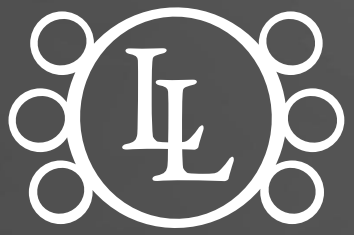


LOSCHMIDT
LABORATORIES

Engineering of enzymes for biotechnological applications

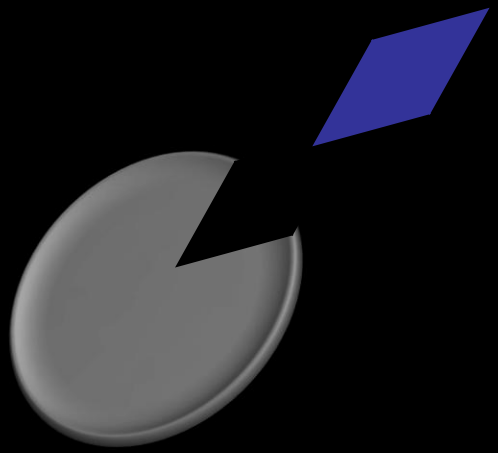
Department of Experimental Biology and Research Centre for Toxic Compounds in the Environment

Masaryk University, Czech Republic

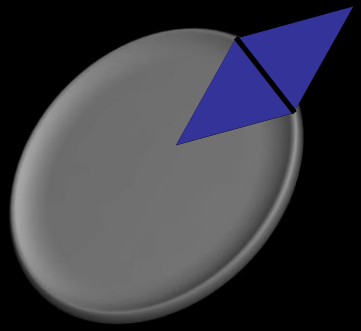
Outline

- Enzymes
- Models
- Software tool
- Engineering activity
- Engineering stability
- Conclusions

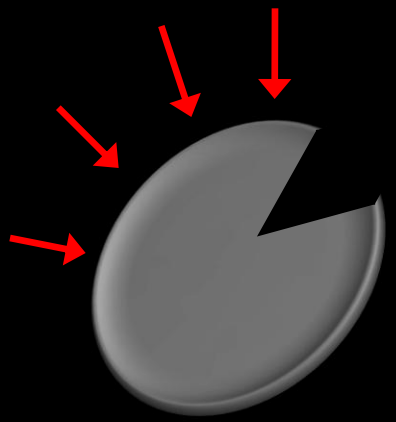
Enzymes



specificity



activity



stability

Enzymes

Food

Medicine

Environment

Household

Agriculture

Textile

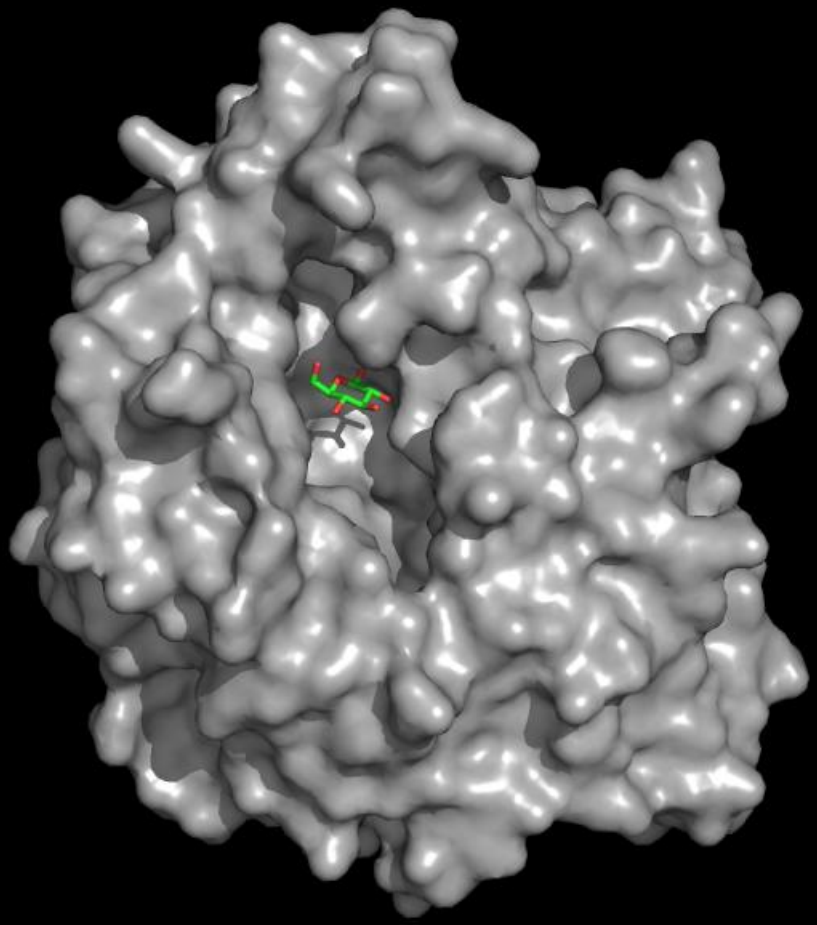
Chemistry

Model lock and key

„To use a picture, I would like to say that enzyme and glucoside have to fit to each other like a lock and key in order to exert a chemical effect on each other."

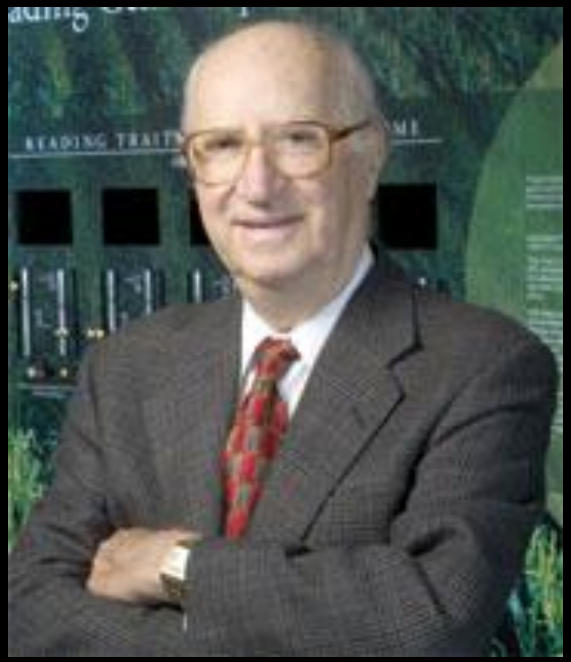
E. Fischer, 1894

Model lock and key



Isorna *et al.*, J. Mol. Biol. 371: 1204 2007

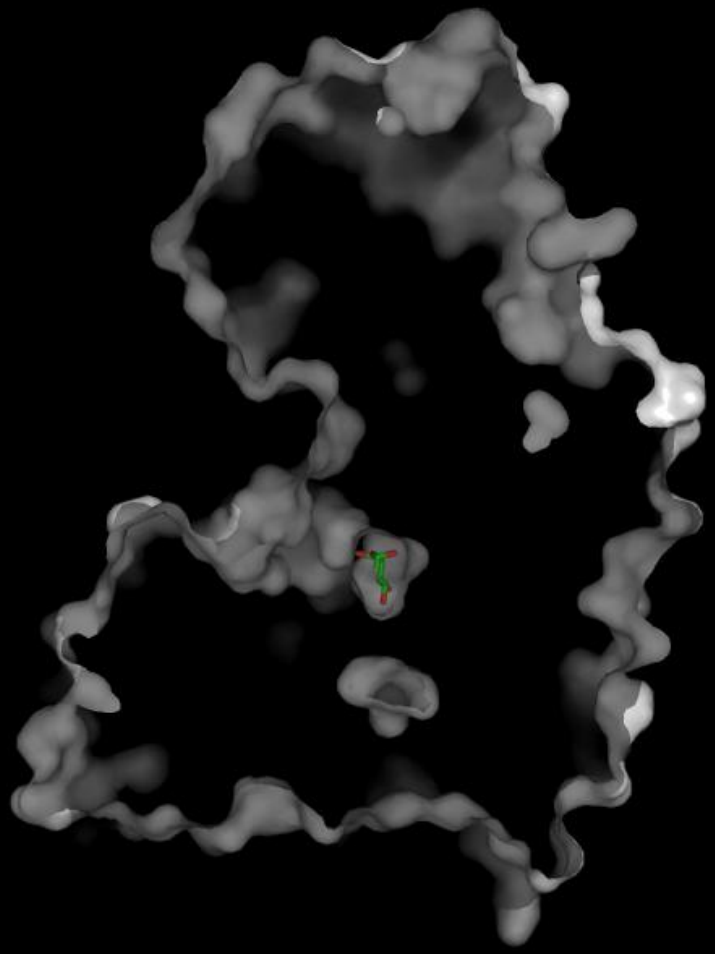
Model induced fit



„The interaction of an enzyme with its substrate resembles the fit of a hand in a glove, with a moderately flexible enzyme (the glove) fitting a moderately flexible substrate (the hand).”

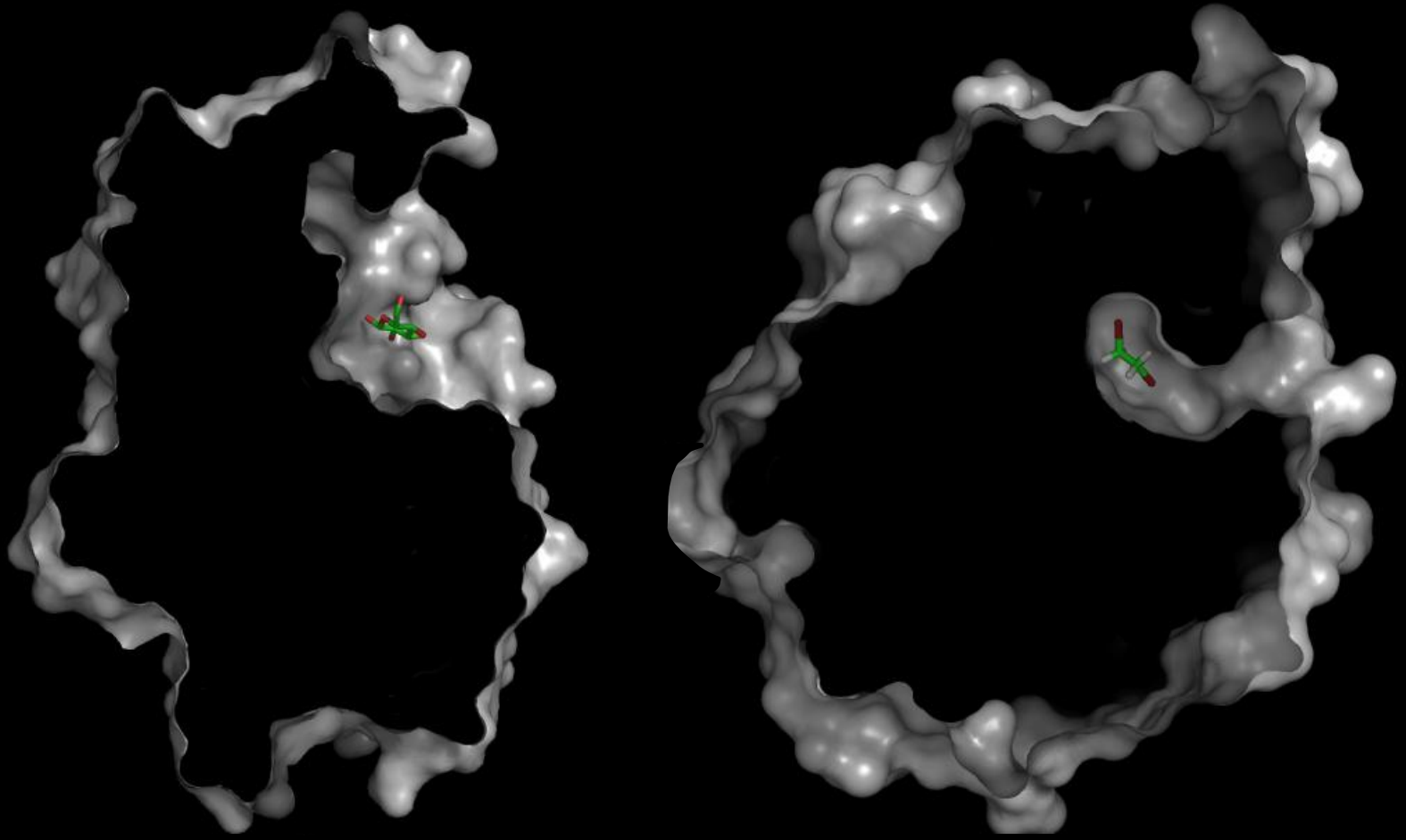
D. Koshland, 1959

Model induced fit

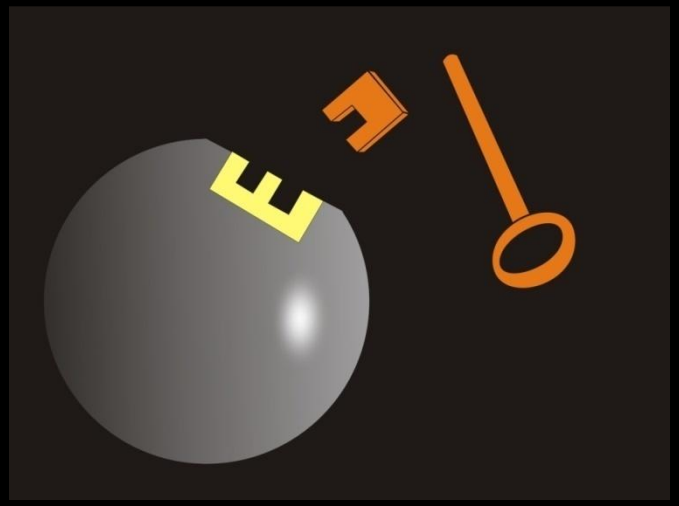


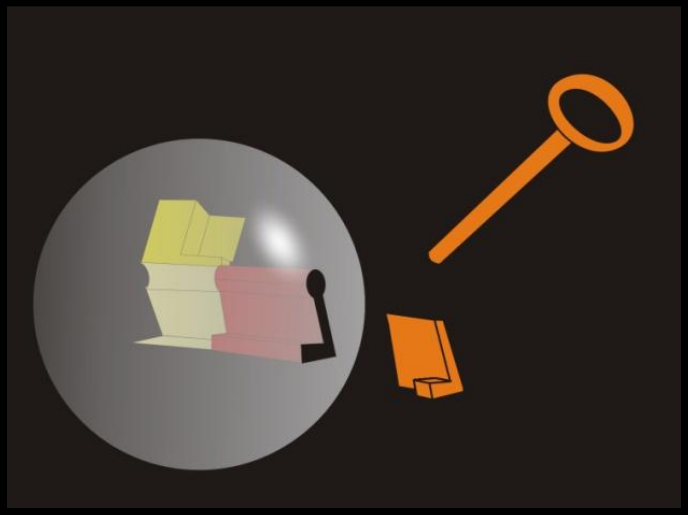
Mulichak *et al.*, Nat. Struct. Biol. 5: 555 1998

Model lock and key



Model keyhole, lock and key



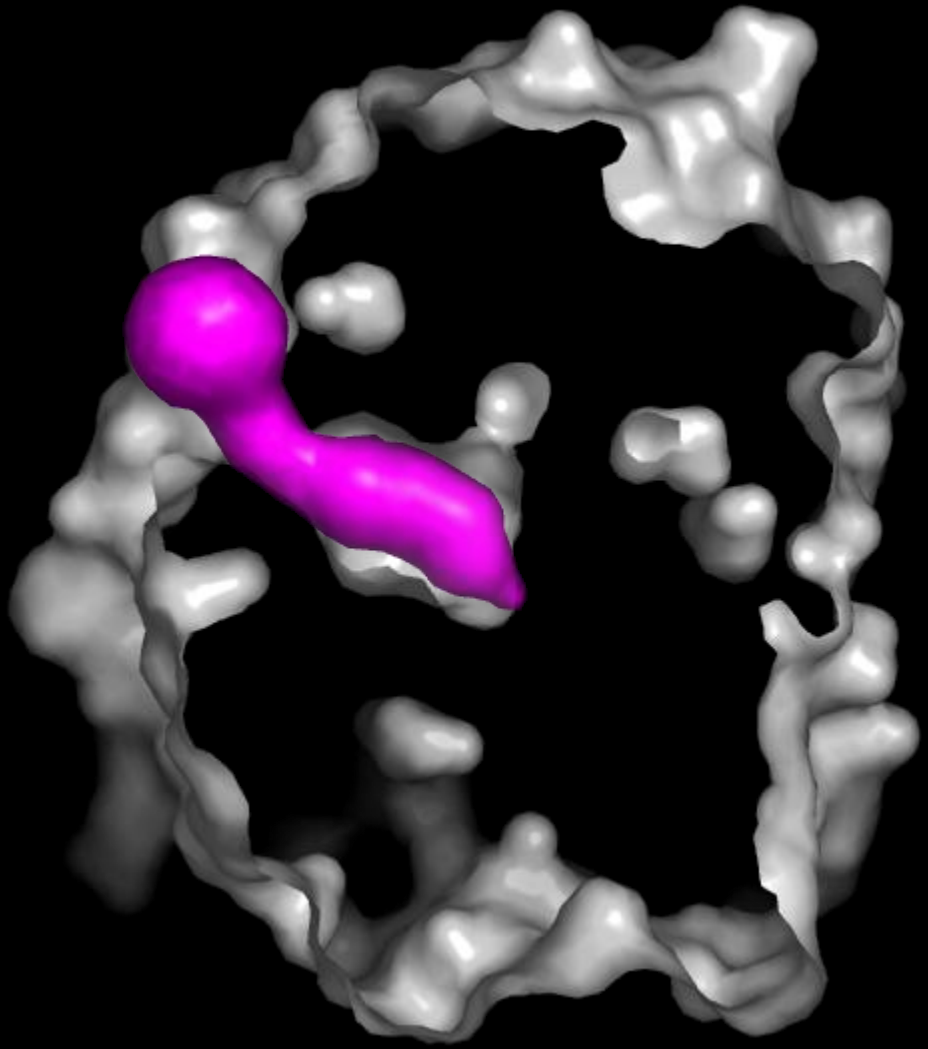


"Size, shape, physico chemical properties and dynamics of access tunnels (keyholes) are as important for catalytic function of enzymes with buried active sites as the match between the substrate and the active site."

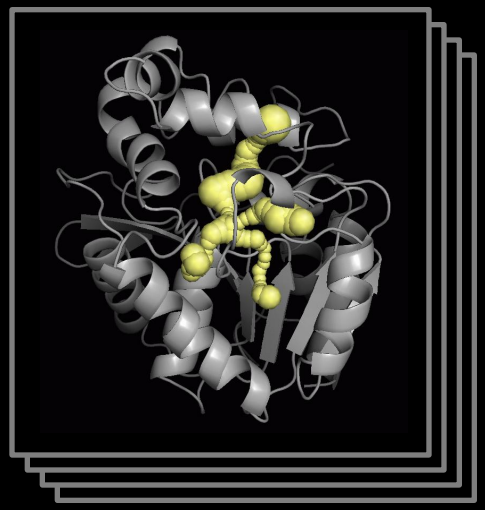
Software tool

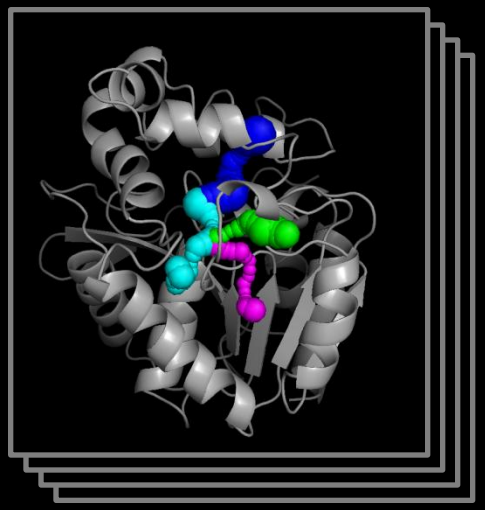
- calculation of tunnels and channels in proteins and nucleic acids
- algorithm based on Voronoi diagram
- fully automated
- 4.650 registered users
- 11.507 downloads

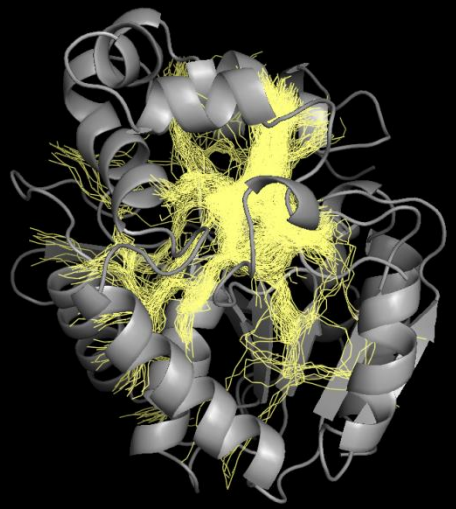
Software CAVER

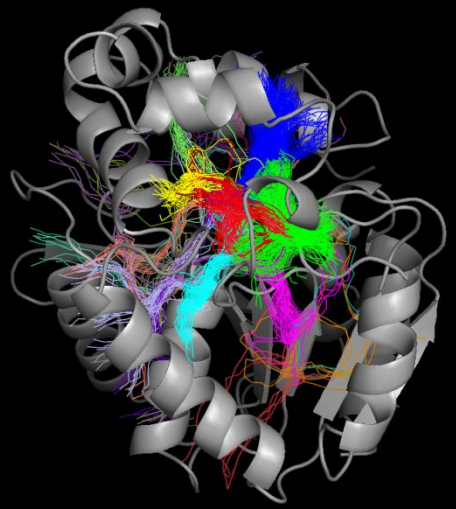


Software CAVER 3.0

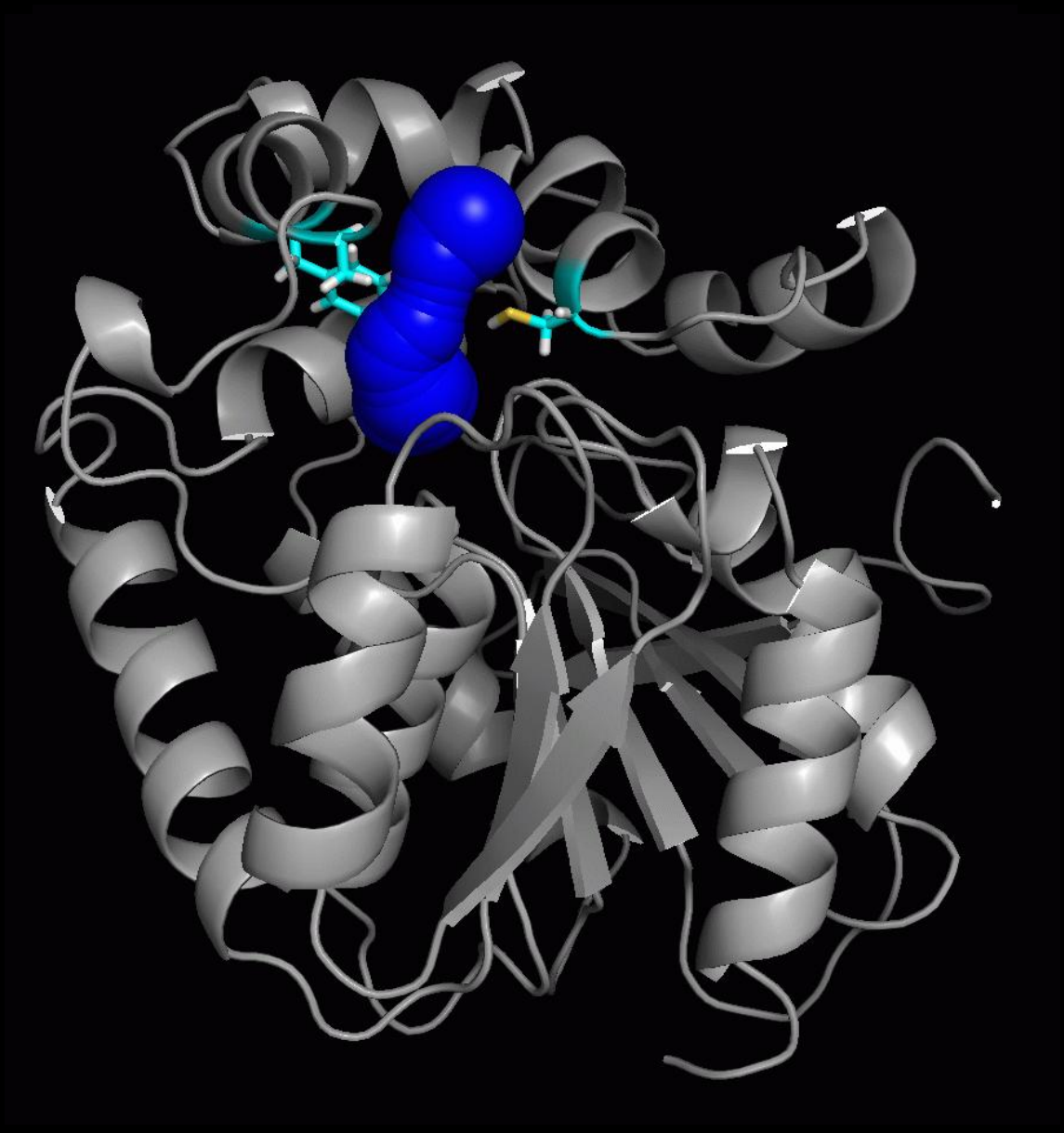








Software CAVER 3.0



Software CAVER 3.0

Chovancova *et al.* Plos Comp. Biol. 8: e1002708 2012

Engineering activity

- haloalkane dehalogenase with enhanced activity towards 1,2,3-trichloropropane (TCP)
- design of sites for mutagenesis by CAVER
- site-directed and saturated mutagenesis
- transient kinetics and molecular dynamics
- engineering of tunnels provided 32x more active and 26x more efficient enzyme

Directed evolution

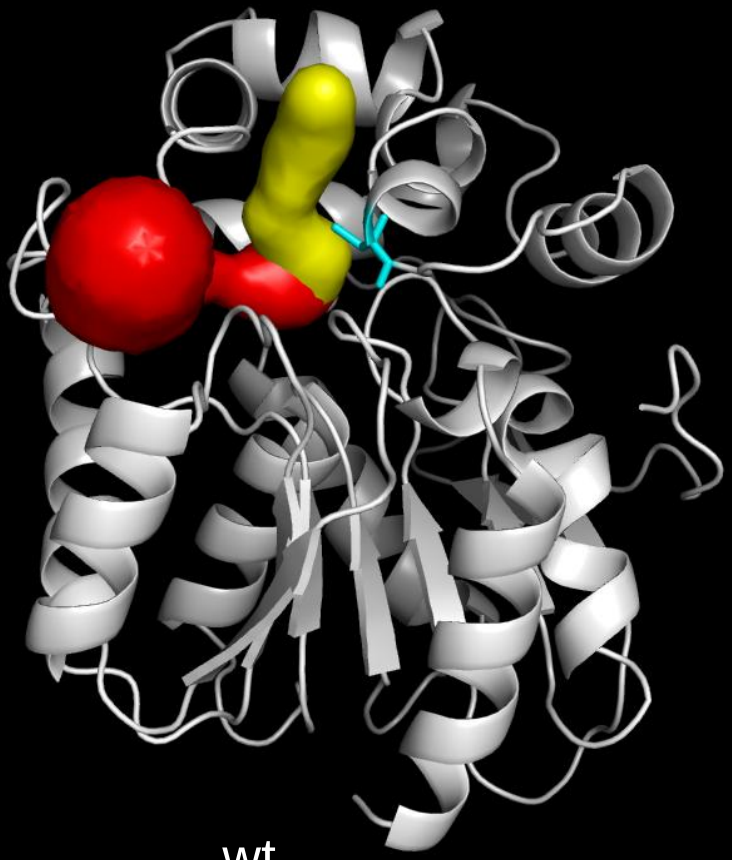
DhaA	Relative activity
wt	1
C176Y+Y273F [1]	3.5
G3D+C176F [2]	4

[1] Bosma *et al. Appl. Environ. Microbiol.* 68: 3582 2002

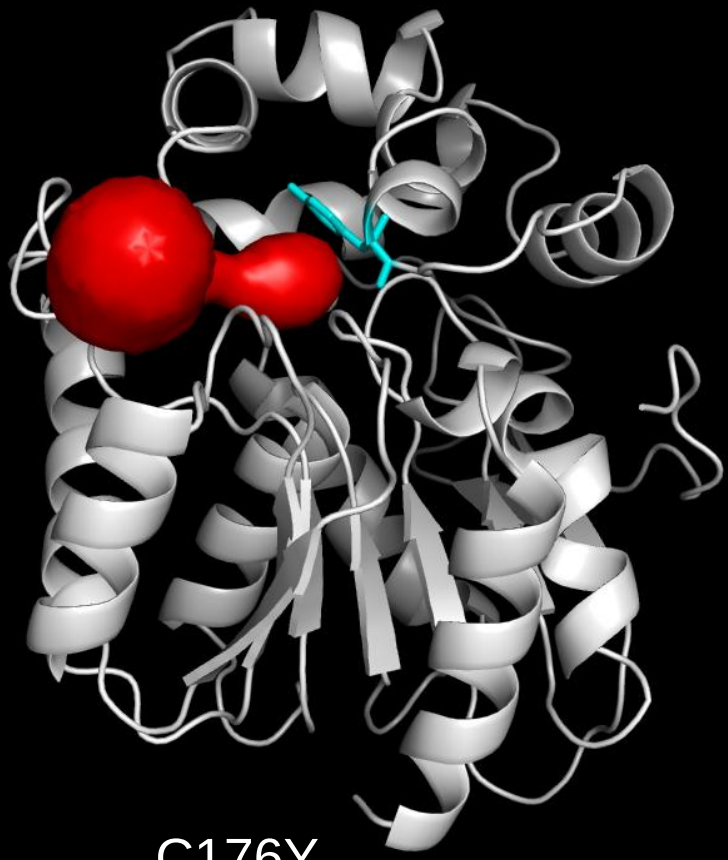
[2] Gray *et al. Adv. Synth. Catal.* 343: 607 2001

Analysis by CAVER

- Main tunnel
- Side tunnel



wt



C176Y

Banas P. *et al.*, J. Comp. Aid.-Mol. Des. 20: 375 2006

Rational design of tunnels

Main tunnel:

- C176Y

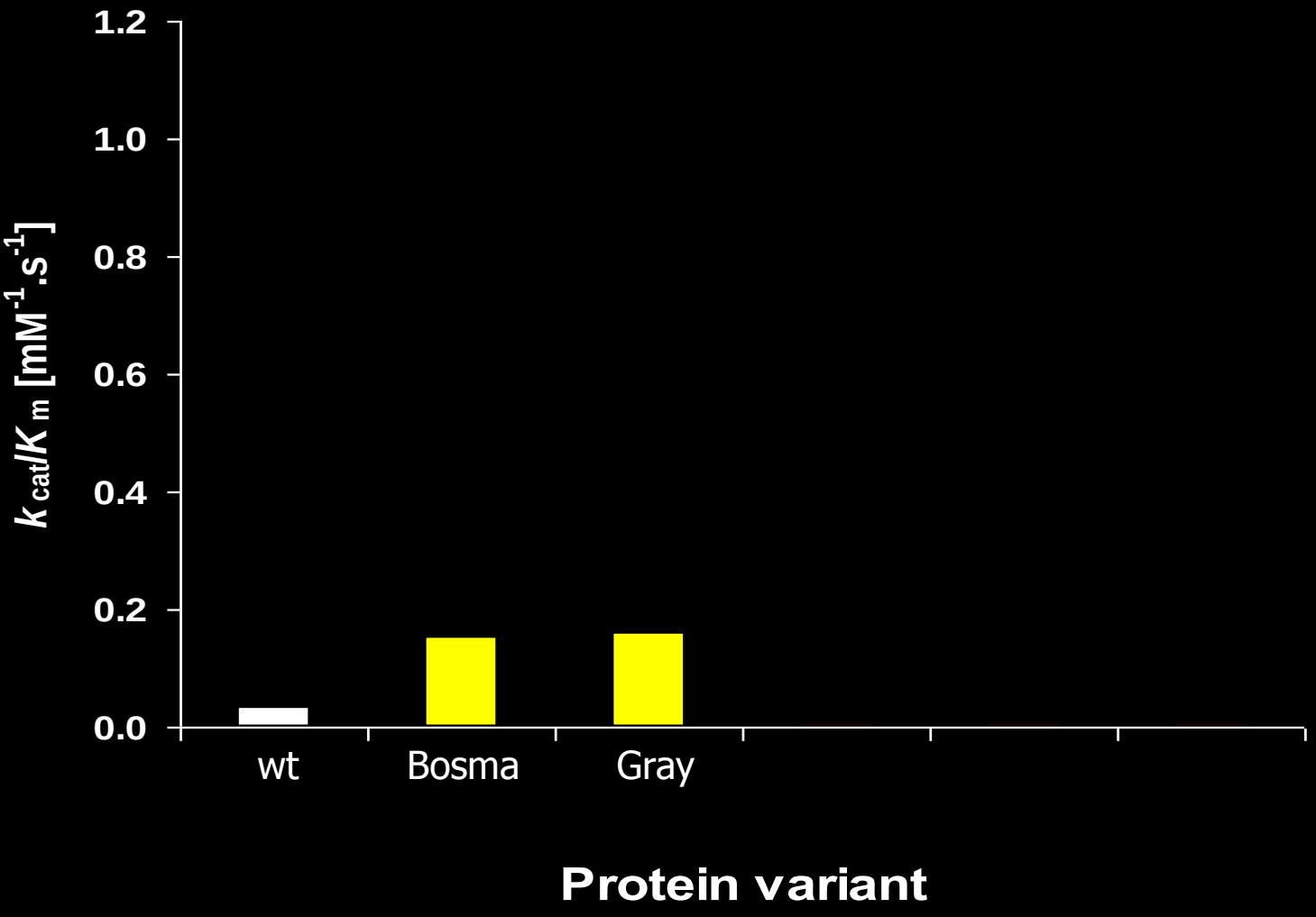
Side tunnel:

- W141X
- I135X
- L245X
- V246X

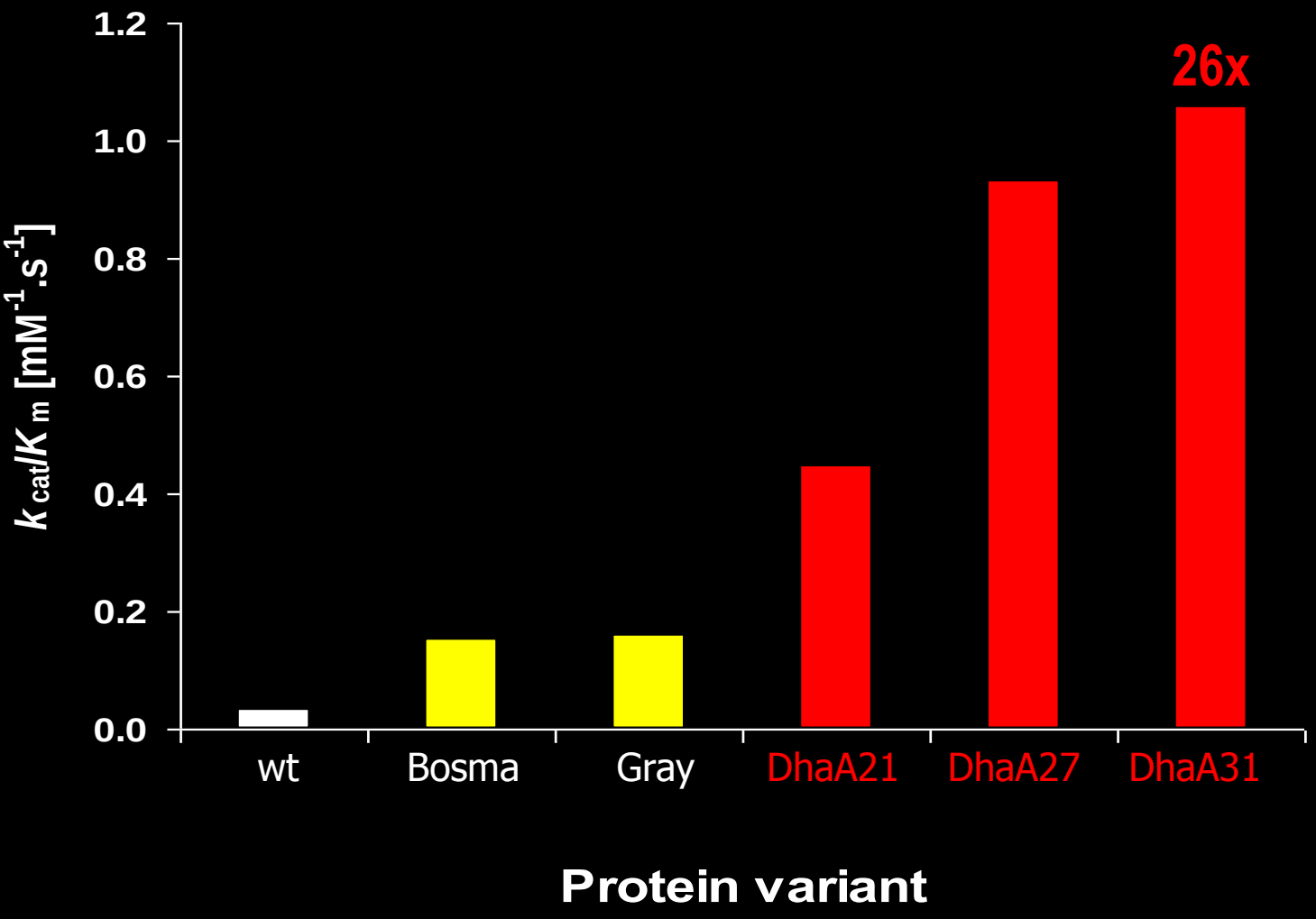
Focused directed evolution of side tunnel

- Site-directed mutagenesis
- Saturated mutagenesis

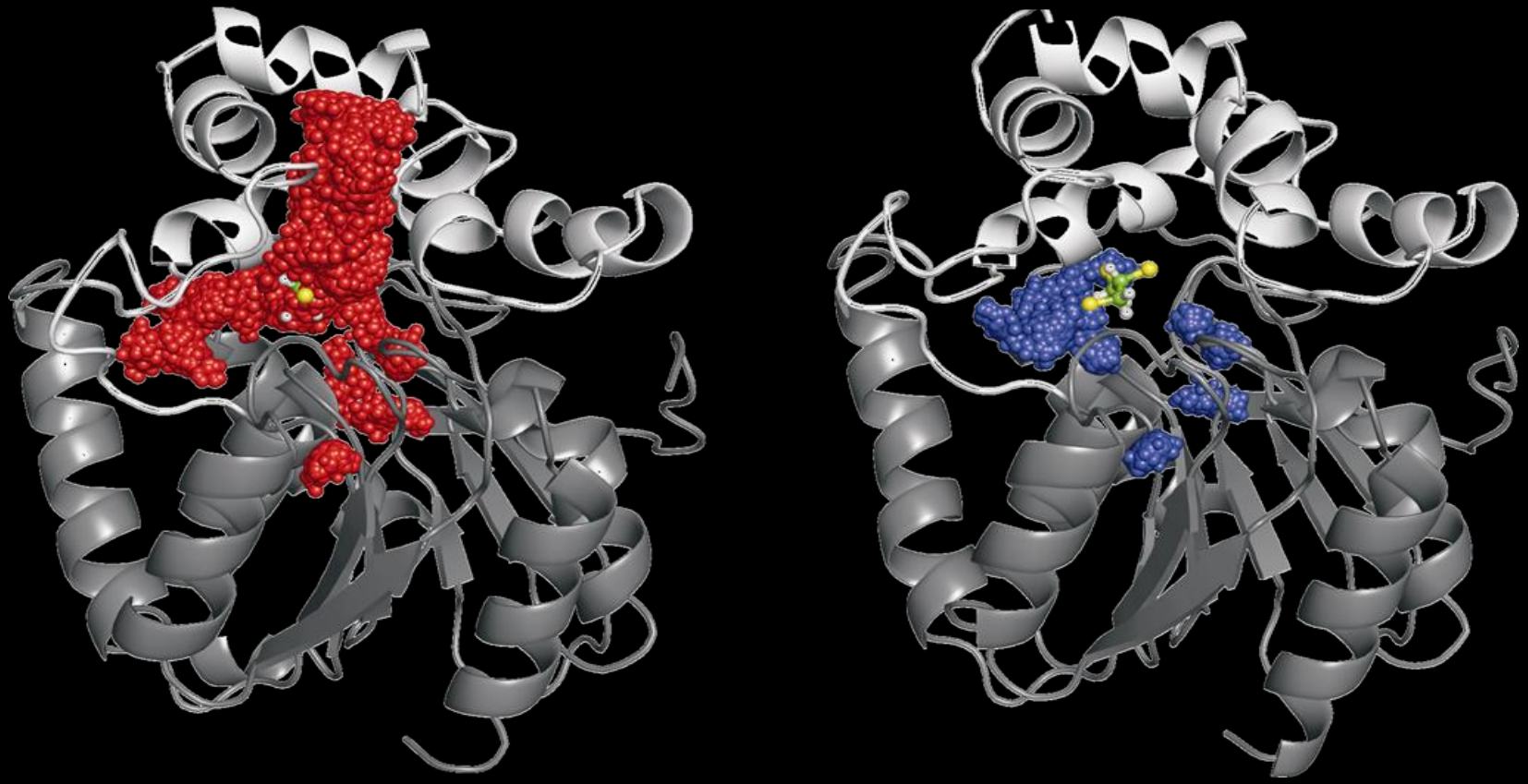
Catalytic efficiency of constructs



Catalytic efficiency of constructs



Solvation of active site by molecular dynamics

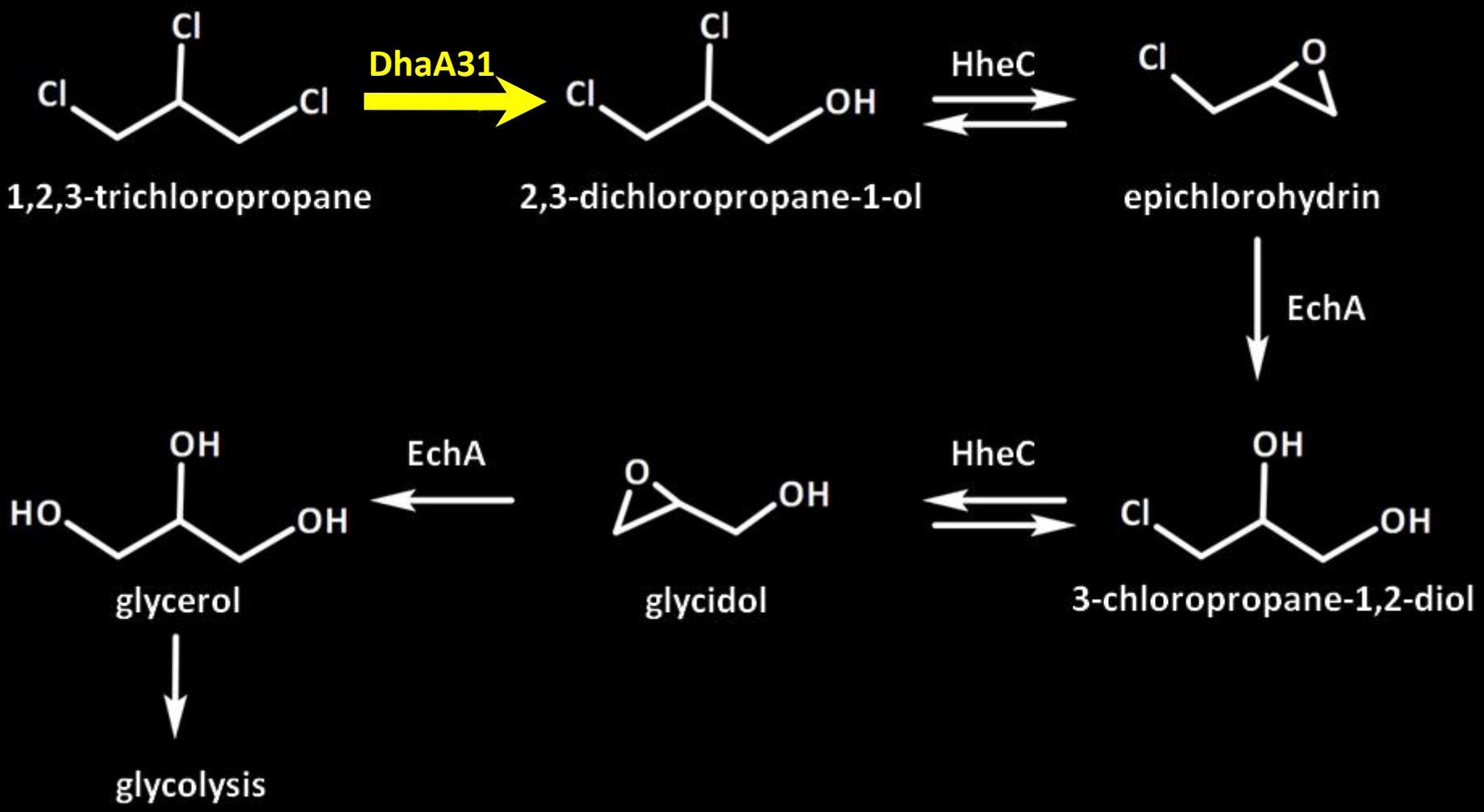


wt

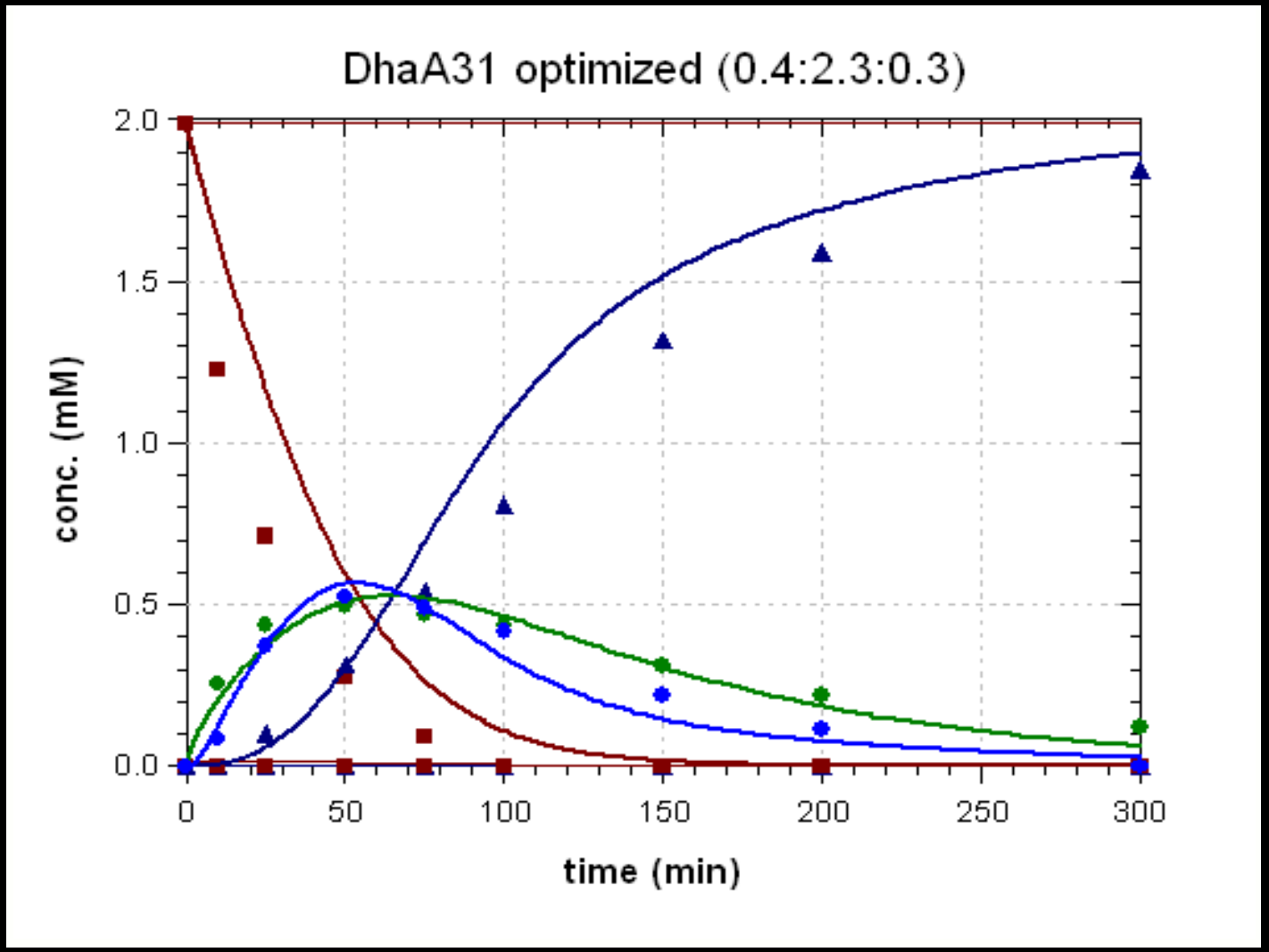
DhaA31

Pavlova *et al.*, Nat. Chem. Biol. 5: 727 2009

Proposed biodegradation pathway



Optimization enzyme stochiometry



Laboratory scale biodegradation experiment

8 liters

10 mM TCP

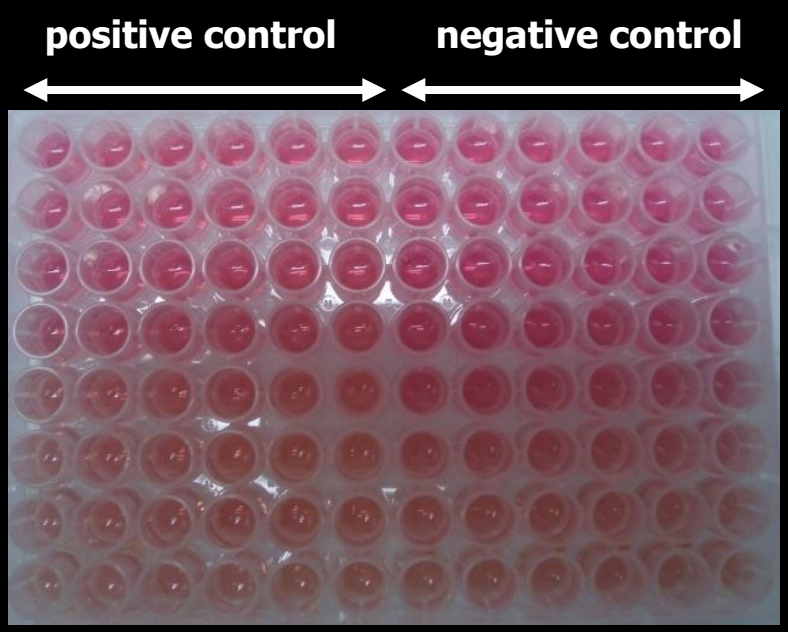
90% efficiency

>7 weeks

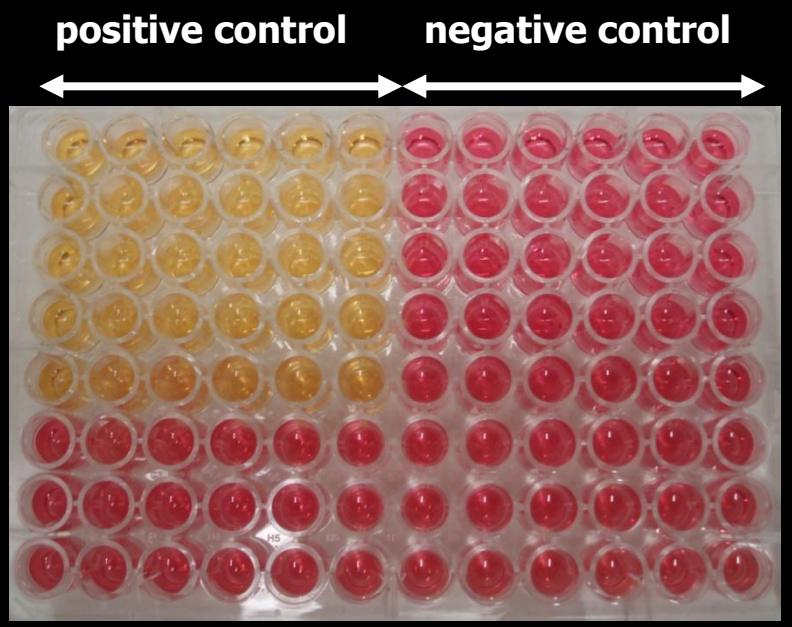
Engineering stability

- haloalkane dehalogenase stable in DMSO
- random and site-directed mutagenesis
- activity with 1,2-dibromoethane in 40% DMSO
- modification of tunnel provided enzyme with 270x higher residual activity, 4000x longer half-life, thermostability 19^{o}C higher

Directed evolution towards stability in DMSO



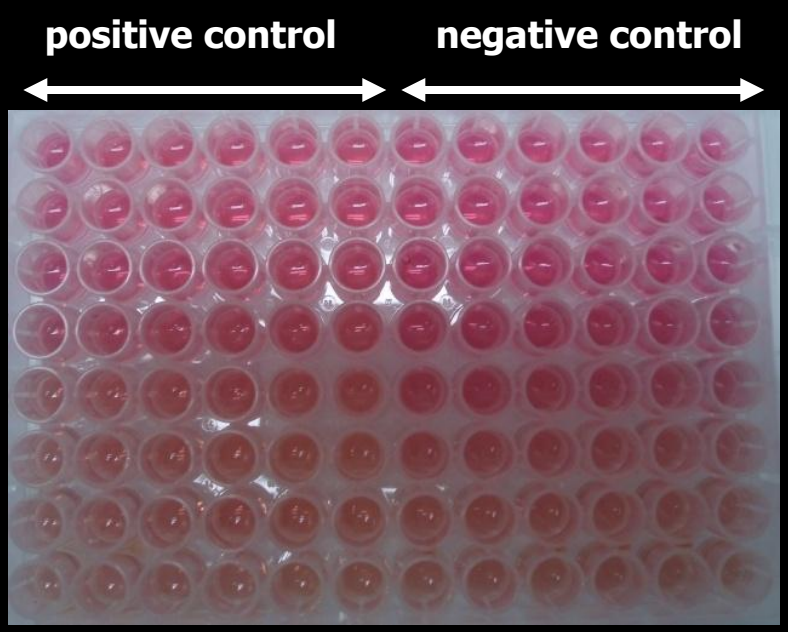
0 %
10 %
20 %
30 %
40 %
50 %
55 %
60 %



before optimization

after optimization

Directed evolution towards stability in DMSO



0 %
10 %
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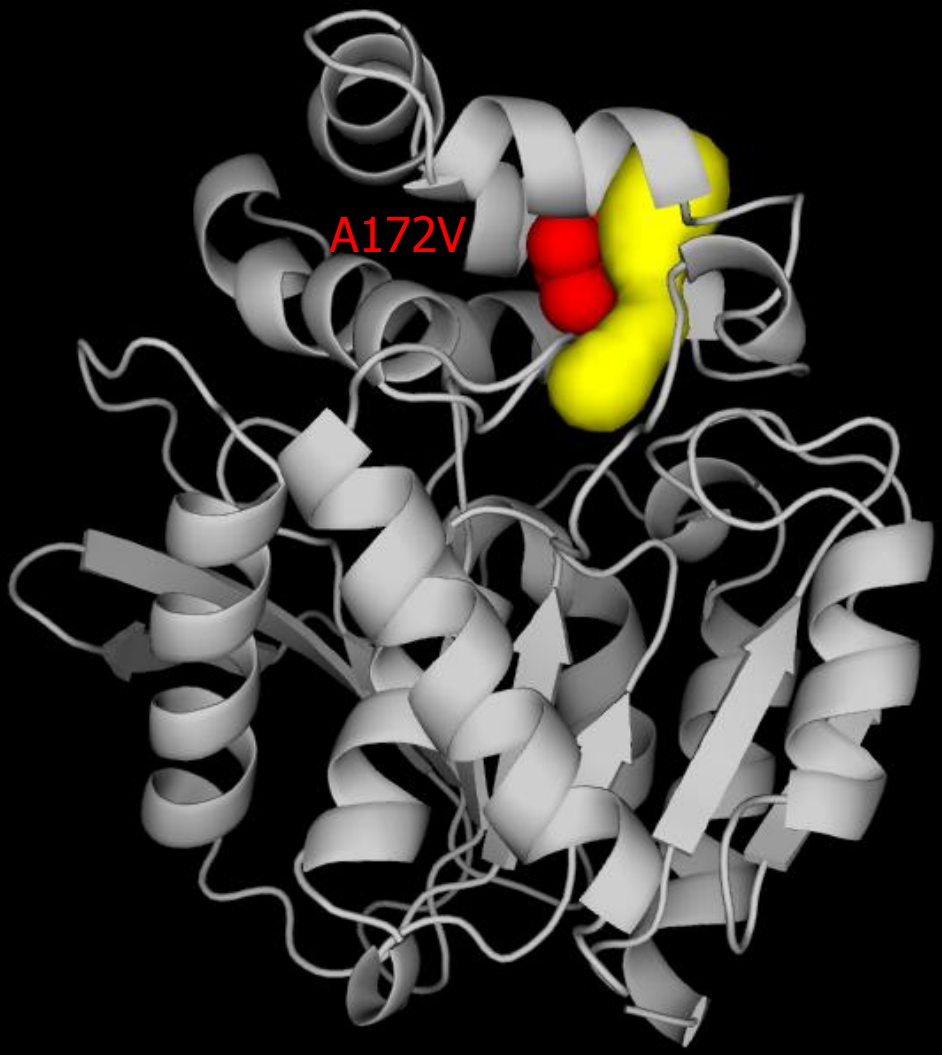
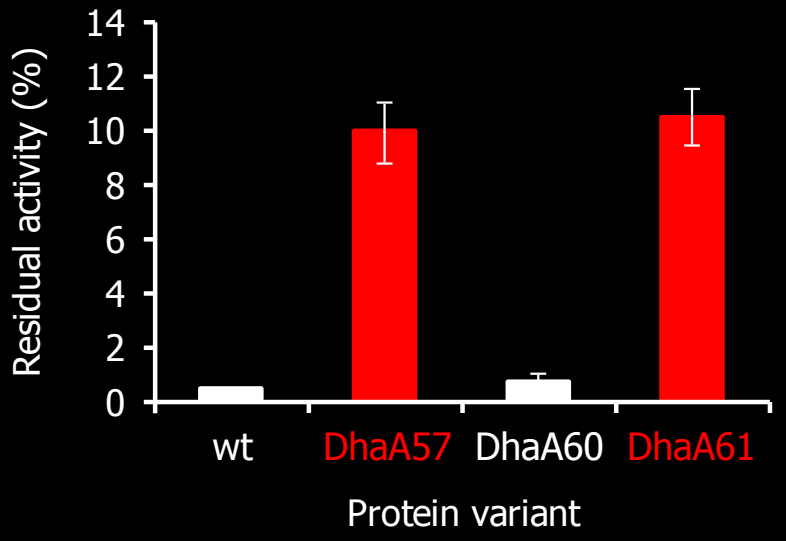
40 %
50 %
55 %
60 %

before optimization

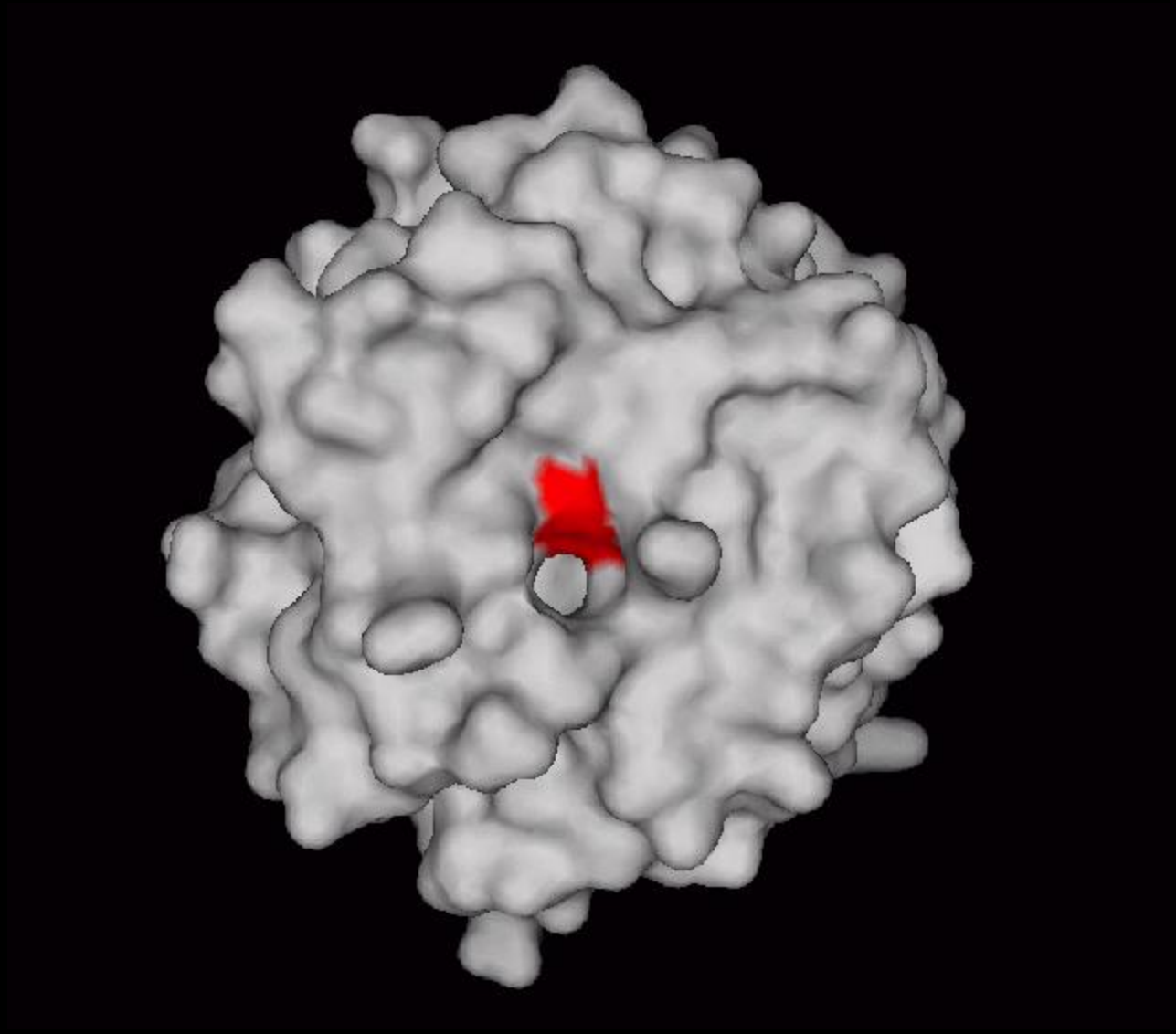
after optimization

Variants with increased stability in DMSO

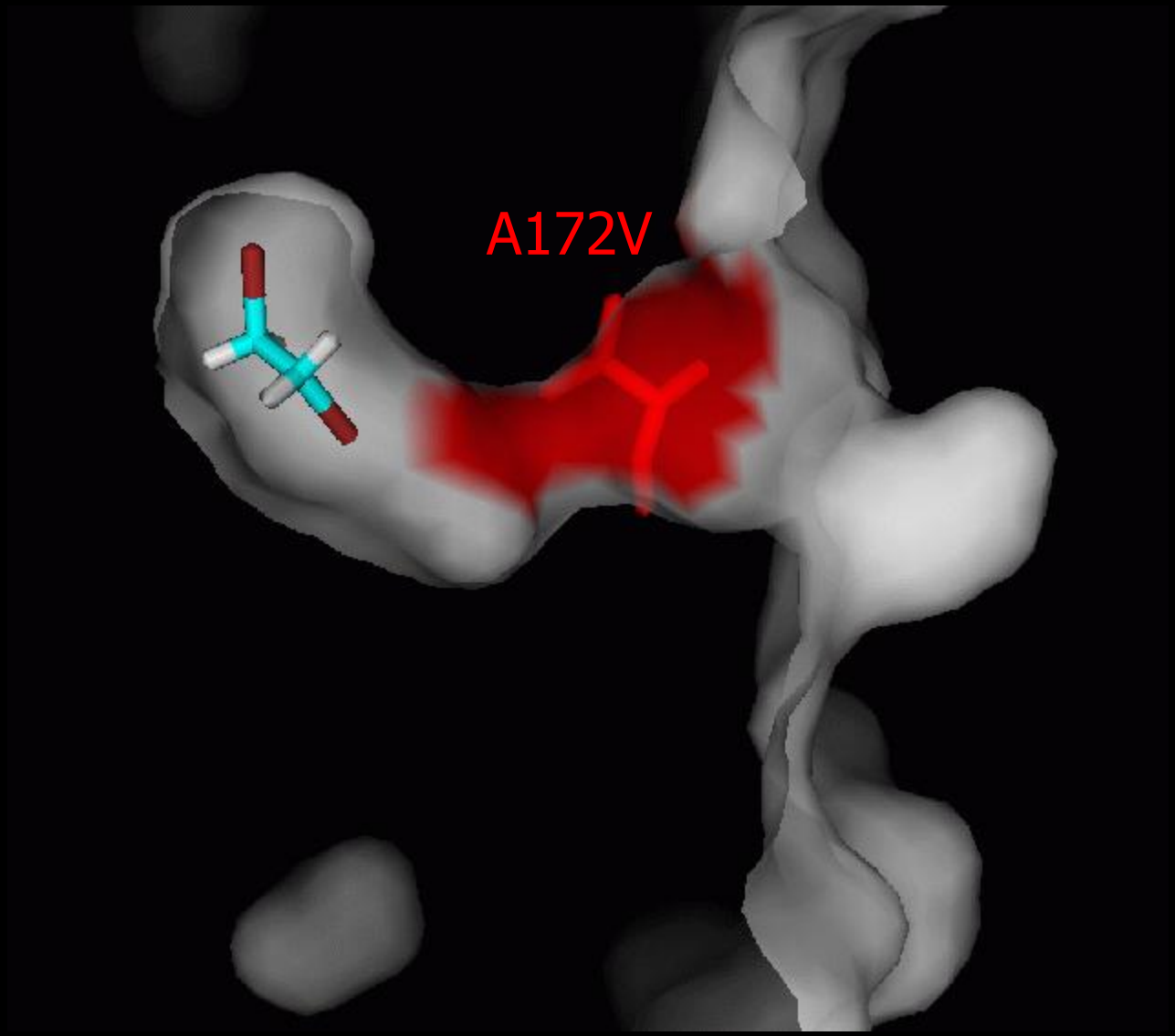




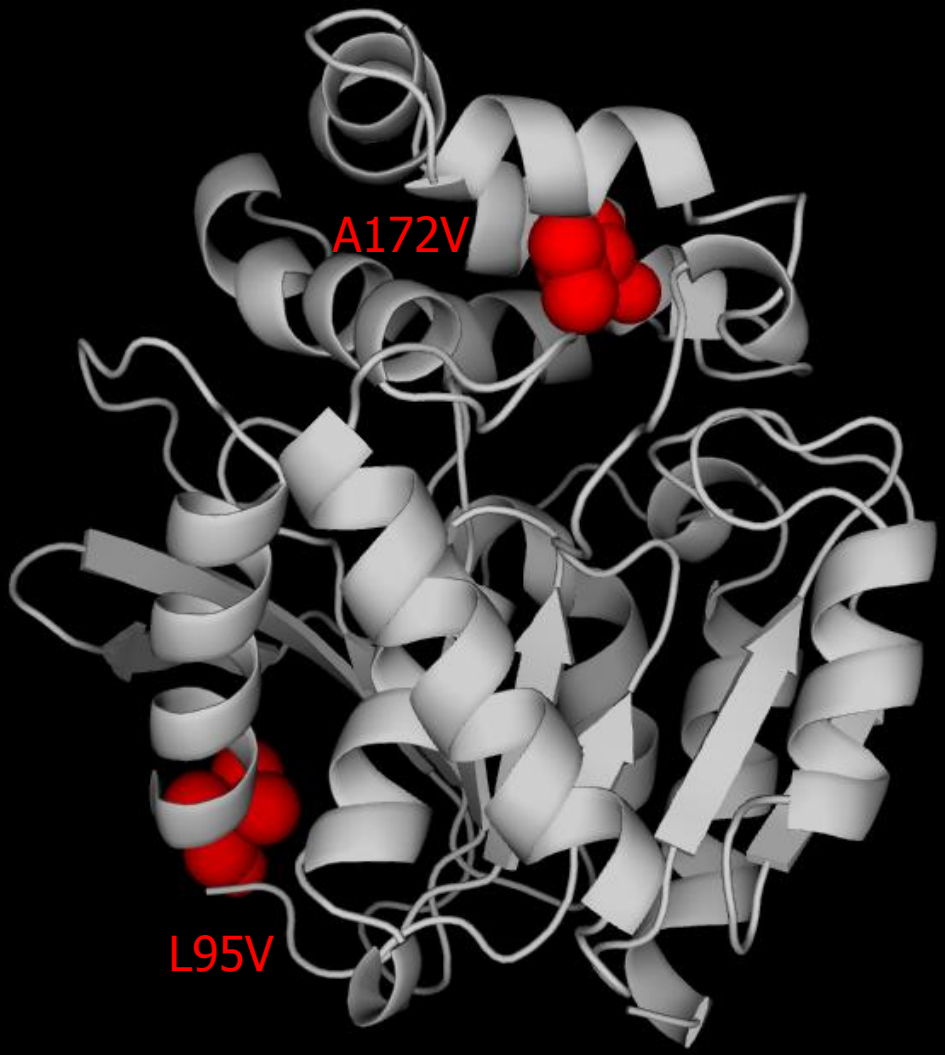
A172 located in tunnel

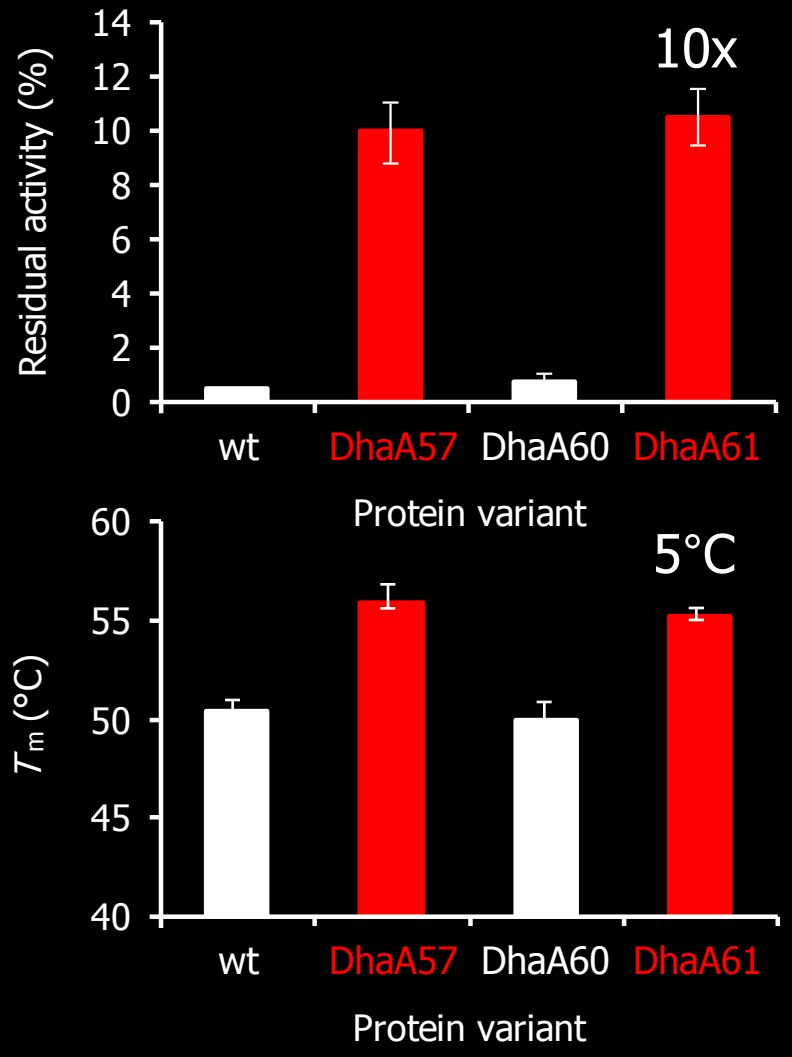


A172 located in tunnel

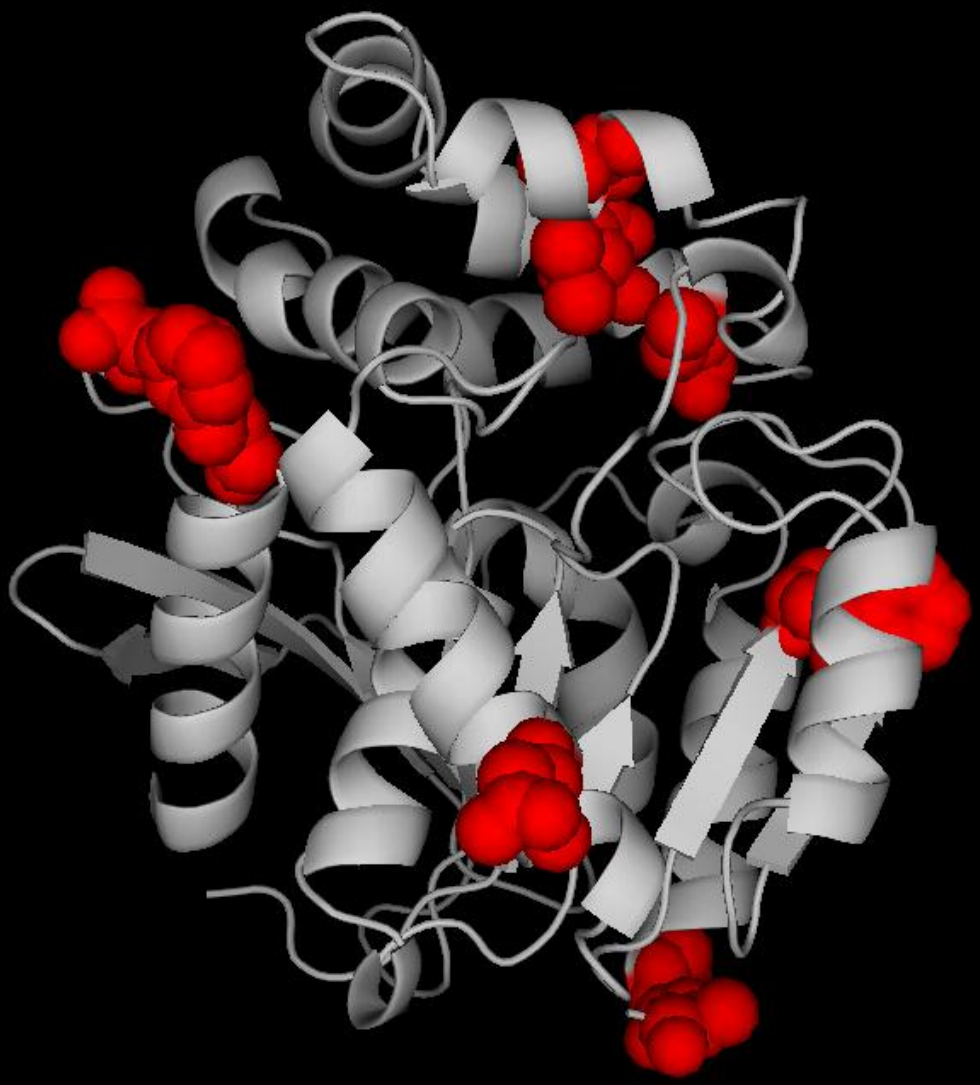


Termostability of variants with increased stability in DMSO





Thermostable mutant

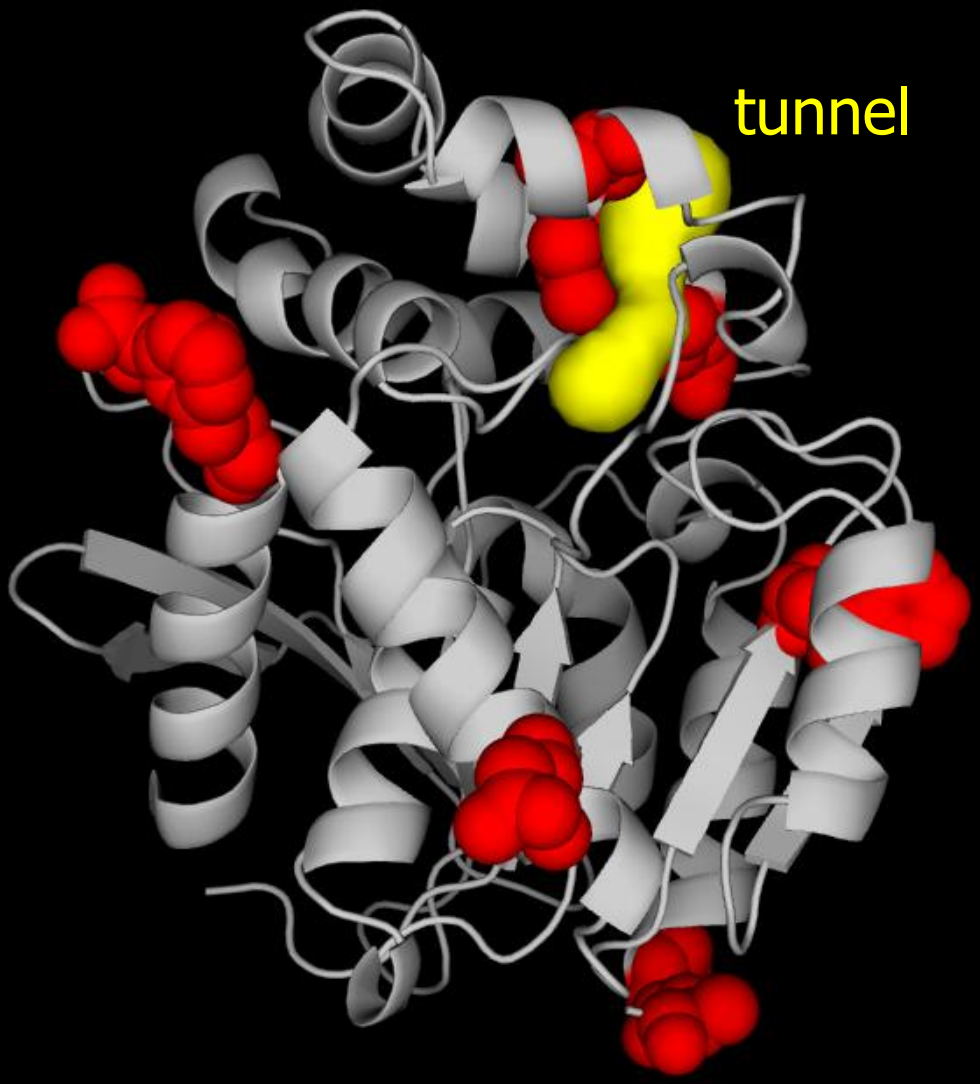


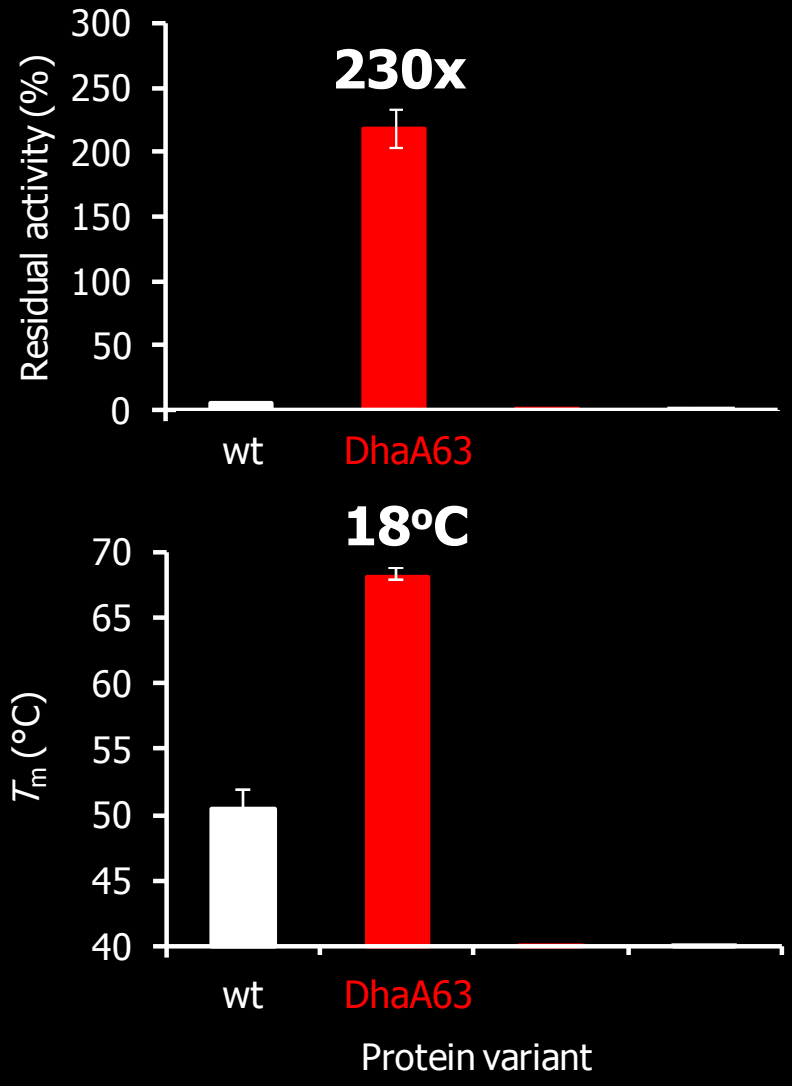
Gray K. and co-workers
Adv. Synth. Catal. 343, 2001

Gene Site Saturation Mutagen.
$T_{1/2}$ (55°C) = 35 h -> 639 h

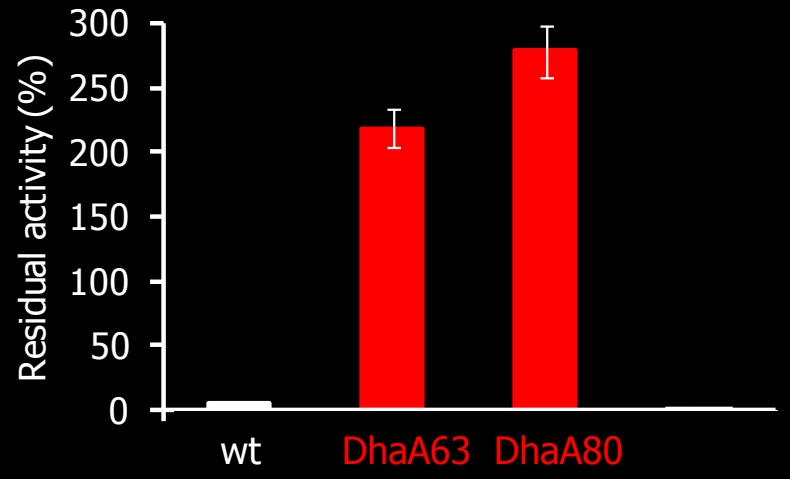
G3D, D78G, F80S, T148L,
G171Q, A172V, C176F, N227T,
W240Y, P291G, A292G

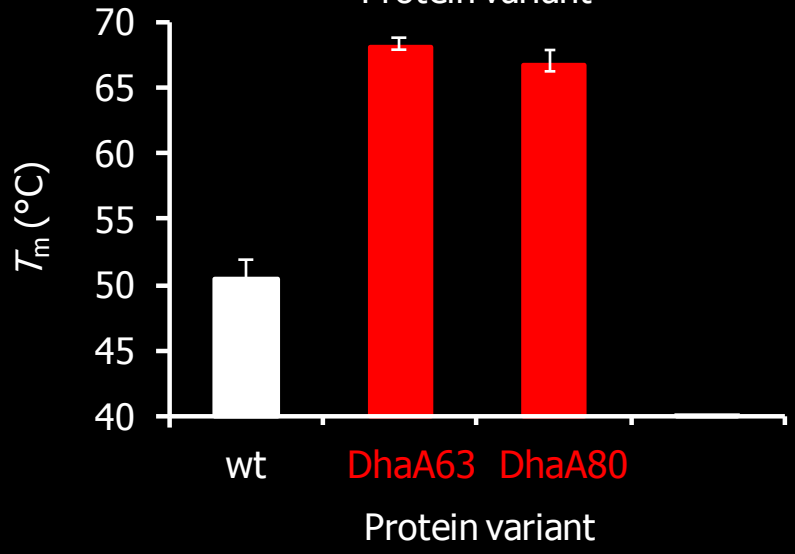
Thermostable mutant



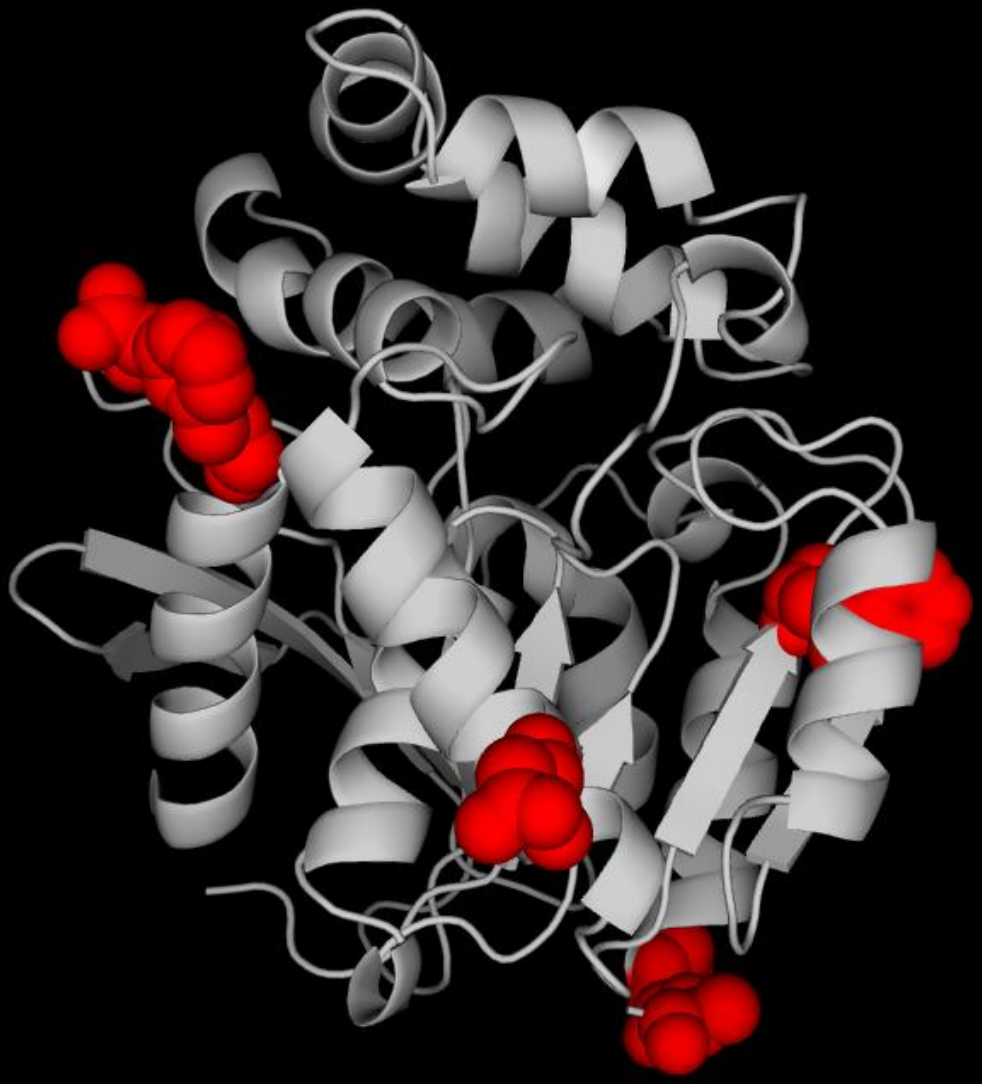


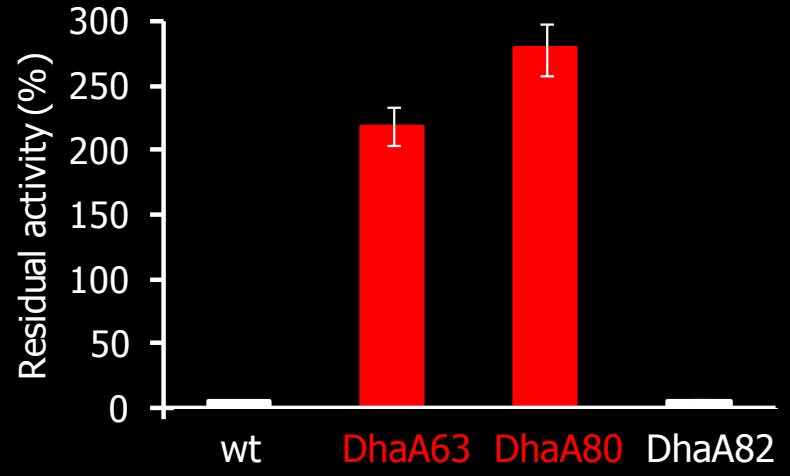
Thermostable mutant

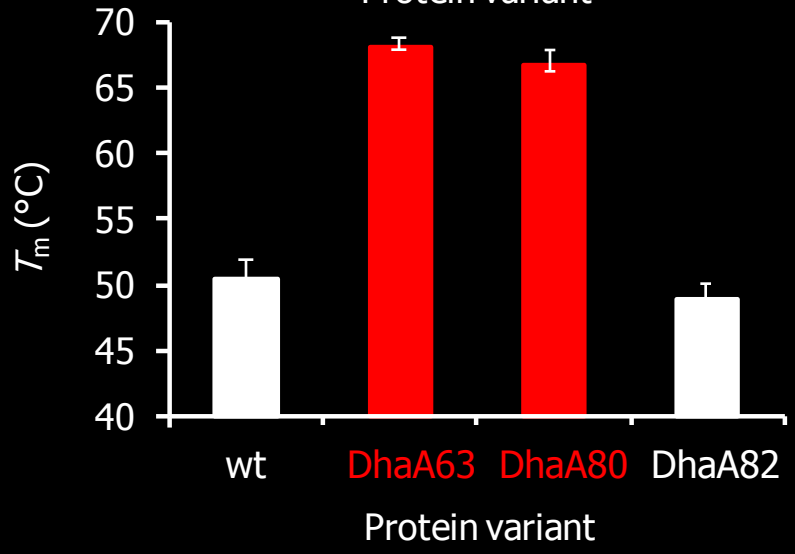


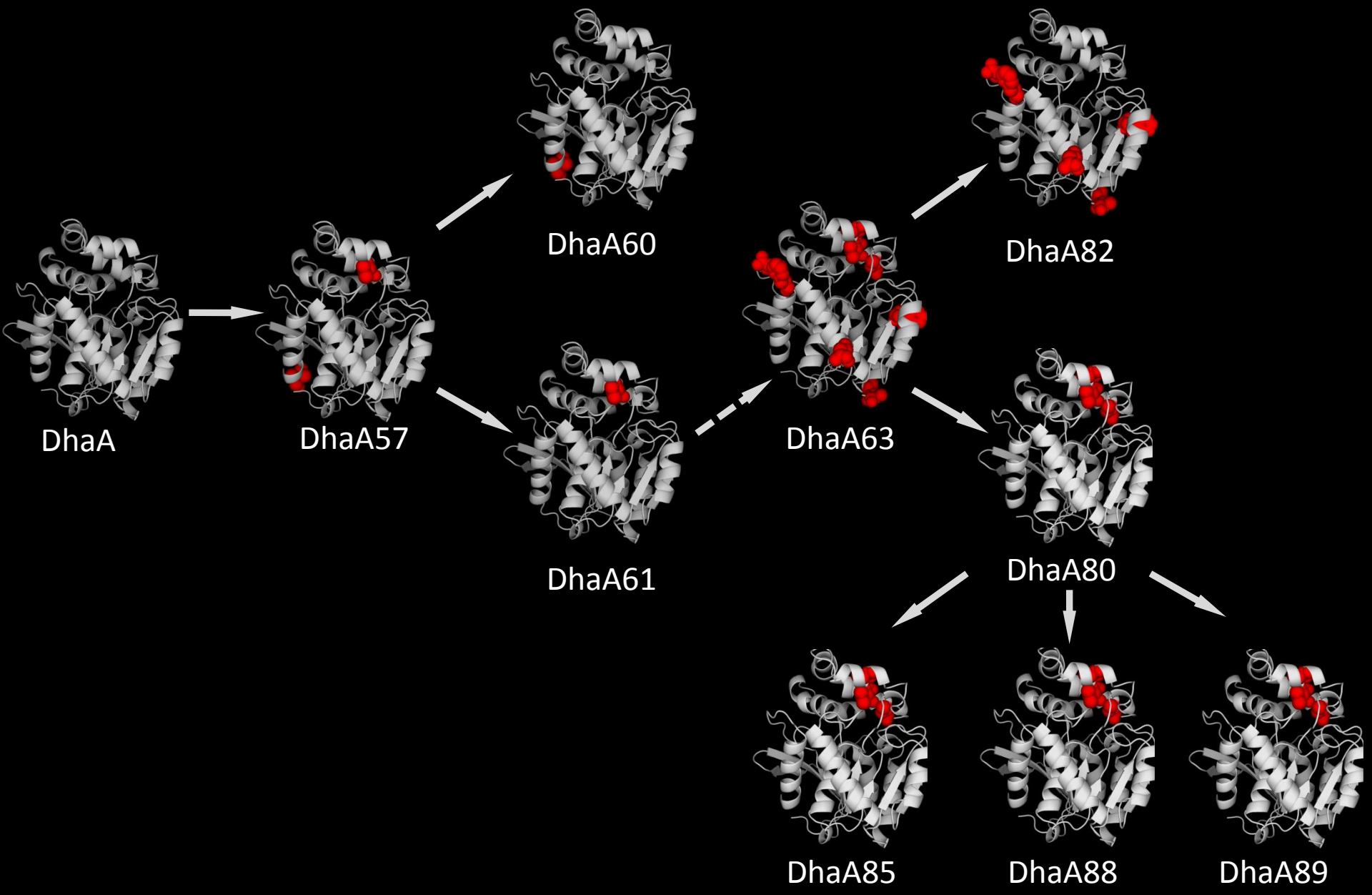


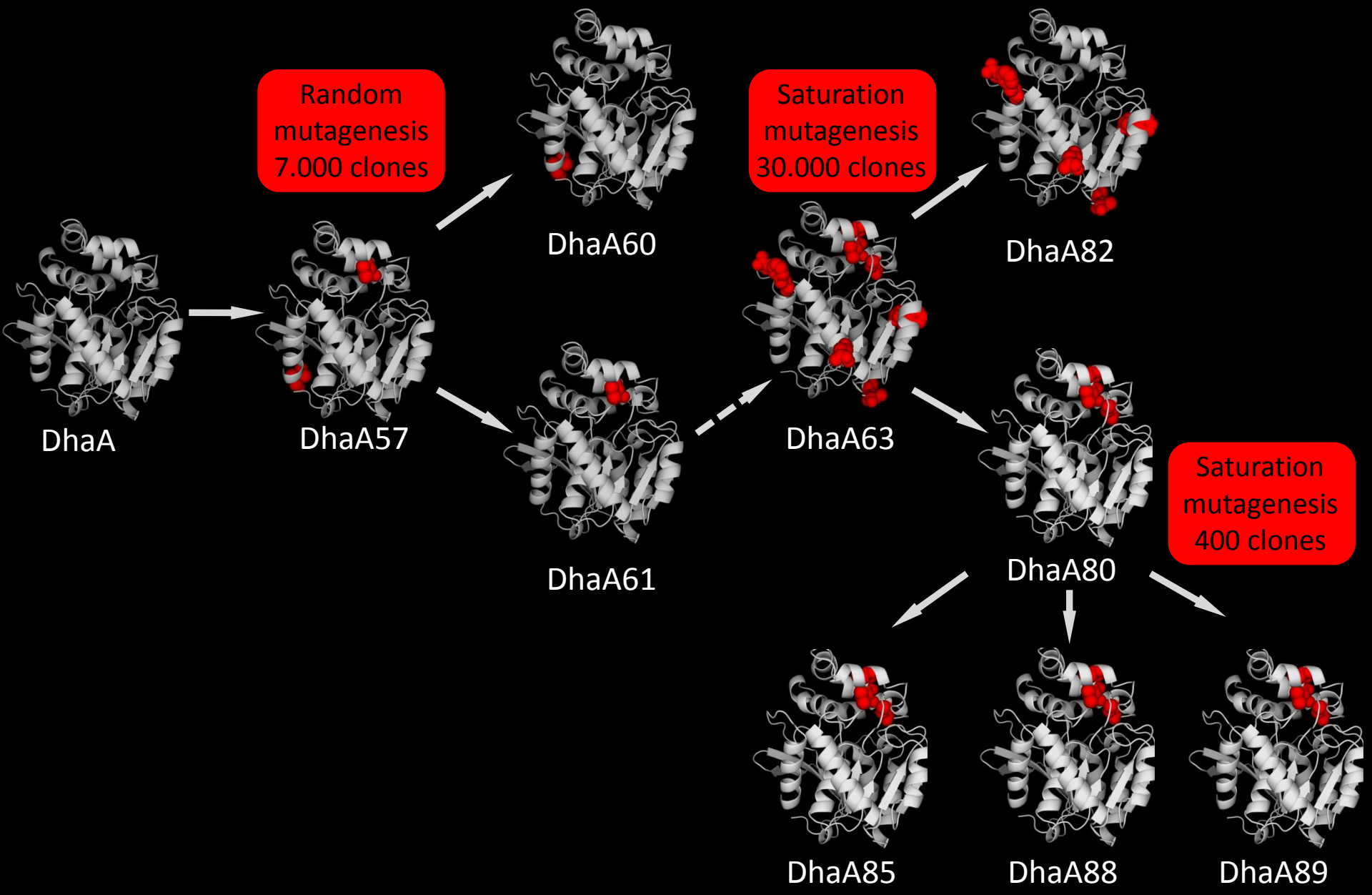
Thermostable mutant





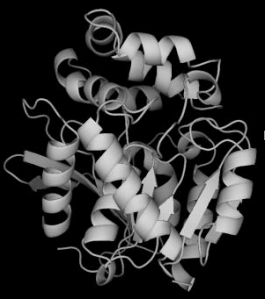



DhaA
DhaA57
DhaA60
DhaA61
DhaA63
DhaA80
DhaA82
DhaA85
DhaA88
DhaA89


Random mutagenesis 7.000 clones
Saturation mutagenesis 30.000 clones
Saturation mutagenesis 400 clones
DhaA
DhaA57
DhaA60
DhaA61
DhaA63
DhaA80
DhaA82
DhaA85
DhaA88
DhaA89

Koudelakova *et al.*, Angewandte Chemie 52: 1959 2013

CAVER



DhaA

Saturation mutagenesis 400 clones

DhaA85

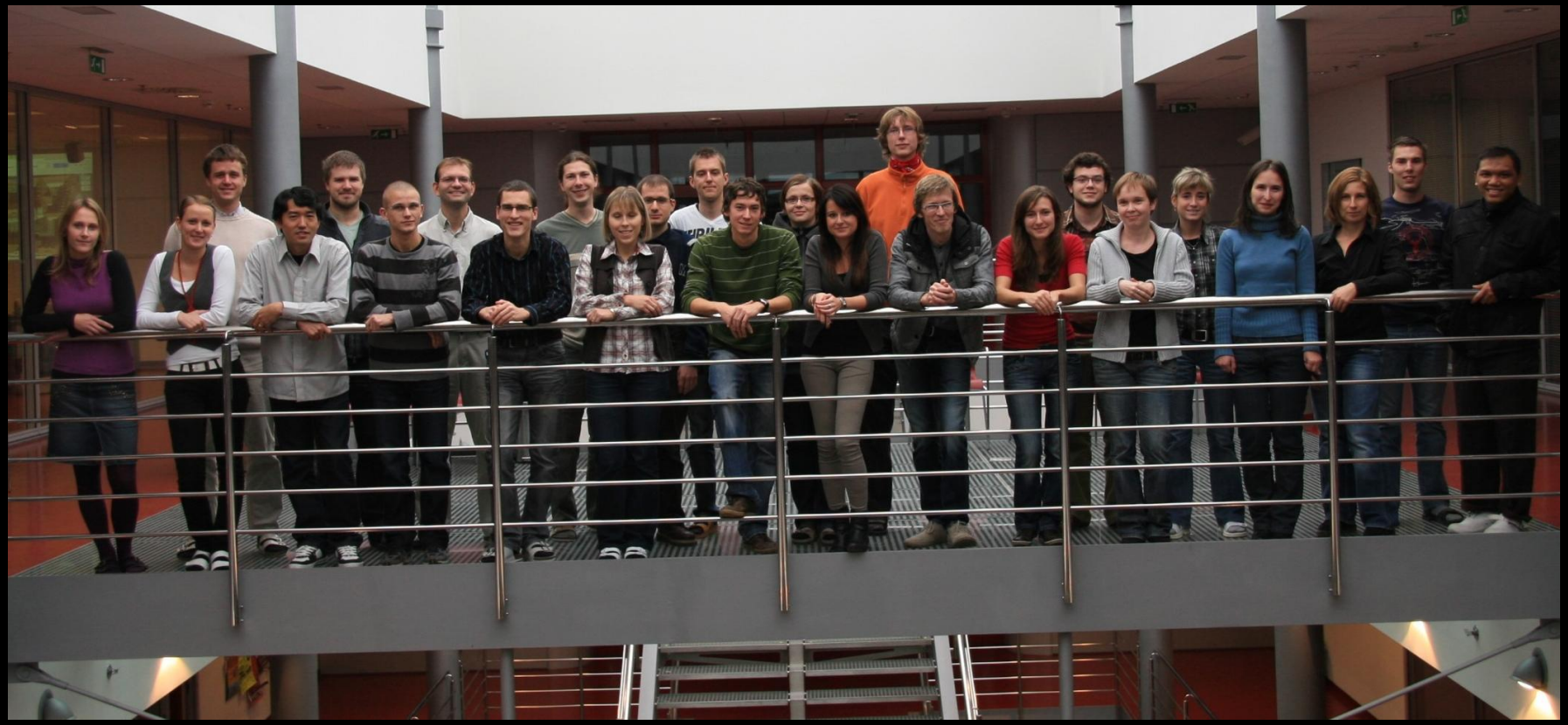
DhaA88

DhaA89

Conclusions

- New model emphasizes importance of tunnels in enzymes with buried active sites.
- CAVER is useful tool for analysis and design of tunnels in proteins.
- Engineering of tunnels provides enzymes with modified activities and stabilities.

Loschmidt Laboratories



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- Jiri Sochor, Vilem Sustr, Barbora Kozlikova, Antonin Pavelka - Masaryk University

- Uwe Bornscheurer, Martin Hesseler - Greifswald University
- Rebecca Wade - EML Research
- Yuji Nagata, Yukari Sato - Tohoku University

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