SUPPORTING INFORMATION

Supplementary Fig. S1. Global analysis of optical spectra versus time data sets of the reaction of saNOS with PN to a three-states model (A→B→C).

Supplementary Fig. S2. Early phase of PN activation by saNOS.

Supplementary Fig. S3 PN concentration-dependence of I435 build-up and decay rates, and of PN decay rates, catalyzed by saNOS.

EXPERIMENTAL PROCEDURES

Stopped-flow experiments − Stopped-flow experiments with saNOS were performed with an Applied-Photophysics SX20 (Applied-Photophysics, Surrey, United Kingdom) equipped with a Xenon light source, a monochromator and a photomultiplier tube for optical measurements. The stopped-flow has a dead-time of 1.3 ms. Multiple single wavelength kinetics were obtained at 5 nm intervals and used to generate sets of data representing Optical spectra (380-460 nm) *vs* time. The buffer used was 100 mM KPi pH 6.4 + 300 μM DTPA. Upon mixing with PN in 0.01N NaOH, the final pH was ~7.0 which is comparable to the pH of the same reaction probed by resonance Raman spectroscopy.

Kinetic analysis of the catalytic intermediate I435 build-up and decay – Three data sets were obtained by mixing 5 μM saNOS with 50, 100, 250, 500 and 700 μM PN in 1:1 volume ratios. Single wavelength kinetic traces obtained at 5 nm intervals from 380-460 nm were used to generate Optical spectra vs Time data sets. These were fitted to a sequential A>B>C kinetic mechanism using the Specfit software. The data obtained by mixing with 500 μM PN are shown in **Figure S1**. Although the model did not perfectly reproduce all the spectral transitions of small amplitude, it provided a reasonable fit to the main transitions that correspond to the conversion of the ferric resting state (A, black) to intermediate I435 (B, blue) and the decay of I435 to a ferric low-spin state (C, red) in panel Fig. S1B. The original data of the early phase of the reaction are showed in **Figure S2**. The rate constants for the formation and decay of intermediate (I) were plotted as a function of PN concentrations (**Figure S3**).

PN decay by stopped-flow electronic optical absorption spectroscopy –PN decay was followed at 302 nm after mixing PN with buffer or with 5 μM saNOS (2.5 μM final concentration after mixing). The rates were obtained from fitting to a mono-exponential function (PN + buffer) or two-exponential functions (PN + saNOS). For the latter, the value of the second exponential was fixed to 0.4 s^{-1}, which is the rate of spontaneous PN decay in these conditions. The rate constant (k_{obs}) of the first exponential term determined from three kinetic traces were averaged and plotted as a function of PN (**Figure S3**).

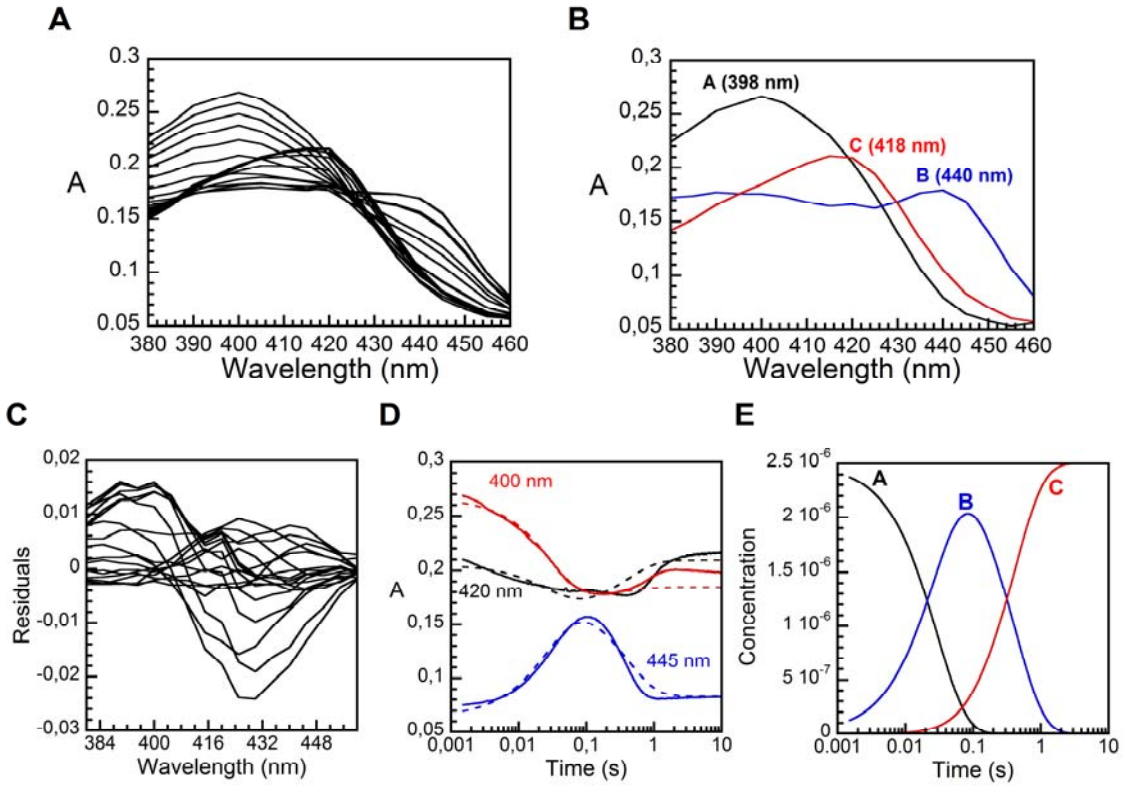

Figure S1. Global analysis of optical spectra versus time data sets of the reaction of saNOS with PN to a three-states model (A→B→C). saNOS (5 μM) in Kpi buffer was mixed 1:1 with 500 μM PN (2.5 μM saNOS and 250 μM PN final concentrations). **Panel A**. Original spectra generated from single wavelength kinetic traces recorded at 5 nm intervals from 380 to 460 nm and over an acquisition time of 10 seconds. **Panel B.** Calculated optical absorption spectra obtained following from global analysis with the three-state model. These three species, initial ferric (A, black), I435 (B, blue) and final ferric (C, red), contribute the most to the overall spectral changes. **Panel C**. Residuals of the fit as a function of wavelengths. The residuals at several times ranging from 1.5 ms to 10 sec are shown. **Panel D.** Kinetic traces recorded at three wavelengths as a function of time (solid lines) and the fits to these transitions (dashed lines). The transitions at 400 nm (red), 420 nm (black) and 445 nm (blue) are showed. **Panel E**. Calculated time course of formation and decay of the three species (A, B and C). Overall, the kinetics show the transient formation of I435 on the time-course of 0 to ~1 s (with a maximum at ~ 100 ms) from an initial ferric enzyme that is high-spin to a final ferric form that is low-spin.

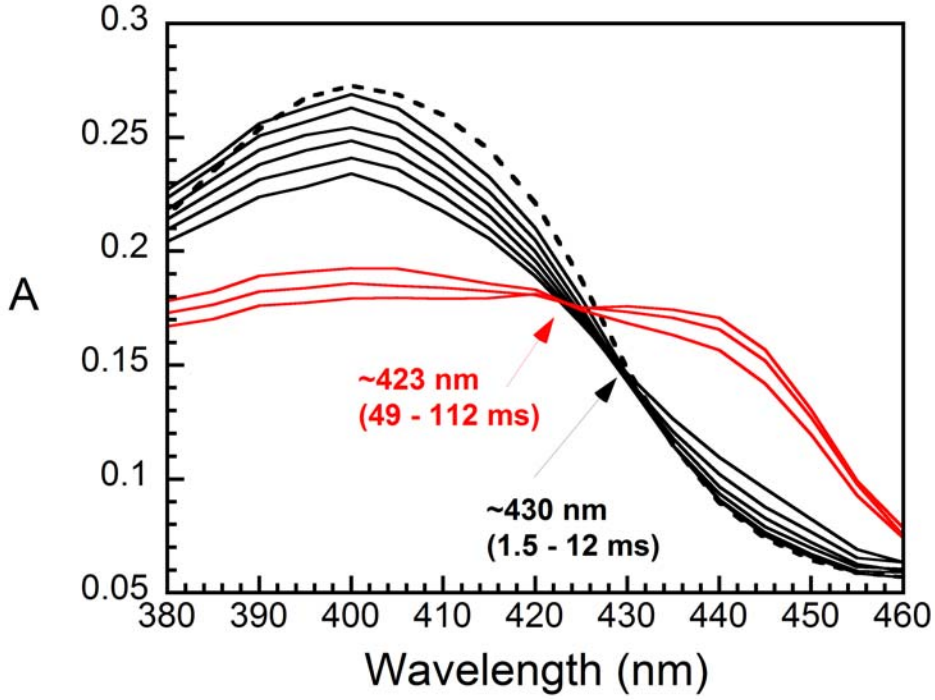

Figure S2. Early phase of PN activation by saNOS. The UV-visible electronic absorption spectra obtained during the early phase of PN activation by saNOS (Data from Figure S1). Spectra at 1.5 ms, 2.5 ms, 3.9 ms, 5.8 ms, 8.5 and 12 ms are shown in black and those recorded at 49 ms, 68 ms and 112 ms are shown in red. The optical spectrum of ferric saNOS (prior to mixing with PN) is also shown (dashed line). The arrows point to the isosbestic points for the 1.5 – 12 ms spectra (black) and for the 49 – 112 ms spectra (red).

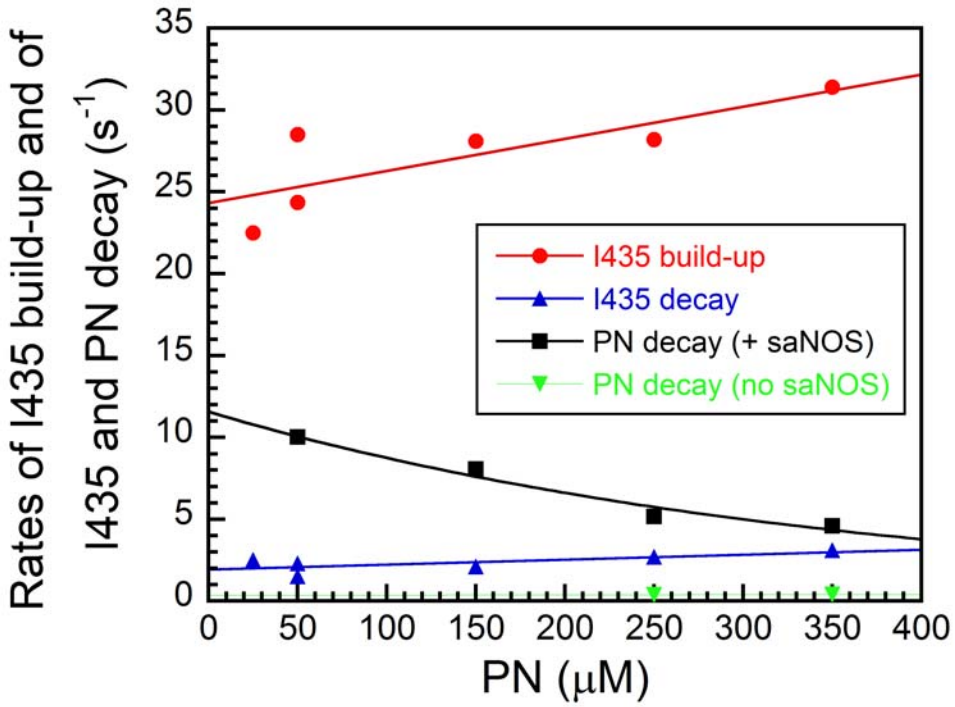

Figure S3. PN concentration-dependence of I435 build-up and decay rates, and of PN decay rates, catalyzed by saNOS. Various concentrations of PN (50 – 700 μM) were rapid-mixed with 5 μM saNOS in anaerobic KPi buffer (red, blue and black) or with KPi buffer alone (green). Apparent rate constants for the build-up and decay of I435 were obtained from the three-state model (A → B → C). They are plotted as a function of PN concentrations for I435 build-up (red) and decay (blue). The linear fits are shown (solid lines). PN decay was followed from single wavelength kinetics recorded at 302 nm. The apparent rates of decay of PN in buffer (kobs = 0.4 s^{-1}, green) was obtained from the fit to a mono-exponential function. The linear fit to these points is showed (green line) to provide the baseline above which PN decay is catalyzed by saNOS. The apparent rates of decay of PN catalyzed by saNOS (black) were obtained from fits to bi-exponential functions with the value of k_2 (spontaneous rate of PN decay) fixed to 0.4 s^{-1}. These apparent rates were fitted to an exponential decay model (black line) which gave a significantly better fit (r^2 = 0.982) than the fit to a linear model (r^2 = 0.948).