AUSTRIAN CENTRE OF INDUSTRIAL BIOTECHNOLOGY

Biocatalytic Synthesis

ENZYMATIC SOLUTIONS
FOR CHEMICAL PROBLEMS

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ENZYMATIC SOLUTIONS FOR CHEMICAL PROBLEMS

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CONTENT

Biocatalytic Synthesis

means that we convert and produce molecules with the help of enzymes.

Biocatalysis at acib involves the conversion and synthesis of known but also new and innovative molecules in order to replace conventional chemical processes with efficient and environmentally-friendly approaches. Beside single reaction formats, acib researchers also focus on multi-step (one-pot) reactions, which allow to reduce the number of process steps and facilitate downstream processing.The complexity of multi-step (one-pot) reactions and whole cell biocatalysis require the integration of molecular techniques such as cell- and protein engineering.

This enables us to replace common chemical processes by efficient and environmental-friendly approaches. Beside single reaction formats that have been successfully implemented in industry, also multi-step reactions in one pot get more and more important! This reduces the number of working steps in a process and requires less purification steps of intermediate products. Means: less CO_2 emissions! But multi-step reactions can get very complex.

To be successful, our researchers of biocatalysis need to work closely with our cell- and protein engineers. Our famous products of this research field are for example oligosaccharides for the food and cosmetic sectors, multi-oxidation reactions for aroma compounds and fine chemicals, or cascade reactions for building blocks in antibiotic production.

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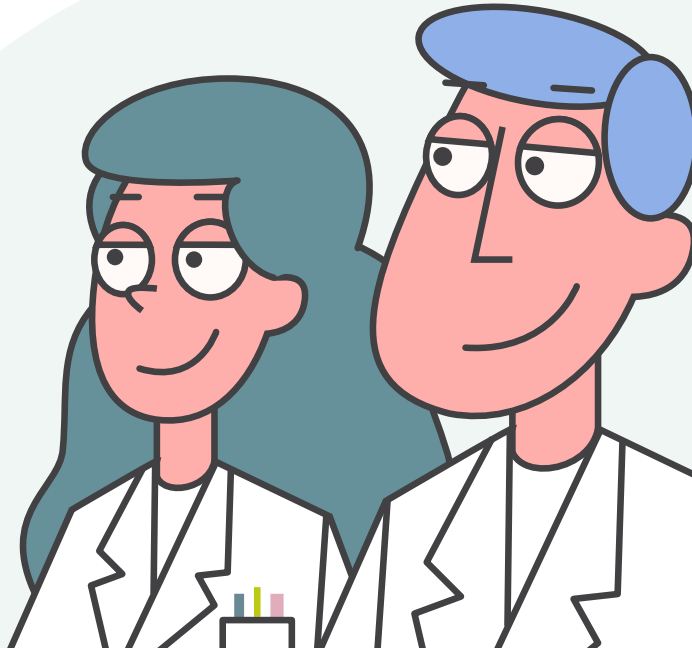
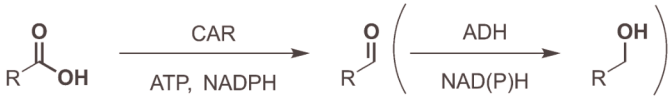


Reduction of Carboxylic Acids



CAR = carboxylate reductase; ADH = alcohol dehydrogenase

K. Napora-Wijata, K. Robins, A. Osorio-Lozada, et al., Whole-Cell Carboxylate Reduction for the Synthesis of 3-Hydroxytyrosol, *ChemCatChem* 6, 1089–1095 (2014).

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M. Winkler, Carboxylic Acid Reductase Enzymes (CARs), *Curr. Opin. Chem. Biol.* 43, 23–29 (2018).

B. Daniel, C. Hashem, M. Leithold, et al., Structure of the Reductase Domain of a Fungal Carboxylic Acid Reductase and its Substrate Scope in Thioester and Aldehyde Reduction, *ACS Catal.* 12, 15668–15674 (2022).

M. Winkler, J. G. Ling, Biocatalytic Carboxylate Reduction – Recent Advances and New Enzymes, *ChemCatChem*14(19), e202200441 (2022).

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M. Horvat, M. Winkler, *In vivo* Reduction of Medium- to Long-Chain Fatty Acids by CAR Enzymes: Limitations and Solutions, *ChemCatChem* 12, 5076–5090 (2020).

D. Schwendenwein, A. K. Ressmann, M. Doerr, et al., Random Mutagenesis Driven Improvement of Carboxylate Reductase Activity by a Substrate Independent High-Throughput Assay in Living Cells, *Adv. Synth. Catal.* 361(11), 2544–2549 (2019).

Reduction of Aldehydes and Ketones

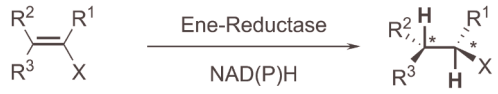
ADH = alcohol dehydrogenase

W. Stampfer, B. Kosjek, C. Moitzi, et al., Biocatalytic Asymmetric Hydrogen Transfer, *Angew. Chem. Int. Ed.* 41, 1014–1017 (2002).

C. V. Voss, C. C. Gruber, W. Kroutil, Deracemization of Secondary Alcohols through a Concurrent Tandem Biocatalytic Oxidation and Reduction, *Angew. Chem. Int. Ed.* 47, 714–745 (2008).

K. Napora, T. M. Wrodnigg, P. Kosmus, et al., *Yarrowia lipolytica* Dehydrogenase/Reductase: An Enzyme Tolerant for Lipophilic Compounds and Carbohydrate Substrates, *Bioorg. Med. Chem. Lett.* 23, 3393–3395 (2013).

Reduction of C=C-Bonds



X = electron-withdrawing group

R. Stuermer, B. Hauer, M. Hall, et al., Asymmetric Bioreduction of Activated C=C Bonds Using Enoate Reductases from the Old Yellow Enzyme Family, *Curr. Opin. Chem. Biol.* 11, 203–213 (2007).

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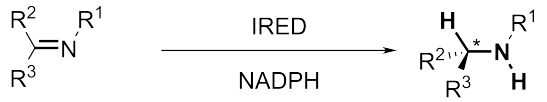
I. Oroz-Guinea, C. K. Winkler, S. M. Glueck, et al., Ene-Reductase Catalyzed Regio- and Stereoselective 1,4-Mono-Reduction of Pseudoionone to Geranylacetone, *ChemCatChem* 14, e202101557 (2022).

Reduction of Imines



IRED = imine reductase

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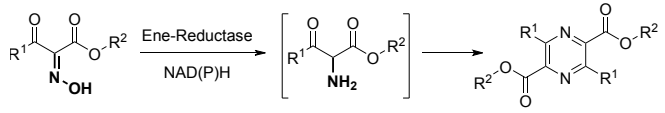
S. Velikogne, V. Resch, C. Dertnig, et al., Sequence-Based *In-silico* Discovery, Characterisation, and Biocatalytic Application of a Set of Imine Reductases, *ChemCatChem* 10, 3236–3246 (2018).

Reduction of Oximes to Amines



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W. B. Breukelaar, N. Polidori, A. Singh, et al., Mechanistic Insights into the Ene-Reductase-Catalyzed Promiscuous Reduction of Oximes to Amines, *ACS Catal.* 13, 2610–2618 (2023).

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Reductive Amination of Aldehydes and Ketones

TA = ω-transaminase

D. Koszelewski, I. Lavandera, D. Clay, et al., Formal Asymmetric Biocatalytic Reductive Amination, *Angew. Chem. Int. Ed.* 47, 9337–9340 (2008).

W. Kroutil, E. M. Fischereder, C. S. Fuchs, et al., Asymmetric Preparation of Prim-, Sec-, and Tert-Amines Employing Selected Biocatalysts, *Org. Process Res. Dev.* 17, 751–759 (2013).

R. C. Simon, N. Richter, E. Busto, et al., Recent Developments of Cascade Reactions Involving ω-Transaminases, *ACS Catal.* 4, 129–143 (2014).

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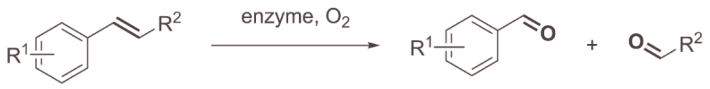
Oxidative Biotransformation

C=C-Bond Cleavage



H. Mang, J. Gross, M. Lara, et al., Biocatalytic Single-Step Alkene Cleavage from Aryl Alkenes: An Enzymatic Equivalent to Reductive Ozonization, *Angew. Chem. Int. Ed.* 45, 5201–5203 (2006).

M. Lara, F. G. Mutti, S. M. Glueck, et al., Oxidative Enzymatic Alkene Cleavage: Indications for a Nonclassical Enzyme Mechanism, *J. Am. Chem. Soc.* 131, 5368–5369 (2009).

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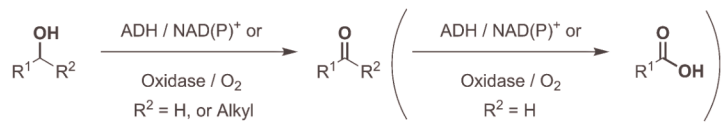
Oxidation of Alcohols / Aldehydes



ADH = alcohol dehydrogenase

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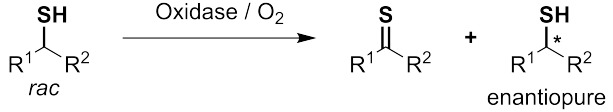
C. V. Voss, C. C. Gruber, W. Kroutil, Deracemization of Secondary Alcohols Through a Concurrent Tandem Biocatalytic Oxidation and Reduction, *Angew. Chem. Int. Ed.* 47, 714–745 (2008).

C. Wuensch, H. Lechner, S. M. Glueck, et al., Asymmetric Biocatalytic Cannizzaro-Type Reaction, *ChemCatChem* 5, 1744–1748 (2013).

S. Gandomkar, R. Rocha, F. A. Sorgenfrei, et al., PQQ-Dependent Dehydrogenase Enables One-Pot Bi-Enzymatic Enantio-Convergent Biocatalytic Amination of Racemic Sec-Allylic Alcohols, *ChemCatChem* 13, 1290–1293 (2021).

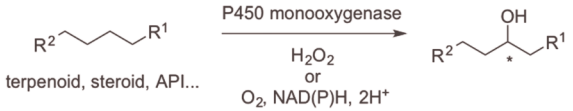
Oxidation of Thiols



TA = transaminase; AO = amine oxidase

M. Pickl, A. Swoboda, E. Romero, et al., Kinetic Resolution of sec-Thiols by Enantioselective Oxidation with Rationally Engineered 5-(Hydroxymethyl)furfural Oxidase, *Angew. Chem. Int. Ed.* 57, 2864–2868 (2018).

Enzymatic Hydroxylation with Cytochrome P450 Monooxygenase



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A. K. Migglautsch, M. Willim, B. Schweda, et al., Aliphatic Hydroxylation and Epoxidation of Capsaicin by Cytochrome P450 CYP505X, *Tetrahedron* 74(43), 6199–6204 (2018).

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C. Rinnofner, B. Kerschbaumer, H. Weber, et al., Cytochrome P450-Mediated Hydroxylation of Ibuprofen Using *Pichia pastoris* as Biocatalyst, *Biocatal. Agric. Biotechnol.* 17, 525–528 (2019).

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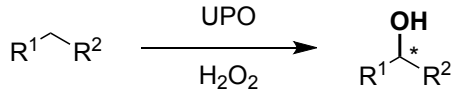
K. Bangert, A. Swoboda, S. Vrabl, et al., Preparative Regio- and Stereoselective α-Hydroxylation of Medium Chain Mono- and Dicarboxylic Fatty Acids, *Green Chem.* 26, 3183–3189 (2024).

Enzymatic Hydroxylation with Unspecific Peroxygenase (UPO)



R^1 and R^2 = alkyl, aryl

A. Swoboda, S. Zwölfer, Z. Duhović, M. Bürgler, K. Ebner, A. Glieder, W. Kroutil, Multistep Biooxidation of 5-(Hydroxymethyl)furfural to 2,5-Furandicarboxylic Acid with H_2O_2 by Unspecific Peroxygenases, *ChemSusChem* 17(11) (2024).

K. Ebner, L. J. Pfeifenberger, V. Schusterbauer, C. Rinnofner, A. Glieder,* M. Winkler*, Discovery and Heterologous Expression of Unspecific Peroxygenases, *Catalysts* 13(1), 206 (2023).

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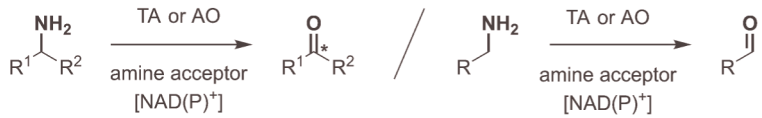
Enzymatic De-Amination



TA = transaminase; AO = amine oxidase

D. Koszelewski, I. Lavandera, D. Clay, et al., Formal asymmetric biocatalytic reductive amination. *Angew. Chem. Int. Ed.*47(48), 9337–9340 (2008).

W. Kroutil, E. M. Fischereder, C. S. Fuchs, et al., Asymmetric preparation of prim-, sec-, and tert-amines employing selected biocatalysts. *Org. Process Res. Dev.* 17(5), 751–759 (2013).

R. C. Simon, N. Richter, E. Busto, et al., Recent developments of cascade reactions involving ω-transaminases. *ACS Catal.* 4(1), 129–143 (2014).

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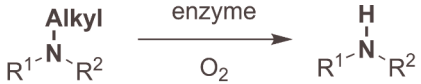
Enzymatic De-Alkylation



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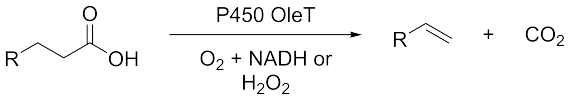
Oxidative Decarboxylation



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I. Zachos, S. K. Gassmeyer, D. Bauer, et al., Photobiocatalytic decarboxylation for olefin synthesis. *Chem. Commun.* 51, 1918–1921 (2015).

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M. Pickl, S. Kurakin, F. G. Cantú Reinhard, et al., Mechanistic studies of fatty acid activation by CYP152 peroxygenases reveal unexpected desaturase activity. *ACS Catal.* 9, 565–577 (2019).

Baeyer-Villiger Oxidation

R^1 = H, or Alkyl
R^2 = H, Alkyl or Aryl

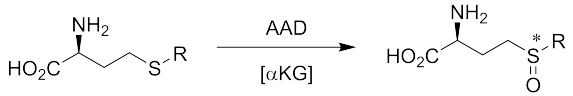
n = 1,2
R = Alkyl

hFMO = human flavin containing monooxygenase

F. Fiorentini, M. Geier, C. Binda, et al., Biocatalytic characterization of human FMO5: Unearthing Baeyer-Villiger reactions in humans. *ACS Chem. Biol.* 11(4), 1039–1048 (2016).

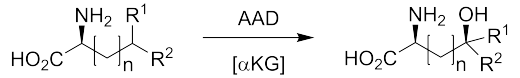
F. Fiorentini, E. Romero, M. W. Fraaije, et al., Baeyer-Villiger monooxygenase FMO5 as entry point in drug metabolism. *ACS Chem. Biol.* 12(9), 2379–2387 (2017).

Oxyfunctionalisation of Amino Acids



R = Me, Et

(*R*,*S*) *or* (*S*,*S*)



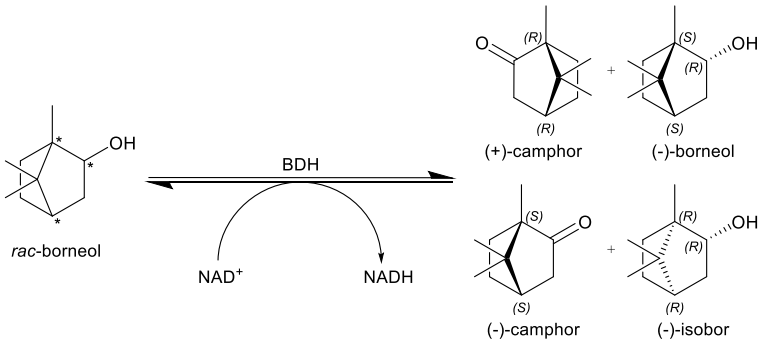
R^1,R^2 = H, alkyl
n = 0,1,2

α,β,γ-hydroxylation

aKG = α-ketoglutarate; AAD = amino acid dioxygenase;

J. Enoki, J. Meisborn, A. C. Müller, et al., A multi-enzymatic cascade reaction for the stereoselective production of γ-oxyfunctionalized amino acids. *Front. Microbiol.* 7, 425 (2016).

Kinetic Resolution of Borneol



BDH = borneol-type dehydrogenase

I. Drienovská, D. Kolanović, A. Chánique, et al., Molecular cloning and functional characterization of two highly stereoselective borneol dehydrogenases from *Salvia officinalis* L. *Phytochemistry* 172, 112227 (2020).

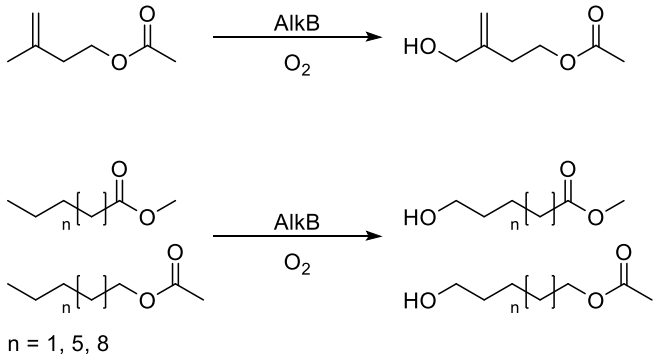
M. Hofer, J. Diener, B. Begander, et al., Engineering of a borneol dehydrogenase from *Pseudomonas putida* for the enzymatic resolution of camphor. *Appl. Microbiol. Biotechnol.* 105, 3159–3167 (2021).

N. Dimos, C.P.O. Helmer, A. Chánique, et al., Rapid high-resolution structure analysis of small, biotechnologically relevant enzymes by cryo-electron microscopy. *bioRxiv* (2021).

A. Chánique, N. Dimos, I. Drienovská, et al., A structural view on the stereospecificity of plant borneol-type dehydrogenases. *ChemCatChem* 13(9), 2262–2277 (2021).

Oxidative Biotransformation

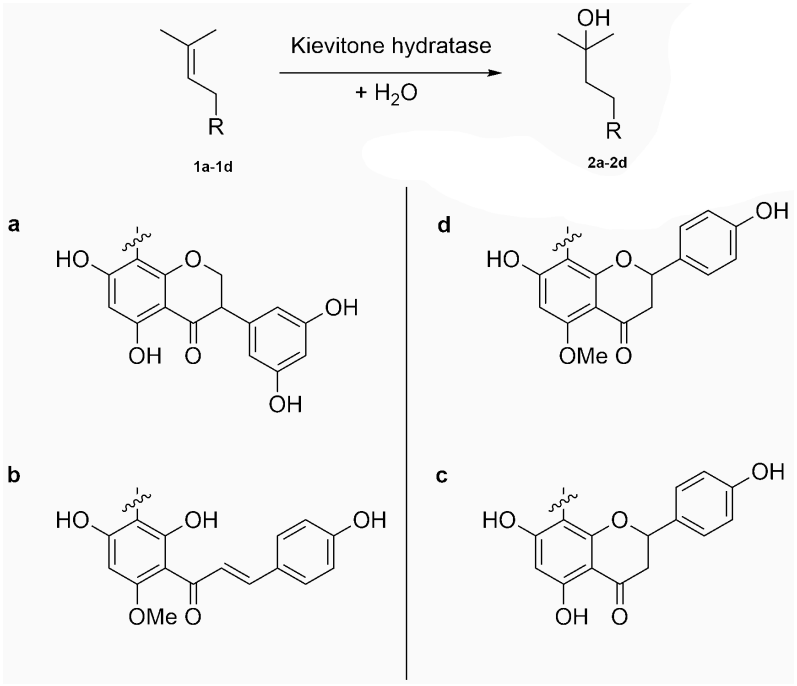
AlkB-Catalyzed Hydroxylation



AlkB = alkane monooxygenase

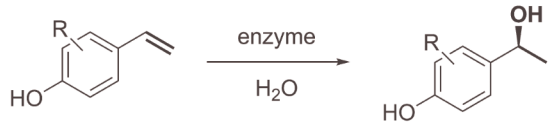
A. Nigl, V. Delsoglio, M. Grgić, et al., Engineering of Transmembrane Alkane Monooxygenases to Improve a Key Reaction Step in the Synthesis of Polymer Precursor Tulipalin A. *bioRxiv* (2024).

Selective Hydration of Flavonoids



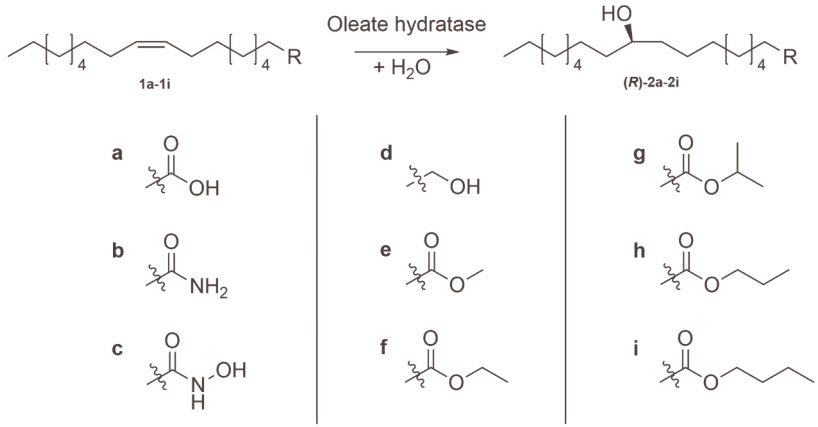
M. Engleder, M. Horvat, A. Emmerstorfer-Augustin, et al., Recombinant expression, purification and biochemical characterization of kievitone hydratase from *Nectria haematococca*. *PLoS One* 13, e0192653 (2018).

Hydration of Hydroxystyrene Derivatives



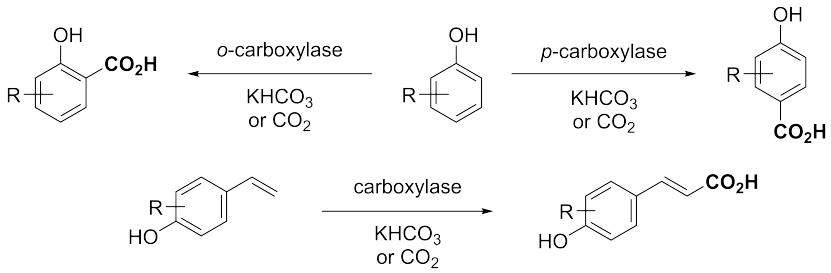
C. Wuensch, J. Gross, G. Steinkellner, et al., Asymmetric enzymatic hydration of hydroxystyrene derivatives. *Angew. Chem. Int. Ed.* 52, 2293–2297 (2013).

Asymmetric Hydration of Olefins



M. Engleder, G. A. Strohmeier, H. Weber, et al., Evolving the promiscuity of *Elizabethkingia meningoseptica* oleate hydratase for the regio- and stereoselective hydration of oleic acid derivatives. *Angew. Chem. Int. Ed.* 58, 7480–7484 (2019).

Bio-Carboxylation



S. M. Glueck, S. Gümüs, W. M. F. Fabian, et al., Biocatalytic carboxylation. *Chem. Soc. Rev.* 39, 313–328 (2010).

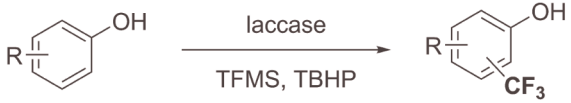
C. Wuensch, S. M. Glueck, J. Gross, et al., Regioselective enzymatic carboxylation of phenols and hydroxystyrene derivatives. *Org. Lett.* 14, 1974–1977 (2012).

C. Wuensch, N. Schmidt, J. Gross, et al., Pushing the equilibrium of regio-complementary carboxylation of phenols and hydroxystyrene derivatives. *J. Biotechnol.* 168, 264–270 (2013).

S. E. Payer, S. A. Marshall, N. Bärland, et al., Regioselective para-carboxylation of catechols with a prenylated flavin dependent decarboxylase. *Angew. Chem. Int. Ed.* 56, 13893–13897 (2017).

M. Nattermann, L. Schulz, R. Zschoche, et al., Enzymatic conversion of CO_2: From natural to artificial utilization. *Chem. Rev.* 123, 5702–5754 (2023).

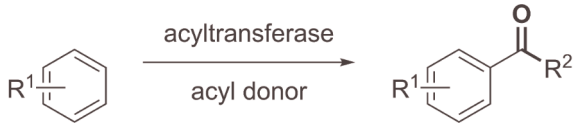
Trifluoromethylation



TFMS = trifluoromethylsulfonic acid; TBHP = tert-butylhydroperoxide

S. E. Payer, S. A. Marshall, N. Bärland, et al., Regioselective para-carboxylation of catechols with a prenylated flavin dependent decarboxylase. *Angew. Chem. Int. Ed.* 56, 13893–13897 (2017).

Bio-Friedel-Crafts Acylation



A. Żądło-Dobrowolska, N. G. Schmidt, W. Kroutil, Thioesters as acyl donors in biocatalytic Friedel-Crafts-type acylation catalyzed by acyltransferase from *Pseudomonas protegens*. *ChemCatChem* 11, 1064–1068 (2019).

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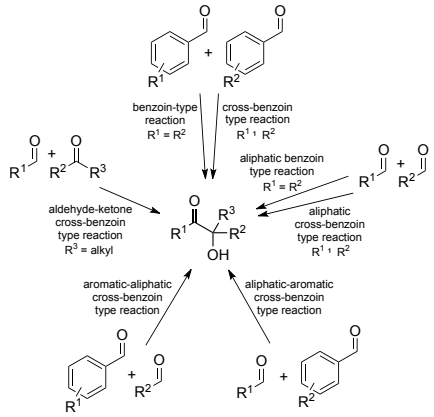
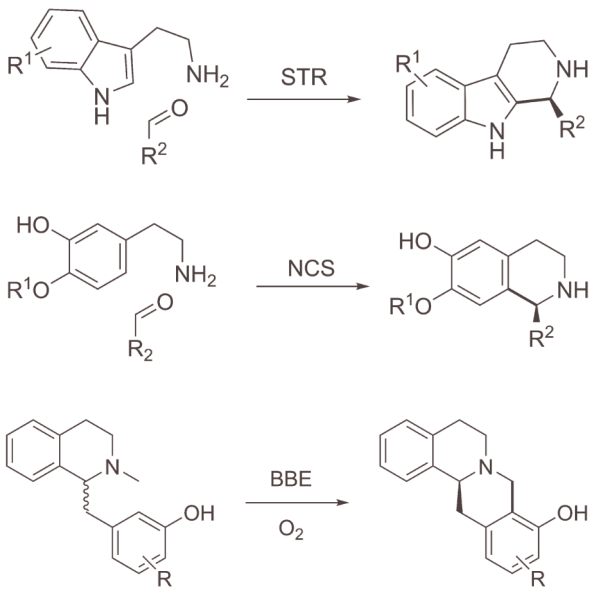
A. Żądło-Dobrowolska, L. Hammerer, T. Pavkov-Keller, et al., Rationally engineered C-acyltransferase transforms sterically demanding acyl donors. *ACS Catal.* 10, 1094–1101 (2020).

Hydroxyketone Synthesis



H. Dobiašová, V. Jurkaš, P. Both, M. Winkler, Recent progress in the synthesis of α-hydroxy carbonyl compounds with ThDP-dependent carboligases. *ChemCatChem* 16, e202301707 (2024).

Biocatalytic Alkaloid Synthesis



BBE = berberine bridge enzyme; STR = strictosidine synthase; NCS = norcoclaurine synthase

W. Kroutil, E. M. Fischereder, C. S. Fuchs, et al., Asymmetric preparation of prim-, sec-, and tert- amines employing selected biocatalysts. *Org. Process Res. Dev.* 17, 751–759 (2013).

J. H. Schrittwieser, V. Resch, J. H. Sattler, et al., Biocatalytic enantioselective oxidative C-C coupling by aerobic C-H activation. *Angew. Chem. Int. Ed.* 50, 1068–1071 (2011).

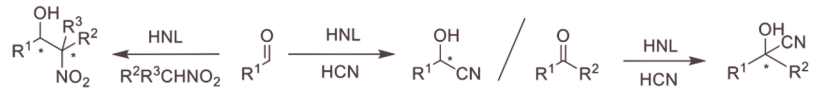
J. H. Schrittwieser, B. Groenendaal, V. Resch, et al., Deracemization by simultaneous bio-oxidative kinetic resolution and stereoinversion. *Angew. Chem. Int. Ed.* 53, 3731–3734 (2014).

D. Pressnitz, E. M. Fischereder, J. Pletz, et al., Asymmetric synthesis of (R)-1-alkyl-substituted tetrahydro-β-carbolines catalyzed by strictosidine synthases. *Angew. Chem. Int. Ed.* 57, 10683–10687 (2018).

E. Cigan, J. Pletz, S. A. Berger, et al., Concise synthesis of (R)-reticuline and (+)-salutaridine by combining early-stage organic synthesis and late-stage biocatalysis. *Chem. Sci.* 14, 9863–9871 (2023).

E. Eger, A. Simon, M. Sharma, et al., Inverted binding of non-natural substrates in strictosidine synthase leads to a switch of stereochemical outcome in enzyme-catalyzed Pictet–Spengler reactions. *J. Am. Chem. Soc.* 142, 792–800 (2020).

Cyanohydrin Synthesis / Henry Reaction



HNL = hydroxynitrile lyase

T. Purkarthofer, K. Gruber, M. Gruber-Khadjawi, et al., A biocatalytic Henry reaction: The hydroxynitrile lyase from *Hevea brasiliensis* also catalyzes nitroaldol reactions. *Angew. Chem. Int. Ed.* 45, 3454–3456 (2006).

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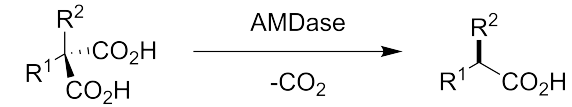
E. Lanfranchi, T. Pavkov-Keller, E. M. Köhler, et al., Enzyme discovery beyond homology: A unique hydroxynitrile lyase in the Bet v1 superfamily. *Sci. Rep.* 7, 46738 (2017).

E. Lanfranchi, B. Grill, Z. Raghoebar, S. V. Pelt, et al., Production of hydroxynitrile lyase from *D. Tyermanii* in *Komagataella phaffii* and its immobilization to generate a robust biocatalyst. *ChemBioChem* 19, 312–316 (2018).

Asymmetric Synthesis of Optically Pure α-Substituted Carboxylic Acids



R^1 = aryl, vinyl
R^2 = Me, OH, NH_2, F

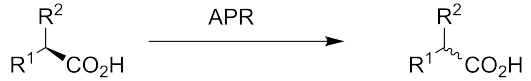
>99% ee
(*R*) *or* (*S*)

AMDase = arylmalonate decarboxylase

S. K. Gaßmeyer, J. Wetzig, C. Mügge, et al., Arylmalonate decarboxylase-catalyzed asymmetric synthesis of both enantiomers of optically pure flurbiprofen. *ChemCatChem.* 8, 916–921 (2016).

K. Miyamoto, R. Kourist, Arylmalonate decarboxylase—a highly selective bacterial biocatalyst with unknown function. *Appl. Microbiol. Biotechnol.* 100(20), 8621–8631 (2016).

Racemization of Arylpropionates



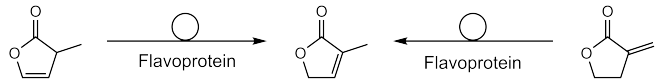
R^1 = aryl, vinyl
R^2 = Et, Me, OH, NH_2, F

AMDase = arylmalonate decarboxylase

S. K. Gaßmeyer, H. Yoshikawa, J. Enoki, et al., STD-NMR-based protein engineering of the unique arylpropionate-racemase AMDase G74C. *ChemBioChem.* 16(13), 1943–1949 (2015).

F. Busch, J. Enoki, N. Hülsemann, et al., Semiempirical QM/MM calculations reveal a step-wise proton transfer and an unusual thiolate pocket in the mechanism of the unique arylpropionate racemase AMDase G74C. *Catal. Sci. Technol.* 6, 4937–4944 (2016).

Isomerisation of C=C-Bond



K. Durchschein, S. Wallner, P. Macheroux, et al., Unusual C=C bond isomerization of an α,β-unsaturated γ-butyrolactone catalyzed by flavoproteins from the Old Yellow Enzyme family. *ChemBioChem.* 13(16), 2346–2351 (2012).

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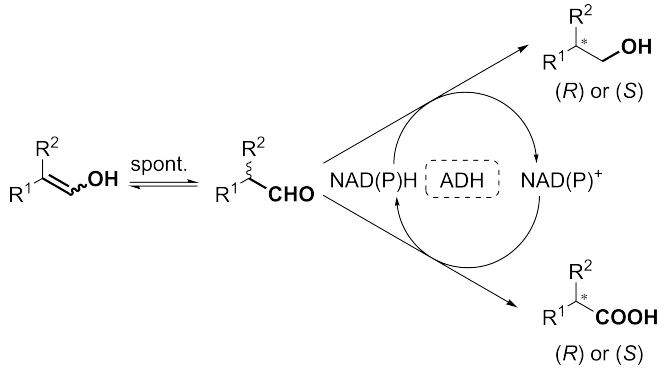
Disproportionation: Biocatalytic Cannizzaro Reaction



ADH = alcohol dehydrogenase

C. Wuensch, H. Lechner, S. M. Glueck, et al., Asymmetric biocatalytic Cannizzaro-type reaction. *ChemCatChem.* 5(7), 1744–1748 (2013).

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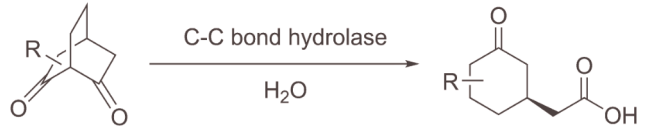
Hydrolysis of Lactams

Z. Assaf, E. Eger, Z. Vitnik, et al., Identification and application of enantiocomplementary lactamases for Vince lactam derivatives. *ChemCatChem.* 6, 2517–2521 (2014).

C-C Bond Hydrolysis

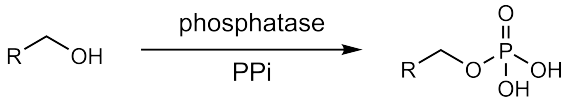


E. Siirola, A. Frank, G. Grogan, et al., C-C hydrolases for biocatalysis. Adv. Synth. Catal. 355, 1677–1691 (2013).

E. Siirola, F. G. Mutti, B. Grischek, et al., Asymmetric synthesis of 3-substituted cyclohexylamine derivatives from prochiral diketones via three biocatalytic steps. *Adv. Synth. Catal.* 355, 1703–1708 (2013).

A. Frank, E. Siirola, W. Kroutil, et al., Mutational analysis of the C-C bond cleaving enzyme phloretin hydrolase from *Eubacterium ramulus*. *Top. Catal.* 57, 376–384 (2014).

Enzymatic Phosphorylation

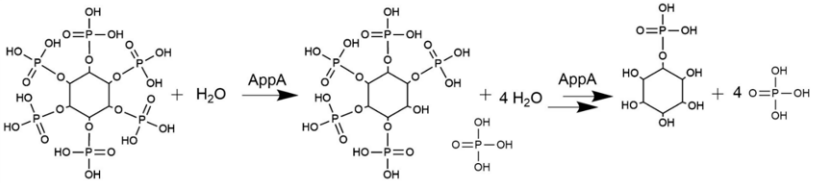


PPi = Pyrophosphate

G. Tasnádi, M. Lukesch, M. Zechner, et al., Exploiting acid phosphatases in the synthesis of phosphorylated monoalcohols and diols. *European J. Org. Chem.* 2016, 45–50 (2016).

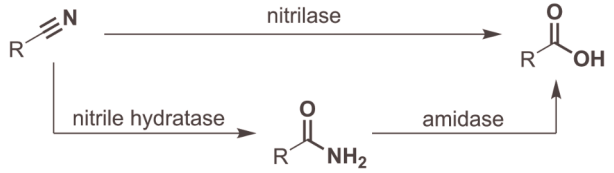
G. Tasnádi, W. Jud, M. Hall, et al., Evaluation of natural and synthetic phosphate donors for the improved enzymatic synthesis of phosphate monoesters. *Adv. Synth. Catal.* 360, 2394–2401 (2018).

Enzymatic Phosphate Hydrolysis



S. Arhar, T. Rauter, H. Stolterfoht-Stock, et al., CO_2-based production of phytase from highly stable expression plasmids in *Cupriavidus necator* H16. *Microbial Cell Factories* 2024, 23, 9.

Nitrile Hydrolysis



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U. Schreiner, G. Steinkellner, J. D. Rozzell, et al., Improved fitness of *Arabidopsis thaliana* nitrilase 2. *ChemCatChem* 2, 263–267 (2010).

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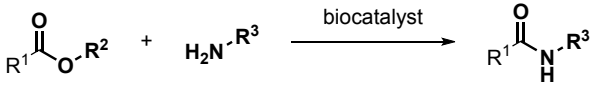
B. Grill, M. Horvat, H. Schwab, et al., Gordonia hydrophobica nitrile hydratase for amide preparation from nitriles. *Catalysts* 11(8), 1287 (2021).

S. Arndt, B. Grill, H. Schwab, et al., The sustainable synthesis of levetiracetam by an enzymatic dynamic kinetic resolution and an ex-cell anodic oxidation. *Green Chem.* 23, 388–395 (2021).

B. Grill, M. Glänzer, H. Schwab, et al., Functional expression and characterization of a panel of cobalt and iron dependent nitrile hydratases. *Molecules* 25(11), 2521 (2020).

Amide Formation

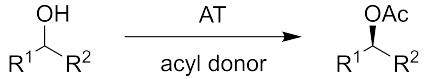


R^1 = alkyl or aryl; R^2 = H or alkyl; R^3 = alkyl or aryl

J. Pitzer, K. Steiner, C. Schmid, et al., Racemization-free and scalable amidation of L-proline in organic media using ammonia and a biocatalyst only. *Green Chem.* 24, 5171–5180 (2022).

Zukic E, Mokos D, Weber M, Stix N, Ditrich K, Ferrario V, Müller H, Willrodt C, Gruber K, Daniel B, Kroutil W. Biocatalytic heteroaromatic amide formation in water enabled by a catalytic tetrad and two access tunnels. *ACS Catal.* 14, 8913–8921 (2024).

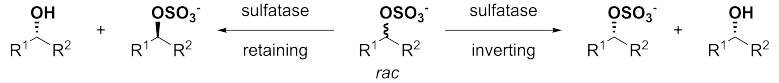
Enantioselective Ester Formation in Aqueous Systems



R^1, R^2 = alkyl

Jost E, Kazemi M, Mrkonjić V, et al. Variants of the acyltransferase from *Mycobacterium smegmatis* enable enantioselective acyl transfer in water. *ACS Catal.* 10, 10500–10507 (2020).

Bio-Mitsunobu-Inversion



S. R. Wallner, M. Bauer, C. Würdemann, et al., Highly enantioselective sec-alkyl sulfatase activity of the marine planctomycete *Rhodopirellula baltica* shows retention of configuration. *Angew. Chem. Int. Ed.* 44(39), 6381–6384 (2005).

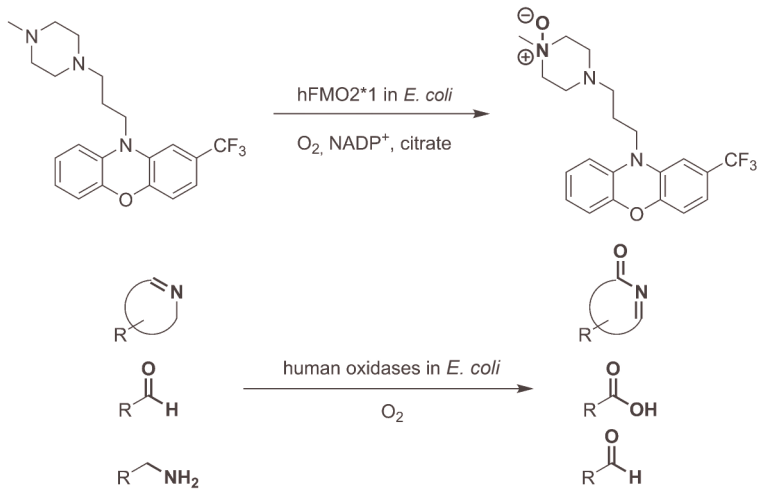
M. Schober, M. Toesch, T. Knaus, et al., One-pot deracemization of sec-alcohols: Enantioconvergent enzymatic hydrolysis of alkyl sulfates using stereocomplementary sulfatases. *Angew. Chem. Int. Ed.* 52(11), 3277–3279 (2013).

M. Fuchs, M. Toesch, M. Schober, et al., Chemoenzymatic asymmetric total synthesis of (R)-lasiodiplo- din methyl ether through a sulfatase-based deracemization process. *European J. Org. Chem.* 2013, 356–361 (2013).

M. Schober, K. Faber, Inverting hydrolases and their use in enantioconvergent biotransformations. *Trends Biotechnol.* 31, 468–478 (2013).

M. Toesch, M. Schober, K. Faber, Microbial alkyl- and aryl-sulfatases: Mechanism, occurrence, screening and stereoselectivities. *Appl. Microbiol. Biotechnol.* 98(4), 1485–1496 (2014).

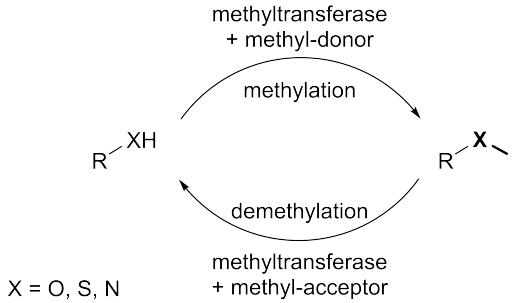
Chemo- & Regioselective Oxidation of Soft Nucleophiles



hFMO2 = human flavin-containing monooxygenase 2

A. Lepak, A. Gutmann, S. T. Kulmer, et al., Creating a water-soluble resveratrol-based antioxidant by site-selective enzymatic glucosylation. *ChemBioChem* 16, 1870–1874 (2015).

Methylation and De-Methylation

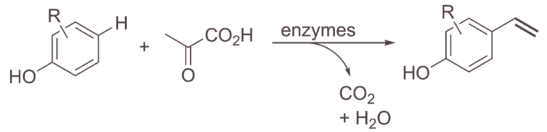


F. Zepeck, J. E. Farnberger, W. Skibar, et al., Biocatalytic methylation and demethylation via a shuttle catalysis concept involving corrinoid proteins. *Commun. Chem.* 1, 82 (2018).

C. Grimm, S. Pompei, K. Egger, et al., Anaerobic demethylation of guaiacyl-derived monolignols enabled by a designed artificial cobalamin methyltransferase fusion enzyme. *RSC Adv.* 2023, 13, 5770–5777 (2023).

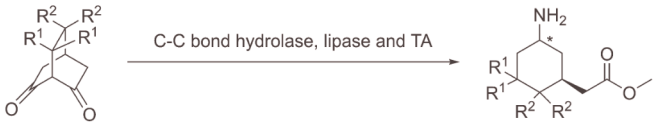
C. Grimm, M. Lazzarotto, S. Pompei, et al., Oxygen-free regioselective biocatalytic demethylation of methylphenyl ethers via methyltransfer employing veratrol-O-demethylase. *ACS Catal.* 2020, 10, 10375–10380 (2020).

Vinylation of Phenols



E. Busto, R. C. Simon, W. Kroutil, Vinylation of unprotected phenols using a biocatalytic system. *Angew. Chem. Int. Ed.* 54, 10899–10902 (2015).

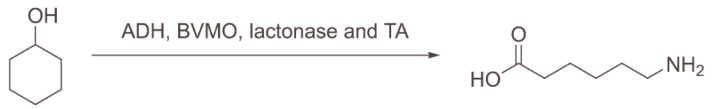
Cyclohexylamines from Diketones



TA = ω-transaminase

E. Siirola, F. G. Mutti, B. Grischek, et al., Asymmetric synthesis of 3-substituted cyclohexylamine derivatives from prochiral diketones via three biocatalytic steps. *Adv. Synth. Catal.* 355, 1703–1708 (2013).

6-Aminohexanoic Acid from Cyclohexanol

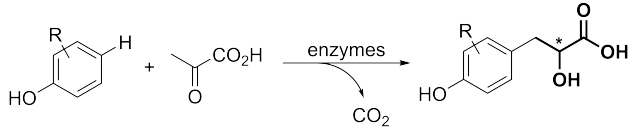


ADH = alcohol dehydrogenase; BVMO = Baeyer-Villiger monooxygenase; TA = ω-transaminase

R. C. Simon, N. Richter, E. Busto, et al., Recent developments of cascade reactions involving ω-transaminases. *ACS Catal.* 4, 129–143 (2014).

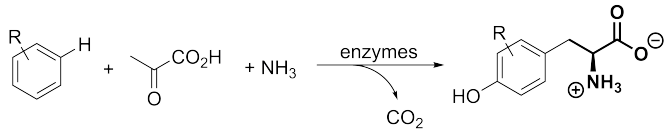
J. H. Sattler, M. Fuchs, F. G. Mutti, et al., Introducing an in situ capping strategy in systems biocatalysis to access 6-aminohexanoic acid. *Angew. Chem. Int. Ed.* 53, 14153–14157 (2014).

Enantiopure Lactic Acids from Phenols



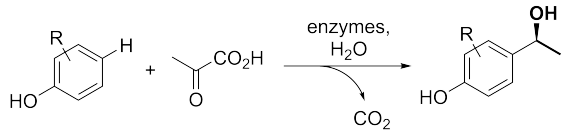
E. Busto, R. C. Simon, N. Richter, et al., One-Pot, Two-Module Three-Step Cascade to Transform Phenol Derivatives to Enantiomerically Pure (R)- or (S)-p-Hydroxyphenyl Lactic Acids. ACS Catal. 6(4), 2393–2397 (2016).

L-Tyrosine Derivatives from Benzenes



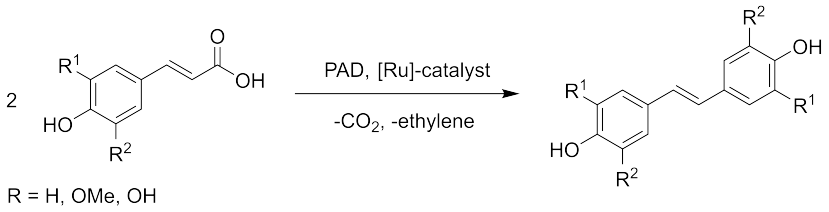
A. Dennig, E. Busto, W. Kroutil, et al., Biocatalytic One-Pot Synthesis of l-Tyrosine Derivatives from Monosubstituted Benzenes, Pyruvate, and Ammonia. *ACS Catal.* 5(12), 7503–7506 (2015).

Hydroxyethylation of Phenols



S. E. Payer, H. Pollak, B. Schmidbauer, et al., Multienzyme One-Pot Cascade for the Stereoselective Hydroxyethyl Functionalization of Substituted Phenols. *Org. Lett.* 20(17), 5139–5143 (2018).

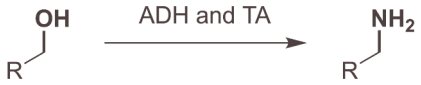
Chemoenzymatic Preparation of Bio-Based Anti-Oxidants



PAD = phenolic acid decarboxylase

Á. Gómez Baraibar, D. Reichert, C. Mügge, et al., A One-Pot Cascade Reaction Combining an Encapsulated Decarboxylase with a Metathesis Catalyst for the Synthesis of Bio-Based Antioxidants. *Angew. Chem. Int. Ed.* 55, 14823–14827 (2016).

Direct Amination of Alcohols



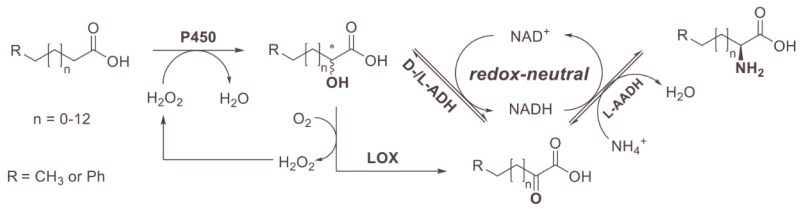
ADH = alcohol dehydrogenase; TA = ω-transaminase

R. C. Simon, N. Richter, E. Busto, et al., Recent developments of cascade reactions involving ω-transaminases. *ACS Catal.* 4, 129–143 (2014).

J. H. Sattler, M. Fuchs, K. Tauber, et al., Redox self-sufficient biocatalyst network for the amination of primary alcohols. *Angew. Chem. Int. Ed.* 51, 9156–9159 (2012).

S. Gandomkar, R. Rocha, F. A. Sorgenfrei, et al., PQQ-dependent Dehydrogenase Enables One-pot Bi-enzymatic Enantio-convergent Biocatalytic Amination of Racemic sec-Allylic Alcohols. *ChemCatChem* 13, 1290–1293 (2021).

Enantioselective α-Oxidation and Amination of Carboxylic Acids



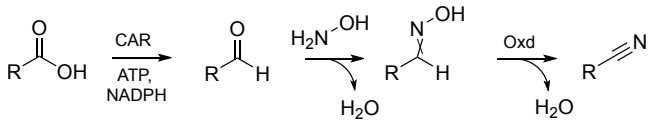
LOX = lactase oxidase; ADH = alcohol dehydrogenase; AADH = amino acid dehydrogenase

S. Gandomkar, A. Dennig, A. Dordic, et al., Biocatalytic Oxidative Cascade for the Conversion of Fatty Acids into α-Ketoacids via Internal H2O2 Recycling. *Angew. Chem. Int. Ed.* 52, 427–430 (2018).

A. Dennig, S. Gandomkar, E. Cigan, et al., Enantioselective biocatalytic formal α-amination of hexanoic acid to l-norleucine. *Org. Biomol. Chem.* 16, 8030–8033 (2018).

A. Dennig, F. Blaschke, S. Gandomkar, et al., Preparative Asymmetric Synthesis of Canonical and Non-canonical α-amino Acids Through Formal Enantioselective Biocatalytic Amination of Carboxylic Acids. *Adv. Synth. Catal.* 361, 1348–1358 (2019).

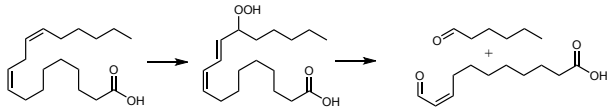
Chemoenzymatic Nitrile Synthesis



M. Winkler, M. Horvat, A. Schiefer, et al., Organic Acid to Nitrile: A Chemoenzymatic Three-Step Route. *Adv. Synth. Catal.* 365, 37–42 (2023).

M. Horvat, V. Weilch, R. Rädisch, et al., Chemoenzymatic One-Pot Reaction from Carboxylic Acid to Nitrile via Oxime. *Catal. Sci. Technol.* 12, 62–66 (2022).

C=C Bond Cleavage

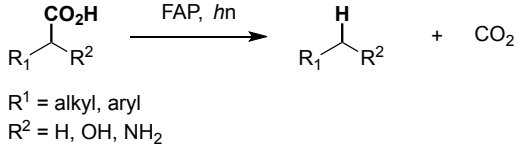


C. Hashem, J. Hochrinner, C. Rinnofner, et al., From Linoleic Acid to Hexanal and Hexanol by Whole Cell Catalysis with a Lipoxygenase, Hydroperoxide Lyase, and Reductase Cascade in *Komagataella phaffii*. *Front. Mol. Biosci.* 9:965315 (2022).

C. Hashem, H. Stolterfoht, C. Rinnofner, et al., Secretion of *Pseudomonas aeruginosa* Lipoxygenase by *Pichia pastoris* Upon Glycerol Feed. *Biotechnol. J.* 15, 2000089 (2020).

H. Stolterfoht, C. Rinnofner, M. Winkler, et al., Recombinant Lipoxygenases and Hydroperoxide Lyases for the Synthesis of Green Leaf Volatiles. *J. Agric. Food Chem.* 67(49), 13367–13392 (2019).

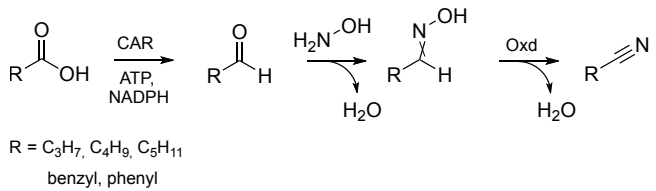
Fatty Acid Photodecarboxylase (FAP)



S. Simić, M. Jaktaitl, W. T. S. Huck, et al., Strategies for Transferring Photobiocatalysis to Continuous Flow Exemplified by Photodecarboxylation of Fatty Acids. *ACS Catal.* 12, 14040–14049 (2022).

C. K. Winkler, S. Simić, V. Jurkaš, et al., Accelerated Reaction Engineering of Photo(Bio)Catalytic Reactions through Parallelization with an Open-Source Photoreactor. *ChemPhotoChem* 5, 957–965 (2021).

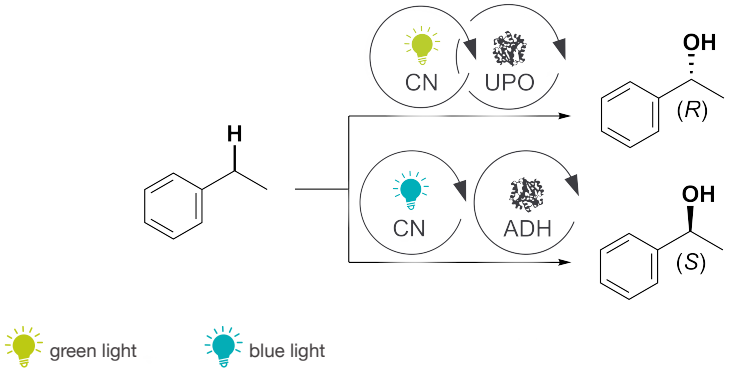
Light-Dependent Protochlorophyllide Oxidoreductase (LPOR)



L. Schmermund, S. Bierbaumer, V. K. Schein, et al., Extending the Library of Light-Dependent Protochlorophyllide Oxidoreductases and their Solvent Tolerance, Stability in Light and Cofactor Flexibility. *ChemCatChem* 12, 4044–4051 (2020).

C. K. Winkler, S. Simić, V. Jurkaš, et al., Accelerated Reaction Engineering of Photo(Bio)Catalytic Reactions through Parallelization with an Open-Source Photoreactor. *ChemPhotoChem* 5, 957–965 (2021).

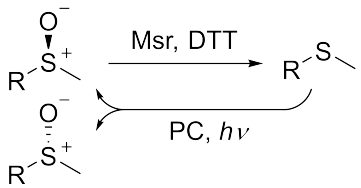
Chromoselective Photochemical-Biocatalytic Cascade



UPO = Unspecific Peroxygenase, ADH = Alcohol Dehydrogenase, CD = Carbon Nitride Photocatalyst

L. Schmermund, S. Reischauer, S. Bierbaumer, et al., Chromoselective Photocatalysis Enables Stereocomplementary Biocatalytic Pathways. *Angew. Chem. Int. Ed.* 60, 6965–6969 (2021).

Photochemical-Biocatalytic Cyclic Deracemization

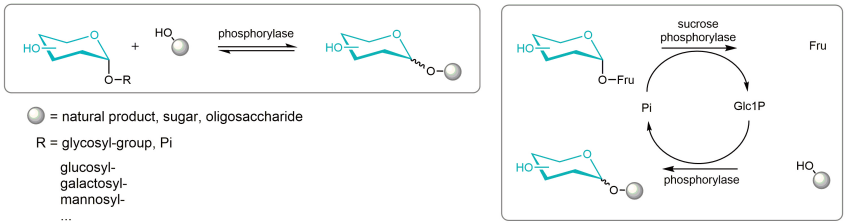


Msr = Methionine Sulfoxide Reductase, DTT = Dithiotreitol, PC = Photocatalyst

S. Bierbaumer, L. Schmermund, A. List, et al., Synthesis of Enantiopure Sulfoxides by Concurrent Photocatalytic Oxidation and Biocatalytic Reduction. *Angew. Chem. Int. Ed.* e202117103 (2022).

Astrid Preisz

Phosphorylase Technology: Direct & Indirect Glucosylation

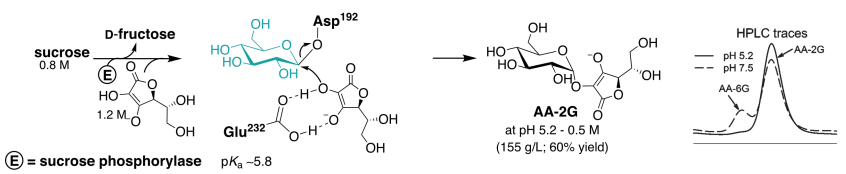


C. Goedl, T. Sawangwan, P. Wildberger, et al., Sucrose phosphorylase: A powerful transglucosylation catalyst for synthesis of α-D-glucosides as industrial fine chemicals. *Biocatal. Biotransformation* 28, 10–21 (2010).

C. Luley-Goedl, B. Nidetzky, Carbohydrate synthesis by disaccharide phosphorylases: Reactions, catalytic mechanisms and application in the glycosciences. *Biotechnol. J.* 5, 1324–1338 (2010).

T. Desmet, W. Soetaert, P. Bojarová, et al., Enzymatic glycosylation of small molecules: Challenging substrates require tailored catalysts. *Chem. - A Eur. J.* 18, 10786–10801 (2012).

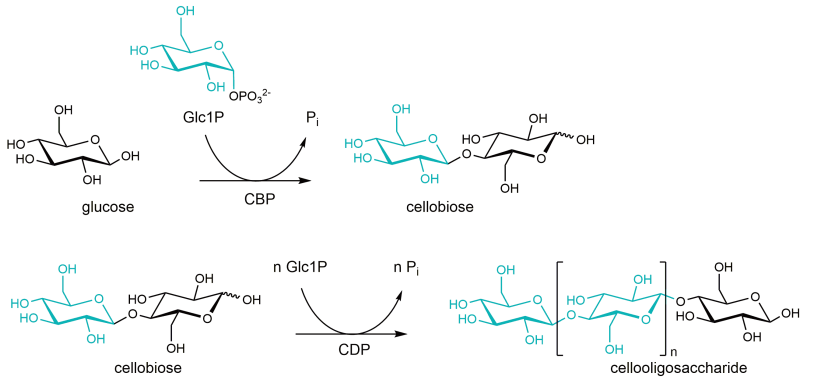
Ascorbic Acid 2-Glucoside



Patent W02017050920 (2017)

R. K. Gudiminchi, B. Nidetzky, Walking a Fine Line with Sucrose Phosphorylase: Efficient Single-Step Biocatalytic Production of L-Ascorbic Acid 2-Glucoside from Sucrose. *ChemBioChem* 18, 1387–1390 (2017).

Cello-oligosaccharides

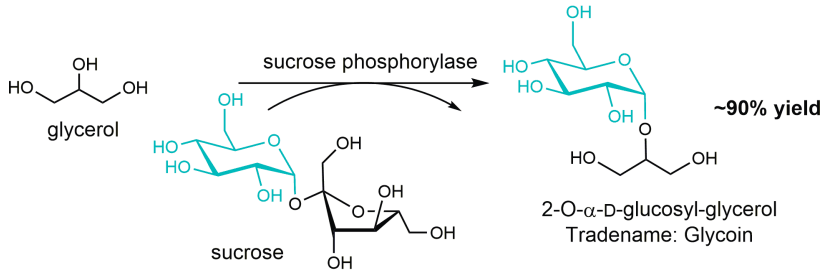


CBP = cellobiose phosphorylase; CDP = cellodextrin phosphorylase

C. Zhong, C. Luley-Goedl, B. Nidetzky, Product solubility control in cellooligosaccharide production by coupled cellobiose and cellodextrin phosphorylase. *Biotechnol. Bioeng.* 116, 2146–2155 (2019).

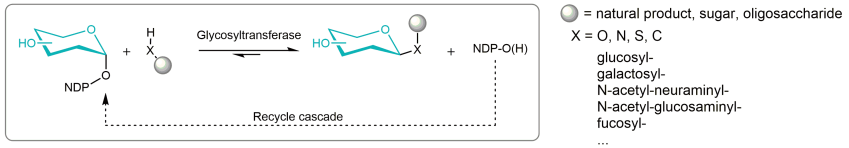
B. Nidetzky, C. Zhong, Phosphorylase-catalyzed bottom-up synthesis of short-chain soluble cellooligosaccharides and property-tunable cellulosic materials. *Biotechnol. Adv.* 51, 107633 (2021).

Glucosylglycerol



C. Goedl, T. Sawangwan, M. Mueller, et al., A high-yielding biocatalytic process for the production of 2-O-(α-D-glucopyranosyl)-sn-glycerol, a natural osmolyte and useful moisturizing ingredient. *Angew. Chem. Int. Ed.* 47, 10086–10089 (2008).

Glycosyltransferase Technology

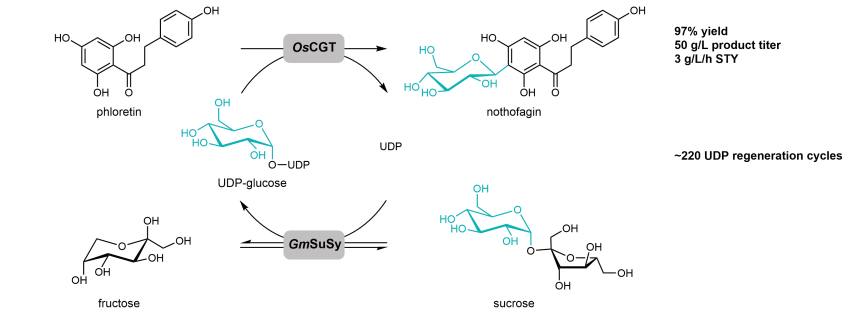


M. Pfeiffer, B. Nidetzky, C-Ribosylating Enzymes in the (Bio)Synthesis of C-Nucleosides and C-Glycosylated Natural Products. *ACS Catalysis*, 13, 15910-15938 (2023).

A. Gutmann, B. Nidetzky, Enzymatic C-glycosylation: Insights from the study of a complementary pair of plant O- and C-glucosyltransferases. *Pure Appl. Chem. 85*, 1865–1877 (2013).

B. Nidetzky, A. Gutmann, C. Zhong, Leloir Glycosyltransferases as Biocatalysts for Chemical Production. *ACS Catal. 8*, 6286–6300 (2018).

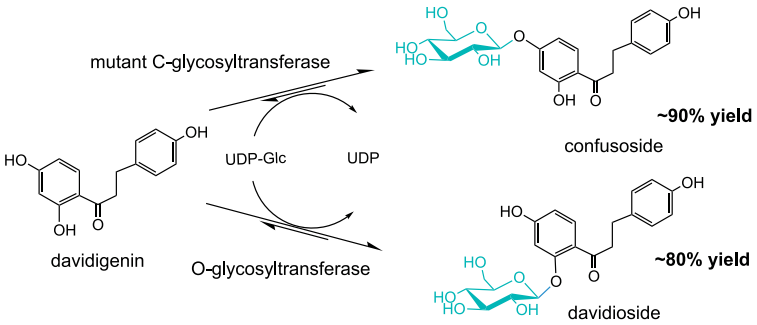
Nothofagin



SuSy = sucrose synthase; CGT = C-glucosyltransferase

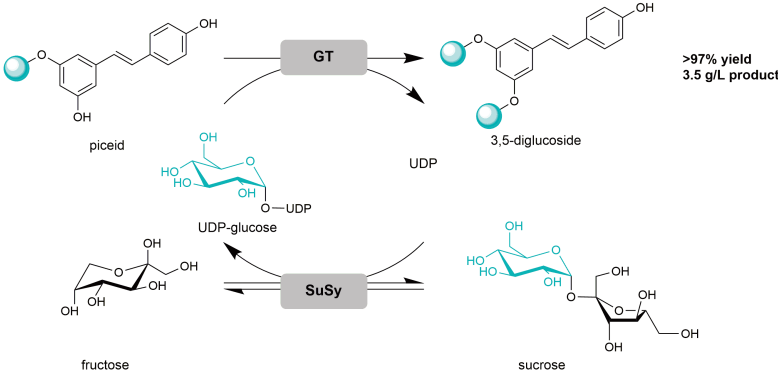
H. Liu, G. Tegl, B. Nidetzky, Glycosyltransferase Co-Immobilization for Natural Product Glycosylation: Cascade Biosynthesis of the C-Glucoside Nothofagin with Efficient Reuse of Enzymes. *Adv. Synth. Catal.* 363, 2157 (2021).

Dihydrochalcone Glucosides



A. Gutmann, L. Bungaruang, H. Weber, et al., Towards the synthesis of glycosylated dihydrochalcone natural products using glycosyltransferase-catalysed cascade reactions. *Green Chem.* 16, 4417–4425 (2014).

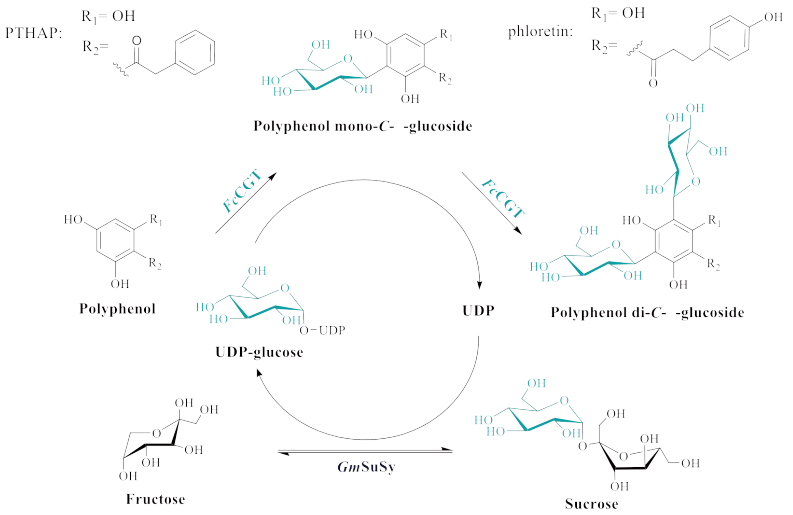
Resveratrol 3,5-β-D-Glucoside



SuSy = sucrose synthase; GT = glycosyltransferase

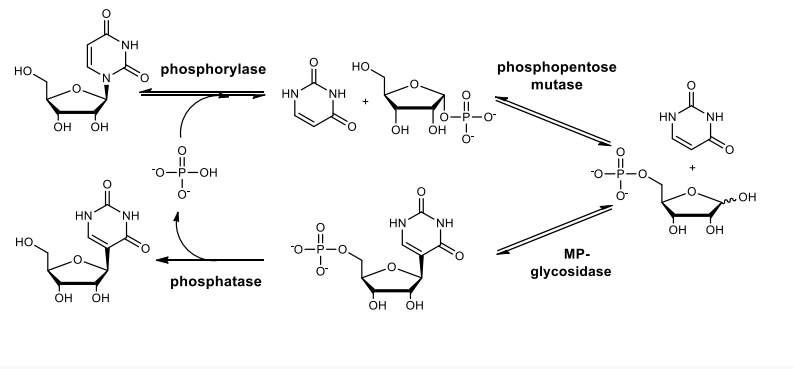
A. Lepak, A. Gutmann, S. T. Kulmer, et al., Creating a Water-Soluble Resveratrol-Based Antioxidant by Site-Selective Enzymatic Glucosylation. *ChemBioChem.* 16, 1870–1874 (2015).

Di-C-Glucosides



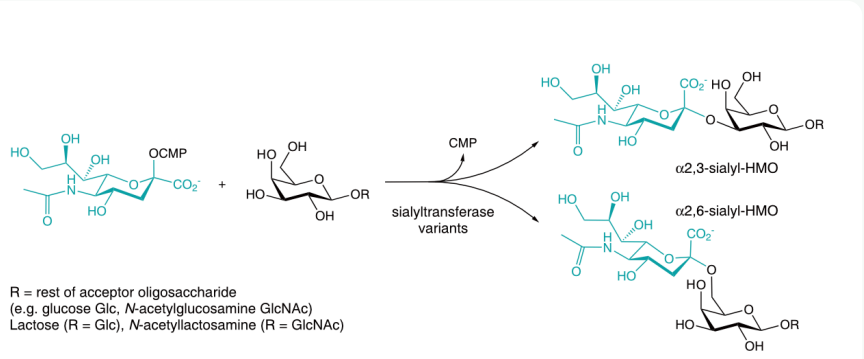
T. Li, A. J. E. Borg, L. Krammer, et al., Reaction intensification for biocatalytic production of polyphenolic natural product di-C-β-glucosides. *Biotechnol. Bioeng.* 120, 1506–1520 (2023).

C-Nucleoside



M. Pfeiffer, A. Ribar, B. Nidetzky, A selective and atom-economic rearrangement of uridine by cascade biocatalysis for production of pseudouridine. *Nat. Commun*. 14, 2261 (2023).

3’- and 6’-sialyl-HMOs

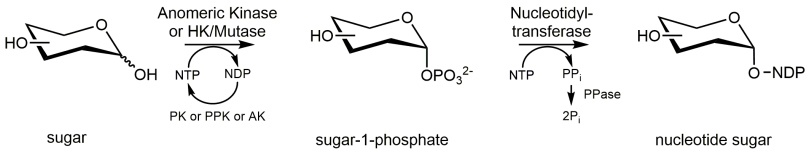


K. Schmölzer, T. Czabany, C. Luley-Goedl, et al., Complete switch from α-2,3- to α-2,6-regioselectivity in *Pasteurella dagmatis* β-D-galactoside sialyltransferase by active-site redesign. *Chem. Commun.* 51, 3083–3086 (2015).

S. Schelch, M. Eibinger, J. Zuson, et al., Modular bioengineering of whole-cell catalysis for sialo-oligosaccharide production: coordinated co-expression of CMP-sialic acid synthetase and sialyltransferase. *Microb. Cell Fact.* 22, 241 (2023).

Astrid Preisz

Kinase & Transferase

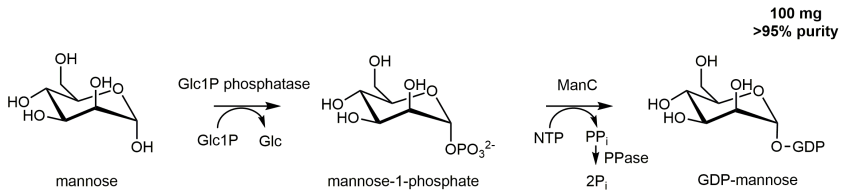


HK = hexokinase, PK = pyruvate kinase; PPK = polyphosphate kinase; AK = acetate kinase; ManC = mannose-1-phosphate guanylyltransferase; PPase = pyrophosphatase

Examples synthesized: GDP-L-fucose, GDP-mannose, UDP-glucose, UDP-galactose, UDP-glucuronic acid, various rare sugar nucleotides

Phosphatase & Transferase

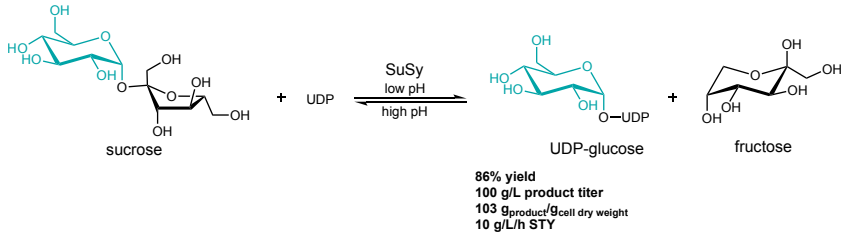
GDP-Mannose



M. Pfeiffer, D. Bulfon, H. Weber, et al., A kinase-independent one-pot multienzyme cascade for an expedient synthesis of guanosine 5′-diphospho-D-mannose. *Adv. Synth. Catal.* 358, 3809–3816 (2016).

Synthase

UDP-Glucose

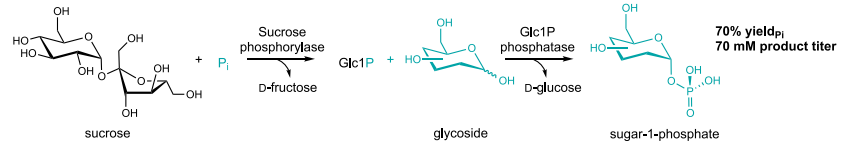


SuSy = sucrose synthase

K. Schmölzer, M. Lemmerer, A. Gutmann, B. Nidetzky, Integrated process design for biocatalytic synthesis by a Leloir glycosyltransferase: UDP-glucose production with sucrose synthase. *Biotechnol. Bioeng.* 114, 924–928 (2017).

Phosphorylase & Phosphatase

Sugar1-Phosphates



P. Wildberger, M. Pfeiffer, L. Brecker, et al., Diastereoselective synthesis of glycosyl phosphates by using a phosphorylase-phosphatase combination catalyst. *Angew. Chem. Int. Ed.* 54, 15867–15871 (2015).

In-silico Search for Novel Biocatalysts

Traditional screening for novel enzymes requires time-consuming experiments and expensive activity assays in the wet-lab. To reduce costs, the prediction and identification of enzyme functionalities is a major challenge of modern bioinformatics. However, the computational annotation of proteins proves to be difficult erroneous and lacks the possibility to identify completely independent novel biocatalysts because they rely on the correlation of (sequence) similarities with the known functions of the template and are bound to find „more of the same“.

CATALOPHORE SEARCH FOR NOVEL ENZYMES

acib-researchers developed a patented bioinformatics method to mine structural databases using three dimensional search templates which cover the arrangement of chemical functional groups or pre-calculated point-clouds representing the „empty space“ of active sites. These search templates are termed „catalophores“ (i.e. carrier of the catalytic function). The searches are independent of structural or sequence similarities to currently employed enzymes. Therefore, these identified enzymes may feature different physico-chemical properties such as stability selectivity or substrate tolerance.
A successful test-case led to the identification of two „novel“ ene-reductases, by searching with patterns obtained from classical old yellow enzymes. The identified enzymes showed significant conversions on typical old yellow enzyme substrates and even allowed access to enantiomers that could not be obtained using current enzyme portfolio although the overall sequence and structural similarity are below 10 %.

G. Steinkellner, C. C. Gruber, T. Pavkov-Keller, et al., Identification of promiscuous ene-reductase activity by mining structural databases using active site constellations. *Nat. Commun.* 5, 4150 (2014).

Kinetic Modeling for Enzymatic Cascade Optimization

To harness the full potential of biotransformations, it is essential to explore the true process boundaries and define the optimal window of operation. Kinetic modeling is a powerful engineering tool in enzyme-based process development, especially in cascade reaction optimization. By using kinetic models, you can adopt a systematic, knowledge-based approach to optimization. These models help unravel the complex network of interconnected factors of cascade process efficiency.

WE OFFER ADVANCED MODEL-BASED REACTION OPTIMIZATION (MECHANISTIC-KINETIC MODELS, HYBRID MODELS) TO UNLOCK THE FULL POTENTIAL OF BIOTRANSFORMATIONS

However, applying mechanistic-kinetic models can be challenging, especially under the actual conditions of biocatalytic synthesis. For instance, high substrate concentrations in synthetic processes can introduce specific and nonspecific effects, complicating the model extension.

To address these challenges, we offer an innovative approach through hybrid modeling. Hybrid models combine mechanistic-kinetic models with empirical descriptions of real process conditions. This approach bridges the gap between mechanistic research and practical application in technologically relevant conditions, providing significant benefits for biocatalytic process development. The modeling approach comprises parameterization, simulation, and optimization. Interfaces with data-driven process analysis methods extend the power of the model-based optimization procedure.

Discover the advantages of kinetic and hybrid modeling with us to elevate your biotransformations to the next level!
with a convolution of specific and nonspecific effects. We offer an approach by hybrid modeling where hybrid models expand the mechanistic-kinetic model by an empirical description of the effect of the real process conditions. Hybrid models can close the gap between mechanistic research and applicability in technologically relevant reaction conditions, such realizing important benefits for biocatalytic process development.

A. Sigg, M. Klimacek, B. Nidetzky, Pushing the boundaries of phosphorylase cascade reaction for cellobiose production I: Kinetic model development. *Biotechnol. Bioeng.* 121, 580–592 (2024).

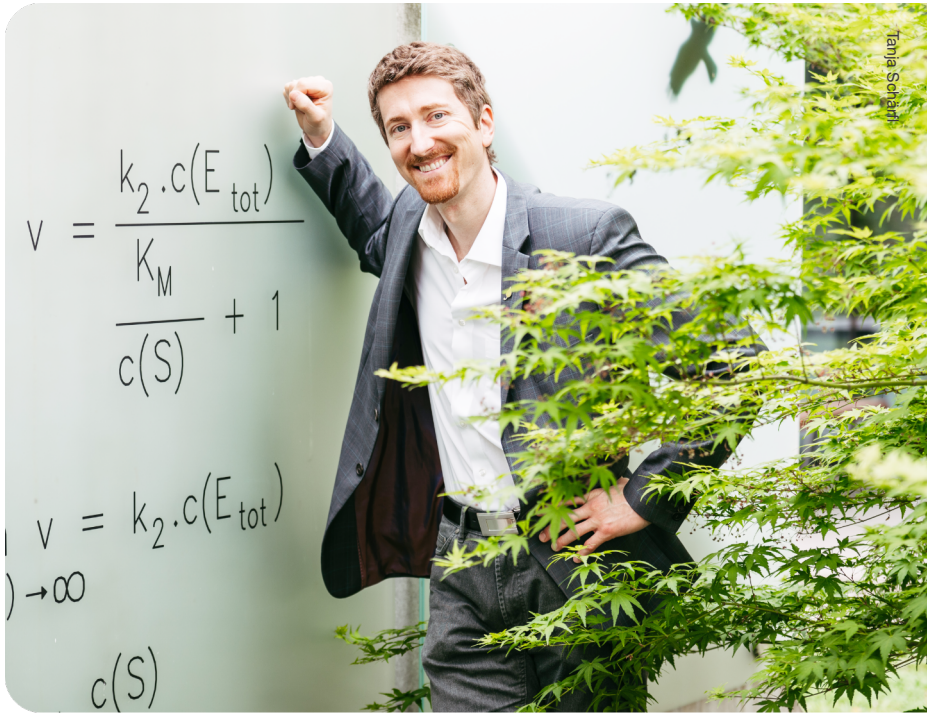
A. Sigg, M. Klimacek, B. Nidetzky, Pushing the boundaries of phosphorylase cascade reaction for cellobiose production II: Model-based multiobjective optimization. *Biotechnol. Bioeng.* 121, 566–579 (2024).

S. Schelch, M. Eibinger, S. Gross Belduma, et al., Engineering analysis of multienzyme cascade reactions for 3'-sialyllactose synthesis. *Biotechnol. Bioeng.* 118, 4290–4304 (2021).

Austrian Centre of Industrial Biotechnology

acib is an international research centre in the field of industrial biotechnology. The centre develops sustainable, and economically and technologically advanced processes for the biotech-, pharmaceutical and chemical industries.

The non-profit organization with its headquarters in Graz has additional sites in Austria, namely in Tulln, Vienna and Innsbruck. acib benefits from a close cooperation to its scientific partners at Austrian Universities such as Graz University of Technology, University of Natural Resources and Life Sciences Vienna (BOKU), University of Technology Vienna, University of Graz or University of Innsbruck for example. acib bundles an international consortium of more than 200 academic and industrial partners. Among the partners are renowned companies such as BASF, Sandoz, Boehringer Ingelheim RCV, Jungbunzlauer, OMV, Validogen, Vogelbusch, and many others.



Tanja Schaar

RESEARCH FIELDS

- Biocatalysis
- Enzyme Technologies & Protein Engineering
- Synthetic Biology
- Bioprocess Technologies
- Bioinformatics & Simulations
- Bioeconomy Technologies

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