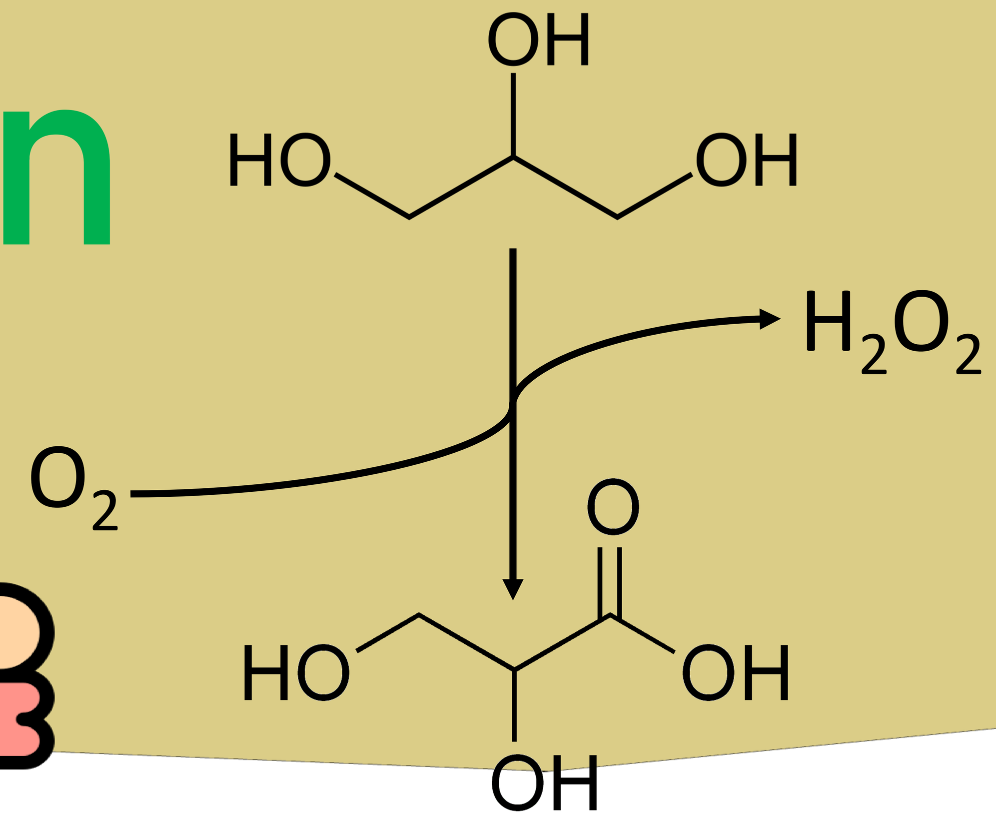
Towards a Green Detergent



Discovery by cell-free protein synthesis and biochemical characterization of thermostable glycerol oxidases

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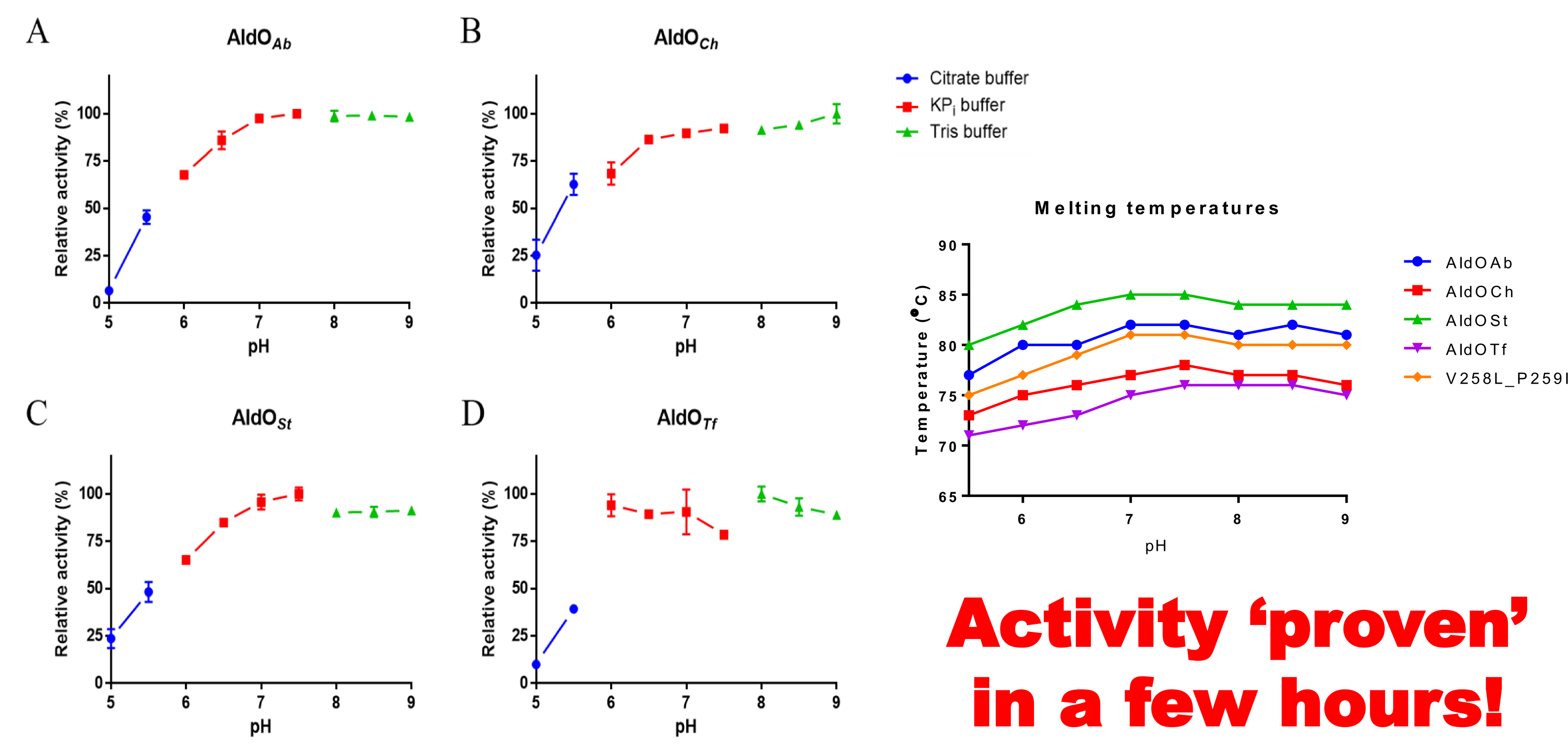
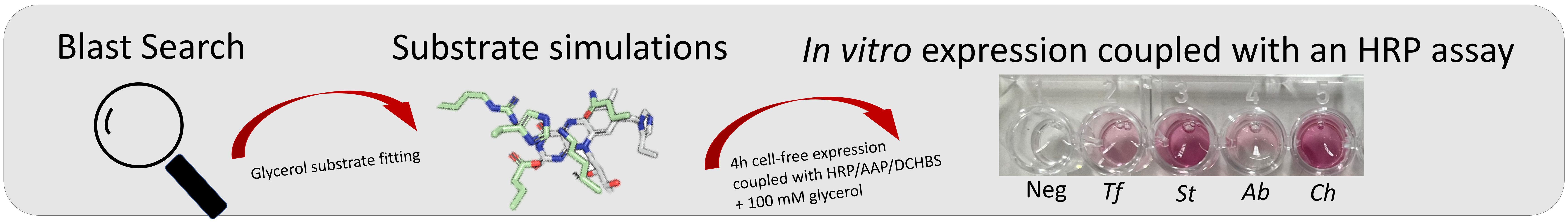
A sticky situation

The market size of detergents in 2020 was roughly 7 billion USD[1]. A substantial portion of these detergents ultimately enters waste-water streams, and owing to their limited biodegradability, they contribute to the decline of natural water quality[2]. Especially chemicals used for antibacterial properties are harmful for the environment. To avoid the use of these chemicals, we sought out to find new glycerol oxidases to create hydrogen peroxide as bactericidal agent in detergents. By integrating in silico biopros-pecting, cell-free protein synthesis and activity screening, an effective pipeline was developed to rapidly identify glycerol oxidases.

Characterization



Activity 'proven' in a few hours!



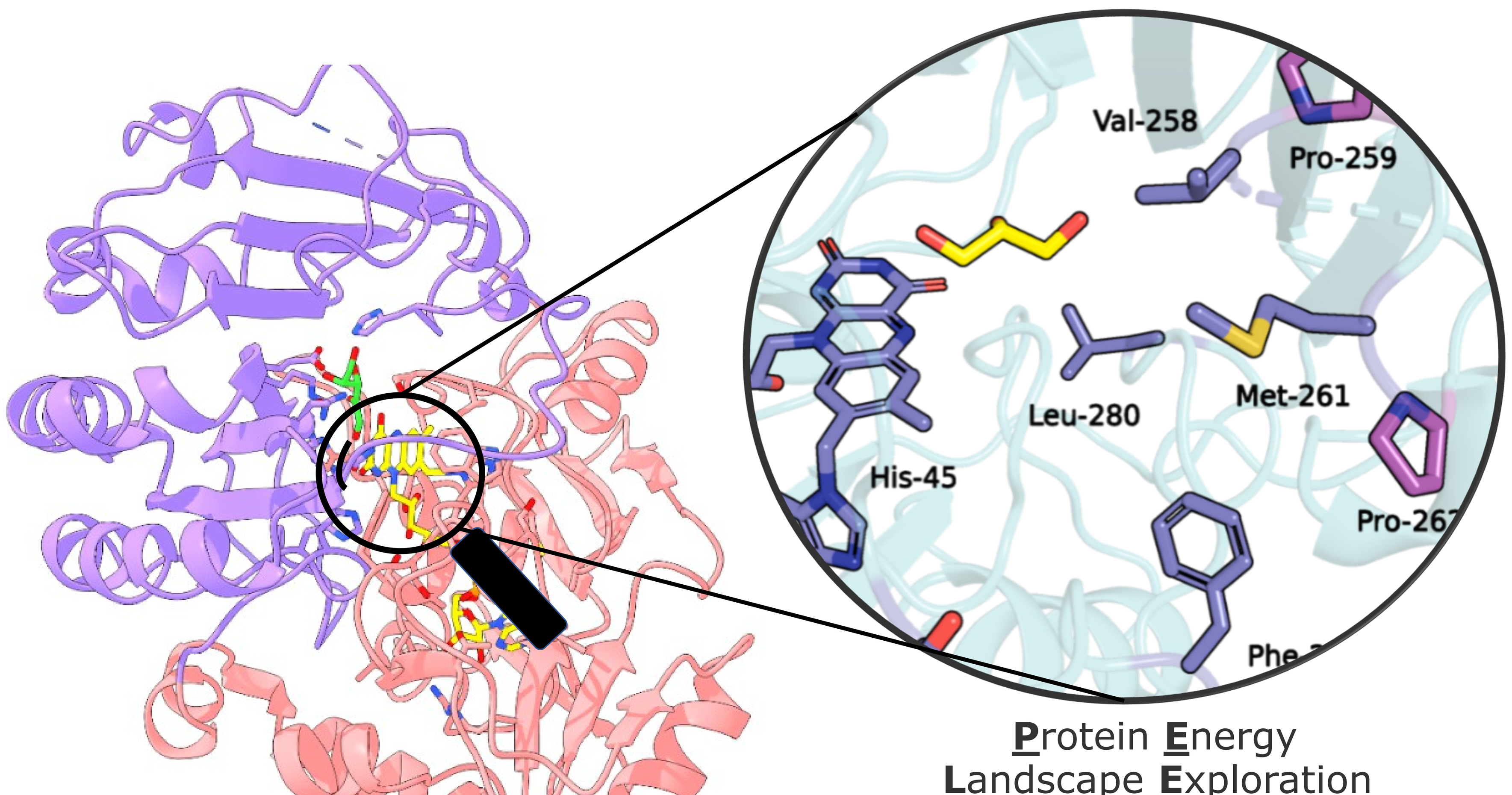
Steady-state kinetics are everything

	Organism of origin	Yield (mg/L culture)	K_M (mM)		k_{cat} (s^{-1})		k_{cat}/K_M ($M^{-1} s^{-1}$)		references
			xylitol	glycerol	xylitol	glycerol	xylitol	glycerol	
$AldO_{Sc}$	*S. coelicolor*	*350* (but a low thermostability)	0.32	350	13	1.6	4.1×10^4	4.6	Heuts et al. 2007[3]
$AldO_{Ac}$	*A. cellulolyticus*	*2,5*	0.07	270	1.9	1.3	2.7×10^4	4.8	Winter et al. 2012[4]
$AldO_{Ab}$	*A. bacterium*	*75*	0.03	184	4.2	2.6	14×10^4	14	this study
$AldO_{Ch}$	*T. chromogena*	*30*	0.04	143	3.5	2.0	12×10^4	14	this study
$AldO_{St}$	*S. thermoviolaceus*	*71*	0.02	523	1.9	4.2	9.5×10^4	8	this study
$AldO_{Tf}$	*T. flexuosa*	*300*	0.03	50	3.1	1.6	10×10^4	32	this study
V258L_P259I $AldO_{Tf}$	*T. flexuosa*	*300*	0.04	41	4.3	4.0	11×10^4	98	this study
V257L_P258I $AldO_{Ab}$	*A. bacterium*	*72*	n.d.	157	n.d.	1.4	n.d.	9	this study

Conclusion

- A new pipeline was developed by integrating computational bioprospecting, in vitro expression and an oxidase assay to quickly discover novel glycerol oxidases.
- Three thermostable alditol oxidases active on glycerol where found (from *Actinobacteria* bacterium, *Streptomyces thermoviolaceus,* and *Thermostaphylospora chromogena).*
- A high resolution crystal structure of the alditol oxidase from the *Actinobacteria* isolate was obtained.
- A structure-inspired double mutant of the alditol oxidase from *Thermopolyspora flexuosa* was engineered and found to be the most efficient glycerol oxidase known.

Room for improvement

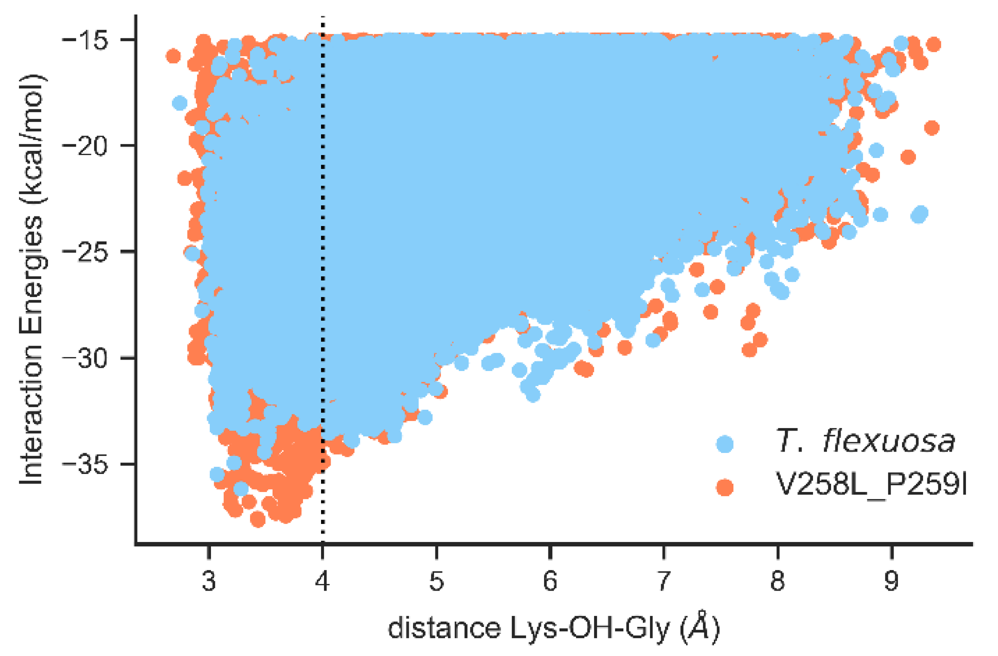


1.5 Å resolution structure of $AldO_{Ab}$

Protein **E**nergy **L**andscape **E**xploration

BSC

V258L P259I



More!

Our Lab

About me

Read details soon!
Manuscript submitted

[1] Liquid Laundry Detergent Market Size Report ID: FBI102962
[2] Mousavi S. A. & Khodadoost F. (2019) Effects of detergent on natural exosystems and wastewater treatment processes: a review. Environmental Science and Pollution research 26, 26439 - 26448
[3] Heuts, D. P. H. M., Hellemond, E. W. van, Janssen, D. B., & Fraaije, M. W. (2007). Discovery, characterization, and kinetic analysis of an alditol oxidase from *Streptomyces coelicolor*. J Biol Chem 282:20283–20291. https://doi.org/10.1074/jbc.M610849200
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