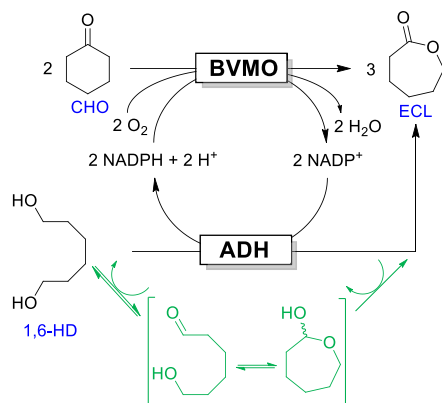


KURZFASSUNG DES POTENTIELLEN VORTRAGS

Title: Engineering strategies towards more efficient biotransformations

Biocatalytic cascades and one-pot multi-step (chemo)-enzymatic reactions have attracted growing interest due to several important advantages: (i) minimal isolation and purification steps required for obtaining more complex molecules, (ii) higher product yields and productivities achieved by such designs, and (iii) the chemo-, regio-, and stereoselectivity of various biocatalysts. The presentation focuses on the development of **novel reaction cascades** and application of **substrate-, medium-, enzyme- and reaction-engineering strategies**, as well as **combinations thereof**, to develop biotransformations that are more efficient. **Substrate engineering** aims at using alternative cosubstrates for enhancing the productivity of the reactions with a minimized environmental impact.



Scheme 1. Synthesis of ECL through a convergent cascade system.^[2]

Cofactor regeneration systems previously proposed with the intent to circumvent the use of stoichiometric amounts of expensive cofactors applied excess amounts of the cosubstrate (e.g., isopropanol, ethanol) and produced stoichiometric amounts of waste. The use of 1,4-butanediol (1,4-BD) as a '**smart cosubstrate**' for the cofactor regeneration was the next step towards more sustainable redox biocatalysis.^[1]

The biocatalytic cascade reactions reported so far fall into four different categories: (i) linear, (ii) orthogonal, (iii) parallel, and (iv) cyclic. Bringing this novel '**smart cosubstrate**' concept one step further, a new class of redox-neutral reactions, called a '**convergent cascade**' was designed, involving bi-substrate and a single product without the formation of an intermediate (Scheme 1). The

convergent cascade was developed for the production of ϵ -caprolactone (ECL), consisting of a Baeyer-Villiger monooxygenase (BVMO) for oxidation of cyclohexanone (CHO), as well as an alcohol dehydrogenase (ADH) for oxidation of the '**double-smart cosubstrate**' 1,6-hexanediol (1,6-HD) and for simultaneous regeneration of the nicotinamide cofactor.^[2]

The next part of the presentation focuses on **medium engineering** for enhancing the productivity of the biotransformations by conducting them in **low-water media** (e.g., in organic solvents or neat substrates). Recently, we reported on highly productive biocatalytic reductions catalyzed by an isolated ADH using the '**smart cosubstrate**' 1,4-BD for cofactor regeneration in water-deficient media. By doing so, a turnover number (TON) of 64,000 could be achieved.^[3] In a follow-up study, this reaction was successfully scaled up to 2 liters, yielding 150 g of isolated enantiopure ($ee \geq 99\%$ (S)) alcohol product.^[4]

In the context of **enzyme- and reaction engineering**, optimization of arylmalonate decarboxylase (AMDase)-catalyzed synthesis of α -arylpropionate derivatives (so-called profenes) is presented. **Enzyme engineering** herein focuses on biocatalyst optimization by means of immobilization. The operational stability of the (S)-selective AMDase variant was improved by a factor of 160 by covalent immobilization.^[5] Characterization of the system in respect to reaction engineering revealed that an efficient process can be developed without *in situ* product removal, despite the fact that the enzyme is competitively inhibited by the product. A highly productive biocatalytic synthesis of (S)-naproxen with a total turnover number (TTN) of 100,000 in 100 mL scale was realized in a stirred tank reactor.^[6]

[1] Kara et al., **2013**. *Green Chem.* 15, 330–335; Kara et al., **2014**. *Appl. Microbiol. Biotechnol.* 98, 1517–1529.

[2] Bornadel et al., **2015**. *ChemCatChem*, 7, 2442–2445; Bornadel et al., **2016**. *Tetrahedron* 72, 7222–7228.

[3] Kara et al., **2014**. *ChemCatChem* 6, 973–976.

[4] Zuhse et al., **2015**. *Org. Process Res. Dev.* 2015, 19, 369–372.

[5] Aßmann et al., **2016**. *Front. Microbiol.* In revision.

[6] Aßmann et al., **2017**. In preparation.