

Supplemental Material for:

Conformational Changes of NADPH-Cytochrome P450 Oxidoreductase are Essential for Catalysis and Cofactor Binding

Chuanwu Xia^{1*}, Djemel Hamdane^{2*}, Anna L. Shen³, Vivian Choi³⁺, Charles B. Kasper³, May Pearl², Haoming Zhang², Sang-Choul Im², Lucy Waskell², and Jung-Ja P. Kim¹

TABLE S1. Strategy for Generation of Cysteine Mutants.

| Mutation | PCR-A | PCR-B | PCR-C | Cloning Sites |
|-----------------|---|--|--|----------------------|
| C136A | 5'-AGTCTTCGCCATGGCCACAT-3' 3'-GGTTTCTGGTTCTCGTAGCT-5' | 5'-ATGTGGCCATGGCGAAGACT-3' 3'-GGCAAGCTTAATGCGGTAGT-5' | 5'-GGCACTGGCTGGTTTCGCTA-3' 3'-GGTTTCTGGTTCTCGTAGCT-5' | BamH1-Pst1 |
| C363T | 5'-TCCGTTCCCACTCCCACCA-3' 3'-GGCAAGCTTAATGCGGTAGT-5' | 5'-TGGTGGGAGTGGGGAACGGA-3' 3'-CCACTGGGAGGAGTCGAGC-5' | 5'-CCACTGGGAGGAGTCGAGC-3' 3'-GGCAAGCTTAATGCGGTAGT-5' | BstY1-Not1 |
| C445L | 5'-GACCACCTGCTTGAGCTGCT-3' 3'-GGCAAGCTTAATGCGGTAGT-5' | 5'-AGCAGCTCAAGCAGGTGGTC-3' 3'-CCACTGGGAGGAGTCGAGC-5' | 5'-CCACTGGGAGGAGTCGAGC-3' 3'-GGCAAGCTTAATGCGGTAGT-5' | BstY1-Not1 |
| C472T1 | 5'-CGTGACATCACTGCCGTGG-3' 3'-GGCAAGCTTAATGCGGTAGT-5' | 5'-CCACGGCAGTGATGTGCACG-3' 3'-CCTGGATGTCATCATGTCTC-5' | 5'-CCTGGATGTCATCATGTCTC-3' 3'-GGCAAGCTTAATGCGGTAGT-5' | Pst1-Not1 |
| C228A | 5'-TGGCCAGCTGTGGCCGAGTTCTT-3' 5'-GGTTTCTGGTTCTCGTAGCT-3' | CLONED DIRECTLY | | Pvu2-Sac1 |
| D147C | 5'-ACCCACGTGCAATGCGCAGG-3' 5'-GGTTTCTGGTTCTCGTAGCT-3' | CLONED DIRECTLY | | BstY1-Sac1 |
| R514C | 5'-ATGTTCTGTGCAAATCTCAG 5'-GGCAAGCTTAATGCGGTAGT-3' | CLONED DIRECTLY | | Not1-Avr2 |

For the mutants, C136A, C363T, C445L, and C472T1, two initial PCRs, designated A and B were carried out. The products of reactions A and B were used as the template for PCR C, using the indicated primers. For C228A, D147C, and R514C, restriction enzyme recognition sites were incorporated into the PCR primers and the resulting PCR fragments were digested and cloned directly.