

# Synthetic enzyme cascades - a modular approach

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Enzymatic multi-step reactions offer significant potential to yield industrially relevant chiral intermediates and building blocks with excellent stereoselectivities. This presentation focuses on the development of synthetic enzyme cascades for the production of pharmaceutically valuable products with multi-chiral centres and strategies to increase ecologic and economic efficiency of such processes.

It is a big advantage of enzymes, that they are highly chemo- and stereoselective. Setting up toolboxes encompassing enzymes with the same reaction type but a varying substrate range or stereoselectivity, a broad range of products with high optical purity is accessible. The flexible combination of enzymes from these toolboxes to cascades enables the access of complex products with multi chiral centres. This flexible combination of enzymes from toolboxes is the big advantage of modular synthetic cascade approach. Exemplarily, the synthesis of all four stereoisomers of pharmaceutically active nor(pseudo)ephedrine<sup>1,2</sup> with high optical purities ( $ee/de >99\%$ / $>98\%$ ) and good conversions (95 %) is presented.

Cascades with highly active and selective catalysts run under optimal reaction conditions might already meet industrial demands of high space-time-yields and excellent product purity. Still, for industrial scale, catalysts production should be predominantly cheaper and product concentrations higher than mostly feasibly with current methods. Therefore we intensely investigate the applicability of whole cell cascade approaches in micro-aqueous reactions systems. Such micro-aqueous systems do not only allow addition of high amounts of poorly water-soluble substrates, but also facilitate downstream processing. The implementation of lyophilised whole cells cuts costs on catalyst production and circumvents the need of cofactor addition. As an example, the production of vicinal diols from cheap aldehydes was run in micro-aqueous organic solvents with product concentration up to 440 mM and space-time-yields up to 330 g L<sup>-1</sup> d<sup>-1</sup> with excellent stereoselectivity ( $ee/de >99\%$ / $>99\%$ )<sup>3</sup>. Furthermore, an easy modularisation of whole cells in teabags enables fast screening of optimal reaction parameters and enzyme combinations<sup>4</sup>. Those teabags are easy storable and recyclable.

Besides enzyme engineering and optimisation of reaction parameters, process engineering and especially the selection of optimal reaction modes and cosubstrates can increase atom- and step-economy immensely. To gain high economic and ecologic efficiency, a recycling cascade mode was developed<sup>1</sup>, in which the given byproduct of the cascades' second step is recycled into the first step of the cascade.

<sup>1</sup> T. Sehl *et al.* (2013). *Angew. Chem. Int. Ed.* 52: 6772-75

<sup>2</sup> T. Sehl *et al.* (2014). *Green Chem.* 16: 3341-48.

<sup>3</sup> A. Jakoblinnert, D. Rother (2014). *Green Chem.* 16: 3472-82

<sup>4</sup> J. Wachtmeister *et al.* (2014). *ChemCatChem* 6: 1051-58