Article:
Kumar, V, Turnbull, WB orcid.org/0000-0002-7352-0360 and Kumar, A (2022) Review on Recent Developments in Biocatalysts for Friedel–Crafts Reactions. ACS Catalysis, 12 (17). pp. 10742-10763. ISSN 2155-5435

https://doi.org/10.1021/acscatal.2c01134

Review on recent developments in biocatalysts for Friedel-Crafts reactions

Vajinder Kumar[1]*, W. Bruce Turnbull[2] and Avneesh Kumar[3]

[1]*Department of Chemistry, Akal University, Talwandi Sabo, Bathinda, Punjab, India*

[2]*School of Chemistry and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK*

[3]*Department of Botany, Akal University, Talwandi Sabo, Bathinda, Punjab, India*

Email ID vkvkumar17@gmail.com

Abstract

The Friedel-Crafts (F-C) reaction has been a fundamental pillar of both academic and industrial synthetic organic chemistry since its discovery in 1873. Its success is based on the versatility and applicability of F-C reactions on a wide range of substrates, and there have been an impressive number of publications and patents describing catalytic F-C reaction methods. The asymmetric version of the reaction was discovered about 100 years after the seminal work by Friedel and Crafts, and has become a major area for research. While chemical methods having much-improved efficacy and scope have been discovered, F-C reactions still suffer limitations. Biocatalysis has the potential to be the best solution to this challenge because of the excellent selectivity (enantio-, chemo, and regioselectivity) displayed by the enzymes. In the last two decades, advancements in molecular biology techniques, bioinformatics, high-throughput screening, directed evolution and process scale-up has led to biocatalysis becoming a mature field. It is therefore not surprizing that researchers around the globe have developed several biocatalysts to do asymmetric F-C reactions. Herein, we review recent developments in the

design and use of catalytic and stereoselective strategies for performing the asymmetric F-C reactions.

Keywords: Biocatalysis, asymmetric Friedel-Crafts reactions, artificial metalloenzymes, DNA-based catalysts, Acyltransferases, methyltransferases, monoamine-oxidases, expanded genetic code methods

Introduction

Since the pioneering work by Charles Friedel and James M. Crafts[1-2], the Friedel Crafts (F-C) reaction has been one of the most powerful synthetic approaches for producing C-C bonds by activation of C-H bonds in aromatics, with the potential for making millions of compounds at both academic and industrial levels.[3] In addition to the petrochemical industries, F-C reactions have broad applicability in pharmaceuticals, dye and agrochemical industries.[4-5] In this typical substitution reaction, an arene acting as a nucleophile is treated with an activated electrophile to form a C-C bond. Traditionally, this would involve an alkyl halide to generate the electrophile in the presence of a strong Lewis acid (aluminium chloride, zinc chloride, boron trifluoride, etc.) or strong Bronsted acid (sulfuric acid etc.). The production of corrosive waste during the workup and use of hazardous solvents has led scientists to explore milder and more environmentally friendly alternatives.[6-7] The development of reusable catalysts such as lanthanide trifluoromethanesulfonate[8-9] showed some practical applications, but the scope has been limited. The development of solid acid catalysts or heterogeneous catalysts (heteroploy acids and their salts[10], resin-supported peptide catalysts[11], zeolites[12], clay montmorillonite[13], mesoporous oxides[14] and ionic liquids immobilized on polyacrylonitrile fibres[15], etc.) by various research groups for performing F-C reactions gained some industrial relevance due to their comparatively easy separation from products and high recyclability, but over-alkylation of arenes and stereochemical configuration can lower the yield of the desired product.[16] More

than 100 years after the work of Friedel and Crafts, Casiraghi and co-workers reported the first example of an asymmetric version of this reaction by developing a method for ortho-specific hydroxyalkylation of phenol,[17] which was then expanded to a variety of aromatic compounds and alkylating agents.[18,19]

Enantioselective asymmetric synthesis is vital in the pharmaceutical, fragrance, and food industries,[20] and in this context, the enzyme-catalyzed chemical processes have shown unparalleled chiral (stereoselectivity) and positional selectivity (regioselectivity) under milder and greener conditions. Fewer by-products, acceptability towards a wide range of substrates, and no need for protection and deprotection strategies to carry out the enantio- and regioselective transformations has attracted chemists to explore potential enzymes for asymmetric F-C reactions.[21] In the last two decades, the field of biocatalysis has developed into a mature technology because of advancements in molecular biology techniques and modern bioinformatics and computer modelling-supported enzyme engineering that can be used to design and produce desired enzymes rapidly.[22–25] These advances have enabled several groups to develop enzymatic asymmetric F-C reactions. Herein, we focus on past and significant recent developments in bio-catalyzed/enzyme-catalyzed F-C reactions on non-natural acceptors. Prenyltransferases are excluded from this article because Mori et al. recently published a detailed review of this class of enzymes.[26]

Artificial metalloenzymes

Artificial matalloenzymes (ArMs) are gaining popularity for the opportunities they present to do new-to-nature reactions.[27,28] As ArMs are, by definition, manufactured rather than benefitting from billions of years of evolution, they showcase how advancement in human understanding can enable the design of enzyme-like activity and selectivity for a particular reaction using a combination of protein/DNA and transition metals.[29] In ArM synthesis, the

careful selection of a protein scaffold (computationally assisted and mechanistically understood) and abiological metal cofactor can provide a rudimentary enzyme which can then be subjected to synthetic biology approaches to develop highly active and selective enzymes for new-to-nature reactions.[30,31] The promiscuity introduced by the abiotic metal ion creates new opportunities to perform a wide range of chemical reactions.[32,33]

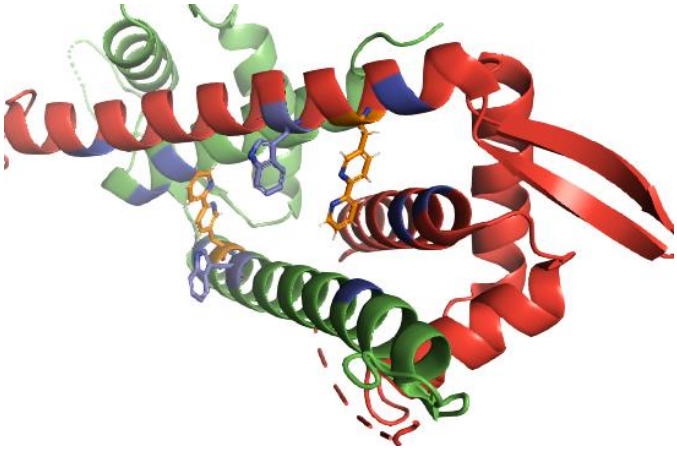
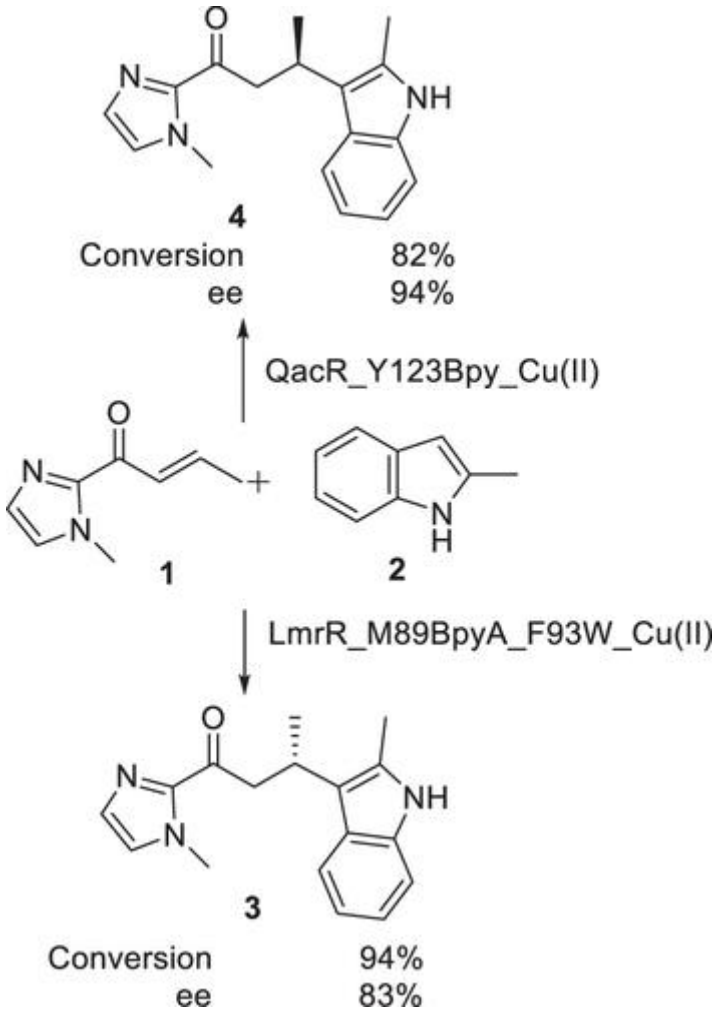


Figure 1. Mutant LmrR protein scaffold was prepared by incorporating BpyAla is highlighted in orange (M89). The blue colour shows the various other position chosen for mutagenesis in addition to BpyAla. The best result was obtained with LmrR_M89BpyAla_F93W. The image was created using PyMOL and PDB ID 3F8F.[38]

One such enzyme, Lactococcal multidrug resistance regulator (LmrR), is a privileged protein scaffold due to its flexibility and adaptability to accommodate a vast range of changes.[34,35] Through chemical modification and expanded genetic code methodologies,[36] Gerard Roelfes and co-workers developed various mutants of LmrR by incorporating metal-binding unnatural amino acids into the protein scaffold.[37] They studied F-C alkylations of indole with α,β-unsaturated acyl-imidazoles by introducing the noncanonical amino acid (2,2'-bipyridin-5yl)alanine (BpyA) at various positions, as shown in Figure 1.[38] The change at position 89 resulted in a mutant that could produce compound **3** in 80% enantiomeric excess. In addition to that, additional mutation at position F93 with tryptophan (F93W) in LmrR protein and

copper (II)-phenanthroline ligand also gave compound **3** in 94% conversion and 83% enantiomeric excess (Scheme 1).[38] In contrast, the same group found that simple covalent anchoring of the metal complex by a cysteine residue did not yield any F-C product.[39,40]



Scheme 1. F-C alkylations reaction catalyzed by ArMs.

For ArM synthesis, other multidrug resistance regulators, such as CgmR, RamR and QacR, from the TetR family, were chosen because they have several aromatic residues surrounding the catalytic site (Figure 2) that could be easily modified by *in vivo* incorporation of noncanonical amino acids by amber stop codon suppression methods. Among these ArMs, the modified QacR protein scaffold, QacR_Y123BpyA, gave proved very successful for catalysing

the F-C reaction giving compound **4** with enantioselectivity of 94% and a conversion rate of 82% (Scheme 1).[41]

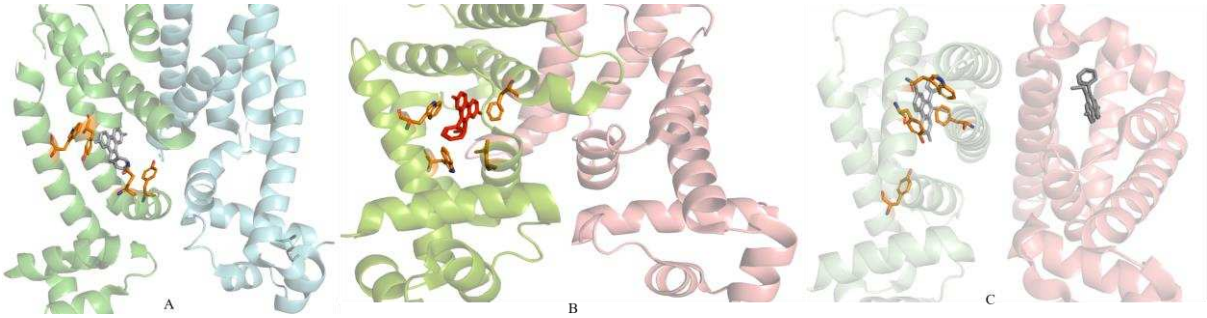


Figure 2. A) Surface representation of protein QacR in complex with ethidium (grey) (PDB ID 3PM1), orange colour shows the amino acids changed by mutagenesis (Y103, Q96, Y123, W61). B) Surface representation of protein CgmR in complex with ethidium (red) (PDB ID 2ZOZ), orange colour shows the amino acids (W63, L100, W113, F147) where modifications were done. C) Surface representation of protein RamR in complex with ethidium (grey) (PDB ID 3VVY), orange colour shows the amino acids (Y59, W89, Y92, F155) where unnatural amino acids were incorporated.

Over the last decade, the catalytic potential of aniline has been widely exploited for the formation of hydrazones and oximes.[42,43] In this form of nucleophilic catalysis, aniline forms a protonated Schiff base intermediate that enables transimination and accelerates the reaction.[44] To expand further the scope of catalysis in F-C alkylation, Roelfes and his group used expanded genetic code strategies to introduce the noncanonical amino acid *para*-aminophenylalanine (pAF) at position 15 of the LmrR protein scaffold to harness the nucleophilic potential of the aniline side chain (Figure 3).[45,46]

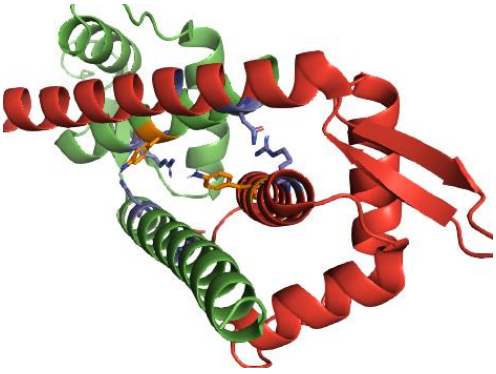
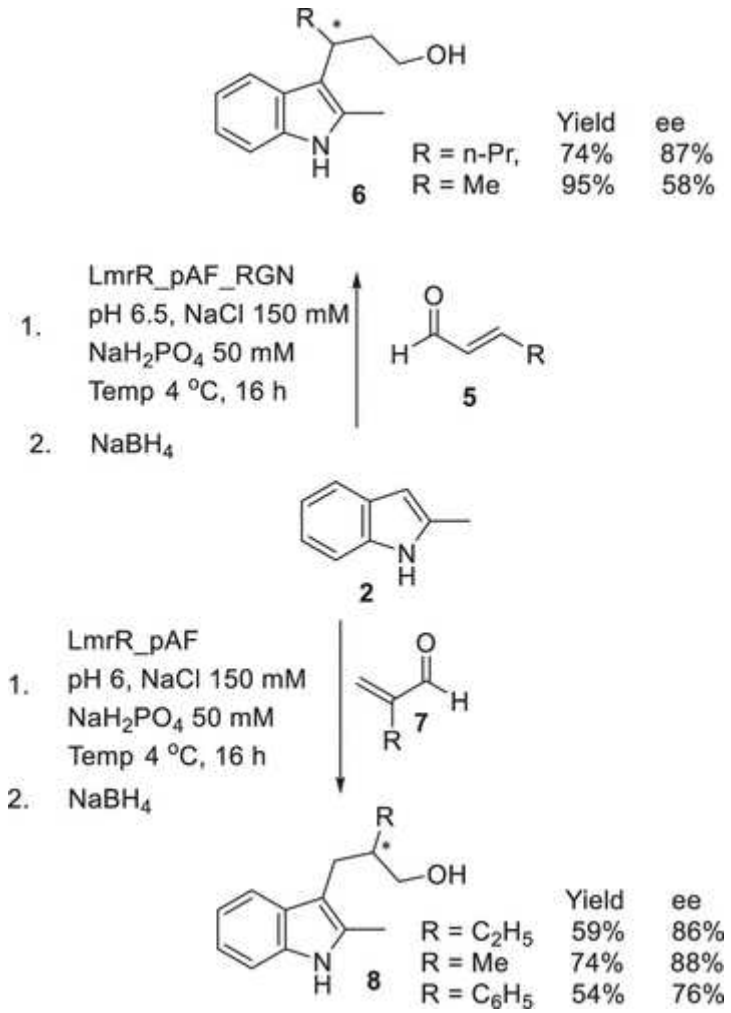


Figure 3. The surface representation of Mutant LmrR, LmrR_V15BpyAF__L18R_S95G_M89N based on PDB ID 3F8F. The noncanonical amino acid, pAF, is highlighted in orange (V15), and other positions (light blue) were chosen for mutagenesis in addition to pAF. This particular mutant gave the best F-C results.

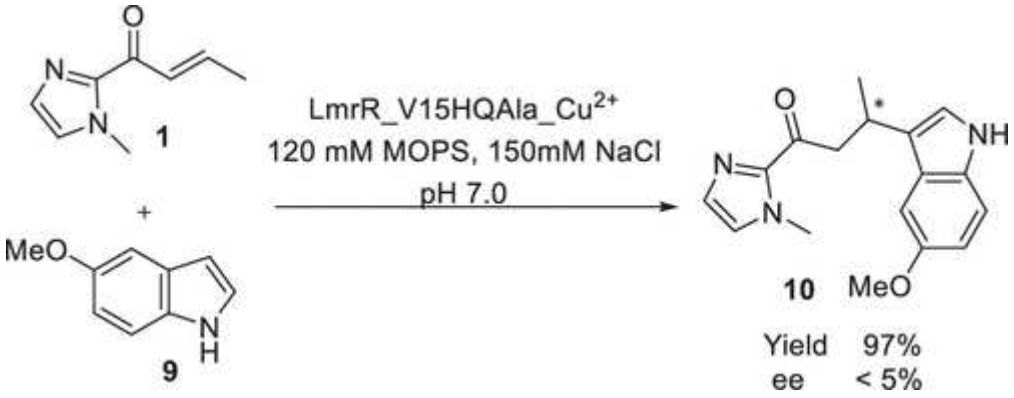
F-C alkylations were studied between α,β-unsaturated aldehydes and indoles using the enzyme without the metal ion and chelating group.[47] They proposed that the pAF residue helped in catalyzing the reaction by forming an intermediate iminium ion species with enal substrates, and the protein scaffold provided stereoselectivity and rate acceleration. Their alanine screening experiments showed the microenvironment created for catalysis by the quartet mutant, LmrR_pAF_L18R_S95G_M89N (LmrR_pAF_RGN), was better than the LmrR_pAF mutant (Figure 3). With hex-2-enal and an indole substrate, the LmrR_pAF_RGN enzyme afforded compound **6** in 74% yield and 87% ee, following reduction. The shorter chain but-2-enal gave an improved yield up to 95%, but ee decreased to 58% (Scheme 2).[47] In one of their recent articles, the same group showed the potential of LmrR_pAF scaffold in performing the challenging tandem F-C alkylation-enantioselective protonation (FC-EP).[48]



Scheme 2. F-C alkylation reaction catalyzed by LmrR_pAF_RGN ArMs.

Another noncanonical amino acid (8-hydroxyquinolin-3-yl)alanine (HQAla) has also been explored for catalysis of F-C reactions. Hydroxyquinoline has been used as an analytical reagent for binding transition metals trhough an N-O bidentate binding mode.[49] [50] Previously, Wang and co-workers incorporated HQAla into a Z-domain protein using amber stop codon methodology and used it as a fluorescent probe and to complex a Zn^{2+} ion which allowed determination the protein structure by single wavelength anomalous diffraction (SAD) phasing.[51] Roelfes and his group introduced HQAla into LmrR protein scaffolds and studied F-C alkylation reactions. With 9 mol% of $Cu(NO_3)_2$ and a slight excess of LmrR_V15HQAla_Cu^{II} catalyst, the final product **10** was obtained with 97% yield but

relatively low enantioselectivity (less than 5%).[52] They hypothesized that the high conversion to product might be because of solvent exposure of HQAla at the mutation site as this could also account for the low enantioselectivity. As HQAla has good affinity towards different metal, ArMs incorporating this amino acid might provide a good approach for other unexplored chemical reactions.

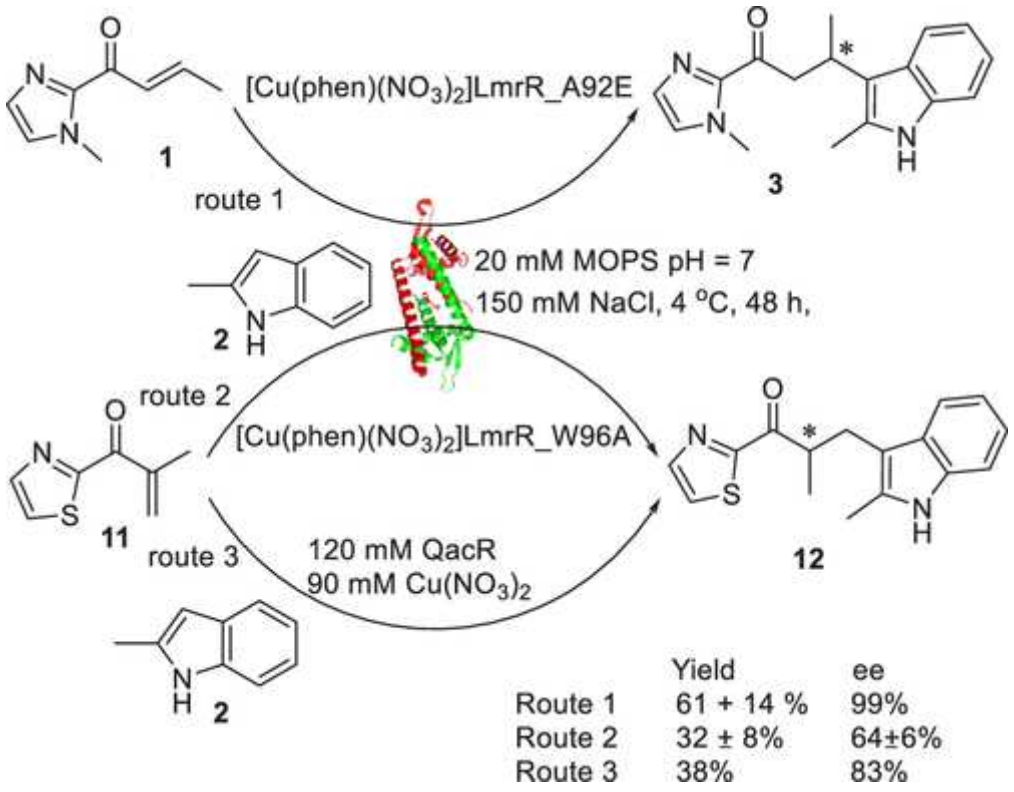


Scheme 3. F-C alkylation reaction catalyzed by LmrR_V15HQAla_Cu^{II} ArMs.

An alternative, more simple approach for manufacturing ArMs is to adopt a supramolecular strategy, in which the system self-assembles upon mixing the protein and metal complex without covalent modification of scaffold. Roelfes' group used this approach to develop LmrR-based ArMs. The two tryptophan moieties (W96 and W96') of this homodimeric protein bind to aromatic compounds by π–stacking, for example showing affinity with Cu^{2+} complex of 1,10-phenanthroline. This supramolecular complex was used for F-C alkylation with substrates shown in scheme 1, and compound **3** was obtained in 100% yield and 94% ee.[53]

Structural dynamics of enzymes/proteins, such as conformational changes (loop motions, partial folding/unfolding, etc.), are essential to provide structural complementarity of the active site to the transition state of the catalyzed reaction.[54,55,56] These changes can sometimes result in alternative or secondary active sites. A subset of structural dynamics is the binding of

cofactors, or their exchange, between various cofactor-binding sites. While this feature is less common in natural enzymes, in ArMs, which are artificial, it is quite possible that the binding of cofactor can happen at more than one location and impact the outcome of the reaction. The dynamic behaviour in terms of positioning the abiological cofactor has been studied in the context of vinylogous F-C and tandem F-C alkylation reactions, as shown in Scheme 4. The difference between these two reactions is that chirality is induced at the conjugate addition step in the vinylogous F-C reaction. Whereas, in tandem F-C reactions, chirality is not rendered at the conjugate addition step but in the subsequent protonation step (Scheme 4).



Scheme 4. Route 1: Vinylogous F-C alkylation catalyzed by LmrR_A92E. Route 2 and 3: tandem F-C alkylation reaction catalyzed by LmrR_W96A and QacR_C72A_C141S mutant ArMs.

In these reactions, the Cu(II)-phen complex sandwiched between two tryptophan residues, W96 and W96', is crucial for F-C reaction. A single point mutation at position A92E resulted in

product **3** of the vinylogous F-C reaction with the highest activity and selectivity (ee up to 99%). MD simulation data indicated that the A92E mutation generated a hydrogen bonding network at the back of the active site and made the necessary structural rearrangements for the Cu(II)-phen ligand to fit better there. However, very low enantioselectivity was observed in the case of tandem F-C reactions, although the use of an inhibitor, Hoechst 33342, which blocks the positioning of Cu(II)-phen ligand, or a point mutation at W96A increased the enantioselectivity of tandem F-C product **12**.[57] Roelfes and co-workers explained this dichotomy by proposing the cofactor dynamics shown in Figure 4, in which most cofactor binds in between the tryptophan residues, which is the primary active site, however a small fraction of cofactor could bind at a secondary active site on the LmrR protein scaffold. Similar trends had been reported in DNA-based catalysis, where cofactors bind at multiple locations reversibly, affecting the catalytic activity and selectivity.[58] In both the F-C and tandem F-C reactions, the outcome of catalysis was dominated by the primary active site, giving the F-C product with high enantioselectivity, but no enantioselectivity in the case of tandem F-C reaction. However, by blocking or eliminating the primary active site, enantiomeric excess of the tandem F-C product was increased. They concluded that these changes in activity and selectivity arise from dynamic interconversion of the binding position of the metal cofactor.[57]

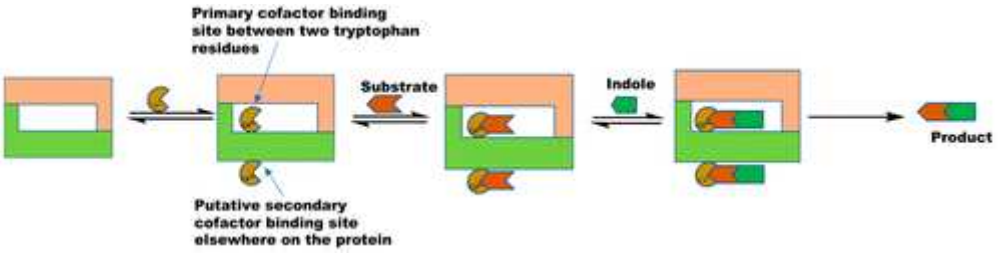


Figure 4. Proposed model about dynamics of cofactors in LmrR based ArMs scaffold. Most of the metal cofactor binds at the primary site between two tryptophan residues, giving high ee in F-C reactions that take place at this site. A small fraction of metal cofactor binds at a secondary site which favours higher ee for the tandem F-C reaction.

The potential of other members the TetR family of multidrug resistance regulator proteins (QacR, CgmR, RamR and their mutants) for catalyzing F-C alkylation by the supramolecular approach has also been explored.[59] These artificial metalloenzymes were created by self-assembly of each protein and Cu(phen)$(NO_3)_2$ complex and resulted in an enantioselective F-C reaction having ee up to 75%,[59] and a QacR_C72A_C141S mutant catalyzed the tandem F-C reaction with 83% enantioselectivity (Scheme 4, route 3).[60] In a recent report from the same group, a supramolecular assembly of LmrR and copper (II)-phenanthroline complex was made in the cytoplasm of *E. coli* cells, demonstrating that a whole-cell system could be active in catalysis of enantioselective F-C alkylation reactions.[61] The incorporation of artificial metalloenzymes into biosynthetic pathways is a significant step toward developing a hybrid metabolism for synthetic cells.

Eppinger and co-workers designed and developed ArMs based on a fluorescent protein scaffold, which they called mTFP*, and a natural amino acid anchoring strategy. [62] The resulting proteins showed high stability towards pH change, temperature and organic solvents. Through site-directed mutagenesis, they developed two variants, $mTFP^{CHH}$ and $mTFP^{EHH}$ (Figure 5), both of which showed high affinities for several metal ions. The F-C alkylation reactions performed with these mutants yielded compound **15** with up to 93% ee (Scheme 5). By introducing amino acid motifs capable of chelating metal ions and creating a binding pocket of adequate size and shape, they showed the catalytic application of a non-catalytic protein scaffold. This strategy can open new pathways for engineering other catalytically active ArMs.

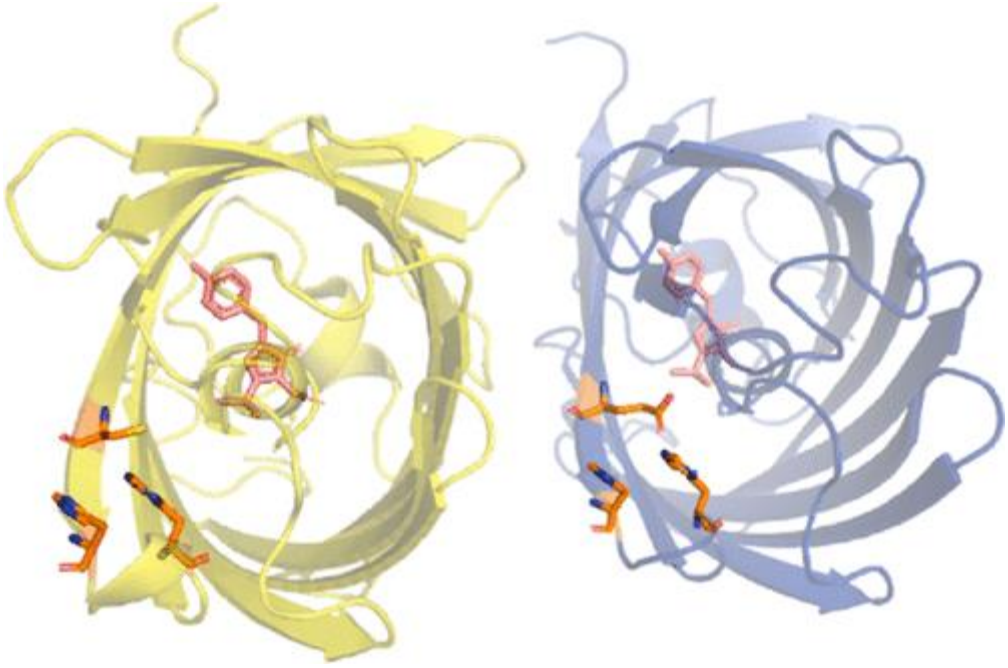
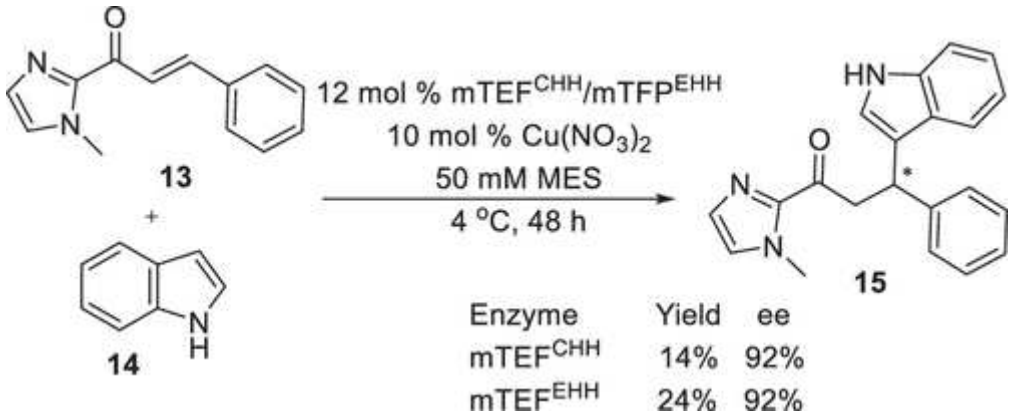


Figure 5. Mutant fluorescent ArMs, named mTEP^{CHH} (yellow) and mTEP^{EHH} (blue) developed by Fischer et al.[62] This image was created in PyMOL using PDB ID 4Q9W. Mutated amino acids are shown in orange [cysteine (I197C), histidine (Y200H) and histidine (Y204H) in the mTEP^{CHH}; glutamic acid (I197E), histidine (Y200H) and histidine (Y204H) in mTEP^{EHH} and the fluorescent chromophore is shown in pink.



Scheme 5. F-C alkylation by fluorescent protein mTFP^{CHH} and mTFP^{EHH}.

Methyltransferases

Methyltransferases (MTase) catalyze the introduction of a methyl group into biologically important molecules such as proteins, DNA/RNA and other small biomolecules.[63] MTases are a large group of enzymes that can be further divided into class I, II, and III proteins.[64] In class I MTases, the Rossmann protein fold helps in binding the cofactor, S-adenosyl-L-methionine (SAM), by interaction with highly conserved acidic residues (aspartic acid/glutamic acid) and a glycine-rich region that adopts a beta turn around the SAM (Figure 6).[65] The CouO and NovO methytransferases from *Streptomyces rishiriensis* and *Streptomyces spheroids,* respectively, use the cofactor SAM to transfer a methyl group for producing antibiotics Coumermycin A and novobiocin.[66,67,68] Gruber-Khadjawi and co-workers showed that the sequence homology between CouO and NovO was up to 84%. Both enzymes accepted a wide variety of substrates (**16**) and chemically modified SAM derivatives (**17**) and the final products (**18**) were obtained with excellent regioselectivity (Scheme 6).[69]

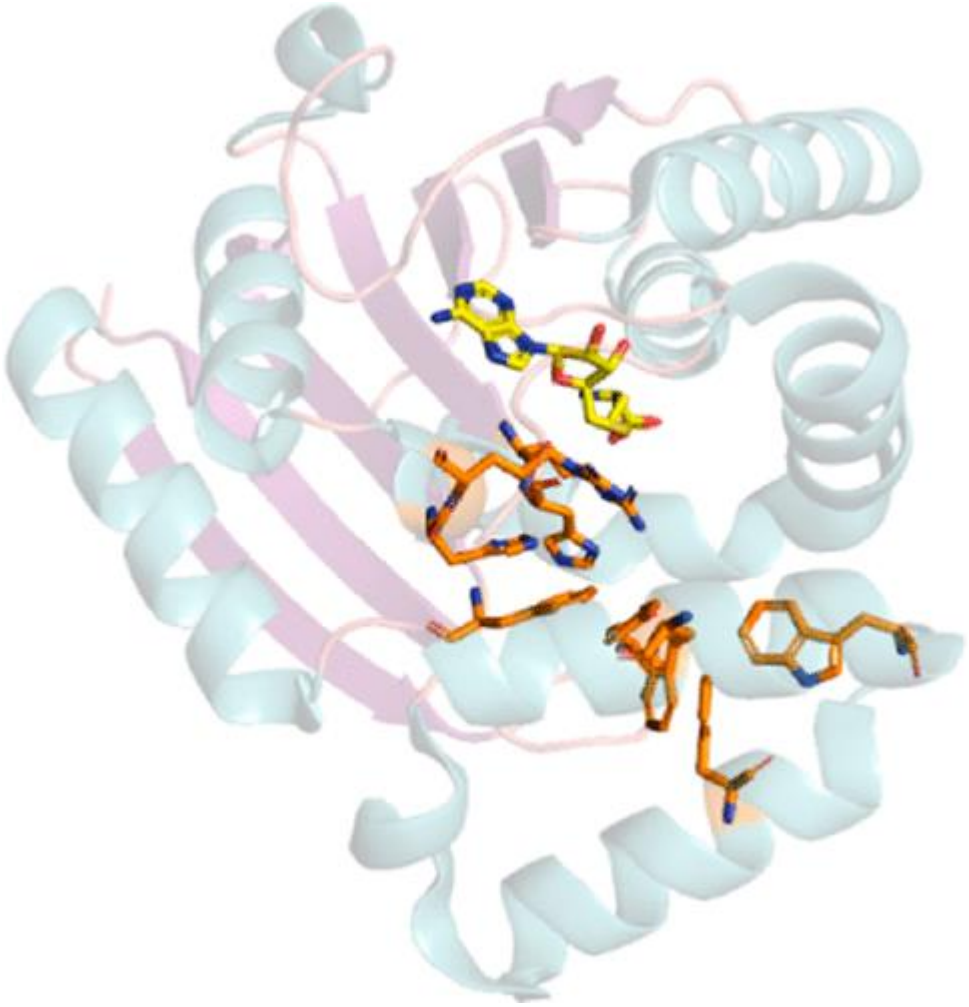
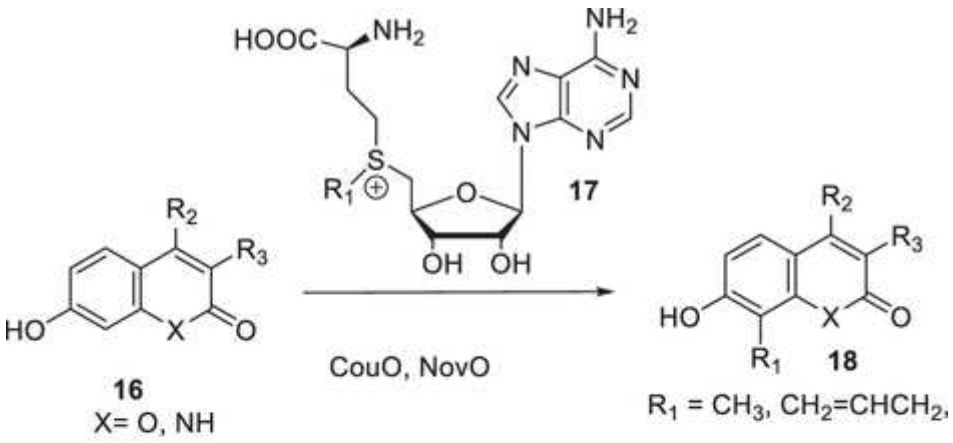
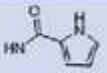
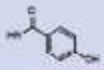


Figure 6. Surface representation of protein methyltransferases from PDB ID 5M58. The binding of SAH cofactor (yellow) by Rossmann-like fold, just above the hydrophobic cavity containing amino acids residues (R116, H117, H120, R121, F147, F164, W170, W178 and Y 216) shown in orange.

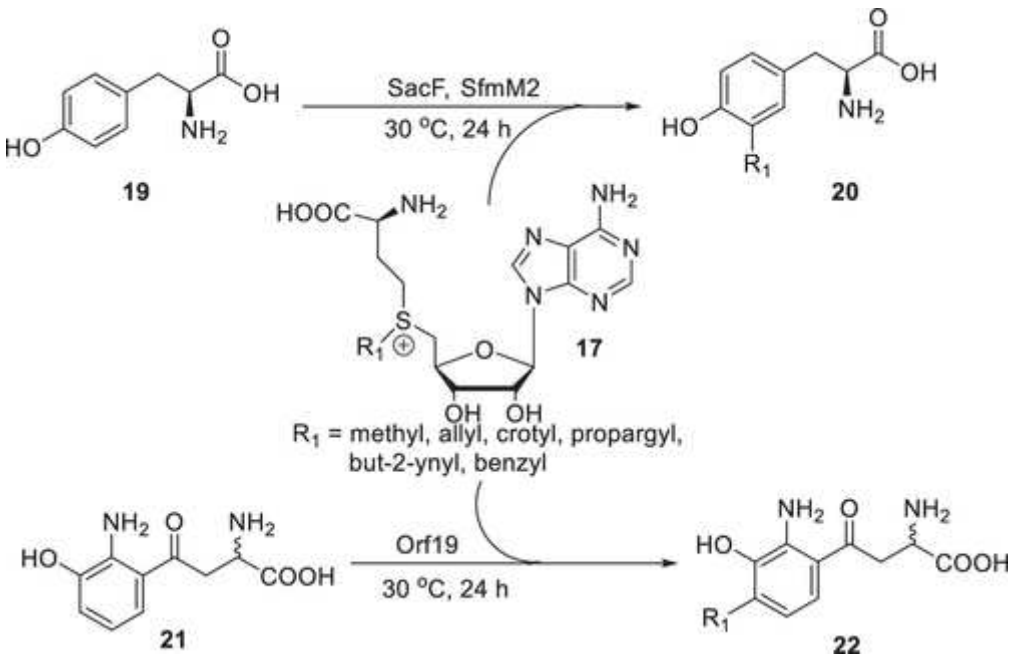


SN	X	R_2	R_3	CouO	NovO
1	O		OH	99%	99%
2	O		OH	99%	96%
3	O		OH	99%	99%
4	O		OH	99%	99%
5	O	CH_2COOH	CH_3	-	30%
6	NH	H	CH_3	-	10%

Scheme 6. F-C alkylation through CouO and NovO

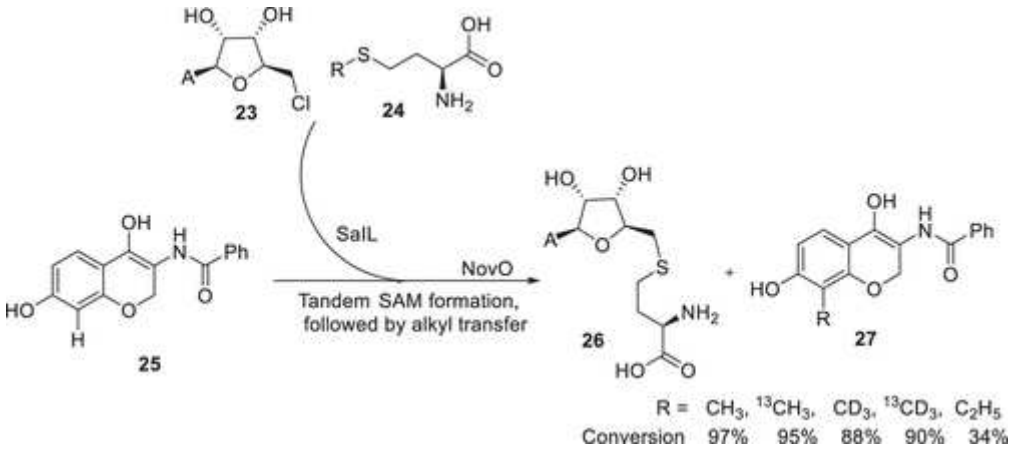
Another article explored the potential of other SAM-dependent methyltransferases such as SfmM2, SacF, SibL and Orf19 (Scheme 7).[70] Compared to CouO and NovO, these enzymes are highly substrate-specific, nonetheless, they can accept a broad range of cofactors. SacF and SfmM2 are involved in the biosynthesis of 3'-methyltyrosine, an essential precursor of tetrahydroisoquinoline moiety in safracin and saframycin antibiotics. Using wide varieties of substrates and cofactors, they showed that these enzymes prefer the natural L-tyrosine substrate, however, they can tolerate D-tyrosine. An increase in the size of the alkyl substituents on SAM analogs decreases the conversion to product. SibL and Orf19 enzymes showed specificity towards only 3-hydroxykynurenine as substrate, however, the enzymes could perform regioselective monoalkylation with a variety of SAM analogs. The limited substrate

selectivity could be overcome by modifying these enzymes using site-directed mutagenesis and expanded genetic code techniques.



Scheme 7. F-C reaction by methyltransferases such as SacF, SfmM2 and Orf19.

Burley and co-workers created a one-pot, two-step scalable biocatalytic platform for F-C methylation or ethylation of aromatics with SaIL and NovO.[71] To address the inherent instability of SAM, diastereomer formation in chemical synthesis and difficulties in purification, they used *in situ* production of the cofactor, SAM, and its analogs.[72] Previously, SAM was synthesized chemoenzymatically with the help of the enzyme SaIL from 5'-deoxy-5-choloroadenosine (**23**) and methionine (**24**), which are relatively cost-effective.[73] For the first time, they utilized SaIL for *in situ* synthesis of SAM analogues, followed by C-methylation of aromatic substrates using NovO in one-pot procedure and obtained F-C product, **27**, in excellent yield. The authors performed their reactions on 20 mg of substrate, which they noted was the first preparative-scale example of a tandem process with *in situ* formation of SAM. The results demonstrate the potential for this scalable enzymatic approach, and also illustrate that there is still some way to go to reach industrial scale reactions.



Scheme 8. One pot, two enzyme processes involving *in situ* formation of SAM by SaIL enzyme and C-methylation by NovO.

Acyltransferases

Acyltransferases play vital roles in numerous biosynthetic pathways that require transfer of acyl moieties to biologically important substrates, such as membrane phospholipids,[74] triglycerol and wax esters,[75] polyketides,[76] lysozyme,[77] etc. Acyltransferases involved in primary and secondary metabolic processes generally utilize acyl-CoA derivatives as donor substrates.[78] For detoxification of antibiotics like chloramphenicol, aminoglycosides,[79] streptothricin,[80] phosphinothricin,[81] etc., CoA-dependent O- and N-acylation processes frequently occur in bacteria. Compared to O- and N-acylation, very few enzymes are known for C-acylation. Kroutil and co-workers discovered that enzymes PpATase and PfATase, isolated from bacteria *Pseudomonas protegens* and *Pseudomonas fluorescens*, catalyze F-C acylation reactions.[82]

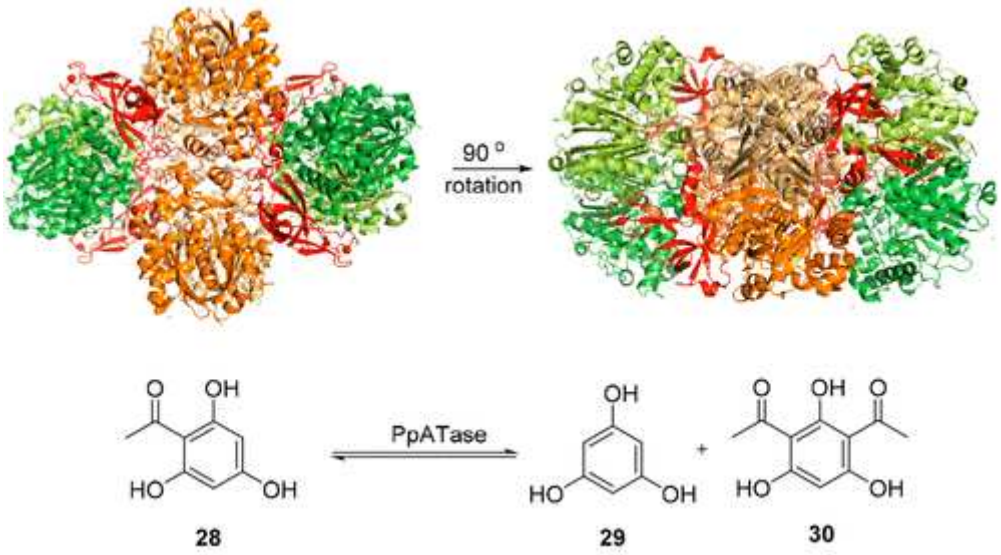
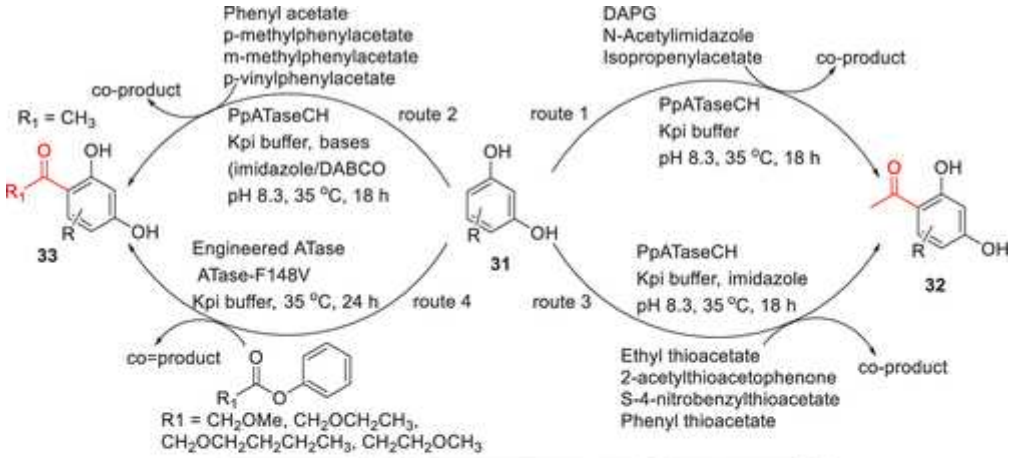


Figure 7. The crystal structure of PpATase protein was drawn using PDB ID 5MG5. Structure of phIACB (PhIA = orange and light orange, PhIB = red and PhIC = green and light green). PhIB connects PhIA and PhIB. Below is the reaction catalyzed by PpATase.

PpATase is composed of three subunits (Figure 7): PhIA, PhIC and PhIB, which are arranged as a $PhI(A_2C_2)_2B_4$ heterododecamer, in which there are four copies of each subunit.[83] The expression of the entire PhIACB operon is necessary for catalytic function since mixing of individual subunits after their expression in *E. coli* did not show any catalytic activity for F-C reaction.[84] It was found that this multi-component enzyme catalyzes a reversible disproportionation reaction between monoacetylphloroglucinol (MAPG), phloroglucinol and diacetylphloroglucinol (DAPG).[85] Moreover, no cofactor is required for its catalytic activity. After structural analysis, Gruber and co-workers showed that F-C acylation was carried out by only the PhIC subunits.[83] In another report, the same group identified various *Pseudomonas* strains that have acyltransferase activities and standardized the conditions for expressing wild type and recombinant proteins.[86] PpATaseCH has been demonstrated to receive a variety of acyl donors and transfer an acetyl moiety to a phenolic acceptor in an F–C acetylation, as shown in Scheme 9 route 1.[82] With DAPG as acyl donor, resorcinol derivatives were converted into

the acylated product **32** with efficiencies up to 99%, and *O*-acylation was not detected. However, with *N*-acylimidazole (*N*-AcIm) derivatives, *O*-acylated products were formed that undergo Fries rearrangement in the presence of the enzyme, yielding final C-acylated F-C products with moderate yield. Another acyl donor, isopropenyl ester (IPEA), led to the formation of an *O*-acylated product similar to *N*-AcIm, but with higher conversion up to 98%.[82] In another article, they explored the potential of vinyl acetate, non-activated aliphatic esters, acetanilide derivatives, phenylacetate and their derivatives as acyl donors, as shown in scheme 9, route 2.[87] No conversion was obtained with non-activated aliphatic esters and acetanilides, but with phenyl esters, 99% conversion to F-C acylated product **33** was observed. The phenylesters did not undergo Fries rearrangement, acting only as acyl donors for acylation of resorcinol.



	Acyl Donor	Yield	Ref
Route 1	DAPG	Up to 99%	82
	N-acetyl imidazole	Up to 97%	
	Isopropenyl acetate	Up to 98%	
Route 2	Phenyl acetate	>99%	87
	p-methyl phenylacetate	>99%	
	m-methyl phenylacetate	>99%	
	p-vinyl phenylacetate	>99%	
Route 3	Ethyl thioester	>99%	88
	Phenyl thioacetate	>99%	
	S-4-nitrobenzylthioacetate	>99%	
	2-acetylthioacetophenone	>99%	
Route 4	Phenyl 2-methoxy acetate	>99%	89
	Phenyl 2-ethoxy acetate	>99%	
	Phenyl 2-butoxy acetate	70%	
	phenyl 2-(2'-methoxyethoxy)acetate	73%	
	Phenyl 2-phenoxyacetate	71%	

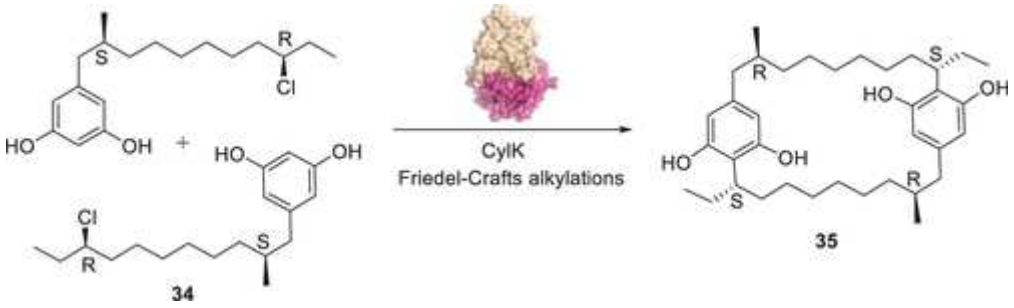
Scheme 9. ATase catalyzed F-C acylation reaction

Imidazole was found to increase conversion to the product. Hence, the effect of other amines, e.g., methyl imidazole and 1,4-diazabicyclo[2.2.2]octane (DABCO), was also studied and it was found that when using DABCO, fewer *O*-acylation products were observed but a more moderate yield (73%) was obtained.[79] The catalytic power of PpATase enzyme for utilization of thioesters as acyl donors was investigated by Kroutil and co-workers (Scheme 9, route 3).[88] Among commercially available thioesters, ethyl thioacetate was the best acyl donor with a conversion to product of 99% (88% isolated yield), but the enzyme was sensitive towards high substrate loading, with lower yields at higher substrate concentration. The addition of imidazole improved the conversion significantly with the butyl-substituted resorcinol substrates. This method tolerated a wide variety of functional groups at the C-6 position of resorcinol (Scheme 9, route 3).[88]

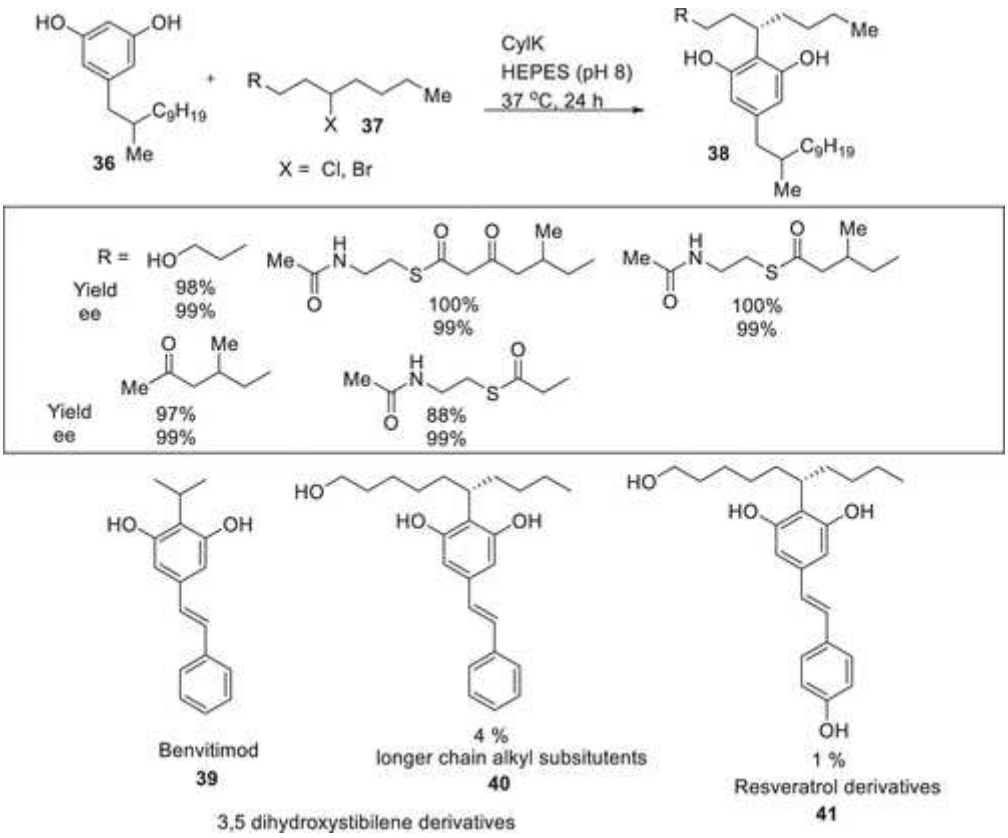
PpATaseCH transfers only acetyl or propionyl groups to resorcinol and its derivatives, with no extended chain acyl donors, such as butanoyl, being accepted by the PpATase enzyme. Crystal structure analysis of PpATaseCH (PDB 5MG5) identified five residues, F148, L300, L383, Y386 and Y298, which were confining the active site space for the acyl group.[89] These selected amino acids were substituted with sterically less demanding amino acids (e.g., alanine or valine) using site-directed mutagenesis. A library of 12 variants was constructed and examined for catalysis of the F-C acylation (Scheme 9, route 4). Notably, only the single point mutants F148V or F148A displayed acceptance of longer chain acyl moieties such as butanoyl phloroglucinol and hexanoyl phloroglucinols. A broad range of phenyl ester derivatives was also investigated as donor substrates to catalyze the acylation reaction with resorcinol. Conversion rates and yields with these sterically crowded acyl donors were moderate, but with alkoxy alkyl phenyl esters, such as methoxyacetyl phenyl ester and ethoxylacetyl phenyl ester, 99% conversion and up to 99% yield was achieved.[89]

Cyl K

Cylindrocyclophanes **35** are cytotoxic natural chemicals produced by photosynthetic cyanobacteria,which exhibit an unusual [7.7] paracyclophane ring structure.[90] A team led by Belakus discovered the enzymatic machinery for their biosynthesis and found the enzyme CylK catalyzed stereospecific C-C bond formation between the aromatic and alkyl parts leading to head to tail dimerization (Scheme 10).[91] They subsequently explored the potential of CylK using non-natural substrates.[92] The study concluded that CylK accepts a wide variety of alkyl halides as electrophiles (Scheme 11), but their acceptance of other aryl substrates (resorcinol derivatives) is minimal.



Scheme 10. Synthesis of cylindrocyclophane F, [7.7] paracyclophane scaffold, through head-to-tail dimerization of (S, R)-33 by CylK.



Scheme 11. CylK mediated F-CAlkylation

Under mild conditions, this unprecedented example of stereospecific and regioselective alkylation at 2-position of resorcinol (**38**) has the potential to streamline the tedious synthesis of benvitimod (**39**) which is currently in clinical trials to treat plaque psoriasis.[93] Its multi-step chemical synthesis involves harsh conditions and as is not stereoselective, making it impractical for preparing derivatives other than the compound with the symmetrical isopropyl group. However, the use of CylK not only overcomes the above-mentioned drawbacks but also shows potential towards other substrates, such as resveratrol derivatives (**41**). While CylK can be used to make chiral versions of benvitimod, so far only an analytical scale of the reaction has been reported with low conversion to the product.[92] While this work represents an advance in accessing stereoselective routes toward such drugs, there is clearly still much work to be done to optimise and scale up such processes to reach an industrial process. Nonetheless, it is

quite realistic to expect that enzymatic engineering techniques can expand their scope by overcoming the existing limitations and giving the pharmaceutical industry a biocatalyst for the stereospecific and regioselective synthesis in the future.

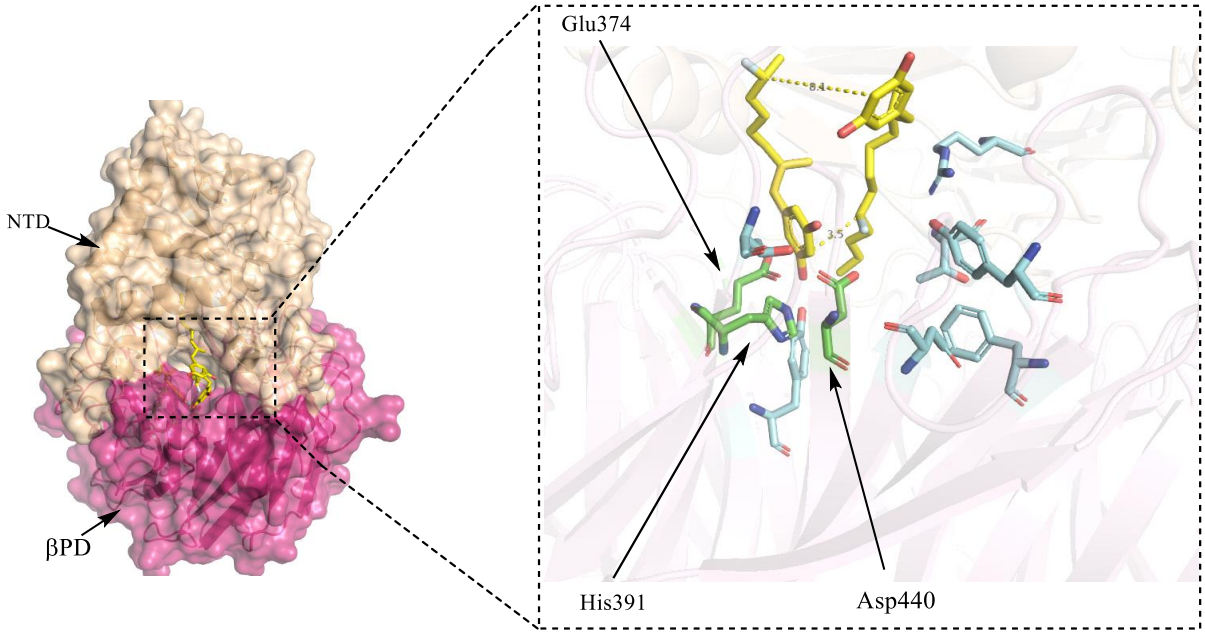


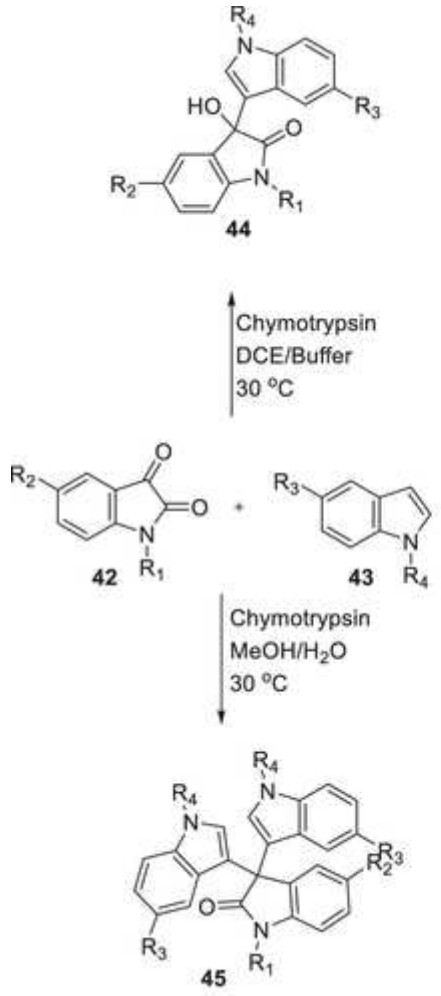
Figure 8. Crystal structure of CylK with ligand drawn using PyMOL from PDB ID 7FH7.[94] It consists of the N-terminal domain (NTD) and β-propeller domain (βPD) and active site at the interface of the NTD and βPD. Inset is an enlarged view of the cavity with two molecules of the substrate arranged in a head-to-tail fashion which brings the electrophilic group of each substrate into close proximity with the nucleophilic benzene ring of the other substrate. The important amino acids (Glu 374, His 391 and Asp 440) for binding and catalyzing the F-C alkylation are shown in green. In addition, other important mutant variants prepared by site-directed mutagenesis of amino acids at various positions [84 (threonine), 105 (arginine), 376 (aspartic acid), 414 (threonine), 457 (asparagine), 473 (tyrosine) and 499 (phenylalanine)] are shown in cyan.

Recently, Xiang and co-workers elucidated the crystal structure and catalytic mechanism of CylK.[94] The structure of CylK proteins consists of an N-terminal domain (NTD) having 237 amino acids and β-propeller domain (βPD) having 415 amino acids. The long, flat and extensive hydrophobic catalytic site is formed at the interface of the NTD and βPD domains. From the crystal structure, free energy simulation and site-directed mutagenesis experiments, they proposed a concerted double activation mechanism of F-C alkylation. They showed that two substrate molecules bind in a head-to-tail fashion in the catalytic site (Figure 8). The C-Cl bond of one substrate is activated by interactions with three key amino acids (Thr84, Arg105 and Tyr473). Simultaneously, another amino acid, Asp440, deprotonates the hydroxyl of the resorcinol ring of a second substrate molecule, resulting in electron enrichment at C-2. This nucleophilic carbon attacks the electrophile from the rear resulting inversion of stereochemistry. In addition, they used mutagenesis experiments to prove that the negatively charged amino acids, Glu374 and Asp440, are essential for the catalysis. This structural view of the reaction mechanism will be helpful in further exploration of the full potential of this enzyme.

α-Chymotrypsin

The α-chymotrypsin enzyme is a member of the hydrolase family generally known for proteolytic and esterolytic activity.[95] Its enzymatic properties and mechanism for hydrolysis of peptide bonds, have been thoroughly explored since 1970.[96] Its low cost, stability for storage, and abundant supply make this enzyme a good catalyst for potential applications.[97] Guan and co-workers used this enzyme to do F-C reactions between indoles and isatins to make 3-hydroxyoxindole and 3,3-bis(indol-3-yl)indolinones.[98] Both are essential scaffolds in the number of natural and pharmaceutical products and have a wide range of biological activities such as antiviral, anticancer, antibacterial and anticonvulsant activity.[99] As demonstrated in

Scheme 12, stereospecific 3-hydroxyoxindole (**44**) and 3,3-bis(indol-3-yl)indolinones) (**45**) were synthesized in dichloroethane and methanol solvent systems, with the final products obtained in up to 97% yield.[98]



Product	R_1	R_2	R_3	R_4	Yield (%)
44a	H	H	H	H	93
44b	Me	H	H	H	90
44c	H	NO_2	H	H	97
44d	H	F	H	H	92
44e	H	Cl	H	H	90
44f	H	H	Br	H	87
44g	H	H	OMe	H	82
44h	H	NO_2	Br	H	93
44i	Me	H	Br	H	81
44j	H	Me	H	H	83
44k	H	Me	Br	H	87
44l	H	OMe	OMe	H	88
44m	H	F	Br	H	96
44n	H	Br	OMe	H	80
44o	H	Me	OMe	H	79
44p	H	Cl	Br	H	99
45a	H	H	H	H	96
45b	H	F	H	H	91
45c	H	NO_2	H	H	91
45d	Me	H	H	H	83
45e	H	H	Me	H	63
45f	H	OMe	Me	H	68
45g	H	Br	Me	H	70
45h	H	H	H	F	93
45i	H	H	H	OMe	86
45j	H	H	H	Br	83
45k	H	F	H	F	87
45l	H	Br	H	OMe	88
45m	Me	H	H	OMe	94
45n	Me	H	H	F	96
45o	H	F	H	OMe	97
45p	H	F	Me	H	78
45q	H	NO_2	H	F	68
45a	H	H	H	H	96
45b	H	F	H	H	91
45c	H	NO_2	H	H	91
45d	Me	H	H	H	83
45e	H	H	Me	H	63
45f	H	OMe	Me	H	68
45g	H	Br	Me	H	70
45h	H	H	H	F	93
45i	H	H	H	OMe	86
45j	H	H	H	Br	83
45k	H	F	H	F	87
45l	H	Br	H	OMe	88
45m	Me	H	H	OMe	94
45n	Me	H	H	F	96
45o	H	F	H	OMe	97

Scheme 12. α-chymotrypsin catalyzed the F-C alkylation reaction between indoles and isatins.

Monoamine Oxidase (MAO-N)

Monoamine oxidase belongs to the flavin-dependent amine oxidases protein family, and it carries out the oxidation of amine-containing substrates lacking an α-carboxyl group.[100,101] In 1995, Schilling and Lerch identified this enzyme (MAO-N) from *Aspergillus niger*.[102] The gene was later cloned and expressed in *E. coli* and oxidation of simple amines was carried out but with low activity.[103] Turner and his team used directed evolution to improve this enzyme

for a broader range of substrates.[104,105] They showed that the five-point mutant variant (Asn336Ser/Met348Lys/Ile246Met/Thr384Asn/Asp385Ser or MAO-N-D5; Figure 9) showed excellent activity towards a variety of substrates.[106]

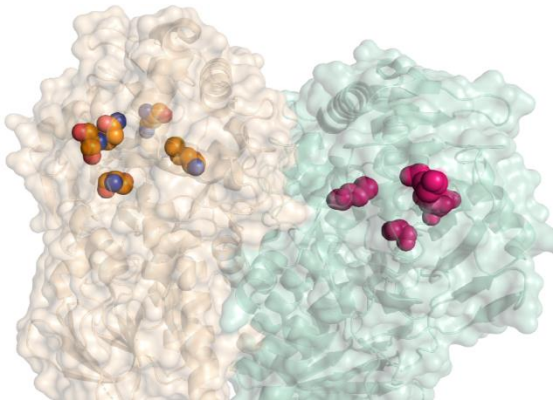
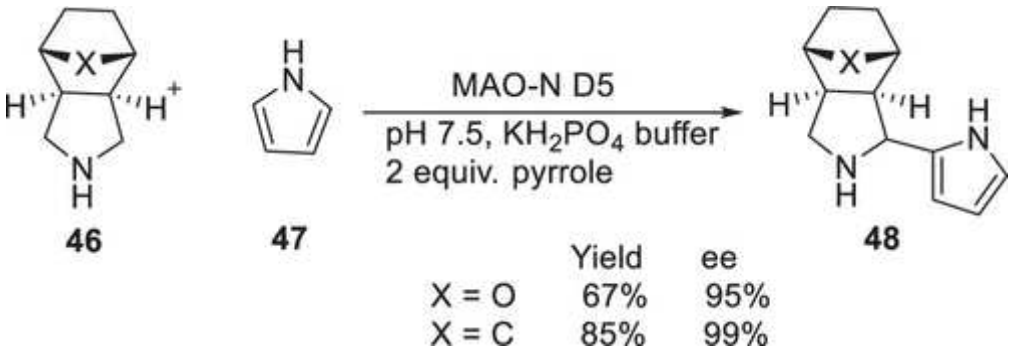


Figure 9. Crystal Structure of MaO-N D5 drawn using PDB 2VVM. The spheres represent the mutation sites (Asn336Ser/Met348Lys/Ile246Met/Thr384Asn/Asp385Ser).[96]

The aza-F-C reaction is an enantioselective addition of aromatic and heteroaromatic compounds to α-imino derivatives and usually needs to be performed in organic solvent and with Lewis/Bronsted acid catalysis. Orru and co-workers displayed the potential of the MAO-N D5 enzyme to accomplish aza-F-C reactions to synthesize chiral 2-substituted pyrrolidine derivatives under green conditions.[107] They developed a one-pot, two-stage protocol in which the amine α-carbon was first activated by biocatalytic oxidation before the addition of the pyrrole derivative C-nucleophile without the need for further addition of catalyst (Scheme 13). The final product, **48**, was obtained as a single diastereomer in good yield (up to 85%) and high enantioselectivity (up to 99%). The reaction was performed under standardized conditions with a variety of other pyrrole and indole derivatives, leading to moderate to good yields with high enantioselectivity. Moreover, *N*-methyl-pyrrolidines also undergo oxidative aza-F-C

reaction but with low enantioselectivity. Nonetheless, this benign method can produce industrially important, highly functionalized chiral small pyrrolidine molecules in one pot.

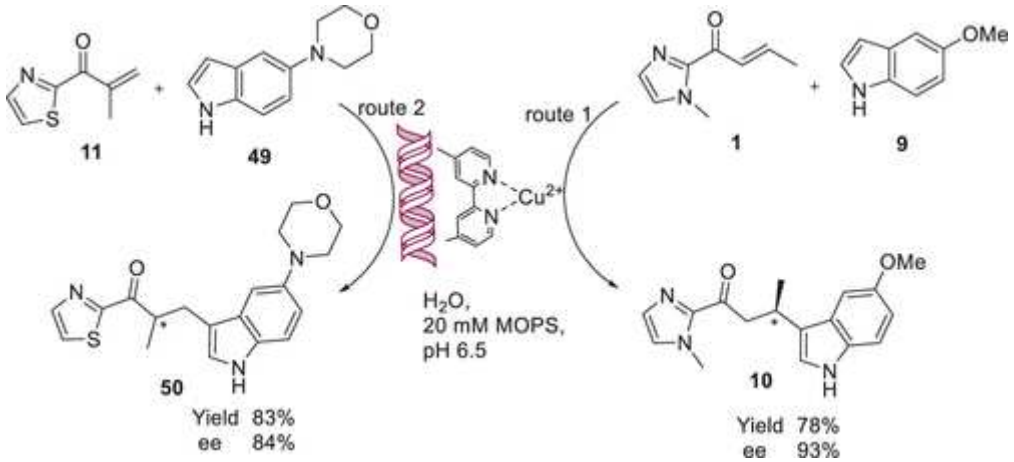


Scheme 13. Chemoenzymatic oxidative Aza-F-C reactions by MAO-N D5.

DNA-based Catalyst and Deoxyribozymes

The concept of DNA-based chirality transfer catalysis was developed by Ben Feringa and his team in 2005.[108] In their approach, they made a hybrid catalyst by combining DNA with a DNA-intercalating transition metal complex that can bind with reactants, stabilize the transition state and transfer the chirality from the DNA helix to the product.[109] Using this hybrid catalyst, the enantioselective Friedel–Crafts alkylation process of 5-methoxyindole **9**, with an α,β-unsaturated 2-acylimidazole **1**, was performed in water (Scheme 14).[110] During optimization of the process, it was found that the DNA sequence was the most crucial variable for success of the reaction. Combining self-complementary oligonucleotide d(TCAGGGCCCTGA)$_2$ with a Cu(4,4'-dimethyl-2,2'-bipyridine) ligand yielded F-C product **10** with 93% ee.[110] They discovered that this DNA-based hybrid catalyst could be used multiple times without decreasing yield and enantioselectivity, achieving 75% yield and 81% ee even in the third run. A kinetic study of F-C alkylation reactions using similar substrates found that DNA not only gave rise to the enantioselectivity, but also increased the reaction rate (9-27 fold) of F-C reactions.[111]

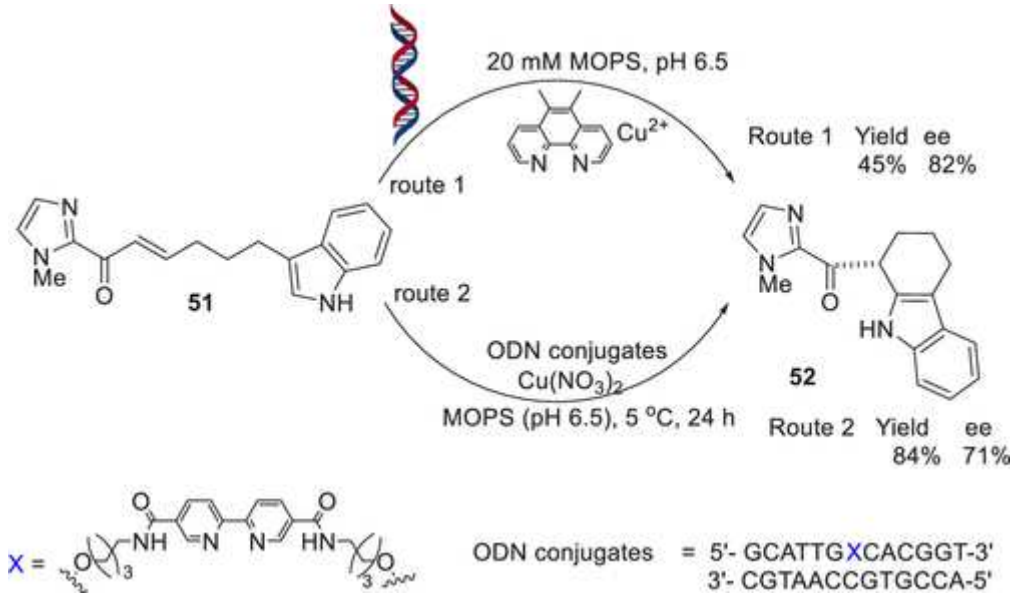
Roelfes and his team members used a thiazole moiety instead of *N*-methyl imidazole as the copper-chelating substrate.[112] F-C alkylations were performed with α-substituted enone [2-methyl-1-(thiazol-2-yl)pro-2-ene-1-one] **11** and various indole derivatives in water. After adding indole at the β-position, subsequent protonation at the prochiral acceptor produces a stereocenter at the α-position. The 5-morpholinoindole derivative **49** gave tandem F-C product **50** with excellent 84% enantioselectivity (Scheme 14 route 2). Fluorescence and UV spectroscopy data showed that 5-morphoindole was also bound to DNA in addition to the metal. No side products were obtained in the presence of DNA, and reactions were significantly faster. They demonstrated a large rate acceleration (at least 700-fold and up to 990-fold) due to the DNA-based bimolecular scaffold.[111] After the kinetic and binding investigations, it was found that the DNA grooves, where the reaction happens,[113,114] act like micelles concentrating the substrates and other reaction components, resulting in acceleration of the reaction rate. The high enantioselectivity of the reaction was attributed to second coordination sphere interactions. These studies demonstrated that DNA-based catalysis could effectively complete chemically challenging processes in water.[112]



Scheme 14. The utilization of a DNA-based hybrid catalyst for enantioselective F-C reactions.

The strong binding of cisplatin complexes to DNA was also exploited to develop a DNA-based hybrid catalyst and applied in the F-C reactions.[115] However, in this covalent anchoring approach, lower activity and enantioselectivity were obtained than the supramolecular approach used previously. Instead of 4,4'-dimethyl-2,2'-bipyridine, Qiao and co-workers used similar bisbenzimidazole derivatives, {*N*,*N*-bis(1H-benzimidazol-2-ylmethyl)-amine(IDB) and *N*,*N*-bis(1H-benzimidazol-2-ylmethyl)-acetamide [IDB(*N*-acetyl)]} as catalytic ligands for F-C asymmetric alkylation reactions (Scheme 14). F-C products were obtained with IDB(*N*-acetyl) as the catalytic ligand, but no F-C alkylation was observed with IDB_Cu^{2+}/st-DNA.[116] Although the conversion rate was good (up to 99%) with IDB(*N*-acetyl) as catalytic ligand, the stereoselectivity was very low (up to 27% ee). A series of spectroscopic assays (CD spectra, UV-Vis titration assay, Raman spectroscopy, singular value decomposition analysis etc.) were used to determine the binding modes of ligand-Cu^{2+} complexes to DNA. They concluded that binding of the ligand [IDB(*N*-acetyl)-Cu^{2+} complex] closer to the centre of the DNA scaffold might create an effective chiral environment for enantioselective synthesis. Overall, this detailed spectroscopic study cast light on dynamics and mechanistic aspects of DNA-based asymmetric catalysis.[116]

Inspired by previous works, Sugiyama and co-workers performed intramolecular F-C reactions.[117] They demonstrated the synthesis of intramolecular F-C products **52** in up to 82% ee with $d(TGTGTGCACACA)_2$ as the oligonucleotide sequence in the presence of copper-5,6-dimethyl-1,10-phenanthroline (5,6-dmp) complex (Scheme 15 route 1).[118] They claimed that Z-DNA, a left-handed chiral scaffold, gave opposite enantioselectivity compared to B-DNA, but with low enantiomeric excess.[118]

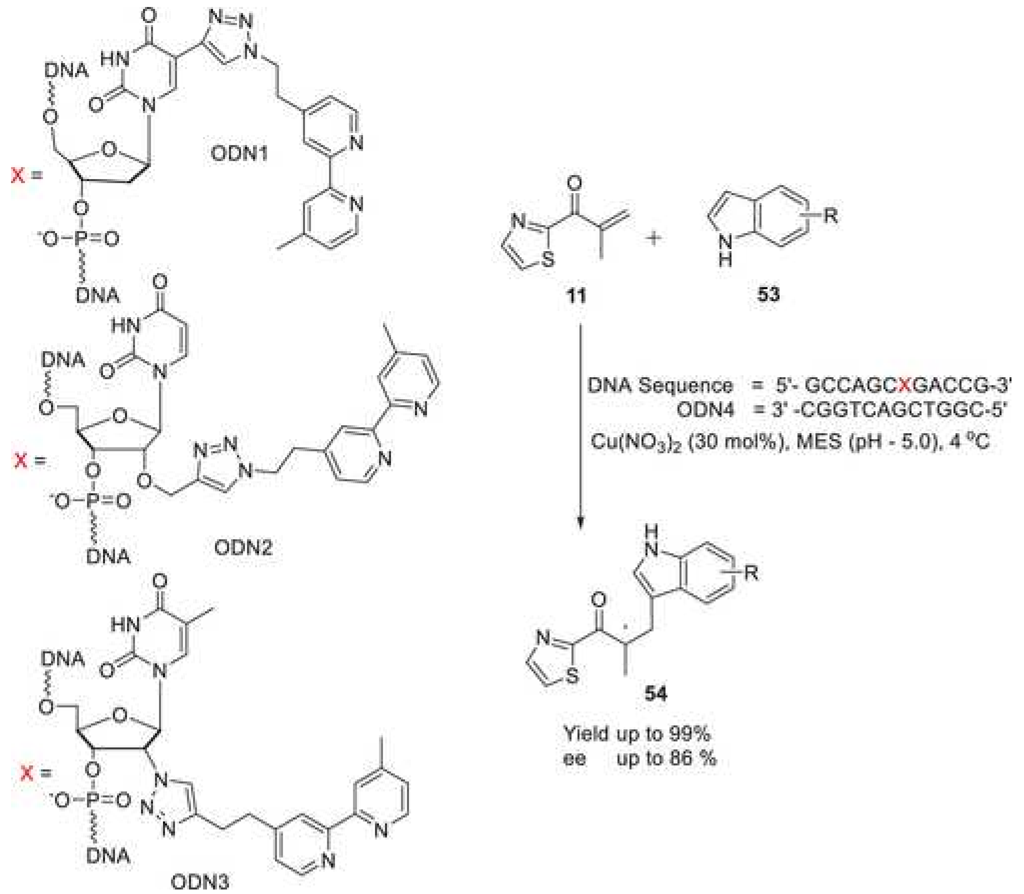


Scheme 15. Intramolecular F-C reaction catalyzed by DNA-based hybrid catalyst.

In the above studies, there is very little information about the position of the metal-ligand complex in the DNA scaffold because controlling its location as a supramolecular complex with DNA is challenging. Without knowing the precise position of the metal-ligand complex in the DNA scaffold, it is difficult to understand structural and mechanistic aspects of the reaction. Therefore, Sugiyama and his team developed new DNA-hybrid catalysts using a covalent anchorage strategy in which a bipyridine derivative was incorporated in the phosphate backbone of a 13-mer oligonucleotide sequence.[119] They applied this advanced catalytic system to intramolecular F-C alkylation, as shown in scheme 15, route 2. They found that the catalytic behaviour depended upon several factors, including the counter base of the ligand, the size of the binding pocket, neighbouring bases, and the disposition of ligands. High enantioselectivity (up to 84%) was obtained with a DNA-based catalyst in which the intrastrand bipyridine ligand was at the centre of DNA duplex, flanked by G and C nucleotides, and with cytosine as the counter base on complementary DNA strand. Molecular modelling of the product **52** and DNA hybrid catalyst indicated that the major groove was likely to be the binding site. Understanding the catalyst structure can be helpful in further designing and tuning DNA-based hybrid

catalysts, and this covalent attachment strategy made rationalization of the DNA-based hybrid system relatively easy.[119]

Interestingly, it has been established that DNA base modifications at the 5-position of pyrimidines are known to put the substituents in the major groove of the double helix, whereas 2-modifications put them in the minor groove.[120,121] Smietana and co-workers have considered the fundamental importance of the metal-chelating ligand's position[122] and developed tailored oligonucleotide sequences (ODN1, ODN2 and ODN3) with a bipyridyl ligand appended to a thymidine/uridine nucleotide. Combining ODNs with complementary strand ODN4 generated DNA duplexes (Scheme 16). To analyze the influence of the position of the metallic cofactor on both reactivity and selectivity, they applied these catalytic systems to tandem F-C conjugate addition/asymmetric protonation (Scheme 16). 2'-Modified bipyridine-containing duplex, ODN2/ODN4, was the best bio-hybrid catalyst for tandem F-C alkylation, giving the tandem F-C product **54** with enantioselectivity up to 86%.[122] Structural examination showed the role of sugar conformation in controlling the stereoselectivity. They proposed that the sugar exists in a puckered ring form in ODN2/ODN4 complex and forms a stable A-shaped cavity to coordinate with a water molecule. The water molecule was sequestered between the 2'- and 3'-*O*-subsitutents by hydrogen bonding and was readily available to protonate a prochiral enolate intermediate.



Scheme 16. Tailored oligonucleotide sequence for tandem F-C conjugate addition/asymmetric protonation reaction.

G4 quadruplex DNA made up of a TTAGGG sequence is found at the end of human telomeres. Mose and co-workers exploited these interesting structures as a chiral scaffolds for catalysis,[123] This aroused the curiosity of Li and co-workers to expand the scope of G4 quadruplex DNA to F-C reactions.[124] They investigated intermolecular F-C reactions with a number of G-quadruplex complexes while using the substrates shown in Scheme 14, route 1, and identified the importance of loop sequence of the G-quadruplex for achieving good enantiomeric excess of product. A G4DNA metalloenzyme, simply assembled from G4DNA with Cu^{2+} ions, showed the highest catalytic conversion (99%) and enantioselectivity up to 75%, while its combination with other metal ions showed poor results. Furthermore, they studied the impact of conformation of the G-quadruplex on enantioselective induction by tuning the concentrations of Na^+, K^+ and PEG200 in the reaction mixtures. Without Na^+ ions, the G4DNA-

Cu^{2+} complex is in a labile antiparallel structure, and F-C product was obtained with 49% ee, but with the addition of Na^{+} ions, a stable and compact antiparallel structure of G4DNA formed resulting in increased in enantioselectivity of up to 75% at 50 mM concentration. However, in the presence of K^{+} ions, G4DNA's conformation was a hybrid type,[125,126] and low enantioselectivity was observed.

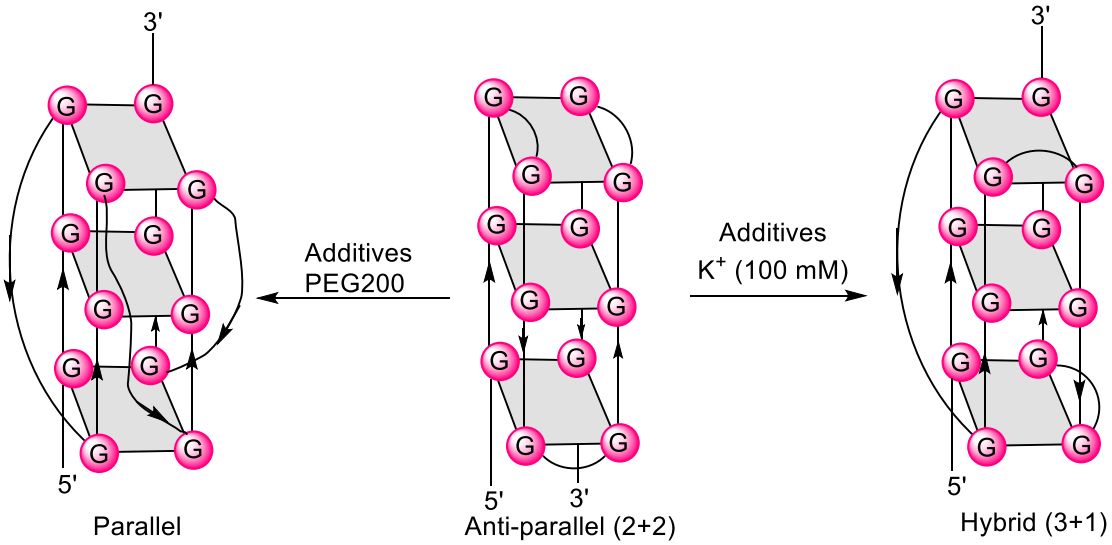


Figure 10. The changes in G4DNA conformation on adding potassium ion (K^{+}) and PEG200 as additives.

Interestingly, the G-quadruplex's conformation changed from antiparallel to parallel on increasing the concentration of PEG200, but the enantioselectivity of final products was low. Among the 21-mer oligodeoxynucleotide loop sequences, 5'-GGG$(TTAGGG)_3$ showed 99% conversion and 75% ee. Hence, they concluded that the DNA and loop sequences are essential factors in obtaining activity and selectivity in F-C chiral catalysis.

Due to electrostatic interactions, Cu^{2+} bound with G-quadruplex sequence in a very nonspecific way. As a result, it is difficult to identify the parameters that influence activity and selectivity in these reactions. Previously, Jaschke and co-workers developed a hybrid catalyst for Michael additions using a site-specific covalent anchoring strategy to introduce Cu(II) ions to two

established G-quadruplex-forming sequences (GQ), namely the human telomeric sequence and a region of c-kit promotor (c-kit) sequence.[127,128] Using a similar approach, they developed modified double-stranded (ds) and G-quadruplex (GQ)DNA-based hybrid catalysts to understand the role of different secondary structures in enantioselective F-C alkylation (Figure 11).[129] In these catalysts, the Cu(II)-coordinated bipyridine ligand was covalently attached to the abovementioned DNA sequences by various sized linkers (Figure 11). Among GQDNA-based catalysts, the best result of F-C catalysis was obtained with pentynyl-bpy-dU12(GQ)+Cu^{2+} quadruplex catalyst where 5-methoxyindole **9** with an α,β-unsaturated 2-acylimidazole **1** gave the (-)-enantiomer (**10**) with 65% ee and 99% conversion, while a dsDNA-based catalyst provided (+)-enantiomers in up to 62% ee and 27% conversion. With an increase in linker length between the modified base and bipyridine metal complex, the conversion rate was increased for the GQDNA-based hybrid catalysts, as shown in Table 1. They proposed that long and flexible linkers can be crucial in developing selective catalysis. Hence, the activity and selectivity of the F-C reactions were modulated by the position of DNA modification, the secondary structure and topology of DNA, and both the nature and length of the ligand.[129]

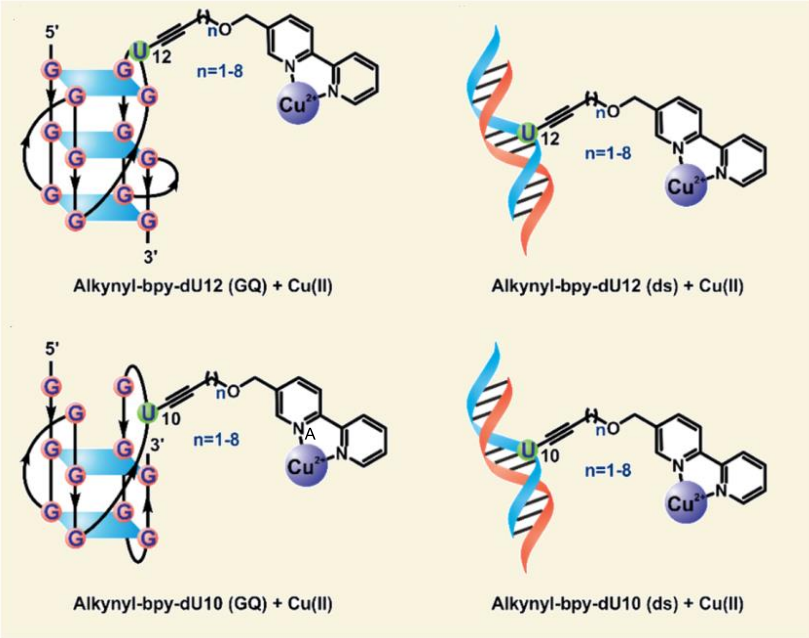
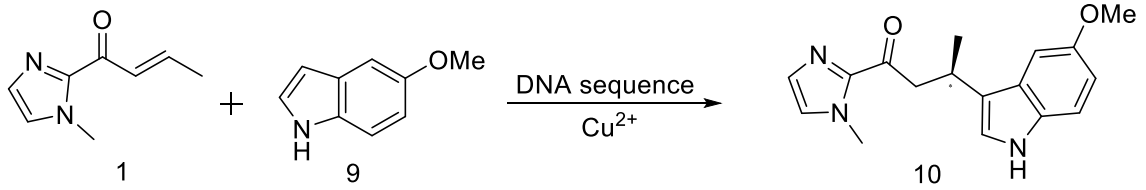


Figure 11. Various modified DNA hybrid catalysts by double standard G-quadruplex structures. This figure is reproduced from reference[129] under a Creative Commons CCBY4.0 licence (http://creativecommons.org/licenses/by/4.0/).

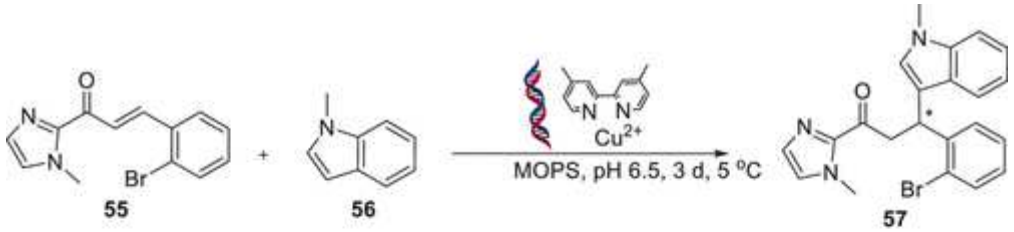
Table 1. F-C reaction catalyzed by modified double-stranded (ds) and G-quadruplex (GQ)DNA-based hybrid catalysts[128]



S.N.	Linker-bipyridine (bpy) complex	n	dU12-modified GQ-DNA		dU12-modified DNA		ds-DNA	dU10-modified GQ-DNA		dU10-modified ds-DNA	
			conv (%)	*ee* (%)	conv (%)	*ee* (%)		conv (%)	*ee* (%)	conv (%)	*ee* (%)
1	Propargyl-bpy	1	64	−23	27	+62		44	+17	23	+37
2	Butynyl-bpy	2	78	−35	48	+16		64	+45	36	+21
3	Pentynyl-bpy	3	99	−65	63	+25		68	+14	49	<+5
4	Hexynyl-bpy	4	99	−19	79	+8		65	+16	61	+31
5	Heptynyl-bpy	5	99	−22	99	−8		67	+10	88	+33

6	Octynyl-bpy	6	99	+29	99	+7	66	+21	89	+40
7	Nonynyl-bpy	7	99	−13	99	−23	64	+8	89	+53
8	Decynyl-bpy	8	99	+8	99	−11	65	+7	91	+38

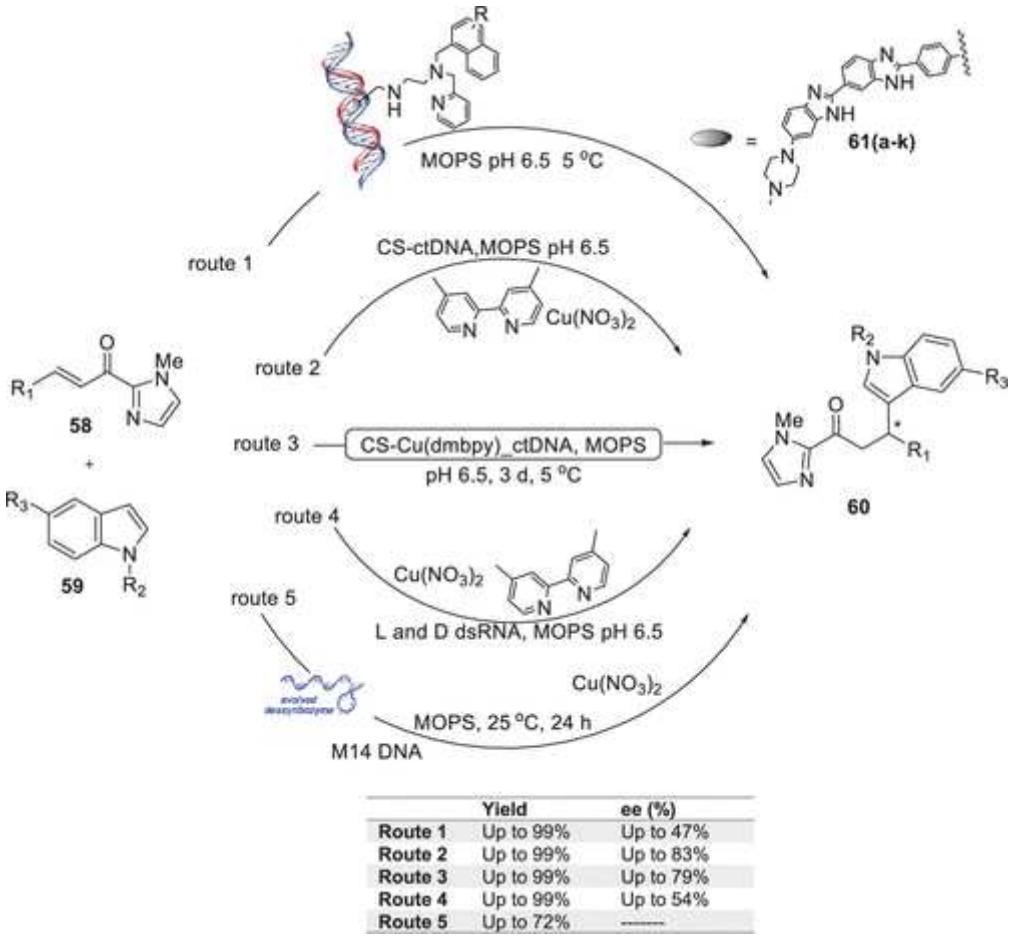
Arseniyadis and his group used L-oligonucleotides (ODN5 and ODN6) and D-oligonucleotides sequences (ODN7 and ODN) to show that L-DNA induced opposite stereoselectivity to D-DNA (Scheme 17).[130] A series of experiments (entries 6-17 in Scheme 17) were carried out by mixing various concentrations of oligonucleotides (ODNs) or salmon testes DNA (st-DNA) to investigate the effect of sequence and length of DNA sequence on enantioselectivity. No chiral bias was observed in the mixing experiments of ODN1 and ODN3 (entries 6-8); however, in other experiments (entries 9-17), chiral bias is clearly marked. These findings show that symmetry breaking could have resulted via the spontaneous production of specially selected sequences from a prebiotic pseudo-racemic pool, as the abundance of left- and right-handed structures is precisely equal.



DNA Sequence	Conversion (%)	ee (%)
DNA = stDNA	61	+77
ODN5 = L-d(TCAGGGCCCTGA)2	73	-83
ODN6 = L-d(CAGTCAGTACTGACTG)2	66	-43
ODN7 = D-d(TCAGGGCCCTGA)2	84	+76
ODN8 = D-dCAGTCAGTACTGACTG)2	65	+47
Mixing experiment		
ODN5/ODN7 (75:25)	75	-44
ODN5/ODN7 (50:50)	65	0
ODN5/ODN7 (25:75)	74	+40
stDNA/ODN5 (75:25)	65	+10
stDNA/ODN5 (50:50)	77	-32
stDNA/ODN5 (25:75)	76	-62
ODN5/ODN8 (75:25)	89	-72
ODN5/ODN8 (50:50)	66	-51
ODN5/ODN8 (25:75)	61	-20
ODN6/ODN7 (75:25)	74	+64
ODN6/ODN7 (50:50)	62	+47
ODN6/ODN7 (25:75)	87	+7

Scheme 17. Asymmetric F-C reactions by L-DNA and D-DNA nucleotide sequence.

To address scalability and industrial use, the same group used commercially available cellulose-supported DNA-based catalysts (CS-ctDNA) to perform enantioselective F-C alkylation of 5-methoxyindole **58** with α,β-unsaturated 2-acylimidazoles **59** (Scheme 18 route 2).[131] Complete conversion and 81% enantiomeric excess of the final product **60** (R_1 = Me, R_2 = H, R_3 = OMe) was obtained. Other indole and α,β-unsaturated 2-acylimidazoles derivatives under the same conditions gave almost quantitative conversion, but the highest enantioselectivity of 83% was achieved with electron-rich indole derivatives. The authors demonstrated that the cellulose material could be reused ten times without adding Cu and dmbpy, underlining the potential cellulose-supported DNA for large-scale applications. They also implemented the method in a single pass, continuous flow process,[132] and discovered that increasing the amount of immobilized DNA-biohybrid catalyst in the chromatography column and a slow flow rate led to 96% conversion and 79% ee of the final product **60** (Scheme 18 route 3).[131]



	Yield	ee (%)
Route 1	Up to 99%	Up to 47%
Route 2	Up to 99%	Up to 83%
Route 3	Up to 99%	Up to 79%
Route 4	Up to 99%	Up to 54%
Route 5	Up to 72%	--------

Scheme 18. a) route 1: F-C alkylations catalysed by DNA linked Hoechst 33258 derivative as ligand.[133] b) route 2: F–C alkylations with Cellulose-supported ct-DNA (CS-ct-DNA) using dmbpy as ligand.[131]. c) route 3: CS-ct-DNA-catalysed F–C alkylations under continuous-flow process[131] by Arseniyadis and co-workers. d) route 4: dsRNA catalysed F-C reactions.[139] e) F-C alkylation with the deoxyribozymes by McNaughton and co-workers.[142]

Several approaches have been discussed thus far for anchoring the metal complex to a DNA chiral scaffold, including: intercalation of coordination complexes into natural dsDNA; second coordination sphere interactions; and covalent attachment of metal ligands directly to DNA. Arseniyadis, Smietana and co-workers developed a new anchoring strategy using the

fluorescent DNA stain Hoechst 33258.[133] Hoechst 33258 binds to the minor groove of dsDNA, preferring adenine and thymine rich regions.[134,135] Hence, they synthesized various bifunctional flexible 'Hoechst-amine' and rigid 'Hoechst-alkyne' type ligands by attaching metal chelating agents to Hoechst 33258 for the coordination to a transition metal (Figure 12). Binding affinity experiments using multiple techniques, such as UV-vis titration, thermal denaturation, fluorescence and circular dichroism spectroscopy, revealed that amine-type ligands had a better affinity towards calf thymus DNA (ct-DNA), poly(d(A-T)$_2$) and various DNA sequences when compared to the alkyne-type ligands. Amine linkers (**61g** and **61h**) and alkyne linker (**61e**) were selected for F-C alkylation with ct-DNA (Figure 12). Fluorescence titration assays showed that DNA sequences containing AATT base pairs were the strongest binding site for the complexes. In contrast, DNA sequences containing TATA sequences were the weakest binding site for the ligand. So, two other oligonucleotide sequences, 5'-d(CG**AATT**CGTTTTCG**AATT**CG)-3' and 5'-d(CG**TATA**CGTTTTCG**TATA**CG)-3' were also synthesized to examine their impact on the enantioselectivity of the F-C reaction (Scheme 18, route 1). They obtained moderate (47% ee) of (+)-enantiomer of product **60** (R_1 = Me, R_2 = H, R_3 = OMe) with ct-DNA and ligand **h**. However, the oligonucleotides having AATT and TATA sequences, preferentially gave the (-)-enantiomer of product **60** in 26% and 23% ee, respectively, with ligand **h**. Similar results were obtained for ligand **g**, while alkyne-linked ligand **e** showed very low enantioselectivity (4-7% ee) with any of the DNA molecules. Sequence-specific targeting ability opens new possibilities in DNA-catalysed enantioselective F-C reactions with Hoechst ligands. (Scheme 18, route 1).

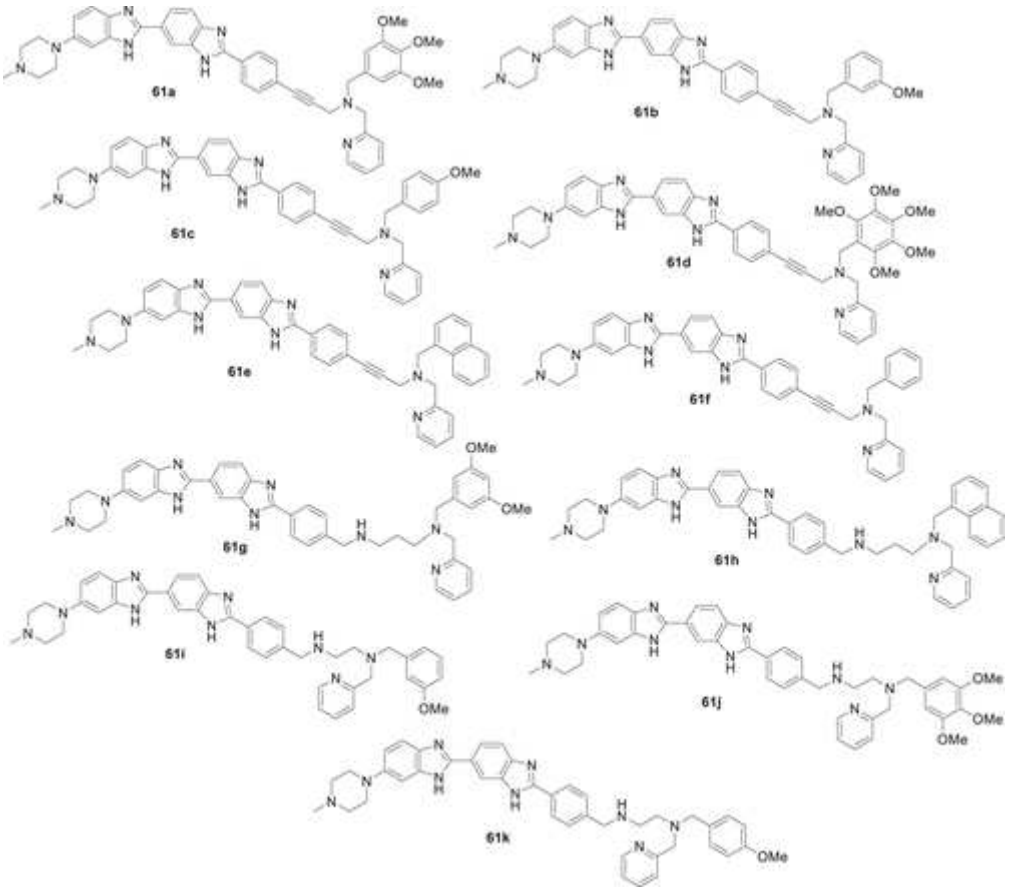


Figure 12. Various bifunctional Hoechst 33258 based alkyne (a-f) and amines (g-k) ligands.

Naturally occurring ribozymes and artificial ribozymes have been used for catalysis of various synthetic transformations, such as Diels-Alder reactions,[136] Michael additions,[137] and aldol condensations.[138] The role of ribonucleic acid (RNA) in asymmetric F-C alkylations was first investigated by Arseniyadis and co-workers.[139] Short and defined synthetic D- and L-oligoribonucleotides sequences were used for asymmetric Cu^{2+}-catalysed F-C alkylation, leading to modest enantioselectivity up to 54% ee with 16mer [5'-$(CAGUCAGUACUGACUG)_2$] RNA sequence in the presence of a 4,4'-dimethyl-2,2'-bipyridine (dmbpy) ligand (Scheme 18, route 4). Further understanding of the factors governing enantioselectivity could help to rationalize and control the RNA chiral environment to create a competitive chiral scaffold.

In examples discussed thus far, DNA is used as a chiral scaffold, but metal complexation is achieved through an added ligand. It is possible for DNA to take on the full role of the enzyme, and DNA enzymes (deoxyribozymes) have also been investigated as catalysts for chemical processes. The majority of deoxyribozymes in the literature are reported for bond-forming and bond-breaking activities in nucleic acids,[140] and Silverman has reviewed the use of DNA for catalyzing chemical reactions.[141] Subsequently, McNaughton and co-workers identified a 72 nucleotide deoxyribozyme catalyzing F-C alkylations using a gel-shift *in vitro* nucleic acid screen (Scheme 18, route 5).[142] They carried out the F-C reactions in two ways: *in cis* and *in trans. In cis* reactions, acylimidazole (**58**) is linked with a particular sequence (M14 DNA) and generated the F-C product with a 32% yield in the presence of Cu^{2+}. However, *in trans* reactions, neither reactant is attached with DNA, and F-C reactions were observed in the presence of M14 DNA and absences of Cu^{2+} ions. The F-C product **60** (R_1 = 4-methyoxyphenyl, R_2 = H, R_3 = OMe) was obtained in 72% yield when using 50 mol% of M14 DNA in the presence of Cu^{2+} ions, but as the amount of M14 DNA decreased, the yield also reduced. Less than 10% product could be detected without $Cu(NO_3)_2$ or M14 DNA or when using a random DNA sequence. Since the formation of the F-C product was dependent on M14DNA, they hypothesized that the catalytic mechanism of deoxyribozymes may be different from the DNA chirality transfer mechanism described above.

Table 2. Summary of all biocatalyst Friedel-Crafts reaction

No.		ArMs	yield % (up to)	ee% (up to)	Ref
1	Vinylogous	LmrR_M89BPyA	92	80	38
2	F-C reactions	LmrR_M89BPyA _F93W	94	83	38
3		QacR_Y123BPyA	82	94	41
2		LmrR_pAF_RGN	74	87	47
5		LmrR_V15HQAla	97	<5	52
6		LmrR_A92E	61	99	57
7	Tandem F-C reactions	LmrR_W96A	32	64	57
8		LmrR_pAF	74	88	48
9		QacR		83	60

10	Vinylogous	$mTEF^{CHH}$	14	92	61
11	F-C reaction	$mTEF^{EHH}$	24	92	61
		Methyltransferases			
12	Regiospecific F-C	CouO	99	---	69
13	methylation	NovO	99	---	69
14	reactions	NovO	97	---	71
		Acyltransferases			
15	F-C acylation	PpATaseCH	99	---	82 87 88
16		ATase-F148V	99	---	89
		CylK			
17	Stereospecific F-C alkylation	Cyl K	100	99	92
		Chymotrypsin			
18	F-C reaction for 3-hydroxyindole	α-chymotrypsin from bovine pancreas	99	---	98
19	F-C reaction for 3,3-bis(indol-3-yl)indolinones	α-chymotrypsin from bovine pancreas	97	---	98
		Monoamine Oxidase			
20		MAO-N D5	85	99	107
		DNA based catalysts deoxyribozymes			
21	F-C alkylation	$d(TCAGGGCCCTGA)_2$	78	93	110
22	Tandem F-C reaction	st-DNA	83	84	112
23	F-C alkylation	$d(TCATGGCCATGA)_2$	84.7	27	116
24	Intramolecular F-C	$d(TGTGTGCACACA)_2$	45	82	118
25	alkylation	5′-GCATTGXCACGGT-3′ 3′-CGTAACCGTGCCA-5′	84	71	119
26	Tandem F-C reaction	5′-GCCAGCXGACCG-3′ 3′-CGGTCGGCTGGC-5′	99	86	122
27	F-C alkylation by modified G-	$5′-GGG(TTAGGG)_3-3′$	99	75	124
28	quadruplex and ds-	Pentaynyl-bpy-dU12(GQ)	99	65	129
29	DNA	Propagyl-bpy-dU12-ds-DNA	27	62	129
30	F-C alkylation	CS-ctDNA	99	83	131
31		CS-ctDNA continuous flow	99	79	131
32		ct-DNA	99	47	133
33		$L-(CAGUCAGUACUGACUG)_2$	99	54	139
34		M14DNA	72	---	141

Summary and outlook

The examples shown here in this article describe progress in the development of biocatalysts for doing asymmetric Friedel-Crafts reactions. This C-C bond formation reaction has been the backbone for synthetic organic chemistry in various industries, especially pharmaceuticals. But the problem associated with the environmental costs of chemical synthesis has led to the investigation of more eco-compatible methods. Biocatalysts present a sustainable approach for

doing complex chemical transformations, and this strategy has also shown the potential to overcome the historical limitation of a lack of regioselectivity and chemoselectivity in F-C reactions. In addition to natural enzymes, modified enzymes have been applied to F-C reactions. We have summarized various biocatalyst F-C reactions in Table 2. The table demonstrates the breadth of reactions that can be achieved with both high yields and high enantioselectivities. Roelfes and his group used the term 'blank canvas' for the LmrR scaffold and proposed that the amino-acid side chains in the LmrR scaffold's pocket can be exploited to promote a variety of catalytic processes.[48] Using ArMs, excellent enantioselectivity up to 99% (LmRr_A92E) was obtained. ArMs are a blossoming concept, and we firmly believe that their industrial potential will be shown up in the near future. The CouO and NovO, methyltransferases, showed broad acceptance substrates (naphthalene derivatives) for methylation, yielding up to 99%.[69] The potential of ATase-F148V to introduce long-chain acyl moieties with a yield of up to 99% has potential to be exploited to produce various antiviral agents.[89] The biologically important scaffold [3-hyroxyindole and 3,3-bis(indol-3-yl)indolinones derivatives] were synthesized in excellent yield using commercially available α-chymotrypsin enzyme pointing toward its pharmaceutical potential.[98] We think in the near future other biocatalysts such as squalene-hopene cyclases[143] and variants of tryptophan synthases[144,145] could also be exploited for doing F-C reactions using non-natural substrates. The high level of enantioselectivity (up to 93%) of DNA-based catalysts is also very promising if the processes can be scaled up.

Indeed, a key step that still needs to be addressed is that of reaction scale. It is common in the literature that authentic samples of products may be prepared by chemical means on scales of up to hundreds of milligrams, to support analysis of enzymatic processes which are themselves investigated on sub-milligram scales. Bridging this scale gap for enzymatic reactions is a key challenge to lead to industrial scale processes. In many cases, progress towards this goal is

being made, for example the development of a scalable platform for biocatalytic F-C reactions using the NovO methyltransferase in with the methylating agent SAM is generated in situ,[71] but still a need remains to take reaction scales from tens of milligrams to gram and kilogram scale processes. It may be that processes that exploit enzymes that are already prepared on an industrial scale like chymotrypsin may prove to be most readily adopted by industry.

While enzymes can also be adapted by site-directed mutagenesis and directed evolution, incorporating noncanonical amino acids, through reprogramming of the genetic code results in new xenobiotic enzymes containing abiological functionalities and expands the catalytic repertoire of biocatalysts to do new-to-nature reactions. Although these revolutionary techniques have made a significant change in biocatalysis, many challenges need to be addressed. For example, improving the yield and efficiency of de novo-designed biocatalysts compared to naturally occurring evolved enzymes, reproducibility and recyclability of biocatalyst, and being able to accurately predict an enzyme's catalytic abilites from its sequence. A synergistic blend of the approaches described in this review will most likely be necessary to address these issues, and successfully create novel enzymes with desired activities. Because of the exciting growth in biocatalysis during the last 7-10 years, it can be expected that these issues will be solved in the near future. These advancements will establish biocatalysis as a greener, cost-effective and atom-efficient method for synthesizing complex molecules for the chemical, pharma, food and fragrance industry.

AUTHOR INFORMATIONS

Corresponding Author

Vajinder Kumar – *Department of Chemistry, Akal University, Talwandi Sabo, Bathinda, Punjab, India.* https://orcid.org/0000-0002-8825-3517

EmailID – vkvkumar17@gmail.com

Authors

Bruce Turnbull - *School of Chemistry and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK.* https://orcid.org/0000-0002-7352-0360

Avneesh Kumar - *Department of Botany, Akal University, Talwandi Sabo, Bathinda, Punjab, India.* https://orcid.org/0000-0002-8907-9047

Funding Source

No funding Source

Notes

The authors declare no competing financial interest.

References

(1) Friedel, C.; Crafts, J. M. Sur Une Nouvelle Méthode Générale de Synthèse d'hydrocarbures, d'acétones, Etc.,. *Hebd. Seances Acad. Sci.* **1877**, *84*, 1392.

(2) Friedel, C.; Crafts, J. M. Sur Une Nouvelle Méthode Générale de Synthèse d'hydrocarbures, d'acétones, Etc.,. *Hebd. Seances Acad. Sci.* **1877**, *84*, 1450.

(3) Olah, G. A.; Reddy, V. P.; Prakash, G. K. S. Friedel-Crafts Reactions. *Kirk-Othmer Encyclopedia of Chemical Technology*; Major Reference Works; 2000. https://doi.org/https://doi.org/10.1002/0471238961.0618090515120108.a01.

(4) Macquarrie, D. J. Industrial Friedel–Crafts Chemistry. In *Catalytic Asymmetric Friedel–Crafts Alkylations*; Wiley Online Books; 2009; pp 271–288. https://doi.org/https://doi.org/10.1002/9783527626977.ch8.

(5) Surburg, H.; Panten, J. *Common Fragrance and Flavor Materials: Preparation, Properties and Uses*, 6th editio.; Wiley-VCH Verlag, 2016 p105.

(6) Tsuchimoto, T.; Tobita, K.; Hiyama, T.; Fukuzawa, S. Scandium(III) Triflate-Catalyzed Friedel−Crafts Alkylation Reactions. *J. Org. Chem.* **1997**, *62* (20), 6997–7005. https://doi.org/10.1021/jo970599u.

(7) Gmouh, S.; Yang, H.; Vaultier, M. Activation of Bismuth(III) Derivatives in Ionic Liquids: Novel and Recyclable Catalytic Systems for Friedel−Crafts Acylation of

Aromatic Compounds. *Org. Lett.* **2003**, *5* (13), 2219–2222. https://doi.org/10.1021/ol034529n.

(8) Kawada, A.; Mitamura, S.; Kobayashi, S. Lanthanide Trifluoromethanesulfonates as Reusable Catalysts: Catalytic Friedel-Crafts Acylation. *J. Chem. Soc. Chem. Commun.* **1993**, No. 14, 1157–1158. https://doi.org/10.1039/C39930001157.

(9) Kobayashi, S.; Nagayama, S. A Microencapsulated Lewis Acid. A New Type of Polymer-Supported Lewis Acid Catalyst of Wide Utility in Organic Synthesis. *J. Am. Chem. Soc.* **1998**, *120* (12), 2985–2986. https://doi.org/10.1021/ja9739289.

(10) Izumi, Y.; Ogawa, M.; Nohara, W.; Urabe, K. Acidic Alkali Metal Salts and Ammonium Salts of Keggin-Type Heteropolyacids as Efficient Solid Acid Catalysts for Liquid-Phase Friedel-Crafts Reactions. *Chem. Lett.* **1992**, *21* (10), 1987–1990. https://doi.org/10.1246/cl.1992.1987.

(11) Akagawa, K.; Yamashita, T.; Sakamoto, S.; Kudo, K. Friedel–Crafts-Type Alkylation in Aqueous Media Using Resin-Supported Peptide Catalyst Having Polyleucine. *Tetrahedron Lett.* **2009**, *50* (40), 5602–5604. https://doi.org/https://doi.org/10.1016/j.tetlet.2009.07.071.

(12) Kantam, M. L.; Ranganath, K. V. S.; Sateesh, M.; Kumar, K. B. S.; Choudary, B. M. Friedel–Crafts Acylation of Aromatics and Heteroaromatics by Beta Zeolite. *J. Mol. Catal. A Chem.* **2005**, *225* (1), 15–20. https://doi.org/https://doi.org/10.1016/j.molcata.2004.08.018.

(13) Cornélis, A.; Laszlo, P.; Shaofang, W. On the Transition State for “Clayzic”-Catalyzed Friedel-Crafts Reactions upon Anisole. *Tetrahedron Lett.* **1993**, *34* (24), 3849–3852. https://doi.org/https://doi.org/10.1016/S0040-4039(00)79244-6.

(14) Chakravarti, R.; Kalita, P.; Selvan, S. T.; Oveisi, H.; Balasubramanian, V. V; Kantam, M. L.; Vinu, A. A Facile Synthesis of Alkylated Nitrogen Heterocycles Catalysed by 3D Mesoporous Aluminosilicates with Cage Type Pores in Aqueous Medium. *Green Chem.* **2010**, *12* (1), 49–53. https://doi.org/10.1039/B914628H.

(15) Shi, X.-L.; Lin, H.; Li, P.; Zhang, W. Friedel–Crafts Alkylation of Indoles Exclusively in Water Catalyzed by Ionic Liquid Supported on a Polyacrylonitrile Fiber: A Simple “Release and Catch” Catalyst. *ChemCatChem* **2014**, *6* (10), 2947–2953. https://doi.org/https://doi.org/10.1002/cctc.201402396.

(16) Bandini, M.; Melloni, A.; Umani-Ronchi, A. New Catalytic Approaches in the Stereoselective Friedel-Crafts Alkylation Reaction. *Angew. Chemie - Int. Ed.* **2004**, *43*

(5), 550–556. https://doi.org/10.1002/anie.200301679.

(17) Bigi, F.; Casiraghi, G.; Casnati, G.; Sartori, G.; Gasparri Fava, G.; Ferrari Belicchi, M. Asymmetric Electrophilic Substitution on Phenols. Enantioselective Ortho-Hydroxyalkylation Mediated by Chiral Alkoxyaluminum Chlorides. *J. Org. Chem.* **1985**, *50* (25), 5018–5022. https://doi.org/10.1021/jo00225a003.

(18) Erker, G.; van der Zeijden, A. A. H. Enantioselective Catalysis with a New Zirconium Trichloride Lewis Acid Containing a "Dibornacyclopentadienyl" Ligand. *Angew. Chemie Int. Ed. English* **1990**, *29* (5), 512–514. https://doi.org/https://doi.org/10.1002/anie.199005121.

(19) Rueping, M.; Nachtsheim, B. J. A Review of New Developments in the Friedel-Crafts Alkylation - From Green Chemistry to Asymmetric Catalysis. *Beilstein J. Org. Chem.* **2010**, *6*, 1–24. https://doi.org/10.3762/bjoc.6.6.

(20) Hughes, D. L. 9.1 Introduction to Industrial Applications of Asymmetric Synthesis; Carreira, E. M., Yamamoto, H. B. T.-C. C., Eds.; Elsevier: Amsterdam, 2012; pp 1–26. https://doi.org/https://doi.org/10.1016/B978-0-08-095167-6.00901-0.

(21) Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M.; Witholt, B. Industrial Biocatalysis Today and Tomorrow. *Nature* **2001**, *409* (6817), 258–268. https://doi.org/10.1038/35051736.

(22) Wu, S.; Snajdrova, R.; Moore, J. C.; Baldenius, K.; Bornscheuer, U. T. Biocatalysis: Enzymatic Synthesis for Industrial Applications. *Angewandte Chemie - International Edition*. Wiley-VCH Verlag January 4, 2021, pp 88–119. https://doi.org/10.1002/anie.202006648.

(23) Turner, N. J.; Kumar, R. Editorial Overview: Biocatalysis and Biotransformation: The Golden Age of Biocatalysis. *Curr. Opin. Chem. Biol.* **2018**, *43*, A1–A3. https://doi.org/https://doi.org/10.1016/j.cbpa.2018.02.012.

(24) de María, P. D.; de Gonzalo, G.; Alcántara, A. R. Biocatalysis as Useful Tool in Asymmetric Synthesis: An Assessment of Recently Granted Patents (2014–2019). *Catalysts*. 2019, pp 802 (1-42). https://doi.org/10.3390/catal9100802.

(25) Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K. Engineering the Third Wave of Biocatalysis. *Nature* **2012**, *485* (7397), 185–194. https://doi.org/10.1038/nature11117.

(26) Mori, T. Enzymatic Studies on Aromatic Prenyltransferases. *Journal of Natural Medicines*. Springer June 1, 2020, pp 501–512. https://doi.org/10.1007/s11418-020-

01393-x.

(27) Schwizer, F.; Okamoto, Y.; Heinisch, T.; Gu, Y.; Pellizzoni, M. M.; Lebrun, V.; Reuter, R.; Köhler, V.; Lewis, J. C.; Ward, T. R. Artificial Metalloenzymes: Reaction Scope and Optimization Strategies. *Chem. Rev.* **2018**, *118* (1), 142–231. https://doi.org/10.1021/acs.chemrev.7b00014.

(28) Jarvis, A. G. Designer Metalloenzymes for Synthetic Biology: Enzyme Hybrids for Catalysis. *Curr. Opin. Chem. Biol.* **2020**, *58* (ii), 63–71. https://doi.org/10.1016/j.cbpa.2020.06.004.

(29) Roelfes, G. LmrR: A Privileged Scaffold for Artificial Metalloenzymes. *Acc. Chem. Res.* **2019**, *52* (3), 545–556. https://doi.org/10.1021/acs.accounts.9b00004.

(30) Thomas, C. M.; Ward, T. R. Artificial Metalloenzymes: Proteins as Hosts for Enantioselective Catalysis. *Chem. Soc. Rev.* **2005**, *34* (4), 337–346. https://doi.org/10.1039/b314695m.

(31) Heinisch, T.; Ward, T. R. Design Strategies for the Creation of Artificial Metalloenzymes. *Curr. Opin. Chem. Biol.* **2010**, *14* (2), 184–199. https://doi.org/https://doi.org/10.1016/j.cbpa.2009.11.026.

(32) Alonso, S.; Santiago, G.; Cea-Rama, I.; Fernandez-Lopez, L.; Coscolín, C.; Modregger, J.; Ressmann, A. K.; Martínez-Martínez, M.; Marrero, H.; Bargiela, R.; Pita, M.; Gonzalez-Alfonso, J. L.; Briand, M. L.; Rojo, D.; Barbas, C.; Plou, F. J.; Golyshin, P. N.; Shahgaldian, P.; Sanz-Aparicio, J.; Guallar, V.; Ferrer, M. Genetically Engineered Proteins with Two Active Sites for Enhanced Biocatalysis and Synergistic Chemo- and Biocatalysis. *Nat. Catal.* **2020**, *3* (3), 319–328. https://doi.org/10.1038/s41929-019-0394-4.

(33) Zhou, Z.; Roelfes, G. Synergistic Catalysis in an Artificial Enzyme by Simultaneous Action of Two Abiological Catalytic Sites. *Nat. Catal.* **2020**, *3* (3), 289–294. https://doi.org/10.1038/s41929-019-0420-6.

(34) Madoori, P. K.; Agustiandari, H.; Driessen, A. J. M.; Thunnissen, A. M. W. H. Structure of the Transcriptional Regulator LmrR and Its Mechanism of Multidrug Recognition. *EMBO J.* **2009**, *28* (2), 156–166. https://doi.org/10.1038/emboj.2008.263.

(35) Takeuchi, K.; Tokunaga, Y.; Imai, M.; Takahashi, H.; Shimada, I. Dynamic Multidrug Recognition by Multidrug Transcriptional Repressor LmrR. *Sci. Rep.* **2014**, *4*, 1–12. https://doi.org/10.1038/srep06922.

(36) Wang, L.; Brock, A.; Herberich, B.; Schultz, P. G. Expanding the Genetic Code of Escherichia Coli. *Science (80-.).* **2001**, *292* (5516), 498–500. https://doi.org/10.1126/science.1060077.

(37) Drienovská, I.; Roelfes, G. Expanding the Enzyme Universe with Genetically Encoded Unnatural Amino Acids. *Nat. Catal.* **2020**, *3* (3), 193–202. https://doi.org/10.1038/s41929-019-0410-8.

(38) Drienovská, I.; Rioz-Martínez, A.; Draksharapu, A.; Roelfes, G. Novel Artificial Metalloenzymes by in Vivo Incorporation of Metal-Binding Unnatural Amino Acids. *Chem. Sci.* **2015**, *6* (1), 770–776. https://doi.org/10.1039/c4sc01525h.

(39) Bos, J.; Fusetti, F.; Driessen, A. J. M.; Roelfes, G. Enantioselective Artificial Metalloenzymes by Creation of a Novel Active Site at the Protein Dimer Interface. *Angew. Chemie - Int. Ed.* **2012**, *51* (30), 7472–7475. https://doi.org/10.1002/anie.201202070.

(40) Bos, J.; García-Herraiz, A.; Roelfes, G. An Enantioselective Artificial Metallo-Hydratase. *Chem. Sci.* **2013**, *4* (9), 3578–3582. https://doi.org/10.1039/c3sc51449h.

(41) Bersellini, M.; Roelfes, G. Multidrug Resistance Regulators (MDRs) as Scaffolds for the Design of Artificial Metalloenzymes. *Org. Biomol. Chem.* **2017**, *15* (14), 3069–3073. https://doi.org/10.1039/C7OB00390K.

(42) Dirksen, A.; Dirksen, S.; Hackeng, T. M.; Dawson, P. E. Nucleophilic Catalysis of Hydrazone Formation and Transimination: Implications for Dynamic Covalent Chemistry. *J. Am. Chem. Soc.* **2006**, *128* (49), 15602–15603. https://doi.org/10.1021/ja067189k.

(43) Dirksen, A.; Hackeng, T. M.; Dawson, P. E. Nucleophilic Catalysis of Oxime Ligation. *Angew. Chemie - Int. Ed.* **2006**, *45* (45), 7581–7584. https://doi.org/10.1002/anie.200602877.

(44) Wendeler, M.; Grinberg, L.; Wang, X.; Dawson, P. E.; Baca, M. Enhanced Catalysis of Oxime-Based Bioconjugations by Substituted Anilines. *Bioconjug. Chem.* **2014**, *25* (1), 93–101. https://doi.org/10.1021/bc400380f.

(45) Drienovská, I.; Mayer, C.; Dulson, C.; Roelfes, G. A Designer Enzyme for Hydrazone and Oxime Formation Featuring an Unnatural Catalytic Aniline Residue. *Nat. Chem.* **2018**, *10* (9), 946–952. https://doi.org/10.1038/s41557-018-0082-z.

(46) Mayer, C.; Dulson, C.; Reddem, E.; Thunnissen, A. M. W. H.; Roelfes, G. Directed Evolution of a Designer Enzyme Featuring an Unnatural Catalytic Amino Acid.

Angew. Chemie - Int. Ed. **2019**, *58* (7), 2083–2087. https://doi.org/10.1002/anie.201813499.

(47) Leveson-Gower, R. B.; Zhou, Z.; Drienovská, I.; Roelfes, G. Unlocking Iminium Catalysis in Artificial Enzymes to Create a Friedel-Crafts Alkylase. *ACS Catal.* **2021**, *11* (12), 6763–6770. https://doi.org/10.1021/acscatal.1c00996.

(48) Leveson-Gower, R. B.; de Boer, R. M.; Roelfes, G. Tandem Friedel-Crafts-Alkylation-Enantioselective-Protonation by Artificial Enzyme Iminium Catalysis. *ChemCatChem* **2022**, *202101875*, 1–7. https://doi.org/10.1002/cctc.202101875.

(49) Ugo, R.; La Monica, G.; Cenini, S.; Bonati, F. Rhodium(I) and Iridium(I) Carbonyl Derivatives with Anionic Chelating Ligands. *J. Organomet. Chem.* **1968**, *11*, 159–166. https://doi.org/https://doi.org/10.1016/0022-328X(68)80031-2.

(50) Kakiuchi, F.; Takano, S.; Kochi, T. Catalytic Reactions of Terminal Alkynes Using Rhodium(I) Complexes Bearing 8-Quinolinolate Ligands. *ACS Catal.* **2018**, *8* (7), 6127–6137. https://doi.org/10.1021/acscatal.8b01286.

(51) Lee, H. S.; Spraggon, G.; Schultz, P. G.; Wang, F. Genetic Incorporation of a Metal-Ion Chelating Amino Acid into Proteins as a Biophysical Probe. *J. Am. Chem. Soc.* **2009**, *131* (7), 2481–2483. https://doi.org/10.1021/ja808340b.

(52) Drienovská, I.; Scheele, R. A.; Gutiérrez de Souza, C.; Roelfes, G. A Hydroxyquinoline-Based Unnatural Amino Acid for the Design of Novel Artificial Metalloenzymes. *ChemBioChem* **2020**, *21* (21), 3077–3081. https://doi.org/10.1002/cbic.202000306.

(53) Bos, J.; Browne, W. R.; Driessen, A. J. M.; Roelfes, G. Supramolecular Assembly of Artificial Metalloenzymes Based on the Dimeric Protein LmrR as Promiscuous Scaffold. *J. Am. Chem. Soc.* **2015**, *137* (31), 9796–9799. https://doi.org/10.1021/jacs.5b05790.

(54) James, L. C.; Tawfik, D. S. Conformational Diversity and Protein Evolution – a 60-Year-Old Hypothesis Revisited. *Trends Biochem. Sci.* **2003**, *28* (7), 361–368. https://doi.org/10.1016/S0968-0004(03)00135-X.

(55) Leveson-Gower, R. B.; Mayer, C.; Roelfes, G. The Importance of Catalytic Promiscuity for Enzyme Design and Evolution. *Nat. Rev. Chem.* **2019**, *3* (12), 687–705. https://doi.org/10.1038/s41570-019-0143-x.

(56) Nobuhiko, T.; S., T. D. Protein Dynamism and Evolvability. *Science (80-.).* **2009**, *324* (5924), 203–207. https://doi.org/10.1126/science.1169375.

(57) Villarino, L.; Chordia, S.; Alonso-Cotchico, L.; Reddem, E.; Zhou, Z.; Thunnissen, A. M. W. H.; Maréchal, J. D.; Roelfes, G. Cofactor Binding Dynamics Influence the Catalytic Activity and Selectivity of an Artificial Metalloenzyme. *ACS Catal.* **2020**, *10* (20), 11783–11790. https://doi.org/10.1021/acscatal.0c01619.

(58) Boersma, A. J.; Klijn, J. E.; Feringa, B. L.; Roelfes, G. DNA-Based Asymmetric Catalysis: Sequence-Dependent Rate Acceleration and Enantioselectivity. *J. Am. Chem. Soc.* **2008**, *130* (35), 11783–11790. https://doi.org/10.1021/ja803170m.

(59) Gutiérrez de Souza, C.; Bersellini, M.; Roelfes, G. Artificial Metalloenzymes Based on TetR Proteins and Cu(II) for Enantioselective Friedel-Crafts Alkylation Reactions. *ChemCatChem* **2020**, *12* (12), 3190–3194. https://doi.org/10.1002/cctc.202000245.

(60) Gutiérrez de Souza, C.; Alonso-Cotchico, L.; Bersellini, M.; Roelfes, G. Unexpected Catalytic Activity of the Regulatory Protein QacR. *ChemRxiv 2020*. https://doi.org/10.26434/chemrxiv.12662003.v1.

(61) Chordia, S.; Narasimhan, S.; Lucini Paioni, A.; Baldus, M.; Roelfes, G. In Vivo Assembly of Artificial Metalloenzymes and Application in Whole-Cell Biocatalysis**. *Angew. Chemie - Int. Ed.* **2021**, *60* (11), 5913–5920. https://doi.org/10.1002/anie.202014771.

(62) Fischer, J.; Renn, D.; Quitterer, F.; Radhakrishnan, A.; Liu, M.; Makki, A.; Ghorpade, S.; Rueping, M.; Arold, S. T.; Groll, M.; Eppinger, J. Robust and Versatile Host Protein for the Design and Evaluation of Artificial Metal Centers. *ACS Catal.* **2019**, *9* (12), 11371–11380. https://doi.org/10.1021/acscatal.9b02896.

(63) Purta, E.; O'Connor, M.; Bujnicki, J. M.; Douthwaite, S. YccW Is the M5C Methyltransferase Specific for 23S RRNA Nucleotide 1962. *J. Mol. Biol.* **2008**, *383* (3), 641–651. https://doi.org/10.1016/j.jmb.2008.08.061.

(64) Schubert, H. L.; Blumenthal, R. M.; Cheng, X. Many Paths to Methyltransfer: A Chronicle of Convergence. *Trends Biochem. Sci.* **2003**, *28* (6), 329–335. https://doi.org/10.1016/S0968-0004(03)00090-2.

(65) Chouhan, B. P. S.; Maimaiti, S.; Gade, M.; Laurino, P. Rossmann-Fold Methyltransferases: Taking a "β-Turn" around Their Cofactor, S-Adenosylmethionine. *Biochemistry* **2019**, *58* (3), 166–170. https://doi.org/10.1021/acs.biochem.8b00994.

(66) Pacholec, M.; Tao, J.; Walsh, C. T. CouO and NovO: C-Methyltransferases for Tailoring the Aminocoumarin Scaffold in Coumermycin and Novobiocin Antibiotic Biosynthesis. *Biochemistry* **2005**, *44* (45), 14969–14976.

https://doi.org/10.1021/bi051599o.

(67) Tengg, M.; Stecher, H.; Remler, P.; Eiteljörg, I.; Schwab, H.; Gruber-Khadjawi, M. Molecular Characterization of the C-Methyltransferase NovO of Streptomyces Spheroides, a Valuable Enzyme for Performing Friedel-Crafts Alkylation. In *Journal of Molecular Catalysis B: Enzymatic*; 2012; Vol. 84, pp 2–8. https://doi.org/10.1016/j.molcatb.2012.03.016.

(68) Pavkov-Keller, T.; Steiner, K.; Faber, M.; Tengg, M.; Schwab, H.; Gruber-Khadjawi, M.; Gruber, K. Crystal Structure and Catalytic Mechanism of Couo, a Versatile C-Methyltransferase from Streptomyces Rishiriensis. *PLoS One* **2017**, *12* (2), e0171056. https://doi.org/10.1371/journal.pone.0171056.

(69) Stecher, H.; Tengg, M.; Ueberbacher, B. J.; Remler, P.; Schwab, H.; Griengl, H.; Gruber-Khadjawi, M. Biocatalytic Friedel-Crafts Alkylation Using Non-Natural Cofactors. *Angew. Chemie - Int. Ed.* **2009**, *48* (50), 9546–9548. https://doi.org/10.1002/anie.200905095.

(70) Tengg, M.; Stecher, H.; Offner, L.; Plasch, K.; Anderl, F.; Weber, H.; Schwab, H.; Gruber-Khadjawi, M. Methyltransferases: Green Catalysts for Friedel-Crafts Alkylations. *ChemCatChem* **2016**, *8* (7), 1354–1360. https://doi.org/10.1002/cctc.201501306.

(71) Sadler, J. C.; Humphreys, L. D.; Snajdrova, R.; Burley, G. A. A Tandem Enzymatic Sp2-C-Methylation Process: Coupling in Situ S-Adenosyl-l-Methionine Formation with Methyl Transfer. *ChemBioChem* **2017**, *18* (11), 992–995. https://doi.org/10.1002/cbic.201700115.

(72) Singh, S.; Zhang, J.; Huber, T. D.; Sunkara, M.; Hurley, K.; Goff, R. D.; Wang, G.; Zhang, W.; Liu, C.; Rohr, J.; Van Lanen, S. G.; Morris, A. J.; Thorson, J. S. Facile Chemoenzymatic Strategies for the Synthesis and Utilization of S-Adenosyl-L-Methionine Analogues. *Angew. Chemie Int. Ed.* **2014**, *53* (15), 3965–3969. https://doi.org/https://doi.org/10.1002/anie.201308272.

(73) Eustáquio, A. S.; Pojer, F.; Noel, J. P.; Moore, B. S. Discovery and Characterization of a Marine Bacterial SAM-Dependent Chlorinase. *Nat. Chem. Biol.* **2008**, *4* (1), 69–74. https://doi.org/10.1038/nchembio.2007.56.

(74) Paoletti, L.; Lu, Y. J.; Schujman, G. E.; De Mendoza, D.; Rock, C. O. Coupling of Fatty Acid and Phospholipid Synthesis in Bacillus Subtilis. *J. Bacteriol.* **2007**, *189* (16), 5816–5824. https://doi.org/10.1128/JB.00602-07.

(75) Kalscheuer, R.; Steinbüchel, A. A Novel Bifunctional Wax Ester Synthase/Acyl-CoA:Diacylglycerol Acyltransferase Mediates Wax Ester and Triacylglycerol Biosynthesis in Acinetobacter Calcoaceticus ADP1. *J. Biol. Chem.* **2003**, *278* (10), 8075–8082. https://doi.org/10.1074/jbc.M210533200.

(76) Cheng, Y. Q.; Tang, G. L.; Shen, B. Type I Polyketide Synthase Requiring a Discrete Acyltransferase for Polyketide Biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100* (6), 3149–3154. https://doi.org/10.1073/pnas.0537286100.

(77) Bera, A.; Herbert, S.; Jakob, A.; Vollmer, W.; Götz, F. Why Are Pathogenic Staphylococci so Lysozyme Resistant? The Peptidoglycan O-Acetyltransferase OatA Is the Major Determinant for Lysozyme Resistance of Staphylococcus Aureus. *Mol. Microbiol.* **2005**, *55* (3), 778–787. https://doi.org/10.1111/j.1365-2958.2004.04446.x.

(78) Hunt, M. C.; Siponen, M. I.; Alexson, S. E. H. The Emerging Role of Acyl-CoA Thioesterases and Acyltransferases in Regulating Peroxisomal Lipid Metabolism. *Biochim. Biophys. Acta - Mol. Basis Dis.* **2012**, *1822* (9), 1397–1410. https://doi.org/10.1016/j.bbadis.2012.03.009.

(79) Favrot, L.; Blanchard, J. S.; Vergnolle, O. Bacterial GCN5-Related N-Acetyltransferases: From Resistance to Regulation. *Biochemistry* **2016**, *55* (7), 989–1002. https://doi.org/10.1021/acs.biochem.5b01269.

(80) Partridge, S. R.; Hall, R. M. Correctly Identifying the Streptothricin Resistance Gene Cassette. *J. Clin. Microbiol.* **2005**, *43* (8), 4298–4300. https://doi.org/10.1128/JCM.43.8.4298-4300.2005.

(81) Stalker, D. M.; McBride, K. E.; Malyj, L. D. Herbicide Resistance in Transgenic Plants Expressing a Bacterial Detoxification Gene. *Science (80-.).* **1988**, *242* (4877), 419–423. https://doi.org/10.1126/science.242.4877.419.

(82) Schmidt, N. G.; Pavkov-Keller, T.; Richter, N.; Wiltschi, B.; Gruber, K.; Kroutil, W. Biocatalytic Friedel-Crafts Acylation and Fries Reaction. *Angew. Chemie Int. Ed.* **2017**, *56* (26), 7615–7619. https://doi.org/10.1002/anie.201703270.

(83) Pavkov-Keller, T.; Schmidt, N. G.; Żądło-Dobrowolska, A.; Kroutil, W.; Gruber, K. Structure and Catalytic Mechanism of a Bacterial Friedel–Crafts Acylase. *ChemBioChem* **2019**, *20* (1), 88–95. https://doi.org/10.1002/cbic.201800462.

(84) Hayashi, A.; Saitou, H.; Mori, T.; Matano, I.; Sugisaki, H.; Maruyama, K. Molecular and Catalytic Properties of Monoacetylphloroglucinol Acetyltransferase from Pseudomonas Sp. YGJ3. *Biosci. Biotechnol. Biochem.* **2012**, *76* (3), 559–566.

https://doi.org/10.1271/bbb.110860.

(85) Yang, F.; Cao, Y. Biosynthesis of Phloroglucinol Compounds in Microorganisms - Review. *Appl. Microbiol. Biotechnol.* **2012**, *93* (2), 487–495. https://doi.org/10.1007/s00253-011-3712-6.

(86) Schmidt, N. G.; Żądło-Dobrowolska, A.; Ruppert, V.; Höflehner, C.; Wiltschi, B.; Kroutil, W. Molecular Cloning, Expression, and Characterization of Acyltransferase from Pseudomonas Protegens. *Appl. Microbiol. Biotechnol.* **2018**, *102* (14), 6057–6068. https://doi.org/10.1007/s00253-018-9052-z.

(87) Schmidt, N. G.; Kroutil, W. Acyl Donors and Additives for the Biocatalytic Friedel–Crafts Acylation. *European J. Org. Chem.* **2017**, *2017* (39), 5865–5871. https://doi.org/10.1002/ejoc.201701079.

(88) Żądło-Dobrowolska, A.; Schmidt, N. G.; Kroutil, W. Thioesters as Acyl Donors in Biocatalytic Friedel-Crafts-Type Acylation Catalyzed by Acyltransferase from Pseudomonas Protegens. *ChemCatChem* **2019**, *11* (3), 1064–1068. https://doi.org/10.1002/cctc.201801856.

(89) Żądło-Dobrowolska, A.; Hammerer, L.; Pavkov-Keller, T.; Gruber, K.; Kroutil, W. Rational Engineered C-Acyltransferase Transforms Sterically Demanding Acyl Donors. *ACS Catal.* **2020**, *10* (2), 1094–1101. https://doi.org/10.1021/acscatal.9b04617.

(90) Nakamura, H.; Schultz, E. E.; Balskus, E. P. A New Strategy for Aromatic Ring Alkylation in Cylindrocyclophane Biosynthesis. *Nat. Chem. Biol.* **2017**, *13* (8), 916–921. https://doi.org/10.1038/nchembio.2421.

(91) Nakamura, H.; Hamer, H. A.; Sirasani, G.; Balskus, E. P. Cylindrocyclophane Biosynthesis Involves Functionalization of an Unactivated Carbon Center. *J. Am. Chem. Soc.* **2012**, *134* (45), 18518–18521. https://doi.org/10.1021/ja308318p.

(92) Schultz, E. E.; Braffman, N. R.; Luescher, M. U.; Hager, H. H.; Balskus, E. P. Biocatalytic Friedel–Crafts Alkylation Using a Promiscuous Biosynthetic Enzyme. *Angew. Chemie - Int. Ed.* **2019**, *58* (10), 3151–3155. https://doi.org/10.1002/anie.201814016.

(93) Lu, S. T.; Kelly, K. A.; Feldman, S. R. An Overview of Benvitimod for the Treatment of Psoriasis: A Narrative Review. *Expert Opin. Pharmacother.* **2022**, *23* (3), 327–333. https://doi.org/10.1080/14656566.2021.2016699.

(94) Wang, H.-Q.; Mou, S.-B.; Xiao, W.; Zhou, H.; Hou, X.-D.; Wang, S.-J.; Wang, Q.;

Gao, J.; Wei, Z.; Liu, L.; Xiang, Z. Structural Basis for the Friedel–Crafts Alkylation in Cylindrocyclophane Biosynthesis. *ACS Catal.* **2022**, *12* (3), 2108–2117. https://doi.org/10.1021/acscatal.1c04816.

(95) Appel, W. Chymotrypsin: Molecular and Catalytic Properties. *Clin. Biochem.* **1986**, *19* (6), 317–322. https://doi.org/https://doi.org/10.1016/S0009-9120(86)80002-9.

(96) Fersht, A. R.; Requena, Y. Mechanism of the .Alpha.-Chymotrypsin-Catalyzed Hydrolysis of Amides. PH Dependence of Kc and Km. Kinetic Detection of an Intermediate. *J. Am. Chem. Soc.* **1971**, *93* (25), 7079–7087. https://doi.org/10.1021/ja00754a066.

(97) Birktoft, J. J.; Blow, D. M.; Henderson, R.; Steitz, T. A. I. Serine Proteinases. The Structure of Alpha-Chymotrypsin. *Philos. Trans. R. Soc. London. Ser. B Biol. Sci.* **1970**, *257* (813), 67–76. https://doi.org/10.1098/rstb.1970.0009.

(98) Xue, J. W.; Guo, J. T.; He, Y. H.; Guan, Z. Solvent-Controlled Enzyme-Catalyzed Friedel–Crafts Reactions of Indoles and Isatins by Using α-Chymotrypsin. *Asian J. Org. Chem.* **2017**, *6* (3), 297–304. https://doi.org/10.1002/ajoc.201600526.

(99) Natarajan, A.; Fan, Y.-H.; Chen, H.; Guo, Y.; Iyasere, J.; Harbinski, F.; Christ, W. J.; Aktas, H.; Halperin, J. A. 3,3-Diaryl-1,3-Dihydroindol-2-Ones as Antiproliferatives Mediated by Translation Initiation Inhibition. *J. Med. Chem.* **2004**, *47* (8), 1882–1885. https://doi.org/10.1021/jm0499716.

(100) Tipton, K. F.; Boyce, S.; O'Sullivan, J.; Davey, G. P.; Healy, J. Monoamine Oxidases: Certainties and Uncertainties. *Curr. Med. Chem.* **2012**, *11* (15), 1965–1982. https://doi.org/10.2174/0929867043364810.

(101) Turner, N. J. Enantioselective Oxidation of C-O and C-N Bonds Using Oxidases. *Chem. Rev.* **2011**, *111* (7), 4073–4087. https://doi.org/10.1021/cr200111v.

(102) Schilling, B.; Lerch, K. Amine Oxidases from Aspergillus Niger : Identification of a Novel Flavin-Dependent Enzyme. *Biochim. Biophys. Acta - Gen. Subj.* **1995**, *1243* (3), 529–537. https://doi.org/https://doi.org/10.1016/0304-4165(94)00183-X.

(103) Sablin, S. O.; Yankovskaya, V.; Bernard, S.; Cronin, C. N.; Singer, T. P. Isolation and Characterization of an Evolutionary Precursor of Human Monoamine Oxidases A and B. *Eur. J. Biochem.* **1998**, *253* (1), 270–279. https://doi.org/10.1046/j.1432-1327.1998.2530270.x.

(104) Alexeeva, M.; Enright, A.; Dawson, M. J.; Mahmoudian, M.; Turner, N. J. Deracemization of α-Methylbenzylamine Using an Enzyme Obtained by in Vitro

Evolution. *Angew. Chemie - Int. Ed.* **2002**, *41* (17), 3177–3180. https://doi.org/10.1002/1521-3773(20020902)41:17<3177::AID-ANIE3177>3.0.CO;2-P.

(105) Dong, J. J.; Fernández-Fueyo, E.; Hollmann, F.; Paul, C. E.; Pesic, M.; Schmidt, S.; Wang, Y.; Younes, S.; Zhang, W. Biocatalytic Oxidation Reactions: A Chemist's Perspective. *Angew. Chemie - Int. Ed.* **2018**, *57* (30), 9238–9261. https://doi.org/10.1002/anie.201800343.

(106) Atkin, K. E.; Reiss, R.; Koehler, V.; Bailey, K. R.; Hart, S.; Turkenburg, J. P.; Turner, N. J.; Brzozowski, A. M.; Grogan, G. The Structure of Monoamine Oxidase from Aspergillus Niger Provides a Molecular Context for Improvements in Activity Obtained by Directed Evolution. *J. Mol. Biol.* **2008**, *384* (5), 1218–1231. https://doi.org/10.1016/j.jmb.2008.09.090.

(107) de Graaff, C.; Oppelaar, B.; Péruch, O.; Vande Velde, C. M. L.; Bechi, B.; Turner, N. J.; Ruijter, E.; Orru, R. V. A. Stereoselective Monoamine Oxidase-Catalyzed Oxidative Aza-Friedel–Crafts Reactions of Meso-Pyrrolidines in Aqueous Buffer. *Adv. Synth. Catal.* **2016**, *358* (10), 1555–1560. https://doi.org/10.1002/adsc.201600038.

(108) Roelfes, G.; Feringa, B. L. DNA-Based Asymmetric Catalysis. *Angew. Chemie - Int. Ed.* **2005**, *44* (21), 3230–3232. https://doi.org/10.1002/anie.200500298.

(109) Boersma, A. J.; Megens, R. P.; Feringa, B. L.; Roelfes, G. DNA-Based Asymmetric Catalysis. *Chem. Soc. Rev.* **2010**, *39* (6), 2083–2092. https://doi.org/10.1039/b811349c.

(110) Boersma, A. J.; Feringa, B. L.; Roelfes, G. Enantioselective Friedel-Crafts Reactions in Water Using a DNA Based Catalyst. *Angew. Chemie - Int. Ed.* **2009**, *48* (18), 3346–3348. https://doi.org/10.1002/anie.200900371.

(111) Dijk, E. W.; Boersma, A. J.; Feringa, B. L.; Roelfes, G. On the Role of DNA in DNA-Based Catalytic Enantioselective Conjugate Addition Reactions. *Org. Biomol. Chem.* **2010**, *8* (17), 3868–3873. https://doi.org/10.1039/c005048b.

(112) García-Fernández, A.; Megens, R. P.; Villarino, L.; Roelfes, G. DNA-Accelerated Copper Catalysis of Friedel-Crafts Conjugate Addition/Enantioselective Protonation Reactions in Water. *J. Am. Chem. Soc.* **2016**, *138* (50), 16308–16314. https://doi.org/10.1021/jacs.6b08295.

(113) Draksharapu, A.; Boersma, A. J.; Leising, M.; Meetsma, A.; Browne, W. R.; Roelfes, G. Binding of Copper(Ii) Polypyridyl Complexes to DNA and Consequences for

DNA-Based Asymmetric Catalysis. *Dalt. Trans.* **2015**, *44* (8), 3647–3655. https://doi.org/10.1039/C4DT02733G.

(114) Draksharapu, A.; Boersma, A. J.; Browne, W. R.; Roelfes, G. Characterisation of the Interactions between Substrate, Copper(Ii) Complex and DNA and Their Role in Rate Acceleration in DNA-Based Asymmetric Catalysis. *Dalt. Trans.* **2015**, *44* (8), 3656–3663. https://doi.org/10.1039/C4DT02734E.

(115) Gjonaj, L.; Roelfes, G. Novel Catalyst Design by Using Cisplatin to Covalently Anchor Catalytically Active Copper Complexes to DNA. *ChemCatChem* **2013**, *5* (7), 1718–1721. https://doi.org/10.1002/cctc.201300082.

(116) Bai, J. K.; Zhou, H.; Sun, X. L.; Chen, D.; Li, C.; Qiao, R. Z. Insight into Stereo-Induction by Minor Modification in the Ligand in DNA-Based Hybrid Catalysis. *Catal. Letters* **2018**, *148* (11), 3315–3324. https://doi.org/10.1007/s10562-018-2549-3.

(117) Park, S.; Sugiyama, H. DNA-Based Hybrid Catalysts for Asymmetric Organic Synthesis. *Angew. Chemie - Int. Ed.* **2010**, *49* (23), 3870–3878. https://doi.org/10.1002/anie.200905382.

(118) Park, S.; Ikehata, K.; Watabe, R.; Hidaka, Y.; Rajendran, A.; Sugiyama, H. Deciphering DNA-Based Asymmetric Catalysis through Intramolecular Friedel–Crafts Alkylations. *Chem. Commun.* **2012**, *48* (84), 10398–10400. https://doi.org/10.1039/c2cc35625b.

(119) Park, S.; Zheng, L.; Kumakiri, S.; Sakashita, S.; Otomo, H.; Ikehata, K.; Sugiyama, H. Development of DNA-Based Hybrid Catalysts through Direct Ligand Incorporation: Toward Understanding of DNA-Based Asymmetric Catalysis. *ACS Catal.* **2014**, *4* (11), 4070–4073. https://doi.org/10.1021/cs501086f.

(120) Kalek, M.; Madsen, A. S.; Wengel, J. Effective Modulation of DNA Duplex Stability by Reversible Transition Metal Complex Formation in the Minor Groove. *J. Am. Chem. Soc.* **2007**, *129* (30), 9392–9400. https://doi.org/10.1021/ja071076z.

(121) Matyasovsky, J.; Perlikova, P.; Malnuit, V.; Pohl, R.; Hocek, M. 2-Substituted DATP Derivatives as Building Blocks for Polymerase-Catalyzed Synthesis of DNA Modified in the Minor Groove. *Angew. Chemie - Int. Ed.* **2016**, *55* (51), 15856–15859. https://doi.org/10.1002/anie.201609007.

(122) Mansot, J.; Aubert, S.; Duchemin, N.; Vasseur, J. J.; Arseniyadis, S.; Smietana, M. A Rational Quest for Selectivity through Precise Ligand-Positioning in Tandem DNA-Catalysed Friedel-Crafts Alkylation/Asymmetric Protonation. *Chem. Sci.* **2019**, *10*

(10), 2875–2881. https://doi.org/10.1039/c8sc05543b.

(123) Roe, S.; Ritson, D. J.; Garner, T.; Searle, M.; Moses, J. E. Tuneable DNA-Based Asymmetric Catalysis Using a G-Quadruplex Supramolecular Assembly. *Chem. Commun.* **2010**, *46* (24), 4309–4311. https://doi.org/10.1039/C0CC00194E.

(124) Wang, C.; Li, Y.; Jia, G.; Liu, Y.; Lu, S.; Li, C. Enantioselective Friedel-Crafts Reactions in Water Catalyzed by a Human Telomeric G-Quadruplex DNA Metalloenzyme. *Chem. Commun.* **2012**, *48* (50), 6232–6234. https://doi.org/10.1039/c2cc31320k.

(125) Lim, K. W.; Amrane, S.; Bouaziz, S.; Xu, W.; Mu, Y.; Patel, D. J.; Luu, K. N.; Phan, A. T. Structure of the Human Telomere in K + Solution: A Stable Basket-Type G-Quadruplex with Only Two G-Tetrad Layers. *J. Am. Chem. Soc.* **2009**, *131* (12), 4301–4309. https://doi.org/10.1021/ja807503g.

(126) Burge, S.; Parkinson, G. N.; Hazel, P.; Todd, A. K.; Neidle, S. Quadruplex DNA: Sequence, Topology and Structure. *Nucleic Acids Res.* **2006**, *34* (19), 5402–5415. https://doi.org/10.1093/nar/gkl655.

(127) Dey, S.; Jäschke, A. Tuning the Stereoselectivity of a DNA-Catalyzed Michael Addition through Covalent Modification. *Angew. Chemie - Int. Ed.* **2015**, *54* (38), 11279–11282. https://doi.org/10.1002/anie.201503838.

(128) Dey, S.; Rühl, C. L.; Jäschke, A. Catalysis of Michael Additions by Covalently Modified G-Quadruplex DNA. *Chem. - A Eur. J.* **2017**, *23* (50), 12162–12170. https://doi.org/10.1002/chem.201700632.

(129) Dey, S.; Jäschke, A. Covalently Functionalized DNA Duplexes and Quadruplexes as Hybrid Catalysts in an Enantioselective Friedel-Crafts Reaction. *Molecules* **2020**, *25* (14). https://doi.org/10.3390/molecules25143121.

(130) Wang, J.; Benedetti, E.; Bethge, L.; Vonhoff, S.; Klussmann, S.; Vasseur, J. J.; Cossy, J.; Smietana, M.; Arseniyadis, S. DNA vs. Mirror-Image DNA: A Universal Approach to Tune the Absolute Configuration in DNA-Based Asymmetric Catalysis. *Angew. Chemie - Int. Ed.* **2013**, *52* (44), 11546–11549. https://doi.org/10.1002/anie.201306232.

(131) Benedetti, E.; Duchemin, N.; Bethge, L.; Vonhoff, S.; Klussmann, S.; Vasseur, J. J.; Cossy, J.; Smietana, M.; Arseniyadis, S. DNA-Cellulose: An Economical, Fully Recyclable and Highly Effective Chiral Biomaterial for Asymmetric Catalysis. *Chem. Commun.* **2015**, *51* (28), 6076–6079. https://doi.org/10.1039/c4cc10190a.

(132) Wegner, J.; Ceylan, S.; Kirschning, A. Flow Chemistry – A Key Enabling Technology for (Multistep) Organic Synthesis. *Adv. Synth. Catal.* **2012**, *354* (1), 17–57. https://doi.org/https://doi.org/10.1002/adsc.201100584.

(133) Amirbekyan, K.; Duchemin, N.; Benedetti, E.; Joseph, R.; Colon, A.; Markarian, S. A.; Bethge, L.; Vonhoff, S.; Klussmann, S.; Cossy, J.; Vasseur, J. J.; Arseniyadis, S.; Smietana, M. Design, Synthesis, and Binding Affinity Evaluation of Hoechst 33258 Derivatives for the Development of Sequence-Specific DNA-Based Asymmetric Catalysts. *ACS Catal.* **2016**, *6* (5), 3096–3105. https://doi.org/10.1021/acscatal.6b00495.

(134) Loontiens, F. G.; Regenfuss, P.; Zechel, A.; Dumortier, L.; Clegg, R. M. Binding Characteristics of Hoechst 33258 with Calf Thymus DNA, Poly[d(A-T)] and d(CCGGAATTCCGG): Multiple Stoichiometries and Determination of Tight Binding with a Wide Spectrum of Site Affinities. *Biochemistry* **1990**, *29* (38), 9029–9039. https://doi.org/10.1021/bi00490a021.

(135) Dervan, P. B. Design of Sequence-Specific DNA-Binding Molecules. *Science (80-.).* **1986**, *232* (4749), 464–471. https://doi.org/10.1126/science.2421408.

(136) Tarasow, T. M.; Tarasow, S. L.; Eaton, B. E. RNA-Catalysed Carbon–Carbon Bond Formation. *Nature* **1997**, *389* (6646), 54–57. https://doi.org/10.1038/37950.

(137) Sengle, G.; Eisenführ, A.; Arora, P. S.; Nowick, J. S.; Famulok, M. Novel RNA Catalysts for the Michael Reaction. *Chem. Biol.* **2001**, *8* (5), 459–473. https://doi.org/https://doi.org/10.1016/S1074-5521(01)00026-6.

(138) Fusz, S.; Eisenführ, A.; Srivatsan, S. G.; Heckel, A.; Famulok, M. A Ribozyme for the Aldol Reaction. *Chem. Biol.* **2005**, *12* (8), 941–950. https://doi.org/https://doi.org/10.1016/j.chembiol.2005.06.008.

(139) Duchemin, N.; Benedetti, E.; Bethge, L.; Vonhoff, S.; Klussmann, S.; Vasseur, J.-J.; Cossy, J.; Smietana, M.; Arseniyadis, S. Expanding Biohybrid-Mediated Asymmetric Catalysis into the Realm of RNA. *Chem. Commun.* **2016**, *52* (55), 8604–8607. https://doi.org/10.1039/C6CC03540J.

(140) Silverman, S. K. Catalytic DNA: Scope, Applications, and Biochemistry of Deoxyribozymes. *Trends Biochem. Sci.* **2016**, *41* (7), 595–609. https://doi.org/10.1016/j.tibs.2016.04.010.

(141) Silverman, S. K. DNA as a Versatile Chemical Component for Catalysis, Encoding, and Stereocontrol. *Angew. Chemie - Int. Ed.* **2010**, *49* (40), 7180–7201.

https://doi.org/10.1002/anie.200906345.

(142) Mohan, U.; Burai, R.; McNaughton, B. R. In Vitro Evolution of a Friedel-Crafts Deoxyribozyme. *Org. Biomol. Chem.* **2013**, *11* (14), 2241–2244. https://doi.org/10.1039/c3ob40080h.

(143) Henche, S.; Nestl, B. M.; Hauer, B. Enzymatic Friedel-Crafts Alkylation Using Squalene-Hopene Cyclases. *ChemCatChem* **2021**, *13* (15), 3405–3409. https://doi.org/10.1002/cctc.202100452.

(144) Boville, C. E.; Romney, D. K.; Almhjell, P. J.; Sieben, M.; Arnold, F. H. Improved Synthesis of 4-Cyanotryptophan and Other Tryptophan Analogues in Aqueous Solvent Using Variants of TrpB from Thermotoga Maritima. *J. Org. Chem.* **2018**, *83* (14), 7447–7452. https://doi.org/10.1021/acs.joc.8b00517.

(145) Boville, C. E.; Scheele, R. A.; Koch, P.; Brinkmann-Chen, S.; Buller, A. R.; Arnold, F. H. Engineered Biosynthesis of β-Alkyl Tryptophan Analogues. *Angew. Chemie - Int. Ed.* **2018**, *57* (45), 14764–14768. https://doi.org/10.1002/anie.201807998.

Graphical Abstract