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Iron-porphyrin Catalyzed Carbene Transfer Reactions – an Evolution from Biomimetic Catalysis towards Chemistry-inspired Non-natural Reactivities of Enzymes

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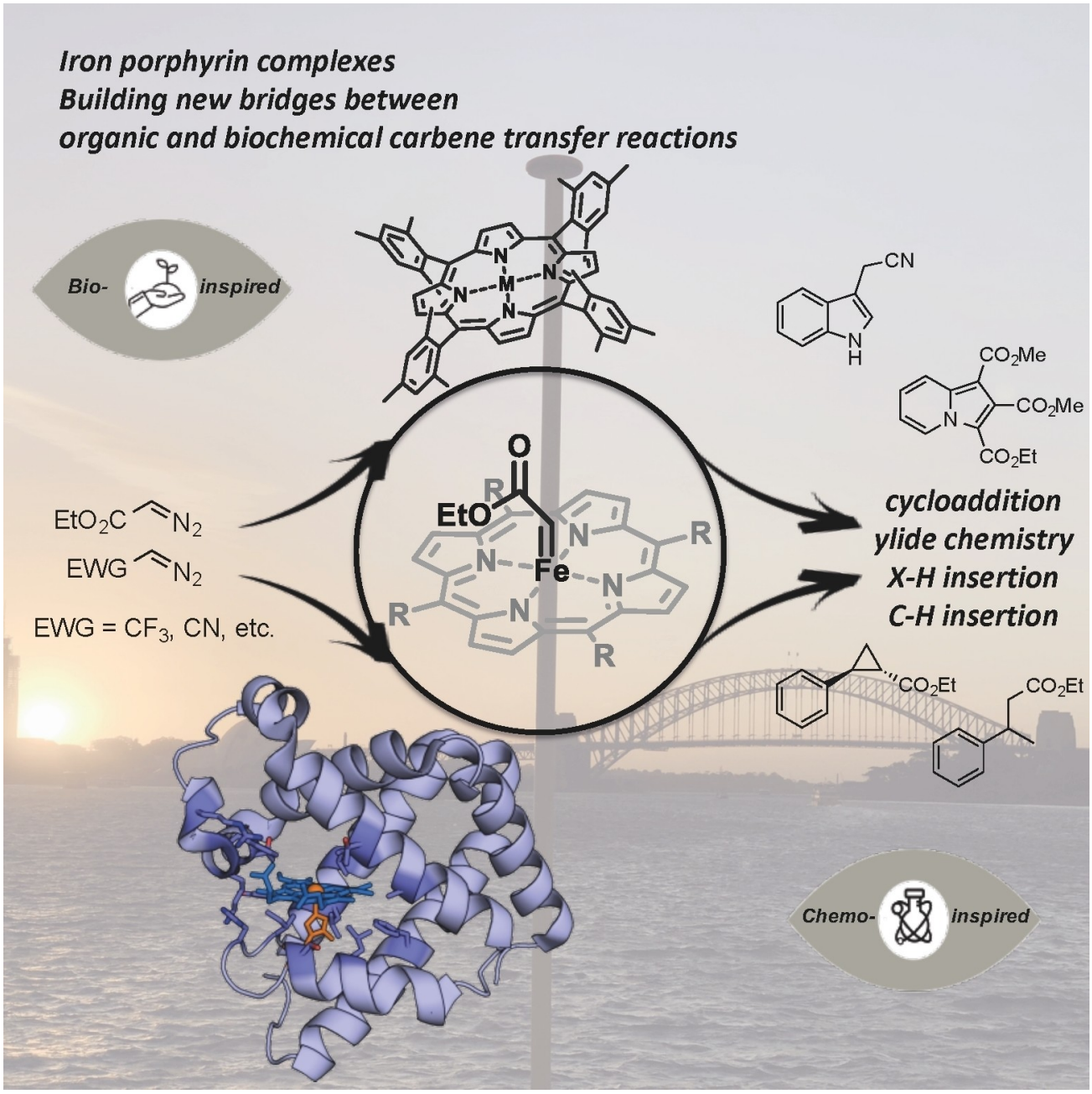

Bioinspired, synthetic porphyrin complexes are important catalysts in organic synthesis and play a pivotal role in efficient carbene transfer reactions. The advances in this research area stimulated recent, "chemo-inspired" developments in biocatalysis. Today, both synthetic iron complexes and enzymes play an important role to conduct carbene transfer reactions. The advances and potential developments in both research areas are discussed in this concept article.

Introduction

In organic chemistry, a variety of reactions and approaches were inspired by biological equivalents.[1,2] Examples for these bioinspired chemical reactions are oxygen transfer reactions, which are catalyzed in nature by P450 monooxygenases, amongst others. These P450 enzymes harbor an iron-heme center and are able to facilitate oxygen-transfer reactions, e.g. in epoxidation or hydroxylation reactions.[2] Over the past decades, a broad variety of oxygen-transfer reactions using synthetic, organometallic complexes were developed (Figure 1)[3] and biomimetic oxygen transfer reactions based on synthetic porphyrin complexes play an important role in the advancement of efficient synthetic oxidation reactions.[4] These reactions can be performed with exquisite enantioselectivity, site-specificity and are commonly used in the total synthesis of complex natural products for the construction of new C–O bonds.[3]

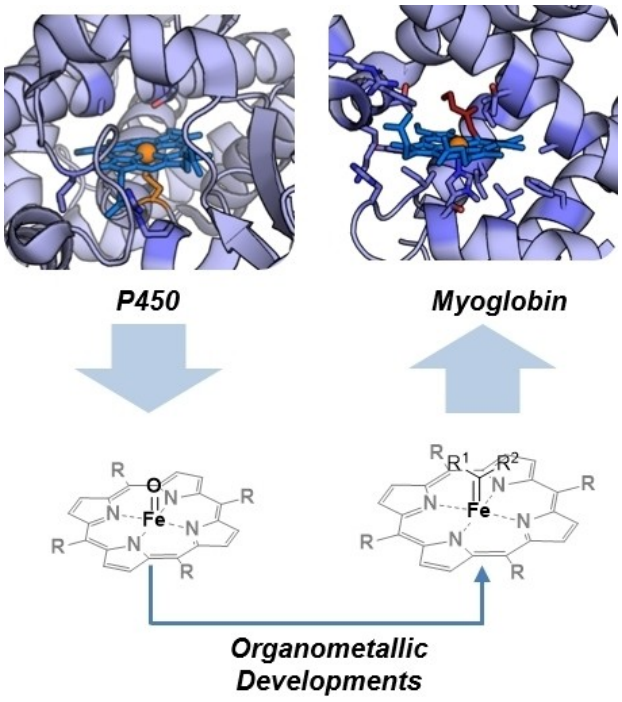

Figure 1. Bio-inspired oxidation reactions and chemo-inspired carbene transfer reactions. The active sites of P450 (PDB: 6H1 L) and myoglobin (PDB: 6G5B) are illustrated. Myoglobin with a heme-carbene complex.

Carbene transfer reactions allow the formation of new C–C bonds and are thus of prime importance for the construction of the core carbon skeleton of small molecules, drugs and complex natural products.[5] They are typically conducted in the presence of precious metal catalysts to control the high reactivity of the carbene intermediate. In this context, Rh(II) paddlewheel complexes are one of the most successful examples to stabilize and control the reactivity of the electronically unsaturated carbene fragment.[2,6] Similarly, complexes based on other transition metals, such as Ru(II), Ir(III), Au(I), Pd(II), or Cu(I), have become important catalysts to conduct efficient carbene transfer reactions.[5] All catalysts have in common that the reactivity of the electronically unsaturated carbene fragment can be controlled by the nature of the metal complex and ligand framework and consequently chemo- and/or stereoselective reactions can be conducted.

Complexes of first-row transition metals, such as cobalt or iron, have only recently emerged as catalysts to conduct more sustainable carbene transfer reactions.[7] Hossain initially reported in 1992 on iron-catalyzed carbene transfer reactions.[8] Since then, and against the background of high costs of both precious metals and ligands, this research area experienced significant advances and today carbene transfer reactions based on cobalt or iron catalysts can be performed with high efficiency. The corresponding porphyrin complexes of iron or cobalt played an important role in the development of these transformations.[7]

Enzymes, containing an iron-heme cofactor, closely resemble synthetic iron-porphyrin complexes and the development of biocatalytic non-natural reactions, inspired by transformations from the classic organic synthesis repertoire – or "chemo-inspired", received significant attention over the past years. A milestone in this research area was uncovered in 2013 by Arnold and co-workers, who could showcase that non-natural cyclopropanation reactions could be achieved using an engineered enzyme.[9,10] More "chemo-inspired" reactions were uncovered over the past years which also allows the discovery of new reactions using enzymatic carbene-transfer reactions.[11,12]

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In this concept article, we will discuss the advances in iron-heme catalyzed carbene transfer reactions from the perspective of both synthetic organic methodology and enzymatic applications. We will highlight advantages of both approaches, describe the "chemo-inspired" examples and conclude with a perspective for future developments.

Iron catalyzed carbene transfer reactions

In the seminal report of Hossain, the cationic $CpFe(CO)_2(THF)BF_4$ complex was demonstrated to be a moderately efficient catalyst in the cyclopropanation reaction of styrene with ethyl diazoacetate. Notably, high selectivity for the *cis*-cyclopropane was observed.[8] Since then, different approaches have been realized to improve the efficiency and selectivity of iron-catalyzed carbene transfer reactions.[7] From the viewpoint of catalyst development, Woo and co-workers reported an important milestone in 1995, when they could showcase the application of iron porphyrin complexes in the *trans*-selective cyclopropanation reaction of styrene derivatives.[13] The reactivity of iron porphyrin complexes can be readily fine-tuned by the substitution pattern of the porphyrin system and high turnover numbers of up to 4300 could be achieved when using an electron-poor pentafluorophenyl-substituted iron porphyrin catalyst.[13] Another important parameter for catalyst optimization lies within the role of the axial ligand of iron porphyrin complexes and several reports suggest favorable effects of nucleophilic additives such as triphenyl phosphine or arsine, dimethylamino pyridine and *N*-methyl imidazole that as a significant influence on the diastereoselectivity of the cyclopropanation reaction.[14] Carreira and Morandi reported the first systematic study on the influence of this additive on the reaction outcome in the reaction of styrene and trifluorodiazoethane.[15] Aviv and Zeev recently reported on their investigations on the closely related iron corrole complexes in carbene transfer reactions, which exhibit slightly higher reactivity compared to the parent porphyrin complex, yet at the expense of diastereoselectivity.[16] In 2002, Che and co-workers were able to crystallize an iron porphyrin complex with a pendant *N*-methyl imidazole and diphenylcarbene ligand. In this study, the authors could reveal that *N*-methyl imidazole significantly weakens the Fe–C bond and ultimately facilitates the carbene transfer by a strong *trans* influence.[14] In the years after, several groups reported on chiral variations of the porphyrin ligand system, essentially by introducing chiral groups into the methine bridge positions,[7b,14,17] yet only moderate enantioselectivities in the cyclopropanation of styrene with ethyl diazoacetate could be achieved (up to 86% ee)[14] and all approaches using iron porphyrin complexes are limited in enantioselectivity.

Further important iron catalysts involve the application of either nitrogen or phosphorous based ligands, including di- and tridentate ligands.[7,18,19] This approach was demonstrated to be very important in the development of enantioselective iron-catalyzed carbene transfer reactions with different nucleophiles. Importantly, in most cases, high catalyst loadings and weakly coordinating anions need to be applied to ensure high catalyst reactivity, which hampers the overall sustainability of these catalysts (Figure 2).[18,19]

Another important catalyst in this context, is the TBA[Fe] ($Bu_4N[Fe(CO)_3(NO)]$) complex that was introduced by Plietker and co-workers. This particular complex features among iron-based carbene transfer catalyst an NO ligand that can act as a non-innocent ligand and thus influence the electronic properties of the metal-carbene complex (Figure 2).[20]

Today, iron complexes have thus emerged as versatile catalysts for carbene transfer reaction, yet the development of catalysts that exhibit both high reactivity and enantioselectivity remains a challenge in synthetic organic methodology.

Rene M. Koenigs obtained his PhD in 2011 from RWTH Aachen University under the guidance of Prof. Magnus Rueping. He subsequently moved to Grunenthal GmbH, working as a medicinal chemist on GPCR and ion channel targets in pain and inflammation research. In 2015 he was appointed as Junior-professor at RWTH Aachen University. His research interests focus on the discovery of new reactivity using metal-catalyzed and photochemical carbene transfer reactions, continuous-flow chemistry and fluorine chemistry.

Martin J. Weissenborn completed his M.Sc in 2008 at the University of Lund, Sweden, and his Chemistry Diploma (Dipl.-Chem.) in 2009 at the University of Kiel, Germany. His diploma thesis was under the supervision of Prof. Thisbe Lindhorst. From 2009–2012 he did his Ph.D. at the Manchester Institute of Biotechnology in the lab of Prof. Sabine Flitsch. In 2012 he moved to Stuttgart, Germany, and did his post-doc in the group of Prof. Bernhard Hauer. In 2016 he had a research-stay in the lab of Prof. Don Hilvert at the ETH Zürich, Switzerland. Since autumn 2016 he is jointly appointed Junior-Professor at the Martin-Luther-University Halle-Wittenberg, Institute of Chemistry, and the Leibniz Institute of Plant Biochemistry. His research interests focus on discovery and directed evolution of novel non-natural enzymatic reactions and the development of fungal peroxygenases for C-H hydroxylations.

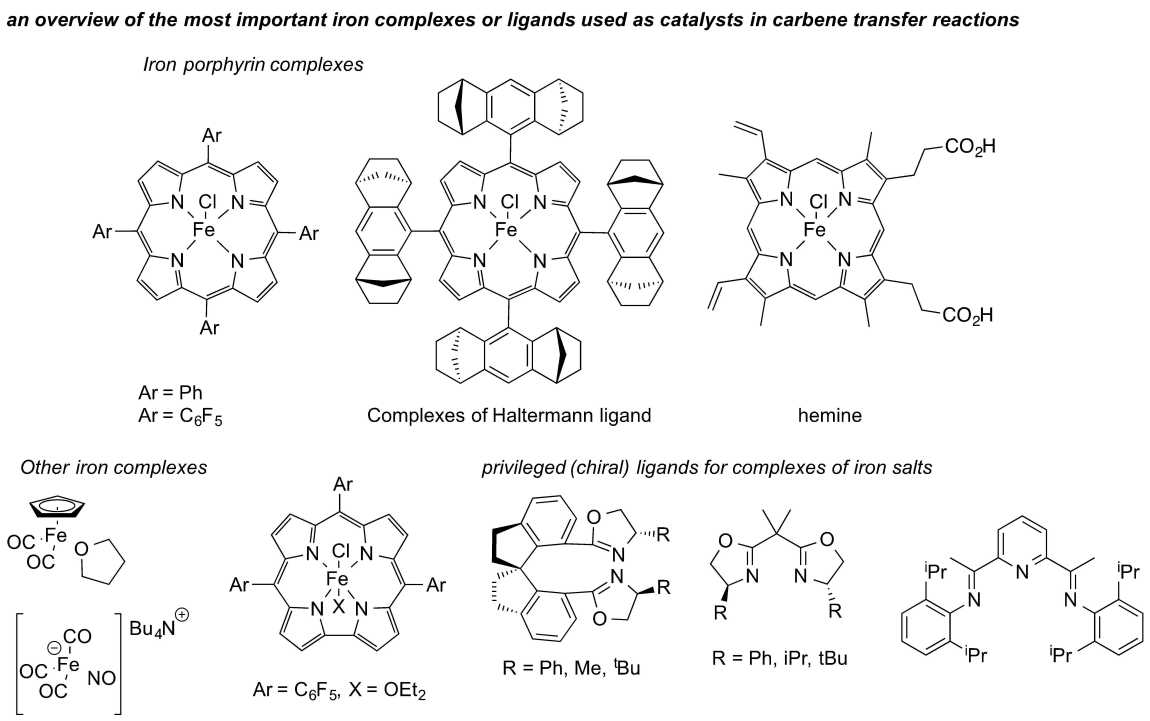


Figure 2. An overview on the most important iron complexes or ligands used in iron catalyzed carbene transfer reactions.

Enzymatic carbene-transfer: inspired by organic synthesis

Derivatives of the cofactor of P450 enzymes, heme b, are regularly used in synthetic organic methodology to conduct carbene transfer reactions that are unknown to nature.[13] The advances on iron porphyrin catalyzed carbene transfer reactions stimulated one of the most rapidly expanding research areas of current chemistry. Arnold and co-workers reported 2013 the first application of an engineered P450 from *Bacillus megaterium* ($P450_{BM3}$) enzyme in cyclopropanation reactions of styrene and ethyl diazoacetate. Enzyme variants, being either *cis*- or *trans*-specific, were engineered with enantioselectivities up 97% *ee* and turnover numbers of 360.[9] Engineering the axial ligand residue of the heme b allowed preparative scale transformations thus solving a long-standing challenge in organic synthesis.[21]

In this section, we give an overview of the protein and cofactor developments with a focus on the reducing agents and give examples that by using different axial ligands or porphyrins no reduction equivalent is required, which now also allows enzymatic carbene transfer reactions without strict exclusion of oxygen.

One major difference between most enzymatic carbene-transfer reactions and the organic methodologies is the requirement of the reducing agent sodium dithionite to obtain Fe(II) in biocatalytic reactions. In $P450_{BM3}$ this reduction is performed by the reductase domain, which carries the cofactors FAD/FMN and transfers one electron from NAD(P)H to the heme-domain for the initial heme reduction step.[22] This electron transfer only occurs after a spin-shift of Fe(III) from low spin to high spin, which is induced by the bound substrate. This shift raises the reduction potential of Fe(III)-to-Fe(II) and thus allows electron transfer from the reductase to the heme domain. As the molecules for the carbene-transfer reactions bind significantly weaker than the natural substrates, this spin-shift does not occur and hence permitting heme-reduction by NAD(P)H. Sodium dithionite ($E^\circ = -660$ mV) is therefore applied as it harbors a significantly higher reduction potential than NAD(P)H ($E = -320$ mV). The use of the recyclable, natural, non-toxic reducing agent NAD(P)H, which is advantageous for metabolic engineering approaches, requires raising the Fe(III)-to-Fe(II) reduction potential. This is achieved by significant mutational changes on the heme-distal side as well as altering amino acid residues fixing the heme prosthetic group. Arnold and co-workers approached it differently and exchanged the axial Cys residue, where the sulfur complexes iron, to the amino acid Ser (oxygen-iron complex). With this modification, heme reduction by NADH even proceeded in absence of the reductase domain (Table 1, Entry 1).[21]

In the organometallic approaches, the axial ligand can be readily altered and the heme environment can be varied by screening solvent and additives. Changing the Fe-coordinating ligand in proteins, as discussed above, requires genetic manipulations and is hence naturally limited to the canonical amino acids. With respect to the axial ligand, it is crucially important to consider the similarity in size to the wildtype amino acid and to provide the ability of coordinating the Fe ion. Most carbene-transferase variants harbor the proximal ligand Cys, His or Ser, *i.e.* the heteroatoms S, N or O.

Table 1. Overview of protein engineering, (non)-canonical axial ligands and cofactor alterations and their influence on oxygen tolerance and reducing agent requirements.

Entry	Reaction	Enzyme (Variant)	Remark	Mutations	Axial Ligand	Conv. [%] (TON)	*ee* [%]	Red. agent	Tolerates O_2	Ref.
1	**Cyclo-propanation**[a]	$P450_{BM3}$ ($P411_{BM3}$-CIS)	purified prot.	14	Ser	70 (437)	98[b]	NADH	yes	[21]
2		Myoglobin (Mb(H64 V,V68 S))	whole cells	2	His	76 (515)	99.9[c]	$Na_2S_2O_4$	yes	[23]
3		Myoglobin (Mb(H64 V, V68 A)(NMH)	purified prot.	3	NMH[d]	80 (800)[e]	>99[c]	none	yes	[11]
4		$P450_{BM3}$ (WIVS-FM T268 A/FeDPIX)	purified prot.	7	Cys	12 (59)	37[c]	$Na_2S_2O_4$	yes	[12a]
5		Myoglobin (Mb(H64 V,V68 A[Fe(Ce6)])	purified prot.	2	His	57 (570)	92[c]	none	yes	[12b]
6	**C–H Functional**	$P450_{BM3}$ (P411-HF T327P A328 S)	whole cells	28[f]	Ser	74 (1670)	-	none	yes	[24]

[a] All selected values based on the reaction of styrene with ethyl diazoacetate, [b] Referring to the *cis* cyclopropane-product, [c] Referring to the *trans* cyclopropane-product, [d] NMH = N_δ-methylhistidine, [e] calculated from bar charts in supporting information, [f] total mutations from $P450_{BM3}$ WT to P411-CIS (14 mutations) to P411-HF (12 mutations – although some are at the same residues as previously mutated in P411-CIS) and T327P A328 S (2 mutations).

Fasan and co-workers showed remarkable activities and gram-scale synthesis of cyclopropane carrying drugs using the natural His ligand (Table 1, Entry 2).[23] As the canonical amino acid alphabet is limited, Hilvert *et al.* introduced the non-canonical amino acid N_δ-methylhistidine (NMH, Figure 3), which is comparable to the organometallic approach by Che and co-workers using N-methyl-histidine (see above).[14] This way Hilvert *et al.* were able to further increase the electrophilicity of the iron and thus improve the activity and selectivity of myoglobin in cyclopropanation reactions. Most remarkably, this myoglobin retained activity in absence of any reducing agent and tolerated oxygen (Table 1, Entry 3).[11]

Since the alteration of the Fe reduction potential is moreover possible by modifying the porphyrin system, Brustad and co-workers evolved $P450_{BM3}$ to selectively incorporate non-proteinogenic Fe deuteroporphyrin IX (Fe-DPIX), which could open up novel opportunities regarding metabolic engineering as well as facilitate directed evolution endeavors with non-natural prosthetic groups.[12a]

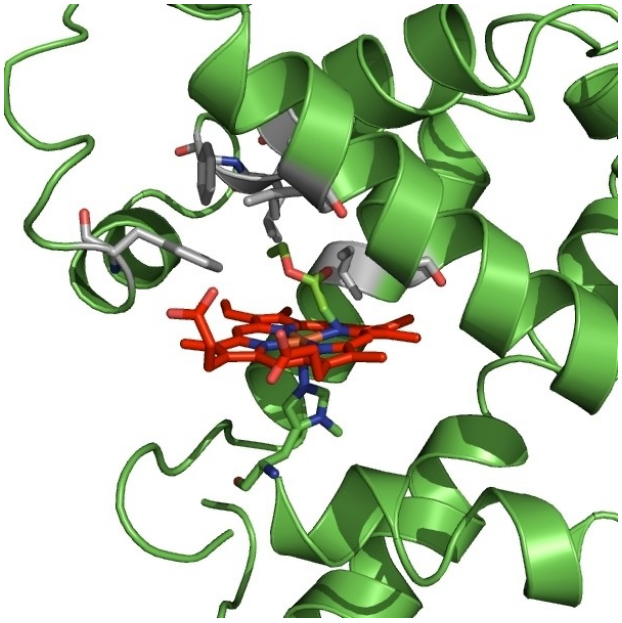


Figure 3. Active site residue of myoglobin variant (H64 V/V68 A) with the heme-iron-carbenoid complex (green). PDB: 6G5B.[11]

Fasan and co-workers showed the incorporation of the Fe-chlorin e6 complex into myoglobin leading to very high catalytic efficiencies in presence of oxygen and performed well in absence of a reducing agent (Table 1, Entry 5).[12b] Arnold and coworkers achieved the independence of a reducing agent and oxygen tolerance by extensive rounds of directed evolution and thereby solely relying on natural amino acids and natural prosthetic groups (Table 1, Entry 6).[24] This highlights the strength of directed evolution and the ability of proteins to be evolved towards required properties. However, the necessary 28 mutations also show the required effort to be invested.

These examples illustrate that biocatalytic approaches towards carbene-transfer reactions were rapidly adopted and in parts solved long-standing methodological limitations as well as identified novel reactivities. As outlined below, further attempts towards enantioselective X–H and in particular C–H insertion reactions represent the challenges in the near future.

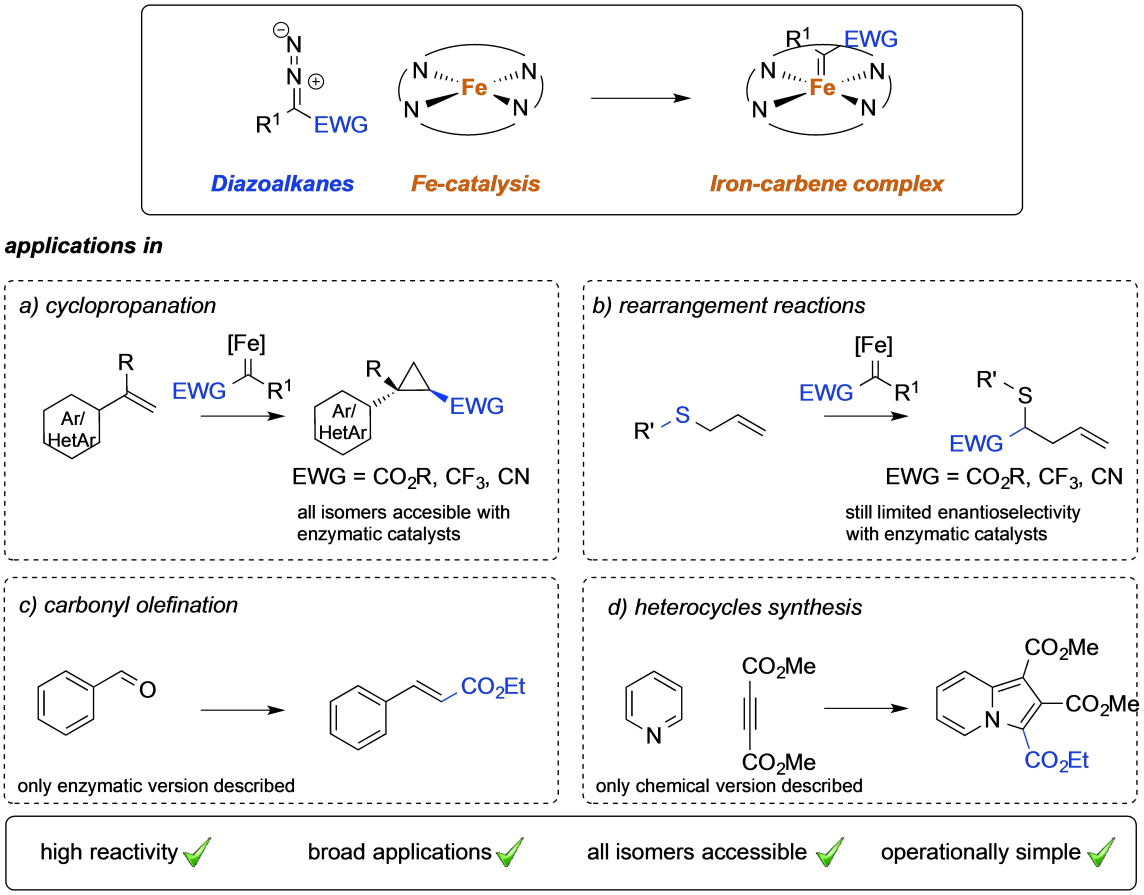
Applications of iron catalysts in carbene transfer reactions

Since the seminal work of Hossain on iron-catalyzed carbene transfer reactions[8] the field has rapidly grown and today iron complexes serve as a versatile catalysts to conduct cyclopropanation reactions as briefly outlined above. With the advent of directed evolution, iron-heme containing enzymes are gaining interest to conduct non-natural reactions with enzymes to obtain high-valued chemicals with broad applications in medicinal chemistry, agrochemistry and process chemistry.[7,10]

Iron-complexes find numerous applications to conduct efficient carbene transfer reactions with mostly acceptor-only diazoalkanes, which could be seen as the major limitation of current iron-porphyrin catalysts. One of the most prominent examples lies within cyclopropanation reactions of ethyl diazoacetate, trifluoro diazoethane, diazomethane and more recently using diazoacetonitrile as reported by the Woo, Carreira, Simmoneaux, Gallo, Koenigs, Gross and other groups.[13,14,15–17,25,26] Ethyl diazoacetate can be readily used as a commercial reagent, trifluorodiazoethane[15] and diazoacetonitrile[25] need to be generated – for safety reasons – *in situ* from the corresponding hydrochloride salts under acidic reaction conditions. Contrarily, diazomethane can be generated *in situ* under basic reaction conditions, which underlines the high efficiency and stability of iron porphyrin complexes.[26] Yet, it should be mentioned that strict exclusion of oxygen needs to be achieved to obtain a high efficiency of iron-heme complexes in carbene transfer reactions.

While the development of enantioselective carbene transfer reactions with synthetic iron complexes in cyclopropanation reactions is still limited and only moderate enantioselectivies can be obtained (up to 86% ee),[14] the evolution of P450 enzyme catalysts now enables enantioselective cyclopropanation reactions with high turnover numbers (up to 67,800).[21] The power of enzymatic catalysts lies in the ability to selectively access all possible isomers in cyclopropanation reactions of styrene with diazoalkanes using the strategy of directed evolution (Scheme 1a). However, a key limitation of enzymatic transfer reactions still lies within the application of acceptor-only diazoalkanes and alkyldiazoacetates.

Similarly, sigmatropic rearrangement reactions[27] can be conducted as initially reported by van Vranken using TMS-diazomethane or ethyl diazoacetate, allylic sulfides and the Fe(II)Cl_2dppe complex as catalyst.[28] Further important advances were reported in the years after by van Vranken,[29] and Plietker using Fe(TBAFe) catalyst.[20] Gross and Aviv described the application of Fe(III) corrole and porphyrin complexes in this transformation.[16] In 2017, Koenigs and co-workers reported on the *in situ* generation of acceptor-only diazoalkanes in Doyle-Kirmse reactions using Fe(III)TPPCl (TPP = *meso*-tetraphenylporphyrin) as a catalyst[30] and more lately in dealkylative rearrangement reactions using an Fe(II)phthalocyanine catalyst.[31] Importantly, catalyst loadings as low as 0.01 mol-% could be used (Scheme 1b). The Dowden group reported In 2016 on the iron-catalyzed ylide formation of ethyl diazoacetate with *N*-heterocycles, which undergo a subsequent [2+3] cycloaddition



Scheme 1. A selected overview of iron porphyrin catalyzed carbene transfer reactions: a) cyclopropanation reactions using synthetic and enzyme-based iron catalysts; b) rearrangement reactions using synthetic and enzyme-based iron catalysts; c) enzymatic carbonyl olefination reactions; d) heterocycles synthesis with synthetic iron catalysts.

reaction with dipolarophiles yielding important indolizidine heterocycles (Scheme 1d).[32] Despite these advances, enantioselective applications using organometallic iron complexes remained elusive and the application of engineered enzymatic catalysts should theoretically enable asymmetric rearrangement reactions. In this context, Fasan *et al.* reported on the only enzymatic sigmatropic rearrangement reaction and could demonstrate proof-of-principle studies on enantioselective Doyle-Kirmse reactions using the Myoglobin variant Mb(L29A, H64V) with turnovers of 390.[33] The same authors thereafter expanded this approach in a systematic study on [2,3]-sigmatropic rearrangement using allylic and propargylic sulfides. The most active variant they identified was the triple-mutant Myoglobin (L29 S, H64 V, V68F) which yielded up to 8820 turnovers and yields of $>99\%$ and up to 71% ee (Scheme 1b).[34] When comparing this result with the latest developments in asymmetric Rh(II), Ni(II) or Cu(I) catalyzed rearrangement reactions, iron-based catalysts or enzymes are still inferior.[35]

The reaction of carbonyl compounds with diazoalkanes in the presence of metal catalysts and triphenyl phosphine was initially reported by Lebel in 2004 and constitutes an organometallic variant of the classic Wittig reaction.[36] In the seminal report on carbonyl olefination reactions, Lebel *et al.* could demonstrate that Wilkinson's catalyst is efficient in the reaction of TMS-diazomethane and aliphatic or aromatic aldehydes to give the corresponding methylenation products. Since then, different catalysts based on precious metals have been employed to further demonstrate the applicability of this transformation, yet, iron complexes have not been employed in this reaction until now. In 2016 Fasan[37] and Weissenborn,[38] achieved the biocatalytic carbonyl olefination employing myoglobin and YfeX variants, respectively. The best myoglobin variant F43 V/V68F up to 185 turnovers with the additive triphenylphosphine. Weissenborn et al. demonstrated with YfeX that the final step of this Wittig-type formation happens outside of the active site, by comparing the diastereoselectivities with synthesized phosphetanes. Excellent selectivities and influence of the protein scaffold on the selectivity of the reaction, however, could be demonstrated by Fasan as well as Weissenborn by employing $AsPh_3$ with up to 99.9% de. Fasan achieved furthermore excellent turnovers of 4230. Weissenborn identified the carbonyl olefination reaction in absence of phosphines/arsines, yet only small turnover numbers (up to 4) were achieved.

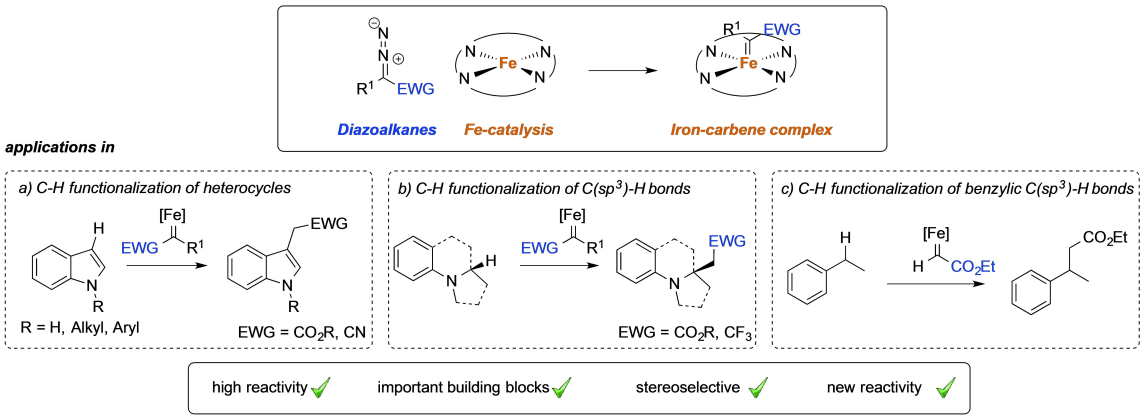
From X-H towards modern C-H functionalization reactions

The direct functionalization of X–H bonds with diazoalkanes furnishes valuable tertiary amines, sulfides, ethers or can be used to introduce boron or silicon functional groups onto a molecular scaffold for further functionalization.[39] Today, X–H functionalization reactions have been described with different synthetic iron catalysts and P450 enzymes, ranging from simple iron salts, iron porphyrin complexes and different alcohols, amines, silanes, boranes and other X–H functional groups.[40] In the presence of chiral ligands or when using P450 enzymes even enantioselective X–H functionalization reactions can be achieved.[41]

The direct functionalization of inert C–H bonds is one of the most challenging reactions in modern organic synthesis. Different strategies, relying either on the activation of a C–H bond by appropriate metal complexes in the presence of directing groups or catalyst-specific direct C–H functionalization reactions are among the key players in this research area. The insertion reaction of carbenes into C–H bonds dates back to seminal reports by Meerwein in 1942 and Doering in 1959,[42,43] yet missing control on the reactivity of free carbene intermediates remained challenging. With the emergence of Rh(II)-paddle-wheel complexes, it is today possible to formally insert a carbene fragment into different C–H bonds in a chemo- and regioselective manner.[5]

Contrarily, iron-catalyzed C–H functionalization reactions remained elusive. Following an initial report on the C–H functionalization of indole heterocycles with donor-acceptor diazoesters using iron complexes bearing a dinitrogen ligand,[44,45] Fasan et al. and Koenigs together with Weissenborn reported on the C–H functionalization of protected and unprotected indole heterocycles using myoglobin, YfeX or Fe(III)TPPCl as catalyst,[46,47] followed by a report by Arnold et al. on the same transformation using a P411 variant (Scheme 2a).[48] These latest examples were all reported almost at the same time and underpin the very closely related progress in the fields of carbene transfer reactions using enzymatic and synthetic iron catalysts.

The C–H functionalization reaction of indole heterocycles occurs in an already activated position of the heterocycle; the direct functionalization of inert aliphatic C–H bonds remained a long-standing challenge. The challenges lie within site-selectivity between C–H bonds at primary, secondary or tertiary carbon atoms. Only recent developments in the area of Rh(II) catalysts could showcase that synthetic catalysts can be used to conduct site-selective C–H functionalization of hydrocarbons. To the best of our knowledge, synthetic iron catalysts remain a curiosity in this research area.[49] Recently, the White group reported in intriguing site-selective intramolecular C–H functionalization reaction using an iron phthalocyanine catalyst.[50] In this research area, the use of engineered enzymes is beneficial and Arnold and co-workers just reported on an intermolecular reaction of fluorinated, acceptor-only diazoalkanes with tertiary amines, which undergo a site-selective and enantioselective C–H functionalization reaction in the α-position of the amine (Scheme 2b).[51] One recent example in enzymatic carbene-transfer reaction of Arnold and co-workers describes direct C–H functionalization reactions on benzylic, allylic, propargylic and α-amino sp^3-carbons. These results were achieved by directed evolution starting from 13 TON and yielding up to 2,020 TON as well as 96.7:3.3 e.r. (Scheme 2c).[52]



Scheme 2. Iron catalyzed C–H functionalization reactions.

Conclusion

Iron complexes have emerged as an important class of catalysts to conduct efficient carbene transfer reactions. Bio-inspired iron complexes serve as privileged catalysts to enable cyclopropanation, rearrangement or X–H and C–H functionalization reactions, yet with the limitation of only moderate enantioselectivity. These chemistry-driven approaches recently resulted in "chemo-inspired" enzymatic carbene transfer reactions using engineered iron-heme containing enzymes to conduct stereoselective cycloaddition reactions and C–H functionalization reactions. A limitation of currently available protocols lies within the specific use of acceptor-only diazoalkanes. Only few applications of other diazoalkanes are reported and it is expected that future developments will address these current limitations. Further developments in this research area are expected to broaden the reactivity of chiral iron complexes to achieve higher catalyst turnovers or machine learning approaches to further expand the possibilities of engineered enzymes.[53,54]

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: iron · carbine · diazoalkane · biocatalysis · directed evolution

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