

Thermodynamics of Proton-Coupled Electron Transfer at Tricopper μ -Oxo/Hydroxo/Aqua Complexes

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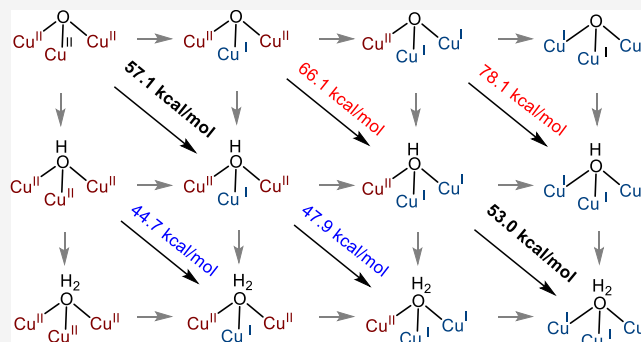


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ABSTRACT: Multicopper oxidases (MCOs) utilize a tricopper active site to reduce dioxygen to water through $4\text{H}^+ 4\text{e}^-$ proton-coupled electron transfer (PCET). Understanding the thermodynamics of PCET at a tricopper cluster is essential for elucidating how MCOs harness the oxidative power of O_2 while mitigating oxidative damage. In this study, we determined the O–H bond dissociation free energies (BDFEs) and pK_a values of a series of tricopper hydroxo and tricopper aqua complexes as synthetic models of the tricopper site in MCOs. Tricopper intermediates on the path of alternating electron and proton transfer (ET-PT-ET-PT-ET) have modest BDFE(O–H) values in the range of 53.0–57.1 kcal/mol. In contrast, those not on the path of ET-PT-ET-PT-ET display much higher (78.1 kcal/mol) or lower (44.7 kcal/mol) BDFE(O–H) values. Additionally, the pK_a of bridging OH and OH_2 motifs increase by 8–16 pK_a units per oxidation state. The same oxidation state changes have a lesser impact on the pK_a of N–H motif in the secondary coordination sphere, with an increase of ca. 5 pK_a units per oxidation state. The steeper pK_a increase of the tricopper center promotes proton transfer from the secondary coordination sphere. Overall, our study shed light on the PCET pathway least prone to decomposition, elucidating why tricopper centers are an optimal choice for promoting efficient oxygen reduction reaction.



INTRODUCTION

Multicopper oxidases (MCOs) are an important class of enzymes found in various organisms, playing crucial roles in diverse biological processes, such as copper homeostasis, electron transfer, and lignin degradation.^{1–4} MCOs couple the oxidation of metal or organic substrates with the four-electron reduction of O_2 to H_2O . This process is carried out with high efficiency without producing reactive oxygen species (ROS) or causing oxidative damage to the protein structure. Understanding the intricate dioxygen reduction at MCOs is of great significance in unveiling how nature harnesses the oxidative power of O_2 .^{4–8}

MCOs contain at least four copper ions: one type 1 (T1) electron-transfer site and a trinuclear copper active site (TNC) with secondary carboxylate motifs (Figure 1A).^{9,10} Dioxygen reduction at MCOs involves the activation of O_2 at fully reduced TNC, followed by $4\text{H}^+ 4\text{e}^-$ proton-coupled electron transfer (PCET) to regenerate the $\text{Cu}(\text{I},\text{I},\text{I})$ state (Figure 1B). The T1 site and secondary carboxylate groups enable well-coordinated PCET during the oxygen reduction reaction (ORR).^{10–15}

A crucial aspect of the MCOs yet to be fully elucidated is the $4\text{H}^+ 4\text{e}^-$ PCET (Figure 1B). Out of the 25 possible tricopper species (five oxidation states and five protonation states), only five have been characterized through spectroscopy: fully reduced (FR), resting oxidized (RO), native intermediate (NI),^{6,16} peroxy intermediate (PI),¹² and alternative resting (AR).¹³

Furthermore, the thermodynamics of PCET, such as pK_a , redox potentials, and O–H bond dissociation free energies (BDFEs), remain elusive. The difficulty in measuring these values within the protein systems motivates us to study these fundamental properties using synthetic tricopper model complexes.

Recently, we reported a tricopper complex that can access all four oxidation states from $\text{Cu}^{\text{II}}\text{Cu}^{\text{II}}\text{Cu}^{\text{II}}$ to $\text{Cu}^{\text{I}}\text{Cu}^{\text{I}}\text{Cu}^{\text{I}}$.^{8,17} In contrast to previous synthetic models of MCOs,^{15,18–23} this tricopper cluster is embedded in a macrocyclic TREN_4 [$\text{TREN} = \text{tris}(2\text{-aminoethyl})\text{amine}$] ligand that provides a rigid coordination environment, reducing the reorganization energy of electron transfer (Figure 1C). Furthermore, we demonstrated a $2\text{H}^+ 3\text{e}^-$ PCET from $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ to $[\text{LHCu}_3(\text{II},\text{II},\text{II})(\text{O})]^{5+}$, where L represents the TREN_4 ligand and LH represents the protonated TREN_4 ligand. Seven intermediates were characterized to mimic the reductive regeneration of the fully reduced trinuclear copper cluster (FR) from the resting oxidized state (RO) in MCOs.²⁴

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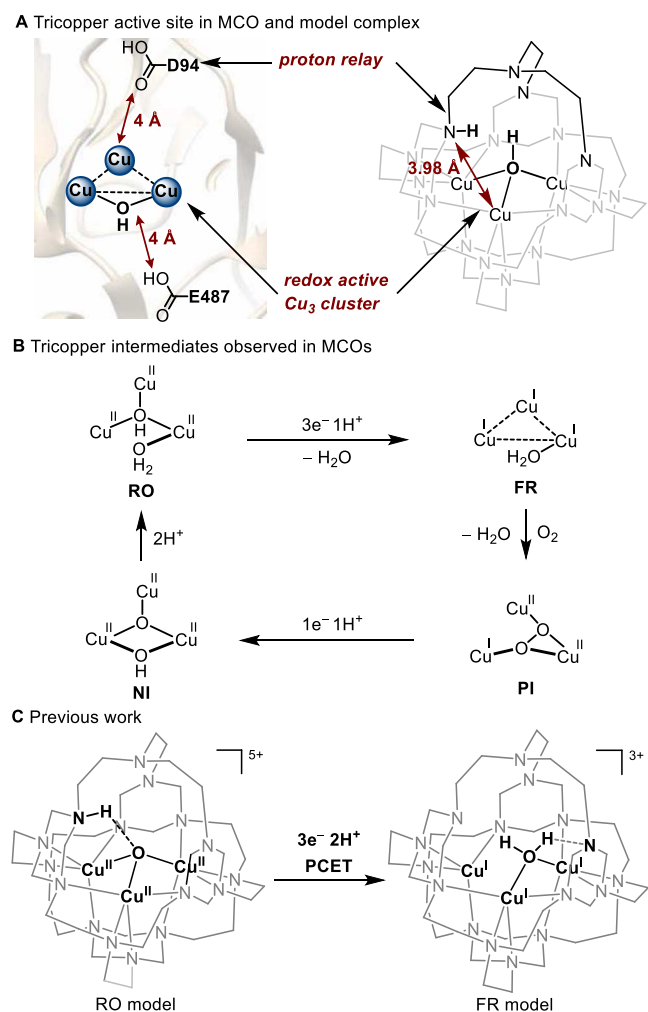


Figure 1. (A) Tricopper active site and (B) observed tricopper intermediates in MCOs. (C) Previous work on modeling the PCET at MCOs with the full encapsulated tricopper complex.^{8,24}

Importantly, a proton can transfer between the top TREN motif in the secondary coordination sphere and the tricopper center (Figure 1C). This intramolecular proton transfer is driven

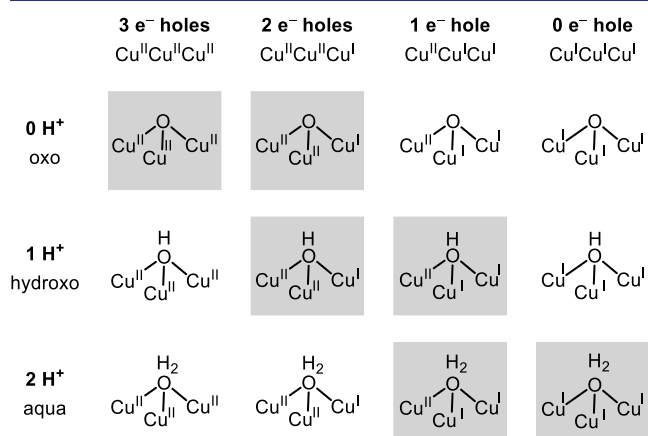


Figure 2. Twelve possible tricopper intermediates for the three-electron two-proton PCET from Cu₃(II,II,II) oxo to Cu₃(I,I,I) aqua species. The gray boxes highlight the ideal path of alternating electron and proton transfer ET-PT-ET-PT-ET.

by the basicity of the tricopper hydroxo/oxo center vs tertiary nitrogen motifs in the secondary coordination sphere, playing an important role in leveling the redox potentials from tricopper-(II,II,II) to tricopper-(I,I,I).^{8,17}

The BDFE(O–H) and pK_a of the copper oxo/hydroxo/aqua complexes can reveal the reaction landscapes of copper oxygen chemistry. Despite their significance, the BDFE(O–H) values of synthetic models of copper active sites have only been sparsely reported (Figure 3),^{25–29} and no BDFE(O–H) of a tricopper complex has been measured.

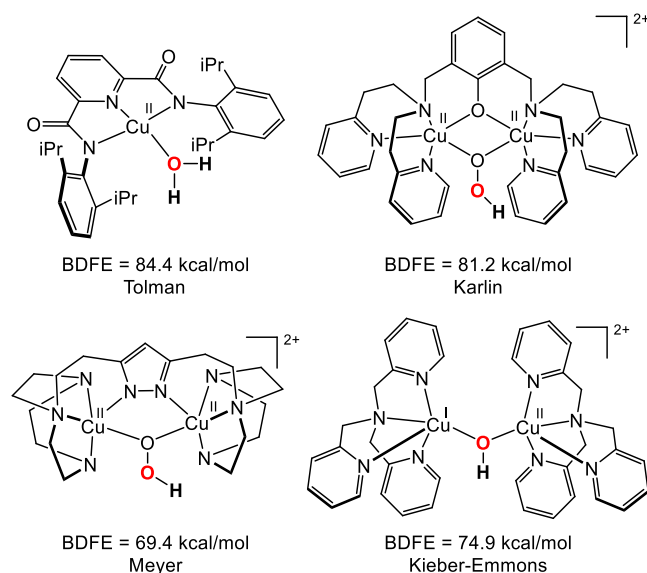


Figure 3. Previous examples of O–H BDFE measurements for mono- and dicopper model complexes.

Determining the BDFE and pK_a of the O–H motifs in a TREN₄ tricopper complex presented a challenge, since the O–H protons are nested in a cage-like structure of the TREN₄ ligand. The N–H/N groups in the secondary coordination sphere complicate the pK_a titration of OH₂/OH/O groups. Due to the relatively lower steric hindrance of the N–H groups, treatment of the tricopper cluster with an acid/base often results in the protonation/deprotonation of the N/N–H group first.

Herein, we report a comprehensive analysis of BDFE and pK_a values of these tricopper hydroxo/aqua model complexes using Mayer's open-circuit potential (OCP) method. We found that the tricopper complexes on the ideal path of alternating electron and proton transfer (ET-PT-ET-PT-ET) have modest BDFE values around 53.0–57.1 kcal/mol (Figure 2, highlighted in gray). In contrast, those tricopper complexes off this ideal path have either high (78.1 kcal/mol) or lower (44.7 kcal/mol) BDFE(O–H) values. The pK_a(O–H) and pK_a(N–H) at different tricopper oxidation states suggest increasing driving forces of proton transfer from the secondary coordination sphere as the tricopper center is reduced from Cu₃(II,II,II) to Cu₃(I,I,I). We discuss the implications of these BDFE and pK_a values in the context of four-electron oxygen reduction at biological tricopper centers.

RESULTS AND DISCUSSION

BDFE(O–H) of Tricopper μ–OH₂ Complexes. In our previous study, we reported the synthesis and characterization of a series of tricopper model complexes at different oxidation and protonation states.^{8,24} Building on this work, we attempt to

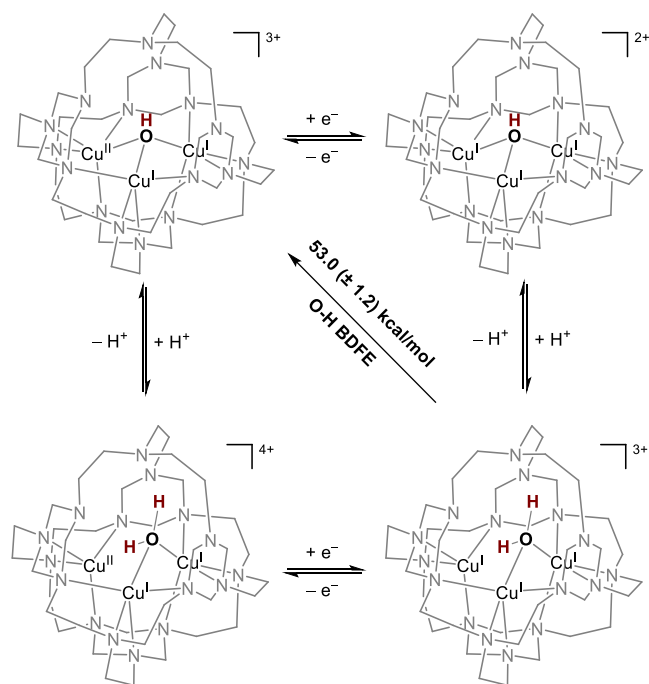
determine the BDFE of O–H and N–H motifs using the Bordwell equation.^{30,31}

$$\text{BDFE}(\text{X} - \text{H}) = 1.37\text{p}K_{\text{a}} + 23.06E^{\circ} + C_{\text{g}} \quad (1)$$

In this equation, $\text{p}K_{\text{a}}$ represents the acid dissociation constant of the acidic proton in the X–H bond, E° represents the standard reduction potential of the redox couple associated with the X–H bond, and C_{g} is a constant that relates to the reduction potential of $\text{H}^+/\text{H}^{\bullet}$ in the solvent of interest.

This method of BDFE analysis is most appropriate for systems with at least three stable species in a square scheme (Scheme 1),

Scheme 1. PCET Square Scheme That Connects Complexes $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ and $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$



allowing for experimental determination of $E_{1/2}$ and $\text{p}K_{\text{a}}$. Although many tricopper intermediates were isolated, we could not measure the $\text{p}K_{\text{a}}$ of O–H and N–H groups using standard acid–base titration because the O–H and N–H motifs are buried inside the cage-like ligand construct. Treatment of the tricopper cluster with an acid or base often leads to disproportionation reactions.

Recent work by Mayer et al. demonstrated an alternative approach to determine the BDFE of the X–H bond using electrochemical OCP measurements.^{32–34} The BDFE of X–H bonds was determined by measuring the OCP of solutions containing varying ratios of reduced and oxidized compounds in a buffer. The purpose of the buffer is to minimize the effects of homoconjugation on the proton activity of the solution and prevent deviations from the expected behavior described by the Nernst equation^{35,36}

$$E = E^{\circ} - \frac{0.0592}{n} \log \frac{[\text{XH}_n][\text{A}^-]^n}{[\text{X}][\text{HA}]^n} - 0.059\text{p}K_{\text{a}} \quad (2)$$

where n represents the number of proton–electron pairs transferred, $[\text{XH}_n]$ and $[\text{X}]$ represent the concentrations of the reduced and oxidized partners, and $[\text{HA}]$ and $[\text{A}^-]$ are the

concentrations of the buffer system; $\text{p}K_{\text{a}}$ is the acid dissociation constant of the buffer used.

Accordingly, the OCP was collected for various ratios of $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}/[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$ in the presence of pyridinium/pyridine buffer ($\text{p}K_{\text{a}} = 12.53$) in acetonitrile. $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ and $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$ were prepared according to literature procedures.^{8,24} These two tricopper clusters are related by the square scheme in Scheme 1. By referencing the potentials measured against the H^+/H_2 couple, the BDFE(O–H) of the tricopper(I,I,I) aqua moiety can be calculated using eq 3

$$\begin{aligned} \text{BDFE}(\text{X} - \text{H}) = & 23.06E_{\text{OCP}}^{\circ}(\text{V vs } \text{H}^+/\text{H}_2) \\ & + \Delta G^{\circ}\left(\frac{1}{2}\text{H}_2(\text{g})/\text{H}_{\text{IM}}\right) \end{aligned} \quad (3)$$

where E_{OCP}° is the OCP measured for a 1:1 mixture of a reduced and oxidized cluster and ΔG° is a constant related to the free energy of homolytic cleavage of H_2 in acetonitrile ($\Delta G^{\circ} = 52.0$ kcal/mol).³² From this calculation, we obtained an BDFE(O–H) of $53.0 (\pm 1.2)$ kcal/mol for $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ in acetonitrile (see Supporting Information, Figure S2).

To further corroborate the $53.0 (\pm 1.2)$ kcal/mol BDFE(O–H) value, we conducted a reactivity study of $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ with various hydrogen atom-accepting reagents in acetonitrile (Figure 4). All HAT reactions were performed for an

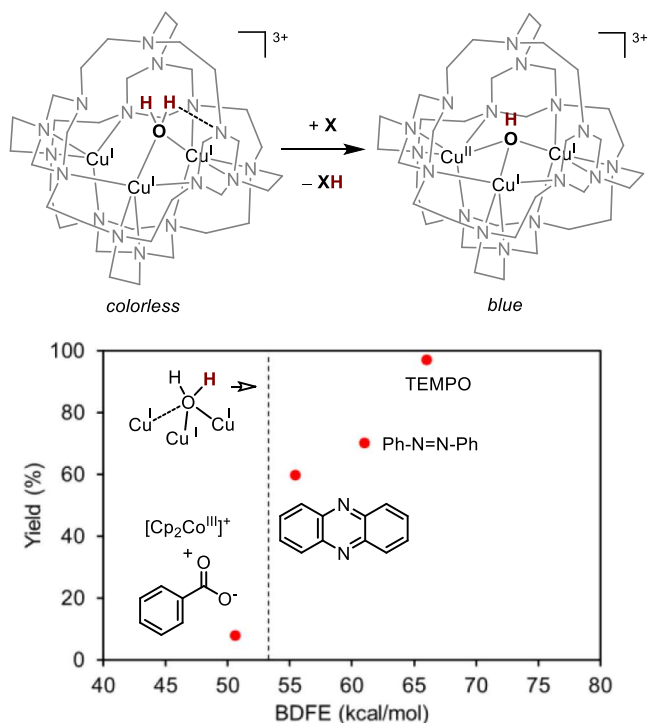


Figure 4. Yields of HAT reaction from $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ to various hydrogen atom acceptors. The dashed line denotes 53.0 kcal/mol, the experimentally determined BDFE of the $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ complex.

extended period of 16 h to ensure that they had reached equilibria (see Supporting Information). Treatment of $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ with one equivalent of TEMPO [BDFE(O–H) of TEMPOH = 66 kcal/mol] at 20 °C leads to a distinct color change from colorless to blue, corresponding to the oxidized cluster $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$. The absorption feature

at 790 nm ($840 \text{ M}^{-1} \text{ cm}^{-1}$) was used to determine the yield as 97%. The formation of TEMPO–H was confirmed by ^1H NMR analysis in 91% yield. Similarly, the reaction of $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ with azobenzene leads to the formation of 1,2-diphenylhydrazine [$\text{BDFE}(\text{N}-\text{H}) = 61 \text{ kcal/mol}$] and the oxidized cluster $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$ in 71% yield. The formation of 1,2-diphenylhydrazine was confirmed by ^1H NMR analysis in 66% yield. Moreover, the reaction of the reduced cluster with phenazine produced the oxidized cluster in 60% yield, indicating that the BDFE of $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ is close to 58 kcal/mol. The formation of a 5,10-dihydrophenazine product [$\text{BDFE}(\text{O}-\text{H}) = 58.7 \text{ kcal/mol}$] was confirmed by ^1H NMR analysis in 63% yield. Finally, we investigated the reactivity of $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ toward the reduction of cobaltocenium hexafluorophosphate ($\text{Cp}_2\text{Co}^{\text{III}}\text{PF}_6$, $E_{1/2} = -1.34$) in the presence of sodium benzoate ($\text{pK}_a = 21.51$) as a base. The combination of these two reagents affords an effective BDFE of 50.6 kcal/mol.^{37,38} As the BDFE(O–H) value of $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ is higher than 50.6 kcal/mol, we did not observe a significant reduction of $\text{Cp}_2\text{Co}^{\text{III}}\text{PF}_6$ (8%) after 16 h. These reactivity studies of $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$ provide additional support to a BDFE(O–H) value of 53.0 kcal/mol.

Redox Potentials of Tricopper μ -OH₂ and μ -OH Complexes. The redox potentials ($E_{1/2}$) of the tricopper cluster were determined by solution cyclic voltammetry (CV) studies. Previously, we reported the CV of tricopper clusters at one-proton and two-proton levels.^{8,24} The intramolecular proton transfer between the tricopper cluster and the secondary coordination sphere N/N–H motif makes certain redox events irreversible. Nevertheless, at high scan rates or at low temperatures, the reversibility improves. This method allowed us to measure seven $E_{1/2}$ values in the PCET square schemes (Table 1; see Supporting Information for details).

Table 1. Summary of Redox Potentials of Tricopper Clusters

	$E_{1/2}^a$ (V)	method	refs
$[\text{LHCu}_3\text{O}]^{5+/4+}$	−0.66	CV at high scan rate	ref 24
$[\text{LHCu}_3\text{O}]^{4+/3+}$	−1.18 ^b	CV at −40°C	this work
$[\text{LCu}_3\text{OH}]^{5+/4+}$	−0.13	CV at −40°C	ref 24
$[\text{LCu}_3\text{OH}]^{4+/3+}$	−0.52	CV at RT	ref 24
$[\text{LCu}_3\text{OH}]^{3+/2+}$	−0.98	CV at RT	ref 24
$[\text{LCu}_3\text{OH}_2]^{4+/3+}$	−0.30 ^b	CV at −40°C	this work
$[\text{LCu}_3\text{OH}_2]^{5+/4+}$	0.01 ^b	CV at −40°C	this work
$[\text{LHCu}_3\text{OH}]^{4+/3+}$	−0.66	CV at RT	ref 24
$[\text{LHCu}_3\text{OH}]^{5+/4+}$	−0.27	CV at RT	ref 24
$[\text{LHCu}_3\text{OH}]^{6+/5+}$	0.17	CV at RT	ref 24

^aReferenced to Fc^+/Fc . ^bIrreversible redox couples. The $E_{1/2}$ values are estimated using the method of inflection potentials.^{39,40}

In this study, we performed additional low-temperature CV studies to estimate the $E_{1/2}$ of three redox couples, namely, $[\text{LHCu}_3\text{O}]^{4+/3+}$, $[\text{LCu}_3\text{OH}_2]^{5+/4+}$, and $[\text{LCu}_3\text{OH}_2]^{4+/3+}$. We prepared the CV sample using the copper clusters that have the correct “protonation state”, cooled the CV sample down to −40 °C to slow down the intramolecular transfer, and then measured their redox potential using CV. For redox couples that do not exhibit reversible CV features, we estimated the $E_{1/2}$ values using the inflection potentials.^{39,40} The resulting redox potentials are summarized in Table 1.

These redox potential values, along with the BDFE(O–H) value of $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$, allowed us to calculate the BDFE(O–H) at other oxidation states using the Bordwell

equation and the redox potentials of clusters (Figure 5A). Additionally, we were able to determine the pK_a values of three tricopper aqua complexes as −3.57 for $[\text{LCu}_3(\text{II},\text{II},\text{I})(\text{OH}_2)]^{5+}$, 5.34 for $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH}_2)]^{4+}$, and 16.8 for $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$, respectively. The basicity of the O–H group increases by 8–11 pK_a units per oxidation state (Figure 5A), providing significant driving forces for intramolecular proton transfer from the secondary coordination sphere as the tricopper center is reduced.

O–H BDFE of Tricopper μ -OH Complexes. Similarly, the BDFE(O–H) of $[\text{LHCu}_3(\text{II},\text{II},\text{I})(\text{OH})]^{5+}$ was determined by OCP measurement of various ratios of $[\text{LHCu}_3(\text{II},\text{II},\text{I})(\text{OH})]^{5+}$ and $[\text{LHCu}_3(\text{II},\text{II},\text{I})(\text{O})]^{5+}$ (Scheme 2) in the presence of 2,4-dinitrobenzenesulfonic acid/2,4-dinitrobenzenesulfonic acid potassium salt buffer ($\text{pK}_a = 3.96$) in acetonitrile. These two tricopper clusters were prepared according to previously reported procedures.^{8,24} Using eq 3, we obtained a BDFE(O–H) for $[\text{LHCu}_3(\text{II},\text{II},\text{I})(\text{OH})]^{5+}$ of 57.1 (± 1.11) kcal/mol in acetonitrile (see Supporting Information, Figure S4). Analogously, this BDFE(O–H) value enabled us to calculate the BDFE(O–H) and $\text{pK}_a(\text{O}-\text{H})$ of the adjacent square schemes using the Bordwell equation (Figure 5B). With each oxidation state change, the basicity of the OH group in tricopper μ -oxo/hydroxo complexes increases by 14–16 pK_a units. This substantial increase in basicity provides the driving force for intramolecular proton transfer from the secondary coordination sphere.

BDFE of N–H Bonds in the Secondary Coordination Sphere. After establishing the BDFE(O–H) of tricopper oxo/hydroxo/aqua species at different oxidation states, we sought to investigate the BDFE(N–H) of the secondary coordination sphere (Figure 5C). Previously, using CV simulation of $[\text{LCu}_3(\text{I},\text{I},\text{I})(\text{OH}_2)]^{3+}$, we estimated the K_{eq} of intramolecular proton transfer between $[\text{LHCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{4+}$ and $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH}_2)]^{4+}$ as 6.5×10^{-6} (Figure 6A).²⁴ Given a $\text{pK}_a(\text{O}-\text{H})$ value of $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH}_2)]^{4+}$ of 5.34 (± 1.21) (Figure 5A), we can estimate the $\text{pK}_a(\text{N}-\text{H})$ of $[\text{LHCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{4+}$ in the range between 8.45 and 11.37 (Figure 6A).

To further validate this $\text{pK}_a(\text{N}-\text{H})$ estimation, we treated $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$ with acids of various strengths and monitored its conversion to $[\text{LHCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{4+}$ (Figure 6B). The formation of $[\text{LHCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{4+}$ can be confirmed using its redox feature at −0.27 V vs Fc^+/Fc .^{8,24} We found that the treatment of $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$ with one equivalent of trichloroacetic acid ($\text{pK}_a = 10.75$) in acetonitrile leads to the formation of $[\text{LHCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{4+}$, as indicated by the cyclic voltammogram (see Supporting Information, Figure S19).^{8,24} In contrast, treatment of $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$ with pyridinium tetrafluoroborate ($\text{pK}_a = 12.53$) in acetonitrile with $[\text{LCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$ does not result in any changes in the voltammogram, indicating that the $\text{pK}_a(\text{N}-\text{H})$ of $[\text{LHCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$ is lower than 12.53 (see Supporting Information, Figure S19). These studies are consistent with the $\text{pK}_a(\text{N}-\text{H})$ between 8.45 and 11.37 estimated by CV simulation.

Using a $\text{pK}_a(\text{N}-\text{H})$ value for $[\text{LHCu}_3(\text{II},\text{I},\text{I})(\text{OH})]^{3+}$, the BDFE(N–H) and $\text{pK}_a(\text{N}-\text{H})$ of the secondary sphere N–H bond at different oxidation states can be calculated using the Bordwell equation. The results are summarized in Figure 5C.

DISCUSSIONS AND CONCLUSIONS

The ability of MCOs to catalyze ORR while avoiding oxidative damage highlights the importance of understanding the

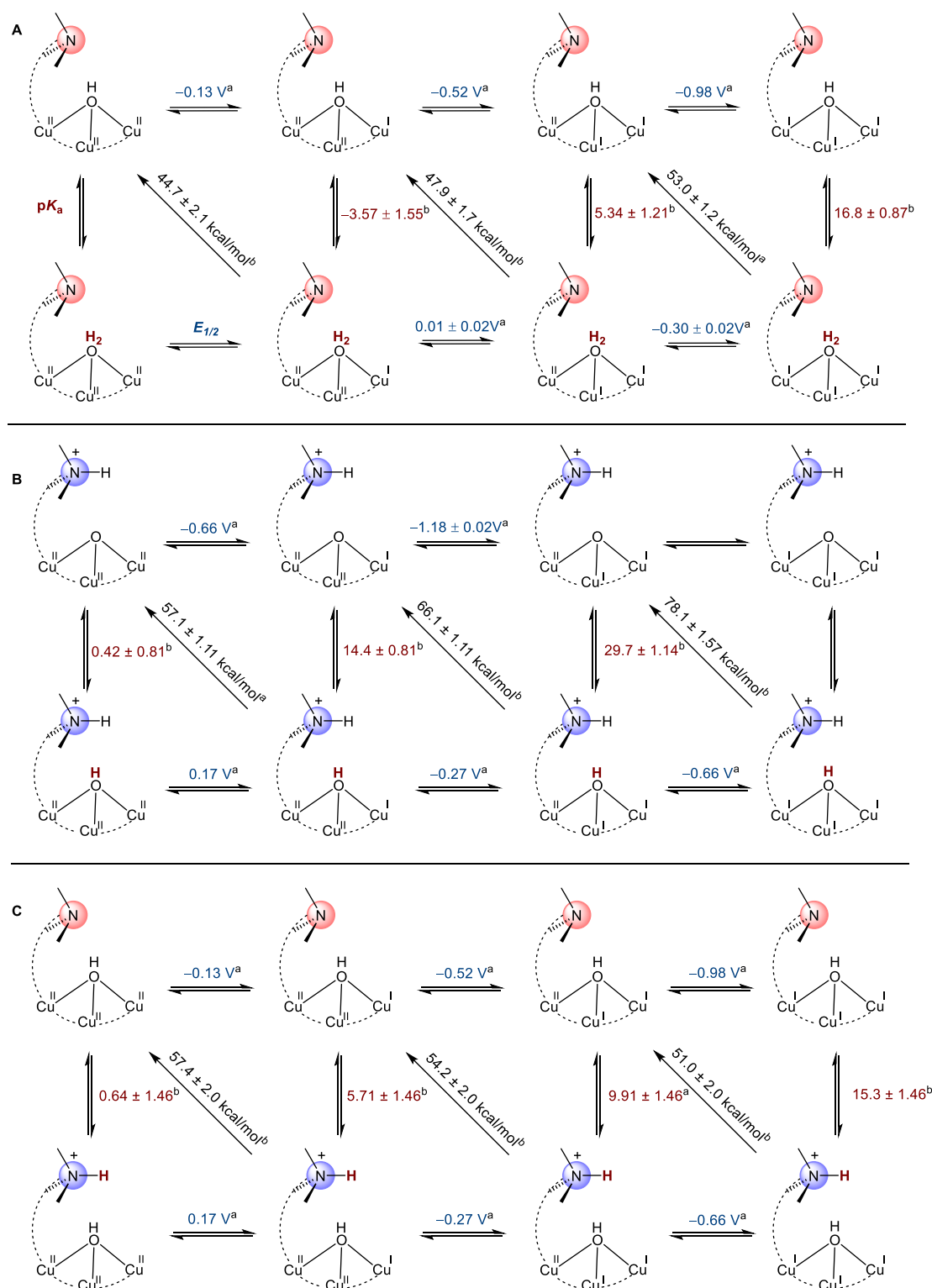


Figure 5. Summary of the BDFE(O–H) (black), pK_a values (red), and redox potentials (blue, vs Fc^+/Fc) at different oxidation states. The TREN_4 ligand is omitted for clarity. The tertiary nitrogen above the cluster indicates the proton storage site in the secondary coordination sphere.^{8,24} (A) PCET at the tricopper complex from $\text{LCu}_3(\text{II},\text{II},\text{II})\text{OH}$ to $\text{LCu}_3(\text{I},\text{I},\text{I})\text{OH}_2$, with tertiary nitrogen being deprotonated. (B) PCET at the tricopper complex from $\text{LHCu}_3(\text{II},\text{II},\text{II})\text{O}$ to $\text{LHCu}_3(\text{I},\text{I},\text{I})\text{OH}$ with tertiary nitrogen being protonated. (C) Multisite PCET at the tricopper complex from $\text{LCu}_3(\text{II},\text{II},\text{II})\text{OH}$ to $\text{LHCu}_3(\text{I},\text{I},\text{I})\text{OH}$, with electron transfer at the tricopper center and proton transfer at tertiary nitrogen at the secondary coordination sphere. ^aValues obtained from direct experimental measurements. ^bValues calculated using the Bordwell equation. The relative uncertainties derived from OCP measurement and estimation of $E_{1/2}$ have been extended to all the calculated values of BDFE and pK_a .

mechanisms of MCOs at the molecular level. Despite the simplicity of the tricopper model complexes, they provide insights into PCET conversion of $\text{Cu}_3(\text{II},\text{II},\text{II})\text{O}$ to $\text{Cu}_3(\text{I},\text{I},\text{I})\text{OH}_2$.

This process mimics the reductive regeneration of RO to FR in MCOs. The top TREN motif, which is ca. 3.98 Å away from the tricopper cluster, serves as a pendant proton relay,

Scheme 2. PCET Square Scheme That Connects Complexes $[\text{LHCu}_3(\text{II,II,I})(\text{OH})]^{5+}$ and $[\text{LCu}_3(\text{II,II,II})(\text{O})]^{3+}$

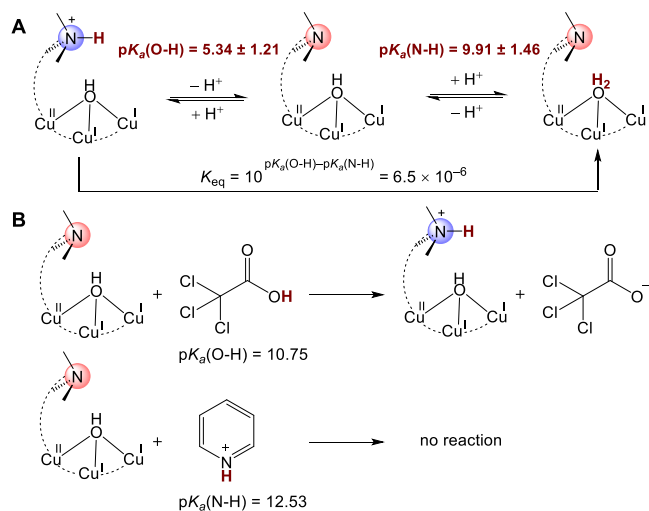
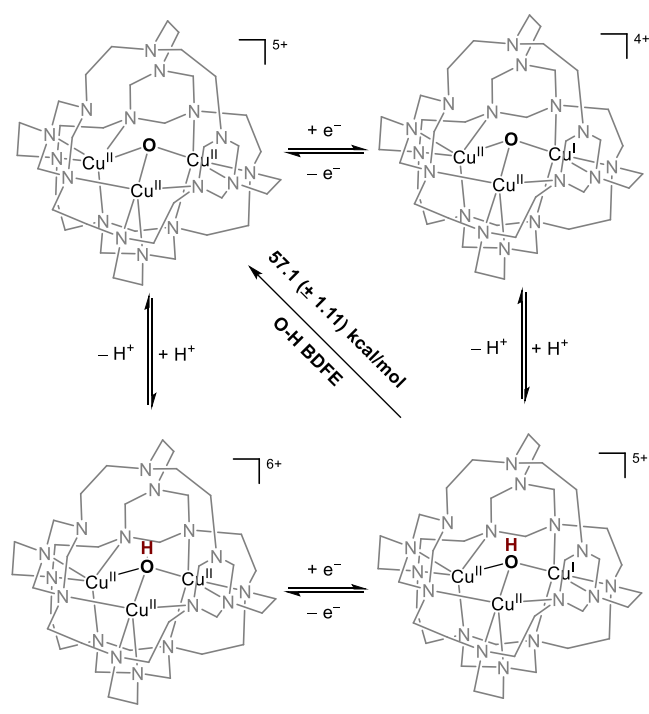


Figure 6. (A) PT equilibria between $[\text{LHCu}_3(\text{II,I,I})(\text{OH})]^{4+}$, $[\text{LCu}_3(\text{II,I,I})(\text{OH})]^{3+}$, and $[\text{LCu}_3(\text{II,I,I})(\text{OH}_2)]^{4+}$. (B) Protonation of $[\text{LCu}_3(\text{II,I,I})(\text{OH})]^{3+}$ with trichloroacetic acid and pyridinium tetrafluoroborate.

mimicking the functions of carboxylate groups adjacent to the biological tricopper center (ca. 4.00 Å, Figure 1A).

The $\text{p}K_a$ values of O–H and N–H motifs determine the protonation states of the tricopper center and the proton relay at the secondary coordination sphere. Although the N–H group is not covalently bonded to the tricopper center, its $\text{p}K_a$ value still increases significantly by ca. 5 $\text{p}K_a$ units per oxidation state (Figure 7, red trace). This observation reflects the electrostatic influences of metallocofactors on the $\text{p}K_a$ of the surrounding amino acid residues.⁴¹

Due to the proximity of OH and OH₂ ligands, their $\text{p}K_a$ values are significantly influenced by the charge/oxidation state of the tricopper center. This is evident in the 14–16 $\text{p}K_a$ increase for

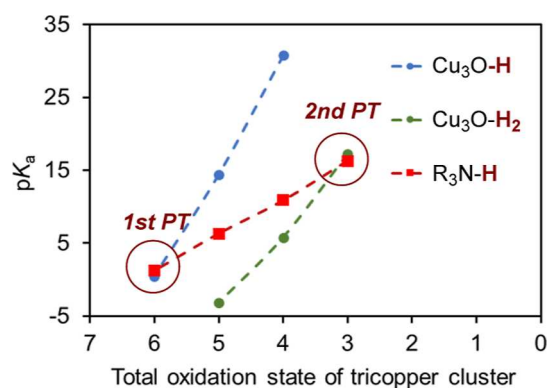


Figure 7. Plots of $\text{p}K_a$ of O–H and N–H motifs against the oxidation state of the tricopper center.

OH (Figure 7, blue trace) and 8–11 $\text{p}K_a$ increase for OH₂ per oxidation state change (Figure 7, green trace). A similar study by Agapie and Reed on the Fe₃Mn aqua cluster and Fe₄ hydroxo cluster shows a change of 4–8 $\text{p}K_a$ (O–H) units per oxidation state.^{42,43}

The consequence of these $\text{p}K_a$ trends in Figure 7 is that, as the tricopper center is reduced, the basicity of the tricopper oxo/hydroxo sites (blue and green traces) increases faster than the secondary coordination sphere N–H motif (red trace), resulting in two intersection points. These two intersection points (red circles, Figure 7) indicate where the basicity of the Cu₃ cluster surpasses that of the N–H motif in the secondary coordination sphere, providing driving forces for proton transfer to the tricopper center.

Worth noting is the trend of BDFE(O–H) along the $3e^- 2H^+$ PCET conversion of $\text{LHCu}_3(\text{II,II,II})\text{O}$ to $\text{LCu}_3(\text{I,I,I})\text{OH}_2$ species. The tricopper species on the ideal path of alternating electron and proton transfer (ET-PT-ET-PT-ET) exhibit mild BDFE(O–H) values around 53.0–57.1 kcal/mol (Figure 8). In

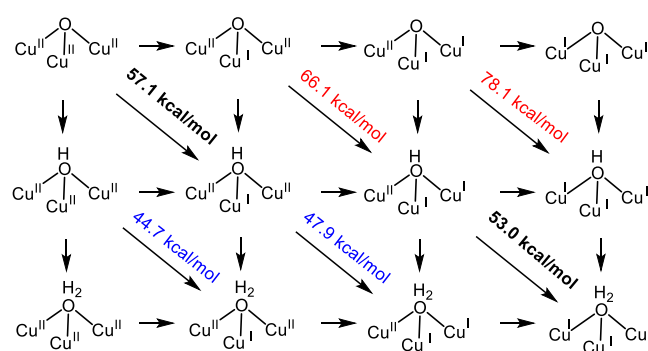


Figure 8. Summary of BDFE(O–H) values at the tricopper cluster.

contrast, those not on the ET-PT-ET-PT-ET path have either much lower (44.7 kcal/mol) or higher (78.1 kcal/mol) BDFE(O–H) values (Figure 8). In the context of biological O₂ reduction at the tricopper center, our study highlights the “safest” PCET pathways with the least reactive tricopper intermediates. The tricopper species along the diagonal pathway tend to be less reactive toward C–H activation (high O–H BDFEs) or production of ROS (low O–H BDFEs), both of which can lead to cellular damage. These results suggest that the BDFE(O–H) should be considered when designing bioinspired ORR catalysts with high efficiency and specificity.

In contrast to the significant changes of BDFE(O–H) values, the BDFE values of the N–H bond in the secondary coordination sphere exhibit minimal variations, ranging from 51 to 58 kcal/mol, despite notable shifts in pK_a (>14 pK_a units) and $E_{1/2}$ changes (>1000 mV). This trend aligns with the findings from Yang et al.'s investigation into the N–H BDFE of manganese imido complexes appended with a crown ether unit containing Lewis acid metal cations.^{44,45} These two studies collectively highlight the efficacy of modifying adjacent charge moieties as a strategy to alter the pK_a values of X–H while preserving BDFE values.

In the tricopper aqua (Cu_3OH_2) system, we observed a decrease in BDFE(O–H) by 4–6 kcal/mol per increment in the oxidation state. In the tricopper hydroxo (Cu_3OH) system, this decrease of BDFE(O–H) is more pronounced, ranging from 9 to 12 kcal/mol per oxidation state increase. These findings are in contrast to those reported by Agapie and Reed, who observed an increase in BDFE(O–H) with higher oxidation states in Fe_4OH clusters and Fe_3MnOH_2 clusters (increments of 4–7 kcal/mol per oxidation state).^{42,43}

To elucidate this difference, we partition the BDFE changes into two key contributing factors: redox potential ($nF\Delta E_{1/2}$, Figure 9, blue bars) and basicity/acidity ($RT\ln\Delta pK_a$, Figure 9, red bars). The cumulative effect of these two opposing influences determines the overall BDFE trend (Figure 9, gray

bars), which trends positive for Fe_3Mn and Fe_4 clusters but trends negative for tricopper clusters. Figure 9 underscores the greater impact of redox potential on the BDFE(O–H) of Fe_3Mn and Fe_4 clusters and the stronger influence of pK_a on the BDFE(O–H) of tricopper clusters. A similar dominance of pK_a contribution to BDFE(O–H) has been noted in the dicopper hydroxo/oxo system studied by Kieber-Emmons.²⁷ These varying trends of BDFE as a function of the cluster oxidation state may arise from (1) inherent differences in mid vs late transition metals, (2) binding mode of oxo/hydroxo/aqua ligands, or (3) the overall charge of the cluster. These factors will be the topic of future investigation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.3c14420>.

Experimental details, including characterization data, spectra, and results (PDF)

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Notes

The authors declare no competing financial interest.

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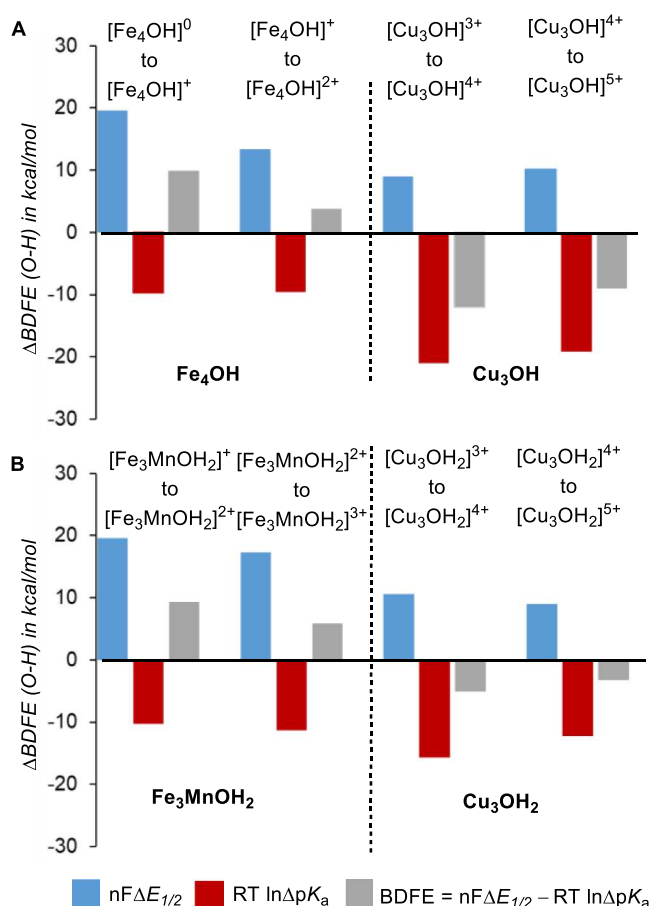


Figure 9. Summary of the contribution of $\Delta E_{1/2}$ (blue) and ΔpK_a (red) toward the overall change in BDFE (gray) at different oxidation states. (A) Comparison of BDFE(O–H) of Fe_4OH vs Cu_3OH at different oxidation states. (B) Comparison of BDFE(O–H) of Fe_3MnOH_2 vs Cu_3OH_2 at different oxidation states.

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