Manual xVis

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Background

Chemical crosslinking combined with mass spectrometry has recently been established as state-of-the-art workflow for the structural analysis of macromolecular protein complexes. The output of the crosslinking analysis is the proximity information between two amino acids linked by the bifunctional chemical agent and is typically summarized in a table format which does not allow an immediate and comprehensive interpretation of the topological data. We developed **xVis**, a server-client-based software solution, to visualize the crosslink derived distance restraints of protein complexes that describe their architecture. **xVis** displays cross-links in clear schematic representations in form of a pie chart, a bar chart or a network diagram. The subunit proteins of a complex are displayed as bars or segments of a circle scaled to the protein lengths, lines connecting specific positions of the bars or the segments indicate the crosslinking sites within the protein sequence from the N- to the C-terminus. The software facilitates the importing of user-defined protein motifs as well as annotated domains from public databases. The graphs reveal the spatial proximity of proteins and their structurally and functionally annotated motifs which is crucial for the initial structural and functional interpretation prior to the more laborious generation of three-dimensional models.

Furthermore, the program offers two options for the qualitative assessment of the crosslink identifications. First, filtering the crosslinks according to the identification score or the false discovery rate allows for the selective representation of restraints below or above a certain threshold value. Second, **xVis** provides a link between each crosslink and the corresponding fragment ion spectrum for the manual inspection of the mass spectrometric data. **xVis** was developed for the representation of crosslinks identified by the software **xQuest**, however, it also supports the visualization of crosslink results obtained from various other search engines providing the appropriate format of the input data.

This software solution represents an easy-to-use tool for the fast and clear representation of distance information on protein complex structures and for the evaluation of the mass spectrometric identification of the crosslinks.

Introduction and Sample Data

The sample data set was acquired by analyzing the multi-subunit chromatin remodeler INO80 in complex with its nucleosome substrate (Tosi et al. 2013 Cell). The INO80 complex was purified by affinity-chromatography from budding yeast and reconstituted in complex with a mono-nucleosome. This supramolecular assembly was crosslinked with isotope-coded BS3 (Bis[sulfosuccinimidyl] suberate). The crosslinked complex was digested with trypsin and the crosslink fractions were analyzed by tandem mass spectrometry (Jennebach et al. 2012 Nucleic Acids Res; Herzog et al. 2012 Science). The resulting spectra were searched by the software **xQuest**

(Walzthoeni et al. 2012 Nat Methods) and visualized by **xVis**.

The dataset is composed of 274 intra-protein and 149 inter-protein crosslinks detected on 18 proteins. The sample data is available under Test Data. For testing **xVis** download the test data and follow the instructions in the manual sections Generating a Plot.

The access of **xVis** without login provides all features and display options. The use of **xVis** with an account (see section File Management) allows storing the settings of the **xVis** analysis, for example, datasets with the preconfigured filter settings. Additionally, the login is required for connecting to the **xQuest** server in order to view and evaluate the fragment ion mass spectra of the respective crosslink. You may enter a URL under *Settings* to connect to xQuest, the search engine that identifies the crosslinks from mass spectrometric data and displays the fragment ion spectra as htmls (see sections: Functions, Settings). For the analysis of the test data we linked the corresponding fragment ion spectra htmls and allocate a test user account (username: test_user; password: user-56).

Input Data

Protein identifier

The protein identifier is used to link protein names to crosslink data and annotations. Therefore, you have to consistently use a unique name in each of the input files. You may choose one of the following three protein identifiers in your input files: UniProt-ID, FASTA-header or user-defined names. The protein name in the crosslink data input file is shown in the representation. It is possible to combine UniProt-IDs and FASTA-header. If you use the FASTA-header in the crosslink data input files only the Entry Name without the accession number is displayed. To generate a plot with user-defined protein names you have to disable *Use UniProt protein lengths* and to upload a *Protein Lengths File* using the same user-defined names as in the crosslink data file (see section: Protein Lengths File).

FASTA-header

The term FASTA-header defines the following part of the UniProt-FASTA-header (see http://www.uniprot.org/help/fasta-headers): *Database*|*UniqueIdentifier*|*EntryName* like *sp*|*P53115*|*INO80_YEAST*. The source Database is ignored. Isoform identifiers like *Q4R572-2* are not allowed. By removing *-2* you get a permissive identifier. You may keep the Entry Name from the UniProt-FASTA-header or use a user-defined name instead. If you use a user-defined name it must follow the rules below (section: User-defined protein name).

UniProt-ID

A UniProt-ID is the Unique Identifier of entries in UniProt (also known as UniProtKB accession number). It consists of 6 or 10 alphanumerical characters. For details see http://www.uniprot.org/help/accession_numbers.

User-defined protein name

The user-defined name may consist of capital and lowercase letters, spaces, minus signs, numbers, brackets and underline characters. Special characters like comma, semicolon, quote or vertical bar are not allowed.

xVis options depend on the appropriate protein identifier

Option	User-defined name	UniProt-ID	FASTA-header
Import protein lengths from UniProt	-*	+	+
Import Secondary structure	-	+	+
Import InterPro annotations	-	+	+
Show user-defined annotations	+**	+**	+**
Show user-defined name	+	-***	+

(- not available, + available)

* If you use user-defined names you cannot import protein lengths from UniProt. In this case, you have to generate a file containing the protein lengths (see section: Protein Lengths File).
** To use user-defined annotations you have to generate an annotations file (see section: Domain File for Annotation).
*** If you do not use FASTA-header in your crosslink data input file and UniProt-IDs in other input files, you may show user-defined names in the diagram.

Crosslink Data File

Minimal Crosslink Data File

A crosslink data file is a comma separated file (csv) that has to contain at least the columns *Protein1*, *Protein2*, *AbsPos1* and *AbsPos2*. The columns may be in any order but you have to use the exact same column headings. *Protein1* and *Protein2* define the two linked proteins with *AbsPos1* and *AbsPos2* indicating the absolute positions of the two crosslinked amino acids within the protein sequences. For intra-links *Protein1* and *Protein2* have to have the same protein identifiers. Additional columns are ignored by **xVis**. If you use the crosslink identification score or the false discovery rate for filtering see section: Medium Crosslink Data File.

Protein1	Protein2	AbsPos1	AbsPos2
sp|P43579|IES1_YEAST	sp|Q03435|NHP10_YEAST	674	32
sp|P32617|IES6_YEAST	sp|P53946|ARP5_YEAST	55	731
sp|Q12464|RUVB2_YEAST	sp|P53115|INO80_YEAST	181	1082
sp|P53115|INO80_YEAST	sp|P40154|IES2_YEAST	1350	275
sp|Q03940|RUVB1_YEAST	sp|P53115|INO80_YEAST	290	1082
sp|Q12464|RUVB2_YEAST	sp|Q03940|RUVB1_YEAST	357	454
sp|Q03940|RUVB1_YEAST	sp|P40154|IES2_YEAST	171	275
sp|P53115|INO80_YEAST	sp|P80428|ARP4_YEAST	478	323
sp|P53115|INO80_YEAST	sp|P40154|IES2_YEAST	1312	275
sp|P80428|ARP4_YEAST	sp|P53115|INO80_YEAST	323	483

Medium Crosslink Data File

The medium crosslink file is comma separated (csv) and needs in addition to the columns *Protein1*, *Protein2*, *AbsPos1* and *AbsPos2* of the minimal crosslink file, the columns of the identification score (Id-Score) and the false discovery rate (FDR) for the filtering option. In contrast to the columns *Protein1*, *Protein2*, *AbsPos1* and *AbsPos2*, the columns of the filter values may have arbitrary headings. For filtering the crosslinks (see section: Menus: Filter) you have to select the filter value in settings and enable displaying the crosslinks above or below the threshold (see section: Settings). If you have selected a filter score column the value is shown for each crosslink on mouse over. The file may contain additional columns which are ignored if they are not chosen in

the settings as filter scores.

Protein1	Protein2	AbsPos1	AbsPos2	Id-Score	fdr
sp|P43579|IES1_YEAST	sp|Q03435|NHP10_YEAST	674	32	41	0
sp|P32617|IES6_YEAST	sp|P53946|ARP5_YEAST	55	731	40.56	0
sp|Q12464|RUVB2_YEAST	sp|P53115|INO80_YEAST	181	1082	40.24	0
sp|P53115|INO80_YEAST	sp|P40154|IES2_YEAST	1350	275	40.09	0
sp|Q03940|RUVB1_YEAST	sp|P53115|INO80_YEAST	290	1082	39.63	0
sp|Q12464|RUVB2_YEAST	sp|Q03940|RUVB1_YEAST	357	454	39.62	0
sp|Q03940|RUVB1_YEAST	sp|P40154|IES2_YEAST	171	275	39.46	0
sp|P53115|INO80_YEAST	sp|P80428|ARP4_YEAST	478	323	39.18	0
sp|P53115|INO80_YEAST	sp|P40154|IES2_YEAST	1312	275	38.83	0
sp|P80428|ARP4_YEAST	sp|P53115|INO80_YEAST	323	483	38.76	0

xQuest generated Crosslink Data File

As the minimal and the medium crosslink file the crosslink file generated by **xQuest** is comma separated (csv) and needs the columns *Protein1*, *Protein2*, *AbsPos1* and *AbsPos2*. To display the corresponding fragment ion spectrum for each crosslink you need the columns *Spectrum*, *Type* and *Id* as well as the name of the **xQuest** output file. Importantly, you have to insert the server location and the user name in settings (see section: Settings). In addition, the file may have one or more columns containing filter score values. They may have an arbitrary heading. For filtering the crosslinks (see section: Menus: Filter) you have to select the respective filter score column in settings and indicate whether crosslinks above or below a threshold value are displayed (see section: Settings). If the column is selected the respective filter score value is shown for each crosslink on mouse over. The file may include additional columns which are ignored.

Id	Protein1	Protein2	Type	Spectrum	AbsPos1	AbsPos2	Id-Score	fdr
VLLNKIER-LSVKR-a5-b4	sp|P43579|IES1_YEAST	sp|Q03435|NHP10_YEAST	xlink	frherzog_M1112_310.c.05146.05146.4_frherzog_M1112_310.c.05129.05129.4	674	32	41	0
SAHYKKPTRR-TVISKK-a6-b5	sp|P32617|IES6_YEAST	sp|P53946|ARP5_YEAST	xlink	frherzog_M1110_159.c.01530.01530.4_frherzog_M1110_159.c.01507.01507.4	55	731	40.56	0
MIDGLTKEK-NPIKYSLPR-a7-b4	sp|Q12464|RUVB2_YEAST	sp|P53115|INO80_YEAST	xlink	frherzog_M1112_311.c.05850.05850.4_frherzog_M1112_311.c.05832.05832.4	181	1082	40.24	0
LDGSSKLEDRR-AGKSR-a6-b3	sp|P53115|INO80_YEAST	sp|P40154|IES2_YEAST	xlink	frherzog_M1110_164.c.03073.03073.4_frherzog_M1110_164.c.03053.03053.4	1350	275	40.09	0
LRQEVNKVVAK-NPIKYSLPR-a7-b4	sp|Q03940|RUVB1_YEAST	sp|P53115|INO80_YEAST	xlink	frherzog_M1112_310.c.04821.04821.5_frherzog_M1112_310.c.04811.04811.5	290	1082	39.63	0
SIIITTKSYNEQEIK-STKILETSANYL-a7-b3	sp|Q12464|RUVB2_YEAST	sp|Q03940|RUVB1_YEAST	xlink	frherzog_M1112_311.c.08108.08108.3_frherzog_M1112_311.c.08409.08409.3	357	454	39.62	0
TISHVIVGLKSAK-AGKSR-a10-b3	sp|Q03940|RUVB1_YEAST	sp|P40154|IES2_YEAST	xlink	frherzog_M1112_310.c.04411.04411.4_frherzog_M1112_310.c.04383.04383.4	171	275	39.46	0
HYDNTYTTIWKDMAR-NDYVPLKR-a11-b7	sp|P53115|INO80_YEAST	sp|P80428|ARP4_YEAST	xlink	frherzog_M1112_310.c.07803.07803.4_frherzog_M1112_310.c.07786.07786.4	478	323	39.18	0
LKSEGHR-AGKSR-a2-b3	sp|P53115|INO80_YEAST	sp|P40154|IES2_YEAST	xlink	frherzog_M1110_163.c.00860.00860.4_frherzog_M1110_163.c.00809.00809.4	1312	275	38.83	0
NDYVPLKR-KDSTK-a7-b1	sp|P80428|ARP4_YEAST	sp|P53115|INO80_YEAST	xlink	frherzog_M1110_163.c.04011.04011.3_frherzog_M1110_163.c.04002.04002.3	323	483	38.76	0

Display options according to crosslink features in different input data formats

Some features depend on the type of the crosslink file (- not available and + available). To apply certain display options you need to use the crosslink data input file supporting this feature.

Option	Minimum	Medium	xQuest
Link to the corresponding fragment ion spectrum	-	-	+
Filtering by Score/FDR	-	+*	+*
Show Score for a crosslink	-	+*	+*

* Options available if input file contains columns for Id-Score and FDR and which have to be selected under settings.

Domain File for Annotation

The domain file is a comma separated (csv) file for annotated protein motifs. **xVis** supports names or even short descriptions as annotations. The *Name* (annotation text) allows capital and lower case letters, numbers, brackets, spaces, minus signs, semicolons, dots and underline characters but no special characters like commas, quotes or vertical bars. The column *Protein* contains a protein identifier (see section: Protein

identifier). The start and the end of the annotation are defined by the amino acid positions in the *Start* and *End* columns. You have to use *Protein*, *Start*, *End* and *Name* as column headings but their order may be altered.

Protein	Start	End	Name
P02299	2	134	Histone-fold
P02299	58	132	Histone core
P32617	114	143	YL1 nuclear, C-terminal
P40154	237	291	INO80 complex subunit B-like conserved region
P53115	467	605	DBINO domain
P53115	709	1017	SNF2-related
P53115	669	935	P-loop containing nucleoside triphosphate hydrolase
P53115	937	1020	P-loop containing nucleoside triphosphate hydrolase
P53115	702	901	Helicase, superfamily 1/2, ATP-binding domain
P53115	1303	1467	Helicase, C-terminal

Protein Lengths File

The comma separated file (csv) includes the protein identifiers (see section: Protein identifier) and the respective protein lengths. The first row may be used for the column headings (ignored by **xVis**). The following rows have to contain a protein identifier and the protein length (identifier, length). If you apply user-defined names as protein identifiers instead of Fasta-headers or UniProt-IDs they have to match the names in the crosslink data input file.

Protein	Length
sp|P43579|IES1_YEAST	692
sp|P32617|IES6_YEAST	166
sp|Q12464|RUVB2_YEAST	471
sp|P53115|INO80_YEAST	1489
sp|Q03940|RUVB1_YEAST	463
sp|P80428|ARP4_YEAST	489
sp|Q12386|ARP8_YEAST	881
sp|Q12345|IES3_YEAST	250
sp|Q08561|IES4_YEAST	116
sp|Q03435|NHP10_YEAST	203

File Management

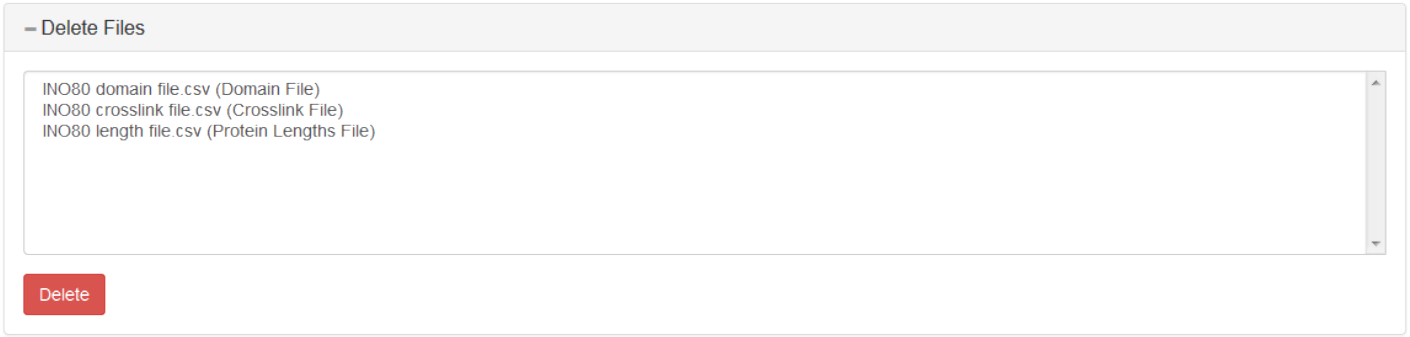
This section describes the upload and handling of input files. The file management option allows the uploading and storing of all types of input files in the user account folder. This option is only available if you are logged in onto the server. For test purposes we offer a test account which has limited storage space and data will be deleted regularly (username: test_user; password: user-56). Without login **xVis** analysis is fully functional, however, input files are only used for generating the plot and are not stored on the server.We recommend to install **xVis** and to distribute user accounts if you connect **xVis** to a **xQuest** server in your IT environment (see section: Installation) .

Upload Files

Here, the different input files are uploaded for generating a plot. You have to select the input file type: crosslink data, domain annotation or protein lengths and the location of the file you want to upload. It is possible to upload more than one file of the same file type. After choosing the files with the file browser you see a list of selected files above as well as their size. You can check if the selected files are assigned to the correct file type. Press the button to upload files to the server.

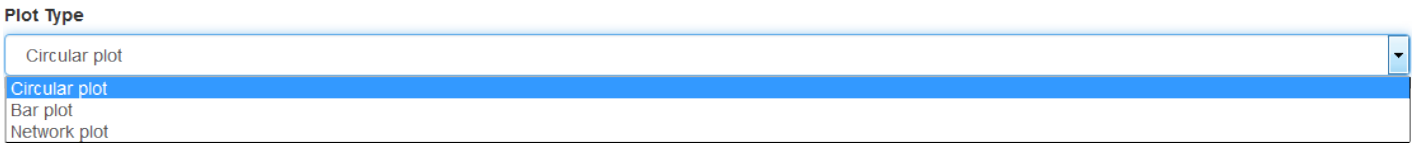
Delete Files

You may select one or more files for deleting. The file type (crosslink data, domain annotation or protein lengths) is indicated in parentheses.

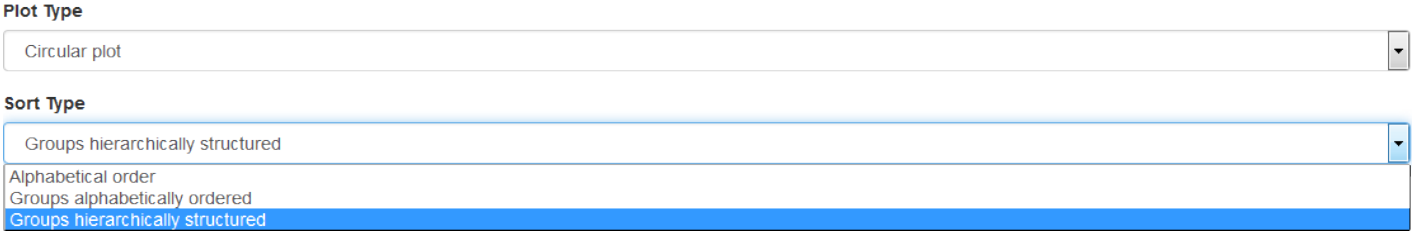


Generating a Plot

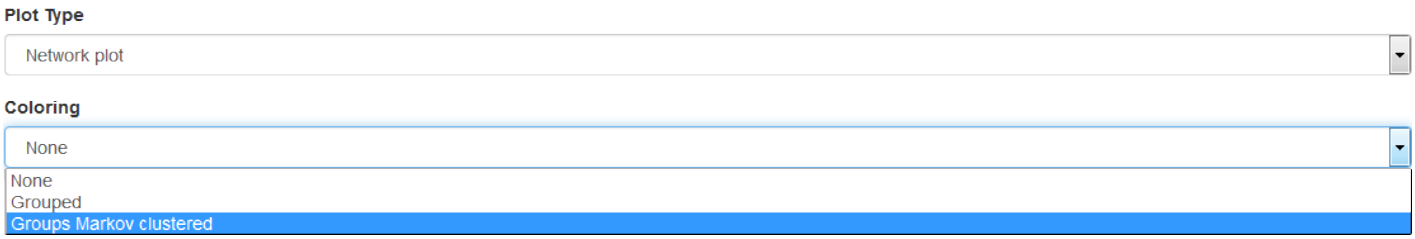
First, you have to choose a plot type. You can choose between circular plot, bar plot and network plot:



Second, you may select a sort type. For the circular plot and the bar plot you can choose between alphabetical order, groups alphabetically ordered and groups hierarchically structured:

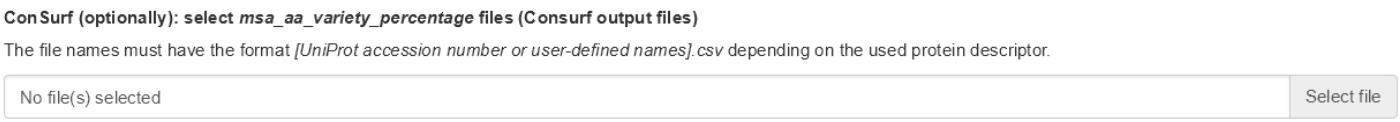


In comparison to the circular plot and the bar plot the network plot offers a drag and drop feature to arrange the pre-arranged proteins. Therefore no sort type option is provided. Instead you may choose a clustering algorithm for coloring the proteins: none (all proteins have the same color), grouped and groups Markov clustered.

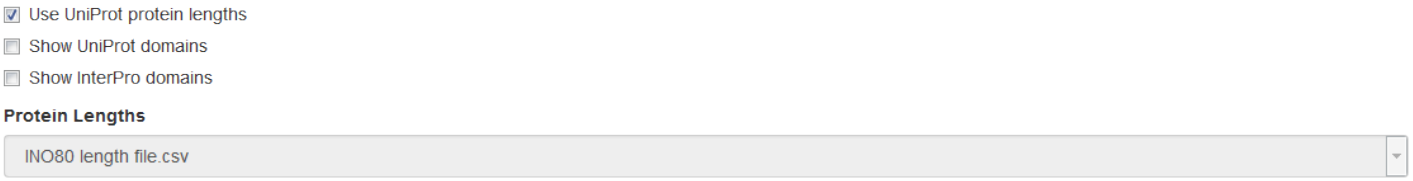


Third, you have to select a crosslink data input file and may select a file with user-defined annotations.

Evolutionary conservation of amino acid positions in the proteins (optional). To integrate on the diagrams amino acid evolutionary conservation generate output files using ConSurf (http://consurf.tau.ac.il/). The file names have to be converted into the format *[UniProt accession number or user-defined names].csv* depending on the used protein identifier. Multiple files can selected for upload.



Furthermore you can decide if you import protein lengths and secondary structures from UniProt (turns, helixes or beta strands) as well as annotations provided by InterPro databases. To use user-defined protein lengths or user-defined protein names you have to deselect this option and chose a protein lengths file below. If you import protein lengths from UniProt you may also import secondary structures provided by UniProt and/or annotations from InterPro. Default: Use UniProt protein lengths.

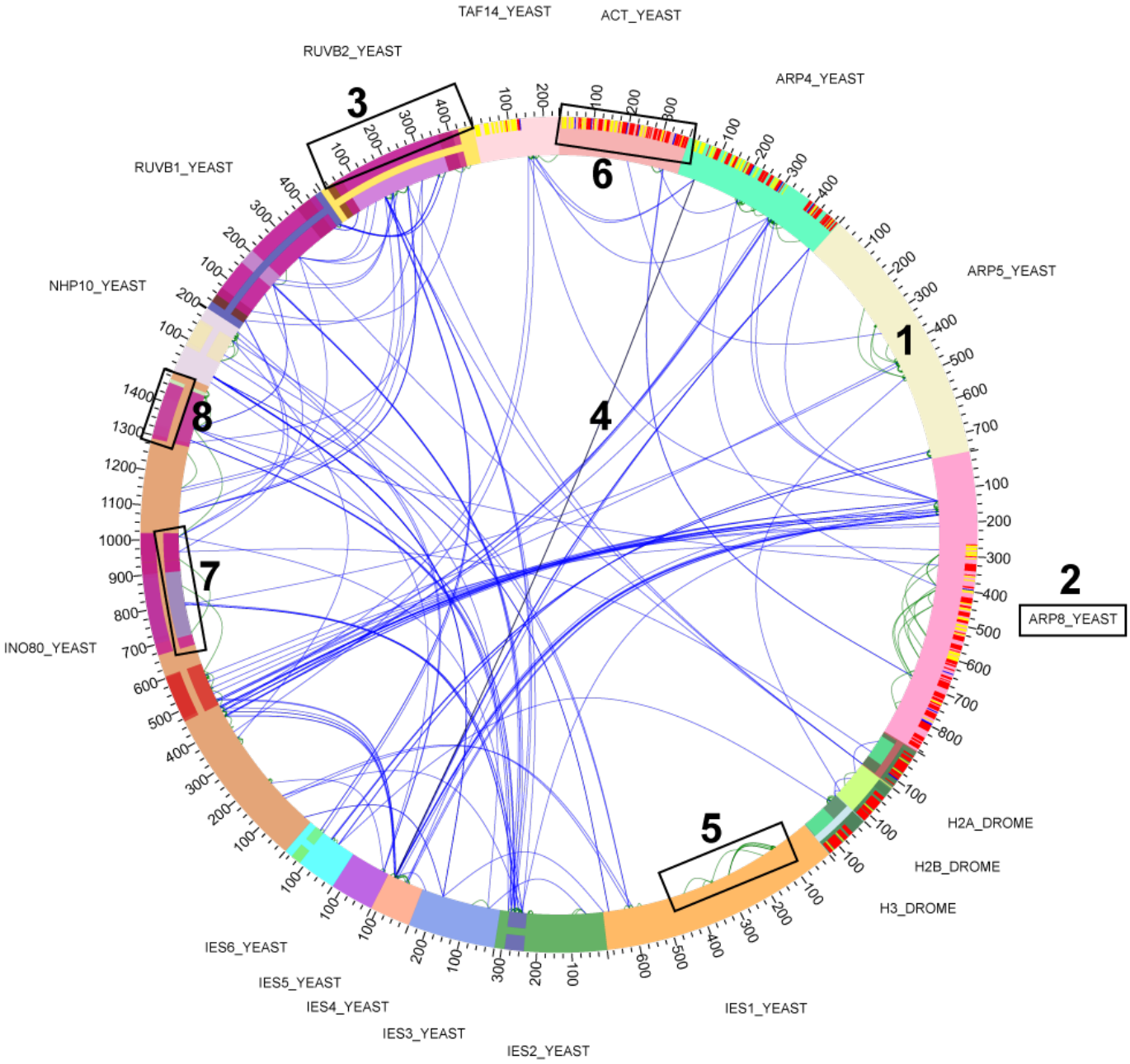


To create a diagram with the chosen features press the Plot button.

Plot Types

Circular Plot

This figure shows a circular plot with all possible annotations. Depending on your input files or choices there could be fewer:



1. protein: each protein has a different color
2. protein name if you use FASTA-header or user-defined names. If you use UniProt-IDs the UniProt-ID is displayed.
3. scale beginning from the first amino acid of the protein
4. inter-links (on mouse over you view the crosslink details; see section:Mouse Over)
5. intra-links (on mouse over you view the crosslink details; see section:Mouse Over)
6. Secondary Structures (turns, helixes and beta strands): available if FASTA-header or UniProt-IDs are used
7. user defined annotations; same color means same annotation (name)
8. InterPro annotations; same color means same annotation. It is possible to hide annotations for example if they overlap.

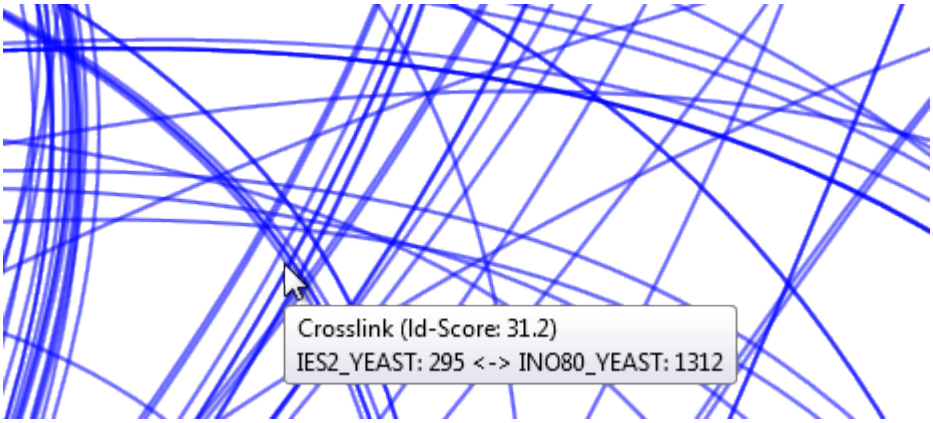
Functions

Zooming and Drag and Drop

The diagram is zoom-able and the position can be adjusted with drag and drop.

Mouse Over

Additional information is displayed by mouse over: mouse over an annotation shows the start and end position and name of it; mouse over a crosslinks shows the names of the crosslinked proteins with the crosslink sites. If a score column is selected in *Settings* (see section: xVis) the Score/FDR of the crosslinks as well as the name of the score are displayed. Mouse over a protein shows the protein name. Default: no score column selected.



On Click

If you click a protein name the color switches between black and the color of the respective protein segment (circular plot) or protein bar (bar plot).

Double Click

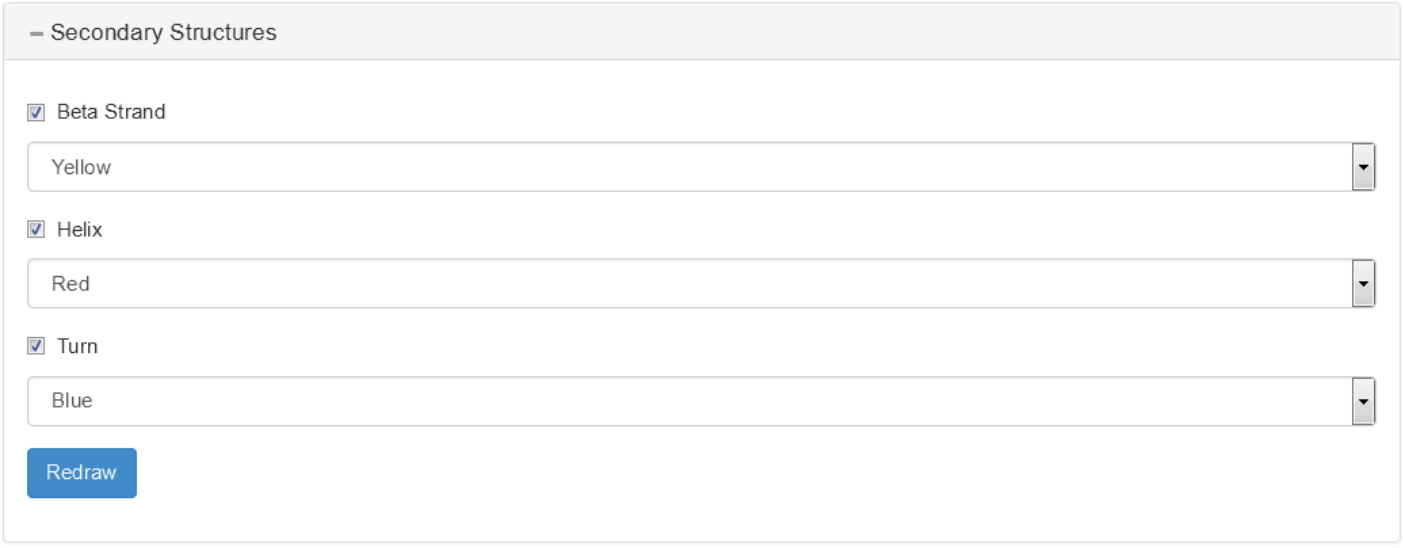
Double clicking a protein field or name opens the corresponding UniProt entry. This feature requires the use of UniProt-IDs or the FASTA-header in the crosslink data input file. Double clicking on a crosslink line links to the login of the **xQuest** viewer and in case you are already logged in to the fragment ion spectrum. This feature requires the **xQuest** generated files and needs the filename given from **xQuest**.

Menus

The menus shown above are collapsible, so their content is displayed after a click on the menu name. New settings are applied by the Redraw button.

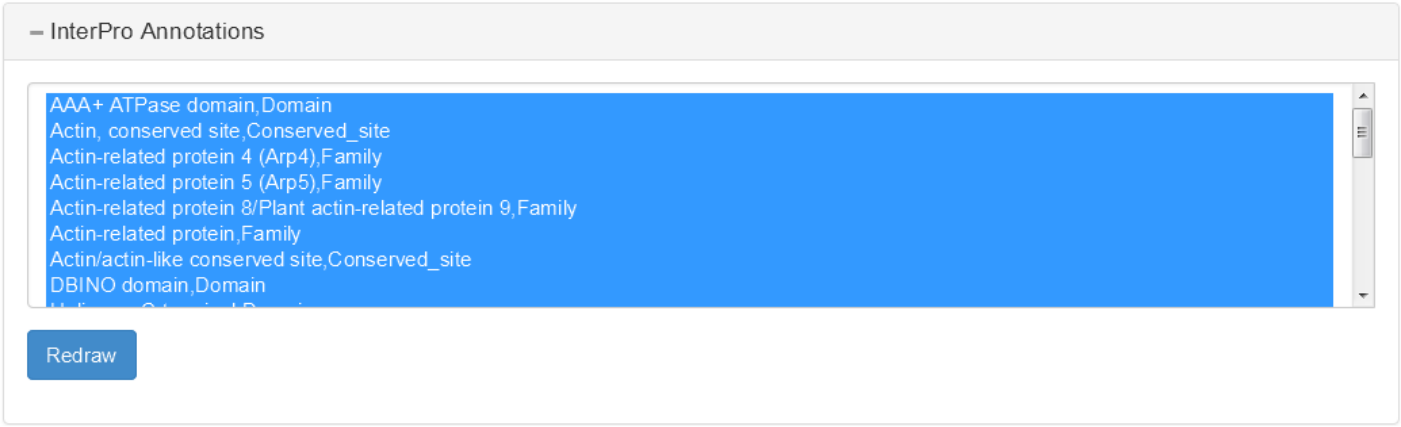
Secondary Structures

You can select each beta strand, helix or turn separately for displaying and assign a color with a dropdown box. Default: all secondary structures are shown; beta strand in yellow, helix in red and turn in blue.



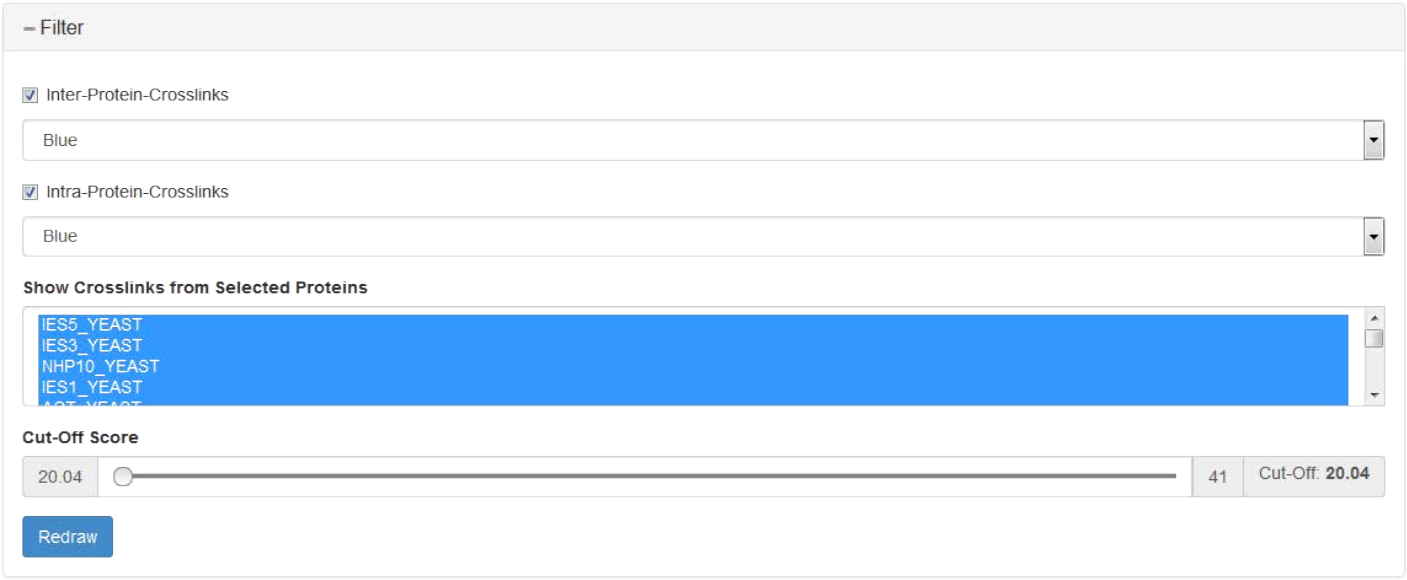
InterPro Annotations

It is possible to select several annotations to be displayed in the plot. The same annotations have the same color in the plot. Default: all annotations downloaded from InterPro are shown.



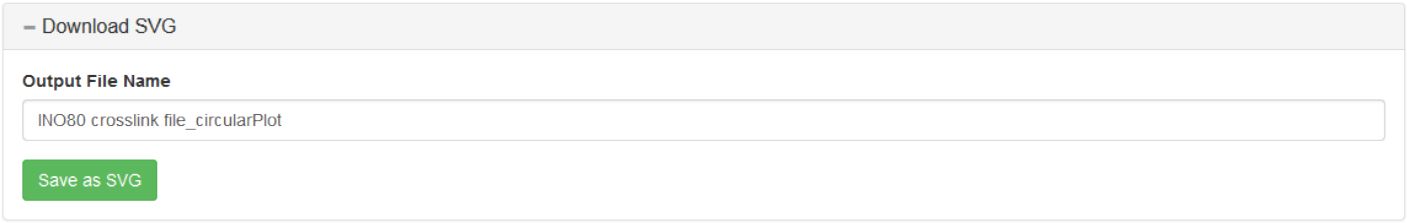
Filter

To distinguish inter- and intra-links it is possible to assign different colors or to selectively hide them. By selecting the involved proteins (multiple selection) you can view this subset of crosslinks. Furthermore the crosslinks can be filtered and displayed according to quality scores. The column of the Id-Score or the FDR can be defined as score column in *Settings* (see section: xVis) and chosing a threshold value facilitates to selectively display crosslinks above or below the cut-off. Default: cross-links to all proteins are shown and inter- and intra-links colored in blue. All crosslinks are shown regardless of their score.



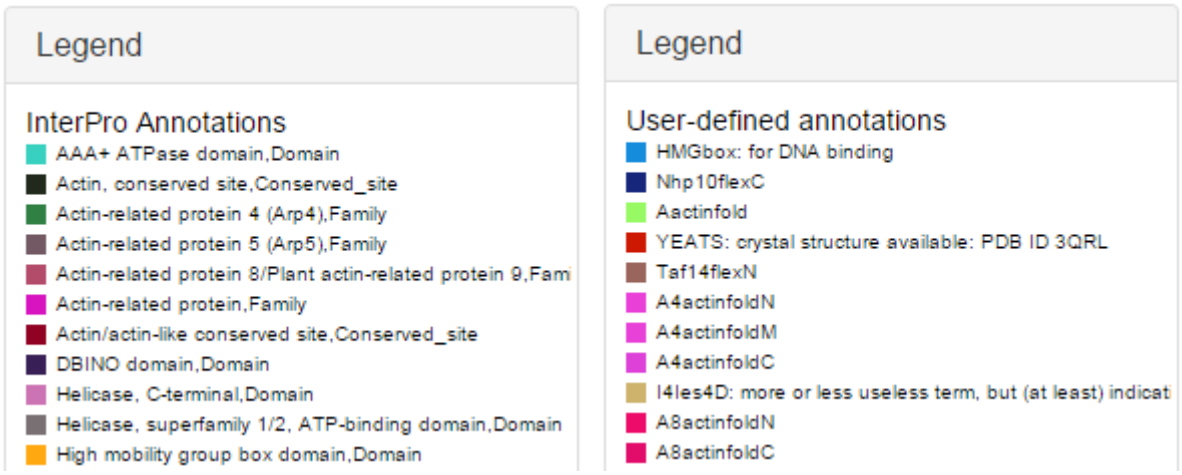
Download

The diagram can be downloaded as SVG (Scalable Vector Graphics). You may define a filename or default filename is used ([crosslink filename]_[plot type]).



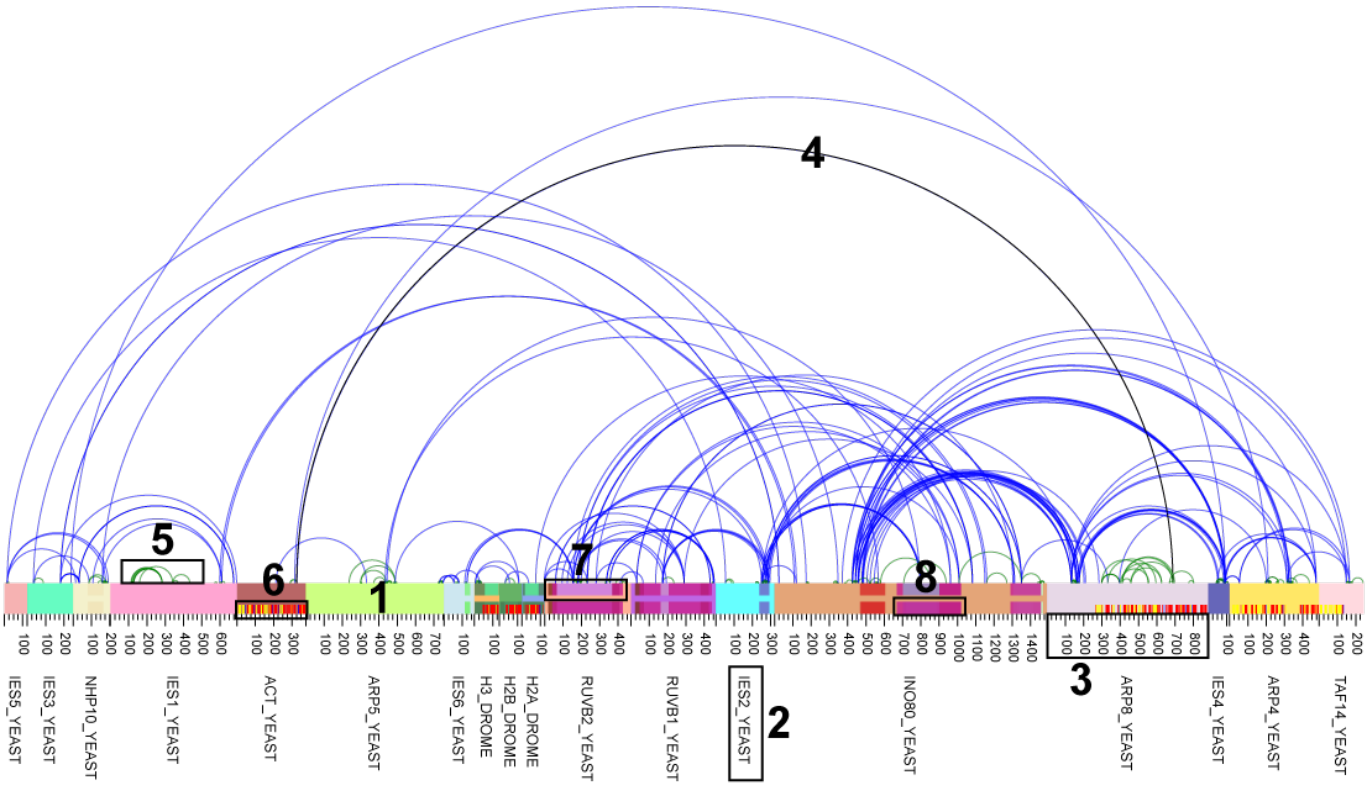
Legends

If InterPro or user-defined annotations are selected an appropriate legend will be displayed on the right panel.



Bar plot

The following diagram shows a bar plot with all possible annotations. Depending on the input file type or choices there are fewer annotations:



1. protein: each protein has a different color
2. protein name if you use FASTA-header or user-defined names. If you use UniProt-IDs the UniProt-ID is displayed.
3. scale beginning from the first amino acid of the protein
4. inter-links (on mouse over you view the crosslink details; see section:Mouse Over)
5. intra-links (on mouse over you view the crosslink details; see section:Mouse Over)
6. Secondary Structures(turns, helixes and beta strands): available if FASTA-header or UniProt-IDs are used
7. user defined annotations; same color means same annotation (name)
8. InterPro annotations; same color means same annotation. It is possible to hide annotations for example if they overlap.

Functions

see Circular Plot

Menus

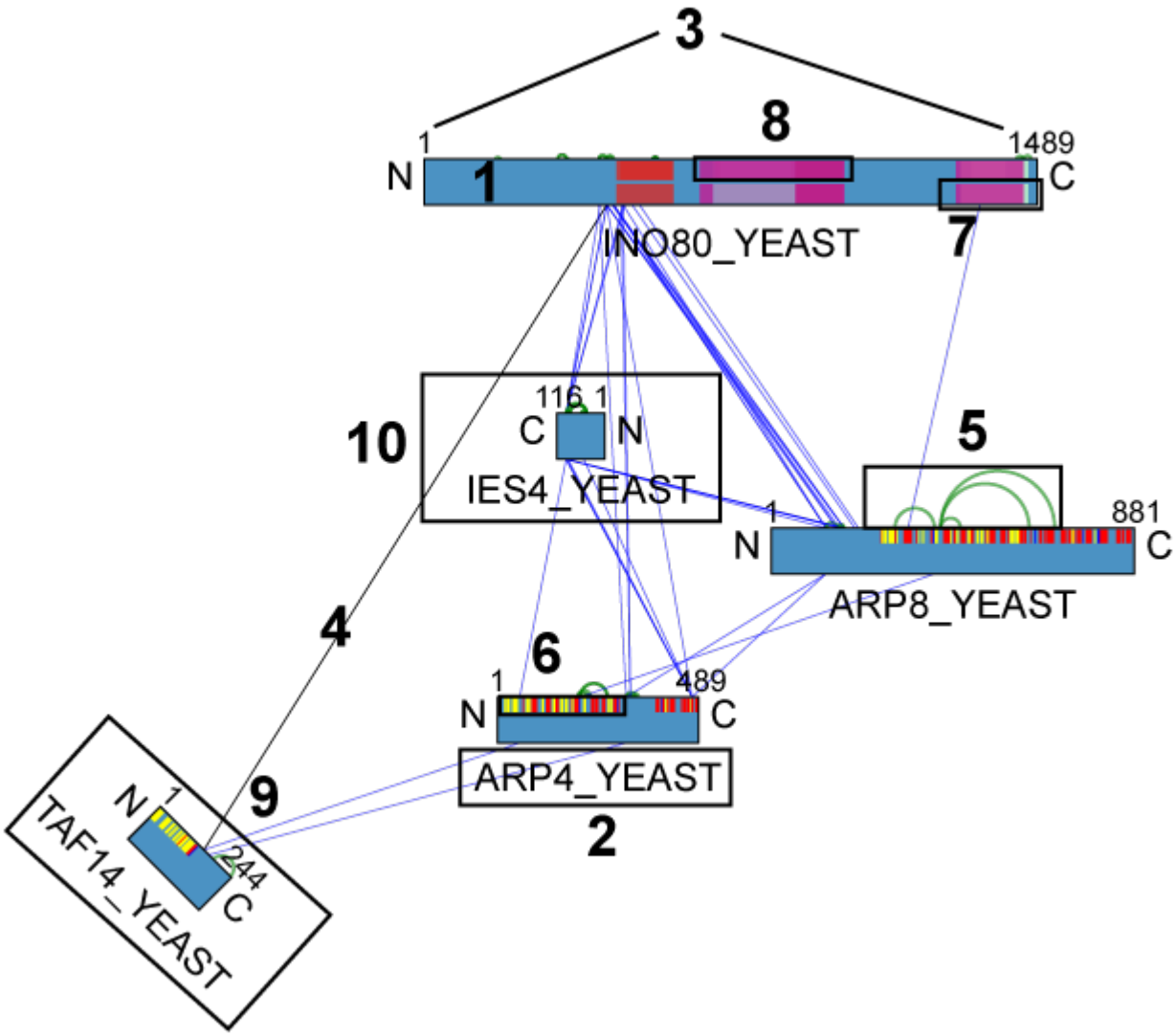
see Circular Plot

Legends

see Circular Plot

Network plot

This diagram shows a part of a network plot with all possible annotations. Depending on the input file type and your choices there are fewer annotations possible:



1. protein
2. protein name if you use FASTA-header or user-defined names. If you use UniProt-IDs the UniProt-ID is displayed.
3. first and last amino acid
4. inter-links (on mouse over you view the crosslink details; see section:Mouse Over)
5. intra-links (on mouse over you view the crosslink details; see section:Mouse Over)
6. Secondary Structures (turns, helixes and beta strands): available if FASTA-header or UniProt-IDs are used

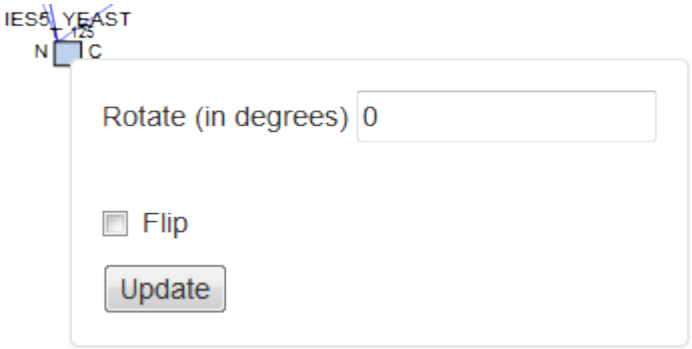
7. user defined annotations; same color means same annotation (name)
8. InterPro annotations; same color means same annotation. It is possible to hide annotations for example if they overlap.
9. rotated protein
10. flipped protein

Functions

In addition to functions described for circular plots (see section: Circular Plot) the network plot offers:

Context Menu

The context menu opens with a right click on a protein. A clicked protein can be mirrored horizontally by choosing *flip* and rotated over a degree value. The degree value has to be positive but can be a decimal number. The new settings are applied upon pressing the button *Update*. Default: not rotated and not mirrored.



Drag and Drop

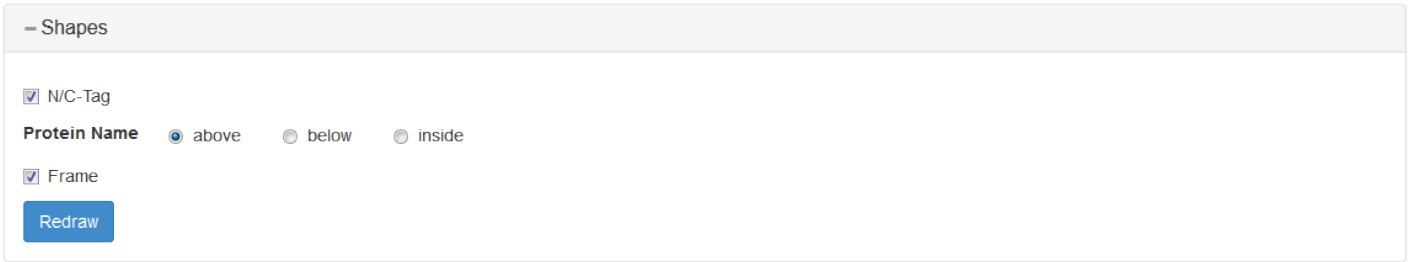
The proteins are moved using drag and drop.

Menus

In addition to menus described for circular plots (see section: Circular Plot) the network plot offers:

Shapes

The *Shapes* menu helps to design the network plot. It is possible to show an N and C for the N- and C-terminus of the protein. In addition the protein name can be displayed above, below or inside the protein bar which can be framed. Default: The protein name is above the bar and the N/C-Tag as well the frame around each bar is shown.



Markov Cluster Settings

Through the Markov Cluster Settings the number of clusters and their size can be determined. High *expansion* values generate a few big cluster and high *inflation* values generate many small cluster. This means the two parameters are counteracting. Protein groups (see section: Markov Clustering) with a lower number of proteins than the *threshold* are not clustered. Default: *expansion 2*, *inflation 1* and *threshold 3*.

– Markov Cluster Settings

Expansion

2

Inflation

3

Threshold (minimum proteins in group)

3

Update Cluster

Legends

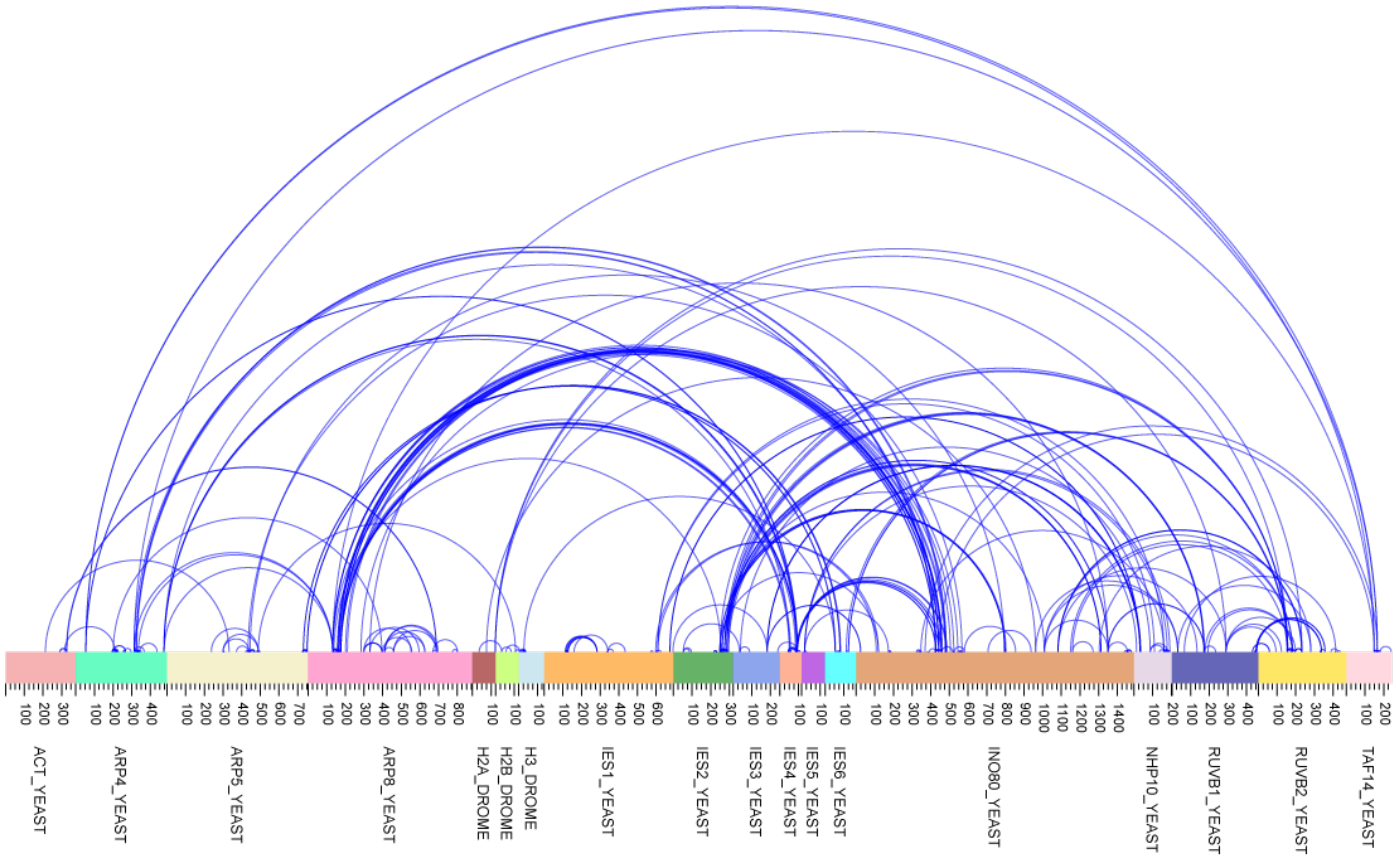
see Circular Plot

Sort type and Coloring

A sort type defines the arrangement of the proteins (available for circular and bar plot) and the coloring of proteins in a network plot.

Alphabetically ordered

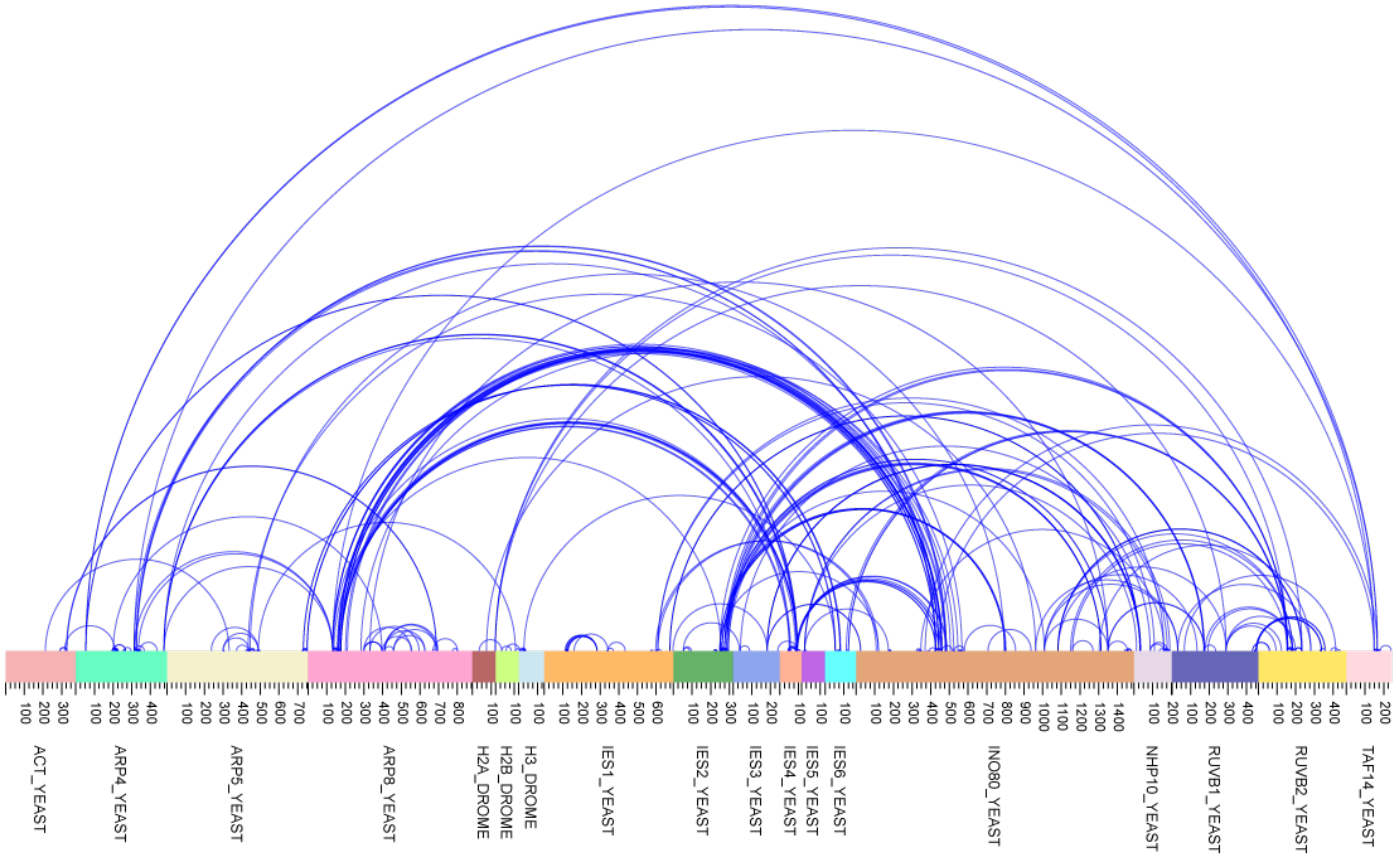
The proteins are ordered alphabetically by protein name if user-defined names or FASTA-header are applied as protein identifier or numerically by UniProt-IDs.



Groups alphabetically ordered

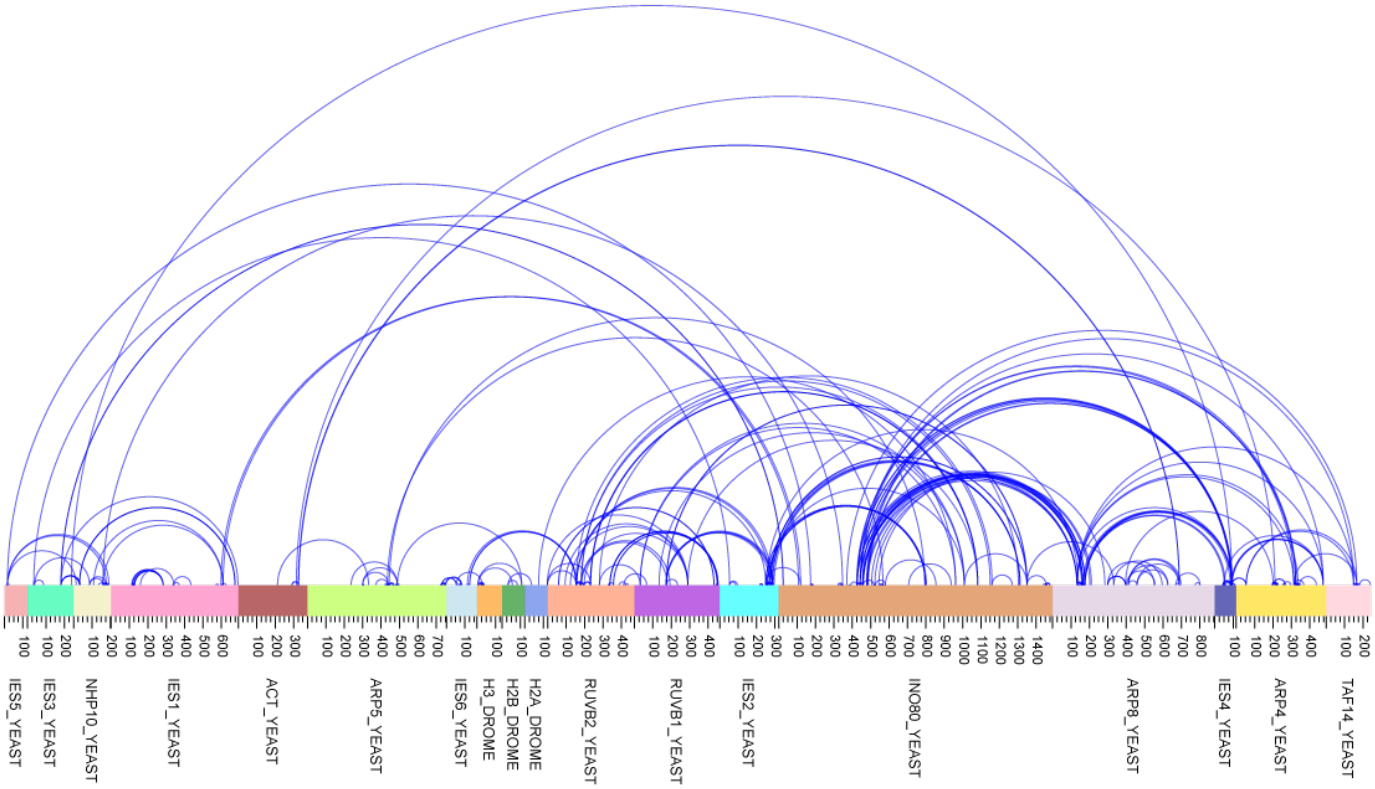
Proteins are separated in clusters comprising a connected group of proteins. In a cluster each protein has to be connected at least to one other protein in the same cluster. A cluster may consist of only one protein if this

protein has no interlinks. After grouping all cluster are sorted alphabetically. In case all proteins are in one cluster this sorting looks like the alphabetically ordering.



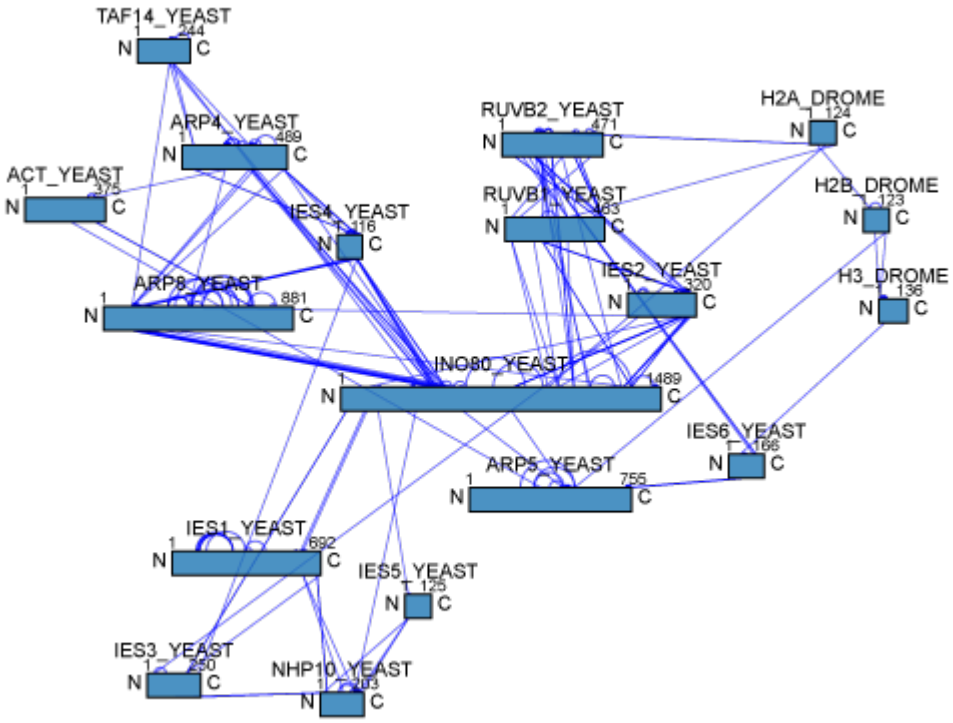
Groups hierarchically ordered

The proteins are grouped like for the alphabetically ordering. Subsequently, a dendrogram depending on the number of crosslinks between the proteins is generated in order to arrange the proteins. In general, many crosslinks between proteins result in a sub complex with fewer crosslinks between them. The dendrogram is converted in a list to arrange the proteins along a line or a circular line.



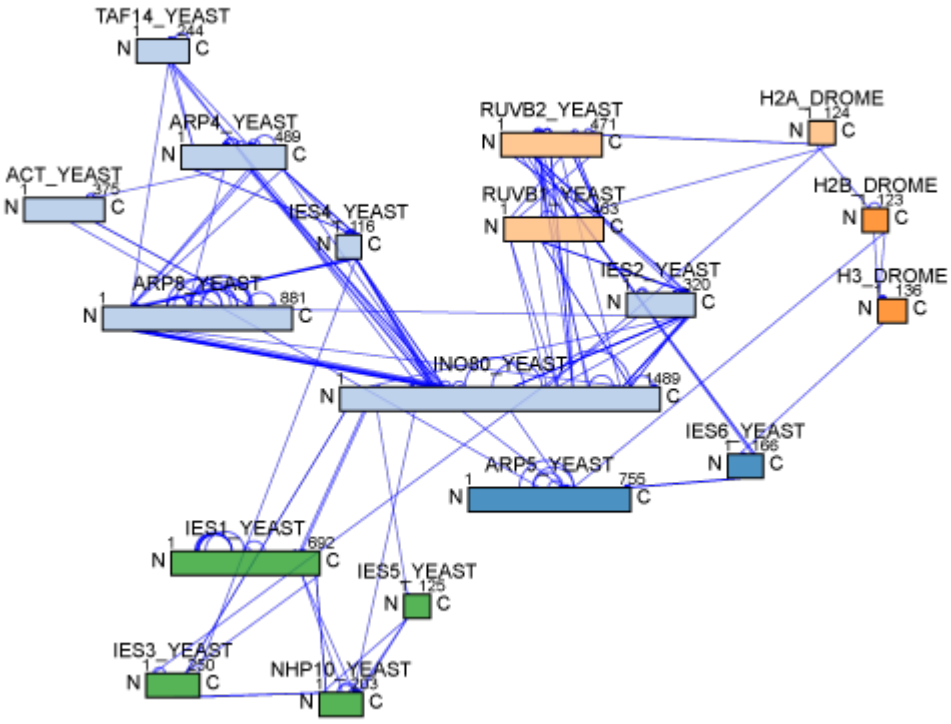
Grouped

The proteins are grouped like for the alphabetically ordering. Subsequently, each group gets a different color in the diagram.



Markov Clustering

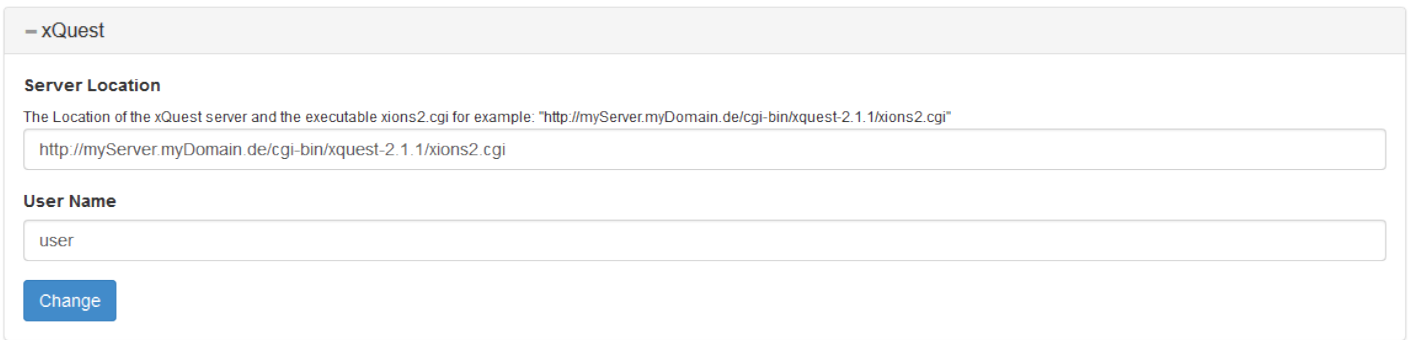
Before the Markov Clustering starts the proteins are grouped like for the alphabetically ordering. Each group is clustered by the Markov Algorithm with default parameters which you may change in the menu *Markov Cluster Settings*. The Markov Clustering Algorithm analyzes the protein network and identifies areas of high connectivity as clusters that are separated by areas with low connectivity from other cluster. It is recommended to optimize the clustering for your protein complex by changing the parameters.



Settings

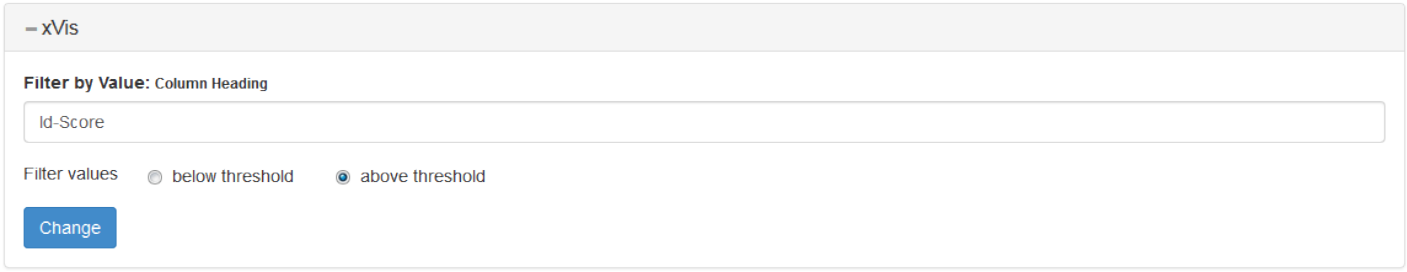
xQuest

Here, you have to define the location of the **xQuest** visualization server as well as your login name for the server if you want to connect crosslinks in **xVis** plots with the **xQuest** fragment ion spectra. As URL you need to insert the whole path to the *xions2.cgi*. Default: no default values



xVis

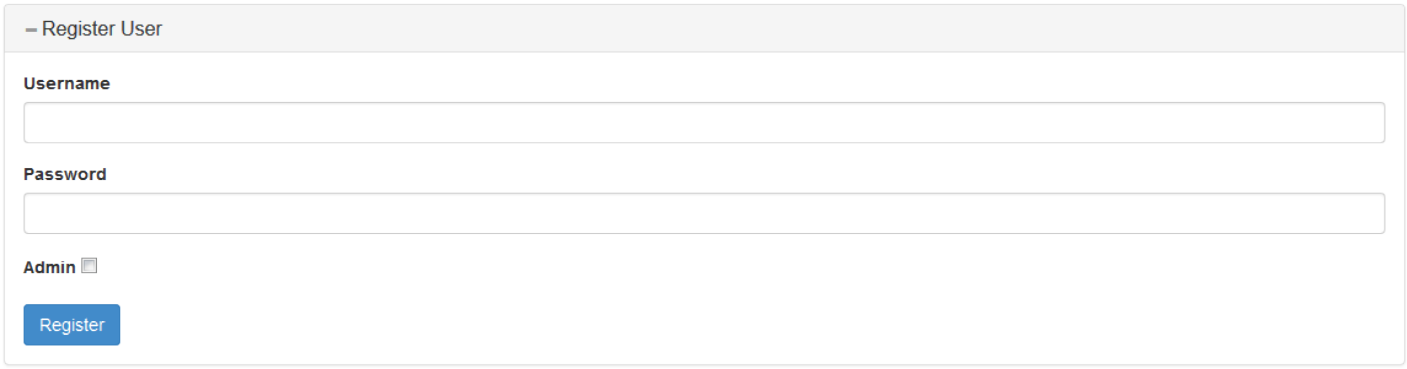
In the xVis menu the *Score column* has to be specified in order to perform the filtering option by Id-Score or FDR. In addition the score range has to be defined by chosing whether crosslinks above or below a defined threshold value are displayed. Default: no score column; score range smaller.



Administrator Setting

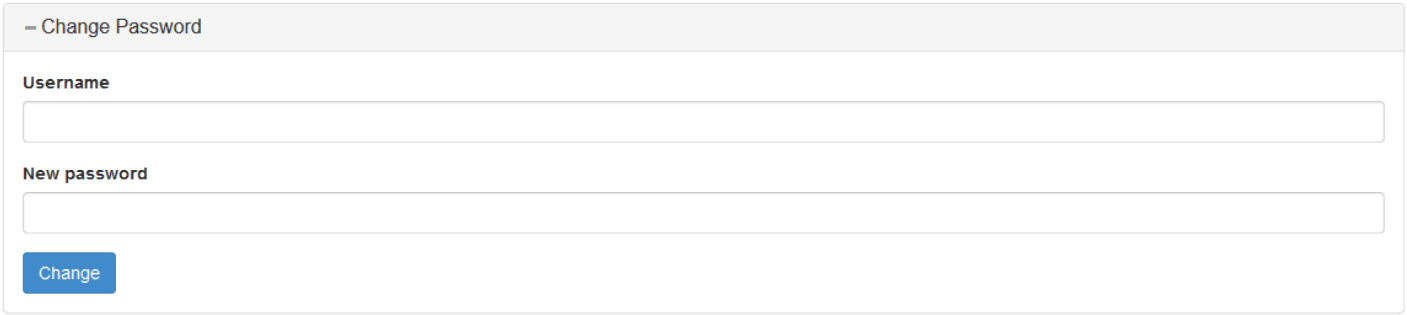
Register User

The easy registration process of new users by the administrator cis possible through the web interface. The user has to enter a username. The name has to be unique in the system. If the name already exists an error will occur. Furthermore, the user has to enter a password in the password field (it shows placeholders instead of the password). If you want to establish a new admin you have to set the *admin* flag. Upon confirmation with the Register button the password gets md5 encrypted and the new user is registered.



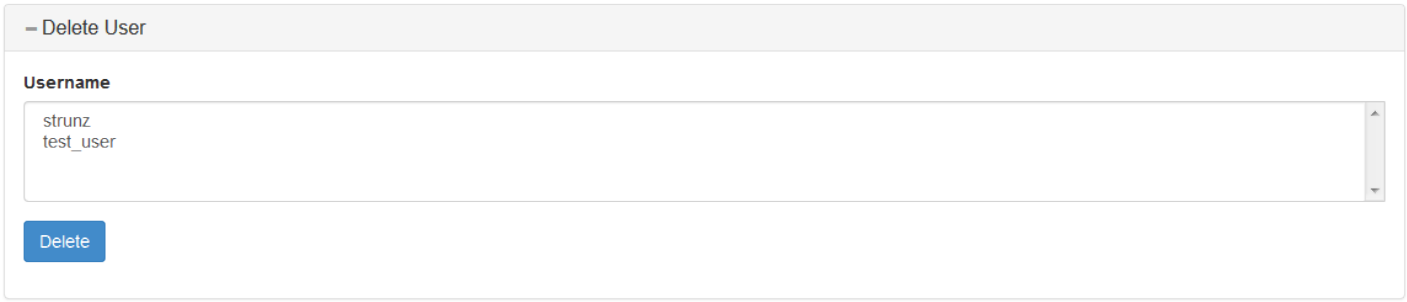
Change Password

The user has to enter the username and a new password. By clicking Change the password gets changed with a modification of the admin status, the settings or files.



Delete User

To delete one or more users select them and press delete. Note if you delete a user you delete also all user settings and the complete folder structure for this user.



Installation

Installation on a web server

1. Verify that your server supports PHP. For more information and to download PHP see http://php.net/downloads.php.
2. Extract the zip file (see xVis source code) and copy source code files in the directory of the server
3. a. If you want to use **xVis** without user accounts delete the folder *xVis/user/test_user*. You should delete the test user to prevent undesired access to the server (**the test user has admin rights!**).
   b. If you want to use **xVis** with user accounts you have to login as *test_user* (password: user-56). Afterwards change the password or create a new admin user and delete *test_user*. Now you are able to create user as well as admin accounts.

Local Installation / Installation of xVis containing XAMPP

This section describes the installation of **xVis** on a local computer as well as the installation if no webserver exist (only available for windows). The provided package contains a predefined Apache server called XAMPP.

1. Extract the zip file (see xVis with XAMPP)and copy the folder xampp into the destination folder.
2. Run *xampp/setup_xampp* and approve the command line application.
3. Run *xampp/apache_start*. You have to rerun this file each time to start the server e.g. after restarting the computer.
4. See *Installation on a web server* section 3
5. It is recommended to change the security settings (http://localhost/security/index.php) of the Apache server if it can be accessed through the internet to prevent malicious access.
6. xVis is accessible by *localhost/xVis* or by *[IP/name of the server]/xVis*