New Concepts and Software Tools for Rational Design of Enzymes

Department of Experimental Biology
Masaryk University, Czech Republic
Location

Brno
Moravian Karst
Outline

- Software development
- Engineering stability

- Theoretical framework
- Engineering activity
Theoretical framework
Lock-key model

Internet sources
Keyhole-lock-key model

Software tools
Software CAVER

Petrek et al., BMC Bioinformatics 7: 316 2006
Tunnels and channels in proteins

- NaK channel
  PDB ID 3E8G

- Candida rugosa lipase
  PDB ID 1CRL

- [NiFeSe]-hydrogenase
  PDB ID 4KL8

- Carbamoyl phosphate synthetase
  PDB ID 1A9X
Software CAVER 3.0
Software CAVER 3.0

CAVER - software tool for protein analysis and visualisation

CAVER is a software tool for analysis and visualisation of tunnels and channels in protein structures. Tunnels are void pathways leading from a cavity, buried in a protein core, to the surrounding solvent. Unlike tunnels, channels lead through the protein structure and their both endings are opened to the surrounding solvent. Studying these pathways is highly important for drug design and molecular enzymology.

www.caver.cz

Chovancova E. et al., PLOS Comp. Biol. 8: e1002708 2012
Kozlikova B. et al., Bioinformatics 30: 2684-2685 2014
Related software

- Pore Walker
- MolAxis
- Caver
- HOLLOW
- MOLE
- Voss Volume Voxelator

Software HOTSPOT WIZARD

loschmidt.chemi.muni.cz/hotspotwizard/
Engineering activity
Genotoxic and persistent 1,2,3-trichloropropane
### Directed evolution

<table>
<thead>
<tr>
<th>DhaA</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type (wt)</td>
<td>1</td>
</tr>
<tr>
<td>C176Y+Y273F $^1$</td>
<td>3.5</td>
</tr>
<tr>
<td>G3D+C176F $^2$</td>
<td>4</td>
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Direct evolution

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Analysis of tunnels by CAVER

- **Main tunnel**
- **Side tunnel**
Analysis of main tunnel by molecular dynamics

Time: 00 ps
Analysis of side tunnel by molecular dynamics

Time: 00 ps
Rational design of side tunnel by HOTSPOT WIZARD

Main tunnel:
- C176Y

Side tunnel:
- W141X
- I135X
- L245X
- V246X
Focused directed evolution of side tunnel

- Site-directed mutagenesis
- Saturated mutagenesis

<table>
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<tr>
<th>Mutant</th>
<th>C176Y</th>
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<th>I135X</th>
<th>V245X</th>
<th>L246X</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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<td>G</td>
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</table>
Kinetic characterization

Protein variant: wt, Bosma, Gray, DhaA21, DhaA27, DhaA31

$k_{cat}/K_m$ [mM$^{-1}$·s$^{-1}$]

- wt: 0
- Bosma: 0.26
- Gray: 0.26
- DhaA21: 0.4
- DhaA27: 1
- DhaA31: 26x

Protein variant
Rate-limiting step by transient kinetics

Chemical reaction:

\[ E + R-Cl \rightleftharpoons E.R-Cl \quad k_1 \]
\[ E.R-Cl \rightleftharpoons E-R.Cl^- \quad k_2 \]

Product release:

\[ E.Cl^-\cdot ROH \rightleftharpoons E + Cl^- + ROH \quad k_4 \]
Active site solvation by molecular dynamics

Pavlova et al., Nat. Chem. Biol. 5: 727 2009
Engineering stability
Directed evolution towards stability in DMSO

positive control

negative control

0 % 10 % 20 % 30 % 40 % 50 % 55 % 60 %

7,000
Variants with increased stability in DMSO

Protein variant

Residual activity (%)

A172V

wt

DhaA57

DhaA60

DhaA61

Protein variant
A172 located in tunnel
A172 located in tunnel

A172V
Termostability of variants with increased stability in DMSO

The image shows a protein structure with highlighted residues A172V and L95V. The graphs depict the residual activity (%) and $T_m$ (°C) for wild type (wt) and variants DhaA57, DhaA60, and DhaA61.
Thermostable variant

Gene Site Saturation Mutagen.
Library size 30,000
T_{1/2} (55°C) = 35 h -> 639 h


Gray K. and co-workers
Thermostable variant

![Thermostable variant protein structure](image)

- **Residual activity (%)**
  - wt
  - DhaA63
  - DhaA80
  - DhaA82

- **$T_m$ (°C)**
  - wt
  - DhaA63
  - DhaA80
  - DhaA82
Structural analysis

wt    DhaA57    DhaA80

Random mutagenesis 7,000 clones

Saturation mutagenesis 30,000 clones

Saturation mutagenesis 400 clones
Damborsky J. et al., Patent US 8,580,932
Conclusions

- Tunnels are important determinants of catalytic properties of enzymes with buried active sites.

- CAVER and HOTSPOT WIZARD are software tools for analysis and design of protein tunnels.

- Engineering of tunnels provides enzymes with modified activities, stabilities and specificities.
Loschmidt Laboratories
Funding

- Czech Ministry of Education
- Czech Grant Agency
- Grant Agency of Academy of Sciences
- North Atlantic Treaty Organization
- EMBO and HHMI
Software tools

Caver 3.0
loschmidt.chemi.muni.cz/caver

Hotspot Wizard 1.7
loschmidt.chemi.muni.cz/hotspotwizard

PredictSNP 1.0
loschmidt.chemi.muni.cz/predictsnp