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Supporting Material

Internal binding of halogenated phenols in dehaloperoxidase-hemoglobin inhibits peroxidase function

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Supporting Information for:

Internal binding of halogenated phenols in dehaloperoxidase-hemoglobin inhibits peroxidase function

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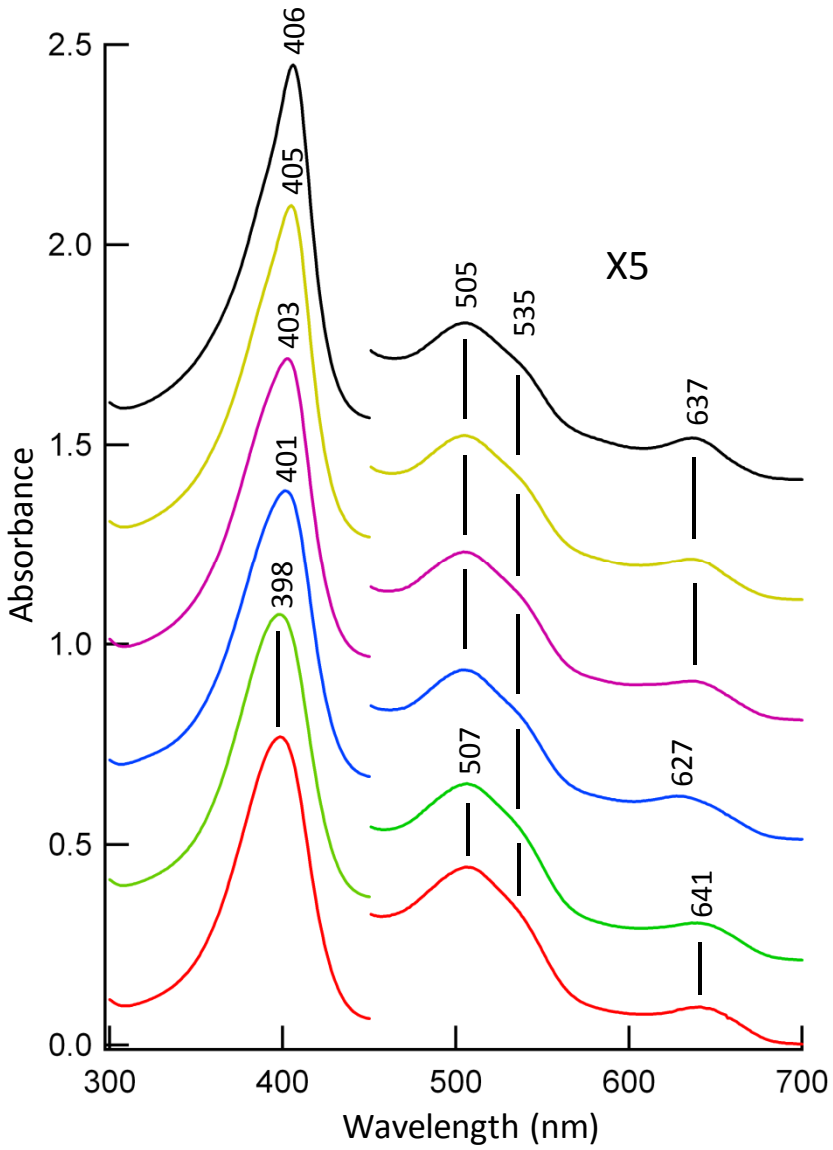

Fig. S1. Electronic absorption spectra of (black) WT-DHP and DHP with (yellow) phenol, (purple) 4-FP, (blue) 4-CP, (green) 4-BP, and (red) 4-IP. The Soret maximum undergoes a systematic blue shift as the substrate halogen is changed and follows the halogen series. The blue shift and a lowered extinction coefficient of the Soret are consistent with a shift from 6cHS to 5cHS heme. For the UV-vis spectroscopic data presented here, DHP concentration was 8 μM and the final concentration of 4-XP was 1 mM (maximum solubility) for 4-IP and 8 mM for 4-BP, 4-CP, 4-FP, and phenol in 150 mM potassium phosphate buffer, pH 6.

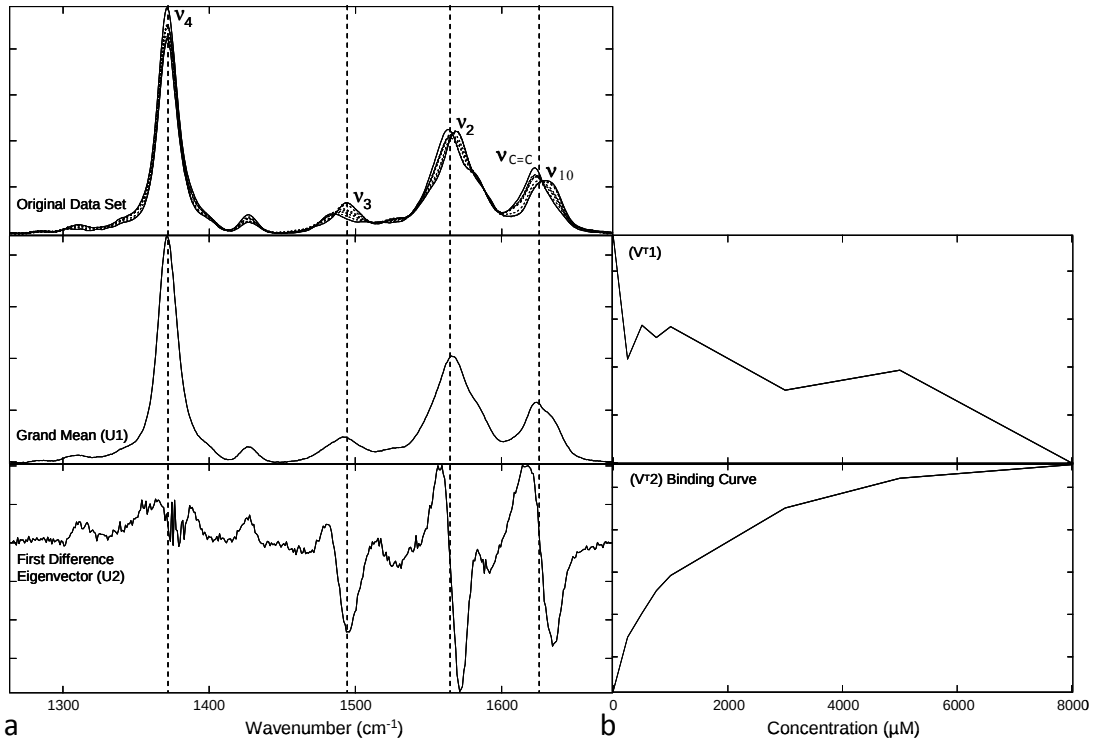

Fig. S2. SVD component analysis. a) Column eigenvectors of SVD showing the grand mean (U1) of the data set and first difference eigenvector (U2) plotted versus wavenumber. The first difference spectrum indicates which peaks shift during the substrate titration. b) Row eigenvectors of SVD displaying well-defined curves corresponding to each of the two column eigenvectors. The VT2 row vector corresponds to the intensity changes and peak shifts of U2 and is used to establish the apparent dissociation constant.

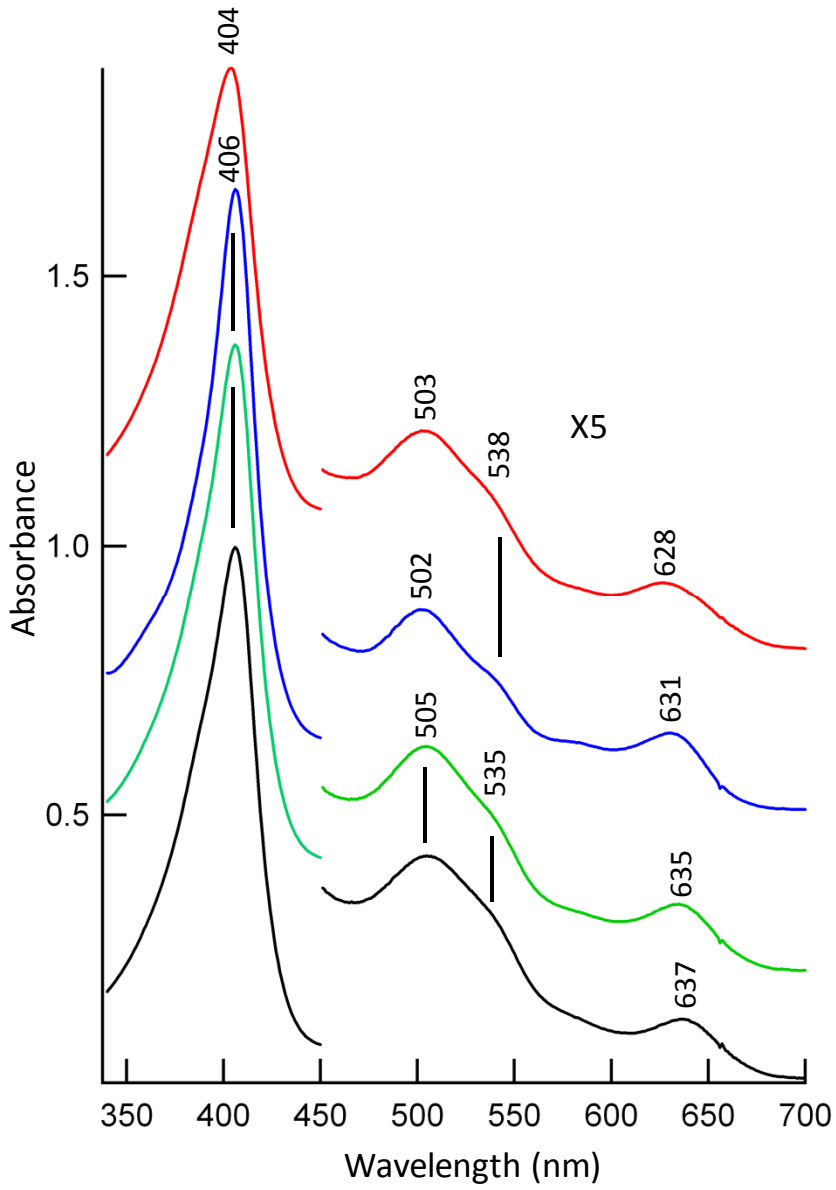

Fig. S3. Electronic absorption spectra of WT-DHP (black), DHP with 2,4,6-TBP (green), DHP with 2,4,6-TCP (blue), and DHP with 2,4,6-TFP (red). The Soret band narrows and the CT1 band blue shifts upon the addition of 2,4,6-TBP and 2,4,6-TCP, consistent with conversion to aquo-6cHS heme. The blue shift of the Soret band upon addition of 2,4,6-TFP can be attributed to the 5cHS population observed in the Raman spectrum. The final concentration of 2,4,6-TBP was 200 μM, and the final concentrations of 2,4,6-TCP and 2,4,6-TFP were 4 mM in 150 mM potassium phosphate buffer, pH 6.

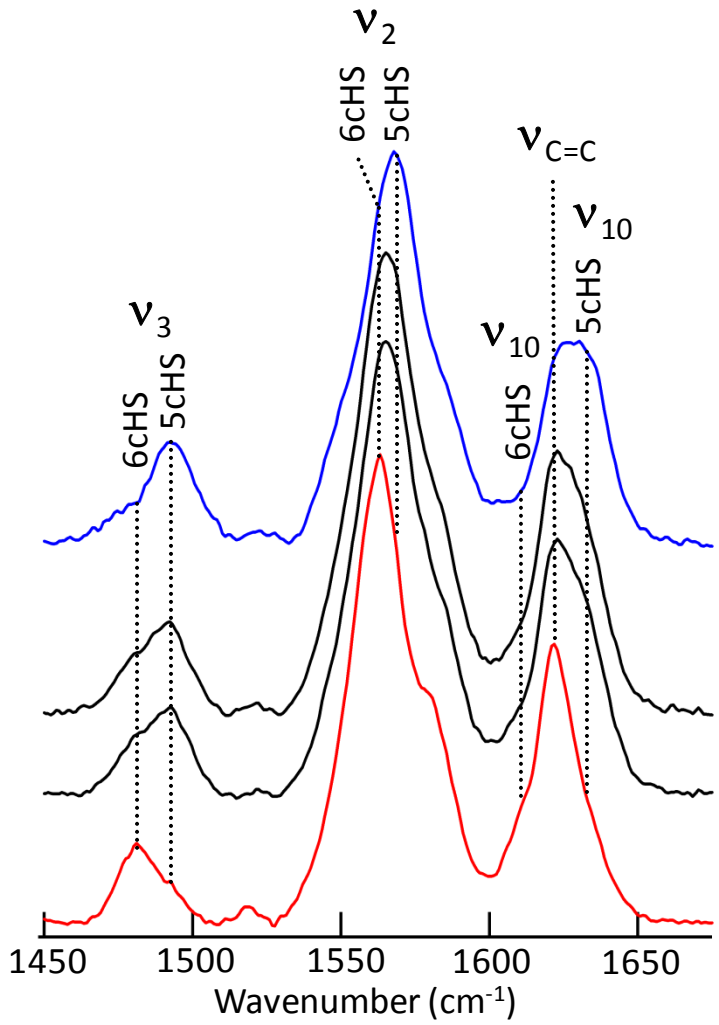

Fig. S4. Resonance Raman spectrum DHP with 3 mM 2,4,6-TCP only (red), DHP with 3 mM 2,4,6-TCP followed by the addition of 5 mM 4-BP (lower black), DHP with 5 mM 4-BP followed by the addition of 3 mM 2,4,6-TCP (upper black), and DHP with 5 mM 4-BP only (blue). The two black spectra, obtained by adding either the substrate or the inhibitor first to DHP, are identical, indicating that the substrate and the inhibitor are in equilibrium with the enzyme. Spectra were obtained in 150 mM potassium phosphate buffer, pH 6. Excitation wavelength was 406 nm; 1.7 cm^{-1} resolution; laser power at the sample 60mW, and 300 s acquisition times.

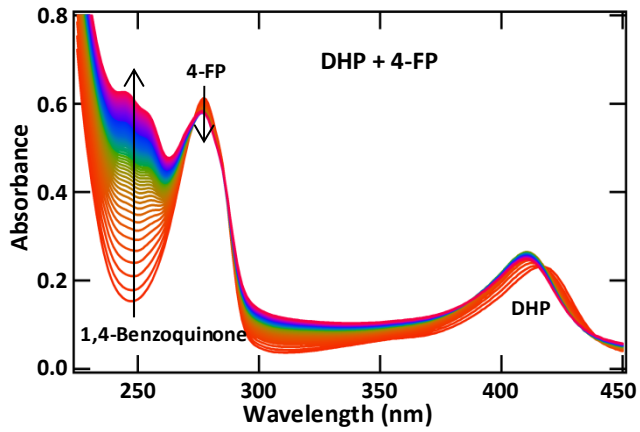

Fig. S5. Time-dependent UV-vis spectra from 0 seconds (red) to 120 seconds (purple). Assay conditions were 2.4 μM DHP, 250 μM 4-FP, and 240 μM H_2O_2 in 100 mM potassium phosphate buffer at pH 7. Unlike 4-IP, 4-BP, and 4-CP, 4-FP has a low affinity to bind internally in DHP and is clearly oxidized to 1,4-benzoquinone.

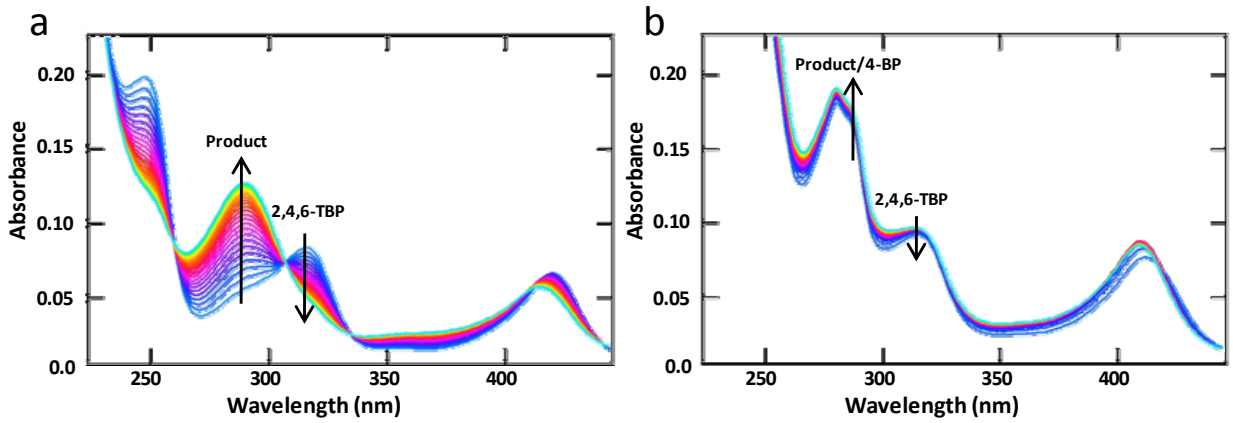

Fig S6. Time-dependent UV-vis spectra from 0 seconds (blue) to 120 seconds (teal). Assay conditions were 2.4 μM DHP, 240 μM H_2O_2, and 120 μM 2,4,6-TBP, pH 7 without (a) and with (b) addition of 240 μM 4-BP. (a) In the absence of 4-BP, the substrate (316 nm) is converted to product (291 nm). (b) In the presence of 4-BP, little product is formed with no significant decrease in the substrate band.

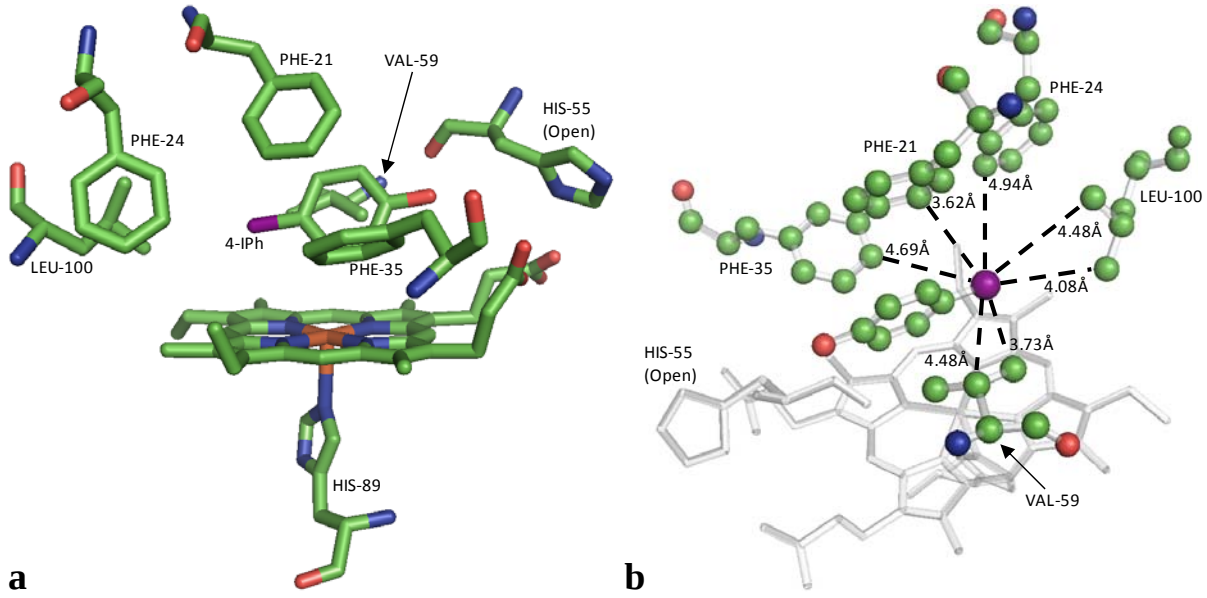

Fig. S7. (a) X-ray crystal structure of the heme active site showing hydrophobic amino acid residues surrounding the distal cavity (PDB 3FNW) (b) The location of the substrate halogen is analogous to the Xe4 binding site in sperm whale met-Mb (1).

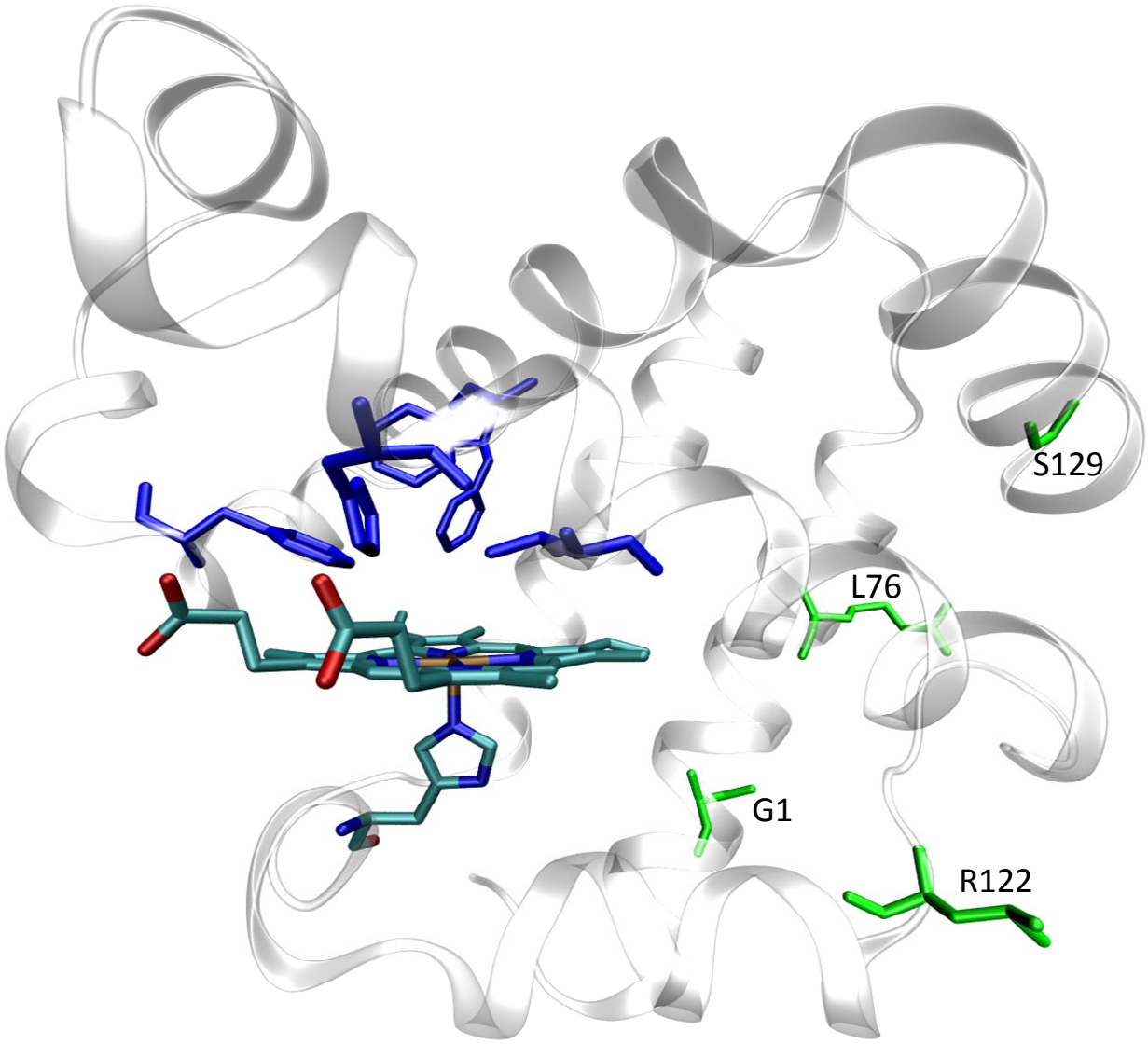

Fig. S8. X-ray crystal structure of the DHP monomer (PDB 2QFK). Blue residues are the distal pocket residues affected by internal binding of the inhibitor (see Figure S7). Green residues are the dimer interface residues affected by substrate binding. Highlighted residues are based on $^{1}H^{-15}N$ HSQC experiments of Davis et al (2).

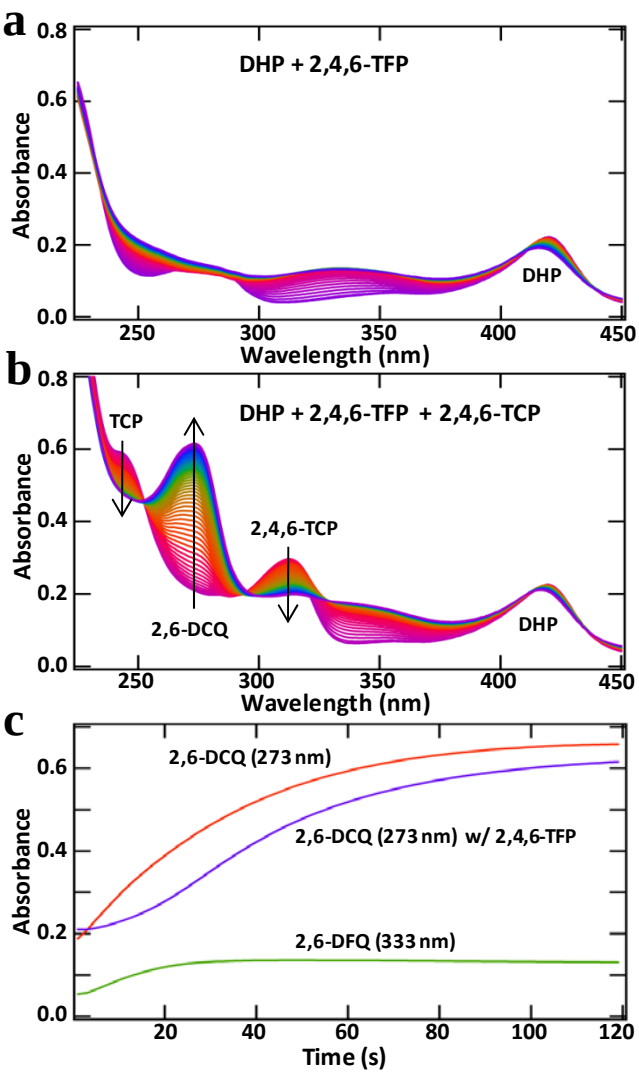

Fig. S9. (a and b) Time-dependent UV-vis spectra from 0 seconds (red) to 120 seconds (purple). Assay conditions were 2.4 μM DHP, 250 μM 2,4,6-TFP, and 240 μM H_2O_2 in 100 mM potassium phosphate buffer at pH 7 (a) and 2.4 μM DHP, 250 μM 2,4,6-TFP, 250 μM 2,4,6-TCP, and 240 μM H_2O_2 in 100 mM potassium phosphate buffer at pH 7 (b). Time traces of product formation (c).

References

1. Tilton, R. F., Jr., I. D. Kuntz, Jr., and G. A. Petsko. 1984. Cavities in proteins: structure of a metmyoglobin xenon complex solved to 1.9 Ã…. Biochemistry 23:2849-2857.
2. Davis, M. F., B. G. Bobay, and S. Franzen. 2010. Determination of Separate Inhibitor and Substrate Binding Sites in the Dehaloperoxidase-Hemoglobin from Amphitrite ornata. Biochemistry 49:1199-1206.