Electronic Supplementary Material (ESI) for Dalton Transactions.

Dioxygen controls the nitrosylation reactions of a protein-bound [4Fe4S] cluster

Daniel B. Grabarczyk,[a]† Philip A. Ash,[a] William K. Myers,[a] Erin L. Dodd,[a] and Kylie A. Vincent*[a]

Electronic Supplementary Information

Table of Contents:

Sequence data for the *A. ferroxidans* HiPIP construct used in this work.

A. ferroxidans HiPIP construct sequence with cluster binding cysteine residues shown in blue. Cysteine residues from the disulfide bridge are shown in green.

MSEKLKETGNAKAGNISRRDMLKGIAITAGVVAAGTVVGVNPIGAAHAAGNCPGTTPKAEVQYQPHPKGK
DQCSVCANFIAPKCCKVVAGPVAPDGYCIAFTPMPA

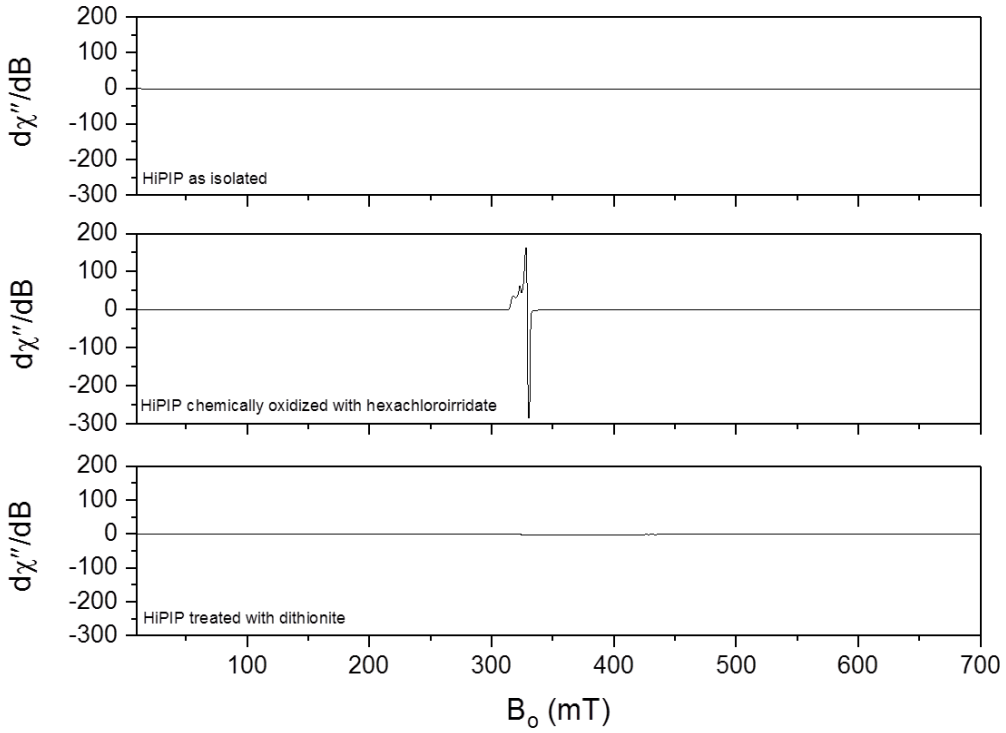

Figure S1: No significant EPR signal seen for HIPIP as isolated or following treatment with $S_2O_4^{2-}$. Wide-range EPR spectra of 120 µM HiPIP as isolated, treated with Na_2IrCl_6 to oxidise, and treated with $Na_2S_2O_4$ to reduce. EPR spectra were recorded at 15K with a microwave power of 10 mW, a field modulation of 1.0 mT and a microwave freq. of 9.3817 GHz.

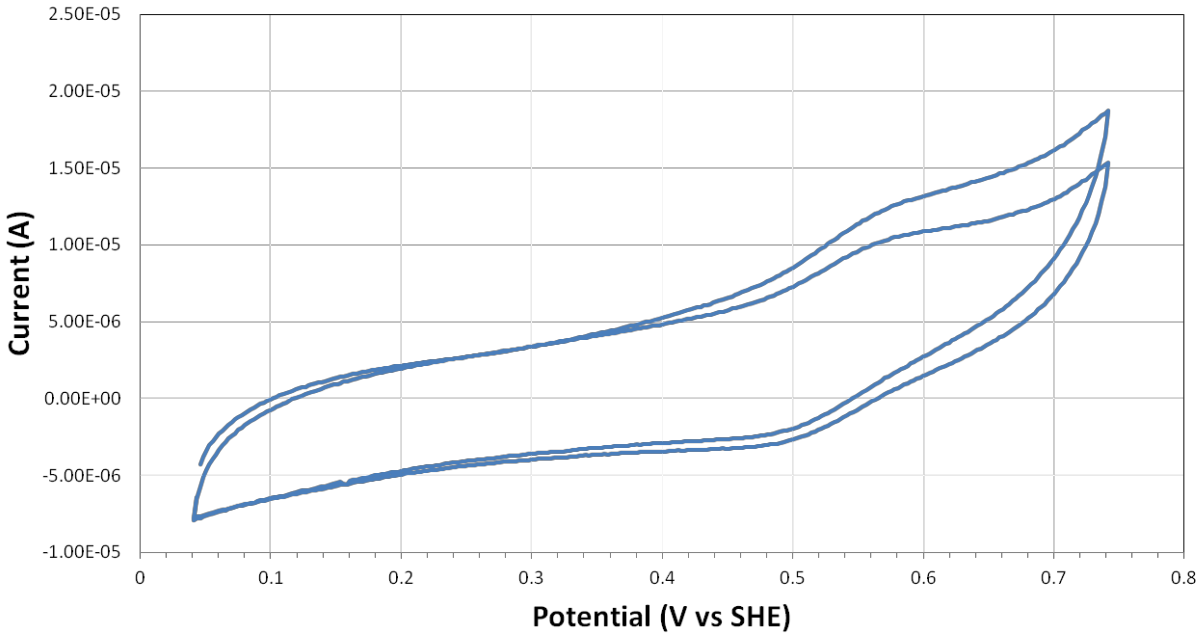

Figure S2: Cyclic voltammetry of HiPIP. Electrochemical experiments were carried out in an anaerobic glovebox (< 1 ppm O_2, Glove Box Technology Ltd) on samples of HiPIP contained within a layer of the polymer electrolyte, Nafion 117 perfluorinated ion exchange resin (Sigma Aldrich, aqueous suspension, 10 % v/v) as previously reported.[1-3] Nafion allows movement of small molecules and ions within hydrated channels, and traps the protein within its structure. The Nafion suspension was mixed with an equal volume of buffer (50 mM HEPES 320 mM NaCl pH 7.5) and then titrated to pH 7.5. An aliquot of HiPIP (0.8 mM, 2 µL) was mixed with 5 µL of the buffered Nafion solution, and placed in contact with a carbon paper (AvCarb P50, Fuel Cell Earth) working electrode in a custom-built small volume electrochemeical cell.[3] Electrochemical control was provided by a PGSTAT 128N potentiostat (Metrohm Autolab) controlled by Nova 1.10 software (EcoChemie). All potentials were recorded relative to a saturated calomel reference electrode (SCE) and were converted to the standard hydrogen electrode (SHE) using the formula $E_{SHE} = E_{SCE} + 0.241$ V at 25 °C.

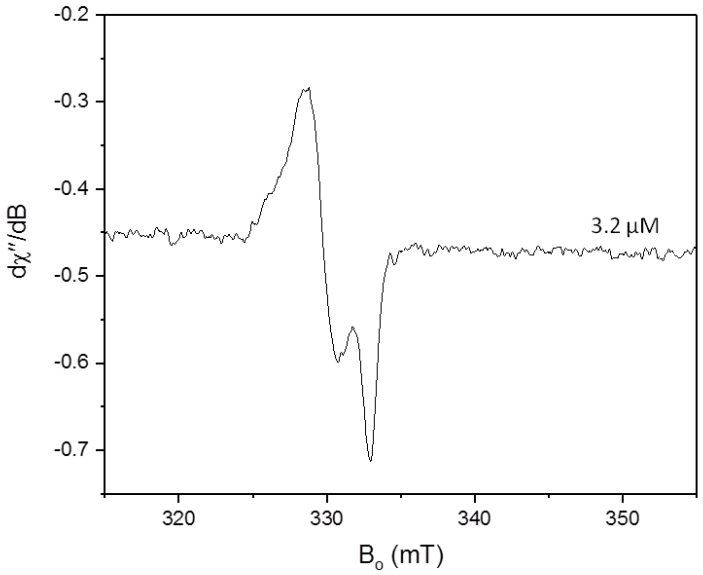

Figure S3: Formation of DNIC products following anaerobic exposure to 10% NO. EPR spectrum at 100K of HiPIP exposed to 10% NO overnight; 200 µW microwave power, 0.4 mT field modulation, and microwave frequencies of 9.3791 GHz.

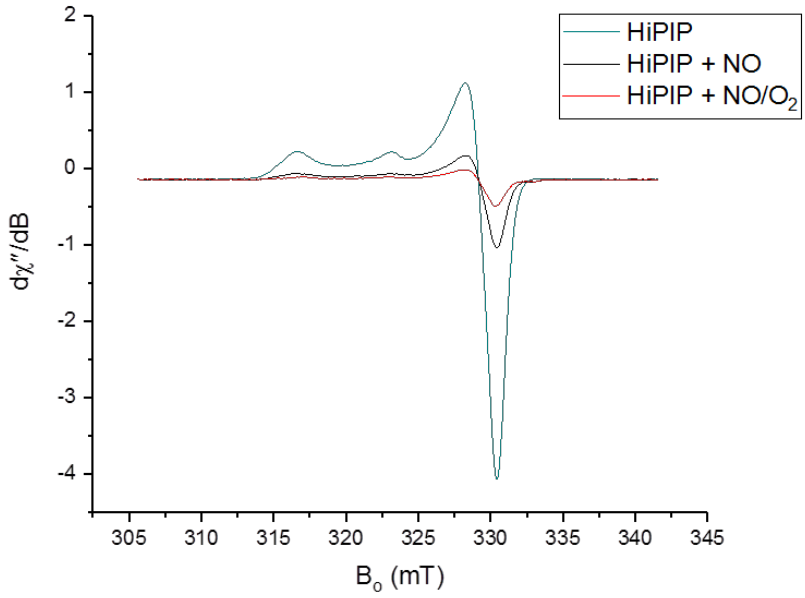

Figure S4: EPR after oxidation by $IrCl_6^{2-}$ shows the relative amounts of remaining unreacted cluster converted to the $[Fe_4S_4]^{3+}$ state. EPR spectra at 15K of HiPIP unreacted (blue); exposed to 10% NO overnight (black) and a 10% NO/ 0.05% O_2 gas mixture for 150 min. (red). EPR measurement conditions were 5 µW microwave power, 0.2 mT field modulation, and microwave frequencies of 9.379 ± 0.002 GHz range.

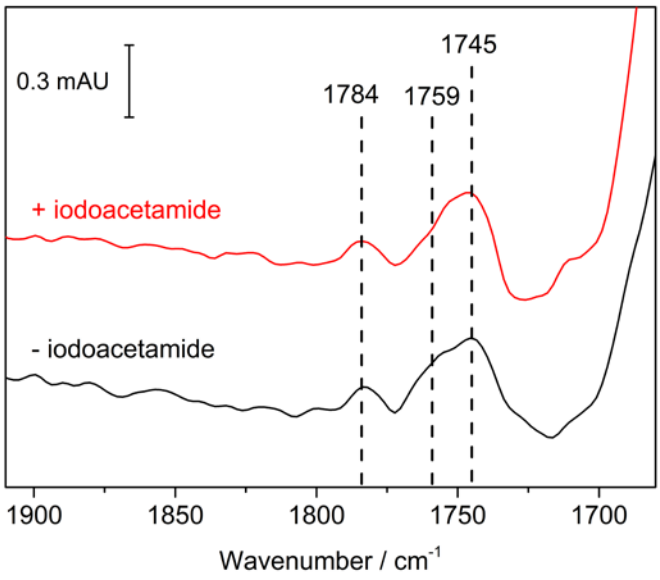

Figure S5: Exposure to NO in the presence of iodoacetamide does not cause a substantial increase in RRE generation. Infrared spectra of HiPIP exposed to 10% NO overnight (black), and exposed to 10% NO overnight in the presence of iodoacetamide (red).

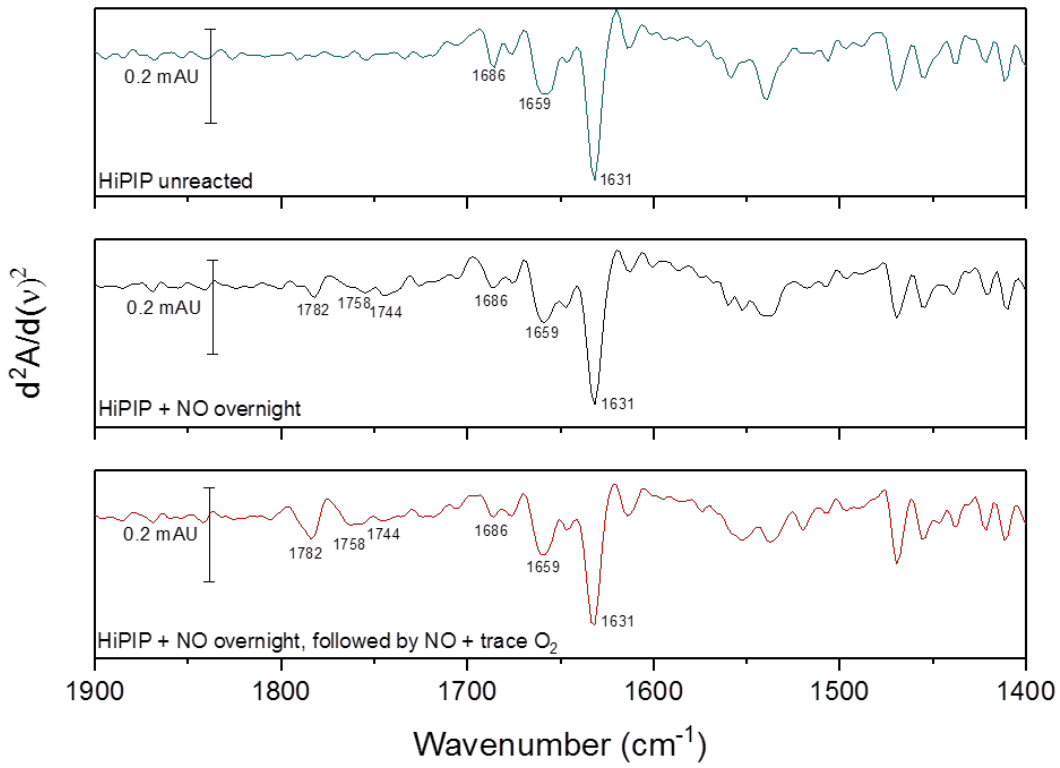

Figure S6: No major changes in the amide backbone region of the spectra (1700-1600 cm-1) indicates no significant increase or decrease in degree of protein folding in the secondary structure. Second derivative plots of infrared spectra of HiPIP unreacted (top), exposed to 10% NO overnight (middle), and exposed to 10% NO overnight (same sample as middle spectra) followed by exposure to a 10% NO/ 0.05% O_2 gas mixture for 150 minutes (bottom).

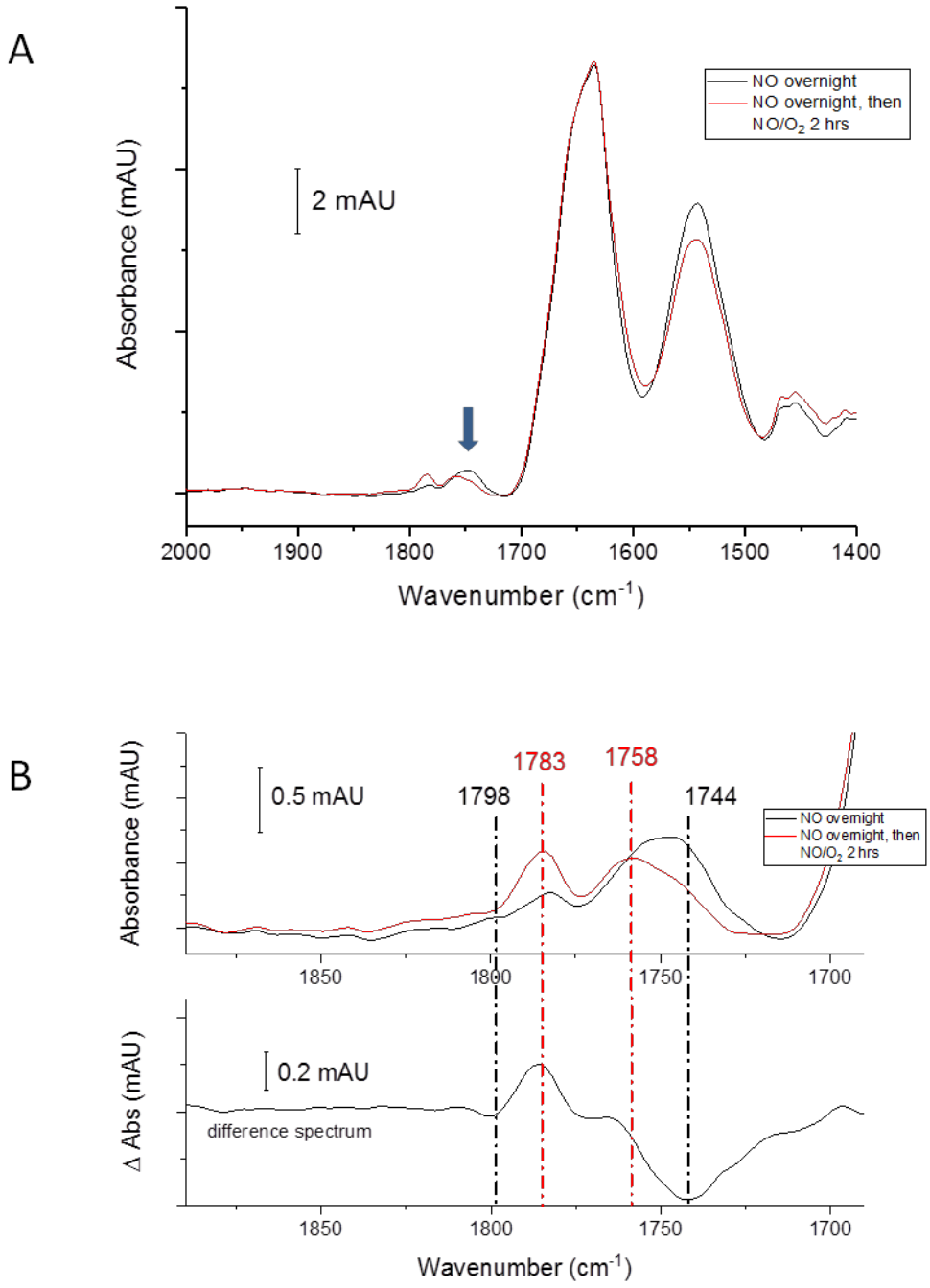

Figure S7: Conversion of RBS-like products to RRE upon addition of trace O_2. A. Comparison of spectra from Fig 4 overlaid: overlay of HiPIP spectra following exposure to NO overnight (black) and the same sample following exposure to NO overnight, then exposure to an NO/trace O_2 mixture for 100 min (red) show a clear loss of intensity in the 1744 cm^{-1} peak. Spectra shown are processed against a background of buffer. **B. Subtraction of spectra shows decrease in RBS band.** Top: nitrosyl range of spectra shown in Figures 4 and S7(A) for comparison. Bottom: Difference spectrum of the two spectra displayed in figure

S7(A) shows growth of intensity at 1783 cm^{-1} and loss of intensity at 1744 cm^{-1} when a HiPIP sample that had been exposed to NO overnight was then exposed to an NO/trace O_2 mixture for 100 min.

In order to determine more clearly whether the observed results represented a conversion or simple increase in the amount of RRE product resulting from nitrosylation of previously unreacted cluster, we carefully subjected each spectrum to an equivalent subtraction of water background and compared the resulting intensities of the nitrosyl bands as compared to the amide I band at 1640 cm^{-1}. Overlaid spectra shows a clear decrease in the intensity of the RBS band at 1744 cm^{-1} as the RRE bands at 1783 cm^{-1} and 1758 cm^{-1} become larger (Figure S7(A)) and the difference spectrum of the same experiment shows a marked increase in intensity at 1783 cm^{-1} and decrease in intensity at 1744 cm^{-1} (Figure S7(B)). The band at 1758 cm^{-1} seems to grow slightly in intensity in the difference spectrum as well, but this growth is dominated by the loss in intensity at 1744 cm^{-1} and the 1758 cm^{-1} peak therefore presents as in inverse shoulder to the RBS band at 1744 cm^{-1} (Figure S7(B)). We conclude from this analysis that there is definitely a decrease in the amount of RBS while the amount of RRE is increasing, which is indicative of loss of RBS as part of this process.

References

1. A. J. Healy, P. A. Ash, O. Lenz and K. A. Vincent, *Phys. Chem. Chem. Phys.,* 2013, 15, 7055-7059.
2. S. V. Hexter, M.-W. Chung, K. A. Vincent and F. A. Armstrong, *J. Am. Chem. Soc.*, 2014, 136, 10470-10477.
3. P. Paengnakorn, P. A. Ash, S. Shaw, K. Danyal, T. Chen, D. R. Dean, L. C. Seefeldt and K. A. Vincent, *Chem. Sci.*, 2017, 8, 1500-1505.