Supplementary material

REGULATION OF FMN SUBDOMAIN INTERACTIONS AND FUNCTION IN NEURONAL NITRIC OXIDE SYNTHASE

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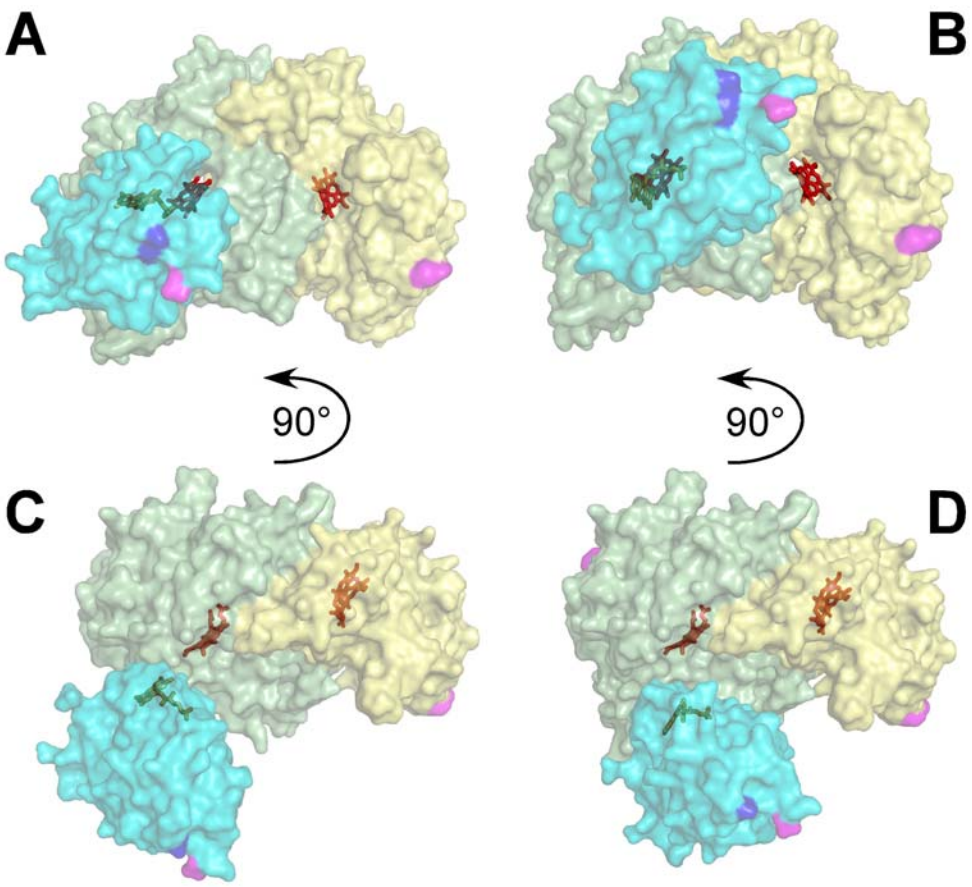

Figure S1. Computer models of the FMN-NOSoxy complex. Panels A and B show the front views of two hypothetical complexes, while Panels C and D are the top view of the complexes in A and B, respectively. Each monomer in the NOSoxy domain is shown in green and yellow, and the FMN domain is shown in cyan. The heme and FMN cofactors are shown as orange (heme) or green (FMN) sticks. The residue 942 (blue) is a terminal residue of a linker sequence that attaches the FMN subdomain to the FNR subdomain (linker H1 in Fig. 1). Likelwise, the magenta residues 716 (NOSoxy) and 750 (FMN subdomain) are covalently bound by a 34-residue linker sequence that contains the CaM binding sequence (H2 in Fig. 1). See Materials and Methods for details.

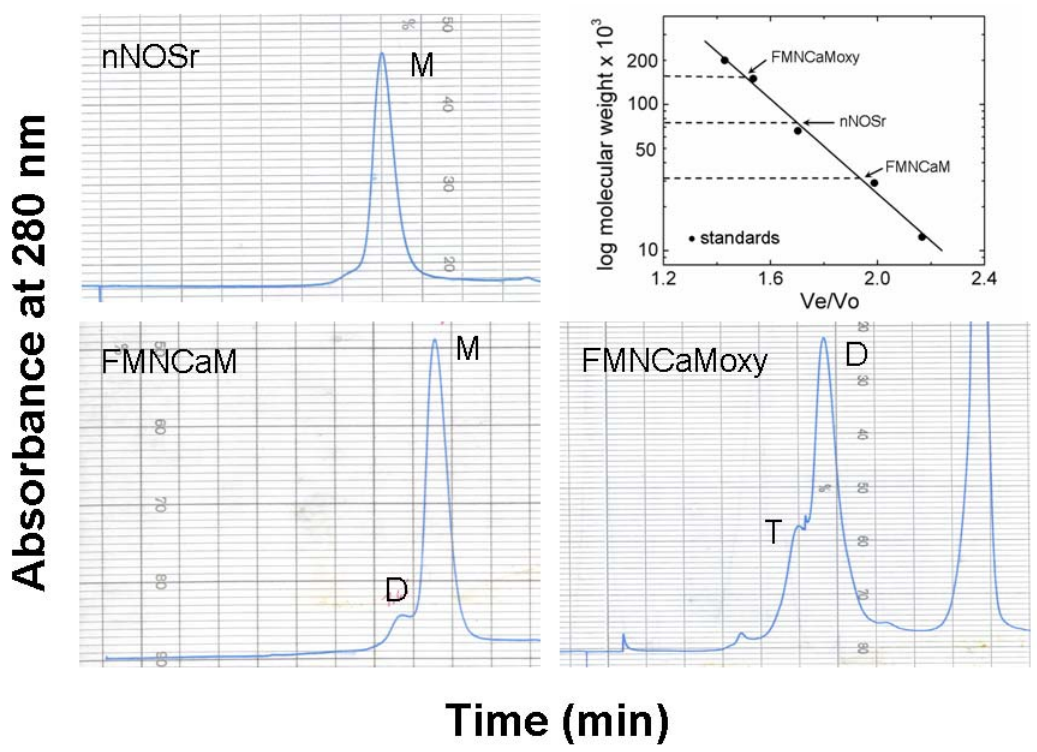

Figure S2. Gel filtration chromatography of nNOSr, FMNCaM, and FMNCaMoxy proteins. Protein absorbance was monitored at 280 nm in the column eluate. Molecular weights of each protein were determined relative to gel filtration molecular weight marker proteins (12,400 to 200,000) (upper right panel). In the chromatograms, M is monomer, D is dimer, and T is tetramer.

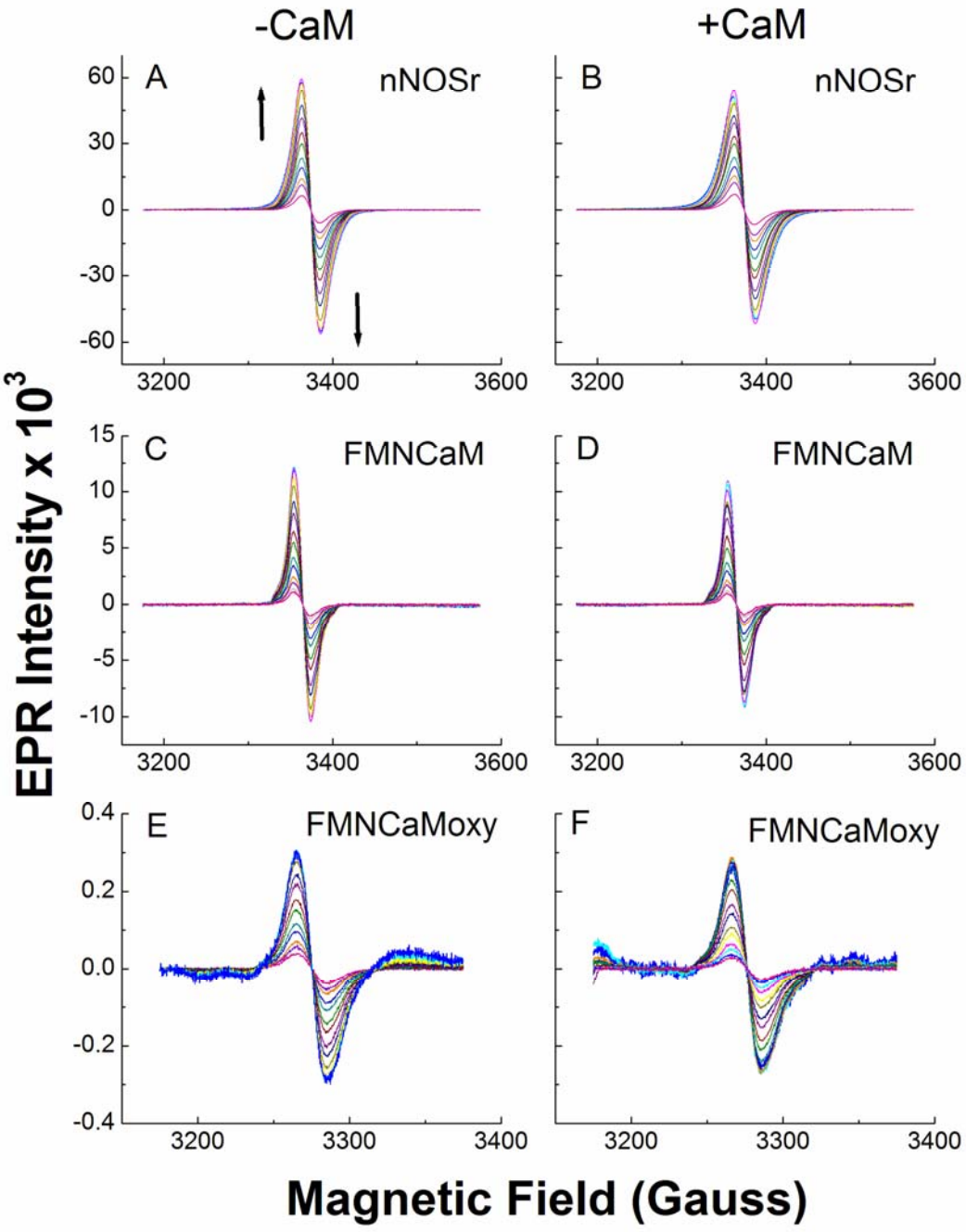

Figure S3. Representative EPR spectra of the various nNOS proteins collected at various microwave power settings. Protein samples contained 13 mM of Dy^{III}-HEDTA in the presence and absence of bound CaM and spectra were recorded at 150 K. Each protein was manipulated as described in Materials and Methods to contain an FMNsq radical before measurements. A total of 17 power settings, ranging from 0.020 to 200 milliwatts, were used for the (A,B) nNOSr, (C,D) FMNCaM, and (E,F) FMNCaMoxy proteins constructs. Arrows indicate increase in EPR signal.

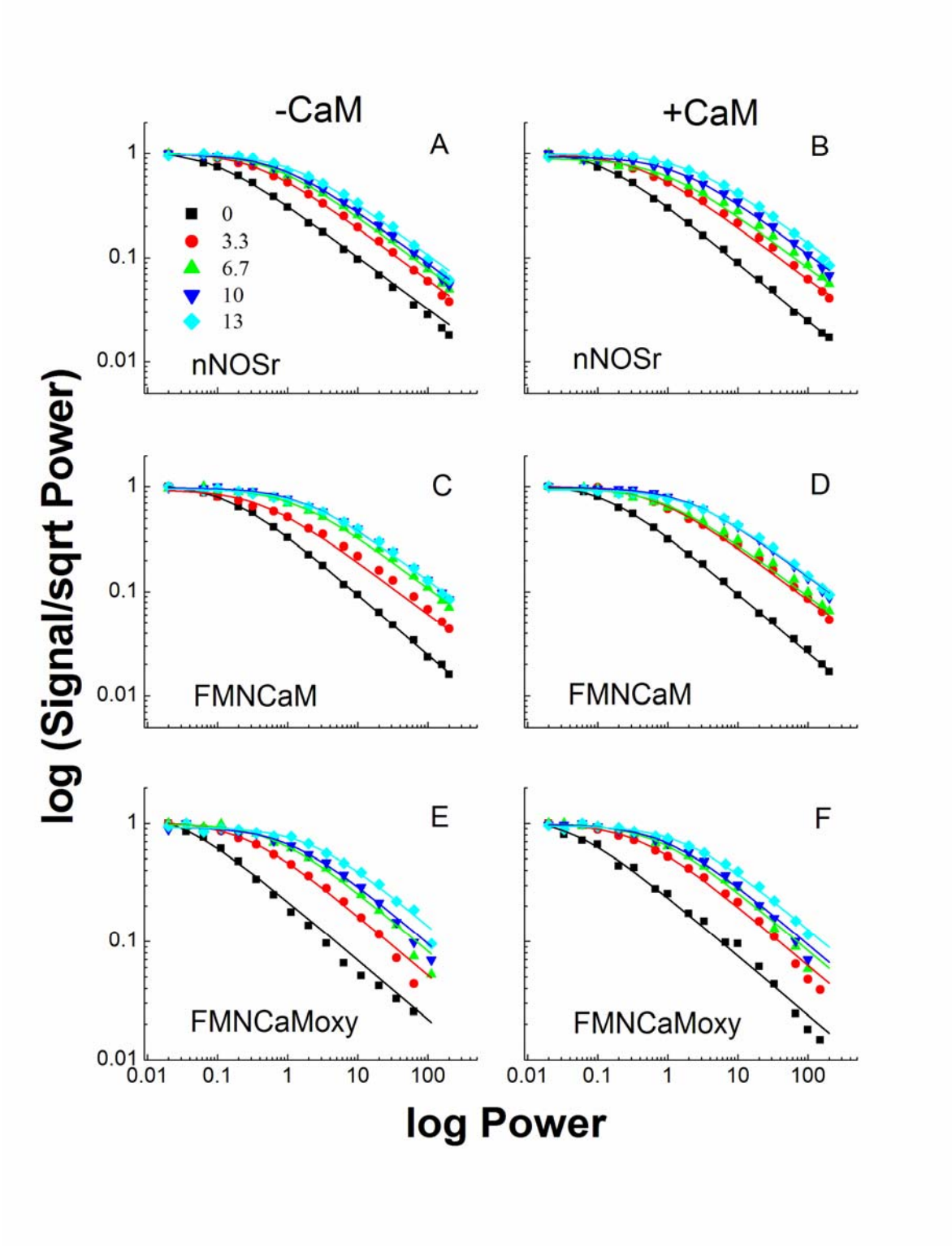

Figure S4. Sequential microwave power saturation curves of the three nNOS proteins. Measurements were done with CaM-free or CaM-bound proteins and in the presence of varying concentrations of Dy^{III}-HEDTA (0, 3.3, 6.7, 10, and 13 mM). (A,B) nNOSr, (C,D) FMNCaM, and (E,F) FMNCaMoxy. Each protein contained a FMNsq radical. The EPR conditions and data fitting are described in Materials and Methods.

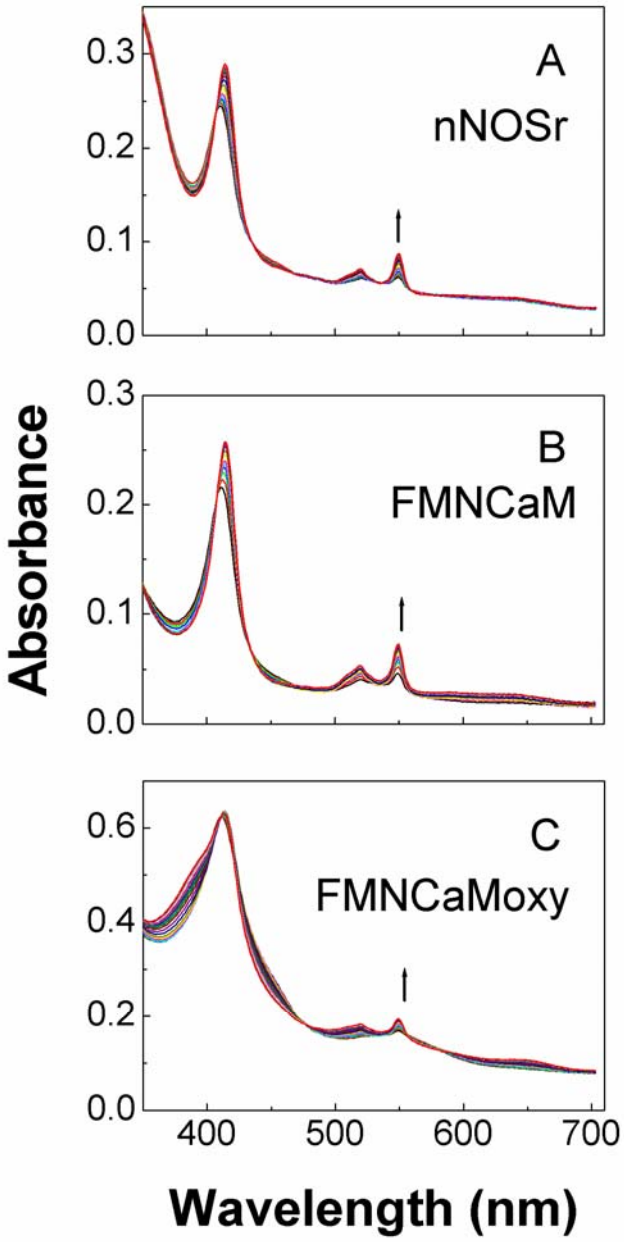

Figure S5. Representative stopped-flow spectra collected at 10 ˚C during anaerobic pre-steady-state cytochrome *c* reduction by a molar excess of each fully-reduced NOS protein: (A) nNOSr, (B) FMNCaM, and (C) FMNCaMoxy as described in Materials and Methods. Arrow indicates the direction of absorbance change at 550 nm.

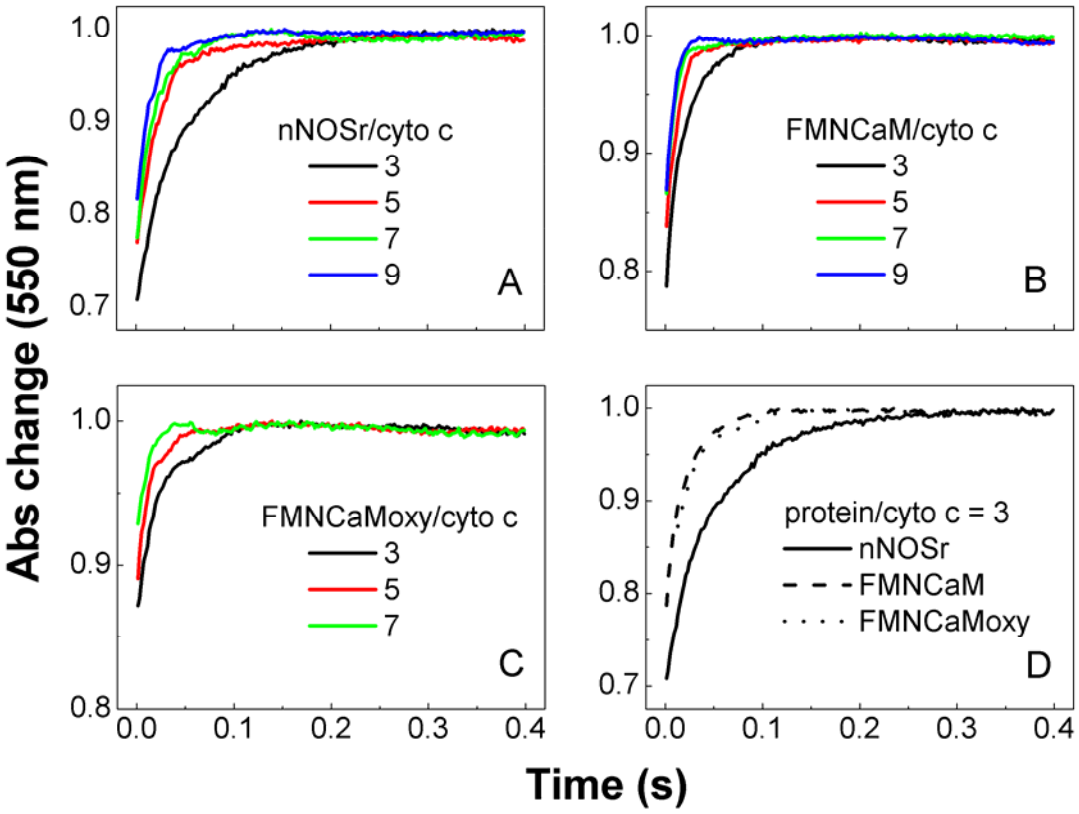

Figure S6. Kinetics of cytochrome *c* reduction by fully-reduced, CaM-free nNOS proteins. Absorbance traces were collected at the indicated NOS protein to cytochrome *c* concentration ratios (panels A-C). Different concentrations of fully-reduced NOS proteins were rapidly-mixed with cytochrome *c* (3 μM) in the stopped-flow spectrometer under anaerobic conditions at 10 ^{o}C. (D) Overlay of the kinetic traces for the different NOS proteins obtained at a NOS protein:cytochrome *c* ratio of 3.

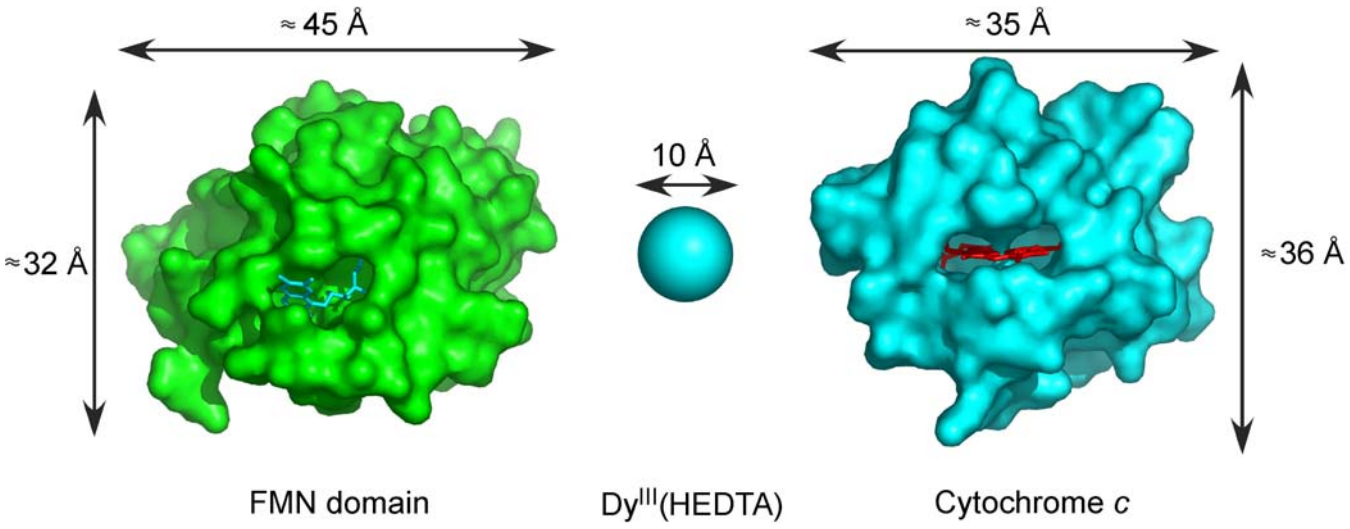

Figure S7. Relative sizes of the FMN domain (left), Cytochrome *c* (right) and the Dy(III)-HEDTA complex (middle). Bound FMN and heme are shown as blue and red sticks, respectively. The FMN and heme cofactors are shown as sticks. The approximate size calculated from structures 1TLL (FMN domain) and 1CRC (Cytochrome *c*) is shown. An estimated diameter of 10 Å was reported for the Dy(III)-HEDTA complex (Oliver, M. E. and Hales, B. J. (1993) *Biochemistry* **32,** 6058-6064).

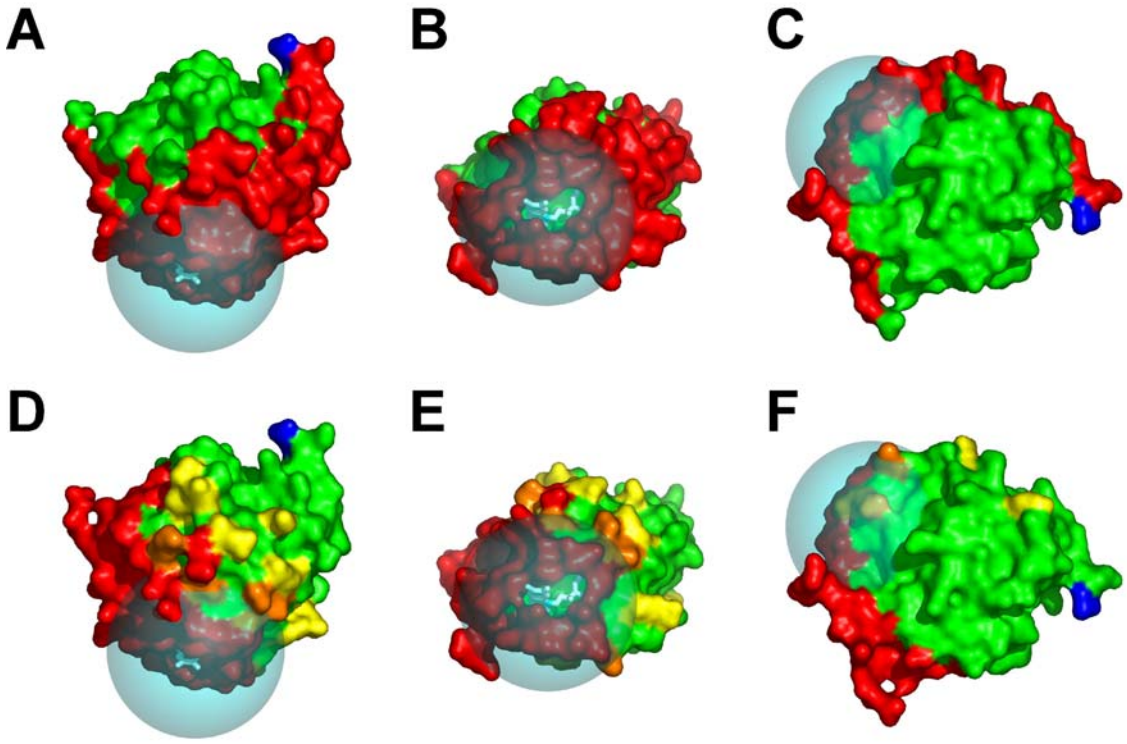

Figure S8. Solvent accessibility of residues on the surface of the FMN domain. Panels A, B, C depict three different views of the FMN subdomain. Each picture indicates the surface residues that either are (red) or are not (green) covered by the FNR domain as indicated in the crystal structure of nNOSr. Panels D, E and F show the same three views of the FMN subdomain, this time indicating the surface residues that are or are not covered by the nNOSoxy domain in four modeled oxy-FMN domain complexes. Residues are colored to indicate they are covered in all four models (red), covered in 2 or 3 models (orange), only in 1 model (yellow), or in no models (green). In all cases, coverage was determined as a decrease in accessible surface calculated with a 10 Å diameter probe, equivalent to a solvated DyIII-HEDTA molecule (see Materials and Methods for details). The semi-transparent cyan sphere indicates a volume of 15 Å radius centered on the FMN cofactor, which approximates the space in which rapid electron transfer involving the FMN cofactor is considered to be possible. The location of the FMN subdomain N-terminal hinge residue 750 is shown in blue to aid viewer orientation.