Enzyme Inhibition

1327-Pos Board B57

Molecular Dynamics of the Dengue Virus NS3/NS2B Protease in Presence of Inhibitor or Substrate

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Dengue is a neglected tropical disease affecting millions of people, and may lead to death. Brazil is the country with the highest number of dengue and dengue hemorrhagic fever (D/DHF) cases combined.[1] However, recent news show that dengue virus reaches areas that had not been hit earlier as the Madeira Island (Portugal) [2], Florida (United States) [3], and in New Caledonia (Oceania) [4]. There are no specific medicines for the treatment of D/DHF and, once infected, the WHO recommendations are limited to observation and symptomatic treatment. Recent efforts have revealed a series of proteins essential to the dengue virus's life cycle, which may be used as targets for new medicines. [5].

The objective of this study is to understand the mechanism of action of the nonstructural protein NS3 protease complexed with the cofactor NS2b (NS3/NS2b), responsible for cleaving the viral polyprotein during the virus replication step, and understanding of interaction of this protein with its substrate and inhibitor through molecular dynamics study.

We will be present molecular dynamics simulations of the NS3/NS2b complex alone, in the presence of the substrate (Ala-Gly-Arg-Lys-Ser-Ile) or an inhibitor (Bzo-Nle-Lys-Arg-Arg-H) in the active site, as well as hybrid QM/MM simulations for understanding the enzymatic mechanism.

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Dynamical Network in HIV-1 Protease and its Mutants: Implications on Drug Resistance

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Drugs targeting HIV protease have long been used in AIDS therapy. However, emergence of multi-drug resistance, due to active and non-active site mutations, intensifies the need to understand the drug resistance mechanism of the enzyme. Here, we employ molecular dynamics (MD) simulations and network analyses to unravel the drug resistance mechanisms. Results from MD simulations suggest that the mutants modulate either the ligand binding envelop in the active site or the dynamics of flaps in HIV-1 protease. While the mutations at the active site or flap region account for drug resistance directly, the mechanism of allosteric mutations could not be explained. Network analyses show that the allosteric mutations affect the functional sites by modulating the stress centrality. Stress interference data ascribe the residues 71, 90 and 93 as the active site modulators and 15, 20 and 36 as the flap modulators. The integrity of the global network, however, was not affected by deletion of the mutant-prone nodes, indicating that the function of HIV-1 protease is preserved.

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Understanding the Molecular Mechanism of Synergistic Inhibition in the Hepatitis C Virus (HCV) Polymerase Using Molecular Dynamics Simulations and Free Energy Calculations

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The Hepatitis C Virus (HCV) affects close to 200 million globally. A major challenge in treating this infection is the emergence of resistance to current treatment regimens. An approach to reducing the rate of drug resistance is to increase the inhibitory effects of allosteric inhibitors by using them in combination to target the HCV polymerase (NS5B). Although recent biochemical studies show the use of multiple allosteric inhibitors has a synergistic inhibitory effect on NS5B, the molecular mechanisms by which this synergistic inhibition occurs still have not been clearly elucidated. To garner insight into the mechanism of synergistic inhibition of NS5B, we employ conventional and temperature accelerated molecular dynamics simulations of the enzyme simultaneously bound to two allosteric ligands. In concert with covariance and principal component analyses, data from the simulations allow us to compare specific structural and dynamic properties of the free and ligand-bound protein. Results thus far suggest that different allosteric ligands induce distinct protein conformations. Furthermore, when two ligands are present we observe that one inhibitor has a dominant impact in determining protein conformation. We have also carried out free energy calculations to understand differences in binding free energy that arise when one or both inhibitors are bound to NS5B. Understanding the molecular mechanism that mediate synergistic inhibition in NS5B may allow us to optimize the inhibitory activity of these compounds against the enzyme. In addition, these studies can provide fundamental insights into how ligand binding regulates protein function. Such information has direct applications in the areas of drug discovery, regulation of metabolic pathways and other signal transduction processes.

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Regulatory Elements of HCV NS5B Polymerase - β -Loop and C-Terminal Tail - are Required for Activity of Allosteric Thumb Site II Inhibitors

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Hepatitis C virus (HCV) chronically infects close to 3% of the world's population, with 30% of carriers expected to develop serious liver-related diseases, including hepatocellular carcinoma. There is an ongoing effort to develop novel antivirals to improve the therapeutic outcome of anti-HCV treatment.

We explore the mechanism of action of nonnucleotide inhibitors (NNIs) of HCV replication that bind to the thumb site II pocket on NS5B polymerase, exemplified by GS-9669 which is in phase II clinical trials. Despite a wealth of enzymatic and structural information regarding NS5B it is not fully understood how binding at this remote pocket inhibits the active site. Stabilization of the closed, inactive enzyme conformation has been suggested, although it has not been described on the mechanistic level.

We employed a combination of enzyme activity, direct binding, and calorimetric studies with GS-9669 and other classes of NNIs and several truncation mutants of NS5B that disrupt critical interactions at the interface of the thumb domain, β-loop and C-terminus. Our studies demonstrated unique binding and inhibition profiles for each NNIs. An intact interface between the β-loop and the C-terminus is critical for inhibitory potency of thumb site II NNIs, although both elements are dispensable for binding. In addition, calorimetric studies revealed the pivotal role of both regulatory elements in communication between polymerase domains. We postulate that binding at the thumb site II pocket locks the entire NS5B in an enzymatically inactive, closed conformation. This stabilization of the closed conformation is a consequence of the communication between the binding pocket on the thumb domain and other domains of polymerase that is mediated by the interactions between two key regulatory elements: the β-loop and the C-terminal tail.

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Computational Thrombin Inhibitor Optimization

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Of the plethora of computational methods for the estimation of binding free energies, molecular dynamics based alchemical approaches provide remarkable accuracy at an affordable computational cost. The computationally less expensive empirical ligand and structure based methods require training a predictive model to yield accurate free energy estimates. In the current study we utilized a combinatorial chemistry library and combined the chemoinformatic and alchemical approaches to optimize a set of known thrombin inhibitors. A confirmed ligand scaffold (D-Phe-Pro) was selected as a basis for modifications. Prior to designing new compounds, we verified the non-equilibrium alchemical free energy calculation setup on a set of inhibitors with experimentally measured binding affinities. Afterwards, a combinatorial chemistry library was constructed by modifying substituents at the ortho, meta and para positions on a benzene ring facing the S1 thrombin pocket. Navigation in the library was guided by training the Partial Least Squares based regression models using topological, structural, physicochemical descriptors and COMFA-like coordinates of the molecular electrostatic surfaces. The predicted potential binders were subjected to the alchemical free energy calculations, and, subsequently, the obtained estimates were used to update the regression models and improve prediction accuracy. Few iterations sufficed to reach convergence towards stronger binders, several of which were predicted to have a higher binding affinity than the strongest inhibitors in the initial compound set. While the newly identified ligands are to be validated in an ITC experiment, the computational workflow utilizing chemoinformatic approaches for navigation in a