

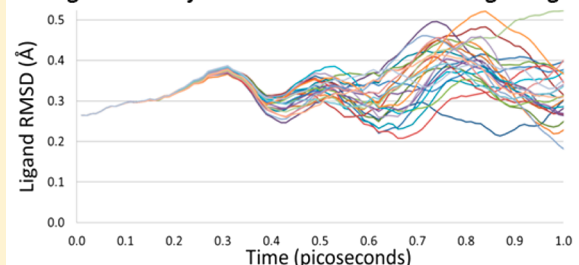
Improved Ligand Binding Energies Derived from Molecular Dynamics: Replicate Sampling Enhances the Search of Conformational Space

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ABSTRACT: Does a single molecular trajectory provide an adequate sample conformational space? Our calculations indicate that for Molecular Mechanics - Poisson–Boltzmann Surface Area (MM-PBSA) measurement of protein ligand binding, a single molecular dynamics trajectory does not provide a representative sampling of phase space. For a single trajectory, the binding energy obtained by averaging over a number of molecular dynamics frames in an equilibrated system will converge after an adequate simulation time. A separate trajectory with nearly identical starting coordinates (1% randomly perturbed by 0.001 Å), however, can lead to a significantly different calculated binding energy. Thus, even though the calculated energy converges for a single molecular dynamics run, the variation across separate runs implies that a single run inadequately samples the system. The divergence in the trajectories is reflected in the individual energy components, such as the van der Waals and the electrostatics terms. These results indicate that the trajectories sample different conformations that are not in rapid exchange. Extending the length of the dynamics simulation does not resolve the energy differences observed between different trajectories. By averaging over multiple simulations, each with a nearly equivalent starting structure, we find the standard deviation in the calculated binding energy to be ~1.3 kcal/mol. The work presented here indicates that combining MM-PBSA with multiple samples of the initial starting coordinates will produce more precise and accurate estimates of protein/ligand affinity.

Small changes (± 0.001 Å) to protein/ligand coordinates yield divergent MD trajectories with different binding energies.



INTRODUCTION

Molecular Mechanics - Poisson–Boltzmann Surface Area (MM-PBSA) is a popular method for estimating the calculated energy change resulting from a ligand binding to a protein,^{1–3} and we routinely use it to help guide our design decisions in drug discovery programs. Molecules that are candidates for organic synthesis are evaluated by MM-PBSA, and those that have more favorable energies of binding are prioritized for synthesis.

In the MM-PBSA method, we run a computer simulation of the room-temperature dynamics of the protein/ligand complex. Once the system has reached equilibrium, this molecular dynamics (MD) trajectory is sampled at regular intervals, and energies are calculated for each of these “frames”. The van der Waals and Coulomb interactions are obtained from the force-field that drives the simulation, while the solvation free energy is estimated by solving the Poisson–Boltzmann equation for the protein/ligand complex and for the free states of both the protein and the ligand. (For small-molecule/protein interactions, the Surface Area term, which represents the nonpolar contribution to solvation, shows little variation between ligands in the same series.) These energies are averaged over many frames of the dynamics trajectory to obtain an overall estimate of the binding energy for that protein/ligand system. Details can be found in several references.^{4–7}

The energies involved in these calculations are very large (tens of kcal/mol or more) and mostly cancel (e.g., a favorable electrostatic interaction will be countered by the dielectric response of the solvent, which will act to reduce the interaction). As a result, the energies computed from individual frames are subject to significant fluctuations. Nevertheless, when averaged over many frames, an MD trajectory yields a stable value (Figure 1).

When our lab was migrating between versions of AMBER, we noticed some discrepancies between MM-PBSA energies calculated from identical starting structures using the same protocols. Further investigation led us to make small variations in the starting coordinates that resulted in significant changes in the calculated MM-PBSA energies for protein/ligand complexes. These effects mirror the chaotic behavior of scores observed by Feher and Williams using standard minimization or docking protocols.^{8–10} In our lab, Amber versions 10 and 11 displayed similar divergent behavior of the trajectories. In each case, the energy averaged over many MD frames appeared to converge but to different values. An example of two such trajectories is shown in Figures 2 and 3.

These unexpected results led us to a more thorough investigation of the effects of starting geometry on the energy

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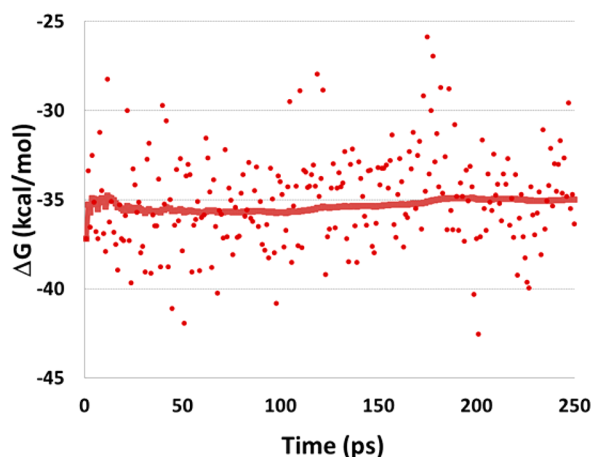


Figure 1. Evolution of the calculated binding energy from an MD trajectory. The cumulative average free energy of binding, calculated at each frame number by averaging all previously sampled frames, is shown as a solid line. Although there is large scatter in the individual binding energies, the cumulative average energy appears to converge to a stable value of -35 kcal/mol.

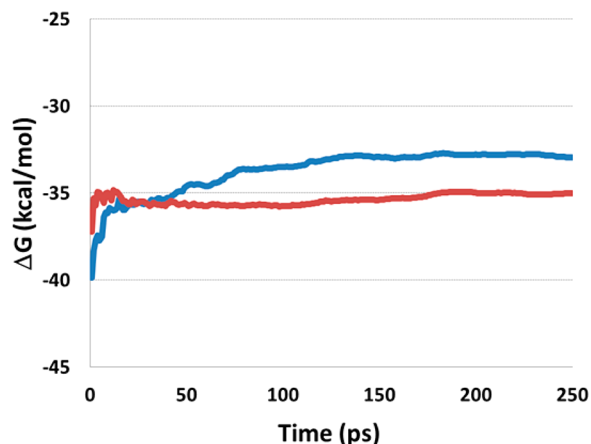


Figure 2. Evolution of the calculated binding energy for two separate MD trajectories. The initial states of the two simulations were identical except for the perturbation of ± 0.001 Å for a randomly selected 1% of the coordinates. The energies for the trajectories are averaged as described in Figure 1.

calculated by the MM-PBSA methodology, the results of which we present here. We show that minute changes (0.001 Å) to the coordinates of the atoms in the initial state of the system lead to variations of the sort seen in Figures 2 and 3. This led us to incorporate a systematic dithering of coordinates to create several nearly identical starting states for the protein/ligand complex. Independent MD trajectories for each of these “replicate” structures yield a distribution of calculated MM-PBSA energies that is approximately Gaussian (Figure 3).

This article presents results from replicate MM-PBSA calculations for four protein/ligand complexes. We show that individual simulations do not yield sufficiently representative average energies. The combination of replicate simulations, however, does obey the expected statistical behavior, demonstrating that more complete sampling is obtained with multiple replicates. We then compare replicate simulations with simulations of longer duration and show that longer simulations are less efficient and still do not provide an adequate sampling of phase space. We further show that minimization of individual

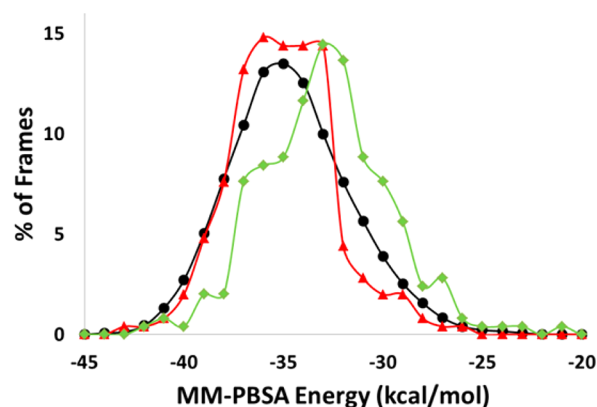


Figure 3. Distribution of energies for the individual MD frames (PLK2/1 system). The black points (●) are the frames pooled from the fifty separate replicates. The red and green points (▲, ◆) are the individual replicates from Figure 2. Note that they clearly deviate from black curve. The t test indicates that the red and green distributions are statistically different ($p > 0.9999$).

frames does not improve the statistical precision. In addition, we use techniques adapted from MD simulations of peptides¹¹ to monitor the propagation of chaotic motion during the initial steps of the MD trajectory. Finally, we show that energies calculated from multiple replicates correlate better with experimental binding affinities than those calculated from single MD trajectories.

RESULTS

1. Individual Replicate Simulations. The replicate simulations all start with the same coordinates for the protein/ligand complex. A small amount of dithering is introduced into each replicate by making random 0.001 Å perturbations in 1% of the coordinates. If these replicate simulations sample the same region of phase space equally well, the differences in average MM-PBSA binding energies should vary in a predictable way. Within a single 250 ps trajectory, the energies have a standard deviation of 3.0 kcal/mol (Figure 1). For each replicate simulation, the predicted precision in energies derived from a single trajectory should follow conventional statistics in which the precision is proportional to $N_f^{-1/2}$, where N_f is the number of independent measurements, frames in this case. Thus increasing the number frames by four-fold should reduce the uncertainty by half. A nine-fold increase in frames provides a three-fold reduction in the uncertainty. Using this formula, the expected value for the deviation in mean energy across replicates should be ± 0.19 kcal/mol (i.e., $3.0 \times 250^{-1/2}$). However, the average energies derived from fifty different replicates showed a much greater variation than predicted, ± 1.20 kcal/mol (Table 1). This

Table 1. Results from 50 Replicate MD Simulations of 250 ps Duration

complex	IC ₅₀ , nM	energy, kcal/mol	SD	t test ^a > 2σ	> 3σ
PLK2/1	5	-34.4	± 1.20	73%	60%
PLK2/2	850	-22.5	± 1.34	81%	71%
PLK2/3	950	-32.7	± 1.38	77%	65%
HIV/KNI-1689	0.8	-43.6	± 1.22	65%	57%

^aPercent of replicate comparisons that fail the t test.

indicates that the replicates do not sample phase space in the same manner. A set of experiments was run in which the size of initial perturbations was varied from 0.01 to 0.0001 Å. This had no measurable effect on the energy distributions of the replicates. There was also no effect observed by changing the fraction of coordinates that are displaced.

The probability that these large variations across multiple samples results from random chance can be estimated by the Student's *t* test.¹² The *t* test assesses whether the means of two samples are statistically different. Failing the *t* test demonstrates that there is a 95% chance (2σ) that the two data sets represent inherently different sample populations. We used the *t* test to compare all 1225 possible pairings of the 50 replicates. Surprisingly, 73% of the pairings failed the *t* test (an example appears in Figure 3). When we used a more rigorous cutoff of 3σ (99.73% confidence level), 60% of pairings failed the *t* test. This result implies that the replicates sample conformational space in ways that are significantly different. Since the energy is derived solely from coordinates of the systems, different energy values must come from alternative conformations. Furthermore, the *t* test results demonstrate that these alternative conformations do not undergo rapid exchange during the simulations.

The observed scatter in the energy values was not unique to the PLK2/1 system. Data from two other PLK-2 ligands (Figure 4) are displayed in Table 1. This table also includes

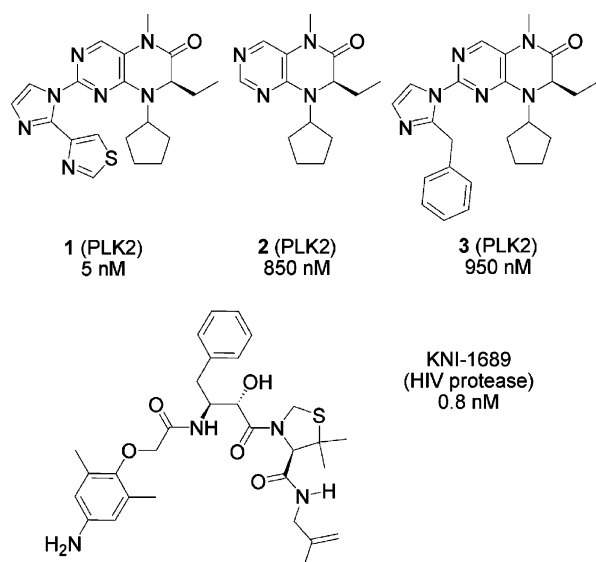


Figure 4. The covalent structure of the inhibitors discussed in this paper. The target protein and the IC_{50} are also listed.

data from another protein/ligand system, which was derived from the crystal structure HIV/KNI-1689, pdb code 3A2O.¹³ The structure was solved to 0.88 Å, and it is one of the highest resolution structures of an enzyme/inhibitor complex. The four different systems in Table 1 show remarkably similar results. The results from all other systems we have tested to date have similar behavior: replicate trajectories consistently show greater spread in energies than expected from conventional statistics.

2. Average Energies from Multiple Replicates.

Averaging the results across replicate simulations does give the expected statistical behavior associated with the adequate sampling of phase space. The precision of average energies is proportional to $N_r^{-1/2}$, where N_r , in this case, is the number of replicates that were averaged together. As a test, we took the 50 replicates for the PLK2/1 simulation and created 25 sets of two replicates. The pairs of replicates were averaged. The variance between 25 averages was ± 0.91 kcal/mol, close to the expected value of ± 0.85 kcal/mol. Similar comparisons were made using the averages of five replicates and averages of ten replicates. The observed variances were ± 0.57 kcal/mol (expected ± 0.54) and ± 0.27 kcal/mol (expected ± 0.40), respectively. Similar results were obtained for the HIV/KNI-1689 system. Thus, using averages of replicate simulations improves the precision of the measured binding energy in a predictable fashion.

In a separate control study, we created 50 artificial replicate trajectories in which the energy for each frame was chosen randomly from the corresponding frame of one of the 50 replicates. In theory, the statistical properties of these artificial replicates should reflect a random sampling from a single distribution (the union of all frames of all replicates). The mean and standard deviation for these scrambled replicates were -34.4 ± 0.2 kcal/mol (the expected value is ± 0.19 kcal/mol, see section 1). The *t* test (at 2σ) showed that only 3% of the pairings were significantly different.

Finally, the combined set of energies yields a distribution that is smooth and normal, as one would expect from a thorough sampling of the accessible states of the system (black circles, Figure 3). The individual replicates do not provide a representative sampling of the available states (red and green lines).

Thus, although the individual MD trajectories do not adequately sample phase space, the combination of replicates yields statistics that are consistent with a thorough sampling. The average energies calculated across these multiple replicates provide more precise measurements of binding energies.

3. Longer Simulations. Our results so far indicate that a single trajectory of 250 ps does not provide an adequate sampling of phase space. There could be several explanations for this phenomenon. One possibility is that there are not enough frames in our simulations. In this case, more frames from independent time points should improve the convergence

Table 2. Results from 50 Replicate MD Simulations of Varying Duration

complex	standard deviation (kcal/mol)				Student's <i>t</i> test > 2σ			
	250 ps	1000 ps	2000 ps	4000 ps	250 ps	1000 ps	2000 ps ^a	4000 ps ^a
PLK2/1	± 1.20	± 0.58	± 0.68	± 0.62	73%	51%	66%	73%
PLK2/2	± 1.34	± 1.02	± 0.68		81%	71%	68%	
PLK2/3	± 1.38	± 1.46	± 1.33		77%	77%	83%	
HIV/KNI-1689	± 1.22	± 1.17	± 0.95		65%	66%	72%	

^a500 frames were collected for the 2000 ps simulations. 4000 ps trajectory used 1000 frames. Therefore the *t* test values cannot be directly compared to the shorter trajectories, which had only 250 frames.

at the rate of $N_f^{-1/2}$, where N_f is the number of sampled frames. Alternatively, there may be slow transitions between different conformational states. Our simulations use a 100 ps pre-equilibrium phase before the production MD run. If the divergence in the energies stems from slow conformational changes, then these changes must be slower than ~ 50 ps or they would equilibrate before the production run. Longer simulations should improve the convergence by a factor of $N_t^{-1/2}$, where N_t is the length time of the simulation. The validity of these theories can be easily tested using longer trajectories.

A new set of 1 ns trajectories was generated for the PLK1/1 and HIV/KNI-1689 systems. The scatter in the MM-PBSA scores was measured using 1000 frames taken at 1 ps intervals. These results were compared to corresponding energies from the same trajectories measured using 250 frames taken at 4 ps intervals. Using 1000 frames improved the convergence by an average of 0.01 kcal/mol. This is not statistically significant. These results indicate that 250 frames provide sufficient averaging of the energy fluctuations shown in Figure 1; additional frames provide no real benefit.

To test the effects of longer trajectories on the convergence, we ran multiple simulations on all four systems (Table 2). There was an 18% reduction in the scatter of the energy values for the 1 ns trajectories. The 2 ns trajectories showed a reduction of 30% compared to the 250 ps data. These improvements were less than half the expected (i.e., $N_t^{-1/2}$) values of 50% and 65% reduction in scatter, respectively. Comparisons between the pairs of replicate trajectories indicate that at least 50% of the pairs failed the t test at 2σ . Furthermore, the four systems did not show a consistent improvement with longer sampling times. The data demonstrate that the longer trajectories do not converge to the same average energy values, and a single trajectory will not provide a precise value for the binding energy.

It is worth noting that these simulations were all based on high-resolution X-ray structures. The X-ray data are consistent with a single well-defined binding position for the ligands. Our computational results also indicate that there are no significant conformational rearrangements during the simulations. Visual inspection of the trajectories did not reveal any slow conformational rearrangements. This is reflected in the ligand coordinates for the PLK2/1 simulations. The average values of these coordinates did not vary by more than 0.05 Å during the 4 ns simulation. The rmsd between ligand coordinates from the 50 replicates remained constant at 0.44 ± 0.02 Å. Similar results were derived from the HIV/KNI-1689 data. Finally, the binding energy remained constant during the longer simulations for all four systems (± 0.1 kcal/mol). Both the experimental and computational results indicate that the system had reached an adequate equilibrium before the start of the production molecular dynamics.

4. Energetics of Minimized Structures. To explore the energy landscape of the replicates, we performed energy minimization on selected frames from the trajectories. Clearly, there is considerable conformational freedom for a system that is equilibrated at 300 °K. In theory, energy measurements derived from minimized structures may show a greater convergence in energies. We took 15 replicates and minimized 75 evenly spaced frames over the 250 ps trajectory. We did not use simulated annealing to cool these structures because we wanted to measure the energy of the nearest local minima. The minimization did reduce the variance of the energies within

each replicate: ± 2.0 kcal/mol for the minimized structures versus ± 3.0 kcal/mol for the same replicates at 300 °K. As expected, the total energy was also lower for the minimized structures, -39.5 kcal/mol versus -34.4 . However, there was a 50% increase in the variation in energy between the replicates: ± 1.78 kcal/mol for the minimized structures versus ± 1.20 for 300 °K measurements. Furthermore, the t test showed that the minimized replicates still sampled distinct regions of conformational space (t test at $2\sigma = 77\%$). The results indicate that the divergence in energy between replicates reflects the complexities of conformational space, and these variations are not an artifact of room temperature MD.

5. Divergent Behavior in the Components of the MM-PBSA Energy. The variation of the individual components of calculated MM-PBSA energies for the 250 ps simulations of PLK2/1 is shown in Table 3. All terms show fluctuations that

Table 3. Data from the 250 ps PLK2/1 Replicates Listing Total Energy and a Selection of Individual Component Energies

energy term ^a	energy, kcal/mol	SD	expected value ^b	t test > 2σ	> 3σ
pbtot	-34.4	± 1.20	0.20	73%	60%
ele	-9.8	± 1.27	0.16	80%	70%
vdw	-56.9	± 0.64	0.13	66%	51%
pbcal	37.9	± 1.53	0.17	82%	72%
gb	29.1	± 1.24	0.15	81%	70%

^aAbbreviations: **pbtot**, total MM-PBSA score; **ele**, columbic term; **vdw**, van der Waals; **pbcal**, Poisson–Boltzmann solvation; **gb**, generalized Born solvation (include for comparison but not incorporated into the total energy values). ^bThe expected value is derived from the standard deviation of the component energy in the 250 frames in the trajectory. The result is then scaled by the expected reduction in variance obtained by averaging the frames together, i.e. $N_f^{-1/2}$, where N_f is the number frames in the trajectory.

are similar to those of the total energy. The standard deviations between the component energy functions are higher than expected considering the frame-to-frame fluctuations within each replicate. Also, the values for the t test indicate that the replicates provide distinct samplings of the component energies. These results indicate that the underlying MD force field contributes to the chaotic behavior of the trajectories.

We also examined the energetics of the extended PLK2/1 simulations shown in Table 2. Based on the van der Waals term, at least 60% of comparisons between replicates failed the Student's t test at 2σ . The electrostatic term revealed an even higher rate of divergence ($>80\%$). The results indicate that the replicates from the longer simulations (1–4 ns) still do not sample the same regions of conformational space.

6. Chaotic Nature of Molecular Dynamics. A new set of experiments was performed in hopes of gaining a better understanding of the divergence in energies. In the initial replicate simulations, the randomization of the coordinates was performed on the starting structures. These coordinate perturbations rapidly spread throughout the structure, and there was significant divergence in coordinates before start of the production MD run (0.4 Å rmsd in the ligand heavy atom coordinates). A new set of 50 replicates was generated from a single structure at the start of the production run. This approach was developed by Braxenthaler et al.¹¹ in their study of peptide dynamics. We selected a replicate that possessed one of the lowest energies in the initial run, -35.9 kcal/mol. This

structure had already been pre-equilibrated at 300 °K for 100 ps. As before, 1% of the coordinates were randomly altered by 0.001 Å. The initial velocities were not changed.

Figure 5 shows the evolution of the coordinates during the first picosecond of the trajectories. The frames were recorded

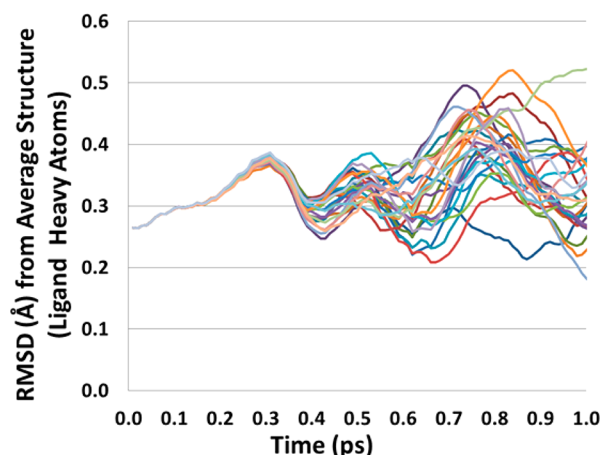


Figure 5. Evolution of the variation in coordinates across 17 different replicates. The coordinates and velocities for the replicates were identical at start of the calculation except for the dithering.

every 10 fs or five steps of molecular dynamics. At 400 fs, the replicates had nearly identical structures (Figure 6a). The average energy of the 50 replicates at this point is -36.0 ± 0.64 kcal. The trajectories show significant divergence at 600 fs. Figure 6b shows five frames that are color coded by energies. In

spite of the differences in calculated energies, -31.3 ± 1.7 kcal/mol, the conformations are very similar, and the rmsd of the ligand coordinates is only 0.17 Å. At 1000 fs, the structures have visually diverged from each (Figure 6c). The energies also diverge, -35.1 ± 2.5 kcal/mol. The divergence in energies at 1 ps is comparable to the divergence seen over the entire 250 ps run (-34.5 ± 3.10 kcal/mol). However, Figure 6c indicates that the replicates still have similar conformations. The protein side chains, including polar hydrogens, still share the same rotamer states. The ligand itself maintains a strong H-bond to the central amide of the hinge (dotted line, Figure 6). Despite considerable effort, we have not established a predictive correlation between conformation and measured binding energy for individual frames.

As trajectories progress beyond 1 ps, the structures rapidly diverge from each other (Figure 6d). Protein side chains adopt alternative conformations, the ligand changes docking positions in response to perturbations in the solvent, etc. This structural divergence is reflected in the spread of average energies from the replicates: -34.6 ± 0.98 kcal/mol, t test = 70% at 2σ . These results are comparable to the data from our canonical PLK2/1 data set: -34.4 ± 1.20 kcal/mol, t test = 73% at 2σ (Table 1). The similarities between the two data sets indicate that the chaotic behavior of the trajectories stems directly from the room temperature molecular dynamics used in our production run.

7. Improved Agreement with Experiment for Replicate Simulations. Figure 7A shows the correlation between the experimental and calculated values of the binding energies for series of PLK2 inhibitors. The calculated binding energies are derived from single trajectories using our 250 ps production

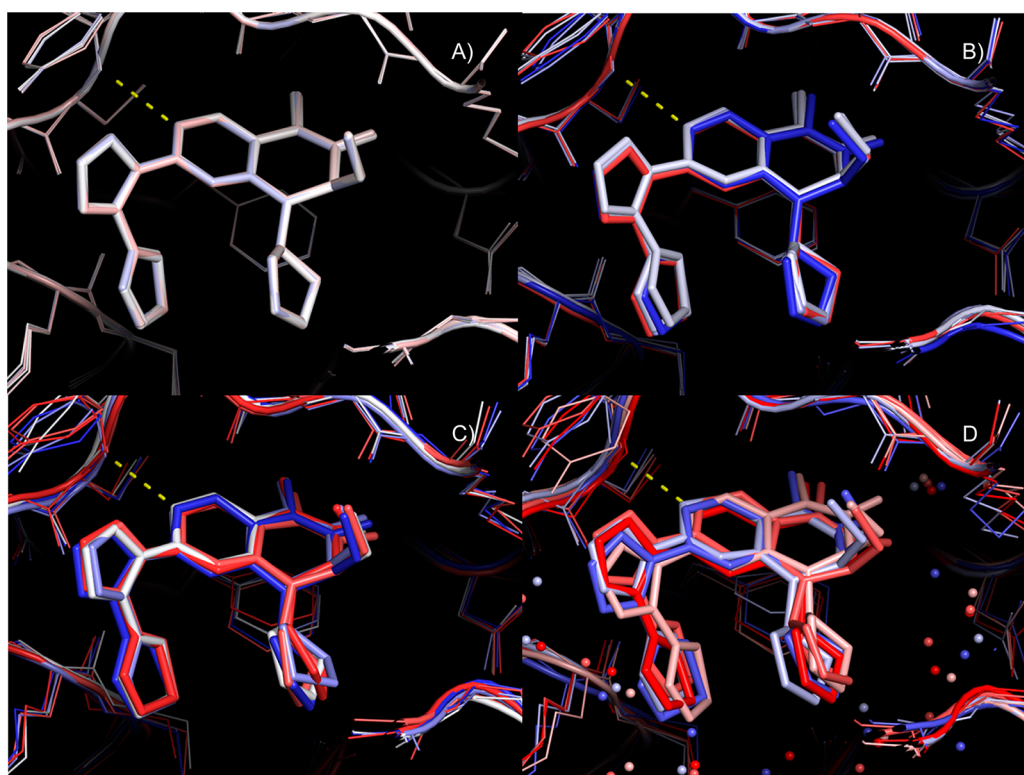


Figure 6. Frames from the 300 °K trajectories that had near identical starting points at time 0: A) 400 fs, B) 600 fs, C) 1000 fs, and D) 5000 fs. Panel D shows the oxygen atoms from the solvent. The replicates are colored blue to red over an energy range of 8 kcal/mol. The energy of the mean value (white structure) is adjusted in each panel to the average of the five structures.

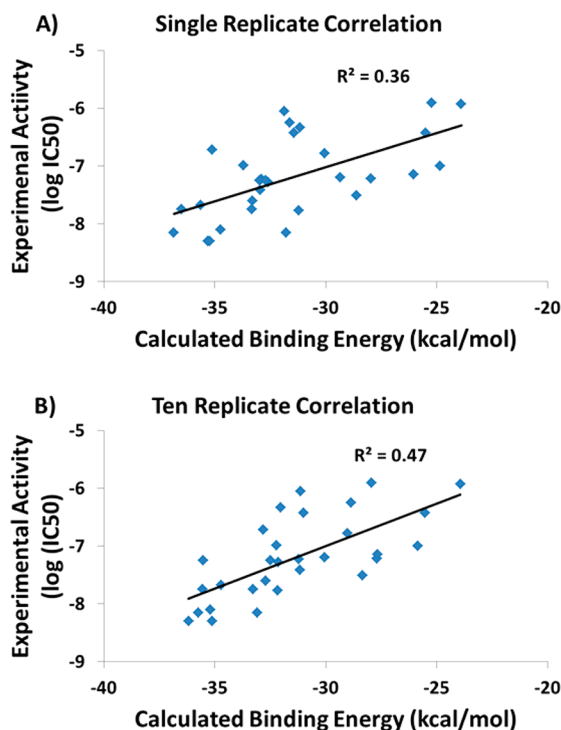


Figure 7. Correlation of calculated binding energies and experimental affinities. A) Single sample for each data point. B) Ten replicates per data point. Plots contain data for 30 compounds with measured IC_{50} values for PLK2 inhibition.¹⁴ Note: Compound 25 from ref 14 was a significant outlier and was not included in the scatter plot.

run. The inhibitors were all taken from Aubele et al.¹⁴ The correlation coefficient (r^2) between the calculated binding energy and the experimentally measured IC_{50} for this series is 0.36.

Figure 7B shows the corresponding results for the same calculation performed using 10 replicates per compound. The r^2 improves to 0.47. There is also improvement in the residuals for calculated energies. In Figure 7A the average in error in the calculated energies is ± 4.8 kcal/mol using single trajectories. The energies derived from replicates have a corresponding error of ± 3.5 kcal/mol. This improvement exceeds the predicted gains from signal averaging (± 0.8 kcal/mol). Although these improvements are modest, the use of replicates improves the precision of the MM-PBSA. This, in turn improves the accuracy.

DISCUSSION

Computational molecular dynamics is a deterministic algorithm. Given a set of starting coordinates and a computer program for calculating atomic interactions, the resulting trajectory is completely reproducible. There are literature examples, however, that demonstrate that minor perturbations to the starting coordinates will produce divergent trajectories. The following quote is from A. Roitberg:¹⁵

Run an MD trajectory with the software, force, temperature and molecule of your choice for X ns. Run a second trajectory with ALL variables the same as above, but with a change in the coordinates of ANY atom in just one component (x,y or z) by ONE unit in the last decimal place in your input file. This is as minimal a perturbation as you can make.

Compare how long does it take for the two trajectories to 'diverge' from each other in some measure (RMSD against each other, frame by frame for instance). It usually takes around 5 ps to get away by as much as 0.5 Å RMSD for 100 atoms.

Note that this is WAY shorter that the MD we run these days.

Braxenthaler et al.¹¹ have done extensive work on the effect of small perturbations on molecular dynamics. They used a solvated 13 residue peptide as a model. Their work shows that perturbations as small as 10^{-9} Å were enough to alter the trajectory in picoseconds. Furthermore, Braxenthaler et al. tested various software packages and observed the same type of chaotic behavior.

The sensitivity to small perturbation observed by these authors demonstrates the chaotic nature of molecular dynamics. These examples, however, were derived from simulations of small solvated molecules that had little internal structure. One could argue that the results from these inherently disordered systems are not relevant to measurements of ligand binding energies. A protein/ligand complex does have a well-defined conformation that can be reproduced by molecular dynamics. An MD simulation should perform an adequate sampling of the local minima. Therefore, any perturbations of the initial coordinates should be averaged out over time. If not, then the length simulation should be increased. Surely, the proper adjustment of the starting parameters should yield an adequate sampling of conformational space with a single trajectory.

The work presented here demonstrates that the simulations of protein/ligand systems are subject to the same chaotic effects observed in less complex systems. The replicates used in this study start with almost identical structures. The size of the coordinate perturbations, 0.001 Å, was well below the level of experimental detection. Figures 5 and 6 demonstrate that these small changes rapidly permeate the system in a single picosecond. The divergent trajectories have unique energy profiles that do not converge at a longer time scale of nanoseconds (Table 2). The resulting spread in energies reflects an inherent uncertainty in the calculations.

It is well-known that protein/ligand complexes have many degrees of freedom, and it is not possible to search all accessible conformations systematically. However, MM-PBSA calculations tacitly assume that a single trajectory performs an adequate search of phase space. The evolution of the average energy shown in Figure 1 is consistent with this assumption. However, our results from the Student's t test indicate that the replicates provide a more thorough search. The divergent behavior of similar trajectories likely stems from the chaotic nature of the energy surface. Once the trajectories diverge, the number of different accessible conformations grows exponentially. The results presented here imply that divergent trajectories proceed into different regions of phase space.

Traditionally, researchers have used longer trajectories in the belief that this will produce an adequate sampling of conformational space. In our hands, extended trajectories did not produce the expected improvements in signal averaging. More importantly, the t test results indicate that the replicates from the longer trajectories still sample different regions of conformational space (Table 2). A single replicate will not provide an accurate value for the binding energy. One working theory is that longer trajectories sample a greater region of conformational space, but this extended region may not have

the same energy signature of the starting coordinates. Because the starting conformations for the simulations presented here were all based on high-resolution crystal structures, it is apparently more efficient and accurate to sample the low energy states nearby, and this is accomplished with a large number of short simulations. Indeed, the cumulative averages shown in Figures 1 and 2 suggest that the duration of the replicates could be significantly shorter than 250 ps.

MM-PBSA is only one of many approaches for predicting ligand binding energies. The PBSA score is primarily used here as a numerical technique for reducing the coordinates down to single value and then using these values to compare replicates. We can only speculate whether dithering would produce similar results with other molecular dynamics based methods for assessing ligand interactions.^{16–18} Perhaps a more comprehensive approach to measuring ligand binding energies might reveal additional terms that dampen energy fluctuations seen in Figures 1 and 3. However, Table 3 demonstrates that the individual components of the energy, such as van der Waals, indicate the trajectories diverge from each other. If two simulations have statistically different values for the van der Waals component, then binding interactions must have been different. These results indicate that single trajectory does not provide an adequate search of conformational space. This, in turn, implies that such chaotic behavior may affect other MD based techniques.

This article demonstrates that there is an inherent uncertainty in MM-PBSA calculations. We do not consider this to be a limitation of the utility of the technique. We have incorporated the use of replicates in our standard operating protocols to improve the precision of the method. Typically, we perform our calculations with eight replicates. For the systems in this study, this increases the precision of each measured binding energy to ~ 0.4 kcal/mol. Furthermore, the improved correlation with experiment shown in Figure 7 is encouraging and suggests that the improved precision obtained through replicate sampling matters.

In the past, important technical refinements in MM-PBSA may have been obscured by random fluctuations in the values measured from single MD simulations. Using replicates could lead to better understanding the individual components of the binding energy. We hope this will lead to innovation.

METHODS

Most of the results presented here were obtained from our studies of polo-like kinase-2 (PLK2), a potential target for Parkinson's disease.¹⁹ The starting protein coordinates for our PLK2 simulations were derived from the crystal structure of PLK2/1 (pdb code 4I6H, 1.9 Å resolution). Compound 1 is part of a series of pteridine-based kinase inhibitors.¹⁴ To prepare the protein complexes for MD simulations, all protein residues were examined to ensure that the correct rotamer and tautomer states were used. The AMBER 10 utility tleap was used to parametrize the protein. A 29 Å sphere of TIP3 waters was added to the complex using the "solvateCap" function in tleap. An in-house computer program was written that identified any unfilled solvent cavities (>3.0 Å radius) on the surface of the protein. The active site waters in the starting model were individually examined and compared against the electron diffraction data for the PLK2/1 complex. Several rounds of testing and refinement were performed to ensure that the MD trajectories accurately reflected the initial crystal structures (see Figure 3 in ref 14).

The starting coordinates of the HIV protease simulation were derived from the 0.88 Å resolution structure 3A2O.¹³ The carboxylate of Asp25 was protonated as recommended by Adachi et al.²⁰ The X-ray waters were hand-inspected to resolve possible overlaps. A 29 Å solvation sphere was created by tleap and used without modification.

The ligand coordinates of compounds 2 and 3 (Figure 4) used in this study were derived from in-house cocrystal structures of these compounds bound to PLK2 (1.9 Å resolution). Active site waters were added to remove voids created by new ligands or removed to avoid steric clashes with the ligand. The rest of the solvated protein system was derived from the PLK2/1 complex. The AMBER utility antechamber was used to parametrize all the ligands. Minor modifications were made to the gaff.dat input file in order to ensure the planarity of aromatic ring nitrogens.

The initial perturbations of the starting structure were generated directly from the AMBER ".crd" files. A random selection of 1% of the coordinates was shifted by ± 0.001 Å. All other files used in the calculations were identical.

The calculations used dielectric constant of 1.0, a nonbonded cutoff of 12 Å, and constant temperature scaling with weak coupling. One ps was used as the time constant for heat bath coupling for the system. Except where noted, AMBER 10 default values²¹ were used for all other parameters. The active site was as defined by 15 Å sphere around the ligand center. Residues within this sphere were allowed to move, and all other protein residues were fixed. The protocol used several minimization steps that were followed by 50 ps warm up period to 300 °K and a 100 ps pre-equilibration period (ref 14, Table 8). SHAKE dynamics were used for MD trajectories.²² A harmonic restraint of 1.5 kcal/mol-Å² was used on the C α for the active site residues during the MD runs. The MM-PBSA energies were calculated from a production run of 250 ps at 300 °K, with frames sampled every picosecond.

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Notes

The authors declare no competing financial interest.

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