

Supporting Information for

Characterization of Iron Dinitrosyl Species Formed in the Reaction of Nitric Oxide with a Biological Rieske Center

*Christine E. Tinberg,^{† §} Zachary J. Tonzetich,^{† §} Hongxin Wang,[‡] Loi H. Do,[†] Yoshitaka Yoda,[‡] Stephen
P. Cramer,^{‡ ¥} and Stephen J. Lippard^{†*}*

[†]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

[‡]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

[‡]Japan Synchrotron Radiation Research Institute, SPring-8, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo
679-5198, Japan

[¥]Department of Applied Science, University of California, Davis, CA 95616

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Table S1. Comparison of 90 K ^{57}Fe Mössbauer parameters for ToMOC-derived and synthetic iron dinitrosyl species recorded at zero-field.

Compound	Isomer Shift, δ (mm/s)	Quadrupole Splitting, ΔE_Q (mm/s)	Linewidth, Γ (mm/s)	Ref
(Et ₄ N)[Fe(NO) ₂ (SPh) ₂] (DNIC)	0.17(2)	0.68(2)	0.26(2)	1
[Fe ₂ (μ -SPh) ₂ (NO) ₄] (RRE)	0.15(2)	0.97(2)	0.29(2)	1
(Et ₄ N)[Fe ₄ (μ_3 -S) ₃ (NO) ₇] (RBS)	0.15(2)	0.81(2)	0.32(2)	1
(Et ₄ N)[Fe ₂ (μ -SPh) ₂ (NO) ₄] (rRRE)	0.23(2)	0.35(2)	0.30(2)	1
ToMOC _{ox} + DEANO	0.15(2)	0.92(2)	0.29(2)	this work
(ToMOC _{ox} + DEANO) _{red}	0.15(2)	0.83(2)	0.31(2)	this work

¹Tonzetich, Z. J.; Wang, H.; Mitra, D.; Tinberg, C. E.; Do, L. H.; Jenney, F. E.; Adams, M. W. W.; Cramer, S. P.; Lippard, S. J. *J. Am. Chem. Soc.* **2010**, *132*, 6914-6916.

Table S2. Dependence on DEANO concentration of rate constants k_{obs1} , k_{obs2} , and k_{obs3} for the reaction of ToMOC_{ox} with DEANO at 25 °C in 25 mM KPi, pH 7.0.

[DEANO] (mM)	k_{obs1} (s ⁻¹)	k_{obs2} (s ⁻¹)	k_{obs3} (s ⁻¹)
0.7	0.092	0.042	0.00038
1.4	0.24	0.080	0.00080
2.0	0.36	0.10	0.00079
3.0	0.45	0.12	0.0013
4.0	0.61	0.13	0.0015

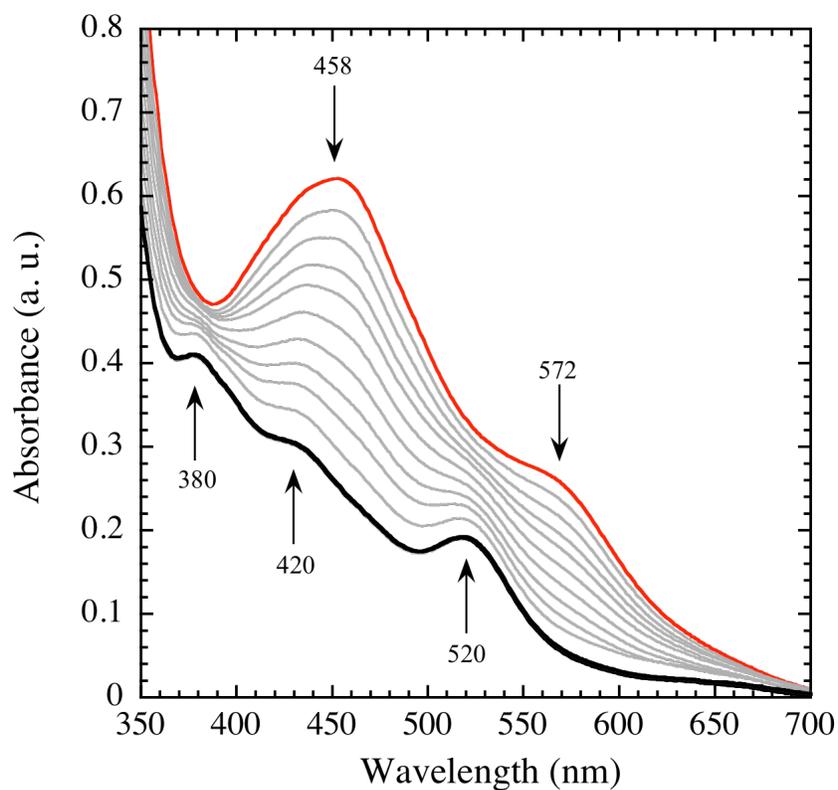


Figure S1. UV-vis spectra of the anaerobic titration of 93 μM ToMOC_{ox} with 937 μM Na₂S₂O₄ at 25 °C in 25 mM KPi buffer, pH 7.0. A solution of Na₂S₂O₄ was added to ToMOC_{ox} in 5 μL aliquots. Spectra of ToMOC_{ox} (red) and ToMOC_{ox} titrated with 5, 10, 15, 20, 25, 30, 35, 40, 45, 55, 60, and 65 (black) μL of Na₂S₂O₄ are depicted. The last three spectra overlap, indicating reaction completion.

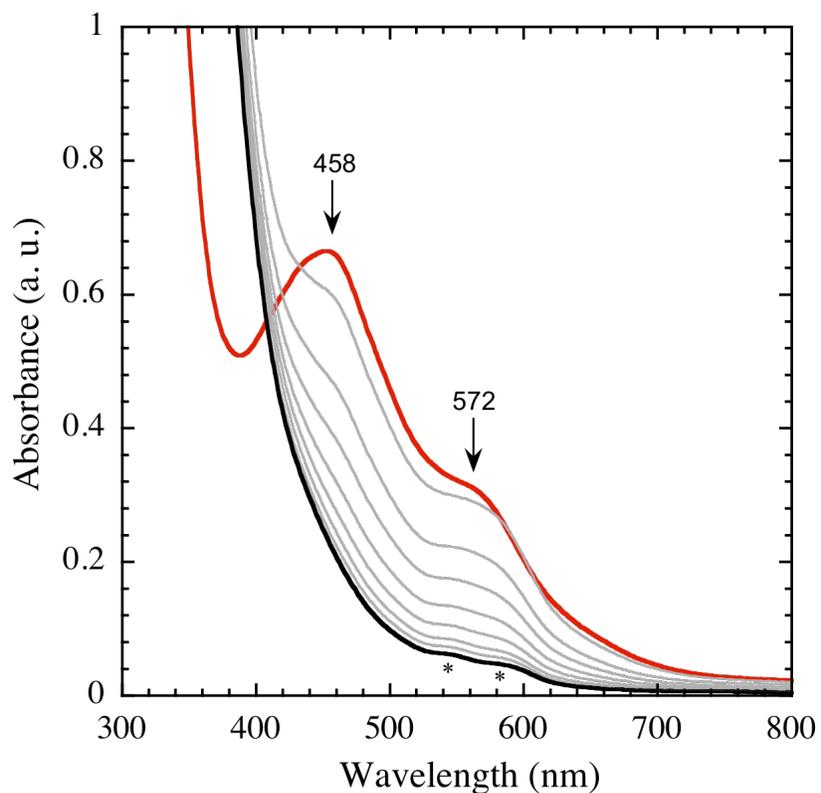


Figure S2. UV-vis spectral changes associated with the anaerobic reaction of 91 μM ToMOC_{ox} with 20 equiv of SNAP at 25 °C in 25 mM KP_i buffer, pH 7.0. Data show the spectra before reaction (red), and at 1 min, 20 min, 40 min, 60 min, 80 min, 100 min, 120 min, and 140 min (black) after reaction initiation. Peaks denoted with an asterisk are due to SNAP.

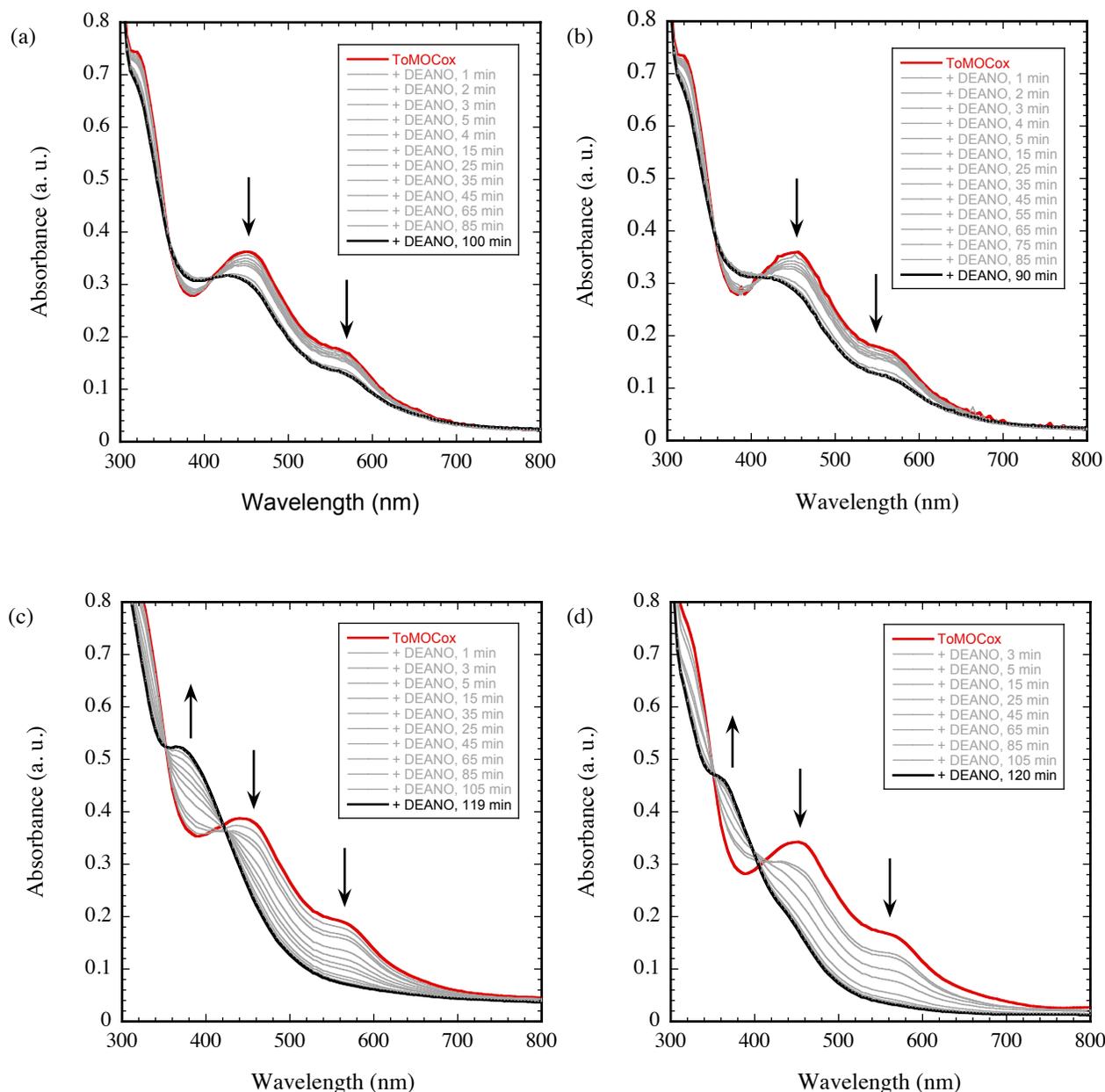


Figure S3. UV-vis spectral changes associated with the anaerobic reaction of 60 μM ToMOC_{ox} with 0.67 equiv DEANO (1 equiv NO) (a), 1.33 equiv DEANO (2 equiv NO) (b), 2.67 equiv DEANO (4 equiv NO) (c), and 6.67 equiv DEANO (10 equiv NO) (d) at 25 °C in 25 mM KP_i buffer, pH 7.0. Data show the spectra before reaction (red), and various times after reaction initiation.

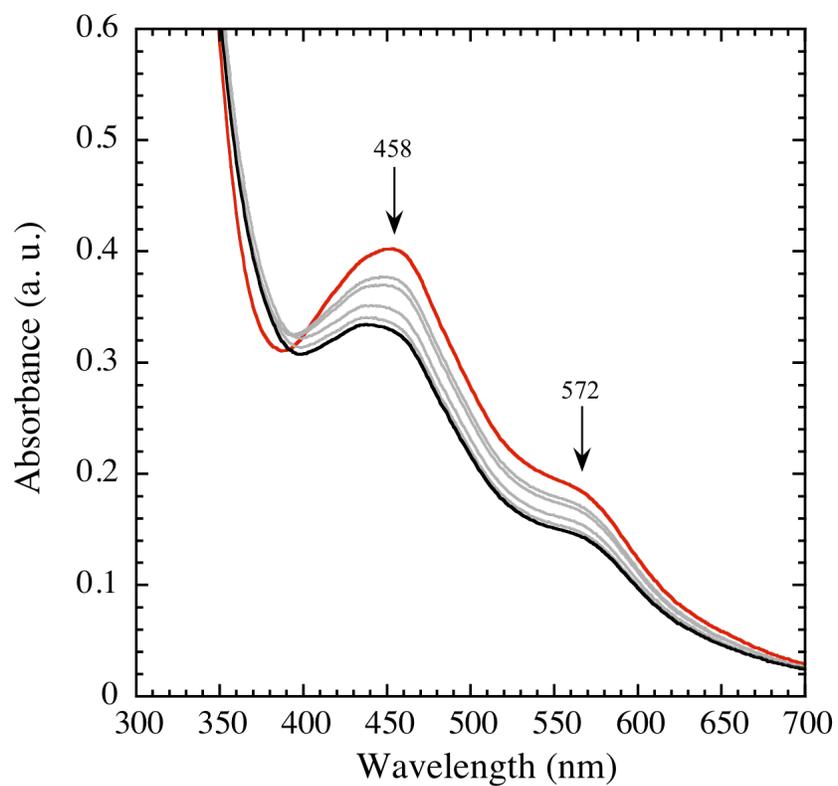


Figure S4. UV-vis spectral changes associated with the anaerobic reaction of 58 μM ToMOC_{ox} with ~ 120 equiv of NO (g) at 25 $^{\circ}\text{C}$ in 25 mM KP_i buffer, pH 7.0. Data show the spectra before reaction (red), and at 10 min, 25 min, 60 min, 80 min, and 90 min (black) after reaction initiation.

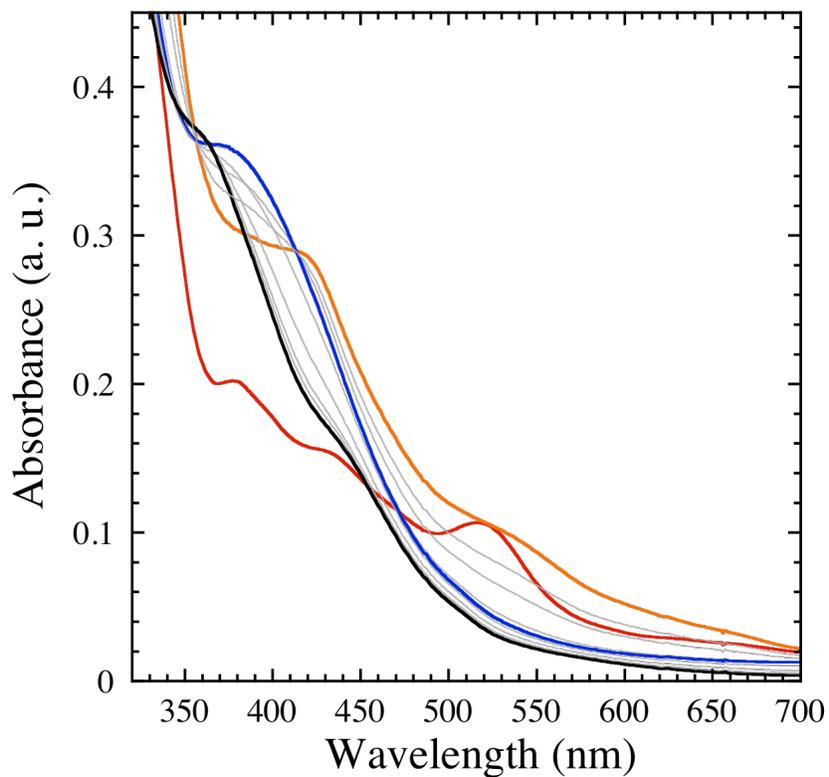


Figure S5. UV-vis spectral changes associated with the anaerobic reaction of 40 μM ToMOC_{red} with 20 equiv of DEANO at 25 °C in 25 mM KP_i buffer, pH 7.0. Data show the spectra before reaction (red), and at intervals after reaction initiation, including 30 s (orange), 5 min (blue), and 64 min (black). The colored spectra reveal clear isosbestic points over the course of the reaction.

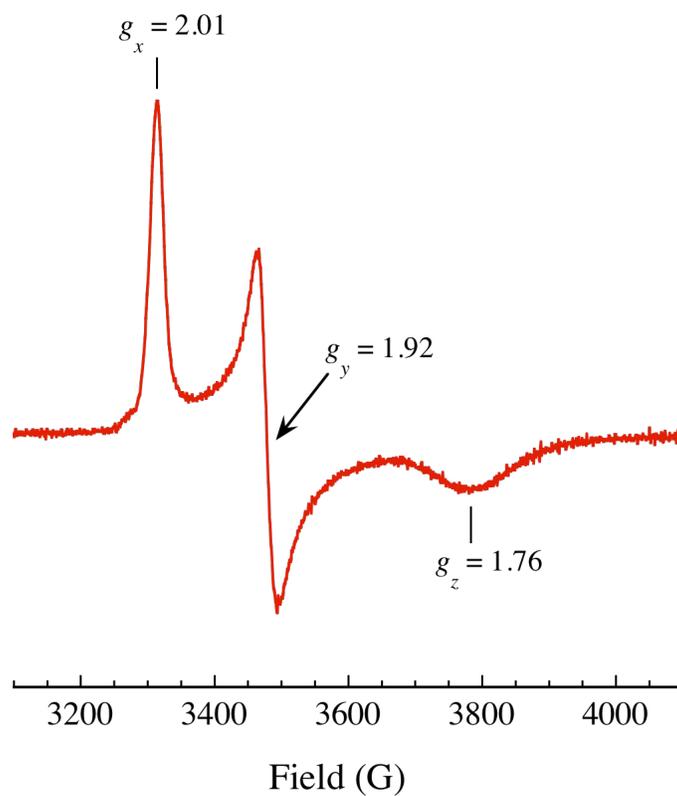


Figure S6. X-Band EPR spectrum (77 K) of 467 μM ToMOC_{red} in 25 mM KP_i, pH 7.0. Instrument parameters: 9.332 GHz microwave frequency; 0.201 mW microwave power; 5.02×10^4 receiver gain; 100.0 kHz modulation frequency; 8.00 G modulation amplitude; 20.480 ms time constant.

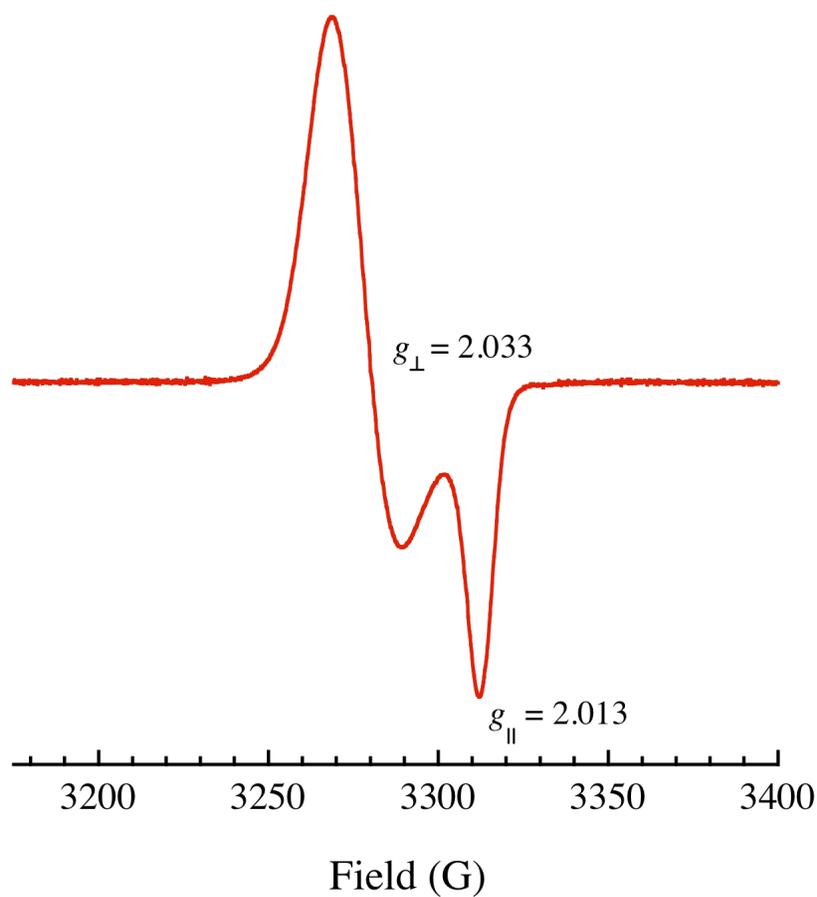


Figure S7. X-Band EPR spectrum (77 K) of the DNIC species formed from reaction of 250 μM ToMOC_{ox} with 20 equiv of SNAP after 1 h at 25 °C in 25 mM KP_i, pH 7.0. Instrument parameters: 9.332 GHz microwave frequency; 0.201 mW microwave power; 5.02×10^3 receiver gain; 100.0 kHz modulation frequency; 8.00 G modulation amplitude; 40.960 ms time constant.

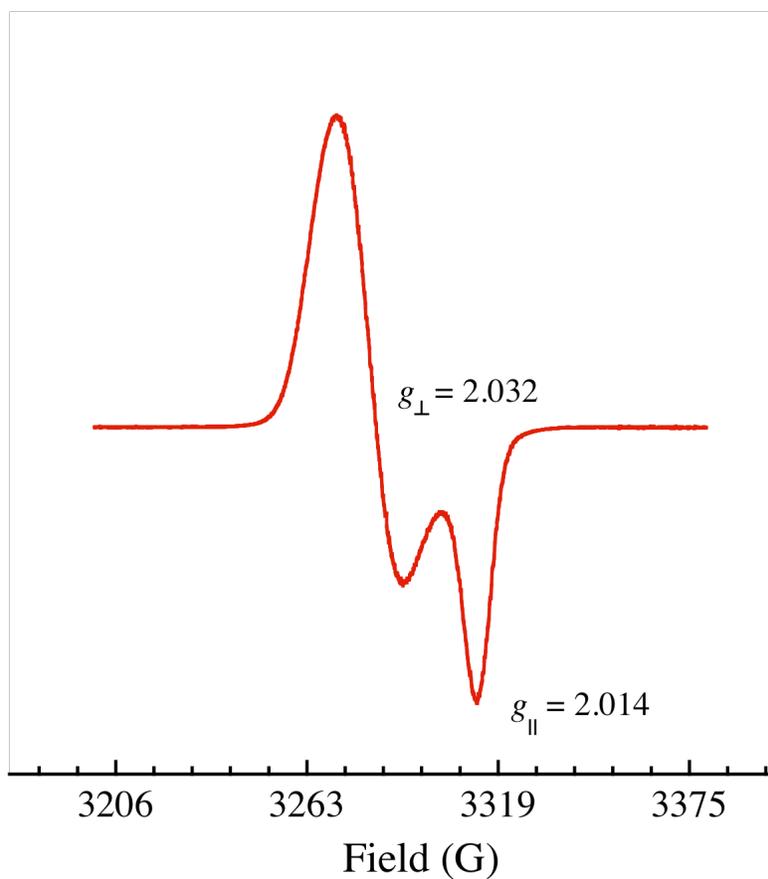


Figure S8. X-Band EPR spectrum (77 K) of the DNIC species formed from reaction of 250 μM ToMOC_{ox} with 100 equiv of NO (g) after 1 h at 25 °C in 25 mM KP_i, pH 7.0. Instrument parameters: 9.332 GHz microwave frequency; 0.201 mW microwave power; 5.02×10^3 receiver gain; 100.0 kHz modulation frequency; 8.00 G modulation amplitude; 40.960 ms time constant.

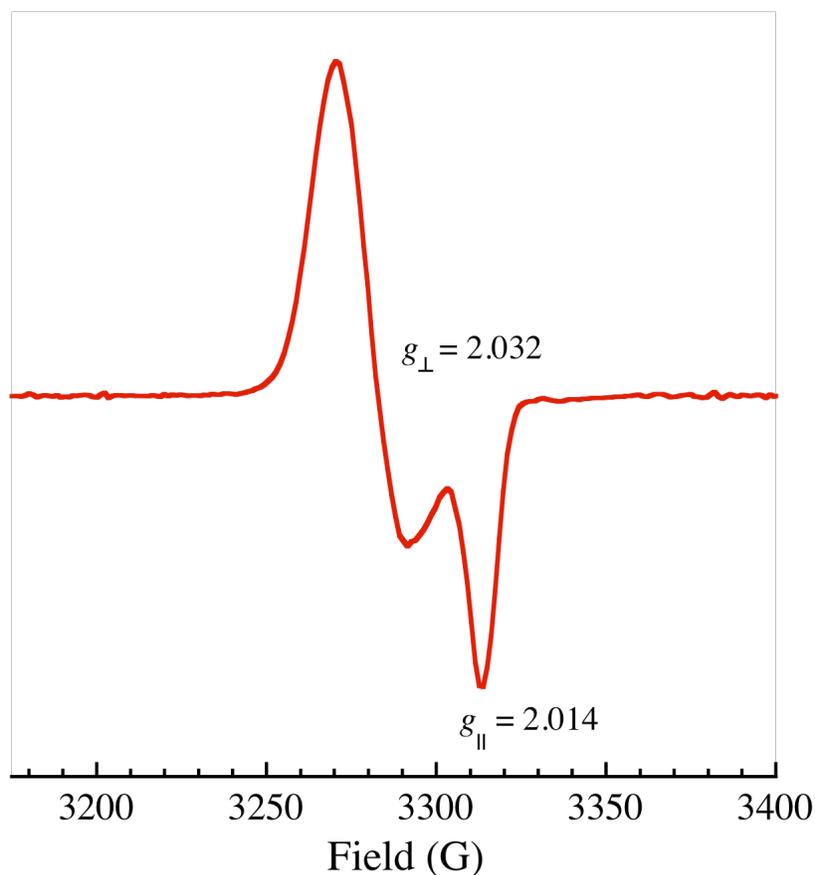


Figure S9. X-Band EPR spectrum (77 K) of the DNIC species formed from reaction of 250 μM $\text{ToMOC}_{\text{red}}$ with 15 equiv of DEANO after 1 h at 25 $^{\circ}\text{C}$ in 25 mM KP_i , pH 7.0. Instrument parameters: 9.332 GHz microwave frequency; 0.201 mW microwave power; 1.00×10^3 receiver gain; 100.0 kHz modulation frequency; 8.00 G modulation amplitude; 40.960 ms time constant.

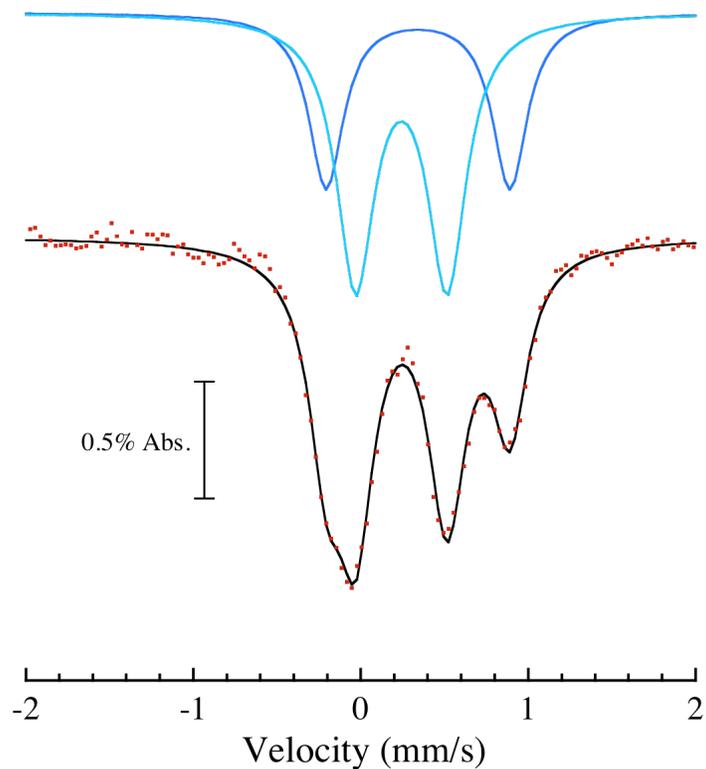


Figure S10. Zero-field ^{57}Fe Mössbauer spectrum (90 K) of $378\ \mu\text{M}$ ^{57}Fe -enriched ToMOC_{ox} in 25 mM KP_i , pH 7.0. Data were fit to two quadrupole doublets with $\delta_1 = 0.34(1)$ mm/s, $\Delta E_{Q1} = 1.10(1)$ mm/s, $\Gamma_1 = 0.25(1)$ mm/s, $\delta_2 = 0.24(1)$ mm/s, $\Delta E_{Q2} = 0.55(1)$ mm/s, $\Gamma_2 = 0.28(1)$ mm/s (black line). Subsites 1 (cyan) and 2 (aqua), shown above the fit, accounted for 42% and 58% of the iron in the sample, respectively.

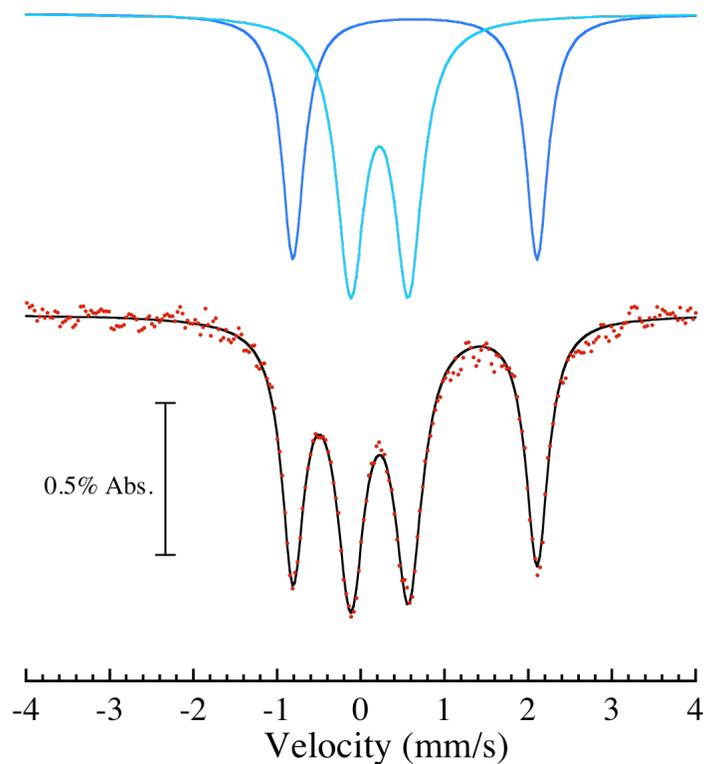


Figure S11. Zero-field ^{57}Fe Mössbauer spectrum (230 K) of 966 μM ^{57}Fe -enriched $\text{ToMOC}_{\text{red}}$ in 25 mM KP_i , pH 7.0. Data were fit to two quadrupole doublets with $\delta_1 = 0.65(1)$ mm/s, $\Delta E_{Q1} = 2.92(1)$ mm/s, $\Gamma_1 = 0.32(1)$ mm/s, $\delta_2 = 0.23(1)$ mm/s, $\Delta E_{Q2} = 0.69(1)$ mm/s, $\Gamma_2 = 0.40(1)$ mm/s (black line). Subsites 1 (cyan) and 2 (aqua), shown above the fit, accounted for 43% and 57% of the iron in the sample, respectively.

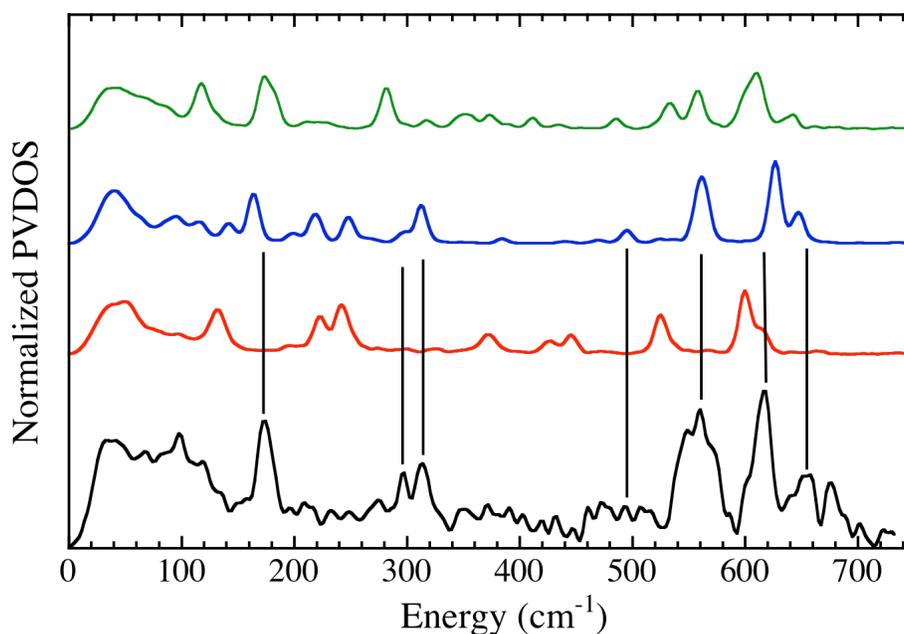


Figure S12. Normalized ^{57}Fe partial vibrational density of states (PVDOS) for 1.66 mM ^{57}Fe -enriched ToMOC_{ox} treated with 10 equiv of DEANO for 1 h at 25 °C in 25 mM KP_i buffer, pH 7.0 (black) compared to those of (Et₄N)[Fe(NO)₂(SPh)₂] (DNIC; red), [Fe₂(μ-SPh)₂(NO)₄] (RRE; blue), and (Et₄N)[Fe₄(μ-S)₃(NO)₇] (RBS; green). Data for the model compounds are taken from Tonzetich, Z. J.; Wang, H.; Mitra, D.; Tinberg, C. E.; Do, L. H.; Jenney, F. E.; Adams, M. W. W.; Cramer, S. P.; Lippard, S. J. *J. Am. Chem. Soc.* **2010**, *132*, 6914-6916.

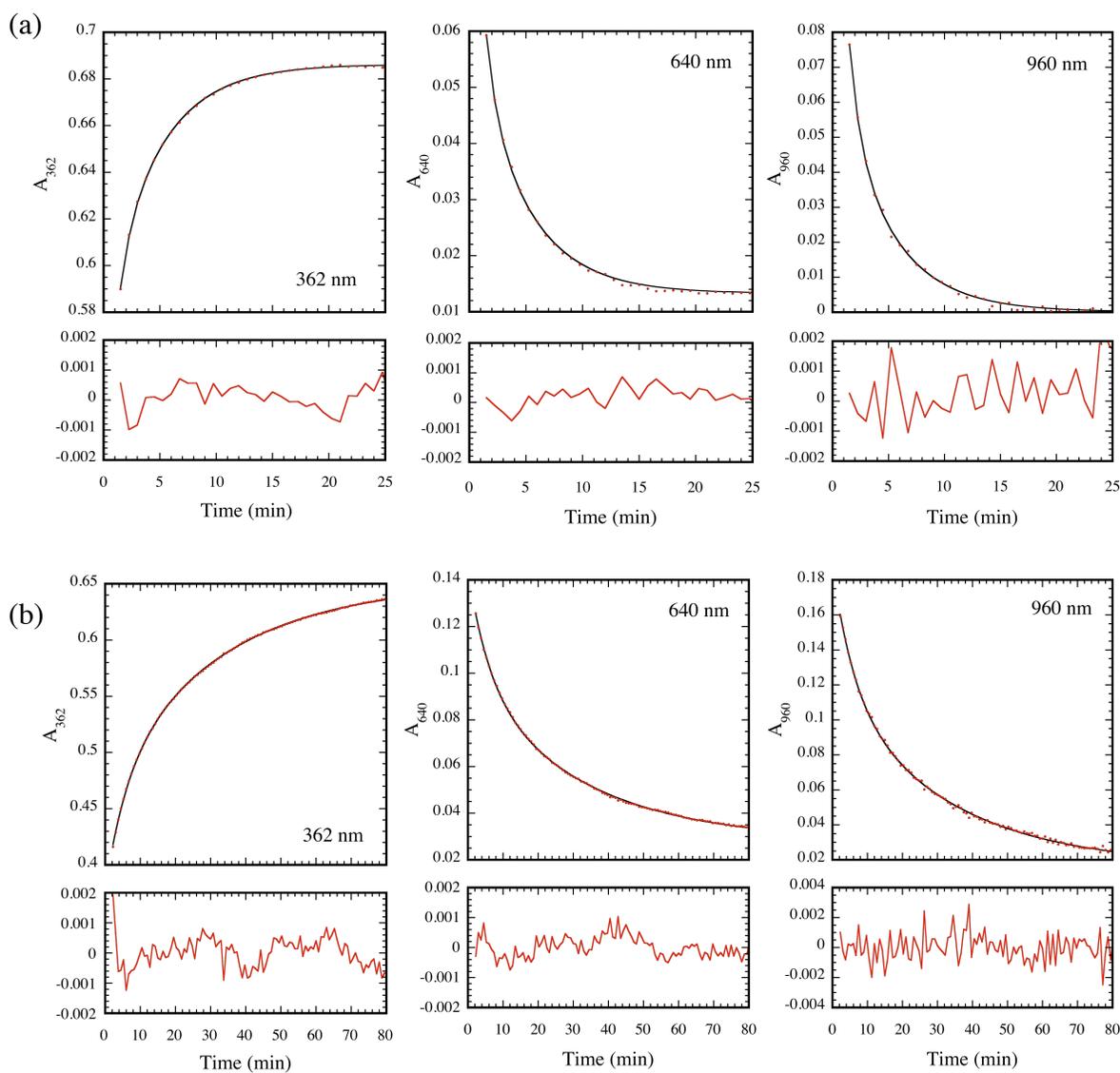


Figure S13. (a) Anaerobic decay kinetics (red points) of the rRRE formed from ToMOC_{NO} generated by reaction of 80 μ M ToMOC_{ox} with 10 equiv DEANO for 1 h at 25 $^{\circ}$ C with 0.5 equiv of Na₂S₂O₄ in 25 mM KP_i, pH 7.0. (b) Anaerobic decay kinetics (red points) of the product of (a) treated a second time with 0.5 equiv of Na₂S₂O₄. All data were fit using the two exponential function $y = A_1 \exp(-k_{\text{obs1}} * t) + A_2 \exp(-k_{\text{obs2}} * t) + \text{Abs}_{t=\infty}$, where t is time, A_1 and A_2 are the pre-exponential factors for the two processes, k_{obs1} and k_{obs2} are the rate constants for the two processes, and $\text{Abs}_{t=\infty}$ is the final absorbance value (black lines). Attempts to fit the data to a one exponential function describing a single process yielded poor fit statistics and residual plots (not shown). Data were fit at 362 nm, 640 nm, and 960 nm simultaneously using shared rate constant parameters. Residual plots are shown in the bottom panels of (a) and (b). For (a), $k_{\text{obs1}} = 1.10 \text{ min}^{-1}$ and $k_{\text{obs2}} = 0.23 \text{ min}^{-1}$. For (b), $k_{\text{obs1}} = 0.17 \text{ min}^{-1}$ and $k_{\text{obs2}} = 0.031 \text{ min}^{-1}$.

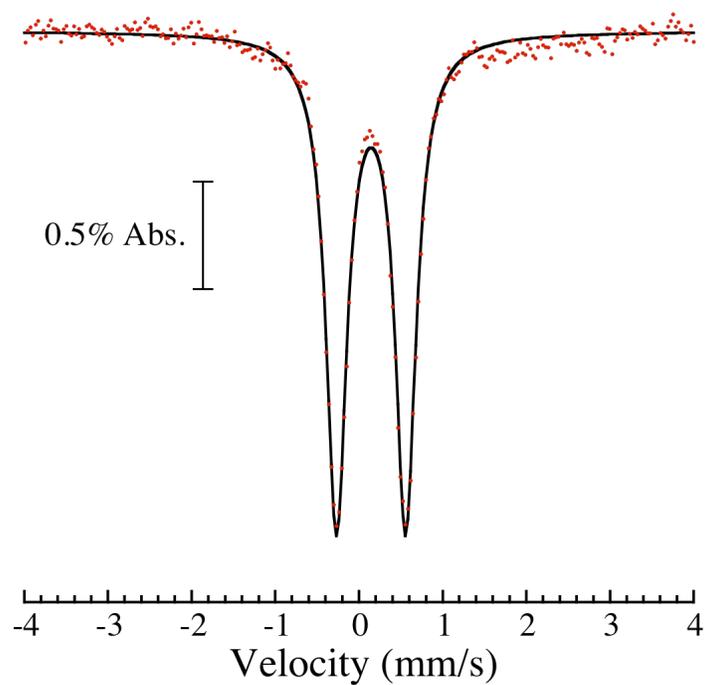


Figure S14. Zero-field ^{57}Fe Mössbauer spectrum (90 K) of the DNIC species formed by incubation of $700\ \mu\text{M}$ ^{57}Fe -enriched ToMOC_{ox} with 34 equiv DEANO for 1 h at $25\ ^\circ\text{C}$ followed by treatment with 1 equiv $\text{Na}_2\text{S}_2\text{O}_4$. Reactions were performed in $25\ \text{mM}$ KP_i , pH 7.0. Data were fit to a single quadrupole doublet with $\delta = 0.15(2)\ \text{mm/s}$, $\Delta E_Q = 0.83(2)\ \text{mm/s}$, and $\Gamma = 0.31(2)\ \text{mm/s}$ (black line).

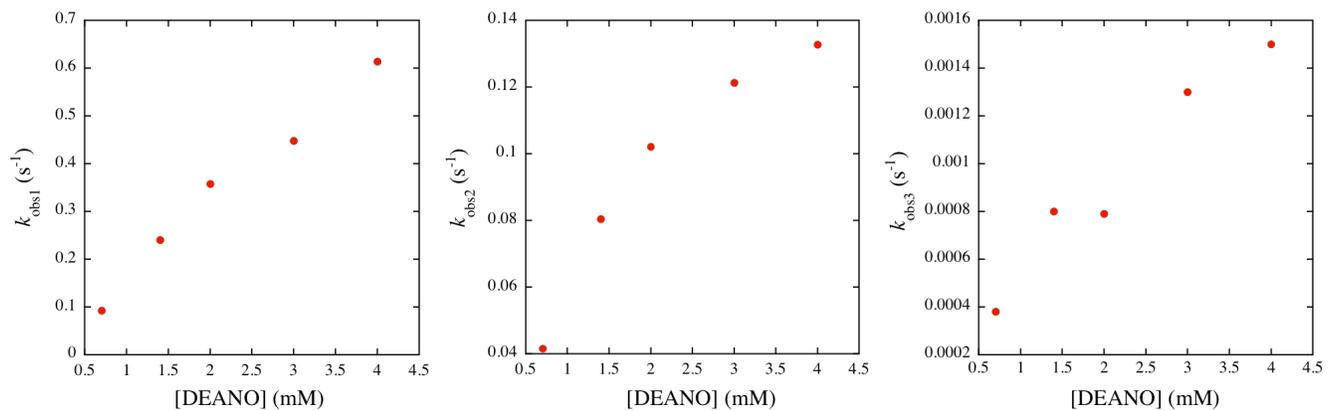


Figure S15. Plots of k_{obs} versus [DEANO] for the reaction of ToMOC_{ox} with DEANO at 25 °C in 25 mM KP_i, pH 7.0.