Enzyme- and Chemo-enzyme-Catalyzed Stereodivergent Synthesis

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Abstract

Multiple stereoisomers can be found when a substance contains chiral carbons in its chemical structure. To obtain the desired stereoisomers, asymmetric synthesis was proposed in the 1970s and developed rapidly at the beginning of this century. Stereodivergent synthesis, an extension of asymmetric synthesis in organic synthesis with the hope to produce all stereoisomers of chiral substances in high conversion and selectivity, enriches the variety of available products and serves as a reference suggestion for the synthesis of their derivatives and other compounds. Since biocatalysis has outstanding advantages of economy, environmental friendliness, high

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efficiency, and reaction at mild conditions, the biocatalytic reaction is regarded as an efficient strategy to perform stereodivergent synthesis. Thus, in this review, we summarize the stereodivergent synthesis catalyzed by enzymes or chemo-enzymes in cases where a compound contains two or three chiral carbons, i.e., at most four or eight stereoisomers are present. The types of reactions, including reduction of substituent ketones, cyclization reactions, olefin addition, and nonredox transesterification reactions, are also discussed for the understanding of the progress and application of biocatalysis in stereodivergent synthesis.

Introduction

In organic synthesis, if chiral carbons are involved in the reactions without regio or stereo control, multiple stereoisomers would be produced. Although several methods, such as high-performance liquid chromatography\(^1\)\(^-\)\(^5\) and optical resolution,\(^6\)\(^-\)\(^8\) can separate the desired products from other isomeric impurities, it still leads to an inevitable decline of yields. To improve the traditional synthesis methods, a new method called asymmetric synthesis began to rise gradually after a seminal article reported by Mosher and colleagues\(^9\)\(^,\)\(^10\) in the 1970s, and rapidly developed at the beginning of this century.\(^11\)\(^,\)\(^12\) During this period, Macmillan and colleagues\(^13\) and List et al\(^14\) independently developed the third type of catalyst—chiral organocatalysts distinct from metal catalysts and enzymes—in 2000, spurring rapid advances in asymmetric synthesis and winning the Nobel Prize in chemistry 2021. The core purpose of asymmetric synthesis represented by metal catalysis, chiral organocatalysis, and enzyme catalysis is to improve the selectivity of the desired product’s configuration in high efficiency, that is, to increase the enantiomeric excess (ee) or diastereomeric excess (de).

Stereodivergent synthesis, established precisely based on asymmetric synthesis, allows access to any given stereoisomer of a product with multiple stereocenters from the same set of starting materials.\(^15\) In the process of stereodivergent synthesis, whether using biocatalysis alone or a combination of biocatalysis and other methods, the reactions performed fall broadly into three categories (Scheme 1). In reaction type 1, there is usually no chiral carbon in the substrate A or the functional group participating in the reaction, such as the reduction of carbonyl groups and C=C double bonds. The two chiral centers of the products are generally produced in successive steps by different catalysts. However, the preparation of (R/S)-A\(_1\) from substrate A can sometimes not be performed due to limitations in technical development. Thus, occasionally enantiomeric or Z/E(cis/trans) isomeric A\(_1\) are also used as the substrates, leading to the preparation of four stereoisomeric products A\(_2\). Reaction type 2 is commonly found in condensation reactions, such as olefin addition reactions, reduction reactions of \(\alpha\)-substituent ketones, and \(\beta\)-substituent ketones as well as transesterification reactions. In addition to olefin addition reactions where the substrate B carries C=C double bonds, the substrate B is usually used in racemic forms in other reactions, which produces the other chiral carbon based on one already existing chiral carbon under the catalysis of different stereoselective catalysts. If the two enantiomers of a racemic substrate B are capable of rapid interconversion spontaneously or by inducers, i.e., maintaining a dynamic equilibrium of configurational proportions, then product B\(_2\) with possible 100% conversion and high selectivity can be obtained by asymmetric reaction of only one enantiomer, which is known as dynamic kinetic resolution (DKR).\(^16\)\(^,\)\(^17\) Otherwise, the conversion of the racemic substrate was ideally at most 50%. What’s more, high percentage preparation of one stereoisomer can also be carry out by epimerization (or chiral inversion\(^18\)) of chiral carbon, which converts other isomers to the desired isomer, shown as reaction type 3. The common methods used in epimerization includes crystallization induction,\(^19\)\(^,\)\(^20\) chemical reagent induction,\(^21\)\(^,\)\(^22\) and biocatalysis.\(^23\)\(^-\)\(^26\)

Reduction of \(\alpha,\beta\)-Unsaturated Ketones

Preparation of Carveols, Dihydrocarvones, and Dihydrocarveols

\(\alpha\),\(\beta\)-unsaturated ketones are valuable additives in the flavor industry.\(^27\)\(^,\)\(^28\) Among related reports, the stereodivergent synthesis of eight stereoisomers of dihydrocarveol (3) catalyzed by two enzymes was first achieved by Guo et al.\(^29\) As shown in Scheme 2, (4R)-1 and (4S)-1 were first reduced by ene-reductases to four stereoisomeric dihydrocarvones (2) with conversions (Conv.) greater than 99% as well as de value ranging from 93 to 96%. Subsequently, dihydrocarvones (2) were further reduced to eight stereoisomeric...
dihydrocarveols (3) with likewise excellent conversions and de values with ketoreductases. The genes encoding the ene-reductases OYE1 and NamA can be obtained from *Saccharomyces pastorianus* and *Bacillus megaterium*, respectively, and the recombinant ketoreductases LfSDR, BmSDR, and BsSDR can be obtained from *Lactobacillus fermentum*, *B. megaterium*, and *Bacillus subtilis*, respectively.

Similarly, four stereoisomeric carveols (4) can also be obtained from enantiomeric carvones (1) catalyzed by ketoreductases (Scheme 3). However, the de of product (2R,4S)-4 was not ideal in this process. Moreover, under the influence of conjugated olefins, the reduction efficiency of the carbon group, i.e., the conversions of (2R,4R)-4 and (2S,4S)-4, is lower than that in Scheme 2.

**Preparation of 4-Methylheptane-3-ols**

4-Methylheptane-3-ol (7) is an insect pheromone that has four stereoisomers and the biological activities of each isomer are quite different.30–34 Although the asymmetric synthesis of (3S,4S)-7 and the resolution of the four
stereoisomers with chromatography or lipase were accomplished as early as the end of the last century, the stereodivergent synthesis of was only reported in recent years by Crotti and colleagues. They achieved stereodivergent synthesis of four stereoisomers of in one pot using a cascade of alcohol dehydrogenases and ene-reductases taken from Pichia stipitis, S. pastorianus as well as Saccharomyces cerevisiae (Scheme 4), while the conversions of reactions ranged from 72 to 99% and de values of obtained products were ranged from 92 to 99%.

Preparation of Michael Addition Products

RA95, first reported by Althoff et al., is an artificial retroaldolase generated by computational design. Building on this, Giger et al. obtained novel mutant RA95.5–8 by directed evolution. Subsequently, Garrabou et al. designed RA95.5–8 into four stereo-complementary catalysts, M-R.R, M-S.R, M-R.S, and M-S.S, capable of catalyzing Michael addition reactions. Under the action of the above four computationally designed enzymes (Scheme 5), the tertiary carbanion reacts with (E)-4-(4-methoxyphenyl)but-3-en-2-one (8) to produce four stereoisomeric ethyl 2-cyano-3-(4-methoxyphenyl)-2-methyl-5-oxohexanoates (9). Since four stereoisomers are inevitably produced in the Michael addition reactions, the isomer percentage was used to characterize the stereoselectivity of these enzymes, rather than de. As indicated in this study, the isomer percentages of the four stereoisomers ranged from 80.4 to 84.7%. Furthermore, the only reported yield among four stereoisomers was 47% for (2S, 3R)-9.

Reduction of α-Substituted Ketones

Preparation of 3-Hydroxyprolines and 3-Hydroxypipecolic Acids

Hydroxyproline and hydroxypipecolic acid are both important intermediates in organic synthesis and privileged pharmacophores in many drugs. In a recent study, Prier et al. prepared four stereoisomers of 3-hydroxyproline (11) in satisfactory yields, as well as ee and de values by DKR of amino-protected methyl 3-oxopyrrolidine-2-carboxylate (10) using commercial ketoreductases (Scheme 6). Similarly, DKR of amino-protected ethyl 3-oxopiperidine-2-carboxylate (12) with similar ketoreductases also produced four stereoisomeric 3-hydroxypipecolic acids (13). Although the catalytic efficiencies of the four ketoreductases used for the six-membered ring substrates were not as high as the five-membered ring substrates, products with high ee values were still accessible.

Reduction of S-(2-Acetamidoethyl) 2-methyl-3-oxopentanethioates and Its Derivatives

Complex polyketides are a broad class of natural products that are synthesized from simple precursors catalyzed by polyketide synthases (PKSs). Among them, EryKR1, TylKR1, AmpKR2, and RifKR7, KR domains derived from erythromycin PKS, tylosin PKS, amphotericin PKS, and

Scheme 4 Stereodivergent synthesis of four stereoisomers of 4-methylheptane-3-ol.

Scheme 5 Stereodivergent synthesis of four Michael addition products.
rifamycin PKS, respectively, have been reported as excellent ketoreductases in the preparation of complex polyketides. Results showed that they can catalyze the DKR of S-(2-acetamidoethyl) 2-methyl-3-oxopentanethioate (14a) and its derivatives (14b, 14c), leading to corresponding four reduced products (15a–c) (Scheme 7). Subsequently, Bailey et al. continued to conduct in-depth research on the protein engineering of these reductases. Their results showed that in EryKR1, the 1,810th amino acid was found to be critical for the conformational shift of the products, and mutation of this residue from leucine to alanine resulted in a shift of the stereoselectivity from the original (2S,3R)-15a–c to (2S,3S)-15a–c with improved conversion as well. In addition, changing the amino acids at other sites also resulted in increased conversions of (2S,3S)-15a–c, among which, the combinatorial mutation EryKR1 D1758A/L1810A exhibited the best catalytic activity. It is worth mentioning that the ee reported in Scheme 7 is not the commonly used ratio of the desired product to its enantiomer, but the desired product to the other three stereoisomers.

Recently, Robles and coworkers also used six ketoreductases, PikKR2, MycKR6, TylKR2, AmpKR3, EryKR3, and EryKR7, to catalyze the DKR of S-(2-acetamidoethyl) 2-methyl-3-oxopentanethioate (16a) and two other derivatives (16b, 16c) and finally obtained the corresponding four stereoisomers (17a–c) with high isomer percentages, respectively (Scheme 8). Unexpectedly, in Scheme 8, a blunted fall in the catalytic selectivity toward the product (2S,3S)-17b by MycKR6 occurs with the isomer percentage of only 46%, which is much lower than those of substrates 17a and 17c. Based on this deficiency, EryKR3 was used to enhance this catalytic reduction reaction, giving the desired product with an isomer percentage of 91%. In addition, the yields for reduction of ethyl substituted 17a and butyl substituted 17b were approximately 65%, while that of 17c was 35%.

Preparation of 2-Amino-2-(5-bromopyridin-3-yl)-1-(2,5-difluorophenyl) ethane-1-ols
2-Amino-2-(5-bromopyridin-3-yl)-1-(2,5-difluorophenyl) ethane-1-ol (19) is a synthetic intermediate for positive

![Scheme 6](image6.png)

**Scheme 6** Stereodivergent synthesis of four stereoisomers of 3-hydroxyproline and 3-hydroxyproline acid.

![Scheme 7](image7.png)

**Scheme 7** Stereodivergent synthesis of 12 reduced products catalyzed by 4 ketoreductases.
allosteric modulators of metabotropic glutamate receptors.\(^5\) During the synthesis of 19, the amino groups in the structure usually need to be protected, and the commonly used protective agents are tert-butoxy carbonyl (Boc),\(^5\) benzyloxy carbonyl, etc. Hanson et al.\(^5\) used three commercial ketoreductases and four microorganisms to catalyze the DKR of amino-protected 2-amino-2-(5-bromopyridin-3-yl)-1-(2,5-difluorophenyl) ethane-1-one (18), obtaining four stereoisomeric chiral alcohols (Scheme 9). It should be emphasized that the *Pichia methanolica* ATCC56508 exhibited excellent catalytic activity and selectivity in the preparation of (1R,2S)-19, even exceeding the screened commercial ketoreductase ES KRED-112.

### Reduction of β-Substituted Ketones

#### Preparation of 3-Phenylcyclohex-1-ols

As mentioned in the Introduction, racemic substrates need to be able to undergo interconversion of configurations in DKR. These spontaneously transformed or able to be induced dynamic centers usually attach active groups, such as hydroxyl group, amino group, carbonyl group, etc., at the chiral carbon.\(^5\) However, Dehovitz et al.\(^5\) broke this restriction and used photocatalysts and commercial ketoreductases to reduce 3-phenylcyclohexan-1-one (20), producing four stereoisomeric 3-phenylcyclohexan-1-ols (21) in good yields (Scheme 10). Besides, the LK-ADH and its mutant, i.e., LK-ADH E145F/F147L/Y190C, used in the synthesis scheme are obtained from *Lactobacillus kefir*.

#### Preparation of 1-(Substituted Phenyl) Butane-1,3-diols

1,3-Diols are important starting materials for the synthesis of pharmacologically active compounds,\(^55\) and these structures also widely exist among natural products.\(^56,57\) In addition, 1,3-diols can also be used to synthesize chiral 1,3-diphosphines that are enantiopure ligands in asymmetric catalysis.\(^58\) In the related reports of 1,3-diols, Baer et al.\(^59\) using organocatalysts as well as alcohol dehydrogenases with R/S-type stereoselectivity (Scheme 11), first completed the stereodivergent synthesis of four stereoisomeric 1-(4-chlorophenyl) butane-1,3-diols (24). As reported in this study, 4-chlorobenzaldehyde (22) and acetone, catalyzed by chiral organocatalysts, were used to synthesize a pair of enantiomeric 4-(4-chlorophenyl)-4-hydroxybutan-2-ones (23). Subsequently, the carbonyl

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**Scheme 8** Stereodivergent synthesis of 12 reduced products catalyzed by 5 ketoreductases.

**Scheme 9** Stereodivergent synthesis of four stereoisomers of chiral alcohol.
groups of 4-hydroxybut-2-ones were stereoselectively reduced by alcohol dehydrogenases in R-type or S-type, respectively, to finally make four 1,3-diols. In addition, the enzymatic activity curve of (S)-ADH in the presence of chiral organocatalyst was also determined. The result shows that the (S)-ADH still retains high activity over a certain range of organocatalyst concentrations and enables the one-pot preparation of (1R,3S)-24.

In addition to the organo-enzymatic catalysis method described above, the method of producing 1,3-diols employing metal-enzyme catalysis was also reported. The process was described by Sonoike et al. using chiral zinc complex catalysts and purchased oxidoreductases to make three structures totaling twelve 1-(substituted phenyl) butane-1,3-diols (Scheme 12, 26a–c).

Cyclization Reaction

Cyclization reactions are a rather important branch in synthetic chemistry and are widely applied in various fields. The multiple types, mechanisms, and corresponding rules of cyclization reactions have been connectively reported in the past half century of research. Here, we mainly report stereodivergent synthesis of three types of cyclized compounds, including intramolecular transesterification reactions as well as nucleophilic substitution reactions and intermolecular epoxidation reactions. Of these, only the cyclopropanation reactions of C=C double bonds with a diazo compound produce chiral centers during the ring formation process, whereas the other chiral cyclization products were obtained based on the already existing chiral centers catalyzed by biocatalysts.

Preparation of Substituted γ-Butyrolactones

Substituted γ-butyrolactones serve both as chiral building blocks for synthetic products and as core groups for many natural compounds and chemical agents. The stereodivergent synthesis of substituted γ-butyrolactones using a one-pot two-enzyme method was first reported by Classen et al. In the one-pot process (Scheme 13), α,β-unsaturated ketones (27) were first reduced by an ene-reductase and subsequently reduced by alcohol dehydrogenase, and finally underwent transesterification with a smooth cyclization to produce a variety of substituted γ-butyrolactones (29). Since the ene-reductase used in the first step of the reaction is not stereoselective, substrate 27 was required in diastereomerically pure form to prepare (S,R)-28 in high optical purity.

In September of the same year, a method for the preparation of substituted γ-butyrolactones utilizing chiral organocatalysts and alcohol dehydrogenases was subsequently reported by Simon et al. In their proposed scheme (Scheme 14), para-
methoxyphenyl-protected ethyl 2-iminoacetate (30) and acetone, catalyzed by proline, undergo the Mannich reaction to give ethyl 2-amino-4-oxopentanoates (31). Subsequently, the enantiomeric products 31 were reduced to four stereoisomers of substituted γ-butyrolactones (32) by the commercial ketoreductases evo-1.1.200 or by ADH-A derived from Rhodococcus ruber along with hydrochloric acid.

Preparation of 2-Methyl-3-pentyloxiranes
Veschambre is one of the pioneers of stereodivergent synthesis utilizing chemical and biocatalytic methods. The preparation of four stereoisomeric 2-methyl-3-pentyloxiranes (37) starting from 2-octanone (34) was accomplished as early as the end of the last century by Veschambre and colleagues. Limited by the lack of development of asymmetric synthesis techniques at that time, 3-bromo-2-octanone (35) was obtained in the racemic form in the preparation process (Scheme 15), so the resultant 3-bromo-2-ols (36) were a mixture of diastereomers and required silica gel column chromatography for product separation to obtain the optically pure products. In addition, the authors also used a similar procedure to prepare four stereoisomeric 3-azido-2-ols. Although the yield of the product now appears to be relatively low, the combination of chemical and enzymatic approaches was undoubtedly groundbreaking at the time and had a profound impact on the subsequently biocatalytic asymmetric synthesis.

Preparation of Ethyl 2-Substituted Cyclopropane-1-carboxylates
Iron (II)-porphyrin, also known as hemoprotein, which is present in cytochrome P450 and hemoglobin, was reported by Brustad and colleagues to be a biocatalyst that can be used for the cyclopropanation of active alkenes (39) such as styrene with ethyl diazoacetate (38). In their subsequent report, the stereodivergent synthesis of four stereoisomers
of ethyl 2-phenylcyclopropane-1-carboxylate (40) and its derivatives could be accomplished under the catalysis of P450BM3 variant from B. megaterium as well as other cytochrome enzymes (Scheme 16).

Stereodivergent cyclopropanation using less active aliphatic alkenes with ethyl diazoacetate (EDA) was first reported by Knight et al.⁷² Four enzymes with excellent activity and catalytic complementation from 11 heme proteins and their mutants extracted from thermophilic and hyper-thermophilic bacteria and archaea were used for catalysis of a wide range of substrates (41a–c, Scheme 17). Among them, ApEPgb AGW and RmaNOD are abbreviations of Aeropyrum pernix protoglobin W59A Y60G F145W and Rhodothermus marinus nitric oxide dioxygenase, respectively.

Scheme 14 Stereodivergent synthesis of four substituted γ-butyrolactones catalyzed by chiral organocatalysts and alcohol dehydrogenases.

Scheme 15 Stereodivergent synthesis of four stereoisomers of 2-methyl-3-pentyloxirane.
P411-UA-V87C and P411-UA-V87F are two variants of the engineered, serine-ligated cytochrome P450BM3. In addition, the total turnover number (TTN) is used to characterize the catalytic activity of these enzymes, and these yields were calculated under conditions designed to demonstrate the catalysts’ potential TTNs.

Other Types of Reactions

The variety of reaction types reported above are reduction, addition, and cyclization reactions that take the monocarbonyl group as the main functional group and simultaneously unite olefins, α-substituent groups, and β-substituent groups, respectively. However, there are other types of reactions, such as olefin addition reaction without carbonyl participation, continuous reduction reaction of poly-carbonyl, amino substitution reaction, and nonredox transesterification reaction, which are also an indispensable part of stereodivergent synthesis technology.

Preparation of 3-Hydroxy-5-methoxyheptanoic Acids

Similar to the stereodivergent reduction of 14a and its various derivatives mentioned above, Keatinge-Clay and colleagues used S-(2-acetamidoethyl) 3-oxopentane-thioate (43) as a substrate and performed sequential reduction of carbonyl groups (Scheme 18) using McyKR6 and TylKR2 from mycolactone PKS and tylosin PKS, respectively, to produce four stereoisomeric 3-hydroxy-5-methoxyheptanoic acids (46). In the scheme, the reductive products 44 were added a carbonyl group and a methyl protecting group after a series of chemical reaction steps. In addition, the protective effect of the methyl group was proved to be more beneficial for the progress of this reaction compared with other protective groups such as an acetyl group.

Preparation of 1-Phenylpropane-1,2-diols and 2-Amino-1-phenylpropan-1-ols

Chiral amino alcohols are a class of chemical structures with outstanding pharmaceutical activity, which are commonly found in hormones, antibiotics, alkaloids, adrenergic blockers, and other drugs. Among them, the enzyme-catalyzed stereodivergent synthesis of four stereoisomeric 2-amino-1-phenylpropan-1-ols (50) that could serve as synthetic intermediates or final drug products was pioneered by Corrado et al. Z/E isomeric prop-1-en-1-ylbenzenes (47) were first oxidized to 2-methyl-3-phenyloxiranes (48) by fused styrene monooxygenase (SMO) from Pseudomonas sp. VLB120 with oxygen (Scheme 19). The fused SMO used was designed to be co-expressed with formate dehydrogenase. Subsequently, 48 were hydrolyzed to four stereoisomeric 1-phenylpropan-1,2-diols (49) by epoxide hydrolases, Sp(S)-EH from Sphingomonas sp. HXN200, and St(R)-EH from Solanum tuberosum, respectively. It is worth mentioning that the above two-step reaction is capable of being performed in one pot. Building on the successful preparation of 49, Corrado et al went on to use multiple sources of alcohol dehydrogenases and transaminases to convert the four isomeric diol compounds into 2-amino-1-phenylpropan-1-ols (50, Scheme 20). In the scheme, Aa-ADH, Ls-ADH, Bs-BDHA (2,3-butanediol dehydrogenase), Brn(S)-ωTA, As(R)-ωTA, At(R)-ωTA, and Cv(S)-ωTA were obtained from Aromatoleum aromaticum, Leifsonia sp., B. subtilis BGSC1A1, B. megaterium SC6394, Arthrobacter sp., Aspergillus terreus, and Chromobacterium violaceum DSM30191, respectively. However, there is a ubiquitous 5 to 20% difference between the conversion and the actual yield of the desired isomer due to the multistep reaction proceeding and the existence of side reaction pathways.

Scheme 16 Stereodivergent synthesis of four stereoisomers of ethyl 2-phenylcyclopropane-1-carboxylate.

Scheme 17 Stereodivergent synthesis of four stereoisomers of three ethyl 2-substituted cyclopropane-1-carboxylates.
that give rise to multiple by-products in the reaction system. In addition, the preparation of diverse 50 from one 49 can also be achieved by combining different alcohol dehydrogenases and transaminases. For example, Ls-ADH and At(\(R\))-\(\omega\)TA could produce \((1R,2R)-50\) rather than \((1R,2S)-49\) with conversion, ee, and de >99%.

**Preparation of 1-Phenylethyl 2-Phenylpropanoates and Its Derivatives**

In asymmetric synthesis, lipases, for their ability to catalyze the hydrolysis of esters in aqueous media, are often used for the resolution of racemic intermediates. However, one lipase derived from Candida antarctica has been reported to be useful for transacylation of secondary alcohols in organic reagents. Based on this, Wu and colleagues successfully constructed four enzymes capable of catalyzing the preparation of four stereoisomeric 1-phenylethyl 2-phenylpropanoates (53a) using a strategy termed “focused rational iterative site-specific mutation” from wild-type C. antarctica lipase B (CALB, Scheme 21). This strategy aims to simplify traditional iterative saturation mutations with fewer but representative amino acids, with the hope of similar good result. Since the racemic substrate 51 used in the protocol cannot interconvert rapidly, conversion of the substrate is at most 50% in an ideal situation where only a single isomer is produced. Isopropyl ether proved to be the best choice for these wild-type and mutant lipases in a variety of organic solvents. In addition, various derivatives of 53a were shown to be produced by the same method. Among them, all four stereoisomers of 53b and 53c and three stereoisomers of 53d, 53e, and 53f can be obtained, respectively.

**Outlook and Conclusion**

Although the application of biocatalysis, especially enzyme catalysis, in asymmetric synthesis is gradually abundant and the related technologies are also gradually maturing, we still have many difficulties to overcome. Summarizing the above reactions, it is not difficult to find that our control over the chiral centers is confined within two-carbon bridges. This limitation is not just present in biocatalysis, but also being discovered in chiral organocatalysis and metal catalysis. Meanwhile, our control over the number of chiral centers is also usually limited to within two, and stereodivergent synthesis containing three and more chiral centers has rarely been reported. In addition, the number of reports on
stereodivergent synthesis catalyzed by biocatalysts only accounts for about one-tenth, which is much less than chiral organocatalysis and metal catalysis. All in all, whether extending the distance of chiral centers, increasing the number of chiral centers, or enriching the reaction substrate types can undoubtedly lead to further refinement of biocatalytic techniques while also improving for stereodivergent synthesis methods.

Here, we present some suggestions for the development of stereodivergent synthesis by biocatalysts based on hot topics that have been reported in recent years. First, deep optimization of directed evolution is needed. Traditional iterative saturation or degenerate codon mutations are more blind strategies for enzyme evolution that bring about an exponential increase in workload. Thus, the use of computer-aided mutation design is a better approach. Based on this, the development of new ancillary software or the optimization of existing algorithms that enable better structural modeling and outcome prediction will undoubtedly benefit the existing biocatalytic approaches. Second, it is also a general trend for the future to develop new enzymes or to mine the functions of known enzymes. Previously, most of the enzymes used in biocatalysis were wild-type enzymes existing in nature or mutant enzymes designed on them. However, the enzymes evolved by organisms to adapt to the environment are difficult to meet our catalytic needs for the synthesis of complex and diverse compounds, which suggests that we need to develop enzymes that do not exist in nature. Under such demand pitfalls described above, de novo protein design, a rational design based on energy function calculations, is precisely the third protein design approach—distinct from structure prediction and fixed backbone design. In addition, there were some valuable enzymes in the existing or extinct organisms, but these enzymes were gradually eliminated by the host in the process of evolution, which also suggests that we can excavate the ancestral sequences buried in history through calculation and derivation, to enrich the types of enzymes. Finally, the combined...
application of multiple methods or catalysts is similarly an effective way to solve the bottleneck of biocatalysis. Photoelectrocatalysis, have been successively reported in recent years, and this approach to selective transformations using visible light can combine the advantages of biocatalysis and photocatalysis, including new reactivity, high stereoselectivity, green synthesis, and high yields. In addition, applications related to the combination of biocatalysis with electrocatalysis, i.e., biophotocatalysis, have been successively reported in recent years.\textsuperscript{85,86}

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**Conflict of Interest**
The authors declare that they have no conflict of interest.

**References**
1. Ali I, Gaitonde VD, Aboul-Enein HY, Hussain A. Chiral separation of \(\beta\)-adrenergic blockers on CelluCoat column by HPLC. Talanta 2009;78(02):458–463
2. He R, Fan J, Tan Q, et al. Enantioselective determination of metconazole in multi matrices by high-performance liquid chromatography. Talanta 2018;178:980–986
3. Czerwenka C, Lämmerhofer M, Lindner W. Micro-HPLC and standard-size HPLC for the separation of peptide stereoisomers employing an ion-exchange principle. J Pharm Biomed Anal 2003;30(06):1789–1800
4. Harada N. HPLC separation of diastereomers: chiral molecular tools useful for the preparation of enantiopure compounds and simultaneous determination of their absolute configurations. Molecules 2016;21(10):1328
5. Beaufour M, Morin P, Ribet JP, Maurizot JC. HPLC quantitation of the four stereoisomers of benzoxathiepin derivatives with cellulose phenyl type chiral stationary phase and circular dichroism detection. J Pharm Biomed Anal 2006;41(02):544–548
6. Xiang DF, Bigley AN, Desormeaux E, Narindoshovii T, Rausch FM. Enzyme-catalyzed kinetic resolution of chiral precursors to anti-viral prodrugs. Biochemistry 2019;58(29):3204–3211
7. Du J, Chu W, Zhang M, Ma C, Feng W. A novel method for preparing Elgilustat through chiral resolution. Biog Med Chem Lett 2020;30(16):127209
8. Turner NJ. Enzyme catalysed deracemisation and dynamic kinetic resolution reactions. Curr Opin Chem Biol 2004;8(02):114–119
9. Yamaguchi S, Mosher HS, Pohland A. Reversal in stereoselectivity depending upon the age of a chiral lithium alkoxaluminohydride reducing agent. J Am Chem Soc 1972;94(26):9254–9255
10. Yamaguchi S, Mosher HS. Asymmetric reductions with chiral reagents from lithium aluminum hydride and \((+)-(2S,3R)-4\)-dimethylamino-3-methyl-1, 2-diphenyl-2-butanol. J Org Chem 1973;38(10):1870–1877
11. Zanoni G, Castronovo F, Franzini M, Vidari G, Gianni E. Toggling enantioselective catalysis—a promising paradigm in the development of more efficient and versatile enantioselective synthetic methodologies. Chem Soc Rev 2003;32(03):115–129
12. Tanaka T, Hayashi M. New approach for complete reversal of enantioselectivity using a single chiral source. Synthesis 2008;2008(21):3361–3376
13. Abrendt KA, Borths CJ, Macmillan DWC. New strategies for organic catalysis: the first highly enantioselective organocatalytic Diels—Alder reaction. J Am Chem Soc 2000;31(31):4243–4244
14. List B, Lerner RA, Barbas CF. Proline-catalyzed direct asymmetric aldol reactions. J Am Chem Soc 2000;122(10):2395–2396
15. Krautwald S, Carreira EM. Stereodivergence in asymmetric catalysis. J Am Chem Soc 2017;139(16):5627–5639
16. Gihani MT, Williams JM. Dynamic kinetic resolution. Curr Opin Chem Biol 1999;3(01):11–15
17. Gustafson JL, Lim D, Miller SJ. Dynamic kinetic resolution of biaryl atropisomers via peptide-catalyzed asymmetric bromination. Science 2010;328(5983):1251–1255
18. Wsöl V, Skálová L, Szoťákóvá B. Chiral inversion of drugs: coincidence or principle? Curr Drug Metab 2004;5(06):517–533
19. Đuriš A, Wiesenganger T, Moravčíková D, et al. Expedient and practical synthesis of CERT-dependent ceramic trafficking inhibitor HPA-12 and its analogues. Org Lett 2011;13(07):1642–1645
20. Boesten WH, Seerden JP, de Lange B, et al. Asymmetric sterrer synthesis of alpha-amino acids via a crystallization-induced asymmetric transformation using (R)-phenylglycine amide as a chiral auxiliary. Org Lett 2001;3(08):1121–1124
21. Park H, Nandhakumar R, Hong J, Ham S, Chin J, Kim KM. Stereocconversion of amino acids and peptides in uryl-pendant binol Schiff bases. Chemistry 2008;14(32):9935–9942
22. Lazzarotto M, Hammerer L, Hetmann M, et al. Chemoenzymatic total synthesis of deoxy-, epi-, and podophyllotoxin and a biocatalytic kinetic resolution of dibenzylbutyrolactones. Angew Chem Int Ed Engl 2019;58(24):8226–8230
23. Castro E, Soraci A, Fogel F, Tapia O. Chiral inversion of R(-) fenoprofen and ketoprofen enantiomers in cats. J Vet Pharmacol Ther 2000;23(05):265–271
24. Zhang R, Wang L, Xu Y, et al. In situ expression of (R)-carbonyl reductase rebalancing an asymmetric pathway improves stereocconversion efficiency of racemic mixture to (S)-phenyl-1,2-ethanediol in Candida parapsilosis CECTC M203011. Microb Cell Fact 2016;15(01):143
25. Ogasawara Y, Dairi T. Pepidine epimerization machineries found in microorganisms. Front Microbiol 2018;9:156
26. Samuel J, Tanner ME. Mechanistic aspects of enzymatic carbohydrate epimerization. Nat Prod Rep 2002;19(03):261–277
27. Brenna E, Fuganti C, Gatti FG, Serra S. Biocatalytic methods for the synthesis of enantiomerically pure biodegradable epoxides. Curr Opin Chem Biol 2011;15(07):4036–4072
28. Bicas JL, Dionísio AP, Pastore GM. Bio-oxidation of terpenes: an approach for the flavor industry. Chem Rev 2009;109(09):4518–4531
29. Guo J, Zhang R, Ouyang J, et al. Stereodivergent synthesis of carvone and dihydrocarvone through ketoreductases/ene-reduction catalyzed asymmetric reduction. ChemCatChem 2018;10(23):5496–5504
30. Mori K. Absolute configuration of \((+)-(2R,3R)-4\)-methylheptan-3-ol, a pheromone of the smaller elm bark beetle, as determined by the synthesis of its \((3R,4R)-(+)\)- and \((3S,4R)-(−)\)-isomers. Tetrahedron 1977;33(07):289–294
31. Blight MM, Wadhams L, Wenham M. The stereoisomeric composition of the 4-ethyl-3-heptanol produced by Scolytus scolytus and the preparation and biological activity of the four synthetic stereoisomers. Insect Biochem 1979;9(05):525–533
32. Ben-Yehuda S, Tolasch T, Francke W, et al. Aggregation pheromone of the almond bark beetle Scolytus amygdali (Coleoptera: Scolytidae). IOBC WPRS Bull 2002;25:1–12
33. Zada A, Ben-Yehuda S, Dunkelblum E, Harel M, Assael F, Mendel Z. Synthesis and biological activity of the four stereoisomers of 4-ethyl-3-heptanol: main component of the aggregation pheromone of Scolytus amygdali. J Chem Ecol 2004;30(03):631–641
34. Attygalle AB, Vostrowsky O, Bestmann HJ, Steghaus-Kovac S, Maschwitz U. (3R,4S)-Methyl-3-heptanol, the trail pheromone.
of the ant *Leptogenys diminuta*. Naturwissenschaften 1988;75 (06):315–317

35 Nakagawa N, Mori K. Pheromone synthesis. Part 68. Synthesis of (3S, 4S)-4-methyl-3-heptanol and its (3S, 4R)-isomer employing asymmetric epoxidation coupled with regioselective cleavage of epoxides with trimethylaluminum. Agric Biol Chem 1984;48(10): 2505–2510

36 Uenius CR, Sandell J, Orrenius C. Enantioselective preparation of the stereoisomers of 4-methylheptan-3-ol using *Candida antarctica* lipase B. Collect Czech Chem Commun 1998;63(04): 525–533

37 Brenna E, Crotti M, Gatti FG, Monti D, Parmeggiani F, Pugliese A. One-pot multi-enzymatic synthesis of the four stereoisomers of 4-methylheptan-3-ol. Molecules 2017;22(10):1591

38 Althoff EA, Wang L, Jiang L, et al. Robust design and optimization of retroaldol enzymes. Protein Sci 2012;21(05):717–726

39 Giger L, Caner S, Obexer R, et al. Evolution of a designed retroaldolase leads to complete active site remodeling. Nat Chem Biol 2013;9(08):494–498

40 Garrabou X, Macdonald DS, Wicky BIM, Hilvert D. Stereodivergent evolution of artificial enzymes for the michael reaction. Angew Chem Int Ed Engl 2018;57(19):5288–5291

41 Miller SP, Zhong YL, Liu Z, et al. Practical and cost-effective manufacturing route for the synthesis of a β-lactamase inhibitor. Org Lett 2014;16(01):174–177

42 Balkovec JM, Hughes DI, Masurekar PS, Sable CA, Schwartz RE, Singh SB. Discovery and development of first in class antifungal caspofungin (CANCIDAS®) – a case study. Nat Prod Rep 2014;31(01):15–34

43 Harper S, McCaulley JA, Rudd MT, et al. Discovery of MK-5172, a macrocyclic hepatitis C virus NS3/4a protease inhibitor. ACS Med Chem Lett 2012;3(04):332–336

44 Prier CK, Lo MMC, Li H, Yasuda N. Stereodivergent synthesis of 3-hydroxyprolines and 3-hydroxypropeptidic acids via ketoreductase-catalyzed dynamic kinetic reduction. Adv Synth Catal 2019;361(22):5140–5143

45 Zhu Y, Burgess K. Filling gaps in asymmetric hydrogenation methods for acyclic stereocontrol: application to chirons for polyketide-derived natural products. Acc Chem Res 2012;45(10):1623–1636

46 Schetter B, Mahrwald R. Modern aldol methods for the total synthesis of polyketides. Angew Chem Int Ed 2006;45(45): 7506–7525

47 Hertweck C. The biosynthetic logic of polyketide diversity. Angew Chem Int Ed Engl 2009;48(26):4688–4716

48 Bailey CB, Pasman ME, Keatinge-Clay AT. Substrate structure-activity relationships guide rational engineering of modular polyketide synthase ketoreductases. Chem Commun (Camb) 2016;52(04):792–795

49 Robles ML. Ketoreductases as Biocatalysts in the Synthesis of Chiral Diketides [M.D. dissertation]. Austin, TX: Texas University; 2020

50 Degnan AP, Huang H, Snyder LB, Yang F, Gillman KW, Parker MF, Ozaaxolidinones as modulators of mGluR5. U.S. Patent 8691821. April, 2014

51 Yang F, Snyder LB, Balakrishnan A, et al. Discovery and preclinical evaluation of BM5-955829, a potent positive allosteric modulator of mGluR5. ACS Med Chem Lett 2016;7(03):289–293

52 Hanson RL, Guo Z, González-Bobes F, Fenster MD, Gossowami A. Enzymatic reduction of α-substituted ketones with concomitant dynamic kinetic resolution. J Mol Catal, B Enzym 2016;133:20–26

53 Huerta FF, Minidis ABE, Bäckvall JE. Racemisation in asymmetric synthesis. Dynamic kinetic resolution and related processes in enzyme and metal catalysis. Chem Soc Rev 2001;30(06):321–331

54 DeGovit JS, Loh YY, Kautzky JA, et al. Static to inducibly dynamic stereocontrol: the convergent use of racemic β-substituted ketones. Science 2020;369(6507):1113–1118

55 Kleemann A, Engels J, Kutscher B, Reichert D. Pharmaceutical Substances; Syntheses, Patents, Applications. 5th ed. Stuttgart: Thieme; 2008

56 Bode SE, Wolberg M, Mueller M. Stereoselective synthesis of 1,3-diols. Synthesis 2006;2006(04):557–588

57 Binder JT, Kirsch SF. Iterative approach to polyketide-type structures: stereoselective synthesis of 1,3-polyols utilizing the catalytic asymmetric Overman esterification. Chem Commun (Camb) 2007;(40):4164–4166

58 Börner A. Phosphorous Ligands in Asymmetric Catalysis—Syntheses and Applications. 1st ed. Weinheim: Wiley-VCH; 2008

59 Baer K, Krausser M, Burda E, Hummel W, Berkesel A, Gröger H. Sequential and modular synthesis of chiral 1,3-diols with two stereogenic centers: access to all four stereoisomers by combination of organo- and biocatalysis. Angew Chem Int Ed Engl 2009;48(45):9355–9358

60 Sonøike S, Itakura T, Kitamura M, Aoki S. One-pot chemoenzymatic synthesis of chiral 1,3-diols using an enantioselective aldol reaction with chiral Zn2+ complex catalysts and enzymatic reduction using oxireductases with cofactor regeneration. Chem Asian J 2012;7(01):64–74

61 Ma S. Electrophilic addition and cyclization reactions of alkenes. Acc Chem Res 2009;42(10):1679–1688

62 Gilmore K, Alabugin IV. Cyclizations of alkynes: revisiting Baldwin’s rules for ring closure. Chem Rev 2011;111(11):6513–6556

63 Saha P, Saikia AK. Ene cyclization reaction in heterocycle synthesis. Org Biomol Chem 2018;16(16):3820–3840

64 Tannert R, Milroy LG, Ellinger B, Hu TS, Arndt HD, Waldmann H. Synthesis and structure-activity correlation of natural-product inspired cyclodepsipeptides stabilizing F-actin. J Am Chem Soc 2010;122(09):3063–3077

65 Tannert R, Hu TS, Arndt HD, Waldmann H. Solid-phase based total synthesis of Jasplakinolide by ring-closing metathesis. Chem Commun (Camb) 2009;(12):1493–1495

66 Fürstner A, Bouchez LC, Morency L, et al. Total syntheses of amphidinolides B1, B4, G1, H1 and structure revision of amphidinolide H2. Chemistry 2009;15(16):3983–4010

67 Classen T, Korpak M, Schöbel M, Pietruszka J. Stereoselective enzyme cascades: an efficient synthesis of chiral γ-butyrolactones. ACS Catal 2014;4(05):1331–1331

68 Simon RC, Busto E, Schrittewieser JH, et al. Stereoselective synthesis of γ-hydroxynorvaline through combination of organo- and biocatalysis. Chem Commun (Camb) 2014;50(99):15669–15672

69 Besser P, Veschambre H. Chemoenzymatic synthesis of “α-bichiral” synthons. Application to the preparation of chiral epoxides. Tetrahedron Asymmetry 1993;4(06):1271–1285

70 Coelho PS, Brustad EM, Kannan A, Arnold FH. Olefin cyclopropanation via carbene transfer catalyzed by engineered cytochrome P450 enzymes. Science 2013;339(6117):307–310

71 Gober JG, Rydeen AE, Gibson-O’Grady EJ, Leuthaeuser JB, Petrow JS, Brustad EM. Mutating a highly conserved residue in diverse cytochrome P450s facilitates diastereoselective olefin cyclopropanation. ChemBioChem 2016;17(05):394–397

72 Knight AM, Kan SBJ, Lewis RD, Brandenberg OF, Chen K, Arnold FH. Diverse engineered heme proteins enable stereodivergent cyclopropanation of unactivated alkenes. ACS Cent Sci 2018;4(03):372–377

73 Zhang Z, Cepeda AJ, Robles ML, et al. General chemoenzymatic route to two-stereocenter triketides employing assembly line ketoreductases. Chem Commun (Camb) 2019;56(01):157–160

74 Breuer M, Ditrich K, Habicter T, et al. Industrial methods for the production of optically active intermediates. Angew Chem Int Ed 2004;43(07):788–824

75 Sehl T, Maugeri Z, Rother D. Multi-step synthesis strategies towards 1, 2-amino alcohols with special emphasis on phenylpropanolamines. J Mol Catal, B Enzym 2015;114:65–71
Gupta P, Mahajan N. Biocatalytic approaches towards the stereo-selective synthesis of vicinal amino alcohols. New J Chem 2018;42 (15):12296–12327

Corrado ML, Knaus T, Mutti FG. Regio- and stereoselective multi-enzymatic aminohydroxylation of β-methylstyrene using dioxygen, ammonia and formate. Green Chem 2019;21(23):6246–6251

Corrado ML, Knaus T, Mutti FG. High regio-and stereoselective multi-enzymatic synthesis of all phenylpropanolamine stereoisomers from β-methylstyrene. ChemBioChem 2021;22(13):2345–2350

Serebryakov EP. Stereodivergent synthesis of chiral low-molecular bioregulators using readily available lipases. Russ Chem Bull 2001;50(11):1984–1997

Wikmark Y, Svedendahl Humble M, Bäckvall JE. Combinatorial library based engineering of Candida antarctica lipase A for enantioselective transacylation of sec-alcohols in organic solvent. Angew Chem Int Ed Engl 2015;54(14):4284–4288

Xu J, Cen Y, Singh W, et al. Stereodivergent protein engineering of a lipase to access all possible stereoisomers of chiral esters with two stereocenters. J Am Chem Soc 2019;141(19):7934–7945

Huang PS, Boyken SE, Baker D. The coming of age of de novo protein design. Nature 2016;537(7620):320–327

Spence MA, Kaczmarski JA, Saunders JW, Jackson CJ. Ancestral sequence reconstruction for protein engineers. Curr Opin Struct Biol 2021;69:131–141

Harrison W, Huang X, Zhao H. Photobiocatalysis for abiological transformations. Acc Chem Res 2022;55(08):1087–1096

Wan L, Heath RS, Megarity CF, et al. Exploiting bidirectional electrocatalysis by a nanoconfined enzyme cascade to drive and control enantioselective reactions. ACS Catal 2021;11(11):6526–6533

Guo K, Qian K, Zhang S, Kong J, Yu C, Liu B. Bio-electrocatalysis of NADH and ethanol based on graphene sheets modified electrodes. Talanta 2011;85(02):1174–1179