPTLQEEQC HI

Color Code: >=80% >=60% >=40% >=20% >=5%

Search parameters

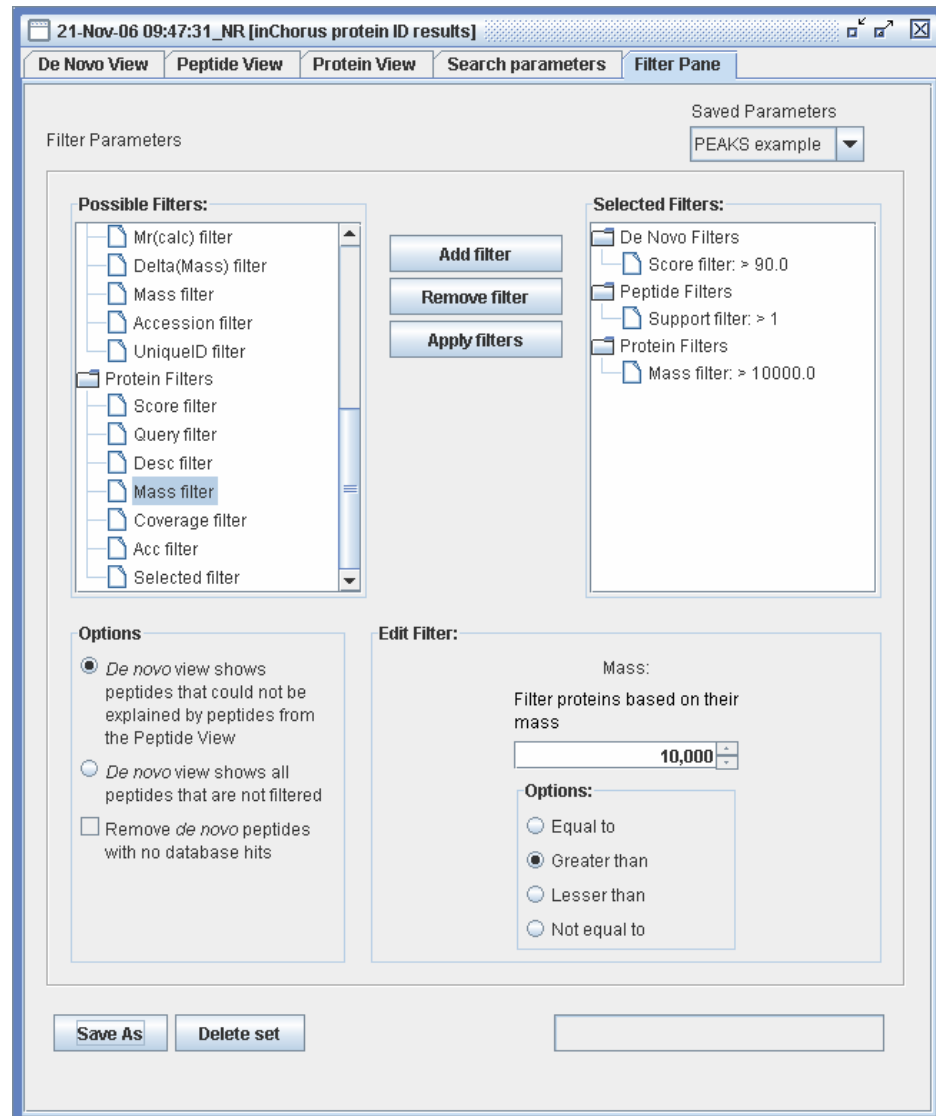
This tab displays the protein identification parameters that were used to guide the search that generated these results.

Filter pane

The Filter Pane allows us to control what to display on the other tabs of the multi-part protein ID report by setting up customizable filters. The various parts of this screen are described as follows:

- **Possible Filters list:** a list of the filters, currently available, that can be used to build a particular filter set.
- **Active Filters list:** a list of the filters, currently selected, that will be used to filter your results.
- **Add Filter Button:** clone the currently selected filter in the Possible Filters listing to the Active Filters.

- **Apply Filter Button:** Apply your filters; any/all selected filters will not be used until this button is actually pressed.
- **Edit Filter Frame:** Edit the settings for the currently selected filter. Filters in the Possible Filters list and the Active Filters list can be edited
- **Options frame:** The options listed here fundamentally change what is listed in the De Novo View, regardless of the specific filters selected.



- **Option 1:** *De Novo View shows peptides that could not be explained by peptides from the Peptide View* – In this mode, the De Novo View only shows peptides for spectra that could not be satisfactorily explained by searching the

**See the section
“Post analysis of
results – preparing
for publication” for
help on using
filters**

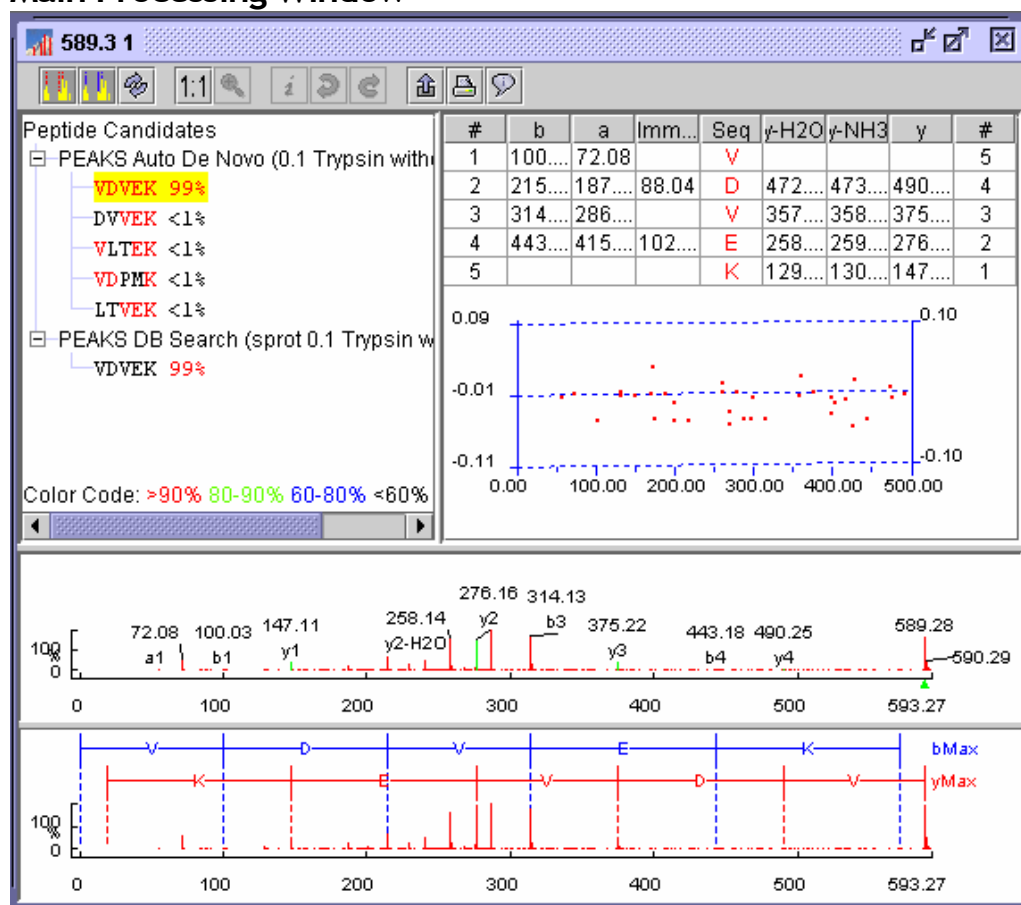
database. As such, removing a peptide/spectrum from the database search results – the Peptide View list – will cause the de novo sequence for that spectrum to **appear on the De Novo View**. The de novo peptides still have to pass the filters defined for the De Novo View.

- **Option 2:** *De Novo View shows all peptides that are not filtered* – In this mode, the De Novo View shows de novo sequences for all spectra that have not been removed by filtering out database peptides or proteins. As such, removing a peptide/spectrum from the database search results – the Peptide View list – will cause the de novo sequence for that spectrum to be **removed from the De Novo View**. The de novo peptides still have to pass the filters defined for the De Novo View.
- **Checkbox:** *remove de novo peptides with no database hits* – This option removes all peptides that have no corresponding database peptide hits. Selecting Option 2 (above) and this checkbox shows essentially the "reverse" of Option 1.
- **Save As Button**– Save and re-use your filters between different sessions of PEAKS. All active filters and selections from the Options Frame are saved.

SCV

The main processing window is used to perform manual *de novo* sequencing, and to examine the results of auto *de novo* sequencing

Main Processing Window



- **Main Processing Window Toolbar:** quick access to many processing functions. See “Toolbars” in the next section.
- **Peptide Candidates Frame (top left):** PEAKS shows peptide sequence candidates, ranked by score for the selected spectrum. Peptide sequences are grouped by the headings: “Auto *de novo*”, “Manual *de novo*”, “user defined result type” and “database search results” depending on how they were derived. For *de novo* results, positional confidence is color coded on each residue. More specific positional confidence appears when the mouse is held over a sequence – this shows the confidence in each of its parts.
- Under the “Manual *de novo*” heading, the masses of the residues are displayed. That is to say, the mass of the whole peptide minus water.

The ions displayed in both modes can be edited. See the section in configuring PEAKS 4.2

- **Ion Table Frame (top right):** The Ion Table shows the proposed ions with their corresponding masses -- i.e. the mass of the b1 ion is shown in the top right corner. The default Ion Table will display b, a, immonium, yH2O, yNH3 and y ions in basic mode; it will display b, b-H2O, a, c, immonium, y, y-H2O, z, z' and y(2+) ions in advanced mode. To switch from basic mode to advanced mode, choose 'Show ion table' from the 'View' menu.

The Ion Table Frame also contains an error plot (*it may be necessary to scroll down to see the error plot*). The error plot shows the confidence each ion is assigned. The most confident results lie on the centerline. Clicking a cell or column in the Ion Table highlights the corresponding points on the error plot and corresponding peaks on the spectrum.

- **Spectrum View Frame (middle):** Shows a graphical representation of the spectrum. Peak masses are labeled, as are the peaks associated with identified ions. We can zoom in on the spectrum by clicking and dragging over an area.
- **Spectrum Alignment Frame (bottom):** Shows a graphical representation of the spectrum. This view always shows the whole spectrum and is used as a tool to help us navigate the spectrum view frame. A blue bar along the horizontal axis of the alignment view indicates the range of the spectrum view in the Spectrum View Frame.

The Spectrum Alignment Frame can also show the positions of major ions that delimit the proposed sequence. By default, the Spectrum Alignment Frame displays b-ion and y-ion peaks and the derived peptide sequence between them. The Spectrum Alignment Frame can also show the position of c-ion and z-ion peaks.

To switch views, choose 'Alignment by' from the 'View menu'

SCV

The Ion Editor is used when performing manual *de novo* sequencing.

Ion Editor

Ion Editor

Please choose ion type:

☒ C Term Ions

☐ N Term Ions

Selected peak information:

mz: 136.0692

intensity: 4.0323

current ion:

x

y

y-H2O

y-NH3

Add

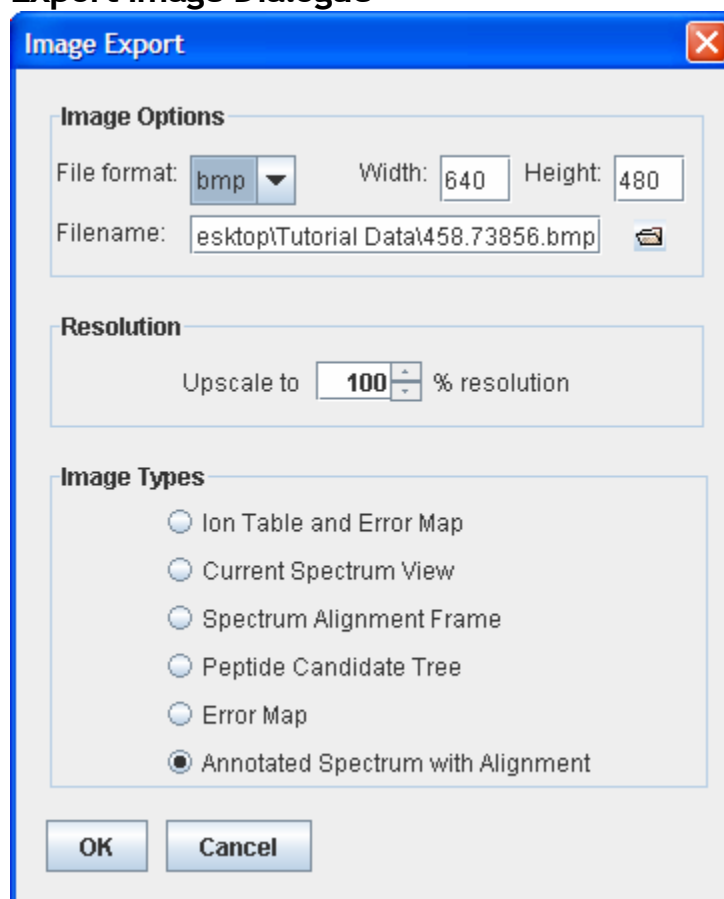
Remove


Comments:

Apply Cancel

- **Selected peak information:** displays information about the currently selected peak.
- Under “**Please choose ion type**”, the radio buttons set whether the ions in the “ion choice list” are C terminal ions or N terminal ions.
- **Ion choice list: (left):** lists the ions we can apply to the selected peak
- **Selected ion list (right):** lists the ions we have selected add or remove them using the “Add” and “Remove” buttons.
- **Apply button:** applies the ions in the “selected ion list” to the selected peak.

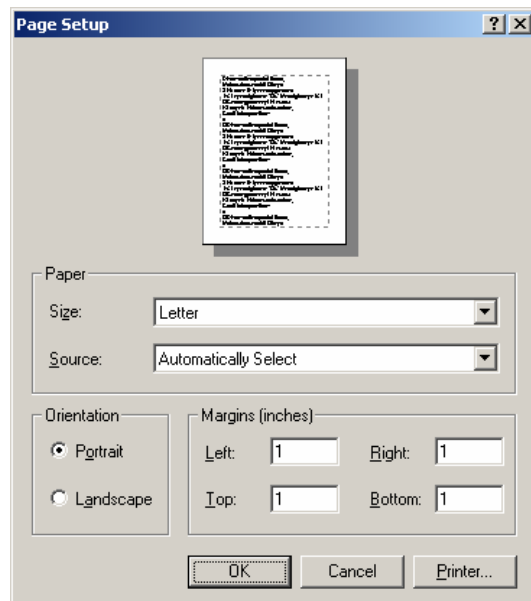
Export Image Dialogue



- **File Format:** select an image file format from the drop down list. Bitmap, JPEG, Portable Network Graphic and Graphics Interchange Format are supported.
- **Width and Height:** together these determine the size of the output image (measured in pixels).
- **Filename:** type in the textbox, or browse to a file , to enter the file name of the image that will be created.
- **Resolution:** in most cases the image resolution can be upscaled for printing large pictures. More resolution (i.e. more pixels) means a higher quality picture when blown up or printed.
- **Image types:** Select one of these options to choose the image that will be output. The 'annotated spectrum with alignment' will suit most purposes.

SCV

Print Image Dialogue



- **Orientation:** paper orientation is shown in the picture at the top. Change this by clicking the “Portrait” or “Landscape” radio buttons.
- **Paper:** Set the paper size and source by selecting from the appropriate dropdown list.
- **“Printer” button:** pressing this will bring up another dialogue where we can select from a list of printers installed on our machine.
- **“OK” button:** this will commence printing.

The default print output is the full spectrum as shown in the spectrum alignment frame. If we wish to print something else, we must use the export image functions and then print the image from another application.

SVC

SCV

Toolbars

Main window toolbar



"Open data file" button: This allows us to open a raw data file built by our mass spectrometer, or a PEAKS data file (in ANZ format) that also contains peptide analysis data. The file should be in PKL, DTA, MGF or ANZ format.



"Close data file" button: Close the selected data file. Press this after selecting a data file in the Peptide Data Frame.



"Save data file" button: Save any changes made to the file (a '*' will appear next to any file that has been changed and not yet saved). The file will be saved in the ANZ format. Press this after selecting a data file in the Peptide Data Frame.



"Save all files" button: Save all files. Any changes to files will be saved in the ANZ format.



"Copy" button: Copy selected spectrum data.



"Cut" button: Cut selected spectrum data.



"Paste" button: Paste spectrum data into the selected data file.



"Manual merge spectra" button: After selecting more than one spectrum in the peptide data tree, this button becomes enabled. Right click it to merge these spectra into a single MS/MS spectrum, and remove the old ones.



"Data Refinement" button: Merge scans of the same peptide, remove noise spectra, preprocess within each MS/MS spectrum and recover peptide charge state. The data refinement options dialogue will allow us to choose and to set parameters for each of these refinement tools.

SC



"Auto *De Novo*" button: perform auto *de novo* for a selected data file, spectrum or list of data files. Press this after selecting one or more data files (or spectra) in the Peptide Data Frame. An auto *de novo* options dialogue will allow us to set parameters before we begin.

SC



"PEAKS Protein ID" button: perform protein identification a selected data file. Press this after selecting one or more data files (or spectra) in the Peptide Data Frame. A protein identification options dialogue will allow us to set parameters before we begin..

SC



"inChorus Protein Identification" button: perform protein identification on a selected data file using multiple search engines. Press this after selecting a data file in the Peptide Data Frame. A protein identification options dialogue will allow us to set parameters before we begin.



"Environment Preference Configuration" button: configure the environment, spectrum color-coding, and manual *de novo* parameters.



"PEAKS Properties Configuration" button: define PTM, Enzymes, and add FASTA protein or EST databases.



"Import Database Wizard" button: help user download and configure database.

Main Processing Window Toolbar



“y ion Alignment” button: toggle (show/hide) the location of PEAKS corresponding to y-ions and the corresponding proposed peptides between them.



“b ion Alignment” button: button: toggle (show/hide) the location of PEAKS corresponding to b-ions and the corresponding proposed peptides between them.



“Deconvolute” button: toggle (on/off) deconvolution of the mass spectrum scale.



“1:1 zoom” button: return spectrum to original 1:1 zoom.



“Undo Zoom” button: return to previous zoom ratio.



“Edit Ion” button: set or edit the type of ion associated with a peak in manual *de novo*. Press this button after having selected a peak in the spectrum view frame.



“Next Peptide” button: redo changes to the peptide in manual *de novo*.



“Previous Peptide” button: undo changes to the peptide in manual *de novo*.



“Export Results” button: export the spectrum view, ion table, or to a picture (bmp, gif, or jpg format) with ions, masses, peaks and peptides marked.



“Print Results” button: print the spectrum view with ions, masses, peaks and peptides marked.



“View Results” button: show, in HTML format: the spectrum view with ions, masses, peaks and peptides marked; peptides and confidence scores; the ion table; and the error plot.

PEAKS Studio Configuration

How to set up PEAKS Studio just the way we like it.


This chapter deals with configuration. PEAKS 4.2 is a versatile and flexible tool. But in order to use the software to its full extent, we must learn how to configure it to make it do what we want it to. Additionally, PEAKS 4.2 allows us to set up many defaults and presets to help us be quick and precise.

We can use PEAKS 4.2 without the need to configure; default settings will be used. However, to increase efficiency we should set environmental preferences, and PEAKS properties. This will enable us to customize the tool to our requirements. It is recommended that we configure PEAKS 4.2 before processing data files.

SCV

PEAKS Properties Configuration

One of PEAKS 4.2 preferences, PEAKS Properties configuration sets the parameters that the algorithm will use while processing our data files. PEAKS properties include: enzyme, PTM and database. **When using PEAKS Client, all PEAKS properties are controlled by the server, please contact your administrator.** PEAKS Studio 4.2 provides tools to edit PEAKS properties for convenient use in *de novo* sequencing and protein identification in PEAKS Studio. To edit PEAKS Properties:

- Click the  icon in the main window toolbar;
- Or from the “Edit” menu, select “Configuration” then “PEAKS Properties”

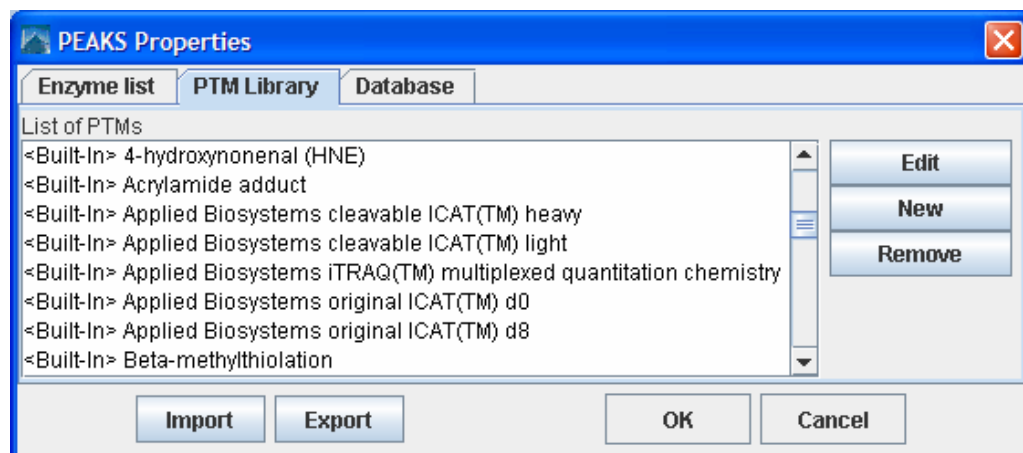
The PEAKS Properties dialogue will then appear. This dialogue box has three tabs: “**Enzyme list**”, “**PTM Library**” and “**Database**”. Clicking a tab will allow us to edit the PEAKS Properties corresponding to that tab. We can also **import** or **export** our preferences to/from a file.

SV

Creating and defining PTM

If we know that our sample protein may have been modified since translation, we need to apply this information to our analysis. To edit the list of PTM available to PEAKS Studio we open the PEAKS Properties dialogue and select the PTM Library tab. **PEAKS Client uses the PTM library defined on the server, editing here will have no effect on the list available during analysis.**

**PTM Post
Translational
Modifications
affect the mass of
modified proteins
and residues**



All PTM are listed here including <built-in> PTM and user defined PTM. From here, we can create a new PTM, edit an existing PTM or remove a PTM from the list. See the sections below for help with these operations. *Built-in PTM cannot be removed from the list, but can be edited.*

Editing a Built in PTM

It is possible to modify a built in PTM. PEAKS will save the modification and treat this PTM as a customized PTM. It will temporarily overwrite the built-in PTM - we will not be able to see the original, built-in, PTM until we remove the customized one. We can remove this customized PTM at any time and the built-in PTM will reappear.

Creating a New PTM

To create a new PTM, we open the PEAKS Properties dialogue, ensure that the 'PTM' tab is selected and click the 'new' button. *To create a new PTM 'on the fly' while setting up PEAKS auto de novo or PEAKS Protein ID, click the "new PTM" button while selecting PTM.* The PTM Editing dialogue will appear:

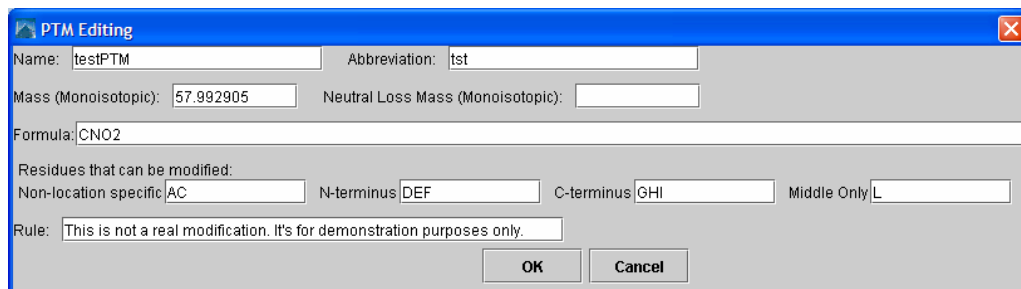


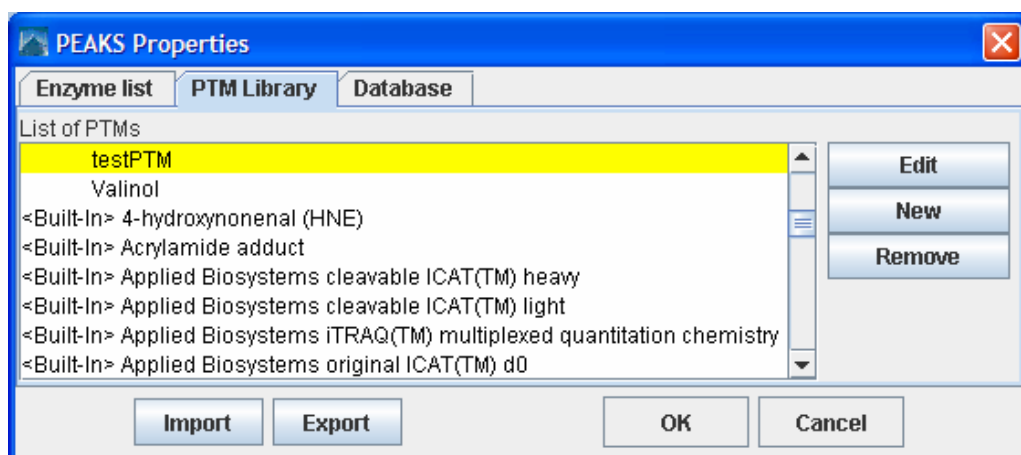
Figure 1 Create new PTM

Now we type information pertaining to our PTM in the appropriate boxes (see above section on **Interface** for a more in depth explanation of these fields). At a minimum, we must enter a name, a mass and one residue that may be modified.

Enter the mass of the modification either by typing in its monoisotopic mass difference directly, or by entering its empirical formula. It is unnecessary to do both; each will override the other.

Click the "OK" button to save changes and create our new PTM, or click the "Cancel" button to exit, discarding changes.

After clicking the "OK" button we return to the PEAKS Properties dialogue to find that our new PTM is listed at the top of the PTM list.



Editing a PTM

To edit a PTM, we open the PEAKS Properties dialogue, ensure that the 'PTM' tab is selected, and select a PTM from the list by clicking on it and click the 'Edit' button. *To edit a PTM 'on-the-fly' while setting up PEAKS auto de novo or PEAKS Protein ID, click the "add/remove PTM" button, to bring up the Modification window for that search, then click the "new PTM" button.* The PTM Editing dialogue will appear. Now we follow the same procedure (see above) as we would if creating a new PTM.

Removing a PTM

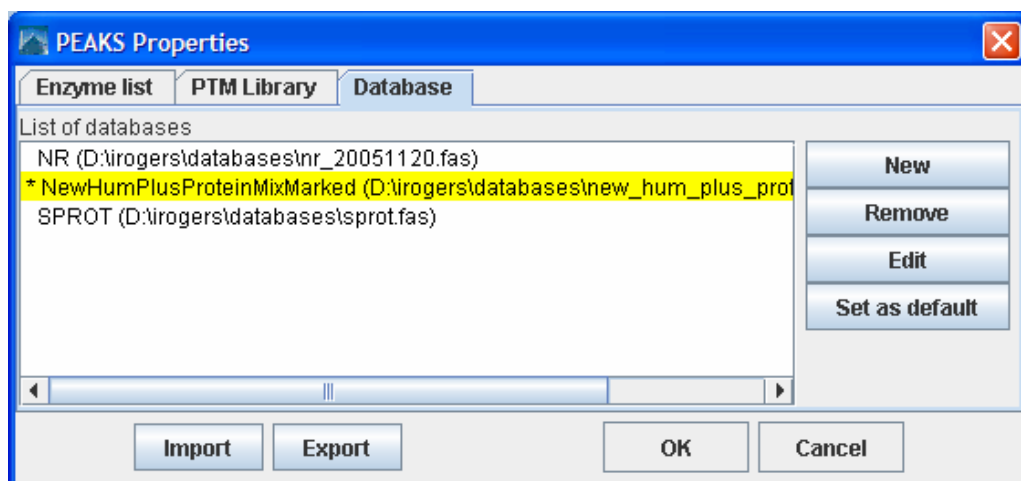
To remove a PTM, we open the PEAKS Properties dialogue, ensure that the 'PTM' tab is selected, select a PTM from the list by clicking on it, and click the 'Remove' button. Any built-in PTM cannot be removed.

S

Database Manager

PEAKS 4.2 needs a protein or EST database (in FASTA format) to identify protein candidates. Since databases are being constantly updated, PEAKS does not ship with a protein or EST database. Thus, we need to download it from the Internet and tell PEAKS where the database is located. PEAKS provides the Database Manager as a tool to help us do this. To see a list of databases available to PEAKS 4.2, load the PEAKS Properties dialogue and click the 'Database' tab. From here we can edit a database's properties, load a new database, or remove an existing database.

PEAKS Client uses the databases configured on the PEAKS Online server.



Load/Configure a new database

For an in depth look at configuring a database, see the Database Configuration section in Chapter 2.

To configure a new database, we open the PEAKS Properties dialogue, ensure that the 'Database' tab is selected and press the "New" button. Now we open up our web browser to find a database to download. Find one, download it, and unpack it. If taxonomy is available for this database, download those files too. Return to PEAKS 4.2 and find the file on our system where we unpacked it. Name the database and select the header format to use (or we can define our own). If taxonomy is available for the database, find those files too. Click "OK". The new database will now appear, listed by our chosen name, in the list of databases.

Remove a Database

To remove a database, we open the PEAKS Properties dialogue, ensure that the 'Database' tab is selected, select a database from the list of databases, and press the "Remove" button. This will not permanently remove it from our system, it may be reloaded (follow the procedure for configuring a new database) at any time.

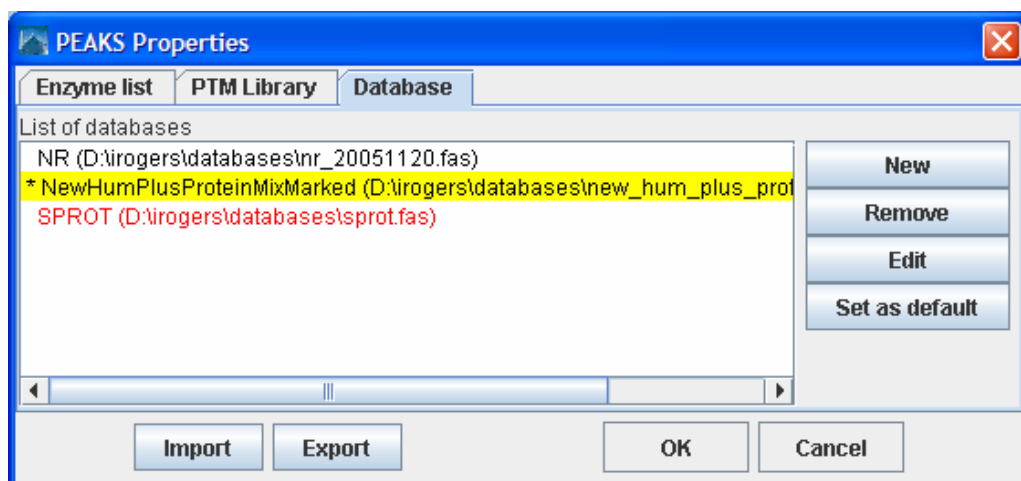
Edit a Database

This tool allows us to change the name that PEAKS 4.2 associates with a database, taxonomy files and the header parsing rules for that database. To edit a database, we open the PEAKS Properties dialogue, ensure that the 'Database' tab is selected, select a database from the list of databases, and press the "Edit" button.

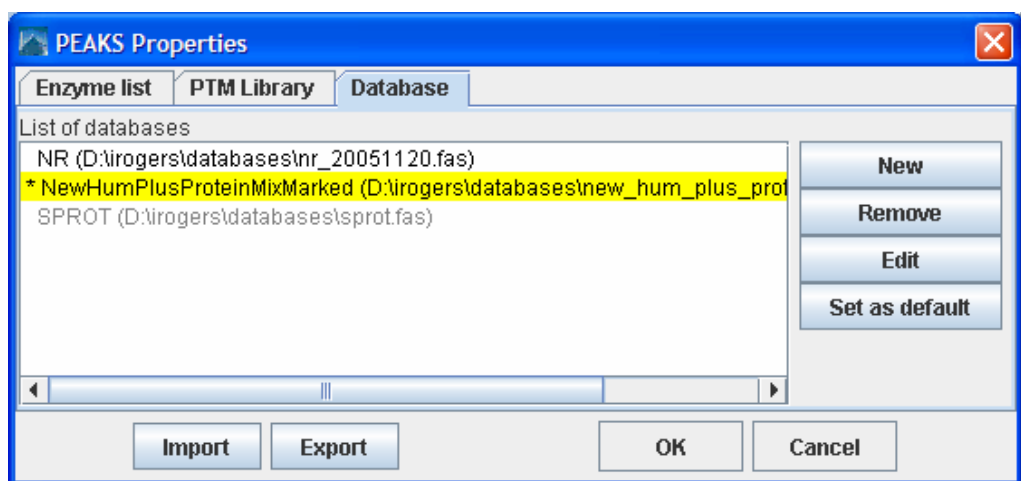
Moving/Updating a Database

If we choose to move a database to another directory, or delete it entirely, we should tell PEAKS. We must remove the database from the list and re-load it. Until we do so, the database name will appear in red in the list of databases and any protein identification using that database will fail.

We can't edit the database itself from within PEAKS 4.2.



If we choose to update the database (perhaps by downloading the latest database file and overwriting the old database file), PEAKS will show the database information in light gray. *A light grey colour could also mean that the database does not have header information.*



SV

Importing and Exporting PEAKS Properties

We may wish to use PEAKS 4.2 on another system. However, if we have a large number of user defined PTM, enzymes and saved parameters it could take a great deal of our time to re-input those. This is where importing and exporting of PEAKS properties is useful.

The export function will save PEAKS Properties information in a XML file. The import function can read a PEAKS properties XML file and overwrite local PEAKS Properties with the information from XML file. If we wish to use our PEAKS properties on a colleague's system, we must remember to export our colleague's properties to a separate file so that it will not be lost and can be imported later.

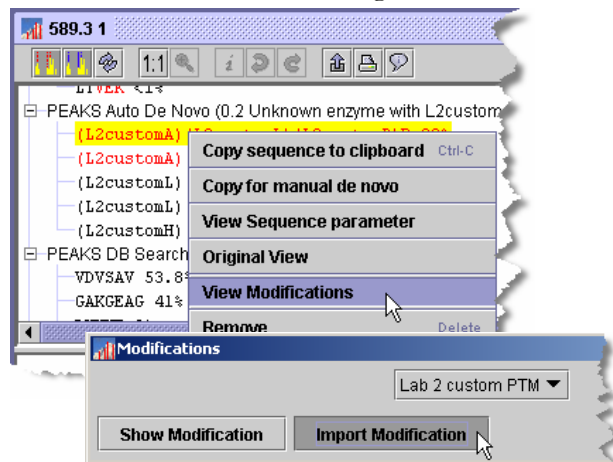
A note on sharing sequences with PTM

Sequence data and protein identification results for a given spectrum are stored in an .ANZ file. Any modifications that were found in the sequence are also included. As such, user-defined modifications will still show up if the file is viewed on another machine. It is not necessary to import all PEAKS properties to view these modifications. Also, user-defined modifications can be extracted from an .ANZ file and added to the local PEAKS properties.

To export PEAKS properties to a file, open the PEAKS Properties dialogue and press the “Export” button. Type in a file name and press the “Save” button.

To import PEAKS properties from a file, open the PEAKS Properties dialogue and press the “Import” button. Select a file or type in a file name and press the “Open” button. This must be a PEAKS configuration file in XML format.


To import a user defined PTM from another user’s .ANZ file, we open the .ANZ file and find a sequence containing the user defined modification. Right click on that sequence to bring up the popup menu. Click the “View Modifications” menu item. This brings up a dialogue box named “Modifications”. Select the PTM of interest from the dropdown list (in this example: ‘Lab 2 custom PTM’) and click the import modification button.



SVC

PEAKS Environment Preference Configuration

One of PEAKS 4.2 preferences, PEAKS Environmental Preference allows us to customize PEAKS 4.2 to our needs. PEAKS Environmental Preferences include: Environment, Color, Manual *de novo*, and Parameters. **To edit PEAKS 4.2 Environment Preferences:**

- Click the  icon in the toolbar;
- Or, from the “Edit” menu, select “Configuration” then “Environment Preference”

The Environment Preferences dialogue will then appear. This dialogue box has seven tabs: **'Parameters'**, **'Basic Ion Table Editor'**, **'Advanced Ion Table Editor'**, **'Environment'**, **'Color'**, **'Display'**, **'Manual *de novo*'** and **'Parameters'**. Clicking a tab will allow us to edit the Environmental Preferences corresponding to that tab.

Environment Preferences

Parameters Basic Ion Table Editor Advanced Ion Table Editor

Environment Colour Display Manual De Novo

Default Data Input Directory:
 C:\Documents and Settings\bsi\Desktop\Tutorial Data
☒ Previous directory ☐ User directory

Default Data Output Directory:
 C:\Documents and Settings\bsi\Desktop\Tutorial Data
☒ Previous directory ☐ User directory

Default Configuration File Directory:
 C:\Documents and Settings\bsi\peaks

Default Peaks Invocation
☒ PEAKS Studio
☐ PEAKS Online Host Name (or IP Address) Port
 User Email

☒ Close the main processing window before loading a new one for a different spectrum.
☒ Load sample files at start up.
☒ Show wiff file options.
☒ Show bruker file options.
☐ Show GNU Public License.

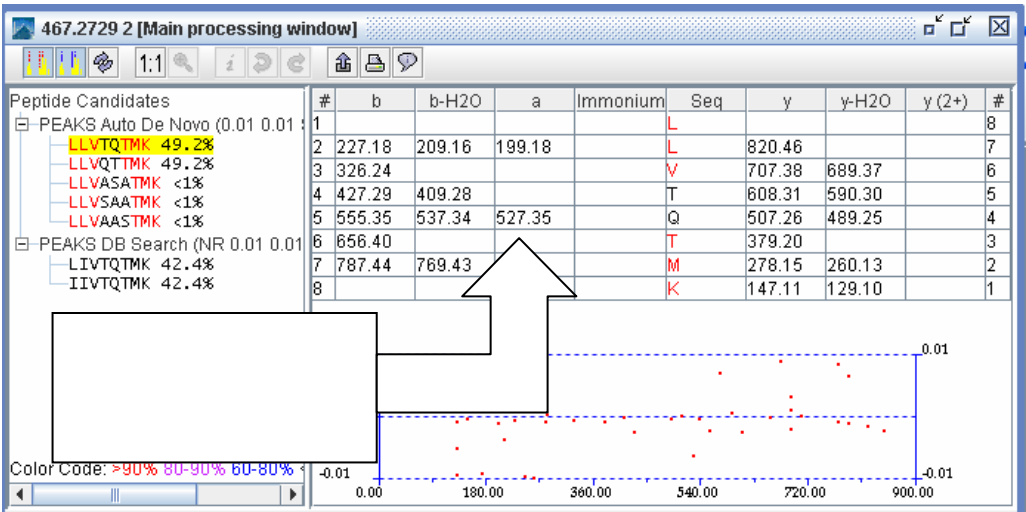
S

Removing Saved Parameters

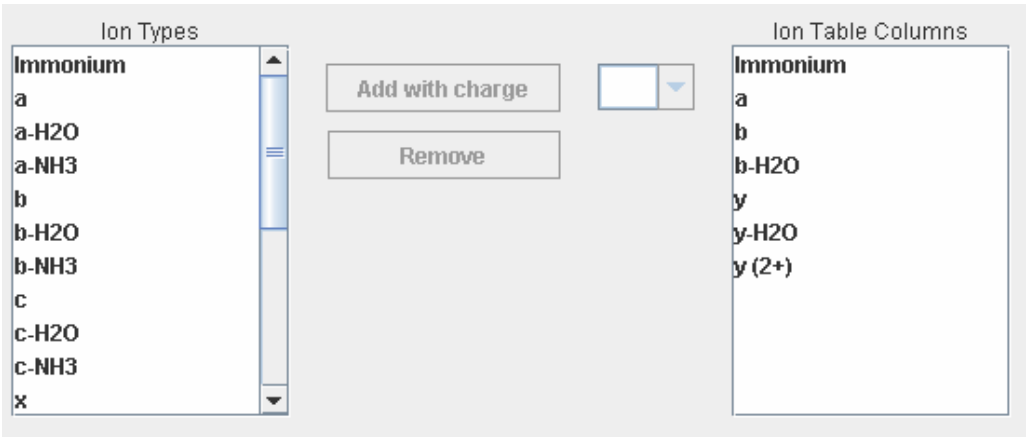
After months of use, our list of parameters saved for use with PEAKS Protein ID or PEAKS auto *de novo* may become cluttered with infrequently used parameter sets. It makes sense to clear them out from time to time. To do so, open the Environment Preferences dialogue and ensure that the 'Parameters' tab is selected. A list is shown for each tool that has savable parameter sets. Select one or more (using shift+click and ctrl+click) and click the adjacent "Remove" button to remove it from the list.

Configuring the ion table

The ion table, displayed in the top right of the main processing window, displays all the ions that were found as evidence for the selected sequence. There are two presets: the Basic Table and the Advanced Table. Select which one to display by choosing “Show Ion Table ►” from the “View” menu.



The Basic Table will display a maximum of 6 ions. The Advanced Table can be configured to display as many as are available. To configure the ion table, we choose “Edit menu” → “configuration” → “environment preferences” and then choose either the ‘Basic Ion Table Editor’ tab, or the ‘Advanced Ion Table Editor’ tab. Both tabs look and work the same way:



The ions types that will be displayed in the ion table are shown on the right. The complete list of ion types available is shown on the left. To add an ion type to the ion table (i.e. add a column to the ion table):

1. Select one or more ions from the list on the left. Use shift+click and ctrl+click to select multiple list items.
2. Select a charge (from 1-4) from the drop down list in the middle.
3. Click the 'Add with charge' button

For example, configure the ion table to display y^{2+} ions by selecting 'y' from the list on the left, and '2' from the dropdown charge list.

Remove ion types from the ion table (i.e. remove columns from the table) by selecting one or more items in the list on the right, and clicking the 'Remove' button.

SCV

Environment - Changing default file handling settings

To change the working environment, we open the Environment Preferences dialogue and ensure that the "Environment" tab is selected.

We can change the environment settings so that when we are browsing our system to find or save data files, PEAKS always starts looking in the folder we specify. The current working folders for data input and data output are shown. PEAKS 4.2 can use the last folder we loaded from (saved to) as the current working folder or by toggling the appropriate radio button to "User directory" we can set it ourselves so that it will be the same each time. The directory where PEAKS stores its preference information cannot be changed.

The option to load a new spectrum view window for each spectrum or open one at a time is available. We can choose to show the sample data at startup. Further, adjustments can be made so that the GNU (General Public) license displays whenever GNU governed software/libraries are called. Click the appropriate checkbox at the bottom of the window.

Once we've chosen from these options, pressing the "OK" button will exit, saving changes. The "Cancel" button will exit discarding changes.

SC

Environment - PEAKS Client to PEAKS Online

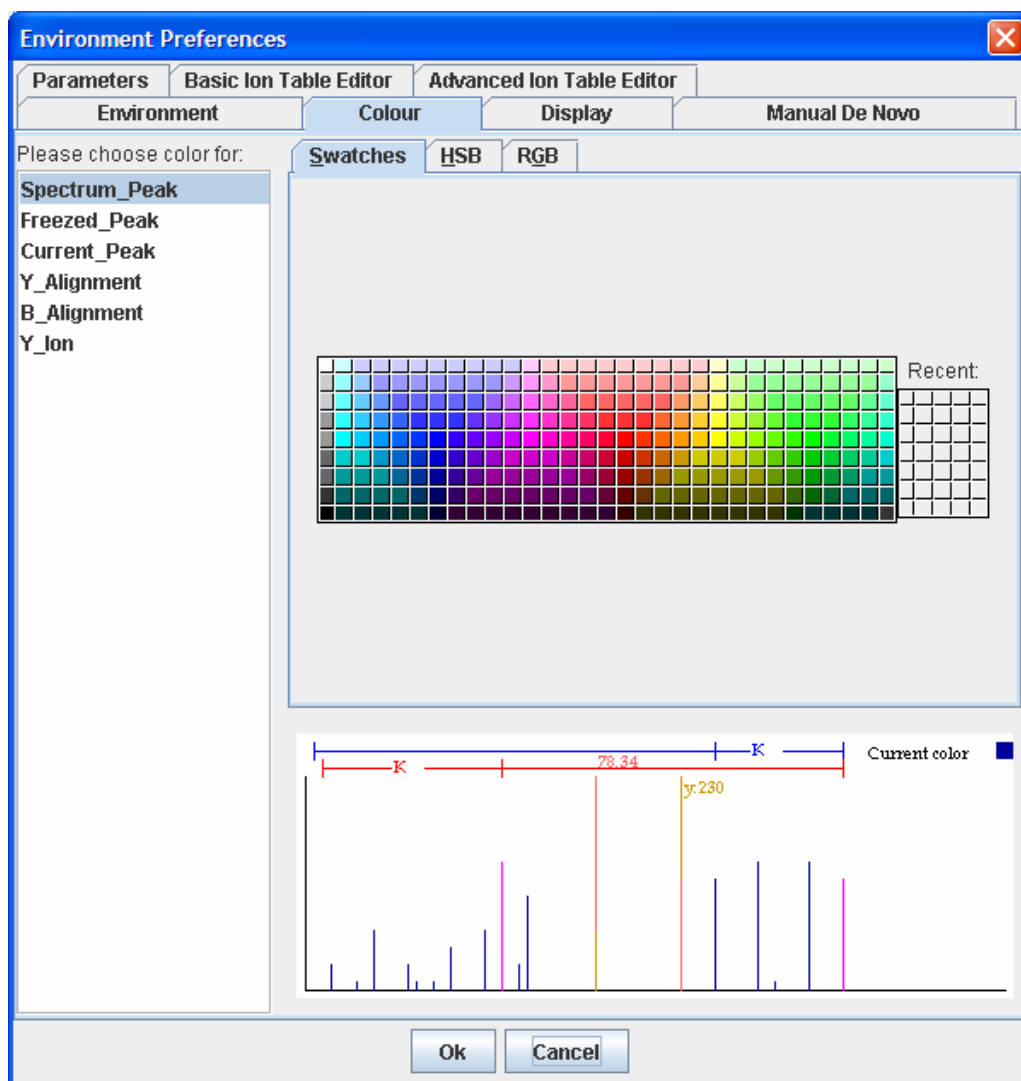
In the **Environment** tab of the Environment preferences dialogue, change the "Default PEAKS Invocation" to "PEAKS Online". Then specify the IP address or URL of the PEAKS Online server, the port number to use, and a username and email address. Press ok. Now we can use PEAKS 4.2 in the same way we normally would, except that processing will occur on the PEAKS Online server. The email is used to notify us that a job has been completed, in the event that we close PEAKS 4.2 before finishing.

SCV

Changing Colours

For ease of viewing, we can choose which colours we would like to represent which items on the spectrum view. To change the colour of an object on the spectrum, we

open the Environment Preferences dialogue and ensure that the 'Colour' tab is selected.

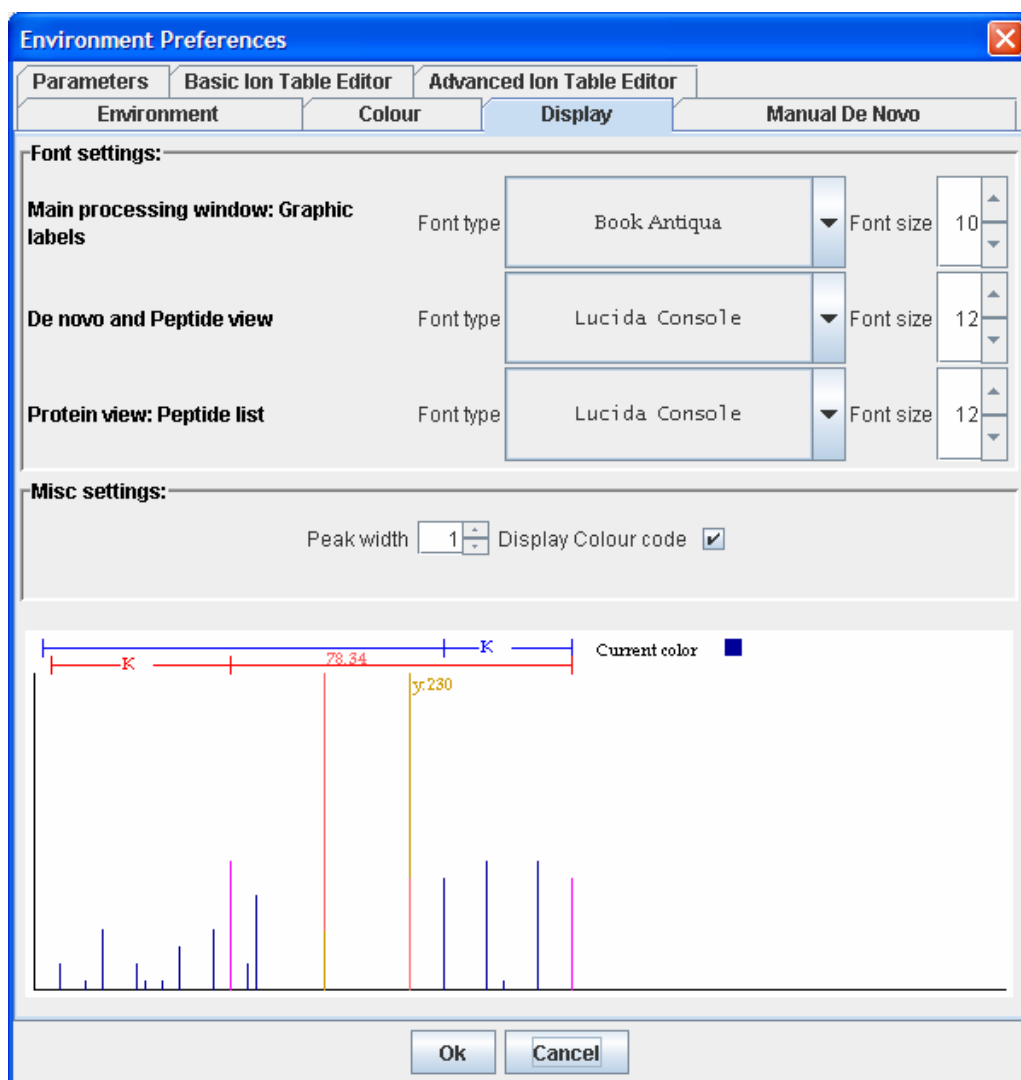


Choose the object whose color we'd like to change from the list at the left. Then we select a swatch to choose the colour. After we've chosen colors we may click the "OK" button to exit and save changes.

SCV

Display - Changing typefaces and peak width

We can customize the various typefaces to use on certain parts of the PEAKS 4.2 interface. To do so, open the Display tab on the Environment Preferences window. Select the typeface from the dropdown list and/or change the font size, then press ok. The width of peaks on the spectrum display can be adjusted from here as well.

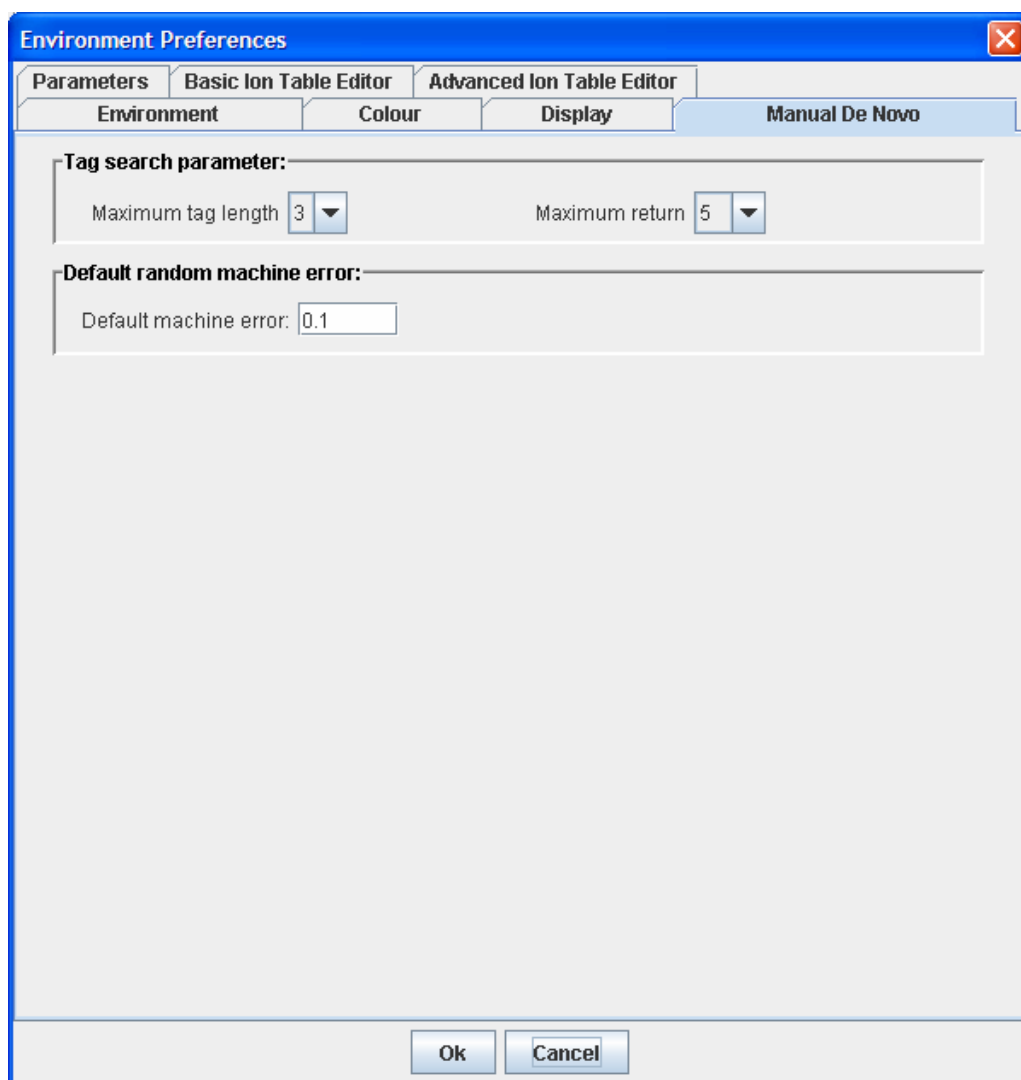


SCV

Changing Manual *de novo* defaults

We may wish to sequence a peptide manually, using spectrum data. PEAKS 4.2 provides us with a set of tools to help us do so. We may need to tweak these tools to adjust for error tolerance and to customize the working environment. This can be done on an individual basis, by right clicking on the sequence we're working on, but we can set defaults.

To adjust Manual *de novo* options, open the Environment Preferences dialogue and ensure that the 'Manual *de novo*' tab is selected.



When sequencing a peptide using the manual *de novo* tools, we can get PEAKS to help us by searching to the left or right of a selected peak and returning a set of possible sequence tags (see the Manual *de novo* section in the next chapter). We can choose how many search results we'd like to see and choose how the maximum (number of amino acid residues) length. To choose how long tags will be, we click on the "Maximum tag length" dropdown list box, and making a selection. To choose the number of search results displayed, click on the "Maximum return" dropdown list and make a selection.

Changing the default machine error sets the amount of error PEAKS will tolerate when tagging a residue. *For example: we have a mass difference of 113.19 between two y-ions that we have labeled. We are fairly confident that this should be tagged L (Leucine, with actual mass of 113.08), but PEAKS is not labeling it for us. This may be because 113.19 is too far out of PEAKS error tolerance for the mass of L.* We can tweak the settings until we get the desired result.

To do so, type a value for error (larger numbers indicate greater tolerance) into the “Default machine error” textbox.

After having made all desired changes, click the “OK” button to save changes and exit the dialogue box. Click the “Cancel” button to exit, discarding changes.

Loading and preparing data

A task based guide to getting our data into PEAKS 4.2.

The following chapters deal with usage. They are broken up into tasks that a typical user might perform. It assumes we can identify parts of the Graphical User Interface and that we are familiar with how PEAKS 4.2 can be configured. The preceding two chapters provide in depth help on these subjects and should be used as a reference. Such detail has occasionally been omitted from this chapter in the interest of succinctness.

SCV

Loading data into PEAKS 4.2


PEAKS 4.2 can be used to process data from any MS/MS instrument, provided the data is accessible, or can be converted to an accessible format. PEAKS handles data files in the following formats:

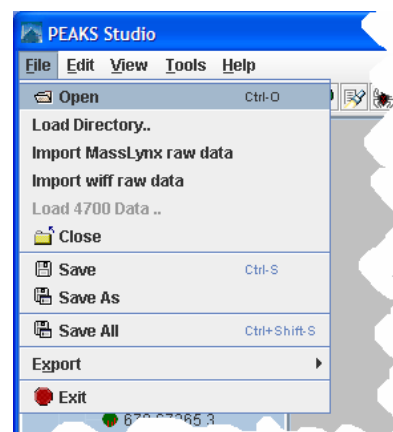
- PKL: The file format associated with Micromass instruments.
- DTA: The file format associated with SEQUEST software
- MGF: The file format associated with Mascot software.
- ANZ – the zip compressed XML based file format associated with PEAKS
- XML format files using the mzXML schema
- XML format files using the mzData schema
- RAW files from Thermo Electron instruments
- WIFF files from ABI QSTAR instruments
- RAW files from Waters QTOF instruments
- .BAF, .YEP and folders of .FID files from Bruker instruments
- XML format files from Waters ProteinLynx software
- DAT files created by BSP's ABI converter software

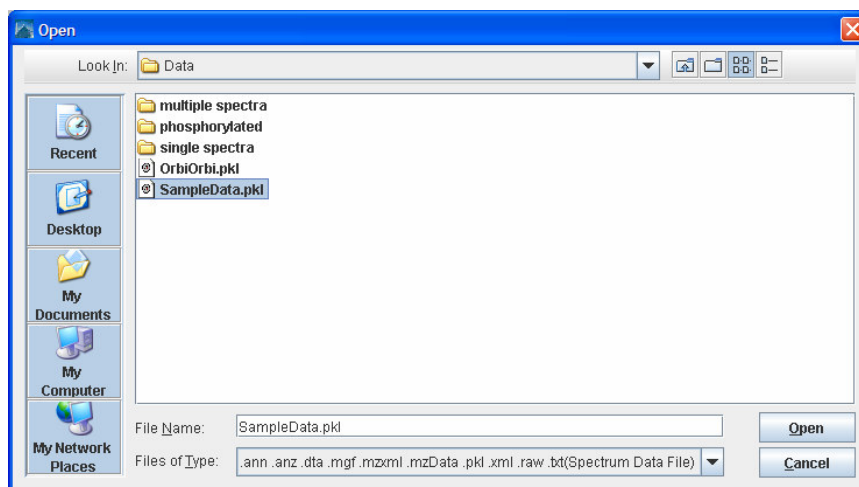
SCV

PEAKS demo data can be found in the DATA sub-directory, located in the PEAKS directory.

Opening data files

In order to do any data processing we must first load our spectrum data into PEAKS 4.2. To open a data file, click the  icon on the toolbar in the upper left corner of the PEAKS window or select “Open” from the “File” menu.





Select a file. Click the Open button.

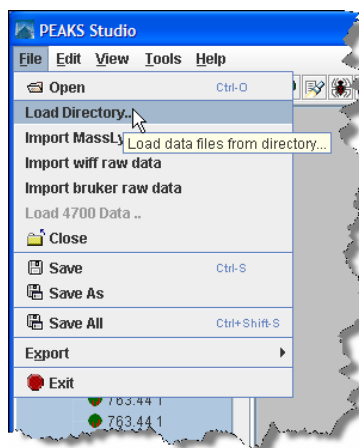
The data file we just opened appears in the Peptide Data Frame on the left. It is represented by its file name. Each spectrum contained in the data file is represented by its precursor ion information (m/z value followed by the charge of the precursor ion that generated the spectrum).

SCV

Loading a directory full of DTA files

DTA spectrum data files can be opened by the same procedure as listed above. However, as we know, some DTA files contain the data for only one spectrum. As such, we may find it useful to import a whole directory (containing DTA MS/MS spectrum data files for a whole MS run) at once and consider it as one MS run. PEAKS 4.2 provides a tool for doing so.

Under the “File” menu, click “Load Directory”. Now browse to the directory we wish to load.



Do not select a file within the directory; rather select the directory itself. Press the Open button.

After loading the spectra, we can choose to sort the spectrum by the source filename or by the precursor m/z value of spectrum. To do so, right click the parent node on the Peptide Data and choose to sort.

The data file we just opened appears in the Peptide Data Frame on the left. It is represented by the folder name. Each spectrum contained in the data file is represented by its precursor ion information (m/z value followed by the charge of the precursor

ion that generated the spectrum) and file name.

SCV

Loading Thermo RAW data

PEAKS 4.2 can load RAW data from our Thermo Electron mass spectrometer; provided that the XCalibur software is installed on the same computer as is PEAKS 4.2. To load Thermo RAW data, simply choose File → Open, and browse to the file.

SCV

Importing Applied Biosystems WIFF data

PEAKS 4.2 can load WIFF data from our Applied Biosystems QSTAR mass spectrometer; provided that the Analyst QS software and the MSX plug-in are installed on the same computer as is PEAKS 4.2. The MSX tool is produced and sold by Infocromics Ltd., and is available (at cost) from Bioinformatics Solutions Inc. Please contact a BSI sales representative to obtain a license. To load QSTAR WIFF data, simply choose File → Import wiff raw data, and browse to the file.

SCV

Importing MassLynx RAW data

PEAKS 4.2 can import RAW data from our Waters/MicroMass QTOF instrument. To do so, we choose “Import RAW data” from the File menu. As above, the file browser appears. Choose the .RAW data, and click the Open button. PEAKS uses a utility called wolf.exe (originally created as part of the Sashimi Project) to access MassLynx libraries and convert the data. PEAKS ships with a version of wolf.exe designed to work with MassLynx 4.0. If we need a different wolf, we can check:

www.bioinfor.com/products/peaks/support/watersmicromass.php

Additionally, we must make sure that the following MassLynx libraries are installed on the same computer as PEAKS and wolf.exe:

Some versions of MassLynx may differ.

- DACServer.dll
- Genutil.dll
- MetaGD32.dll
- raw.dll
- securityAccess.dll
- securitySettings.dll
- securitySignature.dll

They should be stored in the folder C:\MassLynx\ as part of the MassLynx software. If they are not stored here, or MassLynx is installed on another computer, the automatic loading will not work. If the automatic loading is not working for either reason try this:

1. We should be able to find the listed files on our computer, or another computer in our lab. If you can copy them, do so.

2. We can then create a folder called “C:\MassLynx\” on our computer, and place the files we copied here. But we’re not finished, we must also register these files with Windows.
3. We can find a file on our system called regsvr32.exe using the ‘Find’ or ‘Search’ tool in our windows start menu. It is probably in “C:\WINDOWS\System32\”. If it’s not there, substitute the correct location in step 4.
4. Open a command prompt or the Run tool from the start menu and type the following:

```
C:\WINDOWS\System32\regsvr32 C:\MassLynx\DACServer.dll
```

All on one line, with one space in the middle as shown. Press the enter key. If successful, windows will pop up a success message.

Please check the license

The libraries mentioned in this section are part of the MassLynx software, distributed by Waters Corp. Please check the MassLynx license agreement, or contact a Waters representative to make sure it is okay to copy and use the libraries in this way.

SCV

Importing Data from the ABI 4700 or ABI 4800

BSI has created a converter to extract the data from an ABI-Oracle database. If we require this separate, free tool, we must ask a BSI representative. Once installed, we can start up the ABI 4700 Data Extractor from the Start menu.

System Requirements

This extractor can be installed on the same machine as ABI 4700 Explorer and the Oracle database (we will call this machine the 4700 SERVER in the following instructions) or another machine that has direct network access (no firewall, no proxy required) to the 4700 SERVER. Windows 2000 or Windows XP is recommended for use with this tool.

Configuration:

Before using the ABI 4700 Data Extractor, we should configure it. To do so, we can choose “Settings” from the “File” menu. Configuration needs the following:

- **4700 SERVER Name or IP Address:** input “localhost” if the Extractor is running on the 4700 SERVER (this is the default value), otherwise enter the IP address of the 4700 SERVER.
- **The socket used by the 4700 SERVER:** this is the port that the Oracle database listens to (the default is 1521).

- **Username to access the Oracle database:** most likely we do not need to change this (the default is “tsquared”).
- **Password to access the Oracle database:** mostly likely we do not need to change this one either.

Data extraction procedure:

1. Load Spot Set List from the database: (Do it via menu File | Load Spot Set List) The extractor will export the peak list of a spot set into a PKL file.
2. Open a Spot Set: (menu File | Open Spot Set) Spot Set Chooser will help the user to choose a spot set. After selecting a spot set, click 'OK' to open it. The job run information of a spot set will be shown.
3. Select a job run: There is a radio button before each job run, only the MS/MS job run can be selected for export, because we need the precursor information. Select a job run and click 'Convert' to do the extraction.
4. Choose a filename to save: After clicking the 'Convert' button, the user needs to input a file name and the peak lists of the selected job run will be exported.

SC

Refining data before analysis

Since mass spectrometry data often contains noise and redundant data, it makes sense to purify the data before analysis. This will increase the quality of the results, while saving time spent on database searching and/or *de novo* sequencing. MS/MS spectra that are purely noise can be removed from the data:

- peptide charge information can be verified and recovered
- multiple low quality scans of the same peptide can be merged into one scan with intense signal peaks
- the MS/MS scans themselves can be centroided, filtered for noise, and deconvoluted.

When PEAKS is connected to a PEAKS Online server, we'll also save time by uploading smaller, preprocessed data. Data refinement is done locally, before uploading to the server.

To begin the refinement of data from a whole MS/MS run:

1. In the *Peptide Data* Frame, select the data file(s) containing the data that we wish to refine.

2. Click the  Data Refine toolbar icon

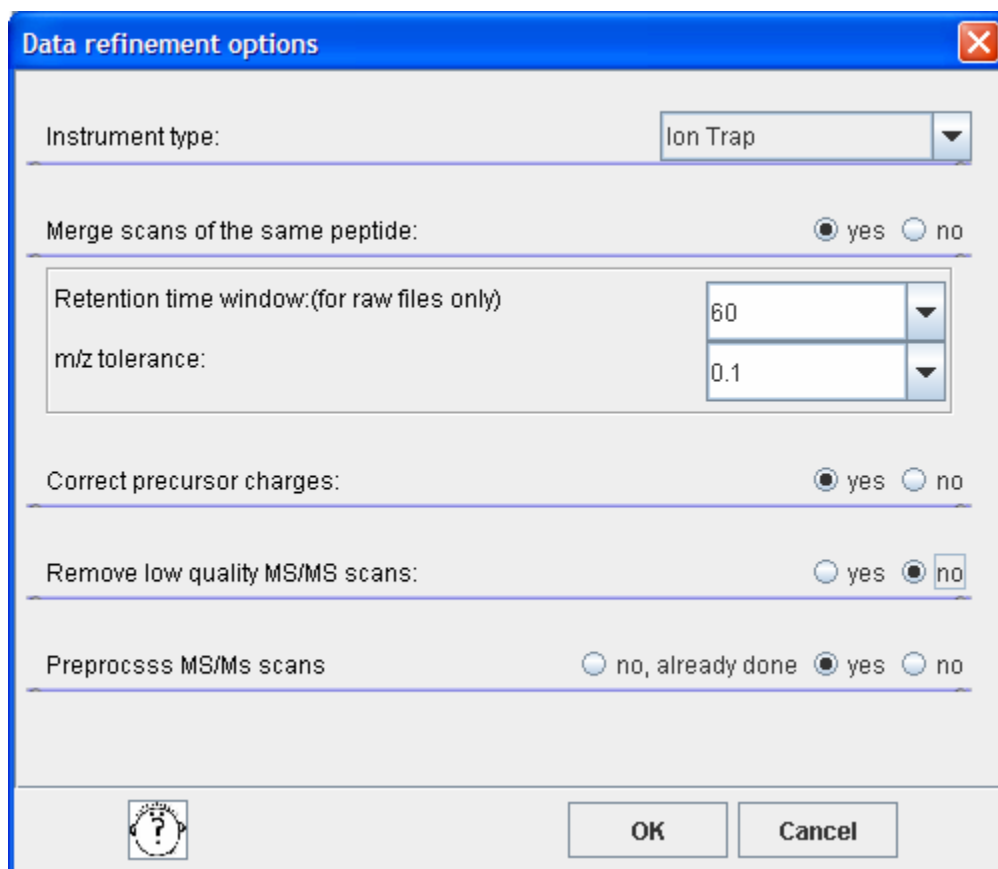
Or

Select “Data Refine” from the “Tools” menu.

Or

Right click on the selected data file and select “Data Refine” from the popup menu

The Data refinement options dialogue appears:



The dialog box titled "Data refinement options" contains the following settings:

- Instrument type: Ion Trap
- Merge scans of the same peptide: ☒ yes ☐ no
- Retention time window: (for raw files only) 60
- m/z tolerance: 0.1
- Correct precursor charges: ☒ yes ☐ no
- Remove low quality MS/MS scans: ☐ yes ☒ no
- Preprocess MS/MS scans: ☐ no, already done ☒ yes ☐ no

Buttons: OK, Cancel

3. Choose the data refinement tools we wish to use by clicking the “yes” radio button next to each one.

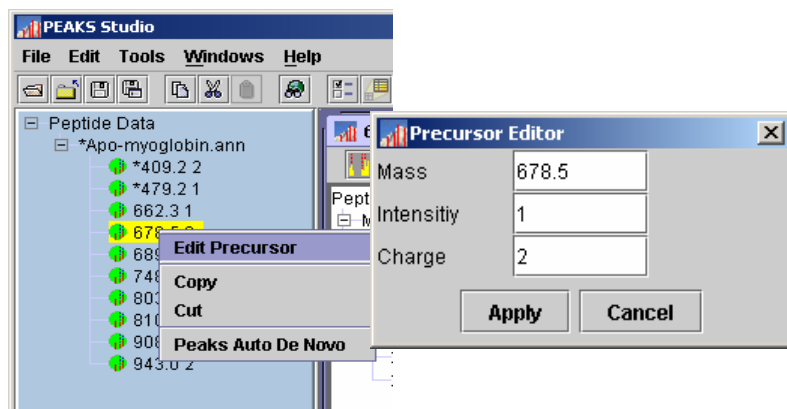
- **Instrument Type:** choose the type of spectrometer that produced the data to be analyzed.
- **Merge Scans of the same peptide:** in DDA mode, a mass spectrometer will often produce several tandem ms (i.e. ms/ms) scans of the same peptide. To increase the intensity of real signal peaks within these scans and to reduce the size of the whole data set, it makes sense to merge ms/ms scans of the same peptide together. To avoid improper merging (of ms/ms scans of different peptides) we make sure that the measured parent ion masses of these peptides are very close and that they have similar retention times in the LC column. The units here are m/z values in Daltons. For retention time, we use whatever units are recorded in the data file (usually minutes or seconds).
- **Correct precursor charges:** Since a mass spectrometer measures mass-to-charge ratios, we must know the charge on a peptide before we can determine its mass. The standard method of finding the charge is to look at the spacing of the isotope ladder in the survey scan. However many Ion-Trap instruments don't have enough resolution for this. So PEAKS will look at the MS/MS data to determine if it's charge 1+, 2+ or 3+. This tool needs to only be used on ion trap data.
- **Remove low quality MS/MS scans:** Scans of contaminants and electrical noise should not be included in analysis. Removing them from the data set will save time, and reduce the risk of random matches to the database. PEAKS presents an effective tool for removing these low quality ms/ms scans. This tool has been designed for use specifically on ion trap data.
- **Preprocess MS/MS scans:** Deconvolution (de-isotoping), centroiding and noise filtering within the MS/MS data. *Data is always saved in the ANZ file along with the PEAKS results. Preprocessing can save hard disk space or upload time. But make sure to have the original data available in case we need to refer to it later.*

A note on preserving data-results integrity

Protein ID and *de novo* sequencing results obtained for a given dataset prior to use of this tool may become invalid, since some spectra are removed/merged/corrected and the data-results relationship may be broken. PEAKS 4.2 will warn us when this may occur and prompt us to save a separate copy.

Manually Manipulating Data Files

Editing Precursor information



It is possible that the precursor information, as listed in the Peptide Data Frame, is incorrect. If the charge listed is wrong or if the m/z listed is even slightly incorrect (more than 0.1 Daltons, depending on the accuracy selected) it could really affect the quality of the results. In this case it is imperative that we change the precursor information. The change will only affect the .ANZ file we are working on.

To edit precursor information, select a spectrum by clicking on its name, then right click the mouse while holding it in position over the name. A small menu will appear; click on “Edit Precursor”.

In the dialogue that follows, type the new precursor information into the appropriate textboxes. Click the “Apply” button when finished to apply the changes. Click the “Cancel” button to exit, discarding changes.


The precursor information will be updated, reflected by a change in the name of the spectrum in the Peptide Data Frame. A * will also appear in front of that name, indicating that there is unsaved information pertaining to that spectrum.

Manually merging MS/MS scans of the same peptide

If we’ve done several MS/MS scans of the same peptide, we may want to reduce the amount of data to process and at the same time, improve the data quality by merging all of a peptide’s MS/MS scans together. Often, we choose to automatically merge appropriate spectra from the whole data file using the Data Refine tool (see above). But this can also be done manually.

To manually merge spectra after opening a data file:

1. Select those spectra we wish to merge together from the Peptide Data Tree (left) using shift+click and ctrl+click.

2. Next, right click in the Peptide Data panel, and choose Merge Spectra from the popup window that appears, OR click the  'Manual Merge Spectra' toolbar button.
3. A dialogue will appear, asking what should be the correct value for the precursor mass and charge. After reviewing and/or correcting the value, press "OK". The spectra will be merged.

SCV



Cutting and Copying Spectrum Data

If we wish to move spectrum data from one data file to another we may do so by copying and pasting it (see below for pasting instructions). Also, we may wish to make a copy of the spectrum in the same data file in order to re-sequence an individual spectrum using different preferences. Cutting spectrum data will remove it completely until pasted. Copying spectrum data will duplicate the spectra when pasted. To cut/copy spectrum data:

Changes made to the original spectrum after duplication will not affect the duplicated spectrum.

Select a spectrum by clicking on its name (select multiple spectra by holding down the 'control' key and clicking on any number of spectrum) in the Peptide Data Frame.

Right click on one of the selected spectra. A small pop-menu will appear. Select "Cut" or "Copy"

Or click the "Copy" button  or "Cut" button  in the main toolbar.

Copied/Cut items will remain on the clipboard until replaced by another copied/cut item. **Warning: Unless pasted, a cut item will be lost, as subsequent cut/copied items will displace it from the clipboard.**

SCV

Pasting Spectrum Data

After having copied or cut spectrum data, we would like to paste it into another data file, or the same data file. To paste spectrum data:

1. Select the data file into which we wish to paste the spectrum (or spectra) by clicking on its name in the Peptide Data Frame. *We may only choose to paste into one data file at a time.*
2. Right click on one of the selected spectra. A small popup menu will appear. Select "Paste" from the popup menu.

Or click the "Paste" button  in the main toolbar.

The pasted spectra will appear in the Peptide Data, under the data file into which we pasted.

Analysing Data with PEAKS 4.2

A task based guide to processing our data with PEAKS 4.2.

This chapter will step us through the analytical techniques available to PEAKS 4.2 users. It is broken up into tasks that a typical user might perform. It assumes we can identify parts of the Graphical User Interface and that we are familiar with how PEAKS 4.2 can be configured. Such detail has occasionally been omitted from this chapter in the interest of succinctness.

Using PEAKS with modifications (PTM)

PEAKS 4.2 provides the most flexible handling of post-translational modifications of any software built for de novo sequencing and protein ID. Users are free to create their own modifications (see the **Creating a New PTM** section), and search for any combination and any number of modifications. Modifications can be considered as part of auto *de novo* sequencing or protein identification. The search is set up the same way for both tools. The options screen for each tool has an area titled “PTM selected for search”. Any modifications to be considered during the search will be shown here (and labeled as ‘Fixed’ or ‘Var’). When we first run PEAKS, the box will be blank, meaning no PTM are selected.

PTM selected for search	
Fixed	Carbamidomethylation
Var	Deamidation
Var	Oxidation
Var	Phosphorylation

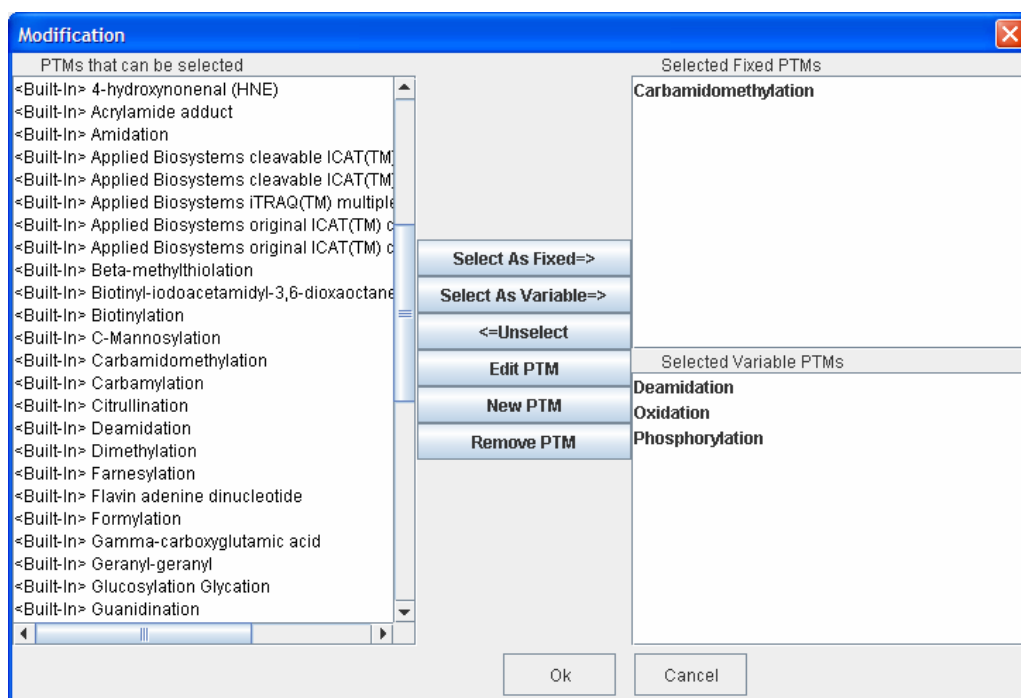
Add/Remove PTM

For PEAKS Client, the PTM library is stored on the server. Ask the administrator to make changes.

To add modifications to this list, click the Add/Remove PTM button. The Modification dialogue appears. The entire **PTM library** – *i.e. the list of all <built in> and user defined modifications that are available to PEAKS* – is displayed in the list on the left. We’ll choose the modifications we need from this list. To add a new modification to the PTM library, click the ‘New PTM’ button. To edit a modification in the PTM library, select it from the list on the left and press the ‘Edit PTM’ button. To remove a modification from the PTM library, select it from the list on the left and press the ‘Remove PTM’ library.

If you remove/edit a PTM, it will be removed or changed from/in any saved parameter set that refers to it.

The lists on the right show what PTM will be enabled for the search. Use the ‘Select as Fixed=>’, ‘Select as Variable=>’ and ‘<=Unselect’ buttons to move them in and out of these lists. Press the ‘OK’ button when finished and the changes we made will be reflected on the protein ID options dialogue.



Generally, the more variable PTM we turn on, the more ambiguous will be the results.

Remember, when doing auto *de novo* sequencing or PEAKS Protein ID on a complex mixture, we will get best results if we choose the correct fixed PTM and a few variable PTM. When using PEAKS Protein ID to characterize a protein, it is best to search against a small database that contains only a few proteins and turn on all modifications. Furthermore, to limit spurious hits, we can assume that it is less likely that a tryptic length peptide will not be modified more than a few times and as such, limit the number of variable modifications that can occur on each peptide.

SC

Auto *de novo* Sequencing

To begin auto *de novo* sequence derivation:

1. In the *Peptide Data* Frame, select the data file(s) containing the spectra that we wish to sequence by Auto *de novo*. We can also select an individual spectrum, or a few spectra within a data file; auto *de novo* will proceed on only the spectra selected.

2. Click the  Automatic *de novo* toolbar icon

Or

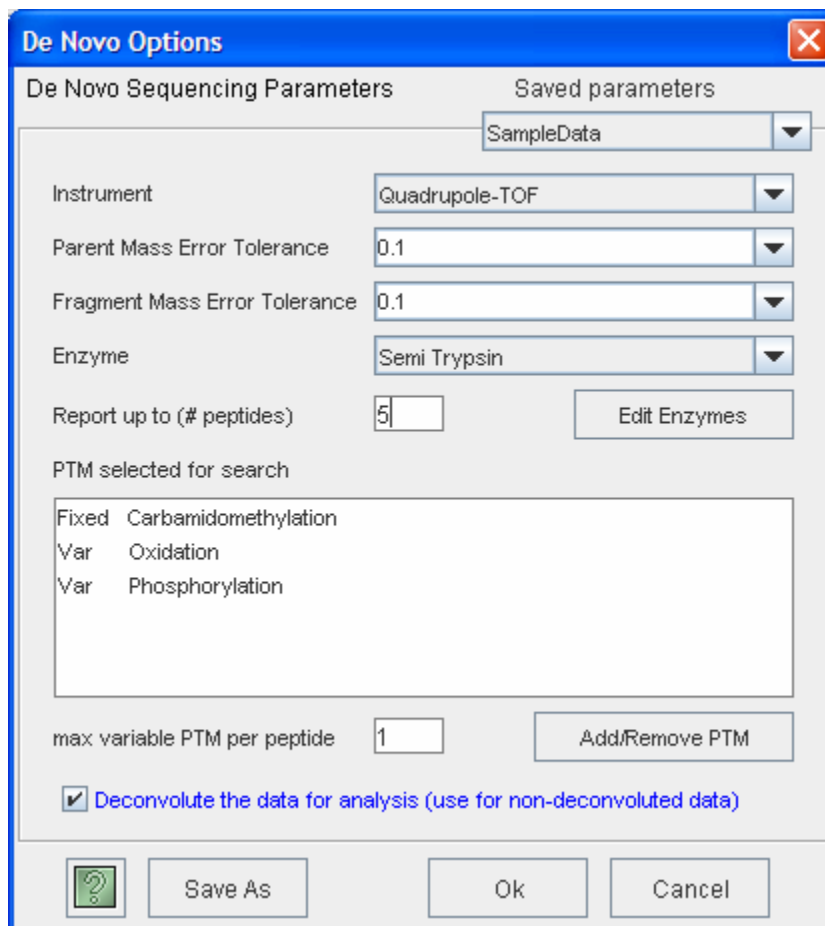
Select “Auto *de novo*” from the “Tools” menu.

Or

Right click on the selected spectra or data files and select “Auto *de novo*” from the popup menu.

The Auto *de novo* Parameters dialogue window will appear:

We should begin by using the suggested error values, then try some slightly higher or lower ones to find the best result.

The image shows a software dialog box titled "De Novo Options". It has a blue title bar with a close button (X) in the top right corner. The dialog is divided into two main sections: "De Novo Sequencing Parameters" and "Saved parameters". The "Saved parameters" section has a dropdown menu currently showing "SampleData". The "De Novo Sequencing Parameters" section contains several settings: "Instrument" is a dropdown menu showing "Quadrupole-TOF"; "Parent Mass Error Tolerance" is a dropdown menu showing "0.1"; "Fragment Mass Error Tolerance" is a dropdown menu showing "0.1"; "Enzyme" is a dropdown menu showing "Semi Trypsin"; "Report up to (# peptides)" is a text input field containing the number "5", with an "Edit Enzymes" button to its right; "PTM selected for search" is a list box containing three items: "Fixed Carbamidomethylation", "Var Oxidation", and "Var Phosphorylation"; "max variable PTM per peptide" is a text input field containing the number "1", with an "Add/Remove PTM" button to its right; and a checkbox labeled "Deconvolute the data for analysis (use for non-deconvoluted data)" which is currently checked. At the bottom of the dialog, there is a help icon (question mark in a green square) and three buttons: "Save As", "Ok", and "Cancel".

3. If we wish to change any of these parameters, we do so now.

- **Instrument Type:** Choose the type of spectrometer that produced the data to be analyzed. If we are using a hybrid instrument, choose a setting that matches our fragment ion mass analyser. For example, if we measure the parent ion in an FT, and the fragment ions in an ion trap, choose the ion trap instrument setting. Fragmentation type can also be chosen from this drop down.
- **Parent mass error tolerance:** Determine how much random and systematic experimental error on the parent/precursor ion PEAKS will allow for in its analysis. Select a tolerance from the dropdown list or type in a value. New



PEAKS users should try setting this a little higher than past experience may suggest.

- **Fragment mass error tolerance:** Determine how much random and systematic experimental error on the fragment/daughter ion PEAKS will allow for in its analysis. Select a tolerance from the dropdown or type in a value. Again, new PEAKS users should try setting this a little higher than past experience may suggest.
- **Enzyme:** Tell PEAKS what kind of enzyme was used to digest the sample. Choose from a dropdown list of enzymes, or if our enzyme (or combination of enzymes) is not in the list, click the "Edit Enzymes" button.
- **Report top:** Set how many peptide sequences PEAKS will report from its de novo sequencing analysis.
- **Max missed cleavages:** Determine the most missed cleavages to allow, internal to the peptide, in a *de novo* sequence. For instance, if we set this to 2, and Trypsin is the enzyme, then PEAKS will return de novo sequences with up to 2 R's or K's internally.
- **PTM selected for search:** This list tells PEAKS what kind of post-translational modifications to include in its analysis. Each is marked Fixed or Variable. To edit this list, click the "Add/Remove PTM" button.
- **Max variable PTM per peptide:** To reduce uncertainty, we can limit PEAKS' de novo sequencing 'vocabulary', by restricting the number of variable PTM we can find on a peptide. Specify a number by typing it into the box. To lift such restrictions, type a very large number (longer than the length of the peptide).
- **Saving/Loading Parameters:** After setting up parameters, we can save them for future use. Click the "Save Parameters" button, and choose a name for future reference when prompted. Don't worry, we can't accidentally overwrite the defaults. Any parameters we save will be available in the drop-down list at the top of the window. To see what's inside, select one and the parameters boxes will be populated.
- **Preprocess before auto de novo:** PEAKS Studio has its own built-in preprocessor for removing noise, centroiding and peak charge recognition from MS/MS data. Check this box to turn preprocessing on.

Notes on pre-processing

BSI highly recommends using PEAKS to preprocess all data, as opposed to using instrument vendor software, if the data is to be used by PEAKS. PEAKS preprocessor should not be used on data that has already been pre-processed as this will have adverse effects on the results (unless it is ion-trap data).

4. Press the “OK” button to commence Auto *de novo* sequencing.

Once a job is submitted to PEAKS 4.2, it is added to the Task Queue for processing. After processing, the job is removed from the task queue list and the dark green  icons beside the spectra (in the Peptide Data Frame) change to light green  and/or an asterisk (*) appears.

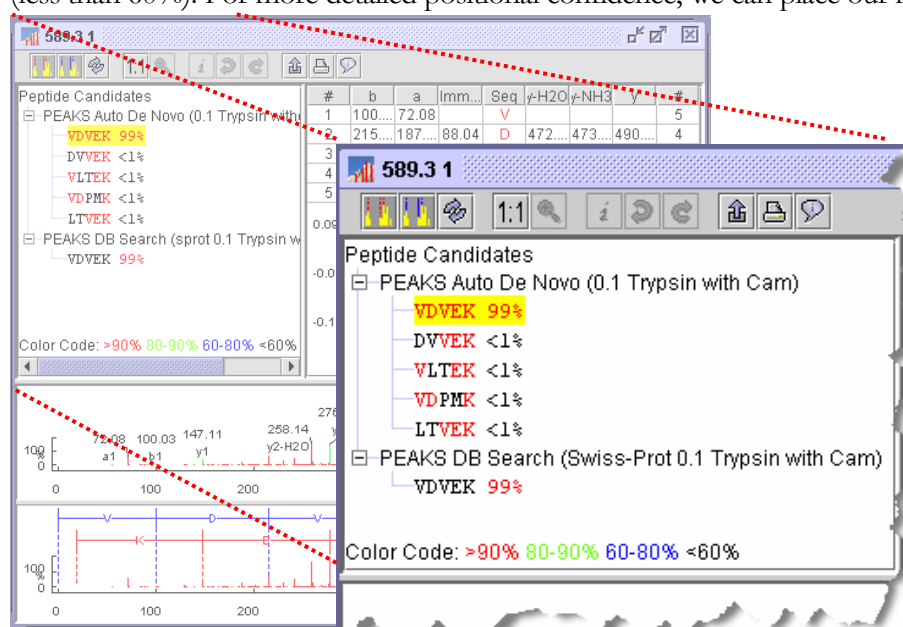
Viewing Auto *de novo* Results

After performing auto *de novo* on a spectrum, we may wish to see what the algorithm determined the peptide sequence to be and review the results for ourselves. To do so, we click on the spectrum of interest in the Peptide Data Frame. This brings up the Main Processing Window for that spectrum.

The most likely peptide sequence candidate, as determined by auto *de novo*, will be automatically selected. This is found in the Peptide Candidates Frame as the top listed candidate under 'PEAKS Auto *de novo*'. In this example, the highlighted sequence is VDVEK. Any modifications that have been found will be shown abbreviated and in sequence before the amino acid residue they are associated with. *If the PTM was defined/created by another PEAKS user on another system, the PTM would still be shown and it can be imported into the local PEAKS configuration as desired.*

Confidence scores are probability based, on a scale of 0 to 100%

Right next to the proposed sequence, the auto *de novo* confidence score is shown. Positional confidences (that is, confidence that the correct residue in each position has been identified) are readily available by color coding. **Red** represents a very high confidence (greater than 90%), **purple** represents a high confidence (80 to 90%) **blue** represents a medium confidence (60 to 80%) and **black** represents a low confidence (less than 60%). For more detailed positional confidence, we can place our mouse over





the sequence of interest. A Position Confidence Table will appear, showing the confidence that each tag/subsequence is correct.

In the Ion Table frame, select a cell from the Ion Table – each cell represents an ion. This will highlight its position on an error plot (scroll the Ion Table frame down if the error plot is not visible). A point close to the centerline indicates a more confident



Zoom in far enough and we may resolve the isotopic ladder (depending on our instrument/data).

result. We can also notice that the peak corresponding to the Ion we selected is highlighted on the Spectrum View. Select a whole column to highlight all the points for that type of ion. The types of ions displayed in the ion table can be configured, choose Configuration → Edit Ion Table from the Edit menu. FTMS users might find this particularly useful when sequencing data acquired using ECD.

By looking at the Spectrum View Frame, we can see the strength of the MS/MS peaks that PEAKS 4.2 has set as ions. The view also displays the mass of the ions at that peak and the type of ion. Click on a peak to mark it and display its information at the top left corner of the Spectrum View Frame.

Zoom in by clicking and dragging horizontally on an area of the Spectrum View. The area over which we dragged will now take up the whole spectrum view. To un-zoom, press the  undo zoom icon or press the  1:1 icon to return to the full spectrum view.

We may also zoom in on the spectrum using the Spectrum Alignment Frame. Again, click and drag horizontally on an area of the Spectrum View. The area over which we dragged will now take up the whole spectrum view. The blue bar beneath the Spectrum Alignment view shows where we are zoomed in. The white portion of the bar represents the area that we are zoomed in on.

We can toggle whether or not we'd like to see the positions of the y-ions and b-ions (and the proposed residues in sequence between them) on the alignment view by pressing the y-ion alignment  (yellow and red) and b-ion alignment  (yellow and purple) icons in the main processing window toolbar.

To view another peptide candidate, as determined by auto *de novo*, click on another peptide in the Peptide Candidates Frame and under 'PEAKS Auto *de novo*'. The information in the Ion Table will change, as will the tags on the spectrum, to reflect the selected peptide candidate's sequence.

SCV

Editing sequencing results (preparation)

We cannot change the results provided by PEAKS auto *de novo* or PEAKS database search. However, we can make a copy of any sequence and edit it using manual *de novo* techniques. To copy a sequence for editing:

1. Select a peptide sequence candidate from within the Peptide Candidates Frame. We can only select one peptide sequence candidate at a time.
2. Right click the mouse button while holding the mouse over that sequence. A pop-up menu will appear.

3. We can select the pop-up menu item “Copy for manual *de novo*”. In this case, the sequence will be automatically placed under the ‘Manual *de novo*’ heading. A ‘Manual *de novo*’ heading will be created if there wasn’t one there already.
4. Now we select our newly copied sequence, under the ‘Manual *de novo*’ heading, to display this sequence in the Ion Table Frame, Spectrum View Frame, and Spectrum Alignment Frame.

Now we are ready to edit the sequence, using manual *de novo* techniques.

SCV

Manual De Novo Sequencing

We can use manual *de novo* sequencing to fine tune the results of an auto *de novo* analysis or to perform our own sequencing analysis from scratch. PEAKS 4.2 provides a set of tools to help us sequence a peptide, using graphic cues from the spectrum.

SCV

Creating a fresh spectrum for sequencing

We cannot change the results provided by PEAKS auto *de novo* or PEAKS database search. Thus, to begin manual *de novo* sequencing, we must either copy a sequenced peptide (see above section: Preparing to edit sequence results) or create a new peptide candidate for sequencing.

To create a new peptide candidate for sequencing:

1. Right click on the 'Peptide Candidates' heading, the 'Manual *de novo*', or any 'user defined type' heading. This will bring up a pop-up menu.
2. Select "New candidate for manual *de novo*" from the pop-up menu

A new candidate will be created under the 'Manual *de novo*' heading, or under the 'user defined type' heading if we selected a user defined type. The new candidate will not have been sequenced, so it will be represented by the mass of the peptide less the mass of water (e.g. [945.15]).

SCV

Manual *de novo* Operations

All operations occur in the Spectrum View Frame of the Main Processing Window.

When the mouse is placed in the Spectrum View Frame, a blue (by default) bar follows the movement of the mouse. This is the *Position Bar* and it is used as a cursor for all manual *de novo* operations. The cursor's position on the m/z scale is enumerated on the top of the *Position Bar*.

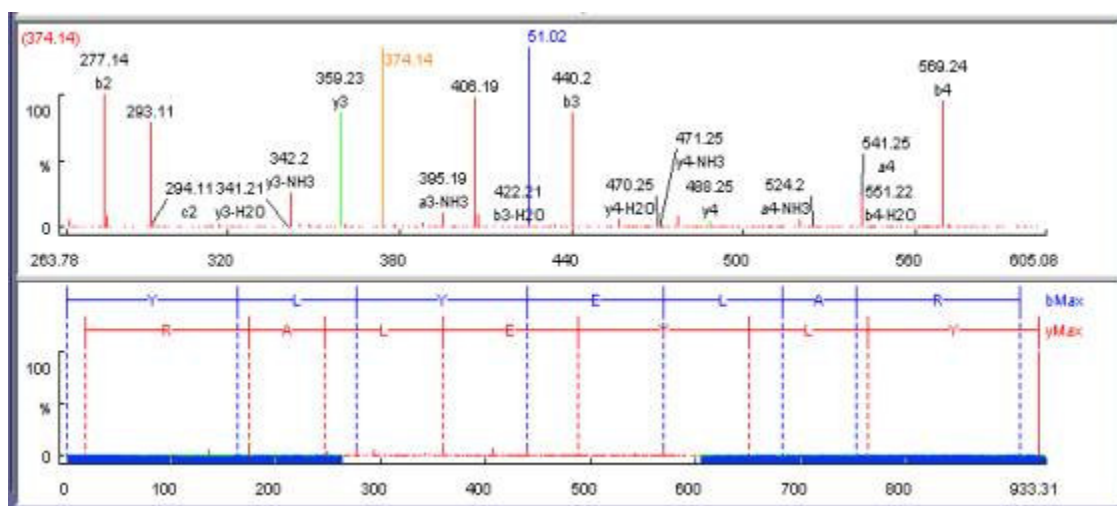
Selecting a peak

To select a peak, click on it. An orange (by default) bar, called *Freeze Bar*, indicates the selected peak.

Alternatively an ion peak can be selected by clicking on its corresponding cell in the Ion Table.

Measuring distance along the m/z scale

Once a peak is selected with the *Freeze Bar*, moving the mouse left or right will display the *Position Bar* along with a value that represents the m/z difference (as an absolute value) between the selected peak (orange) and the *Position Bar* (blue). In the example below, the distance between the selected peak and the position bar is 51.02 Daltons.



Measure the m/z difference between two peaks

Select a peak (orange line by default) with the *Freeze Bar* and move the mouse to the left or right. Hold the *Position Bar* above another peak. The number above the *Position Bar* is the difference between the two peaks.

Deselect a peak

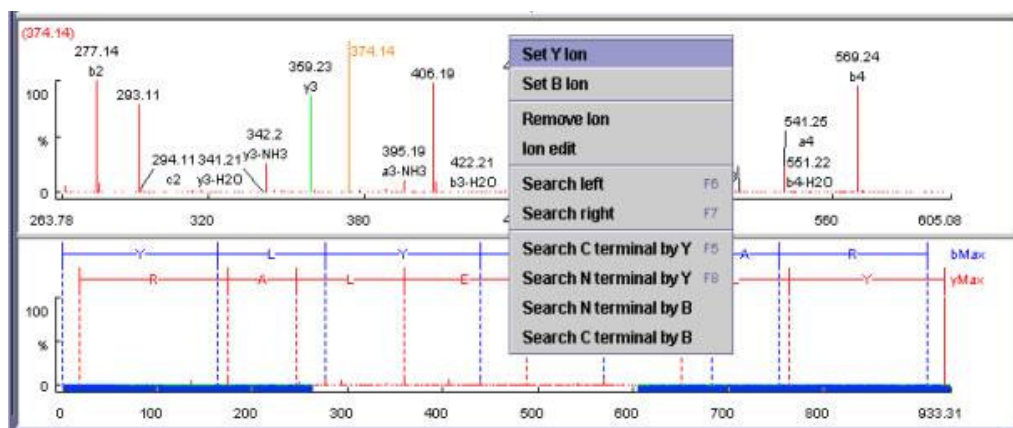
Double click anywhere in the Spectrum View Frame

Zoom in on part of the spectrum

In the Spectrum View Frame or the Spectrum Alignment Frame, click and drag the mouse horizontally. The selected area will be shown in the Spectrum View Frame.

Add (remove) ions to (from) a peak

Select a peak, then right click the mouse anywhere in the Spectrum View Frame. Select “Set Y Ion” from the pop-up menu to designate the peak as a y ion, “Set B Ion” from the pop-up menu to designate the peak as a b ion, Select “Ion Edit” from the pop-up menu to view the Ion Editor dialog box and designate the peak as another ion.



**See PEAKS
Environment
Preference
Configuration to
find out how to
change the
sensitivity of the
residue estimate.**

The Ion Editor dialogue allows us to add or remove ion designations to/from a peak. Select an ion from the ion choice list and press the “Add” button to add it to the selected ion list. Remove an ion from the selected ion list by selecting it and pressing the “Remove” button. We can type any comments we wish to make about the ion/peak, then press the “Apply” button to apply the changes to the selected peak.

Two short-cut keys may also be used to label a peak. Select a peak, then hit the 'y' key to add a y-ion and or the 'b' to add a b-ion to the peak on your keyboard.

After setting an ion, both the alignment view and the peptide sequence candidate name (as displayed in the peptide candidate frame) will change to reflect the mass remaining to be sequenced on either side of the ion. After setting two ions, PEAKS 4.2 will estimate the residue found between them (if a residue corresponds closely to the mass difference). The peptide sequence candidate name (as displayed in the peptide candidate frame) will change to show the residue and the mass remaining to be sequenced on either side of the residue.


Using sequence tags

Searching the C/N terminal by Y/B: right click anywhere in the Spectrum View Frame to trigger the popup menu. From the menu, select the terminal search of interest. PEAKS will select the appropriate terminal tags and show them in the Ion Table Frame. We may test the suitability of a tag by clicking on its radio button; the tag will be shown, in position, on the Spectrum View. We may insert one or more tags by clicking on their checkboxes, then clicking the “Apply” button. Press the “Cancel” button at any time to exit the search discarding changes.


**Two short cut keys
can be used: 'F6'
for searching the
left side and 'F7'
for searching the
right side.**

Search a sequence tag: select a peak with a defined ion (i.e. an ion that has been labeled with a peptide). Right click to trigger the popup menu, then select “Search Right” or “Search Left” to search peptide tags either to the right or left of the selected peak. PEAKS will select the appropriate terminal tags and show them in the Ion Table Frame. We may test the suitability of a tag by clicking on its radio button; the tag will be shown in position on the Spectrum View. We may insert one or more tags by clicking on their checkboxes, then clicking the “Apply” button. Press the “Cancel” button at any time to exit the search discarding changes.

Undoing an edit

If we have made an error in our sequencing it is possible to undo the change. With the Peptide candidate still selected in the Peptide Candidates Frame, click the  previous peptide button to return to the previous peptide sequence. We can click this button multiple times to return to successively earlier stages in our edit.

Redoing an edit

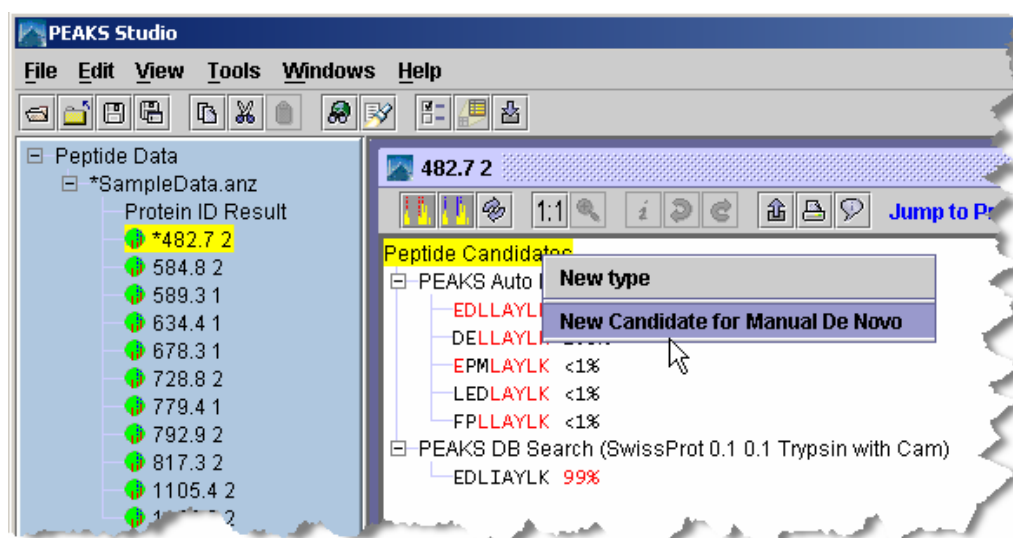
If we have undone one too many changes, we can redo that change by clicking the  next peptide button. We can click this button multiple times to proceed to successively later stages in our edit.

Suggesting a sequence to see how it fits the data

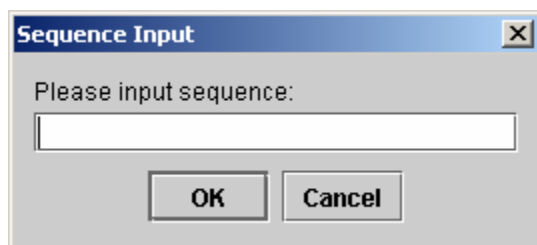
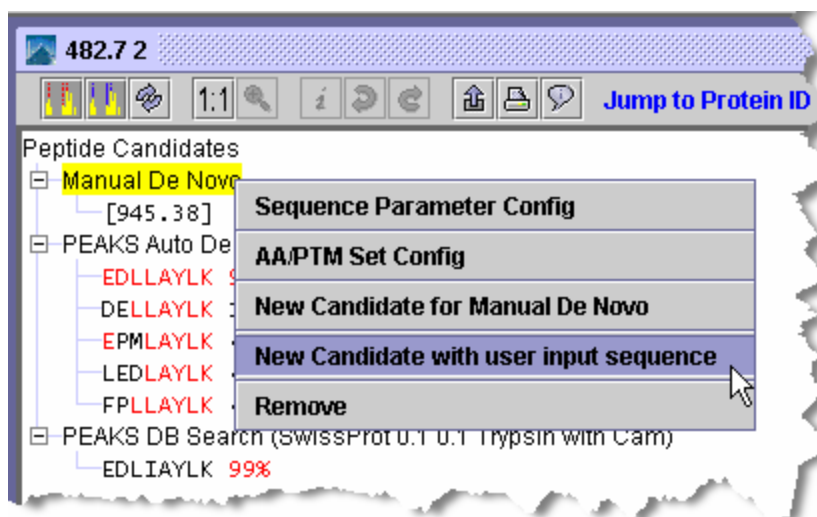
If the data is ambiguous, PEAKS 4.2 may not have displayed a particular candidate that we wish to evaluate, after auto de novo, or protein ID. We may enter this sequence and have PEAKS 4.2 find if there is any evidence for it in the data.

For instance, PEAKS may give the sequence RMYNVHGC(phosphorylationS)K for a particular spectrum, and we may wish to see if there's any evidence for the phosphorylation being on the Tyrosine. As such, we may type in our own version of the sequence and have PEAKS find ions that might support our hypothesis.

To do so, open the spectra in the main processing window, and right click on “Peptide Candidates” (in the Peptide Candidates Frame). Then, from the pop-up menu that appears, choose “New Candidate for Manual De Novo”.



A new node will appear with the heading “Manual De Novo” and beneath it will be the mass of the residues yet to be sequenced (in square brackets). Right-click on this heading. In the pop-up menu that appears, choose “New Candidate with user input sequence”, and the Sequence Input dialogue box will appear.



We can now enter our proposed sequence. The total mass of the residues, modifications, and un-sequenced masses should equal the total mass of the peptide (minus water). We might find the mass calculator tool (Tools menu), useful in this regard.

Short forms for the modifications may also be used.

Enter sequences in the format:

MPELAYLK

[228.09]ELAYLK

DE[226.168]AYLK

EDLLA(phosphorylationY)LK

DE[226.168]A(phosphorylationY)LK

Then press the OK button. The sequence we just entered will appear under the “Manual De Novo” heading and when selected, the ions that PEAKS has found to match the proposed sequence will appear on the spectrum, spectrum alignment view, and ion table.

SC

Protein Identification

The PEAKS protein identification method is unique – an improvement on and the ideal complement to existing tools. The unique approach is a combination of sequence tag searching and fragment ion mass matching.

PEAKS also introduces an amalgamative approach to protein identification called **inChorus**. With inChorus protein identification technology, we can use PEAKS together with several other protein identification software programs. This will deliver more protein coverage and more confidence in results than any one method on its own.


The following two sections deal with usage of PEAKS protein identification on its own and usage of inChorus protein identification.

SCV

PEAKS Protein Identification

The PEAKS Protein ID search engine takes a hybrid approach that uses sequence tag information to filter the protein or EST database before fragment ion fingerprinting. Thus to get useful protein identification results, we must first perform *de novo* sequencing on the spectrum data.

If we already have sequence information for this data, we may use this existing sequence information (manual or auto *de novo* sequences) to filter the database. If we do not have existing sequence information, or if we wish to refine our database search by providing brand new sequence information, we can ask PEAKS to perform auto *de novo* before searching the database. Brand new results will **not overwrite** any existing sequence data that we have.

1. In the *Peptide Data* Frame, we select the data file(s) that we wish PEAKS to use to identify our protein(s). This can be done by clicking on a data file's name in, the peptide data frame.
2. Click the "Protein identification" toolbar icon .

Or

Select "PEAKS Protein ID" from the tools menu.

The Protein Identification Parameters dialogue window will appear

3. If we wish to change any of the protein identification search parameters, we do so now.

- **Instrument Type:** choose the type of spectrometer that produced the data to be analyzed. If we are using a hybrid instrument, choose a setting that matches our fragment ion mass analyser. For example, if we measure the parent ion in an FT, and the fragment ions in an ion trap, choose the ion trap instrument setting. Fragmentation type can also be chosen from this drop down.
- **Parent mass error tolerance:** determine how much random and systematic experimental error on the parent/precursor ion PEAKS will allow for in its analysis. Select a tolerance from the dropdown list, or type in a value. New PEAKS users should try setting this a little higher than past experience would suggest.
- **Fragment mass error tolerance:** determine how much random and systematic experimental error on the fragment/daughter ion PEAKS will allow for in its analysis. Select a tolerance from the dropdown or type in a value. Again, new PEAKS users should try setting this a little higher than past experience would suggest.
- **Enzyme:** Tell PEAKS what kind of enzyme was used to digest the sample. Choose from a dropdown list of enzymes, or if our enzyme (or combination of enzymes) is not in the list, click the "Edit Enzymes" button.
- **Report up to:** set how many peptide sequences PEAKS will report from its de novo sequencing analysis.
- **Max missed cleavages:** determine the most missed cleavages to allow, internal to the peptide, in a de novo sequence. For instance, if we set this to 2, and Trypsin is the enzyme, then PEAKS will return de novo sequences with up to 2 R's or K's internally.
- **PTM selected for search:** this list tells PEAKS what kind of post-translational modifications to include in it's analysis. Each is marked Fixed or Variable. To edit this list, click the "Add/Remove PTM" button.
- **Max variable PTM per peptide:** To reduce uncertainty, we can limit PEAKS' de novo sequencing 'vocabulary', by restricting the number of variable PTM we can find on a peptide. Specify a number by typing it into the box. To lift such restrictions, type a very large number (longer than the length of the peptide).

Best practices for setting modifications (PTM)

The developers have discovered that database searching often returns better results if the auto *de novo* analysis is run with no variable PTM

(perhaps one or two if necessary), but with the correct enzyme and fixed PTM. Modifications should be then turned on for the database search function. m/z tolerance can also be adjusted separately for each phase to allow us to tweak the results.

- **Database to search:** Select from this dropdown list, one of the FASTA databases that we've set up in PEAKS. If the database we'd like to search is not in this list, click the "Load new database" button.
- **Taxonomy selection:** This list displays the taxa we've chosen for our search. If the database selected has taxon information available, we can click the aptly labeled "Add/Remove Taxa" button. Otherwise, the whole database will be searched. The selections correspond to established hierarchy -- i.e. selecting 'Mamalia' will search all of 'horse, cow, rat, mouse, human, etc.'
- **Paste FASTA sequences:** If we already know the sequence of the protein(s) we are studying, we can paste it here (must be FASTA format) and run a search against it. Alternatively, if we search the same sequence regularly, it is recommended to simply create a small text file and configure it as a database for PEAKS.
- **Preprocess before auto de novo:** PEAKS Studio has its own built-in preprocessor for removing noise, centroiding and peak charge recognition from MS/MS data. Check this box to turn preprocessing on.

Notes on pre-processing

BSI highly recommends using PEAKS to preprocess all data, as opposed to using instrument vendor software, if the data is to be used by PEAKS. The PEAKS preprocessor should not be used on data that has already been de-convolved by instrument software as this will have adverse effects on the results (unless it is ion-trap data).

If we choose to do pre-processing 'on the fly' during protein ID (as opposed to using the Data Refine tool beforehand) PEAKS preserves the original data, and does not save the results of its preprocessing.

- **Advanced Options - *de novo*:** We must have some de novo sequences before database searching since PEAKS sequence tags to help in database searching. As such the option of doing de novo prior to protein ID is presented here. In most cases, the same values for instrument, error, enzyme and PTM can be used in de novo and in protein ID, but we have the option of using one of our

saved *de novo* parameter sets for the *de novo* portion. Select one from the drop down list.


- **Saving/Loading Parameters:** After setting up parameters, we can save them for future use. Click the "Save Parameters" button, and choose a name for future reference when prompted. Don't worry, we can't accidentally overwrite the defaults. Any parameters we save will be available in the drop-down list at the top of the window. To see what's inside, just select one, and the parameters boxes will be populated. Note: the Advanced Options selections will not be saved.
4. Press the "OK" button to commence Auto *de novo* (if we have so chosen) and subsequent protein identification.

If we have chosen to perform auto *de novo* prior to our database search, the Auto *De novo* process will appear first in the task queue. Once this is finished the database search will begin. If PEAKS finds protein candidates after searching the database, a Protein Identification Results window will appear:

S

inChorus Protein Identification

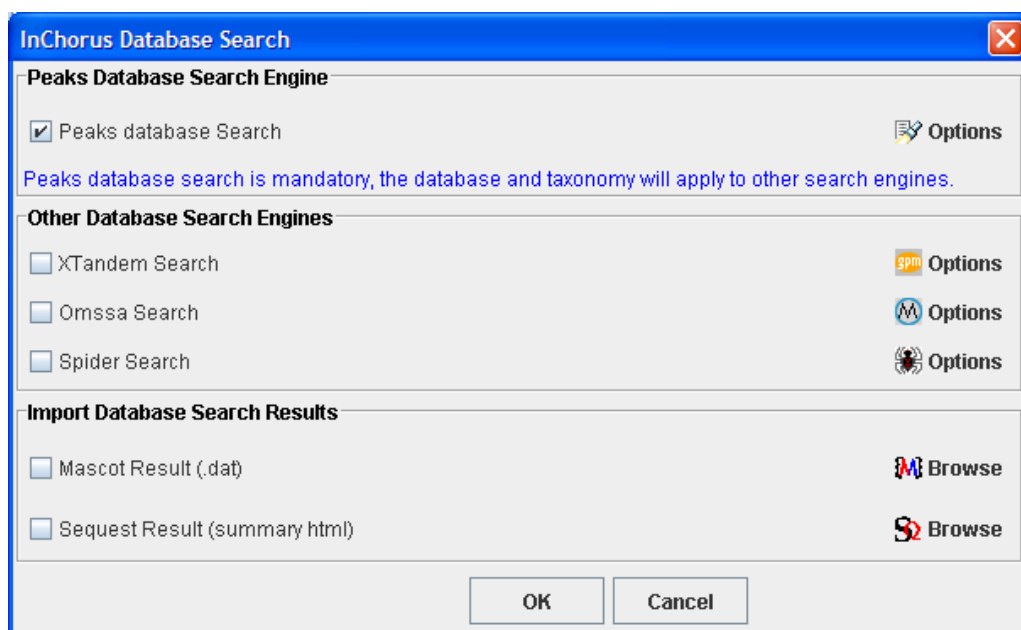
inChorus Protein Identification will call upon several search engines for protein identification. Once we load our data into PEAKS, we can invoke (start searches running on) several search engines at once. When all the results are returned, PEAKS 4.2 will compare the answers and summarize everything in one simple report.

1. In the *Peptide Data* Frame, we select the data file(s) that we wish PEAKS to use to identify our protein(s). This can be done by clicking on a data file's name in the peptide data frame.
2. Click the "Protein Identification" toolbar icon .

Or

Select "inChorus Protein ID" from the tools menu

The inChorus database search launch window will appear:



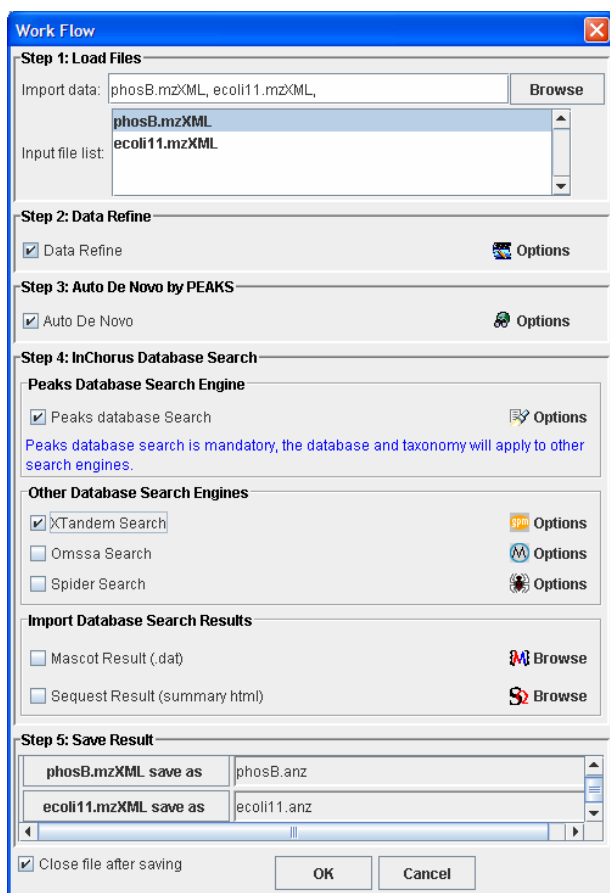
3. First select each of the protein identification tools we would like to use by putting a checkmark in their respective checkboxes. Search parameters for each program can be set by clicking the corresponding Options icon. PEAKS database search engine is mandatory.
4. Then set search parameters for each search engine. Option screens for each of the programs available to inChorus are designed to work in the same way as options screens for the original programs. For help in setting search parameters for each program, please refer to that program's user manual. In the case of PEAKS database search, please refer to the above section.

SC

Creating a high-throughput workflow

In some situations, we may have many data sets that we wish to process all at once, and in the same way. PEAKS 4.2 allows us to do this kind of work, and with minimal effort on our part. By setting up a workflow, we can start a batch process of several data files and not worry about it until it is finished.

It is important to note that all the files we load will be processed in exactly the same way, using exactly the same parameters. If we want to do some differently than others, we must set up another workflow.



Step1: Load Files – Click the browse button to open a file chooser. From the chooser, select several files by shift+click or ctrl+click, and pressing the “OK” button. Load more files by pressing browse again or remove them from the list by right clicking on them.

Step2: Data Refine – Choose how to filter and correct data for maximum utility.

Step3: Auto *de novo* – Choose whether or not to do auto de novo sequencing. Note that PEAKS database search requires some de novo sequencing results.

Step4: inChorus Database Search – Choose which protein identification programs to run the data. PEAKS database search is required.

Step5: Save Results – Saved automatically into an .ANZ file with the same name as the data file. All files will be placed in one folder. Typing in the textboxes or clicking each file’s button changes the name and/or save location. If it’s a large run, we should “close each file after saving”.

SCV

Selecting unmatched de novo results

When working with unknown organisms, abundant but uninteresting proteins (like keratin) can get in the way. We may find it more convenient to eliminate them from the analysis. To do so, we must first identify which peptides belong to those proteins. To do so, run PEAKS 4.2’s protein identification tool. The proteins identified in the sample will be shown in the Protein ID Report.

Click on the Filter tab and make sure that Option 1 (de novo view shows peptides that could not be explained ...) is selected. Then, if desired, add some filtering criteria, then press the Apply Filters button.

Click on the De Novo View tab. This now displays de novo sequences for spectra that could not be explained by the identified proteins (as listed in Protein View). Sort this list, then click and drag to select some de novo sequences...

Ind...	m/z	z	Mass	Peptide	Score(...)
18	933.5426	1	932.53534	LLVTQTMK	99.98
10	674.4233	1	673.416	LPAVFK	99.92
7	597.3386	2	1192.6627	VLVLDTDYK	99.9
5	545.92804	3	1634.7623	TPEVDDEALEKFDK	99.88
14	818.3876	2	1634.7606	TPEVDDEALEKFDK	99.85
4	533.2925	2	1064.5704	VLVLDTDYK	99.41
2	458.73856	2	915.4626	LDALNENK	97.41
20	1065.586	1	1064.5787	ARRRSEYK	94.43
22	1354.1898	2	2706.365	WLKWKDMYASDL SLLDAQSAPLR	9.85
9	673.38513	1	672.37787	LGDLGAK	9.26
9	673.38513	1	672.37787	LGDLGAK	9.26
9	673.38513	1	672.37787	LGDLGAK	79.79
1	452.28497	2	902.5554	KTLLAVPK	7.82

... they appear highlighted in the peptide data tree on the left. We may then chose run auto de novo, or protein id on these spectra, or on everything but these spectra.

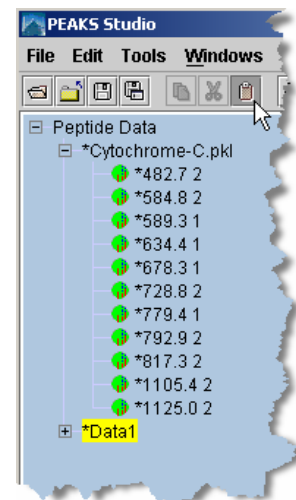
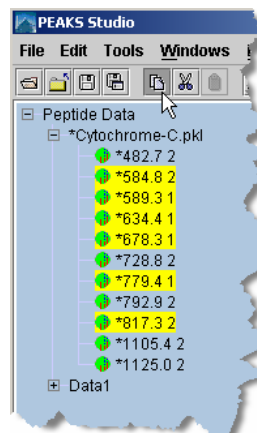
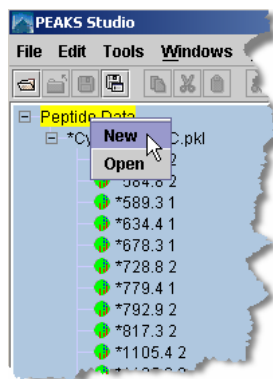
SC

Running protein identification on select spectra

When searching our dataset against a particular database, PEAKS may not have found a hit for certain spectra. If these are good data, we may wish to try searching them against a more general database. Before we do so, we must **create a new data set** with these “good spectra that did not match”. This is essential so that we can organize our data well, and because PEAKS will only run Protein ID on all the spectra in a data node. To create a new data node:

1. Make a new data node by right clicking on the peptide data node. The new node appears as “Data1”.
2. Select the relevant spectra using shift+click and ctrl+click. Then press the cut button.
3. Click the new node and press the paste button. Pressing the + next to Data1 will expand it and reveal the pasted spectra.

After pasting, we must save Data1 to continue

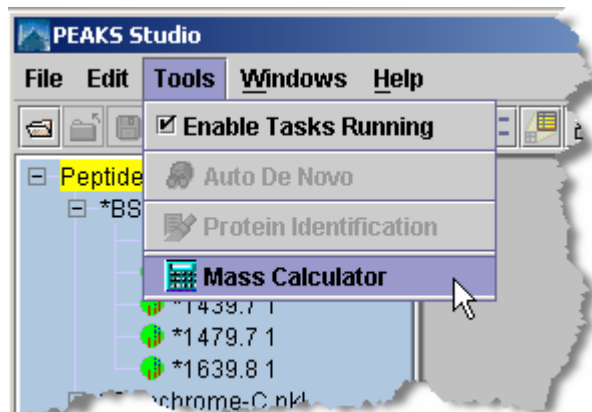


Now we've essentially removed the already matched peptides from our dataset. We can now run protein identification on Data1 or on the remaining spectra in our original dataset. We can save that dataset in a new file or any of the other functions that apply to regular nodes. Make sure the new node is selected before running protein ID (or any other function) on it.

SCV

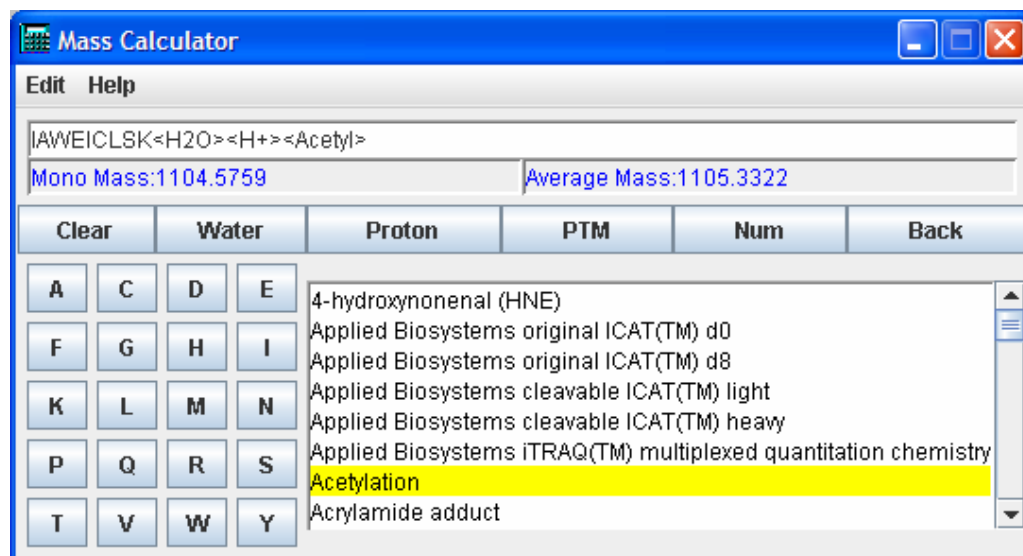
Using the Mass Calculator

The Mass Calculator is a simple tool to help us determine the molecular weight of a peptide. To access the Mass Calculator, open the "Tools" menu and click "Mass Calculator". The Mass Calculator will appear.



We can also load the mass calculator outside of PEAKS. To access the mass calculator without having to load PEAKS, click on the Mass Calculator's icon in the start menu. It will appear in the same program group as PEAKS.

When using the mass calculator, remember to start with water.



We can click any of these buttons multiple times to repeatedly add that mass.

- Amino acids are represented by their single letter symbols. Clicking on an amino acid's button will add it to the 'sequence' above, and add its mass to the mass of the peptide. Note that the peptide's monoisotopic and average masses are both computed.

- Add a proton by clicking the “Proton” button. It will be represented by an <H+> in the ‘peptide’ above.
- To compute the mass of the peptide as if it had been modified, select a PTM from the list, and press the “PTM” button to apply them to the peptide.
- If the PTM we wish to add does not appear in the list, we may wish to enter its mass manually. To add a mass numerically, click the “Num” button and enter a numeric value in the dialogue box that appears. Press the “OK” button on the dialogue and the mass will be added to the sequence.
- To remove a mass that we’ve just added to the peptide, press “Undo”.

Working with Results

Viewing, Post-Analysis, Exporting and Saving of results.

Results are what count. So the PEAKS 4.2 developers have made sure to present them simply, effectively and allow you to get them out of PEAKS easily. This chapter covers how to interpret the results, how to get the most out of them with minimal effort, how to present them to others, and how to save data and results together for future reference.

This chapter assumes that the user is familiar with all preceding chapters.

The walkthrough in Chapter 3 gives a good summary of this topic.

Viewing Protein identification results

To view Protein Identification results for a data file, we must have performed PEAKS protein identification or inChorus protein identification on that data file. The result from each protein identification search is represented by the time stamp and database searched (found just under a data file's "Protein ID Result"). Click on one to display the results report. We can view results by peptide or by protein and check on the search parameters we used to generate these results. We can manipulate the contents of this multi-part report by filtering out what we don't want to keep. Finally we can export the report and sub reports in a number of ways.

Search parameters that we used when generating this report are preserved for future reference and are available by clicking on the search parameters tab.

Peptide View is available by clicking on the Peptide View tab. In tabular format, it displays relevant information about each peptide found. Since two peptides may match to one MS/MS spectrum, they are visually grouped together (using colour) by the MS/MS scan.

When we first load the report, it is sorted by MS/MS index number. This is analogous to the scan number or DTA file name (unless spectra have been merged). In the example above, there was one match returned for MS/MS spectrum #1. For spectrum #2 there were two; LDAINENK and IDALNENK. For spectrum #3, there were also two, and so on. Each peptide is given a score and the protein it matches is displayed for reference under "Accession No." If the report was generated after inChorus protein ID, each peptide that had a search program agree (identify the peptide) is given a checkmark under its column.

The columns themselves are customizable. Right click anywhere in the report and choose Toggle Column from the pop-up menu. The sub-menu that appears shows a checkmark in each of the columns that are currently showing. Click any one of them to show or hide a column. These settings will apply to all our reports.

The peptide view list is sort-able. Click on a column header to sort the list using that column's values. For example, sort by score. Clicking again on the same column header will toggle ascending/descending sort. Use ctrl+click to set a second sort – one can sort by Accession, then by score in this way. *When sorting, the grouping by MS/MS scan number will be retained except when sorting by Accession No.*

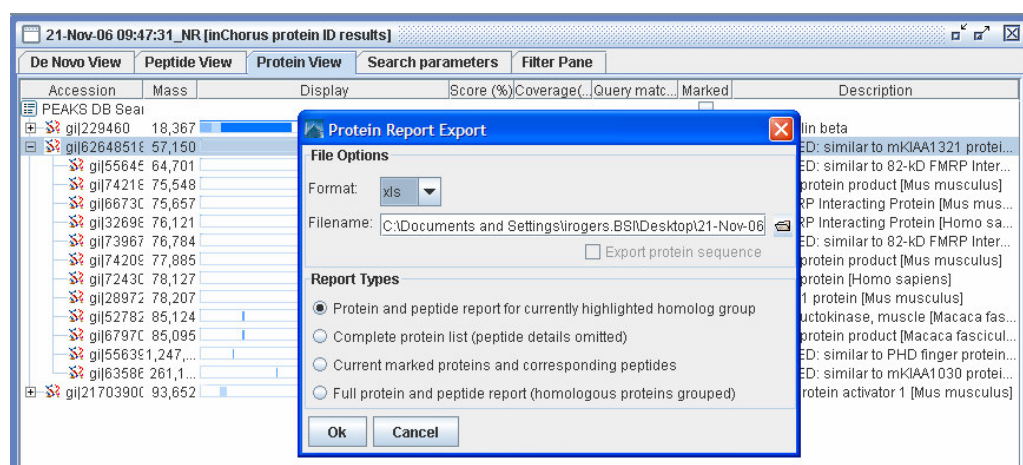
Scores associated with each peptide represent the quality of the match and the number of programs in agreement on the peptide.

21-Nov-06 11:22:04_NR [inChorus protein ID results]									
De Novo View		Peptide View		Protein View		Search parameters		Filter Pane	
Index	m/z	z	Peptide	Mr(Calc)	Score(%)	Start	End	Accession ...	
1	452.28497	2	TKIPAVFK	902.5589	69	76	83	gi 229460	▲
2	458.73856	2	LDAINENK	915.4661	43	84	91	gi 229460	
2	458.73856	2	LDALNENK	915.4661	43	84	91	gi 4388846	
3	467.2729	2	LIVTQTMK	932.5366	42	1	8	gi 229460	
3	467.2729	2	IIVTQTMK	932.5366	42	1	8	gi 72079	
4	533.2925	2	VLVLDTDYK	1,064.5752	87	92	100	gi 229460	
5	545.92804	3	TPEVDDEALEKFDK	1,634.7673	99	125	138	gi 229460	
7	597.3386	2	VLVLDTDYKK	1,192.6702	99	92	101	gi 229460	
8	623.2947	2	TPEVDDEALEK	1,244.5771	99	125	135	gi 229460	
9	673.38513	1	IGDLQK	672.3807	50	504	509	gi 1094913...	
9	673.38513	1	GLDIQK	672.3807	19	9	14	gi 229460	
9	673.38513	1	LGDLQK	672.3807	50	136	141	gi 62510779	
10	674.4233	1	IPAVFK	673.4163	85	78	83	gi 229460	
10	674.4233	1	LPAVFK	673.4163	85	24	29	gi 1145653...	▼

Peptide Details	
Links to retrieve spectrum information:	
458.73856	2
De novo L D A L N E N K	
Database L D A I N E N K	
>gi 229460 prf 732164A lactoglobulin beta	
Matched peptides shown in Red	
1	LIVTQTMKGL DIQKVAGTWY SLAMAASDIS LLDAQSAPLR VYVEELKPTP
51	EGDLEILLQK WENDECAQKK IIAEKTIPA VFK LDAINEN KVLVLDTDYK
101	KYLLFCMENS AEPEQSLVCQ CLVRTPEVDD EALEKFDKAL KALPMHIRLS
151	FNPTLQEEQC HI
NCBI BLAST search of de novo sequence LDALNENK	
NCBI BLAST search of database search sequence LDAINENK	

Clicking on this report will highlight the spectrum in the Peptide Data tree on the left. Select multiple spectra by clicking and dragging, using shift+click or ctrl+click. In this way, the highest scoring peptides may be selected and isolated for further analysis.

Protein View is accessed by clicking on the Protein View tab. It collects all the peptide identifications together, summarizes which proteins were present in the sample, and groups homologous proteins together. The same information is displayed in the Peptide View as in this Protein View; however, the results are organized to best enable us to evaluate at the protein level.



The top section of this view (shown above) behaves like an index, listing each protein found in the sample. Very similar proteins, containing the same set or a subset of the matched peptides, are grouped together. To see the full list of proteins within each grouping click the '+' sign. In the example above, the Bovine Serum Albumin node has been expanded to reveal several similar proteins. Clicking in the same place (now a '—' sign) will collapse the list.

This view is helpful when building a summary that can be sent to a customer/collaborator. Simply right click to export to a MS Excel file. We can export interesting parts of the report or a whole summary. Mark proteins of interest by clicking their checkboxes and export protein and peptide information for those.

- Or highlight a homologue group and export proteins and peptides in that group.
- Or just export the whole report.

The columns themselves are customizable. Right click anywhere in the report and choose 'Toggle Column' from the pop-up menu. The sub-menu that appears shows a checkmark in each of the columns that are currently showing. Click any one of them to show or hide a column. These settings will apply to all our reports.

Whenever we click on a protein, the Matched Peptides panel (bottom) changes to display the spectra (and peptides) that were found to be supporting evidence for that protein. The columns in this list are the same as the ones we choose for 'Peptide View'. It may be necessary to scroll this panel down to see the complete list. Clicking a peptide in this list brings up the Main Processing Window for the corresponding spectrum, and displays the ions that were found in support of this peptide (shown below).

Protein Pane		MSA Pane						
NCBI BLAST search of gi 229460								
Links to retrieve entries containing this sequence from NCBI Entrez:								
Accession/ID	Description							
gi 229460	lactoglobulin beta							
Peptides List								
Index	m/z	z	Mass Peptide	Mr(Calc)	Start	End	UniqueID	Peaks
1	452.28497	2	902.5554 TKIPAVFK	902.5589	76	83	0	<input checked="" type="checkbox"/>
2	458.73856	2	915.4626 LDAINENK	915.4661	84	91	1	<input checked="" type="checkbox"/>
3	467.2729	2	932.5312 LIVTQTMK	932.5366	1	8	3	<input checked="" type="checkbox"/>
4	533.2925	2	1,064.5704 VLVLDTDYK	1,064.5752	92	100	5	<input checked="" type="checkbox"/>
5	545.92804	3	1,634.7623 TPEVDDEALEKFDK	1,634.7673	125	138	6	<input checked="" type="checkbox"/>
7	597.3386	2	1,192.6627 VLVLDTDYK	1,192.6702	92	101	7	<input checked="" type="checkbox"/>
8	623.2947	2	1,244.5748 TPEVDDEALEK	1,244.5771	125	135	8	<input checked="" type="checkbox"/>
9	673.38513	1	672.3779 GLDIQK	672.3807	9	14	10	<input checked="" type="checkbox"/>
10	674.4233	1	673.4160 IPAVFK	673.4163	78	83	13	<input checked="" type="checkbox"/>
12	697.7167	3	2,090.1282 LDAINENKVLVLDTDYK	2,090.1260	84	101	15	<input checked="" type="checkbox"/>
13	771.75635	3	2,312.2473 VYVEELKPTPEGDLLEILLQK	2,312.2517	41	60	17	<input checked="" type="checkbox"/>
14	818.3876	2	1,634.7606 TPEVDDEALEKFDK	1,634.7673	125	138	18	<input checked="" type="checkbox"/>
16	903.1304	3	2,706.3694 VAGTWYSLAMAASDISLLDAQSAPLR	2,706.3687	15	40	19	<input checked="" type="checkbox"/>
17	903.5675	1	902.5602 TKIPAVFK	902.5589	76	83	20	<input checked="" type="checkbox"/>
18	933.5426	1	932.5353 LIVTQTMK	932.5366	1	8	21	<input checked="" type="checkbox"/>
20	1065.586	1	1,064.5787 VLVLDTDYK	1,064.5752	92	100	24	<input checked="" type="checkbox"/>
21	1157.1289	2	2,312.2432 VYVEELKPTPEGDLLEILLQK	2,312.2517	41	60	25	<input checked="" type="checkbox"/>
22	1354.1898	2	2,706.3650 VAGTWYSLAMAASDISLLDAQSAPLR	2,706.3687	15	40	26	<input checked="" type="checkbox"/>
Matched peptides shown in Red								
1	LIVTQTMKGL DIQK VAGTWY SLAMAASDIS LLDAQSAPLR VYVEELKPTP							
51	EGDLEILLQK WENDECAQKK IIAEK TKIPA VFKLDAINEN KVLVLDTDYK							
101	KYLFLCMENS AEPEQSLVCQ CLVR TPEVDD EALEKFDK AL KALPMHIRLS							
151	FNPTLQEEQC HI							
Color Code: >=80% >=60% >=40% >=20% >=5%								

Scrolling down the Matched Peptides panel reveals the complete sequence of the highlighted protein with the matched peptides highlighted in red. The strength of the red colour is a visual indication of the confidence in that portion of the protein. Where an EST database was used, the translated sequence is shown (with all six reading frames concatenated).

De Novo View usually shows de novo sequences for spectra that could not be explained by matching to the identified proteins (as listed in Protein View), but the exact behaviour is customizable. See below for a discussion of filters and how they relate to the De Novo View. The list of peptides in the De Novo View behaves quite like the list of peptides in Peptide View. Differences are as follows:

- Note that since PEAKS 4.2 uses de novo sequences to help out in the database search, it is sometimes useful to see which de novo sequences were used to help in the search. Each sequence that is used in this way has a checkmark in its PID column.
- The parameters used to generate a de novo sequence may be slightly different from the database search parameters. As such, PEAKS 4.2 records how each de novo sequence was generated – this information is in the [Source program] column.
- Positional confidence information is available in de novo sequencing results. Colour codes show the strength of confidence in an individual amino acid. The colour codes are the same as those used in the main processing window

(red is the best, then purple, then blue, then black). Positional confidence can also be seen by holding the mouse over a sequence.

Result filters are accessed by clicking on the 'Filter Pane' tab. These allow us to manipulate the contents of the whole multi-part report. For instance, one may choose to discard any protein hit that was not supported by at least two high scoring peptides. The filters are very flexible, allowing you to create filters on any of the fields (score, mass, search engine, etc) and any number of fields. The filters are also cascading such that removing a protein from the results removes all peptides associated with only that protein and vice versa. But all this can be a bit complicated, so please read on to the section "Post analysis of results – preparing for publication".

SCV

Comparing proteins - multiple sequence alignment

After having identified some proteins, it may be useful to see how their sequences compare. This is particularly useful when trying to identify specific isoforms of proteins or antibodies. To display a multiple sequence alignment between two proteins, click on the time and date stamp underneath a filename in the peptide data tree (left). This brings up a multi-part protein ID report. Click on the **Protein View** tab to see a list of identified proteins. **Mark a few proteins by clicking on their checkboxes** in the 'Marked' column. Then, in the bottom panel of this view, click the **MSA** tab. It will be blank to start with, but click one of the two buttons on this panel to:



generate the MSA and display in the default web browser



generate the MSA and display it on the MSA panel.

The screenshot shows the 'Protein View' tab of a software interface. It contains a table with columns: Accession, Mass, Display, Score, Coverage(%), Query matc..., Marked, and Description. The table lists several protein entries, including 'lactoglobulin beta' and 'Chain B, Bovine Beta-Lactoglobulin, Latti...'. Below the table is the 'MSA Pane' which shows a multiple sequence alignment of two protein sequences (gi|229460 and gi|223780) with a color code at the bottom: Color Code: >=80% >=60% >=40% >=20% >=5%.

Accession	Mass	Display	Score	Coverage(%)	Query matc...	Marked	Description
gi 229460	18,367		99	50.00%	12	<input checked="" type="checkbox"/>	lactoglobulin beta
gi 2194	18,309		99	50.00%	12	<input type="checkbox"/>	Chain B, Bovine Beta-Lactoglobulin, Latti...
gi 4925	18,351		99	50.00%	12	<input type="checkbox"/>	Chain X, The Cys121 ser Mutant Of Beta-L...
gi 7207	18,267		99	50.00%	12	<input type="checkbox"/>	beta-lactoglobulin - water buffalo
gi 7576	18,367		99	50.00%	12	<input type="checkbox"/>	Chain D, The Structure Of Bovine B-Lacto...
gi 2173	18,281		99	50.00%	12	<input type="checkbox"/>	Chain A, Bovine Beta-Lactoglobulin Com...
gi 1627	17,167		99	53.64%	12	<input type="checkbox"/>	beta-lactoglobulin
gi 7245	18,375		99	50.00%	12	<input type="checkbox"/>	Chain A, Structural Changes Accompanyi...
gi 1259	19,883		99	45.51%	12	<input type="checkbox"/>	Beta-lactoglobulin precursor (Beta-LG) (A...
gi 2780	19,921		99	45.51%	12	<input type="checkbox"/>	lactoglobulin, beta [Bos taurus]
gi 3808	20,023		99	45.00%	12	<input type="checkbox"/>	beta-lactoglobulin [Bubalus bubalis]
gi 2237	18,177		99	30.25%	7	<input checked="" type="checkbox"/>	lactoglobulin beta
gi 4949	19,976		99	27.22%	7	<input type="checkbox"/>	beta-lactoglobulin [Capra hircus]

Protein Pane MSA Pane

gi|229460 LIIVTQTMKGLDIQKVAGTWYSLMAASDISLLDAQSAPLRVYVEELKPTPEGDLILLQK

gi|223780 LIIVTQTMKGLDIQKVAGTWYSLMAASDISLLDAQSAPLRVYVEELKPTPEGDLILLQK

gi|229460 WENdECAQKKIIAEKTKIPAVFKLDALNENKVLVLDTDYKKYLLFCMENSAPESQSLVCO

gi|223780 WENgECAQKKIIAEKTKIPAVFKIDALNENKVLVLDTDYKKYLLFCMENSAPESQSLaCQ

gi|229460 CLVRTPEVDDEALEKFDKALKALPMHIRLSFNPTlgEeQCHI

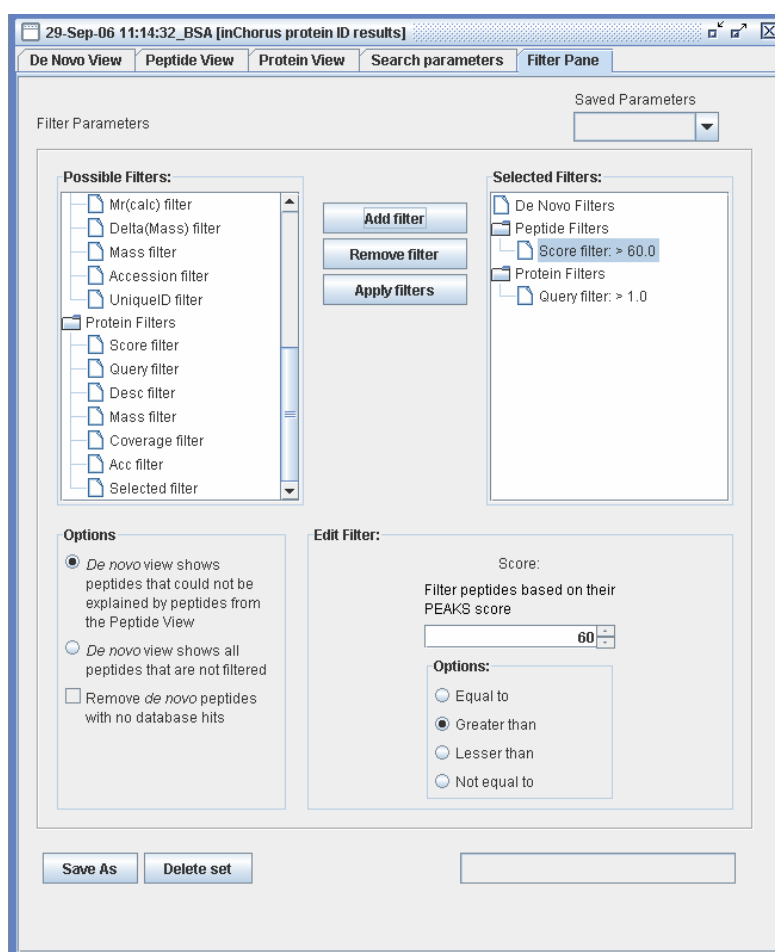
gi|223780 CLVRTPEVDNEALEKFDKALKALPMHIRLAFNPTlEgQCHV

Color Code: >=80% >=60% >=40% >=20% >=5%

Filtering results

PEAKS 4.2 provides us with an exhaustive list of all proteins and peptides that can be found in the sample. But since everyone has their own criteria of what information is required in the report and what is an acceptable result, a good hit, we must have a way of filtering out the less critical information to leave us with the essentials.

In order to manipulate results, we must, of course, have done some protein identification using PEAKS Protein ID or inChorus searching. Click on the time and date stamp associated with this result under its file name in the peptide data panel on the left. Once the report loads, click on the “Filter Pane” tab to bring up the filtering options.



Choose a filter from the ‘Possible Filters’ list on the left by clicking on it. Options for this filter will appear below, in the [Edit filter:] frame. Once we’ve set these options, we press ‘Add filter’ to bring it to the list of ‘Selected Filters’. If we’d like to add another filter, we can repeat the process, continuing to add as many filters as necessary. In this way it is also possible to have two filters on the same property; we can set a range of protein mass, for instance, by applying one filter on the upper bound of the mass and

adding another filter to be the lower bound of the mass. We can also have more complex filters that involve multiple properties. The example above shows filters that allow only proteins with more than one high scoring (greater than 60% score) peptide, a standard requirement for publication.

Filters are grouped into three basic types to reflect what they act on: De Novo filters act to remove proposed de novo sequences, Peptide Filters act to remove peptides found in the database from the report and Protein filters act to remove proteins from the report.

The filters cascade through each view in the multi-part report. For instance, filtering out a protein (by mass perhaps) will remove it from the Protein View and remove all peptides associated with that protein from the Peptide View list. The manner in which the De Novo View is linked can be specified by the user (illustrated on the next page).

Filter sets can be saved and re-used between sessions, but remember to hit Apply Filters to use them.

Once all the filters we need are set up and showing in the Selected Filters list, we must press the Apply Filters button to use them on our results. For larger reports this can take a few seconds, during which time the tabs of the multi-part report will be greyed out. After completion, we can click on any of the tabs to see how the results have been affected.

How filters act on de novo sequences

PEAKS 4.2 automatically relates de novo sequencing results to protein ID results. This makes it easy to see which de novo sequences correspond to known contaminants and which are truly new peptides. The relationship is always drawn back to the data, not necessarily the sequence, which helps highlight important sequence variations. PEAKS 4.2 users can set what is displayed on the list of de novo sequences, according to the following diagrams:

Option Selected (on Filter Pane tab)	What De Novo View Displays (represented in grey)	How Filters are linked
<div><p>Options</p><ul style="list-style-type: none"><input checked="" type="radio"/> De novo view shows peptides that could not be explained by peptides from the Peptide View<input type="radio"/> De novo view shows all peptides that are not filtered<input type="checkbox"/> Remove de novo peptides with no database hits</div>		Filtering out (removing) a de novo result does not affect what is displayed in the Protein View tab or Peptide View tab.
<div><p>Options</p><ul style="list-style-type: none"><input type="radio"/> De novo view shows peptides that could not be explained by peptides from the Peptide View<input checked="" type="radio"/> De novo view shows all peptides that are not filtered<input type="checkbox"/> Remove de novo peptides with no database hits</div>		Filtering out (removing) a de novo result also removes that spectrum from consideration in the Peptide View tab and Protein View tab.
<div><p>Options</p><ul style="list-style-type: none"><input type="radio"/> De novo view shows peptides that could not be explained by peptides from the Peptide View<input checked="" type="radio"/> De novo view shows all peptides that are not filtered<input checked="" type="checkbox"/> Remove de novo peptides with no database hits</div>		Again, filtering out (removing) a de novo result also removes that spectrum from consideration in the Peptide View tab and Protein View tab.

SCV

Filter examples

Any column of information in any part of the Protein ID report can be filtered. Filters cascade throughout the report – i.e removing a protein removes all its associated unique peptides, and removing all peptides from a protein removes that protein too. Each filter can be applied several times over. So it can get a little complex. To illustrate, here are a few examples:

Publication

Show proteins that have two high-scoring hits:

- Add the Protein Filter called Query and in the [Edit Filter] section choose ‘greater than’ and type ‘1’ in the box (without the quotes).
- Then add the Peptide Filter called ‘Score’ and in the [Edit Filter] section choose ‘greater than’ and type ‘50’ in the box (without the quotes).
- Press the ‘Apply Filters’ button.

Digging for a protein by name

To illustrate this process, let us find a protein that contains the word ‘lactoglobulin’ in the database entry’s description.

- Add the Protein Filters called ‘Desc’
- In the [Edit filter] section, we’re required to type in a regular expression (regex). This allows us to use wildcards. A nice, common wild-card is `.*` that means ‘anything of any length’. For example: `.*lactoglobulin.*` Will find anything that contains the word lactoglobulin, with anything before and anything after.
- Press the ‘Apply Filters’ button.

Setting a protein mass range

If we know the approximate mass of the proteins we’re interested in, we should eliminate all proteins that are not close in mass.

- Add two filters: “Protein Filters: Mass >12000” *and* “Protein Filters: Mass < 32000”
- Press the ‘Apply Filters’ button.

SCV

Saving/Loading Filter sets

We’re sure to use some common filters repeatedly. To save a Filter Set, press the ‘Save As’ button on the filter pane. This saves all filters that we’ve added to the ‘Selected Filters’ list. To recall a saved Filter Set, select it from the drop down list in the top right

corner of the filter pane. This will simply populate the 'Selected Filters' list with our saved filters. Don't forget to press the 'Apply Filters' button to apply them.

SCV

The bottom panel in protein view shows the columns selected for peptide view

Toggling columns

Still, the information in the report might be too intensive. Some users might not care to see the start and end position of the peptide in the protein, while some users may wish to see even more information. PEAKS 4.2 allows us to customize which columns are shown. Right-click anywhere in De Novo View or the top section of Peptide View or Protein View. In the pop-up menu that appears, hold the mouse over 'Toggle Column'. A sub-menu will appear showing a checkmark in each of the columns that are currently showing. Click any one of them to show/hide a column. PEAKS will remember which columns we select, and present us with these columns each time we load a report.

SCV

SCV

Exporting Results

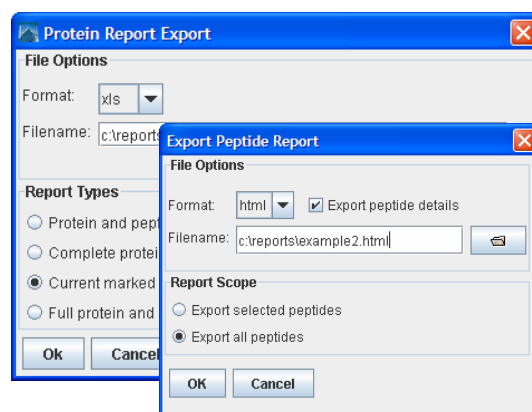
WYSIWYG reports

PEAKS 4.2 allows us to create interactive reports to share with collaborators, colleagues and clients. The reports are available in HTML or Microsoft Excell (.xls) formats and follow a 'What you see is what you get' (WYSIWYG) philosophy. All the information you see on screen in PEAKS 4.2 will appear in the exported report. For this reason, it is important that we complete results filtering and toggling columns before exporting a report.

We can export only one view, one part of the report, at a time.

In order to export a report, first choose the view to export by clicking on the De Novo View, the Peptide View tab or the Protein View tab. Next, right-click anywhere in the top section of the selected view and choose 'Export Report' from the pop-up window that appears.

The report export dialogue will appear. One must choose either HTML or XLS format and specify a file name. PEAKS 4.2 will load the report after it has been created, but it makes sense to always place the report in a place we can find it later on. By default the filename of the report will include the time and date the report was created. To change the location/filename one can either type it in to the Filename box or click the folder icon to browse to a new location.



The bottom part of the Report Export dialogue asks us what we would like to include in the exported report. When exporting from Peptide View or De Novo View we have

the choice of exporting the whole list or the selected ones. When exporting from Protein View we have some more options, allowing us to include peptide details, the complete protein sequence, a listing of proteins containing the same set or a subset of the same peptides, or to include only marked proteins. Press the OK button to complete the export.

SCV

Exporting peak lists

To export data to a PKL file, we select the data file (not an individual spectrum) to export. Then, from the “File” menu, select “Export”, then “Export PKL File”. The spectrum data will be saved in PKL format, but all sequencing and protein data will not be preserved.

SCV

Exporting Sequences by spectrum

For most users, the WYSIWYG reports exporting will be enough, but PEAKS 4.2 also provides other ways to export. To export peptide sequencing results to a FASTA format file, select the data file (not an individual spectrum) to export. Then, from the “File” menu, select “Export”, then “Export Peptide Sequence”. The sequencing data will be saved in FASTA format, but will not retain any spectrum data.

To export peptide sequencing results to an HTML table, select the data file (not an individual spectrum) to export. Then, from the “File” menu, select “Export”, then “Export HTML File”. PEAKS will then ask us which results we would like to export. We can choose from any *de novo* sequencing or Protein ID run we have done. Each will be listed with the parameter set we used. The difference here is only that the results are organized by spectrum rather than by peptide as in the WYSIWYG exporting.

SCV

Exporting Exclusion Lists

If we are repeating an experiment, it can be useful to exclude the peptides we have already scanned and analyzed. In this way, we can focus on some potentially interesting but lower intensity peaks. To tell the instrument to do this we must create an exclusion list. PEAKS 4.2 has the ability to create an exclusion list that will be compatible at least with Waters/Micromass instruments. To create the exclusion list, simply select a few spectra from the peptide data tree on the left, then right click and choose “Export to Exclusion list” from the pop-up menu that appears. The selection of spectra can also be done using by clicking in the Peptide View or De Novo View of a Protein ID report.


SCV

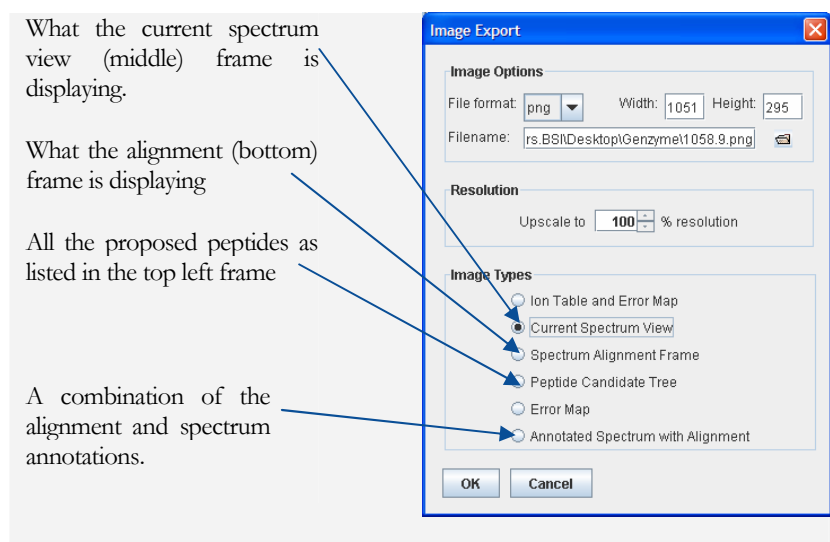
Exporting high-resolution spectral images

It is often useful to provide visual aids in reports, posters and papers. As such, PEAKS 4.2 provides the facility to export high quality images of a spectrum or part of a spectrum zoomed in. In any case, PEAKS allows us to export exactly what’s shown on the screen. So to export an image:

1. Bring up the main processing window for an MS/MS spectrum by double-clicking on its precursor ion *m/z*, or by double clicking a

peptide in any of the reports. The main processing window shows us the spectrum of interest, along with any peptides matched to it.

2. Set up the colour, typeface and size of labels and peaks on the spectrum. To do so, choose Edit menu → Configuration → Environment Preferences. Within the Environment preferences dialogue, choose the 'Colour' or 'Display' tab to get at the relevant settings.
3. *(optional) If we want to export a picture of the Ion Table, we should configure what columns are shown in the ion table before the export. To do so, select one of the Ion Table Editor tabs from the Environment Preferences dialogue.*
4. *(optional) If we want to export a zoomed in region of the spectrum, we must do so now by clicking and dragging horizontally over the area of interest.*
5. Click the  export results button on the main processing window toolbar, or right-click on any frame within the main processing window and choose "Export Image" from the pop-up menu.
6. The Image Export Options Dialogue appears. On this window you can export:



- a. Choose what you want to export.
- b. Select a file type. Four popular formats are supported., but either GIF or PNG formats are recommended.
- c. Choose a file name. We can browse by clicking the folder icon or just type a file name in the box.


- d. *(optional)* Specify width and height dimensions in pixels. In some cases the size is predetermined by the size of the frame on our screen. Otherwise we can increase these numbers to make the picture bigger.
- e. To export high resolution images, increase the resolution by a few hundred percent.
- f. Press ok and you're done.

SCV

Saving Results

Saving results will preserve our work for later use. Saving files in PEAKS's .ANZ format will preserve spectrum data, manual *de novo* sequence information, automatic *de novo* sequence information, protein identification results, and information about any PTM that were found in sequence.

To save the results of our analysis, we first select the data file we wish to save in the Peptide Data Frame.

To save, click the  save icon in the main window toolbar, select "Save" from the "File" menu or right click on the data file and select "save" from the popup menu. This will save the processed spectra in .ANZ format and of the same name as the data file we opened.

To change the name of the ANZ file, choose "Save as" from the "File" menu, or right click on the data file and select "Save as" from the pop-up menu. We may then change the file name.

To save all currently opened data files, select "Save all" from the "File" menu.

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Bioinformatics Solutions Inc. develops advanced algorithms based on innovative ideas and research, providing solutions to fundamental bioinformatics problems. This small, adaptable group is committed to serving the needs of pharmaceutical, biotechnological and academic scientists and to the progression of drug discovery research. The company, founded in 2000 in Waterloo, Canada, comprises a select group of talented, award-winning developers, scientists and sales people.

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Efficient and concentrated research, development, customer focus and market analysis have produced: PEAKS software for protein and peptide identification from tandem mass spectrometry data, RAPTOR and PROSPECT Pro software for threading based 3D protein structure prediction and PatternHunter software for all types of homology search sequence comparison.

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Reference: PEAKS Paper

Please use the following references when publishing a study that involved the use of PEAKS.

Bin Ma, Kaizhong Zhang, Christopher Hendrie, Chengzhi Liang, Ming Li, Amanda Doherty-Kirby, and Gilles Lajoie. [PEAKS: Powerful Software for Peptide De Novo Sequencing by Tandem Mass Spectrometry](#). *Rapid Communication in Mass Spectrometry* 17(20): 2337-2342. 2003