Structural analysis of wild type HIV-1 protease in complex with GRL08613, a new protease inhibitor

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INTRODUCTION

· The **human immunodeficiency viruses** (**HIV**) are two species of *Lentivirus* (a subgroup of retrovirus) that infect humans. Over time, they cause acquired immunodeficiency syndrome (AIDS), a condition in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype.

HIV infects vital cells in the human immune system, such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells. HIV infection leads to low levels of $CD4^+$ T cells through a number of mechanisms, including pyroptosis of abortively infected T cells apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by $CD8^+$ cytotoxic lymphocytes that recognize infected cells When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections, leading to the development of AIDS.

· HIV is a member of the genus *Lentivirus*, part of the family *Retroviridae*. Lentiviruses have many morphologies and biological properties in common. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses. Upon entry into the target cell, the viral RNA genome is converted (reverse transcribed) into double-stranded DNA by a virally encoded enzyme, reverse transcriptase, that is transported along with the viral genome in the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded enzyme, integrase, and host co-factors.

· Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed both lymphadenopathy associated virus (LAV) and human T-lymphotropic virus 3 (HTLV-III). HIV-1 is more virulent and more infective than HIV-2, and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2, compared to HIV-1, implies that fewer of those exposed to HIV-2 will be infected per exposure. Due to its relatively poor capacity for transmission, HIV-2 is largely confined to West Africa.

If left untreated, HIV can lead to the disease AIDS (*acquired immunodeficiency syndrome*).The human body can't get rid of HIV and no effective HIV cure exists. So, once you have HIV, you have it for life.

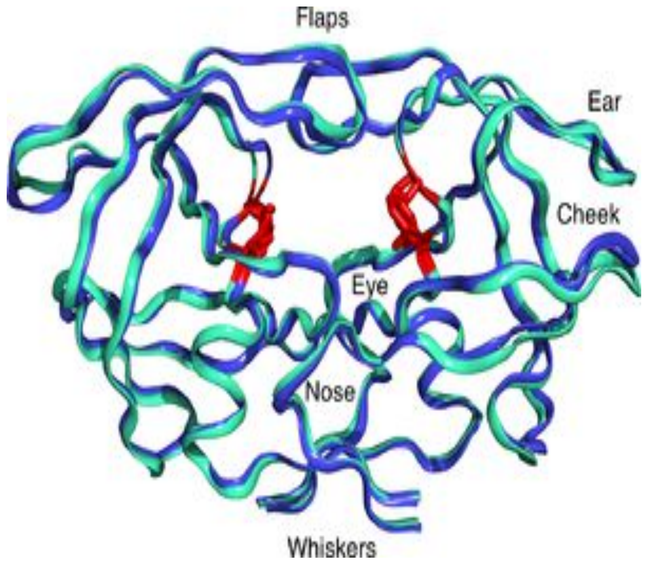
- HIV is transmitted though rblood
- semen
- vaginal and rectal fluids
- breast milk

HIV does NOT transfer through:

- · skin-to-skin contact
  · air or water
  · sharing food or drinks, including drinking fountains
- saliva, tears, or sweat (unless mixed with the blood of a person with HIV)
- sharing a toilet, towels, or bedding
- mosquitoes or other insects

· First identified in 1981, HIV is the cause of one of humanity's deadliest and most persistent epidemics.

- · Both HIV-1 and HIV-2 are believed to have originated in non-human primates in West-central Africa, and are believed to have transferred to humans (a process known as zoonosis) in the early 20th century.
- Antibody tests can take 23 to 90 days to **detect HIV** infection after an exposure. Most rapid tests and the only FDA-approved **HIV** self-**test** are antibody tests. In general, antibody tests that use blood from a vein can **detect HIV** sooner after infection than tests done with blood from a finger prick or with oral fluid.



EXPERIMENTALS

The tools involved in the structural analysis of protein and drug are Protein Data Bank(PDB) and Pymol…

· **PyMOL**, a cross-platform molecular graphics tool, has been widely **used for** three-dimensional (3D) visualization of proteins, nucleic acids, small molecules, electron densities, surfaces, and trajectories. It is also capable of editing molecules, ray tracing, and making movies.

· **PyMOL** is an open source molecular visualization system created by Warren Lyford DeLano. It was commercialized initially by DeLano Scientific LLC, which was a private software company dedicated to creating useful tools that become universally accessible to scientific and educational communities. It is currently commercialized by Schrödinger, Inc. PyMOL can produce high-quality 3D images of small molecules and biological macromolecules, such as proteins.

· PyMOL is one of the few open-source model visualization tools available for use in structural biology. The *Py* part of the software's name refers to the program having been written in the programming language Python. It is also capable of editing molecules, ray tracing, and making movies. This Python-based software, alongside many Python plugin tools, has been developed to enhance its utilities and facilitate the drug design in PyMOL. To gain an insightful view of useful drug design tools and their functions in PyMOL, we present an extensive discussion on various molecular modeling modules in PyMOL, covering those for visualization and analysis enhancement, protein–ligand modeling, molecular simulations, and drug screening: This provides an excellent introduction to present 3D structures visualization and computational drug design in PyMOL.

· The **Protein Data Bank** (**PDB**) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography, NMR spectroscopy, or, increasingly, cryo-electron microscopy, and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organisations . The PDB is overseen by an organization called the Worldwide Protein Data Bank, wwPDB.

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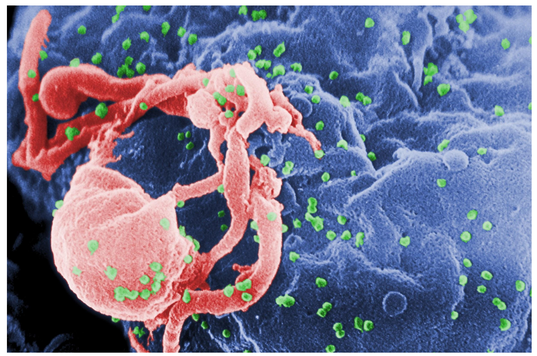

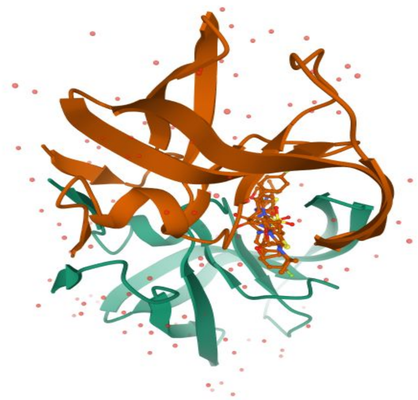

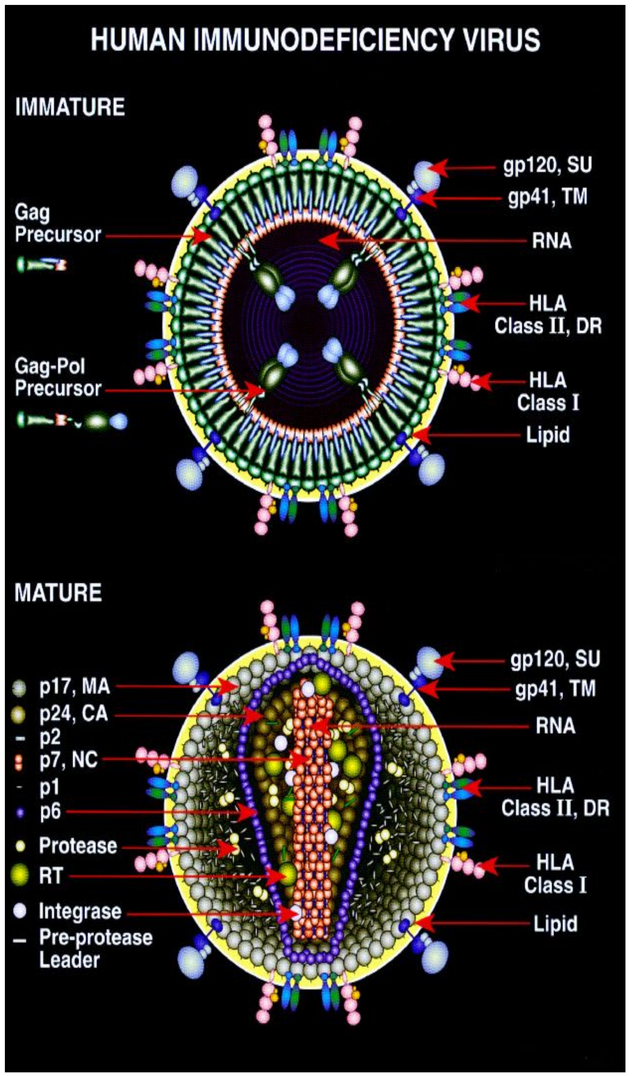

RESULTS & DISCUSSION

HIV-1 protease (PR) is a retroviral aspartyl protease (retropepsin), an enzyme involved with peptide bond hydrolysis in retroviruses, that is essential for the life-cycle of HIV, the retrovirus that causes AIDS.[1] HIV protease cleaves newly synthesized polyproteins (namely, Gag and Gag-Pol at nine cleavage sites to create the mature protein components of an HIV virion, the infectious form of a virus outside of the host cell. Without effective HIV protease, HIV virions remain uninfectious.

As an aspartic protease, the dimerized HIV-1 PR functions through the aspartyl group complex, in order to perform hydrolysis. Of the two Asp25 residues on the combined catalytic active site of HIV-1 PR, one is deprotonated while the other is protonated, due to pKa differences from the micro-environment.

In a general aspartic protease mechanism, once the substrate is properly bound to the active site of the enzyme, the deprotonated Asp25 catalytic amino acid undergoes base catalysis, rendering the incoming water molecule a better nucleophile by deprotonating it. The resulting hydroxyl ion attacks the carbonyl carbon of the peptide bond, forming an intermediate with a transient oxyanion, which is stabilized by the initially protonated Asp25. The oxyanion re-forms a double bond, leading to the cleavage of the peptide bond between the two amino acids, while the initially deprotonated Asp25 undergoes acid catalysis to donate its proton to the amino group, making the amino group a better leaving group for complete peptide bond cleavage and returning to its original deprotonated state.

While HIV-1 PR shares many of the same characteristics as a non-viral aspartic protease, some evidence has shown that HIV-1 PR catalyzes hydrolysis in a concerted manner; in other words, the nucleophilic water molecule and the protonated Asp25 simultaneously attack the scissile peptide bond during catalysis.

With its integral role in HIV replication, HIV protease has been a prime target for drug therapy. HIV protease inhibitors work by specifically binding to the active site by mimicking the tetrahedral intermediate of its substrate and essentially becoming "stuck," disabling the enzyme. After assembly and budding, viral particles lacking active protease cannot mature into infectious virions. Several protease inhibitors have been licensed for HIV therapy.

There are ten HIV-1 PR inhibitors that are currently approved by the Food and Drug Administration. These include indinavir, saquinavir, ritonavir, nelfinavir, lopinavir, amprenavir, fosamprenevir, atazanavir, tipranavir, and darunavir. Many of the inhibitors have different molecular components and thus different mechanistic actions, such as blocking the active site. Their functional roles also extend to influencing circulation concentrations of other inhibitor drugs (ritonavir) and being used only for certain circumstances in which the virus exhibits tolerance of other inhibitors (tipranavir).

The analysis of hiv-1 and drug molecuke involves 23 hydrogen bonds and these are analysed with the help of pymole.

REFERENCES

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e-poster presented online at the ABFR-2020.