Rapid Evaluation of Relative Change in Binding Affinity Using Single Step Free Energy Perturbation (SSFEP)

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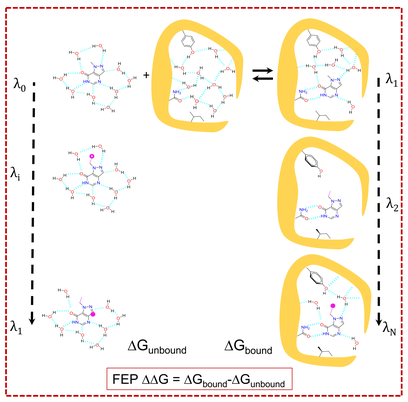
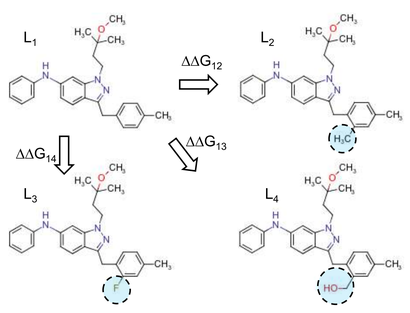
Abstract

Accurate and rapid evaluation of relative change in binding affinity (ΔΔG) associated with a given structural modification to a ligand is an important requirement for computational methods to facilitate and lead the drug-design process. Among the different approaches available, free energy perturbation (FEP) methods are widely adopted due to their overall accuracy. However, they require significant computational resources for each planned modification. Single-step free energy perturbation (SSFEP) is an attractive alternative that is based on repeatedly reusing a pre-computed ensemble of conformations of a parent ligand in explicit solvent and solvated protein environments. This allows for the rapid evaluation of ΔΔGs for a library of small functional group substitutions. Instead of a gradual alchemical transition through non-physical states, SSFEP utilizes the perturbation formulation to evaluate ΔΔG through a single-step transformation between the parent and the modified ligand. Consequently, SSFEP is on the order of one thousand times faster than traditional FEP methods. While originally SSFEP was designed for a single heavy atom substitution, to expand the functional group library that can be evaluated, we report a new protocol where rotatable dihedrals along a modification are explicitly sampled during the transformation. This development allows for evaluating functional group substitutions up to four heavy atoms and three rotatable dihedrals. For ligands targeting three different proteins: Ack1, p38 MAP Kinase and Farnesoid X receptor (FXR), SSFEP's prediction of the directionality of ΔΔG across a defined set of modifications to the parent ligand was found to be competitive with FEP. 5/8, 9/15 and 12/15 modifications to ligands targeting Ack1, p38 MAP Kinase and FXR, respectively, were correctly identified using SSFEP, whereas FEP methods identified 4/8 and 10/15 modifications to ligands targeting Ack1 and p38 MAP Kinase correctly. These results show that SSFEP holds potential as a new computationally tractable strategy that can lead the drug-design process.

Free Energy Perturbation





Separate FEP simulations needed for each of $\Delta\Delta G_{12}$, $\Delta\Delta G_{13}$ & $\Delta\Delta G_{14}$ evaluation.

Computationally very expensive as the data-set size increases (L_1, L_2, ..., L_N).

Single Step Free Energy Perturbation

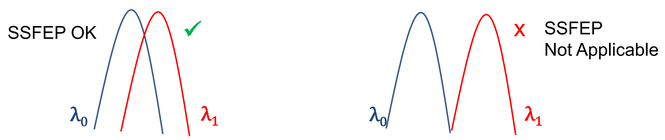
$$\Delta G^{Env}_{L1\to L2} = G_{L2} - G_{L1} = -k_B T \ln\left\langle e^{-\beta(E_{L2}-E_{L1})}\right\rangle^{Env}_{L1}$$

Zwanzig Equation

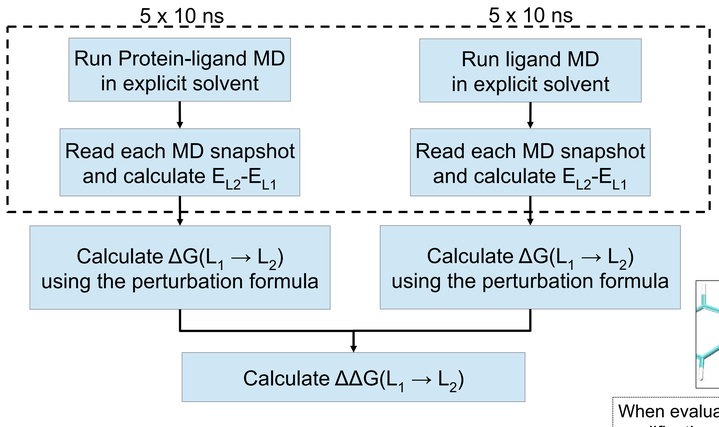
Reasonable if ensemble for **L1** in environment (**Env**), contains sufficient configurations relevant for **L2** in Env.

$$\Delta\Delta G^{\text{bind}}_{L1\to L2} = \Delta G^{\text{protein}}_{L1\to L2} - \Delta G^{\text{water}}_{L1\to L2}$$

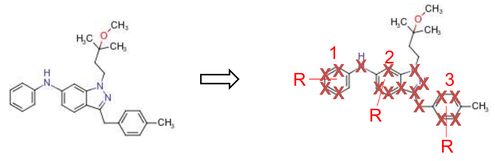
When the size of the modification is such that λ_0 & λ_1 have strong overlaps **directly transition from $\lambda_0 \to \lambda_1$**



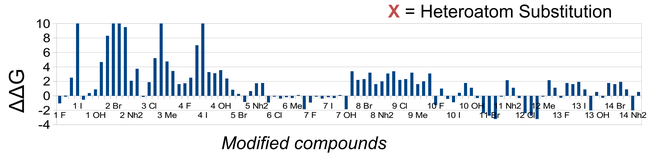
Precompute MD with parent ligand



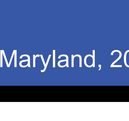
- Single MD simulation with parent Ligand (L_1)
- Directly transform L_1 to L_2 ,L_3 , L_4,.., L_N



R = F, Cl, Br, I, Me, OH, NH2, etc
X = Heteroatom Substitution



Modified compounds

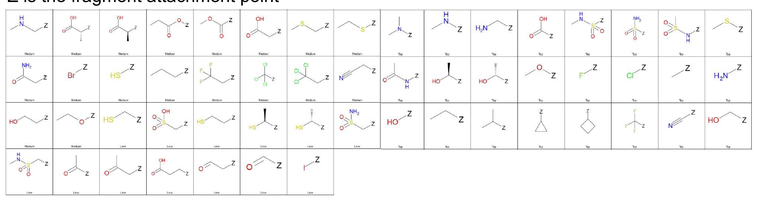


When evaluating a modification with more than one rotatable dihedral, use ΔG with lowest energy conformation obtained by rotating dihedrals through 18 degree increments in every MD snapshot.

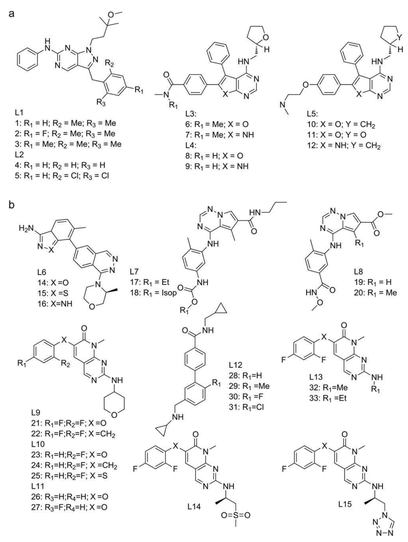
	MD
12 CPU cores (ligand in aq. solution)	**~2** hrs
8 CPU cores+ 1 GPU (ligand-protein complex)	**~2.5 hrs**
1 CPU core 64 ΔΔG evaluations	**~2.5 hrs**

Library of modifications

Z is the fragment attachment point



Ack1, p38 MAP Kinase & Farnesoid X



Raman, Lakkaraju, et al. JCC 38 (2017): 1238-1251.

a) Ack1

Transformation	$\Delta\Delta G_{exp}$	$\Delta\Delta G_{fep}$	$\Delta\Delta G_{ssfep}$
L1/1→2	1.28	1.97	3.00
L1/2→3	2.71	-1.02	4.32
L1/1→3	3.98	0.96	7.32
L2/4→5	-3.76	1.29	4.53
L3/6→7	-0.95	0.93	0.37
L4/8→9	-0.87	0.77	2.00
L5/10→11	0.57	0.45	0.17
L5/10→12	0.11	0.60	2.22

b) p38 MAP Kinase

Transformation	$\Delta\Delta G_{exp}$	$\Delta\Delta G_{fep}$	$\Delta\Delta G_{ssfep}$
L6/14→15	1.12	0.12	-0.32
L6/14→16	2.36	3.33	2.92
L7/17→18	2.85	1.86	2.90
L8/19→20	-2.16	-3.17	38.93
L9/21→22	0.82	-1.09	2.38
L10/23→24	0.61	-0.59	2.55
L10/23→25	1.19	5.54	-0.78
L11/26→27	-1.20	0.04	2.34
L12/28→29	-3.16	-0.94	-1.08
L12/28→30	-1.00	0.72	0.75
L12/28→31	-2.77	-0.68	-0.52
L13/30→31	-1.72	-1.41	-1.27
L13/32→33	-2.44	-1.30	-1.20
L14/rMe→sMe	1.36	-0.76	0.14
L15/rMe→sMe	-1.01	-4.04	1.41

Number of correct predictions in terms of direction of change in ΔΔG

Target	FEP	SSFEP
Ack1	4/8	5/8
p38 MAP Kinase	10/15	10/15
FXR		12/15

FXR data from D3R challenge, 2016.

Summary

- **SSFEP: Precompute ensemble based approaches enable ligand design/optimization.**
- **Rapid scoring of ligand transformations from pre-computed ensembles : ~64 modifications: 1-2 hrs on a single core.**
- **SSFEP predictions competitive with FEP for a diverse range of targets.**

Acknowledgement

Ack1, p38 MAP Kinase projects were performed in collaboration between University of Maryland, School of Pharmacy and Denny A Rajiah, Pfizer, Inc.