Title: Co-expression of an alcohol dehydrogenase and a cyclohexanone monooxygenase for cascade reactions facilitates the regeneration of the NADPH cofactor

Authors: Anna Kohl, Vishnu Srinivasamurthy, Dominique Böttcher, Johannes Kabisch, Uwe T. Bornscheuer

PII: S0141-0229(17)30176-X
DOI: http://dx.doi.org/10.1016/j.enzmictec.2017.09.003
Reference: EMT 9128

To appear in: *Enzyme and Microbial Technology*

Received date: 12-6-2017
Revised date: 22-8-2017
Accepted date: 10-9-2017

Please cite this article as: Kohl Anna, Srinivasamurthy Vishnu, Böttcher Dominique, Kabisch Johannes, Bornscheuer Uwe T.Co-expression of an alcohol dehydrogenase and a cyclohexanone monooxygenase for cascade reactions facilitates the regeneration of the NADPH cofactor.*Enzyme and Microbial Technology* http://dx.doi.org/10.1016/j.enzmictec.2017.09.003

Co-expression of an alcohol dehydrogenase and a cyclohexanone monooxygenase for cascade reactions facilitates the regeneration of the NADPH cofactor

Anna Kohl[a], Vishnu Srinivasamurthy[a], Dominique Böttcher[a], Johannes Kabisch[b], Uwe T. Bornscheuer[a,*]

[a]*Institute of Biochemistry, Department of Biotechnology & Enzyme Catalysis, Greifswald University, Felix-Hausdorff-Str. 4, 17489 Greifswald, Germany*

[b]*Department of Biology, Computer-aided Synthetic Biology, Technische Universität Darmstadt, Schnittspahnstr. 10, 64287 Darmstadt, Germany*

[*]Corresponding author: Institute of Biochemistry, Department of Biotechnology & Enzyme Catalysis, Greifswald University, Felix-Hausdorff-Str. 4, 17489 Greifswald, Germany. Fax: +49 3834 420 794367. *E-mail address*: uwe.bornscheuer@uni-greifswald.de (U. T. Bornscheuer).

Highlights:

- A cyclohexanone monooxygenase (CHMO) and an alcohol dehydrogenase (ADH), were successfully cloned into the vector pRSFDuet.
- Expression of CHMO and ADH in *E. coli* cells using this Duet vector enabled complete substrate conversion without the necessity of cosubstrates for cofactor recycling.
- Ribosomal binding site engineering (RBS) enabled balanced expression levels of CHMO and ADH.
- In whole-cell biocatalysis, a RBS mutant of ADH together with the CHMO exhibited faster substrate conversion and self-sufficient cofactor recycling of NADPH was confirmed.

Abstract

The introduction of a three-enzyme cascade (comprising a cyclohexanone monooxygenase (CHMO), an alcohol dehydrogenase (ADH) and a lipase (CAL-A)) for the production of oligo-ε-caprolactone provided self-sufficiency with respect to NADPH-cofactor regeneration and reduced inhibiting effects on the central CHMO enzyme. For further optimization of cofactor regeneration, now a co-expression of CHMO and ADH in *E. coli* using a Duet™ vector was performed. This led to higher conversion values of the substrate cyclohexanol in whole-cell biocatalysis compared to an expression of both enzymes from two separate