C–H Bond Activation

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Catalytic, Mild, and Selective Oxyfunctionalization of Linear Alkanes: Current Challenges

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Selective catalysts for sustainable oxidation of alkanes are highly demanded because of the abundance of these molecules in the environment, the possibility to transform them into higher-value compounds, such as chemicals or synthetic fuels, and the fact that, kinetically speaking, this is a difficult reaction. Numerous chemical and biological catalysts have been developed in the lasts decades for this purpose, rendering the overview over this field of chemistry difficult. After giving a definition of the ideal catalyst for alkane oxyfunctionalization, this review aims to present the catalysts available today that are closest to ideal.

1. Introduction

Alkanes are saturated hydrocarbons originating from natural gas or crude oil fields, and are widely used as chemical raw materials or fuels.[1] For example, methane and ethane, the principal constituents of natural gas, are primarily used as fuel in private households and in industry. In the industrial sector, methane is mixed with carbon dioxide and dihydrogen to produce other chemicals, such as methanol, acetic anhydride, and acetic acid, under harsh conditions.[1] Other chemicals produced from methane include chloromethanes, acetylene, and syngas.[1] Another example is cyclohexane, the primary use of which is in the production of nylon intermediates, accounting for more than 60%.[2] Longer alkanes can be cracked at high temperatures (above 200°C) in a random way to produce mixtures of smaller alkanes and alkenes.[3] These reactions, aiming to functionalize alkanes to give important building blocks or to generate energy, are either performed under harsh conditions or produce large amounts of carbon dioxide. Hence, alternative methods for functionalization of alkanes, particularly for oxyfunctionalization, are highly demanded.

Oxyfunctionalization of linear alkanes faces several issues in terms of reactivity, regio-, and chemoselectivity.[4] Alkane inertness is commonly illustrated by the fact that *n*-hexane does not react with boiling nitric acid, concentrated sulfuric acid, potassium permanganate, or chromic acid.[1,5] Indeed, the energy of an unactivated C–H bond approaches 400 kJ mol^{-1}, and finding a catalyst that is sufficiently active to break such a strong bond is problematic.[6] Considering a linear alkane, C–H bonds of the terminal methyl positions are approximately 15 kJ mol^{-1} higher in energy than those of adjacent methylene positions.[7] Hence, if the process is not directed through some substrate recognition mechanisms, preferential oxidation will be determined by the bond strength. Consequently, achieving terminal oxidation selectively is typically an even greater challenge. Finally, because oxygenated products are more reactive than the starting alkane, mixtures of overoxidized compounds are often observed.

Taking all these parameters into consideration, a definition of the ideal catalyst for alkane oxyfunctionalization can be given:[8]

1) It should be conveniently produced from cheap, renewable, and harmless sources, such as glucose, glycerol, or even starch,
2) It should enable large turnover numbers (TON) during the highly selective conversion of alkanes into oxidized derivatives (alcohols, aldehydes, ketones, or carboxylic acids), thus, catalyst stability is a key point,

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3) For an easy implementation, it should be operative under "mild" conditions of temperature and pressure. Downstream processing should be kept to minimum by avoiding the use of expensive and/or toxic reagents/solvents,
4) It should be an atom-economical process that produces a minimal amount of non-toxic waste by using an oxidant such as molecular oxygen (from air). Because of its low cost, H_2O_2 might be considered as an environmentally friendly alternative to O_2 in some cases.

Following this definition, researchers have been exploring both chemical and enzymatic strategies for several decades. Important knowledge has been accumulated during this exploration,[9] but unresolved problems are still pending. On the following pages, the most advanced catalysts are presented and possible developments in the area of alkane hydroxylation are discussed.

2. Alkane Hydroxylation Systems Developed by Nature

Alkanes represent a large source of carbon atoms and/or of energy for a wide range of prokaryotic and eukaryotic microorganisms.[10,11] After entering the cell by passive diffusion through the membrane(s), metabolism of these hydrophobic substrates generally involves a common pathway, in which enzymatic hydroxylation occurs as the first step. Once transformed into terminal or subterminal alcohols (including terminal diols), further enzymatic oxidations result in carboxylic acid derivatives that enter the fatty acids β-oxidation metabolic route to generate energy in the cell. Enzymatic systems involved in alkane hydroxylation are very diverse. Owing to the most recent discoveries, the number of known systems is increasing and they are regularly the subject of reviews.[11] Despite the large diversity of enzymatic scaffolds and mechanisms (cytochrome P450 heme iron enzymes, integral membrane non-heme diiron alkane hydroxylases (AlkB), soluble non-heme diiron methane monooxygenases (sMMO), and membrane-bound non-heme copper-containing methane monooxygenases (pMMO)), they all can be classified as transition-metal organocatalysts. The metals found in these alkane monooxygenases are strictly limited to iron and copper. Moreover, most of them share three common characters: they use molecular dioxygen from air as the oxidizing agent, they use reduced nicotinamide dinucleotides (NAD(P)H) as electron suppliers, and they operate under physiological, mild conditions [Eq. (1)].

$$R\text{-}H + O_2 + NAD(P)H + H^+ \rightarrow R\text{-}OH + NAD(P)^+ + H_2O \qquad (1)$$

2.1. Methane Monooxygenases

Methane monooxygenases (MMOs) are found in methanotrophic bacteria.[12] Depending on copper concentration during the cultivation of some of these bacteria, two different types of MMOs can be expressed.[13] The soluble MMO (sMMO, EC 1.14.13.25) expressed under copper-limited conditions is made of a well-characterized carboxylate-bridged bis(μ-oxo)diiron active center[14] (Figure 1). The particulate MMO (pMMO, EC 1.14.18.3) otherwise expressed harbors a dicopper-based active site, which has long been a matter of controversy[15] (Figure 2). The substrate scope of MMOs

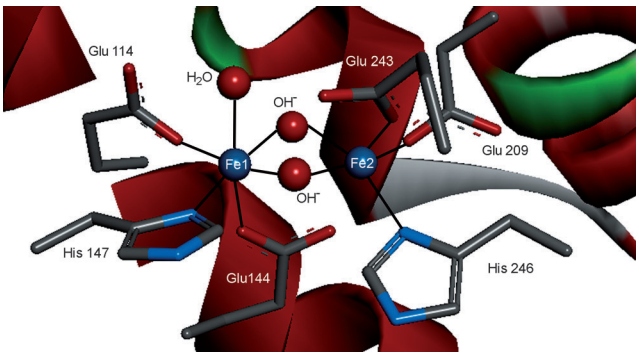

Figure 1. Carboxylate-bridged diiron^{III} resting state of the active site of soluble methane monooxygenase from *Methylococcus capsulatus* (Bath), found in the hydroxylase protein $MMOH_{ox}$ (PDB ID: 1MTY).

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Jullien Drone was born in Blois, France, in 1979. He studied organic chemistry at the University of Nantes and received his Ph.D. degree in 2006. He spent one postdoctoral year with Prof. Manfred T. Reetz at the Max Planck Institute for Coal Research, Germany. In 2007, he started an academic position as an assistant professor at the National Graduate School of Chemistry in Montpellier, France. His main research interests are P450-based biocatalysis and metabolic-pathway engineering for terpene biosynthesis in microorganisms.

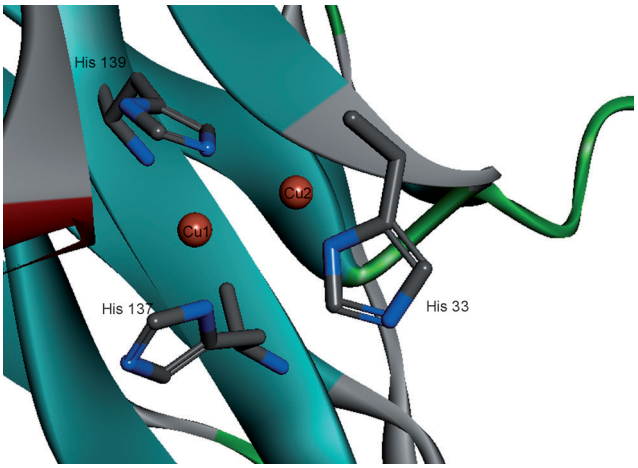

Figure 2. The dicopper active site of particulate monooxygenase from *M. capsulatus* (PDB ID: 1YEW).

ranges from methane to octane, but they are also able to oxidize a vast range of other hydrophobic molecules.[16] These enzymes may seem very promising for in vitro applications, but, as previously noted, the ideal biocatalyst should be easily prepared. An issue with MMOs is the fact that they are multicomponent, because the production of such a recombinant and functional system is extremely difficult.[17] Moreover, pMMO is membrane-bound. A high level of difficulty does not mean an impossible task, as recently demonstrated by different in vitro cutting-edge studies on these enzymes.[15,18–22] In vitro applications are nevertheless strongly limited by these problems. Moreover, these enzymes have been shown to be quite unstable and subject to product inhibition, thus leading to poor productivity[14] (Table 1, entries 1 and 2). Although they appear to be far from ideal catalysts, their binuclear metallic active sites and related mechanisms[23,24] have been for a long time and still are a source of inspiration for chemical-catalyst designers (see Section 5).[25]

2.2. Integral Membrane Non-Heme Diiron Alkane ω-Hydroxylases

The enzyme AlkB (EC 1.14.15.3) was initially isolated from *Pseudomonas putida* GPo1 (*P. oleovorans*), a bacterium able to grow on *n*-octane as the sole source of carbon atoms and energy.[45,46] AlkB-related enzymes can, in fact, be found within several *n*-alkane-degrading bacteria, such as *Alcanivorax*, *Acinetobacter*, *Gordonia*, etc.[47–54] They use a diiron cluster to regioselectively oxidize substrates, generally ranging from pentane to dodecane, in terminal position.[27] In spite of relatively high turnover frequencies (TOF; Table 1, entry 3), AlkBs are not well-suited for in vitro applications

Table 1: Selected catalysts for the mild oxyfunctionalization of linear alkanes.

Entry	Catalyst	Oxidant	Substrate[a]	Product (Selectivity)	TON/TOF[b]	Reference
1	sMMO	O_2	methane	methanol (100%)	n.a.[c]/222	[26]
2	pMMO	O_2	methane	methanol (100%)	18[d]/0.3	[15]
3	AlkB	O_2	octane	1-octanol (100%)	n.a./207	[27]
4	CYP4B1	O_2	heptane	1-heptanol (96%)	n.a./33	[28]
5	CYP52 A3	O_2	hexadecane	1-hexadecanol (98%)	n.a./27	[29]
6	CYP153 A6	O_2	octane	1-octanol (>95%)	n.a./60	[30]
7	CYP153 A[e]	O_2	octane	1-octanol (91%)	55/n.a.	[31]
8	APO1 AaP	H_2O_2	butane	1-butanol (100%)	1200[d]/n.a.	[32]
9	APO1 AaP	H_2O_2	hexane	1-hexanol (53%)	1900[d]/n.a.	[32]
10	LadA[f]	O_2	hexadecane	1-hexadecanol (100%)	n.a./4.4	[33]
11	$P450_{PMO}$ R2	O_2	propane	2-propanol (90%)	45800/370	[34]
12	$P450_{PMO}$ R2	O_2	ethane	ethanol (100%)	2450/n.a.	[35]
13	P450cam[g]	O_2	propane	2-propanol (96%)	n.a./505	[36]
14	P450cam[g]	O_2	ethane	ethanol (100%)	n.a./78	[36]
15	A13-Red	O_2	octane	1-octanol (>99%)	410[h]/57	[37]
16	WT BM3[i]	O_2	propane	2-propanol (100%)	1020/n.a.	[38]
17	WT BM3[i]	O_2	methane	methanol (100%)	2500/n.a.	[38]
18	Mn(TDCPP)Cl	H_2O_2	heptane	ω-1 (64%), ω-2 (28%)[j]	202[d]/n.a.	[39,40]
19	$(FePc tBu_4)_2N$	H_2O_2	methane	formic acid (64%)	29/n.a.	[41]
20	Vanadium POM[k]	H_2O_2	hexane	ω-1 (66%), ω-2 (26%)[l]	22/n.a.	[42]
21	MnAlPO-18	O_2	hexane	ω (66%), ω-1 (32%)[m]	149/n.a.	[43]
22	Cu-ZSM-5	O_2	methane	methanol (>98%)	0.024[d]/n.a.	[44]

[a] Substrates are linear alkanes. [b] TON: turnover number (μmol product/μmol catalyst), TOF: turnover frequency (μmol product/μmol catalyst/min). [c] n.a.: data not available. [d] Calculated from the data available in the reference. [e] CYP153A from *Polaromonas* sp. strain JS666. [f] LadA from *Geobacillus thermodenitrificans* NG80-2 with F146N/N376I mutations. [g] P450cam harboring 9 mutations: F87W/Y96F/T101L/V247L/L1244M/L294M/T185M/L1358P/G248A. [h] By using a NADPH recycling system and in the presence of bovine catalase, the TON of A13-Red on octane reached values above 3000 (unpublished results). [i] Wild-type P450 BM3 was used in the presence of $CF_3(CF_2)_8CO_2H$ and $CF_3(CF_2)_9CO_2H$ in the case of methane and propane, respectively. [j] ω-1: sum of 2-heptanol and 2-heptanone, ω-2: sum of 3-heptanol and 3-heptanone. [k] Vanadium-based polyoxometalate $[(n\text{-}C_4H_9)_4N]_4[\gamma\text{-}HPV_2W_{10}O_{40}]$. [l] ω-1: 2-hexanol, ω-2:3-hexanol. [m] ω: sum of 1-hexanol, 1-hexanal, and hexanoic acid, ω-1: sum of 2-hexanol and 2-hexanone.

because of their membrane-bound and multicomponent nature.[55–57]

2.3. P450 Enzymes

Cytochromes P450 (CYPs) form a superfamily of enzymes widely distributed from prokaryotes to higher eukaryotes.[58,59] They catalyze monooxygenation of C–H bonds on a large variety of hydrophobic molecules, such as alkanes, xenobiotics, steroids, or prostaglandins.[60] Active sites of CYPs ubiquitously consist of a mononuclear iron atom that is complexed by an N_4-tetradentate porphyrin and a proximal cysteinate: the heme prosthetic group.[61] Hydroxylation mechanism occurs through the heme paradigm, in which H· is abstracted from the substrate (R-H) by an oxoiron(IV) reactive species [(Por·$^+$)Fe^{IV}=O)], also known as compound I (Figure 3).[60,62–66]

Selective hydroxylation of alkanes at the terminal methyl position was reported for some mammalian liver P450s, including rabbit liver P450 CYP4B1,[28] and in *Candida* subspecies P450 CYP52A3.[29] However, as AlkB and MMOs, these enzymes are membrane-bound and multicomponent, and their catalytic efficiency is rather low, which dramatically restricts their in vitro applications (Table 1, entries 4 and 5).

Pioneering discoveries by Maier,[67] followed by those from van Beilen[68–70] and Witholt and Funhoff[30] and their respective co-workers have shed light on a novel family of monomeric, soluble (but multicomponent) proteins that regioselectively convert medium-chain alkanes (pentane to dodecane) into the corresponding primary alcohols. These authors collected a significant amount of in vitro data for several members of the CYP153 family (Table 1, entry 6). Alkane hydroxylases of the CYP153 family are at the center of several studies that aim to characterize existing or new members of the family[31,71–74] (Table 1, entry 7), to change their substrate scope[75] or to enhance their efficiency for in vitro applications.[37] This newly discovered family of CYPs is, to date, the most efficient for the regioselective hydroxylation in terminal position. Further studies on this family should focus on the resolution of tridimensional structures to improve our understanding of the molecular mechanisms responsible for their high regio- and chemoselectivities.

2.4. Aromatic Peroxygenases

A few years ago, two novel extracellular fungal peroxidases have been discovered in the agaric mushrooms *Agrocybe aegerita* and *Coprinellus radians*, and have been classified as aromatic peroxygenases (APO).[76–83] These H_2O_2-dependent heme iron thiolate proteins are soluble, have a single component, and combine the unique features of CYPs (oxygen transfer) and of peroxidases, such as the heme

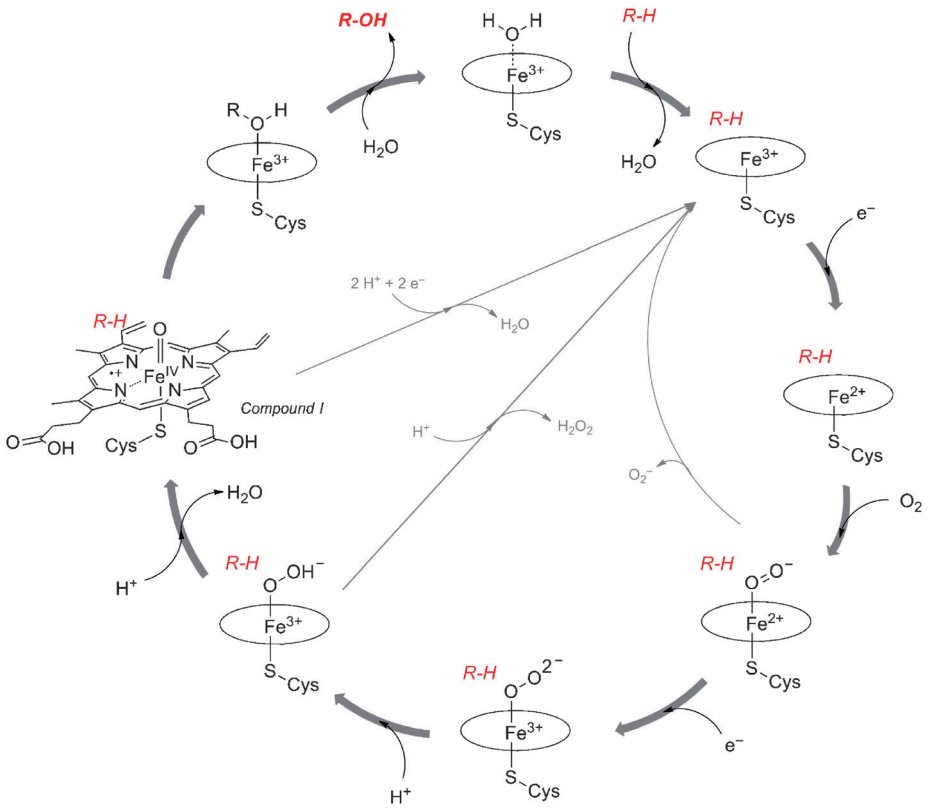

Figure 3. Generic catalytic cycle of cytochrome P450 with the chemical formula of the catalytically active Fe–heme species (compound I). The heme prosthetic group is depicted by an oval and the proximal cysteine ligand is abbreviated CysS.

chloroperoxidase (CPO) from the ascomycete *Caldariomyces fumago*[84] (phenol oxidation, halide oxidation, etc.). It has been established in 2011 that the *A. aegerita* peroxygenase (AaP) is able to hydroxylate alkanes from propane to hexadecane with H_2O_2 as the oxidizing agent. Interestingly, the hydroxylation of propane is 100% selective for 1-propanol, but gives 2- and 3-alcohols from higher alkanes. Moreover, AaP is fairly stable in large amounts of various organic solvents, such as acetone, dichloromethane, or hexane.[32] To date, this enzyme appears to be the closest to ideal biocatalyst for (sub)terminal hydroxylation of short- and medium-chain alkanes under mild conditions. As the enzyme is efficient (Table 1, entries 8 and 9), stable, self-sufficient, and does not require any reduced nicotinamide cofactors, direct applications without protein engineering could be easily implemented. Some efforts should be made on the recombinant production of AaP and homologous APOs as well as on their 3D-structures resolution.

2.5. Long-Chain Alkane Monooxygenase

Isolated in 2007 from *G. thermodenitrificans* NG80-2 (a thermophilic bacterium that metabolizes long-chain alkanes), the soluble enzyme LadA enables the selective but slow oxidation of the terminal position of alkanes from C_{15} to C_{36} (Table 1, entry 10) through an original, metal-free flavoprotein monooxygenase mechanism.[85–87] The crystal structure of LadA was resolved at 2.7 Å in complex with its flavin mononucleotide (FMN) cofactor, and showed a homodimeric organization based on a $(\alpha/\beta)_8$ barrel monomer and a very surprising structural similarity with bacterial luciferase family members.[87] This thermostable biocatalyst requires either the reduced form of FMN ($FMNH_2$) or NADPH, both in stoichiometric amounts to allow substrate oxidation.[33] Hence, LadA is cofactor-dependent, not self-sufficient, and partner enzyme(s), which are not identified yet, are postulated for $FMNH_2$ or NADPH supply and/or recycling. Further biochemical studies on LadA should take an interest in understanding its peculiar mechanism of C–H bond activation without any metallic center.

2.6. Conclusion on Natural Alkane Monooxygenases

Enzymatic systems naturally evolved for alkane hydroxylation are highly specialized and adapted for catalysis in cells, where cofactors can be efficiently regenerated by partner reductase(s). The use of wild-type, cofactor-dependent monooxygenases for in vitro application is consequently drastically limited. The use of whole-cell biocatalysis is an alternative to the use of isolated, cell-free systems, but the viability of this strategy is fundamentally decreased by additional and costly downstream processing.[88] The exception to this rule could be represented by the newly elucidated APOs, but further studies are needed to determine their tridimensional structures[89] and to fully assess their biocatalytic potential. Considering these problems, molecular engineering of natural alkane monooxygenases is a judicious approach to reach the ideal catalyst, as illustrated in the following section.

3. Biological Alkane Hydroxylation Systems Optimized by Molecular Protein Engineering

Protein engineering is a dramatically powerful tool for biocatalysis. Indeed, it provides a way to design enzymes with novel and controllable properties. Several strategies have been developed in the last two decades, and protein engineering has been extensively reviewed.[90–101] In summary, two distinct strategies can be depicted: the random (molecular directed evolution) and the rational strategy. In a directed evolution experiment, a library of randomly mutated genes is created, followed by screening for the desired property at the protein level. Several rounds of randomization/screening are applied, thus mimicking the natural process of the Darwinian evolution. No information on the protein structure is needed with this strategy. On the other hand, rational design of enzymes has proven to be a very efficient strategy as well. It is based on the information provided by the three-dimensional structure of the target protein. The introduction of mutations is perfectly controlled, but requires a good knowledge of the relationships between sequence and activity.

3.1. Directed Evolution of P450 BM3

Cytochrome P450 BM3 is a soluble and single-component fatty acid hydroxylase isolated from *Bacillus megaterium* (CYP102A1). It is the most active of all P450 enzymes, probably because the normally separated components (the hydroxylating and the electron-transfer subunits) are merged in a single polypeptide. This peculiar architecture explains why this CYP is self-sufficient. BM3 catalyzes the subterminal hydroxylation of medium- to long-chain fatty acids.[102,103] Arnold and co-workers have achieved a considerable amount of work to change the specificity of P450 BM3 from fatty acids to short-chain alkanes by using molecular directed evolution.[8,34,104–107] The most evolved variants harbor mutations on both subdomains and show high activity on gaseous alkanes and ultimately on ethane (Table 1, entries 11 and 12). During its evolution, the long, large, and hydrophobic funnel, which is naturally designed to dock fatty acids, was progressively shrinked in order to allow a better interaction with very small molecules.[35] Although still NADPH-dependent, these evolved mutants of BM3 are almost ideal biocatalysts for subterminal alkane hydroxylation, even though they are surprisingly inactive on methane. It might be interesting to evaluate the combination between the mutations of the H_2O_2-dependent mutant 21B3[108] (NAD(P)H independent) with the heme domain of propane-active mutant 35-E11[8] or $P450_{PMO}R2$[34] to obtain a self-sufficient hydrogen peroxide driven propane hydroxylating biocatalyst, an equivalent of APOs.

3.2. Iterative and Rational Design of P450cam

Cytochrome P450cam from *P. putida* (also known as CYP101) is a soluble but multicomponent enzyme that naturally oxidizes camphor.[109–111] On the basis of X-ray structures of the enzyme and some mutants, Wong and co-workers described the progressive shrinking of the large camphor-binding pocket to enable alkane binding and hydroxylation by substituting the original residues with larger or more hydrophobic ones. This series of publications led to the description of a relatively efficient ethane hydroxylase (Table 1, entries 13 and 14) in 2005.[36,112–115] Although very interesting from the fundamental point of view, these variants would probably be difficult to implement in vitro because P450cam is not a naturally self-sufficient CYP.

3.3. Man-made P450 Fusion Enzymes

A limitation to the use of CYPs in vitro is the involvement of redox proteins necessary to shuttle electrons from NAD(P)H to the heme group during the catalytic cycle.[116] Inspired by the original architecture of self-sufficient BM3, and encouraged by the feasibility of artificial fusions between P450-hydroxylase and electron-transfer subunits,[72,117–121] Drone and co-workers generated an artificial single-component P450 for the in vitro regioselective hydroxylation of alkanes in terminal position under mild conditions. In short, they combined the excellent regioselectivity of a CYP153 hydroxylating unit with the catalytic efficiency of a BM3-like architecture (Figure 4). Particularly interesting about the

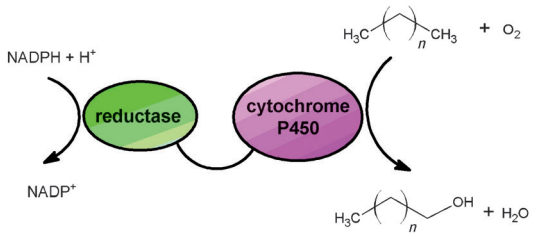

Figure 4. Principle of an artificial fusion between a CYP domain (responsible for the hydroxylation reaction) and a reductase domain (responsible for electron shuttling from NAD(P)H to CYP) leading to a self-sufficient CYP.

P450 unit (CYP153A13a from *Alcanivorax borkumensis* SK2) is its high selectivity toward terminal hydroxylation and the lack of overoxidation activity.[37] This monooxygenase is the most efficient alkane ω-hydroxylase described to date (Table 1, entry 15) and expands the alkane activation toolbox.[122]

This strategy, which enables the construction of self-sufficient systems, had been used to create a functional putidaredoxin reductase-putidaredoxin-cytochrome P450cam triple fusion protein[121] years before P450cam was converted into an ethane monooxygenase. Therefore, it is surprising that no attempt to create the similar triple fusion with the propane- or the ethane-active mutant has been reported to date. This might generate an interesting biocatalyst.

Similarly to the strategy developed by Fraaije and co-workers with Baeyer–Villiger monooxygenases,[123] another type of hybrid system, including the heme, the reductase, and a NAD(P)H-recycling domain on a single polypeptide, could enable access to a new kind of useful self-sufficient alkane hydroxylases.

3.4. Conclusion on Protein Engineering of Alkane Hydroxylases

Wild-type enzymatic systems are versatile and have proven to be remarkable platforms for developing the ideal alkane hydroxylation catalysts using protein engineering. In vitro applications are no longer limited by the absence of suitable enzymes, but more by the cost of NAD(P)H for electron supply.[124] This bottleneck could be circumvented by generating hybrid enzymes, combining the activity of mutant or wild-type heme domains (from P450cam, P450 BM3, or CYP153 family) and the advantages of H_2O_2-dependent APOs. That would be an interesting way to obtain ideal enzymes for hydroxylation of C_2–C_{12} substrates. Based on these hybrids, protein engineering is likely to provide cofactor-independent enzymes active on methane.

4. The Use of Additives for Tuning CYP Activity

The "substrate misrecognition system" is a recent concept originally described in 2007 by Watanabe and co-workers.[125–127] In this strategy, the biocatalyst is tricked by decoy molecules and oxidizes non-natural substrates. Two independent groups have recently reported the efficient and selective hydroxylation of alkanes with the wild-type P450 BM3, using perfluorinated fatty acids as decoy molecules (Table 1, entry 16).[38,128] The long, large, and hydrophobic substrate pocket is, in fact, filled with an inert perfluorinated acid of adequate chain length in order to leave sufficient space for the alkane substrate to dock in and subsequently react with compound I. The principal difference between these studies is that Reetz and co-workers efficiently and selectively oxidized methane to methanol[38] (Table 1, entry 17), while Watanabe and co-workers did not witness activity on alkanes smaller than propane.[128] Notably, it is the very first example of the selective oxidation of methane catalyzed by a P450 enzyme. It represents a real breakthrough, because it was thought for many years that CYPs were unable to perform this reaction. This difference between both studies can probably originate from the different experimental setup. Indeed, Reetz proceeded to use a high-pressure reactor (10 bar) and an NADPH recycling system, while Watanabe performed the catalytic tests in alkane-saturated aqueous buffers with no NADPH recycling system. Thus, it is very interesting to consider the "substrate misrecognition system" strategy, because it presents a less labor-intensive way than a directed-evolution strategy to change the substrate scope of an enzyme. However, a large-scale application would be problematic, because the presence of perfluorinated decoy molecules requires additional downstream processing steps to separate them from the product at the end of the reaction.

Finally, the comparison between these studies shows that experimental conditions can make a large difference between nearly identical enzymes. Using the $P450_{PMO}$ mutants[34] of P450 BM3 in pressurized reactors (with or maybe without decoy molecules) might thus give unexpected results with methane.

5. Chemical Catalysts for Alkane Hydroxylation under Mild Conditions

Even though the number and variety of reports on homogeneous or heterogeneous chemical catalysts for alkane hydroxylation is considerable,[129] only few of them can be considered as ideal, and there are still large gaps in our fundamental knowledge of how to rationally design such catalysts. Actually, all these systems generate high-valent metal–oxo complexes sufficiently active to react with C–H bonds.[61,130,131] Four categories of chemical catalysts for low-temperature (below 60 °C) hydroxylation of alkanes with dioxygen or hydrogen peroxide as terminal oxidant can be depicted: metalloporphyrins, mononuclear non-heme iron complexes, polyoxometalates, and microporous systems. The examples given in this section are willingly restricted to cases, in which no aggressive and/or environmentally harmful oxidants[2] (such as $K_2Cr_2O_7$, CrO_3, $KMnO_4$, PhIO, peracids, alkyl hydroperoxides, OsO_4, SeO_2, oxone, etc.) or prohibitive metals (Au, Pt, Pd, Ru, Ir, etc.) are used.

5.1. Metalloporphyrins: Biomimetic Heme–Iron Complexes

The understanding of mechanisms used by CYPs has inspired the development of biomimetic heme-based catalysts for many years, and metalloporphyrins are some of them.[130,132] During several generations of catalytic systems, the chemical stability of the porphyrin group was progressively improved.[133] Indeed, the porphyrin ligands are susceptible to oxidative self-degradation.[134] Stable iron and manganese porphyrins that use H_2O_2 and are able to sustain alkane hydroxylation with TONs above 200 have emerged, such as *meso*-tetra-(2,6-dichlorophenyl) porphyrin (TDCPP, Figure 5). Cyclohexane and adamantane hydroxylations were almost exclusively used as model reactions at the expense of linear alkanes. Modest conversion of heptane (11.5% with respect to H_2O_2) under mild conditions was reported with Mn(TDCPP)Cl and H_2O_2, giving a mixture of 2-, 3-alcohols and 2-, 3-ketones (Table 1, entry 18).[39,40]

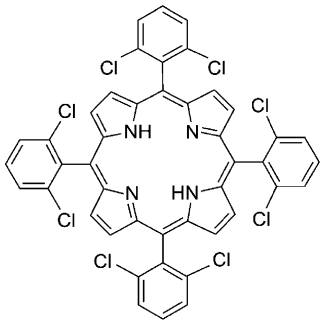

Figure 5. Structure of the biomimetic N_4-tetradentate *meso*-tetra-(2,6-dichlorophenyl)porphyrin or TDCPP ligand.

The use of stable, binuclear, porphyrin-like complexes for the continued challenge of methane oxidation has been described recently by Sorokin and co-workers.[135] The catalysts used in that case are soluble N-bridged diiron phtalocyanines (Figure 6), which are relatively easy to prepare.[135–137] Interestingly, these chemical catalysts combine the porphyrin nature of CYP active site with the diiron nature of sMMO active site. These authors have reported unprecedented catalytic activities on methane to give formic acid with high selectivity[41] (Table 1, entry 19) as well as on propane[138] by using H_2O_2 and performing the reactions under mild conditions (25–60 °C).

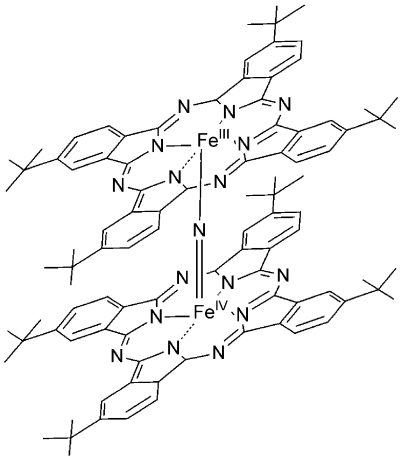

Figure 6. Structure of the N-bridged diiron phtalocyanine $(FePc{t}Bu_4)_2N$ described by Sorokin for methane oxidation under mild conditions.[41]

5.2. Biomimetic Mononuclear Non-Heme Iron Complexes

In a similar way of thinking, non-heme iron complexes were developed with respect to the active sites of mononuclear non-heme monooxygenases (for example, Rieske dioxygenases).[139,140] Iron that is complexed either by the N_4-tetradentate ligands of the tripodal TPA (tris(2-pyridylmethyl)amine)[134] or by the linear BPMEN (*N*,*N*′-bis(2-pyridylmethyl)-*N*,*N*′-dimethyl-1,2-diaminoethane,[141] Figure 7) undergoes alkane hydroxylation in the presence of H_2O_2 at room temperature. For example, Fe^{II}-BPMEN/H_2O_2 is 89% chemoselective for cyclohexanol and achieves 6.3 TONs.[140,142–144] There is almost no documentation of these catalysts with substrates other than cyclohexane and adamantane. Nevertheless, as cyclohexane has a C–H bond dissociation energy of 99 kcal mol^{-1},[145] comparable to that of secondary C–H bonds in linear alkanes,[146] extrapolation of the catalytic activity on these substrates makes sense.

Catalysis with Fe^{II}–TPA/H_2O_2 was further investigated by computational means.[146] Reactivity of the high-valent iron–

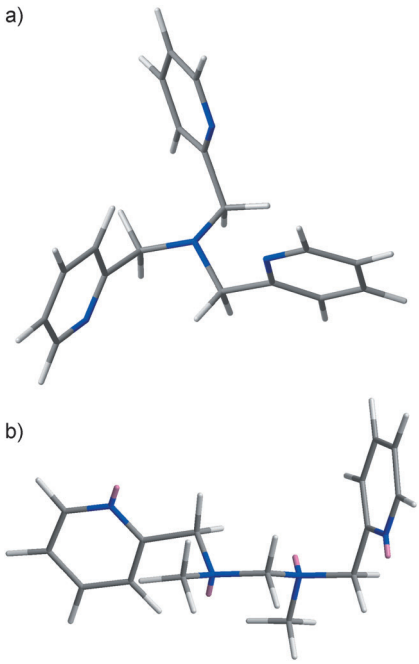

Figure 7. Threedimensional views of a) the N_4-tetradentate, tripodal TPA ligand, and b) the N_4-tetradentate, linear BPMEN ligand.

oxo [HO-(TPA)Fe^{V}=O] species was explored with propane and methane, following the heme paradigm mechanism.[147] It appears that the calculated activation energy for hydrogen abstraction of primary C–H bond in methane is too high for this catalyst. However, it clearly shows that homolytic cleavage of secondary C–H bonds in propane by the reactive species would be experimentally feasible and give 2-propanol.

Based on the catalyst Fe^{II}–TPA, a recent work has attempted to improve its selectivity for terminal hydroxylation. The design of TPA derivatives with long *n*-alkyl chains appears to be an elegant strategy to mimic hydrophobic binding pockets of enzymes, because the linear alkane can be recognized and correctly oriented through a subsequent tunneling effect. Unfortunately, none of these catalysts produced more than 10% of oxygenated products.[148]

5.3. Vanadium-based Polyoxometalates

Polyoxometalates (POMs) are stable clusters of early-transition metals and oxygen anions originally reported in 1826 by Berzelius.[149] Some general properties, such as the possible introduction of a transition metal into their frameworks, make POMs a class of attractive targets for catalysis.[150] Vanadium-based POMs are able to generate strong electrophilic oxidative species in the presence of $HClO_4$ and H_2O_2 (Figure 8, species **C**).[151] This property was recently exploited with a divanadium-substituted phosphotungstate catalyst for the efficient, mild, and selective oxidation of *n*-propane, *n*-butane, and *n*-hexane (Table 1, entry 20).[42,151] Despite modest TONs, this catalyst is highly chemoselective, because it does not overoxidize the alcohols produced.

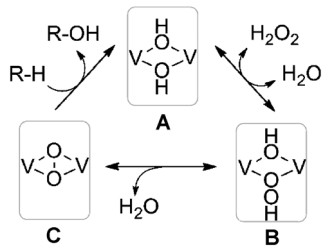

Figure 8. Postulated mechanism for the hydroxylation of alkanes with H_2O_2 catalyzed by vanadium-doped POMs. In the presence of H^+ and hydrogen peroxide, the resting species **A** (bis-(μ-hydroxo) divanadium) is oxidized to give **B** ((μ-hydroperoxo, μ-hydroxo) divanadium), which further dehydrates to **C** ([(μ-η^2:η^2-peroxo) divanadium) believed to be responsible for the oxidation.

5.4. Shape-Selective Oxidation with Microporous Catalysts

Microporous solids contain cages and channels of varying size and shape, and are commonly used as adsorbents and catalysts.[152–154] Under specific conditions, a fraction of the framework's Al^{III} ions of aluminophosphate (AlPO) molecular sieves can be substituted by another metal without notable modifications of their morphology.[155] It has been shown that Mn^{III}-substituted AlPO (in particular Mn-AlPO-18, $Al_{24}P_{24}O_{96}$), with 3.8 Å pores, regioselectively achieves the terminal oxidation of *n*-pentane (39% regioselective), *n*-hexane (65%), *n*-octane (62%), and *n*-dodecane (47%) using air (or O_2) as the unique source of oxygen (Table 1, entry 21).[43,156] In this case, regioselectivity is mainly governed by the vicinity of the terminal methyl position within the cage framework (Figure 9). Thus, there is direct relationship between the pore size of the eight-membered rings of the catalyst and the size of the alkane molecule. However, it should be noted that the chemoselectivity was difficult to control (overoxidized compounds are obtained).[5] Moreover, the conversion rate had to be kept below 10% to avoid deactivation of the catalyst by side products. These results have been contradicted by another team that synthesized the same catalysts and tested their regioselectivity profiles under

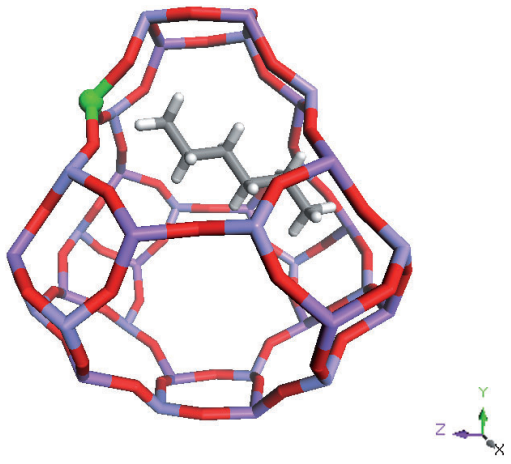

Figure 9. *n*-Hexane docking to the Mn^{III}-substituted AlPO-18 framework.

the same conditions. This second study showed that, in their hands, the terminal oxidation leads to less than 5% of the products, while ω-1 and ω-2 oxidation account for 28 and 33%, respectively.[7]

Some zeolite-based catalysts have been described for the selective methane oxidation to methanol.[44] The aluminosilicate Cu-ZSM-5 (Si/Al = 12, Cu/Al = 0.58) was activated with O_2, then methane was passed through the catalyst at room temperature and selectively gave methanol. This catalyst was also tested with ethane and gave a 4/1 mixture of ethanol/acetaldehyde. The same authors described a zeolite mordenite (Si/Al = 8.8, Cu/Al = 0.43) that resulted in equivalent amounts of methanol per gram of catalyst under identical conditions. Both catalysts were thought to have the adequate architecture to allow the formation of a bis(μ-oxo)dicopper core by mimicking the bis(μ-oxo)diiron sMMO active center. However, strong evidences recently elucidated a bent mono-(μ-oxo)dicupric ($[Cu_2O]^{2+}$ or Cu^{II}-O-Cu^{II}) species to be responsible of C–H bond abstraction.[157] Despite the fact that these systems are not catalytic (TON below the unity, Table 1, entry 22), they brought a significant knowledge in copper-based mechanisms for activation of methane.

5.5. Conclusion on Chemical Catalysts

The development of biomimetic/bioinspired chemical catalysts for alkane hydroxylation has improved our understanding of alkane monoxygenases. It proves that merging these complementary approaches instead of trying to render one more attractive than the other is very important. Chemical catalysts are either regioselective or chemoselective, but both selectivities are not, for instance, found in the same catalyst. Interestingly, while achieving selective transformations for longer alkanes is still problematic, direct, mild, and selective activation of methane, which is the most difficult reaction, is about to be solved by chemical catalysts. Finally, in spite of immense efforts and progresses, the development of methods for the selective oxygenation of alkanes with O_2 still remains a major challenge.

6. Summary and Outlook

Nature has been using and evolving organometallic alkane monooxygenases for at least several hundred million years,[158,159] allowing specialized bacteria to grow independently of photosynthesis, for example, by feeding on methane near cold-gas seeps and hydrothermal vents in oceans.[12] Although membrane-bound and/or multicomponent enzymes clearly appear not to be the right choice for the design of the ideal catalyst, intensive efforts have been invested into the discovery and/or engineering of soluble and single-component enzymes for C(sp^3)–H bond activation. Some of them are fulfilling almost all the criteria of ideality. On the other hand, chemical catalysts are still being studied, engineered, and characterized. Even today, only few of them might be considered to be in agreement with the principles of "green" chemistry, and there is still much to do to reach this ultimate goal. Making comparisons between chemical and enzymatic catalysts for alkane oxyfunctionalization is an extremely useful strategy to gain insights into the fundamental mechanisms of this chemistry. This allows the development of novel catalysts that are able to address the challenges of sustainable oxidation processes.[2,160] It is likely that the efficient, mild, and chemoselective oxidation of methane will be first achieved by chemical catalysts during the next years. Regioselective oxyfunctionalization of longer alkanes will not be possible without the frame of a substrate binding pocket to induce both their recognition and orientation. Single-component, soluble, and H_2O_2-dependent biocatalysts will play a crucial role in this type of transformations, and there is a great chance that such a biocatalyst will be based on the APO/CYP scaffold. Besides biocatalyst design, several practical issues should also be solved, such as low substrate solubility, limited oxygen mass transfer, and product inhibition. As well-illustrated by dedicated reviews,[58,161–166] optimization of reaction parameters to improve productivity, yield, and consequently economic feasibility will allow the development of industrially useful preparative transformations based on these enzymes.

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