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Prediction of the binding affinity of compounds with diverse scaffolds by MP-CAFEE

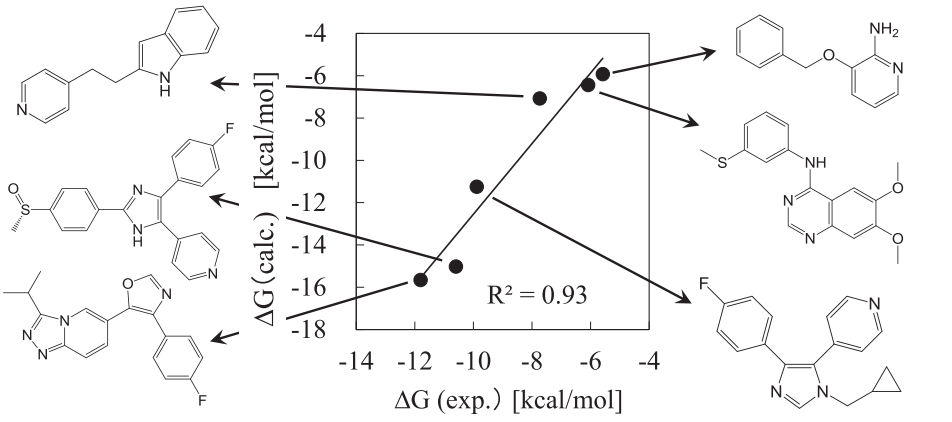
Okimasa Okada [a,*], Hiroshi Yamashita [a], Kei Takedomi [b], Satoshi Ono [b], Shinji Sunada [b], Hideo Kubodera [b]

[a] Medicinal Chemistry Research Laboratories II, Mitsubishi Tanabe Pharma Corporation, Toda, Saitama 335-8505, Japan
[b] Medicinal Chemistry Research Laboratories II, Mitsubishi Tanabe Pharma Corporation, Yokohama, Kanagawa 227-0033, Japan

HIGHLIGHTS

- We predicted binding affinities of six compounds for p38α MAP kinase by MP-CAFEE.
- The compounds have diverse scaffolds and published X-ray co-crystal structures.
- Predicted and experimental binding free energies correlate well ($R^2 = 0.93$).
- We could rank the compounds with different scaffolds using MP-CAFEE.
- We proposed the optimal sample sizes to identify or optimize lead compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

Accurate methods to predict the binding affinities of compounds for target molecules are powerful tools in structure-based drug design (SBDD). A recently developed method called massively parallel computation of absolute binding free energy with a well-equilibrated system (MP-CAFEE) successfully predicted the binding affinities of compounds with relatively similar scaffolds. We investigate the applicability of MP-CAFEE for predicting the affinity of compounds having more diverse scaffolds for the target p38α, a mitogen-activated protein kinase. The calculated and experimental binding affinities correlate well, showing that MP-CAFEE can accurately rank the compounds with diverse scaffolds. We propose a method to determine the optimal number of sampling runs with respect to a predefined level of accuracy, which is established according to the stage in the SBDD process being considered. The optimal number of sampling runs for two key stages—lead identification and lead optimization—is estimated to be five and eight or more, respectively, in our model system using Cochrans sample size formula.

1. Introduction

In structure-based drug design (SBDD), the prediction of binding affinities of compounds for target molecules is one of the most important analyses. Docking calculations have been widely employed for predicting such binding affinities using scoring functions. One of the

Abbreviation: MP-CAFEE, massively parallel computation of absolute binding free energy with a well-equilibrated system.

* Corresponding author at: 2-2-50, Kawagishi, Toda-shi, Saitama 335-8505, Japan. Tel.: +81 48 433 8031.
E-mail address: Okada.Okimasa@mc.mt-pharma.co.jp (O. Okada).

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reasons for utilizing docking software is that the calculation has recently become less time consuming, even with a personal computer. However, the binding affinities predicted by docking simulations are sometimes not accurate or sufficiently reliable to be used in SBDD. Presumably, the inaccuracies occur, because certain factors are neglected in the docking calculations, such as molecular motion, flexibility of target proteins, and enthalpic and entropic effects associated with the surrounding water molecules existing in a real system. To overcome these shortcomings, several methods have been developed. Free energy perturbation (FEP) and thermodynamics integration (TI) methods calculate the binding free energy difference ($\Delta\Delta G$) between initial and final states by changing the molecular structure [1]. However, these methods are not readily applicable to compounds with diverse scaffolds. Molecular mechanics Poisson–Boltzmann solvent accessible surface area (MM-PBSA) [2] calculates the absolute binding free energy (ΔG), but the estimated entropy by normal mode analysis has a large margin of error that introduces significant uncertainty [3].

In 2005, Fujitani et al. proposed a method called massively parallel computation of absolute binding free energy with a well-equilibrated system (MP-CAFEE) to predict the absolute binding free energy [4]. This method is based on Jarzynski's equality [5], which enables the evaluation of the free-energy difference in the equilibrium state from the exponentially weighted average of the non-equilibrium work values. MP-CAFEE utilizes several intermediate states between the initial and final states and simultaneously calculates the free energy differences between adjacent states. This protocol facilitates the massive use of multiple computers to obtain binding affinities within a reasonable time period.

MP-CAFEE was utilized to predict the binding affinities of compounds for three targets, FK506 binding protein (FKBP) [4,6], RNA aptamer [7], and poly (ADP-ribose) polymerase-1 (PARP-1) [8], and the results showed good correlation between the predicted and experimental binding affinities (coefficients of determination (squared correlation coefficients), 0.95, 0.87, and 0.91, respectively). For FKBP, the investigators obtained not only an excellent correlation but also good agreement in the absolute values. Jayachandran et al. pointed out that MP-CAFEE did not consider the standard state correction, and they proposed a modification to correct the standard state based on the binding site volume [9]. However, the coefficient of determination between the predicted and experimental values (0.52) for the target protein FKBP was not as good as that reported by Fujitani et al. (0.95). General also pointed out that MP-CAFEE did not consider the standard state correction [10], although it was successfully used for predicting the binding affinity of compounds [11]. Mitchell et al. showed that the binding affinities of compounds for the target protein FKBP predicted by MP-CAFEE correlated with the experimental values better than those predicted by MM-PBSA, quantum mechanics/molecular mechanics (QM/MM), and fragment molecular orbital methods [12].

The scaffolds of each group of compounds evaluated for two of the three targets mentioned above, FKBP and PARP-1, were similar within their respective target's group, and the binding affinities correlated with the molecular weights (coefficients of determination, 0.80 and 0.51, respectively). However, it is preferable to assemble candidate compounds with diverse scaffolds to avoid the sudden termination of drug discovery efforts due to a scaffold-based problem, such as poor physicochemical properties, toxicity, and lack of selectivity. However, the identification of several promising compounds with diverse scaffolds is not straightforward; therefore a major challenge in drug discovery. To meet this challenge, accurate methods for predicting binding affinities can be effective for selecting promising compounds with alternative scaffolds for synthesis, which is important considering that the synthetic effort required for compounds with diverse molecular frameworks is greater than that for those with similar chemical structures. Therefore, even a simple ranking of the designed compounds with diverse scaffolds using their predicted binding affinities can be very effective for discerning active compounds from inactive ones. In this study, we investigate the applicability of MP-CAFEE for predicting the affinities of structurally diverse compounds for p38α mitogen-activated protein (MAP) kinase, a model target whose inhibitors have therapeutic potential for the treatment of autoimmune diseases. Furthermore, we propose a protocol to estimate the optimal number of sampling runs, considering both the calculation time and accuracy, the level of which depends on the stage of drug discovery being considered.

2. Methods

2.1. Data preparation

We searched the protein data bank (PDB, http://www.pdb.org/pdb/) for X-ray co-crystal structures of p38α MAP kinase with compounds that were bound to the ATP binding site in the catalytically active form. We selected PDB files with fewer missing amino acid residues, especially those located at the ATP binding site, because it is preferable to utilize co-crystal structures with less ambiguity in the molecular dynamics (MD) calculations. We carefully chose six co-crystal structures of p38α with compounds having diverse scaffolds; their PDB IDs are 1A9U (compound **1**), 1BL6 (compound **2**), 1DI9 (compound **3**), 1W7H (compound **4**), 1W84 (compound **5**), and 1ZZL (compound **6**). The chemical structures of these compounds are shown in Fig. 1. Because we need to compare the predicted binding affinities of the compounds

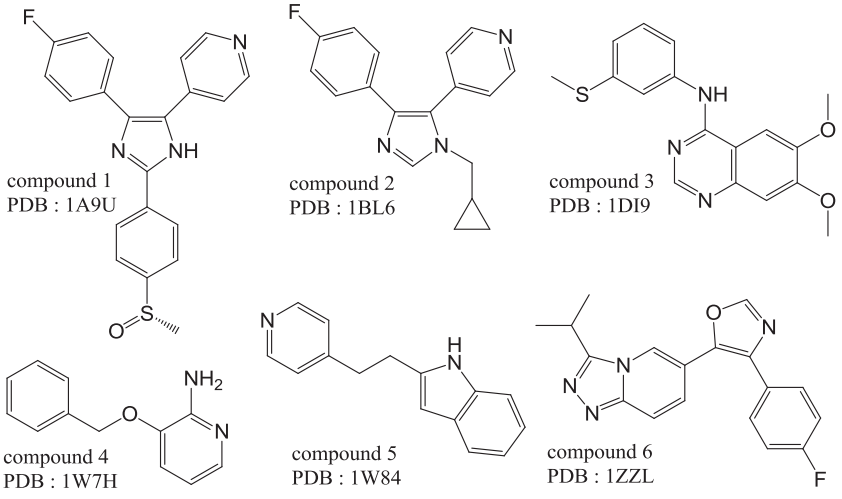

Fig. 1. Molecular structures of compounds used to predict the binding affinities.

with the experimental values, we chose compounds whose binding free energy for p38α has been reported or could be determined by converting the published half-maximal inhibitory concentration (IC_{50}) data obtained from alternative assay conditions. Compound **1** (SB203580) is an inhibitor for MAP Kinase p38α and is often used as a reference compound to compare the binding affinities. The dissociation constant (K_d) values of compounds **1** and **3** are reported in the literature [13] and ΔGs of the two compounds were calculated by the equation $\Delta G = RT \ln K_d$, where R is the gas constant and T is the temperature. The inhibition constant (K_i) value of compound **1** (18 nM) is reported in the literature [14] and is equivalent to the K_d value of compound **1** (20 nM) reported in the literature [13]. The IC_{50} values of compounds **1** and **2** [15], of compounds **1**, **4**, and **5** [16], and of compounds **1** and **6** [17,18] are used to obtain the ΔG values as follows. According to the Cheng–Prusoff equation $K_i = IC_{50} / (1 + [S] / K_m)$ [19], where [S] is the substrate concentration and K_m is the Michaelis constant of the substrate S, K_i is proportional to IC_{50} when the assay conditions are same. Then, we can obtain the K_i values for compound x by the equation, $K_i(\text{compound x}) = K_i(\text{compound } \mathbf{1}) \times (IC_{50}(\text{compound x})/IC_{50}(\text{compound } \mathbf{1}))$. We approximated K_d to equal K_i and calculated the binding free energies of compounds **2**, **4**, **5**, and **6** by the equation $\Delta G = RT \ln K_i$. The experimental binding free energies are shown in Table 1.

2.2. MD calculations

MD calculations were performed using GROMACS ver. 4.5.5 [20]. We used the force field parameter set parm96 in the assisted model building with energy refinement (AMBER) for the protein. The general AMBER force field (GAFF) [21] and TIP3P [22] parameters were used for compounds and water molecules, respectively. The partial charges on the atoms of the compounds were derived by fitting their electrostatic potential energies to those calculated at the HF/6-31G* level after structure optimization at the HF/6-31G level using Gaussian03W [23]. The GROMACS topology format files for the six compounds are available in the Supplementary information.

The particle mesh Ewald method [24] with an interpolation order of 4 was employed to evaluate electrostatic interactions with a real-space cut-off distance of 1.1 nm. A cut-off distance for van der Waals interactions was set to 0.9 nm with a switching region between 0.9 and 1.0 nm. The temperature was controlled by the Nosé–Hoover method [25,26] with a time constant of 0.5 ps, and the pressure was controlled by the Berendsen method [27] at 1 atm with a time constant of 1.0 ps using the compressibility of 4.5×10^{-5} Pa^{-1}. The LINCS algorithm [28] with an order of 8 was employed to constrain all the bonds, and we used a time step of 2 fs.

MP-CAFEE utilizes the double annihilation method, which requires two systems. One system contains a compound in a water box (system 1), and the other contains a protein–compound complex with ions for neutralization in a water box (system 2). Energy minimization was performed for system 2 to remove unfavorable atomic collisions after soaking the complex into a rectangular parallelepiped water box with three-dimensional periodic boundary conditions. The total number of atoms in systems 1 and 2 is approximately 6000 and 60,000, respectively. Initially, the system temperature was gradually increased from 100 to 250 K over 130 ps, and then it was set at 300 K for 1 ns to equilibrate the system. Because co-crystal structures of all compounds were available, we performed only 1-ns equilibration runs. Twelve equilibration MD runs of system 2 for each compound with different initial velocities of atoms were performed to obtain 12 different initial structures and velocities to use in the sampling MD runs, which were processed for 3 ns. We extended the number of sampling runs to 30 for compounds **1**, **4**, and **6** to investigate the optimal number of sampling runs. In this study, we use the term "optimal number" to represent the smallest number that will guarantee the predetermined required accuracy for the predicted binding affinity.

2.3. Binding free energy by MP-CAFEE

MP-CAFEE is based on the double annihilation method and utilizes many intermediate states between the initial solution phase and the final gas phase. The coupling constants for the intermolecular interactions between the compound and other molecules for the intermediate states are gradually decreased to transfer the compound into the gas phase. This process is referred to as annihilation. The free energy difference to transfer the compound from the solution into the gas phase (ΔG_{sol}) is calculated using system 1. The free energy difference to transfer the compound complexed with the protein from the solution into the gas phase ($\Delta G_{complex}$) is calculated using system 2. The binding free energy (ΔG_{bind}) can be obtained by the equation $\Delta G_{bind} = \Delta G_{complex} - \Delta G_{sol}$ (see Eqs. (2)–(4) in Reference [6]). The free energy differences between the adjacent states are evaluated by the instantaneous non-equilibrium work values using the Jarzynski's equality and are summed to obtain the free energy differences ΔG_{sol} and $\Delta G_{complex}$. The instantaneous non-equilibrium work value to transfer from one state to another, where the positions of the atoms are not changed but only the coupling constant is changed, is equal to the intermolecular interaction difference between the two states, because the evaluated free energy difference by the Jarzynski's equality does not depend on the time to transfer the states.

The protocol proposed by Fujitani et al. was used to evaluate the binding free energies of the compounds in this study [6]. First, the electrostatic and then the van der Waals interactions were removed using the soft-core potential. We used the same calculation conditions as those described in the literature [6] except for the initial positions and velocities of atoms for the sampling runs and the amount of time for the equilibration runs. That is, we used the positions and the velocities of the atoms at the last step of the 12 or 30 independent 1-ns equilibration runs, whereas Fujitani et al. selected one equilibrated structure from three independent equilibration runs processed for tens of nanoseconds and performed 12 sampling runs by reassigning 12 different velocity sets to the atoms. The binding free energies were evaluated

Table 1
Experimental binding free energies, predicted binding affinities determined by MP-CAFEE, scores determined by GOLD using a rescoring method, and molecular weights of the compounds. ΔG_{exp} is the experimental value. $\Delta G_{bind} = \Delta G_{complex} - \Delta G_{sol}$, where ΔG_{sol} is the free energy calculated in the solvated compound system, and $\Delta G_{complex}$ is the free energy calculated in the solvated protein–compound complex system. The coefficients of determination with respect to the experimental binding free energies are shown in the bottom row. The values in parentheses for $\Delta G_{complex}$ are the standard deviations.

	ΔGexp [kcal/mol]	ΔGsol [kcal/mol]	ΔGcomplex [kcal/mol] n = 12	ΔGcomplex [kcal/mol] n = 30	ΔGbind [kcal/mol] n = 12	GOLD score	CHEM score	Mw
Compound **1**	−10.59	−19.23	−34.26 (1.26)	−33.97 (1.48)	−15.03	56.41	28.04	377.4
Compound **2**	−9.88	−7.77	−19.02 (0.94)		−11.25	34.84	20.92	293.3
Compound **3**	−6.08	−9.23	−15.67 (1.00)		−6.44	32.01	18.73	327.4
Compound **4**	−5.58	−8.47	−14.39 (0.76)	−14.70 (0.96)	−5.93	42.63	21.59	200.2
Compound **5**	−7.73	−7.75	−14.83 (0.65)		−7.08	52.15	28.14	222.3
Compound **6**	−11.80	−11.39	−27.05 (1.41)	−27.33 (1.42)	−15.66	69.84	31.64	322.3
Determination coefficient					0.93	0.46	0.49	0.37

using the work values obtained during the last 1 ns of the total sampling run time of 3 ns by the Bennett acceptance ratio method [29] implemented in GROMACS ver. 4.5.5.

2.4. Binding affinity by genetic optimization for ligand docking (GOLD)

We predicted the binding affinities of the six compounds for the target protein p38α using the docking software GOLD version 5 [30] and compared the results with those acquired using MP-CAFEE. We used the same initial coordinates obtained from the PDB files for a comparison of GOLD with MP-CAFEE. We employed a rescoring calculation that searches for the binding position of the compound with the highest score near the initial compound position in the co-crystal structure, because this study aims to estimate the binding affinities, not to predict the binding modes.

We prepared six protein structures of the corresponding six compounds using the following procedure. First, we added the hydrogen atoms in an appropriate manner to the protein and compound. Then, only the positions of the hydrogen atoms were optimized by minimizing the potential energy without removing the compound from the binding site. The structure of the compound was optimized in vacuum by minimizing the potential energy and was placed near the original position observed in the X-ray co-crystal structure. We used a force field parameter set called MMFF94x, which is comparable to the GAFF force field [21], for energy minimization. The so-called GOLD and CHEM scores implemented in GOLD were used in the rescoring calculations to predict the binding affinities [30].

2.5. Analysis for the optimal number of sampling runs

The optimal number of sampling runs probably depends on the target and the required accuracy, which is stipulated by the stage of drug discovery being considered. We performed an analysis for the optimal number of sampling runs using Cochran's sample size formula [31]. The required sample size n is expressed as $n = (Zs/d)^2$, where Z is the ratio that determines the confidence level, d is the permissible error, and s is the standard deviation. This formula can be applied to data with a normal distribution. Because the standard deviation of the free energy calculated for the system with a compound in a water box is small, we anticipated that its influence on the standard deviation of the total binding free energy is negligible.

3. Results

3.1. Equilibration of the structures of the complexes in water

Fig. 2A shows the time evolution of the root-mean-square deviations (RMSDs) of the protein backbone calculated by comparing the trajectories of the backbone atoms with the corresponding atoms in the X-ray co-crystal structures. Fig. 2B shows the RMSDs of the compounds calculated by comparing the trajectories, which are obtained by superposition using only the protein backbone atoms, to the corresponding atoms in the X-ray co-crystal structures. Only the first of the 12 runs for each compound is used for plotting the RMSDs. All of the time-

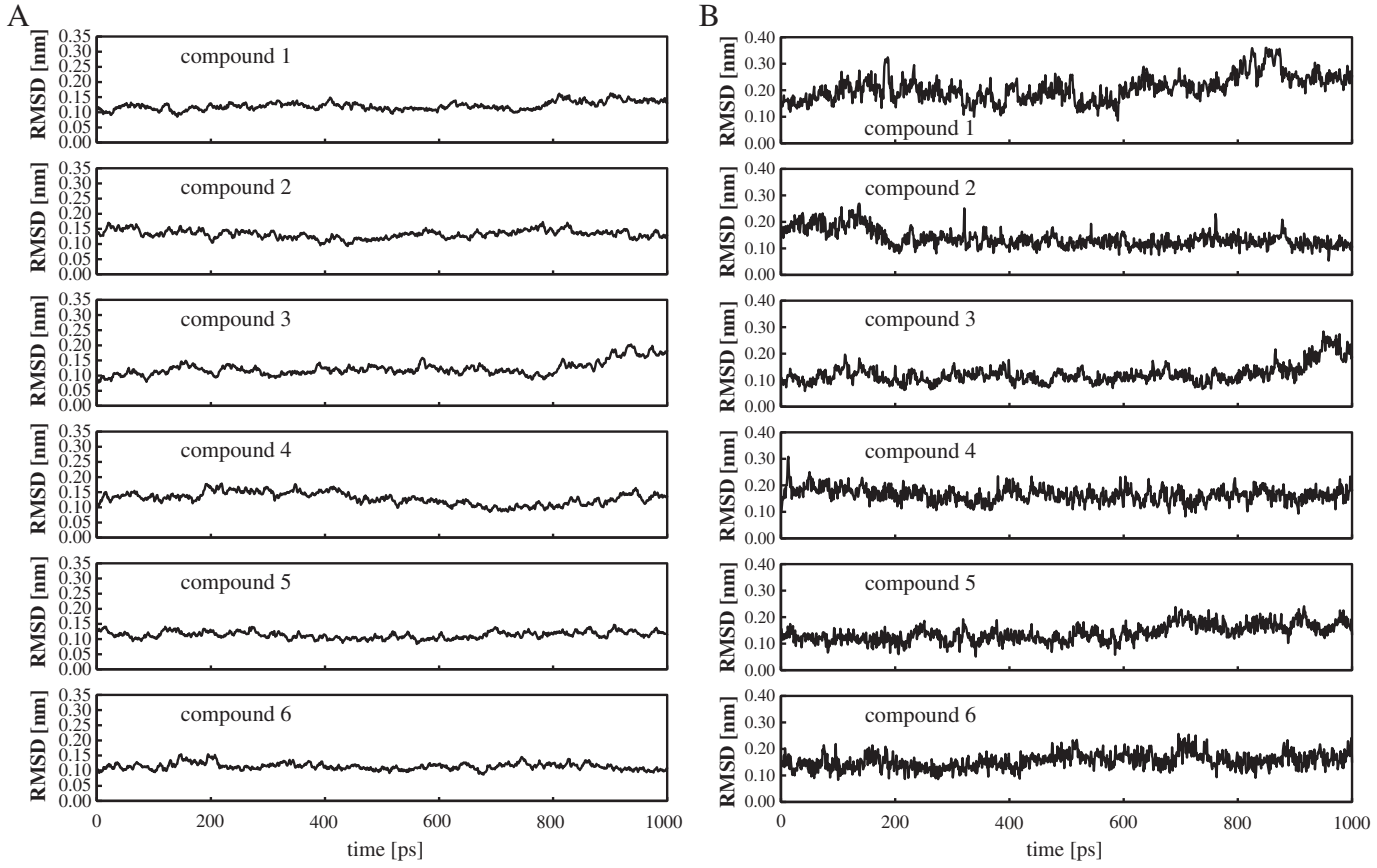

Fig. 2. (A) Time evolution of the RMSDs of backbone atoms of the protein–compound complexes in the binding sites. Only the first of 12 runs for the six compounds is represented. (B) Time evolution of the RMSDs of the compounds calculated by comparing the trajectories, which are obtained by superposition using only the protein backbone atoms, to the corresponding atoms in the X-ray co-crystal structures.

averaged RMSDs of the backbone atoms for the time period between 500 and 1000 ps in the equilibration runs for the six compounds are between 0.097 and 0.20 nm, showing that the tertiary structures are stable. The correspondingly determined time-averaged RMSDs of the compounds are between 0.11 and 0.39 nm, implying that the compounds are located at the binding site. TYR35, located at the tip of the so-called glycine-rich loop, interacts with compounds **1**, **2**, **4**, and **6** in the co-crystal structures largely by changing the conformation of the loop. We directly checked the structures with graphic tools and verified that these four compounds continue to interact with TYR35 (as is the case in the X-ray co-crystal structures) after completing the equilibration runs. Having confirmed that the equilibrated structures of the complexes were reasonable, we utilized these structures and the velocities at the final steps of the equilibration runs for the sampling runs.

3.2. Predicted binding affinities determined by MP-CAFEE and GOLD

The experimental binding free energies and binding affinities predicted by MP-CAFEE and GOLD as well as the molecular weights are listed in Table 1. The coefficients of determination with respect to the experimental binding free energies are also listed. The coefficient of determination between the experimental binding free energies and molecular weights is 0.37, indicating that the correlation is low. Figs. 3A and 3B show the predicted binding affinities of the six compounds as determined by MP-CAFEE and GOLD, respectively, against the experimental values. The coefficient of determination between the binding affinities predicted by MP-CAFEE and the experimental binding free energies is 0.93. In contrast, the corresponding coefficients of determination using GOLD and CHEM scores are 0.46 and 0.49, respectively.

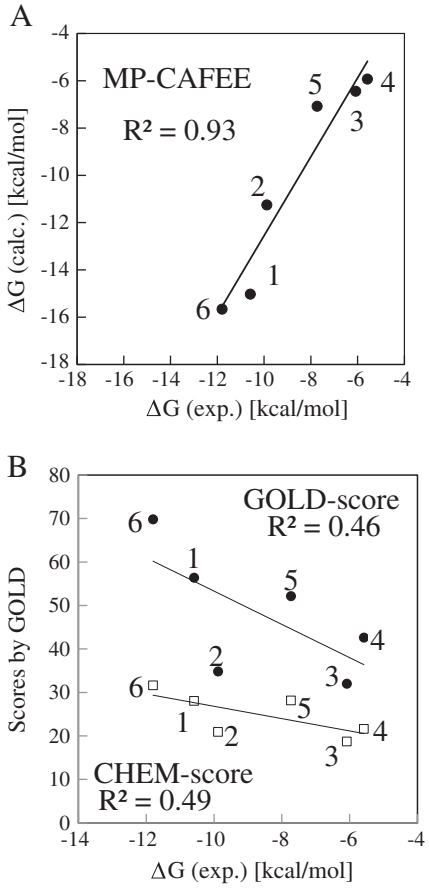

Fig. 3. Predicted binding affinities of the six compounds determined by (A) MP-CAFEE and (B) GOLD (GOLD score: filled circle, CHEM score: open square) as a function of the experimental binding free energies.

3.3. Optimal number of sampling runs for SBDD

We assigned the parameters *d* and *Z* in Cochran's sample size formula as follows. A difference in the binding free energies of 1.38 kcal/mol between two compounds indicates that the binding affinity of one compound is ten times higher than that of the other at room temperature. We examined three grades of accuracy, designated by the permissible errors of ±0.69 (high accuracy), ±1.04 (medium accuracy), and ± 1.38 (low accuracy) kcal/mol, to represent the different stages of drug discovery. To ensure a confidence level of 95%, the *Z* value of Cochran's sample size formula was set to 1.96. The optimal number of sampling runs could then be estimated using the standard deviations along with the parameters *d* and *Z*. The largest standard deviation obtained in this study is 1.48, which is similar to the value of 1.2 reported by Fujitani et al. [6]. The required number of sampling runs for each of the three grades assigned in this study is 18, 8, and 5, for high, medium, and low accuracy, respectively (Table 2).

The binding free energy as a function of the number of sampling runs for each of the six compounds is plotted in Fig. 4A. The order of the sampling runs is meaningless, because they are independent from one another caused by the random assignment of initial velocities to the atoms. The ranges of the binding free energies are overlapped for the pairs of compounds (**1**, **2**), (**1**, **6**), (**3**, **4**), (**3**, **5**), and (**4**, **5**). However, the ranking of the six compounds by the averages is consistent with the experimental binding free energies incidentally at any number of sampling runs. The standard deviation as a function of the number of sampling runs for each of the six compounds is plotted in Fig. 4B to visualize the convergence as the number of runs changes. As the number of sampling runs increases, the standard deviation reaches a point of relative constancy for compounds **1** and **6** (~1.5) and compound **4** (~0.96). Fig. 4C shows histograms of the calculated binding free energies using 30 sampling runs for compounds **1**, **4**, and **6**. These histograms imply that the calculated binding free energies have normal distributions for these three compounds.

4. Discussion

Fujitani et al. developed the MP-CAFEE method and showed that it could successfully predict binding affinities using several targets. Because predicting affinities for compounds with diverse scaffolds is extremely useful in SBDD, in this study, we have investigated its applicability for predicting the binding affinity of six compounds with diverse scaffolds for the target protein p38α.

Before performing sampling runs, we equilibrated two systems: one that contained a compound in a water box, and the other, a complex in a water box. Because the initial structures of the complexes in the water box are important for the sampling runs, we carefully checked the equilibration of the latter system. We verified the stability of the structures of the proteins complexed with each of the six compounds and checked the binding of the compounds to the binding site using the RMSDs. We also directly confirmed that the compounds were located at the binding site in an appropriate manner and the interactions with TYR35 were adequately maintained for compounds **1**, **2**, **4**, and **6**. In SBDD, when new compounds are designed, their binding modes are initially predicted, and then they are further verified by longer equilibration runs. Because all the co-crystal structures of the compounds with

Table 2
Number of sampling runs estimated by standard deviations, permissible errors, and a confidence level.

Permissible error [kcal/mol]	Standard deviation range	Confidence level	Number of required runs
0.69	0.65–1.48	95%(Z = 1.96)	4–18
1.04	0.65–1.48	95%(Z = 1.96)	2–8
1.38	0.65–1.48	95%(Z = 1.96)	1–5

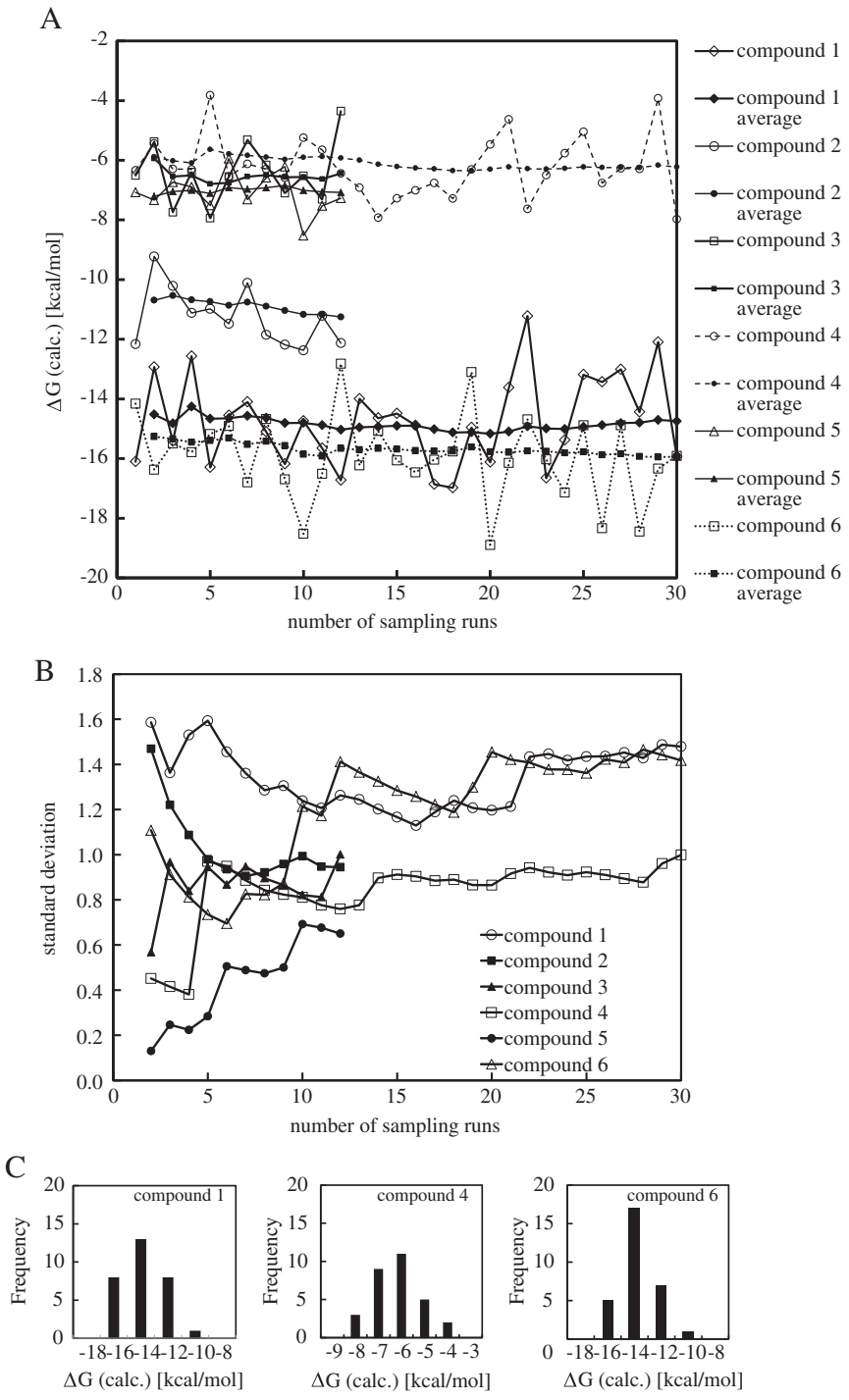

Fig. 4. (A) Calculated binding free energies of six compounds and their averages as a function of the number of sampling runs. (B) Standard deviations for the predicted binding free energies as a function of the number of sampling runs. (C) Histograms of the estimated binding free energies for compounds **1**, **4**, and **6**.

the proteins were available, and our purpose was not to predict the binding modes but to estimate the binding affinities, we chose the equilibration run time of 1 ns, which is shorter than that of tens of nanoseconds employed by Fujitani et al. [4].

After we confirmed that the equilibrated structures of the six compounds were reasonable, we performed sampling runs with MP-CAFEE using the coordinates and velocities of the atoms at the last step of the equilibration runs. Fujitani et al. selected the single most reasonable complex structure from three equilibrated structures obtained by long MD runs. Then, they reassigned 12 different velocities to the coordinates for the 12 sampling runs. Jayachandran et al. commented that careful attention must be paid to choosing the best complex structure as the initial coordinates for sampling runs [9]. We anticipated that a protocol employing both coordinates and velocities obtained by independent equilibration runs would be preferable for two reasons. One reason is that a wider phase space can be sampled using different coordinates. The other is that the dynamics of the molecules can be allowed to smoothly continue without disruption by the reassignment of the velocities. As both protocols seem to have merits and demerits, we cannot judge which is preferable and suggest further rigorous investigation.

The experimental and predicted binding affinities determined by the MP-CAFEE method correlate well, as shown by a high coefficient of

determination of 0.93. Although the predicted values slightly deviate from the absolute binding free energies, ranking the compounds according to the predicted binding affinities is valid, and therefore useful in SBDD. We also evaluated the binding affinities using the docking software GOLD by rescoring the binding modes. The obtained coefficients of determination of 0.46 (GOLD score) and 0.49 (CHEM score) are not as good as that achieved by MP-CAFEE, and the order of the scores is inconsistent for some compounds. These results show that predicting binding affinities with MP-CAFEE is better than with GOLD in this model case.

We investigate the reasons why the absolute values of the predicted binding affinities deviated from the experimental values. Fujitani et al. showed that the force field parameters with partial charges on the atoms influence the prediction [6]. They have reported that the torsional energy maps with respect to the backbone dihedral angles calculated with several force field parameter sets are different from one another [32]. Thus, we suspected that the deviation was caused by the force field parameters, and consequently, we evaluated the torsional energy profiles calculated with parm96 to compare them with other reported torsional energy profiles. The details of the method to evaluate the torsional energy profiles and the obtained results are shown in the Supplementary information.

Fujitani et al. evaluated the torsional energy profiles with high-level ab initio molecular orbital calculations, and they fit the parameters to develop the so-called FUJI force field for backbone dihedral angles. Surprisingly, the torsional energy profiles calculated with parm96 agree well with those evaluated by the high-level ab initio molecular orbital calculations (Fig. S1). Only the barrier height at zero degrees in the torsional energy profile of psi for the glycine dipeptide (GD) model compound significantly deviates from the results of the high-level ab initio molecular orbital calculations. We expect that this deviation does not significantly influence the predicted binding affinities, because the dihedral angles at the four minima are in good agreement with those determined by the ab initio molecular orbital calculations and the barrier height of 2 kcal/mol can hardly be overcome by thermal motion for the folded protein structures. Unfortunately, the reason for the deviation of the absolute values from the experimental ones could not be attributed to the force field parameters. Further rigorous investigations are needed to check the influence of the force field parameters on the absolute binding free energy calculation.

The ranges of the calculated binding free energies are overlapped for some pairs of compounds (Fig. 4A). This implies that the ranking by the averages of the calculated binding free energies with a few sampling runs can be different from that with many sampling runs owing to the random assignment of initial velocities to atoms. Therefore, we performed a statistical analysis to identify the optimal number of sampling runs by recognizing that the number depends on the required accuracy stipulated by the stage of drug discovery being considered. Fujitani et al. performed 12 independent sampling runs for each compound and obtained accurate absolute binding free energies [6]. At the lead optimization stage, the accurate prediction of the binding affinities, as achieved by Fujitani et al., is obviously crucial to the selection of compounds for synthesis from a group of designed compounds. However, at the lead identification stage, the number of sampling runs can probably be reduced, allowing for a short-term estimation of the binding affinities that still guarantees the appropriate level of accuracy.

The application of this protocol in an actual drug discovery setting requires prior validation. To check whether the MP-CAFEE method can accurately predict binding affinities for a particular target, one should evaluate several compounds whose binding affinities have been experimentally measured. It is preferable to select compounds having a wide range of affinities. There is a tendency for compounds with high binding affinities to show large standard deviations, as seen in our results (compounds **1** and **6**, Table 1, and Fig. 4B) as well as those reported by Fujitani et al. [6]. This suggests that the required number of sampling runs should be determined using the compound with the highest binding free energy, since the number of runs is proportional to the square of the standard deviation. Subsequently, the same number of sampling runs estimated with the largest standard deviation can be applied to the other compounds, even though they require fewer runs because of their lower standard deviations.

The distribution of the calculated binding affinities appears to be normal for compounds **1**, **4**, and **6** (Fig. 4C). Thus, we expect that other compounds also have normal distributions and that Cochran's sampling size formula can be applied to the analyses. The largest standard deviation of the binding affinities of the six compounds is 1.48 (compound **1**). We employed three accuracy grades that varied by the permissible error. The required number of sampling runs to obtain binding affinity differences of 10- ($\pm$1.38 kcal/mol), 32- ($\pm$1.04 kcal/mol), and 100-fold ($\pm$0.69 kcal/mol) can be estimated as 5, 8, and 18, respectively. For the lead identification stage, the appropriate number of sampling runs is determined to be 5 in this model study. On the other hand, 8 or 18 runs are needed for lead optimization to provide binding affinity predictions with a medium or high level of accuracy, respectively.

5. Conclusions

We investigated the applicability of MP-CAFEE to predict binding affinities using six compounds with diverse scaffolds. The predicted binding affinities correlated well with the experimental binding free energies with slight deviations in the absolute values. This result implies that MP-CAFEE can be effectively employed in structure-based drug design for ranking compounds with diverse scaffolds according to the predicted binding affinities. Furthermore, we proposed a method to determine the optimal number of sampling runs. The optimal number of sampling runs for the lead identification stage was estimated as five in our model system using Cochran's sample size formula. On the other hand, the optimal number of sampling runs was estimated as eight or more for the lead optimization stage. The number of sampling runs should be carefully determined by considering the level of accuracy required for the specific drug discovery activities.

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References

[1] P.A. Kollman, Free energy calculations: applications to chemical and biochemical phenomena, Chemical Reviews 93 (1993) 2395–2417.
[2] J. Srinivasan, T.E. Cheatham, P.A. Kollman, D.A. Case, Continuum solvent studies of the stability of DNA, RNA and phosphoramidate–DNA helices, Journal of the American Chemical Society 120 (1998) 9401–9409.
[3] http://ambermd.org/tutorials/advanced/tutorial3/April 25th, 2013.
[4] H. Fujitani, Y. Tanida, M. Ito, G. Jayachandran, C.D. Snow, M.R. Shirts, E.J. Sorin, V.S. Pande, Direct calculation of the binding free energies of FKBP ligands, Journal of Chemical Physics 123 (2005) 084108.
[5] C. Jarzynski, Nonequilibrium equality for free energy differences, Physical Review Letters 78 (1997) 2690–2693.
[6] H. Fujitani, Y. Tanida, A. Matsuura, Massively parallel computation of absolute binding free energy with well-equilibrated states, Physical Review E 79 (2009) 021914.
[7] Y. Tanida, M. Ito, H. Fujitani, Calculation of absolute free energy of binding for theophylline and its analogs to RNA aptamer using nonequilibrium work values, Chemical Physics 337 (2007) 135–143.
[8] K. Ohno, T. Mitsui, Y. Tanida, A. Matsuura, H. Fujitani, T. Niimi, M. Orita, Docking study and binding free energy calculation of poly (ADP-ribose) polymerase inhibitors, Journal of Molecular Modeling 17 (2011) 383–389.
[9] G. Jayachandran, M.R. Shirts, S. Park, V.S. Pande, Parallelized-over-parts computation of absolute binding free energy with docking and molecular dynamics, Journal of Chemical Physics 125 (2006) 084901.
[10] I.J. General, A note on the standard state's binding free energy, Journal of Chemical Theory and Computation 6 (2010) 2520–2524.

[11] I.J. General, R. Dragomirova, H. Meirovitch, Calculation of the absolute free energy of binding and related entropies with the HSMD-TI method: the FKBP12-L8 complex, Journal of Chemical Theory and Computation 7 (2011) 4196–4207.
[12] W. Mitchell, S. Matsumoto, Large-scale integrated super-computing platform for next generation virtual drug discovery, Current Opinion in Chemical Biology 15 (2011) 553–559.
[13] J.E. Sullivan, G.A. Holdgate, D. Campbell, D. Timms, S. Gerhardt, J. Breed, A.L. Breeze, A. Bermingham, R.A. Pauptit, R.A. Norman, K.J. Embrey, J. Read, W.S. VanScyoc, W.H.J. Ward, Prevention of MKK6-dependent activation by binding to p38R MAP kinase, Biochemistry 44 (2005) 16475–16490.
[14] R.M. Angell, P. Bamborough, A. Cleasby, S.G. Cockerill, K.L. Jones, C.J. Mooney, D.O. Somers, A.L. Walker, Biphenyl amide p38 kinase inhibitors 1: discovery and binding mode, Bioorganic & Medicinal Chemistry Letters 18 (2008) 318–323.
[15] Z. Wang, B.J. Canagarajah, J.C. Boehm, S. Kassisà, M.H. Cobb, P.R. Young, S. Abdel-Meguid, J.L. Adams, E.J. Goldsmith, Structural basis of inhibitor selectivity in MAP kinases, Structure 6 (1998) 1117–1128.
[16] A.L. Gill, M. Frederickson, A. Cleasby, S.J. Woodhead, M.G. Carr, A.J. Woodhead, M.T. Walker, M.S. Congreve, L.A. Devine, D. Tisi, M. O'Reilly, L.C.A. Seavers, D.J. Davis, J. Curry, R. Anthony, A. Padova, C.W. Murray, R.A.E. Carr, H. Jhoti, Identification of novel p38r MAP kinase inhibitors using fragment-based lead generation, Journal of Medicinal Chemistry 48 (2005) 414–426.
[17] K.F. McClure, Y.A. Abramov, E.R. Laird, J.T. Barberia, W. Cai, T.J. Carty, S.R. Cortina, D.E. Danley, A.J. Dipesa, K.M. Donahue, M.A. Dombroski, N.C. Elliott, C.A. Gabel, S. Han, T.R. Hynes, P.K. LeMotte, M.N. Mansour, E.S. Marr, M.A. Letavic, J. Pandit, D.B. Ripin, F.J. Sweeney, D. Tan, Y. Tao, Theoretical and experimental design of atypical kinase inhibitors: application to p38 MAP kinase, Journal of Medicinal Chemistry 48 (2005) 5728–5737.
[18] M.A. Dombroski, M.A. Letavic, K.F. McClure, J.T. Barberia, T.J. Carty, S.R. Cortina, C. Csiki, A.J. Dipesa, N.C. Elliott, C.A. Gabel, C.K. Jordan, J.M. Labasi, W.H. Martin, K.M. Peese, I.A. Stock, L. Svensson, F.J. Sweeney, C.H. Yu, Benzimidazolone p38 inhibitors, Bioorganic & Medicinal Chemistry Letters 14 (2004) 919–923.
[19] Y. Cheng, W.H. Prusoff, Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction, Biochemical Pharmacology 22 (1973) 3099–3108.
[20] B. Hess, C. Kutzner, D. van der Spoel, E. Lindahl, GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation, Journal of Chemical Theory and Computation 4 (2008) 435–447.
[21] J. Wang, R.M. Wolf, J.W. Caldwell, P.A. Kollman, D.A. Case, Development and testing of a general amber force field, Journal of Computational Chemistry 25 (2004) 1157–1174.
[22] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, R.W. Impey, M.L. Klein, Comparison of simple potential functions for simulating liquid water, Journal of Chemical Physics 79 (1983) 926–935.
[23] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, 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, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian 03, Revision D.01, Gaussian, Inc., Wallingford CT, 2004.
[24] T. Darden, D. York, L. Pedersen, Particle mesh Ewald—an Nlog(N) method for Ewald sums in large systems, Journal of Chemical Physics 98 (1993) 10089–10092.
[25] S. Nosé, A unified formulation of the constant temperature molecular dynamics methods, Journal of Chemical Physics 81 (1984) 511–519.
[26] W.G. Hoover, Canonical dynamics: equilibrium phase-space distributions, Physical Review A 31 (1985) 1695–1697.
[27] H.J.C. Berendsen, J.P. Postma, A. DiNola, J.R. Haak, Molecular dynamics with coupling to an external bath, Journal of Chemical Physics 81 (1984) 3684.
[28] B. Hess, H. Bekker, H.J.C. Berendsen, J.G.E.M. Fraaije, LINCS: a linear constraint solver for molecular simulations, Journal of Computational Chemistry 18 (1997) 1463–1472.
[29] C.H. Bennett, Efficient estimation of free energy differences from Monte Carlo data, Journal of Computational Physics 22 (1976) 245–268.
[30] G. Jones, P. Willet, R.C. Glen, Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation, Journal of Molecular Biology 245 (1995) 43–53.
[31] W.G. Cochran, Sampling Techniques, 3rd ed. John Wiley & Sons, New York, 1977.
[32] H. Fujitani, A. Matsuura, S. Sakai, H. Sato, Y. Tanida, High-level ab initio calculations to improve protein backbone dihedral parameters, Journal of Chemical Theory and Computation 5 (2009) 1155–1165.