

# AlphaMap Tutorial

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Developed by: Eugenia Voytik, Isabell Bludau.

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

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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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## 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 ▼

- 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).

a) Upload a result file:  b) ☒ c) Sample name  d) Prefix / suffix

e) Select samples:

☐ All samples  
☒ raw\_01  
☐ raw\_02

- \* 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:

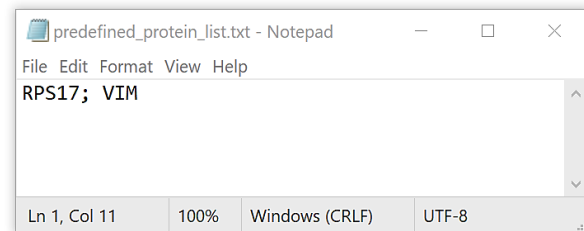
P08670

☒ 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			
<input type="checkbox"/> Select all <input type="checkbox"/> 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 —

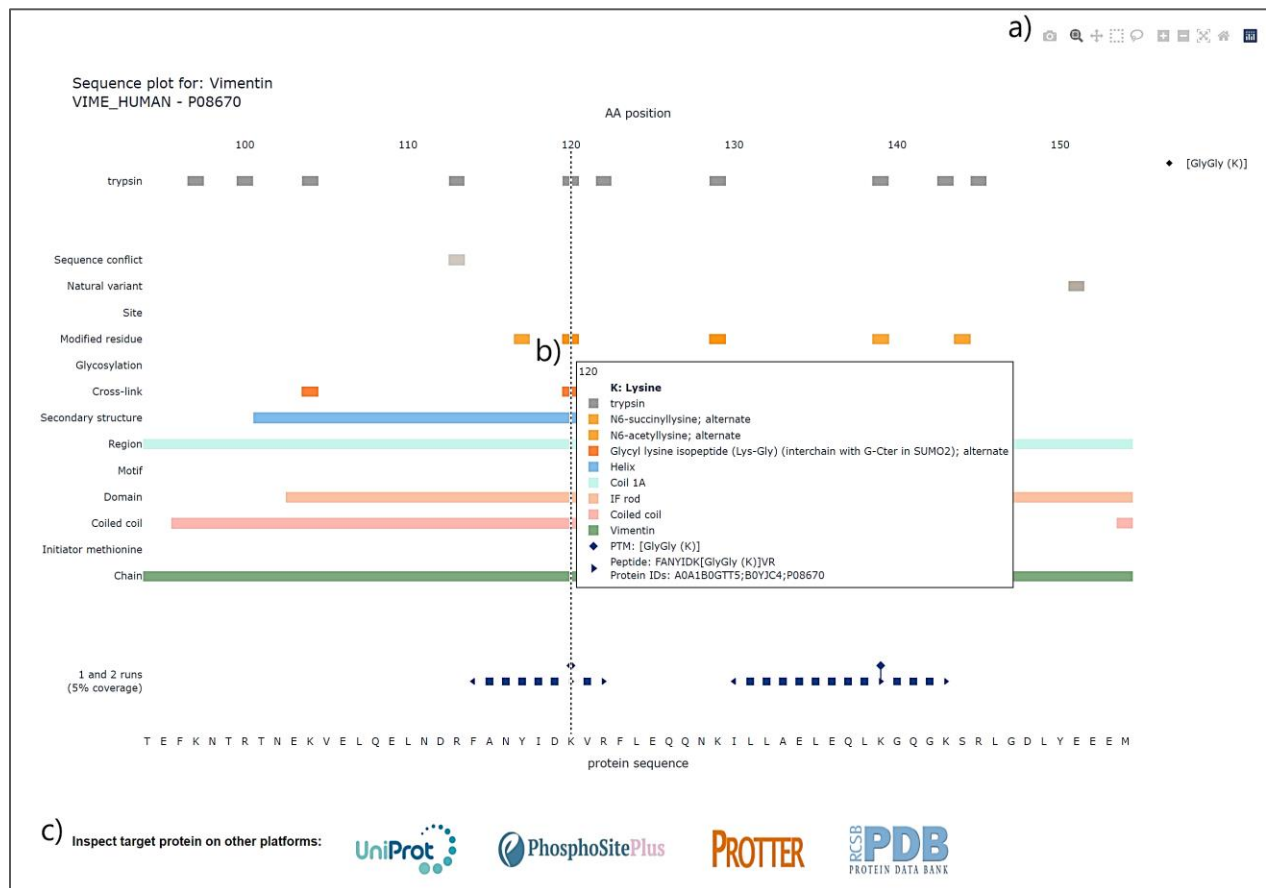
☐ arg-c  
☐ asp-n  
☐ bnps-skatole  
☐ caspase 1  
☐ caspase 2  
☐ caspase 3  
☐ caspase 4  
☐ caspase 5  
☐ caspase 6  
☐ caspase 7  
☐ caspase 8  
☐ caspase 9  
☐ caspase 10  
☐ chymotrypsin high specificity  
☐ chymotrypsin low specificity  
☐ clostripain  
☐ cnbr  
☐ enterokinase  
☐ factor xa  
☐ formic acid  
☐ glutamyl endopeptidase  
☐ granzyme b  
☐ hydroxylamine  
☐ iodosobenzoic acid  
☐ lysc  
☐ ntcbr  
☐ 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!