

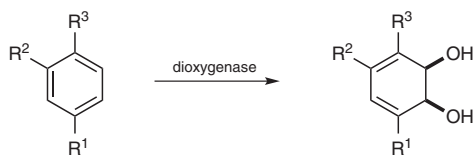
Abstracts

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3.1 Dihydroxylation of Aromatics and Alkenes

C. C. R. Allen

The use of ring-hydroxylating dioxygenase enzymes for the biotransformation of aromatic hydrocarbons, alkenes, and phenols to give chiral *cis*-dihydrodiol metabolites is of significant potential for the preparation of chiral precursors for organic synthesis. Many products are produced with high enantiomeric excess, and a wide number of biotransformations have been studied. This type of biotransformation is typically used to convert readily available starting materials into single enantiomer bioproducts in a single step. The enzymes are multicomponent systems comprising two or more protein subunits. Furthermore, there is a requirement for reducing equivalents (e.g., NADH) and therefore whole-cell biocatalysts are used, either as wild-type strains, mutants, or clones. Recently, there have been significant developments in the use of molecular biology methods to improve these biocatalysts. This review covers the approaches employed to perform specific types of biotransformation, namely arene, alkene, and phenol hydroxylation.



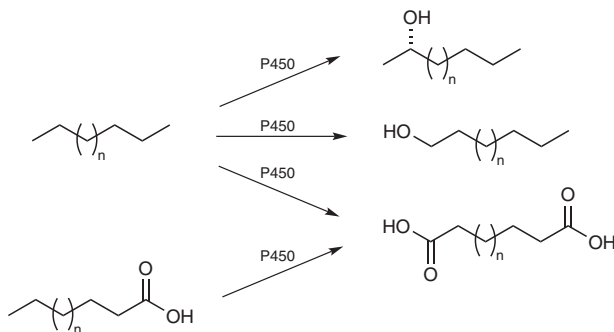
Keywords: biocatalysis · chiral pool · dihydroxylation · enzyme catalysis · diols · phenols

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3.2.1 Cytochrome P450 in the Oxidation of Alkanes

J. C. Nolte and V. B. Urlacher

Selective direct oxidation of relatively cheap alkanes leads to valuable synthons that can be used as building blocks for the chemical and pharmaceutical industry. This chapter describes the hydroxylation of alkanes and fatty acids catalyzed by cytochrome P450 monooxygenases (CYP). It summarizes early and more-recent methods for the selective production of terminal and subterminal alcohols, dicarboxylic acids, and other oxidation products of alkanes using isolated enzymes and whole-cell biocatalysts.

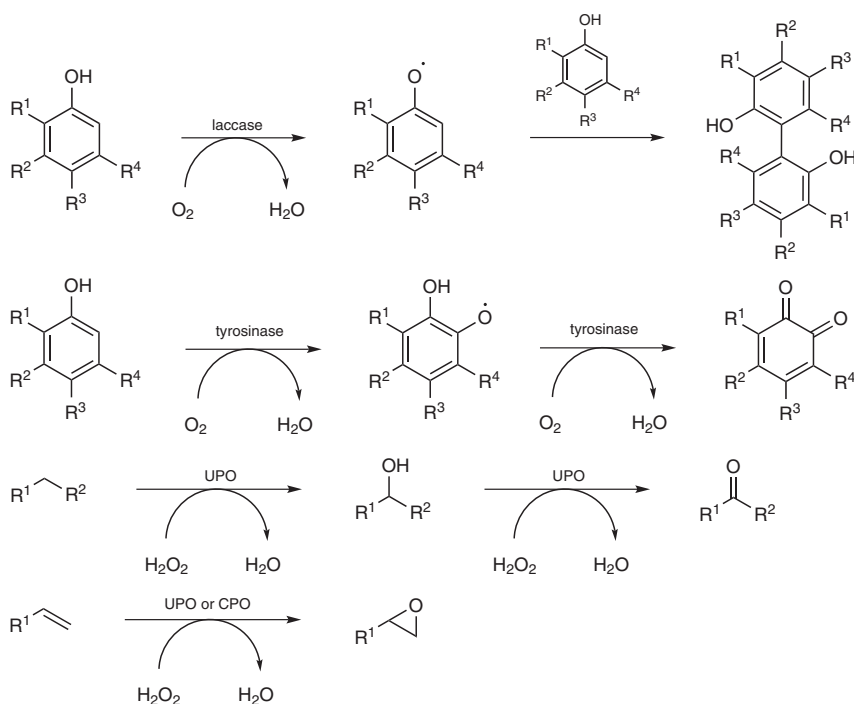


Keywords: alkanes · fatty acids · microbial oxidation · biocatalysts · enzyme catalysis

3.2.2 Oxidation Other Than with Cytochrome P450s

S. Herter and N. J. Turner

The laccase- or tyrosinase-catalyzed oxidation of phenolic compounds leads to the generation of radical cations which subsequently produce quinoid derivatives. Acting as electrophilic Michael acceptors, quinones can undergo successive reactions amongst themselves yielding homomolecular products. In the presence of nucleophiles acting as coupling partners, enzymatically generated quinones undergo hetero-cross-coupling reactions to give novel hybrid molecules. In contrast to laccases, tyrosinase enzymes also catalyze the *ortho*-hydroxylation of monophenols, giving rise to catechols and benzo-1,2-quinones. Unspecific peroxygenases (UPOs) catalyze the hydroxylation of a broad range of C–H containing substrates, including small aromatic compounds, larger polycyclic aromatic hydrocarbons, heteroaromatics, alkanes, and cycloalkanes. A common feature of UPOs and chloroperoxidases (CPOs) is found in the asymmetric epoxidation of alkenes to yield the corresponding epoxides, often with high enantiomeric excess.



Keywords: 1,4-addition reactions · C–C coupling · C–H activation · chloroperoxidases · C–O coupling · Diels–Alder cyclization · domino reactions · epoxidation · homomolecular coupling · hetero-cross-coupling reactions · hydroxylation · laccases · oxidation · quinones · tyrosinases · unspecific peroxygenases

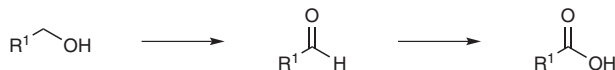
3.3.1 Oxidations Using Dehydrogenases

F. Hollmann

The use of alcohol dehydrogenases has a number of advantages over traditional chemical methods for the oxidation of alcohols. These include the mild reaction conditions, the avoidance of tedious protecting-group strategies, and the high regio- and chemoselectivities. This review highlights the most important alcohol dehydrogenases used for bioca-

talysis and discusses the systems used for cofactor regeneration when employing these enzymes. The oxidation of primary alcohols to aldehydes and further to carboxylic acids is presented, along with examples of subsequent cascade reactions (e.g., oxidation–lactonization of diols). The oxidation of secondary alcohols to ketones, including the application of this reaction in kinetic resolutions and deracemizations, is also described.

selective oxidation to aldehyde or acid



kinetic resolution of racemic alcohols



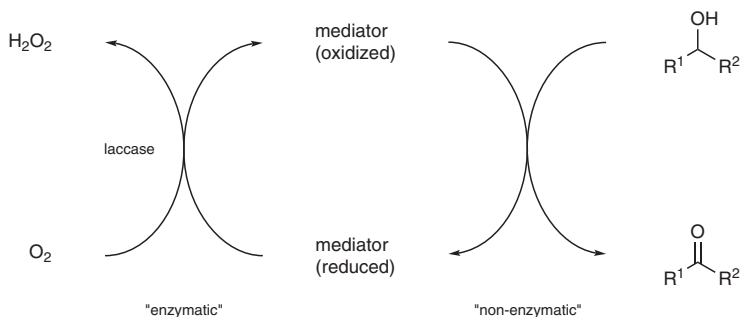
Keywords: alcohol dehydrogenases · oxidation · alcohols · aldehydes · ketones · carboxylic acids · cofactor regeneration · kinetic resolution · deracemization

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3.3.2 Oxidation Using Laccases

S. Herter and N. J. Turner

The oxidation of a diverse range of primary and secondary alcohols to aldehydes and ketones, respectively, can be achieved via the laccase–mediator approach, which operates in aqueous or biphasic systems under mild conditions in the presence of oxygen.



R¹ = H, alkyl, aryl, arylalkyl; R² = alkyl, aryl

Keywords: Achmatowicz reaction · aldehyde synthesis · hydroxyphthalimide · hydroxybenzotriazole · ketone synthesis · laccase-mediated oxidation · primary alcohol oxidation · secondary alcohol oxidation · violuric acid

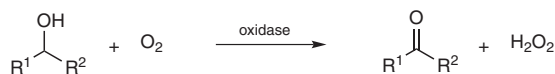
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3.3.3 Oxidations Using Alcohol Oxidases

T. A. Ewing, M. W. Fraaije, and W. J. H. van Berkel

The oxidation of alcohols to carbonyl compounds is one of the most important reactions in organic chemistry. Biocatalysis provides an attractive alternative to traditional methods of alcohol oxidation. Enzyme-catalyzed oxidations are often highly regioselective, enabling the oxidation of polyols without the need for complex protection schemes. Many oxidative enzymes also display exquisite enantioselectivity and thus can be utilized for the preparation of enantiopure secondary alcohols by kinetic resolution or deracemization methods. The use of biocatalysts also has advantages from the point of view of sus-

tainability. This is particularly true for oxidases, which catalyze the oxidation of their substrates using molecular oxygen as the final electron acceptor. This section provides an overview of the known alcohol oxidases, the reactions they catalyze, and, where available, examples of their use for synthetic purposes.



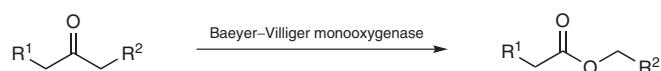
Keywords: alcohols · enzymatic oxidation · oxidase · dehydrogenase · biocatalysis · carbohydrates · regioselectivity · enantioselectivity · flavoproteins

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3.4 Baeyer–Villiger Oxidations

G. de Gonzalo, W. J. H. van Berkel, and M. W. Fraaije

This chapter describes methods for performing biocatalytic Baeyer–Villiger oxidations in which the final compounds are obtained under mild reaction conditions. In particular, reactions that can be performed with typical Baeyer–Villiger monoxygenases are presented that illustrate the high degree of regio- and/or enantioselectivity and good yields obtained with such enzymes for the synthesis of various compounds with high added value.



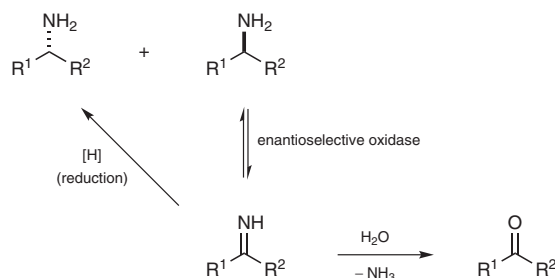
Keywords: Baeyer–Villiger oxidation · lipases · perhydrolysis · monoxygenases · coenzyme regeneration · desymmetrization · lactones · (dynamic) kinetic resolution · regioidivergence · enantioselectivity

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3.5.1 C–N Oxidation with Amine Oxidases and Amino Acid Oxidases

L. Pollegioni and G. Molla

Selective oxidation of amines and amino acids is of utmost importance in synthetic routes toward valuable chemicals. Such reactions can be performed using various enzymes. Here, the focus is on the use of the flavoenzymes monoamine oxidases and amino acid oxidases in the selective oxidation of natural and nonnatural amines and amino acids under mild reaction conditions. A number of recent successful applications, frequently based on protein-engineering studies, are reported.

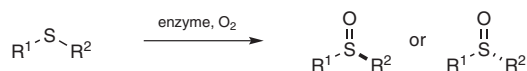


Keywords: amine oxidases · monoamine oxidases · amino acid oxidases · stereoselectivity · deracemization · protein engineering

3.5.2 Oxidation at Sulfur

G. Grogan

The asymmetric biocatalytic oxidation of sulfides can be performed with high enantioselectivity by a number of different enzymes, allowing access to biologically active compounds including flavors and pharmaceuticals, and also chiral auxiliaries for organic synthesis. The application of biocatalysts in asymmetric sulfoxidation has benefited recently from advances in molecular biology that allow the study and application of individual enzymes, either purified or expressed in recombinant strains of *E. coli*. In this chapter, the major contemporary approaches to biocatalytic sulfoxidation, including enzymes such as peroxidases, flavin-dependent monooxygenases, and dioxygenases, are reviewed. In addition, the most user-friendly examples of enzyme-catalyzed sulfoxidation are illustrated using practical exemplar procedures from the relevant literature.

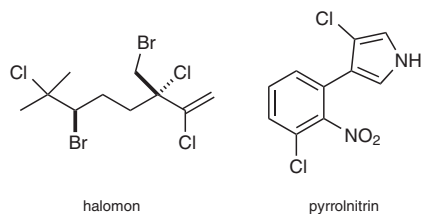


Keywords: Baeyer–Villiger monooxygenase · bovine serum albumin · chloroperoxidase · cytochrome P450 · dioxygenase · flavin-containing monooxygenase · horseradish peroxidase · peroxidase · sulfide · sulfoxidation · sulfoxide · tyrosinase · vanadium bromoperoxidase

3.6 Halogenases

S. Grünschow, D. R. M. Smith, D. S. Gkotsi, and R. J. M. Goss

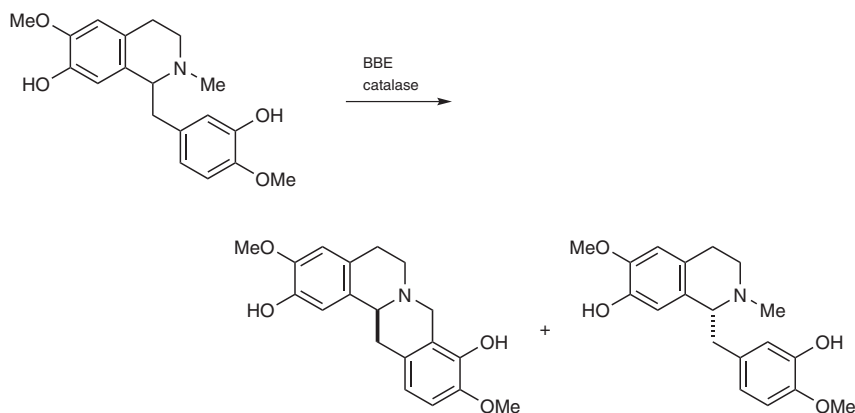
Many halogenated compounds can be found in nature and, of these, a number must have arisen through regio- or stereoselective enzymatic halogenation (e.g., halomon and pyrrolnitrin). In this chapter, the current understanding of halogenating enzymes and their applications is presented. Electrophilic, nucleophilic, and radical halogenation are covered and the mechanism and substrate scope of these enzymatic processes are discussed.



Keywords: bromocyclization · electrophilic substitution · enzyme biocatalysis · flavin-dependent halogenases · halo compounds · halogenases · halogenation · metal-dependent haloperoxidases · nucleophilic fluorination · radical chlorination · *S*-adenosylmethionine halogenases

3.7.1 Isoprenoids, Polyketides, and (Non)ribosomal Peptides*M. B. Quinn, C. M. Flynn, J. J. Ellinger, and C. Schmidt-Dannert*

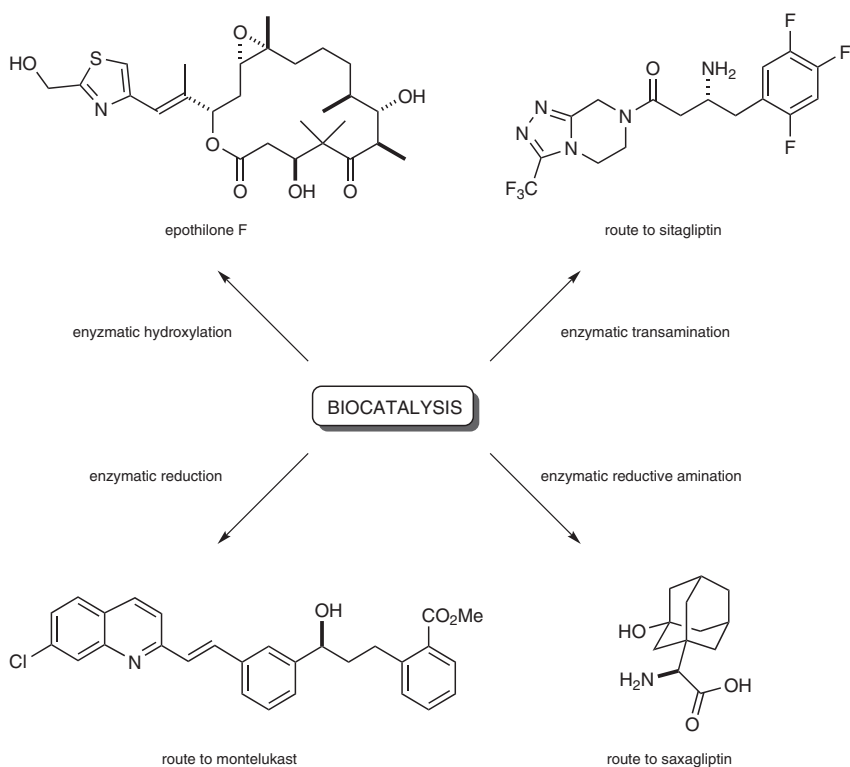
This chapter describes methods for the biosynthesis and biocatalysis of natural products belonging to the isoprenoids, polyketides (acetate pathway), phenylpropanoids (shikimate pathway), alkaloids, and ribosomal and nonribosomal peptides. Recent advances in genome-sequencing technologies and synthetic biology approaches are discussed, and biological approaches are given where available.



Keywords: alkaloids · biocatalysts · carotenoids · chalcones · cinnamic acid derivatives · coumarins · flavanones · flavones · isoprenes · sesquiterpenes · stilbenes

3.7.2 Biocatalytic Key Steps in Semisynthesis and Total Synthesis*R. N. Patel*

Enzyme-catalyzed reactions are highly selective and can be carried out under ambient conditions, thus avoiding the extreme conditions used in chemical reactions which could cause various problems. Enzymes can be cloned and overexpressed and this feature, along with directed evolution of enzymes under desired process conditions, has led to the production of novel and highly efficient biocatalysts for the development of economical processes for pharmaceutical development. This article describes a number of key biocatalytic steps in synthesis and total synthesis.



Keywords: semisynthesis · chiral intermediates · enantioselectivity · regioselectivity · enzymatic deracemization · reductive amination · reduction · desymmetrization · hydroxylation · transamination · condensation · directed evolution · pharmaceutical processes

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3.8.1 Designed Enzymatic Cascades

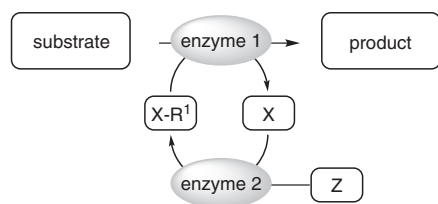
I. Oroz-Guinea, J. Fernández-Lucas, D. Hormigo, and E. García-Junceda

One of the major advantages of enzymes as catalysts is that many of them operate under similar conditions of pH, temperature, etc. and thus can be combined in one-pot multi-step reaction pathways. The joint action of a sequence of enzymes allows the construction of complex structures from simple elements, a reversible process to be made irreversible, or an equilibrium reaction to be shifted in such a way that enantiomerically pure products can be obtained from racemic or prochiral substrates. This chapter highlights recent developments involving multienzyme cascade reactions for the synthesis of various classes of organic compounds.

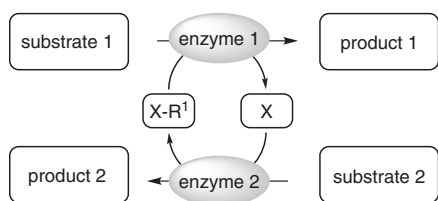
linear cascade



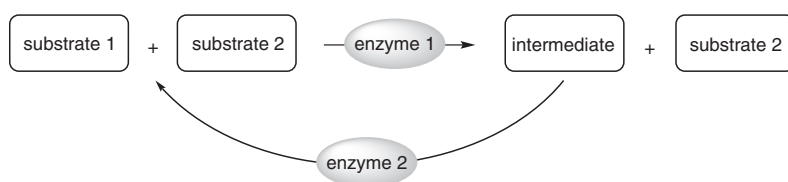
orthogonal cascade



antiparallel cascade



cyclic cascade

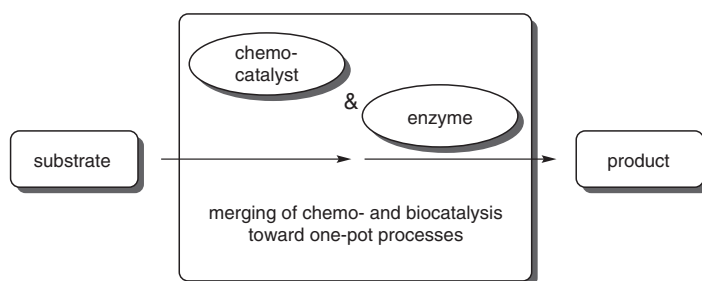


Keywords: enzymatic cascades · multienzyme synthesis · tandem reactions · oxido-reductases · alcohol dehydrogenases · amino acid dehydrogenases · aminotransferases · ammonia lyases · aminomutases · aldolases · glycosyltransferases · glycosynthases · nucleoside phosphorylases · biofuel production · biofuel cells

3.8.2 Merging of Metal, Organic, and Enzyme Catalysis

H. Gröger and W. Hummel

This chapter reviews multistep, one-pot processes through a combination of the catalytic disciplines of enzyme catalysis and chemocatalysis (metal catalysis, organocatalysis), demonstrating that enzymes as catalysts can be compatible with a broad range of man-made chemocatalysts, spanning the range from heterogeneous to homogeneous catalysts and metal catalysts to organocatalysts. Such chemoenzymatic one-pot syntheses, which combine reactions without the need to work-up intermediates, are attractive, for example, with respect to both process efficiency and sustainability.

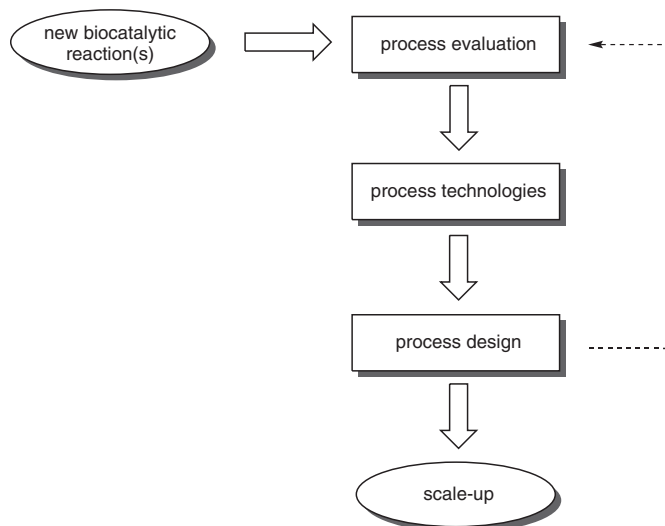


Keywords: asymmetric catalysis · asymmetric synthesis · chemoenzymatic synthesis · chiral compounds · chiral resolution · enantiomeric resolution · enzyme catalysis · green chemistry · metal catalysts · one-pot processes · organocatalysts · tandem reactions

3.9 Scale-Up and Development of Enzyme-Based Processes for Large-Scale Synthesis Applications

J. M. Woodley

This chapter describes the basis for the scale-up and implementation of new biocatalytic processes in industry. Particular emphasis is placed upon the requirements for a commercial process, and the implications for design and choice of the biocatalyst, reactor, and subsequent downstream processing.

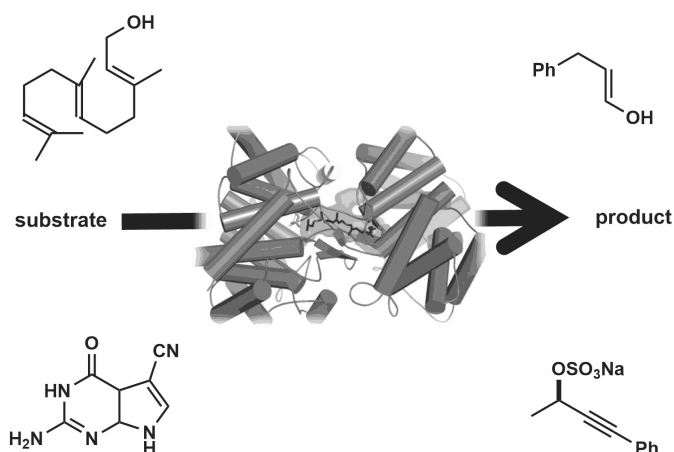


Keywords: biocatalysts · chirality · enzyme catalysis · green chemistry · microbial oxidation · synthesis design

3.10 Emerging Enzymes

K. Faber, S. M. Glueck, S. C. Hammer, B. Hauer, and B. M. Nestl

Nature has developed and adapted a large number of enzyme types. Remarkably, these enzymes may be further used in biocatalysis for synthetic purposes. This chapter provides an overview of emerging cases of novel enzymes. Herein, nitrile reductases, sulfatases, squalene hopene cyclases, and aldoxime dehydratases may provide very powerful novel synthetic approaches in the futures, as they catalyze chemically interesting reactions under very mild reaction conditions and with high selectivities. These biocatalysts comprise a broad field of options, whereby biocatalysis may contribute to the quest for novel chemistry for future applications.



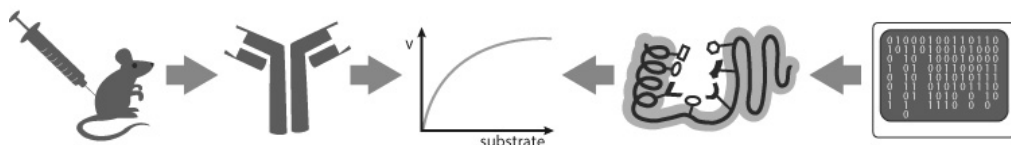
Keywords: emerging enzymes • nitrile reductase • inverting and retaining sulfatase • squalene hopene cyclase • aldoxime dehydratase

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3.11 Creation and Optimization of Artificial Enzymes for Abiological Reactions

R. Obexer, X. Garrabou, and D. Hilvert

Catalytic antibody technology and computational design represent conceptually distinct strategies to artificial enzymes. Both approaches provide significant activities and tailored specificities for mechanistically distinct transformations, including abiological and asymmetric reactions. This review compares the relative strengths and limitations of such de novo catalysts, delineating challenges to overcome in the pursuit of synthetically useful enzymes for any given chemical transformation.



Keywords: catalytic antibodies • computational design • directed evolution • Diels–Alder reaction • Kemp elimination • (retro-)aldol reaction • ester hydrolysis • metalloenzymes • computational redesign