ENGINEERING THE ENANTIOSELECTIVE REDUCTION OF CITRAL ISOMERS IN NCR ENE REDUCTASE

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Ene reductases are members of the Old Yellow Enzyme family catalyzing the asymmetric reduction of activated C=C double bonds with high stereoselectivities. The selective reduction of citral to (*R*)-citronellal is of special interest as it is a cheap precursor for the industrially relevant aroma chemical (-)-menthol. The unsaturated aldehyde citral consists of the two isomers neral (*Z*-isomer) and geranial (*E*-isomer). Enantioconvergent reduction of both substrates to (*R*)-citronellal, the precursor for (-) menthol, is so far unachieved in catalysis. However, the Zymomonas mobilis ene reductase NCR exclusively reduces both citral isomers to (*S*)-citronellal with good activity. Recently, we observed a good mutability in NCR ene reductase. The principal possibility of an enzymatic enantioconvergent citral reduction in NCR inspired us to invert the selectivity by a semi-rational enzyme engineering approach.

As a first approach, a simple hydrophobic active site mutational analysis of the NCR active site disclosed tryptophan 66 as crucial position for alternative (*R*)-selective binding modes. The significance of this position was further analyzed by site-saturation mutagenesis and molecular docking simulation. Interestingly, the variations resulted in quite different responses for the two citral isomers underlying the necessity to screen for both as separate substrates. The preliminary screening provided hot spot regions that have been successfully combined in consecutive mutational approaches. These comprised iterative site-saturations and defined loop variations providing an ene reductase with inverted stereoselectivity.

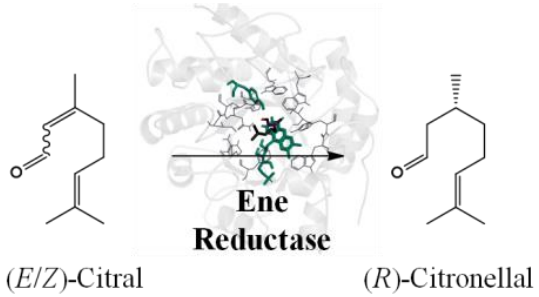

Figure 1 – Ene reductase catalyzed reduction of citral isomers to (R)-citronellal