Insights into the nitric oxide reductase mechanism of flavodiiron proteins from a flavin-free enzyme

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SUPPORTING INFORMATION

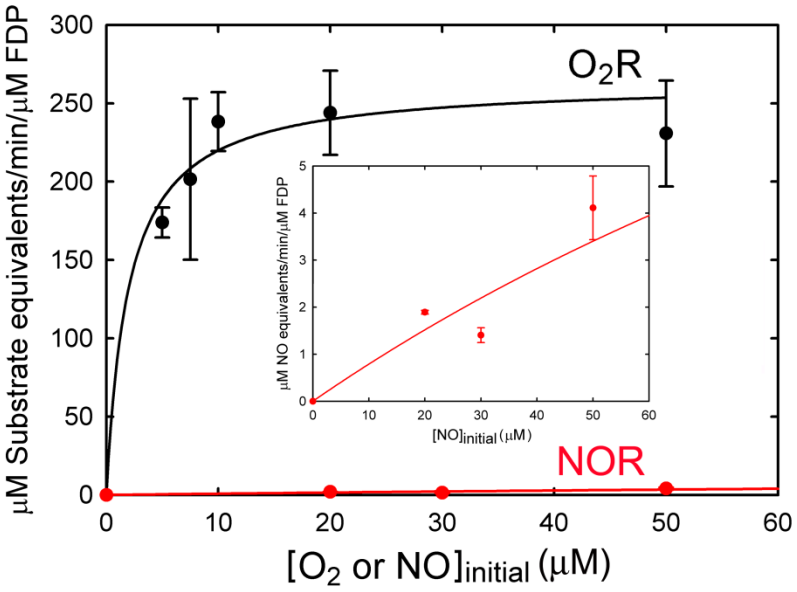

Figure S1. Michaelis-Menten plots for O_2R and NOR activities of as-isolated FDP. All data points represent the average of three determinations with the ranges indicated by the bars. Curves represent least-squares fits of the Michaelis-Menten equation to the data. NADH consumption was converted to substrate equivalents by assuming either 2NADH:O_2 or 1NADH:1NO reaction stoichiometries (see **Scheme 1**).

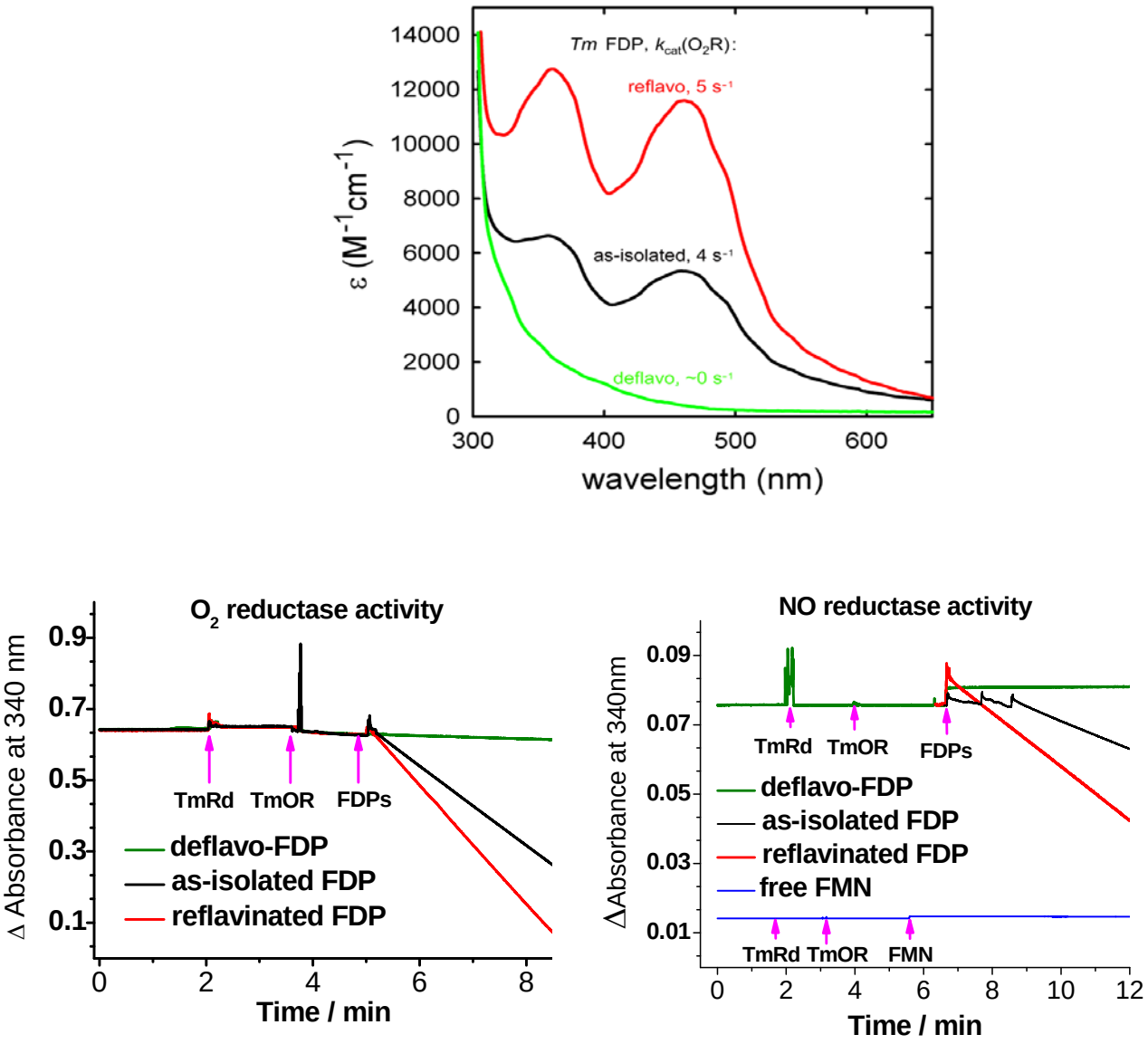

Figure S2. UV-vis absorption spectra (top), O_2R activity assay (bottom, left), and NOR activity assays (bottom, right) of deflavinated, as-isolated (~50% FMN occupancy), and reflavinated *Tm* FDP. Extinction coefficients are based on protein monomer and k_{cat} values for as-isolated and reflavinated *Tm* FDP are based on FMN occupancies. O_2R and NOR activities were determined in air-saturated solutions or NO solutions equilibrated with a headspace containing 0.1 atm NO partial pressure, as described in Materials and Methods. The arrows indicate times of additions of protein components or FMN. To facilitate comparison of O2R and NOR activities, all traces were normalized to 1 μM final concentration in protein monomer or FMN concentration.

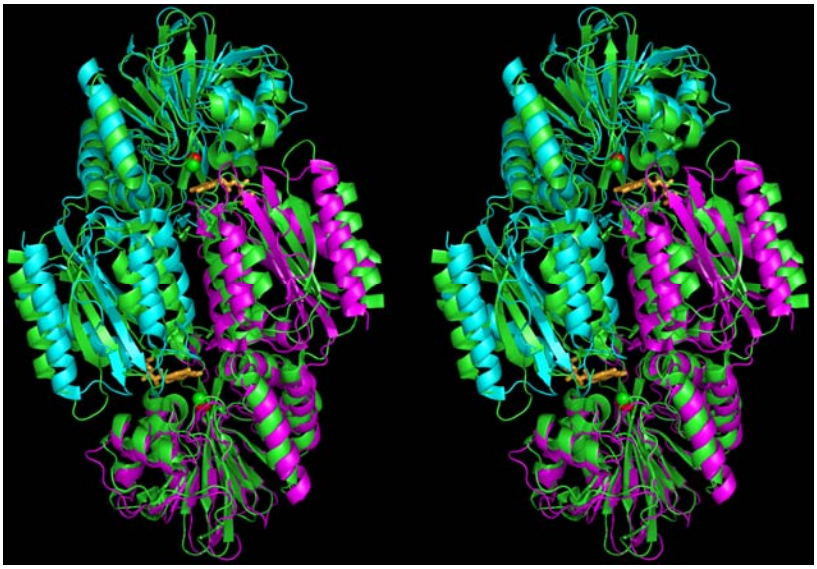

Figure S3. Cross-eyed stereo view of superposition of *Mt* FDP (1YCF) and *Tm* FDP (1VME) homodimer protein backbones. *Mt* FDP subunits are colored cyan and purple, and *Tm* FDP subunits are both colored green. FMN in Mt FDP is represented as orange sticks. Iron atoms are represented as spheres, colored red for *Mt* FDP and green for *Tm* FDP. Superpostioning and image were generated in PyMOL.

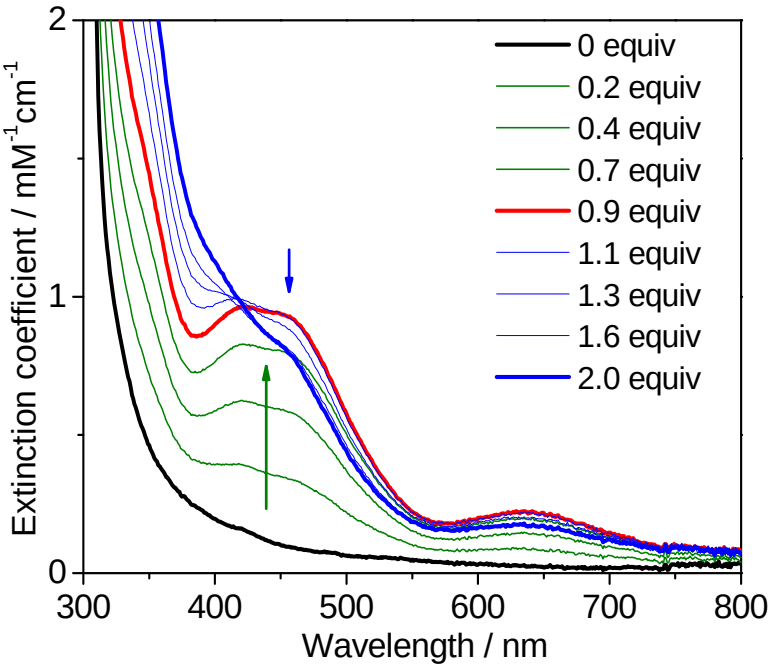

Figure S4. UV-vis spectra monitoring the titration of reduced deflavo-FDP with up to 2.0 equiv of NO (same experimental conditions as in Figure 2).

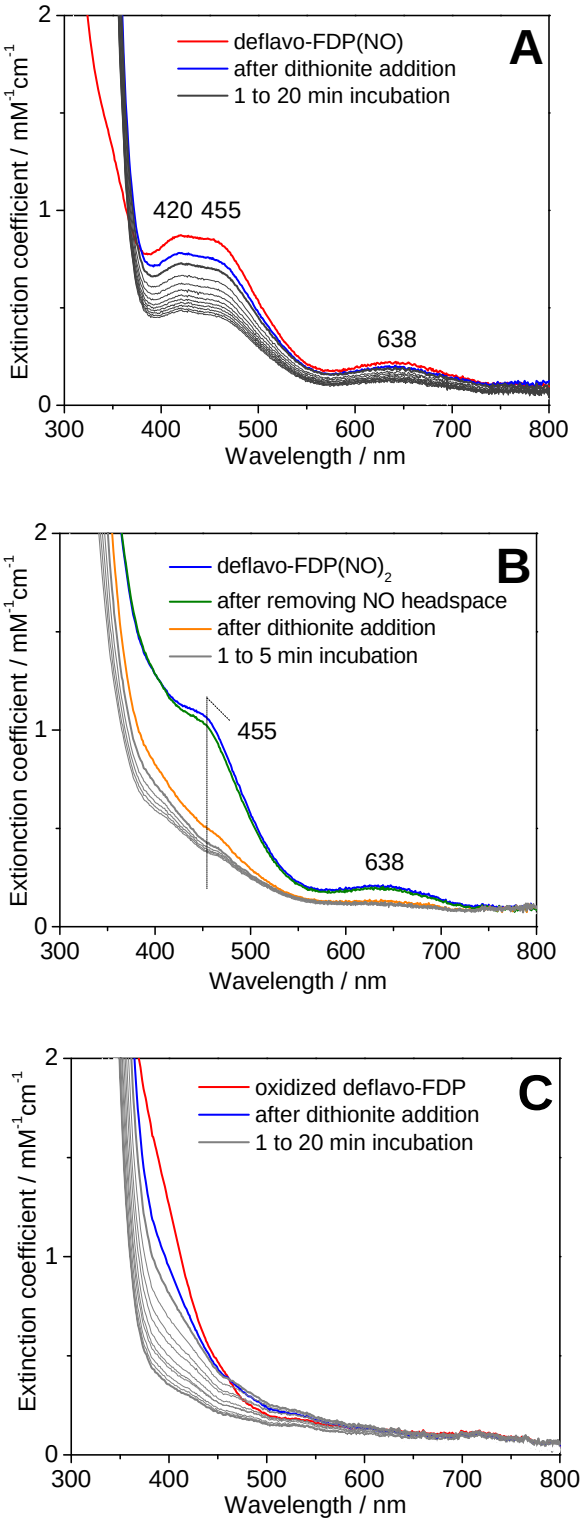

Figure S5. UV-vis spectra of deflavo-FDP(NO) (A), deflavo-FDP(NO)$_2$ (B), and oxidized deflavo-FDP (C), before and after addition of sodium dithionite (initial concentrations [FDP] = 60 μM, [dithionite] = 120 μM).

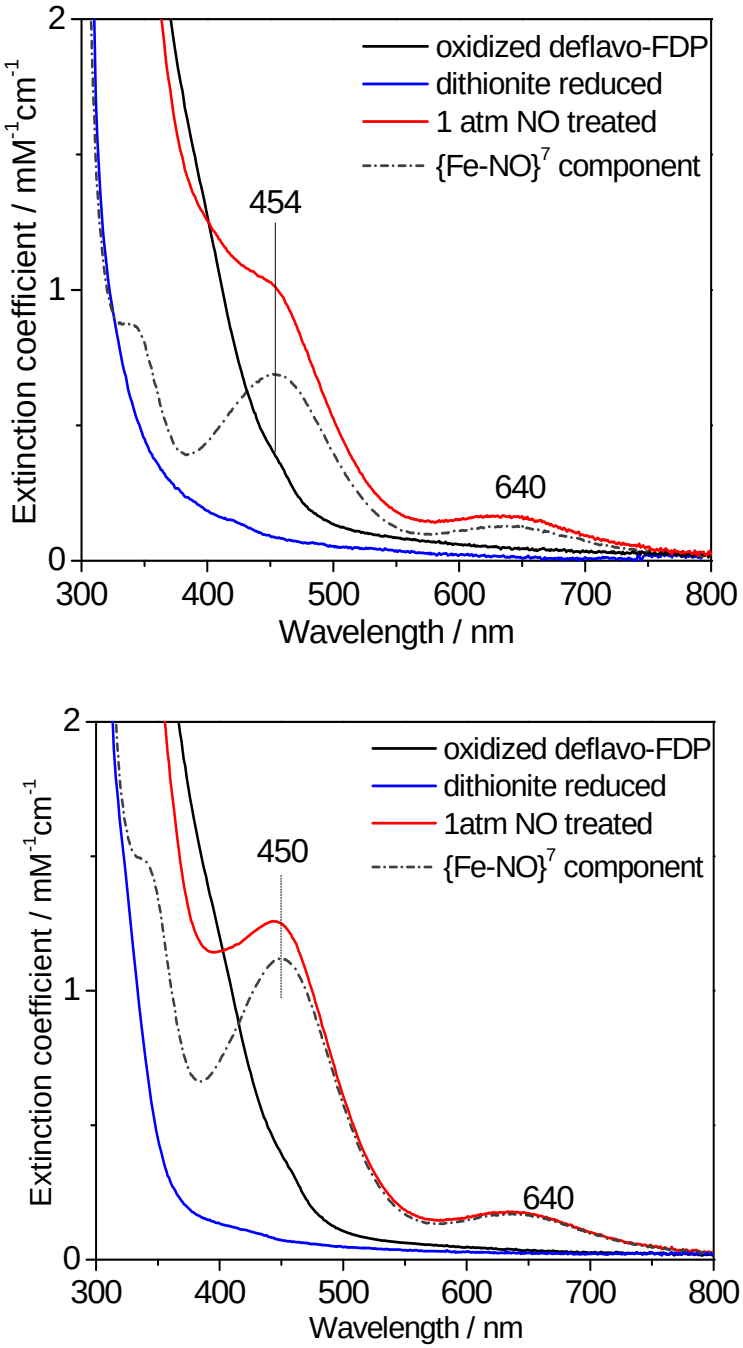

Figure S6. UV-vis spectra of oxidized (black), dithionite reduced (blue), and 1 atm NO treated (red) deflavo-FDP for two different preparations. Also shown, are the difference spectra generated after subtracting contributions from the diferric product spectra (0.65 and 0.4 subtraction factor for the upper and lower panels, respectively). The samples were in 50 mM MOPS pH 7.4 and the extinction coefficients are per diiron site.

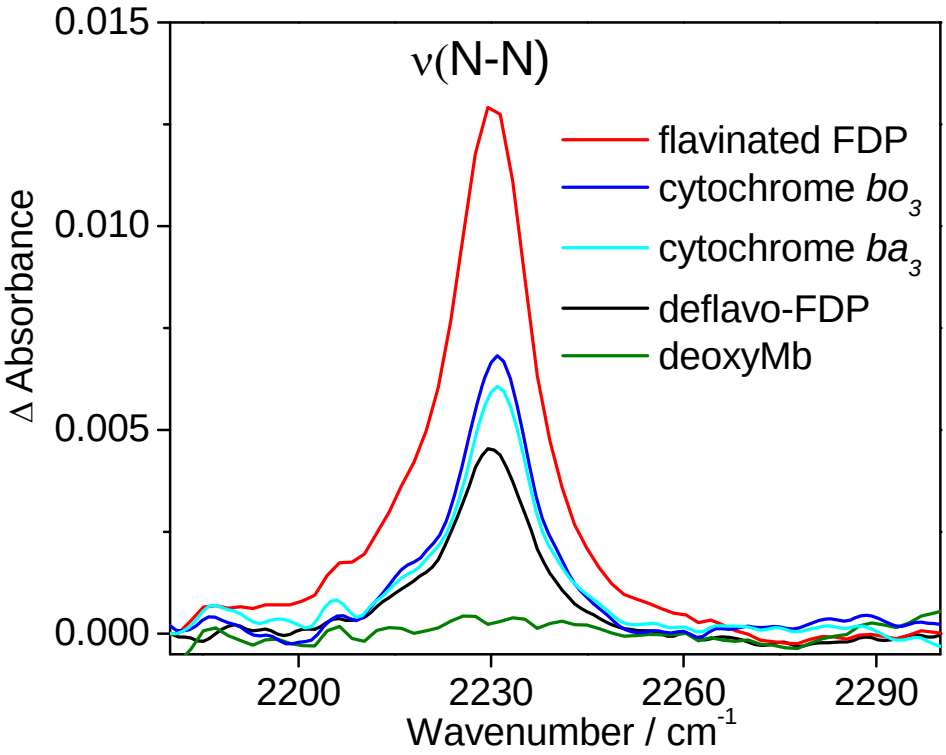

Figure S7. FTIR detection of N_2O produced by reduced flavinated FDP (red: 1 mM, 100 % FMN occupancy), reduced deflavo-FDP (black: 1 mM diiron) deoxymyoglobin (green: 2 mM) after incubation with excess NONOate to produce 2 equiv NO per iron. Also shown, are the FTIR spectra from multi-turnover reactions of reduced cytochrome bo_3 (blue: 50 μM) and ba_3 (light blue: 50 μM) in presence of 10 mM ascorbate and 0.1 mM TMPD and after incubation with 2 mM NO produced from NONOate. Complete reduction of flavinated FDP (~100 μM in 50 mM MOPS pH 7.4) was achieved after 4 h incubation with 1 mM NADH, 4 μM Rd and 0.4 μM NROR. The NADH excess was removed using a spin column inside the glove box and the sample was concentrated down to reach 1 mM in diiron concentration. The NONOate addition was performed just prior to transferring the sample to the FTIR cell.

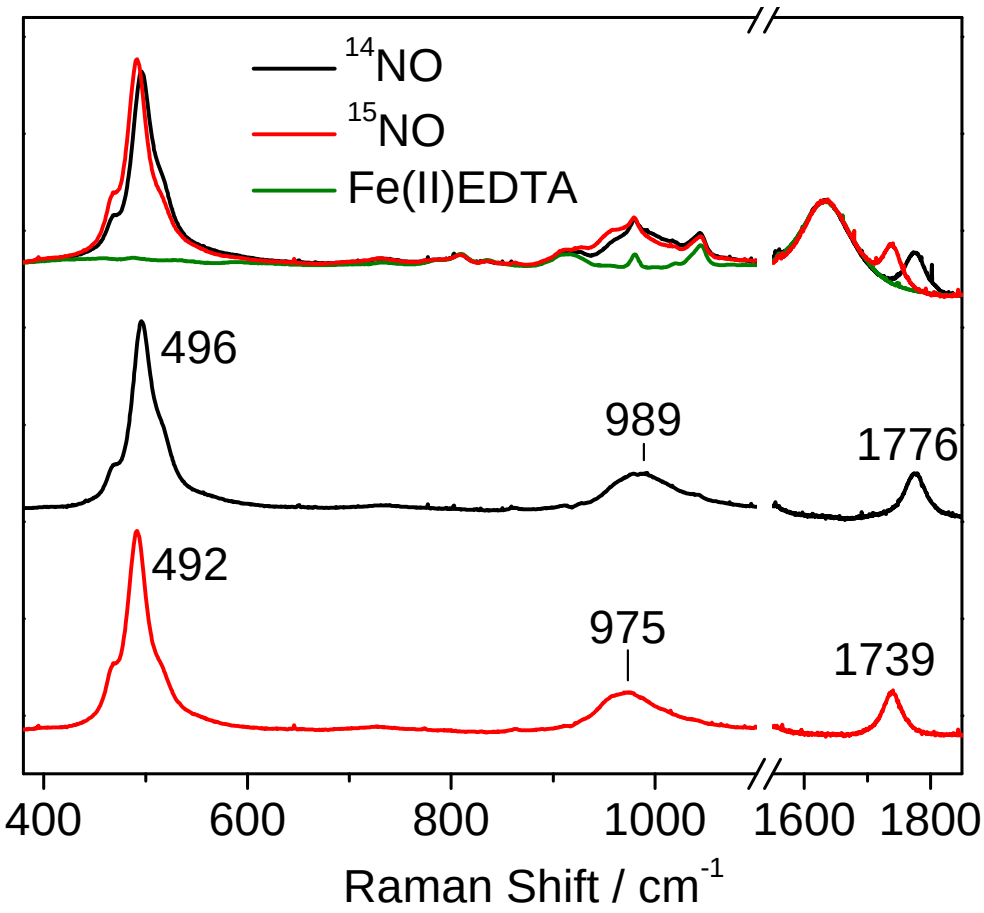

Figure S8. Room temperature resonance Raman spectra of Fe^{II}(EDTA) (green), Fe^{II}(EDTA)^{14}NO (black) and Fe^{II}(EDTA)^{15}NO (red). Also shown are the "iron-nitrosyl" minus "Fe^{II}(EDTA)" difference spectra.