Structural Biology Exercise #1
Insulin

GOALS OF THE PRACTICAL:

1. To learn to look for a structure databank code from a structure paper
2. To get familiar with Pymol, which is used for visualisation of protein structures
3. To learn how Insulin was modified to make it act faster

Pymol is a molecular visualisation program, which is commonly used to make figures. It is originally designed by late Warren DeLano, and since supported by the Schrödinger company.
You can download a freeware version from http://www.pymol.org, but manuals and support are available only for purchased versions.

You have a version of this program on your memory stick, please use it to ***install PyMol*** on your own computer before the practical starts.

1. Getting Started

Open the Biochemistry1994 paper from your Insulin directory. The structure we will use in this demonstration was published in this paper. You can always find the pdb (Protein Data Bank) accession code for the structure in the paper, this time it is on the foot notes on the first page. You can download the structure from the pdb (`http://www.pdb.org`), or directly through the program Pymol.

Open PyMOL by double clicking the icon
Two windows should open.
The smaller window (The PyMOL Molecular Graphics System) contains the menu bar, some command buttons and a command line. This will be called the **Command window** below
The larger window is **PyMOL Viewer** where the molecules are displayed.

From your Command window, find (this is only available in the Windows version!)
`Plugin/PDB Loader Service` and type in the accession code (`1TRZ`).
`OK`
If you do not have the plugin, you can proceed in two ways:

1) download the structure from Protein Data Bank and then open it from the downloaded location `File/Open/1TRZ.pdb`
2) type in the command line of any Pymol window `fetch xxxx ,` where xxxx is the PDB accession code (e.g. fetch 1trz)

Your display window should now contain a lot of colourful lines and crosses. Not very informative ☹. How can we make this better?

Visualising protein structures

In the Viewer, on the right hand side, you see the object buttons.
The first one contains the object name (**1TRZ**) and 5 smaller buttons:
A(actions) **S**(show), **H**(hide), **L**(label) and **C**(colour)

When a molecule is selected in PyMOL, the object button is Grey.

`Click on 1TRZ`. *What happens?*

Select the molecule again by clicking the 1TRZ button.

Lets then get rid of some details:

Next to the 1TRZ button, select `H/nonbonded`. *What happens?*

You hide the water molecules and other nonbonded atoms in the structure

Next to the 1TRZ button, select `H/lines`. *What happens?*

You hide the protein molecule

There is no 'undo'!

If you want to see the 'nonbonded' and 'lines' again, you can do find them from the S(show) tab: `S/nonbonded` and `S/lines`.

Hide (H) both (lines and nonbonded) before you continue.

On `(1TRZ)→` select `S / ribbon`. Now you see the protein structure as a Cα trace.

On `(1TRZ)→` select `S / cartoon`. *Now what do you see?*

Finally, select (`1TRZ)→ A/Preset Publication`*…*

This will colour your molecule from N- to the C-terminus and make a pretty presentation of the structure. Leave this on for now.

2. Lets move!

Below, I will refer to
LMB, when I mean the *left* mouse button
RMB, when I mean the *right* mouse button

Three dimensional molecules are sometimes difficult to understand as a two-dimensional projection, but it helps if you can move the molecule.

Hold on the **LMB and move** the cursor in the window.

Now you can move the molecule around.

Hold on the **RMB** `and move the cursor` in the window. *What happens now?*

Scroll (careful, not too fast!) with the **mouse wheel**.

You can see that back of the molecule dims out. This adjusts the slab in the Z-direction. If you scroll too far, the whole molecule is sliced away. This is however a useful way to reduce detail in the figure. Now scroll back so that you can see the full molecule.

It is easier to work if your **center of rotation** is in the area, which you are interested in.
`Rotate the molecule`. The center of rotation is now in the center of your structure.
Click with `the mouse wheel` anywhere in the structure where you would like to go. Now when you rotate, this area is easier to visualise.

`Hold` down `the wheel` and `move the cursor` in the window. *What happens?*

You can set the center of the rotation back to the center of the molecule by selecting from the buttons `(1TRZ)→ A(actions)/center`.

3. Working in sequence

Insulin is synthesised as a one chain protein, but during maturation, it gets proteolysed into two peptides. These two peptides are held together with disulphide bridges.

The ‘publication’ mode coloured your molecule from the beginning to the end, but sometimes it is easier to understand a structure if you colour the peptides individually. These peptides are easiest to locate in sequence view.

On upper Command window select `Display/sequence`.

Now you see all the residues, which are included in the pdb file. You can select residues from the sequence line. The graphics window shows the selected area on the molecule.

Center on the object `(1TRZ) → A/center`.

`Zoom back` so that you see the whole molecule.

`Select amino acids in sequence view` by clicking on them one by one. You can also hold down the shift key while clicking and select a wider area at the time.

There are a lot of `OOOOOO` residues in the sequence view. These are the waters associated with the protein. You can ignore them for now. Just scroll over.

`Select the whole first A-Chain` (until you come to O water residues)

Now colour this subunit which ever colour you like

`(sele) → C/magentas/deeppurple`.

Now save your selection by renaming it

`(sele) → A/rename selection`,

remove ‘sele’ with backspace and type `chainA <Enter>` on the screen window.

Renaming will save the selection.

If you do not rename then

click on the black background to `deselect the (sele)` object.

New selection will now override your old (sele).

Now in the same way, colour the other the subunit with a different colour.

4. Funny details

In order to understand a protein, often it is not enough just to display a protein fold.

For example, the disulfide bridges help to hold the two insulin peptides together. These are easy to visualize in Pymol.

Choose `(1TRZ) → C/by element`

so that other than carbon atoms get their standard element colour (oxygen red, nitrogen blue).

Note, this will be visible only when you display the side chains.

Choose `(1TRZ) → S/disulfides/sticks`

You see new connections on the peptide backbone, which are coloured yellow in the middle. Two disulfide bonds hold the peptides together and an additional disulphide bonds to give stability to the N-terminal peptides A and C.

(If something went wrong, you can jump here by opening the saved Pymol session in the Insulin directory on the memory stick and `Scene/Recall/F1`)

Other interesting details include essential cofactors and metal ions.

In the `sequence view`, click on the **ZN** residues (there are two).

Choose `(sele) → S/spheres`

Now you see two round metal ions, which seem to be a bit outside of the structure. Their function is easier to understand if we display the full biological insulin unit, a hexamer.

5. All things wonderful

Insulin is stored as a hexamer in our body, and this regulates the release and stability of insulin. The molecule we have been looking at, contains only a dimer, which was the unit during the calculations in structure determination (this is called the asymmetric unit). The biological hexamer is formed by combining three of these dimers, by symmetry in the crystal.
There are two ways to do this. I recommend you do them both before proceeding, so that you know how to choose. I would recommend option 2.!

1. You can generate the symmetry mates (molecules) in PyMol. In case of the Insulin, the program generates so many possible molecules, that a single hexamer is difficult to find.

Choose `(1TRZ) → A/generate/symmetry mates/within 4 A`

This will create 14 new objects, which include the native hexamer. You can undisplay the extra ones by clicking on the object button (1TRZ_0000-1) etc. Most likely the objects 1TRZ_0100000 and 1TRZ_0200000 are the ones which form the hexamer with our original 1TRZ.

2. You can also generate the hexamer in the PISA server and pick it up through the European pdb-server.

Go to `http://www.ebi.ac.uk/pdbe/index.html`

Write `1trz` on the provided box

Click `Quaternary Structure`

The 'Assemblies' page suggests a 12-mer, which is the correct choice (6 x 2 peptides).
Go to the bottom of the page and Click `Download`
`Save the Assembly.pdb` somewhere you can find it

Go back to PyMol
From the Command window:

Find: `File/open` and open the file that you just saved. You can also use the `Insulin_PISA_Assembly.pdb` from the memory stick you were provided.

You can now view it as

`S/cartoon or S/ribbon` and colour it the way you like.

`H/lines`

`C/by chain` might be convenient here. If you want the helices to appear as in the Pretty mode, select in the Command window:

`Setting/Cartoon/Fancy Helices`

Hide the original `1TRZ object` by cliking on its object button on the right.
Now find and display the ZN atoms again:

In the `sequence view`, click on the **ZN** residues (there are two).

Choose `(sele) → S/spheres`

Now you see that the metal ions are actually inside the hexamer.

6. This one I like! (Scene F2 in the prepared PyMol session on the memory stick)

I previously mentioned that here is a view which you can automatically reload, but you can also set your own. The Pymol program does not have an UNDO button, so it is sometimes good to save things before the next step.

Move the molecule so that all six subunits are well visible

On upper Command window select `Scene/Store F2`

You can now come back to these setting any time by selecting `Scene/recall/F1 or F2`.
As long as you do not save something else on top of them...

These scenes are also saved into the PyMol session when you save it. You can do this from the Command window

`File/Save Session As...`

type `Insulin_tutorial`

Now you can come back to this exercise later.

7. Around here (Scene F3)

To view how the Zn ion is coordinated to the Insulin structure, you need to display more than the protein backbone.

`Click on the black background` to in activate any possible (sele) objects you might have.

On `sequence view, select ZN`

`(sele) → A/modify/Expand/by 4A, residues`

`(sele) → S/side chain/sticks`

Deselect the object by clicking anywhere on the background so that the red dots disappear. Zoom in.

The Zn ions are coordinated by Histidine residues from three subunits, thus they help to hold the dimers together in Insulin hexamer.

This view is stored in `Scene/recall/F3` in your readymade Pymol session.

You can also do so in your own practical:

Command window `Scene/Store F3.`

8. How far are we?

Pymol is a common program to prepare figures for publications, and then we might also need to show coordination or hydrogen bonds. This is best done in PyMol by measuring distances.

Use the previous (sele) object , or select the ZN ions again. It is easier to pick objects from the graphics window if if you hide the large spheres.

`(sele) → H/spheres`

`(sele) → S/nb_spheres`

Zoom in so that you can see the Zn ion and the histidines well.

In the Command window, find `Wizard/Measurement`

Click on the `Zn residue` and the closest blue `histidine atom`.

The distance should now be displayed.

You can do this for all three Histidines, one by one.

Click `Done` on the lower right when you do not want to measure more distances.

You now have a new object called **measure01**

Often the numbers are not necessary in a picture.

`(measure01) → H/labels` to hide the numbers.

You can also change the colour of the line from

`(measure01) → C`...

9. Changes (Scene F4)

Rapid acting insulin analogues, are mutated forms of Insulin, where we have engineered the insulin molecule so that it no longer forms dimer or hexamer, and the active, monomeric form of insulin is rapidly absorbed. In these Insulin analogues one noncharged proline (P) residue is mutated to a charged Aspartic residue (D). Where is this in the insulin structure?

Before your do anything, duplicate the hexamer object so that you can make mutations to a different structure. If we make the mutations into the original objects, the scenes we saved will disappear.

On the `assembly` (or `Insulin_PISA_assembly)` object,

select `A/Duplicate` object

`(obj1) → A/rename selection`,

remove 'sele' with backspace and type `Mutated <Enter>` on the screen window.

Hide the the `Insulin_PISA_assembly` by clicking the grey object button black.

From the sequence view, find residue P28 from the B-peptide.

`(sele) → S/side chain/sticks`

This is the residue which has been mutated. You can select and display all 6 prolines (P) in the same way. Rotate the hexamer and see where the proline residues are in the structure. They locate to the interface of the insulin dimer, which we started to work with.

Turn the molecule in such an orientation that you can view two of the prolines.

Click on one of them and center on the (sele) object.

`(sele) → A/center`

`Deactivate (sele)` by clicking on the `background`.

From the Command window, find `Wizard/mutagenesis`.

Click on the Proline in the structure, which you want to mutate (or choose it from the sequence).

From the new pulldown options, which appear on your graphics window on the right:

`No Mutation → Mutate to ASP`

A new residue appears on top of the Proline residue.

Choose a conformation for the new ASP residue with the `arrow keys on your keyboard`.

Zoom in to see better. You can adjust the slab (with the mouse scroll) so that the site is easier to

view. The red dots in the end of the mutated side chain indicate collision with the rest of the structure.

When you are happy with the conformation, click `Apply` and `Done`.

The new Aspartate residue is charged, and it will push apart the two monomers. The effect is enhanced by an identical P28D mutation from the other monomer, on the same interface. Do this mutation also. You can store the view into `Scene F4`

10. Now we take a picture

Although black background works well on a computer screen, in printed pictures lighter background is better.
From the Command window choose

`Display/background/white`.

On the Command window, you can now go through all the nice views you saved:

`Scene/`**`Recall`**`/F1`
`Scene/Recall/F2`
`Scene/Recall/F3`
`Scene/Recall/F4`

Choose one of these views for a figure.

Now we will render the figure surfaces to look nicer. This takes more computer power on the screen so it is practical to do only as a final step.

On the Command window prompt, write `ray <Enter>`.

Wait for the computer to do the calculation; do not move the molecule!
This will render your figure with the screen resolution. If you would want higher resolution, you can try for example:

On the Command window prompt, write `ray 1000 <Enter>`.

What is your image size now?

Save the rasterised image in the Command window (without touching the graphics window):

`File/Save image as/png`
Find your directory
Give a file name, ex. `Insulin.png`

You can view this figure now with any picture viewing program.

As soon as you click on the Viewer window, you image quality is set back to the working mode. If this was a publication figure, it is also good to save it once more to the Scenes, so that you can come back to the same settings again.

Save your session before you quit PyMOL. It will not ask you, and if you forget you will be difficult to create exactly the same views again.

11. Bonus track

You have now created your first image with PyMOL, but sometimes when you get really professional with this, you will want to change more display parameters.
This is done through Command window `Setting` -tab.

You can display the molecule backbone also in the cartoon mode. For this, first select in Command window:

`Setting/cartoon/fancy helices`

Now, in the viewer

`(1TRZ) ) → S/cartoon` and `H/ribbon`.

`Command window: Setting/Edit All`

contains parameters which you can use to adjust bond thickness etc.

Find `cartoon_dumbbell_length` and double click on it.

It is easier to find if you type cartoon on the filter.

Set the `value to 1.0`. Hit `<Enter>`

This will make the helices a bit narrower so that the structure is not so crowded.

Other useful edit all parameters:

`Stick_ball`:	set this on.
`Stick_ball_ratio`:	if you increase the number, residues will be ball-and stick mode.
`Stick_radius`:	adjust thickness of the residues
`cartoon_rect_length`:	adjust the width of the β-strands
`cartoon_rect_width`:	adjust the thickness of the β-strands
`cartoon_loop_radius`:	sets the thickness of the loop structures.
`dash_gap`:	determines the style of the measurement line. If set to 0, you get a solid line.
`dash_lenght`:	

In order to display metal ions, water molecules etc. first create an object, which contains only the metal ion or the desired waters (use the sequence view for the metal ion!). Rename it to something logical. Then select `→ S/spheres`.
The radius of the ball is adjusted from its atomic radius.
You can change size of the ball in `Setting/Edit All`, by changing the `sphere_scale`.