Structural Biology Exercise #2
"Function of Non-steroidal Anti-inflammatory Drugs"

GOALS OF THE PRACTICAL:
1. Get familiar with proteopedia, protein data bank and pymol
2. How to find information about the structure
3. How to visualize and compare structures

1. What is the target molecule of Aspirin? Can we find structural information regarding its function?

PROTEOPEDIA

Mission Statement (quotation)
To collect, organize and disseminate structural and functional knowledge about protein, RNA, DNA, and other macromolecules, and their assemblies and interactions with small molecules, in a manner that is relevant and broadly accessible to students and scientists.

Goals for Proteopedia
- *To serve as a forum for the scientific community to share, retrieve and discuss information related to proteins, macromolecules, and small molecules and chemicals of interest.*
- *To continue to develop the concept of tying text to three-dimensional, interactive images.*
- *To maintain low barriers for contribution.*

Action: Go to Proteopedia (www.proteopedia.org) and search for "aspirin".
As a search hit you find a link that explains briefly how the target protein works and how aspirin affects the target. On the right you see a Jmol window displaying the structure. You can manipulate the view with mouse. While reading the text you can click on green links to update the view of the molecule.

Note the PDB (protein data bank) code 1PTH.

Follow the link to the RCSB PDB Molecule of the Month to read a short but more detailed description about the mechanism and inhibition of the protein.

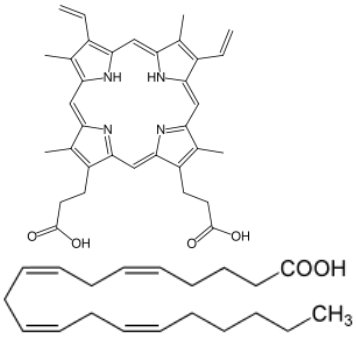
2. Taking a look at the structures yourself.

Go to PDB web site *www.pdb.org*

Search for the PDB code with
- arachidonic acid bound to COX-1 = 1DIY
- aspirin bound to COX-1 = 1PTH
- ibuprofen bound to COX-1 = 1EQG

note the information available on the summary page (primary citation, related pdb entries, ligands, ...) and in other tabs (sequence, annotations, …). What are the three letter identifiers for arachidonic acid, aspirin product and ibuprofen?
ACD, SAL, IBP

Launch pymol
select *file/open session* and select aspirin.pse from the USB stick (file contains already the mentioned three pdb files aligned). You should be able to also just double click the aspirin.pse file in windows.
select 1DIY *A/preset/publication*



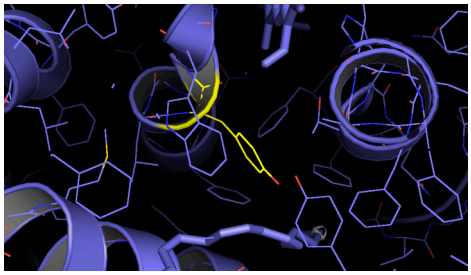
Locate protoporphyrin IX and the bound substrate arachidonic acid shown in the pictures on the right.
(http://en.wikipedia.org/wiki/Protoporphyrin_IX)
(http://en.wikipedia.org/wiki/Arachidonic_acid)

If you have problems recall a stored scene from pull-downs *Scene/Recall/F1* or just press *F1.*

Point at protoporphyrin IX and *RMB/residue/center*
Zoom in with *RMB/up-down*
F2

For 1DIY select *C/by element* and select the color scheme of your choice.

1DIY/S/lines – locate the active site tyrosine situated between heme and arachidonic acid.
(http://en.wikipedia.org/wiki/Amino_acid)



Can not find it? Check the picture on the right.
Still problems? Type “select 1DIY and resid 385” to the command line in the smaller pymol window.

Use mouse scroll to control slab, RMB/up-down to control zoom and make a more clear view.
You can also use MMB to center the view to a atom.
point at this tyrosine (Tyr385) and *RMB/residue/show/sticks*
point at this tyrosine (Tyr385) and *RMB/residue/hide/main chain*
F3

1DIY/H/lines

You can select a different colour sheme for ligands if you want *RMB/residue/color/...*

Save the scene by selecting from the pull-downs *scene/store/F10*
You can return to this scene later by pressing F10 on the keyboard.

Turn on 1PTH by clicking it.
(Note: 1PTH is prealigned in pymol session to 1DIY with *A/align/to molecule/1DIY)*
Can you explain why aspirin inhibits COX?

Center to the arachidonic acid by MMB and look for aspirin and a modified residue.
Point at aspirin and *RMB/residue/show/sticks*
Point at acetylated residue *RMB/residue/show/sticks + RMB/residue/hide/main chain*
Hide lines for 1PTH *H/lines.*
Save the scene by selecting from the pull-downs *scene/store/F11*

Answer: Both aspirin and the acetylated serine are blocking the binding site for arachidonic acid.

Stuck? Press F4

unselect 1PTH and select 1EQG. (It is superposed to 1DIY *A/align/to molecule/1DIY)*
Can you explain why ibuprofen inhibits COX? What is the difference between the inhibition mechanisms?

Point at ibuprofen and *RMB/residue/show/sticks*

Can not find ibuprofen? Press F5
Hide lines and nonbonded atoms for 1EQG. *H/lines & H/nonbonded*
Save the scene by selecting from the pull-downs *scene/store/F12*

Answer: Aspirin is an irreversible inhibitor as it covalently modifies the protein. Ibuprofen only competes for the binding site in the protein with arachidonic acid and is therefore a competitive reversible inhibitor.

3. Make an image from one of the scenes above.

Select which one you want to make an image of and go back to the stored scene.

Select from a pull-down *display/background/white*
Select from a pull-down *settings/transparency/cartoon/60%*

Set the view the way you like it.
Tip: you can also hide the sugars by *RMB/residue/hide/sticks*
Tip: when the cartoon is transparent you can zoom out to get a more complete view of the protein.

Set the view the way you like it and hit the "ray" button.
File/save image as/png and give the name of the image.

4. Too easy...

How about other non-steroidal anti-inflammatory drugs? Do we know how do they bind?

Tips:
What other NSAIDs are there? (google)
Search PDB with the drug name.
Look at related PDB entries.

Example: 3NT1. Load the PDB file using the PDB loader plugin. Align to 1DIY A/align/to molecule/1DIY. Go back to the stored scene. Turn on lines for 3NT1.

As you read from the "molecule of the month" COX-2 is really the target and inhibition of COX-1 leads to harmful effects. Can you find from PDB structures of COX-2 with selective inhibitors.

Example: celecoxib 3LN1