Evaluating Imatinib's Affinities and Specificities for Tyrosine Kinases Using Molecular Dynamics Simulations

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ABSTRACT

Computational chemistry lets us model intermolecular interactions in ways assays cannot. My project focuses on the multi-kinase interactions of the cancer drug, imatinib. Most cancer drugs target one kinase, but some affect multiple kinases. Imatinib treats chronic myeloid leukemia by targeting ABL kinase. Proteomics data reveals it can interact with other kinases, such as KIT to treat gastrointestinal stromal tumors, but the mechanisms are unknown. Imatinib has different affinities for similar kinases, such as a 3000x difference between ABL and SRC, despite sharing 50% structural homology. Here, I investigate the conformational differences between free and imatinib-bound ABL, KIT, and SRC using Molecular Dynamics simulations to understand the key imatinib-kinase interactions. The alignment analysis shows the docked conformations are similar to co-crystal structures in the Protein Data Bank. Root-mean-square-deviation and fluctuation (RMSD and RMSF) analysis show that all simulations converge at 45 ns, with some regions exhibiting differential flexibility. Hydrogen bond analysis across 100 ns simulations show that ABL has one main H-bond, KIT has three main H-bonds, and SRC has no main H-bonds. All the drug-kinase complexes feature at least 15 key salt bridge interactions relevant for structural stability. The dihedral distributions reveal that most residues adopt a single conformation, but some can adopt multiple, increasing the protein flexibility. The entropy results quantify the protein disorder, revealing KIT and SRC favors the apoprotein while ABL favors the complex. This signifies that broad protein similarity does not govern imatinib binding, instead, it is explained by smaller structural details.

KEYWORDS: Drug design, molecular mechanics, kinome, CML, GIST, Off-target

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Dr. Chia-en Chang is a Professor of Chemistry in the Department of Chemistry. She received her Ph.D. from the University of Maryland and conducted post-doctoral research at UC San Diego. Her work focuses on applying computational simulations for biomolecular recognition and drug discovery. She has published over 80 papers on molecular modeling, drug binding, and protein dynamics. She was a previous recipient of the Robert T. Poe Faculty Development award, the Faculty Development Award & Omnibus Travel Award, NSF Career Award, and Chancellor's Award for Excellence in Undergraduate Research. She is also the Vice Chair of the Chemistry Department.

William Troxel

William Troxel is a third-year Biochemistry major. He has conducted computational proteomics research in the Dr. Chia-en Chang lab since January 2020. He is a MARC U STAR Trainee, a former Lead Recruitment Coordinator, and current President of the MARC organization. He delivered an award-winning poster at ABRCMS in November 2021, is a Barry Goldwater Scholarship nominee, and a Phi Beta Kappa invitee. He is conducting an REU at Texas A&M for summer 2022. He will pursue a Ph.D. in Molecular Biophysics to apply computational tools for novel drug discovery.

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INTRODUCTION

Computational protein research allows us to visualize complicated protein-drug interactions in ways conventional protein assays fail to. Drugs designed to target specific signaling proteins may affect other proteins due to shared structures, resulting in unforeseen side-effects and increasing medical applications (Moy et al., 2010). My project focuses on how the cancer drug, imatinib, interacts with ABL, KIT, and SRC kinases. Kinases help reduce the energy needed to transfer phosphate groups from adenosine triphosphate (ATP) to specific acceptor groups on proteins for biochemical reactions. The phosphate charges affect the protein's structure and functions (Koch, 1999). With kinome interaction patterns, kinase profiling data of known inhibitors can predict interactions with help from simulations (Davis et al., 2011).

ABL is a tyrosine kinase involved in cell differentiation, division, and DNA repair, however, overexpression due to ABL-BCR fusion corresponds with chronic myeloid leukemia (CML) for ~95% of reported cases (Aleksandrov and Simonson, 2009) (Ayatollahi, 2018) (Golzarroshan, 2012) (Zagaria, 2015). KIT is a tyrosine kinase involved in cell survival, spread, and differentiation, but overexpression due to KIT mutations corresponds with at least 85% of gastrointestinal stromal tumors (GIST) (Nowain, 2005) (Zhao, 2012). SRC is a tyrosine kinase involved in cell signaling, and while it shares approximately 50% homology with ABL, it has reduced imatinib affinity by 3000x (Aleksandrov and Simonson, 2009) (Ortiz, 2021).

Imatinib is a type II ATP-inhibitor that targets inactive conformations in ABL to treat CML (Smith et al., 2014). It forms six hydrogen bonds (H-bonds) with surrounding residues; E286, T315, M319, I360, H361, and D381 (Dubey, 2011). Van der Waals' interactions on the aromatic ring and a hydrophobic pocket neighboring the piperazinyl-methyl group also contribute to imatinib's ABL affinity (Asaki et al., 2006) (Nagar et al., 2002). Salt bridge interactions have a cumulative effect on protein stability, preventing ATP from binding and suppressing proliferative signals (Eck et al., 2009). This inhibits ABL kinase by reducing phosphorylation of cancer proteins and signal cell transduction in the pathway (Lupino et al., 2014). Researchers later realized imatinib can block PDGF-R and KIT tyrosine kinases, and it is used to treat acute lymphocytic leukemia (ALL), GIST, and dermatofibrosarcoma protuberans (Moy et al., 2010) (Salah et al., 2011) (Seggewiss et al., 2005). This demonstrates the importance of drug and multi-kinase research, as the off-target effects may expand imatinib's purpose and cost-effectiveness. Large-scale proteomics shows imatinib has favorable affinities with many kinases from different families, but the mechanisms remain unknown (Miao et al., 2019).

My objective is to understand imatinib's multi-kinase interactions and selectivities to predict drug efficacy from the bound states and guide structure-based drug design. This will elucidate imatinib's differential affinities and interactions with these kinases based on structural elements and how imatinib's promiscuity changes in different protein environments. If certain kinases share similar structures on the binding site, then the bound complexes should have similar conformations, free-energy, and entropy scores due to their interrelatedness from molecular evolution. If the drug-kinase interactions exhibit different conformations, free-energy, and entropy results, then it would suggest other variables impact drug-kinase binding.

METHODOLOGY

Computational Models

Protein crystal structures are selected from the Protein Data Bank including imatinib-ABL, imatinib-KIT, and imatinib-SRC complexes (PDB: 2HYY, 1T46, 2OIQ). The

disordered residues are resolved using SWISS-MODEL.

MOLECULAR DOCKING

I use molecular docking to study the binding energies and key residue interactions between imatinib and the kinases. Imatinib is removed from the crystal structure using Visual Molecular Dynamics. The imatinib is docked to the three kinases using AutoDockTools with a 22.5x22.5x22.5 cubic angstroms grid box with 20 Lamarckian Genetic Algorithm runs. The energy is reported in kJ/mol, with the average and standard deviation illustrated in table 1.

Molecular Dynamics

I use Molecular Dynamics (MD) simulations to study the conformational changes between the free protein and bound complexes since imatinib interacts with ABL, KIT, and SRC. MD simulations are conducted using the AMBER18 package. The proteins and ligands are parameterized using AMBER ff14sb and Generalized Amber Force Field 2 (GAFF2), respectively (Maier, 2015). They are solvated in a water box using the TIP3P water model with a 12-angstrom buffering distance. To neutralize the systems, 8 sodium ions are added to imatinib-ABL, 5 sodium ions are added to imatinib-SRC, while imatinib-KIT is already neutral. They are minimized starting with hydrogen atoms for 500 steps, then side chains for 5000 steps, then the entire structure for 5000 steps. They are equilibrated in a constant number of molecules, pressure, and temperature (NPT) ensemble, and heated in 50 K increments lasting 200 ps from 100 to 298 K. MD runs are conducted for 100 ns using Langevin thermostat. A 12-angstrom cutoff is used for non-bonded energy calculations, the particle-mesh Ewald method is used for electrostatic interactions, and the SHAKE algorithm is used to constrain covalent bonds involving hydrogen atoms. Trajectories are saved every 2 ps and processed every 20 ps using Amber's cpptraj plug-in. The waters are removed post-production for easier observation. From the MD results, I examine the H-bond and salt bridge frequencies, the dihedral conformations of the residue backbone and side chains, and the entropy for the protein's thermodynamic properties.

Kinase	Docked energy (kJ/mol)
ABL	-10.16 ± 1.13
KIT	-9.29 ± 1.05
SRC	-7.83 ± 1.20

Table 1. Energy results for ABL, KIT, and SRC docking show significant differences for SRC from ABL and KIT.

RESULTS

Molecular Docking Agrees with Experiments

The docking results show that ABL, KIT, and SRC binding have average docking energies of -10.16, -9.29, and -7.83 kJ/mol, respectively. ABL and KIT have statistically similar energies, while SRC is significantly different. The imatinib adopts similar conformations in the binding pocket as the existing crystal structures, demonstrating high accuracy.

RMSD Shows Apoprotein and Complex Stabilization

Figure 1 shows the RMSD for the free apoprotein and imatinib-bound ABL, KIT, and SRC kinases over the 100 ns MD simulation. The ABL equilibrates at 30 ns, KIT at 45 ns, and SRC at 45 ns. The structural stabilization by 45 ns signifies all structures are experimentally appropriate.

RMSF Reveals Flexible Regions of Interest

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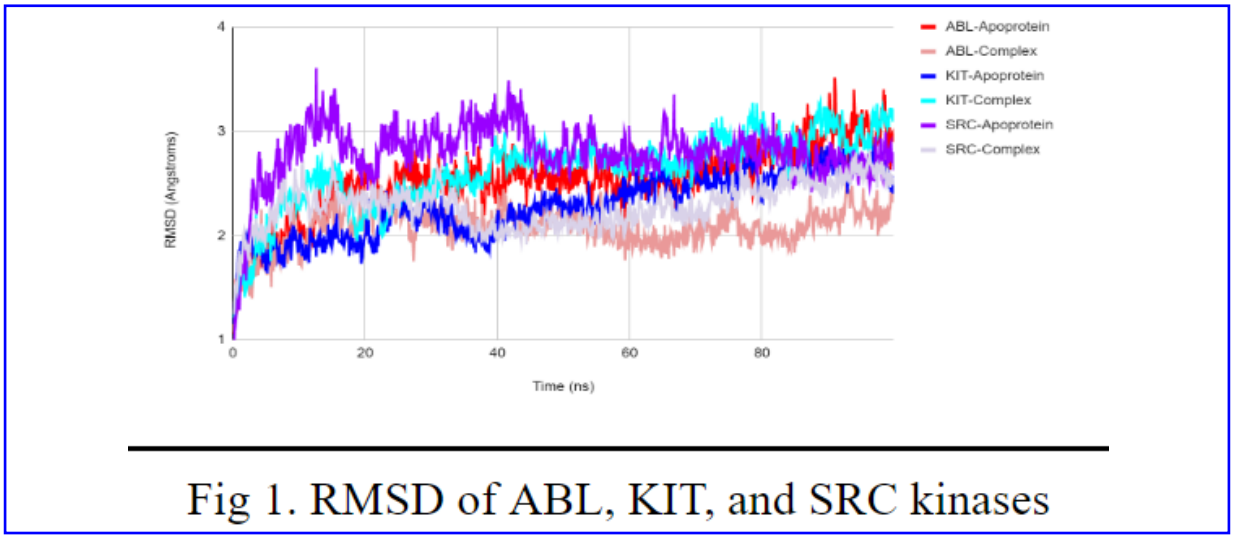

Figure 1. Root-mean-square-deviation for 100 ns ABL, KIT, and SRC apoprotein and imatinib-bound kinases shows stabilization by 45 ns.

The 3D structures for **figures 3, 5, and 7** are colored in accordance with the RMSF plot to reveal the flexible protein regions of interest. **Figure 2** shows broad similarities in the ABL RMSF, except for residues 40-45, which have a greater apoprotein RMSF than the complex. This agrees with the RMSD and ABL's experimentally strong affinity for imatinib. **Figure 4** shows broad similarities in the KIT RMSF, except for residues 127-135 with greater complex RMSF compared to the apoprotein in agreement with the RMSD. **Figure 6** shows that the entire SRC RMSF reveals no significant differences in apoprotein or complex RMSF, aligning with the RMSD and the docking results. This affirms that imatinib has lower binding affinity with SRC.

More H-bonds in ABL Complex and More H-bonds in KIT and SRC Apoprotein

ABL forms 252 intraprotein H-bonds in the complex compared to 240 in the apoprotein. KIT forms 283 intraprotein H-bonds in the apoprotein and 274 in the complex. Finally, SRC forms 243 intraprotein H-bonds in the apoprotein and 237 in the complex. Between the proteins and imatinib, the main H-bond that formed between ABL and imatinib involved M84 for about 20.4 ns. In KIT, there are two main H-bonds between C109 and E76 for 16.8 ns and 11.4 ns, respectively. There are no main H-bonds between SRC and imatinib.

ABL, KIT, and SRC Salt Bridge Role in Apoprotein and Complex Structures

Figure 8 shows that the most substantial salt bridge change forms between E45-R152 in ABL. This salt bridge only forms in the ABL-imatinib complex, but not in the free ABL protein. **Figure 9** shows salt bridges between D143-K129 and E145-K129 in KIT form in the complex but are broken in the apoprotein.

Dihedral analysis reveals residues with multiple rotamers

Most of the dihedrals have a monomodal distribution, meaning the backbone and side chains tend to adopt one conformation. However, several residues express multimodal distributions, representing multiple favorable

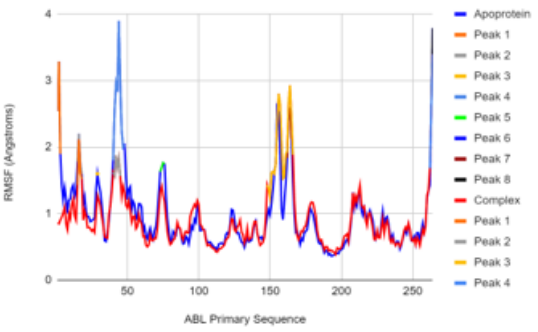

Fig 2. ABL RMSF

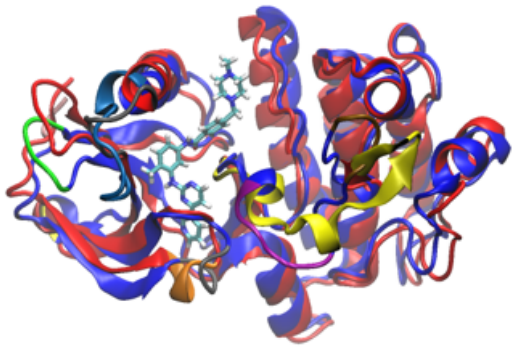

Fig 3. ABL 3D structure

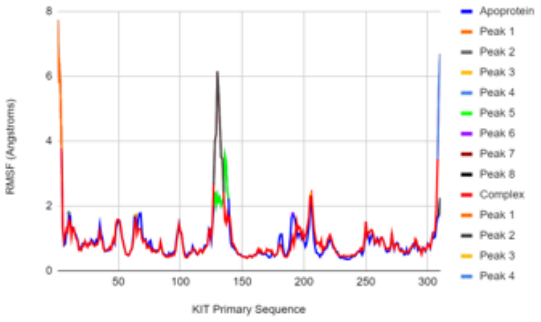

Fig 4. KIT RMSF

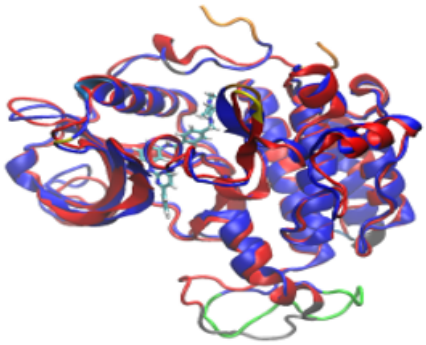

Fig 5. KIT 3D structure

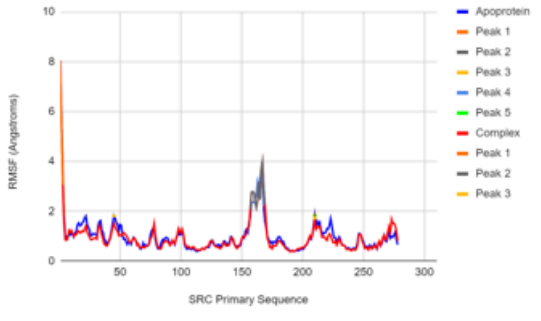

Fig 6. SRC RMSF

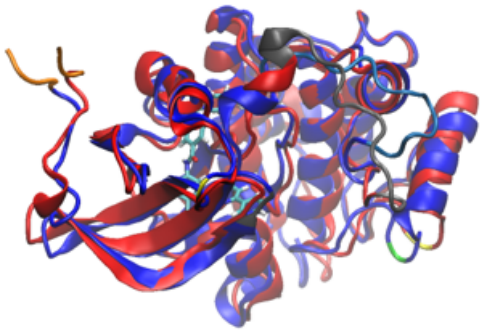

Fig 7. SRC 3D structure

Figures 3, 5, and 7. Superimposed 3D structures for apoprotein (blue) and imatinib-bound complex (red) ABL, KIT, and SRC structures, respectively. **Figures 2, 4, and 6**. Root-mean-square-fluctuation for the ABL, KIT, and SRC apoprotein and imatinib-bound complex revealing flexible protein regions.

Evaluating Imatinib's Affinities and Specificities for Tyrosine Kinases Using Molecular Dynamics Simulations

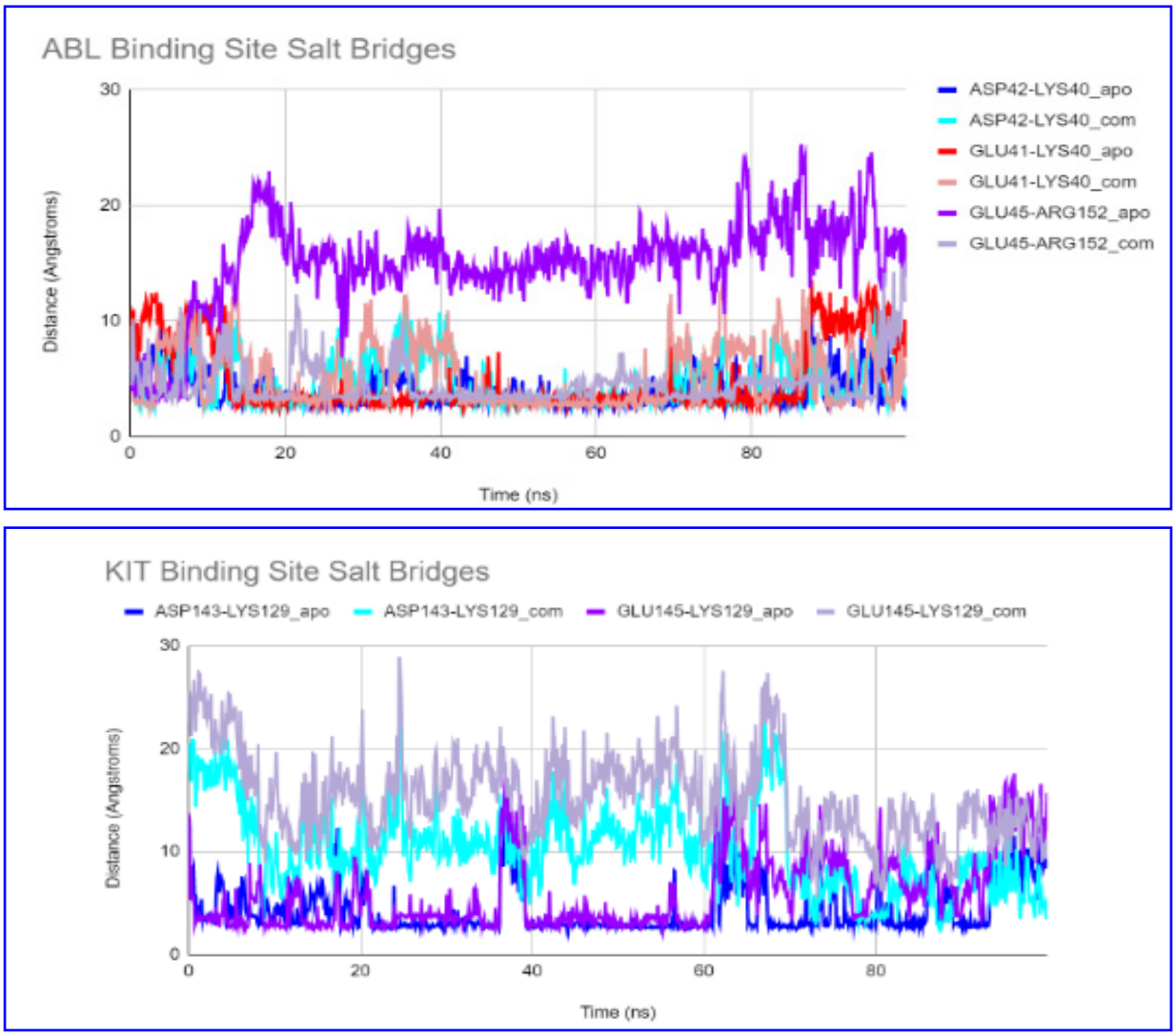

Figure 8 (above) - Salt Bridges on ABL Binding Site. **Figure 9 (left) -** Salt Bridges on KIT Binding Site.**Figures 8 and 9.** Salt bridges that interact with residues within 8 angstroms of imatinib, representing thprotein binding site.

energy wells and conformations the dihedral angle adopts in the protein. This results in higher flexibility, RMSF, and entropy values.

Entropy analysis of the binding site corresponds with RMSF and non-covalent interactions

For the binding site, the most substantial difference is in KIT, with a 2-3 kcal/mol difference favoring the apoprotein structure for the dihedrals. For ABL, the entropy difference is smaller and tends to favor the complex by 0.1-0.3 kcal/mol for the dihedrals. For SRC, the entropy difference favors the apoprotein by 0.4-0.7 kcal/mol for the dihedrals.

DISCUSSION

The purpose of molecular docking is to examine how small molecules bind to larger macromolecules as their interactions are unavailable from the Protein Data Bank. The docking software samples a topological grid to find the global minimum for the drug-protein complex and calculate docking energy values. For the imatinib-kinase trials, the results agree with known results, showing imatinib binds more tightly to ABL and KIT than SRC (Dubey, 2012) (Lin, 2013).

The purpose of RMSD is to show that the structure stabilizes by the end of the simulation. When sampled from the Protein Data Bank, protein structures experience steric hindrance from intramolecular repulsion, so there is an initial increase in the RMSD before it equilibrates. I expect that differences between the bound complex and apoprotein are attributable to the imatinib binding. The RMSD results affirm that imatinib binds tightly with ABL, as it indicates the apoprotein is more flexible than the complex. KIT also exhibits significant change, but with a more flexible apoprotein structure compared to the complex. This is an unusual observation since the apoprotein is typically more flexible. Finally, SRC exhibits similar apoprotein and imatinib-bound RMSD, agreeing with the idea that SRC has a weak affinity for imatinib.

Proteins fold into specific structures to work in the body, so changes in the protein flexibility after ligand binding can affect its bodily function (Teilum et al., 2011). The purpose of RMSF is to discover the flexible residues that contribute most to protein dynamics by examining the positional differences of the structure over the simulation run. ABL and SRC share ~50% of their structure, so it is expected that they share peaks in similar locations. Unlike ABL, SRC has insignificant RMSF differences between the apoprotein and complex structure. This affirms that imatinib's presence in the binding pocket has little effect on SRC's dynamics. KIT peaks in different regions than ABL or SRC, demonstrating that different protein structures can have similar affinities for the same drug while similar structures can have different affinities. This is important as it signifies we cannot rely on the structural similarities alone to judge drug affinities for proteins.

Hydrogen bonds are a crucial non-covalent interaction to maintain protein structures, but they get weaker at longer distances and larger angles. Therefore, I parameterized the H-bond with a maximum length of 3.5 angstroms and angle of 150 degrees for at least 20 ns to categorize it as structurally significant. ABL contains more H-bonds in the complex than the apoprotein, which agrees with the RMSD and RMSF, as more H-bonds help maintain protein rigidity. In contrast, KIT and SRC form more H-bonds in the apoprotein compared to the complex, further agreeing with the RMSD and RMSF. The drug-protein H-bonding shows that ABL and KIT form H-bonds with the imatinib while the SRC has no main H-bonds, explaining the comparatively different docking energies and affirming SRC's lower imatinib affinity compared to KIT or ABL.

Salt bridges weaken as the distance between interacting groups increases. Therefore, I classified a salt bridge when the heavy atoms of D and E are within 4 angstroms distance of K or R residues, where a "significant" salt bridge forms when at least 20 ns of the simulation features a 4-angstrom distance or less (Kumar, 2002). I evaluated the ion pairs that form along the relevant RMSF peaks to investigate how salt bridges change with imatinib binding. Several ion pairs that broke in the apoprotein structure for ABL formed in the complex, and vice versa for KIT. The RMSF for SRC remains similar in the apoprotein and SRC-imatinib complex, meaning the ion pairs likely have little impact on the protein's flexibility after imatinib binds.

I studied the entropy of the binding site residues within 8 angstroms of imatinib for ABL, KIT, and SRC to assess

the relative entropy differences between the apoprotein and bound complex. The KIT and SRC entropy favored the apoprotein structure, with KIT exhibiting a larger difference. For ABL, the entropy difference was also small and favored the complex. The entropy findings are consistent with the observations for protein RMSD, RMSF, and the non-covalent interaction frequencies, and serve as a useful quantitative tool to measure protein flexibility in apoprotein and bound-complex conditions.

FUTURE FOCUS

In the future, I project to simulate the drug-protein interactions of eleven kinases of interest including ABL, KIT, SRC, LCK, p38α, ASK1, AURKA, BRAF, FLT3, CHK1, and GSK3β in the first comprehensive study in atomistic-level details for understanding imatinib binding specificity for future cancer drug design. In addition, I will study the protein structure changes as imatinib unbinds from the kinases. Finally, I will look more closely at the H-bonds and salt bridge interactions that form, break, and reform as imatinib dissociates. From these simulations, I will approximate the affinities based on residence time, the amount of time it takes for the drug to unbind from the protein.

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