CH-314: 2022 Dynamics of biomolecular processes – Structural biology

Investigating protein structures

This exercise should prompt you to explore protein structures using PyMOL

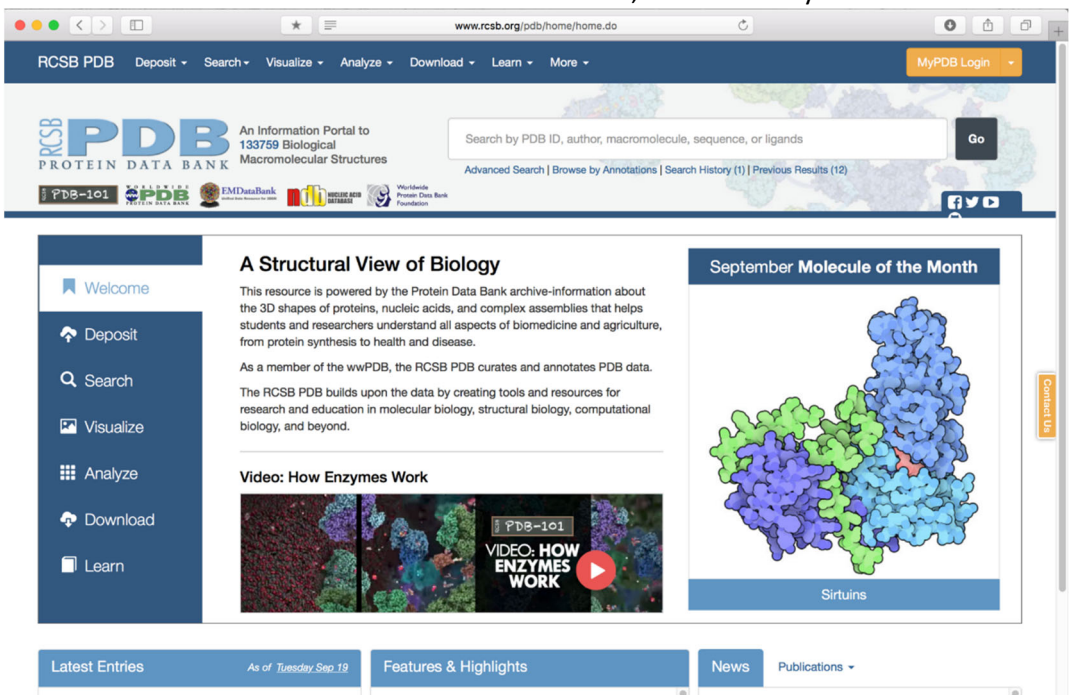
Plan for this exercise:
Analyzing the catalytic mechanism of **sepiapterin reductase.**

Where to find experimental protein structures?

Protein Data Bank: http://www.rcsb.org

Contains more than 133'000 models, from X-ray, NMR & EM method, all are annotated and highly curated

You will also find useful information on all structures, tools for analysis and useful links.



1. Go to http://www.rcsb.org

Search for „sepiapterin reductase"

Which one to choose? We want info on the catalytic mechanism **=> select „1SEP"**

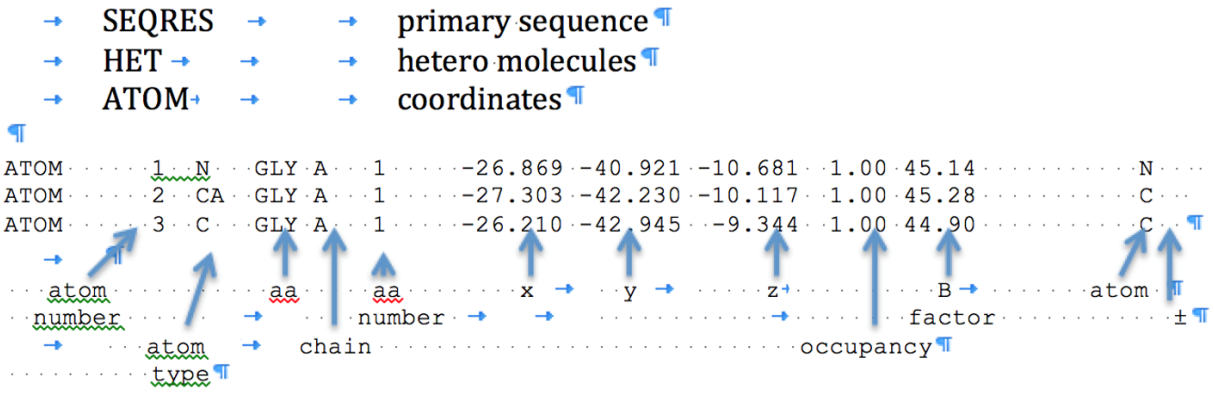
Which infos are available?

- Experimental data Snapshot
- Publications
- Description protein
- Description small molecules
- Interaction small – protein
- Data & Validation

2. Download Files

Select **„PDB format“ => “1SEP.pdb”**

PDB files contain all the 3D coordinates of all the atoms in the structure, about 50 pages of text

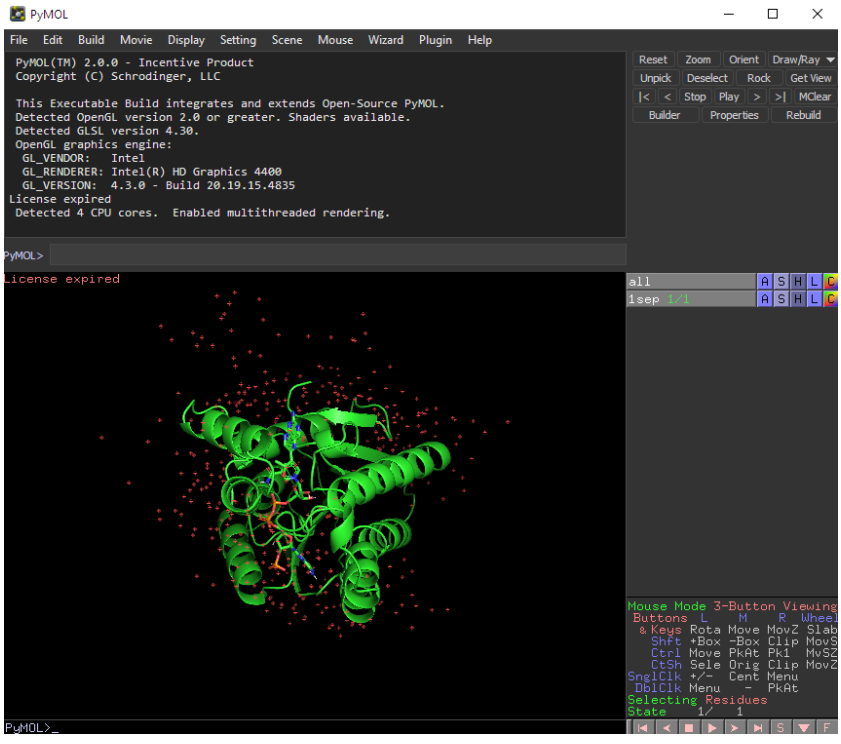


3. Install and start PyMOL

4. Open and display “1SEP.pdb”

via - „Open“ in file menu
- Drag file on the screen

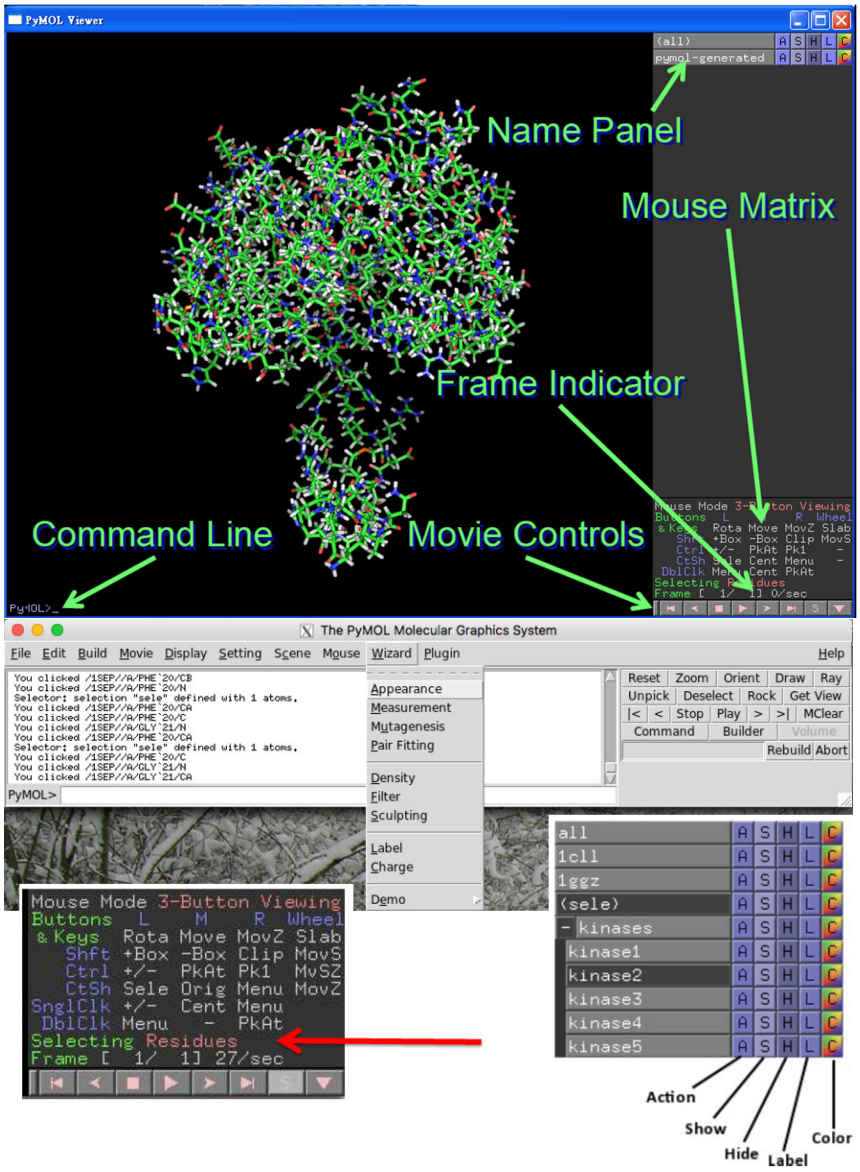
→ protein shows up as a cartoon



-> the red dots are individual water molecules

-> the cofactor is shown as a stick-model

Guide to windows:

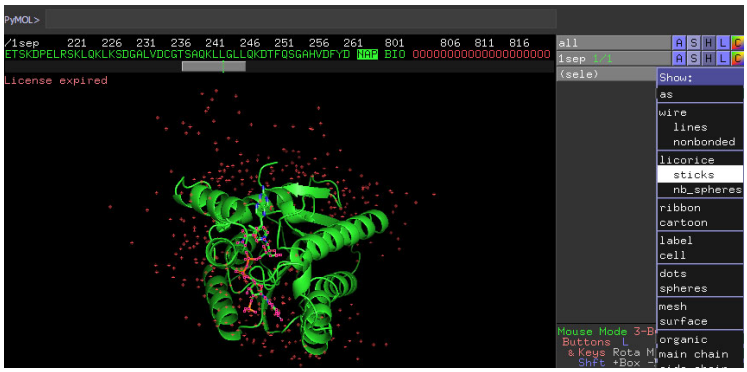


5. Play around with the views:

Left-click and drag with the mouse to rotate the view
Hold right mouse button to zoom in and out
Click on the structure to select something

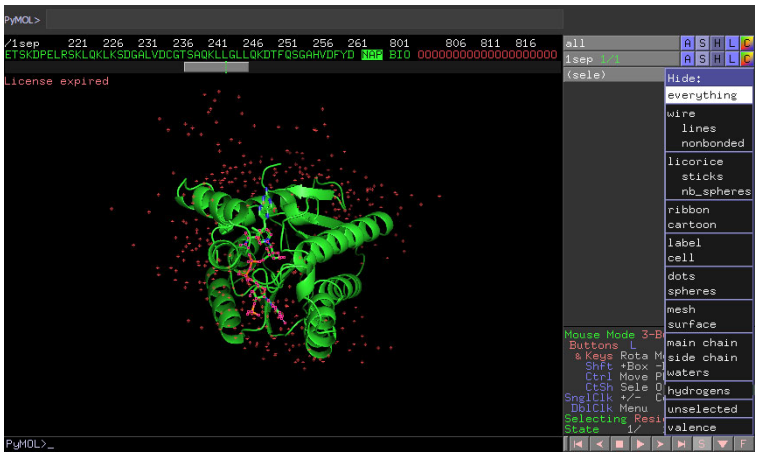
Primary structure

- Under „Display“ choose „Sequence“
- Click on „NAP“, and choose „Sticks“ from „Show“
- Test the same with an amino acid



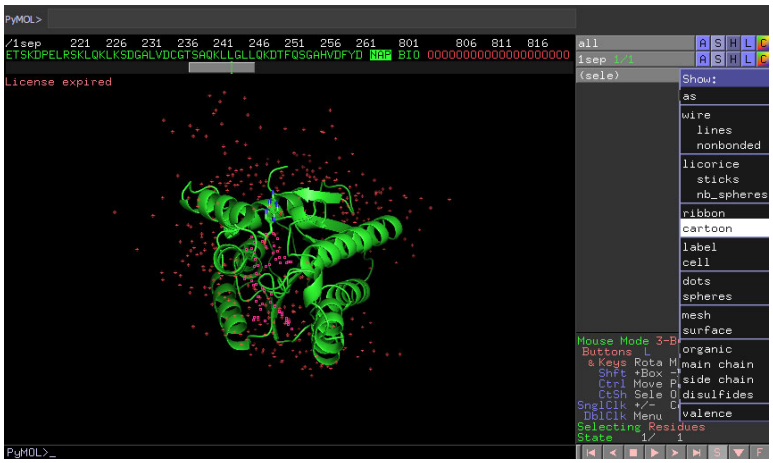
Secondary & tertiary structure

- Hide all by selecting ‘Hide everything’ in the Name panel



- Try under „Show“ the following representations

Lines	=> all protein atoms
Stick	=> idem, but fat
Ribbon	=> Cα-trace
Cartoon	=> stylized
Surface	=> Molecular surface



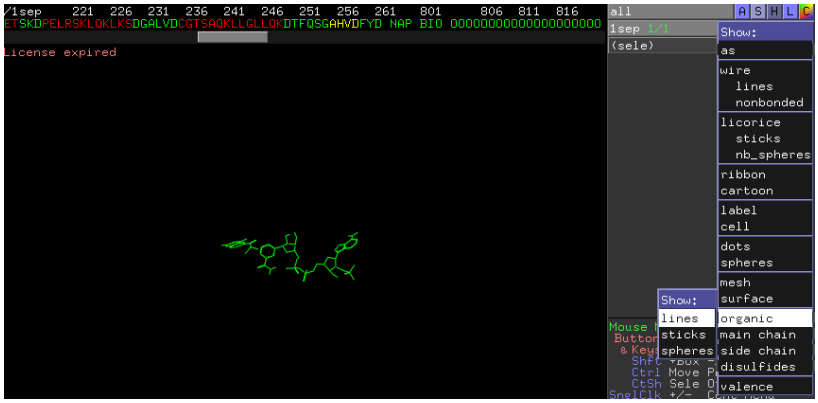
• Try under „Color“ => By ss (secondary structure)

6. Measurements

Geometric parameters are important to evaluate e.g. interactions or conformations

• **Hide all**

„Show => Organics => Lines



• Select with mouse the organics => (sele) => Action => Origin & Center
• Zoom in
• From Wizard menu choose Measurements
• Use mouse matrix to select measurement mode
÷ Distance Click on the 2 atoms of interest
÷ Angle Click on the 3 atoms defining the angle
÷ Dihedral Click on the 4 atoms defining the dihedral

• Click on „Done“ with the measuremens

7. 1SEP in PyMOL - H-bonding of NADP+

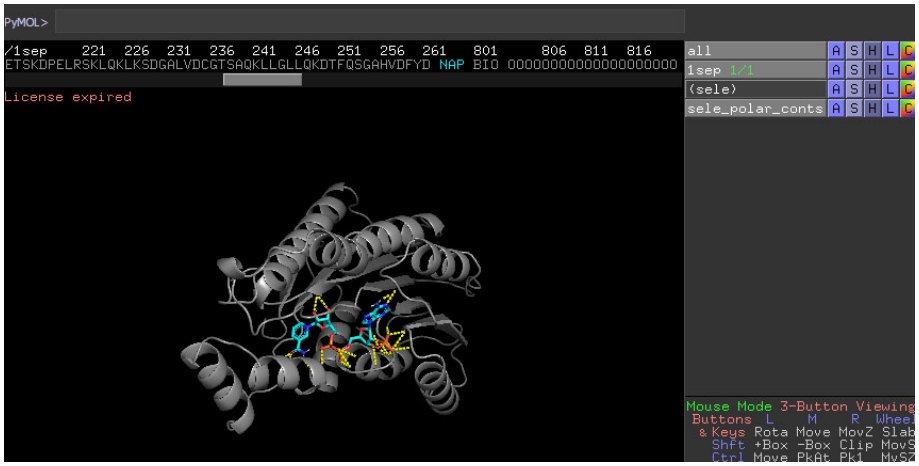
The enzyme SPR

Put as „cartoon“ and color gray

The co-factor

Display=> Sequence & click on NAP to select the co-factor => (sele)
(sele) => Show => Sticks

Color => by element



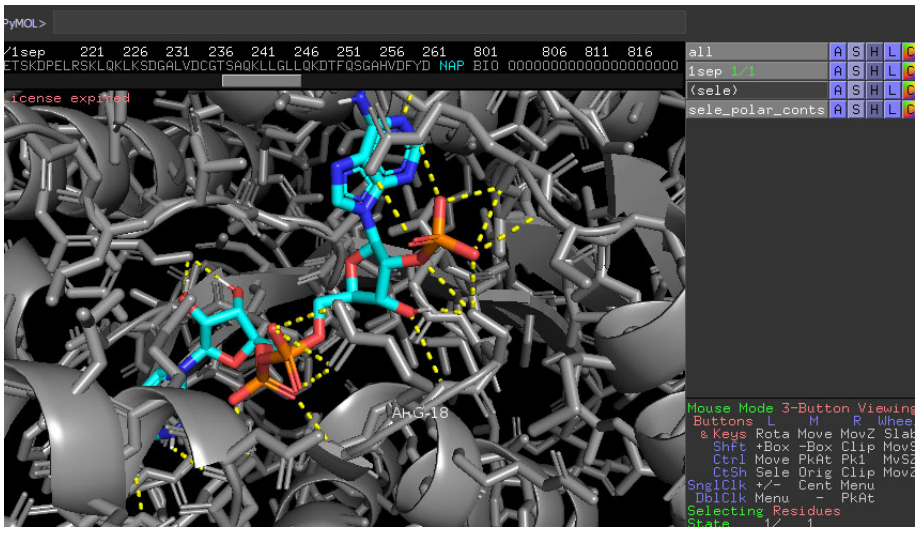
The polar contacts

Use mouse in „residue mode“ to select co-factor

(sele) => Action=> Find=> Polar contacts => to others excluding solvent

Then, for 1SEP => Show => Sticks :: dotted lines indicate H-bonds

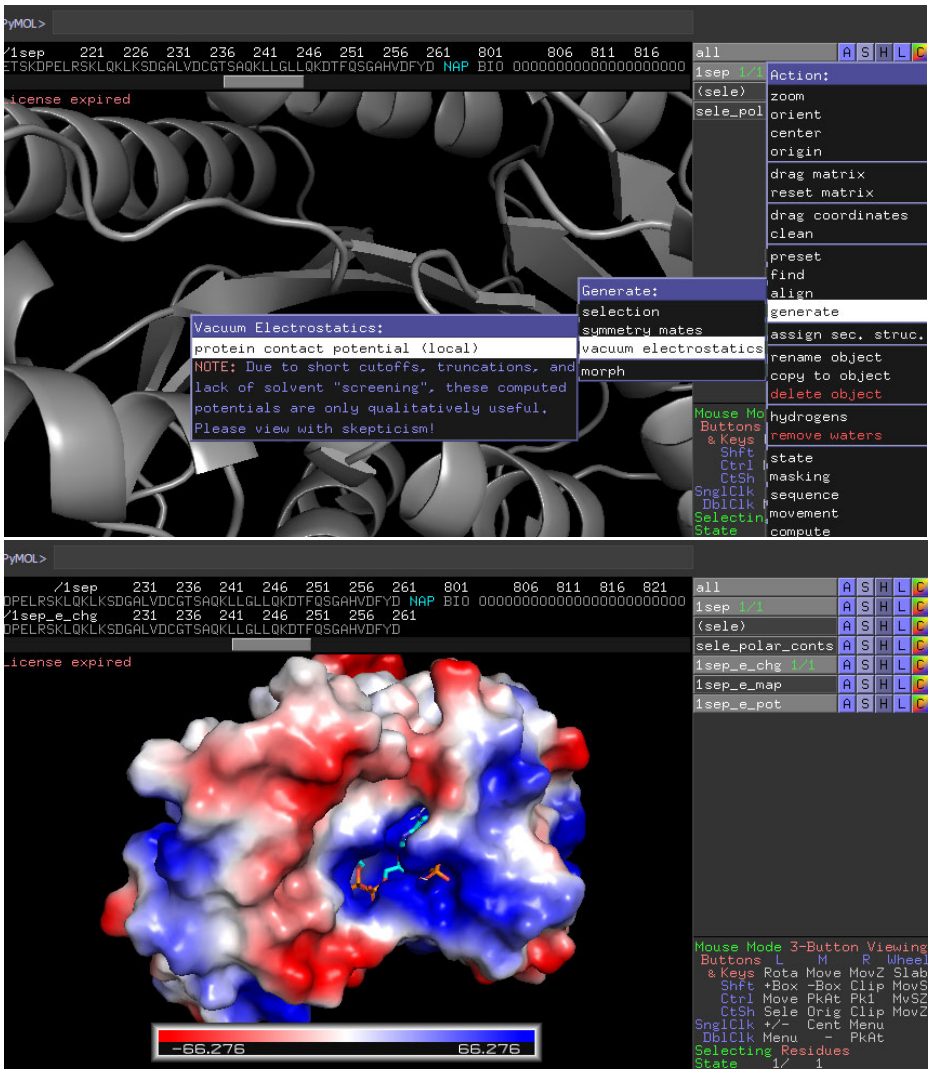
Click on H-bonding residues => (sele) => Label => Residue



8. 1SEP in PyMOL - Electrostatic surface potential

Mouse SPR with substrate & co-factor/substrate

Action => Generate => Vacuum electrostatics



Red for negative, blue for positive

Have a look at the cofactor; what do you notice ?

Relevant for molecular interactions

larger interfaces like	protein-protein
	protein-lipid membrane
	protein-DNA
Small pockets like	substrate-enzyme

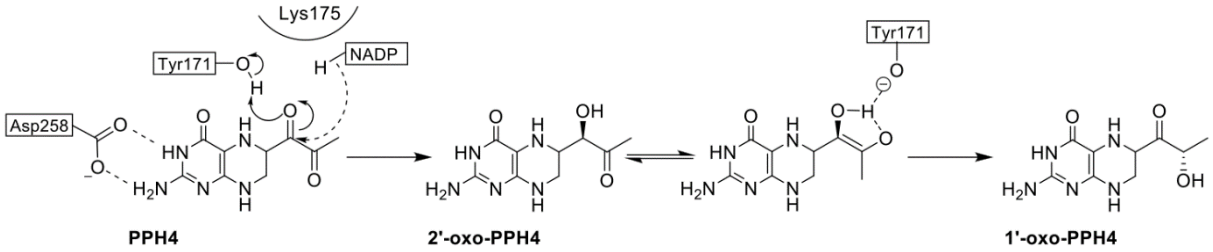
9. PyMOL – Saving your screens

There are several manners to save:

- As molecule => a .pdb file with coordinates
- As image => .png or ray-traced
- As scene => a .pse that rebuilds the current content of your PyMol session

10. PyMOL – Exercises

Investigate the proposed catalytic mechanism:



÷ Are the proposed residues indeed close to the substrate ?
÷ What could be the role of SER_158 ?
÷ How are substrate and co-factor oriented such that one could expect reduction ?
÷ „NAP“ is this the reduced or oxidized co-factor ?
=> look at distances, H-bonds, stacking, ..