

Georgia State University

ScholarWorks @ Georgia State University

Biology Honors Theses

Department of Biology

Fall 12-18-2012

HIV-1 PR P51 Mutant Complex Formation with Inhibitors

Shaquita T. Greene
Georgia State University

Ying Zhang
Georgia State University

Follow this and additional works at: https://scholarworks.gsu.edu/biology_hontheses

Recommended Citation

Greene, Shaquita T. and Zhang, Ying, "HIV-1 PR P51 Mutant Complex Formation with Inhibitors." Thesis, Georgia State University, 2012.

doi: <https://doi.org/10.57709/3555693>

This Thesis is brought to you for free and open access by the Department of Biology at ScholarWorks @ Georgia State University. It has been accepted for inclusion in Biology Honors Theses by an authorized administrator of ScholarWorks @ Georgia State University. For more information, please contact scholarworks@gsu.edu.

HIV-1 PR P51 MUTANT COMPLEX FORMATION WITH INHIBITORS

by

Shaquita T. Greene

Under the Direction of Dr. Irene Weber

ABSTRACT

Human Immunodeficiency Virus (HIV) has become a global pandemic with at least 25 million deaths and no cure. One of the most important targets to inhibit this virus is HIV-1 protease (PR), which is required to cleave the viral proteins needed for maturation of the virus after it invades and replicates in the host cell. There are nine protease inhibitors that are used in AIDS treatment. The virus loses susceptibility to these inhibitors by drug resistance due to mutations. The goal of the project is to examine the highly drug resistant HIV PR P51 in its complex with inhibitors. In this experiment we expressed and purified HIV PR P51 protein. We performed protein crystallization with inhibitors Tipranavir, Amprenavir, Darunavir, and Saquinavir to obtain the structure of the protease and the inhibitors in their complexes. Future analysis of the crystal structures will help with the development of successful therapeutic inhibitors.

INDEX WORDS: Human Immunodeficiency Virus, Inhibitor, Mutant, Protease P51, Drug Resistance, Protein Crystallization

HIV-1 PR P51 MUTANT COMPLEX FORMATION WITH INHIBITORS

by

Shaquita T. Greene

An Honors Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Bachelor of Science

in the College of Arts and Sciences

Georgia State University

2012

Copyright by
Shaquita Tashon Greene
2012

HIV-1 PR P51 MUTANT COMPLEX FORMATION WITH INHIBITORS

by

Shaquita T. Greene

Honors Thesis Director: Dr. Irene Weber
Honors College Associate Dean: Dr. Sarah Cook

Electronic Version Approved:

GSU Honors College
Georgia State University
December 2012

Dedication

I would like to dedicate this paper to my family and all those who have supported me along the way. I greatly appreciate you all and I love you very much.

Acknowledgements

I would like to acknowledge those who have helped me with my research and have helped to edit this paper, Dr. Irene Weber and Ying Zhang. I really appreciate your help and I have enjoyed working with the both of you. I also would like to thank Dr. Johnny (Johnson) Agniswamy. Thank you all so much for your help. I greatly appreciate everything you have done for me.

Table of Contents

Dedication	page v
Acknowledgements	page vi
List of Tables	page viii
List of Figures	page ix
Introduction	page 1
Human Immunodeficiency Virus	page 3
HIV-1 Virion Structure	page 5
HIV Protease	page 7
Drug Resistance	page 7
Experimental Methods	page 11
Protein Expression	page 11
Protein Purification	page 12
Protein Crystallization	page 13
Measurement of Protease Activity and Inhibition	page 13
Results	page 14
Expression of HIV Protease P51 Mutant	page 14
Purification of HIV Protease Mutant P51	page 14
Crystallization of HIV Protease P51 Mutant with Inhibitors	page 14
Enzyme activity and inhibition	page 15
Discussion	page 16

LIST OF TABLES

Table 1: HIV-1 Protease Inhibitors and the Related Drug Resistance Mutations

page 8

LIST OF FIGURES

Figure 1: HIV-1 protease and Gag and Gag-Pol with cleavage sites	page 2
Figure 2: HIV-1 Life Cycle in Host Cell	page 4
Figure 3: HIV-1 structure and structural proteins	page 6
Figure 4: Structure of HIV-1 protease in dimer form	page 9
Figure 5: Crystal Structure of HIV-1 PR Dimer with Sites of Drug Mutation	page 9
Figure 6: Molecular Structure of SQV, APV, DRV, and TPV	page 11
Figure 7: HIV-1 PR p51 Gel Filtration Results	page 17
Figure 8: PR p51 with APV crystals	page 18
Figure 9: PR p51 with DRV crystals	page 18
Figure 10: PR p51 with SQV crystals	page 19
Figure 11: PR p51 with TPV crystals	page 19

Introduction

The number of people currently living with Human Immunodeficiency Virus (HIV) has increased drastically since the virus was first recognized (4). UNAIDS (Joint United Nations Programme on HIV/AIDS), WHO (World Health Organization), and UNICEF (United Nations Children's Fund) have estimated at least 30.6 million adults and 3.4 million children who are infected with HIV at the end of the year of 2012 (17). The development of Highly Active Antiretroviral Therapy (HAART) using a combination of different antiviral drugs to suppress HIV over the years has helped to reduce the effects of the disease on the immune system but still there is no cure (2). Even with the drug therapies available today, multidrug resistance is becoming more of an issue. HIV protease (PR) is an enzyme that is vital to the functioning of HIV (6). PR's catalytic role is an important step in HIV replication because it generates mature infectious viral particles by cleaving viral polyproteins Gag and Gag-Pol (Figure 1) (6). Gag is cleaved into three large proteins called p24, p17, p7 and small proteins called p6, p2, and p1 (6). The large proteins contribute to the structure of the virion and RNA packing and the function of the small proteins are uncertain (6). Gag-pol is cleaved into protease (PR), reverse transcriptase (RT), and integrase (IN) (6). Consequently, HIV protease has been a valuable therapeutic target for HIV/AIDS structure-based drug design for decades (16).

Figure 1. HIV-1 protease and Gag and Gag-Pol with cleavage sites.

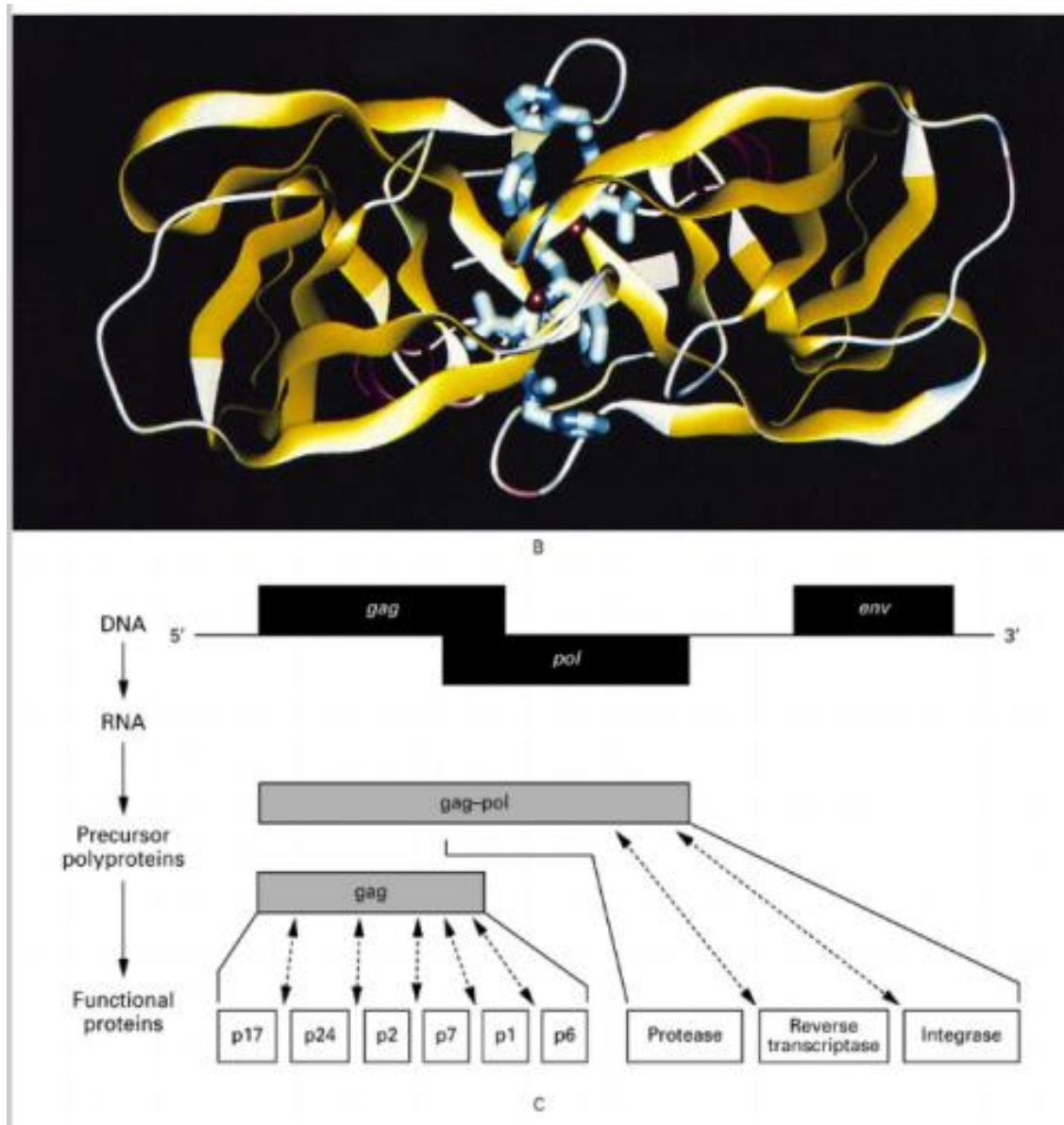


Figure 1. This figure shows the HIV-1 protease at a 180 degree angle (B) and gag and gag-pol genes that are cleaved by HIV-1 protease and at what site these proteins are cleaved (C). [6]

Human Immunodeficiency Virus

Human Immunodeficiency Virus (HIV) is an enveloped retrovirus that leads to the development of Autoimmune Deficiency Syndrome (AIDS) (16). HIV-1 virus can damage the immune system by depleting the CD4+ T cells, also known as T helper cells (13). In order for HIV to successfully destroy the T helper cells, it must first invade the body successfully and undergoes replication. HIV's mode of transmission is through sexual, percutaneous, and perinatal methods but the most common method is sexual contact (9). Some examples of behaviors that can cause the viral infection come from contact with bodily fluids are sharing of needles, syringes, and being born to an infected mother (9). Once it has entered the bloodstream it can begin its phase of replication and destruction of T helper cells. HIV binds to the CD4 and CXCR5 receptors and fuses its envelope with the host cell cellular membrane and liberates its envelope. After that, the proteins and nucleic acids that compose the virus are allowed to enter into the cytoplasm of the cell (8). Then, the ribonucleic acid (RNA) is reverse transcribed into double stranded deoxyribonucleic acid (DNA). The DNA is then integrated into the host DNA via viral integrase (8). The DNA is transcribed into new viral RNA and new viral proteins are made. The proteins with the RNA assemble around the host membrane to bud forming the mature viral particle (8). The new viral particle then continuously invades the host cells and multiplies, taking over the host cell machinery (Figure 2).

Figure 2. HIV-1 Life Cycle in Host Cell.

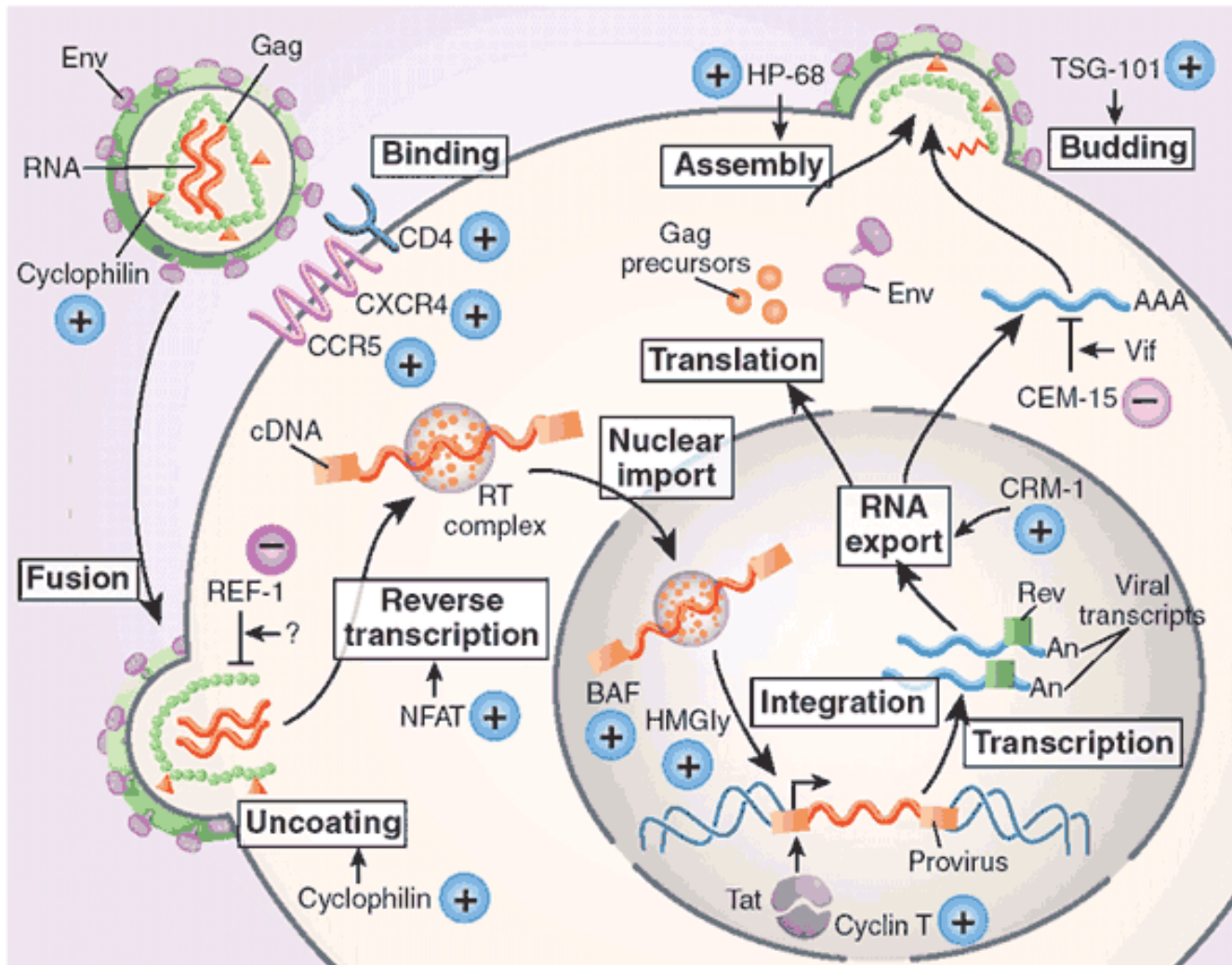


Figure 2. Representation of HIV-1 virus life cycle in the host cell. Positive (+) and negative (-) cellular factors are indicated next to the step in viral replication that they affect. (13)

HIV-1 Virion Structure

The HIV-1 virion shown in Figure 3 (15) is a spherical and compact structure. The viral envelope is the outmost part which is a plasma membrane of host-cell origin. On this membrane is the envelope which includes the surface unit glycoprotein (SU) gp120 and transmembrane envelope (TM) gp41. The viral structure proteins are referred to as Gag proteins: the matrix protein (MA) is located under the envelope and encases the capsid shell; two copies of genomic single-stranded RNA are enclosed by the conical capsid which comprising of capsid protein (CA); the single-strand RNA is tightly bound to the nucleocapsid proteins (NC) (5). Associated with the NC and RNA within the capsid are the Pol gene encoded viral-specific enzymes including the protease (PR), RNA polymerase (RT), integrase (IN) and RNase H (RH). The virus particles also package the accessory proteins, Nef, Vif, and Vpr while the other three additional accessory proteins Rev, Tat and Vpu which function in the host cell do not appear in the virions (15).

Figure 3. HIV-1 structure and structural proteins.

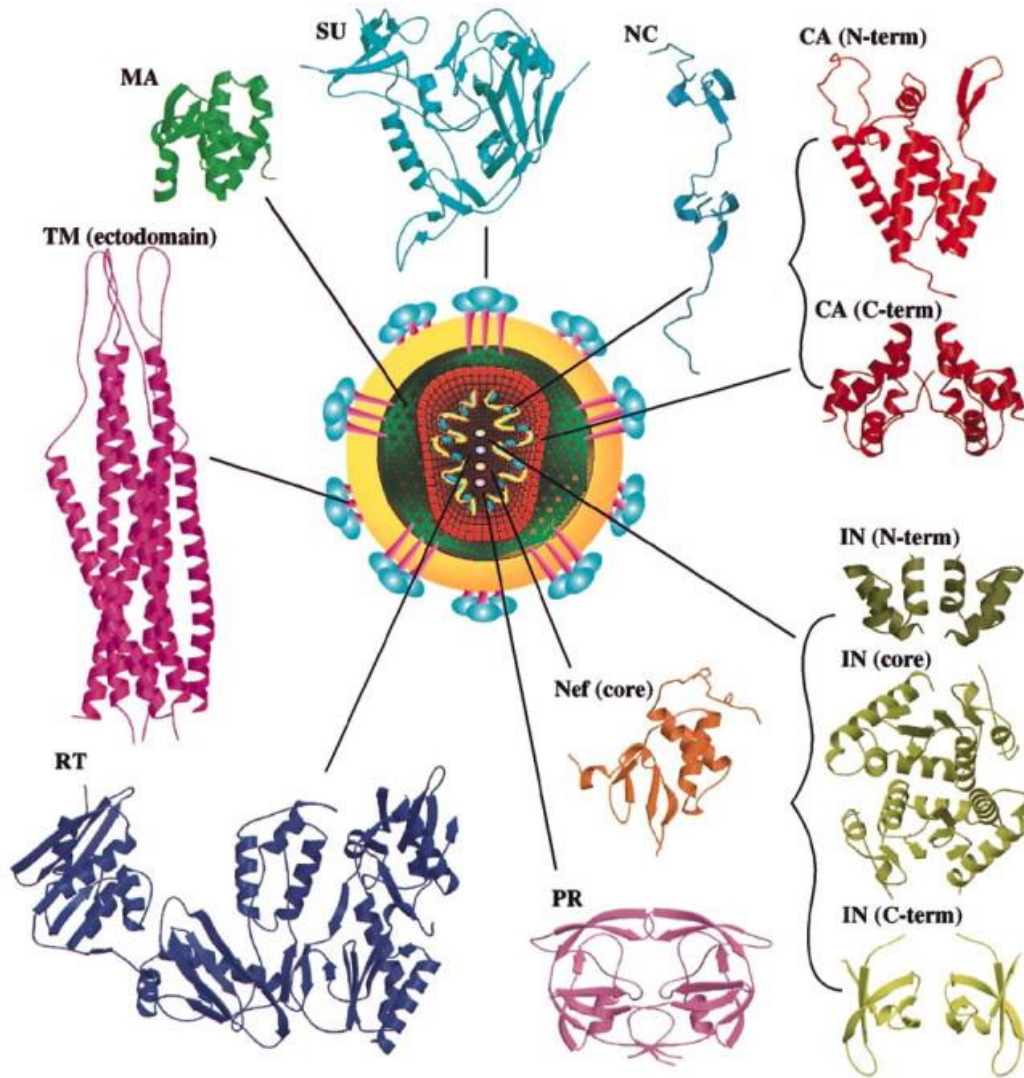


Figure 3. This represents the HIV-1 virion structure and the structural proteins. The structural protein include Surface unit glycoprotein (SU)gp120, transmembrane envelope (TM) gp41, the matrix protein (MA) capsid protein (CA), nucleocapsid proteins (NC), protease (PR), RNA polymerase (RT), integrase (IN) and RNase H (RH). accessory proteins (Nef. Vif. Vpr. Rev. Tat and Vpu). (Turner. et. al)

HIV Protease

Protease is an enzyme that catalyzes the cleavage of peptide bonds between amino acids of a protein (6). HIV-1 protease functions by cleaving peptide bonds of HIV-1 viral proteins. HIV-1 protease is encoded on the 5' end of the 99 amino acid HIV-1 gene gag-pol (6). The protease cleaves amino acid sequences in the Gag and Gag-Pol polyproteins, which is necessary in order for the maturation and replication of virion (6, 8). Lack of an efficient protease causes the development of immature, noninfectious viral particles, which is one of the reasons that the protease is a target for inhibition with many synthetic inhibitors (6). There are ten inhibitors of HIV protease (PR) that are used in antiviral therapy. They are amprenavir (APV), atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, nelfinavir, saquinavir (SQV), tipranavir (TPV), and darunavir (DRV) (6). A protease inhibitor (PI) is designed to inhibit HIV PR based on its structure, mainly its active site (16). These inhibitors interact competitively with the active site and the transition state of the enzyme reaction, inactivating the enzyme function. PIs are very effective in HAART treatment for AIDS and are usually used in a combination of three different antiviral drugs.

Drug Resistance

There is currently no cure for the HIV infection, but there are treatments currently available to slow the progression of the disease. Creation of therapeutic interventions like inhibitors has proven to be successful but have been succeeded by resistance. The creation of these antiviral inhibitors was promising due to the knowledge of the crystal structure of HIV protease and how it binds substrate like inhibitors. One such inhibitor, darunavir (DRV), which is a structure-based designed nonpeptidic ligand (10), has been widely used for the treatment of drug-naïve and multidrug-resistant HIV-1 infected patients and was the most recently approved

PI for drug resistant infections (18-20). Similar to other retroviral polymerases, HIV RT does not have a proofreading function and thus increases the mutational rates of the virus. The emergence of resistance to HIV-1 PIs is associated with the appearance of amino acid substitution mutations in the viral protease gene (Table 1). The mechanism for the development of resistance to PIs is quite complicated (5). The locations of PI-resistant mutations vary from substrate/inhibitor binding site, dimer interface, and flap region to surface of PR (Figure 4)(1). One explanation is that the residues near the active site can directly interfere with the binding of the inhibitor to the enzyme by modifying its specificity and/or affinity (20). Another cause for those mutations which do not directly change the shape or property of the binding cavity is that they may affect the efficiency of catalysis and the stability of the PR, or influence enzyme active site via long-range structural perturbations (11). To date, among the 99 amino acid residues of the protease, 25 or more have been implicated in resistance to PIs (Figure 5) (12).

Table 1. HIV-1 Protease Inhibitors and the Related Drug Resistance Mutations

Major PI Resistance Mutations

	<u>D30</u>	<u>V32</u>	<u>V33</u>	<u>M46</u>	<u>I47</u>	<u>G48</u>	<u>I50</u>	<u>I54</u>	<u>L76</u>	<u>V82</u>	<u>I84</u>	<u>N88</u>	<u>L90</u>
ATV/r			F	IL	V	V	L	VALM		AF	V	S	M
DRV/r		I	F		VA		V	LM	V	F	V		M
FPV/r		I	F	IL	VA		V	LM	V	F	V		M
IDV/r		I		IL	V			VALM	V	AFTS	V	S	M
LPV/r		I	F	IL	VA		V	VALM	V	AFTS	V		M
NFV	N		F	IL	V	V		VALM		AFTS	V	DS	M
SQV/r						V		VALM		TF	V	S	M
TPV/r		I	F	IL	V			VA		TFSL	V		M

Table 1. This table represents known antiviral data for clinical protease inhibitors and their drug resistant mutations. **Bold red** indicates phenotypic evidence for reduced susceptibility in vitro and clinical evidence for reduced virological response. **Underlined bold red** indicates drugs that should be contraindicated when the mutation is present. ((<http://hivdb.stanford.edu/cgi-bin/PIResiNote.cgi>, HIV Drug Resistance Database, Stanford University)

Figure 4. Structure of HIV-1 protease in dimer form.

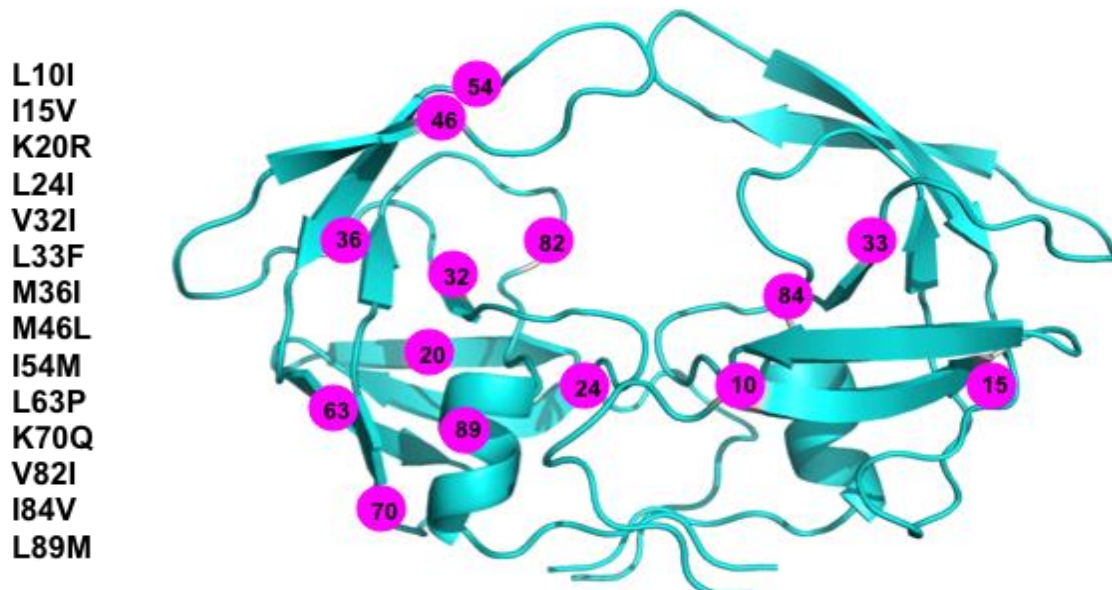


Figure 4. Structure of dimer of HIV-1 protease. Locations of 14 amino acid substitutions in the dimer structure and a list of substitutions in HIV PR P51 (Figure provided by Ying Zhang).

Figure 5. Crystal Structure of HIV-1 PR Dimer with Sites of Drug Mutation.

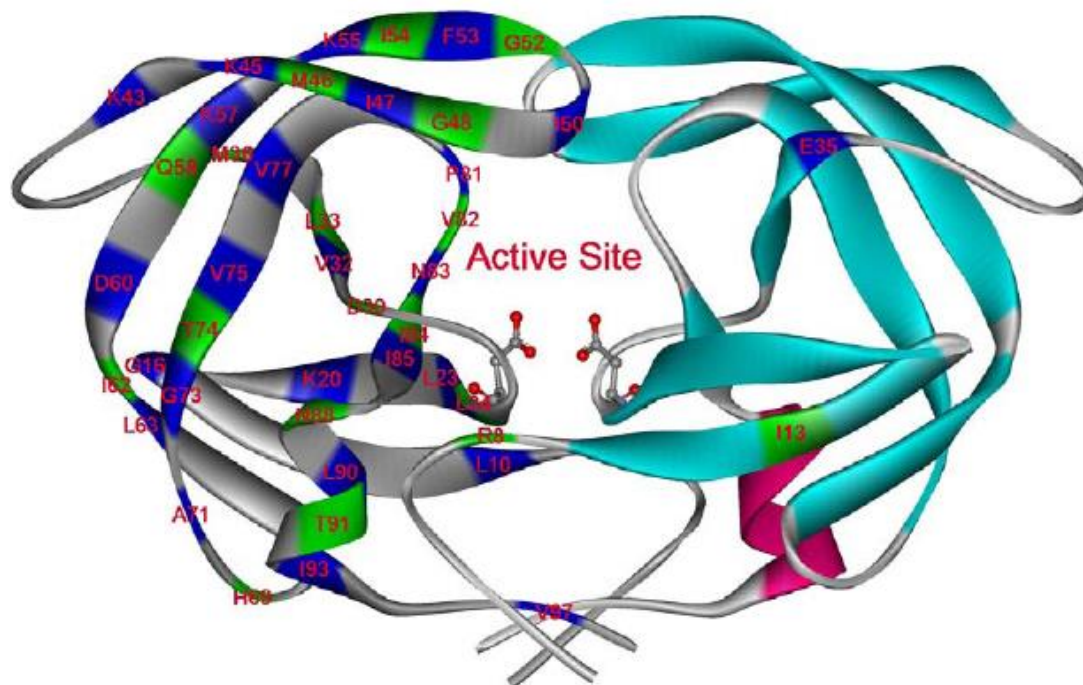


Figure 5. Shaded regions in monomer indicate the secondary structure of the dimeric enzyme. The three mutations Q7K, L33I, and L63I in PR to prevent its intrinsic autoproteolysis and the active site aspartic acid residues of each monomer are shown in a stick representation. Positions of drug-resistant mutations are indicated in blue. (12)

It has been reported that substitution of a variety of amino acids were related to HIV-1 resistance to DRV although it has a potent genetic barrier to the development of HIV-1 resistance and effectively inhibits PR enzymatic activity and dimerization (5). Hence, to further design more effective anti-HIV-1 drugs, highly DRV-resistant HIV-1 variants were acquired to elucidate the mechanism for development of DRV resistance. In Koh's lab, *in vitro* selection of a mixture of 8 highly DRV-susceptible HIV-1 clinical isolates (HIV-1MIX), which contain 9 to 14 PI-resistant mutations, was conducted to be propagated and thus a series of highly DRV-resistant viral populations were produced at different passages (10). The population at passage 51 (HIV-1MIXP51) replicated well at the concentration of 5 μ M DRV containing 14 amino acid substitutions related to HIV-1 resistance (10). HIV-1MIXP51 has shown a high resistance to amprenavir, indinavir, nelfinavir, ritonavir, lopinavir and atazanavir in addition to darunavir (all with EC₅₀s > 1 μ M) and moderate resistance to saquinavir (33 fold increase in EC₅₀) and tipranavir (18 fold increase in EC₅₀) (6). Meanwhile, in the Louis's lab, they assayed the inhibition of protease from HIV-1MIXP51 (P51) and the precursor flanked by the transframe region (TRF) to its N-terminus (7). It has also shown that the binding constant K_L of DRV for PR-P51 was about 7400 fold higher than for wild type protease while the IC₅₀ of TFR- PR-P51 is over 150 fold to TFR-PR. Hence, we are trying to study the crystal structures of HIV PR P51 with DRV, APV, SQV and TPV (molecular structures, Figure 6). Crystallizations of these four complexes are performed as the basis for structural study.

Figure 6. Molecular Structure of SQV, APV, DRV, and TPV

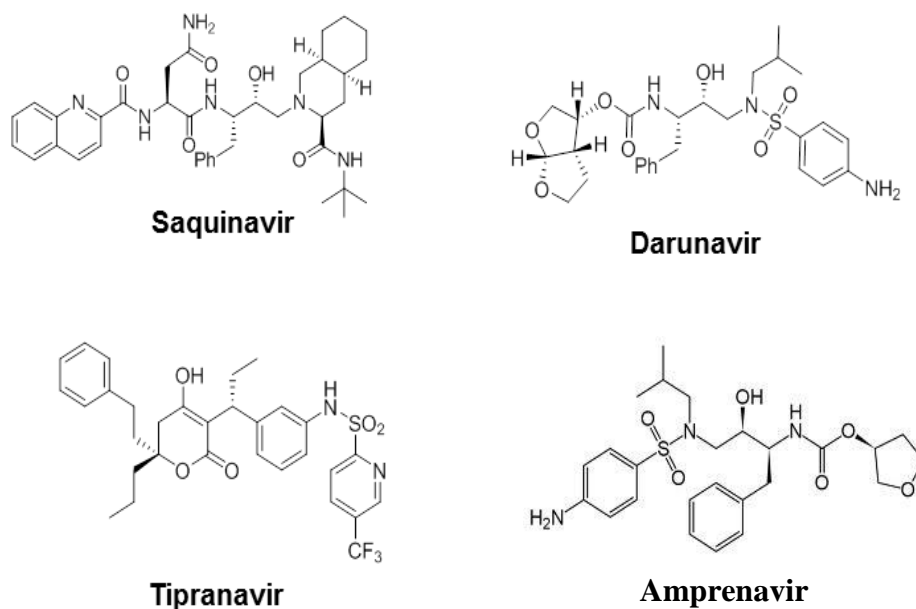


Figure 6. This image shows the molecular structure of the 4 clinical inhibitors for HIV-1 protease that were used in this study.

Experimental Methods and Materials

Protein Expression

In order to express the HIV-1 protease mutant P51, a bacterium is used and the gene of interest is inserted into the plasmid. In this experiment, E-coli bacteria were used as the expression system and pET11A plasmid was used. It contains approximately 5,000-6,000 base pairs (bp) and is ampicillin resistant. In order to express the protein, an expression system was created. The large broth media was created by adding 25 g of Luria broth (solid) to 1 L of distilled water. Mixture was stirred and carbenicillin (CBN) with a final concentration of 100 $\mu\text{g/ml}$ was added to mixture. 25 ml Luria broth was created and CBN with a final concentration

of 100 µg/ml was added to mixture. The cells were inoculated into smaller broth mixture and allowed to grow at 37°C overnight. After stirring overnight, starter culture was added to larger broth 1 L solution and allowed to shake until optical density (OD) value reached approximately 0.5-0.6. Isopropylthio-β-galactoside (IPTG) with a final concentration of 2mM was added to induce protein expression for approximately 3-4 hours. Mixture was then centrifuged at 6,000 rpm for 20 minutes and pellet was retrieved. Pellet was resuspended twice in 50 ml of TE Buffer (50mM Tris and 10mM EDTA in 1L of dH₂O) and centrifuged at 6,000 rpm for 20 minutes. Pellet was resuspended in a 60 ml of TE solution (TE buffer volume is 20 times ml of the cell weight) to prevent desired protein degradation and 0.1 mg/ml lysozyme to break the cell wall of the bacteria. Solution was sonicated for 5-6 cycles and centrifuged at 12,000 rpm for 20 minutes to spin down the inclusion body. Solution was resuspended in Triton Buffer (1% Triton and 2M urea in TE) centrifuged at 12,000 rpm for 20 minutes, pellet was retrieved, and step was repeated again. Solution was resuspended in 8 M guanidine hydrogen chloride and TE buffer. Solution was centrifuged at 12,000 rpm for 20 minutes and supernatant with crude protein was obtained.

Protein Purification

The proteins were purified first by the gel filtration method using size exclusion column (Hiload 26/30, Superdex 75 preparative grade ÄTKA prime) with washing solution of 50mM Tris, 5mM of EDTA, and 3M of Gu•HCl. And further purification was operated on HPLC by a gradient washout of 0.05% TFA in H₂O and 0.05% TFA in CH₃CN (340 µL TFA in 1L of distilled water). The HPLC products of protein was then placed in a dialysis cassette with 10,000 molecular weight cutoff (MWCO) and allowed to undergo dialysis in two steps: first in buffer A of 25 mM formic acid to eliminate the acetonitrile and buffer B of 50 mM NaOAc to refold the protease.

Protein Crystallization

The crystals of HIV-1 protease P51 and D25N mutant and TPV, SQV, DRV, and APV were grown using the hanging drop vapor diffusion procedure with a 1:20 and 1:40 ratio of inhibitor to protein. The hanging drop method entails the addition of a droplet (1 μ L) of the purified protease and inhibitor complex, precipitant, buffer, and additives to a cover slip which is inverted onto a well with similar conditions. The drop is allowed to sit overnight at room temperature to allow for precipitation. The conditions under which these protein crystals were grown contain Potassium sodium tartrate, PEG 8000, HEPES, 0.1 M sodium cacodylate in different pH, 0.1 M ZnAc₂ and 0.1 M CaAc₂. Crystals were then frozen, mounted in crytoprotector with 25% glycerol of the mother liquid, and sent for X-ray crystallography analysis at the Southeast Regional Collaborative Access Team X-ray beamlines of the Advanced Photon Source, Argonne National Laboratory, Argonne, IL.

Measurement of Protease Activity and Inhibition

The TPV inhibition constant for P51 was tried to be measured using a fluorescence assay with the substrate Abz-Thr-Ile-Nle-pNO₂Phe-Gln-Arg-NH₂ (where pNO₂Phe is para-nitrophenylalanine, Nle is norleucine and Abz is anthranilic acid) (Bachem Bioscience Inc., King of Prussia, PA), which is based on the p2/NC cleavage site of the viral polyprotein. 10 μ l PRP51 with a concentration of about 50 μ M with a gradient concentration of TPV was mixed with 100 μ l of reaction buffer [100 mM MES (4-morpholineethanesulfonic acid), pH 5.6, 400 mM NaCl, 1 mM ethylenediaminetetraacetic acid, and 5% glycerol] at 26 °C. The reaction was initiated by adding substrate of a final concentration of 72 μ M by monitoring the increase in fluorescence over 5 min using an excitation wavelength of 340 nm and emission wavelength of 420 nm

(pNO₂Phe as quencher-fluorescent acceptor; Abz as fluorescent donor) with a POLARstar OPTIMA 96-well microplate instrument (BMC Labtech).

Results

Expression of HIV Protease P51 Mutant

Expression had to be repeated since cells were not obtained during the first expression phase. When the cells were transferred to a larger broth, they double in number every 60 minutes. Pellet was successfully centrifuged and approximately 3 grams of cells were grown.

Purification of HIV Protease Mutant P51

HIV PR p51 was purified using High Performance Liquid Chromatography (HPLC) and Gel Filtration (Figure . We obtained about 1.2 mg/ml for monomer.

Crystallization of HIV Protease P51 Mutant with Inhibitors

Protein PR p51 D25N was used because the D25N mutation allowed for deactivation of protein activity. We did not try P51 without the D25N mutation. The concentration of protein that was used was 1.2 mg/ml. The protein crystals that were grown were PR p51 and TPV, PR p51 and APV, PR P51 and SQV, and PR p51 and DRV. In each condition, dH₂O was added until final concentration of the mother liquid equaled 600 µl. One condition in which PR p51 and APV (protein-inhibitor ratio of 1:20) crystals grew were 2M Potassium sodium (KNA)tartrate, 0.1 Sodium cacodylate (NaCaco dylate) pH 6.6, HEPES, 15% PEG 8000, 0.02M CaAc₂, and dH₂O. Another condition in which PR p51 and APV (ratio 1:20) crystals grew was 2M KNA tartrate, 0.1 M NaCaco dylate pH 6.0, HEPES, 20% PEG 8000, 0.02M CaAc₂, and dH₂O. The crystals were

medium sized in both conditions. For PR p51 and APV with a protein-inhibitor ratio of 1:40, one condition in which crystals grew was 2M KNA tartrate, 0.1 NaCaco dylate pH 6.6, 15% PEG 8000, HEPES, 0.02 M CaAc₂, and dH₂O. Another condition in which crystals grew with PR p51 and APV (ratio 1:40) was 2M KNA tartrate, 0.1 NaCaco dylate pH 6.0, 18% PEG 8000, HEPES, 0.02 M CaAc₂, and dH₂O. For PR p51 and DRV (ratio 1:20), one condition in which crystals grew was 2M KNA tartrate, 0.1 M NaCaco dylate pH 6.6, HEPES, 15% PEG 8000, 0.02M ZnAc₂, and dH₂O (Image 1).The crystals that were grown were big in size. For PR p51 and SQV (ratio 1:20), one condition in which crystals grew was 2M KNA tartrate, 0.1 M NaCaco dylate pH 6.0, HEPES, 20% PEG 8000, 0.02M ZnAc₂, and dH₂O. Another condition in which PR p51 and SQV (ratio 1:20) grew crystals was 2M KNA tartrate, 0.1 M NaCaco dylate pH 6.0, HEPES, 10% PEG 8000, 0.02M CaAc₂, and dH₂O. When growing PR p51 and SQV (ratio 1:40) under the following conditions 2M KNA tartrate, 0.1 M NaCaco dylate pH 6.0, HEPES, 15% PEG 8000, 0.02M ZnAc₂, and dH₂O, crystals grew. These crystals were medium sized. Lastly, for PR p51 and TPV, one condition in which crystals grew was 2M KNA tartrate, 0.1 M NaCaco dylate pH 6.0, HEPES, 20% PEG 8000, 0.02M ZnAc₂, and dH₂O (Image 2). Very small sized crystals formed. All crystals listed above were sent to Argonne National Laboratories for diffraction data collection at the Southeast Regional Collaborative Access Team X-ray beamlines. Processing and analysis of the X-ray crystallographic data is in progress.

Enzyme activity and inhibition

The enzyme activity of the protease was tested and there was no activity. Inhibition of the enzyme could not be measured. The concentration used was 1.29 mg/ml. For inhibition constant

measurement, we did not obtain any result due the poor activity of P51. The protein did not show sufficient activity. The expression will need to be repeated with fresh cells and or plasmid DNA.

Discussion

Antiviral drugs have been successful when it comes to reducing the effects of the HIV virus. With the development of drug resistance the virus continues to function and compromise the immune system of HIV/AIDS patients. HIV PR p51 (51 serial passages/ stages) was used in this study because it is one of many mutants that are extremely resistant to drug therapy for HIV. Studying HIV PR P51 in the complex with inhibitors can lead to the development of successful therapeutic agents. To analyze, the complex we expressed and purified the protein and grew crystals to get its crystalline structure. HIV PR P51 was expressed and purified successfully and protein crystals were grown of PR p51 with 4 different inhibitors (TPV, SQV, DRV, and APV). PR p51 and TPV crystals were very small. PR p51 and SQV crystals were good medium sized crystals. PR p51 and APV crystals were also good medium sized crystals. PR p51 and DRV crystals were good and large in size. Enzyme activity and inhibition didn't turn out as planned because the activity of p51 was poor. Therefore we could not measure the inhibition of the enzyme. The next step in research would be to redo the experiment with fresh cells or plasmid DNA to determine the inhibition constant measurement of the protein P51. This research project is a portion of a bigger research project that is currently being conducted. The data obtained will help with the creation of new and successful antiviral drugs against HIV and it will also help better understand the mechanism and molecular origin of drug resistance.

Figure 7. HIV-1 PR p51 Gel Filtration Results

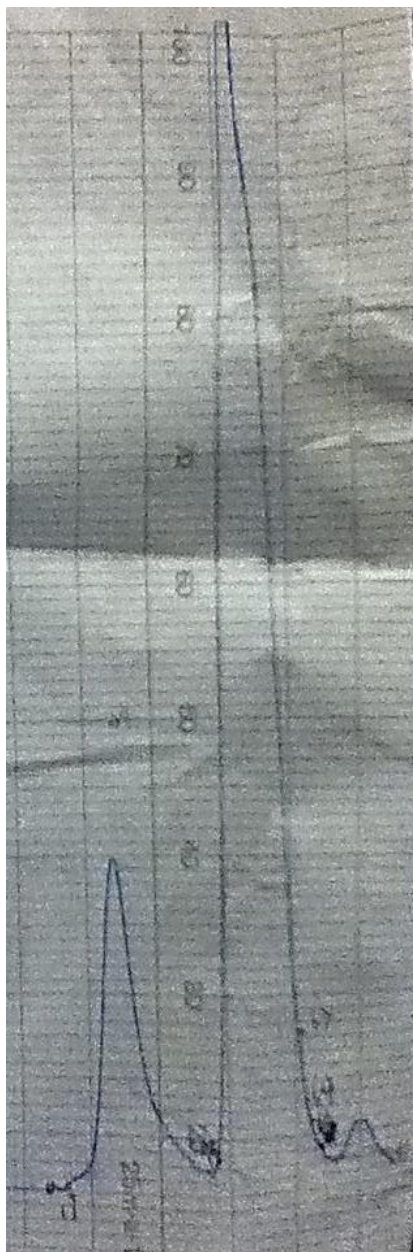


Figure 7. This image represents the results we obtained from gel filtration. The high peak represents the purified protein PR p51 and D25N that we obtained.

Figure 8. PR p51 and APV crystals.

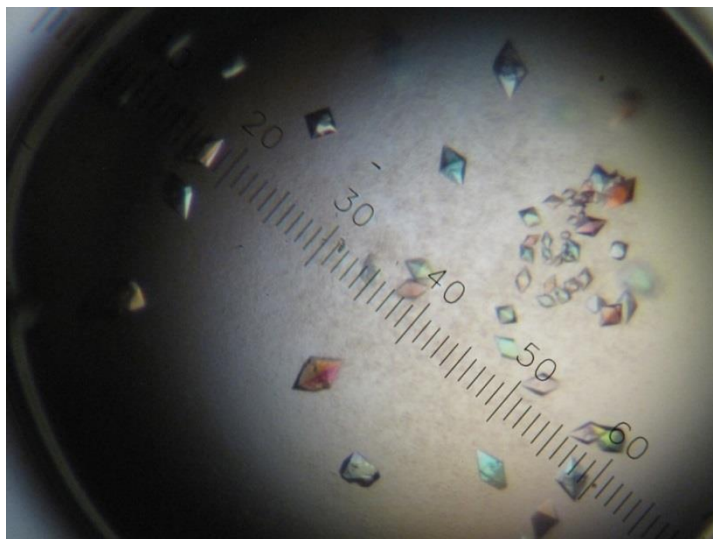


Figure 8. This image shows PR p51 and APV (protein-inhibitor ratio 1:20) crystals that grew under the following conditions: 2M KNA tartrate, 0.1 M NaCaco dylate pH 6.0, HEPES, 20% PEG 8000, 0.02M ZnAc₂, and dH₂O.

Figure 9. PR p51 with DRV crystals

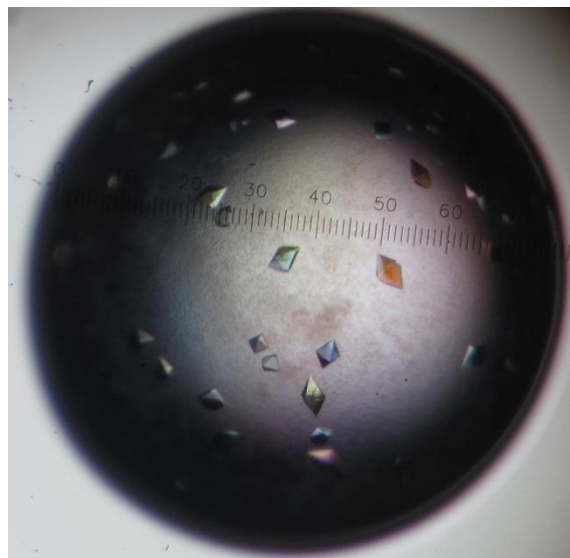


Figure 9. Picture of crystals grown for PR p51 and DRV (protein-inhibitor ratio 1:20). The conditions in which these crystals were grown are 2M KNA tartrate, 0.1 M NaCaco dylate pH 6.6, HEPES, 15% PEG 8000, 0.02M ZnAc₂, and dH₂O.

Figure 10. PR p51 and SQV crystals.

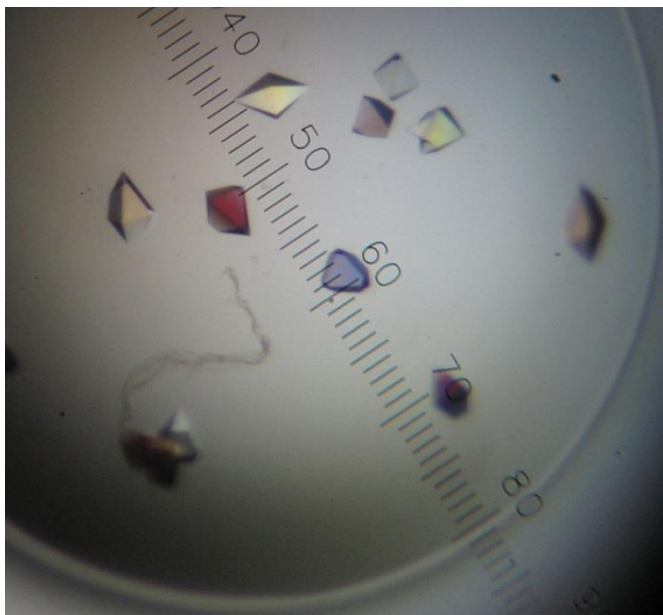


Figure 10. This figure represents crystals from PR p51 and SQV (ratio 1:20) which grew under the following conditions: 2M KNA tartrate, 0.1 M NaCaco dylate pH 6.0, HEPES, 10% PEG 8000, 0.02M CaAc₂, and dH₂O.

Figure 11. PR p51 and TPV crystals

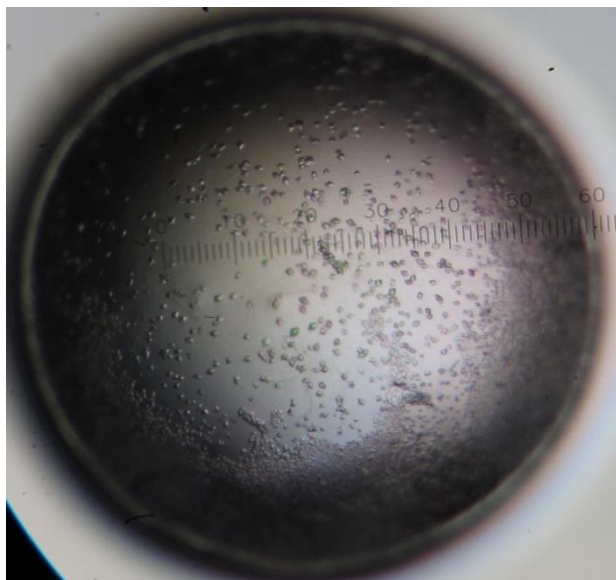


Figure 11. This photo represents PR p51 and TPV (ratio 1:20) crystals grown under the following conditions: 2M KNA tartrate, 0.1 M NaCaco dylate pH 6.0, HEPES, 20% PEG 8000, 0.02M ZnAc₂, and dH₂O.

References

1. Agniswamy, J., Weber, I.T. HIV-1 Protease: Structural Perspectives on Drug Resistance.(2009) *Viruses* 1, 1110-1136.]
2. Antiviral Therapy. (2012). Retrieved October 20, 2012, from World Health Organization: http://www.who.int/topics/antiretroviral_therapy/en/
3. A., W., & J.W., E. (1993). Structure-based inhibitors of HIV-1 protease. *Annual Review of Biochemistry*, 62:543-585.
4. Basic Information about HIV and AIDS. (2012, April 11). Retrieved 2 October, 2012, from <http://www.cdc.gov/hiv/topics/basic/index.htm>
5. Emini, E. A. (2002). "The human Immunodeficiency Virus: Biology, Immunology, and Therapy." Princeton University Press Chapter 1(1-43).
6. Flexner, C. (1998). HIV-Protease Inhibitors. *New England Journal of Medicine*, 338:1281-1293.
7. Freed, E. (1998). HIV-1 gag proteins: diverse functions in the virus life cycle. *Virology*, 251(1):1-15.
8. Goto, T., Nakai, M., & Ikuta, K. (1998). The life-cycle of human immunodeficiency virus type 1. *Micron* , 29(2-3):123-38.
9. Hladik, F., & McElrath, M. (2008). Setting the stage: host invasion by HIV. *Nat Rev Immunol*, 8(6):447-57.
10. Koh, Y., Nakata, H., Maeda, K., Ogata, H., Bilcer, G., Devasamundam, T., Kincaid, J.F., Boross, P., Wang, Y.-F., Tie, Y., Volarath, P., Gaddis, L., Harrison, R.W., Weber, I.T., Ghosh, A.K., Mitsuya, H. Novel bis-tetrahydrofuranylurethane-containing nonpeptidic

- protease inhibitor (PI) UIC-94017 (TMC114) potent against multi-PI-resistant HIV in vitro. (2003) *Antimicrob. Agent Chemother.* 47, 3123-3129.
11. Liu, F., Boross, P. I., Wang, Y. F., Tozser, J., Louis, J. M., Harrison, R. W. and Weber, I. T. (2005). "Kinetic, stability, and structural changes in high-resolution crystal structures of HIV-1 protease with drug-resistant mutations L24I, I50V, and G73S." *J. Mol. Biol.* 354(4): 789-800.
 12. Louis, J. M., Weber, I. T., Tozser, J., Clore, G. M. and Gronenborn, A. M. (2000). "HIV-1 protease: maturation, enzyme specificity, and drug resistance." *Adv. Pharmacol.* 49: 111-146.
 13. Stevenson, M. (2003). HIV-1 pathogenesis. *Nature Medicine* , 9, 853 - 860 .
 14. Tie, Y., Kovalevsky, A. Y., Boross, P. I., Wang, Y. F., Ghosh, A. K., Tozser, J., Harrison, R. W., and Weber, I. T. (2006). "High Resolution Crystal Structures of HIV-1 Protease and Mutants V82A and I84V with Saquinavir." *Proteins: Structure, Function, and Bioinformatics* 67: 232-242.
 15. Turner, B. G. and Summers, M. F. (1999). "Structural biology of HIV." *J. Mol. Biol.* 285(1): 1-32.
 16. Wlodawer, A., & Vondrasek, J. (1998). Inhibitors of HIV-1 protease: a major success of structure-assisted drug design. *Annu Rev Biophys Biomol Struct* , 27:249-84.
 17. *Worldwide HIV/AIDS Statistics Commentary*. (2012). Retrieved November 10, 2012, from AVERT: <http://www.avert.org/worlstatinfo.htm>
 18. De Meyer, S., Azijn, H., Surleraux, D., Jochmans, D., Tahri, A., Pauwels, R., Wigerinck, P., and de Bethune, M. P. (2005) TMC114, a novel human immunodeficiency virus type

- 1 protease inhibitor active against protease inhibitor-resistant viruses, including a broad range of clinical isolates, *Antimicrob Agents Chemother* 49, 2314-2321.
19. Ghosh, A., Sridhar, P., Leshchenko, S., Hussain, A., Li, J., Kovalevsky, A., Walters, D., Wedekind, J., Grum-Tokars, V., and Das, D. (2006) Structure-based design of novel HIV-1 protease inhibitors to combat drug resistance, *Journal of Medicinal Chemistry* 49, 5252-5261.
20. Tie, Y., Boross, P. I., Wang, Y. F., Gaddis, L., Hussain, A. K., Leshchenko, S., Ghosh, A. K., Louis, J. M., Harrison, R. W., and Weber, I. T. (2004) High resolution crystal structures of HIV-1 protease with a potent non-peptide inhibitor (UIC-94017) active against multi-drug-resistant clinical strains, *Journal of molecular biology* 338, 341-352.