Lecture 20: Retroviruses & Inhibitors - HIV Protease.
- Identify potential drug targets based on viral life cycle.
- Compare and contrast serine to aspartyl proteases
- Measure inhibitor binding to characterize drug efficiency.

Human Immunodeficiency Virus (HIV)
- The normal flow of information in cells is: DNA $\rightarrow$ mRNA $\rightarrow$ protein
- In retroviruses, the genetic information is stored in RNA (viral RNA, vRNA) which must be first be copied into DNA: vRNA $\rightarrow$ DNA $\rightarrow$ mRNA $\rightarrow$ viral protein

Central Role of T-helper (T_H) Cells in Immunity:
- B-cells bind to antigen (pathogens = bacteria, viruses) with antibody molecules on their cell surface.
- Peptides from the virus are displayed to T_H (T_H) cells on a membrane protein called MHC.
- T_H cells activate B-cells so that they can differentiate into plasma cells $\rightarrow$ secrete antibodies that inactivate pathogens
- Infection of the T_H cells by HIV results in loss of T_H cells.
- HIV infected individuals cannot make antibodies against relatively harmless pathogens and can die from common infections or rare cancers.

The HIV virus particle contains three essential enzymes for viral replication (these enzymes are coded by the virus)

i) reverse transcriptase,
   Copies vRNA to DNA, error prone $\rightarrow$ drug resistant HIV.

ii) integrase,
   Inserts DNA copy into host cell chromosome.

iii) HIV protease
   Cleaves immature viral proteins to make mature proteins, that assemble into the virus.

HIV Viral Infection of T-Helper Cells:
1. Virus binds to molecules displayed on the T_H cell surface.
2. The virus then fuses with the cell membrane and releases its RNA genome from its lipid envelope.
3. The enzyme reverse transcriptase first makes a double-stranded DNA copy of the viral RNA molecule. This process is error prone, leading to mutations in the virus. These mutations cause drug resistant strains of the virus to arise.
4. The DNA is integrated into the host cell's DNA by an enzyme called integrase.
5. Integrated DNA produces vRNA, the genetic material for new virus particles. mRNA is also made from this DNA, to produce proteins for new particles.
6. Immature viral proteins are made from mRNA using the normal protein synthesizing machinery in the host cell.
7. HIV protease required for maturation of viral proteins, by cleaving them into smaller proteins that form the mature virus.
Drug Targets to Combat the HIV Virus:
1. Reverse transcriptase
2. Integrase
3. HIV Protease
Why are these good targets for inhibitors that can act as anti-virals?

HIV Protease (Aspartyl protease) [https://www.andrew.cmu.edu/user/rule/jmol/hiv_prot.html]
1. An essential enzyme in the maturation of the HIV virus. If inhibited, the virus cannot replicate.
2. The HIV protease is a homo-dimeric protein, containing two catalytic Asp residues, Asp25 and Asp25', the same residue on each chain. However, the pK values of these two differ widely, one is about 4.0 and the other about 6.0. It is a member of the general class of Aspartate proteases.
3. One of the Asp residues must be protonated the other must be deprotonated for full activity.
4. Water is the activated nucleophile, no acyl-intermediate.
5. Prefers hydrophobic substrates due to Val82 plus other non-polar residues in its active site.

Mechanism:
1. Activation of H$_2$O by Asp25'
2. Nucleophilic attack on C=O of substrate
3. Tetrahedral transition state
4. Peptide bond cleavage
5. Protonation of new NH$_2$ by Asp25
Inhibition of HIV Protease (HIV Drugs):
- Most drugs are small peptide-like analogs with non-
  cleavable bonds that resemble peptide bonds.
- Unfortunately, viruses containing mutations in their HIV
  protease arise. These mutants are resistant to current HIV
  drugs.

Drug Design: Compounds A and B (see next page) are
candidates for HIV protease inhibitors. Which of the two
drugs will be more effective at inhibiting the wild-type
protease?

Answer: We will assume that these are competitive
inhibitors. Therefore, we need to compare the $K_i$ values
for each inhibitor binding to the protease, using the
following steps.

1. Select a suitable substrate for steady-state assays.
   The kinetics of HIV protease can be measured using this
   substrate, producing a bright yellow nitrophenolate ion.

https://www.andrew.cmu.edu/user/rule/mmol/hiv_prot_
    drugAB.html

2. Measuring $K_i$ for both Drugs:
   a) Acquire velocity versus substrate, no inhibitor.
   b) Acquire velocity versus substrate, fixed inhibitor.
   c) Plot double reciprocal plots.
   d) Obtain $\alpha$ from ratio of slopes
   e) $K_i = [I]/(\alpha - 1)$

\[
\begin{array}{|c|c|c|c|}
\hline
[S] \mu M & v_{(I=0)} & v_{([A]=10 \text{ nM})} & v_{([B]=10 \text{ nM})} \\
\hline
10 & 7.3 & 3.8 & 0.7 \\
50 & 25.7 & 16.0 & 3.2 \\
\hline
1/\{S\} & 1/v_{(I=0)} & 1/v_{([A]=10 \text{ nM})} & 1/v_{([B]=10 \text{ nM})} \\
\hline
0.10 & 0.138 & 0.263 & 1.513 \\
0.02 & 0.038 & 0.063 & 0.313 \\
\hline
\end{array}
\]

The units of velocity are micromoles product/sec.
$\alpha$ is obtained from the ratio of the slopes in the double
reciprocal plot.

<table>
<thead>
<tr>
<th></th>
<th>Slope</th>
<th>$\alpha = \text{slope}(I&gt;0)/\text{slope}(I=0)$</th>
<th>$K_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Inh.</td>
<td>1.25</td>
<td></td>
<td>$10 \text{ nM}$</td>
</tr>
<tr>
<td>Inhibitor A</td>
<td>2.50</td>
<td>$\alpha = 2.50/1.25 = 2$</td>
<td>$0.9 \text{ nM}$</td>
</tr>
<tr>
<td>Inhibitor B</td>
<td>15.00</td>
<td>$\alpha = 15.00/1.25 = 12$</td>
<td>$10 \text{ nM}/[I] \approx 0.9 \text{ nM}$</td>
</tr>
</tbody>
</table>

Once the $\alpha$ values are found, we can calculate the $K_i$ for each inhibitor using the formula: $K_i = [I]/(\alpha - 1)$. 
Drug resistance & rational drug design:
- Error prone copying of vRNA to DNA introduces changes in the sequence of the viral RNA (mutations), leading to altered amino acids in the viral proteins.
- Changes in the residues that are involved in drug binding may reduce binding.
- The mutant virus is no longer inhibited from growing and will quickly overgrow the wild-type virus.
- A common mutation that arises in many HIV patients is changing \text{Val82} \rightarrow \text{Asn82} in HIV protease.
- The altered HIV protease can be inhibited with modified protease inhibitors. [https://www.andrew.cmu.edu/user/rule/jmol/hiv_prot_mut.html](https://www.andrew.cmu.edu/user/rule/jmol/hiv_prot_mut.html)

*How might you alter the existing inhibitor to be effective at binding to the Asn82 mutation?*

Other HIV Targets:
Reverse Transcriptase:
Reverse transcriptase is an RNA dependent DNA polymerase, it uses the viral RNA as a template to generate a DNA copy.

There are two types of reverse transcriptase inhibitors:
- a) Nucleoside analogs, e.g. AZT

\[
\text{NH} \\
\text{HO} \\
\text{N=N=N}
\]

- b) Non-nucleoside inhibitors (e.g. nevirapine):

\[
\text{O} \\
\text{H}
\]