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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.*