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PBSA_E: A PBSA-Based Free Energy Estimator for Protein−Ligand Binding Affinity

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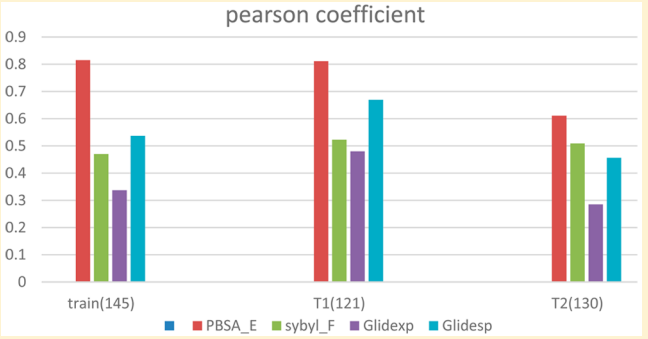
Supporting Information

ABSTRACT: Improving the accuracy of scoring functions for estimating protein−ligand binding affinity is of significant interest as well as practical utility in drug discovery. In this work, PBSA_E, a new free energy estimator based on the molecular mechanics/Poisson−Boltzmann surface area (MM/PBSA) descriptors, has been developed. This free energy estimator was optimized using high-quality experimental data from a training set consisting of 145 protein−ligand complexes. The method was validated on two separate test sets containing 121 and 130 complexes. Comparison of the binding affinities predicted using the present method with those obtained using three popular scoring functions, i.e., GlideXP, GlideSP, and SYBYL_F, demonstrated that the PBSA_E method is more accurate. This new energy estimator requires a MM/PBSA calculation of the protein−ligand binding energy for a single complex configuration, which is typically obtained by optimizing the crystal structure. The present study shows that PBSA_E has the potential to become a robust tool for more reliable estimation of protein−ligand binding affinity in structure-based drug design.



INTRODUCTION

Protein−ligand binding is essential to almost all biological processes, and the underlying physical and chemical interactions determine the specific biological recognition at the molecular level. In drug discovery, one tries to find a molecular ligand that either inhibits or activates a specific protein target through ligand binding. However, finding a ligand that binds a targeted protein with high affinity is a major challenge in early-stage drug discovery. The atomic-resolution structures of protein−ligand complexes, mostly obtained from X-ray crystallography or NMR spectroscopy,[1,2] provide a chemical basis for understanding protein−ligand interactions[3−8] and could be effectively used as the basis for the design of small-molecule drugs for the treatment of diseases.

Computation can help speed up the drug discovery process through simulation and modeling. In structure-based drug design, one calculates the free energies of binding by a range of proposed ligands to a given target protein using three-dimensional atomic structures of the complexes. Thus, accurate and yet efficient evaluation of protein−ligand binding affinity is critical for selecting and designing more effective binders of the target. In terms of the accuracy in evaluating protein−ligand binding, free energy perturbation (FEP) and thermodynamics integration (TI)[5,9−17] are considered high-end methods that are theoretically rigorous but prohibitively expensive when evaluation of a large number of protein−ligand bindings is required, such as in drug screening. At the low end, empirical scoring functions are widely used for fast estimation of protein−ligand binding affinities in large numbers because of their high efficiency. However, evaluation of the binding free energy using a simple empirical scoring function is generally not very reliable. Alternatively, the molecular mechanics with Poisson−Boltzmann surface area (MM/PBSA) approach,[7,18−28] whose accuracy generally lies between those of FEP and empirical scoring functions,[29−32] is a popular method for computing protein−ligand binding affinity. The MM/PBSA method calculates the solvation energy using an implicit-solvation model.[33] In the MM/PBSA approach, however, one is required to perform MD simulation for the protein−ligand

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complex and compute PBSA free energies at multiple snapshots. Such calculations of free energy can become impractical when binding affinities for a large number of protein−ligand complexes are needed, such as in drug screening.

In this study, we aimed to develop a reliable and efficient estimator of protein−ligand binding affinity. The method should be physically based but require no configurational sampling or MD simulation, allowing it to be used for fast evaluation of binding affinities for a large number of protein−ligand systems. To satisfy these two criteria, we chose to utilize the PBSA method to calculate various energy terms in the standard MM/PBSA approach for a fixed structure of a protein−ligand complex. For a given protein−ligand complex, the initial structure is optimized first to alleviate strains, and the PBSA calculation is then performed using this optimized structure. The binding affinity is given by a linear combination of various energy terms from the PBSA calculation. The coefficients of the linear combination were preoptimized using a training set with experimentally known binding affinities. The training set contained a total of 145 complexes whose experimental binding affinities were used to optimize our free energy estimator. The optimized free energy estimator was then tested on two data sets. The first set contained 121 protein−ligand complexes, and the second set contained 130 protein ligand complexes. These test complexes were all selected from the PDBbind database maintained by Wang and co-workers.[34−36] A comparison of the performance of the current method to those of other methods, including some very popular scoring functions,[37−41] is presented and discussed in this paper.

METHODS

Training Set for Protein−Ligand Binding. We first compiled a database containing 145 high-quality protein−ligand complexes with experimentally determined binding affinities from three sources.[37,42,43] These complexes were obtained from the above-referenced data sets by excluding (1) systems with metal ions in the binding site and (2) ligands with more than two charges. It is known that current force fields cannot accurately describe metal-containing proteins. Also, the accuracy of protein−ligand binding energies obtained from MM/PBSA calculations generally decreases with increasing ligand charge.[44] Therefore, these systems were excluded from our training set.

Test Sets for Protein−Ligand Complexes. The above-described training set was used to fit our MM/PBSA-based energy estimator. We prepared two additional data sets as test sets to test and validate the energy estimator. The first test set was obtained by excluding complexes with metal ions in the binding site or with ligands having more than two charges from the "refined set" and "general set" (v2013) of PDBbind of Wang and co-workers.[45] In addition, complexes in the "refined set" with missing residues were also excluded from our test set. These treatments produced a test set denoted as test set 1 (T1) containing 121 complexes. Test set 2 (T2) was extracted from the PDBbind "core set" (v2013)[36] by excluding complexes with metal ions in the binding sites or with ligands having more than two charges, resulting in data set containing 130 complexes. The unit of binding affinity is kcal/mol. All of the complexes along with their Protein Data Bank (PDB) IDs and experimental binding affinities are given in the Supporting Information. There were no redundant complexes in the three data sets, and the structures were downloaded from both the PDB and the PDBbind Web site (http://www.pdbbind-cn.org).[46]

Hydrogen atoms were added to the proteins using the LEAP module in AMBER11,[47] and the Amber force field ff99SB was used in the present work.[48] The amine groups (Lys and Arg residues and the N-terminus) were fully protonated, and the carboxylic groups (Asp and Glu residues and the C-terminus) were deprotonated. All of the His residues were left neutral and then protonated at the ND1 or NE2 position on the basis of the local electrostatic environment. Force field parameters of ligands were obtained using the ANTECHAMBER module[49] based on the generalized Amber force field (GAFF)[50] with the HF/6-31G* RESP charges.[50,51] All of the ab initio calculations were carried out using the Gaussian 09 program.[52]

The training set contained 50 different protein families and a total of 145 systems. Test set 1 contained 49 different protein families, and test set 2 contained 64 different protein families. There were 11 protein families used in the test sets that had close analogues in the training set. This information is provided in Tables S9−S11 in the Supporting Information.

Optimized Structure for the MM/PBSA Calculation. In order to prepare the best structure for the single-configuration MM/PBSA calculation, each protein−ligand complex was immersed in a periodic rectangular box of TIP3P water molecules. The distance from the surface of the box to the closest atom of the solute was set to 10 Å, and counterions were added to neutralize the whole system. Two minimization steps were carried out to optimize the complex structure. In the first step, only the solvent molecules and hydrogen atoms were optimized using the steepest descent algorithm followed by the conjugate gradient method. In the second step, the entire system was energy-minimized until convergence was reached. All of the system optimizations were performed using the AMBER11 program.[47]

Energy Estimator Based on MM/PBSA Calculations. The binding free energy of protein−ligand complex was calculated using a single configuration obtained from the optimization procedure described above. The protein−ligand binding free energy ΔG_{bind} from the MM/PBSA method is given by eq 1:[18,53]

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{pb} + \Delta G_{pbsur} - T\Delta S \quad (1)$$

where the gas-phase interaction energy between the protein and ligand is given by

$$\Delta E_{MM} = \Delta E_{ele} + \Delta E_{vdw} \quad (2)$$

in which ΔE_{ele} and ΔE_{vdw} are the electrostatic and van der Waals energies, respectively. The PB energy ΔG_{pb} was calculated using the PBSA program[26] in AMBER11 with a lattice spacing of 2 grids/Å. In our approach, the number of rotatable bonds of the ligand (N_{rot}) is used to approximate the entropy term upon ligand binding. A conformational penalty of 1 kcal/mol is used for each rotatable bond.[42] The nonpolar solvation term is calculated from the solvent-accessible surface area (SASA)[54] using the formula $\Delta G_{pbsur} = \gamma \cdot \Delta SASA$, where γ = 0.0072 kcal mol^{-1} Å^{-2}.

The proposed energy estimator PBSA_E is given by the following expression

$$PBSA\_E = a_1(\Delta E_{ele} + \Delta G_{pb}) + a_2\Delta E_{vdw} + a_3\Delta G_{pbsur} + a_4 N_{rot} \quad (3)$$

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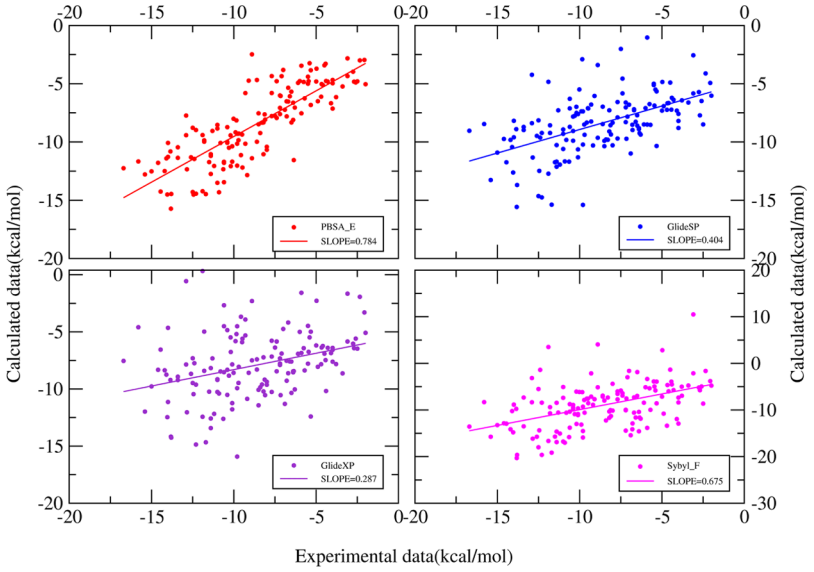

Figure 1. Scatter plots of the binding affinities of the 145 protein−ligand complexes in the training set. In each panel, the *X* axis represents the experimental binding affinities and the *Y* axis the calculated binding affinities obtained by a particular method.

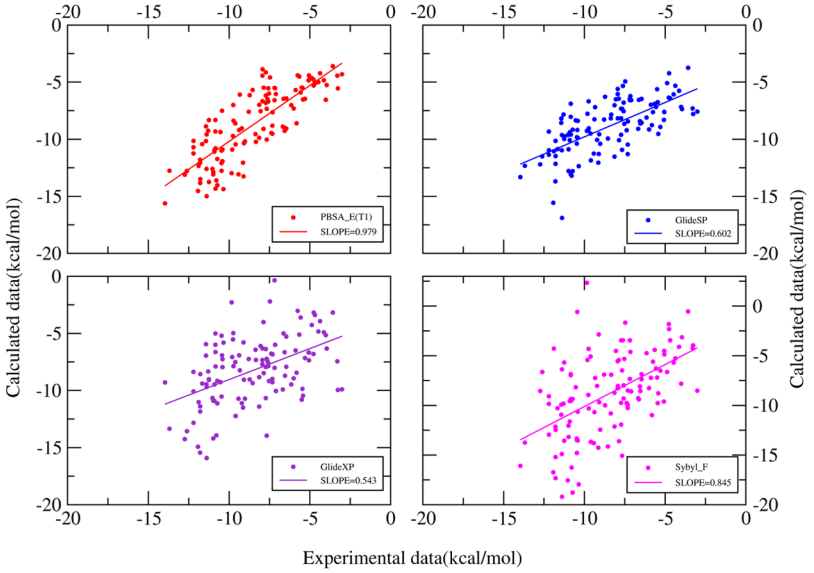

Figure 2. Similar to Figure 1 but for test set 1 (T1).

We calculated the binding affinities for all of the protein−ligand complexes in the given training set (containing 145 complexes as described above) using MM/PBSA. Each MM/PBSA calculation was done using a single configuration optimized from the crystal structure described above. The calculated individual energy terms were put into eq 3, and the coefficients a_1−a_4 were obtained by least-squares fitting to the corresponding experimental binding affinities for all of the complexes in the training set.[19,30,40,55]

The PBSA energy terms in eq 3 can be obtained from MM/PBSA calculations using either a single configuration optimized from the crystal structure or multiple configurations generated from MD simulations. We performed both single- and multiple-configuration MM/PBSA calculations to obtain various energy terms for 20 protein−ligand complexes selected from the test sets. Our results indicated that the energy estimator based on the optimized single-configuration protein−ligand complex structure is superior to that based on multiple configurations generated from MD simulations. Details of the results calculated from MD simulations are given in Tables S7 and S8.

RESULTS AND DISCUSSION

Training Set. The final resulting formula for the energy estimator PBSA_E after the numerical fitting procedure using the training set defined previously in the paper is given by

$$\text{PBSA\_E} = 0.03037(\Delta E_{\text{ele}} + \Delta G_{\text{pb}}) + 0.07791\Delta E_{\text{vdw}} + 1.2193\Delta G_{\text{pbsur}} + 0.1854N_{\text{rot}} \quad (4)$$

Figure 1 shows the relationship between the experimentally determined binding affinities and the calculated PBSA_E values for the 145 protein−ligand complexes in the training set. For comparison, binding affinities converted from the corresponding scoring functions of GlideXP,[37] GlideSP,[41] and Sybyl_F[39,40] are also shown in Figure 1. It is clear that the binding affinities predicted by PBSA_E are in much better agreement with the

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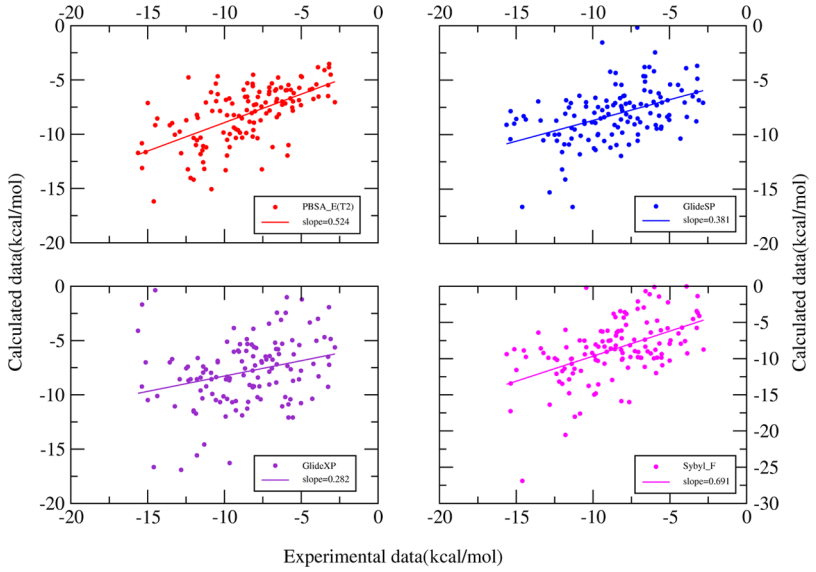

Figure 3. Similar to Figure 1 but for test set 2 (T2).

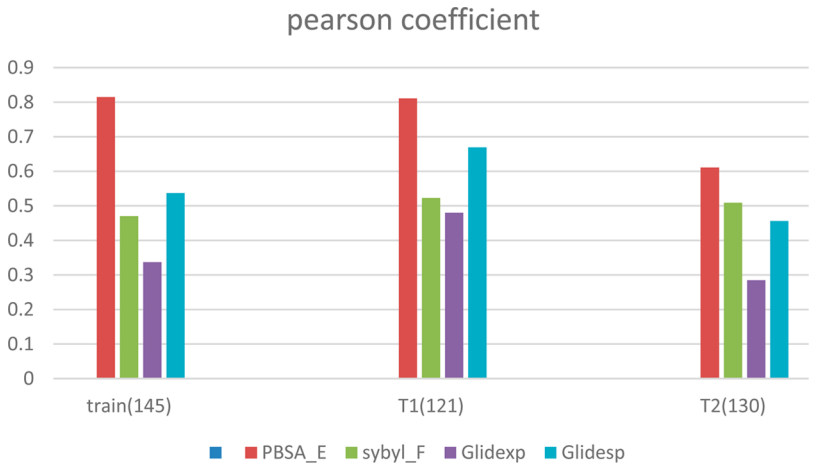

Figure 4. Pearson correlation coefficients (R) for the binding affinities of protein−ligand complexes using different scoring functions corresponding to the training set (train(145)), test set 1 (T1(121)), and test set 2 (T2(130)).

experimental values than their counterparts are. The predicted PBSA_E values are more concentrated around the regression line, while values obtained using GlideXP, GlideSP, and Sybyl_F are more scattered away from the regression line. In another measurement, the slope of the PBSA_E regression line was found to be 0.784, while those for the other three scoring functions are 0.287, 0.404, and 0.675, respectively.

Test Set 1. The performance of PBSA_E had to be validated on test sets in addition to the training set for comparison with other scoring functions. The first test set (T1) contained 121 protein−ligand complexes as described above. Figure 2 plots the relationships of the calculated binding affinities obtained from PBSA_E, GlideXP, GlideSP, and Sybyl_F with the experimentally measured values. Figure 2 shows that the PBSA_E values are more concentrated around the regression line while the values for other three scoring functions are more diffuse. The slopes of the regression lines are 0.979, 0.602, 0.543, and 0.845 for PBSA_E, GlideSP, GlideXP, and Sybyl_F, respectively. We do note in Figure 2 that GlideSP performs relatively better than GlideXP and Sybyl_F and that the latter two scores are more scattered away from the regression line.

Test Set 2. The second test set (T2) contained 130 protein−ligand complexes as described above. Figure 3 shows the relationships of the calculated binding affinities obtained from PBSA_E, GlideXP, GlideSP, and Sybyl_F with the experimentally measured values. Similar to Figure 2 for T1, the PBSA_E values are more concentrated around the regression line than those for the other three scoring functions, and the slopes of the regression lines are 0.524, 0.381, 0.282, and 0.691 for PBSA_E, GlideSP, GlideXP, and Sybyl_F, respectively. Similar to the case of T1, GlideSP also performs relatively better than GlideXP.

Correlation Coefficients and RMSE. The validation of the PBSA_E approach on various data sets can be quantitatively measured by using the correlation coefficient with respect to the corresponding experimental binding affinities. The correlation coefficients for all three data sets (training set, T1, and T2) obtained from PBSA_E and the other three methods are plotted in Figure 4. It is clear that the correlation coefficients for the PBSA_E values are the best among all four scoring function methods. For example, the correlation coefficients of PBSA_E are 0.815, 0.811, and 0.611 for the training set, T1 and T2, respectively, compared with the

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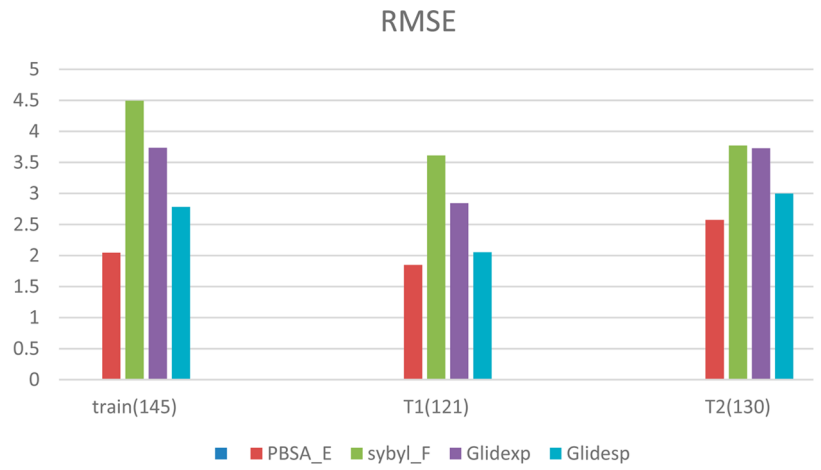

Figure 5. Similar to Figure 4 except for RMSE instead of correlation coefficients.

Table 1. Comparison of PBSA_E Energies (in kcal/mol) with Those Based on Sets of 50 Snapshots Selected from 1 ns MD Simulations: MM/PBSA Energies Were Calculated for These 20 Complexes Using 50 Snapshots Obtained from the First 1 ns MD Run (PBSA_E1) and the Second 1 ns MD Run (PBSA_E2)

system	exp	PBSA_E	PBSA_E1	PBSA_E2	GlideXP	GlideSP	Sybyl_F
2vcj	−10.41	−10.89	−7.64	−7.56	−7.26	−8.40	−10.64
2fwy	−9.09	−10.36	−7.42	−7.41	−9.06	−9.56	−9.68
2qk5	−10.98	−12.88	−9.27	−8.92	−14.20	−12.78	−13.18
2hiz	−9.42	−13.08	−9.43	−9.45	−10.72	−11.26	−13.80
2p83	−10.79	−13.61	−9.84	−9.74	−13.61	−13.93	−16.26
2hm1	−11.79	−13.83	−9.87	−9.04	−11.84	−12.16	−17.33
1o41	−4.14	−4.83	−3.57	−3.33	−4.90	−5.76	−4.11
1o42	−10.91	−11.09	−6.90	−6.88	−10.91	−9.40	−9.56
2ohm	−4.76	−5.30	−4.07	−4.24	−3.18	−4.22	−2.40
2a3c	−6.00	−7.31	−5.84	−5.91	−8.94	−9.14	−8.26
2aac	−3.10	−4.32	−2.73	−2.47	−9.90	−7.58	−8.52
2vv9	−10.54	−9.37	−7.04	−6.52	−9.27	−8.35	−8.18
2w05	−12.20	−10.14	−7.37	−7.51	−12.83	−10.97	−12.95
2w06	−9.60	−10.46	−7.73	−7.21	−12.24	−11.86	−13.68
2q89	−8.544	−5.69	−4.31	−4.10	−9.35	−9.94	−14.14
1ke8	−8.14	−9.88	−7.0	−6.85	−11.41	−11.52	−12.14
1zaf	−10.44	−7.51	−5.84	−5.80	−6.93	−9.19	−0.58
2hb1	−5.15	−6.92	−5.29	−5.29	−8.69	−8.46	−11.34
2b1z	−9.62	−8.61	−6.72	−6.71	−7.52	−9.50	−5.09
3fjg	−5.06	−4.16	−3.60	−3.70	−4.68	−7.62	−4.87
RMSE		**1.83**	**2.49**	**2.63**	**2.62**	**2.24**	**4.20**

corresponding values of 0.537, 0.669, and 0.457 for GlideSP, which is overall better than the other two methods (GlideXP and SYBYL). The correlation coefficients for GlideXP are 0.337, 0.480 and 0.285, respectively (see Figure 4).

We also compared the root-mean-square errors (RMSEs) of the predicted binding free energies obtained using the PBSA_E, GlideXP, GlideSP, and Sybyl_F methods with reference to the experimental values, as shown in Figure 5. Again, the RMSEs of PBSA_E are better than those for the other three methods. For example, the RMSEs of PBSA_E are 2.05, 1.85, 2.58 (all in kcal/mol) for the training set, T1, and T2. This can be compared to the RMSEs of 2.78, 2.05, and 3.00 kcal/mol, respectively, for GlideSP, which is the best of the three comparison methods (GlideSP, GlideXP, and Sybyl_F). Therefore, the PBSA_E method performs consistently better than the three other methods for all three data sets.

Single- versus Multiple-Configuration MM/PBSA Values. The PBSA_E method presented in this paper utilizes MM/PBSA energies calculated for a single protein−ligand configuration that is optimized from the crystal structure without the MD procedure. This leads to the question of whether utilizing MM/PBSA energies from multiple configurations generated by MD simulations would result in more accurate PBSA_E binding energies. To answer this question, we randomly selected 20 protein−ligand complexes from the test sets. We calculated the MM/PBSA energies for these 20 complexes using 50 snapshots obtained from the first 1 ns MD run and 50 more snapshots from the second 1 ns MD run (see Table 1). We then used the ensemble-averaged MM/PBSA energies in eq 4 to obtain PBSA_E scores. These PBSA_E scores are compared to the corresponding values obtained using a single optimized configuration. The results show that the correlation coefficients obtained from these two methods are very similar. However, the RMSEs of the ensemble-averaged PBSA_E energies are 2.49 and 2.63 kcal/mol, respectively, for the first and second 1 ns simulations, compared

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with 1.83 kcal/mol obtained from the single optimized configuration. Thus, there is no real advantage to using multiple configurations to calculate MM/PBSA energies to obtain PBSA_E scores, and the single optimized configuration approach is preferred. We also calculated the RMSE values from GlideXP, GlideSP, and Sybyl_F. As shown in Table 1, the RMSE of GlideSP is 2.24 kcal/mol, which is better than those for the other two scoring functions (GlideXP and Sybyl_F). However, the PBSA_E method based on a single optimized configuration outperforms those empirical scoring functions.

CONCLUSIONS

We have proposed an MM/PBSA-based free energy estimator (PBSA_E) for efficient prediction of protein−ligand binding affinity. The method involves MM/PBSA calculation of the protein−ligand binding energy using a single protein−ligand complex configuration that is optimized from the crystal structure, and no MD simulation is needed. The calculated PBSA energies were fitted to a training set to obtain optimized coefficients for use in a formula (eq 3) to give the free energy prediction (PBSA_E). The performance of this method was validated on two test sets. Explicit comparison with three popular scoring function methods (GlideSP, GlideXP, and Sybyl_F) demonstrated that the PBSA_E is superior to all three of these methods in reliability and accuracy, but it takes more computational effort. The study also showed that using a single optimized protein−ligand configuration is preferred over using multiple configurations generated from MD simulations.

It is useful to give a brief discussion of computational timing. The single-configuration MM/PBSA calculation normally takes 1−2 min for each protein system on one CPU of a standard workstation. If optimization of the complex structure is done in explicit water, it generally takes about 20 min on an eight-CPU workstation (Intel Xeon E5620 2.4 GHz processor). The long optimization time is mostly due to optimization of water molecules. However, our recent study showed that if this optimization is carried out either in the gas phase or using an implicit water model (the GB model), the optimization time could be cut to under 2 min. It should also be mentioned that newer versions of the currently used scoring functions could achieve better correlations than the ones obtained in our study.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jcim.6b00001.

Data concerning the training set, test set 1, and test set 2 and details of scoring in Sybyl, MD simulations, and protein and ligand names (PDF)

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Notes

The authors declare no competing financial interest.

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