ChemCatChem

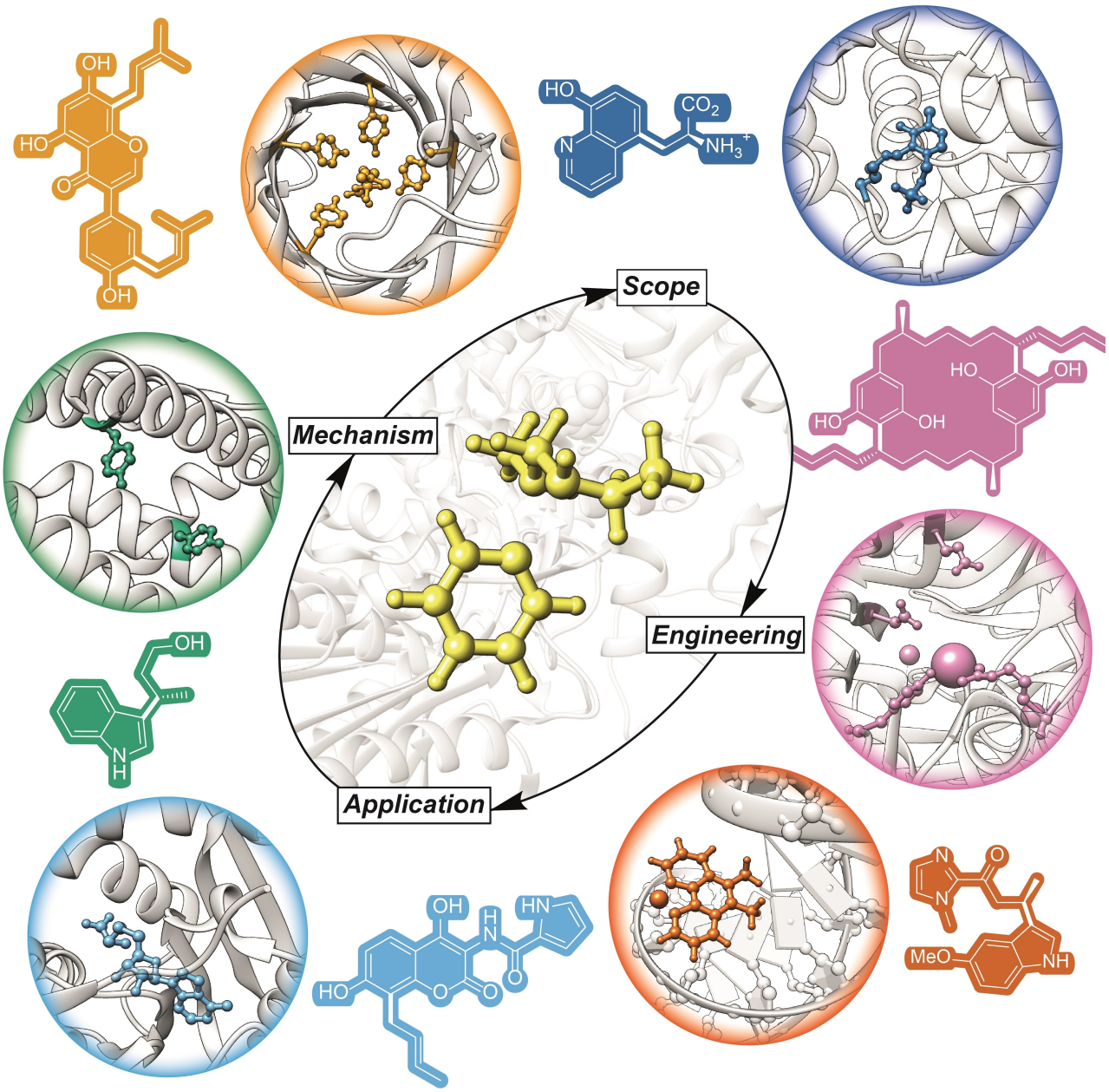
Review
doi.org/10.1002/cctc.202200636

Chemistry Europe
European Chemical Societies Publishing

www.chemcatchem.org

Biocatalytic Friedel-Crafts Reactions

Reuben B. Leveson-Gower[a] and Gerard Roelfes*[a]



Friedel-Crafts alkylation and acylation reactions are important methodologies in synthetic and industrial chemistry for the construction of aryl-alkyl and aryl-acyl linkages that are ubiquitous in bioactive molecules. Nature also exploits these reactions in many biosynthetic processes. Much work has been done to expand the synthetic application of these enzymes to unnatural substrates through directed evolution. The promise of such biocatalysts is their potential to supersede inefficient and toxic chemical approaches to these reactions, with mild operating conditions - the hallmark of enzymes. Complementary work has created many bio-hybrid Friedel-Crafts catalysts consisting of chemical catalysts anchored into biomolecular scaffolds, which display many of the same desirable characteristics. In this Review, we summarise these efforts, focussing on both mechanistic aspects and synthetic considerations, concluding with an overview of the frontiers of this field and routes towards more efficient and benign Friedel-Crafts reactions for the future of humankind.

Introduction

In the late 19th century, seminal work by Friedel and Crafts demonstrated that aryl-alkyl and aryl-acyl C–C bonds could be forged from benzene with alkyl and acyl chlorides respectively, through the use of Lewis acid catalysis.[1] The original conditions reported were harsh, requiring super-stoichiometric quantities of aluminium trichloride and refluxing neat reactions, sometimes for multiple days. The concept laid out in these pioneering studies proved to be a powerful one, whose generality was further honed over the following century. Today, these reactions remain a main-stay of the synthetic organic chemist, thanks, in part, to the development of milder methodologies.[2] In particular, the development of many new metal- and organocatalysed processes has enabled the formation of both benzylic and adjacent stereo-centres, which appear frequently in biologically active compounds.[3] Outside of the research laboratory, both newer and more traditional variants of the Friedel-Crafts reaction are applied in industrial processes for the synthesis of pharmaceuticals, although they feature less frequently in medicinal chemistry than some decades ago.[4] Increasingly, industrial chemical processes are being adapted to adhere with the principles of green chemistry, reducing waste, energy consumption, toxicity and so forth.[5] Many industrial processes for Friedel-Crafts reactions employ stoichiometric amounts of hazardous Lewis acids, require high-temperatures and undesirable organic solvents and as such, they are sub-optimal according to several sustainability metrics, and therefore can benefit significantly from more efficient catalytic methodologies.

The importance of these reactions is not limited to their exploitation by humankind; nature also employs Friedel-Crafts reactions in the biosynthesis of amino acids, antibiotics and alkaloids. The mild reaction conditions under which Friedel-Crafts enzymes operate should translate into a significant reduction in the environmental impact of industrial synthetic methodologies if they can replace the current chemical methods used.[6] In some cases, these enzymes have inherent substrate promiscuity that facilitates their direct application in societally relevant transformations with efficiencies superior to their chemical counterparts, as is demonstrated in the industrial synthesis of L-DOPA.[7] In other cases, their activity towards non-native substrates can be honed through mutagenesis or directed evolution to achieve activities relevant for application in synthesis. Aside from increasing the benignity of these processes, Friedel-Crafts enzymes can also provide regioselectivities that cannot be achieved with chemical catalysis. Furthermore, they often have exquisite compatibility with other enzymes, allowing several reaction steps to be conducted in one pot.[8] This can be leveraged either to increase the simplicity of the substrates added, or the complexity of the products produced.

Our group and others have focused on creating artificial Friedel-Crafts enzymes by combining chemical catalysts with biomolecular scaffolds, creating hybrid systems that facilitate these reactions under the mild operating conditions for which natural enzymes are prized.[9] In this review, we summarise recent work on applying both several classes of natural (and engineered) enzymes, as well as artificial enzymes and DNA catalysts for Friedel-Crafts reactions. We pay particular attention to the mechanisms by which these catalysts operate, which often differ significantly from chemical catalysts. We discuss the relative merits of these enzymes in terms of the substrates they employ for both the arene and electrophilic reactants, and their proclivity towards substrates significantly removed from their natural ones. Finally, we provide our views on the frontiers of this field that, if addressed, will lead the way towards the increasing application of enzymes in Friedel-Crafts reactions to both create a more sustainable future and potentially facilitate access to new useful molecular products.

Tryptophan Synthase and Tyrosine Phenol Lyase

Tryptophan Synthase (TrpS) and Tyrosine Phenol Lyase (TPL) are two pyridoxal 5′-phosphate (PLP) dependent enzymes that can catalyse the formation of the alkyl-aryl linkages present in tryptophan and tyrosine, respectively. Both enzymes exploit an

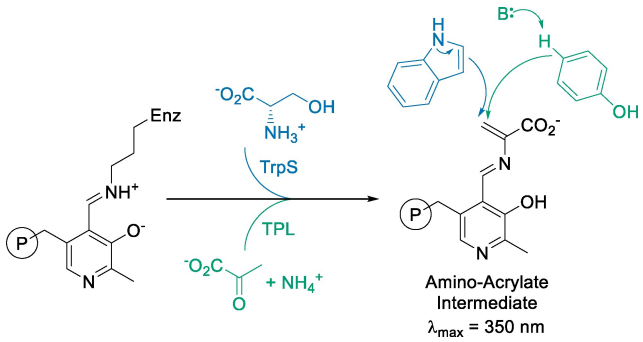
[a] R. B. Leveson-Gower, Prof. G. Roelfes
Stratingh Institute for Chemistry
University of Groningen
9747 AG, Groningen (The Netherlands)
E-mail: j.g.roelfes@rug.nl

This publication is part of a joint Special Collection with EurJOC and EurJIC on the Netherlands Institute for Catalysis Research. Please see our homepage for more articles in the collection.

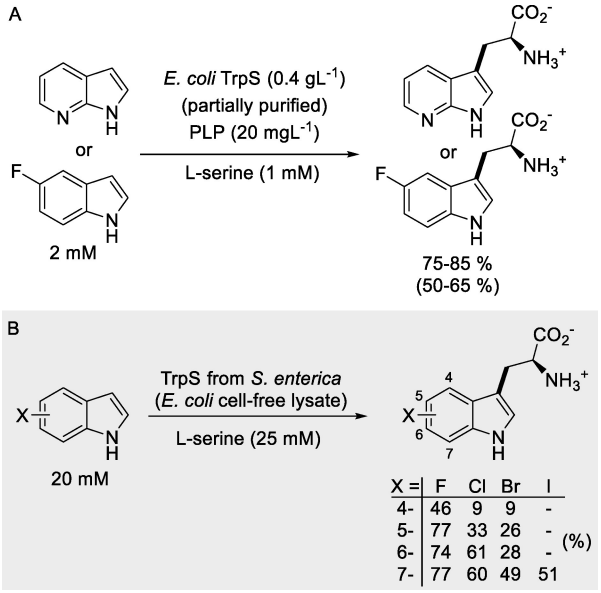
amino-acrylate intermediate activated by the PLP cofactor, which can be observed by UV-vis (ultra-violet and visible) and NMR (nuclear magnetic resonance) spectroscopy,[10] and whose highly electrophilic β-carbon can undergo nucleophilic attack by either indole or phenol (Scheme 1).[11] A notable difference is that whilst TrpS uses serine to form the amino-acrylate intermediate, TPL, whose native activity is tyrosine catabolism, forms the same species instead from pyruvate and ammonia. The indolium resonance structure facilitated by the nitrogen lone-pair in the indole substrate gives significant stabilisation to the Wheland intermediate in TrpS, consistent with kinetic isotope data obtained with a 3-^{2}H-indole substrate.[11a,b] In tyrosine catabolism by TPL, both kinetic isotope and crystallographic studies indicate a concerted process with simultaneous protonation/elimination of the phenol moiety, rather than the formation of a formal Wheland intermediate, despite the fact that keto-resonance structures enabled by the oxygen lone-pair in phenol could stabilise this intermediate.[11c,e] Tryptophan indole-lyase (Trpase, a PLP dependent enzyme involved in tryptophan catabolism) also employs a concerted mechanism for indole cleavage. To the best of our knowledge, kinetic isotope studies with TPL using 4-^{2}H-phenol as substrate have not been performed.[12] Nevertheless, according to the principle of microscopic reversibility, tyrosine synthesis by TPL should also not involve a Wheland intermediate.

Both enzymes have been subject to engineering efforts to expand the scope of arene substrates that they can accept, and, in the case of TrpS, also the substituent at the amino-acrylate β-carbon can be varied.[13] Since the seminal work from Schultz demonstrating the site-selective incorporation of unnatural amino acids through amber-stop-codon suppression, interest in these products has increased drastically, principally for their applications in chemical biology.[14] Moreover, unnatural amino acids have also found application in the design and engineering of natural and artificial enzymes,[15] and also garner interest for their potential applications in therapeutics.[16]

Several examples, starting as early as 1974, leveraged the natural substrate promiscuity of tryptophan synthase using purified, partially-purified or freeze-dried cell-lysates to synthesise tryptophan analogues, including halogenated products (Scheme 2), isolobal tryptophan derivatives with aromatic-carbon-to-nitrogen (Scheme 2A) substitutions and products with other heterocycles containing oxygen, sulphur and



Scheme 1. Mechanism of action of the PLP dependent enzymes TrpS and TPL. For TPL, tyrosine synthesis, as shown, is the reverse process with respect to natural reactivity. Amino acrylate intermediates are formed from either serine or pyruvate and ammonia by reaction with lysine-bound PLP, which can undergo nucleophilic attack by indole or phenol. The amino acid products are released after transimination by the same lysine residue. P = –OPO_3^{2-}.



Scheme 2. Early examples of tryptophan analogue synthesis exploiting the natural substrate promiscuity of tryptophan synthase from *E. coli.* (A) or *S. enterica* (B) for non-native indole derivatives, full reaction conditions can be found in references.[17]

Gerard Roelfes received his PhD from the University of Groningen under the guidance of Prof. Ben L. Feringa, in a collaborative project with Prof. Lawrence Que Jr. (Univ. of Minnesota). Then he went for a postdoctoral stay with Prof. Donald Hilvert at the ETH Zürich. In 2003 he returned to the University of Groningen as a group leader and subsequently rose through the academic ranks and now is Professor of Biomolecular Chemistry and Catalysis. His research interests include designer enzymes, bio-orthogonal catalysis and catalysis in living cells.

Reuben Leveson-Gower graduated from Durham University in 2017, having spent one year working in organometallic chemistry and homogeneous catalysis at SASOL UK laboratory in St. Andrews, headed by Prof. Bob Tooze. He then moved to the University of Groningen to pursue his PhD under the supervision of Gerard Roelfes. His research focusses on the creation and application of artificial enzymes that utilise unnatural amino acids as catalytic residues.

selenium.[17–18] Despite these successes, protein engineering efforts were hampered by the complex hetero-dimeric structure of TrpS that consists of α- and β- subunits. The α-subunit is responsible for the production of indole from indole glycerol phosphate, but also provides allosteric activation to the β-subunit for its Friedel-Crafts alkylating reactivity (Figure 1A). Thus, purification of the TrpB subunit in isolation gives only inactive enzyme.

Given the fact that engineering and application of multimeric enzymes can be inconvenient, and that indoles are far preferable substrates compared to their glycerol-phosphate counterparts from a synthetic standpoint, the identification of a TrpB subunit with "stand-alone" activity was pursued by Arnold, Buller and co-workers.[19] By screening approximately 2,500 clones from EP-PCR (error prone polymerase chain reaction) libraries, mutations were identified in the β-subunit of a thermostable TrpS from *Pyrococcus furiosus* that could recapitulate the allosteric activation normally provided by the α-subunits (Figure 1B). This TrpB variant (*Pf*TrpB^{0B2}) bearing six mutations gave catalytic activities surpassing the natural *Pf*TrpS multimer and was severely inhibited, rather than activated, by addition of *Pf*TrpA. Kinetic investigations revealed that, as with the TrpA subunit, the mutations introduced affect a stabilisation of the amino-acrylate intermediate, indicating a recapitulation of the effect induced by allostery through directed evolution.[19] More recently, a computational study suggested the role of distal mutations in creating paths of interactions throughout the protein structure that tailor the conformation of the active site to favour various steps of the reaction pathway.[20] Moreover, this analysis also allowed the prediction of activating mutations, which were experimentally validated.[21] Remarkably, transferring some or all of the mutations found in the campaign towards *Pf*TrpB^{0B2} to TrpB homologues from other organisms allowed the creation of other active TrpB variants that could address limitations in the substrate scope of *Pf*TrpB^{0B2} to include a broad range 5-substituted indoles (Figure 1C, Scheme 3B) .[22]

By screening the lineage of *Pf*TrpB^{0B2} with threonine instead of serine, an intermediate mutant was identified that could produce quantities of the corresponding β-methyl tryptophan product. Further directed evolution produced the improved *Pf*TrpB^{2B9} mutant (Scheme 3A) .[24] Micklefield and co-workers also demonstrated efficient synthesis of β-methyl tryptophan and derivatives by introducing a single leucine-to-valine mutation into TrpS from *Salmonella typhimurium*.[25] Expanding the substrate scope of TrpB to β-ethyl serine was enabled by a similar mutation in *Pf*TrpB that gave activity sufficient for medium-throughput screening techniques.[26] Once this mutation was made, directed evolution proceeded smoothly to identify a highly active variant that could also accept β-propyl serine. After conducting another directed evolution campaign starting with an intermediate mutant along the *Pf*TrpB^{0B2} lineage for challenging indole-substrates (containing electron-withdrawing groups that destabilise the indolium intermediate), Arnold and co-workers had collected a panel of TrpB mutants from various organisms capable of producing an array of tryptophan derivatives with substituents in the 4-, 5-, 6-, and 7-positions (Scheme 3B).[27] Continuous evolution can also be applied to TrpB knocking out TrpS in a yeast strain to produce a tryptophan auxotroph.[28] The application of this technique to TrpB from *Thermotoga maritima* produced highly mutated variants with differing degrees of substrate selectivity and promiscuity for non-natural indole substrates. Most recently, a variant of *Tm*TrpB was identified that could exploit azulene as nucleophile to create a deep-blue fluorescent amino acid on

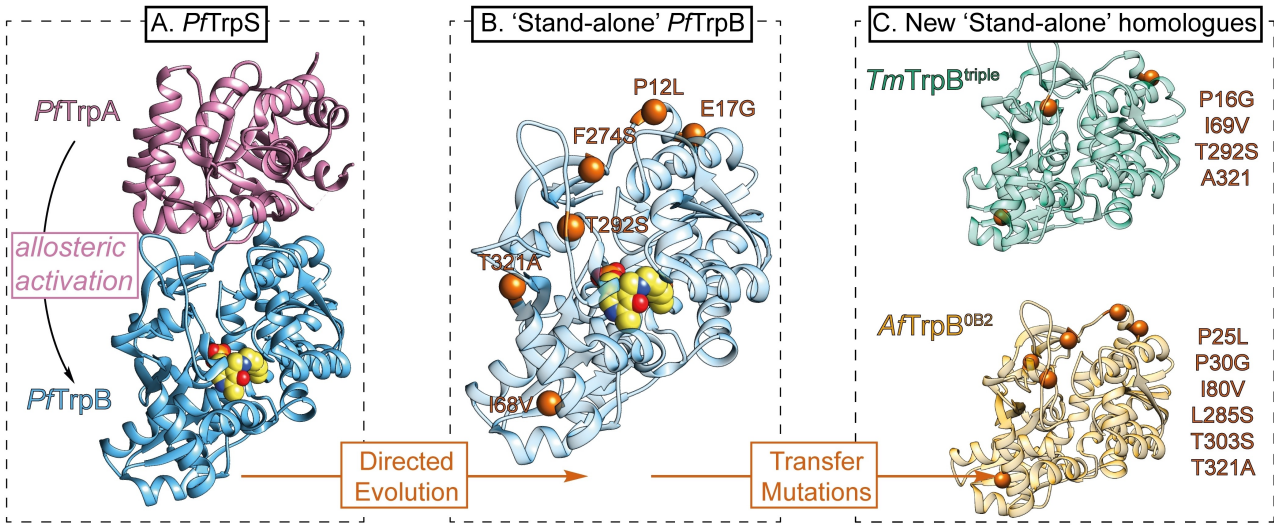
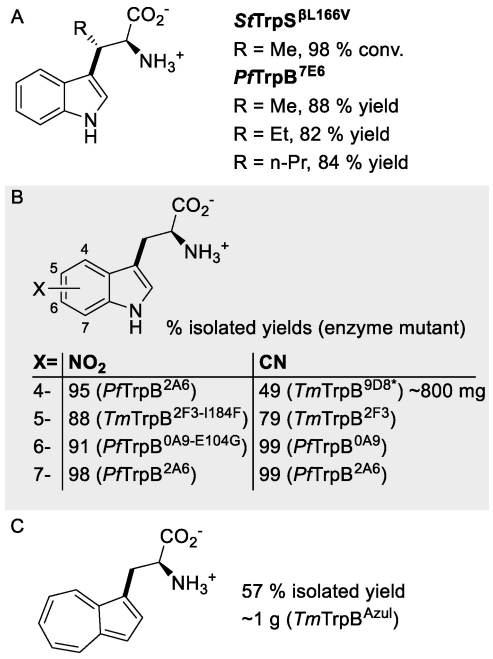


Figure 1. A. TrpA (pink) provides allosteric activation to TrpB (blue), which is responsible for tryptophan synthase activity in the heterodimeric complex from *Pyrococcus furiosus*, the lysine-bound PLP cofactor is shown as yellow spheres with hetero-atoms coloured (PDB: 5E0K).[19] B. Screening libraries of *Pf*TrpB over multiple rounds produced a 'stand-alone' variant bearing six mutations, *Pf*TrpB^{2B9} that does not require the TrpA subunit. Positions of the mutations are shown as spheres on the *Pf*TrpB structure and the lysine-bound PLP cofactor is shown as in (A) (PDB: 5DVZ). C. Some of all of these mutations could be transferred by homology to TrpB homologues from *Thermotoga maritima* (*Tm*TrpB) and *Archaeoglobus fulgidus* (*Af*TrpB) to create further 'stand-alone' TrpB enzymes.[22] *Tm*TrpB already contains alanine at position 321 in the wild-type sequence and thus this mutation did not need to be transferred. Structures shown were produced with AlphaFold, obtained from the uniprot database.[23]



Scheme 3. A. tryptophan analogues with β-alkyl substituents synthesised from e.g., threonine and indole using various tryptophan synthase mutants.[25–26] B. Synthesis of tryptophan derivatives with deactivating substituents on the indole moiety using a variety of engineered TrpB mutants.[27] C. Synthesis of a deep-blue fluorescent amino acid from azulene by an engineered TrpB mutant.[29] See references for reaction conditions and complete description of mutations.

preparative scale (Scheme 3C).[29] The use of TrpB variants is being expanded further to include non-aromatic carbon nucleophiles including nitroalkanes, oxindoles and even ketones.[30]

The products from these engineered TrpB mutants can be further diversified by the concurrent utilisation of other enzymes. Buller and co-workers demonstrated the use of a PLP-dependent tryptophan decarboxylase to produce tryptamine analogues on preparative scale from tryptophan analogues produced *in situ* from indole derivatives by *Pf*TrpB^{2B9}, obtaining the products in 12–100% yields, corresponding to 30–200 mg.[31] Serine-isotopologs can also be accepted by *Pf*TrpB^{2B9}, which can be conveniently produced from deuterated formaldehyde and/or ^{13}C-labelled glycine by L-threonine aldolase to give tryptophan isotopologs with up to 4 isotope substitutions, on an analytical scale, augmenting previously described methods.[32] *Pf*TrpB^{0A9} was applied in a cascade together with a ring-opening tryptophan 2,3-dioxygenase, Tar13, to produce chlorokynurenine and bromokynurenine, the former molecule being a current antidepressant drug candidate.[33] Co-immobilisation of these two enzymes gave a heterogeneous catalyst capable of producing hundreds of milligrams of the products, but activity was rapidly lost upon catalyst-recycling. The same study demonstrated the antidepressant properties of both chlorokynurenine and bromokynurenine in mice.

Examples of the application of TPL to prepare unnatural amino-acids are certainly limited compared with those of TrpB, however non-natural phenol substrates can indeed be accepted in the reverse reaction of TPL for tyrosine-analogue synthesis.[34] Early work on this topic demonstrated synthesis of L-DOPA from catechol, pyruvate and ammonia,[35] a process that is now conducted on an industrial scale with an efficiency besting chemical routes.[7] However, most other substituted phenols showed severely retarded reaction rates compared to the natural substrates.[36] Kroutil and co-workers addressed this limitation through targeted mutagenesis to reduce steric bulk in the active site of TPL from *Citrobacter freundii* identifying a single mutant, M379V, with a particularly broad scope for ortho-substituted phenols which was applied from preparative tyrosine-analogue synthesis (Figure 2A and B).[34] The reasonable organic solvent tolerance of the enzyme allowed substrate concentrations up to 0.24 M, and this biocatalytic approach rendered an otherwise four-step chemoenzymatic synthesis in a single step from commercially available starting materials.

Building on this work, the *in vivo* synthesis of 2-fluoro-tyrosine from 2-fluoro-phenol using TPL and its concurrent incorporation into recombinantly expressed proteins has recently been demonstrated.[38] In a similar vein, Wang and co-

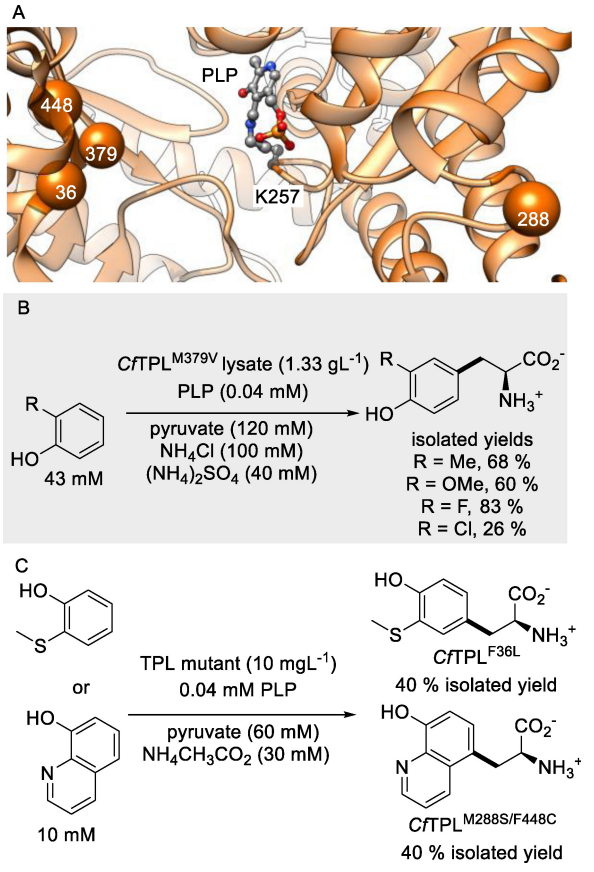


Figure 2. A. active site of *Cf*TPL showing the lysine-bound PLP cofactor as balls and sticks coloured according to the atom type, the positions of important mutations are shown as spheres (PDB: 2EZ1). B. Synthesis of 3-substituted tyrosine derivatives in one pot from ortho-substituted phenols using a rationally designed *Cf*TPL mutant.[34] C. Preparative synthesis of unnatural amino acids with application in biochemical studies employing *Cf*TPL mutants identified by screening site-saturation libraries with TLC.[37] Full details of reaction conditions can be found in the references listed.

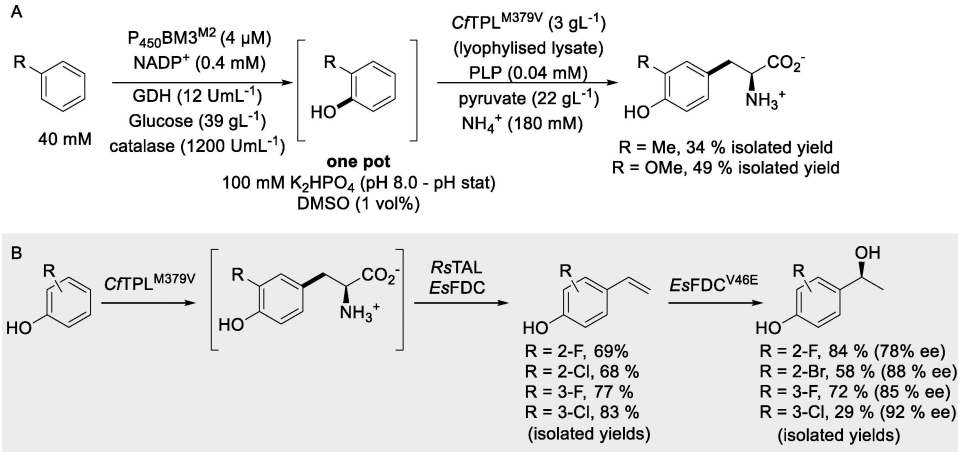
workers screened libraries of TPL variants by TLC (thin-layer chromatography) for their ability to produce two unnatural amino acids with interesting applications in biochemical studies (Figure 2C) .[37] In both cases, the mutants obtained resulted in reduced steric crowding in the active site. When employing 2-(methylthio)phenol as arene substrate, the best mutant contained one apolar-to-apolar side-chain substitution. Contrastingly, the best mutant obtained with 8-hydroxyquinoline introduced new polar residues, but their role in catalysis was not determined. By using the relevant substrates with their identified TPL mutants they produced the corresponding unnatural amino acids on preparative scales that (after HPLC purification) were then incorporated into proteins as a cross-link mimic and fluorescence modulator, respectively, using stop-codon suppression. Notably, the latter amino acid, dubbed HQAla, can also be used to create *de novo* metal binding sites in proteins.[37b,39] Despite this considerable promiscuity of TPL with regard to the phenol substrate, the use of pyruvate analogues with extended chains to produce β-substituted tyrosine derivatives has not been realised. Perhaps the use of high-throughput screens[40] or new variants created through large neutral drifts in continuous evolution experiments can make this a reality.[28]

The combination of TPL with other enzymes, either for *in situ* substrate synthesis or derivatisation of products, has been demonstrated in several studies. Combination of TPL with the P450 BM3 monooxygenase allowed preparative synthesis of tyrosine derivatives starting from mono-substituted benzenes in one-pot (Scheme 4A).[41] Tyrosine ammonia lyase (TAL) from *Rhodobacter spharoides* can be used to produce cinnamic acids from tyrosine derivative products of TPL that can then be converted into styrenes by ferulic acid decarboxylase (FDC) from *Enterobacter sp.* (Scheme 4B).[42] These styrene products can be subsequently hydrated in a Markovnikov fashion in one pot to produce enantioenriched benzylic alcohols by using a rationally designed FDC mutant.[43]

Aromatic Prenyl-Transferases (aPTases)

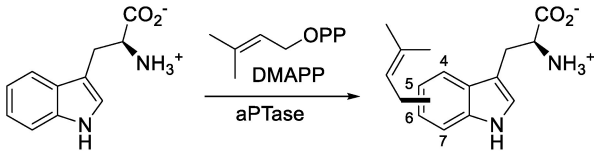
These enzymes transfer prenyl groups to a broad array of aromatic substrates, and play an important role in secondary metabolism in bacteria, plants and fungi[44] including their involvement in the biosynthetic pathway towards ergot alkaloids, which exhibit a range a biological activities.[45] This broad enzyme family is often divided into three categories: Mg^{2+}-dependent membrane-bound UbiA-type enzymes, dimethylallyltryptophan synthase (DMATS)-type enzymes, and ABBA-type enzymes.[46] The first type are found in bacteria, plants and fungi and contain an aspartate-rich motif for metal-ion binding, whilst the latter two types are not found in plants, and contain no metal-binding motif.

Observation of isotope scrambling employing ^{18}O labelled dimethyl-allyl-pyrophosphate (DMAPP) with a tryptophan prenylating enzyme (DMATS) from *Aspergillus fumigatus*, along with ^{2}H KIE experiments with a tryptophan isotopologue deuterated at the prenylation site suggest that these enzymes operate *via* formation and stabilisation of carbocations in the active site.[47] Various homologues of this enzyme have been identified that regioselectively prenylate tryptophan and tryptophan containing compounds in the 4-, 5-, 6-, and 7-positions (Scheme 5A).[48] The resulting products are biosynthetic intermediates for a variety of different indole alkaloids.[44] The prenyl donor and prenylation site can be successfully reprogrammed by rational mutagenesis advised by structural analysis.[49] For instance, just two mutations in the 6-DMATS enzyme from *Micromonospora olivasterospora* are sufficient to shift the regioselectivity of tryptophan dimethylallylation from the 6- to the 5-position and just one mutation gives a ten-fold boost in product yield with the non-native geranyl-pyrophosphate prenyl-donor (GPP). In two studies, single mutations were sufficient to switch the preference of fungal prenyl-transferases from DMAPP to GPP, and in one case even allow the mutant to accept the even longer chain farnesyl-pyrophosphate (FPP) donor.[50] Some aPTases also accept unnatural prenylating-



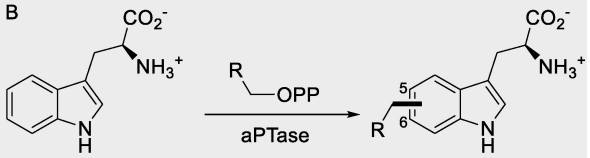
Scheme 4. Cascade reactions involving TPL. A. In situ P450 catalysed synthesis of phenols as substrates for TPL to form 3-substituted tyrosine derivatives.[41] B. Derivatisation of the tyrosine products of TPL by sequential de-amination and decarboxylation to form styrenes that can be optionally further hydrated in a regio- and enantioselective manner.[42–43]

A



aPTase	Position	Organism
FgaPT2	4-	*A. fumigatus*
5-DMATS	5-	*A. clavatus*
6-DMATS	6-	*S. ambofaciens*
HasE	7-	*A. fumigatus*

B



aPTase	R	Position(s)	HPLC yield (%)
FgaPT2	$(CH)_2CH_3$	5- and 6- (1:1)	38
	$(CH)_2CH_2CH_3$	6-	37
	Ph	5-	58
*Ac*5-DMATS	$(CH)_2CH_3$	5- and 6- (1:1)	44
	$(CH)_2CH_2CH_3$	6-	91
*Sa*6-DMATS	$(CH)_2CH_3$	6-	51
	$(CH)_2CH_2CH_3$	6-	91
	Ph	6-	14

Scheme 5. A. aTPases from a variety of fungal organisms that prenylate tryptophan regioselectively at the 4-, 5-, 6- or 7- positions.[48a,b,d,53] B. aTPases that can accept non-natural prenylation substrates.[51–52] See references for detailed reaction conditions. OPP = pyrophosphate.

substrate analogues including methyl-allyl-pyrophosphate (MAPP), 2-pentenyl-pyrophosphate (2-pen-PP) and benzyl pyrophosphate (Scheme 5B).[51] In many cases, the native regioselectivity of the particular aPTase is overridden by the non-native substrate, resulting in convergence in the regioisomer distribution towards 6-substitution of tryptophan with many different aPTases.[52]

In 2017, Dai, Sun and co-workers identified a highly promiscuous DMATS-type aPTase from the *Aspergillus terreus* fungus.[54] This enzyme, called AtaPT, was recombinantly expressed in *E. coli* and the purified enzyme was subsequently screened against 106 pharmaceutically relevant substrates, using DMAPP, geranyl-pyrophosphate GPP and FPP as prenyl donors, finding mono- di- or tri- prenylation with 72 of the substrates tested at either aromatic carbon or hydroxyl sites. Elucidation of the crystal structure of AtaPT identified a ring of tyrosine residues about the prenylating-substrate binding site, thought to form a 'shield' that stabilises carbocation formation, a motif also observed in the crystal structure of another fungal aPTase (Figure 3A).[55] Complexation with a variety of inert substrate analogues revealed multiple conformations of these tyrosine sidechains, as well as of a flexible loop, thought to permit acceptance of both multiple prenylating-substrates and aromatic substrates, respectively. Of particular interest is the modification of genistein by AtaPT, where prenylation with DMAPP or GPP gives derivatives of this compound that have slightly improved potency against three cancer cell lines (Figure 3B). With DMAPP, some O-methylation was observed with wild type AtaPT; a mutant, G326M, gave improved conversion in this reaction and significantly reduced O-methylation.

Aside from tryptophan, tyrosine can also by prenylated at C-3 by a fungal prenyltransferase (Scheme 6).[56] This enzyme, FgaPT2, was found to have a significant promiscuous activity for this transformation, along with its native tryptophan prenylation activity. Structural analysis and rational mutagenesis of FgaPT2 produced a mutant, K174F, with nearly 5-fold higher tyrosine prenylation activity and almost complete loss of native activity. *para*-Amino phenylalanine could also be subjected to the same transformation by FgaPT2 enzyme.[57] This enzyme and other aPTases can also prenylate ortho- and meta-tyrosine, although many give O-prenylation as the sole product with tyrosine.[56] Similar products can be obtained with O-prenyltransferases *via* Claisen rearrangement.[58] One of the above studies attempted to rule this pathway out by HPLC reaction-monitoring, not finding evidence for any intermediates.[57] This suggests that either this pathway does not occur, or else, that it is both rapid and enzyme-mediated. Moreover, some of the regio-isomers obtained cannot be formed by this process, thus

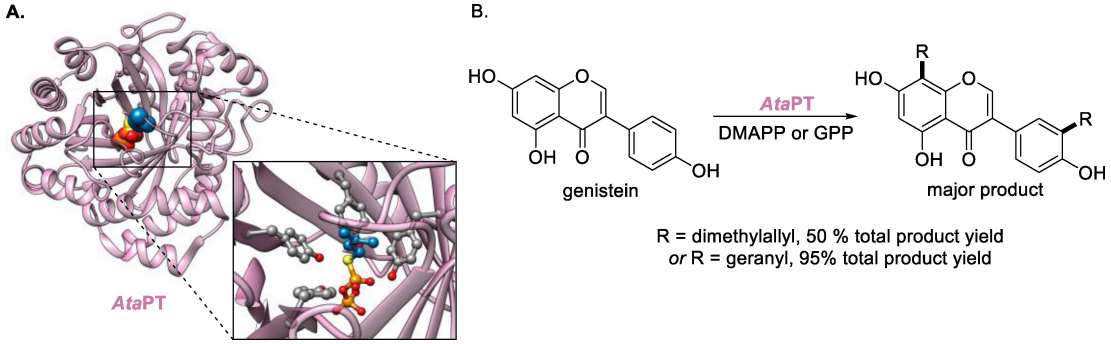
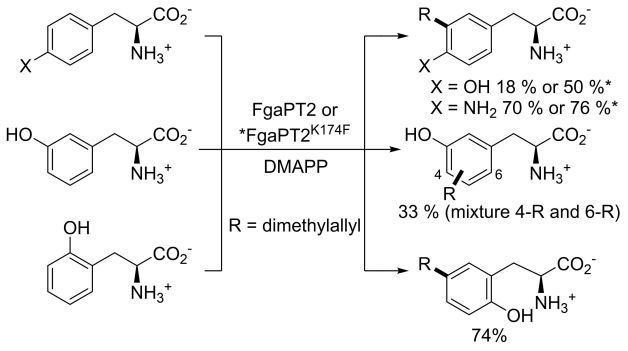


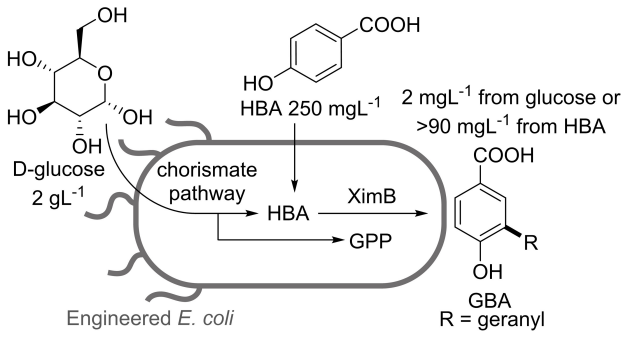
Figure 3. A. crystal structure of *Ata*PT from *Aspergillus terreus* (PDB: 5KCL) with (inlay) tyrosine shield thought to be responsible for substrate activation.[54] The substrate analogue dimethylallyl *S*-thiolodiphosphate (DMASPP) is shown in in blue with the heteroatoms coloured. B. Application of *Ata*PT to modulate the anti-cancer activity of genistein, major products shown, minor products are other regioisomers. See reference for detailed reaction conditions.



Scheme 6. Prenylation of positional isomers of tyrosine as well as para-amino phenylalanine by FgaPT2 and a single mutant.[56–57] See reference for detailed reaction conditions.

a Friedel-Crafts reaction pathway must be responsible for at-least some of the product formation.

Some relatively simple aromatic compounds can also be prenylated, for example fungal aPTases are also known that can prenylate hydroxynapthalenes[59] and acylphloroglucinols.[60] Hydroxy benzoic acid (HBA), that can be produced *in vivo* via the chorismate pathway, can be prenylated by the divalent-metal dependent UbiA-type[61] prenyltransferase XimB (Scheme 7).[62] The authors of this study produced an engineered *E. coli* strain with biosynthetic pathways for the production of GPP and HBA from glucose, although far higher product titres were obtained by direct feeding of HBA. Using this feeding approach, other HBA derivatives were also geranylated *in vivo*. Other prenyl pyrophosate derivates with shorter and longer chain lengths could also be produced *in vivo*, and XimB showed promiscuity for these prenyl donors, and titres of ~2–7 mgL^{-1} of HBA alkylated with dimethylallyl, farnesyl and geranyl-geranyl groups could be obtained. Very recently, Suga and co-workers identified a cyanobacterial prenyltransferase, LimF, which can prenylate histidine, and histidine analogues with altered main chain structures, in peptides at the C-2 position.[63] The creation of a database of prenyl transferase reactions, PrenDB, has allowed the prediction aPTases catalysing prenylation of unexplored aromatic substrates by computer-aided comparison of the similarity to known substrates.[64] This approach was experimentally validated, giving substrate conversion in over half the cases tested, and may help further expand the synthetic potential of these enzymes.



Scheme 7. Geranylation of hydroxy-benzoic acid by XimB, where the substrates can be produced in vivo from glucose by an engineered E. coli strain.[62] See reference for detailed reaction conditions.

A recent study identified the structural basis for catalysis by a new subclass of aPTases, whose folds are most similar to squalene synthase, and whose sequences were annotated as such.[65] These enzymes prenylate carbazole substrates, using active-site-bound magnesium ions to affect the abstraction of pyrophosphate from the prenyl donor. An aspartate residue anchors and stabilises the aromatic substrate by hydrogen bonding with its catechol moiety.

The potential of aPTases to produce biologically active compounds has recently been furthered in their application toward improving heterologous cannabinoid biosynthesis[66] and in the chemoenzymatic synthesis of daptomycin analogues with activity modulation.[67] The discussion on this diverse enzyme family provided here is far from exhaustive, and for further information we direct the reader to recent reviews on the topic.[46,68] The broad range of aromatic substrates identified with various aPTases, as well as their frequent acceptance of alternative and non-natural prenyl-donors, makes them appealing biocatalysts for the preparation of many alkyl-aryl linkages.

Methyl Transferases (MTases)

S-adenosyl methionine (SAM) dependent enzymes are responsible for the methylation of a diverse range of substrates. They have garnered significant interest for their potential to modulate the properties, e.g., lipophilicity, of biologically active compounds, to replace toxic methylating reagents in organic synthesis, and to selectively label DNA and proteins.[69–70] SAM contains a sulfonium centre where the reactive methyl group is attached, and the *S*-adenosyl homocysteine (SAH) forms a leaving group in this methylation reagent. Whilst the majority of MTases catalyse O-, N- and S- methylation, a few MTases catalyse the C-methylation of aromatic substrates. These enzymes include NovO and CouO from *Streptomyces* and Coq5 from *Saccharomyces cerevisiae*, all of which have been structurally and mechanistically characterised, with a high degree of similarity found in both their folds and modes of action.[71] Coq5 is involved in coenzyme Q biosynthesis, and has yet to find a synthetic application on non-natural substrates (Figure 4).[71a] CouO and NovO catalyse methylation steps in the biosynthesis of coumermycin A_1 and novobiocin, respectively. However, they can also methylate a variety of (amino)coumarins, derivatives of their natural substrates, and CouO can also methylate some naphthalene diol isomers.[69,71c,72]

A remarkable aspect of SAM dependent methyl-transferases, including those catalysing Friedel-Crafts alkylation, is their broad acceptance of cofactor analogues with non-natural alkyl-substituents at the sulfonium centre, which can be prepared chemically.[73] As early as 2009, Gruber-Khadjawi and co-workers demonstrated that CouO and NovO could use these unnatural cofactors to install vinyl, crotyl, alkyne-containing and benzyl

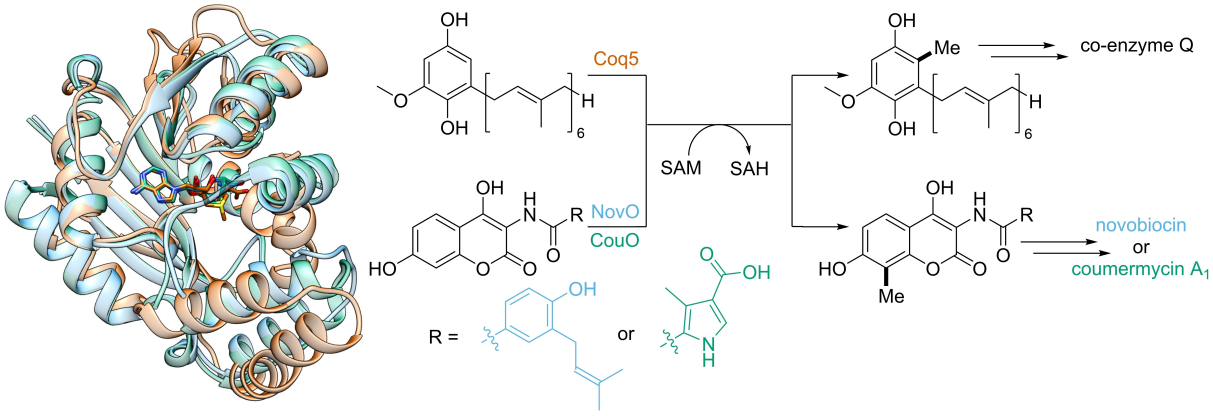
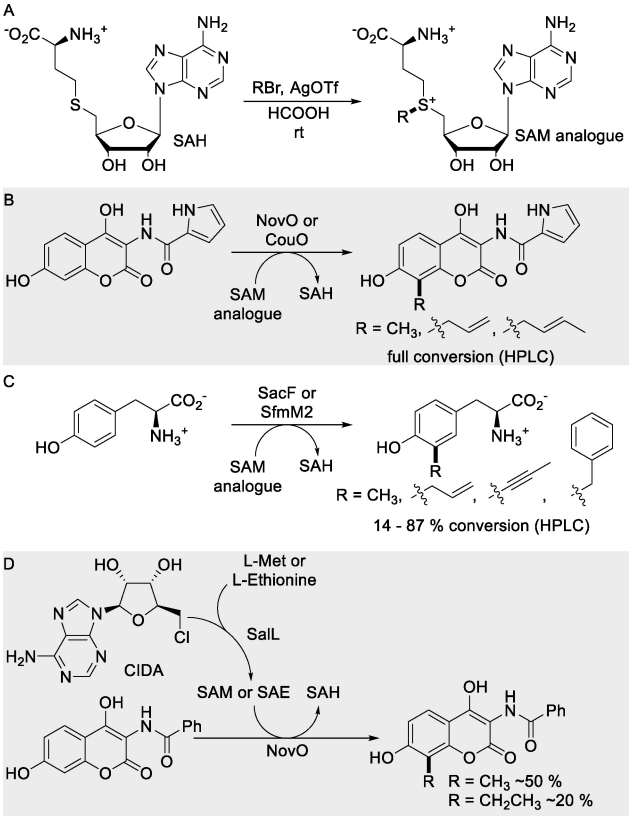


Figure 4. Overlay of the crystal structures of Coq5 (orange, PDB: 4OBW), NovO (blue, PDB: 5MGZ) and (CouO green, PDB: 5 M58) and their native methylation reactivities. CouO is active for methylation at several points through the synthesis of the dimeric coumermycin A_1 biosynthesis, an intermediate with half of the total dimeric-coumarin structure is shown.[69]

groups onto aromatic substrates.[72a] The library of SAM analogues they used were prepared by a general methodology from SAH and seven different alkyl-bromides, using silver(I) triflate as Lewis acid catalyst (Scheme 8A). Coumarin derivatives reminiscent of the native substrates of CouO and NovO were employed (Scheme 8B). In many cases excellent conversions were obtained, and in some cases, their reactivities were complementary, with each filling in gaps in the other's substrate scope. The same group also demonstrated that two MTases, SacF and SfmM2, involved in 3-methyl-tyrosine biosynthesis, could also accept a variety of SAM analogues, to produce tyrosine derivatives with alkyl groups at C-3 (Scheme 8C).[74] SacF performed better than SfmM2 in general and L-DOPA and D-tyrosine could also be methylated, although the former substrate only gave traces of product. Another MTase, Orf19, involved in the biosynthesis of Anthramycin could also be used to alkylate 3-hydroxykynurenine, accepting the SAM analogues with substrate conversions ranging from 3–50%.



Scheme 8. A. Chemical synthesis of SAM analogues by Lewis-acid catalysis. Application of SAM analogues for the alkylation of (B.) coumarin derivatives by CouO and NovO and (C.) tyrosine by SacF and SfmM2.[72a,74] D. In situ synthesis of SAM or SAE from CIDA by SalL, and their concurrent application as substrates in the methylation or ethylation of a coumarin substrate by NovO.[79]

Another class of methyl transferases active on aromatic substrates, exemplified by TsrM, utilise a B_{12} cofactor (cobalamin) to mediate transfer of the methyl moiety from SAM to the aromatic substrate.[75] TsrM is involved in the biosynthesis of thiostrepton, a ribosomally synthesized and post-translationally modified peptide (RiPP) with antibacterial activity against both Gram-negative and some Gram-negative bacteria.[76] Specifically, TsrM catalyses the C-2 methylation of tryptophan, which eventually forms a quinaldic acid moiety in the natural product. This enzyme has an intriguing structure to its catalytic machinery. It contains a [4Fe–4S] cluster, typically involved in radical mechanisms by related enzymes, yet aromatic methylation by TsrM does not involve radical intermediates, as indicated by structural, spectroscopic, and feeding studies.[77] Instead, the mechanism involves nucleophilic action of the cobalt centre to abstract a methyl cation from SAM. The methyl-cobalamin intermediate formed can then be subject to nucleophilic attack from C2 of the tryptophan substrate, affording the C2-methylated product after re-aromatisation (deprotonation). As well as the native tryptophan substrate, a variety of tryptophan and tryptamine derivatives were converted by TsrM.[78] Substituents on the indole ring affected the regioselectivity of the methylation, and some tryptamine substrates were also methylated at the main-chain nitrogen.

In recent years, the biocatalytic, often *in situ*, preparation of SAM analogues has been a burgeoning field of research. For example, methionine adenosyltransferases (MATs) have been used to realise the biocatalytic preparation of SAM analogues from methionine analogues and adenosyl triphosphate (ATP),[80] and can combined with ATP regeneration enzymes to such that only methionine (derivatives) and polyphosphate need to be added to the alkylation reaction.[81] Burley and co-workers demonstrated that the chlorinase SalL could be used to produce SAM derivatives with modified alkyl substituents, nucleobases, and carboxyl moieties exchanged for tetrazoles from methionine analogues and 5′-deoxy-5-chloroadenosine (CIDA) analogues.[79b] These could be subsequently utilised by NovO for methylation and ethylation of coumarin substrates (Scheme 8D).[79] Halide methyltransferases (HMTs) have been applied for SAM synthesis from S-adenosyl homocysteine (SAH) and simple alkyl halides, which facilitates use of catalytic SAH loadings.[82] This methodology has also been expanded to the preparation of SAM analogues containing fluoromethyl, ethyl, propyl and allyl substituents.[83] Enzymatic carboxylation of SAM or S-adenosyl ethionine (SAE) by CmoA has been used to produce unnatural cofactors utilised by MTases to transfer the resulting carboxyl-containing alkyl groups on to nitrogen or oxygen centres.[84] Not all of the diverse array of SAM analogues have been applied with Friedel-Crafts alkylating MTases. In the future we may expect to see, for example, cascades using HMTs and MTases to alkylate aromatic substrates with an array of alkyl chains starting from alkyl-iodides, and using catalytic amounts of SAH.

Acyl Transferases

Friedel-Crafts reactions conducted by chemists are broadly divided into two categories: alkylations and acylations. Whilst the enzymes discussed so far all catalyse the former category of transformations, some natural acyl-transferases (ATases) have recently been discovered that can transfer acyl groups to arene acceptors.[85] In their native reaction, these enzymes transfer an acetyl group from one molecule of mono-acetyl-phlorglucinol (MAPG) to another. This process is effectively a disproportionation reaction, affording di-acetyl-phlorglucinol (DAPG) and phlorglucinol (PG) (Figure 5B). The reaction is reversible; thus DAPG can also act as acetyl donor for the PG substrate. To date, at least four ATases catalysing this reaction have been identified by BLAST searches, with sequence identities of 80–99%, all of which originate from *Pseudomonas* strains.[85b] That from *Pseudomonas brassicacearum* (*Pp*ATase) gives good activities in the cell-free extract upon codon-harmonisation and recombinant expression in *E. coli*. The protein is a hetero trimer, having three stop-codons in the gene sequence, and its complex heterododecameric structure has been determined crystallographically.[86] Computational studies based upon this structural information suggested a mechanism for *Pp*ATase where a catalytic cysteine forms a thioester intermediate upon acylation by the acyl-donor. Two histidines and a tyrosine in proximity to the catalytic cysteine shuttle protons to and from the hydroxyl groups of the substrate to aid the formation and reprotonation of quinoid and phenolate intermediates (Figure 5A).[87]

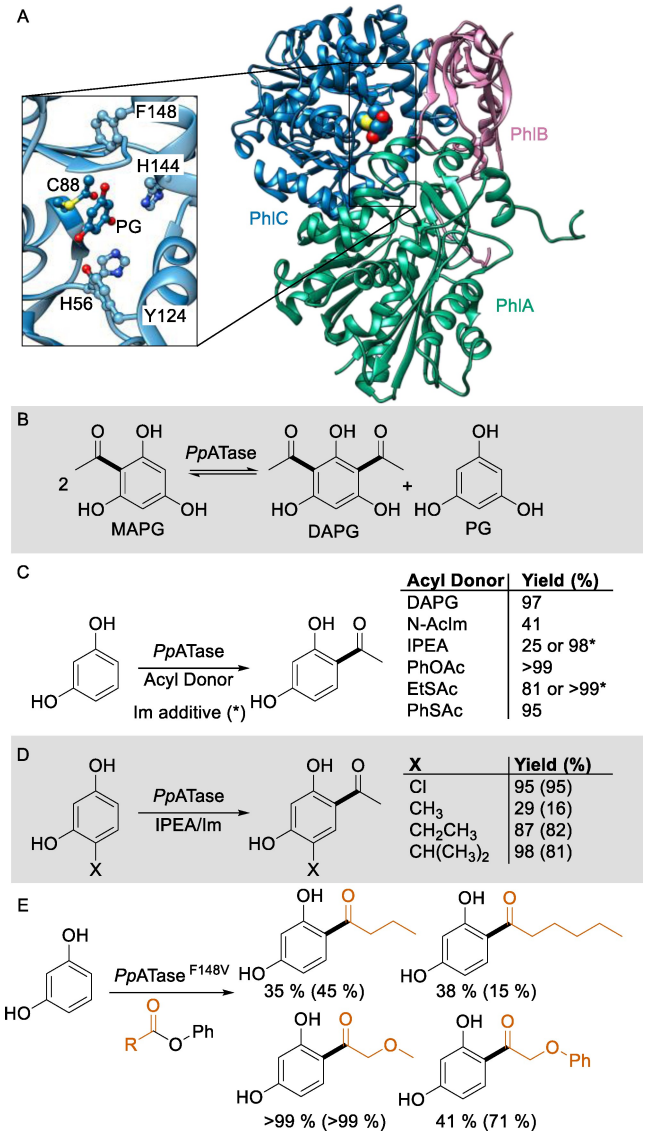


Figure 5. A. Heterotrimer structure (part of the larger heterododecamer) of *Pp*ATase with PG bound, and inlay showing key residues in the active site (PDB: 5MG5).[86] B. Native reaction of *Pp*ATase. Application of *Pp*ATase (C.) with resorcinol and native and non-native acyl-donors, (D.) on a variety of resorcinol derivatives and, (E.) application of a single-mutant for the transfer of bulky acyl-groups to resorcinol.[85b,88] See references for full description of reaction conditions.

*Pp*ATase exhibits promiscuity with regard to the aromatic substrate, and can acetylate a variety of resorcinol derivatives with substituents in the 4- or 5-positions. Most of these substrates give moderate or excellent conversions in the presence of a 1.5-fold excess of a native acetyl donor, DAPG (Figure 5D).[85b] Obtaining high conversions with these non-native substrates suggested that these reactions, unlike the native one, are irreversible. This is a useful property for the application of *Pp*ATase, yet also suggests a strong preference

for the native acyl-donor. The native DAPG acyl-donor is a desirable substrate by comparison to the structurally complex and costly acyl-CoA used by other acyl-transferring enzymes. Nevertheless, DAPG is not commercially available, spurring the search for alternative acyl-donors (Figure 5C). N-acyl-imidazole could serve as a some-what effective DAPG-surrogate, becoming very effective with the addition of an equal concentration of imidazole, affording the products with poor to excellent isolated yields. The imidazole additive proved a general method to switch *Pp*ATase to accepting acetyl donors commonly used with lipases, such as vinyl- and isopropenyl- acetate as well as phenyl acetate and its derivatives.[85b,88a] Interestingly, as revealed by reaction monitoring with HPLC, changing the acetyl donor introduces a new reaction pathway where O-acetylation precedes a Fries-rearrangement reaction to give the C-acetylated products, which is not observed with DAPG. Thioesters could also be employed as acetyl-donors, where, in some cases, only small loses in yield were seen when imidazole was omitted from the reaction.[88b] However, large excesses of thioester were still needed to achieve good product yields. The wild-type *Pp*ATase cannot transfer acyl-groups larger than propionyl. Rational engineering aimed at reducing steric bulk around the catalytic cysteine identified a beneficial mutation, F148V, that allows *Pp*ATase to transfer a range of acyl groups with bulkier side chains to resorcinol substrates using the corresponding phenyl-esters as acyl-donor.[88c] Currently, little progress has been made towards expanding aryl-substrate acceptance of *Pp*ATase; all substrates demonstrated so far are resorcinol derivatives. Whether this is the result of particular intricacies in the substrate binding site, or the result of inherently lower reactivity of the enzyme-acyl intermediate, is yet to be determined.

Squalene Hopene Cyclases (SHCs)

These enzymes are a class of triterpene cyclases that protonate their linear poly-unsaturated substrates using a catalytic aspartic acid residue forming a tertiary carbocation. This starts a poly-carbocyclisation cascade that results in the stereoselective formation of multiple carbon-carbon bonds.[89] With the native squalene triterpene substrate, the catalytic action of these enzymes forms five new C–C bonds in the product, and termination of the process by either deprotonation or hydroxylation gives terminal isopropenyl (hopene) of isopropanol (hopanol) substituents in the products, respectively. The hydrogen bonding network around the catalytic aspartic acid gives the proton an *anti*-orientation with respect to the carbonyl moiety, increasing its acidity to the point where it can protonate these carbon-carbon double bonds that do not possess particular basicity.[90] Of particular interest are the SHCs from *Alicyclobacillus acidocaldarius* (*Aac*SHC) and *Zymomonas mobilis* (*Zmo*SHC) that show both significant substrate promiscuities as well as a proclivity towards laboratory evolution to expand upon these non-native activities (Figure 6A).[91] For instance, when non-native substrate analogues with terminal hydroxyl-, carboxyl-, of keto-groups are employed with this enzyme, very low to moderate degrees of formation of products containing cyclic ether, lactone, or enol-ether moieties are observed by alternative termination mechanisms.[92] *Aac*SHC is particularly well-suited to targeted mutagenesis. Altering the steric bulk at 16 residues within 15 Å of the catalytic D376 created a library single of mutants with differing degrees of non-native activities.[93] When screened on a variety monoterpene derivatives, including those described as inactive with SHCs, hits in the library were found that could effectively form the resulting cyclohexane products with large increases in conversion compared to the vanishingly small conversions obtained with the wild-type, and excellent stereoselectivities (Figure 6B). Again, these products were formed by termination of the carbocation intermediate with an internal accepting moiety.

When the substrate is designed to have an arene moiety, the formed carbocations can alkylate the arene ring, giving the resulting Friedel-Crafts products.[94] A substrate that forms two carbocycles in this process is required for this reaction, and both *Aac*SHC and *Zmo*SHC show low conversions.[94–95] However, for *Aac*SHC, which has good evolvability, the desired reaction is competed by a undesired hydration process that gives an alcohol product without an aryl-alkyl bond.[95] Screening of a library of 66 *Aac*SHC mutants, mostly at the positions previously described, identified some positions important for both con-

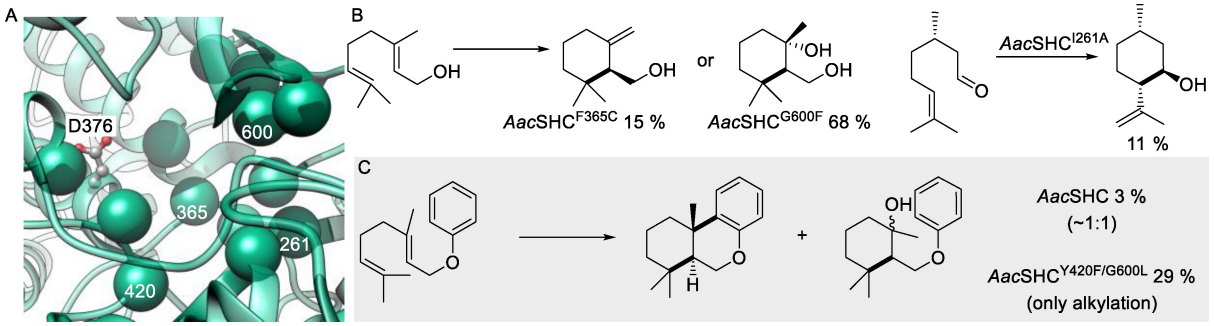


Figure 6. A. Active site structure of *Aac*SHC showing the catalytic aspartic acid residue, and residues targeted for mutagenesis shown as spheres (PDB: 1UMP). B. Unnatural reactions catalysed by *Aac*SHC mutants where the wild-type gives less than 1% conversion.[93] C. Friedel-Crafts alkylation and hydration side-product by wild-type *Aac*SHC and the best performing double mutant identified.[95] See references for detailed reaction conditions.

version as well as product selectivity. Saturation libraries at these positions were screened, and hits that gave improved selectivities were found, although conversion with the most selective mutants was still very low. Eventually, by combining mutations identified from this screening, with ones identified through a consensus-approach from perfectly selective homologues (that gave only low conversions and were less mutable than *Aac*SHC) a double mutant was produced that gave perfect selectivity for alkylation and a conversion of 29%, a more than ten-fold increase compared to the wild-type. To date, only one substrate has been demonstrated, and substrates that could form three or more carbocycles do not undergo Friedel-Crafts alkylation with the SHCs tested.[94] However, the impressive catalytic plasticity of SHCs makes the future discovery of further SHC-catalysed Friedel-Crafts reactions seem likely.

An enzyme from Cylindrocyclophane F Biosynthesis

Cylindrocyclophane F is part of a larger family of cytotoxic compounds of cyanobacterial origin, all of which share a peculiar [7.7]paracyclophane ring structure.[96] In the early 1990s, feeding experiments revealed the biosynthetic origin of their polyketide component, and suggested that the ring structure was formed through a head-to-tail dimerization reaction.[97] The putative enzymes for this reaction catalyse the Friedel-Crafts alkylation of resorcinols with alkyl chlorides, a reaction of striking similarity to its chemo-catalytic counterpart, and thus were thought to hold powerful synthetic potential. In 2017, Balskus and co-workers revealed the identity of one such enzyme, CylK as part of their investigation of the biosynthetic pathway to Cylindrocyclophane F in *Cylindrospermum licheniforme*.[98] *In vitro* assays of CylK with its substrate revealed significant Cylindrocyclophane F formation after 20 hours of incubation, which is formed in a step-wise manner *via* a mono-alkylated intermediate (Figure 7A). When the (*S*,*S*) configured substrate (inverted position of the chloride) was incubated with CylK no reaction occurred, showing that the alkylation occurs in a concerted manner with inversion of stereochemistry, consistent with an S_N2 mechanism.

In a later study, Balskus and co-workers went on to probe the biocatalytic potential of CylK with non-natural substrates.[99] Consistent with the formation of the mono-alkylated intermediate they previously observed, the ability of a substrate to form a [7.7]paracyclophane ring is not necessary for CylK, and non-natural resorcinol and alkyl-chloride substrates can be coupled to one another in high conversions with turnover numbers exceeding one thousand in some instances. Again, the stereocenter was inverted compared with that of the alkyl-chloride substrate, and utilisation of a racemic alkyl chloride substrate gave the product with 88% enantiomeric excess, showing that CylK is selective for a certain substrate absolute configuration. CylK can also convert other alkyl halide substrates with conversion decreasing from chloride to bromide and iodide, however alkyl fluoride substrates were not converted (Figure 7B). Simple resorcinols could also be accepted as substrates, giving low to moderate conversions (Figure 7C). Simple alkyl-chloride substrates were not converted by CylK, neither were phenol nor indole, and conversion of a pharmaceutically relevant stilbene substrate gave conversions of 1–4% depending on the alkyl-chloride substrate.

Two recent studies revealing crystal structures of CylK found it to be a fusion of Ca^{2+} binding and β-propeller domains, with multiple Ca^{2+} binding sites supporting the fold.[100] Through a combination of structural, mutagenesis and simulation studies, the location of the active site was identified in the dimer interface. Here, critical arginine (R105) and tyrosine (Y473) residues are responsible for enabling chloride abstraction and

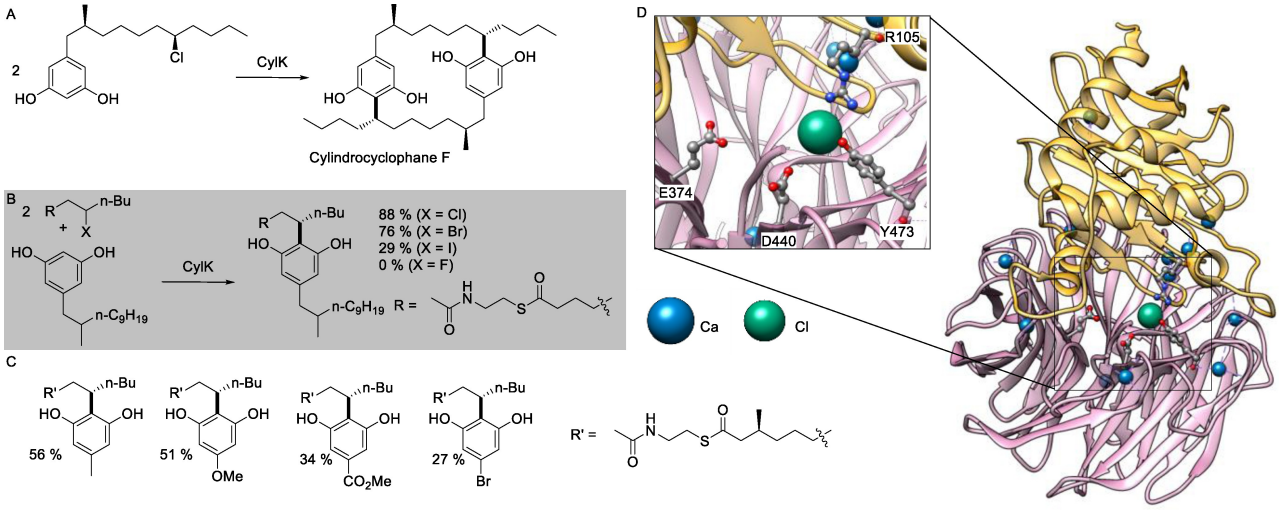


Figure 7. A. Action of CylK in the final biosynthetic step of Cylindrocyclophane F.[98] Application of CylK with unnatural substrates with different halide groups (B.) and (C.) with simple resorcinols.[99] D. Crystal structure of CylK (PDB:7FH6) reveals fused Ca^{2+} binding (light orange) and propeller (pink) domains, supported by many Ca^{2+} binding sites (Ca shown in blue). Important residues in the active site for binding chloride (green) and resorcinols are shown in the insert.

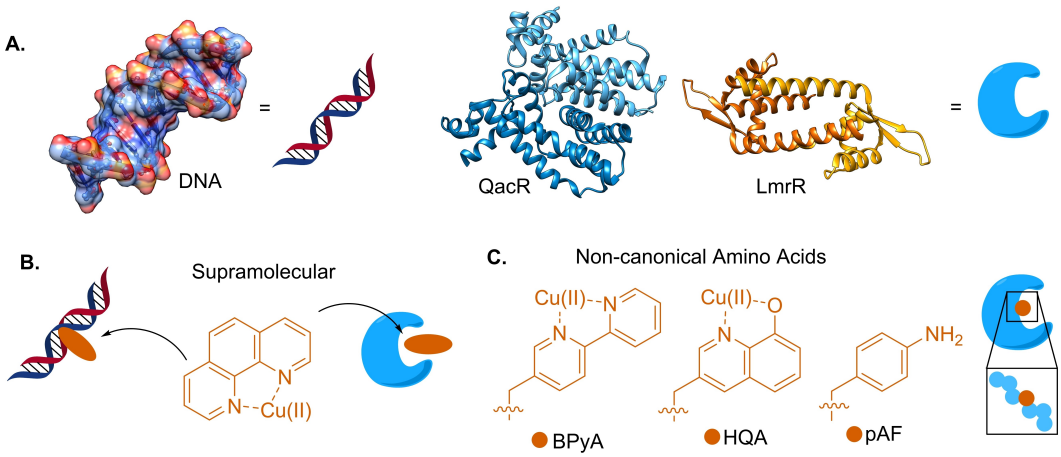


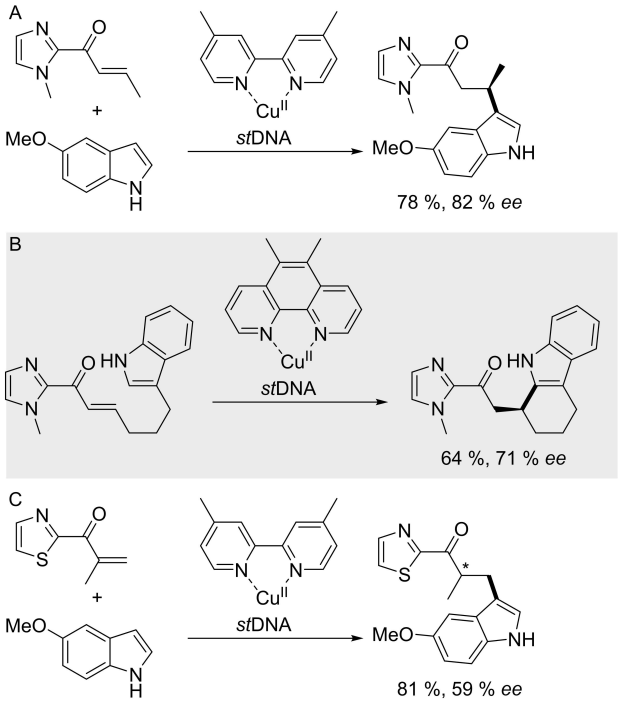
Figure 8. A. Biomolecular scaffolds to create bio-hybrid catalysts including DNA and the proteins QacR (blue, PDB: 1JTY) and LmrR (orange, PDB: 3F8B) whose homodimeric structures are shown in different shades. Methods to assemble the biohybrid catalysts, including (B.) supramolecular assembly, and (C.) the use of unnatural amino acids incorporated into proteins biosynthetically.

bridge the two domains. Glutamic acid (E374) and aspartic acid (D440) residues are essential for positioning and binding of the resorcinol hydroxy groups. Several mildly- or non-deleterious mutations at positions around the active site were also identified, giving good prospects for future engineering of CylK towards activity in more societally relevant transformations.

Artificial Enzymes and DNA Catalysts

As well as natural, promiscuous and engineered enzymes that catalyse the Friedel-Crafts alkylations discussed above, bio-hybrid catalysts that utilise both synthetic (chemical) and biological components have also been described in the literature. These systems take their inspiration from the chemo-catalytic approaches to Friedel-Crafts alkylations and exploit catalytic machinery similar to small molecule catalysts for this reaction. These chemical moieties are incorporated into biological scaffolds such that the scaffold either imparts selectivity, bolsters activity, or provides both of these functions (Figure 8A).[9,101] The biological scaffold can variously be either protein or DNA, and several methods for incorporation of the catalytic moiety exist, including supramolecular assembly and biosynthetic introduction of unnatural amino acids with catalytically relevant side-chains (in the case of protein scaffolds, Figure 8B and C).[15a] In all cases demonstrated to date, and in contrast to most other enzymes discussed in this review, biohybrid catalysts for the Friedel-Crafts reaction exploit a vinylogous mechanism. During the reaction, an α,β-unsaturated bond is subject to a conjugate addition by the arene nucleophile, thus no leaving-group is required, and the reactions have perfect atom economy.

The first bio-hybrid Friedel-Crafts catalyst was reported by our group in 2009, and consisted of a copper (II) complex with 4,4′-dimethyl-2,2′-bipyridine ligand supramolecularly bound (i.e. intercalated) to DNA (Scheme 9A).[102] The copper complex acts as a Lewis acid catalyst, binding an α,β-unsaturated imidazole



Scheme 9. A. Inter-[102] and (B.) intra-molecular[107] Friedel-Crafts reactions catalysed by DNA catalysts consisting of copper(II) complexes bound to DNA. C. Tandem Friedel-Crafts alkylation-enantioselective protonation reaction by DNA catalysis.[110] See references for full reaction conditions.

ketone substrate and lowering its LUMO energy, which allows it to undergo conjugate addition at the β-position by an indole nucleophile. This mechanism bears a similarity to that of TrpB. However, the Michael acceptor moiety in this case is preformed, rather than undergoing dehydration during the activation process. Furthermore, the activation relies on Lewis acid-base, and not covalent, interactions. The imidazole ketone substrate contains an alkyl or aryl group at the β-position and is thus prochiral. The Friedel-Crafts product was formed with enantiomeric

excess despite the copper complex itself being achiral, thus the chirality of the DNA influenced the stereomeric outcome of the reaction. Indeed, the identity of the DNA could have large effects on the enantiomeric excess, with *ee* values ranging from 10 to 93% with single or double-stranded DNA. Decent *ee* values (64-82%) could be obtained with DNA from salmon testes, which is commercially available as a lyophilised powder, and thus allowed preparative scale reactions to be conducted. The system proved efficient, and loadings of the copper complex as low as 0.3 mol% could be employed without decrease in enantiomeric excess, although largely increased reactions times were required for full conversion. According to the binding affinity of the copper complex for DNA, only 16% was bound at these concentrations, however good enantiomeric excess was maintained by the fact that binding of the copper to the DNA causes a 30-fold rate acceleration in catalysis, and thus the unbound majority of the copper species do not contribute significantly to product formation.[103] Rate acceleration by DNA proved a general effect for a variety of substrates in the Friedel-Crafts reaction, although in some other copper-catalysed reactions DNA causes a decrease in rate.[104] The identity of the DNA could affect the apparent rate by around a factor of 2. A variety of organic co-solvents were found to increase the rate of this reaction, which facilitated preparation of some products on a gram-scale.[105] Custom DNA sequences can easily be prepared, including those with nucleic acids featuring the unnatural L-configuration. By using DNA sequences previously described for the Friedel-Crafts reaction, along with their mirror images, Smietana, Arseniyadis and co-workers could selectively prepare either enantiomer of the product.[106] This impressive result showed the versatility of DNA scaffolds in catalysis; however it is not applicable to preparative synthesis.

Intra-molecular Friedel-Crafts reactions have also been demonstrated with bio-hybrid catalysts by Park and co-workers.[107] They employed substrates with both α,β-unsaturated imidazole ketone and indole moieties with a spacer length appropriate to form a 6-membered ring in the resulting product (Scheme 9B). In these reactions, the C–C bond is formed at the less reactive C-2 position of the indole, since the C-3 position is already occupied in the substrate. Here, the ligand used for the copper complex had significant effect on the enantioselectivity and yield, with 5,6-dimethyl-1,10-phenanthroline (5,6-dmp) giving the best results. Computational studies of this reaction concluded that the origin of the observed enantioselectivity (*S*) was increased affinity of the pro-*S* substrate conformer for the DNA scaffold.[108] Further studies into this reaction employed DNA conjugates with bipyridine ligands synthetically incorporated into the phosphate backbone.[109] Through varying the position of the ligand and the surrounding nucleobases, the effect of the DNA scaffold on the copper catalysis was elucidated.

α,β-unsaturated substrates with substituents on the α-position have also been employed in Friedel-Crafts bio-hybrid catalytic reactions, although the N-methyl imidazole moiety must be exchanged with a thiazole moiety to allow substrate chelation at copper and thus a planar conformation (Scheme 9C).[110] In these reactions, after formation of the C–C bond, a prochiral enolate species is formed, and thus a stereocentre is formed by protonation, a process notoriously difficult to control with small molecule catalysts.[111] DNA provides a chiral environment for the copper complex that allows the protonation of the enolate to occur with facial selectivity giving *ee* values up to 84%. In the tandem Friedel-Crafts/enantioselective-protonation process, the DNA also affects a rate acceleration of up to ~10^3, far higher than observed with other Friedel-Crafts reactions. This is thought to be due to a localisation of all the reaction components in close proximity to the DNA helix, resulting in high effective molarity, which provides a major contribution to rate enhancement.

The effect of DNA sequence on both rate-acceleration and enantioselectivity is rather complex. This is partly due to the heterogeneous nature of DNA catalysts: many distinct environments contribute towards the overall activity. Sequences containing G tracts of at least three consecutive G's (or C's) were found to give rise to the highest rate and enantioselectivites in a Diels-Alder reaction.[112] However, in the Friedel-Crafts reaction, DNA sequence gave less significant effects on the enantioselectivity and rate acceleration.[104] Further possibilities for optimization are limited; higher order DNA structures have been used, but they do not provide a clear improvement.[113] Moreover, more complex, protein like structures (DNAzymes) are limited by the fact that free nucleotides (not involved in base pairing) bind the copper, blocking vacant coordination sites and thus severely reduce catalytic activity.[112] In protein engineering, separation of the library members is easily permitted via the production of colonies on solid media, which, in turn, allows a plethora of analysis methods. Such a workflow is not available for engineering DNA sequences, and thus some kind of binding or immobilisation is commonly used to identify improved library members, which limits the scope of reaction that can be screened and may produce sequences with high substrate specificity.[114]

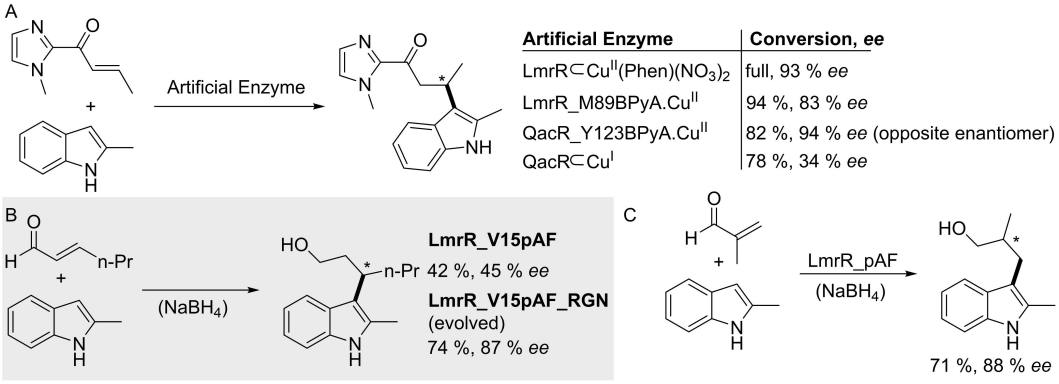
In more recent years, our group has focussed on developing artificial enzymes with protein scaffolds, rather than DNA, several of which also catalyse Friedel-Crafts reactions. The protein scaffold we employ is called the Lactococcal multidrug resistance regulatory protein (LmrR) that features a large and promiscuous hydrophobic binding pocket at the interface of its homo-dimeric structure (Figure 8A).[115] Two tryptophan residues (W96 and W96′) in the centre of this binding pocket give significant contribution to the strength of the ligand interactions. The range of ligands that can be bound there extends to the copper(II) complexes previously employed in DNA catalysis, although their low micromolar binding constants are far higher than some other known ligands.[116] Thus, like with DNA, copper(II) complexes can be incorporated supramolecularly into LmrR, and the resulting artificial enzymes give good conversions in the vinylogous Friedel-Crafts reaction with *ee* values up to 93%. Mutation of tryptophan 96 to alanine abrogates both copper complex binding and enantioselectivity in the reaction, illustrating the role of the chiral protein environment in the reaction. This artificial enzyme can also catalyse the tandem Friedel-Crafts-alkylation enantioselective-protonation process

previously described above, however here the LmrR scaffold contributes in a different and somewhat more cryptic manner.[117] Without the copper complex, LmrR alone gives the opposite enantiomer than in its presence, and the W96A mutation has little effect on the reaction outcome, suggesting that this reaction may employ a different part of the protein environment, rather than the binding pocket.

Aside from supramolecular catalyst assembly, artificial enzymes with LmrR can also be assembled through the incorporation of catalytically relevant UAAs. These UAAs can be incorporated biosynthetically during recombinant expression in *E. coli* through the use of amber stop-codon suppression technology pioneered by Schultz.[14a] In practice, this is easily implemented with a two-plasmid system where one-plasmid encodes the LmrR protein with an in-frame amber stop-codon, and another encodes the orthogonal translation system responsible for UAA incorporation. This concept was first demonstrated in LmrR using (2,2′-bipyridin-5-yl)-alanine (BpyA), which has a 2,2′-bipyridine moiety in its side-chain and thus can bind copper(II) (Figure 8C).[118] When BpyA was incorporated at position methionine 89 in LmrR and copper(II) was added, the *ee* obtained in the Friedel-Crafts reaction was higher than with other positions, up to 83% with the best substrate (Scheme 10A). Another copper-binding UAA, 2-amino-3-(8-hydroxyquinolin-3-yl)propanoic acid (HQA – note, this is a different isomer to the formerly mentioned HQAla) can perform a similar function to BpyA in this context, albeit it with lower conversion and *ee* (Figure 8C, Scheme 10A) .[39a] BpyA could also be used to create active catalytic sites in several other proteins, from the TetR family of MDR (multi-drug resistance) regulatory proteins, producing the opposite enantiomer of some Friedel-Crafts products with up to 94% *ee* when incorporated into QacR.[119] These TetR family proteins were also capable of performing the same reaction with modest *ee* values by simple addition of copper (II) without ligand, and no binding site deliberately incorporated.[120]

The use of unnatural amino acids to create catalytic sites in LmrR where the side-chain participates directly in catalysis, rather than acting as a ligand to bind metals, has also been demonstrated.[121] Here, we took inspiration from organocatalysis, selecting the UAA para-aminophenylalanine (pAF) for its aniline side-chain, which we envisaged could condense aldehyde substrates to form activated iminium-ion intermediates (Figure 8C).[122] Indeed, when pAF was incorporated at position valine 15, forming LmrR_pAF, iminium ion intermediates formed by condensation with benzaldehyde could be trapped by reduction with sodium cyanoborohydride and the adduct observed by mass spectrometry.[121] Initially work focussed on the use of this artificial enzyme for hydrazone formation, a reaction known for aniline small-molecule catalysts, and by using a chromogenic substrate a directed evolution campaign was conducted identifying a triple mutant that had a catalytic efficiency increased by nearly two-orders of magnitude.[123] In order to switch to Friedel-Crafts alkylation reactivity, α,β-unsaturated, rather than aromatic, aldehydes were employed.[124] These substrates create iminium ion intermediates, activated at the β-position for attack by indole nucleophiles. The iminium ion intermediate is reminiscent of the amino-acrylate intermediate in TrpB and TPL, but is more closely related to intermediates produced by MacMillan imidazolidinone catalysts. Despite the considerable structural differences between these organocatalysts and pAF, LmrR_pAF proved an adequate catalyst for this transformation, although the product yields and *ee* were modest (Scheme 10B).[125] Two rounds of directed evolution and one round of rational combination of individual beneficial mutations improved these metrics markedly, with the best triple mutant giving 87% ee, and showing improved reactivity and selectivity with every tested substrate over the parent. This approach was also extended to α-substituted α,β-unsaturated aldehydes, where the stereo centre is formed by enantioselective protonation of an enamine intermediate (Scheme 10C).[126] LmrR pAF affords the product with 88% *ee* without the need for any further mutations.

Several strategies have recently been reported for the creation of artificial enzymes with multiple catalytic components acting either in concert or subsequently, including an artificial Friedel-Crafts alkylase that performs a vinylogous alkylation reaction.[127] The crotonic acid substrate is produced *in situ* from vinyl crotonate by the same enzyme, since it



Scheme 10. A. Friedel-Crafts alkylation catalysed by artificial enzymes based on copper(II) and MDR protein scaffolds.[116,118–120] B. Organocatalytic artificial enzyme catalysed Friedel-Crafts alkylation[125] and (C.) tandem-enantioselective protonation reactions.[126] See references for full reaction conditions.

features a designed catalytic triad based esterase active site.[127b] Design of the second active site of this enzyme made use of the native esterase site to install a copper(II) complex with a suicide-inhibitor motif in the ligand back-bone. This machinery then catalysed the formation of 3-phenylbutyric acid with perfect enantioselectivity, despite the lack of a chelating moiety in the crotonic acid substrate, but the mode of substrate binding to copper was not elucidated. Remarkably, the artificial enzyme operated under mild conditions whilst this reaction otherwise requires long reaction times with refluxing neat substrates with strong Lewis-acids, and copper(II) complexes are not reported to catalyse the reaction. Only one substrate was demonstrated, thus the synthetic potential is, so far, unknown.

Summary and Outlook

Friedel-Crafts enzymes can be compared and contrasted based on a few categories, namely (1) their mechanism of action, (2) scope of alkylation substrates employed, and (3) the scope of arenes that can be alkylated.

(1) Most of the mechanisms employed by the enzymes discussed here differ significantly from those by which the original, and still widely used, chemical Friedel-Crafts reactions operate. They can be divided broadly into two categories: leaving-group strategies and vinylogous (conjugate addition) strategies. Both prenyltransferases and methyltransferases employ the former strategy, using pyrophosphate and SAH as leaving groups to create electrophilic alkylating reagents. CylK too employs this strategy, but rather uses a chloride leaving group, activated in a binding site, and is perhaps thus most reminiscent of the original Friedel-Crafts reactions. By utilising activated esters as acylation substrates, the Friedel-Crafts acylase could also be considered to operate via a leaving group strategy, although the process is markedly different in that it proceeds via a covalently bound complex.

The artificial enzymes described all employ a vinylogous strategy where alkylation occurs at an sp^2-hybridised carbon, activated through Lewis acid or iminium ion chemistry. The Friedel-Crafts reaction catalysed by SHCs can also be considered a conjugate addition reaction, since an arene nucleophile and proton act to desaturate either end of a conjugated system, however, a cyclisation also occurs at the centre of these two processes. The PLP dependent TrpB and TPL have a mechanism that has both characteristics, since both catalyse a formal dehydration (i.e. water as a leaving group) in order to form an electrophilic sp^2 carbon that undergoes a conjugate addition.

One mechanistic feature exhibited by most of the enzymes described here that is perhaps most distinct from Friedel-Crafts reactions as they are known in organic synthesis, is explicit activation of the arene component. In most cases this takes the form of hydrogen-bonding interactions to hetero-atom moieties in the arene, increasing its nucleophilicity, as well as stabilising the subsequent dearomatized intermediates.

(2) Alkylation substrates of Friedel-Crafts enzymes are quite diverse, although typically each particular class of enzyme shows lesser or greater extent of rigidity in accepted substrate structures, in accordance with its particular mechanism. Both prenyl- and methyl-transferases can exhibit a high degree of promiscuity towards alkylating substrates, so long as the appropriate leaving group is present. The complexity of the SAH leaving group means that unnatural nucleosides can also be tolerated. However, for both classes of enzymes, the high costs associated with their substrates renders them unsuitable for large scale synthesis, unless they can be (re)generated *in situ* as is the case with SAM (analogues). Furthermore, these enzymes do not accept substrates with the leaving-group placed on a secondary carbon-centre, and thus cannot be used to impart product stereochemistry.

TPL and TrpB both use abundant and simple alkylation substrates to produce amino acids with standard main-chain structures, but whilst engineered TrpB variants can produce some non-standard main-chain structures, TPL does not accept non-native alkylation substrates. Moreover, including ethyl- or propyl-substrates in the product main-chain with engineered TrpB variants requires the use of expensive non-standard amino acids.

The abundance of chemical and enzymatic methods to prepare alkyl-halides makes enzymes that utilise them for Friedel-Crafts reactions, such as CylK, highly appealing for synthesis. In practice, as with the native substrates of CylK, non-native substrates demonstrated with this enzyme so far all have complex structures, and demonstration of useful products is limited. This may be improved with directed evolution, however the chances of efficient recognition and catalysis with simple chloro-alkanes seems slim.

With squalene hopene cyclases, intramolecular reactions are greatly preferred, thus the alkylation substrate and arene must be linked. This presumably will only permit the production of products with fused polycyclic constructions of six-membered rings. However, the proven malleability of these enzymes through active-site mutagenesis signals good prospects for the selective production of particular stereo- and regio-isomers of such products.

Artificial Friedel-Crafts enzymes often permit large changes in the alkylation substrate to stereoselectively afford products with a variety of alkyl groups. Where these artificial enzymes operate *via* Cu^{II} Lewis-Acid catalysis though, a chelating moiety is always required in the substrate. These are rarely found in useful products, and thus must be further interconverted to reach the target structure. Here, the aldehyde substrates used by the organocatalytic artificial Friedel-Crafts enzyme LmrR_pAF provide an advantage since this functional group can be

interconverted with fewer steps under mild conditions, and possibly even enzymatically.

(3) Whilst almost all of the enzymes discussed here have some degree of promiscuity with regard to the arene substrate, a clear trend, and potential limitation with regard to the production of non-natural products, is the exclusive presence of heteroatoms. These provide stabilisation to the Wheland intermediates that may be formed during the reaction, and also show improved performance in chemical Friedel-Crafts reactions. Indeed, electron poor substrates are challenging for chemical Friedel-Crafts reactions.[128] In Friedel-Crafts enzymes, they also provide hydrogen-bonding handles for substrate binding and thus it is challenging to envisage, for example, how an enzyme that alkylates benzene might be engineered.

With these characteristics in mind, we can identify frontiers for further improvements to Friedel-Crafts enzymes, and considerations for their future applications in (industrial) synthesis. The characteristic of only accepting electron rich arene substrates can be addressed by designing synthetic routes that use these moieties as handles for further functionalisation, e.g. cross-coupling reactions,[129] to obtain the target structure. Moreover, the combination of Friedel-Crafts enzymes with enzymatic aromatic hydroxylation to form the activated arenes *in situ* demonstrates the power of multi-enzyme cascades to form complex products from simple feedstocks.[41] The diversity of alkyl/acyl groups that can be installed varies greatly by enzyme class, and reducing strict specificity in this regard represents an obvious avenue for engineering efforts. However, the substrate used for alkylation must also be considered for its ease of preparation and the handles it provides for further functionalisation. In all, the frontier can be summarised as the creation artificial enzymes, or the engineering of natural ones, that expand the repertoire of Friedel-Crafts enzymes away from chemistry required by nature, and towards that required by modern society.[130] The fact that a Friedel-Crafts enzyme is already applied the industrial synthesis of L-DOPA signals promise in such endeavours, that they may play an important role in the move towards more sustainable chemistry.

Acknowledgements

Support from the Netherlands Ministry of Education, Culture and Science (Gravitation program no. 024.001.035) and the European Research Council (ERC advanced grant 885396) is gratefully acknowledged. We would like to thank Andrew Buller, Peyton Higgins and Jared Lewis for their insightful reviewer comments, which helped to improve this review article. Our thanks also to Lars Longwitz for help checking the article proofs.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Friedel-Crafts Alkylation · Acylation · Biocatalysis · Enzymes · Artificial Enzymes · DNA Catalysis

[1] C. Friedel, J. M. Crafts, *Comptes rendus des séances* **1877**, 1392–1395.
[2] M. M. Heravi, V. Zadsirjan, P. Saedi, T. Momeni, *RSC Adv.* **2018**, *8*, 40061–40163.
[3] a) M. M. Heravi, V. Zadsirjan, M. Heydari, B. Masoumi, *Chem. Rec.* **2019**, *19*, 2236–2340; b) M. Rueping, B. J. Nachtsheim, *Beilstein J. Org. Chem.* **2010**, *6*, 6; c) S. L. You, Q. Cai, M. Zeng, *Chem. Soc. Rev.* **2009**, *38*, 2190–2201; d) M. Bandini, A. Melloni, A. Umani-Ronchi, *Angew. Chem. Int. Ed.* **2004**, *43*, 550–556.
[4] a) D. G. Brown, J. Bostrom, *J. Med. Chem.* **2016**, *59*, 4443–4458; b) O. Dirat, A. J. Bibb, C. M. Burns, G. D. Checksfield, B. R. Dillon, S. E. Field, S. J. Fussell, S. P. Green, C. Mason, J. Mathew, S. Mathew, I. B. Moses, P. I. Nikiforov, A. J. Pettman, F. Susanne, *Org. Proc. Res. Dev.* **2011**, *15*, 1010–1017; c) A. Karmakar, R. Y. Nimje, A. Silamkoti, M. Pitchai, M. Basha, C. Singarayer, D. Ramasamy, G. T. V. Babu, R. Samikannu, S. Subramaniam, P. Anjanappa, M. Vetrichelvan, H. Kumar, A. G. Dikundwar, A. Gupta, A. K. Gupta, R. Rampulla, T. G. M. Dhar, A. Mathur, *Org. Proc. Res. Dev.* **2021**, *25*, 1001–1014; d) F. Peng, L. Tan, L. Chen, S. M. Dalby, D. A. DiRocco, J. Duan, M. Feng, G. Gong, H. Guo, J. C. Hethcox, L. Jin, H. C. Johnson, J. Kim, D. Le, Y. Lin, W. Liu, J. Shen, Y. Wan, C. Xiao, B. Xiang, Q. Xiang, J. Xu, L. Yan, W. Yang, H. Ye, Y. Yu, J. Zhang, *Org. Proc. Res. Dev.* **2021**.
[5] J. B. Zimmerman, P. T. Anastas, H. C. Erythropel, W. Leitner, *Science* **2020**, 397–400.
[6] a) R. A. Sheldon, D. Brady, *ACS Sustain. Chem. Eng.* **2021**, *9*, 8032–8052; b) R. A. Sheldon, D. Brady, *ChemSusChem* **2022**, e202102628.
[7] T. Li, X. Li, *Green Chem.* **2014**, *16*, 4241–4256.
[8] a) J. H. Schrittwieser, S. Velikogne, M. Hall, W. Kroutil, *Chem. Rev.* **2018**, *118*, 270–348; b) M. A. Huffman, A. Fryszkowska, O. Alvizo, M. Borra-Garske, K. R. Campos, K. A. Canada, P. N. Devine, D. Duan, J. H. Forstater, S. T. Grosser, H. M. Halsey, G. J. Hughes, J. Jo, L. A. Joyce, J. N. Kolev, J. Liang, K. M. Maloney, B. F. Mann, N. M. Marshall, M. McLaughlin, J. C. Moore, G. S. Murphy, C. C. Nawrat, J. Nazor, S. Novick, N. R. Patel, A. Rodriguez-Granillo, S. A. Robaire, E. C. Sherer, M. D. Truppo, A. M. Whittaker, D. Verma, L. Xiao, Y. Xu, H. Yang, *Science* **2019**, *366*, 1255–1259.
[9] F. Schwizer, Y. Okamoto, T. Heinisch, Y. Gu, M. M. Pellizzoni, V. Lebrun, R. Reuter, V. Köhler, J. C. Lewis, T. R. Ward, *Chem. Rev.* **2018**, *118*, 142–231.
[10] J. B. Holmes, V. Liu, B. G. Caulkins, E. Hilario, R. K. Ghosh, V. N. Drago, R. P. Young, J. A. Romero, A. D. Gill, P. M. Bogie, J. Paulino, X. Wang, G. Riviere, Y. K. Bosken, J. Struppe, A. Hassan, J. Guidoulianov, B. Perrone, F. Mentink-Vigier, C.-e. A. Chang, J. R. Long, R. J. Hooley, T. C. Mueser, M. F. Dunn, L. J. Mueller, *Proc. Natl. Acad. Sci. USA* **2022**, *119*.
[11] a) A. N. Lane, K. Kirschner, *Eur. J. Biochem.* **1983**, *129*, 571–582; b) M. T. Cash, E. W. Miles, R. S. Phillips, *Arch. Biochem. Biophys.* **2004**, *432*, 233–243; c) D. Milic, T. V. Demidkina, N. G. Faleev, D. Matkovic-Calogovic, A. A. Antson, *J. Biol. Chem.* **2008**, *283*, 29206–29214; d) D. Milic, T. V. Demidkina, N. G. Faleev, R. S. Phillips, D. Matkovic-Calogovic, A. A. Antson, *J. Am. Chem. Soc.* **2011**, *133*, 16468–16476; e) R. S. Phillips, H. Y. Chen, N. G. Faleev, *Biochemistry* **2006**, *45*, 9575–9583.
[12] R. S. Phillips, B. Sundararaju, N. G. Faleev, *J. Am. Chem. Soc.* **2000**, *122*, 1008–1014.
[13] a) E. Watkins-Dulaney, S. Straathof, F. Arnold, *ChemBioChem* **2021**, *22*, 5–16; b) R. S. Phillips, *Tetrahedron: Asym.* **2004**, *15*, 2787–2792.
[14] a) L. Wang, A. Brock, B. Herberich, P. G. Schultz, *Science* **2001**, *292*, 498–500; b) C. C. Liu, P. G. Schultz, *Annu. Rev. Biochem.* **2010**, *79*, 413–444; c) K. Lang, J. W. Chin, *Chem. Rev.* **2014**, *114*, 4764–4806.
[15] a) I. Drienovská, G. Roelfes, *Nat. Catalysis* **2020**, *3*, 193–202; b) J. Zhao, A. J. Burke, A. P. Green, *Curr. Opin. Chem. Biol.* **2020**, *55*, 136–144; c) Y. Yu, C. Hu, L. Xia, J. Wang, *ACS Catal.* **2018**, *8*, 1851–1863.
[16] M. A. Blaskovich, *J. Med. Chem.* **2016**, *59*, 10807–10836.
[17] a) D. R. Smith, T. Willemse, D. S. Gkotsi, W. Schepens, B. U. Maes, S. Ballet, R. J. Goss, *Org. Lett.* **2014**, *16*, 2622–2625; b) M. Wilcox, *Anal. Biochem.* **1974**, *59*, 436–440.
[18] a) M. Lee, R. S. Phillips, *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1563–1564; b) R. S. Phillips, L. A. Cohen, U. Annby, D. Wensbo, S. Gronowitz, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1133–1134; c) M. J. Sloan, R. S. Phillips, *Bioorg. Med. Chem. Lett.* **1992**, *7*, 1053–1056; d) M. Welch, R. S. Phillips, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 647–640; e) R. J. Goss, P. L. Newill, *Chem. Commun.* **2006**, 4924–4925.

[19] A. R. Buller, S. Brinkmann-Chen, D. K. Romney, M. Herger, J. Murciano-Calles, F. H. Arnold, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 14599–14604.
[20] M. A. Maria-Solano, J. Iglesias-Fernández, S. Osuna, *J. Am. Chem. Soc.* **2019**, *141*, 13049–13056.
[21] M. A. Maria-Solano, T. Kinateder, J. Iglesias-Fernández, R. Sterner, S. Osuna, *ACS Catal.* **2021**, *11*, 13733–13743.
[22] J. Murciano-Calles, D. K. Romney, S. Brinkmann-Chen, A. R. Buller, F. H. Arnold, *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 11577–11581.
[23] UniProt Consortium, *Nucleic Acids Res.* **2021**, *49*, D480-D489.
[24] M. Herger, P. van Roye, D. K. Romney, S. Brinkmann-Chen, A. R. Buller, F. H. Arnold, *J. Am. Chem. Soc.* **2016**, *138*, 8388–8391.
[25] D. Francis, M. Winn, J. Latham, M. F. Greaney, J. Micklefield, *ChemBioChem* **2017**, *18*, 382–386.
[26] C. E. Boville, R. A. Scheele, P. Koch, S. Brinkmann-Chen, A. R. Buller, F. H. Arnold, *Angew. Chem. Int. Ed.* **2018**, *57*, 14764–14768.
[27] a) D. K. Romney, J. Murciano-Calles, J. E. Wehrmuller, F. H. Arnold, *J. Am. Chem. Soc.* **2017**, *139*, 10769–10776; b) C. E. Boville, D. K. Romney, P. J. Almhjell, M. Sieben, F. H. Arnold, *J. Org. Chem.* **2018**, *83*, 7447–7452.
[28] G. Rix, E. J. Watkins-Dulaney, P. J. Almhjell, C. E. Boville, F. H. Arnold, C. C. Liu, *Nat. Commun.* **2020**, *11*, 5644.
[29] E. J. Watkins, P. J. Almhjell, F. H. Arnold, *ChemBioChem* **2020**, *21*, 80–83.
[30] a) E. J. Watkins-Dulaney, N. P. Dunham, S. Straathof, S. Turi, F. H. Arnold, A. R. Buller, *Angew. Chem. Int. Ed.* **2021**, *60*, 21412–21417; b) M. Dick, N. S. Sarai, M. W. Martynowycz, T. Gonen, F. H. Arnold, *J. Am. Chem. Soc.* **2019**, *141*, 19817–19822; c) D. K. Romney, N. S. Sarai, F. H. Arnold, *ACS Catal.* **2019**, *9*, 8726–8730.
[31] A. D. McDonald, L. J. Perkins, A. R. Buller, *ChemBioChem* **2019**, *20*, 1939–1944.
[32] a) C. M. Thompson, A. D. McDonald, H. Yang, S. Cavagnero, A. R. Buller, *Org. Biomol. Chem.* **2020**, *18*, 4189–4192; b) J. P. G. Malthouse, T. B. Fitzpatrick, J. J. Milne, L. Grehn, U. Ragnarsson, *J. Pept. Sci.* **1997**, *3*, 361–366; c) A. Osborne, Q. Teng, E. W. Miles, R. S. Phillips, *J. Biol. Chem.* **2003**, *278*, 44083–44090.
[33] Y. Wu, R. Xu, Y. Feng, H. Song, *ACS Catal.* **2022**, 3767–3775.
[34] B. Seisser, R. Zinkl, K. Gruber, F. Kaufmann, A. Hafner, W. Kroutil, *Adv. Synth. Catal.* **2010**, *352*, 731–736.
[35] a) F. Foor, N. Morin, K. A. Bostian, *Appl. Environ. Microbiol.* **1993**, *59*, 3070–3075; b) T. Katayama, H. Suzuki, K. T. H. Kumagai, *Appl. Environ. Microbiol.* **2000**, *66*, 4764–4771.
[36] T. Nagasawa, T. Utagawa, J. Goto, C. Kim, Y. Tani, H. Kumagai, H. Yamada, *Eur. J. Biochem.* **1981**, *117*, 38–40.
[37] a) Q. Zhou, M. Hu, W. Zhang, L. Jiang, S. Perrett, J. Zhou, J. Wang, *Angew. Chem. Int. Ed.* **2013**, *52*, 1203–1207; b) X. Liu, J. Li, C. Hu, Q. Zhou, W. Zhang, M. Hu, J. Zhou, J. Wang, *Angew. Chem. Int. Ed.* **2013**, *52*, 4805–4809.
[38] Y. Won, H. Jeon, A. D. Pagar, M. D. Patil, S. P. Nadarajan, D. T. Flood, P. E. Dawson, H. Yun, *Chem. Commun.* **2019**, *55*, 15133–15136.
[39] a) I. Drienovská, R. A. Scheele, C. Gutiérrez de Souza, G. Roelfes, *ChemBioChem* **2020**, *21*, 3077–3081; b) H. S. Lee, G. Spraggon, P. G. Schultz, F. Wang, *J. Am. Chem. Soc.* **2009**, *131*, 2481–2483.
[40] R. Lauchli, K. S. Rabe, K. Z. Kalbarczyk, A. Tata, T. Heel, R. Z. Kitto, F. H. Arnold, *Angew. Chem. Int. Ed.* **2013**, *52*, 5571–5574.
[41] A. Dennig, E. Busto, W. Kroutil, K. Faber, *ACS Catal.* **2015**, *5*, 7503–7506.
[42] E. Busto, R. C. Simon, W. Kroutil, *Angew. Chem. Int. Ed.* **2015**, *54*, 10899–10902.
[43] S. E. Payer, H. Pollak, B. Schmidbauer, F. Hamm, F. Juricic, K. Faber, S. M. Glueck, *Org. Lett.* **2018**, *20*, 5139–5143.
[44] J. Winkelblech, A. Fan, S. M. Li, *Appl. Microbiol. Biotechnol.* **2015**, *99*, 7379–7397.
[45] N. Steffan, W.-B. Grundmann, A. Yin, S. M. Li, *Curr. Med. Chem.* **2009**, *16*, 218–231.
[46] H. P. Chen, I. Abe, *Synth. Syst. Biotechnol.* **2021**, *6*, 51–62.
[47] L. Y. P. Luk, M. E. Tanner, *J. Am. Chem. Soc.* **2009**, *131*, 13932–13933.
[48] a) I. A. Unsold, S. M. Li, *Microbiology* **2005**, *151*, 1499–1505; b) X. Yu, Y. Liu, X. Xie, X. D. Zheng, S. M. Li, *J. Biol. Chem.* **2012**, *287*, 1371–1380; c) A. Kremer, L. Westrich, S. M. Li, *Microbiology* **2007**, *153*, 3409–3416; d) J. Winkelblech, S. M. Li, *ChemBioChem* **2014**, *15*, 1030–1039.
[49] E. Ostertag, L. Zheng, K. Broger, T. Stehle, S. M. Li, G. Zocher, *J. Mol. Biol.* **2021**, *433*, 166726.
[50] a) P. Mai, G. Zocher, T. Stehle, S. M. Li, *Org. Biomol. Chem.* **2018**, *16*, 7461–7469; b) Y. Xu, D. Li, G. Tan, Y. Zhang, Z. Li, K. Xu, S. M. Li, X. Yu, *Org. Lett.* **2021**, *23*, 497–502.
[51] a) M. Liebhold, X. Xie, S. M. Li, *Org. Lett.* **2013**, *14*, 4882–4885; b) M. Liebhold, S. M. Li, *Org. Lett.* **2013**, *15*, 5834–5837.
[52] J. Winkelblech, M. Liebhold, J. Gunera, X. Xie, P. Kolb, S.-M. Li, *Adv. Synth. Catal.* **2015**, *357*, 975–986.
[53] W. B. Yin, J. A. Baccile, J. W. Bok, Y. Chen, N. P. Keller, F. C. Schroeder, *J. Am. Chem. Soc.* **2013**, *135*, 2064–2067.
[54] R. Chen, B. Gao, X. Liu, F. Ruan, Y. Zhang, J. Lou, K. Feng, C. Wunsch, S. M. Li, J. Dai, F. Sun, *Nat. Chem. Biol.* **2017**, *13*, 226–234.
[55] U. Metzger, C. Schall, G. Zocher, I. Unsöld, H. Stecher, S.-M. Li, L. Heide, T. Stehle, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 14309–14314.
[56] A. Fan, G. Zocher, E. Stec, T. Stehle, S. M. Li, *J. Biol. Chem.* **2015**, *290*, 1364–1373.
[57] A. Fan, X. Xie, S. M. Li, *Org. Biomol. Chem.* **2015**, *13*, 7551–7557.
[58] J. A. McIntosh, Z. Lin, M. D. B. Tianero, E. W. Schmidt, *ACS Chem. Biol.* **2013**, *8*, 877–883.
[59] D. Pockrandt, C. Sack, T. Kosiol, S. M. Li, *Appl. Microbiol. Biotechnol.* **2014**, *98*, 4987–4994.
[60] K. Zhou, L. Ludwig, S. M. Li, *J. Nat. Prod.* **2015**, *78*, 929–933.
[61] Y. Yang, L. Fu, J. Zhang, L. Hu, M. Xu, J. Xu, *PLoS One* **2014**, *9*, e99537.
[62] B. B. He, X. L. Bu, T. Zhou, S. M. Li, M. J. Xu, J. Xu, *ACS Synth. Biol.* **2018**, *7*, 2094–2104.
[63] Y. Zhang, K. Hamada, D. T. Nguyen, S. Inoue, M. Satake, S. Kobayashi, C. Okada, K. Ogata, M. Okada, T. Sengoku, Y. Goto, H. Suga, *Nat. Catal.* **2022**, https://doi.org/10.1038/s41929-022-00822-2.
[64] J. Gunera, F. Kindinger, S. M. Li, P. Kolb, *J. Biol. Chem.* **2017**, *292*, 4003–4021.
[65] R. Nagata, H. Suemune, M. Kobayashi, T. Shinada, K. Shin-Ya, M. Nishiyama, T. Hino, Y. Sato, T. Kuzuyama, S. Nagano, *Angew. Chem. Int. Ed.* **2022**, e202117430.
[66] K. J. H. Lim, Y. D. Hartono, B. Xue, M. K. Go, H. Fan, W. S. Yew, *ACS Catal.* **2022**, *12*, 4628–4639.
[67] E. M. Scull, C. Bandari, B. P. Johnson, E. D. Gardner, M. Tonelli, J. L. You, R. H. Cichewicz, S. Singh, *Appl. Microbiol. Biotechnol.* **2020**, *104*, 7853–7865.
[68] T. Mori, *J. Nat. Med.* **2020**, *74*, 501–512.
[69] M. Pacholec, J. Tao, C. T. Walsh, *Biochemistry* **2005**, *44*, 14969–14976.
[70] a) M. R. Bennett, S. A. Shepherd, V. A. Cronin, J. Micklefield, *Curr. Opin. Chem. Biol.* **2017**, *37*, 97–106; b) B. J. C. Law, A. W. Struck, M. R. Bennett, B. Wilkinson, J. Micklefield, *Chem. Sci.* **2015**, *6*, 2885–2892; c) J. M. Winter, G. Chiou, I. R. Bothwell, W. Xu, N. K. Garg, M. Luo, Y. Tang, *Org. Lett.* **2013**, *15*, 3774–3777.
[71] a) Y. N. Dai, K. Zhou, D. D. Cao, Y. L. Jiang, F. Meng, C. B. Chi, Y. M. Ren, Y. Chen, C. Z. Zhou, *Acta Crystallogr. Sect. D* **2014**, *70*, 2085–2092; b) J. C. Sadler, C. H. Chung, J. E. Mosley, G. A. Burley, L. D. Humphreys, *ACS Chem. Biol.* **2017**, *12*, 374–379; c) T. Pavkov-Keller, K. Steiner, M. Faber, M. Tengg, H. Schwab, M. Gruber-Khadjawi, K. Gruber, *PLoS One* **2017**, *12*, e0171056.
[72] a) H. Stecher, M. Tengg, B. J. Ueberbacher, P. Remler, H. Schwab, H. Griengl, M. Gruber-Khadjawi, *Angew. Chem. Int. Ed.* **2009**, *48*, 9546–9548; b) M. Tengg, H. Stecher, P. Remler, I. Eiteljörg, H. Schwab, M. Gruber-Khadjawi, *J. Mol. Catal. B* **2012**, *84*, 2–8.
[73] T. D. Huber, B. R. Johnson, J. Zhang, J. S. Thorson, *Curr. Opin. Biotechnol.* **2016**, *42*, 189–197.
[74] M. Tengg, H. Stecher, L. Offner, K. Plasch, F. Anderl, H. Weber, H. Schwab, M. Gruber-Khadjawi, *ChemCatChem* **2016**, *8*, 1354–1360.
[75] a) A. Benjdia, S. Pierre, C. Gherasim, A. Guillot, M. Carmona, P. Amara, R. Banerjee, O. Berteau, *Nat. Commun.* **2015**, *6*; b) A. J. Blaszczyk, B. Wang, A. Silakov, J. V. Ho, S. J. Booker, *J. Biol. Chem.* **2017**, *292*, 15456–15467; c) S. Pierre, A. Guillot, A. Benjdia, C. Sandström, P. Langella, O. Berteau, *Nat. Chem. Biol.* **2012**, *8*, 957–959.
[76] C. Bailly, *Eur. J. Pharmacol.* **2022**, *914*, 174661.
[77] a) M. R. Bauerle, E. L. Schwalm, S. J. Booker, *J. Biol. Chem.* **2015**, *290*, 3995–4002; b) A. J. Blaszczyk, A. Silakov, B. Zhang, S. J. Maiocco, N. D. Lanz, W. L. Kelly, S. J. Elliott, C. Krebs, S. J. Booker, *J. Am. Chem. Soc.* **2016**, *138*, 3416–3426; c) P. Zhou, D. Ohagan, U. Mocek, Z. P. Zeng, L. D. Yuen, T. Frenzel, C. J. Unkefer, J. M. Beale, H. G. Floss, *J. Am. Chem. Soc.* **1989**, *111*, 7274–7276.
[78] F. Soualmia, A. Guillot, N. Sabat, C. Brewee, X. Kubiak, M. Haumann, X. Guinchard, A. Benjdia, O. Berteau, *Chem. Eur. J.* **2022**, *28*.
[79] a) J. C. Sadler, L. D. Humphreys, R. Snajdrova, G. A. Burley, *ChemBioChem* **2017**, *18*, 992–995; b) I. J. W. McKean, J. C. Sadler, A. Cuetos, A. Frese, L. D. Humphreys, G. Grogan, P. A. Hoskisson, G. A. Burley, *Angew. Chem. Int. Ed.* **2019**, *58*, 17583–17588.
[80] S. Singh, J. Zhang, T. D. Huber, M. Sunkara, K. Hurley, R. D. Goff, G. Wang, W. Zhang, C. Liu, J. Rohr, S. G. Van Lanen, A. J. Morris, J. S. Thorson, *Angew. Chem. Int. Ed.* **2014**, *53*, 3965–3969.

[81] S. Mordhorst, J. Siegrist, M. Muller, M. Richter, J. N. Andexer, *Angew. Chem. Int. Ed.* **2017**, *56*, 4037–4041.
[82] a) C. Liao, F. P. Seebeck, *Nat. Catal.* **2019**, *2*, 696–701; b) Q. Tang, I. V. Pavlidis, C. P. S. Badenhorst, U. T. Bornscheuer, *ChemBioChem* **2021**, *22*, 2584–2590.
[83] a) J. Peng, C. Liao, C. Bauer, F. P. Seebeck, *Angew. Chem. Int. Ed.* **2021**; b) Q. Tang, C. W. Grathwol, A. S. Aslan-Uzel, S. Wu, A. Link, I. V. Pavlidis, C. P. S. Badenhorst, U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2021**, *60*, 1524–1527.
[84] A. J. Herbert, S. A. Shepherd, V. A. Cronin, M. R. Bennett, R. Sung, J. Micklefield, *Angew. Chem. Int. Ed.* **2020**, *59*, 14950–14956.
[85] a) A. Hayashi, H. Saitou, T. Mori, I. Matano, H. Sugisaki, K. Maruyama, *Biosci. Biotechnol. Biochem.* **2012**, *76*, 559–566; b) N. G. Schmidt, T. Pavkov-Keller, N. Richter, B. Wiltschi, K. Gruber, W. Kroutil, *Angew. Chem. Int. Ed.* **2017**, *56*, 7615–7619; c) F. Yang, Y. Cao, *Appl. Microbiol. Biotechnol.* **2012**, *93*, 487–495.
[86] T. Pavkov-Keller, N. G. Schmidt, A. Zadlo-Dobrowolska, W. Kroutil, K. Gruber, *ChemBioChem* **2019**, *20*, 88–95.
[87] X. Sheng, M. Kazemi, A. Zadlo-Dobrowolska, W. Kroutil, F. Himo, *ACS Catal.* **2020**, *10*, 570–577.
[88] a) N. G. Schmidt, W. Kroutil, *Eur. J. Org. Chem.* **2017**, *2017*, 5865–5871; b) A. Zadlo-Dobrowolska, N. G. Schmidt, W. Kroutil, *ChemCatChem* **2019**, *11*, 1064–1068; c) A. Zadlo-Dobrowolska, L. Hammerer, T. Pavkov-Keller, K. Gruber, W. Kroutil, *ACS Catal.* **2020**, *10*, 1094–1101.
[89] S. C. Hammer, P. O. Syren, M. Seitz, B. M. Nestl, B. Hauer, *Curr. Opin. Chem. Biol.* **2013**, *17*, 293–300.
[90] R. D. Gandour, *Bioorg. Chem.* **1981**, *12*, 169–176.
[91] P. O. Syren, S. Henche, A. Eichler, B. M. Nestl, B. Hauer, *Curr. Opin. Struct. Biol.* **2016**, *41*, 73–82.
[92] M. Seitz, P. O. Syren, L. Steiner, J. Klebensberger, B. M. Nestl, B. Hauer, *ChemBioChem* **2013**, *14*, 436–439.
[93] S. C. Hammer, A. Marjanovic, J. M. Dominicus, B. M. Nestl, B. Hauer, *Nat. Chem. Biol.* **2015**, *11*, 121–126.
[94] S. C. Hammer, J. M. Dominicus, P.-O. Syrén, B. M. Nestl, B. Hauer, *Tetrahedron* **2012**, *68*, 7624–7629.
[95] S. Henche, B. M. Nestl, B. Hauer, *ChemCatChem* **2021**, *13*, 3405–3409.
[96] B. S. Moore, J. L. Chen, G. M. L. Patterson, R. E. Moore, L. S. Brinen, Y. Kato, J. Clardy, *J. Am. Chem. Soc.* **1990**, *112*, 4061–4063.
[97] S. C. Bobzin, R. E. Moore, *Tetrahedron* **1993**, *49*, 7615–7626.
[98] H. Nakamura, E. E. Schultz, E. P. Balskus, *Nat. Chem. Biol.* **2017**, *13*, 916–921.
[99] E. E. Schultz, N. R. Braffman, M. U. Luescher, H. H. Hager, E. P. Balskus, *Angew. Chem. Int. Ed.* **2019**, *58*, 3151–3155.
[100] a) N. R. Braffman, T. B. Ruskoski, K. M. Davis, N. Glasser, C. Johnson, C. Denise Okafor, A. K. Boal, E. P. Balskus, *eLife* **2022**, e75761; b) H.-Q. Wang, S.-B. Mou, W. Xiao, H. Zhou, X.-D. Hou, S.-J. Wang, Q. Wang, J. Gao, Z. Wei, L. Liu, Z. Xiang, *ACS Catal.* **2022**, *12*, 2108–2117.
[101] F. Rosati, G. Roelfes, *ChemCatChem* **2010**, *2*, 916–927.
[102] A. J. Boersma, B. L. Feringa, G. Roelfes, *Angew. Chem. Int. Ed.* **2009**, *48*, 3346–3348.
[103] G. Roelfes, A. J. Boersma, B. L. Feringa, *Chem. Commun.* **2006**, 635–637.
[104] E. W. Dijk, A. J. Boersma, B. L. Feringa, G. Roelfes, *Org. Biomol. Chem.* **2010**, *8*, 3868–3873.
[105] R. P. Megens, G. Roelfes, *Org. Biomol. Chem.* **2010**, *8*, 1387–1393.
[106] J. Wang, E. Benedetti, L. Bethge, S. Vonhoff, S. Klussmann, J. J. Vasseur, J. Cossy, M. Smietana, S. Arseniyadis, *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 11546–11549.
[107] S. Park, K. Ikehata, R. Watabe, Y. Hidaka, A. Rajendran, H. Sugiyama, *Chem. Commun.* **2012**, *48*, 10398–10400.
[108] G. P. Petrova, Z. Ke, S. Park, H. Sugiyama, K. Morokuma, *Chem. Phys. Lett.* **2014**, *600*, 87–95.
[109] S. Park, L. Zheng, S. Kumakiri, S. Sakashita, H. Otomo, K. Ikehata, H. Sugiyama, *ACS Catal.* **2014**, *4*, 4070–4073.
[110] A. García-Fernández, R. P. Megens, L. Villarino, G. Roelfes, *J. Am. Chem. Soc.* **2016**, *138*, 16308–16314.
[111] J. T. Mohr, A. Y. Hong, B. M. Stoltz, *Nat. Chem.* **2009**, *1*, 359–369.
[112] A. J. Boersma, J. E. Klijn, B. L. Feringa, G. Roelfes, *J. Am. Chem. Soc.* **2008**, *130*, 11783–11790.
[113] C. Wang, Y. Li, G. Jia, Y. Liu, S. Lu, C. Li, *Chem. Commun.* **2012**, *48*, 6232–6234.
[114] a) B. Seelig, A. Jaschke, *Chem. Biol.* **1999**, *6*, 167–176; b) B. Seelig, S. Keiper, F. Stuhlmann, A. Jäschke, *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 4576–4579; c) U. Mohan, R. Burai, B. R. McNaughton, *Org. Biomol. Chem.* **2013**, *11*, 2241–2244.
[115] P. K. Madoori, H. Agustiandari, A. J. Driessen, A. M. Thunnissen, *EMBO J.* **2009**, *28*, 156–166.
[116] J. Bos, W. R. Browne, A. J. Driessen, G. Roelfes, *J. Am. Chem. Soc.* **2015**, *137*, 9796–9799.
[117] L. Villarino, S. Chordia, L. Alonso-Cotchico, E. Reddem, Z. Zhou, A. Thunnissen, J. D. Maréchal, G. Roelfes, *ACS Catal.* **2020**, *10*, 11783–11790.
[118] I. Drienovská, A. Rioz-Martínez, A. Draksharapu, G. Roelfes, *Chem. Sci.* **2015**, *6*, 770–776.
[119] M. Bersellini, G. Roelfes, *Org. Biomol. Chem.* **2017**, *15*, 3069–3073.
[120] C. Gutierrez de Souza, M. Bersellini, G. Roelfes, *ChemCatChem* **2020**, *12*, 3190–3194.
[121] I. Drienovská, C. Mayer, C. Dulson, G. Roelfes, *Nat. Chem.* **2018**, *10*, 946–952.
[122] A. Erkillä, I. Majander, P. M. Pihko, *Chem. Rev.* **2007**, *107*, 5416–5470.
[123] C. Mayer, C. Dulson, E. Reddem, A. W. H. Thunnissen, G. Roelfes, *Angew. Chem. Int. Ed.* **2019**, *58*, 2083–2087.
[124] J. F. Austin, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2002**, *124*, 1172–1173.
[125] R. B. Leveson-Gower, Z. Zhou, I. Drienovská, G. Roelfes, *ACS Catal.* **2021**, *11*, 6763–6770.
[126] R. B. Leveson-Gower, R. M. Boer, G. Roelfes, *ChemCatChem* **2022**, *14*, e202101875.
[127] a) Z. Zhou, G. Roelfes, *Nat. Catal.* **2020**, *3*, 289–294; b) S. Alonso, G. Santiago, I. Cea-Rama, L. Fernandez-Lopez, C. Coscolín, J. Modregger, A. K. Ressmann, M. Martínez-Martínez, H. Marrero, R. Bargiela, M. Pita, J. L. Gonzalez-Alfonso, M. L. Briand, D. Rojo, C. Barbas, F. J. Plou, P. N. Golyshin, P. Shahgaldian, J. Sanz-Aparicio, V. Guallar, M. Ferrer, *Nat. Catal.* **2019**, *3*, 319–328; c) F. Christoffel, N. V. Igareta, M. M. Pellizzoni, L. Tiessler-Sala, B. Lozhkin, D. C. Spiess, A. Lledós, J.-D. Maréchal, R. L. Peterson, T. R. Ward, *Nat. Catal.* **2021**, *4*, 643–653.
[128] H. L. Y. Shen, Y. Chen, *J. Org. Chem.* **1990**, *55*, 3961–3962.
[129] Z. Qiu, H. Zeng, C.-J. Li, *Acc. Chem. Res.* **2020**, *53*, 2395–2413.
[130] a) R. B. Leveson-Gower, C. Mayer, G. Roelfes, *Nat. Chem. Rev.* **2019**, *3*, 687–705; b) K. Chen, F. H. Arnold, *Nature Catalysis* **2020**, *3*, 203–213.

Manuscript received: May 17, 2022
Revised manuscript received: July 10, 2022
Accepted manuscript online: July 11, 2022
Version of record online: August 24, 2022