J Comput Aided Mol Des (2013) 27:479–490
DOI 10.1007/s10822-013-9657-3

Estimation of relative binding free energy based on a free energy variational principle for the FKBP-ligand system

Takeshi Ashida · Takeshi Kikuchi

Received: 30 August 2012 / Accepted: 2 June 2013 / Published online: 11 June 2013

Abstract Predicting an accurate binding free energy between a target protein and a ligand can be one of the most important steps in a drug discovery process. Often, many molecules must be screened to find probable high potency ones. Thus, a computational technique with low cost is highly desirable for the estimation of binding free energies of many molecules. Several techniques have thus far been developed for estimating binding free energies. Some techniques provide accurate predictions of binding free energies but high large computational cost. Other methods give good predictions but require tuning of some parameters to predict them with high accuracy. In this study, we propose a method to predict relative binding free energies with accuracy comparable to the results of prior methods but with lower computational cost and with no parameter needing to be carefully tuned. Our technique is based on the free energy variational principle. FK506 binding protein (FKBP) with 18 ligands is taken as a test system. Our results are compared to those from other widely used techniques. Our method provides a correlation coefficient (r^2) of 0.80 between experimental and calculated relative binding free energies and yields an average absolute error of 0.70 kcal/mol compared to experimental values. These results are comparable to or better than results from other techniques. We also discuss the possibility to improve our method further.

Electronic supplementary material The online version of this article (doi:10.1007/s10822-013-9657-3) contains supplementary material, which is available to authorized users.

T. Ashida · T. Kikuchi (✉)
Department of Bioinformatics, College of Life Sciences, Ritsumeikan University, 1-1-1 Nojihigashi, Kusatsu 525-8577, Shiga, Japan
e-mail: tkikuchi@sk.ritsumei.ac.jp

Keywords Binding free energy · Drug design · Free energy variational principle · FKBP · Molecular dynamics simulations

Introduction

Predicting an accurate binding free energy for a target protein with its ligand can help guide the drug discovery process. Several techniques have been developed so far. The free energy perturbation (FEP) method [1] is widely used. For example, Jayachandran et al. [2] applied this technique to FKBP-inhibitor systems and succeeded in predicting binding free energies with an average error of 1.3 kcal/mol from the experimental values, i.e., FEP is fairly good. However, relatively long (200 ns) molecular dynamics (MD) simulations were needed to obtain satisfactory results. Slow computational turnaround can be a severe impediment to its usefulness in a drug discovery environment. Fujiani et al. [3] also used the same method to predict binding free energy for the FKBP-inhibitor system. They made MD simulations for only 1 ns and obtained an rms error from a linear fit of only 0.4 kcal/mol, i.e., very accurate, but their method necessitates rescaling the computed values. Furthermore, the FEP method is not suitable for screening many compounds because it needs to sample intermediate states and hence high computational costs are usually required. To solve this problem, a one-step perturbation method was developed [4]. This method has been applied to calculate solvation free energy of a small molecule [5], binding free energy [6–8] and free energies of DNA [9], but the average error between experimental and calculated values was large.

The MM-GB/SA (Molecular Mechanics–Generalized Born/Surface Area) and MM-PB/SA (Molecular Mechanics–Poisson Boltzmann/Surface Area) methods have been applied to various problems including calculations of binding free

energies with high accuracy [10–23]. In such methods, free energy is calculated by summing up molecular mechanical and solvation energies, and vibrational entropies [24]. Unlike the FEP method, these methods do not need to sample intermediate states or run huge numbers, of MD simulations on a protein, a ligand, and a protein–ligand complex in explicit solvent. Rather, only an MD simulation of a protein–ligand complex in explicit solvent is required. Xu and Wang [12] calculated binding free energies for the FKBP-inhibitor system using the MM-PB/SA method and got a correlation coefficient of 0.93 between experimental and calculated values and a standard deviation of only 0.3 kcal/mol. These methods have been applied to various systems with good results, but calculating entropy values incurs high computational cost [25], and two parameters should be adjusted carefully to calculate solvation free energies well [12].

The linear interaction energy (LIE) method is also often applied to calculate a binding free energy of a ligand to a protein [25–28]. In this method, binding free energy is calculated by considering the changes in the interactions between the ligand and its environment [29, 30]. The LIE method has been applied to various systems with good results. For example, Lamb et al. [31] obtained the binding free energies for the FKBP-inhibitor system by applying the LIE technique with an average absolute error of 0.5 kcal/mol. However, as Wallnoefer et al. [25] pointed out, the quality of results from the LIE method depends on a training set to determine parameters, α, β and γ.

In this paper, we propose a new method to calculate relative binding free energies requiring relatively low computational loads and no parameters to be carefully tuned. The basis of our technique is the free energy variational principle (FEVP) [32]. The FEVP method does not need to make sample intermediate states as in the FEP method or to invoke empirical parameterizations [33]. In our previous study [33], binding free energies for the dihydrofolate reductase—inhibitor system were calculated with our technique. We obtained a high correlation coefficient between experimental and calculated values, but the average absolute error was usually high. The purpose of the present study is to improve our method and produce better results than in our previous study [33].

Method

Free energy variational principle

The FEVP is expressed by the following equation [32].

$$<H_1 - H_0>_1 \leq G_1 - G_0 \leq <H_1 - H_0>_0 \quad (1)$$

In above formula, G_0, H_0, G_1, and H_1 denote free energies and Hamiltonians of non-perturbed and perturbed systems, respectively. $<>_0$ and $<>_1$ signify the ensemble averages taken with the potential surfaces of non-perturbed and perturbed systems, respectively. Thus, according to the Eq. (1), the upper and lower limits of the exact value of the free energy difference can be defined.

Suppose that a process involving ligand L_0 is the non-perturbed process, and a process involving ligand L_1 is the perturbed process. Then the processes of binding a non-perturbed and perturbed ligands to a protein can be expressed by the following equation.

$$P + L_0 \rightarrow PL_0 \quad (2)$$

$$P + L_1 \rightarrow PL_1 \quad (3)$$

P, L_0, PL_0, L_1 and PL_1 denote a protein, ligands and complexes of non-perturbed and perturbed systems, respectively. Binding free energies for non-perturbed and perturbed systems are;

$$\Delta G_{bind\_0} = G_{com\_0} - G_{pro} - G_{lig\_0} \quad (4)$$

$$\Delta G_{bind\_1} = G_{com\_1} - G_{pro} - G_{lig\_1} \quad (5)$$

where ΔG_{bind_1}, G_{com_1}, G_{lig_1}, ΔG_{bind_0}, G_{com_0} and G_{lig_0} indicate a binding free energy, free energy of a complex and a ligand of perturbed and non-perturbed systems. G_{pro} is the free energy of a protein. The relative binding free energy is denoted as

$$\begin{aligned}\Delta\Delta G_{bind} &= \Delta G_{bind\_1} - \Delta G_{bind\_0} \\ &= G_{com\_1} - G_{com\_0} - (G_{lig\_1} - G_{lig\_0}).\end{aligned} \quad (6)$$

The upper and lower limits of the relative binding free energy can be defined according to the FEVP as follows.

$$\begin{aligned}&<H_{com\_1} - H_{com\_0}>_{com\_1} - \\ &<H_{lig\_1} - H_{lig\_0}>_{lig\_1} \leq \Delta\Delta G_{bind} \leq \\ &<H_{com\_1} - H_{com\_0}>_{com\_0} - \\ &<H_{lig\_1} - H_{lig\_0}>_{lig\_0}.\end{aligned} \quad (7)$$

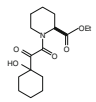
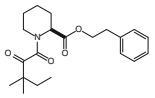
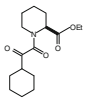
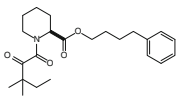
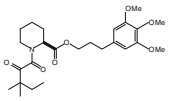
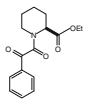
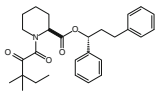
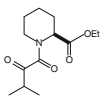
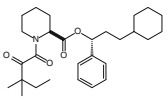
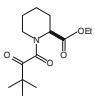
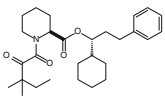
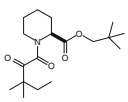
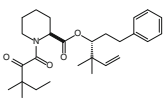
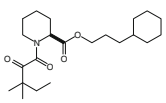
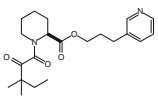
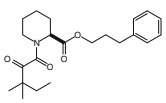
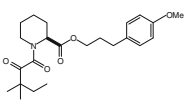
Hence, it is guaranteed in principle by the FEVP that the exact relative binding free energy lies between these limits. However, it is still difficult to pinpoint the exact value between these limits. In this study, we use the first term of the Zwanzig equation [4], Eq. (8), i.e., the average of upper and lower limits, to approximate the exact value.

$$\begin{aligned}\Delta\Delta G_{bind} = [&<H_{com\_1} - H_{com\_0}>_{com\_1} - \\ &<H_{lig\_1} - H_{lig\_0}>_{lig\_1} + \\ &<H_{com\_1} - H_{com\_0}>_{com\_0} - \\ &<H_{lig\_1} - H_{lig\_0}>_{lig\_0}].\end{aligned} \quad (8)$$

Modeling FKBP-Inhibitor System

The 18 ligands used as our test set are shown in Table 1. Their inhibitory constants measured by a binding assay were used [34, 35]. The 3D structures of all ligands were

Table 1 Structures and inhibitory constants of the 18 ligands in this work

No.	Structure	K_i (nM)	No.	Structure	K_i (nM)
L1		1600	L10		340
L2		2000	L11		100
L3		660	L12		12
L4		8000	L13		10
L5		6000	L14		10
L6		2000	L15		7
L7		410	L16		250
L8		186	L17		165
L9		110	L18		70

modeled from the structure of the ligand in the complex structure coded by 1FKG [35] (ligand13 in our study) in PDB. The pipecolyl moiety shown in Fig. 1 is included in all ligands and recognized as important for binding the protein in 1FKG [35] (Fig. 4).

Ligand 13 in 1FKG is regarded as the non-perturbed system and the other 17 ligands are the perturbed systems. It is desirable to take the largest ligand in size as a non-perturbed system because the uncertainty of the modeling structure of a ligand as a perturbed system becomes minimum. Ligand 13 is one of the largest ligands among them. Molecular modeling was performed with the molecular modeling software, Discovery Studio ver. 2.1 from Accelrys Inc. To model a ligand, we use the atomic coordinates of the common pipecolyl moiety in ligand 13 and other parts were modeled manually with Discovery Studio. The molecular parameters of ligands were derived from the program antechamber [36]. Atomic charges in ligands were assigned by the AM1-BCC method [37, 38]. The ff99SB force field [39] was applied to the protein.

MD simulation protocol

All simulations were performed using the AMBER11 molecular dynamics package [40]. The complex and the ligand were soaked in a truncated octahedron of TIP3P [41] water molecules with a margin of 10 Å along each dimension. Cl^- ions were placed as counterions in the complex.

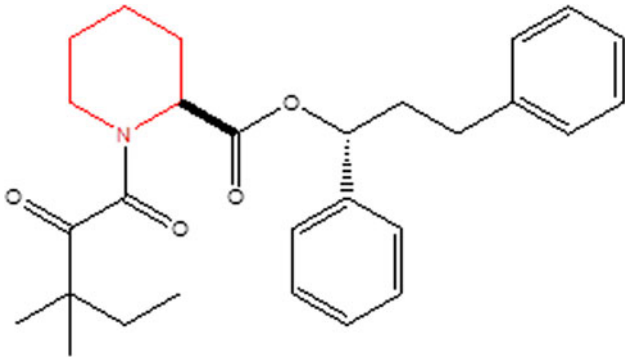


Fig. 1 The structure of ligand included in 1FKG. The *red* color shows pipecolyl moiety which is important to bind the protein

The whole system was energy minimized with QM/MM [42–44] with 2,000 steps of the steepest decent method followed by 1,000 steps of the conjugate gradient method. This procedure was iterated by four times. QM was used in the energy minimization procedure and applied only to a ligand. In each of iteration, a harmonic restraints was applied to each atom in the protein. The following values of the force constant in each iteration were used: 500, 50, 5 and 0 kcal/(mol•Å^2) in the first, second, third and fourth iteration, respectively. The AM1 semiempirical Hamiltonian was used in the molecular orbital calculations.

Then molecular dynamics simulations (MD) were performed. First, the systems were heated form 0 to 300 K for 20 ps and equilibrated at a constant temperature of 300 K and pressure of 1 atm for 20 ps. Harmonic restraints were applied to atoms in the protein during heating and equilibration (force constants used: 20 kcal/(mol•Å^2)). Finally, the systems were subjected to MD simulations at constant temperature of 300 K and pressure of 1 atm for 5.2 ns. The time interval was set to 2 fs with the SHAKE method [45] to constrain all of the covalent bonds involving in hydrogen atoms. The particle mesh Ewald (PME) method [46] was applied to calculate long–range electrostatics interactions. The nonbonded cutoff was set at 8 Å. During this process, harmonic restraints were not applied. The simulation of the ligand was performed with the same protocol, except that the system was energy minimized with QM/MM using 1,000 steps of steepest descent method and 1,000 steps of conjugate gradient method. For all processes, harmonic restraints were not applied.

Calculation of the FEVP

To calculate relative binding free energy, we must calculate values of $< H_{com\_1}–H_{com\_0} >_{com\_0}$, $< H_{com\_1}–H_{com\_0} >_{com\_1}$, $< H_{lig\_1}–H_{lig\_0} >_{lig\_0}$, and $< H_{lig\_1}–H_{lig\_0} >_{lig\_1}$ i.e., the ensemble average of a difference between Hamiltonians of the perturbed and non-perturbed systems. Ensemble averages are calculated with the potential surface of the non-perturbed and perturbed cases when ligands 0 and 1 are the non-perturbed and a perturbed cases, respectively.

The average energies of the ensembles, $< H_{com\_1}–H_{com\_0} >_{com\_0}$, were calculated as follows. First, 1,000 snapshots were taken from the final 5 ns of the MD trajectories of a complex at an interval of 5 ps. (To assure the statistical inefficiency, 5 ps interval is required as observed in Figure S1 in the supplementary material) Potential energies of the complexes with the non-perturbed ligand for 1,000 snapshots were calculated in the vacuum state and in implicit solvent using the GB/SA model. It should be noted that the FEVP method cannot be used with explicit solvent because it requires insertion (by superposition) of the perturbed ligand into simulations of the unperturbed ligand as explained below, which will lead to frequent steric clashes with explicit solvent (steric clashes with the protein also occur, but less frequently, and it is treated by the energetic filtering discussed below; still a few ligands had to be discarded because of this problem). Then, the 3D structure of a perturbed ligand in the complex after some time steps among 1,000 snapshots from the MD trajectory was superimposed on that of the non-perturbed ligand (the ligand from 1FKG) after the same time steps among 1,000 snapshots from the MD trajectory. Thus, we performed 1,000 superimpositions of ligands. The atoms in the non-perturbed ligand used for the superimposition, i.e. pipecolyl moiety, are shown in Fig. 1. It was reported that this moiety corresponds to the binding core [12]. Finally, the non-perturbed ligand was deleted from the complex structure. Then, potential energies of the complexes with the perturbed ligand for 1,000 snapshots were calculated. In the GB/SA model, the dielectric constant of the solute and solvent was set as 4 and 80, respectively. The Linear Combination of Pairwise Overlaps (LCPO) algorithm was used for calculation of a surface area; the default value for the parameter was used.

Only the snapshots satisfying the following condition were used for calculating ensemble averages, i.e., the difference in the potential energies between the non-perturbed and a perturbed complexes had to be less than the difference in the potential energies between two ligands ±22.5 kcal/mol; (Figure S2 in the supplementary material) otherwise snapshots were disregarded because such conformations of the complex would contribute little to the ensemble average. This criterion was justified as follows. When we performed simulations of the non-perturbed and perturbed complexes with the fixed 3D structures of a protein, the energy differences between them are almost always within the energy difference between two ligands ±25 kcal/mol. (The dependency of the results on this cutoff value is shown in Table S1 in the supplementary material. According to this table, the results are not

changed even when the cutoff value is varied between ±20 to ±25 kcal/mol. (The values of correlation coefficients are around 0.8 and the average absolute errors around 1.0 kcal/mol.)) In cases with $|H_{com1} - H_{com0}| >$ the potential energy between two ligands ±25 kcal/mol involve collisions between atoms and/or different binding modes. The potential energy between two ligands was estimated by $< H_{lig\_1} >_{lig\_1} - < H_{lig\_0} >_{lig\_0}$ as follows.

The above process was performed to calculate $< H_{com\_1} - H_{com\_0} >_{com\_0}$. In the same way, $< H_{com\_1} - H_{com\_0} >_{com\_1}$ was calculated. The values of $< H_{lig\_1} - H_{lig\_0} >_{lig\_0}$ and $< H_{lig\_1} - H_{lig\_0} >_{lig\_1}$ were approximated by $< H_{lig\_1} >_{lig\_1} - < H_{lig\_0} >_{lig\_0}$, because their values are very close. The average energy of a free ligand was calculated using isolated 3D structures in 1,000 snapshots from a MD simulation in explicit solvent. In this calculation, vacuum and the GB/SA models were used as solvent models, i.e., $< H_{lig\_1} >_{lig\_1}$ and $< H_{lig\_1} >_{lig\_0}$ were calculated.

Calculation of the MM-GB/SA method

The MM-GB/SA method [24] is a technique to take the solvation free energy into account using the Generalized Born method with accessible surface areas of molecules. This technique has been applied to calculate binding free energy of various protein–ligand systems [10–22], and now this method is widely used to estimate binding free energy of a ligand to a protein. The MM-GB/SA method can be summarized by the following formulas.

$$\Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{protein}} + G_{\text{ligand}}) \quad (9)$$

$$G = E_{\text{gas}} + G_{\text{sol}} - TS \quad (10)$$

$$E_{\text{gas}} = E_{bond} + E_{angle} + E_{torsion} + E_{\text{vdw}} + E_{\text{ele}} \quad (11)$$

$$G_{\text{sol}} = G_{\text{GB}} + G_{\text{SA}} \quad (12)$$

$$G_{\text{SA}} = \beta \times \text{ SASA } + \gamma \quad (13)$$

Here, G_{complex}, G_{protein} and G_{ligand} are the free energies of a complex, a protein and a ligand, respectively. E_{gas} is a standard force field energy including bonds, angles, torsion, van der Waals and electrostatic terms in vacuum. G_{sol} is solvation free energy. G_{GB} and G_{SA} are the solvation free energies of a polar and a nonpolar component. SASA is the solvent-accessible surface area. β and γ are parameters to be determined for the calculation of G_{SA}, and in this study the values for β and γ in the AMBER program were used. The dielectric constants of the solute and solvent were set as 4 and 80, respectively. SASA was computed with a probe radius of 1.4 Å. The calculations of E_{gas} or G_{sol} are also required in the FEVP method depending on whether the FEVP calculations are performed in vacuum or in a continuum solvent. The entropy term was computed by the NMODE module in AMBER. Energy minimizations were carried out with distance dependent dielectric function ($\varepsilon = 4r$) for 50,000 cycles until the root-mean-square of the gradient vector was less 0.0001 kcal/(mol Å^2). As mentioned above, an average energy of an ensemble were calculated with 1,000 snapshots taken from the final 5 ns of the MD trajectory of a complex at an interval of 5 ps. The required MD simulations in the present MM-GB/SA calculations were performed with the same protocol to that described above. The MM-GB/SA calculations were applied to only complex systems.

Calculation by the LIE method

The LIE method was introduced by Åquvist and coworkers [29, 30] for calculations of ligand binding free energy to a protein. Despite the simplicity of the method, it can produce good results by tuning a few parameters [25–28]. This technique requires only two simulations, ligand alone in solvent and solvated ligand–protein system as in Eq. (14). The LIE method can be expressed by the following formula.

$$\Delta G_{bind} = \alpha\left(< V_{vdw} >_{bound} - < V_{vdw} >_{free}\right) + \beta\left(< V_{elec} >_{bound} - < V_{elec} >_{free}\right) + \gamma. \quad (14)$$

Here the meaning of each term is:

$< V_{vdw} >_{bound}$: ensemble average of van der Waals energy of a bound state.
$< V_{vdw} >_{free}$: ensemble average of van der Waals energy of an unbound state
$< V_{elec} >_{bound}$: ensemble average of electrostatic energy of a bound state
$< V_{elec} >_{free}$: ensemble average of electrostatic energy of an unbound state.

It should be noted that these same energy terms are required to calculate E_{gas} in the MMGB/SA method. In the same manner as elsewhere in this study (FEVP method), an average energy of an ensemble were calculated with 1,000 snapshots taken from the final 5 ns of the MD trajectories of a complex and a ligand at an interval of 5 ps. Parameters α, β and γ in LIE were determined using 18 ligands as a training data set.

Results

Free energy variational principle

The calculated relative binding free energies in the vacuum state can be compared with experimental data in Table 2. As mentioned, ligand 13 is taken as the non-perturbed

Table 2 Results for the FEVP in vacuum. The correlation coefficient between the calculated and the experimental is 0.78, and the average absolute error is 1.29 kcal/mol

No.	$<H_1–H_0>_1$	n.s.l	$<H_1–H_0>_0$	n.s.u	$\Delta\Delta G_{calc}$	$\Delta\Delta G_{exp}$	ΔG_{exp}	$\Delta\Delta G_{error}$
L1	−7.24	47	14.63	47	3.70 ± 0.73	3.03	−7.96	0.67
L2	−6.90	20	15.37	39	4.23 ± 0.96	3.16	−7.82	1.07
L3	−5.52	117	14.63	154	4.56 ± 0.51	2.50	−8.48	2.06
L4	−5.66	36	16.26	72	5.30 ± 0.90	3.99	−7.00	1.32
L5	−5.97	11	16.38	109	5.20 ± 1.59	3.81	−7.17	1.39
L6	−3.81	156	16.02	232	6.11 ± 0.48	3.16	−7.82	2.95
L7	−4.19	221	13.40	176	4.61 ± 0.43	2.21	−8.77	2.39
L8	−10.37	87	12.50	90	1.06 ± 0.60	1.74	−9.24	0.68
L9	−7.19	135	13.41	121	3.11 ± 0.51	1.43	−9.55	1.68
L10	−7.44	100	13.66	170	3.11 ± 0.51	2.10	−8.88	1.01
L11	−9.20	111	14.09	98	2.45 ± 0.52	1.37	−9.61	1.07
L12	−10.39	58	13.16	72	1.39 ± 0.72	0.11	−10.87	1.28
L13	0.00	–	0.00	–	0.00	0.00	−10.98	–
L14	−10.36	138	9.97	73	−0.19 ± 0.61	0.00	−10.98	0.19
L15	−11.93	81	9.98	91	−0.97 ± 0.54	−0.21	−11.19	0.76
L16	−10.64	133	13.57	39	1.47 ± 0.67	1.92	−9.06	0.45
L17	−6.54	82	14.05	74	3.75 ± 0.68	1.67	−9.31	2.08
L18	−12.06	84	12.58	197	0.26 ± 0.46	1.16	−9.82	0.90

$<H_1–H_0>_1$: the value of the lower limit

n.s.l: number of samples to calculate the lower limit

$<H_1–H_0>_0$: the value of the upper limit

n.s.u: number of samples to calculate the upper limit

$\Delta\Delta G_{calc}$: the value of relative binding free energy based on the present calculations

The values of the standard errors calculated from the bootstrap standard deviations are added [47]

$\Delta\Delta G_{exp}$: the value of relative binding free energy based on experiment

ΔG_{exp}: the value of binding free energy based on experiment

$\Delta\Delta G_{error}$: $|\Delta\Delta G_{calc} - \Delta\Delta G_{exp}|$

ligand for the calculations of the relative binding free energies in all cases. In the Fig. 2, the abscissa and ordinate denote the calculated value ($\Delta\Delta G_{calc}$) and experimental value ($\Delta\Delta G_{exp}$), respectively. The correlation coefficient between experimental and calculated values, i.e., $\Delta\Delta G_{exp}$ and $\Delta\Delta G_{calc}$, is 0.78, and the average absolute error = $|\Delta\Delta G_{exp} - \Delta\Delta G_{calc}|$ is 1.29 kcal/mol.

In Table 3 and Fig. 3, the calculated values using the GB/SA solvent model for the calculation of the ensemble averages are compared to the experimental values. The correlation coefficient was 0.80, and the average absolute error is 0.70 kcal/mol in this case. It should be noticed that a real value (experimental value) of relative binding free energy always exists between the upper and lower limits for all ligands in the vacuum state as well as with the GB/SA solvent model.

We confirmed that the initial binding mode is maintained after the MD in all cases. The binding mode of FK506 binding protein is shown in Fig. 4.

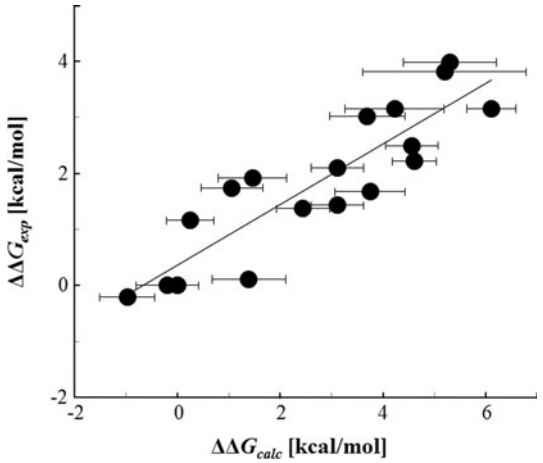


Fig. 2 The *plots* of the experimental ($\Delta\Delta G_{exp}$) versus calculated ($\Delta\Delta G_{calc}$) relative binding free energies with error *bars*. The ordinate and abscissa denote $\Delta\Delta G_{exp}$ and calculated $\Delta\Delta G_{calc}$, respectively. $\Delta\Delta G_{calc}$ were derived in the vacuum state based on FEVP. The correlation coefficient between the calculated and the experimental is 0.78, and the average absolute error is 1.29 kcal/mol

Table 3 Results for the FEVP in GB/SA. The correlation coefficient between the calculated and the experimental is 0.80, and the average absolute error is 0.70 kcal/mol

No.	$<H_1–H_0>_1$	n.s.l	$<H_1–H_0>_0$	n.s.u	$\Delta\Delta G_{calc}$	$\Delta\Delta G_{exp}$	ΔG_{exp}	$\Delta\Delta G_{error}$
L1	−8.54	44	12.99	68	2.22 ± 0.72	3.03	−7.96	0.80
L2	−7.29	16	12.83	68	2.77 ± 0.97	3.16	−7.82	0.39
L3	−7.49	97	12.51	255	2.51 ± 0.52	2.50	−8.48	0.02
L4	−6.67	35	13.80	124	3.56 ± 0.91	3.99	−7.00	0.42
L5	−7.30	8	13.90	235	3.30 ± 0.83	3.81	−7.17	0.51
L6	−6.46	132	12.57	397	3.06 ± 0.45	3.16	−7.82	0.10
L7	−6.90	196	10.51	231	1.81 ± 0.39	2.21	−8.77	0.41
L8	−11.99	81	10.34	107	−0.82 ± 0.55	1.74	−9.24	2.56
L9	−7.94	122	10.94	146	1.50 ± 0.49	1.43	−9.55	0.07
L10	−8.86	84	11.48	245	1.31 ± 0.49	2.10	−8.88	0.79
L11	−10.22	92	11.91	132	0.85 ± 0.50	1.37	−9.61	0.53
L12	−11.56	60	11.55	84	−0.01 ± 0.65	0.11	−10.87	0.11
L13	0.00	–	0.00	–	0.00	0.00	−10.98	–
L14	−10.57	129	9.30	85	−0.63 ± 0.58	0.00	−10.98	0.63
L15	−11.79	76	8.41	95	−1.69 ± 0.53	−0.21	−11.19	1.48
L16	−10.71	130	12.21	43	0.75 ± 0.66	1.92	−9.06	1.17
L17	−8.07	81	11.68	96	1.81 ± 0.61	1.67	−9.31	0.14
L18	−12.41	80	11.21	226	−0.60 ± 0.48	1.16	−9.82	1.76

$<H_1–H_0>_1$: the value of the lower limit

n.s.l: number of samples to calculate the lower limit

$<H_1–H_0>_0$: the value of the upper limit

n.s.u: number of samples to calculate the upper limit

$\Delta\Delta G_{calc}$: the value of relative binding free energy based on the present calculations

The values of the standard errors calculated from the bootstrap standard deviations are added [47]

$\Delta\Delta G_{exp}$: the value of relative binding free energy based on experiment

ΔG_{exp}: the value of binding free energy based on experiment

$\Delta\Delta G_{error}$: $|\Delta\Delta G_{calc} - \Delta\Delta G_{exp}|$

Comparisons with other methods

To examine the effectiveness of the present method, we compare the present results to those produced using the MM-GB/SA and LIE methods. The calculated and experimental relative binding free energies obtained by the MM-GB/SA method are shown in Table 4. We used the parameters in AMBER, i.e., the dielectric constant of solute was 4 and of solvation was 80, when the solvation free energy was calculated. In the Fig. 5, the abscissa and ordinate denote the calculated value ($\Delta\Delta G_{calc}$) and experimental value ($\Delta\Delta G_{exp}$). The correlation coefficient is 0.81, and an average absolute error is 3.89 kcal/mol. (The absolute error of binding free energy (ΔG_{bind}) itself was 8.40 kcal/mol.) The correlation coefficient of 0.83 and an average absolute error of 5.00 kcal/mol were obtained when the entropy effect was omitted, i.e., the average absolute error was getting worse (data not shown). The average absolute error is worse than the result for FEVP, while the amount of correlation coefficient is similar.

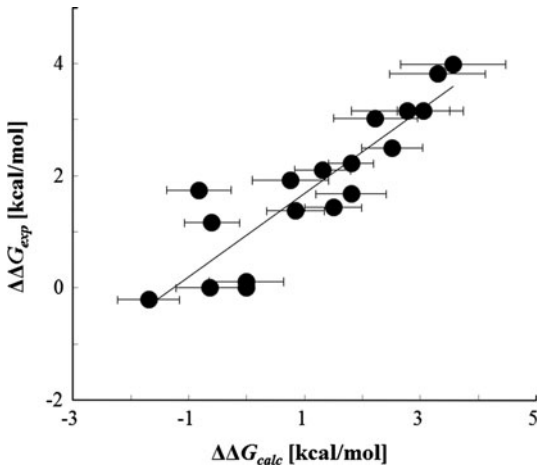


Fig. 3 The *plots* of the experimental ($\Delta\Delta G_{exp}$) versus calculated ($\Delta\Delta G_{calc}$) relative binding free energies with error *bars*. $\Delta\Delta G_{calc}$ were derived in solvent of the GB/SA model based on FEVP. The correlation coefficient between the calculated and the experimental is 0.80, and the average absolute error is 0.70 kcal/mol

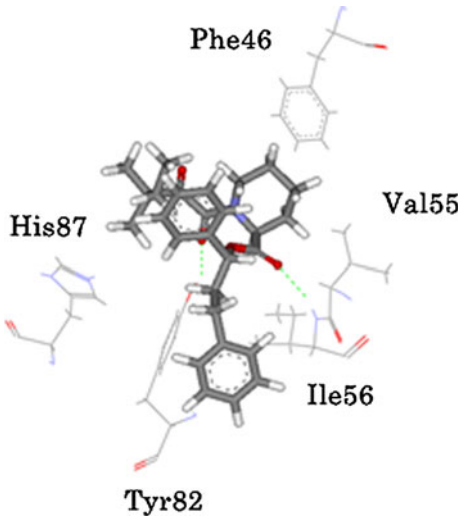


Fig. 4 The binding mode between the ligand 13 and the protein

The calculated and experimental relative binding free energies obtained by the LIE method are shown in Table 5 and Fig. 6. The following formula was used to calculate binding free energies ΔG_{bind}. The coefficients in this formula were determined using 18 ligands as a training data set.

$$\Delta G_{bind} = 1.81\left(<V_{vdw}>_{bound} - <V_{vdw}>_{free}\right) + 0.38\left(<V_{elec}>_{bound} - <V_{elec}>_{free}\right) + 0.98. \quad (15)$$

The calculated values of relative binding free energy ($\Delta\Delta G_{LIE}$) show a correlation coefficient of 0.81, and an average absolute error of 0.44 kcal/mol compared to experimental values. (The parameter γ was adjusted to obtain the ΔG value. In the calculations of $\Delta\Delta G_{LIE}$ values, γ is canceled and becomes inconsequential.) With the standard values of the parameters, i.e., $\alpha = 0.16$, $\beta = 0.50$ and $\gamma = 0$, the correlation coefficient and the average absolute error are 0.72 and 0.57 kcal/mol, respectively. The average absolute error estimated by the LIE method is slightly better than that by the FEVP, while the correlation coefficients in both cases are similar.

These results from the three techniques, i.e., the FEVP, the MM-GB/SA method and LIE method, are summarized in Table 6.

Discussion

By application of the method based on the FEVP to the FKBP-inhibitor system, we can make predictions for the

Table 4 Results for the MM-GB/SA method. The correlation coefficient between the calculated and the experimental is 0.81, and the average absolute error is 3.89 kcal/mol

No.	ΔE_{elec}	ΔE_{vdw}	ΔG_{GB}	ΔG_{SA}	$\Delta G_{MM\text{-}GB/SA}$	$T\Delta S$	ΔG_{total}	$\Delta\Delta G_{total}$
L1	−4.37 ± 0.04	−30.19 ± 0.10	6.19 ± 0.03	−4.14 ± 0.006	−32.51 ± 0.10	−19.85 ± 0.10	−12.66 ± 0.14	10.36
L2	−2.90 ± 0.02	−32.58 ± 0.08	4.98 ± 0.02	−4.33 ± 0.003	−34.83 ± 0.08	−18.89 ± 0.10	−15.94 ± 0.13	7.08
L3	−4.14 ± 0.04	−29.53 ± 0.09	5.58 ± 0.02	−4.09 ± 0.004	−32.18 ± 0.09	−17.64 ± 0.12	−14.54 ± 0.15	8.48
L4	−3.47 ± 0.03	−27.30 ± 0.08	5.45 ± 0.02	−3.98 ± 0.005	−29.30 ± 0.07	−17.44 ± 0.10	−11.86 ± 0.13	11.16
L5	−2.89 ± 0.04	−29.28 ± 0.08	4.86 ± 0.02	−4.09 ± 0.004	−31.40 ± 0.08	−18.38 ± 0.12	−13.02 ± 0.14	10.00
L6	−3.99 ± 0.03	−27.98 ± 0.09	5.44 ± 0.02	−4.01 ± 0.004	−30.54 ± 0.09	−17.87 ± 0.12	−12.67 ± 0.15	10.35
L7	−3.85 ± 0.03	−31.66 ± 0.08	5.59 ± 0.01	−4.30 ± 0.004	−34.22 ± 0.08	−18.27 ± 0.12	−15.95 ± 0.14	7.07
L8	−4.11 ± 0.04	−37.31 ± 0.10	6.04 ± 0.02	−4.80 ± 0.005	−40.18 ± 0.09	−20.42 ± 0.13	−19.76 ± 0.16	3.26
L9	−4.65 ± 0.03	−36.05 ± 0.10	6.45 ± 0.02	−4.78 ± 0.006	−39.03 ± 0.11	−21.57 ± 0.11	−17.46 ± 0.15	5.56
L10	−4.69 ± 0.03	−33.92 ± 0.09	6.46 ± 0.02	−4.38 ± 0.005	−36.53 ± 0.09	−19.60 ± 0.12	−16.93 ± 0.15	6.09
L11	−5.29 ± 0.02	−37.59 ± 0.10	7.12 ± 0.02	−4.79 ± 0.005	−40.55 ± 0.13	−20.50 ± 0.13	−20.05 ± 0.14	2.97
L12	−5.51 ± 0.03	−38.93 ± 0.13	7.72 ± 0.03	−4.94 ± 0.011	−41.66 ± 0.14	−22.10 ± 0.13	−19.56 ± 0.19	3.46
L13	−4.92 ± 0.03	−41.44 ± 0.09	7.18 ± 0.02	−5.10 ± 0.004	−44.28 ± 0.09	−21.26 ± 0.11	−23.02 ± 0.14	0.00
L14	−4.51 ± 0.02	−39.71 ± 0.09	6.54 ± 0.02	−5.11 ± 0.004	−42.79 ± 0.09	−20.73 ± 0.12	−22.06 ± 0.15	0.96
L15	−4.95 ± 0.02	−39.91 ± 0.09	6.95 ± 0.02	−5.05 ± 0.005	−42.96 ± 0.09	−20.31 ± 0.12	−22.65 ± 0.15	0.37
L16	−4.44 ± 0.03	−39.06 ± 0.09	6.63 ± 0.02	−4.97 ± 0.004	−41.84 ± 0.08	−20.45 ± 0.12	−21.39 ± 0.15	1.63
L17	−4.31 ± 0.03	−34.00 ± 0.09	6.33 ± 0.02	−4.56 ± 0.005	−36.54 ± 0.09	−20.26 ± 0.12	−16.28 ± 0.15	6.74
L18	−4.72 ± 0.03	−39.84 ± 0.10	7.16 ± 0.02	−5.12 ± 0.006	−42.52 ± 0.10	−22.68 ± 0.12	−19.84 ± 0.16	3.18

$\Delta G_{MM\text{-}GB/SA} = \Delta E_{elec} + \Delta E_{vdw} + \Delta G_{GB} + \Delta G_{SA}$

$\Delta G_{total} = \Delta G_{MM\text{-}GB/SA} - T\Delta S$

$\Delta\Delta G_{total}$: relative binding free energy

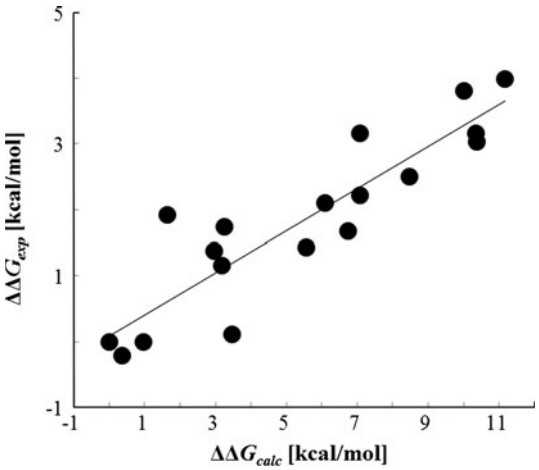


Fig. 5 The *plots* of the experimental ($\Delta\Delta G_{exp}$) versus calculated ($\Delta\Delta G_{calc}$) relative binding free energies. $\Delta\Delta G_{calc}$ were derived in solvent of the MM-GB/SA model. The correlation coefficient between the calculated and the experimental is 0.81, and the average absolute error is 3.89 kcal/mol

relative binding free energies comparable to the results by slower methods developed previously. In spite of the drastic simplification in which a true relative binding free energy value is approximated as the arithmetic mean of the upper and lower bounds, the results are quite good for the FKBP-inhibitor system. Although the FEVP only estimates relative binding affinities, whereas both MM-GB/SA and LIE also give absolute affinities, it is demonstrated that the present method can be applied to different ligands with a common scaffold.

The average absolute error of the present method is better than that of the MM-GB/SA method. However, Xu and Wang have obtained improved results for FKBP by tuning specific parameters in MM/GBSA [12]. In the MM-GB/SA method, it is also crucial to consider the contribution of entropy to the binding free energy. In fact, an accurate estimation of entropy is quite significant in this procedure. However, the calculation of entropy requires high computational cost. Our method does not need to consider the contribution of entropy explicitly if the difference between two ligands can be regarded as a perturbation.

The results by the LIE method were similar in quality to those by the present technique. In the present study, the LIE method with the tuned parameters and with the standard parameters provided very similar results. However, it should be also pointed out that in general the LIE method needs inhibitory constants for ligands in advance to tune parameters for a target system. On the other hand, the FEVP method does not need any parameters to be

Table 5 Results from the LIE method. The correlation coefficient between the calculated and the experimental is 0.81, and the average absolute error is 0.44 kcal/mol

No.	$<E_{vdw}>_{bound}$	$<E_{elec}>_{bound}$	$<E_{vdw}>_{free}$	$<E_{elec}>_{free}$	$\Delta\Delta G_{LIE}$	$\Delta\Delta G_{def}$	$\Delta\Delta G_{exp}$
L1	14.42 ± 0.03	−8.38 ± 0.08	16.69 ± 0.02	6.06 ± 0.07	2.50 ± 0.07	2.88 ± 0.04	3.03
L2	12.38 ± 0.02	−10.51 ± 0.08	13.93 ± 0.02	5.93 ± 0.07	3.05 ± 0.06	1.99 ± 0.03	3.16
L3	14.09 ± 0.02	−7.85 ± 0.08	16.18 ± 0.01	6.96 ± 0.08	2.69 ± 0.06	2.72 ± 0.04	2.50
L4	13.84 ± 0.02	−4.92 ± 0.07	15.63 ± 0.02	7.92 ± 0.06	3.97 ± 0.06	3.76 ± 0.03	3.99
L5	13.20 ± 0.02	−9.88 ± 0.07	14.67 ± 0.02	4.76 ± 0.06	3.87 ± 0.06	2.90 ± 0.03	3.81
L6	13.90 ± 0.02	−7.61 ± 0.08	15.86 ± 0.01	5.88 ± 0.07	3.43 ± 0.06	3.40 ± 0.03	3.16
L7	14.55 ± 0.02	−7.37 ± 0.08	16.44 ± 0.02	7.84 ± 0.08	2.89 ± 0.06	2.55 ± 0.04	2.21
L8	14.49 ± 0.02	−8.90 ± 0.09	16.52 ± 0.02	8.37 ± 0.09	1.86 ± 0.07	1.50 ± 0.04	1.74
L9	13.95 ± 0.02	−7.81 ± 0.09	16.08 ± 0.02	10.04 ± 0.09	1.46 ± 0.06	1.20 ± 0.04	1.43
L10	14.02 ± 0.02	−8.16 ± 0.08	16.29 ± 0.02	8.78 ± 0.09	1.56 ± 0.06	1.63 ± 0.04	2.10
L11	13.52 ± 0.01	−8.83 ± 0.09	15.99 ± 0.01	9.26 ± 0.09	0.78 ± 0.06	1.02 ± 0.04	1.37
L12	12.65 ± 0.02	−9.18 ± 0.10	15.11 ± 0.02	8.73 ± 0.10	0.86 ± 0.07	1.11 ± 0.05	0.11
L13	13.09 ± 0.02	−8.87 ± 0.09	15.56 ± 0.02	11.26 ± 0.10	0.00	0.00	0.00
L14	11.51 ± 0.02	−8.73 ± 0.09	13.67 ± 0.02	9.51 ± 0.10	1.26 ± 0.07	0.99 ± 0.04	0.00
L15	13.01 ± 0.01	−9.03 ± 0.09	15.30 ± 0.02	10.05 ± 0.10	0.73 ± 0.06	0.56 ± 0.04	−0.21
L16	13.69 ± 0.02	−8.21 ± 0.10	15.81 ± 0.01	11.28 ± 0.09	0.86 ± 0.07	0.38 ± 0.04	1.92
L17	11.58 ± 0.02	−5.96 ± 0.09	13.89 ± 0.02	9.65 ± 0.09	1.99 ± 0.07	2.28 ± 0.04	1.67
L18	11.51 ± 0.02	−8.73 ± 0.09	13.67 ± 0.02	9.51 ± 0.09	1.26 ± 0.07	0.99 ± 0.04	1.16

$\Delta\Delta G_{LIE}$: relative binding free energy calculated with $\alpha = 1.81$, $\beta = 0.38$ and $\gamma = 0.98$

$\Delta\Delta G_{def}$: relative binding free energy calculated with $\alpha = 0.16$, $\beta = 0.50$ and $\gamma = 0$

The values of the standard errors calculated from the bootstrap standard deviations are added [47]

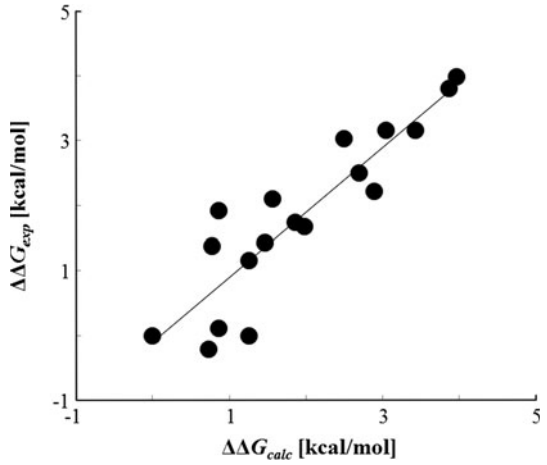


Fig. 6 The *plots* of the experimental ($\Delta\Delta G_{exp}$) versus calculated ($\Delta\Delta G_{calc}$) relative binding free energies. $\Delta\Delta G_{calc}$ were derived using the LIE method for the vacuum state. The correlation coefficient between the calculated and the experimental is 0.81, and the average absolute error is 0.44 kcal/mol

Table 6 Summary of results from the FEVP, the MM-GB/SA and LIE methods

Model	Correlation coefficient (r^2)	Average absolute error (kcal/mol)
FEVP(vac)	0.78	1.29
FEVP(GB/SA)	0.80	0.70
MM-GB/SA	0.81	3.89
LIE	0.81	0.44

determined, although our technique depends on the 22.5 kcal/mol cutoff value and the structure for ligand superposition. It is possible to predict relative binding free energies with only the knowledge of the binding mode and the inhibitory constant of the non-perturbed protein–ligand system in our method.

It is interesting to compare the present results to those by the FEP method. We compare to the result of Jayachandran et al. [2]. They calculated the absolute binding free energy of ligand 2, ligand 3, ligand 9, ligand 12, ligand 13 and ligand 15 to FKBP and obtained the correlation coefficient of 0.96 and average absolute error of 0.48 kcal/mol, whereas the present method gives the correlation coefficient of 0.89 and an average absolute error of 0.41 kcal/mol for these ligands. That is, results comparable to the FEP method are obtained by the present study. It should be pointed out that the present method is used as an end point method and much cheaper than the standard FEP method.

The MMGB/SA, LIE and FEVP methods commonly require the energy calculations by sampling of 3D structures from the MD trajectories. Furthermore, the MMGB/SA method needs the entropy calculation. Further simulations for isolated ligands are necessary in the LIE and FEVP methods. In the FEVP method, the superposition of two ligands should be also done. The most time consuming process is the calculation of entropy. The ligand simulation process is next most time consuming, and the superimposition of ligands is the cheapest process. Overall, the cheapest technique the LIE method, but the FEVP method is almost as cheap.

In the present method, we performed our calculations on the assumption that the trajectory of the non-perturbed system should be similar to that of a perturbed system if a perturbation between the non-perturbed and a perturbed ligands is small. Conversely, if the perturbation is large, the protein structure would change significantly when a perturbed ligand is docked to the protein. As a result, we would obtain a binding free energy deviating largely from the real value. In such cases, we would observe atomic collisions and high potential energies when a perturbed ligand is docked to the protein. We could judge the limitation of the applicability of the present method in such cases.

On the other hand, it is necessary to examine carefully the validity of results from the present method for the case of a large change in the binding mode, i.e., recombinations of electrostatic interactions and hydrogen bonds even when the conformation of the whole protein does not change much during a simulation. Ligand 18 exhibit rather low values compared to experimental values. The binding mode of ligand 13 (non-perturbed ligand) is shown in Fig. 4. In all complexes, this binding mode was maintained, but extra stabilizing interactions that may not exist in the real complexes are observed between the methoxy group in ligand 18 and residues ILE56, ALA81 and TYR82 of the protein (Figure S3a and S3b in the supplementary material). As a result, the calculated values for ligand 18 may indicate more stability than the actual (experimental) values. Hence, ligand 18 could differ significantly from ligand 13 because of exceeding the extent of the perturbation.

We will encounter difficulty in producing accurate results by the present method when the binding modes for two ligands are very different. However, even in such cases, if we can categorize some ligands with the same binding mode as fitting in one group and one of these ligands is selected as the non-perturbed ligand, then our calculations of $\Delta\Delta G_{bind}$ will yield better results.

Acknowledgments The present work is supported by ministry of education, culture, sports, science and technology as a program for the strategic research foundation at private universities, 2010–2014. (Grant No.S1001042).

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