

P.42. Enzymatic Redox Sequences in Asymmetric Synthesis – artificial enzyme cascades *in vitro* and *in vivo*

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Exploiting the diversity of enzymes and their manifold catalytic activities we introduced multi-enzymatic redox reactions in *E. coli* as well *in vitro* as *in vivo*.

The classical 'retrosynthetic' approach [1] is used in the strategic planning of organic syntheses by simplifying a target molecule into precursors by manipulation of functional groups. In a new interpretation [2] different redox enzyme classes are used as tools for functional group transformations and here to design new non-natural enzymatic cascades.

An artificial 'minipathway' starting with oxyfunctionalization of readily available limonene to carveol followed by oxidation to the corresponding α,β -unsaturated ketone via an alcohol dehydrogenase, an enoate reductase for the subsequent reduction of the double bond and concluded by a Baeyer-Villiger monooxygenase for the formation of the corresponding lactone is presented. This extension of the reported cascade [3] was tested in a mixed culture approach as limonene can be specifically hydroxylated by *C. cellulans* to carveol [4]. By this supplement a natural material can be converted to carvolactones. Additionally, we will demonstrate the variability of this approach by the selection of various substrates to depict important aspects of biocatalytic reactions such as achiral transformations, desymmetrization, and kinetic resolutions.

This proof-of-concept study was conducted to demonstrate the principal feasibility and to determine and resolve limiting aspects.

1 Warren, S., Wyatt, P., (2008) Organic Synthesis. The Disconnection Approach. Wiley-VCH, Weinheim

2 Turner, N.J., O'Reilly, E., (2013) Biocatalytic retrosynthesis. Nat Chem Biol 9, 285-288, 10.1038/nchembio.1235.

3 Oberleitner, N., Peters, C., Muschiol, J., Kadow, M., Saß, S., Bayer, T., Schaaf, P., Iqbal, N., Rudroff, F., Mihovilovic, M.D., Bornscheuer, U.T., (2013) An Enzymatic Toolbox for Cascade Reactions: A Showcase for an *In Vivo* Redox Sequence in Asymmetric Synthesis. ChemCatChem 5, 3524-3528, 10.1002/cctc.201300604.

4 Wang, Z., Lie, F., Lim, E., Li, K., Li, Z., (2009) Regio- and Stereoselective Allylic Hydroxylation of D-Limonene to (+)-trans-Carveol with Cellulosimicrobium cellulans EB-8-4. Advanced Synthesis & Catalysis 351, 1849-1856, 10.1002/adsc.200900210.