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## Development of "Sharkavir": A Hypothetical Inhibitor for HIV-1 Protease

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## Research Question

How can molecular models be used to simulate the binding of various HIV-1 protease inhibitors including Darunavir, Ritonavir, and a novel combination of these two named “Sharkavir”?

## Introduction

HIV has infected approximately 37 million people worldwide and results in over 1 million deaths annually. A key component of the HIV life cycle is the enzyme HIV-1 protease, which is involved in cutting newly made protein components of the HIV virion (NIH, 2020). One of the primary forms of treatment for HIV is to inhibit its protease through a variety of competitive inhibitors; however, multiple mutations in HIV-1 protease have caused these inhibitors to be less effective (Figure 1). We collaborated with the CREST (Connecting Researchers, Educators and Students) Program of the Center for Biomolecular Modeling (CBM) to depict this process (Herman et al., 2006; Procko et al., 2019). Our CREST Team prepared a molecular model explaining HIV-1 protease (Figure 2) and two of its potent inhibitors Darunavir (DRV) and Ritonavir (RIT), which are both aspartic proteases that result in peptide cleavage (Figure 3). DRV and RIT are being prescribed as a mixed dose of both drugs as they are shown to be more effective in combination than by administering one of these drugs alone (Mehta et al., 2019). Inspired by this, we designed a hybrid of these two inhibitors, which we named “Sharkavir”, and used our molecular model to show how this inhibitor would bind to HIV-1 protease. The active site of HIV-1 protease (Surleraux et al., 2005) was depicted as a binding box model. The protease inhibitors DRV (Mittal et al., 2012), RIT (Prabu-Jeyabala, et al., 2003), and Sharkavir were 3-D printed to show how they fit into the protease active site.

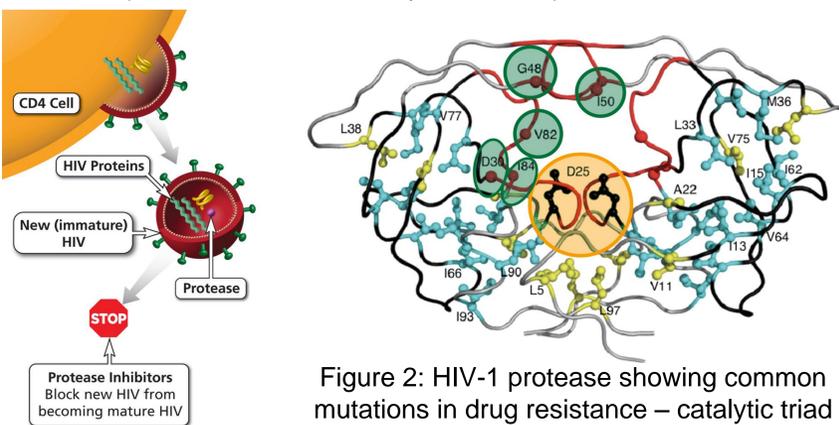


Figure 1: HIV Lifecycle; role of HIV-1 protease (taken from NIH, 2020).

Figure 2: HIV-1 protease showing common mutations in drug resistance – catalytic triad circled in orange; key mutations circled in green to correspond with Figures 5a and b (taken from Mittal et al., 2012).

## Methods

Three protein database files were used to create the molecular models (Berman et al., 2000). These files were 1T3R (HIV-1 Protease), 4DQF (Darunavir) and 1N49 (Ritonavir), Importing these files into Jmol, a protein visualization software, allowed us to manipulate the crystal structures of HIV-1 protease and these inhibitors. The hypothetical drug Sharkavir was created as a molecular combination of DAR and RIT using the program Marvin Sketch. The program AutoDock Vina was used to model the binding of Sharkavir to HIV-1 protease (Morris et al., 2009).

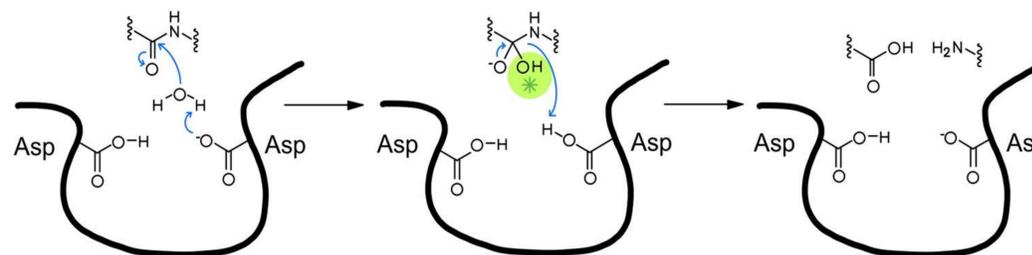


Figure 3: Mechanism of action of peptide cleavage by aspartic proteases including HIV-1 protease (taken from Mahajan and Badgujar, 2010). The interactive hydroxide on the inhibitor is highlighted in green with an asterisk to correspond to Figure 4.

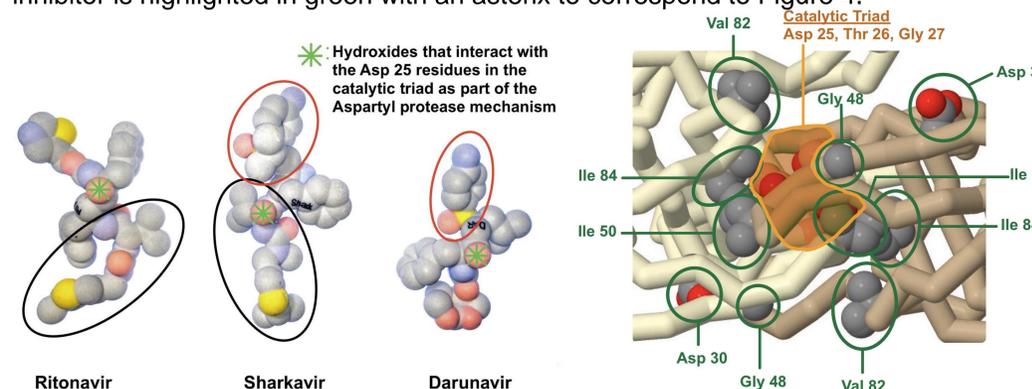


Figure 4: Ritonavir, Darunavir and Sharkavir models prepared in spacefill using light CPK (standard colors). Hydroxides are marked with a green asterisk.

Figure 5a: HIV-1 protease (1T3R) manipulated in Jmol to show key amino acids relevant in substrate binding.

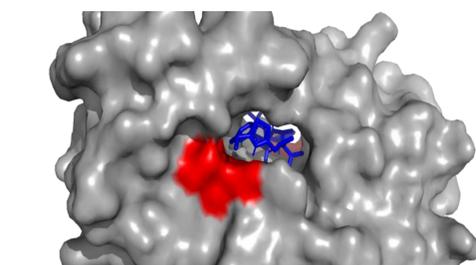


Figure 6: Sharkavir (blue) docked into the active site of HIV-1 protease (1T3R) using AutoDock Vina. Catalytic Asp 25 residues are highlighted in red.

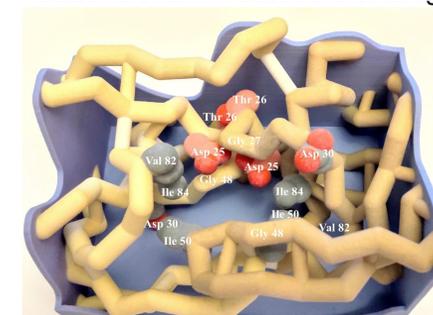


Figure 5b: HIV-1 protease (1T3R) 3-D printed as a 3-D printed protein binding box model with key amino acids labeled.

## Results - Model Information

**Protease Inhibitors (Figure 4):** Darunavir, Ritonavir, and “Sharkavir” (a molecular combination of Darunavir and Ritonavir) were prepared in spacefill mode with light cpk (standard chemical element colors) to show their binding to the active site box model. The hydroxides that interact with the Asp 25 residues in the catalytic triad as part of the aspartic protease mechanism were noted with a green asterisk.

**HIV-1 Protease Active Site Binding Box (Figures 5a and b):**

- Two homodimers of HIV-1 protease shown in light yellow (lemonchiffon) and tan
- Catalytic triad containing **Asp 25, Thr 26, and Gly 27** colored orange/salmon
- Five key amino acids in the active site that often mutate, leading to less specific binding with their substrate inhibitors: **Asp 30, Gly 48, Ile 50, Val 82, and Ile 84**; shown in standard cpk colors
- Sharkavir bound to HIV-1 protease along with catalytic Asp 25 residues (Figure 6)
- Each 3-D printed inhibitor can be manipulated to fit into the HIV-1 protease binding box model.

## Discussion

The development of Sharkavir was based on structures of established inhibitors (DRV and RIT) and can be used as a model towards the *in-silico* development of inhibitory molecules for a wide variety of enzymes. Sharkavir maintains the benzyl ring that is sensed by the specificity pocket consisting of **Ile 84, Ile 50 and Val 82**. The hydroxide that mimics the tetrahedral intermediate of the substrate fits between the catalytic **Asp 25** residues at the dimerization interface. Using the chemical hallmarks present in known inhibitory molecules, combinatory drugs can be modeled, and subsequently synthesized for targeted drug testing. Future steps for this project could involve synthesizing the molecule in the laboratory for inhibitor testing and crystallographic studies. The molecular modeling techniques described are accessible to students and researchers from a wide range of STEM fields and is a scientifically viable first step in the long and costly drug design process. As more undergraduate students are thinking about biochemical research concepts and powerful computational tools become ubiquitous, this style of project is very promising for research labs and as part of Course-Based Undergraduate Research Experiences (CUREs).

## Applications

3-D Molecular Models:

- Visually represent molecular processes assisting in health and student education
- Communicate ideas to others in the scientific community
- Assist in the development of novel drugs that can serve as enzyme inhibitors

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