

Masters Summer Course

Protein Structural Analysis by PyMOL

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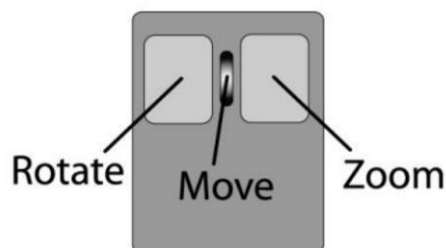
Day 1 & 2: **Introduction and basic PyMOL training**

Topics discussed:

- The paper *PKA-I Holoenzyme Structure Reveals a Mechanism for cAMP-Dependant Activation* will be discussed highlighting the structural features of mammalian PKA activation using the molecular visualization software PyMOL
- Trypanosoma brucei PKAR structure will be analyzed highlighting the 7-CN-7-C-Ino binding features

The original authors of this script were Yuri Volpato and Veronica Ober, modifications done by Qingping Wu

Mouse



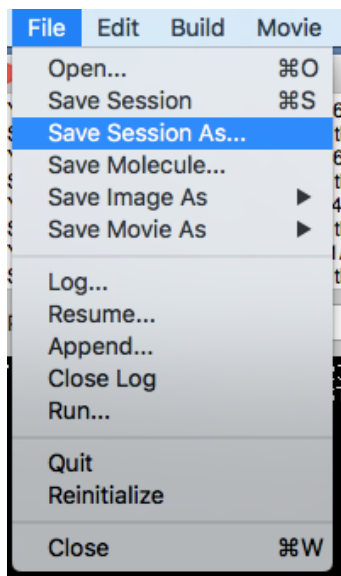
Saving work

PyMOL supports saving your work in various formats. You can save, images, molecules, sessions, movies, etc.

Sessions

A PyMOL sessions retains the state of your PyMOL instance. You can save the session to disk and reload it later. You can setup a complicated scene, with transitions and more, and simply save it as a PyMOL Session (.pse) file. Use, **File=>Save Session** or **Save Session As....**

Loading sessions is just as easy: **File=>Load** and choose your session file.

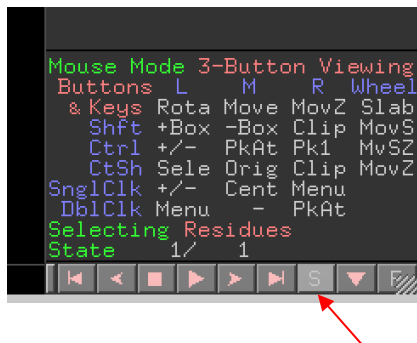


Working with the PKA Holoenzyme structure:

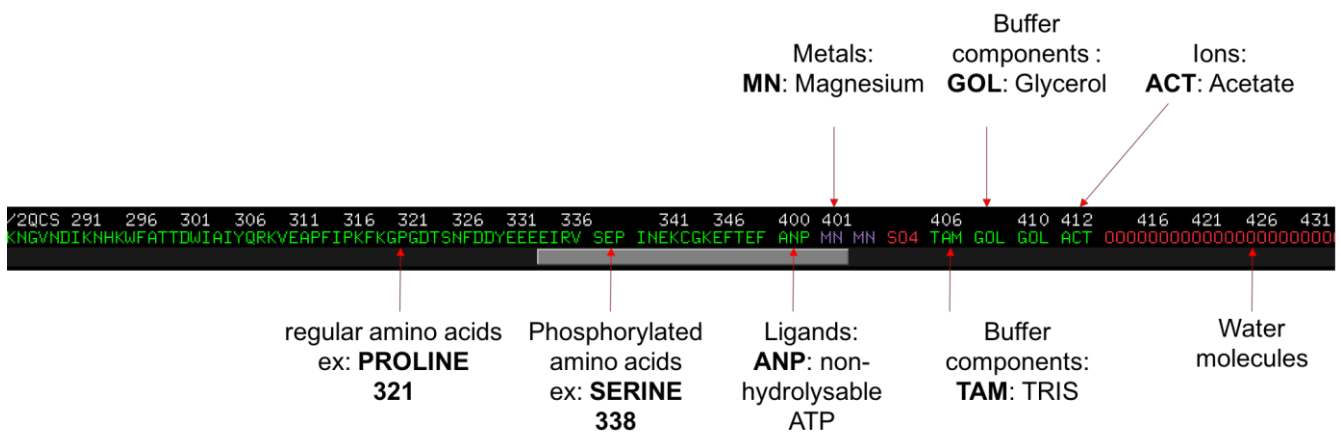
On the command line, type:

```
PyMOL> fetch 2QCS
```

On the bottom right click in **S** for Sequence:



The protein sequence will be displayed on the **top** panel:



There are subpanels in the Right pannel:

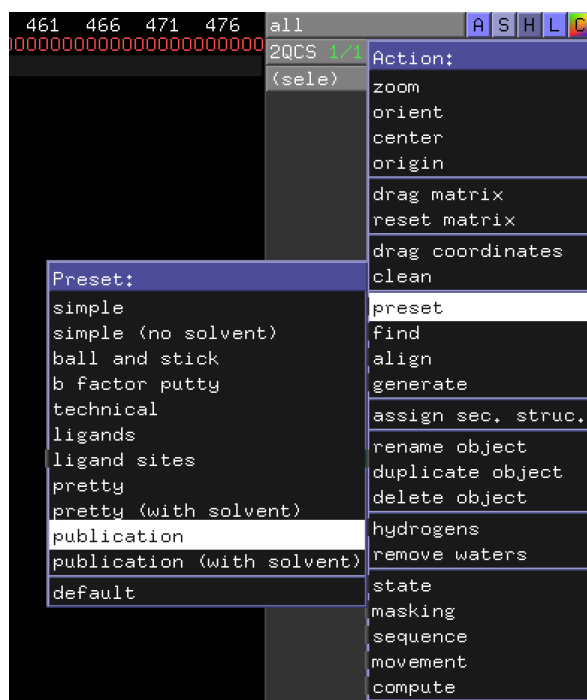
76 281 286 all A S H L C
 TKRFGN LKNGVNDI 2QCS 1/1 A S H L C
 (sele) A S H L C

SELECTION OF A SMALL PEPTIDE (for example)

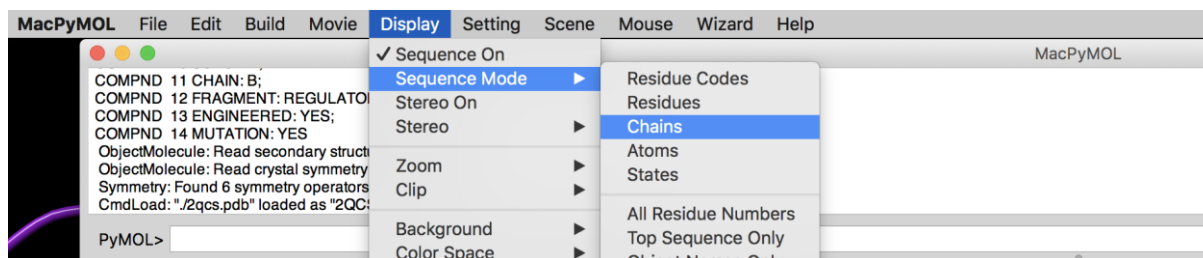
MAIN MOLECULE + SELECTIONS
 MAIN MOLECULE : WHOLE PROTEIN
 SELECTION (PART OF THE PROTEIN)

COLLOR
 LABELL
 HIDE
 SHOW
 ACTION

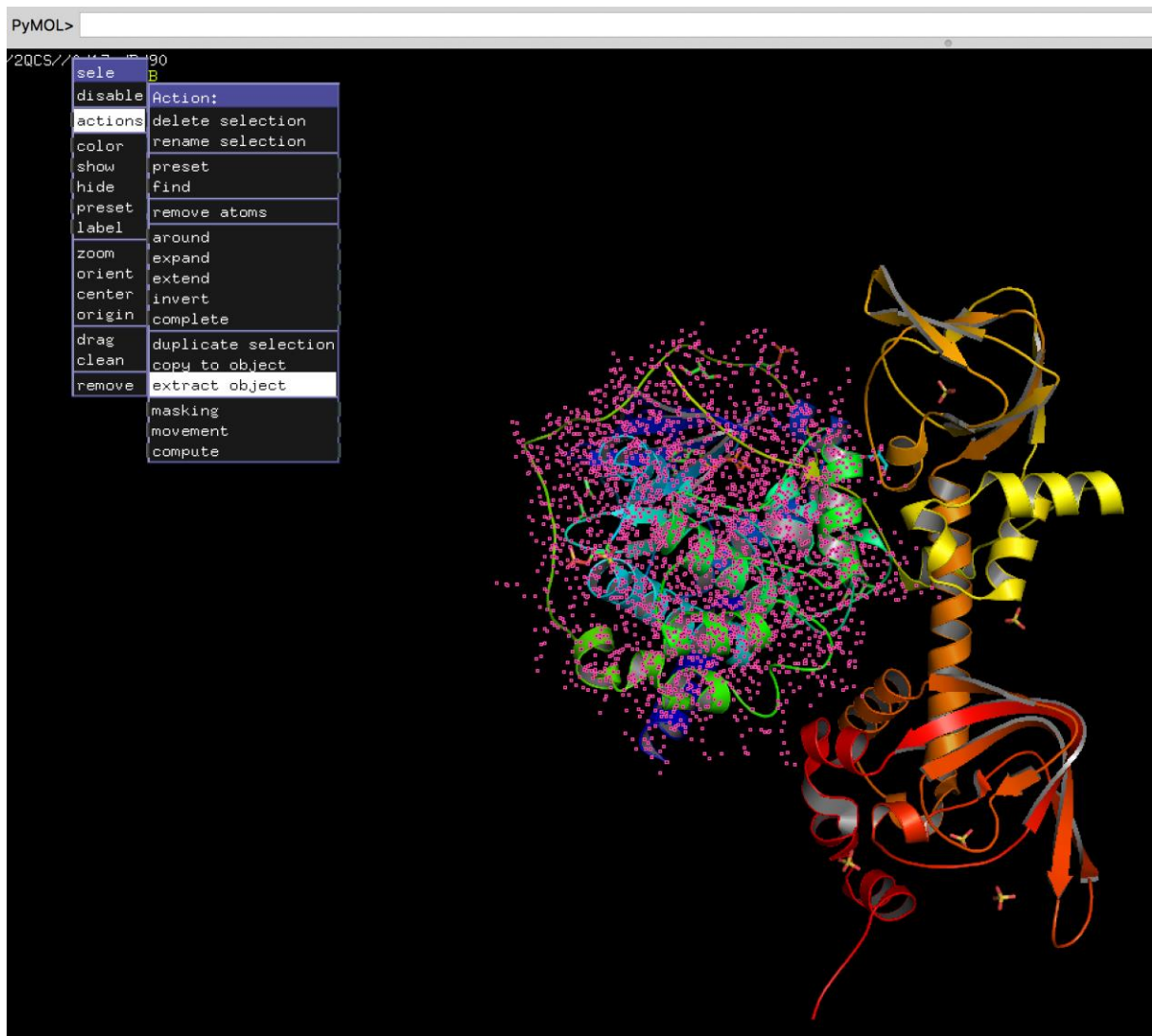
On the right panel up, in the box of the **2QCS** molecule click in **Action (A) > Preset > Publication**:



On the top left click in **Display > Sequence mode > Chains**



Click on chain **A** (PKA Catalytic subunit) > **Actions** > **Extract Object**



Clear the Right Panel by clicking in **all** > make sure nothing is selected and then **select only obj01**. By doing this only PKAC should appear in the visualization area.

all	A	S	H	L	C
2QCS 1/1	A	S	H	L	C
(sele)	A	S	H	L	C
obj01 1/1	A	S	H	L	C

ALL CLEARED UP

all	A	S	H	L	C
2QCS 1/1	A	S	H	L	C
(sele)	A	S	H	L	C
obj01 1/1	A	S	H	L	C

ONLY OBJ01 SELECTED

Rename OBJ01 to PKAC > **Click on OBJ01> Action > Rename object:**



Do the same for PKAR. In the end, it should look like this (figure bellow) in the Right Pannel:

(the content of 2QCS should be empty and the Holoenzyme will be split on two separate entries: PKAR and PKAC)

all	A	S	H	L	C
2QCS 1/1	A	S	H	L	C
(sele)	A	S	H	L	C
PKAC1 1/1	A	S	H	L	C
PKAR 1/1	A	S	H	L	C

***You can also rename things using this command:**

```
set_name old_name, new_name
```

Change the name of the entry 2QCS to Holoenzyme by typing: (just to see how it works)

```
set_name 2QCS, Holoenzyme
```

Now issue the command **dss** for PKAR and PKAC. In the command line, type:

dss PKAR, press Enter

dss PKAC, press Enter

```
PyMOL> dss PKAR
```

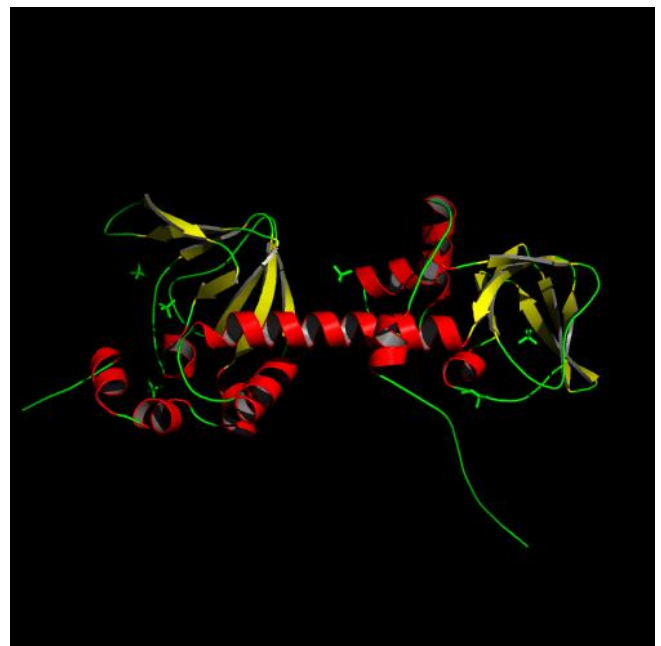
(This command will assure secondary structure assignment is correct for both subunits, it makes the delimitations of alpha helixes and beta sheet more accurate)

Collouring and secondary structures:

Hint: If at any moment you loose track of the molecule you are working with just click on the molecule you lost > (PKAR, for example) > **Action** > **Orient** and it will come to the center of the screen again.



Now we will **color** PKAR according to **secondary structure (ss)** elements. Click on **PKAR > Color > by ss > Helix Sheet Loop** and the protein should look like the image bellow:



Represent the protein sequence again in the form of single letter residue codes by clicking in : **Display > Sequence Mode > Residue Codes** on the top left.

Now should

Display	Setting	Scene	Mouse	Wizard	Help
✓ Sequence On					
Sequence Mode			Residue Codes		
Stereo On			Residues		

the aminoacid sequence be coloured according to the elements of secondary structure, amino acids belonging to alpha helix in red, amino acids belonging to beta sheet in yellow and amino acids belonging to loops in green, like in the Figure bellow:

```
PKAR//B/90 96 101 106 111 116 121 126 131 136 141 146 151 156 161 166 171 176 181 186 191
KGRPRRGATSAEVYTEEDAASYVRKVIPKDYKTMAALAKATEKNVLFSLDDNEPSDIFDAMFPVSTIAGETVIQOGDEGDNFYVIDQCEMDVYVNNWATSVGEC
```

Alpha helix structure:

Select the following region of the protein: **R226 to K250** (it's a long alpha helix called **helix alpha B/C** that should be in red)

Right click on the selection and go to > Actions > Copy it to Object:

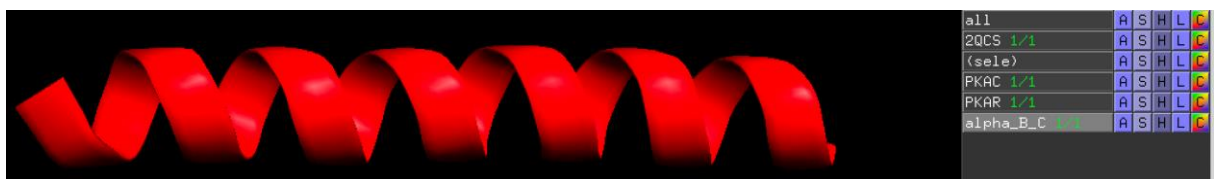
SELECT THIS REGION

R226 K250

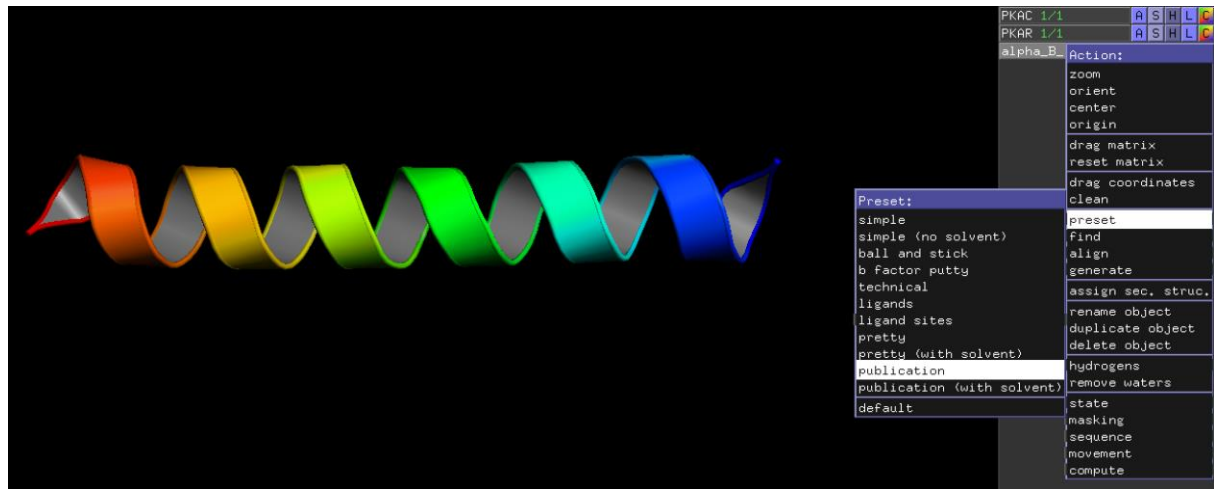
RIGHT CLICK ON THE SELECTION:

- > ACTIONS
- > COPY TO OBJECT

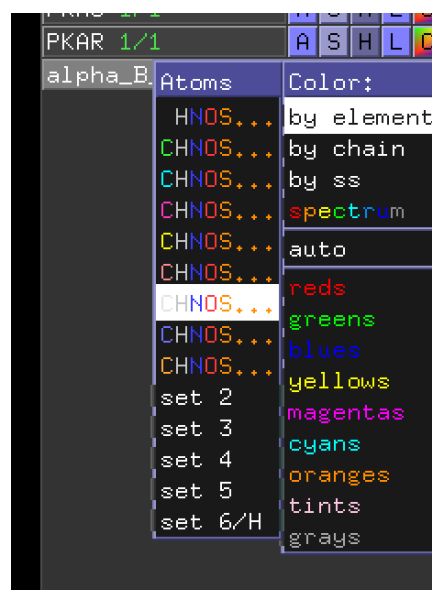
Rename it to helix_alpha_B_C:



Actions > Preset > Publication:



Color > By Element > Carbons in Grey (7th option of color scheme):



alpha B/C > Show > **Side Chain** > Sticks:

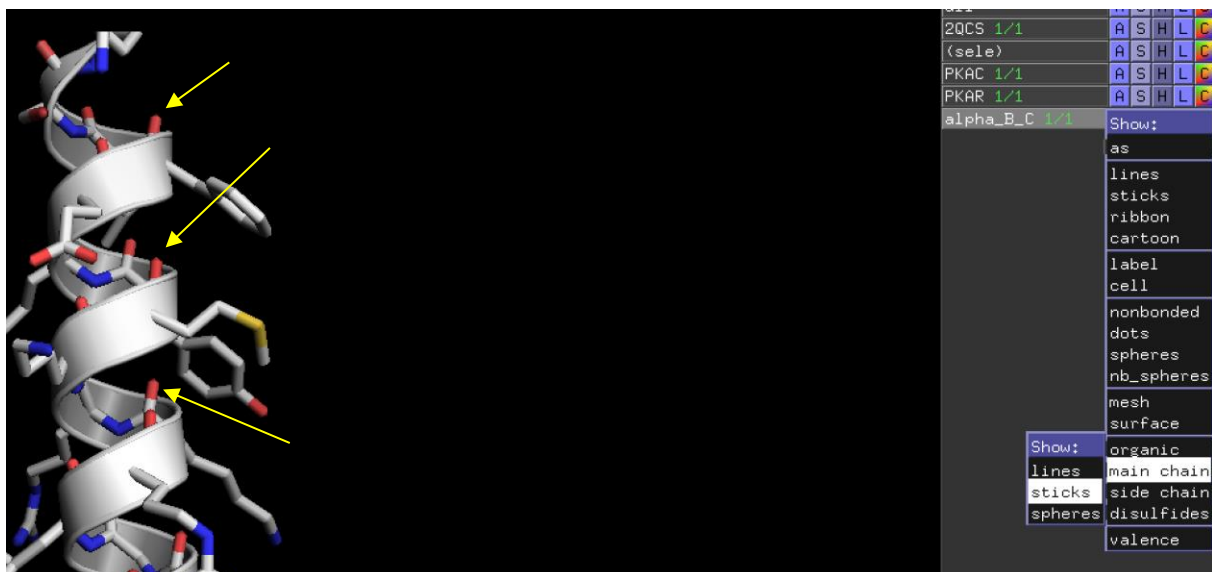


How many hydrophilic residues are there in this helix?

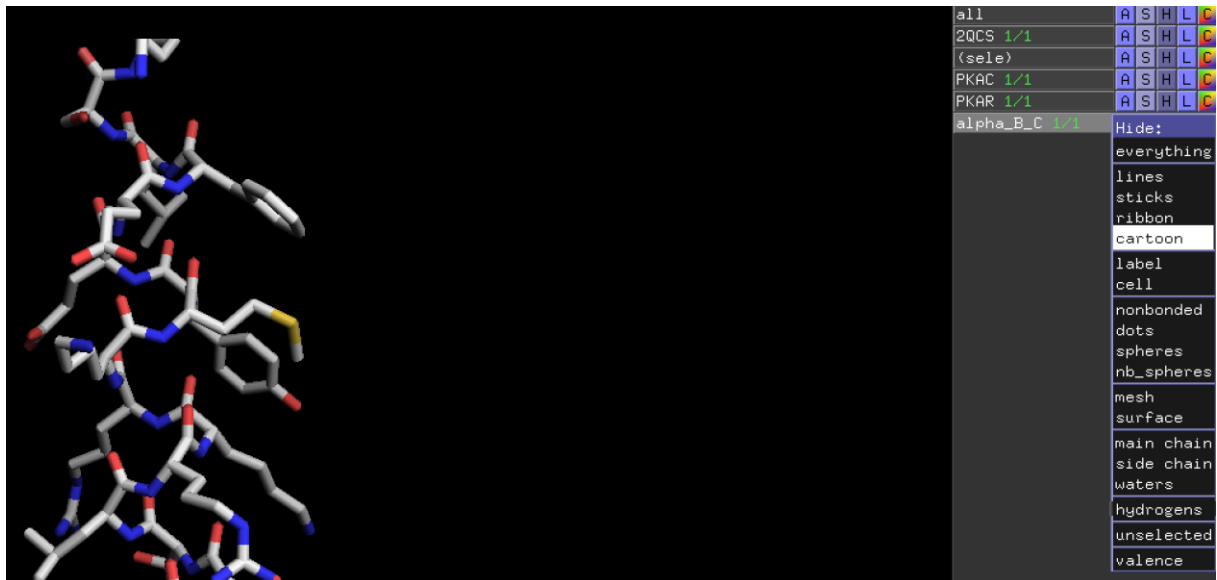
How many hydrophobic?

Try to think of a reason for this difference.

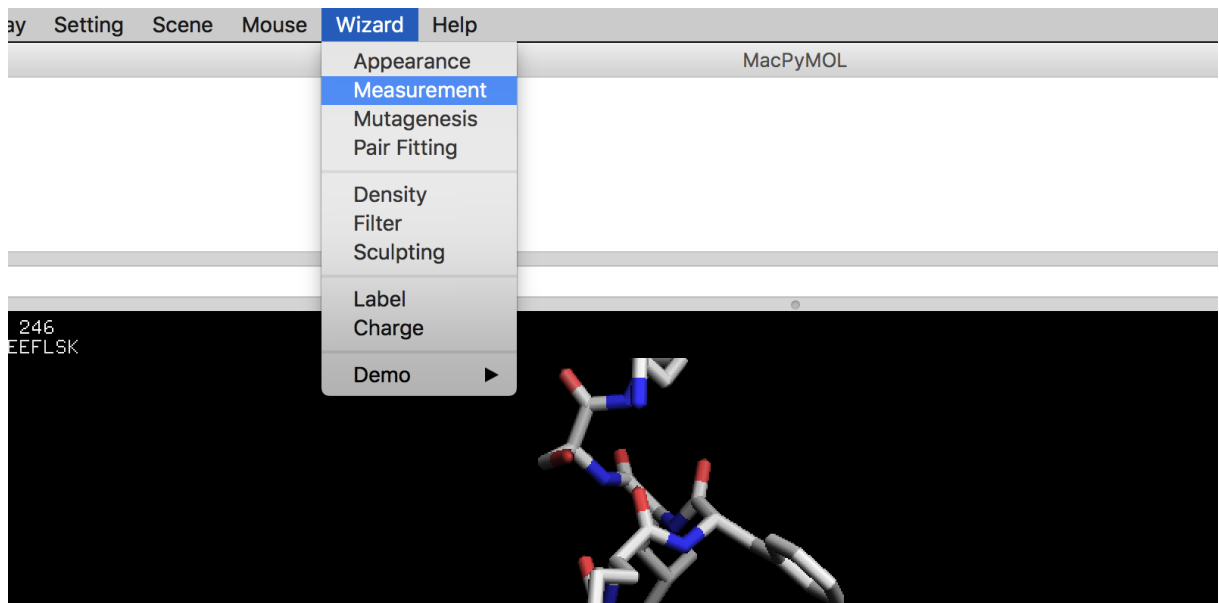
Show > Main Chain > Sticks:



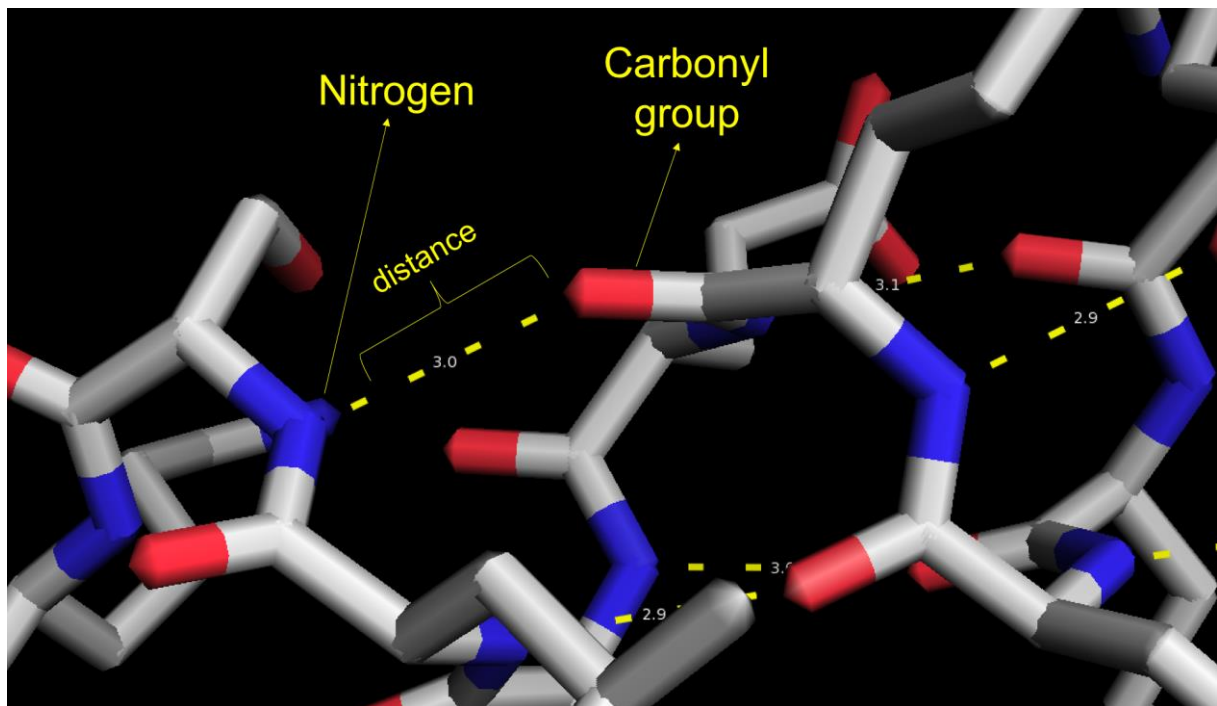
Hide > Cartoon:



Wizard > **Measurement**:

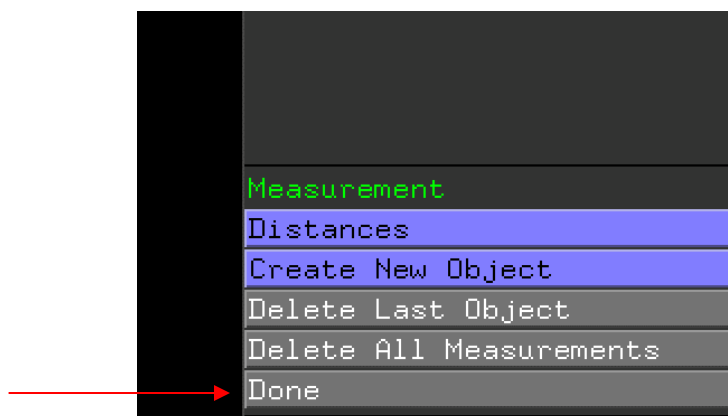


In order to make a measurement you have to click in two atoms from where you want to know the distance. In this case we will measure the distance between the **carbonyl groups (C=O)** of the alpha helix main chain and the respective **main chain nitrogen (-NH-)** to which they hydrogen bond.

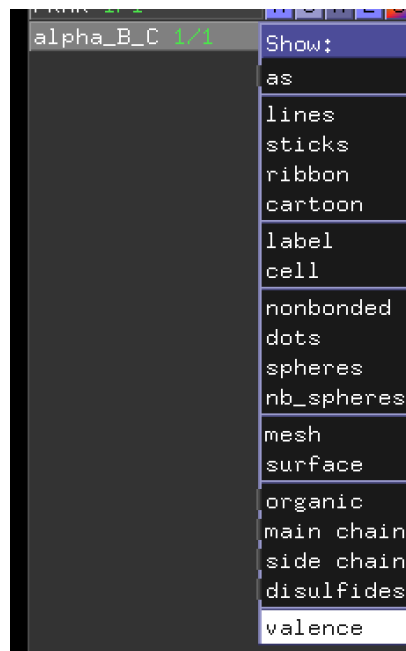


You should be able to find around **18 hydrogen bonds** that have around 3.0 Å in length.

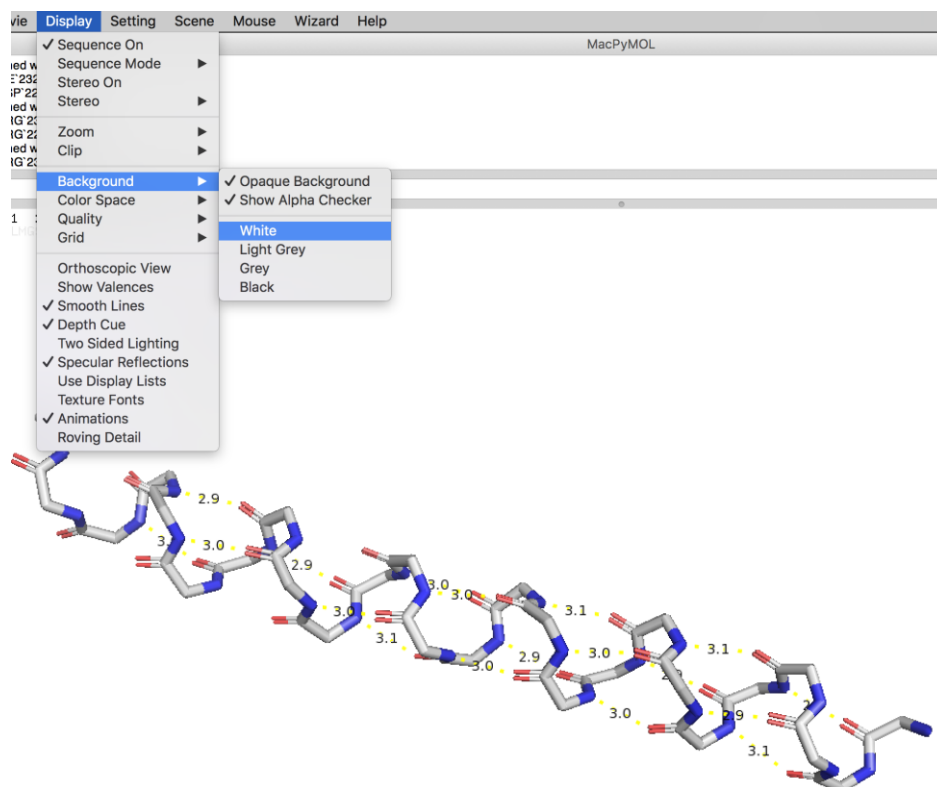
When you finish click in **DONE** :



On the alpha B/C entry > Show > Valence : (this will make the double bond between carbon and oxygen appear)



Now hide the amino acids side chains by clicking > Hide > Side chain
Display > Background > White:



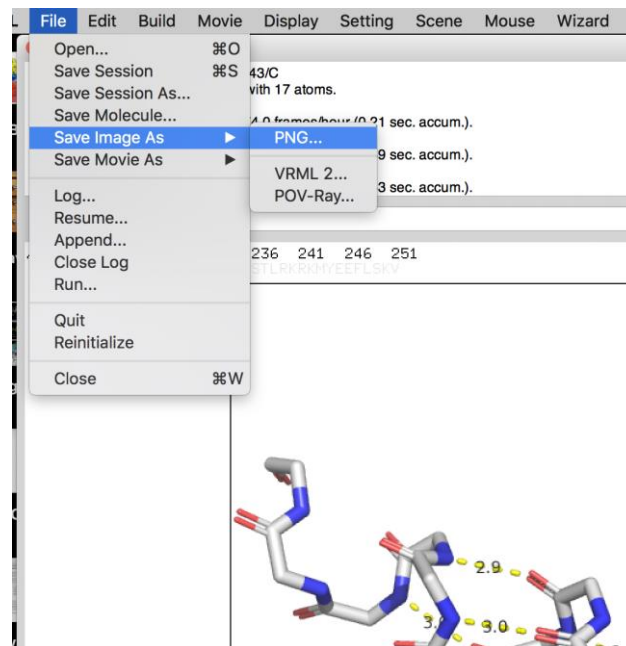
Now let's make pictures using the **ray** command. The number coming after the command ray in the command line is the size of the picture in pixels. For example, if you want to make an

image that has 3000 x 2000 pixels you have to type:

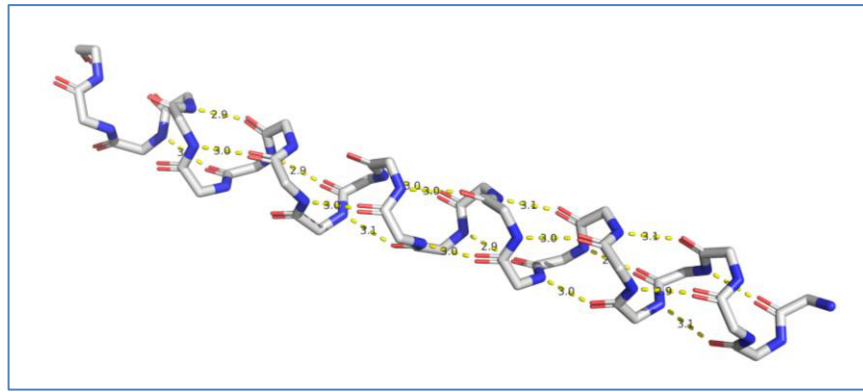
> ray 3000, 2000

```
PyMOL> ray 3000, 2000
Ray: render time: 0.65 sec. = 5520.0 frames/hour (1.82 sec. accum.).
ScenePNG: wrote 3000x2000 pixel image to file "/Users/yuri/Desktop/Kurs
PyMOL> ray 3000, 2000
/alpha_B_C//B/226 231 236 241 246 251
RDSYRRILMGSTLRKRKMYEEFLSKV
```

- Then without clicking in the visualization area (if you do you loose the image) click in:
- File > Save Image As > PNG:



- Save it with the name of **Exercise1**. We will use this name sequence (Exercise 2, 3, 4 ...) to save the images related to the tasks performed during the course. Save all those images in the same folder (name it PyMOL Exercises – *your_name_*) and keep it with you until the end of the course.



Exercise 01: Ball and stick representation of the helix alpha B/C backbone showing the atomic distances between the (C=O / N-H) pairs involved in the formation of the helix secondary structure.

Dipole Moment in the alpha helix:

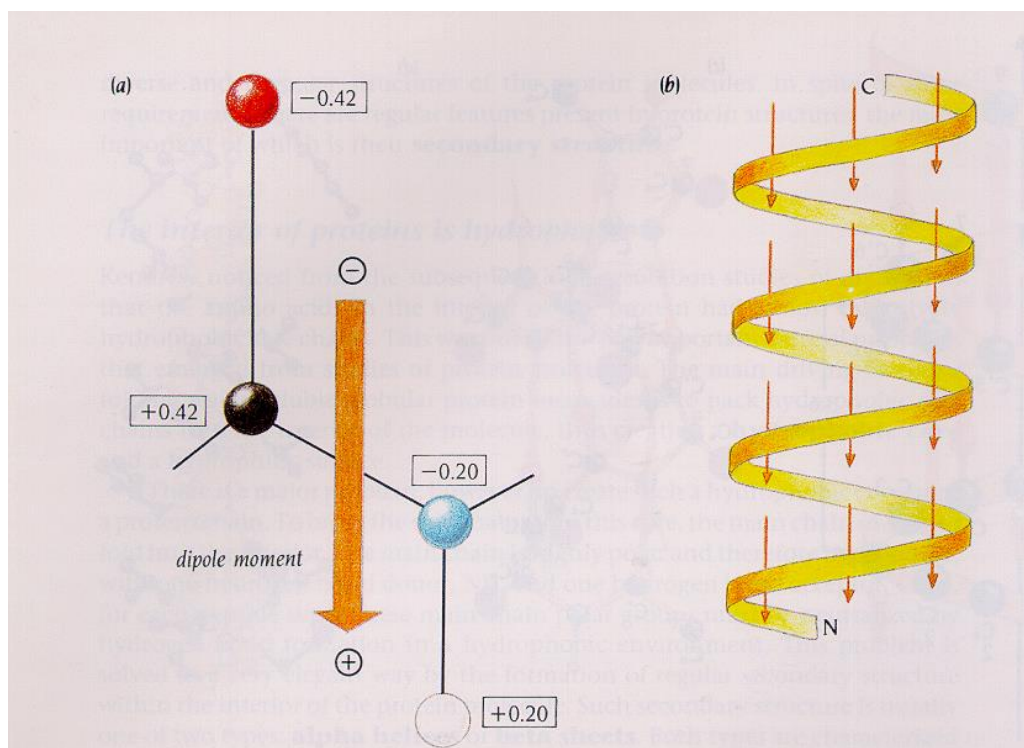
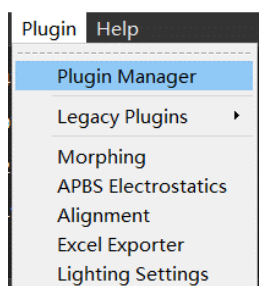


Figure 01: **a)** Electrostatic charge of the main chain atoms: oxygen (red), carbon (black), nitrogen (blue) and hydrogen (white) followed by the dipole moment arrow from the oxygen (more negative) to the nitrogen (less negative) **b)** The alpha helix is **negative in the C terminus** and **positive in the N terminus**. (Figure credits: <http://www.cryst.bbk.ac.uk/>)

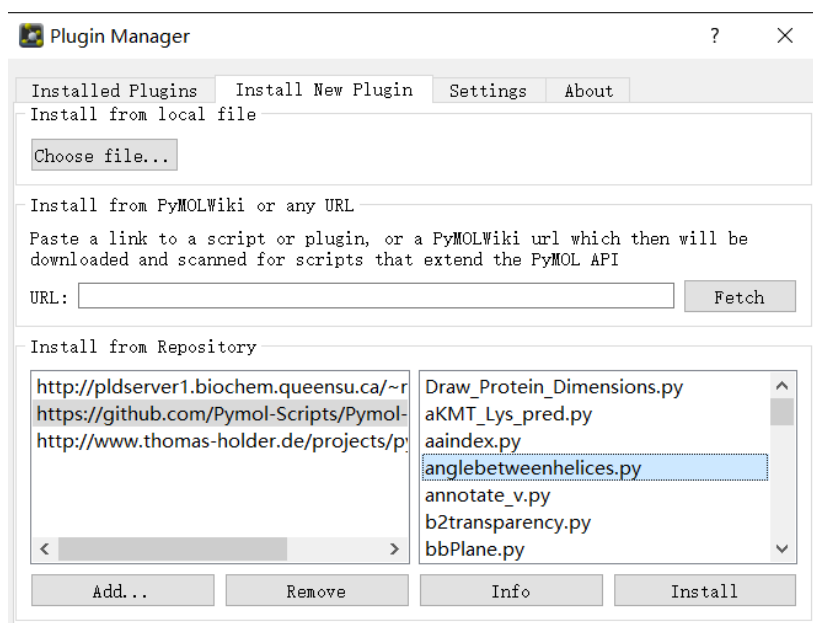
- PyMOL does not calculate the dipole moment of helices (Schrödinger does, we will see in the end of the course) but there is another function called **angle_between_helix** that gives an idea of how this vector might look like, with the difference that the orientation vector has the opposite direction of the dipole moment vector (pointing from N to C terminus instead of C to N terminus).

To install the angle-between-helices module, **select -> Plugin -> Plugin Manager**



In the pup-up window, under Install New Plugin choose the second link (<https://github.com/Pymol-Scripts/...>) on the Repository panel and find the module `anglebetweenhelices.py`, click install.

If an error message informing you should install the module in a 'default' folder coming out, just agree with it, otherwise the installation will be failed.



Just do the same for other modules like `findseq.py`.

Now type:

```
select hel1, alpha_B_C*
```

***this has to be exactly the same name as your helix is called in the right panel. If you named it for example alphaB/C with slashes (/) instead of underline (_) stick to the name it was given in the first place otherwise the command won't work.**

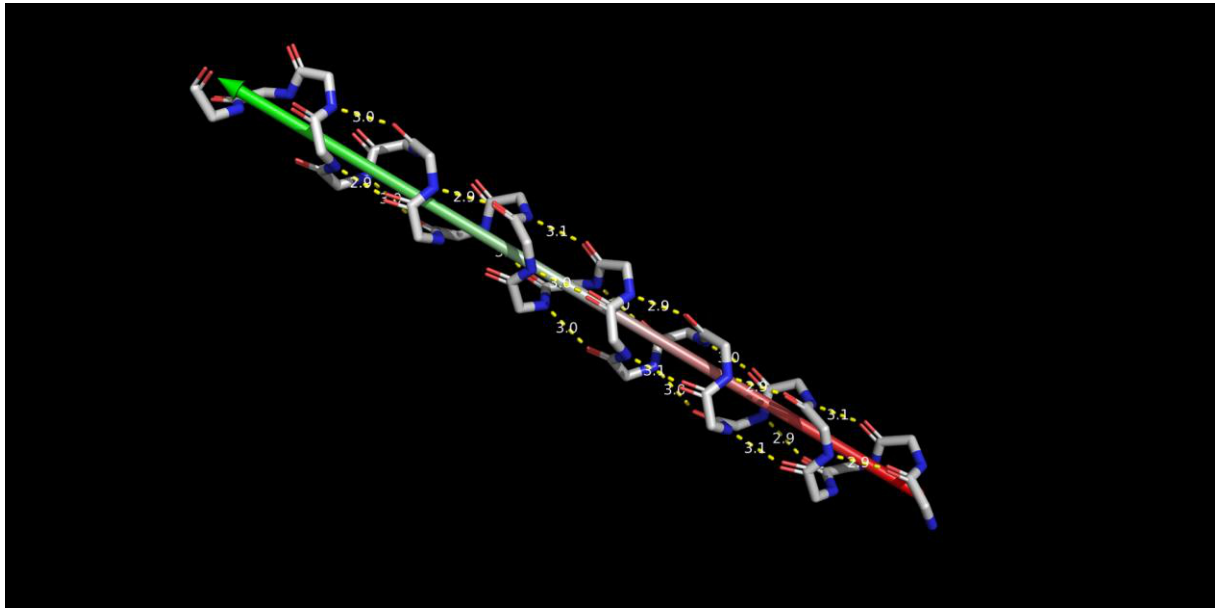
```
PyMOL> select hel1, alpha_B_C|  
/alpha_B_C//B/226 231 236 241 246 251  
RDSYRRILMGSTLRKRKMYEEFLSKV
```

After this command a new element (hel1) was created and selected as you can check in the right panel.

Now type:

```
PyMOL> helix_orientation_hbond hel1  
/alpha_B_C//B/226 231 236 241 246 251  
RDSYRRILMGSTLRKRKMYEEFLSKV
```

An orientation vector entry will appear in the right panel and you should be able to visualize it inside the helix. Make a picture (ray 2000, 1000) and save it as Exercise 2.



Exercise 2: Orientation vector defining an alpha-helix

Now let's clean a bit the workspace for Exercise 3:

Delete all the measurements:

Wizard > Measurements >

On the bottom right click in "Delete all measurements"

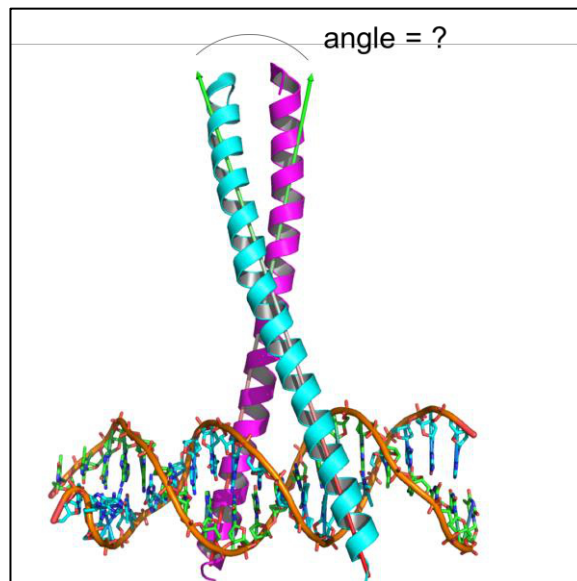


Delete the Orientation vector > Action > Delete

Exercise 3: Using the command :

```
angle_between_helices hel1, hel2
```

calculate the angle between the α -1 and α -2 in the Leucine Zipper DNA binding protein (PDB: 1YSA) and **generate the figure below** (Fig Ex. 3). Save it as **Exercise 3** in your exercise folder. You are supposed to use all the information you learned so far to generate this Figure.



Exercise 03: Using the PDB 1YSA you should be able to generate this figure and calculate the angle between the blue and pink alpha helix.

Vizualizing Beta sheets:

In the PKAR entry select residue 270 to 349 and copy them as an object to a new entry. **Rename-it as CNB_B**. Issue the command:

```
dss CNB_B
```

*CNB-B stands for Cyclic Nucleotide Domain: B

Choose representation to show only main chain (side chains should be hidden) and color to be carbon coloured as grey (colour > by element > 7th option).

On the **Show button**, click in **Show > valence**.

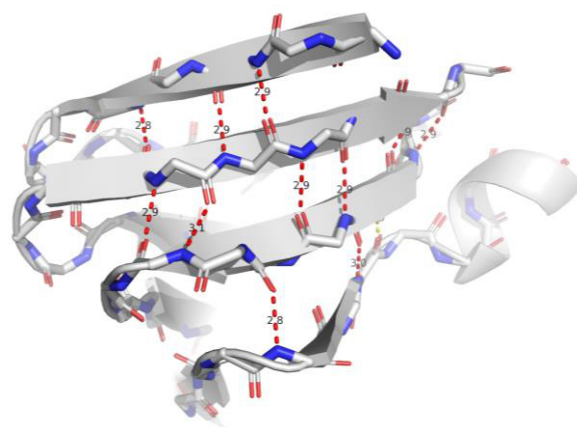
Now measure the distance between the carbonyl group in one sheet and the NH group in the next sheet (distances should be within the range of 3Å).

You should find around 30 hydrogen bonds that compose this beta barrel.

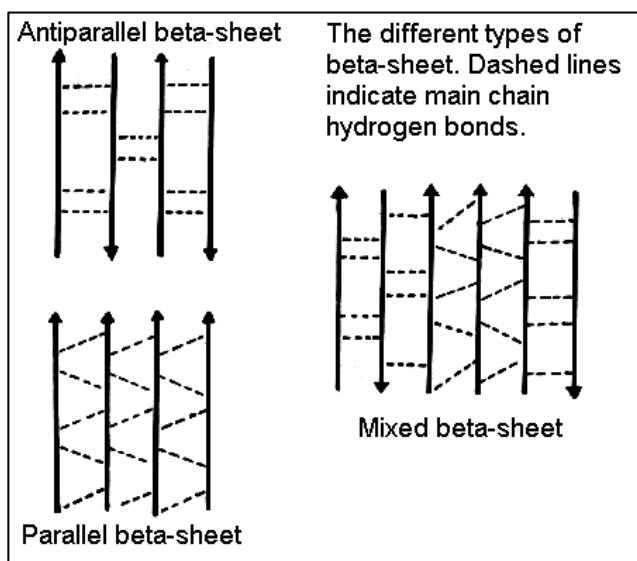
If you did everything right your screen should look more or less like this:

(dash colour is arbitrary)

- Question: are those beta sheets parallel or antiparallel!?



Here are the types of beta sheet arrangement found in proteins:



(image source: <http://www.cryst.bbk.ac.uk/>)

In case your measurement colour was not in red before change the color to red using the command:

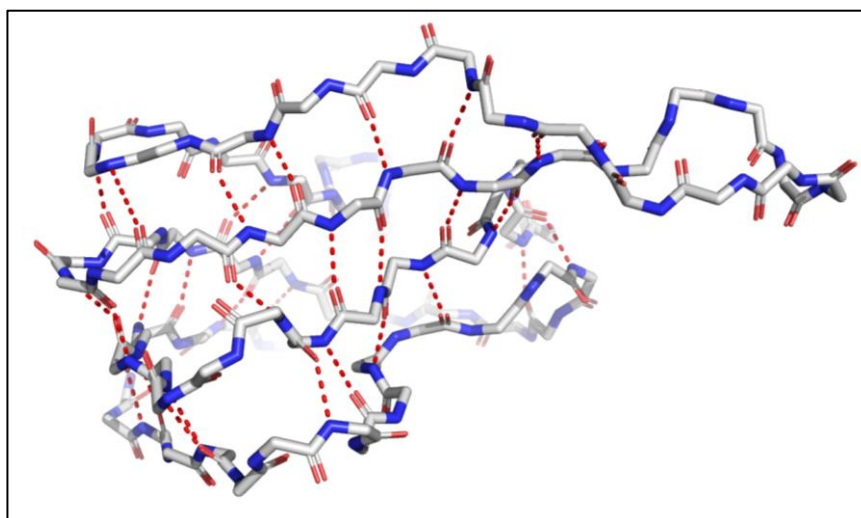
```
set dash_color, red
```

And hide all the labels with the command:

```
hide labels
```

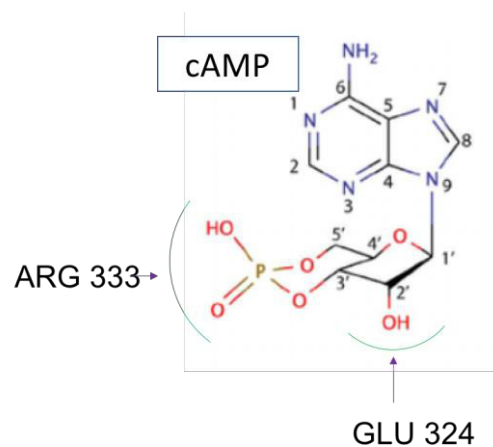
And you should be able to generate the figure related to **Exercise 04**:

(Hint – to fade the back of the image use the middle scrolling button of the mouse rolling it upwards)

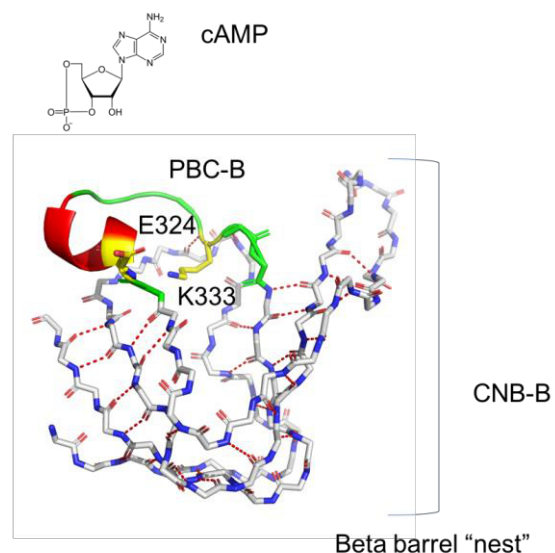
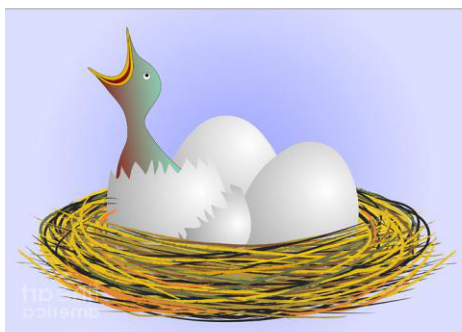


Exercise 04: Beta barrel structure of the **Cyclic Nucleotide Binding site B (CNB: B)** of the regulatory subunit of protein kinase A (PKAR) / (ray 2000, 1000 was used)

We will now visualize the cAMP binding domain, also known as PBC (Phosphate Binding Cassete), that sits on top of the CNB-B domains (on both A and B sites, but here we will focus the analysis on the B-site). The PBC-B sequence is: GEIALLMNRPRAA where the two important amino acids for cAMP binding are the glutamate 324 and the arginine 333 (GLU 324 and ARG 333). The glutamate interacts with position 2'OH in the ribose ring of cAMP and the arginine (which is positively charged) interacts and stabilizes the negative charge of cAMP's exocyclic phosphate (which is negatively charged):



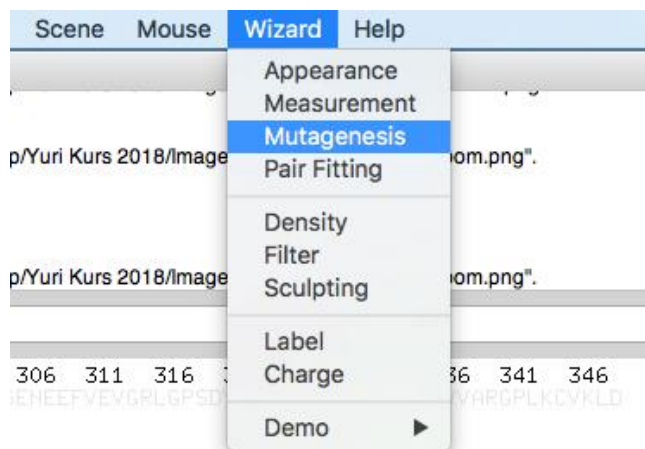
The CNB-B (Cyclic Nucleotide Binding domain B), which is comprised of a beta barrel structure functions as a nest to accommodate the PBC-B on top of it. The function of the PBC-B is to “capture” a molecule of cAMP.



But if you look carefully in the Holoenzyme structure there is a Lysine in place of an Arginine on position 333. Try to think of a reason for this mutation, related of how the protein was purified (read in the paper methods).

Because we have knowledge about the protein sequence we will now mutate this aminoacid back to Arginine.

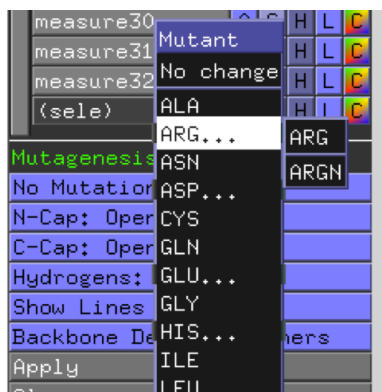
Click on Wizard > Mutagenesis:



Click on LYS 333

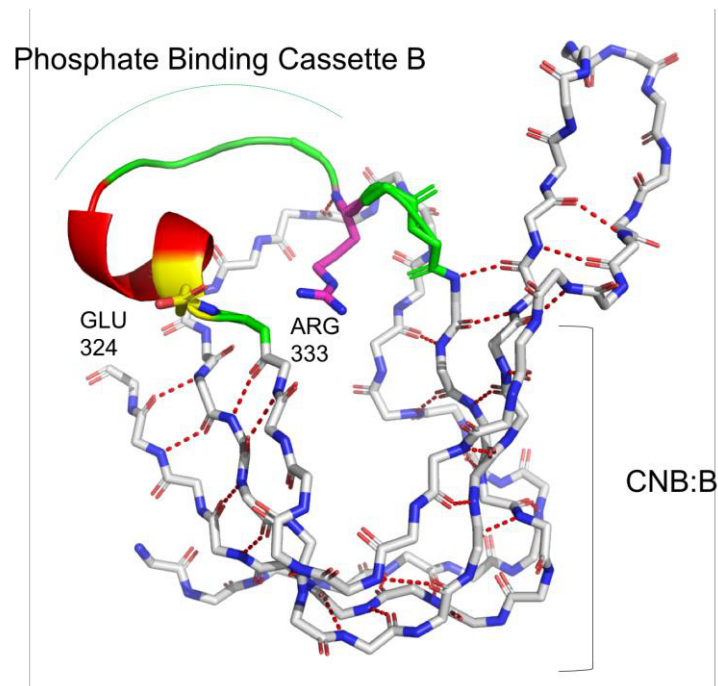
Change from **No Mutation** to **ARG**

Select the best conformer (Selecting States) > State 15



- browse for conformers here
- pick up conformer 15

Now use the skills you learned so far generate the figure related to **Exercise 05** (use powerpoint to generate labels) :



Exercise 05 : Beta barrel of CNB:B (main chain representation) highlighting the PBC sequence responsible for binding cAMP (GEIALLMNRPRAA) sitting on top of it. The PBC sequence is represented in cartoon and colour coded according to secondary structure.

Using the command findseq.py:

Copy the file **findseq.py** to the folder: pymol > modules

(This command will find the specified sequence, select it and creat an antry called "foundseq __ (number) __")

On the command line type:

```
import findseq
```

Now we will open the structure of a bacterial porin (*S. typhimurium*) formed by a 18-strand β barrel which's function is the diffusion of sucrose from the outside to the inside of the cellular membrane.

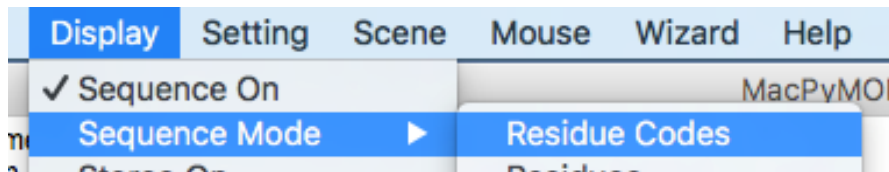
```
fetch 1A0S
```

***it's a zero not an O**

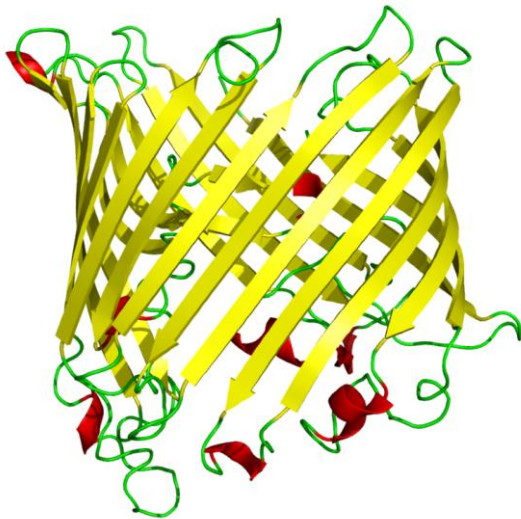
Open the structure, which is composed of 3 chains (P, Q and R) and copy one of the chains to another object renaming it **chain_P**

Color > by ss > **Helix** **Sheet** Loop

Make the sequence mode go back to one letter residue codes:



You should have something like this by now:



Issue the command the following command to find the Phenylalanines (F), Tryptophanes (W) and Tyrosines (Y) in the protein:

```
findseq F, chain_P
```

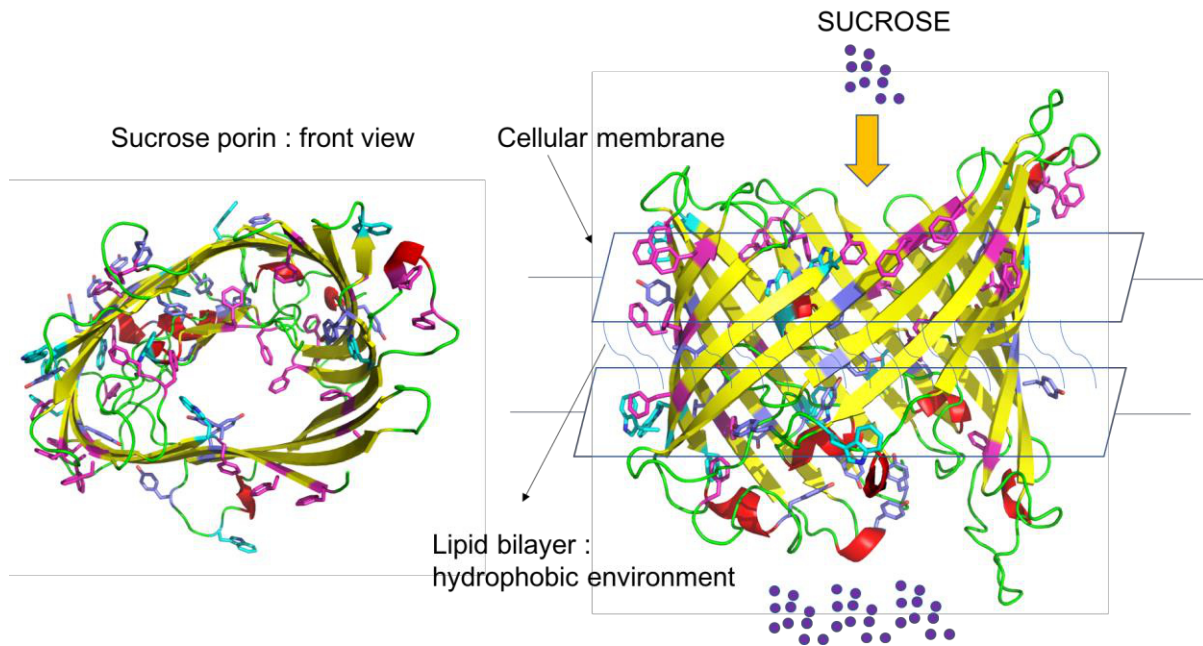
```
findseq W, chain_P
```

```
findseq Y, chain_P
```

Rename the foundSeq---- entries to **F**, **W** and **Y**, respectively:

(FoundSeq4122)	A	S	H	L	C
(F)	A	S	H	L	C
(W)	A	S	H	L	C
(Y)	A	S	H	L	C

Colour those residues in **Magenta**, **Cyano** and **Purple**, respectively.
Save the image as **Exercise 06**. (ray 2000, 1000)



Exercise 06: Sucrose porin showing the aminoacids F, Y and W (magenta, purple and cyan) buried in the hydrophobic environment of the cellular membrane. **Save only the left part of the figure as Exercise 06. Drawing the membrane is not necessary.**

The architecture of the Catalytic Subunit (PKAC)

Before we start delete the entries 1YSA, 1A0S, CNB_B and **clean the workspace**.

To **clean** the workspace from **all the measurements**, use the command:

```
delete measure*
```

(It makes all easier so you don't have to delete measurements one by one!)

Now open the PDB entry 3FJQ by typing:

```
fetch 3FJQ
```

This PDB entry (3FJQ) contains the catalytic subunit of PKA bound to a peptide inhibitor (PKAI 6-25) that mimics its original substrate. We will use electron density maps downloaded from the PDB to reproduce the bound state of this peptide and understand how it traps an ATP molecule and two magnesium ions inside the catalytic subunit. (Literature: Comparative surface geometry of the protein kinase family. Thompson et. al, 2009, Protein Science)

In the PDB website (<http://www.rcsb.org/>) enter 3FJQ and download the files **2fo-fc MAP (DSN6)** and **fo-fc MAP (DSN6)** (save it into a separate folder)

The screenshot shows the RCSB PDB website for entry 3FJQ. The entry title is "Crystal structure of cAMP-dependent protein kinase catalytic subunit with peptide inhibitor PKI alpha (6-25)". The classification is TRANSFERASE, and the organism is Mus musculus. The expression system is Escherichia coli. The deposition date is 2008-12-15, and the release date is 2009-08-04. The deposition author is Kim, C.

The 'Download Files' dropdown menu is open, showing the following options:

- FASTA Sequence
- PDB Format
- PDB Format (gz)
- PDBx/mmCIF Format
- PDBx/mmCIF Format (gz)
- PDBML/XML Format (gz)
- Biological Assembly 1
- Structure Factors (CIF)
- Structure Factors (CIF - gz)
- 2fo-fc Map (DSN6)
- fo-fc Map (DSN6)

Red arrows point to the '2fo-fc Map (DSN6)' and 'fo-fc Map (DSN6)' options in the dropdown menu.

Split the entry (3FJQ) into 4 objects (extract to object) and rename them:

- **PKAC**
- **PKAI (+ its waters)**
- **ATP**
- **MN (2 Magnesium ions)**

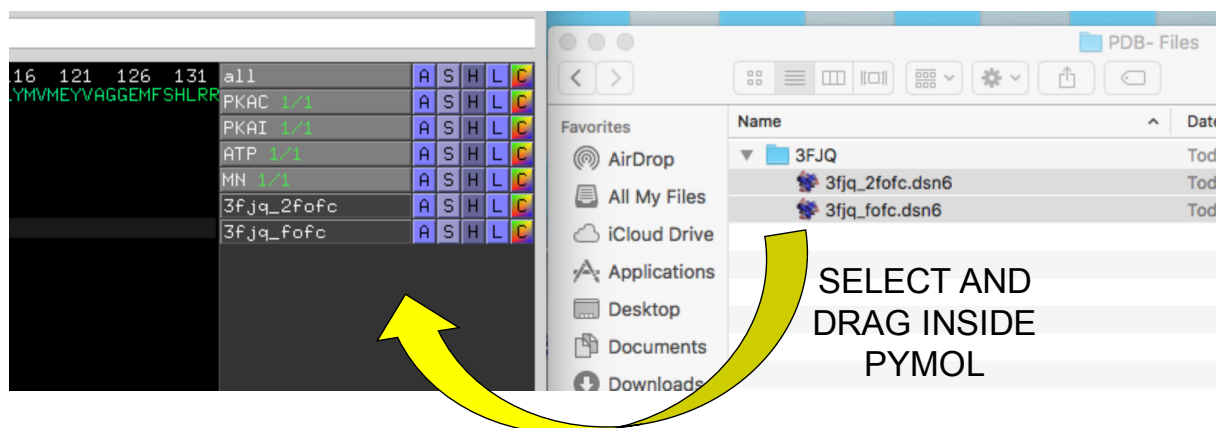
Remember that for re-naming objects you can use the command:

```
set name old name, new name
```

After finished your header should look like this:

PyMOL> 


Now load the files you just downloaded from the PDB inside pymol:



Rename them to 2FoFc and FoFc :

all	A	S	H	L	C
PKAC 1/1	A	S	H	L	C
PKAI 1/1	A	S	H	L	C
ATP 1/1	A	S	H	L	C
MN 1/1	A	S	H	L	C
2FoF _o	A	S	H	L	C
FoF _o	A	S	H	L	C

```
isomesh msh1, 2FoFc, 1.0
```

```
isomesh msh2, 2FoFc, 1.0
```

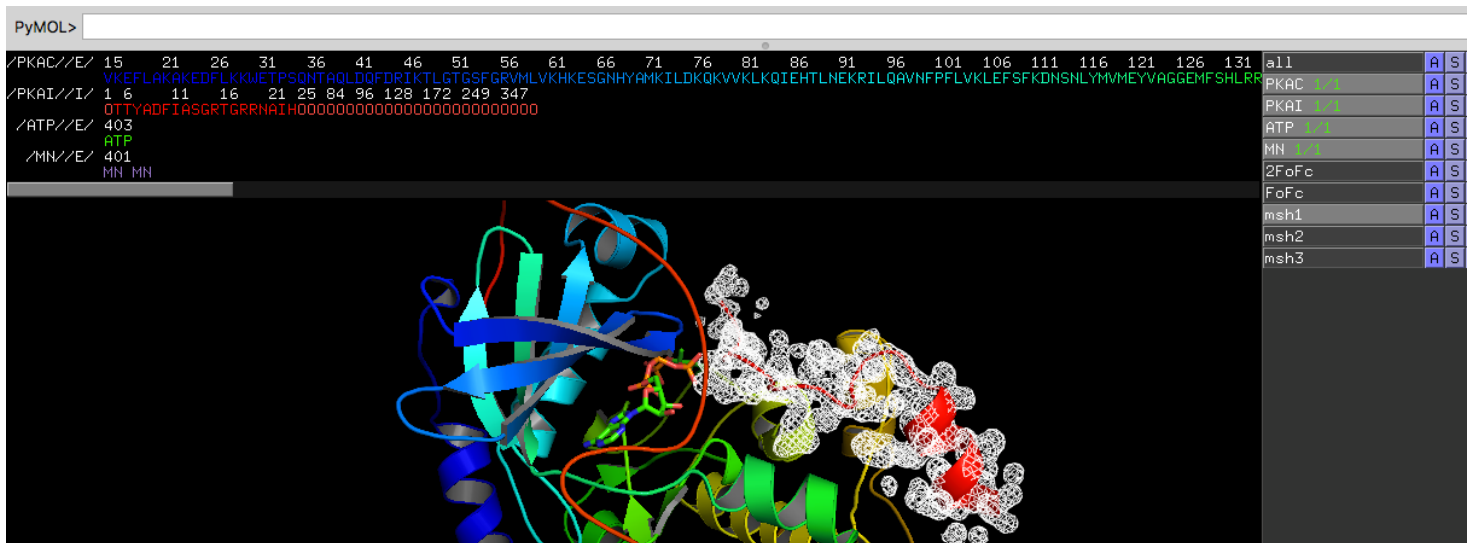
```
isomesh msh3, 2FoFc, 1.0
```

In the Right panel deselect all the 3 msh entries, **make sure they are “off”** :

all	A	S	H	L	C
PKAC 1/1	A	S	H	L	C
PKAI 1/1	A	S	H	L	C
ATP 1/1	A	S	H	L	C
MN 1/1	A	S	H	L	C
2FoFo	A	S	H	L	C
FoFo	A	S	H	L	C
msh1	A	S	H	L	C
msh2	A	S	H	L	C
msh3	A	S	H	L	C

```
isomesh msh1, 2FoFc, 1.0, PKAI, carve=1.5
```

The screen should be like this:



For the PKAI entry:

Hide Cartoon

Show Sticks

Colour > By Element > 2nd Option: Carbon coloured ad green

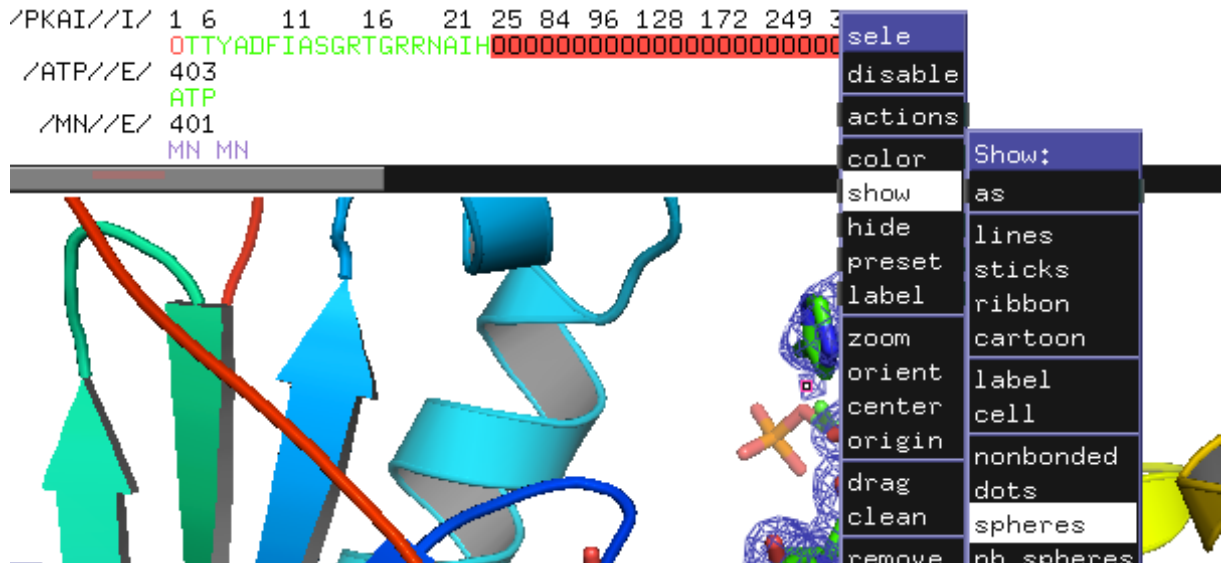
For the msh1 entry:

Colour > blues > deepblue

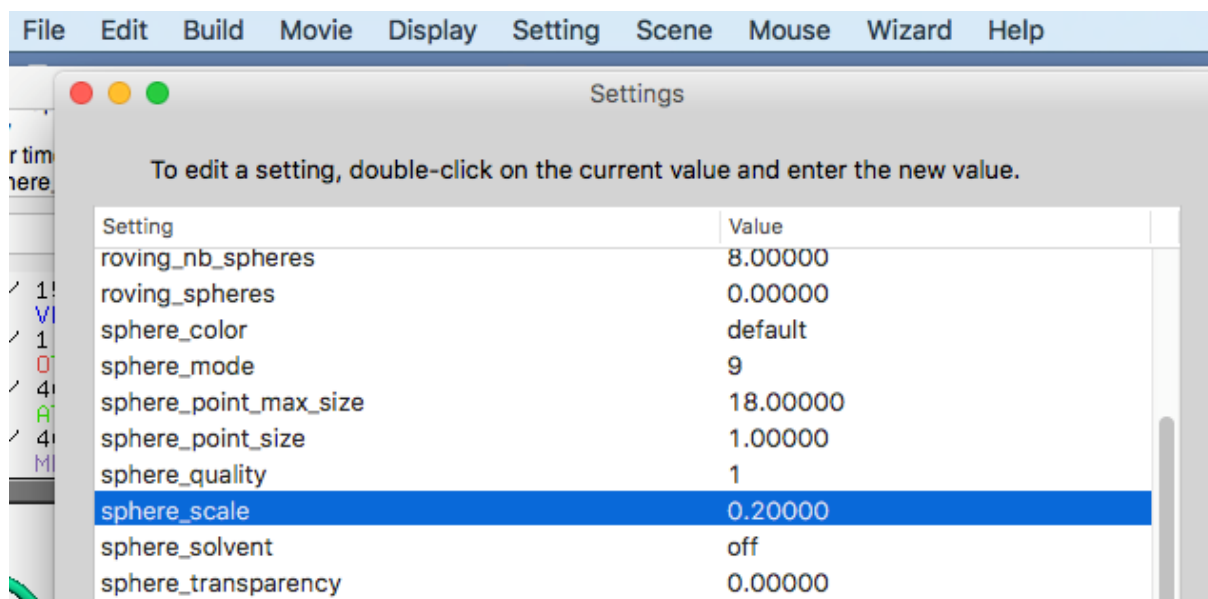


and change Background to white

Select the waters (00000000000) of PKAI and show as spheres:



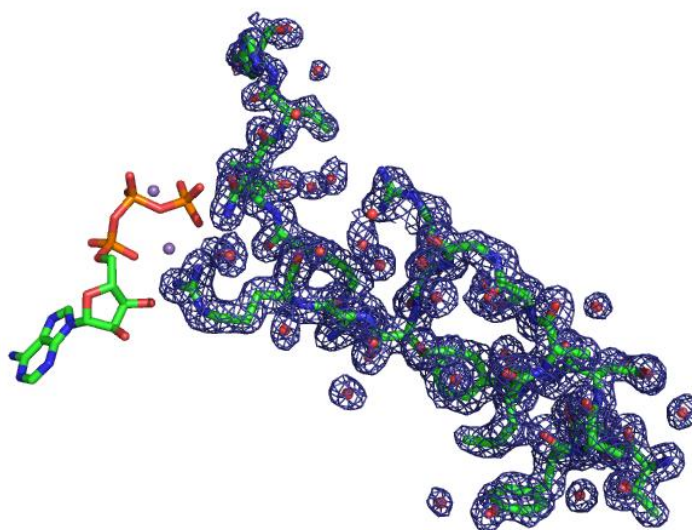
Go into Settings > Edit All > change **sphere_scale** to **0.2**



Turn off the PKAC entry

Turn on the MN (Magnesium ions) entry and show it as spheres (purple)

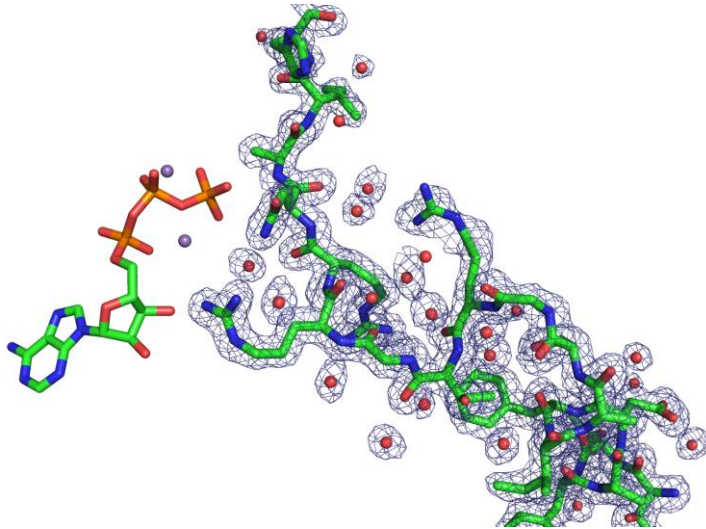
If you take a picture now (ray 1000, 500) it will look like this:



and the mesh width is too thick so we will change it:

```
set mesh_width, 0.3
```

Now it should look like this:

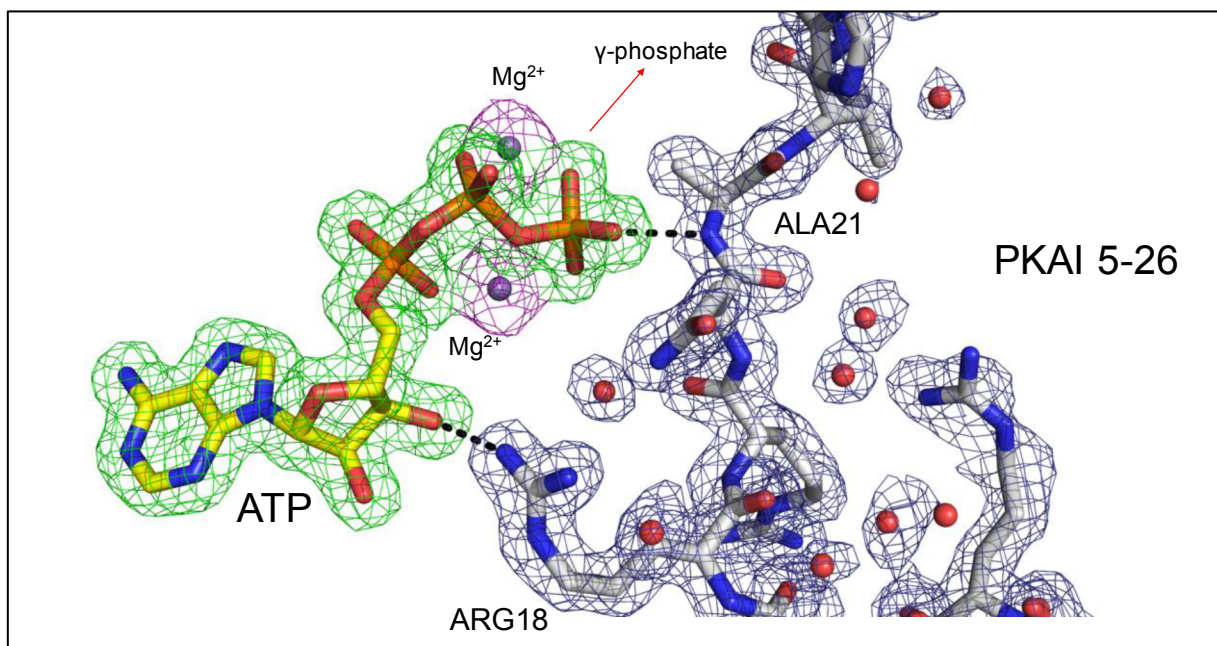


Using the same principle applied to generate the electron density map of PKAI now you should generate the maps for the cAMP and the Magnesium ions and **save it as Exercise 07** (ray 2000, 1000).

Hint: you have to use again the command:

```
isomesh msh, 2FoFc, 1.0, object, carve=1.5
```

where **msh** and **object** are variables.



Exercise 07: Interaction between PKAI 5-26 and the ATP molecule in the ATP-binding site of PKAC (PDB: 3FJQ). Electron density maps (2Fo-Fc) for ATP, PKAI and magnesium ions are shown in green, blue and purple, respectively.

Question for Exercise 07: Why can't the gamma-phosphate of ATP be transferred to the Inhibitor Peptide? What would happen if this were possible? (A bit googling about kinases might help)

Exercise 08:

The PDB entry 1LVB shows a protein known as TEV protease. It is used as a molecular scissor to cleave tags that comes fused to proteins after affinity purification steps. This protease recognizes the peptide sequence ENLYFQ/S and cleaves it.

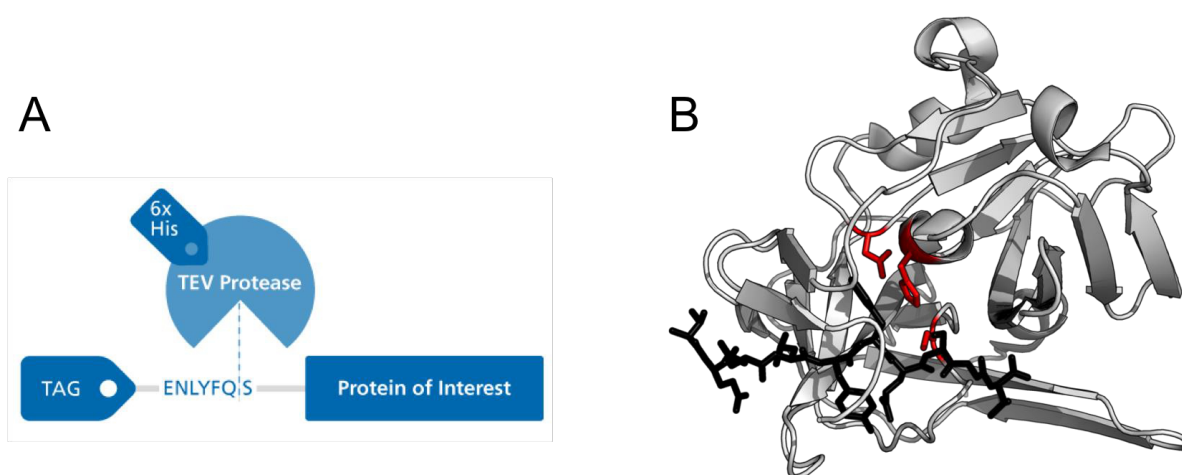


Figure 02: A) TEV protease acting as a molecular scissor cleaving a substrate-like sequence between the protein of interest and its TAG. B) TEV protease (white) complexed with peptide substrate (black) with active site triad residues (red) based on 1LVB.

- For **Exercise 08** represent Figure 02 B showing the **electron density of the peptide substrate** and also the **electron density of the aminoacids taking part in the catalytic triad that cleaves it**. Represent all the hydrogen bonds involved in the binding of this peptide (literature research might help). Image it as ray 2000, 1000 and save it.

Question for Exercise 08: Which mutation in the protein made possible the crystallization of this peptide substrate? If this mutation were not present what would have happened? Which are the 3 amino acids involved in the catalytic triad?
(**Hint:** Use the literature available in the PDB related to the entry 1LVB to answer those questions)

Mapping the subdomains of PKAC:

We will now explore some of the important domains of PKAC that will help us to understand how a single **phosphorylation on Threonine 197** turns the kinase active and how this active kinase can be inhibited by interaction with the R subunit forming the Holoenzyme complex.

The PKAC sequence is: (from *Mus Musculus*, as in the Holoenzyme structure)

MGNAAAAKKGSEQESVKEFLAKAKEDFLKKWETPSQNTAQLDQFDRIKTLGTGSF
 GRVMLVKHKESGNHYAMKILDKQKVVKLKQIEHTLNEKRILQAVNFPFLVKLEFSFK
 DNSNLYMVMEYVAGGEMFSLRRIGRFSEPHARFYAAQIVLTFEYLHSLDLIYRDLK
 PENLLIDQQGYIQVTDFGFAKRVKGRTWLTCGTPEYLAPEIILSKGYNKAVDWWALG
 VLIYEMAAGYPFFADQPIQIYEKIVSGKVRFP SHFSSDLKDLLRNLLQVDLTKRFGN
 LKNGVNDIKNHKWFATTDWIAIYQRKVEAPFIPKFKGPGDTSNFDDYEEEEIRVSINE
 KCGKEFTEF

Glycine-Rich Loop (Subdomain I):

- positions the gamma phosphate of ATP to be transferred

(Subdomain II):

- This subdomain contains **Lysin 72** (...K...) marked as **bold** that interacts with **Glutamate 91** in the C-Helix in order to position ATP for γ -phosphate transfer.

C-Helix:

- Only helical element in the kinase small lobe. Interacts with the inhibitory sequence of the R subunit .

Subdomain V:

- Links the small lobe (N-terminus) and big lobe (C-terminus) via a linker region (GG –glycines are good linkers for being flexible) that connects one beta strand to one alpha helix.

Catalytic Loop:

- Formed by a small loop between two beta sheets (b6 and b7)
- Essential for catalysis (transfer for the gamma phosphate to the substrate protein)

Activation Loop:

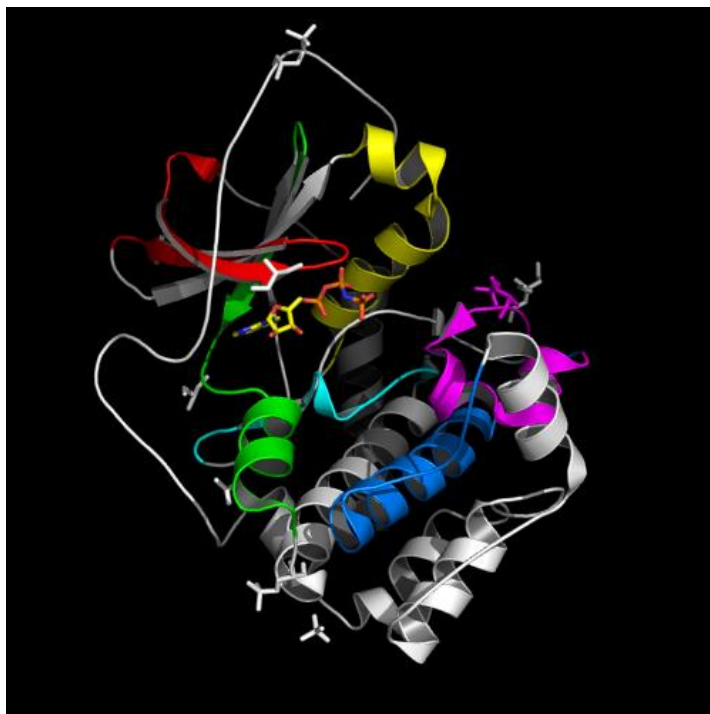
- Contains Threonine 197 (T) that gets phosphorylated and therefore turns the protein into an active kinase.

F-Helix :

- Very hydrophobic helix, all the kinase is assembled around this helix, it's a very important one. It links to the two kinase hydrophobic spines (regulatory and catalytic) and turns into an active protein.

Exercise 09: Using the colour scheme from the protein sequence describe above and the command **findseq** generate the figure bellow and save it as exercise 09. You should change the name of the findseq entries to the names of the respective subdomains of PKAC. For the Activation Loop the command will not work because of the phosphorylated threonine therefore you have to select this sequence and then click on Actions> Rename Selection.

Your workspace and right panel should look like this in the end. For Exercise 09 you should generate the figure bellow.



(ActivationLoop)	A	S	H	L	C
PKAC_Holo 1/1	A	S	H	L	C
PKAR_Holo 1/1	A	S	H	L	C
(GlyRichLoop)	A	S	H	L	C
(C-Helix)	A	S	H	L	C
(SubdomainV)	A	S	H	L	C
(Catalytic_Loop)	A	S	H	L	C
(F-Helix)	A	S	H	L	C

The Catalytic Mechanism: positioning of alpha and beta phosphates of ATP

We will now visualize the interaction of the pair **K72:E91** forming a salt bridge (remember lysine is positive and glutamate is negative) that stabilizes the alpha and beta phosphates of ATP so that the gamma phosphate can be transferred to a substrate.

For Exercise 10:

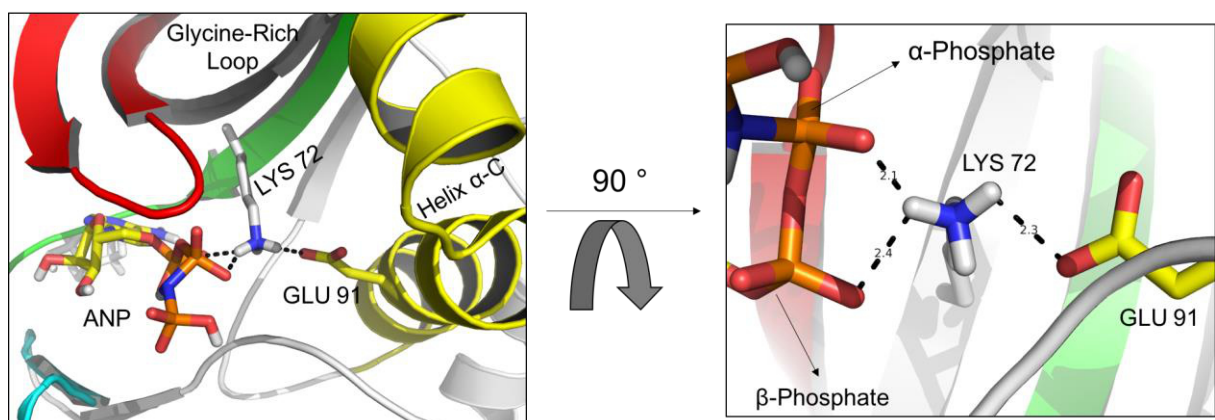
In order to add hydrogens, use the command:

```
h_add (selection)
```

To hide the other hydrogens (apart from the ones involved in H bonds) select the amino acid (or ligand) and click in **Hide > Hydrogens > Non-Polar**.

A common way of representing important interactions is to turn the image and make a zoom highlighting the HB described in the paper. We use an arrow indicating in how many degrees the protein was twisted in order to get a closer look into the interaction we want to show.

Using the commands above assemble **Exercise 10** bellow (use power point for the labels). This is a typical Figure you will find in structural papers and when you understand the color code and the functions of each part of the protein from the beginning understanding such papers does not look so complicated!



Exercise 10: PKAC co-crystallized with ANP showing the salt bridge formed by K72:E91. In the zoom K72 is shown positioning the phosphates alpha and beta of ANP.

Question for Exercise 10: What is ANP? (look for it on the PDB website > search > ligands. All ligands in the PDB are given a 3-letter code) and why was the Holoenzyme co-crystallized with ANP instead of ATP?

Role of Glycin-Rich Loop in positioning the gamma phosphate of ATP for transfer

The **Glycin-Rich Loop** (marked in red in the protein sequence) is a very **mobile motif** that closes over the ATP molecule in order to position the gamma phosphate for catalysis. In order to see its mobility, we have to compare two structures of PKAC, one without ATP inside (also known as APO structure) and another one with ATP inside, that is already the one we have. The APO structure we will use is in the PDB 4NTS. Do the following:

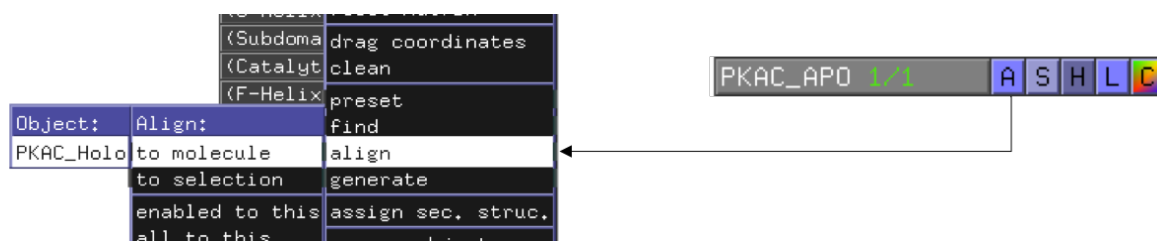
fetch 4NTS

rename it to PKAC_APO

change color to palegreen

select the glycin rich loop and change color to blue

On the PKAC_APO click in **Action > Align > To molecule > PKAC_Holo**:

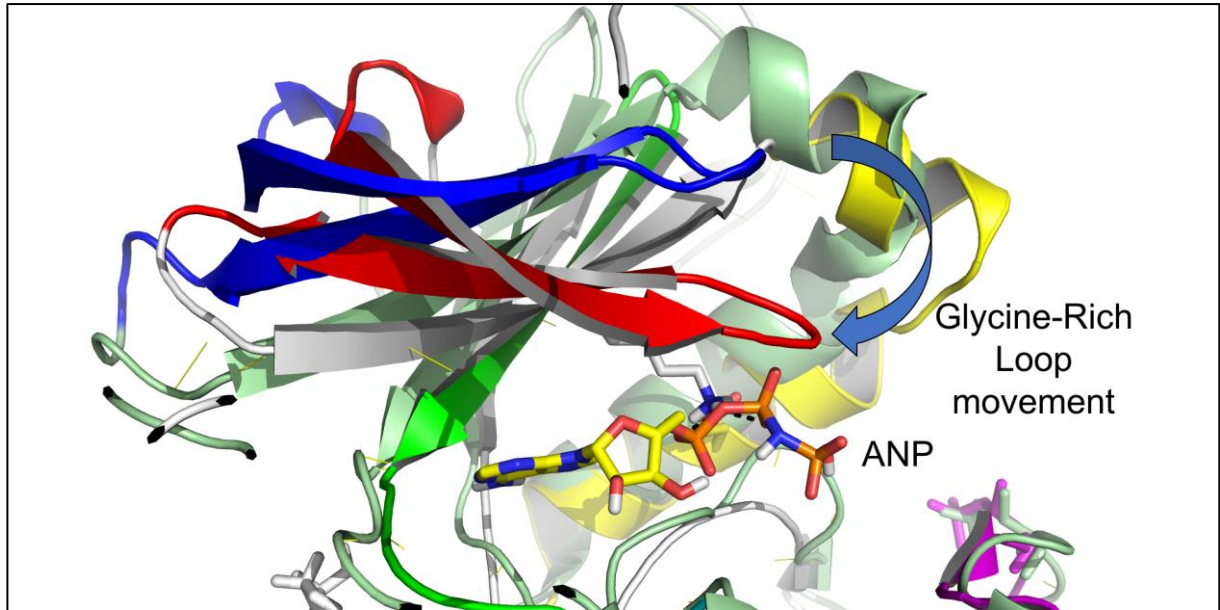


and you will be able to read in the script (above the command line) the RMS (root mean square) deviation of the alignment of the two structures. Something like this should appear:

- Executive: RMS = 0.982 (256 to 256 atoms)

Now that the structures are overlayed you can compare the Glycin-Rich Loop in its open conformation (blue) with its closed conformation (red). This is also a very common way of representation in structural papers, showing the transition of a side chain of a protein from one state (ligand-bound state).

Save the movement of the Glycine-Rich-Loop as **Exercise 11** (ray 2000, 1000):

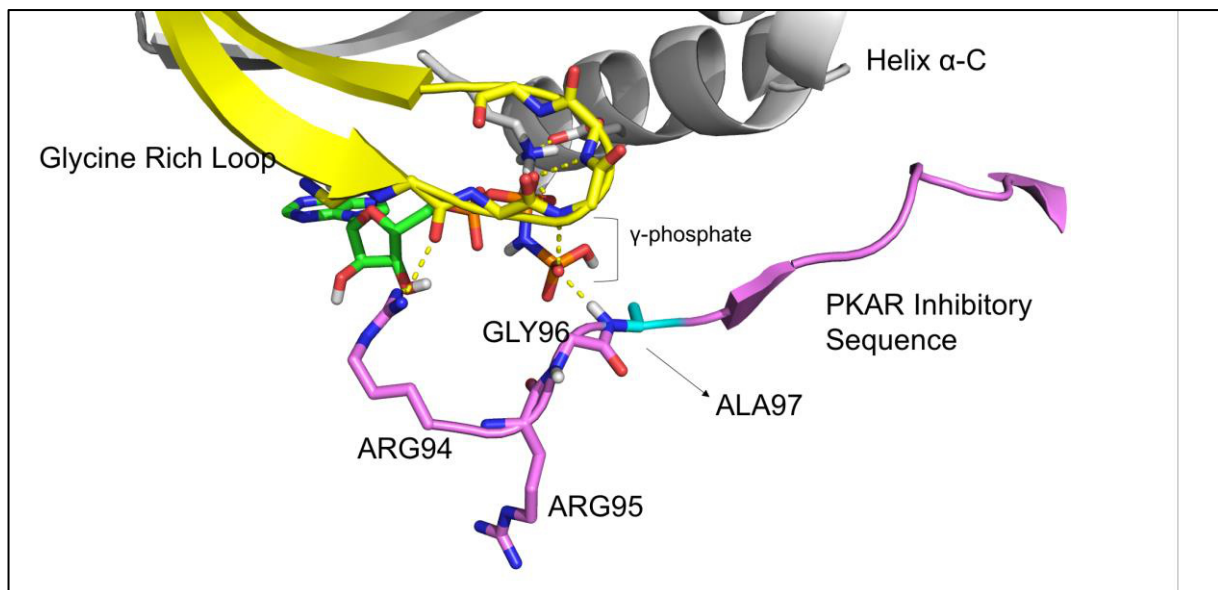


Exercise 11: Alignment of PKAC APO (PDB: 4NTS) with PKAC in the Holoenzyme conformation (PDB: 2QCS, chain B) showing the movement of the Glycine-Rich Loop closing under the ATP analog in order to position the gamma phosphate to be transferred.

Interaction between R subunit and C subunit:

How PKAR inhibits PKAC in the Holoenzyme complex

Based in the structural insides you have got so far and based in the literature about PKA and PKA Holoenzyme make the figure bellow (as Exercise 12) and explain the mechanism of how the inhibitory sequence of PKAR interacts with PKAC and why it does not get phosphorylated by it.



Exercise 12:

Exercise 12

- Reproduce the figure above (variations are allowed)
- Show all the HBs you can find between the Glycin Rich Loop, ANP and the IS of PKAR
- Show how the the Glycin-Rich-Loop positions the Gamma phosphate for being transferred
- Write a legend to the figure
- Give an explanation for how PKAR inhibits PKAC

Exercise 13:

Make a Figure that explains the Holoenzyme activation mechanism in mammalian PKA. Suppose you are the scientist who solved the PKA holoenzyme crystal structure and managed to figure it out how the binding of cAMP to PKAR triggers a conformational change that releases PKAC. Now you have to communicate your discovery to the scientific community using a very **clean, clear and understable step by step figure** (comprised of 4 parts A B C D). Write a legend to you figure and save it as Exercise 13.

(You can use the PKAR bound to cAMP structure (PDB:1RGS) in order to compare the cAMP-bound state of PKAR with the Holoenzyme state)

Don't copy anything from the Holoenzyme Paper! (Just use it as a reference)

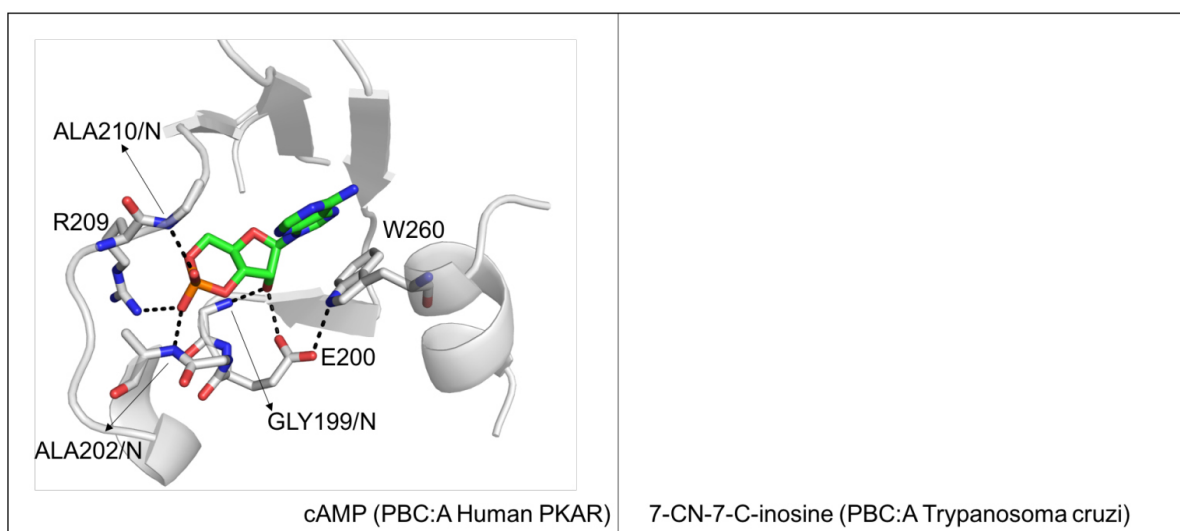
A	B	C
	D	

Exercise 13: PKA Holoenzyme activation by cAMP(legend).....

The size of each part (A B C D) can be different from the model above.

Exercise 14: Representation of ligands binding to proteins:

We will now analyze the difference in the binding of cAMP to HumanPKAR versus 7-cyano-7-deaza-inosine binding to T. cruzi PKAR in order to understand how each protein can be specific for its own ligand. Using the PDB of TcPKAR bound to 7-CN-7-C-inosine (PDB: 6FTF) **make a binding diagram to the right side of the figure bellow**. Use as example the left side of the figure so they can be easily comparable.



Exercise 14: Comparing the binding of cAMP to HumanPKAR versus 7-CN-7-C-inosine to T.cruzi PKAR

Questions for Exercise 14:

Which interactions are the same between those proteins?

Which are different?

What confers ligand specificity to each protein? Why does cAMP cannot bind T.cruzi PKAR and why 7-CN-7-C-inosine cannot bind Human PKAR? (as our data have shown)

Exercise 15: Presentation of a structural paper

Please select a structural paper (if you do not find any, ask your supervisor for suggestions). You should read it and tell its story in 3-5 figures (self made with pymol). Include these figures together with legends in your power point presentation.

Protocol

1. Please finish the exercises mentioned above and save the PNG images as well as the session (.psy).

once you lose the session file, you have to do it from scratch.

2. Create a power point presentation and insert the PNG images into it. Please also write down the answers of the questions asked in between of each exercise.

You can use Powerpoint to add labels