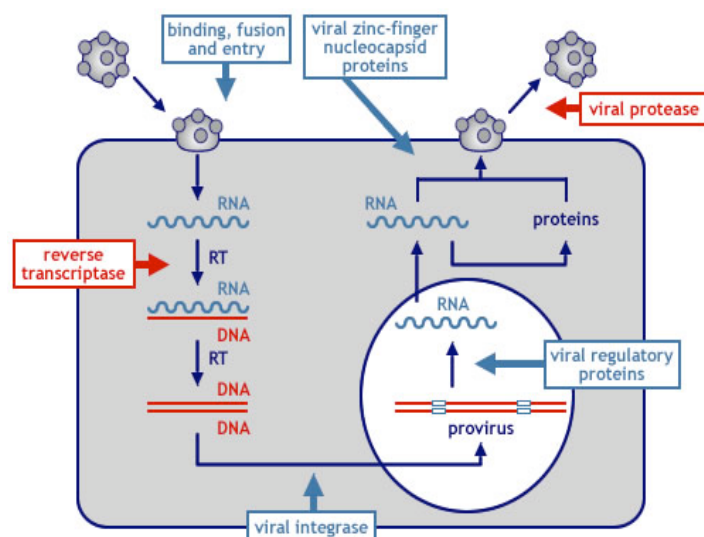


## Implications for Drug Resistance



Source: <http://www.hivtools.com/ARV.php>

**Figure 1:** The viral life cycle showing at which step the viral enzymes act.

Drug resistance is a major challenge in developing effective treatments against HIV. The high mutational rate of HIV has helped the virus in evading the optimal efficacy of antiretroviral therapy. What would normally seem like a fault in the virus's machinery is actually what gives it its greatest advantage. The viral enzyme reverse transcriptase (RT) synthesizes a DNA copy of the viral RNA at such a low fidelity that it introduces about 1 mutation per replication cycle.<sup>1</sup> In an untreated patient, RT can produce 10 billion copies of HIV in one day alone.<sup>1</sup> Unlike DNA polymerases, RT lacks proofreading capacity, thus enabling the virus to accumulate a number of mutations, few being truly advantageous to the virus. The low fidelity of RT and high replication rate of HIV, therefore, allows for the generation of numerous naturally occurring genetic polymorphisms in the *pol* gene, thus introducing mutations in the enzymes reverse transcriptase, integrase, and protease- all of which are targets for antiretroviral drugs. Inhibition in any of these enzymes would prevent replication at specific points in the viral life cycle (see **Figure 1**).

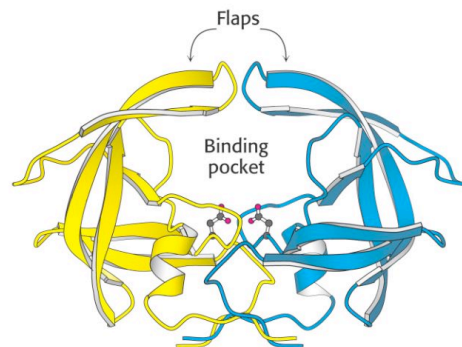
There are currently seven FDA approved protease inhibitors (PIs): aprenavir, indinavir, saquinavir, fosamprenavir, ritonavir, atazanavir, and nelfinavir.<sup>4</sup> Inhibitors designed against the viral protease were developed using *in vitro* studies on HIV-1 subtype B isolates. However, subtype B predominates only in highly developed industrialized countries and accounts for only 12% of all infections. While past studies have suggested that the available inhibitors are just as effective against non-B subtypes, more studies need to be carried out concerning how they respond to antiretroviral drugs and differences in the actual mechanisms of drug resistance between different subtypes. Previous studies have noted that some viruses develop subtype-specific polymorphisms or mutations that are more prevalent in a certain subtype.<sup>5</sup> This implies that subtypes may be responding differently to the same treatments and conversely suggesting differing routes of drug resistance. This is significant in the evolution of the virus and the development of novel inhibitors that accommodate for forthcoming changes in the biochemical structure of the protease that may lower inhibitor affinity.

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## Protease Structure

The HIV-1 protease is a relatively small enzyme, composed of two identical 99 amino acid monomers that are stabilized by a  $\beta$ -sheet.<sup>6</sup>

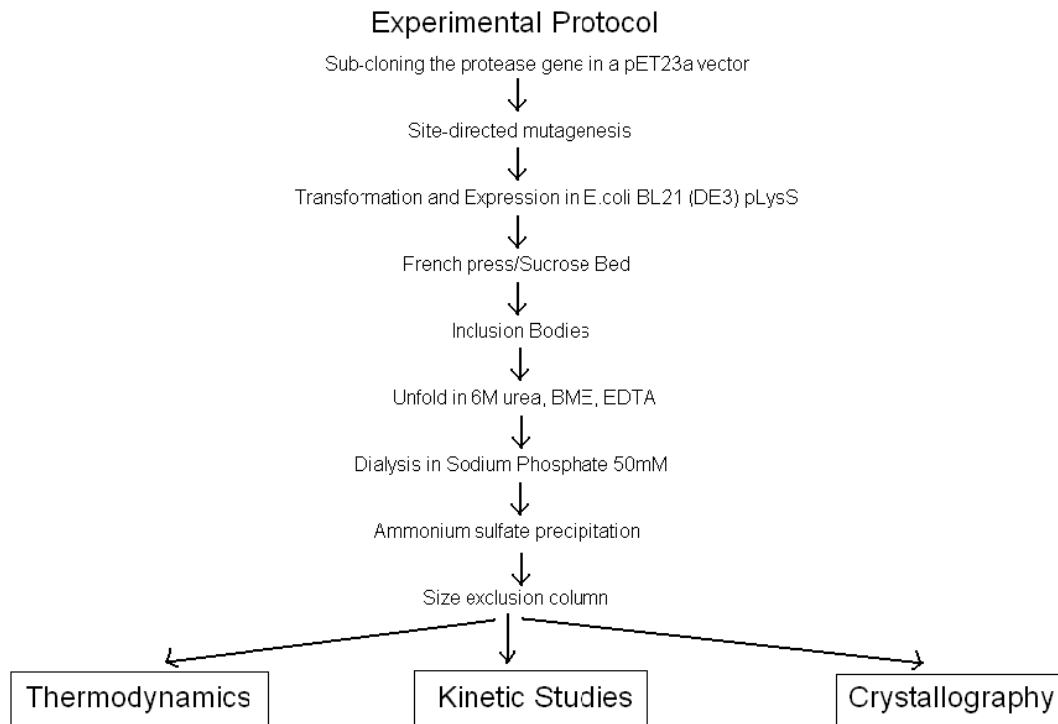
Within the active site are two aspartic residues that function in the enzyme's catalytic activity. These attack a protein chain by removing a proton from a water molecule resulting in cleavage.<sup>7</sup> To gain access to the active site, the flaps undergo a large conformational change, with the tip of the flap opening as much as 20Å.<sup>7</sup> This allows the substrate to enter into the cleft while the flaps then enclose upon it tightly.<sup>7</sup>



Source:  
<http://oregonstate.edu/instruction/bb450/stryer/ch09/Slide33.jpg> **Figure 2:** 3D structure of the HIV-1

## Methods

In this study, we plan to crystallize the HIV-1 subtype F protease in order to solve its 3-dimensional structure. In its native form, the HIV subtype F protease has heightened catalytic activity, such that it cleaves itself before a sufficient amount of intact protease can be obtained for crystallization. To work around this challenge, we will mutate its amino acid sequence at the sites of auto-cleavage through site-directed mutagenesis using PCR. There are three such sites: Q7K, L33I, and I63L. After introduction of these three mutations into the DNA and confirming this by DNA sequencing, we will clone the HIV-1 Subtype F protease in *Escherichia coli* cells (Star DE3 pLysS), use a French press to lyse the cells and a sucrose bed to extract the inclusion bodies. The inclusion bodies will then be added to 8M urea to denature the protein. The protein will be dialyzed in different buffers and combinations of buffers, at various temperatures, and at different pHs to determine the optimal conditions at which the protease folds. Amounts of the inactive protease will be used for crystallization. We will experiment with different concentrations of protease and buffers and also adjust the pH in order to achieve crystallization. The following is an overall outline of the experimental protocol:



**Figure 3.** Outline of the experimental protocol

The second half of the project will be to investigate the HIV F protease's kinetic behavior with different inhibitors and to compare it to Subtype B and Subtype C. We explore differences in affinity for several available inhibitors. These studies can help in deciding which of the available inhibitors should be used for this subtype.

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