**N2O Reduction by the μ4-Sulfide-Bridged Tetranuclear CuZ Cluster Active Site**

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Keywords: bioinorganic chemistry · copper · electronic structure · metalloenzymes · structure–activity relationships

1. Introduction

Selective oxidation of organic substrates is a significant challenge.[1] Reagents energetically capable of performing useful oxidations are often so reactive that they offer no substrate selectivity; others produce byproducts that lead to undesirable side reactions. A potentially attractive oxo-transfer reagent to oxidize organic substrates is nitrous oxide (N2O + 2H+ + 2e⁻ → N2 + H2O, E° = 1.76 V),[2] which provides a clean reaction since the sole byproduct is N2. However, N2O is a kinetically inert molecule, which is reflected in the approximately 59 kcal mol⁻¹ activation barrier for its thermal decomposition (a unimolecular, spin-forbidden process).[3] Reduction of N2O in homogeneous systems normally requires transition metals, such as Ti, V, Ni, Zr, Ru, Hf,[4–9] as activation centers, where the required two electrons are either derived from the metal centers, which results in terminal[10,11] or bridged[4] metal-oxide products, or from the ligands through insertion of the oxygen atom into the metal–ligand bond.[5–7,12] To date, none of the reported metal/N2O complexes has been structurally determined crystallographically. The N2O unit in the [(NH3)5Ru(N2O)]²⁺ complex has been found from spectroscopy to coordinate to the ruthenium atom in a linear end-on mode by its terminal nitrogen atom.[13] Additional terminal oxygen coordination to metal sites has also been implicated in the formation of the {Ru(N2O)Ru} dimer[10,14] and has been spectroscopically identified in N2O adsorption on α-Cr2O3.[15]

The reduction of N2O in biological systems is accomplished by the multicopper-center containing enzyme nitrous oxide reductase (N2OR). This reaction is the last step in the denitrification process of the bacterial nitrogen cycle and is accomplished by the enzyme nitrous oxide reductase, whose active site consists of a μ4-sulfide-bridged tetranuclear CuZ cluster which has many unusual spectroscopic features. Recent studies have developed a detailed electronic-structure description of the resting CuZ cluster, determined its catalytically relevant state, and provided insight into the role of this tetranuclear copper cluster in N2O activation and reduction.

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CuA and CuZ centers are from different subunits in the C-terminal domain of each subunit. The neighboring (2.4 Å–C) was later solved for N2OR from spectroscopic results.[21,22] A low-resolution crystal structure assigned as a binuclear copper center based on some initial structural motif composed of a bridged tetranuclear copper cluster and is located in the N-terminal domain in each subunit of the dimeric protein, while the CuA center is located in the C-terminal domain of each subunit. The neighboring CuA and CuZ centers are from different subunits in the dimeric protein (Figure 2). Quantitative elemental analysis combined with spectroscopic arguments soon indicated that the CuZ center is in fact a μ4-sulfide bridged tetranuclear copper cluster.[24] This was later confirmed by the high-resolution crystal structure of N2OR from Paracoccus denitrificans (Pd) (1.6 Å), and the original Pn N2OR structure was then revised.[25,26]

The Cu4S core of the CuZ structure has approximate C2 symmetry with Cu-S-Cu angles defining the mirror plane (see Figure 2). The Cu-S-Cu angle is approximately 160°, and the other Cu-S-Cu angles are close to orthogonal. All the Cu–S bond lengths are approximately the same, about 2.3 Å. However, the Cu–Cu separations are very different, with the three copper centers, CuII, CuIII, CuIV (Figure 2), closer to each other and Cu more distant (Cu–Cu close to 3.4 Å, Cu–Cu close to 2.6 Å, Cu–Cu close to 2.9 Å). The whole [CuS] cluster is coordinated to the protein backbone by seven histidine ligands, and there is an additional ligand L at the Cu/Cu edge. This Cu/Cu edge is believed to be the substrate binding site.[23] To date the exact nature of this ligand (O2, OH, H2O) is unclear from the crystal-structure studies.

Much spectroscopic data have been published on the resting CuZ center,[27] including its absorption spectrum (Figure 3a, solid line) which shows an intense charge-transfer band at about 640 nm (≈ 15660 cm−1), and the low-temperature magnetic circular dichroism (MCD) spectrum (Figure 3a, broken line) which has an intense feature in this 640 nm region.[21,22,24,28] The EPR spectrum of resting CuZ has also been reported, it exhibits a very small gH value and complicated hyperfine-coupling pattern.[18,21,28] However, because of the lack of accurate quantification of the number of copper atoms in the enzyme, their oxidation states, and

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structural information, a reasonable understanding of these spectral features and the enzymatic mechanism had not been possible. Herein, we focus on our recent efforts to understand these spectroscopic features and develop an electronic-structure description of the CuZ site of the N2OR enzyme.[29–31]

Insight into the contribution of the electronic structure to N₂O reduction and the possible mechanism for this reaction by the CuZ center are also presented.

2. The Electronic Structure of the CuZ Center

The intensity of the MCD signals shown in Figure 3a are temperature and magnetic-field dependent (contribution of the MCD C-term) and increases with lower temperature and higher magnetic field. The MCD intensity will eventually saturate at high field (≈7 T) and low temperature (≈2 K). Different spin-state systems have different saturation behaviors.[32] Variable temperature, variable field (VTVH) saturation MCD can thus determine the spin state of the resting CuZ center, which is the state of the crystallographically defined CuZ cluster. The VTVH MCD saturation of resting CuZ is shown in Figure 3b along with theoretically calculated saturation curves for \( S = \frac{1}{2}, 1, \) and \( \frac{3}{2} \) systems, which show that the resting CuZ center has an \( S_{\text{total}} = \frac{1}{2} \) ground state.[29]

Since the CuZ center is a tetraneutral copper cluster, there are two possibilities for the copper oxidation states to give \( S_{\text{total}} = \frac{1}{2} \), either 1CuII/3CuI or 3CuII/1CuI (CuII, oxidized; CuI, d°, reduced). In the latter case, two of the oxidized copper atoms would have to be antiferromagnetically coupled. Cu K-edge X-ray absorption spectroscopy (XAS) was used to distinguish between these two possibilities. CuI complexes have an intense characteristic absorption feature at approximately 8984 eV, which is the electric-dipole-allowed CuI \( 1s \rightarrow 3p \) transition, while CuII complexes have no intense feature below 8985 eV. Instead they have a very weak transition at about 8979 eV, which is the electric-dipole-forbidden CuI \( 1s \rightarrow 3d \) transition.[33] The copper K-edge X-ray...
absorption spectrum of resting CuZ is given in Figure 4, along with simulated spectra for the 1 CuII/3 CuI and 3 CuII/1 CuI configurations. The simulations show that the 1 CuII/3 CuI model is far better than the 3 CuII/1 CuI alternative, which indicates that there is only one oxidized CuII in the CuZ cluster with a single spin (a single Cu d-hole).

EPR spectroscopy was used to determine the distribution of the single spin (from one CuII center) over the CuZ center. The Q-band ($\nu \approx 35 \text{ GHz}$) EPR spectrum of resting CuZ (Figure 5a) shows an axial pattern with $g_1 \approx 2.16 > g_2 \approx 2.04 > 2.0$, indicating that the single spin resides in a Cu d$_{x^2-y^2}$ orbital. The $g_1$ value of resting CuZ is very small as compared to the approximately 2.3–2.4 for normal tetragonal CuII complexes (e.g. CuSO$_4$).[35] This could be due to extensive covalent character in the metal–ligand bonding or high d–d transition energies.[36] High transition energies affect the $g$ values as the spin-orbit coupling of these excited states with the ground state leads to the $g$ values deviating from 2.0023, and this deviation will decrease as the energies of these excited states increase. In correlating the Q-band to the lower frequency X-band ($\nu \approx 9.3 \text{ GHz}$) EPR spectrum of resting CuZ, metal hyperfine coupling is resolved in the $g_2$ region (Figure 5b). Mapping the $g$ values determined from the Q-band EPR onto the X-band EPR spectrum, the $g_1$ value ($\approx 2.16$) coincides with a hyperfine feature indicating metal hyperfine pattern with an odd number of lines (Figure 5b). This is in contrast to the EPR hyperfine pattern of normal mononuclear CuII complexes, which is a four-line pattern (Cu nuclear spin $= \frac{3}{2}$). To account for the X-band metal hyperfine pattern, two copper centers are required for the hyperfine coupling. One copper center dominates the hyperfine splitting ($A_1 = 61 \times 10^{-4} \text{ cm}^{-1}$) and a second copper center contributes somewhat less with $A_2 = 24 \times 10^{-4} \text{ cm}^{-1}$. The ratio of the hyperfine coupling constants gives the approximate ratio of spin densities on the two copper atoms, approximately 5:2. Therefore, resting CuZ in Pr N$_2$OR can be described as a partially delocalized mix-valent system with the single spin predominantly in a d$_{x^2-y^2}$ orbital on one copper atom.[29]

XAS at the Cu K-edge was used to determine the copper oxidation states of resting CuZ. XAS at the sulfur K-edge (S K-edge) was also used to determine the bridging-sulfide covalency in the ground state of resting CuZ.[36] The pre-edge transition at the S K-edge is the S 1s$^2$–gound state SOMO transition, where the SOMO is a linear combination of Cu 3d and S 3p orbitals (Figure 6a). The intensity of this pre-edge transition is directly proportional to the sulfide covalency $\alpha^2$ in the ground-state wave function (see Equation in Figure 6a).[36] Figure 6b presents the S K-edge spectrum of resting CuZ,[37] where the S pre-edge transition occurs at approximately 2469 eV and its weak intensity corresponds to around 15–22% sulfur character in the ground-state wave function. For comparison, the S K-edge spectrum of the Cu$_{\delta}$ center (also given in Figure 6b) has a much stronger pre-edge feature at about 2470 eV. The intensity of this Cu$_{\delta}$ pre-edge transition corresponds to approximately 46% S character in the ground state, which is well documented.

Density functional theory (DFT) calculations were used to obtain a detailed description of the resting CuZ ground-state wave function. Figure 7 and Table 1 give the optimized structures and atomic spin densities of the spin-doublet state of the Cu$_{\delta}$ cluster with different Cu$_{\delta}$/Cu$_{\gamma}$-edge ligands. These calculations indicate that the ground-state wave function and the spin-density distribution of the Cu$_{\delta}$ cluster are sensitive to the nature of the ligand at the Cu$_{\delta}$/Cu$_{\gamma}$ edge. In general, Cu$_{\delta}$ is the predominantly oxidized copper center (Table 1). This situation is consistent with Cu$_{\delta}$ having a four-coordinate structure and the other copper atoms having lower coordination numbers (see Figures 2 and 7). The spin distribution between the Cu$_{\delta}$ and Cu$_{\gamma}$ atoms in the cluster with L = H$_2$O and the spin distribution between the Cu$_{\delta}$ and Cu$_{\delta}$ atoms in

![Figure 4. Cu K-edge XAS spectrum of resting CuZ from Pr N$_2$OR and simulated spectra assuming 1 CuII/3 CuI and 3 CuII/1 CuI oxidation state configurations.](image)

![Figure 5. Experimental and simulated EPR spectra of resting CuZ from Pr N$_2$OR at a) Q-band and b) X-band.](image)
the cluster with \( L = \text{HO-H-OH}^- \) have an approximately 2:1 ratio. This ratio is in agreement with the Q/X-band EPR results which indicate an approximately 5:2 ratio. The complexes with other water-derived ligands show a spin distribution different from 5:2. This observation suggests \( \text{H}_2\text{O} \) or the \( \text{HO-H-OH}^-/\text{CO} \) ion as the edge ligand in the resting form of the \( \text{CuZ} \) cluster. In these species, the \( \mu_4 \)-bridging sulfide group contributes around 13–16% to the ground-state wave function, which is also consistent with the S covalency determined from the S K-edge results (Figure 6b).

Electron delocalization in the \( \text{CuZ} \) cluster is very important because it contributes to a low reorganization energy during redox processes of the \( \text{CuZ} \) center and leads to stabilization of the oxidized form of \( \text{CuZ} \) after \( \text{N}_2\text{O} \) reduction.

The description obtained from the ground-state wave function greatly facilitates the understanding of other characteristic spectroscopic features of resting \( \text{CuZ} \) cluster. Figure 8a presents the resonance Raman spectrum of resting \( \text{CuZ} \) clusters excited at 624.4 nm.\[30\] Three dominant features are observed, at 366, 386, and 415 cm\(^{-1}\), all of which shift to lower frequencies upon \( ^{34}\text{S} \) isotope labeling.\[39\] Based on the frequencies and \( ^{34}\text{S} \) isotope shifts, these three vibrations can be assigned as \( \text{Cu-S} \) based stretching vibrations. The vibration modes observed can be understood by using a \( \text{Cu}_4 \text{S} \) cluster model, which has an approximate \( \text{C}_5 \) symmetry with \( \text{CuI-S-CuII} \) defining the mirror plane (Figure 2).\[25\] There are four \( \text{Cu-S} \) bonds in this cluster and thus a total of four \( \text{Cu-S} \) based stretching modes: two symmetric (A' symmetry in the \( \text{C}_5 \) point group) in-plane modes from \( \text{CuI-S/CuII} \) vibrations and two out-of-plane modes, one the symmetric (plus)

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Ratio(^{[a]})</th>
<th>( \text{Cu}_{\text{II}} )</th>
<th>( \text{Cu}_{\text{III}} )</th>
<th>( \text{Cu}_{\text{IV}} )</th>
<th>( \text{Cu}_{\text{V}} )</th>
<th>( \text{S} )</th>
<th>( \text{O}_{\text{a}} )</th>
<th>( \text{O}_{\text{b}} )</th>
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<tbody>
<tr>
<td>( \text{O}^- )</td>
<td>5.7 (19)</td>
<td>0.31 (0.40)</td>
<td>0.02 (0.01)</td>
<td>0.01 (0.01)</td>
<td>0.06 (0.02)</td>
<td>0.11 (0.13)</td>
<td>0.48 (0.42)</td>
<td>–</td>
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<tr>
<td>( \text{OH}^- )</td>
<td>3.4 (5.2)</td>
<td>0.41 (0.45)</td>
<td>0.07 (0.08)</td>
<td>0.05 (0.07)</td>
<td>0.12 (0.01)</td>
<td>0.22 (0.26)</td>
<td>0.07 (0.06)</td>
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</tr>
<tr>
<td>( \text{H}_2\text{O} )(^{[b]})</td>
<td>2.4 (2.0)</td>
<td>0.42 (0.39)</td>
<td>0.18 (0.19)</td>
<td>0.07 (0.07)</td>
<td>0.04 (0.05)</td>
<td>0.15 (0.16)</td>
<td>0.01 (0.01)</td>
<td>–</td>
</tr>
<tr>
<td>( \text{O-H-OH}^- )</td>
<td>5.1</td>
<td>0.47</td>
<td>0.00</td>
<td>0.01</td>
<td>0.09</td>
<td>0.11</td>
<td>0.28</td>
<td>0.01</td>
</tr>
<tr>
<td>( \text{HOO}^- )(^{[c]})</td>
<td>1.4</td>
<td>0.24</td>
<td>0.00</td>
<td>0.00</td>
<td>0.34</td>
<td>0.08</td>
<td>0.04</td>
<td>0.28</td>
</tr>
<tr>
<td>( \text{HO-H-OH}^- )</td>
<td>1.7 (3.6)</td>
<td>0.41 (0.51)</td>
<td>0.06 (0.06)</td>
<td>0.02 (0.02)</td>
<td>0.24 (0.14)</td>
<td>0.13 (0.11)</td>
<td>0.02 (0.05)</td>
<td>0.04 (0.01)</td>
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\(^{[a]}\) The calculations were performed for the spin-doublet ground state at the spin unrestricted B3LYP/6-311G** level of theory using the BP86/LANL2DZ and B3LYP/6-311G** optimized structures. The spin densities obtained using the B3LYP/6-311G** optimized structures are shown in parenthesis.\(^{[b]}\) Ratio of the atomic spin density between the copper atoms with the largest and second-largest values.
combination of CuI$^-$S/CuIV$^-$S, and the other the antisymmetric (minus, A'' symmetry) combination of CuII$^-$S/CuIV$^-$S. The antisymmetric mode should not be resonance enhanced.\[40\] Therefore, only three symmetric resonance Raman modes are expected and experimentally observed (Figure 8a). Using the observed vibrational frequencies and $^{34}$S isotope shifts, individual Cu$^-$S bond strengths can be determined from a normal coordinate analysis.\[30\] The CuI$^-$S bond is the strongest (ca. 3.3 mdynÅ$^\text{-1}$), the CuII$^-$S bond is next in strength (ca. 3.1 mdynÅ$^\text{-1}$), and the CuIII$^-$S/CuIV$^-$S bonds are the weakest (ca. 1.3 mdynÅ$^\text{-1}$). The bond-strength pattern of the four Cu$^-$S bonds reflects the electronic structure description of the resting Cu Z site with L = H$_2$O, where CuI is the predominantly oxidized center, CuII gains some oxidized character through electron delocalization from CuI through the bridging sulfide, and CuIII and CuIV are mostly reduced.

The excitation profiles of the observed resonance Raman modes are shown in Figure 8b, overlaid on the absorption spectrum of resting CuZ.\[30\] All three Cu$^-$S based vibrations are resonance enhanced by excitation in the region of the broad CT absorption envelope centered at approximately 640 nm ($\approx$ 15 650 cm$^{-1}$), which indicates their S$^\text{-}$Cu CT nature. Importantly, three individual electronic transitions are resolved in the resonance Raman excitation profiles, at about 14 300, 15 700, and 16 500 cm$^{-1}$. These three transitions can be associated with excitations from the three p orbitals of the µ$^\text{-}$bridging sulfide.

Returning to the absorption and MCD spectra of resting CuZ, mentioned in the Introduction (Figure 3a), a number of transitions can be experimentally resolved by a Gaussian analysis of the bands (Figure 9).\[30\] The ground-state wave function (spin-down LUMO) is the o-antibonding combination of mainly the CuI d$_{x^2-y^2}$ and S p$_{z'}$ orbitals and is the acceptor orbital for all the electronic transitions. Three S$^\text{-}$Cu CT bands have been identified in the resonance Raman excitation profiles (Figure 8b, and bands 5, 6, 7 in Figure 9). Band 6 is the strongest among the three transitions in the absorption spectrum and can be assigned as the S p$_{z'}$ spin-down LUMO transition. In this transition, the donor orbital is the direct bonding counterpart to the acceptor orbital and thus this transition has the largest donor–acceptor orbital overlap and is the most intense in the absorption spectrum. Band 5 is the weakest S$^\text{-}$Cu CT transition and can be assigned to the transition from the S p$_{z'}$ donor orbital. This S p$_{z'}$ orbital is out of the Cu d$_{x^2-y^2}$ plane and orthogonal to this acceptor orbital (i.e. poor donor–acceptor overlap), which leads to its low intensity in the absorption spectrum. Band 7 is intermediate in intensity in the absorption spectrum and can be assigned to the CT transition out of the S p$_{z'}$ orbital. Since only CuI is dominantly oxidized in resting CuZ, only this center exhibits d–d transitions (Bands 1, 3, 4, and 8, Figure 9).

These d–d transitions have a large MCD/absorption intensity ratio, which is due to the large spin-orbit coupling on the copper center ($\xi$(Cu$^\text{II}$) $\approx$ 830 cm$^{-1}$).\[41\] Interestingly, the d$_{x^2-y^2}$ spin-down LUMO transition is high in energy at approximately 18 000 cm$^{-1}$, relative to its position in normal tetragonal Cu$^\text{II}$ complexes.\[30\] Its high energy comes from the ligand geometry of the CuI center which has two histidine and one sulfide ligand forming a T-shaped environment with one histidine distorted out of the molecular plane.\[30\] The high energy of the d$_{x^2-y^2}$ transition is the reason for the small $g_z$ value (ca. 2.16) observed for the resting CuZ cluster, since the covalency of the bridging sulfide is low (ca. 15–22% S char-
acter in the ground state, Figure 6b) and cannot account for the observed $g_k$ value. Additionally, band 2 in the absorption spectrum of the resting CuZ cluster has been identified as an intervalence charge transfer (IT) transition. This IT transition is unidirectional and thus does not give rise to MCD intensity at low-temperature (Figure 9b). It formally corresponds to an electron transfer \( \text{Cu}^{II}(d^9) \rightarrow \text{Cu}^{I}(d^{10}) \), and reflects the electronic coupling and electron delocalization between the copper atoms mediated by the bridging sulfide unit. The higher energy bands (9–13, Figure 9) can be assigned as histidine–Cu CT transitions. They are in fact low in energy relative to CT transitions in tetragonal copper–imidazole model complexes. This is due to the low coordination number (three-coordinate) of the CuI center in the CuZ cluster.

3. Catalytically Relevant State of the CuZ Center

Past studies indicate that N2OR can be activated in vitro to reduce N2O by incubation with dithionite-reduced methyl viologen, and prolonged pre-incubation can result in higher enzyme activity. To identify the catalytically relevant form of CuZ, the X-band EPR spectrum of N2OR was measured after different incubation times in excess methyl viologen and dithionite solution (Figure 10a). At time zero, the spectrum shows the characteristic EPR signal of resting CuZ with $g_k = 2.16$. The signal intensity decreases gradually with increasing incubation time. Since resting CuZ is at the 1CuII/3CuI state with only one electron hole, the loss of the CuZ EPR signal in this reducing environment indicates that the CuZ cluster is reduced to the 4CuI form. Parallel activity measurements at similar incubation times show that the enzyme activity increases with increasing incubation time and the increase of activity is directly correlated to the decrease of the resting CuZ EPR signal (Figure 10b). This direct correlation of enzyme activity and reduction of resting CuZ indicates that the catalytically relevant form of CuZ is the fully reduced 4CuI state. Recent measurements using absorption and nitrogen labeling are consistent with these findings.

4. N2O Activation and the Role of the Tetranuclear Cu4S Cluster

Having developed a description of the electronic structure of the resting CuZ center and determined the catalytically relevant form of CuZ, we could gain insight into the reaction catalyzed by this cluster, which is the two-electron reduction of N2O to N2. The catalytically relevant form of CuZ is an electron-rich site with four reduced CuI centers. At the substrate-binding CuI/CuIV edge (Figure 2), the N2O substrate could interact with both CuI and CuIV, possibly in a bridged binding mode (Figure 11). Two electrons could be simultaneously donated from CuI and CuIV to overcome the reaction barrier for N2O reduction. Additionally, there are good electron-transfer pathways from the neighboring CuI center in the second subunit of the dimeric N2OR protein to CuIII and CuIV.[29] Together with the delocalized electronic structure of the CuZ cluster, these could allow rapid re-reduction of the CuZ center during enzymatic turnover. The electron delocalization over the bridging sulfide contributes to a low...
reorganization energy during redox processes and stabilization of the oxidized form of the CuZ center after N₂O reduction.

Computational methods were used to investigate the possible interaction of the N₂O substrate with the catalytic 4Cu⁺ form of the CuZ center. The lowest energy structure of the CuZ(4Cu⁺)-N₂O complex is shown in Figure 12, where

Figure 12. DFT optimized geometry of the N₂O complex with the 4Cu⁺ form of CuZ.[31]

N₂O binds at the Cu⁴/Cu⁵ edge in a bent μ-1,3 bridging mode (α=139°) with the terminal nitrogen atom coordinating to Cu⁴. (Other binding modes of N₂O to the CuZ cluster are higher in energy.) The bending of the bound N₂O unit in the CuZ(4Cu⁺)-N₂O complex results in a 2 eV splitting of the doubly degenerate LUMO of free N₂O into two nondegenerate π* orbitals. The π* LUMO in the N-N-O plane is stabilized by approximately 3 eV owing to its loss in antibonding character (Figure 13b). This change shifts the π* orbital of N₂O close to the fully occupied d orbitals of the Cu₂(4Cu⁺) cluster and makes the N₂O ligand a very good electron acceptor.

The LUMO of the Cu₂(4Cu⁺)-N₂O complex (Figure 13a) has mostly N₂O π* character (54%) with d orbital contributions of 12% from Cu⁴ and 10% from Cu⁵, which indicates significant backbonding interactions from the fully reduced CuZ center to the bound N₂O. This substantial Cu–N₂O π* backbonding interaction is reflected in the ~0.53 charge of the bound N₂O ligand and the elongation of the N–N and N–O bonds (+0.03 Å and +0.07 Å, respectively). This strong Cu₂(4Cu⁺)-to-N₂O back donation is only present when N₂O binds at the Cu⁴/Cu⁵ edge in a bent μ-1,3 bridging mode. Other binding modes of N₂O exhibit much weaker back donation (4–5 times small).

The electronic interactions between the fully reduced CuZ cluster and the bound N₂O ligand play a crucial role in N₂O activation and reduction which leads to N–O bond cleavage. The Cu–N₂O π* backbonding interaction significantly weakens the N–O bond which may facilitate the direct N–O bond cleavage through simultaneous transfer of two electrons from CuZ to the μ-1,3 bridged N₂O (Scheme 1 path A). This backbonding interaction also increases the electron density on the oxygen atom of the bound N₂O molecule (oxygen charge = −0.5), and would activate it for electrophilic attack by a proton (Scheme 1 path B). The HOMO-2 of the Cu₂(4Cu⁺)-N₂O complex, which lies close to HOMO and has significant O character (Figure 13a), can serve as the donor frontier molecular orbital for protonation. This and other possible reaction channels (Scheme 1 path C) leading to the reductive cleavage of the N–O bond in N₂O are presently being evaluated. Our DFT calculations[50] indicate that path B is a very favorable reaction channel: oxygen-atom protonation of the coordinated N₂O causes the barrierless N–O bond cleavage.

5. Summary

Spectroscopic methods combined with density functional calculations have been used to define the spin state, copper...
oxidation state, spin distribution, and ground-state wave function of the resting CuZ center, and to understand its unusual vibrational and optical spectral features. The electronic-structure description developed for the resting CuZ cluster and the determination that the catalytically relevant form of CuZ is the fully reduced state provide the basis for understanding the role of the μ 3-sulfide bridged tetranuclear copper cluster in the activation of N₂O for two-electron reduction. Strong back donation into the N₂O ligand in the CuZ(4CuI)-N₂O complex where N₂O binds in as bent copper cluster in the activation of N₂O for two-electron reduction. Note that the slow rate of reduction of the resting CuZ center with excess dithionite solution, the CuA center in the enzyme is with excess dithionite solution, the CuA center in the enzyme is thus reduced to the spectroscopically silent Cu⁺/Cu²⁺ state and does not contribute to absorption, MCD, and EPR signals. See refs. [28,30].


[38] Previous calculations of CuZ with L = H₂O were performed on a simplified model where the histidine ligands were modeled as NH₃ (see refs. [29,30]). The results for the L = H₂O species presented here are similar to the previous values.


[48] Note that the slow rate of reduction of the resting CuZ center (1Cu⁺/3Cu²⁺) indicates that this species can not take part in the catalytic cycle, and the rate of re-reduction of the N₂O-oxidized CuZ center must be fast in enzymatic turnover.
