

# PYMOLE LEVEL 3 – ANALYSIS

We will analyze protein structures with PyMol. Before starting each analysis in this tutorial, initialize PyMol by **File**→**Reinitialize**, or `reinitialize` command.

## Change PyMOL's current working directory.

Run a PyMol command,

```
cd \documents and settings\username\desktop
```

(replace *username* by your login id (UA NetID)). With this command you change your *current working directory*, i.e., where you are working (reading and writing data). Commands that save file, such as `fetch` and `png`, will save the results in the *current working directory*. `fetch` command works once you are in a good *current working directory*.

## Electrostatic calculation

PyMOL can calculate a qualitative electrostatic potential on the surface of proteins. Let's try to calculate the electrostatic potential of the phosphate binding pocket of Alkaline Phosphatase. Please download a PDB file of AP with bound phosphate, *1ED8*

```
fetch 1ED8
```

First calculate electrostatic potential on the AP surface, by selecting **A**(ction) of **1ED8** object → **generate** → **vacuum electrostatics** → **local** (it may take a minute).

Protein surface is colored according to the distribution of local charges; blue indicates positive charges and red negative charges. What charge does the phosphate group have? how should the Pi binding pocket should be?

Find the phosphate, Mg(II), and Zn(II) pockets (it looks like a hole in the structure). Residue name of the ions are listed in the PDB file as:

```
HETNAM      ZN  ZINC  ION
HETNAM      MG  MAGNESIUM ION
HETNAM      P04 PHOSPHATE ION
HETNAM      S04 SULFATE ION
```

Select the atoms and display it as sphere:

```
enable 1ED8                (or click 1ed8 from menu)
select phosphate ion, resname P04
select magnesium ion, resname MG
select zinc ion, resname ZN
select sulfate ion, resname S04
show sphere, P04           (or show sphere from menu)
```

continue to display the other ions as spheres as well.

Use **C** - Color: Change the color of atoms and groups. Change it "by element".

Go to **Display** and change **Background** to **Black**. you could actually see a unit cell around the molecule which can help you uncover the surface (by rolling the mouse wheel).

*Note: electrostatic calculation by PyMol is qualitative and not precise. For detailed analysis of electrostatic property of proteins, APBS is recommended ([http://www.pymolwiki.org/index.php/Protein\\_contact\\_potential](http://www.pymolwiki.org/index.php/Protein_contact_potential)).*

## Contact between residues

The interactions made by the phosphate, Zinc, Magnesium, and sulfate are worth taking a closer look using PyMol. One could also take a look at the disulfide bridges made by the cysteines.

Open PDB ID **1ED8**.

One of the easiest way to find contacts is: **1ed8**→**A**(ction)→**find**→**polar contacts**→**within selection**.

Dotted lines indicate the contact between polar groups.

*Note: Many of identified contacts would be hydrogen bonds, however PyMol cannot tell which are hydrogen bonds. Most of PDB data are from X-ray crystallography and X-ray crystallography usually cannot identify the positions of hydrogen atoms (they are too small and X-ray cannot see them). You have to examine each contact to identify hydrogen bonds if you want.*

Now add residue labels: **1ed8**→**L**(abel)→**residues**.

```
select phosphate ion, rename P04
select magnesium ion, rename MG
select zinc ion, rename ZN
select sulfate ion, rename S04
```

To find the residues, open selection tool: Click **S** button, the lower-right corner of the Viewer, next to the animation controls. Set selecting mode to **Residue** by clicking **Selecting** (just above S button) until the right mode appears (it is set to residue by default).

**Now go to** : **1ed8**→**S**(elect)→**disulfides**.

Click **on the cysteines** to select. Then **(sele)**→**A**(ction)→**zoom**. You should see the residues in the display.

Zoom out a bit to see the surrounding residues (right click and drag). Adjust *slab* by turning the mouse wheel.

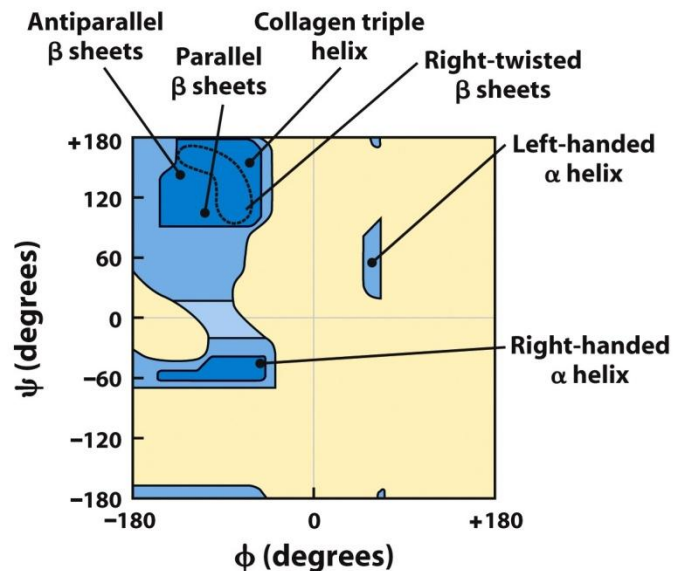


Figure 4-8a  
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## Ramachandran plot

Reinitialize. And open 1ED9 (this does not have phosphate)

AP is a  $\alpha$ + $\beta$  protein.

Show cartoon to see the secondary structure.

**H**(ide) → **side chain** and **H**(ide) → **water** to focus on the backbone structure.

Add **residue** labels.

Pick an Ala residue in the first  $\alpha$ -helix. From **Selection tool**, select **Ala18** and zoom into it.

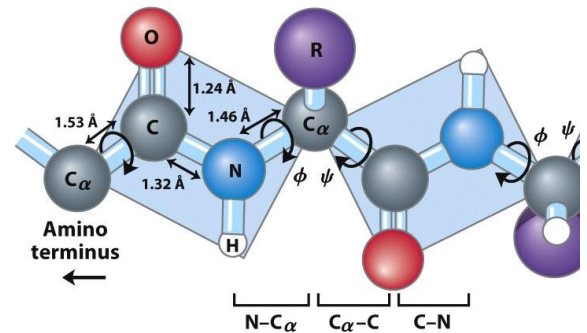
The dihedral angle  $\phi$  of Ala is defined by 4 atoms: C of the previous residue and N, C $\alpha$ , C of the **Ala18**.

Click **Wizard** → **Measurement**. Measurement tool will appear on the right side. Click **Distances** and change *Measurement mode* to **Dihedrals**.

First, hide Cartoon (easier to select atoms). Then click 4 atoms. Measured dihedral angle should appear (you need to click 4 atoms in the above order. Check the console window to see if you clicked the correct atoms. If you did mistake, click Delete Last Object and try again).

Also measure  $\psi$  angle. Where does it locate in Ramachandran plot?

Ala 90 is in  $\beta$ -strand. Measure its  $\Phi$ ,  $\psi$  angles. Where is it in Ramachandran plot?



**Figure 4-2b**  
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## Structure comparison

Often we compare protein structures from different species or mutations. Here we will try to compare the structures of AP (with Pi) (PDB ID 1ED8) and AP (without Pi) (PDB ID 1ED9).

First, load 2 PDB files 1ED8 and 1ED9.

To make structure comparison easier, remove water molecules by **all** → **A**(ction) → **remove waters**.

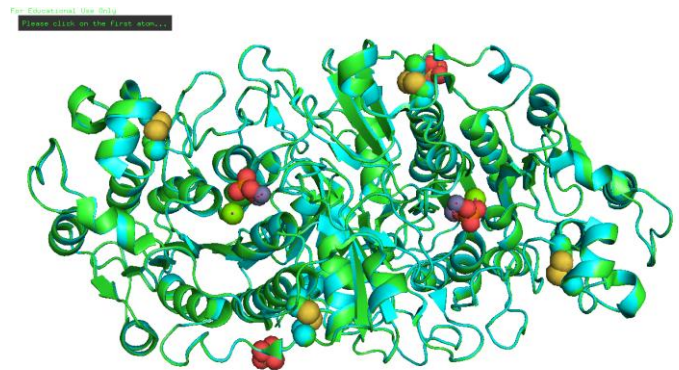
To see the protein backbone better, hide side chains, by **all** → **H**(ide) → **side chain**.

To align AP (W/o Pi) to AP (with Pi)

**1ed8** → **A**(ction) → **align** → **to 1ed9**

In the console window, you will find the *RMSD* value between 1ed8 and 1ed9 (see the note below).

Display the phosphate group and compare the two structures. You can zoom into the fitted structures as well.



## HOMEWORK

Go to [rcsb.org](http://rcsb.org) and look at other structures of AP (mutants) and repeat the above exercise.

## RMSD value

The root mean square deviation (RMSD) is a frequently-used measure of the differences between structures.

$$R = \frac{\sqrt{\sum_{i=1}^N \Delta \mathbf{r}_i^2}}{N}$$

where  $N$  is the number of atoms and  $\Delta \mathbf{r}_i$  is the deviation in the positions of atom  $i$  between two fitted structures. Smaller RMSD indicates high similarity between two structures.