Supporting Information

Flooding Enzymes: quantifying the contributions of interstitial water and cavity shape to ligand binding using Extended Linear Response free energy calculations

Katie L. Whalen[1,2,3] and M. Ashley Spies[1,2]

[1]College of Pharmacy, Division of Medicinal and Natural Products Chemistry, and [2] Carver College of Medicine, Department of Biochemistry, the University of Iowa, Iowa City, Iowa; [3]Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois

Table S1. Primers used in site-directed mutagenesis to create desired GR mutations.

Desired Mutation	Template DNA	Primer	Primer Sequence (5' to 3')
N75A	*pET15b-racE_WT*	RacEN75Afor	gctcgtgatcgcctgtgctacagcaacagcgatcg
		RacEN75Arev	cgatcgctgttgctgtcgaacaggcgatcacgagc
N75L*	*pET15b-racE_C74A*	RacEN75AC74Afor	caaaatgctcgtgatcgccgctaatacagcaacagcgatc
		RacEN75AC74Arev	gatcgctgttgctgtattagcggcgatcacgagcattttg

*The production of GR-N75L was a serendipitous result of mis-priming in PCR during parallel attempts to construct *racE-C74A-N75A* with the listed primers and *pET15b-racE-C75A* as template. Sequencing confirms *racE-N75L* is the only resulting mutation. This result was highly repeatable.

Table S2. Active site analysis of inhibitor-bound GR complexes via MOE Site Finder. *

Enzyme	Inhibitor	Size	PLB	Hyd	Side	Residues
GR-WT	BISA	189	2.49	18	140	D10 S11 G12 V13 G14 G15 T37 C40 P41 Y42 G43 A73 C74 N75 T76 A77 V96 T118 N120 T121 V149 E153 G184 C185 T186 H187 F246 I249
	CA	192	2.43	21	147	D10 S11 G12 V13 G14 G 15 T37 C40 P41 Y42 G43 A73 C74 N75 T76 A77 T118 N120 T121 V149 E153 C815 T186 H187 Q245 I249
GR-N75L	BISA	187	2.94	16	141	D10 S11 G12 V13 G14 G15 T37 C40 P41 Y42 G43 A73 C74 L75 T76 A77 V96 G117 T118 N120 T121 V149 E153 G184 C185 T186 H187 Q245 I249
	CA	156	3.02	11	117	D10 S11 G12 V13 G14 G15 C40 P41 Y42 G43 P44 C74 A75 T76 A77 V96 G117 T118 N120 T121 V149 E153 G184 C185 T185 H187 Y188
GR-N75A	BISA	131	2.98	10	93	D10 S11 G12 G14 G15 P41 Y42 G43 A73 C74 A75 T76 A77 G117 T118 N120 T121 V149 G184 C185 T186 H187
	CA	127	2.86	8	93	D10 S11 G12 V13 G14 G15 C40 P41 Y42 G43 P44 C74 A75 T76 G117 T118 N120 T121 V149 P150 E153 G184 C185 T186 H187

* Final snapshots from MD simulation were imported to MOE v2011.10. "Size" indicates the number of contact atoms in the receptor. "PLB" corresponds to the Propensity for Ligand Binding [Soga 2007] score. "Hyd" indicates the number of hydrophobic contact atoms in the receptor. "Side" indicates the number of sidechain contact atoms in the receptor. Finally, "Residues" indicates the residues that make up the calculated site.

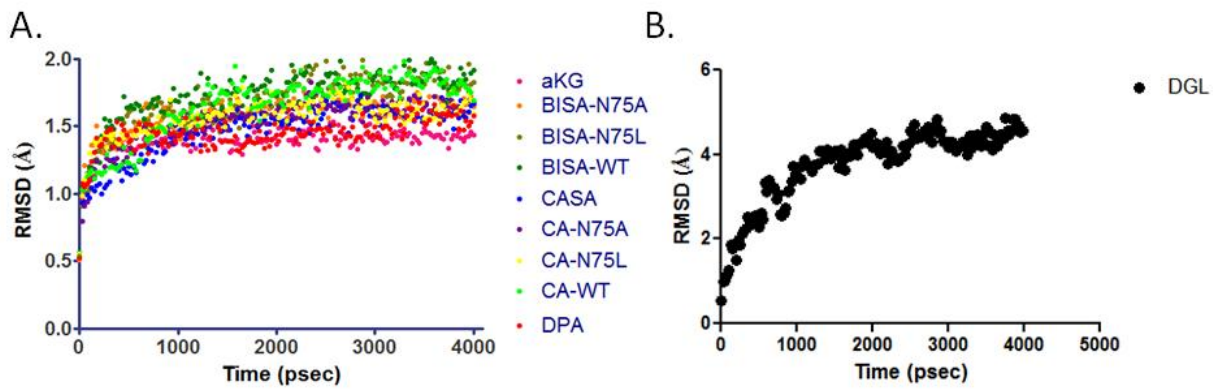

Figure S1. Cα-atom RMSD plots for GR inhibitors (A) and substrate (B) over the course of each complex's 4-ns MD simulation.

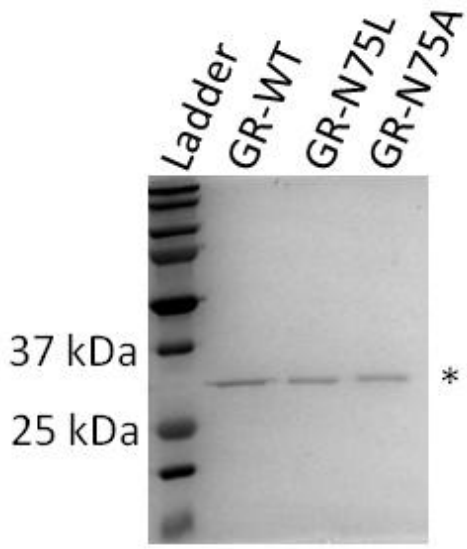

Figure S2. SDS-PAGE analysis of purified recombinant RacE (*B. subtilis*). Molecular weight is approximately 31,000 Da with the 6X-His-tag.

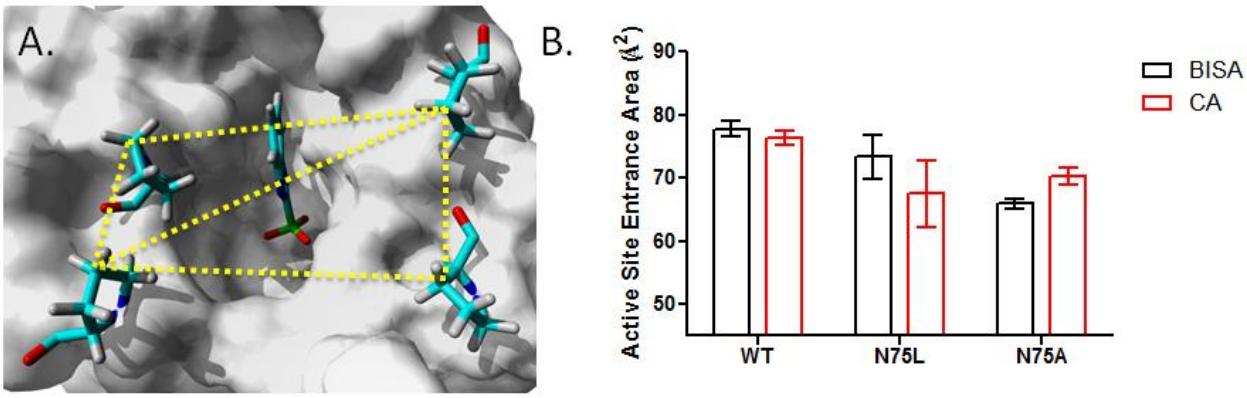

Figure S3. Quantification of the active site cleft for BISA (**1**) and CA (**2**) in complex with GR-WT, GR-N75L, or GR-N75A. Cleft measurements made by calculating the area between four points which form a circumference about the active site entrance (A). Measurements made for the final three snapshots of each complex simulation with standard deviations shown (B).

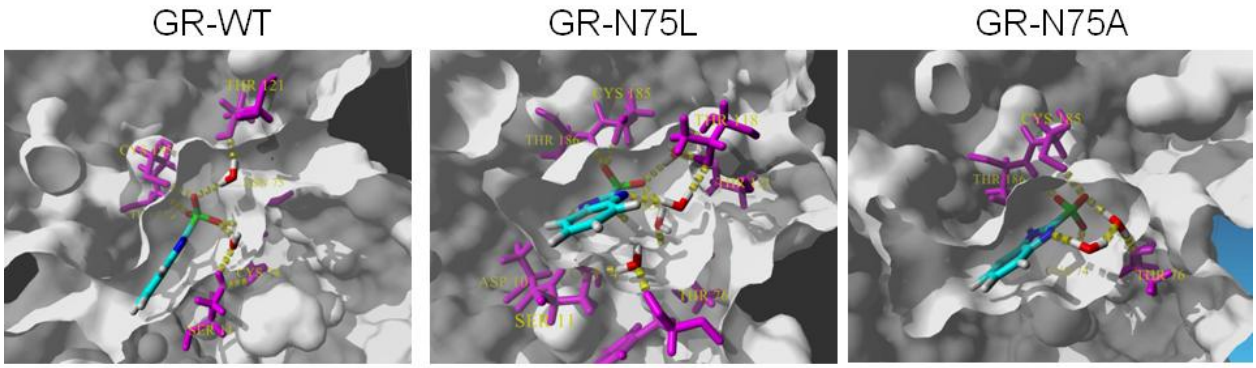

Figure S4. Molecular surface representations of GR-BISA complexes. Structures were obtained from the final snapshot of each 4-ns MD simulation. Solvent accessible surface area is rendered in white using the YASARA Structure package. Surfaces are cut away to show the ligand binding pocket shape. Key residues interacting with the ligand (directly or via interstitial bonds) are shown (stick, magenta). BISA is shown bound to the active site (stick, elemental coloring). Hydrogen bonds are shown (yellow, dotted line), and all interstitial waters are shown (stick, elemental coloring). Each structure is oriented with the entrance to the active site positioned at the left of the image.

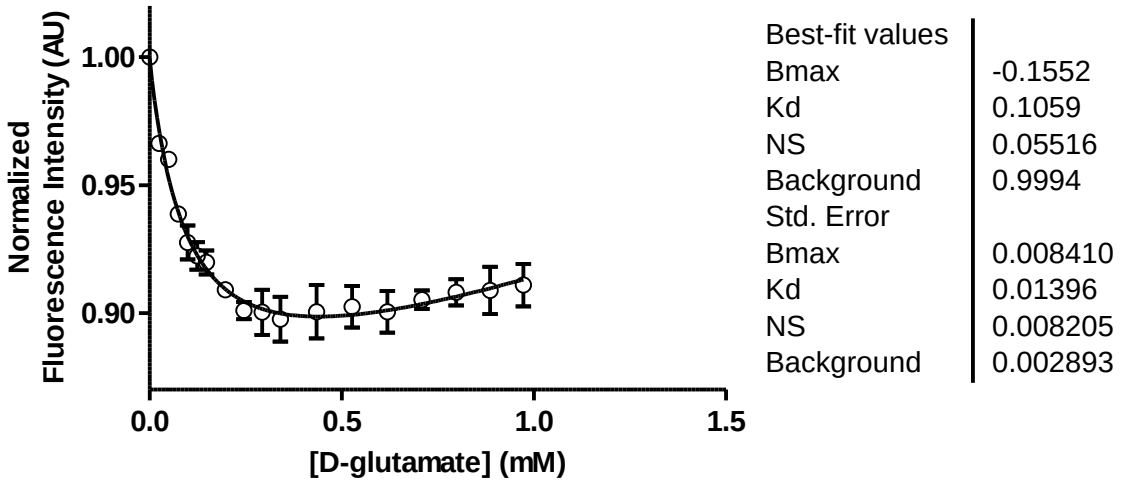

Figure S5. Titration of D-glutamate to RacE-C74A for determination of dissociation constant. RacE-C74A is a functionally-inactive form of glutamate racemase that can bind D-glutamate, but cannot abstract the Cα proton. Intrinsic tryptophan/tyrosine fluorescence (excitation at 280 nm, emission at 343 nm) was measured continuously at 25°C using a Cary Eclipse Fluorescence Spectrophotometer (Agilent). A 90 μM solution of purified RacE-C74A in 50 mM Tris-HCl, 100 mM NaCl, pH 8.0, 2 mM DTT was incubated in a quartz cuvette (pathlength = 10 mm) for 10 min at 25°C prior to the first injection. A 50 mM solution of D-glutamate in the same buffer was used for substrate injections. The titration was repeated three times. Data was fit to a "Total binding - one site" model with GraphPad Prism v5.0. The dissociation constant is within error of the reported K_M value.

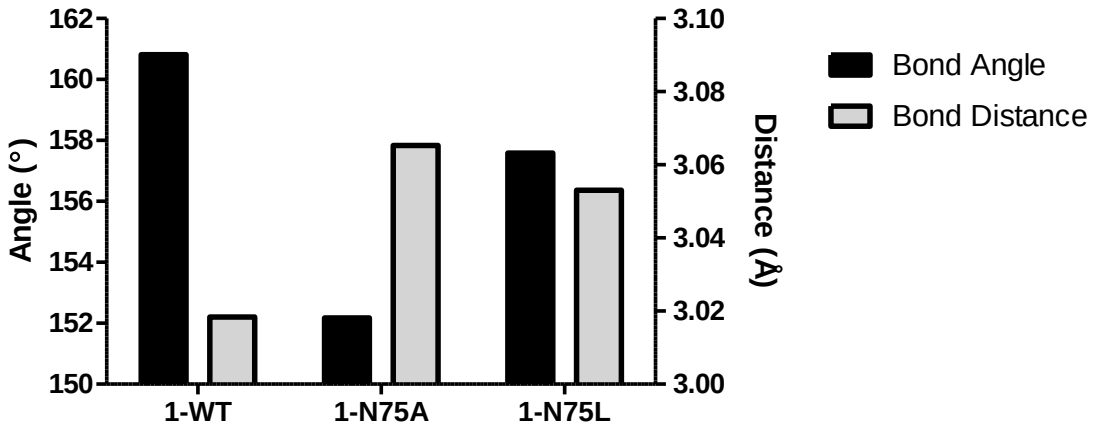

Figure S6. Average angle and distance measurements for ligand-protein hydrogen bonds observed in the final 3 snapshots of MD simulation. Measurements made for 3 individual snapshots per simulation. Each complex contained between 3-4 hydrogen bonding interactions between ligand and protein. The **1**-WT complex possessed the most optimal overall bonding distances and angles, where both **1**-N75A and **1**-N75L complexes suffer from increased bond distances and decreased bond angles.