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Sulfur Oxygenation in Biomimetic Non-Heme Iron-Thiolate Complexes

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Abstract

The *S*-oxygenation of cysteine with dioxygen to give cysteine sulfinic acid occurs at the non-heme iron active site of cysteine dioxygenase. Similar *S*-oxygenation events occur in other non-heme iron enzymes, including nitrile hydratase and isopenicillin N synthase, and these enzymes have inspired the development of a class of $[N_xS_y]\text{-Fe}$ model complexes. Certain members of this class have provided some intriguing examples of *S*-oxygenation, and this article summarizes these results, focusing on the non-heme iron(II/III)-thiolate model complexes that are known to react with O_2 or in some cases other O-atom transfer oxidants, to yield sulfur oxygenates. Key aspects of the synthesis, structure, and reactivity of these systems are presented, along with any mechanistic information available on the oxygenation reactions. A number of iron(III)-thiolate complexes react with O_2 to give *S*-oxygenates, and the degree to which the thiolate sulfur donors are oxidized varies among the different complexes, depending upon the nature of the ligand, metal geometry, and spin state. The first examples of iron(II)-thiolate complexes that react with O_2 to give selective *S*-oxygenation are just emerging. Mechanistic information on these transformations is limited, with isotope labeling studies providing much of the current mechanistic data. The many questions that remain unanswered for both models and enzymes provide strong motivation for future work in this area.

Introduction

Enzymes containing iron in their active sites provide a wide range of functions in biological systems. Several of them play a key role in dioxygen binding and activation. Reactions of O_2 with many organic compounds are thermodynamically favored, but proceed slowly due to a spin state barrier and the low one-electron reduction potential of O_2 .¹ In the presence of iron-containing oxygenases, however, the iron can either activate dioxygen or the substrate, or both, to catalyze oxidation chemistry. A typical mechanism for non-heme enzymes of this type includes O_2 binding to the iron center, followed by electron-transfer from Fe to O_2 to afford either iron-superoxo ($\text{Fe}^{\text{III}}\text{-O}_2^-$), iron-peroxo ($\text{Fe}^{\text{III}}\text{-O}_2^{2-}$), or iron-oxo ($\text{Fe}^{\text{IV/V}}\text{=O}$) species.^{2–7} These oxygen intermediates are invoked as the active oxidants in various biologically relevant, catalytic oxidations.

In this Perspective article, we focus on progress that has been made in constructing synthetic models of a small subset of these systems. This subset is comprised of mononuclear, non-heme iron enzymes that incorporate thiolate ligands in their coordination sphere and generate *S*-oxygenated products. Mononuclear non-heme iron enzymes that contain thiolate donors include nitrile hydratases (NHase), isopenicillin N synthase (IPNS), and thiol dioxygenases. Nitrile hydratases, which contain both iron(III) and cobalt(III) centers, catalyze the hydrolysis of a wide variety of nitriles to the corresponding amides.^{8–10}

Isopenicillin N synthase (IPNS) utilizes an iron(II) center to catalyze the oxidation of the thiolate-containing tripeptide δ -(L- α -aminoadipoyl)-L-cysteinyl-D-valine (ACV) to generate isopenicillin N.^{11–14} Finally, cysteine dioxygenase (CDO) is a non-heme mononuclear iron(II) enzyme which activates dioxygen to catalyze the oxidation of cysteine to cysteine sulfinic acid.¹⁵

Nitrile hydratase has been studied and reviewed elsewhere,^{8,16–18} but it is important to note here the unique donor set of the iron(III) center. The iron(III) ion is coordinated by two deprotonated carboxamido nitrogens and three cysteine thiolate donors, two of which are posttranslationally modified, one to a sulfenic acid group (Cys-SO) and one to a sulfinic acid group (Cys-SO₂).¹⁷ In the inactive form, NO is bound in the sixth site, but upon photoactivation, water is believed to displace the NO ligand (Figure 1a). This unprecedented coordination environment at iron, along with the industrial importance of nitrile hydrolysis, has inspired much effort in the synthesis of analogs that mimic both the structure and function of NHase. While the catalytic cycle of nitrile hydrolysis does not involve O₂ activation or thiolate oxygenation, the efficacy of this enzyme is dependent upon the posttranslational S-oxygenation of the cysteine residues.^{16,18–20} It has been suggested that O₂, in the presence of the iron(III) ion of the unmodified enzyme, is the oxidant responsible for the oxidation of both cysteinate moieties, although the mechanism of S-oxygenation is not known.¹⁶

In the case of IPNS, on the other hand, the substrate thiolate binds to the iron center in a position *cis* to the proposed O₂ binding site. The active site of IPNS contains the characteristic 2-His-1-carboxylate binding motif of O₂-dependent iron(II) enzymes. The metal ion of IPNS is bound to two His residues and one Asp residue, along with the carbonyl group of a Gln residue and two water molecules in the resting state.^{21,22} The iron(II)-substrate complex was crystallized with NO as a substitute for O₂, and its structure showed that Gln and H₂O were displaced by substrate thiolate and NO, respectively (Figure 1b).²³ The mechanism has been studied and reviewed elsewhere,^{23,24} but the initial steps consist of iron(II) oxidation by O₂ to generate an Fe^{III}-superoxo intermediate, which then abstracts a hydrogen atom from the cysteinyl β -carbon atom to give a thioaldehyde and a hydroperoxo-iron(II) intermediate. Subsequent cleavage of the O-O bond of this hydroperoxo species with concomitant deprotonation of the amide N-H allows simultaneous cyclization of the β -lactam ring and oxidation to an Fe^{IV}-oxo species. This ferryl species activates the C-H bond of the tertiary valine β -carbon to generate an iron(III)-hydroxide intermediate and a carbon-centered radical, which then attacks the cysteine thiolate to close the thiazolidine ring of the final isopenicillin N product, followed by regeneration of the starting iron(II) species. Thus, addition of the native ACV substrate to this enzyme does not result in sulfur oxygenation. However, addition of a series of substrate analogs to IPNS was used to help determine the sequence of oxidative cyclization.^{24–27} One substrate analog, (1-(*S*-carboxy-2-thiomethyl)ethyl ester (ACOmC), was oxidized by IPNS to give a sulfenato (singly oxygenated sulfur) derivative (Figure 1c).²⁸ The iron(II) site of IPNS is normally five-coordinate upon addition of substrate or substrate analogs prior to the binding of O₂, but ACOmC coordinates in a bidentate fashion, with the *S*-methyl sulfur bound in the proposed oxygen binding site. This IPNS/ACOmC complex was turned over upon exposure to O₂ despite being 6-coordinate, which resulted in hydroxylation at the β -carbon of the third residue, as well as formation of an intriguing side-on metal-bound sulfenate, which was definitively characterized by X-ray crystallography (Figure 1c).²⁸

Thiol dioxygenases consist of cysteine dioxygenase (CDO), cysteamine dioxygenase (ADO, 2-aminoethanethiol dioxygenase), as well as the bacterial 3-mercaptopropionate dioxygenase (3MPDO). All three enzymes are iron-dependent and add molecular oxygen to a sulfhydryl group to form a sulfinic acid (Figure 2).²⁹ Cysteine dioxygenase and

cysteamine dioxygenase are important enzymes in the metabolism of cysteine in mammals. CDO catalyzes the oxidation of cysteine to cysteine sulfinic acid. This product can either proceed to form hypotaurine through decarboxylation by cysteine sulfinic decarboxylase, or 3-sulfinylpyruvate, which spontaneously decomposes to pyruvate and sulfite, eventually producing sulfate.³⁰ Meanwhile, ADO catalyzes the oxidation of cysteamine, a decarboxylated cysteine formed in the process of coenzyme A synthesis, to hypotaurine.²⁹ Once hypotaurine is generated, it is further oxidized to taurine. Finally, 3-MPDO is a homolog of cysteine dioxygenase found in bacteria and is responsible for the catabolism of 3-mercaptopyruvate (3MP) to generate 3-sulfinopyruvate.³¹

While the biological role of CDO has been studied for several decades,³² the first crystal structure of CDO was not reported until Phillips and co-workers described the Ni^{II}-substituted active site of mouse CDO in 2006 (Figure 3a).³³ Shortly thereafter, another group reported the crystal structure of rat CDO containing the native iron center.³⁴ The active site of cysteine dioxygenase is atypical of non-heme iron(II) dioxygenases, which generally consist of a 2-His-1-carboxylate facial triad. A third histidine residue replaces the carboxylate ligand in CDO to give three His residues in a facial triad, with a water molecule occupying the fourth coordination site to provide a pseudo-tetrahedral environment around iron (Figure 3b). A subsequent crystal structure obtained from human CDO was reported along with the substrate cysteine bound via its amino nitrogen and thiolate sulfur (Figure 3c).³⁵ In all of these crystal structures, a cross-linked cysteine-tyrosine adduct is observed near the iron(II) center. Some evidence indicated that the thioether linkage of this cofactor was unnecessary for catalysis.^{35,36} However, recently, the non-crosslinked CDO protein was expressed in the absence of O₂ and was proven to have very low activity for the generation of cysteine sulfinic acid.³⁷ The cross-link is formed during the course of cysteine oxidation due to exposure to O₂, which in turn increases the activity of the enzyme, indicating a direct correlation between the amount of cross-linked cysteine-tyrosine and activity.³⁷ The proton of the modified tyrosine is more acidic, and is suggested to possibly play a significant role in hydrogen-bonding to potential oxygen intermediates, as well as providing a source of protons.³⁰

Cysteine dioxygenase employs a high-spin iron(II) ion with few distinguishing spectral features, with the exception of features in the Mössbauer spectra, which have been exploited to discern tight binding of cysteine to the enzyme active site in solution.³⁸ The absence of spectroscopic evidence supporting any dioxygen-bound intermediates in the catalytic cycle of cysteine oxidation by CDO creates difficulties in assigning a mechanism. However, the three crystal structures, along with mutagenesis studies, led to the proposal of several mechanisms for the catalytic cycle.^{33–35} In addition, EPR spectroscopic and computational studies of mouse CDO with NO coordinated to Fe as a surrogate for O₂ support a mechanism where Cys binds prior to O₂.³⁹ Theoretical calculations conducted by de Visser and coworkers provide some further insight into the possible mechanism of CDO.^{40–42} The results of these DFT and QM/MM computations predict that an Fe^{III}-superoxo moiety should form upon initial O₂ binding to the iron(II) center. In a following step, the formation of a cyclic four-membered Fe-O-O-S ring structure is energetically favorable, after which O-O cleavage to generate an Fe^{IV}(O)-sulfenate species occurs (Scheme 1). The S-O bond rotation and subsequent oxo-transfer steps generate the Fe-sulfinate complex prior to release of cysteine sulfinic acid.

The former computational mechanism is reasonable in light of what is known regarding other non-heme iron oxygenase enzymes and their reliance upon high-valent ferryl (Fe=O) intermediates. However, one wonders whether it is necessary to form such a powerful oxidizing agent for the oxidation of a sulfur ligand, which is thermodynamically easier to oxidize than the typical C-H substrates of other non-heme Fe enzymes. The structural

characterization of an iron-persulfenate adduct (Figure 3d) suggests another possible mechanism for the oxidation pathway in CDO.⁴³ The persulfenate moiety was trapped by soaking crystals of rat CDO in cysteine under aerobic conditions. It is not known if this species is in fact a true intermediate of the catalytic cycle or just a byproduct from a side reaction, but it is suggestive of a mechanism where the cysteine sulfur attacks an iron-dioxygen intermediate at the Fe-bound proximal oxygen atom. In this case, instead of generating a four-membered cyclic peroxo complex by attack of the distal oxygen atom on the thiolate sulfur, the proximal oxygen atom, thiolate sulfur and iron ion participate in a three-membered cyclic persulfenic complex. Rearrangement of this complex could lead to the final sulfinic acid product without the participation of a ferryl intermediate. However, calculations indicate that formation of this iron-persulfenate intermediate involves a high-energy pathway, leading to the conclusion that this pathway is unlikely to participate in the catalytic cycle of CDO.⁴⁰

Pierce and coworkers recently targeted superoxide (O_2^-) addition to the catalytically inactive Fe^{III} -CDO to probe the feasibility of an Fe^{III} -superoxo intermediate being involved in the catalytic cycle.⁴⁴ Upon treatment of resting Fe^{II} -CDO with potassium hexachloroiridate(IV) (K_2IrCl_6), the catalytically inactive Fe^{III} -CDO was generated in ~95% yield. Addition of cysteine resulted in an intense, relatively stable sulfur-to-iron(III) charge transfer (LMCT) band at 650 nm ($\epsilon = 2200 \text{ M}^{-1} \text{ cm}^{-1}$) indicative of direct substrate binding. Superoxide was generated in solution by mixing xanthine oxidase with xanthine and O_2 prior to addition to Cys-bound Fe^{III} -CDO. Superoxide addition resulted in the generation of a transient intermediate with a feature at 565 nm, which showed complete decay within 10 min. Parallel-mode EPR analysis led to the assignment of a high-spin iron(III) ion ($S = 5/2$) ferromagnetically coupled to a superoxide radical ($S = 1/2$) to give an overall spin state of $S = 3$. The decay of the proposed iron(III)-superoxo intermediate led to the concomitant formation of cysteine sulfinic acid and the recovery of active Fe^{II} -CDO, providing support for the involvement of an iron(III)-superoxo species during native turnover with O_2 . However, a slower rate of formation of product was observed in the superoxide experiments, which may be due to an inherent difference in the local environment of the active site of Cys-bound Fe^{III} -CDO relative to the untreated ferrous enzyme.

Although the above computational and experimental work on the mechanism of CDO is limited, it provides some of the most detailed mechanistic information to date for S -oxygenation in non-heme iron enzymes. Thus there clearly remains much to be learned regarding O_2 -mediated S -oxygenation promoted by non-heme iron centers. In this regard, the study of synthetic analogs of non-heme iron-thiolate enzyme active sites has the potential to make significant progress in helping to predict mechanistic pathways as well as provide synthetic precedent for proposed, key bond-making/bond-breaking mechanistic steps. While great strides have been made in the use of nitrogen-ligated iron model complexes to understand the O_2 reactivity of non-heme iron oxygenases,^{45–48} the synthesis and study of synthetic analogs containing iron-thiolate bonds remains relatively rare, and their reactivity with O_2 is even less understood. Given the interest in S -oxygenation at non-heme iron centers, we have focused in this Perspective article on summarizing the state of knowledge regarding biomimetic analogs of non-heme iron enzymes that incorporate thiolate ligands and react with O_2 to give S -oxygenated products. We have attempted to highlight commonalities in synthesis, structure, spectroscopic features, reactivity and possible mechanisms.

Dioxygen-driven S -oxygenation in iron complexes

The first evidence for the sulfur oxygenation of a synthetic iron-thiolate complex by dioxygen was not reported until Chottard and coworkers' description in 1999.⁴⁹ This work

was part of efforts to model the active site of nitrile hydratase. A pentadentate ligand was designed to contain three aliphatic thiol groups and two aliphatic amide donors to reproduce the first coordination sphere of the iron center in NHase. Deprotonation of the thiol groups with NaH in DMF followed by addition of FeCl₃ at -5 °C and addition of Et₄NCl led to the isolation of a red precipitate under anaerobic conditions. This material was redissolved in CH₃CN and exposed to air, leading to the formation of the sulfinato complex (NEt₄)₂[Fe^{III}(L-O₂)] shown in Scheme 2. This product was characterized by X-ray crystallography, providing the first example of a sulfinato-iron bond. The structure revealed that the sulfinato group was coordinated to the iron(III) ion through one of the oxygen atoms, not the sulfur donor, with an Fe^{III}-O distance of 2.005(6) Å, while the other thiolato and amido groups were bound as designed to give a 5-coordinate complex. Analysis by EPR spectroscopy and magnetic susceptibility showed (NEt₄)₂[Fe^{III}(L-O₂)] was an intermediate spin (*S* = 3/2) iron(III) complex. Analysis by IR spectroscopy gave the S-O stretching band at 1040 cm⁻¹, whereas resonance Raman (RR) spectroscopy revealed ν(S-O) = 978 and 1160 cm⁻¹. Excitation profiles from RR identified the electronic transition at 475 nm observed in the UV-vis spectrum of this complex as a sulfinate-to-iron(III) charge-transfer (CT) band. Although the apical sulfur donor was clearly oxygenated in (NEt₄)₂[Fe^{III}(L-O₂)], the precursor complex, which is presumably an [Fe^{III}(N₂S₃)]²⁻ species, could not be isolated under anaerobic conditions. Thus the mechanism of oxygenation in this work is not clear. Several possible pathways can be envisioned, including O₂ activation at the metal center or direct attack of the thiolate donor by O₂.

Another example of O₂-mediated *S*-oxygenation in iron chemistry that followed soon after the initial report of Chottard and coworkers involved a similar mixed amido/thiolato ligand set. In this work, Mascharak and coworkers were also targeting NHase model complexes. The ligand employed was PyPSH₄, which contained an N₃S₂ donor set, made up of two amido and one pyridine nitrogen donor, and two phenylthiolate donors (Scheme 3).⁵⁰ This ligand led to the synthesis of the electron-rich, 5-coordinate Fe^{III} complex (Et₄N)[Fe^{III}(PyPS)] from (Et₄N)[Fe^{III}Cl₄] and deprotonated PyPS⁴⁻ in DMF, which was characterized by X-ray crystallography and shown to be high-spin (*S* = 5/2) by EPR and magnetic susceptibility. Addition of a limited amount of O₂ to this complex in acetone led to the oxygenation of both phenylthiolate ligands upon stirring for several days, to give a high-spin iron(III) bis(sulfinato) product, (Et₄N)[Fe^{III}(PyP{SO₂}₂)] (Scheme 3a). Structural identification revealed two O-bonded sulfates, as seen in Chottard's complex, and a single S-O stretch was observed by IR (ν(S-O) = 1070 cm⁻¹), consistent with the RSO₂-Fe bonding mode. Electrochemical measurements comparing the redox potentials of [Fe^{III}(PyPS)]⁻ and [Fe^{III}(PyP{SO₂}₂)]⁻ (E_{1/2} = -0.65 V and -0.36 V vs SCE, respectively) indicated the sulfinato donors reduced the stability of the iron(III) center.

Although there is little mechanistic information on the formation of [Fe^{III}(PyP{SO₂}₂)]⁻, electrospray ionization mass spectrometry (ESIMS) provided some details on the time-course of oxygenation. Evidence for single O-atom addition to [Fe^{III}(PyPS)]⁻ was obtained by ESIMS, where ions corresponding to oxygenated species with one, two, three, and four O atoms were observed over 48 h. This observation suggests the initial formation of a sulfenato intermediate, as shown in Scheme 3a, and argues against a dioxygenase-like mechanism in which one O₂ molecule adds to a single sulfur site.

The high-spin (*S* = 2) Fe^{II} complex (Et₄N)₂[Fe^{II}(PyPS)] was isolated under anaerobic conditions and crystallographically characterized, providing a comparison between Fe^{II} and Fe^{III} with regards to O₂ reactivity. Although this issue was not examined in detail by Mascharak and co-workers, it was reported that the Fe^{II} complex readily converted to the Fe^{III} analog following exposure to O₂ in DMF (Scheme 3b). Thus it appears as though no oxygenation of the Fe^{II} complex occurs, but rather outer-sphere oxidation takes place to give

the Fe^{III} complex, which can then react further with O₂ to give the bis(sulfinato) product. This result is somewhat surprising given the open site on the iron(II) center available for binding of O₂ and the electron-rich environment of (Et₄N)₂[Fe^{II}(PyPS)].

The third instance of *S*-oxygenation reported for an Fe-thiolate complex and the first example of oxygenation of an Fe-thiolate complex by O₂ to give an *S*-bound, as opposed to O-bound sulfinate, was described by Artaud and co-workers.⁵¹ The starting material in this case was also an iron(III) complex, and contained a tetradentate ligand with a similar donor set to the previous NHase models by Mascharak and Chottard. The ligand was comprised of bis(arylamido), bis(alkylthiolato) donors, and was reacted with Fe^{III} in DMF to give an intermediate-spin (*S* = 3/2) complex of formula (Et₄N)₂[Fe^{III}(N₂S₂Cl₂)Cl] (Scheme 4). Although no X-ray structure was obtained for this material, it was prepared in the same manner as a structurally characterized analog, (Et₄N)₂[Fe^{III}(N₂S₂)Cl], which differed only in the fact that the Cl substituents on the phenyl group were absent.⁵² The complex [Fe^{III}(N₂S₂Cl₂)Cl]²⁻ proved unreactive to O₂. However, addition of a strongly donating CN⁻ ligand afforded an air-sensitive complex that was purified by gel filtration chromatography and formulated as [Fe^{III}(N₂S₂Cl₂)CN]²⁻ based on elemental analysis and IR. Interestingly, evidence for a mixture of mono- and di-cyano complexes was observed by EPR in frozen CH₃CN (20 K), in which signals corresponding to both intermediate-spin Fe^{III} (*S* = 3/2) and low-spin Fe^{III} (*S* = 1/2) were apparent. The intermediate-spin signal was assigned to the mono-cyano complex, and the low-spin signal was assigned to the di-cyano complex, suggesting an equilibrium exists between the two forms in CH₃CN solution. Exposure of [Fe^{III}(N₂S₂Cl₂)CN]²⁻ to excess O₂ leads to decomposition of this complex, but the same reaction in the presence of an added equivalent of Et₄NCN resulted in the isolation of a product where oxygenation occurred at both thiolato donors. Precipitation of this product, followed by recrystallization from Et₂O/CH₃CN, yielded X-ray quality crystals which revealed a low-spin Fe^{III} complex (Et₄N)₃[Fe^{III}(N₂Cl₂{SO₂}₂)(CN)₂]. From the crystal structure it was clear that the thiolate donors had been converted to two *S*-bound sulfinato ligands. Two bands were observed at 1166 and 1050 cm⁻¹ in the IR spectrum for this product and assigned to S-O stretching modes for the *S*-bound sulfinato ligands.

In this study it was determined that the addition of exogenous CN⁻ was crucial for O₂ reactivity with this bis(amido)bis(thiolato) iron(III) complex. It was shown that the mono-cyano complex [Fe^{III}(N₂S₂Cl₂)CN]²⁻ decomposes upon O₂ addition, but addition of a second equivalent of CN⁻ in the presence of O₂ leads to the isolation of the bis(sulfinato) complex. However, it is not clear how this second equivalent of CN⁻ functions in the *S*-oxygenation mechanism. One possibility is that the second CN⁻ coordinates prior to reaction with O₂, inducing a spin-state change from intermediate-spin to low-spin, and it is this di-cyano, low-spin complex that is reactive toward O₂ (Scheme 4, pathway a). A second possibility is that *S*-oxygenation does occur for the mono-cyano complex, but the second equivalent of CN⁻ is necessary for stabilization and isolation of the final bis(sulfinato) product (Scheme 4, pathway b). These mechanistic questions remain unanswered, but it is apparent that there is a requirement for an additional strong-field donor (CN⁻) for O₂ reactivity, despite the presence of the strongly electron-donating (N₂S₂)⁴⁻ chelate. Interestingly, attempted oxidations with stronger oxidants such as H₂O₂ or dioxirane only resulted in complex decomposition.

A more recent example of *S*-oxygenation at an Fe^{III}-thiolate site was provided by Kovacs and co-workers.⁵³ These workers prepared an iron(III) complex with a bis(amido)tris(thiolato) (N₂S₃) ligand (Scheme 5) whose structure was similar to that of Chottard (Scheme 2) except for the position of the amide carbonyl groups. As in Chottard's work, the ligand was designed to give 5-coordinate iron complexes as models of NHase.⁴⁹ In this case, a mononuclear iron(III) complex was isolated prior to exposure to dioxigen.

The crystal structure of this iron(III) complex, $(\text{Me}_4\text{N})_2[\text{Fe}^{\text{III}}((\text{tame-N}_2\text{S})\text{S}_2^{\text{Me}_2})]$, revealed a 5-coordinate Fe^{III} center with the ligand coordinating through the thiolato and amido donors as designed. It was found to be intermediate-spin ($S = 3/2$) by magnetic susceptibility and EPR spectroscopy. The pentaanionic ligand highly stabilizes the iron(III) state, as evidenced by the absence of any reduction process down to -1.8 V vs SCE in the cyclic voltammogram, which indicates that the iron(II) state is essentially inaccessible with this ligand environment.

Exposure of $[\text{Fe}^{\text{III}}((\text{tame-N}_2\text{S})\text{S}_2^{\text{Me}_2})]^{2-}$ to dry O_2 in CH_3CN results in the addition of two O atoms to the apical thiolate donor to give a monosulfinate iron(III) complex (Scheme 5). The final product was characterized by ESIMS and X-ray crystallography, and the crystal structure revealed that the sulfinato group was *O*-bound to the iron(III) ion. The metal ion remained an intermediate-spin iron(III) center following oxygenation. Only one of three thiolate donors is oxygenated in this case, and it is suggested that the longer Fe-S bond distance observed for the apical thiolate makes this site susceptible to attack by O_2 . Interestingly, this pattern of O_2 reactivity also matches that seen for the close analog prepared by Chottard nine years earlier. The same regioselectivity is seen for the addition of O_2 to Chottard's complex, where only the apical thiolate is oxygenated.

All of the former examples of *S*-oxygenation occurred with ancillary ligands bearing electron-rich amido donors in combination with two or more thiolate donors, resulting in strongly electron-donating 4- or 5- polydentate ligands when fully deprotonated. However, prior to work done in our laboratory, there was only one report of *S*-oxygenation by O_2 of an iron complex supported by a ligand bearing neutral amino N atom donors and a charge lower than 4-. Grapperhaus and co-workers prepared an iron(III) complex with a pentadentate ligand comprised of two amine, one thioether, and two thiolate groups to give $(\text{N}_2\text{S}_3)\text{Fe}^{\text{III}}(\text{X})$ ($\text{X} = \text{Cl}^-, \text{CN}^-$) (Scheme 6) as models for NHase.⁵⁴ The cyanide derivative was crystallographically characterized and shown to be a low-spin ($S = 1/2$) Fe^{III} complex.⁵⁵ This purple complex reacts slowly with an atmosphere of O_2 in MeOH over the course of several days to give a dark green complex that precipitates from the reaction mixture. Although it was not possible to characterize this species by X-ray crystallography, the compound was characterized by a combination of IR and ESIMS. IR bands at 1221, 947, and 477 cm^{-1} were observed to shift upon substitution with $^{18}\text{O}_2$, and were consistent with metal-coordinated *S*-oxygenates.⁵⁴ The degree of oxygenation was determined by ESIMS, where a molecular ion ($+ \text{K}^+$) peak corresponding to the bis(sulfonato)-iron(III) complex was detected. In support of the bis(sulfonato) assignment, a nickel(II) analog ($[(\text{N}_2\text{S}_3)\text{Ni}^{\text{II}}]$) of $[(\text{N}_2\text{S}_3)\text{Fe}^{\text{III}}(\text{X})]$ was synthesized and oxidized by H_2O_2 to give the Ni^{II} (bis(sulfonato)) derivative, which was characterized by single-crystal X-ray diffraction. Comparison of the IR spectra between the Ni^{II} (bis(sulfonato)) complex and the Fe^{III} product following O_2 oxidation led to the conclusion that the Fe^{III} product likely contained sulfonato donors.

The chloride analog, $[(\text{N}_2\text{S}_3)\text{Fe}^{\text{III}}(\text{Cl})]$, was also tested with regard to its O_2 reactivity.⁵⁴ This complex was not definitively characterized by an X-ray structure, but was used as the precursor to the cyanide derivative. EPR spectroscopy showed the chloride adduct was high-spin iron(III), and this complex reacts with O_2 within one hour to give a product that was analyzed by IR and ESIMS. The spectroscopic data suggested formation of iron-oxo clusters and ligand disulfide products, but no evidence for *S*-oxygenation was obtained. In a previous report, the authors presented the structural characterization of an iron(III) oxide-alkoxide cluster with fourteen iron centers generated upon reaction of $[(\text{N}_2\text{S}_3)\text{Fe}^{\text{III}}(\text{Cl})]$ with O_2 .⁵⁶ DFT calculations were performed to shed light on the dramatic differences in reactivity between the cyanide (low-spin) and chloride (high-spin) iron(III) complexes. The DFT analysis pointed to more covalent bond character for the Fe-S(thiolate) interactions in the low-spin complex as opposed to the high-spin complex. Thus it was concluded that stronger

covalent interactions in low-spin iron(III)-thiolate complexes favor *S*-oxygenation, whereas the more ionic character calculated for the high-spin Fe^{III}-S bonds favor disulfide formation in a fashion similar to simple thiolate salts.

Synthetic models of the active site of NHase that incorporate nitrosyl ligands were prepared by Liaw and co-workers and shown to react with O₂ to give *S*-oxygenated products (Schemes 7 and 8).^{57,58} As is the case for metal-nitrosyl complexes, the metal-oxidation states in this chemistry are difficult to assign, and the Enemark-Feltham notation is adopted to indicate electron counts of the {Fe-NO} unit.⁵⁹ The bis(benzenedithiolate) {Fe-NO}⁶ complex, [PPN][(NO)Fe(S,S-C₆H₄)₂], reacts slowly with O₂ (one week) in THF/CH₂Cl₂ to yield the *S*-bound monosulfinate [(NO)Fe(S,SO₂-C₆H₄)(S,S-C₆H₄)]⁻, which was characterized by X-ray crystallography and IR (Scheme 7). Two bands in the IR spectrum of the sulfinato complex are assigned to the S-O stretching modes (1196 cm⁻¹ and 1060 cm⁻¹), as they shift upon ¹⁸O-labeling to 1154 cm⁻¹ and 1019 cm⁻¹, respectively. Interestingly, the starting {Fe-NO}⁶ complex can be regenerated by reacting the monosulfinate complex with two equivalents of PPh₃, to yield OPPh₃, or through photolysis under N₂ purge. Moreover, the monosulfinate [(NO)Fe(S,SO₂-C₆H₄)(S,S-C₆H₄)]⁻ complex can further react with O₂ in the presence of [PPN][NO₂] to generate a dimeric bis(sulfinato) complex, [PPN]₂[(NO)Fe(SO₂,SO₂-C₆H₄)(S,S-C₆H₄)₂], which was also characterized by X-ray crystallography and IR. The role of the addition of [PPN][NO₂] for the synthesis of this dimer was not discussed. Bands assigned to S-O stretching shifted to 1212, 1067, and 1057 cm⁻¹ upon dimerization. Interestingly, when the fifth coordination site is occupied by THF as found in the iron(III) starting material [PPN][(C₄H₈O)Fe(S,S-C₆H₄)₂], there is no reaction with O₂ to yield any *S*-oxygenated products (Scheme 7).

Two subsequent reports from Liaw showed that derivatives of [PPN][(NO)Fe(S,S-C₆H₄)₂] could be synthesized with different substituents (Me, Cl) on the benzenedithiolate ligands.^{58,60} These complexes reacted with O₂ in a similar manner with regard to *S*-oxygenation, resulting in the formation of the sulfinato group (Scheme 8). In the case of the 3,6-dichloro-substituted benzenedithiolate complex, reaction with O₂ generates a bis(thiolate)-bridged dimeric Fe₂ complex with monosulfinato modification to two of the six available terminal sulfur donors. In contrast, the Me or parent H-substituted complexes yield mononuclear sulfinato derivatives. Both mononuclear and dinuclear sulfinato complexes react with PPh₃ to remove the O atoms and regenerate the starting {Fe-NO}⁶ complex. UV-vis and magnetic measurements on both the mononuclear and dinuclear sulfinato-bound complexes indicated that instead of these complexes retaining an {Fe-NO}⁶ configuration, they were best described as {Fe-NO}⁷ species with one electron being transferred from the non-innocent benzenedithiolate moiety [S,S-C₆H₃R]²⁻, in turn giving a dithio-*o*-semibenzoquinone(-) radical. These {Fe-NO}⁷ complexes exhibited nearly linear Fe-NO angles, consistent with their formulation as formally Fe^I(NO⁺). Reaction of the former complexes with EtS⁻ or *o*-aminothiophenolate led to the one-electron reduction of the dithio-*o*-semibenzoquinone(-) radical, and interestingly, also induced a configuration change at the metal to give Fe^{II}(NO•) (Scheme 8).

Although we are focused on mononuclear iron complexes here, a related dinuclear iron complex with bridging arylthiolate donors is worthy of mentioning. Sellmann and coworkers reported the synthesis of a series of electron-rich mononuclear and dinuclear Fe and Ru complexes with mixed N/S(thiolate)/S(thioether) pentadentate ligands.⁶¹ In one case the dinuclear Fe^{II} complex [{Fe(Et₂NpyS₄)₂] was synthesized with a pentadentate ligand (Et₂NpyS₄²⁻) that contains one pyridine N donor, two thioether donors, and two arylthiolate donors (Scheme 9). Although no X-ray structure was reported for this compound, it was described as a dimer with one thiolate group per ligand forming a bridge between iron atoms to give an Fe₂S₂ core. Reaction of [{Fe(Et₂NpyS₄)₂] with air over 72 h in CHCl₃ afforded

the *S*-oxygenated product $[\{\text{Fe}(\text{Et}_2\text{NpyS}_4\text{-O}_2)\}_2]$, in which both terminal thiolates were converted to sulfinate groups. The structure of the *S*-oxygenated product was confirmed by X-ray crystallography and revealed that both sulfinate groups were bound through the sulfur atoms to the metal. The $\nu_a(\text{SO}_2)$ and $\nu_s(\text{SO}_2)$ bands were observed in the IR spectrum at 1238, 1185, 1133, and 983 cm^{-1} . Although the starting material here is presumed to be a dinuclear complex, and therefore is not directly relevant to the reactivity of mononuclear iron-thiolate species, it appears to be the only iron(II) complex prior to our own work that reacts with O_2 to give an *S*-oxygenated product. No information on the mechanism of formation of the bis(sulfinato) diiron product was reported, including any direct evidence for O_2 being the source of oxygen in the final product.

The previous examples of sulfur oxygenation in iron thiolate model complexes were reported with ligand sets containing more than one thiolate donor. Additionally, they were synthesized to model the active site of nitrile hydratase, which contains two cysteine residues that are post-translationally modified to both the cysteine sulfenato and cysteine sulfinato moieties. In contrast, there is a noticeable lack of cysteine dioxygenase (CDO) models reported in the literature. New experimental and theoretical data on the active site, and the lack of many mechanistic details, make it an important target for structural and functional model complexes. In our lab, we have made efforts to model the structure and function of the active site of CDO as well as understand the mechanistic factors that allow for S- vs Fe-oxygenation.

As shown in Figure 3, CDO contains three neutral N donors from histidine and one cysteine thiolate donor upon the proposed coordination of substrate cysteine to the metal active site. We initiated our studies with ligands based on the bis(imino)pyridine (BIP) framework. These ligands have proven to yield versatile systems for a number of applications in catalysis, demonstrating their robust nature and convenient use.^{62–66} Metal complexes with BIP-derived ligands have also been used to stabilize Fe^{II} , and we speculated that they should provide good steric protection to prevent the formation of O- or S-bridged Fe dimer complexes. Incorporation of a thiolate donor was achieved by metal-templated synthesis of LN_3S .⁶⁷ Following Scheme 10, the unsymmetrical ketone 2-($\text{O}=\text{CMe}$)-6-(2,6-($i\text{Pr}_2$ - $\text{C}_6\text{H}_3\text{N}=\text{CMe}$)- $\text{C}_5\text{H}_3\text{N}$) was synthesized by treatment of 2,6-diacetylpyridine with 0.9 equivalents of 2,6-diisopropylaniline in the presence of formic acid in methanol at room temperature. Reaction of the unsymmetrical ketone with 2-aminothiophenol in the presence of $\text{Fe}^{\text{II}}(\text{OTf})_2$ and Et_3N at 80 °C in ethanol yields the high-spin, dark brown complex $[\text{Fe}^{\text{II}}(\text{LN}_3\text{S})(\text{OTf})]$, which was characterized by X-ray crystallography. The three neutral N donors and the phenylthiolate S donor are bound to the Fe^{II} ion in a distorted square pyramidal geometry ($\tau = 0.12$) with the triflate anion occupying the axial position (Figure 4). The thiolate sulfur donor is coordinated in a position *cis* to the potential open site for binding of O_2 . Steric shielding of the metal center is provided by the diisopropylphenyl substituents, which likely prevents the formation of thiolate-bridged structures.

The reactivity of $[\text{Fe}^{\text{II}}(\text{LN}_3\text{S})(\text{OTf})]$ with O_2 was examined. Dioxygen was added to a solution of $[\text{Fe}^{\text{II}}(\text{LN}_3\text{S})(\text{OTf})]$ in CH_2Cl_2 , leading to an immediate color change from black to brown. Analysis by laser desorption ionization mass spectrometry (LDIMS) after 24 h showed the complete loss of starting material at m/z 484.2 ($[\text{Fe}^{\text{II}}(\text{LN}_3\text{S})]^+$) and the appearance of a prominent ion at m/z 532.1, consistent with the triply oxygenated species $[\text{Fe}^{\text{II}}(\text{LN}_3\text{SO}_3)]^+$ (Scheme 11). Addition of $^{18}\text{O}_2$ to $[\text{Fe}^{\text{II}}(\text{LN}_3\text{S})(\text{OTf})]$ results in the fully labeled $[\text{Fe}^{\text{II}}(\text{LN}_3\text{S}^{18}\text{O}_3)]^+$ by LDIMS, whereas no ^{18}O incorporation is obtained when the oxygenation reaction is run in the presence of excess H_2^{18}O . These data show that O_2 is the source of the oxygen atoms in the final product. A crystal suitable for X-ray crystallography could not be obtained for the sulfonato product, but by carrying out the template reaction with 2- $\text{H}_2\text{N}-\text{C}_6\text{H}_4\text{SO}_3\text{H}$ as opposed to 2-aminothiophenol, we were able to isolate X-ray

quality crystals and show that the sulfonato group coordinates to the iron(II) center as expected in the O₂ reaction. A yield of the sulfonato product from the O₂ reaction was obtained by demetalation and acid hydrolysis, followed by quantitative reversed-phase HPLC. The hydrolyzed phenylsulfonato 2-H₂N—C₆H₄SO₃H product was obtained in 60% yield. Assignment of the oxidation state for the iron(II) sulfonato complex derived from O₂ was made by EPR spectroscopy (EPR-silent, as expected for high-spin Fe^{II}), as well as colorimetric titration with 1,10-phenanthroline.

To help elucidate the mechanism of oxygenation for [Fe^{II}(LN₃S)(OTf)], a mixed isotopic labeling study was conducted. There are two reasonable scenarios for the incorporation of three oxygen atoms into the *S*-oxygenated sulfonato product. In the first scenario, an intact molecule of O₂ is incorporated either before or after the addition of a single O atom, which we call the “2 + 1” mechanism. In the second scenario, the reaction proceeds by addition of single O atoms from different molecules of O₂, or a “1 + 1 + 1” mechanism. These two mechanisms could be distinguished by reaction of [Fe^{II}(LN₃S)(OTf)] with a mixture of ¹⁸O₂/¹⁶O₂ (~49/51), followed by LDIMS analysis. The theoretical distribution pattern expected for the isotopic cluster of [Fe^{II}(LN₃SO₃)]⁺ (*m/z* 532.1) differs significantly for the 2 + 1 versus the 1 + 1 + 1 mechanistic pathways in this mixed-label experiment. Comparison of the experimental LDIMS pattern for [Fe^{II}(LN₃SO₃)]⁺ from ¹⁸O₂/¹⁶O₂ with a statistical simulation of the isotopic distribution pattern showed that the 2 + 1 mechanism is the dominant pathway of *S*-oxygenation. In addition, mass spectral analysis of reaction mixtures prior to completion of the reaction showed no ion corresponding to the singly oxygenated sulfenato species, but instead showed a minor peak corresponding to the doubly oxygenated complex, suggesting that first dioxygenation occurs, and then in a subsequent step, the third O atom is added. Thus, at least the initial oxygenation step mimics the dioxygenase activity of the enzyme.

A definitive role for the Fe^{II} ion in the *S*-oxygenation reaction of [Fe^{II}(LN₃S)(OTf)] has not been established. However, we were able to compare the O₂ reactivity of the Zn^{II} analog [Zn^{II}(LN₃S)(OTf)], which was synthesized independently.⁶⁷ It is safe to assume that the Zn^{II} ion is redox inactive and unable to directly activate O₂ by the transfer of electrons, whereas Fe^{II} is redox active. Exposure of [Zn^{II}(LN₃S)(OTf)] to excess O₂ in CH₂Cl₂ for up to 7 d showed no evidence for oxygenation, as determined by ¹H NMR and LDIMS. This result suggests that iron(II) is crucial for the activation of dioxygen, leading to the *S*-oxygenation of [Fe^{II}(LN₃S)(OTf)]. Thus, some mechanistic information on the *S*-oxygenation of [Fe^{II}(LN₃S)]⁺ has been obtained, but no oxygen intermediates for this reaction have yet been characterized. Despite the lack of mechanistic information, these results were significant in that they described the first example of a monomeric Fe^{II}-thiolate complex reacting with O₂ to afford an *S*-oxygenated product.

For further insight into the mechanism of oxidation in this CDO model complex, as well as further comparison with the enzyme, computational studies were conducted.⁶⁸ The overall mechanism obtained from the computational studies on [Fe^{II}(LN₃S)]⁺ + O₂ is quite similar to that derived for the enzyme (Scheme 1). One key difference is that the rate determining step for the model complex is O-O bond cleavage to generate the sulfenate-ligated iron(IV)-oxo complex, whereas the rate determining step for the enzyme is S-O bond formation to generate the cyclic Fe-O-O-S species. Also, direct attack of dioxygen on the thiolate ligand was shown to be unlikely through additional calculations, which showed the approach of molecular oxygen to [Fe^{II}(LN₃S)]⁺ strongly favors formation of a stable iron(III)-superoxo complex prior to S-O bond formation.

Following this initial study, two new Fe^{II}-thiolate complexes were synthesized in our lab with the related bis(imino)pyridine (BIP) ligand, although in this work, an exogenous

thiolate donor was employed, as opposed to the covalently tethered thiolate donor found in LN_3S^- .⁶⁹ Following Scheme 12, the complexes $[(iPr\text{BIP})\text{Fe}^{\text{II}}(\text{SPh})(\text{Cl})]$ and $[(iPr\text{BIP})\text{Fe}^{\text{II}}(\text{SPh})(\text{OTf})]$ were synthesized by displacing one anionic donor X ($\text{X} = \text{Cl}^-$ or OTf^-) of the parent $[(iPr\text{BIP})\text{Fe}^{\text{II}}\text{X}_2]$ complex with one equivalent of PhS^- in THF. Both Fe^{II} complexes were characterized by X-ray crystallography, which revealed five-coordinate Fe^{II} ions in a distorted square pyramidal geometry with bond lengths and angles consistent with those of high-spin iron(II). Interestingly, the position of the phenylthiolate ligand differs in these two complexes. When $\text{X} = \text{Cl}^-$, the PhS^- group occupies the axial position *trans* to the open site on the Fe^{II} center. Conversely, when $\text{X} = \text{OTf}^-$, the thiolate S donor is bound in a pseudoequatorial position *cis* to the open coordination site (Scheme 12).

Both $[(iPr\text{BIP})\text{Fe}^{\text{II}}(\text{SPh})(\text{Cl})]$ and $[(iPr\text{BIP})\text{Fe}^{\text{II}}(\text{SPh})(\text{OTf})]$ exhibited O_2 reactivity, although they followed different oxidation pathways. Addition of a slight excess of dry O_2 to the chloro analog led to a color change from dark-blue to green, with a $\lambda_{\text{max}} = 690 \text{ nm}$ ($\epsilon \approx 1500 \text{ M}^{-1} \text{ cm}^{-1}$). Analysis of the reaction mixture by LDIMS provided a dominant ion at m/z 588, consistent with the formula for an $\text{Fe}(\text{O})$ complex, $[(iPr\text{BIP})\text{Fe}(\text{O})(\text{Cl})]^+$, which shifts to $m/z = 590$ upon addition of $^{18}\text{O}_2$ (80% ^{18}O incorporation). The thiolate ligand is oxidized to disulfide during this reaction, as determined by ^1H NMR spectroscopy ($\text{PhS}-\text{SPh}$, 85%). Reaction of excess PPh_3 with this intermediate resulted in the formation of OPPh_3 , as well as the regeneration of $[(iPr\text{BIP})\text{Fe}^{\text{II}}(\text{SPh})(\text{Cl})]$. These data led us to conclude that the chloro complex reacts with O_2 to give oxygenation exclusively at the metal center, not the phenylthiolate sulfur atom.

Surprisingly, we observed different products in the reaction of $[(iPr\text{BIP})\text{Fe}^{\text{II}}(\text{SPh})(\text{OTf})]$ with O_2 , which paralleled those we have seen in the tethered $[\text{Fe}^{\text{II}}(\text{LN}_3\text{S})(\text{OTf})]$ complex. Upon addition of stoichiometric amounts of O_2 to $[(iPr\text{BIP})\text{Fe}^{\text{II}}(\text{SPh})(\text{OTf})]$, an immediate color change from dark-blue to brown was observed. When analyzed by LDIMS, the ion peak corresponding to an $\text{Fe}(\text{O})$ complex was not seen. Instead, a cluster at m/z 694 corresponding to the *S*-oxygenated product $[\text{Fe}^{\text{II}}(iPr\text{BIP})(\text{PhSO}_3)]^+$ was observed. We showed that the O atoms of the sulfonate ligand come from O_2 by reacting with $^{18}\text{O}_2$, which resulted in ~90% incorporation of ^{18}O . After acid hydrolysis of the reaction mixture, the identity of the benzenesulfonic acid product was confirmed by ^1H NMR and quantified by reversed-phase HPLC (30% yield of PhSO_3H based on total Fe). This work showed that *S*-oxygenation could occur at an Fe^{II} center with an appropriate environment, even if the thiolate donor is not tethered and potentially susceptible to disulfide formation.

The oxidation pathway differs among $[(iPr\text{BIP})\text{Fe}^{\text{II}}(\text{SPh})(\text{Cl})]$ and $[(iPr\text{BIP})\text{Fe}^{\text{II}}(\text{SPh})(\text{OTf})]$ despite similar electronic properties, which led us to consider the role of the position of the thiolate ligand in O_2 reactivity. The most obvious difference between these two complexes is the arrangement of the PhS^- ligand in relation to the sixth, open coordination site on the iron(II) center. A mechanistic proposal for the observed O_2 reactivity is shown in Scheme 13. In both cases, the Fe^{II} center first reacts with O_2 to generate a putative Fe^{III} -superoxo moiety. When the S donor is *trans* to the coordinated O_2 , as in the chloro analog, intramolecular attack of an $\text{Fe}-\text{O}_2$ intermediate on the sulfur donor is strongly disfavored. However, the $\text{Fe}-\text{O}_2$ intermediate of the triflate complex is generated *cis* to the thiolate ligand, which can then follow the *S*-oxygenation pathway.

In the previous work we also showed that the presence of the thiolate ligand is critical for O_2 activation. The related non-thiolate-ligated complexes, $[\text{Fe}^{\text{II}}(iPr\text{BIP})\text{Cl}_2]$ and $[\text{Fe}^{\text{II}}(iPr\text{BIP})(\text{OTf})_2]$, were found to be completely unreactive toward O_2 in both solution (CH_2Cl_2 and CH_3CN) and the solid state. A significant clue regarding the difference in reactivity between the thiolate- and non-thiolate-ligated analogs comes from comparing the redox potentials of these complexes. These potentials, together with other Fe^{II} complexes, are given in Table 1.

As can be seen in Table 1, upon coordination of a thiolate ligand, the redox potential greatly decreases in both the chloro and triflate analogs to < -0.1 V (vs Fc^+/Fc), and this cut-off appears to be a prerequisite for O_2 activation in non-heme Fe^{II} complexes.⁴⁵ Thus, displacing one anionic donor (e.g., Cl^- or OTf^-) with a phenylthiolate donor tunes the redox potential of the ($^{\text{Pr}}\text{BIP}$) Fe^{II} complexes to permit O_2 activation.

Recent work done by Limberg and co-workers provided another biomimetic model of CDO.⁷⁰ The well-known trispyrazolylborato ligand system was utilized in this study to mimic the 3 His coordination sphere of resting CDO, as well as an exogenous cysteine model, cysteine ethyl ester. Similar to the enzyme, the trispyrazolylborato ligand binds in a facial arrangement and the cysteinato ligand coordinates in a bidentate fashion to generate the iron(II) complex $[\text{Tp}^{\text{Me}}, \text{PhFeCysOEt}]$ (Scheme 14). Reaction of this model complex with O_2 in dichloromethane results in a slow color change from pale yellow to dark green within 7.5 h. After 12 h, analysis of the reaction by ESI-TOF showed an intense peak at m/z 742.19, corresponding to the sodium adduct of the doubly oxygenated complex $[\text{Fe}(\text{Tp}^{\text{Me}}, \text{PhCysOEt} + \text{O}_2) + \text{Na}]^+$. Isotopic labeling studies were conducted, which indicated the O atoms of the final product came from the same O_2 molecule. In the absence of a crystallographically characterized final product, the authors determined that dioxygenation takes place selectively at the cysteinato ligand by ^1H NMR analysis of the demetalated and hydrolyzed product.

Current efforts in our laboratory have resulted in the synthesis of a new model of CDO with an improved design incorporating three N donors bound in a facial arrangement about the iron(II) ion.⁷¹ The N_4Py ligand established for previous non-heme iron models allowed for the characterization and isolation of several key iron-oxygen [e.g., $\text{Fe}=\text{O}$, $\text{Fe}-\text{OO}(\text{H})$] intermediates, and provided the scaffold upon which our new N_3PySH ligand was built.^{72–74} Reaction of the protected phenylthiolate 3-(2-bromomethylphenylsulfanyl)propionitrile with N -[di(2-pyridinyl)methyl]- N -(2-pyridinylmethyl)amine in the presence of K_2CO_3 at 60°C in CH_3CN resulted in the isolation of the protected pentadentate ligand $\text{N}_3\text{PySEtCN}$. Deprotection with tBuOK in THF afforded N_3PySH in moderate yield after workup (Scheme 15). Metalation with $\text{Fe}^{\text{II}}(\text{BF}_4)_2 \cdot 6\text{H}_2\text{O}$ in the presence of 1 equiv of Et_3N in acetonitrile afforded the dark-red iron(II) complex $[\text{Fe}^{\text{II}}(\text{N}_3\text{PyS})(\text{CH}_3\text{CN})]\text{BF}_4$. Structure determination by X-ray crystallography indicated that the pentadentate ligand is bound to the iron(II) ion in a pseudo-square-pyramidal geometry as designed, with the thiolate donor held cis to the sixth site occupied by a labile acetonitrile ligand. This N_4S coordination corresponds closely to that of the cysteine-bound active site in CDO.

The reactivity of $[\text{Fe}^{\text{II}}(\text{N}_3\text{PyS})]^+$ with O_2 was studied. The iron(II) complex $[\text{Fe}^{\text{II}}(\text{N}_3\text{PyS})]^+$ is low-spin in acetonitrile, but upon dissolution in methanol, it is converted to a high-spin iron(II) complex. Addition of excess dioxygen to high-spin $[\text{Fe}^{\text{II}}(\text{N}_3\text{PyS})]^+$ in MeOH resulted in a color change from dark-brown to a new, relatively stable deep-green species. In order to isolate a crystalline product, 1 equiv of SCN^- was added as a potentially good ligand for the sixth site on iron, affording a color change from green to brown. Vapor diffusion of diisopropyl ether resulted in the isolation of crystals within 6 days. Structure determination by X-ray crystallography revealed double oxygenation of the sulfur atom bound to the low-spin iron(II) center, as well as a thiocyanate anion bound in the sixth coordination site to give $[\text{Fe}^{\text{II}}(\text{N}_3\text{PySO}_2)(\text{NCS})]$ (Scheme 16). Infrared spectroscopy of the crystalline Fe^{II} -sulfinate complex revealed two bands at 1129 and 1012 cm^{-1} , corresponding to the symmetric and asymmetric S-O stretching modes. Mass spectral analysis revealed a prominent isotopic cluster centered at m/z 581.7, which corresponds to the K^+ adduct $[(\text{K})(\text{Fe}^{\text{II}}(\text{N}_3\text{PySO}_2)(\text{NCS}))]^+$.

Labeling studies were conducted to elucidate the source of O atoms as well as the mechanism of sulfur oxygenation. Addition of $^{18}\text{O}_2$ to $[\text{Fe}^{\text{II}}(\text{N3PyS})]^+$ resulted in a shift in the isotopic labeling pattern, indicating that 14% of the Fe^{II} -sulfinate product contained one labeled O atom ($\text{S}^{16}\text{O}^{18}\text{O}$) while approximately 2% of the product was labeled in both O atom positions (S^{18}O_2). While these results suggested that O_2 is a source of O atoms in the S-oxygenation mechanism, exchange with water in solution was likely. To test this hypothesis, we ran the reaction in the presence of excess H_2^{18}O , and analysis by ESI-MS revealed that 70% of the final sulfinate complex was labeled in both O atom positions (S^{18}O_2) and 28% of the product contained one labeled O atom ($\text{S}^{16}\text{O}^{18}\text{O}$). Thus, these results implicated one or more intermediates along the sulfur oxygenation pathway that are susceptible to undergo O-atom exchange with H_2O . A likely intermediate is a sulfenato-iron species $[\text{RS}(\text{O})\text{-Fe}]$, as metal sulfenato complexes have been shown to undergo O-atom exchange with water. Reactivity of the green species with PPh_3 and dimedone, a known selective sulfenic acid trap, was suggestive of the presence of a sulfenato-iron intermediate with an exchangeable O-atom, although the presence of other intermediates at this stage has not been ruled out.

This work showed that the re-design of the ligand to give a facial 3 N arrangement appears to favor sulfinato over sulfonato formation as found in the BIP-based systems. It also provided the first example, to our knowledge, of a crystallographically characterized Fe^{II} -sulfinato complex.

Synthetic models of S-oxygenation by other oxidants

Although our main interest for this Perspective article focuses on S-oxygenation mediated by biologically relevant, mononuclear iron complexes and their reaction with O_2 , a few cases in which O_2 has been replaced by another oxidant are relevant to the present discussion. Que and co-workers reported a mechanism for sulfur oxygenation of the alkylthiolate-ligated cyclam derivative $[\text{Fe}^{\text{II}}(\text{TMCS})]^+$ with *m*-CPBA (*m*-chloroperbenzoic acid) as oxidant.⁷⁵ The formation of an iron(IV)-oxo complex $[\text{Fe}^{\text{IV}}(\text{O})(\text{TMCS})]^+$ was previously reported by reaction of $[\text{Fe}^{\text{II}}(\text{TMCS})]^+$ with *m*-CPBA in the presence of base,⁷⁶ however in the absence of base, $[\text{Fe}^{\text{II}}(\text{TMCS})]^+$ is rapidly converted to the S-oxygenated sulfinato complex $[\text{Fe}^{\text{II}}(\text{TMCSO}_2)]^+$ upon addition of 2 equiv *m*-CPBA in methanol. This complex is then further oxidized to a ferryl species, $[\text{Fe}^{\text{IV}}(\text{O})(\text{TMCSO}_2)]^+$, with the addition of a third equivalent of *m*-CPBA (Scheme 17). While no X-ray quality crystals were obtained for either of these products, a number of spectroscopic methods were used to characterize these complexes. The titration of successive equivalents of oxidant was followed by UV-Vis, Mössbauer, and mass spectrometry, and these data indicated a rapid and quantitative conversion of the Fe^{II} -thiolate complex to an Fe^{II} -sulfinato complex with up to 2 equivalents of oxidant. The addition of the third equivalent of *m*-CPBA results in the gradual formation (30 min) of the Fe^{IV} -oxo-sulfinato, with features at $\lambda_{\text{max}} = 330 \text{ nm}$ ($\epsilon = 9000 \text{ M}^{-1} \text{ cm}^{-1}$), 830 nm ($\epsilon = 170 \text{ M}^{-1} \text{ cm}^{-1}$), and 990 nm ($\epsilon = 170 \text{ M}^{-1} \text{ cm}^{-1}$). The latter two near-IR features are characteristic of previously reported $[\text{Fe}^{\text{IV}}(\text{O})(\text{L})]$ complexes.

The importance of H^+ and the identity of the oxidant for sulfur oxidation was investigated in detail, starting from either the Fe^{II} -thiolate or the $\text{Fe}^{\text{IV}}(\text{O})$ -thiolate complexes. The addition of 6 equiv H_2O_2 instead of *m*-CPBA results in the formation of $[\text{Fe}^{\text{IV}}(\text{O})(\text{TMCS})]^+$. However, the same reaction carried out in the presence of 10 equiv benzoic acid (PhCO_2H) results in the formation of $[\text{Fe}^{\text{IV}}(\text{O})(\text{TMCSO}_2)]^+$. When $[\text{Fe}^{\text{II}}(\text{TMCS})]^+$ was exposed to iodosylbenzene (PhIO), no sulfur oxidation products were observed unless the reaction was carried out in the presence of excess PhCO_2H , which produced $[\text{Fe}^{\text{II}}(\text{TMCSO}_2)]^+$. These results suggested that the availability of H^+ controls whether the thiolate ligand undergoes S-oxidation upon treatment of the starting Fe^{II} -thiolate with an oxidant such as H_2O_2 or PhIO .

Interestingly, treatment of $[\text{Fe}^{\text{IV}}(\text{O})(\text{TMCS})]^+$ with acid results in the rapid decomposition of the iron(IV)-oxo species with subsequent formation of $[\text{Fe}^{\text{II}}(\text{TMCSO}_2)]^+$ (47%) as well as another Fe^{IV} species assigned to the conjugate acid of $[\text{Fe}^{\text{IV}}(\text{O})(\text{TMCS})]^+$ (i.e., $[\text{Fe}^{\text{IV}}(\text{O})(\text{TMCSH})]^+$) (Scheme 18). This new $\text{Fe}^{\text{IV}}(\text{O})$ complex is suggested to be a much better oxygen atom transfer reagent than the parent $[\text{Fe}^{\text{IV}}(\text{O})(\text{TMCS})]^+$ species. Thus, the authors suggest a mechanism in which the first two equivalents of *m*-CPBA react with the iron(II) center of $[\text{Fe}^{\text{II}}(\text{TMCS})]^+$ to generate $[\text{Fe}^{\text{IV}}(\text{O})(\text{TMCSH})]^+$, which in turn transfers the O atom to the thiol of a second molecule of $[\text{Fe}^{\text{IV}}(\text{O})(\text{TMCSH})]^+$ to yield the sulfur oxygenated product $[\text{Fe}^{\text{II}}(\text{TMCSO}_2)]^+$. However, treatment of the redox-inactive complex $[\text{Zn}^{\text{II}}(\text{TMCS})]^+$ with 2 equiv *m*-CPBA resulted in the formation of a sulfinate complex $[\text{Zn}^{\text{II}}(\text{TMCSO}_2)]^+$, suggesting that a direct *S*-oxidation mechanism by *m*-CPBA cannot be totally excluded.

The only iron-sulfenate model complex to be structurally characterized was reported by Kovacs and co-workers,⁷⁷ and was the result of the use of an oxaziridine oxidant. The low-spin Fe^{III} complex, $[\text{Fe}^{\text{III}}(\text{ADIT})_2]^+$, is comprised of two tridentate ligands, each with an alkylthiolato S donor, an imino N donor, and an amino N donor (Scheme 19). Addition of 1.4 equiv *tert*-butyl *N*-sulfonyloxaziridine to $[\text{Fe}^{\text{III}}(\text{ADIT})_2]^+$ in methanol results in a color change from green to grape-purple. The addition of a single oxygen atom to the coordination sphere was determined by ESIMS, and the structure was confirmed by X-ray crystallography to contain a singly oxygenated thiolate sulfur, or sulfenate, bound to the Fe^{III} ion to generate $[\text{Fe}^{\text{III}}(\text{ADIT})(\text{ADIT-O})]^+$ (Scheme 19). An IR band at 931 cm^{-1} was assigned to the S=O stretch of the metal-bound sulfenate. Oxygen atom addition to the thiolato donor does not change the spin-state of the iron(III) center as determined by EPR and magnetic susceptibility, and as expected, causes a slight anodic shift of the redox potential by 75 mV. The oxygen atom of $[\text{Fe}^{\text{III}}(\text{ADIT})(\text{ADIT-O})]^+$ can be abstracted upon addition of triethylphosphine (PEt_3) to regenerate $[\text{Fe}^{\text{III}}(\text{ADIT})_2]^+$, and triethylphosphine oxide (OPEt_3) is detected by mass spectrometry. In the case of this coordinatively saturated Fe^{III} -thiolate complex, sulfur oxygenation is observed in place of iron oxygenation.

Reversible protonation of the sulfenate oxygen atom was observed by UV-Vis upon the addition of 1.07 equiv HBF_4 at $-40\text{ }^\circ\text{C}$ in CH_3CN followed by subsequent addition of 1.02 equiv Et_3N . The resulting red-shift from 575 nm corresponding to the sulfenate $[\text{Fe}^{\text{III}}(\text{ADIT})(\text{ADIT-O})]^+$ to 622 nm corresponding to the sulfenic acid complex $[\text{Fe}^{\text{III}}(\text{ADIT})(\text{ADIT-OH})]^{2+}$ (Scheme 20) differed from the blue-shift upon protonation of the parent $[\text{Fe}^{\text{III}}(\text{ADIT})_2]^+$ to generate $[\text{Fe}^{\text{III}}(\text{ADIT})(\text{ADIT-H})]^{2+}$ (718 nm to 640 nm). This distinction led to the suggestion that the protonation site is different in the dithiolate and the sulfenate/thiolate complexes. The resulting protonated complex, $[\text{Fe}^{\text{III}}(\text{ADIT})(\text{ADIT-OH})]^{2+}$, remains low-spin but is thermally unstable. Addition of 1.8 equiv ZnCl_2 to the sulfenate in CH_3CN results in a color change from grape-purple to midnight-blue, represented by a red-shift from 575 nm to 610 nm. A crystal structure reveals the electrophilic Zn^{2+} ion coordinates to the sulfenate oxygen of $[\text{Fe}^{\text{III}}(\text{ADIT})(\text{ADIT-O})]^+$ (Scheme 20). The Cl^- counterion present in the sulfenate complex binds to the Zn^{2+} ion to complete its tetrahedral coordination sphere. The presence of a Lewis acid slightly activates the S-O bond, indicated by its longer bond length, as well as its lower $\nu(\text{S-O})$ stretching frequency (893 cm^{-1} vs 931 cm^{-1} of the sulfenate).

S-oxygenation by an oxaziridine derivative has also been described by Mascharak and co-workers.⁷⁸ A tetradentate bis(arylamido), bis(arylthiolato) ligand was employed (Scheme 21), similar to that of $[\text{Fe}^{\text{III}}(\text{N}_2\text{S}_2)\text{Cl}]$ previously described by Artaud and co-workers.⁵¹ Substitution of Cl^- for dimethylaminopyridine (DMAP) afforded the intermediate-spin iron(III) ($S = 3/2$) complex $[\text{Fe}^{\text{III}}(\text{Cl}_2\text{PhPepS})(\text{DMAP})]^-$. Treatment of this complex with 1 equiv NO gas in CH_3CN at $-40\text{ }^\circ\text{C}$ yields $[\text{Fe}(\text{Cl}_2\text{PhPepS})(\text{NO})(\text{DMAP})]^-$, a formally $\{\text{Fe}$ -

NO}⁶ system, which was structurally characterized by X-ray crystallography. In work aimed at models of the inactive “dark form” of NHase, efforts were made to oxidize the S donors of this system. Addition of either O₂ or H₂O₂ did not result in S-oxygenation. However, when [Fe(Cl₂PhPepS)(NO)(DMAP)][−] was treated with 4 equiv (1*S*)-(+)-(10-camphorsulfonyl)oxaziridine in CHCl₃, a color change from red to pale-orange was observed. The IR spectrum of the product displayed bands at 1078, 1046, and 1007 cm^{−1}, consistent with S-oxygenation. The vibrational data, along with mass spectral analysis (*m/z* = 721.1), led to the assignment of this species as [Fe(Cl₂PhPep{SO₂})₂(NO)(DMAP)][−] (Scheme 21). Interestingly, the NO ligand of the starting thiolato complex [Fe(Cl₂PhPepS)(NO)(DMAP)][−] proved labile *in vacuo*, as well as upon irradiation with visible or UV light, provided it was dissolved in coordinating solvents. Upon S-oxidation, rapid photorelease of NO was observed, generating the low-spin (*S* = ½) complex [(Cl₂PhPep{SO₂})₂Fe^{III}(DMAP)₂][−].

The addition of O₂ to [Fe^{III}(PyPS)][−] results in the O-bound sulfinato complex [Fe^{III}(PyP{SO₂})₂][−] (see Scheme 3),⁵⁰ and the reaction of a related complex with H₂O₂ was also reported (Scheme 22).⁷⁹ The ligand in this study, PyPepSH₂, utilizes one amido and one pyridine nitrogen donor, as well as a phenylthiolate sulfur donor. The low-spin (*S* = ½) iron(III) center is coordinated by two equivalents of this tridentate ligand to afford six-coordinate (Et₄N)[Fe^{III}(PyPepS)₂]. Addition of H₂O₂ to a cold (−40 °C) solution of [Fe^{III}(PyPepS)₂][−] in DMF results in a color change from red to green with a λ_{max} at 690 nm. Structural characterization of the sulfinato species was possible upon addition of an equivalent of NaClO₄ to the green solution to afford Na[Fe^{III}(PyPepSO₂)₂]. Analysis of the structure of the oxidized product by X-ray crystallography indicates both thiolate donors are oxidized to sulfinato donors bound to the iron(III) center through the sulfur atom. The band at 1184 cm^{−1} in the IR spectrum is assigned to the S-O stretching mode of the S-bound sulfinato complex [Fe^{III}(PyPepSO₂)₂][−].

Iron versus sulfur oxygenation

Finally, it is instructive to examine some key examples of thiolate-iron(II) complexes in which S-oxygenation does not result upon reaction with O₂ or other oxidants, but Fe-oxygenation is favored instead. Darensbourg and co-workers synthesized two Fe^{III}-O-Fe^{III} complexes, [(bme*-daco)Fe]₂O and [(bme-daco)Fe]₂O, which were both crystallographically characterized (Figure 5).⁸⁰ The authors described two pathways to obtain [(bme*-daco)Fe]₂O (Scheme 23), one in which the isolated dimeric [(bme*-daco)Fe]₂ reacts slowly (1 week) with O₂, and the other in which Fe(acac)₃, the ligand H₂-bme*-daco, and O₂ react in one pot to generate the μ-oxo dimer. The related [(bme-daco)Fe]₂ is much more sensitive to O₂ and, in fact, decomposes upon exposure. The [(bme-daco)Fe]₂O complex is instead generated from the O-atom donor 4-chloropyridine *N*-oxide prior to isolation of the [(bme-daco)Fe]₂ dimer (Scheme 24). In both cases, evidence of sulfur oxygenation is not observed. The authors suggest [(bme-daco)Fe]₂ is air sensitive while [(bme*-daco)Fe]₂ is not, because the thiolate donors of [(bme*-daco)Fe]₂ are sterically hindered by methyl groups, which are believed to help prevent disulfide formation.

Another example of μ-oxo dimer formation was reported by Kovacs and co-workers.⁸¹ When [Fe^{II}(S^{Me}₂N₄(tren))]PF₆ is exposed to air in methanol, a brick-red powder is obtained upon the removal of solvent. Analysis by X-ray crystallography reveals a μ-oxo dimer, [Fe^{III}(S^{Me}₂N₄(tren))]₂(μ-O)(PF₆)₂ (Scheme 25). Reaction of [Fe^{II}(S^{Me}₂N₄(tren))] with other oxidants, such as superoxide (O₂[−]), led to the formation of an Fe^{III}-OOH species. Previous studies indicating the inner-sphere mechanism for O₂[−] reduction by [Fe^{II}(S^{Me}₂N₄(tren))] suggest O₂ reduction occurs via the initial coordination of O₂ to the iron(II) center.⁸² Inserting bulkier ethyl groups into this ligand system in order to prevent μ-

oxo dimer formation inhibits O₂ activation altogether. The redox potential of [Fe^{II}(S^{Me}₂N₄(tren-Et₄))]⁺ is anodically shifted +510 mV vs [Fe^{II}(S^{Me}₂N₄(tren))]⁺, indicating a much less stable Fe^{III} oxidation state for the more sterically encumbered analog.

A glutathione oxidation was reported by Kodanko and co-workers using a high-valent non-heme iron complex.⁸³ In an aqueous buffer solution, excess GSH was added to [Fe^{IV}(O)(N4Py)]²⁺, resulting in the rapid formation of a green intermediate with λ_{max} = 650 nm, followed by a slower decomposition to regenerate [Fe^{II}(OH₂)(N4Py)]²⁺. Based on EPR, UV-vis and mass spectral data, the authors assigned the green intermediate as [Fe^{III}(SG)(N4Py)]²⁺. Final disulfide GSSG production was quantitated to be 90 ± 20% based on [Fe^{IV}(O)(N4Py)]²⁺ with no other oxidized GSH products detected, such as the sulfenic, sulfinic, or sulfonic acids. Homolysis of the Fe^{III}-SG bond and the dimerization of two GS radicals to form GSSG are proposed as the mechanism for disulfide formation. Thus in this case a high-valent ferryl does not induce S-oxygenation, but rather leads only to the production of disulfide with the biologically important glutathione substrate.

Conclusions

Our goal in this Perspective is to provide an overview of discrete, non-heme iron-thiolate centers and their reactivity toward dioxygen to give *S*-oxygenation. This chemistry is important from a synthetic modeling perspective because of the direct relevance to a number of non-heme iron enzymes that undergo *S*-oxygenation, and is also of considerable importance given the current interest in controlling metal-mediated O₂ oxidations for catalytic applications. A small number of Fe^{III}-SR complexes exhibit reactivity with O₂ to give *S*-oxygenates, and these complexes appear to have some structural commonalities in that they typically contain multiple thiolate ligands and/or strongly electron-donating amidate donors. Thus the iron-thiolate units in these complexes are generally quite electron-rich. Evidence for production of singly oxygenated sulfenato complexes via Fe^{III}-SR + O₂ is scarce, as may be expected from the inherent instability of sulfenates, whereas the doubly oxygenated sulfinato and triply oxygenated sulfonate complexes are more common products. Little direct information is available on the mechanism of *S*-oxygenation in these complexes, and it is therefore not clear at this time what factors control the outcome of *S*-oxygenation for iron(III)-thiolate species. It is reasonable to speculate that the mechanism may involve direct reaction between O₂ and the coordinated thiolate donor, in analogy to the well-known *S*-oxygenation that occurs for Ni^{II}-thiolate complexes and O₂.^{84,85} As with Ni^{II}, the Fe^{III} ion is not readily susceptible to oxidation by O₂, and in an electron-rich complex, the thiolate ligands may serve as potent nucleophiles for direct attack on electrophilic O₂. For iron(II)-thiolate species the situation is significantly more complex because both the Fe and S centers are potential sites for direct oxidation by O₂. Synthetic systems are just beginning to emerge that exhibit controlled *S*-oxygenation of an Fe^{II}-SR unit, favoring this pathway over Fe-oxygenation (e.g. Fe-O-Fe formation). Initial model studies from our lab suggest that a redox potential below -0.1 V and an open site for O₂ binding *cis* to the thiolate donor are required for *S*-oxygenation. It is not clear yet whether a high-valent ferryl is a necessary intermediate in Fe^{II}-SR/O₂ reactivity as is predicted by calculations, but direct attack of O₂ on the thiolate donor of an iron(II)-thiolate species appears unlikely from the limited available data and calculations on both enzymes and models. A more reasonable mechanistic proposal at this point involves coordination and activation of O₂ by the ferrous center, as described for other non-thiolate-ligated non-heme oxygenase-type reactions. Determining the key factors that control the oxygenation pathways, such as ligand environment, spin state, and redox potential, remains an area ripe for further research.

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Biographies

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David P. Goldberg received his B.A. degree from Williams College in 1989, and his Ph.D. from the Massachusetts Institute of Technology in 1995 working in the field of bioinorganic chemistry under the direction of Prof. Stephen J. Lippard. He was then an NIH postdoctoral fellow at Northwestern University from 1995–1998 with Prof. Brian M. Hoffman. He joined the Department of Chemistry at The Johns Hopkins University in 1998, where he is now a professor of Chemistry. His research focuses on the synthesis, reactivity and mechanism of both heme- and non-heme coordination complexes as models of enzyme active sites as well as bio-inspired systems for catalytic applications.

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Figure 1.

Depictions of the active sites of a) catalytically active ($\text{X} = \text{H}_2\text{O}$) and inactive ($\text{X} = \text{NO}$) nitrile hydratase; b) ACV-bound isopenicillin N synthase crystallized with NO ; and c) the isolated sulfenate complex generated upon reaction of O_2 with the IPNS/ACOmC complex.

Figure 2.

Reactions catalyzed by a) cysteine dioxygenase (CDO), b) cysteamine dioxygenase (ADO), and c) 3-mercaptoprionate dioxygenase (3MPDO).

Figure 3. Depiction of the active sites of CDO derived from X-ray crystallography for a) the Ni^{II} -substituted center from mouse CDO, b) the Fe^{II} resting state from rat CDO, c) the Cys-bound center from human CDO, and d) a trapped persulfenate complex.

Figure 4.
Displacement ellipsoid plot for [Fe^{II}(LN₃S)(OTf)].

Figure 5.
Depictions of μ -oxo complexes derived from O_2 .

Scheme 1.

Proposed mechanism for the sulfur oxygenation of cysteine by CDO.

Scheme 2.
Synthetic scheme for (NEt₄)₂[Fe^{III}(L-O₂)].

Scheme 3.

Oxidation pathways of $[\text{Fe}^{\text{II/III}}(\text{PyPS})]^{2-/ -}$ by O_2 ; a) production of $[\text{Fe}^{\text{III}}(\text{PyP}\{\text{SO}_2\}_2)]^-$ with proposed sulfenato intermediate starting from $[\text{Fe}^{\text{III}}(\text{PyPS})]^-$; b) outersphere oxidation by O_2 of $[\text{Fe}^{\text{II}}(\text{PyPS})]^{2-}$.

Scheme 4. Possible pathways for the *S*-oxygenation of $[\text{Fe}^{\text{III}}(\text{N}_2\text{S}_2\text{Cl}_2)\text{CN}]^{2-}$.

Scheme 5.

O-bound sulfinate complex $[\text{Fe}^{\text{III}}((\text{tame-N}_2\text{SO}_2)\text{S}_2^{\text{Me}_2})]^{2-}$ derived from the oxygenation of $[\text{Fe}^{\text{III}}((\text{tame-N}_2\text{S})\text{S}_2^{\text{Me}_2})]^{2-}$.

Scheme 6.
Oxygenation pathways for $[(N_2S_3)Fe^{III}(Cl)]$ and $[(N_2S_3)Fe^{III}(CN)]$.

Scheme 7.

Synthetic scheme for the generation of monosulfinate $[(\text{NO})\text{Fe}(\text{S},\text{SO}_2-\text{C}_6\text{H}_4)(\text{S},\text{S}-\text{C}_6\text{H}_4)]^-$ and bis(sulfinate) dimer $[(\text{NO})\text{Fe}(\text{SO}_2, \text{SO}_2-\text{C}_6\text{H}_4)(\text{S},\text{S}-\text{C}_6\text{H}_4)]_2^{2-}$.

Scheme 8. Sulfur oxygenation and reduction pathways for derivatives of $[(NO)Fe(S,S-C_6H_4)_2]^-$.

Scheme 9.
Generation of $[\{\text{Fe}(\text{Et}_2\text{NpyS}_4\text{-O}_2)\}_2]$

Scheme 10.
Synthesis of $[\text{Fe}^{\text{II}}(\text{LN}_3\text{S})(\text{OTf})]$.

Scheme 11.

Generation of $[\text{Fe}^{\text{II}}(\text{LN}_3\text{SO}_3)]^+$ upon addition of dioxygen to $[\text{Fe}^{\text{II}}(\text{LN}_3\text{S})(\text{OTf})]$.

Scheme 12.

Synthetic scheme and displacement ellipsoid plots for $[(iPr)_2BIP]Fe^{II}(SPh)(Cl)$ and $[(iPr)_2BIP]Fe^{II}(SPh)(OTf)$.

Scheme 13.

Proposed oxidation pathways for $[(iPr)BIP]Fe^{II}(SPh)(Cl)$ and $[(iPr)BIP]Fe^{II}(SPh)(OTf)$.

Scheme 14.
Sulfur oxygenation of CDO model complex $[\text{Tp}^{\text{Me,Ph}}\text{FeCysOEt}]$.

Scheme 15.
Synthesis of N3PySH and $[\text{Fe}^{\text{II}}(\text{N3PyS})(\text{CH}_3\text{CN})]\text{BF}_4$.

Scheme 16.

Displacement ellipsoid plots of $[\text{Fe}^{\text{II}}(\text{N3PyS})(\text{CH}_3\text{CN})]^+$ and $[\text{Fe}^{\text{II}}(\text{N3PySO}_2)(\text{NCS})]$.

Scheme 17.
Reaction of $[\text{Fe}^{\text{II}}(\text{TMCS})]^+$ with $m\text{-CPBA}$.

Scheme 18.

Possible mechanisms for the oxidation of the thiolate of $[\text{Fe}^{\text{II}}(\text{TMCS})]^+$ using $m\text{-CPBA}$ as oxidant.

Scheme 19.
Synthesis of [Fe^{III}(ADIT)(ADIT-O)]⁺.

Scheme 20.
Addition of Brønsted and Lewis acids to $[\text{Fe}^{\text{III}}(\text{ADIT})(\text{ADIT-O})]^+$.

Scheme 21.

Oxidation of $[(Cl_2PhPepS)Fe(NO)(DMAP)]^-$ to $[(Cl_2PhPep{SO_2}_2)Fe(NO)(DMAP)]^-$.

Scheme 22.

Oxidation of $[\text{Fe}^{\text{II}}(\text{PyPepS})_2]^-$ by H_2O_2 to generate $[\text{Fe}^{\text{III}}(\text{PyPepSO}_2)_2]^-$.

Scheme 23. Stepwise and *in situ* generation of $[(\text{bme}^*\text{-daco})\text{Fe}]_2\text{O}$.

Scheme 24.
Anaerobic generation of $[(\text{bme-daco})\text{Fe}]_2\text{O}$.

Scheme 25.

Oxidation of $[\text{Fe}^{\text{II}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))]\text{PF}_6$ by O_2 to generate $[\text{Fe}^{\text{III}}(\text{S}^{\text{Me}_2}\text{N}_4(\text{tren}))]_2(\mu\text{-O})(\text{PF}_6)_2$.

Table 1Redox potentials for non-heme iron(II) complexes and O₂ reactivity

Compound	$E_{1/2}$ (ΔE_{pp}) ^a	O ₂ reactivity
[(^{Pr} BIP)Fe ^{II} (SPh)(Cl)]	−0.173 ^b (0.114) (r)	yes
[(^{Pr} BIP)Fe ^{II} (SPh)(OTf)]	−0.372 ^b (0.149) (r)	yes
[(^{Pr} BIP)Fe ^{II} (Cl) ₂]	0.025 ^b (0.153) (r)	no
[(^{Pr} BIP)Fe ^{II} (OTf) ₂]	0.613 ^{b, c} (ir)	no
[(TMC)Fe ^{II} (OTf) ₂] ^{d, e}	−0.14 ^f (qr)	yes
[(TMC)Fe ^{II} (OTf) ₂] ^{d, e}	0.02 ^g (r)	no
[(TPA)Fe ^{II}] ²⁺ , ^{e, h}	0.36 ⁱ (r)	no

^aValues in V vs Fc⁺/Fc. ΔE_{pp} = peak-to-peak separation; r = reversible, ir – irreversible, qr = quasi-reversible.^bIn CH₂Cl₂ at a scan rate of 0.1 V/s from ref 69.^cAnodic peak potential.^dTMC = tetramethylcyclam.^eData taken from ref 45.^fIn 1:1 MeCN/THF.^gIn 1:1 MeCN/CH₂Cl₂.^hTPA = tris(2-pyridylmethyl)amine.ⁱIn neat MeCN.