Project Title:

Prediction of protein-ligand binding affinities using the free-energy perturbation and generalized-ensemble methods

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1. Background and purpose of the project, relationship of the project with other projects

Abl kinase (Abelson tyrosine kinase) is a cytoplasmic non-receptor tyrosine kinase involved in cellular signaling, cytoskeletal dynamics, and DNA damage response. In chronic myeloid leukemia (CML), the BCR-Abl fusion protein drives uncontrolled kinase activity, leading to cancer progression, making it an important drug target. However, like other protein kinases, its conformational flexibility and reliance on key motifs make it a difficult target for drug design. Imatinib (Gleevec) is a small-molecule tyrosine kinase inhibitor (TKI) that specifically targets BCR-Abl. It binds to the inactive conformation of the ATP-binding site, preventing ATP from phosphorylating downstream signaling proteins. By blocking this phosphorylation, imatinib effectively inhibits aberrant cell proliferation. Mutations in the Abl kinase domain are a key factor in imatinib resistance in CML. While their exact mechanism remains unclear, they disrupt imatinib's ability to stabilize the inactive conformation of Abl, reducing drug binding affinity and allowing kinase activity to persist, ultimately leading to treatment failure.

This project is part of a broader study investigating the relationship between conformational states, ligand binding affinity, key structural motifs, and mutations in kinases. Here, we focus on the interaction between Abl and imatinib to evaluate how mutations in Abl impact their binding free energy. The primary objective is to apply Free Energy Perturbation (FEP) calculations to the Abl-Imatinib complex, quantifying how specific mutations alter binding affinity.

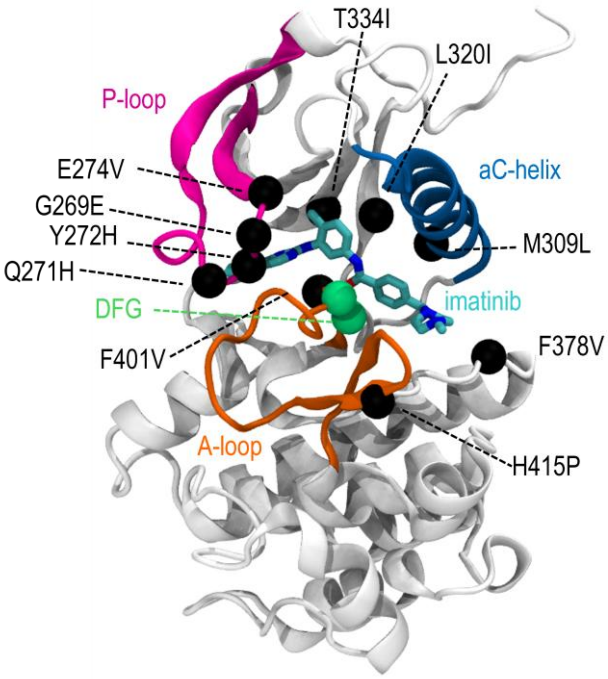

Figure 1. Structure of Abl kinase bound to imatinib used in the study. Important structural motifs and mutations are marked.

2. Specific usage status of the system and calculation method

We performed mutation-FEP simulations to evaluate the impact of 10 known oncogenic mutations (T334I, H415P, Y272H, E274V, F378V, Q271H, G269E, M309L, L320I, and F401V) on imatinib binding affinity.

To obtain the difference in binding free energy between the wild-type (WT) and a mutant (e.g.,

T334I), we follow the thermodynamic cycle depicted in Figure 2, where horizontal processes represent binding and vertical processes represent mutations. The binding free energy difference ($\Delta\Delta G_b$(T334I)) is given by:

$$\Delta\Delta G_b(T334I)=\Delta G_b(T334I)-\Delta G_6(WT)=\Delta G_{mut}^{complex}-\Delta G_{mut}^{apo}$$

We perform mutation-FEP simulations for each mutation in two separate environments, the complex state ($\Delta G_{mut}^{complex}$) and the apo state of Abl (ΔG_{mut}^{apo}). The Abl kinase-imatinib WT complex (PDB: 1IEP) was used as the structural template. Calculations were performed using GENESIS 2.0 MD software (Jung et al. *JPCB* 128.25 (2024): 6028-6048.) with the AMBER ff99SB-ILDN force field for the protein and GAFF for imatinib (with AM1-BCC), employing a dual topology approach for FEP. To enhance sampling efficiency, FEP simulations at different λ values were coupled using the Hamiltonian replica exchange method. A total of 21 λ windows (=replicas) were used, with each window running for 50 ns, and exchanges attempted every 10 ps.

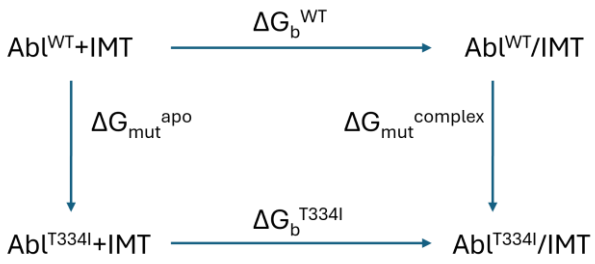

Figure 2. Thermodynamic cycle used in this study to calculate the effect of mutation on binding affinity between Abl and imatinib.

3. Result

So far, we completed the first set of simulations, obtaining $\Delta G_{imut}^{complex}$ in the complex state for five of the ten mutations (Figure 3).

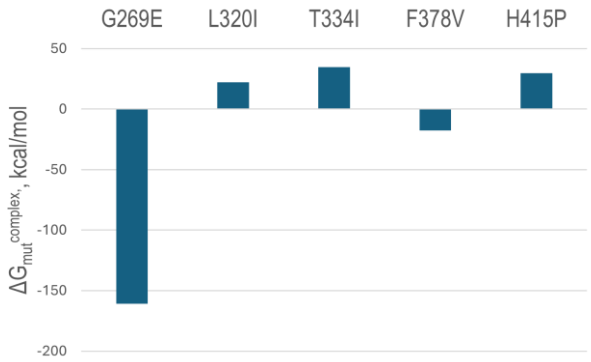

Figure 3. Differences in free energies between the WT and mutants for the Abl-imatinib complex.

The charge-changing G269E mutation exhibits a significantly larger energy difference compared to other mutations, suggesting a potential sampling insufficiency. Further analysis is needed to confirm convergence. For the other mutations, the energy differences are smaller. However, we need to wait for the apo FEP results to determine their effect on ΔΔG.

4. Conclusion

We performed mutation-FEP simulations to evaluate how 10 oncogenic mutations in Abl kinase affect imatinib binding affinity. So far, we completed simulations for the complex state, revealing that the charge-changing G269E mutation shows a much larger energy difference, possibly due to insufficient sampling. The effect of other mutations appears smaller, but final conclusions depend on the apo FEP results.

5. Schedule and prospect for the future

Next, we will perform the apo FEP simulations to obtain $\Delta\Delta_bG$ and complete calculations for the remaining five mutations. Since the structure used is in the active state, and mutations can shift the active-inactive equilibrium, this must be considered in our analysis. By combining these results with additional simulations sampling the conformational energy landscape of Abl, both with and without imatinib, we aim to elucidate the relationship between conformational states, ligand binding affinity, key structural motifs, and mutations in kinases.