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Expanding the synthetic scope of biocatalysis by enzyme discovery and protein engineering

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This paper is dedicated to the memory of Jon Williams who was a pioneer of chemoenzymatic catalysis, a field which has grown significantly thanks to his vision and ideas.

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ABSTRACT

Despite the growing impact of enzyme catalysis in industrial chemistry, the full potential of this technology is yet to be unlocked. Accessing new chemistries and expanding the scope of existing reactions is necessary in order to make biocatalysis a pivotal technology in the manufacturing of chemicals across the whole industrial spectrum. This review highlights how the biocatalytic toolbox for synthetic chemistry has recently been expanded by extending the scope of industrially relevant reactions, and the addition of new reactions via enzyme discovery or protein engineering.

1. Introduction

Enzymes are Nature's catalysts and have evolved to accelerate chemical reactions by several orders of magnitude displaying exquisite selectivity under mild reaction conditions. The implementation of enzymes in industrial processes covers many sectors of the chemical industry, although the main focus has usually been on the synthesis of chiral intermediates for pharmaceutical applications [1,2]. Associated with this are the high costs and time of enzyme engineering campaigns that are often needed to meet economical and process requirements. However, over the coming decade, a profound redesign in manufacturing processes will be necessary to enable the transition into a greener and more sustainable society. In this context, biocatalysis should be a pivotal technology for this change despite being traditionally hampered by the narrow range of chemical transformations compared to the outstanding versatility of organocatalytic reactions and transition metal (TM) chemistry. Recent remarkable advances have overcome this limitation by constructing artificial metalloenzymes which combine the optimal catalytic component [3], the incorporation of unnatural amino acids to escape inherent chemical limitations of canonical amino acids [4], manipulating cofactors to trigger new reactivities [5] or the *de novo* design of enzymes [6]. Some of these emerging strategies have been recently reviewed by Hyster et al. [7]. and will not be extensively considered in this survey. Herein, we review recent research, with particular emphasis on articles published in the past 5 years, focused on expanding the biocatalytic toolbox towards both new chemistries and broader substrate scopes by either enzyme discovery or natural metalloenzyme engineering. An emphasis has been placed on reactions with a high impact in the wider industrial chemistry community, which are emerging or have been incorporated into the field of biocatalysis, including halogenation, amide bond formation, or different strategies in C–H activation.

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2. New reactions through enzyme discovery

2.1. Carbon-halogen bond formation. Flavin-dependent halogenases

The incorporation of a halogen on to an aromatic ring is one of the most important transformations in organic chemistry as the products are starting materials for key reactions such as the Buchwald-Hartwig amination or the Suzuki-Miyaura or Heck couplings [8], as well as having an effect on the bioactivity or metabolic stability of a given molecule [9]. Conventional chemical methods often rely on hazardous chemicals and harsh conditions, therefore halogenases have emerged as promising alternatives to the production of halogenated compounds. Through a more sustainable manner as they operate under mild reaction conditions often in high selectivity and using simple and harmless halide salts as the halogen source [10,11]. Halogenases can be classified by the mechanism they proceed through and, in recent years, flavin-dependent halogenases (FDHs) have been demonstrated to have a larger potential for their use in organic synthesis.

2.1.1. Chlorination and bromination

The enzyme-mediated incorporation of chlorine and bromine into organic molecules has been the focus of extensive research in the last decade and nowadays biocatalysis offers a range of enzymes to perform these reactions in a highly selective and effective manner (Fig. 1a). In 2013, Payne et al. explored the synthetic utility of the tryptophan 7-halogenase from *Lechevaliera aerocolonigenes* (RebH) [12] by screening a panel of different indole derivatives and naphthalenes [13]. The regioselective chlorination and bromination was achieved in high yields up to a 100 mg scale. The combination of a tryptophan synthase with RebH was employed by Frese et al. to access a set of 22 halogenated tryptophan derivatives from indoles [14]. The same group applied this enzyme on a gram scale for the production of 7-bromo tryptophan demonstrating its potential for large scale synthesis [15]. In 2015, the Lewis group evolved a variant of RebH previously obtained with increased stability [16] to expand the substrate scope via random mutagenesis [17]. After three rounds of evolution, three variants showed increased substrate scope towards bulkier substrates showing up to a 67-fold increase in activity over wild-type RebH. Beyond RebH, regiocomplementary halogenases PyrH from *Streptomyces rugosporus* and PrnA from *Pseudomonas fluorescens* BL915 have also been the focus of recent studies. In 2015, the group of Micklefield altered both the activity and regioselectivity of PyrH and PrnA through structure-guided engineering [18]. Crystal structures permitted the targeted mutagenesis of various active site residues. Multiple variants were obtained with increased activity towards aromatic compounds. Remarkably, some variants showed altered regioselectivity as well, demonstrating that regiocomplementary variants can be accessed from the same wild-type template.

2.1.2. Iodination

Recently, the Goss group reported the discovery of a new flavin-dependent halogenase from a marine cyanophage that shows an activity previously not described [19]. Following a bioinformatics approach, they searched for different motifs known to be present in flavin-dependent halogenases as well as a new motif that had been previously overlooked. They identified a putative enzyme from the cyanophage Syn10 (VirX1) which shares low sequence similarity to other halogenases such as PrnA and RebH. They initially assessed the scope of this new enzyme by screening a panel of 400 substrates using halide salts (Fig. 1b). Generally, the enzyme showed poor chlorination activity whilst good bromination and, remarkably, iodination activity was detected. A total of 32 substrates could be iodinated in conversions that ranged from poor to excellent (5–95%). Kinetic studies indicated a preference for iodide over bromide and a crystal structure provided insight into the distinct features that enable the enzyme to bind and use bulkier halides.

2.1.3. Fluorination

The fluorinase from *Streptomyces cattleya* (Fig. 1c) [20] has also been the focus of recent studies [21] although the substrate scope is very narrow, hence its application in general synthetic chemistry is still very limited. Nevertheless, we envisage that new technologies in enzyme discovery such as metagenomics platforms or targeted bioinformatics could be powerful tools to find and deliver new

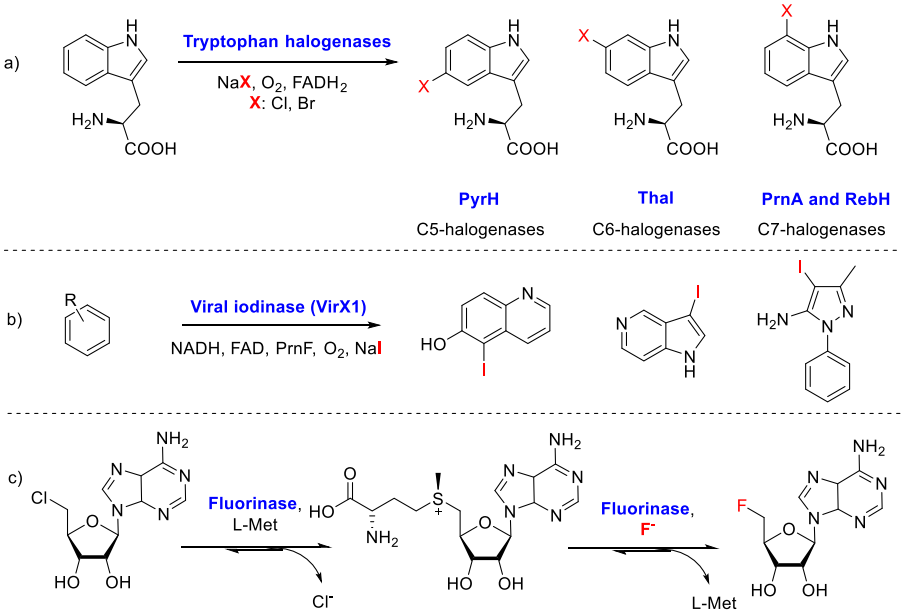

Fig. 1. Novel halogenases for the insertion of different halogens into organic compounds: a) different Tryptophan halogenases for the regioselective insertion of Cl and Br, **b)** halogenase from the cyanophage Syn10 displaying iodination activity, and **c)** the fluorinase from *Streptomyces cattleya*.

fluorinases for general use in industrial biotechnology in the coming years.

2.2. Carbon–carbon bond formation

The generation of new carbon-carbon bonds is arguably the most valuable transformation in organic chemistry although unfortunately it still constitutes one of the main challenges in biocatalysis. Other catalytic approaches excel in this area and they clearly outperform enzymatic catalysis to date. Lyases such as aldolases [22] and cyclases [23], as well as certain transferases have been traditionally used. In recent years promising studies on new enzyme families and strategies for the formation of carbon-carbon bonds have been reported and represent an area of research with huge potential in the coming decade.

2.2.1. Biocatalytic Friedel-Crafts and fries reactions

The Friedel-Crafts acylation is an aromatic electrophilic substitution reaction developed by Charles Friedel and James Crafts in 1877, in which the electrophile is an acyl halide activated by a strong Lewis acid such as $AlCl_3$, which is usually employed in stoichiometric amounts and under harsh reaction conditions. In 2017, Schmidt et al. reported a biocatalytic version of the Friedel-Crafts acylation using a multi-component acyl transferase (ATase) from *Pseudomonas protegens* [24]. In this manner, a series of resorcinol derivatives could be prepared in yields up to 99% using 2,4-diacetylphloroglucinol (DAPG) as acyl donor (Fig. 2a). Remarkably, for *o*-acyl derivatives, a rearrangement leading to the c-acylated product was observed in the presence of the ATase, which represents a formal Fries reaction and the first biocatalytic example. The influence of different additives [25] and acyl donors [26] was also studied and will inspire future research in this area that will enable the development of greener alternatives to this relevant reaction.

2.2.2. Trifluromethylation

As mentioned above, the introduction of halogen atoms into organic molecules can impact different pharmacokinetic properties. This is particularly interesting for fluorine introduction [27] and given the current limited synthetic scope of fluorinases, the insertion of the trifluoromethyl (CF_3) moiety can be an alternative and useful strategy for this purpose. In 2016, Simon et al. exploited the ability of laccases to generate phenol-derived radicals and combined it with the CF_3 radical generated *in situ* using conventional oxidants (Fig. 2b) [28]. Trifluoromethylation was tested against a series of substituted phenols which were successfully transformed in excellent conversions and isolated in moderate to good yields (32–62%), displaying a preference for the *meta*-substituted isomers in most cases. Additionally, and due to the mild reaction conditions, this method proved to be tolerant to different functional groups such as aldehydes, esters, ketones and nitriles. We envisage that this process can be coupled with further enzymatic steps to build artificial cascades, which can be applied in the preparation of synthetically relevant compounds.

2.2.3. (De)carboxylases

CO_2 fixation is key in different biosynthetic pathways although the enzymes involved, carboxylases, present a high substrate specificity and, therefore, their synthetic potential is quite limited [29]. Conversely, enzymatic decarboxylation is amongst one of the most common transformations in Nature and the enzymes responsible for this reaction can be found in many different degradation pathways. It is therefore in these biosynthetic routes where enzymes displaying a broader substrate scope are likely to be found [30]. There are different types of decarboxylases depending on the cofactor employed to overcome the inherent thermodynamic limitations of this reaction. This process is reversible, and under the appropriate reaction conditions, it can be performed in the synthetic direction to generate new carbon-carbon bonds using CO_2 as the carbon source. The group of Faber have done extensive research on metal dependent (Zn^{2+} and Mn^{2+}) decarboxylases in the past decade, especially on the synthetic applications of benzoic acid decarboxylases. For instance, in 2014, they studied three decarboxylases from *Aspergillus oryzae* (2,3-DHBD_Ao), *Trichosporon moniliiforme* (SAD_Tm) and *Rhizobium sp.* (2,6-DHBD_Rs) to develop a biocatalytic version of the Kolbe-Schmidt reaction [31]. A series of structurally diverse phenols were converted into the corresponding benzoic acids in moderate to good conversions, finding exquisite regioselectivity towards the *ortho* substitution with respect to the phenolic moiety. This study was further extended to other phenols with industrial interest such as resveratrol or phloretin [32]. Zhang et al. carried out a comprehensive biochemical characterisation and substrate profiling of the 2, 3-dihydroxybenzoic acid decarboxylase from *Fusarium oxysporum* (2,3-DHBD_*Fo*) looking at several parameters such as substrate loading, organic solvent tolerance or the effect of different additives [33]. The same group has recently reported the crystal structure of this enzyme which can provide insight into future engineering to expand its synthetic applications [34]. The potential of cofactor independent decarboxylases has also been explored. Phenolic acid decarboxylases (PADs) for instance have been used for the carboxylation of styrene derivatives [35], although the low conversions were obtained as well as the need of a *para*-substituted hydroxy group limiting the synthetic utility of this enzyme.

Enzymes from the UbiD family have recently shown to possess a huge potential for the carboxylation of organic molecules due to the broad substrate scope they display. These enzymes use a prenylated flavin cofactor (prFMN) which is synthesised by a flavin transferase (UbiX) [36]. In 2017, the synthetic utility of the decarboxylase from *Enterobacter cloacae* (*Ec*AroY) was explored towards different catechols employing either 3M potassium bicarbonate or pressurised CO_2 (30 bar) displaying modest activities and a higher preference for *para* substitution [37]. In 2018, Aleku et al. demonstrated that ferulic acid decarboxylases (FDCs) possessed a remarkably broad substrate scope for the decarboxylation of α,β-unsaturated carboxylic acids [38]. Despite the low conversions observed in the carboxylation reaction, the same authors have very recently shown that the ferulic acid decarboxylase from *Aspergillus niger* (*An*FDC) can be used to functionalise non-activated alkenes or aromatic systems. This was undertaken using CO_2 at near stoichiometric concentrations by coupling the carboxylation reaction with a series of enzymes to access primary alcohols, amines or amides (Fig. 2c) [39]. This report reveals that, despite the thermodynamic limitations, decarboxylases can be a valuable tool to generate synthetic complexity using CO_2 as a C1 synthon under mild reaction conditions.

2.3. Amide bond formation. ATP-dependent enzymes

The synthesis of an amide bond is one of the most important reactions in organic chemistry as this moiety is present in many chemicals in the pharmaceutical, polymer, and agrochemical industries. Classical methodologies consist of the use of coupling reagents to activate the carboxylic acid that is normally employed in stoichiometric amounts leading to processes with poor atom economy [40]. Different catalytic strategies have been recently developed although they often rely on the use of organic solvents and harsh reaction conditions therefore amide bond formation still represents a challenge in sustainable chemistry [41,42]. In this context, biocatalysis nowadays offers a good portfolio of enzymes

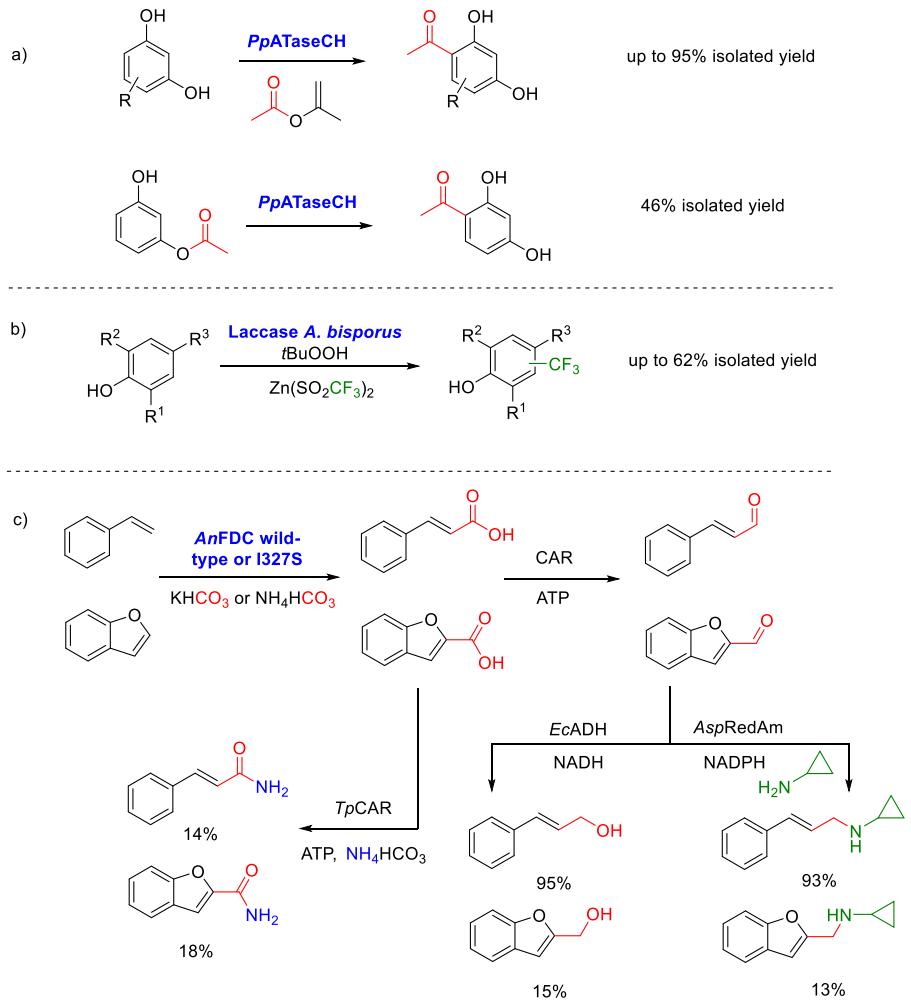

Fig. 2. New enzymatic strategies for C–C bond formation. a) Biocatalytic Friedel-Crafts and Fries reactions catalysed by a multi-component acyl transferase (ATase) from *Pseudomonas protegens*, **b)** Laccase-mediated trifluoromethylation of phenols and **c)** cascades involving the ferulic acid decarboxylase from *Aspergillus niger* (*An*FDC) for the functionalisation of non-activated alkenes and aromatics.

available for this task [43], with ATP-dependent enzymes emerging as promising catalysts to access these molecules in recent years. In the same way as conventional chemical approaches, they use ATP to activate the carboxylic acid via two different pathways, i.e. phosphorylation and adenylation. CoA ligases (CLs) for instance have been the focus of recent studies. These enzymes catalyse the formation of thioesters in two steps. Firstly, the acid is activated with ATP to form an acid-AMP intermediate that is then transformed into the corresponding thioester upon CoA addition. In 2018, Philpott et *al.* developed a whole-cell two-enzyme cascade system for the synthesis of amides by combining ATP-dependent CLs and *N*-acyltransferases (NATs) [44]. They initially screened a panel of 9 CLs towards a series of synthetically relevant carboxylic acids finding substrate complementary enzymes to take into the next step. A panel of 31 NATs was then screened towards a series of amines and different thioesters to assess the product scope of their collection, finding several NATs with good substrate promiscuity and allowing a diverse range of amide products to be accessed in high conversions. The synthetic potential of this *in vivo* system was assessed by synthesising an intermediate of the API (active pharmaceutical ingredient) lismapimod. *E. coli* cells expressing the 4-chlorobenzoate CL from *Alcaligenes sp.* and a serotonin hydroxycinnamoyl transferase from *Capsicum annuum* catalysed the coupling of 6-chloronicotinic acid and neopentylamine in 83% conversion and 74% isolated yield (Fig. 3a). A different strategy to access amides using CLs has been recently reported by Zaparucha and coworkers. In this case, the authors envisaged that in the absence of CoA and by working at higher temperatures, the acid-AMP intermediate can be transformed into the corresponding amide via non-catalysed amine addition over the activated acid intermediate [45]. In this manner, they designed a CoA-free system to synthesise *N*-methylbutyrylamide from butyric acid and methylamine (10 eq.) in excellent conversion (95%) and good yield (77%) using a thermophilic CL from *Metallosphaera sedula* in combination with a two-enzyme ATP regeneration system. Following a similar mechanism, carboxylic acid reductases (CARs) catalyse the reduction of carboxylic acids to the corresponding aldehydes [46,47] via adenylation to form an acyl adenylate intermediate that is subsequently thiolated to be finally reduced by a nicotinamide cofactor and yield the corresponding aldehyde. In 2017, Wood et al. envisaged that, in the absence of NADPH, the substrate can still be activated but not reduced, and it could then potentially react with different nucleophiles [48]. In this manner, promising conversions up to 25% to the corresponding primary amide with ammonia as the nucleophile were obtained using the CARs from *Mycobacterium marinum* (CAR*mm*) towards a series of benzoic and cinnamic acid

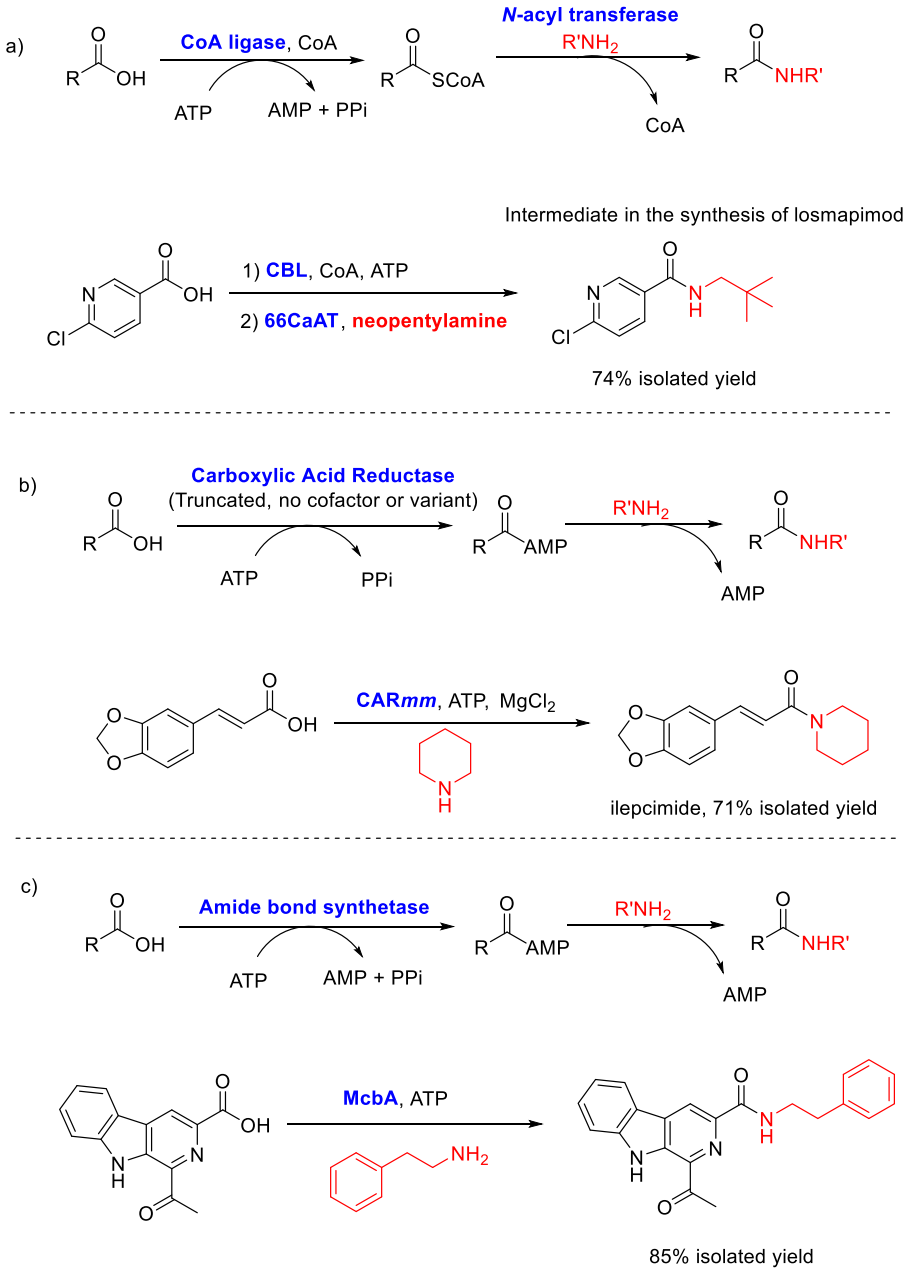

Fig. 3. New biocatalytic strategies for the synthesis of amides employing ATP-dependent enzymes with selected examples carried out at a preparative scale: **a)** cascade process by combining ATP-dependent CoA ligases and *N*-acyltransferases, **b)** use of truncated carboxylic acid reductases followed by amine addition, and **c)** use of amide-bond synthetases.

derivatives. Other amine-coupling partners were also attempted finding that CAR*mm* can also accept primary and secondary amines such as methylamine or piperidine respectively. To demonstrate the synthetic potential, the anticonvulsant API ilepcimide was synthesised in 96% conversion 71% isolated yield (Fig. 3b). Very recently, Lubberink et al. demonstrated the versatility of CAR truncated constructs for amide bond synthesis by accessing a remarkable set of pharmaceutically relevant amides from diamines [49]. Another system that has recently shown potential in organic synthesis is amide bond synthetases. Amongst them, the amide-bond synthetase from *Marinactinospora thermotolerans* (McbA) has been the focus of recent studies. It was firstly described by Chen et al. in 2013 [50] and employed to prepare some products bearing the ß-carboline scaffold. More recently, the Grogan group reported the use of McbA for the synthesis of pharmaceutically relevant amides (Fig. 3c) [51]. Several ß-carboline derived carboxylic acids as well as other aromatic acids were screened along with several aromatic amines. This enzyme exhibited a broad substrate scope with respect to the acid and 2-phenylethylamine which was found to be the best amine-coupling partner. Employing this particular amine, several preparative-scale reactions were carried out to synthesise a series of pharmaceutically relevant amides in good to excellent isolated yields (50–85%). The use of this enzyme for the preparation of amide-derived APIs has been further exemplified by the same authors [52]. Using a new ATP regeneration system involving two polyphosphate kinases (AjPPK2-II, SmPPK2-I) and polyphosphate, the synthesis of the antidepressant moclobemide from 4-chlorobenzoic acid and 4-(2-aminoethyl)morpholine was

achieved in 64% isolated yield. Another adenylating enzyme that has recently shown potential for its use in industrial biotechnology is TamA. This enzyme is key in the biosynthesis of the natural product Tambjamine YP1 which was found to have antimicrobial, antimalarial, and cytotoxic activities. Recently, Marchetti et al. successfully obtained its adenylating domain and its ability for the synthesis of long-chain amides was assessed by coupling a range of fatty acids with different amines and amino acids [53]. Although the conversions obtained were modest, these promising results demonstrate the potential of this system for the preparation of pharmaceutically relevant amides.

2.4. Carbon–oxygen bond formation

The biocatalytic toolbox to create new C–O bonds is quite extensive, with a broad range of oxygenases and peroxygenases available for this task. A recent and comprehensive revision by Hollmann and coworkers reviews the use of these enzymes, which involves different redox processes, from a synthetic perspective so they will not be the focus of this survey [54]. Hydratases on the other hand, catalyse the redox neutral reversible addition of H_2O to non-activated C–C double bonds, opening a new route to the formation of C–O bonds under mild reaction conditions.

2.4.1. Hydratases

Hydration of double bonds to access alcohols is often carried out under harsh conditions which also result in a lack of selectivity. As a promising alternative, hydratases or hydro-lyases catalyse the reversible addition of water to both activated and non-activated C–C double bonds in high regio-, stereo- and enantioselectivity (Fig. 4a). Several cofactor-dependent enzymes have been already described [55] although in recent years there has been some growing interest towards cofactor independent hydratases due to their potential industrial applications [56]. Most hydratases are limited by their high substrate specificity, however in the past 4 years, interesting developments have been made with fatty acid hydratases. Initial investigations on the substrate scope of the oleate hydratase from *Elizabethkingia meningoseptica* (*Em*OAH) revealed that the scope is not only restricted to fatty acids but, remarkably, alkenes can also be hydrated to yield chiral secondary alcohols [57]. Recently, the same group identified a residue in position 248 as responsible for substrate recognition. An Ala248Leu variant was found to be an efficient catalyst for the preparation of a series of chiral secondary alcohols starting from non-activated alkenes (Fig. 4b) [58]. Very recently, an oleate hydratase from *Paracoccus aminophilus* DSM 8538 was combined in a cascade process with an alcohol dehydrogenase to synthesise 10-oxostearic acid from oleic acid [59]. The process was scaled up to 1L obtaining the target product in 96% conversion and a space-time yield up to 552 g L^{-1} d^{-1}, demonstrating the great potential of these enzymes for large scale use.

3. New reactions through enzyme engineering

3.1. New to nature chemistry in heme-dependent proteins

The re-purposing and tuning of enzymes towards abiological chemistries has been completely underpinned by the power of directed evolution [60]. Iterative rounds of mutagenesis through PCR-based methods gives rise to genetic variation which is complemented with (commonly chromatographic-based) screening towards a desired function selecting the most appropriate candidate. Desired functions include optimisation of stereoselectivity, or enhanced performance and stability under diverse sets of reaction conditions. However, drawing from small-molecule catalysis (chemomimetic biocatalysis), directed evolution has been pivotal in unlocking a whole repertoire of biocatalysts where such reactions cannot be found in Nature, often starting from minimal promiscuous activity. At the forefront of such research are heme-dependent proteins, including P450 monooxygenases and myoglobin proteins. P450s are a truly versatile class of enzymes, which through a natural iron-oxene intermediate species carry out a range of oxygenation reactions including C–H hydroxylations, dealkylations, sulfoxidations, and epoxidations [60]. Transition metal-based metalloporphyrin complexes developed by synthetic chemists sought to mimic P450 oxene transferase activity through carbene and nitrene transfers to access non-natural transformations, however, these complexes failed to match stereoselectivity offered by biocatalysts [60]. Utilising this knowledge, heme-dependent proteins have been, over the last decade, evolved to perform carbene and nitrene transfers installing valuable chemistries in proteins with no biological counterparts.

3.2. Carbene transferases furnishing carbocycles

Carbenes are highly reactive species that enable the formation of new carbon-carbon or carbon-heteroatom bonds and its transfer reaction has proven to be a useful transformation in synthetic chemistry [61]. Enzymatic carbene transfers proceed through a high-valent iron-carbenoid intermediate species (Fig. 5) [60], and in recent years, enormous efforts by the groups of Arnold, Fasan, and Hartwig have been undertaken for the formation of carbocyclic rings, namely cyclopropanes, using engineered heme proteins typically through whole-cell based biotransformations (Fig. 5a). A

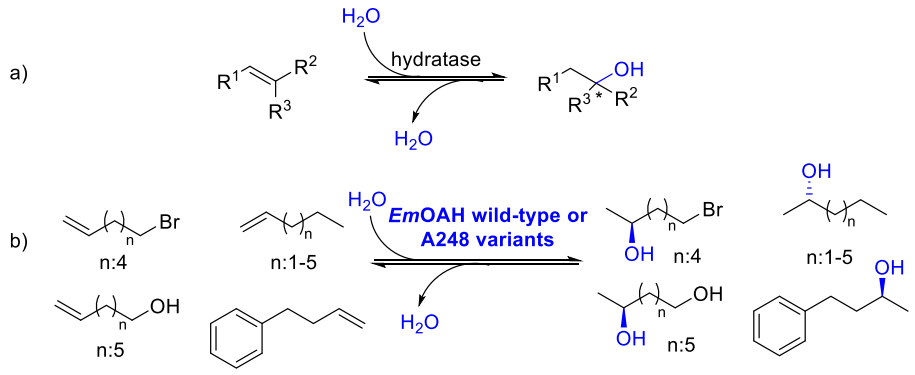

Fig. 4. New hydratases for the synthesis of chiral alcohols from non-activated alkenes: a) General scheme for the hydration of alkenes catalysed by hydratases. **b)** Substrate scope displayed by the oleate hydratase from *Elizabethkingia meningoseptica* (*Em*OAH) wild-type and A248 variants.

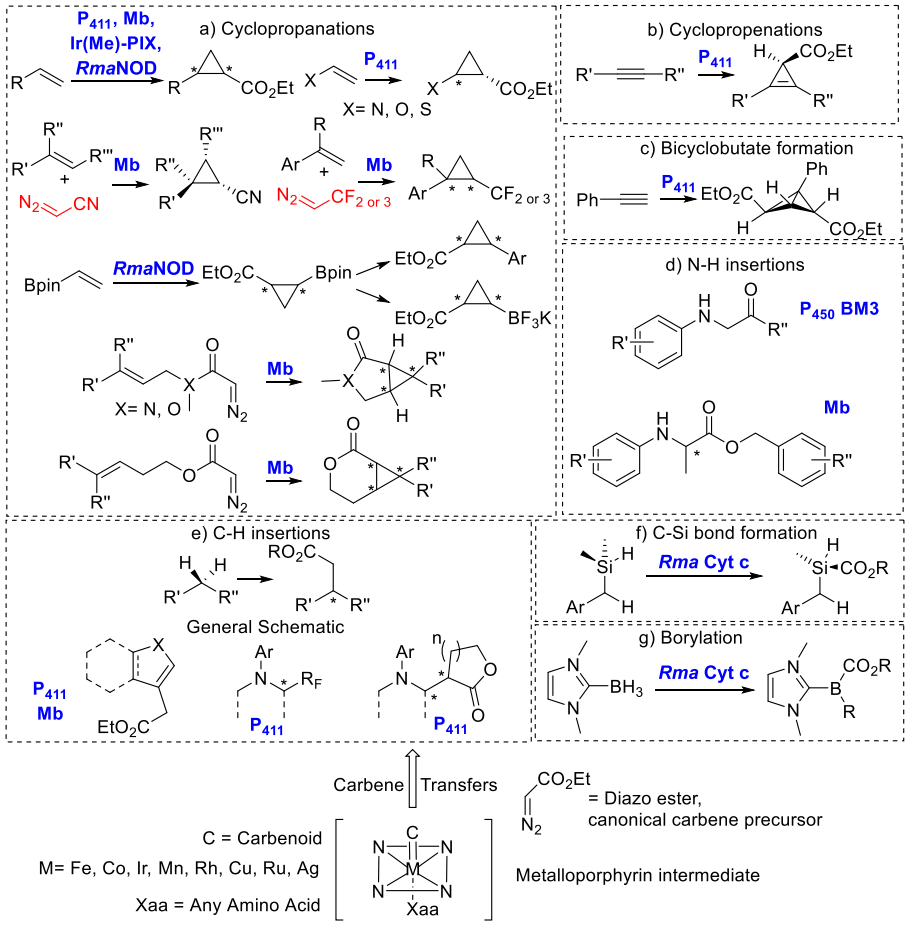

Fig. 5. A recent snapshot of new-to nature carbene transfer chemistries in engineered heme-dependent proteins. Boxes **a-g** represent the recent scope of carbene transfers where upon addition of the diazoester reagent this forms carbenoid intermediate species with metal (co-ordinated in the porphyrin ring) where N_2 is released (not shown). This carbene is then transferred from the metallo-intermediate to the corresponding reagent. Box **a** where various cyclopropanation reactions are shown, atypical diazoester substrates are highlighted in red. P411: serine ligated P450. Box **d** shows the products of carbene N—H insertions. P411: Engineered serine ligated P450s. Box **e** gives the general schematic for sp^3 C—H insertion with specific products given below. P411: Engineered serine ligated P450s. Mb: myoglobin variants. Ir(Me)-PIX: iridium containing P450s. *Rma*NOD: *Rhodothermus marinus* nitric oxide dioxygenase. *Rma* Cyt C: Cytochrome c from *Rhodothermus marinus*.

key histidine to serine mutation for heme ligation in $P450_{BM3}$ from *Bacillus megaterium* gave rise to powerful cytochrome P411s for carbene transfers yielding cyclopropane products starting from inexpensive styrene [62]. Following the evolutionary trajectories of P411s, challenging and precise synthesis of highly strained cyclopropenes and bicyclobutanes have recently been enabled from terminal alkynes (Fig. 5b and c respectively) in excellent yields, offering further substrate diversification to these enzymes [63]. This was further extended to include internal alkynes [64], where no previous systems existed for the cyclopropenenation.

To extend the olefin cyclopropanation scope, engineering campaigns on P411s, wild-type *Rhodothermus marinus* nitric oxide dioxygenase (*Rma*NOD), and a protoglobin allowed for the acceptance of unactivated and electron-deficient alkenes, which are attractive feedstocks to such reactions [65]. Further engineering on P411s was recently conducted by the Arnold group to access heteroatom-substituted cyclopropanes, which are present in several relevant chemicals with *N*-, S- and O- containing moieties such as the anti-viral drug Grazoprevir (Fig. 5a) [66]. Advancing the incorporation and stereospecificity of biocatalysis towards early drug development, the Arnold lab applied *Rma*NOD towards the synthesis of pinacolboronate (Bpin) substituted cyclopropane diastereo-divergent products on multi-gram scale (Fig. 5a). Suzuki—Miyaura coupling was further employed to generate aromatic cyclopropane products in up to 92% yield with >99% *ee* [67].

To expand the toolbox of non-natural transformations with heme-dependent proteins, Fasan and co-workers engineered a myoglobin protein towards carbene transfers [60]. Myoglobins, with their natural function being an oxygen reservoir, are smaller compared to P450s, with a mass of approximately 17 kDa, offering reduced complexity for evolution. Engineered myoglobin carbene transferases have been sought to synthesise a wide range of cyclopropane scaffolds to encompass tricyclic [68], nitrile-substituted [69], difluoro- and trifluoromethyl-substituted (Fig. 5a) [70,71]. Nitrile and trifluoromethylated cyclopropane products serve as important functional handles for further derivatisation. Reduction with lithium aluminium hydride followed by *N*-benzyolation produced a methylamino derivative from a nitrile-substituted cyclopropane substrate, where this methylamino scaffold can be found in the antidepressant Levomilnacipran [72].

For the neat synthesis of cyclopropane fused γ-lactones (Fig. 5a), site saturation mutagenesis was carried out on myoglobin variants towards intra-molecular stereocomplementary cyclopropanation with allylic diazoacetate derivatives through cyclisation, with a wide range of substituents tolerated on the phenyl group [73]. Gram scale synthesis of dimethyl cyclopropane 3-oxabicyclohexan-2-one was carried out in 83% yield enabling the total synthesis of

pyrethroid natural products. Following this, Ren and co-workers employed allylic diazoacetamides to access cyclopropane fused γ-lactams [74]. Excellent selectivity was observed across all 13 substrates; although turnover numbers were relatively low (200 TON). In a similar system, over multiple rounds of directed evolution the sperm-whale myoglobin biocatalyst was able to furnish cyclopropane fused δ-lactones (Fig. 5a) [75], forming the scaffold of the antidepressant Dinardokanshone B. The fused δ-Lactones were synthesised in up to 99% yield highlighting the efficiency of such a system, where few poorly selective transition-metal based approaches have been previously described.

Both P450s and myoglobin catalysts have also been applied to carbene N–H insertions in forming C–N bonds, a highly prevalent (chiral) bond in many new chemical entities. $P450_{BM3}$ was first reported by the Arnold lab to undertake carbenoid N–H insertions [76], where this had previously been reported with metalloporphyrins in organic solvents, however suffered from poor chemoselectivity. Aniline combined with ethyl diazoacetate (EDA) as the precursor was chosen as the model substrate (Fig. 5d), which was then expanded to a variety of substituted anilines. Recently, the Fasan lab expanded this scope using myoglobin variants to a variety of aromatic amines with benzylayted 2-diazopropanoate esters and various derivatives for asymmetric carbene N–H insertions (Fig. 5d) [77]. It was observed that a combination of protein engineering combined with engineering of *para*-, *meta*- and *ortho*-substituents of the aromatic 2-diazopropanoate esters heavily influenced the stereoinduction of the carbene transfer. The products of these carbene transfers offer a sustainable route and overcome challenges associated chemocataytic methods in the synthesis α-amino acid derivatives.

Replacing iron for iridium (an abiological noble metal) in the porphyrin ring of heme-proteins such as P450s, garners different activities whilst still maintaining protein structure. Hartwig and co-workers applied Ir(Me)-PIX enzymes for the cylcopropanation across a broad set of substrates to include natural terpenes and derivatives, as well as aliphatic alkenes, with several acyclic substrates achieving excellent selectivity with numerous diazo ester precursors [78].

3.3. Carbene transferases enabling X–H formation and functionalisation

Nature's toolbox for sp^3 C–H functionalisation consists of mainly alkyltransferases, but in recent years carbene transfer reactions enabled by engineered heme proteins proved to be particularly powerful (Fig. 5e). For instance, engineered Ir(Me)-PIX CYP119 variants were generated for the selective meta- and *para*-functionalisation of phthalan derivatives, overcoming the poor chemoselectivity of small molecule catalysis where multiple C–H insertions is often observed [79]. Such derivatives were chosen as they contain two sp^3 centres with similar steric and electronic environments where multiple functionalisations may occur. Moreover, iron containing P411s have been shown to perform sp^3 C–H functionalisation across a range of benzylic, allylic, propargylic and alkyl amines substrates [80]. To show that P411s are not limited to EDA, fluoroalkylation was also achieved with 2,2,2-trifluoro-1-diazoethane and derivatives as the carbene precursor (Fig. 5e) [81]. As highlighted previously, fluoroalkylated compounds are prevalent in the field of medicinal chemistry where such carbene transfers are also unachievable with small molecule catalysis. Recently, the use of P411s was also expanded to lactone-carbene precursors to generate γ-lactone derivatives (Fig. 5e) [82]. In addition, upon screening thousands of variants over 17 rounds of directed evolution [83], P411–HF (heterocycle functionalisation), and variants derived from the P411–CIS [62] were able to selectively functionalise sterically demanding heterocycles (indoles and pyrroles) with high catalytic efficiencies (Fig. 5e). Similarly, but displaying lower catalytic efficiencies than P411 variants, myoglobin carbene transferases were able to selectively functionalise unprotected indoles, and their synthetic potential was shown by developing a chemoenzymatic synthesis of the anti-inflammatory indomethacin [84].

Installing the abiological element silicon through carbene transfers highlights the proficiency of engineering heme-dependent biocatalysts. In 2016, Kan and co-workers engineered the Cytochrome c from *Rhodothermus marinus* (*Rma* cyt c) to introduce a range of carbon-silicon bonds generating 20 silicated

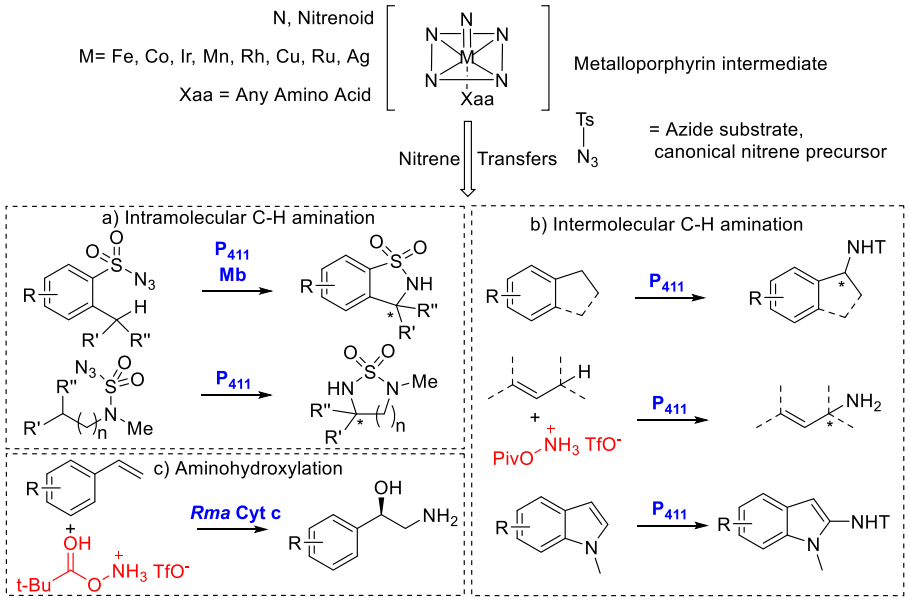

Fig. 6. A recent snapshot of new-to nature nitrene transfer chemistries in engineered heme-dependent proteins. Boxes **a-c** represent the recent scope of nitrene transfers. Nitrene transfer proceeds in a similar mechanism to that of the carbene transfers. Hydroxylamine precursors (**b** and **c**) over azide substrates are highlighted in red. P411: Engineered serine ligated P450s. Mb: myoglobin variants. *Rma* Cyt C: Cytochrome c from *Rhodothermus marinus*.

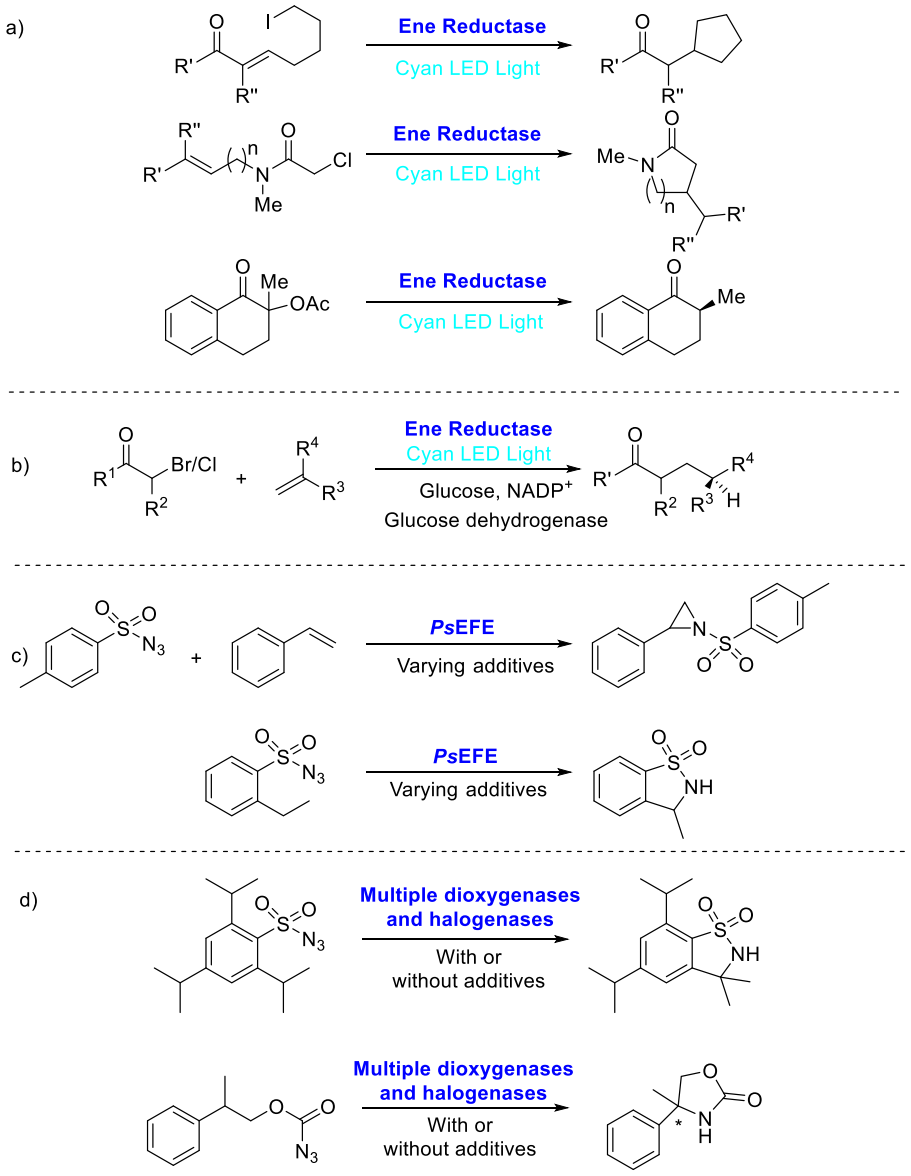

Fig. 7. A recent snapshot of new-to nature chemistries in non-heme dependent proteins. a, Represents the work of the Hyster lab through photoexcitation of ene-reductases to carry out varied chemistries (radical dehalogenation and cyclisation, deacetoxylation). **b**, radical intermolecular hydroalkylation to access γ-chiral carbonyl compounds. **c** and **d,** nitrene transfers in non-heme dependent iron containing proteins. *Ps*EFE: ethylene forming enzyme from *Pseudomonas savastanoi.*

products using primarily ethyl 2-diazopropanoate (Me-EDA) as the carbene precursor (Fig. 5f) [85]. This was achieved with up to exceptional TTNs (47−8210 TTNs), particularly when compared to small-molecule catalysts (<100 TTNs).

As a biocatalytic first, *Rma* cyt c was further engineered for carbon-boron bond formation through carbene transfers (Fig. 5g) [86], this borylation platform recently expanded the range of carbene precursors, with 5-, 6-, and 7-membered lactone carbenes being accepted with up to 24,000 TTN in a similar substrate trajectory to that of P411 C−H functionalisation. Structurally diverse and bulky trifluorodiazo precursors were also accommodated by *Rma* cyt c BOR-CF_3 [87], and molecular dynamic simulations demonstrated that the beneficial mutations (M103D and Y44I) in the active site facilitated the stabilisation of the diazo and carbene intermediates. It was found that the large diazo substituents were exposed towards the solvent and the trifluoro group towards the heme pocket. Such constrained conformation therefore allowed for the highly selective carbene transfer.

3.4. Nitrene transferase chemistry

Proceeding through a similar Fe^{III}/Fe^{II} nitrenenoid intermediate, nitrene transfers offer a further expansion of hemeproteins' abilities to perform abiological chemistries (Fig. 6). The Arnold lab first engineered $P450_{BM3}$ towards the intramolecular C−H aminations of azide substrates through nitrene transfers [60], then in 2017 expanded towards the synthesis of intermolecular amination across a range of aryl substrates [88]. Again demonstrating that engineered hemeproteins can outcompete small molecule catalysts, an engineered platform of P411 variants were applied to the synthesis of a huge architecture of enantioenriched cyclic sulfamides (Fig. 6a) [89], which were further processed to give the diamine through primary, secondary and previously unattainable tertiary sp^3 C−H amination. Rivalling the scope of other chiral amine biocatalysts (transaminases, amine dehydrogenases, imine reductases), $P411_{B/APA}$ - referring to benzylic and allylic primary aminase-yielded a range of aliphatic primary unprotected amines

in high yields (up to 93%) from hydroxylamine precursors without necessitating the pre-oxidised substrate (Fig. 6b) [90]. However, regioselectivity could be a challenge across further substrate diversification.

A hindrance in nitrene transfers is often observed in the reduction of the azide substrate to the corresponding sulfonamide. By reducing the rate of electron transfer with a W1046P mutation, Brandenburg and co-workers managed to tightly control such selectivity in the amination of indoles. This reduced the sulfonamide side product yield by 10% and increased the amidation yield by 10%, generating C_2-aminated indoles with up to 91% yield and 8000 TTN (Fig. 6b) [91]. In an effort to push the activity of nitrene transferases to comparable levels observed in P450 native monooxygenase activity, the Fasan group following a structure guided approach to engineer three P450s (XplA and BezE, and FL#62 a variant of $_{BM3}$) coupled with deep mechanistic studies through kinetic isotopic effect experiments achieving up to 14,000 TTNs for intramolecular C–H amination (Fig. 6a) [92]. By targeting structurally conserved elements, it was determined that, with sulfonyl azides, activation is the rate limiting step in the formation of the niterenoid and disruption of the electron relay aids C–H aminase activity, consistent with other studies.

Recently, following site saturation mutagenesis, *Rma* cyt c was used as template to generate a 7-point variant TQL which was applied to the synthesis of olefin derived chiral 1,2-amino alcohols (Fig. 6c) [93]. Such reactions were performed on a 2.0 mmol scale achieving up to 61% yield. Fluctuations in selectivities were observed across the scope, however this offers a new route to aminoalcohols bypassing multi-enzyme cascades in a single step [94].

3.5. Abiological chemistries in non-heme dependent proteins

Although not covered in this review comprehensively (see Chen and Arnold for a detailed survey) [60] it is worth mentioning that non-heme dependent proteins have also incorporated a wide range of further non-natural chemistries into their scopes. Recently, the Hyster lab through photoexcitation of the nicotinamide cofactor (NADPH) to generate a single electron reductant has allowed the introduction of (new) radical chemistries into proteins. These include radical dehalogenation and cyclisations [95], ketone reductions, and deacetoxylations all in the well-established family of ene-reductases (EREDs) (Fig. 7a) [7]. In 2020, Huang and co-workers pushed the radical photoreactivity scope of EREDs to allow for the intermolecular hydroalkylation furnishing chiral γ-carbonyl compounds with perfect enantioselectivities (Fig. 7b) [96]. Chiral γ-carbonyl moieties are found in a number of bioactive compounds such (*R*)-1-hydroxyboivinianin belonging to the family of Sesquiterpenes.

Nitrene transfers have also been explored in non-heme dependent mononuclear proteins. The class mononuclear proteins are representative of many families of enzymes, including Rieske oxygenases and α-ketoglutarate dependent dioxygenases [97], and are capable of performing a range of natural oxidation reactions implemented in degradation pathways of xenobiotics. These mononuclear proteins also possess a nitrenoid intermediate analogous to that observed in engineered P450s and other heme-dependent proteins. The Arnold group first demonstrated nitrene transfers in engineered non-heme dependent enzymes through directed evolution of an ethylene forming enzyme from *Pseudomonas savastanoi* (*Ps*eEFE) for olefin aziridination (Fig. 7c) [98]. In the same study this protein was further mutated to incorporate nitrene transfers for intramolecular C–H insertions with aromatic sulfonyl azide substrates. Also capable of such transfers over several rounds of active site engineering of a naphthalene diooxygenases (NDO), this enzyme was capable of performing gram-scale intramolecular C–H amination on aromatic sulfonyl azide (Fig. 7d) [99]. With both engineered enzymes lacking the coordinating heme complex it was noticed that in the case of *Ps*eEFE the selectivity and activity could easily be modified with simple additives, such as acetate or *N*-oxalylglycine instead of αKG. These non-heme dependent iron containing enzymes offer promising routes coupled with further engineering towards previously unattainable nitrene or carbene transfers. Also in a recent study, mononuclear nonheme-dependent hydroxylases were repurposed for nitrile formation with the assistance of an azido group expanding this families reaction scope [100].

4. Conclusions and outlook

Over the last decade, by either enzyme discovery or protein engineering, the portfolio of reactions that can be accessed via biocatalytic strategies has greatly expanded. In this report, we have reviewed recent and selected examples of new biocatalytic reactions based on their potential impact on synthetic chemistry. However, other important transformations not surveyed in this article have recently been added to the biocatalytic toolbox such as nitrations [101–104], cyclisations [105–107], Diels-Alder reaction [108–110], and SAM-dependent alkylations [111–113], which we envision they will also be the focus of extensive research in the coming years and highlights the broad scope currently offered through enzyme catalysis. We also envisage that new and diverse enzyme discovery platforms such as metagenomics combined with efficient screening strategies or retrosynthetic tools will enable the discovery or application of new reactions that can be useful in industrial chemistry. Moreover, advances in computational tools, *de novo* design of enzymes, novel artificial metalloenzymes, incorporation of non-canonical amino acids, and the combination of these areas will lead to new chemistries and hence a greater uptake of industrial biocatalysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] S. Wu, R. Snajdrova, J.C. Moore, K. Baldenius, U. Bornscheuer, Biocatalysis: enzymatic synthesis for industrial applications, Angew. Chem. Int. Ed. (2020), https://doi.org/10.1002/anie.202006648.
[2] P.N. Devine, R.M. Howard, R. Kumar, M.P. Thompson, M.D. Truppo, N.J. Turner, Extending the application of biocatalysis to meet the challenges of drug development, Nat. Rev. Chem. 2 (2018) 409–421, https://doi.org/10.1038/s41570-018-0055-1.
[3] H.J. Davis, T.R. Ward, Artificial metalloenzymes: challenges and opportunities, ACS Cent. Sci. 5 (2019) 1120–1136, https://doi.org/10.1021/acscentsci.9b00397.
[4] C. Mayer, Selection, addiction and catalysis: emerging trends for the incorporation of noncanonical amino acids into peptides and proteins in vivo, Chembiochem 20 (2019) 1357–1364, https://doi.org/10.1002/

cbic.201800733.
[5] M.A. Emmanuel, N.R. Greenberg, D.G. Oblinsky, T.K. Hyster, Accessing non-natural reactivity by irradiating nicotinamide-dependent enzymes with light, Nature 540 (2016) 414–417, https://doi.org/10.1038/nature20569.
[6] L. Jiang, E.A. Althoff, F.R. Clemente, L. Doyle, D. Röthlisberger, A. Zanghellini, J.L. Gallaher, J.L. Betker, F. Tanaka, C.F. Barbas, D. Hilvert, K.N. Houk, B.L. Stoddard, D. Baker, De novo computational design of retro-aldol enzymes, Science 319 (2008) 1387–1391, https://doi.org/10.1126/science.1152692.
[7] B.A. Sandoval, T.K. Hyster, Emerging strategies for expanding the toolbox of enzymes in biocatalysis, Curr. Opin. Chem. Biol. 55 (2020) 45–51, https://doi.org/10.1016/j.cbpa.2019.12.006.
[8] A. Biffis, P. Centomo, A. Del Zotto, M. Zecca, Pd metal catalysts for cross-couplings and related reactions in the 21st century: a critical review, Chem. Rev. 118 (2018) 2249–2295, https://doi.org/10.1021/acs.chemrev.7b00443.
[9] Z. Xu, Z. Yang, Y. Liu, Y. Lu, K. Chen, W. Zhu, Halogen bond: its role beyond drug-target binding affinity for drug discovery and development, J. Chem. Inf. Model. 54 (2014) 69–78, https://doi.org/10.1021/ci400539q.
[10] C. Schnepel, N. Sewald, Enzymatic halogenation: a timely strategy for regioselective C–H activation, Chem. Eur J. 23 (2017) 12064–12086, https://doi.org/10.1002/chem.201701209.
[11] D.S. Gkotsi, J. Dhaliwal, M.M. McLachlan, K.R. Mulholand, R.J. Goss, Halogenases: powerful tools for biocatalysis (mechanisms applications and scope), Curr. Opin. Chem. Biol. 43 (2018) 119–126, https://doi.org/10.1016/j.cbpa.2018.01.002.
[12] E. Yeh, S. Garneau, C.T. Walsh, Robust in vitro activity of RebF and RebH, a two-component reductase/halogenase, generating 7-chlorotryptophan during rebeccamycin biosynthesis, Proc. Natl. Acad. Sci. U. S. A 102 (2005) 3960–3965, https://doi.org/10.1073/pnas.0500755102.
[13] J.T. Payne, M.C. Andorfer, J.C. Lewis, Regioselective arene halogenation using the FAD-dependent halogenase RebH, Angew. Chem. Int. Ed. 52 (2013) 5271–5274, https://doi.org/10.1002/anie.201300762.
[14] M. Frese, P.H. Guzowska, H. Voß, N. Sewald, Regioselective enzymatic halogenation of substituted tryptophan derivatives using the FAD-dependent halogenase RebH, ChemCatChem 6 (2014) 1270–1276, https://doi.org/10.1002/cctc.201301090.
[15] M. Frese, N. Sewald, Enzymatic halogenation of tryptophan on a gram scale, Angew. Chem. Int. Ed. 54 (2015) 298–301, https://doi.org/10.1002/anie.201408561.
[16] C.B. Poor, M.C. Andorfer, J.C. Lewis, Improving the stability and catalyst lifetime of the halogenase RebH by directed evolution, Chembiochem 15 (2014) 1286–1289, https://doi.org/10.1002/cbic.201300780.
[17] J.T. Payne, C.B. Poor, J.C. Lewis, Directed evolution of rebh for site-selective halogenation of large biologically active molecules, Angew. Chem. Int. Ed. 54 (2015) 4226–4230, https://doi.org/10.1002/anie.201411901.
[18] S.A. Shepherd, C. Karthikeyan, J. Latham, A.W. Struck, M.L. Thompson, B.R.K. Menon, M.Q. Styles, C. Levy, D. Leys, J. Micklefield, Extending the biocatalytic scope of regiocomplementary flavin-dependent halogenase enzymes, Chem. Sci. 6 (2015) 3454–3460, https://doi.org/10.1039/c5sc00913h.
[19] D.S. Gkotsi, H. Ludewig, S.V. Sharma, J.A. Connolly, J. Dhaliwal, Y. Wang, W.P. Unsworth, R.J.K. Taylor, M.M.W. McLachlan, S. Shanahan, J.H. Naismith, R.J.M. Goss, A marine viral halogenase that iodinates diverse substrates, Nat. Chem. 11 (2019) 1091–1097, https://doi.org/10.1038/s41557-019-0349-z.
[20] C. Dong, F. Huang, H. Deng, C. Schaffrath, J.B. Spencer, D. O'Hagan, J.H. Naismith, Crystal structure and mechanism of a bacterial fluorinating enzyme, Nature 427 (2004) 561–565, https://doi.org/10.1038/nature02280.
[21] H. Sun, W.L. Yeo, Y.H. Lim, X. Chew, D.J. Smith, B. Xue, K.P. Chan, R.C. Robinson, E.G. Robins, H. Zhao, E.L. Ang, Directed evolution of a fluorinase for improved fluorination efficiency with a non-native substrate, Angew. Chem. 128 (2016) 14489–14492, https://doi.org/10.1002/ange.201606722.
[22] C.L. Windle, M. Müller, A. Nelson, A. Berry, Engineering aldolases as biocatalysts, Curr. Opin. Chem. Biol. 19 (2014) 25–33, https://doi.org/10.1016/j.cbpa.2013.12.010.
[23] P.O. Syrén, S. Henche, A. Eichler, B.M. Nestl, B. Hauer, Squalene-hopene cyclases - evolution, dynamics and catalytic scope, Curr. Opin. Struct. Biol. 41 (2016) 73–82, https://doi.org/10.1016/j.sbi.2016.05.019.
[24] N.G. Schmidt, T. Pavkov-Keller, N. Richter, B. Wiltschi, K. Gruber, W. Kroutil, Biocatalytic friedel–crafts acylation and Fries reaction, Angew. Chem. Int. Ed. 56 (2017) 7615–7619, https://doi.org/10.1002/anie.201703270.
[25] N.G. Schmidt, W. Kroutil, Acyl donors and additives for the biocatalytic friedel–crafts acylation, Eur. J. Org Chem. (2017) 5865–5871, https://doi.org/10.1002/ejoc.201701079.
[26] A. Żądło-Dobrowolska, N.G. Schmidt, W. Kroutil, Thioesters as acyl donors in biocatalytic friedel-crafts-type Acylation catalyzed by acyltransferase from Pseudomonas protegens, ChemCatChem 11 (2019) 1064–1068, https://doi.org/10.1002/cctc.201801856.
[27] H.J. Böhm, D. Banner, S. Bendels, M. Kansy, B. Kuhn, K. Müller, U. Obst-Sander, M. Stahl, Fluorine in medicinal chemistry, Chembiochem 5 (2004) 637–643, https://doi.org/10.1002/cbic.200301023.
[28] R.C. Simon, E. Busto, N. Richter, V. Resch, K.N. Houk, W. Kroutil, Biocatalytic trifluoromethylation of unprotected phenols, Nat. Commun. 7 (2016) 1–6, https://doi.org/10.1038/ncomms13323.
[29] S.M. Glueck, S. Gömös, W.M.F. Fabian, K. Faber, Biocatalytic carboxylation, Chem. Soc. Rev. 39 (2010) 313–328, https://doi.org/10.1039/b807875k.
[30] S.E. Payer, K. Faber, S.M. Glueck, Non-oxidative enzymatic (De)Carboxylation of (Hetero)Aromatics and acrylic acid derivatives, Adv. Synth. Catal. 361 (2019) 2402–2420, https://doi.org/10.1002/adsc.201900275.
[31] C. Wuensch, J. Gross, G. Steinkellner, A. Lyskowski, K. Gruber, S.M. Glueck, K. Faber, Regioselective ortho-carboxylation of phenols catalyzed by benzoic acid decarboxylases: a biocatalytic equivalent to the Kolbe-Schmitt reaction, RSC Adv. 4 (2014) 9673–9679, https://doi.org/10.1039/c3ra47719c.
[32] K. Plasch, V. Resch, J. Hitce, J. Popłoński, K. Faber, S.M. Glueck, Regioselective enzymatic carboxylation of bioactive (Poly)phenols, Adv. Synth. Catal. 359 (2017) 959–965, https://doi.org/10.1002/adsc.201601046.
[33] X. Zhang, J. Ren, P. Yao, R. Gong, M. Wang, Q. Wu, D. Zhu, Biochemical characterization and substrate profiling of a reversible 2,3-dihydroxybenzoic acid decarboxylase for biocatalytic Kolbe-Schmitt reaction, Enzym. Microb. Technol. 113 (2018) 37–43, https://doi.org/10.1016/j.enzmictec.2018.02.008.
[34] M. Song, X. Zhang, W. Liu, J. Feng, Y. Cui, P. Yao, M. Wang, R.T. Guo, Q. Wu, D. Zhu, 2,3-Dihydroxybenzoic acid decarboxylase from Fusarium oxysporum: crystal structures and substrate recognition mechanism, Chembiochem (2020) 1–8, https://doi.org/10.1002/cbic.202000244.
[35] C. Wuensch, S.M. Glueck, J. Gross, D. Koszelewski, M. Schober, K. Faber, Regioselective enzymatic carboxylation of phenols and hydroxystyrene derivatives, Org. Lett. 14 (2012) 1974–1977, https://doi.org/10.1021/ol300385k.
[36] K.A.P. Payne, M.D. White, K. Fisher, B. Khara, S.S. Bailey, D. Parker, N.J.W. Rattray, D.K. Trivedi, R. Goodacre, R. Beveridge, P. Barran, S.E.J. Rigby, N.S. Scrutton, S. Hay, D. Leys, New cofactor supports α,β-unsaturated acid decarboxylation via 1,3-dipolar cycloaddition, Nature 522 (2015) 497–501, https://doi.org/10.1038/nature14560.
[37] S.E. Payer, S.A. Marshall, N. Bärland, X. Sheng, T. Reiter, A. Dordic, G. Steinkellner, C. Wuensch, S. Kaltwasser, K. Fisher, S.E.J. Rigby, P. Macheroux, J. Vonck, K. Gruber, K. Faber, F. Himo, D. Leys, T. Pavkov-Keller, S.M. Glueck, Regioselective para-carboxylation of catechols with a prenylated flavin dependent decarboxylase, Angew. Chem. Int. Ed. 56 (2017) 13893–13897, https://doi.org/10.1002/anie.201708091.
[38] G.A. Aleku, C. Prause, R.T. Bradshaw-Allen, K. Plasch, S.M. Glueck, S.S. Bailey, K.A.P. Payne, D.A. Parker, K. Faber, D. Leys, Terminal alkenes from acrylic acid derivatives via non-oxidative enzymatic decarboxylation by ferulic acid decarboxylases, ChemCatChem 10 (2018) 3736–3745, https://doi.org/10.1002/cctc.201800643.
[39] G.A. Aleku, A. Saaret, R.T. Bradshaw-Allen, S.R. Derrington, G.R. Titchiner, I. Gostimskaya, D. Gahloth, D.A. Parker, S. Hay, D. Leys, Enzymatic C–H activation of aromatic compounds through CO2 fixation, Nat. Chem. Biol. (2020), https://doi.org/10.1038/s41589-020-0603-0.
[40] J.R. Dunetz, J. Magano, G.A. Weisenburger, Large-scale Applications of amide coupling reagents for the synthesis of pharmaceuticals, Org. Process Res. Dev. 20 (2016) 140–177, https://doi.org/10.1021/op500305s.
[41] M.T. Sabatini, L.T. Boulton, H.F. Sneddon, T.D. Sheppard, A green chemistry perspective on catalytic amide bond formation, Nat. Catal. 2 (2019) 10–17, https://doi.org/10.1038/s41929-018-0211-5.
[42] M.C. Bryan, P.J. Dunn, D. Entwistle, F. Gallou, S.G. Koenig, J.D. Hayler, M.R. Hickey, S. Hughes, M.E. Kopach, G. Moine, P. Richardson, F. Roschangar, A. Steven, F.J. Weiberth, Key Green Chemistry research areas from a pharmaceutical manufacturers' perspective revisited, Green Chem. 20 (2018) 5082–5103, https://doi.org/10.1039/c8gc01276h.
[43] M.R. Petchey, G. Grogan, Enzyme-catalysed synthesis of secondary and tertiary amides, Adv. Synth. Catal. 361 (2019) 3895–3914, https://doi.org/10.1002/adsc.201900694.
[44] H.K. Philpott, P.J. Thomas, D. Tew, D.E. Fuerst, S.L. Lovelock, A versatile biosynthetic approach to amide bond formation, Green Chem. 20 (2018) 3426–3431, https://doi.org/10.1039/c8gc01697f.
[45] C.M. Lelièvre, M. Balandras, J.L. Petit, C. Vergne-Vaxelaire, A. Zaparucha, ATP regeneration system in chemoenzymatic amide bond formation with thermophilic CoA ligase, ChemCatChem 12 (2020) 1184–1189, https://doi.org/10.1002/cctc.201901870.
[46] S.R. Derrington, N.J. Turner, S.P. France, Carboxylic acid reductases (CARs): an industrial perspective, J. Biotechnol. 304 (2019) 78–88, https://doi.org/10.1016/j.jbiotec.2019.08.010.
[47] M. Winkler, Carboxylic acid reductase enzymes (CARs), Curr. Opin. Chem. Biol. 43 (2018) 23–29, https://doi.org/10.1016/j.cbpa.2017.10.006.
[48] A.J.L. Wood, N.J. Weise, J.D. Frampton, M.S. Dunstan, M.A. Hollas, S.R. Derrington, R.C. Lloyd, D. Quaglia, F. Parmeggiani, D. Leys, N.J. Turner, S.L. Flitsch, Adenylation activity of carboxylic acid reductases enables the synthesis of amides, Angew. Chem. Int. Ed. 56 (2017) 14498–14501, https://doi.org/10.1002/anie.201707918.
[49] M. Lubberink, C. Schnepel, J. Citoler, S.R. Derrington, W. Finnigan, M.A. Hayes, N.J. Turner, S.L. Flitsch, M. Lubberink, C. Schnepel, J. Citoler, S.R. Derrington, W. Finnigan, A. Hayes, N.J. Turner, S.L. Flitsch, Biocatalytic Monoacylation of Symmetrical Diamines and its Application to the Synthesis of Pharmaceutically Relevant Amides Biocatalytic Monoacylation of Symmetrical Diamines and its Application to the Synthesis of Pharmaceutically Relevant Amides, 2020, https://doi.org/10.1021/acscatal.0c02228.
[50] Q. Chen, C. Ji, Y. Song, H. Huang, J. Ma, X. Tian, J. Ju, Discovery of McbB, an enzyme catalyzing the β-carboline skeleton construction in the marinacarboline biosynthetic pathway, Angew. Chem. Int. Ed. 52 (2013)

9980–9984, https://doi.org/10.1002/anie.201303449.
[51] M. Petchey, A. Cuetos, B. Rowlinson, S. Dannevald, A. Frese, P.W. Sutton, S. Lovelock, R.C. Lloyd, I.J.S. Fairlamb, G. Grogan, The broad aryl acid specificity of the amide bond synthetase McbA suggests potential for the biocatalytic synthesis of amides, Angew. Chem. Int. Ed. 57 (2018) 11584–11588, https://doi.org/10.1002/anie.201804592.
[52] M.R. Petchey, B. Rowlinson, R.C. Lloyd, I.J.S. Fairlamb, G. Grogan, Biocatalytic synthesis of moclobemide using the amide bond synthetase McbA coupled with an ATP recycling system, ACS Catal. (2020) 4659–4663, https://doi.org/10.1021/acscatal.0c00929.
[53] P.M. Marchetti, S.M. Richardson, N.M. Kariem, D.J. Campopiano, Synthesis of: N-acyl amide natural products using a versatile adenylating biocatalyst, Medchemcomm 10 (2019) 1192–1196, https://doi.org/10.1039/c9md00063a.
[54] J.J. Dong, E. Fernández-Fueyo, F. Hollmann, C.E. Paul, M. Pesic, S. Schmidt, Y. Wang, S. Younes, W. Zhang, Biocatalytic oxidation reactions: a chemist's perspective, Angew. Chem. Int. Ed. 57 (2018) 9238–9261, https://doi.org/10.1002/anie.201800343.
[55] A. Hiseni, I.W.C.E. Arends, L.G. Otten, New cofactor-independent hydration biocatalysts: structural, biochemical, and biocatalytic characteristics of carotenoid and oleate hydratases, ChemCatChem 7 (2015) 29–37, https://doi.org/10.1002/cctc.201402511.
[56] R.M. Demming, M.P. Fischer, J. Schmid, B. Hauer, (De)hydratases — recent developments and future perspectives, Curr. Opin. Chem. Biol. 43 (2018) 43–50, https://doi.org/10.1016/j.cbpa.2017.10.030.
[57] R.M. Demming, K.B. Otte, B.M. Nestl, B. Hauer, Optimized reaction conditions enable the hydration of non-natural substrates by the oleate hydratase from Elizabethkingia meningoseptica, ChemCatChem 9 (2017) 758–766, https://doi.org/10.1002/cctc.201601329.
[58] R.M. Demming, S.C. Hammer, B.M. Nestl, S. Gergel, S. Fademrecht, J. Pleiss, B. Hauer, Asymmetric enzymatic hydration of unactivated, aliphatic alkenes, Angew. Chem. Int. Ed. 58 (2019) 173–177, https://doi.org/10.1002/anie.201810005.
[59] Y.X. Wu, J. Pan, H.L. Yu, J.H. Xu, Enzymatic synthesis of 10-oxostearic acid in high space-time yield via cascade reaction of a new oleate hydratase and an alcohol dehydrogenase, J. Biotechnol. X. 2 (2019) 100008, https://doi.org/10.1016/j.btecx.2019.100008.
[60] K. Chen, F.H. Arnold, Engineering new catalytic activities in enzymes, Nat. Catal. (2020), https://doi.org/10.1038/s41929-019-0385-5.
[61] M.P. Doyle, D.C. Forbes, Recent advances in asymmetric catalytic metal carbene transformations, Chem. Rev. 98 (1998) 911–935, https://doi.org/10.1021/cr940066a.
[62] P.S. Coelho, Z.J. Wang, M.E. Ener, S.A. Baril, A. Kannan, F.H. Arnold, E.M. Brustad, A serine-substituted P450 catalyzes highly efficient carbene transfer to olefins in vivo, Nat. Chem. Biol. 9 (2013) 485–487, https://doi.org/10.1038/nchembio.1278.
[63] K. Chen, X. Huang, S.B.J. Kan, R.K. Zhang, F.H. Arnold, Enzymatic construction of highly strained carbocycles, Science 360 (2018) 71, https://doi.org/10.1126/science.aar4239.
[64] K. Chen, F.H. Arnold, Engineering cytochrome P450s for enantioselective cyclopropenation of internal alkynes, J. Am. Chem. Soc. 142 (2020) 6891–6895, https://doi.org/10.1021/jacs.0c01313.
[65] A.M. Knight, S.B. Jennifer Kan, R.D. Lewis, O.F. Brandenberg, K. Chen, F.H. Arnold, Diverse engineered heme proteins enable stereodivergent cyclopropanation of unactivated alkenes, ACS Cent. Sci. 4 (2018) 372–377, https://doi.org/10.1021/acscentsci.7b00548.
[66] O.F. Brandenberg, C.K. Prier, K. Chen, A.M. Knight, Z. Wu, F.H. Arnold, Stereoselective enzymatic synthesis of heteroatom-substituted cyclopropanes, ACS Catal. 8 (2018) 2629–2634, https://doi.org/10.1021/acscatal.7b04423.
[67] B.J. Wittmann, A.M. Knight, J.L. Hofstra, S.E. Reisman, S.B. Jennifer Kan, F.H. Arnold, Diversity-oriented enzymatic synthesis of cyclopropane building blocks, ACS Catal. 10 (2020) 7112–7116, https://doi.org/10.1021/acscatal.0c01888.
[68] D.A. Vargas, R.L. Khade, Y. Zhang, R. Fasan, Biocatalytic strategy for highly diastereo- and enantioselective synthesis of 2,3-dihydrobenzofuran-based tricyclic scaffolds, Angew. Chem. Int. Ed. 58 (2019) 10148–10152, https://doi.org/10.1002/anie.201903455.
[69] A.L. Chandgude, R. Fasan, Highly diastereo- and enantioselective synthesis of nitrile-substituted cyclopropanes by myoglobin-mediated carbene transfer catalysis, Angew. Chem. Int. Ed. 57 (2018) 15852–15856, https://doi.org/10.1002/anie.201810059.
[70] A. Tinoco, V. Steck, V. Tyagi, R. Fasan, Highly diastereo- and enantioselective synthesis of trifluoromethyl-substituted cyclopropanes via myoglobin-catalyzed transfer of trifluoromethylcarbene, J. Am. Chem. Soc. 139 (2017) 5293–5296, https://doi.org/10.1021/jacs.7b00768.
[71] A.R. Fasan, D.M. Carminati, J. Decaens, S. Couve-bonnaire, P. Jubault, Biocatalytic strategy for the highly stereoselective synthesis of CHF2- containing trisubstituted cyclopropanes, Angew. Chem. Int. Ed. (2020), https://doi.org/10.1002/anie.202015895.
[72] R. Mago, R. Mahajan, M.E. Thase, Levomilnacipran: a newly approved drug for treatment of major depressive disorder, Expet Rev. Clin. Pharmacol. 7 (2014) 137–145, https://doi.org/10.1586/17512433.2014.889563.
[73] A.L. Chandgude, X. Ren, R. Fasan, Stereodivergent intramolecular cyclopropanation enabled by engineered carbene transferases, J. Am. Chem. Soc. 141 (2019) 9145–9150, https://doi.org/10.1021/jacs.9b02700.
[74] X. Ren, A.L. Chandgude, R. Fasan, Highly stereoselective synthesis of fused cyclopropane-γ-lactams via biocatalytic iron-catalyzed intramolecular cyclopropanation, ACS Catal. 10 (2020) 2308–2313, https://doi.org/10.1021/acscatal.9b05383.
[75] X. Ren, N. Liu, A.L. Chandgude, R. Fasan, An enzymatic platform for the highly enantioselective and stereodivergent construction of cyclopropyl-δ-lactones, Angew. Chemie Int. Ed. n/a (2020), https://doi.org/10.1002/anie.202007953.
[76] Z.J. Wang, N.E. Peck, H. Renata, F.H. Arnold, Cytochrome P450-catalyzed insertion of carbenoids into N–H bonds, Chem. Sci. 5 (2014) 598–601, https://doi.org/10.1039/C3SC52535J.
[77] V. Steck, D.M. Carminati, N.R. Johnson, R. Fasan, Enantioselective synthesis of chiral amines via biocatalytic carbene N–H insertion, ACS Catal. 10 (2020) 10967–10977, https://doi.org/10.1021/acscatal.0c02794.
[78] H.M. Key, P. Dydio, Z. Liu, J.Y.-E. Rha, A. Nazarenko, V. Seyedkazemi, D.S. Clark, J.F. Hartwig, Beyond iron: iridium-containing P450 enzymes for selective cyclopropanations of structurally diverse alkenes, ACS Cent. Sci. 3 (2017) 302–308, https://doi.org/10.1021/acscentsci.6b00391.
[79] Y. Gu, S.N. Natoli, Z. Liu, D.S. Clark, J.F. Hartwig, Site-selective functionalization of (sp3)C–H bonds catalyzed by artificial metalloenzymes containing an iridium-porphyrin cofactor, Angew. Chem. Int. Ed. 58 (2019) 13954–13960, https://doi.org/10.1002/anie.201907460.
[80] R.K. Zhang, K. Chen, X. Huang, L. Wohlschlager, H. Renata, F.H. Arnold, Enzymatic assembly of carbon–carbon bonds via iron-catalysed sp 3 C–H functionalization, Nature 565 (2019) 67–72, https://doi.org/10.1038/s41586-018-0808-5.
[81] J. Zhang, X. Huang, R.K. Zhang, F.H. Arnold, Enantiodivergent α-amino C–H fluoroalkylation catalyzed by engineered cytochrome P450s, J. Am. Chem. Soc. 141 (2019) 9798–9802, https://doi.org/10.1021/jacs.9b04344.
[82] A.Z. Zhou, K. Chen, F.H. Arnold, Enzymatic lactone-carbene C–H insertion to build contiguous chiral centers, ACS Catal. 10 (2020) 5393–5398, https://doi.org/10.1021/acscatal.0c01349.
[83] O.F. Brandenberg, K. Chen, F.H. Arnold, Directed evolution of a cytochrome P450 carbene transferase for selective functionalization of cyclic compounds, J. Am. Chem. Soc. 141 (2019) 8989–8995, https://doi.org/10.1021/jacs.9b02931.
[84] D.A. Vargas, A. Tinoco, V. Tyagi, R. Fasan, Myoglobin-Catalyzed C–H functionalization of unprotected indoles, Angew. Chem. Int. Ed. 57 (2018) 9911–9915, https://doi.org/10.1002/anie.201804779.
[85] S.B.J. Kan, R.D. Lewis, K. Chen, F.H. Arnold, Directed evolution of cytochrome c for carbon–silicon bond formation: bringing silicon to life, Science 354 (2016) 1048, https://doi.org/10.1126/science.aah6219.
[86] S.B.J. Kan, X. Huang, Y. Gumulya, K. Chen, F.H. Arnold, Genetically programmed chiral organoborane synthesis, Nature 552 (2017) 132–136, https://doi.org/10.1038/nature24996.
[87] X. Huang, M. Garcia-Borràs, K. Miao, S.B. Jennifer Kan, A. Zutshi, K.N. Houk, F.H. Arnold, A biocatalytic platform for synthesis of chiral α-trifluoromethylated organoborons, ACS Cent. Sci. 5 (2019) 270–276, https://doi.org/10.1021/acscentsci.8b00679.
[88] C.K. Prier, R.K. Zhang, A.R. Buller, S. Brinkmann-Chen, F.H. Arnold, Enantioselective, intermolecular benzylic C–H amination catalysed by an engineered iron-haem enzyme, Nat. Chem. 9 (2017) 629–634, https://doi.org/10.1038/nchem.2783.
[89] Y. Yang, I. Cho, X. Qi, P. Liu, F.H. Arnold, An enzymatic platform for the asymmetric amination of primary, secondary and tertiary C(sp3)–H bonds, Nat. Chem. 11 (2019) 987–993, https://doi.org/10.1038/s41557-019-0343-5.
[90] Z.-J. Jia, S. Gao, F.H. Arnold, Enzymatic primary amination of benzylic and allylic C(sp3)–H bonds, J. Am. Chem. Soc. 142 (2020) 10279–10283, https://doi.org/10.1021/jacs.0c03428.
[91] O.F. Brandenberg, D.C. Miller, U. Markel, A. Ouald Chaib, F.H. Arnold, Engineering chemoselectivity in hemoprotein-catalyzed indole amidation, ACS Catal. 9 (2019) 8271–8275, https://doi.org/10.1021/acscatal.9b02508.
[92] V. Steck, J.N. Kolev, X. Ren, R. Fasan, Mechanism-guided design and discovery of efficient cytochrome P450-derived C–H amination biocatalysts, J. Am. Chem. Soc. 142 (2020) 10343–10357, https://doi.org/10.1021/jacs.9b12859.
[93] I. Cho, C.K. Prier, Z.-J. Jia, R.K. Zhang, T. Görbe, F.H. Arnold, Enantioselective aminohydroxylation of styrenyl olefins catalyzed by an engineered hemoprotein, Angew. Chem. Int. Ed. 58 (2019) 3138–3142, https://doi.org/10.1002/anie.201812968.
[94] S. Wu, Y. Zhou, T. Wang, H.-P. Too, D.I.C. Wang, Z. Li, Highly regio- and enantioselective multiple oxy- and amino-functionalizations of alkenes by modular cascade biocatalysis, Nat. Commun. 7 (2016) 11917, https://doi.org/10.1038/ncomms11917.
[95] P.D. Clayman, T.K. Hyster, Photoenzymatic generation of unstabilized alkyl radicals: an asymmetric reductive cyclization, J. Am. Chem. Soc. 142 (2020) 15673–15677, https://doi.org/10.1021/jacs.0c07918.
[96] X. Huang, B. Wang, Y. Wang, G. Jiang, J. Feng, H. Zhao, Photoenzymatic enantioselective intermolecular radical hydroalkylation, Nature (2020), https://doi.org/10.1038/s41586-020-2406-6.
[97] E.G. Kovaleva, J.D. Lipscomb, Versatility of biological non-heme Fe(II) centers in oxygen activation reactions, Nat. Chem. Biol. 4 (2008) 186–193, https://doi.org/10.1038/nchembio.71.
[98] N.W. Goldberg, A.M. Knight, R.K. Zhang, F.H. Arnold, Nitrene transfer catalyzed by a non-heme iron enzyme and enhanced by non-native small-molecule ligands, J. Am. Chem. Soc. 141 (2019) 19585–19588, https://doi.org/10.1021/jacs.9b11608.

[99] M.A. Vila, V. Steck, S. Rodriguez Giordano, I. Carrera, R. Fasan, C–H amination via nitrene transfer catalyzed by mononuclear non-heme iron-dependent enzymes, Chembiochem 21 (2020) 1981–1987, https://doi.org/10.1002/cbic.201900783.
[100] M. Davidson, M. McNamee, R. Fan, Y. Guo, W. Chang, Repurposing nonheme iron hydroxylases to enable catalytic nitrile installation through an azido group Assistance, J. Am. Chem. Soc. 141 (2019) 3419–3423, https://doi.org/10.1021/jacs.8b13906.
[101] S.M. Barry, J.A. Kers, E.G. Johnson, L. Song, P.R. Aston, B. Patel, S.B. Krasnoff, B.R. Crane, D.M. Gibson, R. Loria, G.L. Challis, Cytochrome P450-catalyzed L-tryptophan nitration in thaxtomin phytotoxin biosynthesis, Nat. Chem. Biol. 8 (2012) 814–816, https://doi.org/10.1038/nchembio.1048.
[102] S.C. Dodani, J.K.B. Cahn, T. Heinisch, S. Brinkmann-Chen, J.A. McIntosh, F.H. Arnold, Structural, functional, and spectroscopic characterization of the substrate scope of the novel nitrating cytochrome P450 TxtE, Chembiochem 15 (2014) 2259–2267, https://doi.org/10.1002/cbic.201402241.
[103] S.C. Dodani, G. Kiss, J.K.B. Cahn, Y. Su, V.S. Pande, F.H. Arnold, Discovery of a regioselectivity switch in nitrating P450s guided by molecular dynamics simulations and Markov models, Nat. Chem. 8 (2016) 419–425, https://doi.org/10.1038/nchem.2474.
[104] R. Zuo, Y. Zhang, C. Jiang, J.C. Hackett, R. Loria, S.D. Bruner, Y. Ding, Engineered P450 biocatalysts show improved activity and regio-promiscuity in aromatic nitration, Sci. Rep. 7 (2017) 1–9, https://doi.org/10.1038/s41598-017-00897-z.
[105] S.C. Hammer, A. Marjanovic, J.M. Dominicus, B.M. Nestl, B. Hauer, Squalene hopene cyclases are protonases for stereoselective Brønsted acid catalysis, Nat. Chem. Biol. 11 (2015) 121–126, https://doi.org/10.1038/nchembio.1719.
[106] S.C. Hammer, P.O. Syrén, B. Hauer, Substrate pre-folding and water molecule organization matters for terpene cyclase catalyzed conversion of unnatural substrates, Chemistry 1 (2016) 3589–3593, https://doi.org/10.1002/slct.201600572.
[107] S.A. Bastian, S.C. Hammer, N. Kreß, B.M. Nestl, B. Hauer, Selectivity in the cyclization of citronellal introduced by squalene hopene cyclase variants, ChemCatChem 9 (2017) 4364–4368, https://doi.org/10.1002/cctc.201700734.
[108] C.O. Marsh, N.R. Lees, L.C. Han, M.J. Byrne, S.Z. Mbatha, L. Maschio, S. Pagden-Ratcliffe, P.W. Duke, J.E.M. Stach, P. Curnow, C.L. Willis, P.R. Race, A natural diels-alder biocatalyst enables efficient [4+2] cycloaddition under harsh reaction conditions, ChemCatChem 11 (2019) 5027–5031, https://doi.org/10.1002/cctc.201901285.
[109] D. Tan, C.S. Jamieson, M. Ohashi, M.C. Tang, K.N. Houk, Y. Tang, Genome-mined diels-alderase catalyzes formation of the cis-octahydrodecalins of varicidin A and B, J. Am. Chem. Soc. 4–8 (2019), https://doi.org/10.1021/jacs.8b12010.
[110] M.J. Byrne, N.R. Lees, L.C. Han, M.W. Van Der Kamp, A.J. Mulholland, J.E.M. Stach, C.L. Willis, P.R. Race, The catalytic mechanism of a natural diels-alderase revealed in molecular detail, J. Am. Chem. Soc. 138 (2016) 6095–6098, https://doi.org/10.1021/jacs.6b00232.
[111] L.C. Ward, H.V. McCue, A.J. Carnell, Carboxyl methyltransferases: natural functions and potential applications in industrial biotechnology, ChemCatChem (2020) 1–9, https://doi.org/10.1002/cctc.202001316.
[112] M.E.Q. Tang, C.W. Grathwol, A.S. Aslan-Üzel, S. Wu, A. Link, I.V. Pavlidis, C.P.S. Badenhorst, U.T. Bornscheuer, Directed evolution of a halide methyltransferase enables biocatalytic synthesis of diverse SAM analogs, Angew. Chem. Int. Ed. (2020), https://doi.org/10.1002/anie.202013871.
[113] A.J. Herbert, S.A. Shepherd, V.A. Cronin, M.R. Bennett, R. Sung, J. Micklefield, Engineering orthogonal methyltransferases to create alternative bioalkylation pathways, Angew. Chem. Int. Ed. 59 (2020) 14950–14956, https://doi.org/10.1002/anie.202004963.

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