Supporting Information

Regulatory Role of Glu546 in Flavin Mononucleotide – Heme Electron Transfer in Human Inducible NOS

Wenbing Li [†], Li Chen [†], Changyuan Lu [‖], Bradley O. Elmore [†], Andrei V. Astashkin [⊥], Denis L. Rousseau [‖], Syun-Ru Yeh [‖], Changjian Feng [†,*]

[†] College of Pharmacy, University of New Mexico, Albuquerque, NM 87131, USA;

[‖] Department of Physiology and Biophysics, Albert Einstein College of Medicine, NY 10461, USA

[⊥] Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ 85721, USA

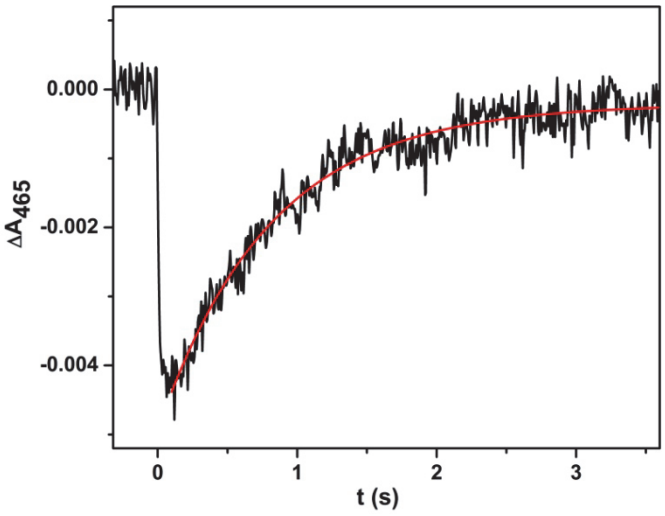

Figure S1. Transient trace at 465 nm from 0 to 4.0 s obtained for the [Fe(II)-CO][FMNH$^{\bullet}$] form of the human iNOS oxyFMN E546N mutant flashed by a 446 nm laser excitation. The optical change is due to rebinding of CO to Fe(II). The sample temperature was set at 21 °C. The anaerobic solution contained ~ 10 μM NOS protein, ~ 20 μM dRF and 5 mM fresh semicarbazide in pH 7.6 buffer (40 mM Bis-Tris propane, 400 mM NaCl, 2 mM L-arginine, 20 μM 6*R*-5,6,7,8-tetrahydrobiopterin, 1 mM Ca^{2+} and 10 % glycerol). The solid line corresponds to the best single-exponential fit to the data.

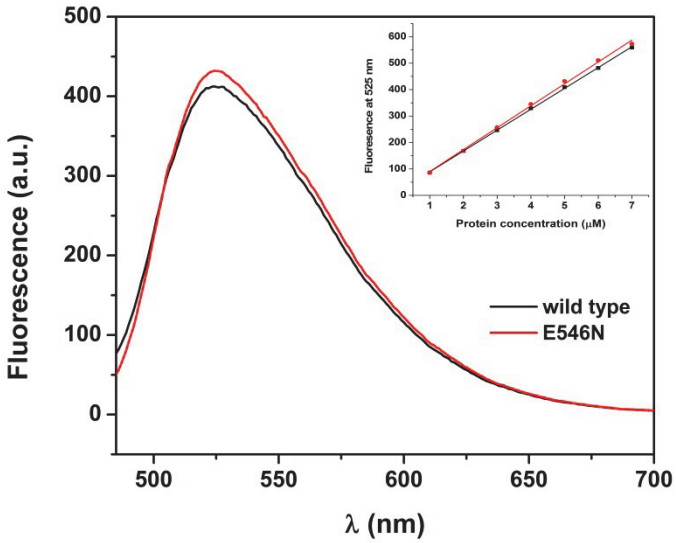

Figure S2. Flavin fluorescence spectra of wild type and E546N human iNOS oxyFMN proteins. The protein concentrations were 5 μM each, and the measurements were performed under the same conditions; excitation wavelength: 446 nm. Inset: plot of flavin fluorescence intensity (at 525 nm) versus the protein concentration (1 − 7 μM). Buffer: 40 mM Bis-Tris propane, 400 mM NaCl, 2 mM L-arginine, and 10 % glycerol, pH 7.6. Data are representative of two experiments.

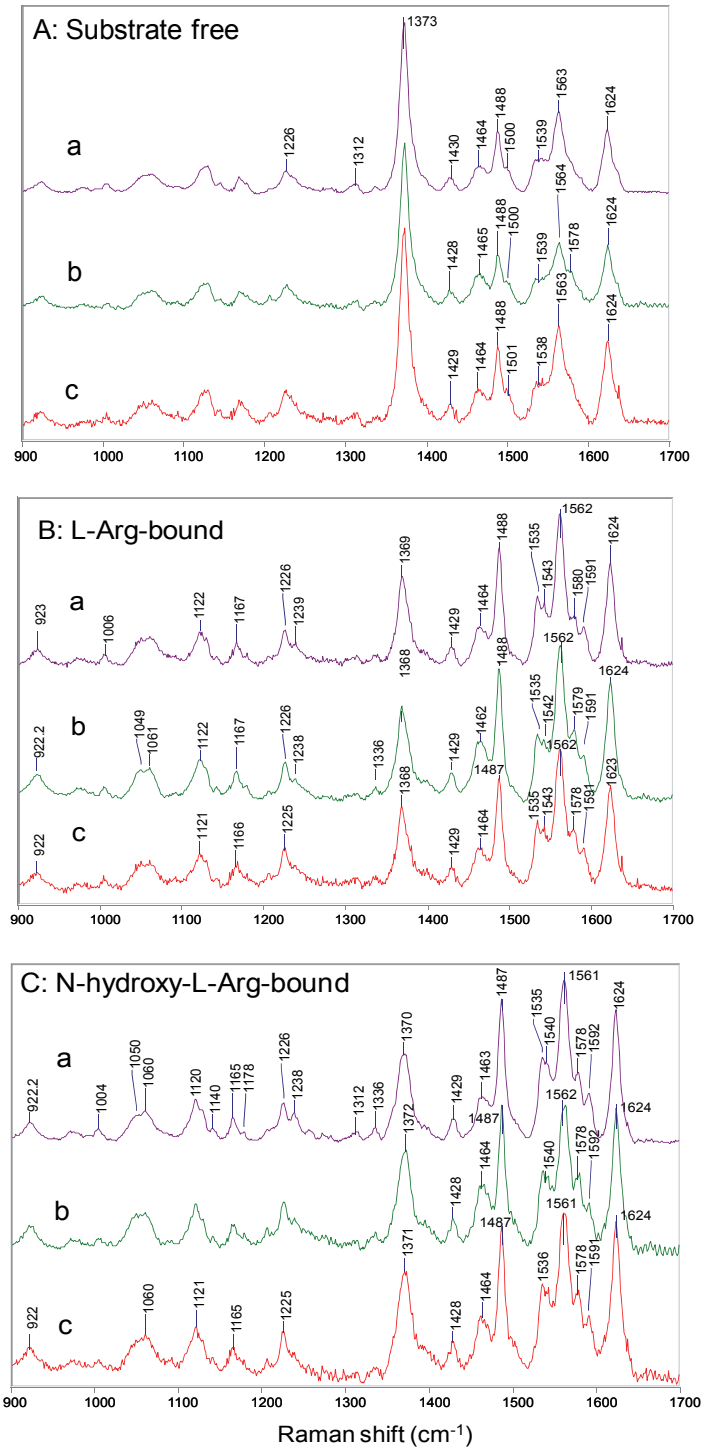

Figure S3. Resonance Raman spectra of the substrate-free (A), L-arginine-bound (B) and N-hydroxy-L-arginine-bound (C) complexes of the ferric derivatives. In each panel spectrum (a) is the wt iNOSoxy; spectrum (b) is the wt oxyFMN construct; spectrum (c) is the E546N oxyFMN construct. The right panel shows their associated $^{12}C^{16}O$-$^{13}C^{18}O$ isotope difference spectra. The excitation wavelength was 441.6 nm.

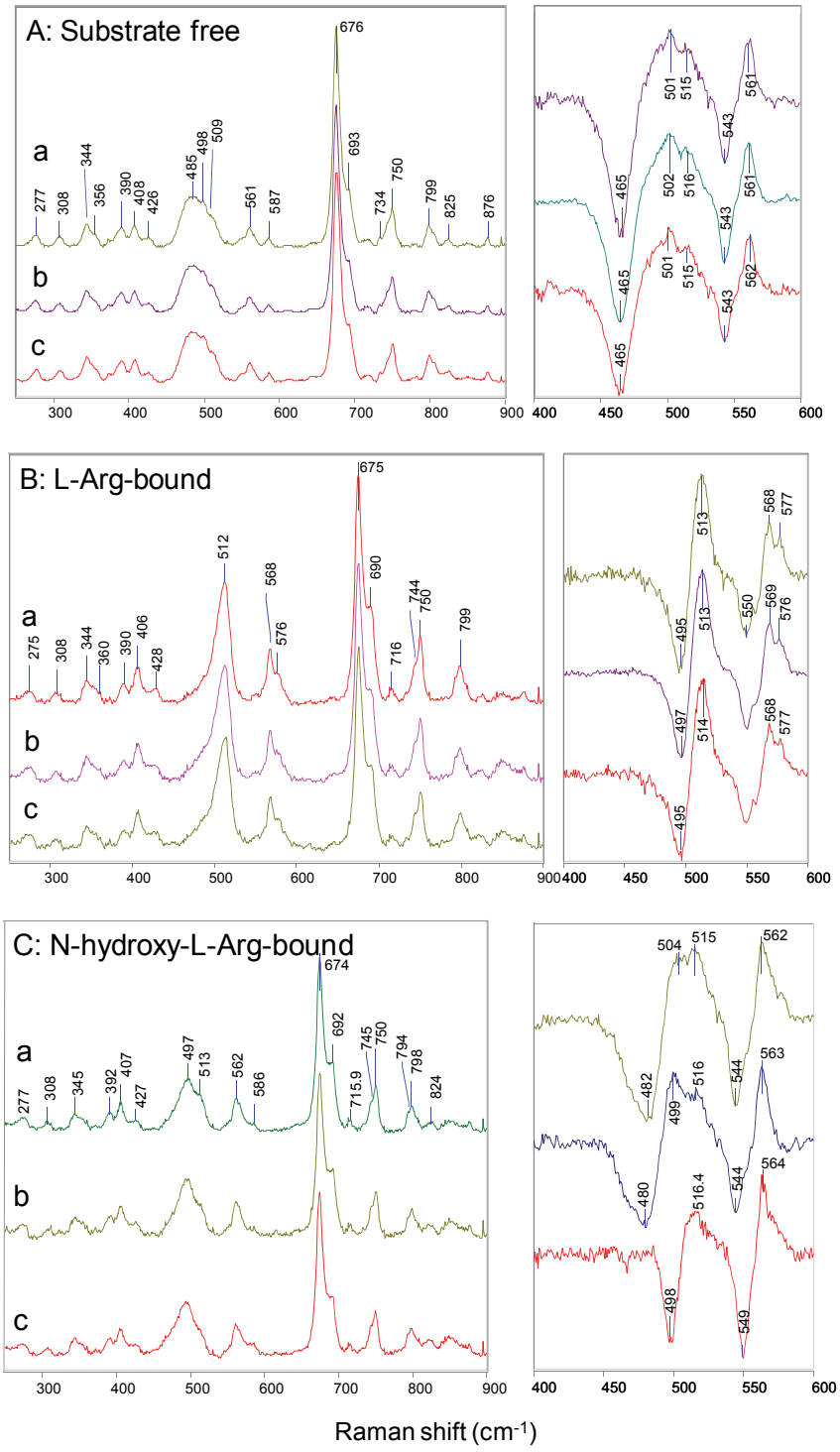

Figure S4. Resonance Raman spectra of the substrate-free (A), L-arginine-bound (B) and N-hydroxy-L-arginine-bound (C) complexes of the ferrous-CO derivatives. In each panel spectrum (a) is the wt iNOSoxy; spectrum (b) is the wt oxyFMN construct; spectrum (c) is the E546N oxyFMN construct. The right panel shows their associated $^{12}C^{16}O$-$^{13}C^{18}O$ isotope difference spectra. The excitation wavelength is 441.6 nm.

Table S1. The FMN−heme IET rate constants, k_{et}, of human iNOS oxyFMN proteins over the 283 to 304 K temperature range.

Temperature (K)	k_{et} (s^{-1})	
	E546N	wild type [a]
283.15	72 ± 4	142 ± 3
288.15	89 ± 7	238 ± 15
294.15	139 ± 3	343 ± 11
299.15	194 ± 6	579 ± 4
304.15	294 ± 21	863 ± 6

[a] Data taken from previous work (ref. 20 in main text).