Supporting Information for "Inhibition Mechanisms of *Rhodococcus Erythropolis* 2'-Hydroxybiphenyl-2-Sulfinate Desulfinase (*DszB*)"

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Figure S.1. SDS PAGE for IMAC Purified DszB. "Cell Pellet" and "Supernatant" samples were taken after cell lysis centrifugation. The "Column Wash" sample was taken from the cumulative column flow through prior to addition of elution buffer. "DszB Elution Fractions" were taken from 1 ml fractions throughout the elution processes. Elution fractions contain some impurities due to a close association with co-expressed molecular chaperones groES and groEL.

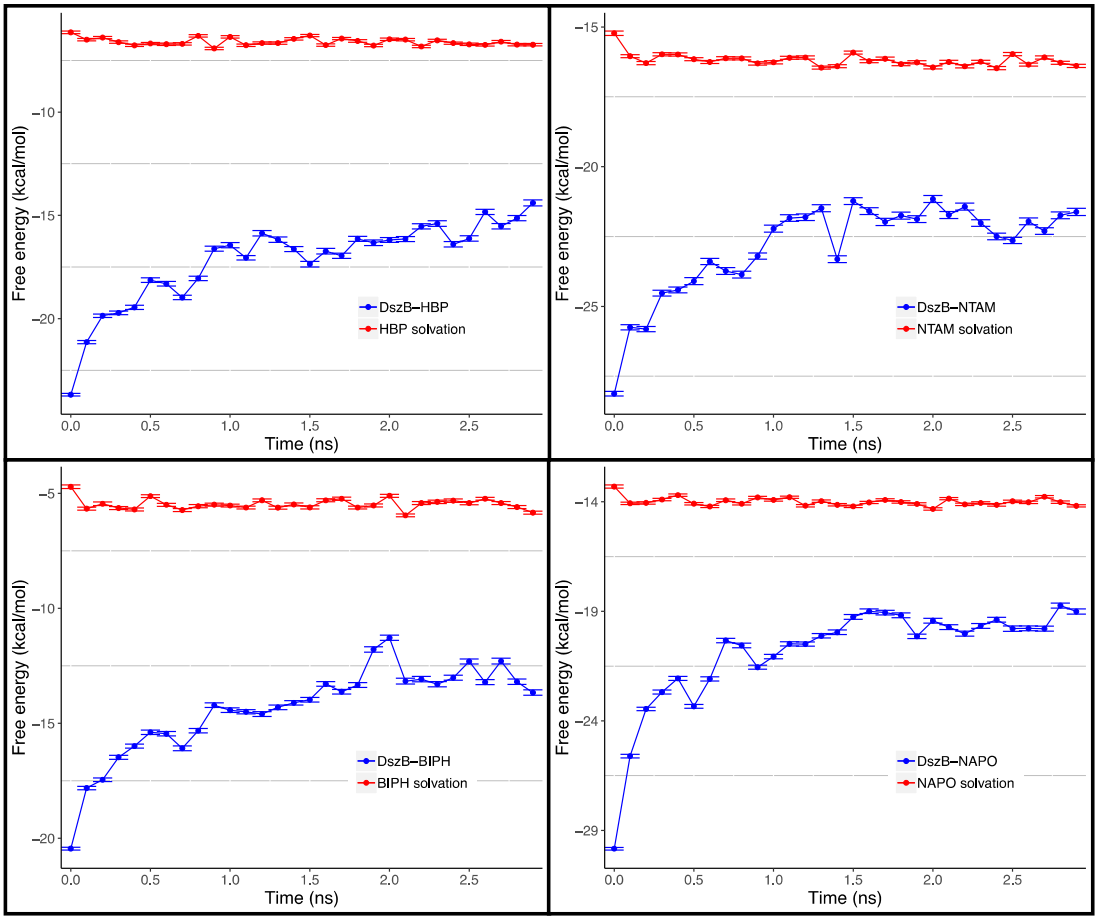

Figure S.2. Gibbs free energy over 30 consecutive 0.1-ns simulations, as determined using FEP/λ-REMD for selected systems. Blue lines represent the decoupling of the bound ligand from solvated DszB to a vacuum. Red lines represent ligand solvation free energy. Convergence was assessed based on time progression of the discrete free energy values. The last 1 ns data was used for all determinations of change in free energy.

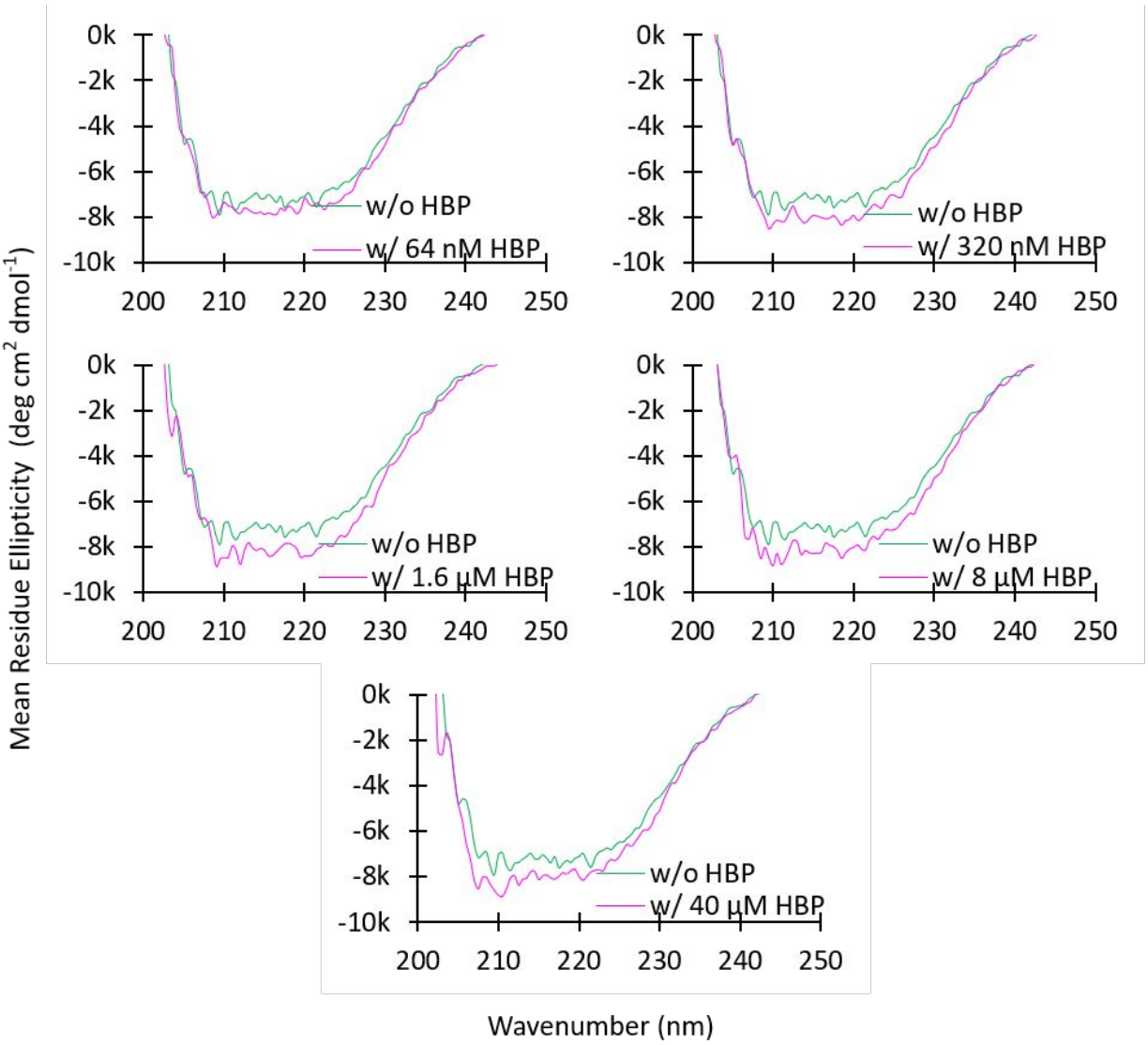

Figure S.3. Circular dichroism spectra for DszB interacting with HBP (fuchsia) and unbound (green) for multiple concentrations of HBP (64 nM, 320 nM, 1.6 µM, 8 µM, and 40 µM) over a wavenumber range indicative of helical structure (200 to 250 nm).

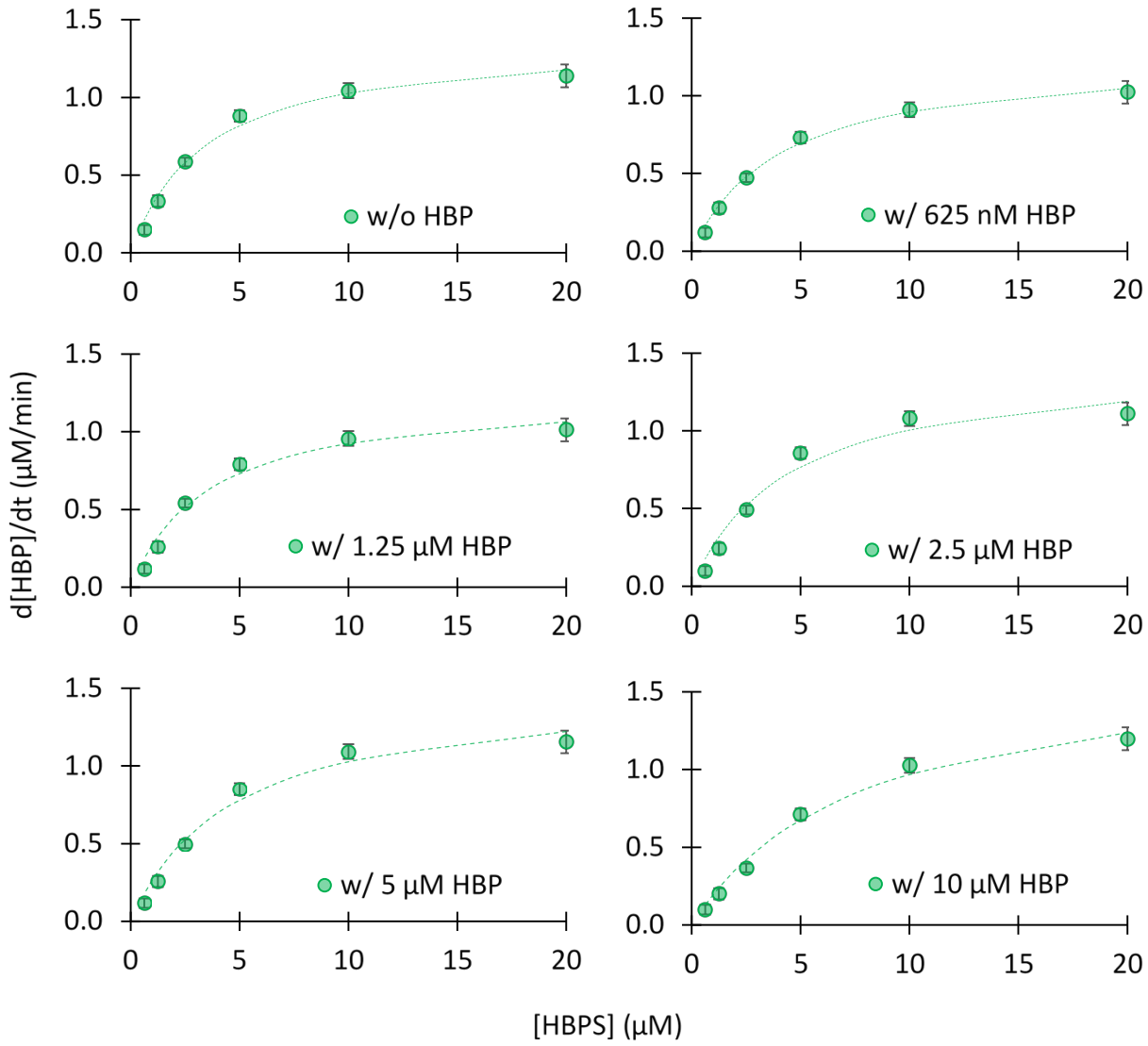

Figure S.4. Michaelis-Menten saturation curves for pure DszB (w/o HBP) and DszB with varied concentrations of HBP (w/ 0.625, 1.25, 2.5, 5, and 10 μM). Dashed lines indicate curve fitting results to the standard Michaelis-Menten equation. Error bars denote standard error with N=3.

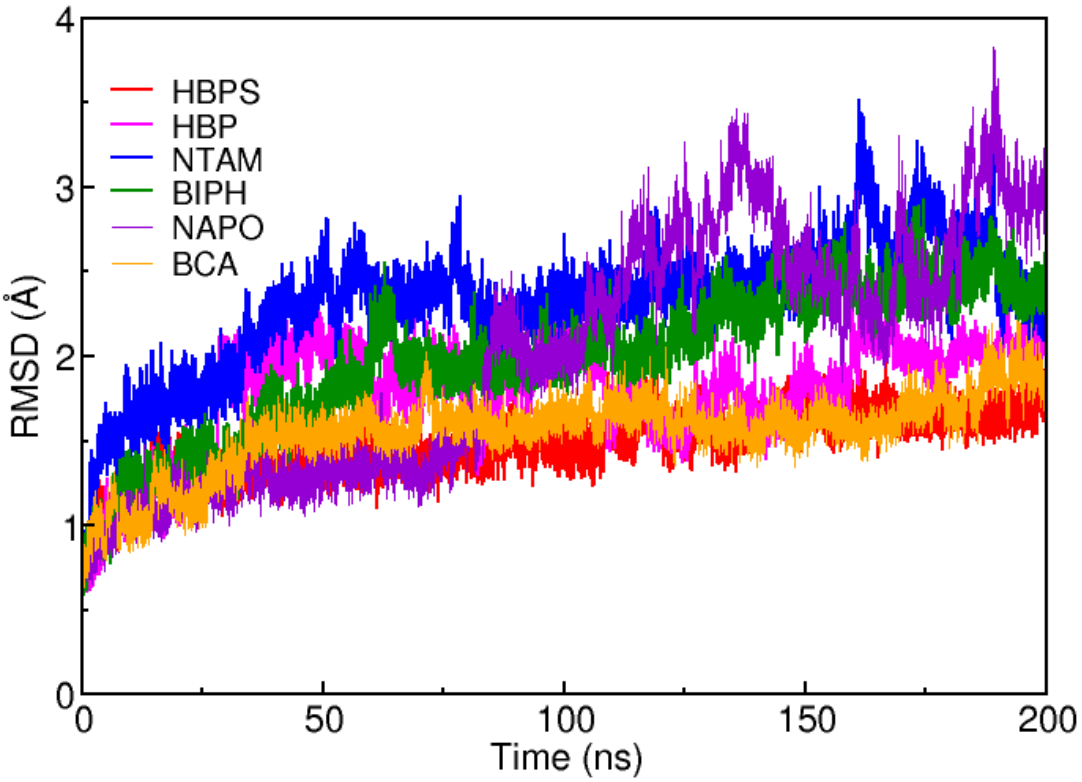

Figure S.5. Root mean square deviation (RMSD) of the protein backbone when bound with HBPS, HBP, NTAM, BIPH, NAPO, and BCA, determined over the course of 200-ns MD simulation. Note that NAPO is a non-inhibitory molecule; when DSZB was bound with NAPO, the protein backbone fluctuated more relative to the other complexes as a result of ligand movement within the binding site.

Table S.1 *Occupancy of each hydrogen bond formed between a ligand and DszB. Inhibitors, including HBP, NTAM, and BIPH, are highlighted in blue, and non-inhibitory molecules, NAPO and BCA, are highlighted in gray.*

	HBPS			HBP		
Inhibitor	Donor	Acceptor	Occupancy (%)	Donor	Acceptor	Occupancy (%)
	R70-side	HBPS-O3	77.31	R70-side	HBP-O	1.05
	R70-side	HBPS-O2	55.31	HBP-O	H60-side	2.51
	G73-main	HBPS-O2	49.05	H60-side	HBP	1.01
	NTAM			BIPH		
	Donor	Acceptor	Occupancy (%)	Donor	Acceptor	Occupancy (%)
	G73-main	NTAM-O2	7.71	G73-main	BIPH-O1	4.55
	R70-side	NTAM-O1	1.05	G73-main	BIPH-O2	5.46
	G73-main	NTAM-O1	1.01	-	-	-
Non-inhibitory	NAPO			BCA		
	Donor	Acceptor	Occupancy (%)	Donor	Acceptor	Occupancy (%)
	-	-	-	R70-side	BCA-O2	1.57
	-	-	-	BCA-O1	H60-side	5.45

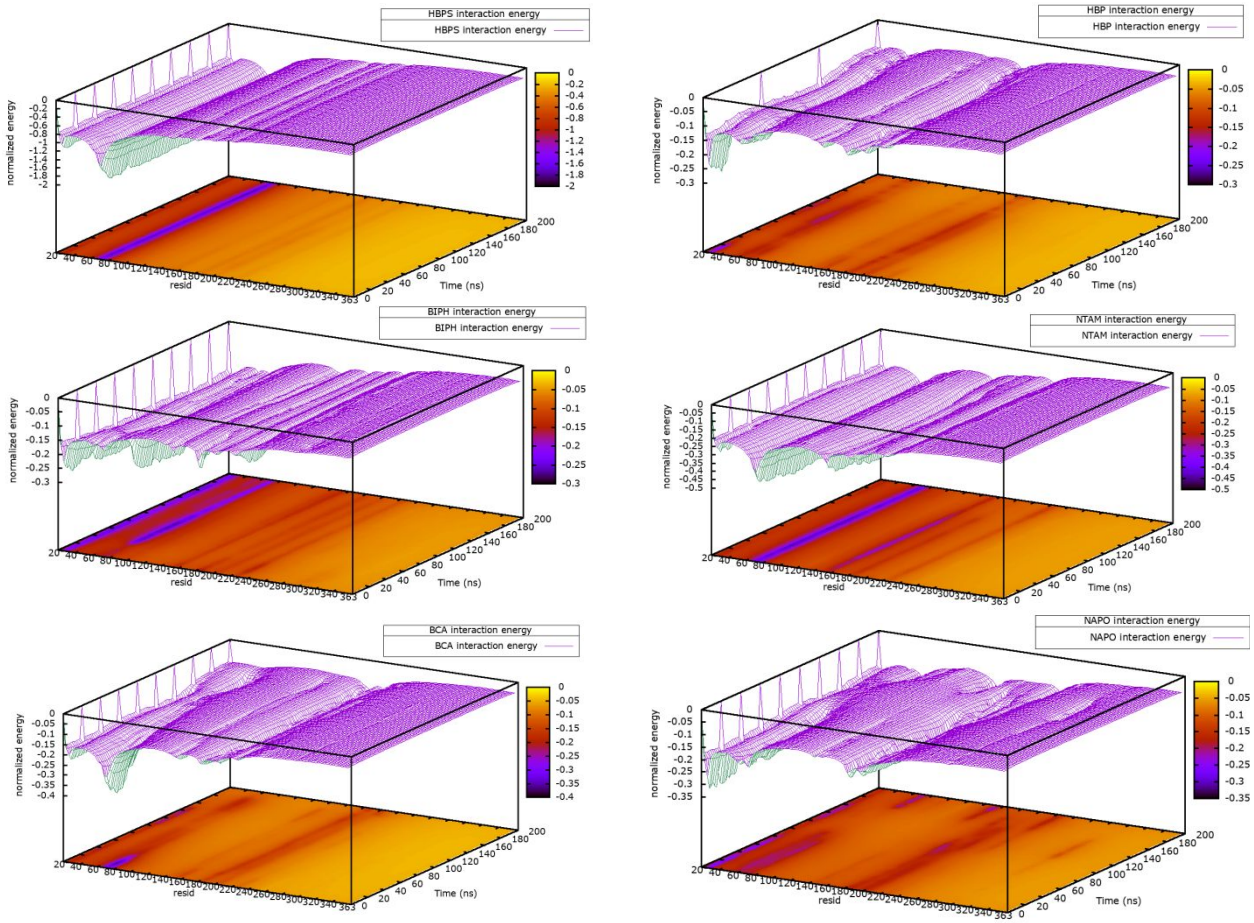

Figure S.5. Total nonbonded interaction energy between a ligand and *DszB* with respect to time. The energy value was calculated as a weighted average using a grid size of 100. The *x*-axis represents residue number, ranging from 20 to 363. The *y*-axis is the time series from 0 to 200 ns.