Supporting Information

EPR-ENDOR of the Cu(I)NO Complex of Nitrite Reductase

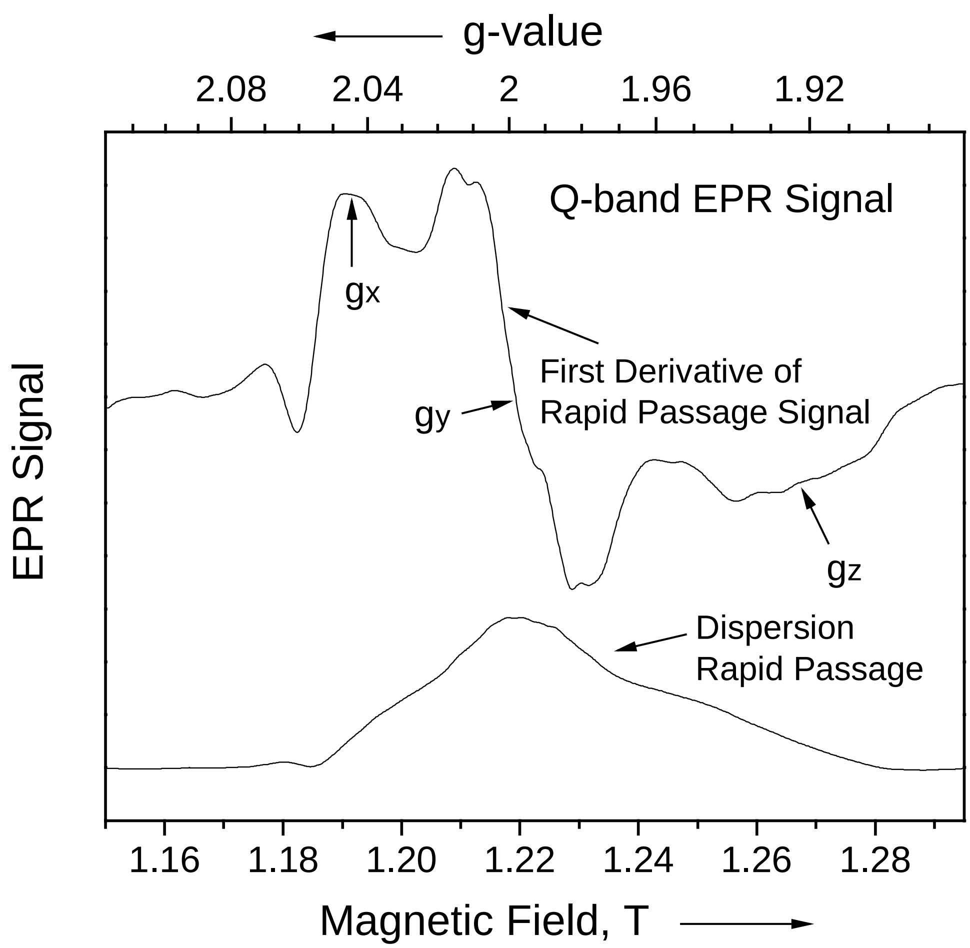
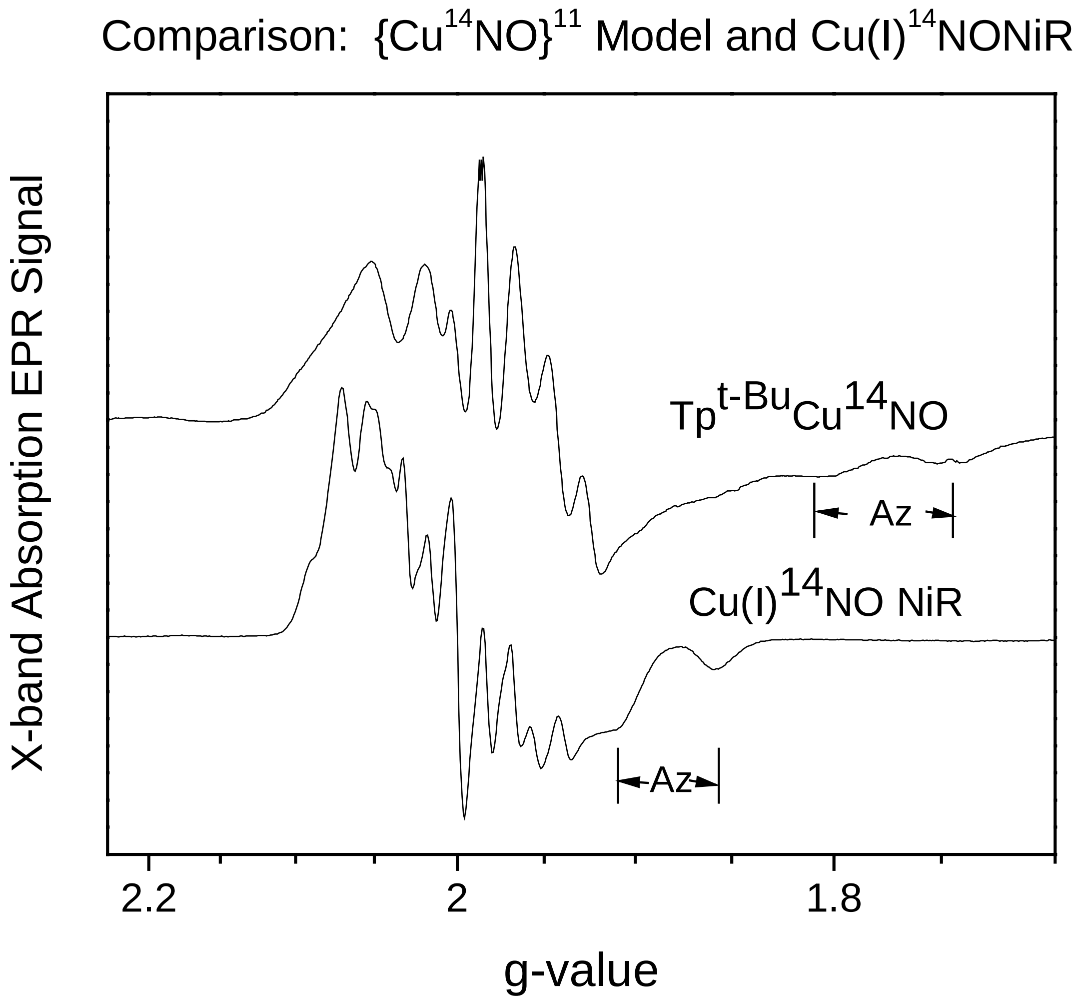
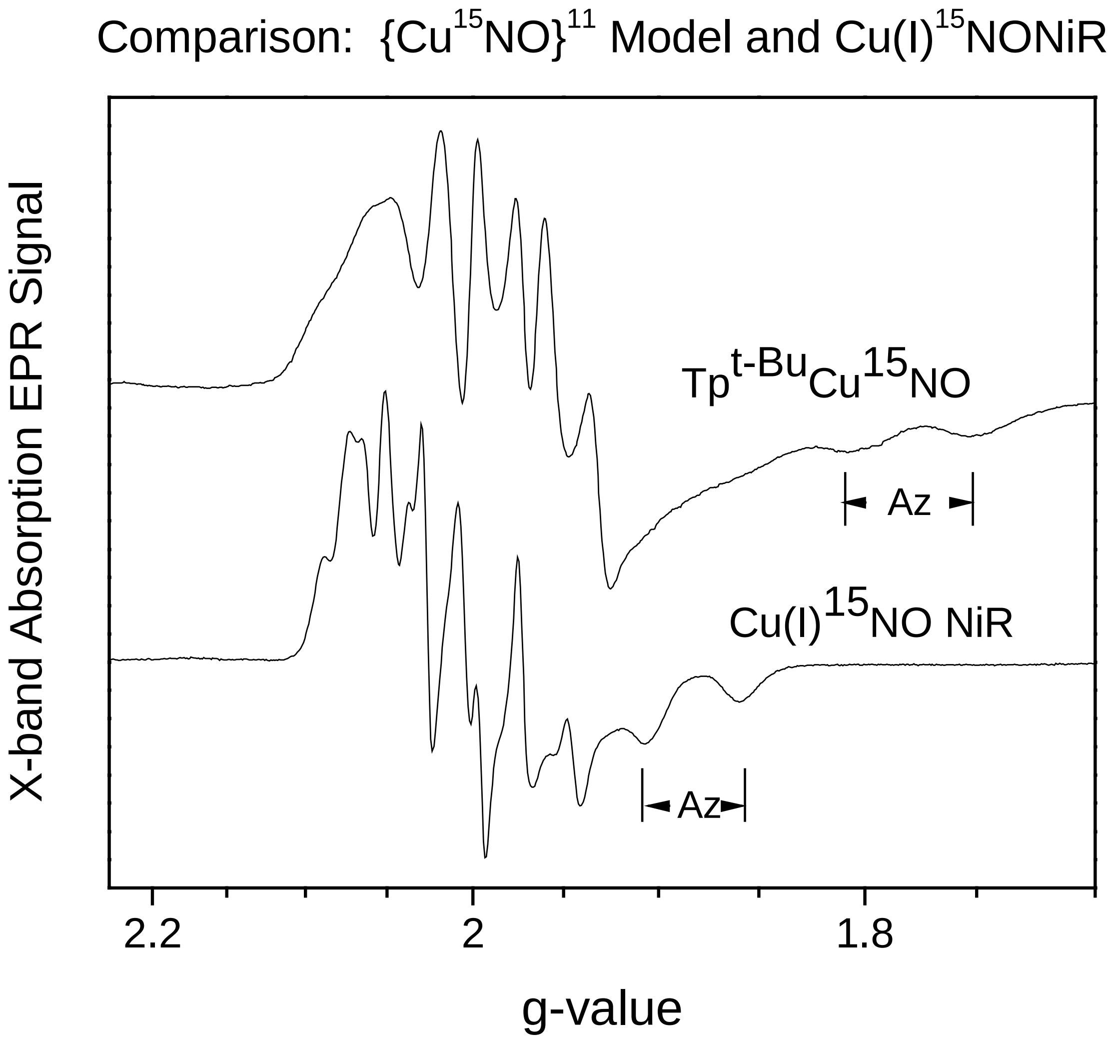


Figure 1S. The Q-band first derivative EPR spectrum of Cu(I)^{14}NONiR is shown as the upper spectrum. It was obtained by taking a numerical first derivative (by the Savitzky-Golay smoothing method) from the Q-band rapid passage signal which is shown as the lower spectrum. The rapid passage spectrum was obtained under the following conditions: T = 1.8 K, microwave power = 7.8 µW, 100 KHz field modulation = 0.1 mT, ν_{EPR} = 34.10 GHz. The primary purpose of the Q-band derivative spectrum is to obtain empirical estimates g-values, which are g_x = 2.044 ± 0.003, g_y = 1.998 ± 0.002, g_z = 1.923 ± 0.005. There is a small distortion to the rapid passage signal near 1.18 T.



Spectrum 2SA. This figure compares the X-band EPR spectrum of the model $\{Cu(I)^{14}NO\}^{11}$ $Tp^{t\text{-}Bu}Cu^{14}NO$ complex of Ruggiero et al.[1] with the EPR spectrum of $Cu(I)^{14}NONiR$ taken at 9.525 GHz. [$Tp^{t\text{-}Bu}$ is tris(3-t-Bu, 5-H-pyrazolyl)hydroborate] The X-band EPR spectrum for $Tp^{t\text{-}Bu}Cu^{14}NO$ were taken in 1993[1] with an EPR frequency of 9.234 GHz and a temperature of 30 K. The conditions for $Cu(I)^{14}NONiR$ are those of Figure 2A except that the sample contains Cu in isotopic natural abundance. The hyperfine coupling A_z for Cu(I)NONiR is 88 Gauss. The hyperfine coupling A_z for the model is reported to be 105 Gauss.



Spectrum 2SB. This figure compares the X-band EPR spectrum of the model $\{Cu(I)^{15}NO\}^{11}$ $Tp^{t\text{-}Bu}Cu^{15}NO$ complex of Ruggiero et al.[1] with the EPR spectrum of $Cu(I)^{15}NONiR$. [$Tp^{t\text{-}Bu}$ is tris(3-t-Bu, 5-H-pyrazolyl)hydroborate] The X-band EPR spectrum for $Tp^{t\text{-}Bu}Cu^{15}NO$ were taken in 1993[1] with an EPR frequency of 9.234 GHz and a temperature of 30 K. The conditions for $Cu(I)^{15}NONiR$ are as for Figure 2B except that the sample contains Cu in isotopic natural abundance.

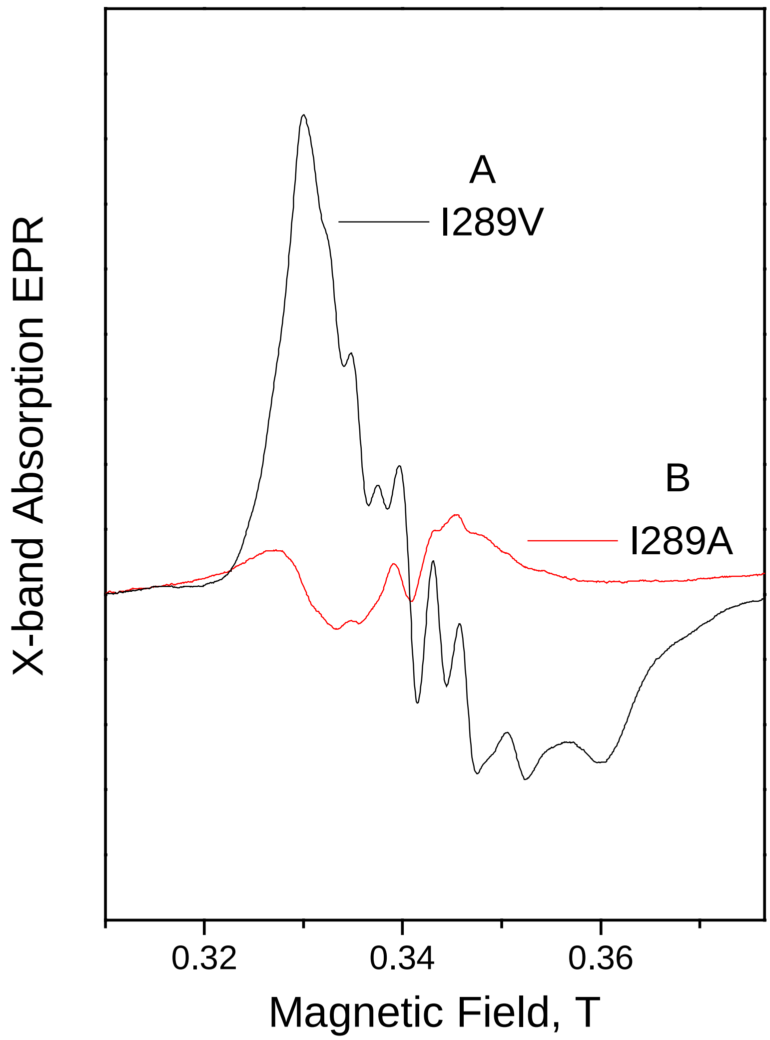


Figure 3S. Comparison of X-band EPR spectra of Cu(I)NONiR prepared from (**A**) I289V (black) and (**B**) I289A (red). Samples were ~0.5 mM in NiR subunits. ~0.05 mL in volume, and prepared using ^{14}N-nitrite and Cu in natural isotopic abundance. The spectra were recorded at T = 15 K, 0.6 mT field modulation, 100 s signal averaging over 70 mT sweep, 2 mW microwave power, EPR frequency= 9.525 GHz.

Estimating Spin Density on ^{14}N of NO from the ^{14}NO Hyperfine Couplings

The values for $^{14}A_x$ and $^{14}A_y$ and are respectively provided as 46 and 80 MHz in Table 1. The value for $^{14}A_z$ has been too small to measure at X-band. However, evidence from preliminary S-band EPR data (kindly provided by W. Antholine) provides a very rough estimate of $^{14}A_z$, as follows: At S-band in the second harmonic presentation a difference in peak-to-peak linewidths of 19.45 and 17.5 Gauss respectively has been observed between the $^{63}Cu(I)^{14}NONiR$ and $^{63}Cu(I)^{15}NONir$. The contribution to the square linewidth from unresolved hyperfine structure is $[(4/3)I(I+1)A^2]$,[2] where I is the nuclear spin and A is the hyperfine coupling for a particular nucleus. The difference in square linewidths, in $[\text{Gauss}]^2$, is $(19.45)^2 - (17.5)^2 = 72 = (8/3)(^{14}A_z^{\,2}) - (^{15}A_z^{\,2})$. $= (0.696)\,(^{14}A_z^{\,2})$ where the 1.404 ratio of ^{15}N to ^{14}N magnetic moments has been used. The resultant estimate for $^{14}A_z$ is 10.2 Gauss or 27 MHz. This is a crude estimate for $^{14}A_z$, but it should be noted that neither $^{14}A_x$ nor $^{14}A_z$ has previously been resolved for $\{CuNO\}^{11}$ complexes.

We write our ^{14}N hyperfine tensor as :

$$^{14}A_x = A_{iso} - A_{\pi*y'} + 2A_{\pi*x'} \quad = 46\text{ MHz}$$

$$^{14}A_y = A_{iso} + 2A_{\pi*y'} - A_{\pi*x'} \quad = 80\text{ MHz}$$

$$^{14}A_z = A_{iso} - A_{\pi*y'} - A_{\pi*x'} \quad = 27\text{ MHz}$$

Neglecting core polarization, A_{iso} (= 51 MHz) can be approximated as being proportional to the fraction of unpaired electron spin density, f_{2s} in the nitrogen 2s orbital. Using the information in Footnote 3 below, we calculate that there is 3 % spin in the nitrogen 2s orbital.

$A_{\pi*y'}$ and $A_{\pi*x'}$ are dipolar couplings proportional to the respective electron 2p spin density, $f_{\pi*y'}$ and $f_{\pi*x'}$, in the $\pi*y'$ and $\pi*x'$ orbitals. $A_{\pi*y'}$ and $A_{\pi*x'}$ are respectively 17.7 and 6.3 MHz. Using the information in Footnote 4 below, $f_{\pi*y'}$ and $f_{\pi*x'}$ are respectively 37 % and 13 % spin. Thus the total spin on the N is 53 %. This result is a very approximate estimate of total spin on N of NO.

It might be argued that instead of incorporating spin in the $\pi*_{y'}$ and the $\pi*_{x'}$ orbitals, we should have incorporated spin in the $\pi*_{y'}$ and n orbitals where the major axis for the hyperfine coupling of the n orbital would be along the z axis. This approach led to unrealistic negative spin density in the 2p part of the n orbital.

Estimating Spin Density in the d Orbitals from the Anisotropic Character of the Cu Hyperfine Tensor

We apply methods and equations developed by Sojka et al.[5] (pp. 4836-4837) to estimate the orbital coefficients of the copper d(z^2) and d(yz) orbitals. We first write the copper hyperfine tensor in terms of its isotropic part ${}^{Cu}A_{iso}$, and its anisotropic, traceless part, ${}^{Cu}\mathbf{T}$. Couplings are taken from our data in Table 1.

${}^{Cu}A_x = {}^{Cu}A_{iso} + {}^{Cu}T_{xx} = 102$ MHz

${}^{Cu}A_y = {}^{Cu}A_{iso} + {}^{Cu}T_{yy} = 124$ MHz

${}^{Cu}A_z = {}^{Cu}A_{iso} + {}^{Cu}T_{zz} = 238$ MHz

Thus ${}^{Cu}A_{iso} = 154.7$ MHz and ${}^{Cu}T_{xx} = -52.7$ MHz, ${}^{Cu}T_{yy} = -30.6$ MHz, and ${}^{Cu}T_{zz} = 83.3$ MHz.

According to the methods of Sojka et al.,[5] expressions for ${}^{Cu}T_{xx}$, ${}^{Cu}T_{yy}$, and ${}^{Cu}T_{zz}$ are written in terms of the respective admixture coefficients, c_1 and c_2, of the d(z^2) and d(yz) orbitals (where $|c_1|^2 + |c_2|^2 = 1$). The net 3d spin population is ρ^{3d}. $P = g_e g_{Cu} \beta_e \beta_n \langle r^{-3} \rangle_{3d}$, and it is used in estimating the dipolar coupling of an electron spin in a copper 3d orbital to the copper nucleus. P is provided from theory and P = 1080 MHz or 360 X 10^{-4} cm^{-1}. In the Sojka et al.[5] approach an estimate of the ratio of $|c_2|^2$ to $|c_1|^2$ is initially made graphically (Figure 8 in Sojka et al.) to fit the ratioed values of ${}^{Cu}T_{zz}$, ${}^{Cu}T_{xx}$, and ${}^{Cu}T_{yy}$.

$${}^{Cu}T_{xx} = (2P/7)\, \rho^{3d} \left[-|c_1|^2 - 2\,|c_2|^2 \right]$$

$${}^{Cu}T_{yy} = (2P/7)\, \rho^{3d} \left[(-|c_1|^2 + |c_2|^2)\cos^2\beta + (2|c_1|^2 + |c_2|^2)\sin^2\beta + (2\sqrt{3}) c_1 c_2 \sin\beta \cos\beta \right]$$

$$^{Cu}T_{zz} = (2P/7)\,\rho^{3d}[\,(-|c_1|^2 + |c_2|^2)\sin^2\beta + (2|c_1|^2 + |c_2|^2)\cos^2\beta - (2\sqrt{3})c_1c_2\sin\beta\cos\beta]$$

$$\tan 2\beta = -2c_2/(3c_1)$$

(It should be pointed out that these formulas are those of Eq. 9 on p.4836 of Sojka et al.,[5] but with the "x" and the "y" labels interchanged to accommodate the fact that our 3*d* electron admixture is of the d(z^2) and d(yz) orbitals rather than d(z^2) and d(xz).).

The hyperfine tensor, $^{Cu}T_{xx}$, $^{Cu}T_{yy}$, $^{Cu}T_{zz}$ was fit with the following parameters: $|c_1|^2 = 0.75$, $|c_2|^2 = 0.25$, $\beta = -10.5°$, and $\rho^{3d} = 0.14$. *Thus the copper anisotropic hyperfine coupling is fit by 14 % 3d copper character, where 10.5 % is in the d(z^2) orbital and 3.5 % is in the d(yz) orbital.*

References and Footnotes

(1) Ruggiero, C. E.; Carrier, S. M.; Antholine, W. E.; Whittaker, J. W.; Cramer, C. J.; Tolman, W. B., *J. Am. Chem. Soc.* **1993,** *115*, 11285-11298

(2) McElroy, J. D.; Feher, G.; Mauzerall, D. C., *Biochim Biophys Acta* **1972,** *267*, 363-374

(3) The Fermi coupling in MHz is related to the fraction of unpaired spin in a *2s* orbital on ^{14}N as follows: $A_{Fermi} = (16 \text{ X } 10^{-6}) f_{2s} g_n \beta_e \beta_n |\psi_{o2s}|^2 \pi/(3h) = (1.59 \text{ X } 10^3)\, f_{2s}$ (MHz), Where f_{2s} is the fraction of unpaired electron spin in the nitrogen *2s* orbital, g_n is the ^{14}N nuclear g-value (= 0.40347), β_e and β_n are the electron and nuclear Bohr magnetons, $|\psi_{o2s}|^2 = 33.4 \text{ X } 10^{24}$ cm^{-3} (Hartree, D. R.; Hartree, W., *Proc. R. Soc. London, Ser. A.* **1949,** 193, 299-304.) is the *2s* wave function at the nitrogen nucleus, and h is Planck's constant.

(4) The anisotropic contribution from a *2p* electron on ^{14}N is related to the fraction of unpaired spin in that orbital as follows: $A_p = (4 \text{ X } 10^{-6})\, f_{2p} g_n \beta_e \beta_n \langle r^{-3} \rangle_{2p}/(5h) = (48.1)\, f_{2p}$ (MHz), Where f_{2p} is the fraction of unpaired electron spin in a particular nitrogen *2p* orbital, g_n is the ^{14}N nuclear g-value (= 0.40347), β_e and β_n are the electron and nuclear Bohr magnetons, $\langle r^{-3} \rangle_{2p} = 21.1 \text{ X } 10^{24}$ cm^{-3} (Hartree, D. R.; Hartree, W., *Proc. R. Soc. London, Ser. A.* **1949,** 193, 299-304.) is the expectation value of r^{-3} for a nitrogen *2p* orbital, h is Planck's constant.

(5) Sojka, Z.; Che, M.; Giamello, E., *J. Phys. Chem. B* **1997,** *101*, 4831-4838