Supporting Information:

Pulsed EPR Determination of Distance between Heme Iron and FMN Centers in a Human Inducible Nitric Oxide Synthase

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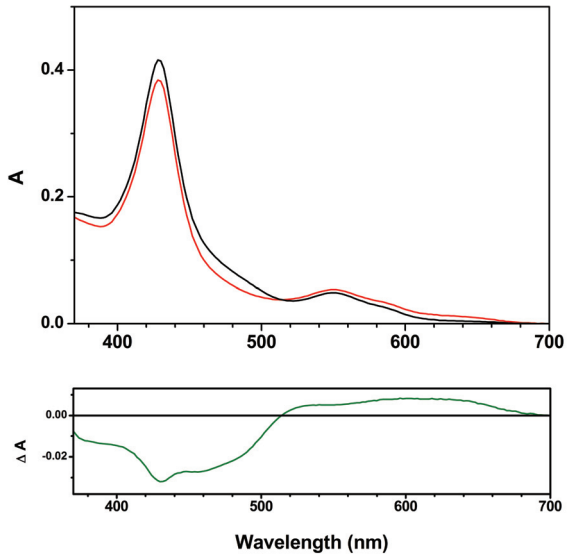
(a)



(b)

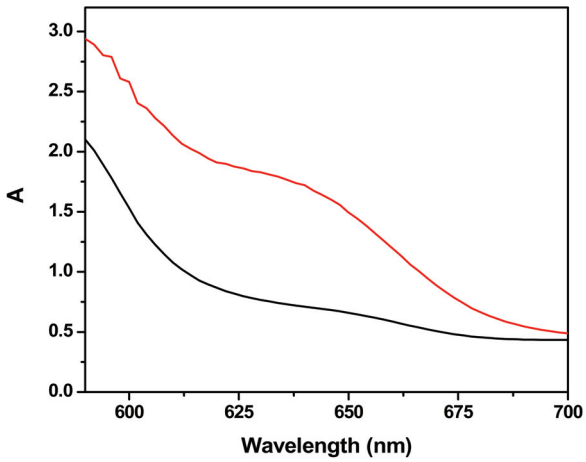


Figure S1. (a) Top, UV-vis spectra of 1.5 μM imidazole-bound human iNOS oxyFMN construct during dithionite titration (black trace, as-isolated protein; red trace, partially reduced protein); bottom, difference spectrum (partially reduced minus as-isolated) showing formation of the $FMNH^{\bullet}$ in the partially reduced sample. (b) Absorption spectra of 324 μM iNOS sample during the dithionite titration: black trace, as-isolated protein; red trace, partially reduced sample that has maximum buildup of the $FMNH^{\bullet}$. The final sample (red trace, panel b) was immediately frozen in liquid nitrogen for pulsed EPR studies. Buffer: 100 mM bis-Tris-propane, 200 mM NaCl, 3 mM imidazole, 42% ethylene glycol, pH 7.6.

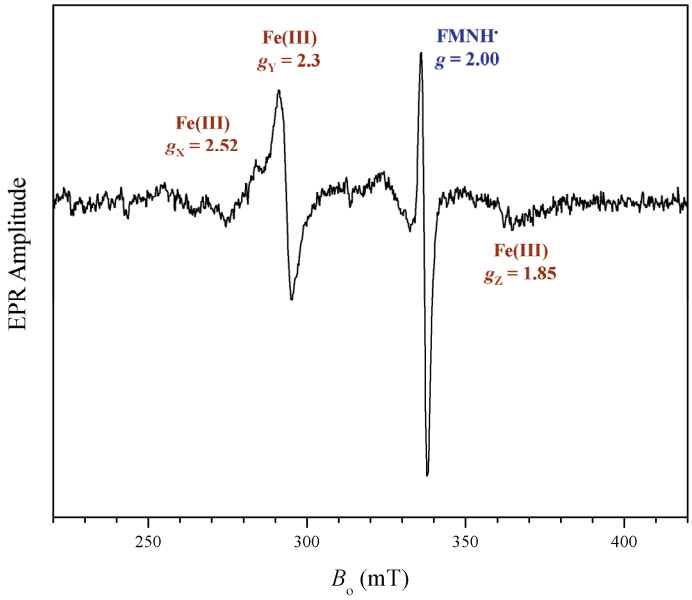


Figure S2. X-band continuous wave EPR spectrum of the [Fe(III)][FMNH$^\bullet$] form of imidazole-bound human iNOS oxyFMN. The principal g-values of the FMNH$^\bullet$ and low spin Fe(III) centers are indicated. This spectrum confirms that the [Fe(III)][FMNH$^\bullet$] form can be prepared at high yield by using the dithionite titration procedure (see Experimental Section). Experimental conditions: mw frequency, 9.453 GHz; mw power, 2 mW; modulation amplitude, 0.5 mT; temperature, 77 K.

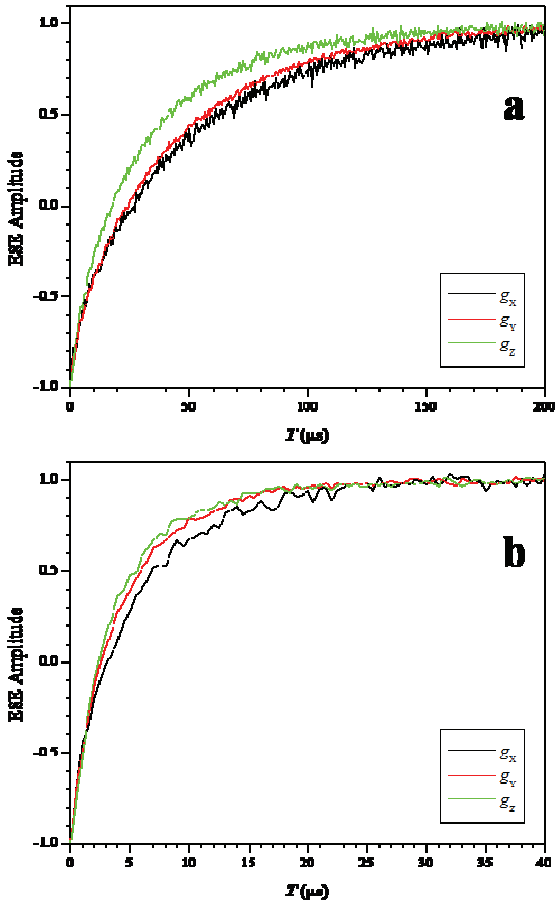


Figure S3. Inversion recovery traces for the Fe(III) heme center of iNOS recorded at 15 K (a) and 25 K (b). The black, red and green traces correspond to g_X, g_Y, and g_Z EPR turning points, respectively. Experimental conditions: mw frequency, 29.454 GHz; magnetic fields, B_o = 845.2 mT (g_X), 914.8 mT (g_Y), and 1124.8 mT (g_Z); inversion mw pulse, 15 ns; observation mw pulses, 20 ns and 40 ns.

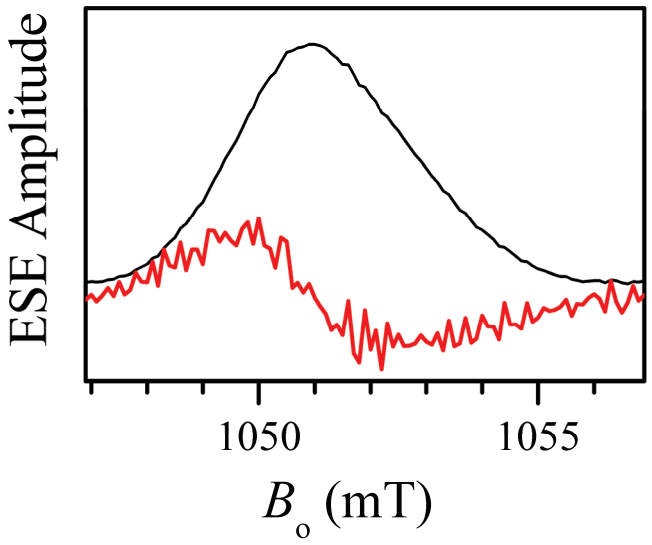


Figure S4. Two-pulse ESE field sweep spectrum of $FMNH^{\bullet}$ (black trace) and numerical first derivative of the spectrum (red trace). Experimental conditions: mw frequency, 29.454 GHz; mw pulses, 9 and 15 ns; pulse repetition rate, 10 Hz; temperature, 15 K.

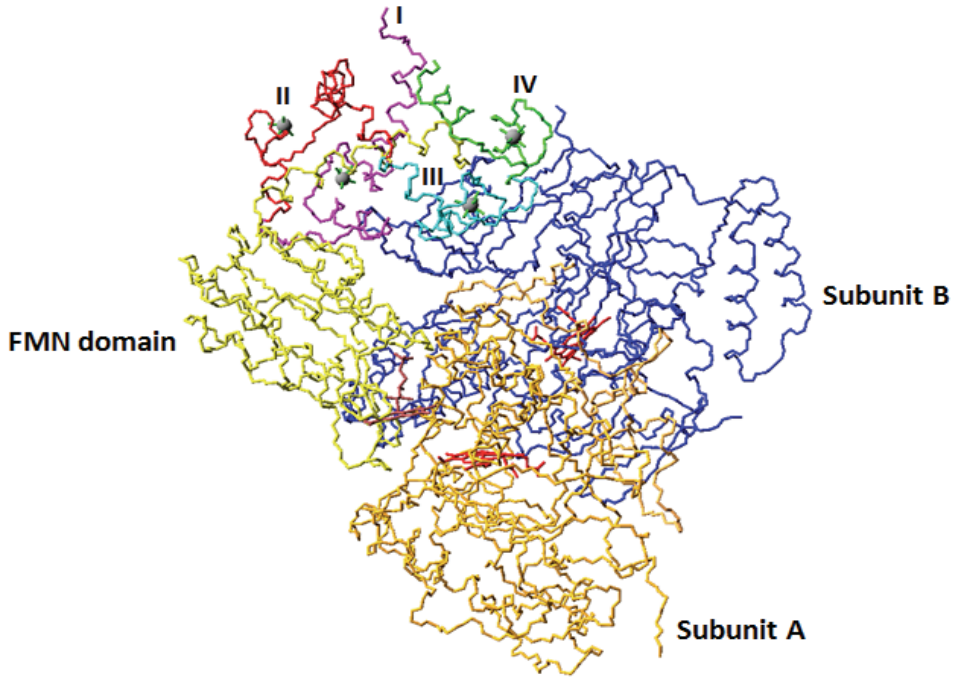


Figure S5. Model of a complex between the human iNOS oxygenase (orange and blue) and FMN (yellow) domains; CaM is added to the ZDOCK solution number 6. The CaM EF hands I, II, III and IV are shown in magenta, red, cyan and green, respectively. Note that the EF hand III interacts significantly with NOS oxygenase domain. The FMN and heme cofactors are shown in sticks, and calcium ions are gray spheres. The structure is orientated to better show the CaM domains.

Table S1. Parameters for the nine ZDOCK solutions [a]

Complex	ZDOCK score	Fe···N_5 (Å)	R511···E502 [b] (Å)
1	40.73	18.5	78.1
2	40.42	26.7	68.5
3	38.32	14.7	58.0
4	38.19	24.1	105.1
5	38.14	15.2	72.0
6	36.79	20.7	18.6
7	36.77	14.0	80.2
8	36.57	22.5	31.7
9	36.08	20.7	6.0

[a] Based on the two constraints (*i.e.*, Fe···N_5 distance being close to 18.8 Å, and R511···E502 distance being within a reasonable distance), model #6 is selected among the nine solutions.

[b] FMN domain docks to one oxygenase subunit (A), and is covalently connected to another oxygenase subunit (B) through the FMN domain residue R511 and oxygenase subunit B residue E502; see main text and Figure 9.