

Modeling the Effects of Mutation on Substrate Binding Affinity within HIV Protease

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Recently, our lab has developed and applied information theory based tools to analyze amino acid sequence patterns within the Stanford HIVdb, a database of HIV protease and reverse transcriptase sequences extracted from AIDS patients. These tools have identified unusual conservation patterns within the HIV protease of untreated patients (shown in Fig. 1), which we suggest are linked to the function of the enzyme. These patterns include the strong conservation of amino acids far from the active site. The link between these sequence positions and the function of the protease is unknown. Developing models to generally explain and predict the effect of mutations on protein function has broad significance to the fields of protein engineering, molecular evolution, and nanotechnology. A simple hypothesis that must be explored is that the strong sequence conservation patterns in amino acids distant from the HIV protease active site arise due to the impact of mutations on the ability of the protease to bind natural substrates. To test this hypothesis, students will start with crystal structures of inactive HIV protease (D25N) bound to five natural substrates of the protease. These structures have been recently deposited within the protein databank (PDB). Students will then use the MMTSB tool set to mutate the nonfunctional asparagine residues within the active site to the wild-type aspartic acids. These model structures will be optimized using standard minimization algorithms contained within the CHARMM molecular modeling package. An approximate modeling approach, based on molecular dynamics simulations and a post-processing analysis of molecular mechanics and implicit solvation energy terms (MMPB/SA), will be used to estimate the binding free energy between the wild-type HIV protease and each of the natural substrates. Again, using the MMTSB tool set, mutations will be made in the protease reflecting unobserved changes within the HIVdb at conserved sites within the protein. Reapplying MMPB/SA analysis, students will characterize the changes in binding free energy due to mutations in these sites. Using this approach, students will evaluate the validity of the hypothesis that mutations in conserved sites distant from the active site cause disruptions in substrate binding within the protease.

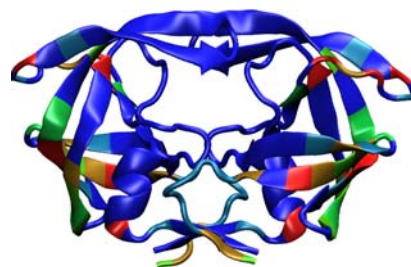


Figure 1. HIV Protease colored according to conservation in HIVdb. Blue is most conserved.