CAVER ANALYST

ENGINEERING OF ENZYMES BY MODIFICATION OF ACCESS TUNNELS

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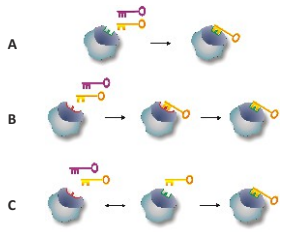
INTRODUCTION

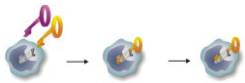
Models of enzymatic catalysis



Traditional models of enzymatic catalysis: *lock-key* model (A), *induced-fit* model (B), *selected-fit* model (C) [1,2,3]

***Keyhole-lock-key* model** – enzymes with active sites buried inside the core and connected with a surface by tunnels [4,5]

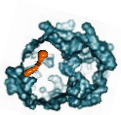
- Key = ligand
- Lock = active site
- Keyhole = **tunnel**



Size, shape, physico-chemical properties and dynamics of a tunnel are as important as fit between a ligand and the active site.

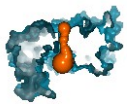
Tunnels in enzymes

cytochrome CYP3A4
EC: 1.14.14.1



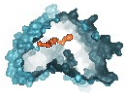
undecaprenyl pyrophosphate synthase EC: 2.5.1.31

haloalkane dehalogenase DhaA
EC: 3.8.1.5



chondroitinase
EC: 4.2.2.5

squalene-hopene cyclase
EC: 5.4.99.17

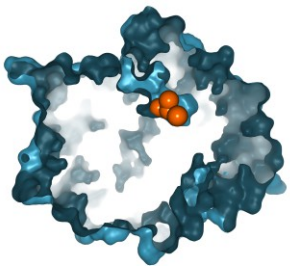


asparagine synthetase
EC: 6.3.1.1

Tunnels are present in all six classes of enzymes.

Engineering implications

Recognition of the substrate by the enzyme with buried active site can be seen as a two-step process: (i) passage of the substrate via the tunnel and (ii) fit to the active site [5].



Targeting tunnels by site-directed or saturation mutagenesis has wide practical applications in protein engineering.

Altering the size, physico-chemical properties or dynamics of tunnels can lead to significant changes in activity, specificity, stereoselectivity and stability.

Compared to mutations in the active site, engineering of tunnel residues provide higher chances of obtaining functional variants.

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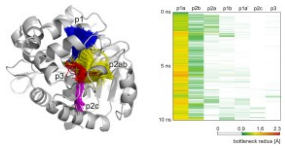
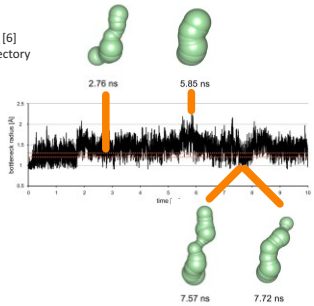
CAVER Analyst - Tunnel computation

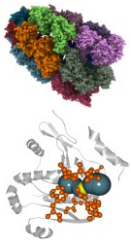
- computation of all tunnels by state-of-the-art algorithms [6]
- clustering of tunnels throughout molecular dynamic trajectory
- analysis of tunnel properties
  - average radius, length, curvature
  - maximum and average bottleneck
  - residues lining given tunnel and its bottleneck
  - tunnel profiles, heat plots

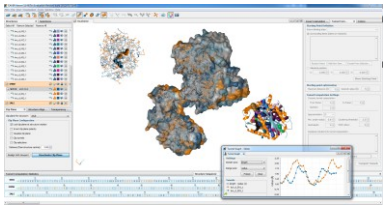


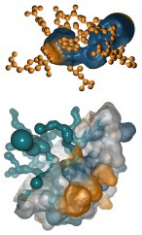


CAVER Analyst - Tunnel visualization

Software for the analysis of tunnels in static and dynamic protein structures employing **CAVER 3.0 algorithms** [6].



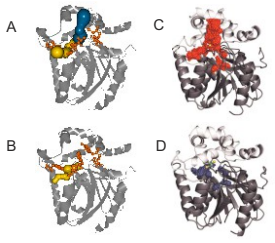




CASE STUDIES

Engineering enzyme activity

Redesigning access tunnels of the haloalkane dehalogenase DhaA provides 32-fold increase in activity with toxic pollutant 1,2,3-trichloropropane (TCP). The results demonstrate the power of combining rational design with directed evolution focused to the access tunnels [9].

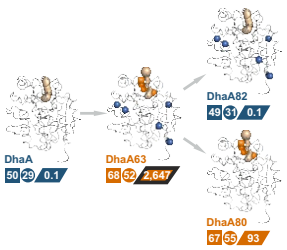


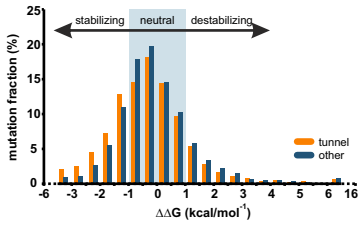
Mutations	Method	Effect	Library size	Reference
C176Y + Y273F	random mutagenesis	3.5-times higher activity with TCP	10 000 clones	[7]
G3D + C176F	random mutagenesis	4.0-times higher activity with TCP	10 000 clones	[8]
I135F + C176Y +V245F + L246I + Y273F	focused mutagenesis	32-times higher activity with TCP	5 000 clones	[9]
K175M + C176G + Y273L	combinatorial mutagenesis	10 000-times higher binding rate of fluorescent probe	not reported	[10]

The active site of wild type DhaA (A) is an occluded cavity with two major access tunnels (main tunnel - blue, slot tunnel - yellow). Mutants carrying substitutions in the main tunnel (C176Y/F) show higher activity towards TCP. The mutant DhaA31 (B) has narrowed also the slot tunnel (mutated positions - orange). Water molecules in the active compete with TCP and prevent formation of the activated complex (C). Narrowing both main and slot tunnels shielded the active site from bulk solvent and stabilized the activated complex (D).

Engineering enzyme stability

Modification of residues lining the access tunnel of the haloalkane dehalogenase DhaA increased its melting temperature by 19°C and resistance to co-solvent DMSO 4000-fold. Mutations in the tunnel improved structural and kinetic stability, while the surface mutations did not contribute to protein stabilization [11].





Thermostability, structural resistance to DMSO and kinetic stability in 40% (v/v) DMSO were quantified by the melting temperature (square), half-concentration (circle) and the half-life (rhombus). Orange color signifies substantial improvement, blue color no improvement.

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

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