Arcadiate Help

For interactive help when using Arcadiate activate the Assistant Window via the command **Window** > **Assistant Window**.

Introduction to Arcadiate
Import various types of data.

Arcadiate Windows
Description of Arcadiate windows.

Data Collections
Work with a specific subset of your data.

Views
Work within various views to inspect and evaluate your data.

Data Processing
Search here when you look for some specific tasks to be performed.

Preference Settings
Use the preference settings to influence appearance and some specific behavior of Arcadiate.

Normalisation of Quantity Values
An explanation what kind of normalisation procedures are available within Arcadiate.

Introduction to Arcadiate

Commands:
Edit > Cut

Arcadiate is a database oriented program for working with mass spectrometric data. It supports mass spectrometric data generated by Data Dependent and All Ion Fragmentation acquisition regimes. It can import individual mass spectra, mass spectrometric chromatograms, Mascot and MaxQuant search results, ion groups, groups of proteins and molecular markers. In addition the library can store objects created within the program in its Object Store.

Its newest data type are All Ion Fragmentation data using several simultaneous fragmentation windows which alternate from scan to scan – randomly multiplexed selection windows for fragmentation. This is a particularly interesting data set because it represents essentially a multilevelled chromatographic space, one for intact molecules and many other for fragment ions with a specific selection window.

Data can be deleted from the library by selecting it in its table and choosing the command **Edit > Cut.**

Import of Mass Spectrometric Data
Import of spectra and chromatograms.

Import of Search Results
Import Mascot or MaxQuant Search Results to correlate it with your fragment spectra.

Import of Ion Groups
Import Ion Groups to quantify specific chromatographic ions.

Import of Molecular Markers
Import Molecular Markers to detect them in All Ion Fragmentation (MS^E) chromatograms.

Import of Proteins
Import Proteins to quantify them in chromatograms.

Arcadiat Viewer Window

Commands:
File > Arcadiate Viewer
File > New Collection Window
File > Import
File > Export
File > Show in Finder
File > Recover from Validation Error
Window > Drawer
Window > Open Assistant
Window > Residue Library
Window > Isotopic Distribution
Window > Tasks

The command **File > Arcadiate Viewer** generates the main Arcadiate window. The window gives access to all the objects stored in its database and allows to create an arbitrary number of Data Collections – the environments to display and use mass spectrometric data sets and associated information.

The Library section gives access to imported data sets and to the Object Store.
Data is imported into Arcadiate using the command **File > Import**. Imported files populate one of the Library groups, Spectra, Chromatograms, Database Searches, Ion Groups or the Object Store.
The Object Store contains objects that are created in Data Collections and copied from there via drag & drop into the Object Store – for instance protein groups. From the different sections of the Library data objects can be dragged to Data Collections to be used there. Imported data objects are linked, not copied. Objects from the Object Store are copied.

When deleting imported objects from the Library they are removed from all data collections and deleted.

The command **File > Show in Finder** displays the data library in the Finder.

The command **File > Recover from Validation Error** deletes erroneous data from the Arcadiate database.
Pressing the button + - adds or removes a Data Collection.

The commands under **File > Export** allow to export a variety of content. The command refers always to the currently active view.

- **> Spectrum Description**... exports the spectrum description file. This file describes the characteristics of MS and tandem MS spectra and is required to import centroided data from the same instrument or type of

instrument.

- > **Protein Quantifications**... export protein quantification results either as tab separated text, xml or MaxQuant proteinGroups file format.
- > **Spectrum**... exports a spectrum as tap separated text of a Spectrum View.
- > **Precursors**... exports the currently displayed precursor list of a Precursor View.
- > **Ions**... exports an ion group of an Ion Group View and its quantities as tap separated text.
- > **Peptide List**... exports the list of all identified peptides sorted by score of a Database Search Result View as tap separated text.
- > **Proteins**... exports the list of all proteins from a Protein Group View as a tab separated text file.
- > **Molecular Markers**... exports the list of all molecular markers from a Molecular Marker Group View as a tab separated text file.
- > **Chromatogram as mzML**... exports a chromatogram in mzML format. It is possible to export a chromatogram with non-identified fragment spectra only.
- > **Database Search Result as xml**... exports a database search result in xml format.
- > **Fragment Spectra for Mascot Search**... exports all centroided fragment spectra as a text file in Mascot Generic Format (mgf) for submission to a data base search.

The command **Window** > **New Collection Window** creates a new window similar to the Arcadiate Viewer which represents the currently selected data collection.

The command **Window** > **Drawer** displays the Drawer – a window with the hidden views of the currently selected Data Collection.
The command **Window** > **Open Assistant** displays the Assistant Panel – a panel with two sections: 1. information about possible actions in the currently active view and 2. the properties of the database object displayed in the currently active view.
The command **Window** > **Residue Library** shows the amino acid residue library that is currently in use.
The command **Window** > **Isotopic Distribution** shows a graphic display of the isotopic distribution of selected ions in a table which are linked to chromatograms. This window gives a rapid overview whether ions display the expected isotopic distribution or whether its peaks are overlaid with other ions. Peptide ions can be excluded on a per ion base from being considered for the quantity calculation of their protein. By clicking onto an isotope distribution graph the corresponding peaks are displayed in the main window.
The command **Window** > **Tasks** shows a list of very specific tasks.

Data Collections

Data Collections are environments to work with a subset of all the data in the library. They are created and deleted by using the +/- button  at the bottom of the Arcadiate window. You can add data objects by dragging them from the library onto the data collection icon.

A data collection has two display modes, a list and a view mode. You choose one using the view toggle button  at the bottom of the Arcadiate window. The list mode displays all library data objects that had been added to the collection in the upper table. The lower table shows all data objects created within the data collection that are not part of the library for instance amino acid sequences or copies from the Object Store. These objects can be dragged onto the Object Store icon  of the library to store them there permanently.

The view mode shows the current working environment of the collection. You add a view to the view mode by pressing the Add View button of the data object in list mode.

Deleting a view from the collection does not delete its data object from the collection. Deleting a data object from a collection does not delete it from the library.

A data collection does not restrict access exclusively to its data objects. The entire data library remains accessible. In some cases two imported data objects are inherently linked – like a Database Search Result and its chromatogram. In this case the Database Search Result will allow access to the chromatogram even if only the Database Search is part of the collection.

A data collection can be displayed in its own window by choosing the command **File > New Collection Window** or by control-clicking onto a data collection name.

Generating views
Generate views to display the data content of the data collection.

Removing views
Remove views to simplify the data display. You can hide or delete views.

Moving views
Move a view to organize a data collection display.

Linking views
Link views to establish a relationship between the views and their data objects.

Copying Views
Copy views to the clipboard for using them in drawing applications.

Using the Drawer

Use the Drawer panel to access hidden views.

Using the Assistant Panel

Use the Assistant panel to get information about activities in the currently selected view and see the properties of the displayed database object.

Views

Views offer many ways to manipulate the displayed data. It is recommended to activate the Assistant panel via the command **Window** > **Open Assistant** to take full advantage of them.

General Commands
Explains activities which are not dependent on any specific view.

Spectrum View
Use a Spectrum view to explore mass spectrometric data in detail.

Sequence View
Use a Sequence view to work with amino acid sequences.

Protein View

- Protein Group View
- Peptide View

The Protein view displays amino acid sequences grouped by proteins. It can be used to edit sequences and quantify peptides and proteins.

Precursor View
Use the Precursor view to access all fragment spectra and filter them.

Database View

- Database Protein View
- Database Peptide View

The Database Search Result view displays all identified proteins and their peptides. It can be used to quantify peptides and proteins.

Database Group View

- Database Group Protein View
- Database Group Peptide View

The Database Group View is used to combine several databases for quantifying protein based on peptides identified in several chromatographic runs.

Ion Group View
Use an Ion Group view to determine the ion volume of specific chromatographic ions.

Normalization Ion Group View
Use an Normalization Ion Group view to normalize the ion intensities of

chromatogram(s) via a set of norm-ions.

Info Text View

Use an Info Text view to store and access information of a data collection or a specific chromatogram.

Chromatogram Group View

Use a Chromatogram Group view to follow ion volumes of specific ions over several similar chromatograms.

Chromatogram Alignment View

Use a Chromatogram Alignment view to view and edit the alignment of two chromatograms.

Chromatogram Map View

Use a Chromatogram Map view get an overview of an entire chromatogram.

Molecular References View

Use a Molecular Reference View to describe the isotopic labeling of peptides in a chromatogram.

Data Graph View

Use a Data Graph View to display and edit (x,y) data in a graphical way, for instance the intensity normalization function of a chromatogram.

Molecular Marker Group View

- Marker Group View
- Marker Group Set View

Use a Molecular Marker Group view to define molecular marker for detection in an All Ion Fragmentation (MS^E) chromatogram.

Fragment Ion Group View

Use a Fragment Ion Group view to define fragment masses for simultaneous detection in an All Ion Fragmentation (MS^E) chromatogram.

Introduction to Arcadiate

Commands:
File > Import > Arcadiate xml...
File > Import > Arcadiate txt...
File > Import > Mass Data...
File > Import > Database Searches...
File > Import > IonGroups...
File > Import > Molecular Markers...
File > Import > Proteins...

File > Export > Arcadiate xml...
File > Export > Arcadiate txt...
File > Export > Spectrum Description...
File > Export > Protein Quantifications...
,File > Export > Spectrum...
File > Export > Precursor...
File > Export > Ions...
File > Export > Peptides...
File > Export > Proteins...
File > Export > Molecular Markers...
File > Export > Chromatogram as mzML...
File > Export > Database Search Result as xml...
File > Export > Fragment Spectra for Mascot Search...

Edit > Cut

Arcadiate supports two generic export and import file formats that allow the exchange of database objects like chromatograms or database search results between two different users: the arcXML and the arcTxt format (**File > Export > Arcadiate xml..., File > Export > Arcadiate txt... , File > Import > Arcadiate xml... , File > Import > Arcadiate txt...**). The arcTxt format can serve as a template how to build a file from external information to import it into Arcadiate – like a Molecular Marker Group.

arcXML and arcTxt files do not capture relationships between objects – like fragment spectra assignments to peptide sequences or quantifications of proteins. To export those various export commands had been implemented which most often export in tabular form (**File > Export > Protein Quantifications..., File > Export > Ions..., File > Export > Peptides.., File > Export > Proteins..., File > Export > Molecular Markers...**).

Some export commands follow public standards to support interactivity with other programmes (**File > Export > Chromatogram as mzML..., File > Export > Database Search Result as xml..., File > Export > Fragment Spectra for**

Mascot Search, File > Export > Spectrum...).

With the help of a Spectrum Description file exported from a chromatogram that contains profile intact ion and fragment ion spectra centroided spectra can be imported (**File > Export > Spectrum Description...)**. A Spectrum Description file captures the peak shape, the x-axis spacing and the resolution of mass spectra and describes therefore the mass spectrometer used. For some mass spectrometers such files can be downloaded from the Arcadiate Web Page.

Several import commands read in data from external sources. They are a starting point for using Arcadiate. Some use public standard format (**File > Import > Mass Data... , File > Import > Database Searches...**), some simple tabular data (**File > Import > Mass Data...** for spectra, **File > Import > IonGroups...**), others use more arbitrary tabular formats (**File > Import > Molecular Markers... , File > Import > Proteins...**). These arbitrary formats reflect the problem to reflect tree-like data structures in tabular form. An xml data structure is the appropriate text format to reflect trees. The arcTxt format solves this problem systematically for simple trees and is the recommended import format for external data which is not xml.

Import of Mass Spectrometric Data
Import of spectra and chromatograms.

Import of Search Results
Import Mascot or MaxQuant Search Results to correlate it with your fragment spectra.

Import of Ion Groups
Import Ion Groups to quantify specific chromatographic ions.

Import of Molecular Markers
Import Molecular Markers to detect them in All Ion Fragmentation (MS^E) chromatograms.

Import of Proteins
Import Proteins to quantify them in chromatograms.

Data Processing

Commands:
Chromatogram > Centroid...
Spectrum > Centroid
Arcadiate > Preferences... > Spectrum > Expand Centroided Data
Window > Tasks

Specific experimental situations require specific data processing methods. Some are listed here.

The commands **Chromatogram > Centroid...** and **Spectrum > Centroid** generate a centroided chromatogram resp. spectrum. Centroiding can reduce the file size considerably. By using the preference option **Arcadiate > Preferences... > Spectrum > Expand Centroided Data** centroided spectra can be displayed as profile data. This mode can not perfectly reproduce the original spectra but it simulates them to a reasonable degree.

Tasks

Use the command **Window > Tasks** to activate the tasks window. The task window lists a series of data processing methods.

Preference Settings

Commands:
Arcadiate > Preferences...

Use the command **Arcadiate > Preferences...** to access the preference settings.

Spectrum

- Color of Spectrum
- Color of Chromatogram
- Color of Overlay: an overlay is generated by dropping a spectrum onto another spectrum view using the view icon
- Color of MS/MS Chromatogram: color of a chromatogram generated from fragment ions – applicable for All Ion Fragmentation (MS^E) data
- Automatic and manual peak labelling
- Expanding centroided data: centroided data is displayed in profile mode
- Lowest expanded peak intensity: this value influences centroiding procedures. Only peaks whose expanded peak intensity is beyond this value will be retained when centroiding the data
- Chromatographic m/z broadening: This factor affects the m/z range of automatically generated chromatograms. The range proposed from the measured spectrum characteristics is multiplied by this factor.

Quantification

- **Quantify within Chromatograms:** by peak matching or by integration – peak matching means that the elution profile is matched against a characteristic peak form. The matched peak is integrated. This quantification method can be more robust against overlapping chromatographic peaks. It is the default option. By Integration means that the intensity profile itself is integrated.
- **Molecular Reference Quantity:** Molecular reference are labeled molecules whose intensity serve as normalization for the quantity of the actually measured molecule
  - **Determination:** by chromatographic peak comparison – the intensity of both the reference molecule and the measured molecule are determined by integrating their resp. ion chromatogram. The ratio between the two integrals is the actual result
  - **Determination:** by spectral peak comparison – the intensity of the reference molecule and the measured molecule are measured both within the same spectra. The ratios between the peak intensities are averaged and is the actual result. This mode requires that molecular reference and measured molecule have the same

retention time. If this is the case this method is more robust than the chromatographic peak comparison

- **Display:** relative or absolute – the intensity of the measured molecule can be displayed as a relative value to its molecular reference or as an absolute value.

- **Peptide Chromatogram:**

- constructed using only the first isotope
- constructed using several isotopes – this option requires the presence of all considered isotopes to yield a chromatographic intensity.

- **Protein Quantity Calculation:** Protein quantities are calculated from their peptide quantities. The average of the three most abundant peptides reflects that the digesting enzyme does not digest proteins in a homogeneous way. Only the most abundant peptides might reflect the chemical concentration of the protein. Calculating the protein quantity as the sum of all peptides divided by the protein mass is statistically a more robust value and the default choice when relative protein changes between experiments is the important observation.
- **Time resolution of Chromatogram Normalizations:** This parameter controls the density of normalisation points when normalising chromatograms to reference molecules. The default value is 10 minutes.
- **Normalise Chromatograms by their Total Ion Count:** This parameter declares whether two chromatograms should be normalised so that they have the same total ion count. This is often use to compensate for differences in the total amount of material used for mass spectrometric runs.
- **Display Normalized Protein Quantities:** Decides whether protein quantities in Database Search Result views are displayed as relative values, normalized to the quantities of a norm-protein defined by dropping a protein-line from a Database Search View onto a chromatogram map or spectrum.

Miscellaneous

- **Width of Chromatogram for Display:** this parameter limits the reconstruction of chromatograms to this time window. 0 means that chromatograms will be reconstructed over the complete time range.
- **Insert Modified Amino Acid Code when Importing Sequences:** Database search engines can determine whether some amino acids in the identified peptide were modified. They often can not specify their location. If this option is chosen and the import detects that a peptide sequence is modified it replaces the first occurrence of the unmodified amino acid code by its modified amino acid code. The detection of a specific modification is based on descriptors associated with the modified amino acid in the residue library.
- **Display Tool Tips:** toggles the on-screen display of tool tips
- **Reset Dialogs:** some dialogs offer the possibility not to show them any longer. Pressing this button tells all these dialogs to reappear.

Normalization of Quantity Values

Generally electrospray ion intensities do not reflect directly the quantity of a molecule in solution. For obtaining ion concentrations or quantities normalisation procedures have to be applied.
Arcadiate allows two level of normalisations, the ion or peptide level and the protein level.

Ion level normalisation:
The ion intensities in a chromatogram can be normalized by two means:

- using isotopically labelled standards
- using a set of molecules whose concentration is known or always the same – a set of internal standard molecules

a) Isotopically labelled standards
When using isotopically labelled standards the ion intensity of a molecule is compared to the ion intensity of a structurally mostly identical molecule that carries some heavy isotopes. Isotope standards can be defined by defining Molecular References in a **Molecular References View**. The measurement expresses the quantity of the molecule in relation to the quantity of the isotopic standard – as a fold change.

b) A set of standard molecules, an internal standard
In cases when isotopic labels cannot be used the ion intensities of a chromatogram can be normalised to the ion intensities of a set of molecules whose concentration is known or is always the same. It is preferable that the retention time distribution of these ions covers a wide range of the analytically useful separation width. This set of ions can be defined using a **Normalization Ion Group View**. Ions can be transferred from an Ion Group to a Normalization Ion Group using copy – paste. Every ion in the Normalization Ion Group should have a Normalized Quantity. By dropping the link ion onto a chromatogram the ions actual quantities in this chromatogram are measured. With the actual and the normalized quantities available a set of normalisation factors is calculated that brings the measured quantities onto the level of the normalized ion quantities. These factors are at the origin of the normalisation function that covers the chromatogram. The function's parameter are displayed in the lower part of the Normalization Ion Group View. The normalisation factors and the normalisation function can be displayed graphically by dropping the Normalization Views link icon onto an empty Data Graph View. The Data Graph View allows the editing of individual normalisation function values.

The normalisation function is not automatically applied to the chromatogram. To apply it its respective column in the lower part of the Normalization Ion

Group View has to be selected and the Apply has to be pressed. After activating the function all ion quantities determined in this chromatogram will be multiplied with the retention time depending normalisation factor of the normalisation function. The exceptions are ion quantities displayed in Normalization Ion Groups. They always represent ion quantities as they have been measured by the mass spectrometer.

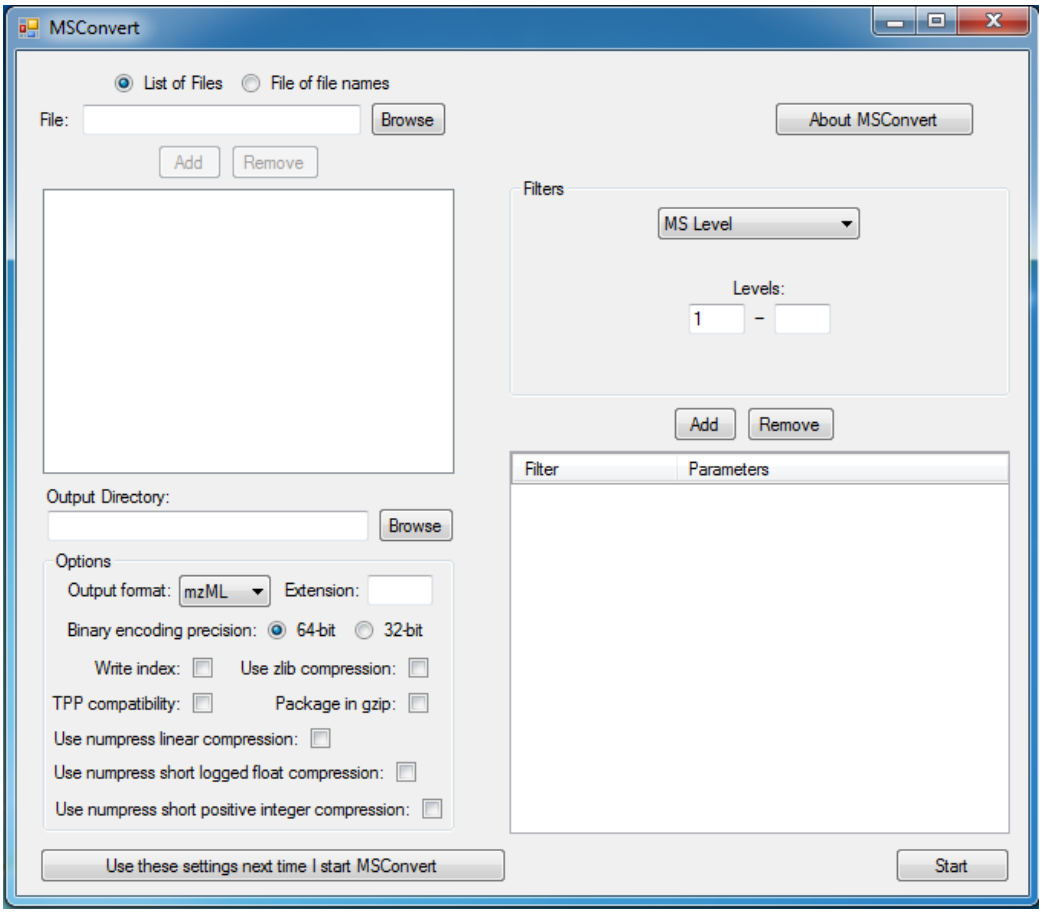
When employing internal standards it is possible to use the **total ion count** as an additional normalisation. The total ion count in a proteomic experiment can be a good estimate for the total amount of sample applied to the chromatogram. Normalising it to a given value can compensate for slight variations in the total material used to run different mass spectrometric investigations.

Protein level normalisation:
All protein quantifications can be seen in relation to the level of a chosen protein. This protein should be a member of a Database Search Result View. Its line in the protein table should be dropped onto a chromatogram map or chromatogram spectrum to set the norm-protein of the chromatogram. Whether protein level normalisations are used is controlled by a preference setting: **Arcadiate** > **Preferences**... > **Quantification** > **Display Normalized Protein Quantities**.

Import of Mass Spectra

Commands:
File > Import > Mass Data...
File > Export > Spetrum Description...

Spectra and Chromatograms
Arcadiate imports individual spectra as [m/z (tab) intensity] lists in ASCII format. Chromatographic data is imported in mzData, mzXML or mzML format. Mass spectrometric chromatographic data can be very complex. Currently Arcadiate supports only the most simple format: MS spectra mixed with tandem MS spectra. Both types should be homogeneous. This means all MS spectra and all tandem MS spectra should be each a group of spectra with the same mass spectrometric characteristic (see below). A good programme for converting mass spectrometric data to xml format is MSConvert in the Proteowizard Package.
The best is to convert the data in 64 bit mode without any other parameter selected:



Several files can be selected using the command **File > Import > Mass Data....**
Arcadiate attempts to automatically detect the file type and displays the Import Window. The Import Window contains a table of all the files to be imported with several parameters describing the data. These parameter should correctly reflect the data characteristics.
Centroided data can only be imported when a *spectrum description file* is available for this type of data. A *spectrum description* is a central element in how Arcadiate stores and displays mass spectrometric data. When profile data is imported two spectrum descriptions are generated automatically, one for the MS and a second for the tandem MS data. A spectrum description describes the m/z-axis value spacing, the typical peak shape and how this peak shape

changes with m/z. A *spectrum description file* can be exported using the command **File** > **Export** > **Spetrum Description....** Take care that the profile data contains tandem MS spectra so that the two spectrum descriptions for MS and tandem MS data are generated and exported. The exported file has to be used to import centroided mass spectra. The centroided data is treated and displayed in the same way as the profile data the spectrum description was generated from.

Import of Mascot Results

Commands:
File > Import > Database Searches...

In general fragment spectra are processed (often centroided), exported and searched against a protein sequence data bank to identify the underlying proteins. The search result is sent back to the user.

Mascot Search Results
When the Mascot search engine is used it is possible to export the search result in form of an xml file. The command **File > Import > Database Searches...** imports Mascot Search xml files. To allow the correlation with original data the Mascot xml file must contain fragment spectra information. The option to include the "MS/MS peak list" or "Input query data" must have been chosen when generating the xml file from Mascot.

MaxQuant Search Results
In case the MaxQuant search engine is used the MaxQuant search generates a folder called "txt". This entire folder has to be selected when importing the MaxQuant search result into Arcadiate.

Import of Ion Groups

Commands:
File > Import > Ion Groups...

Chromatographic ions are characterized by their m/z value and retention time.

Ion Groups
The command **File > Import > Ion Groups...** allows to import text files representing groups of chromatographic ions. A text file should contain a list of [m/z (separator) retention time [min]] values. There could be additional columns which will be ignored. The Import Window allows to choose the columns that represent the m/z and retention time values amongst the columns detected. Individual ions can be added or removed after import.

Import of Molecular Markers

Commands:
File > **Import** > **Molecular Markers...**

A Molecular Marker has a name, a specific m/z value, a series of fragment ions characterised by their m/z values and can have an associated info text.

Molecular Markers
The command **File** > **Import** > **Molecular Markers...** allows to import text files representing groups of molecular markers. The expected file format is a text file with header and separated columns. Every line represents one molecular marker with its fragment ions. The import operation tries to interpret the contents of each column. These assignments can be edited before the import starts. Individual markers or fragment ions can be added or removed after import.
After import the **Molecular Marker Group** is listed in the **Object Store**.

Import of Proteins

Commands:
File > **Import** > **Proteins...**

A Protein has a name, an associated info text and has several peptides. A peptide has an amino acid sequence, a charge state, a chromatographic retention time (in minutes) and an info text assigned to it.

Proteins
The command **File** > **Import** > **Proteins...** allows to import text files representing groups of proteins. The expected file format is a text file with header and separated columns. The import operation tries to interpret the contents of each column. These assignments can be edited before the import starts. Each protein and each of its peptides occupy a separate line. Their characteristics appear in different columns. Individual proteins or peptides can be added or removed after import.
After import the **Protein Group** is listed in the **Object Store**.

Residue Library Window

Commands:
Window > Residue Library

The command **Window > Residue Library** displays the Residue Library Window. The residue library stores the amino acid and modified amino acid symbols, masses and descriptors and allows to define peptide termini. This is the place where the type of fragment ions that are displayed in Sequence Views are defined.
All the information is saved in a residue library file. A saved residue library file is copied to the Arcadiate database file location. The copied version of the file is the actually used one (see command **File > Show in Finder)**

The window has four sections: Standard Amino Acids, Modified Amino Acids, Termini and Fragment Ion Series. Separate residue libraries can be saved and opened.
Standard Amino Acids: The Standard Amino Acid page defines the standard residues, their names, short codes, average and mono-isotopic masses.
Modified Amino Acids: The Modified Amino Acids page defines modified residues that are based on standard residues. The descriptor of a modified amino acid is the precise text which is used in the output file of database search programmes to describe a modified amino acid. When importing database search files modified amino acid codes are inserted into the peptide sequence based on these descriptors when the option "Insert Modified Amino Acid Code when importing Sequences" in the Arcadiate Preferences is selected (see command **Aracdiate > Preferences**..., tab **Miscellaneous**).
Termini: The Termini page defines left and right terminus of the sequences (for amino acids, the N- and C-terminus).
Fragment Ion Series: The Fragment Ion Series page defines which fragment ions will be displayed in the sequence view tables, whether the masses are mono-isotopic or average, their charge state and the polarity.

Generating Views

Commands:
View > Ion Group
View > Normalization Ion Group
View > Info Text
View > Data Graph
View > Molecular References
Chromatogram > Chromatogram Map
Chromatogram > Precursors
Chromatogram > Chromatogram Group > New
Chromatogram > Chromatogram Group > Alignment
Sequences > Sequence View
Sequences > Database Group > Database Group View
Sequences > Protein Group > Protein Group View

- **Sequences > Create Protein Group View**
- **Sequences > Create Molecular Markers**
- For All Ion Fragmentation Data:
- **MS/MS Chromatogram > Marker Views > Fragment Ion Group**
- **MS/MS Chromatogram > Marker Views > Marker Group**

Views of data objects in the collection can be generated by pressing the **Add View** button of the data object in list view of the data collection.
Views can as well be generated by the menu commands:

- **View > Ion Group:** Ion group view – this command creates a new, empty ion group in the library and the collection.
- **View > Normalization Ion Group:** Normalization Ion group view – this command creates a new, empty normalization ion group in the collection. A Normalization Ion Group is used for defining a set of ions that are used to normalize the intensity of a group of chromatograms.
- **View > Info Text:** Text view – displays chromatogram or Data Collection associated text.
- **View > Data Graph:** Data Graph view – used to display and edit graphically an arbitrary set of data – like the retention time relationship of two aligned chromatograms.
- **View > Molecular References:** Molecular Reference view – view to define amino acid based molecular references.
- **Chromatogram > Chromatogram Map:** Chromatogram Map – a two dimensional display of a chromatogram
- **Chromatogram > Precursors:** Precursor view – a list of all fragmented precursors of a chromatogram

- **Chromatogram > Chromatogram Group > New**: Chromatogram group – for collecting related chromatograms
- **Chromatogram > Chromatogram Group > Alignment**: Chromatogram Alignment – for displaying and editing chromatogram alignments
- **Sequences > Sequence View:** Sequence view – to display a single amino acid sequences and their fragment masses
- **Sequences > Database Group > Database Group View:** Database Group View – to display a collection of database search results.
- **Sequences > Protein Group > Protein Group View:** Protein group view – to display a collection of amino acid sequences grouped by proteins.
- **Sequences > Create Protein Group View:** Creates from a Database Search Result a Protein Group View. Protein Groups can be edited Database Search Results only in a limited way.
- **Sequences > Create Molecular Markers:** Creates a Molecular Marker Group from a Database Search whose peptides are linked to fragment spectra.
- **MS/MS Chromatogram > Marker Views > Fragment Ion Group:** Creates a Fragment Ion Group to trace their simultaneous appearance in an All Ion Fragmentation chromatogram
- **MS/MS Chromatogram > Marker Views > Marker Group:** Creates a Molecular Marker Group to trace the simultaneous appearance of intact ions m/z values and their associated fragments in an All Ion Fragmentation chromatogram.

Of the many views in the Arcadiate window the main view is the one with a white background. Some of the view commands relate to primary data objects. They are only active when the main view allows to determine this primary data object.
Nearly all views support zooming by pinch gestures.
Many views are generated automatically by a variety of activities in other views.

Removing Views

Commands:
View > Focus View
View > Unfocus View
View > Hide View
View > Show All
Window > Drawer
Edit > Cut

Views can be hidden or deleted.

Hiding views
The command **View > Hide View** hides the main view. Hidden views are moved to the Drawer of the window. The command **Window > Drawer** opens and closes it. A hidden view can be displayed again by clicking onto the view in the Drawer. All hidden views can be displayed by choosing the command **View > Show All**.
Views can be linked to other views. The command **View > Focus View** hides all views that are not related to the main view. Since view linking can go over several levels using the command several times successively hides more views which are only indirectly related to the main view. The command **View > Unfocus View** reverses step by step the Focus View command.
The Drawer allows not only to add a specific hidden view to the displayed views but by holding the **Alt** key while clicking onto a hidden view displays this view and hides all unrelated views.

Deleting views
The main view can be deleted using the command **Edit > Cut** or by dragging the view icon to the trash. Deleting a view does not remove an associated data object from the collection. However there are data structures that are only associated with views and are not part of the library like calculated spectra. These objects are deleted together with their views.

Moving Views

Moving views

By dragging the view icon a view can be moved to a different location within the Arcadiate window.

By dragging the icon and pressing the Alt-key ⌥ the view is copied to a different position.

Linking Views

Many views can linked to other views to represent a relationship between the underlying data structures. Such a link can initiate considerable activity within Arcadiate and the results are often saved to the library.

Linking views

Dropping the link symbol of one view onto another view establishes a relationship between the two views and their data structures.

- Spectrum view ⟷ Spectrum view: links their x-axis
- Spectrum view ⌥ ⟷ Spectrum view: links their x- and y-axis
- Spectrum view ⟷ Chromatogram view: displays the location of the spectrum in the chromatogram
- Spectrum view ⟷ Chromatogram map view: displays the location of the spectrum on the map
- Chromatogram view ⟷ Chromatogram group view: the chromatogram is added to the chromatogram group

- Chromatogram view ⟷ Chromatogram map view: displays the location of the chromatogram on the map
- Precursor view ⟷ Fragment spectrum view: uses the fragment spectrum view to display other fragment spectra when changing the selection in the precursor table

- Precursor view ⟷ Chromatogram map view: displays the location of the precursors on the map
- Sequence view ⟷ Spectrum view: projection of all selected fragment ions onto the spectrum

- Database search view ⟷ Chromatogram view: fragment spectra are associated with peptide sequences, the ion volumes of the first isotope of the precursors are determined
- Database search view ⌥ ⟷ Chromatogram view: re-establishment of the peptide - fragment spectrum association
- Database search view ⟷ Chromatogram group view: determines the ion volumes of the first isotope of all precursors in all chromatograms
  The chromatograms must have been aligned and the Mascot search must have been related to one of them. This is required because it defines the retention times of the precursors in relationship to the other chromatograms.
- Database search view ⟷ Chromatogram map view: determines the ion volume of the first isotope of all precursors and displays their location on the map

Database search view ⌥ Chromatogram group view: re-determines the ion volumes of the first isotope of all precursors in all chromatograms

- Database search view ⌥ Database search group view: joining the sequences of the database search view to the group

- Ion or Protein group view Chromatogram view: determination of the ion volumes in this chromatogram
- Ion or Protein group view ⌥ Chromatogram view: re-determination of the ion volumes in this chromatogram
- Ion or Protein group view Chromatogram group view: determines the ion volumes in all chromatograms
  The chromatograms must have been aligned and the ion group must have been related to one of them. This is required because it defines the retention times of the ions in relationship to the other chromatograms.
- Ion or Protein group view ⌥ Chromatogram group view: re-determines the ion volumes in all chromatograms
- Ion or Protein group view Chromatogram map view: quantifies the ions and displays their location
- Text view Chromatogram view: display of the chromatogram info text
- Text view itself: display of the data collection info text
- Chromatogram map view Chromatogram group view: adds the chromatogram to the group
- Molecular reference view &nbs Chromatogram map view: assigns the molecular references to the chromatogram
- Molecular reference view Spectrum view: assigns the molecular references to the underlying chromatogram
- Molecular reference view Chromatogram group view: assigns the molecular references to every chromatogram of the group
- Molecular reference view ⌥ Chromatogram group view, Chromatogram map view, Spectrum view: removes an assigned molecular reference group
- Normalization ion group view Chromatogram view: determination of the ion volumes in this chromatogram and calculation of the normalization factors
- Normalization ion group view ⌥ Chromatogram view: re-determination of the ion volumes in this chromatogram and re-calcuation of the normalization factors
- Normalization ion group view Chromatogram group view: determines the ion volumes and normalization factors for all chromatograms
  The chromatograms must have been aligned and the ion group must have been related to one of them. This is required because it defines the retention times of the ions in relationship to the other chromatograms.
- Normalization ion group view ⌥ Chromatogram group view: re-determines the ion volumes and normalization factors for all chromatograms
- Normalization ion group view Chromatogram map view: determines the ion volumes and normalization factors for the

chromatogram and displays their location

- Normalization ion group view Data graph view: shows the normalization factors over time in an editable graphic way
- Chromatogram group view Data graph view: shows the retention time transformations of aligned chromatograms
- Molecular marker group view Spectrum view, Map view or Chromatogram group view: characterises all molecular marker in the underlying chromatograms if these chromatograms represent All Ion Fragmentation (MS^E) acquisitions.
- Database search group view Chromatogram group view: correlates all sequences of the database searches to their fragment spectra in the specific chromatograms where the sequences were identified and quantifies all correlated sequences in all chromatograms.

Unlinking views

Clicking the unlink symbol  unlinks a view and its data structure from all linked views.

Copying Views

Commands:
Edit > Copy
Edit > Copy All Views

The command **Edit > Copy** copies the currently active view as a pdf object to the clipboard to use it in drawing applications.
The command **Edit > Copy All Views** copies all views of the Arcadiate window as a pdf object to the clipboard.

Drawer

Commands:
Window > Drawer

The Drawer panel contains the hidden views. It is opened and closed using the command **Window > Drawer**. By clicking onto a hidden view the view is added to the displayed views. By holding the Alt-key ⌥ pressed and clicking a view the view is displayed and other unrelated views are hidden.

Arcadiate Help

Commands:
Window > Open Assistant

The Assistant panel shows information about view related activities and the properties of the database object displayed in the view. It is opened and closed using the command **Window > Open Assistant** resp. **Window > Close Assistant**. It can be sized manually by dragging its border. Its scrollable **Activities view** is the main source of information how to interact with the content of a view and how to work with Arcadiate. Its **Properties view** displays information about the database object displayed and its relationship to other objects. This can be very helpful in placing the data into an overall context.

Arcadiate Help

Commands:
Pressing the z-key on the keyboard
Pinch gesture on the trackpad
View > Customise Table...
View > Filter Table...
View > Resize Table

Many activities common to all views are described in the Data Collection section.

All views can be temporarily zoomed to take the size of the entire window by pressing and holding the **z-key on the keyboard**.
Many views allow to zoom their content by using a **pinch gesture on the trackpad**.
Tables in an active view can be manipulated:

- Columns can be hidden or shown using the **View > Customise Table...** command
- Rows can be filtered with **View > Filter Table...**
- Columns can be resized to the width of their header with the **View > Resize Table** command.

Spectrum View

Commands:
Edit > Label Selected Peaks
Edit > Remove All Labels
Edit > Copy
View > Select & Scale
View > Zoom
View > Create Subview
View > Display Data
Spectrum > Display
Spectrum > Centroid
Spectrum > Calculate Protein Mass
Spectrum > Reconstruct Protein Mass...
Spectrum > Set Protein Mass...
Spectrum > Hide Protein Mass
File > Export > Spectrum...
File > Export > Spectrum Description...
Chromatogram > Display...
Chromatogram > Stop Reconstruction
Chromatogram > Centroid...
MS/MS Chromatogram > Show Matched Spectrum
Context Menu > MS/MS Spectrum
Context Menu > MS Spectrum
Spectrum > Calibrate...

A spectrum view displays spectral data.

There are three types of spectra: mass spectra, fragment spectra and chromatographic spectra.

Maneuvering within a Spectrum
Maneuvering within a spectrum is identical for all types of spectra:

- Zoom the x-axis: **Mouse Down and drag** below the x-axis
- Display full x-range: **Double Click** below the x-axis
- Zoom to a specific region of the spectrum: **⌥-Mouse Down and drag** within the spectrum
- Display full y-range: **Double Click** left of the y-axis
- Display full y-range from 0: **⌥-Double Click** left of the y-axis
- Display former range: **Double Click** into the spectrum (up to three former ranges are stored)
- Slide x-axis up resp. down: **⌥-Left Arrow resp. ⌥-Right Arrow**
- Jump x-axis up resp. down: **^-Left Arrow resp. ^-Right Arrow**
- Scroll spectrum view: **Mouse Scroll** horizontally and vertically
- Scroll spectrum view horizontally: **⇧-Mouse Scroll**

- **Using the scroller** at the bottom of the document window scrolls the currently active spectrum
- Holding down the ⌥- **key** while clicking into the scroller at the bottom of the window zooms the spectrum around the current selection. If there is more than one selection or no selection at all the spectrum will be zoomed around its middle m/z value.
- A **pinch gestures** zooms the x-axis of the spectrum

Mass Spectra

There are two type of spectra, those that are alone-standing and those that are part of a chromatographic data set. In the latter case the spectrum offers an entry-port to the entire chromatogram.

Activities:

- Chromatogram generation: holding ⌥ ⌘ **keys while click-dragging** over a m/z range of the spectrum
- Measuring within the spectrum: ⌘ **key while click-dragging** over a spectral range
- Setting a selection: **click-dragging** over a spectral range
- Adding a selection: ⇧ **click-dragging**
- Labeling peaks: the command **Edit** > **Label** Selected Peaks labels the most abundant peak in every selection. Labels are editable.
- Removing labels: deleting their text removes individual labels. The command Edit > Remove All Labels removes all.
- Precisely define selections: command **View** > **Select & Scale**
- Magnify regions: the command **View** > **Select & Scale**
- Precisely define the displayed spectrum region: command **View** > **Zoom**
- Creating a spectrum from a range: command **View** > **Create Subview**
- Investigating data points: the command **View** > **Display Data** creates a table with all the data points covered by the selection(s).
- Display a spectrum of a defined time range from a chromatogram: command **Spectrum** > **Display**
- Calculate a centroided spectrum: command **Spectrum** > **Centroid**
- Calculate a protein mass from several multiply charged ions: the command **Spectrum** > **Calculate Protein Mass** calculates protein masses from the highest intensity points in the selections. At least two selections have to be set. The methods assumes that multiply charged ions are of the form $(M_{Protein} + nH)^{n+}$. Several protein masses can be calculated simultaneously but the selections for different proteins should not be intercalated.
- Reconstruct a protein mass spectrum: the command **Spectrum** > **Reconstruct Protein Mass…** reconstructs a neutral mass spectrum of specified range from the displayed range of the spectrum. The ions are assumed to have the form of $(M_{Protein} + nH)^{n+}$.
- Display multiply charged ions of a protein: **Spectrum** > **Set Protein Mass…** projects the location of multiply charged ions of the form $(M_{Protein} + nH)^{n+}$ onto the spectrum.
- Remove the projected multiply charged ions of a protein: **Spectrum** >

Hide Protein Mass hides the projections

- Export a spectrum: the command **File** > **Export** > **Spectrum...** exports the spectrum in form of a tab separated m/z – intensity list.
- Exporting a spectrum description: the command **File** > **Export** > **Spectrum Description...** exports a spectrum description file. The spectrum description file encodes a general description of the spectrum quality – like the typical peak shape in a normalized form. If the current spectrum is derived from a chromatogram with fragment spectra the spectrum description file encodes two spectrum descriptions, one for the mass spectra and a second for the fragment spectra of the chromatogram.
- Copying the spectrum view: the command **Edit** > **Copy** copies the displayed spectrum view as a pdf-object to the clipboard.
- Calibrating a spectrum: the command **Spectrum** > **Calibrate...** starts a calibration work flow. Currently only 1 point and 2 point calibrations are supported. For a chromatographic data set all intact ion spectra use the same calibration.
- For All Ion Fragmentation (MS^E) data: The context menu (ctrl-Click) command **MS/MS Spectrum** allows to display the fragment ion spectrum acquired at the same time.

The drag symbol is used for moving or copying the spectrum view (see Moving Views). The link symbol is used for linking the spectrum view to other views (see Linking Views).

Fragment Spectra

Fragment spectra provide the same working environment like mass spectra only that they allow the generation of mass chromatograms only for datasets acquired in All Ion Fragmentation (MS^E) mode.

Activities:

- Sequence views can be linked to fragment spectra to project the location of different fragment ions onto them.
- Calibrating a fragment spectrum: the command **Spectrum** > **Calibrate...** starts a calibration work flow. Currently only 1 point and 2 point calibrations are supported. For a chromatographic data set all fragment ion spectra use the same calibration.
- For All Ion Fragmentation (MS^E) data: ion chromatograms from the fragments can be generated in the same way as for mass spectra.
- For All Ion Fragmentation (MS^E) data: The context menu (ctrl-Click) command **MS Spectrum** allows to display the intact ion spectrum acquired at the same time.

Chromatographic Spectra

Chromatographic spectra are calculated spectra. The x-axis displays the time in minutes over which the chromatographic analysis was acquired. The y-axis represents an intensity value extracted from the spectrum acquired at the specific time. For the base peak chromatogram it is the highest intensity, for the

total ion chromatogram it is the sum of all intensities and for mass chromatograms it is the sum of the intensities over a specific mass range of the spectrum at the specific time points.
Generally chromatographic provide give the same working environment as mass spectra only that instead of allowing the generation of mass chromatograms they generate summed mass spectra.

- Spectrum generation: holding ⌥ ⌘ keys while click and/or dragging over a time range of the chromatogram generates a summed spectrum of the covered time range
- Changing the type of the displayed chromatogram: the command **Chromatogram > Display...** allows to define precisely the type of chromatogram
- Stopping the reconstruction of a chromatogram: command **Chromatogram > Stop Reconstruction**
- Centroiding all spectra of a chromatogram: command **Chromatogram > Centroid...**
- Defining a norm-protein to normalise all protein quantities determined from this chromatogram to the quantities of this proteins: drag a protein-line from a Database Search View onto the spectrum

Chromatographic Spectra generated from All Ion Fragmentation (MS^E) data sets:
All Ion Fragmentation data sets allow the construction of chromatograms from fragments or from intact ions. Since no particular precursor is selected to generate the fragments a general question for All Ion Fragmentation data is: which fragment ions belong to a specific precursor and which precursor produced a particular fragment ion. This fragment - precursor assignments are based on their identical chromatographic profile.

If in a chromatographic spectrum generated from a particular fragment ion a peak is selected the command **MS/MS Chromatogram > Show Matched Spectrum** displays a spectrum of intact ions present at the time of the chromatographic peak. First, this spectrum is empty but it can be successively populated by peaks. The peaks are sorted according to their chromatographic profile. The peaks with the most similar profile appear first. Like this the precursor which generated the fragment ion can be identified. When selecting the chromatographic peak it is important to include the low-level environment of the peak so that this information is available for the chromatographic peak comparison.

The same procedure can be done in a chromatogram of an intact ion to find the fragments generated from this ion. Select a peak in the chromatogram of the intact ion and choose the command **MS/MS Chromatogram > Show Matched Spectrum.** A fragment spectrum will be displayed which first is empty but can be successively populated with fragment ions to find the fragment ions with the most similar chromatographic trace.

Sequence View

Commands:
Sequences > Sequence
Window > Residue Library

A sequence view displays a single amino acid sequence and its calculated fragment ions. It is used to project its fragment ions in a related fragment spectrum.

Sequence views are generated by the command **Sequences > Sequence View.** Sequence views are used to work with individual amino acid sequences in Arcadiate. They depend on an external residue library.

Sequence View
A sequence view allows to type in an amino acid sequence. The text field only accepts letters that are defined in the associated residue library. The residue library is accessed by pressing the Properties button or by using the command **Window > Residue Library.** Codes of residues consisting of serveral letters have to be bracketed by round brackets. A sequence view can be linked to a spectrum using the link icon . The fragment masses of selected columns will be projected over the spectrum.

Arcadiate Help

Commands:
Sequences > Protein Group > Protein Group View
Sequences > Create Protein Group View
Sequences > Protein Group > Peptide View
Sequences > Protein Group > Unlink Peptides
Sequences > Protein Group > Create Normalization Ion Group
Sequences > Protein Group > 18O Labelling...
Sequences > Replace Amino Acids...
Sequences > Recalculate Protein Quantities
Sequences > Peptide Chromatogram
Sequences > m/z Chromatogram
Window > Residue Library
File > Export > Peptide List
File > Export > Protein Quantifications...

A protein group view is used to display and edit several proteins each one having a set of peptides. Proteins and peptides can be quantified by relating them to a chromatogram.

Protein Group views are generated by the command **Sequences > Protein Group > Protein Group View** or by the command **Sequences > Create Protein Group View.** Protein Group views are used to work with many amino acid sequences in Arcadiate. They depend on an external residue library (see command **Window > Residue Library**).

Protein Group View
The command **Sequences > Protein Group > Protein Group View** creates an empty protein group. Proteins and individual amino acid sequences can be added or deleted by using the + - button. Using the command **Sequences > Create Protein Group View** creates a populated protein group view from all the proteins and peptide sequences of a database search result.
A protein group can be viewed in two forms, grouped or ungrouped by proteins. The command **Sequences > Protein Group > Peptide View** displays the ungrouped form whereas the command **Sequences > Protein Group > Protein Group View** switches back to the protein grouped form.

Activities:

- Associating the protein group with a chromatogram and quantifying all peptides: dropping the link icon onto a chromatogram spectrum or map view.
- Displaying the chromatogram used for quantification: clicking on a blue coloured quantity.

- Reassigning the protein group to a chromatogram: ⌥-link icon dropping onto a chromatogram spectrum or map view.
- Defining a norm-protein of a chromatogram to normalise all protein quantifications to the amounts of this protein: Drag and drop a protein line onto a chromatogram, a chromatogram map or a chromatogram spectrum.
- Displaying the quantities of proteins over several chromatograms: Drag and drop several protein lines onto a data graph view.
- Replacing amino acid codes by other codes or inserting special N- or C-terminal codes: **Sequences** > **Replace Amino Acids...**
- Unlinking a protein group from a chromatogram: using the command **Sequences** > **Protein Group** > **Unlink Peptides**.
- Quantifying all peptides and proteins in several chromatograms: dropping the link icon onto an aligned chromatogram group view
- Recalculating all protein quantities: using the command **Sequences** > **Recalculate Protein Quantities**.
- Displaying the chromatogram of the first peptide isotope: selecting a peptide and using the command **Sequences** > **m/z Chromatogram**.
- Displaying the chromatogram of significant isotopes: selecting a peptide and using the command **Sequences** > **Peptide Chromatogram**. A peptide chromatogram shows the time points where all expected isotopes of the peptide are present simultaneously. This is an improved method to detect the presence of a peptide in a mass chromatogram.
- Peptides can be defined as being partially 18O labelled at their C-terminus. The degree of 18O labelling is set with the command **Sequences** > **Protein Group** > **18O Labelling...**.The labelling affects the isotopic distribution of the peptides and the way how peptide chromatograms as multiple isotope chromatograms are constructed.
- Peptides can be unlinked from chromatograms using the command **Sequences** > **Protein Group** > **Unlink Peptides.**
- Generate a Normalization Ion Group: using the command **Sequences** > **Create Normalization Ion Group** creates a normalization ion group which can be used to normalize quantities in chromatograms (see Normalization Ion Group).
- Exporting the peptides in text format: the command **File** > **Export** > **Peptide List** is used to export peptides as text file.
- Exporting the protein quantifications: in the protein groups view the command **File** > **Export** > **Protein Quantifications...** exports the quantified proteins in text, xml or MaxQuant Protein Groups format.

Precursor View

Commands:
View > Precursors
Window > Residue Library
View > Filter
File > Export > Precursors...

A precursor view shows all fragmented m/z ions of the chromatogram and gives access to the fragment spectra.

A Precursor view can be generated with the command **View > Precursors** if the main view is related to a chromatogram with fragment spectra. The Precursor view gives access to all fragment spectra.

Activities:

- Displaying a fragment spectrum: changing the selection in the precursor table
- Displaying a fragment spectrum with its identified amino acid sequence: changing the selection in the precursor table to a blue colored precursor
- Filtering the precursors: the command **View > Filter**
  - for a data dependent analysis allows to limit the display to identified precursors or to fragment spectra whose summed intensity beyond the m/z value (selected precursor + 1) is larger or smaller a specified value
  - for an all ion fragmentation analysis allows to display exclusively the fragment spectra acquired for a specific precursor selection window

Exporting precursors: the command **File > Export > Precursors...** exports the currently displayed precursors as a tab separated list.
The precursor view can be linked to other chromatograms using the link symbol  to display their fragment spectra. It can be linked to a chromatogram map for a general overview.

Database Search View

Commands:
Sequences > Database Protein View
Sequences > Database Peptide View
Sequences > Create Protein Group View
Sequences > Unlink Fragment Spectra...
File > Export > Peptides...
File > Export > Protein Quantifications...
File > Export > Database Search Result as xml...
Sequences > Create Protein Group View
Sequences > Create Molecular Markers...
Sequences > Replace Amino Acids...
Sequences > Reset Determ. Retention Times and MZs
Sequences > Recalculate Protein Quantities
Sequences > Peptide Chromatogram
Sequences > m/z Chromatogram
Sequences > Unlink Fragment Spectra

A database search view displays the identified proteins each with its set of identified peptides. They are used for quantifying identified proteins.

A Database Search Result view is generated by clicking its Add View button in the data collection list view. The Database Search Result view displays all the identified proteins and their associated peptide sequences. A Database Search can be displayed with the peptides grouped by protein with the command **Sequences > Database Protein View** or as a list of all peptides with the command **Sequences > Database Peptide View**.

Activities:

- Associating fragment spectra with peptide sequences: dropping the link icon onto a chromatogram view
- Displaying the fragment spectrum with its peptide sequence: clicking on a blue coloured amino acid sequence
- Removing an association between a database search and the fragment spectra: command **Sequences > Unlink Fragment Spectra...**
- Determining the ion volume of the first isotope of identified peptides in a chromatogram: dropping the link icon onto a chromatogram view
- Displaying the noise reduced chromatogram of the first isotope of an identified peptide: clicking on a blue coloured ion volume figure
- Editing the precursor ion volume: displaying its chromatogram and changing its selection by ⌘ click-dragging over the chromatogram
- Re-establishing the default ion volume figure: clicking on a blue coloured ion volume figure of the peptide
- Determining the first isotope ion volumes of identified precursors in

several chromatograms: dropping the link icon onto a chromatogram group whose chromatograms are aligned. The database search has to be linked with a chromatogram that is part of the group to define the retention times of the precursors in the other chromatograms.

- Defining a norm-protein of a chromatogram to normalise all protein quantifications to the amounts of this protein: Drag and drop a protein line onto a chromatogram, a chromatogram map or a chromatogram spectrum.
- Displaying the quantities of proteins over several chromatograms: Drag and drop several protein lines onto a data graph view.
- Exporting peptides: the command **File** > **Export** > **Peptides...** exports all peptides as a tab separated list
- Exporting quantification results: the command **File** > **Export** > **Protein Quantifications...** allows to export the ion volumes and sequences of all selected proteins in a structured way as a tab separated list
- Exporting the Database Search Result in Mascot XML format: **File** > **Export** > **Database Search Result as xml...**
- Accessing the URL of the original Database Search Result: clicking the URL-button URL
- Editing the URL of the original Database Search Result: ⌥-clicking the URL-button URL
- Creating a protein group whose sequences can be edited: **Sequences** > **Create Protein Group View**.
- Creating a molecular marker group: The command **Sequences** > **Create Molecular Markers...** creates a molecular marker group from peptides which are linked to their fragment spectra.
- **Sequences** > **Replace Amino Acids...** allows to replace all occurrences of specific amino acids by for instance modified amino acids
- The command **Sequences** > **Reset Determ. Retention Times and MZs** resets the retention times and m/z values of peptides as they were determined when associating them with fragment spectra to the original retention times and m/z values as they were recorded in the database search results.
- With **Sequences** > **Recalculate Protein Quantities** all protein quantities are recalculated in accordance with the actual settings (see Arcadiate > Preferences > Quantification and the different normalisation settings).
- The command **Sequences** > **Peptide Chromatogram** displays a peptide chromatogram of the currently selected peptide. A peptide chromatogram can consider several isotopes.
- **Sequences** > **m/z Chromatogram** displays the chromatogram of the first isotope of the selected peptide.
- **Sequences** > **Unlink Fragment Spectra** unlinks the peptides from their fragment spectra.

Database Search Group View

Commands:
Sequences > Database Group View
Sequences > Database Group Peptide View
Sequences > Unlink Fragment Spectra...
File > Export > Peptides...
File > Export > Protein Quantifications...
Sequences > Create Protein Group View
Sequences > Create Molecular Markers...
Sequences > Replace Amino Acids...
Sequences > Reset Determ. Retention Times and MZs
Sequences > Recalculate Protein Quantities
Sequences > Peptide Chromatogram
Sequences > m/z Chromatogram
Sequences > Unlink Fragment SpectraEdit > Cut

A database search group allows to combine several database searches. Proteins and peptides are not merely copied but combined such that duplicate peptides and proteins are avoided. Database search groups can be edited. They are used to quantify proteins based on all peptides found in several different chromatographic runs.

A Database Search Group view is generated by using the command **Sequences > Database Group View**. The Database Search Group view displays all the proteins and peptides of the database search results in its group. When adding a database search result peptide sequencing belonging to a protein already present in the group are added without duplicating the protein entry. All the peptides can be displayed as a list by using the command **Sequences > Database Group Peptide View** or ordered by proteins via the command **Sequences > Database Group View.**

Activities:

- Associating fragment spectra with peptide sequences: dropping the link icon onto a chromatogram group view
- Displaying the fragment spectrum with its peptide sequence: clicking on a blue coloured amino acid sequence
- Removing an association between a database search and the fragment spectra: command **Sequences > Unlink Fragment Spectra...**
- Determining the ion volume of the first isotope of identified peptides in a chromatogram: dropping the link icon onto a chromatogram group view
- Displaying the noise reduced chromatogram of the first isotope of an identified peptide: clicking on a blue coloured ion volume figure
- Editing the precursor ion volume: displaying its chromatogram and

changing its selection by ⌘ click-dragging over the chromatogram
- Re-establishing the default ion volume figure: clicking on a blue coloured ion volume figure of the peptide
- Determining the first isotope ion volumes of identified precursors in several chromatograms: dropping the link icon  onto a chromatogram group whose chromatograms are aligned.
- Defining a norm-protein of a chromatogram to normalise all protein quantifications to the amounts of this protein: Drag and drop a protein line onto a chromatogram, a chromatogram map or a chromatogram spectrum.
- Displaying the quantities of proteins over several chromatograms: Drag and drop several protein lines onto a data graph view.
- Exporting peptides: the command **File** > **Export** > **Peptides...** exports all peptides as a tab separated list
- Exporting quantification results: the command **File** > **Export** > **Protein Quantifications...** allows to export the ion volumes and sequences of all selected proteins in a structured way as a tab separated list
- Creating a protein group whose sequences can be edited: **Sequences** > **Create Protein Group View**.

- Creating a molecular marker group: The command **Sequences** > **Create Molecular Markers...** creates a molecular marker group from peptides which are linked to their fragment spectra.
- **Sequences** > **Replace Amino Acids...** allows to replace all occurrences of specific amino acids by for instance modified amino acids
- The command **Sequences** > **Reset Determ. Retention Times and MZs** resets the retention times and m/z values of peptides as they were determined when associating them with fragment spectra to the original retention times and m/z values as they were recorded in the database search results.
- With **Sequences** > **Recalculate Protein Quantities** all protein quantities are recalculated in accordance with the actual settings (see Arcadiate > Preferences > Quantification and the different normalisation settings).
- The command **Sequences** > **Peptide Chromatogram** displays a peptide chromatogram of the currently selected peptide. A peptide chromatogram can consider several isotopes.
- **Sequences** > **m/z Chromatogram** displays the chromatogram of the first isotope of the selected peptide.
- **Sequences** > **Unlink Fragment Spectra** unlinks the peptides from their fragment spectra.
- Database search group views are editable - with **Edit** > **Cut** the selected proteins, selected peptides or the view itself can be deleted.

Ion Group View

Commands:
View > Ion Group
View > Unlink Ion Group
View > Charge State Determination
Sequences > m/z Chromatogram
Arcadiate > Preferences... > Quantification

An ion group lists chromatographic ions defined by their m/z value and retention time. They are used to quantify targeted ions.

An Ion Group view can be generated by clicking the Add View button in the list view of a data collection or by using the command View > Ion Group. An ion group is used to determine ion volumes of specific chromatographic ions.

Activities:

- Determination of the ion volumes in a specific chromatogram: dropping the link icon onto a chromatogram view
- Displaying the quantitative chromatogram, a chromatogram of a centroided, noise reduced ion: clicking onto the blue colored ion volume number of an ion
- Editing the ion volume number: displaying its chromatogram and changing its selection by ⌘ click-dragging over the chromatogram (only when the ion volume was determined by integration – see **Arcadiate > Preferences... > Quantification**: by integration).
- Re-establishing the default ion volume figure: clicking on a blue colored ion volume figure of the ion
- Unlinking the ion group from a chromatogram: command **View > Unlink Ion Group**
- Determining the ion volumes in several chromatograms: dropping the link icon onto a chromatogram group whose chromatograms are aligned. The ion group has to be linked with a chromatogram that is part of the group to define the retention times of the ions in the other chromatograms.
- Charge state determination of ions in a chromatogram: the command **View > Charge State Determination** if the ion group is associated with a chromatogram
- Display the m/z chromatogram of a selected ion: **Sequences > m/z Chromatogram**

Normalization Ion Group View

Commands:
View > Ion Group
View > Unlink Ion Group
View > Charge State Determination
Sequences > m/z Chromatogram

A normalization ion group lists several chromatographic ions and allows to set their expected intensity. It is used to normalise chromatographic runs.

A Normalization Ion Group view is generated by using the command **View > Normalization Ion Group**. A Normalization Ion Group is used to normalize the ion intensities within chromatograms so that a set of norm ions has the same intensity in all of them. See Normalization for a detailed discussion of normalizations.

Activities:

- Adding or removing ions to the group by pressing the + - button or by copying them from an Ion Group View.
- Determination of the ion volumes in a specific chromatogram: dropping the link icon onto a chromatogram view.
- Filling in the Normalized Quantity column by typing in the values line by line or be selecting an ion volume volume and copy-pasting all values over to the Normalized Quantity column.
- Displaying the quantitative chromatogram, a chromatogram of a centroided, noise reduced ion: clicking onto the blue colored ion volume number of an ion
- Editing the ion volume number: displaying its chromatogram and changing its selection by ⌘ click-dragging over the chromatogram (only when the ion volume was determined by integration - see **Arcadiate > Preferences... > Quantification**: by integration).
- Re-establishing the default ion volume figure: clicking on a blue colored ion volume figure of the ion
- Display the m/z chromatogram of a selected ion: **Sequences > m/z Chromatogram**
- Unlinking the ion group from a chromatogram: command **View > Unlink Ion Group**
- Determining the ion volumes in several chromatograms: dropping the link icon onto a chromatogram group whose chromatograms are aligned. The ion group has to be linked with a chromatogram that is part of the group to define the retention times of the ions in the other chromatograms.
- Charge state determination of ions in a chromatogram: the command

View > **Charge State Determination** if the ion group is associated with a chromatogram

- Applying normalizations to chromatograms: after the ion group was linked to chromatograms their normalization factors had been calculated in the Norm-values Table. These values can be applied to the chromatograms by selecting the columns and pressing the Apply button.
- Removing the normalizations from chromatograms: Selecting the respective columns in the Norm-values Table and press the Reset button.
- Using Total Ion Count Normalization: Total ion count normalization is a function that the intensities in the chromatograms are multiplied by a factor which ensures that the total ion count of the normalized chromatograms is the same. The value in **Arcadiate** > **Preferences**... > **Quantification**: Normalize Chromatograms by their Integrated Ion Count defines the default value. For every specific normalization the function can be switched on or off in the Total Ion Count Normalization table.
- Visualizing and editing the normalization functions: linking the Ion Group Normalization view to a Data Graph view displays the normalization function in the Data Graph view and allows editing.

Info Text View

Commands:
View > Info Text

An info text view displays and stores arbitrary information about data collections and chromatograms.

An Info Text view can be generated by the command **View > Info Text**. Info texts are available for every chromatogram and data collection.

Activities:

- Displaying the info text of a chromatogram: dropping the link icon onto a chromatogram view or a spectrum view associated with a chromatogram
- Displaying the info text of a data collection: dropping the link icon onto the text view itself
- Deleting a text view: dragging the view icon to the trash. Deleting the view does not delete the text.

Chromatogram Group View

Commands:
View > Chromatogram Group
Edit > Cut
View > Chromatogram Group > Alignment

A chromatogram group collects several related chromatographic runs. It is used to align their time axis to each other, to normalise them in the same way or to quantify ions and proteins in all chromatograms simultaneously.

A Chromatogram Group view is generated by the command **View > Chromatogram Group**. A chromatogram group is used to align the time axis of related chromatograms.

Activities:

- Adding a chromatogram to the group: dropping the link icon of a spectrum view related to a chromatogram or of a chromatogram map view onto the chromatogram group view
- Removing a chromatograms from a group: selecting the chromatograms and using the command **Edit > Cut**
- Storing text information about the group: dragging the lower border of the table upwards opens a text field
- Changing the name of the chromatogram group: editing the name field at the lower border of the table
- Time-aligning un-aligned chromatograms: by using the align button Align the non-aligned chromatograms are aligned
- Re-aligning all chromatograms: by pressing the Alt key and the align button ⌥ Align all chromatograms are re-aligned

The alignment procedure calculates for every chromatogram a time-axis transformation to transform its eigen-time to a norm-time. For the chromatogram that is displayed in bold red its eigen-time is identical to the norm-time. Even if this chromatogram is deleted the functionality of the alignment remains intact. Via the alignment the retention time of every chromatogram in the group can be transformed into the corresponding retention time of any other chromatogram.

The alignment can be inspected in two ways:

- The alignment function between reference chromatogram time and the aligned chromatogram time can be visualised by dragging the link icon onto a Data Graph view. This function is not directly editable.
- The points used for alignment can be inspected and edited by using the

command **View** > **Chromatogram Group** > **Alignment** while the chromatogram group is the activated view. This displays an chromatogram alignment view.

Chromatogram Alignment View

Commands:
View > Chromatogram Group > Alignment
View > New Anchor Points
View > Delete All Anchor Points

A chromatogram alignment view is used to display and edit the time-alignments of two chromatograms from a chromatogram group.

A Chromatogram Alignment view is generated by the command **View > Chromatogram Group > Alignment**. A chromatogram alignment view is used to display and edit the alignment of two chromatograms.

Activities:

- Most of the functionality associated with Chromatogram Map Views is present in the alignment view.
- Clicking into the grey region around an anchor point edits this anchor point.
- Clicking the button New Anchor Points or chosing the command **View > New Anchor Points** enters the anchor point editing mode.
- Clicking the Delete All button or choosing the command **View > Delete All Anchor Points** deletes all anchor points.
- Clicking the button Apply applies the changes of the edited anchor points, leaves the anchor point editing mode and recalculates the alignment function.
- Editing the x- and y-shift values shifts the Reference Map against the Aligned Map.
- Dropping a line of a chromatogram group onto the alignement view exchanges the displayed aligned chromatogram map.

Chromatogram Map View

Commands:
View > Chromatogram Map
Edit > Label Selected Peaks
Edit > Remove All Labels
File > Export > Chromatogram as mzML...

A chromatogram map view displays a two dimensional intensity map of a chromatographic run. It gives a good overall impression of the chromatographic resolution and the location of fragmented and identified peptides or ions.

A Chromatogram Map view is generated by using the Add View button of a chromatogram data object in the data collection list view or by using the command **View > Chromatogram Map**. A chromatogram map gives an overview over an entire chromatographic data set.

Activities:

- Zooming in: holding the Alt key ⌥ and dragging the mouse over the map to define the region to zoom in to
- Zooming in with constant axis ratio: holding the keys ⌥ ⇧ and dragging the mouse over the map
- Restoring the former view: double mouse click into the map region. Up to three views are stored.
- Generate a selection for peak labelling: click-drag mouse of a map region
- Add a selection for peak labelling: ⇧ click-drag mouse of a map region
- Label the most abundant peak in every selection: command **Edit > Label Selected Peaks**
- Remove a label: by deleting its text
- Remove all labels: command **Edit > Remove All Labels**
- Generating a chromatogram: ⌥ ⌘ click-dragging the mouse generates a mass chromatogram of the chosen x-axis interval
- Generating a single spectrum: ⌥ ⌘ mouse click into the map generates a spectrum at the chosen time coordinate
- Generating a summed spectrum: ⌥ ⌘ click-dragging the mouse generates a summed spectrum of the chosen y-axis interval
- Measuring within the map: ⌘ click-dragging the mouse - care is advisable because a lot of data might be accessed
- Moving within a zoomed map: the arrow keys ↑, ↓, →, ← move the map in the chosen direction. Keeping the Alt key pressed ⌥↑, ⌥↓, ⌥→, ⌥← rapidly shifts the map in the chosen direction. The map can be scrolled.
- Changing the staining intensity: Shifting the slider changes the ion intensity for the most intense staining, ⌥ shifting the slider changes the ion intensity for the lowest staining

- Displaying all precursors that had been selected for fragmentation: dragging the link icon of the chromatogram's precursor view onto the map – clicking onto a precursor marker displays the fragment spectrum
- Displaying all ions of an ion group on the map: dragging the link icon of an ion group onto the map – clicking onto a ion marker displays the ion's chromatogram
- Displaying all precursors of identified peptides: dragging the link icon of a Mascot search result view onto the map – clicking the precursor marker displays the fragment spectrum and the peptide sequence
- Defining a norm-protein to normalise the quantities of all proteins determined from this chromatogram to its quantities: dragging a protein line from a Database Search View onto the map.
- Dropping a chromatogram from an aligned chromatogram group that contains the chromatogram of the map onto the map overlays the map with the second chromatogram's map in its aligned form
- Several chromatograms can be overlaid simultaneously
- A menu allows to set the top-most chromatogram – with two sliders the transparency and the staining level of the top-most chromatogram can be regulated
- The command **Edit** > **Cut** removes the top-most chromatogram overlay
- Exporting the entire chromatogram in mzML format: **File** > **Export** > **Chromatogram as mzML**...

Molecular Reference View

Commands:
View > Molecular References

A molecular reference view allows to define isotopic labels attached to peptides and declare within the program that isotopically labeled peptides are present in a chromatographic run. By this they influence how quantities extracted from the chromatogram are calculated and displayed. Isotopically labeled peptides are often used in a mixture with un-labeled peptides to allow precise relative quantification of many peptides and proteins.

A Molecular Reference view is generated by using the command **View > Molecular References**. A Molecular Reference view allows to define mass labels which are used to differentiate different sets of otherwise identical peptides in one sample. Molecular references are used for relative quantifications (see Normalization).

Activities:

- Pressing the button + - adds or removes a molecular reference from the group.
- Molecular references are based upon labelled amino acids or a labelled peptide terminus.
- Often they consists of heavy isotopes that contain an additional number of neutrons.
- Their additional mass can be edited directly in the added mass column.
- For each molecular mass a colour can be defined that will be used to display associated peptide quantities in Mascot Search views or ion group views.
- Dropping the link symbol onto a chromatogram or chromatogram map assigns the molecular references to the chromatogram. In further quantifications the chromatogram is treated as containing the molecular references as defined in the molecular references group.
- Pressing the ⌥ key and dropping the link symbol onto a chromatogram or chromatogram map removes the molecular reference assignment from the chromatogram.

Data Graph View

Commands:
View > Data Graph
Chromatogram > Show Normalization

A data graph view is used to display arbitrary data in graphical form. Some data sets can be edited by via their graphical representation.

A Data Graph view is generated by using the command **View > Data Graph**. A Data Graph view is used to display arbitrary x-, y- value based data. In some instances the individual data points can be edited.

Activities:
Maneuvering within a data graph

- Zoom the x-axis: **Mouse Down and drag** below the x-axis
- Display full x-range: **Double Click** below the x-axis
- Zoom to a specific region of the spectrum: **⌥-Mouse Down and drag** within the data graph
- Display full y-range: **Double Click** left of the y-axis
- Display full y-range from 0: **⌥-Double Click** left of the y-axis
- Display former range: **Double Click** into the data graph (up to three former ranges are stored)
- Slide x-axis up resp. down: **⌥-Left Arrow resp. ⌥-Right Arrow**
- Jump x-axis up resp. down: **^-Left Arrow resp. ^-Right Arrow**
- Scroll the data graph view: **Mouse Scroll** horizontally and vertically
- Scroll the data graph view horizontally: **⇧-Mouse Scroll**

Editing data graph properties: Double click onto a graph object
The label, point style, line width, colour, display status and whether the data points are connected can be edited. The editing of x- and y-values is controlled by the context the data graph has been generated with.

Choosing a graph object via the Graph menu in the graph property window automatically hides unrelated graph objects. They can be shown again using the context menu of the data graph view. The context menu is displayed when pressing **^ mouse click** in the data graph.

Data graph views are filled with data when linking an aligned Chromatogram Group or a Normalization Ion Group with a data graph view. Using the command **Chromatogram > Show Normalization** displays the current chromatogram normalisation in a data graph view if the normalisation is based on a normalisation ion group.

Molecular Marker Group View

Commands:
MS/MS Chromatogram > Marker Views > Marker Group
MS/MS Chromatogram > Marker Views > Marker Group Set
File > Import > Molecular Markers...
MS/MS Chromatogram > Marker Chromatogram
MS/MS Chromatogram > Fragment Chromatogram
MS/MS Chromatogram > Unlink Molecular Markers
MS/MS Chromatogram > Modify Masses...
MS/MS Chromatogram > Show Matched Spectrum

Molecular Marker Views allow to define molecular markers for detection in a chromatogram consisting of All Ion Fragmentation data (MS^E).

All Ion Fragmentation data have the characteristic that chromatograms can be generated from fragment masses since fragment spectra from all precursors within the analytical range are acquired continuously .
The main purpose of a Molecular Marker View is to relate it to an All Ion Fragmentation chromatogram to trace the marker molecules amongst all the molecules analysed. A molecular marker is considered to be detected when its m/z value and all of its fragment m/z values are detected at the same time. This is indicated by a green dot in the marker's detection column. If some of its fragment masses are not detected at the time of maximum correlation between the marker's m/z value and the fragment's m/z values the marker's detection column will show an orange dot. If there is no correlation between the marker's m/z value and it's fragments m/z values the detection column shows a red dot.

A Molecular Marker view is generated by clicking the Add View button of a Molecular Marker Group in the data collection list view or by using the command **MS/MS Chromatogram > Marker Views > Marker Group.** The MS/MS Chromatogram menu structure is only visible when the active view is based on a chromatographic data set generated by All Ion Fragmentation (MS^E).
Molecular Marker Groups can be generated either by importing a corresponding text file via **File > Import > Molecular Markers...** or by calculating it from a Database Search Result or a Database Search Result Group that is correlated with its fragment spectra (see Sequences > Create Molecular Markers...).
A Molecular Marker consists of the m/z value of the intact molecule and several m/z values of fragment ions generated by the marker upon fragmentation.

Molecular Markers can be organised in sets – like peptides that belong to specific proteins. The command **MS/MS Chromatogram > Marker Views > Marker Group Set** generates an empty set-structured Molecular Marker Group. When calculating a marker group from a database search result the proteins assignment of peptides remains intact by generating for every protein a set. A

set-structured marker group can be displayed as Marker Group Set or as conventional Marker Group using the commands **MS/MS Chromatogram > Marker Views > Marker Group Set** and **MS/MS Chromatogram > Marker Views > Marker Group.**

For overlays fragment chromatograms are individually and dynamically scaled. The order of their intensities corresponds to the acquired data. Their intensity ratios are changed for display purposes.

Activities:

- Adding or removing Molecular Markers to / from a group: selecting the Marker table in the view and pressing the + - button
- Adding or removing Fragment ions to / from a molecular marker: selecting the molecular marker, selecting the fragment ion table and pressing the + - button
- Detecting the molecular marker in a chromatogram: dropping the link icon onto a chromatogram spectrum or map view
- Detecting the molecular markers in a group of chromatograms: dropping the link icon onto a chromatogram group view
- Clicking onto the blue intensity value of the chromatogram column of the marker table: displays the m/z chromatogram of the intact marker molecule
- Alt-Clicking onto the blue intensity value of the chromatogram column of the marker table: displays the m/z chromatogram of the intact marker molecule and toggles the quantification method between peak fitting and area integration.
- Clicking onto a blue value of the retention time: displays the marker molecule m/z chromatogram overlaid with all fragment m/z chromatograms
- Clicking onto a blue m/z value in the fragment ion table: displays the marker ion m/z chromatogram overlaid with the fragment m/z chromatogram
- Clicking onto a quantity value of a fragment displays the fragment chromatogram
- Alt-Clicking onto a quantity value of a fragment displays the fragment chromatogram and toggles the quantification method between peak fitting and area integration.
- A marker chromatogram from the selected molecular marker can be generated by choosing the command **MS/MS Chromatogram > Marker Chromatogram**
- A fragment chromatogram from the selected fragment can be generated by choosing the command **MS/MS Chromatogram > Fragment Chromatogram**
- The command **MS/MS Chromatogram > Unlink Molecular Markers** unlinks the molecular marker table from all chromatograms
- The command **MS/MS Chromatogram > Modify Masses...** allows to modify the m/z values of all molecular markers or fragments in the selected group
- Starting with a chromatographic peak from an intact molecule resp. a fragment ion the command **MS/MS Chromatogram > Show Matched**

Spectrum displays a spectrum of fragment masses resp. intact ion masses which have a similar chromatographic profile (see Spectrum View)

Fragment Ion Group View

Commands:
MS/MS Chromatogram > Marker Views > Fragment Ion Group

A fragment ion group view allows to define a set of fragment ions for detection in a chromatographic data set that reflects All Ion Fragmentation (MS^E) data. This analysis is only supported for All Ion Fragmentation data sets with only one static selection window.

All Ion Fragmentation data have the characteristic that chromatograms can be generated from fragment masses since like the intact ion data fragment spectra are acquired continuously without selecting a specific precursor mass. If the All Ion Fragmentation chromatograms use multiple selection windows you have to use a Molecular Marker Group.

A Fragment Ion Group view is generated by clicking the Add View button of a Fragment Ion Group in the data collection list view or by using the command **MS/MS Chromatogram > Marker Views > Fragment Ion Group.** The MS/MS Chromatogram menu structure is only visible when the active view is based upon a chromatographic data set generated by All Ion Fragmentation (MS^E).

Activities:

- Adding or removing fragment ions to / from a group: pressing the + - button
- Detecting the fragment ions in a chromatogram: dropping the link icon onto a chromatogram spectrum. The fragment chromatogram is a correlative chromatogram. Only locations where all fragments are present simultaneously are shown.

Tasks

Commands:
Tools > Tasks > Precursor
Tools > Tasks > Ion Group
Tools > Tasks > Mascot Search
View > Unlink Fragment Spectra

The tasks window lists a series of data processing methods.

Simulation of Precursor Selection
The command Tools > Tasks > Precursor opens the precursor selection simulation pane. This task simulates the selection of ions for fragmentation throughout a chromatographic tandem MS run.

- The **m/z Range** is the m/z range from which an ion will be selected.
- The **Intensity Threshold** (**absolute** or **relative**) is the intensity threshold for selection. No ion with an intensity below the higher of the two values will be selected for fragmentation.
- The **Precursor Exclusion** defines which ions will not be selected based on their m/z values.
- The **dynamic** exclusion time defines a time for which the m/z values of all formerly selected ion m/z values will not be selected for a second time.
- The **fixed** exclusion list defines m/z values that will never be selected.
- The **Charge State** options define which potential ion will be excluded or selected based on their charge state as determined in real time on the available data. "Real time" charge state determination might not always be correct.
- The **Process Simulation** options list in preselected precursors how many precursors will be fragmented before a new mass spectrum is acquired.
- The **acquisition time** is the time the mass spectrometer will require (on the average) to acquire a single fragment spectrum.

Ion Group Comparison
The command Tools > Tasks > Ion Group opens the ion group comparison pane. This task compares two ion groups in the active Arcadiate data collection.

- The **Reference Group** is selected by activating an ion group in a data collection and pressing the Active Group button.
- The **Compared Group** is selected in the same way.
- **Tolerance in m/z** is the m/z window within which two ions are considered to have the same m/z value
- **Tolerance in Time** is the width of the time window within which two ions are considered to have the same retention time

Database Search Significance Level

The command **Tools** > **Tasks** > **Mascot Search** activates the database search significance panel. This task determines a score threshold for a given false positive rate.

- Database searches are selected by activating a Database Search Result view in a data collection and pushing the button Active Search
- The **Tolerated False Positive Rate** gives the percentage of statistically tolerated false positives in the results from the real database

All peptide identifications with a score higher than the determined score threshold will be considered to be significant identifications. The score threshold is calculated by comparing the search in the real database with the search in a decoy database. Using a tolerated false positive rate the score threshold is reached when the number of identifications in the decoy database with scores higher than the threshold divided by the number of identifications in the real database with scores higher than the threshold remains just below the tolerated false positive rate.

The determined score threshold can be used with the command **View** > **Unlink Fragment Spectra** to dissociate all sequences from their assigned fragment spectra below this score. This type of significance level determination is appropriate for search results with many identifications.