



# **User Guide**

## **Version 2.0 beta**

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# 1 Introduction

## 1.1 About CAVER Analyst

CAVER Analyst 2.0 is a software program for the analysis of access pathways to buried active sites in proteins in long molecular dynamics (MD) simulations. 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 2.0 assists with calculation, analysis, visualization and rational re-design of the ligand access pathways in MD.

CAVER Analyst 2.0 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 of long MD simulations.
- Specialized visualization techniques for exploration of tunnel profile, tunnel bottleneck, and surrounding residues over time.
- Measurement of distances and angles between atoms.
- Mutation of individual residues, using rotamer libraries.
- Multiple clip planes for exploration of protein inner structure.

## 1.2 Hardware and Software Requirements

### Minimal configuration:

- Java 1.8
- 2 GB RAM
- 32-bit architecture
- Intel HD family graphics adapter

### Recommended configuration:

- Java 1.8 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 7, 8, 10
- Mac OS X 10.7.5 or later
- Linux major distributions including Fedora Core, Red Hat and Ubuntu

## 1.3 User Support

Almost 6,000 users world-wide have downloaded some version of CAVER (almost 80,000 individual downloads) 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 write to us at [caver@caver.cz](mailto:caver@caver.cz).

## 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), to manage existing selections (Structure Selections window) and to manage computed cavities (Cavities Overview window). The bottom of the left panel is reserved for working with various tools, e.g., structural and sequence alignment and controlling probe size and transparency of protein and tunnel surface.

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, tunnels, cavities, 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). It contains also the Console window for controlling the main functions of the application using the command line. It also offers a new visualization feature for exploration of tunnel profile over time (Residue Graph window).

The right panel (**computation panel**) allows users to choose input parameters for tunnel computation and to launch their calculation (Tunnel Computation window). Furthermore, it contains the Cavity Computation window for setting input parameters and calculation of cavities. 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. More detailed information about given structure is present in the Structure Statistics window. This panel also contains the Mutagenesis window for designing mutations of residues on static structures.

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), for displaying log messages (Log window), and for novel representation of tunnel cross cut and its evolution over time (Contours window).

#### 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.

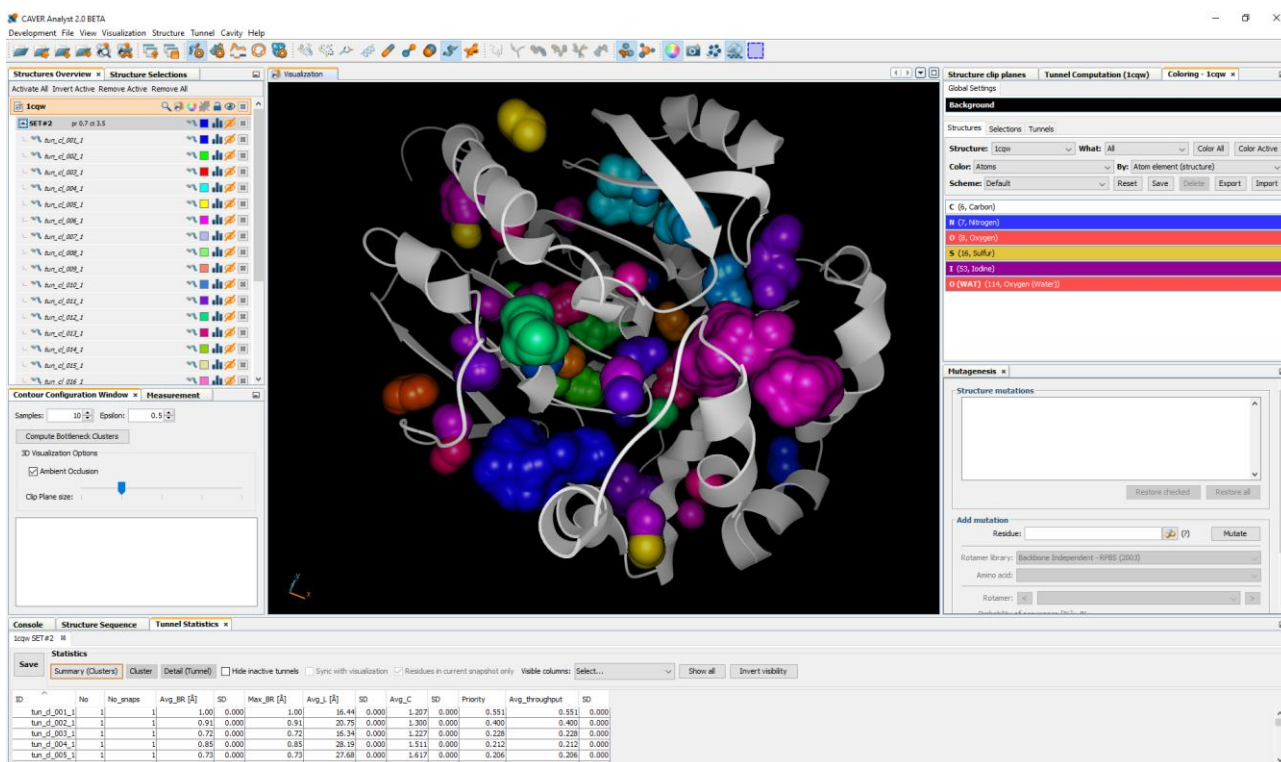


Figure 2.1: CAVER Analyst work area

## 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.
  - **Convert Dynamics...**  
Allows the user to convert the molecular dynamics from the AMBER, GROMACS, or CHARMM file format to the set of PDB files.
  - **Load Workspace...**  
Allows the user to load sessions previously saved on the hard drive (in .cws format).
  - **Save Workspace...**



Saves the current CAVAR 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.
- **Application Settings**  
Opens the dialog window with general settings of the application.
- **Console**  
Opens the Console window where the user can control the main functions of the application using the command line.
- **Exit**  
Closes the CAVAR 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, tunnel surface, and selection surface, and define 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.
- **Reset Structures**  
Resets all the loaded structures to their original position after loading to the application.
- **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.
  - **Cavity Visualization** – shows/hides icons related to visualization of cavities.
  - **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 activated protein structures, computed tunnels, or cavities. If no tunnels or cavities 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.
  - **Spheres**  
Shows the tunnel as a set of intersecting spheres. 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.
- **Tunnel Asymmetric Surface**  
Shows the asymmetric shape of the tunnel surface.

***Cavity Visualization Styles:***

- **Surface**  
Shows the surface of detected cavities.
- **Locked Probes**  
Shows the locked probes, i.e., sites, where a probe of given size cannot move, it is locked in its position. More details in section 3.8.

- **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 3.10.
- **Alignment**  
Opens the Alignment window in the bottom left panel. Details in section 3.16.
- **Search in Structure...**  
Opens the dialog window where the user can search for specific parts of the protein by stating their ID. Details will be described in section 3.19.
- **Delete from Structure...**  
Opens the dialog window where the user can delete specific parts of the protein. Details will be described in section 3.20.
- **Clip Planes**  
Opens the Structure Clip Planes window in the top right panel. Details will be described in section 3.17.
- **Sequence**  
Opens the bottom panel containing a sequential representation of all loaded proteins. More details in section 3.9.
- **Dynamics**  
Opens the bottom panel containing control buttons for animating molecular dynamics. More details in section 3.11.
- **Properties**  
Opens the top right panel, which contains the summarized information about the active structure. More details in section 3.12.
- **Statistics**  
Opens the top right panel containing information about the constitution of active structure. More details in section 3.13.
- **Measurement**  
Opens the bottom left panel enabling the user to perform distance and angle measurements between atoms. More details in section 3.21.

- **Mutagenesis**  
Opens the bottom right panel enabling to design mutations of selected residues for static structures. More details in section 3.22.
- **Tunnel**
  - **Computation**  
Opens the top right panel, enabling the users to enter parameters for tunnel computation. More details in section 3.3.
  - **Asymmetric Tunnels**  
Opens the top right panel, enabling the users to define parameters for computation of asymmetric tunnels. More details in section 3.23.
  - **Residue Graph**  
Opens the bottom panel enabling the users to explore the tunnel profile over time, along with the surrounding residues and their properties. More details in section 3.24.
  - **Contours**  
Opens the central panel enabling the user to explore the contour shape of a selected cross section of a tunnel (i.e., its bottleneck) over time, along with the surrounding residues and their properties. More details in section 3.25.
  - **Statistics**  
Opens the bottom panel with detailed statistics about computed tunnels – clusters, lining residues, bottlenecks, etc. More details in section 3.13.
  - **Graph**  
Opens the center panel containing a window which displays various tunnel statistics. More details in section 3.14.
  - **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.
- **Cavity**
  - **Computation**  
Opens the top right panel, enabling to set the input parameters for cavities computation. More details in section 3.6.
  - **Overview**  
Opens the top left panel containing the list of detected cavities. More details in section 3.7.
  - **Locked Probes**  
Opens the top right panel enabling the users to set the parameters of locked probes. More details in section 3.8.
- **Help**
  - **Automatic Updates**  
Enables the user to turn on and off automatic updates of the application.
  - **User Guide**  
Opens this user guide.
  - **Report Bug**  
Opens the dialog window where the user can report a bug or desired feature.
  - **About CAVER Analyst**  
Information about product version, authors and copyright.

## 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.
- **Convert Dynamics...**  
Opens a dialog window for converting the molecular dynamics in the AMBER, GROMACS, or CHARMM format to the sequence of PDBs.
- **Search in Structure...**  
Opens the dialog window for selecting parts of the protein by stating their ID.
- **Delete from Structure...**  
Opens the dialog window for deleting parts of the protein by stating their ID.
- **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.
- **Tunnel Computation**  
Opens the Tunnel Computation panel.
- **Asymmetric Tunnels**  
Opens the panel for setting the parameters for computation of asymmetric tunnels.
- **Residue Graph**  
Opens the panel containing the visualization of the tunnel profile and its surrounding residues in molecular dynamics.
- **Contours**  
Opens the panel containing the visualization of the tunnel cross cut contour shape and its surrounding residues in molecular dynamics.
- **Cavity Computation**  
Opens the Cavity Computation panel.

*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 3.18.

- **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 Spheres**

Tunnels are represented by intersecting spheres positioned on the tunnel centerline. Also suitable for visualizing tunnel dynamic changes.

- **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 3.18.

- **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.

- **Tunnel Asymmetric Surface**

Tunnels are represented as a surface describing the tunnel void space more precisely, based on the user-defined parameters.

- **Cavity Surface**

Enables the visualization of detected cavities.

- **Locked Probes**

Enables to define the parameters of the locked probes.

*Other functions:*

- **Coloring**

Opens the Coloring window for changing colors of different protein parts (see section 3.15).

- **Screen Capture**

Allows the user to store current snapshot of the visualization window in the .png, .bmp or .jpg format.

- **Video Capture**

Opens the panel for capturing the video of the main visualization window.

- **Fog**

Allows the user to turn fog on and off in the scene. Fog can enhance the perception of depth in the scene.

- **Selection Mode**

Enables to define the selection mode is then used for direct selection of protein parts in the main visualization window.

# 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.

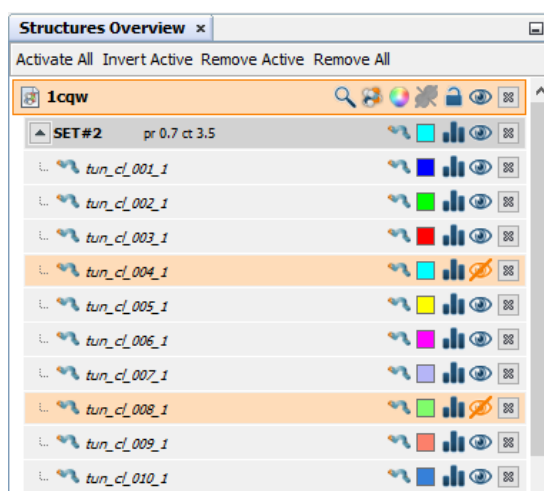


Figure 3.1.1: Structures Overview Window example

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### 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, the 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):

- **Zoom**  
Positions the structure to the center of the visualization window.
- **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.
- **Structure Symmetries**  
If the protein has some defined symmetries in the PDB file, these can be turned on and off using this button.
- **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:

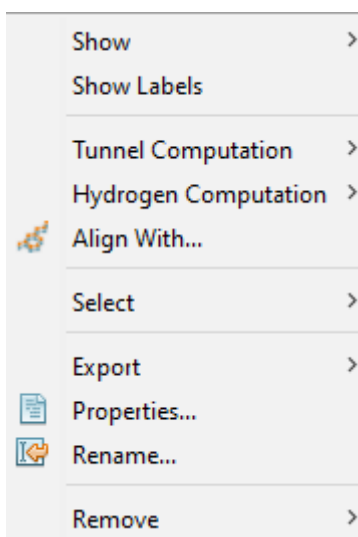


Figure 3.1.2: Menu for each structure

- **Show**  
Enables to define which parts of the loaded PDB structure should be visible in the visualization window.
  - **Amino Acids**
  - **Hydrogens**
  - **Waters**
  - **Ligands**
- **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 Alignment window (see details in section 3.16).
- **Select**  
Creates a selection containing all instances of the type below present in the PDB file of given structure.
  - **Amino Acids**
  - **Backbone**
  - **Waters**
  - **Ligands**
  - **Donors**
  - **Acceptors**
- **Export**  
Allows the user to save the current structure in the given format to an external file.
  - **Export to PDB...**
  - **Export Trajectory to PDB...**
  - **Export to FASTA...**
  - **Export to OBJ...**
  - **Export to PLY...**
  - **Export to STL...**
- **Properties...**  
Opens the Structure Properties window (see details in section 3.12).
- **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.

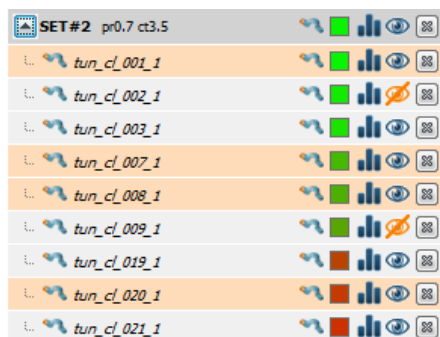
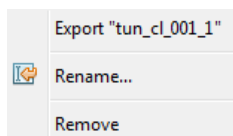


Figure 3.1.3: Example of tunnel set record

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 3.14).
- **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.

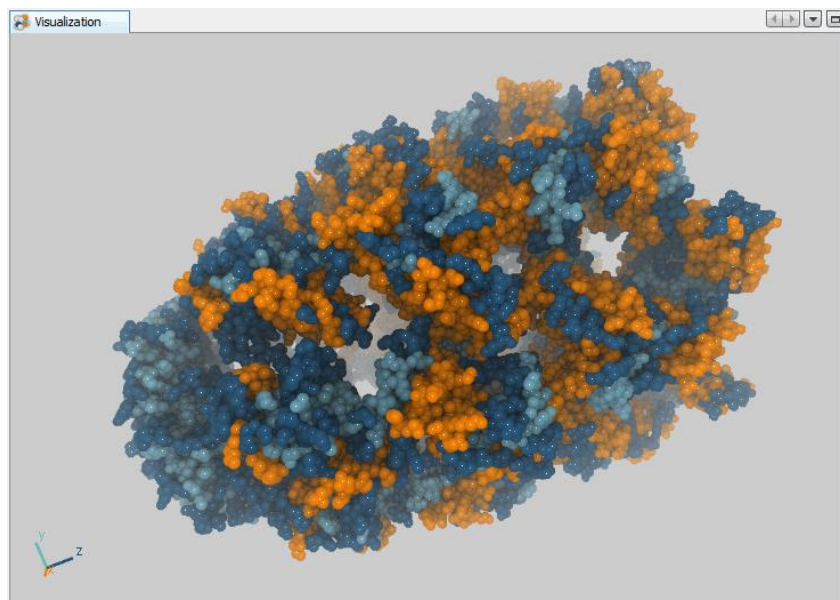


Figure 3.2: Visualization window with gray background color and enabled fog

### 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 3.3.1) – setting the binding site position, important parameters, etc. This panel is activated by selecting the desired structure in the Structure Overview window. The panel has two modes – the basic and the advanced one. The advanced mode can be activated using the checkbox on the top of the panel.

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”.

The screenshot shows the 'Tunnel Computation (1cqw)' window in its advanced mode. The 'Advanced' checkbox is checked. The 'Starting Point' section has 'Binding sites: Site #1 (CSA)' selected. Under 'Surroundings', three residues are listed: '1cqw: A-116 HIS', '1cqw: A-117 ASP', and '1cqw: A-52 ASN'. Below this are buttons for 'Restore', 'Add...', 'From Selection...', and 'To Selection'. The 'Absolute position' section shows X: 20.647, Y: 95.816, and Z: 38.176, with a 'Show Starting Point' button and a 'Copy to Another Structure...' button. The 'Starting Point Optimization' section has 'Maximum distance (Å): 3' and 'Desired radius (Å): 5'. The 'Settings' section includes 'Dynamic tunnel computation' with 'From frame: 1' and 'To frame: 1', 'Sparsity: 1', 'Approximation: 12', 'Min. probe radius: 0.9', 'Clustering threshold: 3.5', 'Shell depth: 4', and 'Shell radius: 3'. Under 'Residues included', there are checkboxes for 'HOH' (unchecked), 'AA' (checked), and 'IOD' (unchecked). The 'Output directory' is set to 'C:\Users\Bobina\caver\caver3'. At the bottom are 'Compute Tunnels' and 'Import Tunnels' buttons, along with icons for file operations.

Figure 3.3.1: The advanced mode of the 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 3.3.2).

First, 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 the 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 (e.g., 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.

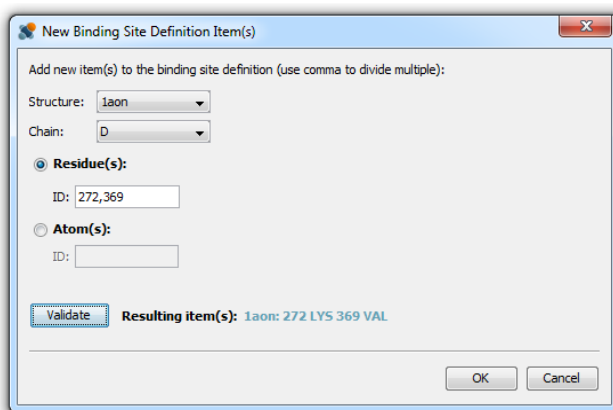


Figure 3.3.2 Definition of a binding site

- **From Selection...**

The binding site can be also defined by a selection (see figure 3.3.3). 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.

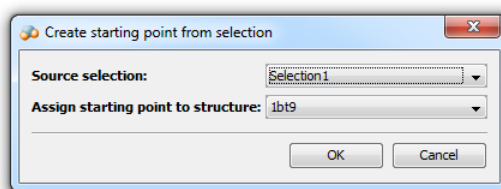


Figure 3.3.3 Creating the starting point from selection

- **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.
- **Copy to Another Structure...**  
A given binding site can be reused for another structure (see figure 3.3.3). This can perform the structure alignment and stores the starting point for selected structures as absolute position.

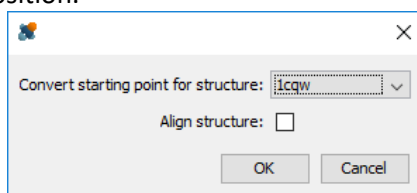


Figure 3.3.3 Conversion of a starting point

- **Starting Point Optimization** (available only in the advanced mode)
  - **Maximum distance (Å)**  
Specifies the maximum distance of the calculation starting point from the initial starting point (for more details see CAVER 3.0.2 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 representing the input structure, which will be used as the starting point for the calculation of tunnels (for more details see CAVER 3.0.2 user guide).
- **Settings**
  - **Dynamic tunnel computation** (available only in the advanced mode)  
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** (available only in the advanced mode)  
The number of frames defining the first and last frame for tunnel computation (for more details see CAVER 3.0.2 user guide).
    - **Sparsity** (available only in the advanced mode)  
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 CAVER 3.0.2 user guide).

- **Approximation**  
Specifies the number of balls which will be used to approximate individual atoms in the input structure (for more details see CAV-ER 3.0.2 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 CAV-ER 3.0.2 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 CAV-ER 3.0.2 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 CAV-ER 3.0.2 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 CAV-ER 3.0.2 user guide).
- **Residues included**  
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.
- **Compute Tunnels**  
Button for launching the tunnel computation.
- **Import Tunnels**  
Enables to load the tunnels computed by the standalone CAV-ER tool.
- 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



### 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 3.0.2 user guide.

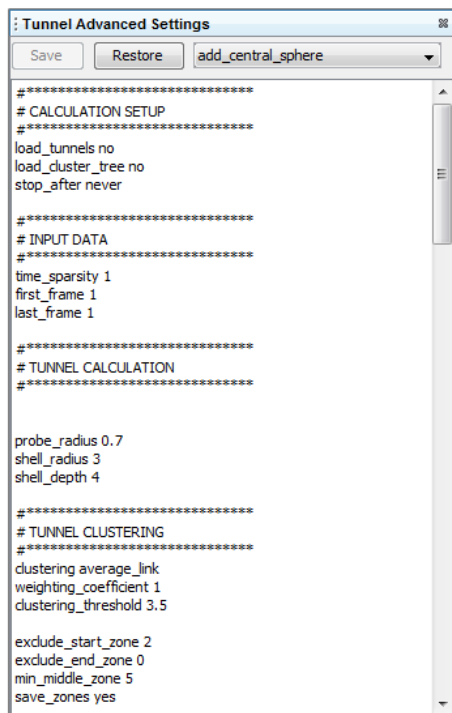


Figure 3.4 Tunnel advanced settings panel

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).

1cqw SET #4											
Statistics											
Save	Summary (Clusters) Cluster Detail (Tunnel) <input type="checkbox"/> Hide inactive tunnels <input type="checkbox"/> Synchronize with visualization <input checked="" type="checkbox"/> residues in current snapshot only Visible columns: Select... Show all Invert visibility										
ID	No	No_snaps	Avg_BR [Å]	SD	Max_BR [Å]	Avg_L [Å]	SD	Avg_C	SD	Priority	Avg_throughput
tun_d_001_1	1	1	10.72	0.000	0.71	17.65	0.000	1.175	0.000	0.432	0.432
tun_d_002_1	1	1	10.72	0.000	0.71	18.98	0.000	1.747	0.000	0.327	0.327
tun_d_003_1	1	1	10.72	0.000	0.71	21.49	0.000	1.338	0.000	0.190	0.190
tun_d_004_1	1	1	10.72	0.000	0.71	22.58	0.000	1.574	0.000	0.186	0.186
tun_d_005_1	1	1	10.72	0.000	0.71	21.86	0.000	2.179	0.000	0.185	0.185
tun_d_006_1	1	1	10.72	0.000	0.71	32.35	0.000	1.740	0.000	0.095	0.095
tun_d_007_1	1	1	10.72	0.000	0.71	32.28	0.000	1.805	0.000	0.093	0.093

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 3.11), it enables to animate also the statistics table.
- **Residues in current snapshot only**  
In molecular dynamics, it displays only residues relevant to tunnels only in given snapshot.
- **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).

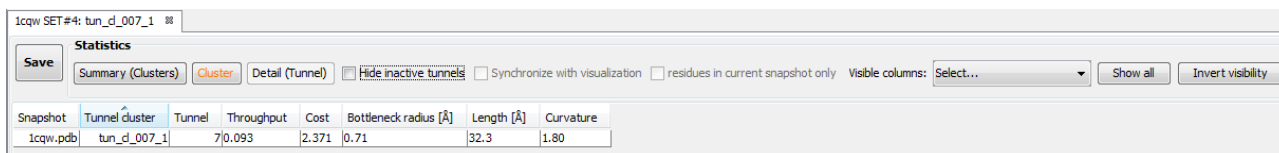
Individual columns have the following meaning:

- **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).



The screenshot shows a software interface with a 'Statistics' window. It has tabs for 'Summary (Clusters)', 'Cluster', and 'Detail (Tunnel)'. The 'Cluster' tab is active. Below the tabs is a table with the following data:

Snapshot	Tunnel cluster	Tunnel	Throughput	Cost	Bottleneck radius [Å]	Length [Å]	Curvature
1cqw.pdb	tun_d_007_1	7	0.093	2.371	0.71	32.3	1.80

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  $\text{length}/\text{distance}$ , 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).

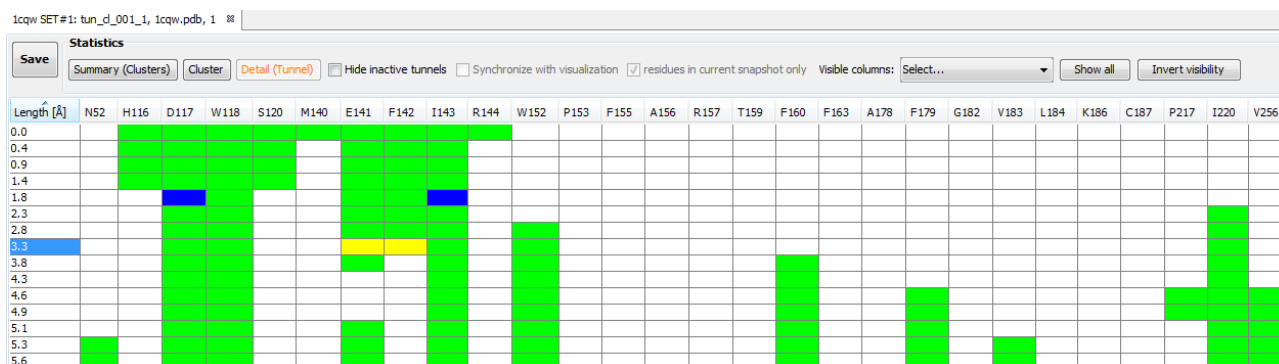


Figure 3.5.3 Table containing information about individual tunnel

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 center.
- **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).

Tunnel Statistics

sg SET#2: tun\_d\_002\_11cqw SET#4 residues

Save

☐ Side chain only

Minimum residue occurrence0%

Visible columns: Select...

Show all

Invert visibility

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).

1cqw SET#1: tun\_d\_001\_1, 1cqw.pdb, 1 1cqw SET#1 residues 1cqw SET#1 bottlenecks

Save

Minimum bottleneck occurrence 0%

Visible columns: Select...

Show all

Invert visibility

Snapshot	Cluster	F21	P53	I63	P64	D117	I143	I180	F204	L248	L249	F250	L257
1cqw.pdb	tun_d_001_1												
1cqw.pdb	tun_d_002_1												
1cqw.pdb	tun_d_003_1												
1cqw.pdb	tun_d_004_1												
1cqw.pdb	tun_d_005_1												
1cqw.pdb	tun_d_006_1												
1cqw.pdb	tun_d_007_1												
1cqw.pdb	tun_d_008_1												
1cqw.pdb	tun_d_009_1												
1cqw.pdb	tun_d_010_1												
1cqw.pdb	tun_d_011_1												
1cqw.pdb	tun_d_012_1												
1cqw.pdb	tun_d_013_1												
1cqw.pdb	tun_d_014_1												
1cqw.pdb	tun_d_015_1												
1cqw.pdb	tun_d_016_1												
1cqw.pdb	tun_d_017_1												

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).
- **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 Cavity Computation Window

The Cavity Computation window (see figure 3.6.1) controls the computation of pockets and cavities and the visualization of their solvent-excluded surface (SES). When changing parameters, the surface is updated in real time. Individual cavities and pockets are distinguished from each other by random colors attached to them.

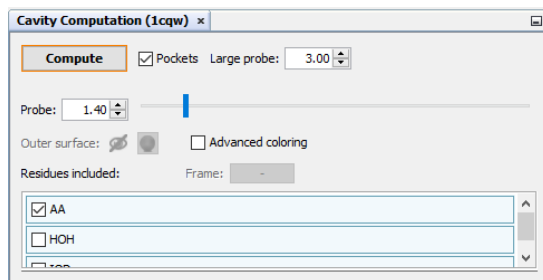


Figure 3.6.1 Cavity Computation window

Pockets can be defined with respect to the radii of a pair of spherical probes (a big probe and a small probe) as the regions of empty space, into which the small probe can get from the outside but the large probe cannot and where the small probe cannot collide with the large probe. Cavities are regions of empty space, which can be occupied by the small probe but from which the probe cannot escape to the outside. A very similar definition of pockets was introduced by Kwabata and Go in their article from 2007 [1].

The window contains the following parts and options:

- **Compute/Reset**  
This action performs the initial computation or resets already computed cavities and pockets. When the computation is finished, the resulting cavities appear in the visualization window and their details in the Cavities Overview window. The computation may take a while for larger structures. Ongoing computations can be canceled in the progress bar.
- **Pockets**  
This option enables or disables the computation of pockets and it can only be changed prior to the computation or after resetting the result of the last computation. Its default value can be changed in application settings.  
*Warning:* The computation of pockets is more intensive and more sensitive to numerical errors than the computation of cavities. The computation can be restarted several times with slightly perturbed coordinates if numerical errors are detected.
- **Large probe**  
The radius of the large probe for the computation of pockets. Its default value can be changed in application settings. Increasing this value will have a negative effect on performance.
- **Probe**  
The radius of the probe for the computation of cavities and pockets. If the computation of pockets was disabled, it is the radius the single probe for the detection of cavities. If the computation of pockets was enabled, it is the radius of the second (small) probe. When cavities/pockets are already computed, this radius can be changed interactively.
- **Outer surface**  
Enables or disables the visualization of the outer surface and allows to change its color. It is the surface, which can be reached by the probe from the outside. This option is available only if the com-

putation of pockets is disabled.

- **Advanced coloring**

By default, different cavities and pockets are distinguished by random colors. This option enables or disables advanced coloring schemes according to the strategy chosen in the Coloring window. Colors are interpolated over the whole surface from colors on the atoms.

- **Residues included**

This list contains residue types that are present in the current molecular structure (amino-acids, solvent molecules, ligands and non-standard residues). Residue types selected in this list will be included in the computation of cavities and pockets. The list of included residues can only be changed prior to the computation or after resetting the result of the last computation. The height of this GUI component can be changed manually.

- **Frame**

This button is for dynamic molecular structures only. It displays the frame number in which cavities or pockets were computed and allows to move the dynamics to the frame.

Illustrative examples of cavities, pockets and the outer surface are depicted in Figures 3.6.2 and 3.6.3.

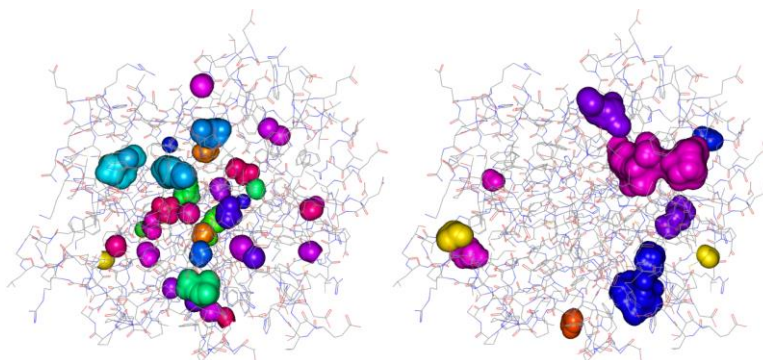


Figure 3.6.2 Left: All cavities detected for the probe radius 1.4 Å. Right: All pockets detected for the large probe radius 3 Å and the small probe 1.4 Å. PDB: 1CQW.

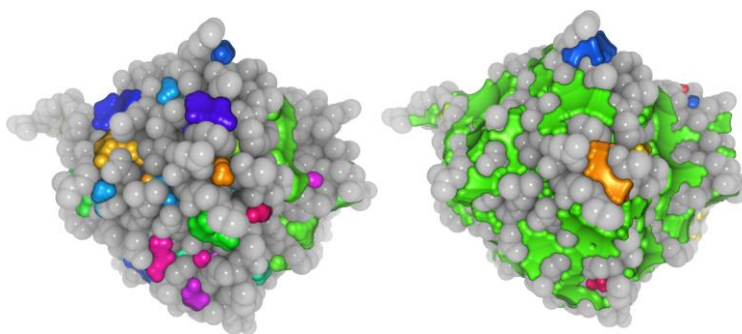


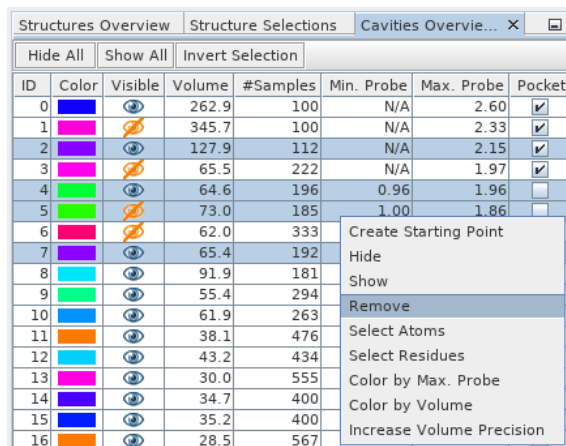
Figure 3.6.3 Pockets detected for the large probe radius 5 Å and various radii of the small probe. Left: Small probe radius 1 Å. Right: 0.6 Å. PDB: 1CQW.

[1] Kawabata, T. and Go, N. (2007), Detection of pockets on protein surfaces using small and large probe spheres to find putative ligand binding sites. *Proteins*, 68: 516–529. doi:10.1002/prot.21283.



## 3.7 Cavities Overview Window

This window (see figure 3.7.1) displays detailed information about computed cavities and pockets, and allows to select, hide, and perform also other actions. The table with cavities and pockets can be sorted by clicking on a column header.



The screenshot shows the 'Cavities Overview' window with a table of 17 rows (ID 0 to 16). The table columns are ID, Color, Visible, Volume, #Samples, Min. Probe, Max. Probe, and Pocket. A context menu is open over row 6, showing options: Create Starting Point, Hide, Show, Remove, Select Atoms, Select Residues, Color by Max. Probe, Color by Volume, and Increase Volume Precision.

ID	Color	Visible	Volume	#Samples	Min. Probe	Max. Probe	Pocket
0	Blue	Visible	262.9	100	N/A	2.60	✓
1	Magenta	Hidden	345.7	100	N/A	2.33	✓
2	Magenta	Visible	127.9	112	N/A	2.15	✓
3	Magenta	Hidden	65.5	222	N/A	1.97	✓
4	Green	Visible	64.6	196	0.96	1.96	☐
5	Green	Hidden	73.0	185	1.00	1.86	☐
6	Magenta	Hidden	62.0	333			
7	Purple	Visible	65.4	192			
8	Cyan	Visible	91.9	181			
9	Green	Visible	55.4	294			
10	Blue	Visible	61.9	263			
11	Orange	Visible	38.1	476			
12	Cyan	Visible	43.2	434			
13	Magenta	Visible	30.0	555			
14	Purple	Visible	34.7	400			
15	Blue	Visible	35.2	400			
16	Orange	Visible	28.5	567			

Figure 3.7.1 Cavities Overview window and the context pop-up menu

The window contains the following parts and options:

- **Hide All**

This action hides all cavities and pockets **currently listed in the table**. Hidden cavities will still appear in the table and can be selectively made visible by switching the eye icon in the visibility column. If the probe radius is manipulated in the Cavity Computation window, hidden cavities and pockets will still be hidden, but new cavities or pockets can be detected and they will be visible by default.

- **Show All**

This action makes visible all cavities and pockets **that have ever been hidden**, even those that have been hidden in the past for a different choice of the probe radius. If the probe radius parameter is manipulated in the Cavity Computation window and the cavity will be detected again, it will be visible.

- **Invert Selection**

Inverts the selection of cavities and pockets currently listed in the table. Selected items will be deselected and deselected items will be selected.

- **ID**

Zero-based indices of detected items (cavities and pockets).

- **Color**

The color assigned to each item. It cannot be changed to a custom color.

- **Visible**  
The icon indicates whether the item is visible (eye icon) or invisible (crossed eye). A mouse click on the icon will switch the visibility.
- **Volume**  
A very **rough estimation** of the volume, in cubic Å. The volume of each item (cavity or pocket) is computed by random sampling the bounding box of filling balls. Multiplying a rough estimate of the hit probability by the bounding box volume gives an estimation of the real volume. **The number of samples has to be increased manually to get a better estimation.**
- **#Samples**  
This is the number of samples used for the volume estimation. The initial number of samples is usually very low, hence the first estimation is imprecise. To get a better estimation, double click on the number of samples and enter a custom value or select the item, open a pop-up menu and choose “Increase volume precision”, which will add 5000 samples to selected cavities.
- **Min. Probe**  
The limiting probe radius, for which the cavity will open up and disappear. Its surface will become accessible from the outside and merge with the outer surface. The value is rounded to two decimal places, so the precision is plus/minus 0.01 Å. This value is meaningful only for cavities. Pockets have N/A in this field, which means that the value is “Not Available” (the small probe which has been used to detect a pocket can always escape from the pocket).
- **Max. Probe**  
The radius of the largest possible sphere, which fits inside the cavity / pocket. The value is rounded to two decimal places, so the precision is plus/minus 0.01 Å.
- **Pocket**  
This column indicates which items represent pockets and which represent cavities.

When clicking with the right mouse button on a row in the table, the following context menu appears:

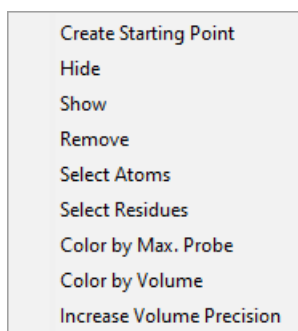


Figure 3.7.2 Context menu

The context menu offers the following functionality:

- **Create Starting Point**  
This action creates new starting points for the computation of tunnels. The starting point will be generated in the center of the maximal sphere that fits in the cavity/pocket.
- **Hide**  
This action hides all selected items. The items will still be listed in the table, only their visibility will be turned off.

- **Show**  
This action shows all selected items.
- **Remove**  
This action removes all selected items. The removed items will no longer be available in the table until the next re-computation of cavities/pockets.
- **Select Atoms**  
This action selects the atoms that touch the surface of the cavity/pocket, but only those which have been used for the computation. Selected items can be manipulated in the Structure Selections window.
- **Select Residues**  
This action selects the residues of the atoms that touch the surface of the cavity/pocket, but only those which have been used for the computation. Selected items can be manipulated in the Structure Selections window.
- **Color by Max. Probe**  
This action changes the coloring of cavities according to their “Max. Probe” values. This is a simple way of highlighting large cavities/pockets.
- **Color by Volume**  
This action changes the coloring of cavities according to their volume estimation. This is another way of highlighting large cavities/pockets.
- **Increase Volume Precision**  
This action increases the precision of the estimated volume of selected cavities/pockets by using next 5000 samples per each cavity/pocket. The number of samples is in the column #Samples and it can also be increased manually by double-clicking the field and entering a custom value.

Advanced settings of cavities can be found in the Application Settings (File – Application Settings – Cavities). There are the following options related to rendering the surface of cavities:

- **Pixel Artifacts Correction**  
This option enables or disables a post-processing step in rendering that reduces minor pixel artifacts caused by the analytic computation of solvent-excluded surface on GPU. Disabling this option may improve performance but some pixels can be rendered incorrectly.
- **Face Culling**  
This option enables or disables back-face culling. Enabling this option may improve performance.
- **Rendering Strategies**  
This section allows to choose a rendering strategy for elements of the solvent excluded surface.
  - Point Sprites  
This technique should be the fastest one but problematic on some graphic cards.
  - Rectangles  
This technique can be used instead of Point Sprites.
  - Bounding Boxes  
The slowest one but most robust technique.

The category Default Values contains several options, which will be used by default in the Cavities Computation window for newly opened molecular structures. These options decide whether to allow the computation of pockets, the default radii of the large and small probes, etc.

## 3.8 Locked Probes Window

When cavities are computed, it is also possible to show locked probes. This may help to indicate the positions of active sites in enzymatic proteins. Locked probes are largest possible empty spheres touching atoms of the molecular structure (see Figure 3.8.1). Such probes are held by the atomic balls they touch. They cannot be moved and cannot be expanded without hitting some atomic ball.

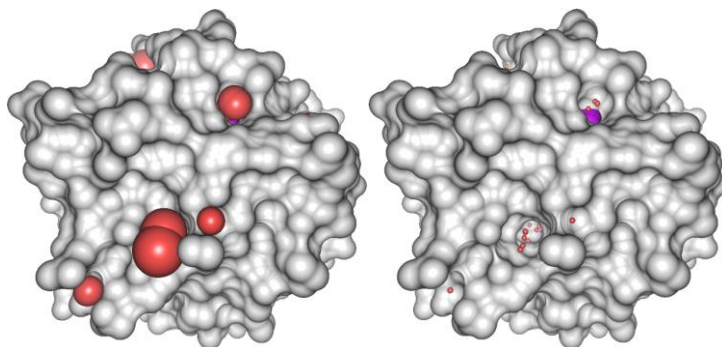


Figure 3.8.1 Locked probes - spheres and centers

The parameters for the locked probes can be set in the Locked Probes window (see Figure 3.8.2) and their visualization can be enabled by activating the corresponding button in the toolbar.

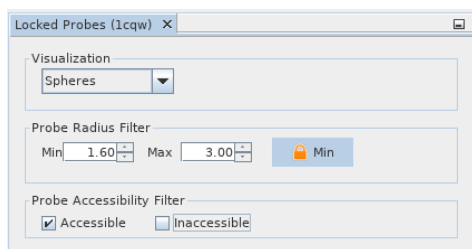


Figure 3.8.2 Locked Probes window

The window contains the following parts and options:

- **Visualization**  
Switches the visualization of locked probes – either spheres or centers of the spheres.
- **Probe Radius Filter**  
This filter limits the locked probes to spheres having the radii in the interval given by its endpoints – the minimum and the maximum. The button with the Min label and a lock icon activates or deactivates the synchronization of the minimum of the interval and the probe radius in the Cavities Computation window.
- **Probe Accessibility Filter**  
This filter limits the locked probes to accessible or inaccessible with respect to the probe radius in the Cavity Computation window. The spheres of inaccessible locked probes are trapped in cavities.

## 3.9 Structure Sequence Window

This window shows the primary structure of all loaded molecules (see figure 3.9). 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.

Structure Sequence																																																																																																							
1CQW	<	A	I	G	T	G	F	P	F	D	P	H	Y	V	E	L	G	R	M	H	Y	V	D	V	G	P	R	D	G	T	P	V	L	F	L	H	G	N	P	T	S	S	Y	L	W	R	N	I	I	P	H	V	A	P	S	H	R	C	I	A	P	D	L	I	G	M	G	K	S	D	K	P	D	L	D	Y	F	F	D	D	H	V	R	Y	L	D	A	F	I	E	A	L	G	L	E	E	V	V	L	V	I	H	
1AON	<	A	A	A	K	D	V	K	F	G	N	D	A	R	V	K	M	L	R	G	V	N	V	L	A	D	A	V	K	V	T	L	G	P	K	G	R	N	V	I	D	K	S	F	G	A	P	T	I	T	K	D	G	V	S	V	A	R	E	I	E	L	E	D	K	F	E	N	M	G	A	Q	M	V	K	E	V	A	S	K	A	N	D	A	A	G	D	G	I	T	T	A	T	V	L	A	Q	A	I	I	T	E	G
1EHY	<	A	A	I	R	R	P	E	D	F	K	H	Y	E	V	Q	L	P	D	V	K	I	H	Y	V	R	E	G	A	G	P	T	L	L	L	H	G	W	P	G	F	W	E	W	S	K	V	I	G	P	L	A	E	H	Y	D	V	I	V	P	D	I	R	G	F	G	D	S	E	K	P	D	L	N	D	L	S	K	Y	S	I	D	K	A	A	D	D	Q	A	A	L	I	D	A	L	G	I	E	K	A	Y	V	

Figure 3.9: Structure Sequence Window

## 3.10 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.
- **None**  
Disables the selection.
- **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.
- **SS**  
Allows the user to select whole secondary structures.

Each selection has its own record. The record contains:

- **Selection name**  
Displays the name of a given selection.
- **Zoom**  
Zooms and centers the given selection in the visualization window.
- **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.
- **Color selection**  
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.

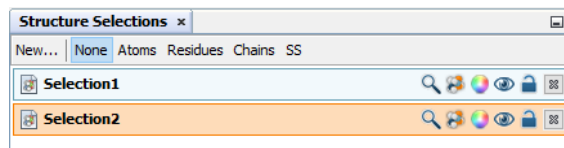


Figure 3.10.1: Structure Selections Window

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

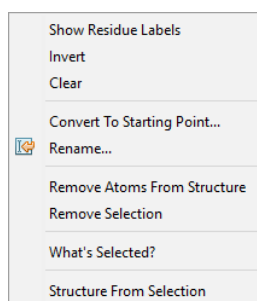


Figure 3.10.2: Menu for each selection

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 Atoms From Structure**  
Deletes the selected atoms from the structure.
- **Remove Selection**  
Removes the given selection.
- **What's selected?**  
Shows the list of atoms/residues/chains present in the selection.
- **Structure From Selection**  
Creates a standalone structure from the given selection. The new structure will appear in the list of structures in the Structure Overview panel.

## 3.11 Structure Dynamics Window

This window controls the playback of molecular dynamics (see figure 3.11). It is active only when dynamics are loaded and active.

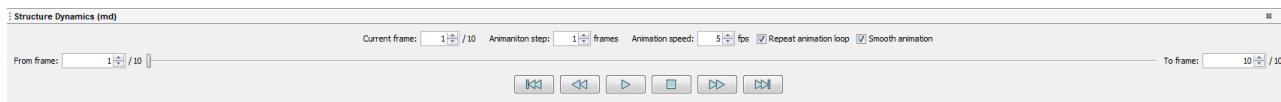


Figure 3.11: Structure Dynamics Window

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.
- **Smooth animation**  
Enables the interpolation between frames.
- **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.)

## 3.12 Structure Properties Window

This window shows more information about active structure (see figure 3.12). 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 3.12: Structure Properties Window

### 3.13 Structure Statistics Window

This window show detailed information about the constitution of given structure (see figure 3.13). It contains the list of present atoms, their count in the structure and their radii. Moreover, the list of present residues and their count is presented.

Structure Statistics (1cqw)					
Atoms					
Name	Symbol	Count	VdW	Covalent R	
Carbon	C	1532	1.7	0.68	
Nitrogen	N	398	1.55	0.68	
Oxygen	O	813	1.52	0.68	
Sulfur	S	9	1.8	1.02	
Iodine	I	2	1.98	1.4	
Residues					
Name	Identifier	Shortcut	Count		
Glutamic Acid	GLU	E	23		
Methionine	MET	M	5		
Tyrosine	TYR	Y	8		
Glutamine	GLN	Q	4		
Proline	PRO	P	30		
Aspartic Acid	ASP	D	19		
Alanine	ALA	A	21		
Lysine	LYS	K	8		
Serine	SER	S	9		
Threonine	THR	T	9		
Isoleucine	ILE	I	20		
Histidine	HIS	H	11		
Valine	VAL	V	22		
Water	H <sub>2</sub> O	H <sub>2</sub> O	394		
Glycine	GLY	G	23		
Phenylalanine	PHE	F	15		
Arginine	ARG	R	17		
Cysteine	CYS	C	4		
Leucine	LEU	L	29		
Tryptophan	TRP	W	9		
Asparagine	ASN	N	9		

Figure 3.13: Structure Statistics Window

### 3.14 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).



### 3.14.1 Static case

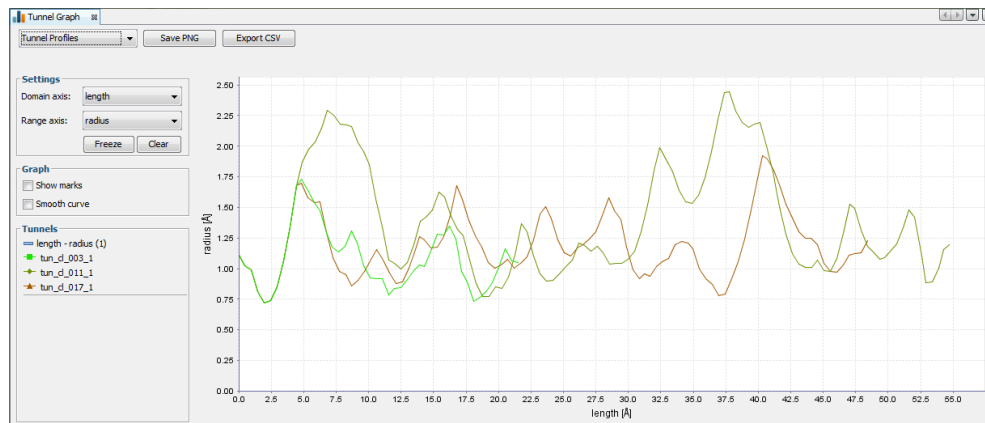


Figure 3.14.1.1: Tunnel Graph window for static case

In the static case (figure 3.14.1.1), 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 3.14.1.2).

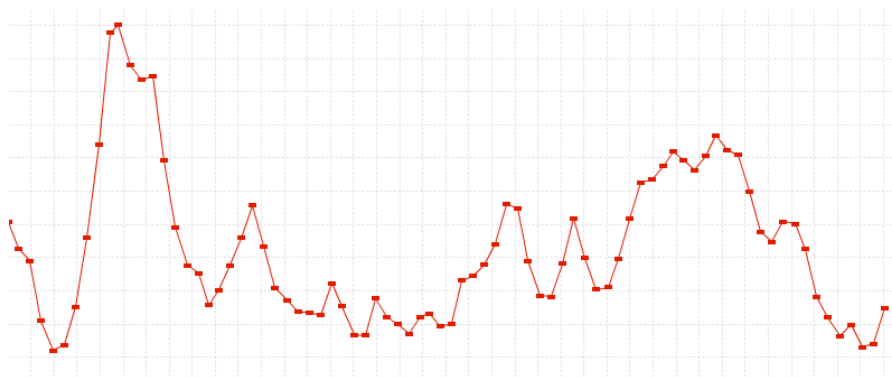
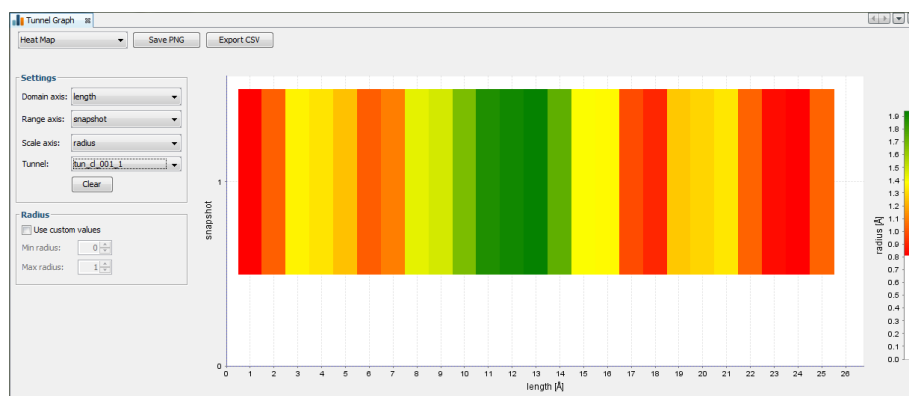


Figure 3.14.1.2: Graph with highlighted marks on the curve

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).

### 3.14.2 Dynamic case

In the dynamic case (see figures 3.14.2.1 and 3.14.2.2), 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).

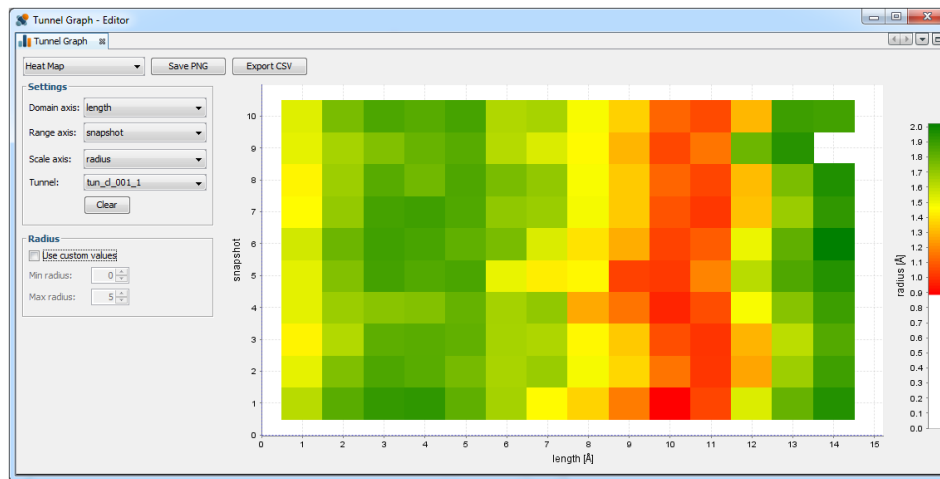


3.14.2.1 Tunnel Graph window for molecular dynamics

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.



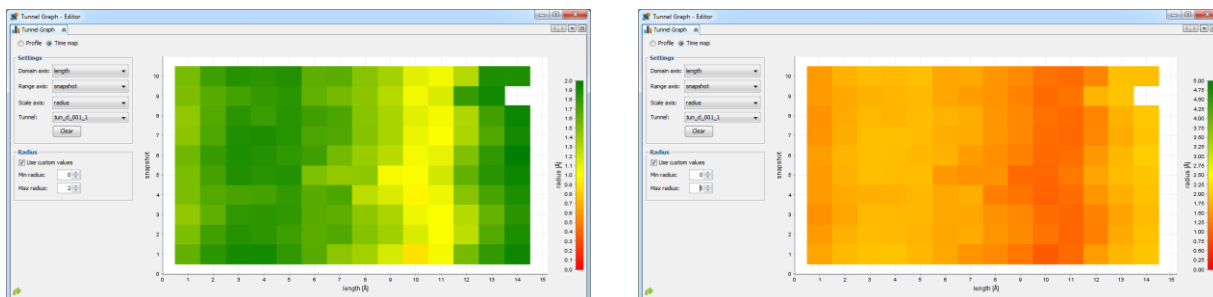
3.14.2.2: Example of heat plot (heat map)

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 3.14.2.3).

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



3.14.2.3: 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.

## 3.15 Coloring Window

The Coloring window (see figure 3.15) is used for coloring the scene background, structures and their parts, selections, and tunnels. The Global Settings part enables to change the background color of the main visualization window. There are three main tabs for changing the coloring schemes of structures, selections, and tunnels.

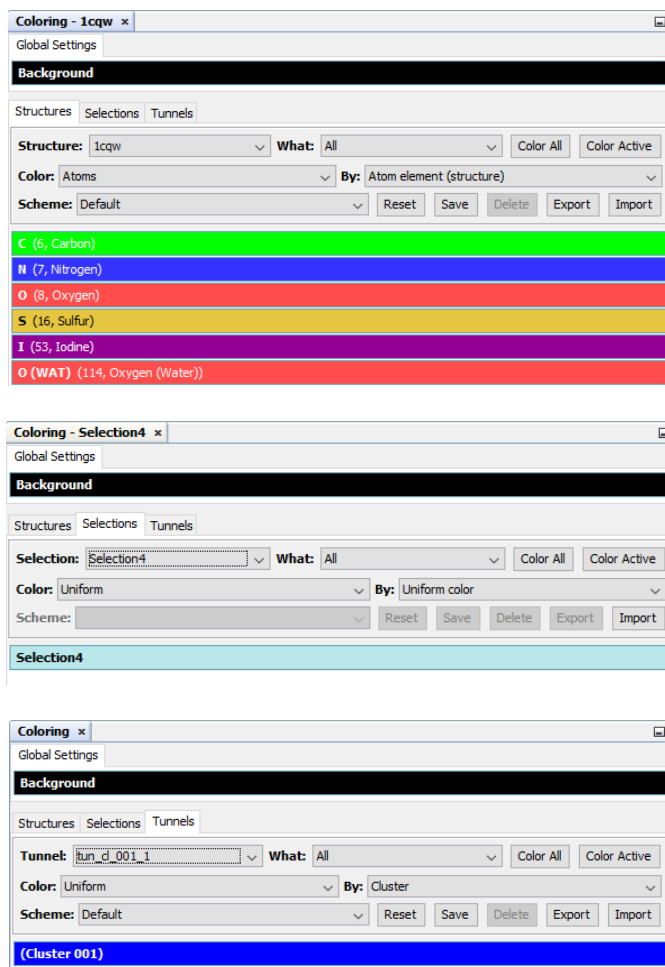


Figure 3.15: Coloring Window for structures, selections, and tunnels

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).

The panel contains the following options:

- **Structure (Selection, Tunnel)**  
Enables to select a structure (selection, or tunnel) which will be influenced by the color change.
- **What**  
Defines which representations will be influenced by the color change.

- **Color All**  
This button transfers the color settings to all loaded structures.
- **Color Active**  
This button transfers the color settings to all active structures.
- **Color**  
Defines which parts of the structure will be used for coloring (atoms, residues, chains, secondary structures, uniform coloring of the whole structure).
- **By**  
For each type in the Color mode, it determines the coloring mode (i.e., hydrophobicity for residues).
- **Scheme**  
Enables to select a predefined coloring scheme.
- **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.
- **Delete**  
Deletes the given scheme.
- **Export**  
Exports the given scheme in the .xml format.
- **Import**  
Imports a scheme from the .xml format.

## 3.16 Alignment Window

This function allows the user to perform structure or sequence alignment.

In structure alignment, we define two structures to align. The result is shown in the visualization window and the computed RMSD (Root Mean Square Deviation) value is displayed (see figure 3.16). Users can select the first and second structure for alignment and press the “Align” 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.

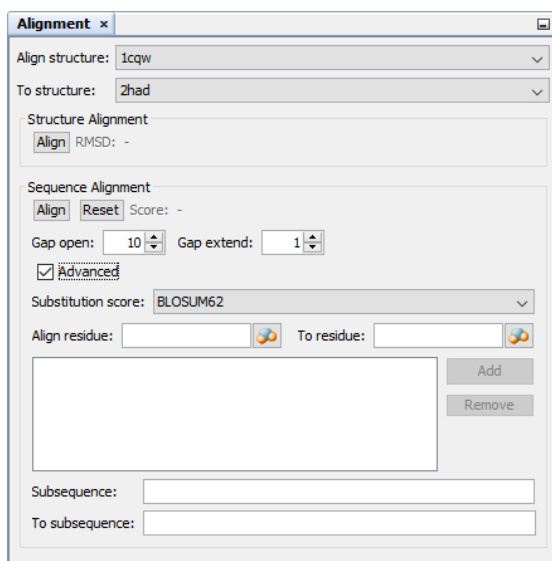


Figure 3.16: Structure Alignment Window

The sequence alignment enables the user to align two structures according to their sequential constitution. The basic parameters are the allowed sizes of inserted gaps. The alignment can be applied using the “Align” button or cancelled using the “Reset” button. The sequence alignment offers also the Advanced mode, where we can refine the parameters for alignment. We can select the score used, define IDs of residues or subsequence, which should correspond in the alignment.

### 3.17 Structure Clip Planes Window

This window allows the user to activate work with several clip planes and slices of clip planes for each structure, which can stress important features of proteins as well as tunnels (see figure 3.17).

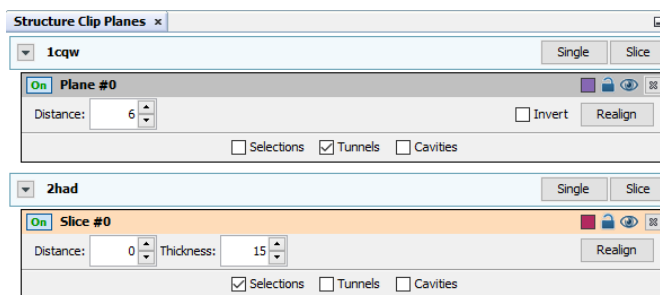


Figure 3.17: Clip Planes Window

The Clip Planes window allows the user to activate several clip planes and slices for each structure and configure the following settings and functions:

For clip planes:

- Activate or deactivate the clip plane using the On/Off button.
- Set to color of the clip plane.
- Lock the clip plane, i.e., all transformations applied to the structure are applied to the clip plane as well.

- Show or hide the clip plane representation (colored rectangle).
- **Distance**  
Defines the distance of the clip plane from the camera.
- **Invert**  
Inverts the visibility of the clipped structure.
- **Realign**  
In case when the clip plane is locked, this button resets the clip plane position to be perpendicular to the camera.
- Define if the clip plane should be applied also to Selections, Tunnels, and Cavities.

For slices we have one extra option:

- **Thickness**  
Defines the thickness of the slice.

## 3.18 Surface Configuration Window

The Surface Configuration window (see figure 3.18) directly influences the computed and visualized surface of a selected structure, tunnel, or selection. It enables to define the structure for which we are changing the surface parameters and determine the surface type. Then we can change the transparency of a surface for the structure, its tunnels and selections, and change the probe size for the structure's surface and also for the Detailed Surface method for tunnel visualization.

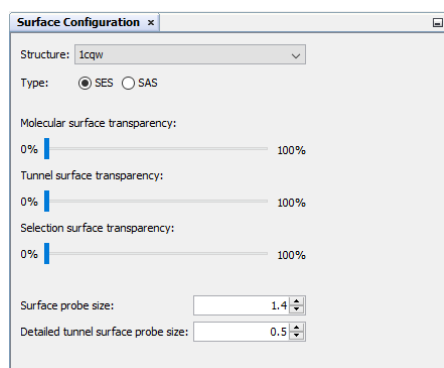


Figure 3.18: Surface Configuration Window

## 3.19 Search in Structure

The Search in Structure window (see figure 3.19) enables the user to select a specific part of the structure by defining the identifiers of residues and/or atoms. First, the user defines the structure in which the selection should be performed (can be applicable also to all structures in the scene), then defines the chain identifier and the identifiers of residues and atoms (individual numbers divided by comma, ranges). Then the selected residues and/or atoms can be added to an existing selection or can form a new selection. The “Select” button finishes the process. The “Center & Zoom” button centers and zooms the selection in the visualization window. The “Close” button closes the window without any action.

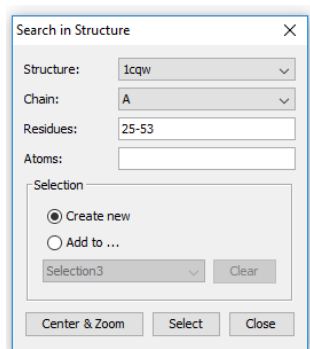


Figure 3.19: Search in Structure Window

## 3.20 Delete from Structure

The Delete from Structure window (see figure 3.20) serves for deleting the user-specified part of structure. The user can delete a selection or specify the structure, its chain, and identifiers of residues and/or atoms, which should be deleted. The “Remove” button finishes the process. The “Center & Zoom” button centers and zooms the selection in the visualization window. The “Close” button closes the window without any action.

Warning: This process is irreversible, after deleting a given part of structure the structure will contain a gap.

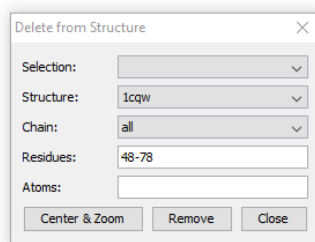


Figure 3.20: Delete from Structure Window

## 3.21 Measurement Window

The Measurement window (see figure 3.21) enables the user to measure distances, angles, and dihedral angles between atoms. First, the user has to select the type of measurement in the top part of the panel. Then, by clicking to the scene (Ctrl + left mouse click on a selected atom) or specifying the atom identifier directly in the text field, the user defines a given number of atoms – two for distance, three for angle, four for dihedral angle. After adding the last atom, the measurement is automatically performed and it is listed in the panel. The measurement is also visualized in the main window as a line connecting the corresponding atoms and with the resulting measured number next to the line. The user can change the color of the line, show measurement graph, show/hide the measurement, and delete the measurement.

When performing the atom selection, the given icon next to the atom field has to be selected (it is stated by blue background of the icon).



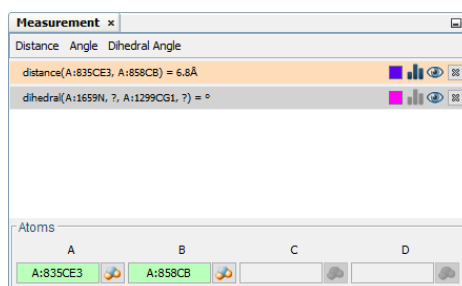


Figure 3.21: Measurement Window

## 3.22 Mutagenesis Window

The Mutagenesis window (see figure 3.22) enables the user to perform mutations of individual residues on static structures. The panel contains the list of performed mutations, which can be cancelled by using the “Restore checked” or “Restore all” button.

The Add mutation section serves for specification of the mutation. First the user defines the residue identifier (numerical value). Then the rotamer library has to be selected which will be used for searching the rotamers. Then the user specifies the type of new amino acid and selects the desired rotamer from the given list. Here the decision can be based on the score of individual rotamers, based on their Probability of occurrence, Standard deviation, and Collision penalty score. The “Apply mutation” button performs the mutation and creates an item in the list of mutations. The “Cancel mutation” button cancels the operation. The “Show collisions” button displays the collision of the selected rotamer with the rest of the structure in the visualization window.

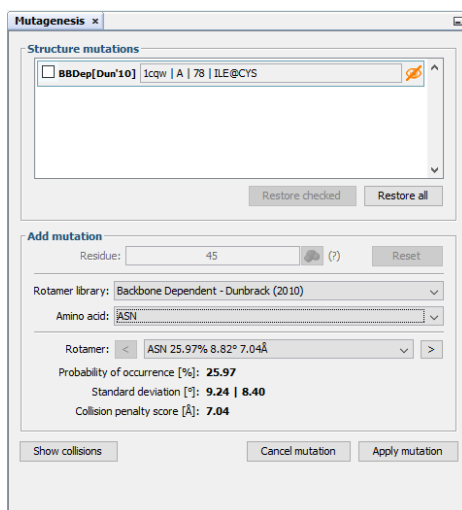


Figure 3.22: Mutagenesis Window

## 3.23 Asymmetric Tunnels Window

The Asymmetric Tunnels window (see figure 3.23.1) helps to specify parameters for the asymmetric representation of tunnels (see figure 3.23.2). This representation can be calculated either automatically based on the surrounding atoms or can be controlled by the user-defined parameter, specifying the enlargement of the probe size used for the tunnel computation. This method searches for tunnel surface in highly asymmetric cavities and calculates the tunnel volume. This is the difference between the asymmetric tunnel and the detailed surface representation, available in the list of possible tunnel representations. The tunnel detailed surface is a simple extension of the probe size used for tunnel computation. It is set automatically and the user does not have any control of the appearance. When the tunnel is highly asymmetric, this representation does not cover completely the bulky parts of the tunnel. The detailed surface is used for the computation of contours in cross cuts, used in the Contours window (see section 3.25).

The Asymmetric Tunnel Computation window (see figure 3.23.1) contains the following items:

- **Extrusion** – defines the tunnel surface enlargement. It can be automatic (then it searches for the tunnel-lining residues and their atoms) or manual (enlargement of radii of spheres forming the tunnel computed by the CAVER tool).
- **Voxel size** – defines the density of the grid which is used for searching for the tunnel-lining residues.
- **Probe radius** – size of the probe. Can be defined manually by the user or the tool can automatically use the size of the bottleneck as a probe size.
- **Change settings** – recalculates the asymmetric tunnel after changing the parameters.
- **Select lining residues** – selects the closest residues lining the tunnel (a selection will be created which will be displayed in the visualization window).

Then follows the list of calculated asymmetric tunnels, along with their volume.

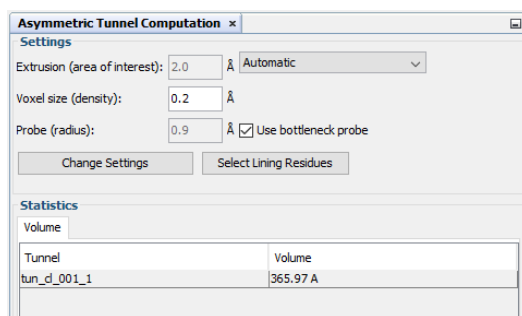


Figure 3.23.1: Asymmetric Tunnel Computation window

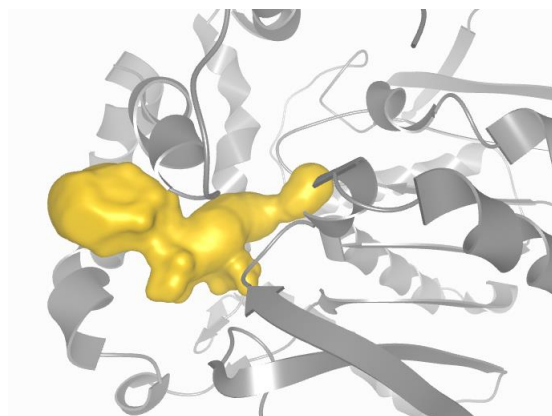


Figure 3.23.2: Asymmetric representation of a tunnel

## 3.24 Residue Graph Window

The Residue Graph window (see figure 3.24.1) enables the user to visually explore the evolution of a selected tunnel over time in detail. The window consists of the following parts:

- **Manipulation panel**

In this top part of the window, the user selects the structure and one of its tunnels, which should be explored in the bottom parts. The computation of the tunnel profile and its surrounding residues is performed using the “Compute” button.

- Analyse Selected tunnels – enables to explore more tunnels at once.
- Import Contour Data – enables to import the stored data about the contours, computed using the Contours window (see section 3.25).
- Graph – enables to color the tunnel profile below based on different properties – time, tunnel length, tunnel maximal radius, and tunnel ID.
- Residues – enables to color the surrounding residues based on their different properties – hydrophobicity, partial charge, donors and acceptors, tunnel ID, and influence of the tunnel by these residues.
- Detailed – changes the representation of the surrounding residues below from an interpolated representation (default) to line-by-line representation (each line corresponds to one timestep).

- **Tunnel profile**

This graph representation plots the tunnel profile (width) along its centerline. On the left side is the starting point of the tunnel in the active site, the right side represents the molecular surface (the tunnel gorge). Each line represents the tunnel in one timestep. From the graph, the user can clearly detect the narrowest parts of the tunnel (bottlenecks) and the most stable and unstable parts of the tunnel over time.

- **Surrounding residues**

The bottom part of the window shows the list of all residues contributing to the tunnel. Each of them is represented by a thick colored line, and the residue identifier is positioned on the left side of the line. The color corresponds to a selected property (i.e., hydrophobicity). The horizontal position of the color in the line shows which part of the tunnel this residue influences (related to the

tunnel profile above). The longer the colored line, the more it influences the tunnel.

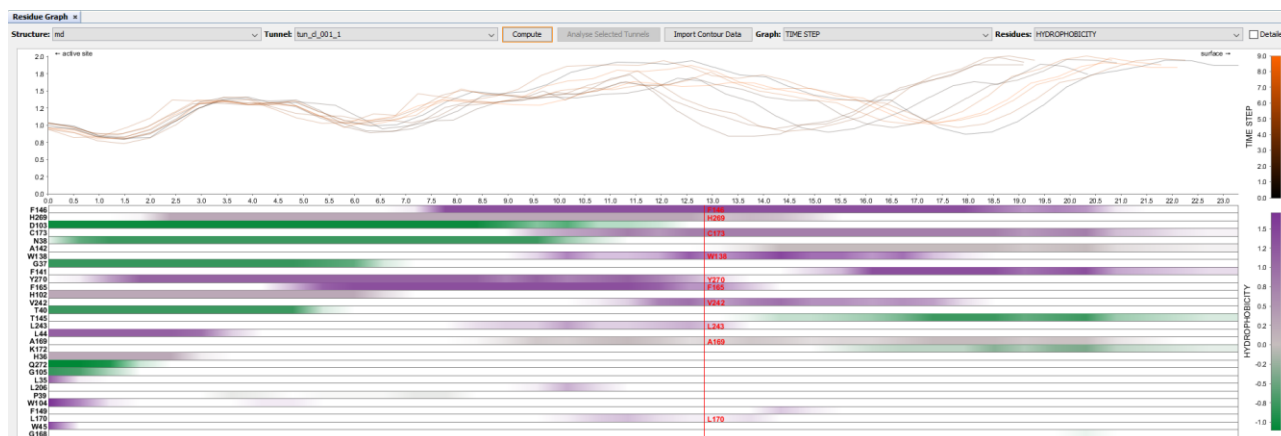


Figure 3.24.1: Residue Graph window

The vertical red slider shows the constitution of the tunnel (its surrounding residues) in a given position along the tunnel centerline. By clicking on a specific site, the user launches the calculation of the detailed cross cut of the tunnel in this site, which can be explored in detail using the Contours window (see section 3.25).

Right mouse button click in the window opens the menu containing the following items (see figure 3.25.2):

- Properties... – opens a window where the user can set various properties influencing the appearance of the tunnel plot.
- Copy – enables to save the content of the window (without the top panel) to clipboard.
- Save as – enables to save the content of the window in the PNG format.
- Clear Selection & Filters – removes all applied filters (shows data for all timesteps).
- Show Filters – enables to use filtering based on selected values of the residue properties.
- Sort Residues – enables to sort residues according to a selected property (6 options available).

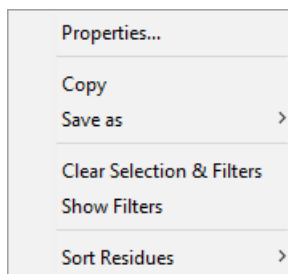


Figure 3.24.2: Menu with advanced options

Details about the design rationale of the Residue Graph and examples of usage can be found in [2].

[2] Byska, J., Le Muzic, M., Gröller, E. M., Viola, I., Kozlikova, B. AnimoAminoMiner: Exploration of Protein Tunnels and their Properties in Molecular Dynamics. IEEE Transactions on Visualization and Computer Graphics, IEEE, 2016, 22(1), 747-756. ISSN 1077-2626. doi:10.1109/TVCG.2015.2467434.

## 3.25 Contours Window

The Contours window (see figure 3.25.1) enables the user to visually explore the evolution of a selected tunnel cross cut (i.e., bottleneck) over time in detail.



Figure 3.25.1: Contours window

The window consists of the following parts:

- **Manipulation panel**
  - Colored by – changes the coloring of the contours according to time or contour area.
  - Show Circles – shows the “coordinate system” with a measure.
  - Show 3D – projects the contour to the 3D main visualization window so the user can observe simultaneously the 3D representation of the tunnel, its cross cut, and the contour representation.
- **Contour visualization**

The main part of the window contains the 2D contour representation. Each contour represents one timestep. It can be colored according to time or area occupied. The surroundings of the contours is filled with bars representing individual residues forming the tunnel in this cross cut part. The position of the bars corresponds to their actual positions around the cross cut. Each residue can be represented by more bars, arranged in a co-circular manner – each bar represents one selected property (in figure 3.25.1 hydrophobicity and partial charge). When a bar is filled only partially with a color, it means that this residue did not contribute to this tunnel cross cut in all timesteps.

Right mouse button click in the window opens the menu containing the following items (see figure 3.25.2):

- Properties... – opens a window where the user can set various properties influencing the appearance of the tunnel plot.
- Copy – enables to save the content of the window (without the top panel) to clipboard.
- Save as – enables to save the content of the window in the PNG format.
- Clear Selection & Filters – removes all applied filters (shows data for all timesteps).
- Show Filters – enables to use filtering based on selected values of the residue properties.

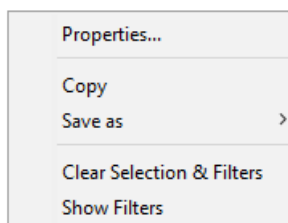


Figure 3.25.2: Menu with advanced options

The Contour window is interactively connected with the Residue Graph window (see section 3.24).

Details about the design rationale of the Contours and examples of usage can be found in [3].

[3] Byska, J., Jurcik, A., GRÖLLER, E. M., Viola, I., Kozlikova, B. MoleCollar and Tunnel Heat Map Visualizations for Conveying Spatio-Temporo-Chemical Properties Across and Along Protein Voids. Computer Graphics Forum, Wiley-Blackwell, 2015, 34(3), 1-10. ISSN 0167-7055. doi:10.1111/cgf.12612.

## 3.26 Console Window

The Console window (see figure 3.26) enables the user to control the most important functions of CAVER Analyst using the command line.

When typing *help* to the command line, the list of all commands appears.

Using *help xxx*, where *xxx* stands for one of the commands, displays the description of usage of the given command.

```

C:\Users\Bobina\caver $ help
cd          changes working directory
center      centers the camera to selected structure (set of structures)
color       colors specified elements of a structure
delete      deletes specified object
download    downloads structure file from the PDB database
exit        closes the application
fetch       downloads structure file from the PDB database
help        prints help
hide        disables the visualization style of the structure
label       creates a label for specified atoms/residues
list        lists all available CAVER Analyst's variables
load        loads structure file
ls          prints files in the current directory
pore        computes pore in the structure
print       prints the current value of a variable
remove      removes specified atoms from model
rename      renames specified object in the scene
renumber    rennumbers identifiers inside the structure(s)
run         runs a command script
screenshot  takes a screenshot
select      creates or modifies a selection
set         sets a variable
show        enables the visualization style of the structure
sleep       waits for a given period of time
tunnels     computes tunnels for the structure
unlabel     removes a label for specified atoms/residues
workspace   saves/loads the workspace to/from the specified location
write       writes a given text to console
zoom        zooms the camera to selected element of the structure
C:\Users\Bobina\caver $

```

Figure 3.26: Console Window with the list of all supported commands