SUPPLEMENTAL DATA

The distal heme pocket residues of a B-type Dye-decolorizing peroxidase: arginine but not aspartate is essential for peroxidase activity

Rahul Singh[1], Jason C. Grigg[1], Zachary Armstrong[1], Michael E.P. Murphy[1], Lindsay D. Eltis[1]*

[1]Department of Microbiology and Immunology, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada.

Table of Contents

Page S-1 – Supplemental Experimental Procedures
Page S-2 – Supplemental Table S1
Page S-3 – Supplemental Table S2
Page S-4 – Supplemental Figures S1and S2
Page S-5 – Supplemental Figure S3
Page S-6 – Supplemental Figure S4
Page S-7 – Supplemental Figure S5
Page S-8 – Supplemental Figure S6
Page S-9 – Supplemental Figure S7
Page S-10 – Supplemental Figure S8
Page S-11 – Supplemental Figure S9

Supplemental Experimental Procedures

Construction of DypB variants: The plasmids carrying genes coding for the D153H (pETDB153H), D153A (pETDB153A), N246H (pETDB246H) and N246A (pETDB246A) were constructed using pETDYB1 as the template DNA and the following 5' phosphorylated forward primers:

1. oD153A: GGATTCGTC**GCG**GGCACCGAGAAT
2. oN246A: CCTCCGCGAC**GCG**ATGGCATTCG
3. oD153H: GGATTCGTC**CAC**GGCACCGAGAAT
4. oN246H: CCTCCGCGAC**CAC**ATGGCATTCG

The plasmid carrying the gene encoding the D153A/N246A variant (pETDB153A/246A) was constructed using pETDB153A as the template DNA and the primer oN246A. Mutagenesis was performed using the QuikChange^{TM} PCR protocol (Agilent Technologies) with minor modifications. Thus, only forward primer was used for the mutagenesis reaction and the resulting PCR products were digested with DpnI and transformed into *E. coli* Nova Blue electro-competent cells (*recA*$^-$, *endA*$^-$, *lacI*^{q}). The desired mutation was confirmed by sequencing the complete gene length

Supplemental Table S1. UV-vis electronic absorption spectroscopy of DypB and the variants. The protein samples were in 20 mM MOPS and 50 mM NaCl at pH 7.5.

Enzyme	Soret (nm)	*Rz* (Soret/280)	ε (mM^{-1} cm^{-1})
DypB[1]	404	2.7	84
D153H	410	2.8	91
D153A	404	3.0	120
R244L[2]	410	1.8	80
N246H	410	4.2	92
N246A	404	2.6	120
D153A /N246A	404	2.5	98

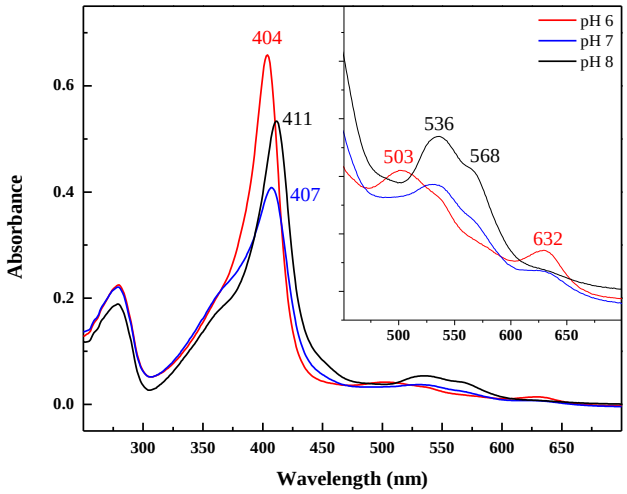
[1]Obtained from the reference 7

[2]At pH 6, the Soret was recorded at 404 nm and the *Rz* was 2.9 (Supplemental Figure S1).

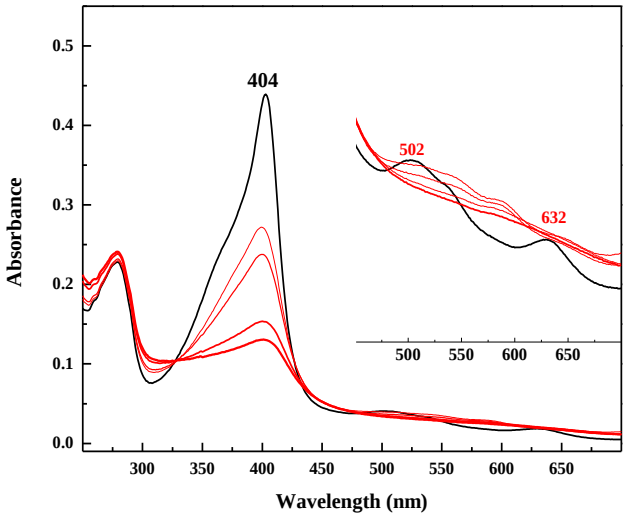
Supplemental Table S2. Data collection and refinement statistics.

	DypB D153A	DypB D153H	DypB N246A	DypB N246H	DypB R244L
Data collection[a]					
Wavelength (Å)	0.97952	0.97952	0.97952	0.97952	0.97952
Resolution range (Å)	40.0 – 2.6 (2.64 – 2.60)	40.0 – 2.5 (2.54 – 2.50)	40.0 – 2.4 (2.44 – 2.40)	45.0 – 2.64 (2.69 – 2.64)	53.0 – 2.35 (2.43 – 2.35)
Space group	$P3_221$	$P3_221$	$P3_221$	$P3_221$	$P3_221$
Unit cell dimensions (Å)	*a* = 132.4, *b* = 132.4, *c* = 160.6	*a* = 132.6, *b* = 132.6, *c* = 159.6	*a* = 132.9, *b* = 132.9, *c* = 161.0	*a* = 132.3, *b* = 132.3, *c* = 158.8	*a* = 133.2, *b* = 133.2, *c* = 158.9
Unique reflections	50668	56625	64436	49698	67842
Completeness (%)	99.1 (100)	99.6 (99.5)	98.0 (98.6)	99.5 (99.8)	99.9 (100)
Average I/σI	21.6 (2.7)	19.5 (2.3)	22.2 (3.5)	19.7 (2.9)	15.6 (3.6)
Redundancy	5.0 (4.7)	5.3 (4.3)	6.3 (6.4)	5.5 (5.2)	6.0 (6.0)
R_{merge}	0.062 (0.543)	0.069 (0.627)	0.070 (0.588)	0.074 (0.566)	0.096 (0.516)
Wilson B ($Å^2$)	57.7	54.7	50.0	60.0	44.2
Refinement					
R_{work} (R_{free})	0.166 (0.213)	0.184 (0.218)	0.184 (0.218)	0.156 (0.205)	0.186 (0.222)
B-factors ($Å^2$)					
All atoms	51.6	48.9	44.1	51.3	39.1
Protein	51.9	49.2	44.3	51.6	39.1
Heme	42.8	37.9	33.3	38.7	33.4
Water	44.3	42.1	41.0	45.3	38.2
r.m.s.d. bond length (Å)	0.013	0.013	0.013	0.013	0.013
In most-favorable region	90.5	91.2	91.8	90.0	92.7
In disallowed regions	0.0	0.1	0.4	0.0	0.1
PDB Accession Codes	3VEC	3VED	3VEE	3VEF	3VEG

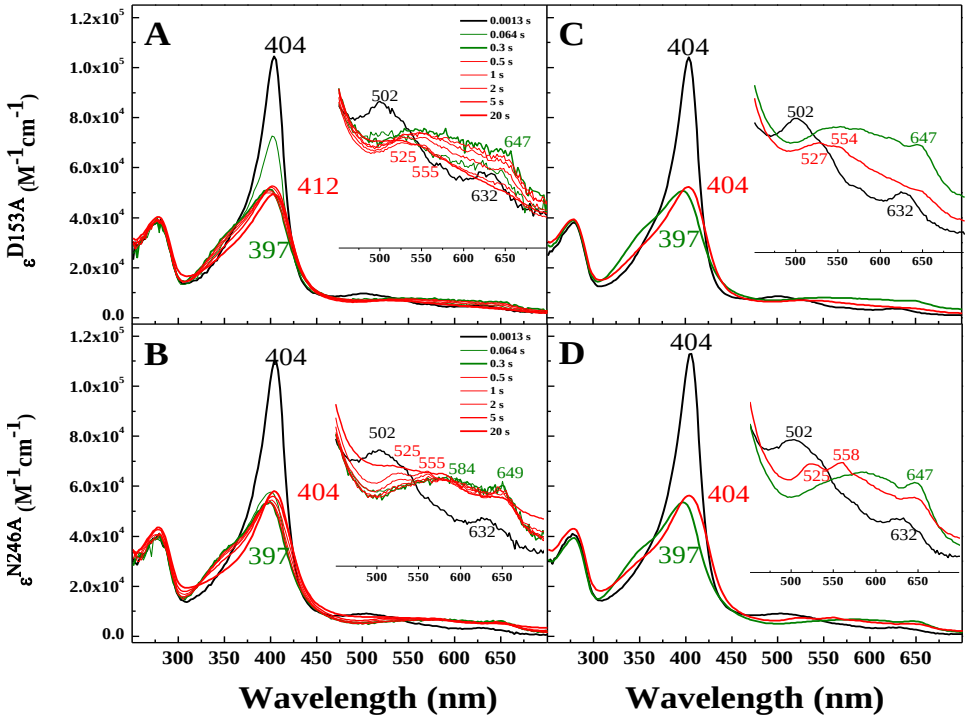
[a]values for the highest resolution shell are shown in parenthesis



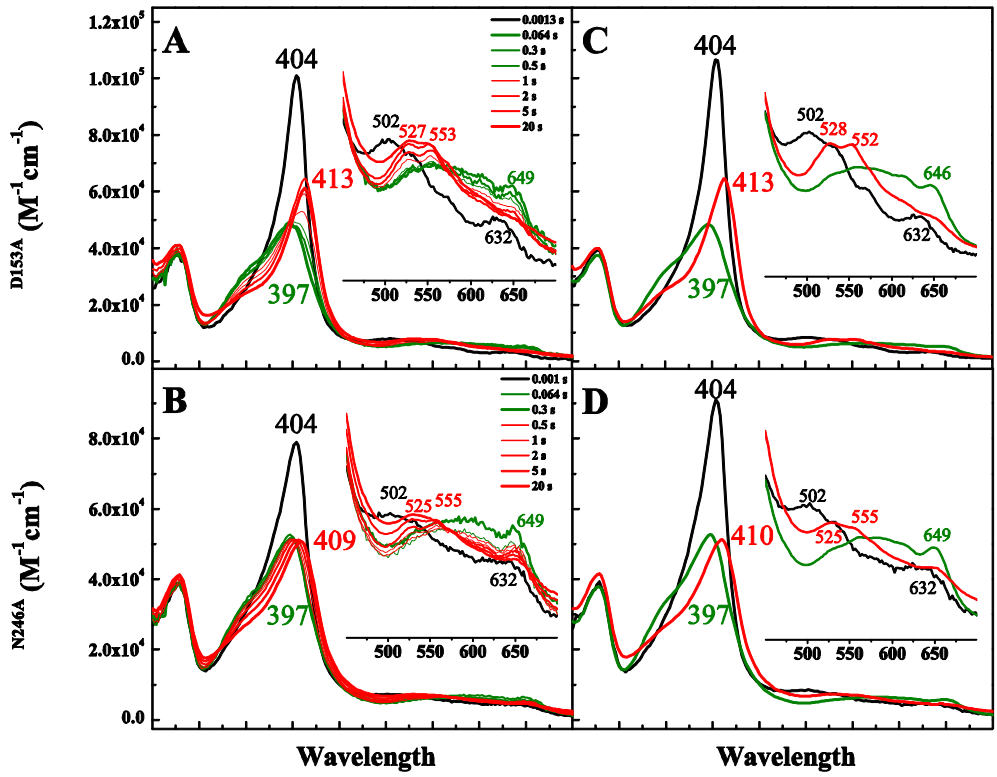
Supplemental Figure S1. UV-vis electronic absorption spectrum of purified R244L at pH 8 (black), 7 (blue), and 6 (red). The region between 450-700 nm is shown at greater magnification in the inset.



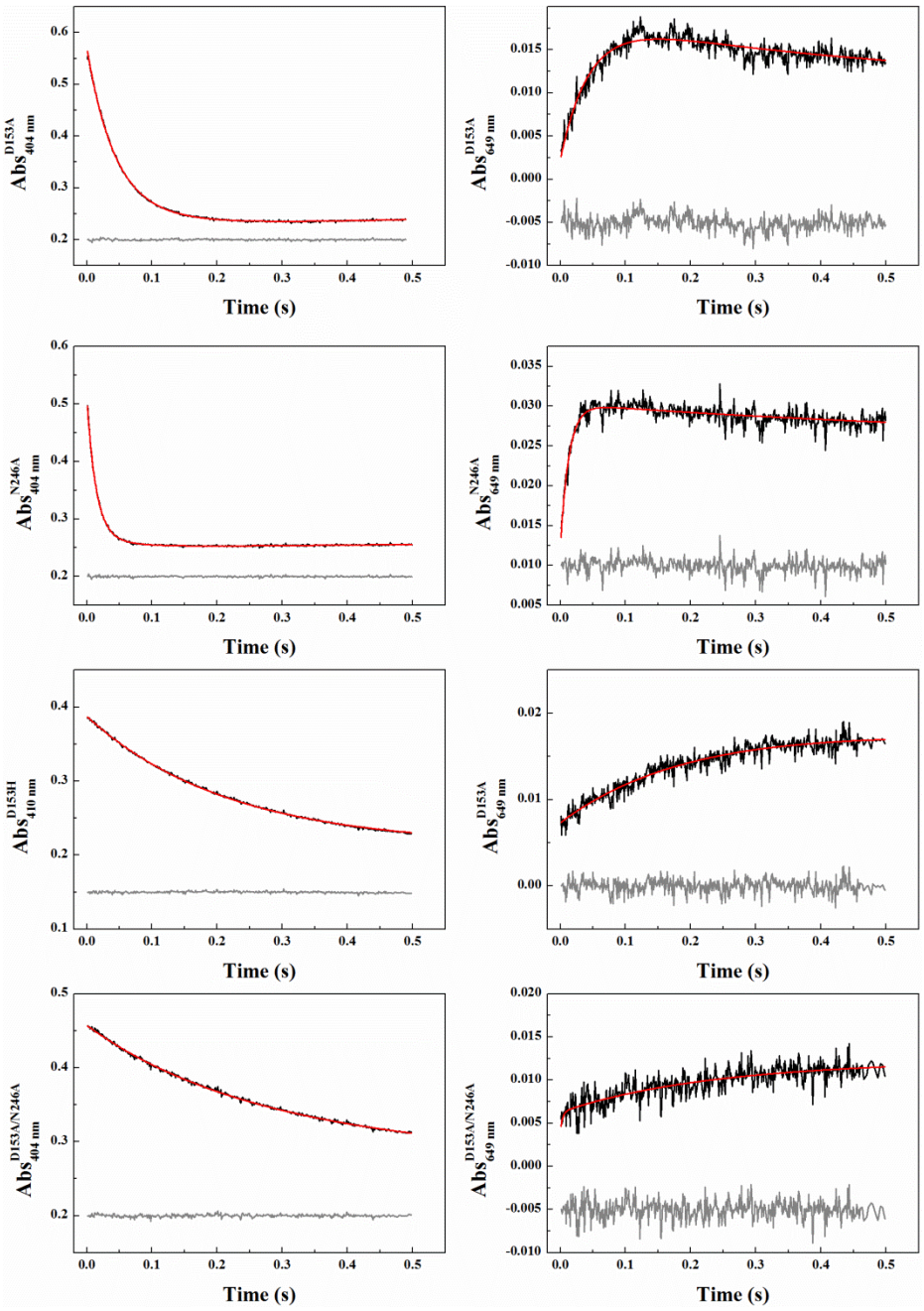
Supplemental Figure S2. Reaction of R244L with 1 equivalent of H_2O_2 (50 mM sodium succinate, pH 5, 25 °C). Reaction of 5 µM R244L (black) with 5 µM H_2O_2. The spectra (thin red to thick red) were recorded at the following time intervals (s): 5, 120,360, and 1200. The region between 450-700 nm is shown at greater magnification in the inset.



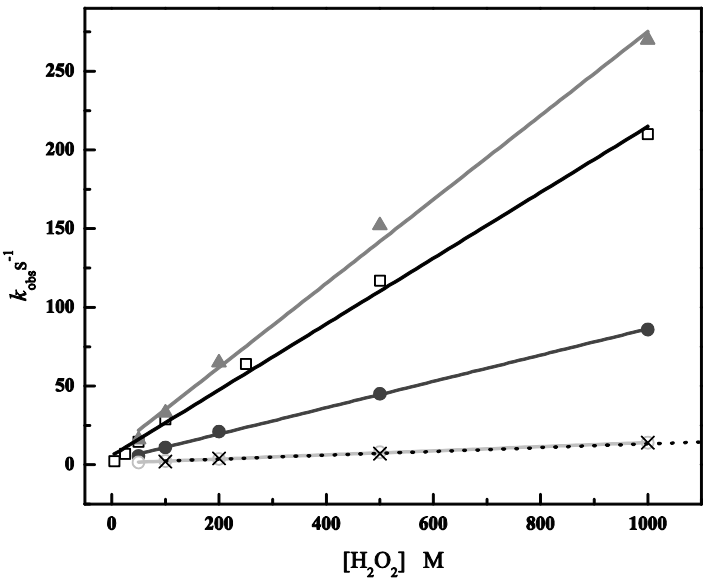
Supplemental Figure S3. Stopped-flow analyses of the reaction of D153A and N246A with H_2O_2 (50 mM sodium acetate, pH 5.5, 25 °C). Reaction of 10 µM D153A (A) or N246A (B) with an equal volume of 200 µM H_2O_2. Calculated spectra for D153A (C) and N246A (D) derived from the SVD analysis of the data from A and B. The region between 450-700 nm is shown at greater magnification in the inset.



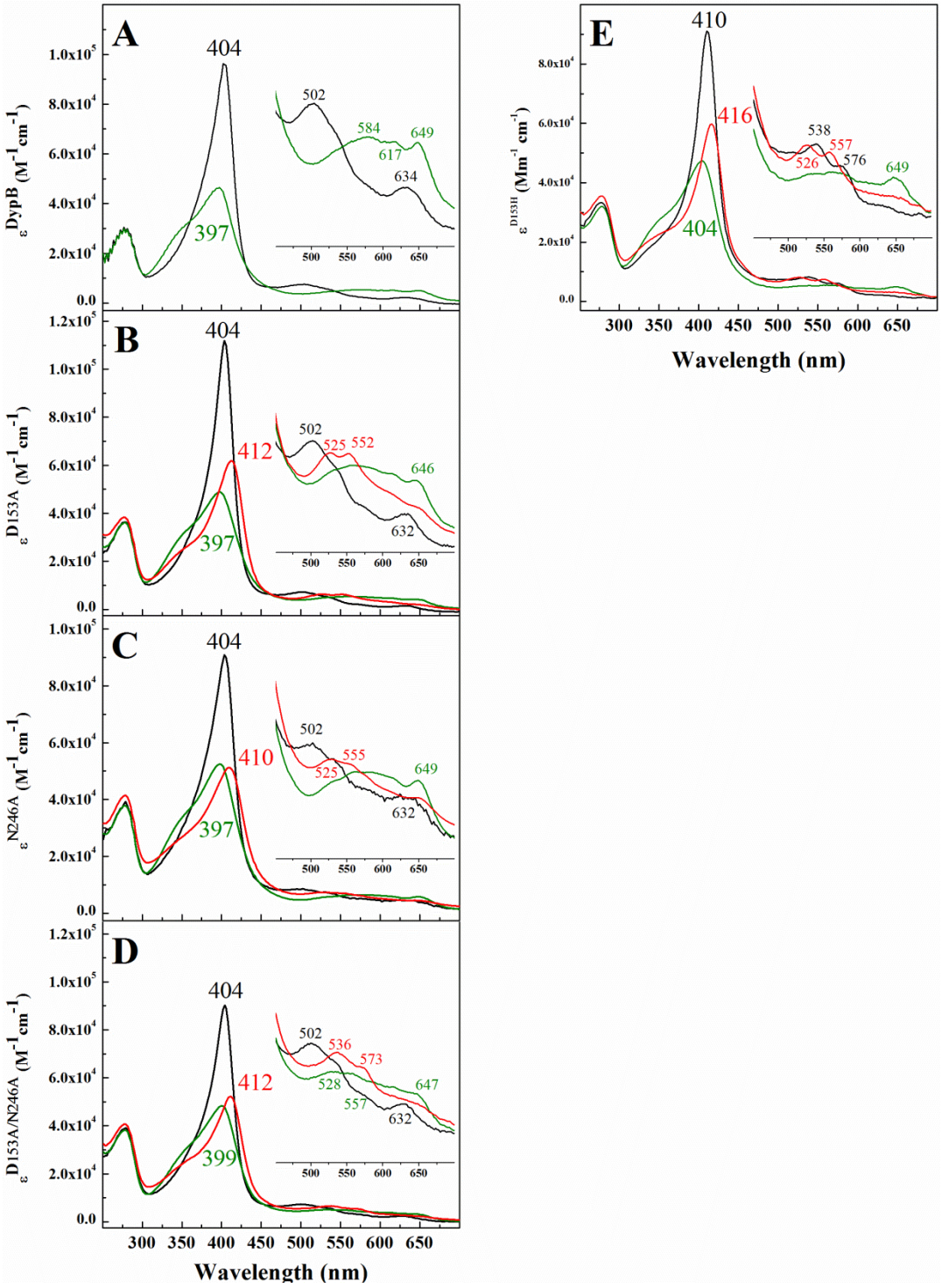
Supplemental Figure S4. Stopped-flow analyses of the reaction of D153A, N246A and D153A/N246A with excess of H_2O_2 (20 mM MOPS, 50 mM NaCl, pH 7.5, 25 °C). The right panel shows the reaction of 10 µM D153A (A) or N246A (B) with an equal volume of 1000 µM H_2O_2. The left panel shows the calculated spectra for D153A (C) or N246A (D) using SVD analysis. The region between 450-700 nm is shown at greater magnification in the inset.



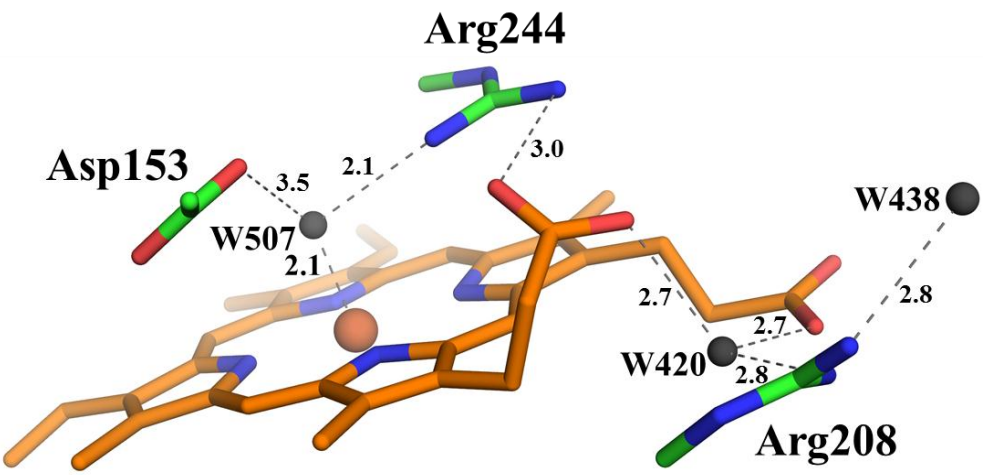
Supplemental Figure S5. Time traces of the reaction of DypB variants with H_2O_2. The reactions were followed at 404 nm (410 nm for the D153H variants) (left panel) and 649 nm (right panel) by after mixing 10 µM enzyme with 200 µM H_2O_2. Red curves show the fits of single-exponential decays to the data and the residual is shown in grey.



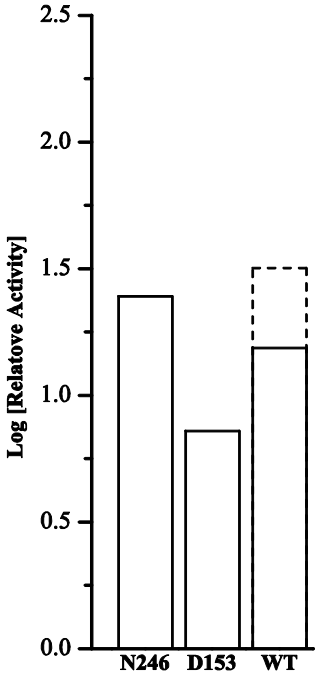
Supplemental Figure S6. Plots of k_{obs} vs. [H_2O_2] to determine second order rate constants at 404 nm for DypB (Black open square), D153A (Dark gray close circle), D153H (Black cross) N246A (Gray close triangle) and D153A/N246A (Light-gray open circle).



Supplemental Figure S7. Calculated spectra of the data shown in Figure 3 using singular-value decomposition (SVD). The data for DypB (A) was fitted to two step model while the data for D153A (B), N246A (C) and D153A/N246A (D) was fitted to three step model. The black spectrum corresponds to the ferric enzyme. The transitions to the first and second intermediates are shown in green and red, respectively. The region between 450-700 nm is shown at greater magnification in the inset.



Supplemental Figure S8. Conserved hydrogen bond network in DypB involving Asp153, Water (W) 507, Arg244, Heme propionates, W420, Arg208 and W438. Only side chains of the residues are shown. Non-covalent bonds are shown in grey as dashed lines and the atomic distances are shown in Å. The atoms are colored as follows: heme carbon, orange; residue carbons, cyan; nitrogen, blue; oxygen, red; iron, brown; and solvent species oxygen, gray. The figure was generated using coordinates from PDB ID 3QNS.



Supplemental Figure S9. Inverse analysis of Asp153 and Asn246 on Compound I formation. The log of the k_1 values of each variant and WT *versus* D153A/N246A are plotted. The dashed bars plotted for WT DypB represent what the relative k_1 values of the WT and the D153A/N246A would be if the effects of the two sets of substitutions was additive.