Catalyst design in oxidation chemistry; from $KMnO_4$ to Artificial metalloenzymes

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Abstract:
Oxidation reactions are an important part of the synthetic organic chemist's toolkit and continued advancements have, in many cases, resulted in high yields and selectivities. This review aims to give an overview of the current state-of-the-art in oxygenation reactions using both chemical and enzymatic processes, the design principles applied to date and a possible future in the direction of hybrid catalysts combining the best of chemical and natural design.

Oxidation reactions are an important part of the synthetic organic chemist's toolkit and have been known for over 160 years, with the oxidative properties of potassium permanganate reported in 1851.[1] By the mid-20th Century it had also been established that nature conducts many redox reactions, and we now know that many biological processes from cellular respiration to photosynthesis are centered on redox reactions.[2] Oxidation reactions such as the Sharpless epoxidation, Swern oxidation and ozonolysis enable chemists to perform a myriad of different transformations, and continued advancements have in many cases resulted in high yields and selectivities. This review aims to give an overview of the current state-of-the-art in oxygenation reactions using both chemical and enzymatic processes, the design principles applied to date, and a possible future in the direction of hybrid catalysts combining the best of chemical and natural design.

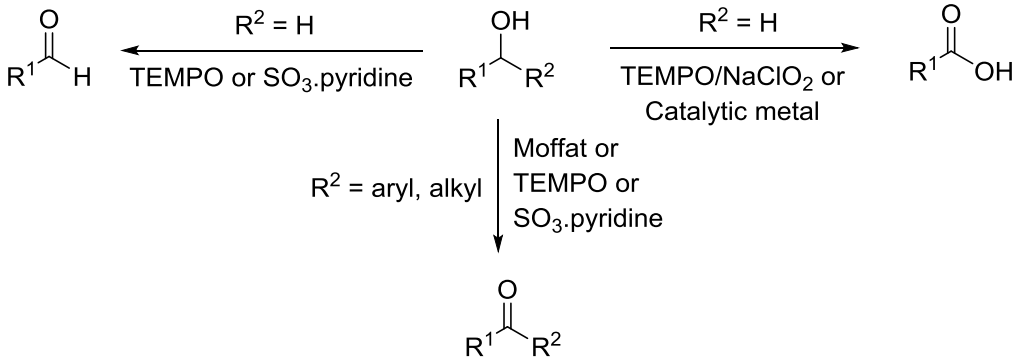
1. Chemical oxidation

One of the main challenges faced in the optimization of any type of reaction is controlling the selectivity. In oxidation chemistry, one quickly encounters the inherent difficulty in controlling the site-, chemo- and enantioselectivity. Frequently the initially formed oxidation products will be more susceptible to oxidation than the initial starting material,[3] which renders chemoselectivity challenging. Alkane oxidation is prone to over-oxidation at multiple positions, to the extent that catalytic experiments are frequently performed with 100 or even 1000 fold excess of the substrate to minimise over-oxidation to ketone, aldehyde and carboxylic acid side products.[4,5] Each of these problems is further complicated by the presence of other oxidation-prone functional groups, which is a particular challenge for functionalities lacking efficient protecting group strategies (alkenes), or in C-H oxidation where multiple oxidation sites exist for many substrates. Another challenge facing the chemical industries is the desire to make processes as 'green' and economical as possible. Many oxidations rely on oxidants and co-oxidants, which are not atom economical, such as hypervalent iodine reagents. Catalytic oxidation employing O_2 or H_2O_2 represents a great step forward, and exciting developments towards this have been reported.[6]

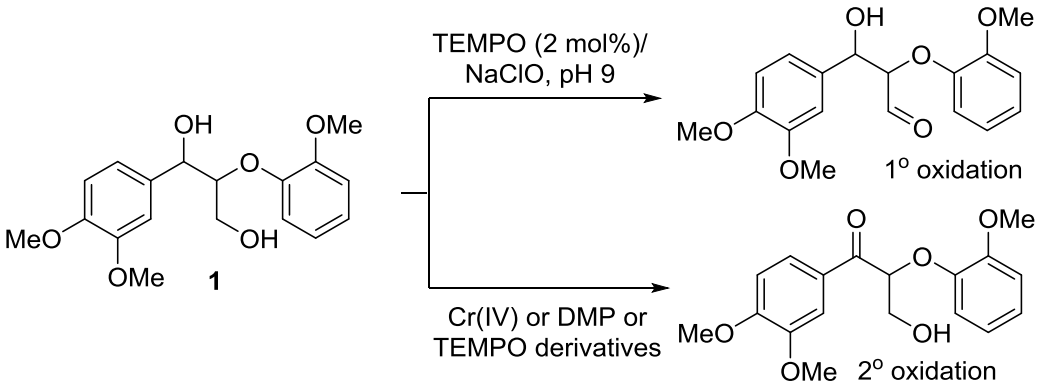
The most common substrates for oxidation, in either academic or industrial contexts, are primary and secondary alcohols,[7] sulfides,[8] alkenes (employed both for epoxidation and dihydroxylation)[9] and alkanes.[10] Each substrate carries its own particular challenges for selective oxidation and we will discuss the most promising methods currently available to overcome these issues.

1.1. Oxidation of alcohols

There are a whole plethora of methods available to oxidize alcohols to the corresponding ketone, aldehyde or acid.[11] The preferred oxidant in industry is TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy), followed by procedures involving sulfur compounds (Swern Oxidation, Moffat or SO_3.pyridine) or transition metal catalysis (*e.g.* Ley-Griffith oxidation[12]) (Scheme 1).[11] These methods allow the oxidation of primary alcohols to be stopped at the intermediate aldehyde product rather than progressing further to carboxylic acid formation. More challenging is the oxidation of primary alcohols in the presence of secondary alcohols. This is illustrated nicely by work towards the selective oxidation of lignin.[13] Lignin is a biopolymer that contains both primary and secondary alcohols and is an important potential renewable feedstock for aromatic chemicals.[14,15] A model compound of lignin (**1**) was tested with a variety of oxidants and it was found that the oxidation of the benzylic alcohol can be obtained selectively with a variety of oxidants, even when applied on lignin itself. However, bleach in the presence of catalytic TEMPO was the only system resulting in the selective oxidation of the primary alcohol in **1** (Scheme 2).

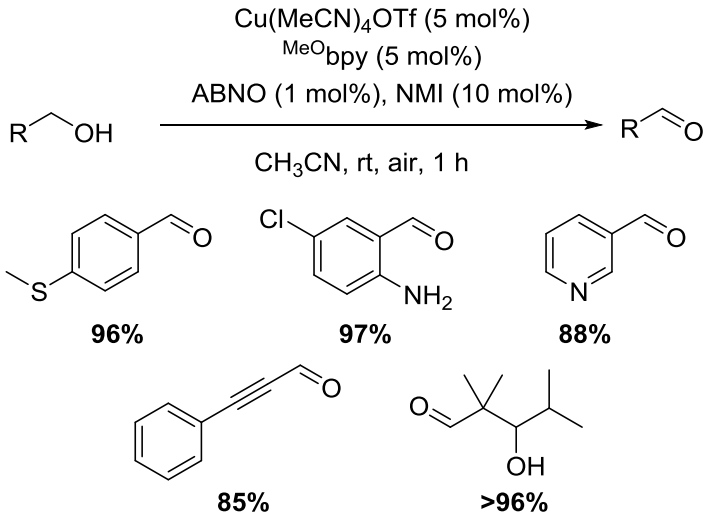


Scheme 1: The preferred choices for oxidation of primary and secondary alcohols.[11]



Scheme 2: Selective oxidation of a lignin model compound.[13]

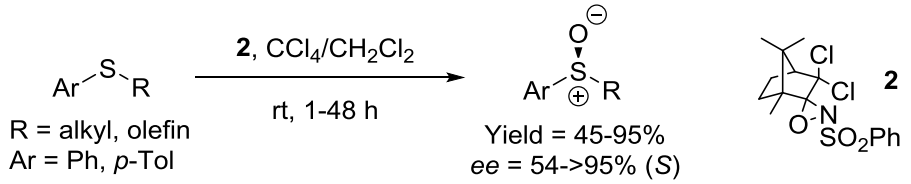
The quest to improve atom efficiency has led to work focusing on the use of oxygen to oxidize alcohols. The most successful approach to date has been the use of catalytic TEMPO/metal systems under an aerobic atmosphere.[7] The chemoselective oxidation of primary alcohols using Cu(I)/TEMPO or Cu(I)/ABNO (ABNO = 9-azabicyclo[3.3.1]nonane *N*-oxyl radical) catalyst systems has been reported.[16] This methodology tolerates other oxidizable functional groups such as alkenes, alkynes, heterocycles, thioethers, and aryl halides and can selectively oxidize diols (Scheme 3). A continuous-flow process provides full conversion of phenyl and alkynyl alcohols within 5 minutes with catalyst loadings as low as 0.25 mol% copper.[17] That this work has reached the level of process development on a research scale certainly reveals its promise for future large-scale application and the replacement of stoichiometric oxidants.



Scheme 3: Cu(I)/ABNO system for selective alcohol oxidation with wide functional group tolerance (selected examples) (NMI = *N*-methylimidazole, ^{MeO}Bpy = 4,4'-dimethoxy-2,2'-bipyridine).[16]

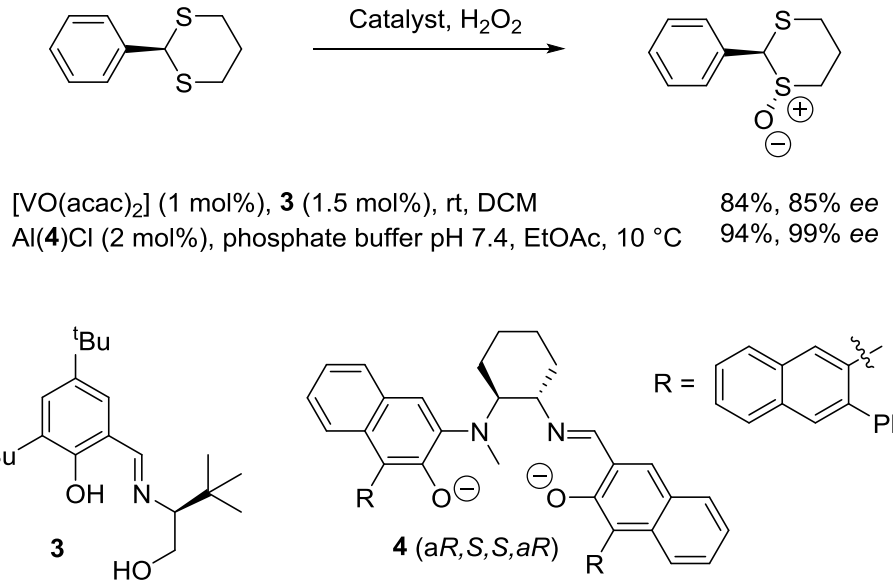
1.2. Asymmetric sulfoxidation

Chiral sulfoxides have become highly desired compounds over the last 25 years due to their renowned bioactivity and their potential as chiral ligands for transition metals.[8] Early methods of asymmetric sulfoxidation were based on the titanium catalysts used by Sharpless for epoxidation and were often limited to aryl methyl sulfides.[18,19] One exception to this was the use of **2,** the oxaziridine derivative of camphor, which gave good to excellent enantioselectivities for a range of aryl alkyl and aryl alkenyl sulfides (Scheme 4).[20]



Scheme 4: Organocatalytic sulfoxidation of sulfides using oxaziridine, 2.[20]

Ligand development has led to catalysts with broadened reaction scope.[21] Of particular note are the Schiff base complexes reported by the groups of Bolm and Katsuki. The vanadium complexes of Bolm are efficient catalysts for the oxidation of aryl alkyl sulfides (54-94% yield, up to 70% *ee*),[22] thioacetals (Scheme 5) and disulfides.[23] Katsuki has reported a variety of metal salen, salalen and salan complexes. Of these, the aluminium salalen complex Al(**4**)Cl has emerged as a state of the art reagent in the sulfoxidation of cyclic alkyl dithioacetals (Scheme 5).[24] The conformational flexibility of the substrate is important in determining the selectivity of the reaction: acyclic dithioacetals delivered very low selectivities and yields relative to the cyclic substrates. Furthermore, di-sulfoxidation was difficult to avoid for several substrates, leaving room for further optimisation and broadening of substrate scope. Dialkyl and sterically hindered sulfides remain a challenge with catalysts suffering from poor activity.[25]



Scheme 5: Vanadium and Al-salalen catalysts for highly selective oxidation of cyclic dithioacetals (acac = acetylacetonate).[22,24]

1.3. Olefin epoxidation and dihydroxylation

The use of epoxides and 1,2-diols in synthetic and industrial applications has led to the fast development of these transformations over the last 40 years. Classic reagents such as *meta*-chloroperoxybenzoic acid (*m*CPBA) and H_2O_2 are still often the regents of choice when there is no requirement for regio- or enantioselectivity. The pioneering work of Sharpless and Katsuki in the early 1980s on asymmetric epoxidation of allyl alcohols led to the development of titanium catalysts derived from Ti(O*i*-Pr)$_4$ and either (+) or (-) diethyl tartrate (DET).[26] This method is a gold standard in producing asymmetric epoxyalcohols using *tert*-butyl hydroperoxide, however it is limited to alkenes with directing groups such as alcohols. Subsequent work by Jacobsen and Katsuki developed Mn(III) salen catalysts for a more general epoxidation of *cis*-disubstituted and trisubstituented alkenes.[27,28] Meanwhile, Shi demonstrated the potential of organocatalysis in the epoxidation of terminal, *cis*- and *trans*-disubstituted, and trisubstituted alkenes (Figure 1).[29]

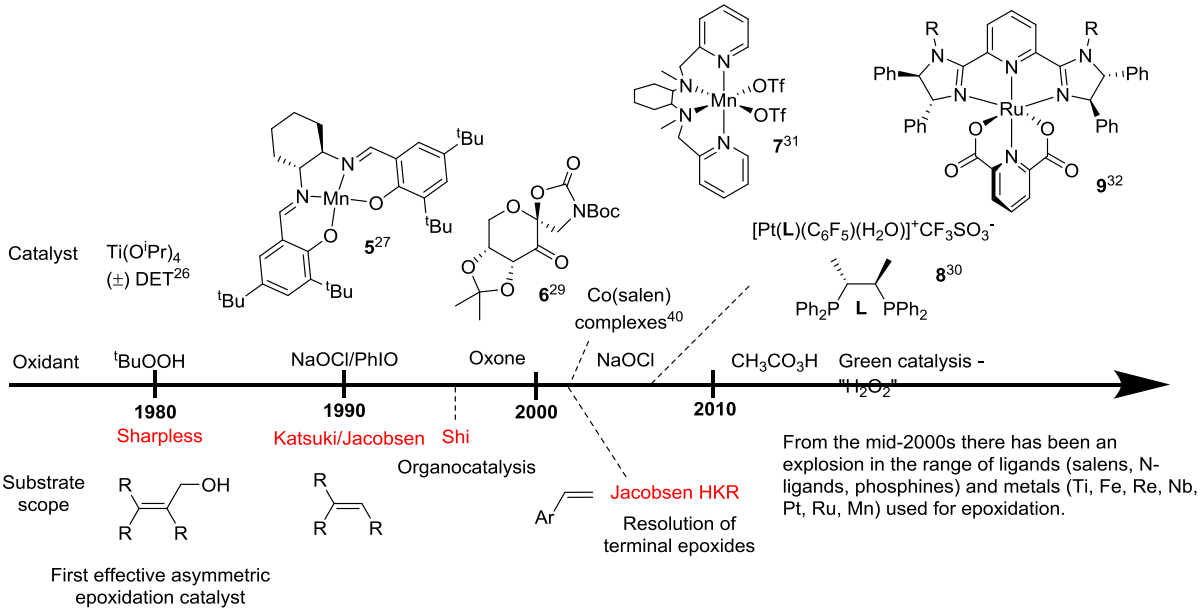
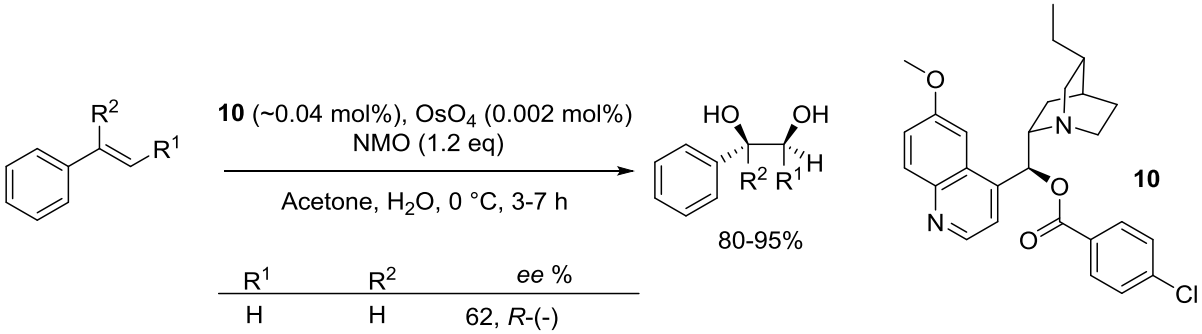


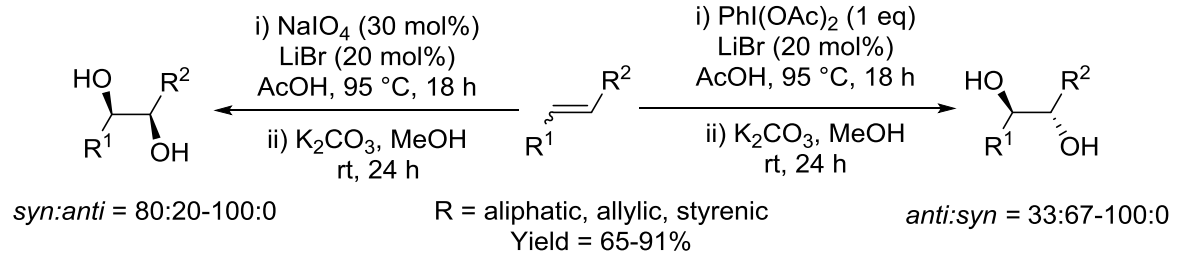
Figure 1: Timeline of discoveries in epoxidation reactions.[30,31,32]

The classic reagent for dihydroxylation is OsO_4, and the use of *N*-methylmorpholine *N*-oxide (NMO) by Upjohn has rendered this methodology catalytic in osmium.[33] The development of chiral cinchona alkaloid ligands, such as **10**, by Sharpless provided the first breakthrough in asymmetric dihydroxylation (Scheme 6).[34] Since then several modifications have been reported.[35] Despite the high cost and toxicity of OsO_4, catalytic osmium remains one of the most effective methods for carrying out this transformation. Although, osmium free-approaches have been developed (Scheme 7)[36], OsO_4 remains the method of choice in natural product synthesis.[37,38]



R^1	R^2	*ee* %
H	H	62, *R*-(-)
H	Me	33, *R*-(-)
Me	H	65, *R,R*-(-)
Ph	H	88, *R,R*-(+)
CH_2OAc	H	76, *R,R*-(+)

Scheme 6: *Cis*-dihydroxylation using Cinchona ligands.[34]



syn:anti = 80:20-100:0 R = aliphatic, allylic, styrenic Yield = 65-91% *anti:syn* = 33:67-100:0

Scheme 7: Conditions employed for *cis* and *trans* olefin dihydroxylation without OsO_4.[36]

Recent advances in both epoxidation and dihydroxylation have focused on the use of hydrogen peroxide as a 'green oxidant' in conjunction with metal catalysts or organocatalysts.[6] Whilst impressive results have been obtained that allow the enantioselective epoxidation and dihydroxylation of many alkenes, styrenes and symmetrical (or near symmetrical) alkenes remain challenging substrates. Other limitations to substrate scope include the requirement of a chelating or electron withdrawing activating group in the substrate for synthetically useful activity. Additionally, halogenated solvents are often used which is undesirable from an industrial standpoint.[39]

Another approach worthy of mention for the synthesis of enantiopure epoxides is the development of hydrolytic kinetic resolution (HKR) using Co(II) salen catalysts to enable racemic mixtures of a terminal epoxide to be enriched in favour of one enantiomer, with the enantiopure hydrolysis product of the other enantiomer also being produced.[40] More recently, enzymes such as galactose oxidase have been used in this manner (see Section 2.3). Dynamic kinetic resolution offers a less wasteful method for procuring enantiopure epoxides but very few effective catalysts for this approach exist.[41]

1.4. Alkane oxidation

The oxidation of C-H bonds is highly desirable, both as a way of generating platform chemicals from petrochemical feedstocks and for late-stage modification of complex molecules. The oxidation of methane and other alkanes has been the focus of intense research.[42,43] The challenges to overcome are the chemical inertness of the C-H bond and overoxidation ultimately to CO_2. The discovery by Shilov that Pt(II) could readily oxidize

methane to methanol,[44] and its subsequent optimization by Periana leading to the 'Periana-Catalytica' system, illustrated that even the most inert chemical substrate of all could be selectively oxidized.[45] In order to keep selectivity high and avoid overoxidation the product concentration must be kept below 1 M. Advancements have been made with the whole plethora of late-transition metal catalysts, however few of these catalysts have reached the activities and stabilities needed for general use.[46,10] Heteropoly acids have also been extensively studied for alkane oxidation, but low yields (often less than 10%) and poor selectivities have hindered commercialization.[47]

By contrast microporous solids have provided a number of more successful oxidation catalysts. The rigid framework of the material serves to restrict the conformational flexibility of the substrate at the active site, restricting oxidation to the least sterically hindered sites. The strength of this approach has been shown by Tolman's Fe-Pd zeolite which is one of the best C-1 selective oxidation catalysts for *n*-octane to date with 67% selectivity for primary C-H bonds over secondary C-H bonds.[139] These results are particularly impressive considering that the catalyst operates *via* Fenton chemistry, which generates extremely reactive hydroxyl radicals, as opposed to the more selective metal-*oxo* type mechanism. Radical reactions show a preference for C-H bonds with lower bond dissociation energies (BDE $3^o<2^o<1^o$), though more reactive radicals, such as hydroxyl radicals, give lower selectivities (*e.g.* low $3^o:2^o$ ratios for the hydroxylation of alkanes such as adamantane). A manganese zeolite reported by Iglesia and co-workers achieved primary selectivity of 42% in the oxidation of *n*-hexane.[48]

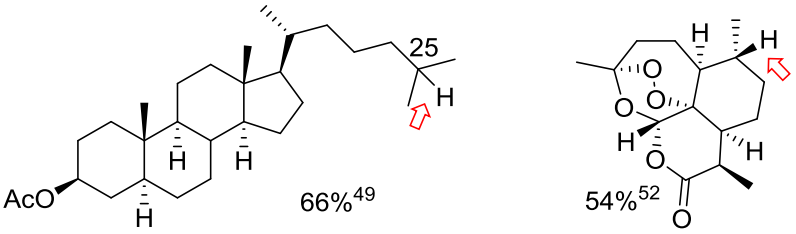
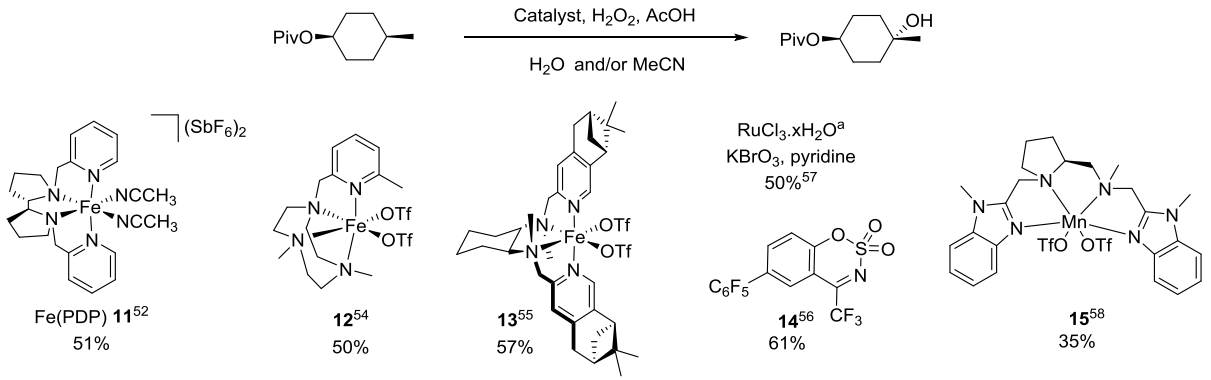


Figure 2: Examples of late stage oxidation of complex molecules.[49,52]

Arguably greater success has been experienced in the oxidation of more complex molecules (Figure 2).[50] Both organic oxidants and metal complexes (especially iron, Scheme 8) in conjunction with oxidants such as H_2O_2 have successfully been used to oxidize a wide range of structurally complex substrates in moderate yields, but with high regio- and chemoselectivity.[49,52] Both radical and metal-*oxo* mechanisms have been proposed for the iron catalysts used to date.[42] Subtle ligand tuning effects are observed to change to change selectivities, for example the alcohol to ketone ratios of aliphatic C-H bonds or the $3^o/2^o$ selectivitiy in adamantane.[51] The catalyst Fe(PDP) **11** appears to follow the selectivity expected of radical or electrophilic intermediates, as tertiary positions are oxidized to alcohols in preference to secondary C-H bonds (which are often oxidized all the way to ketones), and electron rich positions are favoured over electron deficient sites. This catalyst operates on the basis of substrate control, with selectivity predicted on the basis of each substrate's steric, electronic and stereoelectronic properties.[52] White and co-workers have shown that increasing the steric bulk of the PDP ligand (seen in **11**, Scheme 8) by adding 2,6-(CF_3)Ph groups in the *ortho* position leads towards reactions proceeding under catalyst control, and therefore overrides some of the innate substrate control.[53] The results

obtained to date are impressive, though in many cases the yields are only moderate and simpler substrates such as alkanes undergo low conversion even when used in excess. On more complex substrates, predictable late stage functionalization of C-H bonds adds a powerful tool to the synthetic chemist's arsenal. Despite encouraging breakthroughs, combining high selectivity with synthetically relevant activity remains elusive in this field, even after over 50 years of research. Continued advancements in ligand design may allow for further development for selective catalysts, though developments in other fields such as biocatalysis (see below) may eclipse this work.



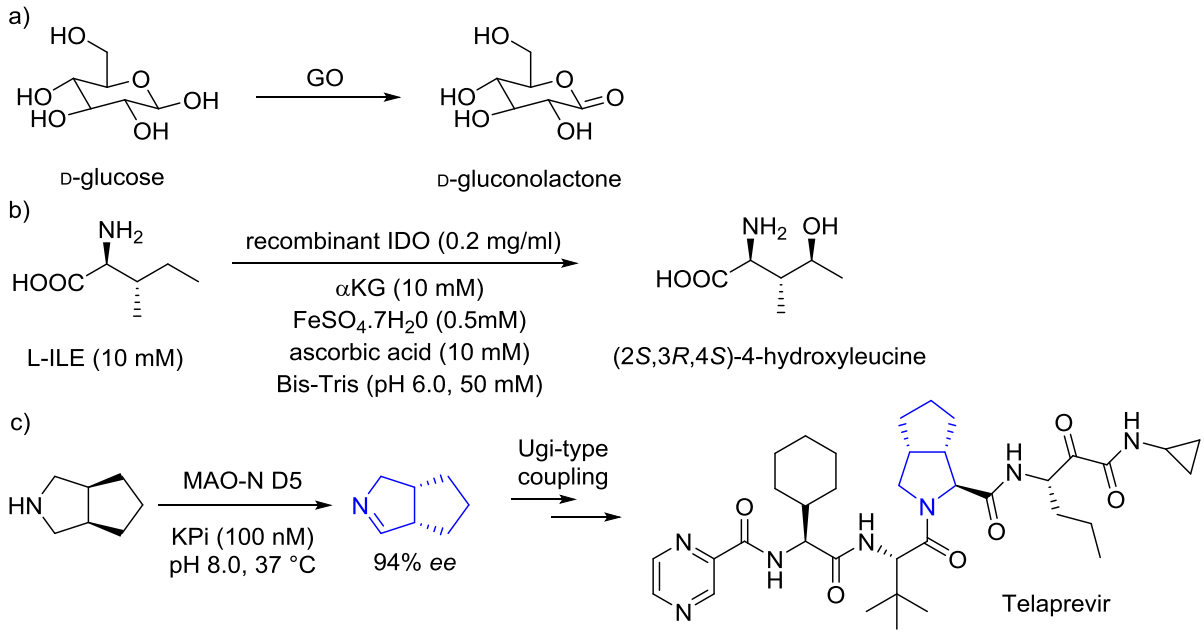
Scheme 8: Systems used for 3° hydroxylation. ([a] no H_2O_2 or AcOH). [52,54,55,56,57,58]

2. Enzymatic Oxidation

Over billions of years nature has evolved its biosynthetic technology to catalyze difficult reactions. Much of the present research in oxidation catalysis has been inspired by naturally occurring metalloenzymes, which predominately employ iron or copper ions within their active sites.[2] Nature has explored the use of proteins as macromolecular ligands successfully outperforming the low molecular weight iron complexes used by chemists. There is a wide range of different enzyme types that are known to catalyze oxidation reactions.[59] As well, they have the advantage of being able to utilize molecular oxygen, the 'greenest oxidant', efficiently under mild reaction conditions. Metalloenzyme catalyzed oxidations often demonstrate high substrate specificity alongside high levels of enantio- and regioselectivity.[60] Naturally occurring enzymes have been shown to perform oxidative reactions including hydroxylation, dihydroxylation of aromatic and aliphatic C-H bonds, epoxidation, heteroatom oxidation, Baeyer-Villiger oxidation of ketones to lactones, and halohydrin formation from alkenes.[61]

Enzymatic oxidation reactions have emerged on an industrial scale.[64] For example, the oxidation of D-glucose to D-gluconolactone using the enzyme glucose oxidase (GO) has been used in the food and wine industry (Scheme 9a).[62] Another example is the production of hydroxy amino acids using L-isoleucine dioxygenase (IDO) (Scheme 9b).[63] Hydroxy amino acids are promising compounds for drugs and functional foods due to antidiabetes activity. The GO and IDO examples are interesting because these utilise the wild type enzymes whereas a significant number of current examples of industrial applications use designed mutants. More recent examples have appeared in medicinal chemistry. These include the use of a mutant monoamine oxidase in the production of cyclic imine intermediates for the

synthesis of telapravir, a pharmaceutical drug used for the treatment of hepatitis C (Scheme 9c).[64]



Scheme 9: a) Oxidation of D-glucose,[62] b) Production of hydroxyl amino acids,[63] c) Synthesis of telapravir. (αKG = *alpha*ketoglutarate, KPi = potassium phosphate buffer).[64]

However, there are still significant limitations to the application of enzymes in an industrial scale processes.[65] The most crucial are enzyme stability and productivity. Advances in protein engineering provide a vast range of methods to tailor biocatalysts to overcome these limitations. Summarised below are the major classes of oxidative enzymes and examples are given where bioengineering has led to extraordinary results.

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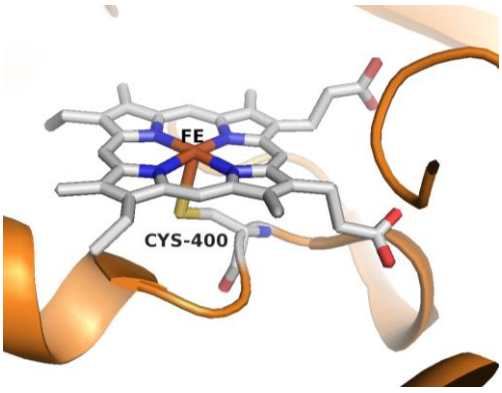

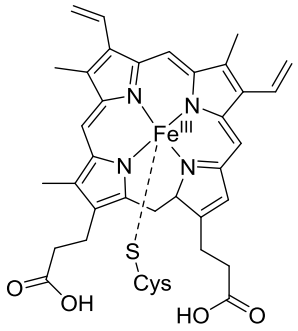

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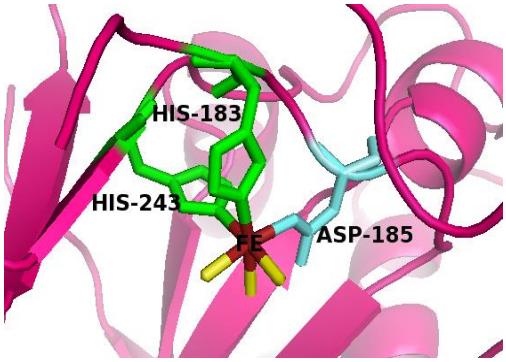

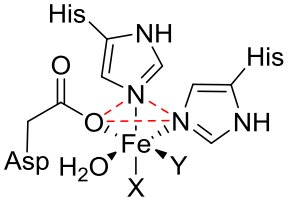

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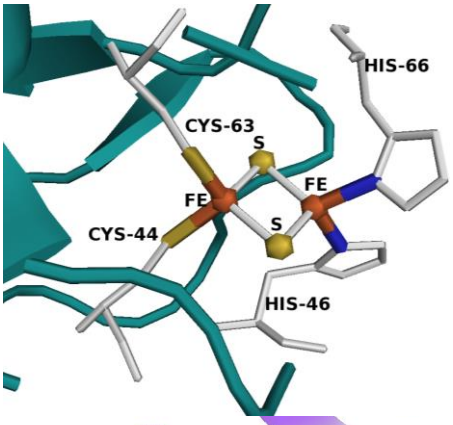

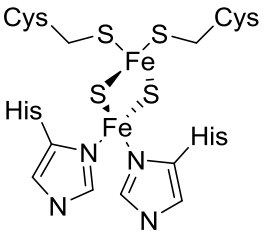

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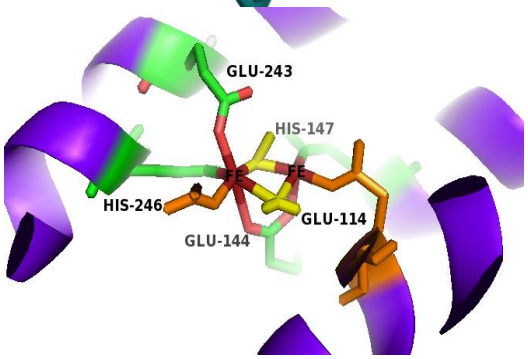


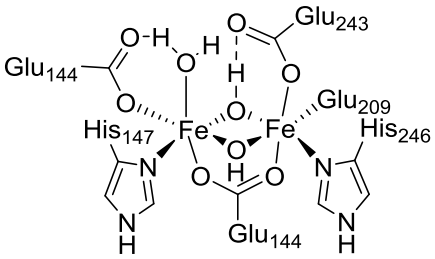
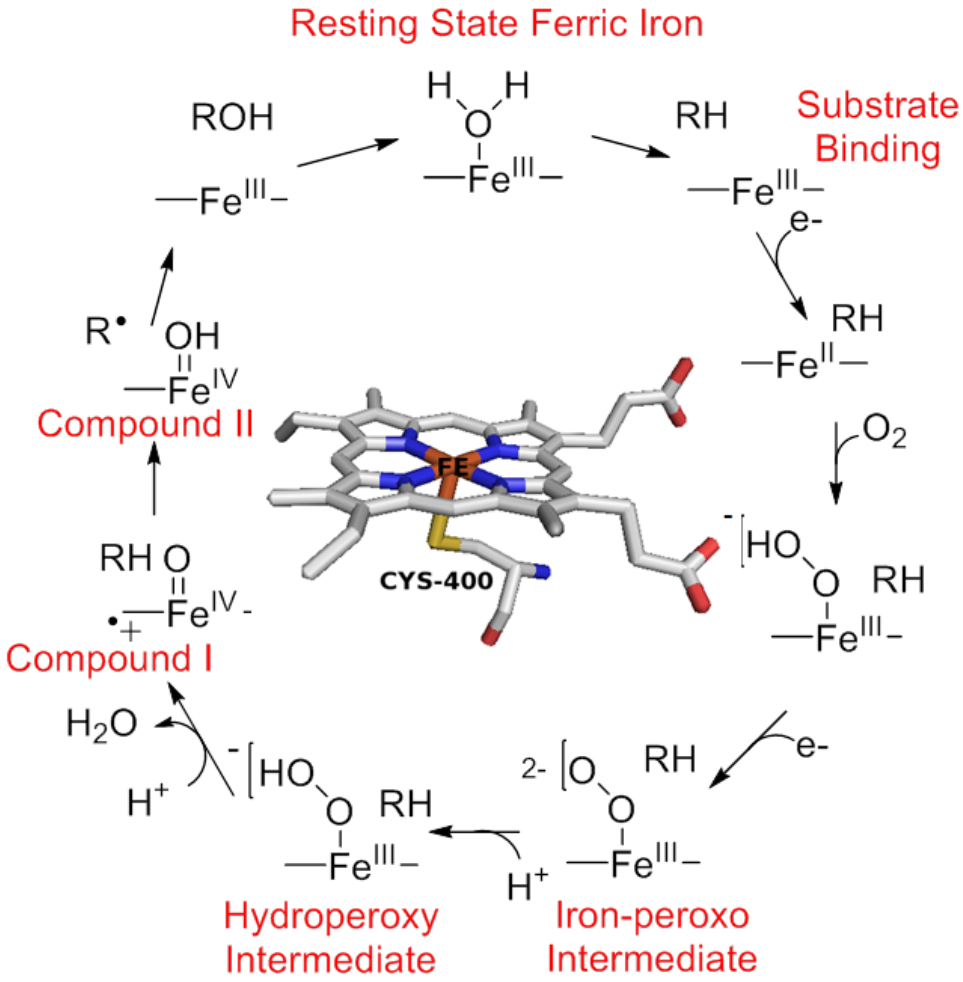


Figure 3: Diagrams to represent the different Iron containing active sites found within nature a) P450 Heme Active Site (PDB 1JPZ) b) 2-His-1-carboxlate facial triad motif, His(Green) Asp (Blue) Water (Yellow) (PDB 1RXF) c) [2Fe-2S] Cluster (PDB 1UUW) d) Di-iron center seen in monooxygenases Glu (Orange) His (Green) Water (Yellow) (PDB 1MTY)

2.1 Heme Containing Oxidative Enzymes

Heme containing enzymes are one of the most studied classes of oxidative enzymes; examples within this family are peroxidases, catalases and P450s. These redox enzymes can be used to activate hydrogen peroxide or molecular oxygen, and transfer oxygen to the substrate *via* high-valent iron-*oxo* intermediates (Scheme 10).[66] The active site comprises of a porphyrin ring anchored to the protein backbone through a histidine or cysteine residue (Figure 3a)[67] and held in place *via* ionic, van der Waals and hydrogen bonding interactions. An important intermediate of the heme enzyme's catalytic cycle is Compound I, an *oxo*iron(IV) porphyrin π cation radical, which has the ability to oxidize a variety of substrates, a factor often exploited in biomimetic approaches to oxidative catalysis.[68,69]



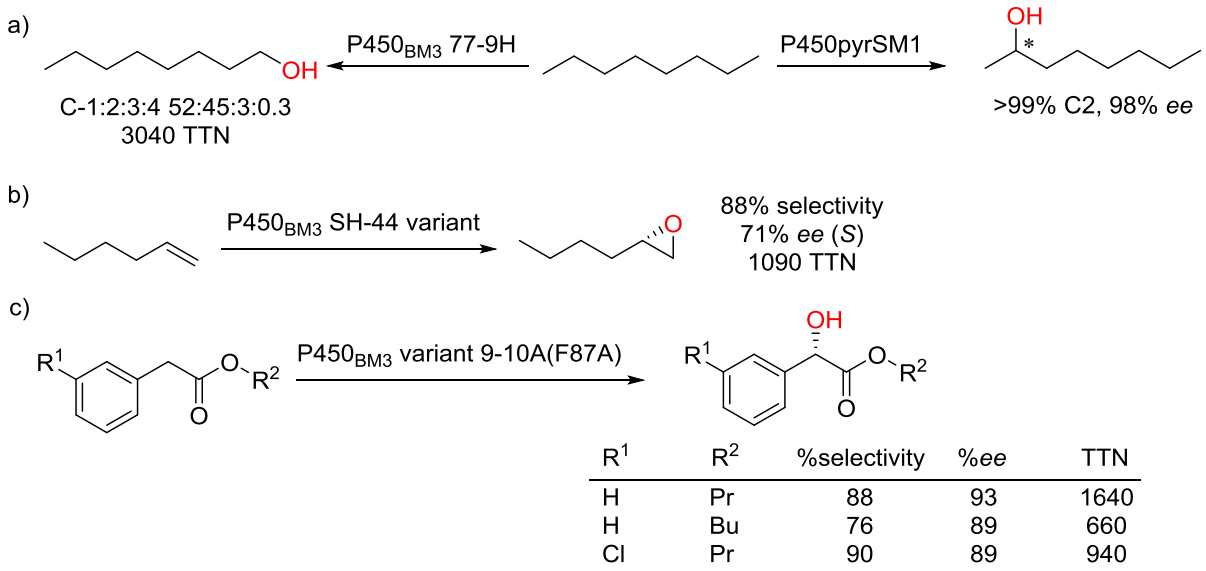
Scheme 10: The P450 catalytic cycle highlighting important intermediates. Centre of cycle indicates the active site with a conserved threonine and cysteine.

The most extensively studied type of heme containing enzymes is the cytochrome P450 family.[70,71,72] Cytochrome P450s are a wide family of oxidative enzymes that play a role in a diverse span of processes. Some of the most significant reactions catalyzed by P450s are monooxygenation of aliphatic and aryl C-H bonds, alkene epoxidation and sulfoxidation. The potential of P450s to catalyze the oxidation of unactivated C-H bonds under mild conditions using molecular oxygen make this family of enzymes attractive from a synthetic point of view.[73] Although the complexity of P450s creates limitations in utilizing these enzymes for in industrial contexts or for non-native transformations, progress has been made to tune the reactivity of these enzymes and to expand their substrate scope.[73]

Key advances in DNA and gene synthesis have provided a vast range of methods to tailor biocatalysts by protein engineering and design. These advances occur *via* rational, semirational or even evolutionary approaches. Examples lie in the areas of directed evolution, bioinformatics tools (*e.g.* multiple sequence alignments and BLAST), computational modelling screening and gene shuffling.[74,75] In some cases metabolic pathway engineering can also be used to disable the pathways to unwanted products. $P450_{BM3}$ and $P450_{cam}$ are naturally occurring enzymes that represent major targets for

engineering due to their high catalytic activity, high solubility, high expression in *Escherichia coli*, and available structural information on their monooxygenase components.[76] $P450_{BM3}$ is the first identified self-sufficient P450, composed of P450 monooxygenase and an NADPH diflavin reductase ($NADP^+$ = Nicotinamide adenine dinucleotide phosphate, NADPH = reduced form of $NADP^+$).

Bioengineering of P450s has been used to generate enzymes that showed impressive regioselectivities in alkane oxidation. The selective oxidation of *n*-octane has been achieved by the $P450_{BM3}$ mutant 77-9H which was obtained by directed evolution (Scheme 11a). This enzyme gave rise to 52% selectivity for the terminal position, while typically the wild type $P450_{BM3}$ enzymes show less than 10% selectivity for 1-octanol. The regioselectivity was shown to be octane specific as this high level of selectivity was not seen with other hydrocarbon chain lengths.[77] The same authors have also targeted the hydroxylation of more complex target molecules,[78,79] as well as epoxidation,[80] utilizing the chirality in the protein scaffold to give rise to enantioselective transformations (Scheme 11b and c). The P450 mutants changed the proteins' active site in particular ways, for example the F87A mutation in $P450_{BM3}$ 9-10A carves out a space in the active site to allow a wider range of substrates to bind.[78]



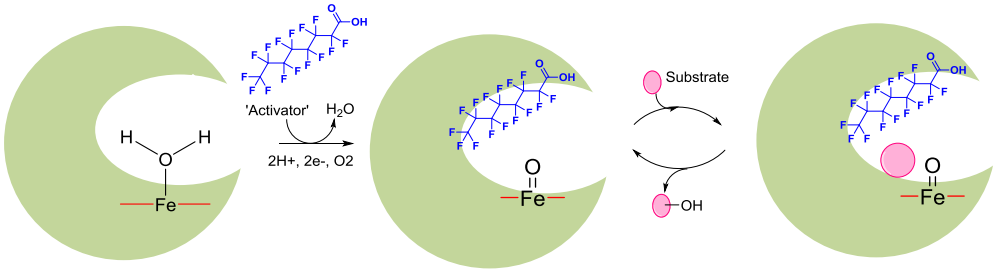
R^1	R^2	%selectivity	%*ee*	TTN
H	Pr	88	93	1640
H	Bu	76	89	660
Cl	Pr	90	89	940

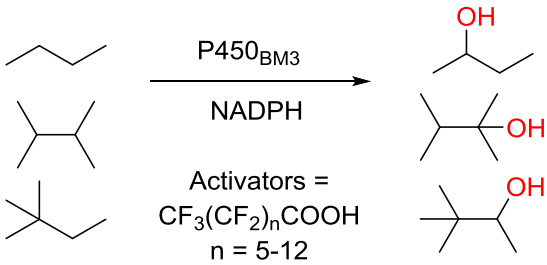
Scheme 11: a) Hydroxylation of Octane using $P450_{BM3}$ 77-9H,[77] b) Enantioselective Epoxidation of Terminal Alkenes Scheme,[80] c) Oxidation of α–aryl-acetic acid esters.[78] (TTN = total turnover number).

Regioselective and enantioselective hydroxylation of the C2 position of *n*-alkanes has been achieved using the sextuple mutant P450pyrSM1. *n*-Octane was hydroxylated exclusively (>99%) at the C2 position with 98% *ee*.[81] The P450pyrSM1 mutant is the first enzyme to show this high regioselectivity in alkane hydroxylation (Scheme 11a). In a similar way, another sextuple mutant P450pyrSM2 hydroxylated propylbenzene with 95% *ee* and 98% selectivity to give almost exclusively (*S*)-1-phenyl-2-propanol.[81]

One limitation of using P450s for alkane oxidation is that the P450 binding pocket is significantly larger than small chain alkanes, so the binding constant of these alkanes is

probably too low to bring them into the correct orientation for rapid oxidation to occur. An elegant approach to tackle this issue and control the substrate binding employs wild type $P450_{BM3}$ in the presence of chemically inert perfluorinated carboxylic acids (Scheme 12).[82] Computational studies suggested that the high turnover was attributable to the perfluorinated decanoic acid occupying the optimum volume in an apolar binding pocket (Scheme 12).





	TON	
	no activator	activator
	527	3632
	20	3246
	111	891

Scheme 12: Activator based strategy for modulating P450 reactivity using perfluoro carboxylic acids, turnover numbers for variety of hydrocarbon oxidation reactions given.[82]

2.2 Non-heme Iron Oxidative Enzymes

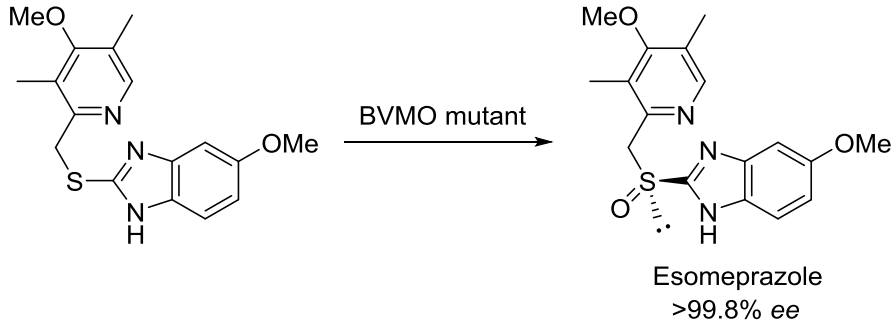
Monooxygenases are a widely studied class of non-heme iron enzymes, they catalyze the selective oxidation of a variety of substrates depending on the subtype of enzymes. In all cases they use molecular oxygen as the oxidant.[83] Several of these enzymes are of particular interest for potential chemical applications and will be discussed below.

Methane monooxygenases (MMOs), of which soluble MMO (sMMO) is the most widely studied and understood, catalyze the selective oxidation of methane to methanol. sMMO is a three subunit system in which the active site contains a non-heme diiron centre (Figure 3d). Oxidation of methane using oxygen occurs at a carboxylate-bridged diiron centre of the enzyme's active site in the α-subunit of the enzyme. This subunit is a hydroxylase known as MMOH. The other two subunits are a reductase and a coupling protein. To enable efficient catalysis all three components are needed, though the MMOH subunit has the capability to activate dioxygen by itself.[84]

The most inert of all alkanes (methane) is readily oxidised to methanol by methane monooxygenases (MMO's). Additionally, the substrate scope of wild type sMMO includes

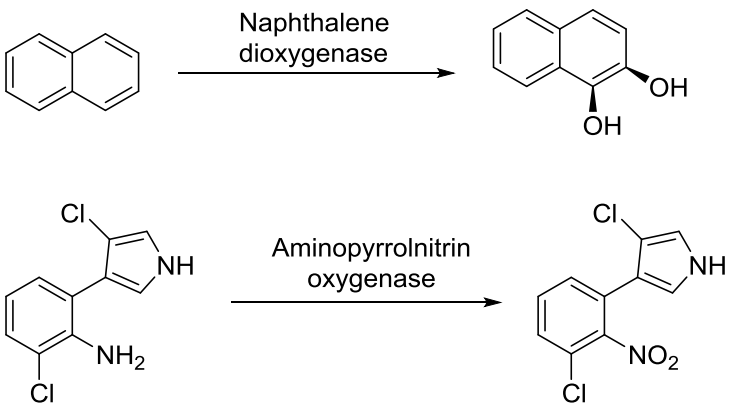
both branched and linear alkanes and tolerates alkene chains up to eight carbons, along with aromatic, heterocyclic and chlorinated compounds.[83] The application of sMMO on large scale seems unlikely as these enzymes are usually multicomponent enzymes and recombinant production is problematic. They are also often unstable and prone to product inhibition causing low levels of productivity.[85]

A class of monooxygenases that has found use within the pharmaceutical industry are the Baeyer-Villiger monooxygenases (BVMO), which are capable of oxidation of both linear and cyclic ketones to esters and lactones using molecular oxygen. BVMOs have been applied within microbes, as enzyme extracts and as recombinant enzymes in organic synthesis.[86] Several reviews have appeared in recent years describing the use of BVMOs in organic reactions such as steroid transformations, degradation of ketones, heteroatom oxidation, aldehyde oxidation and epoxidation.[87,88] However, these reactions are complicated due to the need for regeneration and retention of the flavin cofactor.[87] Significant progress has been made in co-factor recycling by continuous substrate feeding, resins and using biphasic reactions. A recently developed example involved protein engineering on a BVMO to invert the enantioselectivity and improve the activity, stability and chemoselectivity of the enzyme. This novel BVMO catalyzes a sulfoxidation reaction to give enantiomerically pure esomeprazole (Scheme 13).[85,89] Nevertheless, more progress is needed on improving the stability and efficiency of BVMO enzymes for their effective use within organic synthesis.



Scheme 13: BVMO sulfoxidation to synthesise Esomeprazole.[81]

Rieske non-heme iron oxygenases (RO) catalyze stereo- and regiospecific reactions and are known to catalyze the generation of *cis*-dihydroxylated metabolites (a common first step in bacterial degradation of many aromatic compounds).[90] RO enzymes have the ability to carry out additional oxidation reactions; some of these include oxidative ring closure, desaturation, oxidative catechol cleavage, oxidation of anilines and arene-*cis*-hydroxylation (Scheme 14).[91]



Scheme 14: Examples of reactions catalyzed by different RO enzymes.[91,93]

Rieske proteins contain a multicomponent iron-sulfur cluster (Fig. 3c), in which the [2Fe-2S] cluster transfers an electron from either the ferredoxin or the reductase component to the mononuclear iron for catalytic reactions. The mononuclear iron(II) centre acts as the active site of oxidation and is bound in a 2-His-1-carboxylate facial triad motif (Figure 3b). This motif is found commonly throughout non-heme iron enzymes. Coordination at this site exposes the iron to the large hydrophobic active site, where oxygen can bind and thus allows it to interact with the substrate permitting the reaction to occur.[92] This trigonal unit is often used as inspiration for biomimetic work (see Section 3.2) because it allows these enzymes to catalyze more complex reactions in comparison to heme enzymes. Progress with RO enzymes in comparison to other enzyme groups has been limited due to the lack of structural data until fairly recently.[93]

2.3 Copper Containing Oxidative Enzymes

Similar to the iron containing enzymes, copper containing oxidases and oxygenases bind and activate oxygen to oxidize organic substrates. The active sites within these copper containing enzymes contain a varied number of copper ions. Laccases are the largest subgroup of blue multicopper oxidases (MCO). They have the capability to catalyze the oxidation of various aromatic substrates including *ortho*- and *para*-diphenols, aminophenols, polyphenols, polyamines, aryl diamines, and inorganic ions.[94] They contain four copper atoms in the active site, which are organized into three different copper centres (Figure 4).[95] Most substrates used by laccase enzymes are phenols with redox potentials similar to that of the laccase itself, hence their ability to reduce the T1 centre.[96]

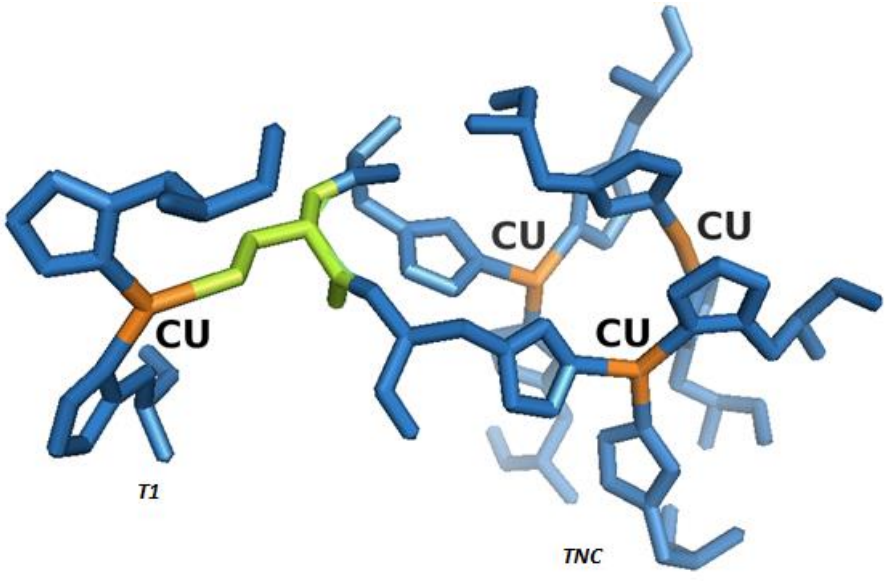
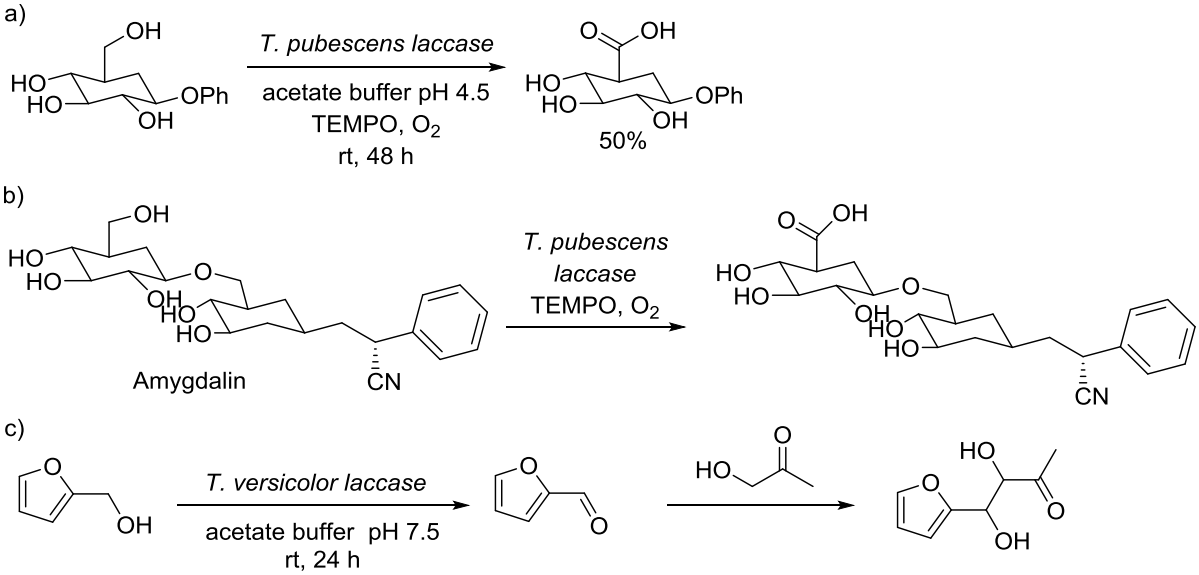


Figure 4: Laccase Active Site (PDB 25HU) illustrating the trinuclear cluster (TNC) of copper atoms and the single copper atom known as a T1 site (His in blue, Cys in green).

Laccase proteins are able to maintain stability at 60 °C, which is beneficial for large-scale applications. However, they have narrow substrate specificity and the enzyme is difficult to recycle. The redox potential of laccases prevents the enzyme from oxidizing primary alcohols but a biphasic system of laccase with a chemical mediator, TEMPO, has been successfully developed for this reaction.[97] This system has the advantage that TEMPO is regenerated by the laccase, and therefore the methodology is not restricted by the substrate selectivity of the enzyme.[98] The laccase/TEMPO system has been shown to oxidize the primary hydroxyl group in monosaccharides and natural glycosides to acids under mild conditions (Scheme 15a and b).[99] A laccase/TEMPO system was applied for the formation of C-C bonds *via* an aldol addition, involving the *in situ* generation of the acceptor aldehyde (Scheme 15c).[98]



Scheme 15: Laccase catalysed oxidation of a) a monosaccharide,[99] b) Amygdalin[99] and c) an acceptor aldehyde.[98]

Galactose oxidase (GAO) is a well-studied radical copper oxidase that uses an unusual copper (II)-tyrosyl radical unit to undergo two electron redox chemistry for the oxidation of alcohols to aldehydes.[100] The native enzyme has a relatively narrow substrate scope only oxidizing D-galactose to D-galacto-hexodialdose. Using directed evolution methods, galactose oxidase has been engineered for the kinetic resolution of secondary alcohols. (*R*)-enantiomers were oxidized with up to 50% conversion and the remaining (*S*)-substrates were obtained in 99% *ee*.[101]

3. Design strategies towards selective oxidation

In the sections above we have highlighted examples of successful chemical and enzymatic approaches to oxidative transformations. Behind these examples, a wide variety of design strategies have been utilized to improve the selectivity and activity of catalysts for oxidation reactions.

3.1 From empirical methods to ligand design

Early oxidation reactions were initially developed on an empirical basis, employing reagents known to be powerful oxidizing agents. Trial and error along with mechanistic understanding gave rise to a variety of successful stoichiometric oxidants that are still routinely employed in research labs around the world, such as $NaClO_4$, *m*CPBA, oxalyl chloride (Swern Oxidation), Dess-Martin periodinane, and TEMPO.[11] However, the drive towards more environmentally benign procedures in the chemical industries has seen the implementation of catalytic methodologies. The use of NMO to regenerate the oxidant serves as an effective method of minimising the quantities of oxidant from stoichiometric to catalytic, and has been used in the Ley-Griffith oxidation and Upjohn dihydroxylations. Whilst the use of NMO to generate catalytic methodology is useful for application in alcohol oxidation, it is less useful in enantioselective reactions such as sulfoxidation, epoxidation and dihydroxylation.

Among others, the developments of the Noyori, Knowles, Sharpless and Jacobsen catalysts led to the huge rise in rational ligand design.[102] The Jacobsen epoxidation represents an early example of the possibilities of designing ligands that can impart predictable selectivity upon a given substrate. To overcome the challenge of epoxidizing unfunctionalised alkenes Katsuki[28] and Jacobsen[103] independently arrived at chiral Mn(III) salen catalysts. Both systems used steric bulk on the aryl groups to affect enantioselectivity, through obstructing the approach of one face of the alkene over the other. This built on the concept of 'quadrants', namely, that the approach of a substrate to a reactive metal centre can be simplified to two of four open 'quadrants'. The other two quadrants are 'closed off' to approach by steric bulk, which gives rise to a powerful method for preparing and optimising asymmetric catalysts (Figure 5). Continuing fine tuning of ligand design and greater understanding of reaction mechanisms has led to a number of privileged chiral ligands, including the salen (see **5**, Figure 1) and cinchona ligand classes (see **10**, Scheme 6) used in asymmetric epoxidation, dihydroxylation and sulfoxidation.[104] The rational design of ligands has been very successful in the field of asymmetric catalysis, though often the rational used for the design is challenged by subsequent results. Without increasing understanding of reaction mechanism and all the factors affecting reaction outcomes, chances of successful ligand design may become small.

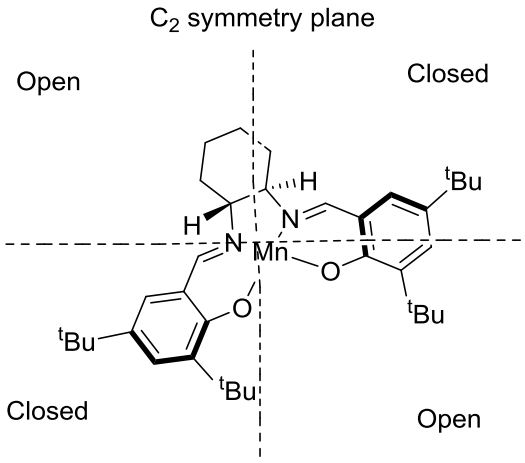


Figure 5: Quadrant concept as exemplified by the Jacobsen catalysts.

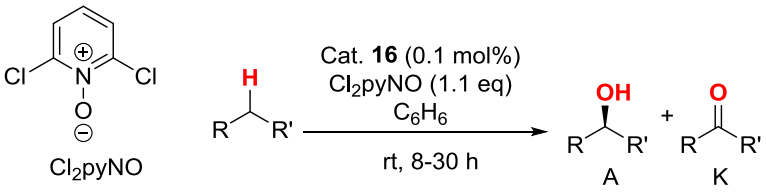
3.2 Biomimetic and bioinspired approaches

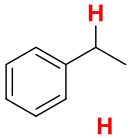
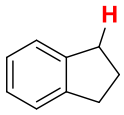
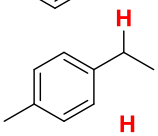
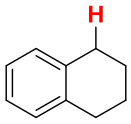
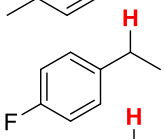
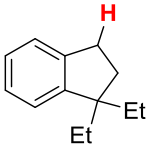
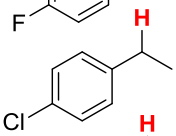
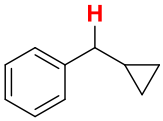
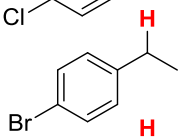
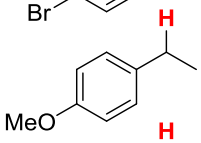
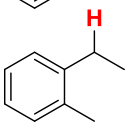
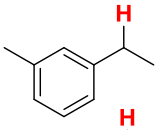
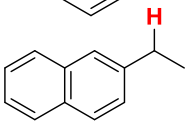
The way nature addresses the challenge of oxidation provides an inspiration and a starting point for the synthetic chemist. The study of enzymes has led to biomimetic design approaches in which the first coordination sphere of a metalloenzyme is mimicked using ligand design.[105,106] The enzymes most often used for inspiration are cytochrome P450s, sMMO and Rieske dioxygenases (see section 2, Figures 3 for illustrations of their active sites). These enzymes are able to carry out highly selective oxidations, however, their substrate scope can be narrow, and several issues hinder their use in the chemical industries, including the need for complex co-factors and their instability towards harsh reaction conditions.

Biomimetic work aims to provide a greater understanding of the mechanism of oxidation reactions both in nature and in the laboratory. In a number of cases the intermediates in oxidation reactions have not been isolated and the mechanism of these systems is still the source of controversy. For example, since Fenton's observation that simple iron salts oxidized samples of tartrate in the presence of H_2O_2, the exact nature of the reaction has been debated.[107,108,109] It is generally accepted that 'Fenton chemistry' operates *via* the generation of extremely reactive hydroxyl radicals.[110] This makes selective C-H oxidation extremely difficult, as H radicals will be abstracted indiscriminately and with little control over the extent of oxidation, giving poor regio- and chemoselectivity.[111] In contrast, Barton's 'Gif' system is commonly held to proceed *via* a high-valent metal-*oxo* intermediate involving C-H abstraction quickly followed by 'oxygen rebound', as evidenced by its unusual reactivity and the lack of mechanistic evidence for radicals.[112] The proposed mechanism bears strong similarities to the 'push-pull' concept proposed for P450s (Scheme 10), and is an elegant example of how biological understanding can enhance and direct chemical design. [113]

Early biomimetic work focused on metalloporphyrins due to a greater understanding of the mechanism of heme proteins compared to others at that time. This led to these systems being among the first exploited in bioinspired organic synthesis focusing on oxidation chemistry.[114] Early examples included epoxidation and some of the first reported alkane oxidation catalysts.[115] Later development of porphyrin based catalysts concentrated heavily on restricting the conformational flexibility of the substrate (discussed in 3.3), and

developing asymmetric catalysts. These catalysts have been used in asymmetric C-H hydroxylation and epoxidation, albeit with limited substrate scope and high variability in yield and *ee* (see Table 1).[116] These limitations, alongside the synthetic challenges in preparing these catalysts, prevent these systems from reaching mainstream synthetic applications and have led to the focus switching to non-heme inspired systems in recent years.[117,6]



substrate	conv. (%)	*ee* (*S*)	A:K	substrate	conv. (%)	*ee* (*S*)	A:K
	13	72	1.68:1		54	12	1.91:1
	20	76	3.00:1		42	12	1.50:1
	11	72	1.58:1		36	76	2.33:1
	23	74	0.40:1		30	76	0.40:1
	14	74	1.75:1				
	15	62	2.03:1				
	5	38	9.00:1				
	12	48	1.50:1				
	15	75	1.63:1				

Catalyst **16**

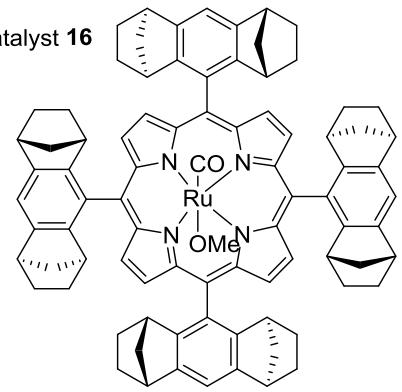


Table 1: C-H activation using ruthenium porphyrin catalysis.[116]

Attempts have also been made to mimic the intriguing diiron center of sMMO to gain a better understanding of the enzyme's mode of action, and to develop novel synthetic catalysts. This work has centred on the use of carboxylate or *N*-ligands such as tris(2-pyridylmethyl) amine (TPA) with dinuclear metal cores. Disappointingly, the mimics reported thus far often show only low levels of oxidation activity and have not led to a synthetically useful methodology. However, these structural mimics (Figure 6)[118] have been highly influential in determining the nature of the active species and providing mechanistic details of oxidation using MMOs.[119,120]

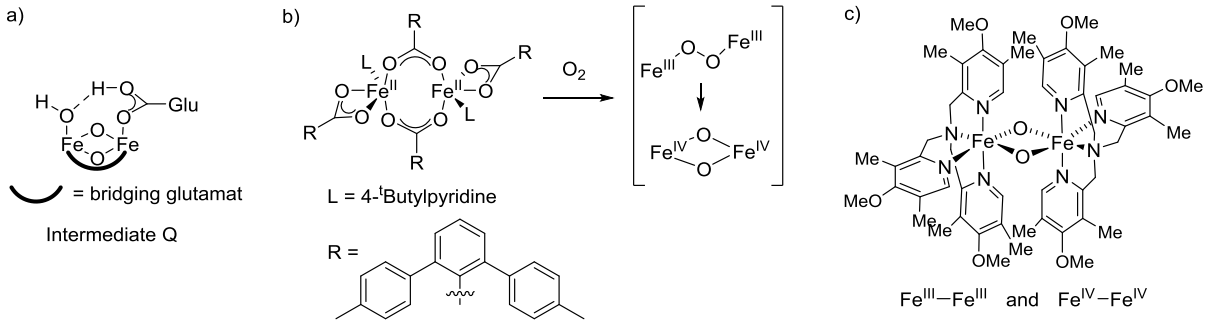
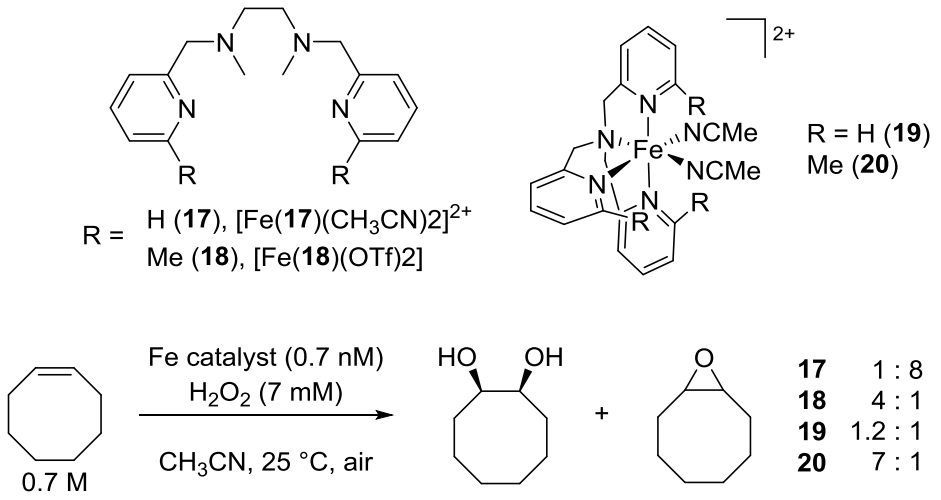


Figure 6: a) Intermediate Q from the catalytic cycle of sMMO[119] and mimics of intermediate Q: b) derived from dicarboxylate ligands[121] and c) derived from TPA derivatives.[119]

More recent work has therefore focused on the active sites of Rieske dioxygenases (Figure 3b) and its 2-His-1-carboxlate facial triad motif. This has led to a diverse range of bioinspired oxidation catalysts being reported. Early examples appeared from Que and co-workers, who in 1997 reported TPA iron complexes capable of regio-selective alkane oxidation.[122] The best performing non-heme ligands are typically tetradentate nitrogen donors, as illustrated by the novel catalysts reported in the groups of Britovsek,[123] Costas,[54] White,[52] and Goldsmith.[124]

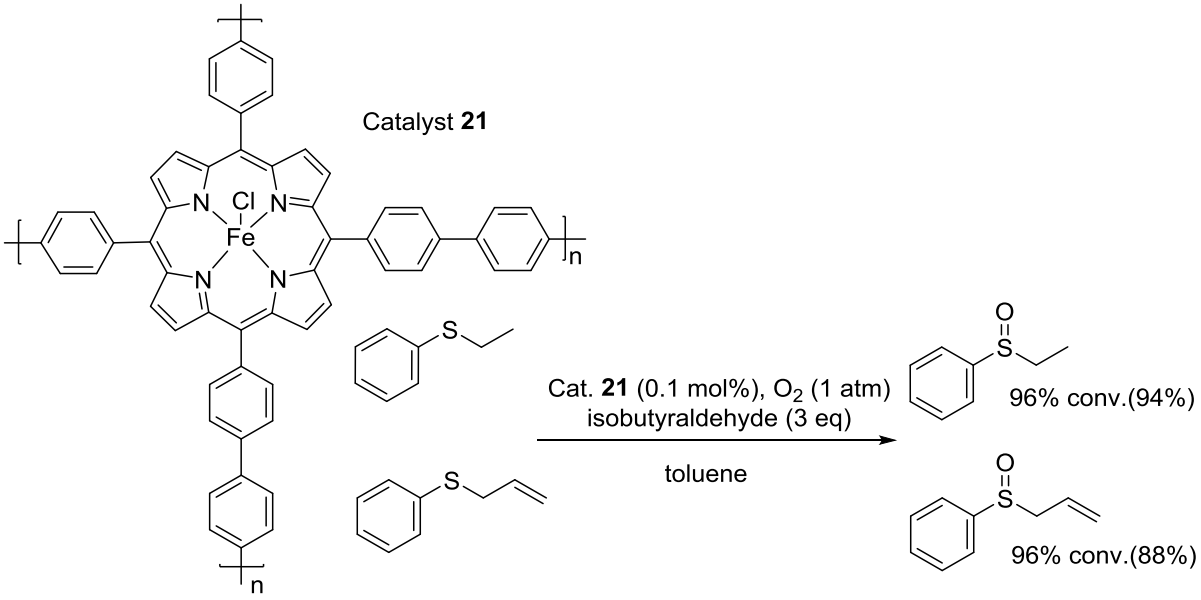
As part of their work on biomimetic oxidation, Que and co-workers conducted detailed investigations into the TPA ligand class. They demonstrated mechanistic evidence of a high valence, non-heme iron *oxo* species believed to effect oxygen transfer.[51] Later, they established that the outcome of a non-heme iron catalyzed oxidation of a given alkene (epoxidation *vs*. dihydroxylation) was highly dependent upon the ability of the initially formed iron(III) centre to maintain a low spin configuration (Scheme 16).[125] Increased steric bulk at the α-pyridyl position (**18** and **20** vs **17** and **19**) was suggested to increase preference for a high spin iron(III) intermediate, which led to dihydroxylation becoming the favoured outcome. Subsequent work by Costas and co-workers showed that the electron donating properties of *para*-pyridyl substituents had a direct influence on enantioselectivity in the epoxidation of conjugated alkenes.[126]



Scheme 16: The influence of *N*-pyridyl substituents on diol:epoxide ratios.[125]

One of the features of both the sMMO and Rieske dioxygenase enzymes is the presence of carboxylate ligands in the first coordination sphere. The above work has focused on nitrogen donor ligands, though in a number of cases carboxylic acid additives are found to play a key role in enhancing selectivity and activity. Recent mechanistic studies provide a putative explanation for the role of carboxylic acids in accelerating these reactions, producing EPR evidence of an acylperoxo iron(III) species which promotes O-O bond cleavage. This has further guided the optimization of these processes.[127,128] Mechanistic studies also suggest that the oxygenation step occurs in a concerted fashion from a metal *oxo* species, as the stereochemistry was completely retained in these reactions, with 91% incorporation of ^{18}O from radiolabelled $H_2{}^{18}O_2$.[129]

Some heterogeneous catalysts, like enzymes, exploit the concept of encapsulating the substrate in a defined macroscopic environment. We will discuss shape selective approaches in the next section. In some cases, structural motifs direct from nature such as metalloporphyrins have been combined with macroscopic heterogeneous scaffolds to make use of their reactivity. Micro- and mesoporous frameworks consisting of iron porphyrins delivered extraordinary turnover numbers of over 97,000 in the oxidation of thioanisole under ambient temperature and atmospheric pressure with O_2 as the oxidant (Scheme 17).[130] When the catalyst was tested across a wider range of substrates under similar conditions, both product selectivity for the sulfoxide over the sulfone (99:1) and activity were retained.[130] Heterogeneous material made from amino acids is another bioinspired approach. L-leucine polymers capped with phosphonates for titanium coordination were effective (up to 99%) for enantioselective formation of styrene glycol from racemic styrene oxide.[131] Additionally, a similar L-phenylglycine based material was successfully employed for the epoxidation and hydration of styrene, the latter with excellent enantioselectivity (>99%).[132]



Scheme 17: Cross linked porphyrins employed for catalytic sulfoxidation (value in brackets gives product selectivity for the sulfoxide over sulfone).[130]

3.3 Shape selective approaches

One of the most important aspects of enzymes responsible for their selectivity is the influence of the secondary coordination sphere on substrate binding and orientation. In Section 2 we have seen examples of how this can be modified in enzymes either by mutation or the use of additives such as perfluorinated carboxylic acids. An alternative way to improve reaction selectivity with chemical catalysis is to use shape selective approaches.[3] An early design concept based upon this was the so-called 'picket fence' idea postulated by Collman (Figure 7). This focused on restricting the conformational flexibility of the substrate at the porphyrin based catalytic centre. This would serve to direct selectivity in favour of sterically less hindered sites, as well as minimise catalyst deactivation by the formation of *oxo*-bridged dimers. This approach was employed throughout the 1980s and early 1990s in several groups, including those of Groves,[133] Nam,[134] Khenkin,[135] Suslick[136] and Mansuy.[137] One of the more promising examples of these so-called 'bis pocket' porphyrins was tetrakis(triphenylpheny)l porphyrin (TTPPP, Figure 7), which gave some of the best regioselectivities known at the time for linear alkanes (21:48:16:15 for C-1:2:3:4 in the hydroxylation of *n*-octane).[136] By anchoring a manganese porphyrin to four cyclodextrin moieties, Breslow and co-workers achieved 73% conversion, and 100% selectivity for the hydroxylation of C-7 of a steroid (Figure 7).[138]

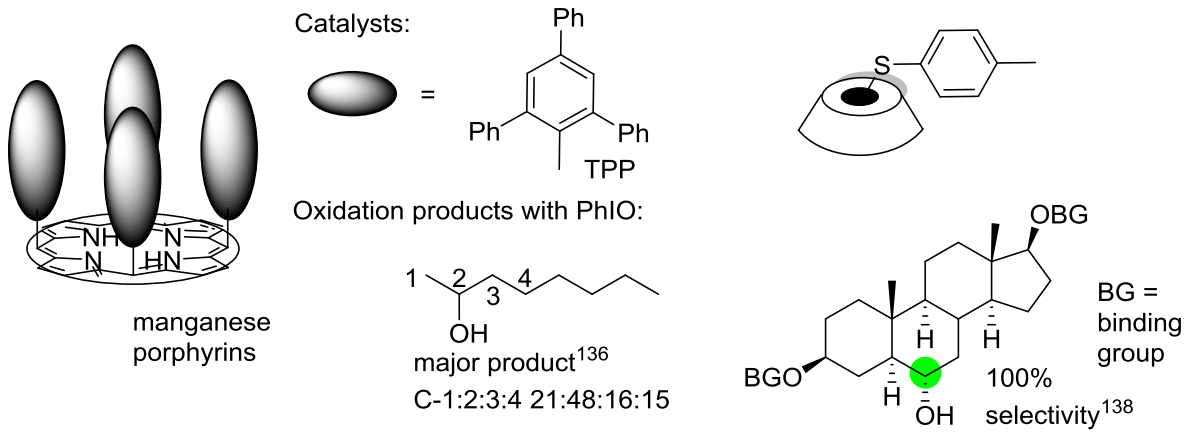
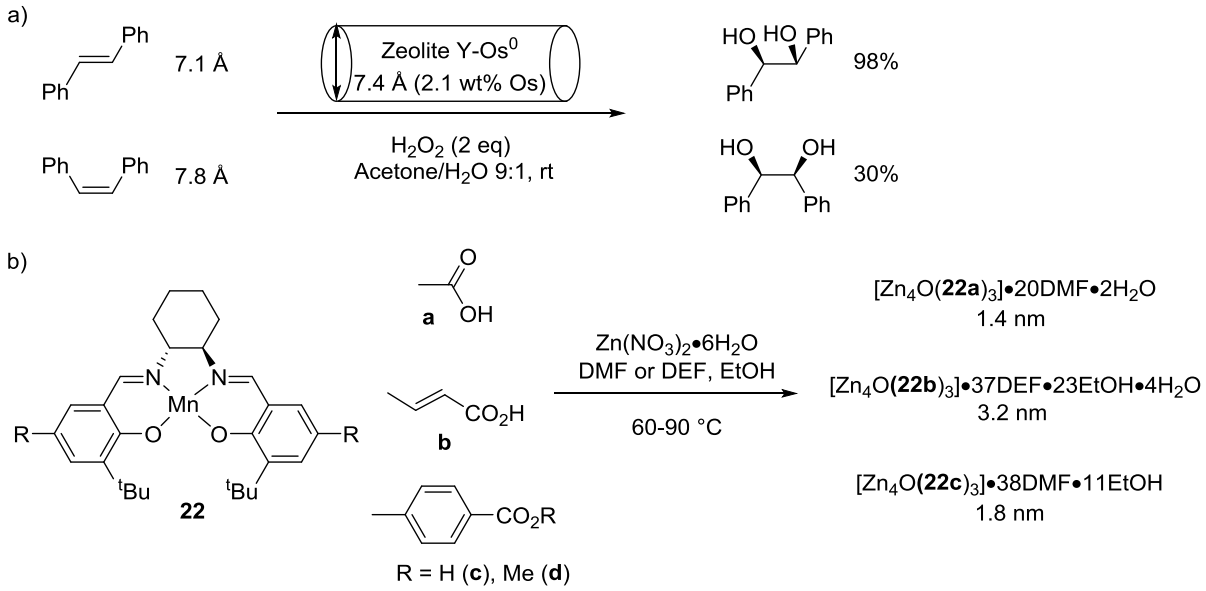


Figure 7: Porphyrins designed on the 'picket fence' concept.

A common feature of catalyst systems such as these, however, is that regioselectivity is generally found to be dependent upon the length of the alkane substrate.[136] This is indicative of a lack of control over the conformational flexibility of the substrate at the metal centre, which can be overcome using heterogeneous systems.[139] Alongside offering more rigid systems, porous materials such as zeolites withstand harsher reaction conditions and are therefore less susceptible to degradation under oxidizing conditions.[140] Tolman elegantly showed this with a Fe-Pd zeolite, which operated *via* supposedly 'unselective' Fenton chemistry, yet showed excellent primary C-H selectivity (0.67 primary *vs.* secondary C-H) in the oxidation of linear alkane, superior to the best homogeneous catalysts at the time.[139] Moreover, this selectivity was preserved irrespective of alkane chain length, and in the presence of a competitive cyclic substrate (cyclohexane). This is in contrast to the bis pocket porphyrins.[139] Thomas and co-workers reported utilizing molecular sieves impregnated with cobalt and manganese to achieve primary C-H selectivity of up to 0.32 in the oxidation of *n*-octane.[141]

A zeolite catalyst consisting of osmium(0) clusters encapsulated in zeolites carried out *syn*-dihydroxylation across a broad range of alkenes in yields of up to 98% (Scheme 18a).[142] Selectivity was seen for substrates of certain sizes depending on the zeolite pore size. For example, *trans*-stilbene could be hydroxylated in high yields, but *cis*-stilbene is too large to enter the pores and therefore could only be oxidized on the surface sites giving a reduced yield (Scheme 18b). The use of metal-organic frameworks (MOFs) allows chiral metal centers to be inserted into a heterogenous framework. Through changing the metal linkers the pore size can be modified (1.4 nm to 3.2 nm), leading to rates of reaction equivalent to homogenous catalysts for the larger MOFs.[143] The enantioselectivities were similar to those obtained with the free ligand, **22d**. The heterogeneous approach does however suffer from the harsh temperatures and pressures employed, and the use of O_2 at such pressures does present safety concerns.



Scheme 18: a) Dihydroxylation using osmium impregnated Zeolite,[142] b) Manganese salen MOFs.[143] (DMF = dimethylformamide, DEF = diethylformamide).

4. Design, synthesis and application of artificial metalloenzyme hybrid catalysts for oxidation reactions

Despite the wide array of design principles and optimization tools at our disposal both in chemistry and biology, numerous challenges still remain to be solved. This includes highly selective catalysts at industrially viable activities across a broad range of substrate scope and utilizing mild conditions (*e.g.* oxygen as the oxidant). Alkane oxidation in particular is still in its infancy. Chemical based approaches using small molecule catalysts, whilst extremely successful in many fields, has not provided answers to some of the great chemical challenges such as alkane oxidation despite 50 years of research. Biocatalysis may provide one answer, though their reactions are limited to those employing bioavailable metals, and industrialization still poses numerous challenges. We have seen above how mutations to enzymes have allowed chemists to increase the substrate scope and tolerance of reactions, as well as unlocking new reaction pathways. In the long term the ultimate aim would be to utilize any substrate of choice, and to design and synthesize the required catalyst for the desired chemical transformation. As such, new catalyst design concepts are still required for oxidation of substrates that demand specific chemo-, regio- and enantioselectivity or a combination of these. One avenue of exploration lies in the development of hybrid catalysts which take the best from nature (use of oxygen, high activities and substrate specificity) and synthetic chemistry (broad substrate scope, non-natural activities).

Catalysts that have been designed by using templates and scaffolds provided by nature offer many possibilities for tackling such selectivity problems, as they are intensively studied and advanced engineering tools are available. A wide range of new catalysts can be synthesized by combining "unnatural" metal centres within protein environments yielding artificial metalloenzymes. A chemogenetic approach allows for the application of the full plethora of chemical and biochemical techniques for optimization of these catalysts.[144,145,146] This means that for a given transition metal chosen on the basis of activity, two levels of

optimization can be envisioned. The first level is the optimization of the first coordination sphere by the incorporation of an artificial metal binding site *via* site-directed mutation in an enzyme binding site or by introduction of synthetic cofactors. The second level is by fine-tuning the protein scaffold using protein engineering tools such as mutagenesis or directed evolution to allow for optimal substrate binding and orientation leading to enhanced rates and selectivities (Figure 8).

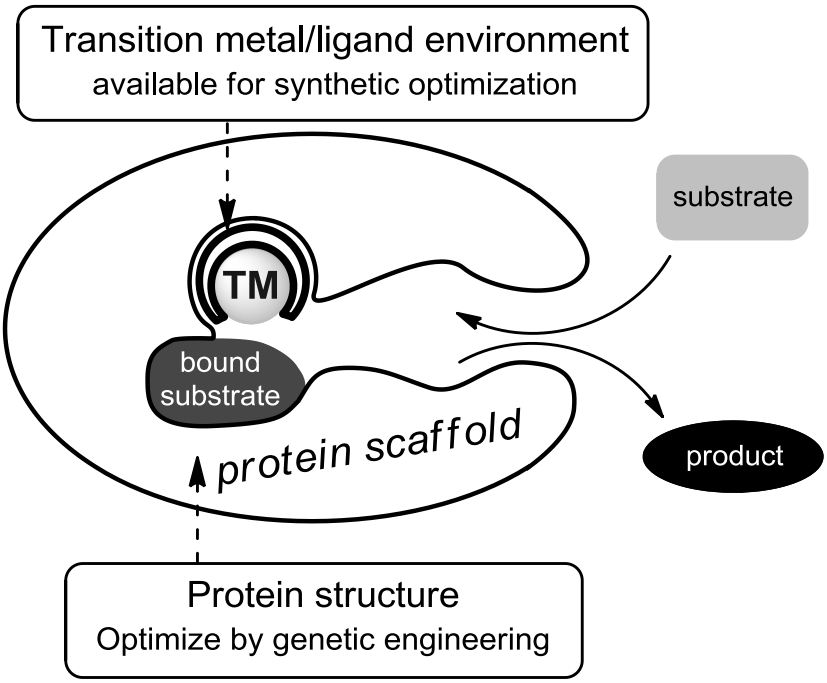


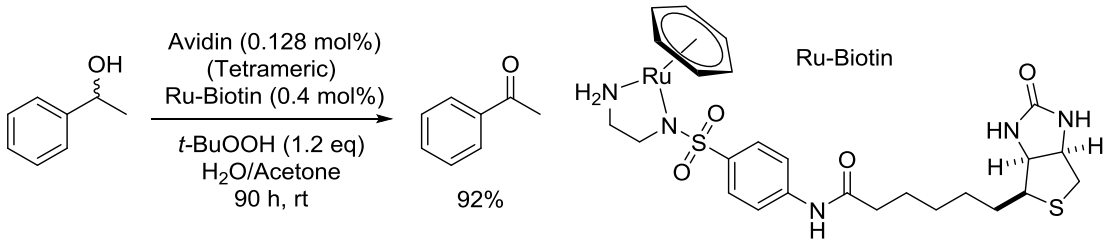
Figure 8: Concept of artificial metalloenzymes and opportunities for catalyst optimization.

There are several approaches for the design and synthesis of artificial metalloenzymes for chemical transformations not performed by natural enzymes.[60,144,146,147] Since the pioneering work of Kaiser and Whitesides in the 1970s on the synthesis of such hybrid catalysts,[148,149] the design, synthesis and application of artificial metalloenzymes has made impressive progress.[150,151] The many different design strategies of these hybrid catalysts can be categorized into dative association, supramolecular anchoring, and covalent modification. Dative association relates to the introduction of a non-native active metal into the protein *via* native amino acid residues present in a metalloezyme binding site, or *via* amino acids introduced by mutagenesis of an existing protein with the desired structural characteristics. Supramolecular anchoring involves making use of the natural affinity of a selected protein for a cofactor that can be synthetically modified with a transition metal binding site. An example is exploitation of the strong interaction between biotin and (strept)avidin (dissociation constant in the order of 10^{-14} M) to create artificial metalloenzymes by application of transition metal-modified biotin molecules.[152] The covalent modification of protein structures is performed by site selective modification of the protein with a transition metal complex, for example by making use a nucleophilic amino acid residue. Protein hosts can be selected based on desired characteristics such as substrate binding, stability and shape of the binding site. This approach is not limited to using natural occurring proteins as hosts, as demonstrated by Harada *et al.* who raised monoclonal antibodies for a rhodium diphosphine complex. Highly enantioselective

hydrogenation catalysts were obtained by combining such an antibody with the host complex.[153] Additionally, *de novo* design allows for the design of completely new protein structures with desired characteristics based on ever evolving modelling software.[154,155] Moreover, the design is not limited to proteins. Other structurally defined biopolymers such as DNA[156,157,158] have also been utilized. Several elegant approaches for the design and application of artificial hybrid oxidation catalysts are highlighted below based on the type of oxidation reactions performed.

4.1. Alcohol oxidation

Several elegant bioinspired innovations for the oxidation of alcohols have been reported using hydrogen transfer pathways,[159,160,161] but the application of artificial metalloenzyme catalysts is rarer. One of the earliest reports on artificial metalloenzymes demonstrated the oxidation of ascorbic acid to dehydroascorbic acid.[149] Since then, only a few examples have been reported. Hybrid catalysts based on streptavidin and avidin have been developed using a supramolecular anchoring strategy with different ruthenium, iridium and rhodium modified biotin complexes and these have been used for the oxidation of 1-phenylethanol (Scheme 19).[162] Benzyl alcohol and cyclohexanol could also be converted into the corresponding aldehyde and ketone respectively (68% and 80% conversion).



Scheme 19: Benzyl alcohol oxidation using ruthenium amino-sulfamide biotin complexes anchored *via* supramolecular interaction to streptavidin.[162]

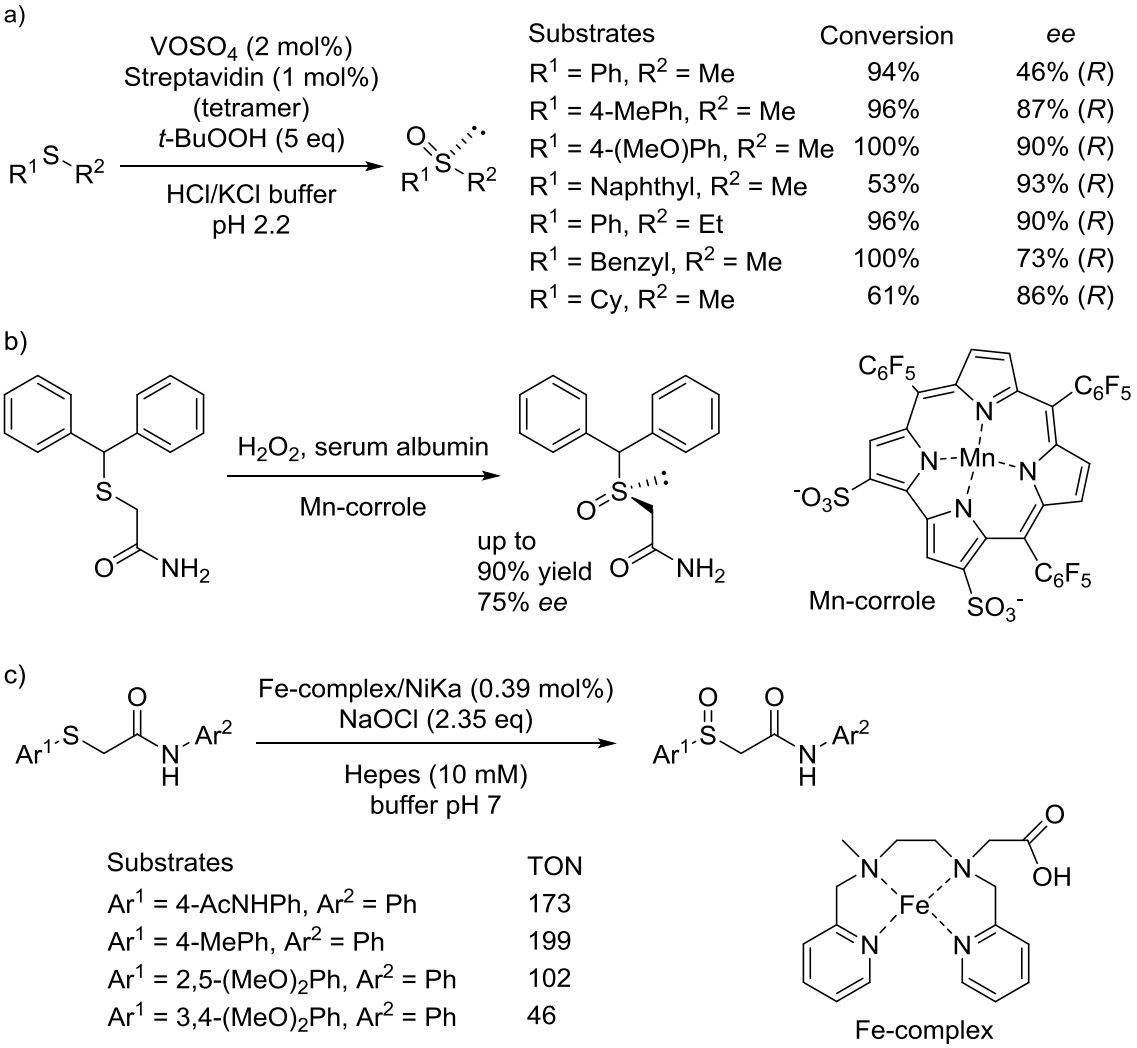
Recently, an artificial metalloenzyme for the oxidation of catechol derivatives has been designed.[163] The zinc in metallo-β-lactamase from *Stenotrophomonas maltophilia* can be replaced by copper simply by overexpressing the protein in *E. coli* in the presence of $CuSO_4$. Subsequently, a triple mutant was designed based on computer-assisted structural analysis of the binding site in order to effectively incorporate a dinuclear copper site. This triple mutant contained three strategically placed proximal histidines to achieve this. The obtained catalyst system showed a significant rate increase for the oxidation of catechols.

4.2. Sulfoxidation

Asymmetric sulfoxidation of methyl phenyl sulfide and similar substrates is a benchmark reaction for assessment of hybrid oxidation catalysts. The emphasis is typically on obtaining enantioselectivity, as this demonstrates the influence of chiral environment of the protein host on the reaction. Ward and co-workers created a highly selective catalyst system for this reaction reaching up to 93% *ee* for naphthalene methyl sulfide using *tert*-butyl hydroperoxide as oxidant. They employed the dative approach incorporating a vanadyl ion in the biotin binding site of streptavidin (Scheme 20a).[164] They reasoned that the hydrogen bonds that allow biotin to bind strongly are perfectly suited for coordinating the vanadyl ion while the hydrophobic residues involved in biotin binding are left free for substrate

interactions. The choice of oxidant also appeared important for the selectivity in the reaction, as hydrogen peroxide gave no enantioselectivity while cumene hydroperoxide provided low enantioselectivity towards the opposite enantiomer.

Many more examples of artificial metalloenzymes for the (asymmetric) sulfoxidation of methyl phenyl sulfide and analogues have been reported. Vanadate was combined with phytase[165] and analogous proteins[166] to obtain hybrid catalysts for the same reaction. The group of Ward attempted to create enantioselective artificial metalloenzymes for the oxidation of methyl phenyl sulfide by anchoring a biotin-manganese salen complexes in streptavidin.[167] However, these only reached enantioselectivities of up to 13% *ee*, significantly lower than the 46% *ee* obtained in the same reaction using the vanadyl streptavidin described above. Myoglobin anchored managanese and chromium salphen complexes have been used to yield up to 30% *ee* for enantioselective oxidation of methyl phenyl sulfide with hydrogen peroxide.[168,169] Other examples include the use of Xylanase A combined with an iron porphyrin;[170] manganese salen complexes supramolecularly anchored in human serum albumin;[171] and myoglobin in combination with manganese and iron porphycene and porphyrins.[172] In all cases, once again no improvements in enantioselectivity over the vanadyl streptavidin system were obtained, though the latter example showed high TOFs (up to 142 $mol \cdot mol^{-1} \cdot h^{-1}$). Application of the supramolecular interaction between antibodies and metal complexes to obtain enantioselective sulfoxidation catalysts has also been reported (*ee*'s up to 45% *R*, and 43% *S*).[173,174,175] Additionally, covalent anchoring has been applied to combine manganese salphen complexes with myoglobin giving up to 60% *ee*.[176,177]



Scheme 20: (Enantioselective) sulfoxidation by a) complex resulting from the interaction of streptavidin with $VOSO_4$[164] b) Supramolecularly anchored manganese corroles into serum albumins[179] c) Iron-nitrogen complexes supramolecularly anchored in nickel-binding protein (NiKa).[180]

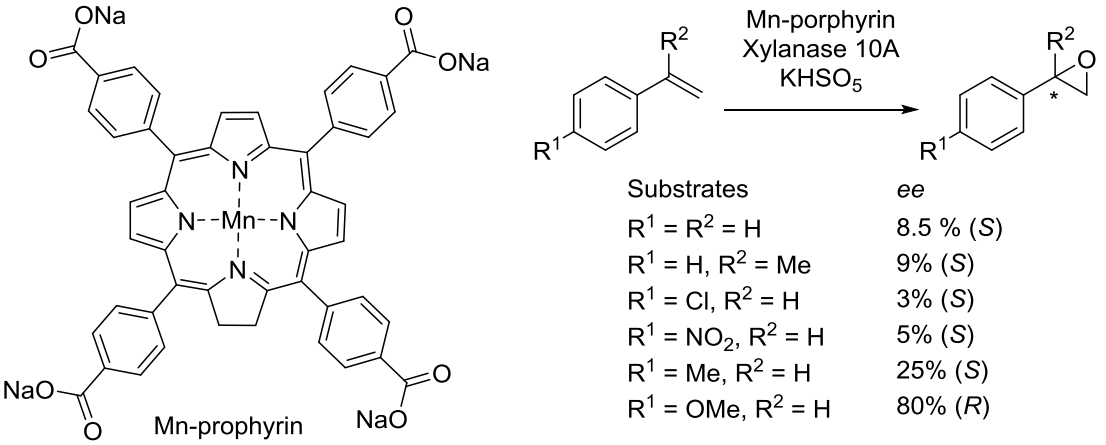
One advantage of using artificial metalloenzymes is that they can outperform traditional catalytic metal centres containing chiral ligands in ease of synthesis, activity and enantioselectivity.[178] Classic asymmetric ligands for transition metals such as chiral manganese and iron corrole complexes can be difficult to synthesize, compared to achiral derivatives. Inspired by work that had demonstrated how effective sulfoxidation catalysts could be obtained by mixing amphiphilic manganese corroles with serum albumins,[179] a small library of achiral manganese corroles was applied in combination with a small library of proteins. Protein/Mn-corrole combinations were identified that gave the sulfoxide product in good yield (up to 71%) and good enantioselectivity (up to 65% *ee*), outperforming the synthetic chiral corrole complexes. Application of this approach combined with further optimization of the reaction conditions yielded a good catalyst for the enantioselective synthesis of Armodafinil through asymmetric sulfoxidation (Scheme 20b).

One of the challenges in the design of artificial metalloenzymes is gaining structural information about the active site and translating that to catalyst improvement. The use of crystal structures with docking experiments is one way to identify suitable substrates for sulfoxidation. Crystal structures were obtained of a hybrid sulfoxidation catalyst consisting of iron-dipyridine-diamine complexes with the periplasmic nickel-binding protein NiKa (Scheme 20c).[180] These showed that the metal complexes were supramolecularly anchored into the binding site *via* salt bridges and π-stacking with arginines and a tryptophan respectively.[181,182] It was found that when substrates containing an Ar1-S-CH_2-CONH-Ar2 motif are docked into the binding site this results in an ideal Fe-O---S transition state for this reaction. Several of these substrates were tested and gave high conversions and showed a good relationship between the substrate specificity and results from the docking studies. In these systems the protein environment did not seem to induce high stereospecificity as only very low enantioselectivity (up to 10% *ee*) was observed. Sodium perchlorate was essential for the activity as other oxidants such as hydrogen peroxide gave no activity.

4.3. C=C and C-H bond oxygenation

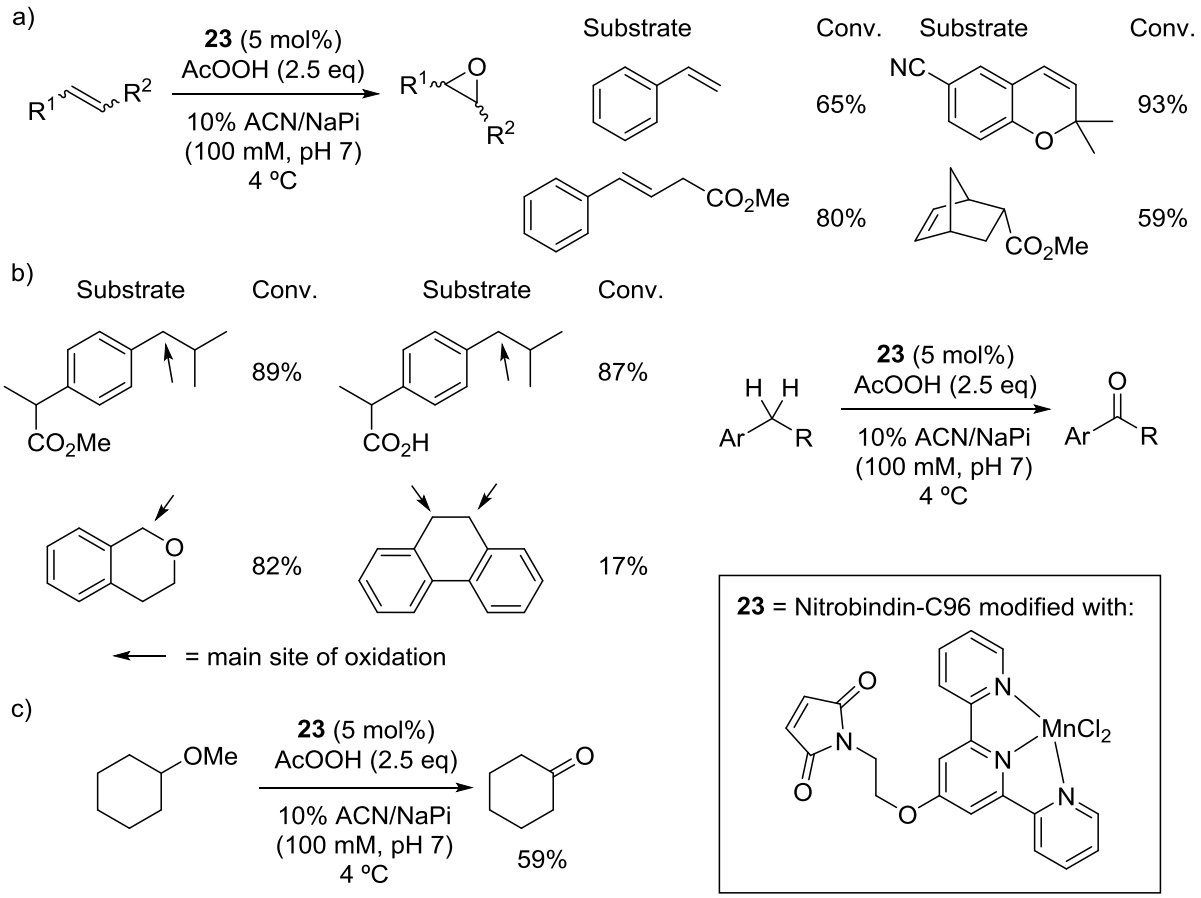
Following an early report on artificial metalloenzymes for enantioselective epoxidation of alkenes using manganese-salen covalently modified papain,[183] several groups have reported more selective hybrid catalysts for this reaction. The group of Kazlauskas reported the use of carbonic anhydrase in which the native bound zinc ion was replaced by manganese.[184] Enantioselectivities of up to 67% *ee* were reported for the epoxidation of styrene derivatives using a mixture of hydrogen peroxide with hydrogen carbonate buffer, which is postulated to generate peroxymonocarbonate (HCO_4^-) *in situ*. Other substrates, like *trans*-β-methylstyrene and aliphatic alkenes, were epoxidized with moderate enantioselectivity. However, a very low TON (up to 10 mol substrate/mol catalyst) was observed. Importantly no aldehyde side products were observed. Similar results for the epoxidation of alkenes using a nearly identical catalytic system have been reported.[185]

The group of Mahy reported artificial heme containing enzymes which are able to oxidise guaiacol and *o*-dianiside with hydrogen peroxide.[186] These were prepared from Xylanase A, an enzyme that is commercially applied in the hydrolysis of xylan and therefore readily available. The same group associated manganese porphyrins into Xylanase A for the epoxidation of styrene derivatives using potassium peroxymonosulfate (Scheme 21).[187] With this catalytic system low enantioselectivity was found for the epoxidation of styrene (8.5% *ee*). However, *p*-methoxystyrene gave good enantioselecitivty (80% *ee*). Conversion was low (up to 17%) but higher compared to hybrid catalyst systems described earlier in this section.



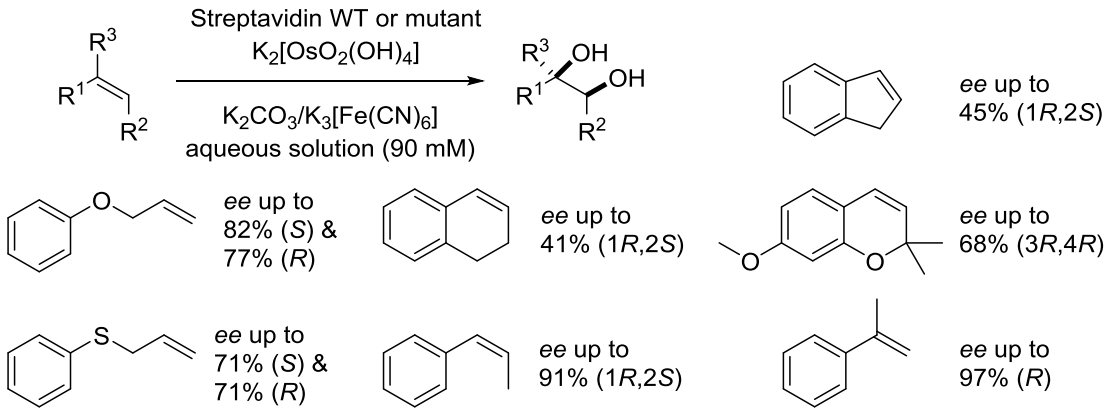
Scheme 21: Enantioselective alkene epoxidation using Mn-porphyrins supramolecularly anchored into Xylanase 10A.[187]

High conversion for the epoxidation of styrene derivatives (up to 93%) with an artificial metalloprotein was accomplished by covalently modifying a unique cysteine in nitrobindin, a hemebinding protein with a large hydrophobic cavity, with a maleimide containing manganese terpyridine cofactor (Scheme 22a).[188] Using peroxyacetic acid in phosphate buffer, high epoxide yields were obtained at low temperature. However, only a minimal preference for one of the enantiomers was reported (*er* 53.5:46.5). Benzylic C-H bonds could be oxidized to the corresponding ketones using the same catalytic system and conditions (Scheme 22b). Additionally, oxidation of cyclohexyl methyl ether was performed to obtain cyclohexanone *via* ethereal α-C-H oxidation followed by hemiacetal decomposition using the same catalyst and reaction conditions (Scheme 22c). Hayashi and co-workers incorporated manganese porphycene into myoglobin and the obtained artificial metalloenzymes were able to selectively oxidize ethylbenzene to 1-phenylethanol with hydrogen peroxide.[172] They showed using kinetic isotope effects that hydrogen abstraction from the substrate is the likely rate determining step and that the C-H activation partially includes an electron transfer process.



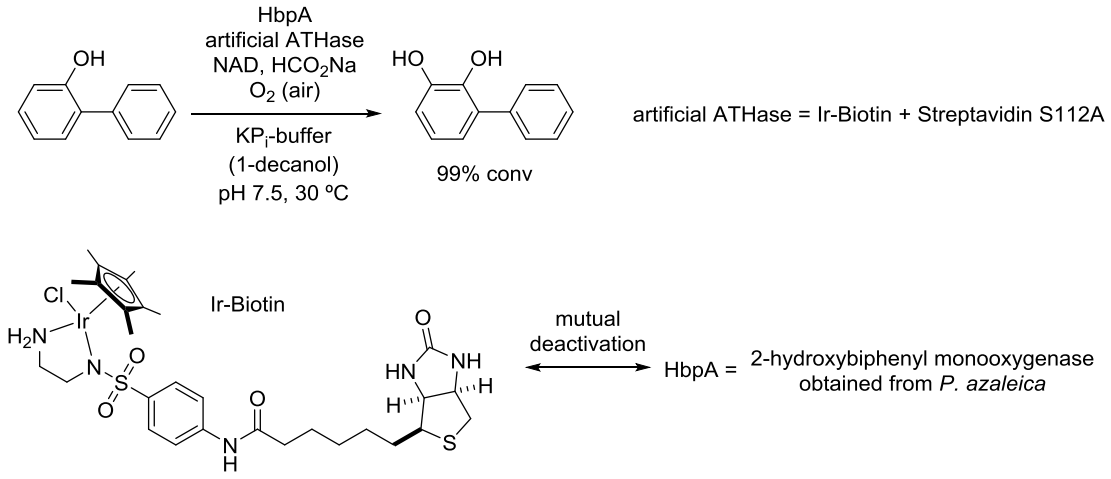
Scheme 22: Manganese terpyridine covalently modified nitrobindin[188] for a) alkene epoxidation with b) benzylic oxidation and c) etheral C-H oxidation.

Asymmetric dihydroxylation of alkenes using artificial metalloenzymes was already reported in 1983 by Okano *et al*.[189] They utilized the strong affinity of bovine serum albumin (BSA) for metal ions to create an enantioselective *cis*-hydroxylation catalyst based on osmium tetraoxide. A promising 68% *ee* for the *cis*-hydroxylation of α-methylstyrene was obtained. Recently, this early work has been used as the basis for the development of osmium tetraoxide-streptavidin based hybrid catalysts for the same reaction (Scheme 23).[190] Streptavidin was demonstrated to be the most effective host protein for OsO_4 achieving high selectivity and activity for the *cis*-hydroxylation of α-methylstyrene (95% *ee R*, TON = 27 mol substrate/mol $K_2[OsO_2(OH)_4]$), though BSA also performed well (77% *ee S*, TON = 4 mol substrate/mol $K_2[OsO_2(OH)_4]$). The enantioselectivity could be improved to 97% *ee* (*R*) using site directed mutagenesis. Other alkene substrates were also dihydroxylated with good enantioselectivity. Remarkably, almost complete inversion of the enantioselectivity could be achieved for allyl phenyl ether by two mutations (from 82% *S* to 77% *R*). This is an indication of the power of genetic optimisation for these catalytic hybrid systems. Nevertheless, no mutations have thus far yielded more active catalysts compared to the wild-type streptavidin based catalytic system.



Scheme 23: Enantioselective dihydroxylation of alkenes by an artificial metalloenzyme created by the dative interaction of streptavidin mutants and OsO_4.[190]

Natural enzymes are able to catalyze complex cascades of chemical reactions in living cells, even in the presence of many other enzyme clusters. This is partly due to efficient compartmentalization of the different active sites protecting them from mutual deactivation. Being able to mimic such tandem reactions would be highly desirable. One-pot combinations of enzyme catalyzed reactions with transition metal catalyzed reactions have already been reported[191] but the development of such systems is very challenging and only a few examples exist which employ artificial metalloenzymes.[192] Several such tandem reactions, based on an artifical transfer hydrogenase that can act in a cascade with enzymatic reactions, have been reported (Scheme 24).[193] The artificial transfer hydrogenase was assembled by combining the high affinity of streptavidin for biotin with a modified iridium-d^6-pianostool complex. This resulted in compartmentalization of the hydrogen transfer catalyst and the NADH dependent monooxygenase, allowing regeneration of the NADH. If the reaction was run without streptavidin, the iridium catalyst was quickly deactivited demonstrating the need for compartmentalization.



Scheme 24: Cascade reaction for the NADH dependent enzymatic hydroxylation of 2-hydroxybiphenyl coupled to a NADH regeneration process based on an artificial transfer hydrogenase (ATHase).[193]

4.4. Other Oxidation reactions

Site selective cleavage of DNA is an important field where artificial metalloenzymes are applied using sequence specific DNA-binding proteins. A recently reported elegant construct for oxidative site selective cleavage of DNA makes use of an existing a trimeric ring shaped DNA-clamp that is part of bacteriophage T4 replisome (gp45) (Figure 9).[194] Covalent modification with a manganese porphyrin *via* a single cysteine, introduced by site-directed mutagenesis, gives rise to an enzyme that selectively cleaves one strand of double stranded DNA containing three consecutive AT base pairs forming aldehyde residues. The resulting aldehydes were visualized with atomic force microscopy by reaction with hydroxylamine modified biotin followed by association to streptavidin. Utilization of the clamp showed clusters of streptavidin labels on the plasmid. This indicated processive catalytic behaviour, meaning the performance of consecutive reactions at the initial binding site. Addition of an octapeptide that blocks the clamping behaviour of the trimeric protein resulted in disruption of the clamp indicated by the observation of no streptavidin clusters, which leads to distributive catalysis.

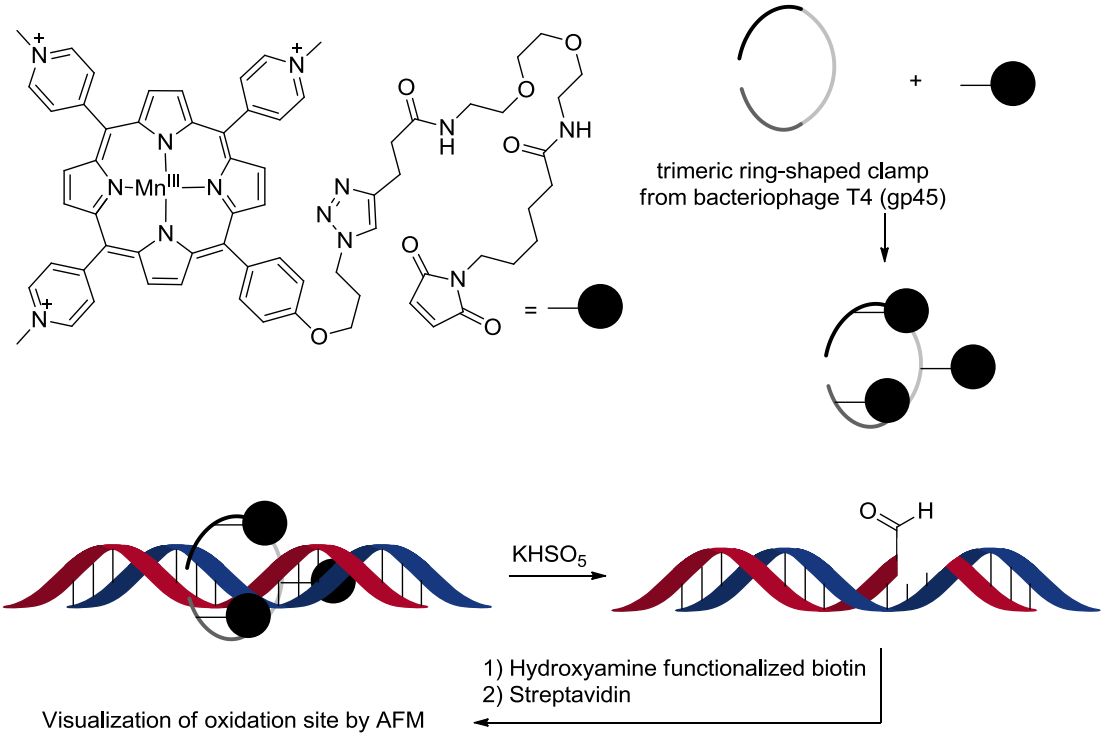


Figure 9: Metalloenzyme construct for site selective single stand cleavage of double stranded DNA and subsequent visualization methodology.[194]

Metalloenzymes designed for site-specific DNA cleavage were also created using unnatural amino acids. A bipyridine substituted alanine was introduced into a catabolite activator protein. Upon addition of copper, an artificial metalloenzyme was created which could be applied in site selective cleavage by sequence specific association of this complex to double stranded DNA.[195]

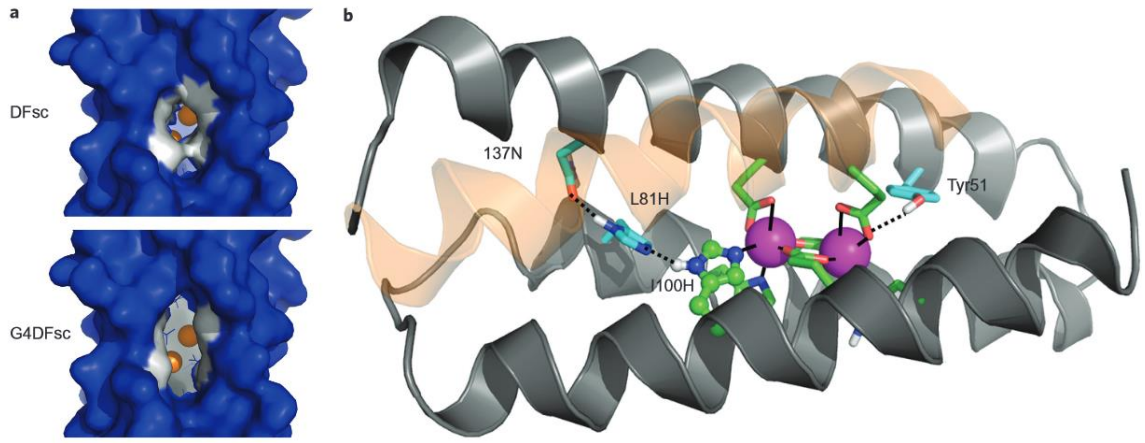


Figure 10: a) Surface models of DFsc (DFsinglechain) (top) and G4DFsc (bottom) based on the initial computational design. The four Ala to Gly substiturtions (shown in white) open the substrate access channel. b) Illustration of the 3-His-G2DFsc variant that highlights the added active site His residue (H100) Reprinted with permission from ref197. Copyright (2014) Nature Chemistry.

The most challenging aspect of artificial metalloenzymes is the *de novo* design of peptide-transition metal catalysts. Several of the examples mentioned above already come close to this concept such as site directed mutagenesis guided by rational computer aided active site design. An elegant example of this so called *de novo* design for oxidation catalysts is the design of novel 'Due Ferri' (DF) diiron complex containing proteins. DeGrado and co-workers designed a four-helical bundle containing a diiron binding site consisting of four glutamate and two histidine residues.[196,197] In order to create an effective phenol oxidation catalyst based on the original four-helical bundle four leucine residues were replaced by glycines (G4DFsc) to allow better substrate access to the active site (Figure 10). The subsequent helical destabilization caused by these mutations was counteracted by further mutations aimed at stabilizing the helix. The resulting artificial diiron enzyme was an effective catalyst for the oxidation of 3,4-di-*tert*-butylcatechol and 4-aminophenol. Recently, the same group reprogrammed this artificial metalloenzyme to perform *N*-hydroxylation of activated aryl amines.[198] The change in activity was achieved *via* several site-directed mutagenesis steps based on rational design and molecular modelling. An additional iron binding histidine was introduced based on the active site to mimic a similar active site in *p*-aminobenzoate *N*-oxygenase (AurF). The latter mutation (3His-G2DFsc) resulted in steric clashes necessitating two further second-shell mutations and one third-shell mutation. The resulting catalyst showed similar hydroxylation activity compared to Aurf with complete loss of hydroquinone oxidation activity.

5. Conclusions

Transition metal oxidation catalysts and natural enzymes perform oxidation reactions that play a crucial role in chemical synthesis, with many examples of industrial application. Both have their distinctive advantages and often they are complementary to one another. In addition, enzymes also display high levels of enantio- and regioselectivity. As shown in the examples in this review metalloenzyme catalyzed oxidation reactions often demonstrate high substrate specificity. Nevertheless, most laboratory scale and many industrial scale

oxidation reactions are performed using active transition metal salts due to the wide number of reactions they catalyze, their activity and ease of use, even though selectivity issues mean additional steps (*e.g.* the introduction of protecting groups) is needed.[60]

While the naturally occurring enzymes provide many possibilities, there are still many limitations to using them on an everyday basis. Enzymes are often superior to synthetic catalysts due to highly efficient substrate recognition and orientation leading to selectivity. However, they often have limited diversity in reaction and substrate scope, being confined to biologically relevant reactions and naturally occurring substrates. Application can often be non-trivial due to restraints on reaction conditions, required use of coenzymes and non-trivial production and purification procedures. Additionally, for many desired oxidative transformations there are no enzyme catalysts currently available. Synthetic catalysts have the advantage that they possess good activity over a wide span of reactions, but they have low selectivity and limited means to differentiate between substrates. Combining these catalytic systems is a logical line of research to obtain catalysts that overcome their inherent limitations.

In the last decade many reports have been dedicated on the development of methodology to create artificial metalloenzymes and for implementation in model reactions, to overcome the inherent limitations of both chemical and enzymatic catalysis. These advancements were made possible by the increasing understanding of how enzymes function, which has been advanced by developments in structural characterization techniques and mechanistic studies. As more is known about the enzymes functionality and the influence of the second coordination sphere on reactions, an array of interesting starting points to develop the naturally found enzymes into ideal catalysts presents themselves. The design of such catalysts will be aided by advanced molecular modelling of the active sites to select mutations that will give optimal substrate binding or transition state stabilization. Additionally, *de novo* design of novel amino acid scaffolds for assembly of artificial metalloenzymes can lead to completely new structural designs. The application of accurate structure prediction software is essential for the development of such catalytic systems. The demonstration of the application of artificial metalloenzymes in tandem reactions also begins to unravel their true potential. Compartmentalized catalysts could provide significant advantages over traditional synthetic catalysts. We anticipate these advancements will increase the variety of ideal catalysts available for demanding oxidative transformations, as well as greatly increasing our knowledge of how enzymes function.

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