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 (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 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.


1328-Pos  Board B58
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 envelope 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.

1329-Pos  Board B59
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 mediates 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.
combinatorial chemistry library and first principles based alchemical calculations for accurate free energy estimates appears to be a powerful approach for ligand optimization.

1332-Pos  Board B62
Probing the Ligand Binding Mechanism of Mnk Inhibitors by Docking and Molecular Dynamics Simulations
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Mitogen-activated protein kinases-interacting kinases 1 and 2 (MNK1 and MNK2) belong to the group of CaMK (calmodulin-dependent kinases (CaMK)) that phosphorylate Eukaryotic initiation factor 4E (eIF4E) [Luc Furic., et al., PNAS, 2010, 107 (32):14134-14139]. Therefore, Mnk1/2 inhibitors could be effective therapeutic agents for the treatment of cancers driven by an overexpression of eIF4E.

Overexpression of eIF4E has been associated with tumorigenesis and studies have indicated that eIF4E phosphorylation is oncogenic [Jinqing Hou, et al., Oncotarget, 2012, 3:118-131]. Therefore, Mnk1/2 inhibitors could be effective therapeutic agents for the treatment of cancers driven by an overexpression of phosphorylated eIF4E.

In the current study we have carried out molecular docking combined with molecular dynamics simulations to study the interaction between ligand and Mnk1/2 kinase catalytic domains. Three dimensional structures of both Mnk1 and Mnk2 were built using comparative modeling methods. A series of Mnk kinase inhibitors were docked to the ensemble of representative structures extracted from a clustering analysis of the MD simulations. The predicted bound conformations were further studied in explicit solvent by MD simulations.

Our combined computation approach identified key residues that are important for the protein - inhibitor interactions, provides detailed understanding of the mechanism of these kinds of inhibitors and will be useful for the rational design of Mnk inhibitors.

1333-Pos  Board B63
Development of Novel Xanthine Oxidase Inhibitors with Radical Scavenging Properties for the Prevention of Reperfusion Injuries
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Reperfusion injuries can cause severe damage in hypoxic tissue after the blockage of oxygen supply has been relieved. The condition frequently occurs after ischemic events or surgery and is caused by a combination of inflammation and the generation of harmful reactive oxygen species (ROS). In an effort to gain access to agents capable of combating the damaging effects of ROS, we developed compounds with dual properties capable of preventing the generation of ROS and of absorbing ROS already formed. By combining two beneficial properties in a single molecule, we expected these compounds to be more flexible and effective than those that feature only one of the two activities. Based on the scaffolds of the natural products chalcone and caffeic acid phenethyl ester (CAPE), we synthesized and tested a selection of compounds capable of inhibiting Xanthine oxidase (XO), a major source of ROS production, and of absorbing ROS. The predictors of success employed in this study measured inhibitory potency against XO activity, radical scavenging ability, and the capacity to increase cell viability under oxidative stress.

In addition, computational docking was performed to elucidate XO/inhibitor interactions at the molecular level. Structure-activity relationships were established that identified correlations between molecular structure and the two bioactivities under investigation and that can guide the future synthesis of materials with improved properties.

1334-Pos  Board B64
Data Mining the Pdb: Phosphorylation Can Directly Interfere with Drug Binding
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As many as 30% of all proteins are phosphorylated. Protein kinases play a central role in controlling cell proliferation, differentiation, cell cycle progression, and angiogenesis - processes which frequently become dysregulated during carcinogenic transformation. Many experimental therapeutics are developed using purified protein and may be less effective against targets that are post-translationally modified in vivo. To test whether protein phosphorylation may decrease drug binding and increase resistance, we examined 310 unique drug-bound protein structures mapped from the DrugBank database to the Protein Data Bank. We cross-referenced the sites with recorded phosphorylation sites found in the PhosphoSitePlus database.

“Hits” are defined as target proteins that have phosphorylated residues within 12 Å of their drug binding sites. For these proteins, phosphorylation could directly interfere with drug binding in vivo. The hits fell into two classes. Class I hits are targets for which the drug compound competes with natural substrate in the active site. The phosphorylation site is also in the active site, and phosphorylation results in inactivation of the protein. One would not expect a large reduction in drug efficacy for these proteins, as phosphorylation simultaneously causes drug resistance and inactivates the protein target. For Class II hits, the drug compounds bind to allosteric sites outside the active site of the target protein, and the documented phosphorylation either activates the target or only moderately decreases its activity. We hypothesize that Class II compounds are more likely to encounter resistance when used as therapy, especially in cancer where there are larger populations of aberrantly phosphorylated proteins.

1335-Pos  Board B65
Effect of Natural Product Extracts on Lipoygenase, Cyclooxygenase, and Protein Tyrosine Phosphatase 1B
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The purpose of this research project is to examine the inhibition of enzymes linked to inflammation and associated diseases by plants traditionally as anti-inflammatory medicines: *Tussilago farfara*, *Grindelia squarrosa*, and *Urtica dioica*.

Arachidonic acid is metabolized in the body through two main metabolic pathways with the enzymes: cyclooxygenases (COX) and lipoygenases (LOX). Elevated levels of prostanooids and leukotrienes, products of the two respective pathways, have been linked to inflammatory diseases. Finding a dual inhibitor of COX and LOX is promising in preventing the inflammation and diseases that are linked to the overproduction of both pathways while minimizing the side effects associated with inhibition of individual pathways.

Furthermore, the enzyme protein tyrosine phosphatase 1B (PTPβ) is linked between inflammation and metabolic disease through the leptin receptor-associated Janus Kinase (JAK). PTPβ is a negative regulator of insulin and leptin receptors thus being explored as a possible therapeutic for type II diabetes and obesity.

Crude methanolic extracts of *Tussilago farfara*, *Grindelia squarrosa*, and *Urtica dioica* were taken to approximate the plants’ components released in the body upon consumption. The bioactivities of the standardized extracts were then determined using enzymatic assay kits for COX I and II, LOX, and PTPβ. T. farfara is the strongest LOX inhibitor at 60% inhibition of 15-LOX, followed by *U. dioica* and *G. squarrosa* at 45% and 39%, respectively. Studies for COX I and II as well as PTPβ inhibition are ongoing, and the results will be presented.

1336-Pos  Board B66
Structure-Thermodynamics Correlations of Fluorinated Benzensulfonamides as Inhibitors of Human Carbonic Anhydrases
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The carbonic anhydrases (CA) are established as therapeutic targets [1]. There are 12 catalytically active CA isozymes in human body. At least 30 CA sulfonamide inhibitors have been used as drugs to treat glaucoma, epileptic seizures, altitude sickness, and as diuretics. However, most of them exhibit poor selectivity towards target isozymes and result in various side effects.

In this work, a class of 4-substituted-2,3,5,6-tetrafluorobenzensulfonamides as inhibitors of CA I and II as well as PTP1B was reported. Crystal structures of CAI, CAXII and CAXIII bound with the fluorinated compounds were solved and provided structural details of inhibitor binding. The binding affinity to carbonic anhydrases I, II, VII, XII and XIII was measured by isothermal titration calorimetry and the fluorescent thermal shift assay, and inhibition was determined by stopped-flow CO2 hydration assay. The combined use of these methods has provided a detailed picture of protein-ligand interactions. Experimentally obtained binding data usually depends on various factors including temperature, pH, buffer, etc. In this study, we present intrinsic parameters of binding that are independent of experimental conditions. Structure-thermodynamics correlations were studied using intrinsic parameters. All used biophysical methods have confirmed that fluorinated sulfonamides bind stronger to CA than non-fluorinated, because of the presence of electronegative substituents that decrease the pKa of sulfonamide group and this correlates with an increase in the CA inhibitory properties [2]. A large group of fluorinated compounds possessed nanomolar affinity for selected CAs and several of them were selective towards CAI.

1337-Pos  Board B67
An Asymmetric Pattern in Binding of Prostaglandin Endoperoxide H Synthases to Their Inhibitors and Its Implications for Enzyme Catalysis and Allosteric Regulation
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Proteins experience some conformational changes upon ligand bindings and their effects on protein functions are displayed in various ways. Crystallographic data of