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High-Spin and Low-Spin States in \{\text{FeNO}\}^7, \text{Fe}^{\text{IV}}=\text{O}, \text{and Fe}^{\text{III}}–\text{OOH} Complexes and Their Correlations to Reactivity

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15.1 Introduction

The mononuclear nonheme iron (NHFe) enzymes given in Scheme 15.1 catalyze a broad spectrum of critical biological and health-related functions. They are involved in antibiotic [1–4] collagen [5] and natural product biosynthesis [6, 7] and regulation of leukotrienes in asthma and atherosclerosis [8], a number of neurological disorders [9–11], hypoxia regulation [12], DNA repair [13], bioremediation [14, 15], and include the related anticancer drug bleomycin (BLM) [16, 17]. All members of this large NHFe family are involved in $^{3}$O$_{2}$ reactions with organic substrates ($S = 0$) that, if uncatalyzed, are spin-forbidden and thus kinetically slow. These enzymes can be divided into two groups depending on whether an Fe$^{\text{III}}$ site activates a substrate for reaction with O$_{2}$, or whether an Fe$^{\text{II}}$ site is utilized for O$_{2}$ activation for reaction with a substrate [18]. The latter strategy is adapted by most mononuclear NHFe enzymes.

The group of substrate-activating NHFe$^{\text{III}}$ enzymes includes the lipoxygenases and the intradiol dioxygenases (Scheme 15.1). For lipoxygenases, activation of the fatty acid substrates is thought to proceed via H-atom abstraction (HAA) by Fe$^{\text{III}}$–OH, which leads to the formation of the Fe$^{\text{II}}$–OH$_{2}$ intermediate and a substrate radical that undergoes subsequent reaction with O$_{2}$ to give the final hydroperoxidated product [19]. For intradiol dioxygenases, the catecholate dianionic substrate binds to the Fe$^{\text{III}}$ site, triggering a concerted
ligand-to-metal charge-transfer mechanism of substrate activation, where the change in the ligand field on the Fe\textsuperscript{III} overcomes the spin-forbidden formation of a peroxy-bridged intermediate that goes on to form the intradiol-cleaved product [20].

The group of O\textsubscript{2}-activating NHFe\textsuperscript{II} enzymes is large and can be divided into six classes (Scheme 15.1): (1) The extradiol dioxygenases. These activate O\textsubscript{2} by two electrons from the catecholate substrate via a putative peroxy-quinone-bridged Fe\textsuperscript{II} intermediate that undergoes a Criegee rearrangement, eventually leading to a seven-membered lactone ring that collapses to form the extradiol cleaved product [21]. (2) The pterin-dependent and (3) α-ketoglutarate (αKG)-dependent enzymes. Both of these classes utilize an Fe\textsuperscript{II} center and a cofactor to reduce O\textsubscript{2} by four electrons (through the formation of a peroxo bridge between the metal and an electron-donating cofactor) that cleaves to give a high-spin (HS) (S = 2) Fe\textsuperscript{IV}=O intermediate reactive in H-atom abstraction (αKG dependent) and electrophilic aromatic substitution (pterin dependent) [22, 23]. (4) The Rieske dioxygenases (RDOs). These contain the Fe\textsubscript{2}S\textsubscript{2} Rieske center in addition to the catalytic NHFe\textsuperscript{II}, activate O\textsubscript{2} by two-electron reduction, one electron from the Rieske center and another from the Fe\textsuperscript{II}; this generates a high-spin (S = 5/2) Fe\textsuperscript{III}=OOH intermediate that performs C–H bond activation [24] and electrophilic cis-dihydroxylation of aromatic compounds [25]. (5) The anticancer drug Bleomycin (BLM). This metalloglycopeptide activates O\textsubscript{2} by two-electron reduction (one electron from Fe\textsuperscript{II} and another exogenous
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Figure 15.1 The general mechanistic strategy for \( \text{O}_2 \) activation by NHFe\(^{II} \) active sites [18, 35]. Reproduced from [35] with permission from American Chemical Society.

electron), which forms a low-spin (LS) \((S = 1/2)\) Fe\(^{III}–\text{OOH} \) intermediate (activated bleomycin [ABLM]) capable of H-atom abstraction from DNA for strand cleavage in its anticancer activity [26]. (6) A diverse class of enzymes that do not require a cofactor and have redox inactive substrates (e.g., isopenicillin-N-synthase [IPNS], cysteine dioxygenase [CDO]). For this subclass, the NHFe\(^{II} \) center, as the only redox center available, is able to perform the thermodynamically difficult \( \text{1e}^{-} \) reduction of \( \text{O}_2 \) to generate an Fe\(^{III}–\text{O}_2^{\cdot-} \) intermediate that performs C–H bond activation or oxygenation of the substrate [27, 28].

From the above summary, the generation of \( S = 2 \) Fe\(^{IV}=\text{O} \) or Fe\(^{III}–\text{OOH} \) intermediates are utilized by most of the NHFe\(^{II} \) enzymes. Parallel to studies directed toward trapping and characterization of the geometric and electronic structures of these enzymatic intermediates, there have been significant advances in the preparation and investigation of their synthetic analogues. Thus, \( S = 1 \) and \( S = 2 \) Fe\(^{IV}=\text{O} \) and \( S = 5/2 \) and \( S = 1/2 \) Fe\(^{III}–\text{OOH} \) model complexes have been structurally defined with non-biological ligand sets [29–32].

Except for ABLM, which is of low spin because of its strong 5-nitrogen ligand set, all the NHFe enzyme oxygen intermediates are of high spin. The ferrous NHFe enzymes have the canonical 2His/1carboxylate facial triad ligation and additional coordination positions occupied by water ligands in the resting state. However, it has recently been found that a number of these enzymes have a 3His ligand set [33] and several of the \( \alpha \)KG-dependent enzymes are halogenases [34], where \( \text{Cl}^{-} \) or \( \text{Br}^{-} \) replaces the carboxylate ligand of the facial triad.

An important common theme for these NHFe\(^{II} \) species is their general mechanistic strategy for \( \text{O}_2 \) activation elucidated by variable temperature variable field (VTVH) magnetic circular dichroism (MCD) [18]. In most of the classes in Scheme 15.1, the resting Fe\(^{II} \) site and the Fe\(^{II} \) site in the presence of only substrate or cofactor are 6-coordinate, coordinatively saturated, and relatively stable in the presence of \( \text{O}_2 \). However, the simultaneous presence of all of the reaction partners involved in catalysis leads to an open coordination position on the Fe\(^{II} \) site (by releasing a labilized water ligand). This allows \( \text{O}_2 \) binding and activation (Figure 15.1) [35]. This strategy ensures effective coupled catalysis, avoiding uncoupled turnover and side reactions of auto-oxidation and self-hydroxylation.
Since the initial Fe–O₂ intermediates are not trappable for spectroscopic characterization in any NHFe enzyme except for the extradiol dioxygenases, the methodology has been developed in which NO is used as an analogue for O₂ in order to experimentally probe the early stages of O₂ activation and the role of the coordinated substrate (IPNS) or cofactor (in the αKG-dependent enzymes). NO binds reversibly to the FeII site, forming a stable species that is amenable to a range of spectral methods. These Fe–NO complexes, termed as {FeNO}³ in the notation of Enemark and Feltham [36] (\(7 = \) number of metal d-electrons + number of NO valence electrons), are one-electron deficient relative to putative \{FeO₂\}⁸ intermediates and generally have \(S = 3/2\) ground states. These have an electronic structure best described as high-spin FeIII (\(S = 5/2\)) anti-ferromagnetically coupled to NO⁻ (\(S = 1\)) [37], although in some cases \(S = 1/2\) species with either an FeIII (\(S = 3/2\)) anti-ferromagnetically coupled to NO⁻ (\(S = 1\)) or a low-spin FeII (\(S = 0\)) NO⁺ (\(S = 1/2\)) electronic structure has been observed [28, 38, 39]. The spectroscopic features of these \{FeNO\}³ complexes, correlated to density functional theory (DFT) calculations, provide a calibration of the DFT functional and basis set for computationally replacing NO with O₂ to define the O₂ activation reaction coordinate of the mostly experimentally inaccessible \{FeO₂\}⁸ species. This \{FeNO\}³/{FeO₂}⁸ methodology has been applied to the α-keto acid (αKA)-dependent enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) [40] and IPNS [27] to elucidate their O₂ activation reaction coordinates.

In this chapter, we present a detailed discussion of high-spin and low-spin states in \{FeNO\}³ (\(S = 3/2, 1/2\)), FeIV=O (\(S = 2, 1\)), and FeIIIOOH (\(S = 5/2, 1/2\)) complexes, their frontier molecular orbitals (FMOs, that is, low-lying unoccupied molecular orbitals (MOs) with proper orbital character and orientation for attack on substrate), and their contributions to reactivity in model and enzyme systems.

15.2 High- and Low-Spin \{FeNO\}³ Complexes: Correlations to O₂ Activation

15.2.1 Spectroscopic Definition of the Electronic Structure of High-Spin \{FeNO\}³

Five-coordinate ferrous sites in nonheme Fe enzymes generally have low reactivity with O₂ unless both substrate and cofactor necessary for the reaction are present (cf. Figure 15.1). Once all co-substrates are bound, O₂ can bind to the coordinatively unsaturated Fe, generating a mostly unobserved \{FeO₂\}⁸ intermediate that then reacts with substrate. In contrast, NO binds reversibly to these sites, even in the absence of cofactor and substrate, generating an \{FeNO\}³ complex. These \{FeNO\}³ complexes thus provide a useful probe for understanding the spectral perturbations introduced by co-substrate binding that turns on O₂ reactivity in NHFeII enzymes. \{FeNO\}³ complexes, in both models and enzymes, can exist in total spin states of \(S = 3/2\) and \(1/2\). \(S = 3/2\), being the common spin state for these complexes, is the main focus of this review. A brief discussion of \(S = 1/2\) \{FeNO\}³ complexes and their possible relevance in several enzymatic reactions is presented at the end of this section. There are the five d orbitals on Fe and two \(\pi^*\) orbitals on NO (\(\pi_{ip}^*\) in the Fe–N–O plane and \(\pi_{op}^*\) out of the Fe–N–O plane) available for bonding, and many proposals for the electronic structure of the \{FeNO\}³ unit based on possible distributions of the seven FeNO electrons (six from the FeII and one from the NO⁺) in these orbitals exist in the literature [41].

The electronic structure of \(S = 3/2\) \{FeNO\}³ complexes was first unambiguously determined through a combination of spectroscopic studies [37] and calculations [42] on two complexes, \[\text{Fe(Me}_3\text{TACN)}(\text{N}_3)_2(\text{NO})\] (1; \(\text{Me}_3\text{TACN} = \text{N},\text{N}',\text{N}''\text{-trimethyl-1,4,7-triazacyclononane}\)) and \[\text{Fe(EDTA)}(\text{NO})\] (2; EDTA = ethylenediaminetetraacetic acid). The geometric structure of 1 was known from crystallography (Figure 15.2a) [43], and 2 was structurally defined through extended X-ray absorption fine structure (EXAFS) measurements (including multiple-scattering analysis of the data using the software package GNXAS for determination of the Fe–N–O angle) [44]. For 1, the Fe–N bond length is 1.74 Å and the Fe–N–O angle is 155°, and for 2 these are 1.78 Å and 156° respectively.
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The electronic structures of both complexes were investigated using X-ray Absorption (XAS), Electron Paramagnetic Resonance (EPR), Resonance Raman (RR), UV/Vis Absorption (Abs), and MCD spectroscopies [37]. XAS data on both complexes showed these to be high-spin ferric species. The EPR spectra for \(1\) and \(2\) (Figure 15.3a) show typical \(S = 3/2\) behavior, with \(g\)-effective values of \(\sim 4\) and \(\sim 2\). These spectra were fit to the spin Hamiltonian in equation (15.1):

\[
H = D[S^2 - S(S + 1)/3] - E(S_x^2 - S_y^2) + g_0\beta SH
\]  

(15.1)

where \(D\) is the axial zero-field splitting (ZFS) parameter, \(E\) is the rhombic ZFS parameter, and \(g_0 = 2.0\). The \(E/D\) value can range from 0 to 0.33 and denotes the rhombicity of the site, with 0 being the axial limit. The signal at \(g\)-effective \(\sim 4\) will split as \(E/D\) deviates from the axial limit (0 for \(1\) and 0.016 for \(2\)). \(D\)-values of \(+20 \pm 2\) cm\(^{-1}\) (\(1\)) and \(+12 \pm 1\) cm\(^{-1}\) (\(2\)) were obtained for these complexes through fitting magnetic susceptibility data. Thus, both of these \(S = 3/2\) \{FeNO\}\(^7\) complexes have axial EPR spectra \((E/D < 0.02)\) and large, positive ZFS \(D\).

Resonance Raman spectroscopic studies of \(1\) and \(2\) prepared with \(^{14}\)N\(^{16}\)O, \(^{15}\)N\(^{16}\)O, and \(^{14}\)N\(^{18}\)O, shown in Figure 15.3b, revealed isotope-sensitive features in both complexes. For \(1\), peaks at 1712, 497, and 436 cm\(^{-1}\) were found to shift to 1681, 489, and 435 cm\(^{-1}\) with \(^{15}\)N\(^{16}\)O substitution, and to 1672, 490, and 434 cm\(^{-1}\) with \(^{14}\)N\(^{18}\)O substitution. These are assigned as the \(\nu(\text{NO})\), \(\nu(\text{FeN})\), and \(\delta(\text{FeNO})\), respectively, based on the expected isotope shifts of these features in bent nonheme metal–nitrosyl complexes (Figure 15.3b, lower) [37, 45]. By a similar argument, for \(2\), features at 1776 and 496 cm\(^{-1}\) are assigned respectively as \(\nu(\text{NO})\) and \(\nu(\text{FeN})\). The N–O stretching frequency for both complexes falls between those of triplet \(\text{NO}^-\) in the gas phase (1363 cm\(^{-1}\)) [46] and \(\text{NO}^0\) in NO gas (1876 cm\(^{-1}\)) [47]. The force constants for \(\nu(\text{NO})\) derived from a normal coordinate analysis based on the RR data exhibit a similar trend: 12.6 and 13.2 mdyn/Å for \(1\) and \(2\) respectively that fall between the values of 8.6 mdyn/Å for \(\text{NO}^-\) and 15.5 mdyn/Å for \(\text{NO}^0\). These observations are consistent with partial \(\text{NO}^-\) character that is decreased due to its donor bonding to the \(\text{Fe}^{III}\).

Taken together, the experimental data show that these \{FeNO\}\(^7\) complexes are best described as \(\text{Fe}^{III}--\text{NO}^-\) complexes, with an \(S = 1\) \(\text{NO}^-\) anti-ferromagnetically coupled to an \(S = 5/2\) \(\text{Fe}^{III}\) to give an overall \(S = 3/2\) ground state [37]. The \(S = 5/2\) on the \(\text{Fe}^{III}\) arises from exchange stabilization of the five \(\alpha-\delta\)-electrons, and the \(S = 1\) on the \(\text{NO}^-\) arises from stabilization due to the overlap of the spin-down \(\text{NO}^- \pi^+\) orbitals with the \(\text{Fe}^{III}\)
Figure 15.3 Spectroscopy of complexes 1 and 2. (a) EPR data showing E/D of 0 for 1 and 0.016 for 2; (b) Resonance Raman data with $^{14}$N$^{16}$O (solid), $^{14}$N$^{18}$O (dashed), and $^{15}$N$^{16}$O (dotted); (c) Abs, MCD, and RR profile data (triangles and unfilled squares correspond to $\nu$(FeN) intensity; crosses and filled squares correspond to $\nu$(NO) intensity) with bands numbered. Reproduced from [37] with permission from American Chemical Society.
d_{xz} and d_{yz} levels (the z-axis is defined along the Fe–N bond, x is in the Fe—N–O plane, y is perpendicular to the plane, and the FeNO plane bisects cis-L–M–L bonds), which opposes the splitting of \( \pi_{ip}^\ast \) and \( \pi_{op}^\ast \) and disfavors spin pairing. This has become the generally accepted electronic structure description for \( S = 3/2 \) \{FeNO\} \(^7\) complexes [41, 45].

UV-Vis Absorption, MCD, VTVH MCD, and RR profile data for 1 and 2 are presented in Figure 15.3c. The spectra for each have been simultaneously fit to five bands involving the FeNO unit, with Bands 3–5 exhibiting resonance enhancement of \( \nu(\text{NO}) \) and \( \nu(\text{FeN}) \) for both complexes. Using the experimentally defined electronic structure, these are assigned as NO\(^−\) to Fe\(^{III}\) charge transfer (CT) transitions on the basis of this resonance enhancement and their \( z \)-polarization from VTVH MCD (vide infra), with Bands 3–5 assigned as the NO\(^−\) \( \beta-\pi_{ip}^\ast \rightarrow \) Fe\(^{III}\) \( d_{z^2} \), NO\(^−\) \( \beta-\pi_{ip}^\ast \rightarrow \) Fe\(^{III}\) \( d_{x^2−y^2} \), and NO\(^−\) \( \beta-\pi_{op}^\ast \rightarrow \) Fe\(^{III}\) \( d_{y^2} \). CT transitions, respectively, with the last transition being most intense due to its greater amount of NO\(^−\) \( \pi \) donating character relative to the two transitions originating from the NO\(^−\) \( \pi_{ip}^\ast \) orbitals. Bands 1 and 2 are assigned as Fe\(^{III}\) ligand-field (LF) transitions. Note that these are spin-forbidden in high-spin Fe\(^{III}\) but become allowed in the anti-ferromagnetically coupled system. Important to note is that the spectral features reported here for model systems are also observed in the \{FeNO\} \(^7\) complexes of NO bound to the resting 2His/1carboxylate facial triad in NHFe\(^{II}\) enzymes such as protocatechuate 4,5-dioxygenase [48] and HPPD [40], providing an important benchmark for the studies reviewed in Section 2.3.

15.2.2 Computational Studies of \( S = 3/2 \) \{FeNO\} \(^7\) Complexes and Related \{FeO\} \(^8\) Complexes

To develop a more detailed understanding of the electronic structure of \{FeNO\} \(^7\) complexes, and to determine an appropriate DFT methodology for describing them, a series of calculations using a variety of functionals and basis sets were carried out on 1 [42], which was chosen because it has a well-defined crystal structure [43]. It was found that a hybrid functional consisting of the BP86 functional with 10\% Hartree–Fock exchange (HFX) contribution (BP86 + 10\% HFX) along with a split basis set of triple-\( \zeta \) 6-311G* on the FeNO unit and double-\( \zeta \) 6-31G* on the other atoms (for computational efficiency) gave an optimized geometry in best agreement with the crystal structure. In addition, this combination of functional and basis set gave good agreement with the experimental N–O stretching frequency, and NO\(^−\) → Fe\(^{III}\) CT transition energies (from \( \Delta \)SCF calculations) that agreed well with energies from Abs and MCD data. Calculations with more HFX (>10\%) gave poor agreement with experiment. Analysis of the unoccupied molecular orbitals for 1 calculated using this level of DFT (the unoccupied orbitals are used here as these reflect uncompensated electron density from the occupied orbitals, which often are strongly mixed due to their energy stabilization from spin polarization) showed two unoccupied \( \alpha \)-MOs with significant NO \( \pi^* \) character (\( \pi_{ip}^* \) and \( \pi_{op}^* \)) and five unoccupied \( \beta \)-MOs with Fe 3d orbital character, consistent with the experimentally defined Fe\(^{III}\) (\( S = 5/2 \)) anti-ferromagnetically coupled to NO\(^−\) (\( S = 1 \)) electronic structure description (Figure 15.4a and b). From these MOs, a bonding description for 1 was defined. There are two types of bonding interactions reflected in the amount of NO character in the unoccupied \( \beta \) Fe d-orbitals, as well as in the NO \( \pi^* \) → Fe d-CTs (both reflecting donation from the NO\(^−\) to the Fe\(^{III}\)): (i) \( \sigma \)-bonding interactions between NO \( \pi_{ip}^* \) and Fe \( d_{x^2−y^2} \) (using the axis system defined above) (Figure 15.4b, panels C, D, and G) and, importantly, (ii) a strong \( \pi \)-bonding interaction between NO \( \pi_{op}^* \) and Fe \( d_{y^2} \) (Figure 15.4b, panel E). These interactions lead to a strong Fe\(^{III}\)–NO\(^−\) bond.

This experimentally calibrated \( S = 3/2 \) \{FeNO\} \(^7\) methodology was then extended to the \{FeO\} \(^8\) analogue of complex 1 (complex 3, Figure 15.2b) in order to develop a basis for understanding \( O_2 \) activation and reactivity in nonheme iron enzymes. DFT geometry optimizations were performed on the three possible spin states, \( S = 1, 2, \) and 3, with the electronic structure of the \( S = 1 \) corresponding to low-spin Fe\(^{III}\) ferromagnetically coupled to \( O_2^{−−} \), \( S = 2 \) to high-spin Fe\(^{III}\) anti-ferromagnetically coupled to \( O_2^{−−} \), and \( S = 3 \) to high-spin Fe\(^{III}\) ferromagnetically coupled to \( O_2^{−−} \). The \( S = 2 \) spin state was computationally lowest in energy.
Figure 15.4  Energy diagrams (a and c) and MO contours (b and d) with significant Fe or NO/O₂ character in 1 and its O₂ analogue 3 respectively. Both complexes have a high-spin Fe center with five unoccupied β d-orbitals. The primary difference between the two is that the α π⁺ orbital (b, panel A) is unoccupied in 1 but occupied in 3 (d, panel A), leading to a difference in NO/O₂ bond strength. All other depicted MOs are unoccupied. Reproduced from [42] with permission from American Chemical Society.
by ~10 kcal/mol; thus the following discussion focuses on this $S = 2$ ground state. The Fe–O bond length in 3 is 1.88 Å (versus 1.72 Å in 1), and the Fe–O–O angle is 130° (versus 148° in 1). The $\Delta G^0$ of formation for 3 from the putative 5C Fe$^{II}$(Me$_3$)TACN(N$_3$)$_2$ derivative is ~4.0 kcal/mol. In contrast, the $\Delta G^0$ of formation of the NO complex 1 is highly exergonic at ~25 kcal/mol, despite the fact that NO is more difficult than O$_2$ to reduce by one electron [49, 50]. Thus, the more favorable formation of 1 is due to the formation of a stronger Fe$^{III}$–NO bond relative to Fe$^{III}$–O$_2$–$^*$. As with 1, the FeO$_2$ system 3 is calculated to have five unoccupied $\beta$ MOs with Fe d-orbital character (Figure 15.4d). Its $\alpha$-manifold differs in that 3 has one more electron, which occupies the $\alpha$ O$_2 \pi^*_{ip}$ orbital (Figure 15.4c), while the $\alpha$ O$_2 \pi_{op}^*$ orbital is unoccupied, leading to the Fe$^{III}$ ($S = 5/2$) anti-ferromagnetically coupled to O$_2$–$^-$ ($S = 1/2$) for an $S = 2$ ground state electronic structure described above. The additional electron and the change from NO$^-$ to O$_2$–$^-$ lead to a significant difference in the bonding description between 1 and 3. In 3, the $\beta \pi^*$ O$_2$ orbitals are lower in energy due to the increased electronegativity, which also causes the coefficient on the atom bound to Fe to decrease. Both of these factors decrease the strength of the Fe–O$_2$ bond relative to that of the Fe–NO bond. Further, the extra electron in the $\alpha$ O$_2 \pi_{ip}^*$ orbital leads to repulsion between the $\alpha$ and $\beta$ electrons of this doubly occupied MO, which raises the energy of $\beta \pi_{ip}^*$ and leads to stronger mixing of this orbital relative to $\beta \pi_{op}^*$ with the unoccupied $\beta$ d-orbitals, which stabilizes it in energy in Figure 15.4d. The resultant electronic structure reflects $\sigma$ donation between $\beta \pi_{ip}^*$ and $d_{e2}$ and only weak $\pi$ donation between $\pi_{ip}^*$ and $d_{xz}$ (Figure 15.4d, panels E and G), with essentially no $\pi$ donation of the $\beta \pi_{op}^*$ with the $dx^2$ orbitals (Figure 15.4c and Figure 15.4d, panel C). Lack of a strong $\pi$ donor bonding interaction leads to a longer Fe–O$_2$ bond relative to the Fe–NO bond (1.88 vs. 1.72 Å), a steeper Fe–O–O angle relative to the Fe-N-O angle (130° vs. 148°) in order to maximize the $\sigma$ overlap, and a weaker Fe–O$_2$ bond.

This result provides insight into the experimental observation that 5-coordinate resting ferrous sites in enzymes, such as extradiol dioxygenase DHBD (2,3-dihydroxybiphenyl 1,2-dioxygenase), react only very slowly with O$_2$ [51] but form strong Fe–NO bonds [52]. While the calculation presented above predicts slightly favorable O$_2$ binding at ~4.0 kcal/mol, this is not the case for 5C NHFe$^{II}$ enzyme sites, likely because the Fe$^{II}$ reactant to form 3 is destabilized by the rigid TACN chelation. In the NHFe$^{II}$ enzymes, the calculated energetics of O$_2$ binding to the 2His/1carboxylate 5-coordinate Fe$^{II}$ facial triad generating an $S = 2$ $\{\text{FeO}_2\}^8$ was found to have a $\Delta G^0$ of +18.9 kcal/mol [27], consistent with the lack of reactivity for 5C NHFe$^{II}$ active sites in the absence of co-substrates.

### 15.2.3 Extension to IPNS and HPPD: Implications for Reactivity

This methodology was extended to the study of O$_2$ activation and reactivity by the members of the two classes of NHFe$^{II}$ enzymes: IPNS [27] and HPPD [40]. Geometry-optimized structures of the substrate-bound $\{\text{FeNO}\}^7$ complexes of these active sites are presented in Figure 15.5. IPNS catalyzes the oxidative ring closure of δ-(1-α-aminoasipitinyl)-1-cysteinyld-valine (ACV) [53]. This involves H-atom abstraction (see Scheme 15.1) from the substrate by a putative Fe$^{III}$–superoxo intermediate (vide infra) to generate isopenicillin N in the biosynthesis of penicillins and cephalosporin [1]. As the substrate is not redox-active and there are no additional cofactors to provide electrons, the Fe$^{III}$–superoxide likely forms through electron transfer from Fe$^{II}$ to O$_2$, a process that would generally be unfavorable (vide supra). HPPD catalyzes the formation of homogentisate from (4-hydroxyphenyl)pyruvate (HPP), which has both the substrate and the αKA necessary for the reaction linked covalently [54]. For both of these enzymes, the resting Fe$^{II}$ site is unreactive with O$_2$ but substrate binding to the Fe$^{II}$ turns on O$_2$ reactivity (in accordance with the mechanism in Figure 15.1). Therefore, the $\{\text{FeNO}\}^7 \rightarrow \{\text{FeO}_2\}^8$ methodology was applied to understand new electronic structure features arising from substrate directly coordinating to the $\{\text{FeNO}\}^7$ species that turn on the reactivity in $\{\text{FeO}_2\}^8$ complexes.
In IPNS, the Fe–ACV–NO analogue (substrate bound to Fe through the cysteinyl S) was studied through a combination of variable temperature EPR, Abs, CD/MCD, and VTVH MCD spectroscopies. Fitting the variable-temperature EPR intensity in Figure 15.6a to equation (15.2):

\[
\text{Intensity} = \frac{C}{T} \left[ \frac{1}{1 + \exp(-2D/kT)} \right],
\]

where \(C\) is the Curie constant, \(T\) is the temperature, and \(k\) is the Boltzmann’s constant, gives an axial ZFS \(D\) parameter of \(+12.5 \pm 0.5\) cm\(^{-1}\), similar to the model complexes discussed above and reduced from the value for the NO-bound resting facial triad \(+16\) cm\(^{-1}\) \[40\]. Fitting of the EPR spectrum gives an \(E/D\) value of 0.035, indicating an axial system with a rhombic perturbation. In Abs/CD/MCD (Figure 15.6), two different spectral features relative to the models (and the \(\{\text{FeNO}\}^7\) facial triad without substrate bound) were identified at 13,100 cm\(^{-1}\) (Band 1) and 19,800 cm\(^{-1}\) (Band 5) (Figure 15.6b).

Band 5 is intense in absorption and has negative MCD intensity (Figure 15.6b, lower). The negative intensity of this band raises the possibility that it interacts (through spin-orbit coupling [SOC]) with another positive MCD band. The VTVH MCD saturation behavior of a band in MCD is dependent on the polarization of that band relative to the ZFS tensor \[55\]. The VTVH MCD data (Figure 15.6c) show that Band 1 has mixed \(z\) and \(x/y\)-polarization (with the \(z\)-axis of the ZFS tensor along the strong Fe–NO bond), indicating that it has both NO\(^-\) and RS\(^-\) to Fe\(^{III}\) CT character. In contrast, Band 6 is purely \(z\)-polarized and thus defined as an intense NO\(^-\) \(\rightarrow\) Fe\(^{III}\) CT transition at \(~22000\) cm\(^{-1}\). These results correlate well with electronic structure calculations.

The density functional theory calculations of this IPNS-ACV-NO complex (structure in Figure 15.5a), using the basis set and functional combination determined above for the model systems, revealed a generally similar electronic structure to that of the models (two unoccupied NO \(\pi^*\) orbitals in the \(\alpha\) manifold and five unoccupied Fe d-orbitals in the \(\beta\) manifold (Figure 15.7a).

However, a major difference between the electronic structure of ACV-bound IPNS and the models is that these orbitals also reflect the interaction of the bound ACV thiolate 3p orbitals with the FeNO unit. Abs and
High-Spin and Low-Spin States in $\{\text{FeNO}\}_7^+$, $\text{Fe}^{\text{IV}}=\text{O}$, and $\text{Fe}^{\text{III}}–\text{OOH}$ Complexes

MCD data coupled to $\Delta$SCF calculations enabled the assignment of the CT bands in IPNS–ACV–NO. Bands 2 and 3 are LF transitions analogous to those in the models. Band 4 was assigned as the $\text{NO}^- \pi_{\text{ip}}^* \rightarrow \text{Fe}^{\text{III}} d_{xz}$ CT and Band 6 as the $\text{NO}^- \pi_{\text{op}}^* \rightarrow \text{Fe}^{\text{III}} d_{yz}$ CT (using the coordinate system defined above for the models, $z$ along the Fe–N bond, $x$ in and $y$ perpendicular to the Fe-N-O plane), consistent with its $z$-polarization from VTVH MCD and the assignment of the corresponding bands in 1. Because of its strong absorption intensity and based on overlap between S $\pi$-orbitals and Fe d-orbitals, Band 5 is assigned as the S $\pi \rightarrow \text{Fe} d_{12-\gamma 2}$ CT transition at an energy consistent with that predicted from the $\Delta$SCF calculations. Its negative MCD intensity raises the possibility that this transition couples through SOC to a CT transition with positive MCD intensity and perpendicular (to the S–Fe bond) polarization to generate a pseudo-A term [55]. This would require the two transitions to share either the same donor or acceptor orbital. $\Delta$SCF calculations predict the $\text{NO}^- \pi_{\text{ip}}^* \rightarrow \text{Fe} d_{12-\gamma 2}$ CT transition at 13100 cm$^{-1}$, much lower in energy than calculated for 1 (16,450 cm$^{-1}$). This transition shares an acceptor orbital with Band 5, has a perpendicular transition moment (i.e., along the Fe–NO axis), and can SOC to it in a mutually orthogonal direction (through the $d$-character in the donor orbitals),

Figure 15.6  (a) Variable-temperature EPR data for IPNS–ACV–NO. Fitting of the temperature dependence of the signal intensity to a Curie law dependence gives an axial ZFS $D$ parameter of 12.5 cm$^{-1}$. (b) Abs, CD, and MCD data for IPNS–ACV–NO with arrows indicating new transitions relative to Fe–EDTA–NO (inset). (c) VTVH MCD isotherms (inset) collected on Bands 1 and 6 at energies indicated by the arrows. Reproduced from [27] with permission from American Chemical Society.
Figure 15.7  (Top) MO diagrams and (bottom) contours of unoccupied orbitals for (left) IPNS–ACV–NO and (right) IPNS–ACV–O₂. Note different energy ordering of the NO/O₂ \(\pi^*\) orbitals relative to the models in Figure 15.4a and c arising from configuration interaction between the S pseudo-\(\sigma\) orbital and the \(\pi_{ip}^*\) orbital. Reproduced from [27] with permission from American Chemical Society.

defining a pseudo-A term interaction with Band 1 and providing a mechanism for the negative MCD intensity of Band 5.

Both the donor and acceptor orbitals of the NO\(^-\) \(\pi_{ip}^*\) → Fe \(d_{x^2-y^2}\) CT transition (donor is not shown and acceptor is in panel G in Figure 15.7b) have \(\sim 10\%\) S character (S \(\pi\) character in the acceptor and S pseudo-\(\sigma\) character in the donor, where pseudo-\(\sigma\) is a \(\sigma\)-like orbital in the Fe–S–C\(_{\text{substrate}}\) plane that is skewed off of the Fe–S bond axis due to the obtuse Fe–S–C angle). This S character will mix \(x/y\)-polarized intensity into this transition, leading to the mixed \(z\)- and \(x/y\)-polarization observed for Band 1 in the VTVH MCD data. A configuration interaction (CI) of the occupied NO\(^-\) \(\pi_{ip}^*\) with the S pseudo-\(\sigma\) orbital raises its energy
High-Spin and Low-Spin States in \( \{\text{FeNO}\}^7, \text{Fe}^{IV}=\text{O}, \text{and Fe}^{III}–\text{OOH Complexes} \)

**Figure 15.8** Energetics of NO and \( \text{O}_2 \) binding to the NHFe\( ^{\text{II}} \) facial triad (here that of phenylalanine hydroxylase) versus binding to (IPNS)Fe\( ^{\text{II}}–\text{ACV} \). Substrate binding makes \( \text{O}_2 \) binding more favorable by 26 kcal/mol, and NO binding more favorable by 16.5 kcal/mol. Reproduced from [27] with permission from American Chemical Society.

(Figure 15.7a) and thus lowers the energy of the \( \text{NO}^- \ \pi_{\text{ip}}^* \rightarrow \text{Fe} \ \text{d}_{x^2-y^2} \) transition, Band 1, relative to its energy in the models.

For the \( \{\text{FeO}_2\}^8 \) IPNS–ACV–\( \text{O}_2 \) complex (which has an \( S = 5/2 \) \text{Fe}^{\text{III}} \) anti-ferromagnetically coupled to \( S = 1/2 \) \text{O}_2^- \) electronic structure leading to an \( S = 2 \) ground state), DFT calculations gave a \( \Delta G^0 \) of \( \text{O}_2 \) binding of \( -7 \) kcal/mol (cf: \( -22 \) kcal/mol for NO), as shown in Figure 15.8. Thus, substrate binding has turned on \( \text{O}_2 \) activation at the superoxide level, as \( \text{O}_2 \) binding to the resting facial triad (\textit{vide supra}) is endergonic (+19 kcal/mol, blue line in Figure 15.8).

Relative to the resting facial triad, the calculated oxidation of \text{Fe}^{\text{II}} to \text{Fe}^{\text{III}} prior to NO/\( \text{O}_2 \) binding has become more favorable by \( \sim \)49 kcal/mol with the thiolate of the substrate bound to the Fe (Figure 15.8), and the overall binding energy has become more favorable by 25.9 kcal/mol. This stabilization is due to thiolate charge donation, which is stronger for \text{Fe}^{\text{III}} than for \text{Fe}^{\text{II}} and makes the overall formation of \text{Fe}^{\text{III}}–\( \text{O}_2^- \) species exergonic.

Thiolate binding also had an effect on the FMO of the putative \( \{\text{FeO}_2\}^8 \) intermediate in IPNS. These calibrated DFT calculations show that the lowest unoccupied orbital in the \( \alpha \) manifold is the \( \text{O}_2^- \ \pi_{\text{op}}^* \) orbital (Figure 15.7d, panel E) (rather than the \( \pi_{\text{op}}^* \) orbital as seen in the \( \{\text{FeO}_2\}^8 \) model 3, Figure 15.4d). This difference is due to the strong CI between the S pseudo-\( \sigma \) orbital and the \( \text{O}_2^- \ \pi_{\text{ip}}^* \) orbital (observed experimentally in the low energy of Band 1 for the ACV-NO complex as compared to the model complex 1, 13,100 vs. 17,400 cm\(^{-1}\)), destabilizing the latter relative to \( \pi_{\text{op}}^* \). From Figure 15.9, this \( \text{O}_2^- \) FMO is well oriented for H-atom abstraction from ACV, providing an explanation for the HAA/oxidase activity of this enzyme.

The \( \{\text{FeNO}\}^7 \rightarrow \{\text{FeO}_2\}^8 \) methodology thus elucidated the mechanism of \( \text{O}_2 \) activation in IPNS. Substrate binding stabilizes \text{Fe}^{\text{III}} relative to the \text{Fe}^{\text{II}} through charge donation (as experimentally observed from the
S $\pi \rightarrow$ Fe $d_{x^2-y^2}$ CT transition, Band 5); thus, the substrate binding turns on the 1e$^-$ reduction of O$_2$ to generate a superoxide intermediate. Due to the strong thiolate pseudo-$\sigma$ configuration interaction with the $\pi^*$ orbital described above, this intermediate has an FMO for selective H-atom abstraction from the $\beta$H–C substrate bond.

For the enzyme HPPD, VT EPR and Abs/MCD/VTUH MCD data were collected on the HPPD–NO resting form (i.e., NO bound to the resting facial triad of HPPD) and the substrate-bound (bidentate through the $\alpha$KA moiety) HPPD–HPP–NO complex (Figure 15.10) [40].

Similar to IPNS, substrate binding led to new spectral features and changes relative to the $\{\text{FeNO}\}_7$ model complexes: (i) the appearance of a low-energy LF band in MCD (Figure 15.10c, marked by arrow), (ii) a decrease in axial ZFS by a factor of 2 (from 16 to 8.4 cm$^{-1}$, determined by VT EPR, Figure 15.10b), (iii) an appearance of a substrate $\rightarrow$ Fe$^{\text{III}}$ CT at 29,000 cm$^{-1}$ in Abs (Figure 15.10a) and MCD (Figure 15.10c), and (iv) the NO$^-$ $\rightarrow$ Fe$^{\text{III}}$ CTs with mixed $z/xy$-polarization (obtained from VTVH MCD data, Figure 15.10c, inset). All of these features are due to the strong $\sigma$-donor interaction of the $\alpha$KA with the Fe, and are reproduced using the calibrated DFT approach described above.

The $\{\text{FeNO}\}_7$ HPPD–$\alpha$KA–NO complex calculations (with $\alpha$-ketoglutarate bound to the Fe rather than the specific HPP substrate for computational generality) were extended to the $\{\text{FeO}_2\}_8$ HPPD–$\alpha$KA–O$_2$ complex to understand how the $\alpha$KG binding to the Fe$^{\text{II}}$ leads to the O$_2$ reactivity of this enzyme. The energetics of O$_2$ binding were calculated for $S = 1, 2,$ and 3 states, with $S = 3$ leading to the formation of an end-on O$_2^{\ddagger}$– ferromagnetically coupled to high-spin ($S = 5/2$) Fe$^{\text{III}}$, $S = 2$ to the formation of an end-on O$_2^{\ddagger}$– anti-ferromagnetically coupled to $S = 5/2$ Fe$^{\text{III}}$, and $S = 1$ giving rise to a low-spin Fe$^{\text{IV}}$O$_2^{2-}$ intermediate. The $S = 1$ pathway is lowest in energy by 6 kcal/mol, and the strong $\sigma$–donor interactions of the $\alpha$KA with the Fe (which raises the energy of the Fe $d_\sigma$–orbital as evidenced by the new LF transition in the MCD data for the $\{\text{FeNO}\}_7$ HPPD/HPP) facilitate electron transfers from the Fe$^{\text{II}}$ center to the approaching O$_2$ molecule that,
via a transient Fe^{III}−superoxide state, produces an end-on Fe^{IV}−peroxide system. The peroxide is activated in nucleophilic attack (due to its high-energy occupied O_2^{2−} π* orbital) on the unoccupied π* carbonyl orbital of the αKA, forming a peroxy-bridged Fe^{IV}−O_2^{2−}−αKA S = 1 complex (Figure 15.11i). The first observed intermediate after O_2 attack in the related αKG-dependent nonheme Fe enzyme reactions is an S = 2 Fe^{IV}=O complex generated after the αKA decarboxylates; thus, the reaction coordinate generating an S = 2 Fe^{IV}=O from the S = 1 peroxy-bridged complex was evaluated. An [S = 1]/[S = 2] spin surface crossing was found on the 2D reaction coordinate corresponding to increasing C−C (substrate) and Fe−O (CO_2) bond lengths (Figure 15.11, left). This spin crossing point has a barrier of 17 kcal/mol, consistent with the experimental ΔG^≠ of 14 kcal/mol.

At the crossing point, an αKA electron has been transferred to the Fe (Figure 15.11ii), leading to a bridged Fe^{III}−O_2^{2−} electronic structure. After the spin-crossover to the S = 2 surface (Figure 15.11iii), the C−C bond cleaves, the αKA decarboxylates, and a second αKA electron transfers to iron, generating a bridged Fe^{III}−O_2^{2−} species (as shown in Figure 15.11iv). This intermediate then undergoes O−O bond cleavage with a barrier <1 kcal/mol to generate the S = 2 Fe^{IV}=O intermediate (Figure 15.11v, experimentally observed in the αKG-dependent enzymes), which then goes on to react with the substrate via electrophilic aromatic substitution using its σ FMO (see Section 3).

Thus, application of the \{(FeNO)^7 \rightarrow \{(FeO_2)^8 methodology to IPNS−ACV−NO and HPPD−HPP−NO has elucidated new spectral features associated with strong donor interactions between the coordinated substrate and the Fe that lead to the activation of O_2. In IPNS, charge donation from the substrate cysteine S to Fe stabilizes the one-electron transfer from Fe^{III} to O_2 to form an Fe^{III}−O_2^{2−} S = 2 species with an FMO well oriented for HAA from the substrate. In HPPD, charge donation from the αKA promoted the formation of an S = 1 Fe^{IV}−O_2^{2−}−αKA-bridged species, which undergoes a spin crossover to the S = 2 surface during the course of substrate decarboxylation to generate an S = 2 Fe^{IV}=O intermediate that is well oriented for EAS.
Figure 15.11  Left: 2D reaction coordinate for spin-crossover in generation of the \( S = 2 \) \( \text{Fe}^{IV} = \text{O} \) intermediate from the \( S = 1 \) peroxo-bridged complex. Right: Geometric and electronic structure schematic of the \( \text{Fe--O}_2 = \text{aKA} \) along the substrate \( \text{C--C} \) cleavage reaction coordinate. Reproduced from [40] with permission from American Chemical Society. (See colour plate section)
15.2.4 Correlation to \{FeNO\}$_7$ $S = 1/2$

Some nonheme FeNO model complexes have a low spin ($S = 1/2$) ground state. This is also reported for at least one enzyme, CDO with thiolate substrate bound in a bidentate mode and in a 3His ligand set [28]. In the enzymes it is interesting to consider whether this spin-state difference ($S = 1/2$ vs. $S = 3/2$) can contribute to reactivity. Thus, it becomes important to define the electronic structure of these low-spin \{FeNO\}$_7$ complexes, as well as the factors that influence adoption of this spin state as opposed to the more common $S = 3/2$, and how this would relate to O$_2$ activation. To investigate this, sulfur K-edge spectroscopy (since thiolates are bound in both IPNS and CDO) coupled to TD-DFT calculations was applied to both 6-coordinate high-spin [($S_{Me2N4(tren)}$)Fe(NO), tren = tris(2-aminomethyl)amine] and 5-coordinate low-spin [(bme-daco)Fe(NO), bme = $\beta$-mercaptoethanol; daco = 1,5-diazacyclooctane] thiolate ligated \{FeNO\}$_7$ complexes [38]. Correlation of sulfur K-edge data to TD-DFT calculations showed that the high-spin complex had the Fe$^{III}$ ($S = 5/2$) anti-ferromagnetically coupled to NO$^{-}$ ($S = 1$) electronic structure described above (Scheme 15.2, 1st column), while the low-spin complex had an intermediate spin Fe$^{III}$ ($S = 3/2$) anti-ferromagnetically coupled to NO$^{-}$ ($S = 1$) electronic structure due to the square pyramidal ligation, which splits the energy of $d_{x^2-y^2}$ and $d_{z^2}$ orbitals resulting in the intermediate spin on the Fe$^{III}$ (Scheme 15.2, 2nd column).

Computationally adding an axial CN$^{-}$ ligand to the 5C low-spin complex led to a predicted electronic structure of Fe$^{II}$ ($S = 0$) NO$^{-}$ ($S = 1/2$) – Scheme 15.2, 4th column. This additional axial ligand destabilizes the $d_{z^2}$ orbital and favors spin-pairing in the $d_{x^2-y^2}$ orbital that would initially lead to a low-spin Fe$^{III}$ ($S = 1/2$) anti-ferromagnetically coupled to NO$^{-}$ ($S = 1$) structure (Scheme 15.2, 3rd column). However, this would eliminate the metal-centered exchange stabilization and have poor anti-ferromagnetic coupling between the Fe $S = 1/2$ and the NO$^{-}$ $S = 1$. Thus, the difference in energy between the higher-energy NO $\pi^*$ and the Fe $d_{x^2-y^2}$ orbitals resulting in electron transfer from NO$^{-}$ to Fe$^{III}$ and an Fe$^{II}$($S = 0$)–NO$^{-}$ ($S = 1/2$) electronic structure (Scheme 15.2, 4th column).

This computational prediction was supported experimentally by later studies on the 6C low-spin model complex (N$_3$PyS)Fe(NO), where Mössbauer and sulfur K-edge spectroscopy coupled to DFT calculations supported an Fe$^{II}$ ($S = 0$) NO$^{-}$ ($S = 1/2$) electronic structure, with some mixing-in of Fe$^{III}$ ($S = 1/2$)/NO$^{-}$ ($S = 0$) character due to back-bonding [39]. The change in ligand field on the Fe changes its spin, which changes the 2e$^{-}$ exchange, and thus the e$^{-}$ distribution over the \{FeNO\}$_7$ unit, from Fe$^{III}$–NO$^{-}$ to Fe$^{II}$–NO$^{-}$. This change in e$^{-}$ distribution with the LF on the Fe may contribute to the O$_2$ reactivity of CDO, which
forms a low-spin \( \{\text{FeNO}\} \) \(^7\) \( S = 1/2 \) enzyme-substrate complex, in contrast to the high-spin complex found in substrate-bound IPNS, and activates \( \text{O}_2 \) for sulfur oxidation while IPNS activates \( \text{O}_2 \) for H-atom abstraction.

15.3 Low-Spin \( (S = 1) \) and High-Spin \( (S = 2) \) \( \text{Fe}^{IV}=\text{O} \) Complexes

There has been great success in the preparation and characterization of biomimetic ferryl \( (\text{Fe}^{IV}=\text{O}) \) model complexes \([29, 30]\). These systems were found to be in either low-spin \( (S = 1) \) or high-spin \( (S = 2) \) ground states\(^1\); the \( \text{Fe}^{IV}=\text{O} \) intermediates that have thus far been found in NHFe enzymes have \( S = 2 \) ground states. Thus, there has been a growing body of crystallographic, kinetic, electrochemical, spectroscopic, and theoretical data that reveal parallels and differences between \( S = 1 \) and \( S = 2 \) NHFe\(^{IV}=\text{O} \) chemistry and have important implications for NHFe enzymology \([29a, 30a, 56–58]\). There are now two known NHFe enzyme classes that use a reactive \( S = 2 \) \( \text{Fe}^{IV}=\text{O} \) intermediate: the \( \alpha \)KG- and pterin-dependent enzymes (Scheme 15.1).

For the \( \alpha \)KG-dependent enzymes, \( \text{Fe}^{IV}=\text{O} \) intermediates were trapped and spectroscopically characterized in four enzymes: taurine dioxygenase (TauD) \([59, 60]\), prolyl-4-hydroxylase (P4H) \([61]\), cytoticin halogenase (CytC3) \([62, 63]\), and syringomycin halogenase (SyrB2) \([64]\), while for the pterin-dependent enzymes experimentally observed intermediates have been trapped for tyrosine hydroxylase (TyrH) \([65]\) and phenylalanine hydroxylase (PheH) \([66]\). From the range of \( S = 1 \) ferryl model complexes, the electronic properties of two structurally defined 6-coordinate \( \sim \text{C}_4 \) symmetric complexes, \([\text{Fe}^{IV}=\text{O}(\text{N}_4\text{Py})]\) \([29c]\) [\( \text{N}_4\text{Py}: \) a pentadentate N,N-bis(2-pyridylmethyl)-N-bis(2-pyridyl)methylamine chelate] and \([\text{Fe}^{IV}=\text{O}(\text{CH}_3\text{CN})(\text{TMC})]\) \([29b]\) [\( \text{TMC}: \) a tetradentate 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane chelate] are discussed here and compared to the electronic structure of one of the two 5-coordinate \( \sim \text{C}_3 \) crystallographically defined \( S = 2 \) NHFe\(^{IV}=\text{O} \) complexes, \([\text{Fe}^{IV}=\text{O}(\text{TMG}_3\text{tren})]\) \([30a]\) [\( \text{TMG}_3\text{tren}: \) a tetradentate 1,1,1-tris\{2-N2-(1,1,3,3-tetramethylguanidino)ethyl\}amine chelate]. The model complexes considered here, whose crystal structures along with d-orbital splittings are shown in Figure 15.12, were prepared and characterized by Que and colleagues (University of Minnesota). Analysis of the MCD spectra of the models has elucidated the FMOs available for reactivity in each spin state, and nuclear resonance vibrational spectroscopy (NRVS) coupled to DFT calculations has defined the geometric contributions to HAA and oxygen-atom transfer (OAT) reactivity in the models and the HAA reactivity of the \( \text{Fe}^{IV}=\text{O} \) enzyme intermediate in SyrB2.

15.3.1 \( \text{Fe}^{IV}=\text{O} \) \( S = 1 \) Complexes: \( \pi^* \) FMO

Low temperature (LT) MCD spectroscopy (combined with Abs spectroscopy as well as DFT and multi-reference \( ab \text{ initio} \) calculations) is a powerful technique that provides unique insight into the nature of the low-lying excited states, and thus allows for definition of FMOs of these NHFe\(^{IV}=\text{O} \) species. For both \( S = 1 \) systems, the near-infrared (NIR) LT MCD spectra show similar spectral features comprised of three overlapping bands—two of these are positively signed and one negative with a well-resolved vibronic structure (labeled as I, II, and III in Figure 15.13, left panels). For \([\text{Fe}^{IV}=\text{O}(\text{CH}_3\text{CN})(\text{TMC})]\), these three bands are centered at \( \sim 10,400, \sim 10,600, \) and \( \sim 12,900 \) \( \text{cm}^{-1} \), while for \([\text{Fe}^{IV}=\text{O}(\text{N}_4\text{Py})]\) these are shifted to higher energies: \( \sim 14,000, \sim 12,600, \) and \( \sim 15,100 \) \( \text{cm}^{-1} \) respectively. Considering the axial ZFS \( D \) relative to the direction of the magnetic field, the analysis of the temperature dependence of the MCD intensities allows for decoupling these bands and determination of their polarizations. For a large positive \( D \)-axis, as is the case for both \( S = 1 \) NHFe\(^{IV}=\text{O} \) complexes (+22 \( \text{cm}^{-1} \) for \([\text{Fe}^{IV}=\text{O}(\text{N}_4\text{Py})]\) and +29 \( \text{cm}^{-1} \) for \([\text{Fe}^{IV}=\text{O}(\text{CH}_3\text{CN})]\)

\(^1\) An \( \text{Fe}^{IV}=\text{O} \) \( S = 0 \) is of course possible; this would require extreme \( \pi \) anisotropy (\textit{vide infra}) and has not yet been reported.
High-Spin and Low-Spin States in \{\text{FeNO}\}^7, \text{Fe}^{IV} = \text{O}, \text{and Fe}^{III} – \text{OOH} \text{ Complexes}

Figure 15.12 Three crystallographically characterized NHFe\textsuperscript{IV} = O complexes (crystal structures from [29b, 29c, and 30a]). The d-manifold splittings in both C\textsubscript{\text{4v}}- and C\textsubscript{\text{3v}}-like symmetric structures, along with electron occupations, are qualitatively depicted.

(TMC) [29a] and a magnetic field \((H)\) of 7T, a \(z\)-polarized transition gains MCD intensity only for \(H\) perpendicular to the molecular \(z\)-axis. This intensity is highest at the lowest temperature \((T)\) and decreases with increasing \(T\). An \(x/y\)-polarized band has MCD intensity for \(H\) parallel to the molecular \(z\)-axis. In this case, the MCD intensity is low at the lowest \(T\) and first increases and then decreases with increasing \(T\). For details on the theory and application to Fe\textsuperscript{IV} = O \(S = 1\), see Reference [67]. Thus, Band I in Figure 15.13 was found to be \(z\)-polarized, while Bands II and III are \(x/y\)-polarized. In correlating TD-DFT calculations to these MCD data, Bands I, II, and III were assigned as the ligand-field \(d_{xy} \rightarrow d_{x^2-y^2}\), \(d_{xy} \rightarrow d_{xz/yz} p^*\) and \(d_{xz/yz} p^* \rightarrow d_{x^2-y^2}\) transitions respectively. Among these, Band II involves the excitation of an \(e^-\) from a nonbonding \(d_{xy}\) to the anti-bonding \(d_{xz/yz} \pi^*\) orbital. This is associated with an MCD vibronic progression (Band II) that provides the Fe–O bond length and associated Fe–O stretching frequency in this excited state (Figure 15.13, second-column panels). This quantifies the \(\pi\) donor contribution to the Fe–O bond. This \(\pi\) donor interaction puts oxo character into the low-energy unoccupied \(\beta\) Fe \(d\pi^*\)-orbitals, activating these for reactivity.

A vibronic progression in the Franck–Condon (FC) approximation is given by a Poisson distribution according to equation (15.3):

\[
\Delta \varepsilon = \sum_n \Delta \varepsilon_{0 \rightarrow n} \frac{S^n}{n!} \exp \left( -\frac{2.733}{\Delta_{\text{FWHM}}^2} \left( n \nu + \nu_{0 \rightarrow 0} \right)^2 \right)
\]

where \(S\) is the Huang–Rhys factor defined by the equation \(S^n/n! = \Delta \varepsilon_{0 \rightarrow n}/\Delta \varepsilon_{0 \rightarrow 0}\) reflecting the progression bandshape, \(\nu\) is the frequency of the Fe–O stretching mode (in \(\text{cm}^{-1}\)), \(\Delta_{\text{FWHM}}\) is the full width at half maximum of each individual peak in a progression (in \(\text{cm}^{-1}\)), and \(\nu_{0 \rightarrow 0}\) and \(\Delta \varepsilon_{0 \rightarrow 0}\) are the energy (in \(\text{cm}^{-1}\)) and the intensity (in \(\text{M}^{-1} \text{cm}^{-1}\)), respectively, of the origin, that is, the first peak in the progression. These parameters (obtained from the Franck–Condon fits to the MCD data plotted as positive progressions in Figure 15.13, second-column panels) can be used to construct parabolic representations of the excited-state
**Figure 15.13** The variable-temperature NIR MCD spectra of two S = 1 NHFeIV=O model complexes, each comprising three bands (I–III) along with band polarizations (determined from the temperature behaviors of I–III as indicated by vertical arrows) and the assignments are shown (left panels). The Franck–Condon fits of the MCD Band II that is plotted positive (second-column panels) give the information on the potential-energy surfaces of low-lying excited states (third-column panels). The MO diagram is obtained from the interaction of the oxo p with the Fe d-orbitals in the ∼C₄ᵥ geometry. Ground-state electron configuration for the S = 2 state is also displayed [67]. Reproduced from [35] with permission from American Chemical Society. (See colour plate section)
High-Spin and Low-Spin States in \( \{\text{FeNO}\}^7, \text{Fe}^{IV}=\text{O}, \) and \( \text{Fe}^{III}–\text{OOH} \) Complexes

Figure 15.14  The \( \pi^* \) FMO and its interaction with the \( \sigma \) C–H orbital (left) defines the H-atom abstraction coordinates for the \( S=1 \) NH\( \text{Fe}^{IV}=\text{O} \) systems (right). Reproduced from [67] with permission from American Chemical Society.

potential-energy surface (PES) along the Fe–O stretching coordinate (Figure 15.13, third-column panels) that is given by equation (15.4):

\[
E_{\text{PES}} = 1.4825 \times 10^{-2} \mu \nu^2 \left[ Q - (Q_0 + \Delta Q) \right]^2 + E_{\text{min}}
\]  

(15.4)

where \( \nu \) (in cm\(^{-1}\)) is as defined above, \( \mu \) (in amu) is the reduced mass of the Fe–O stretching mode, \( \Delta Q \) (in Å) is the distortion of the excited state relative to the ground-state equilibrium Fe–O bond length, \( Q_0 \), and is determined from the Franck–Condon fit using \( \Delta Q = 8.213 \sqrt{S/\mu \nu} \), \( E_{\text{PES}} \) is the energy of the PES (in cm\(^{-1}\)) along \( Q \) that corresponds to the Fe–O bond length (in Å); \( E_{\text{min}} \) is the energy of the excited-state PES minimum (in cm\(^{-1}\)), which is determined as \( \nu_{0\to0} + (\nu_{\text{GS}} - \nu) / 2 \), where \( \nu_{\text{GS}} \) is the frequency of the Fe–O stretching mode in the ground state (\( \nu_{\text{GS}} \) values for \( \text{Fe}^{IV}=\text{O(CH}_3\text{CN)(TMC)} \) and \( \text{Fe}^{IV}=\text{O(N}_4\text{Py)} \) are taken from the IR and NRVS spectra and are 343 and 824 cm\(^{-1}\) respectively [29b, 68]).

From the Franck–Condon-derived PESs (Figure 15.13, third-column panels), it is clear that upon excitation of a \( \beta \)-electron from the nonbonding \( d_{xy} \) into the anti-bonding \( d_{xz/yz} \) \( \pi^* \) orbitals (key orbitals displayed in Figure 15.13, right) the Fe–O bond in \( \text{Fe}^{IV}=\text{O(N}_4\text{Py)} \) becomes weaker and more distorted than the corresponding bond in \( \text{Fe}^{IV}=\text{O(CH}_3\text{CN)(TMC)} \). The excitation is shifted to higher energies in \( \text{Fe}^{IV}=\text{O(N}_4\text{Py)} \) than in \( \text{Fe}^{IV}=\text{O(CH}_3\text{CN)(TMC)} \) and indicates stronger anti-bonding interactions between the Fe center and the equatorial ligands, while the wider and more distorted excited-state PES in \( \text{Fe}^{IV}=\text{O(N}_4\text{Py)} \) reflects stronger anti-bonding interaction between oxo \( p_{xy} \) and Fe \( d_{xz/yz} \) orbitals in the \( \pi^* \) LUMO, correlating with a stronger and more covalent \( \pi \)-contribution to the Fe–O bond. This results in a larger oxo character in \( \pi^* \) orbital and increased electrophilicity. In both \( S=1 \) systems, the energetically accessible \( \pi^* \) orbital is the FMO for HAA from a substrate. Therefore, an increased electrophilicity of the \( \pi^* \) FMO along with a smaller chelate steric hindrance provided an explanation for the increased HAA reactivity observed for \( \text{Fe}^{IV}=\text{O(N}_4\text{Py)} \) as compared to \( \text{Fe}^{IV}=\text{O(CH}_3\text{CN)(TMC)} \) (\( k_2 = 2.0 \) vs. 0.016 M\(^{-1}\) s\(^{-1}\) for reaction with dihydroanthracene at \( T = -30^\circ\text{C} \) [69]). Importantly, both \( S=1 \) systems possess a \( \pi^* \) FMO that, due to a strong \( \pi \) donor interaction has oxo character oriented perpendicular to the Fe–oxo bond, defining the optimal reaction coordinate for HAA (Figure 15.14).

Such a coordinate corresponds to the perpendicular approach of the substrate C–H bond relative to the Fe–oxo bond axis, and thus defines the \( \pi \) channel reactive pathway. If the equatorial steric hindrance of the chelating ligand excludes this perpendicular access of the C–H bond, there is the possibility (based on DFT calculations) for a two-state \( S=1/S=2 \) mechanism of HAA [70]. This spin crossover is viable, provided an \( S=2 \) state, arising from the excitation of a \( \beta-d_{xy} \) \( e^- \) into the \( \beta-d_{z^2-y^2} \) orbital, is reasonably low in energy. Such a spin crossover along the reaction coordinate would, as described below for the \( S=2 \) Fe\( ^{IV}=\text{O} \) ground state, lead to a large stabilization of the \( \sigma-d_{z^2} \) \( \sigma^* \) FMO orbital, allowing for HAA reactivity along the Fe–oxo bond axis. This would provide a \( \sigma \) channel for reactivity [67, 71].
15.3.2 FeIV=O S = 2 Sites: π* and σ* FMOs

The \( S = 2 \) [FeIV=O(TMg)] \(_3\) tren] model complex (Figure 15.12, right) has an Abs and MCD spectrum as shown in Figure 15.15a (and VT MCD in Figure 15.15b). As for the \( S = 1 \) systems, the MCD spectrum of this \( S = 2 \) complex is information-rich with vibronic structure. The derivative-shaped (i.e., pseudo-A term) MCD feature at \( \sim 11,000 \text{ cm}^{-1} \) in fact comprises three \( x/y \)-polarized vibronic progressions: two negatively signed bands centered at \( \sim 10,500 \) and \( \sim 12,350 \text{ cm}^{-1} \) and one positive band at \( \sim 10700 \text{ cm}^{-1} \), and one overlapping sharp positive peak \( \sim 12,500 \text{ cm}^{-1} \) (Figure 15.15c).

From the MCD/abs intensity, this is an LF transition, and from its derivative shape and VT MCD, it is \( x/y \)-polarized. \textit{Ab initio} calculations correlate well with these MCD data and assign this feature as the \( d_{x2y2z2} \) \( \pi^* \rightarrow d_{x2} \sigma^* \) LF transition to the \( 5E \) excited state (MO diagram in Figure 15.15e). This \( 5E \) undergoes intra-state SOC. This gives rise to the lower-energy positive and the higher-energy negative component of the NIR MCD feature. In addition, the negative component is affected by a strong inter-state SOC with a nearby \( S = 1 \) excited state that distorts its PES and produces two negative vibronic progressions with different vibrational frequencies. The positive MCD component of the \( 5E \) interacts through a weak SOC with another \( S = 1 \) state, which gives a sharp positive peak overlapping the positive FC progression (Figure 15.15c). Note from Figure 15.15a that this sharp MCD peak correlates to a dip in the corresponding Abs band. This is a Fano anti-resonance, whose shape (peak vs. derivative vs. dip) derives from the ratio of the transition-dipole matrix elements that contribute to the SOC-modified sharp (\( S = 1 \)) state relative to the interacting fraction of the broad (\( 5E \)) state \([56, 71]\). As discussed above, the vibronic structure contains information on the excited state PESs through the Franck–Condon parameters: the Huang–Rhys factor, \( S \), and the vibronic spacing, \( \nu \) [see equations (15.3) and (15.4)]. For the FeIV=O \( S = 2 \) system, these three NIR vibronic progressions associated with the \( 5E \) LF transition, along with the vibronically resolved MCD band centered at \( \sim 19,500 \text{ cm}^{-1} \) (Figure 15.15b), assigned as oxo \( p_{x,y} \rightarrow d_{x2y2z2} \) \( \pi^* \) CT based on its \( z \)-polarization from VT MCD and large decrease in \( \nu_{Fe-O} \) from \( 820 \text{ cm}^{-1} \) in the ground state to \( 490 \text{ cm}^{-1} \) in the FC progression) can be translated into parabolic \( S = 2 \) PESs as displayed in Figure 15.15d (the top graph).

The experimentally derived PESs (associated with the ground state, and two low-lying LF \( 5E \) and CT states) were found to be in good agreement with those obtained from \textit{ab initio} calculations (\textit{cf}: upper and lower panels in Figure 15.15d), and thus allowed definition of FMOs in this FeIV=O \( S = 2 \) species. These PESs further revealed how the electronic structure evolves in going to the Fe–O bond lengths that are relevant to the transition state (TS) for HAA by this complex (Figure 15.16). In contrast to the \( S = 1 \) systems, where only the \( \pi^* \) FMO is energetically accessible, in the \( S = 2 \) NHFeIV=O complex there are two low-lying \( \pi^* \) FMOs and one \( \sigma^* \) FMO that provide three different channels for HAA (see Figure 15.16): (i) the LF \( 5E \)-related \( \pi \) channel that is facilitated through generation of the FeIII–oxyl character at the TS and yields five unpaired \( \alpha \)-electrons on the FeIII center and one \( \pi^- \) hole in the oxo \( p_{x,y} \) orbitals – [\( \pi(\text{Fe}_S = s/2O^-) \)]; (ii) the LMCT-related \( \pi \) channel that is facilitated by FeIII–oxyl with three unpaired \( \alpha \)-electrons on the FeIII center – [\( \pi(\text{Fe}_S = 3/2O^-) \)] and an oxo \( p_{\pi} \) hole; and (iii) the ground-state \( \sigma \) channel that generates five unpaired electrons on the Fe center and oxyl character at the TS – [\( \sigma(\text{Fe}_S = s/2O^-) \)] with the hole in the oxo \( p_z \) orbital. Note that the \( \sigma \) channel requires that the substrate C–H bond approaches along the Fe–oxo bond. These findings have important implications for the role of the FeIV=O \( S = 2 \) state in biology. While the \( S = 1 \) state has only one \( \pi \) channel available for HAA, there are three possible \( S = 2 \) channels (two \( \pi \) and one \( \sigma \)), giving an important flexibility to NHFeIV=O active sites to control reactivity efficiently in enzymes such as SyrB2, which catalyzes either halogenation or hydroxylation depending on the substrate (\textit{vide infra}), or in HmaS and HPPD, different enzymes which use the same substrate to perform HAA and hydroxylation or electrophilic aromatic substitution, respectively \([35]\).
Figure 15.15  (a) The 233 K absorption and 2K MCD spectrum of the $S = 2 \{\text{Fe}^{IV} = \text{O}(\text{TMG}_3\text{tren})\}$ model complex (upper and lower panel respectively).  (b) The variable-temperature MCD spectra.  Polarization and the assignment of the bands are also shown.  For the CT band, the Franck–Condon parameters, that is, the Huang–Rhys factor ($S$) and spacing ($v$), characterizing its vibronic structure are included.  (c) The Franck–Condon fits of the vibronically resolved NIR MCD feature.  (d) Upper panel: The PESs derived from the Franck–Condon fits from panels b and c (labeled accordingly); Lower panel: The calculated PESs (for computational details, see [71]) with the same labeling as in panels c and upper d.  (e) MO diagram obtained from the interaction of the oxo p with the Fe d-orbitals in the $\sim C_3v$ geometry.  Ground-state electron configuration for the $S = 2$ state is also displayed.  Reproduced from [57] and [71] with permission from Proceedings of the National Academy of Sciences.
Figure 15.16  The calculated potential energy surfaces for three lowest $S = 2$ states. Characteristics for these PESs fit well with those obtained from Franck–Condon analyses of vibronically resolved MCD features. In addition, the wavefunction evolutions along these PESs reveal dominant oxyl character on the oxo group in going from the ground-state geometry to transition state for HAA, with an e$^-$ hole in oxo $p_z$ that defines one $\sigma$ channel for HAA, or with an e$^-$ hole in oxo $p_{x/y}$ that defines two $\pi$ channels leading to five or three $\alpha$ electrons on the Fe$^{III}$ center. These $\sigma/\pi$ channels require different orientations of the Fe$^{IV}$=O bond with respect to the attacked C–H bond. Reproduced from [57] and [71] with permission from Proceedings of the National Academy of Sciences.

15.3.3 Contributions of FMOs to Reactivity

Nuclear resonance vibrational spectroscopy correlated with DFT calculations elucidated geometric contributions to different HAA reactivities of $S = 1$ and $S = 2$ Fe$^{IV}$=O sites and provided insight into the structure/reactivity correlation in SyrB2 [72]. NRVS uses the third-generation synchrotron to probe the Mössbauer $^{57}$Fe nuclear excitation and its vibrational sidebands that correspond to normal modes involving Fe motion [73]. This allowed definition of the most characteristic modes in both $S = 1$ and $S = 2$ complexes (Figure 15.17) [68, 74]. Among these NRVS-active modes, the trans-axial bends are usually the most intense ones as they have significant Fe motion (Figure 15.17, bottom). However, in the TMG$_3$tren complex, these were found to be affected by the axial steric wall associated with the guanidinium groups of the chelate that is large in this $S = 2$ Fe$^{IV}$=O complex, and thus hinders motion. This is reflected by a decrease of the NRVS intensity in the trans-axial bends and the increase in intensity of the low-energy trans-axial shear modes (as compared to $S = 1$ [Fe$^{IV}$=O(N4Py)] complex with a low steric wall around the oxo group) (Figure 15.17). This axial steric contribution in the TMG$_3$tren complex affects its reaction coordinate on the $S = 2$ surface (Figure 15.17,
Figure 15.17 NRVS spectra of the $S = 2$ and $S = 1$ Fe$^{IV}$=O model complexes (upper and lower left panels respectively). Two types of vibrational modes whose NRVS intensities are significantly changed in going from the $S = 2$ to $S = 1$ system are also included. Energy profiles ($\Delta E/\Delta G$ in kcal mol$^{-1}$) for reactions of the $S = 2$ and $S = 1$ complexes with 1,4-cyclohexadiene (upper and lower right panels). Decomposition of the activation barrier into steric and electronic components is also shown. Reproduced from [68] and [74] with permission from John Wiley & Sons.

Recently, this model-calibrated NRVS approach was also applied to the $S = 2$ Fe$^{IV}$=O intermediate of the halogenase SyrB2 that had been generated by the Bollinger/Krebs group [72]. The halide-perturbed NRVS data (Figure 15.18) show a three-peak pattern with intensity shifting from high to low frequency modes with the Cl → Br perturbation. In combination with DFT modeling, these data showed that the Br/Cl–Fe$^{IV}$=O right). Removal of the axial steric contribution to the total barrier for HAA gives an intrinsic barrier of ~7 kcal/mol. Interestingly, the $S = 1$ [Fe$^{IV}$=O(N4Py)] also has a steric barrier, in this case associated with the equatorial chelate due to the required perpendicular approach of substrate to the Fe$^{IV}$=O center associated with its $\pi$ channel. Removal of this steric effect gives an electronic contribution to the barrier of ~4 kcal/mol, similar to the electronic barrier found for $\sigma$ attack in the $S = 2$ complex. Thus, these findings show that the $S = 2$ $\sigma$-pathway is not intrinsically more reactive toward the HAA reaction than the $\pi$ channel in $S = 1$ but it represents an additional channel available to the $S = 2$ species that can make the oxo group more accessible and thus more versatile in NHFe enzymes.
intermediate has a 5-coordinate, trigonal bipyramidal structure with the oxo along the \( \sim C_3 \) axis. Modeling the reaction coordinate with the native substrate \( L\)-threonine generated an intermediate structure correlating well with the NRVS data with the Fe–oxo-oriented perpendicular to the C–H bond of the substrate. This orientation allows attack through the \( \pi \)-channel and leads to a first intermediate (Cl–FeIII–OH) with the OH\(^-\) oriented away from the C radical but the halide oriented well for the less thermodynamically favored halogenation.

In contrast, the \( \sigma \)-orientation has been computationally suggested for the non-native substrate, \( L\)-norvaline, which is hydroxylated rather than halogenated (Figure 15.18). For this orientation, H-atom abstraction generates a first intermediate with the halide oriented away but the OH\(^-\) toward the C radical, leading to the observed hydroxylation reactivity and demonstrating the versatility afforded to \( S = 2 \) Fe\(^{IV}\)=O complexes by the availability of both \( \sigma \) and \( \pi \) channels.

In addition to HAA reactivity, the \( S = 1 \) and \( S = 2 \) NHFe\(^{IV}\)=O systems are also capable of OAT reactions that are of importance for the pterin-dependent NHFe enzymes (Scheme 15.1) that perform electrophilic aromatic substitutions. Factors controlling OAT vs. HAA pathways in \( S = 1 \) vs. \( S = 2 \) systems were investigated for another \( S = 1 \) model complex, [Fe\(^{IV}\)=O(CH\(_3\)CN)(TBC)] [TBC: a tetradentate 1,4,8,11-tetrazenyl-1,4,8,11-tetraazacyclotetradecane chelate] [75]. This complex surprisingly exhibits a rate enhancement of two orders of magnitude over [Fe\(^{IV}\)=O(CH\(_3\)CN)(TMC)] for both HAA and OAT reactions, despite the more bulky benzyl substituents on the cyclam framework as compared to the methyl groups in TMC [75]. From spectroscopic and computational results, the increased steric interactions in [Fe\(^{IV}\)=O(CH\(_3\)CN)(TBC)] lead to a larger distortion of the cyclam ring that weakens the axial and equatorial ligand fields. This stabilizes the \( d_{x^2-y^2} \) and \( d_{z^2} \) orbitals and lowers the energy gap between the \( S = 2 \) and the \( S = 1 \) ground states. This decreased energy difference is reflected in the larger axial ZFS \( D \) parameter. Thus, the greater accessibility of the \( S = 2 \) state (in particular accessibility of the \( d_{z^2} \sigma^* \) channel in this state) in the more bulky [Fe\(^{IV}\)=O(CH\(_3\)CN)(TBC)] provides insight into its increased reactivity toward HAA and OAT as compared to [Fe\(^{IV}\)=O(CH\(_3\)CN)(TMC)]. For the single-electron HAA process, the chelate steric hindrance in both systems prohibits the \( S = 1 \) \( \pi \) channel so that the
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Figure 15.19  Two-electron process of OAT from the [Fe\text{IV}=\text{O(CH}_3\text{CN})(\text{TBC})] complex to the substrate sulfur for the \(S=2\) and \(S=1\) states (panels a and b respectively). The donor \(b_1(S)\) orbital in the reactant along with two acceptor Fe \(d_{z^2}\) and \(d_{xz/yz}\) orbitals in TS are also shown. The \(S=2\) and \(S=1\) transition states are schematically depicted in insets of both panels. Reproduced from [75] with permission from American Chemical Society.

only alternative pathway involves the \(S = 1/S = 2\) spin crossover allowing for the \(S = 2\) \(\sigma\) channel and axial reactant that is more accessible in [Fe\text{IV}=\text{O(CH}_3\text{CN})(\text{TBC})].

In contrast, the OAT process involves two-electron transfer (one \(\alpha\) and one \(\beta\)) from the substrate to the Fe\text{IV}=O center and thus requires both \(\sigma\)- and \(\pi\)-attack on the substrate with the participation of both the Fe \(d_{z^2}\)\(\sigma^*\) and \(d_{xz/yz}\)\(\pi^*\) FMOs (Figure 15.19). According to spectroscopically calibrated DFT analyses, the \(S = 2\) TS for OAT is considerably lower in energy than the corresponding \(S = 1\) TS, which is in line with the stabilization of the \(\alpha-d_{z^2}\) orbital and increased axial reactivity in going from \(S = 1\) to \(S = 2\). As a result, the electronic structure at the \(S = 2\) TS is characterized as the early transfer of an \(\alpha\)-electron in the \(d_{z^2}\) that weakens both the Fe–oxo and trans-axial Fe–acetonitrile (ACN) bonds, which in turn allows the Fe–oxo bond to move out of the cyclam plane and decreases the chelate steric hindrance for “subsequent” \(\beta\)-electron transfer through \(\pi\)-attack on the substrate (\(~70\% \alpha\)-spin vs. \(~20\% \beta\) transferred at the TS) – see Figure 15.19a. At the \(S = 1\) TS, these \(\sigma\)- and \(\pi\)-attacks are more concerted (\(~30\% \alpha\) vs. \(~45\% \beta\) electron transferred at the TS), which is
reflected by much smaller distortion of the Fe–oxo bond from the cyclam plane, and thus larger chelate steric contribution to the barrier for $S = 1$ OAT (Figure 15.19b). These differences in structure of the TS for HAA ($\sigma^*$ FMO) and oxo transfer ($\sigma^*$ and $\pi^*$ FMOs) may play an important role in the different trends observed for these reactivities over a series of Fe$^{IV}$=O complexes [76].

15.4 Low-Spin ($S = 1/2$) and High-Spin ($S = 5/2$) Fe$^{III}$–OOH Complexes

15.4.1 Spin State Dependence of O–O Bond Homolysis

As shown in Figure 15.1, two-electron activation of $O_2$ occurs in two classes of NHFe$^{II}$ active sites to generate reactive Fe$^{III}$–OOH intermediates capable of H-atom abstraction and electrophilic aromatic substitution. Two different ground spin states have been identified for these intermediates: the $S = 1/2$ state (in ABLM, performing HAA) and the $S = 5/2$ state (in the RDOs, performing EAS) [25, 26]. In parallel, a number of synthetic $S = 5/2$ and $S = 1/2$ Fe$^{III}$–OOH model complexes have been prepared [18, 31, 32, 77]. Among these are two structurally related systems, $S = 1/2$ [Fe$^{III}$–OOBu(OH)$_x$(TPA)] and $S = 5/2$ [Fe$^{III}$–OOBu(OH)$_x$(6-Me$_3$TPA)] [TPA: tris(2-pyridylmethyl)amine, Bu: tert-butyl and $x = 1, 2$; 6-Me$_3$TPA: methylated TPA] prepared by Kim et al. [78]. These were subjected to spectroscopic/electronic structure studies that allowed elucidation of the spin-state dependence of O–O activation [79]. These were correlated to differences in reactivity in O–O bond homolysis; in particular in comparing the $S = 5/2$ and $S = 1/2$ Fe$^{III}$–OOH systems, only the latter was experimentally found to undergo homolytic cleavage of its O–O bond [80].

Abs, MCD, and RR spectra were taken for both of these TPA-based complexes [79]. By using $^{18}$O isotope perturbations, RR showed the Fe–O stretch in the Fe$^{III}$–OO[R] $S = 5/2$ complex to be at 637 cm$^{-1}$, while the O–O bond stretch was found to be centered at $\sim$859 cm$^{-1}$ (in fact, there are two isotope-sensitive peaks at 842 and 876 cm$^{-1}$ resulting from mixing of the O–O stretch with the symmetric C–C stretch of the alkyl peroxide) (Figure 15.20, upper panel). In going to the low-spin ($S = 1/2$) Fe$^{III}$–OOR complex, the Fe–O stretch shifted to higher frequency ($\Delta \nu_{Fe–O} = +59$ cm$^{-1}$), whereas the O–O stretching mode shifted down in energy ($\Delta \nu_{O–O} = -63$ cm$^{-1}$) (Figure 15.20, lower panel). Importantly, as derived from these RR data, in the $S = 1/2$ Fe$^{III}$–OOR system, the force constant for the O–O bond is, in fact, weaker than that for the Fe$–$O bond (i.e., 2.92 vs. 3.53 mdyn/Å respectively) [79a]; the opposite is the case for the $S = 5/2$ cognate (3.55 vs. 2.87 mdyn/Å for the O–O vs. Fe–O bond [79b]). These differences in O–O/Fe–O bond strengths between the low- and high-spin Fe$^{III}$–OOH system (weaker O–O, stronger Fe–O in LS) correlate to their different reactivities (i.e., $S = 1/2$ O–O vs. $S = 5/2$ Fe–O homolysis).

From spectroscopically calibrated DFT calculations, this difference in bonding is reflected in the larger activation barrier for the homolytic O–O bond cleavage in the Fe$^{III}$–OOH $S = 5/2$ system relative to the low-spin complex ($\Delta E^\neq \sim 32$ kcal mol$^{-1}$ vs. 20 kcal mol$^{-1}$, see Figure 15.21a) [79b, 81]. This results from the allowed crossing of an $\alpha$-spin occupied metal d$_\sigma$ donor orbital with the OOBu$^i$ $\sigma^*$ acceptor orbital (i.e., one electron is transferred from the Fe$^{III}$ to the hydroperoxy $\sigma^*$ orbital along this reaction coordinate to homolytically cleave the O–O bond to form Fe$^{IV}$=O and $^*$OH) (Figure 15.21b). Alternatively, for the $S = 1/2$ system, the d$_\sigma$ orbital on the low-spin Fe$^{III}$ is unoccupied. Thus, an e$^-$ is transferred from $\alpha$-spin-occupied d$_\pi$ orbital into the OOBu$^i$ $\sigma^*$ acceptor orbital along the O–O reaction coordinate. There is now a strong CI between these two interacting redox-active orbitals (Figure 15.21c) that lowers the activation barrier for OO homolysis in the Fe$^{III}$–OOBu$^i$ $S = 1/2$ complexes (by $\Delta E^\neq \sim 12$ kcal/mol).

In light of the above results, it was interesting that temperature-dependent kinetic data for two other Fe$^{III}$–OOH model complexes, $S = 1/2$ [Fe$^{III}$–OOH(N4Py)] and $S = 5/2$ [Fe$^{III}$–OOH(TMC)] [N4Py and TMC are defined in Section 3] showed that O–O homolysis in the $S = 5/2$ occurs at 298 K with $\Delta G^\neq$ of 18.7 kcal/mol.
High-Spin and Low-Spin States in \{\text{FeNO}\}_7^7\text{ Fe}^{IV}=\text{O}, \text{ and Fe}^{III}–\text{OOH Complexes}

Figure 15.20  Resonance Raman spectrum of the high-spin TPA-based complex with natural abundance isotopes (the first row) and \(^{18}\text{O}\) substituted at the coordinating oxygen (the second row). The RR spectrum of the low-spin TPA-based complex with natural abundance isotopes (the third row) and \(^{18}\text{O}\) substituted at the coordinating oxygen (the fourth row). Note, the RR profiles revealed that, for \(S = 1/2\), the O–O and Fe–O stretches are resonance enhanced with respect to the absorption band at \(\sim 16,700\text{ cm}^{-1}\). For \(S = 5/2\), the corresponding RR-active band is at \(\sim 17,900\text{ cm}^{-1}\). These bands were assigned as \(^{1}\text{BuOO}^–\text{to-Fe}^{III}\) charge-transfer transitions. The label \(S\) stands for the signal due to the solvent. Reproduced from [79a] and [79b] with permission from American Chemical Society.

Figure 15.21  (a) DFT PESs for O–O homolysis at different spin states; allowed and avoided orbital crossing for the O–O homolysis of the high- and low-spin Fe\(^{III}\)--OOR complexes (panels b and c, respectively). Reproduced from [35] and [81] with permission from American Chemical Society.
O-O homolysis involving spin crossover

![Diagram showing PESs for different spin states of Fe(III)-OOH complex](image)

Figure 15.22 2D PESs of the $S = 5/2$ spin state (transparent red) and the $S = 1/2$ spin state (blue) of the [Fe$^{III}$–OOH(TMC)]$^-$ for the O–O bond homolysis, with the acetonitrile as a trans-axial ligand to the OOH$^-$ group. These PESs are scanned along two coordinates: the O–O and Fe–ACN bonds (in Å). All energies are calculated relative to the $S = 5/2$ [Fe$^{III}$–OOH(TMC)]$^-$ global minimum with the O–OH and Fe–ACN bond lengths of 1.48 and 4.37 Å, respectively (its position represented by red solid dot). Reproduced from [81] with permission from American Chemical Society.

(from Eyring plots) that is 2.2 kcal/mol lower than the corresponding free-energy barrier for the $S = 1/2$ complex [81]. The apparent difference in reactivity toward O–O bond cleavage is, in fact, due to ACN solvent coordination that leads to a spin crossover of the $S = 5/2$ to the $S = 1/2$ surface along this multidimensional O–O cleavage reaction coordinate. This axial coordination occurs before the O–O bond is cleaved, leading to an Fe$^{III}$OOH $S = 1/2$ that actually undergoes the homolysis. This results in the six-coordinate $S = 1$ [Fe$^{IV}$=O(CH$_3$CN)(TMC)] product complex that is observed experimentally (Figure 15.22).

15.4.2 Fe$^{III}$–OOH $S = 1/2$ Reactivity: ABLM

Activated BLM is the last observable intermediate before H-atom abstraction from DNA that is responsible for the cytotoxicity of this drug. From DFT simulations of the NRVS spectral data shown and assigned in Figure 15.23a, ABLM is a monoprotonated low-spin Fe$^{III}$–OOH complex with the geometry-optimized structure shown in Figure 15.23b [82]. Experimentally this low-spin Fe$^{III}$–OOH site has been found to directly abstract the H-atom from the C$_4'$ site of DNA with a relatively low primary and high secondary KIE (3.6 ± 0.9 and 1.7 ± 0.2, respectively) [83]. Thus, ABLM was subjected to the reaction coordinate calculations in Figure 15.24a, which is a two-dimensional PES [83]. One is, of course, H-atom abstraction by the distal O, while the second dimension, which is very important, is the O–O bond cleavage. A TS was found that has a low activation energy ($\Delta E^\ddagger \sim 13$ kcal mol$^{-1}$) and reproduces the KIEs. Importantly, it is late in O–O cleavage (O–O bond elongates from 1.50 Å in the Fe$^{III}$OOH reactant to 2.73 Å at the TS) and early in H-atom abstraction (Figure 15.24b). This reflects the FMO of the low-spin Fe$^{III}$–OOH, which is the hydroperoxide $\sigma^*$ orbital (Figure 15.25a).
High-Spin and Low-Spin States in \{FeNO\}^{7}, Fe^{IV}=O, and Fe^{III}–OOH Complexes

Figure 15.23 (a) Nuclear resonance vibrational spectrum of ABLM and four major normal modes contributing to this spectrum (b) and (c). Structure of ABLM whose calculated NRVS spectrum correlates with the experimental NRVS data. Reproduced from [82] with permission from Proceedings of the National Academy of Sciences.

At the late TS, the $\sigma^b/\sigma^*$ is lost and the two spin holes in the $\sigma^*$ polarize—first one to the distal oxygen, effectively making it an hydroxyl well oriented for H-atom abstraction; the second one localizes on the proximal O, leading to an Fe^{IV}−O $S=1$ (Figure 15.25b) that is effective in performing a second H-atom abstraction leading to the double strand cleavage that is important in its toxicity.

15.4.3 Fe^{III}–OOH Spin State-Dependent Reactivity: FMOs

These HAA studies were then directed to high-spin and low-spin Fe^{III}–OOH model complexes to explore the spin dependence of HAA in collaboration with Nam and colleagues [81]. Both $S=1/2$ [Fe^{III}–OOH(N4Py)] and $S=5/2$ [Fe^{III}–OOH(TMC)] were experimentally found to perform direct HAA reactions (with xanthene) but, as defined by DFT calculations, these follow very different reaction coordinates. For [Fe^{III}–OOH(N4Py)], the $S=1/2$ TS was found to be late along the O−O bond cleavage coordinate and early in C−H abstraction with Fe^{IV}=O and $^*$OH character, consistent with the above results for ABLM. Thus, the $^*$OH group directly attacks the substrate for the H-atom abstraction. In contrast to the $S=1/2$ systems, the TS for HAA by the $S=5/2$ [Fe^{III}–OOH(TMC)] complex is early in the O−O coordinate and late in the C−H abstraction. This reflects the higher barrier for the $S=5/2$ O−O homolysis reaction described earlier (Figure 15.21a). This leads to very different TS electronic structures for the $S=1/2$ and $S=5/2$ HAA processes. As shown in Figure 15.26a (upper), there is a substantial electron transfer from the xanthene substrate to the Fe^{III}–OOH LUMO at the $S=5/2$ TS (i.e., 0.33 e$^-$), but no electron transfer at the $S=1/2$ TS (Figure 15.26b, upper), the latter reflecting the formation of the hydroxyl radical (and Fe^{IV}=O). This difference correlates with the nature and energies of the redox-active molecular orbitals (RAMOs) involved in the $S=5/2$ vs. $S=1/2$ HAA (Figure 15.26a vs. b, lower). For the high-spin Fe^{III}–OOH complex, the $d_{\beta e}$-based RAMO, which accepts a $\beta$ e$^-$ from the substrate during HAA, is stabilized by a bonding interaction with the unoccupied OOH$^−$ $\sigma^*$ orbital. Therefore, this RAMO exhibits a higher electron affinity than that of the low-spin system, for which
the RAMO is destabilized by an anti-bonding interaction with the occupied OOH$^{-}$ $\pi^*$ orbital [81]. Thus, the $S = 5/2$ system has a higher reduction potential relative to $S = 1/2$ complex. This allows $S = 5/2$ Fe$^{III}$OOH complexes to be active in electrophilic reactions without prior O–O bond cleavage, but with significant charge transfer from the substrate to the Fe center at the TS. This has important implications for the RDOs, which catalyze the \textit{cis}-dihydroxylation of aromatic rings and proceed through a putative $S = 5/2$ Fe$^{III}$-hydroperoxo-level intermediate [22, 84, 85]. Current mechanistic proposals for the reactivity of this intermediate include either direct EAS attack of the Fe$^{III}$-hydroperoxide on the substrate or initial O–O bond cleavage to form
Figure 15.26  Transition states of the $S = 5/2$ and $S = 1/2$ Fe$^{III}$–OOH in the H-atom abstraction coordinate (upper schematic in panels A and B), respectively. Schematic in lower part of panel A: the RAMO in the $S = 5/2$ system is the LUMO $\beta$-spin $d_{xz} + \sigma^*$ orbital that is stabilized by CI between the lower-energy Fe$^{III}$ $d_{yz}$ and the higher-energy OOH$^{-} s^*$ orbital. Schematic in lower part of panel B: the RAMO for $S = 1/2$ is $\beta$-spin $d_{xz} - \pi^*$ that is destabilized by CI between the lower-energy OOH$^{-} \pi_v^*$ and the higher-energy Fe$^{III}$ $d_{xz}$. Reproduced from [81] with permission from American Chemical Society.

an high-valent HO–Fe$^V$=O prior to substrate attack [86]. If the RDO intermediate is an $S = 5/2$ Fe$^{III}$–OOH species, these model studies support the feasibility of the direct electrophilic attack mechanism with a TS early along the O–O reaction coordinate.

As discussed above, an important observation from these model studies is that the high-spin TS exhibits significant electron transfer from the substrate to the Fe, while the low-spin TS does not. Because of this, the HAA reactivity of the high-spin Fe$^{III}$–OOH should be more dependent on substrate than low-spin Fe$^{III}$–OOH. To evaluate this, DFT calculations were employed to define TSs for reactions of these $S = 5/2$ and $S = 1/2$ complexes with substrates of varying ionization potentials (xanthene and xanthene derivatives fluorinated at six different positions) and C–H bond strengths (xanthene and a dioxine derivative) [81]. These calculations found a positive linear correlation of the TS energy with both substrate ionization potential (Figure 15.27, black) and bond strength (the C–H bond strength and TS energy for xanthene: 70.0 and 18.4 kcal/mol; for dioxane: 91.0 and 25.3 kcal/mol) for the high-spin Fe$^{III}$–OOH reaction. Importantly, the low-spin Fe$^{III}$–OOH TS energy is independent of the substrate ionization potential (Figure 15.27, red) and very weakly dependent on C–H bond strength (i.e., C–H bond in dioxine is 21 kcal mol$^{-1}$ stronger than in xanthene, while the TS with dioxine is only 0.8 kcal mol$^{-1}$ above that with xanthene). This suggests that $S = 5/2$ Fe$^{III}$–OOH systems should be more selective (depending on the substrate), and thus more effective in controlling biochemical and environmental processes than their low-spin cognates.

15.5 Concluding Remarks

Our understanding of the electronic structures of $\{\text{FeNO}\}^7/\{\text{FeO}_2\}^8$, Fe$^{IV}$=O, and FeOOH species and their dependencies on spin state, which is controlled by the ligand field, has greatly advanced in recent years. A
change in spin state can (i) change the electron distribution in \{\text{FeNO}\}^7/\{\text{FeO}_2\}^8species, (ii) change the FMOs available in FeIV=O species (\(\pi\) in \(S = 1\) vs. \(\sigma\) and \(\pi\) channels in \(S = 2\)), and (iii) change the O–O bond activation and nature of the transition state in FeIII–OOH species.

It is now of fundamental importance to evaluate and define how these spin-state contributions to O2 activation contribute to and control their biological functions. This requires the extension of the above model studies to detailed and strongly coupled spectroscopic and electronic structure studies on enzyme intermediates and their analogues.

### 15.6 Acknowledgments

This work was supported by the National Institutes of Health (Grant No. GM 40392).

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