

Engineering of Tunnels in Proteins with Buried Active Sites

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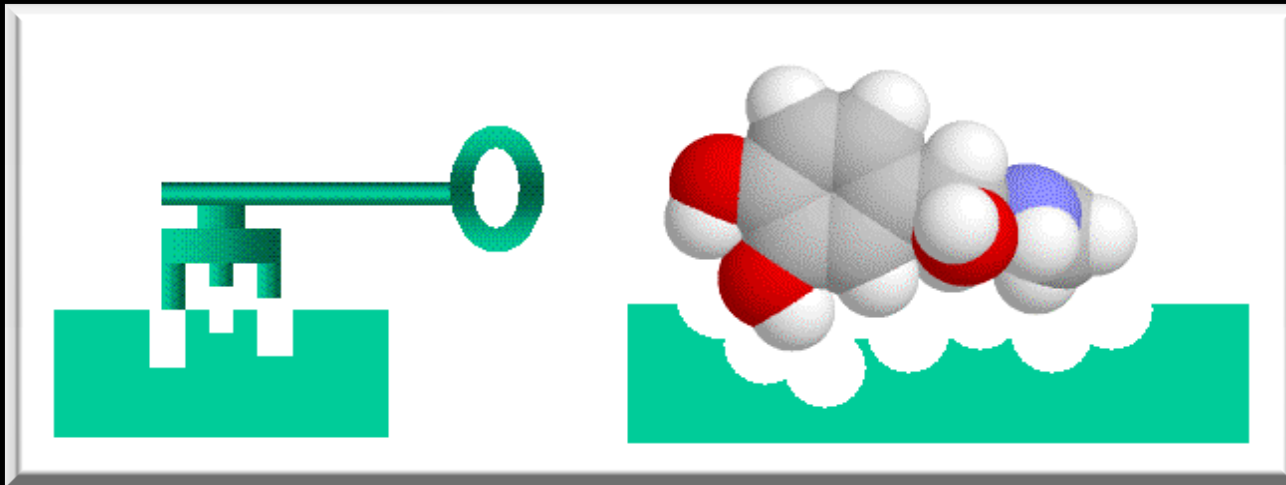
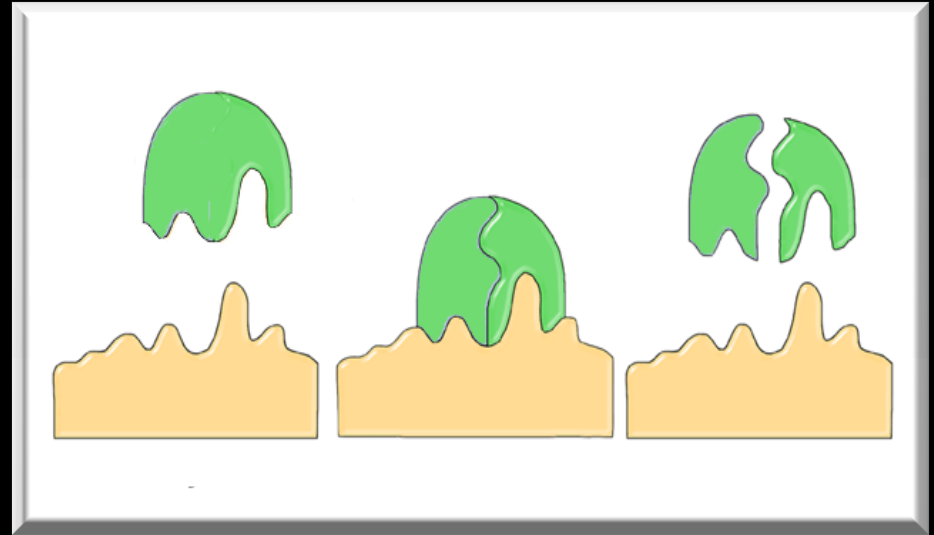
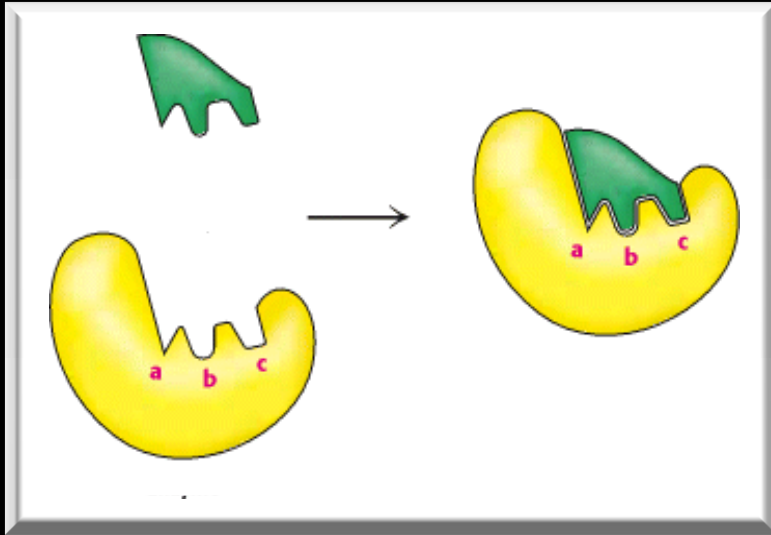
Lock-key model



*“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

Lock-key model



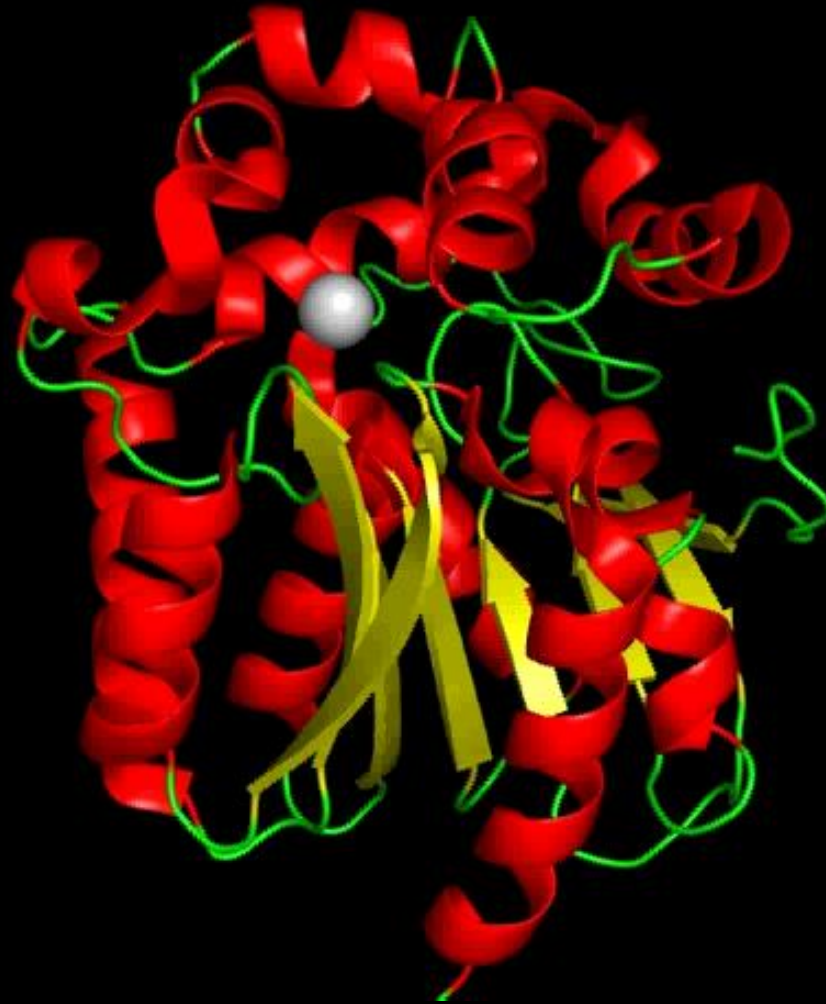
Outline

- New concept – keyhole-lock-key
- Tools – CAVER and HOTSPOT WIZARD
- Case – design of efficient biocatalyst

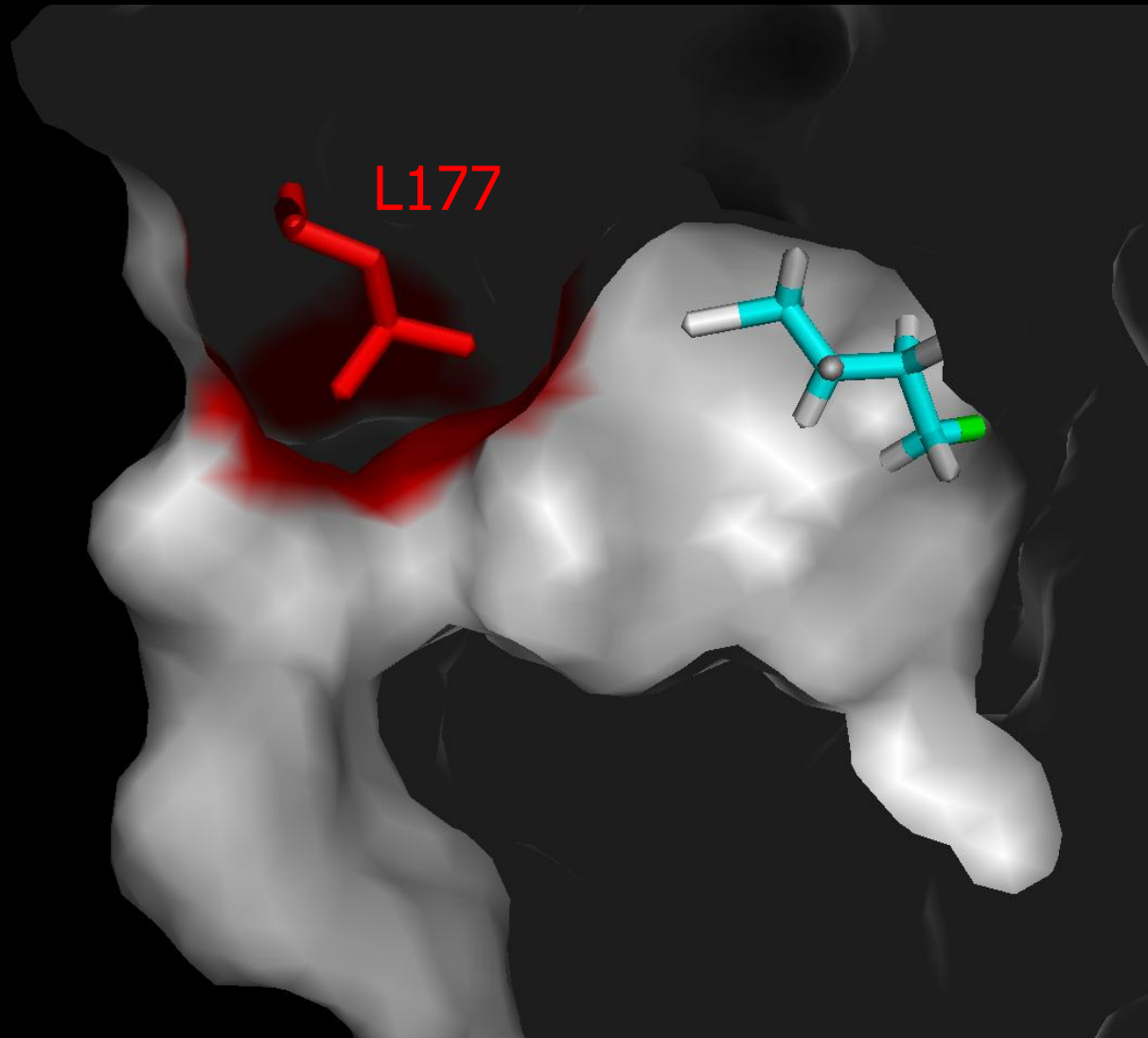
New concept

- haloalkane dehalogenases
- evolutionary analysis
- identification of hot spots
- construction exhaustive set of mutants
- kinetic characterization
- multivariate statistical analysis

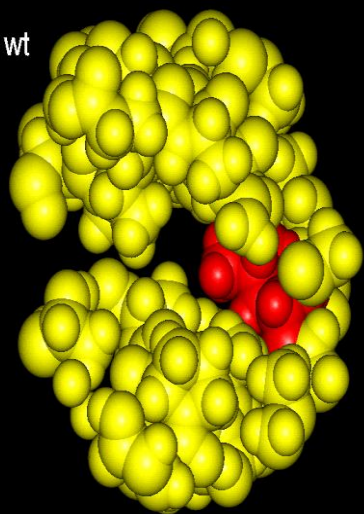
Haloalkane dehalogenase



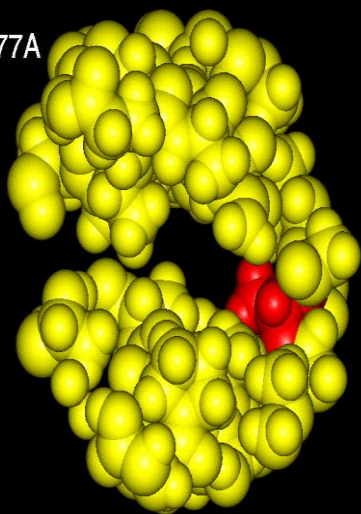
L177 located in tunnel



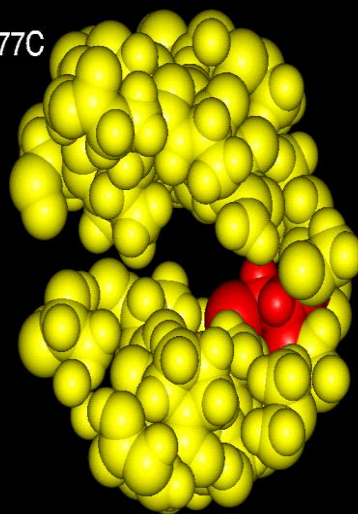
wt



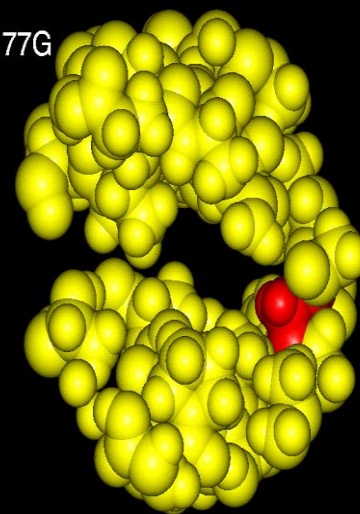
L177A



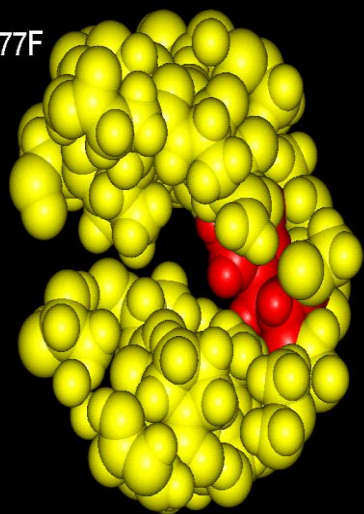
L177C



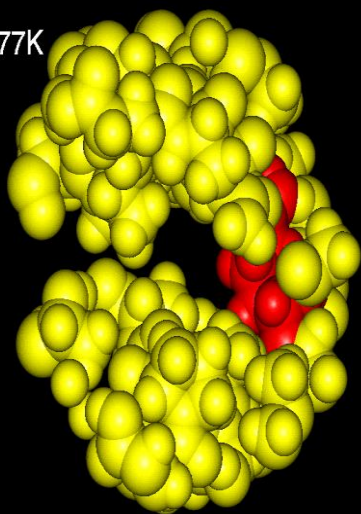
L177G



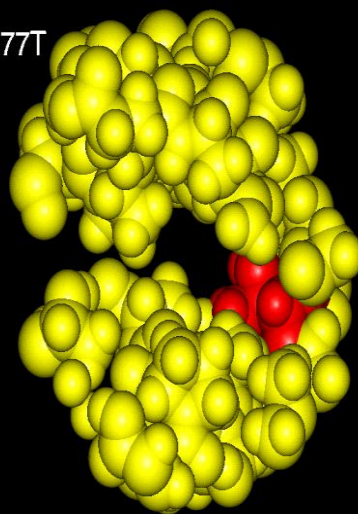
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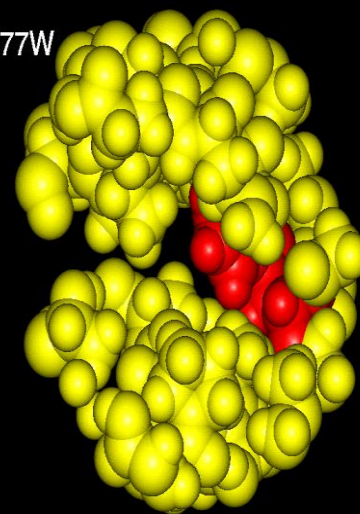
L177K



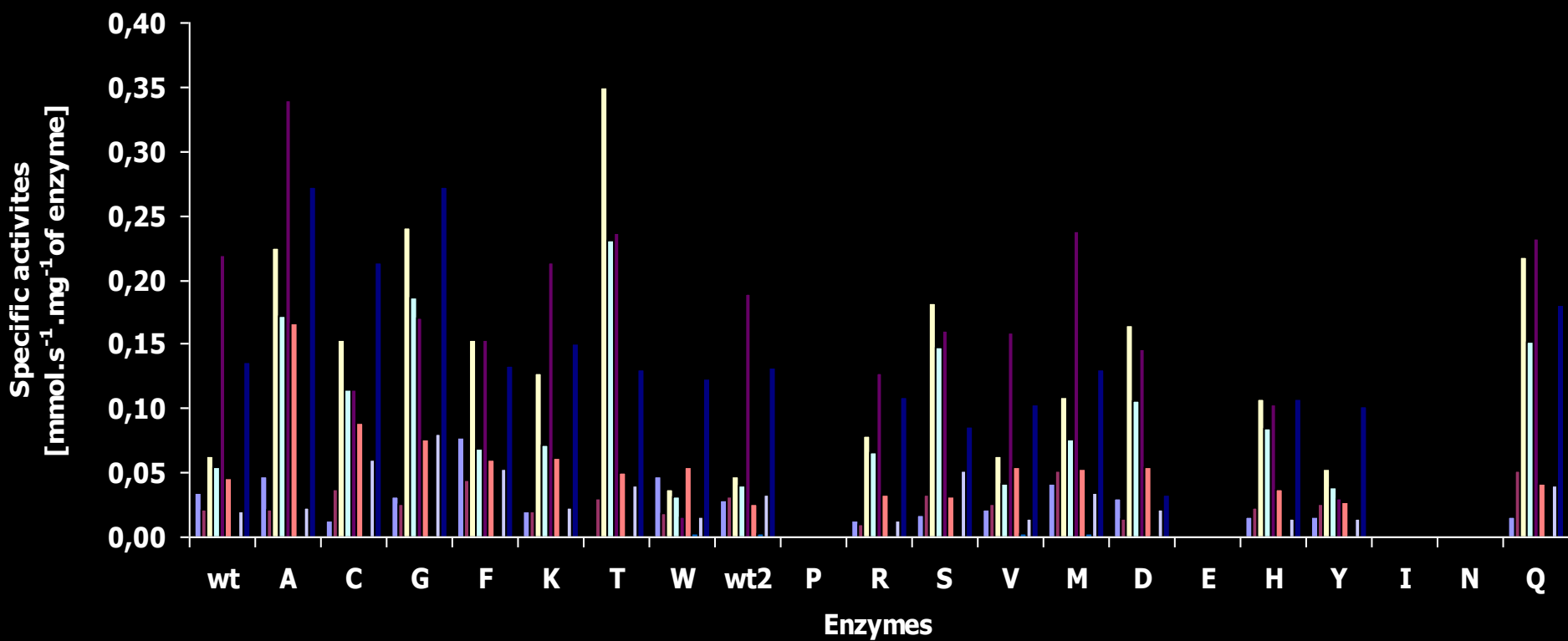
L177T



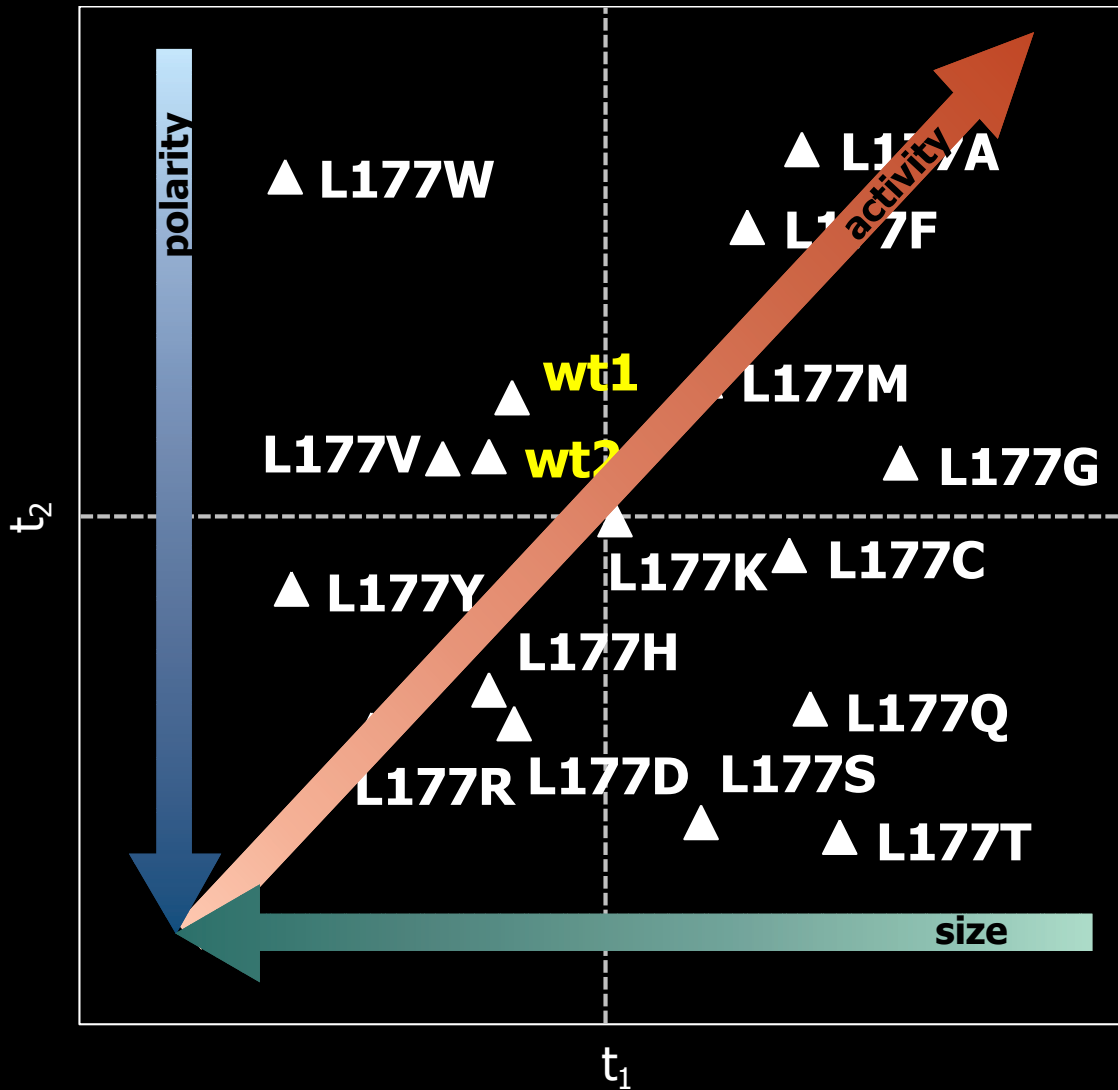
L177W



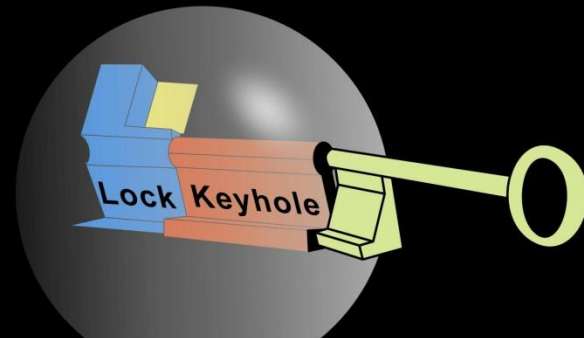
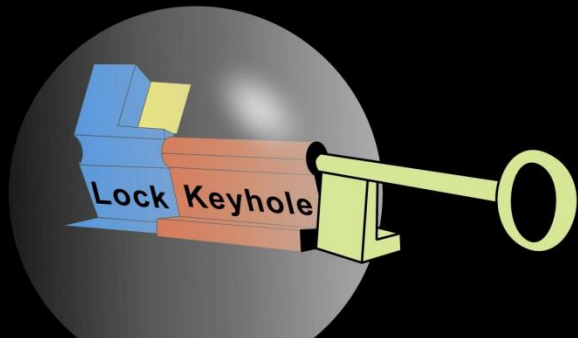
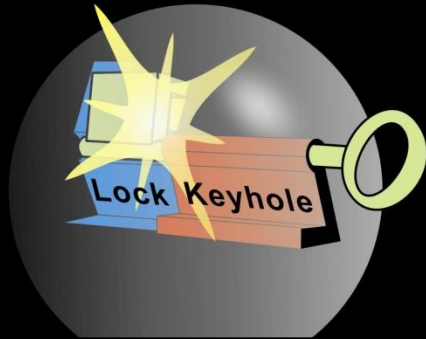
- 1-chlorobutane
- 1-chlorohexane
- 1-bromobutane
- 1-iodobutane
- 1,2-dibromoethane
- 1,3-diiodopropane
- chlorocyclohexane
- bromocyclohexane
- 3-chloro-2-methylpropene



Principal component analysis



Keyhole-lock-key model



Implications for cell biology

- two-level discrimination of ligands
- discrimination based on protein dynamics
- elimination of non-productive complexes
- enhanced evolvability of enzymes

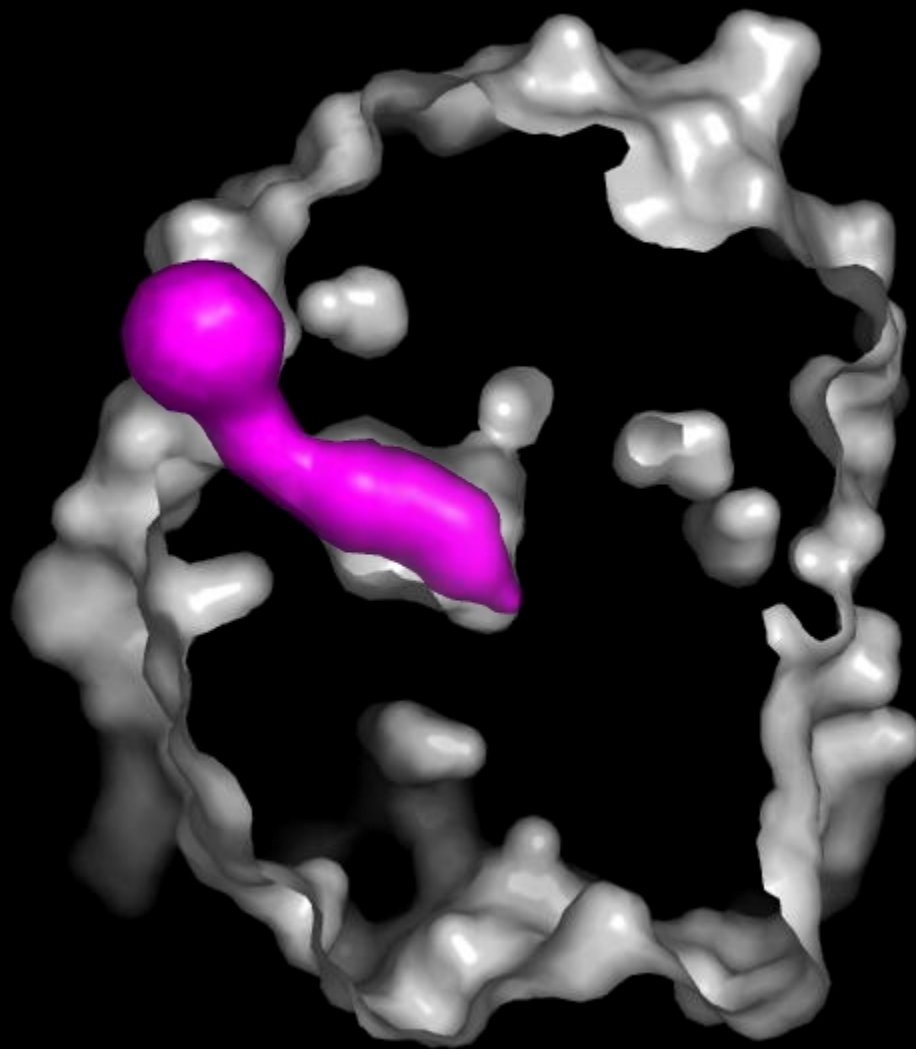
Implications for synthetic biology

- **optimization** of elements for biorecognition, biosensing, bioconjugation & biocatalysis
 - engineering **affinity**
 - engineering **catalytic efficiency**
 - engineering **selectivity**
 - engineering **orthogonality**

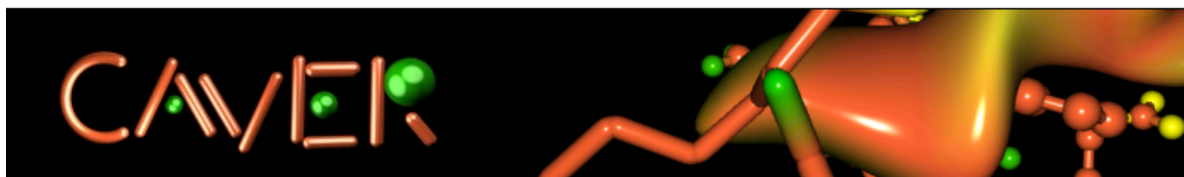
Software tools

- **CAVER** – automated calculation of tunnels in proteins and nucleic acids
- **HOTSPOT WIZARD** – automated prediction of hot spots for mutagenesis

Software CAVER



Software CAVER



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News

09/03/2009 10:52:24
[CAVER plugin 2.0 v0.003](#)

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Unique IPs: 5110
Registered users: 1530

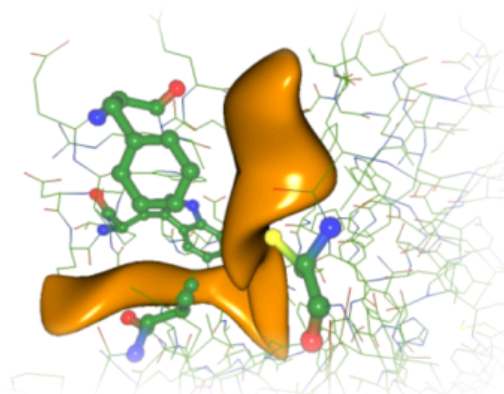
1530

CAVER - secure caving in the world of biomolecules ...

CAVER provides rapid, accurate and fully automated calculation of pathways leading from buried cavities to outside solvent in protein structures. Study of these pathways is important in drug design and molecular enzymology.

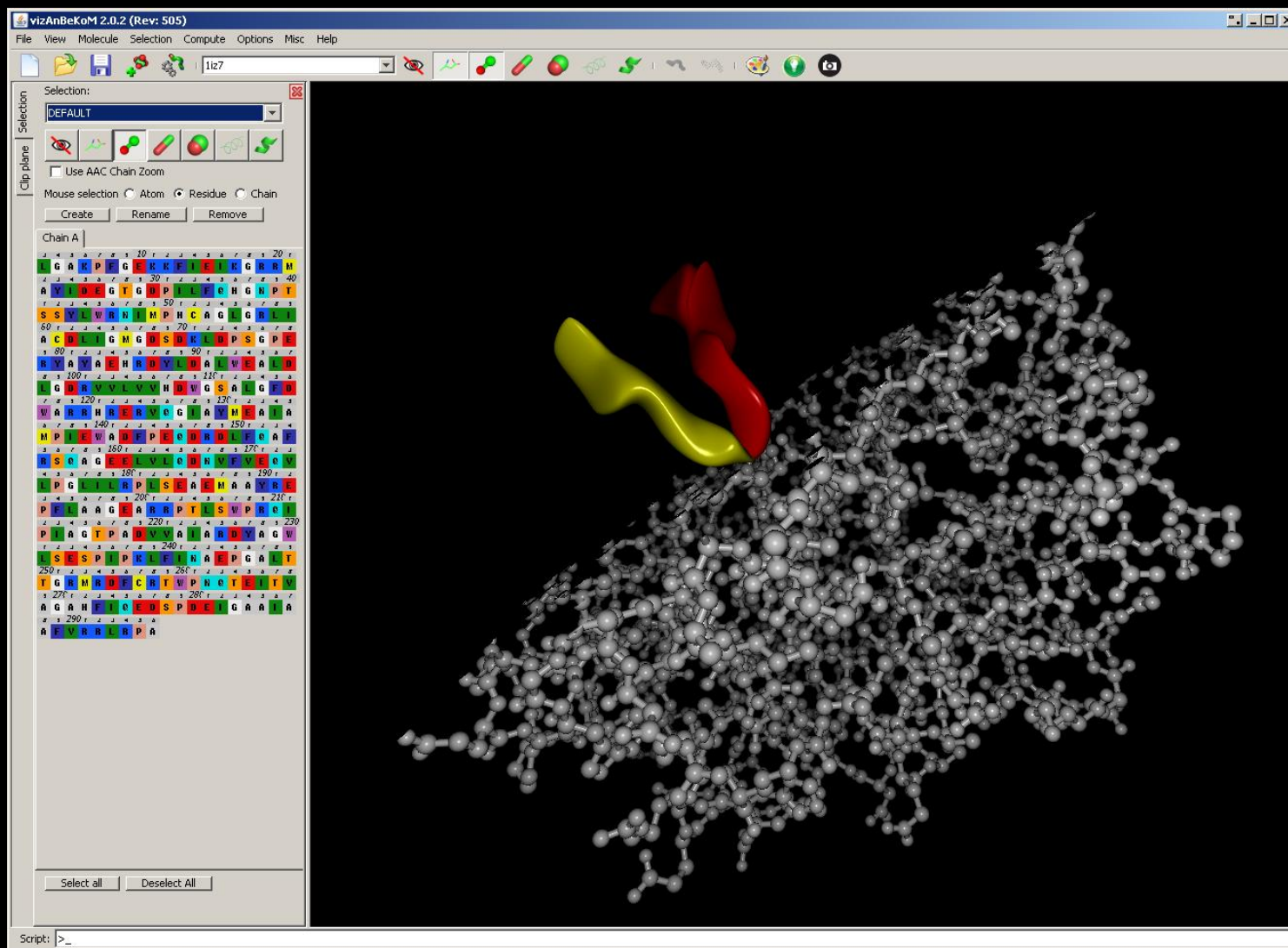
CAVER facilitates analysis of any molecular structure including proteins, nucleic acids, inorganic materials, and is available as online version or PyMOL plugin.

CAVER Viewer enables efficient visualization of calculated pathways and can be run online using Java Web Start technology.

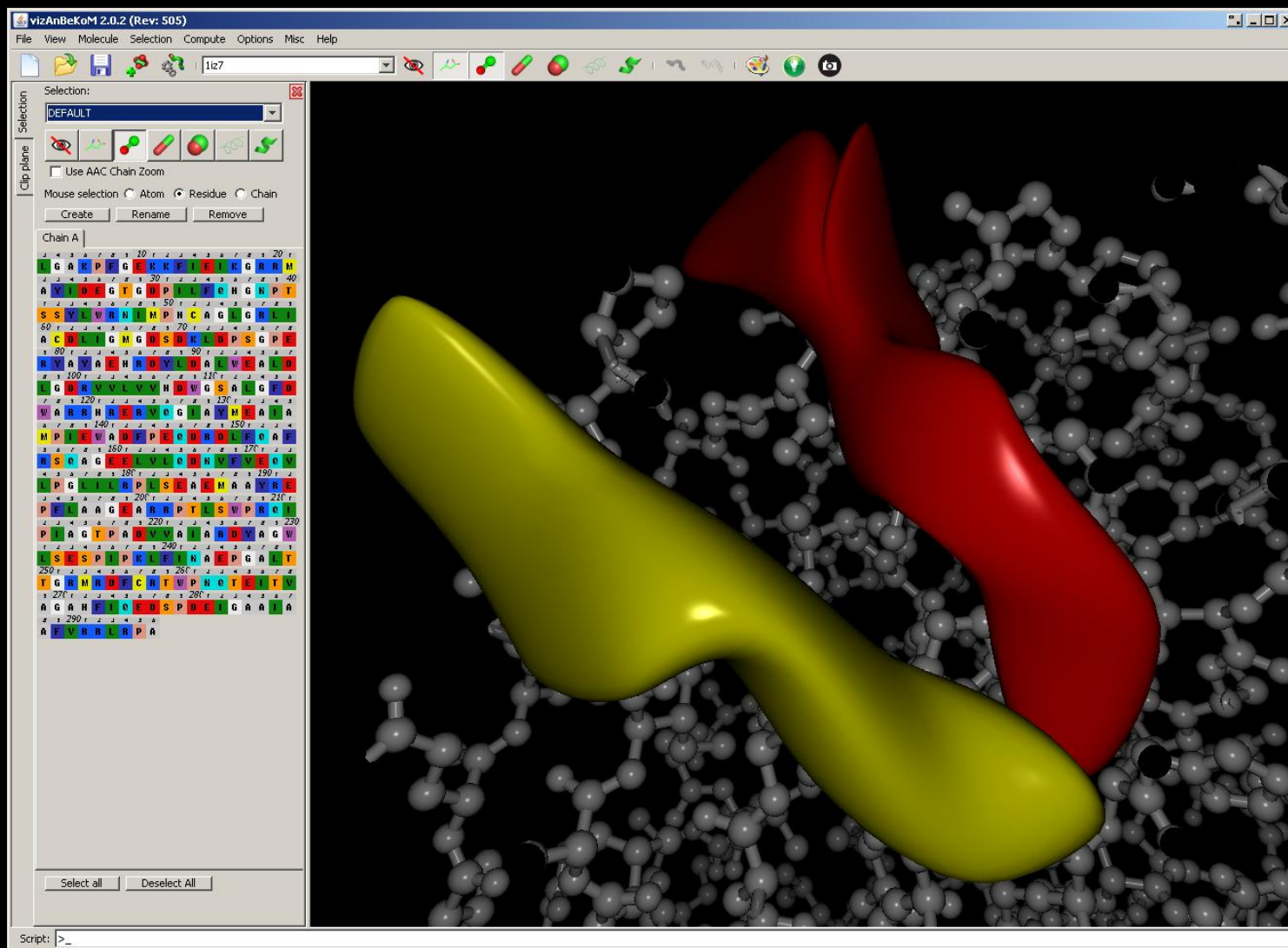


© 2008 Piotr Halmó

Software CAVER 2.0

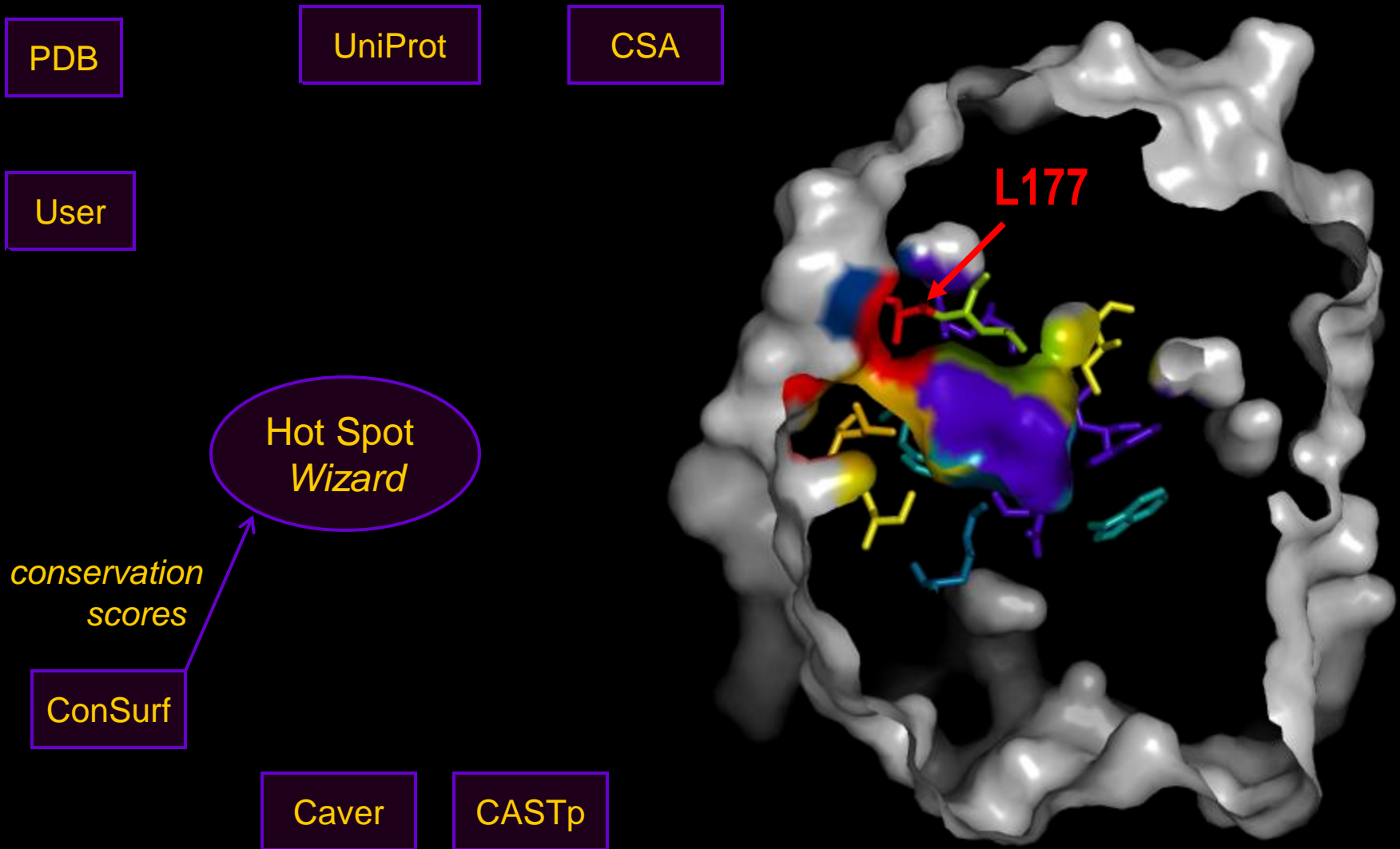


Software CAVER 2.0



[http:// loschmidt.chemi.muni.cz/caver/](http://loschmidt.chemi.muni.cz/caver/)

Software HOTSPOT WIZARD



Software HOTSPOT WIZARD

Controls

Select: center

Render: on thick thin only off

Color:

Color by:

Tunnel:

Debug: ar log

Sequence

1cv2

Chain A:

```
10 20 30 40 50
| | | |
GAKPFGEKKFIEIKGRRMAYIDEGTGDPIILFQHGMP TSSYLWENIMPHCA
60 70 80 90 100
| | | |
GLGRLIACDLIGMDSKLDPSGPERYAYAEHRDYLDALWEALDLGDRVV
110 120 130 140 150
| | | |
LVVHDTGSGALGFDWARRHREKRVQGIAYMEAIAMP IEWADFPEQDRDLFQA
160 170 180 190 200
| | | |
FRSQAGEELVLQDNV FVEQVLPGLILRPLSEAEAAATREPFLAAGEARRP
210 220 230 240 250
| | | |
TLSWFRQIP IAGTPADVVAIARDYAGMLSESP IPKLFINAEPGALTTGRM
260 270 280 290
| | | |
RDFCRTWPNQTEITVAGAHF IQEDSPDEIGAAIAAFVRELRPA
```

Active Pocket and Tunnel

146 Q conservation grade 1, variable
6 L, 4 Q, 3 D, 3 P, 2 A, 2 F, 2 K, 1 G, 1 I, 1 L, 1 M,
1 N, 1 R, 1 S, 1 T, 1 V

143 F conservation grade 1, variable
12 F, 7 L, 5 M, 4 W, 3 A, 3 T, 3 V, 2 P, 2 Q, 2 S,
1 D, 1 G, 1 I

147 D conservation grade 3, variable

Jmol 0:88

Jmol script terminated

<http://loschmidt.chemi.muni.cz/hotspotwizard/>

Case

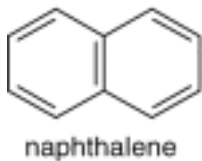
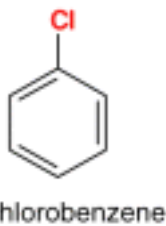
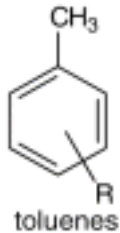
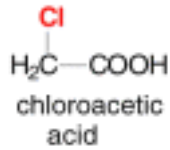
- 1,2,3-trichloropropane
- identification of tunnels by CAVER
- analysis of ligand release by simulations
- mutations by focused directed evolution
- screening by biochemical assay
- characterization by transient kinetics

degradable (aerobic)

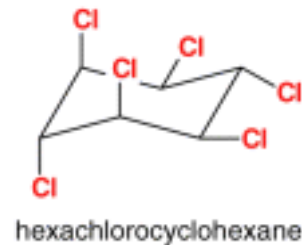
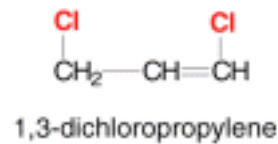
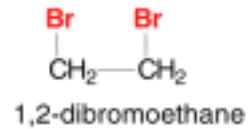
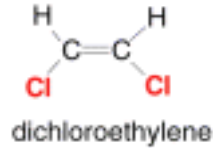
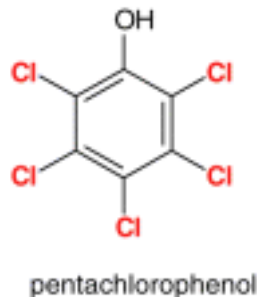
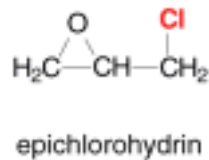
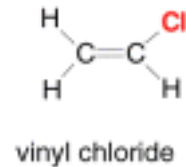
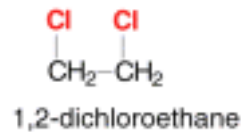
difficult

impossible

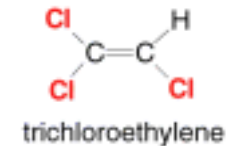
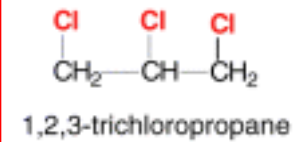
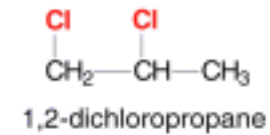
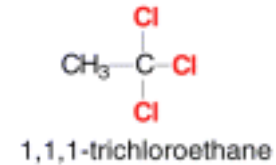
CH_3Cl
methylchloride



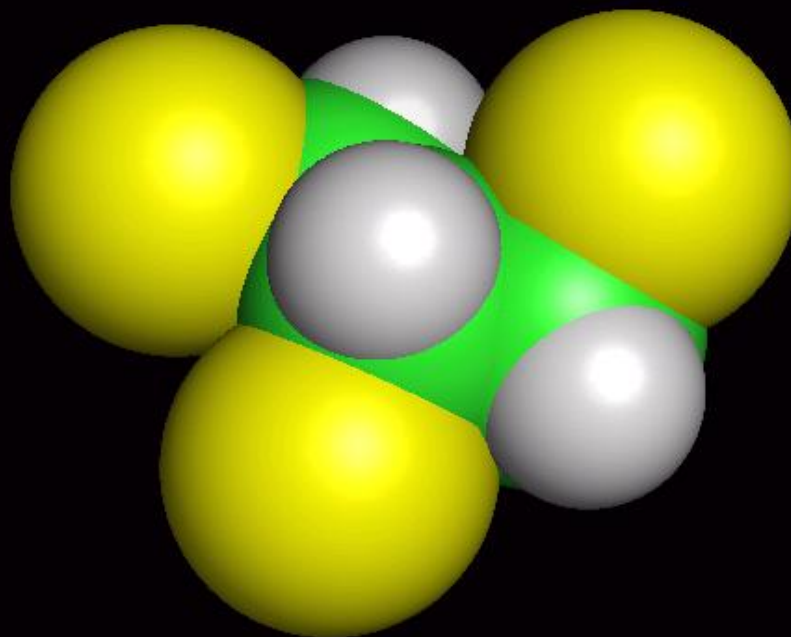
CH_2Cl_2
methylene chloride



CHCl_3
chloroform



1,2,3-Trichloropropane



Directed evolution

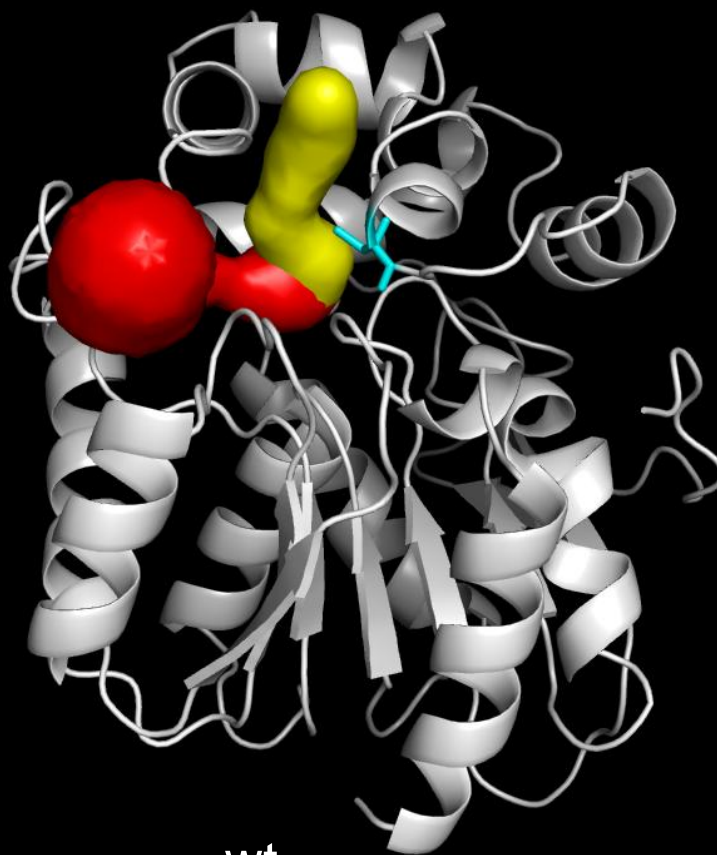
DhaA	Relative activity
wt	1
C176Y+Y273F ¹	3.5
G3D+C176F ²	4

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

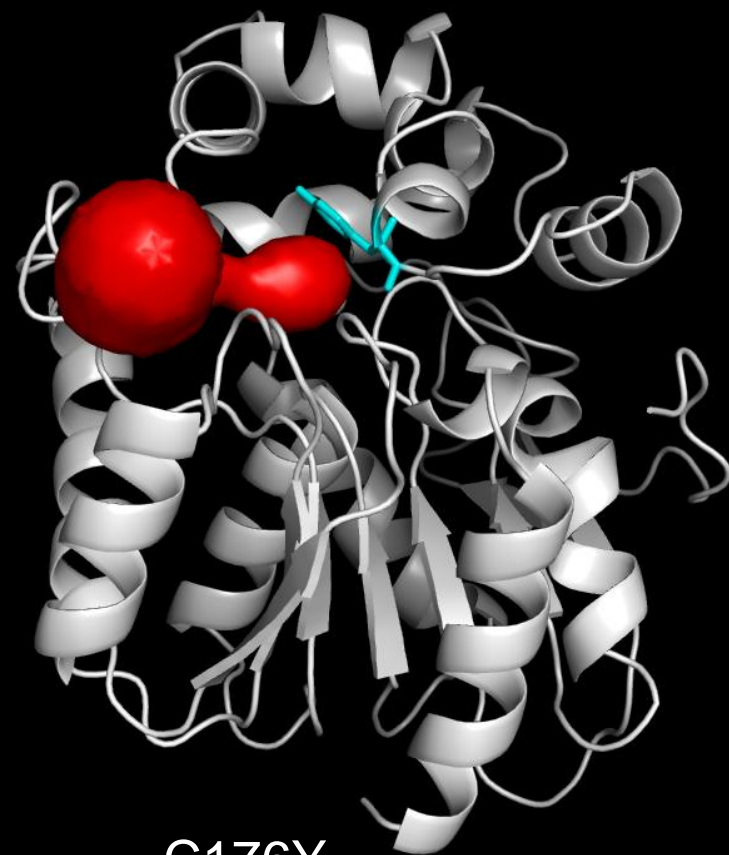
² Gray *et al.* Adv. Synth. Catal. 343: 607 2001

Analysis of tunnels by CAVER

- Main tunnel
- Side tunnel

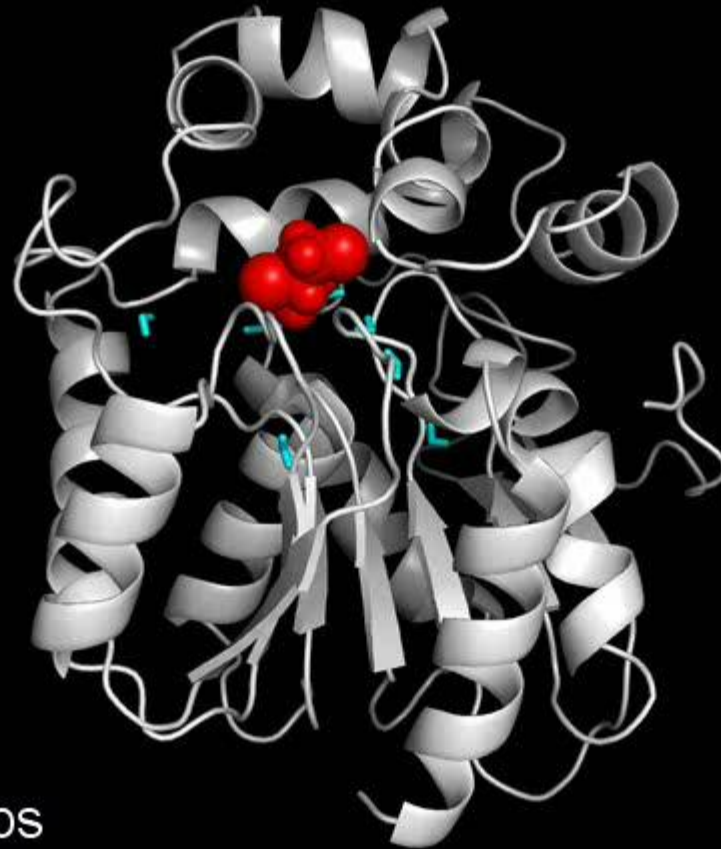


wt



C176Y

Modeling of ligand release by computer simulation



Time: 00 ps

Rational design of tunnels

Main tunnel:

● C176Y

Side tunnel:

● W141F

● I135X

● L245X

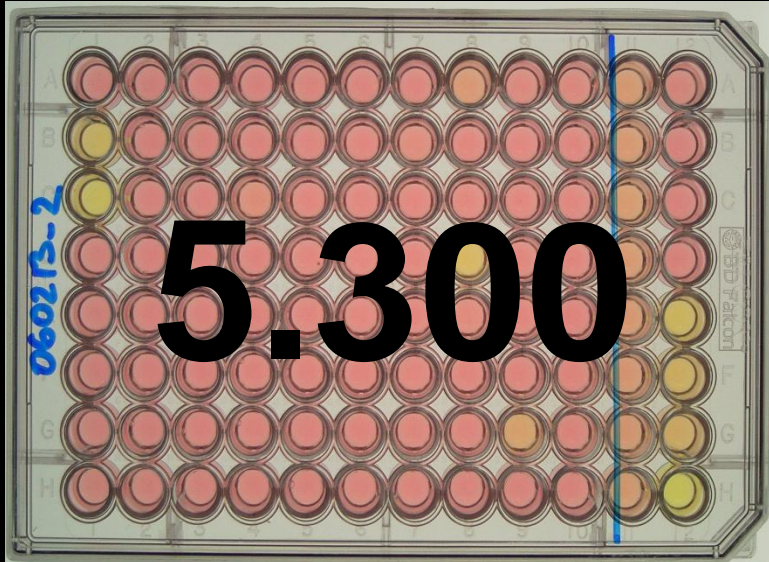
● V246X



Modification of tunnels by focused directed evolution

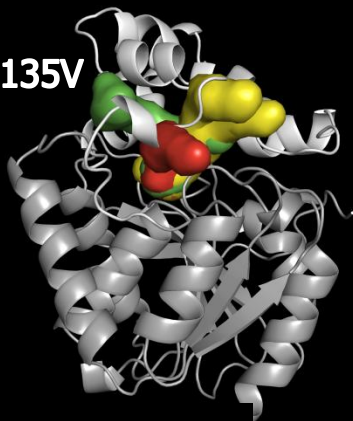
- Site-directed mutagenesis
- Saturated mutagenesis

Mutant	C176Y	W141X	I135X	V245X	L246X
A	✓				
B	✓				
C	✓				
D	✓				
E	✓				
F	✓				

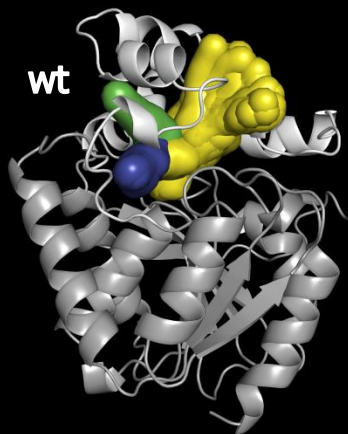


Constructs with modified tunnels

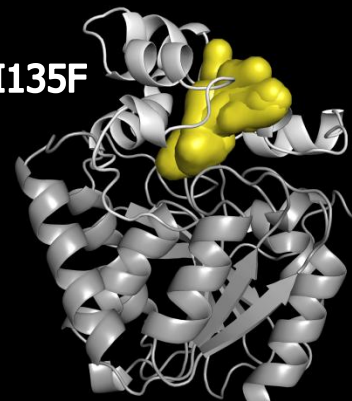
+I135V



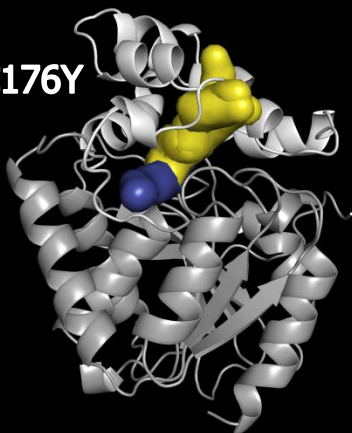
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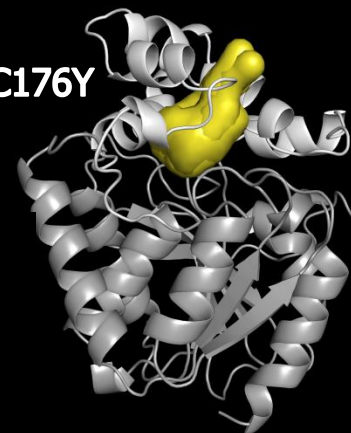
+I135F



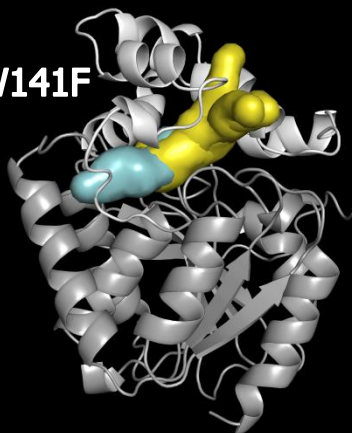
+C176Y



+C176Y



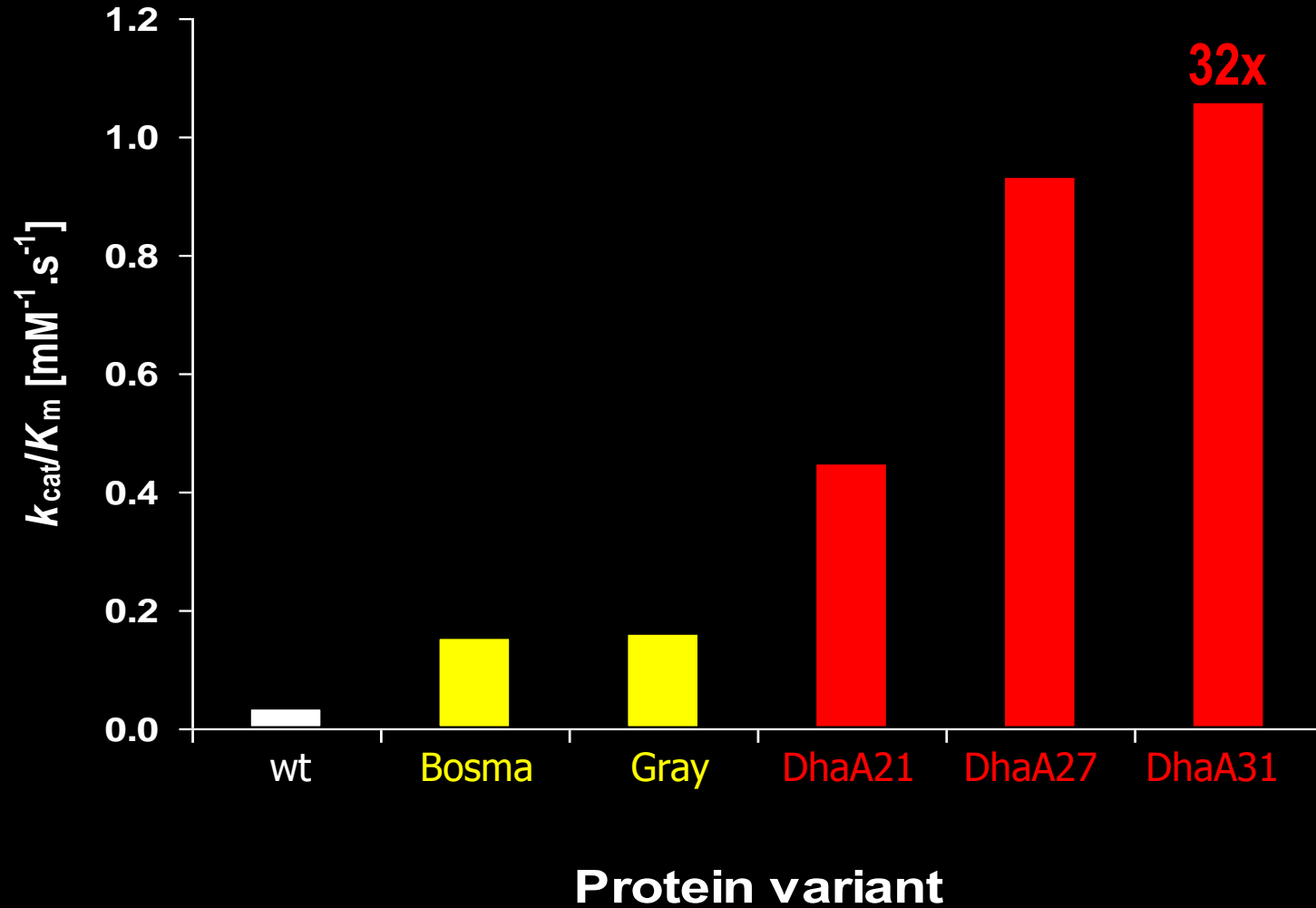
+W141F



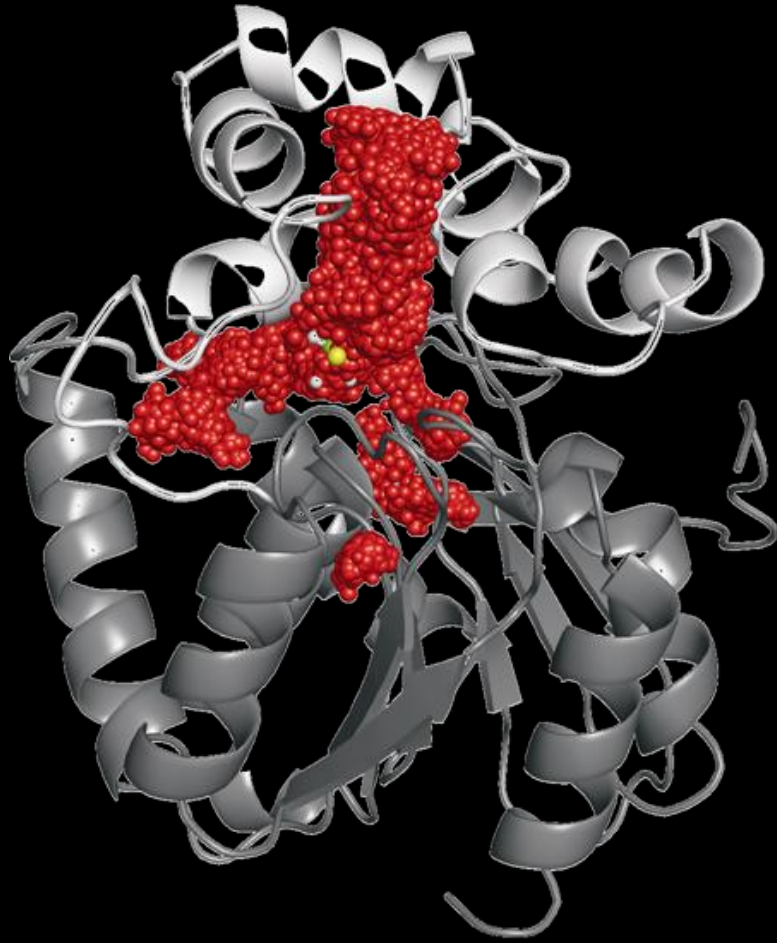
+A172F



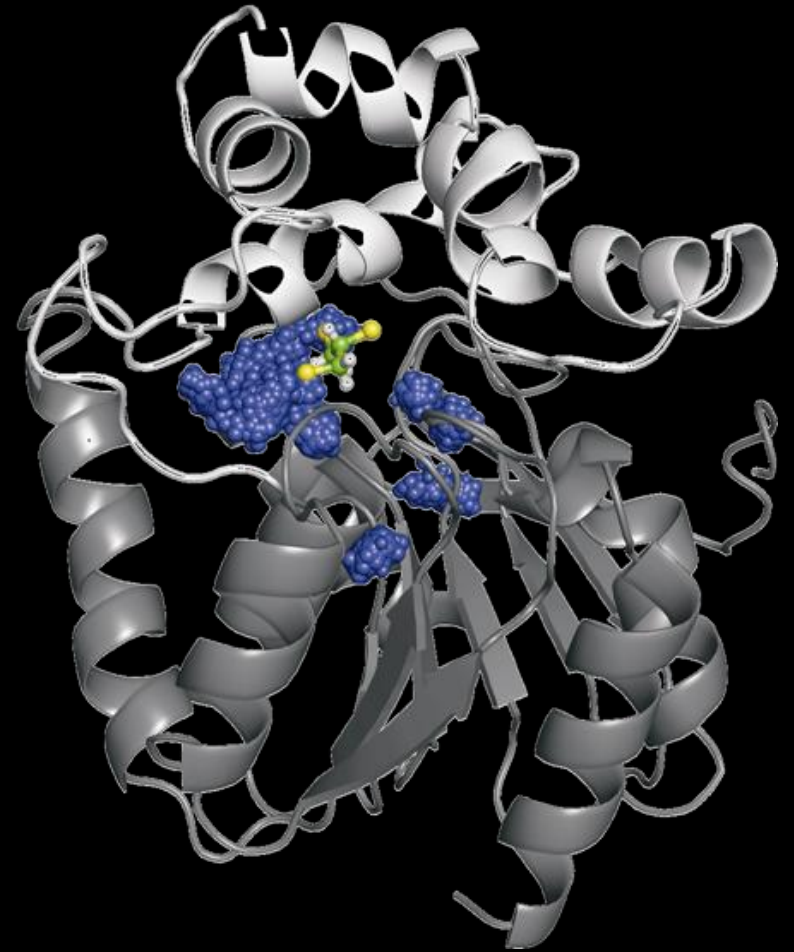
Catalytic efficiency of constructs



Desolvation of active site pocket



wt



DhaA31

Conclusions

- **Keyhole-lock-key** – new concept for catalysis by enzymes with buried sites
- **CAVER & HOTSPOT WIZARD** – software tools for protein engineering
- **Engineering tunnels** – optimized catalytic and biorecognition elements for SB

Collaborators

Modelling

- Jiri Sochor (Masaryk University, Czech Republic)
- Michal Otyepka (University Palackeho, Czech Republic)
- Rebecca Wade (EML, Germany)

Experiments

- Martin Hof (Heyrovsky Institute, Czech Republic)
- Yuji Nagata (Tohoku University, Japan)

Genetic team



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Bioinformatics team



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Chovancová



Antonín
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Petr
Jaša



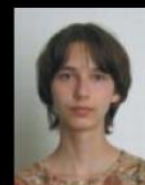
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Wolný



Jiří
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Mozga



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Hasan



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Veronika
Štěpánková



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Assistant



Monika Straková
Technician

Modelling team



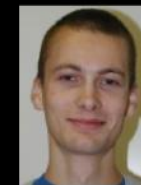
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Jan
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Biedermannová



Martin
Klvaňa



Lukáš
Daniel