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CAVER Analyst 1.0: graphic tool for interactive visualization and analysis of tunnels and channels in protein structures

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ABSTRACT

Summary: The transport of ligands, ions or solvent molecules into proteins with buried binding sites or through the membrane is enabled by protein tunnels and channels. CAVER Analyst is a software tool for calculation, analysis and real-time visualization of access tunnels and channels in static and dynamic protein structures. It provides an intuitive graphic user interface for setting up the calculation and interactive exploration of identified tunnels/channels and their characteristics.

Availability and Implementation: CAVER Analyst is a multi-platform software written in JAVA. Binaries and documentation are freely available for non-commercial use at http://www.caver.cz.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

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1 INTRODUCTION

Binding sites of many proteins are deeply buried in the protein core and are connected with the surrounding environment by access tunnels (Prokop *et al*., 2012). The protein channels are found in transmembrane proteins and are important for the traffic of small molecules through the membranes. Mutations in these structures can lead to serious hereditary diseases. Shape, physicochemical properties and dynamics of tunnels/channels determine the accessibility of binding sites to ligands, ions or solvent molecules and their biological activity (Damborsky *et al*., 2007; Prokop *et al*., 2012). Tunnels/channels can be found in a wide range of proteins, making their analysis important and useful. Owing to the intrinsic protein dynamics, the tunnels/channels significantly change their shape and properties over time (Chovancova *et al*., 2012; Karplus *et al*., 2002; Klvana *et al*., 2009). Interactive calculation and visualization of tunnels/channels with their characteristics can facilitate the study of important biochemical phenomena, as well as the design of new catalysts or effective drugs (Gora *et al*., 2013; Koudelakova *et al*., 2013; Pavlova *et al*., 2009; Prokop *et al*., 2012). To meet these needs, we have developed the CAVER Analyst (Fig. 1), which complements the recently published command-line CAVER 3.0 for identification of tunnels in static and dynamic protein structures (Chovancova *et al*., 2012). Unlike other available graphic interfaces for tunnel analysis MolAxis (Yaffe *et al*., 2008), PoreWalker (Pellegrini-Calace *et al*., 2009) and MOLEonline 2.0 (Berka *et al*., 2012), which are limited to the tunnel analysis in a single static structure (Brezovsky *et al*., 2013), the CAVER Analyst enables comparative analysis of tunnels/channels in homologous structures and to study their dynamics.

Tunnels/channels calculation: CAVER Analyst integrates CAVER 3.0 for identification of tunnels in static structures and molecular dynamic trajectories. The calculation can be set and performed directly using the CAVER Analyst interface. The most important calculation settings are available through the Tunnel Computation window (Supplementary Fig. S1), while the advanced parameters can be set in the Tunnel Advanced Settings window or loaded from the configuration file. The starting point for the calculation can be derived from (i) the catalytic residues automatically loaded from the databases, (ii) automatically identified cavities, (iii) interactive selections or (iv) manually specified atoms, residues or coordinates. The starting point can be manually adjusted.

Cavity calculation: CAVER Analyst integrates an efficient algorithm for computation and visualization of molecular surfaces and cavities. The algorithm is based on the additively weighted Voronoi diagram and allows users to access a real-time analysis of cavities in static structures (Supplementary Fig. S2).

Visualization: Protein structures can be visualized using all standard visualization styles and colored according to various criteria (Supplementary Fig. S3). The visualization of molecular surface can be customized by setting the level of the surface refinement and transparency. For exploration of the inner protein structure, CAVER Analyst offers clip planes for cutting off parts of the molecule. Tunnels and channels can be visualized by (i) center lines indicating tunnel location and curvature, (ii) spheres showing approximate tunnel geometry, omitting asymmetrical

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†The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

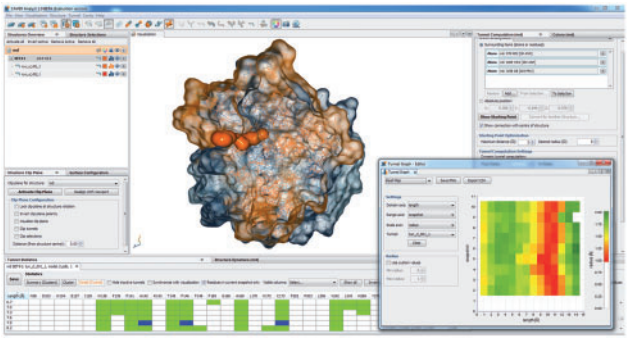

Fig. 1. The graphic user interface of the CAVER Analyst 1.0

parts of the tunnel and (iii) detailed surface, which presents the tunnel geometry more accurately, including the asymmetrical parts (Supplementary Fig. S3). Tunnels/channels identified throughout the trajectory can also be visualized in one snapshot as their center lines.

Statistics: The summary table lists the statistics of individual tunnel clusters, including their priority, frequency, average bottleneck radius, length, curvature and throughput (Supplementary Fig. S4). By selecting a particular cluster, characteristics of all individual tunnels/channels from a given cluster are depicted. Furthermore, each individual tunnel/channel can be explored at the level of its profile, showing changes in its radius and neighboring residues along its length. Additionally, the tables listing the tunnel-lining residues and the bottleneck residues are provided for each tunnel cluster. All tables can be sorted by user-selected characteristics, and data can be exported as comma-separated values (CSV) or text files. All tables are interactively interconnected with the visualization window, enabling the interpretation of the results in the context of the 3D protein structure. The tunnels and residues selected in the tables are automatically highlighted in the structure and thus can be simultaneously explored in the tables and visualization window.

Graphs: Characteristics of individual tunnels/channels can be plotted in the form of 2D graphs. Users can plot together into one graph the profiles of different tunnels or the profiles of the same tunnel identified in different structures or snapshots, which significantly facilitate tunnel comparison (Supplementary Fig. S5). For molecular dynamics, the tunnel/channel profiles can be animated by switching between individual snapshots. Besides the profiles, this module enables users to display a time evolution of its characteristics (bottleneck and average radius, length and curvature) in the form of 2D graphs or heat plots (Supplementary Fig. S6). All graphs can be saved as images or exported as raw data.

Other: Besides the features specific to the tunnel/channel analysis, the CAVER Analyst provides a number of additional features: (i) structure alignment by combinatorial extensions (Shindyalov *et al.*, 1998), (ii) addition of hydrogen atoms, (iii) changing protonation of titratable residues (Case *et al.*, 2012), (iv) selections, (v) coloring and labeling, (vi) managing of workspaces, etc.

2 IMPLEMENTATION

CAVER Analyst is a multi-platform JAVA-based software. It can run on both 32- and 64-bit system architectures with JAVA 1.7 or a later version (Supplementary Material for implementation).

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Conflict of Interest: B.K., V.S., O.S., P.B., J.D. and J.S. are founders of the spin-off company CaverSoft Ltd.

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