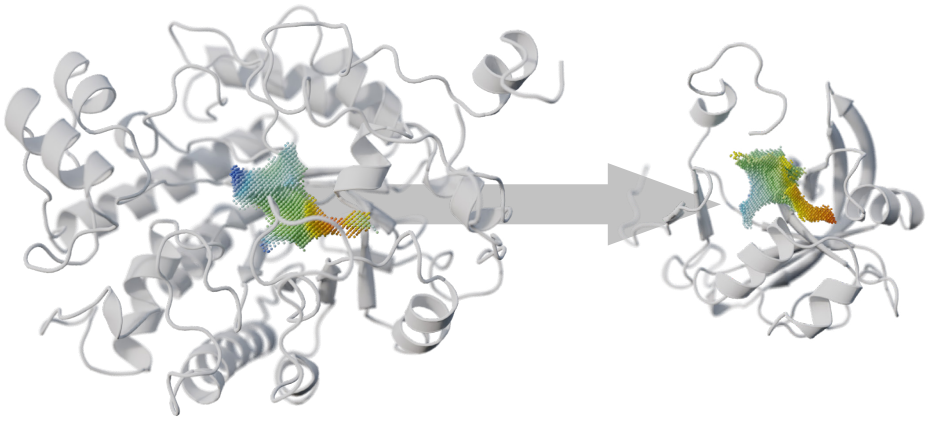
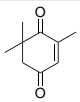
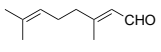
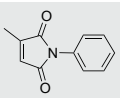


Overall structure and point cloud of search template (left) compared to newly identified enzyme (right).

Unexpected **Enzymatic Activity**

Back in 2014, we developed a structural bioinformatics method for predicting catalytic activities of enzymes based on three-dimensional constellations of functional groups in active sites ('catalophores'). As a proof-of-concept, we identified two enzymes with predicted promiscuous ene-reductase activity (reduction of activated C=C double bonds) and compared them with known ene reductases. Despite completely different amino acid sequences (sequence identity of 9%), overall structures and protein folds, high-resolution crystal structures revealed equivalent binding modes of typical Old Yellow Enzyme substrates and ligands and comparable catalytic activities. It was also possible to identify enzymes with switched enantioselectivity.

	***Ph*ENR**		***Tt*ENR**	
	c (%)	e.e.(%)	c (%)	e.e.(%)
	17	–	3	–
	28	87 (*S*)	81	4 (*S*)
	>99	15 (*S*)	45	12 (*S*)
	19	96 (*R*)	<1	n.d.
	77	62 (*S*)	86	77 (*S*)

c, conversion; e.e., enantiomeric excess; n.d., not determined

Selected substrates used to verify the ene reductase activity of the newly found enzymes (*Ph*ENR = ene reductase from *Pyrococcus horikoshii*, *Tt*ENR = ene reductase from *Thermus thermophilus*).

G. Steinkellner, C. C. Gruber, T. Pavkov-Keller, A. Binter, K. Steiner, C. Winkler, A. Lyskowski, O. Schwamberger, M. Oberer, H. Schwab, K. Faber, P. Macheroux, K. Gruber, *Nat. Commun.* 2014, **5**, 4150.