Supporting Information for

Structural and kinetic study of an internal binding site of the native substrate of dehaloperoxidase-hemoglobin A from *Amphitrite ornata*

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Figure S1. View of the distal side of the heme pocket of DHP A, showing the position occupied by the substrate 2,4,6-tribromophenol (2,4,6-TBP) bound to the protein. In this structure (PDB 4FH6) the 2,4,6-TBP was dissolved in DMSO, and final DMSO concentration was 10%. The substrate location is determined with the help of anomalous X-ray scattering of bromine at its K electron absorption edge as described in the text. The anomalous map colored red, and is contoured at 3.3 σ electron density level. The three Br atoms and heme Fe can be observed.

Figure S2. Comparison of substrate and inhibitor binding sites in the distal pocket. A. The 2,4,6-TBP substrate binding site (purple) is shown with an O_2 molecule bound to the heme Fe (red). B. the 4-BP inhibitor binding site is shown with the inhibitor (black) directly above the heme Fe enforcing a five-coordinate geometry.

Figure S3. Stereoview of the binding of 2,4,6-TBP to DHP A (magenta) and 1,3,5-trichlorobenzene (TCB) to cytochrome P 450_{cam} mutant F87W/Y96F/V247L (blue). DHP A heme, 2,4,6-TBP and some residues forming the distal pocket (PDB entry 4FH7) are shown colored magenta, whereas heme from cytochrome P450_{cam}, TCB and the distal pocket residues are shown in blue with the methyl groups colored green. Only the residues from the DHP A structure are labeled.

Table S1. Residues of the distal heme pocket of subunit A with less than 4 Å contacts to the 2,4,6-TBP substrate

Residue atom	Substrate atom	Distance (Å)
Ile20 CG2	Br4	3.43
Phe21 CD1	C5	3.58
	Br6	3.17
Phe24 CD2	C5	3.29
	Br6	3.44
Val59 CG1	Br2	3.10
Phe60 CZ	C3	3.73
Met63 CE	Br2	3.10
Leu100 CD1	C3	3.03
Hem139 CAB	Br2	3.10
	Br6	3.40
	OH	3.08
	OH	2.89
	OH	2.66
	OH	3.73

Table S2. Position of the substrate relative to heme iron in DHP A chain A and in cytochrome P450cam F87W/Y96F/V247L mutant molecule A and molecule B

	DHPA		CytP450cam F87W/Y96F/V247L mutant	
	Chain A		Molecule A	Molecule B
Fe—Substrate				
Bend angle	166.99		133.5	143.5
Tilt angle	42.8		6.9	9.5
Fe—Ring distance	C1	5.07	--	--
	OH	3.75		
	C2	--	4.0	3.9

Figure S4. The real and imaginary contributions to the anomalous scattering cross-section for Fe are shown. The K-edge for Fe is observed at 1.74 Å.

Figure S5. The real and imaginary contributions to the anomalous scattering cross-section for Br are shown. The K-edge for Br is observed at 0.922 Å.

Figure S6. Soret band shift upon addition of organic solvent. The concentration of DHP A $\sim 10 \mu\text{M}$ in KP_i with added solvents 10% MeOH, 10% iPrOH and 10% DMSO (left panel). In the presence of 10% DMSO in KP_i, the Soret band shifts to 410 nm and 420 nm when the concentration of DHP A declined to 3.5 μM and 2.4 μM respectively (right panel).

Figure S7. Arrhenius Plot obtained by linear fit of $\ln(k_{cat}/K_m)$ vs $1/T$. The assay conditions were ferric DHP A 2.4 μ M, H_2O_2 1200 μ M in the KPB with 10% MeOH.

Figure S8. Single wavelength kinetics of DHP catalyzed 2,4,6-TBP oxidation as a function of substrate concentration and enzymatic reaction initial rate in KPB with 10% and 2-PrOH. The assay conditions were ferric DHP A 2.4 μ M, H_2O_2 1200 μ M.

Figure S9. Resonance Raman spectra of core size marker band region for DHP A in 100 mM KPB, pH 7.0 (Black), DHP A in 100 mM KPB, pH 7.0 with 10% MeOH (Blue), DHP A in 100 mM KPB, pH 7.0 with 20% MeOH (Red). The final concentration of DHP A were 100 μ M. Excitation wavelength, 406 nm; resolution, 0.9 cm^{-1} ; acquisition time, 1200 s.

Solution of the modified Michaelis-Menten diffusion equation

The modified Michaelis-Menten diffusion equation is:

$$k_s([S_0] - [S]) = \frac{V_{max}[S]}{K_m + [S] + \frac{[S]^2}{K_i}}$$

Introducing the dimensionless substrate concentrations:

$$\beta_0 = \frac{[S_0]}{K_m}, \quad \beta = \frac{[S]}{K_m}$$

the dimensionless substrate modulus

$$\mu = \frac{V_{max}}{k_s K_m}$$

and the dimensionless inhibition constant

$$\kappa = \frac{K_m}{K_i}$$

the equation can be simplified as:

$$\beta - \beta_0 = \frac{\mu}{1 + \frac{1}{\beta} + \kappa\beta}$$

$$\kappa\beta^3 + (1 - \kappa\beta_0)\beta^2 + (1 + \mu - \beta_0)\beta - \beta_0 = 0$$

can be solved for β . The only real solution establishes the relationship between β_0 and β . To further simplified the solution we assume that $\kappa \approx 1$, which means $K_m \approx K_i$. The only real solution is:

$$\begin{aligned} \beta &= \frac{1}{3\sqrt[3]{2}} \left(2\beta_0^3 + 3\beta_0^2 + \sqrt{4(-\beta_0^2 - \beta_0 + 3\mu + 2)^3 + (2\beta_0^3 + 3\beta_0^2 - 9\beta_0\mu + 15\beta_0 + 9\mu + 7)^2} - 9S\mu + 15\beta_0 \right. \\ &\quad \left. + 9\mu + 7 \right)^{\frac{1}{3}} \\ &\quad - \frac{\sqrt[3]{2}(-\beta_0^2 - \beta_0 + 3\mu + 2)}{3 \left[2\beta_0^3 + 3\beta_0^2 + \sqrt{4(-\beta_0^2 - \beta_0 + 3\mu + 2)^3 + (2\beta_0^3 + 3\beta_0^2 - 9\beta_0\mu + 15\beta_0 + 9\mu + 7)^2} - 9\beta_0\mu + 15\beta_0 + 9\mu + 7 \right]^{\frac{1}{3}}} \\ &\quad + \frac{\beta_0 - 1}{3} \end{aligned}$$

Thus, rate equation can be expressed in terms of S_0 , the bulk solution substrate concentration and coded for fitting the data.