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Spin Interconversion of Heme-Peroxo-Copper Complexes Facilitated by Intramolecular Hydrogen-Bonding Interactions

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Abstract

Synthetic peroxo-bridged high-spin (HS) heme-($\mu\text{-}\eta^2\text{:}\eta^1\text{-O}_2^{2-}$)-Cu(L) complexes incorporating (as part of the copper ligand) intramolecular hydrogen-bond (H-bond) capabilities and/or steric effects are herein demonstrated to affect the complex's electronic and geometric structure, notably impacting the spin state. An H-bonding interaction with the peroxo core favors a low-spin (LS) heme-($\mu\text{-}\eta^1\text{:}\eta^1\text{-O}_2^{2-}$)-Cu(L) structure, resulting in a reversible temperature-dependent interconversion of spin state (5 coordinate HS to 6 coordinate LS). The LS state dominates at low temperatures, even in the absence of a strong trans-axial heme ligand. Lewis base addition inhibits the H-bond facilitated spin interconversion by competition for the H-bond donor, illustrating the precise H-bonding interaction required to induce spin-crossover (SCO). Resonance Raman spectroscopy (rR) shows that the H-bonding pendant interacts with the bridging peroxide ligand to stabilize the LS but not the HS state. The H-bond (to the Cu-bound O atom) acts to weaken the O–O bond and strengthen the Fe–O bond, exhibiting $\nu(\text{M–O})$ and $\nu(\text{O–O})$ values comparable to analogous known LS complexes with a strong donating trans-axial ligand, 1,5-dicyclohexylimidazole, (DCHIm)heme-($\mu\text{-}\eta^1\text{:}\eta^1\text{-O}_2^{2-}$)-Cu(L). Variable-temperature (–90 to –130 °C) UV–vis and ²H NMR spectroscopies confirm the SCO process and implicate the involvement of solvent binding. Examining a case of solvent binding without SCO, thermodynamic parameters were obtained from a van't Hoff analysis, accounting for its contribution in SCO. Taken together, these data provide evidence for the H-bond group facilitating a core geometry change and allowing solvent to bind, stabilizing a LS state. The rR data, complemented by DFT analysis, reveal a stronger H-bonding interaction with the peroxo core in the LS compared to the HS complexes, which enthalpically favors the LS state. These insights enhance our fundamental understanding of secondary coordination sphere influences in metalloenzymes.

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ASSOCIATED CONTENT

Supporting Information

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Synthetic and analytical details (methodologies and UV–vis, rR, NMR, spectra with DFT analysis) (PDF)

The authors declare no competing financial interest.

Graphical Abstract

INTRODUCTION

Metalloenzymes with d^4 – d^7 metal centers can manifest both low- and high-spin (LS and HS, respectively) electronic states, which can be nearly degenerate and result in spin crossover (SCO) phenomena; these occur most prominently in protein-bound hemes.^{1–4} SCO has also been implicated in multiple reaction mechanisms, in some contexts referred to as two-state reactivity.^{5–12} For example, during the hydroxylation of C–H substrates by a high-valent non-heme metal-oxo species, SCO from the LS to the HS potential energy surface is said to be required due to the high steric activation barrier of the former.^{13–15} Many divalent first-row transition-metal complexes also exhibit SCO behavior, and these have been extensively studied, particularly for non-heme iron(II) complexes.^{2,4,16–26}

A complex's predilection for SCO behavior may be influenced and controlled by external factors including temperature, pressure, light, and magnetic/electric fields as well as properties (often interdependent) such as ligand type, coordination geometry, intermolecular interactions, and secondary bonding interactions.^{4,17,19,21,23,27–32} Thus, synthetic model coordination complexes inspired by metalloenzymes are designed for systematically and independently probing the influence of each aspect through synthetic input of ligand and/or metal ion and variations in environment.^{33–36} Fundamental chemical insight into how these components impact electronic structure, which dictates magnetic, structural, and physical properties, is essential for promising fields of study such as molecular switching materials for nanotechnology applications and thin-film (or metal–organic framework) studies of SCO complexes for molecular electronics and spintronics applications.^{16,17,21,25,27,37–47}

Examples of SCO-facilitated reactivity are prevalent throughout biology.^{10,48–56} Electron-transfer reactions of heme proteins are often accompanied by SCO of the iron metal center, such as the oxygenation and carbonylation of myoglobin and hemoglobin.^{22,48}

Computational studies have shown that deoxy-heme and oxy-heme contain nearly degenerate spin states, allowing for fast and reversible SCO upon dioxygen binding (Figure 1A).⁵⁷ A spin-state equilibrium also regulates substrate binding and the $\text{Fe}^{\text{III/II}}$ redox potential in cytochrome P450 monooxygenase (cyt P450), wherein substrate binding drives SCO of Fe^{III} from a LS to HS state, which facilitates one-electron reduction to yield a HS Fe^{II} state, that is necessary to bind O_2 and initiate its catalytic cycle (Figure 1B).^{1,58} Additionally, the high-pH form of met-myoglobin (Mb) has been shown to undergo SCO,

where at room temperature it is in a HS state and at lower temperatures it is in a LS ground state (Figure 1C).⁵⁹ The influence of distal hydrogen bonding (H-bonding) in Mb,^{60–68} hemoglobin,^{68–72} cytochrome P450,^{73–79} and cytochrome *c* oxidase^{80–85} (CcO) has been examined by experimental (biochemical) and density functional theory (DFT) calculations. Investigations involving biomimetic synthetic models have already contributed and will continue to be key for understanding the influence of distal H-bonding interactions on the behavior of reactive intermediates in enzymatic cycles, for example, ferric superoxo,⁵⁷ ferric hydroperoxo,^{86–88} and heme-peroxo-copper species.^{33,89–92}

Extensive examples of SCO in enzymatic and synthetic non-heme systems^{2,4,16–18,25,26} have been investigated, though only a handful of studies with enzymatic and especially synthetic heme systems have been published.^{49–51,55,56,93–98} Notable examples include those by the laboratories of de Visser and Rath, along with Dey, who reported on the reversible SCO in synthetic iron(III) complexes in the presence of inter- and intramolecular H-bonding, respectively.^{94,95} In synthetic heme systems, the conversion from HS to LS iron(III) is most often associated with a strong σ -donor ligand (e.g. imidazole) binding axially to the iron, which raises the Fe d_z^2 orbital energy.⁹⁹ However, of the few accounts of SCO in synthetic 5-coordinate (5C) or 6-coordinate (6C) heme systems as well as the exhaustive list of enzymes capable of SCO, there has been little comprehensive thermodynamic analysis of the SCO process.^{49–51,100} Therefore, detailed studies are warranted to understand the nature of H-bonding networks that are able to dictate the electronics of the active site metal ion and its subsequent reactivity.

As a prime example, it has been reported in enzymes such as CcO, which utilize a heterobinuclear active site comprising of a heme and copper moiety, that H-bonding interactions facilitate the reduction of O₂ to water in all eukaryotes, by weakening the O–O bond after O₂ binding. To investigate this concept, CcO-inspired synthetic models have been prepared by pairing various copper(I) complexes coordinated to TMPA-based ligands (TMPA = tris(2-pyridylmethyl)amine) bearing substituents in the 6-pyridyl position(s) (i.e., [^XTMPA]Cu^I) with the tetrakis(2,6-difluorophenyl)porphyrinate (F₈) heme (Scheme 1). TMPA has long been employed in numerous studies^{91,101–106} because its infrastructure can be easily manipulated synthetically to probe various stereoelectronic effects. For instance, Masuda and co-workers synthesized various TMPA-based ligands (including ^{NH₂}TMPA, the system of interest in this current study) and demonstrated their H-bonding capabilities, which help stabilize dicopper(II) *trans*-peroxide complexes.^{107,108} Similarly, F₈ has also long been utilized in heme models^{10,101,109,110} due to its ease in synthesis and therefore provides a direct comparison with analogues in the literature. Following previously established methods, dioxygen was bubbled through an equimolar mixture of the reduced metal–ligand complexes¹¹¹ at –90 °C in 2-methyltetrahydrofuran (MeTHF) as solvent to generate HS heme-peroxo-copper complexes (Scheme 1) and subsequent addition of 1 equiv of an exogenous base has been shown to form LS heme-peroxo-copper analogues (Scheme 1, Pathway 1).^{33,90,111,112} Recent work investigating the H-bonding capabilities of LS (axial base)heme-(O₂²⁻)-Cu(L) complexes (L = ^XTMPA), employing strongly donating 1,5-dicyclohexylimidazole (DCHIm) as a *trans*-axial base (Scheme 1), have shown an interaction between the O_{Cu} and the amino group resulting in elongation of the O–O peroxo

core, demonstrating a remarkable utility of this ligand scaffold as a probe of intramolecular interactions.^{33,89}

Herein, it is observed that intramolecular H-bonding interactions facilitate thermally induced spin-state changes in synthetic heme-peroxo-copper complexes. This study evaluates the HS heme-peroxo-bridged-copper complexes by spectroscopic (including UV-vis, EPR, ²H NMR, and resonance Raman) and computational methods to understand how secondary coordination sphere effects, particularly intramolecular H-bonding, affect the geometric and electronic structure of the O-O peroxo core and induce a SCO (5C HS to 6C LS), an essential process in multiple critical enzymatic transformations. The effects of intramolecular H-bonding are examined in this work, including the utilization of the single amino-substituted ligand ^{NH}₂TMPA (Scheme 1), which mimics the established H-bonded array of water molecules frequently depicted in the active site of CcO or the H-bonding of the nearby tyrosine residue (covalently cross-linked to a Cu-coordinated His), both of whose H-bonded effects have been established to be critical in the reduction of dioxygen to water.^{113–116} Conclusions drawn also apply to numerous other metalloenzymes which utilize secondary coordination H-bonding in their catalytic cycles (e.g., cytochrome P450 and catalase), offering possible insight into the evaluation of these effects in enzymes that undergo SCO when binding and activating O₂.^{117–119}

It is shown that lowering the temperature of a solution of HS [(F₈)Fe^{III}-(μ-η²:η¹-O₂²⁻)-Cu^{II}(^{NH}₂TMPA)]⁺ from -90 to -130 °C results in gradual SCO between -100 and -130 °C, yielding the solvent-bound LS complex [(MeTHF)(F₈)-Fe^{III}-(μ-1,2-O₂²⁻)-Cu^{II}(^{NH}₂TMPA)]⁺. The involvement of a solvent (MeTHF or THF) binding process in the HS state for [(F₈)Fe^{III}-(O₂²⁻)-Cu^{II}(^{NH}₂TMPA)]⁺ while decreasing temperature from -90 to -100 °C (at higher temperature than observed for the onset of SCO) is suggested by (1) a blue shift in the λ_{max} of the Q-band from a split absorbance at 560 nm to a sharp peak at 533 nm with no low-energy features, (2) a similar blue shift is observed for [(F₈)Fe^{III}-(O₂²⁻)-Cu^{II}(^{NH}₂.CH₃TMPA)]⁺, which does not undergo SCO, (3) ²H NMR spectral evidence that the species at -100 °C remains HS, and (4) variable-temperature (VT) UV-vis data in two different solvent mixtures (MeTHF vs 2:3 THF:MeTHF), showing that THF induces spectral changes earlier upon cooling (i.e., at higher temperatures) than MeTHF, consistent with THF serving as a better coordinating ligand. The second process, thermally induced SCO facilitated by H-bonding, upon cooling from -100 to -130 °C is supported by (1) the appearance of low-energy (700–800 nm) absorption features in the UV-vis spectra with decreasing temperature, (2) a change in spin state, from HS to LS, observed in the ²H NMR spectra, (3) similar spectral (UV-vis and ²H NMR) changes do not occur for ligands without H-bonding moieties, (4) inhibition of these spectral changes occurs upon addition of 1 equiv of various Lewis bases, (5) rR data at -196 °C exhibiting vibrational modes characteristic of a LS species (including M-O and O-O modes of the peroxo core as well as heme marker bands, and (6) DFT calculations which provide good agreement with the rR data and support that SCO is preferentially favored for the complex with a pendant H-bonding group.

RESULTS AND DISCUSSION

Impact of Temperature and Exogenous Lewis Bases on Spin-State Monitored by UV-vis and NMR Spectroscopies.

The HS heme-peroxo-copper complexes $[(F_8)Fe^{III}-(O_2^{2-})-Cu^{II}(^XTMPA)]^+$ (**HS-^XTMPA**), varying in substitution on the ^XTMPA copper ligand where X = H, NH₂, CH₃, (NH₂,CH₃), and (CH₃)₂ (see Scheme 1), exhibit similar UV-vis spectra at -90 °C, with Q-bands centered around 560 nm, consistent with the absorption signature of previously reported HS heme-peroxo copper species (Figures 2A, S1, and S24–S26).^{91,111,120} Initial studies done on the parent HS-TMPA complex, with in-depth characterization using UV-vis, rR, XAS, EXAFS, NMR, and Mössbauer spectroscopies, provide a strong chemical foundation from which further studies can be expanded upon, as in this study which probes 6-pyridyl substituents on the TMPA framework.^{120–123} All of these HS complexes are EPR silent (perpendicular mode) arising from antiferromagnetic coupling between the $S = 5/2$ Fe^{III} and $S = 1/2$ Cu^{II} ion, giving a total $S = 2$ at -90 °C in MeTHF, as was proven for previously examined HS heme-peroxo-copper complexes.¹¹¹

However, interesting behavior is observed for the heme-peroxo-copper complex bearing the NH₂TMPA ligand, where cooling to -130 °C results in a significant blue shift in the Q-band λ_{max} from 560 to 533 nm and the appearance of unique low-energy (i.e., near IR region) charge-transfer bands around 700–850 nm (Figures 2A and S2, and Scheme 1, Pathway 2), similar to the absorption spectra observed for the previously reported LS heme-peroxo-copper complexes bearing the strong trans-axial donor ligand on the heme, DCHIm (e.g., $[(DCHIm)(F_8)Fe^{III}-(O_2^{2-})-Cu^{II}(TMPA)]^+$, **LS(DCHIm)-TMPA**, Scheme 1, Pathway 1; $R^1 = R^2 = H$).^{90,111} The change in spin state (from 5C HS to 6C LS) upon cooling is supported by ²H NMR spectroscopic data (Figure 2B), using the pyrrole-deuterated *d*₈-F₈ porphyrin analog, indicating conversion from a paramagnetic to diamagnetic ground state as evidenced by the 93.4 ppm peak observed at -90 °C moving upfield to the diamagnetic region to give a somewhat broadened peak centered around 3.85 ppm at -130 °C; spectral deconvolution shows this to consist of two peaks (Figure 2B) attributed to the LS peroxo complex plus a ferric superoxo species ($[(MeTHF)(F_8)Fe^{III}(O_2^{\bullet-})]$), established as a minor innocent iron-only impurity formed concomitantly with the heme-peroxo-copper complex. (Note: This was previously shown to be a LS species due to the binding of an axial solvent (THF) molecule.)^{111,124}

Upon warming to -90 °C, the characteristic HS ferric heme UV-vis features and paramagnetic ²H NMR signals returned, demonstrating that the temperature-dependent interconversion is reversible. In contrast, this temperature-dependent spin change was not observed for the parent heme-peroxo-copper complex (**HS-TMPA**) nor for the analogous methyl-substituted complexes (**HS-CH₃TMPA**, **HS-NH₂,CH₃TMPA**, **HS-(CH₃)₂TMPA**) (Scheme 1), evidenced by the ²H NMR spectroscopic data showing these complexes remain paramagnetic and thus HS upon cooling to the very low cryogenic temperatures (-130 °C) (*vide infra* and see Figures S27–S29). This is further supported by the UV-vis data, which lack the appearance of the diagnostic visible region low-energy ($\lambda_{max} \sim 750$ nm) charge-transfer bands (Figure 2A).¹²⁵ Overall, these observations implicate H-bonding in

facilitating the spin-state change with the NH_2TMPA heme-peroxo-copper complex, which will be further demonstrated by experiments described below.

To further probe how this H-bond-facilitated SCO can be controlled by external interactions, a series of Lewis bases (i.e., exogenous H-bond acceptors) were added to $\text{LS}(\text{MeTHF})\text{-NH}_2\text{TMPA}$ at $-130\text{ }^\circ\text{C}$ in MeTHF (Scheme 2). Interestingly, addition of 1 equiv of the Lewis bases (1,8-bis(dimethylamino)naphthalene (Proton Sponge, PS), triethylphosphine oxide (TEPO), or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)) resulted in inhibition of the spin conversion, as demonstrated by UV-vis and ^2H NMR spectroscopies (Figures 3 and S21–S23). That is, upon addition of the Lewis base, the 533 nm Q-band (associated with the LS complex) decreased concomitantly with an increase at 560 nm and the disappearance of the low-energy charge-transfer bands. Likewise, a paramagnetic signature around 90 ppm (and no upfield paramagnetic to diamagnetic shift) is observed in the ^2H NMR spectra at $-130\text{ }^\circ\text{C}$ for this reaction. This shows that the Lewis base competes favorably for the ligand arm associated H-bonding NH_2 -group of $\text{LS}(\text{MeTHF})\text{-NH}_2\text{TMPA}$, weakening the $\text{O}_2^{2-}\cdots\text{amino}$ H-bonding interaction, resulting in the formation of $\text{HS-NH}_2\text{TMPA}$. Together, these observations further implicate the need for H-bonding to the O–O peroxo core in order for SCO to occur.

Insight Into Thermally Dependent Solvent Binding and Spin Conversion from UV-vis and ^2H NMR Spectroscopies.

To more closely examine the SCO process occurring when cooling from -90 to $-130\text{ }^\circ\text{C}$, UV-vis spectroscopy data were collected while incrementally lowering temperature. As shown in Figure 4, the absorption spectrum of $\text{HS-NH}_2\text{TMPA}$ changes biphasically. This is emphasized by the Inset in Figure 4 depicting the absorbances at either the diagnostic LS λ_{max} 533 nm (red) or HS λ_{max} 560 nm (blue) over the temperatures -90 to $-130\text{ }^\circ\text{C}$, demonstrating two regions with different slopes. Cooling from -90 to $-100\text{ }^\circ\text{C}$ results in the disappearance of the 560 nm band associated with the HS (and higher-temperature) species and the appearance of a narrow band around 533 nm. However, no absorption bands are observed around 750 nm (charge-transfer bands uniquely characteristic of the LS state), suggesting that the species at $-100\text{ }^\circ\text{C}$ is still predominantly in the HS state. As a control, the parent HS-TMPA heme-peroxo-copper complex was also characterized by VT UV-vis spectroscopy (Figure S1); no spectral changes were observed.

To determine the spin state of these complexes at various temperatures, ^2H NMR data were obtained with the $\text{d}_8\text{-F}_8$ pyrrole deuterated analog. For the heme-peroxo-copper complex bearing TMPA , the NMR signature remained unchanged from $-90\text{ }^\circ\text{C}$ to $-130\text{ }^\circ\text{C}$ (Figure 5A), showing only two peaks that are ascribed to the paramagnetic HS-TMPA species (shifting from ~ 97 to ~ 135 ppm as temperature is lowered) and the diamagnetic minor ferric superoxide impurity at ~ 9.9 ppm (denoted by asterisks in Figure 5A).^{111,124} Interestingly, the UV-vis data of $\text{HS-NH}_2\text{TMPA}$ collected at $-100\text{ }^\circ\text{C}$ (which lacks the low-energy bands, *vide supra*) are supported by ^2H NMR data showing a paramagnetic peak around 94 ppm (Figure 5B), indicating that the dominant species present is in the HS state. Finally, a LS species forms upon further cooling from -105 to $-130\text{ }^\circ\text{C}$ as indicated by a gradual appearance of the low-energy bands ($\lambda_{\text{max}} \sim 750\text{ nm}$) (as previously reported for

LS(DCHIm)-NH₂TMPA).⁹⁰ Accordingly and in support, when the **HS-NH₂TMPA** heme-peroxo-copper complex is cooled down to very low cryogenic temperatures and the low-energy absorption feature grows in, the NMR data show a gradual spin-state conversion from HS to LS. As shown in Figures 4 and 5B, the percentage of HS species present decreases upon cooling, concomitant with an increase in the LS species, until -130 °C where the HS component is no longer detected by ²H NMR (Figure 2A). The VT ²H NMR data indicate that the paramagnetic to diamagnetic conversion begins around -100 °C (Figure 5B).

Given that the temperature-dependent spectral changes are gradual and reversible, it can be assumed that the system is in a thermal equilibrium. This implicates a process under entropic control, as enthalpy is largely temperature independent. Therefore, interpreting the biphasic temperature dependence to indicate that two distinct entropy-dependent processes are occurring, it was hypothesized that solvent binding may be contributing to the spectral changes. To probe the involvement of solvent binding, similar experiments were carried out in 2:3 THF:MeTHF (Figures 6 and S8–S10, Tables S4 and S5), as THF should be a better axial ligand donor to the heme-iron atom since the O atom lone pairs are less sterically hindered (resulting in more enthalpically favorable binding). The VT UV–vis data in this solvent mixture likewise show biphasic behavior (Figure 6, inset), with the change in the Q-band occurring at higher temperature. This observation is consistent with a greater enthalpic driving force for THF binding compared to MeTHF. Furthermore, complete conversion to the high-temperature species (i.e., dissociation of the axially bound solvent molecule) required higher temperatures in the 2:3 THF:MeTHF mixture (-85 °C for the THF mixture vs -90 °C in pure MeTHF) (see the Figure 6 inset compared to the Figure 4 inset), suggesting that THF binds more strongly.

Interestingly, while the parent **HS-TMPA** complex does not show solvent or temperature dependence by UV–vis, NMR, or rR spectroscopies (Figures 5A and S1, Table 1), the complexes **HS-CH₃TMPA** and **HS-NH₂,CH₃TMPA** do appear to exhibit a partially shifted Q-band (similar to that observed for solvent binding to **HS-NH₂TMPA**) at -130 °C, which occurs to a greater extent in 2:3 THF:MeTHF than in MeTHF. However, the ²H NMR data indicate that these complexes (presumably solvent-bound) remain fully HS at -130 °C (Figures S24 and S26–S29). The spectral change between the two HS forms may reflect a difference in symmetry of the porphyrin ring, as the intensity of the α band (the lower energy Q-band feature) is known to decrease for higher heme symmetry (e.g., planarity).¹²⁶ Since the UV–vis spectrum of **HS-NH₂,CH₃TMPA** in 2:3 THF:MeTHF indicates almost complete solvent binding at -130 °C, a van't Hoff analysis was applied to the VT UV–vis data to estimate the thermodynamic parameters of THF binding. As shown in Figure S3, this yielded values of $H_{\text{bind}} = -5.6$ kcal/mol and $S_{\text{bind}} = -34.6$ cal/(mol K), in excellent agreement with thermodynamics typical for solvent binding (the entropy term ($T \Delta S$) for binding would be -10.3 kcal/mol at room temperature).¹²⁷

A similar analysis for solvent binding could not be directly obtained for **HS-NH₂TMPA** due to spectral overlap of the HS and LS species as well as overlap of the solvent binding and spin conversion processes (modeling the data assuming negligible overlap would overestimate H and S , see SI Section II, Figure S4). However, the solvent binding parameters obtained for **HS-NH₂,CH₃TMPA** provide a valuable estimate for the analogous

process in **HS-NH₂TPMA**. Inferring from the NMR data that the spin conversion with the **NH₂TPMA** ligand occurs gradually over the temperature range -100 to -130 °C, we have modeled the UV-vis data as a thermally dependent equilibrium between three species (5C HS, 6C HS, and 6C LS) to consider a rough approximation of the thermodynamics for spin conversion (see Figure S30). We obtain a reasonable fit to the data in both MeTHF and 2:3 THF:MeTHF solvents (i.e., the data shown in Figures 4 and 6, respectively), with values of $H_{\text{bind}} = -6.1$ kcal/mol and $S_{\text{bind}} = -35$ cal/(mol K) for solvent binding (5C to 6C), and $H_{\text{SC}} = -4.1$ kcal/mol and $S_{\text{SC}} = -25$ cal/(mol K) for spin conversion (6C HS to 6C LS). The H_{SC} and S_{SC} are found to be solvent independent, (these values yield a similar quality fit for both solvent mixtures, within error).

The H_{SC} and S_{SC} in SCO of **HS-NH₂TPMA** are comparable to other HS/LS conversions reported for 6C enzymatic heme systems in the literature (in general, $H_{\text{SC}} = -1$ to -6 kcal/mol and $S_{\text{SC}} = -10$ to -25 cal/(mol K)),¹²⁸ where the change in entropy is primarily attributed to the shorter bonds (i.e., the vibrational contribution to the entropy) around the Fe^{III} center in the LS structure.^{97,129,130} The H_{SC} and S_{SC} for **HS-NH₂TPMA** are larger than most SCO systems in the literature, which may derive from factors such as altering the core geometry, strengthening bonds to the Cu ion, strengthening the interaction with an axial ligand (solvent), and strengthening (or forming) an H-bond with the amine substituent. As will be presented in detail below, resonance Raman data indicate that each of these factors may be contributing to SCO in **HS-NH₂TPMA**. As a possible biological comparison, the reported spin conversion of cytochrome P450 (with camphor bound in the enzymatic pocket) under slightly acidic conditions (protonic activity (p_{aH}) of 6) gave values much closer to those reported herein ($H_{\text{LS/HS}} = -10$ kcal/mol and $S_{\text{LS/HS}} = -45.2$ cal/(mol K))¹³¹. Increasing to slightly alkaline protonic activity ($p_{\text{aH}} = 7.6$) resulted in a decrease in both the enthalpic and entropic terms, implicating the putative importance of a nearby protonated amino acid residue (potentially analogous to the H-bonding substituent of **NH₂TPMA** in our heme-peroxo-copper study) in promoting SCO.¹³¹

Using the thermodynamic parameters (H and S) for spin conversion given above, the HS state is calculated to be 0.2 kcal/mol lower in G at -100 °C, while at -130 °C, the LS state is favored by 0.5 kcal/mol. Considering this narrow difference in G , it is unsurprising that altering the H-bonding abilities (either with exogenous Lewis bases or different ligand substitution) can turn off the SCO behavior. Nevertheless, the combined VT UV-vis and ²H NMR data demonstrate that while **HS-CH₃TPMA** and **HS-NH₂.CH₃TPMA** exhibit temperature-dependent spectral changes attributable to solvent binding, only the complex possessing intramolecular H-bonding donors (amine), but not steric (methyl) groups, is capable of favoring a LS electronic structure in a weakly binding solvent.

²H NMR Spectral Deconvolution Analysis To Determine Species in Solution.

The NMR signature at -105 °C is a mixture of **HS(MeTHF)-NH₂TPMA**, **LS-(MeTHF)-NH₂TPMA**, and the previously established minor ferric superoxo impurity [(MeTHF)(F₈)Fe^{III}(O₂^{•-})] (Figure 5B).^{111,124} This is supported by deconvolution analysis of the spectrum at -105 °C (Figure S6), which shows that two peaks are required to fit the diamagnetic region (i.e., fitting two peaks allowed for no residual peaks in the difference

spectrum between the fit and experimental data), indicating the presence of two diamagnetic species (**LS(MeTHF)-NH₂TPMA** and the ferric superoxo impurity). Deconvolution analysis of the paramagnetic region at -105 °C (Figure S6) confirms the presence of a HS component, exhibiting a broad peak around 100 ppm (close to that observed at -100 °C in Figure 5B). Similar NMR deconvolution analyses were carried out (Figure S5) for the spectra obtained at -110 to -130 °C, which indicate that three species are present from -110 to -120 °C (**HS(MeTHF)-NH₂TPMA**, **LS(MeTHF)-NH₂TPMA**, and a minor amount of a ferric superoxo species). However, at -130 °C there is only a mixture of **LS(MeTHF)-NH₂TPMA** and ferric superoxo species in the ²H NMR spectrum, and no paramagnetic peak is observed. As expected, the NMR signal intensity is greater for **HS-TPMA** (Figure 5A) than for **NH₂TPMA** (Figure 5B), particularly at lower temperatures. Due to **HS-TPMA** remaining fully HS (and thus paramagnetic) at all temperatures, the faster relaxation rate associated with paramagnetic species (vs diamagnetic species) results in the greater accumulation of signal. Thus, the decreased signal-to-noise ratio (S/N) upon cooling **HS-NH₂TPMA** is due to the increase in the percentage of diamagnetic species present in solution, further supporting that SCO is occurring. The broadening associated with paramagnetic signals and the increased broadening at low temperatures complicates quantitative determination of the relative HS/LS composition via NMR spectroscopy due to the low S/N. In order to accurately integrate peaks, the S/N of the signal should be approximately 200. At -130 °C, the S/N is <20, therefore, about 100 times more scans would be required to obtain the desired S/N. This would extend the experimental time to be more than 100 h in some cases for this study, which is not feasible with our low-temperature setup; thus, we conclude that UV-vis spectroscopy provides a reliable means of quantifying the ratio of **HS(MeTHF)-NH₂TPMA** to **LS-(MeTHF)-NH₂TPMA**, as has been described above.

Insights into Core Geometries and H-Bonding Influences: rR Spectroscopy.

Resonance Raman spectroscopy presents a particularly valuable platform for studying the spin conversion process, offering a direct probe of bond strengths, spin-state and ligation of the heme, and even the nature of absorption bands. The binding mode of the peroxide ligand in the parent **HS-TPMA** complex has been formulated as a $\mu\text{-}\eta^2\text{:}\eta^1$ bridge (Scheme 1), which exhibits fairly low $\nu(\text{M-O})$ frequencies and a ν_2 (spin-state marker) band characteristic of HS ferric heme (Table 1).¹²⁰⁻¹²² In contrast, **LS-TPMA** has the peroxide end-on to iron and copper, exhibits a much stronger Fe-O bond (now having an unoccupied antibonding Fe d_z^2 orbital) and higher frequency ν_2 band, characteristic of LS ferric heme.^{99,111} In agreement with the UV-vis and NMR data (Figures 2, S24 and S27), the complexes with **CH₃TPMA** (at -196 °C) and **NH₂TPMA** (at -90 °C) show a HS ν_2 band, low $\nu(\text{M-O})$ frequencies, and spectra that are overall similar to that of **HS-TPMA** (blue and green spectra in Figure 7A,B,E; see also Figures S9 and S10, Table 1). It is very interesting, however, that **HS-CH₃TPMA** and **HS-NH₂TPMA** (at -90 °C) exhibit nearly identical $\nu(\text{M-O})$ and $\nu(\text{O-O})$ frequencies, suggesting the amino group of **NH₂TPMA** has a negligible impact on the bonding in the peroxo core (i.e., no indications of significant H-bonding are observed) (Table 1 and Figure 7). Although the isotope-sensitive mode at 526 cm⁻¹ (assigned as $\nu(\text{Fe-O})$) is less strongly enhanced for **HS-NH₂TPMA** with 413 nm excitation, (Figure 7), this could result from slight variations in mode coupling or even absorption bands masked by the

Soret absorption. Furthermore, the mode at $\sim 475\text{ cm}^{-1}$ (assigned as $\nu(\text{Cu-O})$) exhibits a much smaller isotope shift ($^{16}\text{O}_2\text{-}^{18}\text{O}_2 = 11\text{ cm}^{-1}$) than predicted for a simple harmonic oscillator ($^{16}\text{O}_2\text{-}^{18}\text{O}_2 = 21\text{ cm}^{-1}$), which indicates substantial coupling with nearby modes. The observation of this unusually small isotope shift for both **HS-CH₃TMPA** and **HS-NH₂TMPA** therefore further suggests that they are structurally very similar. Therefore, the difference in core bond strengths of **HS-CH₃TMPA** and **HS-NH₂TMPA** relative to **HS-TMPA** (lower $\nu(\text{M-O})$ s but comparable $\nu(\text{O-O})$) can be attributed to *steric*, rather than *H-bonding* effects.

To better understand the rR spectral differences observed between **HS-TMPA** and **HS-CH₃TMPA** (or **HS-NH₂TMPA**), particularly the changes in M-O modes, rR data were collected for **HS-TMPA** and **HS-CH₃TMPA** using $^{54}\text{Fe}/^{57}\text{Fe}$ isotope labeling. As shown in Figure S13, the **HS-TMPA** features at 538 cm^{-1} and 515 cm^{-1} both shift with Fe isotope substitution (to 540 cm^{-1} and 516 cm^{-1} with ^{54}Fe , and 537 cm^{-1} and 513 cm^{-1} with ^{57}Fe , respectively), indicating Fe motion in the vibration. These modes were previously assigned¹²³ as Fe-O₂ and Fe-O-Cu, in excellent agreement with these findings. In contrast, the data for **HS-CH₃TMPA** reveal that only the 527 cm^{-1} mode shifts with $^{54}\text{Fe}/^{57}\text{Fe}$ labeling (from 528 cm^{-1} (^{54}Fe) to 525 cm^{-1} (^{57}Fe)), comparable to the shift for the 538 cm^{-1} mode in **HS-TMPA**, while the 475 cm^{-1} feature is insensitive to the Fe substitution. This suggests that the latter may correspond to the Cu-O stretch that is not significantly coupled to Fe motion. Such changes in the nature of vibrational modes (e.g., amount of Fe motion) for **HS-TMPA** versus **HS-CH₃TMPA** (or **HS-NH₂TMPA**) may therefore indicate differences in bond angles in the peroxo core, as this would impact coupling between vibrational modes.

Upon cooling to $-196\text{ }^\circ\text{C}$, (liq. N₂), a clear spectral change is observed for the **NH₂TMPA** complex (blue to red in Figure 7B,D) consistent with conversion from HS to LS, including an upshift in the ν_2 band, and the appearance of $\nu(\text{M-O})$ and $\nu(\text{O-O})$ frequencies that are comparable to [(DCHIm)(F₈)-Fe^{III}-(O₂²⁻)-Cu^{II}-(NH₂TMPA)]⁺ (black in Figures 7E,F and S12, Table 1). The intensity and position of the ν_3 band suggests that the heme is 6C (with MeTHF bound axially)¹³² in agreement with the interpretation of the VT UV-vis data given above (*vide supra*). Additionally, laser excitation into the low-energy band ($A_{\text{ex}} = 780\text{--}900\text{ nm}$, Figures S14 and S15) revealed selective enhancement of all three core modes, strongly implicating absorption feature(s) in this region have ligand to metal charge-transfer (LMCT) character that is diagnostic of LS heme-peroxo-Cu complexes possessing a $\mu\text{-}\eta^1\text{:}\eta^1$ peroxo core structure. Notably, the $\nu(\text{Fe-O})$ frequency (632 cm^{-1}) of this LS species (likely MeTHF-bound) is among the highest that has been observed in synthetic heme-peroxo-Cu complexes to date (Table 1).

Considering the spectral similarity between the LS complexes [(MeTHF)(F₈)Fe^{III}-(O₂²⁻)-Cu^{II}-(NH₂TMPA)]⁺ and [(DCHIm)-(F₈)Fe^{III}-(O₂²⁻)-Cu^{II}-(NH₂TMPA)]⁺ (red and black in Figure 7F, respectively), and that an H-bonding interaction with the peroxo core was demonstrated in the latter,⁹⁰ it can be inferred that a similar H-bonding interaction is present in the former. Since the data therefore indicate that the amine is interacting with the peroxo ligand in the LS structure, but not the HS analog (*vide supra*), these results suggest that the H-bonding interaction facilitates spin conversion by stabilizing the LS state more than the HS state. It should be recognized, however, that the weakened M-O bonds in **HS-CH₃TMPA**

and **HS-NH₂TMPA** (relative to **HS-TMPA**) may indicate that the sterics of monosubstitution are acting to destabilize the HS state.

It is interesting to consider the case of **HS-NH₂,CH₃TMPA**, which remains HS upon cooling to -130 °C (Figures S26 and S29), despite its DCHIm-ligated LS complex possessing an intramolecular H-bonding interaction with the peroxo core.⁹⁰ It is worth noting that the $\nu(\text{Fe-O})$ stretch for **HS-NH₂,CH₃TMPA** is higher than typically observed for side-on bound peroxo complexes, while the $\nu(\text{Cu-O})$ is exceptionally low; this may be due to either the peroxo binding end-on to the HS Fe or the sterics of the disubstituted TMPA weakening donation from the peroxo moiety into Cu and strengthening donation into Fe. Nevertheless, comparison of the rR data for **HS-NH₂,CH₃TMPA** to **HS-(CH₃)₂TMPA** (Figure S11, Table 1) suggests that the amine substituent is impacting the peroxo core in the HS structure, in contrast to **HS-NH₂TMPA** (Figure 7). Therefore, the H-bond in **HS-NH₂,CH₃TMPA** may also be stabilizing the HS structure, disfavoring SCO. An alternative possibility is that the methyl substituent lessens the degree to which the H-bond can stabilize the LS state (since sterics have been found to weaken the M-O and O-O bonds in the LS complexes).⁹⁰

To examine the effect of solvent binding in the HS state, rR data were collected for **HS-NH₂,CH₃TMPA** in 2:3 THF:MeTHF at -90 °C and -130 °C, where solvent binding was most evident based on the VT UV-vis and NMR data (*vide supra*). As shown in Figure S16, the “solvent-bound” complex at -130 °C has a $\nu(\text{Fe-O})$ that is 5 cm⁻¹ lower (564 cm⁻¹) than the “unbound” complex at -90 °C (569 cm⁻¹) and a $\nu(\text{Cu-O})$ (442 cm⁻¹) that is 11 cm⁻¹ lower than the complex at -90 °C (453 cm⁻¹). In contrast, the $\nu(\text{O-O})$ is unchanged between the two forms, although its intensity appears greater at -130 °C. In support of the structural assignments for -90 °C and -130 °C as 5C HS and 6C HS, respectively, the high-frequency ν_3 heme band exhibits a shift down in energy (1500 to 1494 cm⁻¹) and increases in intensity upon going from -90 °C to -130 °C, comparable to behavior seen in 5C vs 6C HS forms in peroxidase enzymes.^{133,134}

Since the VT UV-vis and NMR data on **HS-NH₂TMPA** suggest that solvent-dependent absorption spectrum changes occur at higher temperatures than SCO, rR data for **HS-NH₂TMPA** in both MeTHF and 2:3 THF:MeTHF were also collected at several temperatures from -90 °C to -110 °C (Figures S18 and S19). While minor spectral changes were observed over this range, they appear to be attributable to a gradual conversion from the HS to the LS species, which occurs to a greater extent in 40% THF compared to MeTHF. In agreement with the NMR data, the rR data show that the solution is still predominantly HS at -110 °C. Thus, no spectral features assignable to a 6C HS complex were observed, suggesting that the changes observed in UV-vis between -90 °C and -100 °C are not associated with a significant impact on the HS peroxo core. *Nevertheless, the results for NH₂TMPA at lower temperature show that an H-bonding interaction can provide a unique stabilization of the LS state relative to the HS state, thereby favoring a LS electronic structure even in the absence of a strong trans-axial donor ligand.*

Understanding Geometric and Electronic Structure in Spin Crossover: DFT Calculations.

The data presented above provide experimental insight into key components of the SCO process, including geometric and electronic structure, H-bonding and steric effects, HS vs

LS stabilization, and thermodynamics of axial ligand binding. Therefore, this presents an excellent opportunity to apply well-calibrated DFT calculations to both elucidate details of the electronic nature of SCO and understand H-bonding contributions to reactivity for future studies. As it is well-known that spin-state energetics and metal–ligand bond strengths are highly functional dependent (although our calculations employ the B3LYP* functional shown to perform well for SCO), to understand the effect of H-bonding on SCO, our analysis focuses on comparisons between the different ^XTMPA ligands, placing a smaller emphasis on the absolute energies (which overstabilize the LS and solvent-bound forms).

In agreement with the experimental findings, DFT calculations (additional details are given in the SI) show that the conversion from **HS**-^{NH₂}TMPA to **LS**(THF)-^{NH₂}TMPA is thermodynamically favorable at –130 °C ($G = -9.1$ kcal/mol, Table S7) and that this has a greater driving force for SCO than the analogous complexes of ^{CH₃}TMPA and **TMPA** (by 4.1 and 1.1 kcal/mol, respectively). Furthermore, axial THF binding is more favorable in the LS state than the HS state (by ~3 kcal/mol), indicating that (1) the LS complex will be THF bound in the weakly coordinating solvent and (2) solvent binding provides an additional driving force for SCO.¹³⁵ Although the calculations suggest that binding of an axial ligand trans to the peroxo may not be *required* to stabilize the 5C LS complex (although it is evidently overstabilized in the calculations), we are unable to synthesize these complexes in noncoordinating solvents to evaluate this possibility experimentally. Nevertheless, while SCO for **HS** to **LS**(THF) is energetically more favorable for ^{NH₂}TMPA than ^{CH₃}TMPA and **TMPA** by only a small margin, the results obtained from the VT UV–vis analysis suggest that the **LS**(THF)-^{NH₂}TMPA complex is favored over the HS state by 0.5 kcal/mol at –130 °C (*vide supra*). In other words, disfavoring SCO by only ~1 kcal/mol would be sufficient to prevent a LS complex from being observed.

The DFT-optimized structures (Figure 8) correlate well with the rR data in two key ways: First, the **HS**-^{NH₂}TMPA and **HS**-^{CH₃}TMPA complexes both exhibit $\mu\text{-}\eta^2\text{:}\eta^1$ cores with similar bond lengths (in agreement with the similarity in their core vibrations by rR), including a minimal interaction of the amine H-bond with the peroxo (Figure 8A,B, Table 2). Second, the lowest-energy structure for **LS**(THF)-^{NH₂}TMPA exhibits a $\mu\text{-}\eta^1\text{:}\eta^1$ peroxo core and an H-bonding interaction with the O_{Cu} atom (which is sterically more accessible in the $\mu\text{-}\eta^1\text{:}\eta^1$ core, Figure 8D,E), comparable to its DCHIm-ligated analog recently reported.⁹⁰ These calculations show that in the LS structure, the H-bond lowers the energy of the peroxide orbitals and draws net electron density away from the metals (Table S9). However, the key consequence of the H-bonding interaction is a slight increase in σ -donation into Fe, in line with the exceptionally strong Fe–O bond observed by rR (Figure 7 and Table 1) and shorter Fe–O bond length in **LS**(THF) complex with ^{NH₂}TMPA compared to **TMPA** and ^{CH₃}TMPA (Table S8). Importantly, this behavior (observed in rR spectroscopy and DFT calculations) is corroborated in the comparison of **LS**(DCHIm)-**TMPA** and **LS**-(DCHIm)-^{NH₂}TMPA, where experimental data of both LS complexes are available.⁹⁰ Thus, the DFT calculations reproduce the rR data by showing that the H-bond in **LS**(THF)-^{NH₂}TMPA serves to weaken (elongate) the O–O bond, strengthen (shorten) the Fe–O bond, and weaken (elongate) the Cu–O bond (Table S8), similar to the previously reported DCHIm-ligated complexes. To evaluate whether the F atoms of the F₈ porphyrin could be could be

impacting the SCO behavior by interacting with the -NH_2 group of NH_2TMPA , the structures in Figure 8 (as well as LS-TMPA) were reoptimized with the F atoms on the top of the phenyl rings removed; however, this had little impact on the effects of H-bonding on vibrational frequencies/bond lengths in the LS complexes and HS vs LS energetics. Instances of an Fe–O bond strengthened by H-bonding have been observed in other LS systems involving intramolecular and intermolecular H-bonding to the peroxo bridging ligand.^{33,89,136} The enhanced donation into the unoccupied Fe d_z^2 orbital may therefore be key to the H-bond driving conversion to the LS state in the absence of a strong trans-axial donor (Figure 7). Considering recently published results highlighting the importance of Fe–O bond covalency in O–O cleavage chemistry,⁸⁹ these findings may present critical insight for predicting or understanding reactivity patterns.

Drawing from the findings above for NH_2TMPA , DFT was further applied to investigate why **HS**- $\text{NH}_2\text{CH}_3\text{TMPA}$ does not undergo SCO, even though it (1) contains an H-bonding substituent which has been suggested to interact with the peroxo core in **LS(DCHIm)**- $\text{NH}_2\text{CH}_3\text{TMPA}$ (based on UV-vis, rR, and DFT)⁹⁰ and (2) appears to bind THF solvent at lower temperatures. The lowest energy structure for the 5C complex (Figure 8C) exhibits a $\mu\text{-}\eta^2\text{:}\eta^1$ peroxo core, while end-on ($\mu\text{-}\eta^1\text{:}\eta^1$) binding is calculated to be higher in energy by 1.7 kcal/mol (G at -130°C). The -NH_2 group is H-bonded to the O_{Cu} atom, with an $\text{N(H)}\cdots\text{O}_{\text{Cu}}$ distance of 2.72 Å (compared to 2.89 Å for **HS**- NH_2TMPA). The Cu ligand geometry is square pyramidal ($\tau_5 = 0.16$), which minimizes steric clash with the porphyrin ring. This suggests that the H-bonding in the HS state is stronger with $\text{NH}_2\text{CH}_3\text{TMPA}$ than with NH_2TMPA due to changes in the Cu ligand structure required by sterics. Geometry optimization with a THF molecule added to bind axially to the HS Fe shows that THF binding is thermodynamically favorable ($G = -3.6$ kcal/mol at -130°C , lowest energy structure shown in Figure S20), with the $\mu\text{-}\eta^2\text{:}\eta^1$ peroxo core again found to be more stable than $\mu\text{-}\eta^1\text{:}\eta^1$ (by $G = 1.8$ kcal/mol). Similar to the optimized **HS**- $\text{NH}_2\text{CH}_3\text{TMPA}$, this **HS(THF)**- $\text{NH}_2\text{CH}_3\text{TMPA}$ structure has a short $\text{N(H)}\cdots\text{O}_{\text{Cu}}$ H-bond distance of 2.70 Å.

In contrast, optimization of a THF-bound LS complex, **LS(THF)**- $\text{NH}_2\text{CH}_3\text{TMPA}$, yields an end-on peroxo structure (Figure S20) that is 3.0 kcal/mol lower in G (at -130°C) than the **HS(THF)**- $\text{NH}_2\text{CH}_3\text{TMPA}$ complex. For comparison, the conversion of **HS(THF)**- NH_2TMPA to **LS(THF)**- NH_2TMPA is $G = -3.7$ kcal/mol. The -NH_2 group in **LS(THF)**- $\text{NH}_2\text{CH}_3\text{TMPA}$ is again H-bonded to the O_{Cu} atom, with an $\text{N(H)}\cdots\text{O}_{\text{Cu}}$ distance of 2.75 Å, nearly identical to the H-bond in the optimized structures for the LS complexes **LS(DCHIm)**- $\text{NH}_2\text{CH}_3\text{TMPA}$ (2.75 Å) and **LS(THF)**- NH_2TMPA (2.76 Å). This indicates that in the case of $\text{NH}_2\text{CH}_3\text{TMPA}$, the H-bonding interaction with the peroxo moiety is stronger in the HS state, which stabilizes the HS over the LS state, such that **HS**- $\text{NH}_2\text{CH}_3\text{TMPA}$ does not undergo SCO.

Finally, to understand the thermodynamics involved in the overall SCO process, the changes in ligation (5C to 6C(THF)), peroxide binding mode ($\mu\text{-}\eta^2\text{:}\eta^1$ to $\mu\text{-}\eta^1\text{:}\eta^1$), and spin state (HS to LS) of complexes with TMPA, CH_3TMPA , NH_2TMPA , and $\text{NH}_2\text{CH}_3\text{TMPA}$ ligands for the copper ion were systematically evaluated by DFT. Summarized in Table 3, the entropic cost in SCO (which is similar across the different Cu ligands) arises primarily from solvent binding, and to a lesser extent from spin change. The results suggest that NH_2TMPA is more

favorable than the other copper-ligands in SCO thermodynamics in the conversion from side-on to end-on peroxo binding, which is enthalpically favorable if a THF molecule is bound. The additional driving force with NH_2TMPA is attributable to the $\text{N(H)}\cdots\text{O}_{\text{Cu}}$ H-bond distance shortening from 2.89 to 2.79 Å (in contrast, the change in binding mode for $\text{HS(THF)-NH}_2\text{CH}_3\text{TMPA}$ causes the H-bond to elongate from 2.71 to 2.83 Å and is enthalpically uphill). Upon conversion from HS (end-on) to LS (end-on), the H-bond distance in the NH_2TMPA containing complex shortens further to 2.76 Å, supporting that the LS, solvent-bound structure is enthalpically most favorable for NH_2TMPA , and therefore dominant at low temperatures. In all, these results demonstrate that the SCO behavior observed with NH_2TMPA is the result of an H-bonding interaction enabled in the end-on peroxo core (but not the side-on), where the LS state is energetically favored over the HS when an axial ligand trans to the peroxo is available to bind.

CONCLUSION

While SCO is central to reactivity in numerous heme-based metalloenzymes, such behavior has scarcely been reported for discrete coordination complexes that are synthetically derived. Employing H-bond donor groups in the ligand scaffold contributes valuable insight into key factors in SCO. It is demonstrated that an H-bonding group in the second coordination sphere can facilitate SCO from HS to LS as temperature is lowered, even in the absence of a strong trans-axial donor ligand. This is ascribed to the H-bond stabilizing the LS state where the LS form is associated with an exceptionally strong Fe–O bond in the $\mu\text{-}\eta\text{:}\eta^1$ peroxy-bridged structure, based on rR spectroscopic data and DFT calculations. Furthermore, an analysis of the temperature and solvent dependence of the electronic and ^2H NMR spectra suggests that solvent binding occurs in the HS state prior to SCO as temperature is lowered and can even occur in HS complexes that do not undergo SCO. Analysis of solvent binding to a HS complex provided thermodynamic parameters for this process, which can be applied to decouple the thermodynamics of solvent binding and SCO. In all, the wealth of experimental data available using these derivatives of the TMPA ligand on the Cu ion present an excellent avenue to evaluate critical factors in SCO, reliably correlate experiment to computation, and provide insight into H-bonding and spin-state contributions to mechanism and reactivity.

EXPERIMENTAL SECTION

General.

All reagents and solvents purchased and used were of commercially available quality except as noted. MeTHF and THF were distilled over Na/benzophenone under Ar. The ligands XTMPA ($\text{X} = \text{H}, -\text{NH}_2, -\text{NH}_2\text{CH}_3, -\text{CH}_3$, and $-(\text{CH}_3)_2$) and complexes $[(\text{XTMPA})\text{Cu}^{\text{I}}]\text{BAR}^{\text{F}}$ ($\text{BAR}^{\text{F}} = \text{B}(\text{C}_6\text{F}_5)_4^-$), $(\text{F}_8)^{56}\text{Fe}^{\text{II}}$, $(\text{F}_8)^{57}\text{Fe}^{\text{II}}$, and $d_8\text{-F}_8\text{Fe}^{\text{II}}$ were synthesized as previously described.^{107,108,123,124,138–142} Preparation and handling of air-sensitive complexes were performed in a Vac atmosphere OMNI-LAB drybox or under argon atmosphere using standard Schlenk techniques. Solvent deoxygenation was achieved by bubbling Ar through the desired solvent for 45 min via an addition funnel connected to a receiving Schlenk flask. UV–vis measurements were carried out by using a Carey-50 Bio

spectrophotometer with a 10 mm path quartz cell. The spectrometer was equipped with Cary WinUV Scanning Kinetics software and a Unisoku thermostat cell holder for low-temperature experiments. All NMR spectra were recorded in 9 in., 5 mm o.d. NMR tubes on Bruker 300 NMR instrument equipped with a tunable deuterium probe to enhance deuterium detection. The ^2H chemical shifts are calibrated to natural abundance deuterium solvent peaks. Resonance Raman samples were excited at a variety of wavelengths (413 nm excitation unless otherwise noted with data), using either a Coherent 190C-K Kr^+ ion laser, a Coherent 25/7 Sabre Ar^+ ion laser, or an Ar^+ pumped Coherent Ti:Saph laser while the sample was immersed in a liquid nitrogen cooled (77 K) EPR finger Dewar (Wilmad). For data collection at temperatures between $-110\text{ }^\circ\text{C}$ and $-90\text{ }^\circ\text{C}$, the finger dewar was filled with various solvent/ N_2 cooling baths, using a thermocouple thermometer to monitor the temperature at sample point in situ. Power was $\sim 2\text{ mW}$ at the sample for the high-energy lines and $>200\text{ mW}$ at the low-energy lines. Data were recorded while rotating the sample to minimize photodecomposition. The spectra were recorded using a Spex 1877 CP triple monochromator with either a 600, 1200, or 2400 grooves/mm holographic spectrograph grating and detected by an Andor Newton CCD cooled to $-80\text{ }^\circ\text{C}$ (for high-energy excitation) or an Andor IDus CCD cooled to $-80\text{ }^\circ\text{C}$ (for the low-energy excitation). Spectra were calibrated on the energy axis to toluene. Excitation profiles were intensity calibrated to the solvent (MeTHF) by peak fitting in the program Origin. EPR spectra were collected with an ER 073 magnet equipped with a Bruker ER041 X-Band microwave bridge and a Bruker EMX 081 power supply: microwave frequency = 9.42 GHz, microwave power = 0.201 mW, attenuation = 30 dB, modulation amplitude = 10 G, modulation frequency = 100 kHz, temperature = 10 K.

Synthesis.

$^{54}\text{Fe}^{\text{II}}\text{Cl}_2$ and the novel compounds $(\text{F}_8\text{TPP})^{54}\text{Fe}^{\text{III}}\text{-Cl}$ and $(\text{F}_8\text{TPP})^{54}\text{Fe}^{\text{II}}$ were synthesized using the same literature methods by Karlin and co-workers to make the ^{57}Fe derivative,¹²³ with the only difference being that $^{54}\text{Fe}^{\text{II}}\text{Cl}_2$ was utilized in the metalation of F_8 .

$(\text{F}_8\text{TPP})^{54}\text{Fe}^{\text{III}}\text{-Cl}$.

UV-vis (CH_2Cl_2): 411, 503, 639, 772. ^1H NMR (CDCl_3): δ 80.1 (s, br, 8 H), 13.8 (s, 4 H), 12.5 (s, 4 H), 7.6 (s, 4 H). Calculated for $\text{C}_{44}\text{H}_{20}\text{ClF}_8\text{FeN}_4$: C, 62.32; H, 2.38; N, 6.61. Found: C, 62.44; H, 2.51; N 6.58.

$(\text{F}_8\text{TPP})^{54}\text{Fe}^{\text{II}}$.

UV-vis (MeTHF): 422, 542. ^1H NMR ($\text{THF}-d_8$): δ 56.1 (s, 8 H), 8.4 (s, 4H), 7.2 (s, 8H). Calculated for $\text{C}_{44}\text{H}_{20}\text{F}_8\text{FeN}_4$: C, 65.04; H, 2.48; N, 6.90. Found: C, 64.56; H, 2.83; N 6.87.

UV-vis.

Heme-peroxo-copper complexes were generated by taking a MeTHF solution or a 2:3 THF:MeTHF solution containing 3.0 mL of an equimolar (0.01 mM or 0.1 mM) mixture of $(\text{F}_8)\text{Fe}^{\text{II}}$ and $[(^X\text{TMPA})\text{Cu}^{\text{I}}]\text{BAR}^{\text{F}}$. quartz Schlenk cuvette that was prepared in a glovebox and sealed with a rubber septum before being cooled in the cryostat chamber to temperatures between $-90\text{ }^\circ\text{C}$ to $-130\text{ }^\circ\text{C}$. Dioxygen was bubbled through the solution until full formation

of the species was observed (30 s), that is, no further spectroscopic changes occurred. After dioxygen was added, vacuum/Ar bubbling cycles were performed to remove excess O₂.

Variable-temperature UV–vis spectroscopy measurements were replicated in three or four trials. The heme-peroxo-copper complexes were typically generated starting at –130 °C, followed by progressively warming up every 5 to 10 °C and waiting 20–30 min for the reaction mixture to come to temperature equilibrium. The relative concentrations of LS and HS species for the ^{NH}₂**TMPA** heme-peroxo-copper complex were determined at each temperature for the van't Hoff analysis in order to obtain thermodynamic parameters ΔS and ΔH to understand the SCO process. The van't Hoff analysis assumed that at –130 °C, 100% of the solution was LS with MeTHF coordinated (**LS(MeTHF)**), supported by no UV–vis spectral change when cooling to –135 °C and at –90 °C, 100% of the solution is HS that is side-on to the iron and end-on to the copper (HS). Using extinction coefficients at λ_{max} = 535 and 560 nm at these two temperatures (–130 and –90 °C), concentrations of **LS(MeTHF)** and **HS-NH₂TMPA** were calculated at each temperature (Table S2). The same calculations were done for the 2:3 THF:MeTHF solvent mixture (Table S3). These values were used to create the van't Hoff plots (Figure S3) with fitting done using the program Origin.

For reactivity studies, 1 equiv of Lewis base (e.g., 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), triethylphosphine oxide (TEPO), or proton sponge (PS)) was added to **LS-(MeTHF)-NH₂TMPA** at –130 °C. Over the course of 1 h, the 533 nm LS peak disappeared, and 559 nm HS peak grew in, indicating that there was an interaction between the H-bonded moiety and the Lewis base which reverted back to the original HS state.

Lewis bases were also added to the **HS-NH₂TMPA** complex at –90 °C and allowed to react for 1 h. Subsequently, the solution was cooled to –130 °C, and no UV–vis change was observed indicating that the interaction between the Lewis base successfully interfered with the H-bonds interaction with the O–O bond of the peroxo core thus preventing SCO.

EPR.

Heme-peroxo-copper complexes were generated by bubbling 5 mL of dioxygen through a MeTHF solution containing an equimolar (1 mM) mixture of (F₈)Fe^{II} and [(^XTMPA)Cu^I]BAR^F (total volume = 0.6 mL) at –90 °C (acetone/liquid N₂ bath) or –130 °C (pentane/liquid N₂ bath) in a 5 mm outer diameter quartz EPR tube (and the tube was frozen in N₂(liq)). In each case, after addition of dioxygen, the solution was bubbled with Ar for 60 s to remove excess O₂. After complexes were generated, tubes were frozen in N₂(liq), and all spectra were recorded at 10 K. All complexes were EPR silent (X-band) consistent with antiferromagnetic coupling between the iron and copper moieties.

²H NMR.

The heme-peroxo-copper complexes with ^X**TMPA** ligation were generated using the pyrrole-deuterated porphyrin, F₈-d₈,¹²⁴ and 1 equiv of the [(^XTMPA)Cu^I]BAR^F complex at 5.0 mM concentrations in MeTHF or in 2:3 THF:MeTHF with a total volume of 0.6 mL at –90 °C (acetone/liquid nitrogen bath) or at –130 °C (pentane/liq. N₂ bath) in a 9 in., 5 mm,

rubber septum-capped, NMR tube. Dioxygen was added to the reduced complexes via bubbling needle for 30 s, followed by bubbling with Ar for 30 s to remove ($X = -H, -NH_2, -NH_2CH_3, -CH_3$, and excess oxygen).¹¹¹ VT NMR studies were typically carried out starting at $-130\text{ }^{\circ}\text{C}$ and gradually warming up every $5\text{--}10\text{ }^{\circ}\text{C}$ ending at $-90\text{ }^{\circ}\text{C}$. At each new temperature, the solution was allowed to come to temperature equilibrium for 20 to 30 min before collecting spectrum, and each VT study was repeated at least three times. VT studies were also cooled back down from $-90\text{ }^{\circ}\text{C}$ to $-130\text{ }^{\circ}\text{C}$ showing that the NH_2 TMPA heme-peroxo-copper complex ability to undergo SCO is reversible. VT NMR spectra were taken of **HS-TMPA** as a control, in order to show that without a H-bonding moiety the NMR peak remains in the paramagnetic region.

Lewis bases were added to the **HS- NH_2 TMPA** complex at $-90\text{ }^{\circ}\text{C}$ and allowed to react for 1 h. Subsequently, the solution was cooled to $-120\text{ }^{\circ}\text{C}$ (where a distinct paramagnetic peak could be seen), and no chemical shift change was observed indicating that the interaction between the Lewis base successfully interfered with the H-bonds interaction with the O–O bond of the peroxo core thus preventing SCO.

A total of 6600–18944 scans were collected using a recycle delay of 0.01 s. Peak broadening was observed as the reaction mixture was cooled to $-130\text{ }^{\circ}\text{C}$ possibly due to a slower tumbling rate and a more viscous solution. The chemical shifts were referenced to the $-CH_3$ group of MeTHF, which is the most upfield field peak at 1.2 ppm. The HS (99 ppm) and LS (5 ppm) parent TMPA complexes were previously reported by Karlin and co-workers.¹¹¹ Some samples may have the previously reported (MeTHF)(F₈)Fe^{III}(O₂^{•-}) as a minor impurity and exhibits a signal 9.2 ppm.¹¹¹

Deconvolution analysis was performed using MNova NMR package from Mestrelab Research. To ensure the best fits, baseline correction on the spectra was first performed using either the multipoint correction method or the Whittaker smoother method. The peaks were then manually deconvoluted based on knowing at least the approximate chemical shift of the diamagnetic (MeTHF)-(F₈)Fe^{III}(O₂^{•-}) impurity.

rR.

The HS and LS complexes with X TMPA ligation were generated in the same fashion as EPR samples, above, at 1.0 mM concentrations with a total volume of 0.6 mL at $-90\text{ }^{\circ}\text{C}$ (acetone/liq. N₂ bath) in a 9 in., 5 mm, rubber septum-capped, NMR tube using either ¹⁶O₂ or ¹⁸O₂ and mixed by bubbling Ar. The tubes were then frozen and flame-sealed. Unless otherwise noted, spectra were obtained with spinning tubes at 77 K, using 413 nm excitation. For data collection at $-90\text{ }^{\circ}\text{C}$, $-93\text{ }^{\circ}\text{C}$, $-96\text{ }^{\circ}\text{C}$, and $-110\text{ }^{\circ}\text{C}$, sealed samples were first transferred to a solvent/liq. N₂ bath (solvent = acetone, methanol, ethanol, and *n*-pentane, respectively), allowed to equilibrate for 1 h, and then transferred to an EPR finger dewar filled with the same cooling bath for data acquisition, monitoring temperature at sample point with an Omega Engineering 650 thermocouple thermometer.

DFT Calculations.

DFT calculations were performed with the Gaussian 16 software package.¹⁴⁰ All calculations were done using the B3LYP* functional (15% HF exchange) including GD3BJ dispersion corrections, within the spin-unrestricted formalism and broken-symmetry singlet surface (for LS complexes). Geometry optimizations employed a split basis set as follows: 6-311g* for Fe/Cu/N/O; 6-31+ +g** for H-bonding H atoms, and 6-31g for all remaining atoms (H/C/F). Tight SCF convergence and an ultrafine integration grid were used as well as a PCM solvation model (THF solvent). Optimized structures yielded no imaginary frequencies. SCF energies were computed using single-point calculations with Def2-TZVP basis set on all atoms. Bonding and orbital analyses were obtained using NBO v3.1 contained within the Gaussian software. Thermodynamic parameters were taken from frequency calculations. Additional details and results using the B3LYP functional are given in the SI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

SCO in hemoglobin (A) or in cytochrome P450 (B). Myoglobin has been reported to have a spin-state temperature dependence (C).

Figure 2.

(A) UV-vis and (B) ²H-NMR spectra at -90 (blue) and -130 °C (red). **HS**-NH₂**TPMA** [(F₈)Fe^{III}-(O₂²⁻)-Cu^{II}(NH₂TPMA)]⁺ exhibits UV-vis absorbances and NMR resonances at 560 nm and 93.4 ppm, respectively. **LS(MeTHF)**-NH₂**TPMA** [(MeTHF)(F₈)Fe^{III}-(O₂²⁻)-Cu^{II}(NH₂TPMA)]⁺ shows resonances at 533/750 nm and 3.85 ppm. For (B), an asterisk denotes the ferric superoxo impurity [(MeTHF)(F₈)Fe^{III}(O₂^{•-})] at ~9.9 ppm that can be more easily seen at -90 °C (vs -130 °C) as previously reported.¹¹¹ Deconvolution analysis (see inset and the Supporting Information (SI) for extensive details) supports that

the diamagnetic range (i.e., the pyrrole deuterium signals) at $-130\text{ }^{\circ}\text{C}$ represents a mixture of the **LS(MeTHF)-^{NH₂}TPPA** peroxo complex at 3.85 ppm along with (previously reported)¹¹¹ ferric superoxo impurity at ~9.9 ppm.

Figure 3.

UV-vis spectra monitoring the addition of 1 equiv DBU to **LS(MeTHF)-NH₂TMPA** (red) at -130 °C in MeTHF and after allowing the reaction mixture to react for 1 h, **HS-NH₂TMPA** (blue) forms, evidenced by the red shift from 533 to 560 nm and the loss of the low-energy bands around 750–800 nm. See SI for complementary ²H NMR spectra showing the diamagnetic signature of **LS-(MeTHF)-NH₂TMPA** (red) shifts to the paramagnetic region ascribed to **HS-NH₂TMPA** (blue). Also, see the SI for data (UV-vis and NMR spectroscopy) associated with using PS and TEPO.

Figure 4.

UV-vis spectra at 0.1 mM for the NH_2TMPA heme-peroxo-copper complex in MeTHF at various cryogenic temperatures. Inset: Absorbances of the bands at 533 (LS complex) and 560 nm (HS-complex) at varying temperatures. This shows that two processes are occurring, one between -90 and -100 °C and a second one between -105 and -130 °C. No hysteresis in the data is observed upon warming and cooling.

Figure 5.

(A) VT 2H NMR spectra for the parent **HS-TMPA** in MeTHF, which remains paramagnetic even at very low temperatures. (B) VT 2H NMR spectra for the NH_2 **TMPA** heme-peroxo-copper complex in MeTHF. Mixtures of the HS and LS species are observed at -105 °C to -120 °C and only until cooling to the very low temperature of -130 °C is the solution completely LS (based on the absence of the paramagnetic peak). For (B), deconvolution analysis (see SI for details) supports that the diamagnetic range (i.e., the pyrrole deuterons) in the temperature range from -105 to -120 °C represents a mixture of the **LS(MeTHF)- NH_2 TMPA** peroxo complex along with (the previously reported)¹¹¹ ferric superoxo impurity $[(MeTHF)(F_8)Fe^{III}(O_2^{\bullet-})]$. Asterisks (*) denote this ferric superoxo impurity at 9.9 ppm in (A) and (B) in pure HS solutions. Double bars (||) denote a mixture of **LS(MeTHF)- NH_2 TMPA** and ferric impurity in diamagnetic region is observed. The SCO process for $[(F_8)Fe^{III}-(O_2^{2-})-Cu^{II}(NH_2TMPA)]^+$ is reversible with temperature, indicated by the arrows shown at the right. Also, see the text.

Figure 6.

UV-vis spectra at 0.1 mM for the NH_2TMPA heme-peroxo-copper complex in 2:3 THF:MeTHF at temperatures ranging from $-130\text{ }^{\circ}\text{C}$ to $-85\text{ }^{\circ}\text{C}$. Similar UV changes are observed as in Figure 4. Interestingly, in the 2:3 THF:MeTHF solution, the full **HS**- NH_2TMPA species forms at $-85\text{ }^{\circ}\text{C}$ (instead of $-90\text{ }^{\circ}\text{C}$ in pure MeTHF). Inset: Absorbances of the bands at 533 and 560 nm at varying temperatures. This shows that two processes are occurring, one between -90 and $-100\text{ }^{\circ}\text{C}$ and a second one between -105 and $-130\text{ }^{\circ}\text{C}$.

Figure 7.

Comparison of rR spectra of heme-peroxo-copper complexes, showing overlaid $^{16}\text{O}_2$ (dark lines) and $^{18}\text{O}_2$ (light lines) spectra (A, B, D, E), and $^{16}\text{O}_2$ - $^{18}\text{O}_2$ data for the HS (C) and LS (F) complexes. Difference spectra for **HS-CH₃TMPA** (green) and **HS-NH₂TMPA** (blue, data collected at 183 K) (C) and for **LS(MeTHF)-NH₂TMPA** (red, data collected at 77 K) and **LS-(DCHIm)-NH₂TMPA** (black) (F) show similarities and confirm spin states of the NH₂TMPA complex at 183 K vs 77 K. Data were collected with Soret excitation (413 nm), yielding strong enhancement of (isotope-insensitive) heme bands; the high similarity of

these bands for the two HS (A vs B) and the two LS (D vs E) complexes illustrates the structural similarity within each pair. Asterisks denote the $\nu(\text{Fe}-\text{O})$ of ferric superoxo impurity. Data collected at 77 K unless otherwise specified.

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Figure 8.

DFT-optimized structures for **HS-CH₃TMPA** (A), **HS-NH₂TMPA** (B), **HS-NH₂,CH₃TMPA** (C), **LS(THF)-NH₂TMPA** (D), and **LS(DCHIm)-NH₂TMPA** (E). Structural parameters are given in the Tables 2 and S8. τ_5 values are indications of Cu geometry, with $\tau_5 = 1$ being trigonal bipyramidal and $\tau_5 = 0$ indicative of square pyramidal geometry.¹³⁷

Scheme 1. Generation of HS Heme-Peroxo-Copper Complexes [(F₈)Fe^{III}-(O₂²⁻)-Cu^{II}(^XTMPA)]⁺ (HS-^XTMPA) at -90 °C in MeTHF^a

^aFormation of the analogous LS species at -90 °C LS-(DCHIm)-^XTMPA has been previously studied⁹⁰ (Pathway 1). This study explores the difference in spin state when the heme-peroxo-copper species is made at -90 °C (HS-^XTMPA) versus when it is prepared at -130 °C (LS(MeTHF)-^XTMPA (Pathway 2).

Scheme 2. Proposed Interaction of LS(MeTHF)-NH₂TMPA with a Lewis Base (here, DBU) Which Hinders Interaction between the H-Bonding Group (-NH₂) with the Peroxo O-O Bond^a

^aThe H-bond with the peroxo had facilitated the spin-state change from 5C HS to 6C LS; also, see the text.

Table 1.Resonance Raman Data for Heme-Peroxo-Copper Complexes^{a,b,c}

identity of Cu-ligand, L	isotope-sensitive bands and heme marker bands ¹⁶ O ₂ (cm ⁻¹)		
	HS	LS (MeTHF) ^d	LS (DCHIm) ^e
TMPA	$\nu(\text{O-O}) = 804$	N/A	$\nu(\text{O-O}) = 812$
	$\nu(\text{Fe-O}) = 538$		$\nu(\text{Fe-O}) = 623$
	$\nu(\text{Cu-O}) = 515$		$\nu(\text{Cu-O}) = 535$
	$\nu_2; \nu_4 = 1558; 1360$		$\nu_2; \nu_4 = 1572; 1364$
NH₂TMPA	$\nu(\text{O-O}) = 806$	$\nu(\text{O-O}) = 784$	$\nu(\text{O-O}) = 775$
	$\nu(\text{Fe-O}) = 526$	$\nu(\text{Fe-O}) = 632$	$\nu(\text{Fe-O}) = 625$
	$\nu(\text{Cu-O}) = 475$	$\nu(\text{Cu-O}) = 537$	$\nu(\text{Cu-O}) = 533$
	$\nu_2; \nu_4 = 1558; 1361$	$\nu_2; \nu_4 = 1571; 1365$	$\nu_2; \nu_4 = 1570; 1364$
CH₃TMPA	$\nu(\text{O-O}) = 807$	N/A	$\nu(\text{O-O}) = 789$
	$\nu(\text{Fe-O}) = 527$		$\nu(\text{Fe-O}) = 612$
	$\nu(\text{Cu-O}) = 476$		$\nu(\text{Cu-O}) = 525$
	$\nu_1; \nu_4 = 1559; 1361$		$\nu_2; \nu_4 = 1570; 1363$
NH₂,CH₃TMPA	$\nu(\text{O-O}) = 786$	N/A	$\nu(\text{O-O}) = 765$
	$\nu(\text{Fe-O}) = 567$		$\nu(\text{Fe-O}) = 610$
	$\nu(\text{Cu-O}) = 445$		$\nu(\text{Cu-O}) = 510$
	$\nu_2; \nu_4 = 1560; 1361$		$\nu_2; \nu_4 = 1570; 1367$
(CH₃)₂TMPA	$\nu(\text{O-O}) = 789$	N/A	$\nu(\text{O-O}) = 776$
	$\nu(\text{Fe-O}) = 556$		$\nu(\text{Fe-O}) = 593$
	$\nu(\text{Cu-O}) = 456$		$\nu(\text{Cu-O}) = 501$
	$\nu_2; \nu_4 = 1560; 1361$		$\nu_2; \nu_4 = 1570; 1363$

^a All data collected at -196 °C except **HS-NH₂TMPA**, which was at -90 °C.^b All complexes are EPR-silent (perpendicular mode) collected at 10 K, consistent with being of integer spin electronic spin state and antiferromagnetic coupling of the Fe and Cu through the peroxo-bridge.^c Please refer to the Experimental Section or SI for additional details; such as (¹⁶O₂-¹⁸O₂) values and for the raw rR spectra of the **HS-TMPA**, **HS-NH₂,CH₃TMPA**, and **HS-(CH₃)₂TMPA** complexes.^d _{LS(THF)}: $\nu(\text{O-O}) = 782 \text{ cm}^{-1}$, $\nu(\text{Fe-O}) = 635 \text{ cm}^{-1}$, $\nu(\text{Cu-O}) = 537 \text{ cm}^{-1}$.^e Ref 90.

Table 2.

Structural Parameters from DFT-Optimized Structures in Figure 8

complex	O-O (Å)	Fe-O (Å)	Cu-O (Å)	Fe-Cu (Å)	N(H)···O (Å)	angle of N-H-O °
HS-CH₃TMPA (A)	1.45	1.93 2.03	1.88	3.8	N/A	
HS-NH₂TMPA (B)	1.46	1.93 2.02	1.87	3.8	2.89 [156.8]	
HS-NH₂CH₃TMPA (C)	1.45	1.95 2.05	1.92	3.73	2.72 [148.7]	
LS(THF)₂-NH₂TMPA (D)	1.41	1.77	1.89	4.29	2.76 [162.3]	
LS(DCHIm)-NH₂TMPA (E)	1.41	1.80	1.89	4.30	2.78 [161.5]	

Calculated Thermodynamic Parameters for SCO from HS (with $\mu\eta^2:\eta^1$ Peroxide Binding) to LS ($\mu\eta^1:\eta^1$, with THF Solvent Bound)

Table 3.

geometric/electronic change	calculated thermodynamics H (kcal/mol) [ΔS] [cal/(mol K)]			
	XTMPAX = H	X = CH ₃	X = NH ₂	X = NH ₂ , CH ₃
5C HS $\rightarrow$ 6C HS ($\mu\eta^2:\eta^1$, binding THF)	-10.0 [-46.3]	-10.5 [-38.9]	-11.5 [-42.4]	-10.0 [-45.2]
side-on $\rightarrow$ end-on (6C HS)	-1.1 [+3.0]	-0.3 [-2.1]	-1.6 [-0.1]	+1.4 [+5.5]
6C HS (end-on) $\rightarrow$ 6C LS	-4.3 [-8.2]	-2.5 [-17.9]	-3.7 [-11.3]	-5.1 [-10.3]
total (5C HS $\rightarrow$ 6C LS)	-15.4 [-51.6]	-13.4 [-58.9]	-16.8 [-53.7]	-13.7 [-49.9]
5C HS $\rightarrow$ 5C LS	-1.7 [-8.6]	+0.1 [-7.8]	-2.6 [-6.2]	+2.9 [-3.7]