mMass 5.5.0 User's Guide

by **Martin Strohalm**

Contents

1. Preface

mMass is the fruit of years of study and development. While I've put a lot of energy into making this program as stable and reliable as possible, *mMass* comes with no warranty of any kind. You are however welcome to read the code, modify it and send me any suggestions or patches. *mMass* development still continues, so any ideas, function requests or bug reports are more than welcome. Feel free to contact me through the *mMass's* web page http://www.mmass.org or join the forum at http://forum.mmass.org.

1.1. Motivation

While tools for the automated analysis of MS and LC-MS/ MS data are continuously improving, it is still often the case that at the end of an experiment, the mass spectrometrists will spend time carefully examining individual spectra. Current software support is mostly provided only by the instrument vendors, and the available software tools are often instrument-dependent. Such software can only be used to analyze data from a specific instrument, and this causes serious problems for laboratories that use more than one instrument. This tight software-instrument relationship also causes problems for laboratories that do not possess their own instruments, and have to obtain mass spectra from other, collaborating, laboratories. To provide a solution to these limitations I have started to develop *mMass* - open source multi-platform tool for precise mass spectrometric data analysis and interpretation.

1.2. Authors

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http://www.mmass.org

I wish to express my thanks to all the people involved in the excellent *Python* language, *wxPython* and *NumPy* libraries, and namely to the people helping me to make *mMass* still better:

Daniel Kavan: C programing
Laboratory of Molecular Structure Characterization
Institute of Microbiology, Prague
Academy of Sciences of the Czech Republic
http://ms.biomed.cas.cz

Timo Niedermeyer: Custom-type sequence handling and fragmentation design
Institute of Pharmaceutical Biology,
EMA-University Greifswald, Germany
Cyano Biotech GmbH, Germany

Sebastian Gibb: OpenSUSE packages
Universität Leipzig, Germany

Filippo Rusconi: Debian packages
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Petr Novák:
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1.3. Publications to Cite

- Niedermeyer THJ and Strohalm M
  mMass as a Software Tool for the Annotation of Cyclic Peptide Tandem Mass Spectra
  PLoS ONE **7** (9), e44913 (2012)
  DOI:10.1371/journal.pone.0044913

- Strohalm M, Kavan D, Novák P, Volný M, Havlíček V:
  mMass 3: A Cross-Platform Software Environment for Precise Analysis of Mass Spectrometric Data.
  Anal Chem **82** (11), 4648-51 (2010)
  DOI:10.1021/ac100818g

- Strohalm M, Hassman M, Košata B, Kodíček M
  mMass Data Miner: an Open Source Alternative for Mass Spectrometric Data Analysis.
  Rapid Commun Mass Spec **22** (6), 905-908 (2008)
  DOI:10.1002/rcm.3444

1.4. Technicalities

At the very beginning, *mMass* started as a simple *PHP*-based tool to compare peak differences within mass spectrum. Fortunately, *PHP* wasn't powerful enough for calculations and I stated to learn *Python*...

Current version of *mMass* is written mostly in *Python* programing language (http://www.python.org) and uses *wxPython* libraries (http://www.wxpython.org) for graphical user interface (GUI) and *NumPy* module (http://www.numpy.org) for faster computing of mathematical tasks.

Since resolution of modern mass spectrometers grows up, resulting data sets become very large. Even *Python* powered by *NumPy* is not powerful enough for some calculations, therefore a piece of code written in *C* has been added into *mMass* to speed-up some processing functions.

1.5. Typographical Conventions

Following typographical conventions are used throughout this document:

Names of mMass's components and modules, names of dialog values etc.

Menu items and buttons.

```
Terminal commands, scripts and other pieces of code.
```

Advanced tips and hints.

Important notes and warnings.

2. Version History

Version 5.5.0 (released July 1, 2013)

ADDED Peak list can now be exported into ASCII with column headers.
ADDED *Mass to Formula* tool is now able to calculate negative compositions (element losses).
IMPROVED Form parameters updated in *Protein Prospector* tools.
FIXED *Mass to Formula* tool crashed when no composition was found.
FIXED Correct scan number is now parsed from mzML format.

Version 5.4.1 (released Dec 25, 2012)

CHANGED Internal server has been disabled for OS X.
FIXED An issue with crashing plot canvas.
FIXED An issue with crashing *Envelope Fit* tool.

Version 5.4.0 (released Nov 18, 2012)

ADDED Multiple documents overlay in *Mass Defect Plot* panel.
IMPROVED On Linux, system user's folder is now used for configs and presets.
FIXED Incorrect handling of C-terminal modification in *Peptide Fragmentation* panel.
FIXED Incorrect calculation of some mass defects.
FIXED An issue with peak picking crashing on some documents.
FIXED An issue with mzML files with incorrect base peak intensity.
FIXED An issue with mzML files with unspecified retention time units.
FIXED Incorrect zooming style in *Scan Selection* dialog.
FIXED Updated link to *ChemSpider* from *Mass to Formula* panel.
FIXED Updated link to *Human Metabolom Database* from *Mass to Formula* tool.

Version 5.3.0 (released Aug 27, 2012)

ADDED *Mass Defect Plot* to clusterize peaks based on their mass defect.
ADDED *Swap Data* function is now available in the *Batch Processing* panel.
ADDED Line style can be set for profile mass spectra.
IMPROVED Open multiple documents via command line.
FIXED An issue with negatively charged ions matching.

Version 5.2.0 (released Jul 1, 2012)

ADDED New spectrum canvas view option to allow ranges outside current documents boundaries.
ADDED File association now works on all platforms via listening on port 65456.
IMPROVED More detailed *Spectrum Ruler* info for charge and neutral mass.
FIXED In some cases, document selection in batch processing was unpredictable.

Version 5.1.0 (released May 17, 2012)

ADDED *Mass to Formula* tool now compares theoretical isotopic pattern with acquired data.
ADDED Isotopic pattern can be modeled using either symmetrical or asymmetrical peak shapes.
ADDED Artificial spectra can be modeled using either symmetrical or asymmetrical peak shapes.
ADDED *Compare Peak Lists* tool now shows additional table with combined peak list.
ADDED *Compare Peak Lists* tool now enables intensity ratio threshold for highlighting peaks.

ADDED Unmatched peaks can now be removed from *Peak Differences* tool table.
ADDED Neutral mass can be calculated and shown by *Spectrum Ruler* tool.
IMPROVED Peak list generated by *Isotopic Pattern* tool is calculated from final envelope.
CHANGED Baseline shift removed from normalization.
FIXED Preview in *Math Operations* tool did not work correctly.
FIXED An issue with re-creating configuration files.
FIXED Peak list updating in *Match* panel plot.

Version 5.0.1 (released Mar 28, 2012)

IMPROVED Molecular formula of the peptide is shown in custom-type *Sequence Editor*.
FIXED *Sequence Search* tool did not work.
FIXED Sequence info did not update correctly while switching sequences.
FIXED Applying processing tools with no document selected may cause application to crash.
FIXED An issue with mzML files with different data array bits.

Version 5.0.0 (released Mar 2, 2012)

ADDED *Mass to Formula* tool to generate molecular formula from given m/z value.
ADDED *Batch Processing* tool to apply multiple processing steps to multiple documents at once.
ADDED Minor axis ticks can be shown in spectrum canvas.
ADDED Peak area can be calculated and shown by *Spectrum Ruler* tool.
ADDED Protein charge state can be calculated and shown by *Spectrum Ruler* tool.
ADDED *Overlay* function added to *Math Operations* panel.
ADDED *Combine all, Average all* and *Overlay all* functions added to *Math Operations* panel.
ADDED Photoshop-like method to see/enable just one document while hiding others.
ADDED Sequence modifications can be applied specifically to *N* or *C* terminus.
ADDED New view option to show/hide labels' *Group* name.
IMPROVED Part of the mMass's processing core is now written in *C*.
IMPROVED Faster *Math Operations*.
IMPROVED Faster loading of single-scan documents from mzML, mzXML and mzData.
IMPROVED Information shown by *Spectrum Ruler* tool can be set via application menu View.
IMPROVED Drawing of individual peaks in pattern simulator can be disabled to speed up calculation.
FIXED Multiple files selection in *Open* dialog.
FIXED Application crashed when peaks were labeled with *Sequence* panel opened.

Version 4.0.0 (released Dec 2, 2011)

ADDED New *Sequence Editor* to define custom peptide sequence with non-standard amino acids.
ADDED Peptide sequence can be set as linear or cyclic.
ADDED N-terminal modifications can be saved in presets.
ADDED Internal monomer library with more that 500 building blocks from NORINE database.
ADDED *Monomer Library Editor* for user-defined monomers and corresponding neutral losses.
ADDED Improved *Peptide Fragmentation* tool with more fragmentation pathways.
ADDED Gaussian smoothing filter.
ADDED Overall matched intensity is calculated and shown in *Match* panel and *Sample Report*.
ADDED *Envelope Fit* tool to determine heavy atoms exchange (e.g for HDX experiments).
ADDED Monoisotopic mass determination for large proteins with isotopically resolved envelope.
ADDED Charge state is shown in the bottom bar for *Measure Distance* tool and current difference.
ADDED *Save All* feature to save all opened documents at once.
ADDED Current document's path is shown in *Document Info* panel.
ADDED Charge column is shown for annotations and sequence matches in *Sample Report*.
ADDED User's Guide PDF is accessible via application menu *Help*.

CHANGED Definition of the z-series ions according to Mascot.
CHANGED Polished icons to better fit into OS X Lion.
FIXED An issue with mzData time parameter.

Version 3.12.1 (released Aug 28, 2011)

FIXED Application sometimes crashed while closing last document.
FIXED Some peaks were skipped when peak picking height was set to 100%.
FIXED Calibration curve sometimes did not fit well.

Version 3.12.0 (released Jul 14, 2011)

ADDED *Deconvolution* tool to generate deconvoluted peak list from peaks with assigned charge.
ADDED New document can be created directly from clipboard data.
ADDED Different application layouts are available via *Window* menu.
ADDED Group name parameter can be specified for any peak via *Peak Editor*.
ADDED Group name column can be shown in *Peaklist Panel*.
ADDED For *Label Envelope* tool, intensity can be calculated as envelope max, sum or average.
IMPROVED Faster calculation of complex isotopic distributions.
IMPROVED *Spectrum Generator* tool is using native peak's FWHM value to generate spectra.
CHANGED Unified toolbar for all platforms.
FIXED Isotopic envelope was not calculated correctly for single-isotope compounds.
FIXED Peak list was not updated correctly after applying math functions.
FIXED Some toolbars were not drawn correctly in floating panels.
FIXED Peak lists concatenation (A+B) did not work correctly.

Version 3.11.0 (released May 10, 2011)

ADDED Simple *Spectrum Generator* tool for artificial mass spectra generation.
ADDED Cancel button for long-running tasks.
ADDED All the peak picking tools are using baseline according to *Baseline Correction* panel.
ADDED Live threshold line preview is shown for *Peak Picking* tool according to current settings.
ADDED *Label Envelope* tool to label isotopes or envelope centroid or to "measure" peak charge.
ADDED *Isotope mass shift* correction parameter in *Deisotoping* panel.
ADDED Support for MGF (Mascot Generic Format) data format.
ADDED User annotations and sequence matches can be shown as "labels" in *Spectrum Viewer*.
ADDED *Peaklist Panel* columns can be enabled or disabled.
ADDED A.i. value can be shown in *Peaklist Panel*.
ADDED Neutral mass is calculated and can be shown in *Peaklist Panel*.
ADDED Left-click+Alt/Ctrl on a document title in *Documents Panel* hides other documents.
ADDED Scale y-axis simply by mouse scroll with the cursor positioned in the y-axis area.
ADDED Sequence accession number can be specified in *Sequence Editor*.
ADDED Direct links to protein databases are available in *Report* via protein accession numbers.
ADDED H_3PO_4 neutral loss added to *Sequence Fragmentation* panel.
ADDED Semi-specific mass search in *Sequence Panel* can be enabled.
IMPROVED Various speed improvements for high-resolution data.
IMPROVED Application closing is much faster.
CHANGED Parameters of manual peak picking tools were moved to corresponding processing panels.
FIXED Documents loading queue.
FIXED *Sequence Editor* popup menu now behaves correctly.
FIXED Specifity filter in *Sequence Modifications* panel correctly handles terminal amino acids.
FIXED Metadata (e.g. operator, contact) are correctly loaded from mzData, mzXML and mzML.
FIXED Resolved an issue causing axis labels overlapping.

Version 3.10.0 (released Feb 23, 2011)

ADDED *Mass Filter* tool to annotate or remove contaminant masses.
ADDED *Set Mass Range* tool to specify current mass range in *Spectrum Panel*.
ADDED Font and line scaling can be specified in *Export Spectrum Image* tool.
ADDED Peak list filter can be specified in *Match* panel and protein identification tools.
ADDED IDs for PubChem Compounds and NORINE peptide database are recognized in *Report*.
ADDED New items in *Peptide Fragmentation* panel.
CHANGED Data normalization tool moved to *Processing Panel*.
CHANGED All annotations and sequence matches are removed when processing tools are applied.

Version 3.9.0 (released Dec 15, 2010)

ADDED *Compare Peak Lists* tool to compare peak lists between multiple documents.
ADDED Simple math functions are now available to add, subtract and multiply spectra.
ADDED Peptides, fragments or compounds lists can be filtered to show matched/unmatched items.
ADDED Sequences from digest, fragments or peptide search lists can be copied into clipboard.
ADDED Duplicate copy of selected document can be make via pop-up menu in *Documents Panel*.
ADDED Distance measurement tool shows current distance in both *m/z* and *ppm* (bottom bar only).
ADDED Multiple documents can be opened at once using *Open* dialog.
FIXED Correct drawing of spectra with no continuous baseline (typically for SIM experiments).
FIXED FWHM and resolution columns in a peak list can now be copied into clipboard as well.
FIXED Option to enable/disable notation marks now works correctly.

Version 3.8.0 (released Aug 20, 2010)

ADDED TIC and BPC chromatograms are now available in scan selection dialog.
ADDED Support for *mzML* data format.
ADDED Spectrum intensity offset (visual only) can be set either manually or using new mouse tool.
ADDED Intensity bar is now available in *Spectrum Panel*.
ADDED Height of position bar and intensity bar can now be set in *Canvas Properties*.
ADDED New view option to show/hide spectrum data points.
ADDED Peak width is assigned automatically when overlaying theoretical isotopic patterns.
FIXED Incorrect charge calculation in *Deisotoping* module.
FIXED Incorrect file extension for files converted by *CompassXport*.

Version 3.7.0 (released Jul 19, 2010)

ADDED Simplified interface for *Protein Prospector* tools *MS-Fit* and *MS-Tag*.
ADDED FWHM and resolution is now calculated for each peak and shown in *Peaklist Panel*.
ADDED Global modifications can now be stored as presets.
ADDED Spectrum polarity can now be set in *Document Info* panel.
FIXED Application crashed when "None" document was sent into *Calibration Panel*.
FIXED Some of the potential peaks were skipped in *Shoulder Peaks Removal* algorithm.
FIXED Data matching for negative spectra was not possible with *Ignore charge* option unchecked.

Version 3.6.0 (released Jul 5, 2010)

ADDED *Match Summary* is now available for each data matching.
ADDED *Remove shoulder peaks* option is now available for FTMS data peak-picking.
ADDED All annotations and sequence matches can now be removed at once from *Document panel*.
ADDED Compounds and reference masses can now be imported using corresponding library editor.
ADDED Spectrum flipping is now accessible from the *View* menu.

IMPROVED Faster peak-picking, especially when *Adaptive threshold* option is used.
FIXED Loading of some *mzXML* files with parameter *"compressionType=none"* was not possible.
FIXED Application crashed on a single right-click in *Isotopic pattern* panel.

Version 3.5.0 (released Jun 4, 2010)

ADDED Possibility to vertically flip spectra in *Spectrum Viewer*.
ADDED -H_2O can now be searched in *Compounds Search* tool.
FIXED Normalized view did not worked for centroid spectra.

Version 3.4.0 (released May 12, 2010)

ADDED Radical ions can now be calculated in *Mass Calculator* and *Compounds Search* tools.
ADDED Single-point linear calibration has been enabled.
ADDED Calibration curve is now shown in the calibration error plot.
ADDED Current peak list is now shown as a background in error plots (calibration, data matching).
ADDED New view option to enable/disable normalized view of opened documents.
ADDED New view option to enable/disable notation marks.
ADDED All annotations and matches can now be highlighted by selecting a document root.
ADDED Theoretical and matched sequence coverage is now shown in *Protein Digest* tool.
ADDED Final composition of ion is validated in ion series calculations.
ADDED Automatic checking for available updates has been added.
ADDED Application preferences for updates and *CompassXport* utility has been added.
FIXED *Mass Calculator* - calculation of ions with negative agent charge.
FIXED *Mass Calculator* - pattern modeling with complex charging agent.
FIXED Normalization now includes baseline shift.

Version 3.3.0 (released Apr 9, 2010)

ADDED *Periodic Table* of the elements has been added.
ADDED Bruker's raw data are imported using *CompassXport* tool (MSW only).
FIXED Small fixes in manual peak picking.
FIXED Dropping documents to *mMass* caused the source window to freeze until loading finished.
FIXED *Mass Search* tool - Enzyme endings selection did not worked.
FIXED *Mass Search* tool - *Max charge* has been replaced by *Charge.*
IMPROVED *Mass Search* tool - better filtering.

Version 3.2.0 (released Mar 14, 2010)

ADDED *Isotopic Pattern* panel is now collapsable.
ADDED Hidden modifications can now be enabled in *Mascot Search* interface.
ADDED Modified documents are marked by asterisk.
IMPROVED Relative intensity for annotations and matches is now calculated on-the-fly.

Version 3.1.0 (released Mar 3, 2010)

ADDED Lipid database (by *LIPID MAPS Consortium*) has been added to *Compounds Search* tool.
ADDED *ProFound Search* interface has been implemented.
ADDED Sequences can now be imported form *mMass's* and *FASTA* files.
ADDED Manually labeled peak can now be automatically set as monoisotopic.
ADDED Specific cursor images for spectrum tools has been added.
ADDED Scan list buffer is now used to speed-up loading of multi-scan documents.
FIXED Multiple fixes in *Mascot Search* interface.

IMPROVED Peak-picking algorithm has been improved.
IMPROVED More "Pythonic" syntax in the *mspy* module.
IMPROVED Spectrum tools are now accessible from the main menu as well.

Version 3.0.0 (released Feb 1, 2010)

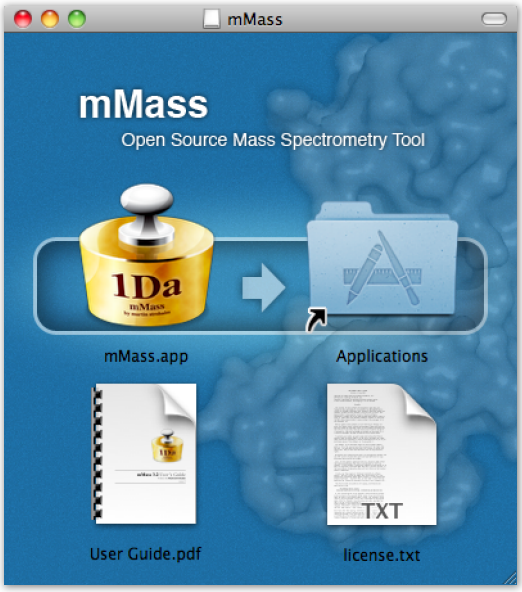
Complete *mMass* redesign - new user interface, new features and new source code architecture.

3. License

4. Installation

4.1. Mac OS X

As usually, there is no special installation procedure for Mac OS X. *mMass* is available as a regular disk image containing the *"mMass.app"* application. This is a standalone package containing all the necessary modules and libraries. To install *mMass* just open the disk image and drag the *mMass.app* to your application folder. After first run *mMass* creates the specific folder *"~/Library/Application Support/mMass/"* to store all your presets and objects libraries. Current version of *mMass* was successfully tested on the Intel based computers with Mac OS X 10.5 and 10.6.



mMass's installation disk image for Mac OS X.

4.2. MS Windows

In most cases, installation on MS Windows is very easy. *mMass* is available as regular ZIP archive containing the *"mMass"* application folder. There are no installation steps needed to run *mMass* under MS Windows. This means that you can run the program directly after unpacking downloaded archive. Simply run the *"mmass.exe"*. Current version of *mMass* was successfully tested on XP SP3, Vista and Windows7.

⚠ *Please note that you must have the privileges for writing to the "mMass" folder, otherwise you will not be able to store any program presets such as startup defaults, modifications etc.*

For older versions of MS Windows, *mMass* sometimes doesn't want to start up showing the following message instead: ***The application failed to start because the application configuration is incorrect. Reinstalling the application may fix this problem.*** In such a case you'll need to download and install the runtime components of Visual C++ Libraries from Microsoft's website.

http://www.microsoft.com/downloads/details.aspx?familyid=9B2DA534-3E03-4391-8A4D-074B9F2BC1BF&displaylang=en

4.3. Linux

I have to say that I'm not very familiar with Linux platform and therefore there is no special build of *mMass* available for Linux. Nevertheless, I am always testing and debugging *mMass* on my virtual version of *Debian* distribution to make it work properly and to retain application native look. If you want to run *mMass* on Linux see the following chapter to run it from the source code.

4.4. Running from Source

mMass belongs to the wide family of open source software so why not to modify it? All you need to do is download and unpack the *mMass's* source ZIP archive and then do what you can. There are some additional modules and libraries needed to run *mMass*, however, all of them are available for free and easy to install.

Dependencies

Python (http://www.python.org) *"Python is a programming language that lets you work more quickly and integrate your systems more effectively. You can learn to use Python and see almost immediate gains in productivity and lower maintenance costs."*

wxPython (http://www.wxpython.org) *"wxPython is a GUI toolkit for the Python programming language. It allows Python programmers to create programs with a robust, highly functional graphical user interface, simply and easily. It is implemented as a Python extension module (native code) that wraps the popular wxWidgets cross platform GUI library, which is written in C++."*

NumPy (http://www.numpy.org) *"NumPy is the fundamental package needed for scientific computing with Python. It contains among other things: a powerful N-dimensional array object, sophisticated (broadcasting) functions, tools for integrating C/C++ and Fortran code, useful linear algebra, Fourier transform, and random number capabilities."*

⚠ *Please note that current version of mMass was developed and tested using Python 2.7, wxPython 2.8.12.1 and NumPy 1.5. I cannot guarantee that mMass will work with different version of particular libraries.*

Compiling C-code

In addition to installing all of the above libraries and modules you'll need to compile a piece of *C* code used to speed up data processing. It can be done very easily with the *"setup.py"* script located in the *"mspy"* folder.

On Mac OS X go to the *"mspy"* folder and run compilation command in *Terminal*, then locate resulted *"calculations.so"* file in the build folder and move it to *"mspy"*. The compilation command should be like:

```
python setup.py build
```

On MS Windows go to the *"mspy"* folder and run compilation command in *Command Prompt*, then locate resulted *"calculations.pyd"* file in the build folder and move it to *"mspy"*. If you are using *MinGW* (http://www.mingw.org) the compilation command should be like:

```
python setup.py build --compiler=mingw32
```

On Linux go to the *"mspy"* folder and run compilation command in *Terminal*, then locate resulted *"calculations.so"* file in the build folder and move it to *"mspy"*. The compilation command should be like this:

```
python setup.py build
```

⚠ *Please note that in order to compile the C part of mMass on Linux, you need to have the "-dev" package of Python installed.*

Running Application

To start up *mMass* go to *mMass's* main folder and simply run the following command:

```
python mmass.py
```

Making Application Bundle

If you want to make your own application bundle for Mac OS X or MS Windows you need to have *py2app* (http://pypi.python.org/pypi/py2app) or *py2exe* (http://pypi.python.org/pypi/py2exe) respectively. After installing the corresponding utility you can make the bundle simply by using *"setup.py"* script located in *mMass's* main folder. Just go to the folder and run the following command:

On Mac OS X

```
python setup.py py2app
```

On MS Windows

```
python setup.py py2exe
```

5. User Interface

5.1. Application Layout

Beside the standard elements, like toolbar etc., *mMass's* user interface is divided into the three main parts: *Documents Panel, Spectrum Viewer* and *Peaklist Panel*. You can choose from several different application layouts via Window menu. All processing and analyzing modules and tools are available in the form of floating panels to enable direct validation of results and acquired data.

mMass's user interface.

5.2. Main Menu

File

New - creates a blank document with no data. In those cases where data are available in the form of printed peak list only you can use the blank document to manually define peak list and to analyze it in *mMass*. This feature can also be used to create a document containing just a protein or peptide sequence to simulate digestion, fragmentation or isotopic pattern or to make a personal sequence database.

New from Clipboard - creates new document from clipboard data.

Open - opens document.

Open Recent - opens one of the recent documents.

Close - closes selected document.

Close All - closes all opened documents.

Save - saves selected document into *mMass's* native format.

Save As - saves selected document as a new file.

Save All - saves all opened documents.

Export - exports current spectrum image, spectrum points or peak list.

Print Spectrum - prints current spectrum view.

Analysis Report - generates analysis report for selected document.

Document Info - shows document information panel for selected document. Various items can be specified such as *analysis title, operator's name, contact, institution, instrument, spectrum parameters* and *analysis notes*. It is generally good idea to fill up this information if you want to share the analysis with other people.

Preferences - shows application preferences dialog. (In the *mMass* menu for OS X)

Quit - quits application. (In the *mMass* menu for OS X)

View

Spectrum Canvas → Legend - shows or hides legend in spectrum viewer.

Spectrum Canvas → Gridlines - shows or hides gridlines in spectrum viewer.

Spectrum Canvas → Minor Ticks - shows or hides minor axes ticks in spectrum viewer.

Spectrum Canvas → Data Points - shows or hides spectrum data points on detailed zoom.

Spectrum Canvas → Position Bars - shows or hides position bars in spectrum viewer.

Spectrum Canvas → Gel View - shows or hides gel-view in spectrum viewer.

Spectrum Canvas → Gel View Legend - shows or hides legend in gel-view.

Spectrum Canvas → Cursor Tracker - shows or hides cursor tracker in spectrum viewer.

Spectrum Canvas → Check Limits - block ranges outside documents boudaries.

Labels → Show/Hide Labels - shows or hides peak labels in spectrum viewer.

Labels → Show/Hide Label Ticks - shows or hides ticks for peak labels.

Labels → Label Charge - shows or hides charge value for peak labels.

Labels → Label Group - shows or hides group value for peak labels.

Labels → Label Background - shows or hides a solid white background for peak labels.

Labels → Horizontal/Vertical Labels - shows peak labels horizontally or vertically.

Labels → Allow Overlapping - enables or disables automatic managing of labels overlaps.

Labels → Labels in All Documents - shows or hides peak labels in all visible spectra.

Notations → Show/Hide Notations - shows or hides annotated or matched peaks.

Notations → Notation Marks - shows or hides marks for annotated or matched peaks.

Notations → Notation Labels - shows or hides labels for annotated or matched peaks.

Notations → Notation m/z - shows or hides m/z for annotated or matched peaks.

Spectrum Ruler → m/z - shows or hides cursor m/z position in bottom bar.

Spectrum Ruler → Distance - shows or hides cursor distance value in bottom bar.

Spectrum Ruler → ppm - shows or hides cursor distance ppm value in bottom bar.

Spectrum Ruler → Charge - shows or hides calculated charge in bottom bar.

Spectrum Ruler → Mass - shows or hides calculated neutral mass in bottom bar.

Spectrum Ruler → Area - shows or hides calculated cursor selection area in bottom bar.

Peak List Columns - shows or hides peak list columns.

Autoscale Intensity - enables or disables automatic scaling of intensity axis.

Normalize Intensity - enables or disables normalization of intensity axis for all spectra.

Set Mass Range - defines current mass range in spectrum canvas.

Flip Spectrum - flips selected spectrum vertically.

Offset Spectrum - specifies intensity offset for current spectrum.

Clear All Offsets - clears intensity offsets for all spectra.

Canvas Properties - additional options for spectrum canvas (gel height, mass precision, font sizes etc.).

Processing

Undo - reverts back last operation. Most of the operations in *mMass* can be undone such as all processing functions or editing of labels, matches and annotations. However, only one step back is currently available.

Peak Picking - automatically finds and labels peaks in selected document.

Deisotoping - automatically assigns charges and deletes isotopes in current peak list.

Deconvolution - makes duplicated document with deconvoluted peak list.

Correct Baseline - corrects baseline in selected spectrum.

Smooth Spectrum - smooths selected spectrum.

Crop - crops spectrum points and peak list in selected document.

Math Operations - normalize, combine, subtract or multiply spectra.

Batch Processing - apply processing steps in batch.

Calibration - calibrates data in selected document using internal, external or statistical calibration.

Swap Data - swaps data between profile spectrum and peak list. For some formats it is not possible to see whether data should be handled like spectrum points or peak list. This function can be used to swap spectrum points to peak list data if the default decision was wrong.

Sequence

New - creates new sequence object in selected document.

Import - imports sequence object from a file into selected document.

Edit Sequence - shows sequence editor with selected sequence.

Edit Modifications - shows sequence modifications editor with selected sequence.

Digest Protein - generates digestion peptides from selected sequence.

Fragment Peptide - generates fragments from selected sequence.

Mass Search - searches for peptide corresponding to given m/z value within selected sequence.

Show Isotopic Pattern - sends current sequence into *Mass Calculator* tool.

Send to Envelope Fit - sends current sequence into *Envelope Fit* tool.

Calibrate by Matches - uses current sequence matches as reference masses for data re-calibration.

Delete All Matches - deletes all the matches for selected sequence.

Delete Sequence - deletes selected sequence object.

Sort By Titles - sorts all sequences in selected document by titles.

Tools

Spectrum Ruler - sets mouse tool to measure distances, charge or area in a spectrum.

Label Peak - sets mouse tool to label peaks in selected spectrum.

Label Point - sets mouse tool to label exact points in selected spectrum.

Label Envelope - sets mouse tool to precisely label monoisotopic peak, isotopes or envelope centroid.

Delete Label - sets mouse tool to delete labels in selected spectrum.

Offset Spectrum - sets mouse tool to offset spectrum intensity.

Periodic Table - shows periodic table of elements.

Mass Calculator - calculates ion series and isotopic pattern for specified molecular formula.

Mass to Formula - generates elemental composition for given m/z value.

Mass Defect Plot - clusterizes and visualizes peaks based on their mass defect.

Mass Filter - removes or annotates contaminant masses.

Compounds Search - searches for specified compounds and adducts within selected document.

Peak Differences - generates table of peak differences to searches for amino acids or any m/z difference.

Compare Peak Lists - compare multiple peak lists.

Spectrum Generator - generates artificial spectrum from current peak list.

Envelope Fit - determine heavy atoms exchange by fitting theoretical profiles to measured data.

Mascot PMF - sends selected peak list to Mascot's Peptide Mass Fingerprint tool.

Mascot MS/MS Ion Search - sends selected peak list to Mascot's MS/MS Ion Search tool.

Mascot Sequence Query - sends selected peak list to Mascot's Sequence Query tool.

ProFound Search - sends selected peak list to ProFound Search tool.

Protein Prospector MS-Fit - sends selected peak list to Protein Prospector MS-Fit Search tool.

Protein Prospector MS-Tag - sends selected peak list to Protein Prospector MS-Tag Search tool.

Libraries

Compounds - shows compounds library editor.

Modifications - shows modifications library editor.

Monomers - shows monomers library editor.

Enzymes - shows shows enzymes library editor.

Reference Masses - shows reference masses library editor.

Mascot Servers - shows Mascot servers library editor.

Presets - shows presets library editor.

Links

Direct links to popular tools, databases and knowledge servers related to mass spectrometry.

Help

Homepage - opens *mMass's* website in a default web browser.

Support Forum - opens *mMass's* forum website in web browser.

Twitter - opens *mMass's* Twitter account in web browser.

Papers to Cite - shows related papers in web browser.

Make a Donation - shows donation page in web browser.

Check for updates - checks for available updates.

5.3. Toolbar

 - Open document dialog

 - Save current document

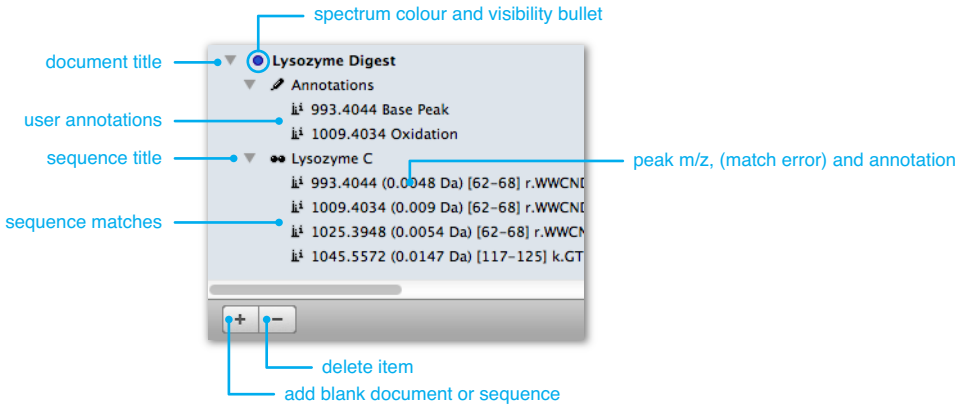
 - Print current *Spectrum Viewer* content

 - *Processing* tools panel

- *Calibration* tool panel
- *Sequence* tools panel
- *Periodic Table* panel
- *Mass Calculator* tools panel
- *Mass to Formula* tool panel
- *Mass Defect Plot* tool panel
- *Mass Filter* tool panel
- *Compound Search* tool panel
- *Peak Differences* tool panel
- *Compare Peak Lists* tool panel
- *Spectrum Generator* tool panel
- *Envelope Fit* tool panel
- *Mascot Search* tools panel
- *ProFound Search* tool panel
- *Document Info* panel
- *Analysis Report*
- *Export* tools panel

5.4. Documents Panel

The main purpose of *Documents Panel* is to provide a structured view of loaded documents with all its sub-elements. In this panel, each element of selected document can be edited by double-clicking or processed via specific command or context menu. Most of the sequence related functions are also available via the main menu Sequence.



Documents Panel.

Document

➡ **To add blank document:**
Press + button from the bottom toolbar and select New Document, or choose File → New from the main menu.

➡ **To select document for analysis:**
Click on any sub-element of the document you want to select. Selected document is marked by a bold title.

➡ **To make a document copy:**
Right-click on the document title and choose Duplicate Document.

➡ **To temporarily hide document in spectrum viewer:**
Click on the color bullet next to document title.

➡ **To temporarily show only one document:**
Click on the document title or bullet while holding Alt or Ctrl key. Click again to revert all documents to the previous state.

➡ **To change document title, information or notes:**
Double-click on the document title, or choose File → Document Info from the main menu to show up *Document Info* panel. See *Document Information* chapter for more details.

➡ **To vertically flip document in spectrum viewer:**
Right-click on the document title and choose Flip Spectrum, or choose View → Flip Spectrum from the main menu.

➡ **To offset spectrum intensity in spectrum viewer:**
Right-click on the document title and choose Offset Spectrum, or choose View → Offset Spectrum from the main menu to show up offset dialog. Set *Intensity offset* and press Offset button.

➡ **To clear spectrum offset:**
Right-click on the document title and choose Clear Offset. Choose View → Clear Offsets from the main menu to clear offset for all opened documents.

➡ **To change document colour:**
Right-click on the document title and choose Change Colour.

➡ **To change spectrum line style:**
Right-click on the document title and choose a style from the Line Style menu.

➡ **To delete all annotations and sequence matches:**
Right-click on the document title and choose Delete All Notations.

➡ **To close document:**
Right-click on the document title and select Close Document, press - button from the bottom toolbar and choose Close Document, or choose File → Close from the main menu.

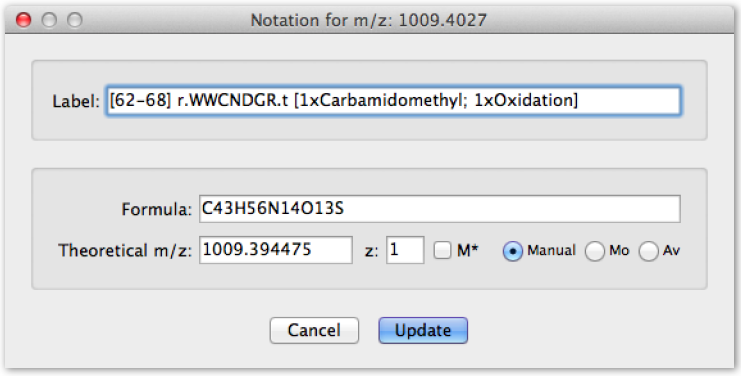
➡ **To close all documents:**
Right-click on any document title and select Close All Documents, press - button from the bottom toolbar and choose Close All Documents, or choose File → Close All from the main menu.

⚠ *Please note that spectrum offset is applied only if normalization is disabled.*

⚠ *Please note that normalization and spectrum offset do not modify any document data. These are just visualization tools.*

User Annotations

It is possible to add a user annotation to any peak in a peak list. You can specify annotation *Label*, which is then shown in *Documents Panel*, and assign molecular *Formula* and *Theoretical m/z* value. All the annotations with theoretical m/z value can be used as calibration standards to re-calibrate your data. If the molecular formula is specified you can generate its isotopic pattern to see the difference between theoretical and measured peak.



Annotation dialog.

➡ **To add user annotation:**
Double-click on the peak in *Peaklist Panel* or press button from the bottom toolbar of *Peaklist Panel* to show up annotation dialog and type your annotation.

➡ **To edit user annotation:**
Double-click on the annotation to show up annotation dialog and edit the annotation.

➡ **To delete user annotation:**
Right-click on any annotation and select Delete Annotation, or press button from the bottom toolbar and choose Delete Annotation.

➡ **To delete all user annotations:**
Right-click on any annotation or the annotations root and select Delete All Annotations, or press button from the bottom toolbar and choose Delete All Annotations.

➡ **To highlight user annotation in spectrum viewer:**
Click on the annotation and spectrum moves to the m/z value. Small red arrow shows up on m/z axis of the spectrum.

➡ **To show or hide user annotation labels in spectrum viewer:**
Choose View → Notations → Show/Hide Notations from the main menu and click on the annotations root. You can specify the information to show using View → Notations menu.

➡ **To show isotopic pattern of user annotation:**
Right-click on the annotation and select Show Isotopic Pattern. *Mass Calculator* panel shows up with the annotation formula and corresponding peak charge. This function is available only if molecular formula and charge is specified in annotation dialog. See *Mass Calculator* chapter for more information.

➡ **To generate molecular composition for annotated m/z:**
Right-click on the annotation and select Send to Mass to Formula. *Mass to Formula* panel shows up with the annotation m/z and corresponding peak charge. See *Mass to Formula* chapter for more information.

➡ **To re-calibrate data by user annotations:**
Right-click on any single annotation or annotations root and select Calibrate by Annotations. All the

user annotations will be send to *Calibration* panel. Only those annotations with specified theoretical m/z value can be used for data calibration. See *Calibration* chapter for more information.

Sequence

In order to use some *mMass's* tools such as *Protein Digest* or *Peptide Fragmentation,* specific sequence object must be defined. All sequence matches are stored in the similar way as user annotations and there are the same features available as well. See *Sequence Tools* chapter for more information about sequence editing and processing.

➡ **To add new sequence:**
Press [+] button from the bottom toolbar and select New Sequence, or choose Sequence → New Sequence from the main menu. See *Sequence Tools* chapter for more information.

➡ **To edit sequence:**
Double-click on the sequence title and *Sequence Editor* shows up. See *Sequence Tools* chapter for more information.

➡ **To delete sequence:**
Click on the sequence title and press Command+Backspace or Delete, or press [-] button from the bottom toolbar and choose Delete Sequence.

➡ **To edit sequence match:**
Double-click on the sequence match to show up annotation dialog and edit the annotation.

➡ **To delete sequence match:**
Right-click on any sequence match and select Delete Match, or press [-] button from the bottom toolbar and choose Delete Match.

➡ **To delete all sequence matches:**
Right-click on any sequence match or the sequence title and select Delete All Matches, or press [-] button from the bottom toolbar and choose Delete All Matches.

➡ **To highlight sequence match in spectrum viewer:**
Click on the sequence match and spectrum moves to the m/z value. Small red arrow shows up on m/z axis of the spectrum.

➡ **To show or hide sequence match labels in spectrum viewer:**
Choose View → Notations → Show/Hide Notations from the main menu and click on the sequence root. You can specify the information to show using View → Notations menu.

➡ **To show isotopic pattern of sequence match:**
Right-click on the sequence match and select Show Isotopic Pattern. *Mass Calculator* panel shows up with the peptide formula and corresponding peak charge. See *Mass Calculator* chapter for more information.

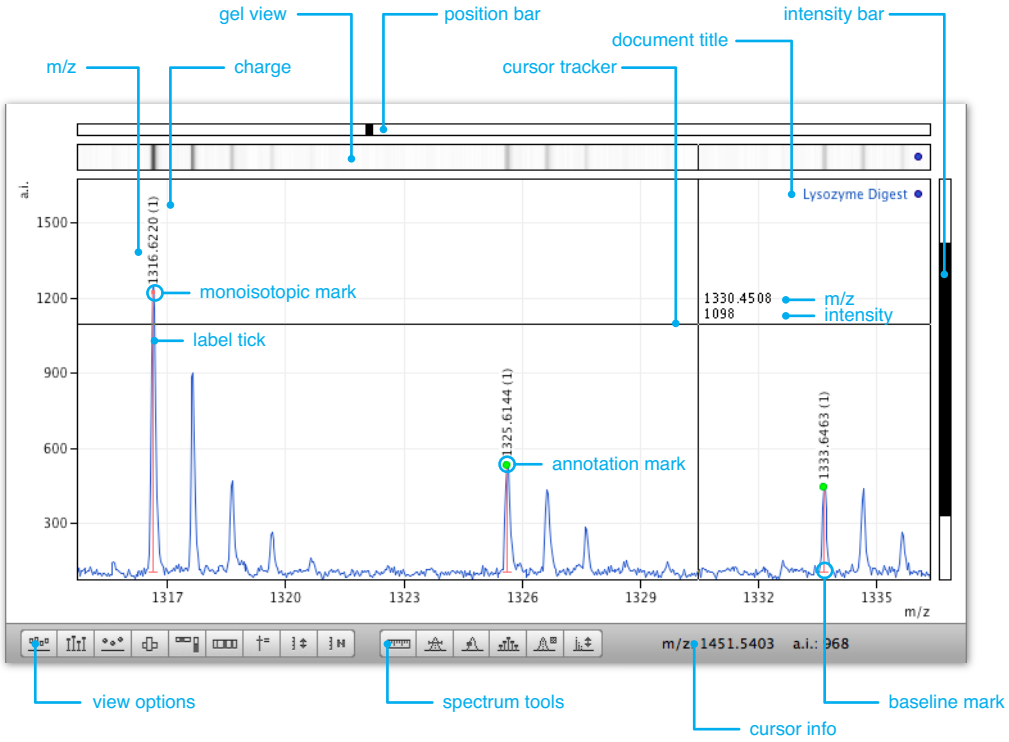
➡ **To send sequence match formula to Envelope Fit:**
Right-click on the annotation and select Send to Envelope Fit. *Envelope Fit* panel shows up with the peptide formula and corresponding peak charge. See *Envelope Fit* chapter for more information.

➡ **To re-calibrate data using sequence matches:**
Right-click on any single match or sequence title and select Calibrate by Matches. All the matches will be send to *Calibration* panel. See *Calibration* chapter for more information.

5.5. Spectrum Viewer

Since most time in data interpretation is spent manipulating spectra (e.i. moving, scaling, zooming etc.), Attention was paid to make these functions as easy and user friendly as possible. Everything is accessible by mouse, keyboard or combination of both. Position bars always indicate your current position, zoom and relative intensity within the context of shown data. Vertical flipping, spectrum offset and gel view can

be used to effectively compare your samples with a standard or blank spectrum. In addition, *Spectrum Viewer* offers some other useful features such as peak labeling tools and distance, charge or peak area measurements.



Spectrum Viewer panel.

Spectrum Manipulations

➡ **To zoom spectrum range:**
Select the m/z range with right or middle mouse button pressed.

➡ **To zoom continuously from cursor position:**
Move the mouse cursor to desired m/z position and scroll the mouse wheel while holding Alt or Ctrl key.

➡ **To zoom from center of current view:**
Use Left and Right arrows keys on your keyboard while holding Alt key.

➡ **To zoom from beginning of current view:**
Drag the m/z axis horizontally with right mouse button pressed.

➡ **To move spectrum horizontally:**
Scroll the mouse wheel or drag the m/z axis horizontally with left mouse button pressed. If you prefer using keyboard, use Left, Right, PageUp, PageDown, Home and End keys.

➡ **To scale intensity axis:**
Scroll the mouse wheel while holding Shift key or the cursor placed within the y-axis area or drag the intensity axis vertically with right mouse button pressed.

➡ **To scale intensity axis automatically to fit current spectrum range:**
Press button from the bottom toolbar, or choose View → Autoscale Intensity from the main menu.

➡ **To show full mass range:**
Double-click on the m/z axis.

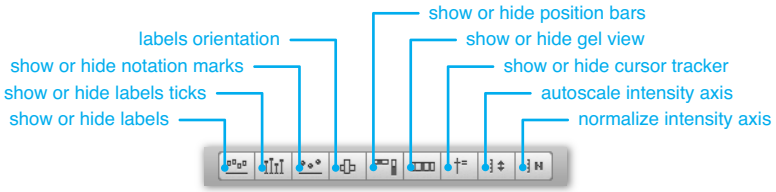
➡ **To show full intensity range:**
Double-click on the intensity axis.

➡ **To show full spectrum:**
Double-click anywhere in the spectrum.

➡ **To show specified mass range:**
Choose View → Set Mass Range from the main menu. Specify the range and press OK button.

View Options

There are different view options available for *Spectrum Viewer*. Those commonly used are available directly from the bottom toolbar, others can be accessed via the application main menu View.



Bottom toolbar with the most common Spectrum Viewer options.

➡ **To show or hide gridlines:**
Choose View → Spectrum Canvas → Show/Hide Gridlines from the main menu.

➡ **To show or hide legend:**
Choose View → Spectrum Canvas → Show/Hide Legend from the main menu.

➡ **To show or hide position and intensity bar:**
Press button from the bottom toolbar, or choose View → Spectrum Canvas → Show/Hide Position Bars from the main menu.

➡ **To show or hide gel view:**
Press button from the bottom toolbar, or choose View → Spectrum Canvas → Show/Hide Gel View from the main menu.

➡ **To show or hide gel view legend:**
Choose View → Spectrum Canvas → Show/Hide Gel View Legend from the main menu.

➡ **To show or hide cursor tracker:**
Press button from the bottom toolbar, or choose View → Spectrum Canvas → Show/Hide Cursor Tracker from the main menu.

➡ **To enable or disable data points:**
Choose View → Spectrum Canvas → Show/Hide Data Points from the main menu. Data points are typically visible on detailed zoom only.

➡ **To show or hide labels:**
Press button from the bottom toolbar, or choose View → Labels → Show/Hide Labels from the main menu.

➡ **To show or hide labels ticks:**
Press button from the bottom toolbar, or choose View → Labels → Show/Hide Label Ticks from the main menu.

➡ **To show or hide charge in labels:**
Choose View → Labels → Show/Hide Label Charge from the main menu.

➡ **To show or hide solid background for labels:**
Choose View → Labels → Show/Hide Label Background from the main menu.

➡ **To change labels orientation:**
Press button from the bottom toolbar, or choose View → Labels → Horizontal/Vertical Labels from the main menu.

➡ **To allow overlapping labels:**
Choose View → Labels → Allow Overlapping from the main menu.

➡ **To show labels in all documents:**
Choose View → Labels → Labels in All Documents from the main menu. Only labels from the selected document will be shown if this feature is disabled.

➡ **To show or hide notations:**
Press button from the bottom toolbar, or choose View → Notations → Show/Hide Notations from the main menu.

➡ **To show or hide notation marks:**
Choose View → Notations → Notation Marks from the main menu.

➡ **To show or hide notation labels:**
Choose View → Notations → Notation Labels from the main menu.

➡ **To show or hide notation m/z:**
Choose View → Notations → Notation M/Z from the main menu.

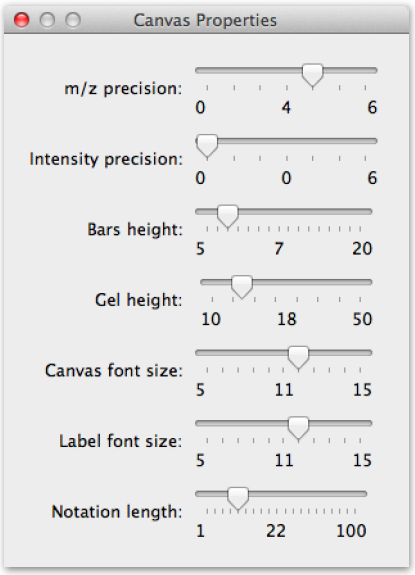
➡ **To enable intensity autoscale:**
Press button from the bottom toolbar, or choose View → Autoscale Intensity from the main menu.

➡ **To enable intensity normalization:**
Press button from the bottom toolbar, choose View → Normalize Intensity from the main menu.

➡ **To flip spectrum vertically:**
Right-click on the document title in *Documents Panel* and choose Flip Spectrum.

➡ **To offset spectrum intensity:**
Right-click on the document title in *Documents Panel* and choose Offset Spectrum, or choose View → Offset Spectrum from the main menu to show up offset dialog. Set *Intensity offset* and press Offset button. You can choose *Offset spectrum* tool from the bottom toolbar as well.

➡ **To clear spectrum offset:**
Right-click on the document title and choose Clear Offset. Choose View → Clear Offsets from the main menu to clear offset for all documents.



Canvas Properties dialog.

➡ **To set m/z precision (number of digits):**
Choose View → Canvas Properties from the main menu to show up *Canvas Properties* dialog and move the *m/z precision* slider. This settings is used for all occurrences of mass throughout the application.

➡ **To set intensity precision (number of digits):**
Choose View → Canvas Properties from the main menu to show up *Canvas Properties* dialog and move the *Intensity precision* slider. This settings is used for all occurrences of intensity throughout the application.

➡ **To set gel height:**
Choose View → Canvas Properties from the main menu to show up *Canvas Properties* dialog and move the *Gel height* slider.

➡ **To set position and intensity bars height:**
Choose View → Canvas Properties from the main menu to show up *Canvas Properties* dialog and move the *Bars height* slider.

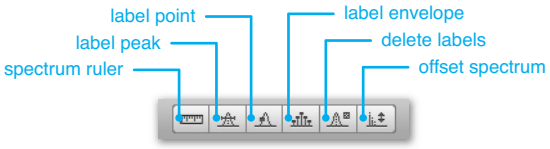
➡ **To set axis and legend font size:**
Choose View → Canvas Properties from the main menu to show up *Canvas Properties* dialog and move the *Canvas font size* slider.

➡ **To set label font size:**
Choose View → Canvas Properties from the main menu to show up *Canvas Properties* dialog and move the *Label font size* slider.

➡ **To set max length of notation label:**
Choose View → Canvas Properties from the main menu to show up *Canvas Properties* dialog and move the *Notation label size* slider. Longer notations will be cropped and with "..." tailing.

Spectrum Tools

There are several mouse tools available for *Spectrum Viewer* such as *Spectrum Ruler, Label Peak, Label Point, Label Envelope, Delete Labels* and *Offset Spectrum*. All the tools parameters are specified in *Baseline Correction, Peak Picking* and *Deisotoping* panels.



Bottom toolbar with Spectrum tools.

➡ **To measure distance in spectrum:**
Press button from the bottom toolbar and drag mouse in the spectrum with left mouse button pressed. Several values can be shown in the bottom toolbar according to the View menu settings.

➡ **To label peak:**
Press button from the bottom toolbar and select desired peak in the spectrum. Only the most intense peak in the selection will be labeled. See *Data Processing* chapter for more information.

➡ **To label point:**
Press button from the bottom toolbar and click at desired point in the spectrum. A cursor cross mark tracks the spectrum line until you release the mouse button. See *Data Processing* chapter for more information.

➡ **To label isotopes or envelope centroid:**
Press button from the bottom toolbar and position the mouse cursor at first isotope of a cluster. Scroll the mouse wheel while holding Shift button to set charge or Shift+Ctrl (Shift+Alt on Mac OS X) to set isotopes involved in calculations. Positions of involved isotopes are marked by black circles in *Spectrum Viewer*. Depending on the value specified in *Deisotoping* panel, 1st selected isotope, monoisotopic peak, all isotopes or envelope centroid is labeled after left mouse button click.

➡ **To delete labels:**
Press button from the bottom toolbar and select area where the labels should be deleted. Only those peaks/labels where its m/z and intensity values fall into the selected area will be deleted.

➡ **To automatically set labeled peak as monoisotopic:**
From the main menu choose Processing → Deisotoping and check the *Set labels as monoisotopes* checkbox.

➡ **To measure charge state and neutral mass in spectrum:**
Press button from the bottom toolbar and drag mouse in the spectrum with left mouse button pressed. Drag between two adjacent isotopes or drag between adjacent protein peaks. Corresponding charge state and neutral mass will be shown in the bottom toolbar if enabled in the View menu.

➡ **To measure peak area in spectrum:**
Press button from the bottom toolbar and drag mouse in the spectrum with left mouse button pressed to define area edges. Corresponding area is shown in the bottom toolbar if enabled in the View menu. Current baseline settings is used to determine bottom edge. Open *Baseline Correction* panel to adjust the baseline.

➡ **To enable or disable cursor info/spectrum ruler values:**
Check the values in the main menu View → Spectrum Ruler or right-click in the bottom toolbar and select items to show.

➡ **To offset spectrum intensity:**
Press button from the bottom toolbar and drag mouse in the spectrum with left mouse button pressed. Distance shows in the bottom toolbar and next to the cursor if *Cursor Tracker* is enabled. Current spectrum will be shifted after releasing the mouse button. Choose View → Offset Spectrum from the main menu to set spectrum offset manually.

See *Senko M. et al. 1995 JASMS* for more information about monoisotopic peak determination.

It is sometimes little bit tricky to use *Label Envelope* tool, especially for high-resolution data. Try to use wider zoom range to see whole envelope and set the charge state and number of isotopes involved. Then zoom to the first isotope and click onto its center. All the isotopes should be labeled correctly. If not, try to use *Isotope mass shift* correction in *Deisotoping* panel.

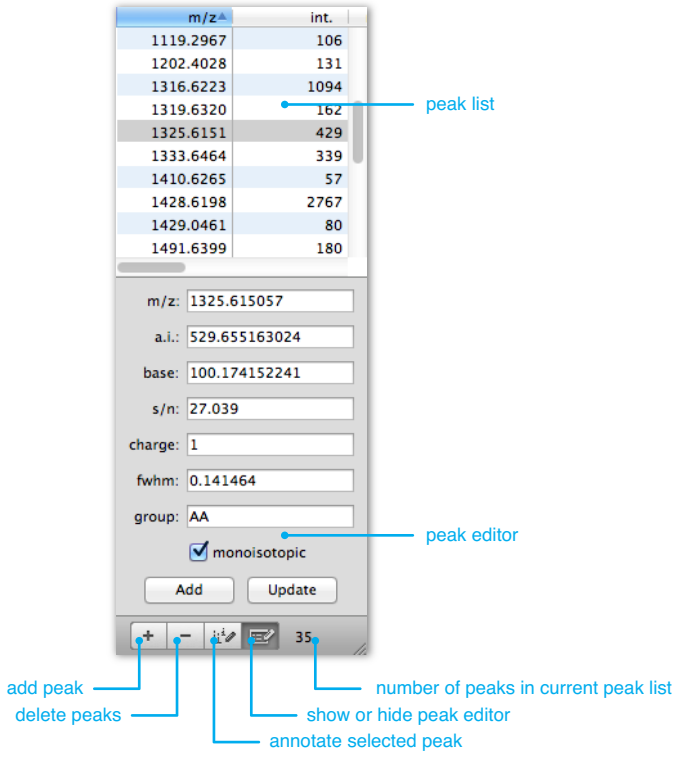
Please note that for Spectrum Ruler tool the calculated charge is shown as float number, however, its rounded value is used for calculation of corresponding neutral mass.

Please note that spectrum offset is just a visualization tool and no real data are changed.

Please note that spectrum offset cannot be applied when normalized view is turned on.

5.6. Peaklist Panel

Peaklist Panel provides a table of all labeled peaks in selected document. For each peak, various parameters can be shown, according to current settings. In addition, new peaks can be manually added, edited or removed or any user annotation can be specified.



Peaklist Panel with peak editor enabled.

- *m/z* - peak's m/z
- *a.i.* - peak's native intensity (no baseline correction)
- *base* - peak's baseline intensity
- *int.* - peak's corrected intensity (baseline subtracted)
- *r. int.* - peak's relative intensity (in % of the most intense peak)
- *s/n* - peak's signal-to-noise ratio
- *z* - peak's charge

- *mass* - peak's recalculated neutral mass
- *fwhm* - peak's full width at half maximum
- *resol.* - peak's resolution
- *group* - peak's group name

➡ **To set visible columns:**
Right-click on the peak list header and choose column to show / hide or choose View → Peak List Columns from the main menu.

➡ **To manually add new peak:**
Press button from the bottom toolbar to show and clear peak editor, fill-up the peak parameters and press Add button.

➡ **To edit peak parameters:**
Press button from the bottom toolbar to show peak editor and click on the peak. Edit peak parameters and press Update button.

➡ **To duplicate peak with different parameters:**
Press button from the bottom toolbar to show peak editor and click on the peak. Change peak parameters and press Add button.

➡ **To delete peaks:**
Select the peaks you want to delete and press Command+Backspace or Delete, or press button from the bottom toolbar and choose Delete Selected. You can use *Delete Labels* tool in *Spectrum Viewer* as well.

➡ **To delete peaks by threshold:**
Press button from the bottom toolbar and choose Delete by Threshold to show up threshold dialog. Set *Minimal value* and *Threshold type* and press Delete button. Another way is to sort peak list by specific value, selecting and deleting unwanted peaks.

➡ **To delete all peaks:**
Press button from the bottom toolbar and choose Delete All.

➡ **To annotate peak:**
Double-click the peak or press button in the bottom toolbar to show up annotation dialog and type your annotation.

➡ **To highlight peak in spectrum viewer:**
Click on the peak and spectrum moves to the m/z value. Small red arrow shows up on m/z axis of the spectrum. Current zoom range remains the same.

➡ **To copy selected peaks into clipboard:**
Select the peaks you'd like to copy and press Command+C (Ctrl+C on MS Windows and Linux).

➡ **To export peak list:**
Press button from the main toolbar, or choose File → Export from the main menu to show up *Export* dialog, and select *Export Peak List* tool. See *Export* chapter for more information.

6. Document Basics

6.1. Supported Formats

mMass supports several mass spectrometry formats. Popular XML-based formats like *mzData* (http://www.psidev.info), *mzXML* (http://tools.proteomecenter.org) and *mzML* (http://www.psidev.info), which are open source and well documented, are fully supported. The *MGF (Mascot Generic Format)* data format is supported in its standard form. Mass spectra and peak list data can also be imported from an *ASCII* file consisting of *m/z* and *intensity* columns separated by a *tab*, *space*, *comma* or *semicolon*.

Most of the native vendors' software also support copying *m/z* and *intensity* values directly into system clipboard from which they can be easily pasted into mMass as a new document.

Since it is often impossible to obtain the manufacturer's description of their native file formats, they are not currently supported. However, if you have a *Bruker's CompassXport* tool installed on your computer it is automatically used to convert and open raw data from all *Bruker's* instruments. This tool is available for free at www.bdal.de, unfortunately, for MS Windows platform only. In *mMass's* preferences you can specify whether *mzData*, *mzXML* or *mzML* format will be used for conversion and whether profile spectrum or peak list only will be extracted.

mMass is so-called a *"single spectrum editor"* therefore experiments such as LC-MS runs cannot be fully analyzed by *mMass*. However, it is possible to open any selected scan from such runs to analyze it separately. Sure, it does not make sense for lots of LC-MS experiments but it can be useful in many cases.

6.2. Open Document

➡ **To open document:**
Drag the document file into *mMass's* main window or choose File → Open from the main menu.

➡ **To open selected scan from LC-MS run:**
Drag the document file into *mMass's* main window or choose File → Open from the main menu. If document contains more scans, *Select Scan* dialog shows up. Select scan(s) you'd like to open and press Open button. *Total ion chromatogram* and *base peak chromatogram* is available if corresponding parameters are found in the data.

➡ **To open spectrum from Bruker's instruments:**
Drag the spectrum folder into *mMass's* main window or choose File → Open from the main menu and locate the *analysis.baf*, *analysis.yep* or *fid* file. You need to have *CompassXport* tool installed. This function is currently available for MS Windows platform only.

➡ **To import spectrum from clipboard:**
Be sure to copy only *m/z* and *intensity* columns into clipboard. Than choose File → New from Clipboard from the main menu. If *mMass* detects some problem while reading your data a small text dialog appears to let you correct the errors. Typically, this happens when you copy your data with column headers.

For *ASCII XY* and some *mzXML* documents *mMass* is unable to determine whether the document contains spectrum points or just centroides of peaks and chooses spectrum as default. If this decision isn't correct you can swap the data between line spectrum and peak list by choosing Processing → Swap Data from the main menu.

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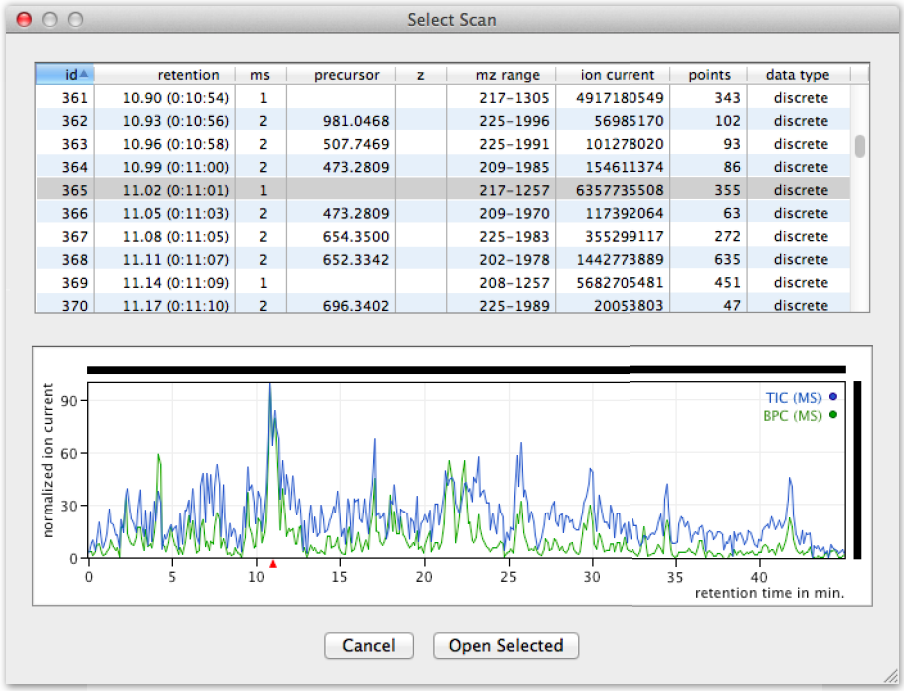
mMass allows for file association using server-client approach listening on port 65456. If you encounter any conflict using this particular port, change the "serverPort" value in config.xml file. On Mac OS X, all configuration files can be found under "~/Library/Application Support/mMass/". On MS Windows and Linux systems, all configuration files can be found under "configs" folder,

located directly under the mMass's main folder. This feature is not necessary on Mac OS X and the server is disabled by default, however, file association still works correctly.

⚠ *Please note that CompassXport tool does not allow non-ascii characters in a document path.*



Select Scan dialog.

6.3. Blank Document

One of the unique features of *mMass* is the ability to make a blank document and manually create peak list. This feature is particularly useful in those cases where raw spectrum is unavailable and user only has a spectrum image or printed list of labelled peaks. Such is often the situation in laboratories that do not have their own instruments and have to send their samples to other laboratories. This feature gives such laboratories the chance to analyze MS data themselves. Once the peak list is prepared, all interpretation tools can be used. (See *Peaklist Panel* chapter for more information about peak list editing.) Blank documents can also be used for protein digest or peptide fragmentation simulations or to make a personal database of sequences which can be easily imported into any document.

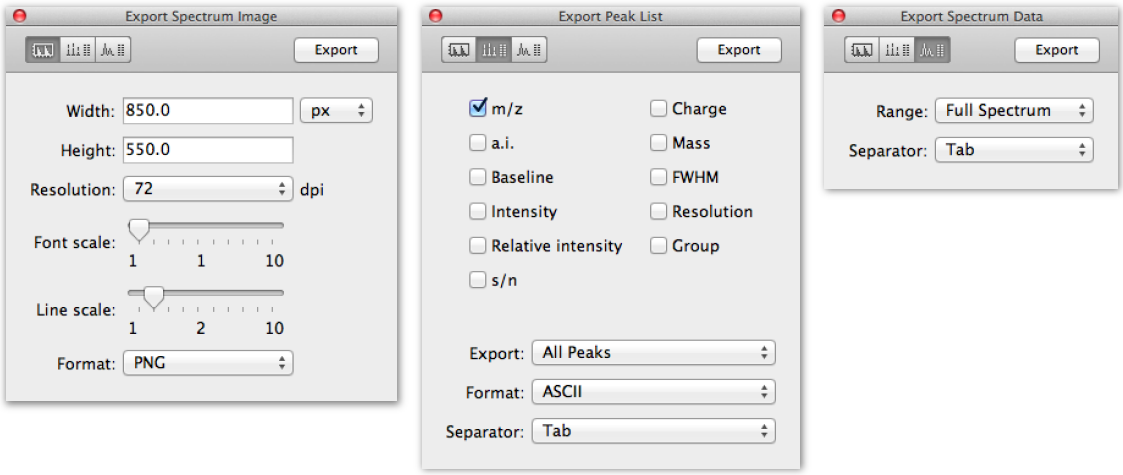
➡ **To open blank document:**
Press + button from the bottom toolbar and select New Document or choose File → New from the main menu.

6.4. Save Document

To retain all the information related to analysis such as protein sequence or user's annotations *mMass* has its own document format to store the data (*mSD* format). This format is XML-based to be easily processed by other software.

➡ **To save document:**
Select the document in *Documents Panel* and choose File → Save from the main menu.

6.5. Export



Export tools.

➡ **To export spectrum image:**
Press button from the main toolbar or choose File → Export from the main menu to show up *Export* dialog, and select *Export Spectrum Image* tool. Specify export parameters and press Export button.

➡ **To export spectrum points:**
Press button from the main toolbar or choose File → Export from the main menu to show up *Export* dialog, and select *Export Spectrum Data* tool. Specify export parameters and press Export button.

➡ **To export peak list:**
Press button from the main toolbar or choose File → Export from the main menu to show up *Export* dialog and select *Export Peak List* tool. Specify export parameters and press Export button.

➡ **To export spectrum image to vector format:**
On Mac OS X and Linux platform you can use native support for PDF creation to export spectrum into vector format. From the main menu choose File → Print and select PDF. On MS Windows you need to have some PDF convertor installed first. To be able to specify the image size you have to define appropriate paper.

To export spectrum images for screen presentation only, use 72 dpi. To export images for printing or publications use higher resolution and set the font and line scaling according to image size.

6.6. Print Spectrum

➡ **To print spectrum:**
Choose File → Print Spectrum from the main menu and current *Spectrum Viewer* contents will be printed.

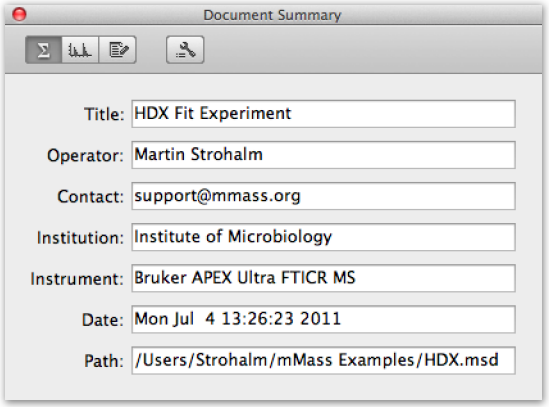
Please note that whole spectrum canvas is redrawn internally to fill paper size. While *m/z* range is retained, *intensity* range can be slightly different if *Autoscale Intensity* option is enabled. To be sure the intensity range is the same as in the *Spectrum Viewer* just disable *Autoscale Intensity* option when printing.

6.7. Document Info

Document Info panel provides some information about selected document and place to write your own description of the sample, analysis and results. It is generally good idea to fill up this information if you want to share the analysis with other people.

Document Summary

In the *Document Summary* part of *Document Info* panel you can specify document *Title*, *Operator's* name, *Contact* and *Institution*, used *Instrument* and measurement *Date*. You can save current values as a preset to use it later again.



Document Summary.

➡ **To show document summary:**
Press button from the main toolbar or choose File → Document Info from the main menu to show up *Document Info* panel. Then press Σ button from the panel toolbar.

➡ **To save current values as presets:**
Write the information you'd like to save as presets. Press button from the panel toolbar and choose Save as Presets. In the dialog write presets name and press Save button.

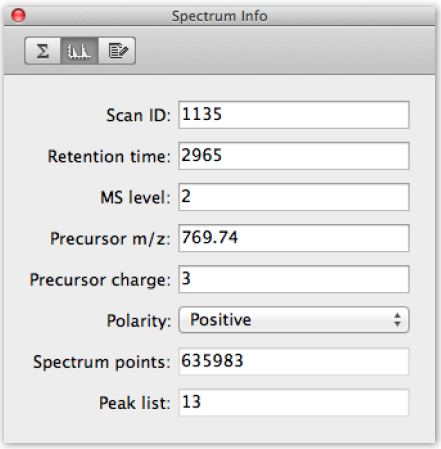
➡ **To use saved presets:**
Press button from the panel toolbar and choose any of your presets from the pup-up menu.

➡ **To delete saved presets:**
Choose Libraries → Presets from the main menu to show up *Presets Library* editor. Choose the presets you'd like to delete and press Delete button.

➡ **To rename saved presets:**
Choose Libraries → Presets from the main menu to show up *Presets Library* editor. Choose the presets you'd like to rename, type the new name and press Rename button.

Spectrum Info

In the *Spectrum Info* part of *Document Info* panel you can specify *Scan ID, Retention time, MS level Precursor m/z, Precursor charge* and *Polarity*. In addition, number of spectrum points and total number of peaks in peak list are shown.

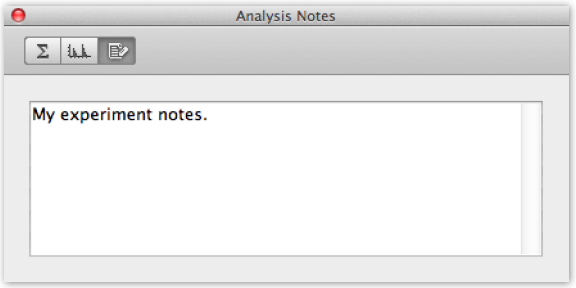


Spectrum Info.

➡ **To show spectrum info:**
Press button from the main toolbar or choose File → Document Info from the main menu to show up *Document Info* panel. Then press button from the panel toolbar.

Analysis Notes

Analysis Notes provides a place to type you personal notes about sample preparation, sample analysis and interpretation results. These information will be also shown in analysis report. See *Analysis Report* chapter for more information.

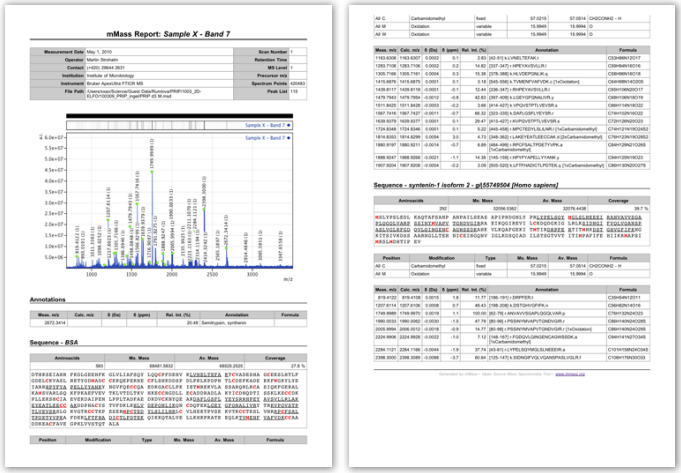


Analysis Notes.

➡ **To show analysis notes:**
Press button from the main toolbar or choose File → Document Info from the main menu to show up *Document Info* panel. Then press button from the panel toolbar.

6.8. Analysis Report

Analysis report can be generated from selected document and all available information such as document info, current *Spectrum Viewer* content, user's notes and annotations, sequence, modifications and matches will be included. This report is generated in *HTML* format and automatically shows up in your web browser.



Analysis report.

➡ **To make analysis report:**

Press $ button from the main toolbar or choose File → Analysis Report from the main menu. Report shows up in your web browser.

➡ **To sort data in report tables:**

Click on appropriate column header in the report. Please note that this feature needs to have *JavaScript* enabled in your web browser.

It is generally good practice to write your analysis notes and results into document using *Document Info* tool prior to report generation. See *Document Info* chapter for more information.

On Mac OS X and Linux platform you can use native support for PDF creation to make PDF report. When the report shows up in your web browser choose File → Print and select PDF. On MS Windows you need to have some PDF convertor installed first.

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7. Data Processing

mMass offers number of common data processing functions such as mathematical operations, cropping, baseline correction, smoothing, peak picking, charging, deisotoping and deconvolution. In order to speed up data processing from different instruments you can define your own processing presets and use it frequently. In addition, you can process multiple documents at once using *Batch Processing* tool.

➡ **To open data processing tool:**
Press button from the main toolbar and select the tool or choose specific tool from Processing menu.

➡ **To undo last processing step:**
From the main menu choose Processing → Undo.

➡ **To save current values as presets:**
Press button from the panel toolbar and choose Save as Presets. In the dialog type the presets name and press Save button.

➡ **To use saved presets:**
Press button from the panel toolbar and choose any of your presets from the pup-up menu.

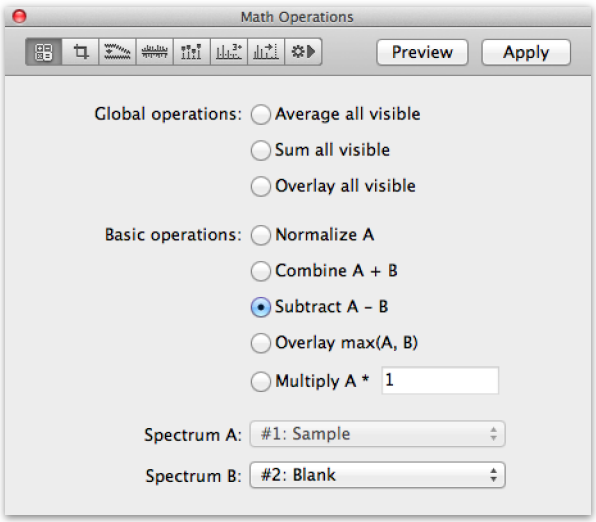
➡ **To delete saved presets:**
Choose Libraries → Presets from the main menu to show up *Presets Library* editor. Choose the presets you'd like to delete and press Delete button.

➡ **To rename saved presets:**
Choose Libraries → Presets from the main menu to show up *Presets Library* editor. Choose the presets you'd like to rename, type the new name and press Rename button.

⚠ *Please note that presets contain parameters from all the processing tools. If you load any presets, parameters for all the tools will be changed.*

7.1. Math Operations

Simple math operations are available to normalize, combine, subtract overlay or multiply documents. Most of the operations can be applied to spectrum points only while a peak list is removed.



Math Operations tool.

- *Global operation* - math operation to be applied to all visible documents. New document is generated a result.
- *Basic operation* - math operation to be applied to currently selected document.
- *Spectrum A* - document currently selected.
- *Spectrum B* - spectrum to be added or subtracted from current document.

➡ **To open math operation tool:**
Press button from the main toolbar, then press button from the panel toolbar or choose Processing → Math Operations from the main menu.

➡ **To average all document:**
Show/enable all documents to be averaged in the *Documents Panel*. In the *Math Operations* tool select *"Average all visible"* and press Apply button to generate averaged spectrum.

➡ **To sum all document:**
Show/enable all documents to be summed in the *Documents Panel*. In the *Math Operations* tool select *"Sum all visible"* and press Apply button to generate summed spectrum.

➡ **To overlay all document:**
Show/enable all documents to be overlaid in the *Documents Panel*. In the *Math Operations* tool select *"Overlay all visible"* and press Apply button to generate overlaid spectrum.

➡ **To normalize selected document:**
Select a document in the *Documents Panel*. In the *Math Operations* tool select *"Normalize"* and press Apply button to apply operation on selected document.

➡ **To add spectrum to selected document:**
Select a document in the *Documents Panel*. In the *Math Operations* tool select *"Combine A + B"* and the document to be added (*Spectrum B*). Press Apply button to apply operation on selected document.

➡ **To subtract spectrum from selected document:**
Select a document in the *Documents Panel*. In the *Math Operations* tool select *"Subtract A - B"* and the document to be subtracted (*Spectrum B*). Press Apply button to apply operation on selected document.

➡ **To overlay spectrum with selected document:**
Select a document in the *Documents Panel*. In the *Math Operations* tool select *"Overlay max(A, B)"* and the document to be overlaid (*Spectrum B*). Press Apply button to apply operation on selected document.

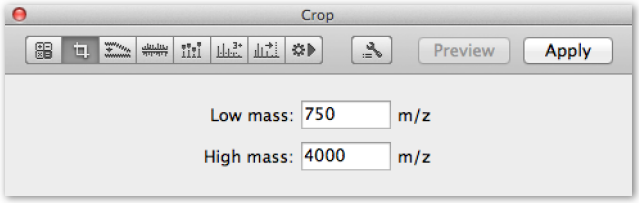
➡ **To multiply selected document:**
Select a document in the *Documents Panel*. In the *Math Operations* tool select *"Multiply A *"* and specify multiplier value. Press Apply button to apply operation on selected document.

⚠ *To be able to sum-up or subtract two spectra, both must have the same x-raster. Unfortunately, this is not often the case, therefore additional points needs to be added and resulted spectrum can easily became very huge and further processing very time-consuming! Since x-raster is mostly changed by different calibrations, it is generally good idea to apply this processing prior to any re-calibration.*

⚠ *Please note that all of the user's annotations and sequence matches will be removed after applying math operation. It is generally good idea to apply processing functions prior to any data interpretation.*

7.2. Crop

This function simply discards all the spectrum data points, labeled peaks, annotations and sequence matches which are out of the m/z range specified by *Low mass* and *High mass* parameters.



Crop tool.

- *Low mass* - data below this limit will be removed.
- *High mass* - data above this limit will be removed.

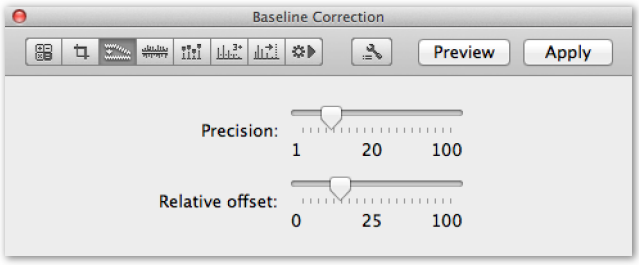
➡ **To open crop tool:**
Press button from the main toolbar, then press button from the panel toolbar or choose Processing → Crop from the main menu.

➡ **To crop data:**
Using *Low mass* and *High mass* values specify the m/z range to keep and press Apply button.

Using *Crop* tool to remove "matrix area" from MALDI-TOF mass spectra can significantly reduce number of spectrum data points and speed up further processing.

7.3. Baseline Correction

Especially for MALDI-TOF mass spectra of proteins a strong baseline distortion is common problem which needs to be corrected quite often. Using *Baseline Correction* tool, this processing step can be done very easily. Baseline is calculated from the spectrum noise as a median of all data points. Using *Precision* slider, baseline can be set to trace a spectrum shape precisely. In addition, you can specify *Relative offset* to move calculated baseline down a bit. You can see calculated baseline while changing the parameters and when ready a preview can be generated. Specified baseline settings is also used for automatic *Peak Picking* tool to calculate baseline for each labeled peak.



Baseline Correction tool.

- *Precision* - baseline shape precision (1 = straight line).
- *Relative offset* - baseline shift.

➡ **To open baseline correction tool:**
Press button from the main toolbar, then press button from the panel toolbar or choose Processing → Smooth Spectrum from the main menu.

➡ **To preview corrected data:**
Specify all the parameters and press Preview button. A temporary (red) corrected spectrum appears in *Spectrum Viewer*.

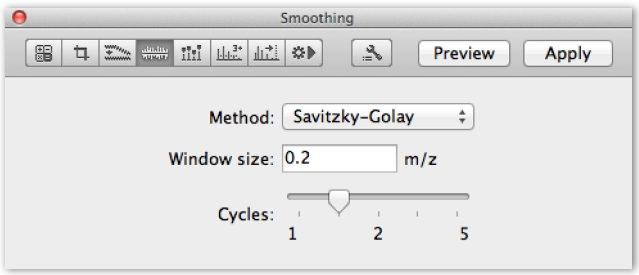
➡ **To subtract baseline from data:**
Specify all the parameters and press Apply button.

Please note that calculated baseline is shows in *Spectrum Viewer* while moving *Precision* or *Relative offset* slider. This feature can be very helpful to set baseline precisely.

Please note that current peak list and all the user's annotations and sequence matches will be removed after applying baseline correction. It is generally good idea to apply processing functions prior to any data interpretation.

7.4. Smoothing

You can use this function to smooth the noise which distorts peak shape. There are three different smoothing *Methods* available - *Moving Average*, *Gaussian* and *Savitzky-Golay*. In general, *Moving Average* and *Gaussian* are much faster but causes significant intensity loss for sharp peaks. These methods should be preferentially used to smooth high-mass spectra where peaks are broader. On the other hand, *Savitzky-Golay* filter is very slow but intensity loss is much lower. This method should be preferentially used to smooth low-mass spectra where peaks are sharp. After setting all the parameters you can see a preview before processing your data.



Smoothing tool.

- *Method* - smoothing algorithm.
- *Window size* - smoothing window.
- *Cycles* - number of smoothing repetitions.

➡ **To open smooth tool:**
Press button from the main toolbar, then press button from the panel toolbar or choose Processing → Smooth Spectrum from the main menu.

➡ **To preview smoothed data:**
Specify all the parameters and press Preview button. A temporary (red) smoothed spectrum appears in *Spectrum Viewer*.

➡ **To smooth data:**
Specify all the parameters and press Apply button.

Always check the intensity loss for different peaks along the entire mass range. Use smaller *Window size* if the intensity loss is too strong.

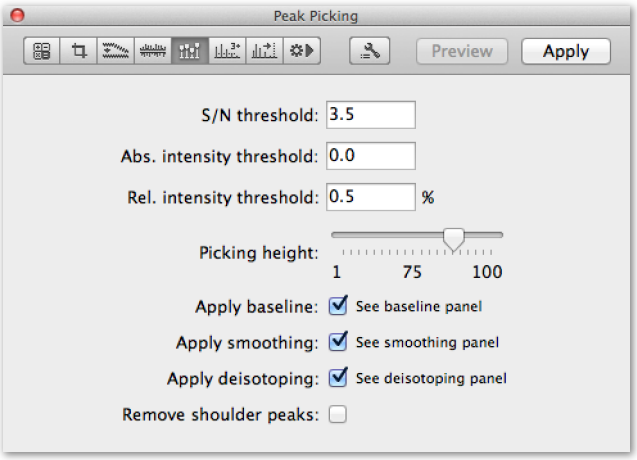
⚠ *Please note that at least 4 data points within the smoothing window are needed for smoothing. No smoothing is applied if Window size is too narrow.*

⚠ *Please note that current peak list and all the user's annotations and sequence matches will be removed after applying smoothing. It is generally good idea to apply processing functions prior to any data interpretation.*

7.5. Peak Picking

As mentioned earlier in *Spectrum Viewer* chapter, there are specific tools to label peaks manually. However, this could be very annoying especially for data-rich spectra like those from FTICR MS or Orbitrap. Fortunately, an algorithm is incorporated into *mMass* to facilitate automatic peak picking. There are couple of basic steps involved in the peak picking algorithm:

- *Baseline correction* - if enabled, baseline and noise widths are calculated and used for each peak.
- *Smoothing* - if enabled, raw spectrum is smoothed temporarily to eliminate peak noise.
- *Searching for local maxima* - local maxima are temporarily labeled as potential peaks.
- *Filtering by threshold* - peaks bellow user intensity or S/N threshold are removed.
- *Centroides calculation* - peak centroides are calculated and unresolved peaks are grouped together.
- *Filtering by threshold* - peaks bellow user intensity or S/N threshold are removed.
- *Removing shoulder peaks* - if enabled, "shoulder peaks" for FTMS data are removed.
- *Deisotoping* - if enabled, deisotoping is applied to remove isotopes and calculate peak charges.



Peak Picking tool.

- *S/N threshold* - peaks bellow the signal to noise threshold will not be labeled.
- *Abs. intensity threshold* - peaks bellow the absolute intensity (peak intensity - baseline) threshold will not be labeled.
- *Rel. intensity threshold* - peaks bellow the relative intensity threshold will not be labeled.
- *Picking height* - picking height for centroides.
- *Apply baseline* - for each peak its baseline and noise width is calculated according to current baseline settings. See *Baseline Correction* chapter for more information.
- *Apply smoothing* - data will be smoothed prior to peak picking according to current smoothing settings. See *Smoothing* chapter for more information.
- *Apply deisotoping* - peak isotopes will be removed and charge calculated according to current deisotoping settings. See *Deisotoping* chapter for more information.

- *Remove shoulder peaks* - small surrounding peaks called "shoulder peaks" occurring in FTMS data will be removed.

➡ **To open peak picking tool:**
Press button from the main toolbar, then press button from the panel toolbar or choose Processing → Peak Picking from the main menu.

➡ **To label peaks:**
Specify all the parameters and press Apply button. Follow the threshold line showed in *Spectrum Viewer* while changing the parameters.

Set current baseline in *Baseline Correction* panel before peak picking is applied.

Use smoothing for MALDI-TOF data of any kind.

If smoothing is enabled, don't forget to check intensity loss for different peaks along the spectrum.

Smoothing significantly decreases number of local maxima, therefore speeds up peak picking.

If baseline, smoothing or deisotoping is enabled, don't forget to check corresponding panels.

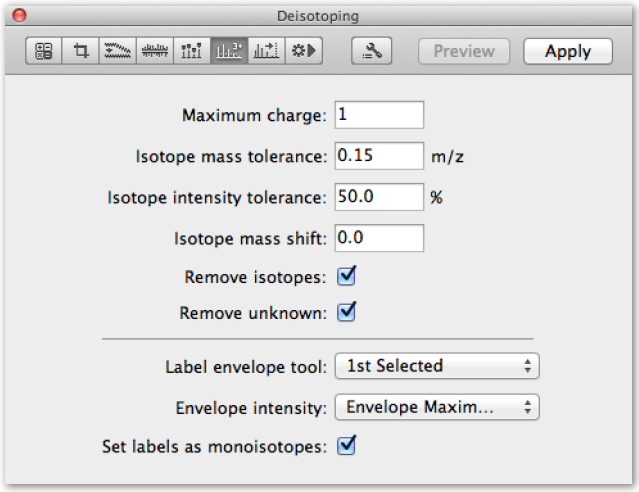
Please note that baseline, peak picking and deisotoping parameters are used for manual peak picking tools as well.

Please note that all the user's annotations and sequence matches will be removed after applying peak picking. It is generally good idea to apply processing functions prior to any data interpretation.

7.6. Deisotoping

The main purpose of this tool is to remove unwanted peak isotopes after peak picking. However, in order to find the isotopes a peak charge needs to be calculated, therefore this tool can also be used for charge determination.

Starting from specified *Maximum charge*, for every peak its isotopes are searched using corresponding isotopic mass difference *(1.00287/abs(z))* ± *Isotope mass tolerance*. If at least one isotope is found, parent peak is set as the monoisotopic with current charge state. If no isotope is found, current charge state is decreased *(abs(z) - 1)* and search cycle starts again for the same peak. Because of possible peak overlaps, theoretical isotopic pattern needs to be taken into account. Intensity of every isotope is compared with its theoretical value. If the intensity is matching theoretical value ± *Isotope intensity tolerance*, corresponding isotope is discarded from any subsequent search cycle. If the difference is over tolerance, corresponding isotope will be used as possible parent (monoisotopic) peak in subsequent search cycle. Since *mMass* is mostly used for proteomic data interpretation, *the averagine (C4.9384 N1.3577 O1.4773 S0.0417 H7.7583)* is used to approximate theoretical isotopic patterns (Senko *et al.* 1995 AJMS).



Deisotoping tool.

- *Maximum charge* - maximum charge state to be searched.
- *Isotope mass tolerance* - tolerance for mass difference between adjacent isotopes.
- *Isotope intensity tolerance* - tolerance for intensity check between theoretical and measured isotope.
- *Isotope mass shift* - mass correction to the default isotope distance used (1.00287).
- *Remove isotopes* - all identified isotopes will be removed after processing.
- *Remove unknown* - all unknown peaks will be removed after processing.
- *Label envelope tool* - function applied to *Label Envelope* tool.
- *Envelope intensity* - method used to calculate final intensity of the envelope.
- *Set labels as monoisotopes* - manually labeled peaks are automatically set as monoisotopic.

➡ **To open deisotoping tool:**
Press button from the main toolbar, then press button from the panel toolbar or choose Processing → Deisotoping from the main menu.

➡ **To deisotope peak list:**
Specify all the parameters and press Apply button.

➡ **To apply deisotoping automatically after peak picking:**
In *Peak Picking* panel enable *Apply deisotoping*.

➡ **To edit peak charge:**
Press in the *Peaklist Panel* bottom toolbar to show peak editor and click on the peak in the list. Set the new charge and press Replace button.

➡ **To manually set peak as monoisotopic:**
Press in the *Peaklist Panel* bottom toolbar to show peak editor and click on the peak in the list. Check *monoisotopic* checkbox and press Replace button.

Use the *Isotope mass shift* to correct deuterium effect in H/D exchange experiments.

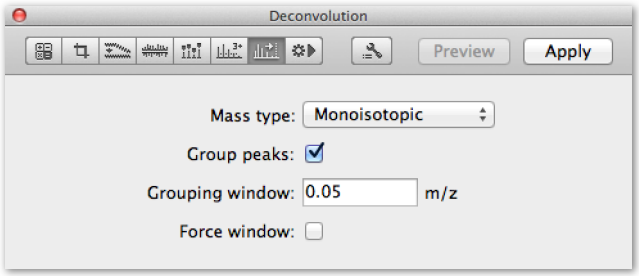
Please note the relationship between Maximum charge and Isotope mass tolerance. Isotope mass tolerance must be lower than $1.00287 \times (1/(z-1) - 1/z)$ to successfully distinguish z and z-1 charge.

Please note that the combination of averagine intensity distribution and higher Isotope intensity tolerance is generally applicable to wide range of organic compounds but can be very problematic if atoms like Cl, Fe, Hg, Pt etc. are incorporated.

⚠ *Please note that all the user's annotations and sequence matches will be removed after applying deisotoping. It is generally good idea to apply processing functions prior to any data interpretation.*

7.7. Deconvolution

ESI mass spectra typically contain same species in the form of different charge states, which can complicate data interpretation. Therefore a simple process called *deconvolution* is often used to recalculate all the multiply-charged species into singly-charged form. In addition, related peaks, originated from the same species but with different charge state, can be grouped together using either known peak's FWHM value or default m/z window. By applying *Deconvolution*, copy of current document is made with deconvoluted peak list.



Deconvolution tool.

- *Mass type* - mass type to be used for charge re-calculation.
- *Grouping window* - default window to group peaks with unknown FWHM value.
- *Group peaks* - apply grouping to neighboring peaks.
- *Force grouping window* - use default window even if FWHM value is known.

➡ **To open deconvolution tool:**
Press button from the main toolbar, then press button from the panel toolbar or choose Processing → Deconvolution from the main menu.

➡ **To deconvolute data:**
Specify all the parameters and press Apply button. New document with deconvoluted peak list will be generated.

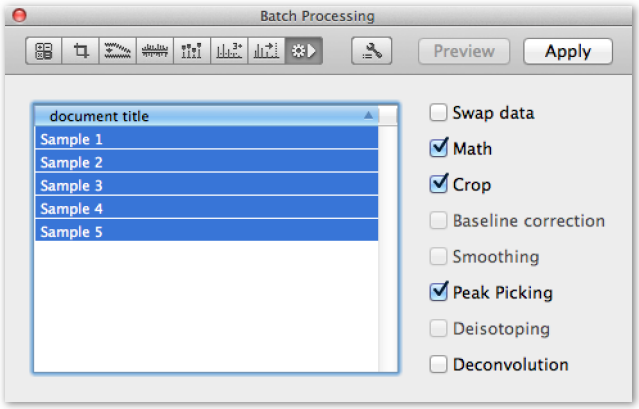
Use *Spectrum Generator* tool to make artificial mass spectrum from deconvoluted peak list.

Use *Label Envelope* tool to manually label peak clusters which are hard to process automatically.

⚠ *Please note that peaks' charge state must be set prior to deconvolution. Use either automatic peak picking followed by deisotoping or manually set charge in Peak Editor.*

7.8. Batch Processing

In order to save time, you can apply same processing steps to multiple documents at once using *Batch Processing* tool.



Batch Processing tool.

➡ **To open batch processing tool:**
Press button from the main toolbar, then press button from the panel toolbar or choose Processing → Batch Processing from the main menu.

➡ **To apply processing to multiple documents:**
Select documents to be processed, check all the processing steps and press Apply button.

To prevent double baseline correction, double smoothing or double deisotoping, these options are disabled if corresponding processing step is checked in *Peak Picking* panel. However, please note that for *Peak Picking* tool, baseline correction and smoothing is applied only temporarily.

7.9. Utilities

Swap Data

For *ASCII XY* and some *mzXML* documents *mMass* is unable to determine whether document contains line spectrum points or just centroides of peaks and chooses line spectrum as default. If this decision isn't correct you can swap the data between line spectrum and peak list.

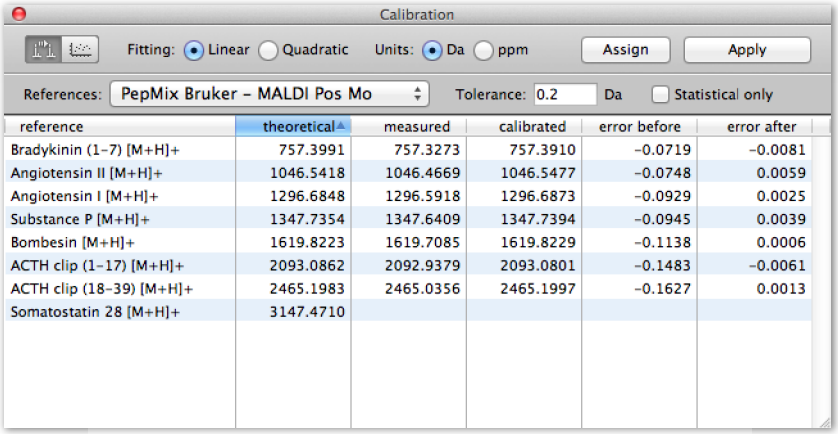
➡ **To swap data between spectrum and peak list:**
Choose Processing → Swap Data from the main menu.

8. Calibration

In order to re-calibrate you data *mMass* enables two principal approaches - standard calibration and statistical calibration. In the case of standard calibration any reference list must be selected first and reference values must be assigned to measured peaks. Reference values are assigned automatically using *Tolerance* value. You can use either *Linear* or *Quadratic* fitting to calculate calibration constants. In general, linear fitting is much safer if you want to re-calibrate an m/z range outside of your assigned references. Always see a calibration plot before recalculating your data.

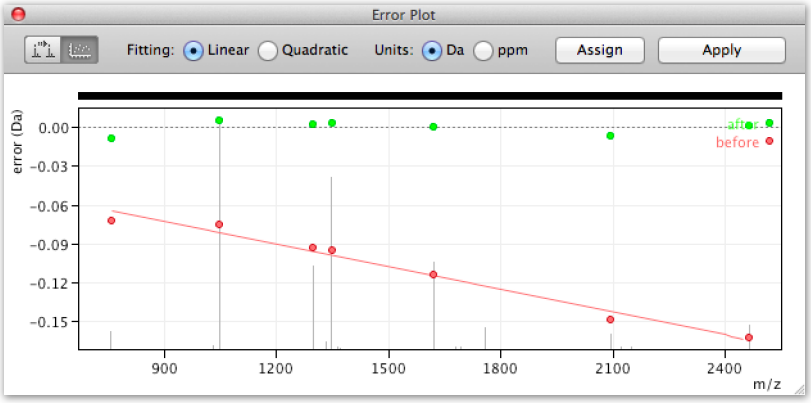
Statistical calibration, sometimes called "self-calibration" is a special method for peptide mass spectra only. It does not need any external reference points since it is based on the fact that monoisotopic masses of peptides are distributed in a very regular way. Decimal fraction of the peptide mass can be calculated with sufficient accuracy just by multiplying their integral part by factor *1.00048*. For this method, every peak above 700 m/z limit is recalculated and used as reference mass.

It is sometimes good to use other known peaks as internal calibration standards. Once you have the theoretical masses defined within your annotations or sequence matches, you can use them to re-calibrate your data.



reference	theoretical	measured	calibrated	error before	error after
Bradykinin (1–7) [M+H]+	757.3991	757.3273	757.3910	–0.0719	–0.0081
Angiotensin II [M+H]+	1046.5418	1046.4669	1046.5477	–0.0748	0.0059
Angiotensin I [M+H]+	1296.6848	1296.5918	1296.6873	–0.0929	0.0025
Substance P [M+H]+	1347.7354	1347.6409	1347.7394	–0.0945	0.0039
Bombesin [M+H]+	1619.8223	1619.7085	1619.8229	–0.1138	0.0006
ACTH clip (1–17) [M+H]+	2093.0862	2092.9379	2093.0801	–0.1483	–0.0061
ACTH clip (18–39) [M+H]+	2465.1983	2465.0356	2465.1997	–0.1627	0.0013
Somatostatin 28 [M+H]+	3147.4710				

Calibration tool.



Calibration Error Plot.

➡ **To show calibration tool:**
Press button from the main toolbar or choose Processing → Calibration from the main menu.

➡ **To show error plot:**
Press button from the panel toolbar.

➡ **To assign reference masses:**
Specify *Tolerance* value and *Units* and press Assign button.

➡ **To exclude assigned reference from calculation:**
Double-click on the reference in the list. Corresponding line turns grey and italic.

➡ **To highlight reference mass in spectrum viewer:**
Click on the reference in the list and spectrum moves to theoretical m/z value. Small red arrow shows up on m/z axis of the spectrum. Current zoom range remains the same.

➡ **To use statistical calibration:**
Check *Statistical only* checkbox. Reference masses and calibration constants are automatically calculated from the current peak list.

➡ **To define new reference list:**
Choose Libraries → Reference Masses from the main menu to show up *Reference Masses Library* editor. See *Libraries* chapter for more information.

➡ **To re-calibrate data by user annotations:**
Right-click on any single annotation or the annotations root in *Documents Panel* and select Calibrate by Annotations. All the user annotations will be send to *Calibration* panel. Please note that only those annotations with specified theoretical m/z value can be used.

➡ **To re-calibrate data by sequence matches:**
Right-click on any single match or sequence title in *Documents Panel* and select Calibrate by Matches. All the matches will be send to *Calibration* panel.

➡ **To apply same calibration to multiple documents:**
Once the calibration is calculated you can apply it to multiple documents. Simply select another document in *Documents Panel* and press Apply Recent button.

Always check *Error plot* to see whether the calibration is correct.

In general, use *Linear* fitting if assigned references do not cover entire m/z range you'd like to calibrate.

Single-point calibration can be used for linear fitting.

User annotations and sequence matches are re-calibrated as well.

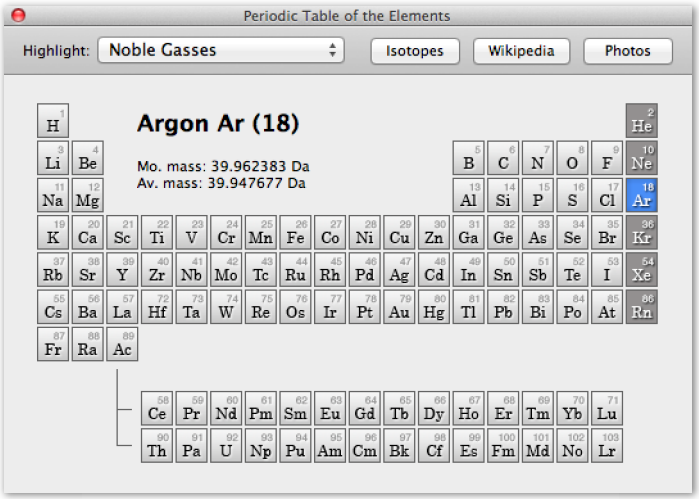
Please note that at least 3 reference mass points are needed for quadratic fitting.

Please note that the calibration is calculated using absolute m/z errors. Therefore shown calibration curve cannot be liner if you have "ppm" units selected, even if linear model is used.

Please note that statistical calibration can be used for peptides only! Any non-peptide contamination or modification could have disturbing effect to the calibration and should be discarded from calculations.

9. Periodic Table of Elements

Periodic Table of Elements provides some basic information about the elements. Different groups can be highlighted and element name, mass and isotopic pattern can be shown. For each element its detailed information can be seen on *Wikipedia* (en.wikipedia.org/wiki/Periodic_table) or *The Photographic Periodic Table of the Elements* (www.periodictable.com) using direct links.



Periodic Table of Elements.

➡ **To show periodic table:**
Choose Tools → Periodic Table from the main menu.

➡ **To highlight group of elements:**
Select the group name from the *Highlight* combo box.

➡ **To see element name and mass:**
Press the element button.

➡ **To see isotopic pattern of element:**
Select the element in the table and press Isotopes button. Element pattern will be shown in *Mass Calculator* tool.

➡ **To see detailed information about element:**
Select the element in the table and press Wikipedia button. Corresponding wiki page shows up in your web browser.

➡ **To see element photos:**
Select the element in the table and press Photos button. Corresponding photo page shows up in your web browser.

10.Mass Calculator

Mass Calculator provides a tools for calculation of molecular masses of compounds, generation of ion series and isotopic pattern simulation. Molecular formula can be typed manually or can be sent from various *mMass's* tools such as *Protein Digest, Peptide Fragmentation* etc. Theoretical isotopic profile can be easily overlaid with measured data or stored as regular document.

➡ **To show mass calculator:**
Press button from the main toolbar or choose Tools → Mass Calculator from the main menu.

10.1. Formula Syntax

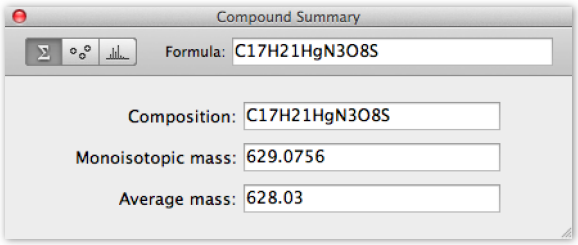
All known elements and their isotopes are defined within *mMass's* library and can be used to define any compound. Use the following syntax rules to specify compound formula:

- Use common syntax to type simple formula: C16H32O2.
- Use parenthesis "()" to define groups: CH3(CH2)14COOH
- Use negative "-" values to remove atoms: CH3(CH2)14COOH(NaH-1)
- Use braces "{}" to define specific isotope of the element: C{13}16H32O2

⚠ *Please note that using negative values for groups such as C16H32O2(OH)-1 does not work.*

10.2. Compound Summary

Compound Summary panel provides a fast preview of the compound defined in *Formula* field. *Composition*, *Monoisotopic* and *Average masses* are shown.

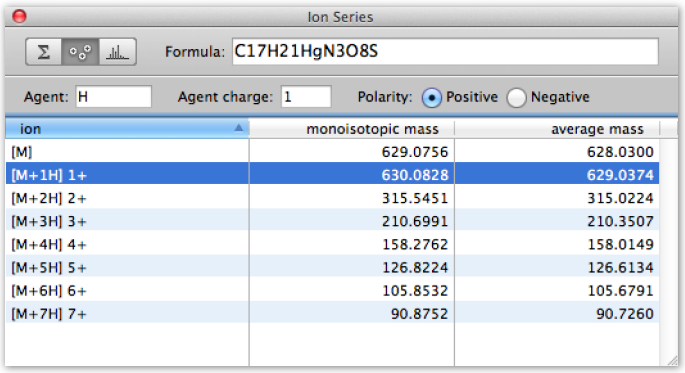


Compound Summary.

➡ **To show compound summary:**
Press button from the main toolbar or choose Tools → Mass Calculator from the main menu to show up *Mass Calculator* panel. Then press Σ button from the panel toolbar and write your compound into *Formula* field.

10.3. Ion Series

Using *Ion Series* panel you can see all the ions for the compound defined in *Formula* field. Corresponding monoisotopic and average masses are calculated with respect to polarity settings. By default, a proton is used as a "charging agent" but you can specify your own formula or *"e"* for radical ions.



Ion Series.

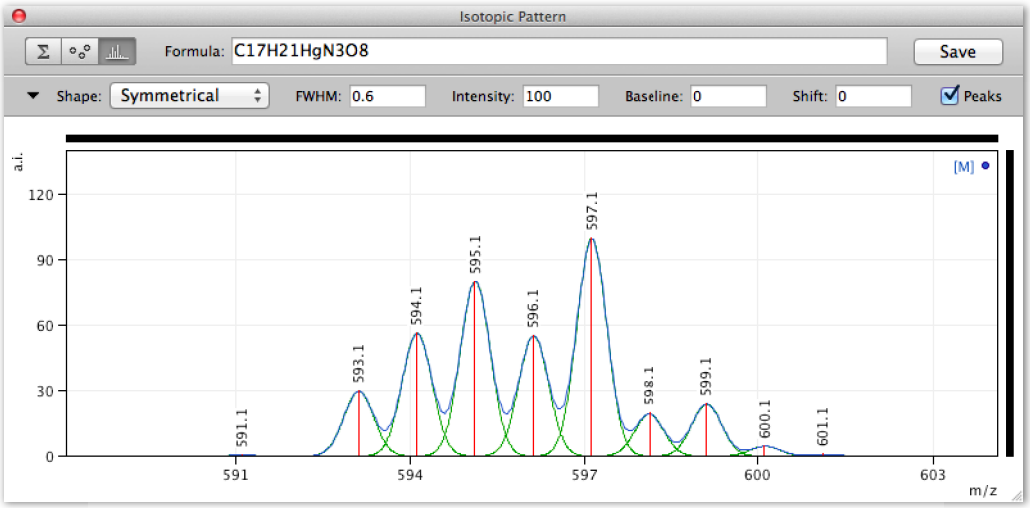
➡ **To show ion series:**
Press button from the main toolbar or choose Tools → Mass Calculator from the main menu to show up *Mass Calculator* panel. Then press button from the panel toolbar and type your compound into *Formula* field. Ions are generated automatically.

➡ **To copy ion series into clipboard:**
Click into the ions list and press Command+C (Ctrl+C on MS Windows and Linux).

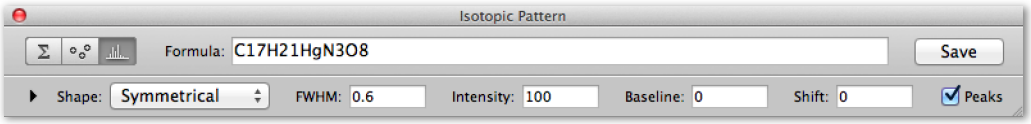
10.4. Isotopic Pattern

Isotopic Pattern panel provides a simple tool for generating theoretical isotopic pattern from compound formula. Isotopes are calculated and grouped together with respect to specified peak width *(FWHM)*. For each isotope, peak shape can be modeled as either symmetrical (Gaussian) or asymmetrical (half-Gaussian, half-Lorentzian) and final profile is calculated as a sum of all the peaks.

Theoretical isotopic profile can be overlaid with measured data and positioned precisely using *FWHM, Intensity, Baseline* and m/z *Shift*. You can save theoretical profile as regular document as well.



Isotopic Pattern within Mass Calculator tool.



Collapsed version of Isotopic Pattern panel.

➡ **To show isotopic pattern:**
Press button from the main toolbar or choose Tools → Mass Calculator from the main menu to show up *Mass Calculator* panel. Then press button from the panel toolbar and type your compound into *Formula* field.

➡ **To change the ion type for current isotopic patter:**
Press button from the panel toolbar and select desired ion type in the list.

➡ **To overlay isotopic pattern with measured data:**
Press button from the panel toolbar and select desired ion type in the list. Theoretical pattern is then overlaid with measured data. Set *Intensity, Baseline* and *Shift* values to position the pattern precisely.

➡ **To save current isotopic pattern as regular document:**
Press Save button from the panel toolbar.

➡ **To collapse pattern panel:**
Press the small triangle on the left side of the panel toolbar.

➡ **To show individual peaks:**
Check *Show peaks* checkbox in the panel toolbar.

Use asymmetrical peak shape to model isotopic pattern of TOF instruments.

Use the same spectrum manipulation conventions in isotopic pattern window as in the main *Spectrum Viewer*.

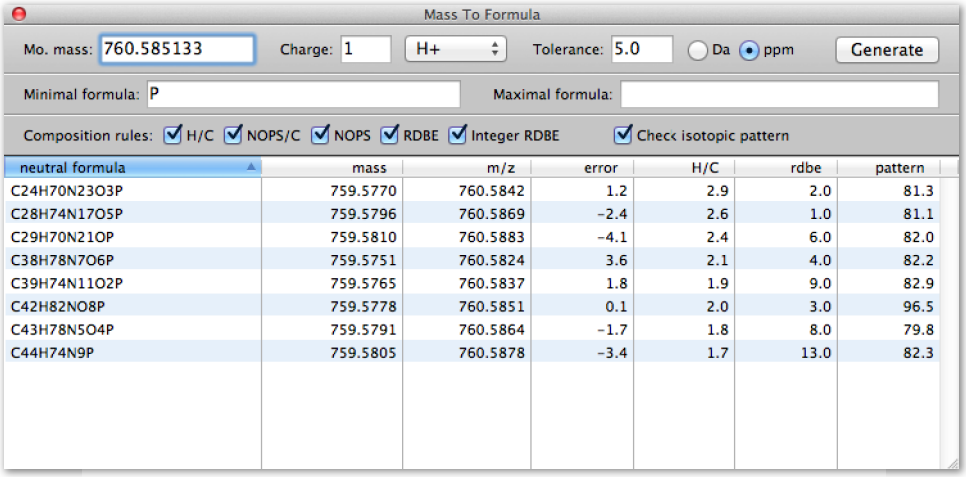
Turn off the *Peaks* checkbox to speed up calculation of complex patterns with ultra-high resolution.

Use collapsed panel while overlaying theoretical profile and acquired data. This provides you more space to look at your data but all the positioning parameters are still available.

Please note that when overlaying theoretical profile and acquired data, offset and flipping is applied automatically according to selected document.

11. Mass to Formula

Modern high-accuracy instruments allows for calculation of molecular formulae from a single measured mass. *Mass To Formula* tool generates all possible molecular formulae corresponding to given mass, tolerance and composition limits. To eliminate nonsense formulae, several composition rules can be applied and theoretical isotopic pattern can be automatically compared with measured profile. In addition, result can be directly searched within several public databases such as PubChem, METLIN, ChemSpider, HMDB or Lipid MAPS.



neutral formula	mass	m/z	error	H/C	rdbe	pattern
C24H70N23O3P	759.5770	760.5842	1.2	2.9	2.0	81.3
C28H74N17O5P	759.5796	760.5869	-2.4	2.6	1.0	81.1
C29H70N21OP	759.5810	760.5883	-4.1	2.4	6.0	82.0
C38H78N7O6P	759.5751	760.5824	3.6	2.1	4.0	82.2
C39H74N11O2P	759.5765	760.5837	1.8	1.9	9.0	82.9
C42H82NO8P	759.5778	760.5851	0.1	2.0	3.0	96.5
C43H78N5O4P	759.5791	760.5864	-1.7	1.8	8.0	79.8
C44H74N9P	759.5805	760.5878	-3.4	1.7	13.0	82.3

Mass to Formula tool.

- *Mo. mass* - m/z value of ion or neutral compound to be calculated.
- *Charge* - current charge and ionization specie of an ion.
- *Tolerance* - mass tolerance value and units for calculated compounds.
- *Minimal formula* - minimal composition for calculated compounds.
- *Maximal formula* - maximal composition for calculated compounds.
- *Composition rules* - rules to be applied to filter nonsense compounds.
- *Check isotopic patter* - calculate similarity value (in %) between theoretical and measured profile.

You can specify limits for each element allowed for the resulting formulae, however, limits for the elements like C, H, N and O are calculated automatically (if not specified) using the following rules.

$$C < 40, H < 80, N < 20, O < 20 \text{ if neutral mass } < 500$$
$$C < 80, H < 130, N < 30, O < 30 \text{ if neutral mass } 500 - 1000$$
$$C < 160, H < 250, N < 40, O < 70 \text{ if neutral mass } 1000 - 2000$$
$$C < 180, H < 300, N < 60, O < 90 \text{ if neutral mass } > 2000$$

In order to show relevant compositions only, following rules can be applied:

- *H/C rule* - if checked, only the compounds with following hydrogen/carbon ratio are shown:

$$0.1 \leq H/C \leq 3.0$$

- *NOPS/C rule* - if checked, only the compounds with following heteroatom/carbon ratios are shown:

$$N/C < 4,\ O/C < 3,\ P/C < 2 \text{ and } S/C < 3$$

- *NOPS rule* - if checked, only the compounds with following heteroatom counts are shown:

$$N < 10, O < 20, P < 4, S < 3 \text{ if NOPS each} > 1$$
$$N < 11, O < 21, P < 6 \text{ if NOP each} > 3$$
$$O < 14, P < 3, S < 3 \text{ if OPS each} > 1$$
$$N < 3, P < 3, S < 4 \text{ if NPS each} > 1$$
$$N < 19, O < 14, S < 8 \text{ if NOS each} > 6$$

- *RDBE rule* - if checked, only the compounds with following *ring and double-bond equivalent* for neutral compound are shown:

$$-1 \leq RDBE \leq 40$$

- *Integer RDBE rule* - if checked, only the compounds with integer value of *ring and double-bond equivalent* for neutral compound are shown. It corresponds to the neutral compounds with even electron configuration.

➡ **To generate formula from mass:**
Press button from the main toolbar or choose Tools → Mass to Formula from the main menu to show up *Mass to Formula* panel. Specify all the parameters and press Generate button.

➡ **To highlight compound mass in spectrum viewer:**
Click on the compound and spectrum moves to theoretical m/z value. Small red arrow shows up on m/z axis of the spectrum. Current zoom range remains the same.

➡ **To show isotopic pattern of compound:**
Double-click on the compound to show up *Mass Calculator* panel. Compound formula appears in *Formula* field and corresponding ion type is selected automatically. See *Mass Calculator* chapter for more information.

➡ **To search compound in public databases:**
Right-click on the compound and choose the database to use. *HTML* page will be generated and sent to specified server. Results show up in your default web browser.

➡ **To copy compounds into clipboard:**
Click into the list and press Command+C (Ctrl+C on MS Windows and Linux) or use pop-up menu.

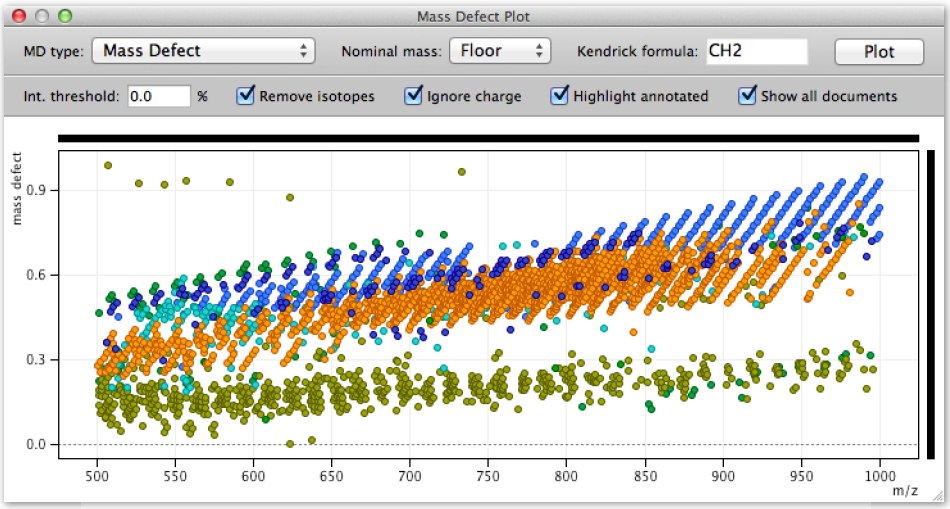
See *Kind T. at al. 2007 BMC Bioniformatics* for more information about the composition rules.

Please note that there is an internal limit of 1000 compounds to be calculated at most.

Please note that isotopic pattern can be compared with profile data only.

12.Mass Defect Plot

Mass Defect Plot serves as a simple tool to visualize peaks in selected document based on their *Fractional Mass* or various types of mass defect. In a generated plot, similar compounds tend to group into clusters. Standard *Mass Defect* is defined as the difference between a compound's or peaks's exact mass and its nominal mass. Here the nominal mass can be calculated as *Round*, *Floor* (round down) or *Ceil* (round up). Similarly, the *Relative Mass Defect* is caclulated by dividing the standard mass defect by current *m/z* of the peak and multiplied by *1e6*. *Kendrick Mass Defect* can be utilized to group all the compounds differing just in the number of CH_2 groups (or any user-specified formula).



Mass Defect Plot tool.

- *MD type* - mass defect type to show.
- *Nominal mass* - function to use for nominal mass calculation (exact mass rounding).
- *Kendrick formula* - molecular formula to use for Kendrick mass defect.
- *Int. threshold* - hides peaks below specified relative intensity limit.
- *Remove isotopes* - removes assigned isotopes (deisotoping step needs to be applied before).
- *Ignore charge* - do not recalculate multiply charged items and use acquired m/z instead.
- *Highlight annotated* - highlights all annotated peaks and sequence matches.
- *Show all documents* - overlay all visible documents in single plot.

➡ **To generate mass defect plot:**
Press button from the main toolbar or choose Tools → Mass Defect Plot from the main menu to show up *Mass Defect Plot* panel. Specify all the parameters and press Plot button.

➡ **To highlight annotated peaks:**
In the panel toolbar check the *Highlight annotated* checkbox. All the annotated peaks show in red.

➡ **To show all visible documents:**
In the panel toolbar check the *Show all documents* checkbox. All the opened and visible documents will be show.

➡ **To highlight mass in the spectrum viewer:**
Left-click within the plot area to show selected mass in the main spectrum viewer. Spectrum moves to selected position and highlights it using a small red arrow on x-axis.

See *Sleno L. 2012 J Mass Spectrom* for more information about the mass defect in MS.

13.Sequence Tools

mMass provides an internal *Sequence Editor*, which can be used to make any protein or peptide sequence available for other tools. Any modification can be applied either as fixed or variable. *Protein Digest* tool can be used to generate a list of peptides resulting from *in silico* enzymatic or chemical digestion of specified protein sequence. Similarly, *Peptide Fragmentation* tool generates a list of common peptide fragments. In both cases, all possible combinations of variable modifications are calculated and results can be easily compared with measured data. In addition, sequence can be searched for a sub-peptide mass by *Mass Search* tool to identify any non-specific or semi-specific cleavages.

➡ **To add new sequence:**
Press [+] button from the *Documents Panel* bottom toolbar and select New Sequence or choose Sequence → New from the main menu. *Sequence Editor* shows up. Type your sequence manually or use copy/paste.

➡ **To import sequence:**
Choose Sequence → Import from the main menu and select your sequence file. If more than one sequence are available in the file, a selection dialog shows up. Select one or more sequence and press Import button. *mMass* and *FASTA* documents are currently supported.

➡ **To edit sequence:**
Double-click on the sequence title in *Documents Panel* and *Sequence Editor* shows up.

➡ **To delete sequence:**
Click on the sequence title in *Documents Panel* and press Command+Backspace or Delete, or press [-] button from the *Documents Panel* bottom toolbar and choose Delete Sequence.

➡ **To generate isotopic pattern of sequence:**
In *Sequence Editor* press Pattern button.

If you are using some of the sequences frequently, you can make a personal sequence library by saving the sequences into a single *mMass's* document.

⚠ *Please note that only the valid sequences are shown in the import dialog.*

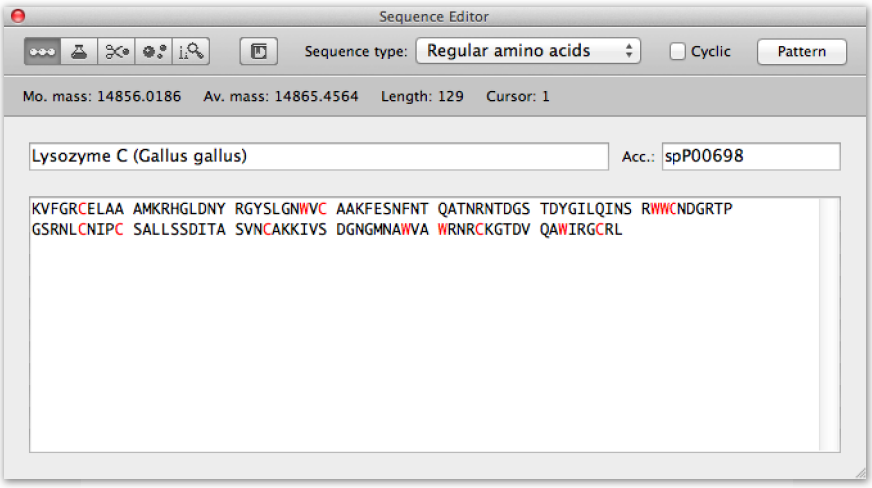
13.1. Sequence Editor

Internal *Sequence Editor* provides a tool to define protein or peptide sequence to be available for other tools. While typical protein sequence consists of regular proteinogenic amino acids, with some optional modifications, this is not the case for amount of naturally occurring peptides, where various building blocks (monomers) are used as well. For this reason, *mMass* provides two different sequence editors, which can be switched by the *Sequence type* combo box. In addition, sequence structure can be set as linear or cyclic.

⚠ *Please note that some tools are not available for custom-type or cyclic sequences.*

Standard Sequence Editor

Using the standard sequence editor, a sequence can be manually typed, pasted from clipboard or imported from any *mMass* or *FASTA* document. Every input is automatically checked to contain regular amino acids only. If any modification is set using *Modifications* panel, modified amino acids are shown in red.

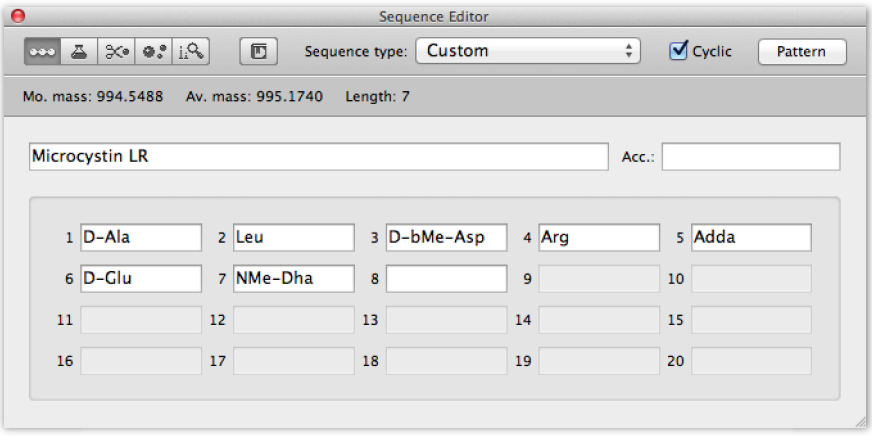


Standard Sequence Editor.

If a sequence is pasted from the clipboard all white spaces and numbers are removed automatically. This can be very useful if you copy/paste a sequence from web sites.

Custom-Type Sequence Editor

Using the custom-type sequence editor, any non-standard peptide sequence consisting of up to 20 user-definable monomers can be created. Sequence monomers can be manually typed into corresponding fields or dropped from *Monomer Library* panel.



Custom Sequence Editor

➡ **To show available monomers:**
Press button from the *Sequence Editor* toolbar to show up *Monomer Library* panel.

➡ **To compose custom sequence:**
Drag and drop any monomer from *Monomer Library* panel or type particular abbreviation manually.

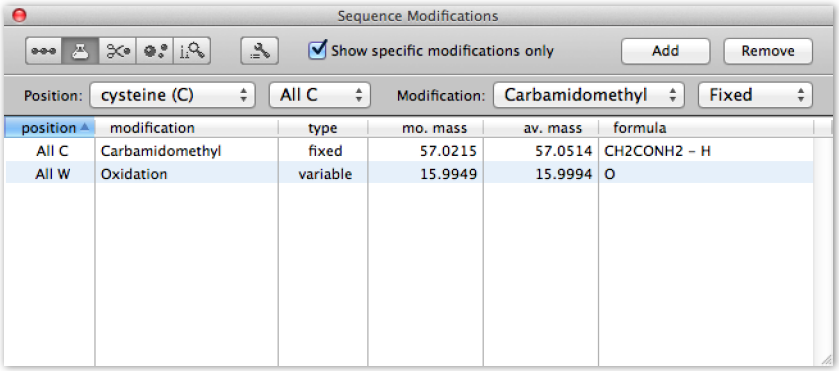
➡ **To add / edit monomer definition:**
Choose Libraries → Monomers from the main menu to show up *Monomers Library* editor. See *Libraries* chapter for more information.

Use the search field of *Monomer Library* panel to quickly find a monomer you are looking for.

⚠ *Please note that each sequence is automatically checked to contain defined monomers only. If any error or gap is found in the sequence, the remaining part is ignored.*

13.2. Modifications

In *Sequence Modifications* panel any post-translational modification can be set to sequence terminus, a single amino acid or to selected type. Beside a position, for each modification you can specify whether it should be *Fixed* or *Variable* and all possible variants are then calculated in the sequence related tools. In order to avoid permanent assignment of the same modification set you can define your own presets and use it frequently.



Sequence Modifications.

- *Show specific modifications only* - show modifications specific for selected amino acid.
- *Position* - amino acid type and position.
- *Modification* - modification name and type.

➡ **To add modification:**
Press button from the *Sequence* panel toolbar to show up *Sequence Modifications*. Select amino acid type, position, modification and modification type and press Add button.

➡ **To remove modification:**
Press button from the *Sequence* panel toolbar to show up *Sequence Modifications*. Select desired modification in the list and press Remove button.

➡ **To add / edit modification definition:**
Choose Libraries → Modifications from the main menu to show up *Modifications Library* editor. See *Libraries* chapter for more information.

➡ **To save current global modifications as presets:**
Press button from the panel toolbar and choose Save as Presets. In the dialog type the presets name and press Save button.

➡ **To use saved presets:**
Press button from the panel toolbar and choose any of your presets from the pup-up menu.

➡ **To delete saved presets:**
Choose Libraries → Presets from the main menu to show up *Presets Library* editor. Choose the presets you'd like to delete and press Delete button.

➡ **To rename saved presets:**
Choose Libraries → Presets from the main menu to show up *Presets Library* editor. Choose the presets you'd like to rename, type the new name and press Rename button.

⚠ *Please note that multiple modifications on a single residue are not allowed.*

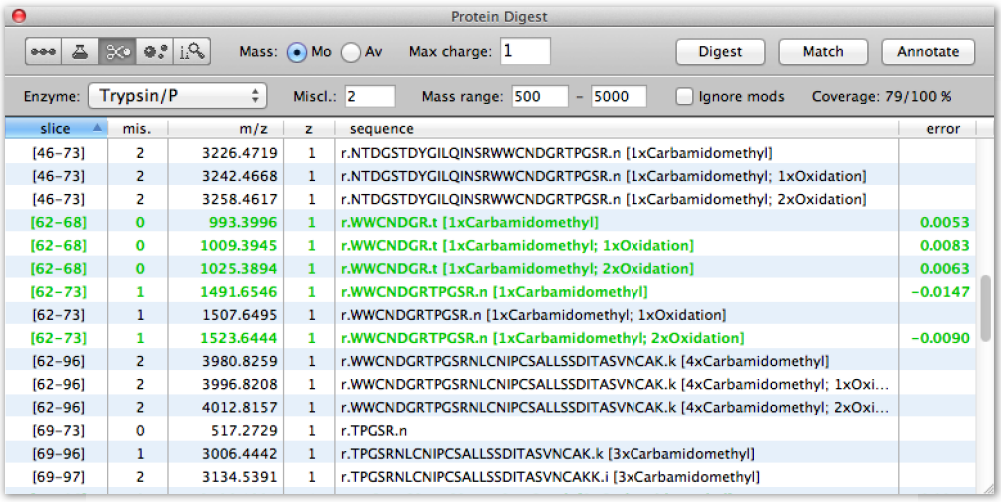
⚠ *Please note that terminal modification are removed upon cyclization.*

⚠ *Please note that global and terminal modifications can only be stored in presets.*

⚠ *Please note that modifications are not available for custom-type sequences.*

13.3. Protein Digest

Many experiments, involving detection of modifications, protein validation etc., apply the specific enzymatic digestion of a protein with a known sequence. *Protein Digest* panel provides a tool which can be used to generate a list of peptides resulting from *in silico* digestion of a sequence. Masses of these peptides can then be compared with current peak list within a specified tolerance to see any matches. In addition, theoretical isotopic patterns can be generated and overlaid with the data to validate matches.



slice	mis.	m/z	z	sequence	error
[46-73]	2	3226.4719	1	r.NTDGSTDYGILQINSRWWCNDGRTPGSR.n [1xCarbamidomethyl]	
[46-73]	2	3242.4668	1	r.NTDGSTDYGILQINSRWWCNDGRTPGSR.n [1xCarbamidomethyl; 1xOxidation]	
[46-73]	2	3258.4617	1	r.NTDGSTDYGILQINSRWWCNDGRTPGSR.n [1xCarbamidomethyl; 2xOxidation]	
[62-68]	0	993.3996	1	r.WWCNDGR.t [1xCarbamidomethyl]	0.0053
[62-68]	0	1009.3945	1	r.WWCNDGR.t [1xCarbamidomethyl; 1xOxidation]	0.0083
[62-68]	0	1025.3894	1	r.WWCNDGR.t [1xCarbamidomethyl; 2xOxidation]	0.0063
[62-73]	1	1491.6546	1	r.WWCNDGRTPGSR.n [1xCarbamidomethyl]	-0.0147
[62-73]	1	1507.6495	1	r.WWCNDGRTPGSR.n [1xCarbamidomethyl; 1xOxidation]	
[62-73]	1	1523.6444	1	r.WWCNDGRTPGSR.n [1xCarbamidomethyl; 2xOxidation]	-0.0090
[62-96]	2	3980.8259	1	r.WWCNDGRTPGSRNLCNIPCSALLSSDITASVNCAK.k [4xCarbamidomethyl]	
[62-96]	2	3996.8208	1	r.WWCNDGRTPGSRNLCNIPCSALLSSDITASVNCAK.k [4xCarbamidomethyl; 1xOxi...	
[62-96]	2	4012.8157	1	r.WWCNDGRTPGSRNLCNIPCSALLSSDITASVNCAK.k [4xCarbamidomethyl; 2xOxi...	
[69-73]	0	517.2729	1	r.TPGSR.n	
[69-96]	1	3006.4442	1	r.TPGSRNLCNIPCSALLSSDITASVNCAK.k [3xCarbamidomethyl]	
[69-97]	2	3134.5391	1	r.TPGSRNLCNIPCSALLSSDITASVNCAKK.i [3xCarbamidomethyl]	

Protein Digest tool.

- *Mass* - mass type to be used for calculations.
- *Max charge* - maximum charge to be calculated.
- *Enzyme* - enzyme to be used to simulate digestion.
- *Miscl.* - maximum number of enzyme miss cleavages.
- *Mass range* - m/z range for peptides to be calculated.
- *Ignore mods* - ignore modifications in cleavage site.
- *Coverage* - matched / theoretical sequence coverage.

➡ **To digest protein:**
Double-click on the sequence title in *Documents Panel* to show up *Sequence* tools and press button for *Protein Digest* tool. Specify all the parameters and press Digest button.

➡ **To highlight peptide mass in spectrum viewer:**
Click on the peptide and spectrum moves to theoretical m/z value. Small red arrow shows up on m/z axis of the spectrum. Current zoom range remains the same.

➡ **To show isotopic pattern of peptide:**
Double-click on the peptide to show up *Mass Calculator* panel. Peptide formula appears in *Formula* field and corresponding ion type is selected. See *Mass Calculator* chapter for more information.

➡ **To match peptides to peak list:**
Generate theoretical peptides and press Match button in the panel toolbar to show up *Match Peptides* panel. Matched peptides shows green and bold in the list. See *Data Matching* chapter for more information.

➡ **To annotate peaks by matched peptides:**
Press Annotate button in the panel toolbar. All the matched items show up in *Documents Panel* under current sequence.

➡ **To copy peptide sequence into clipboard:**
Right-click on any peptide and choose *Copy Selected Sequence* from pop-up menu.

➡ **To copy peptides into clipboard:**
Click into the list and press Command+C (Ctrl+C on MS Windows and Linux) or use pop-up menu.

➡ **To see matched/unmatched peptides only:**
Right-click into the list and select view option from pop-up menu.

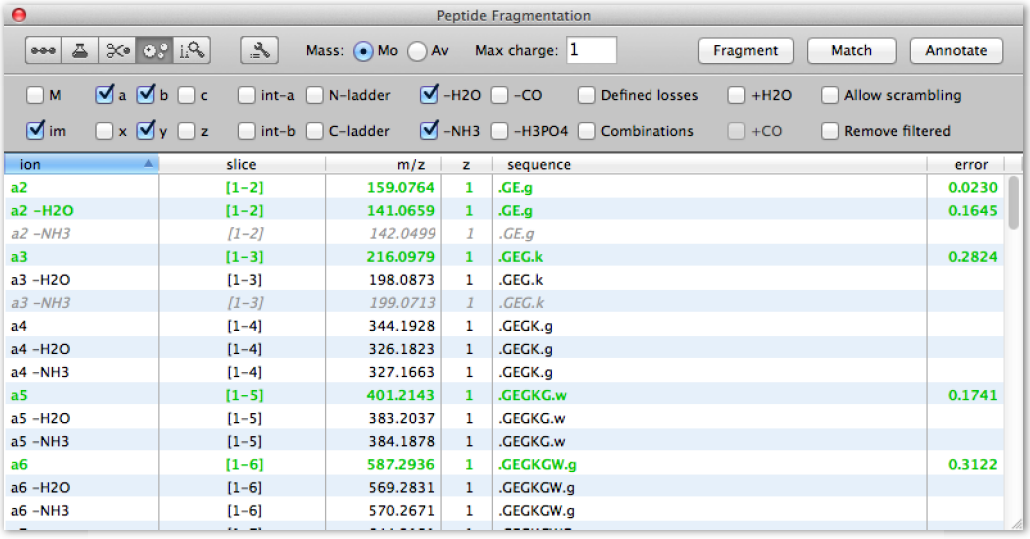
➡ **To add / edit enzyme definition:**
Choose Libraries → Enzymes from the main menu to show up *Enzymes Library* editor. See *Libraries* chapter for more information.

Use theoretical sequence coverage to select the right enzyme for particular protein and mass range.

Please note that this tool is not available for custom-type and cyclic sequences.

13.4. Peptide Fragmentation

Different types of fragmentation techniques can be used for peptide sequence validation, as well as for the detection, localization and characterization of post-translational modifications. For these types of experiments, common fragments of a peptide sequence can be generated *in silico* using *Peptide Fragmentation* tool. Depending on a fragmentation technique used, different fragments can be generated. Some of the peptide fragments are theoretical only and can be removed using *Remove filtered* check box.



ion	slice	m/z	z	sequence	error
a2	[1-2]	159.0764	1	.GE.g	0.0230
a2 -H2O	[1-2]	141.0659	1	.GE.g	0.1645
a2 -NH3	*[1-2]*	*142.0499*	*1*	*.GE.g*	
a3	[1-3]	216.0979	1	.GEG.k	0.2824
a3 -H2O	[1-3]	198.0873	1	.GEG.k	
a3 -NH3	*[1-3]*	*199.0713*	*1*	*.GEG.k*	
a4	[1-4]	344.1928	1	.GEGK.g	
a4 -H2O	[1-4]	326.1823	1	.GEGK.g	
a4 -NH3	[1-4]	327.1663	1	.GEGK.g	
a5	[1-5]	401.2143	1	.GEGKG.w	0.1741
a5 -H2O	[1-5]	383.2037	1	.GEGKG.w	
a5 -NH3	[1-5]	384.1878	1	.GEGKG.w	
a6	[1-6]	587.2936	1	.GEGKGW.g	0.3122
a6 -H2O	[1-6]	569.2831	1	.GEGKGW.g	
a6 -NH3	[1-6]	570.2671	1	.GEGKGW.g	

Peptide Fragmentation tool.

- *Mass* - mass type to be used for calculations.
- *Max charge* - maximum charge to be calculated.

➡ **To fragment peptide:**
Double-click on the sequence title in *Documents Panel* to show up *Sequence* tools and press button for *Peptide Fragmentation* tool. Specify all the parameters and press Fragment button.

➡ **To highlight fragment mass in spectrum viewer:**
Click on the fragment and spectrum moves to theoretical m/z value. Small red arrow shows up on m/z axis of the spectrum. Current zoom range remains the same.

➡ **To show isotopic pattern of fragment:**
Double-click on the fragment to show up *Mass Calculator* panel. Fragment formula appears in *Formula* field and corresponding ion type is selected. See *Mass Calculator* chapter for more information.

➡ **To match fragments to peak list:**
Generate theoretical fragments and press Match button in the panel toolbar to show up *Match Fragments* panel. Matched fragments shows green and bold in the list. See *Data Matching* chapter for more information.

➡ **To annotate peaks by matched fragments:**
Press Annotate button in the panel toolbar. All the matched items show up in *Documents Panel* under current sequence.

➡ **To copy fragments into clipboard:**
Click into the list and press Command+C (Ctrl+C on MS Windows and Linux) or use pop-up menu.

➡ **To see matched/unmatched fragments only:**
Right-click into the list and select view option from pop-up menu.

➡ **To save fragments settings as presets:**
Press button from the panel toolbar and choose Save as Presets. In the dialog type the presets name and press Save button.

➡ **To use saved presets:**
Press button from the panel toolbar and choose any of your presets from the pup-up menu.

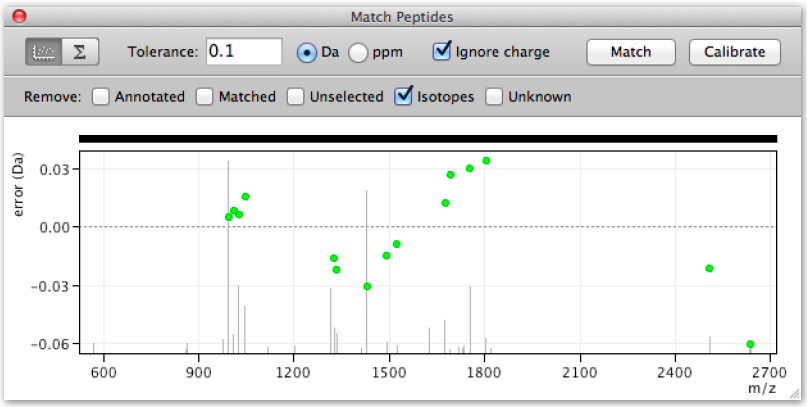
➡ **To delete saved presets:**
Choose Libraries → Presets from the main menu to show up *Presets Library* editor. Choose the presets you'd like to delete and press Delete button.

➡ **To rename saved presets:**
Choose Libraries → Presets from the main menu to show up *Presets Library* editor. Choose the presets you'd like to rename, type the new name and press Rename button.

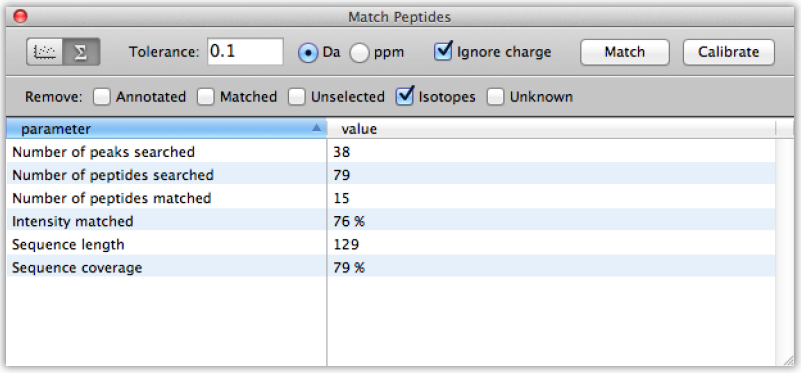
To decrease number of false-positive matches, be sure to calculate only the relevant fragmentation pathways for particular fragmentation technique used.

13.5. Data Matching

Both, theoretical peptides and fragments lists can be compared with the peak list of current document by pressing Match button in corresponding tool. If some of the peptides or fragments are matched successfully you can use them for annotations or data recalibration.



Match tool.



Match summary.

- *Tolerance* - mass tolerance for data matching.
- *Da, ppm* - units for tolerance value.
- *Ignore charge* - ignore peak charge while data matching.
- *Annotated* - don't use peaks with user's annotation.
- *Matched* - don't use peaks matched to sequence peptide or fragment.
- *Unselected* - use only peaks selected in the *Peaklist Panel*.
- *Isotopes* - don't use isotopes.
- *Unknown* - don't use peaks with no isotope number or charge assigned.

➡ **To match peptides to peak list:**
Generate theoretical peptides in *Protein Digest* tool and press Match button in the panel toolbar to show up *Match Peptides* panel. Specify the parameters and press Match button.

➡ **To match fragments to peak list:**
Generate theoretical fragments in *Peptide Fragmentation* tool and press Match button in the panel toolbar to show up *Match Fragments* panel. Specify the parameters and press Match button.

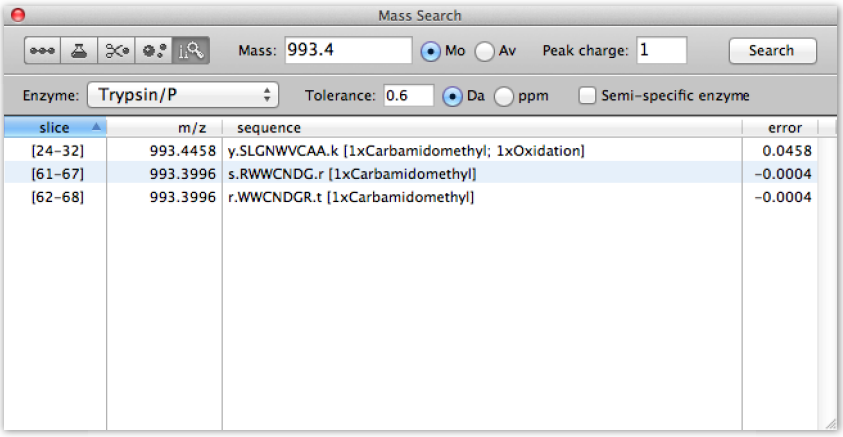
➡ **To ignore peak charge and use only m/z value while data matching:**
Check *Ignore charge* checkbox.

➡ **To re-calibrate data using matches:**
Press Calibrate button in the panel toolbar to send matched items into *Calibration* tool. See *Calibration* chapter for more information.

Use the same spectrum manipulation conventions in the error plot as in the main *Spectrum Viewer*.

13.6. Mass Search

Using sequence *Mass Search* tool you can search a sequence for any peptide of specified m/z. This tool can be very useful for identification of the peaks resulting from non-specific or semi-specific cleavage of a protein.



Mass Search tool.

- *Mass* - peptide mass to be searched.
- *Mo, Av* - mass type to be searched.
- *Max charge* - maximum charge to be calculated for peptides.
- *Enzyme* - enzyme to use for peptide "caps" (e.g. H and OH) and semi-specificity.
- *Tolerance* - mass tolerance for searching.
- *Da, ppm* - units for tolerance value.
- *Semi-specific enzyme* - only the peptides resulted from partially specific digest are searched (otherwise the non-specific peptides are searched).

➡ **To search sequence:**
Double-click on the sequence title in *Documents Panel* to show up *Sequence* tools and press button for *Mass Search* tool. Specify all the parameters and press Search button. List of matched peptides will be shown.

➡ **To highlight peptide mass in spectrum viewer:**
Click on the peptide and spectrum moves to theoretical m/z value. Small red arrow shows up on m/z axis of the spectrum. Current zoom range remains the same.

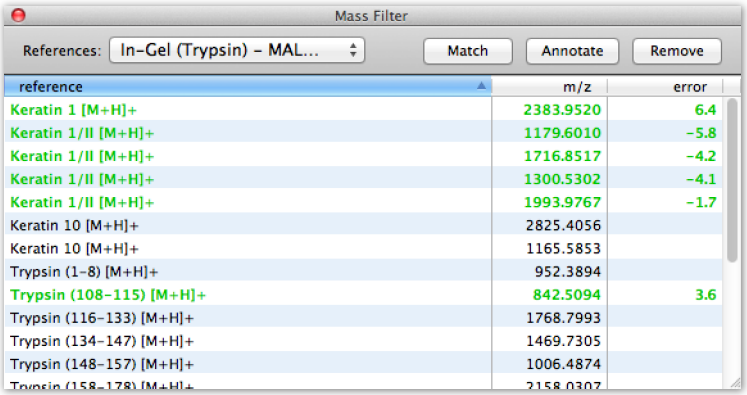
➡ **To show isotopic pattern of peptide:**
Double-click on the peptide to show up *Mass Calculator* panel. Peptide formula appears in *Formula* field and corresponding ion type is selected. See *Mass Calculator* chapter for more information.

➡ **To copy peptide sequence into clipboard:**
Right-click on any peptide and choose *Copy Selected Sequence* from pop-up menu.

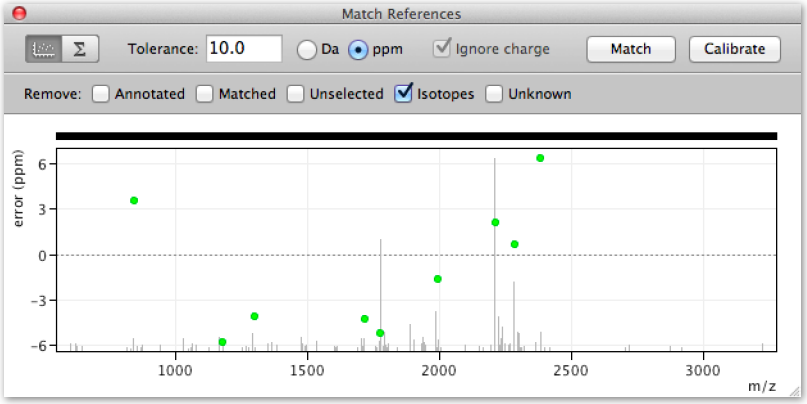
➡ **To copy peptides into clipboard:**
Click into the list and press Command+C (Ctrl+C on MS Windows and Linux) or use pop-up menu.

14.Mass Filter

In many mass spectra, common contaminant masses are observed and can have a negative effect on data interpretation. Keratin and trypsin autolysis peptides are the most know examples in protein identification experiments. Using *Mass Filter* tool, such contaminants can be easily annotated or removed completely from a peak list. In addition, matched peaks can be used as an internal standard for data re-calibration.



Mass Filter tool.



Match References tool.

- *Tolerance* - mass tolerance for data matching.
- *Da, ppm* - units for tolerance value.
- *Ignore charge* - ignore peak charge while data matching.
- *Annotated* - don't use peaks with user's annotation.
- *Matched* - don't use peaks matched to sequence peptide or fragment.
- *Unselected* - use only peaks selected in the *Peaklist Panel*.
- *Isotopes* - don't use isotopes.
- *Unknown* - don't use peaks with no isotope number or charge assigned.

➡ **To filter contaminant masses:**
Press button from the main toolbar or choose Tools → Mass Filter from the main menu.. Select *References* list name and press Match button to show up *Match References* tool.

➡ **To annotate matched peaks:**
When the mass list is matched to your data press Annotate button in the *Mass Filter* panel toolbar.

All the matched items show up in *Documents Panel* under *Annotations*. If you want to exclude any matched item just double-click the item in *Mass Filter* list.

➡ **To remove matched peaks:**
When the mass list is matched to your data press Remove button in the *Mass Filter* panel toolbar. All the matched peak will be removed from the peak list. If you want to exclude any matched item just double-click the item in *Mass Filter* list.

➡ **To re-calibrate data using matches:**
Press Calibrate button in the *Match References* panel toolbar to send matched items into *Calibration* tool. See *Calibration* chapter for more information.

➡ **To define a new list of reference masses:**
Choose Libraries → Reference Masses from the main menu to show up *Reference Masses Library* editor. See *Libraries* chapter for more information.

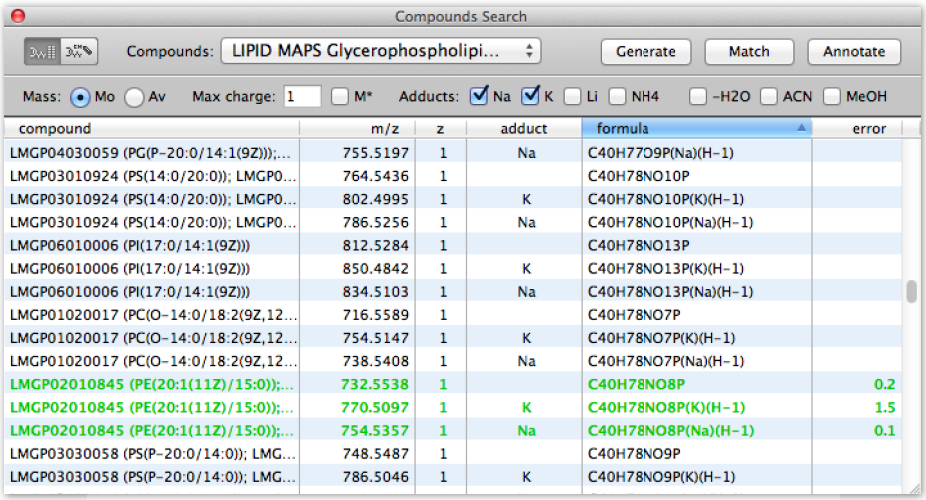

Use the same spectrum manipulation conventions in the error plot as in the main *Spectrum Viewer*.


See match summary panel for more information about current match.


Please note that since the reference masses don't have the charge value defined, the Ignore charge switch doesn't have any effect in this tool.

15.Compounds Search

Using *Compounds Search* tool you can search for any user-specified compound or even a list of compounds in a peak list. Every compound is specified in *mMass's* library as molecular formula, title and description, therefore both monoisotopic or average masses can be search with any charge. In addition common adducts can be searched automatically as well. All the matched peaks can be annotated and then shown or printed within analysis report.



compound	m/z	z	adduct	formula	error
LMGP04030059 (PG(P-20:0/14:1(9Z)));...	755.5197	1	Na	C40H77O9P(Na)(H-1)	
LMGP03010924 (PS(14:0/20:0)); LMGP0...	764.5436	1		C40H78NO10P	
LMGP03010924 (PS(14:0/20:0)); LMGP0...	802.4995	1	K	C40H78NO10P(K)(H-1)	
LMGP03010924 (PS(14:0/20:0)); LMGP0...	786.5256	1	Na	C40H78NO10P(Na)(H-1)	
LMGP06010006 (PI(17:0/14:1(9Z)))	812.5284	1		C40H78NO13P	
LMGP06010006 (PI(17:0/14:1(9Z)))	850.4842	1	K	C40H78NO13P(K)(H-1)	
LMGP06010006 (PI(17:0/14:1(9Z)))	834.5103	1	Na	C40H78NO13P(Na)(H-1)	
LMGP01020017 (PC(O-14:0/18:2(9Z,12...	716.5589	1		C40H78NO7P	
LMGP01020017 (PC(O-14:0/18:2(9Z,12...	754.5147	1	K	C40H78NO7P(K)(H-1)	
LMGP01020017 (PC(O-14:0/18:2(9Z,12...	738.5408	1	Na	C40H78NO7P(Na)(H-1)	
LMGP02010845 (PE(20:1(11Z)/15:0));...	732.5538	1		C40H78NO8P	0.2
LMGP02010845 (PE(20:1(11Z)/15:0));...	770.5097	1	K	C40H78NO8P(K)(H-1)	1.5
LMGP02010845 (PE(20:1(11Z)/15:0));...	754.5357	1	Na	C40H78NO8P(Na)(H-1)	0.1
LMGP03030058 (PS(P-20:0/14:0)); LMG...	748.5487	1		C40H78NO9P	
LMGP03030058 (PS(P-20:0/14:0)); LMG...	786.5046	1	K	C40H78NO9P(K)(H-1)	

Compounds Search tool.

- *Compounds* - compounds list to be searched.
- *Formula* - compound formula to be searched.
- *Mass* - mass type to be searched.
- *Max charge* - maximum charge to be calculated.
- *M** - calculate radical ions.
- *Adducts* - type of adduct to be searched.

➡ **To search for list of compounds:**
Press button from the main toolbar or choose Tools → Compounds Search from the main menu and select tool in the panel toolbar. Select *Compounds* list name, set the parameters and press Generate button. Then press Match button to show up *Match Compounds* tool.

➡ **To search for specified formula:**
Press button from the main toolbar or choose Tools → Compounds Search from the main menu and select tool in the panel toolbar. Type compound *Formula*, set the parameters and press Generate button. Then press Match button to show up *Match Compounds* tool. See *Mass Calculator* chapter for more information about formula syntax.

➡ **To highlight compound mass in spectrum viewer:**
Click on the compound and spectrum moves to theoretical m/z value. Small red arrow shows up on m/z axis of the spectrum. Current zoom range remains the same.

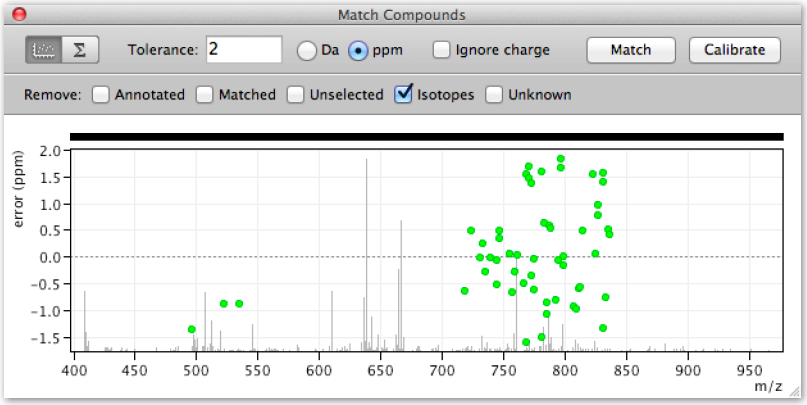
➡ **To show isotopic pattern of compound:**
Double-click on the compound to show up *Mass Calculator* panel. Compound formula appears in *Formula* field and corresponding ion type is selected automatically. See *Mass Calculator* chapter for more information.

➡ **To copy compounds into clipboard:**
Click into the list and press Command+C (Ctrl+C on MS Windows and Linux) or use pop-up menu.

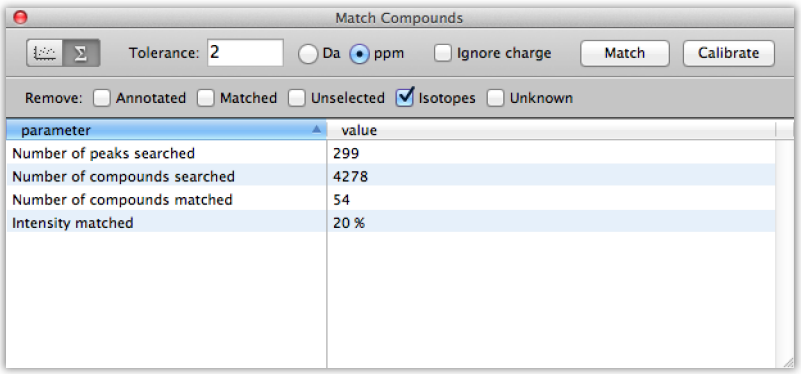
➡ **To see matched/unmatched compounds only:**
Right-click into the list and select view option from pop-up menu.

➡ **To define a new list of compounds:**
Choose Libraries → Compounds from the main menu to show up *Compounds Library* editor. See *Libraries* chapter for more information.

➡ **To match compounds to peak list:**
Generate theoretical compounds and press Match button in the panel toolbar to show up *Match Compounds* panel.



Match Compounds tool.



Match Compounds summary.

- *Tolerance* - mass tolerance for data matching.
- *Da, ppm* - units for tolerance value.
- *Ignore charge* - ignore peak charge while data matching.
- *Annotated* - don't use peaks with user's annotation.
- *Matched* - don't use peaks matched to sequence peptide or fragment.
- *Unselected* - use only peaks selected in the *Peaklist Panel*.
- *Isotopes* - don't use isotopes.
- *Unknown* - don't use peaks with no isotope number or charge assigned.

➡ **To annotate peaks by matched compounds:**
Press Annotate button in panel toolbar. All the matched items show up in *Documents Panel* under *Annotations*.

➡ **To ignore peak charge while data matching:**
Check *Ignore charge* checkbox.

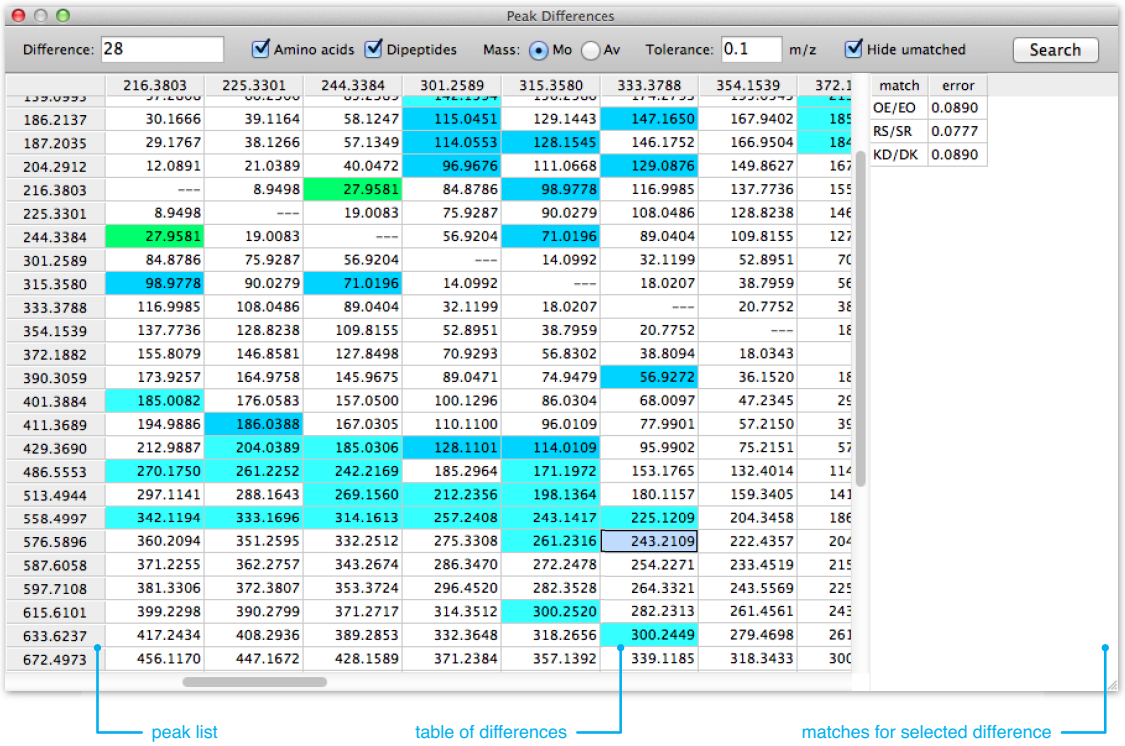
➡ **To re-calibrate data using matches:**
Press Calibrate button in the panel toolbar to send matched items into *Calibration* tool. See *Calibration* chapter for more information.

Use the same spectrum manipulation conventions in the error plot as in the main *Spectrum Viewer*.

See match summary panel for more information about current match.

16.Peak Differences

Interpretation of mass spectra typically involves a seemingly never-ending check of the differences between all peaks in a spectrum. However, *Peak Differences* tool is able to simply generate a table of all the differences between the peaks in the peak list. This table can then be used to automatically compare, within a specified tolerance, each difference with the respective masses of all amino acids, calculated dipeptides or specified m/z value. *Peak Differences* tool therefore provides a useful utility for *de novo* peptide sequencing or PTM search.



Peak Differences tool.

- *Difference* - user defined m/z difference to searched.
- *Amino acids* - all available amino acids will be searched.
- *Dipeptides* - all calculated dipeptides will be searched.
- *Mass* - mass type to be used for amino acids and dipeptides calculation.
- *Tolerance* - mass tolerance for searching.
- *Hide umatched* - if checked, only the matched peaks will be shown.

➡ **To show peak differences tool:**
Press button from the main toolbar or choose Tools → Peak Differences from the main menu.

➡ **To search:**
Specify all the parameters and press Search button from the panel toolbar. Click on any highlighted differences to see corresponding matches.

If the specified mass is matched, corresponding difference turns green. If at least one amino acid is matched, corresponding difference turns dark blue. If at least one dipeptide is matched, corresponding difference turns light blue.

17.Compare Peak Lists

Similarly as the *Peak Differences* tool, the *Compare Peak Lists* tool can easily be utilized to compare peak lists or annotations between multiple documents. Using this tool, one of the most typical and annoying manual task can be automatically performed within a few clicks. Once the tool is shown, all documents are loaded and marked by its own spectrum color and can be compared within the specified mass tolerance or even using intensity ratio threshold. For each peak identified in a different document, corresponding document color is highlighted next to the *m/z* value. By selecting any *m/z* value in the peak lists table, all the matched peaks are shown followed by error and intensity ratios (*a* - selected peak, *b* - matched peak). In addition to individual peak lists, separate table for combined peak list is also available.

Compare Peak Lists tool.

- *Compare* - data type to be compared.
- *Tolerance* - mass tolerance for comparison.
- *Da, ppm* - units for tolerance value.
- *Ignore charge* - ignore peak charge while comparing peaks.

➡ **To show compare peak lists tool:**
Press button from the main toolbar or choose Tools → Compare Peak Lists from the main menu.

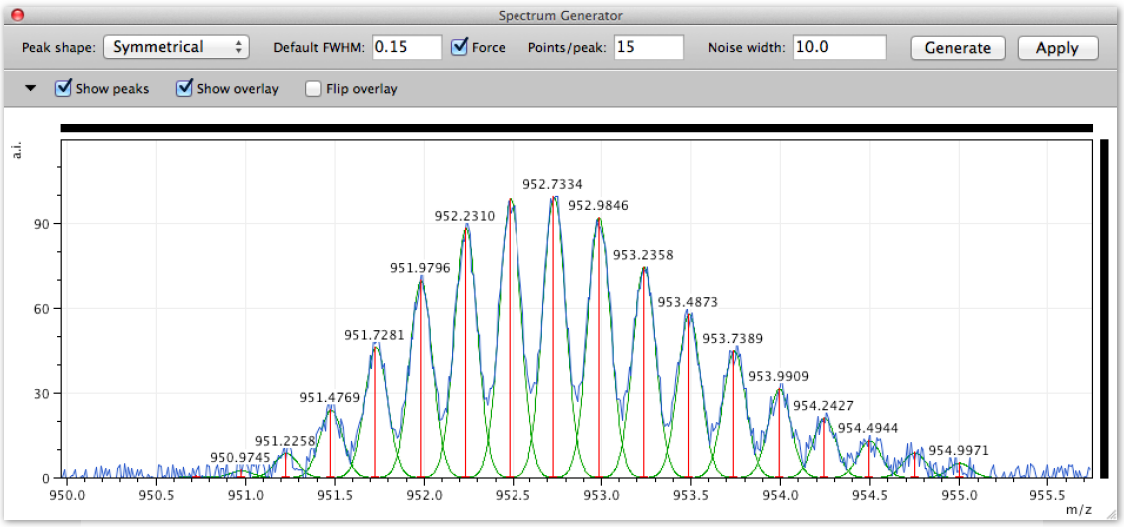
➡ **To compare:**
Specify all the parameters and press Compare button from the panel toolbar. Click on any *m/z* value in the peak lists table to see corresponding matches.

➡ **To remove peak list temporarily:**
Disable document by clicking the corresponding document bullet in the main *Documents Panel*.

➡ **To copy tables into clipboard:**
Click into the table you'd like to copy and press Command+C (Ctrl+C on MS Windows and Linux).

18.Spectrum Generator

In various cases, such as identification of peak overlaps or validation of different algorithms, it might be useful to generate an artificial mass spectrum to compare with acquired data or to test an algorithm capability. Using *Spectrum Generator* tool you can model each peak of the peak list of selected document as either symmetric (Gaussian) or asymmetric (half-Gaussian, half-Lorentzian) curve with corresponding intensity, specified width (FWHM) and number of points per peak. All the peaks are combined together to make final profile spectrum, which can be overlaid with *Spectrum Viewer* content or saved to selected document. To add more realistic look you can apply some random noise of specified width.



Spectrum Generator tool.

- *Peak shape* - symmetric (Gaussian) or asymmetric (half-Gaussian, half-Lorentzian) peak shape.
- *Default FWHM* - default FWHM value used if peak's own value is unknown.
- *Force* - use default FWHM value even if peak's own value is known.
- *Points/peak* - approximated number of points to draw each peak.
- *Noise width* - width of randomly generated noise.
- *Show peaks* - show individual peak shapes in generated spectrum.
- *Show overlay* - show generated profile in the main spectrum viewer.
- *Flip overlay* - show overlaid profile flipped in the main spectrum viewer.
- *Generate* - generate spectrum according to current parameters.
- *Apply* - apply generated profile spectrum to current document.

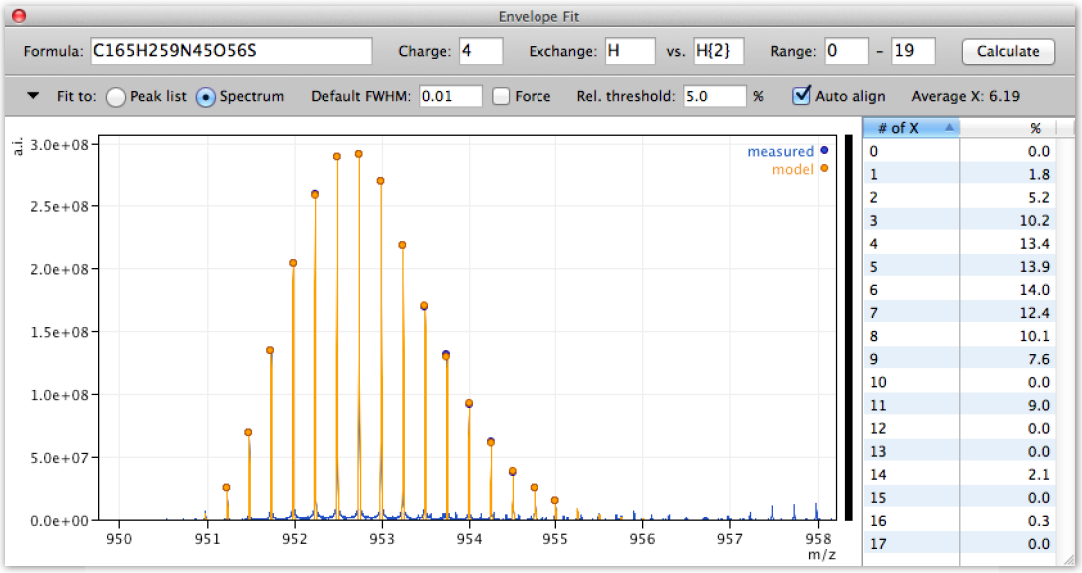
➡ **To model artificial mass spectrum:**
Make a new blank document by choosing File → New from the main menu. Press button from the *Peaklist Panel* bottom toolbar to show peak editor and define a peak list you'd like to model. Press button from the main toolbar or choose Tools → Spectrum Generator from the main menu to show *Spectrum Generator* tool. Specify all the parameters and press Generate button.

➡ **To save generated spectrum to current document**
Generate artificial spectrum and press Apply button. Current document's spectrum will be replaced by the newly generated. This operation can be undone.

Use asymmetrical peak shape to model TOF instrument spectra.

19.Envelope Fit

Envelope Fit serves as a simple tool to determine incorporation of heavy atoms into a molecule. Hydrogen/deuterium exchange experiment might be a typical example. In general, it calculates all the possible isotopic patterns for specified *Formula* and *Exchange* loss/gain formulae, and uses their linear combination and least-square fitting to fit modeled patterns to acquired data. Percentage of each individual state as well as average incorporation are shown as a result.



Envelope Fit tool.

- *Formula* - basic formula to use for modeling.
- *Charge* - charge state of the peak to fit to.
- *Exchange* - atoms to be exchanged (loss vs. gain).
- *Range* - minimum and maximum number of exchanged atoms.
- *Fit to* - acquired data type to fit to.
- *Default FWHM* - default FWHM value used if peak's own value is unknown.
- *Force* - use default FWHM value even if peak's own value is known.
- *Rel. threshold* - relative intensity threshold for peaks involved (to basepeak of relevant mass range).
- *Auto align* - automatically align acquired data with modeled envelope.
- *Average X* - calculated average number of exchanged atoms.

➡ **To show envelope fit tool:**
Press EX button from the main toolbar or choose Tools → Envelope Fit from the main menu.

➡ **To calculate peptide sequence deuteration:**
Use *Sequence Editor* to define a sequence. Right-click on the sequence title in *Documents Panel* and choose *Send to Envelope Fit...* to send data to *Envelope Fit* panel. Specify all the parameters and press Calculate button. (Use *"H"* and *"H{2}"* for the *Exchange* values.)

Sequences, sequence matches and annotations can be sent to *Envelope Fit* by right-clicking the item in *Documents Panel* and choosing *Send to Envelope Fit...* Similarly, any generated peptide can be used as well from *Protein Digest* or *Mass Search* panels.

Use relevant exchange *Range* to speed up calculation or discard unwanted peaks.

Use *Rel. threshold* or make your own peak list then *Fit to Peak list* to discard unwanted peaks.

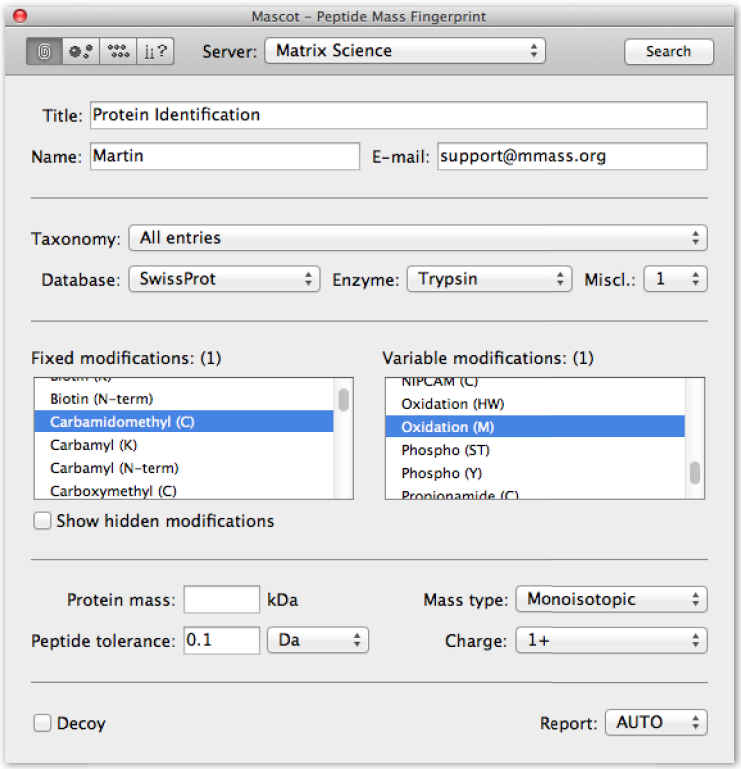
If a sequence object is sent to *Envelope Fit* tool and H/H{2} are set for exchange, corresponding deuteration range is calculated automatically.

Auto align feature should be turned on in most cases. It is used to ensure that corresponding peak maxima are used for each isotope fitting.

Be sure to have a correct charge specified!

20.Mascot Search

mMass provides an interface that allows data to be directly sent to the main tools available on *Mascot* website (http://www.matrixscience.com); *Peptide Mass Fingerprint, Sequence Query* and *MS/MS Ion Search*. When Search button is pressed, temporary *HTML* page is generated containing all the parameters and using *JavaScript*, the page is automatically sent to selected server. Please see the *Mascot* website for more information about the tools and the form fields.



Peptide Mass Fingerprint search tool.

➡ **To show peptide mass fingerprint tool:**
Press button from the main toolbar to show Mascot tools and press button from the panel toolbar or choose Tools → Mascot Peptide Mass Fingerprint from the main menu.

➡ **To show MS/MS ion search tool:**
Press button from the main toolbar to show Mascot tools and press button from the panel toolbar or choose Tools → Mascot MS/MS Ion Search from the main menu.

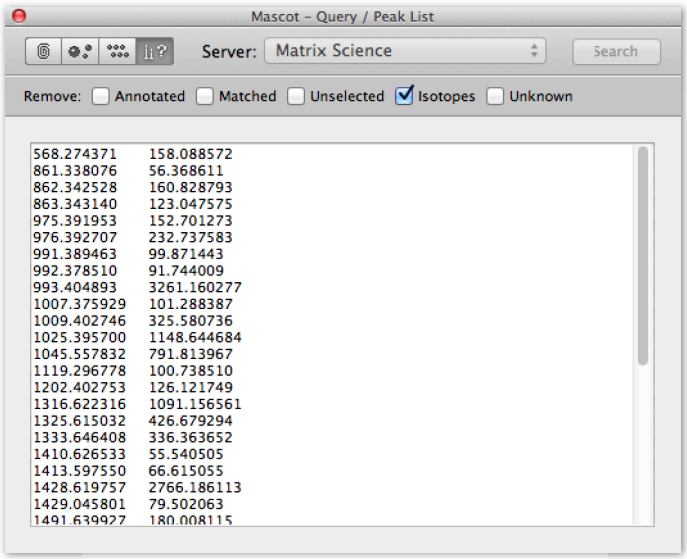
➡ **To show sequence query tool:**
Press button from the main toolbar to show Mascot tools and press button from the panel toolbar or choose Tools → Mascot Sequence Query from the main menu.

➡ **To change server:**
Select the *Server* in the panel toolbar.

➡ **To send data to server:**
Specify all the parameters and press Send button in the panel toolbar. *HTML* page will be generated and sent to specified server. Results show up in your default web browser.

➡ **To define new server:**
Choose Libraries → Mascot Servers from the main menu to show up *Mascot Servers Library* editor. See *Libraries* chapter for more information.

➡ **To specify peak list or query:**
Press from the panel toolbar and type your query manually or peak list filters.



Query / Peak List.

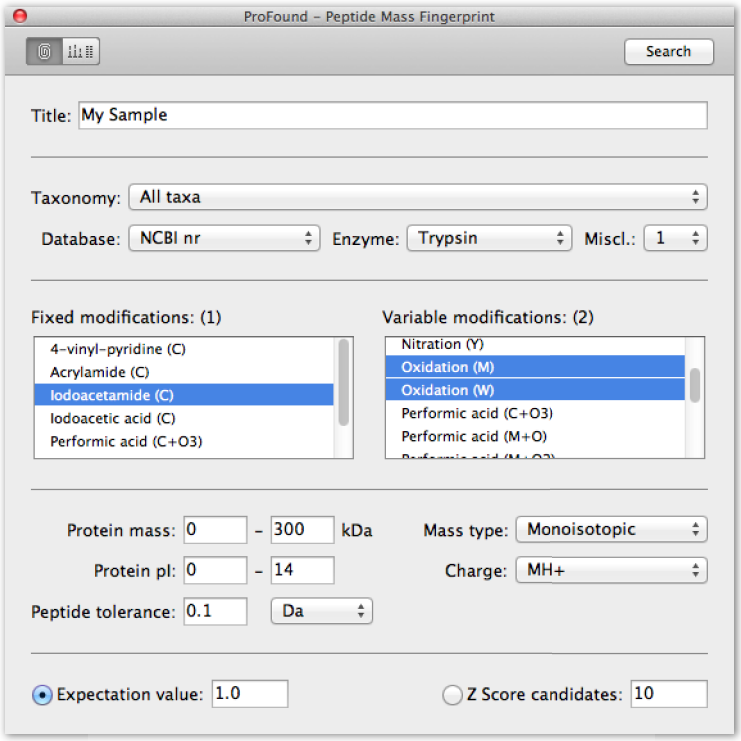
- *Annotated* - remove peaks with user's annotation.
- *Matched* - remove peaks matched to sequence peptide or fragment.
- *Unselected* - use only peaks selected in the *Peaklist Panel*.
- *Isotopes* - remove isotopes.
- *Unknown* - remove peaks with no isotope number or charge assigned.

See http://www.matrixscience.com website for more information about *Mascot* tools and form parameters.

Please note that while changing server, most of the form fields changed as well. Check if the form is filled correctly.

21. ProFound Search

mMass provides an interface that allows data to be directly sent to *ProFound* search tool available on a website of Professor Brian T. Chait group (http://prowl.rockefeller.edu). When Search button is pressed, temporary *HTML* page is generated containing all the parameters and using *JavaScript*, the page is automatically sent to *ProFound* server. Please see the *ProFound* website for more information about the tool and the form fields.



ProFound search tool.

➡ **To show ProFound tool:**
Press PRO FOUND button from the main toolbar or choose Tools → ProFound from the main menu.

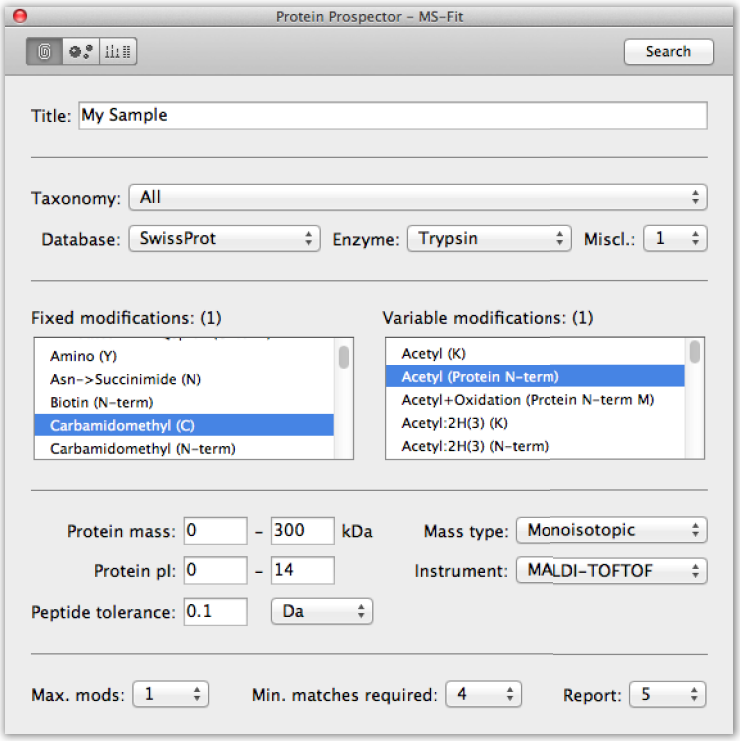
➡ **To send data to server:**
Specify all the parameters and press Send button in the panel toolbar. *HTML* page will be generated and sent to *ProFound* server. Results show up in your default web browser.

➡ **To specify peak list:**
Press from the panel toolbar and use peak list filters.

See http://prowl.rockefeller.edu website for more information about *ProFound* tool and form parameters.

22.Protein Prospector Search

mMass provides a simplified interface that allows data to be directly sent to the main tools available on *Protein Prospector* website (http://prospector.ucsf.edu); *MS-Fit* and *MS-Tag*. When Search button is pressed, temporary *HTML* page is generated containing all the parameters and using *JavaScript*, the page is automatically sent to *Protein Prospector* server. Please see the *Protein Prospector* website for more information about the tools and the form fields.



MS-Fit search tool.

➡ **To show MS-Fit tool:**
Choose Tools → Protein Prospector MS-Fit from the main menu.

➡ **To show MS-Tag tool:**
Choose Tools → Protein Prospector MS-Tag from the main menu.

➡ **To send data to server:**
Specify all the parameters and press Send button in the panel toolbar. *HTML* page will be generated and sent to *Protein Prospector* server. Results show up in your default web browser.

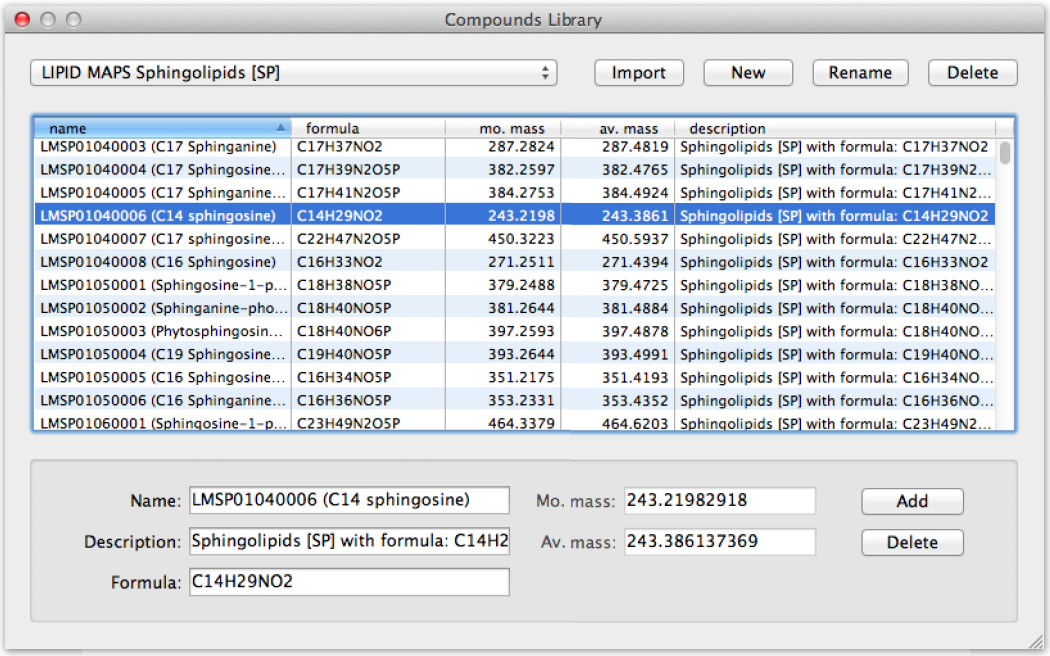
➡ **To specify peak list:**
Press from the panel toolbar and use peak list filters.

See http://prospector.ucsf.edu website for more information about *ProteinProspector* tools and form parameters.

23. Libraries

All *mMass's* libraries and configuration files are located within a single configuration folder having different locations, depending on your platform. On Mac OS X, all configuration files can be found under *"~/Library/Application Support/mMass/"*. On MS Windows and Linux systems, all configuration files can be found under *"configs"* folder, located directly under the *mMass's* main folder. All files are in XML-based formats and can be easily edited manually, however, it is strongly recommended to use dedicated *mMass's* tools.

23.1. Compounds



Compounds Library editor.

- *Name* - unique compound name.
- *Description* - brief description.
- *Formula* - compound formula
- *Mo. mass* - resulting monoisotopic mass.
- *Av. mass* - resulting average mass.

➡ **To show compounds library editor:**
Choose Libraries → Compounds from the main menu.

➡ **To import compounds lists:**
Open *Compounds Library* editor and press Import button on top of the panel. Select your compounds library and press Open button. Select compounds lists you'd like to import and press Import button. See above for location of all the libraries on your system.

➡ **To add new compounds list:**
Open *Compounds Library* editor and press New button on top of the panel. Type the name and press OK button.

➡ **To rename compounds list:**
Open *Compounds Library* editor, select the list you'd like to rename and press Rename button on top of the panel. Type the name and press OK button.

➡ **To delete compounds list:**
Open *Compounds Library* editor, select the list you'd like to delete and press Delete button on top of the panel.

➡ **To add compound:**
Open *Compounds Library* editor and select the list for the compound. Specify all the compound parameters and press Add button down on the panel.

➡ **To edit compound:**
Open *Compounds Library* editor and select the list containing the compound you'd like to edit. Change the compound parameters and press Add button down on the panel.

➡ **To rename compounds:**
Open *Compounds Library* editor and select the list containing the compound you'd like to rename. Change the *Name* and press Add button. New compound will be added and the old one can be deleted.

➡ **To delete compound:**
Open *Compounds Library* editor, select the list containing the compound you'd like to delete and press Delete button down on the panel.

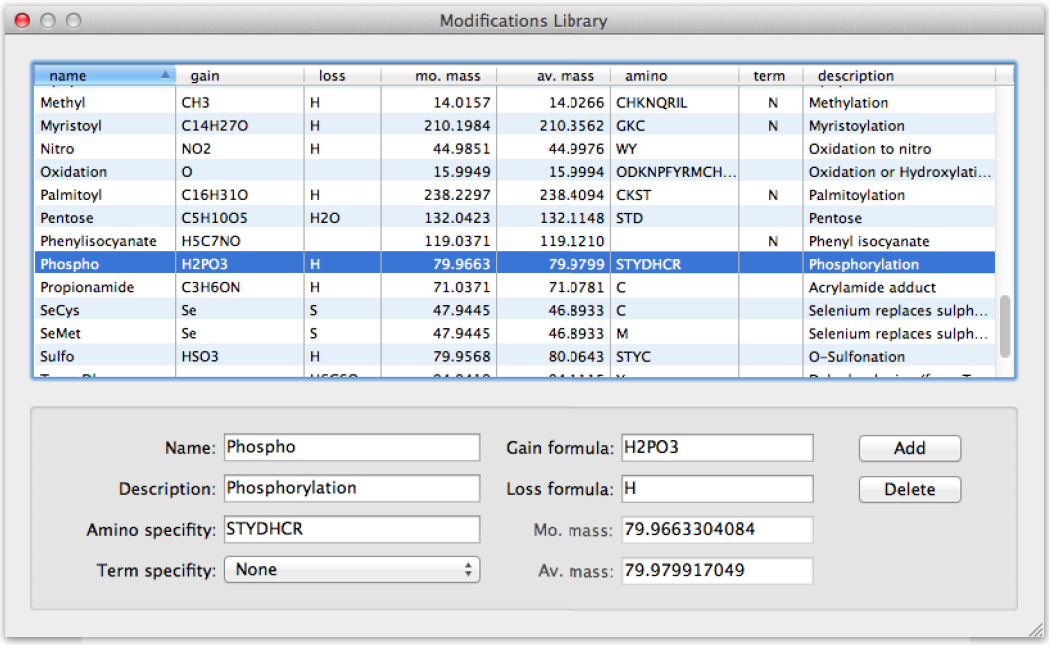
See *Mass Calculator* chapter for more information about formula syntax.

Monoisotopic and average masses are calculated automatically as you type the formula.

Please note that the group name must be unique for each group.

Please note that the compound name must be unique for each compound.

23.2. Modifications



Modifications Library editor.

- *Name* - unique modification name.
- *Description* - brief description.
- *Amino specifity* - amino acids which can be modified.
- *Term specifity* - sequence terminus which can be modified.
- *Gain formula* - formula to be added to a sequence.
- *Loss formula* - formula to be removed from a sequence.
- *Mo. mass* - resulting monoisotopic mass.
- *Av. mass* - resulting average mass.

➡ **To show modification library editor:**
Choose Libraries → Modifications from the main menu.

➡ **To add modification:**
Open *Modifications Library* editor, specify all the modification parameters and press Add button.

➡ **To edit modification:**
Open *Modifications Library* editor, select the modification you'd like to edit, change the parameters and press Add button. If the *Name* remains the same you will be asked to Replace modification definition.

➡ **To rename modification:**
Open *Modifications Library* editor, select the modification you'd like to rename, change the *Name* and press Add button. New modification will be added and the old one can be deleted.

➡ **To delete modification:**
Open *Modifications Library* editor, select the modification you'd like to delete and press Delete button. It is not possible to delete modification which is used in one of the opened documents.

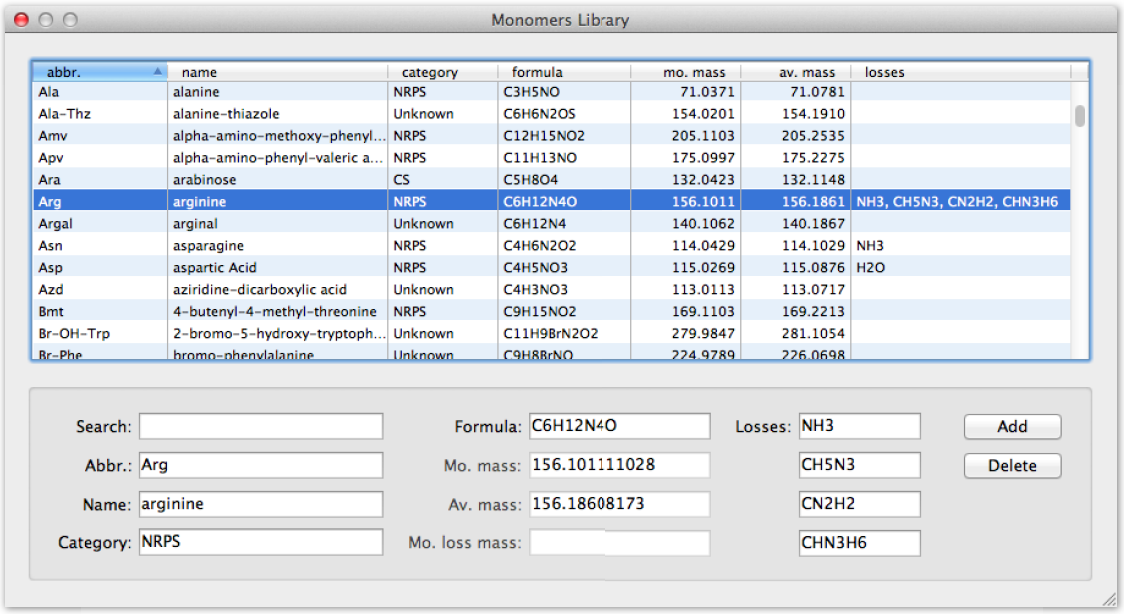

See *Mass Calculator* chapter for more information about formula syntax.


Monoisotopic and average masses are calculated automatically as you type the formula.


If you open a document with undefined modification this modification is added into your library automatically, however, the *Amino specifity* is set to ACDEFGHIKLMNPQRSTVWY.


Please note that the modification name must be unique for each modification.

23.3. Monomers



Monomers Library editor.

- *Search* - search tool.
- *Abbr.* - monomer unique abbreviation.
- *Name* - monomer name.
- *Category* - monomer category.
- *Formula* - monomer residual molecular formula (without water).
- *Mo. mass* - calculated monoisotopic mass.
- *Av. mass* - calculated average mass.
- *Mo. loss mass* - calculated monoisotopic mass of selected neutral loss.
- *Loss* - molecular formula of possible neutral loss.

➡ **To show monomer library editor:**
Choose Libraries → Monomers from the main menu.

➡ **To add monomer:**
Open *Monomers Library* editor, specify all the monomer parameters and press Add button.

➡ **To edit monomer:**
Open *Monomers Library* editor, select the monomer you'd like to edit, change the parameters and press Add button. If the *Abbr.* remains the same you will be asked to Replace monomer definition.

➡ **To delete monomer:**
Open *Monomers Library* editor, select the monomer you'd like to delete and press Delete button. It is not possible to delete monomer which is currently used in one of the opened documents.


See *Mass Calculator* chapter for more information about formula syntax.

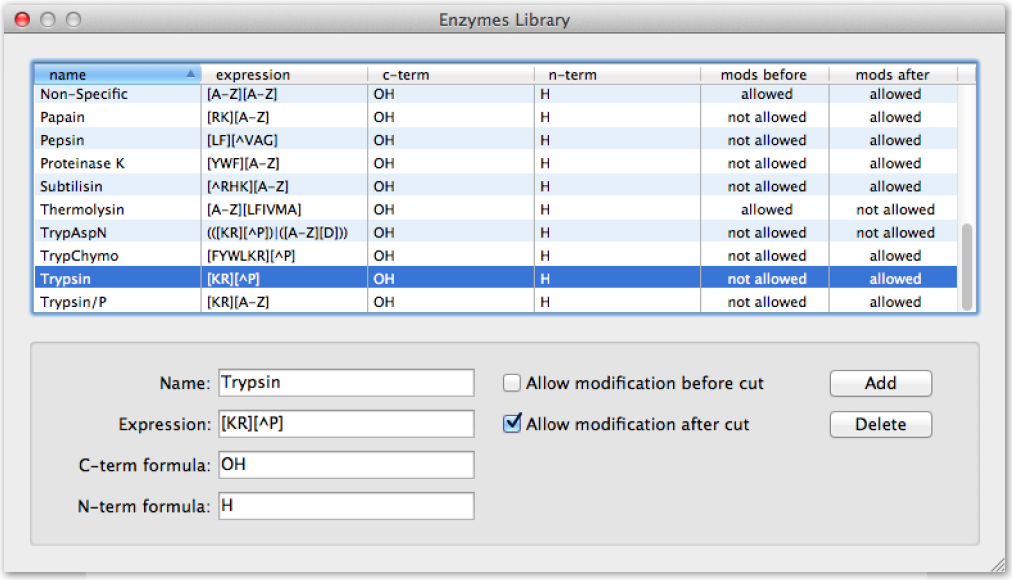

Monoisotopic and average masses are calculated automatically as you type the formula.

If you open a document with undefined monomer this monomer is added into your library automatically, however, only the *Abbr* and *Formula* parameters are set.

Use the *Search* field to quickly find a monomer you are looking for.

Please note that the monomer abbreviation must be unique for each monomer.

23.4. Enzymes



Enzymes Library editor

- *Name* - unique enzyme name.
- *Expression* - enzyme regular expression.
- *C-term formula* - formula to be added at new C-terminus.
- *N-term formula* - formula to be added at new N-terminus.
- *Allow modification before cut* - allow modifications before cleavage site.
- *Allow modification after cut* - allow modifications after cleavage site.

➡ **To show enzymes library editor:**
Choose Libraries → Enzymes from the main menu.

➡ **To add enzyme:**
Open *Enzymes Library* editor, specify all the enzyme parameters and press Add button.

➡ **To edit enzyme:**
Open *Enzymes Library* editor, select the enzyme you'd like to edit, change the parameters and press Add button. If the *Name* remains the same you will be asked to Replace enzyme definition.

➡ **To rename enzyme:**
Open *Enzymes Library* editor, select the enzyme you'd like to rename, change the *Name* and press Add button. New enzyme will be added and the old one can be deleted.

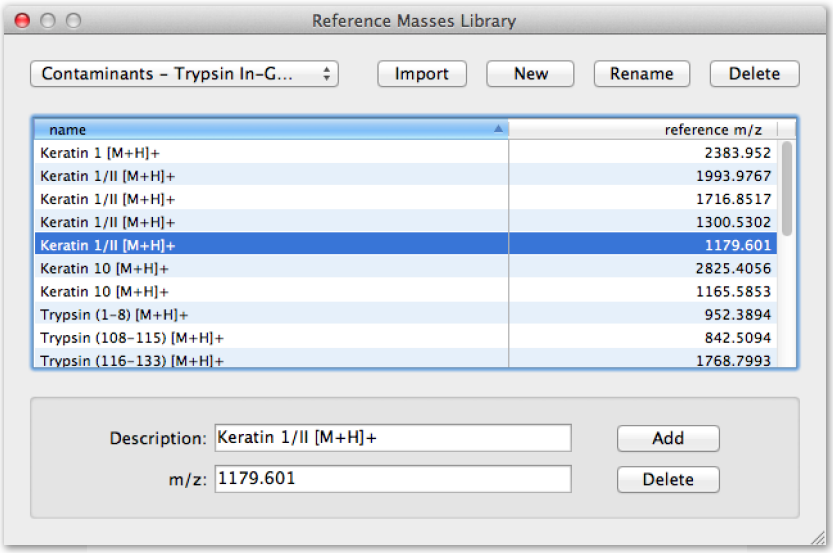
➡ **To delete enzyme:**
Open *Enzymes Library* editor, select the enzyme you'd like to delete and press Delete button.

The enzyme expression uses regular expression syntax where both, amino acids before and after cleavage must be defined. For example *[KR][A-Z]* for the Trypsin/P, where *[KR]* means that lysine or arginine must be before cleavage and *[A-Z]* means that any amino acid (i.e. character in the sequence) is allowed after cleavage. To block any amino acid use "^" like in the regular Trypsin *[KR][^P]*. See the http://docs.python.org/howto/regex.html#regex-howto for more information about regular expression syntax.

See *Mass calculator* chapter for more information about formula syntax.

Please note that the enzyme name must be unique for each enzyme.

23.5. Reference Masses



Reference Masses Library editor.

- *Description* - reference name.
- *m/z* - reference m/z value.

➡ **To show reference masses library editor:**
Choose Libraries → Reference Masses from the main menu.

➡ **To import reference lists:**
Open *Reference Masses Library* editor and press Import button on top of the panel. Select your references library and press Open button. Select references lists you'd like to import and press Import button. See above for location of all the libraries on your system.

➡ **To add new reference list:**
Open *Reference Masses Library* editor and press New button on top of the panel. Type the name and press OK button.

➡ **To rename reference list:**
Open *Reference Masses Library* editor, select the list you'd like to rename and press Rename button on top of the panel. Type the name and press OK button.

➡ **To delete reference list:**
Open *Reference Masses Library* editor, select the list you'd like to delete and press Delete button on top of the panel. Type the name and press OK button.

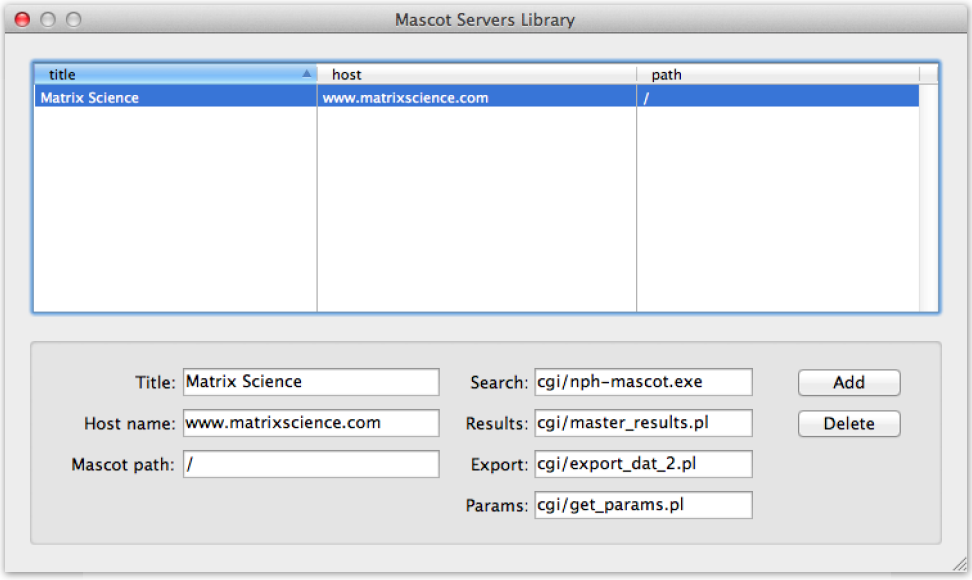
➡ **To add reference mass:**
Open *Reference Masses Library* editor and select appropriate reference list. Specify all the parameters and press Add button down on the panel.

➡ **To edit reference mass:**
Open *Reference Masses Library* editor and select the list containing the mass you'd like to edit. Change the parameters and press Add button down on the panel.

➡ **To rename reference mass:**
Open *Reference Masses Library* editor and select the list containing the mass you'd like to rename. Change the *Description* and press Add button. New reference mass will be added and the old one can be deleted.

➡ **To delete reference mass:**
Open *Reference Masses Library* editor, select the list containing the mass you'd like to delete and press Delete button down on the panel.

23.6. Mascot Servers



Mascot Servers Library editor.

- *Title* - server title.
- *Host name* - IP address or domain.
- *Mascot path* - path to the Mascot's main folder ("/" or "/mascot/" etc.).
- *Search* - path to the main search script (relative to the Mascot's main folder).
- *Results* - path to the results viewer script (relative to he Mascot's main folder).
- *Export* - path to the results export script (relative to he Mascot's main folder).
- *Params* - path to the form parameters values (relative to he Mascot's main folder).

➡ **To show mascot servers library editor:**
Choose Libraries → Mascot Servers from the main menu.

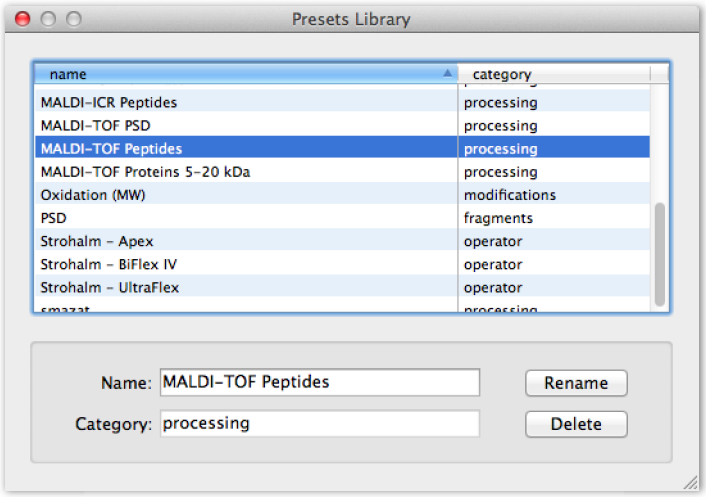
➡ **To add server:**
Open *Mascot Servers Library* editor, specify all the server parameters and press Add button.

➡ **To edit server:**
Open *Mascot Servers Library* editor, select the server you'd like to edit, change the parameters and press Add button. If the *Title* remains the same you will be asked to Replace server definition.

➡ **To rename server:**
Open *Mascot Servers Library* editor, select the server you'd like to rename, change the *Title* and press Add button. New server will be added and the old one can be deleted.

➡ **To delete server:**
Open *Mascot Servers Library* editor, select the server you'd like to delete and press Delete button.

23.7. Presets



Presets Library editor.

- *Name* - presets name.
- *Category* - presets category.

➡ **To show presets library editor:**
Choose Libraries → Presets from the main menu.

➡ **To rename presets:**
Open *Presets Library* editor, select the presets you'd like to rename, change the *Name* and press Rename button.

➡ **To delete presets:**
Open *Presets Library* editor, select the presets you'd like to delete and press Delete button.