

BMB/Bi/Ch 173 – Winter 2018

Homework Set 6.2 – Assigned 2-15-18, due 2-20-18 by 10:30 a.m

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Office hours – Friday Feb 16 3:00pm - 5:00pm (in SFL 220) and Monday Feb 19 4:00pm - 5:30pm (in SFL 229), or by appointment

In this problem set, you will use Coot to fit a protein segment to an electron density map. Since we had an in class tutorial for installing coot, I expect that most of you have coot properly installed. In case there are any issues please refer to the instructions at the end of this problem set, and if that does not help do not hesitate to email with questions.

Navigating in Coot

Detailed instructions on using Coot are available at:

<https://www2.mrc-lmb.cam.ac.uk/Personal/pemsley/coot/web/docs/coot.html>

Once a PDB file has been opened, the most common navigation methods are:

Left-mouse drag	Rotate view
Ctrl + left-mouse	Translate view
Shift + left-mouse	Label atom
Right-mouse drag	Zoom in and out
Ctrl + shift + right-mouse drag	Rotate view around screen
Middle-mouse	Center on atom
Scroll wheel	Increase and decrease map contour level

Coot can directly download coordinates and electron density maps from the PDB (Note: If this does not work for you, please just proceed to the problem set.) View the X-ray crystal structure of GFP to familiarize yourself with the controls:

File > Get PDB & Map using EDS.

Enter 1GFL and click 'Get it'. Wait a few seconds for it to download. You will be presented with coordinates and two maps, a $2|F_o| - |F_c|$ and a $|F_o| - |F_c|$ map. Hide the $|F_o| - |F_c|$ map.

Draw > Display Manager.

Uncheck the display box for the second map, labeled FOF CWT. Try zooming in and out, centering on various atoms, and changing the contour level of the map. The contour level sets the minimum electron density that must be present at an xyz point for the gridded map to be drawn around it. The sigma value indicated in the top right corner as you change contour level is signal over noise, so be careful interpreting maps with sigma less than 1.

Model building

Before proceeding, delete the GFP map and coordinates:

File > Close Molecule/Map.

Select everything and click 'Delete Marked Molecules & Maps'.

Download the electron density map.

You are given a 2.0 Å resolution electron density map and a structure that is partially built into the map (Download BMB173_2018_PS6.2.zip from <http://saf.bio.caltech.edu/bmb173/>. Extract the files somewhere on your computer to use for this problem set). It was generated using experimental intensities and calculated phases from a refined model.

File > Open Coordinates.

Select coordinates.pdb and click OK.

File > Open Map.

Select map.map and click OK.

Edit > Map Parameters.

Increasing the map radius to 20 Å is recommended, although this may cause the program to lag on some computers, in which case you may not want to increase the radius as much.

The first thing you will probably want to do upon viewing the map is to adjust the contour level to an appropriate level. As you move the scroll wheel up or down (avoid doing this quickly) you will see a value displayed at the top of the screen that is changing which represents the contour. A good value will be around $1.3753 \text{ e}/\text{Å}^3$ or 15.03 rmsd.

Zoom in and out of the fragment to familiarize yourself with its shape. This region is a 21-residue sequence of an α -helix and β -strand that is located within the following sequence: MTLGLDLKGGRLRIMAAGDVFRAAASNELQIWAARTGCEIVVIRQPNDQYQF. Starting from this idealized polyaniline helix, you will need to build the structure of the rest of the peptide. First, we will mutate the residues of the helix so that they match the actual sequence. To do this we must identify the side-chain density that most obviously matches a specific amino acid. Once we mutate the identified residue to this amino acid, we can infer the identity of neighboring amino acids based on the sequence provided above. To make the initial mutation do the following:

Calculate > Model/Fit/Refine.

This will bring up a tool box in a new window. When using an interface in a separate window keep in mind that the window will sometimes disappear behind the main coat window, and also that you will often need to click things multiple times because a first click is needed to highlight the window you are clicking on.

Select Mutate & Autofit

Click on a residue and select the new amino acid from the list that appears. After you do this for the first residue based on the appearance of the density, you should be able to infer identity of every amino acid in the helix. As you go through and mutate all of these, you will need to refine the placement of the amino acids with the following commands:

Select Real Space Refine Zone

Click on a residue and then a second residue further down the chain (it's usually best to select a range of 4-5 residues). Check the presented model and make any changes if necessary by clicking on and pulling the white model. Click OK when you are done. Real space refinement helps find the best possible conformation of the selection for fitting into the electron density, but often results in unfavorable conformations. It is usually best to fix the conformation afterwards:

Select Regularize Zone

Click on a residue and then a second residue further down the chain (it's usually best to select a range of 4-5 residues). Check the presented model and make any changes if necessary by clicking on and pulling the white model. Click OK when you are done. Regularize zone helps find the most favorable conformation of the selection, but may not fit the electron density perfectly.

After mutating all of the residues in the helix to match the sequence, we need to add all of the additional residues in the sequence to the N- and C-terminus of the helix we have in place:

Select Add Terminal Residue

Click the residue that you want to build from (lysine for the first case). A white alanine residue will appear in the window. You can position the residue by clicking and dragging, and when you are happy with its location hit OK in the pop-up window.

Using the above command you should be able to gradually build the complete structure by adding alanine residues to the N- and C-terminus, mutating them, and then refining their position.

Once you have finished building the model we will go over a few of the features in coot that we can use for confirming the validity of the model. To check your work, you can evaluate the geometry of your building by examining the Ramachandran plot:

Validate > Ramachandran Plot.

Select the structure which should be the only option in the sub-menu. Check to make sure your amino acids have phi and psi angles that fall in the preferred regions of the map. Amino acids that fall in the allowed regions are acceptable, but outliers should be fixed. The map will expand to allow more phi and psi angles if the cursor is pointed at a glycine. Conversely, the map will contract to exclude certain phi and psi angles if a proline is selected.

Coot also features tools that evaluate how well the model fits the density:

Validate > Density Fit Analysis.

The resulting graph will plot the errors in density fit. Manipulate your structure to minimize errors in the density fit.

You will be evaluated on the completeness and correctness of your model, the Ramachandran plot, and the Density Fit Analysis (these graphs can be generated using only your submitted pdb file and the density map, so there is no need to turn these in). When you are finished with your structure, go to File >> Save Coordinates, click on Select Filename, and save your model as firstnamelastname.pdb (I would be taylorstevens.pdb). Email me your coordinate file (tstevens@caltech.edu).

In addition to giving an introduction to coot, which is a very common tool for building structural models in both crystallography and cryo-EM, the goal of this problem set is to demonstrate how tricky model-building can be for data-sets around the very common resolution of 3 Å.

Installation

Mac (10.6 or higher):

First, you will need to download and install XQuartz (unless you are working on OS 10.7 or lower) at the following link: <https://www.xquartz.org/>. Next, go to <http://www.ccp4.ac.uk/download/index.php#os=mac> and download the CCP4 program suite v7.0 (NOT the SHELX version). Once you have downloaded the package you will need to source the setup script in your .bash_profile. To do this you will need to first find out the full file path of the folder where CCP4 has been installed (make sure it's the directory which contains /lib, /bin and so on, there may be a folder called ccp4 with another folder called ccp4{version-number} in it which is the one you should use; I'll refer to this as \$CCP4_HOME) and then add the following line of text to the bottom of the file called .bash_profile in your home directory (can be accessed as ~/.bash_profile):

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source $CCP4_HOME/bin/ccp4.setup-sh
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After you do this, you can close out of terminal, reopen it, and then you should be able to simply type 'coot' into the command line and hit enter to launch coot.

Linux:

If you happen to be running a linux, you probably have already figured this out.... otherwise send me an email with your distro and I'll point you to the right version (if there is one).

Windows:

Go to <http://www.ccp4.ac.uk/download/index.php#os=windows> and download the standalone coot package at the bottom of the three available installation options.