Strategies and software tools for engineering protein tunnels and dynamical gates

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Web-based software tools
enzyme engineering

Department of Experimental Biology
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Framework
From concepts to web tools
Solvent exposed *versus* burried active sites

Software tools
Software CAVER
analysis of protein tunnels and channels

Petrek et al. BMC Bioinformatics 7: 316 2006
Software analysis of dynamical protein tunnels and channels
Software analysis of dynamical protein tunnels and channels
Software analysis of ligand transport via protein tunnels
Software analysis of ligand transport via protein tunnels

Filipovic J. et al. IEEE/ACM in press 2019
Vavra O. et al. Bioinformatics 1: btz386 2019
Software

Hot Spot Wizard

design of safe mutations and smart libraries
Amgen, AstraZeneca, Biomin, Novozymes, Codexis, Genentech, GSK Pharma, EngIZyMe, Danisco, Novo Nordisk, Contipro Pharma, FEI, Genia, Enzymicals, C-Lecta, FluidOps, BRAIN, Asaclean, Amano Enzyme, Mitsubishi Chemical Holdings, Ingenza, Axis Shield, Amicogen, Clariant, Verdezyne, Pfizer

Prediction from sequence
Stability analysis of mutations

17,000 jobs

De novo tunnel design
Main tunnel closure

LinB-C

D147C
L177C
### Substrate Specificity

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Specific Activity [µmol.s(^{-1}).mg(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>LinB(^{-})C</td>
<td>0.00</td>
</tr>
<tr>
<td>LinB(^{-})C</td>
<td>0.05</td>
</tr>
<tr>
<td>LinB(^{-})C</td>
<td>0.10</td>
</tr>
<tr>
<td>LinB(^{-})C</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**Diagram:**
- **X-axis:** Substrates
- **Y-axis:** Specific activity [µmol.s\(^{-1}\).mg\(^{-1}\)]
- **Legend:**
  - wt
  - LinB\(^{-}\)C

**Plot Description:**
- The graph shows the specific activity of substrates under two conditions: wt and LinB\(^{-}\)C.
- The y-axis represents the specific activity in units of [µmol.s\(^{-1}\).mg\(^{-1}\)].
- The x-axis lists different substrates.
- The graph includes a scatter plot with substrate labels on the right side, indicating their corresponding specific activities.
Tunnels distribution by CAVER

LinB-C oxidative

LinB-C reductive
Tunnels distribution by CAVER

LinB-C oxidative

LinB-C reductive

0.0       0.5       1.0       1.5       2.0      2.5 Å
0          150       300 ns

p1
p2
p3

p1
p2
p3

Arrows indicate the direction of the tunnel distribution.

The color scale represents the tunnel distribution with darker colors indicating a higher density of tunnels.

The graph shows the distribution of tunnels for different protein regions (p1, p2, p3) for oxidative and reductive conditions.
Tunnel analysis by CAVER
Focused directed evolution by Hotspot Wizard

LinB-C

4.160
Focused directed evolution to open *de novo* tunnel

F143L
F143V
I211V
W140L+F143L
W140L+I211L
F143Y+I211L
F143V+I211L
F143L+I211L
W140L+F143V+I211L
W140L+F143Y+I211L
**W140A+F143L+I211L**
W140T+F143L+I211L

LinB-O
Tunnels distribution by CAVER

LinB-C

LinB-O
Disulfide bridge replaced by bulky tryptophan

Brezovsky J. *et al.* ACS Catalysis 6: 7597 2016
Tunnel functionality
Crystallographic analysis

wt

LinB-C

LinB-O

0.7 Å

0.6 Å

1.0 Å
Multiple molecular dynamics

LinB-O

LinB-O

LinB-O
Analysis of ligand transport by CaverDock

Vavra O. et al. Bioinformatics 1: btz386 2019
Conclusions

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

- **Caver, CaverDock, CaverWeb and Hotspot Wizard** are software tools for rational design proteins.

- Engineering of **gated tunnels** provides enzymes with modified activities and specificities.
Loschmidt Laboratories
Collaborators & Funding

- David Baker – University Washington, USA
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- Florian Hollfelder – University Cambridge, UK
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- Barbora Kozlikova, Jiri Filipovic – Masaryk University, CZ
- Dieter Moll, Markus Aleschko, Sebastian Fruhauf – Biomin, A
- Pablo Nickel – Biosustain, DK
<table>
<thead>
<tr>
<th>Software tools</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caver 3.0, CaverAnalyst 2.0</td>
<td>analysis of protein tunnels and channels</td>
</tr>
<tr>
<td>CaverDock 1.0</td>
<td>energetic analysis of passage of ligands</td>
</tr>
<tr>
<td>Hotspot Wizard 3.0</td>
<td>safe mutagenesis and smart libraries</td>
</tr>
<tr>
<td>FireProt 1.0</td>
<td>design of thermostabilizing mutations</td>
</tr>
<tr>
<td>EnzymeMiner 1.0</td>
<td>mining of novel enzymes in genetic databases</td>
</tr>
<tr>
<td>SoluProt 1.0</td>
<td>prediction of protein solubility</td>
</tr>
</tbody>
</table>

http://loschmidt.chemi.muni.cz/software