Rationalizing the enantioselectivity of aldoxime dehydratases

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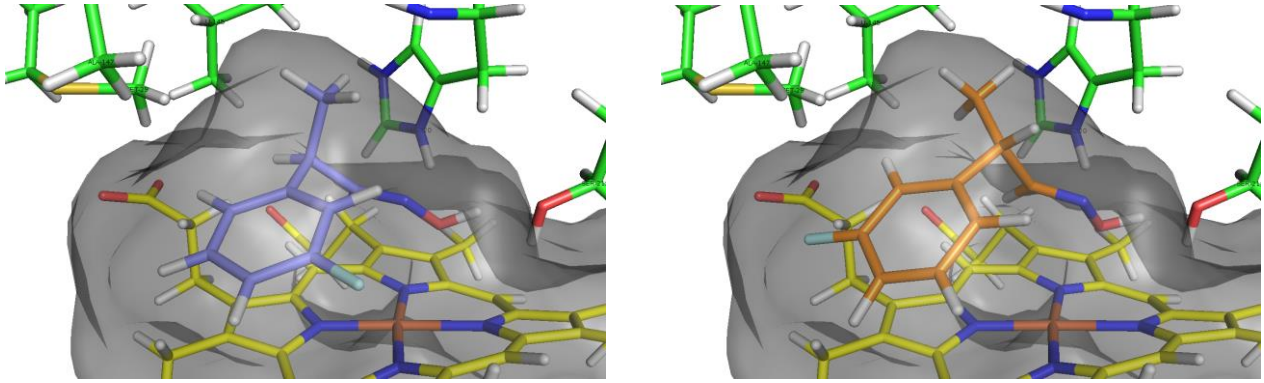
The advantages of enzyme-catalysis such as high enantioselectivity and mild reaction conditions are well known. In order to increase the potential of biocatalysis further, gaining a deep insight into the mechanism and catalytic properties of enzymes appears to be of high importance. Toward this end, *in silico* assays can be a powerful tool for protein engineering approaches. Latest experiments from *Betke et al.* [1] showed an unexpected phenomenon for the enantioselective dehydration of aldoximes under formation of nitriles: in dependency of the *E*- or *Z*- conformation of a racemic aldoxime, a switch of the enantiopreference was observed. Thus, starting from the same racemic aldehyde and albeit using the same aldoxime dehydratase as an enzyme, both enantiomers are accessible. Based on a general postulated mechanism for an aldoxime dehydratase by *Nomura et al.* [2], we focused on rationalizing this unusual switch in enzyme selectivity by means of docking experiments. As a software MOE (*Molecular Operating Environment*) was used to find suitable ligand-protein conformations. First, we defined *cut off*-values, which were determined by using the co-crystal from *Sawai et al*. [3] and considering *van-der-Waals*-radii of the interacting atoms being included in the postulated mechanism. All 28



ΔG= -6,420 kcal/mol
3-(*E*)-*R*-FPPOX

Δ Δ G= 0,367 kcal/mol
81 %ee

ΔG= -6,786 kcal/mol
3-(E)-*S*-FPPOX

Figure 1: Comparison of docked structure with OxdRE and 3-(*E*)-fluoro-phenylpropanal oxime (FPPOX) in *R* - and *S*- conformation.

phenylpropanal-oxime (PPOX) derivatives, which were used for the docking studies showed a privileged conformation in the active site. The methyl-group of these structures were nearly always localized inside a small cavity in the active site pocket. Furthermore, the ΔΔG values of the transition states when starting from the (*E*)- or (*Z*)- isomers were determined, thus enabling a prediction of the formed enantiomers. The experimental data are consistent with the docking result for example, 3-(*E*)-*rac*-FPPOX could be converted to 3-*S*-FPPN with 49 % conv. and 81 %ee. A hypothesis for the enzyme selectivity is that the methyl-group in the cavity causes (mainly) this energy difference.

[1] T. Betke, P. Rommelmann, K. Oike, Y. Asano and H. Gröger, *Angew.. Chem. (In. Ed.)* **2017**, 56, 12361-12366

[2] J. Nomura, H. Hashimotob, T. Ohtac, Y. Hashimotoa, K. Wadaa, Y. Naruta, K. Oinuma, and M. Kobayashi, *PNAS* **2013**, *110*, 2810-2815.

[3] H. Sawai, H. Sugimoto, Y. Kato, Y. Asano, Y. Shiro, and S. Aono, *J. Biol. Chem.* **2009**, *284*, 32089.