

L3-3 An enzymatic 'in vivo' toolbox for RedOx cascade reactions: from feedstock limonene to biorenewable polyesters

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Biocatalysis, mainly based on single-step transformations, has gained significant scientific attention over the last decades. Exploiting the manifoldness of enzymes and their different catalytic activities we present a 'general concept' study for the introduction of multi-enzymatic redox reactions *in vivo*.

The design of artificial biosynthetic pathways composed of naturally non-related enzymes is based on a classical 'retrosynthetic' approach. This technique is used in the strategic planning of organic syntheses by transforming a target molecule into simpler precursor whereas molecular complexity is reduced by manipulation of functional groups. The power of retrosynthetic analysis becomes evident in the design of a synthesis. In this particular case different redox enzyme classes are used as tools for such functional group transformations to design new non-natural enzymatic cascades.



Figure 1: Illustration of the developed artificial mini-pathway in *E. coli* towards biorenewable polyesters

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. Starting from the renewable feedstock limonene, a high valuable chiral carveolactone product, which has recently been investigated as monomer useful for the production of functional biorenewable polyesters was synthesized (Figure 1).

This particular sequence was realized fully *in vivo* as a proof-of-concept study to demonstrate the principal feasibility and to determine and resolve limiting aspects. Additionally, we will demonstrate the variability of this approach by the selection of various substrates to depict all important aspects of biocatalytic reactions such as achiral transformations, desymmetrization, and kinetic resolutions.