



User Guide

Version 1.0 beta

CaverSoft, Ltd.

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1 Introduction

1.1 About CAVER Analyst

CAVER Analyst 1.0 is a professional software program for the analysis of access pathways to buried active sites in proteins. Many proteins possess deeply buried binding sites in the protein core. The size, shape, physico-chemical properties and dynamics of pathways leading from the protein surface to the core are of the utmost importance for the accessibility and biological activity. Accessibility of the pathways for the ligands is as important as the ligand's fit to the binding site itself. CAVER Analyst 1.0 assists with calculation, analysis, visualization and rational re-design of the ligand access pathways.

CAVER Analyst 1.0 offers a number of features assisting analysis of the access pathways in protein structures:

- Visualization of all identified access pathways.
- Frequency and average overall cost of individual pathways.
- Average radius, length, volume and curvature of a given pathway.
- Maximum and average bottleneck of a given pathway.
- Residues lining a given pathway and bottleneck.
- Detailed pathway profiles.
- Pathway dynamics by animation and heat plots.

1.2 Hardware and Software Requirements

Minimal configuration:

- Java 1.7
- 1 GB RAM
- 32-bit architecture
- Intel HD family graphics adapter

Recommended configuration:

- Java 1.7 or later
- 8 to 16 GB RAM
- 64-bit architecture
- AMD Radeon or NVIDIA GeForce family graphics card

Regardless of the recommended configuration, CAVER Analyst is platform-independent; therefore the following platforms are supported:

- Windows XP, Vista, 7 or 8
- Mac OS X 10.6 or later
- Linux major distributions including Fedora Core, Red Hat and Ubuntu

1.3 User Support

More than 5,000 users world-wide have downloaded some version of CAVER and many of them have provided invaluable feedback. Users' comments and recommendations have always been instrumental for the development of the software thus we are grateful for any kind of feedback from your experience with the CAVER Analyst.

To obtain more information, provide recommendation, request new features or report bugs in CAVER Analyst, please register at our webpage at www.caver.cz, or write us at caver@caver.cz.

Users of the commercial version can also utilize a user support provided by CaverSoft, Ltd. at info@caversoft.com.

2 Software Features

2.1 Workspace

The workspace of the CAVER Analyst application consists of the main menu, toolbar and four main panels (see figure 2.1). The top part of the left panel (**control panel**) is used to control loaded structures (Structures Overview window) and to manage existing selections (Structure Selections window). The bottom of the left panel is reserved for working with various tools, e.g. clip planes (Structure Clip Plane window), structure alignment of two structures (Structure Alignment window) and controlling probe size and transparency of protein surface (Surface Configuration window).

The main menu and toolbar provide access to all functions of the application. The toolbar has a shortcut to the most often used functions, such as loading the molecule from a disc, downloading it online from the PDB database, saving and loading the current session, changing the visualization style of active structures and tunnels and others. Full descriptions will be provided later in this document.

The bottom panel (**explorer panel**) allows users to explore primary protein structure and make residue selections (Structure Sequence window), to control the animation playback when a dynamic simulation is loaded (Structure Dynamics window) and to explore various tunnel statistics – lining residues, bottlenecks, etc. (Tunnel Computation Statistics window).

The right panel (**computation panel**) allows users to choose input parameters for tunnel computation and to launch their calculation (Tunnel Computation window). This area also contains the Color window for changing color schemes for individual atoms, residues or chains for visualization purposes. When you want to see more information about loaded structures, you can use the Structure Properties window, which will also be displayed in the right panel.

Finally, the central **visualization panel** serves for visualization and view control (Visualization window, visualizing and setting of graph statistics of computed tunnels (Tunnel Graph window) and for displaying log messages (Log window).

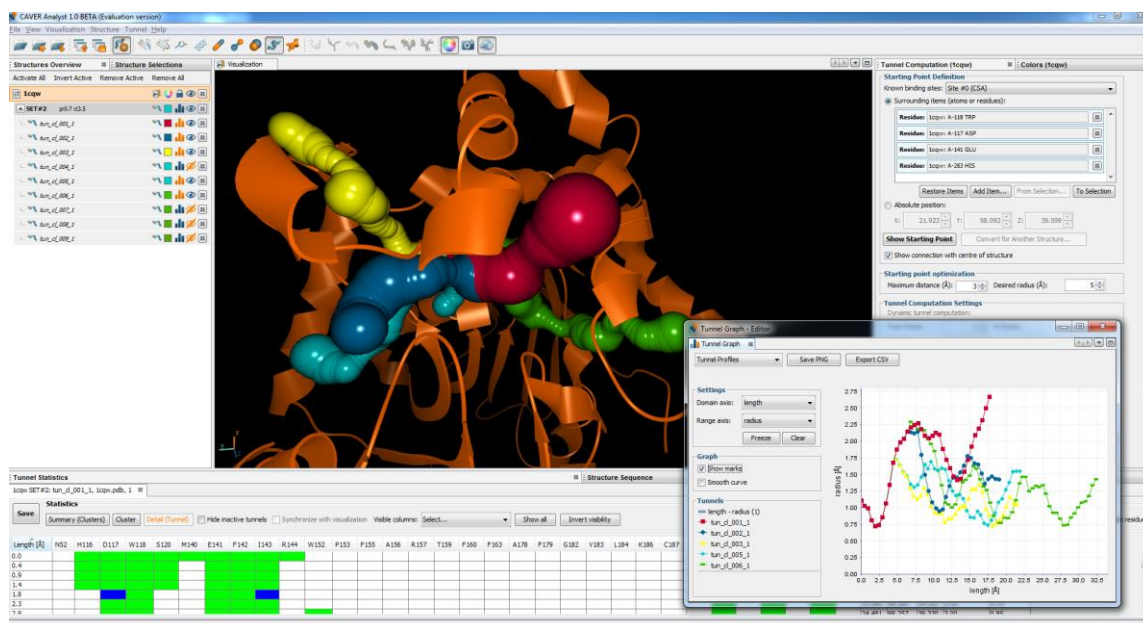


Figure 2.1: CAVER Analyst work area

IMPORTANT NOTE

Windows in one panel can be switched by clicking their title header. Each panel can be closed or reopened via the Main Menu. Their size can be deliberately altered by dragging. Users can also design their own layout by dragging the windows to the desired panel (drag the title header). They can return to the default layout and size in the main menu: View/Reset Windows. Windows can also be undocked and separated from the workspace.

2.2 Main Menu

Top main menu contains the following categories:

- **File**
 - **Open Structure(s)...**
Allows the user to open a structure from the PDB file stored on the hard drive. CAVER Analyst creates its own *caver* folder (in Windows in C:/Documents and Settings/username/caver). This is where all downloaded PDB files are stored.
 - **Open Molecular Dynamics...**
Allows the user to open a set of PDB files representing molecular dynamics stored on the hard drive.
 - **Download Structure...**
Opens the dialog window enabling the user to enter the PDB ID of the molecule, which will then be downloaded from the PDB database.
 - **Load Workspace...**
Allows the user to load sessions previously saved on the hard drive (in .cws format).
 - **Save Workspace...**
Saves the current CAVER Analyst session (all loaded structures, computed tunnels and their settings) to the hard drive (.cws format).
 - **Application Log**
Opens the center panel with information about performing actions.
 - **Exit**
Closes the CAVER Analyst application.
- **View**
 - **Visualization Window**
Opens the center panel containing a visualization of the scene. More details in section 3.2.
 - **Highlight Selection**
Enables/disables the highlighting of atoms, residues or chains (according to the settings in the Selection window) when passing over them with the cursor.
 - **Surface Configuration**
Opens the Surface Configuration window in the bottom left panel. It allows the user to change the transparency of the molecular surface and the probe size, which controls the preciseness of the surface. It has no effect on other visualization styles.
 - **Scene Fog**
Enables/disables visualization of fog, enhancing the perception of depth in the scene.

- **Scene Drag Rotation**
When enabled, users can launch automatic rotation of the whole scene. The direction and speed of rotation is determined by dragging the mouse.
- **Orthographic Projection**
Allows the user to change the projection type. Orthographic projection does not distort the scene.
- **Reset Camera**
Resets the scene so the loaded structures are centered to the viewport.
- **Toolbars**
Here users can customize the Top Toolbar section – show/hide groups of related icons, change icon size or customize the toolbar.
 - **File** – shows/hides icons related to opening or downloading structures.
 - **Workspace** – shows/hides icons related to loading or saving current session.
 - **Computation** – shows/hides icon related to tunnel computation.
 - **Structure Visualization** – shows/hides icons related to changing visualization modes of structures.
 - **Tunnel Visualization** – shows/hides icons related to changing visualization modes of tunnels.
 - **Tools** – shows/hides icons related to additional tools (currently color chooser and making screenshots).
 - **Small Toolbar Icons** – changes the icon size.
 - **Reset Toolbars** – sets all toolbars to their default settings.
 - **Customize** – add arbitrary icons to the Top Toolbar section.
- **Reset Windows**
All windows are returned to their default position and size.
- **Visualization**
Allows the user to change the visualization style of an activated protein structure or computed tunnels. If no tunnels for a given structure have been computed, changing their visualization style is disabled.
Structure Visualization Styles:
 - **Points**
Visualizes molecule as crosses positioned into the center of each atom.
 - **Dots**
Displays atoms as dotted spheres of van der Waals radii.
 - **Wireframe**
Represents a molecule with its bonds visualized as lines.
 - **Alpha Trace**
Visualizes the polypeptidic chain of a protein by connecting the C-alpha atoms of amino acids.
 - **Sticks**
Displays molecular bonds as three-dimensional sticks.
 - **Balls & Sticks**
Shows both atoms and bonds of the protein.
 - **Van der Waals Radii**
Molecules are represented by a set of spheres with van der Waals radii.

- **Cartoon**
Representation of secondary structures.
- **Surface**
Visualization of the protein surface. Its transparency can be set using the Visualization/Transparency function available from the main menu.

Tunnel Visualization Styles:

- **Points**
Displays tunnels using Tunnel Subdivision Surface, where the surface is represented by dots. This method can also be used for visualizing tunnels in molecular dynamics.
- **Centerline**
Shows a line representing the centerline of a tunnel. This method can also be used for visualizing tunnels in molecular dynamics.
- **Dots**
Displays tunnels as a dotted surface. This method can also be used for visualizing tunnels in molecular dynamics.
- **Spheres**
Shows the tunnel as a set of intersecting spheres. This method can also be used for visualizing tunnels in molecular dynamics.
- **Tube Surface**
Represents the tunnel as a tube along the centerline. This method can also be used for visualizing tunnels in molecular dynamics.
- **Detailed Surface**
This is the widest and most precise tunnel representation. This method takes more computation time, thus it is used only in a static case.
- **Clusters (all snapshots in one)**
This method is available only when computed tunnels in dynamics are available. It displays the centerlines for all tunnels computed for all snapshots at once. Centerlines are colored according to their related clusters.

- **Structure**

- **Overview**
Opens the top left panel containing a list of all loaded structures. More details in section 3.1.
- **Selections**
Opens the top left panel containing a list of all created selections. More details in section 3.7.
- **Alignment**
Opens the Structure Alignment window in the bottom left panel. Details in section 3.11.
- **Clip Plane**
Opens the Structure Clip Plane window in the bottom left panel. Details will be described in section 3.12.
- **Sequence**
Opens the bottom panel containing a sequential representation of all loaded proteins. More details in section 3.6.
- **Dynamics**
Opens the bottom panel containing control buttons for animating molecular dynamics.

- More details in section 3.8.
 - **Properties**
Opens the top right panel, which contains detailed information about the active structure. More details in section 3.9.
- **Tunnel**
 - **Computation**
Opens the top right panel, enabling entry parameters for tunnel computation. More details in section 3.3.
 - **Statistics**
Opens the bottom panel with detailed statistics about computed tunnels – clusters, lining residues, bottlenecks, etc. More details in section 3.4.
 - **Graph**
Opens the center panel containing a window which displays various tunnel statistics. More details in section 3.10.
 - **Advanced Settings**
Opens the top right panel, enabling the user to view and edit all parameters influencing the CAVER algorithm for tunnel computation. More details in section 3.4.
- **Help**
 - **About CAVER Analyst**
Information about product version and copyright.
 - **User guide**
Opens this user guide in the pdf format.

2.3 Top Toolbar

The top toolbar contains buttons with shortcuts to the most frequently used functions (see figure 2.3).



Figure 2.3: Toolbar icons

Functions of icons (from left to right):

- **Open Structure(s)...**
Opens a dialog window for selecting a PDB file from the hard disc.
- **Open Molecular Dynamics...**
Opens a dialog window for selecting a set of PDB files the from hard disc which represent molecular dynamics.
- **Download Structure...**
Allows the user to enter a 4-character PDB ID for a molecule, which will then be downloaded from the PDB database.
- **Load Workspace...**
Opens a dialog window for selecting a previously stored working session in .cws format.
- **Save Workspace...**
Opens a dialog window where the user selects the destination for saving the current working session in .cws format.

Changing visualization styles of molecules and tunnels (they influence only the “active” structures and tunnels – highlighted in the Structure window and more styles can be activated simultaneously):

- **Structure Points**
Molecules are represented by crosses in the centers of all atoms.
- **Structure Dots**
Atoms are represented by dotted spheres with van der Waals radii.
- **Structure Wireframe**
Molecules are rendered by lines representing bonds between atoms.
- **Structure Alpha Trace**
C-alpha atoms of all amino acids are connected and interpolated – it displays the polypeptidic chain in a simple manner.
- **Structure Sticks**
Molecules are represented by 3D sticks showing bonds between atoms.
- **Structure Balls & Sticks**
The most detailed representation of a molecule. It displays all atoms and bonds.
- **Structure Van der Waals Radii**
Atoms are represented by spheres of van der Waals radii.
- **Structure Cartoon**
Molecules are displayed using secondary structures – helices, sheets, coils.
- **Structure Surface**
Displays the molecular surface of the protein. Surface preciseness can subsequently be refined – see section 3.14.
- **Tunnel Points**
Tunnels are visualized using a dotted subdivision surface. It can also be animated.
- **Tunnel Centerline**
Tunnels are displayed as a line representing their centerline. Also suitable for moving tunnels in molecular dynamics.
- **Tunnel Dots**
Tunnels are displayed as a smooth surface (Tunnel Tube Surface) represented by dots.
- **Tunnel Spheres**
Tunnels are represented by intersecting spheres positioned on the tunnel centerline. Also suitable for visualizing tunnel dynamic changes.
- **Tunnel Tube Surface**
A novel method for tunnel visualization using a smooth surface. It is also possible to use this representation in molecular dynamics.
- **Tunnel Detailed Surface**
The most exact representation of tunnel boundaries in surface form. It is also possible to use this representation in molecular dynamics. Moreover, the user can change the preciseness of the tunnel surface by changing the probe size – see section 3.14.
- **Tunnel Clusters (all snapshots in one)**
This representation is suitable only for tunnels computed in a trajectory. It displays tunnels as centerlines and visualizes tunnels from all snapshots at once. The centerline color determines individual clusters detected in the computation phase.

Other functions:

- **Colors**
Opens the Colors window for changing colors of different protein parts (see section 3.9).
- **Screen Capture**
Allows the user to store current snapshot of the visualization window in the .png, .bmp or .jpg format.
- **Fog**
Allows the user to turn fog on and off in the scene. Fog can enhance the perception of depth in the scene.

3 Windows and their Features

3.1 Structures Overview Window

This window allows the user to organize all structures currently loaded into the application (see figure 3.1.1). The basic principle of manipulation with structures is as follows. When the user wants to work with a given structure, the structure must be **ACTIVE**. This means that it is selected in the Structures Overview window. A selected structure is highlighted in orange. More structures can be activated at once. Using Ctrl + Left Mouse Button, the user can select or deselect any structure. The Shift + Left Mouse Button combination allows the user to select or deselect more structures between two selected structures (similar as in other, common applications).

All structures can be selected using the “Activate All” button at the top of the window. The “Invert Active” button switches between selected and deselected items. The “Remove Selected” and “Remove All” buttons can remove structures from the application.

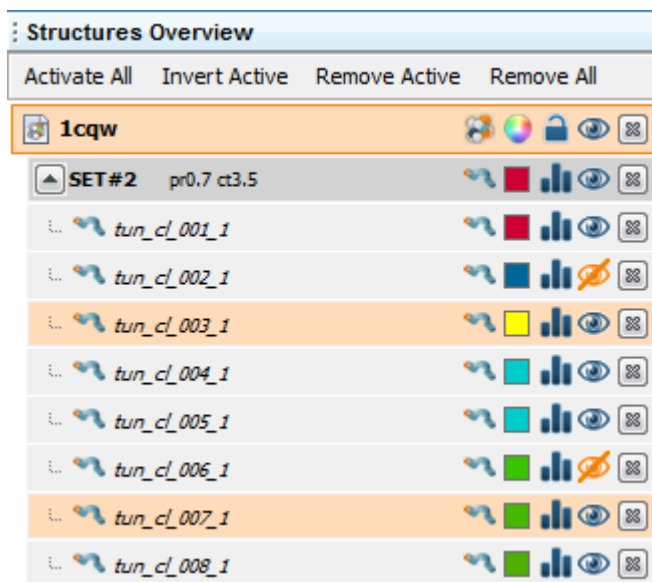


Figure 3.1.1: Structures Overview Window example

IMPORTANT NOTE:

WHEN SOME FUNCTIONALITY OF CAVER ANALYST APPEARS TO BE DISABLED, PLEASE VERIFY THAT YOUR DESIRED STRUCTURE IS ACTIVE!

Detailed Description of Structures Overview Window

In the Structures Overview Window loaded structures and tunnels are displayed as a list of individual rows. Each structure row contains the 4-character PDB ID of the structure and a set of icons for direct manipulation of the structure. The icons are (from left to right):

- **Changing Visualization Style**
Shows the changing style window very similar to the window which can be activated in the main menu – Visualization.
- **Colors**
Switches to the Colors window for changing coloring styles.
- **Lock Structure**
Allows the user to lock the structure in a given position so that the user cannot rotate and move with the structure within the scene.
- **Show/Hide Structure**
Shows/hides the structure in the visualization window.
- **Close Structure**
Removes the structure from the application.

When clicking on a structure with the right mouse button, the following menu appears:

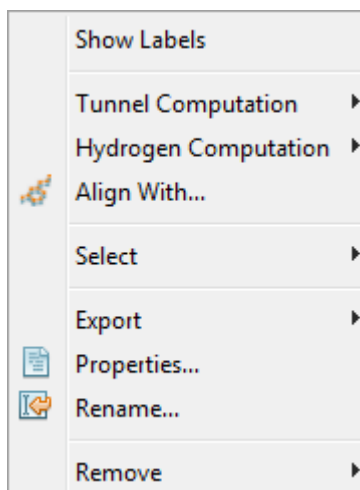


Figure 3.1.2: Menu for each structure

- **Show Labels**
Shows labels on all atoms in the scene.
- **Tunnel Computation**
 - **Compute Tunnels...**
Opens the Tunnel Computation window (see details in section 3.3).

- **Import Tunnels...**
Allows the user to import already computed tunnels from the hard drive in the .pdb format (stored previously using tunnel export – see below).
- **Import Caver 3 Outputs...**
Allows the user to load the whole CAVER 3 output directory containing computed tunnels and all statistics.
- **Hydrogen Computation**
 - **Compute Hydrogens**
Launches a computation of hydrogen atoms which are assigned to a given structure and visualized.
 - **Configure Protonation of Titratable Residues...**
Allows the user to change the protonation of titratable residues when computing hydrogen atoms.
- **Align With...**
Opens the Structure Alignment window (see details in section 3.12).
- **Select**
 - **Waters** – creates a selection containing all waters present in the PDB file of given structure.
- **Export**
 - **Export to PDB** – allows the user to save the current structure in PDB format to an external file.
- **Properties...**
Opens the Structure Properties window (see details in section 3.9).
- **Rename..**
Allows the user to rename the structure (the new name appears in the given structure row).
- **Remove**
 - **Structure** – removes the structure from the application.
 - **Waters** – allows the user to remove water molecules from the structure. Please note that this function cannot be undone!
 - **Hydrogens** – removes hydrogens from the given structure. Recommended usage: when some hydrogens are already present in the PDB file of the structure, first remove them and then launch Compute Hydrogens.
 - **All Tunnels** – removes all tunnels of a given structure from the application.
 - **Ligands...** – if the input PDB file of a given structure also contains a ligand, it is recognized in the loading phase and stored separately. This function opens the dialog window with all ligands present in the structure and the user can select which ones should be removed.

All tunnels computed in one pass of the CAVER algorithm are grouped into one tunnel set, marked as SET#X. Each tunnel (tunnel cluster, to be more specific) has also its own record (see figure 3.1.3). The tunnel set is assigned to its structure and its name also contains the settings of two major computation parameters – *probe radius* and *clustering threshold*. Moreover, individual tunnels are also named (starting with *tun_cl_#* - abbreviation for *tunnel cluster*). They are sorted – a smaller tunnel number indicates its greater relevance.

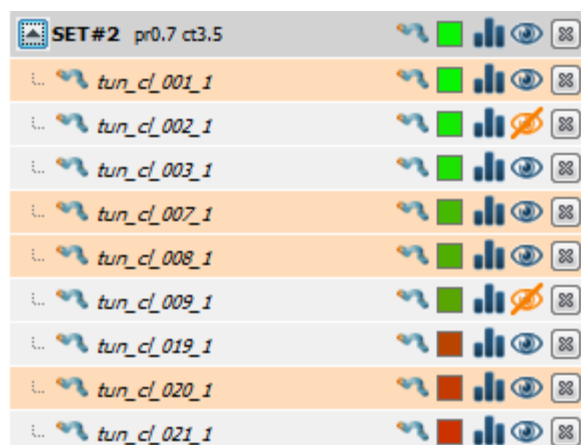
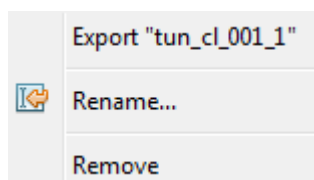


Figure 3.1.3: Example of tunnel set record

Each tunnel set and tunnel record contains the following functions controlled by icons (from left to right):

- **Changing Visualization Style**
Shows the changing style window where the user can change the visualization style of the tunnel (same as in Toolbar or Visualization in main menu).
- **Tunnel Color**
Allows the user to change the color of given tunnel.
- **Show Tunnel Graph**
Opens the Tunnel Graph window (see section 3.9).
- **Show/Hide Tunnel**
Shows/hides the tunnel in the visualized scene.
- **Close Tunnel**
Removes the tunnel from the application.

When clicking with the right mouse button on a specific tunnel, the following menu appears:



3.1.4: Menu for each tunnel record

- **Export "tun_cl_#"**
Allows the user to store a given tunnel in .pdb format to the hard drive.
- **Rename...**
Shows the dialog window for renaming a given tunnel.
- **Remove**
Removes a given tunnel from the application.

3.2 Visualization Window

The visualization window displays all loaded structures (if they are not explicitly hidden) and allows the user to control them via a standard interface. The user can rotate (left mouse button click + mouse drag), scale (right mouse button click + mouse drag) or translate them (mouse wheel button click + drag), see figure 3.2.

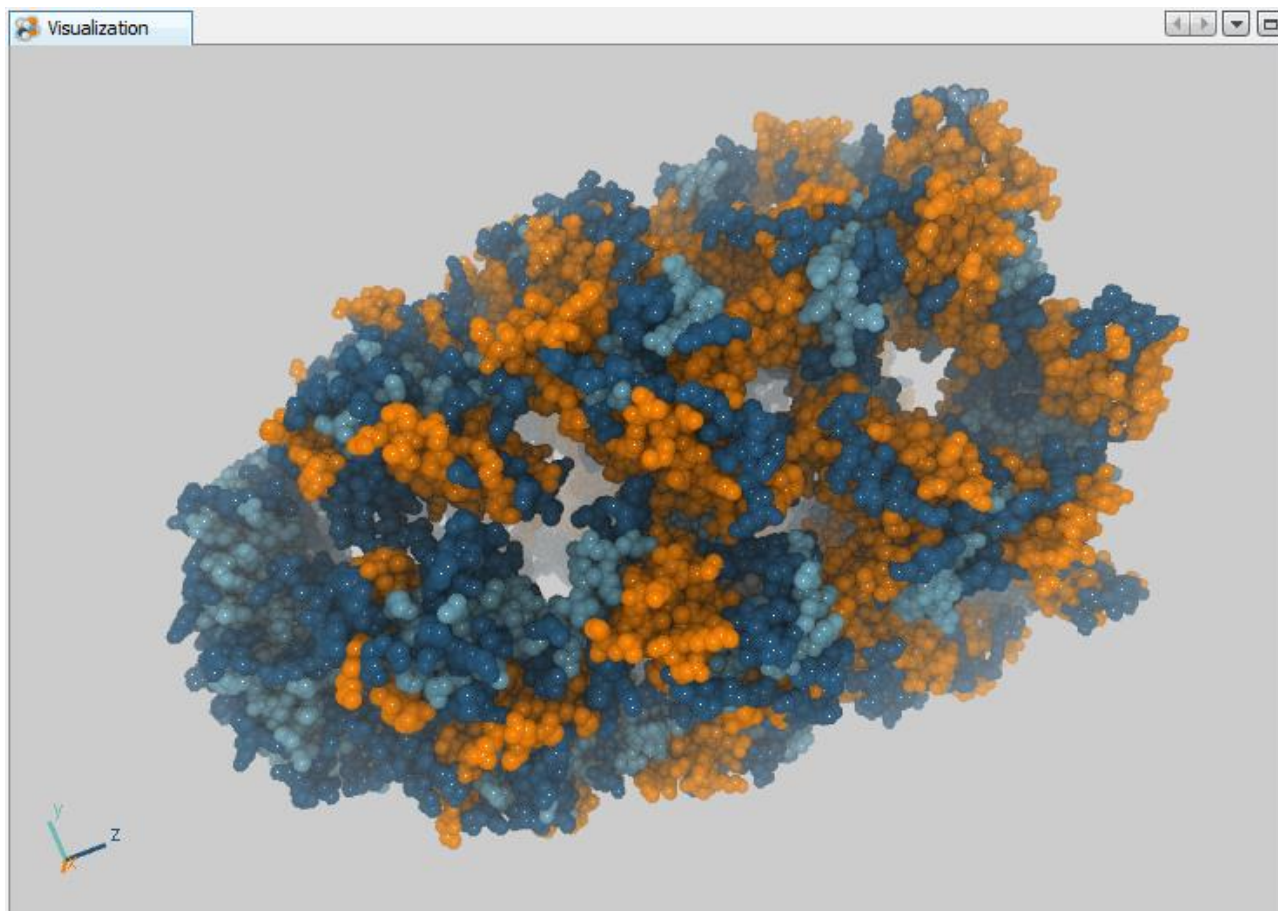


Figure 3.2: Visualization window with gray background color and enabled fog

3.2.1 Manipulation with the Scene

The scene can be controlled using two different approaches:

- **Global manipulation** – users can manipulate (zoom, rotate, move) the whole scene (with all loaded structures at once). This can be done by using three mouse buttons.
 - *Left mouse button* – rotation with all structures (active and inactive) around the scene center.
 - *Right mouse button* – scales the whole scene along the Z-axis.
 - *Middle mouse button* – translates the whole scene.
- **Local manipulation** – users can only manipulate active structures (highlighted in the Structures Overview window). This manipulation is activated by pressing **CTRL** + mouse buttons.

- *CTRL + left mouse button* – rotation with active structures around their local center (defined by the bounding box).
- *CTRL + right mouse button* – scales the ACTIVE structures in the Z-axis.
- *CTRL + middle mouse button* – translates the ACTIVE structures.

3.3 Tunnel Computation Window

The Tunnel Computation window controls the computation of tunnels (see figure 3.3) – setting the binding site position, important parameters, etc. This panel is activated by selecting the desired structure in the Structure Overview window. In the first section, the user specifies the starting point – the initial position of binding (or active) site. The application first searches the active site in the Catalytic Site Atlas, according to the structure PDB ID code. When the search fails or if the user decides to select other starting point, the active site can be set manually (by determining the surrounding atoms and/or residues, or X, Y, Z coordinates). This starting point can be subsequently visualized in the display window using the “Show Starting Point” button. After that, the user chooses the desired number of tunnels they want to compute and items included into computation (waters are excluded by default) and clicks “Compute Tunnels”.

Figure 3.3: Tunnel Computation window

Proper descriptions of each function of the Tunnel Computation window are as follows:

- **Starting point definition**

This section allows the user to set the position of the binding site.

- **Known binding sites**

Shows the list of active sites for a given structure. This list is loaded from the Catalytic Site Atlas (CSA) database. If no records for a given structure are present in CSA, this section remains empty.

- **Surrounding items (atoms or residues)**

Displays the list of atoms or amino acids surrounding the active site. Users can manipulate this list by adding and removing atoms and/or residues. The “Restore Items” button resets the list to initial values (loaded from CSA or empty). The “Add Item” button opens the dialog window for setting parameters of a new atom and/or residue included into the active site definition (see figure 3.3.1).

Firstly, the user can select a structure for which they want to define the binding site. If this structure contains more chains, they can also choose one containing the desired atom/residue. Then they can select atoms or residues for binding site definition. In both cases the user must know the ID of the atom or residue. The user can add more atoms or residues at one time by dividing them by comma (eg. 123,247,256).

Then the user must check if a given ID is present in the given structure and chain by pressing the “Validate” button. If it succeeds, the user obtains a list of selected atoms/residues along with their name abbreviation.

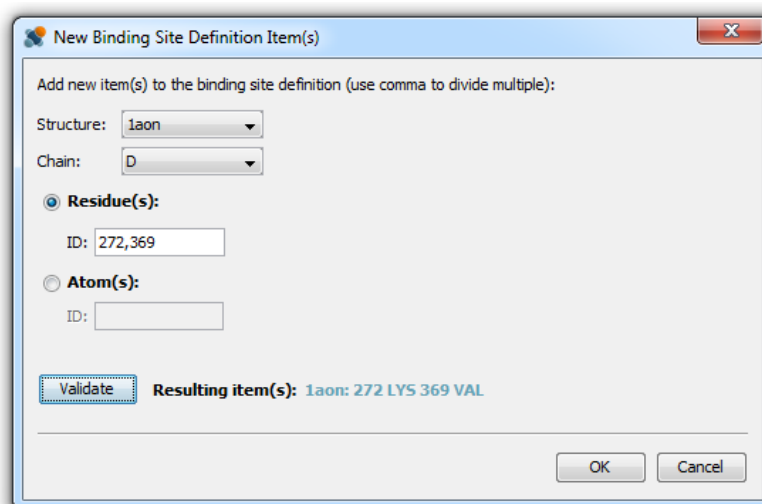


Figure 3.3.1 Definition of a binding site

- **From Selection...**

The binding site can be also defined by a selection (see figure 3.3.2). The user can choose from a list of created selections (present in Structure Selections window) and specify if the starting point should be defined by atoms or residues of the given selection. Finally the user assigns the starting point to one of the loaded structures.

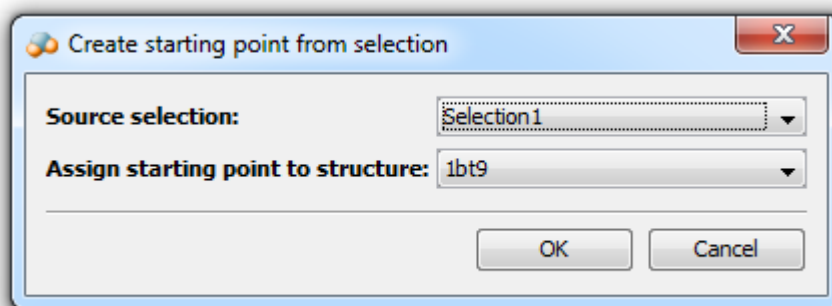


Figure 3.3.2 Creating starting point from selection

- **To Selection**
This option adds the list of surrounding items into the selection. If a selection is active, it adds the items to this selection. Otherwise it creates a new selection.
- **Absolute position**
Users can set the absolute X, Y, Z coordinates of the active site.
- **Show Starting Point**
Button activating visualization of a cross representing the active site.
- **Convert For Another Structure...**
A given binding site can be reused for another structure (see figure 3.3.3). This performs the structure alignment and stores the binding site for selected structures as absolute position.

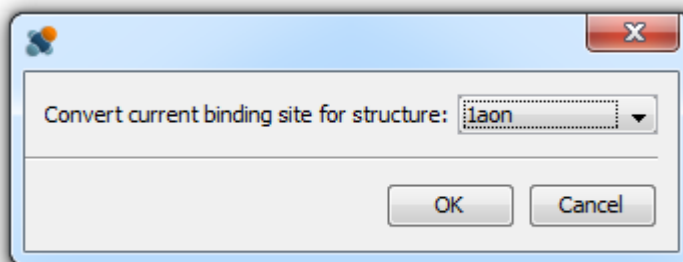


Figure 3.3.3 Conversion of a binding site

- **Show connection with centre of structure**
This checkbox displays the orange cross in the geometrical centre of the structure and connects it by a dashed line with the starting point. This serves for better orientation in the space.
- **Starting Point Optimization**
 - **Maximum distance (Å)**
Specifies the maximum distance of the calculation starting point from the initial starting point (for more details see CAVER 3.0 user guide).
 - **Desired radius (Å)**
The closest Voronoi vertex to the initial starting point, which is located within a specified distance from the initial starting point and at least a *desired_radius* far from the balls rep-

representing the input structure, which will be used as the starting point for the calculation of tunnels (for more details see CAVAR 3.0 user guide).

- **Tunnel Computation Settings**

- **Dynamic tunnel computation**

- Allows the user to set the range and sparsity of snapshots for which tunnels should be computed. Active only for dynamic structures.

- **From frame, To frame**

- The number of frames defining the first and last frame for tunnel computation (for more details see CAVAR 3.0 user guide).

- **Sparsity**

- Defines which snapshots tunnels will be computed for. For example, a sparsity value of 5 means that tunnels will be computed in each fifth snapshot from the trajectory (for more details see CAVAR 3.0 user guide).

- **Approximation**

- Specifies the number of balls which will be used to approximate individual atoms in the input structure (for more details see CAVAR 3.0 user guide). A higher number means more precise results but also greater memory consumption.

- **Min. probe radius**

- Defines the desired minimal radius of computed tunnels (for more details see CAVAR 3.0 user guide).

- **Clustering threshold**

- Specifies the level of detail at which the tree hierarchy of tunnel clusters will be cut, and thus influences the size of resulting clusters (for more details see CAVAR 3.0 user guide).

- **Shell depth**

- Specifies the maximum depth of a surface region, i.e., a part of the input structure located below the bulk solvent region (for more details see CAVAR 3.0 user guide).

- **Shell radius**

- Specifies the radius of the shell probe which is used to define which parts of the Voronoi diagram represent the bulk solvent (for more details see CAVAR 3.0 user guide).

- **Residues included into tunnel computation**

- In this section the user can find all structures which are present in a given structure (amino acids, ligands, waters, etc.). The user can select which of these structures should be involved in a tunnel calculation.

- This section includes two checkboxes:

- **Detailed list**

- Displays the list of all amino acids present in the structure (along with their ID).

- **Exclude active selections**

- Excludes selections which are highlighted as active in the Structure Selections window.

- **Output directory**

- Allows the user to select the destination folder for the results of tunnel computation.

- Set of icons for:
 - **Save computation settings to an external file**
 - **Load computation settings from an external file**
 - **Get computation settings from previously computed results**
- **Compute Tunnels**
 Button for launching the tunnel computation.

3.4 Tunnel Advanced Settings Window

This window allows the user to edit all parameters which influence the results of tunnel computation (see figure 3.4). Changing or adding arbitrary parameters is further described in the CAVER 3.0 user guide.

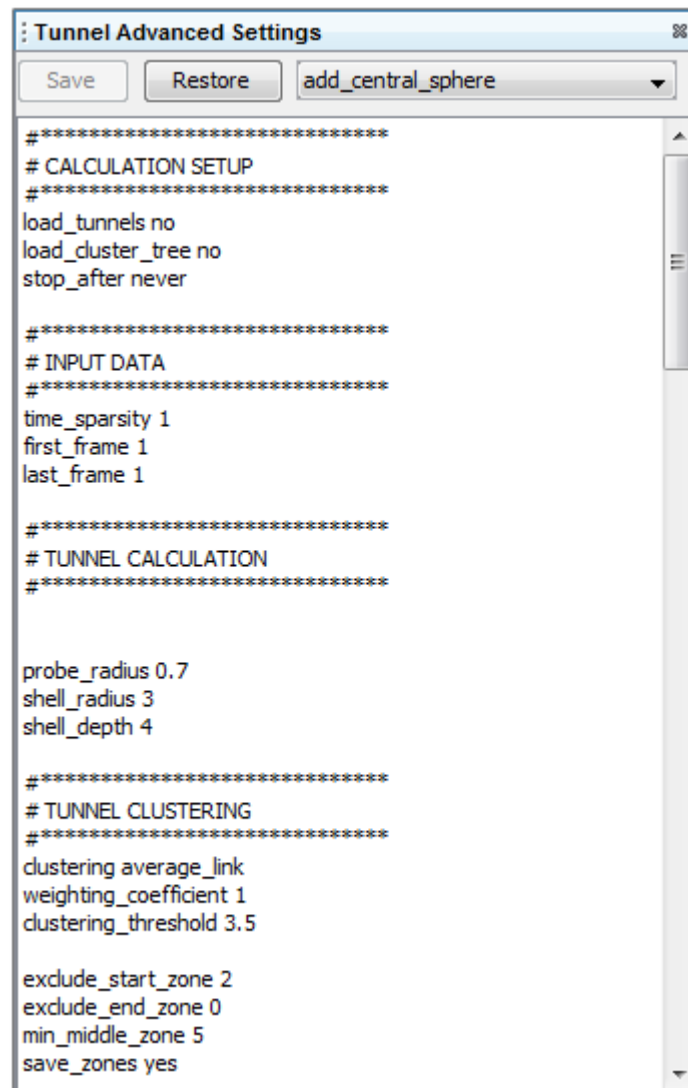


Figure 3.4 Tunnel advanced settings panel

After changing a parameter, the “Save” button is enabled. To return to the default settings, the user can use the “Restore” button. For a better orientation in the parameters, the user can jump to a section containing the desired parameter using the combo box at the top right corner of the panel.

3.5 Tunnel Statistics Window

The Tunnel Statistics window allows the user to display statistical information about computed tunnels at different levels of detail.

The first table displays a summary for computed tunnel clusters (see figure 3.5.1).

ID	No	No_snaps	Avg_BR [Å]	SD	Max_BR [Å]	Avg_L [Å]	SD	Avg_C	SD	Priority	Avg_throughput	SD
tun_d_001_1	1		10.72	0.000	0.71	17.65	0.000	1.175	0.000	0.432	0.432	0.000
tun_d_002_1	1		10.72	0.000	0.71	18.98	0.000	1.747	0.000	0.327	0.327	0.000
tun_d_003_1	1		10.72	0.000	0.71	21.49	0.000	1.338	0.000	0.190	0.190	0.000
tun_d_004_1	1		10.72	0.000	0.71	22.58	0.000	1.574	0.000	0.186	0.186	0.000
tun_d_005_1	1		10.72	0.000	0.71	21.86	0.000	2.179	0.000	0.185	0.185	0.000
tun_d_006_1	1		10.72	0.000	0.71	32.35	0.000	1.740	0.000	0.095	0.095	0.000
tun_d_007_1	1		10.72	0.000	0.71	32.28	0.000	1.805	0.000	0.093	0.093	0.000
tun_d_008_1	1		10.72	0.000	0.71	41.71	0.000	1.811	0.000	0.084	0.084	0.000

Figure 3.5.1 Table summarizing tunnel cluster statistics

The user can open more tunnel cluster statistics at once and they’ll be displayed as individual tabs. Each tab is named according to the related tunnel set, tunnel cluster or individual tunnel. Each tab contains a set of buttons and other controls for manipulation within the table.

- **Save**
Allows the user to save the table in the .csv format to a desired location on the hard drive.
- **Summary (Clusters)**
This button allows for navigation in the table. When the user switches to detailed tables containing individual clusters (second level of detail) or tunnels (third level of detail) (see below), this button returns to this table summarizing information about clusters.
- **Cluster**
This button has a similar function as the Summary (Clusters) button. It returns the user from the third level of detail – information about individual tunnels – to the second level containing cluster information.
- **Detail (Tunnel)**
Informative button, always disabled. It informs the user about the level of detail in the statistics panel.
- **Hide inactive tunnels**
When this checkbox is checked, the Visualization window shows only active tunnel clusters (activated by clicking into the table on a corresponding row or activating them in the Structures Overview window – see section 3.1).
- **Synchronize with visualization**
Active only for molecular dynamics. When the dynamics is animated (animation can be launched using the Structure Dynamics window – see section 3.8), it enables to animate also the statistics table.

- **Visible columns**
Shows the list of all available columns and allows the user to show/hide them using their checkbox.
- **Show all**
Displays all available columns, including the hidden ones.
- **Invert Visibility**
Inverts the visibility of columns (the visible ones will be invisible and vice versa).

Individual columns have the following meaning:

- **ID** – identification of a given tunnel cluster; ranks a given cluster based on their priority.
- **No** – total number of tunnels belonging to a given cluster.
- **No_snaps** – number of snapshots with at least one tunnel with a radius \geq parameter *min_probe_radius*
- **Avg_BR [Å]** – average bottleneck radius.
- **SD** – standard deviation (present more times in the table, always corresponds to preceding column).
- **Max_BR[Å]** – maximum bottleneck radius.
- **Avg_L[Å]** – average tunnel length.
- **Avg_C** – average tunnel curvature.
- **Priority** – tunnel priority calculated by averaging tunnel throughputs over all snapshots (zero value used for snapshots without tunnels).
- **Avg_throughput** – average tunnel throughput.

By **double-clicking** on a desired tunnel cluster (corresponding row in the table) the user can open the details about the given cluster (see figure 3.5.2).

Snapshot	Tunnel cluster	Tunnel	Throughput	Cost	Bottleneck radius [Å]	Length [Å]	Curvature
1cqw.pdb	tun_d_001_1	1	0.432	0.840	0.71	17.7	1.18
1cqw.pdb	tun_d_001_1	3	0.296	1.218	0.71	19.3	1.54
1cqw.pdb	tun_d_001_1	5	0.222	1.507	0.71	19.6	1.33
1cqw.pdb	tun_d_001_1	9	0.182	1.703	0.71	21.1	1.53

Figure 3.5.2 Table containing information about individual cluster

This table activates the “Cluster” button. To return to the summary about all clusters, the user can press “Summary (Clusters)” button. This table contains the following columns:

- **Snapshot** – name of the input structure, in which the cluster was identified.
- **Tunnel cluster** – the ID of the tunnel cluster to which a given tunnel belongs (corresponds to the Tunnel cluster ID in the summary.txt).
- **Tunnel** – ID of a given tunnel in a given snapshot.
- **Throughput** – the throughput of a given tunnel ($\text{throughput} = e^{-\text{cost}}$).
- **Cost** – the cost of a given tunnel defined as the balance between the width and length of the tunnel.

- **Bottleneck radius [Å]** – the radius of the bottleneck, i.e., the narrowest part, of a given tunnel.
- **Length [Å]** – the length of a given tunnel.
- **Curvature** – the curvature of a given tunnel which is calculated as *length/distance*, where *length* is the length of the tunnel (distance from the calculation starting point to the tunnel ending point calculated along the tunnel axis) and *distance* is the shortest possible distance between the calculation starting point and the tunnel ending point.

By **double-clicking** on desired tunnel (corresponding row in the table) the user can open the details about given tunnel (see figure 3.5.3).

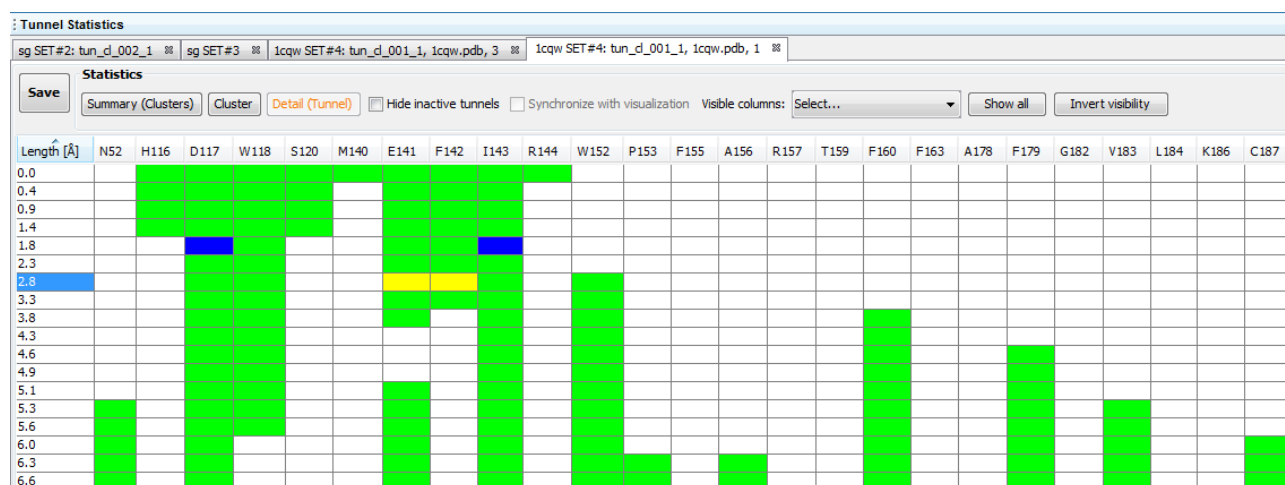


Figure 3.5.3 Table containing information about individual tunnel

This table provides detailed information about an individual tunnel. It displays tunnel lining residues along with their exact position in the tunnel. If a given residue influences the width of the tunnel in a position given by the Distance parameter (counted from the binding site position to the outer surface), it is marked by a green square.

Moreover, if the tunnel analysis was performed with the *compute_bottleneck_residues* parameter set to yes, this table also shows the bottleneck residues which line the narrowest part of the tunnel. These residues are marked with a blue square.

A yellow square represents the selected residue (it is also added to the active selection). It can be selected using the left mouse button. Users can also utilize the combination with Shift and Ctrl having the standard meaning. Selection can be deselected using the Ctrl+D.

If the bottleneck residues were not calculated, the user can start their calculation in the Structure window. By clicking with the right mouse button on a desired tunnel set and choosing “Statistics → Bottlenecks” the user can also compute the bottleneck residues. Reopening the statistics table with details about clusters (choosing “Statistics → Tunnels” in the same window as with bottlenecks) displays blue squares representing bottleneck residues.

The rows of the table represent one sphere contained in the tunnel representation. It starts from the binding site and ends at the protein surface. The green squares in each row signify their presence around the sphere.

The columns of the table have the following meaning:

- **Length [Å]** – the Euclidean distance between the first sphere of the tunnel and the current sphere (in given table row).
- **Individual tunnel lining residues** – a set of columns; each column represents one residue which was present at least once at the tunnel neighborhood. Each column is named according to the one-letter amino acid abbreviation and it's PDB ID (e.g. A156).
- **X, Y, Z** – coordinates of the corresponding sphere centre.
- **Distance [Å]** – represents the distance of the current sphere from the first sphere of the tunnel, calculated along the tunnel centerline. It depends on the *tunnel_sampling_step* parameter.
- **Radius [Å]** – radius of the corresponding sphere.

Residues table

This table provides detailed information about the tunnel lining residues of a given tunnel set (see figure 3.5.4).

Cluster	I15	G16	F19	P20	F21	D22	P23	H24	Y25	V26	E27	V28	L29	E31	M33	H34	Y35	V36	D37	V38	P40	R41	D42	G43	T44	P45	V46	L47	F48	L49	H50
tun_d_004_1																															
tun_d_005_1																															
tun_d_006_1																															
tun_d_007_1																															
tun_d_008_1																															
tun_d_009_1																															
tun_d_010_1																															
tun_d_011_1																															
tun_d_012_1																															
tun_d_013_1																															
tun_d_014_1																															
tun_d_015_1																															
tun_d_016_1																															
tun_d_017_1																															
tun_d_018_1																															
tun_d_019_1																															
tun_d_020_1																															
tun_d_021_1																															

Figure 3.5.4 Table containing information about residues lining given tunnel set

Each row of the table represents one tunnel cluster.

Each column of the table represents an amino acid which was marked as a tunnel lining residue at least once throughout the whole tunnel set.

The green squares mark those residues which line the corresponding tunnel cluster. The yellow squares determine the selected residues.

The table contains the following controls:

- **Save**
Allows the user to save the table in the .csv format to a desired location on the hard drive.
- **Side chain only**
Shows residues where at least one side chain atom of a given residue lines a given tunnel. For this purpose, all atoms except those named H, N, C, O, CA or HA are considered as side chain atoms.
- **Minimum residue occurrence**
This parameter is enabled only when analyzing trajectories of molecular dynamics. It signifies the percentage of a given residue which lines a tunnel during the whole dynamics. For

example, when this number is set to 60%, the user obtains a set of residues which formed the tunnel in at least 60% of the snapshots of the trajectory (so the less frequent ones are filtered out).

- **Visible columns**
Shows the list of all available columns and allows the user to show/hide them using their checkbox.
- **Show all**
Displays all available columns, including hidden ones.
- **Invert Visibility**
Inverts the visibility of columns (the visible ones will be invisible and vice versa).

Bottlenecks table

This table contains detailed information about the bottlenecks of a given tunnel set (see figure 3.5.5).

Tunnel Statistics																										
sg SET#2: tun_d_002_1		1cqw SET#4 bottlenecks																								
Save		Minimum bottleneck occurrence		0%		Visible columns:		Select...		Show all		Invert visibility														
Snapshot	Cluster	Tunnel	Throughput	Cost	X	Y	Z	Radius (Å)	F21	T44	P53	I63	P64	H70	R71	I73	I103	L108	D117	R133	F142	I143	R144	I180	F204	L213
1cqw.pdb	tun_d_001_1	1 0.432	0.840	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_001_1	3 0.296	1.218	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_001_1	5 0.222	1.507	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_001_1	9 0.182	1.703	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_002_1	2 0.327	1.119	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_002_1	4 0.286	1.252	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_003_1	6 0.190	1.662	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_004_1	7 0.186	1.682	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_004_1	10 0.127	2.067	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_005_1	8 0.185	1.687	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_005_1	11 0.110	2.210	21.460	109.012	41.143	0.70																			
1cqw.pdb	tun_d_005_1	19 0.058	2.849	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_006_1	12 0.095	2.352	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_007_1	13 0.093	2.371	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_008_1	14 0.084	2.472	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_008_1	15 0.083	2.493	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_008_1	18 0.059	2.829	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_008_1	27 0.037	3.302	23.689	99.469	39.357	0.71																			
1cqw.pdb	tun_d_008_1	30 0.035	3.354	25.319	85.391	33.366	0.70																			

Figure 3.5.5 Table containing information about bottlenecks of given tunnel set

The controls of the table are similar to the Residues table but some have slightly different meanings.

- **Save**
Allows the user to save the table in the .csv format to a desired location on the hard drive.
- **Minimum bottleneck occurrence**
Takes into account all tunnels in a given cluster and their bottleneck residues. This number signifies the frequency of occurrence of these residues in the cluster. For example, a value of 30% means that a given row remains when all three bottleneck residues have the occurrence in the corresponding tunnel cluster of at least 30%.
- **Visible columns**
Shows the list of all available columns and allows the user to show/hide them using their associated checkboxes.
- **Show all**
Displays all available columns, including hidden ones.
- **Invert Visibility**
Inverts the visibility of columns (the visible ones will be invisible and vice versa).

Each row of the table contains information about one tunnel and its bottleneck. The columns have the

following meaning:

- **Snapshot** – name of the input structure.
- **Cluster** – ID of a tunnel cluster to which a given tunnel belongs (corresponds to the Tunnel cluster ID in the summary.txt).
- **Tunnel** – ID of a given tunnel in a given snapshot.
- **Throughput** – throughput of a given tunnel ($\text{throughput} = e^{-\text{cost}}$).
- **Cost** – the cost of a given tunnel defined as the balance between width and length of the tunnel.
- **X, Y, Z** – coordinates of the center of the sphere forming the bottleneck.
- **Radius [Å]** – radius of the sphere forming the bottleneck.
- **Individual bottleneck lining residues** – residues located within the specified distance from the bottleneck of a given tunnel. They are ordered from the closest to the most distant ones.

3.6 Structure Sequence Window

This window shows the primary structure of all loaded molecules (see figure 3.6). Each row consists of the following sections:

- **Structure name**
- **List of one-letter abbreviations of residues**

Clicking the right button mouse on a residue shows the list of all atoms of a given residue.

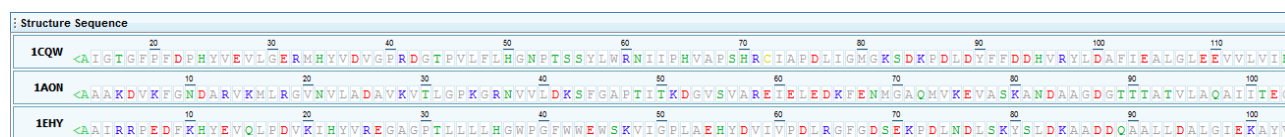


Figure 3.6: Structure Sequence Window

3.7 Structure Selections Window

The selections window allows the user to create a selection of atoms, residues and chains. This selection can be performed within one molecule or can cover parts of more structures. The Selections window has the following functions:

- **New...**
Allows the user to create a new selection with a user-specified name.
- **Atoms**
Allows the user to select individual atoms.
- **Residues**
Allows the user to select whole residues.
- **Chains**
Allows the user to select whole polypeptidic chains.

Each selection has its own record. The record contains:

- **Selection name**
Displays the name of a given selection.
- **Changing visualization style**

Allows the user to change the visualization style of a given selection. Supported styles are: Dots, Sticks, Balls & Sticks, Van der Waals Radii, Surface.

- **Selection color**
Allows the user to change the color of a given selection in the scene.
- **Show/Hide selection**
Shows or hides a given selection in the scene.
- **Lock selection**
Allows the user to lock the selection in a given position so the user cannot rotate and move the selection in the scene.
- **Close**
Removes a selection.

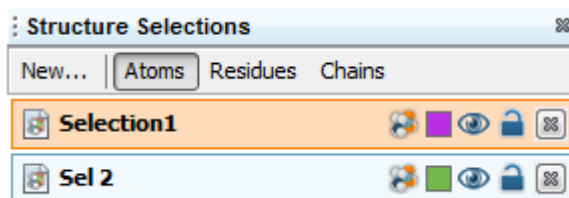


Figure 3.7.1: Structure Selections Window

When clicking with the right mouse button on a selection, the following menu appears:

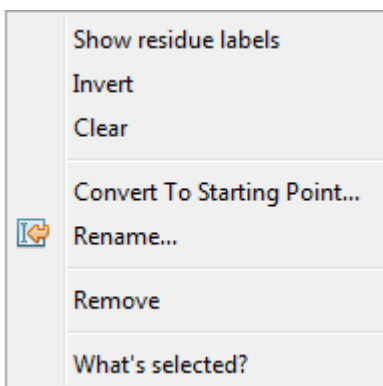


Figure 3.7.2: Menu for each selection

It has the following functions:

- **Show residue labels**
Shows labeling of residues in a selection.
- **Invert**
Changes the selection to include all atoms in the scene which were not selected.
- **Clear**
Deselect all structures of a selection.
- **Convert To Starting Point...**
Opens the same dialog window as the "From Selection..." button in the Tunnel Computation window (see section 3.3).

- **Rename...**
Opens the dialog window for renaming a -selection.
- **Remove**
Removes a selection from the application.
- **What's selected?**
Shows the list of atoms/residues/chains present in the selection.

3.8 Structure Dynamics Window

This window controls the playback of molecular dynamics (see figure 3.8). It is active only when dynamics are loaded and active.

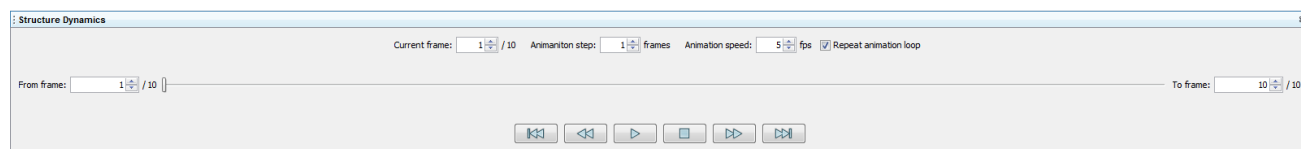


Figure 3.8: Structure Dynamics Window

The Structure Dynamics window contains the following features:

- **Current frame**
Shows the number of currently displayed snapshots during the animation.
- **Animation step (in frames)**
Defines which snapshots will be included in animation (e.g. a value of 2 means that every second frame will be included).
- **Animation speed (in frames per second)**
Controls the animation speed.
- **Repeat animation loop**
Defines whether the entire animation will be played back in a loop.
- **From frame, To frame**
Defines the starting and ending snapshot of the animation.
- **Animation control buttons**
Standard buttons for controlling the playback of the animation (e.g. start, stop, pause, fast forward, etc.)

3.9 Structure Properties Window

This window shows more information about active structure (see figure 3.9). It contains the PDB ID of the structure, its classification and inner structure – the number of chains, all residues, amino acid residues, water molecules, ligands, atoms, hetero atoms and atoms with alternate locations. The number of loaded frames of the structure is also shown.

Structure Properties	
Property	Value
Code	10GS
Classification	COMPLEX (TRANSFERASE/INHIBITOR)
Source file name	10gs.pdb
Chains	4
Residues	592
Amino acid residues	418
Water residues	169
Other residues (ligands)	5
Atoms	3521
Hetero atoms	241
Atoms with alternate location	0
Frames (snapshots)	1

Figure 3.9: Structure Properties Window

3.10 Tunnel Graph Window

This window provides settings, visualization, manipulation and animation of graph statistics of computed tunnels. Basically, there are two types of graphs: those for static snapshots and those for molecular dynamics (profiles and heat plots).

3.10.1 Static case

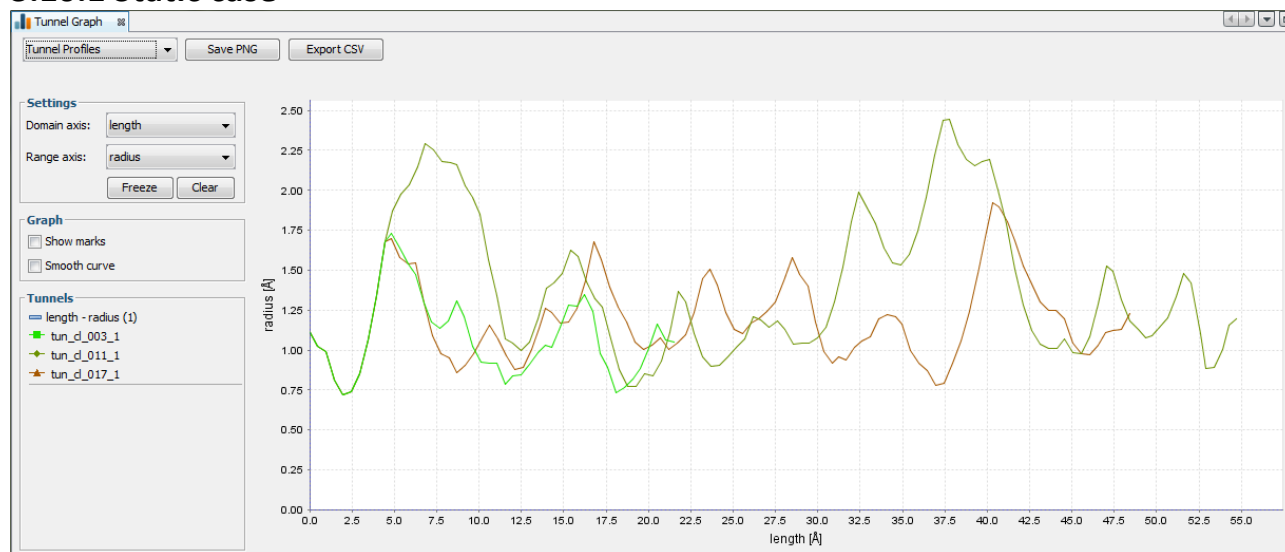


Figure 3.10.1.1: Tunnel Graph window for static case

In the static case (figure 3.10.1.1), users can:

- **Save PNG**
Saves the graph as a picture in the PNG format.

- **Export CSV**
Saves the graph as a table in the CSV format.

Users can also set the following parameters:

- **Domain axis**
Allows the user to select the feature which will be mapped to the X-axis.
- **Range axis**
Allows the user to select the feature which will be mapped to the Y-axis.
- **Freeze**
This function locks the currently displayed graph. It is useful when users want to combine more graphs into one. E.g. in one graph the user can display the relationship between tunnel length and width and also between length and distance.
- **Clear**
Clears the data from the Tunnel Graph window.

The Graph section contains the “Show marks” checkbox, which adds marks to the curve (see figure 3.10.1.2).

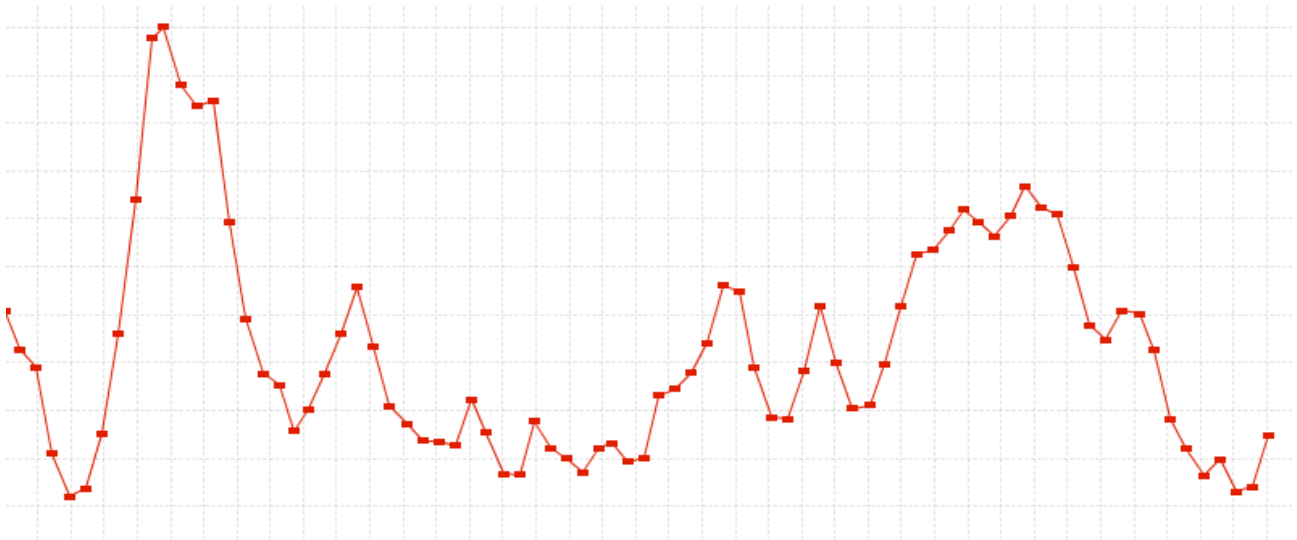
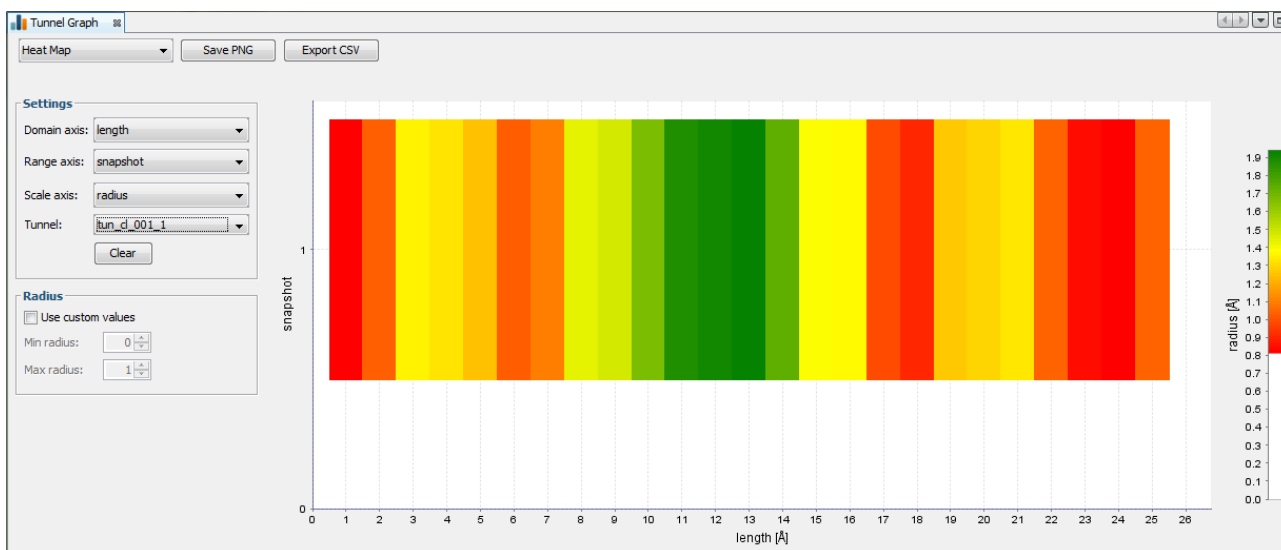


Figure 3.10.1.2: Graph with highlighted marks on the curve

The Tunnels section then shows the legend of displayed curves. The color of each curve corresponds to the color of the corresponding tunnel in the Structures window (where the color of tunnels and their graph curves can also be changed).

3.10.2 Dynamic case

In the dynamic case (see figures 3.10.2.1 and 3.10.2.2), users can choose between a similar representation as the static case and heat plots of computed tunnels. Switching between these two representations can be obtained by choosing between **Tunnel Profiles** and **Heat map** (heat plot).

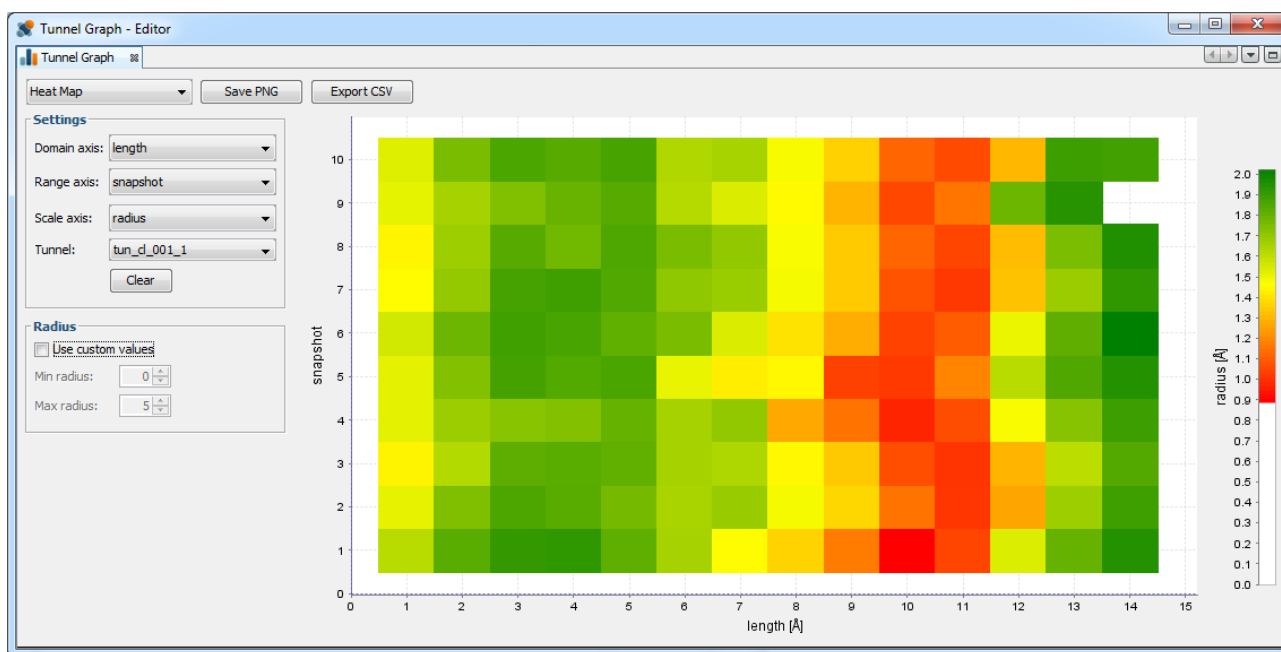


3.10.2.1 Tunnel Graph window for molecular dynamics

The visualization of dynamic tunnels using profiles has the same settings as profiles in the static case. It also contains the following dynamics controls:

- **Snapshot**

Displays the currently processed and visualized snapshot. Checking the **current** checkbox tightly connects the graph to Structure Dynamics window. When user starts playback in that window, profiles of tunnels also animate.



3.10.2.2: Example of heat plot (heat map)

With the Heat map (also known as heat plot), the user can set the following parameters:

- **Domain axis**

Allows the user to select the feature which will be mapped to the X-axis.

- **Range axis**

Allows the user to select the feature which will be mapped to the Y-axis.

- **Scale axis**

Allows the user to select the feature which will be mapped to colors specified by the range displayed on the right side of the heat plot.

- **Tunnel**

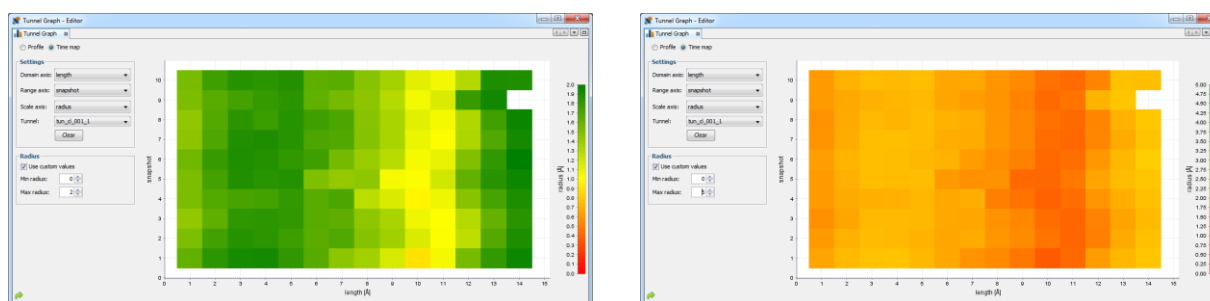
Allows the user to switch between heat plots of individual detected tunnels.

- **Clear**

Clears the data from the Tunnel Graph window.

In the Radius section, the user can customize the color range of tunnel radii in the heat plot (see figure 3.10.2.3).

The user can manually set the range of radii and the color palette changes according to these new values.



3.10.2.3: Example of different settings of custom color values of tunnel radii

By clicking with the right mouse button on the graph, menu with advanced settings appears.

3.11 Colors Window

The Colors window (see figure 3.11) is used for coloring loading structures and their parts. There are four panes representing the main coloring methods according to atoms, residues, chains, secondary structure and the background of the whole scene. Structure is colored according to atom elements by default. For every method there are several color schemes available, for example hydrophobicity of residues.

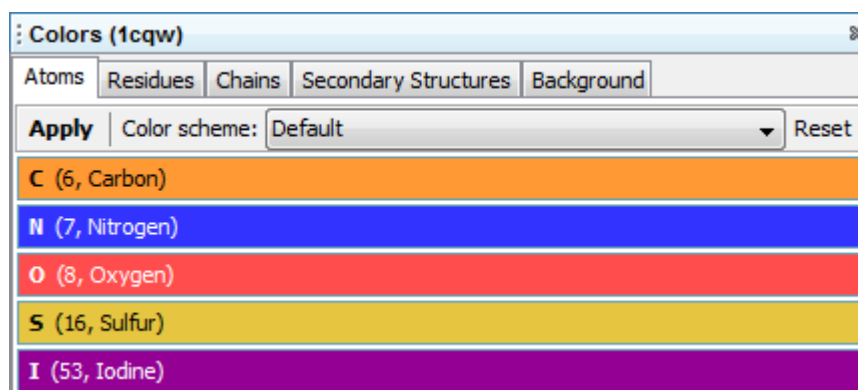


Figure 3.11: Colors Window

In this window the user can choose between coloring individual atoms (according to their chemical elements), residues, chains or secondary structures.

In all cases the user can choose between predefined **Color schemes**. Users can also change the colors of individual atoms (residues, chains or secondary structures) by clicking on the desired chemical element (its colored row).

- **Apply**
When changing color schemes from one type (e.g. atoms) to another (e. g. secondary structures), this button confirms the change.
- **Reset**
Returns colors to the currently selected type and color scheme.
- **Save...**
Allows the users to save their own color scheme using the current settings.

3.12 Structure Alignment Window

This function allows the user to align two structures, shows the result in the visualization window and computes the RMSD (Root Mean Square Deviation) value (see figure 3.12).

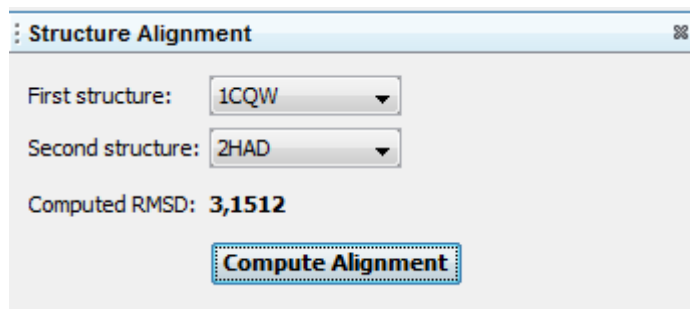


Figure 3.12: Structure Alignment Window

Users can select the first and second structure for alignment and press the “Compute Alignment” button.

TIP: To align more structures, it is necessary to choose one “reference” structure as a First structure and then select a Second structure for alignment.

3.13 Structure Clip Plane Window

This window allows the user to activate work with a clip plane which can stress important features of proteins as well as tunnels (see figure 3.13).

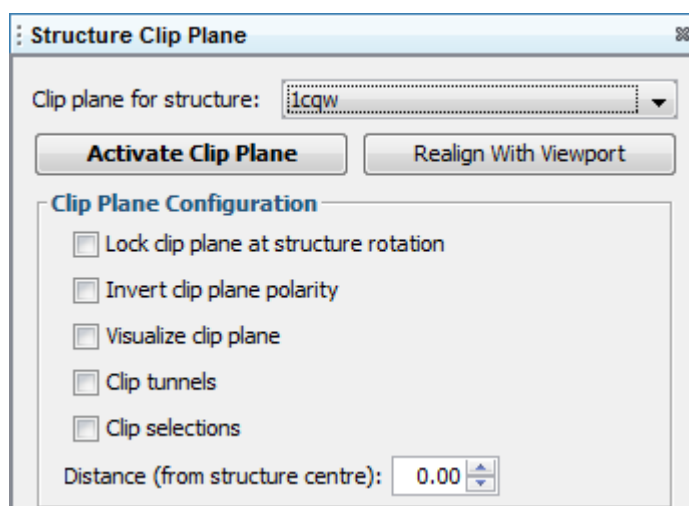


Figure 3.13: Clip Plane Window

The Clip Plane window allows the user to configure the following settings and functions:

- **Clip plane for structure:**
Allows the user to select the structure on which the clip plane will be applied.
- **Activate/Deactivate clip plane**
Activates and deactivates the clip plane.
- **Realign with viewport**
Allows the user to orient the clip plane to the initial position (perpendicular to the user's viewing area).
- **Lock clip plane at structure rotation**
When selecting this tool, the clip plane will rotate along with the molecule according to the user's command.
- **Invert clip plane polarity**
The opposite site of the molecule will be clipped.
- **Visualize clip plane**
Allows the user to transparently visualize the clip plane.
- **Clip tunnels**
This function allows the user to enable or disable clipped tunnels present in the clipped part of the molecule.
- **Clip selections**
This function allows the user to enable or disable clip selections present in the clipped part of the molecule.
- **Distance (from structure centre):**
Shows and allows the user to set the distance between the centre of the molecule and the clip plane.

3.14 Surface Configuration Window

The Surface Configuration window (see figure 3.14) directly influences the computed and visualized surface of a selected structure. It is able to change the transparency of a surface for a selected structure and change the probe size for a structure's surface and also for the Detailed Surface method for tunnel visualization.

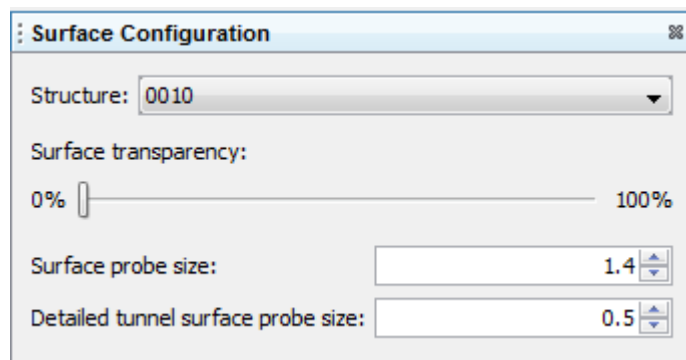


Figure 3.14: Surface Configuration Window

4 Guided Examples

4.1 Example 1 – Static Case

This example will describe how to load a structure to CAVER Analyst 1.0, change its features and compute tunnels.

The workspace for the scenario containing this example can be found in the *static.cws* file provided with this supporting documentation and can be loaded by choosing “File → Load Workspace...” and selecting the file.

A) Loading the structure

First, the user downloads the molecule from the Protein Data Bank (internet connection is required). Choose “File → Download Structure...” and the following window appears (figure 4.1):

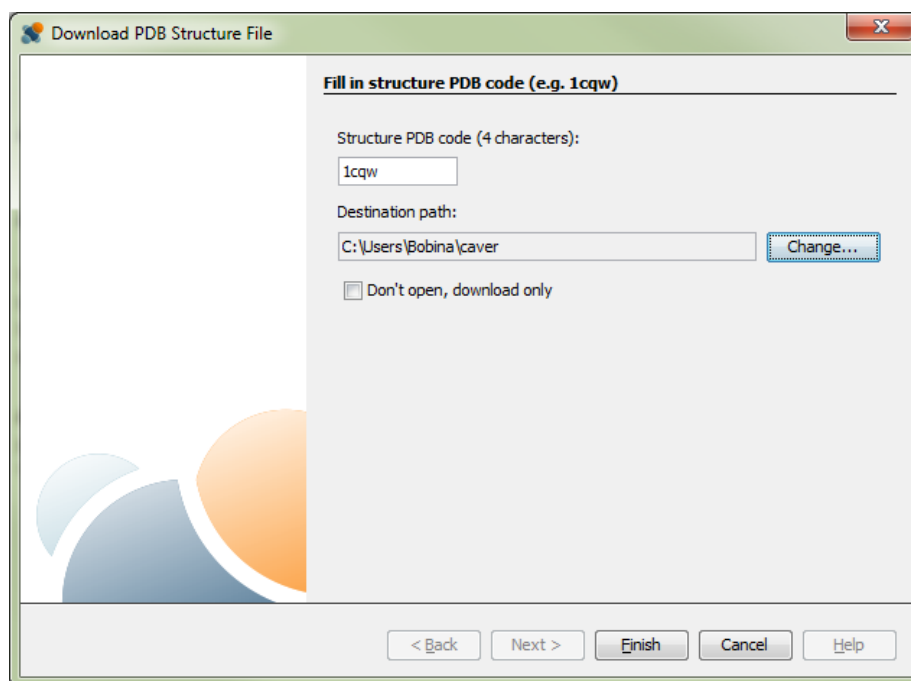


Figure 4.1 Dialog window for downloading the structure

Here, the user enters the PDB code of the structure (1cqw in this case) and selects the destination path where the copy of the structure .pdb file will be stored. The user then clicks “Finish”.

The structure downloads and then appears in the Visualization window. It is also listed in the left panel – the Structures window. By default, the currently loaded structure is marked as active – it is signified by the orange color of the structure record in the Structures window (figure 4.2):



Figure 4.2 Activated record of the loaded structure in the Structures window

B) Changing the visualization style

By default the structure is visualized as a Wireframe. This can be changed by clicking on the icon marked with the blue circle in figure 4.3 and choosing desired representation.



Figure 4.3 Icon for changing the structure visualization style (marked with blue circle)

C) Setting the binding site for tunnel computation

Now the user prepares the structure for tunnel computation. First, the user opens the panel for tunnel computation – “Tools → Tunnel Computation”. The panel opens on the right side of the application window.

Then the user must define the position of the starting point (binding site) of the calculation. In this case, the active site of 1cqw structure is present in the CSA (Catalytic Site Atlas) database so residues surrounding the binding site are automatically loaded into the Tunnel Computation panel (figure 4.4).

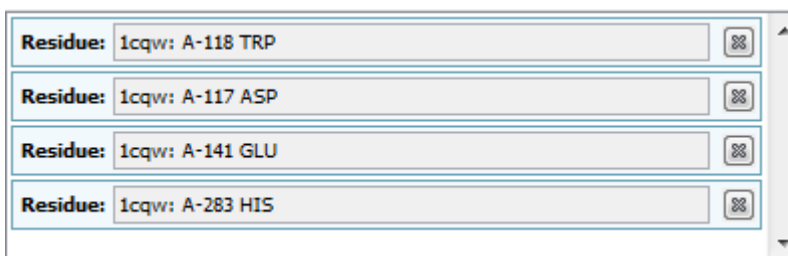


Figure 4.4 List of residues defining the starting point for 1cqw structure, loaded from the CSA database

The user can change this list of residues (add, remove residues, add atoms). To visualize the starting point in the Visualization window, click on the “Show Starting Point” button. It displays a cross with three axes (figure 4.5) which can be used for fine correction of the starting point position (by dragging the mouse).

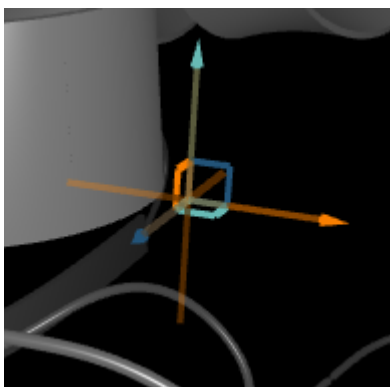


Figure 4.5 Crossing with X, Y, Z axes displaying the position of starting point

D) Launching tunnel computation

Now the user can change input parameters for tunnel computation. The most important ones are present in the Tunnel Computation window (see section 3.3); the rest can be edited in the CAVER 3 Computation

Settings window (see section 3.6).

For the 1cqw structure, it is necessary to set the *Min. probe radius* to 0.7 or less (this represents the minimal tunnel width). The smaller the value of this parameter, the more tunnels will be computed. By pressing the “Compute Tunnels” button, the user launches the tunnel computation using the CAVER 3.0 algorithm.

E) Exploring results

Once the tunnels are computed, they appear in the Structure window under the corresponding structure (figure 4.6). All tunnel clusters from one computation are grouped into the tunnel set. All individual tunnel clusters are in this set.

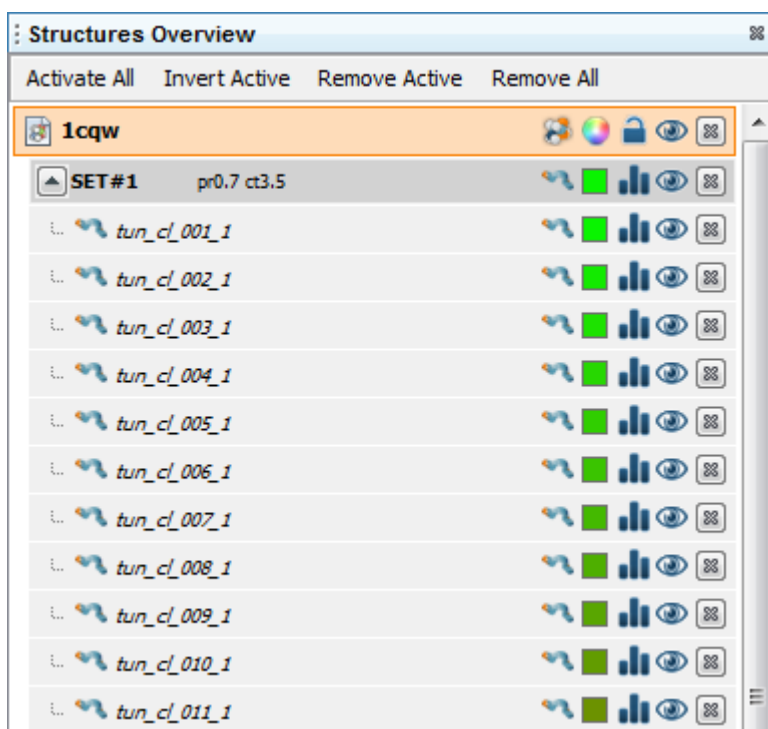


Figure 4.6 Records for computed tunnels in the Structures window

Each set and tunnel clusters contain a set of icons for changing their visualization style, color and visibility. The user can generate a graph of one cluster by clicking on the icon marked with blue circle in figure 4.7.

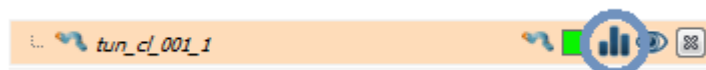


Figure 4.7 Icon for activating the graph marked with blue circle

When activated, the icon changes its color to orange and the Tunnel Graph window appears (figure 4.8).

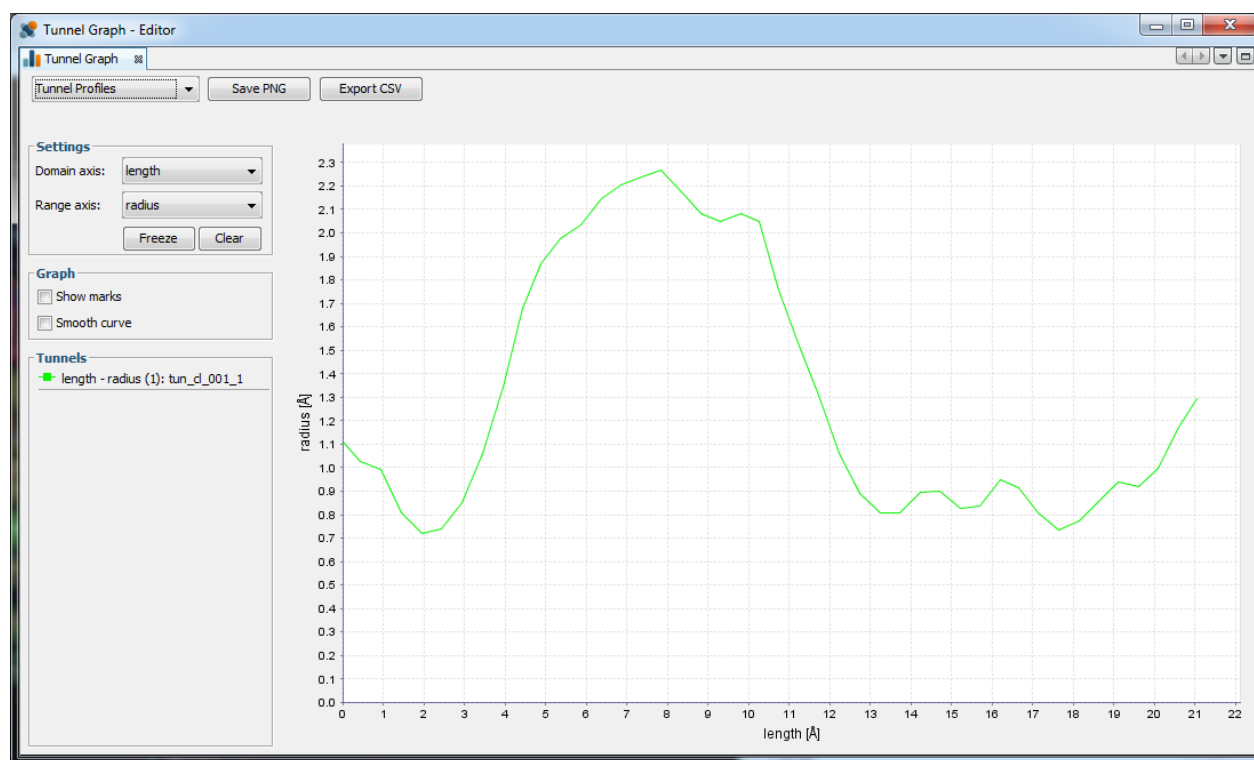


Figure 4.8 Graph representation of the tunnel cluster

4.2 Example 2 – Dynamic Case

This is a similar scenario but with a trajectory of molecular dynamics case.

The workspace of this example scenario is a part of this supporting material. It is found in the *dynamic.cws* file and can be loaded by choosing “File → Load Workspace...” and selecting the file.

A) Loading the structure

In this example, the trajectory must be stored on the hard drive. Choose “File → Open Molecular Dynamics...” and the following window appears (figure 4.9):

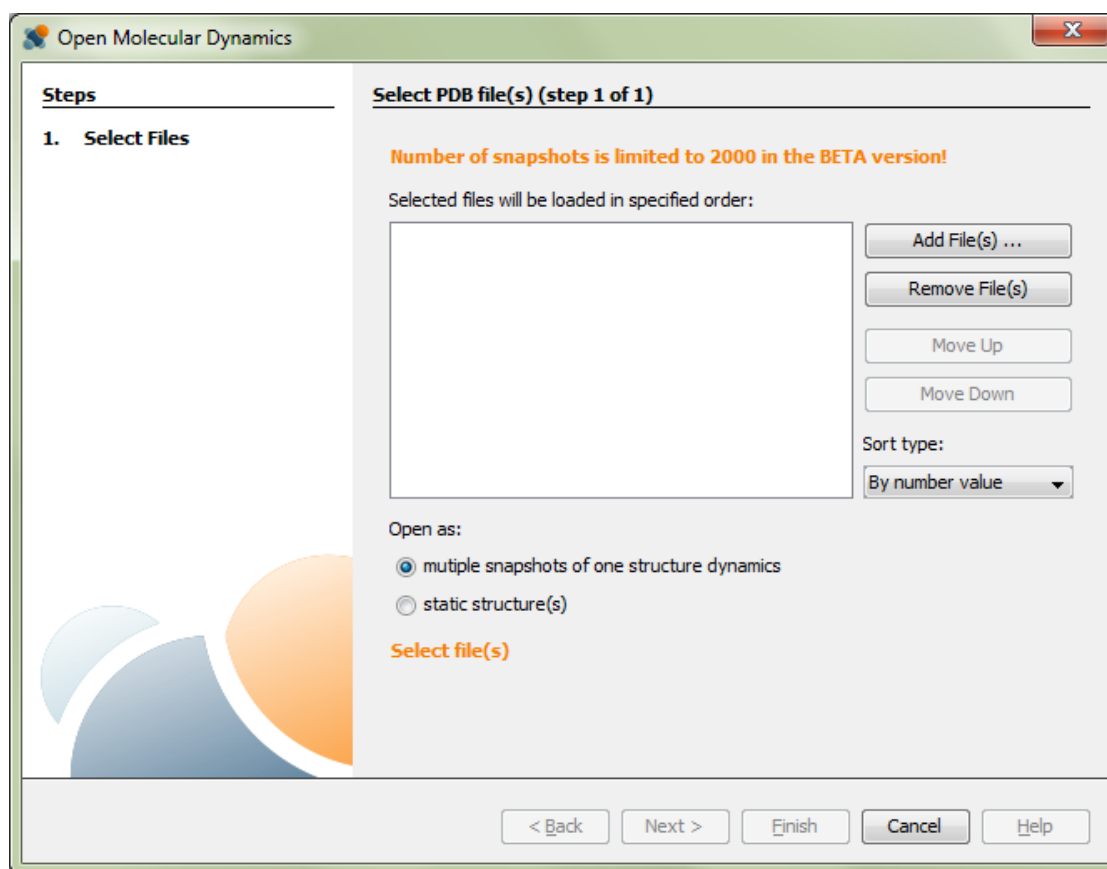


Figure 4.9 Dialog window for loading trajectory of molecular dynamics

Using “Add File(s),” the user chooses a set of PDB files representing the trajectory from the hard drive. By clicking on the “Finish” button, the user launches the loading phase. The supplementary material contains the example of short molecular dynamics called “*md*”.

After loading, the Structure Dynamics window (see section 3.8) opens automatically. It is used for animating and navigation in the trajectory.

B) Setting the binding site for tunnel computation

This phase is very similar to that in the static case. However, the CSA database does not contain any records for trajectories of molecular dynamics. So the user must define the starting point manually – either by defining the surrounding atoms or residues or by setting an absolute position using X, Y, Z coordinates. In case of “*md*” trajectory, the starting point is defined by atoms with ID’s 578, 1609, 3258.

C) Launching tunnel computation

Again, the user can influence the results of the computation by changing parameters present in the Tunnel Computation window (see section 3.3) or the Tunnel Advanced Settings (see section 3.6). In this case, the Min. probe radius is set to 0.9 and in the Residues included into tunnel computation the following items should be checked: AA, HIE, HID. The computation is launched using the “Compute Tunnels” button.

D) Exploring results

Computed tunnels can be explored by animating the trajectory using the Structure Dynamics window.

Again, the user can generate a graph for the results by clicking on the icon marked by the blue circle (figure 4.10).

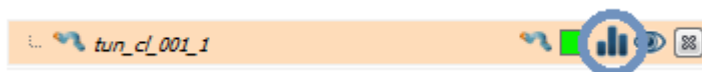


Figure 4.10 Icon for activating the graph marked with blue circle

By checking the “current” snapshot (figure 4.11) and playing the animation in the Structure Dynamics window the graph is changing according to current snapshot.

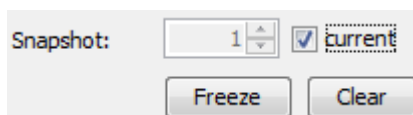


Figure 4.11 Activating the graph animation

For molecular dynamics, the user can also open the heat map of the trajectory – see figure 4.12.

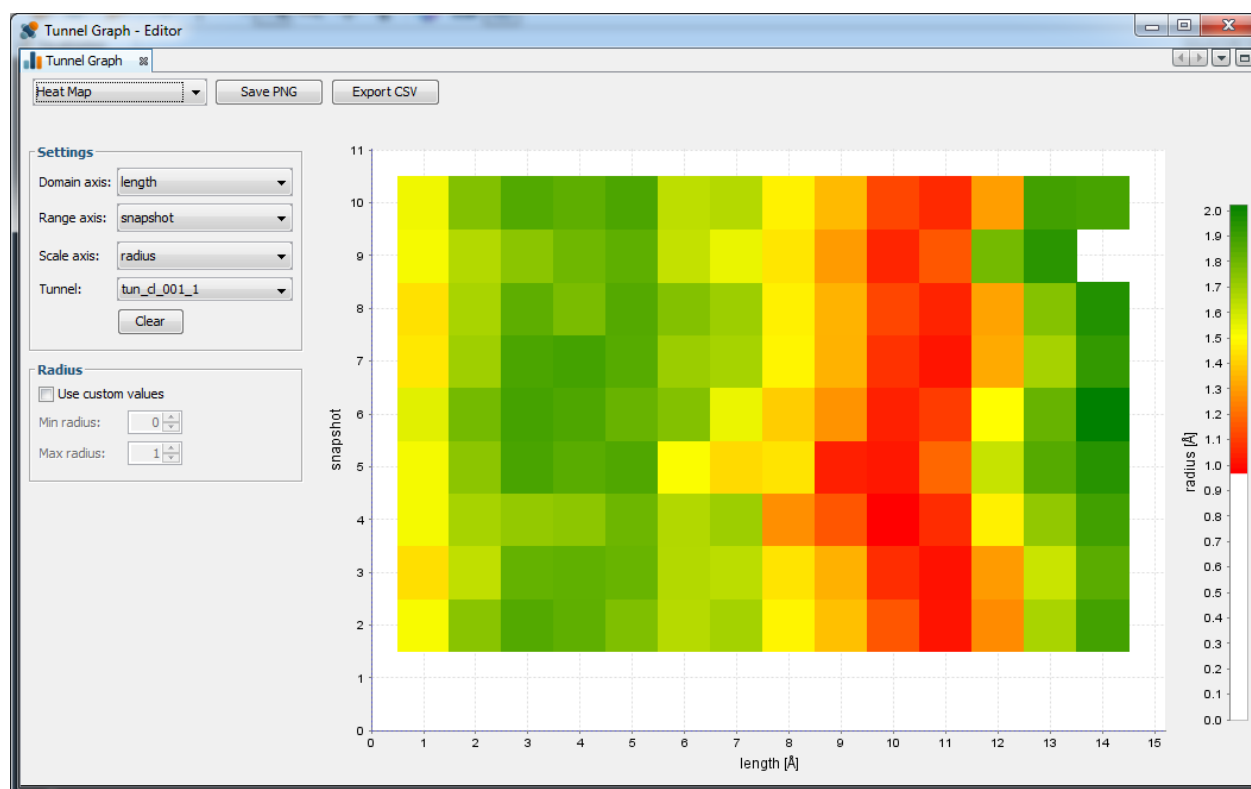


Figure 4.12 Heat plot for tunnels in the trajectory, activated by the radiobutton marked with blue circle

4.3 Example 3 – Case Study – Engineering Enzyme Activity

This example shows more complex utilization of CAVER Analyst which can be utilized for engineering enzyme activity.

Redesigning access tunnels of the haloalkane dehalogenase DhaA provides 32-fold increase in activity with toxic pollutant 1,2,3-trichloropropane (TCP). The results demonstrate the power of combining rational design with directed evolution focused to the access tunnels. For more information see [1].

A) Loading the structures

The study was performed on the wild type and the C176Y mutation of haloalkane dehalogenase DhaA. For this purpose users can load the *wt_dhaa.pdb* and *m1_dhaa.pdb* files which are also included in the supporting documentation.

B) Setting the binding site for tunnel computation

The CSA database does not contain any records for these structures so the user must define the starting point manually – by defining the surrounding residues. In both structures the active site is defined by residues with ID's 106, 107, 130 and 272. They can be set using the Add Item... button in the Tunnel Computation window, section Surrounding items. It opens the following window:

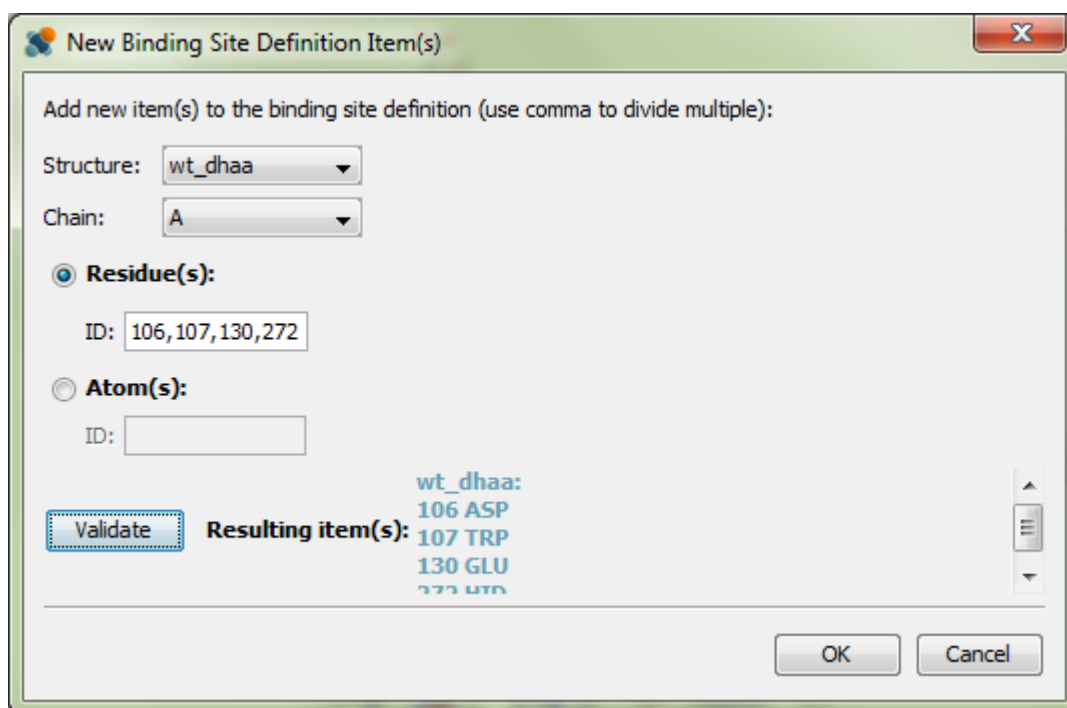


Figure 4.13 Definition of the starting point

C) Launching tunnel computation

In this case the parameters present in the Tunnel Computation window (see section 3.3) or the CAVER 3 Computation Settings (see section 3.6) should have the following values:

- Maximum distance (Å) = 5
- Min. probe radius = 0.7

The computation is then launched using the “Compute Tunnels” button.

This is performed for both structures and then their structure alignment can be launched – using Structure → Alignment (see section 3.12).

D) Interpretation of results

The active site of wild type DhaA is an occluded cavity with two major access tunnels (main tunnel - blue, slot tunnel - yellow). Mutant carrying substitution in the main tunnel (C176Y) shows higher activity towards TCP.

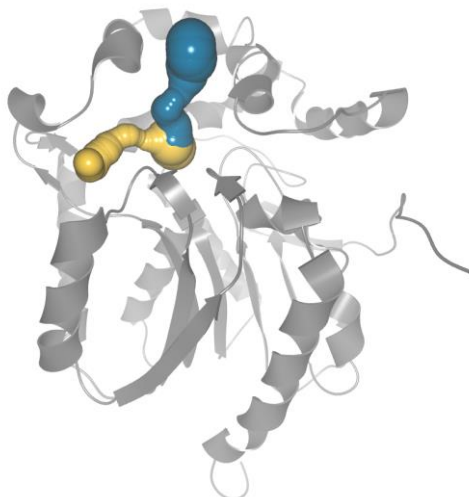


Figure 4.14 Main and slot tunnels of the DhaA haloalkane dehalogenase

4.4 Example 4 – Case Study – Engineering Enzyme Stability

This example shows more complex utilization of CAVER Analyst which can be utilized for engineering enzyme stability.

In this case, the modification of residues lining the access tunnel of the haloalkane dehalogenase DhaA increased its melting temperature by 19°C and resistance to co-solvent DMSO 4000-fold. Mutations in the tunnel improved structural and kinetic stability, while the surface mutations did not contribute to protein stabilization. For more information see [2].

A) Loading the structures

The study was again performed on the wild type of haloalkane dehalogenase DhaA and its mutations – DhaA57 and DhaA80. All structures corresponding to this case are stored in the *1cqw.pdb*, *4f5z.pdb* and *4f60.pdb* files which are also included in the supporting documentation.

B) Setting the binding site for tunnel computation

The starting point for the *1cqw.pdb* structure is automatically loaded from the CSA database. This point can be copied to the other structures using the “Convert for Another Structure...” button in the Tunnel Computation window (see section 3.3).

C) Launching tunnel computation

The parameters for tunnel computation are the following:

- For all structures the Maximum distance (Å) parameter is set to 5
- For the *1cqw* and *4f5z* structures the Min. probe radius is set to 0.7

- For the *4f60* structure the Min. probe radius is set to 0.5

In the computed tunnel sets, the main tunnel is the first tunnel in the *1cqw* and *4f5z* structures and the second tunnel in the *4f60* structure. In the attached workspace the other tunnels were removed.

D) Interpretation of results

In a static case, the main tunnel bottleneck of DhaA57 (yellow) increased to 1.7 Å in comparison with wild type (1.4 Å) (blue). The bottleneck of the main tunnel in DhaA80 (red) decreased to 0.6 Å. Here we can conclude that DhaA57 mutation extends the tunnel bottleneck.

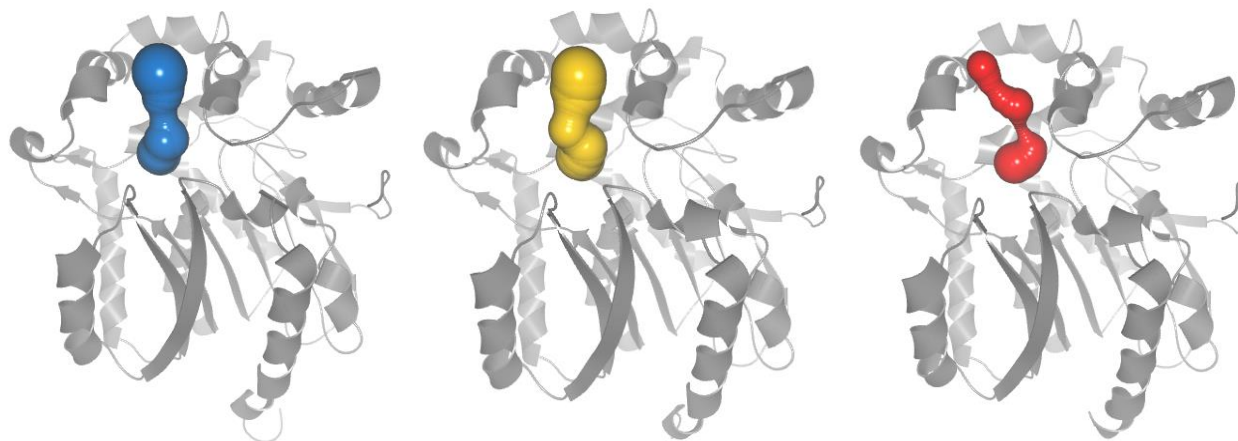


Figure 4.15 Main tunnel of wild type DhaA and its mutations DhaA57 and DhaA80

Predicted stability effects of all possible single point mutations (227 924) in 26 different proteins from all 6 enzyme classes. Targeting the tunnel residues has 2-times higher chance to produce protein variants with significantly improved stability than mutagenesis targeting other protein regions.

E) Graphs for tunnels

The graph statistics of computed tunnels can be visualized using the icon for graph visualization – see figure 4.16.



Figure 4.16 Blue circle shows the icon for launching the graph visualization of given tunnel set

This icon opens the Tunnel Graph window with the main tunnel from the *1cqw* structure. To be able to add the tunnel from another structure to the same graph, users have to press the “Freeze” button. Then another tunnel can be added by pressing the same icon at figure 4.16 for the second structure. The same process is used for the third structure. Figure 4.17 shows the resulting graph comparison of the three studied tunnels.

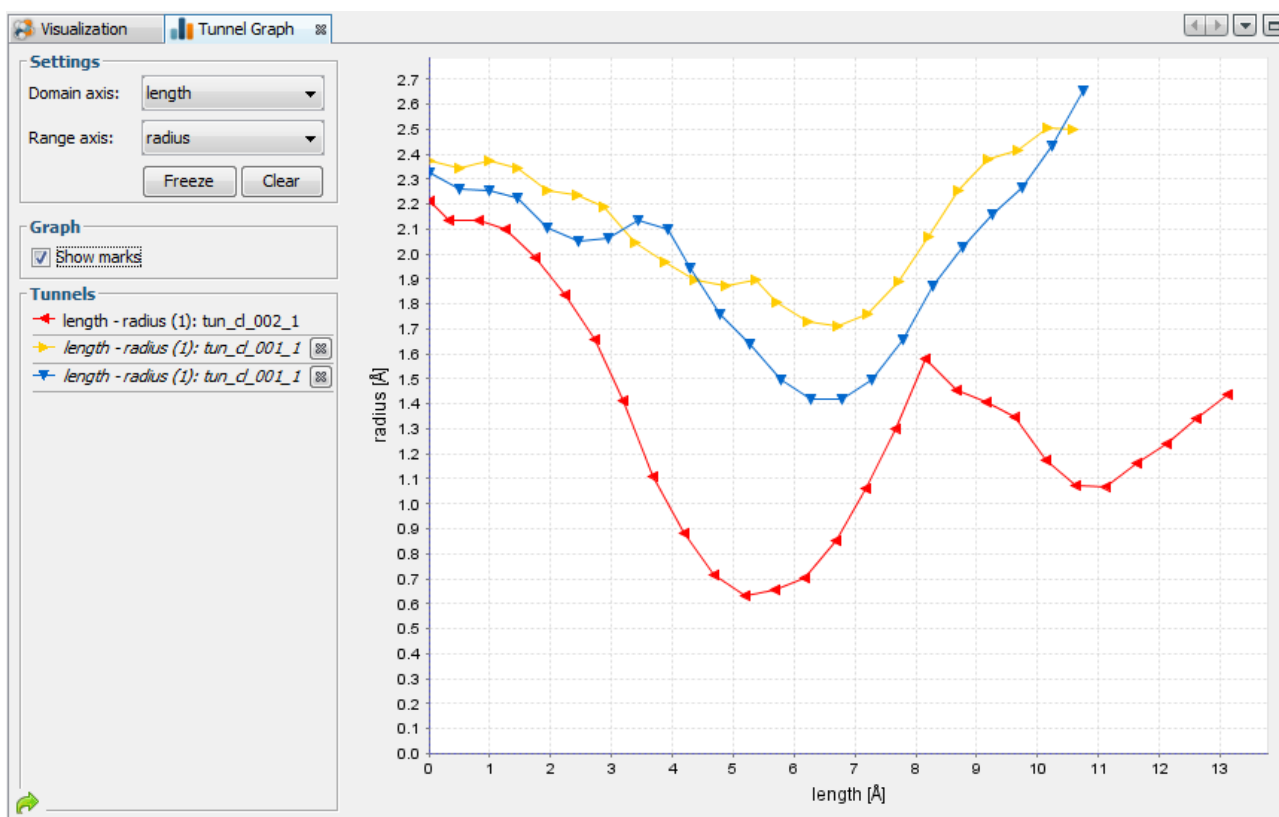


Figure 4.17 Tunnel graph

4.5 References

- [1] Pavlova, M., Klvana, M., Prokop, Z., Chaloupkova, R., Banas, P., Otyepka, M., Wade, R.C., Tsuda, M., Nagata, Y., Damborsky, J., 2009: Redesigning Dehalogenase Access Tunnels as a Strategy for Degrading an Anthropogenic Substrate. *Nature Chemical Biology* 5: 727-733.
- [2] Koudelakova, T., Chaloupkova, R., Brezovsky, J., Prokop, Z., Sebestova, E., Hesseler, M., Khabiri, M., Plevaka, M., Kulik, D., Kuta Smatanova, I., Rezacova, P., Ettrich, R., Bornscheuer, U. T., Damborsky, J., 2013: Engineering Enzyme Stability and Resistance to an Organic Cosolvent by Modification of Residues in the Access Tunnel. *Angewandte Chemie International Edition* 52: 1959-1963.