Supporting information

Site Specific Fragment Optimization Guided by Single Step Free Energy Perturbation Calculations

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Figure S1 (a) Convergence data for the thermodynamic integration (TI) calculations to determine relative hydration free energies of benzene analogues. The sum of the alchemical TI calculations in the forward and reverse directions, which ideally should be 0, serves as a convergence metric. **(b)** Relative hydration free energies ΔΔG computed using TI vs. experiment. The units are kcal/mol.

a

	ΔΔG exp	ΔΔG TI avg	ΔΔG TI vaccum (fwd+rev)	ΔΔG TI solution (fwd+ rev)
chlorobenzene	-0.26	-0.15	0.01	0.00
fluorobenzene	0.06	-0.53	0.02	0.02
bromobenzene	-0.60	-0.56	0.09	0.14
iodobenzene	-0.88	-0.61	0.28	0.04
phenol	-5.75	-4.33	0.02	0.07
toluene	-0.03	0.76	0.04	0.21
aniline	-4.63	-4.70	0.00	0.05
pyridine	-3.83	-4.66	0.00	0.21

b

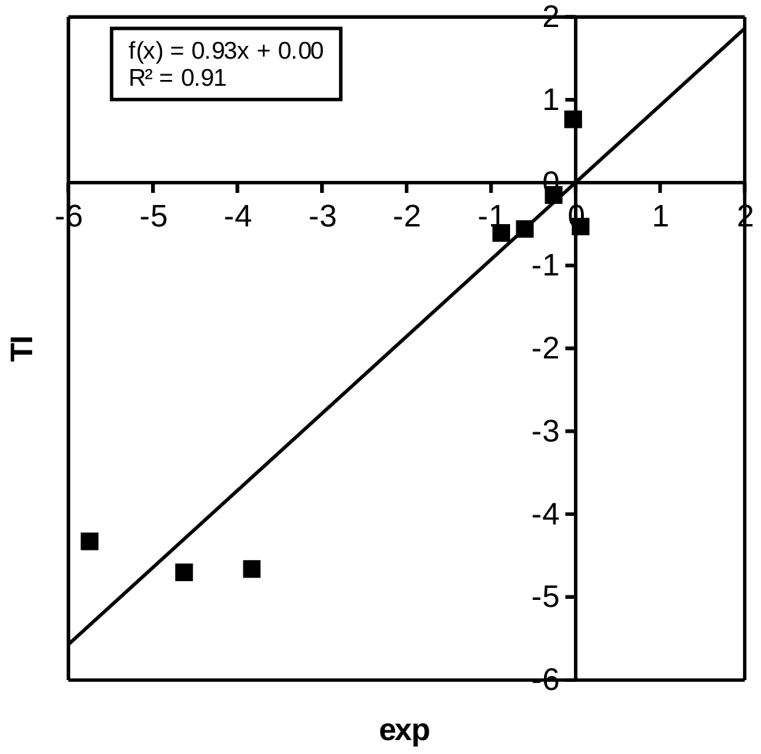

Figure S2. SSFEP computed vs. experimental relative binding free energies of **(a)** thrombin and **(b)** P38 MAP kinase ligands. The SSFEP computation was performed without removing the rotation of the phenyl ring conformation with respect to the reference conformation. P38MK data shown in panel b is from the protein-restrained simulation. Predicted values for two P38MK ligands(#12 and 13) fall off the scale and are not shown in the plot. The units are kcal/mol.

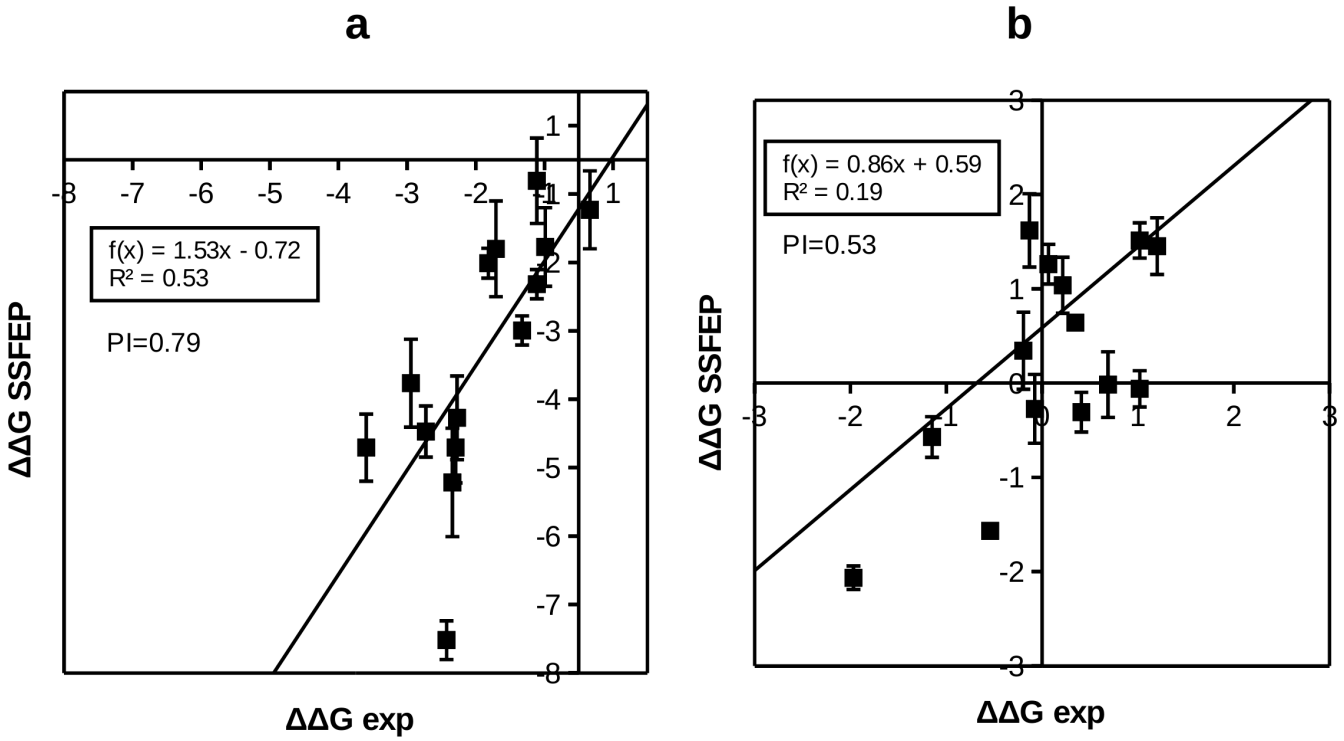

Figure S3. Correlation between predicted ΔΔG values of benzene analogues in thrombin S1-pocket computed using the SSFEP protocol using four different reference structures. Ref1 (blue) is the benzene from the crystal conformation of the phenyl ring in the inhibitor ATI used throughout the manuscript. Ref2 (red) and Ref3 (grey) are arbitrarily chosen conformations from the SILCS simulations. Ref4 (orange) is chosen based on best overlap with the benzene FragMap constructed from the SILCS simulations. The inter-benzene 1-2, 1-3 and 1-4 RMSDs are 0.98 1.25 and 1.30 Å respectively.