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PhD Thesis

Title: Theoretical Studies of HIV-1 Enzymes

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Abstract

This dissertation has three major purposes: to investigate reaction mechanism catalyzed by the protease (PR), to provide an insight into specific interactions between inhibitors and the reverse transcriptase (RT) and to describe reaction mechanism taking place in the integrase (IN) active site. These enzymes are responsible for HIV-1 (Human Immunodeficiency Virus) life cycle which in turn leads to AIDS (Acquired Immunodeficiency Syndrome). To achieve these goals the quantum chemistry tools need to be applied, especially hybrid Quantum Mechanics/Molecular Mechanics (QM/MM) simulations developed to study complex biochemical systems.

In order to describe the mechanism of peptide bond breaking catalyzed by the aspartyl protease of HIV-1, a few different pathways have been explored. Based on obtained energy barriers and kinetic isotope effects (KIEs), compared with experimental values, the most favorable reaction path was indicated with its rate-limiting transition state (TS) structure. So far in the literature role of the protease was limited to nucleophilic activation of a water molecule. However, our analysis explained that the whole protein creates electrostatic field inside the active site leading to peptide bond breaking. Moreover, dynamic effect of the protease from heavy enzyme calculations and quantum tunneling effects has been described as being negligible in this particular case.

Molecular mechanics (MM) and quantum mechanics (QM) molecular dynamics (MD) simulations of the HIV-1 reverse transcriptase with different inhibitors docked in different sites

of the enzyme were carried out to reveal ligand-enzyme interactions. Initially, available theoretical tools were evaluated based on Food and Drug Administration (FDA) approved drugs to develop the proper protocol. Our methodology was then applied to novel triazole-based inhibitors. The potential energy of interactions between inhibitors and protein amino acids were obtained through QM/MM dynamic simulations. Furthermore, inhibitors affinity was sorted by the free energy of binding brought by Alchemical Free Energy Perturbation (FEP) method. Finally, Binding Isotope Effects (BIEs) analysis was used to indicate the site where putative inhibitors were bound to.

HIV-1 integrase tetramer was studied along 60 ns of molecular dynamics simulation to get insight into crucial interactions, to confirm the stability of structure with bounded DNA chains and the stability of the active site. Due to the fact that the integrase active site is remarkably complex, the proper level of theory had to be found. A few semi-empirical and density functional theory (DFT) methods have been tested to propose few different reaction pathways of 3'-end processing of the phosphodiester bond catalyzed by integrase.

The detailed description of the protease and the integrase reaction mechanisms, as well as inhibitors-reverse transcriptase interactions analysis, supplemented gaps in general understanding of HIV-1 action and inhibition, which in turns may allow improving rational drug development.

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