Parameterization, Docking, and Molecular Dynamics Generated Binding Affinity for SHP2 *in silico*

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Abstract

In order to find optimal inhibitors of SHP-2, small organic molecules were parameterized using CHARMM General Forcefield. AutoDockFR was used to find docking affinity and orientation and optimal docking orientations were used as inputs for steered molecular dynamics simulations to find protein-ligand binding energy. Parameterization of novel molecules remains a challenge, but successful molecular dynamics simulations of already parameterized ligands and relative docking affinity scores show promise.

Purpose

Protein tyrosine phosphatase SHP-2 is unique due to its involvement in pathways that can both stimulate and shut down growth. Diseases such as Leopard Syndrome, Noonan Syndrome, and childhood leukemia are linked to mutations in SHP-2, further increasing the importance of SHP-2's study. In cooperation with the Leonard lab, the McCullough lab designs and computationally simulates ligands to inhibit SHP-2 and close its signaling pathways, allowing the pathways in question to be studied. This approach promises savings in time and money over brute force syntheses and *in vitro* analysis. Molecular dynamics software is used to test the efficacy of an inhibitor by simulating protein-ligand interactions.

Parameterization and CHARMM

Parameterization of potential inhibitors is necessary in order to accurately simulate binding with SHP-2 in solvent. Potential inhibitors are parameterized using the CHARMM philosophy and using the CHARMM General Force Field (Fig 1). Parameters are assigned by analogy to similar molecules and refined so that molecular dynamics simulations reproduce quantum mechanical properties. This is done using the ffTK plugin for VMD. Accurate parameterization is one of the more daunting and inherently difficult parts of this project, and our efforts are ongoing.

$$E_{\text{bonded}} = \sum_{\text{bonds}} K_b(b-b_0)^2 + \sum_{\text{angles}} K_\theta(\theta-\theta_0)^2 + \sum_{\text{imprprs}} K_\varphi(\varphi-\varphi_0)^2 + \sum_{\text{dhdrls}} \sum_{n=1}^{6} K_{\phi,n}(1+\cos(n\phi-\delta_n)$$

$$E_{\text{nonbnd}} = \sum_{\text{nonbnd pairs } i,j} \frac{q_i q_j}{4\pi D|\boldsymbol{r}_i-\boldsymbol{r}_j|} + \sum_{\text{nonbnd pairs } i,j} \epsilon_{ij}\left[\left(\frac{R_{\min\, i,j}}{|\boldsymbol{r}_i-\boldsymbol{r}_j|}\right)^{12} - 2\left(\frac{R_{\min\, i,j}}{|\boldsymbol{r}_i-\boldsymbol{r}_j|}\right)^{6}\right]$$

Figure 1. The CHARMM General Force Field potential energy function, which contains terms for interatomic bonds, angles, impoper and proper dihedrals, as well as electrostatic and Leonard-Jones potentials.

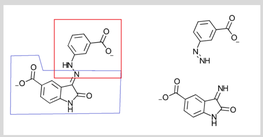


Figure 2. Potential inhibitor with individually parameterized substructures.

Docking

Docking programs simulate electrostatic interactions within the protein-ligand system to find probable binding sites and relative affinity. By testing many different positions, orientations, and conformations of both the ligand and protein, Autodock Flexible Receptors (ADFR) attempts to find the most stable protein-ligand complex. The ADFR software creates reasonable starting points for molecular dynamics simulations and streamlines the process of finding binding energies.

Figure 3. SHP2 with highlighted active site (green) and WPD loop (red).

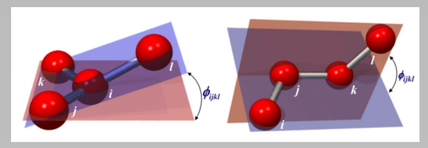


Figure 4. Improper (left) and proper (right) dihedrals. Image from http://cbio.bmt.tue.nl

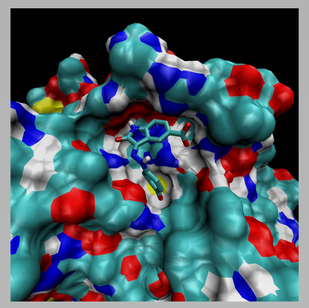


Figure 5. Favorable protein-ligand complex proposed by docking simulation.

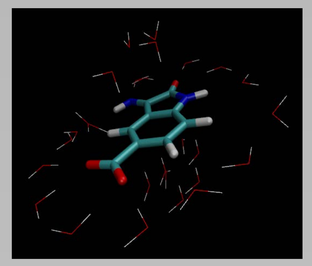


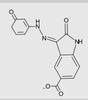
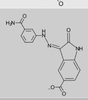
Figure 6. Water interactions used for charge parameterization.

Molecular Dynamics

Molecular Dynamics (MD) and Steered Molecular Dynamics (SMD) are used to obtain approximate binding energies for solvated protein-ligand complexes in order to compare inhibitor success. Best docking orientations for the protein-ligand complex are simulated for a realistic *in vitro* environment for several hundred picoseconds before running many slow, fixed-velocity SMD simulations to remove the bound ligand. Path integrals of the resultant forces are then averaged and translated into approximate binding energy through use of the Jarzynski inequality.

Results and Future Direction

Docking affinity scores yield an approximate ranking of potential inhibitors, but need validation through molecular dynamics studies. Results to date suggest that the active site interacts favorably with large negative charges, a finding supported by other researchers[5]. The SMD method of obtaining approximate binding energies for inhibitors was used successfully and is ready for application with novel molecules pending satisfactory parameterization.

Ligand	ADFR Score
	-14.676
	-15.178
	-13.264

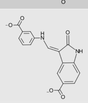
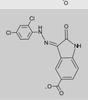
Ligand	ADFR Score
	-13.366
	-15.119
	-13.577

Figure 7. Novel SHP-2 inhibitors and their AutoDock affinity scores. More negative scores indicate a better binding affinity and therefore better inhibitors.

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