Supporting Information

Pulsed ENDOR determination of relative orientation of g- and molecular frames of imidazole-coordinated heme center of iNOS

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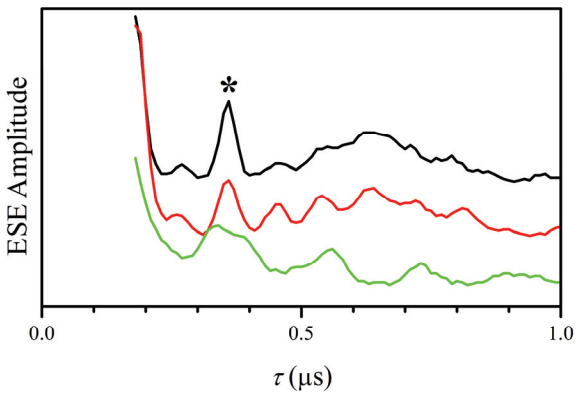

Figure S1. Three-pulse (stimulated) ESE decays recorded for the imidazole-coordinated heme of human iNOS oxyFMN at the EPR turning points as a function of the time interval τ between the 1st and 2nd pulses. Experimental conditions: mw frequency, 29.966 GHz; mw pulses, 3×11 ns; time interval between the 2nd and 3rd mw pulses, T = 4 μs; B_o = 864.8 mT (g_{lf}, black trace), 928.8 mT (g_{if}, red trace), and 1141.8 mT (g_{hf}, green trace); temperature, 10 K. The asterisk corresponds to τ = 360 ns used in Mims ENDOR measurements.

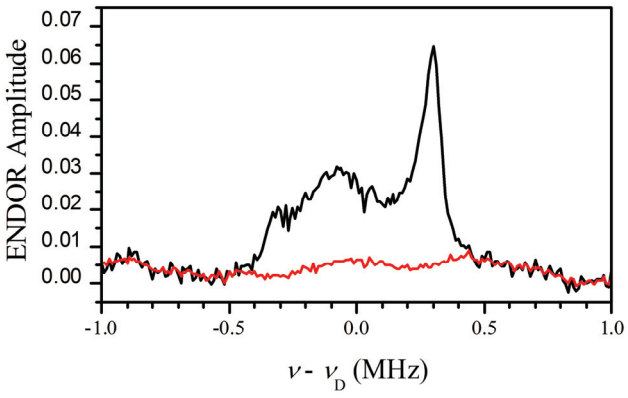

Figure S2. Mims ENDOR spectra of imidazole-coordinated heme in the human iNOS oxyFMN construct. Black and red traces correspond to the samples prepared with Im-D and Im-H, respectively. The spectra were normalized by the ESE signal amplitude without RF. Experimental conditions: mw frequency, 29.966 GHz; B_o = 8928.8 mT (g_{if}); mw pulses, 3×11 ns; time interval between the first and second mw pulses, τ = 360 ns; time interval between the second and third mw pulses, T = 60 μs; RF pulse, 55 μs; temperature, 10 K.

Table S1. The wavefunction coefficients describing the contributions of the d_{XY}, d_{XZ} and d_{YZ} orbitals into the singly occupied orbital of the Im-coordinated Fe(III) heme of iNOS as obtained from analysis of the principal g-values using the formalism of Taylor (Taylor, C. P. S. *Biochim. Biophys. Acta* **1977**, *491*, 137; ref. 57 of the manuscript). The subscripts at the *d*-orbitals above and in the Table refer to the axes of the proper coordinate system. The proper quantization axis, Z_Q, for the Fe(III) *d*-orbital set corresponds to $|g_{hf}| = 1.86$, and thus coincides with the g-frame axis X_g as defined in the main text. The other two proper axes, X_Q and Y_Q, correspond to $|g_{lf}| = 2.52$ and $|g_{if}| = 2.3$, and *vice versa*. The ratio of the rhombic and tetragonal splittings is the same for all of the solutions, $|V/\Delta| = 0.449$. The values shown in the Table correspond to the assignment $X_Q \rightarrow |g_{lf}| = 2.52$ and $Y_Q \rightarrow |g_{if}| = 2.3$. Given the ENDOR results of this work, the "proper" orbitals d_{XY} and d_{XZ} are actually the d_π orbitals because their planes are perpendicular to the heme plane. The solutions with the opposite assignment of X_Q and Y_Q are similar (only the values of $|a_{XZ}|$ and $|a_{YZ}|$ have to be interchanged).

g_X	g_Y	g_Z	$\lvert a_{XY}\rvert$	$\lvert a_{XZ}\rvert$	$\lvert a_{YZ}\rvert$	$\Sigma\lvert a_{ij}\rvert^2$
-2.52	2.3	-1.86	0.991	0.136	0.090	1.009
-2.52	2.3	1.86	0.659	0.599	0.569	1.117
-2.52	-2.3	1.86	0.054	1.074	0.108	1.168
2.52	2.3	1.86	0.061	0.182	1.148	1.355