Supporting Information

Structural Evidence: A Single Charged Residue Affects Substrate Binding in Cytochrome P450 BM-3

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R47K	5'-ctttaaattcgaggcgcctggtaaggtaacgcgctacttatcaagtc-3'
R47K_antisense	5'-gacttgataagtagcgcgttaccttaccaggcgcctcgaatttaaag-3'

R47Q	5'-attcgaggcgcctggtcaggtaacgcgctacttatc-3'
R47Q_antisense	5'-gataagtagcgcgttacctgaccaggcgcctgaat-3'

R47E	5'-tttaaattcgaggcgcctggtgaggtaacgcgctacttatcaagt-3'
R47E_antisense	5'-acttgataagtagcgcgttacctcaccaggcgcctcgaatttaaa-3'

Table S1 - Primers both forward (sense) and reverse (antisense) used to sequence R47K, R47Q, and R47E mutants of BM-3 and BMP.

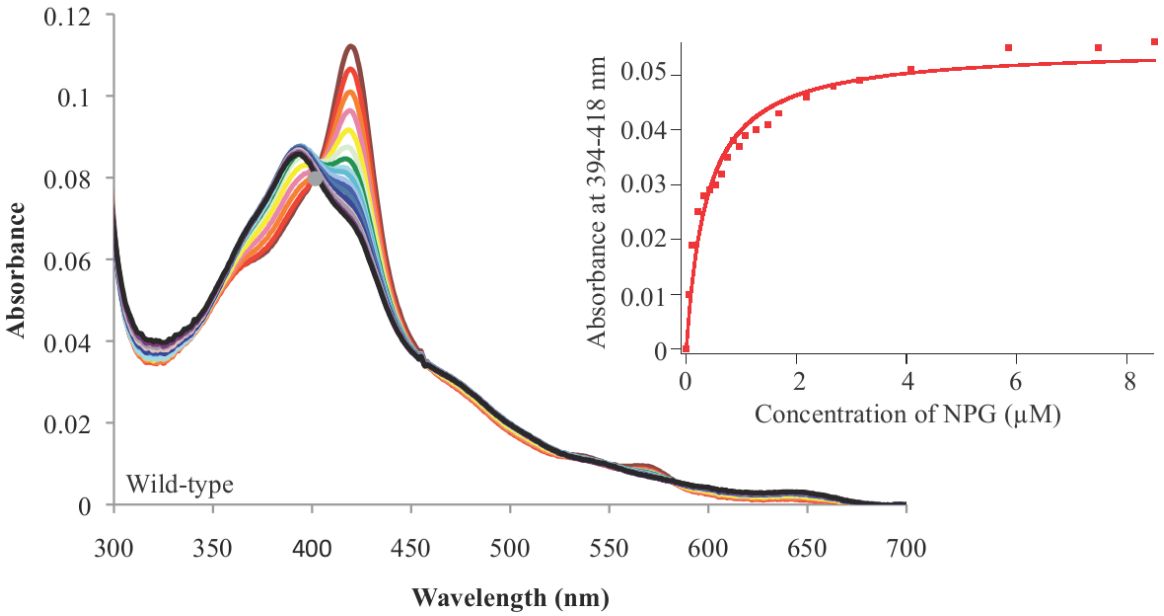

Figure S1 - Titration of NPG into BM-3 (wild-type) at room temperature. The dark red spectrum is at the start of the titration with zero NPG and the black spectrum is at saturation conditions of NPG. The optical absoption spectra of wild-type BM-3 has typical type 1 binding, an increase in absorabance at 394 nm upon the addition of NPG and a decrease at 418 nm indicating a change in spin state upon ligand binding. The inlay in the absorbance spectrum is the fit of the change in absorbance between 394nm and 418nm plotted against the change in concentration of NPG to determine the K_d. The K_d of wild-type was 0.37± 0.05 μM.

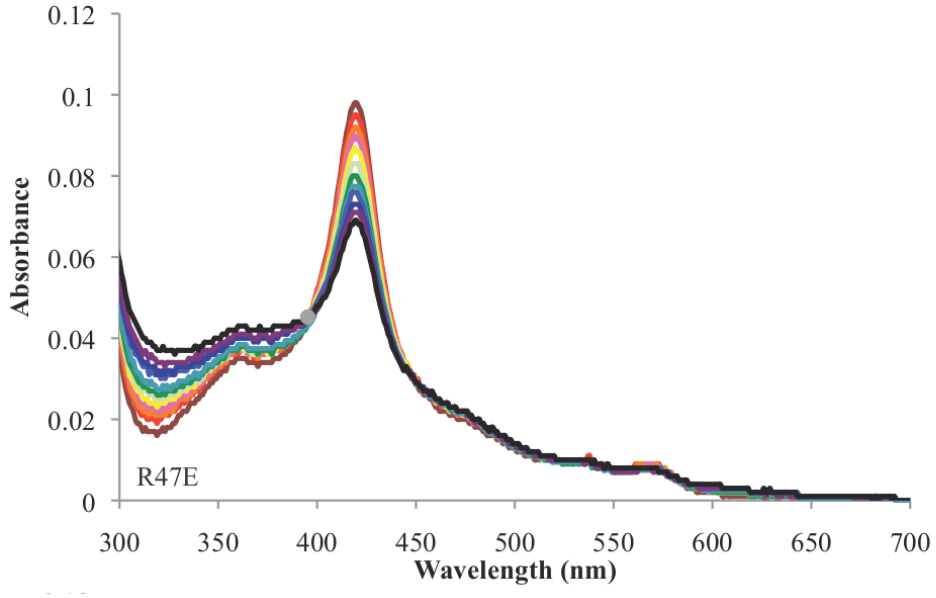

Figure S2 - Titration of NPG into R47E BM-3 at room temperature. The dark red spectrum is the start of the titration with zero NPG and the black spectrum is at saturation conditions of NPG. In the optical absorption spectra of R47E the same type I binding is not observed as in the wild-type and the isospectic point (gray circle) is different (wt 406 nm and R47E 399 nm), therefore a K_d was not determined.

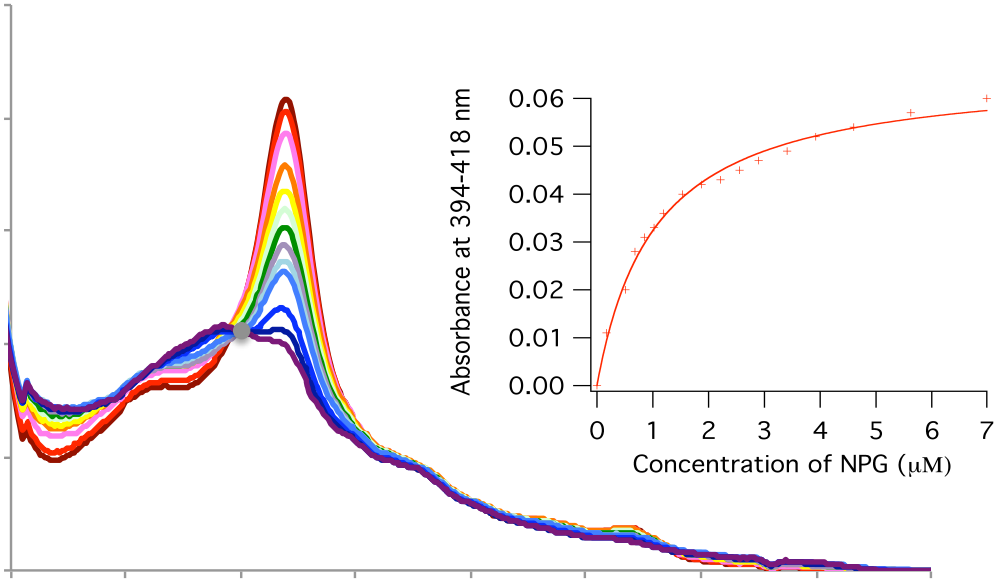

Figure S3 - Titration of NPG into R47K BM-3 at room temperature. The dark red spectrum is at the start of the titration with zero NPG and the purple spectrum is at saturation conditions of NPG. In the optical absorption spectra of R47K type I binding is observed with the same the isospectic point (gray circle) as the wild-type (406 nm). The inlay in the absorbance spectrum is the fit of the change in absorbance between 394nm and 418nm plotted against the change in concentration of NPG to determine the K_d. The K_d of 1.2 ± 0.1 μM for R47K was determined, which is three times weaker than the wild-type.

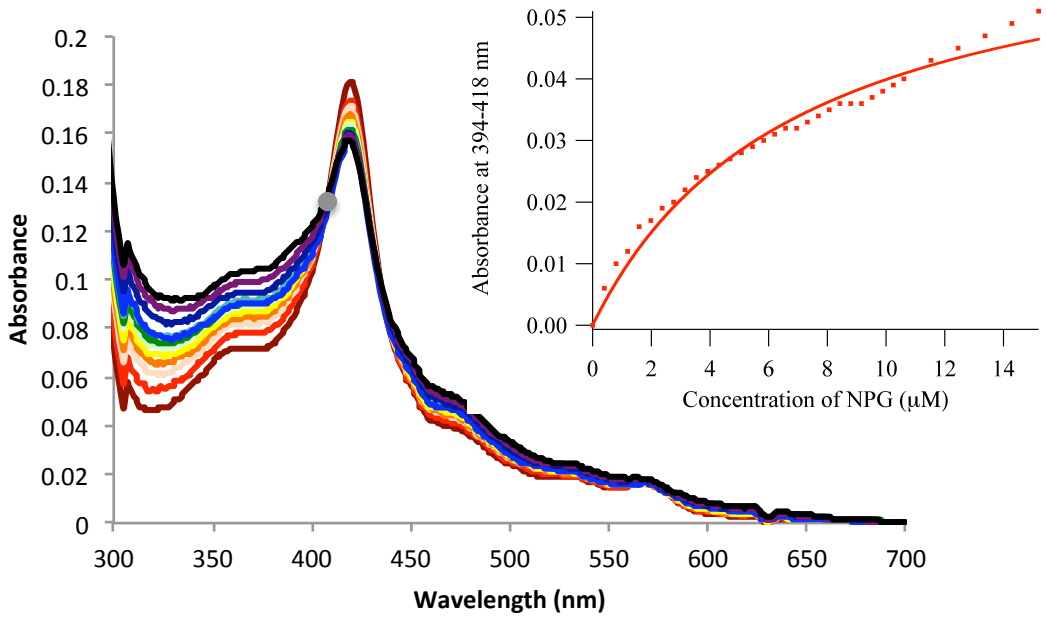

Figure S4 - Titration of NPG into R47Q BM-3 at room temperature. The dark red spectrum is the start of the titration with zero NPG and the black spectrum is at saturation conditions of NPG. In the optical absorption spectra of R47Q type I binding is observed with the same the isospectic point (gray circle) as the wild-type (406 nm). The inlay in the absorbance spectrum is the fit of the change in absorbance between 394nm and 418nm plotted against the change in concentration of NPG to determine the K_d. The K_d of 7.0 ± 0.7 μM for R47Q was determined, which is approximately twenty times weaker than the wild-type.

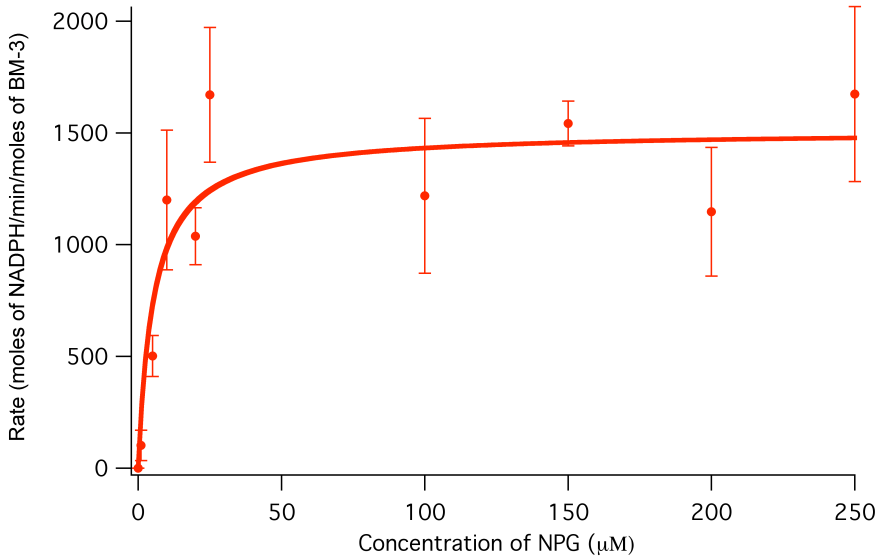

Figure S5- A fit of the rate of catalysis in moles of NAPDH/min/moles of BM-3 versus the concentration of NPG. The K_m was determined at 1500 ± 140 and k_{cat} at 5 ± 3 for at room temperature.

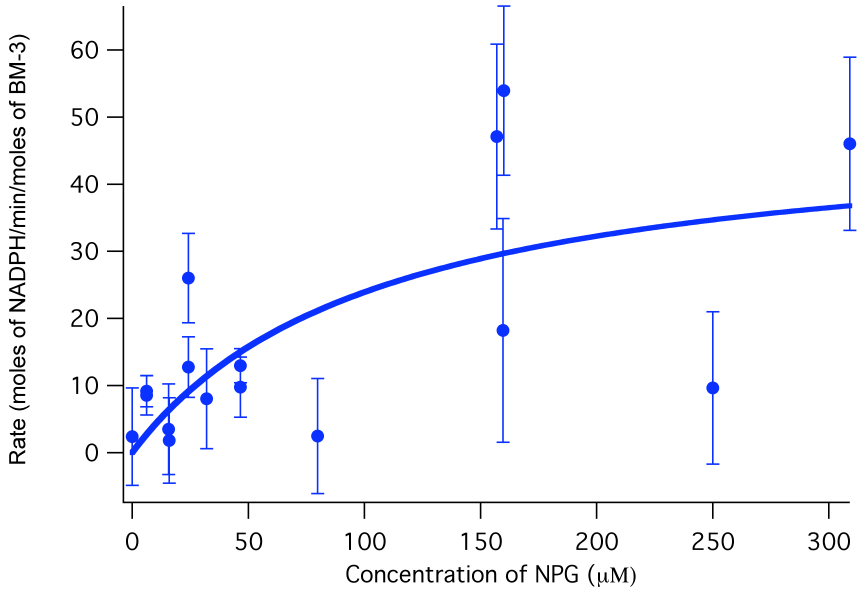

Figure S6- A fit of the rate of catalysis in moles of NAPDH/min/moles of R47E BM-3 versus the concentration of NPG. The K_m was determined at 90 ± 24μM and k_{cat} at 42 ± 8 for R47E at room temperature. At low concentrations of NPG, barely any catalysis is occurring since non-coupled electron transfer was measured at 5 ± 4 min^{-1}.

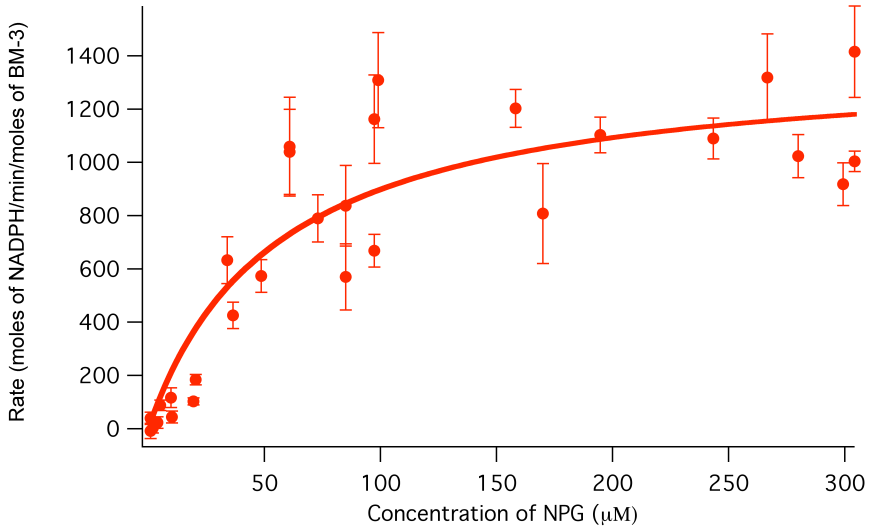

Figure S7- A fit of the rate of catalysis in moles of NAPDH/min/moles of R47K BM-3 versus the concentration of NPG. The K_m was determined at 55 ± 17 μM and k_{cat} at 1390 ± 140 for R47K at room temperature. The K_m was approximately ten times weaker than the wild-type. However, the k_{cat} was within error of the wild-type.

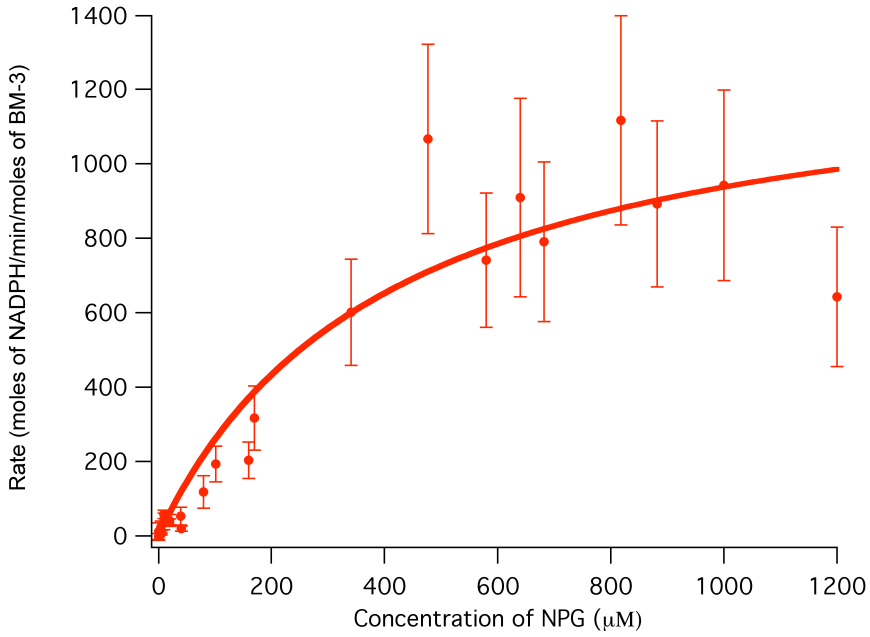

Figure S8- A fit of the rate of catalysis in moles of NAPDH/min/moles of R47Q BM-3 versus the concentration of NPG. The K_m was determined at 411 ± 165 μM and k_{cat} at 1320 ± 214 for R47Q at room temperature. The K_m was approximately eighty times weaker than the wild-type, showing R47's importance in binding. However, the k_{cat} was within error of the wild-type.