Modelling of Product Release and Identification of Export Routes in Haloalkane Dehalogenase DhaA

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1,2,3-Trichloropropane (TCP)

Dangerous environmental pollutant

Propylene → epichlorohydrin (93 %) + TCP (7 %)

Biodegradation by haloalkane dehalogenase DhaA

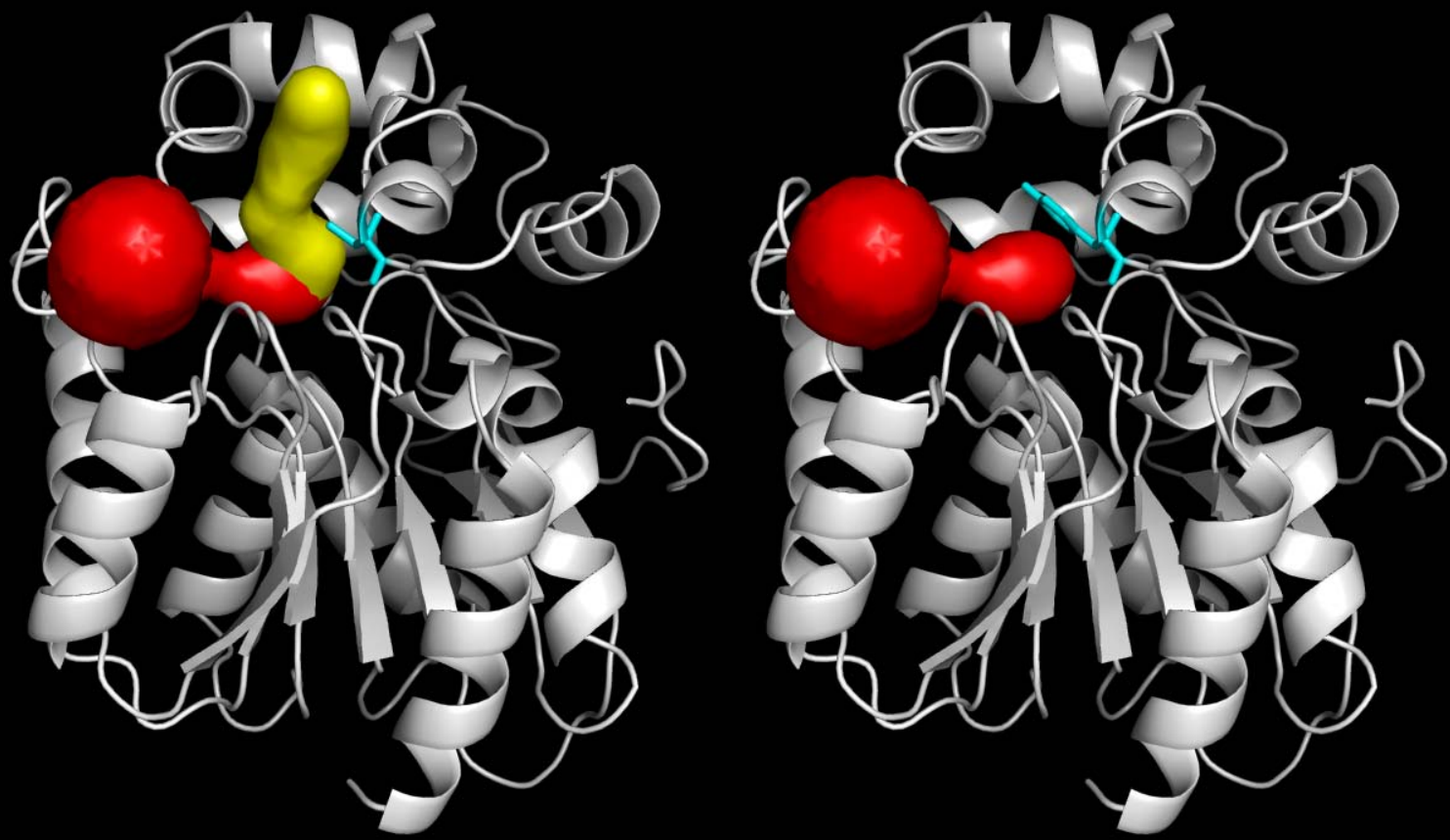
Low activity for commercial use

DhaA variant	Relative activity
WT	1
Bosma[1]	3.5
Gray[2]	4

[1]Bosma et al. (2002) *App.Environ.Microbiol. 68*, 3582-3587.
[2]Gray et al. (2001) *Adv.Synth.Catal. 343*, 607-616.

Why Export Routes?

- Main tunnel
- Slot



C176 (WT)

Y176 (Bosma, Gray)

Export of Cl^- through the Main Tunnel

1. Equilibration MD

Time: 1700 ps

Export of DCL through the Main Tunnel

2 RAMD

Export of DCL through the Slot

2 RAMD

Time: 00 ps

Re-design of Export Routes

Main tunnel:

- C176Y

Slot:

- W141F
- I135X
- L245X
- V246X

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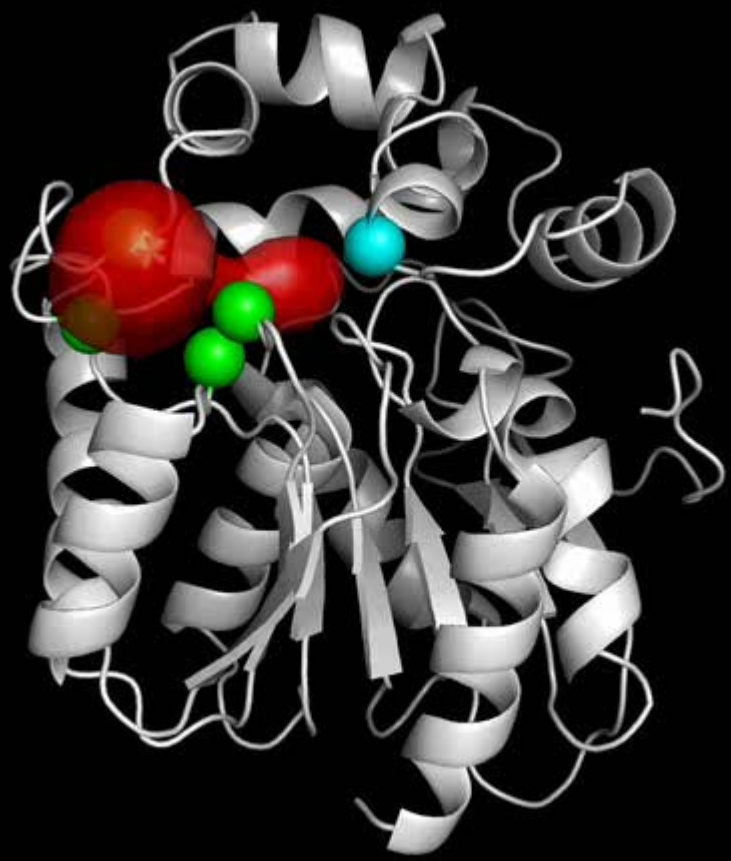
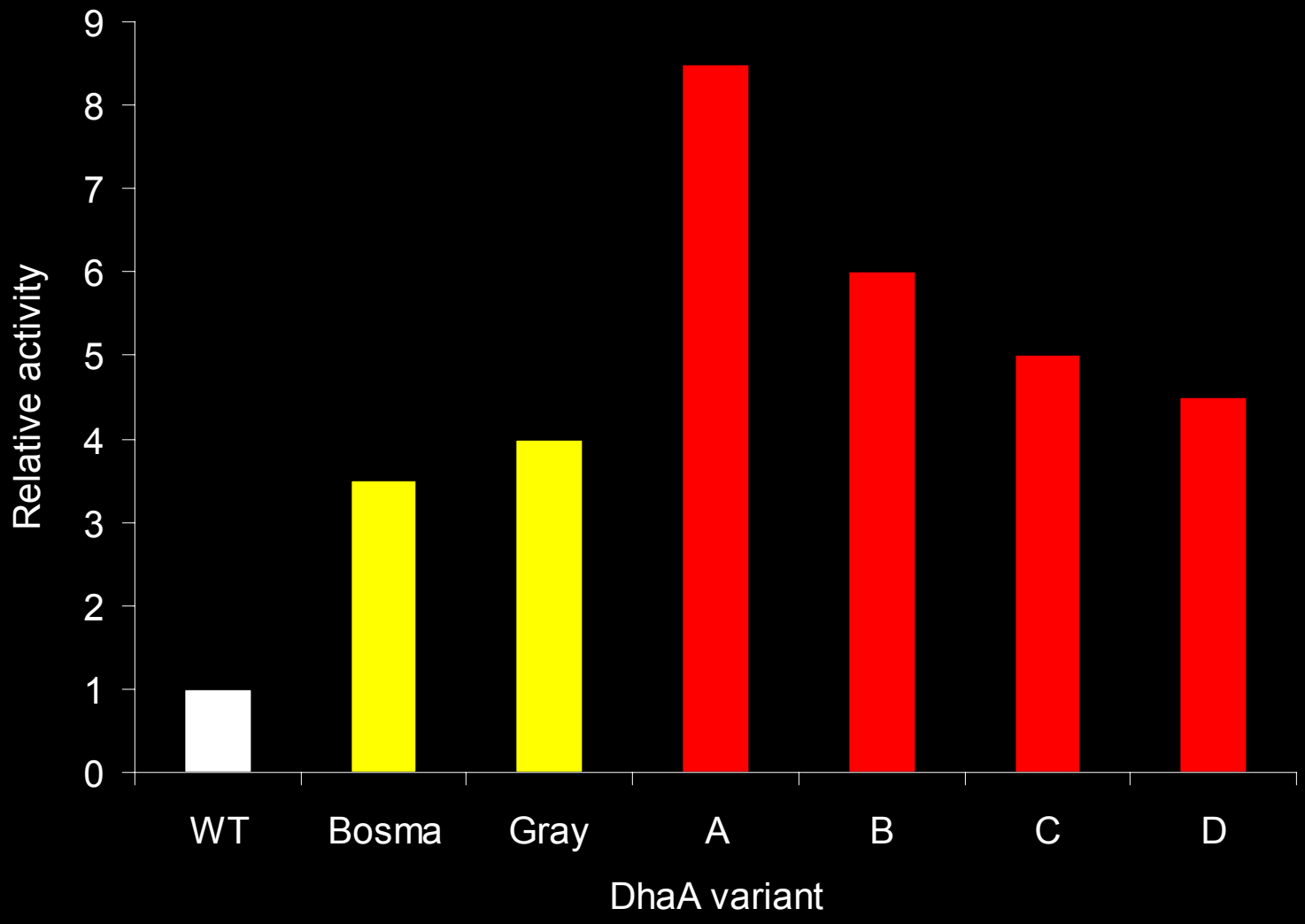


Mutagenesis

- Site-directed mutagenesis
- Directed evolution

Mutant	C176Y	W141F	I135X	V245X	L246X
A	✓	✓	F	M	I
B	✓	✓	Y	M	I
C	✓	✓	F	F	I
D	✓	✓	L	F	I

Mutagenesis



Conclusions

RAMD provides useful information for protein design

Order of product release is 1. Cl^-, 2. DCL

Export route for Cl^- is main tunnel

Export route for DCL is main tunnel and slot

Slot residues selected for mutagenesis are I135, W141, V245 and L246

The best mutant has 2-fold higher activity than mutant of Gray *et al.*