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Utilizing the Trispyrazolyl Borate Ligand for the Mimicking of O_2-Activating Mononuclear Nonheme Iron Enzymes

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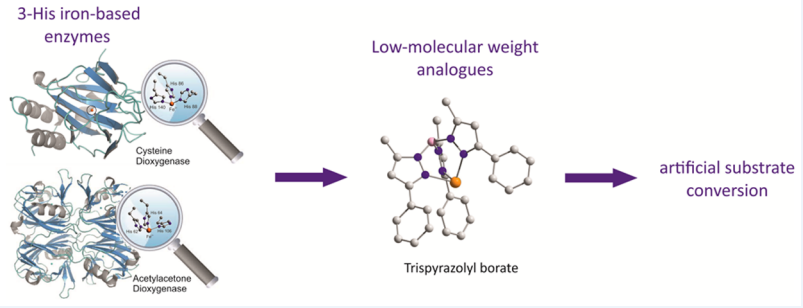


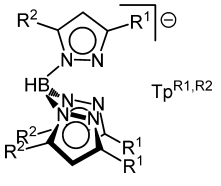
CONSPECTUS: Mononuclear, O_2-activating nonheme iron enzymes are a fascinating class of metalloproteines, capable of realizing the most different reactions, ranging from C−H activation, via O atom transfer to C−C bond cleavage, in the course of O_2 activation. They can lead us the way to achieve similar reactions with comparable efficiency and selectivity in chemical laboratories, which would be highly desirable aiming at accessing value-added products or to achieve degradation of unwanted compounds. Hence, these enyzmes motivate attempts to construct artificial low-molecular weight analogues, mimicking structural or functional characteristics. Such models can, for instance, provide insights about which of the features inherent to an active site are essential and guarantee the enzyme function, and from this kind of information the minimal requirements for a biomimetic or bioinspired complex that may be applied in catalysis can be derived. On the other hand, they can contribute to an understanding of the enzyme functioning. In order to create such replicates, it is important to faithfully mimic the surroundings of the iron centers in their active sites. Most of them feature two histidine residues and one carboxylate donor, while a few exhibit a deceptively simple $(His)_3Fe$ active site. For the simulation of these, the trispyrazolyl borate ligand (Tp) particularly offers itself, as the facial arrangement of three pyrazole donors is reminiscent of the three histidine-derived imidazole donors. The focus of this Account will be on bioinorganic/biomimetic research from our laboratory utilizing Tp ligands to develop molecular models for (i) two representatives of the $(His)_3Fe$-enzyme family, namely, the cysteine dioxygenase (CDO) and acetyl acetone dioxygenase (Dke1), (ii) a related but less well-explored variant of the CDO—the 2-aminoethanethiol dioxygenase—as well as (iii) the 2-His-1-carboxylate representative 1-aminocyclopropane-1-carboxylic acid oxidase (ACCO). The CDO catalyzes the dioxygenation of cysteine with O_2 to give cysteine sulfinic acid, which could be mimicked at TpFe units in a realistic manner. Furthermore, the successful dioxygenation of 2-aminoethanethiol at the same complex metal fragments lends further support to the hypothesis that the active sites of CDO and the one of 2-aminoethanethiol dioxygenase, whose structure is unknown, are quite similar.

Dke1 is capable of cleaving diketones and ketoesters to give the corresponding carboxylic acids and α-keto aldehydes, and Tp-based models have achieved comparable C−C bond cleavage reactions. The ACCO develops ethylene from ACC in the course of oxidation, and recently this has been achieved the first time for a TpFe model, too.

1. INTRODUCTION

Since the first description of the parent hydrido-trispyrazol-1-ylborate, Tp, and first complexes almost 50 years ago, this system has developed to a rather popular ligand in coordination chemistry,[1] especially after the development of the hexamethylated version Tp* (R^1, R^2 = Me) and the second Tp generation: In 1986, Tp-derivatives were introduced, which contained bulky substituents in the 3-positions of the pyrazole units and thus allowed steric control.[1]

Initially, one research line focused on a comparison of Tp and Tp* with cyclopentadiende anions, Cp and Cp*, in



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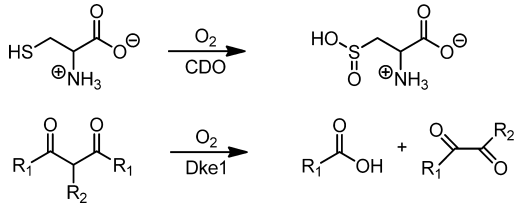
organometallic conversions including catalysis.[1] Indeed, both Tp and Cp carry a single negative charge, donate six electrons to a metal center, and occupy three facial coordination sites but apart from that both ligands should be seen individually, with their specific advantages and disadvantages. One special feature of Tp is that the facial arrangement of three pyrazole donors is reminiscent of the three histidine-derived imidazole donors which coordinate metal ions in certain enzymes. Hence, Tp has been employed increasingly also in the area of bioinorganic chemistry to mimic active sites containing His_3M moieties (M = Zn (carbonic anhydrase), Mn (superoxide dismutase), Cu (tyrosinase, catechol oxidase, hemocyanin)). The earliest example of enyzme modeling using Tp ligands has been provided by Lippard and co-workers, who aimed at mimicking hemerythrin, a dinuclear iron enzyme where two carboxylate-bridged iron centers are coordinated by five histidine residues.[2]

Later on, also Tp-based replicates of *mononuclear* iron proteins were developed,[3] in particular for dioxygenases, that is, representatives which activate O_2 for the transfer of both O atoms to a substrate, and in the following some of these enzymes will be discussed in more detail.

Among the nonheme iron dioxygenases, the most prominent family features a single iron atom coordinated by two histidine donors and one amino acid derived carboxylate function (aspartate/glutamate), the so-called 2-His-1-carboxylate facial triad.[3] Although the Tp ligand provides three N donors, it has also been employed successfully to imitate the 2-His-1-carboxylate facial triad. The disadvantage of one mismatched donor atom is compensated by an adequate charge situation, and hence models, for various members of this family, including for instance, also catechol-1,2-dioxygenase or the α-keto acid dependent enzymes, could be developed.[4,5b]

There are, however, also dioxygenases, where the iron center is bound exclusively by three histidine residues. The first enzyme of that type which has been characterized structurally is the cysteine dioxygenase (CDO).[6,7] It occurs in the cells of mammals and catalyzes the irreversible oxidation of cysteine with dioxygen to cysteine sulfinic acid (see Scheme 1), which represents the last step of the cysteine catabolism.[6]

Scheme 1. Two Nonheme Iron Oxygenases with 3-His Ligand Spheres in Their Active Sites



A further example of a dioxygenase with a $(His)_3Fe$ core is the acetylacetone dioxygenase (Dke1), which mediates the degradation of acetylacetone with O_2 to give acetate and methylglyoxal,[8] Both the CDO and the Dke1 have been the subject of intense research efforts in recent years.[7]

The focus of this Account will be on bioinorganic/biomimetic research from our lab utilizing Tp ligands to develop low-molecular-weight analogues (i) of these two enzymes (CDO/Dke1), (ii) of a related but less well-explored 86 variant of the CDO (cysteamine oxidase), as well as (iii) of the 2-His-1-carboxylate representative 1-aminocyclopropane-1-carboxylic acid oxidase. In general, one of the main driving forces for the construction of metalloenzyme replicates is the gain of structural or functional insights. They can, for instance, provide information about which of the features inherent to an active site are essential and guarantee the enzyme function, and from this kind of information the minimal requirements for a biomimetic or bioinspired complex that may be applied in catalysis can be derived. On the other hand, such models can contribute to an understanding of the enzyme functioning. The following sections outline what has been achieved in this respect employing Tp ligands in comparison with other systems for the mimicking of the above-mentioned iron enzymes.

2. THE CYSTEINE DIOXYGENASE CDO

a. Structure and Function

The first crystal structure of an unmodified CDO isolated from a rat (*Rattus norvegicus*) was reported in 2006. It featured an Fe^{II} ion within the active site, which was coordinated by three histidine residues and a water molecule, so that a distorted tetrahedral coordination geometry resulted.[9] In 2007, finally Rao et al. succeeded in determining the structure of a human substrate-bound CDO (see Figure 1).[10]

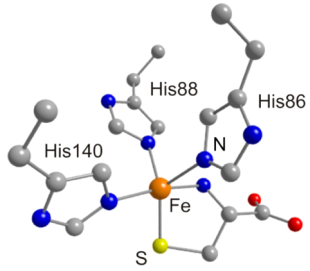


Figure 1. Structure of the CDO substrate complex.

In the substrate complex, a cysteinate ligand binds as a chelating ligand at the His_3Fe unit, via both the thiolate and amino functions, so that the iron center resides in a pentagonal coordination sphere and still possesses one vacant coordination site for the potential activation of O_2. While it is thus commonly accepted that in the first step of the O_2 reaction an iron(III) superoxide species is formed, there is no experimental information on the subsequent steps and corresponding intermediates, as these are rather short-lived. In 2007, theoretical studies were been performed by de Visser and co-workers,[11] and the mechanism derived on the basis of the results is depicted in Figure 2 (mechanism I).

After an end-on coordination of dioxygen at the iron(II) ion (A), the distal O atom attacks at the S atom of the cysteinate ligand, resulting in a structure with a four-membered S−Fe−O−O ring (B). Subsequently an O−O bond cleavage occurs to give sulfoxide and an iron(IV) oxido species (C), which then oxygenates the sulfoxide unit to the sulfinate (D). An alternative suggestion was made by Karplus and co-workers in 2008 based on the results of crystallographic data. Their mechanism included a persulfenate intermediate (E).[12] Hydrogen bonds between the phenol function of Tyr157 and the distal O atom were proposed to support the formation of an S−O bond between the cysteine S atom and the proximal O atom, so that a ring structure (F) is formed, which yields a sulfoxide intermediate (G) in course of the O−O bond cleavage. A rearrangement finally leads to the cysteine sulfinate (D).

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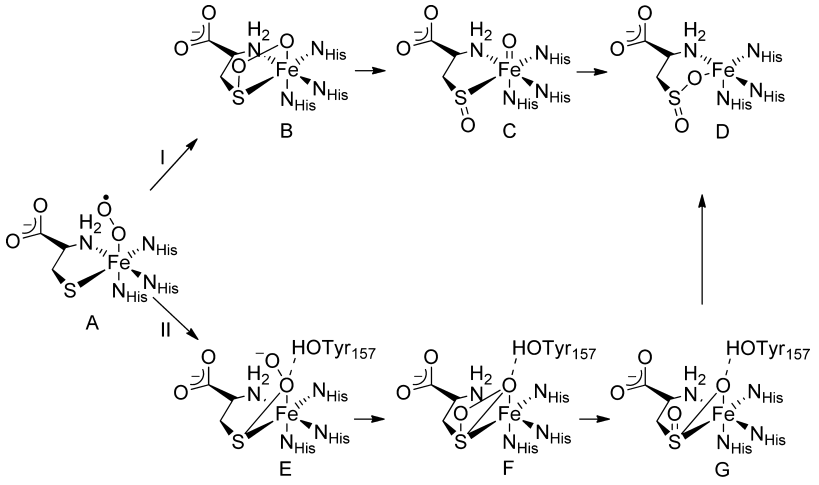

Figure 2. Postulated mechanisms after O_2 activation at the CDO active site according to quantum mechanical calculations (mechanism I)[11] and crystallographic results[12] (mechanism II).

This triggered a further theoretical study in 2011 by de Visser and co-workers, the results of which raised doubts on the occurrence of a persulfenate intermediate (E):[13] It was shown that an attack of the proximal O atom of the Fe^{III}-superoxide to form the postulated persulfenate species and the subsequent reaction steps are associated with much higher energy barriers (>30 kcal mol^{-1}) than reaction path I, on both the singlet and the triplet potential energy surfaces. Hence, path I appeared more realistic. This was supported experimentally in 2013 by Jameson and co-workers studying crystals of persulfenate bound CDO. Persulfenate was proposed to be formed in a side reaction of CDO, blocking the active site, effectively inhibiting it.[14]

b. Model Systems

Until 2010, compounds that could be considered as models for the CDO all contained iron(III) and/or reacted via unclear mechanisms. In attempts to model the CDO function through reactions of RS−Fe^{II} complexes with O_2, often the formation of Fe^{III}−O−Fe^{III} compounds instead of oxygenated sulfur-containing species was observed.[15] In 2010, Goldberg et al. showed for the first time that in an adequate ligand environment an Fe^{II}−SR compound, namely [Fe^{II}(LN_3S) (OTf)] (**1**, Figure 3), can indeed react with O_2 selectively via S-oxygenation. A sterically demanding bis(imino)pyridine (BIP) framework had been chosen to avoid formation of O- or S-bridged compounds in course of oxidation and the SR function had been tethered to the ligand system. Its oxygenation did not lead to a sulfinate, though, but to a sulfonate, so that finally ([Fe^{II}(LN_3SO_3) (OTf)], **2**, was isolated (Figure 3).[16]

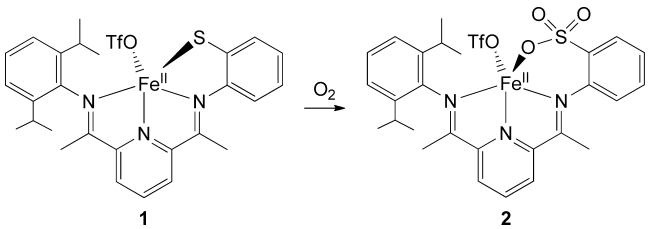

Figure 3. Reactivity of [Fe^{II}(LN_3S) (OTf)] (**1**) toward dioxygen.[16]

Subsequent studies showed that, using BIP as the spectator ligand, also the S-oxidation of terminal, untethered thiolate ligands could be achieved, and it was pointed out that a chelating binding mode as proposed for cysteine binding to the CDO (with strong support from the results of the above-mentioned crystal structure determination[10] of a cysteine complex) is not necessary to accomplish S oxidation.[17] Figure 4 shows the synthesis of the respective precursor complex [(^{iPr}BIP)Fe^{II}(SPh) (OTf)] (**4**) and of an analogue [(^{iPr}BIP)-Fe^{II}(SPh) (Cl)] (**3**), that reacts differently. In both complexes, the iron(II) ions are coordinated in square pyramidal fashions, but in **3** the thiolate is located in the axial position *trans* to the open site of the iron center, while in **4** it is found in pseudoequatorial position *cis* to the open site. Both complexes react with O_2, but **3** leads to the formation of the disulfide indicating that the oxidation occurs at the metal, while in case of **4** (as for **1**) triple oxidation of the S atom was observed. It was thus concluded that a *cis* orientation of the thiolate to the activated O_2 is essential to guarantee S oxygenation.

Beyond that, model studies by Nam, Que, and co-workers, also from 2010, with a cyclam−iron−thiolate complex and a peracid as the oxidant are noteworthy.[18] In dependence on the presence of protons, different reactivities were observed (Figure 5): strongly basic conditions led the iron(IV)-thiolate species, **5**, while in a weakly acidic milieu in the presence of 2 equiv of peracid the iron(II) sulfinate species **6** was formed, which in turn was readily oxidized by an additional equivalent of *m*-

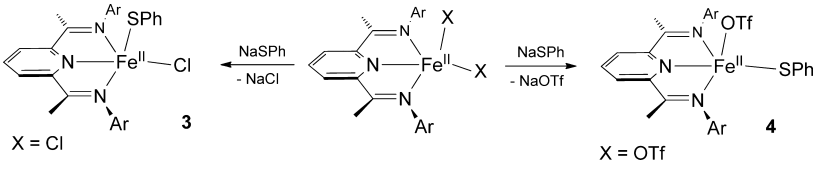

Figure 4. Synthesis of **3** and **4**.[17]

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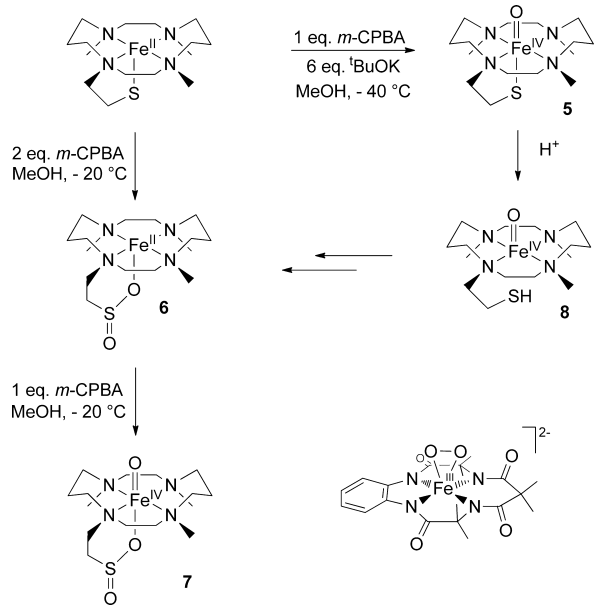

Figure 5. Reaction of an iron(II)-cyclam-thiolate with *m*-CPBA[18] and a structurally characterized iron(III) superoxide.[28]

CPBA to give **7**. The results of experiments probing the mechanism of S oxidation showed that the oxoiron(IV) unit of **5** via **8** could be involved in the oxidation of thiolate to sulfinate, from which it was deduced that an oxoiron(IV) intermediate is plausible in the catalytic mechanism of the CDO.[18]

Recently, for the first time, a synthetic model for an iron(III) superoxide intermediate, proposed to form in the first step of this mechanism, has been structurally characterized, which was also based on a macrocyclic ligand system (see Figure 5 bottom).[28]

We decided to test the Tp ligand system for the simulation of the 3-His coordination sphere at the iron center of the CDO. To create a protective reaction pocket approaching the situation in enzymes and also to prevent the formation of Fe−O−Fe units, phenyl groups at the 3-positions of the pyrazolate units were chosen. Hence, from the palette of TpFe precursor compounds, which we had made accessible,[19] complex $Tp^{Me,Ph}FeCl$ (**9**) was chosen and reacted with L-cysteine ethyl ester hydrochloride (L-HCysOEt*HCl) to yield the desired cysteinato complex [$Tp^{Me,Ph}$FeCysOEt] (**10**, Figure 6).[20]

As in the structure of the substrate complex of CDO,[10] the cysteinate-unit binds as a chelate ligand via the amine and thiolate functions of the S-deprotonated cysteine ethylester, and altogether the resulting immediate (distorted trigonal bipyramidal) coordination spheres of the Fe centers in the CDO and in **10** are very similar (Figure 7).

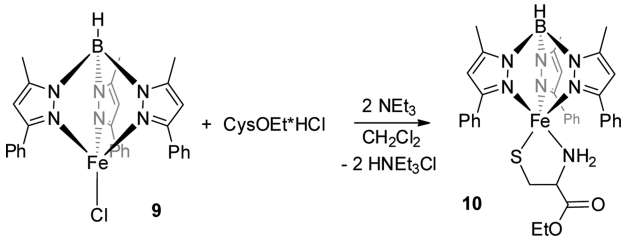

Figure 6. Synthesis of [$Tp^{Me,Ph}$FeCysOEt] (**10**).[20]

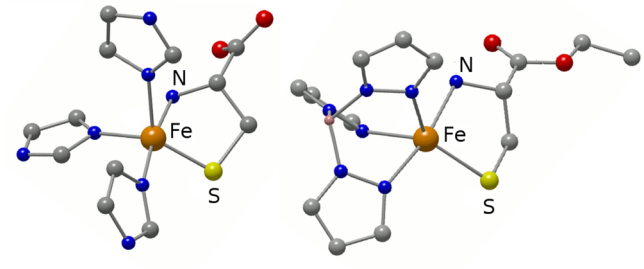

Figure 7. Comparison of the immediate coordination spheres of the iron centers in the substrate complex of CDO (left) and **10** (right). The protein environment and the substituents at the Tp ligand have been omitted for clarity.

To test the potential of **10** to also act as a functional model, a solution in dichloromethane was treated with dioxygen. Subsequently, an analysis by ESI-TOF showed that dioxygenation had occurred, which was supported by using ^{18}O-enriched dioxygen. Through employment of a $^{16}O_2/^{18}O_2$-mixture (~50/50), it could be shown that in this process both O atoms came from the same O_2 molecule. Monitoring of the reaction by paramagnetic NMR revealed that the reaction proceeds slowly (within 8 h) but fairly selectively, yielding mainly one product with iron in the oxidation state + II as confirmed by EPR. Due to the instability of the product all attempts to grow single crystals that would have allowed a characterization via X-ray diffraction have failed so far.

To further clarify the site where oxygenation had occurred and to therefore reveal the constitution of the product a workup procedure was developed for the reaction mixture, which finally allowed for the isolation of the cysteinate part of the reaction product. This was identified as cysteine sulfinic acid ethyl ester (Figure 8) by means of NMR spectroscopy.

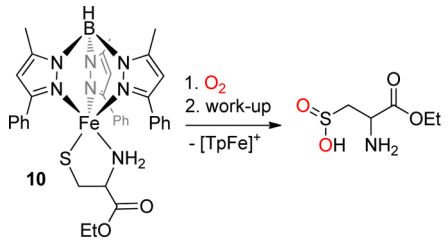

Figure 8. Reactivity of [$Tp^{Me,Ph}$FeCysOEt] toward dioxygen.[20]

Hence, the composition [TpFe($O_2S-CH_2-CH(NH_2)(CO_2Et)$], **11**, was derived for the initial product complex, for which accordingly structural information was pursued, especially as there are no experimental data available for the product complex of the natural enzyme itself. The results of the above-mentioned DFT calculations on its structure[11] had led to the conclusion that the coordination of the sulfinate function proceeds via a η^2-*O,O* binding mode, and indeed η^2-*O,O* binding was predicted for **11**, too (Figure 9),[21] underlining the model character of **10** and motivating investigations to confirm this result experimentally, namely, by solution XANES and EXAFS investigations. EXAFS data supported a η^2-*O,O* binding mode in the model **11** as predicted by theory, and bearing in mind that theory suggests such a coordination also for the

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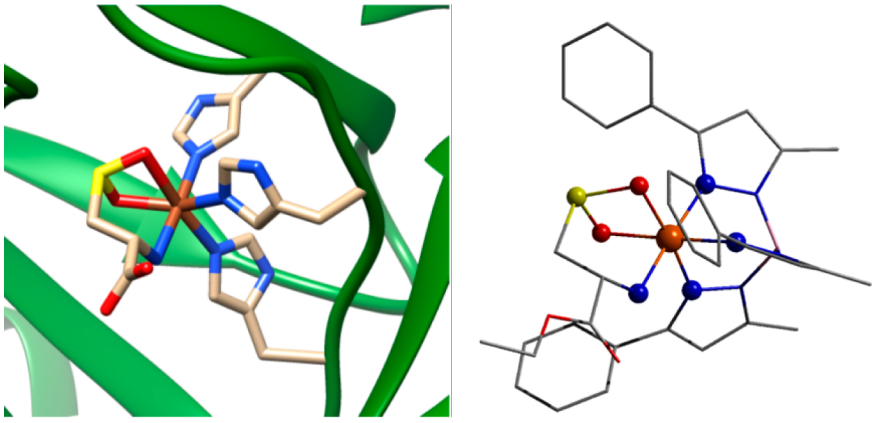


Figure 9. Comparison of the quintet structure calculated by DFT for **11** (right) with the QM/MM optimized structure of the quintet spin CDO product complex as determined in ref 13.

enzymatic product, the experimental model chemistry in turn supports this proposal, too.[21]

10 thus represents a rather realistic model for the active site of cysteine dioxygenase, as it meets two important criteria: (1) The structural similarity: Although it carries a charge, the $Tp^{Me,Ph}$ ligand nicely mimics the facial 3-His coordination sphere of the Fe^{II} center, and the cysteine substrate was only slightly modified by esterification. (2) Also the function is simulated: Treatment with dioxygen leads to dioxygenase activity and a sulfinato complex very similar to the one of the CDO, according to the above-mentioned results likely even with an analogous structure. The theoretical analysis could rationalize that the reaction of **10** with O_2 is comparatively slow: O_2 binding was found to be endergonic and thus represents the rate-determining step.[21] An independent theoretical work (using similar methods) published very recently arrived at similar conclusions, although specific energies and structures varied somewhat.[22]

In 2012, Goldberg et al. presented a further iron(II) thiolate complex that reacted with O_2 via dioxygenation at the S atom.[23] Again, the thiolate function was part of a polydentate ligand system that was used to prepare the precursor $[Fe^{II}(N_3PyS)(CH_3CN)]BF_4$, **12**, and the resulting product **13** (Figure 10) could also be investigated via single crystal X-ray diffraction revealing a coordination of the sulfinate unit via the S atom.

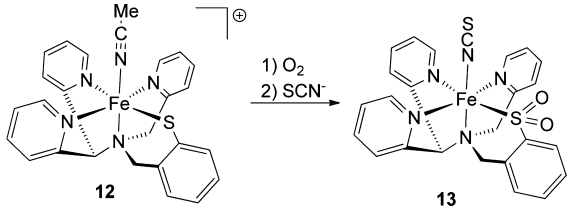


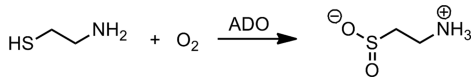
Figure 10. O_2 reactivity of $[Fe^{II}(N_3PySO_2)(CH_3CN)]$, **12**, dissolved in methanol.[23]

Comparison with the previous findings for the systems depicted in Figures 3 and 4 featuring meridional N-donor ligands, which had led to trioxygenation, it was concluded that a facial arrangement of the N donors about the iron center, as in the enzyme, biases the O_2 reactivity toward production of the biologically relevant sulfinato product,[23] and this matches the findings made for **10**.

c. A Close Analogue of the CDO—Cysteamine Dioxygenase?

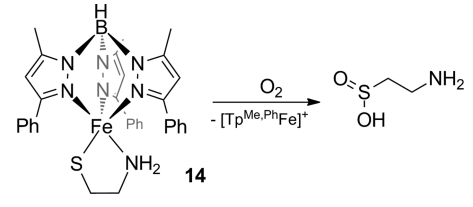
Apart from the cysteine dioxygenase, there is only one other thiol dioxygenase known to be active in mammals: the cysteamine (2-aminoethanethiol) dioxygenase (ADO), which catalyzes the conversion of 2-aminoethanethiol into hypotaurine using dioxygen as the oxidant (Scheme 2).[24]

Scheme 2. Oxidation of Cysteamine to Hypotaurine Catalyzed by the ADO[24]



Compared to the CDO, the ADO has received comparatively little attention, though. It is known to contain iron in its active site, whose nature, however, remains unclear and thus also its functioning. The fact that CDO and ADO both dioxygenate a thiol substrate led to the hypothesis that there is a phylogenetic connection between them.[24,7] Bearing in mind that in our previous work a $TpFe^{II}$ complex with a cysteinate ethyl ester ligand had proved capable of mimicking the CDO both structurally and functionally,[20,21] an investigation on the oxidation of cysteamine at the $Tp^{Me,Ph}Fe^{+}$ scaffold suggested itself to learn more about a potential functioning of the ADO. Hence, we recently synthesized a complex $Tp^{Me,Ph}Fe(SCH_2CH_2NH_2)$, **14**, as a speculative model for the ADO.[25] Indeed, its reaction with O_2 led to the dioxygenation of the S atom and thus to hypotaurine (see Scheme 3). This result lends further support to the hypothesis that the active sites of CDO and ADO are quite similar.

Scheme 3. Reaction of 14 with O_2



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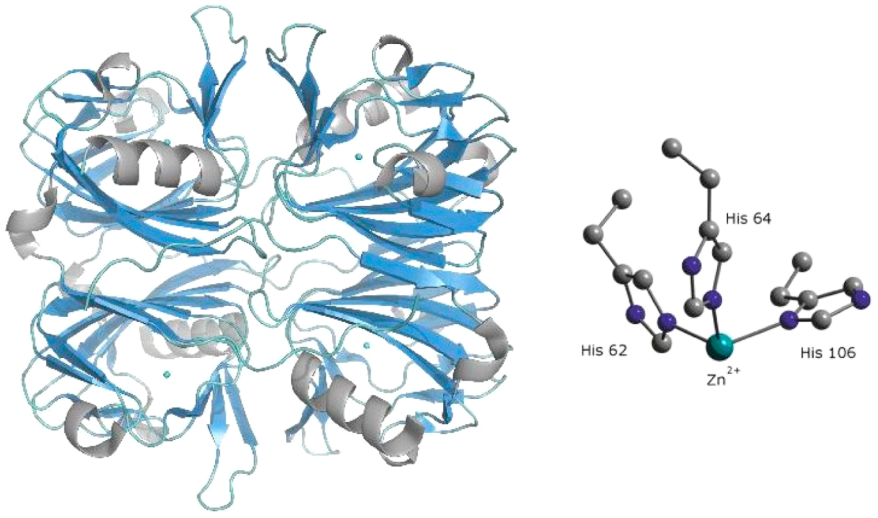

Figure 11. Structure of Dke1 (left) from *A. johnsonii* (PDB code 3BAL) as well as its active site after replacement of Fe^{II} by Zn^{II} (right).[7,27]

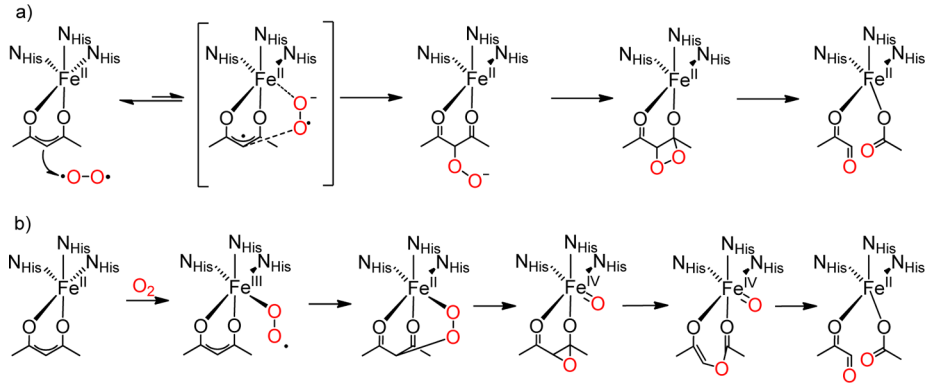

Figure 12. Postulated mechanism for the Dke1 activity after (a) experimental reaction coordinate analysis employing a variety of substrates[30] and (b) according to DFT studies.[31]

3. THE ACETYLACETONE DIOXYGENASE

a. Structure and Function

Acetylacetone (acac) is an important bulk precursor compound for chemical synthesis both in academia and industry.[26] On the other hand acac is toxic for mammals, marine creatures, and microorganisms. The biological conversion of acac to less toxic degradation products is thus of great scientific interest.[26]

In 2002, Straganz et al. reported for the first time about the identification of a bacterium (*Acinetobacter johnsonii*) which decomposes acac with O_2 to give acetate and methyl glyoxal.[27]

The enyzme catalyzing this reaction is the acetylacetone dioxygenase also called Dke1 (diketone cleaving enzyme).[8] Its structure could be revealed by X-ray diffraction of single crystals grown of the enzyme with zinc(II) bound in place of iron(II) (Figure 11).

In the active site, as in the CDO, the iron(II) ion is coordinated facially by three histidine residues. Acetylacetone is assumed to bind in a bidentate fashion, so that a pentagonal coordination sphere results in the substrate bound state, and consequently one coordination site remains open for the potential binding and activation of O_2. Reactivity studies showed that, for each equivalent of acac, which is oxidatively cleaved by the Dke1, one equivalent of O_2 is consumed.[8] With the aid of isotope labeling studies, it could be confirmed that a dioxygenation takes place.[29] Besides acac, the Dke1 is capable of cleaving a series of 1-, 3-, or 5-substituted diketones and ketoesters to give the corresponding carboxylic acids and α-keto aldehydes, however, the dicarbonyl structural motif is essential for activity.[8,27] Based on the results of the above-mentioned first isotopic labeling experiments, an initial deprotonation of acetylacetone followed by attack of O_2 or superoxide at the C_α position to give an organoperoxide unit has been suggested.[29] Contemplating potential subsequent steps, it was noted that the pattern of C−C bond scission in diketone conversion depends strongly on electronic substituent effects with favorable C−C bond cleavage adjacent to the more electron withdrawing group. As this hints to a negatively charged transition state during C−C bond fission, a subsequent nucleophilic attack of the superoxides terminal O atom at the carbonyl C atom with formation of a dioxetane species was suggested; the latter can be expected to decompose to the final cleavage products.[29] In the course of subsequent investigations, it was shown that the apparent turnover number is governed by the electron-donating ability of the substrate. Moreover, from further reactivity studies it was concluded that O_2 reduction and C−O bond formation take place in one single kinetic step and that O_2 thus attacks directly at the substrate, without being bonded to the iron center first (see Figure 12a). In this mechanism the iron(II) center is mainly needed for the breakup of the spin-forbiddance for the reaction of triplet dioxygen with the singlet substrate.[30]

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The results of extensive DFT calculations performed 2011 by Solomon et al. led to a different mechanistic proposal, which, however, is still in line with the experimental results outlined above.[31] It starts with a step which has been found to play a decisive role in conversions of most oxygenating heme and nonheme iron enzymes: the binding of O_2 at the Fe^{II} center under formation of a $Fe^{III}-O_2\cdot$ entity.[3,13] Based on the results of reactivity studies with synthetic models, such a step had also been suggested for the Dke1 (see below[32]), although the apparent strong coupling of O_2 reduction with C−O bond formation as descried above had argued against this[30] (see above). However, the superoxide was found by DFT to be high in energy, thus rationalizing the latter results.[31]

According to computational analysis, the terminal O atom of the superoxide then attacks at the C3 carbon atom, generating a peroxidate intermediate, whose O−O bond cleaves to yield a Fe^{IV}=O species and an epoxide intermediate. The latter rearranges into an ester compound first before it is attacked by the Fe^{IV}=O species and cleaved to the final products (Figure 12, path b).[31]

b. Model Systems

Considerations to model systems can also be found in ref 26. The first compound that can be discussed as a model, $Tp^{iPr2}Fe(acac)$, **15**, was published by Kitajima et al. in 1993.[33] The Dke1 was not known at that time, and hence, the work was not motivated by a biological background or discussed in this context, but obviously the Tp ligand suitably imitates the 3-His coordination sphere of the iron center within the Dke1, and acac represents one of its substrates.

On exposure to air, **15** decomposed within 1 week to yield a trinuclear iron(III) complex (**16**) containing μ-oxo-bis-μ-acetato and μ-hydroxo-bis-μ-acetato ligand constellations (Figure 13).[33] Doubtlessly, the bridging acetato ligands had

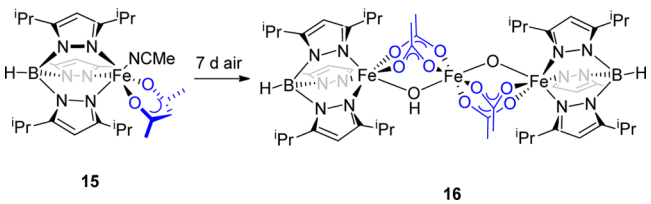

Figure 13. Decomposition of **15** in air to give **16**.[33]

their origin in the acac ligands employed. However, we were able to show for a derivative of **15**, where the isopropyl residues at the ligand were replaced by methyl groups, $[Tp^{Me2}Fe^{II}(acac)]$, that *dried* O_2 does not react with **15** under cleavage of the acac ligand;[32] only upon addition of water to the reaction mixture iron complexes containing acetato ligands could be substantiated, which suggests that the acetato ligands of the previous studies are at least partly due to the water present under aerobic conditions (hydrolysis of the acac ligand following oxidation of the complex).[32] Hence, **15** can be considered as a structural model but not as an adequate functional model.

In 2008, we reported that treating a dinuclear iron(II) complex of the ligand "Xanthmal" featuring two malonato binding pockets (**17**) with O_2 led to the oxygenation of the latter: at the C_α positions, monooxygenation and peroxide formation occurred, partially accompanied by C−C bond cleavage to yield α-keto ester functions (Figure 14).[34]

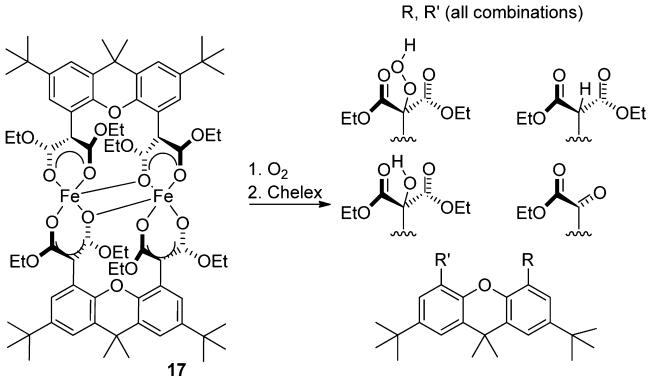

Figure 14. Reaction of $[Fe_2(Xanthmal)_2]$ with O_2 and the products obtained after workup.[34]

The results of mechanistic investigations could be explained postulating the formation of high-valent Fe intermediates, and the mechanistic scheme derived includes several steps that mimic the (suggested) functioning of nonheme iron enzymes. Observation of the oxidative cleavage of aliphatic C−C bonds belonging to the dicarbonyl units with formation of an α-ketoester (Figure 14) moreover stimulated the use of malonates in Dke1 modeling studies with mononuclear TpFe complexes.

As discussed above for the enzyme, the cleavage rate increases with increasing potential of the substrate to act as a donor, and since obviously, unlike the $[His_3Fe]^{2+}$ unit, the $[TpFe]^+$ moiety does not mediate the cleavage of acac,[33,32] most likely for electronic reasons, employment of a more electron rich substrate, like a malonate, promised reactivity. At the same time, malonates are related to β-ketoesters that are cleaved by Dke1, too. To avoid further reaction of the envisaged α-dicarbonyl cleavage product at the C_α position, we employed a derivative with a phenyl substituent in the C_α position (HPhmal).

Hence, the complex $Tp^{Me2}Fe(Phmal)$, **18** (Figure 15), was synthesized and a structural investigation showed that a vacant coordination site was available at the Fe^{II} center for the complexation and activation of O_2.

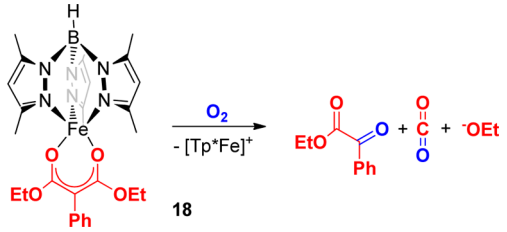

Figure 15. Acetylacetone dioxygenase related activity of $[Tp^{Me2}Fe^{II}(Phmal)]$ (**18**).[32]

Treatment of an acetonitrile solution of **18** with dry dioxygen at r.t. was shown to lead to ethyl benzoylformate, which is one of the products expected in case of a dioxygenase activity of **18** analogously to the function of Dke1 (compare Scheme 1). The second one, $EtOCO_2^-$, apparently decomposed already during the reaction to give ethanolate and CO_2. Isotopic labeling studies with ^{18}O-enriched dioxygen revealed the expected incorporation of one ^{18}O atom into the α-keto ester with an efficiency of 94%, while the other one was incorporated into the CO_2.[32] **18** even proved to catalyze the selective reaction of

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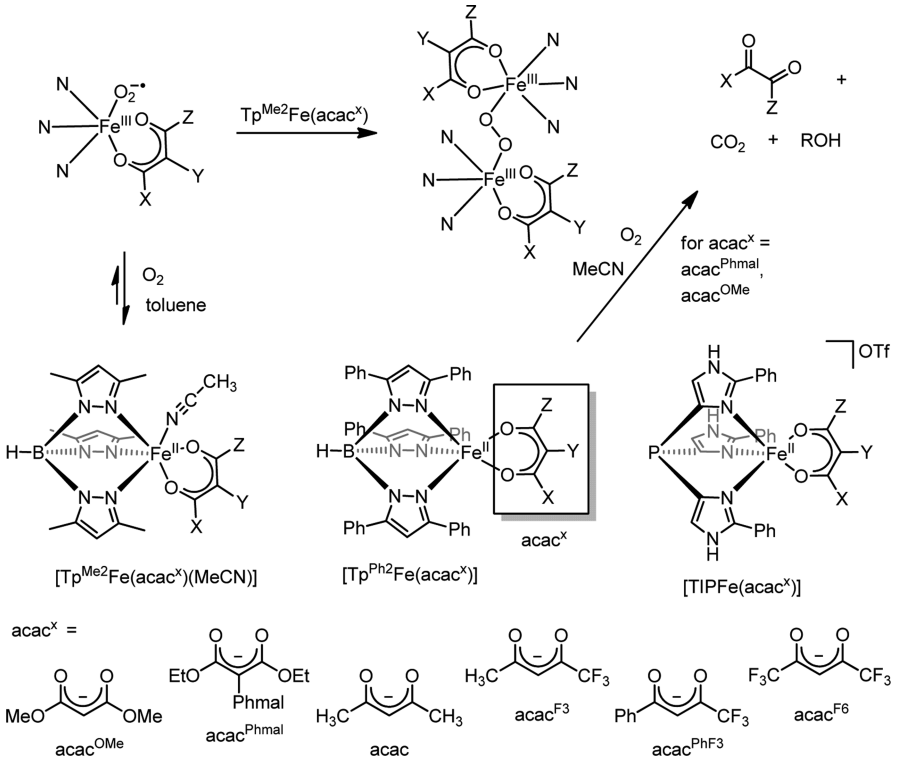

Figure 16. Iron(II) acac^x precursors based on the ligand systems Tp^{Me2} (left), Tp^{Ph2} (middle), and TIP (right) as used by Fiedler et al. as well as their O_2 reactivity.[35,37]

PhmalLi and O_2 to ethyl benzoylformate, CO_2, and EtO^- with a TOF of 55 h^{-1}.

To address the question of whether the malonate cleavage requires an activation of O_2 at the iron(II) center, LiPhmal was reacted with O_2, too, but was found to be unreactive, so that apparently the C═C cleavage requires more than just a Lewis acidic metal center (like Li^+ or in **18** Fe^{2+}). Moreover, **18** was oxidized with $NOPF_6$ and the resulting Fe^{III} compound treated with O_2. Again no cleavage products were formed suggesting that the reaction of **18** takes place via initial activation of O_2 at the Fe^{II} center to yield an iron(III)-superoxido species. Interestingly, rather recently O_2 binding to a five-coordinated TpFe complex with formation of an iron(III) superoxide could be demonstrated spectroscopically, after employment of a strongly electron donating, tightly binding and robust (with respect to oxidative degradation) coligand.[5a] Hence, there is now also direct evidence for the feasibility of such a step.

On the basis of the results gathered for **18**, superoxide formation in the initial step was postulated for the Dke1, too,[32] and indeed 3 years later this idea was supported through the above-mentioned results of calculations for the enzyme.[31]

In 2011, Fiedler et al. published a series of iron-β-diketonato complexes for the structural modeling of the substrate complexes of the Dke1.[35] The 3-His structural motif was mimicked inter alia also using Tp ligands, and the diketonato substrate, acac^x, was varied in its electronic and steric properties (Figure 16) to evaluate the effect such variations have on the structural and spectroscopic features of the resulting complexes and to get detailed insights into their electronic structures. The findings were in line with the observation that variations in the facial triad give rise to only modest spectral perturbations for the Dke1.[36] The results of DFT calculations indicated only a small amount of unpaired spin density on the acac^X ligands in the models and revealed that the frontier MOs are exclusively iron-based, thus again supporting the previous suggestion[31,32] that reaction with O_2 is more likely at Fe than at the ligand.

In subsequent work, it was shown that toluene solutions of the [Tp^{Me2}Fe(acac^x) (MeCN)] series of complexes, with the exception of the acac^{F6} compound, reacted with O_2 at low temperatures instantaneously to yield green intermediates, identified by low-temperature techniques as peroxide species (Figure 16).[37] When the reactions were carried out in acetonitrile as the solvent (with trace amounts of water), some [Tp^{Me2}Fe(acac^x)] complexes finally yielded in trinuclear compounds with bridging carboxylate, oxido, and hydroxido ligands, related to **16**.[33]

Unlike the [Tp^{Me2}Fe(acac^x)] series, complexes with Tp^{Ph2} or TlP ligands exhibited a significantly lower reactivity upon exposure to O_2. The increased steric demand of the phenyl residues at the Tp ligand was, however, excluded as the origin, as NO was shown to bind at the metal center, so that O_2 should have access, too.[37] Interestingly, the O_2 reactions with Tp^{Ph2}Fe(acac^{Phmal}) and Tp^{Ph2}Fe(acac^{OMe}) led to oxidative cleavage, as already observed for the complex **18** by us (Figure 16), although CV measurements showed that Tp^{Ph2}Fe(acac^{Phmal}) was actually harder to oxidize than Tp^{Ph2}Fe(acac), which failed to react with O_2, though. Within the Tp^{Ph2}Fe(acac^X) series, it thus appeared that O_2 reactivity was primarily governed by the relative energies of acac^x π-electrons, not the Fe redox potentials.[37]

Concluding, it was confirmed that only activated, electron-rich substrate ligands (malonates) can be cleaved in model systems for the Dke1 in the absence of supporting secondary interactions.

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4. 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE, ACCO

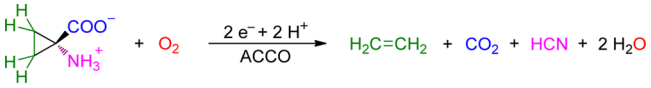
Recently, an oxidase belonging to the 2-His 1-carboxylate family, the ACCO, has intrigued us and motivated investigations to test the potential of the Tp ligand in this context.

a. Structure and Function

As the CDO, the ACCO also oxidatively converts an amino acid, namely, 1-amino-cyclopropane-1-carboxylic acid, to produce ethylene, which is used by plants as a key hormone for development and defense.

The reaction proceeds at an 2-His-1-asp iron center[38] with dioxygen as the oxidant, and ascorbate is needed as the coreductant.[3] The mechanism, by which the complex conversion of Scheme 4 is realized, is still discussed controversially, but it is generally assumed that it follows a radical mechanism.[3] Functional models which (setting out from a related structure) convert complexed ACC in the presence of O_2 to produce ethylene may open the way for further studies that contribute to an improved mechanistic understanding. However, until recently, such models did not exist.[3,39]

Scheme 4. ACCO Conversion



b. Model Studies

Setting out from **9**, we succeeded in preparing [$Tp^{Me,Ph}$FeACC], **19**, which, unlike other published compounds containing ACC as a ligand, at the same time (i) contains iron, (ii) is mononuclear, and (iii) features ACC coordinated in a bidentate fashion (as proposed for the enzymatic substrate complex)[3,38] as well as an open coordination site for the potential binding of O_2. It thus represents an excellent structural model, and indeed also the function could be mimicked: **19** is the first known ACC complex that reacts with O_2 to produce ethylene (Figure 17).

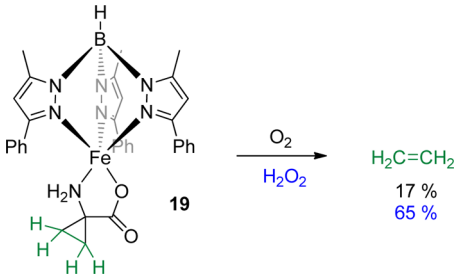


Figure 17. Functional model for the ACCO.

As FeOOH species had been suggested as intermediates of the catalytic cycle, H_2O_2 was tested as the oxidant, too, and indeed evolution of ethylene proceeded even more rapidly with 65% yield.

5. CONCLUSIONS

Tp-based ligands are well-suited for mimicking the 3-His or 2-His-1-carboxylate ligand spheres, which iron ions experience in oxidizing nonheme iron enzymes, as demonstrated in this Account by some examples from our laboratory and others. Functional low-molecular-weight analogues have been developed, which, in combination with independent model studies, biological studies, and quantum mechanical investigations, have contributed to an understanding of mechanistic issues, experimental findings, and structure–function correlations. The synthetic models were often found to convert the substrates relatively slowly despite quite faithful replications of the ligand spheres, and peroxide formation has been observed as a nonbiomimetic competing pathway. Hence, the protein environments probably play several important roles in significantly lowering the intrinsic barriers for the initial step (superoxide formation) and the postsuperoxide steps. For the preparation of biomimetic catalysts, it will be important in the future to unravel these roles. In this context, an interesting question remains: Why do the particular enzymes discussed here favor 3-His coordination spheres over the well-known 2-His 1-carboxylate structural motif?[7]

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Notes

The authors declare no competing financial interest.

Biographies

Madleen Sallmann started her chemistry studies in 2004 at the Christian-Albrechts-Universität zu Kiel, where she received her Diploma in 2009 under the supervision of Felix Tuczek. From January 2010 until October 2014, she worked on her Ph.D. thesis in the group of Christian Limberg at the Humboldt-Universität zu Berlin. Her research is focused on activation of dioxygen with bioinspired mononuclear iron and nickel complexes.

Christian Limberg received his Ph.D. in 1992 in Bochum and subsequently performed postdoctoral work together with A. J. Downs at Oxford University. After completion of his Habilitation in Heidelberg (1999), he was appointed full professor at the Humboldt-Universität zu Berlin in 2002. His research spans the design of molecular metal/oxygen systems inspired by the surfaces of oxide materials/catalysts as well as by the active sites of oxygenating enzymes and the development of homo- and heterobimetallic complexes for the activation of small (inert) substrates (e.g., H_2, N_2, CO_2).

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