BIO-315
Biomolecular Structure and Mechanics

How to interpret crystallographic electron density maps

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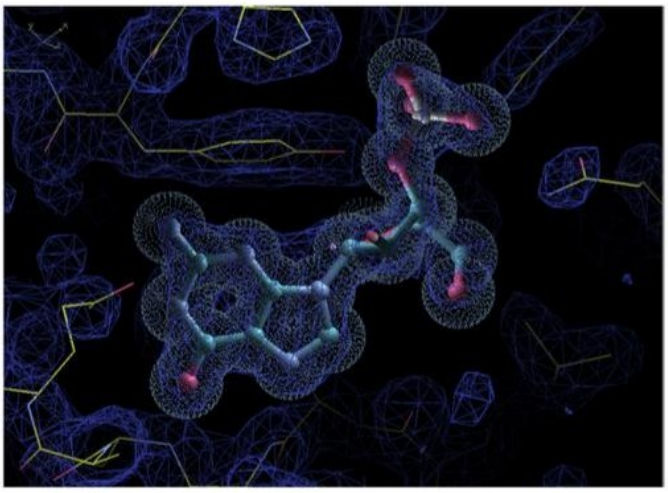

1. Technical operations

In order to set everything up for this computational lab, do the following: 1. Download and install the COOT program on your laptop.

Windows: http://bernhardcl.github.io/coot/wincoot-download.html

Linux: https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/

MacOSX: you will need to install two programs.

a. Please install the CCP4 package that includes coot and makes it ready to use on a macbook; https://www.ccp4.ac.uk/download/#os=mac (go to the CCP4 Program Suite v8.0.018 and agree to licence and then download and move into your applications).
b. Please install Xquarz to be able to use coot on your computer. https://www.xquartz.org/
c. In order to open Coot now on the MacBook open Xquarz and open a terminal window under Applications - Terminal
d. Start Coot by typing /Applications/ccp4-8.0/bin/coot in the terminal (Unless the folder is stored somewhere else on your computer)
e. Coot should start now

2.Download all the PDB and MTZ files for this homework which can be found on Moodle.

You should now be ready to start !

Note: All questions are homework questions.

2. Introduction

I-DmoI is an enzyme from the thermostable archaea bacterium Desulfurococcus mobilis. I-DmoI belongs to the family of the LAGLIDADG homing endonucleases. These enzymes recognize and cleave long double stranded DNA (dsDNA) sequences of around 25 base pairs, that is why they are also called meganucleases. The homing endonucleases have been studied with the aim of using them for genetic engineering. Amino acids in the meganuclease can be mutated in order to change the DNA sequence it cleaves, i.e., its specificity. This new DNA sequence of choice can be part of a human gene that contains a disease-causing mutation. If the meganuclease is inserted in a cell containing this genetic mutation, it will cut the DNA at only this site, causing a double strand break in the DNA. This site will be then repaired by the cell using homologous recombination using the wt DNA sequence as template and the disease-causing mutation will be corrected.

3.Get to know I-DmoI

The PDB file wtCa.pdb shows the structure of the wild type (wt) protein bound to the target DNA sequence and Ca^{2+} ions. Open it in Pymol or VMD. You can observe 3 complexes in the asymmetric unit and many water molecules. Observe how the DNA is bent as compared to normal B-double stranded DNA. The Ca^{2+} inhibits the enzymatic activity, therefore the DNA in this crystal structure is not cut.

Question 1: Note down the sequences of the dsDNA in the wtCa.pdb structure, in the 5'-3' direction for both strands. Do the strands contain a phosphate atom at the 3' or 5' end?

Question 2: Locate the Ca^{2+} ions within one complex. This way you have located the active site. How many Ca^{2+} are bound in each complex?

4. Crystal structures

We have solved the structure of the wt I-DmoI and a mutant version (I52F, L95Q, let's call it "Mutant") in complex with DNA and Mn^{2+}. Your job in this tutorial is to use the electron density maps from both crystals to find out the main differences between wt I-DmoI with Ca^{2+} or Mn^{2+} and between wt and mutant structures.

Let's start with the structure of wt I-DmoI in the presence of DNA and Mn^{2+}. The DNA sequence is the same as in wtCa.pdb. The space group and the crystal packing are also the same as in wtCa.pdb. The resolution of the data set is 2.1˚A.

Question 3: Find the cell parameters and space group for the wtCa.pdb (hint: use a text editor to open the PDB file). You will not use this information in this tutorial but it is useful to know where to find it in case you want to compare two crystal structures and their crystal contacts and their crystallographic and non- crystallographic symmetries.

a. Crystallographic symmetry is determined by the space group. By applying the crystallographic symmetry to the asymmetric unit, we can recon- struct the whole crystal
b. Non-crystallographic symmetry is the symmetry found INSIDE the asymmetric unit- in this case we don't have non-crystallographic symmetry because the three I-DmoI-DNA complexes are not perfectly symmetrically arranged.

5. Structure analysis with COOT

5.1 Working in COOT

We have solved the structures of "wtMn" and "mutant" data sets by Molecular Replacement.

We covered a little bit of Molecular Replacement (MR) in the lecture yesterday with the example of the Aurora kinase and the small-molecule inhibitor. MR is a method to solve the phase problem and be able to obtain an electron density map from the diffraction pattern data. MR uses a model which has a structure similar to the one you want to solve. MR performs 3D searches, i.e., rotates and translates the model in 3D within the asymmetric unit, until the calculated amplitudes from the calculated diffraction pattern from the relocated model (A_c) fit the experimental diffraction data amplitudes (A_0): i.e., MR finds a "solution". This "solution" is used to calculate an initial estimate of the calculated phases: α_c. These phases are combined with the experimental diffraction data amplitudes (A_0) in a new mtz file ($F_c = A_0\, e^{2\pi i \alpha_c}$). This mtz file can be used to generate the first electron density maps ($\rho(x,y,z) = FT^{-1}(A_0\, e^{2\pi i \alpha_c})$).

Question 4: What is the disadvantage of using Molecular Replacement to obtain the first phase estimate? How can you avoid problems?

COOT is used to display and manipulate atomic models of macromolecules and electron density maps. Specifically, it focuses on building and validating atomic models into three-dimensional electron density maps obtained by X-ray crystallography methods. For more information, check COOT on Wikipedia (https://en.wikipedia.org/wiki/Coot_(software))

We have used the wt-Ca^{2+} complex structure as model for the MR job. We have deleted

all water molecules and all ions, and kept the DNA and protein atoms, since those are the common ones in both crystals. The solution found by MR is the pdb ”wtMn.pdb”. The mtz file with the experimental amplitudes (A_0) and first estimate for the phase (α_c) is ”wtMn.mtz”. Follow these steps:

- Open the wtMn.pdb in COOT using File → Open coordinates.
- Notice, that as the space group and cell parameters are similar to the wt structure, there are also three complexes in the asymmetric unit.Spend some time getting familiar with the mouse rotating, zooming and centering into the structure (left click to rotate structure; right click to zoom; ctrl+leftclick to move structure around). Also, you can go to ”dis- play manager” and choose to display it or not, and in which representation (e.g., change visualization between ’CAs+ligand’ and ’Bonds’).
- With the command ”Draw-go to atom”, choose to center at a particular residue.
- With the command Draw→Cell and symmetry, then check Yes in Mas- ter Switch: Show Symmetry Atoms? and click ”Apply”. The neighbor molecules in the crystal lattice will appear as all atom representation. You can also show them as CA traces as follows: click on ”Symmetry by Molecule” and then on ”Display as CAs”. Then change the ”Symmetry Atom Display Radius” to 20A, if you want to see more molecules.
- For the next step, chose the ”Display Sphere” option in ”Symmetry by Molecule” and click ”Apply”.

5.2 Visualizing and analysing electron density maps

Now we are going to use the wtMn.mtz to display the electron density maps. In COOT, create these two maps using ”Auto open mtz” and select wtMn.mtz. Once you have done that, you will find a blue mesh and a green/red mesh displayed. Also, you will find them in Display Manager as ”wtmn.mtz FWT PHWT” for the $2F_o$-F_c map (in blue) and ”wtMn.mtz DELWT PHDELWT” for the F_o-F_c difference map (in red and green).

The blue map is called $2F_o$-F_c map, which is roughly:

$$\rho(x,y,z) = FT^{-1}((2A_0 - A_c)e^{2\pi i\alpha_c}) \approx FT^{-1}(A_0 e^{2\pi i\alpha_c})$$

and it is used to see the overall electron density within the

asymmetric unit.

The red and green map is called the difference map or F_o-F_c map:

$$\rho(x,y,z) = FT^{-1}((A_0\text{-}A_c)e^{2\pi i\alpha_c}$$

and shows negative difference density (where there should not be any density) in red and positive difference density (where there should be density) in green. Both regions correctly reveal the difference between the starting model and the correct model.

In Display Manager, you can choose to display them or not and you can also select which map is ”active” by clicking on ”scroll”. ”Scroll” refers to changing the sigma value of the map, i.e., the contour level. By moving the wheel of the mouse, you can change the contour level of each map, and you will see a value in the top right of the screen changing (for example ”map 1 level = 0.3062e/A^3 (1.69rmsd)”). The ”1.69 rmsd” is the sigma value or contour level of the map.

• In order to visualize the electron density maps correctly and avoid visualizing noise, adjust the contour level of the $2F_o$-F_c map to 1 and the F_o-F_c map to 3.

The intensity of the map changes depending on the part of the structure you are looking at. In well-ordered regions the map is stronger (i.e., still visible at high rmsd values)

than in flexible regions. Changing the contour level of the map allows you to adjust accordingly and understand which parts of the structure are more rigid/flexible/disordered.

Play with the sigma value – contour level, how to switch from one map to the other. You can also play with the map parameters in ”Edit – Map Parame- ters” changing the Map display radius, for example. Move around the structure.

For the sake of simplicity, to answer the following questions, stay within the complex composed of chains A for the protein and B and C for the DNA strands.

• Move to the active site. Go to residue A21, using the command ”Draw → go to atom”.

There is a huge mess with the maps! Lots of green and red blobs, showing that there are differences between our model (wtMn.pdb) and the real structure. This is a very well-ordered area of the crystal structure and the electron density map is very strong. Therefore, please increase the contour levels rmsd values to 2.9 and 12, for the $2F_o$-F_c and Fo-F_c maps, respectively.

Question 5: Take a screen shot of the active site, by ”draw - screenshot” and include it in your homework.

Let’s look for the Mn^{2+} ions. Mn^{2+} ions are very electron-rich. Since our model has no ions at all, the difference map will ask for extra density (i.e., will be green) at their position. You will be looking for very strong (rmsd > 12) green spheres.

Question 6: How many Mn^{2+} ions are there in the active site?

Question 7: Try to place the Mn^{2+} ions in their place:

1. Using the left mouse button and pressing Control at the same time you can move the pointer that indicates the center of the screen. Move it to the center of one green blob. You will have to rotate the structure to be sure it is at the center. You can also increase the contour level of the difference map to make the blob smaller.
2. Then go to the commands at the right hand side of the screen and find ”Place Atom at Pointer”. Click and choose as ”Pointer Atom Type” - Other – Mn. ”Pointer Atom Added to Molecule” - wtMn.pdb. Do the same for all the Mn in the active site of chain A, only.
3. Save the pdb file: ”File – Save coordinates” – save it as ”wtMn2.pdb”
4. NOTE: Glu117 might have been moved into the Mn density. . .

Question 8: Very well-ordered water molecules can also be seen as big spheres in the electron density maps but are weaker than the Mn^{2+} ions. When can you resolve water molecules solvating a biomolecule? In this case, can you see a water molecule? Hint: play with the contour level of the difference map to around rmsd=6. If so, place it in the pdb file as well. Save coordinate file.

Now let’s investigate if the DNA strands are cleaved or not. Mn^{2+} ions, unlike calcium, support cleavage. Inspect the $2F_o$-F_c map at 2.9-3.5 contour level between bases 14-17 of both chains B and C:

Question 9: Is the DNA cut? Both strands ? Take screen shots that show where you think the density is broken, indicating that the DNA is cut.

Question 10: Where (in between which bases) is the cut? Write down the sequence of the product DNA

strands. Are the product DNA strands having a 3’ or 5’ phosphate after the reaction?

Compare with the mutant structure

Now let’s have a look at the mutant.pdb structure. I-DmoI is an enzyme of a thermostable archaea and its optimal temperature for cleavage is 70°C, having almost no activity at 37°C (human cell temperature). Therefore, the wt protein could not be used for human gene repair. Mutated versions of the protein were generated in a random manner and tested for cleavage activity at 37°C in a cell-based assay. The structure was solved of the mutant with best activity at 37°C. A data set was collected to 2.75˚A resolution, also in the presence of Mn^{2+} ions and the same DNA duplex. As before, we have used the wt-Ca^{2+} complex structure as model for the Molecular Replacement job. As before, we have deleted all water molecules and all ions.

Follow the steps above: load the mutant.pdb structure and create the maps using the mutant.mtz file.

Question 11: Go to the complex made of chains A, B and C. Set up the contour level of the $2F_o$-F_c map to 1. Locate the two mutations I52F, L95Q. Looking into the commands at the right-hand side of the screen find ”Mutate and autofit”. Make the corresponding mutations ONLY for chain A and save the coordinate file.

Go to the active site of Chain A. Using similar settings for the contour levels of the maps as before (2.9 and 10, for the $2F_o$-F_c and F_o-F_c maps, respectively):

Question 12: How many Mn^{2+} ions are there in the active site of chain A?

Question 13: Place the Mn^{2+} ions and water molecules in the active site of chain A and save the coordinate file.

Question 14: Is the DNA cut?

Note 1: Since the structures of wt I-DmoI and mutant-I-DmoI are essen- tially the same, studying the proteins by x-ray crystallography was not enough to explain why the mutant had activity at 37°C. Molecular Dy- namic Simulation experiments are being performed to better understand the cleavage mechanism and how the mutations might affect the dynamics of catalysis.

Note 2: For all the I-DmoI variants, when crystallized in the presence of Mn^{2+} ions, many crystals were obtained. However, in some crystals the DNA was cut while in others it was not. Perhaps longer incubation times mixing the protein and DNA before setting up the crystallization experiment would have yield more crystals with the DNA cut.