Part 1: HIV protease inhibitors: HIV\_protease.pse

This file contains the structure of HIV protease with several lead compounds as well as with two FDA approved drugs.

1) Several companies (including Agouron, Upjohn, and Roche) were working on developing HIV protease inhibitors, they each used structure-based design. Structures of the protease with several lead compounds (labeled cmpnd1-4) are in this file, and their KI (concentration of inhibition of 50% of activity) are shown in the table to the left. Compare the surface of the protease with the regions of the inhibitors that are modified. Is “bigger” always better (a lower KI is better)? What regions seem to matter most?

|  |  |
| --- | --- |
| **Drug** | **KI** |
| Cmpnd 1 | 560 nM |
| Cmpnd 2 | 160 nM |
| Cmpnd 3 | 41 nM |
| Cmpnd 4 | 0.4 nM |
| *Tipranivir* | 8 pM (0.008 nM) |

2) Compare the lead compounds with the eventual FDA-approved Upjohn drug, Tipranivir. Which lead compound is it most similar to. Try to see what the chemists learned from probing each of the regions of the compound (note that they made over 100 versions; we're just looking at 5 of them).

3) Compare Tipranivir with Agouron's inhibitor, nefranivir. What residues are they contacting in the protein? (one way to see this is to create selections using the command line: e.g. activate only the tipranivir structure and **select nearTPV, visible and byresidue resname TPV around 3.5** or activate only the nefranivir structure and **select nearNEF, visible and byresidue resname 1UN** then show sticks for them). Note: you can figure out the residue name ('resname') of a ligand by clicking on it and seeing what prints out in the top window. The PDB database also has a tool to do this: <https://www.rcsb.org/3d-view/1OHR?preset=ligandInteraction&sele=1UN> (where 1OHR the ID of a PDB file, in this case the Nefranivir structure and 1UN is the resname of the Nefranivir in the structure).

Part 2: Influenza virus neuraminidase inhibitors: Flu\_neuraminidase.pse

This file contains the structure of various isolates of flu neuraminidase (including from H1N1 and the 1918 spanish flu).

1) Neuraminidases cleave sialic acid from glycoproteins, releasing flu virus from the host cell so it can infect the next cell. Scientists solved the structure of the flu enzyme in complex with sialic acid (the NA\_sialate object). Note that neuraminidase is, itself, a glycoprotein, and sugars (covalently attached to asparigine residues) are shown in stick representation. Take a look at these (relatively simple) sugar modifications, and compare their size to that of the protein. Because of their size sugars, often serve to protect proteins from degradation, or, in the case of flu and bacteria, from attack by the immune system.

2) Locate the active site (it has a sialic acid bound in its center). Middle click on the sialate to center and zoom in. Take a look at the surface of NA\_sialate protein in comparison to the bound sialate. When scientists solved this, they noticed that there were “pockets” that they could build into around the sialate. Can you identify which positions look to be good places to 'build out' from the sialic acid?

3) Show some of the lead compounds (1B9S/1B9T/1B9V; you can hide the cartoons for them – if you make a mistake and need to show the sticks for just the drugs, you can type “show sticks, not polymer” in the command line portion). Why do you think some regions of the sialic acid remain unmodified in each of these molecules?

4) Now compare the Tamiflu and Relenza structures to those of the lead compound and to each other. How are they similar, how are they different?

5) The H274Y mutation (or H275Y in some strains) renders neuraminidases resistant to Tamiflu (1000-fold drop in EC50; effective concentration for 50% activity), but not to Relenza. In the Tamiflu structure, show the cartoon of the protein, then show sticks for only His274 (and for the Tamiflu). This can be done by showing the sequence and clicking on His274 to select it, or by command line (show sticks, resid 274 and visible). You can simulate this mutation using the mutagenesis wizard (see the PyMOL basics ppt). Try mutating His274 to Tyr. What does it look like.

6) Now look at the actual Relenza structure with His274. Why might Relenza still bind, while Tamiflu doesn't (hint: there are two potential reasons). If you were a medicinal chemist, how might you modify Tamiflu to overcome this inhibition (without making it look exactly like Relenza!).