

Supplemental Figure S1

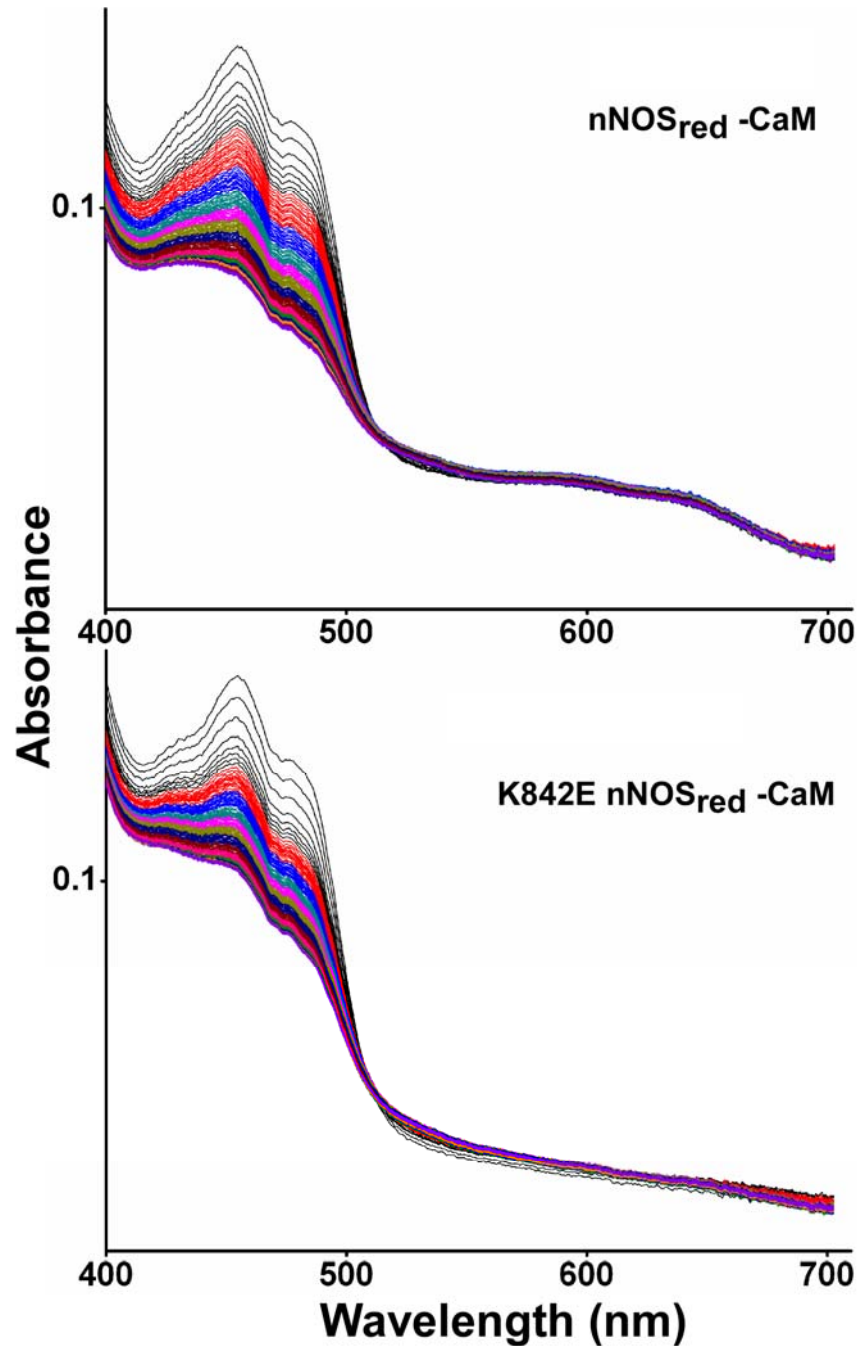


Fig. S1. Kinetics of anaerobic flavin reduction in CaM-free WT and K842E nNOS_{red} by excess NADPH. Stopped flow diode array traces were collected for initial 0.4 sec after rapidly mixing 7-8 μ M CaM-free oxidized WT nNOS_{red} or K842E nNOS_{red} with 10-fold excess of NADPH at 10 °C as described under “Experimental Procedures”. *Upper panel* shows the spectral traces for WT nNOS_{red} whereas the *lower panel* shows spectral traces for K842E nNOS_{red} mutant.

Supplemental Figure S2

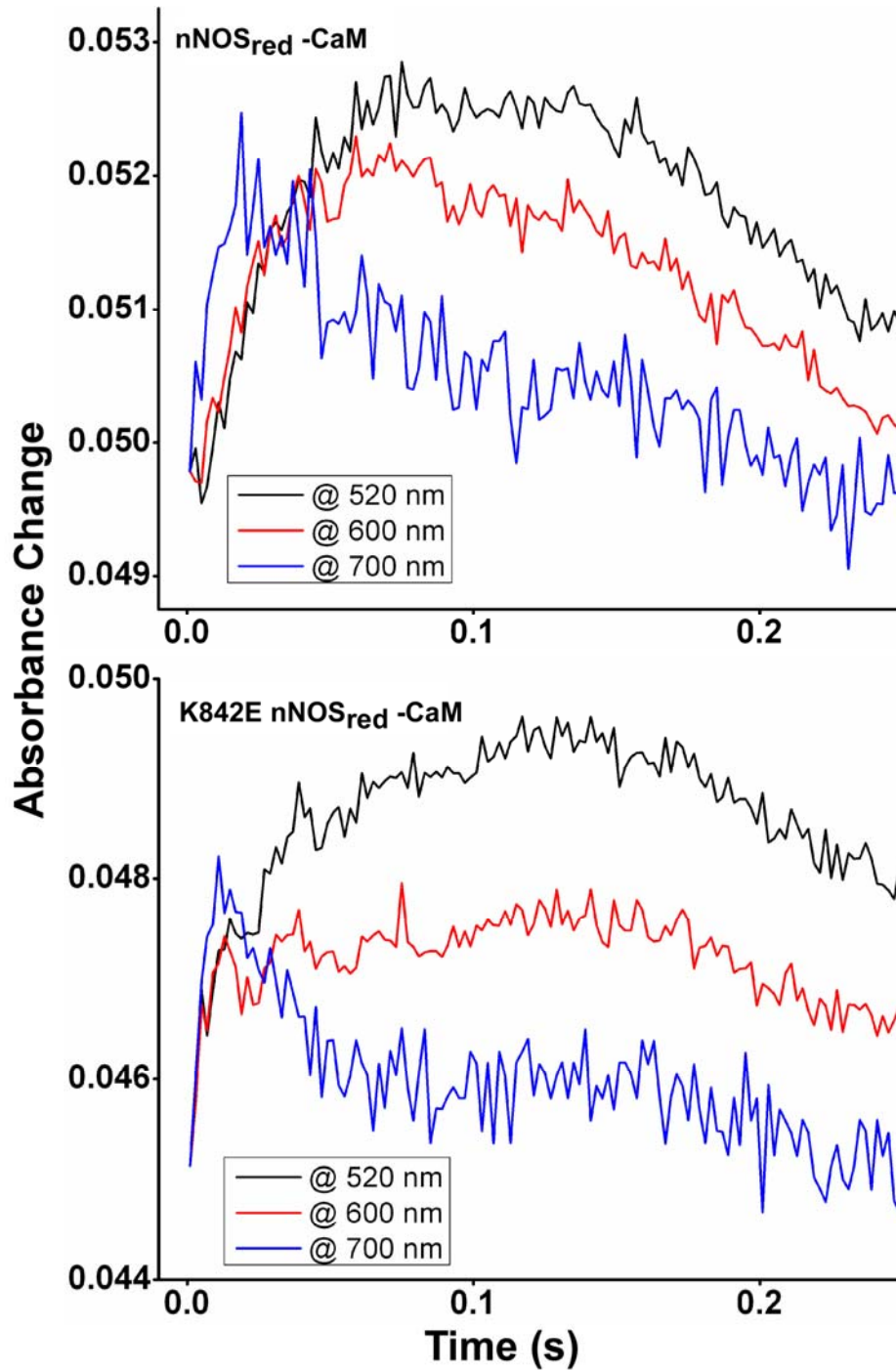


Fig. S2. Kinetics of anaerobic flavin reduction in CaM-free WT and K842E nNOS_{red} by excess NADPH. Stopped flow traces were collected after rapidly mixing 7-8 μ M CaM-free oxidized WT nNOS_{red} or K842E nNOS_{red} with 10-fold excess of NADPH at 10 °C as described under “Experimental Procedures”. *Upper panel* shows the spectral traces for WT nNOS_{red} whereas the *lower panel* shows spectral traces for K842E nNOS_{red} mutant.

Supplemental Figure S3

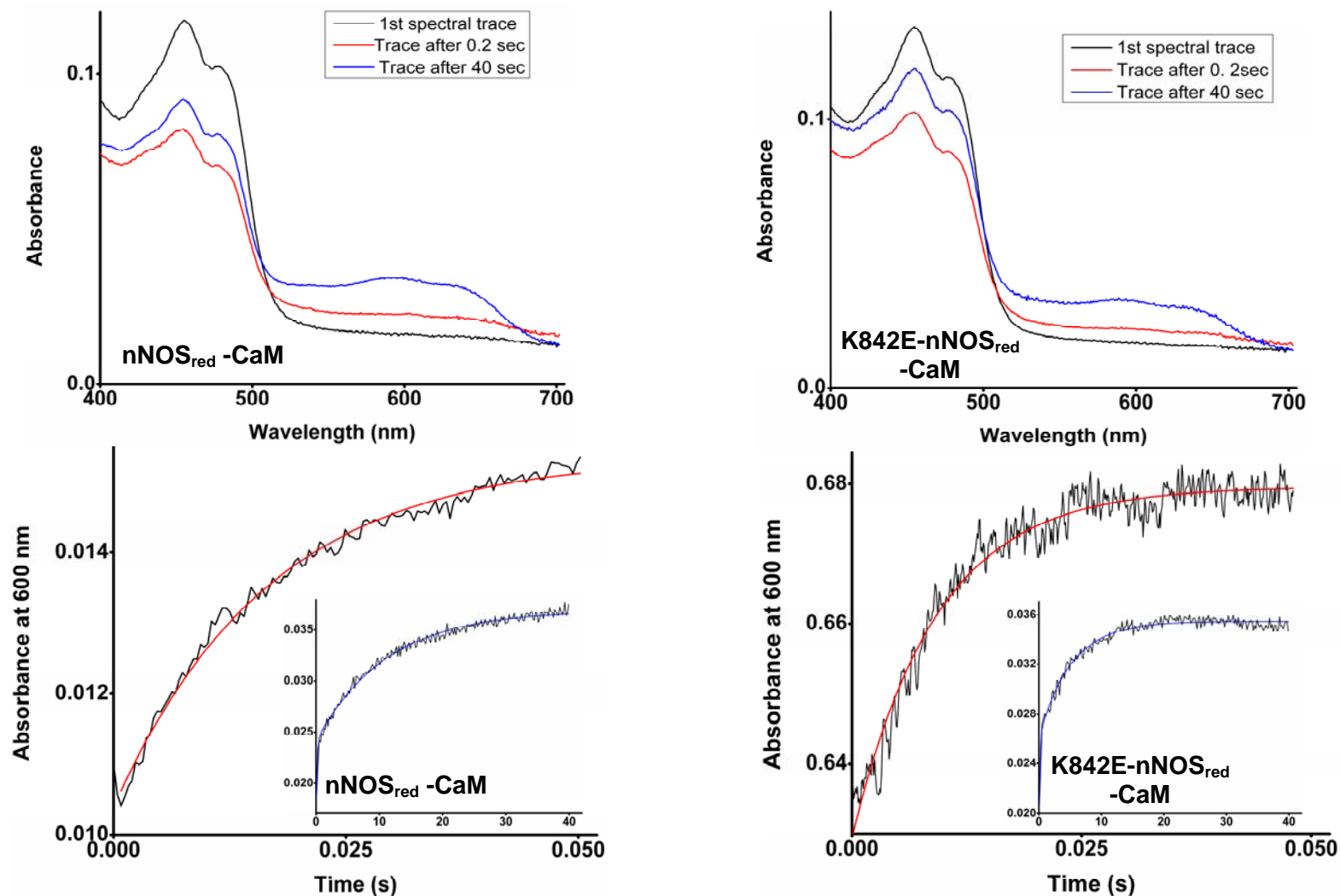


Fig. S3. Kinetics of anaerobic flavin reduction in WT and K842E nNOS_{red} by a single molar equivalent of NADPH. Stopped flow traces were collected after rapidly mixing oxidized WT nNOS_{red} (*Left panels*) and K842E nNOS_{red} (*Right panels*) with a single molar equivalent of NADPH at 10 °C as described under “Experimental procedures”. Rapid-scanning diode array experiments for both WT and K842E nNOS_{red} (upper panels) illustrate the spectral changes and the corresponding changes at 600 nm. Data was obtained by using 8-9 μ M nNOS_{red} proteins prior to mixing. The absorbance changes were separated into initial fast phases and subsequent slow phases (insets) with representative traces given. Rate constants obtained by fitting these data are reported in Supplemental Table 1.

Supplemental Figure S4

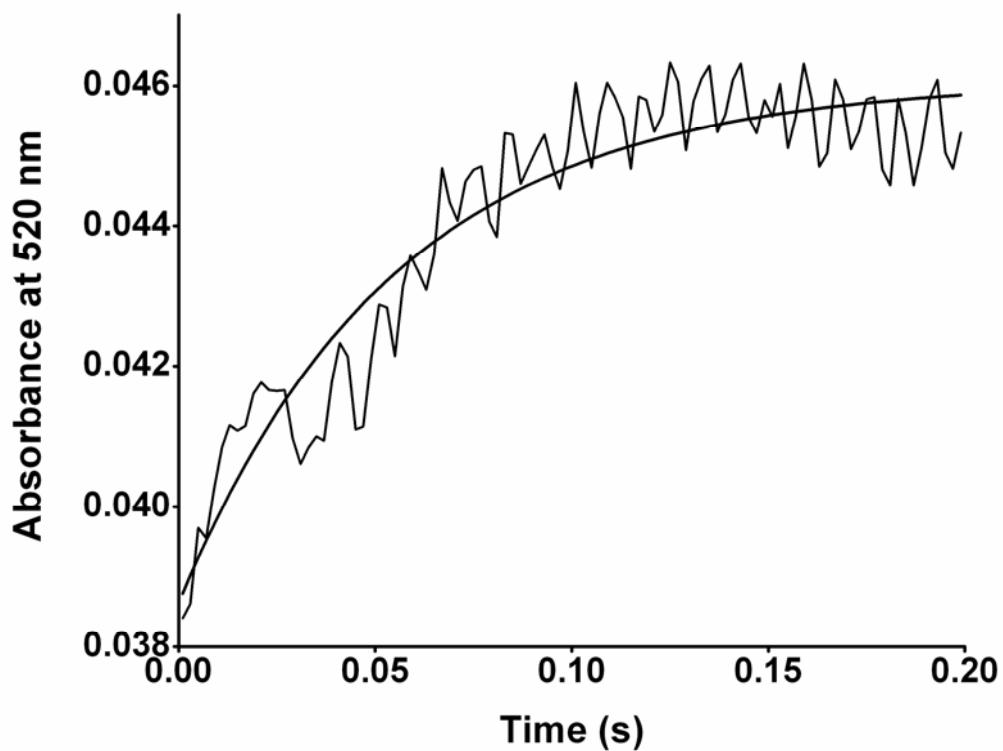


Fig. S4. Absorption change at 520 nm in CaM-bound K842E nNOS_{red}. Stopped flow traces were obtained after rapidly mixing the air-stable semiquinone forms of CaM-bound K842E nNOS_{red} with 10-fold excess of NADPH at 10 °C as described under “Experimental Procedures”. The calculated best fit curves are plotted as *solid lines*.