Biocatalytic Synthesis
enzymatic solutions for chemical problems

Enzyme Engineering & Technology
enzymes designed for industry

science is our success
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Reductive Biotransformations

Reduction of Carboxylic Acids

\[
\text{CAR} \quad \text{R}^1\text{OH} \xrightarrow{\text{ATP, NADPH}} \text{R}^1\text{COOH}
\]

\[
\text{ADH} \quad \text{R}\xrightarrow{\text{NAD(P)H}} \text{R}\text{OH}
\]


Reduction of Aldehydes and Ketones

\[
\text{ADH} \quad \text{R}^1\text{R}^2\xrightarrow{\text{NAD(P)H}} \text{R}^1\text{R}^2\text{OH}
\]

\[
\text{ADH} \quad \text{R}^1\xrightarrow{\text{NAD(P)H}} \text{R}^1\text{OH}
\]


Reduction of C=C-Bonds

\[
\text{Ene-Reductase} \quad \text{R}^2\text{R}^1\text{X} \xrightarrow{\text{NAD(P)H}} \text{R}^2\text{R}^1\text{H}^*\text{R}^3\text{H}^*
\]


Reduction of Imines

\[
\text{IRED} \quad \text{R}^2\text{R}^1\text{N}^*\text{R}^3 \xrightarrow{\text{NADPH}} \text{R}^2\text{R}^1\text{H}^*\text{R}^3\text{H}
\]

Reductive Biotransformations

Reductive Amination of Aldehydes and Ketones

\[ \begin{align*}
\text{O} & \quad \text{amine donor} & \quad \text{N} & \quad \text{amine donor} \\
\text{R}^1 & \quad \text{R}^2 & \quad \text{[NAD(P)]H} & \quad \text{[NAD(P)]H}
\end{align*} \]

\[ \begin{align*}
\text{R} & \quad \text{R}^1 & \quad \text{NH}_2 & \quad \text{NH}_2 \\
\end{align*} \]

\( (\text{TA} = \omega\text{-transaminase}; \text{Angew. Chem., Int. Ed.} \ 2008, \ 47, \ 9337; \text{Angew. Chem., Int. Ed.} \ 2012, \ 27, \ 1; \text{Org. Process Res. Dev.} \ 2013, \ 17, \ 751; \text{ACS Catal.} \ 2014, \ 4, \ 129. ) \)

Structure of an Ene-Reductase
Oxidative Biotransformations

Oxidation of Alcohols / Aldehydes

\[
\begin{align*}
\text{OH} & \quad \text{ADH / NAD(P)⁺ or Oxidase / O₂} \\
R¹R² & \quad \rightarrow \quad \text{O₂} \\
R¹R² & \quad \text{ADH / NAD(P)⁺ or Oxidase / O₂} \\
R¹R² & \quad \text{O} \\
\end{align*}
\]


C=C-Bond Cleavage

\[
\begin{align*}
\text{R¹} & \quad \text{enzyme, O₂} \\
\text{R²} & \quad \rightarrow \quad \text{O₂} \\
\text{R¹} & \quad + \quad \text{O₂R²} \\
\end{align*}
\]


Enzymatic De-Amination

\[
\begin{align*}
\text{R¹} & \quad \text{TA or AO amine acceptor [NAD(P)⁺]} \\
\text{R²} & \quad \rightarrow \quad \text{O₂} \\
\text{R¹} & \quad \text{TA or AO amine acceptor [NAD(P)⁺]} \\
\text{R²} & \quad \rightarrow \quad \text{O₂} \\
\end{align*}
\]


Enzymatic De-Alkylation

\[
\begin{align*}
\text{Alkyl} & \quad \text{enzyme} \\
\text{R¹} & \quad \text{O₂} \\
\text{R²} & \quad \rightarrow \quad \text{H} \\
\text{R¹} & \quad \text{O₂} \\
\text{R²} & \quad \rightarrow \quad \text{H} \\
\end{align*}
\]

\( (\text{Angew. Chem. Int. Ed. 2015, 54, 15051}). \)

Enzymatic Hydration

\[
\begin{align*}
\text{HO} & \quad \text{enzyme} \quad \text{H₂O} \\
\text{OH} & \quad \rightarrow \quad \text{OH} \\
\end{align*}
\]

\( (\text{Angew. Chem., Int. Ed. 2013, 52, 2293}). \)
Oxidative Biotransformations

Oxidative Decarboxylation

\[
\begin{align*}
\text{R}^1\text{R}^2\text{R}^3\text{O} &\xrightarrow{\text{P450 OleT}} \text{R}^1\text{R}^2\text{R}^3\text{O}^2 + \text{CO}_2 \\
\text{R}^1\text{R}^2\text{R}^3\text{OH} &\xrightarrow{\text{O}_2 + \text{NADH or H}_2\text{O}_2} \text{R}^1\text{R}^2\text{R}^3\text{O} + \text{CO}_2
\end{align*}
\]


Enzymatic Hydroxylation

\[
\text{R}^1\text{R}^2\text{R}^3\text{O} \xrightarrow{\text{P450 monoxygenase}} \text{R}^1\text{R}^2\text{R}^3\text{OH}
\]


Baeyer-Villiger Oxidation

\[
\begin{align*}
\text{R}^1\text{R}^2\text{O} &\xrightarrow{\text{hFMO}} \text{R}^1\text{R}^2\text{O}^2 \\
\text{R}^1\text{R}^2\text{R}^3\text{O} &\xrightarrow{\text{hFMO}} \text{R}^1\text{R}^2\text{R}^3\text{O}^2
\end{align*}
\]

(hFMO = human flavin containing monoxygenase; ACS Chem. Biol., 2016, 11, 1039-1048.)

Oxyfunctionalisation of Amino Acids

(aKG = α-ketoglutarate; AAD = amino acid dioxygenase; Front. Microbiol., 2016, 7, 425.)
C-C Bond Formation

Bio-Carboxylation

Bio-Carboxylation

\[
\begin{align*}
\text{Ph} & \quad o\text{-carboxylase} & \quad \text{KHCO}_3 & \quad \text{Ph} \quad o\text{-carboxylase} & \quad \text{KHCO}_3 \\
\text{Ph} & \quad p\text{-carboxylase} & \quad \text{KHCO}_3 & \quad \text{Ph} \quad p\text{-carboxylase} & \quad \text{KHCO}_3
\end{align*}
\]


Bio-Friedel-Crafts Acylation

Bio-Friedel-Crafts Acylation

\[
\begin{align*}
\text{Ph} & \quad \text{acyltransferase} & \quad \text{acyl donor} & \quad \text{Ph} \quad \text{acyl donor}
\end{align*}
\]

(unpublished results)

Biocatalytic Alkaloid Synthesis

Biocatalytic Alkaloid Synthesis

\[
\begin{align*}
\text{Ph} & \quad \text{STR} & \quad \text{NCS} & \quad \text{Angew. Chem., Int. Ed. 2011, 50, 1068; Org. Process Res. Dev. 2013, 17, 751; Angew. Chem., Int. Ed. 2014, 53, 3731.}
\end{align*}
\]

Trifluoromethylation

Trifluoromethylation

\[
\begin{align*}
\text{Ph} & \quad \text{laccase} & \quad \text{TFMS, TBHP} & \quad \text{Ph} \quad \text{CF}_3
\end{align*}
\]

(TFMS = trifluoromethylsulfonic acid; TBHP = tert-butylhydroperoxide; Nat. Commun. 2016, in press, DOI: 10.1038/ncomms13323.)
C-C Bond Formation

Cyanohydrin Synthesis/Henry Reaction

\[
\begin{array}{c}
\text{R}^1\text{R}^2\text{R}^3\text{CHNO}_2 \xrightarrow{\text{HNL}} \text{R}^1\text{H} \xrightarrow{\text{HNL}} \text{R}^1\text{R}^2\text{R}^3\text{CN} \\
\end{array}
\]


C-C Bond Breaking

Asymmetric Synthesis of Optically Pure \(\alpha\)-Substituted Carboxylic Acids

\[
\begin{array}{c}
\text{R}^1\text{R}^2\text{CO}_2\text{H} \xrightarrow{\text{AMDase}} \text{R}^1\text{R}^2\text{CO}_2\text{H} \\
\end{array}
\]

R\(^1\) = aryl, vinyl
R\(^2\) = Me, OH, NH\(_2\), F

>99% ee
(R) or (S)

Enzymatic Isomerisation

Racemization of Arylpropionates

\[
\begin{align*}
\text{APR} & \quad \text{R}^1 \text{CO}_2\text{H} \quad \rightarrow \quad \text{R}^2 \text{CO}_2\text{H} \\
\text{R}^1 & = \text{aryl, vinyl} \\
\text{R}^2 & = \text{Et, Me, OH, NH}_2, \quad \text{F}
\end{align*}
\]

\[\text{(APR = arylpropionate racemase; ChemBioChem, 2016, 16, 1943; Cat. Sci. Technol., 2016, 6, 4397.)}\]

Isomerisations of C=C-Bonds


Disproportionation: Biocatalytic Cannizzaro Reaction

\[\text{(ADH = alcohol dehydrogenase; ChemCatChem, 2013, 5, 1744–1748.)}\]
Hydrolysis / Esterification

Hydrolysis of Lactams

\[ \text{H}_2\text{N} - \text{C}=\text{O} \rightarrow \text{H}_2\text{N} - \text{C}=\text{O} + \text{H}_2\text{O} \]

(ChemCatChem 2014, 6, 2517.)

Bio-Mitsunobu-Inversion

\[ \text{OH} + \text{OSO}_3\text{H} \rightarrow \text{OSO}_3\text{H} + \text{OH} \]


C-C Bond Hydrolysis

\[ \text{R} - \text{C}=\text{O} \rightarrow \text{R} - \text{C}=\text{O} + \text{H}_2\text{O} \]


Nitrile Hydrolysis

\[ \text{RCN} \rightarrow \text{RCONH}_2 \rightarrow \text{RCOOH} \]

Hydrolysis / Esterification

Enantioselective Ester Formation in Aqueous Systems

\[
\begin{align*}
&\text{OH} \\
&\text{R}^1 \quad \text{R}^2 \\
&\text{at} \\
&\text{acyl donor} \\
&\text{OAc} \\
&\text{R}^1 \quad \text{R}^2
\end{align*}
\]

\( R^1, R^2 = \text{alkyl} \)

\((\text{AT} = \text{acyl transferase}; \text{to be published.})\)

Enzymatic Phosphorylation

\[
\begin{align*}
&\text{OH} \\
&\text{R} \\
&\text{phosphatase} \\
&\text{PPi} \\
&\text{R} \quad \text{O} \quad \text{P} \quad \text{O} \quad \text{OH}
\end{align*}
\]

\((\text{PPi} = \text{phyrophosphate}; \text{Eur. J. Org. Chem.}, 2016, 45–50.)\)

Enzymatic Cascades

Chemoenzymatic Preparation of Bio-Based Anti-Oxidants

\[
\begin{align*}
&\text{2} \\
&\text{2R}^1 \quad \text{2R}^2 \\
&\text{HO} \\
&\text{HO} \\
&\text{PAD, [Ru]-catalyst} \\
&\text{-CO}_2, \text{-ethylene} \\
&\text{R}^1 \quad \text{R}^2 \quad \text{OH} \\
&\text{R}^1 \quad \text{R}^2
\end{align*}
\]

\((\text{PAD} = \text{phenolic acid decarboxylase}; \text{Angew. Chem., Int. Ed.}, 2016, \text{in press}, \text{DOI: 10.1002/anie.201607777.})\)
Enzymatic Cascades

Direct Amination of Alcohols

\[
\begin{align*}
\text{OH} & \quad \text{ADH and TA} \quad \text{NH}_2 \\
R & \quad \text{R'}
\end{align*}
\]

\((ADH = \text{alcohol dehydrogenase}; \ TA = \omega\text{-transaminase}; \text{Angew. Chem., Int. Ed. 2012, 51, 9156; ACS Catal. 2014, 4, 129.})\)

6-Aminohexanoic Acid from Cyclohexanol

\[
\begin{align*}
\text{OH} & \quad \text{ADH, BVMO, lactonase and TA} \quad \text{NH}_2 \\
\text{HO} & \quad \text{HO} \\
\text{NH}_2 & \quad \text{NH}_2
\end{align*}
\]

\((ADH = \text{alcohol dehydrogenase}; \ BVMO = \text{Baeyer-Villiger monoxygenase}, \ TA = \omega\text{-transaminase}; \text{Angew. Chem., Int. Ed. 2014, 53, 14153; ACS Catal. 2014, 4, 129.})\)

Cyclohexylamines from Diketones

\[
\begin{align*}
\text{R}_2 \quad \text{C-C bond hydrolase, lipase and TA} \quad \text{NH}_2 \\
\text{R}_1 \quad \text{R}_1 \quad \text{R}_2 \quad \text{R}_2 \\
\text{NH}_2 \quad \text{NH}_2
\end{align*}
\]


Vinylation of Phenols

\[
\begin{align*}
\text{HO} & \quad \text{CO}_2 \quad \text{H} \quad \text{CO}_2 \\
\text{HO} & \quad \text{CO}_2 \quad \text{H} \quad \text{CO}_2
\end{align*}
\]

\((\text{Angew. Chem. Int. Ed. 2015, 45, 10899.})\)
Glycozyme Technology

Regioselective Glycosyltransfer

(Siocat. Biotrans. 2010, 28, 10; Pure Appl. Chem. 2013, 85, 1865; PCT Int. Appl. 2015, WO 2015001033.)

Small Molecule Glycosylation


Regioselective Glucosylation – Flavonoid Glycosylation

(Green Chem. 2014, 16, 4417.)
**Glycozyme Technology**

**Nucleotid Sugar Synthesis**

![Chemical Reaction Diagram]

(SPase = sucrose phosphorylase; AGP = α-D-glucose 1-phosphate phosphatase; PPase = pyrophosphatase; NTPase = nucleotide transferase; GTP = guanosine 5′-triphosphate; D-Glc = D-glucose; D-Fru = D-fructose; Pi = inorganic phosphate; Adv. Synth. Catal., submitted.)

**Diasterioselective Synthesis of Glycosyl Phosphates**

![Chemical Reaction Diagram]

(SPase = sucrose phosphorylase; AGP = α-D-glucose 1-phosphate phosphatase; D-Glc = D-glucose; D-Fru = D-fructose; Pi = inorganic phosphate; Angew. Chem., Int. Ed., 2015, 54, 15867.)
API-Modification with Human Enzymes

Chemo- & Regioselective Oxidation of Soft Nucleophiles

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\(^1\) 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\(^2\), 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%.

Catalophore used for the identification of the old-yellow enzyme example; a) schematic mechanism of the reaction mechanism; b) 3D active site constellation (“catalphore”); c) catalophore motif indicating used atom types; d) geometrical representation of the search motif used for the database search.

about acib

next step in industrial research

The Austrian Centre of Industrial Biotechnology (acib) is an international research center with 200+ employees based in Austria with collaborative links to Germany, Slovenia, Italy, Spain and Poland. acib can draw on 25+ years of experience to apply sophisticated, new methods in industrial production – focused on biocatalysis, enzymes, polymers, (pharmaceutical) protein production & purification, cell line development, bioinformatics and synthetic biology.

Together with 130+ industrial and scientific partners acib adopts the tools and methods of nature for i) new production processes & products with improved ecological efficiency, ii) new production processes with higher economic efficiency, iii) products with higher quality and purity, iv) functional innovative products for everyday use and v) sophisticated computational methods for the health care, pharmaceutical or chemical industry...

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