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(49) Grobe S., Badenhorst C., Bayer T., Hamnevik E., Wu S., Grathwol C, Link A, **Koban S., Brundiek H.**, Großjohann B., Bornscheuer U. **Engineering Regioselectivity of a P450 Monooxygenase Enables the Synthesis of Ursodeoxycholic Acid via 7b-Hydroxylation of Lithocholic Acid, (2020), doi.org/10.1002/anie.202012675**

Abstract: *We engineered the cytochrome P450 monooxygenase CYP107D1 (OleP) from Streptomyces antibioticus for the stereo- and regioselective 7-hydroxylation of lithocholic acid (LCA) to yield ursodeoxycholic acid (UDCA). OleP was previously shown to hydroxylate testosterone at the 7-position but LCA is exclusively hydroxylated at the 6-position, forming murideoxycholic acid (MDCA). Structural and 3DM analysis, and molecular docking were used to identify amino acid residues F84, S240, and V291 as specificity-determining residues. Alanine scanning identified S240A as a UDCA-producing variant. A synthetic "small but smart" library based on these positions was screened using a colorimetric assay for UDCA. We identified a nearly perfectly regio- and stereoselective triple mutant (F84Q/S240A/V291G) that produces 10-fold higher levels of UDCA than the S240A variant. This biocatalyst opens up new possibilities for the environmentally friendly synthesis of UDCA from the biological waste product LCA.*

(45) Grobe S., Wszołek A., **Brundiek H.**, Fekete M., Bornscheuer U.T.
Highly selective bile acid hydroxylation by the multifunctional bacterial P450 monooxygenase CYP107D1, (2020), doi: 10.1007/s10529-020-02813-4

Abstract: *AA textile-based reaction system for new peroxidase reactions in non-native media was implemented. The epoxidation of cyclohexene by the commercial peroxidase MaxiBright® was realized with the textile-immobilized enzyme in an adapted liquid-liquid two-phase reactor. A commercially available polyester felt was used as low-price carrier and functionalized with polyvinyl amine. The covalent immobilization with glutardialdehyde lead to an enzyme loading of 0.10 genzyme/gtextile. The textile-based peroxidase shows a high activity retention in the presence of organic media. This catalyst is shown to enable the epoxidation of cyclohexene in various solvents as well as under neat conditions. A model reactor was produced by 3D printing which places the textile catalyst at the interphase between the liquid reaction phase and the product extracting solvent.*

(37) Oroz-Guinea I., Zorn K., **Brundiek H.**,
Protein engineering of enzymes involved in lipid modification, chapter 2 in Lipid Modification by Enzymes and Engineered Microbes, 2018, pp 11–43

Abstract: *This chapter provides an overview of the most important protein engineering tools and strategies applied to enzymes involved in lipid modification. Thus, application of several methodologies is discussed, including directed evolution, rational protein design, construction of chimeric enzymes, and de novo design, together with the utility of bioinformatic tools along the protein engineering process. Additionally, different approaches for the creation of semirational libraries and the potential use of different high-throughput screenings for the detection of improved protein variants are described. The examples provided cover the recent achievements in the utilization of these techniques on a broad variety of enzymes. This comprises not only engineered lipases with altered fatty acid chain length selectivity, fatty acid specificity, and improved performance in esterification reactions, but also successfully altered phospholipases, lipoxygenases, P450 monooxygenases, decarboxylating enzymes, and fatty acid hydratases.*

(29) Sehl T., Bock S., Marx L., Maugeri Z., Walter L., Westphal R., Vogel C., **Menyes U.**, Erhardt M., Muller M., Pohl M., Rother D. (2016), Asymmetric synthesis of (S) phenylacetylcarbinol – closing a gap in C-C bond-formation, Green Chemistry, Green Chem., 2017,19, 380-384, DOI: 10.1039/C6GC01803C

Abstract: *(S)-Phenylacetylcarbinol [(S)-PAC] and its derivatives are valuable intermediates for the synthesis of various APIs (active pharmaceutical ingredients), however their selective synthesis is challenging. As no highly selective enzymes or chemical catalysts for the stereoselective synthesis of (S)-PAC were available, we tailored a potent biocatalyst by semi-rational enzyme engineering to a stereoselectivity of >97 % for the (S)-PAC synthesis by enzyme design. Together with reaction- and process optimisation industrially relevant product concentrations >48 g/L (up to 320 mM) could be gained. In addition, the best enzyme variant gave access to a broad range of ring-substituted (S)-PAC derivatives with high stereoselectivity, especially for meta-substituted products.*

(27) Zorna K., Oroz-Guinea I., **Brundiek H.**, Bornscheuer U. T. (2016)
Engineering and Application of Enzymes for Lipid Modification, an Update, Progress in Lipid Research, 2016 Jul;63:153-64. DOI:10.1016/j.plipres.2016.06.001

Abstract: *This review first provides a brief introduction into the most important tools and strategies for protein engineering (i.e. directed evolution and rational protein design combined with high-throughput screening methods) followed by examples from literature, in which enzymes have been optimized for biocatalytic applications. This covers engineered lipases with altered fatty acid chain length selectivity, fatty acid specificity and improved performance in esterification reactions. Furthermore, recent achievements reported for phospholipases, lipoxygenases, P450 monooxygenases, decarboxylating enzymes, fatty acid hydratases and the use of enzymes in cascade reactions are treated.*

(26) Brundiek H.., Höhne M. (2016)
„Transaminases–A Biosynthetic Route for Chiral Amines." in: Applied Biocatalysis: From Fundamental Science to Industrial Applications, Wiley-VCH, ISBN 9783527336692

Abstract: *No abstract available*

(25) Gand M., Thöle Ch., Müller H., **Brundiek H.**, Bashiric G., Höhne M. (2016)
A NADH-accepting imine reductase variant - immobilization and cofactor regeneration by oxidative deam¬ination, Journal of Biotechnology, Volume 230, 20 July 2016, Pages 11–18.

Abstract: *Engineering cofactor specificity of enzymes is a promising approach that can expand the application of enzymes for biocatalytic production of industrially relevant chemicals. Until now, only NADPH-dependent imine reductases (IREDs) are known. This limits their applications to reactions employing whole cells as a cost-efficient cofactor regeneration system. For applications of IREDs as cell-free catalysts, (i) we created an IRED variant showing an improved activity for NADH. With rational design we were able to identify four residues in the (R)-selective IRED from Streptomyces GF3587 (IR-Sgf3587), which coordinate the 2'-phosphate moiety of the NADPH cofactor. From a set of 15 variants, the highest NADH activity was caused by the single amino acid exchange K40A resulting in a 3-fold increased acceptance of NADH. (ii) We showed its applicability using an immobilisate obtained either from purified enzyme or from lysate using the EziG™ carriers. Applying the variant and NADH, we reached 88% conversion in a preparative scale biotransformation when employing 4% (w/v) 2-methylpyrroline. (iii) We demonstrated a one-enzyme cofactor regeneration approach using the achiral amine N-methyl-3-aminopentanone as a hydrogen donor co-substrate.*

(22) Müller J., Sowa M., Fredrich B., **Brundiek H.**, Bornscheuer U.T., (2015)
Enhancing the Acyltransferase Activity of Candida antarctica Lipase A by Rational Design, ChemBioChem, DOI: 10.1002/cbic.201500187

Abstract: *Keywords: Acyltransferase; CAL-A; enzyme catalysis; ester synthesis; immobilization*
Few lipases like the Candida antarctica lipase A (CAL-A) are known to possess an acyltransferase activity. This activity enables the enzyme to synthesize fatty acid esters from natural oils and alcohols even in the presence of bulk water. Unfortunately, still fatty acids are formed in these reactions as undesired side product. To reduce the amount of fatty acids, several CAL-A variants were rationally designed based on its crystal structure. These variants were expressed in Escherichia coli and Pichia pastoris, purified and investigated concerning their acyltransferase/hydrolase activity via various biocatalytic approaches. Among the investigated variants, the single mutant Asp122Leu showed a significant decrease in the hydrolytic activity, reducing the side product yield during acylation reactions. As desired, this variant maintained process relevant features like pH-profile or thermostability similar to the wild-type.

(19) Gand M., Müller H., **Wardenga R.**, Höhne M.,(2014)
Characterization of three novel enzymes with imine reductase activity, Journal of Molecular Catalysis B: Enzymatic, 110, 126-132.

Abstract: *Imine reductases (IRED) are promising catalysts for the synthesis of optically pure secondary cyclic amines. Three novel IREDs from Paenibacillus elgii B69, Streptomyces ipomoeae 91-03 and Pseudomonas putida KT2440 were identified by amino acid or structural similarity search, cloned and recombinantly expressed in E. coli and their substrate scope was investigated. Beside the acceptance of cyclic amines, also acyclic amines could be identified as substrates for all IREDs. For the IRED from Pseudomonas putida, a crystal structure (PDB-code 3L6D) is available in the database, but the function of the protein was not investigated so far. This enzyme showed the highest apparent E-value of approximately Eapp=52 for (R) methylpyrrolidine of the IREDs investigated in this study. Thus, an excellent enantiomeric purity of >99%eeP and 97% conversion was reached in a biocatalytic reaction using resting cells after 24h. Interestingly, a histidine residue could be confirmed as a catalytic residue by mutagenesis, but the residue is placed one turn aside compared to the formally known position of the catalytic Asp187 of Streptomyces kanamyceticus IRED.*

(18) Schallmey, M., Koopmeiners, J., Wells, E., **Wardenga, R.**, Schallmey, A. (2014)
Expanding the halohydrin dehalogenase enzyme family: Identification of novel enzymes by database mining. Applied and environmental microbiology, 80(23), 7303-7315.

Abstract: *Halohydrin dehalogenases are very rare enzymes which are naturally involved in the mineralization of halogenated xenobiotics. Due to their catalytic potential and promiscuity, many biocatalytic reactions have been described which have led to several interesting and also industrially important applications. Nevertheless, only a handful of these enzymes have been made available through recombinant techniques and hence it is of general interest to expand the repertoire of these enzymes to enable novel biocatalytic applications. After identification of specific sequence motifs, 37 novel enzyme sequences were readily identified in public sequence databases. All enzymes which could be heterologously expressed also catalyzed typical halohydrin dehalogenase reactions. Phylogenetic inference for enzymes of the halohydrin dehalogenase enzyme family confirmed that all enzymes form a distinct monophyletic clade within the short chain dehydrogenase/reductase superfamily. In addition, the majority of novel enzymes are substantially different to previously known phylogenetic subtypes. Consequently, four additional phylogenetic subtypes were defined which expand the halohydrin dehalogenase enzyme family at large. We show that the enormous wealth of environmental and genome sequences present in public databases can be tapped for the in silico identification of very rare but nonetheless biotechnologically important biocatalysts. Our findings help to readily identify halohydrin dehalogenases in ever growing sequence databases and, in consequence, make even more members of this interesting enzyme family available to the scientific and industrial community.*

(15) Schallmey, A., Schallmey, M., **Wardenga R.** (2013)
Identifikation neuartiger Halohydrin-Dehalogenasen. Biospektrum, 19 (7), 816-817.

Abstract: *Halohydrin dehalogenases (HHDHs) are biotechnologically relevant enzymes that can be applied as biocatalysts for the selective synthesis of various ß-substituted alcohols. Despite that fact, only very few HHDHs are currently available. In an attempt to identify novel ones, database mining of publicly available sequence databases was performed using HHDH-specific sequence information. As a result, 19 novel HHDHs were obtained all exhibiting true HHDH activity.*

(11) Mallin, H., **Menyes, U.**, Vorhaben, T., Höhne, M., Bornscheuer, U.T. (2013),
Immobilization of two (R)-amine transaminases on an optimized chitosan support for the enzymatic synthesis of optically pure amines, ChemCatChem, 5, 588-593.

Abstract: *Two (R)-selective amine transaminases from Gibberella zeae (GibZea) and from Neosartorya fischeri (NeoFis) were immobilized on chitosan as a carrier to improve their application in the biocatalytic synthesis of chiral (R)-amines. An (S)-selective enzyme from Vibrio fluvialis (VfTA) was used for comparison. After improving the immobilization conditions, all enzymes could be efficiently immobilized. Additionally, the thermal stability of GibZea and NeoFis could be improved and also a slight shift of the pH optimum was observed for GibZea. All enzymes showed good activity in the conversion of α-methylbenzylamine. In the asymmetric synthesis of (R)-2-aminohexane from the corresponding ketone, a 13.4-fold higher conversion (>99 %) was found for the immobilized GibZea compared to the free enzyme. Hence, the covalent binding with glutaraldehyde of these enzymes on chitosan beads resulted in a significant stabilization of the amine transaminases investigated.*

(9) Brundiek, H., Padhi, S.K., Evitt, A., Kourist, R., Bornscheuer, U.T. (2012),
Altering the scissile fatty acid binding site of Candida antarctica lipase A by protein engineering for the selective hydrolysis of medium chain fatty acids, Eur. J. Lipid Sci. Technol., 114, 1148-1153.

Abstract: *Candida antarctica lipase A (CAL-A) is the first representative of a new subclass of lipases because of its unique cap domain. The acyl-binding tunnel – having a short alternative binding region – is mainly formed by this domain. In order to create CAL-A variants with a high specificity for medium chain length (MCL) fatty acids (C6–C12), we used rational protein design to block the primary acyl-binding tunnel of CAL-A at position G237, which is near the junction to the alternative binding pocket. By closing the junction to the main tunnel, CAL-A variants (G237A/L/V/Y) have been created, which are highly specific for medium chain fatty acids (MCFAs) as determined by chain length profiles with p-nitrophenyl esters and triacylglycerides. Especially the CAL-A variants G237L/V/Y, in which the junction to the primary tunnel is completely closed, show a distinct preference for the hydrolysis of hexanoate esters. Hydrolytic activity for substrates with a chain length >C6 is suppressed extensively in mutants G237L/V/Y. Therefore, these highly MCL specific CAL-A variants may represent interesting biocatalysts for the production of MCL-derived esters for the food, flavor, and fragrance industry.*

Practical application: Since medium chain fatty acids (MCFAs: C6-C10) and their corresponding triacylglycerides (MCTs) provide quick access to energy and have been considered to be less implicated in the accumulation of body fat than long chain fatty acids, they represent interesting food additives. As functional oils, they are part of weight loss diets or are used in clinical nutrition. In the food industry MCTs are also utilized as storage stabilizing agents in cooking products, as release agents in food processing or as flavor diluent. Another interesting field of application of MCFA derived compounds, especially of C6 esters and alcohols, is as ingredients in flavors and fragrances. The CAL-A variants described in this study can be used for the biocatalytic synthesis of these compounds.

(8) Brundiek, H., Sass, S., Evitt, A., Kourist, R., Bornscheuer, U.T. (2012),
The short form of the recombinant CAL-A-type lipase UM03410 from the smut fungus Ustilago maydis exhibits an inherent trans fatty acid selectivity, Appl. Microb. Biotechnol., 94, 141-150; erratum: 94, 285.

Abstract: *The Ustilago maydis lipase UM03410 belongs to the mostly unexplored Candida antarctica lipase (CAL-A) subfamily. The two lipases with [...] the highest identity are a lipase from Sporisorium reilianum and the prototypic CAL-A. In contrast to the other CAL-A-type lipases, this hypothetical U. maydis lipase is annotated to possess a prolonged N-terminus of unknown function. Here, we show for the first time the recombinant expression of two versions of lipase UM03410: the full-length form (lipUMf) and an N-terminally truncated form (lipUMs). For comparison to the prototype, the expression of recombinant CAL-A in E. coli was investigated. Although both forms of lipase UM03410 could be expressed functionally in E. coli, the N-terminally truncated form (lipUMs) demonstrated significantly higher activities towards p-nitrophenyl esters. The functional expression of the N-terminally truncated lipase was further optimized by the appropriate choice of the E. coli strain, lowering the cultivation temperature to 20 °C and enrichment of the cultivation medium with glucose. Primary characteristics of the recombinant lipase are its pH optimum in the range of 6.5–7.0 and its temperature optimum at 55 °C. As is typical for lipases, lipUM03410 shows preference for long chain fatty acid esters with myristic acid ester (C14:0 ester) being the most preferred one. More importantly, lipUMs exhibits an inherent preference for C18:1Δ9 trans and C18:1Δ11 trans-fatty acid esters similar to CAL-A. Therefore, the short form of this U. maydis lipase is the only other currently known lipase with a distinct trans-fatty acid selectivity.*

(7) Brundiek, H.B., *Evitt, A.S., Kourist, R., Bornscheuer, U.T. (2012),*
Creation of a lipase highly selective for trans fatty acid by protein engineering, Angew. Chem. Int. Ed., 51, 412-414; Erzeugung einer für trans-Fettsäuren hochselektiven Lipase durch Protein-Engineering, Angew. Chem., 124, 425-428.

Abstract: *Keywords: enzyme catalysis; fatty acids; high-throughput screening; lipases; protein engineering biocatalytic process concept for ε-caprolactone*
Sorting out: Protein engineering of lipase CAL-A led to the discovery of mutants with excellent chemoselectivity for the removal of trans and saturated fatty acids from partially hydrogenated vegetable oil. These fatty acids, identified as a major risk factor for human health, can now be removed by enzyme catalysis.

(6) Leipold, F., **Wardenga, R.**, Bornscheuer, U.T. (2012),
Cloning, expression and characterisation of an eukaryotic cycloalkanone monooxygenase from Cylindrocarpon radicicola ATCC 11011, Appl. Microb. Biotechnol., 94, 705-717.

Abstract: *In this study, we have cloned and characterized a cycloalkanone monooxygenase (CAMO) from the ascomycete Cylindrocarpon radicicola ATCC 11011 (identical to Cylindrocarpon destructans DSM 837). The primary structure of this Baeyer–Villiger monooxygenase (BMVO) revealed 531 residues with around 45% sequence identity to known cyclohexanone monooxygenases. The enzyme was functionally overexpressed in Escherichia coli and investigated with respect to substrate spectrum and kinetic parameters. Substrate specificity studies revealed that a large variety of cycloaliphatic and bicycloaliphatic ketones are converted by this CAMO. A high catalytic efficiency against cyclobutanone was observed and seems to be a particular property of this BVMO. The thus produced butyrolactone derivatives are valuable building blocks for the synthesis of a variety of natural products and bioactive compounds. Furthermore, the enzyme revealed activity against open-chain ketones such as cyclobutyl, cyclopentyl and cyclohexyl methyl ketone which have not been reported to be accepted by typical cyclohexanone monooxygenases. These results suggest that the BVMO from C. radicicola indeed might be rather unique and since no BVMOs originating from eukaryotic organisms have been produced recombinantly so far, this study provides the first example for such an enzyme.*

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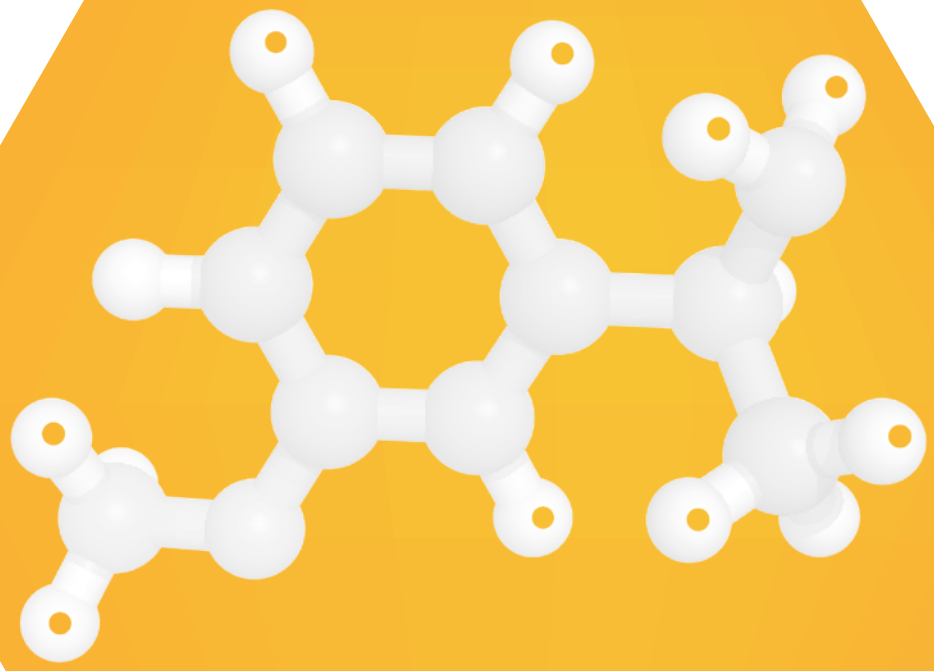

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(52) Calderini E., Süss P., Hollmann F., Wardenga R., Schallmey A. Two (chemo-)enzymatic cascades for the production of opposite enantiomers of chiral azidoalcohols (2021), doi.org/10.3390/catal11080982

Abstract: *Multi-step cascade reactions have gained increasing attention in the biocatalysis field in recent years. In particular, multi-enzymatic cascades can achieve high molecular complexity without workup of reaction intermediates thanks to the enzymes' intrinsic selectivity; and where enzymes fall short, organo- or metal catalysts can further expand the range of possible synthetic routes. Here, we present two enantiocomplementary (chemo)-enzymatic cascades composed of either a styrene monooxygenase (StyAB) or the Shi epoxidation catalyst for enantioselective alkene epoxidation in the first step, coupled with a halohydrin dehalogenase (HHDH)-catalysed regioselective epoxide ring opening in the second step for the synthesis of chiral aliphatic non-terminal azidoalcohols. Through the controlled formation of two new stereocenters, corresponding azidoalcohol products could be obtained with high regioselectivity and excellent enantioselectivity (99% ee) in the StyAB-HHDH cascade, while product enantiomeric excesses in the Shi-HHDH cascade ranged between 56 and 61%.*

(48) Ingenbosch K., Quint S., Dyllick-Brenzinger M., Wunschik D., Kiebist J., **Süss P.**, Liebelt U., Zuhse R., **Menyes U.**, Scheibner K., Mayer C., Opwis K., Gutmann J., Hoffmann-Jacobsen K.
Singlet-Oxygen Generation by Peroxidases and Peroxygenases for Chemoenzymatic Synthesis, (2020), doi.org/10.1002/cbic.202000326

Abstract: *Singlet oxygen is a reactive oxygen species undesired in living cells but a rare and valuable reagent in chemical synthesis. We present a fluorescence spectroscopic analysis of the singlet-oxygen formation activity of commercial peroxidases and novel peroxygenases. Singlet-oxygen sensor green (SOSG) is used as fluorogenic singlet oxygen trap. Establishing a kinetic model for the reaction cascade to the fluorescent SOSG endoperoxide permits a kinetic analysis of enzymatic singlet-oxygen formation. All peroxidases and peroxygenases show singlet-oxygen formation. No singlet oxygen activity could be found for any catalase under investigation. Substrate inhibition is observed for all reactive enzymes. The commercial dye-decolorizing peroxidase industrially used for dairy bleaching shows the highest singlet-oxygen activity and the lowest inhibition. This enzyme was immobilized on a textile carrier and successfully applied for a chemical synthesis. Here, ascaridole was synthesized via enzymatically produced singlet oxygen.*

(44) Sabry H., Younes H., Tieves F., Lan D., Wang Y., **Süss P., Brundiek H.**, Wever R., Hollmann F.
Chemoenzymatic Halocyclization of γ,δ-Unsaturated Carboxylic Acids and Alcohols (2019), doi.org/10.1002/cssc.201902240

Abstract: *A chemoenzymatic method for the halocyclization of unsaturated alcohols and acids by using the robust V-dependent chloroperoxidase from Curvularia inaequalis (CiVCPO) as catalyst has been developed for the in situ generation of hypohalites. A broad range of halolactones and cyclic haloethers are formed with excellent performance of the biocatalyst.*

(43) Sviatenko O., Ríos-Lombardía N., Morís F., González-Sabín J., Venkata Manideep K., Merdivan S., Günther S., **Süss P.**, Höhne M.
One-pot Synthesis of 4-Aminocyclohexanol Isomers by Combining a Keto Reductase and an Amine Transaminase (2019), DOI 10.1002/cctc.201900733

Abstract: *The efficient multifunctionalization by one-pot or cascade catalytic systems has developed as an important research field, but is often challenging due to incompatibilities or cross-reactivities of the catalysts leading to side product formation. Herein we report the stereoselective preparation of cis- and trans-4-aminocyclohexanol from the potentially bio-based precursor 1,4-cyclohexanedione. We identified regio- and stereoselective enzymes catalyzing reduction and transamination of the diketone, which can be performed in a one-pot sequential or cascade mode. For this, we identified regioselective keto reductases for the selective mono reduction of the diketone to give 4-hydroxycyclohexanone. The system is modular and by choosing stereocomplementary amine transaminases, both cis- and trans-4-aminocyclohexanol were synthesized with good to excellent diastereomeric ratios. Furthermore, we identified an amine transaminase that produces cis-1,4-cyclohexanediamine with diastereomeric ratios >98 : 2. These examples highlight that the high selectivity of enzymes enable short and stereoselective cascade multifunctionalizations to generate high-value building blocks from renewable starting materials.*

(42) Marx L., Rios-Lombardia N., **Süss P.**, Höhne M., Moris F., Gonzales-Sabin J., Berglund P.
Chemoenzymatic Synthesis of Sertraline (2019), DOI 10.1002/ejoc.201901810

Abstract: *A chemoenzymatic approach has been developed for the preparation of sertraline, an established anti-depressant drug. Ketoreductases (KREDs) were employed to yield a key chiral precursor. The bioreduction of the racemic tetralone exhibited excellent enantioselectivity (>99% ee) and diastereomeric ratio (99:1) at 29% conversion (the maximum theoretical yield is 50%) after 7 hours. The resulting (S,S)-alcohol was efficiently oxidized to an enantiopure (S)-ketone, an immediate precursor of sertraline, by using sodium hypochlorite as oxidant and 2-azaadamantane N-oxyl (AZADO) as organocatalyst. Alternative routes aiming at the direct biocatatalytic amination using imine reductases and transaminases were unsuccessful.*

(41) *Calderini E., Wessel J.,* **Süss P.**, *Schrepfer P.,* **Wardenga R.**, *Schallmey A.*
Selective Ring-Opening of Di-substituted Epoxides Catalysed by Halohydrin Dehalogenases; ChemCatChem (2019), DOI 10.1002/cctc.201900103

Abstract: *Halohydrin dehalogenases (HHDHs) are valuable biocatalysts for the synthesis of β-substituted alcohols based on their epoxide ring-opening activity with a number of small anionic nucleophiles. In an attempt to further broaden the scope of substrates accepted by these enzymes, a panel of 22 HHDHs was investigated in the conversion of aliphatic and aromatic vicinally di-substituted trans-epoxides using azide as nucleophile. The majority of these HHDHs was able to convert aliphatic methyl-substituted epoxide substrates to the corresponding azido-alcohols, in some cases even with absolute regioselectivity. HheG from Ilumatobacter coccineus exhibited also high activity towards sterically more demanding di-substituted epoxides. This further expands the range of β-substituted alcohols that are accessible by HHDH catalysis.*

(40) Zhang W., Fernandez Fueyo E.F., Hollmann F., Leemans-Martin L., Pesic M., **Wardenga R.**, Höhne M., Schmidt S., (2018)
Combining Photo-Organo Redox- and Enzyme Catalysis Facilitates Asymmetric C-H Bond Functionalization, European Journal of Organic Chemistry DOI: 10.1002/ejoc.201801692

Abstract: *In this study, we combined photo-organo redox catalysis and biocatalysis to achieve asymmetric C–H bond functionalization of simple alkane starting materials. The photocatalyst anthraquinone sulfate (SAS) was employed to oxyfunctionalise alkanes to aldehydes and ketones. We coupled this light-driven reaction with asymmetric enzymatic functionalisations to yield chiral hydroxynitriles, amines, acyloins and α-chiral ketones with up to 99% ee. In addition, we demonstrate functional group interconversion to alcohols, esters and carboxylic acids. The transformations can be performed as concurrent tandem reactions. We identified the degradation of substrates and inhibition of the biocatalysts as limiting factors affecting compatibility, due to reactive oxygen species generated in the photocatalytic step. These incompatibilities were addressed by reaction engineering such as, applying a two-phase system or temporal and spatial separation of the catalysts. Using a selection of eleven starting alkanes, one photo-organo catalyst and 8 diverse biocatalysts, we synthesized 26 products and report for the model compounds benzoin and mandelonitrile >97% ee at gram scale.*

(39) Meissner M.P., **Süss P., Brundiek H.**, Woodley J.M., v. Langermann J.
Scoping the Enantioselective Desymmetrization of a Poorly Water-Soluble Diester by Recombinant Pig Liver Esterase, Org. Process Res. Dev., Just Accepted Manuscript, Org. Process Res. Dev. 2018, 22, 1518–1523.

Abstract: *Previously, the biocatalytic desymmetrization of dimethyl cyclohex-4-ene-cis-1,2-dicarboxylate to (1S,2R)-1-(methoxycarbonyl)cyclohex-4-ene-2-carboxylic acid, an important intermediate towards the synthesis of biologically active molecules, had been well-characterized in terms of pH and temperature optima and several aspects of process performance. Eventually this promising reaction could convert 200 mM (40 g·L-1) of substrate with > 99.5% e.e. using the recombinant pig liver esterase, ECS-PLE06, at a scale of 8.8 L. However, the precise influence of substrate concentration and the poorly water-soluble nature of the substrate (approximately 60 mM in water at 25 °C for structurally similar dimethyl 1,4-cyclohexanedicarboxylate) remained elusive. Therefore, this work focuses on using a recently published methodology based on reaction trajectory analysis to identify mass transfer limitations in this reaction. With the constraints of mass transfer on space-time yield considered, it was possible to evaluate and improve biocatalyst yield (mass of product per mass of biocatalyst) through the use of higher substrate concentrations. Ultimately the complete conversion of approximately 75 g·L-1 substrate was achieved in 3.65 h yielding an excellent productivity of 20 g·L-1·h-1 with a biocatalyst yield of 4.36 g·g¬¬biocat-1. This work also highlights the simplicity of applying a reaction trajectory analysis methodology, importance of scale during reaction characterizations and identifies future directions for reaction improvement to address substrate supply and product inhibition/deactivation.*

(36) Schoenenberger B., **Wszolek A.**, Meier R., **Brundiek H.**, Obkircher M., Wohlgemuth R. (2018).
Recombinant AroL-catalyzed Phosphorylation for the efficient Synthesis of Shikimic acid 3-phosphate. Biotechnology journal, 1700529/biot.201700529

Abstract: *Shikimic acid 3-phosphate, as a central metabolite of the shikimate pathway, is of high interest as enzyme substrate for 5-enolpyruvoyl-shikimate 3-phosphate synthase, a drug target in infectious diseases and a prime enzyme target for the herbicide glyphosate. As the important substrate shikimic acid 3-phosphate is only accessible via a chemical multi-step route, a new straightforward preparative one-step enzymatic phosphorylation of shikimate using a stable recombinant shikimate kinase has been developed for the selective phosphorylation of shikimate in the 3-position. Highly active shikimate kinase is produced by straightforward expression of a synthetic aroL gene in Escherichia coli. The time course of the shikimate kinase-catalyzed phosphorylation is investigated by 1H- and 31P-NMR, using the phosphoenolpyruvate/pyruvate kinase system for the regeneration of the ATP cofactor. This enables the development of a quantitative biocatalytic 3-phosphorylation of shikimic acid. After a standard workup procedure, a good yield of shikimic acid 3-phosphate, with high HPLC- and NMR purity, is obtained. This efficient biocatalytic synthesis of shikimic acid 3-phosphate is superior to any other method and has been successfully scaled up to multi-gram scale.*

(34) R. Wardenga (2017)
Biocatalytic access to a novel class of Mannich catalysts; Manufacturing Chemist, October 2017, pp 52-53

Abstract: *Enzymicals AG highlights the possibility to synthesize novel diastereomers of 5-benzyl-C□-methyl-□-proline by stereoselective hydrolysis of branched malonate diesters. Application of recombinant pig liver esterase isoenzymes was the key to success to gain the (3S,5S)-diastereomer which shows activity as anti-Mannich catalyst.*

(33) B. Schoenenberger, **A. Wszolek**, R. Meier, **H. Brundiek**, M. Obkircher,and R. Wohlgemuth (2017),
Biocatalytic asymmetric Michael addition reaction of L-arginine to fumarate for the green synthesis of N-(([(4S)-4-amino-4-carboxy-butyl]amino) iminomethyl)-L-aspartic acid lithium salt (L-argininosuccinic acid lithium salt) RCS-Advances, 2017, 7, 48952; DOI: 10.1039/c7ra10236d

Abstract: *The basic natural amino acid L-argininosuccinate containing two chiral centers occurs in L-alanine, L-arginine, L-aspartate, L-glutamate and L-proline metabolic pathways and plays a role in the biosynthesis of secondary metabolites and other amino acids. It is a precursor for arginine in the urea cycle or the citrulline–NO cycle as well as a precursor to fumarate in the citric acid cycle via argininosuccinate lyase. We aimed to run part of the urea cycle in reverse by catalyzing not the elimination but the addition reaction of L-arginine to fumarate in order to synthesize L-argininosuccinate. Argininosuccinate lyase (ASL) from Saccharomyces cerevisiae has been chosen as the catalyst for this addition reaction. The selected ARG4 gene was synthesized and homogeneously expressed in E. coli leading to a highly active argininosuccinate lyase. The ASL-catalyzed addition reaction of L-arginine to fumarate has been successfully developed at gram scale. After a standard workup procedure the pure final product L-argininosuccinate has been isolated in good yield and high purity.*

(32) Kotapati H.K., Robinson J., Lawrence D., Fortner K., Stanford C., Powell D., **Wardenga R.**, Bornscheuer U. (2017),
Diastereoselective hydrolysis of branched malonate diesters by Porcine Liver Esterase: Synthesis of 5-benzyl substituted Cα-methyl-β-proline and catalytic evaluation, European Journal of Organic Chemistry, DOI: 10.1002/ejoc.201700605

Abstract: *Malonate diesters with highly branched side chains containing a preexisting chiral center were prepared from optically pure amino alcohols and subjected to asymmetric enzymatic hydrolysis by Porcine Liver Esterase (PLE). Recombinant PLE isoenzymes have been utilized in this work to synthesize diastereomerically enriched malonate half-esters from enantiopure malonate diesters. The diastereomeric excess of the product half-esters was further improved in the later steps of synthesis either by simple recrystallization or flash column chromatography. The diastereomerically enriched half-ester was transformed into a novel 5-substituted Cα-methyl-β-proline analogue (3R, 5S)-1c, in high optical purity employing a stereoselective cyclization methodology. This β-proline analogue was tested for activity as a catalyst of the Mannich reaction. The β-proline analogue derived from the hydrolysis reaction by the crude PLE appeared to catalyze the Mannich reaction between an α-imino ester and an aldehyde providing decent to good diastereoselectivities. However, the enantioselectivities in the reaction was low. The second diastereomer of the 5-benzyl substituted Cα-methyl-β-proline, (3S, 5S)-1c was prepared by enzymatic hydrolysis using PLE isoenzyme 3 and tested for its catalytic activity in the Mannich reaction. Amino acid, (3S, 5S)-1c catalyzed the Mannich reaction between isovaleraldehyde and an α-imino ester yielding the „anti" selective product with an optical purity of 99%ee.*

(30) Schoenenberger B., **Wszolek A.**, Milesi T., Obkircher M., **Brundiek H.**, Wohlgemuth R. (2016),
Synthesis of Nω-Phospho-L-arginine by Biocatalytic Phosphorylation of L-Arginine, Chemcatchem, 10.1002/cctc.201601080, Volume 9, Issue 1, January 9, 2017, Pages 121–126

Abstract: *The Nω-Phospho-L-arginine energy-buffering system is mainly present in invertebrates for regulating energy requirements when it is highly needed, as in the flight muscle of an insect or when energy supply fluctuates, as in the medically important protozoa Trypanosoma brucei, Trypanosoma cruzi and Leishmania major. The lacking availability of this important metabolite was due to a tedious chemical procedure, by which Nω-phospho-L-arginine was prepared up to now over 5 reaction steps in a low yield. Therefore, we aimed at improving the synthetic methodology for the preparation of this important metabolite. As site- and enantioselective kinases have been very useful catalysts for biocatalytic phosphorylations in straightforward syntheses of phosphorylated metabolites, a stable and selective arginine kinase has been selected for the selective phosphorylation of L-arginine. The arg gene has been cloned and expressed in E.coli and a highly active arginine kinase has been prepared. A simple synthesis of Nω-phospho-L-arginine has been developed by arginine kinase-catalyzed phosphorylation of L-arginine combined with the recycling of the phosphorylating agent ATP using the phosphoenolpyruvate/pyruvate kinase system. After standard workup the desired product Nω-Phospho-L-arginine has been obtained in gram quantities and in one step.*

(23) Kohls H., Sowa M., Anderson M., Dickerhoff J., Weisz K., Córdova A.; Berglund P., Bornscheuer U.T., **Brundiek H.**, Höhne M. (2015)
Selective Access to All Four Diastereomers of a 1,3-Amino Alcohol by Combination of a Keto Reductase- and an Amine Transaminase-Catalysed Reaction, Advanced Synthesis & Catalysis, 357 (8), 1808–1814

Abstract: *The biocatalytic synthesis of chiral amines has become a valuable addition to the chemists' toolbox. However, the efficient asymmetric synthesis of functionalised amines bearing more than one stereocentre, such as 1,3-amino alcohols, remains challenging. By employing a keto reductase (KRED) and two enantiocomplementary amine transaminases (ATA), we developed a biocatalytic route towards all four diastereomers of 4-amino-1-phenylpentane-2-ol as a representative molecule bearing the 1,3-amino alcohol functionality. Starting from a racemic hydroxy ketone, a kinetic resolution using an (S)-selective KRED provided optically active hydroxy ketone (86% ee) and the corresponding diketone. Further transamination of the hydroxy ketone was performed by either an (R)- or an (S)-selective ATA, yielding the (2R,4R)- and (2R,4S)-1,3-amino alcohol diastereomers. The remaining two diastereomers were accessible in two subsequent asymmetric steps: the diketone was reduced regio- and enantioselectively by the same KRED, which yielded the (S)-configured hydroxy ketone. Eventually, the subsequent transamination of the crude product with (R)- and (S)-selective ATAs yielded the remaining (2S,4R)- and (2S,4S)-diastereomers, respectively.*

(16) Sehl, T., Hailes, C. H., Ward, J. M., **Menyes, U.**, Pohl, M., Rother, D. (2014)
Efficient 2-step biocatalytic strategies for the synthesis of all nor(pseudo)ephedrine isomers. Green Chem., 2014, 16, 3341-3348

Abstract: *Chiral 1,2-amino alcohols are important building blocks for chemistry and pharmacy. Here, we developed two different biocatalytic 2-step cascades for the synthesis of all four nor(pseudo)ephedrine (N(P)E) stereoisomers. In the first one, the combination of an (R)-selective thiamine diphosphate (ThDP)-dependent carboligase with an (S)- or (R)-selective ω-transaminase resulted in the formation of (1R,2S)-NE or (1R,2R)-NPE in excellent optical purities (ee >99% and de >98%). For the synthesis of (1R,2R)-NPE, space–time yields up to 26 g L–1 d–1 have been achieved. Since a highly (S)-selective carboligase is currently not available for this reaction, another strategy was followed to complement the nor(pseudo)ephedrine platform. Here, the combination of an (S)-selective transaminase with an (S)-selective alcohol dehydrogenase yielded (1S,2S)-NPE with an ee >98% and a de >99%. Although lyophilized whole cells are cheap to prepare and were shown to be appropriate for use as biocatalysts, higher optical purities were observed with purified enzymes. These synthetic enzyme cascade reactions render the N(P)E-products accessible from inexpensive, achiral starting materials in only two reaction steps and without the isolation of the reaction intermediates.*

(14) Wardenga, R., Bednarczyk, A., Höhne, M. (2013),
Asymmetric synthesis of chiral amines from ketones. How to apply biocatalysis and find a suitable enzyme. PharmaChem, 12, 22-25.

Abstract: *Optically active amines play an important role in the pharmaceutical, agrochemical, and chemical industries. They are frequently used as synthons for the preparation of various pharmaceutically active substances. Consequently, there is a need for efficient methods to obtain the desired enantiomer of a given target structure in an optically pure form. Beside a range of chemical methods using for example, asymmetric synthesis with transition metal catalysts, enzymes represent a useful alternative to access this important class of compounds. This article focusses on the biocatalytic transaminase approach with emphasis on how to screen for suitable catalysts for the asymmetric synthesis starting from prostereogenic ketones.*

(13) Sehl, T., Hailes, H. C., Ward, J. M., **Wardenga, R.**, von Lieres, E., Offermann, H., Westphal, R., Pohl, M., Rother, D., (2013)
Two Steps in One Pot: Enzyme Cascade for the Synthesis of Nor(pseudo)ephedrine from Inexpensive Starting Materials. Angewandte Chemie International Edition vol. 52 (26) 6772-6775.

***Abstract**: Keywords: asymmetric synthesis; biocatalysis; enzyme cascades; phenylpropanolamine; ω-transaminase*
Two steps in one pot: An enzyme cascade consisting of a lyase and an (R)- or (S)-selective ω-transaminase (TA) provides (1R,2R)-norpseudoephedrine and (1R,2S)-norephedrine in only two steps. The intermediate is not isolated in this one-pot reaction and the products are obtained in high enantio- and diastereomeric purity. Moreover, the by-product from the second reaction can be recycled to serve as the substrate for the first reaction.

(12) Staudt, S., Bornscheuer, U.T., **Menyes, U.**, Hummel, W., Gröger, H. (2013),
Direct biocatalytic one-pot-transformation of cyclohexanol with molecular oxygen into ε-caprolactone, Enzyme Microb. Technol., 53, 288-292.

Abstract: The development of a biocatalytic process concept for ε-caprolactone, which directly converts cyclohexanol as an easily available industrial raw material into the desired ε-caprolactone in a one-pot fashion while only requiring air as sole reagent, is reported. The desired product ε-caprolactone was obtained with 94–97% conversion when operating at a substrate concentration in the range of 20–60 mM. At higher substrate concentrations, however, a significant drop of conversion was found. Subsequent detailed studies on the impact of the starting material, intermediate and product components revealed a significant inhibition and partial deactivation of the BVMO by the product ε-caprolactone (in particular at higher concentrations) as well as an inhibition of the BVMO by cyclohexanol and cyclohexanone.

(10) Smith, M.E., Banerjee, S., Shi, Y., **Schmidt, M.**, Bornscheuer, U.T. Masterson, D.S. (2012),
Investigation of the cosolvent effect on six isoenzymes of PLE in the enantioselective hydrolysis of selected a,a-disubstituted malonate esters, ChemCatChem, 4, 472-475

***Abstract:** Keywords: cosolvent effects; enantioselectivity; enzymes; inversion of chirality; synthesis*
Six pigs in a pot: Pig liver esterase (PLE) is among the most widely studied esterase enzymes utilized in organic synthesis. Here we illustrate that the six recombinantly produced isoenzymes of PLE exhibit varying enantioselectivity during the hydrolysis of α,α-disubstituted malonate esters in cosolvent mixtures. We have observed a rare cosolvent-induced reversal of enantioselectivity for isoenzyme PLE 6 in the hydrolysis of a phthalimide-containing α,α-disubstituted malonate ester.

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(51) Neuburger J., Helmholz F., Tiedemann S., Lehmann P., Süss P., Menyes U., Langermann J. Implementation and scale-up of a semi-continuous transaminase-catalyzed reactive crystallization for the preparation of (S)-(3-methoxyphenyl)ethylamine, (2021), doi.org/10.1016/j.cep.2021.108578

Abstract: *Unfavorable equilibrium positions are often a critical factor in the process development of biocatalytic reactions and usually require secondary solutions for efficient synthesis strategies. This explicitly applies to transaminase-catalyzed reactions, which can be used for the synthesis of enantiomerically pure amines. Overcoming the unfavorable equilibrium situation in these biocatalytic reactions, especially with a focus on efficient product isolation, has only been partly studied for preparative applications especially with higher concentration of a product amine. In this study, we present the process development of a transaminase-catalyzed reaction with the specific integration of an in situ- product crystallization, which allows downstream-processing via filtration in addition to the required equilibrium shift. This approach highlights a semi-continuous reaction concept with intermediate substrate addition and product removal in the form of a suspension-to-suspension reaction, supported by a light vacuum to remove the by-product acetone. Using the methodology up to 1.2 mol L-–1 product amine could be obtained by an iterative process development. Thus, the presented method represents a high potential for the preparative application of amine-based biocatalytic reactions at larger scales.*

50) Švarc A., Fekete M., Hernandez K., Clapés P., Findrik Z., Szekrenyi A., Skendrović D., Vasić-Rački D., Charnock S., Presečki A.
An innovative route for the production of atorvastatin side-chain precursor by DERA-catalysed double aldol addition, (2020), doi.org/10.1016/j.ces.2020.116312

Abstract: *A multi-enzyme route for the production of an atorvastatin side-chain was proposed. The approach includes the consecutive double aldol addition of acetaldehyde to the phenylacetamide amino-protected propanal by 2-deoxyribose-5-phosphate aldolase (DERA), the oxidation by ketoreductase, the lipase-catalysed acylation and amino group deprotection by penicillin G-acylase. To explore the feasibility of the process, the DERA reaction was studied into detail. Based on the kinetic and stability studies, a mathematical model was developed and validated in a batch and fed-batch reactor. The highest productivity was obtained in repetitive batch reactor (229.1 g/(L day)). The highest final product concentration of 124 g/L was obtained in fed-batch reactor. The mathematical model-based optimisation provided insight into the possibilities for process metrics improvement. Further, the second step was explored. The results showed that the DERA reaction and the oxidation reaction can be carried out as the multi-enzyme one pot process in the sequential manner.*

(47) Meyer L-E, **Brundiek H.** , Langermann J.
Integration of ion exchange resin materials for a downstream-processing approach of an imine reductase-catalyzed reaction, (2020), doi:10.1002/btpr.3024

Abstract: *In this study, an ion exchange resin-based downstream-processing concept for imine reductase (IRED)-catalyzed reactions was investigated. As a model reaction, 2-methylpyrroline was converted to its corresponding product (S)-2-methylpyrrolidine with >99% of conversion by the (S)-selective IRED from Paenibacillus elgii B69. Under optimized reaction conditions full conversion was achieved using a substrate concentration of 150 and 500 mmol/L of d -glucose. Seven commercially available cation- and anion-exchange resins were studied with respect to their ability to recover the product from the reaction solution. Without any pretreatment, cation-exchange resins Amberlite IR-120(H), IRN-150, Dowex Monosphere 650C, and Dowex Marathon MSC showed high recovery capacities (up to >90%). A 150-ml preparative scale reaction was performed yielding ~1 g hydrochloride salt product with >99% purity. Any further purification steps, for example, by column chromatography or recrystallization, were not required.*

(46) Wunschik D. S., Ingenbosch K. N., **Süss P.**, Liebelt U., Quinte S., Dyllick-Brenzinger M., Zuhse R., **Menyes U.**, Hoffmann-Jacobsen K., Opwis K., Gutmann J .S.; Enzymatic epoxidation of cyclohexene by peroxidase immobilization on a textile and an adapted reactor design, (2020), doi: 10.1016/j.enzmictec.2020.109512

Abstract: *Objective: Regio- and stereoselective hydroxylation of lithocholic acid (LCA) using CYP107D1 (OleP), a cytochrome P450 monooxygenase from the oleandomycin synthesis pathway of Streptomyces antibioticus.* ***Results:*** *Co-expression of CYP107D1 from S. antibioticus and the reductase/ferredoxin system PdR/PdX from Pseudomonas putida was performed in Escherichia coli whole cells. In vivo hydroxylation of LCA exclusively yielded the 6β-OH product murideoxycholic acid (MDCA). In resting cells, 19.5% of LCA was converted to MDCA within 24 h, resulting in a space time yield of 0.04 mmol L-1 h-1. NMR spectroscopy confirmed the identity of MDCA as the sole product.* ***Conclusions:*** *The multifunctional P450 monooxygenase CYP107D1 (OleP) can hydroxylate LCA, forming MDCA as the only product.*

(38) Hülsewede D., Tänzler M., **Süss P.**, Mildner A., **Menyes U.**, v. Langermann J.,
Development of an in situ-Product Crystallization (ISPC)- Concept to Shift the Reaction Equilibria of Selected AmineTransaminase-Catalyzed Reactions, European Journal of Organic Chemistry, 18, 2130-2133 (2018)

Abstract: *The synthesis of enantiopure amines via amine transaminases involves several challenges including unfavorable reaction equilibria and product inhibition. Described here is a non-catalytic approach to overcome such problems by using an in situ-product crystallization (ISPC) to selectively remove a targeted product amine from an amine transaminase-catalyzed reaction. The continuous removal of the product amine from its reaction solution as a barely soluble salt effectively yields a displacement of the reaction equilibrium towards the products and facilitates a simple downstream processing approach via filtration. The targeted product amine is eventually obtained from the salt, while the counterion compound can be easily recycled.*

(35) Hülsewede D., Tänzler M., Süss P., Mildner M., **Menyes U.**, v. Langermann J. (2018),
Development of an in situ-Product Crystallization (ISPC)-Concept to Shift the Reaction Equilibria of Selected Amine Transaminase-Catalyzed Reactions, Eur. J. Org. Chem. 10.1002/ejoc.201800323

Abstract: *The synthesis of enantiopure amines via amine transaminases involves several challenges including unfavorable reaction equilibria and product inhibition. Described here is a non-catalytic approach to overcome such problems by using an in situ-product crystallization (ISPC) to selectively remove a targeted product amine from an amine transaminase-catalyzed reaction. The continuous removal of the product amine from its reaction solution as a barely soluble salt effectively yields a displacement of the reaction equilibrium towards the products and facilitates a simple downstream processing approach via filtration. The targeted product amine is eventually obtained from the salt, while the counter ion compound can be easily recycled.*

(31) Wardenga R., Rother D. (2017)
Efficient Chiral Chemistry by Application of Stereoselective Biocatalysts in Micro-Aqueous Reaction Systems, Speciality Chemicals Magazine, volume 31, issue 01, February 2017, pages 16-17

Abstract: *Dr Rainer Wardenga of Enzymicals and Dr Dörte Rother of Forschungszentrum Jülich discuss the applicability of diverse enzymes in micro-aqueous reaction systems, enabling the conversion of hydrophobic or water-unstable substrates while maintaining the stereoselectivity of the biocatalysts.*

(28) Hinze J., **Süss P.**, Strohmaier S., Bornscheuer U. T., **Wardenga R.**, v. Langermann J. (2016)
Recombinant Pig Liver Esterase-Catalyzed Synthesis of (1S,4R)-4-Hydroxy-2-cyclopentenyl Acetate Combined with Subsequent Enantioselective Crystallization, DOI: 10.1021/acs.oprd.6b00093, Org. Process Res. Dev. 2016, 20, 1258–1264

Abstract: *The recombinant pig liver esterase catalyzed hydrolysis of cis-1,4-diacetoxy-2-cyclopentene forming (1S,4R)-4-hydroxy-2-cyclopentenyl acetate was investigated and realized at preparative scale. Relevant reaction conditions were examined and optimized to achieve full conversion with an enantiomeric excess of about 86% ee. Enantiopure product was then obtained after enantioselective crystallization, which required further studies of the solid phase behavior, including its binary melting point phase diagram.*

(21) Schmidt S., Scherkus C., Muschiol J., **Menyes U.**, Winkler T., Hummel W., Gröger H., Liese A., Herz H.G., Bornscheuer U.T.,
An Enzyme Cascade Synthesis of ε-Caprolactone and its Oligomers, Angew. Chem. Int. Ed. 2015, 54, 2784-2787

Abstract: *Poly-ε-caprolactone (PCL) is currently produced only chemically on industrial scale in spite of the need for hazardous peracetic acid as oxidation reagent. Although Baeyer-Villiger monooxygenases (BVMO) allow in principle the enzymatic synthesis of ε-caprolactone (ε-CL) directly from cyclohexanone with molecular oxygen, current systems suffer from low productivity and entail substrate and product inhibition. In this work, we overcame major limitations for such a biocatalytic route to produce this bulk chemical by combining an alcohol dehydrogenase with a BVMO to enable an efficient oxidation of cyclohexanol to ε-CL. Key to success was a subsequent direct ring-opening oligomerization of in situ-formed ε-CL in an aqueous phase using lipase A from Candida antarctica, thus solving efficiently the product inhibition problem and leading to formation of oligo-ε-CL at >20 g/L when starting from 200 mm cyclohexanol. This oligomer could easily be polymerized chemically to PCL.*

(20) Scherkus C., Liese A., Gröger H., Kragl U., Bornscheuer U. T., **Menyes U.**, (2014)
Prozessentwicklung zur enzymatischen Synthese eines biologisch abbaubaren Polymers, Chemie Ingenieur Technik, 86 (9), 1424–1425

Abstract: *No abstract available*

(17) Süss, P., Illner, S., v. Langermann, J., **Borchert, S.**, Bornscheuer, U.T., **Wardenga, R.**, Kragl, U. (2014)
Scale-Up of a Recombinant Pig Liver Esterase-Catalyzed Desymmetrization of Dimethyl Cyclohex-4-ene-cis-1,2-dicarboxylate. Org. Process Res. Dev., 18, 897-903

Abstract: *A recombinant isoenzyme of pig liver esterase was used for the highly enantioselective desymmetrization of dimethyl cyclohex-4-ene-cis-1,2-dicarboxylate. The selected recombinant esterase showed a significant advantage in enantioselectivity over the commonly used esterase from the mammalian source. The process was scaled up to yield 265 g of product with a simplified pH control, and the target molecule was obtained with an enantiopurity of >99.5% ee.*