Prediction of the Binding Affinities of PSD95 PDZ Domain in Complex with the CRIPT Peptide

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***Abstract*—In this work, we have applied the state of art molecular dynamics simulations in combination with solvation free energy and conformational entropy calculations to predict the binding affinities of PSD95 PDZ domain in complex with the CRIPT peptide. Four diffident computational protocols were evaluated on reproducing the relative binding free energies of the wild type PDZ and its five mutants. The protocol of MM-GB/SA in combination with normal mode analysis (NMA), which has a correlation coefficient square of 0.84, apparently outperforms the others especially for the two MM-PB/SA-based protocols. Free energy decomposition was also performed in order to identify the hot spots that contribute significantly to the binding.**

***Index Terms*—MD Simulations, MMPB/SA, MMGB/SA, NMA, WSAS, PDZ Domain**

I. Introduction

A. Binding Free Energy Calculations Using Continuum Models

It is of great interest in modern drug design to accurately calculate the free energies of protein-ligand or nucleic acid-ligand binding. MM-PBSA (Molecular Mechanics-Poisson Boltzmann Surface Area) [1]-[3] and MM-GBSA (Molecular Mechanics-Generalized Born Surface Area)[4]-[6] have gained popularity in this field.[7]-[16] In MM-PB/GBSA theory, the free energy of a molecule is calculated with Eqs. 1-3, and the binding free energy is calculated using Eq. (4).

$$G = \langle H_{gas} \rangle + \langle G_{solv} \rangle - T\langle S_{conf} \rangle \quad (1)$$

$$G_{solv} = G_{solv}^{pol} + G_{solv}^{nonpol} \quad (2)$$

$$S_{conf} = S_{trans} + S_{rot} + S_{vib} \quad (3)$$

$$\Delta G_{binding} = G_{AB} - (G_A + G_B) \quad (4)$$

The first term in Eq. 1, H_{gas}, is replaced with E_{gas}, the gas phase MM energy, as the PV term is negligible for a molecule in condensed phase. <> indicates those energy terms are ensemble averages. The second term in Eq.1, G_{solv} consists of two components, the polar and nonpolar solvation free energies (Eq. 2). The polar solvation energy is evaluated either by a PB or a GB model; while the nonpolar solvation free energy is typically estimated with the solvent accessible surface area (SAS) assuming that the non-polar contribution is proportional to SAS. The nonpolar term accounts for the entropy penalty associated with the reorganization of solvent molecules around the solute and the van der Waals interaction between the solute and solvent. The reorganization energy is usually very small and therefore omitted in calculations. The third term in Eq. (1), the conformational entropy, is further decomposed into three parts, the translational, the rotational and the vibrational entropies (Eq. 3). The translational entropy (S_{trans}) and the rotational entropy (S_{rot}) can be approximated using the standard equations for rigid body translation and rotation, and the vibrational part of conformational entropy (S_{vib}) is typically estimated by normal mode analysis assuming that the vibrational movement around the energy well is harmonic. The S_{vib} term can also be obtained by conducting a quasi-harmonic analysis using the MD trajectories in the sampling phase. The S_{vib} term is the bottleneck of the MM-PBSA and MM-GBSA methods as thoroughly minimized structures are perquisite for performing normal mode analysis. However, minimizing the root-mean-square force of protein structures to a low level, say the converge criterion of the gradient being set to 0.0001 kcal/(molÅ), may take tens of thousands steps of conjugated gradient minimization followed by hundreds of Newton Raphson minimization. However, the TS term is needed to calculate the absolute binding free energies. For the sake of computational efficiency, we have recently developed a fast approach to estimate the conformational entropy based upon solvent accessible surface area calculations.[17] In our approach, the conformational entropy of a molecule, *S*, can be obtained by summing up the contributions of all atoms, no matter they are buried or exposed. Each atom has two types of surface areas, solvent accessible surface area (SAS) and buried SAS (BSAS). The two types of surface areas are weighted to estimate the contribution of an atom to *S*. Atoms having the same atom type share the same weight and a general parameter *k* is applied to balance the contributions of the two types of surface areas. The WSAS (Weighted SAS) model has been extensively evaluated in rational protein design and rational drug

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design. The overall performance of WSAS is comparable to that of normal mode analysis, but WSAS is much cheaper than NMA and it does not need to minimize the protein structures.

Eq. 4 is used to calculate the binding free energy, $\Delta G_{binding}$ of A + B $\rightarrow$ AB reaction. In a typical binding free energy calculation using MM-PBSA or MM-GBSA, the molecular system of interest is first immersed in a water box and several to tens of nanoseconds molecular dynamics simulations are performed to collect MD snapshots for the post-MM-PBSA analysis. In most scenarios, the ligand or substrate binding does not lead to a dramatic conformational change and the "single trajectory" sampling protocol, for which G_A and G_B of Eq. 4 are calculated using the MD snapshots sampled for AB, is preferred as it can achieve a better error cancellation.[18] On the other hand, if the bound and unbound states are dramatically different, the "individual trajectories" sampling protocol must be employed, i.e. G_A, G_B and G_{AB} of Eq. 4 are calculated using their own MD snapshots sampled in separate MD simulations.

In the second stage of post free energy analysis, the free energies of A, B and AB are calculated using Eqs. 1-3.

Critical assessment of the two techniques on modeling protein-ligand binding begins to emerge.[19]-[24] It is generally agreed that the MM-PBSA and MM-GBSA methods performed relatively better than most docking scoring functions.[19], [25] Although they are not reliable methods for calculating the absolute binding free energies, MM-PBSA and MM-GBSA can usually predict the relative binding free energies of structurally similar ligands with a satisfactory performance.[18] We have conducted systematic studies to evaluate the MM-PBSA and MM-GBSA methods in protein-ligand bindings.[21], [22] In this work, we presented a case study to evaluate the two methods using a protein-peptide system, the PSD95 PDZ domain.

The PDZ (Post-synaptic density 95, Discs Large, Zona Occludens 1) domains are a ubiquitous family of protein interaction domains and play a central role in signal pathways by anchoring trans-membrane proteins to the cytoskeleton and holding signaling complexes together.[26] The biological importance of PDZ domains is further underscored by the identification of various PDZ-containing proteins as human disease and pathogen effector targets. To date, several hundred PDZ domain sequences have been identified [27] and up to 200 experimental structures have been resolved. The PDZ domains typically containing 80-90 amino acids. Though many basic facts on PDZ domains are clear, it is still an enormous challenge to fully understand how PDZ domains regulate signal transmission. Recently, McLaughlin Jr *et al*. have measured the binding free energies of the wild type and 86 single-residue mutants of PSD 95 PDZ in complex with the CRIPT peptide (TKNYKQTSV-COOH).[28] Taking the advantage of the released data, in this case study, we set out to evaluate the MM-PBSA and GBSA methods in modeling the energies of protein-peptide binding.

II. Methodologies

A. MD Simulations

The crystal structure of PSD95 PDZ/CRIPT (PDB Code 1BE9)[29] was downed from Protein Data Bank (www.pdb.org).[30] In Fig. 1, the CRIPT peptide is colored in magenta and the five cherry-picked mutation sites were shown in stick and colored differently. The surrounding residues of the CRIPT peptide are shown in the right panel of Fig. 1.

The Parm99SB biomolecular force field[31],[32] was used for all the molecular mechanics calculations. All MD simulations were performed with the periodic boundary condition to produce isothermal-isobaric ensembles at 298 K using the Sander program in AMBER11. [33] The Particle Mesh Ewald (PME) method [34]-[36] was used to calculate the full electrostatic energy of a unit cell in a macroscopic lattice of repeating images. The integration of the equations of motion was conducted at a time step of 2 femtoseconds. The covalent bonds involving hydrogen atoms were frozen with the SHAKE algorithm.[37] Temperature was regulated using the Langevin dynamics[38] with the collision frequency of 5 ps^{-1}.[39]-[41] Pressure regulation was achieved with isotropic position scaling and the pressure relaxation time was set to 1.0 picosecond. There are three phases in MD simulations, i.e. the relation phase, the equilibration phase and the sampling phase. In the relaxation phase, the main chain atoms of protein were restrained to the positions of crystal structures with gradually decreased force constants from 20 to 10, 5, and 1 $kcalmol^{-1}Å^{-2}$. The total simulation length of the first stage is 2 nano seconds. Then the systems were equilibrated for 14 nano seconds and 80 snapshots were evenly collected from the following 8 nano second sampling phase.

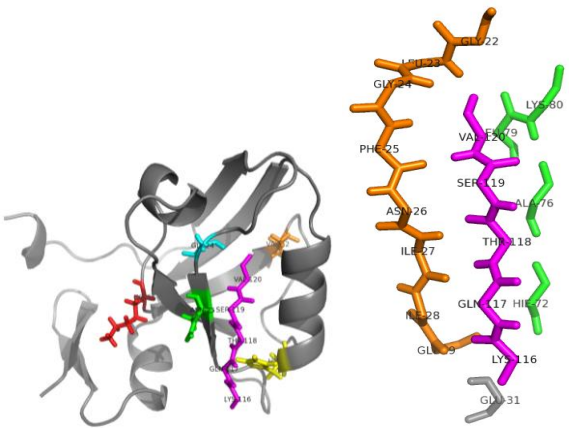

Figure 1. The cartoon representation of the crystal structure of PSD95 PDZ/CRIPT (left panel) and the stick representation of the surrounding residues of the peptide ligand (right panel). The CRIPT peptide is colored in magenta and the mutated residues are color-coded: K55-red, G24-cyan, N25-green, V62-brown, and H72-yellow.

B. MMPB/GBSA Analysis

The computational details of MM-PBSA and MM-GBSA binding free calculations were presented in our previous work.[17],[21] In brief, a modified GB model

developed by Onufriev et al. was applied to calculate the GB solvation free energies; [42] the PB solvation energies were calculated with the Delphi software package;[2] the nonpolar part of solvation free energies were estimated by weighting the solvent accessible surface areas using the default surface tension parameters suggested by the developers of the solvation models; normal mode analysis were performed using the NAB module of AMBER11 package,[33] after thoroughly minimization with the RMS gradient equal to or smaller than 2.0×10^{-12}; WSAS entropies were calculated followed the protocol described in Ref. 17. Finally, the free energy decomposition was performed with the same GB model using the Sander module of AMBER11 package.[33]

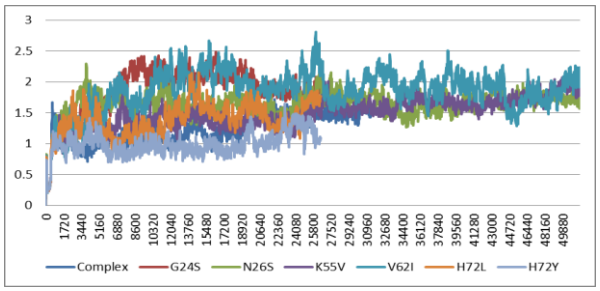

Figure 2. Root-mean-square displacements of the main chain atoms of PSD95 PDZ domain along the MD simulation time (in picosecond)

III. Results and Discussion

A. MD Simulations

As shown in Fig. 2, all the seven MD simulations are well equilibrated after around 5 nano seconds. For K55V, N26S and V62I, MD simulations were extended to 50 nano seconds, the trajectories are very stable during the course of 50 nano second MD simulations.

B. Binding Free Energy Calculations

TABLE I. Experimental Relative Binding Free Energies and Gas Phase Interaction Energies (kcal/mol)

Mutant	Expt. Binding Free Energy	MM_{gas}
WT	0.00	-355.02±10.18
G24S	1.67	-209.29±5.51
N26S	-0.31	-358.69±6.03
V62I	0.81	-284.14±5.63
H72L	1.78	-336.59±9.44
H72Y	3.14	-359.24±8.41

As the CRIPT peptide (KQTSV-COOH) in the crystal structure (1BE9) is four residues shorter from the N-terminal than the one studied by McLaughlin Jr et al. in measuring binding affinity. The absolute binding free energies for the truncated CRIPT peptide are not available. The relative experimental binding free energies for the truncated CRIPT peptide listed in Table I were estimated with an assumption that the four additional N-terminal residues have the same effect on the binding. This is a reasonable assumption since the key residues that define the function types of PDZ domains are the C-terminal residues.[26] In addition, as shown in Fig. 1, the additional N-terminal residues are unlikely have different interactions with the receptor for the six PDZ proteins in study.

In Tables II and III, the polar and nopolar components of solvation free energies are listed for the GBSA and PBSA solvation models, respectively. In Table IV, the conformation entropies by both normal mode analysis and the WSAS are listed. It is clear that the TΔS calculated by the two approaches have a good correlation (square of correlation coefficient is 0.72). As we have two solvation and two entropy models, there are in total four computational models evaluated, which are MM-GBSA-NMA, MM-GBSA-WSAS, MM-PBSA-NMA and MM-PBSA-WSAS. The binding free energies are listed in Tables V and VI. The correlations between the experimental and calculated relative binding free energies were calculated and the correlation coefficient squares are 0.84, 0.72, 0.07 and 0.11 for the above-mentioned four models, respectively. If the entropy contribution is ignored, the correlation coefficient squares become 0.64 and 0.15 for MM-GBSA and MM-PBSA, respectively. The correlation between the experimental and MM-GBSA-NMA relative binding free energies is shown in Fig. 3.

TABLE II. MM-GBSA Solvation Free Energies (kcal/mol)

Mutant	E_{GB}	G_{SA}	G_{GBSA}
WT	312.85±10.12	-6.22±0.07	-48.39±0.66
G24S	175.10±5.66	-5.27±0.08	-39.47±0.55
N26S	314.83±6.14	-5.94±0.10	-49.79±1.64
V62I	247.50±5.13	-5.69±0.04	-42.33±0.92
H72L	301.12±9.18	-5.60±0.07	-41.07±1.48
H72Y	323.75±7.30	-6.17±0.05	-41.66±1.36

TABLE III. MM-PBSA Solvation Free Energies (kcal/mol)

Mutant	E_{PB}	G_{SA}	G_{PBSA}
WT	328.05±9.46	-2.44±0.05	-29.41±0.94
G24S	182.10±5.96	-1.69±0.03	-28.88±1.12
N26S	323.98±6.16	-2.24±0.05	-36.95±1.00
V62I	261.16±5.17	-2.19±0.03	-25.17±0.87
H72L	304.35±9.33	-1.81±0.04	-34.05±1.18
H72Y	333.82±7.85	-2.04±0.04	-27.45±0.73

TABLE IV. Conformational Entropies Predicted by Normal Mode Analysis and Wsas

Mutant	TΔS by NMA	TΔS by WSAS
WT	-32.37±1.17	-23.86±0.13
G24S	-28.39±0.80	-21.75±0.15
N26S	-32.74±0.62	-23.28±0.24
V62I	-31.13±1.06	-22.51±0.07
H72L	-29.26±0.41	-22.60±0.19
H72Y	-32.75±0.49	-23.11±0.12

Although MM-PBSA has a more solid physical ground than MM-GBSA, unfortunately, the performance of MM-PBSA is far unsatisfactory in relative binding free energy calculations for this protein-peptide system. Interestingly, the similar conclusion was drawn when we evaluated MM-PBSA and MM-GBSA for protein-ligand bindings.[21], [22] As to how to improve the two types of solvation models, more discussions were presented in our previous publications.[17],[18]

TABLE V. TOTAL BINDING FREE ENERGIES BY USING GBSA

Mutant	MM-GBSA-NMA		MM-GBSA-WSAS	
	ΔG	ΔΔG	ΔG	ΔΔG
WT	-16.01±0.67	0.00	-24.53±0.66	0.00
G24S	-11.08±0.70	4.93	-17.71±0.52	6.82
N26S	-17.06±1.32	-1.04	-26.52±1.66	-1.98
V62I	-11.19±1.50	4.82	-19.81±0.87	4.72
H72L	-11.81±1.43	4.20	-18.47±1.49	6.07
H72Y	-8.91±1.36	7.11	-18.55±1.32	5.98

TABLE VI. TOTAL BINDING FREE ENERGIES BY USING PBSA

Mutant	MM-PBSA-NMA		MM-PBSA-WSAS	
	ΔG	ΔΔG	ΔG	ΔΔG
WT	2.96±1.37	0.00	-5.56±0.82	0.0
G24S	-0.49±0.98	-3.45	-7.12±1.07	-1.57
N26S	-4.21±0.87	-7.18	-13.67±1.06	-8.12
V62I	5.96±1.31	3.00	-2.66±0.89	2.90
H72L	-4.79±1.21	-7.75	-11.45±1.24	-5.89
H72Y	5.30±0.83	2.34	-4.34±0.69	1.21

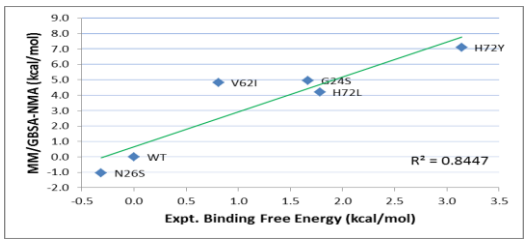

Figure 3. Correlation between the experimental and calculated relative binding free energies (kcal/mol)

C. Free Energy Decomposition

Free energy decomposition was performed for MM-GBSA for wide type PDZ and its five mutants. Hot spots, which make significant contribution to PDZ/CRIPT peptide binding, were then identified. In this work, a hot spot is recognized when its binding free energy to the peptide is equal to or smaller than -0.5 kcal/mol. Residues that have large adverse effect (binding free energy larger than +0.1 kcal/mol) were also selected. The contributions of hot spots and adverse spots are shown in Fig. 4. It is interesting that H72L and H72Y mutations do not lead to adverse interaction at Position 72, although the total MM-GBSA binding free energies are worse than that of the wild type.

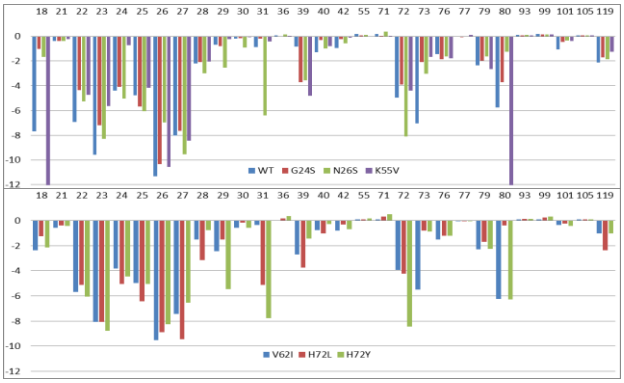

Figure 4. Hot spots and adverse spots of the PDZ protein that make significant contribution to the CRIPT peptide binding predicted by MM-GBSA. Vertical axis: MM-GBSA binding free energies, horizontal axis: residue positions.

IV. Conclusion

In this case study, we have performed MM-GB/SA and MM-PB/SA analysis for PSD95 PDZ domain. In agreement with our previous findings, MM-GBSA-NMA achieves an encouraging performance in reproducing the relatives binding free energies of wild type and five single residue mutants with a regression coefficient square of 0.84. This is an encouraging performance given the fact that the prediction of the binding free energy change due to mutagenesis is a more difficult problem than ranking similar ligands sharing the same binding mode.

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