Industrial Application of 2-Oxoglutarate-Dependent Oxygenases

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Abstract: C–H functionalization is a chemically challenging but highly desirable transformation. 2-oxoglutarate-dependent oxygenases (2OGXs) are remarkably versatile biocatalysts for the activation of C–H bonds. In nature, they have been shown to accept both small and large molecules carrying out a plethora of reactions, including hydroxylations, demethylations, ring formations, rearrangements, desaturations, and halogenations, making them promising candidates for industrial manufacture. In this review, we describe the current status of 2OGX use in biocatalytic applications concentrating on 2OGX-catalyzed oxyfunctionalization of amino acids and synthesis of antibiotics. Looking forward, continued bioinformatic sourcing will help identify additional, practical useful members of this intriguing enzyme family, while enzyme engineering will pave the way to enhance 2OGX reactivity for non-native substrates.

Keywords: 2-oxoglutarate-dependent oxygenases; industrial biocatalysis; hydroxylation; alpha-ketoglutarate; amino acid modification; antibiotic synthesis

1. Introduction

Alkanes are important, easily obtainable building blocks for the chemical industry. They stem from resources, such as gas and crude oil, but are also found in algae, bacteria, and plants and serve as precursors for a wide range of agrochemicals and pharmaceuticals. However, the activation of alkanes remains challenging in an industrial context due to the fact that carbon–hydrogen bonds are relatively inert. Additionally, when employing substrates with multiple C–H bonds, obtaining satisfactory chemo-, regio- and stereoselectivity remains problematic, while, at the same time, the oxidation state of the final product can be difficult to control using conventional chemical catalysis [1].

Nature, in contrast, has perfected oxidation reactions, which are an integral part of the biosynthesis of a diverse set of compounds, especially of secondary metabolites [2,3]. Nature’s repertoire of oxidative biocatalysts can be mechanistically classified into two categories: dehydrogenation and oxyfunctionalization catalysts [4]. Dehydrogenation catalysts abstract a hydrogen atom via an acceptor molecule, such as a nicotinamide or flavin cofactor, but do not involve an active oxygen intermediate. Oxyfunctionalization catalysts, on the other hand, reductively activate molecular oxygen or rely on hydrogen peroxide for subsequent electrophilic substitution into the starting material. Synthetically, oxyfunctionalization catalysts are more intriguing, as they can introduce new functional groups by catalyzing the selective insertion of oxygen into C–H, C–C, and C=C bonds [4]. Harnessing the power of precisely placed cofactors in the enzyme active site, C–H activating biocatalysts derivatize their substrates regio- and stereospecifically at ambient temperature and pressure in aqueous solutions, thus eliminating the challenges typically associated with chemical approaches.
Today, biocatalytic C–H functionalization is already of substantial industrial importance for chemical production processes ranging from the milligram production of drug metabolites [5–7] to the biosynthetic manufacture of complex biomolecules in microorganisms [8–10]. In these biooxidation applications, the most prominently used class of enzymes are the P450 monooxygenases (P450s). Due to their ability to perform challenging regioselective and stereoselective activations of remote, unactivated C–H, P450s have a long history of use in the pharmaceutical industry [7,11], a prominent example being the use of fungal P450s for the production of steroids [12]. In addition, P450s are exploited for the late-stage functionalization of added-value molecules [13,14] and in this context, an engineered P450 enzyme has been recently employed in the total synthesis of nigelladine A, a norditerpenoid [15]. Thanks to significant advances in (semi-) rational protein engineering and directed evolution (2018 Nobel Prize in Chemistry), P450 variants performing new-to-nature C–H transformations, such as C–N and C–C bond-forming processes, have been developed in the last years [16]. However, despite the impressive successes in P450 engineering the preparative use of P450 enzymes is still limited to selected examples because enzyme performance can be affected by inefficient electron transfer, uncoupling, and low stability and activity [17,18].

Another heme-dependent enzyme family that has been associated with great potential for C–H activation are the so-called ‘unspecific peroxygenases’ (UPOs), which use H₂O₂ instead of O₂ as the oxidant to drive numerous oxygenation reactions on a broad range of substrates without the need for an electron delivery system [19]. These enzymes have been described to carry out reactions such as alkane hydroxylations, aromatic hydroxylations, and epoxidations, as well as catalyzing oxidative decarboxylations and the Achmatowicz reaction [20].

Besides the well-studied heme-dependent oxygenases, non-heme Fe(II)-dependent oxygenases, in particular 2-oxoglutarate-dependent oxygenases (2OGXs), are an interesting alternative to carry out desirable C–H activation chemistry [2,21]. 2OGXs constitute a diverse protein superfamily with members from bacteria, fungi, plants, and vertebrates. Their biological function includes collagen biosynthesis, plant and animal development, transcriptional regulation, nucleic acid modification/repair, fatty acid metabolism, and secondary metabolite biosynthesis [22]. In plants and animals, 2OGXs catalyze reactions during the biosynthesis of most of the major classes of secondary metabolites, including peptides, alkaloids, terpenoids, carbohydrates, and mixed-origin molecules [23]. In microbes, 2OGXs also have additional roles in sulfur and phosphate metabolism [2]. Catalyzed reactions cover a wide range, including hydroxylation, dealkylation, desaturation, epoxidation, epimerization, cyclization, and halogenations [2,24] (Figure 1). Industrially, this class of enzymes has been used mainly for the derivatization of amino acids and in the biosynthesis of antibiotics, while other biotransformation processes have been less explored to date. However, due to their chemical versatility, the biocatalytic use of 2OGX enzymes is continuously explored in industry [25] and academia [21,26,27] and is considered to have high potential for sustainable, robust, and scalable chemical synthesis [28].
Structurally, 2OGX enzymes share distinctive features. Crystallographic studies show that the enzymes contain a double-stranded β-helix core fold (DSBH, also known as a jelly roll, cupin, or jumonji C fold) composed of eight antiparallel β-strands forming a β-sandwich structure, which supports two His residues and (with exception of the halogenases) a carboxylate Glu or Asp residue [43]. This motif, also called the 2-His-1-carboxylate facial triad, is responsible for binding the Fe(II) center, whereas the other face of the metal coordinates three water molecules [44]. Aik et al. proposed that beyond housing the active site, the β-strands also assist in providing selectivity for binding the primary substrates [45].

In the consensus view of the 2OGX reaction mechanism for hydroxylation activity (Figure 2), the most well-studied reactivity of 2OGX enzymes, two water molecules are displaced upon 2-oxoglutarate (2OG) chelation. 2OG binds to the Fe center in a bidentate configuration, in which the keto group is oriented opposite the Asp/Glu and its carboxylate opposite one of the His residues [46]. The binding of the primary substrate proximal to the metal center leads to the displacement of the remaining water molecule triggering the binding of dioxygen. In this way, the formation of the Fe(III)-superoxo species
takes place, which initiates oxidative decarboxylation of 2OG to form succinate, CO$_2$, and an Fe(IV) oxo species, also known as the ferryl intermediate. This ferryl intermediate abstracts a hydrogen from an unactivated C(sp$^3$)–H bond in the primary substrate yielding Fe(III)-OH and the substrate radical. Hydroxyl radical rebound yields the hydroxylated product, which dissociates to complete the catalytic cycle [24,47,48]. In cases where the carbon undergoing hydroxylation is bound to a heteroatom, the initially formed product may spontaneously react further [24].

Notably, in 2OGX catalysis the electrons required for oxyfunctionalization of the unactivated C(sp$^3$)–H bond stem from the cheap co-factor 2-oxoglutarate. In biocatalytic reactions, 2-oxoglutarate can be added from external sources; however, microbial metabolism may also provide this redox cofactor. The central carbon metabolism fuels 2OGX enzymes with 2-oxoglutarate and in turn assimilates and recycles the coproduct of the enzymatic reaction, namely succinate, through the tricarboxylic acid (TCA) cycle. Thus, metabolic engineering of microbial hosts to increase 2-oxoglutarate levels and force flux through the 2OGX biocatalyst is an attractive strategy to generate optimized cell factories. Using this approach, Theodosiou et al. and Smirnov et al. engineered the TCA cycle in Escherichia coli by deleting succinate-producing enzymes, thus optimizing 2OG flux for the production of trans-4-hydroxy-L-proline and trans-4-hydroxy-L-isoleucine, respectively [49,50]. Beyond their use as chassis strains for amino acid hydroxylation, these constructs also principally enable the selection of active 2OGX variants as the sole producers of succinate [49].

In this review, we will highlight the biocatalytic utility of 2OGX enzymes by outlining the applications of 2OGXs in the hydroxylation of amino acid derivatives and of antibiotics (for a comprehensive overview of halogenation reactions please refer to [51,52]). Future implications of the rapidly increasing number of
available genomes, as well as the increasing sophistication of enzyme engineering approaches, will also be discussed.

2. Biocatalytic Applications of 2-oxoglutarate-Dependent Oxygenases

Amino acid hydroxylation is the most common industrial application of 2-oxoglutarate-dependent oxygenases to date (Figure 3). Several filed patents attest to the enzyme family’s potential for the biocatalytic production of derivatized amino acids, which serve as building blocks for the chemical industry [25,53–59]. Beyond amino acid hydroxylation, 2-oxoglutarate-dependent oxygenases are explored for the synthesis of antibiotics, in the chemo-enzymatic total synthesis of complex natural products, and as part of enzymatic cascade reactions for the production of valuable building blocks.

**Figure 3.** Overview of selected amino acid hydroxylations by 2-oxoglutarate-dependent enzymes. The enzymes typically accept free-standing amino acids as a substrate. In cases where the substrate is part of a more complex molecule, the enzyme name is marked with an asterisk.

2.1. Aliphatic Amino Acid Oxygenases

2.1.1. L-isoleucine 4-hydroxylase or L-isoleucine Dihydroxygenase (IDO)

L-isoleucine 4-hydroxylase from *Bacillus thuringiensis* converts L-isoleucine stereoselectively into (2S,3R,4S)-4-hydroxyisoleucine (4-HIL) [60], which was isolated for the first time from seeds of fenugreek (*Trigonella foenum-graceum*) [61]. The IDO-catalyzed conversion of L-isoleucine into 4-HIL (Figure 4) is patented by the Japanese company Ajinomoto Co. Inc. [58] and the Chinese company Henan Julong Bio-Engineering Co. Ltd. [62]. Studies on the properties of the hydroxylation product associate 4-HIL with anti-diabetic effects [63] and show that it is an effective drug for body weight control, glycemia, and insulinemia [64]. In *Bacillus thuringiensis*, 4-HIL is further converted into (2S,3R)-2-amino-3-methyl-4-ketopentanoic acid, a vitamin B12 antimetabolite with antibiotic
properties [65]. To boost 4-HIL production titers, an engineered E. coli strain lacking the isocitrate lyase and isocitrate dehydrogenase kinase/phosphatase was designed channeling endogenously produced co-substrate 2-oxoglutarate toward the 2OGX [50]. Using this strain, 4-HIL yields were found to be 82%, employing 100 mmol L-isoleucine as a substrate [50]. Recently, Zhang et al. enhanced the activity of IDO by introducing four mutations, which were identified by error-prone PCR [66]. Using this approach, the kcat/Km value of IDO could be increased 1.5-fold to reach approximately 33 L/mmol/min, and the 4-HIL production doubled in a small-scale biocatalysis reaction [66]. In a study to investigate IDO’s substrate acceptance beyond L-isoleucine, recombinantly expressed IDO was successfully tested for the hydroxylation of the aliphatic amino acids L-leucin, L-norleucine, and L-norvaline and for the (S)-sulfoxidation of L-methionine and L-ethionine [67]. Exploiting this substrate promiscuity, the Kourist group established a stereoselective three-enzyme cascade for the synthesis of diasteromerically pure γ-oxyfunctionalized α-amino acids by combining IDO with an N-acylamino acid racemase (NAAAR) and an L-selective aminocarboxylase from Geobacillus thermoglucosidasius. In a one-pot strategy, 5 mM racemic N-acetylmethionine was quantitatively converted into L-methionine-(S)-sulfoxide with a 97% yield and 95% diastereomeric excess [68].

![Figure 4](image-url)

**Figure 4.** Enzymatic L-isoleucine hydroxylation. Right: L-isoleucine dihydroxygenase (IDO)-catalyzed derivatization of L-isoleucine to form (2S,3R,4S)-4-hydroxyisoleucine; Left: Subsequent reaction of L-isoleucine with HillA and HillB to generate the dehydroxylated 4,4′-dihydroxyisoleucine.

### 2.1.2. L-isoleucine-4′-dioxygenase

In contrast to the C4 selectivity of IDO, the 2-oxoglutarate-dependent oxygenase HillA from Pantoea ananatis hydroxylates L-isoleucine on the C3 methyl group to produce 2-amino-3-(hydroxymethyl)pentanoic acid or 4′-hydroxyisoleucine [69,70]. In the native organism a second 2OGX, named HillB, further processes 4′-hydroxyisoleucine into dihydroxylated 4,4′-dihydroxyisoleucine (Figure 4). In another study, Smirnov et al. investigated the substrate scope of HillA and showed promiscuous activity for the conversion of L-valine into L-4-hydroxyvaline [70].

#### 2.1.3. L-leucine 5-hydroxylase or L-leucine Dioxygenase from Cyanobacteria

To date, three L-leucine dioxygenases from various cyanobacteria have been investigated. The first enzyme, LdoA, was isolated from Nostoc punctiforme PCC73102 [71]. The characterization of its substrate acceptance highlighted the capability of this enzyme to hydroxylate L-leucine into (2S,4S)-5-hydroxyxyleucine and L-norleucine into (2S)-5-hydroxyxylornelueine and to carry out sulfoxidation of L-methionine and L-ethionine [71]. Recently, oxygenase NpLDO from Nostoc piscinale possessing 72% sequence identity to LdoA [72] and AvLDO from Anabaena variabilis [73] were analyzed. While the studies highlighted that both enzymes exhibit a similar substrate spectrum to LdoA, e.g., hydroxylation of L-isoleucine into (2S,4S)-5-hydroxyxyleucine and sulfoxidation of L-methionine, AvLDO shows a differing enantiopreference and forms L-methionine-(R)-sulfoxide in contrast to the (S)-enantiomer formed with L-isoleucine 4-hydroxylase (IDO).

#### 2.1.4. L-leucine 5-hydroxylase from Streptomyces

Manzacidins A–C, a family of bromopyrrole alkaloids with suspected biological activities, were first isolated from the Okinawan sponge Hymeniacidon sp. by Kobayashi et al. in 1991 [74,75]. Manzacidins possess a unique structure consisting of an ester-linked bromopyrrolecarboxylic acid
and a 3,4,5,6-tetrahydropropyrimidine ring in which one of the amino groups is attached to the C6 quaternary carbon center [75]. Several strategies for the total synthesis of manzacidin A and C were developed in the early years of the 21st century, generating mg amounts of the final product [75]. In contrast to the purely chemical approach, Renata and co-workers investigated a chemo-enzymatic route to produce manzacidin C, harnessing the 2-oxoglutarate-dependent leucine 5-hydroxylase GriE from *Streptomyces* sp. DSM40835 [76]. GriE has been implicated in the biosynthesis of the anti-tuberculosis molecules griselimycins found in *Streptomyces* sp. [77] by converting L-leucine into (2S,4R)-5-hydroxyleucine [78]. Investigations of GriE’s substrate scope demonstrated the enzyme’s ability to hydroxylate at least 13 amino acid derivatives with useful turnovers. While substitutions at the γ position of the amino acid analogs were generally accepted, the enzyme proved to be sensitive to substitution at the β position [76]. For the total chemo-enzymatic synthesis of manzacidin C (Figure 5), photocatalytically produced azidoleucine (134 mg) was selectively δ-hydroxylated with 95% conversion using purified enzyme. Next, three additional chemical steps yielded the final product manzacidin C with complete stereocontrol shortening the known chemical routes substantially [76]. In an alternative set-up, the same group highlighted the possibility of generating manzacidin C via the gram scale hydroxylation of L-leucine by whole cell biocatalysis. The total synthesis consisting of nine steps led to the desired product in 11% total yield from L-leucine [79].

![Figure 5. Total chemo-enzymatic synthesis of manzacidin C and cavinafungin B.](image)

In elevated concentrations, GriE can convert L-leucine into the corresponding imine by double oxidation at C5. The resulting imine can be reduced to (2S,4R)-4-methylproline [76], an important building block for the synthesis of depsipeptide antibiotics or the antifungal agent cavinafungin B [80]. Cavinafungin B, an aldehyde lipopeptide isolated from *Colispora cavincola* in 2015 [81], is a close analogue to cavinafungin A, which was recently shown to possess antiviral properties against the Dengue virus and the Zika virus [82]. The total synthesis of cavinafungin B (37% final yield) was carried out via a 10-step Fmoc-based solid-phase peptide synthesis (SPPS) using chemo-enzymatically generated (2S,4R)-4-methylproline as a key building block (Figure 5) [80].

2.1.5. N-Succinyl L-leucine 3-hydroxylase

The 2OGX SadA from *Burkholderia ambifaria* AMMD has a unique amino acid sequence possessing no close homologue within the class of amino acid hydroxylases. SadA catalyzes the reaction of...
N-succinyl-L-leucine into N-succinyl-L-threo-β-hydroxyleucine with 99% diastereoselectivity [83]. No activity could be detected for the conversion of L-leucine or N-succinyl-D-leucine, but N-formyl, N-acetyl, and N-carbamyl-L-leucine, as well as N-succinyl-L-valine, N-succinyl-L-isoleucine and N-succinyl-L-phenylalanine were accepted as substrates [84,85]. Furthermore, the enzyme was shown to have very low activity for the hydroxylation of N-succinyl-L-3,4-dimethoxyphenylalanine (NSDOPA) to yield N-succinyl-L-threo-3,4-dimethoxy-phenylserine (NSDOPS), a precursor of the psychoactive drug L-threo-3,4-dihydroxyphenylserine (Droxidopa) [86]. Using structure-based protein engineering, two mutations could be identified (G79A and F261R/W), which enhanced the activity for conversion of NSDOPA six-fold [86].

Notably, SadA was also found to have application in an enzymatic cascade reaction for the production of β-hydroxy amino acids. In this study, SadA was coupled with the N-succinyl L-amino acid desuccinylase (LasA) in a sequential mode [87]. First, 50 mM N-succinyl-L-leucine was converted by SadA into N-succinyl L-threo- β-hydroxyleucine in 24 h (97 % conversion) before incubation with LasA yielded L-threo- β-hydroxyleucine (93% conversion, >99% diastereomeric excess) [87].

2.2. Dioxygenase for Auxinoxidation and Fumitremorgin Oxygenases

Tryptophan or indole-derived natural products are common in nature, and the literature provides evidence that 2-oxoglutarate-dependent enzymes are involved in their biosynthesis. In plants, for example, the dioxygenase for auxinoxidation (DAO) from Arabidopsis hydroxylates the natural product auxin indole-3-acetic acid into the inactive 2-oxoindole-3-acetic acid [33–35], while the microbial FtmOx1 from Aspergillus fumigatus catalyzes the conversion of fumitremorgin B into the mycotoxin verruculogen. FtmOx1 operates by inserting an endoperoxide bond between two prenyl moieties [41,42]. This enzymatic activity is dependent on a specific residue, Tyr 244, which has been identified by mechanistic and structural studies [88,89]. However, despite their interesting activities, these enzymes have not yet featured in biocatalytic applications.

2.3. L-proline Hydroxylases

In nature, hydroxyprolines are the building blocks of small molecules, such as actinomycin 1 or etamycin, but also occur as part of proteins. A class of 2-oxoglutarate-dependent oxygenases named proline hydroxylases (PHs) can catalyze the conversion of L-proline either into 3-hydroxy-L-proline or 4-hydroxy-L-proline [51]. The first described PH was isolated from Streptomyces griseoviridus P8648 (SgP4H) in 1994. It selectively epoxidizes 3,4-dehydro-L-proline, yielding trans-3,4-epoxy-L-proline [90]. Since then, a number of additional PHs have been discovered and characterized, and today, all four monohydroxy-isomers of L-proline can be produced enzymatically (Table 1). However, it should be noted that trans-3-hydroxy-L-proline is only formed as a side product by the enzymes HtyE and GloF, two enzymes, which are known to preferentially produce trans-4-hydroxyproline [91].

| Product                  | Name                  | Source Organism          |
|--------------------------|-----------------------|--------------------------|
| cis-3-hydroxy-L-proline  | Cis-P3H1 (type I)     | Streptomyces sp. Th1 [92,93] |
|                          | Cis-P3H2 (type II)    | Streptomyces sp. Th1 [94] |
|                          | GetF                  | Streptomyces sp. [95]    |
|                          | PiFa                  | Frankia alni [95]        |
| cis-4-hydroxy-L-proline  | SmP4H                 | Sinorhizobium meliloti [96,97] |
|                          | MIP4H                 | Mesorhizobium loti [96]  |
|                          | SrPH                  | Streptosporangium roseum [98] |
|                          | CaPH                  | Catenulispora acidiphila [98] |
Table 1. List of proline hydroxylases classified according to their substrate preference (not exhaustive).

| Product                      | Name     | Source Organism                          |
|------------------------------|----------|------------------------------------------|
| trans-4-hydroxy-L-proline    | AlP4H    | Alteromonas mediterranea [29]            |
|                              | MiP4H    | Micromonospora sp. CNB394 [29]           |
|                              | ScP4H    | Sorangium cellulosum [29]                |
|                              | UbP4H    | Uncultured bacterium esnapd13 [99]       |
|                              | DaP4H    | Dactylosporangium sp. strain RH1 [100,101] |
|                              | HtyE     | Aspergillus pachycristatus [91] 2        |
|                              | GloF     | Glarea lozoyensis [91,102,103] 2         |
|                              | P4HP     | Pseudomonas stutzeri [104]               |
|                              | P4HB     | Bordetella bronchiseptica [104]          |
| trans-3-hydroxy-L-proline    | HtyE     | Aspergillus pachycristatus [91] 2        |
|                              | GloF     | Glarea lozoyensis [91,102,103] 2         |

1 mainly cis-4 hydroxyproline. 2 mainly trans-4 hydroxyproline.

The company Kyowa Hakko Kogyo has patented several PH-based processes. In an initial patent, the company protects the use of a 2-oxoglutarate-dependent oxygenase, namely cis-P3H1, for the production of cis-3-hydroxy-L-proline [55]. They describe the use of the purified enzyme to convert L-proline (20 mM) into cis-3-hydroxy-L-proline (18mM) in 30 min. Additional patents of the same company cover the production of cis-4-hydroxy-L-proline using SmP4H [56] and the manufacture of trans-4-hydroxy-L-proline with DaP4H [54]. In the latter case, strain optimization took place, and by expressing DaP4H with a tryptophan tandem promotor, the desired product trans-4-hydroxy-L-proline could be obtained in titers of up to 41 g/L (87% yield) in 100 h [101]. Beyond Kyowa Hakko, the Japanese company API Corp holds a patent for the production of cis-5-hydroxy-pipeolic acid using a proline hydroxylase from Xenorhabdus sp. [53].

Other proline hydroxylases also accept the industrially important structural homolog piperolic acid. Klein et al. used Cis-P3H1, SmP4H, and DaP4H for the synthesis of hydroxy-pipeolic acid isomers [105]. To improve SmP4H’s regioselectivity in the conversion of piperolic acid, Codexis Inc. optimized the enzyme by protein engineering, yielding a variant capable of a more selective conversion of L-pipeolic acid into cis-5-hydroxy-pipeolic acid [25,57]. Interestingly, the fungal enzymes GetF and PiFa preferentially accept piperolic acid, whereas L-proline is converted only to a very small extent. Using site-directed mutagenesis, the group of Hüttel attempted to shift enzyme activity towards L-proline, albeit with little success [95].

In some cases, the biocatalytic use of proline hydroxylases can be hampered by their low temperature stability. In a recent study, Liu et al. addressed this problem for enzyme UbP4H by loop grafting and site-directed mutagenesis improving T_m from 43 °C to 46 °C [99].

2.4. L-Hydroxyasparagine Oxygenases

Calcium-dependent antibiotics (CDAs) are structurally related acidic lipopeptide antibiotics, which possess a decapptide lactone or a lactam motif containing non-proteinogenic amino acids and feature N-terminal fatty acid side chains [106]. The most prominent representative of this antibiotic class is daptomycin [107]. L-3-hydroxy-asparagine is an important building block for the biosynthesis of CDAs in Streptomyces coelicolor [106]. The hydroxylation of L-asparagine is catalyzed by asparagine oxygenase (AsnO) [108]. By solving the crystal structure of AsnO with a resolution of 1.45 Å, Striecker and coworkers demonstrated that only free L-asparagine can be converted by the enzyme, whereas the late-stage functionalization of CDA or the acceptance of an asparagine bound to a peptidyl carrier protein was excluded [109]. Using protein engineering, the group succeeded in switching substrate acceptance to L-aspartic acid. Upon the introduction of only one mutation (D241N), the enzyme produced L-3-hydroxy-aspartic acid exclusively, albeit with a loss of catalytic efficiency (the wildtype
has a 300-time higher \( k_{\text{cat}} \) [110]. The preparative synthesis of \( L \)-3-hydroxy-aspartic acid was also demonstrated on the scale of 18 mg with a purified protein [110].

2.5. \( L \)-Arginine Oxygenase

The tuberactinomycins (Tbms) are nonribosomal peptide antibiotics including viomycin (tuberactinomycin B) and the capreomycins, which exhibit activity against *Mycobacterium tuberculosis*. The compounds exhibit a range of biological activities that center on their ability to bind RNA and disturb bacterial protein biosynthesis [111]. The gene cluster that directs viomycin biosynthesis from *Streptomyces vinaceus* ATCC 11861 comprises the 2-OGX VioC [112]. VioC is involved in the production of the building block \( (2S,3R) \)-capreomycinide by hydroxylation of \( L \)-arginine into \( (2S,3S) \)-hydroxyarginine [113], which is then converted by the PLP-dependent VioD into \( (2S,3R) \)-capreomycinide [114]. Apart from \( L \)-arginine, VioC can also hydroxylate \( L \)-homoarginine and \( L \)-canavanine [115] and, interestingly, also accepts \( D \)-arginine, catalyzing an oxidative deamination into the corresponding 2-oxo-5-guanidinopentanoate (2O5GP) [116].

In a biocatalytic context, VioC has been used together with an arginase and an ornithine cyclodeaminase to convert \( L \)-arginine into trans-3-hydroxy-\( L \)-proline, which is difficult to selectively produce using proline hydroxylases [117]. Despite successfully setting up the enzymatic cascade, Hara et al. concluded that long reaction times (72 h) and low ornithine cyclodeaminase activity make industrial applications unlikely at this time [117].

2.6. \( L \)-Lysine Hydroxylase

In 2015, two groups independently discovered a non-ribosomal tetrapeptide (JBIR-126 [118] or tambromycin [119]) containing a characteristic indole substructure, an oxazoline moiety, and a novel pyrrolidine-containing amino acid (named “tambroline”, which stands for two-amino-beta-homoprolinate) coupled with a C-terminal \( \alpha \)-methylserine residue.

The protected building block tambroline can be synthesized by selective C3 hydroxylation of \( L \)-lysine, employing the 2-OXG lysine hydroxylase KDO1 from *Catenulispora acidiphila* (DSM 44928) [120,121] followed by an additional four chemical steps [122]. The biocatalytic transformation was established on a 4 g scale (35 mM) using a crude cell lysate of KDO1 (>99% conversion) [122]. The chemo-enzymatic total synthesis of tambromycin consisted of 10 steps and led to the desired product with an overall yield of 2.4%. By comparison, in a recently published purely chemical synthesis consisting of 13 steps, tambromycin was obtained with an overall yield of 1.3% [123], positively underscoring the potential of chemo-enzymatic setups.

Apart from KDO1, six additional lysine hydroxylases (K3H1, K3H2, K4H1, K4H2, K4H3, and K4H4) are known for the regio- and stereoselective hydroxylation of the C-3 or C-4 position in \( L \)-lysine [124]. To explore their biocatalytic usefulness, Hara et al. conducted several multi-gram scale hydroxylation reactions: Using K3H1 from *Kineococcus radiotolerans* (SRS0216) and K4H4 from *Chryseobacterium gleum* (ATCC 35910), the desired products \( (2S,3S) \)-3-hydroxylysine (531 mM; 43 g/L, 88% molar conversion in 52h) and \( (2S,4R) \)-4-hydroxylysine (265 m; 43 g/L, 88% molar conversion in 24h), respectively, could be synthesized in batch-scale experiments [124]. All of these enzymes are mentioned in a patent by API Corp. protecting the production of hydroxylysine [59].

2.7. \( L \)-Glutamate Hydroxylase

Two 2-oxoglutarate-dependent enzymes, namely KtzO and KtzP, are involved in the natural synthesis of the antifungal and antimicrobial kutznerides [125]. In this specific case, the substrate \( L \)-glutamate is bound to a peptidyl carrier to enable conversion to threo-\( L \)-hydroxyglutamic acid by KtzO or erythro-\( L \)-hydroxyglutamic acid by KtzP [125]. Both enzymes cannot convert free-standing \( L \)-glutamate.
2.8. Clavaminic Synthase

Clavulamic acid is an effective β-lactamase inhibitor and therefore often used in combination with β-lactam antibiotics to overcome antibiotic resistances [126]. The trifunctional 2-oxoglutarate-dependent clavaminic synthase (CAS) from Streptomyces clavuligerus catalyzes three distinct steps in the compound’s biosynthesis, namely hydroxylation of deoxyguanidino-proclavaminic, cyclization of proclavaminic acid, and desaturation of dihydroclavaminic acid (Figure 6) [40]. In Streptomyces clavuligerus, two isoenzymes CS1 and CS2 were identified and structurally and mechanistically characterized [127–130]. Interestingly, fermentation optimization of Streptomyces clavuligerus is a field of ongoing study [131–133], and to date, the production of clavulamic acid by fermentation has not yet substantially exceeded 1 g/L [131].

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\text{deoxyguanidino-proclavaminic acid} \xrightarrow{\text{CAS}} \text{guanidino-proclavaminic acid} \xrightarrow{\text{Hydrolase}} \text{proclavaminic acid}
\]

\[
\text{clavulamic acid} \xrightarrow{\text{CAS}} \text{clavamic acid} \xrightarrow{\text{CAS}} \text{dihydroclavamic acid}
\]

**Figure 6.** Biosynthesis of clavulamic acid. The tri-functional oxygenase CAS sequentially carries out a hydroxylation, ring formation, and desaturation step.

2.9. Deacetoxycephalosporin C Synthase (DAOCS) and Deacetylcephalosporin C Synthase (DACS)

Cephalosporins are a class of beta-lactam antibiotics originally derived from the fungus Acremonium [134]. The ring expansion of the penicillin N amide thiazolidine ring to form the dihydrothiazine caphem ring, a key step in the biosynthesis of cephalosporins, is catalyzed by the 2-oxoglutarate-dependent eacetoxycephalosporin C synthase (DAOCS) before the second 2-oxoglutarate-dependent deacetylcephalosporin C synthase (DACS) hydroxylates at C-3′ position to form deacetylcephalosporin C (Figure 7) [39]. In eukaryotic microorganisms, a single bifunctional enzyme called DAOCS/DACS catalyzes both reactions [135], whereas in prokaryotes two distinct enzymes are needed [136,137].

\[
\text{penicillin N} \xrightarrow{\text{DAOCS}} \text{deacetoxycephalosporin C} \xrightarrow{\text{DACS}} \text{deacetylcephalosporin C}
\]

**Figure 7.** Reactions catalyzed by DAOCS and DACS in the biosynthesis of cephalosporins.

Industrially, penicillin G is a more abundantly available starting material than penicillin N, which is the natural substrate of DAOCS. However, wildtype DAOCS accepts penicillin G poorly, a fact that triggered a number of enzyme engineering studies to improve the activity of DAOCS for this non-natural substrate [28,138–140]. Single mutations have already had a profound effect on DAOCS
activity; for example, mutation N304K increases the $k_{\text{cat}}/K_m$ value for the conversion of penicillin G from 18 $\text{M}^{-1}\text{s}^{-1}$ to 256 $\text{M}^{-1}\text{s}^{-1}$ [141]. In a different study, error-prone PCR yielded a quadruple DAOCS mutant (C155Y/Y184H/V275I/C281Y) with a 40-fold improvement in $k_{\text{cat}}/K_m$ [142], while further engineering generated a variant with a 118-fold improved $k_{\text{cat}}/K_m$ value compared with the wildtype [143].

3. Conclusions

Due to the explosion of available genome information, bioinformatic studies continuously identify additional members of the 2OGX superfamily in bacteria, metazoa, and plants [144–146]. This wealth of available enzyme sequences will lead to the characterization of additional, industrially promising 2OGX variants with desirable substrate spectra. Already today, 2-oxoglutarate-dependent oxygenases are employed for a variety of industrially and academically relevant transformations, ranging from the production of amino acid derivatives to the chemo-enzymatic total synthesis of complex natural products (vide supra). As attested by recent enzyme engineering studies on 2-oxoglutarate-dependent oxygenases [25,99,140], the progress made in the field of protein design and evolution over the last few decades [147,148] facilitates the development of robust and active C–H functionalization biocatalysts [149]. As the integration of automation and the use of advanced computer algorithms continue to facilitate enzyme engineering, the industrial implementation of 2OGX-driven processes will be further accelerated.

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References

1. Turner, N.J.; Humphreys, L. Biocatalysis in Organic Synthesis: The Retrosynthesis Approach; Royal Society of Chemistry: London, UK, 2018.
2. Islam, M.S.; Leissinger, T.M.; Chowdhury, R.; Hopkinson, R.J.; Schofield, C.J. 2-Oxoglutarate-Dependent Oxygenases. *Annu. Rev. Biochem.* 2018, 87, 585–620. [CrossRef] [PubMed]
3. Croteau, R.; Kutchan, T.M.; Lewis, N.G. Natural products (secondary metabolites). In *Biochemistry & Molecular Biology of Plants*; Buchanan, B., Gruissem, W., Jones, R., Eds.; American Society of Plant Physiologists: Rockville, MD, USA, 2000; pp. 1250–1318.
4. Dong, J.; Fernandez-Fueyo, E.; Hollmann, F.; Paul, C.E.; Pesci, M.; Schmidt, S.; Wang, Y.; Younes, S.; Zhang, W. Biocatalytic oxidation reactions: A chemist’s perspective. *Angew. Chem. Int. Ed.* 2018, 57, 9238–9261. [CrossRef] [PubMed]
5. Zanger, U.M.; Schwab, M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol. Ther.* 2013, 138, 103–141. [CrossRef] [PubMed]
6. Peters, F.T.; Bureik, M.; Maurer, H.H. Biotechnological synthesis of drug metabolites using human cytochrome P450 isoforms heterologously expressed in fission yeast. *Bioanalysis* 2009, 1, 821–830. [CrossRef] [PubMed]
7. Schroer, K.; Kittelmann, M.; Lütz, S. Recombinant human cytochrome P450 monooxygenases for drug metabolite synthesis. *Biotechnol. Bioeng.* 2010, 106, 699–706. [CrossRef] [PubMed]
8. Galanie, S.; Thodey, K.; Trenchard, I.J.; Filsinger Interrante, M.; Smolke, C.D. Complete biosynthesis of opioids in yeast. *Science 2015*, 349, 1095–1100. [CrossRef] [PubMed]
9. McLean, K.J.; Hans, M.; Meijrink, B.; van Scheppingen, W.B.; Vollebregt, A.; Tee, K.L.; van der Laan, J.M.; Leys, D.; Munro, A.W.; van den Berg, M.A. Single-step fermentative production of the cholesterol-lowering drug pravastatin via reprogramming of *Penicillium chrysogenum*. *Proc. Natl. Acad. Sci. USA* 2015, 112, 2847–2852. [CrossRef] [PubMed]
10. Paddon, C.J.; Westfall, P.J.; Pitera, D.J.; Benjamin, K.; Fisher, K.; McPhee, D.; Leavell, M.D.; Tai, A.; Main, A.; Eng, D.; et al. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* 2013, 496, 528–532. [CrossRef] [PubMed]

11. Buller, R.; Hecht, K.; Mirata, M.A.; Meyer, H.-P. An appreciation of biocatalysis in the Swiss manufacturing environment. In *Biocatalysis: An Industrial Perspective*; The Royal Society of Chemistry: Cambridge, UK, 2018; pp. 1–43.

12. Durairaj, P.; Hur, J.-S.; Yun, H. Versatile biocatalysis of fungal cytochrome P450 monooxygenases. *Microb. Cell Fact.* 2016, 15, 125. [CrossRef] [PubMed]

13. Kolev, J.N.; O’Dwyer, K.M.; Jordan, C.T.; Fasan, R. Discovery of potent parthenolide-based antileukemic agents enabled by late-stage P450-mediated C—H functionalization. *ACS Chem. Biol.* 2014, 9, 164–173. [CrossRef] [PubMed]

14. Negretti, S.; Narayan, A.R.H.; Chiou, K.C.; Kells, P.M.; Stachowski, J.L.; Hansen, D.A.; Podust, L.M.; Montgomery, J.; Sherman, D.H. Directing group-controlled regioselectivity in an enzymatic C–H bond oxygenation. *J. Am. Chem. Soc.* 2014, 136, 4901–4904. [CrossRef] [PubMed]

15. Loskot, S.A.; Romney, D.K.; Arnold, F.H.; Stoltz, B.M. Enantioselective total synthesis of nigelladine A via late-stage C–H oxidation enabled by an engineered P450 enzyme. *J. Am. Chem. Soc.* 2017, 139, 10196–10199. [CrossRef] [PubMed]

16. Zhang, R.K.; Huang, X.; Arnold, F.H. Selective CH bond functionalization with engineered heme proteins: New tools to generate complexity. *Curr. Opin. Chem. Biol.* 2019, 49, 67–75. [CrossRef] [PubMed]

17. Bernhardt, R.; Urlacher, V.B. Cytochromes P450 as promising catalysts for biotechnological application: Chances and limitations. *Appl. Microbiol. Biotechnol.* 2014, 98, 6185–6203. [CrossRef] [PubMed]

18. O’Reilly, E.; Köhler, V.; Flitsch, S.L.; Turner, N.J. Cytochromes P450 as useful biocatalysts: Addressing the limitations. *Chem. Commun.* 2011, 47, 2490–2501. [CrossRef] [PubMed]

19. Hofrichter, M.; Ullrich, R. Oxidations catalyzed by fungal peroxygenases. *Curr. Opin. Chem. Biol.* 2014, 19, 116–125. [CrossRef] [PubMed]

20. Wang, Y.; Lan, D.; Durrani, R.; Hollman, F. Peroxygenases en route to becoming dream catalysts. What are the opportunities and challenges? *Curr. Opin. Chem. Biol.* 2017, 37, 1–9. [CrossRef] [PubMed]

21. Herr, C.Q.; Hausinger, R.P. Amazing diversity in biochemical roles of Fe(II)/2-oxoglutarate oxygenases. *Trends Biochem. Sci.* 2018, 43, 517–532. [CrossRef] [PubMed]

22. Schofield, C.; Hausinger, R. 2-Oxoglutarate-Dependent Oxygenases; The Royal Society of Chemistry: Cambridge, UK, 2015.

23. Hausinger, R.P. Biochemical diversity of 2-oxoglutarate-dependent oxygenases. In *2-Oxoglutarate-Dependent Oxygenases*; The Royal Society of Chemistry: Cambridge, UK, 2015; pp. 1–58.

24. Martinez, S.; Hausinger, R.P. Catalytic mechanisms of Fe(II)- and 2-oxoglutarate-dependent oxygenases. *J. Biol. Chem.* 2015, 290, 20702–20711. [CrossRef] [PubMed]

25. Chai, F.; Bong, Y.K.; Cabirol, F.L.; Prafulchandra, A.G.; Li, T.; Moore, J.C.; Quintanar-Audelo, M.; Hong, Y.; Collier, S.J.; Smith, D. Biocatalysts and Methods for Hydroxylation of Chemical Compounds. U.S. Patent US2017/0121744 A1, 4 May 2017.

26. Hibért, M.; Ogawa, J. Characteristics and biotechnology applications of aliphatic amino acid hydroxylases belonging to the Fe(II)/alpha-ketoglutarate-dependent dioxygenase superfamily. *Appl. Environ. Microbiol.* 2014, 80, 3869–3876.

27. Zhang, X.; Dong, L.B.; Yang, L.C.; Rudolf, J.D.; Shen, B.; Renata, H. Harnessing the biocatalytic potential of PtmO6, an α-ketoglutarate-dependent dioxygenase from platsensimycin biosynthesis, for the chemoenzymatic synthesis of highly oxidized ent-kaurane diterpenes. *ChemRxiv* 2019. accepted. [CrossRef]

28. Hüttel, W. Biocatalytic production of chemical building blocks in technical scale with α-ketoglutarate-dependent dioxygenases. *Chem. Ing. Tech.* 2013, 85, 809–817. [CrossRef]

29. Wang, X.-C.; Liu, J.; Zhao, J.; Ni, X.-M.; Zheng, P.; Guo, X.; Sun, C.-M.; Sun, J.-B.; Ma, Y.-H. Efficient production of trans-4-hydroxy-L-proline from glucose using a new trans-proline 4-hydroxylase in *Escherichia coli*. *J. Biosci. Bioeng.* 2018, 126, 470–477. [CrossRef] [PubMed]

30. Hillwig, M.L.; Liu, X. A new family of iron-dependent halogenases acts on freestanding substrates. *Nat. Chem. Biol.* 2014, 10, 921–923. [CrossRef] [PubMed]
31. Ishikawa, N.; Tanaka, H.; Koyama, F.; Noguchi, H.; Wang, C.C.; Hotta, K.; Watanabe, K. Non-heme dioxygenase catalyzes atypical oxidations of 6,7-bicyclic systems to form the 6,6-quinolone core of viridicatin-type fungal alkaloids. Angew. Chem. Int. Ed. 2014, 53, 12880–12884. [CrossRef] [PubMed]

32. Porco, S.; Penčík, A.; Rashed, A.; Voß, U.; Casanova-Sáez, R.; Bishopp, A.; Golebiowska, A.; Bhsale, R.; Swarup, R.; Swarup, K.; et al. Dioxygenase-encoding AtlDAO1 gene controls IAA oxidation and homeostasis in Arabidopsis. Proc. Natl. Acad. Sci. USA 2016, 113, 11010–11015. [CrossRef] [PubMed]

33. Zhao, Z.; Zhang, Y.; Liu, X.; Zhang, X.; Liu, S.; Yu, X.; Ren, Y.; Zheng, X.; Zhou, K.; Jiang, L.; et al. A role for a dioxygenase in auxin metabolism and reproductive development in rice. Dev. Cell 2013, 27, 113–122. [CrossRef] [PubMed]

34. Bollinger, J.M.; Price, J.C.; Hoffart, L.M.; Barr, E.W.; Krebs, C. Mechanism of taurine: -lactam biosynthesis. J. Am. Chem. Soc. 2006, 128, 4848–4849. [CrossRef] [PubMed]

35. Steffan, N.; Grundmann, A.; Afiyatullov, S.; Ruan, H.; Li, S.-M. FtmOx1, a non-heme Fe(ii) and non-heme iron(II) enzymes. Eur. J. Inorg. Chem. 2012, 22, 691–700. [CrossRef] [PubMed]

36. Hegg, E.L.; Que, L. The 2-His-1-carboxylate facial triad—An emerging structural motif in mononuclear non-heme iron(II) enzymes. Eur. J. Biochem. 1997, 250, 625–629. [CrossRef] [PubMed]

37. Clifton, I.J.; McDonough, M.A.; Ehrismann, D.; Kershaw, N.J.; Granatino, N.; Schofield, C.J. Structural studies on 2-oxoglutarate oxygenases and related double-stranded β-helix fold proteins. J. Inorg. Biochem. 2006, 100, 644–669. [CrossRef] [PubMed]

38. Elkins, J.M.; Ryle, M.J.; Clifton, I.J.; Hotopp, J.C.D.; Lloyd, J.S.; Burzlaff, N.I.; Baldwin, J.E.; Hausinger, R.P.; Roach, P.L. X-ray crystal structure of Escherichia coli taurine/α-ketoglutarate dioxygenase complexed to ferrous iron and substrates. Biochemistry 2002, 41, 5185–5192. [CrossRef] [PubMed]

39. Bollinger, J.M.; Price, J.C.; Hoffart, L.M.; Barr, E.W.; Krebs, C. Mechanism of taurine: α-ketoglutarate dioxygenase (TauD) from Escherichia coli. Eur. J. Inorg. Chem. 2005, 4245–4254. [CrossRef] [PubMed]

40. Krebs, C.; Fujimori, D.G.; Walsh, C.T.; Bollinger, J.M. Non-heme Fe(IV)-oxo intermediates. Acc. Chem. Res. 2007, 40, 484–492. [CrossRef] [PubMed]

41. Steffan, N.; Grundmann, A.; Afiyatullov, S.; Ruan, H.; Li, S.-M. FtmOx1, a non-heme Fe(ii) and non-heme iron(II) enzymes. Eur. J. Inorg. Chem. 2012, 22, 691–700. [CrossRef] [PubMed]
50. Smirnov, S.V.; Kodera, T.; Samsonova, N.N.; Kotlyarova, V.A.; Rushkevich, N.Y.; Kivero, A.D.; Sokolov, P.M.; Hibi, M.; Ogawa, J.; Shimizu, S. Metabolic engineering of *Escherichia coli* to produce (2S,3R,4S)-4-hydroxyisoleucine. *Appl. Microbiol. Biotechnol.* **2010**, *88*, 719–726. [CrossRef] [PubMed]

51. Gao, S.-S.; Naowarojna, N.; Cheng, R.; Liu, X.; Liu, P. Recent examples of α-ketoglutarate-dependent mononuclear non-haem iron enzymes in natural product biosyntheses. *Nat. Prod. Rep.* **2018**, *35*, 792–837. [CrossRef] [PubMed]

52. Latham, J.; Brandenburger, E.; Shepherd, S.A.; Menon, B.R.K.; Micklefield, J. Development of halogenase enzymes for use in synthesis. *Chem. Rev.* **2018**, *118*, 232–269. [CrossRef] [PubMed]

53. Enoki, J.; Meisborn, J.; Müller, A.-C.; Kourist, R. A multi-enzymatic cascade reaction for the stereoselective production of γ-oxyfunctionalized amino acids. *Front. Microbiol.* **2016**, *7*, 425. [CrossRef] [PubMed]

54. Ozaki, A.; Mori, H.; Shibasaki, T.; Ando, K.; Chiba, S. Process for Producing trans-4-hydroxy-L-proline. U.S. Patent US7238501B2, 3 July 2007.

55. Ozaki, A.; Mori, H.; Shibasaki, T.; Ando, K.; Ochiai, K.; Chiba, S.; Uosaki, Y. Process for Producing cis-3-hydroxy-L-proline. U.S. Patent US6413748B1, 2 July 2002.

56. Kino, K.; Hara, R. L-proline cis-4-hydroxylase and Use thereof to Produce cis-4-hydroxy-L-proline. U.S. Patent US8854120B2, 24 September 2013.

57. Chen, H.; Bong, Y.K.; Cabirol, F.; Gohel, A.; Li, T.; Moore, J.C.; Quintanar-Audelo, M.; Yang, H.; Collier, S.J.; Smith, D. Biocatalysts and Methods for Hydroxylation of Chemical Compounds. European Patent EP2847327B1, 7 May 2013.

58. Kodera, T.; Smirnov, S.V.; Samsonova, N.N.; Kotliarova, V.A.; Rushkevich, N.Y.; Kozlov, Y.I.; Shimizu, S.; Ogawa, J.; Hibi, M. Method for Producing 4-hydroxy-L-iso-leucine. U.S. Patent US8367381B2, 5 February 2013.

59. Kino, K.; Hara, R.; Miyake, R.; Kawabata, H. Method for Producing hydroxy-L-lysine Employing an L-lysine Hydroxylase and Method for Producing hydroxy-L-pipecolic Acid. European Patent EP2889378B1, 18 February 2014.

60. Kino, K.; Hara, R.; Miyake, R.; Kawabata, H. Method for Producing hydroxy-L-lysine Employing an L-lysine Hydroxylase and Method for Producing hydroxy-L-pipecolic Acid. European Patent EP2889378B1, 18 February 2014.

61. Fowden, L.; Pratt, H.M.; Smith, A. 4-hydroxyisoleucine from seed of *Trigonella foenum-graecum*. *Phytochemistry* **1973**, *12*, 1707–1711. [CrossRef]

62. Cao, H.; Liu, S.; Li, J.; Yue, G.; Zhang, M. Production L-4-hydroxy-isoleucine Conversion Method of a Microbial Enzyme Method. Chinese Patent CN 105779522 B, 17 May 2016.

63. Broca, C.; Gross, R.; Petit, P.; Sauvain, Y.; Manteghetti, M.; Tournier, M.; Masielo, P.; Comis, R.; Ribes, G. 4-Hydroxyisoleucine: Experimental evidence of its insulino-tropic and anti-diabetic properties. *Am. J. Physiol. Endocrinol. Metab.* **1999**, *277*, E617–E623. [CrossRef] [PubMed]

64. Jette, L.; Harvey, L.; Eugeni, K.; Levens, N. 4-Hydroxyisoleucine: A plant-derived treatment for metabolic syndrome. *Curr. Opin. Investig. Drugs* **2009**, *10*, 353–358. [PubMed]

65. Ogawa, J.; Kodera, T.; Smirnov, S.V.; Hibi, M.; Samsonova, N.N.; Koyama, R.; Yamanaka, H.; Mano, J.; Kawashima, T.; Yokozeki, K.; et al. A novel L-isoleucine dioxygenating enzyme, L-isoleucine dioxygenase from *Bacillus thuringiensis*, produces (2S,3R,4S)-4-hydroxyisoleucine. *Biochem. Biophys. Res. Commun.* **2009**, *390*, 506–510. [CrossRef] [PubMed]

66. Zhang, C.; Ma, J.; Li, Z.; Liang, Y.; Xu, Q.; Xie, X.; Chen, N. A strategy for L-isoleucine dioxygenase screening and 4-hydroxyisoleucine production by resting cells. *Bioengineered* **2018**, *9*, 72–79. [CrossRef] [PubMed]

67. Hibi, M.; Kawashima, T.; Kodera, T.; Smirnov, S.V.; Sokolov, P.M.; Sugiyama, M.; Shimizu, S.; Yokozeki, K.; Ogawa, J. Characterization of *Bacillus thuringiensis* L-isoleucine dioxygenase for production of useful amino acids. *Appl. Environ. Microbiol.* **2011**, *77*, 6926–6930. [CrossRef] [PubMed]

68. Enoki, J.; Meisborn, J.; Müller, A.-C.; Kourist, R. A multi-enzymatic cascade reaction for the stereoselective production of γ-oxyfunctionalized amino acids. *Front. Microbiol.* **2016**, *7*, 425. [CrossRef] [PubMed]

69. Sokolov, P.M.; Smirnov, S.V.; Kodera, T.; Sugiyama, M.; Yokozeki, K.; Hibi, M.; Ogawa, J.; Shimizu, S. A novel family of bacterial dioxygenases that catalyze the hydroxylation of free L-amino acids. *FEMS Microbiol. Lett.* **2012**, *331*, 97–104.
70. Smirnov, S.V.; Sokolov, P.M.; Kotlyarova, V.A.; Samsonova, N.N.; Kodera, T.; Sugiyama, M.; Torii, T.; Hibi, M.; Shimizu, S.; Yokozeki, K.; et al. A novel L-isoleucine-4′-dioxygenase and L-isoleucine dihydroxylation cascade in *Pantoea ananatis*. *Microbiologiya* 2013, 2, 471–481. [CrossRef] [PubMed]
71. Hibi, M.; Kawashima, T.; Sokolov, P.V.; Smirnov, S.V.; Kodera, T.; Sugiyama, M.; Shimizu, S.; Yokozeki, K.; Ogawa, J. *L*-Leucine 5-hydroxylase of *Nostoc punctiforme* is a novel type of Fe(II)/α-ketoglutarate-dependent dioxygenase that is useful as a biocatalyst. *Appl. Microbiol. Biotechnol.* 2013, 97, 2467–2472. [CrossRef] [PubMed]
72. Sun, D.; Gao, D.; Xu, P.; Guo, Q.; Zhu, Z.; Cheng, X.; Bai, S.; Qin, H.-M.; Lu, F. A novel *L*-leucine 5-hydroxylase from *Nostoc piscinale* unravels unexpected sulphoxidation activity toward *L*-methionine. *Protein Expr. Purif.* 2018, 149, 1–6. [CrossRef] [PubMed]
73. Correia Cordeiro, R.S.; Enoki, J.; Busch, F.; Mügge, C.; Kourist, R. Cloning and characterization of a new delta-specific *L*-leucine dioxygenase from *Anabaena variabilis*. *J. Biotechnol.* 2018, 284, 68–74. [CrossRef] [PubMed]
74. Kobayashi, J.; Kanda, F.; Ishibashi, M.; Shigemori, H. Manzacidins A-C, novel tetrahydropyrimidine alkaloids from the Okinawan marine sponge *Hymeniacidon* sp. *J. Org. Chem.* 1991, 56, 4574–4576. [CrossRef]
75. Hashimoto, T.; Maruoka, K. Syntheses of manzacidins: A stage for the demonstration of synthetic methodologies. *Org. Biomol. Chem.* 2008, 6, 829–835. [CrossRef] [PubMed]
76. Zwick, C.R.; Renata, H. A novel *L*-leucine dioxygenase from *Burkholderia ambifaria* unravels unexpected sulphoxidation activity toward *L*-methionine. *Protein Expr. Purif.* 2018, 149, 1–6. [CrossRef] [PubMed]
77. Kling, A.; Lukat, P.; Almeida, D.V.; Bauer, A.; Fontaine, E.; Sordello, S.; Zaburannyi, N.; Herrmann, J.; Wenzel, S.C.; König, C.; et al. Targeting DnaN for tuberculosis therapy using novel griselimycins. *Science* 2015, 348, 1106–1112. [CrossRef] [PubMed]
78. Lukat, P.; Katsuyma, Y.; Wenzel, S.; Binz, T.; König, C.; Blankenfeldt, W.; Brönstrup, M.; Müller, R. Biosynthesis of methyl-proline containing griselimycins, natural products with anti-tuberculosis activity. *Chem. Sci.* 2017, 8, 7521–7527. [CrossRef] [PubMed]
79. Zwick, C.R.; Renata, H. Evolution of biocatalytic and chemocatalytic C–H functionalization strategy in the synthesis of manzacidin C. *J. Org. Chem.* 2018, 83, 7407–7415. [CrossRef] [PubMed]
80. Zwick, C.R.; Renata, H. A one-pot chemoenzymatic synthesis of (2S, 4R)-4-methylproline enables the first total synthesis of antiviral lipopeptide cavinafungin B. *Tetrahedron* 2018, 74, 6469–6473. [CrossRef]
81. Ortiz-López, F.J.; Monteiro, M.C.; González-Menéndez, V.; Tormo, J.R.; Genilloud, O.; Bills, G.F.; Vicente, F.; Zhang, C.; Roemer, T.; Singh, S.B.; et al. Cyclic colisporifungin and linear cavinafungins, antifungal lipopeptides isolated from *Colispora cavincola*. *J. Nat. Prod.* 2015, 78, 468–475. [CrossRef] [PubMed]
82. Estoppey, D.; Lee, C.M.; Janoschke, M.; Lee, B.H.; Wan, K.F.; Dong, H.; Mathys, P.; Filippuzzi, I.; Schuhmann, T.; Riedl, R.; et al. The natural product cavinafungin selectively interferes with Zika and Dengue virus replication by inhibition of the host signal peptidase. *Cell Rep.* 2017, 19, 451–460. [CrossRef] [PubMed]
83. Qin, H.-M.; Miyakawa, T.; Nakamura, A.; Xue, Y.-L.; Kawashima, T.; Kasahara, T.; Hibi, M.; Ogawa, J.; Tanokura, M. Expression, purification, crystallization and preliminary X-ray analysis of a novel N-substituted branched-chain L-amino-acid dioxygenase from *Burkholderia ambifaria* AMMD. *Acta Crystallogr. F* 2012, 68, 1067–1069. [CrossRef] [PubMed]
84. Hibi, M.; Kawashima, T.; Kasahara, T.; Sokolov, P.M.; Smirnov, S.V.; Kodera, T.; Sugiyama, M.; Shimizu, S.; Yokozeki, K.; Ogawa, J. A novel Fe(II)/α-ketoglutarate-dependent dioxygenase from *Burkholderia ambifaria* has β-hydroxylating activity of *N*-succinyl *L*-leucine. *Lett. Appl. Microbiol.* 2012, 55, 414–419. [CrossRef] [PubMed]
85. Qin, H.M.; Miyakawa, T.; Jia, M.Z.; Nakamura, A.; Ohtsuka, J.; Xue, Y.L.; Kawashima, T.; Kasahara, T.; Hibi, M.; Ogawa, J.; et al. Crystal structure of a novel N-substituted L-amino acid dioxygenase from *Burkholderia ambifaria* AMMD. *PLoS ONE* 2013, 8, e63996. [CrossRef] [PubMed]
86. Qin, H.-M.; Miyakawa, T.; Nakamura, A.; Hibi, M.; Ogawa, J.; Tanokura, M. Structural optimization of SadA, an Fe(II)- and α-ketoglutarate-dependent dioxygenase targeting biocatalytic synthesis of *N*-succinyl-*L*-threo-3,4-dimethoxyphenylserine. *Biochem. Biophys. Res. Commun.* 2014, 450, 1458–1461. [CrossRef] [PubMed]
87. Hibi, M.; Kasahara, T.; Kawashima, T.; Yajima, H.; Kozono, S.; Smirnov, S.V.; Kodera, T.; Sugiyama, M.; Shimizu, S.; Yokozeki, K.; et al. Multi-enzymatic synthesis of optically pure β-hydroxy α-amino acids. *Adv. Synth. Catal.* 2015, 357, 767–774. [CrossRef]

88. Yan, W.; Song, H.; Song, F.; Guo, Y.; Wu, C.-H.; Sae Her, A.; Pu, Y.; Wang, S.; Naowarojna, N.; Weitz, A.; et al. Endo-protoxide formation by an α-ketoglutarate-dependent mononuclear non-haem iron enzyme. *Nature* 2015, 527, 539. [CrossRef] [PubMed]

89. Ji, J.-N.; Chen, S.-L. Asymmetric abstraction of two chemically-equivalent methylene hydrogens: Significant enantioselectivity of endo-protoxide presented by fumitremorgin B endo-protoxidase. *Phys. Chem. Chem. Phys.* 2018, 20, 26500–26505. [CrossRef] [PubMed]

90. Baldwin, J.E.; Lawrence, C.C.; Robinson, J.K.; Schofield, C.J. Substrate specificity of proline 4-hydroxylase. *Chem. Biol.* 2005, 12, 2650–26505. [CrossRef] [PubMed]

91. Shibasaki, T.; Mori, H.; Ozaki, A. Cloning of an isozyme of proline 3-hydroxylase and its purification from *Glarea lozoyensis* pneumocandin-producing fungus. *Biosci. Biotechnol. Biochem.* 2000, 64, 746–750. [CrossRef] [PubMed]

92. Shibasaki, T.; Mori, H.; Chiba, S.; Ozaki, A. Microbial proline 4-hydroxylase screening and gene cloning. *Appl. Environ. Microbiol.* 1999, 65, 4028–4031. [PubMed]

93. Shibasaki, T.; Mori, H.; Ozaki, A. Cloning of an isozyme of proline 3-hydroxylase and its purification from recombinant *Escherichia coli*. *Biotecnol. Lett.* 2000, 22, 1967–1973. [CrossRef]

94. Shibasaki, T.; Mori, H.; Ozaki, A. Purification and cloning of a proline 3-hydroxylase, a novel enzyme which hydroxylates free L-proline to cis-3-hydroxy-L-proline. *J. Bacteriol.* 1997, 179, 5677–5683. [CrossRef] [PubMed]

95. Shibasaki, T.; Mori, H.; Chiba, S.; Ozaki, A. Microbial proline 4-hydroxylase screening and gene cloning. *Appl. Environ. Microbiol.* 1999, 65, 4028–4031. [PubMed]

96. Shibasaki, T.; Mori, H.; Ozaki, A. Enzymatic production of proline 3-hydroxylase that discriminates L-proline from D-proline. *Chem. Biol.* 2009, 16, 1523–1528. [CrossRef] [PubMed]

97. Koketsu, K.; Shomura, Y.; Moriwaki, K.; Hayashi, M.; Mitsuhashi, S.; Hara, R.; Kino, K.; Higuchi, Y. Characterization of novel 2-oxoglutarate dependent dioxygenases catalyzing selective cis-hydroxylation of proline and pipecolic acid from actinomycetes. *J. Bacteriol.* 2014, 196, 55–58. [CrossRef] [PubMed]

98. Hara, R.; Kino, K. Identification and characterization of 2-oxoglutarate-dependent dioxygenases catalyzing selective cis-hydroxylation of proline and pipecolinic acid from actinomycetes. *J. Bacteriol.* 2014, 196, 55–58. [CrossRef] [PubMed]

99. Shibasaki, T.; Mori, H.; Chiba, S.; Ozaki, A. Microbial proline 4-hydroxylase screening and gene cloning. *Appl. Environ. Microbiol.* 1999, 65, 4028–4031. [PubMed]

100. Shibasaki, T.; Mori, H.; Ozaki, A. Microbial proline 4-hydroxylase screening and gene cloning. *Appl. Environ. Microbiol.* 1999, 65, 4028–4031. [PubMed]
108. Neary, J.M.; Powell, A.; Gordon, L.; Milne, C.; Flett, F.; Wilkinson, B.; Smith, C.P.; Micklefield, J. An asparagine oxygenase (AsnO) and a 3-hydroxyasparaginyl phosphotransferase (HasP) are involved in the biosynthesis of calcium-dependent lipopeptide antibiotics. Microbiology 2007, 153, 768–776. [CrossRef] [PubMed]

109. Strieker, M.; Kopp, F.; Mahlert, C.; Essen, L.-O.; Marahiel, M.A. Mechanistic and structural basis of stereospecific Cβ-hydroxylation in calcium-dependent antibiotic, a daptomycin-type lipopeptide. ACS Chem. Biol. 2007, 2, 187–196. [CrossRef] [PubMed]

110. Marrero, P.; Cabañas, M.J.; Modolell, J. Induction of translational errors (misreading) by tuberactinomycins and capreomycins. Biochem. Biophys. Res. Commun. 1980, 97, 1047–1052. [CrossRef]

111. Yi, X.; McPhail, K.L.; Kim, K.-J.; Zabriskie, T.M. VioC is a non-heme iron, α-ketoglutarate-dependent oxygenase that catalyzes the formation of 35-hydroxy-L-arginine during viomycin biosynthesis. ChemBioChem 2004, 5, 1274–1277. [CrossRef] [PubMed]

112. Yin, X.; McPhail, K.L.; Kim, K.-J.; Zabriskie, T.M. Formation of the nonproteinogenic amino acid 2S,3R-capreomycidine by VioD from the viomycin biosynthesis pathway. ChemBioChem 2004, 5, 1278–1281. [CrossRef] [PubMed]

113. Yin, X.; O’Hare, T.; Gould, S.J.; Zabriskie, T.M. Identification and cloning of genes encoding viomycin biosynthesis from Streptomyces violaçus and evidence for involvement of a rare oxygenase. Gene 2003, 312, 215–224. [CrossRef]

114. Goering, A.W.; McClure, R.A.; Doroghazi, J.R.; Albright, J.C.; Haverland, N.A.; Zhang, Y.; Ju, K.-S.; Thomson, R.J.; Metcalfe, W.W.; Kelleher, N.L. Metabologenomics: Correlation of microbial gene clusters with metabolites drives discovery of a nonribosomal peptide with an unusual amino acid monomer. ACS Cent. Sci. 2016, 2, 99–108. [CrossRef] [PubMed]

115. Helmertag, V.; Samel, S.A.; Thomas, M.G.; Marahiel, M.A.; Essen, L.-O. Structural basis for the erythro-stereospecificity of the L-arginine oxygenase VioC in viomycin biosynthesis. FEBS J. 2009, 276, 3669–3682. [CrossRef] [PubMed]

116. Izumikawa, M.; Kawahara, T.; Kagaya, N.; Yamamura, H.; Hayakawa, M.; Takagi, M.; Yoshida, M.; Doi, T.; Shin-ya, K. Pyrrolidine-containing peptides, JBIR-126, -148, and -149, from Streptomyces sp. NBRC 111228. Tetrahedron Lett. 2015, 56, 5333–5336. [CrossRef]

117. Goering, A.W.; McClure, R.A.; Doroghazi, J.R.; Albright, J.C.; Haverland, N.A.; Zhang, Y.; Ju, K.-S.; Thomson, R.J.; Metcalfe, W.W.; Kelleher, N.L. Metabologenomics: Correlation of microbial gene clusters with metabolites drives discovery of a nonribosomal peptide with an unusual amino acid monomer. ACS Cent. Sci. 2016, 2, 99–108. [CrossRef] [PubMed]

118. Baud, D.; Saaid, P.-L.; Monfrin, A.; Harari, M.; Cuccaro, J.; Fossey, A.; Besnard, M.; Debard, A.; Mariage, A.; Pellouin, V.; et al. Synthesis of mono- and dihydroxylated amino acids with new α-ketoglutarate-dependent dioxygenases: biocatalytic oxidation of C-H bonds. ChemCatChem 2014, 6, 3012–3017. [CrossRef]

119. Bastard, K.; Isabet, T.; Stura, E.A.; Legrand, P.; Zaparusha, A. Structural studies based on two lysine dioxygenases with distinct regioselectivity brings insights into enzyme specificity within the clavaminate synthase-like family. Sci. Rep. 2018, 8, 16587. [CrossRef] [PubMed]

120. Zhang, X.; King-Smith, E.; Renata, H. Total synthesis of tambromycin by combining chemocatalytic and biocatalytic C–H functionalization. Angew. Chem. Int. Ed. Engl. 2018, 57, 5037–5041. [CrossRef] [PubMed]

121. Miley, G.P.; Rote, J.C.; Silverman, R.B.; Kelleher, N.L.; Thomson, R.J. Total synthesis of tambromycin enabled by indole C–H functionalization. Org. Lett. 2018, 20, 2369–2373. [CrossRef] [PubMed]

122. Hai, R.; Yamagata, K.; Miyake, R.; Kawabata, H.; Uehara, H.; Kino, K. Discovery of lysine hydroxylases in the clavaminic acid synthase-like superfamily for efficient hydroxylysine bioproduction. Appl. Environ. Microbiol. 2017, 83, e0093-17. [CrossRef] [PubMed]

123. Strieker, M.; Nolan, E.M.; Walsh, C.T.; Marahiel, M.A. Stereospecific synthesis of trans- and erythro-beta-hydroxyglutamic acid during kutzneride biosynthesis. J. Am. Chem. Soc. 2009, 131, 13523–13530. [CrossRef] [PubMed]

124. Saudagar, P.S.; Survase, S.A.; Singhal, R.S. Clavulanic acid: A review. Biotechnol. Adv. 2008, 26, 335–351. [CrossRef] [PubMed]
127. Pavel, E.G.; Zhou, J.; Busby, R.W.; Gunsior, M.; Townsend, C.A.; Solomon, E.I. Circular dichroism and magnetic circular dichroism spectroscopic studies of the non-heme ferrous active site in clavaminate synthase and its interaction with α-ketoglutarate cosubstrate. *J. Am. Chem. Soc.* 1998, 120, 743–753. [CrossRef]

128. Zhou, J.; Kelly, W.L.; Bachmann, B.O.; Gunsior, M.; Townsend, C.A.; Solomon, E.I. Spectroscopic studies of substrate interactions with clavaminate synthase 2, a multifunctional α-KG-dependent non-heme iron enzyme: Correlation with mechanisms and reactivities. *J. Am. Chem. Soc.* 2001, 123, 7388–7398. [CrossRef] [PubMed]

129. Zhang, Z.; Ren, J.; Stammers, D.K.; Baldwin, J.E.; Harlos, K.; Schofield, C.J. Structural origins of the selectivity of the trifunctional oxygenase clavaminic acid synthase. *Nat. Struct. Biol.* 2000, 7, 127. [PubMed]

130. Zhang, Z.; Ren, J.-S.; Harlos, K.; McKinnon, C.H.; Clifton, I.J.; Schofield, C.J. Crystal structure of a clavaminate synthase–Fe(II)–2-oxoglutarate–substrate–NO complex: Evidence for metal centred rearrangements. *FEBS Lett.* 2002, 517, 7–12. [CrossRef]

131. Ser, H.-L.; Law, J.W.-F.; Chaiyakunapruk, N.; Jacob, S.A.; Palanisamy, U.D.; Chan, K.-G.; Goh, B.-H.; Lee, L.-H. Fermentation conditions that affect clavaminic acid production in *Streptomyces clavuligerus*: A systematic review. *Front. Microbiol.* 2016, 7, 522. [CrossRef] [PubMed]

132. Ramirez-Malule, H.; Junne, S.; Nicolas Cruz-Bournazou, M.; Neubauer, P.; Rios-Estepa, R. *Streptomyces clavuligerus* shows a strong association between TCA cycle intermediate accumulation and clavamidine acid biosynthesis. *Appl. Environ. Microbiol.* 2018, 102, 4009–4023. [CrossRef] [PubMed]

133. Rodrigues, K.C.S.; Souza, A.T.d.; Badino, A.C.; Pedrolli, D.B.; Cerri, M.O. Screening of medium constituents and functions in bacteria. *Plant J.* 2003, 36, 1–14. [CrossRef] [PubMed]

134. Abraham, E.P. Cephalosporins 1945–1986. *Drugs* 1987, 34, 1–14. [CrossRef] [PubMed]

135. Samson, S.M.; Dotzlaf, J.E.; Slisz, M.L.; Becker, G.W.; Van Frank, R.M.; Veal, L.E.; Yeh, W.-K.; Miller, J.R.; Abraham, E.P.; Cephalosporins 1945–1986. *Appl. Environ. Microbiol.* 2003, 69, 619–633. [CrossRef] [PubMed]

136. Samson, S.M.; Dotzlaf, J.E.; Slisz, M.L.; Becker, G.W.; Van Frank, R.M.; Veal, L.E.; Yeh, W.-K.; Miller, J.R.; Queener, S.W.; Ingolia, T.D. Cloning and expression of the fungal expandase/hydroxylase gene involved in cephalosporin biosynthesis. *Biochemistry* 1987, 5, 1207–1214. [CrossRef]

137. Kovacevic, S.; Miller, J.R. Cloning and sequencing of the β-lactam hydroxylase gene (*cefF*) from *Streptomyces clavuligerus*. *Appl. Environ. Microbiol.* 1989, 55, 7809–7812. [CrossRef] [PubMed]

138. Kovacevic, S.; Miller, J.R. Cloning, characterization, and expression of the gene encoding deacetoxycephalosporin C synthetase. *J. Bacteriol.* 1991, 173, 398–400. [CrossRef] [PubMed]

139. Fan, K.; Lin, B.; Tao, Y.; Yang, K. Engineering deacetoxycephalosporin C synthase as a catalyst for the bioconversion of penicillins. *J. Ind. Microbiol. Biotechnol.* 2017, 44, 705–710. [CrossRef] [PubMed]

140. Goo, K.-S.; Chua, C.-S.; Sim, T.-S. Directed evolution and rational approaches to improving *Streptomyces clavuligerus* deacetoxycephalosporin C synthase for optimal ring expansion activity toward penicillin G. *Appl. Environ. Microbiol.* 2009, 75, 743–753. [CrossRef]

141. Ji, J.; Fan, K.; Tian, X.; Zhang, X.; Zhang, Y.; Yang, K. Iterative combinatorial mutagenesis as an effective strategy for generation of deacetoxycephalosporin C synthase with improved activity toward penicillin G. *Appl. Environ. Microbiol.* 2012, 78, 7809–7812. [CrossRef] [PubMed]

142. Wei, C.-L.; Yang, Y.-B.; Wang, W.-C.; Liu, W.-C.; Hsu, J.-S.; Tsai, Y.-C. Engineering *Streptomyces clavuligerus* deacetoxycephalosporin C synthase for optimal ring expansion activity toward penicillin G. *Appl. Environ. Microbiol.* 2003, 69, 2306–2312. [CrossRef] [PubMed]

143. Wei, C.-L.; Yang, Y.-B.; Deng, C.-H.; Liu, W.-C.; Hsu, J.-S.; Lin, Y.-C.; Liaw, S.-H.; Tsai, Y.-C. Directed evolution of *Streptomyces clavuligerus* deacetoxycephalosporin C synthase for enhancement of penicillin G expansion. *Appl. Environ. Microbiol.* 2005, 71, 8873–8880. [CrossRef] [PubMed]

144. Hsu, J.-S.; Yang, Y.-B.; Deng, C.-H.; Wei, C.-L.; Liaw, S.-H.; Tsai, Y.-C. Family shuffling of expandase genes to enhance substrate specificity for penicillin G. *Appl. Environ. Microbiol.* 2004, 70, 6257–6263. [CrossRef] [PubMed]

145. Kawai, Y.; Ono, E.; Mizutani, M. Evolution and diversity of the 2-oxoglutarate-dependent dioxygenase superfamily in plants. *Plant J.* 2014, 78, 328–343. [CrossRef] [PubMed]

146. Jia, B.; Jia, X.; Kim, K.H.; Jeon, C.O. Integrative view of 2-oxoglutarate/Fe(II)-dependent oxygenase diversity and functions in bacteria. *Biochim. Biophys. Acta Gen. Subj.* 2017, 1861, 323–334. [CrossRef] [PubMed]
146. Jia, B.L.; Tang, K.; Chun, B.H.; Jeon, C.O. Large-scale examination of functional and sequence diversity of 2-oxoglutarate/Fe(II)-dependent oxygenases in Metazoa. *Biochim. Biophys. Acta Gen. Subj.* 2017, 1861, 2922–2933. [CrossRef] [PubMed]

147. Zeymer, C.; Hilvert, D. directed evolution of protein catalysts. *Annu. Rev. Biochem.* 2018, 87, 131–157. [CrossRef] [PubMed]

148. Arnold, F.H. Directed evolution: Bringing new chemistry to life. *Angew. Chem. Int Ed. Engl.* 2018, 57, 4143–4148. [CrossRef] [PubMed]

149. Frey, R.; Hayashi, T.; Buller, R.M. Directed evolution of carbon–hydrogen bond activating enzymes. *Curr. Opin. Biotechnol.* 2019, 60, 29–38. [CrossRef] [PubMed]

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