Supporting Information:

Heme-Copper Assembly Mediated Reductive Coupling of Nitrogen Monoxide (•NO)

Jun Wang, Mark P. Schopfer, Amy A. N. Sarjeant and Kenneth D. Karlin *
Department of Chemistry, The Johns Hopkins University, Baltimore, MD 21218.

*Corresponding Author: karlin@jhu.edu

Contents:

1. Materials and Methods.

Unless otherwise stated all solvents and chemicals used were of commercially available analytical grade. Nitrogen monoxide (•NO) gas was obtained from Matheson Gases and passed multiple times through a column containing KOH pellets and through an acetone/liquid N_2 (-78 ºC) cooled trap to remove impurities. The purified $•NO_{(g)}$ was passed into a Schlenk flask placed in liquid N_2, to freeze. For use in reactions, this frozen gas was briefly warmed with the -78 ºC bath and allowed to pass into an evacuated Schlenk flask (typically 50 mL) fitted with a septum. Addition of $•NO_{(g)}$ to metal complex solutions was effected by transfer via a three-way long syringe needle. Dinitrogen oxide (N_2O) gas as a was purchased from Airgas as a custom mixture, at a concentration of 250 ppm,, balanced with dinitrogen at 1 atm. Dichloromethane (CH_2Cl_2), acetonitrile (MeCN), methanol (MeOH) and pentane were used after passing them through a 60 cm long column of activated alumina (Innovative Technologies, Inc.) under argon. Tetrahydrofuran (THF) and acetone were purified and dried by distillation from sodium/benzophenone ketyl. Preparation and handling of air sensitive compounds were performed under an argon atmosphere using standard Schlenk techniques or in an MBraun Labmaster 130 inert atmosphere (<1 ppm O_2, <1 ppm H_2O) drybox filled with nitrogen. Deoxygenation of solvents was effected by either repeated freeze/pump/thaw cycles or bubbling with argon for 30 - 45 minutes. UV-vis spectra were recorded on a Hewlett-Packard Model 8453A diode array spectrophotometer equipped with a two-window quartz H.S. Martin Dewar filled with cold MeOH (25 °C to -85 °C) maintained and controlled by a Neslab VLT-95 low temp circulator. Spectrophotometer cells used were made by Quark Glass with column and pressure/vacuum side stopcock and 1 cm path length. Electron paramagnetic resonance (EPR) spectra were recorded on a Bruker EMX spectrometer controlled with a Bruker ER 041 X G microwave bridge operating at X-band (~9.4 GHz). ESI mass spectra were acquired using a Finnigan LCQDeca ion-trap mass spectrometer equipped with an electrospray ionization source (Thermo Finnigan, San Jose, CA). The heated capillary temperature was 250 °C and the spray voltage was 5 kV. X-ray diffraction was performed at the X-ray diffraction facility at the Johns Hopkins University. The X-ray intensity data were measured on an Oxford Diffraction Xcalibur3 system equipped with a graphite monochromator and an Enhance (Mo) X-ray Source ($\lambda = 0.71073$Å) operated at 2 kW power (50 kV, 40 mA) and a CCD detector. The frames were integrated, scaled and corrected for absorption using the Oxford Diffraction CrysAlisPRO software package. Gas chromatography analysis was performed on a Varian CP-3800 instrument equipped with a 1041 manual injector, electron conductivity detector, and a 25m 5Å molecular sieve capillary column. Ion chromatography analysis was performed on a Dionex DX-120 Ion chromatograph, with an AS40 automated sampler, and an IonPac AS14 (4*250 mm) column. The eluent is 3.5 mM Na_2CO_3, 1.0 mM $NaHCO_3$.

2. Synthesis of $(F_8)Fe(NO)$.

This synthesis is a modified version of that reported earlier for tetraphenylporphinato-iron-**(NO).**[a] Under Ar, 300 mg $(F_8)Fe^{III}Cl$ [b] was dissolved in 30 mL degassed solvent mixture of CH_2Cl_2 and MeOH (10:1). Purified nitrogen monoxide (•NO) was bubbled into the solution through the purifier (see above) for 2 min. The solvent was concentrated in vacuo, and addition of 70 mL deoxygenated pentane precipitated $(F_8)Fe(NO)$ as a purple solid. This precipitate was redissolved in 8 mL CH_2Cl_2 and precipitated twice with the addition of 80 mL pentane, yielding a purple powder in 67 % yield (200 mg). IR (KBr, cm^{-1}) ν_{NO} = 1683. UV-vis (λ_{max}, nm): THF, 410 (Soret), 546; CH_2Cl_2, 400 (Soret), 472, 541; acetone, 399(Soret), 472, 541. EPR spectrum (**Figure S1**) in Acetone at 77 K: $g_1 = 2.024$, $g_2 = 2.011$, $g_3 = 1.999$. ESI-MS (**Figure S2**) (812.47, $(F_8)Fe$; 843.51, $(F_8)Fe(NO)$+H). Anal. Calcd. For $\{(F_8)Fe(NO)+0.4CH_2Cl_2\}$; $C_{44.4}H_{20.8}Cl_{0.8}F_8FeN_5O$: C, 60.84; H, 2.39; N, 7.99. Found: C, 61.21; H, 2.18; N, 7.79.

[a] Scheidt, W. R.; Frisse, M. E. *J. Am. Chem. Soc.* **1975**, *97*, 17-21.
[b] Karlin, K. D.; Nanthakumar, A; Fox, S.; Murthy, N.N.; Ravi, N.; Huynh, B.H.; Orosz, R.D.; Day, E.P. *J. Am. Chem. Soc.* **1994**, *116*, 4753-4763.

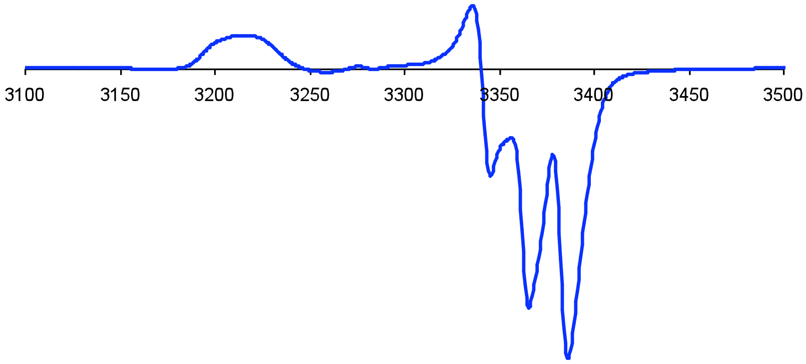

Figure S1. EPR spectrum of $(F_8)Fe(NO)$ in Acetone at 77 K

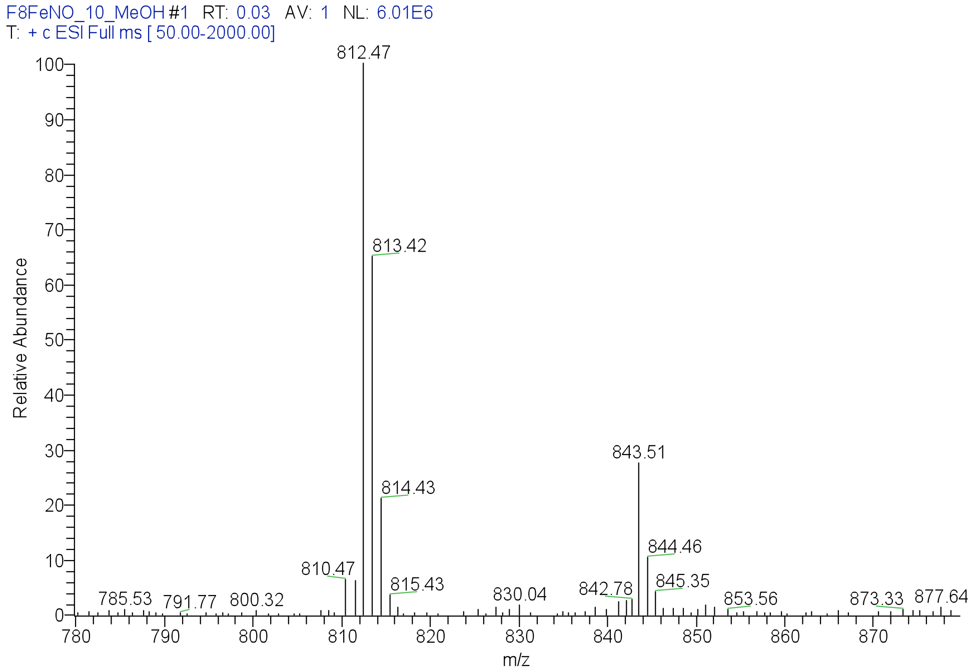

Figure S2. ESI-MS spectrum of $(F_8)Fe(NO)$

3. Crystal structure of $(F_8)Fe(NO)$.

X-ray (**Figure S3**) analysis of $(F_8)Fe(NO)$ indicates that NO binds to the five coordinated iron (II) ion via a bent mode.

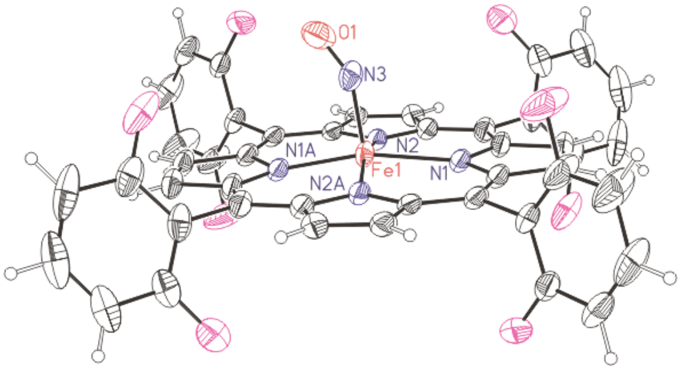

Figure S3. ORTEP of $(F_8)Fe(NO)$ showing ellipsoids at 50% probability level. A minor component of disorder is not shown.

Table S1. Selected Distance and Angles for $(F_8)Fe(NO)$

Bond Lengths (Å)		**Bond Angles (deg.)**	
Fe(1)-N(3)	1.738(5)	Fe(1)-N(3)-O(1)	140.4(5)
N(3)-O(1)	1.167(9)	N(1)-Fe(1)-N(2A)	91.78(12)
Fe(1)-N(1)	2.064(3)	N(2A)-Fe(1)-N(1A)	90.10(13)
Fe(1)-N(1A)	1.934(3)	N(1A)-Fe(1)-N(2)	87.46(11)
Fe(1)-N(2)	1.996(3)	N(1)-Fe(1)-N(2)	87.10(12)
Fe(1)-N(2A)	2.019(3)		

4. Synthesis of (^{6}L)Fe(NO) (1).

This synthesis is a modified version of that reported earlier for tetraphenylporphinato-iron-**(NO).**[a] Under Ar, 200 mg (^{6}L)FeOH [c] was dissolved in a 20 mL degassed solvent mixture of CH_2Cl_2 and MeOH (10:1). Purified nitrogen monoxide (•NO) was bubbled into the solution through the purifier for 2 min. The solvent was concentrated in vacuo, and addition of 70 mL deoxygenated pentane precipitated **1** as a purple solid. This precipitate was redissolved in 8 mL CH_2Cl_2 and precipitated twice using 80 mL pentane. Decantation of the solvent, washing with pentane and drying in vacuo yielded a purple powder (150 mg, 75% yield). Anal. Calcd. For (^{6}L)Fe(NO) (**1**); $C_{63}H_{40}F_6FeN_9O_2$: C, 67.27; H, 3.58; N, 11.21. Found: C, 67.16; H, 3.64; N, 11.05. IR (KBr, cm^{-1}) ν_{NO} = 1687. UV-vis (λ_{max}, nm): THF, 411 (Soret), 547; CH_2Cl_2, 402 (Soret), 472, 541; acetone, 400 (Soret), 472, 541. EPR spectrum (Figure S4) in acetone at 77 K: g_1 = 2.024, g_2 = 2.014, g_3 = 2.004. ESI-MS (Figure S5) (1125.26, (^{6}L)Fe(NO)+H; 1147.25, (^{6}L)Fe(NO)+Na).

[c] Obias, H. V.; van Strijdonck, G. P. F.; Lee, D.-H.; Ralle, M.; Blackburn, N. J.; Karlin, K. D. *J. Am. Chem. Soc.* **1998**, *120*, 9696-9697.

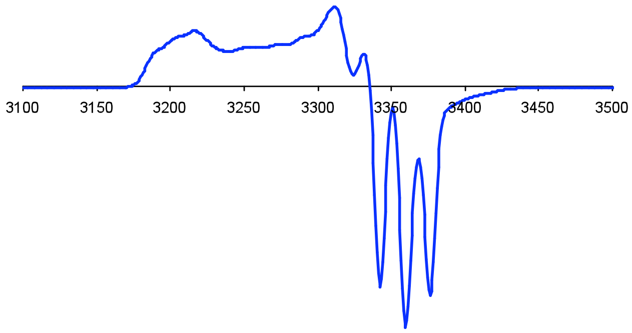

Figure S4. EPR spectrum of (^{6}L)Fe(NO) (1) in Acetone at 77 K

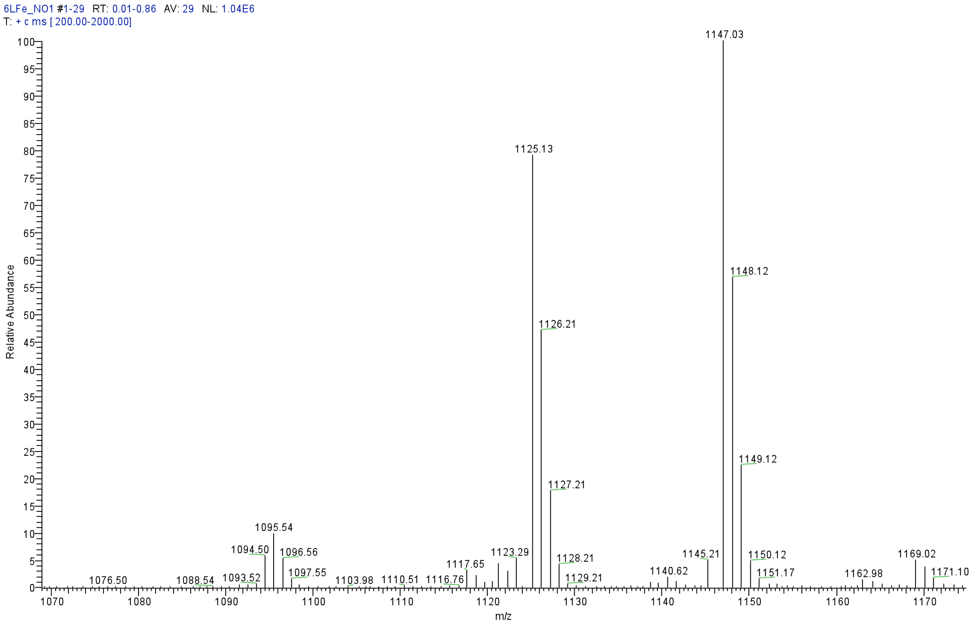

Figure S5. ESI-MS spectrum of (^{6}L)Fe(NO) (1)

5. Generation of (^{6}L)Fe(NO)$_2$ (2).

A 5 mL acetone solution of (^{6}L)Fe(NO) (**1**) was taken in a UV-vis cuvette assembly under argon which was cooled to –80ºC and an initial spectrum was recorded. Excess NO$_{(g)}$ was bubbled through the cold solution using a three-way long needle syringe, and the resulting new spectrum was recorded, **Figure S6**, λ_{max} = 427 (Soret), 540 nm. Additional bubbling with •NO$_{(g)}$ did not change the spectrum further. This resulting product species (^{6}L)Fe(NO)$_2$ (**2**) at –80 ºC released NO when warming up to RT., and (^{6}L)Fe(NO)$_2$ (**2**) was regenerated when cooling to –80 ºC or re-adding more NO$_{(g)}$. Complex (^{6}L)Fe(NO)$_2$ (**2**) in acetone (77 K) is EPR silent.

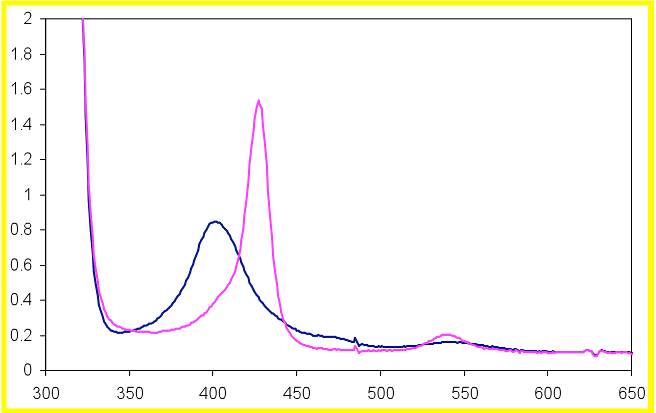

Figure S6. UV-vis spectrum of NO reversible binding to (^{6}L)Fe(NO) (**1**)

Titration of (^{6}L)Fe(NO)$_2$ (**2**) with (F$_8$)Fe^{II} ^{d} was performed in acetone at –80 ºC under Ar. (^{6}L)Fe(NO)$_2$ (**2**) (3.47 x 10^{-5} mol/L, 10 mL) was generated at –80 ºC in acetone and excess NO$_{(g)}$ was removed via six vacuum/purge cycles. Then, one equivalent (F$_8$)Fe^{II} (1.72 x10^{-4} mol/L, 2 mL) was added to the cold solution and the solution mixture was allowed to warm up slowly to room temperature. The yield of (^{6}L)Fe(NO) (**1**) and (F$_8$)Fe(NO) (1:1) was essentially 100% (**Figure S7**). ^{d} Kopf, M.-A.; Neuhold, Y.-M.; Zuberbuhler, A. D.; Karlin, K. D. *Inorg. Chem.* **1999**, *38*, 3093-3102.

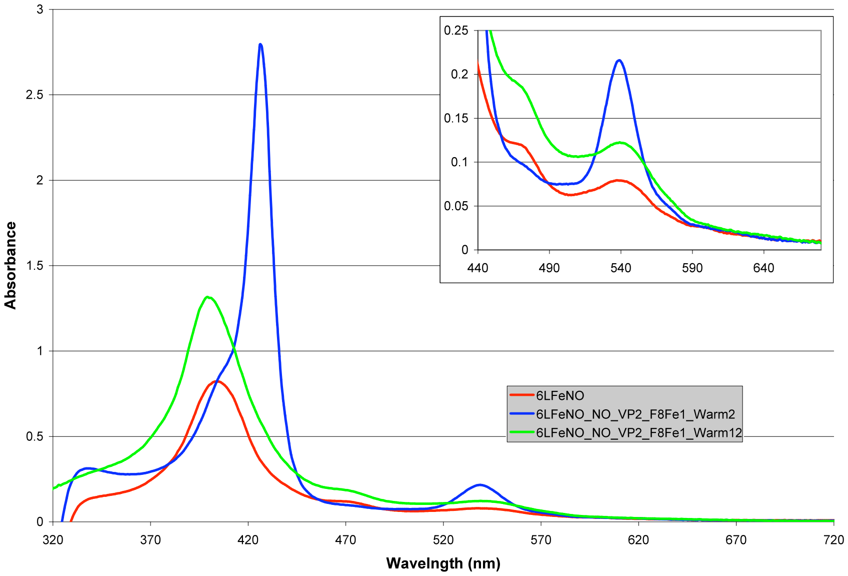

Figure S7. UV-vis spectrum showing a titration of (^{6}L)Fe(NO)$_2$ (**2**) with (F$_8$)Fe^{II} in Acetone, revealing that **2** contains two equiv •NO.

6. Reaction of $(^6L)Fe(NO)_2$ (2) with $[Cu^I(MeCN)_4][B(C_6F_5)_4]$ and acid.

Complex $(^6L)Fe(NO)_2$ (**2**) (0.050 g, 0.044 mmol) was dissolved in 10 mL acetone in a 50 mL Schlenk flask and then cooled to -80ºC using an acetone/dry-ice bath. Excess $NO_{(g)}$ was bubbled through the cold solution using a three-way syringe. And then the excess removed via vacuum purge cycles. After stirring for 30 min, the orange solution was added to the chilled $[Cu^I(MeCN)_4][B(C_6F_5)_4]$ [e] in 10 mL acetone/MeCN (1:1) (0.041 mg, 0.045 mmol) solution via a two-way syringe needle. After stirring for ten minutes, a 5 mL acetonitrile solution of $[H(C_2H_5OC_2H_5)_2][B(C_6F_5)_4]$ (HBArF) [f] (0.074 mg, 0.089 mmol) was added to the cold solution mixture. The warmed-up solution turned to brown at RT. The solution was concentrated in vacuo, and addition of deoxygenated pentane led to brown solid. An EPR spectrum of the brown solid (or that redissolved in acetone) revealed that a high-spin heme-Fe^{III} and Cu^{II} (tetragonal complex) were both present (**Figure S8**). EPR quantification in acetone at 77 K, employing authentic $[(TPP)Fe^{III}]PF_6$ [g] together with $[Cu^{II}(TMPA)(MeCN)](ClO_4)_2$ [h], indicated the presence of a 1:1 mixture of heme-Fe^{III} ($g = 6.01$) and Cu^{II} ($g_{||} = 2.26$, $g_{\perp} = 2.05$, $A_{||} = 150$ G, $A_{\perp} = 36$ G), thus complex $[(^6L)Fe^{III}\ldots Cu^{II}]^{3+}$ (**3**). A UV-vis spectrum gives $\lambda_{max} = 396$ (Soret), 515 nm in THF. An ESI-MS spectrum (**Figure S9**) of the brown solid obtained from evaporation shows that **3** has not unexpectedly converted to the μ-oxo complex $[(^6L)Fe^{III}\text{-O-}Cu^{II}]^+$ (**4**).[c]

A 20 mL dichloromethane solution of the brown product mixed with 20 mL aqueous NaCl solution (400 uM), and stirred for half an hour. Ion chromatography analysis of this upper aqueous layer dilution gave no signal for nitrite ion (NO_2^-). Gas chromatography analysis of the head space of the product mixture revealed a $N_2O_{(g)}$ yield of 80%, corresponding to the NOR stoichiometry of $2\ NO + 2\ H^+ + 2\ e^- \rightarrow N_2O + H_2O$. Thus, the binuclear brown solid is formulated as $[(^6L)Fe^{III}\ldots Cu^{II}]^{3+}$ (**3**), while $N_2O_{(g)}$ is the gaseous product.

[e] Liang, H.-C.; Kim, E.; Incarvito, C. D.; Rheingold, A. L.; Karlin, K. D. *Inorg. Chem.* **2002**, *41*, 2209-2212.

[f] Jutzi, P.; Muller, C.; Stammler, A.; Stammler, H.-G. *Organometallics* **2000**, *19*, 1442-1444.
[g] Reed, C.A.; Mashiko, T.; Bentley, S. P.; Kastner, M. E.; Scheidt, W. R.; Spartalian, K.; Lang, G. *J. Am. Chem. Soc.* **1979**, *101*, 2948-2958.
[h] Fox, S.; Nanthakumar, A.; Wikstrom, M.; Karlin, K. D.; Blackburn, N. J. *J. Am. Chem. Soc.* **1996**, *118*, 24-34.

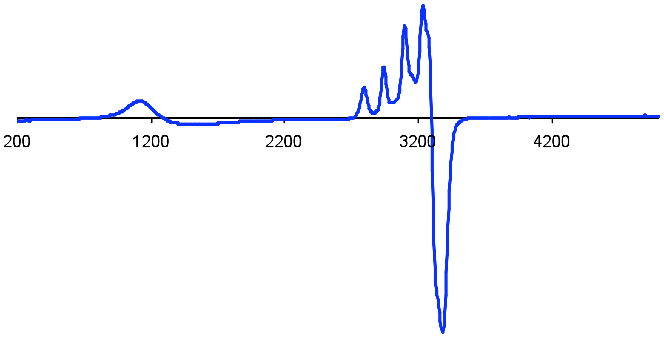

Figure S8. EPR spectrum of $[(^6L)Fe^{III}\ldots Cu^{II}]^{3+}$ (**3**) in acetone at 77 K.

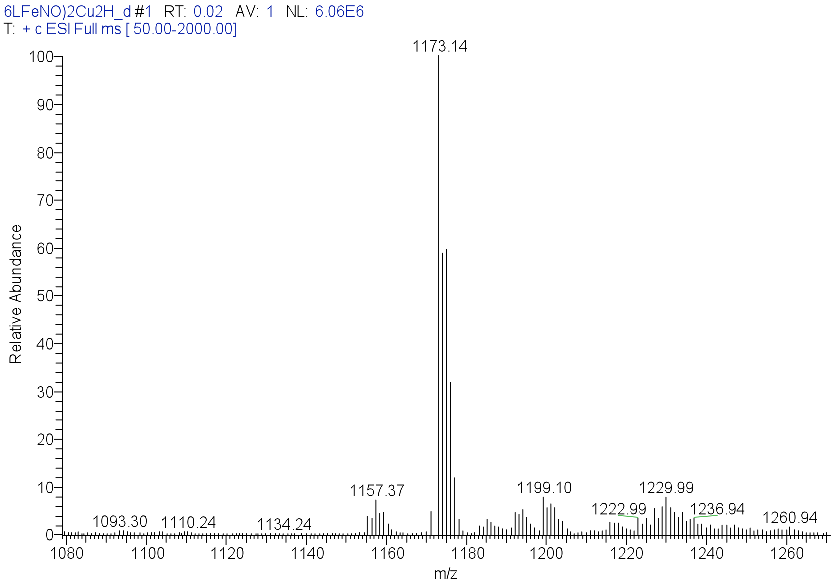

Figure S9. ESI-MS spectrum of $[(^6L)Fe^{III}\ldots Cu^{II}]^{3+}$ (**3**) in MeCN, m/z = 1173. The sample was derived from the brown solid obtained in the reaction of $(^6L)Fe(NO)_2$ (**2**) with $[Cu^I(MeCN)_4][B(C_6F_5)_4]$ and 2 equiv HBArF, indicating it converts to $[(^6L)Fe^{III}\text{-}O\text{-}Cu^{II}]^+$ (**4**) in the ESI instrument. Complex **3** also chemically converts to **4** with addition of Et_3N/H_2O, see text.

7. $[(^6L)Fe^{III}...Cu^{II}]^{3+}$ (3) conversion to $[(^6L)Fe^{III}\text{-O-}Cu^{II}]^+$ (4).

A THF solution of the product $[(^6L)Fe^{III}...Cu^{II}]^{3+}$ (**3**) (λ_{max}, nm: 396, 515), obtained from the reaction of $(^6L)Fe(NO)_2$ (**2**) with $[Cu^I(MeCN)_4][B(C_6F_5)_4]$ and $HB(C_6F_5)_4$, was reacted with one equiv triethylamine/H_2O producing $[(^6L)Fe^{III}\text{-O-}Cu^{II}]^+$ (**4**),[c] λ_{max}, nm: 442, 558 (**Figure S10**).

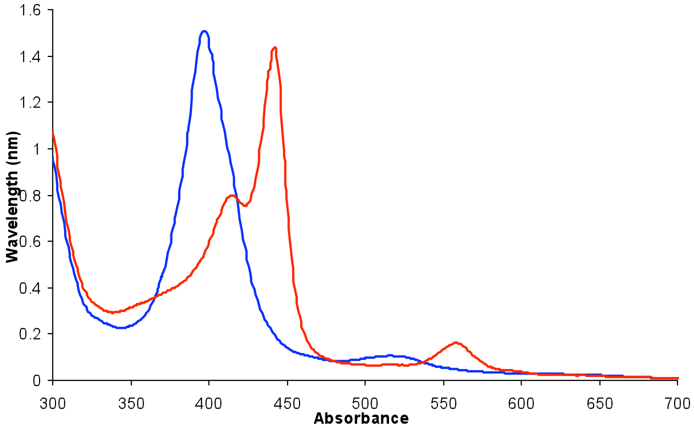

Figure S10. UV-vis spectrum of $[(^6L)Fe^{III}...Cu^{II}]^{3+}$ (**3**) (Blue) conversion to $[(^6L)Fe^{III}\text{-O-}Cu^{II}]^+$ (**4**) (Red) in THF at R.T.

8. Reaction of $(^6L)Fe(NO)_2$ (2) with $[Cu^I(MeCN)_4][B(C_6F_5)_4]$.

Complex $(^6L)Fe(NO)_2$ (**2**) (0.050 g, 0.044 mmol) was dissolved in 10 mL acetone and this solution was cooled to -80ºC using an acetone/dry-ice bath. Excess $•NO_{(g)}$ was bubbled through the cold solution using a three-way syringe. After stirring for 30 min, the cold orange solution of **2** was added to a cold $[Cu^I(MeCN)_4][B(C_6F_5)_4]$ (0.041 mg, 0.045 mmol) solution in 10 mL acetone/MeCN (1:1) via a two-way syringe needle, and the mixture was allowed to stir for 30 min. After warming, the solution was concentrated in vacuo, and addition of 70 mL deoxygenated pentane precipitated **1** as a purple solid. An EPR spectrum matched to a mixture of $(F_8)Fe(NO)$ and Cu^{II} species (**Figure S11**) while an IR spectrum of this solid (Nujol mull) indicates •NO is bound to the $(^6L)Fe$ fragment, $\nu_{NO} = 1687\ cm^{-1}$. UV-vis (λ_{max}, nm): THF, 411 (Soret), 547; CH_2Cl_2, 402 (Soret), 472, 541. A 20 mL dichloromethane solution of the brown product mixed with 20 mL aqueous NaCl solution (400 uM), and stirred for half an hour. Ion chromatography analysis of this upper aqueous layer dilution indicated nitrite (NO_2^-) was present. Gas chromatography analysis of the head space of the reaction products reveal a $N_2O_{(g)}$ yield of 90%, for the reaction stoichiometry: 3 •NO + $[(L)Cu^I]^+$ → N_2O + $[(L)Cu^{II}(NO_2^-)]^+$. Thus, the binuclear purple solid is formulated as $[(^6L)Fe(NO)\ldots Cu(NO_2)]^+$ (**5**), when $N_2O_{(g)}$ is the gaseous product.

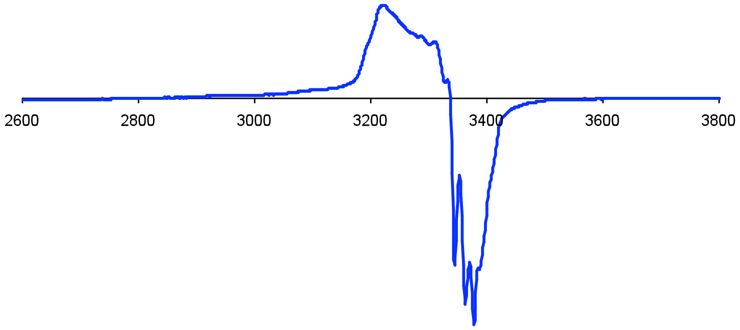

Figure S11. EPR spectrum of $[(^6L)Fe(NO)\ldots Cu(NO_2)]^+$ (**5**) in acetone at 77 K from the reaction of $(^6L)Fe(NO)_2$ (**2**) with $[Cu^I(MeCN)_4][B(C_6F_5)_4]$, indicating it is the mixture of a (heme)Fe-NO and a copper complex. A separate experiment of copper(I) complexes of the tris(2-pyridylmethyl)amine moiety found in ^6L reacting with $•NO_{(g)}$, EPR of the product gave typical Cu^{II} spectrum.

9. Analysis of gaseous products.

Calibration curves were generated in the concentration range needed by injecting various volumes of 250.0 ppm N_2O balanced with nitrogen at 1 atm (purchased from Airgas). The absolute number of moles of N_2O in each injection volume was calculated using the ideal gas relation PV = nRT. Headspace GC analysis of all the above reaction products in **Section 6** and **8** was performed on a Varian CP-3800 instrument equipped with a 1041 manual injector, electron conductivity detector, and a 25m 5Å molecular sieve capillary column. All standard samples were prepared and injected from another separated Schlenk flask with the same volume, containing the same amount N_2O based on the ideal theoretical calculation, either from the NOR stoichiometry of $2\ NO + 2\ H^+ + 2\ e^- \rightarrow N_2O + H_2O$, **or** 3 •NO + $[(L)Cu^I]^+$ → N_2O + $[(L)Cu^{II}(NO_2^-)]^+$. When the oven temperature is 175°C and flow rate equals to 6 mL/min, the retention times are 9.01 (very sharp peak) and 11.75 min for N_2O and CO_2, respectively. Nitrogen monoxide gas (•NO) possessed a retention time of 8.9 (broad leading edge).