Validation of AMBER/GAFF for Relative Free Energy Calculations

Lin Frank Song[a], Tai-Sung Lee[b], Chun-Zhu[a], Darrin M. York[b], and Kenneth M. Merz Jr.[a,c]

a. Department of Chemistry and the Department of Biochemistry and Molecular Biology, Michigan State University, 578 S. Shaw Lane, East Lansing, MI 48824

b. Laboratory for Biomolecular Simulation Research, Center for Integrative Proteomics Research and Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey 08854, United States

c. Institute for Cyber Enabled Research, Michigan State University, 567 Wilson Road, Room 1440, East Lansing, MI 48824

Corresponding author: Kenneth M. Merz Jr.

Phone: (517) 355-9715

Fax: (517) 353-7248

E-mail: kmerz1@gmail.com

Abstract: With renewed interest in free energy methods in contemporary structure-based drug design there is a pressing need to validate against multiple targets and force fields to assess the overall ability of these methods to accurately predict relative binding free energies. We computed relative binding free energies using GPU accelerated Thermodynamic Integration (GPU-TI) on a dataset originally assembled by Schrödinger, Inc.. Using their GPU enabled free energy code (FEP+) and the OPLS2.1 force field combined with REST2 enhanced sampling approach, these authors obtained an overall MUE of 0.9 kcal/mol and an overall RMSD of 1.14 kcal/mol. In our study using GPU-TI of AMBER with the AMBER14SB/GAFF1.8 force field but without enhanced sampling, we obtained an overall MUE of 1.17 kcal/mol and an overall RMSD of 1.50 kcal/mol for the 330 mutations contained in this data set. More detailed of analyses of our results suggested that the observed differences between the two studies arise from differences in sampling protocols along with differences in the force fields employed, otherwise these two GPU-accelerated free energy programs would deliver comparable results. Future work should address the problem of establishing benchmark quality results with robust statistical error bars obtained through multiple independent runs and enhanced sampling, which soon may be possible with forthcoming GPU-accelerated features in AMBER.

1. Introduction

Protein-ligand binding affinity calculation has drawn a lot of attention for many years because of its ability to significantly accelerate drug discovery by focusing experimental effort on high quality leads.[1-6] Using molecular dynamics (MD) or Monte Carlo algorithms to perform the necessary sampling, many methods have been developed to address this task[7-23]: These include the linear interaction energy (LIE) approach[7-8], the Molecular Mechanics-Poisson−Boltzmann/Surface Area (MM-PBSA) and Molecular Mechanics-Generalized Born/Surface Area (MM-GBSA)[9-12] approaches, and physical pathway methods such as the Umbrella Sampling (US) method[13-14] combined with Weighted Histogram Analysis (WHAM)[24-25] or variational free energy profile (vFEP) methods[26-27].

Another class of methods are the alchemical methods, where the thermodynamic path between two end states is defined and the free energy change is calculated based on statistical mechanical analysis of the simulations. Based on the theoretical foundation of Kirkwood, Zwanzig and Bennett among others,[17-23] three widely used alchemical methods are Thermodynamic Integration (TI),[18] Free Energy Perturbation (FEP),[22] and Bennett Acceptance Ratio (BAR).[17, 21] In these approaches, the alchemical transformation from the initial state to the final state usually is characterized by a coupling parameter λ varying from 0 to 1 and the free energy difference is calculated as the summation of alchemical transformation between fixed-λ states. Another notable method is λ-dynamics,[19] pioneered by Brooks and co-workers. It combines the idea of alchemical methods and US method by treating the λ variable dynamically, and generates a potential of mean force in λ-space. The initial application studies of alchemical methods were performed a few decades ago by a number of groups.[3-4, 28] Since this time, more and more significant applications in both academia and industry have been reported.[29-35]

However, to more reliably drive decisions in lead optimization, there are two major issues that alchemical methods should deal with, namely, adequate sampling of relevant configurations and force field accuracy.[5] To address the sampling issues methods like Hamiltonian exchange and replica exchange have been developed,[36-38] while improvements in force fields have been an ongoing effort and will continue to be for the foreseeable future.[2, 39-42]

Recently, an FEP protocol that gives excellent performance in predicting relative binding affinity over a broad range of protein-ligand complexes was reported.[1] This approach used replica exchange with solute tempering (REST2) in combination with the OPLS2.1 force field which was carefully parameterized using an extensive training set of relevant torsional and covalent interactions.[1] The AMBER/GAFF class of force fields[42-43] are widely used, but have not been extensively validated against such a large data set (8 systems and 330 mutations). To fill this gap we employed the recently implemented[44] GPU-accelerated TI module of Amber18 to repeat the calculations for these systems in order to assess the capabilities of another force field model on this same data set. Overall, our computed results are comparable to the FEP+ result**,** albeit with slightly larger average errors, indicating that the GPU-TI module of AMBER using the AMBER/GAFF force field is an emerging alternative choice for high-throughput relative binding free energy calculations.

2. Methods

All of the protein and ligand PDBs were obtained from the SI from the Wang *et al.* publication.[1] The protonation states of all the charged residues were maintained as reported in Wang *et al*.. The AMBER FF14SB force field[45] was employed to describe the proteins and GAFF (version

1.8) was[42] used for the ligands. Restrained electrostatic potential (RESP) charges for the ligands were calculated at the HF/6-31G(d) level of theory using the Gaussian 09 program[46] and AMBERTools16. The parmchk utility from AMBERTools16 was used to generate the missing parameters for the ligands. The systems were solvated using the SPCE[47] water model using cubic simulation cells. The resulting solvated protein/ligand systems were then charge neutralized by adding Na^+ or Cl^- ions[48]. The particle mesh Ewald (PME) method[49-50] was used to treat the long-range electrostatic interactions. All bonds involving hydrogen atoms were constrained using SHAKE[51]. The AMBER16 package[52] was used to run the MD simulations. MD simulations for each protein-ligand system were performed to equilibrate the systems. The equilibration process involved 100000 steps of energy minimization, 1 ns of NVT followed by 5 ns of NPT equilibration using a 12.0 Å non-bonded interaction cutoff. The second ligand (the ligand to be mutated to) was then added and the dual topology for the TI simulation was then generated using the parmed.py utility.

TI simulations. As shown in Figure 1, the relative binding free energy ($\Delta\Delta G$) can be calculated as the difference between the free energies (ΔGs) of mutating one ligand to the other in the protein matrix and in solution. Therefore, TI simulations for both process 1 and 2 were performed. For each process, a one-step protocol was adopted, i.e. disappearing one ligand and appearing the other ligand simultaneously. The detailed TI simulation protocol is as follows: First, using the dual topology parameter file, 50000 steps of steepest descent minimization was performed, followed by 1 ns NVT where the system was gradually heated up from 0 K to 300 K which was then followed by 1 ns NPT equilibration at 300 K and 1 bar. These simulations were performed at λ=0.5 to equilibrate the system[53-54]. No restraint was applied for these simulations and all

structures were visually checked. For some mutations, multiple runs had to be performed in order to obtain a stable starting structure. Afterwards the equilibrated structure was used as the starting structure for 12 λ windows. For each λ, 1 ns of NVT equilibration was performed followed by a 5 ns NVT simulation from which δU/δλ data were collected. The efficient 12-point Gaussian quadrature was used for the numerical integration of ∂U/∂λ to obtain all necessary ΔG values. The non-bonded interaction cutoff was 9.0 Å and a softcore potential was used. The NPT equilibration was performed using the CPU version of pmemd from the Amber14 package. All TI simulations used the Berendsen thermostat except for the NPT equilibration step, which used a Langevin thermostat. The obtained results for all eight systems can be found in the Supplemental Information (SI).

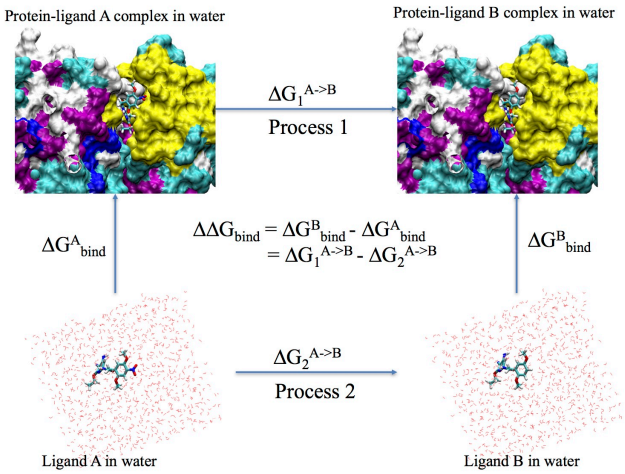

Figure 1. Thermodynamic cycle used for the calculation of the relative binding free energy between protein-ligand system A and protein-ligand system B.

3. Results and Discussion

Overall result. The ΔΔG values directly obtained from TI calculations can be found in SI. The mean unsigned error (MUE) and root mean square deviation (RMSD) of these values compared to experiment are summarized in SI Table 1. After obtaining the ΔΔG values, we employed the

cycle closure convergence strategy described previously[55] and obtained our final ΔΔG values. Thus the following analysis is based on the cycle-closure ΔΔG values. Table 1 summarizes the MUE and RMSD compared to experiment. The overall MUE obtained with GPU-TI of AMBER using the AMBER FF14SB/GAFF1.8 force field (AMBER for short) is 1.17 kcal/mol (0.27 kcal/mol larger than FEP+. Similarly, the RMSD is a bit higher for AMBER: 1.50 kcal/mol *versus* 1.14 kcal/mol for FEP+. Moreover, in our current work, we did not apply replica exchange, which could help enhance the overall sampling and facilitate equilibrium between different λ simulations. Future work will explore the role sampling (both in λ-space and phase space) plays on these systems versus the effect of force field errors.

Table 1. Summary of the MUE and RMSD of the eight systems based on cycle closure ΔΔG values.

System	# of ligands	# of perturbations	FEP+/OPLS 2.1 (kcal/mol)		AMBER GPU-TI/AMBER FF14SB + GAFF(1.8) (kcal/mol)		Difference* (kcal/mol)	
			MUE	RMSD	MUE	RMSD	MUE	RMSD
Thrombin	11	16	0.76	0.93	0.46	0.62	-0.30	-0.31
Tyk2	16	24	0.75	0.93	1.07	1.27	0.32	0.34
Jnk1	21	31	0.78	1	1.07	1.45	0.29	0.45
CDK2	16	25	0.91	1.11	0.97	1.13	0.06	0.02
PTP1B	23	49	0.89	1.22	1.06	1.40	0.17	0.18
BACE	36	58	0.84	1.03	1.20	1.47	0.36	0.44
MCL1	42	71	1.16	1.41	1.52	1.83	0.36	0.42
P38a	34	56	0.8	1.03	1.20	1.56	0.40	0.53
Overall	199	330	0.90	1.14	1.17	1.50	0.27	0.36

* The difference is calculated as AMBER MUE or RMSD minus Schrödinger MUE or RMSD.

We also converted the ΔΔG to ΔG's for each ligand and plotted them against experimental values in Figure 2. We can see AMBER fared a little worse than FEP+. Out of the 199 ligands, 5 ligands (2.5%) for Schrödinger and 18 ligands (9.0%) for AMBER are more than 2kcal/mol off from experiment.

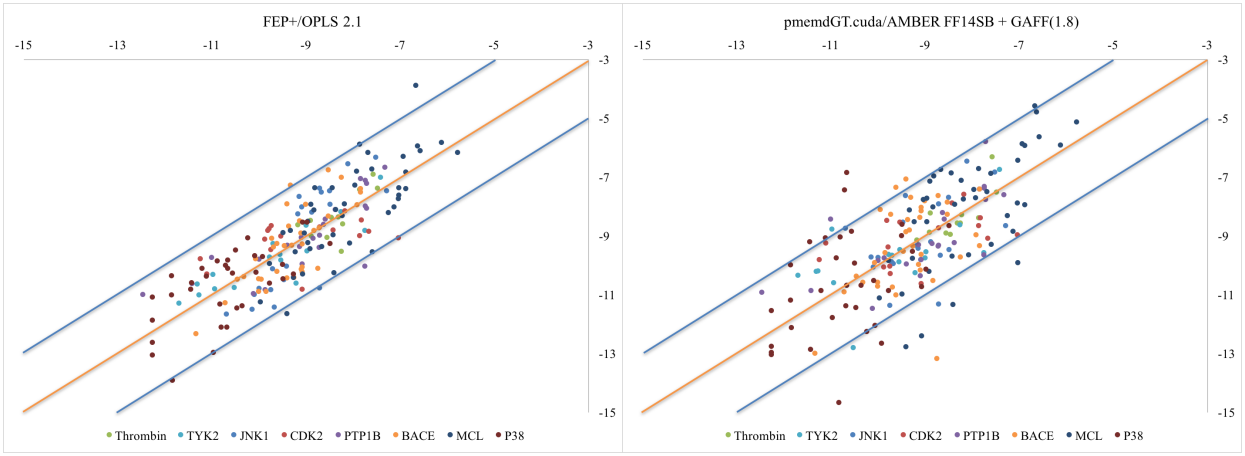

Figure 2. Correlation between predicted binding free energies and experimental data for the eight systems.

Discussion. In SI (see Trend.xlsx) we summarized all of the 330 mutations. Overall, we find that AMBER performs reasonably well for mutations between halogens and H/CH3/CH2CH3: 44 of 49 mutations have error less than 2 kcal/mol, 34 of which have an error less than 1 kcal/mol. Mutations involving large van der Waals radii changes, like Br to H or I to H, tend to have larger errors. We further analyzed the mutations based on the "size" of the mutation; whether there is ring appearance/disappearance or whether there is ring type change (for example, pyridine to benzene). We classified mutations that involve 3 heavy atoms changing or more as "big change" mutations, and the others as "small change" mutations. AMBER performs well for "big change" as well as "small change" mutations : 151 of the 194 "big change" mutations (~80%) have errors less than 2 kcal/mol, 99 of which have errors less than 1 kcal/mol; 107 of the 136 "small change" mutations have errors less than 2 kcal/mol, 99 of which have errors less than 1 kcal/mol.

Compared to "big change" mutations, a larger percentage of "small change" mutations have errors less than 1 kcal/mol: 69% for "small change" vs 51% for "big change" mutations. What's more, ring disappearance/appearance and ring type change are also often seen in mutation studies and they're present in this data set also. From our analysis, we find AMBER performs well for both: 54 of 68 ring disappearance/appearance mutations have errors less than 2 kcal/mol, 35 of which have an error less than 1 kcal/mol; 52 of 78 ring type change mutations have errors less than 2 kcal/mol, 29 of which have an error less than 1 kcal/mol.

4. Conclusions

We repeated the relative binding free energy calculations on the data set described in previous work.[1] Comparing to the Schrödinger FEP/OPLS 2.1 force field, GPU TI with AMBER FF14SB and the GAFF force field performs reasonably well on this data set, with errors above those seen using the FEP/OPLS 2.1 force field. For the 330 mutations, AMBER has MUE and RMSDs of 1.17 kcal/mol and 1.50 kcal/mol, which is comparable to Schrödinger's 0.90 kcal/mol and 1.14 kcal/mol. For the 199 ligands, most of the binding free energy values are within 2 kcal/mol, except 18 ligands and 5 ligands for AMBER and Schrödinger, respectively. Future work will explore the use of replica exchange and other new features in AMBER to enhance the sampling (both in λ-space and orthogonal degree of freedom), compare capabilities of the next generation GAFF2 and possibly other force fields, and test procedures for creating benchmark quality results with meaningful error estimates that can be used as a baseline for other comparisons.

ACKNOWLEDGEMENTS

This work was supported by NIH grants GM107485 (DMY). Computational resources were provided by the MSU HPC.

Reference:

1. Wang, L.; Wu, Y. J.; Deng, Y. Q.; Kim, B.; Pierce, L.; Krilov, G.; Lupyan, D.; Robinson, S.; Dahlgren, M. K.; Greenwood, J.; Romero, D. L.; Masse, C.; Knight, J. L.; Steinbrecher, T.; Beuming, T.; Damm, W.; Harder, E.; Sherman, W.; Brewer, M.; Wester, R.; Murcko, M.; Frye, L.; Farid, R.; Lin, T.; Mobley, D. L.; Jorgensen, W. L.; Berne, B. J.; Friesner, R. A.; Abel, R., Accurate and Reliable Prediction of Relative Ligand Binding Potency in Prospective Drug Discovery by Way of a Modern Free-Energy Calculation Protocol and Force Field. *Journal of the American Chemical Society* **2015,** *137* (7), 2695-2703.
2. Jorgensen, W. L.; Maxwell, D. S.; TiradoRives, J., Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. *Journal of the American Chemical Society* **1996,** *118* (45), 11225-11236.
3. Mccammon, J. A.; Gelin, B. R.; Karplus, M., Dynamics of Folded Proteins. *Nature* **1977,** *267* (5612), 585-590.
4. Merz, K. M.; Kollman, P. A., Free-Energy Perturbation Simulations of the Inhibition of Thermolysin - Prediction of the Free-Energy of Binding of a New Inhibitor. *Journal of the American Chemical Society* **1989,** *111* (15), 5649-5658.
5. Mobley, D. L.; Gilson, M. K., Predicting Binding Free Energies: Frontiers and Benchmarks. *Annu Rev Biophys* **2017,** *46*, 531-558.
6. Mobley, D. L.; Klimovich, P. V., Perspective: Alchemical free energy calculations for drug discovery. *Journal of Chemical Physics* **2012,** *137* (23).
7. Aqvist, J.; Luzhkov, V. B.; Brandsdal, B. O., Ligand binding affinities from MD simulations. *Accounts Chem Res* **2002,** *35* (6), 358-365.
8. Guitierrez-de-Teran, H.; Aqvist, J., Linear Interaction Energy: Method and Applications in Drug Design. *Methods Mol Biol* **2012,** *819*, 305-323.
9. Kollman, P. A.; Massova, I.; Reyes, C.; Kuhn, B.; Huo, S. H.; Chong, L.; Lee, M.; Lee, T.; Duan, Y.; Wang, W.; Donini, O.; Cieplak, P.; Srinivasan, J.; Case, D. A.; Cheatham, T. E., Calculating structures and free energies of complex molecules: Combining molecular mechanics and continuum models. *Accounts Chem Res* **2000,** *33* (12), 889-897.
10. Kuhn, B.; Kollman, P. A., Binding of a diverse set of ligands to avidin and streptavidin: An accurate quantitative prediction of their relative affinities by a combination of molecular mechanics and continuum solvent models. *J Med Chem* **2000,** *43* (20), 3786-3791.
11. Li, Y.; Liu, Z. H.; Wang, R. X., Test MM-PB/SA on True Conformational Ensembles of Protein-Ligand Complexes. *J Chem Inf Model* **2010,** *50* (9), 1682-1692.
12. Rastelli, G.; Del Rio, A.; Degliesposti, G.; Sgobba, M., Fast and Accurate Predictions of Binding Free Energies Using MM-PBSA and MM-GBSA. *J Comput Chem* **2010,** *31* (4), 797-810.
13. Souaille, M.; Roux, B., Extension to the weighted histogram analysis method: combining umbrella sampling with free energy calculations. *Comput Phys Commun* **2001,** *135* (1), 40-57.
14. Torrie, G. M.; Valleau, J. P., Non-Physical Sampling Distributions in Monte-Carlo Free-Energy Estimation - Umbrella Sampling. *J Comput Phys* **1977,** *23* (2), 187-199.
15. Velez-Vega, C.; Gilson, M. K., Overcoming Dissipation in the Calculation of Standard Binding Free Energies by Ligand Extraction. *J Comput Chem* **2013,** *34* (27), 2360-2371.
16. Ytreberg, F. M., Absolute FKBP binding affinities obtained via nonequilibrium unbinding simulations. *Journal of Chemical Physics* **2009,** *130* (16).

17. Bennett, C. H., Efficient Estimation of Free-Energy Differences from Monte-Carlo Data. *J Comput Phys* **1976,** *22* (2), 245-268.
18. Kirkwood, J. G., Statistical mechanics of fluid mixtures. *Journal of Chemical Physics* **1935,** *3* (5), 300-313.
19. Kong, X. J.; Brooks, C. L., lambda-Dynamics: A new approach to free energy calculations. *Journal of Chemical Physics* **1996,** *105* (6), 2414-2423.
20. Lee, F. S.; Chu, Z. T.; Bolger, M. B.; Warshel, A., Calculations of Antibody Antigen Interactions - Microscopic and Semimicroscopic Evaluation of the Free-Energies of Binding of Phosphorylcholine Analogs to Mcpc603. *Protein Eng* **1992,** *5* (3), 215-228.
21. Shirts, M. R.; Chodera, J. D., Statistically optimal analysis of samples from multiple equilibrium states. *Journal of Chemical Physics* **2008,** *129* (12).
22. Zwanzig, R. W., High-Temperature Equation of State by a Perturbation Method .1. Nonpolar Gases. *Journal of Chemical Physics* **1954,** *22* (8), 1420-1426.
23. Zwanzig, R. W.; Kirkwood, J. G.; Oppenheim, I.; Alder, B. J., Statistical Mechanical Theory of Transport Processes .7. The Coefficient of Thermal Conductivity of Monatomic Liquids. *Journal of Chemical Physics* **1954,** *22* (5), 783-790.
24. Kumar, S.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A.; Rosenberg, J. M., The Weighted Histogram Analysis Method for Free-Energy Calculations on Biomolecules .1. The Method. *J Comput Chem* **1992,** *13* (8), 1011-1021.
25. Kumar, S.; Rosenberg, J. M.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A., Multidimensional Free-Energy Calculations Using the Weighted Histogram Analysis Method. *J Comput Chem* **1995,** *16* (11), 1339-1350.
26. Lee, T. S.; Radak, B. K.; Pabis, A.; York, D. M., A New Maximum Likelihood Approach for Free Energy Profile Construction from Molecular Simulations. *J Chem Theory Comput* **2013,** *9* (1), 153-164.
27. Lee, T. S.; Radak, B. K.; Huang, M.; Wong, K. Y.; York, D. M., Roadmaps through Free Energy Landscapes Calculated Using the Multidimensional vFEP Approach. *J Chem Theory Comput* **2014,** *10* (1), 24-34.
28. Kollman, P., Free-Energy Calculations - Applications to Chemical and Biochemical Phenomena. *Chem Rev* **1993,** *93* (7), 2395-2417.
29. Luccarelli, J.; Michel, J.; Tirado-Rives, J.; Jorgensen, W. L., Effects of Water Placement on Predictions of Binding Affinities for p38 alpha MAP Kinase Inhibitors. *J Chem Theory Comput* **2010,** *6* (12), 3850-3856.
30. Steinbrecher, T.; Case, D. A.; Labahn, A., A multistep approach to structure-based drug design: Studying ligand binding at the human neutrophil elastase. *J Med Chem* **2006,** *49* (6), 1837-1844.
31. Stembrecher, T.; Hrenn, A.; Dormann, K. L.; Merfort, I.; Labahn, A., Bornyl (3,4,5-trihydroxy)-cinnamate - An optimized human neutrophil elastase inhibitor designed by free energy calculations. *Bioorgan Med Chem* **2008,** *16* (5), 2385-2390.
32. Lawrenz, M.; Wereszczynski, J.; Amaro, R.; Walker, R.; Roitberg, A.; McCammon, J. A., Impact of calcium on N1 influenza neuraminidase dynamics and binding free energy. *Proteins-Structure Function and Bioinformatics* **2010,** *78* (11), 2523-2532.

33. Reddy, M. R.; Erion, M. D., Calculation of relative binding free energy differences for fructose 1,6-bisphosphatase inhibitors using the thermodynamic cycle perturbation approach. *Journal of the American Chemical Society* **2001,** *123* (26), 6246-6252.
34. Palma, P. N.; Bonifacio, M. J.; Loureiro, A. I.; Soares-da-Silva, P., Computation of the Binding Affinities of Catechol-O-methyltransferase Inhibitors: Multisubstate Relative Free Energy Calculations. *J Comput Chem* **2012,** *33* (9), 970-986.
35. Erion, M. D.; Dang, Q.; Reddy, M. R.; Kasibhatla, S. R.; Huang, J.; Lipscomb, W. N.; van Poelje, P. D., Structure-guided design of AMP mimics that inhibit fructose-1,6-bisphosphatase with high affinity and specificity. *Journal of the American Chemical Society* **2007,** *129* (50), 15480-15490.
36. Jiang, W.; Roux, B., Free Energy Perturbation Hamiltonian Replica-Exchange Molecular Dynamics (FEP/H-REMD) for Absolute Ligand Binding Free Energy Calculations. *J Chem Theory Comput* **2010,** *6* (9), 2559-2565.
37. Khavrutskii, I. V.; Wallqvist, A., Improved Binding Free Energy Predictions from Single-Reference Thermodynamic Integration Augmented with Hamiltonian Replica Exchange. *J Chem Theory Comput* **2011,** *7* (9), 3001-3011.
38. Wang, L.; Berne, B. J.; Friesner, R. A., On achieving high accuracy and reliability in the calculation of relative protein-ligand binding affinities. *P Natl Acad Sci USA* **2012,** *109* (6), 1937-1942.
39. Harder, E.; Damm, W.; Maple, J.; Wu, C. J.; Reboul, M.; Xiang, J. Y.; Wang, L. L.; Lupyan, D.; Dahlgren, M. K.; Knight, J. L.; Kaus, J. W.; Cerutti, D. S.; Krilov, G.; Jorgensen, W. L.; Abel, R.; Friesner, R. A., OPLS3: A Force Field Providing Broad Coverage of Drug-like Small Molecules and Proteins. *J Chem Theory Comput* **2016,** *12* (1), 281-296.
40. Storer, J. W.; Giesen, D. J.; Cramer, C. J.; Truhlar, D. G., Class-Iv Charge Models - a New Semiempirical Approach in Quantum-Chemistry. *J Comput Aid Mol Des* **1995,** *9* (1), 87-110.
41. Shivakumar, D.; Harder, E.; Damm, W.; Friesner, R. A.; Sherman, W., Improving the Prediction of Absolute Solvation Free Energies Using the Next Generation OPLS Force Field. *J Chem Theory Comput* **2012,** *8* (8), 2553-2558.
42. Wang, J. M.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A., Development and testing of a general amber force field. *J Comput Chem* **2004,** *25* (9), 1157-1174.
43. D.A. Case, R. M. B., D.S. Cerutti, T.E. Cheatham, III, T.A. Darden, R.E. Duke, T.J. Giese, H. Gohlke,; A.W. Goetz, N. H., S. Izadi, P. Janowski, J. Kaus, A. Kovalenko, T.S. Lee, S. LeGrand, P. Li, C.; Lin, T. L., R. Luo, B. Madej, D. Mermelstein, K.M. Merz, G. Monard, H. Nguyen, H.T. Nguyen, I.; Omelyan, A. O., D.R. Roe, A. Roitberg, C. Sagui, C.L. Simmerling, W.M. Botello-Smith, J. Swails,; R.C. Walker, J. W., R.M. Wolf, X. Wu, L. Xiao and P.A. Kollman, AMBER 2016, University of California, San Francisco. **2016**.
44. Lee, T. S.; Hu, Y.; Sherborne, B.; Guo, Z.; York, D. M., Toward Fast and Accurate Binding Affinity Prediction with pmemdGTl: An Efficient Implementation of GPU-Accelerated Thermodynamic Integration. *J Chem Theory Comput* **2017,** *13* (7), 3077-3084.
45. D.A. Case, V. B., J.T. Berryman, R.M. Betz, Q. Cai, D.S. Cerutti, T.E. Cheatham, III, T.A. Darden, R.E.; Duke, H. G., A.W. Goetz, S. Gusarov, N. Homeyer, P. Janowski, J. Kaus, I. Kolossváry, A. Kovalenko,; T.S. Lee, S. L., T. Luchko, R. Luo, B. Madej, K.M. Merz, F. Paesani, D.R. Roe, A. Roitberg, C. Sagui,; R. Salomon-Ferrer, G. S., C.L. Simmerling, W. Smith, J. Swails, R.C.

Walker, J. Wang, R.M. Wolf, X.; Kollman, W. a. P. A., AMBER 14, University of California, San Francisco. **2014**.
46. M. J. Frisch, G. W. T., H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian 09 (Gaussian, Inc., Wallingford CT, 2009).
47. Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P., The Missing Term in Effective Pair Potentials. *J Phys Chem-Us* **1987,** *91* (24), 6269-6271.
48. Joung, I. S.; Cheatham, T. E., Determination of alkali and halide monovalent ion parameters for use in explicitly solvated biomolecular simulations. *J Phys Chem B* **2008,** *112* (30), 9020-9041.
49. Darden, T.; York, D.; Pedersen, L., Particle Mesh Ewald - an N.Log(N) Method for Ewald Sums in Large Systems. *Journal of Chemical Physics* **1993,** *98* (12), 10089-10092.
50. Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G., A Smooth Particle Mesh Ewald Method. *Journal of Chemical Physics* **1995,** *103* (19), 8577-8593.
51. Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C., Numerical-Integration of Cartesian Equations of Motion of a System with Constraints - Molecular-Dynamics of N-Alkanes. *J Comput Phys* **1977,** *23* (3), 327-341.
52. D.A. Case, R. M. B., D.S. Cerutti, T.E. Cheatham, III, T.A. Darden, R.E. Duke, T.J. Giese, H. Gohlke,; A.W. Goetz, N. H., S. Izadi, P. Janowski, J. Kaus, A. Kovalenko, T.S. Lee, S. LeGrand, P. Li, C.; Lin, T. L., R. Luo, B. Madej, D. Mermelstein, K.M. Merz, G. Monard, H. Nguyen, H.T. Nguyen, I.; Omelyan, A. O., D.R. Roe, A. Roitberg, C. Sagui, C.L. Simmerling, W.M. Botello-Smith, J. Swails,; R.C. Walker, J. W., R.M. Wolf, X. Wu, L. Xiao and P.A. Kollman (2016), , AMBER 2016, University of California, San Francisco.
53. Ucisik, M. N.; Hammes-Schiffer, S., Relative Binding Free Energies of Adenine and Guanine to Damaged and Undamaged DNA in Human DNA Polymerase eta: Clues for Fidelity and Overall Efficiency. *Journal of the American Chemical Society* **2015,** *137* (41), 13240-13243.
54. Kaus, J. W.; Pierce, L. T.; Walker, R. C.; McCammon, J. A., Improving the Efficiency of Free Energy Calculations in the Amber Molecular Dynamics Package. *J Chem Theory Comput* **2013,** *9* (9), 4131-4139.
55. Wang, L. L.; Deng, Y. Q.; Knight, J. L.; Wu, Y. J.; Kim, B.; Sherman, W.; Shelley, J. C.; Lin, T.; Abel, R., Modeling Local Structural Rearrangements Using FEP/REST: Application to Relative Binding Affinity Predictions of CDK2 Inhibitors. *J Chem Theory Comput* **2013,** *9* (2), 1282-1293.