$[4Fe4S]^{2+}$ Clusters Exhibit Ground-State Paramagnetism

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Supporting Information

Supplementary Materials and Methods

Cell Growth and Protein Purification. All *A. vinelandii* strains were grown in 180 L batches in a 200 L New Brunswick fermentor in Burke's minimal medium supplemented with 2 mM ammonium acetate. The growth rate was measured by cell density at 436 nm. After ammonia consumption, the cells were de-repressed for 3 h and subsequently harvested by using a flow-through centrifugal harvester (Cepa, Lahr/Schwarzwald, Germany). The cell paste was washed with 50 mM Tris-HCl (pH 8.0). All proteins used in this work were purified by methods described previously (*S1*, *S2*).

Redox Titration Experiments. Redox titration was performed at ambient temperature in a glove box under anaerobic conditions, using 20 mM Tris-HCl buffer (pH 8.0) that contained 55% glycerol. Redox mediator dyes, including methyl viologen, benzyl viologen, safranin O and phenosafranin, were added to a final concentration of 10 μM. The Δ*nifB* NifEN sample (10 mg/mL) was first reduced by excess sodium dithionite followed by oxidative titration with 1 mM potassium ferricyanide. Reduction potentials were monitored with a platinum working electrode and a saturated Ag/AgCl reference electrode and reported relative to normal hydrogen potential

(NHE). After reaching the desired potential, a 280 μL aliquot was transferred to a calibrated EPR tube and frozen immediately in liquid nitrogen. In parallel, a 2.5 mL aliquot was quickly concentrated to ~70 mg/mL, filled in a MCD sample cell and immediately frozen in a liquid-nitrogen/pentane slush. MCD and EPR measurements were performed as described below. The reduction potentials of the MCD and EPR samples were reported in Fig. 2A.

EPR Spectroscopy. All EPR spectra were recorded at perpendicular-mode by using a Bruker ESP 300 E_z spectrophotometer (Bruker, Billerica, MA), interfaced with an Oxford Instruments ESR-9002 liquid helium continuous flow cryostat (Oxford Instruments, Oxon, U.K.). All spectra were recorded at 10 K by using a power of 50 mW, a frequency of 9.62 or 9.39 GHz, a gain of 5 x 10^4, a modulation frequency of 100 kHz and a modulation amplitude of 5 G, and 10 scans were averaged for one sample. Spin quantification of EPR signals was carried out as described earlier (*S3*). The relative spectral intensity of Δ*nifB* NifEN in the reduced (S = ½, 2 spins per protein) or oxidized (EPR-silent) state was plotted against the reduction potential of the corresponding sample (see above) to generate a Nernst curve in Fig. 2A.

MCD Spectroscopy. Sample cells were constructed of optical quality quartz (170-2200 nm, 1 mm path length, Model BS-1-Q-1, Starna®, Model SUV R-1001). Each cuvette was cut to the appropriate dimensions to fit the sample holder (1.8 cm × 0.45 cm), resulting in a sample volume of ~160 μL. MCD spectra were recorded with a CD spectropolarimeter (Model J-710; Jasco, MD) interfaced with a superconducting magnet (Model Spectromag 400-7T; Oxford, U.K.) as previously described (*S4*). All spectral intensities have been corrected for depolarization effects. MCD magnetization curves were analyzed in Fig. 1D using a fit/simulation program created by Neese and Solomon (*S5*), where B = magnetic field, β = Bohr magneton, k = Boltzmann constant and T = absolute temperature.

References

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S3. Hu, Y.; Fay, A. W.; Dos Santos, P. C.; Naderi, F.; Ribbe, M. W. *J. Biol. Chem.* **2004**, *279*, 54963-54971.

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S5. Neese, F.; Solomon, E. I. *Inorg. Chem.* **1999**, *38*, 1847-1865