

# AlphaMap Tutorial

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

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## Table of Contents

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Program Description .....	- 1 -
Installation .....	- 2 -
- Windows .....	- 2 -
- MacOS .....	- 4 -
- Linux .....	- 5 -
How to use AlphaMap .....	- 6 -

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## Program Description

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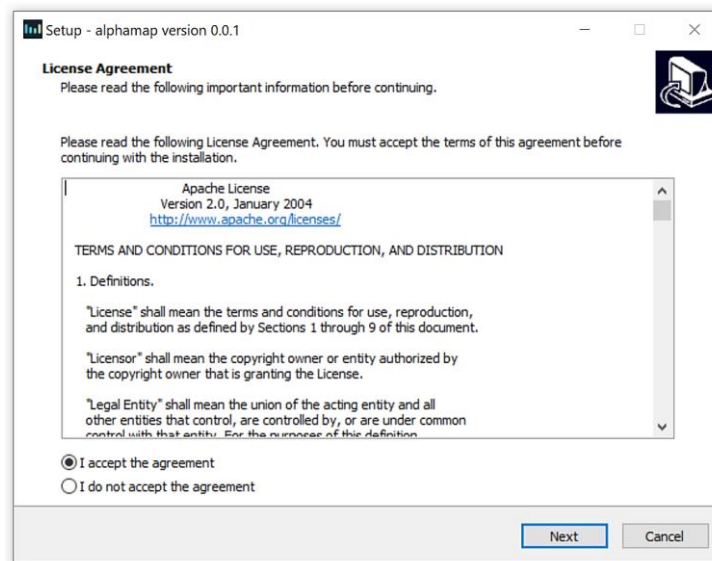
This website enables the exploration of proteomic datasets on the peptide level. It is possible to evaluate the sequence coverage of any identified protein and its post-translational modifications (PTMs). AlphaMap further integrates all available UniProt sequence annotations as well as information about proteolytic cleavage sites.

# Installation

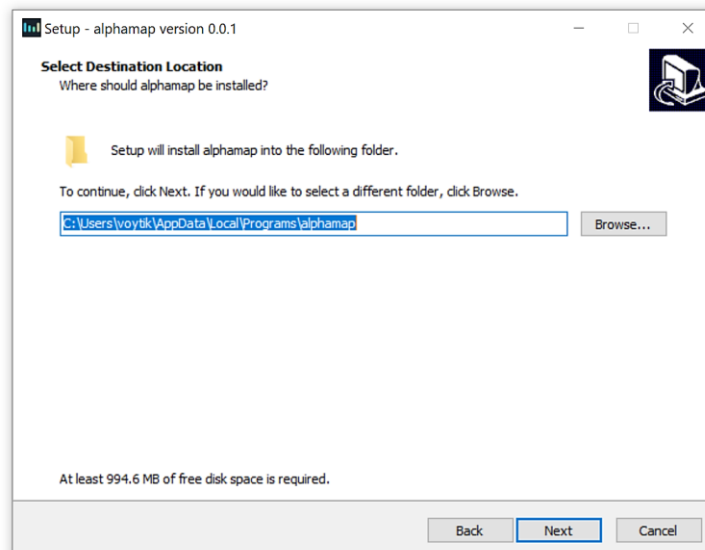
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## Windows:

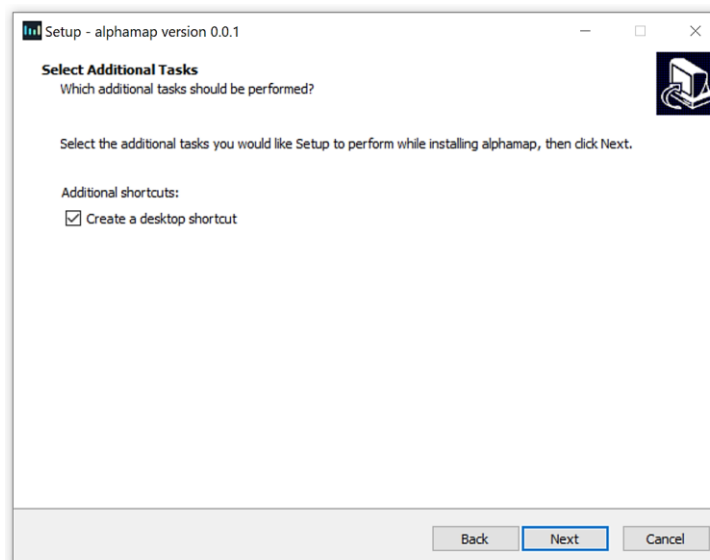
1. Download [the latest release](#) for Windows (alphamap\_installer\_windows.exe) from the GitHub repository and open the .exe file.
2. In the “User Account Control” dialog asking about permission for the app to make changes to your device press the “Yes” button.
3. In the appearing “Setup – alphamap version X.X.X” dialog window accept the License Agreement and press the “Next” button.



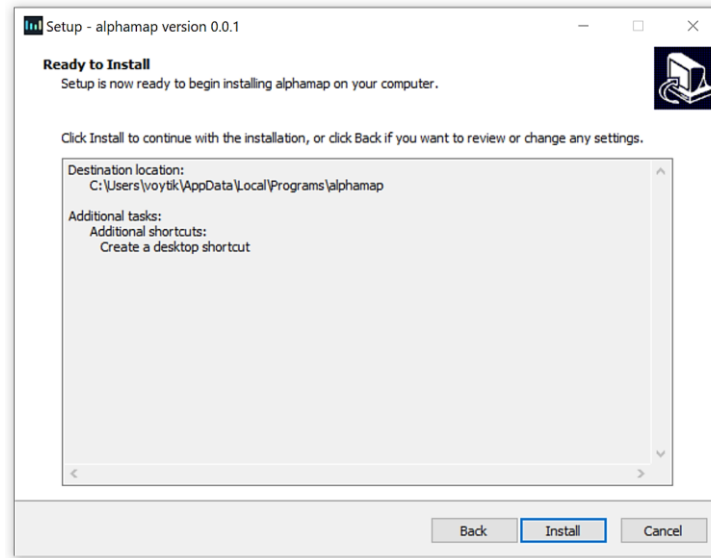
4. Select the destination location for the installation of AlphaMap software (the size of the whole package is 994.6 MB) and press the “Next” button.



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 alphamap” 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 AlphaMap tool on your PC.
9. Check your default browser (Google Chrome or Mozilla Firefox are suggested for the fast running of the AlphaMap) and start working with the tool.

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

## MacOS:

1. Download [the latest release](#) for macOS (alphamap.app.zip) from the GitHub repository, unzip the file and move it to your applications folder. By doing it, you accept the terms of the AlphaMap license agreement and all third-party licenses.
2. Launch the file and take into account that the first opening of the tool on macOS takes a long time to load. The loading time will be significantly reduced upon the second launch.
3. Check your default browser (Google Chrome or Mozilla Firefox are suggested for the fast running of the AlphaMap) and start working with the tool.

\* If nothing happens when you launch AlphaMap, you might need to grant it permissions by going to the macOS menu "System Preferences | Security & Privacy | General". If the problem still persists, it is possible that macOS already quarantined the AlphaMap app. It can be

removed from quarantine by running `<xattr -dr com.apple.quarantine alphamap.app>` (copy everything between <>) in your terminal in the application folder where alphamap.app is located.

## Linux:

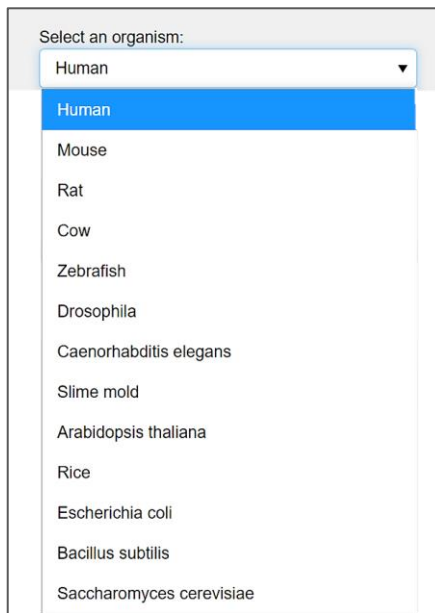
1. Download [the latest release](#) for Linux (alphamap) from the GitHub repository to the desired location. By doing it, you accept the terms of the AlphaMap license agreement and all third-party licenses.
2. To run it, drag and drop the folder in the terminal and the GUI will open as a tab in your default browser. Google Chrome or Mozilla Firefox are suggested for the fast running of AlphaMap.

\* If permissions are wrong, run `<chmod +x alphamap>` (copy everything between <>) in the terminal (at the right location).

# How to use AlphaMap

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1. Select the organism of your proteomic study. Currently, the [13 most popular organisms based on UniProt](#) are available for selection, including: Human [Taxon identifier=9606], Mouse [10090], Rat [10116], Cow [9913], Zebrafish [7955], Drosophila [7227], Caenorhabditis elegans [6239], Slime mold [44689], Arabidopsis thaliana [3702], Rice [39947], Escherichia coli (strain K12) [83333], Bacillus subtilis (strain 168) [224308], Saccharomyces cerevisiae (strain ATCC 204508 / S288c) [559292].



Select an organism:

- Human
- Mouse
- Rat
- Cow
- Zebrafish
- Drosophila
- Caenorhabditis elegans
- Slime mold
- Arabidopsis thaliana
- Rice
- Escherichia coli
- Bacillus subtilis
- Saccharomyces cerevisiae

2. Upload your proteomic datasets analyzed by AlphaPept, MaxQuant or Spectronaut:
  - a) Provide the filepath to the result file in the "Upload a result file:" field, e.g. "D:\spectronaut\_output.csv".
  - b) Wait for samples to be displayed in the "Select samples" field. The loading process is indicated by a spinner symbol.
  - c) (optional) Select either all samples (default) or any specific sample(s) to visualize together as one trace.
  - d) (optional) Choose a name by which the selected sample(s) will be displayed in the figure. If no name is provided, the original names of all selected samples will be concatenated by semicolon. If 'all samples' were selected, the filename will be the default name.

- e) (optional) Provide a prefix or suffix to be removed from the original names of the selected samples. This option only applies if no user defined name is provided (see d).

The screenshot shows a web interface with four main sections: a) Upload a result file: A text input field containing 'D:\alphamap\testdata\test\_spectronaut\_input.csv'. b) A radio button, currently unselected. c) Sample name: A text input field containing '1 and 2 runs'. d) Prefix / suffix: An empty text input field. e) Select samples: A list box with a scroll bar containing the following items: 'All samples', 'raw\_01', and 'raw\_02'. The 'raw\_01' and 'raw\_02' items are highlighted in grey.

- \* Up to three datasets or sets of selected samples can be visualized together. For this, use the “Upload additional result files” option. If you would like to choose different samples from the same result file, you need to provide the same filepath and select the different samples.
- \* If you cannot upload the selected file, please take a look at the detailed instructions for Spectronaut and Maxquant input formats.

Spectronaut instructions

The data needs to be exported in the **normal long** format as .tsv or .csv file.

It needs to include the following columns:

- PEP.AllOccurringProteinAccessions
- EG.ModifiedSequence
- R.FileName

To ensure the correct export format from Spectronaut, you can download and apply the provided export scheme “spectronaut\_export\_scheme.rs”.

Download spectronaut\_export\_scheme.rs

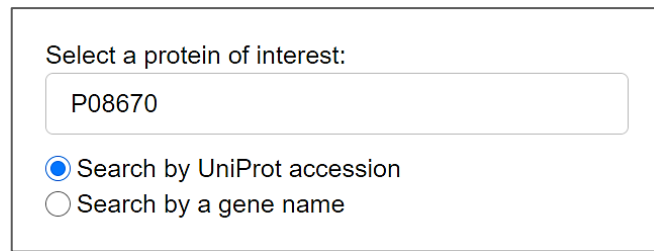
MaxQuant instructions

To visualize the proteins which were analyzed by the MaxQuant software please use the **evidence.txt** file.

The following columns from the file are used for visualization:

- Proteins
- Modified sequence
- Raw file

3. Press the "Upload Data" button. The loading process is indicated by a spinner symbol.
4. Select a protein of interest. Per default, you can choose from all UniProt accessions. Click the "Search by a gene name" option to select proteins by their gene name.

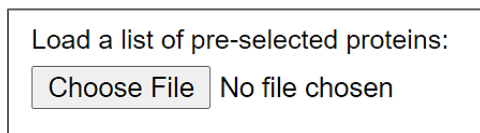


Select a protein of interest:

P08670

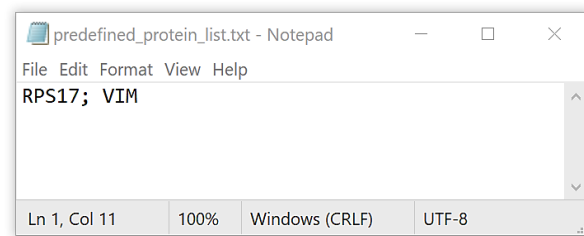
☒ Search by UniProt accession  
☐ Search by a gene name

5. (optional) Load a list of pre-selected proteins. This can be a .txt file containing either UniProt accessions or gene names separated by semicolons. This will reduce the options available in the selection of proteins of interest (step 4).



Load a list of pre-selected proteins:

No file chosen



6. Select annotation options for the sequence visualization.
  - All sequence annotations from UniProt are available and displayed per default. You can choose a customized set of displayed annotations in the "UniProt annotation" selection.



UniProt annotations						
Molecule processing						
Chain	Initiator methionine	Peptide	Propeptide	Signal peptide	Transit peptide	
Post-translational modification						
Cross-link	Disulfide bond	Glycosylation	Lipidation	Modified residue		
Family & Domain						
Coiled coil	Compositional bias	Domain	Motif	Region	Repeat	Zinc finger
Subcellular location						
Intramembrane		Topological domain		Transmembrane		
Function						
Active site	Binding site	Calcium binding	DNA binding	Metal binding	Nucleotide binding	Site
Sequence						
Alternative sequence	Natural variant	Non-adjacent residues	Non-standard residue	Non-terminal residue	Sequence conflict	Sequence uncertainty
Other options						
Secondary structure			Mutagenesis			

☐ Select all
 ☐ Clear all

- All theoretical cleavage sites for the most common proteases can be shown. Trypsin is selected by default. Alternative or additional proteases can be selected in the “Protease cleavage sites” selection.

Protease cleavage sites —

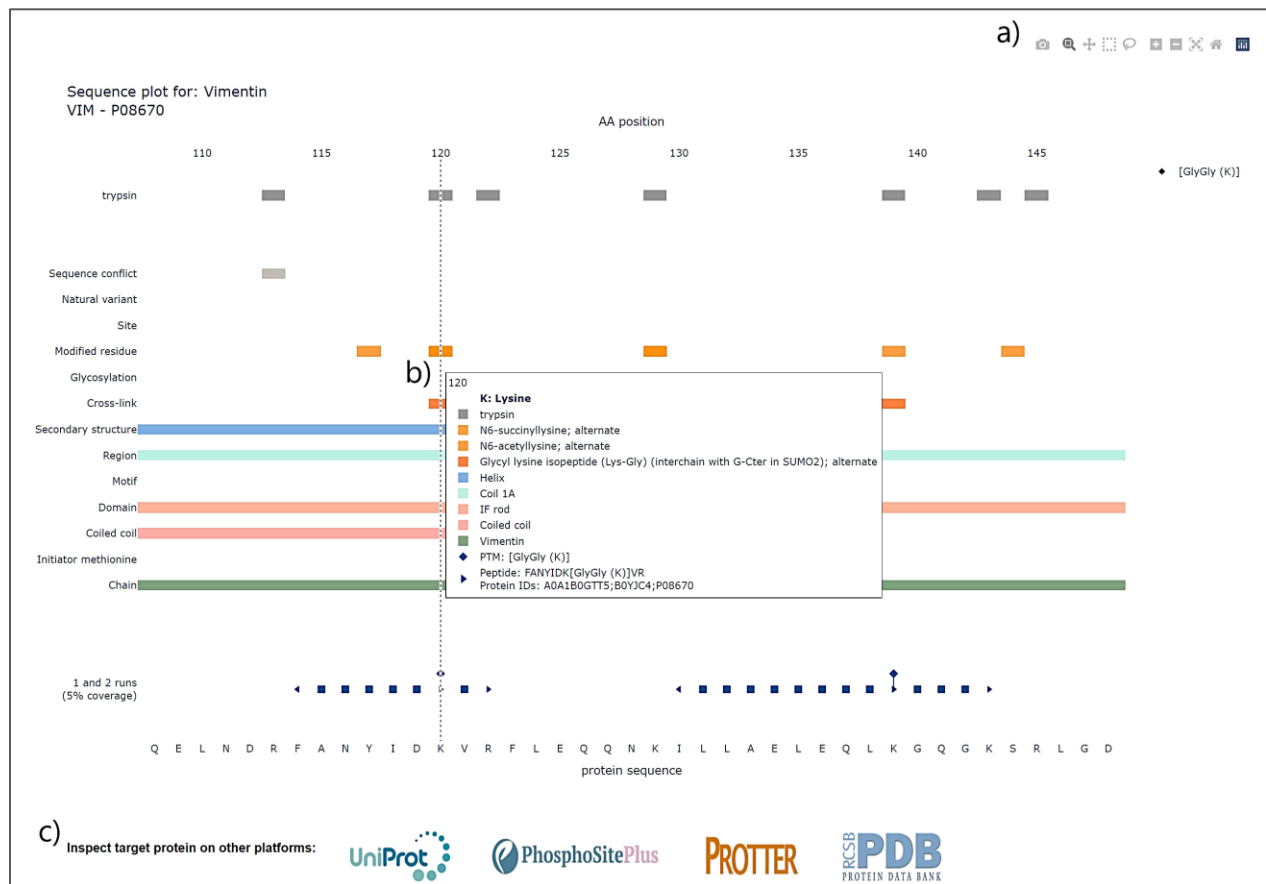
☐ arg-c  
☐ asp-n  
☐ bnps-skatole  
☐ caspase 1  
☐ caspase 2  
☐ caspase 3  
☐ caspase 4  
☐ caspase 5  
☐ caspase 6  
☐ caspase 7  
☐ caspase 8  
☐ caspase 9  
☐ caspase 10  
☐ chymotrypsin high specificity  
☐ chymotrypsin low specificity  
☐ clostripain  
☐ cnbr  
☐ enterokinase  
☐ factor xa  
☐ formic acid  
☐ glutamyl endopeptidase  
☐ granzyme b  
☐ hydroxylamine  
☐ iodosobenzoic acid  
☐ lysc  
☐ ntcb  
☐ pepsin ph1.3  
☐ pepsin ph2.0  
☐ proline endopeptidase  
☐ proteinase k  
☐ staphylococcal peptidase i  
☐ thermolysin  
☐ thrombin  
☐ trypsin\_full  
☐ trypsin\_exception  
☐ non-specific  
☒ trypsin

☐ Select all
 ☐ Clear all

\* You can use “Select all” or “Clear all” checkboxes to speed up the selection process.

7. Press the "Visualize Protein" button. The loading process is indicated by a spinner symbol.

- a) You can use the interactive toolbar to for example zoom in and out or to highlight specific sequence regions. Press the little camera icon to download a high-resolution .svg image of the currently displayed protein and sequence region.
- b) If you hover over the sequence, all annotation information for the current sequence position of the cursor will be displayed.
- c) You can directly visit other websites for further exploration of details on the selected protein of interest. UniProt, PhosphoSitePlus, Protter and PDB are available for direct access.



8. Enjoy exploring your data!

9. Please, press “Quit” button when your working session in AlphaMap is finished.

**IMPORTANT WARNING!** If you just close the browser tab and do not press the "Quit" button, AlphaMap will keep running in the background (potentially using a significant amount of RAM memory). This is especially important for **macOS**.