SUPPORTING INFORMATION for:

The *In Vivo* Potential-Regulated Protective Protein of Nitrogenase in *Azotobacter vinelandii* Supports Aerobic Bioelectrochemical Dinitrogen Reduction *In Vitro*

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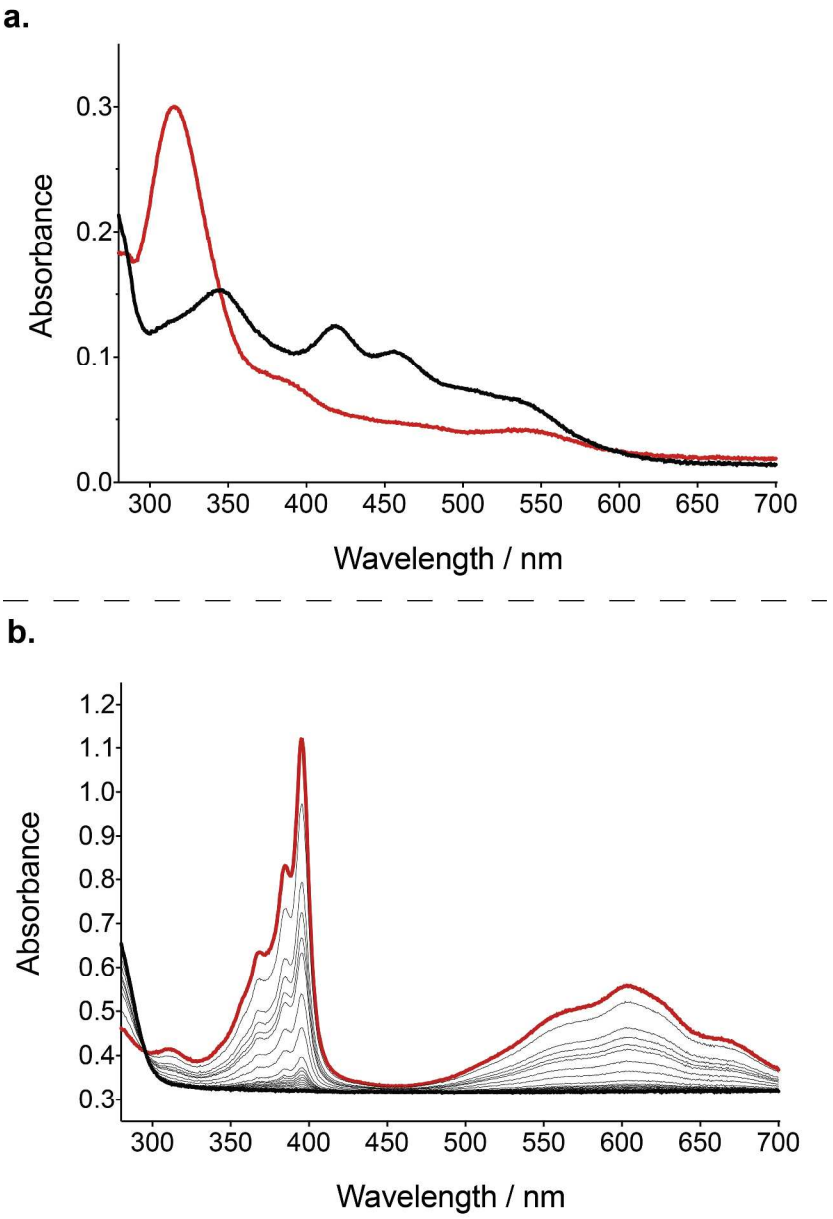

Figure S1. (a) UV/visible absorption spectra of FeSII as oxidized by O_2 (black) and reduced by 10x excess DT (red). (b) Spectroelectrochemical redox of the $MV^{2+}/MV^{\bullet+}$ redox couple. Initially MV was reduced at -0.61 V (*vs.* SHE) until stable (red), at which time an oxidative potential of -0.01 V (*vs.* SHE) was applied and the oxidation of $MV^{\bullet+}$ was followed by recording its UV/visible absorption at 1 minute intervals. Complete oxidation (black line) was achieved in < 20 minutes.

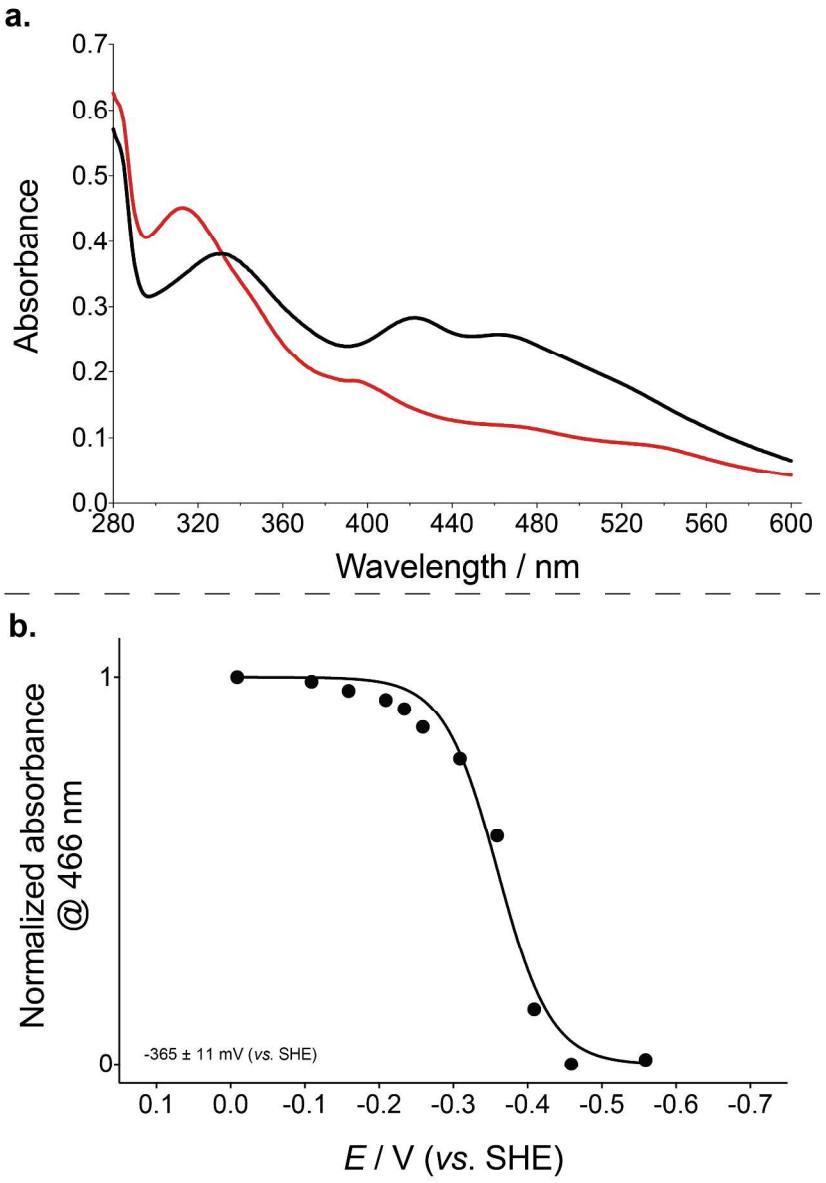

Figure S2. (a) UV/visible absorption spectra of FeSII as oxidized by O_2 (black) and reduced by 2x excess DT (red). (b) Spectroelectrochemical determination of the reduction potential of petF ferredoxin (200 µM), using MV (80 µM) as the electron mediator.

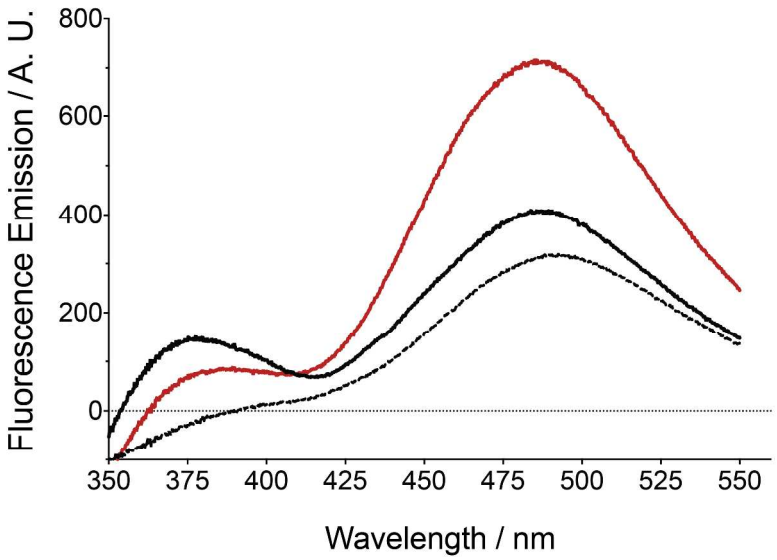

Figure S3. FRET of the double-mutant (CtW/K80C) FeSII protein labeled with AEDANS. Initially (solid red line) W was specifically excited at 295 nm and fluorescence emission with a maxima ~485 nm confirmed FRET between the W donor and AEDANS acceptor couple. A control experiment (black dashed line) consisting of wild-type (WT) FeSII protein at the same concentration in the presence of stoichiometric AEDANS (~67 % labeling was determined for the FeSII CtW/K80C + AEDANS mutant) confirmed that W was able to act as a FRET donor to AEDANS. An additional control (solid black line) experiment consisted of the addition of CtW/K80C FeSII void of AEDANS labeling to determine the extent of diffusion-mediated FRET, where the decrease in the emission of the AEDANS acceptor indicated significant FRET quenching, which we hypothesize to arise from the wide-ranging absorbance of the [2Fe-2S] cluster of the FeSII protein.

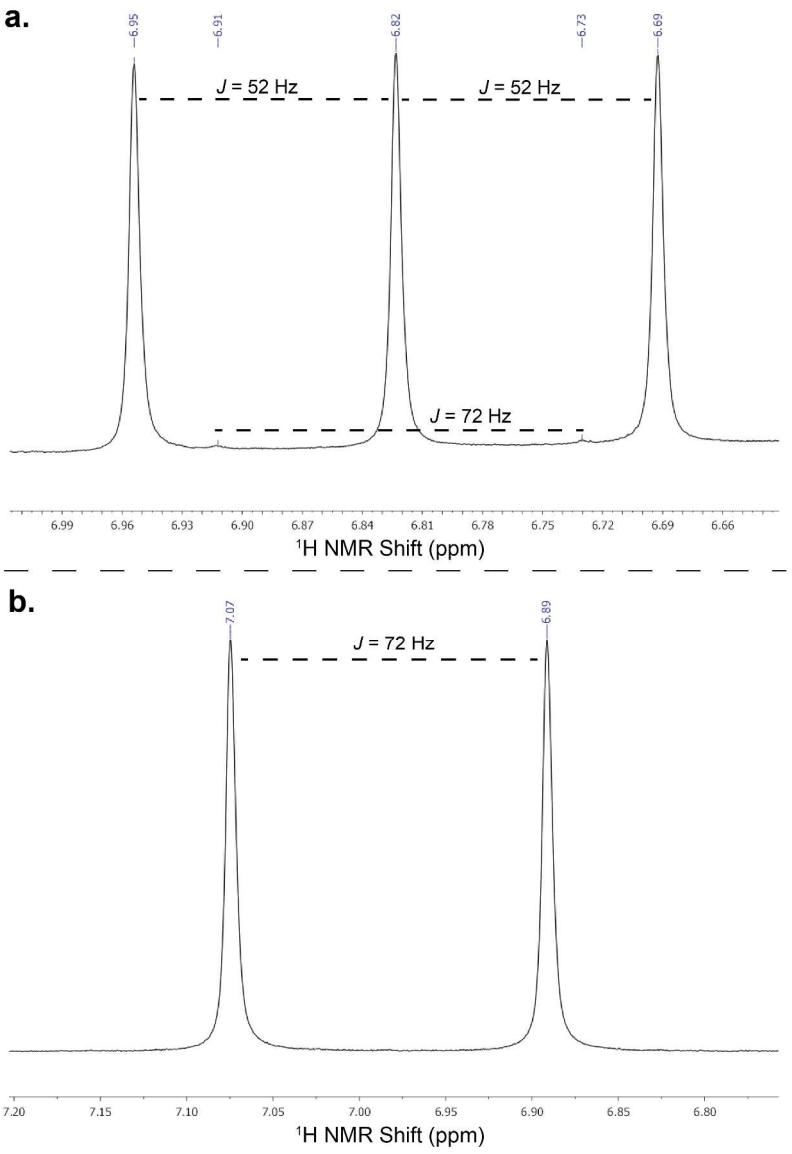

Figure S4. (a) ^{1}H NMR (400 MHz) spectrum of $^{14}NH_4Cl$ in 1 M HCl (D_2O capillary). The peaks coupled by 52 Hz arise from $^{14}NH_4^+$, whereas the peaks coupled by 72 Hz arise from naturally-abundant $^{15}NH_4^+$. (b) ^{1}H NMR spectrum of $^{15}NH_4Cl$ in 1 M HCl (D_2O capillary).

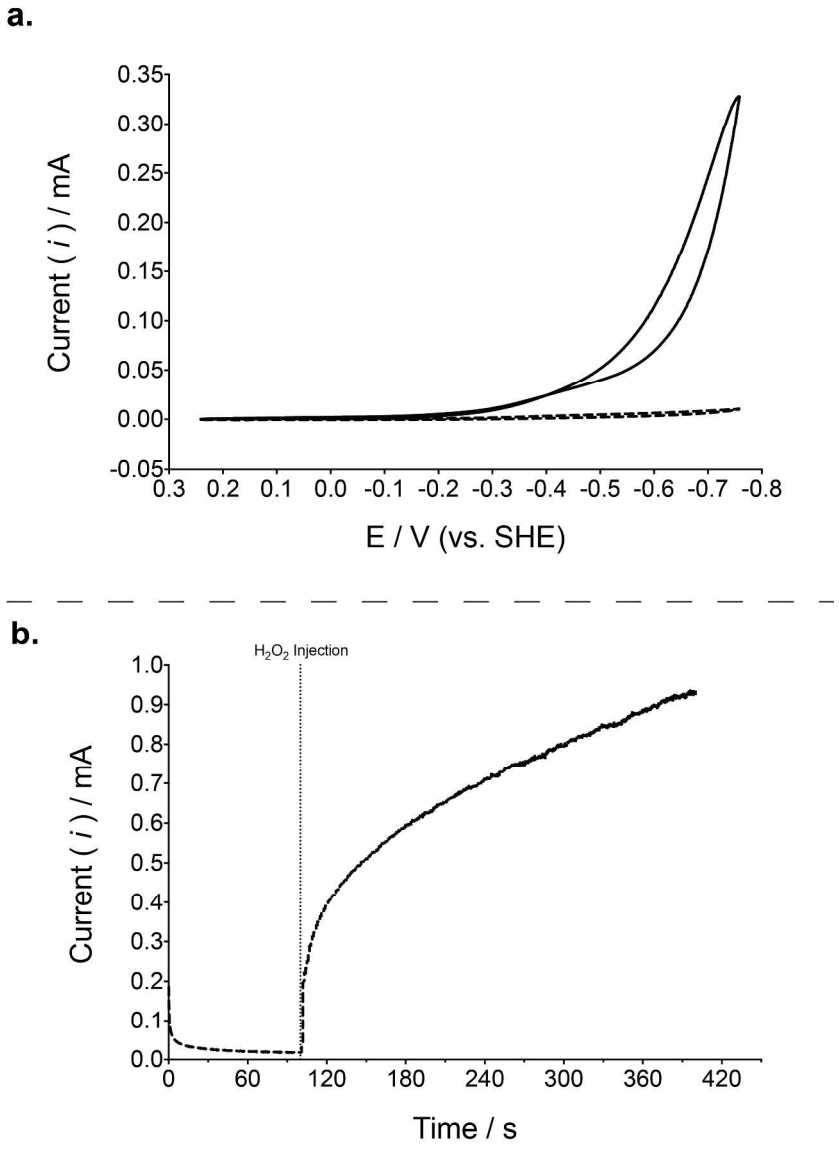

Figure S5. Electrochemical reduction of H_2O_2 at a carbon foam working electrode (r = 0.45 cm, h = 1.5 cm) by (a) cyclic voltammetry at 2 mV s^{-1} and (b) steady-state amperometric i-t at -0.76 V *vs.* SHE, performed in anoxic (< 0.5 ppm O_2) MOPS buffer, pH 7.0 100 mM.

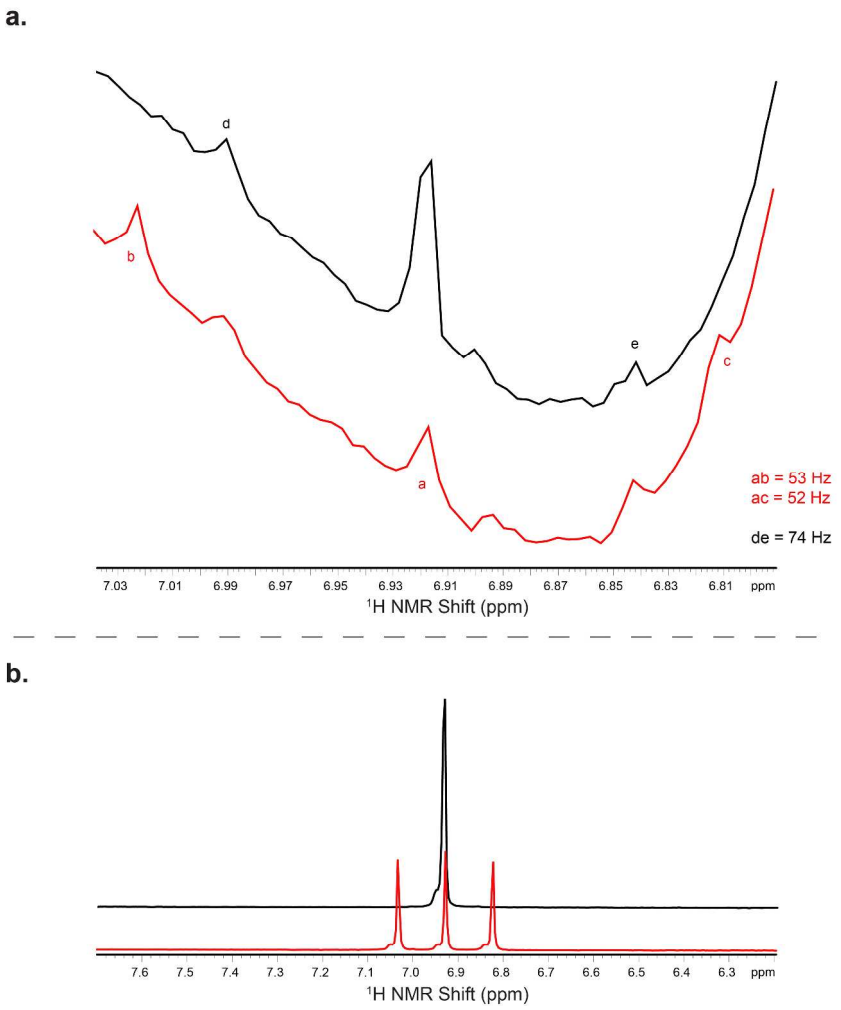

Figure S6. (a) ^{1}H NMR (500 MHz) spectroscopic determination of ^{14}N and ^{15}N NH_4^+ produced by nitrogenase bioelectrosynthesis following the addition of artificial air (containing equivalent $^{15}N_2$ in the place of quiescent $^{14}N_2$) under coupled (red) or ^{14}N-decoupled (black) conditions. Peaks "d" and "e" are present due to the reduction of $^{15}N_2$ by nitrogenase. (b) ^{1}H-^{14}N coupled (red) and decoupled (black) NMR spectra of a 10 mg mL^{-1} $^{14}NH_4Cl$ standard in 1M HCl (with a D_2O capillary).