Computational Binding Affinities of Disheveled PDZ Protein-Ligand Complexes

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ABSTRACT:

Wnt/ß-catenin signaling is critical for cell growth and development, with its hyperactive dysregulation implicated in the development of cancer. Current therapeutic research on inhibition of Wnt/ß-catenin signaling is impeded by the high cost of experimentally determining binding affinities. Consequently, interest has risen in screening potential inhibitors binding affinities with computational tools to reduce costs. Here, we test the validity of a computational molecular dynamics simulator, Binding Free Energy Estimator 2 (BFEE2), for determining peptide ligand affinity for Wnt/ß-catenin signaling. We focus on the Dishevelled (DVL) PDZ domain, a key mediator in WNT signaling through its ability to bind to various peptide ligands. We analyze the binding affinities of several DVL PDZ domain-peptide and domain-ligand complexes against previously established results to determine the validity of computational analysis. We conclude that computational molecular dynamics simulations were successful for peptide-ligand complexes with mixed results for small-molecule scenarios.

1. INTRODUCTION

The Wnt signalling pathway is crucial in cell growth, differentiation, and development. In the canonical cascade, Wnt ligands bind Frizzled receptors, which recruit Dishevelled-1 (DL1); DV1 subsequently promotes B-catenin stabilization [1]. However, overactivation of DVL1 can drive excessive B-catenin accumulation and uncontrolled cell proliferation. This hyperproliferation contributes to tumorigenesis, in particular retinoblastomas. Identifying potent small-molecule inhibitors that target the DV1 PDZ domain is critical in restoring normal Wnt signaling and cellular growth [2,3]. A convenient pre-screening tool would enable more efficient evaluation of new ligands, helping to prioritize those with stronger binding affinities to the Dishevelled protein.

Binding Free Energy Estimator 2 (BFEE2) was tested for validity as a pre-screening tool for the DVL domain. BFEE2 uses the geometric-restraint route via fixing the ligand's orientation with distance, angle, and dihedral restraints, samples the restrained complex while gradually decoupling the ligand. The restraints are then

analytically released to yield the standard-state binding free energy [4]. The DVL domain contains 22 protein ligand molecules which were considered for analysis, with 4 peptide-ligand complexes and 7 small molecule complexes meeting inclusion criteria. Complex structure files were sourced from RCSB Protein Data Bank (RCSB) and were parameterized according to standard NAMD procedure. Parameterized complexes were analyzed with BFEE2 for computational binding affinity output. In silico binding affinity obtained from BFEE2 was compared to published experimental values to assess for convergence. We found peptide-ligand complex experimental and computational binding affinities were convergent while most small molecule complexes had divergent binding affinities. We conclude that computational tool BFEE2 can adequately calculate peptide-ligand complex binding affinities; however, we find this method inadequate for small molecule binding affinity analysis without manual parameterization.

2. METHODS

2.1 Inclusion Criteria

DVL domain complexes were sourced from RCSB Protein Data Bank [5]. DVL domain complexes which lacked ligands were excluded for analysis given BFEE2 is a tool for modeling ligand affinity interactions. DVL domain complexes which had incomplete structure or no peer-reviewed experimental binding affinities were excluded from analysis.

2.2 Pre-Processing

Artifact water and solvent ions were pruned from complex protein database files to reduce X-Ray/NMR noise. Dimerized complexes were converted into monomers by analyzing the protein from one monomer and a ligand from the other monomer to create a single protein-ligand complex.

Atomic structure topology was generated by standard NAMD analysis. Molecular topology files from NAMD were used to construct an atomic connectivity file in a .psf format for analysis with Visual Molecular (VMD) AutoPSF. The complex .pdb and .psf molecule files were padded with 10 Angstroms to prevent interactions with periodic images and allow for realistic solvation. The system was solvated to 0.15 M NaCl to mimic biologic conditions [6,7].

2.3 BFEE2 Analysis

The CHARMM36 peptide parameter file was obtained from NAMD for the peptide complexes, and the parameter files for the set of small molecule ligands were each run and obtained from CGenFF, an online small molecule parameterization software [8–10].

Each step of the BFEE2 protocol targets a specific component of the ligand's binding affinity by applying Colvars (software library used in MD simulations to bias runs)

restraints to sample the energy landscape. Three different forms of restraints were used: harmonic, angular, and RMSD. A harmonic distance restraint keeps the ligand near the binding pocket by constraining the center-of-mass distance between the protein and ligand. Angular restraints limit the ligand's rotational freedom relative to the protein, enabling systematic sampling of orientational degrees of freedom such as eulerTheta, eulerPhi, eulerPsi, polarTheta, and polarPhi. The RMSD restraint ensures the ligand remains within a defined structural envelope, distinguishing bound from unbound states [4,11].

As part of Colvars, BFEE2 used the Adaptive Biasing Force (ABF) method to provide enhanced sampling. ABF identifies which degrees of freedom, like ligand rotation or separation, are energetically unfavorable then applies a small, adaptive biasing force to help the molecule overcome these energy barriers and explore the configurational space better.

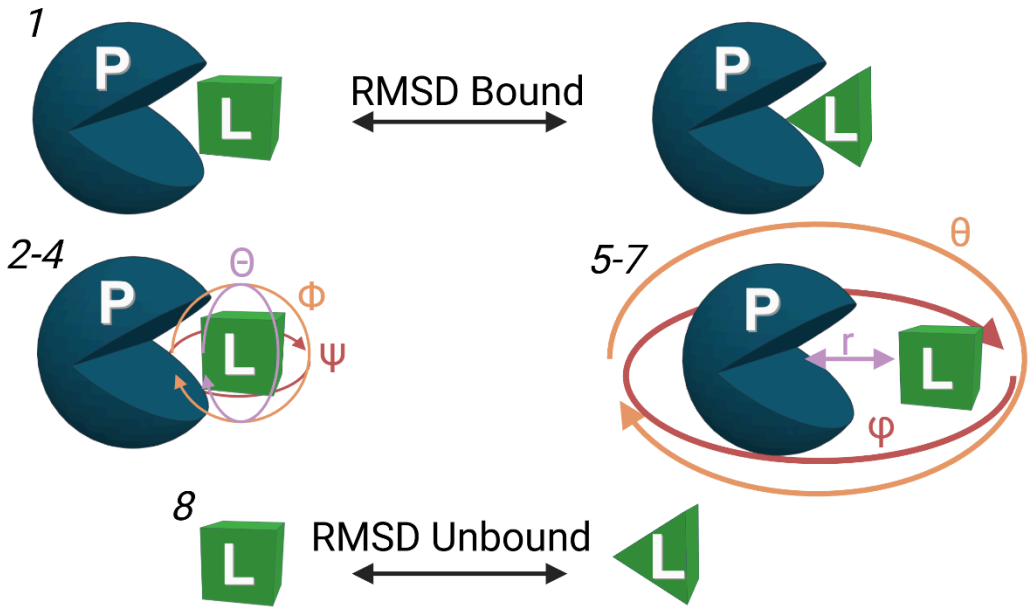

Figure 1. BFEE2 geometric route setup adapted from BFEE2 paper; steps 1-8 along with their respective restraints [11].

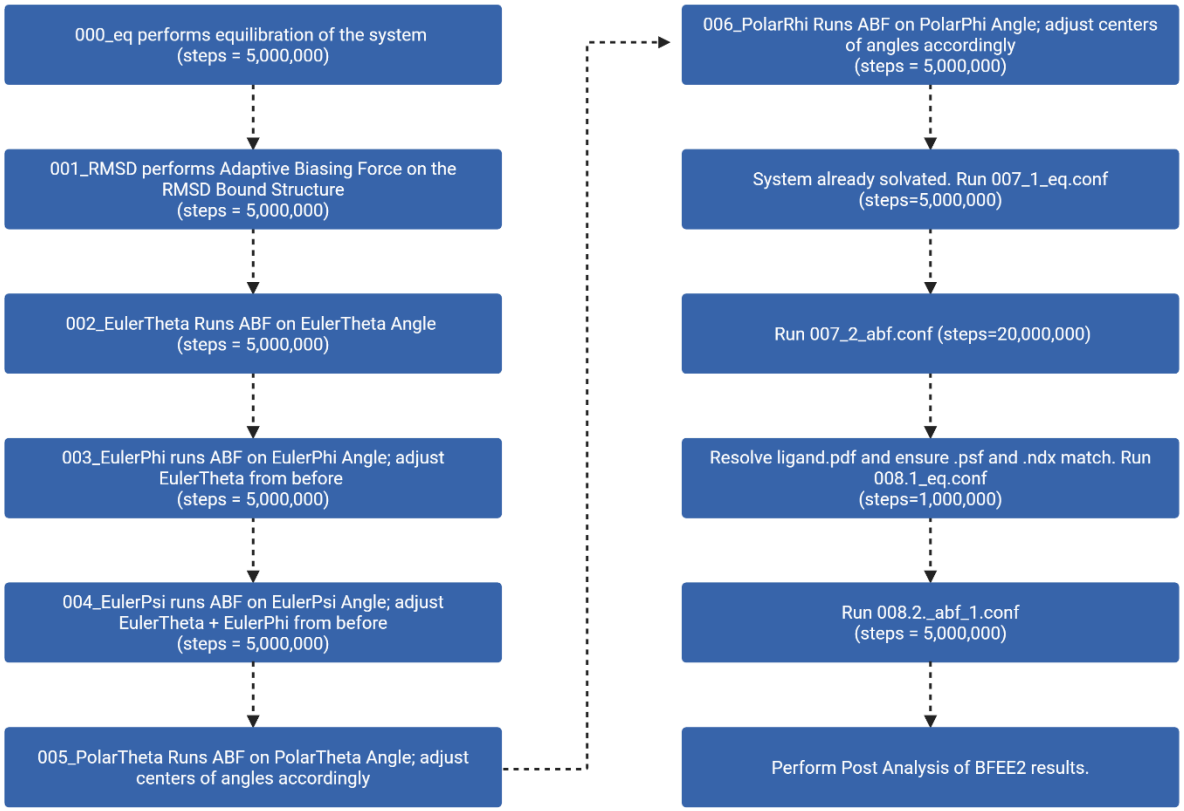

Figure 2. The default parameters of each step of the BFEE2 simulation are outputted with their corresponding steps. For small protein ligand complexes, the default parameterization approximately reaches RMSD convergence.

After running 5,000,000 steps in a BFEE2, a potential of mean force (PMF) is generated, displaying how the free energy changes along a selected collective variable (e.g. distance, angle, or orientation between protein and ligand). The binding free energy is then calculated using Boltzmann-weighted integration, which evaluates the probability of the ligand being bound versus unbound to yield the absolute binding free energy. Each step of the BFEE2 simulation corresponds to the free energy associated with a specific restraint, and summing up these values gives the total binding free energy of the ligand to the protein. To ensure the simulation ran properly, two key checkpoints were used. First, in the 000_eq (equilibration) folder, the DCD trajectory file was loaded into VMD alongside the corresponding PSF structure file to visually confirm that the ligand remained in the binding pocket and did not drift or unbind prematurely, which is a sign of an equilibrated system. Second, for each simulation window, the RMSD plot was inspected to verify convergence, which is indicated by RMSD values flattening out gradually towards run completion (Figure 2).

Computational binding affinities were compared to experimentally obtained values obtained from the published data on RCSB. Differences in binding affinities less than 1 kcal/mol were considered to be convergent while differences outside this range were considered to be divergent [4].

3. Results

22 DVL protein complexes were considered for analysis. 11 complexes (2F0A, 3FY5, 2REY, 6LCA, 1MC7, 6LCB, 8WWR, and 8YR7) were excluded due to lack of ligand complexes. An additional 2 proteins were excluded, 3CBZ for incomplete publicly available PDB structure, and 2MX6 for lack of verified experimentally determined binding affinity. 4 peptide-ligand complexes (1L6O, 3CC0, 3CBY, 3CBX) and 7 small molecules (6ZC6, 6ZBZ, 6ZBQ, 6ZC3, 6ZC4, 6ZC7, 2KAW) were considered for full analysis.

Peptide-ligand complex binding affinities calculated in silico were found to be congruent with verified experimental values, within ±1 kcal/mol.

PDB ID	**ΔGbind(calc) (kcal/mol)**	**ΔGbind(exp) (kcal/mol)**	**Convergent, Δ (kcal/mol)**
1L6O	-8.06	-7.08	Yes, (0.98)
3CC0	-7.86	-7.33	Yes, (0.53)
3CBY	-9.10	-8.13	Yes, (0.97)
3CBX	-8.37	-8.45	Yes, (0.08)

Table 1. Comparison of computational vs experimental binding affinities for all protein-ligand complexes [2].

Only 1 of 7 small molecule complex binding affinities calculated in silico were found to be convergent with verified experimental values.

PDB ID	**ΔGbind(calc) (kcal/mol)**	**ΔGbind(exp) (kcal/mol)**	**Convergent**
6ZC6	0.50	-6.67	No, (7.17)
6ZBZ	3.42	-5.29	No, (8.71)
6ZBQ	-3.12	-5.62	No, (2.48)

6ZC3	-0.64	Negligible affinity (>1000[ITC])	No, (undetermined)
6ZC4	8.44	-6.43	No, (14.87)
6ZC7	-5.93	-6.42	Yes, (0.49)
2KAW	6.61	-6.97	No, (13.58)

Table 2. Protein–ligand complexes for which the small-molecule force-field parameters were not fully validated. The calculated binding free energies (ΔG_bind(calc)) show poor agreement with experimental measurements (ΔG_bind(exp)), consistent with inaccurate parameterization. These data are included for completeness and to guide future reparameterization and benchmarking [12].

4. Discussion

BFEE2 binding affinity simulations were found to be relatively accurate when applied to peptide-PDZ complexes. This simulation validates the BFEE2 protocol for peptide ligand complexes. This accurate parameterization allows simulation to generate experimental measurements when only peptide-PDZ complexes are considered. Analysis of peptides with BFEE2 is likely accurate across different domains due to peptides simply being a collection of amino acids. Amino acid forcefields have been rigorously studied, and consequently BFEE2 simulations conducted with peptides are the most promising.

Unlike peptide-PDZ complexes, in silico and experimental results diverge when small molecule ligands are used. This divergence indicates the need for better force field parameter files, a well-known issue in the MD community. Small-molecule inhibitors will most likely require manual parameterization following CGenFF with programs like VMD FFTK(force field toolkit) that require quantum-mechanical calculations to obtain parameters.

5. Conclusion

We conclude BFEE2 is a useful pre-screening tool for dishevelled peptide ligands with significant correlation with experimental data. Use of BFEE2 tools for investigation of disheveled peptide proteins has been optimized and thus lacks the need for manual parameterization. We theorize that study of peptide binding affinities in general. Researchers investigating disheveled peptide proteins or other peptide ligands should have access to a good GPU or supercluster in order to conveniently run the NAMD simulations. Researchers should not need extensive manual intervention with parameterization or BFEE2.

Future research would involve trying the alchemical option on BFEE2 to see how binding affinity data compares to the geometric option already tested and manually parameterizing the set of small molecule parameter files to get better BFEE2 results.

SUPPLEMENTARY MATERIALS

Below outlines the issues encountered with each protein-ligand complex. This is included for future work on the peptides and small molecule ligand complexes particularly.

PDB ID	Pitfalls
1L6O	Peptide complex ran with no issues
6ZC6	Lone pair caused 008_RMSDUnbound to produce a “rattle shake algorithm” and crash. LP kept for 001-007 and taken out for 008_RMSDUnbound with just ligand
3CC0	Peptide complex ran with no issues
3CBY	Peptide complex ran with no issues
3CBX	Peptide complex ran with no issues
6ZBZ	CGenFF penalty errors low but still existent
6ZBQ	CGenFF penalty errors low but still existent
6ZC3	CGenFF penalty errors low but still existent
6ZC4	Lone pair caused 008_RMSDUnbound to produce a “rattle shake algorithm” and crash. LP kept for 001-007 and taken out for 008_RMSDUnbound with just ligand
6ZC7	Lone pair caused 008_RMSDUnbound to produce a “rattle shake algorithm” and crash. LP kept for 001-007 and taken out for 008_RMSDUnbound with just ligand
2KAW	Sulindac generated higher penalty errors.

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