SUPPORTING INFORMATION

X-ray Crystal Structures of *Moorella thermoacetica* FprA. Novel Diiron Site Structure and Mechanistic Insights into a Scavenging Nitric Oxide Reductase[†]

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FIGURE 1S. UV-vis absorption spectrum of as-isolated *M. thermoacetica* FprA crystals recorded in mother liquor (containing pH 6.5 precipitant) at room temperature. This spectrum is representative of the highly reproducible spectra recorded separately on several as-isolated FprA crystals whose long axes were oriented along several directions in a plane approximately perpendicular to the light beam. Lower left inset: photograph of as-isolated *M. thermoacetica* FprA crystals in mother liquor. Top right inset: solution UV-vis absorption spectrum of as-isolated (iron-containing) *M. thermoacetica* FprA and Zn-containing FprA in solution (reproduced from Silaghi-Dumitrescu, R., Coulter, E. D., Das, A., Ljungdahl, L. G., Jameson, G. N., Huynh., B. H., Kurtz, D. M., Jr. (2003) A flavodiiron protein and high molecular weight rubredoxin from *Moorella thermoacetica* with nitric oxide reductase activity, *Biochemistry* 42, 2806-2815.).

FIGURE 2S: Left: $1F_{\text{O}}-F_{\text{C}}$ composite omit map contoured at -3σ for reduced *M. thermoacetica* FprA crystals with the Fe1-Fe2 distance fixed at 2.9 Å in the calculated map. Right: $1F_{\text{O}}-F_{\text{C}}$ map contoured at -4σ for the reduced FprA crystals with the Fe1-Fe2 distance fixed at 3.8 Å in the calculated map. Maps are superimposed on the wireframe model for the diiron site. Atoms are color-coded as listed in the legend to Figure 2.

FIGURE 3S. NO concentration dependence of NADH:NO oxidoreductase activity of *M. thermoacetica* Hrb + FprA variants, H25F or Y195F at saturating Hrb and NADH. Assay solutions contained 10 μ M Hrb, 160 μ M NADH, and either 190 nM Y195F FprA or 400 nM H25F FprA in 50 mM phosphate pH 7. All rates were measured at room temperature (~ 23 $^{\circ}$ C) as initial rates of NADH-dependent NO consumption monitored using an NO-sensitive electrode, as described in Silaghi-Dumitrescu, R., Coulter, E. D., Das, A., Ljungdahl, L. G., Jameson, G. N., Huynh., B. H., Kurtz, D. M., Jr. (2003) A flavodiiron protein and high molecular weight rubredoxin from *Moorella thermoacetica* with nitric oxide reductase activity, *Biochemistry* 42, 2806-2815. The solid traces represent fits of the experimental data (solid circles) to the Michaelis-Menten equation using parameters listed in the text.