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Exploring the Effectiveness of Binding Free Energy Calculations

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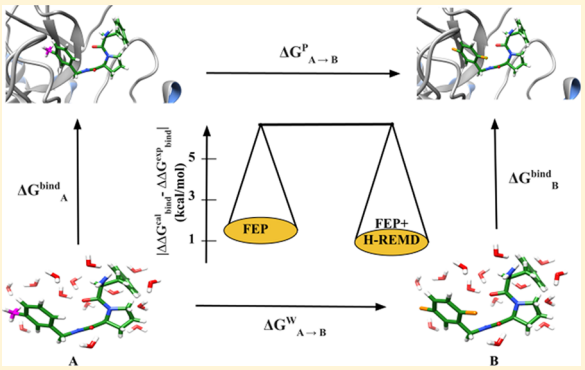
ABSTRACT: Increasing the accuracy of the evaluation of ligand-binding energies is one of the most important tasks of current computational biology. Here we explore the accuracy of free energy perturbation (FEP) approaches by comparing the performance of a "regular" FEP method to the one using replica exchange to enhance the sampling on a well-defined benchmark. The examination was limited to the so-called alchemical perturbations which are restricted to a fragment of the drug, and therefore, the calculation is a relative one rather than the absolute binding energy of the drug. Overall, our calculations reach the 1 kcal/mol accuracy limit. It is also shown that the accurate prediction of the position of water molecules around the binding pocket is important for FEP calculations. Interestingly, the replica exchange method does not significantly improve the accuracy of binding energies, suggesting that we reach the limit where the force field quality is a critical factor for accurate calculations.



1. INTRODUCTION

Accurately calculating binding free energies is a challenging problem in biomolecular simulations. A great deal of progress has been made toward developing new force fields to accurately predict the physical properties of protein systems and perhaps more importantly in refining methods for enhancing the efficiency of sampling to explore the configurational space. In spite of these advancements, the unsigned errors in relative free binding energy (RBFE) calculations are still about 1 kcal/mol.[1,2] Furthermore, errors of 1.0 kcal/mol can result in a wrong prediction of up to a 5-fold difference in binding affinity. While recent force field developments have enabled us to represent interactions among different segments of a system in a relatively reliable way, it is assumed that to accurately calculate RBFEs, efficient sampling is still the biggest bottleneck. Apparently, it is not always possible to sample the relevant configurational space by using conventional molecular dynamics (MD) or Monte Carlo (MC) methods. Thus, different enhanced sampling methods, such as umbrella sampling,[3] replica exchange,[4] para-dynamics,[5] and simulated annealing,[6] have been developed to improve sampling. However, force field definitions and sampling efficiency sometimes are not the only barriers to accurately calculating RBFEs: the representation of a proper thermodynamic cycle and how the system of interest is described is also important. Therefore, a reliable method and proper description of the system of interest should be considered together with the sampling problem.

Because evaluation of absolute binding energy is still very challenging,[7,8] we focus in this work on the less demanding alchemical free energy perturbation (FEP) methods to get the relative binding free energies of molecular fragments. In addition to a proper thermodynamic cycle for alchemical binding free energy calculations, we used modified Lennard Jones potential when creation/annihilation of atoms are attempted to perform the FEP MDs rigorously. We then started examining the problem of enhancing the sampling efficiency. Replica exchange is one of the most successful sampling methods for FEP calculations, in which information is exchanged between noninteracting "replicas" of the system that are run in parallel. We have used Hamiltonian replica exchange MDs (H-REMD)[9] to see if the accuracy of alchemical FEP calculations can be improved with increased sampling.

We have taken a series of thrombin inhibitors with systematic chemical differences as a model system to examine how our alchemical FEP calculations performed, with and without H-REMD-enhanced sampling, and compared the accuracies to ref 1 and the experimental data.

Thrombin is a well-known drug target for many cardiovascular diseases. Numerous efforts have been made to produce high-affinity drugs for thrombin inhibition, with some direct thrombin inhibitors already available in the market. Note that we are not trying to design a new thrombin inhibitor, but instead use a well-established experimental data set to validate our results. For our calculations, we selected a series of D-Phe-Pro-based thrombin inhibitors[10] that differ only in the portion that binds to the S1 pocket (see Figure 1).

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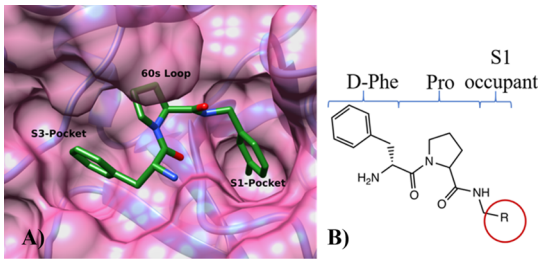

Figure 1. (A) The binding pocket of thrombin, where the ligand is represented in stick (atom-based colorings are used) and protein as blue ribbons. The vdw surface (pink) is calculated using Chimera.[11,12] Different parts of the ligand binding pocket are clearly shown. (B) A generic ligand (D-Phe-Pro-based thrombin inhibitor), where the chemical groups in the ligand are marked according to the portion of the binding pocket where it binds.

The selected small molecule thrombin inhibitors are good as a model system, because of the congeneric nature of the inhibitor series. Thus, calculations of relative binding free energy can be used in the lead optimization stage to choose the most potent drug candidate. To perform alchemical FEP calculations, we started from two different reference ligands and alchemically converted a part of these reference ligands to other ligands in the set. Our results show that the proposed method is reasonably accurate, and the estimated relative binding free energies are within 1 kcal/mol in most cases.

2. THEORETICAL BACKGROUND AND METHODS

Relative binding free energy (RBFE) can be calculated using the thermodynamic cycle described in Figure 2 and eq 1.

$$\Delta\Delta G_{A\to B} = \Delta G_B^{bind} - \Delta G_A^{bind} = \Delta G^P_{A\to B} - \Delta G^W_{A\to B} \quad (1)$$

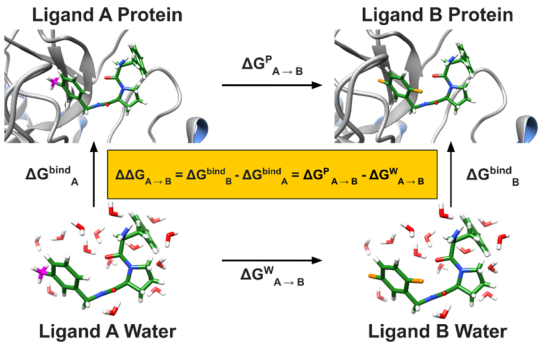

Figure 2. A thermodynamic cycle for relative binding free energy calculations using the alchemical FEP method.

In eq 1, $\Delta\Delta G_{A\to B}$ is the difference in binding free energies of ligand A and B, whereas, $\Delta G^W_{A\to B}$ and $\Delta G^P_{A\to B}$ represent the change in free energy of converting ligand A to B in a water and protein environment, respectively. The terms $\Delta G^W_{A\to B}$ and $\Delta G^P_{A\to B}$ can be calculated using the alchemical FEP method as discussed below. In alchemical changes, we start from ligand A and try to change a part of it to get ligand B, which may require creation or annihilation of atoms. While annihilation of bonds/angle/dihedrals can happen along with the annihilation of atoms but replacing real atoms with "dummy" atoms can solve the problem of explicitly treating the annihilation of the bonds/angle/dihedrals. Unless large changes are made, this replacement (atom type conversion) procedure can work for the desired alchemical changes. To convert atoms of one type to the other (dummy or other atom types) we change the charge distribution of the ligand and the nonbonding parameters of the atoms which are undergoing changes.

We have used FEP adiabatic charging (AC) of the Enzymix module from Molaris-XG software[13] for all the calculations. The FEP[14] calculations are done by using a mapping potential of eq 2.

$$\epsilon_m(\lambda_m) = U_1(1-\lambda_m) + U_2\lambda_m \quad (2)$$

where in eq 2 U_1 and U_2 are the potential surfaces of the system when the ligand is in its initial and final state, respectively, and λ_m is the mapping weight that varies between $(0 \le \lambda_m \le 1)$. The change in free energy associated with the change of ϵ_m is given by

$$\Delta G(\lambda_m \to \lambda_{m+1}) = -\frac{1}{\beta}\exp\langle -(\epsilon_{m+1} - \epsilon_m)\beta\rangle_m \quad (3)$$

where $\langle\rangle_m$ denotes that the average is evaluated by propagating trajectories over ϵ_m. Thus, the overall change in free energy can be obtained by changing ϵ_1 to ϵ_2 in n equal increments and evaluating using eq 4.

$$\Delta G(U_1 \to U_2) = \sum_{m=0}^{n-1} \Delta G(\lambda_m \to \lambda_{m+1}) \quad (4)$$

In the case of creation or annihilation of atoms using alchemical FEP methods, the simulations suffer huge convergence problem because of the inability of proper sampling as λ approaches zero (creation) or one (annihilation) when we use conventional LJ/electrostatic potentials for nonbonding interactions.[15] To avoid this, different schemes have been developed.[15−17] A widely used solution to the problem is to introduce a soft-core potential, which modifies the LJ potential (see ref 15) given in eq 5, where i is the atom to be created and j is the other unaltered atom in the system. The terms A_i and A_j or B_i and B_j are the LJ parameters A or B for atom i and j, respectively. λ is the FEP mapping weight, and α is a positive constant that is parameterized to 0.5 in our current study.

$$U^{LJ}_{ij}(r^{ij};\lambda) = \lambda\frac{(B_i \times B_j)^2}{(A_i \times A_j)}\left(\frac{1}{\left[\alpha(1-\lambda)^2 + \frac{r_{ij}^6}{((B_i\times B_j)/(A_i\times A_j))}\right]^2} - \frac{1}{\left[\alpha(1-\lambda)^2 + \frac{r_{ij}^6}{((B_i\times B_j)/(A_i\times A_j))}\right]}\right) \quad (5)$$

No modified soft-core potential for coulomb interaction is used as we have used a separate step to change the partial charge distributions of the ligands. The schematic in Figure 3 explains the pathway of alchemical change for converting a ligand with the $-CH_3$ group to a ligand with the $-Cl$ group. The conversion in this case occurs in three steps instead of the one step. One-step conversion suffers from the problem of proper sampling if the parameterization of different soft-core

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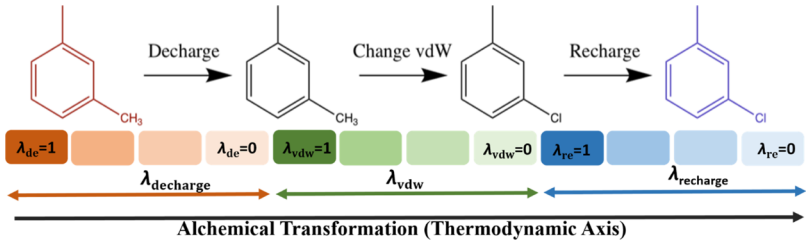

Figure 3. Schematic of the alchemical free energy change pathway. The part of a ligand containing a methyl group is shown to be replaced to a ligand with the Cl group. At the first step the partial charges of the depicted region are converted to zero. In the second step the atom types are changed (C → Cl; H → dummy) and in the last step the partial charges of the Cl-containing ligand are regenerated. The color in the boxes represents different steps and the color gradient is the representation of the percentage of the initial state on the corresponding FEP window. $\lambda_{decharge}$, λ_{vdw}, and $\lambda_{recharge}$ are the mapping constants for the decharging, atom type conversion, and recharging steps, respectively.

potentials is not performed appropriately. When both decharging/recharging and changes in nonelectrostatic interactions (to create/annihilate atoms) are performed simultaneously in alchemical free energy changes, the pair potential function (coulomb and LJ potential) takes an unusual shape at the intermediate λ-values (see ref 18), leading to a sampling problem.

In most cases the alchemical FEP method can be used to calculate accurately relative binding free energies; however, sometimes the accuracy is not enough, which is usually a result of insufficient sampling of relevant configuration spaces.

H-REMD has been used as a method of choice to overcome the sampling problem in FEP calculations for systems of large size. There exists many variations of H-REMD, but we have used a replica exchange method where exchanges are attempted between adjacent replicas (different λ-values) along the alchemical transformation axis. It is important to note that exchange attempts are made within replicas of a single transformation step. The replica-exchange algorithm follows Metropolis MC exchange criterion[19]

$$P(\lambda_i \rightarrow \lambda_j) = \min\{1,\ e^{-\beta\Delta U}\}$$

where,

$$\Delta U = [U_i(X_j) + U_j(X_i) - U_i(X_i) + U_j(X_j)] \quad (6)$$

$U_j(X_i)$ denotes the potential energy of the system having configuration X_i and the energy is calculated using the force field of the replica j. In the current implementation, the replica exchanges are attempted after every 2 ps between adjacent replicas. The exchanges are attempted in such a way that for an intermediate replica, the configuration of the system can be exchanged with both of its neighboring replicas. On odd swap cycles, the exchanges are performed between (λ_1, λ_2) (λ_3, λ_4) (λ_5, λ_6) frames, whereas on even swap cycles (λ_2, λ_3) (λ_4, λ_5) (λ_6, λ_7) frames are involved in the exchange. Therefore, in every 4 ps, for the intermediate replicas, the system configuration can possibly be exchanged with their neighboring replicas (see Figure 4).

In this work we have taken ten ligands (see Figure 5) which differ only in the portion that binds to the S1 pocket of thrombin. In order to perform the alchemical transformation, we chose the ligand with R = 3-methylbenzyl or 3,6-dimethylbenzyl group (see Figure 1) as the starting state and converted that to other R groups (see the transformation maps in Figure 5).

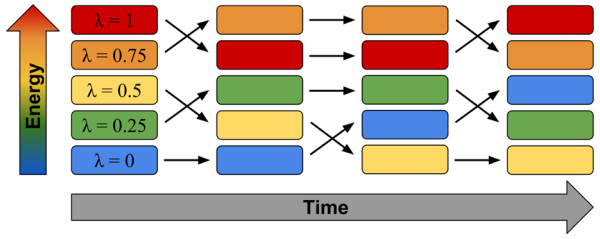

Figure 4. Schematic representation of the Hamiltonian Replica Exchange sampling. For λ = 0.25 if in an even cycle the exchange is attempted with λ = 0.50, in the next odd cycle the exchange would be attempted with λ = 0.0.

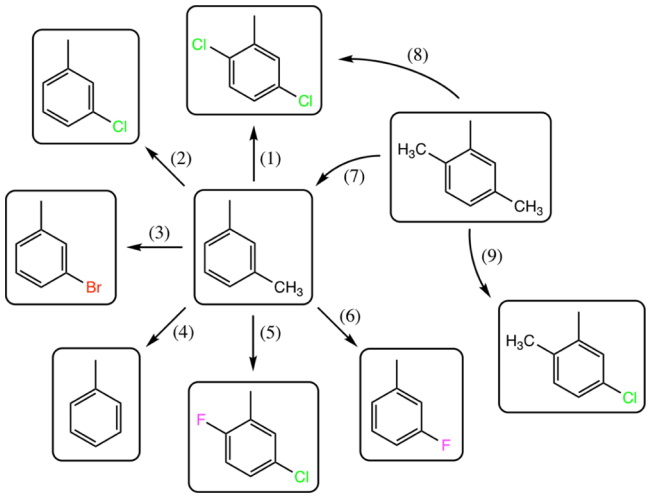

Figure 5. Alchemical Transformation network. Staring from (R═ CH_3) the alchemical transformation is used to convert to ligands (R = Cl, Br, H, F in step 2, 3, 4, and 6, respectively, and R, R′ = (Cl, Cl); (Cl, F) in step 1 and 5, respectively). The same type of alchemical transformation is used from R, R′ = (CH_3, CH_3) to (CH_3, H), (Cl, Cl) and (CH_3, Cl) in step 7, 8 and 9, respectively.

2.1. TRANSFORMATION FROM 3-METHYLBENZYL (−CH_3)

All calculations are performed using the Enzymix[20] force field of Molaris-XG. We have used PDB: 2ZF0[10] as a starting structure for alchemical transformation. All the nonprotein atoms except the ligand were removed from the PDB file before proceeding to the next step, where the missing residues were added using Modeller 9.17.[21] The modeled structure was selected based on the low Modeler objective function score. The modeler-generated structure was then used for relaxation using Molaris-XG. The protein system was immersed in a

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spherical solvent sphere of radius 18 Å (measured from the center of the ligand). The center of the ligand was taken as the center of the simulation system. The boundary water molecules of the spherical solvent were subject to polarization and radial restraint according to the surface constraint all-atom solvent model.[22] These surface constraints are introduced to make the finite system behave like as if it is part of an infinite system. The long-range electrostatic interactions were treated using the local reaction field approach.[23] The initial system was heated slowly from 10 to 300 K for 200 ps with a time step of 0.001 ps, while constraining the ligand at its initial position with a force constant of 5 kcal/mol. The constrain force was then relaxed further from 5 to 0.3 kcal/mol in 2×10^5 steps with step size 0.001 ps. Throughout the relaxation process a position constrain of 0.03 kcal/mol was applied on the system (excluding the ligand) within 18 Å from the center of the system. The system outside 18 Å was kept at the original positions. The relaxed systems were used for further relaxations to generate restart files to perform several independent simulations. Before starting the alchemical transformation simulations, we checked the probable water configurations near the binding pocket of the thrombin ligands using our water flooding approach[24] [chemical potential (B) = −12.0 kcal/mol]. The importance of water positioning in ligand-binding studies has been discussed extensively in our works previously.[25,26] The water flooding method has shown to be effective in a previous study on drug resistance by HCV proteases.[27] In the case of R = 3-methylbenzyl ligand, no extra water molecules can be added to the binding pocket. It is worth mentioning that the restart files generated after the last step were used in all the decharging steps of the alchemical transformation axis for all transformations. We have used the B3LYP functional and 6-31G(d,p) basis set to calculate the partial charges of all the ligands.

In our adiabatic changing (AC) calculations[28] without H-REMD, we have used 21 FEP windows with a total MD simulation time of 420 ps (0.001 ps step size) for each step of alchemical transformation (Figure 3). In our AC/H-REMD simulations, 11 replicas were used, and 100 swaps were intended with an interval of 2 ps. The results were obtained by taking an average of the last 50 swaps because the free energy change of the transformation converges within the first half of the AC/H-REMD simulations for all transformation steps (see Figure 6 for an example). It is worthwhile mentioning that even in these calculations the alchemical free change in water is calculated using "regular" FEP/MD.

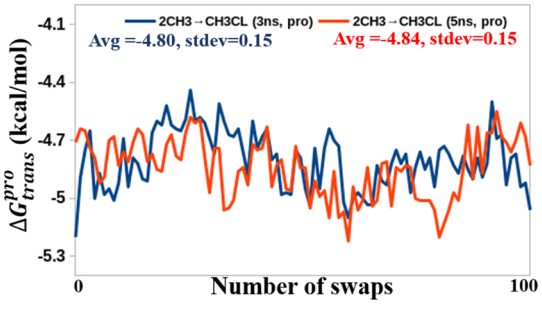

Figure 6. Plot of change in the free energy of transformation with the number of swaps attempted in the FEP/H-ERMD simulation.

2.2. TRANSFORMATION FROM 3,6-DIMETHYLBENZYL (−2CH$_3$)

In order to check the accuracy of the method, we started from a different initial state where R = 3,6-dimethylbenzyl group. In this case the crystal structure was unavailable, so we mutated the 3-methylbenzyl group containing the ligand of the modeler-generated structure to generate the starting structure for relaxation. The relaxation and AC calculation protocols are the same as mentioned above. Interestingly, here one extra water molecule has been found after the water flooding simulation. Thus, we have tried to perform our alchemical free energy transformations both with and without water-flooding generated structures. The comparison between the last two systems would help us to justify how important water configurations could be in predicting the ligand binding energy accurately.

3. RESULTS AND DISCUSSION

The alchemical transformation protocol used for the relative binding energy calculations was validated by comparing the calculated relative binding energies with experimental values. Isothermal titration calorimetric values in Table 1 of ref 10 were converted to relative binding free energies using eq 7.

$$\Delta\Delta G^{exp}_{1\to 2} = \Delta G_2 - \Delta G_1 \quad (7)$$

The results of our alchemical free energy calculation are presented in Table 1. The calculated relative free energies

Table 1. Comparison between Calculated and Experimental Relative binding Free Energies[a]

conversion	$\Delta\Delta G^{cal}_{bind}$	$\Delta\Delta G^{exp}_{bind}$
−CH$_3$ → −Cl	−1.64	−0.14
−CH$_3$ → −2Cl	−0.83	−0.86
−CH$_3$ → −ClF	−1.12	−0.59
−CH$_3$ → −Br	−1.96	−0.24
−CH$_3$ → −H	0.73	0.74
−CH$_3$ → −F	0.59	0.84
−CH$_3$ → −CH$_3$	−0.02	0.00
−2CH$_3$ → −CH$_3$	0.66	−0.10
−2CH$_3$ → −CH$_3$	−0.02[b]	−0.10
−2CH$_3$ → −CH$_3$Cl	0.17	−0.67
−2CH$_3$ → −CH$_3$Cl	−0.70[b]	−0.67
−2CH$_3$ → −2Cl	0.31	−0.96
−2CH$_3$ → −2CH$_3$	0.01	0.00

[a]The relative binding energies are calculated using the conventional FEP method. [b]With water-flooding calculations (see main text).

agree with the experimental findings, but the relative error in the calculated values for some cases are still more than 1 kcal/mol. There is always a question of convergence in FEP calculations and we converted −CH$_3$ to −CH$_3$ and −2CH$_3$ to −2CH$_3$ (see Table 1) to verify that the simulation trajectories for our alchemical calculations were sufficiently long and the applied perturbation was sufficiently small.

We have further tried the H-REMD, as mentioned in the above section, to check the improvement of the conventional FEP calculations. The results of FEP/H-REMD calculations are given in Table 2. It can be seen that the mean error in relative binding energy calculations has decreased in most of the cases compared to the conventional FEP method. We have also compared our FEP/H-REMD results with that of ref. 1,

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Table 2. Comparison of Our Calculated Relative Binding Free Energies (Using FEP/H-REMD) with Those of the Experimental and the Calculated Results of ref 1

conversion	$\Delta\Delta G_{bind}^{cal}$	$\Delta\Delta G_{bind}^{cal\_ref1}$	$\Delta\Delta G_{bind}^{exp}$
$-CH_3 \rightarrow -Cl$	−1.31	−0.02	−0.14
$-CH_3 \rightarrow -2Cl$	−0.76	−0.27	−0.86
$-CH_3 \rightarrow -ClF$	−1.09	−0.12	−0.59
$-CH_3 \rightarrow -Br$	−1.27	−0.70	−0.24
$-CH_3 \rightarrow -H$	1.07	1.45	0.74
$-CH_3 \rightarrow -F$	1.16	0.98	0.84
$-CH_3 \rightarrow -CH_3$	0.08		0.00
$-2CH_3 \rightarrow -CH_3$	0.71[a]	0.24	−0.10
$-2CH_3 \rightarrow -CH_3$	0.03[a,b]	0.24	−0.10
$-2CH_3 \rightarrow -CH_3Cl$	0.16	−0.41	−0.67
$-2CH_3 \rightarrow -CH_3Cl$	−0.68[b]	−0.41	−0.67
$-2CH_3 \rightarrow -2Cl$	−0.29	−0.03	−0.96
$-2CH_3 \rightarrow -2CH_3$	0.00		0.00

[a]For the atom-type changing step, the free energy of the change is taken as same as that of in the FEP/MD. [b]With water-flooding calculations (see main text).

where FEP/REST have been used[29] for enhancing the sampling efficiency and the OPLS2.1 force field for accurately representing the system. It can be seen from Table 2 that in most cases the absolute error in predicting the relative binding free energies by our method and ref 1 (see Figure 7) are comparable. The convergence of the FEP results were also checked by calculating the alchemical transformation of $-CH_3$ to $-CH_3$ and $-2CH_3$ to $-2CH_3$.

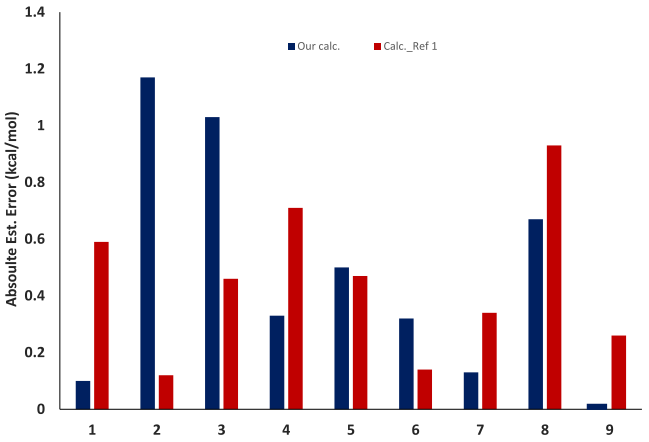

Figure 7. A comparison of absolute estimated errors in predicting relative binding energies between our calculations and that of ref 1. The blue and red bars correspond to the absolute estimated error in our calculations and those of ref 1, respectively. The *X*-axis represents the transformations and the number corresponds to steps in the transformation network in Figure 5.

Interestingly, in the cases of $-2CH_3$ the relative binding energy prediction accuracy has been increased after using the structures that are generated from water flooding calculations (see Tables 1 and 2). This shows the importance of water positioning in binding free energy calculations.

Our overall results are encouraging. The estimated error in our relative binding free energy prediction is less than 1 kcal/mol in most cases (see Figure 7). By comparing with the experimentally obtained relative binding free energy values (see Table 2), the calculated relative binding energy is systematically overestimated, while the errors in most cases are low. It is difficult to assess the actual reason for such an overestimation, a probable reason being the force field parameters used in our calculations, especially the partial charges of the ligand. In most of our studied cases, the energy contributions in decharging and recharging processes are observed to be higher compared to the vdW parameter changing step. It is also observed that in few cases the fluctuation of the total potential energy change, in steps where annihilation of atoms are carried out, is considerable (in case of FEP/H-REMD), which could also contribute to the overestimations.

Furthermore, the conventional FEP calculation is relatively accurate in all cases, and the FEP/H-REMD has only helped slightly to improve the results. The effect of the implemented replica exchange is not drastic as can be seen from Figure 8. It

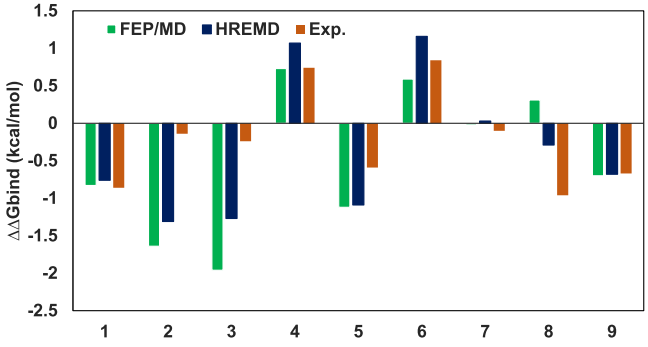

Figure 8. A bar diagram that compares the calculated relative bind free energy with and without using H-REMD.

is possible that the exploration of the conformational space by H-REMD is not sufficiently robust. A replica exchange protocol in the torsional space can be added to the current protocol (orthogonal to the thermodynamic axis) to improve the performance of H-REMD.[30]

4. CONCLUSIONS

The accuracy of relative binding free energy calculations is still an unsettled issue. While many reasonable approaches have been proposed, no single method has been shown to be highly successful for a wide range of protein ligand complexes (namely, going below 1 kcal/mol), and ref 1 reached this limit. Although our main interest has been in calculations of the absolute binding free energies, we decided to explore the enhanced sampling type used in ref 1 using the Molars-XG software framework to calculate relative binding free energies. Our method has shown to be accurate on a set of thrombin inhibitors, and the absolute error in predicting the relative binding free energy is also similar (in most cases) to that of ref 1. While these results are encouraging, we still observe a systematic overestimation in our calculated results and even enhanced sampling with H-REMD has not been able to reduce it considerably.

It is found that replica exchange is not the most crucial element in obtaining accurate binding energies. This might mean that the convergence limit has been approached. If this is true, we might have reached a limit where the force field quality starts to become a major factor in the accuracy of free energy calculations.

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Notes

The authors declare no competing financial interest.

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