

## Technical Report

# Identification of tunnels in proteins, nucleic acids, inorganic materials and molecular ensembles

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The knowledge of the access paths connecting interior of molecular systems with surrounding environment is important for the understanding of structure function relationships and engineering of molecules for biotechnological applications. CAVER is a computer program developed for calculations of tunnels, channels or pores in the biomolecules, inorganic materials and molecular ensembles. The algorithm performs a skeleton search based on a reciprocal distance function grid. The algorithm is implemented in the stand-alone version, web version and as plug-in for PyMol. CAVER is available from the website <http://loschmidt.chemi.muni.cz/caver>.

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## 1 Introduction

Tertiary structures of macromolecules represent complex objects containing many clefts, protrusions and cavities. Functional sites are often located in the core of the molecules, but must be accessible to small ligands, *e.g.*, substrates, co-substrates, inhibitors, co-factors, or effectors. Accessibility is enabled by the access paths connecting the core with surrounding environment. These access paths, called tunnels, channels or pores, possess certain size, shape, flexibility and physicochemical properties, which determine whether a ligand can penetrate to the macromolecule, bind to the functional site and make a response, react, inhibit or otherwise express its function. Knowledge of the molecular properties of the access paths and their changes in time is essential for analysis of structure-function relationships and engineering of molecules for various biotechnological applications.

## 2 Description of the program

The program CAVER was developed to provide rapid, accurate and automated identification of paths leading from buried functional sites in dynamic and static structures to the outside solvent [1]. The algorithm performs a skeleton search based on a reciprocal distance function grid. Initially, a user specifies starting point for CAVER search, which is typically the point located deeply in the pocket. CAVER traverses grid points in an empty space of a pocket/tunnel and prefers those points that have more empty space around. This is done by an evaluation of a given cost-function embodied to the main chain of the Dijkstra's algorithm [2]. The cost function gives higher penalty to points that are closer to protein atoms, *i.e.*, preferred points have a small penalty value. Calculation ends when the searching process reaches protein's convex boundary pre-computed by the program Qhull [3]. Calculations can be performed using discrete structures from crystallographic analyses as well as molecular ensembles from molecular dynamics simulations from NMR experiments. The fully functional program is available as a stand-alone

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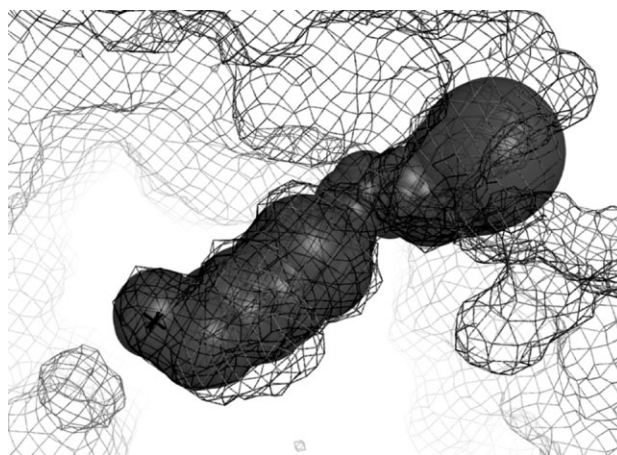
**Figure 1.** Screen-shot of the online version of CAVER. PDB file containing the coordinates and a “starting point” located in the functional/active site are required to submit the calculation. Computation is processed in a few minutes and results are returned by email.

version and as plug-in for the molecular modeling program PyMol [4]. Selected functions are available in an online version via the internet (Fig. 1).

### 3 Algorithm

The geometrically most favorable path from the molecule void to the bulk solvent has to be found by systematic exploration of the molecule interior, to calculate the access route gorge radius. In our model, a molecule consists of hard sphere atoms with assigned van der Waals radii [1]. The molecule body is modeled on a discrete 3-D grid space. Nodes located in the interior of the molecule, *i.e.*, inside atomic van der Waals radii, and nodes located outside the molecule convex hull are eliminated and not used in further calculations. An attention is paid to nodes that lie on a boundary of the molecule's convex hull. These nodes are potential end-stops of the grid search algorithm because each boundary node can be treated as a putative outfall of the channel. The mathematical object, which is called a vertex-weighted graph, is constructed and the Dijkstra's algorithm [2] applied to identify the shortest low-cost path. Each possible path from the active site to the exterior is evaluated as a positive value. This value represents the relative cost to navigate each path in what could be described as a “highway-toll”. The cost  $C(P)$  is defined for the given path  $P$  as the sum of node-price-function values calculated for all nodes forming the path  $P$ . The single node-price-function  $c(x)$  fulfills two requirements: (i) is positive for each node and (ii) has low value for nodes that are surrounded by large empty space. The graph-searching algorithm then establishes the lowest

cost path from the active site to the external environment preferentially selecting the low-price nodes and short paths. The bottleneck of the found path can be identified, and the radius representing the largest ball that could be inscribed into the tunnel gorge can be determined. The found path can be easily visualized using graphical programs (Fig. 2). Detailed mathematical description of CAVER algorithm has been published elsewhere [1].



**Figure 2.** A tunnel connecting the active site of a protein with its exterior. Mesh represents the protein surface, star indicates the “starting point” and the balls define the tunnel. The high-resolution structure of haloalkane dehalogenase LinB from *S. paucimobilis* UT26 [5] was used in these calculations (PDB ID 1MJ5).

## 4 Examples

CAVER was primarily developed for proteins, but the algorithm is sufficiently fast and robust to allow analysis of any molecular system, including nucleic acids, inorganic materials or molecular ensembles.

### 4.1 Proteins

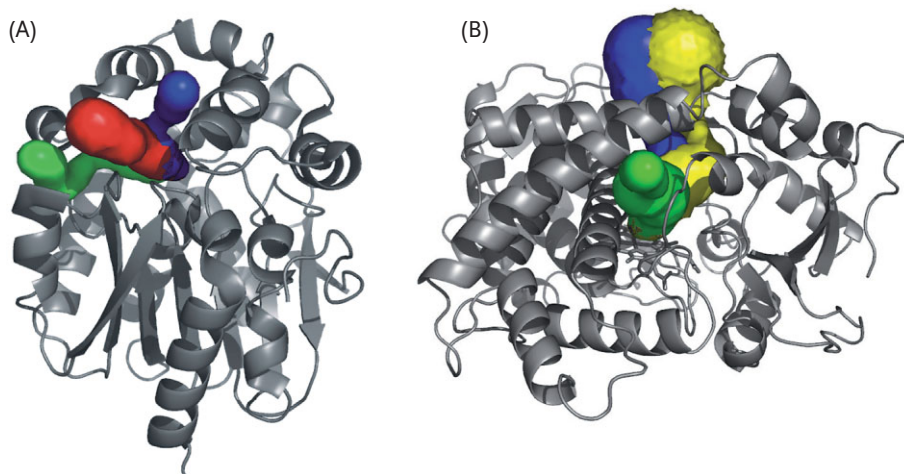
Proteins involved in catalysis of chemical reactions, *i.e.*, enzymes, find their use in a variety of biotechnological applications, including biotransformation, biocatalysis, biosensing and detoxification. Recent developments in protein engineering have provided important tools for the efficient development of enzymes with improved properties for established technical applications, and production of new enzymes tailor-made for entirely new areas of application. Here we present examples from the use of CAVER for identification of tunnels in two protein families studied in our laboratories. Enzyme haloalkane dehalogenase LinB is the 1,3,4,6-tetrachloro-1,4-cyclohexadiene halohydrilase, which is involved in the degradation of gamma-hexachlorocyclohexane in *Sphingomonas paucimobilis* UT26 [6]. LinB catalyses the cleavage of the carbon-halogen bond in more than 100 different halogenated compounds [7] by a hydrolytic mechanism leading to the formation of corresponding alcohol, a halide ion and a proton. Haloalkane dehalogenases are obvious target for the engineering projects as they can find their use in bioremediation [8], biocatalysis [9, 10], biosensors [11] and degradation of warfare agents [12, 57]. The active site of the enzyme is deeply buried in the protein core and is connected with the surrounding solvent by at least three different tunnels (Fig. 3A). Engineering of these tunnels provides the enzyme variants with significantly modified specificities and activities [13]. Computer-assisted design and engineering of the tunnels in the haloalkane dehalogenase DhaA [14] resulted in the biocatalyst with 32-

fold improved catalytic efficiency towards severe environmental pollutant 1,2,3-trichloropropane (Pavlova M. *et al.*, in preparation).

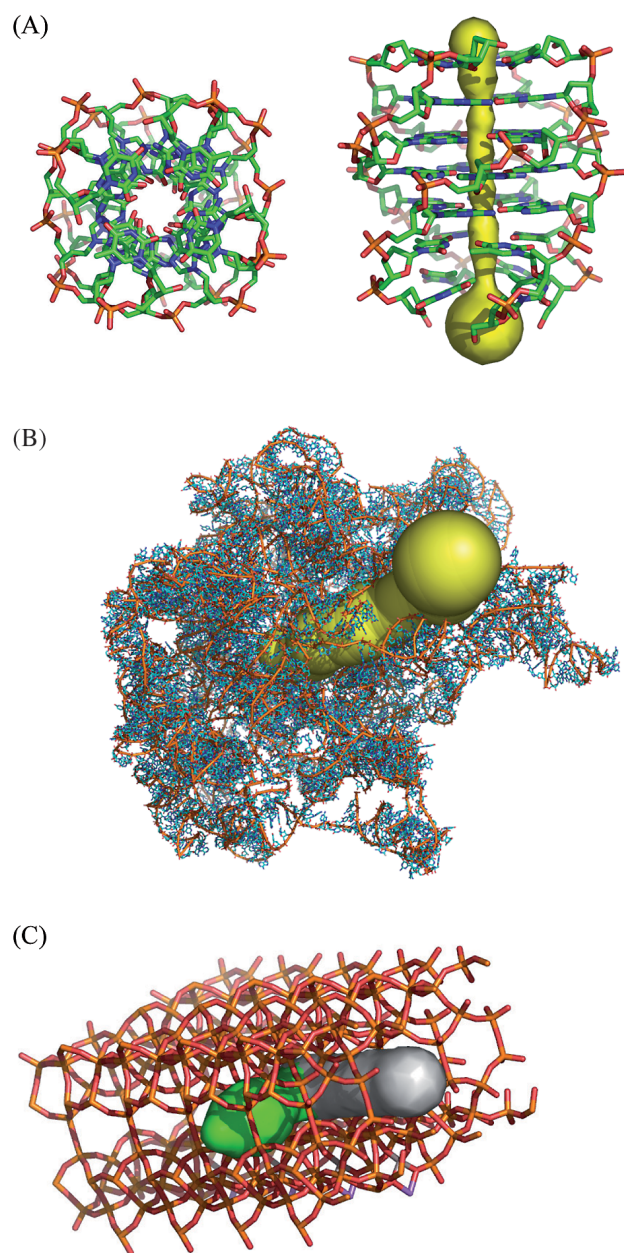
Cytochrome P450s are monooxygenases widely spread in living organisms on the Earth and they act as oxidizing agents with a broad capacity to oxidize structurally diverse substrates [16]. The high diversity of catalyzed reactions and structural variety of substrates makes cytochrome P450s versatile biocatalyst for many biotechnology processes [17]. The active site of cytochrome P450 is deeply buried in the protein interior and several access paths connect it with the protein surface [18–20]. The substrate specificity of cytochrome P450s can be determined by both active site properties and access paths size, flexibility and chemical composition [21, 22]. The CAVER has been recently applied to characterize cytochrome P450 access paths [23, 24] (Fig. 3B).

### 4.2 Nucleic acids

DNA strands preferentially form canonical Watson-Crick double helix but can also adapt a number of other structures. Among them, the quadruplex structures formed by guanine-rich nucleic acid sequences have received significant attention recently because of growing evidence for their role in important biological processes and as therapeutic targets [26–31]. G-quadruplex DNA has been suggested to regulate DNA replication and may control cellular proliferation. The quadruplex structure is stabilized by monovalent cations bound in the quadruplex central channel (Fig. 4A). The natural role and biological validation of these structures is starting to be explored. The quadruplex structures as well as other forms of DNA, holds some promise also for nanotechnologies [32–35]. Rigid DNA structures that serve as scaffolds for the organization of matter at the molecular scale, and can build simple DNA-computing devices, diagnostic machines and DNA motors.



**Figure 3.** (A) Three tunnels identified in the haloalkane dehalogenase LinB from *S. paucimobilis* UT26 [15] using CAVER (PDB ID 1CV2). (B) Three tunnels identified in the human microsomal cytochrome P450 2C9 [25] using CAVER (PDB ID 1OG2).



**Figure 4.** (A) The G-quadruplex central channel [36] identified using CAVER (PDB ID 139D). (B) The largest tunnel inside the 23S ribosomal RNA [43] identified using CAVER (PDB ID 1C2W). (C) Two pores inside the periodic structure of the ferrierite [48] calculated using CAVER.

#### 4.3 Ribonucleic acids

The functional form of single-stranded RNA molecules frequently requires a specific tertiary structure. The scaffold for this structure is provided by secondary structural elements, like hairpin loops, bulges and internal loops. Several types of RNA, such as tRNA and rRNA, contain a great deal of secondary structure, which help promote

stability. Knowledge of the geometry of tunnels (Fig. 4B) is essential for understanding of the function of rRNA molecules [37]. Most biologically active RNAs are extensively base paired to form double-stranded helices. Unlike DNA, this structure is not limited to long double-stranded helices but rather collections of short helices packed together into structures akin to proteins. In this fashion, RNAs can achieve chemical catalysis, like enzymes. This feature also makes them potential building blocks for the bottom-up fabrication of nanodevices [38]. RNA is unique in nanoscale fabrication due to its amazing diversity of function and structure. Small RNA molecules are also key elements of a machinery that trigger chromosomal modifications, post-transcriptional gene silencing and protein translational blockade and holds great potential for gene therapy [39–42].

#### 4.4 Inorganic materials

Zeolites are inorganic material with unique properties making them broadly applicable in many technology processes as molecular sieves and catalysts [44–46]. The zeolite materials are periodic structures with many inner pores. Catalytic and sorption properties are determined by their composition and structure, including the size of the pores (Fig. 4C). Inorganic composites synthesized in consideration of pore size and 3-D structure are suitable as new chromatographic carriers. Some of them can be used for the purification of proteins according to physico-chemical principles, *pI*, molecular weight and shape [47].

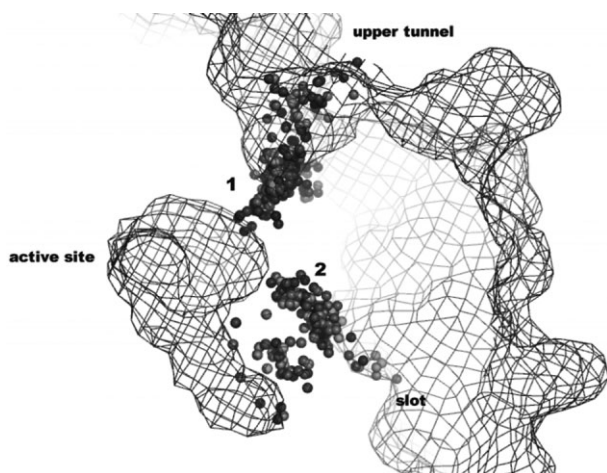
#### 4.5 Molecular ensembles

Molecular structures are not rigid but fluctuate around their mean positions and may even reshape in the presence of ligand. These motions can have significant impact on the function of biomolecules [49, 50]. Nuclear magnetic resonance and the molecular dynamics simulation are broadly used to sample thermal motions and conformational changes providing the large ensembles of structures. CAVER is sufficiently fast to allow analysis of a large number of snapshots, which is needed for effective monitoring of changes in size and geometry of tunnels during these motions (Fig. 5).

### 5 Related software

CAST [52, 53] is the program for identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules. It measures analytically the area and volume of each pocket and cavity, both in solvent-accessible surfaces and molecular surfaces. It also measures the number of mouth openings, area of the openings, circumference of mouth lips, in both type of surfaces for each pocket. The program





**Figure 5.** Population of the preferred tunnel gorges in 400 structures from 1 ns molecular dynamics simulations of the haloalkane dehalogenase DhaA from *Rhodococcus* sp. [51] analyzed using CAVER (PDB ID 1CQW). Two preferred paths with the population 64% and 36%, respectively, were assigned as the upper tunnel and the slot [14]. Molecular surface is represented by wire, tunnel gorges are represented by the balls. Intensity of color corresponds to the size of the gorge (wider is darker).

is freely available from the website <http://cast.engr.uic.edu/cast/>.

PASS [54] is a simple computational tool that uses geometry to characterize regions of buried volume in proteins and to identify positions likely to represent binding sites based upon the size, shape, and extent of burial of these volumes. The program can be used as a predictive tool for binding sites, and identification is tested by predicting known binding sites of proteins in the PDB using both complexed macromolecules and their corresponding apo-protein structures. The program is freely available from the website <http://www.ccl.net/cca/software/UNIX/pass/overview.shtml>.

PSCAN (unpublished) is a small but practical tool for finding out any potential binding site of a protein. It can picture the size and appearance of a binding site, and point out the possible pharmacophore. The program is freely available from the website <http://home.pchome.com.tw/team/gentamicin/mol/mol.htm>.

QHULL [3] is the program for calculation of the convex hull, Delaunay triangulation, Voronoi diagram, halfspace intersection about a point, furthest-site Delaunay triangulation, and furthest-site Voronoi diagram. The source code runs in two, three, four, and higher dimensions. QHULL implements the QUICKHULL algorithm for computing the convex hull. It computes volumes, surface areas, and approximations to the convex hull. The program is available from the website <http://www.qhull.org/>.

Q-SITE FINDER [55] is an energy-based method for the prediction of protein-ligand binding sites. It uses the interaction energy between the protein and a simple van der Waal's probe to locate energetically favorable binding

sites. Energetically favorable probe sites are clustered according to their spatial proximity and clusters are then ranked according to the sum of interaction energies for sites within each cluster. The program is available from the website <http://www.bioinformatics.leeds.ac.uk/qsitefinder/>.

VOIDOO [56] is the program for detection of cavities in macromolecular structures. It uses an algorithm that makes it possible to detect different types of cavities that are connected to surrounding environment. Three different types of cavity can be handled by VOIDOO: Van der Waal's cavities, probe-accessible cavities and molecular surface probe-occupied cavities. The program is available from the website <http://www.bioinformatics.leeds.ac.uk/qsitefinder/>.

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