[](https://www.biochem.mpg.de/mann)[](https://github.com/MannLabs/alphamap)[](https://www.biochem.mpg.de/en)**AlphaViz Tutorial**

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This step-by-step guide helps you to get started with our software AlphaViz.

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# Description

Software tools such as AlphaPept, MaxQuant or DIA-NN identify and quantify high amounts of proteins. After downstream processing in Perseus, MSstats or the Clinical Knowledge Graph, differentially expressed proteins become possible candidates for biomarker discovery. AlphaViz is an automated visualization pipeline to link these identifications with the original raw data and easily assess their individual quality or the overall quality whole samples.

Data that was processed by any of the above-mentioned software tools can be uploaded to AlphaViz and proteins of interests can be selected to explore all available information about the identified peptides. It shows the position of each peptide on the protein sequence, its extracted ion chromatogram or the spectra with the identified b- or y-ions. Additionally, it visualizes the position where the precursor was peaked for sequencing on MS1 and MS2/PASEF frames.

# Installation

## Windows

**Prerequisites: Windows 10** (a system update might be necessary in case older versions do not work).

**Important:** To prevent installation errors on Windows, we recommend uninstalling any previous AlphaViz version before installing a new one.

1. Download [the latest release](https://github.com/MannLabs/alphaviz/releases) for Windows (alphaviz\_gui\_installer\_windows.exe) from the GitHub repository and open the .exe file.
2. In the “User Account Control” dialog asking about permission for the app to make changes to your device press the “Yes” button.
3. In the appearing “Setup – alphaviz version X.X.X” dialog window accept the License Agreement and press the “Next” button.
4. Select the destination location for the installation of AlphaViz software (the size of the whole package is around 800 MB) and press the “Next” button.
5. In the next dialog window mark the “Create a desktop shortcut” check box and press the “Next” button.
6. Check the setting and if everything is correct, press “Install” button. You may go back to change some settings using the “Back” button or “Cancel” the installation.
7. Wait till the installation process is finished and with the marked “Launch alphaviz” check box press the “Finish” button.
8. In the appearing “Windows Security Alert” dialog window press the “Allow access” button that will prevent the Windows Defender Firewall from blocking the AlphaViz tool on your PC.
9. Check your default browser (Google Chrome or Mozilla Firefox is recommended for fast running and correct display of AlphaViz visualization) and start working with the tool.

\* If you install AlphaViz for all users, you might need admin privileges to run it (right-click on the AlphaViz logo on your desktop and select "Run as administrator").

## MacOS

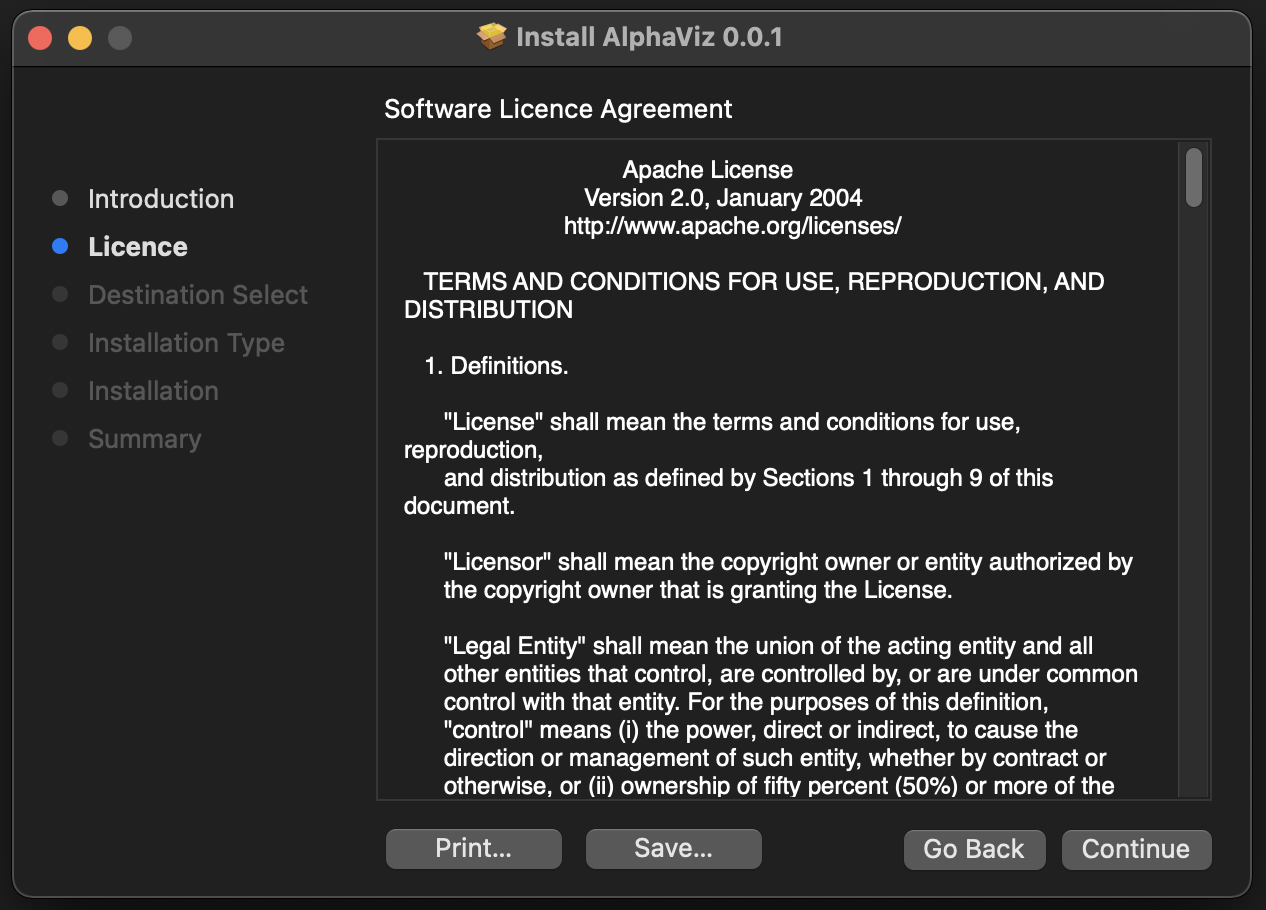
**Prerequisites: at least macOS Catalina (10.15) or higher (a system update might be necessary in case older versions do not work).**

**IMPORTANT WARNING:** Since AlphaViz uses AlphaTims to read LC-TIMS-Q-TOF data from Bruker’s timsTOF pro instrument (Bruker Daltonik), some calibration functions for it are provided by Bruker as libraries and are only available on Windows and Linux. Therefore, to avoid any problems with MS2 spectra quality assessment please use .hdf files into which .d folders can be converted using the AlphaTims’s CLI on Windows or Linux machines as described [in the AlphaTims CLI manual](https://github.com/MannLabs/alphatims/blob/master/alphatims/docs/cli_manual.pdf).

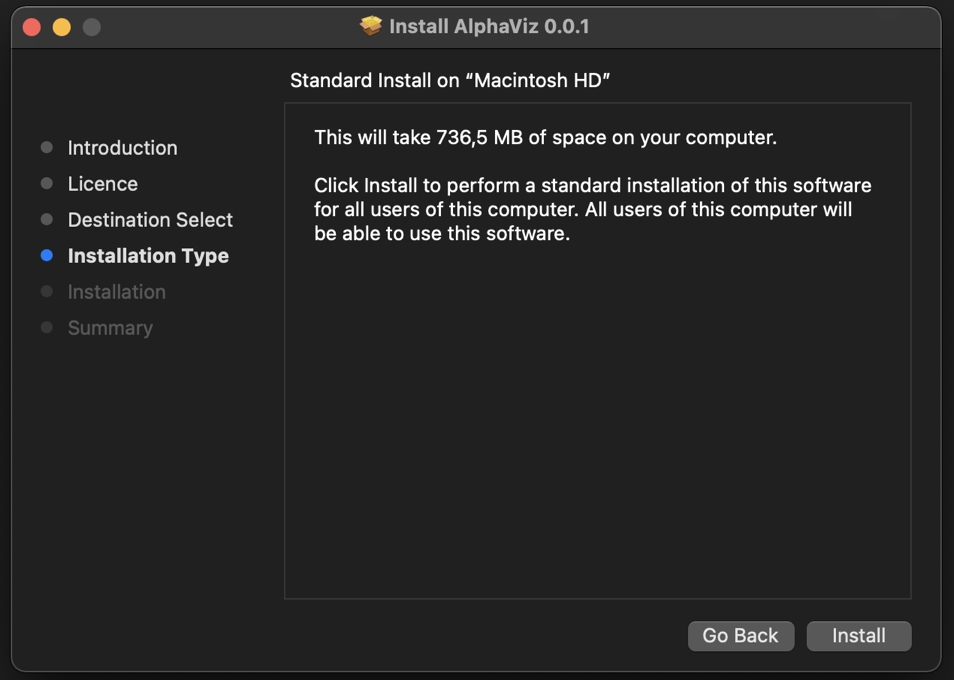
1. Download [the latest release](https://github.com/MannLabs/alphaviz/releases) for macOS (alphaviz\_gui\_installer\_macos.pkg) from the GitHub repository and open the .pkg file.
2. Click “Continue” on the appearing “Install AlphaViz X.X.X” dialog window.



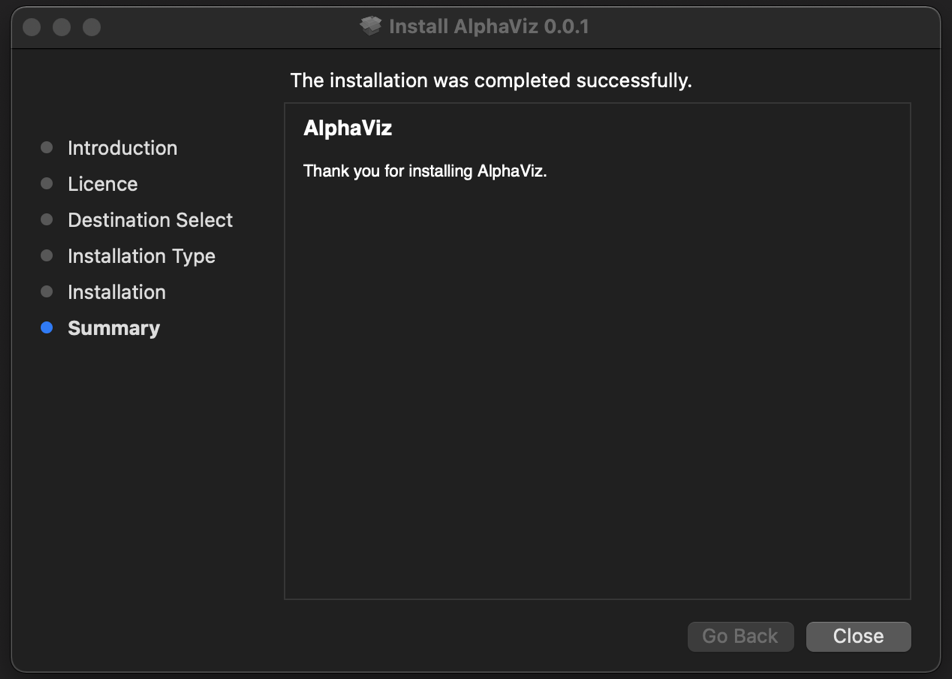
1. In the **License** section the Software License Agreement (Apache License) will be shown for you. To continue the installation, press "Continue" button and in the appeared pop-up window you need to agree with the regulations of the license.



1. Press “Install” to start the installation (the size of the whole package is around 800 MB). This might take a few minutes.

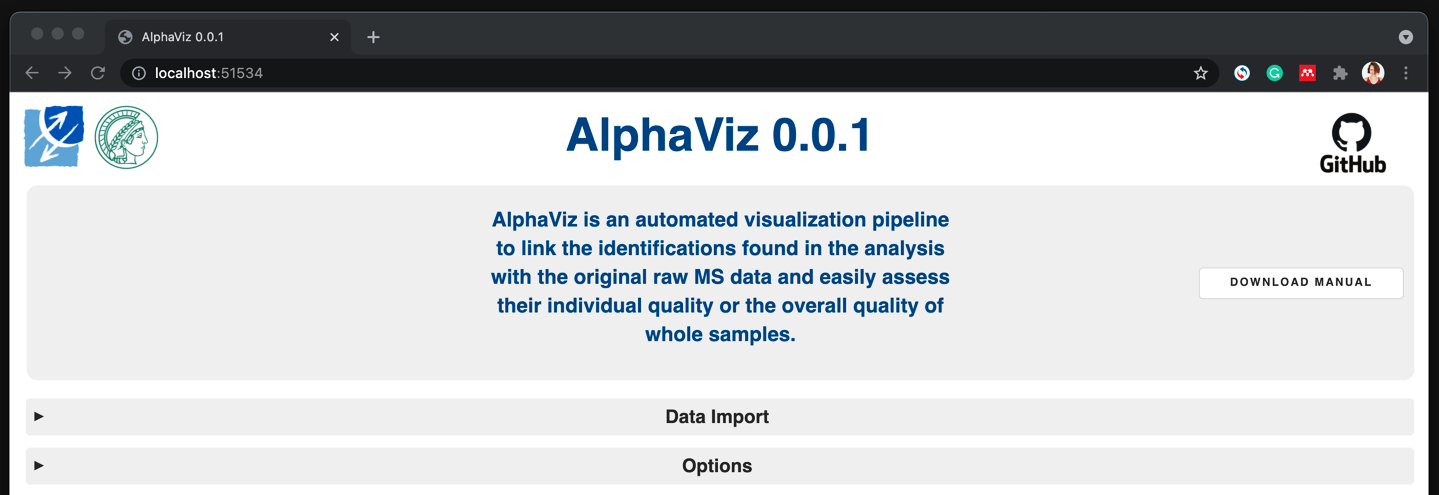


1. Click “Close” to quit the installation menu. AlphaViz is now available in the applications folder on your MacOS.



\* In rare cases, you might get an error message that the “alphaviz\_gui\_install\_macos.pkg” cannot be opened because it is coming from an unidentified developer. This error can be avoided by pressing the OPTION key or by going to ‘Security and Privacy’ under ‘System Preferences’ and enable AlphaViz installation.

# How to use AlphaViz

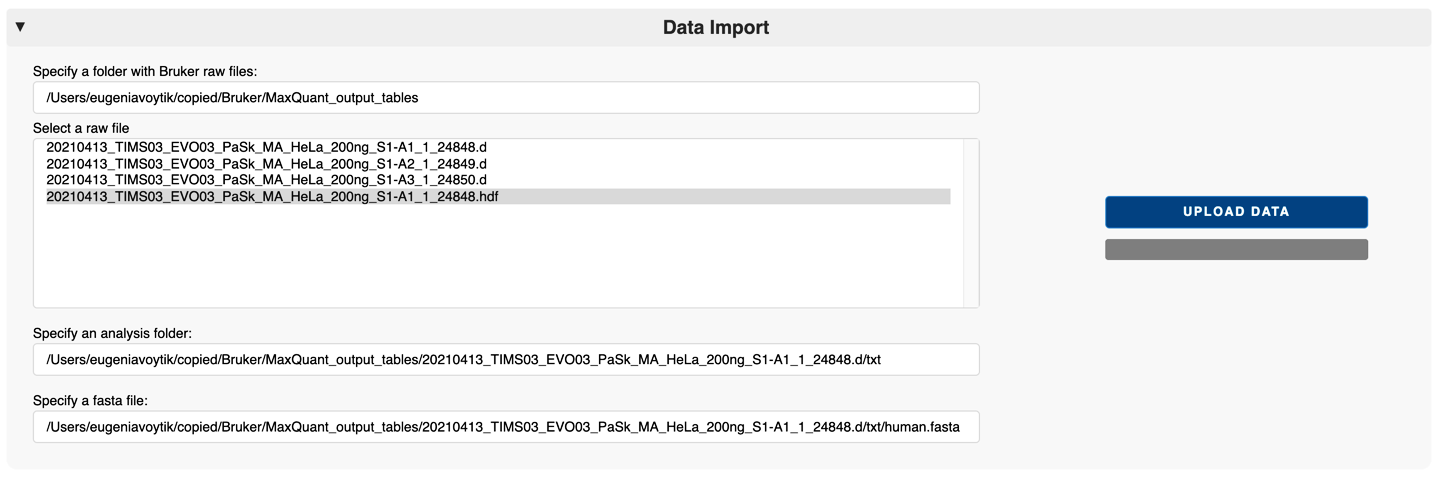


After launching AlphaViz, firstly a terminal window will open containing the tool’s background information and after that you can access a new tab called “AlphaViz X.X.X” in the default browser with the “localhost:XXXXX” URL. For better performance, we recommend to use Google Chrome or Mozilla Firefox. To close the tool, just close the opened “AlphaViz X.X.X” tab or press “Ctrl + c” in the running terminal window.

Pressing “Download manual” button you can also download and check out this GUI manual.

## Data import

To start working with AlphaViz, you need to upload your proteomic dataset analyzed by MaxQuant and the fasta file that was used for the data analysis.



a

b

e

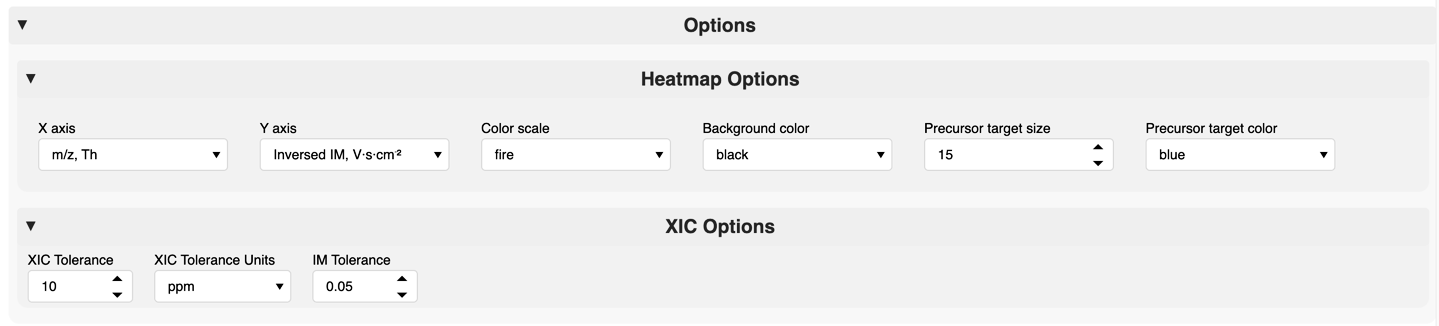
d

c

* 1. Provide the filepath to the folder with the .d or .hdf files in the “Specify a folder with Bruker raw files:” field, e.g. “D:\Bruker\MaxQuant\_output\_tables” (Windows) or “/Users/eugeniavoytik/copied/Bruker/MaxQuant\_output\_tables” (MacOS).
  2. Once the previous step is completed, the “Select a raw file” field will automatically display all .d and .hdf files present in the specified folder. By default, the first file out of all present is selected (highlighted in grey), but you can click on any other file to select it.
  3. In the “Specify an analysis folder:” field you need to enter the path to the MaxQuant’s output “txt” folder. If this “txt” folder is inside the .d folder specified in step b), this path will be filled in automatically. Otherwise, just manually copy and paste the path to the “txt” folder into this field.
  4. In the “Specify a fasta file:” field the path to the .fasta file that was used for analysis should be specified. As in step c), this field can also be filled in automatically if this .fasta file is located inside the .d folder.
  5. Press the “Upload data” button. The loading process is indicated by a progress bar. Once the data has been uploaded, the “Data Import” panel will automatically collapse and new tabs will appear in AlphaViz.

## Options

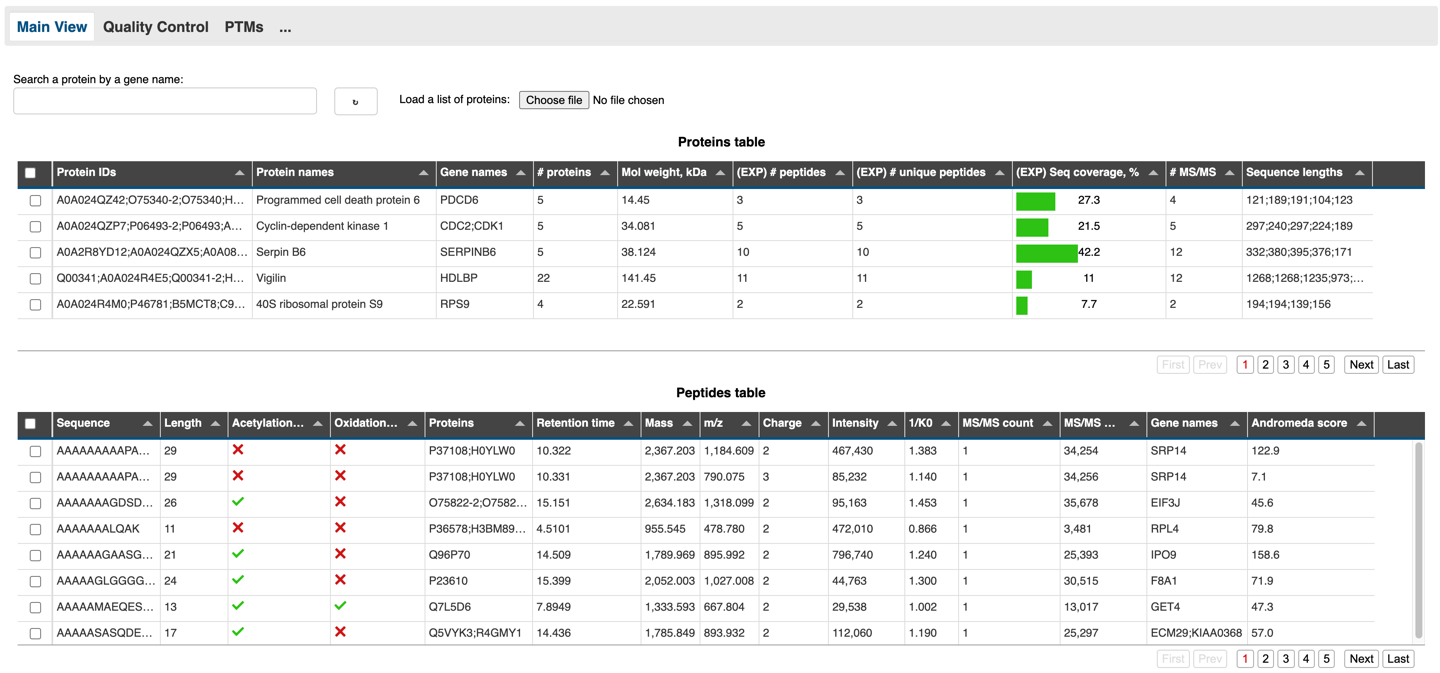
To customize the visualization of all available graphs in AlphaViz, you can change their options in the “Options” panel. For all available graphs you can find separate option sections such as “Heatmap Options”, “XIC Options”, etc.



## Data Visualization

Once the data has been uploaded to AlphaViz, several new tabs will appear. By default the “Main View” tab is opened (highlighted in white), but you can switch between tabs by clicking on them.

### “Main View” tab



a

b

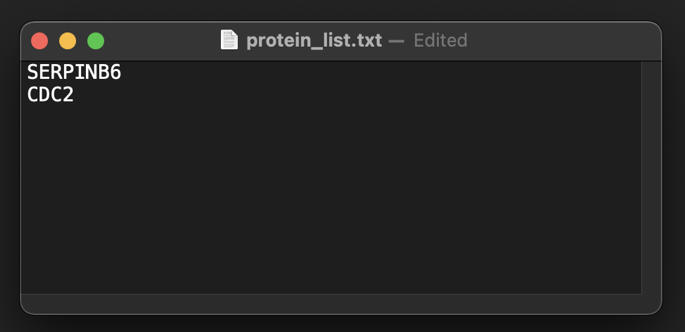
c

d

e

To start a visual inspection, simply select the protein of interest. This can be done in several different ways:

1. Start typing the gene name of the protein of interest in the “Search a protein by a gene name:” field. After entering the first three letters you will get a list of all the available gene names’ of proteins to choose from. The protein table (d) will be filtered based on this name.
2. To filter the protein table (d) based on the list of proteins, load a list of pre-selected proteins by pressing the “Choose file” button. In doing so, you can upload a .txt file containing a list of gene names one identifier per line.

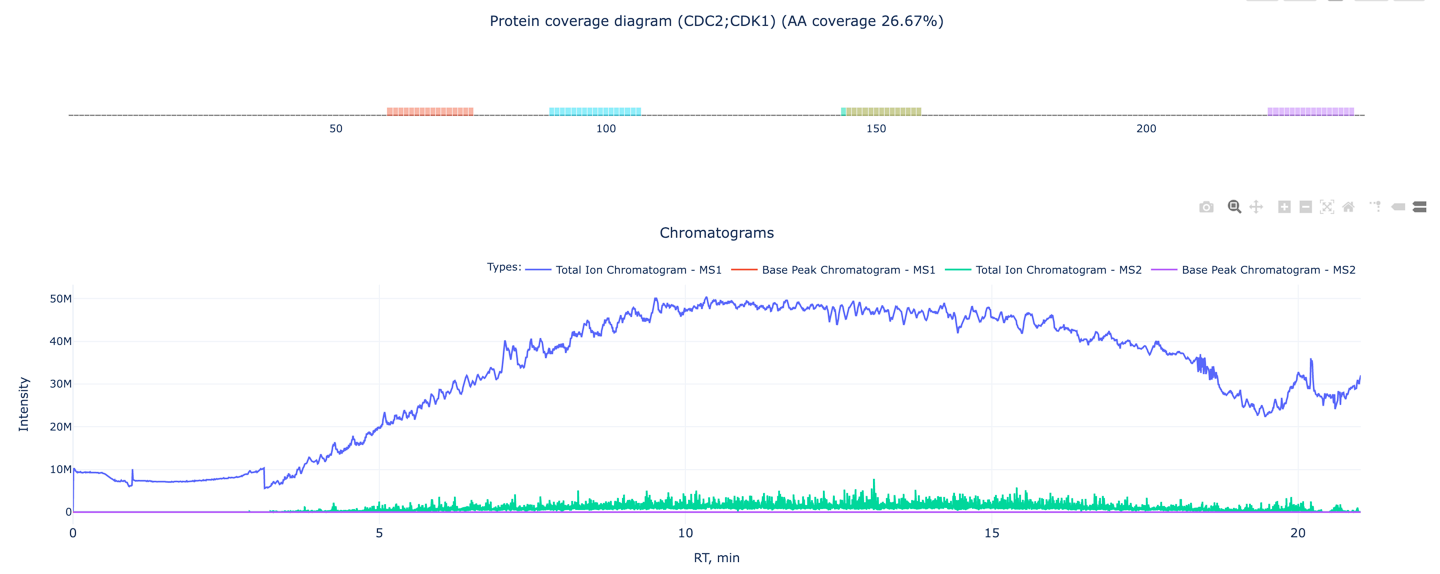


1. To return the original protein list displayed in the protein table (d) press the “Undo” button.
2. The protein table can be pre-filtered as described above to make it easier to use. To navigate through the table, click on the page number or the “Next”|“Last” buttons. To select a protein for subsequent visualization, check/uncheck the box for the individual protein.

IMPORTANT! Only one protein can be used for visualization at a time. If more than one protein is checked in the protein table, only the first selected will be used later.

1. After checking the protein in the previous step, the peptides table will be filtered for all peptides of that protein identified in the MaxQuant analysis.

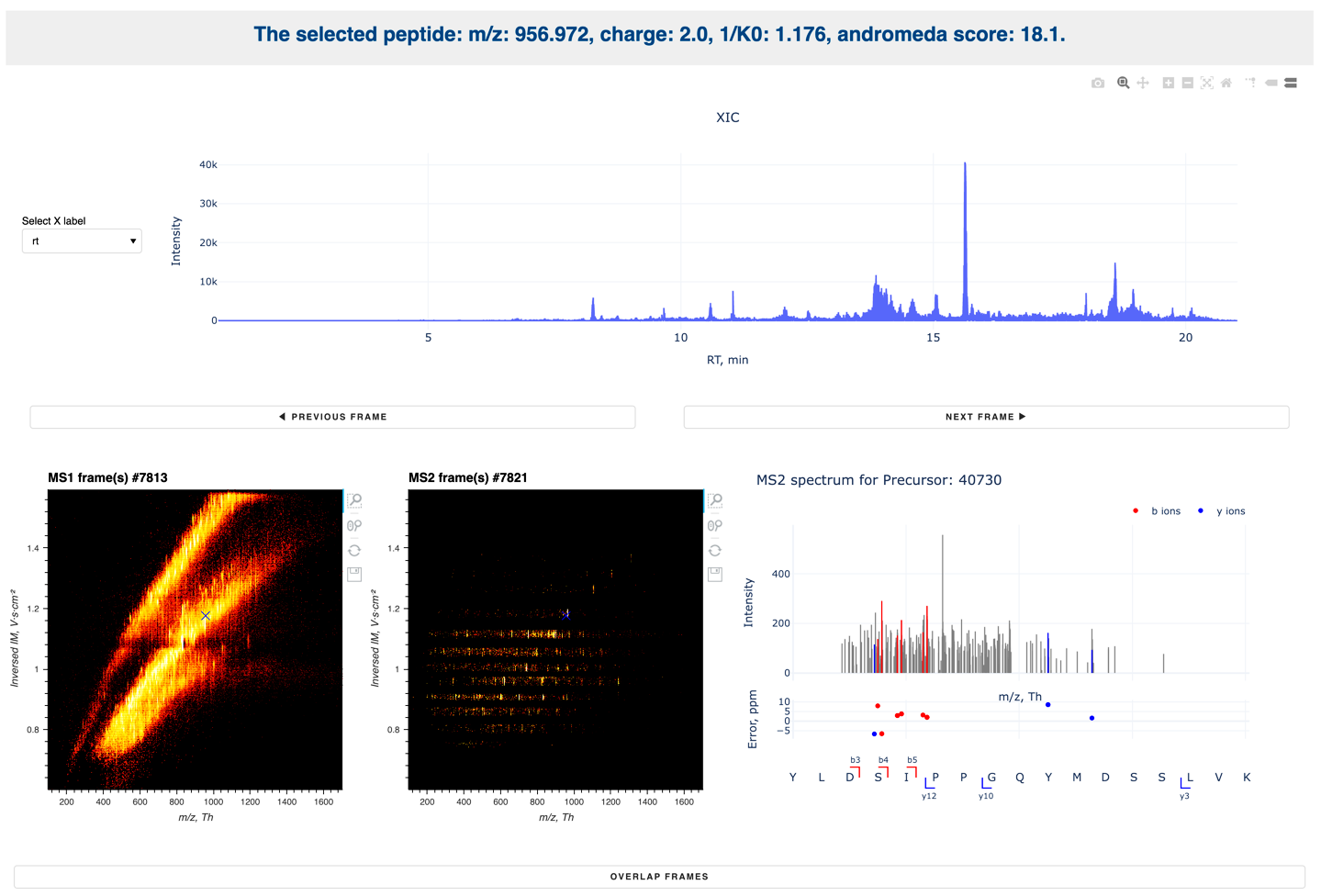
At the same time the first plots will be shown below the tables. The first graph(f) shows a protein coverage diagram for the peptides of the selected proteins where the position of the peptides on the protein sequence and the percentage of amino acid coverage can be seen. Various types of chromatograms (g) are also shown including total ion chromatograms and base peak chromatograms for all precursor (MS1) and fragment (MS2) ions during the whole run.



g

f

To assess the quality of raw data for each peptide separately check the individual peptide in the peptide table (e).



l

k

j

i

h

For the peptide selected in the peptide table (e) we can examine:

1. A header (h) containing important information about the peptide, such as its m/z, charge, ion mobility and the score calculated in the analysis software, e.g. the andromeda score of MaxQuant software.
2. A line plot showing either an extracted ion chromatogram (XIC) or mobilogram depending on whether "rt" or "mobility" is chosen from the “Select X label” drop-down menu. XIC parameters can be set in the Options > XIC Options panel.
3. M/z vs. IM heatmaps of the intensity values for frames MS1 (j) and MS2 (k). The positions of the peptide in the frames are marked as ‘X’. To switch between the frames in which the peptide was analyzed, press the “Previous frame” or “Next frame” buttons. To overlap the frames, press the “Overlap frames” button. Heatmap parameters can be set in the Options > Heatmap Options panel.
4. A combined graph (l) showing the interactive MS2 spectrum highlighting the b- and y-ions identified by the analysis software, a mass error plot for the identified b- and y-ions and the peptide sequence showing which ions have been identified.

In the selected example, the visualization of the mass spectrum explains the low andromeda score, as only a few b- and y-ions were identified for the peptide, which is usually insufficient for a confident identification.

### “Quality Control” tab

Hereby you can interactively examine the quality control plots of the entire sample.

