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Applications Note

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CAVER Analyst 2.0: analysis and visualization of channels and tunnels in protein structures and molecular dynamics trajectories

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Abstract

Motivation: Studying the transport paths of ligands, solvents, or ions in transmembrane proteins and proteins with buried binding sites is fundamental to the understanding of their biological function. A detailed analysis of the structural features influencing the transport paths is also important for engineering proteins for biomedical and biotechnological applications.

Results: CAVER Analyst 2.0 is a software tool for quantitative analysis and real-time visualization of tunnels and channels in static and dynamic structures. This version provides the users with many new functions, including advanced techniques for intuitive visual inspection of the spatiotemporal behavior of tunnels and channels. Novel integrated algorithms allow an efficient analysis and data reduction in large protein structures and molecular dynamic simulations.

Availability and implementation: CAVER Analyst 2.0 is a multi-platform standalone Java-based application. Binaries and documentation are freely available at www.caver.cz.

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

1 Introduction

The importance of access tunnels in proteins has been demonstrated by many studies in the last decade (Kingsley *et al.*, 2015; Marques *et al.*, 2017). Their examination in dynamical protein ensembles became a standard technique for studying important biochemical phenomena, designing new biocatalysts, materials or drugs (Brezovsky *et al.*, 2016; Gora *et al.*, 2013; Koudelakova *et al.*, 2013; Liskova *et al.*, 2015; Yu *et al.*, 2013). With the current computational capacity, it becomes affordable to obtain molecular dynamics (MD) trajectories up to the microsecond time scales. This trend requires new approaches to explore

the large datasets, as it becomes impracticable to observe such simulations in a frame-by-frame manner. Feature extraction and aggregation techniques, giving a guidance and overview of interesting sites and properties of tunnels over time, are therefore necessary. To follow this trend, we are introducing CAVER Analyst 2.0, which enables visual exploration of protein tunnels and channels even in microsecond-long MD simulations. This was achieved by introducing novel visualization approaches and other advanced functions, which enhance the manipulation of such simulation data. CAVER Analyst 2.0 introduces significant changes and improvements, focusing especially on large data processing, but also on providing the users with a complete description of the structural and biophysical features of protein tunnels and channels.

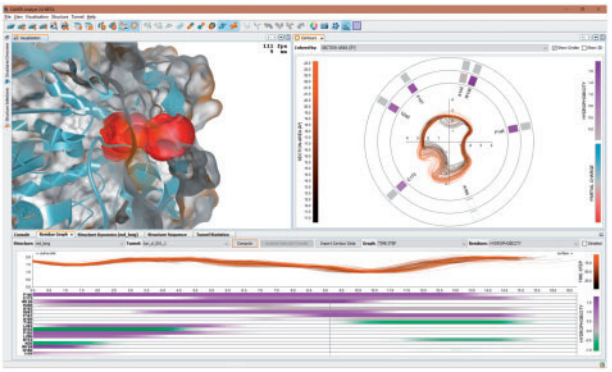

Fig. 1. CAVER Analyst 2.0 user interface

2 Features

Tunnel, channel and cavity calculation: CAVER Analyst 2.0 integrates the most up-to-date CAVER tool with the set of algorithms for: (i) identification of tunnels and channels in proteins, (ii) analysis of tunnels and channels in large MD simulations and (iii) identification of protein pockets and inner cavities. The algorithms are being continuously developed to provide the most accurate and computationally efficient description of these specific structural features. The tunnel calculation can be launched directly from the CAVER Analyst interface, which offers the basic and advanced calculation settings modes. For compatibility reasons, we keep the user interface of the Tunnel Computation window consistent with the version 1.0 (Kozlikova *et al.*, 2014). We have also improved the algorithm for the cavity detection (Manak *et al.*, 2017).

Visual analysis of tunnels: New visualization techniques present an important contribution to CAVER Analyst 2.0. They were mostly designed with the purpose of tunnel exploration in long MD trajectories (in AMBER, GROMACS, CHARMM formats), focusing on the changes of the tunnel properties and its surrounding residues over the time. Both techniques aggregate the spatial information to a single overview image so the user can get the information about the main trends in the tunnel behavior, regardless the MD simulation length. The first technique (Byska *et al.*, 2015) focuses on the visual representation of the shape of tunnel cross-cut at a specific site, e.g. its bottleneck. It shows its changes over time and physico-chemical properties of the amino acids lining that section (Fig. 1 and Supplementary Fig. S2). The central part is formed by the contour, which is defined by the cross-cut through a given tunnel. Each time step generates one contour and their overlay shows the shape of the cross-cut over the time. The rectangular bars surrounding the contours represent the respective lining amino acids colored by their physico-chemical properties. The second technique (Byska *et al.*, 2016) shows the width profile of a selected tunnel along the tunnel centerline (Fig. 1 and Supplementary Fig. S1). The amino acids forming the tunnel boundary are presented below the profile using a set of lines. The length of these lines illustrates the portion of the tunnel influenced by a particular amino acid. When dealing with dynamic ensembles, the lines represent the residues and their relative influence averaged over the entire simulation. Using a vertical slider, the user can specify a given section of the tunnel, for which the contour representation is calculated and visualized. The Supplementary Material demonstrates the applicability of these visualizations with two case studies focused on the engineering of tunnels aimed at improving protein stability and catalytic activity.

Mutagenesis: Engineering proteins typically requires the design and modeling of mutations. CAVER Analyst 2.0 supports this task by the new Mutagenesis Window (Supplementary Fig. S3). It offers the possibility to design one or more mutations in selected positions of a static molecule structure, which can be further used to recalculate the tunnels and visually compare the differences with the template. The newly designed molecule can be exported, upon which additional modeling studies, such as MD simulations, can be performed. The obtained trajectories can be loaded again to CAVER Analyst 2.0 and visually explored. The mutagenesis may use two different libraries of residue rotamers (Dunbrack *et al.*, 2011; http://bioserv.rpbs.univ-paris-diderot.fr/software.html).

Buffering: CAVER Analyst 2.0 enables to manipulate MD simulations of arbitrary length instantly, which ensures that the tool will be usable with simulations containing orders of magnitude higher number of time steps than now.

Other features: CAVER Analyst 2.0 offers advanced Measurement Window, the Clip Plane Window enabling to operate several independent clip planes and slices at once (Supplementary Fig. S4), improved manipulation of the protein structure, e.g. removing selected atoms, exporting structures from selected objects, video recording, high-resolution screenshots and the accessibility to common actions via the command line.

3 Implementation

CAVER Analyst 2.0 is a multi-platform JAVA-based software. It can run on both 32- and 64-bit system architectures with JAVA 1.8 (see Supplementary Material for implementation details).

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Conflict of Interest: none declared.

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