Applied Biochemistry and Biotechnology (2020) 192:146–179
https://doi.org/10.1007/s12010-020-03272-3

Synthesizing Chiral Drug Intermediates by Biocatalysis

Wei Jiang[1] · Baishan Fang[2,3,4]

Received: 23 October 2019 / Accepted: 13 February 2020 /
Published online: 22 April 2020

Abstract

The chiral feature is a critical factor for the efficacy and safety of many therapeutic agents. At present, about 57% of marketed drugs are chiral drugs and about 99% of purified natural products are chiral compounds. There has been a tremendous potential of functional microorganisms and biocatalysts derived from them for the bioconversion of synthetic chemicals into drugs with high enantio-, chemo-, and regio-selectivities. Biocatalysis is becoming a key subassembly in the medicinal chemist's toolbox. In fact, the intermediates of many important therapeutic agents such as sitagliptin, pregabalin, ragaglitazar, paclitaxel, epothilone, abacavir, atorvastatin, rosuvastatin, and omapatrilat have been successfully synthesized via biocatalysis. In this review, various biocatalytic systems that enable to synthesize these chiral drug intermediates are updated and discussed regarding their potential application in the pharmaceutical industry. Further development and increased utilization of biocatalysis for production of drugs with emphasis on green chemistry can be expected.

Keywords Biocatalysis · Chiral drug intermediate · Anti-Alzheimer's drugs · Synthetic biology · Metabolic engineering

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12010-020-03272-3) contains supplementary material, which is available to authorized users.

✉ Wei Jiang
wjiang@hqu.edu.cn

✉ Baishan Fang
fbs@xmu.edu.cn

[1] Department of Bioengineering and Biotechnology, College of Chemical Engineering, Huaqiao University, Xiamen 361021 Fujian, China

[2] Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China

[3] The Key Lab for Synthetic Biotechnology of Xiamen City, Xiamen University, Xiamen 361005, China

[4] The Key Laboratory for Chemical Biology of Fujian Province, Xiamen University, Xiamen 361005 Fujian, China

Introduction

The production of single enantiomers, which can be obtained by either biocatalytic or chemical synthesis of chiral intermediates, has become increasingly important in the development and use of pharmaceuticals and agrochemicals [56, 99, 100, 163, 209]. Biocatalysis is now keeping to maintain good growth momentum and is becoming a key constituent part in the toolbox for the chemists, and it has a place alongside chromatographic separations and chemocatalysis [183]. Biocatalysis systems (both whole-cell and isolated enzyme systems) are increasingly used as a synthetic route to produce complex molecules in industrial fields, such as synthesis of high value-added chemicals and drug intermediates [14, 18, 22, 28, 53, 55, 132, 176, 183, 232]. Further, the bioconversion field has gained more interest for the synthesis of chiral building blocks same as biologically active compounds [41], providing the development and employment of more economically and environmentally attractive processes. In contrast to the chemical synthesis, the biocatalysis shows the advantages of the reduction of environmental pollution and the high regio-, chemo-, and enantio-selectivity. And these biocatalyst-catalyzed reactions can be conducted at atmospheric pressure and ambient temperature to avoid the use of extreme conditions, which often could cause problems with racemization, isomerization, rearrangement of the compound, and epimerization. Therefore, biocatalysis possesses many appealing characteristics in the field of green chemistry particularly for the synthesis of chiral intermediates, which are subsequently used to produce food additives or enantiopure drugs [152]. And further development and employment of biocatalysis for high-productivity processes and green chemistry can also be expected [183].

Enzymes and functional microbes can be modified, evolved, redesigned, immobilized, and reused for many times. Overexpressed target enzymes and directed evolution of biocatalysts with ameliorative activity, stability, and selectivity can be tailor-made. Thus, biocatalysis makes the biotransformation process economically feasible and highly efficient. And the ways of designing biocatalysts, enzymes, and functional microbes will change the face of synthesis. Recent review articles about organic synthesis using various enzymes and biosystems [21, 48, 73, 101, 103, 115, 142, 154, 161, 164, 183, 187, 195, 201, 213, 214, 219, 229, 231, 272, 70, 218]have been reported, which summarized the main routes of producing single enantiomers of key drug intermediates by biocatalysis (Fig. 1), and some case studies and examples on the use of biocatalysis to synthesize biologically active compounds and pure building blocks in the development and utilization of pharmaceuticals.

Key Enzymes

Hydrolytic enzymes such as esterases, lipases, dehalogenases, proteases, nitrilases, acylases, epoxide hydrolases, amidases and decarboxylases, aminotransferases, dehydrogenases, aldolases, dioxygenases, and monooxygenases are the biocatalysts generally chosen for the biosynthesis of compounds with stereogenic centers [163, 215]. Some approaches have been executed, such as oxidative deaminization, enzymatic deracemization, reductive amination, and dynamic resolution, to achieve high e.e. and more than 50% yield through combination of chemo- and/or biocatalysts by a single biocatalysis or in sequential reactions. Dioxygenases have been successfully used in the production of chiral dihydrodiols through chemoenzymatic routes while monooxygenases were used in epoxidation, enantioselective hydroxylation, sulfoxidation, and Baeyer-Villiger reactions, and regioselective hydroxylation is utilized for

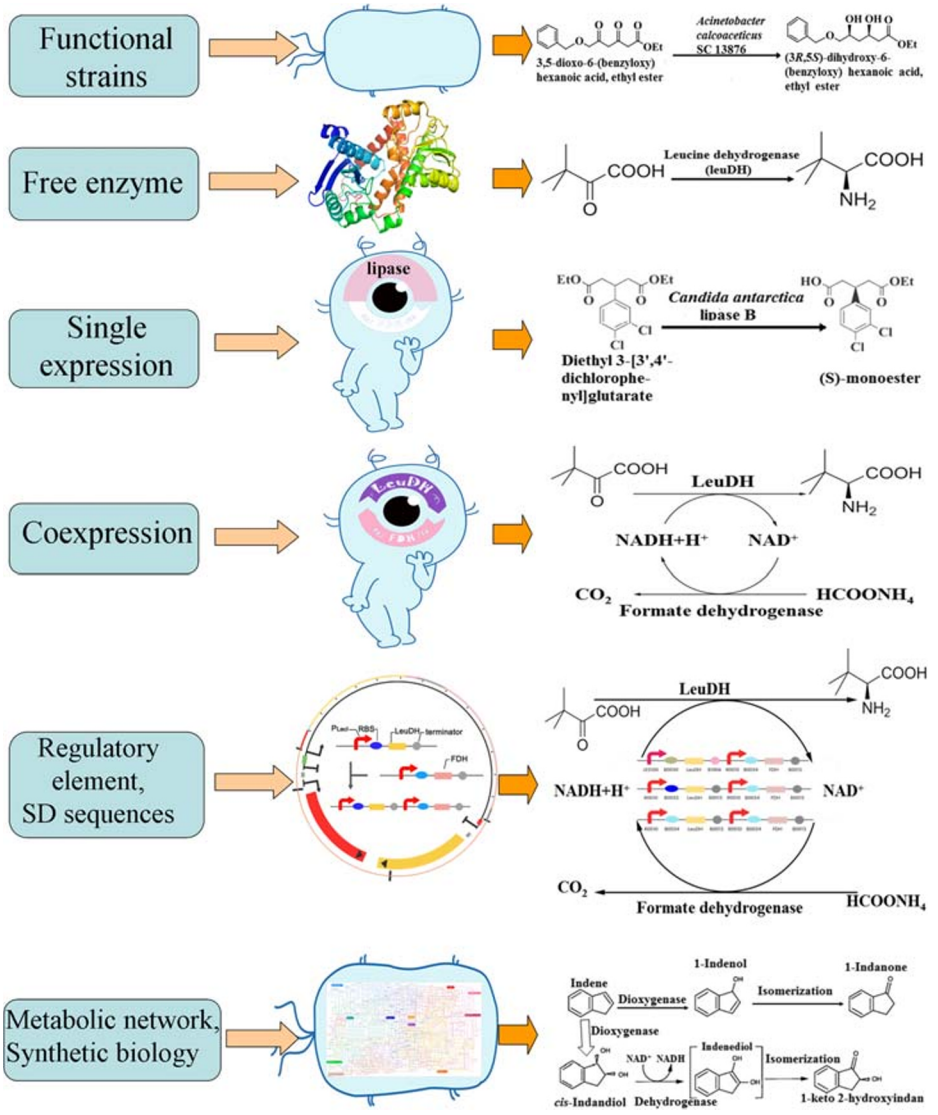

Fig. 1. The diagrammatic sketch of main methods for the synthesis of chiral drug intermediate by biocatalysis

synthesizing chiral compounds. Unlike the dioxygenases and monooxygenases, the decarboxylases and aldolases have been effectively used by acyloin condensation and aldol condensation reactions in the asymmetric synthesis progress. Cofactor regeneration is very economical and important for the cofactor-dependent progress due to the high cost of adding cofactors, such as NADH and NADPH [109, 234]. As the key enzymes, aminotransferases and dehydrogenases have been effectively used in biosynthesis of amino acids, amino alcohols, amines, and chiral alcohols with cofactors or cofactor regeneration. The hydrolytic enzymes have been used for synthesis of high value-added chiral compounds through the asymmetric synthesis of prochiral compounds or the kinetic resolution of racemic compounds.

Abundant access to various microbial cultures and enzymes applied in organic synthesis of key chiral intermediates and target drug products has been opened up through substrate engineering; medium engineering; protein engineering; biocatalyst engineering like directed evolution, random and site-directed mutagenesis of biocatalysts, biocatalytic cascade processes, cell-free synthetic biology; and reactor engineering and downstream processing like fermentation technology with high cell density for a long time in the past decade and future. The biocatalysis for biosynthesis or with chemosynthesis of chiral compounds looks promising in the future.

Functional Bacterial Strains

Under some circumstances, using whole microbial cells, natural functional bacteria or engineering bacteria, as biocatalysts is smarter rather than using isolated enzymes for cofactor regeneration due to instability of free enzyme outside the cell [29], and the multi-enzyme-catalyzed composite reactions and the whole-cell processes are cheaper to implement upstream for there is no need to isolate the enzyme. And whole cells should show prominent advantages in multistep conversion processes like side-chain cleavage of the β-sitosterol by using the whole cells of *Mycobacterium* sp. NRRL B-3805 [217]. It is just like redox enzymes which are frequently used, since the internal cellular metabolism is contributing to the regeneration of cofactors by this method. Whole cells (natural functional bacteria or engineering bacteria as "bags of enzymes") are also employed when multi-enzyme-catalyzed step reactions are expected and designed for the formation of a multi-enzyme cluster. Compared with using isolated enzymes, using whole cells as the biocatalysts is economical and more attractive. Nowadays, 75% of routes for industrially executed redox bioconversions are using whole cells [92, 93]. Herein, we will describe some cases of the use of natural functional bacteria or engineering bacteria whole cells in the synthesis of chiral precursors of drugs.

Natural Functional Bacteria

Oxidative biocatalysts (oxidases, dehydrogenases, oxygenases, and peroxidases) possess many advantages in contrast to their chemical counterparts as the problems, such as uncontrollable and unpredictable final product structures, can be overcome for oxidation routes in traditional chemical catalyzing and the use of oxidative biocatalysts is economical [215]. Some representative cases implemented by oxidative biocatalysts are listed in Table S1. The 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro-[1, 2, 4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one (OTPP) was converted into the (*S*)-3-hydroxy-1-(3-(trifluoromethyl)-5,6-dihydro-[1, 2, 4]triazolo[4,3-a]pyrazine-7(8H)-yl)-4-(2,4,5-trifluorophenyl)butan-1-one ((*S*)-HTPP). A key intermediate for the preparation of sitagliptin employed to cure type 2 diabetes [128] was produced by using the *Pseudomonas pseudoalcaligenes* XW-40 (*P. pseudoalcaligenes* XW-40) with > 99.9% enantiomeric excess and > 99.5% yield [245] (entry 1, Table S1). And the *Rhizopus microsporus* var. rhizopodiformis ZJPH1308, a new fungus which showed the ability of catalysis OTPP to (*S*)-HTPP with > 99.9% e.e. value and 93.2% yield, was screened and identified [222] (entry 1, Table S1). The herbicide precursor, (*R*)-2-(4-hydroxyphenoxy)propanoic acid, was obtained by parahydroxylating the raw material (*R*)-2-phenoxypropionic acid as a representative case of the bio-oxidation-mediated hydroxylations designed by the BASF AG company [49] (entry 3, Table S1). A route by using whole cells was developed for the synthesis of the (*S*)-2-amino-6-

hydroxyhexanoic acid, a key chiral intermediate for the preparation of an antihypertensive metalloendopeptidase inhibitor and a vasopeptidase inhibitor [159] (entry 4, Table S1). The (3*S*,4*R*,5*S*)-1,3,4,5-tetrahydroxy-6-(alkyl-amino)hexan-2-one, an important intermediate for preparing the oral α-glucosidase inhibitors which is used against retroviral infections or cure carbohydrate metabolism disorders, was obtained through catalyzed *N*-protected 1-amino-D-sorbitol by using the *Gluconobacter oxydans*' whole cells in the Bayer AG company [126] (entry 5, Table S1). Dioxygenases and monooxygenases, which can introduce oxygen atom into the substrate, as the ideal biocatalysts to synthesize 2-quinoxalinecarboxylic acid which was used in the production of various biologically active compounds [253] (entry 6, Table S1)), sodium (3*R*,5*R*)-3,5-dihydroxy-7-((1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-((*S*)-2-methylbutanoyloxy)-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)heptanoate) [156] (entry 7, Table S1), and *cis*,*cis*-muconic acid are used as important intermediates to synthesize a variety of agrochemicals and pharmaceuticals [264] (entry 8, Table S1).

The stereoselective reduction of ketones (also well-known as stereoselective reduction of a C=O bond into its corresponding alcohol) to form a stereogenic center (*CH–OH) is an important process in the asymmetric synthesis because of its wonderful atom economy in the preparation of bioactive molecules and pharmaceuticals [93, 208]. And this route, the asymmetric hydrogenation of ketones by biocatalysis, can be regarded as an ideal greener strategy in contrast to the chemical catalysts. Here, some preparative examples and cases of bio-reductions catalyzed by whole cells are listed in Table S2. An important chiral precursor, (*S*)-1-(benzo[d][1, 3]dioxol-5-yl)propan-2-ol [2] (entry 1, Table S2), in the synthesis of benzodiazepines like Talampanel™ and a key intermediate, (1*R*,4*S*,6*R*)-6-hydroxybicyclo[2.2.2]octan-2-one [109] (entry 2, Table S2), for the production of antitumoral taxane derivatives were synthesized by using whole cells. A key alcohol intermediate for the synthesis of the atazanavir (an HIV endopeptidase inhibitor and an acyclic aza-peptidomimetic), the *tert*-butyl ((2*S*,3*R*)-4-chloro-3-hydroxy-1-phenylbutan-2-yl)carbamate, was obtained by using *Rhodococcus erythropolis* SC 13854 (*R. erythropolis* SC 13854) with 95% yield and 99% e.e. value [173] (entry 3, Table S2). The atazanavir was approved by the Food and Drug Administration to treat autoimmune diseases (AIDS) [13, 191]. The (*1S*,2*R*)-[3-chloro-2-hydroxy-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethylethyl ester, an important chiral intermediate for the preparation of the HIV protease inhibitor atazanavir, was synthesized by using *Hansenula*, *Rhodococcus*, and *Brevibacterium* strains with e.e. of 99.4% [173] (entry 4, Table S2). And this route was further improved by mutation and screening of desired mutant for the synthesis of the important intermediate for the preparation of the atazanavir [20]. The (1*S*)-[3-chloro-2-oxo-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethylethyl ester was catalyzed to its corresponding chiral alcohol, a key intermediate for chemical synthesis of a class of HIV protease inhibitors [7], by using the *Mortierella ramanniana* SC 13850 and *Streptomyces nodosus* SC 13149 (*S. nodosus* SC 13149) [170]. And 67% reaction yield and 99.9% e.e. value were gained by employing the cells of *S. nodosus* SC 13149 while 54% reaction yield and 99.9% e.e. value were obtained by employing the cells of *M. ramanniana* SC 13850 [170] (entry 5, Table S2). Chemical reduction of the chloroketone (1*S*,2*R*)-[3-chloro-2-hydroxy-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethylethyl ester often generates the undesired chlorohydrin diastereomer [257]. The key intermediate (*S*,*E*)-methyl 2-(2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-hydroxypropyl)phenyl)-2-methyl-propanoate for the production of anti-asthma montelukast was implemented by using *Microbacterium campoquemadoensis* [205] (entry 6, Table S2). The *Aureobasidium pullulans* was used in stereoselective reduction of the a-keto ester ethyl 2-

oxo-2-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethyl-6′-naphthalenyl)acetate, which was a key intermediate for product retinoic acid receptor modulators used as anticancer and dermatological drugs with 94% yield and 97% enantioselectivity [172] (entry 7, Table S2). The production of the (*S*)-(3-(cyclopentyloxy)-4-methoxyphenyl)(phenyl)methanol, an ideal precursor to synthesize the phosphodiesterase inhibitor which is selected to treat asthma, was implemented by the Merck company [30] (entry 8, Table S2). The cell suspensions of the *Acinetobacter calcoaceticus* SC 13876 (*A. calcoaceticus* SC 13876) were used for the synthesis of the (3*S*,5*R*)-dihydroxy-6-(benzyloxy) hexanoic acid ethyl ester, a key important intermediate for the production of cholesterol-lowering agents, such as rosuvastatin and atorvastatin, with e.e. value of 97% and reaction yield of 85% [145, 169] (entry 9, Table S2).

Chiral alcohols are widely used as the key drug intermediate for the synthesis of some important pharmaceuticals. The *Aspergillus niger* (*A. niger*) was employed to produce the (*R*,*S*)-3-(2-methoxyphenoxy)propane-1,2-diol, a β-blocker used for the treatment of certain arrhythmias and hypertension, with 90% e.e. value and 50% conversion rate [134] (entry 1, Table S3). Then, the (*R*,*S*)-3-(2-methoxyphenoxy)propane-1,2-diol was converted to (*S*)-moprolol through chemical synthesis with two steps [134]. The *Geotrichum candidum* SC 5469 (*G. candidum* SC 5469) was used for the synthesis of the (*S*)-4-chloro-3-hydroxybutanoic acid methyl ester, an important chiral intermediate for the preparation of a HMG-CoA reductase inhibitor [106, 244], with 95% reaction yield and 96% e.e. value (entry 2, Table S3). And the e.e. value of the (*S*)-4-chloro-3-hydroxybutanoic acid methyl ester increased to 98% by using cell suspensions with heat treatment [166, 174]. The *Mortierella ramanniana* ATCC 38191 (*M. ramanniana* ATCC 38191) and *Pullularia pullulans* ATCC 16623 (*P. pullulans* ATCC 16623) were screened from various microorganisms to introduce the enantioselective reduction of 1-(4-fluorophenyl)4-[4-(5-fluoro-2-pyrimidinyl)1-piperazinyl]-1butanone to (*R*)-1-(4-fluorophenyl)4-[4-(5-fluoro-2-pyrimidinyl)1-piperazinyl]-1-butanone, which was used for the development of a new class of antipsychotic pharmaceuticals [58, 59, 111, 110], with a 100% reaction yield and a 98.9% e.e. value [168] (entry 3, Table S3). The (*S*)-5-hydroxyhexanenitrile and ethyl-(*S*)-5-hydroxyhexanoate are key and ideal chiral intermediates in the preparation of anti-Alzheimer's drugs [184]. And *Pichia methanolica* SC 16116 was employed to synthesize both of these chiral compounds by enantioselective reduction of 5-oxohexanenitrile and ethyl-5-oxohexanoate [151] (entry 4, Table S3). Furthermore, the *Candida antarctica* (*C. antarctica*) lipase was also employed in the preparation of anti-Alzheimer's drugs, and a 42% reaction yield and a > 99% e.e. value were achieved [167] (entry 5, Table S3). Organisms from genus Baker's yeast, *Pichia*, *Candida*, *Rhodotorula*, *Sphingomonas*, *Hansenula*, and *Saccharomyces* were used for the preparation of the key chiral intermediates, (*S*)-methyl 4-(2′-acetyl-5′-fluorophenyl)-butanol and (*S*)-1-(2′-bromo-4′-fluorophenyl)ethanol, for the production of several potential anti-Alzheimer's drugs [199, 173] (entry 6, Table S3). It was approved by the FDA that paclitaxel is used for the treatment of metastatic breast cancer and ovarian cancer, and paclitaxel is also used for curing other various kinds of cancers [94, 117, 158]. The cell suspensions of *Hansenula fabianii* SC 13894 (*H. fabianii* SC 13894) and *H. polymorpha* SC 13865 were selected to synthesize the (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine ethyl ester, an important precursor for the semi-synthetic process of the paclitaxel with over 94% of e.e. value and over 80% of reaction yield [167] (entry 7, Table S3). The *Rhodococcus globerulus* SC 16305 (71.8% enantiomeric excess) and *H. polymorpha* SC 13824 (73.8% enantiomeric excess) were identified to synthesize the (*S*)-2-chloro-1-(3-chlorophenyl)ethanol which is required in the production of a IGF-1 receptor inhibitor (an anticancer program) [252] (entry 8, Table S3). The

Rhodotorula piliminae ATCC 32762 (*R. piliminae* ATCC 32762) and *P. delftensis* MY 1569 were introduced in the synthesis of the key chiral alcohol for the chloroketone and keto ester, and the 97% yield of the (*S*)-alcohols was reached [125] (entry 10, Table S3). The *Candida parapsilosis* IFO 1396 was screened for the preparation of the (*R*)-4-chloro-3-hydroxybutanoate and (*R*)-1, 3-butanediol, which is of great significance in the synthesis of azetidinone derivatives utilized for the production of carbapenem antibiotics and penem [105] (entry 11, Table S3). The 4,5-dihydro-4-(4-methoxyphenyl)-6-(trifluoromethyl)-1H-1-benzazepin-2,3-dione was used for the generation of the 1,3,4,5-tetrahydro-3-hydroxy-4-(4-methoxyphenyl)-6-(trifluromethyl)-2H-1-benzazepin-2-one, a key intermediate for the synthesis of the diltiazem commonly used for the treatment of angina and hypertension [181], with 96% reaction yield and 99.8% e.e. value by using *Nocardia salmonicolor* SC 6310 (*N. salmonicolor* SC 6310) [165] (entry 12, Table S3).

Depression and anxiety as psychiatric disorders are major health problems worldwide. Though lots of treatments have been developed for these two orders, the reduction of side effect and the increment of efficacy are still required [104, 155]. The (*R*)-1-cyclopropylethylamine and (*R*)-sec-butylamine are important chiral intermediates used to synthesize the CRF-1 receptor antagonists which are taken for the novel pharmacological treatments for stress disorders, depression, and anxiety [66, 129, 155, 224]. And the deacylation or acylation reactions for the preparation of racemic amines were executed by using oxidases or lipases and proteases [71, 72, 183]. The enantioselective reduction of 6-oxobuspirone to either (*S*)- or (*R*)-6-hydroxybuspirone was established by screening about 150 microorganisms [67, 175]. And the 6-oxobuspironeto is a key material for the preparation of the buspirone which is used as a drug for curing depression and anxiety through binding the serotonin 5HT1A receptor [107, 144, 262]. *Bacillus megaterium* (*B. megaterium* SC 6394) isolated from several soil samples was used to synthesize the (*R*)-1-cyclopropylethylamine and (*R*)-sec-butylamine [85] (entry 1, Table S4). The epothilones, a novel kind of natural product cytotoxic compounds, were obtained by using myxobacterium *Sorangium cellulosum*; it showed ideal result in the trigger apoptosis progress [62, 11] (entry 3, Table S4). Using *Amycolatopsis orientalis* SC 15847 (*A. orientalis* SC 15847), a microbial hydroxylation route was executed for the preparation of epothilone F with epothilone B as the precursor (entry 4, Table S4). After that, the key gene, ferredoxin gene from *A. orientalis* SC 15847, was expressed in the *Streptomyces rimosus* for the synthesis of the epothilone F from epothilone B with 80% yield [8] (entry 5, Table S4).

Engineered Bacteria

Using genetic engineering to produce target engineering bacteria with specific function is an ideal route to equip more active biocatalysts. Using genetic engineering protocols, the *E. coli* cells containing the NADPH-dependent carbonyl reductase, which was discovered through genome mining, from *Yarrowia lipolytica* ACA-DC 50109 (*Y. lipolytica* ACA-DC 50109), were employed to catalyze the ethyl 4-chloro-3-oxobutanoate to ethyl (*S*)-4-chloro-3-hydroxybutanoate, a key intermediate for the preparation of statins (blockbuster drugs) with 90% yield and 99% e.e. value [259] (entry 1, Table S5). And the *Escherichia coli* cells co-expressing the NADPH-dependent carbonyl reductase, which was also found through genome mining, from *Rhodococcus pyridinivorans* and glucose dehydrogenase (GDH), were employed to produce ethyl (*S*)-4-chloro-3-hydroxybutanoate with 91% yield and 99% e.e. value [258] (entry 2, Table S5). The recombinant *E. coli* which contains a formate

dehydrogenase (FDH) from *Mycobacterium* sp. and a carbonyl reductase from *Pichia finlandica* was used as a biocatalyst to synthesize a key chiral intermediate for anticholesterol drugs, (2*R*,3*S*)-ethyl 2-chloro-3-hydroxybutanoate, with 85% yield and 99% e.e. value [113] (entry 3, Table S5), and the FDH (a dehydrogenase for cofactor regeneration) and carbonyl reductase (a ketoreductase to reduce the target substrate) were inserted in the *E. coli* system in this case. The recombinant *E. coli* which contains an enoate reductase (also known as old yellow enzyme) from *Candida macedoniensis* was used for stereoselective reduction C=C bond to produce 6R-levodione, an important intermediate in the preparation of some flavors and carotenoids [114] (entry 4, Table S5). The (*S*)-methyl 4-chloro-3-hydroxybutanoate (entry 5, Table S5) and (*S*)-ethyl 4-chloro-3-hydroxybutanoate (entry 6, Table S5) were synthesized by bio-reduction to generate the lateral chain of atorvastatin, a blockbuster drug for the treatment of lowering blood cholesterol [159, 118]. Using whole cells as biocatalysts, the two enzymes, a modified form of phenylalanine dehydrogenase (PheDH) from *Thermoactinomyces intermedius* and an engineered FDH expressed in single recombinant *E. coli*, were used for the synthesis of the (*S*)-3-hydroxyadamantylglycine, a key chiral intermediate for the synthesis of saxagliptin, an inhibitor of dipeptidyl peptidase-4 and an antidiabetic drug (an antihyperglycemic agent) with 100% e.e. and 98% yield [196, 239, 83] (entry 7, Table S5). The glucose-6-phosphate dehydrogenase from *Saccharomyces cerevisiae*, NADPH-dependent *R* reductase from *H. polymorpha* SC 13845, NADH-dependent *S* reductase from *Pseudomonas putida* SC 16269, and FDH from *Pichia pastoris* were selected and expressed in *E. coli*, respectively. Then, these four recombinant *E. coli* strains were used for the conversion of the 6-ketobuspirone to (*R*)- or (*S*)-6-hydroxybuspirone, a key material for the preparation of the buspirone which is used as a drug for resistant anxiety and depression through binding the serotonin 5HT1A receptor [107, 144, 262], with > 99.9% e.e. value and > 98% yields [67] (entry 8, Table S5). A ketoreductase III from *Acinetobacter* sp. 13874 was identified, cloned in the *E. coli* expression system for the synthesis of (3*S*,5*R*)-dihydroxy-6-(benzyloxy) hexanoic acid, ethyl ester. And this reaction was executed by using the recombinant *E. coli*'s cells or extracts with 99% yield [68, 75] (entry 10, Table S2).

Using the *E. coli* system to overexpress the phenylalanine dehydrogenase (PheDH) from *Sporosarcina* sp., the (*S*)-2-amino-5-(1,3-dioxolan-2-yl)-pentanoic acid (one of the three building blocks for the synthesis of the Vanlev) was synthesized through reductive amination of the corresponding a-keto acid with 98% enantioselectivity and 91% yield. By using *E. coli* cells overexpressing PheDH from *Sporosarcina* sp., 197 kg of the products was obtained in three batches of 1600 L with 91% yield and 98% enantioselectivity [159] (entry 9, Table S5). The recombinant *E. coli* which contained a transaminase from *B. megaterium* SC 6394 was employed for the synthesis of the (*R*)-1-cyclopropylethylamine and (*R*)-sec-butylamine by the recombinant cells with 42% isolated yield and 99% e.e. value [85] (entry 2, Table S4). A ketoreductase III from *Acinetobacter* sp. 13874 was identified, cloned in the *E. coli* expression system for the synthesis of (3*S*,5*R*)-dihydroxy-6-(benzyloxy) hexanoic acid ethyl ester. And this reaction was executed by using the recombinant *E. coli*'s cells or extracts with 99% yield [68, 75] (entry 10, Table S2). And an extract of *E. coli* which has two recombinant enzymes, a glucose-6-phosphate dehydrogenase from *Saccharomyces cerevisiae* (*S. cerevisiae*) and a ketoreductase from *H. polymorpha* SC 13824, was used to produce (*S*)-2-chloro-1-(3-chloro-4-fluorophenyl)ethanol with 100% e.e. value and 89% yield [83] (entry 9, Table S3). The stereoselective reduction of C=N bond by amino acid dehydrogenases is a great exciting bio-reduction to prepare high value-added amino acids and their derivatives [159]. The leucine dehydrogenase (LeuDH) from *Bacillus cereus* and the FDH from *Candida boidinii* were

expressed in one genetic circuit in the *E. coli* system to synthesize L-*tert*-leucine (L-Tle), a building block to prepare many drugs with different activities (antitumoral agents or HIV endopeptidase inhibitors), with high enantiopure form (> 99% e.e.) and yield (approximately 100%) [109] (entry 10, Table S5). Using recombinant *E. coli* to express LeuDH from *Thermoactinomyces intermedius* and FDH from *P. pastoris*, the L-Tle was synthesized with 99.5% stereoselectivity and 95% yield [124] (entry 11, Table S5). The ethyl (*R*)-4-cyano-3-hydroxybutyric acid, an ideal intermediate for the production of carnitine, was synthesized by using the glucose dehydrogenase from *Bacillus subtilis*, halohydrin dehydrogenase from *Agrobacterium tumefaciens* (*A. tumefaciens*), FDH from *C. boidinii*, and ketoreductase from *Candida magnoliae*. These four enzymes were separately cloned and expressed into *E. coli* BL21, and were subsequently isolated and characterized to synthesize the ethyl (*R*)-4-cyano-3-hydroxybutyric acid [38, 39] (entry 12, Table S5).

Single Expression or/and Isolated Enzymes

Using oxidoreductases to synthesize high-value chiral building blocks is a powerful and highly efficient tool to aid chemists for designing more productive and sustainable synthesis of drugs. Actually, reductions or oxidations as ideal biocatalysts involved about 30% of the industrial processes as redox is a greatly efficient and attractive choice to the point of being environmentally friendly compared with the use of chemical catalysts [208]. Even though whole-cell catalysis (natural functional bacteria or engineering bacteria as "bags of enzymes") has many advantages, it also showed some drawbacks [55]: (1) limited substrates or product permeability; (2) substrate or product inhibition, such as inhibition of the cell growth and biocatalyst activities; (3) low total output, low enzymatic activities, purification and separation of the target product are time-consuming and complicated, and the values of the environmental factor E would increase [208]; (4) the multiple reductases are often present in the whole cell which lead to the insufficiency of enantiomeric excess in this route [29]; (5) degradation and consumption of the product or substrate through cellular metabolism resulting in low enantiomeric excesses and yields; and (6) generation of unwanted by-products for the presence of multiple enzymes. And these shortcomings of the whole cells can be overcome by using isolated enzymes, which is an ideal method to generate a highly efficient coenzyme recycling system for industrial and lab scale, especially important for the former progress [12]. Compared with whole cells, one-step reactions catalyzed by isolated enzymes provide significant benefits of direct access of substrates and avoiding side reactions. A large number of new enzymes and biosynthetic pathways have been reported by genome sequencing, increasing considerably the field of enzymology and permitting the development of drug design [10]. In this review, we will show some cases of the use of single expression or/and isolated enzymes in the synthesis of chiral precursors of pharmaceuticals.

Oxidoreductase

Pharmaceutical production will benefit greatly from the selectivity earnings associated with the enzymatic catalysis by using biocatalysts such as oxidoreductase. Bio-oxidation reactions and bio-reduction reactions have been widely used in the pharmaceutical field. For example, boceprevir and telaprevir (both hepatitis C virus protease inhibitors) [120, 130] and the esomeprazole (the proton-pump inhibitor) [15] were manufactured industrially by using

oxidases while sitagliptin was synthesized by using reductase for combating the type 2 diabetes [128]. The mutant D11 obtained by using rational design, directed evolution, and high-throughput screening with the monoamine oxidase from *A. niger* as the wild type was employed to synthesize the important pharmaceutical intermediates for the preparation of solifenacin and levocetirizine with > 97% e.e. value [63, 64] (entry 1, Table S6). And the monoamine oxidase mouton D5 was employed to synthesize the TRPV1 inhibitor and serotonin receptor inhibitor with 99% e.e. value [42] (entry 2, Table S6). The cyclohexanone monooxygenase was applied in the preparation of the 2-(benzhydrylsulfinyl) acetic acid, an important material for the preparation of the armodafinil. And it was approved by FDA that the armodafinil can be used as a stimulant-like pharmaceutical to cure shift work disorder, obstructive sleep apnea, narcolepsy, and other sleep disorders [3] (entry 3, Table S6). Bacterial laccases and the vanillyl alcohol oxidase from *Penicillium simplicissimum* (*P. simplicissimum*) were used for the synthesis of pinoresinol, which shows a protective effect of combating multiple health disorders, by a two-step one-pot synthesis in vitro [188] (entry 4, Table S6). Then, the pinoresinol reductase from *Arabidopsis thaliana* (*A. thaliana*) and pinoresinol lariciresinol reductase from *Forsythia intermedia* (*F. intermedia*) were employed for the establishment of a one-pot "two-cell" system to produce the enantiopure pinoresinol with more than 97% e.e. value [189] (entry 5, Table S6). The aromatic amino alcohols and *N*-carbobenzyloxy (Cbz)-protected aliphatic compound were oxidized to their corresponding α-hydroxyaldehydes by the galactose oxidase mutants [90]. Two engineered carbonyl reductases, P170H/L174Y and P170R/L174Y, were used to catalyze the 3-(dimethylamino)-1-(2-thienyl)-propan-1-one and 3-(dimethylamino)-1-phenylpropan-1-one to 3-(dimethylamino)-1-(2-thienyl)-1-ol and 3-(dimethylamino)-1-phenylpropan-1-ol, respectively. And the 3-(dimethylamino)-1-(2-thienyl)-1-ol and 3-(dimethylamino)-1-phenylpropan-1-ol are key intermediates for the production of some antidepressant drugs [270] (entries 6 and 7, Table S6). A new carbonyl reductase (KlAKR) from *Kluyveromyces lactis* (*K. lactis*) was discovered and employed to asymmetrically reduce t-butyl 6-cyano-(5*R*)-hydroxy-3-oxohexanoate to t-butyl 6-cyano-(3*R*,5*R*)-dihydroxyhexanoate, the crucial chiral precursor for the synthesis of the Lipitor® (atorvastatin calcium) [135] (entry 8, Table S6). Then, the KlAKR was engineered by using a semi-rational design method based on molecular docking and homology modeling, and a mutant Y295W/W296L which exhibited 11.25-fold catalytic efficiency (k_{cat}/K_m) than that of a wild type was obtained [136]. The (*R*)-alpha-hydroxyketal, a great chiral precursor of an important pharmaceutical intermediate used to synthesize a chemokine receptor inhibitor, was synthesized by using a recombinant ketoreductase with > 99% e.e. value [121] (entry 9, Table S6). Coupling with a GDH for NADPH cofactor regeneration, an economical route was developed to produce the (*R*)-α-hydroxyketal with a 96–98% yield [121] (entry 10, Table S6). The (*R*)-tetrahydrothiophene-3-ol (entry 11 Table S6) or (*S*)-2-butanol (entry 11, Table S6), an important component for a potent antibacterial, sulopenem, was synthesized by using the native ketoreductase from *Lactobacillus kefir* and the engineered enzyme with directed evolution [76, 131]. The (*S*)-3, 5-bistrifluo-romethylphenyl ethanol, a raw material for synthesis of antagonists of receptors K-1, was produced by using the alcohol dehydrogenase (ADH) from *R. erythropolis* by Merck & Co. Inc. (entry 12, Table S6). The genetically modified ketone reductases, which were generated by directed evolution and were 3000 times than that of the native enzyme from *Microbacterium campoquemadoensis* MB5614, were used in the synthesis of (*S*,*E*)-methyl 2-(3-(3-(2-(7-chloroquinolin-2-yl)-vinyl)phenyl)-3-hydroxypropyl)benzoate, montelukast (an anti-asthma drug), with 90% yield and 99% e.e. value [131] (entry 13, Table S6). The ketoreductase KRED1001 was employed in the

preparation of the (*R*)-2-hydroxy-3, 3-dimethylbutanoic acid, a key chiral intermediate for the synthesis of a thrombin inhibitor [131]. The GDH was introduced for cofactor NADPH regeneration, and the (*R*)-2-hydroxy-3,3-dimethylbutanoic acid was saponified without epimerization with > 99.5% e.e. value and 82% isolated yield [197] (entry 14, Table S6). Multifarious protein engineering technologies, such as mutation approach, substrate walking, and modeling, were employed in modifying a transaminase to enhance the productivity of the (*R*)-3-amino-1-(3-(trifluoromethyl)-5,6-dihydro-[1, 2, 4]triazolo[4,3-a]pyrazin-7(8H)-yl)-4-(2,4,5-trifluorophenyl)butan-1-one-itagliptin (sitagliptin, used as an oral antihyperglycemic agent) [122, 212] (entry 15, Table S6) or some other chiral amines [198]. An engineered transaminase was used in the preparation of sitagliptin with 99% enantiomeric excess and 92% yield (entry 16, Table S6), and this route was of more productivity and efficiency of synthesis of the target product compared with the corresponding chemical route [198]. And by using a chiral biocatalyst, sitagliptin was synthesized by enantioselective hydrogenation of an enamine [80, 89, 88]. A FDH for cofactor regeneration and a D-lactate dehydrogenase (D-LDH) for reduction reaction were selected to synthesize the (*R*)-3-(4-fluorophenyl)-2-hydroxy propionic acid, a key building block for the production of the rhinovirus protease inhibitor AG7088 [52, 265] (entry 17, Table S6), through the use of a membrane reactor [228]. The L-lysine ε-aminotransferase from *Sphingomonas paucimobilis* SC 16113 (*S. paucimobilis* SC 16113) was selected and overexpressed in *E. coli* for the synthesis of the [(4*S*)-(4*a*,7*a*,10*ab*)]-1-octahydro-5-oxo-4-[[(phenylmethoxy)-carbonyl]amino]-7H-pyrido-[2,1-b][1, 3]thiazepine-7-carboxylic acid, an important intermediate in the production of the omapatrilat which is used as an antihypertensive drug by inhibiting neutral endopeptidase and angiotensin-converting enzyme [171, 192] (entry 18, Table S6). (*R*)-Amino acid, an important material for the preparation of antimigraine drugs (also known as calcitonin gene–related peptide receptors) or other pharmaceuticals such as anticoagulants and fertility drugs [53, 87, 147, 230, 261], can be synthesized by combining with (*R*)-hydantoinases with (*R*)-acylases or (*R*)-carbamoylases, (*R*)-amidases, (*R*)-transaminases, and HNO_2. And an (*S*)-amino acid was synthesized by using an (*S*)-amino acid dehydrogenase and an (*R*)-amino acid oxidase with a precursor, racemic amino acid [82]. The (*R*)-2-amino-3-(7-methyl-1H-indazol-5-yl)-propanoic acid, an important intermediate for the production of antagonists of a calcitonin gene–related peptide receptors (for the preparation of antimigraine drugs and other pharmaceuticals) [31, 32], was prepared by using a (*S*)-amino acid oxidase, which was obtained from *Proteus mirabilis* (*P. mirabilis*) and expressed in *E. coli*, and a commercial (*R*)-transaminase with 68% isolated yield and > 99% e.e. value [86] (entry 19, Table S6). Then, the (*R*)-transaminase from *B. thuringiensis* was expressed in *E. coli* and employed in this progress, leading to a nearly complete bioconversion without requirement of additional biocatalysts (enzymes) for removing the pyruvate [86]. Two enzymes, (*S*)-aminotransferase from *Sporosarcina ureae* and (*R*)-amino acid oxidase from *Thamniochloris variabilis* expressed in *E. coli*, respectively, were put together for the synthesis of the (*S*)-amino-3-[3-{6-(2-methylphenyl)}ridyl]-propionic acid (a key material for glucagon-like peptide). Meanwhile, a (*S*)-aminotransferase from *Burkholderia* sp. was expressed in the *E. coli* system and applied in this route, and the isolated yield reached 99.4% and with e.e. value of more than 99.4% [160] (entry 20, Table S6). The (*R*)-cyclohexylalanine, another important (*R*)-amino acid which is an important chiral material for the production of thrombin inhibitor inogatran [77], was obtained by using the modified meso-diaminopimelate (*R*)-dehydrogenase from *Corynebacterium glutamicum* with > 99% e.e. value and 98% yield [149, 235] (entry 21, Table S6). And the modified enzyme form with nicotinamide cofactor dependence, high stereoselectivity, and broad substrate range was obtained through random

and rational mutagenesis [235]. The LeuDH from *Bacillus sphaericus* ATCC 4525 was used in the synthesis of the α-keto-β-hydroxyisovalerate, a key material for the synthesis of the (*S*)-β-hydroxyvaline which is an important chiral intermediate for the production of tigemonam (orally active monobactam) [69], with a 98% reaction yield and a 99.8% enantiomeric excess [81] (entry 22, Table S6). And either GDH from *B. megaterium* or FDH from *C. boidinii* was employed in this progress for NADH regeneration, which is necessary in this reaction [81].

Aldolases

Aldolases are often used as the ideal choice to catalyze aldol additions to the corresponding asymmetric compounds with high catalytic efficiency and selectivity [35]. The *N*-acetyl-D-neuraminic acid aldolase either from *Clostridium perfringens* (*C. perfringens*) or *E. coli* was applied to produce *N*-acetyl-D-neuraminic acid, an important intermediate to synthesize a selective and potent influenza virus sialidase inhibitor Relenza which is an important drug for curing the two types most responsible for flu epidemics [5, 44, 116, 139] (entry 1, Table S7). Then, this progress was improved by implementing the reaction in an excellent enzyme membrane reactor for enhancing the productivity of *N*-acetyl-D-neuraminic acid synthesis [123]. And the *N*-acetyl-D-neuraminic acid aldolase from *E. coli* was immobilized to synthesize quantities of *N*-acetyl-D-neuraminic acid [40, 140]. And immobilized enzyme technology would benefit from the enzyme reusability [27]. The 2-deoxyri-bose-5-phosphate aldolase was selected to synthesize the 2,4-dideoxyhexose derivative which acts as a key role in the production of some cholesterol-lowering agents and HMG-CoA reductase inhibitors with a 96% diastereomeric excess and more than a 99% e.e. value by Diversa Corporation, and the aldolase was expressed in the *E. coli* system [215] (entry 2, Table S7).

Lipases

Lipases are general candidates of biocatalyst for the biosynthesis of chiral compounds by the kinetic resolution of amides and esters or the enantioselective enzymatic desymmetrization of amines and secondary alcohols to synthesize the corresponding amides or chiral esters due to their good stability and high activity in organic solvents. Several labs or industrial processes have already used the lipase as catalysts in the synthesis of pharmaceuticals as described. The lipase from *Thermomyces lanuginosus* (*T. lanuginosus*) was immobilized to prepare (*S*)-indanol for the synthesis of the rasagiline mesylate, an active key ingredient of the medicine AZILECT® which is used to combat Parkinson's disease, with > 99% e.e. value [60, 223] (entry 3, Table S7). The lipase from *Candida rugosa* (also called *C. cylindracea* lipase) as the ideal candidate has been employed as serine hydrolases in the production of (*S*)-2-arylpropionic acids (active isomer) and non-steroidal anti-inflammatory drugs (NSAIDs), like ibuprofen ((*S*)-2-(4-isobutylphenyl)propanoic acid, (*S*)-ibuprofen) (entry 4, Table S7). The (*S*)-ibuprofen was synthesized in industrial scale by the Pfizer, and this route was achieved by *C. rugosa* lipase through enantioselective hydrolysis of the racemic methoxyethyl ester (entry 4, Table S7), and the *C. rugosa* lipase was immobilized in a membrane reactor [206, 207]. Two isoenzymes from *C. antarctica*, A (CALA) and B (CALB) which presented several differences, have been described. CALB was used as the biocatalyst to synthesize the antithrombotic compound lotrafiban S-16 by GlaxoSmithKline Pharmaceuticals (entry 5, Table S7), and the enzyme was immobilized in aqueous medium [241]. CALB has been also used in the synthesis of (*S*)-monoester, (*S*)-3-(3,4-dichlorophenyl)-5-ethoxy-5-oxopentanoic acid (entry 6,

Table S7), by the Schering-Plough company, and (*S*)-3-(3,4-dichlorophenyl)-5-ethoxy-5-oxopentanoic acid is a key chiral intermediate for the production of antagonists of tachykinin receptors NK1 and NK2, which were used in the treatment of arthritis, asthma, and migraine in various biological processes like vasodilatation, secretion, inflammation, and pain transmission [95, 237]. By using DNA shuffling, a chimeric lipase B with high activity for the diethyl 3-[3′, 4′-dichlorophenyl]glutarate was created with three homologous lipases from *Hyphozyma* sp. CBS 648.91, *C. antarctica* ATCC 32657, and *Cryptococcus tsukubaensis* ATCC 24555 as the female parent. And the activity of the lipase was improved 20-fold than that of the parents [220] while the thermostability of the lipase B was enhanced by directed evolution [266] (entry 7, Table S7). The (*S*)-tert-butyl 2-carbamoyl-2,3-dihydro-1H-pyrrole-1-carboxylate (entry 8, Table S7), an important intermediate for the production of the oral hypoglycemic agent saxagliptin, was synthesized by CALB in Bristol-Myers Squibb [65]. And the (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid (entry 9, Table S7), an important intermediate for the generation of a chemokine receptor's potential modulator, was synthesized by immobilized *C. antarctica* CALB (Novozyme 435@) in Bristol-Myers Squibb (69). The Novozyme 435 (Chirazyme L-2 or *C. antarctica* lipase B) was selected for the preparation of the ribavirin, an antiviral agent for the treatment of hepatitis C [57, 180, 225] (entry 10, Table S7). The lipase from *Pseudomonas cepacia* or BMS lipase (extracellular enzyme obtained from *Pseudomonas* sp. SC 13856), which showed good selectivity of secondary alcohols' transesterification or esters' hydrolysis and was immobilized through polypropylene Accurel, was employed to synthesize the potent anticancer agent paclitaxel (Taxol) [6] (entry 11, Table S7), an antimitotic agent for the treatment of breast and ovarian cancer. Semi-synthetic pathways of paclitaxel were much more attractive alternatives as the 1 kg of paclitaxel was isolated consuming around 3000 tree barks. The porcine pancreatic lipase was immobilized to synthesize (*R*)-oxiran-2-yl-methanol, which was used as an important chiral intermediate for the synthesis of β-blockers, by the DSM company [207] (entry 12, Table S7). *Pseudomonas fluorescens* lipase as one of the most ideal enzymes in the resolution of racemic compounds was used in the synthesis of a chiral building block, (3*S*,3a*R*,6a*R*)-3-hydroxy-3,3a,4,6a-tetrahydro-2H-cyclopenta[b]furan-2-one, which is required for the preparation of the nucleoside analogue carbovir, an antiviral agent used for combating AIDS [54] (entry 13, Table S7). Lipase from *Serratia marcescens*, such as *S. marcescens* Sr41 8000, was applied in the synthesis of a key chiral intermediate for the calcium canal blocker diltiazem by Tanabe Pharmaceutical Co. [143] (entry 14, Table S7). Pregabalin [(*S*)-3-(aminomethyl)-5-methylhexanoic acid], a γ-aminobutyric acid (a lipophilic GABA analogue) was developed to cure some central nervous system disorders, such as social phobia, epilepsy, generalized anxiety disorder, neuropathic pain, and chronic pain like spinal cord injury and fibromyalgia [127, 204] (entry 15, Table S7). A new greater manufacturing route for synthesis of pregabalin was developed by using a commercially available lipase compared with the traditional methods of chemical synthesis.

Transaminases

Transaminases are widely used in the chiral amines or chiral amino synthesis and the chiral amines or chiral amino acids are key building blocks for lots of bioactive pharmaceuticals and natural products, such as sitagliptin and atazanavir [177]. Sitagliptin was developed by spiroindolone compounds and Codexis for malaria treatment [37, 198]. The transaminase mutant ATA-303 was evolved based on ATA-013 transaminase to produce the key chiral

amine intermediate for the synthesis of vernakalant, which is used as an antiarrhythmic agent to treat the atrial fibrillation and most common cardiac arrhythmia, with > 99.5% e.e. value and 85% assay yield [133] (entry 16, Table S7). Monoamine oxidases and x-transaminases were employed to synthesize the 2,5-disubstituted pyrrolidines, key scaffolds for various natural products, and pharmaceutical compounds, with > 94% excellent enantioselectivity and > 98% diastereoselectivity [153] (entry 17, Table S7). Modular cascade biocatalysis was established to synthesize (*S*)-phenylethanolamine and its derivatives, key chiral precursors for lots of bioactive compounds like antitumor potential drugs BMS-577098 [236] and BMS-536924 [252], with high yields and high e.e. value [255] (entry 18, Table S7). And five enzymes containing x-transaminase were employed for the establishment of two enzyme modules and were overexpressed in *E. coli* to produce (*S*)-amino alcohols in one-pot conversion [255] (entry 19, Table S7). The transaminase ATA-036 was employed to synthesize the 1-((2*R*,4*R*)-2-(1H-benzo[d]imidazol-2-yl)-1-methylpiperidin-4-yl)-3-(4-cyanophenyl)urea, a smoothened receptor inhibitor which was confirmed to have a potential impact on a variety of blood-related cancers by Pfizer [150] (entry 20, Table S7), with 99% e.e. value and 85% yield [179]. An x-transaminase mouton from *Arthrobacter* sp. was obtained to produce 17-a-amino steroids with 99% e.e. value and > 83% yield [78] (entry 21, Table S7). *Ruegeria* sp. TM1040 transaminase was evolved for the preparation of some important bulky chiral amines and it showed high stereoselectivity and 8900-fold higher activity than that of the wild type [78, 178] (entry 22, Table S7). Rational design was used to broaden the substrate spectrum of the amine transaminase from *Vibrio fluvialis* (*V. fluvialis*), and the ideal mutant, W57F/R88H/V153S/K163F/I259M/R415A/V422A, was obtained with > 1716 times higher reaction rate and > 99% e.e. value of pure (*S*)-amine [51] (entry 23, Table S7).

Lyases and Hydrolase

Lyases are used for the preparation of chiral compounds as they can catalyze their substrate to generate new double bonds through removing the groups from the substrates by methods other than oxidation and hydrolysis. The (+)-γ-lactamase from *Bradyrhizobium japonicum* USDA 6 (*B. japonicum* USDA 6) was discovered and overexpressed in *E. coli* [273]. Then, the (+)-γ-lactamase was applied in the synthesis of the (+)-γ-lactam, an important chiral intermediate for abacavir and carbovir (the antiviral carbocyclic nucleoside drugs) which were used in curing the "swine flu" and HIV infection, respectively, with excellent enantioselectivity (> 99.8% e.e. value) and high yield (> 49.9%) [61, 62, 223] (entry 24, Table S7). The γ-lactam 2-azabicyclo[2.2.1]hept-5-en-3-one, an ideal intermediate for the synthesis of the abacavir (also known as Ziagen, a 2-aminopurine nucleoside analogue) which is selected as a reverse transcriptase inhibitor to treat hepatitis B viruses and human HIV, was synthesized using the *Rhodococcus* NCIMB40213 and *Pseudomonas solanacearum* NCIMB40249 as these two microorganisms have γ-lactamase [54, 141] (entry 25, Table S7). And some commercially available enzymes were involved for the preparation of the target products. The whole *Erwinia herbicola* (*E. herbicola*) cells containing a tyrosine phenol lyase were applied in the synthesis of the L-3,4-dihydroxyphenylalanine, an important drug predominantly applied in curing Parkinson's disease [162] (entry 26, Table S7). Lyases were also used in the synthesis of other pharmaceutical intermediate and pharmaceuticals [223]. A tyrosine phenol lyase and a monooxygenase P450 BM3 were used for the establishment of a one-pot two-step cascade scheme to synthesize 3-methoxy-L-tyrosine, which is more stable than L-3,4-dihydroxyphenylalanine and has positive effects on the cure of Parkinson's disease, with >

97% e.e. value and an output of 2 g/L [45] (entry 27, Table S7). The whole-cell catalyst, *E. coli* cells consisting of the cellobiose 2-epimerase from *Caldicellulosiruptor saccharolyticus* (*C. saccharolyticus*), was applied in the production of the lactulose, which is generally applied in nutraceuticals, pharmaceuticals, and food industries [243], with 65.1% conversion yield [243] (entry 28, Table S7). Then, the best mutant G4-C5, R5M/I52V/A12S/K328I/F231L which was obtained through high-throughput screening after four cycles of random mutagenesis, was used for the preparation of lactulose with the elevated conversion yield of 76% [211] (entry 29, Table S7). *N*-Succinylamino acid racemase and D-succinylase were applied in the synthesis of D-phenylalanine with 91.1% conversion yield and 86.7% e.e. value [221] (entry 30, Table S7). The *N*-succinylamino acid racemase from *Geobacillus kaustophilus* CECT4264 (*G. kaustophilus* CECT4264) and L-carbamoylase from *Geobacillus stearothermophilus* CECT43 were immobilized to produce a panel of unnatural and natural L-amino acids [216]. D-Phenylalanine and its derivatives are important building blocks for many drugs and natural products, such as D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone and nateglinide [156]. D-Substituted phenylalanines were synthesized by using an engineered phenylalanine ammonia lyase with 98% e.e. value and the phenylalanine ammonia lyase was obtained from cyanobacteria *Anabaena variabilis* (*A. variabilis*) [157] (entry 31, Table S7). Phenylalanine ammonia lyase was also used for the synthesis of unnatural L-amino acids [193]. The phenylalanine ammonia lyase from *Rhodotorula graminis* was evolved to synthesize L-Br-phenylalanine with a > 99% e.e. value and a 94% yield [193] (entry 32, Table S7). The (*S*)-b- or (*S*)-a-arylalanines was synthesized by using the ammonia lyase EncP from *Scirpus maritimus* and its rationally designed mutants [246] (entry 33, Table S7).

Endopeptidases

Endopeptidases, such as serine endopeptidases, are a kind of hydrolases, hydrolyze proteins, which bio-catalyze the hydrolysis of peptidic bonds. These enzymes are ideal biocatalysts because they not only unrequired consuming cofactors but also are very regio-, enantio-, and chemo-selective. And the reaction is often implemented under mild reaction conditions [185]. The alkaline endopeptidase (also called as subtilisin A or subtilisin Carlsberg) from *Bacillus licheniformis* was used in the preparation of (*S*)-2-benzyl-3-(2-methyl-1-morpholino-1-oxopropan-2-ylsulfonyl)propanoic acid, a chiral intermediate for the synthesis of ciprokiren and renin inhibitor remikiren, with 99% e.e. value and 50% yield [50] (entry 34, Table S7).

Isomerases

Isomerases have great potential in synthetic medicinal chemistry and pharmaceuticals as they can transform cheap, abundant sugars into rare, expensive sugars [9]. The mannose-6-phosphate isomerase and L-arabinose isomerase were used in the preparation of L-ribose, a key precursor for the preparation of pharmaceutically L-form sugar which was used in the production of those targeting human cytomegalovirus, HIV, and hepatitis B/C virus [260] (entry 35, Table S7). The L-arabinose isomerase from *Paenibacillus polymyxa* (*P. polymyxa*) was immobilized on nanomaterials to produce the L-ribulose with 61.8% conversion rate [260] (entry 36, Table S7). A high-efficiency three-step separation-integrated route was built by combining a D-xylose isomerase, a D-tagatose epimerase, and an invertase to synthesize D-psicose which shows the ability to enhance D-glucose tolerance and insulin sensitivity in type 2

diabetes [96], with 89% yield and 99.9% purity [240] (entry 37, Table S7). Two mutants, Var8 and IDF10-3, were obtained by the directed evolution of the D-tagatose epimerase and employed in the synthesis of the D-psicose in an enzyme membrane reactor [19] (entry 38, Table S7).

Other Enzymes

Although oxidoreductase, transaminases, aldolases, lactamase, and lyases are the most commonly used biocatalysts in the biocatalytic synthesis of chiral drug intermediates and chiral pharmaceuticals, some other enzyme cases also deserved to be described, as listed in Table S7. The cytidine deaminase from recombinant strain *E. coli* was immobilized to synthesize the Epivir [(2′*R-cis*)-2′-deoxy-3-thiacytidine], which is approved by the FDA to cure HIV, and it was used more than 15 cycles [139, 140] (entry 39, Table S7). The nitrilases, which can bioconvert a variety of nitriles, were also widely employed as the ideal biocatalyst in chemical transformations and one of the most representative cases was shown in the production of (*R*)-3-hydroxy-4-cya-nobutyric acid, an important intermediate in the synthesis of the Lipitor (a cholesterol-lowering agent) with 98% yield and 95% e.e. value [46] (entry 40, Table S7). And a nitrilase and its improved version, which was obtained by using site-saturation mutagenesis, were used for the synthesis of 3-hydroxyglutaronitrile [46, 47] and it was demonstrated that nitrilases can be used to synthesize various chiral carboxylic acid [48, 119]. The (*S*)-2-ethoxy-3-(4-hydroxyphenyl)propanoic acid, a key intermediate for the synthesis of ragaglitazar (a new antidiabetic drug), was synthesized using the esterase from *Aspergillus oryzae* with 99.6% enantiomeric purity and 48% yields [54] (entry 41, Table S7). The phenylpyruvate decarboxylase from *Achromobacter eyrydice* (*A. eyrydice*) was used for the synthesis of the $PhCH_2COCH(OH)R$, the α-hydroxyketones (acyloin) which is an important class of chiral intermediates in organic synthesis [54, 74] (entry 42, Table S7). Semi-synthetic pathways, as an ideal approach, were used in overcoming satisfactory chemical and pharmacological limitations of β-lactam antibiotics, one of the generally employed and the most important pharmaceuticals. 7-Aminocephalosporanic acid (7-ACA), 6-amin-openicillanic acid (6-APA), and 7-amine-3-desacetoxycephalosporanic acid (7-ADCA) have been prepared by hydrolysis of cephalosporin G and penicillin G. The 7-ADCA was obtained from cephalosporin G by using penicillin G acylase from *Actinomyces viscosus* or *E. coli with* 93% yields (entry 43, Table S7). And the Dr. Vig Medicaments has successfully prepared the 6-APA by using penicillin G acylase from immobilized *B. megaterium*, *A. viscosus*, or *E. coli* [132] (entry 44, Table S7).

Co-expression of the Key Enzymes

The most prominent routes for the synthesis of chiral alcohol, chiral amino acid, and chiral amine were executed and usually required cofactor NADH or NADPH. Coenzyme regeneration is a very ideal method for the coenzyme-dependent progress due to the addition of the costly cofactor [234] and it is an ideal selection to co-express the key enzyme for coenzyme regeneration to reduce costs and help achieve green production. In the last years, scientists spent a lot of energy on building cofactor regeneration systems and realizing the regeneration of cofactor by using different enzymatic, electrochemical, and chemical approaches [12], as less than 0.1 mol% cofactor used can be taken as economically acceptable [102]. However,

these effects do not achieve the preparative relevance. Enzyme-coupled (one enzyme catalyzes the synthesis of the target product, and another enzyme (auxiliary enzyme) is used to regenerate the coenzyme) and substrate-coupled (an enzyme catalyzes oxidation and reduction reactions with cofactor regeneration, such as a ketone substrate and a cheap alcohol were used as the substrate-coupled materials) coenzyme regeneration, two enzymatic regeneration strategies, were established. FDH is the best-known ideal enzyme for regeneration of cofactor as the reaction is practically irreversible, very easy, and the carbon dioxide can be eliminated in a timely manner, which is beneficial to the separation of products and helpful reaction to the direction of generating coenzymes. In some cases, the phosphite dehydrogenase was also used as the auxiliary enzyme [238]. And the GDH is also used for generating coenzymes for the high activity of GDH and practical irreversibility of the reaction [102, 215]. Using cofactor coupling to synthesize valuable molecules is becoming a great attractive methodology [269]. And it could be used to synthesize the high value-added products, such as L-Tle and 1, 3-dihydroxiacetone, by using different enzymes to catalyze a reductive and an oxidative reaction at the same time with raw materials, trimethylpyruvic acid, and glycerol.

There are many enantiopure molecules synthesized by using enzyme-coupled or substrate-coupled route. The FDH from *C. boidinii* and ADH from *R. erythropolis* used the enzyme-coupled to produce (*S*)-1-phenylpropan-2-ol, a key intermediate to prepare the amphetamines (sympathomimetics), and this progress was developed by the Forschungszentrum Jülich GmbH Co. (entry 1, Table S8). The (*S*)-3-hydroxyadamantylglycine, a key chiral intermediate for the synthesis of saxagliptin, an inhibitor of dipeptidyl peptidase-4, and an antidiabetic drug, was synthesized by using a modified form of PheDH from *T. intermedius* and expressed in *P. pastoris* with an engineered FDH for cofactor recycling [197] (entry 8, Table S4). The reductive amination route was scaled up to synthesize saxagliptin by using an engineered PheDH and FDH which were expressed in a single *E. coli* cell with 98% yield and 100% e.e. value [84, 239]. And the modified PheDH has 12 amino acid extensions in the C-terminus area and two amino acid mutations at the same area [84, 239]. The (*R*)-4-chloro-3-hydroxybutanoate esters (methyl ester and ethyl ester), which were used to generate the lateral chain of atorvastatin, can be prepared by using a recombinant ketoreductases with GDH and glucose (entry 2, Table S8) or by using the whole cells of *E. coli* [138, 159] (entry 2, Table S8). An engineered ketoreductase was used for the synthesis of the (*S*)-3-(*N*,*N*,dimethylamino)-1-(thien-2-yl)propan-1-ol, which was used as a precursor for duloxetine (an antidepressant drug), and using GDH and glucose for cofactor regeneration [199] (entry 3, Table S8). The recombinant *E. coli*, which co-expresses both secondary alcohol dehydrogenase from *P. finlandica* and FDH from *Mycobacterium* sp., was employed to produce (*S*)-4-chloro-3-hydroxybutonoate, an important chiral intermediate for the preparation of a HMG-CoA reductase inhibitor [106, 244], with 99% e.e. value and 98.5% yield [208] (entry 4, Table S8).

Fusion expression forms a multifunctional biocatalyst that was also used in the synthesis of drug intermediate. The functional fusion with two or more enzymes is built to aim at offering several advantages than individual enzymes in reaction kinetics and enzymatic catalysis. Proper linker peptides can reduce folding interference from each other for fusion enzymes making it function as independently as possible by inserting between individual enzymes [36]. A FDH from *C. boidinii* and a PheDH from *Bacillus halodurans* were tethered by using a linker peptide for constructing a cofactor regeneration system to rapid analysis of phenylketonuria (PKU) and high-efficiency synthesis of L-phenylalanine, an important material for the preparation of aspartame (sweetener), with 99% conversion rate [108] (entry 5, Table S8). Peptide linkers were used for the establishment of bifunctional LeuDH and FDH to generate

enzymatic complex for L-*tert*-leucine (L-Tle) biotransformation and efficient cofactor regeneration [271] (entry 6, Table S8).

Regulatory Element and SD Sequences for Biocatalytic Synthesis

One of the most significant decisions when designing and planning a manufacturing process employing biocatalysis method is whether the conversion is executed using one or more biocatalysts (enzymes) contained within an isolated enzyme or whole-cell extract [183]. A regulatory expression system to coordinate enzymes by cascade connection of protein-protein expressions and regulation of strength of RBS was established for the preparation of L-Tle, an important chiral drug intermediate for the preparation of the drugs telaprevir (HCV) and Reyataz (HIV), with approximately 100% yield and > 99% enantiopure form [109] (entry 1, Table S9). And the schematic diagram and flow chart of this experiment was shown as Fig. 2. A trienzyme CLEA including a non-selective nitrilase from *P. fluorescens* EBC 191, a (*S*)-hydroxynitrile lyase from *Manihot esculenta* (*M. esculenta*), and an amidase from *R. erythropolis* was employed for the construction of a biocatalytic cascade system to produce the (*S*)-mandelic acid with > 99% e.e. value and 90% yield [108, 33] (entry 2, Table S9). And the rate of the biocatalytic cascades was higher than that of admixtures of the respective cross-linked enzyme aggregates [33]. Three different routes were developed to synthesize the chiral intermediates for the preparation of omapatrilat 1: (1) 2-keto-6-hydroxyhexanoic acid was conversed to L-6-hydroxynorleucine by using beef liver glutamate dehydrogenase, and D-amino acid oxidase from *T. variabilis* or porcine kidney was employed to synthesize (1) the 2-keto-6-hydroxyhexanoic acid for the chemical synthesis of the 2-keto-6-hydroxyhexanoic acid since it was lengthy; (2) the PDH from *T. intermedius* was selected to synthesize the (*S*)-2-amino-5-(1,3-dioxolan-2-yl)-pentanoic acid and the PDH was overexpressed in *P. pastoris* and *E. coli*; and (3) the L-lysine-aminotransferase from *S. paucimobilis* SC 16113 was employed to produce the [4*S*-(4a,7a,10ab)]1-octahydro-5-oxo-4[[(phenylmethoxy)carbonyl]amino]-7H-pyrido-[2,1-b] [1, 3]thiazepine-7-carboxylic acid, and a *E. coli* which contain the enzyme was also used in this synthetic process. Therefore, there are some biocatalysts that are applied to the synthesis of the chiral intermediates of omapatrilat 1. Chemoenzymatic cascade reactions and biological cascade reactions also continue to attract enhancing attention and make significant progress [43, 242]. The safety-based process development and smart design ensure the productivity and quality of the pharmaceuticals manufactured are also the increasing key challenges in pharmaceutical synthesis [248]. And the yield of the target product would significantly increase while the regulatory elements or SD sequences were involved in multi-enzyme-catalyzed composite reaction to balance the speed between the reactions and the cost. Taken together, it is envisaged that the regulatory element and SD sequences for catalytic synthesis will become more generally used in the production of pharmaceutical intermediates and pharmaceuticals in the future.

Metabolic Network (Predesign)

We foresee the enhanced application of synthetic biology and metabolic engineering as an ideal strategy for the fast implementation of biocatalysis routes into drug development, and this strategy will give an exciting future for biocatalytic routes, such as in multistep processes using

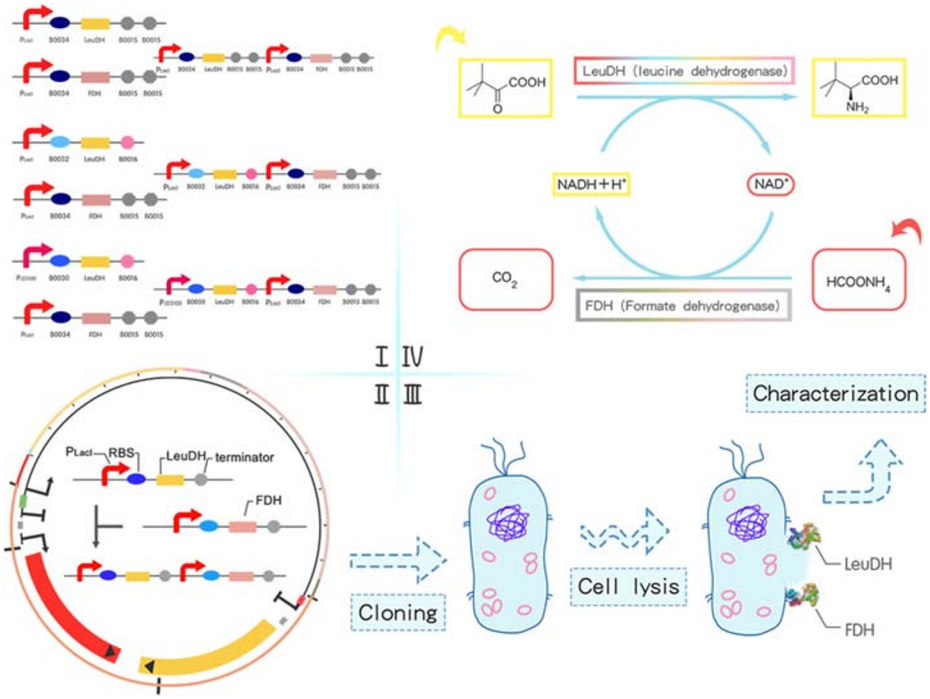

Fig. 2. The schematic diagram and flow chart for the construction of the regulatory expression system. I: Design genetic circuit; II: construct genetic circuit; III: expression and regulation of the target protein; IV: a tunable multi-enzyme-coordinate expression coenzyme regeneration system was constructed for biosynthesis of L-*tert*-leucine

one designed whole cell to express multiple enzymes. Lately, cell-free systems and redesigned recombinant whole cell were designed and developed to execute novel complex biocatalytic reactions in vitro [17, 25, 91, 146, 263]. Telescoping multistep synthesis to generate one-pot catalytic cascades is ultimate in green conversion methodologies and the soul of synthesis as this system could avoid the purification of intermediates and time-consuming isolation. Such catalytic cascade syntheses, which are built by truly emulating the enzymatic progresses in the metabolic pathways in living cell, show several advantages: drive equilibria go in the direction of product and avoiding the use for excess reagents, the incompatibility of the catalysts with each other can be overcome, less solvent, fewer unit operations, reactor volume, higher space-time yields and volumetric, shorter cycle times, and less waste, which mean substantial environmental and economic benefits [209]. The envisaged environmental and economic benefits of biocatalytic cascade processes (also known as the cell-free synthetic biology) make these progresses become a focus of attention in the last few years [210]. Free enzymatic bioconversion pathways are designed and engineered through assembling more than ten purified enzymes by using synthetic biology, and the coenzymes, which were used for the synthesis of the target products, could not be prepared by using a single enzyme. Cell-free synthetic biological systems (biocatalytic cascade processes) are more efficient, easier to build, and allow greater engineering flexibility than the modification of primitive functional cells as they are not influenced by complexity, cell viability, cellular membranes, physiology, and/or cell walls [263, 268], solving equilibrium issues [92] and substrate and/or product inhibition [93].

Construction of de novo metabolic pathways has placed great expectations to biosynthesize useful advanced intermediates. And it is predicted that novel biosynthetic pathways will be predesigned and assembled to generate an ideal metabolic pathway for a one-step synthesis of many semi-synthetic natural products in the future [24]. A metabolic pathway was constructed and optimized for the production of *cis*-(1*S*,2*R*)-indandiol and *trans*-(1*S*,2*R*)-indandiol by using metabolic engineering [23] (entry 3, Table S9). Indene was oxidized to mixtures of *trans*- and *cis*-indandiols and related metabolites by *Rhodococcus* sp. and *P. putida* isolates, and its metabolism was consistent with dioxygenase and monooxygenase activity. The *P. putida* was applied in the synthesis of the *cis*-(1*S*,2*R*)-indandiol, a key intermediate for the preparation of CRIXIVAN (indinavir sulfate) which was a protease inhibitor for the cure of AIDS. It was confirmed that the dihydrodiol dehydrogenase was required to resolve racemic mixtures of *cis*-indandiol while the toluene dioxygenase from *P. putida* was cloned in *E. coli* [23]. These synthetic biological systems show prominent use for the synthesis of biofuels, e.g., hydrogen, ethanol and butanol [263, 267, 268], organic acids and amino acids [249], and chiral pharmaceuticals [23, 24, 109, 233].

Conclusions and Future Perspectives

Due to the increasing strict environmental, quality, economical, and safety requirements of industrial synthetic procedures, as well as the growing demand of compounds which are enantiomerically pure, the development of more healthy, sustainable, and economically and environmentally attractive strategies for the product of chiral biologically active molecules, such as chiral drug intermediate and chiral drug, still remains a great challenge in the pharmaceutical industry. Biocatalysis is an ideal option to create or resolve one or more chiral centers [183]. Biocatalysis has been successfully used in almost all kinds of bioconversion reactions in the last few decades, and it showed benefits for the pharmaceutical and fine chemical research industries [223]. Many biocatalysis routes have been successfully built, which is more sustainable and environment friendly than that of chemical synthetic progress. It is no doubt that using biocatalysts to synthesize the key enantiopure molecules is an ideal and potential route from the cases and examples presented in this review. And it will enhance the usefulness of bioconversion progresses inside the frame of industrial biotechnology with the increasing demands of greener synthetic progresses; the great recent advances in protein chemistry, biochemistry, molecular cloning, random and site-directed mutagenesis, DNA shuffling, and direct evolution of biocatalyst; the achievement in stabilization and immobilization of enzymes or cells, bioprocess engineering, and modeling techniques [183]; and the successfully acquired new enzyme, such as silico enzymes [112], with the ability to perform a catalytic reaction that has never been done before. For example, the protein engineering can modify the biocatalyst to match a programmed synthesis or conquer process limitations like product or inhibition [16, 26]. The bioconversion field, which is executed by biocatalyst, has emerged as a good alternative for the traditional synthetic method, because of the exquisite regio-, enantio-, and chemo-selectivities commonly displayed by function cells or enzymes; thus, biocatalysis is becoming a universal methodology for the biosynthesis of chiral pure compounds in laboratory scale as well as in industrial scale. Additionally, the use of biocatalysts often circumvents the requirement for functional group activation, hence avoiding deprotection and protection steps usually required in conventional organic syntheses. And these properties afford courses which generate less or shorter waste, and are, hence, both economically and environmentally smarter than traditional routes.

It is still required to engineer function microorganisms and enzymes to enhance their stability, activity, selectivity, and productivity under the mild reaction conditions [227]. There are many routes to enhance robustness of biocatalysts and function microorganism, among them (1) immobilization may possibly be one of the most conventional methods used and studied [79]; (2) the extreme conditions have been used to screen the extremophiles for excavation of novel biochemical pathways and new enzymes [87, 250, 256, 4, 205, 60, 34, 179, 182, 96, 216]; (3) the use of enzyme molecular engineering techniques, substrate engineering techniques, like directed evolution [1, 84, 137, 142, 186, 187, 194, 202, 203, 226, 251, 266, 272, 200], and DNA shuffling [97, 98, 148, 220] has vastly contributed to the new function of gene cluster or biocatalysts, able to work effectively in experimental conditions greatly different from the "natural" ones, in terms of substrate spectrum, presence of organic solvents, pH, temperature, etc.; (4) the use of regulatory elements [109], such as promoter and ribosome bind site (RBS) [196], to regulate and coordinate the expression of key enzymes which are the key catalyst for the synthesis of target products, and regulation and optimization of metabolic network through gene engineering and protein engineering [247]; (5) using synthetic biology, computational systems biology, and metabolic engineering to design and optimize artificial metabolic network to synthesize high-value chemicals such as omega-3 LCPUFAs [254] and docosahexaenoic acid (DHA) [190] and chiral drug intermediates such as L-Tle [109], *cis*-(1*S*,2*R*)-indandiol [23], and *trans*-(1*S*,2*R*)-indandiol [23]. And we expect that the holistic approach of the methods described in this review will play an ideal and important enabling role in the development of chiral drug intermediates, high value-added chemicals, and chiral pharmaceuticals.

Acknowledgments We thank Prof. Fengwu Bai from Shanghai Jiao Tong University for providing us with valuable advice and model modification.

Authors' Contributions This review was conceived, researched, and written by Wei Jiang. Wei Jiang and Baishan Fang participated in devising the study, the discussions, the critical review of the manuscript draft, and revision of the manuscript. All authors read and approved the final manuscript.

Funding Information This work was supported by the National Natural Science Foundation of China (No. 21808073), the high-level personnel activation fee of Huaqiao University (No. 600005-Z17Y0072), and Quanzhou City Science & Technology Program of China (No. 2018C008), the State Key Program of National Natural Science Foundation of China (No. 21336009), and the Public Science and Technology Research Fund Projects of Ocean (No.: 201505032-6).

Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

Ethics Approval and Consent to Participate Not applicable.

References

1. Agresti, J. J., Antipov, E., Abate, A. R., Ahn, K., Rowat, A. C., Baret, J.-C., Marquez, M., Klibanov, A. M., Griffiths, A. D., & Weitz, D. A. (2010). Ultrahigh-throughput screening in drop-based microfluidics

for directed evolution. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 4004–4009.
2. Anderson, B. A., Hansen, M. M., Harkness, A. R., Henry, C. L., Vicenzi, J. T., & Zmijewski, M. J. (1996). Application of a practical biocatalytic reduction to an enantioselective synthesis of the 5H-2,3-benzodiazepine LY300164. *Journal of the American Chemical Society, 117*, 12358–12359.
3. Ang EL, Oscar A., Behrouzian B, et al.2016. Biocatalysts and methods for the synthesis of armodafinil, US20160264945 A1 .
4. Antranikian, G., Vorgias, C. E., & Bertoldo, C. (2005). Extreme environments as a resource for microorganisms and novel biocatalysts. *Marine Biotechnology, 1*, 219–262.
5. Augé, C., David, S., & Gautheron, C. (1984). Synthesis with immobilized enzyme of the most important sialic acid. *Tetrahedron Letters, 25*, 4663–4664.
6. Banerjee, A., Ko, R. Y., Howell, J. M., Li, W. S., & Partyka, R. A. (1994). Enzymic preparation of (3*R*-cis)-3-(acetyloxy)-4-phenyl-2-azetidinone: a taxol side-chain synthon. *Biotechnology and Applied Biochemistry, 20*, 23–33.
7. Barrish, J. C., Gordon, E., Alam, M., Lin, P. F., Bisacchi, G. S., Chen, P., Cheng, P. T., Fritz, A. W., Greytok, J. A., & Hermsmeier, M. A. (1994). Aminodiol HIV protease inhibitors. 1. Design, synthesis, and preliminary SAR. *Journal of Medicinal Chemistry, 37*, 1758–1768.
8. Basch, J., Chiang, S., Liu, S., Nayeem, A. and Sun, Y. (2004) The epothilone B hydroxylase and ferredoxin genes of Amycolatopsis and their use in the development of strains for the manufacture of hydroxylated epothilones. PCT Int. Appl.
9. Beerens, K., Desmet, T., & Soetaert, W. (2012). Enzymes for the biocatalytic production of rare sugars. *Journal of Industrial Microbiology & Biotechnology, 39*, 823–834.
10. Begley, T. P., & Tsai, M. D. (2003). Biocatalysis and biotransformation enzymology in the genomics era. *Current Opinion in Chemical Biology, 7*, 228–229.
11. Benigni, D., Tully, T. P. and Davis, B. L. (2011). Methods for the preparation, isolation and purification of epothilone B, and X-ray crystal structures of epothilone B. Google Patents.
12. Berenguer-Murcia, A., & Fernandez-Lafuente, R. (2010). New trends in the recycling of NAD(P)H for the design of sustainable asymmetric reductions catalyzed by dehydrogenases. *Current Organic Chemistry, 14*, 1000.
13. Bold, G., Fassler, A., Capraro, H. G., Cozens, R., Klimkait, T., Lazdins, J., Mestan, J., Poncioni, B., Rosel, J., Stover, D., Tintelnot-Blomley, M., Acemoglu, F., Beck, W., Boss, E., Eschbach, M., Hurlimann, T., Masso, E., Roussel, S., Ucci-Stoll, K., Wyss, D., & Lang, M. (1998). New aza-dipeptide analogues as potent and orally absorbed HIV-1 protease inhibitors: candidates for clinical development. *Journal of Medicinal Chemistry, 41*, 3387.
14. Bommarius, A. S. and Riebel-Bommarius, B. R. (2004). Biocatalysis: fundamentals and applications. ed. John Wiley & Sons.
15. Bong, Y. K., Clay, M.D., Collier, S. J., Mijts, B., Vogel, M., Zhang, X., Zhu, J., Nazor, J., Smith, D., Song, S. (2013). Synthesis of prazole compounds.
16. Bornscheuer, U. T. (2005). in Biotechnology for the future, Springer, 181-203.
17. Bornscheuer, U. T., & Buchholz, K. (2010). Highlights in biocatalysis-historical landmarks and current trends. *Engineering in Life Sciences, 5*, 309–323.
18. Bornscheuer, U. T., Kazlauskas, R. J. (2006). Hydrolases in organic synthesis: regio-and stereoselective biotransformations. ed. John Wiley & Sons.
19. Bosshart, A., Wagner, N., Lei, L., Panke, S., & Bechtold, M. (2016). Highly efficient production of rare sugars D-psicose and L-tagatose by two engineered D-tagatose epimerases. *Biotechnology and Bioengineering, 113*, 349–358.
20. Bowers, N. I., Skonezny, P. M., Stein, G. L., Franceschini, T., Chiang, S. J., Anderson, W. L., You, L., Xing, Z. (2008). A process for preparing (2R,3S)-1,2-epoxy-3-(protected)amino-4-substituted butane and intermediates thereof.
21. Brustad, E. M., & Arnold, F. H. (2011). Optimizing non-natural protein function with directed evolution. *Current Opinion in Chemical Biology, 15*, 201–210.
22. Buchholz, K., Kasche, V. and Bornscheuer, U. T. (2012). Biocatalysts and enzyme technology. ed. John Wiley & Sons.
23. Buckland, B. C., Drew, S. W., Connors, N. C., Chartrain, M. M., Lee, C., Salmon, P. M., Gbewonyo, K., Zhou, W., Gailliot, P., Singhvi, R., Olewinski Jr., R. C., Sun, W. J., Reddy, J., Zhang, J., Jackey, B. A., Taylor, C., Goklen, K. E., Junker, B., & Greasham, R. L. (1999). Microbial conversion of indene to indandiol: a key intermediate in the synthesis of CRIXIVAN. *Metabolic Engineering, 1*, 63–74.
24. Buckland, B. C., Robinson, D. K., & Chartrain, M. (2000). Biocatalysis for pharmaceuticals–status and prospects for a key technology. *Metabolic Engineering, 2*, 42–48.

25. Bujara, M., Schümperli, M., Billerbeck, S., Heinemann, M., & Panke, S. (2010). Exploiting cell-free systems: implementation and debugging of a system of biotransformations. *Biotechnology and Bioengineering, 106*, 376–389.
26. Burton, S. G., Cowan, D. A., & Woodley, J. M. (2002). The search for the ideal biocatalyst. *Nature Biotechnology, 20*, 37–45.
27. Cao, L. (2005). Immobilised enzymes: science or art? *Current Opinion in Chemical Biology, 9*, 217–226.
28. Cao, L., Schmid, R. (2005). D. Carrier-bound immobilized enzymes. Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany.
29. Carballeira, J., Quezada, M., Hoyos, P., Simeó, Y., Hernaiz, M., Alcantara, A., & Sinisterra, J. (2009). Microbial cells as catalysts for stereoselective red–ox reactions. *Biotechnology Advances, 27*, 686–714.
30. Chartrain, M., Lynch, J., Choi, W. B., Churchill, H., Patel, S., Yamazaki, S., Volante, R., & Greasham, R. (2000). Asymmetric bioreduction of a bisaryl ketone to its corresponding (S)-bisaryl alcohol, by the yeast *Rhodotorula pilimanae* ATCC 32762. *J Mol Catal B-Enzym, 8*, 285–288.
31. Chaturvedula, P. V., Chen, L., Civiello, R., Degnan, A. P., Dubowchik, G. M., Han, X., Jiang, X. J. J., Karageorge, G. N., Luo, G. and Macor, J. E. (2007). Calcitonin gene related peptide receptor antagonists. Google Patents.
32. Chaturvedula, P. V. D., G M., Degan, A. P., Han, X., Conway, D., Cook, C., Davis, R., Denton, R., Macci, N. R., Mathias, S., Pin, L., Signor, G., Thalody, R., Schartman, K. A., Widmann, C., Xu, C., Macor, J. E. (2007). Abstracts of papers; 234th National Meeting of the American Chemical Society, Boston, MA, August 19 23, American Chemical Society: Washington.
33. Chmura, A., Rustler, S., & Paravidino, M. (2013). The combi-CLEA approach: enzymatic cascade synthesis of enantiomerically pure (*S*)-mandelic acid. *Tetrahedron-Asymmetry, 24*, 1225–1232.
34. Chou, C.-J., Shockley, K. R., Conners, S. B., Lewis, D. L., Comfort, D. A., Adams, M. W. W., & Kelly, R. M. (2007). Impact of substrate glycoside linkage and elemental sulfur on bioenergetics of and hydrogen production by the hyperthermophilic archaeon *Pyrococcus furiosus*. *Applied and Environmental Microbiology, 73*, 6842–6853.
35. Claps, P., & Garrabou, X. (2011). Current trends in asymmetric synthesis with aldolases. *Advanced Synthesis and Catalysis, 353*, 2263–2283.
36. Crasto, C. J., & Feng, J.-a. (2000). LINKER: a program to generate linker sequences for fusion proteins. *Protein Engineering, 13*, 309–312.
37. Crowe, M., Foulkes, M., Francese, G., Grimler, D., Kuesters, E., Laumen, K., Li, Y., Lin, C., Nazor, J., & Ruch, T. (2015). Chemical process for preparing spiroindolones and intermediates thereof. *WO*.
38. Davis, S., Grate, J., Gray, D., Gruber, J., Huisman, G., Ma, S., Newman, L., Sheldon, R. and Wang, L. (2004). Halohydrin dehalogenases and method for production of 4-cyano-3-hydroxybutyric acid esters and amides. United States Patent Appl.
39. Davis, S. C., Grate, J. H., Gray, D. R., Gruber, J. M., Huisman, G. W., Ma, S. K., Newman, L. M., Sheldon, R., Wang, L. A. (2006). Enzymatic processes for the production of 4-substituted 3-hydroxybutyric acid derivatives. Google Patents.
40. Dawson, M. (1997). PCT WO. 9429476. 1994.
41. de Carvalho, C. C. (2011). Enzymatic and whole cell catalysis: finding new strategies for old processes. *Biotechnology Advances, 29*, 75–83.
42. de Graaff, C., Bensch, L., Boersma, S. J., Cioc, R. C., van Lint, M. J., Janssen, E., Turner, N. J., Orru, R. V. A., & Ruijter, E. (2015). Asymmetric synthesis of tetracyclic pyrroloindolines and constrained tryptamines by a switchable cascade reaction. *Angewandte Chemie (International Ed. in English), 54*, 14133–14136.
43. Denard, C. A., Hartwig, J. F., & Zhao, H. (2013). Multistep one-pot reactions combining biocatalysts and chemical catalysts for asymmetric synthesis. *ACS Catalysis, 3*, 2856–2864.
44. Deninno, M. P. (1991). The synthesis and glycosidation of N-acetylneuraminic acid. *Synthesis, 1991*, 583–593.
45. Dennig, A., Busto, E., Kroutil, W., & Faber, K. (2015). Biocatalytic one-pot synthesis of l-tyrosine derivatives from monosubstituted benzenes, pyruvate, and ammonia. *ACS Catalysis, 5*, 1–5.
46. DeSantis, G., Zhu, Z., Greenberg, W. A., Wong, K., Chaplin, J., Hanson, S. R., Farwell, B., Nicholson, L. W., Rand, C. L., & Weiner, D. P. (2002). An enzyme library approach to biocatalysis: development of nitrilases for enantioselective production of carboxylic acid derivatives. *Journal of the American Chemical Society, 124*, 9024–9025.
47. DeSantis, G., Wong, K., Farwell, B., Chatman, K., Zhu, Z., Tomlinson, G., Huang, H., Tan, X., Bibbs, L., & Chen, P. (2003). Creation of a productive, highly enantioselective nitrilase through gene site saturation mutagenesis (GSSM). *Journal of the American Chemical Society, 125*, 11476–11477.
48. DiCosimo, R. (2007). *Nitrilases and nitrile hydratases* (pp. 1–26). Boca Raton: CRC Press.
49. Dingler, C., Ladner, W., Krei, G. A., Cooper, B., & Hauer, B. (2015). Preparation of (R)-2-(4-hydroxyphenoxy) propionic acid by biotransformation. *Pesticide Science, 46*, 33–35.

50. Doswald, S., Estermann, H., Kupfer, E., Stadler, H., Walther, W., Weisbrod, T., Wirz, B., & Wostl, W. (1994). Large scale preparation of chiral building blocks for the P 3 site of renin inhibitors. *Bioorganic & Medicinal Chemistry, 2*, 403–410.
51. Dourado, D. F. A. R., Pohle, S., Carvalho, A. T. P., Dheeman, D. S., Caswell, J. M., Skvortsov, T., Miskelly, I., Brown, R. T., Quinn, D. J., & Allen, C. C. R. (2016). Rational design of a (*S*)-selective-transaminase for asymmetric synthesis of (1*S*)-1-(1,1'-biphenyl 2-yl)ethanamine. *ACS Catalysis, 6*, 7749–7759.
52. Dragovich, P. S., Prins, T. J., Zhou, R., Webber, S. E., Marakovits, J. T., Fuhrman, S. A., Patick, A. K., Matthews, D. A., Lee, C. A., & Ford, C. E. (1999). Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 4. Incorporation of P1 lactam moieties as L-glutamine replacements. *Journal of Medicinal Chemistry, 42*, 1213–1224.
53. Drauz, K., Gröger, H. and May, O. (2012). Enzyme catalysis in organic synthesis: a comprehensive handbook. John Wiley & Sons.
54. Evans, C. T., Roberts, S. M., Shoberu, K. A., & Sutherland, A. G. (1992). Potential use of carbocyclic nucleosides for the treatment of AIDS: chemo-enzymatic syntheses of the enantiomers of carbovir. *Journal of the Chemical Society, Perkin Transactions*, 589–592.
55. Faber, K. (2011). Biotransformations in organic chemistry: a textbook.Springer Science & Business Media.
56. Federsel, H. J. (2005). Asymmetry on large scale: the roadmap to stereoselective processes. *Cheminform, 36*, 685–697.
57. Ferenci, P., Brunner, H., Nachbaur, K., Datz, C., Gschwantler, M., Hofer, H., Stauber, R., Hackl, F., Jessner, W., & Rosenbeiger, M. (2001). Combination of interferon induction therapy and ribavirin in chronic hepatitis C. *Hepatology, 34*, 1006–1011.
58. Ferralli, P., & a., John Duick Egan, a. and Floyd Lester Erickson, a. (2007). Making Taq DNA polymerase in the undergraduate biology laboratory. *Bios, 69*.
59. Ferris, C. D., Hirsch, D. J., Brooks, B. P., & Snyder, S. H. (1991). σ receptors: from molecule to man. *Journal of Neurochemistry, 57*, 729.
60. Fonseca, T. d. S., Silva, M. R. d., de Oliveira, M. d. C. F., Lemos, T. L. G. d., Marques, R. d. A., & de Mattos, M. C. (2015). Chemoenzymatic synthesis of rasagiline mesylate using lipases. *Appl Catal A, 492*, 76–82.
61. Frock, A. D., Notey, J. S., & Kelly, R. M. (2010). The genus thermotoga: recent developments. *Environmental Technology, 31*, 1169.
62. Gao, S., Zhu, S., Huang, R., Lu, Y., & Zheng, G. (2015). Efficient synthesis of the intermediate of abacavir and carbovir using a novel (+)-γ-lactamase as a catalyst. *Bioorganic & Medicinal Chemistry Letters, 25*, 3878–3881.
63. Gerth, K., Pradella, S., Perlova, O., Beyer, S., & Müller, R. (2003). Myxobacteria: proficient producers of novel natural products with various biological activities-past and future biotechnological aspects with the focus on the genus Sorangium. *Journal of Biotechnology, 106*, 233–253.
64. Ghislieri, D., Green, A. P., Pontini, M., Willies, S. C., Rowles, I., Frank, A., Grogan, G., & Turner, N. J. (2013). Engineering an enantioselective amine oxidase for the synthesis of pharmaceutical building blocks and alkaloid natural products. *Journal of the American Chemical Society, 135*, 10863–10869.
65. Gill, I., & Patel, R. (2006). Biocatalytic ammonolysis of (5S)-4, 5-dihydro-1H-pyrrole-1, 5-dicarboxylic acid, 1-(1, 1-dimethylethyl)-5-ethyl ester: preparation of an intermediate to the dipeptidyl peptidase IV inhibitor Saxagliptin. *Bioorganic & Medicinal Chemistry Letters, 16*, 705–709.
66. Gilligan, P. J., Clarke, T., He, L., Lelas, S., Li, Y.-W., Heman, K., Fitzgerald, L., Miller, K., Zhang, G., & Marshall, A. (2009). Synthesis and structure-activity relationships of 8-(pyrid-3-yl) pyrazolo [1, 5-a]-1, 3, 5-triazines: potent, orally bioavailable corticotropin releasing factor receptor-1 (CRF1) antagonists. *Journal of Medicinal Chemistry, 52*, 3084–3092.
67. Goldberg, S. L., Nanduri, V. B., Chu, L., Johnston, R. M., & Patel, R. N. (2006). Enantioselective microbial reduction of 6-oxo-8-[4-[4-(2-pyrimidinyl)-1-piperazinyl] butyl]-8-azaspiro [4.5] decane-7, 9-dione: cloning and expression of reductases. *Enzyme and Microbial Technology, 39*, 1441–1450.
68. Goldberg, S., Guo, Z., Chen, S., Goswami, A., & Patel, R. N. (2008). Synthesis of ethyl-(3*R*,5*S*)-dihydroxy-6-benzyloxyhexanoates via diastereo- and enantioselective microbial reduction: cloning and expression of ketoreductase III from *Acinetobacter* sp. SC 13874. *Enzyme and Microbial Technology, 43*, 544–549.
69. Gordon, E. M., Ondetti, M. A., Pluscec, J., Cimarusti, C. M., Bonner, D. P. and Sykes, R. B. (1983). ChemInform abstract: O-sulfated β-lactam hydroxamic acids (monosulfactams). novel monocyclic β-lactam antibiotics of synthetic origin. J Am Chem Soc*,* 14, no-no.

70. Goswami, A., & Kissick, T. P. (2009). Enzymatic desymmetrization of dimethyl cylcohex-4-ene-cis-1, 2-dicarboxylate to (1 *S*, 2 *R*)-2-(methoxycarbonyl) cyclohex-4-ene-1-carboxylic acid. *Organic Process Research and Development, 13*, 483–488.
71. Gotor, V. (1999). Non-conventional hydrolase chemistry: amide and carbamate bond formation catalyzed by lipases. *Bioorganic & Medicinal Chemistry, 7*, 2189–2197.
72. Gotor-Fernandez, V., & Gotor, V. (2006). Enzymatic aminolysis and ammonolysis processes in the preparation of chiral nitrogenated compounds. *Current Organic Chemistry, 10*, 1125–1143.
73. Gotor-Fernández, V., Brieva, R., & Gotor, V. (2006). Lipases: eseful biocatalysts for the preparation of pharmaceuticals. *Journal of Molecular Catalysis B: Enzymatic, 40*, 111–120.
74. Guo, Z., Goswami, A., Mirfakhrae, K. D., & Patel, R. N. (1999). Asymmetric acyloin condensation catalyzed by phenylpyruvate decarboxylase. *Tetrahedron-Asymmetry, 10*, 4667–4675.
75. Guo, Z., Chen, Y., Goswami, A., Hanson, R. L., & Patel, R. N. (2006). Synthesis of ethyl and t-butyl (3*R*, 5*S*)-dihydroxy-6-benzyloxy hexanoates via diastereo- and enantioselective microbial reduction. *Tetrahedron-Asymmetry, 17*, 1589–1602.
76. Gupta, A., Tschentscher, A. and Bobkova, M. (2008). 2-butanol production method. US.
77. Gustafsson, D., Elg, M., Lenfors, S., Börjesson, I., & Teger-Nilsson, A. (1996). Effects of inogatran, a new low-molecular-weight thrombin inhibitor, in rat models of venous and arterial thrombosis, thrombolysis and bleeding time. *Blood Coagulation Fibrinolysis, 7*, 69–79.
78. Hailes, H., Richter, N., Simon, R. C., Kroutil, W., & Ward, J. M. (2014). Synthesis of pharmaceutically relevant 17-[small alpha]-amino steroids using an [small omega]-transaminase. *Chemical Communications, 50*, 6098–6100.
79. Hanefeld, U., Gardossi, L., & Magner, E. (2009). Understanding enzyme immobilisation. *Chemical Society Reviews, 38*, 453–468.
80. Hansen, K. B., Hsiao, Y., Xu, F., Rivera, N., Clausen, A., Kubryk, M., Krska, S., Rosner, T., Simmons, B., & Balsells, J. (2009). Highly efficient asymmetric synthesis of sitagliptin. *Journal of the American Chemical Society, 131*, 8798–8804.
81. Hanson, R. L., Singh, J., Kissick, T. P., Patel, R. N., Szarka, L. J., & Mueller, R. H. (1990). Synthesis of l -β-hydroxyvaline from α-keto-β-hydroxyisovalerate using leucine dehydrogenase from *Bacillus species*. *Bioorganic Chemistry, 18*, 116–130.
82. Hanson, R. L., Schwinden, M. D., Banerjee, A., Brzozowski, D. B., Chen, B.-C., Patel, B. P., McNamee, C. G., Kodersha, G. A., Kronenthal, D. R., & Patel, R. N. (1999). Enzymatic synthesis of L-6-hydroxynorleucine. *Bioorganic & Medicinal Chemistry, 7*, 2247–2252.
83. Hanson, R. L., Goldberg, S., Goswami, A., Tully, T. P., & Patel, R. N. (2005). Purification and cloning of a ketoreductase used for the preparation of chiral alcohols. *Advanced Synthesis and Catalysis, 347*, 1073.
84. Hanson, R. L., Goldberg, S. L., Brzozowski, D. B., Tully, T. P., Cazzulino, D., Parker, W. L., Lyngberg, O. K., Vu, T. C., Wong, M. K., & Patel, R. N. (2007). Preparation of an amino acid intermediate for the dipeptidyl peptidase IV inhibitor, saxagliptin, using a modified phenylalanine dehydrogenase. *Advanced Synthesis and Catalysis, 349*, 1369–1378.
85. Hanson, R. L., Davis, B. L., Chen, Y., Goldberg, S. L., Parker, W. L., Tully, T. P., Montana, M. A., & Patel, R. N. (2008). Preparation of (*R*)-amines from racemic amines with an (*S*)-amine transaminase from Bacillus megaterium. *Advanced Synthesis and Catalysis, 350*, 1367–1375.
86. Hanson, R. L., Davis, B. L., Goldberg, S. L., Johnston, R. M., Parker, W. L., Tully, T. P., Montana, M. A., & Patel, R. N. (2008). Enzymatic preparation of a D-amino acid from a racemic amino acid or keto acid. *Org Proc Res Dev, 12*, 1119–1129.
87. Hauptmann, J. (2002). Pharmacokinetics of an emerging new class of anticoagulant/antithrombotic drugs. *European Journal of Clinical Pharmacology, 57*, 751–758.
88. Herbert, R. A. (1992). A perspective on the biotechnological potential of extremophiles. *Trends in Biotechnology, 10*, 395–402.
89. Herman, G. A., Bergman, A., Yi, B., Kipnes, M., & Group, S. S. (2006). Tolerability and pharmacokinetics of metformin and the dipeptidyl peptidase-4 inhibitor sitagliptin when co-administered in patients with type 2 diabetes. *Current Medical Research and Opinion, 22*, 1939–1947.
90. Herter, S., McKenna, S. M., Frazer, A. R., Leimkühler, S., Carnell, A. J., & Turner, N. J. (2015). Galactose oxidase variants for the oxidation of amino alcohols in enzyme cascade synthesis. *ChemCatChem, 7*, 2313.
91. Hold, C., & Panke, S. (2009). Towards the engineering of in vitro systems. *J R Soc Interface, 6*(Suppl 4), S507–S521.
92. Hollmann, F., Arends, I. W. C. E., Buehler, K., Schallmey, A., & Bühler, B. (2011). Enzyme-mediated oxidations for the chemist. *Green Chemistry, 13*, 226–265.
93. Hollmann, F., Arends, I. W. C. E., & Holtmann, D. (2011). Enzymatic reductions for the chemist. *Green Chemistry, 13*, 2285–2314.

94. Holton, R., Biediger, R., Joatman, P. (1995) Semisynthesis of taxol and taxotere. In Taxol: Science and Application; Suffness, M., CRC press: NewYork, pp. 97–123.
95. Homann, M. J., Vail, R., Morgan, B., Sabesan, V., Levy, C., Dodds, D. R., & Zaks, A. (2001). Enzymatic hydrolysis of a prochiral 3-substituted glutarate ester, an intermediate in the synthesis of an NK1/NK2 dual antagonist. *Advanced Synthesis and Catalysis, 343*, 744–749.
96. Hossain, M. A., Kitagaki, S., Nakano, D., Nishiyama, A., Funamoto, Y., Matsunaga, T., Tsukamoto, I., Yamaguchi, F., Kamitori, K., & Dong, Y. (2011). Rare sugar D-psicose improves insulin sensitivity and glucose tolerance in type 2 diabetes Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biochemical and Biophysical Research Communications, 405*, 7–12.
97. Hough, D. W., & Danson, M. J. (1999). Extremozymes. *Current Opinion in Chemical Biology, 3*, 39–46.
98. Hu, H., Qian, J., Chu, J., Wang, Y., Zhuang, Y., & Zhang, S. (2009). DNA shuffling of methionine adenosyltransferase gene leads to improved S-adenosyl-l-methionine production in Pichia pastoris. *Journal of Biotechnology, 141*, 97–103.
99. Huang, X., Garcia-Borràs, M., Miao, K., Kan, S. J., Zutchi, A., Houk, K. N. and Arnold, F. H. (2019). A biocatalytic platform for synthesis of chiral α-trifluoromethylated organoborons. ACS Central Science.
100. Huisman, G. W., & Collier, S. J. (2013). On the development of new biocatalytic processes for practical pharmaceutical synthesis. *Current Opinion in Chemical Biology, 17*, 284–292.
101. Huisman, G. W. and Lalonde, J. J. (2007). 30 enzyme evolution for chemical process applications.
102. Huisman, G. W., Liang, J., & Krebber, A. (2010). Practical chiral alcohol manufacture using ketoreductases. *Current Opinion in Chemical Biology, 14*, 122–129.
103. Ishige, T., Honda, K., & Shimizu, S. (2005). Whole organism biocatalysis. *Current Opinion in Chemical Biology, 9*, 174–180.
104. Ising, M., Zimmermann, U. S., Künzel, H. E., Uhr, M., Foster, A. C., Learned-Coughlin, S. M., Holsboer, F., & Grigoriadis, D. E. (2007). High-affinity CRF_1 receptor antagonist NBI-34041: preclinical and clinical data suggest safety and efficacy in attenuating elevated stress response. *Neuropsychopharmacology, 32*, 1941–1949.
105. Iwata, H., Tanaka, R., & Ishiguro, M. (1990). Structures of the alkaline hydrolysis products of penem antibiotic, SUN5555. *The Journal of Antibiotics, 43*, 901–903.
106. Jagoda, E., Stouffer, B., Ogan, M., Tsay, H. M., Turabi, N., Mantha, S., Yost, F., & Tu, J. I. (1993). Radioimmunoassay for hydroxyphosphinyl-3-hydroxybutanoic acid (SQ 33,600), a hypocholesterolemia agent. *Therapeutic Drug Monitoring, 15*, 213–219.
107. Jajoo, H., Mayol, R., LaBudde, J., & Blair, I. (1989). Metabolism of the antianxiety drug buspirone in human subjects. *Drug Metabolism and Disposition, 17*, 634–640.
108. Jiang, W., & Fang, B.-S. (2016). Construction and evaluation of a novel bifunctional phenylalanine–formate dehydrogenase fusion protein for bienzyme system with cofactor regeneration. *Journal of Industrial Microbiology & Biotechnology, 43*, 1–8.
109. Jiang, W., & Fang, B. (2016). Construction of a tunable multi-enzyme-coordinate expression system for biosynthesis of chiral drug intermediates. *Scientific Reports, 6*, 30462–30462.
110. Johanson, T., Carlquist, M., Olsson, C., Rudolf, A., Frejd, T., & Gorwagrauslund, M. F. (2008). Reaction and strain engineering for improved stereo-selective whole-cell reduction of a bicyclic diketone. *Applied Microbiology and Biotechnology, 77*, 1111–1118.
111. Junien, J. L., & Leonard, B. E. (1989). Drugs acting on sigma and phencyclidine receptors: a review of their nature, function, and possible therapeutic importance. *Clinical Neuropharmacology, 12*, 353–374.
112. Justin, B. S., Alexandre, Z., Helena, M. L., Gert, K., Abigail, R. L., Jennifer, L. S. C., Jasmine, L. G., Donald, H., Michael, H. G., Barry, L. S., Kendall, N. H., Forrest, E. M., & David, B. (2010). Computational design of an enzyme catalyst for a stereoselective bimolecular Diels-Alder reaction. *Science, 309*.
113. Kaluzna, I. A., Feske, B. D., Wittayanan, W., Ghiviriga, I., & Stewart, J. D. (2005). Stereoselective, biocatalytic reductions of alpha-chloro-beta-keto esters. *The Journal of Organic Chemistry, 70*, 342–345.
114. Kataoka, M., Kotaka, A., Thiwthong, R., Wada, M., Nakamori, S., & Shimizu, S. (2004). Cloning and overexpression of the old yellow enzyme gene of *Candida macedoniensis*, and its application to the production of a chiral compound. *Journal of Biotechnology, 114*, 1–9.
115. Kazlauskas, R. J. (2005). Enhancing catalytic promiscuity for biocatalysis. *Current Opinion in Chemical Biology, 9*, 195–201.
116. Kim, M. J., Hennen, W. J., Sweers, H. M., & Chi, H. W. (1988). Enzymes in carbohydrate synthesis: N-acetylneuraminic acid aldolase catalyzed reactions and preparation of N-acetyl-2-deoxy-D-neuraminic acid derivatives. *Journal of the American Chemical Society, 110*, 6481–6468.
117. Kingston, D. (1995). Natural taxoids: structure and chemistry. In M. Suffness (Ed.), *Taxol: science and application* (pp. 287–317). New York: CRC press.

118. Kizaki, N., Yasohara, Y., Hasegawa, J., Wada, M., Kataoka, M., & Shimizu, S. (2001). Synthesis of optically pure ethyl (*S*)-4-chloro-3-hydroxybutanoate by Escherichia coli transformant cells coexpressing the carbonyl reductase and glucose dehydrogenase genes. *Applied Microbiology and Biotechnology, 55*, 590–595.
119. Kobayashi, S., Kamiyama, K., Iimori, T., & Ohno, M. (1984). Creation of novel chiral synthons with enzymes and applications to natural product synthesis. 15. Efficient introduction of chiral centers into cyclohexane ring. *Tetrahedron Letters, 25*, 2557–2560.
120. Köhler, V., Bailey, K. R., Znabet, A., Raftery, J., Helliwell, M., & Turner, N. J. (2010). Enantioselective biocatalytic oxidative desymmetrization of substituted pyrrolidines. *Angewandte Chemie, International Edition, 49*, 2182–2184.
121. Kosjek, B., Ntigyabaah, J., Telari, K., Dunne, L., & Moore, J. C. (2008). Preparative asymmetric synthesis of 4,4-dimethoxytetrahydro-2H-pyran-3-ol with a ketone reductase and in situ cofactor recycling using glucose dehydrogenase. *Organic Process Research and Development, 12*, 584–588.
122. Koszelewski, D., Clay, D., Rozzell, D., & Kroutil, W. (2009). Deracemisation of α-chiral primary amines by a one-pot, two-step cascade reaction catalysed by ω-transaminases. *European Journal of Organic Chemistry, 2009*, 2289–2292.
123. Kragl, U., Gygax, D., Ghisalba, O., & Wandrey, C. (1991). Enzymatic two-step synthesis of N-acetylneuraminic acid in the enzyme membrane reactor. *Angewandte Chemie, International Edition, 30*, 827–828.
124. Kragl, U., Vasic-Racki, D., & Wandrey, C. (1996). Continuous production of L-*tert*-leucine in series of two enzyme membrane reactors. *Bioprocess Engineering, 14*, 291–297.
125. Krulewicz, B., Tschaen, D., Devine, P., Lee, S. S., Roberge, C., Greasham, R., & Chartrain, M. (2009). Asymmetric biosynthesis of key aromatic intermediates in the synthesis of an endothelin receptor antagonist. *Biocatalysis and Biotransformation, 19*, 267–279.
126. Landis, B. H., Mclaughlin, J. K., Heeren, R., And, R. W. G., & Wang, P. T. (2002). Bioconversion of N-butylglucamine to 6-deoxy-6-butylamino sorbose by Gluconobacter oxydans. *Organic Process Research and Development, 6*, 547–552.
127. Lauria-Horner, B. A., & Pohl, R. B. (2003). Pregabalin: a new anxiolytic. *Expert Opinion on Investigational Drugs, 12*, 663–672.
128. Lee, M., & Rhee, M. K. (2015). Sitagliptin for type 2 diabetes: a 2015 update. *Exp Rev Cardiovasc Ther, 13*, 597–610.
129. Lelas, S., Wong, H., Li, Y.-W., Heman, K. L., Ward, K. A., Zeller, K. L., Sieracki, K. K., Polino, J. L., Godonis, H. E., & Ren, S. X. (2004). Anxiolytic-like effects of the corticotropin-releasing factor1 (CRF1) antagonist DMP904 [4-(3-pentylamino)-2, 7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1, 5-a]-pyrimidine] administered acutely or chronically at doses occupying central CRF1 receptors in rats. *The Journal of Pharmacology and Experimental Therapeutics, 309*, 293–302.
130. Li, T., Liang, J., Ambrogelly, A., Brennan, T., Gloor, G., Huisman, G., Lalonde, J., Lekhal, A., Mijts, B., Muley, S., Newman, L., Tobin, M., Wong, G., Zaks, A., & Zhang, X. (2012). Efficient, chemoenzymatic process for manufacture of the Boceprevir bicyclic [3.1.0]proline intermediate based on amine oxidase-catalyzed desymmetrization. *Journal of the American Chemical Society, 134*, 6467–6472.
131. Liang, J., Mundorff, E., Voladri, R., Jenne, S., Gilson, L., Conway, A., Krebber, A., Wong, J., Huisman, G., & Truesdell, S. (2010). Highly enantioselective reduction of a small heterocyclic ketone: biocatalytic reduction of tetrahydrothiophene-3-one to the corresponding (*R*)-alcohol. *Organic Process Research and Development, 14*, 188–192.
132. Liese, A., Seelbach, K. and Wandrey, C. (2006). Industrial biotransformations. John Wiley & Sons.
133. Limanto, J., Ashley, E. R., Yin, J., Beutner, G. L., Grau, B. T., Kassim, A. M., Kim, M. M., Klapars, A., Liu, Z., Strotman, H. R., & Truppo, M. D. (2014). A highly efficient asymmetric synthesis of vernakalant. *Organic Letters, 16*, 2716–2719.
134. Liu, W., Ma, H., Luo, J., Shen, W., Xu, X., Li, S., Hu, Y., & Huang, H. (2014). Efficient synthesis of l- tert-leucine through reductive amination using leucine dehydrogenase and formate dehydrogenase coexpressed in recombinant *E. coli*. *Biochemical Engineering Journal, 91*, 204–209.
135. Luo, X., Wang, Y.-J., & Zheng, Y.-G. (2015). Cloning and characterization of a NADH-dependent aldo-keto reductase from a newly isolated *Kluyveromyces lactis* XP1461. *Enzyme and Microbial Technology, 77*, 68–77.
136. Luo, X., Wang, Y.-J., Shen, W., & Zheng, Y.-G. (2016). Activity improvement of a Kluyveromyces lactis aldo-keto reductase KlAKR via rational design. *Journal of Biotechnology, 224*, 20–26.
137. Lutz, S., & Bornscheuer, U. T. (2012). *Protein engineering handbook* (ed ed.). John Wiley & Sons.
138. Ma, S. K., Gruber, J., Davis, C., Newman, L., Gray, D., Wang, A., Grate, J., Huisman, G. W., & Sheldon, R. A. (2010). A green-by-design biocatalytic process for atorvastatin intermediate. *Green Chemistry, 12*, 81–86.

139. Mahmoudian, M. D., & M., J. (1997). In W. R. Strohl (Ed.), *Biotechnology of anti-biotics* (2nd ed., p. 753). New York: Marcel Dekker Inc..
140. Mahmoudian, M., Noble, D., Drake, C., Middleton, R., Montgomery, D., Piercey, J., Ramlakhan, D., Todd, M., & Dawson, M. (1997). An efficient process for production of N-acetylneuraminic acid using N-acetylneuraminic acid aldolase. *Enzyme and Microbial Technology, 20*, 393–400.
141. Mahmoudian, M., Lowdon, A., Jones, M., Dawson, M., & Wallis, C. (1999). A practical enzymatic procedure for the resolution of N-substituted 2-azabicyclo [2.2. 1] hept-5-en-3-one. *Tetrahedron-Asymmetry, 10*, 1201–1206.
142. Manfred T. R. (2016). Directed evolution of selective enzymes: catalysts for organic chemistry and biotechnology ed. John Wiley & Sons.
143. Matsumae, H., Furui, M., & Shibatani, T. (1993). Lipase-catalyzed asymmetric hydrolysis of 3-phenylglycidic acid ester, the key intermediate in the synthesis of diltiazem hydrochloride. *Journal of Fermentation and Bioengineering, 75*, 93–98.
144. Mayol, R. F. (2000). Anxiety method. Google Patents.
145. McTaggart, F., Buckett, L., Davidson, R., Holdgate, G., McCormick, A., Schneck, D., Smith, G., & Warwick, M. (2001). Preclinical and clinical pharmacology of rosuvastatin, a new 3-hydroxy- 3-methylglutaryl coenzyme A reductase inhibitor11CRESTOR is a trademark, the property of AstraZeneca PLC. Research discussed in this article was supported by AstraZeneca. *The American Journal of Cardiology, 87*, 28–32.
146. Menzel, A., Werner, H., Altenbuchner, J., & Gröger, H. (2004). From enzymes to "designer bugs" in reductive amination: a new process for the synthesis of L-*tert*-leucine using a whole cell-catalyst. *Engineering in Life Sciences, 4*, 573–576.
147. Merviel, P., Najas, S., Campy, H., Floret, S., & Brasseur, F. (2005). Use of GNRH antagonists in reproductive medicine. *Minerva Ginecolog, 57*, 29–43.
148. Merz, A., Yee, M. C., Szadkowski, H., Pappenberger, G., Crameri, A., Stemmer, W. P., Yanofsky, C., & Kirschner, K. (2000). Improving the catalytic activity of a thermophilic enzyme at low temperatures. *Biochemistry, 39*, 880–889.
149. Moriguchi, M., & Ideta, K. (1988). Production of D-aminoacylase from *Alcaligenes denitrificans* subsp. xylosoxydans MI-4. *Applied and Environmental Microbiology, 54*, 2767–2770.
150. Munchhof, M. J., Li, Q., Shavnya, A., Borzillo, G. V., Boyden, T. L., Jones, C. S., Lagreca, S. D., Martinezalsina, L., Patel, N., & Pelletier, K. (2011). Discovery of PF-04449913, a potent and orally bioavailable inhibitor of smoothened. *ACS Medicinal Chemistry Letters, 3*, 106.
151. Nanduri, V. B., Hanson, R. L., Goswami, A., Wasylyk, J. M., LaPorte, T. L., Katipally, K., Chung, H.-J., & Patel, R. N. (2001). Biochemical approaches to the synthesis of ethyl 5-(*s*)-hydroxyhexanoate and 5-(*s*)-hydroxyhexanenitrile. *Enzyme and Microbial Technology, 28*, 632–636.
152. Nestl, B. M., Nebel, B. A., & Hauer, B. (2011). Recent progress in industrial biocatalysis. *Current Opinion in Chemical Biology, 15*, 187–193.
153. O'Reilly, E., Iglesias, C., Ghislieri, D., Hopwood, J., Galman, J. L., Lloyd, R. C., & Turner, N. J. (2014). A regio- and stereoselective ω-transaminase/monoamine oxidase cascade for the synthesis of chiral 2,5-disubstituted pyrrolidines. *Angewandte Chemie (International Ed. in English), 53*, 2447.
154. Otey, C. R., Bandara, G., Lalonde, J., Takahashi, K., & Arnold, F. H. (2006). Preparation of human metabolites of propranolol using laboratory-evolved bacterial cytochromes P450. *Biotechnology and Bioengineering, 93*, 494–499.
155. Overstreet, D. H., & Griebel, G. (2004). Antidepressant-like effects of CRF1 receptor antagonist SSR125543 in an animal model of depression. *European Journal of Pharmacology, 497*, 49–53.
156. Park, J. W., Lee, J. K., Kwon, T. J., Yi, D. H., Kim, Y. J., Moon, S. H., Suh, H. H., Kang, S. M., & Park, Y. I. (2003). Bioconversion of compactin into pravastatin by *Streptomyces* sp. *Biotechnology Letters, 25*, 1827–1831.
157. Parmeggiani, F., Lovelock, S. L., Weise, N. J., Ahmed, S. T., & Turner, N. J. (2015). Synthesis of d- and l-phenylalanine derivatives by phenylalanine ammonia lyases: a multienzymatic cascade process. *Angewandte Chemie, International Edition, 127*, 4691–4694.
158. Patel, R. N. (1998). Tour de paclitaxel: biocatalysis for semisynthesis. *Annual Review of Microbiology, 52*, 361–395.
159. Patel, R. N. (2001). Biocatalytic synthesis of intermediates for the synthesis of chiral drug substances. *Current Opinion in Biotechnology, 12*, 587–604.
160. Patel, R. N. (2006). *Biocatalysis in the pharmaceutical and biotechnology industries* (ed ed.). CRC press.
161. Patel, R. N. (2006). Biocatalysis: synthesis of chiral intermediates for pharmaceuticals. *Current Organic Chemistry, 10*, 1289–1321.
162. Patel, R. N. (2008). Synthesis of chiral pharmaceutical intermediates by biocatalysis. *Coordination Chemistry Reviews, 252*, 659–701.

163. Patel, R. N. (2011). Biocatalysis: synthesis of key intermediates for development of pharmaceuticals. *ACS Catalysis, 1*, 1056–1074.
164. Patel, R. N. (2018). Biocatalysis for synthesis of pharmaceuticals. *Bioorganic & Medicinal Chemistry, 26*, 1252–1274.
165. Patel, R. N., Robison, R. S., Szarka, L. J., Kloss, J., Thottathil, J. K., & Mueller, R. H. (1991). Stereospecific microbial reduction of 4,5-dihydro-4-(4-methoxyphenyl)-6-(trifluoromethyl-1H-1)-benzazepin+ ++-2-o ne. *Enzyme and Microbial Technology, 13*, 906–912.
166. Patel, R. N., McNamee, C. G., Banerjee, A., Howell, J. M., Robison, R. S., & Szarka, L. J. (1992). Stereoselective reduction of β-keto esters by *Geotrichum candidum*. *Enzyme and Microbial Technology, 14*, 731–738.
167. Patel, R. N., Banerjee, A., Howell, J. M., McNamee, C. G., Brozozowski, D., Mirfakhrae, D., Nanduri, V., Thottathil, J. K., & Szarka, L. J. (1993). Microbial synthesis of (2*R*,3*S*)-(−)-N-benzoyl-3-phenyl isoserine ethyl ester-a taxol side-chain synthon. *Tetrahedron-Asymmetry, 4*, 2069–2084.
168. Patel, R. N., Banerjee, A., Liu, M., Hanson, R., Ko, R., Howell, J., & Szarka, L. J. (1993). Microbial reduction of 1-(4-fluorophenyl)-4-[4-(5-fluoro-2-pyrimidinyl)-1- piperazinyl]butan-1-one. *Biotechnology and Applied Biochemistry, 17*(Pt 2), 139.
169. Patel, R. N., Banerjee, A., Mcnamee, C. G., Brzozowski, D., Hanson, R. L., & Szarka, L. J. (1993). Enantioselective microbial reduction of 3,5-dioxo-6-(benzyloxy) hexanoic acid, ethyl ester. *Enzyme and Microbial Technology, 15*, 1014–1021.
170. Patel, R. N., Banerjee, A., Mcnamee, C. G., Brzozowski, D. B., & Szarka, L. J. (1997). Preparation of chiral synthon for HIV protease inhibitor: stereoselective microbial reduction of N-protected α-aminochloroketone. *Tetrahedron-Asymmetry, 8*, 2547–2552.
171. Patel, R. N., Banerjee, A., Nanduri, V. B., Goldberg, S. L., Johnston, R. M., Hanson, R. L., McNamee, C. G., Brzozowski, D. B., Tully, T. P., & Ko, R. Y. (2000). Biocatalytic preparation of a chiral synthon for a vasopeptidase inhibitor: enzymatic conversion of N 2-[N-Phenylmethoxy) carbonyl] L-homocysteinyl]-L-lysine (1-> 1')-disulfide to [4S-(4I, 7I, 10aJ)] 1-octahydro-5-oxo-4-[phenylmethoxy) carbonyl] amino]-7H-pyrido-[2, 1-b][1, 3] thiazepine-7-carboxylic acid methyl ester by a novel L-lysine ϵ-aminotransferase. *Enzyme Microb Technol, 27*, 376–389.
172. Patel, R. N., Chu, L., Chidambaram, R., Zhu, J., & Kant, J. (2002). Enantioselective microbial reduction of 2-oxo-2-(1′,2′,3′,4′-tetrahydro-1′,1′,4′,4′-tetramethyl-6′-naphthalenyl)acetic acid and its ethyl ester. *Tetrahedron-Asymmetry, 13*, 349–355.
173. Patel, R. N., Chu, L., & Mueller, R. (2003). Diastereoselective microbial reduction of (S)-[3-chloro-2-oxo-1-(phenylmethyl)propyl]carbamic acid, 1,1-dimethylethyl ester. *Tetrahedron-Asymmetry, 14*, 3105–3109.
174. Patel, R. N., Goswami, A., Chu, L., Donovan, M. J., Nanduri, V., Goldberg, S., Johnston, R., Siva, P. J., Nielsen, B., Fan, J., He, W., Shi, Z., Wang, K. Y., Eiring, R., Cazzulino, D., Singh, A., & Mueller, R. (2004). Enantioselective microbial reduction of substituted acetophenones. *Tetrahedron-Asymmetry, 15*, 1247–1258.
175. Patel, R., Chu, L., Nanduri, V., Li, J., Kotnis, A., Parker, W., Liu, M., & Mueller, R. (2005). Enantioselective microbial reduction of 6-oxo-8-[4-[4-(2-pyrimidinyl)-1-piperazinyl] butyl]-8-azaspiro [4.5] decane-7, 9-dione. *Tetrahedron-Asymmetry, 16*, 2778–2783.
176. Patel, R. N., Chu, L. N. H., Johnson, R. M., Guo, Z., Chen, Y., Goldberg, S. L., Hanson, R. L., Goswami, A. and Katipally, K. (2008). Stereoselective reduction process for the preparation of pyrrolotriazine compounds. Google Patents.
177. Paul, C. E., Rodriguez-Mata, M., Busto, E., Lavandera, I., Gotor-Fernandez, V., Gotor, V., Garcia-Cerrada, S., Mendiola, J., de Frutos, O., & Collado, I. (2014). Transaminases applied to the synthesis of high added-value enantiopure amines. *Organic Process Research and Development, 18*, 788–792.
178. Pavlidis, I. V., Weiß, M. S., Genz, M., Spurr, P., Hanlon, S. P., Wirz, B., Iding, H., & Bornscheuer, U. T. (2016). Identification of (*S*)-selective transaminases for the asymmetric synthesis of bulky chiral amines. *Nature Chemistry, 8*, 1076–1082.
179. Peng, Z., Wong, J. W., Hansen, E. C., Puchlopekdermenci, A. L. A., & Clarke, H. J. (2014). Development of a concise, asymmetric synthesis of a smoothened receptor (SMO) inhibitor: enzymatic transamination of a 4-piperidinone with dynamic kinetic resolution. *Organic Letters, 16*, 860–863.
180. Pianko, S., & McHutchison, J. G. (2000). Treatment of hepatitis C with interferon and ribavirin. *Journal of Gastroenterology and Hepatology, 15*, 581–586.
181. Pinder, R. M., Brogden, R. N., Speight, T. M., & Avery, G. S. (1977). Maprotiline: a review of its pharmacological properties and therapeutic efficacy in mental depressive states. *Drugs, 13*, 321.
182. Podar, M., & Reysenbach, A.-L. (2006). New opportunities revealed by biotechnological explorations of extremophiles. *Current Opinion in Biotechnology, 17*, 250–255.

183. Pollard, D. J., & Woodley, J. M. (2007). Biocatalysis for pharmaceutical intermediates: the future is now. *Trends in Biotechnology, 25*, 66–73.
184. Prasad, C. V. C., Noonan, J. W., Sloan, C. P., Lau, W., Vig, S., Parker, M. F., Smith, D. W., Hansel, S. B., Polson, C. T., Barten, D. M., Felsenstein, K. M., & Roberts, S. B. (2004). Hydroxytriamides as potent γ-secretase inhibitors. *Bioorganic & Medicinal Chemistry Letters, 14*, 1917–1921.
185. Rao, M. B., Tanksale, A. M., Ghatge, M. S., & Deshpande, V. V. (1998). Molecular and biotechnological aspects of microbial proteases. *Microbiology and Molecular Biology Reviews, 62*, 597–635.
186. Manfred T. Reetz (2011). Gerichtete evolution stereoselektiver enzyme: eine ergiebige Katalysator-Quelle für asymmetrische Reaktionen[J]. Angewandte Chemie, 123(1): 144–182. Gerichtete Evolution stereoselektiver Enzyme: Eine ergiebige Katalysator-Quelle für asymmetrische Reaktionen. Angew Chem Int Ed, **123,** 144–182.
187. Reetz, M. T., Torre, C., Eipper, A., Lohmer, R., Hermes, M., Brunner, B., Maichele, A., Bocola, M., Arand, M., & Cronin, A. (2004). Enhancing the enantioselectivity of an epoxide hydrolase by directed evolution. *Organic Letters, 6*, 177–180.
188. Ricklefs, E., Girhard, M., Koschorreck, K., Smit, M. S., & Urlacher, V. B. (2015). Two-step one-pot synthesis of pinoresinol from eugenol in an enzymatic cascade. *ChemCatChem, 7*, 1857.
189. Ricklefs, E., Girhard, M., & Urlacher, V. B. (2016). Three-steps in one-pot: whole-cell biocatalytic synthesis of enantiopure (+)- and (-)-pinoresinol via kinetic resolution. *Microbial Cell Factories, 15*, 78–78.
190. Robert, S. S., Singh, S. P., Zhou, X. R., Petrie, J. R., Blackburn, S. I., Mansour, P. M., Nichols, P. D., Liu, Q., & Green, A. G. (2005). Metabolic engineering of Arabidopsis to produce nutritionally important DHA in seed oil. *Func Plant Biol, 32*, 473–479.
191. Robinson, B. S., Riccardi, K. A., Gong, Y. F., Guo, Q., Stock, D. A., Blair, W. S., Terry, B. J., Deminie, C. A., Djang, F., Colonno, R. J., & Lin, P. F. (2000). BMS-232632, a highly potent human immunodeficiency virus protease inhibitor that can be used in combination with other available antiretroviral agents. *Antimicrobial Agents and Chemotherapy, 44*, 2093–2099.
192. Robl, J. A., Sun, C.-Q., Stevenson, J., Ryono, D. E., Simpkins, L. M., Cimarusti, M. P., Dejneka, T., Slusarchyk, W. A., Chao, S., & Stratton, L. (1997). Dual metalloprotease inhibitors: mercaptoacetyl-based fused heterocyclic dipeptide mimetics as inhibitors of angiotensin-converting enzyme and neutral endopeptidase. *Journal of Medicinal Chemistry, 40*, 1570–1577.
193. Rowles, I., Groenendaal, B., Binay, B., Malone, K. J., Willies, S. C., & Turner, N. J. (2016). Engineering of phenylalanine ammonia lyase from Rhodotorula graminis for the enhanced synthesis of unnatural l-amino acids. *Tetrahedron, 72*, 7343–7347.
194. Rozzell, D., Liang, J.. (2008). Speciality Chemicals Magazine, 36.
195. Rubin-Pitel, S. B., & Zhao, H. (2006). Recent advances in biocatalysis by directed enzyme evolution. *Combinatorial Chemistry & High Throughput Screening, 9*, 247–257.
196. Salis, H. M., Mirsky, E. A., & Voigt, C. A. (2009). Automated design of synthetic ribosome binding sites to control protein expression. *Nature Biotechnology, 27*, 946–950.
197. Savage, S. A., Jones, G. S., Kolotuchin, S., Ramrattan, S. A., Vu, T., & Waltermire, R. E. (2009). Preparation of saxagliptin, a Novel DPP-IV Inhibitor. *Organic Process Research and Development, 13*, 1169–1176.
198. Savile, C. K., Janey, J. M., Mundorff, E. C., Moore, J. C., Tam, S., Jarvis, W. R., Colbeck, J. C., Krebber, A., Fleitz, F. J., & Brands, J. (2010). Biocatalytic asymmetric synthesis of chiral amines from ketones applied to sitagliptin manufacture. *Science, 329*, 305–309.
199. Savile, C., Gruber, J. M., Mundorff, E., Huisman, G. W. and Collier, S. J. (2014). Ketoreductase polypeptides for the production of a 3-aryl-3-hydroxypropanamine from a 3-aryl-3-ketopropanamine. pp. 42-46. WO.
200. Schenk, D., Games, D., & Seubert, P. (2001). Potential treatment opportunities for Alzheimer's disease through inhibition of secretases and Aβ immunization. *Journal of Molecular Neuroscience, 17*, 259–267.
201. Schmidt, M., Baumann, M., Henke, E., Konarzycka-Bessler, M., & Bornscheuer, U. T. (2004). Directed evolution of lipases and esterases. *Methods in Enzymology, 388*, 199–207.
202. Schmidt, M., Hasenpusch, D., Kähler, M., Kirchner, U., Wiggenhorn, K., Langel, W., & Bornscheuer, U. T. (2006). Directed evolution of an esterase from Pseudomonas fluorescens yields a mutant with excellent enantioselectivity and activity for the kinetic resolution of a chiral building block. *Chembiochem, 7*, 805–809.
203. Schmidt-Dannert, C., & Arnold, F. H. (1999). Directed evolution of industrial enzymes. *Trends in Biotechnology, 17*, 135–136.
204. Selak, I. (2003). Pregabalin: a new anxiolytic. *Current Opinion in Investigational Drugs, 12*, 663–672.
205. Shafiee, A., Motamedi, H., & King, A. (1998). Purification, characterization and immobilization of an NADPH-dependent enzyme involved in the chiral specific reduction of the keto ester M, an intermediate in

the synthesis of an anti-asthma drug, Montelukast, from *Microbacterium campoquemadoensis* (MB5614). *Applied Microbiology and Biotechnology, 49*, 709.
206. Shaw, A. J., Podkaminer, K. K., Desai, S. G., Bardsley, J. S., Rogers, S. R., Thorne, P. G., Hogsett, D. A., & Lynd, L. R. (2008). Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 13769–13774.
207. Sheldon, R. (1993). Chirotechnology Marcel Dekker. *New York, 365*.
208. Sheldon, R. A. (2008). E factors, green chemistry and catalysis: an odyssey. Chemical Communications, 3352–3365.
209. Sheldon, R. A., & Pereira, P. C. (2017). Biocatalysis engineering: the big picture. *Chemical Society Reviews, 46*, 2678–2691.
210. Sheldon, R. A., & Woodley, J. M. (2018). Role of biocatalysis in sustainable chemistry. *Chemical Reviews, 118*, 801–838.
211. Shen, Q., Zhang, Y., Yang, R., Pan, S., Dong, J., Fan, Y., & Han, L. (2016). Enhancement of isomerization activity and lactulose production of cellobiose 2-epimerase from *Caldicellulosiruptor saccharolyticus*. *Food Chemistry, 207*, 60–67.
212. Shin, J.-S., & Kim, B.-G. (2002). Exploring the active site of amine: pyruvate aminotransferase on the basis of the substrate structure- reactivity relationship: how the enzyme controls substrate specificity and stereoselectivity. *The Journal of Organic Chemistry, 67*, 2848–2853.
213. Simeo, Y., Kroutil, W., & Faber, K. (2007). *Biocatalytic deracemization: dynamic resolution, stereoinversion, enantioconvergent processes, and cyclic deracemization*. Boca Raton: CRC Press.
214. Simons, C., Hanefeld, U., Arends, I. W., Maschmeyer, T., & Sheldon, R. A. (2006). Towards catalytic cascade reactions: asymmetric synthesis using combined chemo-enzymatic catalysts. *Topics in Catalysis, 40*, 35–44.
215. Solano, D. M., Hoyos, P., Hernáiz, M., Alcántara, A., & Sánchez-Montero, J. (2012). Industrial biotransformations in the synthesis of building blocks leading to enantiopure drugs. *Bioresource Technology, 115*, 196–207.
216. Soriano-Maldonado, P., Las Heras-Vazquez, F. J., Clemente-Jimenez, J. M., Rodriguez-Vico, F., & Martínez-Rodríguez, S. (2015). Enzymatic dynamic kinetic resolution of racemic N-formyl- and N-carbamoyl-amino acids using immobilized L-N-carbamoylase and N-succinyl-amino acid racemase. *Applied Microbiology and Biotechnology, 99*, 283–291.
217. Sousa, T., Paterson, R., Moore, V., Carlsson, A., Abrahamsson, B., & Basit, A. W. (2008). The gastrointestinal microbiota as a site for the biotransformation of drugs. *International Journal of Pharmaceutics, 363*, 1–25.
218. Staebler, A., Cruz, A., van der Goot, W., Pinheiro, H. M., Cabral, J. M. S., & Fernandes, P. (2004). Optimization of androstenedione production in an organic–aqueous two-liquid phase system. *Journal of Molecular Catalysis B: Enzymatic, 29*, 19–23.
219. Steinreiber, A., & Faber, K. (2001). Microbial epoxide hydrolases for preparative biotransformations. *Current Opinion in Biotechnology, 12*, 552–558.
220. Suen, W. C., Zhang, N., Xiao, L., Madison, V., & Zaks, A. (2004). Improved activity and thermostability of *Candida antarctica* lipase B by DNA family shuffling. *Protein Engineering, Design & Selection, 17*, 133–140.
221. Sumida, Y., Iwai, S., Nishiya, Y., Kumagai, S., Yamada, T., & Azuma, M. (2016). Identification and characterization of D-succinylase, and a proposed enzymatic method for D-amino acid synthesis. *Advanced Synthesis and Catalysis, 358*, 2041.
222. Sun, J., Huang, J., Ding, X., & Wang, P. (2016). Efficient enantioselective biocatalytic production of a chiral intermediate of Sitagliptin by a newly filamentous fungus isolate. *Applied Biochemistry and Biotechnology, 180*, 695–706.
223. Sun, H., Zhang, H., Ang, E. L., & Zhao, H. (2017). Biocatalysis for the synthesis of pharmaceuticals and pharmaceutical intermediates. *Bioorganic & Medicinal Chemistry*.
224. Taché, Y., Martinez, V., Wang, L., & Million, M. (2004). CRF1 receptor signaling pathways are involved in stress-related alterations of colonic function and viscerosensitivity: implications for irritable bowel syndrome. *Br J Phamacol, 141*, 1321–1330.
225. Tamarez, M., Morgan, B., Wong, G. S., Tong, W., Bennett, F., Lovey, R., McCormick, J. L., & Zaks, A. (2003). Pilot-scale lipase-catalyzed regioselective acylation of ribavirin in anhydrous media in the synthesis of a novel prodrug intermediate. *Organic Process Research and Development, 7*, 951–953.
226. Tao, H., & Cornish, V. W. (2002). Milestones in directed enzyme evolution. *Current Opinion in Chemical Biology, 6*, 858–864.
227. Tao, J. A., Kazlauskas, R. J. (2011). Biocatalysis for green chemistry and chemical process development. John Wiley & Sons.

228. Tao, J., & McGee, K. (2002). Development of a continuous enzymatic process for the preparation of (*R*)-3-(4-fluorophenyl)-2-hydroxy propionic acid. *Organic Process Research and Development, 6*, 520–524.
229. Tao, J., & Xu, J.-H. (2009). Biocatalysis in development of green pharmaceutical processes. *Current Opinion in Chemical Biology, 13*, 43–50.
230. Taylor, P. P., Pantaleone, D. P., Senkpeil, R. F., & Fotheringham, I. G. (1998). Novel biosynthetic approaches to the production of unnatural amino acids using transaminases. *Trends in Biotechnology, 16*, 412–418.
231. Turner, N. J. (2004). Enzyme catalysed deracemisation and dynamic kinetic resolution reactions. *Current Opinion in Chemical Biology, 8*, 114–119.
232. Turner, N. J., & O'Reilly, E. (2013). Biocatalytic retrosynthesis. *Nature Chemical Biology, 9*, 285–288.
233. Tyo, K. E., Alper, H. S., & Stephanopoulos, G. N. (2007). Expanding the metabolic engineering toolbox: more options to engineer cells. *Trends in Biotechnology, 25*, 132–137.
234. van der Donk, W. A., & Zhao, H. (2003). Recent developments in pyridine nucleotide regeneration. *Current Opinion in Biotechnology, 14*, 421–426.
235. Vedha-Peters, K., Gunawardana, M., Rozzell, J. D., & Novick, S. J. (2006). Creation of a broad-range and highly stereoselective D-amino acid dehydrogenase for the one-step synthesis of D-amino acids. *Journal of the American Chemical Society, 128*, 10923–10929.
236. Velaparthi, U., Saulnier, M. G., Wittman, M. D., Liu, P., Frennesson, D. B., Zimmermann, K., Carboni, J. M., Gottardis, M., Li, A., & Greer, A. (2010). Insulin-like growth factor-1 receptor (IGF-1R) kinase inhibitors: SAR of a series of 3-[6-(4-substituted-piperazin-1-yl)-4-methyl-1H-benzimidazol-2-yl]-1H-pyridine-2-one. *Bioorganic & Medicinal Chemistry Letters, 20*, 3182–3185.
237. Veronesi, B., Carter, J., Devlin, R., Simon, S., & Oortgiesen, M. (1999). Neuropeptides and capsaicin stimulate the release of inflammatory cytokines in a human bronchial epithelial cell line. *Neuropeptides, 33*, 447–456.
238. Vrtis, J. M., White, A. K., Metcalf, W. W., & van der Donk, W. A. (2002). Phosphite dehydrogenase: a versatile cofactor-regeneration enzyme. *Angewandte Chemie (International Ed. in English), 41*, 3257–3259.
239. Vu, T., Brzozowski, D., Fox, R., Godfrey Jr., J., Hanson, R., Kolotuchin, S., Mazzullo Jr., J., Patel, R., Wang, J., & Wong, K. (2004). *Preparation of cyclopropyl-fused pyrrolidine-based inhibitors of dipeptidyl peptidase IV.* Appl: PCT Int.
240. Wagner, N., Bosshart, A., Failmezger, J., Bechtold, M., & Panke, S. (2015). A separation-integrated cascade reaction to overcome thermodynamic limitations in rare-sugar synthesis. *Angewandte Chemie, 54*, 4182–4186.
241. Walsgrove, T. C., Powell, L., & Wells, A. (2002). Lotrafiban, ((2S)-7-(4,4'-Bipiperidinylcarbonyl)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-Benzodiazepine-2-acetic acid) utilising an enzymic resolution as the final step. *Organic Process Research and Development, 6*, 488–491.
242. Wang, Y., & Zhao, H. (2016). Tandem reactions combining biocatalysts and chemical catalysts for asymmetric synthesis. *Catalysts, 6*, 194.
243. Wang, M., Yang, R., Hua, X., Shen, Q., Zhang, W., & Zhao, W. (2015). Lactulose production from lactose by recombinant cellobiose 2-epimerase in permeabilised *Escherichia coli* cells. *International Journal of Food Science and Technology, 50*, 1625–1631.
244. Wang-Iverson, D., Arnold, M. E., Jemal, M., & Cohen, A. I. (1992). Determination of SQ 33,600, a phosphinic acid containing HMG CoA reductase inhibitor, in human serum by high-performance liquid chromatography combined with ionspray mass spectrometry. *Biological Mass Spectrometry, 21*, 189–194.
245. Wei, Y., Xia, S., He, C., Xiong, W., & Xu, H. (2016). Highly enantioselective production of a chiral intermediate of sitagliptin by a novel isolate of *Pseudomonas pseudoalcaligenes*. *Biotechnology Letters, 38*, 841–846.
246. Weise, N. J., Parmeggiani, F., Ahmed, S. T., & Turner, N. J. (2015). The bacterial ammonia lyase EncP: a tunable biocatalyst for the synthesis of unnatural amino acids. *Journal of the American Chemical Society, 137*, 12977–12983.
247. Weiwei, Z., Lough, A. J., Young Feng, L., & Morris, R. H. (2013). Amine(imine)diphosphine iron catalysts for asymmetric transfer hydrogenation of ketones and imines. *Science, 342*, 1080–1083.
248. Wells, A. S., Finch, G. L., Michels, P. C., & Wong, J. W. (2012). Use of enzymes in the manufacture of active pharmaceutical ingredients-A science and safety-based approach to ensure patient safety and drug quality. *Organic Process Research and Development, 16*, 1986–1993.
249. Wendisch, V. F., Bott, M., & Eikmanns, B. J. (2006). Metabolic engineering of *Escherichia coli* and *Corynebacterium glutamicum* for biotechnological production of organic acids and amino acids. *Current Opinion in Microbiology, 9*, 268–274.
250. Wilson, Z. E., & Brimble, M. A. (2009). Molecules derived from the extremes of life. *Natural Product Reports, 26*, 44–71.

251. Wiseman, A., Goldfard, P. S., Woods, L., & Ridgway, T. (2001). Novel biocatalytic enzymes by directed evolution. *Trends in Biotechnology, 19*, 382–382.
252. Wittman, M., Carboni, J., Attar, R., Balasubramanian, B., Balimane, P., Brassil, P., Beaulieu, F., Chang, C., Clarke, W., & Dell, J. (2005). Discovery of a (1H-benzoimidazol-2-yl)-1H-pyridin-2-one (BMS-536924) inhibitor of insulin-like growth factor I receptor kinase with in vivo antitumor activity. *Journal of Medicinal Chemistry, 48*, 5639.
253. Wong, J. W., Watson, H. A., Bouressa, J. F., Burns, M. P., Cawley, J. J., Doro, A. E., Guzek, D. B., Hintz, M. A., McCormick, E. L., Scully, D. A., Siderewicz, J. M., Taylor, W. J., Truesdell, S. J., & Wax, R. G. (2002). Biocatalytic oxidation of 2-methylquinoxaline to 2-quinoxalinecarboxylic acid. *Organic Process Research and Development, 6*, 477–481.
254. Wu, G., Truksa, M., Datla, N., Vrinten, P., Bauer, J., Zank, T., Cirpus, P., Heinz, E., & Qiu, X. (2005). Stepwise engineering to produce high yields of very long-chain polyunsaturated fatty acids in plants. *Nature Biotechnology, 23*, 1013–1017.
255. Wu, S., Zhou, Y., Wang, T., Too, H.-P., Wang, D. I. C., & Li, Z. (2016). Highly regio- and enantioselective multiple oxy- and amino-functionalizations of alkenes by modular cascade biocatalysis. *Nature Communications, 7*, 11917–11917.
256. Xiang, Z., Birrien, J.-L., Fouquet, Y., Cherkashov, G., Jebbar, M., Querellou, J., Oger, P., Cambon-Bonavita, M.-A., Xiang, X., & Prieur, D. (2009). Pyrococcus CH1, an obligate piezophilic hyperthermophile: extending the upper pressure-temperature limits for life. *The ISME Journal, 3*, 873.
257. Xu, Z., Singh, J., Schwinden, M. D., Zheng, B., Kissick, T. P., Patel, B., Humora, M. J., Quiroz, F., Dong, L., & Hsieh, D.-M. (2002). Process research and development for an efficient synthesis of the HIV protease inhibitor BMS-232632. *Organic Process Research and Development, 6*, 323–328.
258. Xu, G. C., Tang, M. H., & Ni, Y. (2016). Asymmetric synthesis of lipitor chiral intermediate using a robust carbonyl reductase at high substrate to catalyst ratio. *Journal of Molecular Catalysis B: Enzymatic, 123*, 67–72.
259. Xu, Q., Tao, W. Y., Huang, H., & Li, S. (2016). Highly efficient synthesis of ethyl (*S*)-4-chloro-3-hydroxybutanoate by a novel carbonyl reductase from *Yarrowia lipolytica* and using mannitol or sorbitol as cosubstrate. *Biochemical Engineering Journal, 106*, 61–67.
260. Xu, Z., Wang, R., Liu, C., Chi, B., Gao, J., Chen, B., & Xu, H. (2016). A new L-arabinose isomerase with copper ion tolerance is suitable for creating protein-inorganic hybrid nanoflowers with enhanced enzyme activity and stability. *RSC Advances, 6*, 30791–30794.
261. Yagasaki, M., & Ozaki, A. (1998). Industrial biotransformations for the production of D-amino acids. *Journal of Molecular Catalysis B: Enzymatic, 4*, 1–11.
262. Yevich, J. P., New, J. S., Lobeck, W. G., Dextraze, P., Bernstein, E., Taylor, D. P., Yocca, F. D., Eison, M. S., & Temple Jr., D. L. (1992). Synthesis and biological characterization of. alpha.-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol and analogs as potential atypical antipsychotic agents. *Journal of Medicinal Chemistry, 35*, 4516–4525.
263. YHP, Z. (2010). Production of biocommodities and bioelectricity by cell-free synthetic enzymatic pathway biotransformations: challenges and opportunities. Biotechnology and Bioengineering
264. Yoshikawa, N., Ohta, K., Mizuno, S., & Ohkishi, H. (1993). Production of cis,cis-muconic acid from benzoic acid. *Bioprocess Technology, 16*, 131.
265. Zalman, L., Brothers, M., Dragovich, P., Zhou, R., Prins, T., Worland, S., & Patick, A. (2000). Inhibition of human rhinovirus-induced cytokine production by AG7088, a human rhinovirus 3C protease inhibitor. *Antimicrobial Agents and Chemotherapy, 44*, 1236–1241.
266. Zhang, N., Suen, W.-C., Windsor, W., Xiao, L., Madison, V., & Zaks, A. (2003). Improving tolerance of *Candida antarctica* lipase B towards irreversible thermal inactivation through directed evolution. *Protein Engineering, 16*, 599–605.
267. Zhang, Y. H. P., Evans, B. R., Mielenz, J. R., Hopkins, R. C., & Adams, M. W. W. (2007). High-yield hydrogen production from starch and water by a synthetic enzymatic pathway. *PLoS One, 2*, e456–e456.
268. Zhang, Y. H. P., Sun, J., & Zhong, J.-J. (2010). Biofuel production by in vitro synthetic enzymatic pathway biotransformation. *Current Opinion in Biotechnology, 21*, 663–669.
269. Zhang, Y., Gao, F., Zhang, S.-P., Su, Z.-G., Ma, G.-H., & Wang, P. (2011). Simultaneous production of 1, 3-dihydroxyacetone and xylitol from glycerol and xylose using a nanoparticle-supported multi-enzyme system with in situ cofactor regeneration. *Bioresource Technology, 102*, 1837–1843.
270. Zhang, D., Chen, X., Chi, J., Feng, J., Wu, Q., & Zhu, D. (2015). Semi-rational engineering a carbonyl reductase for the enantioselective reduction of β-amino ketones. *ACS Catalysis, 5*, 150309095209002.
271. Zhang, Y., Wang, Y., Wang, S., & Fang, B. (2017). Engineering bi-functional enzyme complex of formate dehydrogenase and leucine dehydrogenase by peptide linker mediated fusion for accelerating cofactor regeneration. *Engineering in Life Sciences, 17*, 989.

272. Zhao, H., Chockalingam, K., & Chen, Z. (2002). Directed evolution of enzymes and pathways for industrial biocatalysis. *Current Opinion in Biotechnology, 13*, 104–110.
273. Zhu, S., Gong, C., Song, D., Gao, S., & Zheng, G. (2012). Discovery of a Novel (+)- gamma -lactamase from *Bradyrhizobium japonicum* USDA 6 by rational genome mining. *Applied and Environmental Microbiology, 78*, 7492–7495.