

**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:**

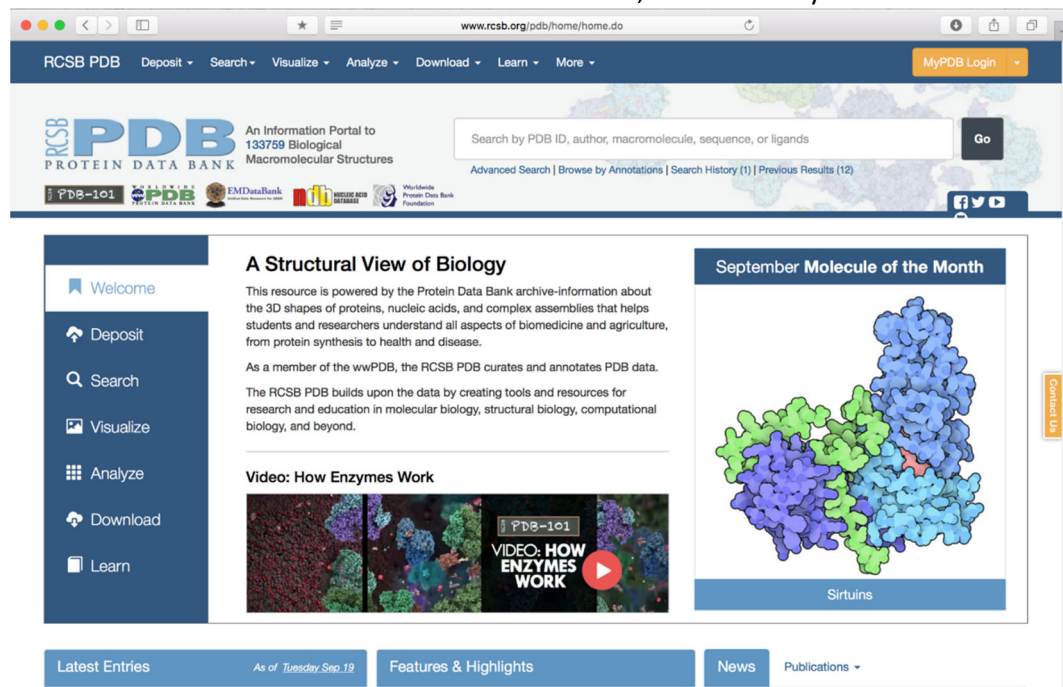
Analyzing the catalytic mechanism of **sepiapterin reductase**.

**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

→	SEQRES	→	→	primary sequence	¶
→	HET	→	→	hetero molecules	¶
→	ATOM	→	→	coordinates	¶

ATOM	1	N	GLY	A	1	-26.869	-40.921	-10.681	1.00	45.14	N
ATOM	2	CA	GLY	A	1	-27.303	-42.230	-10.117	1.00	45.28	C
ATOM	3	C	GLY	A	1	-26.210	-42.945	-9.344	1.00	44.90	C

atom	number	aa	number	x	y	z	factor	atom
→	→	→	→	→	→	→	→	→
→	→	→	→	→	→	→	→	→
→	→	→	→	→	→	→	→	→

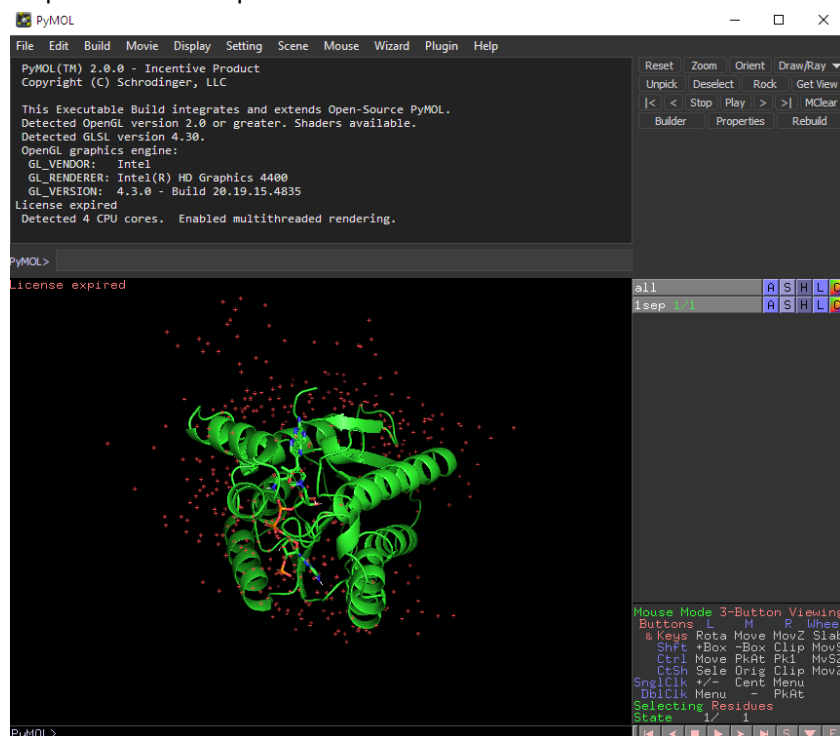
## 3. Install and start PyMOL

## 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:

The image shows the PyMOL Molecular Graphics System interface. The main window displays a 3D molecular model of a protein-ligand complex. The interface includes several panels and controls:

- Name Panel:** Located at the top right, it lists the objects in the scene, including 'all' and 'pymol-generated'.
- Mouse Matrix:** Located at the bottom right, it displays a table of mouse actions and their corresponding keyboard shortcuts for various viewing modes.
- Frame Indicator:** Located at the bottom center, it shows the current frame number and the total number of frames in the movie.
- Command Line:** Located at the bottom left, it displays the command history and the current command being executed.
- Movie Controls:** Located at the bottom center, it includes buttons for playing, pausing, and navigating through the movie frames.

The interface also includes a menu bar (File, Edit, Build, Movie, Display, Setting, Scene, Mouse, Wizard, Plugin, Help) and a toolbar with various icons for object manipulation and display settings.

**Mouse Matrix:**

Buttons	L	M	R	Wheel
& Keys	Rota	Move	MovZ	Slab
Shift	+Box	-Box	Clip	MovS
Ctrl	+/-	PkAt	Pk1	MvSZ
CtSh	Sele	Orig	Menu	MovZ
SingleClick	+/-	Cent	Menu	
DoubleClick	Menu	-	PkAt	

**Selecting Residues:**

Frame [ 1 / 11 27/sec

**Object List:**

Object	A	S	H	L	C
all					
icll					
lggz					
(sele)					
- kinases					
kinase1					
kinase2					
kinase3					
kinase4					
kinase5					

**Action Legend:**

- Action
- Show
- Hide
- Label
- Color

### 5. Play around with the views:

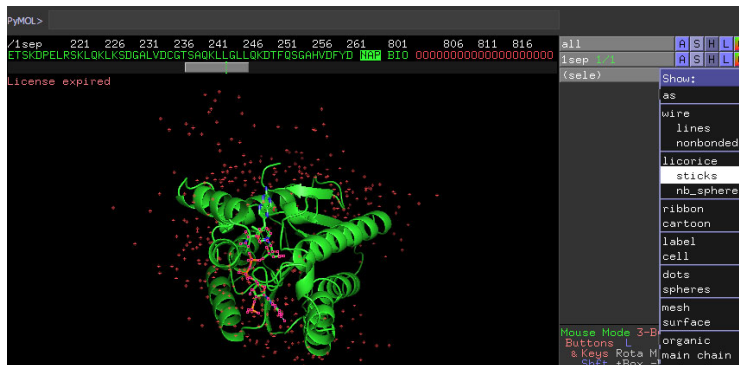
Left-click and drag with the mouse to rotate the view

Hold right mouse button to zoom in and out

Click on the structure to select something

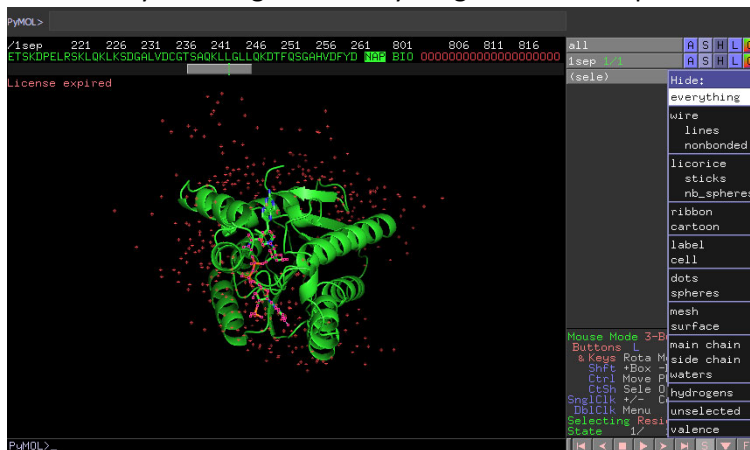
#### Primary structure

- 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

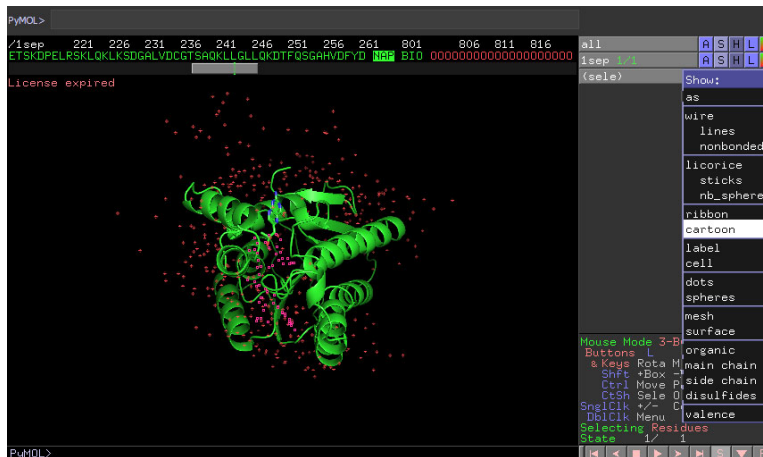
Lines => all protein atoms

Stick => idem, but fat

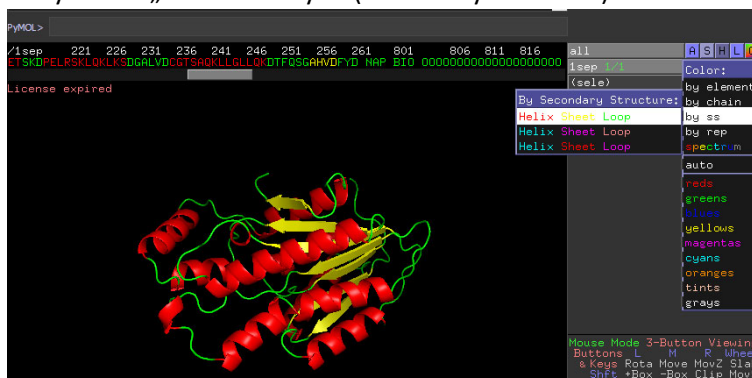
Ribbon => Cα-trace

Cartoon => stylized

Surface => Molecular surface



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

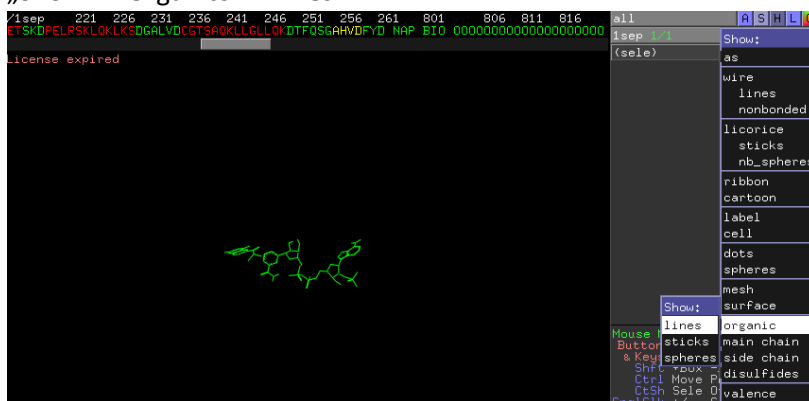


## 6. Measurements

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 measurements

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

### The enzyme SPR

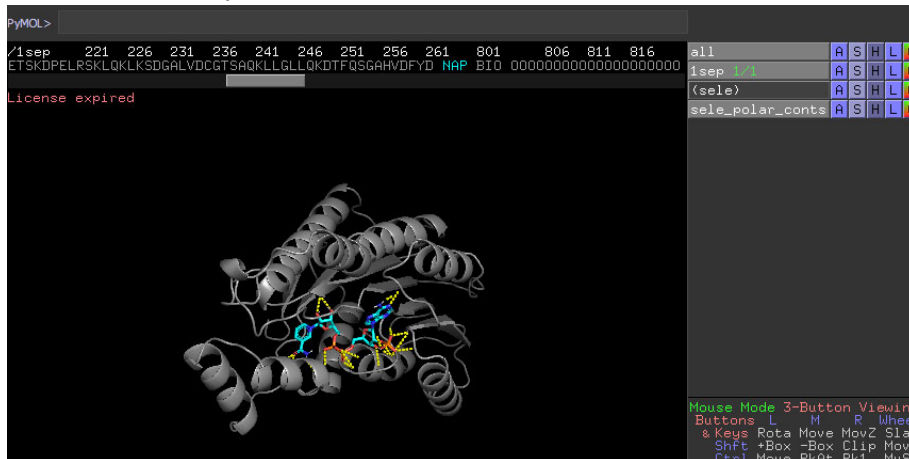
Put as „cartoon“ and color gray

### The co-factor

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

(sele) => Show => Sticks

Color => by element



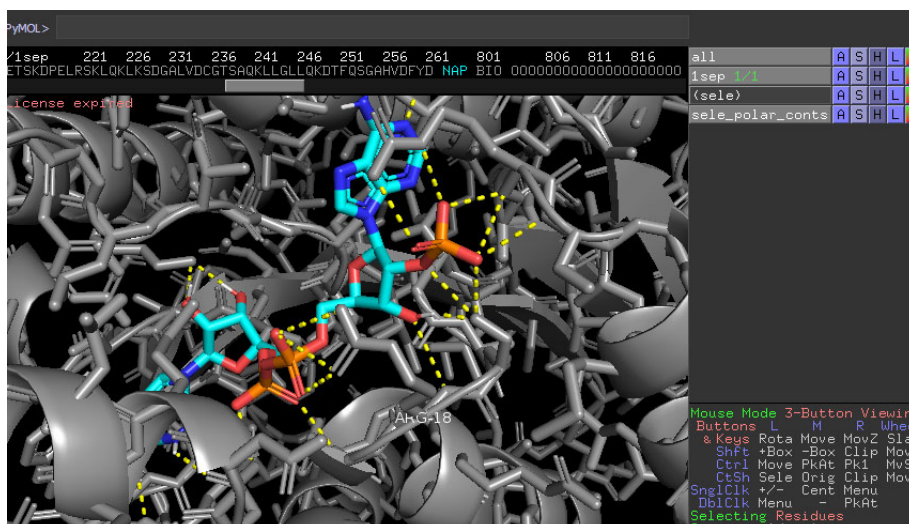
### The polar contacts

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

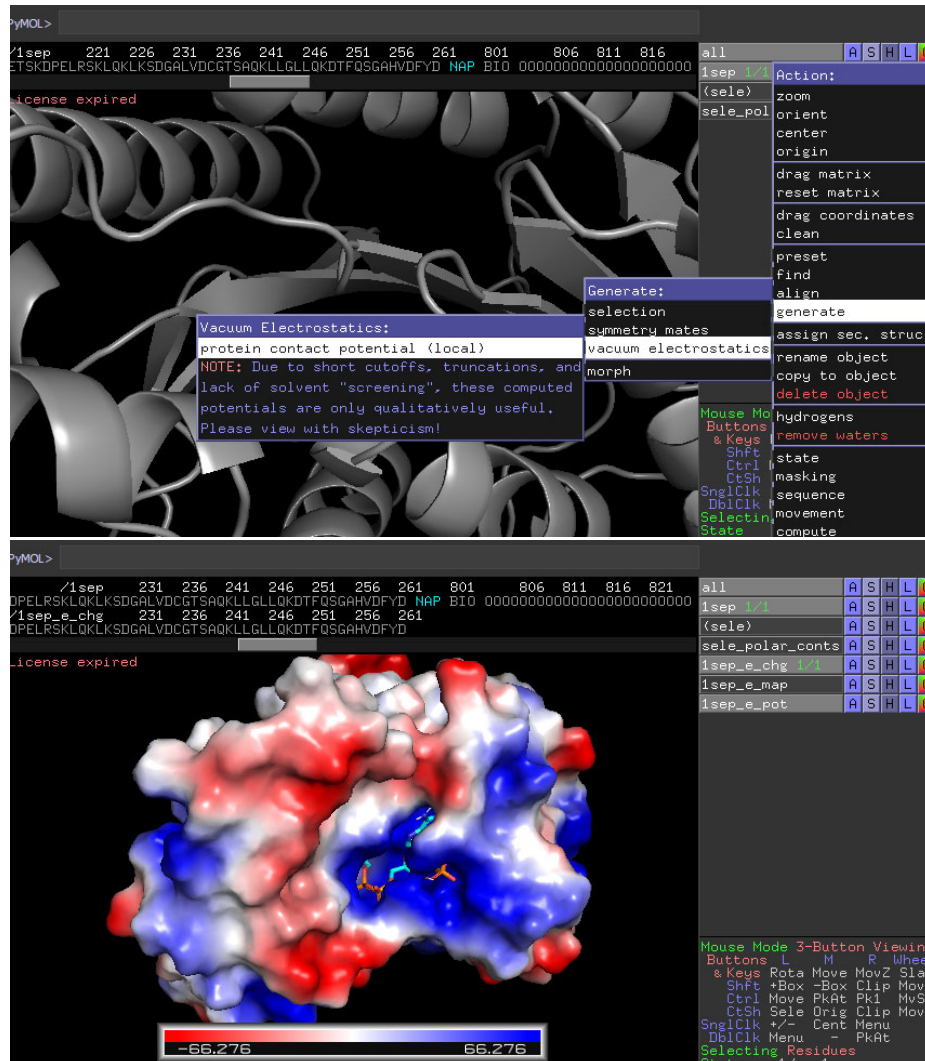




## 8. 1SEP in PyMOL - Electrostatic surface potential

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

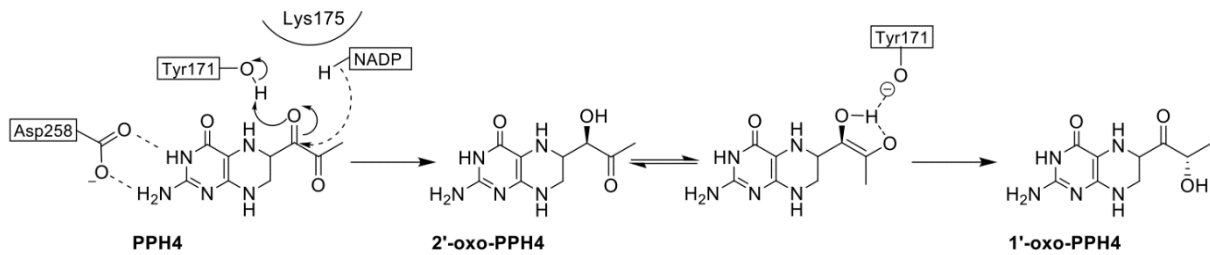
## 9. PyMOL – Saving your screens

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, ..