Excited state potential energy surfaces and their interactions in Fe$^{IV}=O$ active sites

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The non-heme ferryl active sites are of significant interest for their application in biomedical and green catalysis. These sites have been shown to have an $S = 1$ or $S = 2$ ground spin state; the latter is functional in biology. Low-temperature magnetic circular dichroism (LT MCD) spectroscopy probes the nature of the excited states in these species including ligand-field (LF) states that are otherwise difficult to study by other spectroscopies. In particular, the temperature dependences of MCD features enable their unambiguous assignment and thus determination of the low-lying excited states in two prototypical $S = 1$ and $S = 2$ NHFe$^{IV}=O$ complexes. Furthermore, some MCD bands exhibit vibronic structures that allow mapping of excited-state interactions and their effects on the potential energy surfaces (PESs). For the $S = 2$ species, there is also an unusual spectral feature in both near-infrared absorption and MCD spectra – Fano antiresonance (dip in Abs) and Fano resonance (sharp peak in MCD) that indicates the weak spin–orbit coupling of an $S = 1$ state with the $S = 2$ LF state. These experimental data are correlated with quantum-chemical calculations that are further extended to analyze the low-lying electronic states and the evolution of their multiconfigurational characters along the Fe–O PESs. These investigations show that the lowest-energy states develop oxyl Fe$^{III}$ character at distances that are relevant to the transition state (TS) for H-atom abstraction and define the frontier molecular orbitals that participate in the reactivity of $S = 1$ vs. $S = 2$ non-heme Fe$^{IV}=O$ active sites. The $S = 1$ species has only one available channel that requires the C–H bond of a substrate to approach perpendicular to the Fe–oxo bond (the $\pi$ channel). In contrast, there are three channels (one $\sigma$ and two $\pi$) available for the $S = 2$ non-heme Fe$^{IV}=O$ system allowing C–H substrate approach both along and perpendicular to the Fe–oxo bond that have important implications for enzymatic selectivity.

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Shaun Wong received his B.S. in chemistry from UC Berkeley and his Ph.D. from Stanford University. His work centered on elucidating the electronic structures and reaction mechanisms of low- and high-spin Fe($n$)-oxo species in enzymes and model complexes.
Introduction

Mononuclear non-heme iron (NHFe) enzymes, ubiquitous in living organisms, are involved in many vital biological processes including the regulation of hypoxia, demethylation of DNA, antibiotic, and natural product biosynthesis and bioremediation, and are related to disease states.1,2 Most of these enzymes use an S = 2 Fe4+ center to activate O2 to perform various ‘difficult’ and formally spin-forbidden reactions including hydroxylation, halogenation, desaturation, and electrophilic aromatic substitution on unreactive singlet organic substrates that require the cleavage of strong aliphatic or aromatic C–H bonds. The mononuclear NHFe enzymes can be divided into six classes3 based on their O2 activation mechanism (Table 1; here we omit classes where substrates are cleaved by a peroxy-quinone-bridged FeII intermediate.5). Provided the active species in using dσ* vs. e from the non-bonding dxy orbital into the α-dπ *,–* orbital leads to a considerable electron–electron exchange stabilization (referred in the literature to as spin polarization20 or exchange enhancement21) that shifts the α-MO manifold down in energy; thus the unoccupied α-dσ* becomes energetically comparable to the dσ* FMOs and accessible for reactivity.21 The flexibility of S = 2 species in using dσ* and/or dx* FMOs were shown to be key to the reaction selectivity in SyrB212 where dx* vs. dσ* control halogenation vs. hydroxylation of different substrates, and in HmaS vs. HPPD,29 where dx* vs. dσ* participate in HAA vs. electrophilic aromatic substitution (EAS) on the same substrate.

From the experimental perspective, these FMOs can be studied through electronic spectroscopy particularly of the ligand-field (LF) transitions. These correspond to excitations into low-lying unoccupied d orbitals that get significant oxo character (hence their electrophilicity) through covalent bonding. However, these absorption (Abs) spectra are generally quite featureless due to band overlaps and thus not very informative. By contrast, the variable-temperature magnetic circular dichroism (VT MCD) spectra are much more resolved and thus give unique insight into these excited states and thus the FMOs that are key to electrophilic reactivity.

In this perspective, we focus on our spectroscopic results on the first structurally defined –C2π, symmetric S = 1 FeIV=O glutarate as a cofactor, the formation of high-spin S = 2 FeIV=O (ferryl) intermediates proceed through the four-electron reduction of O2 (2e– from Fe3+ and 2e– from the cofactor).7 Note these NHFe oxygen intermediates, in spite of their elusive nature, have attracted much attention for their reactivities and selectivities as exemplified by studies on the high-spin (S = 2) FeIV=O intermediate in syringomycin halogenase (SyrB2).10–13

Efforts to understand the general physico-chemical factors that control reaction mechanisms of NHFe enzymes have also catalyzed interest in the syntheses and crystallographic characterizations of model NHFe complexes that include low-spin (S = 1) and high-spin (S = 2) FeIV=O complexes.14–19 These enable an important step toward the elucidation of geometric and electronic properties contributing to NHFe function: the spectroscopic and quantum-chemical elucidation of the frontier molecular orbitals (FMOs) and their contributions to reactivity in model S = 1 and S = 2 FeIV=O systems.20–28 For the S = 1 FeIV=O species, the pair of singly-occupied dπ* FMOs, resulting from the strong antibonding interaction between oxo pπ and Fe dx2-y2 orbitals, were shown to define the π channel for H-atom abstraction (HAA) which requires for reactivity the perpendicular orientation of the substrate C–H bond with respect to the Fe–oxy bond.21 For the S = 2 FeIV=O species, two types of FMOs were defined, i.e., dσ* and dπ*. The dσ* defines a π channel similar to the S = 1 FeIV=O species, while dπ* arises from the strong σ antibonding interaction of the oxo pπ with the Fe dx2-y2 orbital and defines the σ channel for HAA that requires collinear orientation of the substrate C–H bond relative to FeIV=O bond.22 This additional σ channel in S = 2 complexes was elucidated on the basis of spin unrestricted MO theory as given in Fig. 1. In going from an S = 1 to an S = 2 FeIV=O system, the excitation of a β e– from the nonbonding dxy orbital into the α-dπ *,–* orbital leads to a considerable electron–electron exchange stabilization (referred in the literature as spin polarization20 or exchange enhancement21) that shifts the α-MO manifold down in energy; thus the unoccupied α-dσ* becomes energetically comparable to the dσ* FMOs and accessible for reactivity.21 The flexibility of S = 2 species in using dσ* and/or dx* FMOs were shown to be key to the reaction selectivity in SyrB212 where dx* vs. dσ* control halogenation vs. hydroxylation of different substrates, and in HmaS vs. HPPD,29 where dx* vs. dσ* participate in HAA vs. electrophilic aromatic substitution (EAS) on the same substrate.

Edward I. Solomon grew up in North Miami Beach, Florida, received his Ph.D. at Princeton (with D. S. McClure), and was a postdoctoral fellow at The Ørsted Institute (with C. J. Ballhausen) and then at Caltech (with H. B. Gray). He was a Professor at the Massachusetts Institute of Technology until 1982, when he joined the faculty at Stanford University, where he is now the Monroe E. Spaght Professor of Humanities and Sciences and Professor of Photon Science at SLAC National Accelerator Lab. He has been an Invited Professor in Argentina, Australia, Brazil, China, France, India, and Japan. Professor Solomon’s research is in the fields of Physical-Inorganic and Bioinorganic Chemistry with emphasis on the application of a wide range of spectroscopic methods combined with QM calculations to elucidate the electronic structure of transition metal sites and its contribution to physical properties and reactivity.
complex chelated by the tetradentate 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane (TMC) ligand with an axially bound acetonitrile and denoted as (CH$_3$CN)(TMC)Fe$^{IV}$O; see Fig. 2] and the first structurally defined $C_{3v}$-like symmetric Fe$^{IV}$O complex chelated by the tetradentate 1,1,1-tris-[2-[(N$_2$H$_2$)$_2$-(1,1,3,3-tetramethylguanidino)]ethyl]amine (TMG$_3$tren)
The Abs and MCD spectra of the $S = 1$ (CH$_3$CN)(TMC)Fe$^{IV}$=O model complex are shown in Fig. 3A and B, respectively. While the Abs spectrum clearly shows three spectral features (at $\sim$12 250, $\sim$17 500 (weak) and 24 900 cm$^{-1}$ (strong)), the VT MCD spectra are much more resolved such that the near-infrared (NIR) feature centered at 12 250 cm$^{-1}$ is composed of three overlapping bands (one having vibronic structure), two of which have positive MCD intensity (bands labelled I and III at $\sim$10 400 and $\sim$12 900 cm$^{-1}$ in Fig. 3B) while one is negative (band II at $\sim$10 600 cm$^{-1}$ with sharp features). Additionally, the temperature dependence of these bands gives valuable information on the polarizations of the electronic transitions in frozen solution, where all orientation of the molecules are present. These polarizations allow the ground state to be coupled to the excited states leading to (along with the analysis of $C_D/D_0$ ratios) the assignment of the MCD/Abs bands. This is given by the selection rules for MCD transitions from a zero-field-split (ZFS) $S = 1$ ground spin state that is further split due to interaction with the external magnetic field (Fig. 4C and ref. 20, 21).

Using the phenomenological spin Hamiltonian that includes the ZFS term $D(S^2 - S(S + 1)/3) + E(S^2_x - S^2_y)$ with axial $D$ and rhombic $E$ ZFS parameters, the analysis of magnetic Mössbauer spectra of the (CH$_3$CN)(TMC)Fe$^{IV}$=O complex showed $E$ to be negligible and $D = +$29 cm$^{-1}$. As depicted in Fig. 3C, for this positive $D$ value, the ground triplet state splits into two sublevels with two-fold degenerate $M_S = \pm 1$ lying above the $M_S = 0$ sublevel. From the selection rules for MCD intensity, the magnetic field ($H$) must be perpendicular to the polarization direction of the transition. Thus a $z$-polarized transition requires $H$ to be perpendicular to the molecular $z$ axis (coinciding with the Fe–oxo bond). In this case, the Zeeman-induced mixing between $M_S = -1$ and $M_S = 0$ results in significant $M_S = -1$ character in the ground sublevel at $H = 7$ T. From this MCD-active sublevel, the $z$-polarized transition exhibits the highest MCD intensity at lowest temperature, $T$ ($\sim$2 K) that decreases with increasing $T$ because of increased Boltzmann populations of higher-energy sublevels: the MCD-inactive $M_S = 0$ and opposite-signed $M_S = +1$. For an ($xy$)-polarized transition, $H$ is required to be parallel to the molecular $x$ axis. This leaves the MCD-inactive $M_S = 0$ as the ground sublevel at $H = 7$ T. As a consequence, the intensity of a ($xy$)-polarized MCD band is low at low temperature and increases with increasing $T$ because of an increased Boltzmann population of the first excited MCD-active $M_S = -1$ sublevel. Further increasing the $T$ decreases the MCD intensity because the third sublevel, $M_S = +1$, becomes populated, producing MCD intensity of opposite sign.

From these selection rules, band I in Fig. 3B was shown to be $z$-polarized (decreasing in intensity with increasing $T$), while bands II and III are ($xy$)-polarized (both first increase and then decrease with increasing $T$). In particular, the different polarizations (and thus temperature behavior) of the two overlapping bands I and II allowed these to be decoupled and band II to be unambiguously associated with the MCD vibronic structure (plotted as a positive band in Fig. 3D). From group theory and DFT calculations, band II was assigned as the lowest-energy ($xy$)-polarized $d_{xy} \rightarrow \pi^*_{xz/yz}$ ligand-field (LF) transition (MOs shown in Fig. 3E; band I and III, IV and V are assigned as indicated in Fig. 3B). Importantly, this transition,
due to its vibronic progression in the Fe–oxo stretch, is a direct experimental probe of how the dx* FMO changes when occupied by a nonbonding e\(^-\) (the d\(_y\)) nonbonding in C\(_{4v}\)). A Frank-Condon (FC) analysis of this vibronic progression provides important parameters that characterize the PES of this excited state in the vicinity of ground-state equilibrium geometry: (i) the frequency of the Fe–oxo stretching mode in this excited state, which corresponds to the spacing between two adjacent peaks in the vibronic progression (\(v_{\text{FeO}} = 620 \text{ cm}^{-1}\)); and (ii) the excited-state distortion relative to the ground-state equilibrium structure. The vibronic progression in Fig. 3D gives \(\Delta r_{Fe-O} (\approx 0.14 \text{ Å})\), which was determined from eqn (1):

\[
\Delta r_{\text{Fe-O}} = \sqrt{\hbar S_{\text{Fe-O}}/4\pi \mu_{\text{Fe-O}}}
\]

where \(S_{\text{Fe-O}} (\approx 3.0)\) is the Huang-Rhys parameter given by eqn (2):

\[
S = \sqrt{n!(\Delta \varepsilon_{\text{Fe-O}}/\Delta \varepsilon_{0-n})}
\]

with \(\Delta \varepsilon_{0-n}\) and \(\Delta \varepsilon_{0-n}\) being the intensity of the first and (\(n + 1\))-th peak in the progression, and \(\mu\) (\(\approx 15\) amu) is the reduced mass of the (ligand-)Fe–oxo stretching mode. The frequency of the Fe–oxo stretching mode in the ground state is known from the IR and NRVS spectra (~830 cm\(^{-1}\)).\(^{30,33}\) From these data, PESs of the ground and excited state along Fe–oxo stretching mode can be defined experimentally (Fig. 3F). Clearly, the excited state is associated with a distorted PES having its minimum shifted to longer Fe–oxo bond length as compared to the PES of the ground state, which reflects the weaker Fe–oxo bond due to excitation of a nonbonding d \(e^-\) into the antibonding Fe–oxo dx* FMO. This strong \(\pi^b/\pi^*\) interaction results in significant oxo \(\sigma\) character in the dx* orbital that is key to activating it for reactivity. Note that the low-energy two-fold degenerate dx* orbital set is much lower in energy than Fe–oxo dx* (the latter is associated with the band V at ~24 900 cm\(^{-1}\) in Fig. 3B and displayed in Fig. 3E). These results have important implications for the H-atom abstraction (HAA) reaction coordinate for \(S = 1\) Fe\(^{IV}\)=O systems. HAA must involve this low-energy Fe dx* orbital that accepts the \(e^-\) from the substrate requiring good overlap with the C–H \(\sigma\) orbital. Since the dx* is oriented perpendicular to the Fe–oxo bond (thus having significant oxo \(p_{xy}\) \(\pi\) character), a perpendicular orientation of the C–H relative to the Fe–oxo bond is required for an accessible HAA trajectory (Scheme 1). Note, in the model complexes, there are likely steric effects of the equatorial chelate that hinder this \(\pi\)-channel in \(S = 1\) Fe\(^{IV}\)=O sites (see ref. 34).

Alternatively to the \(S = 1\) \(\pi\) channel, several computational works suggested a possible spin crossover from \(S = 1\) to \(S = 2\) in the HAA process.\(^{35,36}\) The feasibility of such a two-state mechanism depends on the accessibility of a \(S = 2\) state that is obtained by the excitation of a \(\beta-d_{xy}\) \(e^-\) into the \(\alpha-d_{x^2-y^2}\) orbital. This would lead to a large stabilization of the \(\alpha-d_{xy}, \sigma^*\) orbital (Fig. 1). This stabilization would provide the \(\sigma^*\) FMO for HAA, which requires the collinear orientation of the substrate C–H relative to Fe–oxo bond and thus could overcome the chelate steric hindrance in some \(S = 1\) systems.

**Scheme 1** The dx* FMO interacting with the electron-donating C–H \(\sigma\) orbital (left) and thus defining H-atom abstraction reaction coordinate for the \(S = 1\) NHFe\(^{IV}\)=O species (right).
The Abs spectrum of the prototypical \( S = 2 \) ferriyl complex, \((\text{TMG}_3\text{tren})\text{Fe}^\text{IV}=\text{O}\), exhibits three noticeable spectral features (Fig. 4A). Within the 6000–16 000 cm\(^{-1}\) region, there is a weak Abs band centered at 12 500 cm\(^{-1}\) (labelled as band I) having a dip in its intensity at \( \sim 12 000 \) cm\(^{-1}\), while the 16 000–30 000 cm\(^{-1}\) (UV-vis) region is characterized by an intense broad band at 25 000 cm\(^{-1}\) (band III) with a weak shoulder at 19 000 cm\(^{-1}\) (band II). These bands become much more revealing in the LT MCD spectrum. Thus, as shown in Fig. 4B, the NIR feature I in fact consists of three overlapping bands: a lower-energy positive and higher-energy negative vibronic progression with a spacing of 490 cm\(^{-1}\), respectively, and one positive sharp peak at the same energy as the dip in Abs (dashed vertical line in Fig. 4A and B). Additionally, the MCD reveals that four bands contribute to the Abs envelope in the UV-vis region (bands II, III, IV and V in Fig. 4A and B), where the negatively signed band II has well-resolved vibronic structure with a spacing of 490 cm\(^{-1}\).

These MCD features (I–III) clearly exhibit two different VT behaviors that reflect different band polarizations. Feature I first increases and then decreases in intensity with increasing temperature, while bands II and III decrease in intensity with increasing \( T \) (Fig. 4D). These behaviors are well fit for the axial \( D \) ZFS parameter of \( +7 \)–8 cm\(^{-1}\). For a given ZFS of the \( S = 2 \) ground state that is further split in the presence of the magnetic field (Fig. 4D) and following the theory described in section 2, the temperature dependences show that feature I is \((x,y)\)-polarized, and bands II and III are \( z \)-polarized.

Features I–V were also characterized in terms of their \( C_0/D_0 \) ratios \(^{33} \) (included in Fig. 4B), with a large value for I (0.250) and significantly smaller values for II–V (<0.040). These indicate that feature I corresponds to a LF transition, while bands II–V are charge-transfer (CT) transitions. These experimental data, further correlated to TD-DFT calculations (Fig. 4E), led to the unambiguous assignment of the three lowest-energy features: Band I is the two-fold degenerate \((x,y)\)-polarized \( \alpha \) \( d\pi^*_{x'y'z'} \rightarrow d\sigma^*_{z^2} \) LF transition (\( i.e., \) \( ^5A_1 \rightarrow ^5E \)), and bands II–III are the \( z \)-polarized \( \beta \) oxo \( \pi_{x'y'} \rightarrow \sigma^*_{z^2} \) CT transitions. The molecular orbitals involved along with the transitions to the lowest-energy excited state \( LF^5E \) are schematically depicted in Fig. 5A and B, respectively.

MCD feature I associated with the lowest excited state \( LF^5E \) has a derivative band shape (Fig. 4B) that is primarily due to spin–orbit coupling (SOC) within the \( LF^5E \) state. This gives rise to two oppositely signed C-term MCD features forming a pseudo-A term with the right circularly polarized (RCP; \( i.e., \) negative) component at higher energies (Fig. 5C). The in-state SOC between the spatially two-fold degenerate components of the \( LF^5E \) state (depicted in Fig. 5B) is effectively controlled by the \( z \)-component of the SOC operator \( \lambda S_z \) (where \( \lambda \) is a many-electron state-specific SOC constant) by acting on wavefunctions \( |M^\text{eff}_L = \pm 1, M_S = 0, \pm 1, \pm 2 \rangle \), where the effective magnetic quantum number \( M^\text{eff}_L \) reflects the one-electron configuration subspace \( d_{x^2}^I d_{y^2}^I, d_{z^2}^I d_{\pi^*}^I \) of the \( LF^5E \). This SOC produces two-fold degenerate sublevels of the \( LF^5E \) state with an energy separation between the lower-energy LCP and higher-energy RCP state of \( 2\lambda = 2(\xi_{3d}^\text{II} + 1) = 260 \) cm\(^{-1}\) (\( \xi_{3d} \) is the one-electron SOC constant for \( \text{Fe}^{III} \); 520 cm\(^{-1}\)) \(^{38} \) as shown in Fig. 5C. The relation \( \lambda = \xi/4 \) for this case is given by eqn (3):

\[
\langle M^\text{eff}_L = +1, M_S | \lambda S_z | M^\text{eff}_L = +1, M_S = 2 \rangle = \left\langle \frac{1}{\sqrt{2}} (d_{x^2} + id_{y^2}) \alpha \xi_{3d}^\text{II} S_z | \frac{1}{\sqrt{2}} (d_{x^2} - id_{y^2}) \alpha \right\rangle
\]

Note these in-state SOC-perturbed MCD-active (LCP and RCP) states would have potential energy surfaces that do not differ in their shape. However, this is different from what is
observed from the NIR MCD pseudo-A term spectra that exhibit two very distinct vibronic progressions (Fig. 4B, right). These reflect a large difference in the frequency of the Fe–oxo stretching mode in the LCP vs. RCP component of the LF \( ^5\text{E} \) excited state (\( \nu_{\text{Fe-O}} = 710 \text{ vs. 880 cm}^{-1} \)); hence there must be a difference in the shapes of the PESs associated with these states. Since the latter value of \( \nu_{\text{Fe-O}} \) is even higher than the Fe–oxo frequency in the ground state (820–840 cm\(^{-1}\))\(^{11,14} \), the LF \( ^5\text{E} \)-based RCP state must undergo a strong SOC interaction with a nearby and spectroscopically spin-forbidden state. This would produce two RCP-active PESs, where the higher-energy PES is associated with the higher \( \nu_{\text{Fe-O}} \) relative to its lower-energy cognate.

Indeed, the NIR MCD pseudo-A term is best fit by three (one positive and two negative) FC progressions and a sharp peak (Fig. 6), where the higher-energy RCP FC progression (green) has a larger \( \nu_{\text{Fe-O}} \) than its lower-energy RCP FC cognate (dark blue) that is largely masked by the more intense LCP FC progression (dotted red). As was the case for band II of the \( S = 1 \ \text{Fe}^{\text{IV}=\text{O}} \) species in MCD (section 2), all of the FC fits contributing to this pseudo-A term (as well as the FC fit of the negative MCD band II associated with the oxo-to-Fe CT state in Fig. 4C, 3rd panel) are characterized in terms of the zero-vibronic 0–0 transition (\( E_{\text{lo}} \)), the \( \nu_{\text{Fe-O}} \) and the Huang–Rhys factor (\( S \)), which is defined by eqn (2). These are given in Fig. 6.

There is an energy equivalence between the sharp positive peak in the \( ^5\text{E} \) MCD spectrum (solid red line in Fig. 6) and the dip in the Abs spectrum (Fig. 7A and B). This peak, due to its sharpness (the full width at half maximum is \(< 400 \text{ cm}^{-1} \)) and its positive MCD intensity, indicates a weak SOC interaction of the spin-allowed (broad) LCP component of the LF \( ^5\text{E} \) with a close-lying, spectroscopically spin-forbidden triplet state. This very unusual spectral feature (Abs dip [antiresonance] vs. MCD peak [resonance]) is a Fano interference effect. According to the Fano theory (ref. 39, 40 and 41), the spectral band shape can be fit to the four-parameter equation:

\[
R(\nu) = \frac{\alpha(\nu)}{\alpha_0(\nu)} = 1 + p \frac{\nu^2 + 2q(\nu - \nu_0)\gamma^{-1} - 1}{1 + (\nu - \nu_0)^2\gamma^{-2}}
\]

where

\[
p = \frac{\alpha(\nu_0)}{\alpha_0(\nu_0)} = \frac{\langle \Psi_\text{GS} | \hat{\mu} | \Psi_\text{GS} \rangle^2}{\langle \Psi_\text{GS} | \hat{\mu}^\dagger \Psi_\text{GS} \rangle^2};
\]

\[
q = \pi \langle \Psi_\text{S} | \hat{H}_\text{int} | \Psi_\text{GS} \rangle \langle \langle \Psi_\text{GS} | \hat{\mu} | \Psi_\text{S} \rangle \rangle;
\]

\[
\gamma = \pi \langle \Psi_\text{GS} | \hat{H}_\text{int} | \Psi_\text{S} \rangle^2.
\]

\( \alpha_0 \) is line shape of the broad “continuum” (background) band \( (\alpha_0 = \epsilon_0 + \Delta \epsilon_0 \text{ in Abs and MCD, respectively}) \), \( \alpha(\nu_0) \) is the absorption at the center of the interference, \( \Psi_\text{GS} \) is the ground state, \( \Psi_\text{S} \) and \( \Psi_\text{S'} \) are broad (spectroscopically allowed) and sharp (spectroscopically forbidden) excited states, \( \Psi_\text{S} \) is the modified sharp state that results from the interaction of \( \Psi_\text{S} \) with \( \Psi_\text{S'} \) (here the interaction Hamiltonian \( \hat{H}_\text{int} \) is SOC, \( \psi_{S'} \) is a certain fraction of the background \( \Psi_\text{S} \) state connected to \( \Psi_\text{S} \) and \( \hat{\mu} \) is the electric dipole transition moment.

The Fano feature, \( R(\nu) \), is experimentally defined for Abs by the ratio \( \epsilon_\text{observed}/\epsilon_\text{background} \) and for MCD by \( \epsilon_\text{observed}/\epsilon_\text{background} \) (Fig. 7). Theoretically, this is determined by the parameters \( \nu_0, p, \lambda \) and \( q \) (eqn (4)) that control its energy, height, width
and shape (e.g., \( q = \infty \), 1 and 0 correspond to a Lorentzian peak, dispersion-like curve and Lorentzian dip, respectively). The parameter \( p \) reflects a fraction of the broad state (LF \( 3E \)) that weakly interacts via SOC with the sharp state. The parameter \( q \) is related to the ratio of the transition-dipole matrix elements that contribute excitations into the SOC-modified sharp state and the fraction of the broad state, while the parameter \( r \) is a measure of the SOC between the sharp and the broad state (scaled down by the overlap of the sharp peak with the broad LF \( 5E \)-based continuum).

Fig. 7A and B give the analyses of the Abs and MCD Fano features. The \( \gamma \) and \( p \) parameters remain essentially unchanged in going from Abs to MCD. This is in line with the expectation that both the SOC strength (related to \( \gamma \)) and the parameter \( p \) (in eqn (5)) are independent of the Abs/MCD selection rules. Note the inter-state SOC off-diagonal matrix element is estimated from \( \gamma \) to be \( \sim 100 \text{ cm}^{-1} \), indicating a weak SOC interaction. However, there is a significant difference between Fano \( q_{\text{Abs}} \) and \( q_{\text{MCD}} \) parameters (0.15 and 5.0) that reflects the different selection rules in Abs vs. MCD. For Abs, all of the populated sublevels of the \( S = 2 \) ground state, \( M_s = 0, \pm 1 \) and \( \pm 2 \), contribute to the spin-allowed transition to the LF \( 3E \) excited state. Among these only the \( M_s = 1 \) sublevel contributes to the spin-forbidden Fano transition. For MCD, from all the ground-state \( M_s = 0, \pm 1 \) and \( \pm 2 \) sublevels, only the \( M_s = -1 \) sublevel contributes to both the Fano spin-forbidden and spin-allowed positive MCD intensity. Therefore, the fraction of the ground-state sublevels contributing to the Fano transition is larger in MCD than in Abs, which results in \( q_{\text{MCD}} > q_{\text{Abs}} \) (eqn (6)).

The above FC (and Fano) analyses enable the experimental characterization of the low-lying, spectroscopically spin-allowed and spin-forbidden, excited states in the \( S = 2 \) Fe\(^{3+} \)=O complex along with their in-state and inter-state SOC interactions. As a result, the experimental PESs of these excited states can be plotted along the Fe-oxo coordinate (Fig. 8A). Correlating \textit{ab initio} multireference (CASPT2) calculations with these experimental data, the lowest-energy \( S = 2 \) (LF \( 3E \)) excited state along with two close-lying \( S = 1 \) excited states (LF \( 5E \) and \( 3A_1 \)) were identified. In fact, the LF \( 3E \)= with the LF \( 5E \)-based LCP component (Fig. 8A) and the \( 3A_1 \) with the LF \( 5E \)-based LCP component (Fig. 8B), a strong inter-state SOC interaction of the \( 3E \) excited state interacting with two \( 3E \)=O complex along with their in-state and inter-state SOC interactions. In going from non-relativistic to relativistic (CASPT2-based) calculations (from \textit{left to right column} in Fig. 8B), a strong inter-state SOC interaction of the \( \pi \) state with the RCP component of the \( 3E \) state at the CASPT2 equilibrium geometry (left column in Fig. 8B). Owing to strong SOC between the \( \pi \) and \( \sigma \) component of LF \( 3E \) and \( 2E \) component, two RCP-active MCDs are produced (inset). D. Evolution of the two dominant electronic configurations contributing to the multiconfigurational wavefunction characters of each electronic state (\( 3A_1 \) ground state and LF \( 5E \)). Configurations depicted correlate with the MOs diagram given in Fig. 5A. Note, throughout the entire figure, labels for states and their PESs (1 through 4) are the same as those used for the corresponding FC progressions and the sharp peak in Fig. 6 (the oxo-to-Fe CT state is state 5 and corresponds to MCD feature II in Fig. 4B).

### Table 2 Experimental and theoretical parameters for \( \sigma \rightleftharpoons \pi \rightleftharpoons \sigma \) CT excited states

<table>
<thead>
<tr>
<th>State (^a)</th>
<th>( \Delta E_{\text{vertical}}^{[b]} ) cm(^{-1})</th>
<th>( \Delta E_{\text{E-A}}^{[c]} ) cm(^{-1})</th>
<th>( \Delta E_{\text{vertical}}^{[d]} ) cm(^{-1})</th>
<th>( \Delta E_{\text{E-A}}^{[e]} ) cm(^{-1})</th>
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<tr>
<td>Ground state</td>
<td>0.0</td>
<td>820(^b)</td>
<td>0.0</td>
<td>940</td>
</tr>
<tr>
<td>1</td>
<td>10 460</td>
<td>620</td>
<td>0.16</td>
<td>13 775</td>
</tr>
<tr>
<td>2</td>
<td>10 700</td>
<td>710</td>
<td>0.13</td>
<td>13 860</td>
</tr>
<tr>
<td>3</td>
<td>12 355</td>
<td>880</td>
<td>0.08</td>
<td>14 100</td>
</tr>
<tr>
<td>4</td>
<td>19 500</td>
<td>490</td>
<td>0.21</td>
<td>28 714(^c)</td>
</tr>
</tbody>
</table>

\(^a\) States are labeled as in Fig. 8. \(^b\) Electronic vertical transition from \( 3A_1 \) ground state to highest-intensity component of Frank-Condon progression. \(^c\) Frequency of excited-state Fe-oxo stretching mode. \(^d\) Distortion of excited-state geometry along Fe-oxo stretching mode calculated from eqn (1) in the text. \(^e\) Electronic vertical transition from \( 3A_1 \) ground state at CASPT2 equilibrium geometry (with Fe-oxo bond length of 1.6 Å). \(^f\) Estimated from 1/2 \( \sqrt{k/\mu} \) where \( k \) obtained from fit of CASPT2 potential energy surfaces with third-order Taylor expansion surfaces (fitted SOC-perturbed PESs from Fig. 8). \(^g\) The \( \Delta E_{\text{E-A}}^{[c]} \) taken as difference between equilibrium geometries of \( 3A_1 \) state and excited state. \(^h\) Taken from ref. 34. \(^i\) Taken from Table S2 in ref. 22.
the ground-state equilibrium geometry produces PESs 1 and 3 (green and blue lines in Fig. 8C), the latter of which is much less shifted and distorted as compared to the first; (ii) PES 2 (red line), whose shape is unaffected by weak SOC with state 4 (red bar), is less distorted than PES 1 and more than PES 3; PES 5 (magenta line), which corresponds to the oxo-to-Fe CT state, is the most distorted and shifted among all of the PESs investigated. More quantitative evaluations of the calculated PESs and their comparisons with experiment are provided in Table 2.

These spectroscopically-calibrated calculations can be further extended to analyses of the characters in the ground- and excited-state wavefunctions, and their evolutions along the Fe–O bond coordinate. This inspection reveals important electronic configuration interactions that contribute to the characters of the ground $^3A_1$ and the lowest excited $^5E$ state. For the equilibrium Fe–O bond length, the $^3A_1$ is characterized by the dominant Fe$^{IV}$($S=2$)$O^{2-} \pi_2^2 \pi_2^2 \pi_2^2 \pi_2^2 d_{yz} d_{xz} d_{xz} d_{y^2}$ configuration (depicted in Fig. 5A) with a minor contribution from the Fe$^{III}$($S=5/2$)O$^{--}$ configuration that results from spin polarization of the $\alpha e^-$ from the oxo $\sigma$ into the $d_{z^2}$ orbital (Fig. 8D, top panel left). In elongating the Fe–O bond, the weight of the first and latter configuration decreases and increases, respectively, such that the latter becomes dominant at Fe–O distances longer than 1.88 Å (Fig. 8D, top panel right). Thus for this elongation (relevant for TS in HAA), the $^3A_1$ state gains strong oxyl character with the unpaired $\alpha e^-$ in the oxo $\sigma$ orbital (directed along the $z$ axis of the Fe–O bond). For the $^5E$ state at the Fe–O bond length of the ground state, the character is mostly the Fe$^{IV}$($S=2$)$O^{2-} \pi_2^2 \pi_2^2 \pi_2^2 \pi_2^2 d_{yz} d_{xz} d_{xz} d_{y^2}$ configuration (Fig. 5B), blended with a minor component of the Fe$^{III}$($S=5/2$)O$^{--}$ $\pi_2^2 \pi_4^1 \pi_2^2 d_{yz} d_{xz} d_{xz} d_{y^2}$ configuration (Fig. 8D, lower panel left shown only for the LCP component). The weights of these two configurations evolve such that the latter becomes dominant for distances larger than 1.72 Å (Fig. 8D, lower panel right). Thus, this $^3E$ state becomes strongly oxyl in character at the TS for HAA with the unpaired $\alpha e^-$ in the oxo $\pi$ orbital (perpendicular to the axis of the Fe–O bond) with five $\alpha e^-$ on the Fe$^{III}$ center. Finally, the second excited state, the oxo-to-Fe CT state, retains its character over entire Fe–O PES, i.e., this CT
state has oxyl character with the unpaired $\alpha$ $e^-$ in the oxo $\pi_{xy}$ orbital with three $\alpha$ $e^-$ on the Fe$^{III}$ center. This corresponds to an oxyl Fe$^{III}$ of intermediate spin ($S_{Fe} = 3/2$) at the TS. Thus these PESs define FMOs that have directionality ($\pi$ vs. $\sigma$; see Scheme 2) and reactivity in being holes on the oxo at the TS as required for efficient overlap for HAA.

Concluding comments

LT MCD spectroscopy is a powerful method for investigation of transitions into low-lying excited states of both the $S = 1$ and $S = 2$ NHFe$^{IV}$=O active sites, particularly of LF (d–d) transitions. The great advantage of MCD lies in decoupling of the overlapping bands due to their different selection rules that gives different signs of bands as well as their temperature behaviors that further provide information on the polarization of transitions and thus their unambiguous assignments. In addition, the MCD spectra exhibit vibronic structures that provide information on the excited-state PESs along the Fe–O coordinate and their (SOC) interactions. These spectroscopic results, in combination with $ab$ initio multireference calculations, reveal the nature of the FMOs of $S = 1$ and $S = 2$ NHFe$^{IV}$=O species that provide fundamental mechanistic insight into their reactivities (Scheme 3). In particular, the spectroscopically calibrated analyses show that the lowest-energy electronic states (and associated FMOs) evolve along the Fe–O PESs giving rise to oxyl Fe$^{III}$ character at Fe–O distances relevant to the TS for H-atom abstraction. For these TS-active sites, a hole is generated only in the oxo-based $p_z$ orbitals and thus, due to spin polarization, the $d\pi^*$ orbitals become reactive towards HAA. This defines the $\pi$ channel for HAA in these $S = 1$ systems (that can be hindered by equatorially chelate sterics in models). In contrast, the biologically relevant $C_{3v}$ $S = 2$ NHFe$^{IV}$=O species can generate a hole in the oxo-based $p_{xy}$ or $p_z$ orbitals and thus $d\sigma^*$ and $d\pi^*$ FMOs, respectively, can be active in HAA, providing flexibility to an NHFe$^{IV}$=O active site in controlling reaction selectivity as in the halogenase SyrB2$^{11}$ and in the HPPD/HmaS enzyme systems.$^{29}$

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References


32 Ratio of the MCD transition dipole moment ($C_0$) and dipole strength ($D_0$). Experimentally, this ratio is estimated using $C_0/D_0 = k_B T/\mu_B H (\Delta \varepsilon/\varepsilon)_{\text{max}}$, where $k_B$ and $\mu_B$ are Boltzmann constant and Bohr magneton, $T$ is the temperature, $H$ is the applied magnetic field, $\varepsilon$ is molar extinction coefficient obtained from absorption and $\Delta \varepsilon$ is the difference between the molar extinction coefficients for left- and right-circularly polarized light. In general, $C_0/D_0$ is >0.1 for ligand-field transitions, while charge-transfer transitions are characterized by $C_0/D_0 < 0.1$.


42 To estimate of the inter-state SOC matrix element between the broad and sharp state, we used eqn (7) considering that only 7% of the broad positive band overlaps with the sharp peak (Fig. 6): $\langle \Psi_b | H_{\text{int}} | \Psi_b \rangle = (0.07)^{-1} \sqrt{T/\pi}$. 

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Perspective

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