Refmac tutorial

Download tutorial file from the website:

www.ysbl.york.ac.uk/refmac/refmac_tutorial.tar.gz

Find the file (It should be in the Download directory). Create a subdirectory where you usually work and copy tutorial file to this directory.
mkdir refmac
cd refmac
mv whereever_tutorial_file/refmac_tutorial.tar.gz .

tar xzvf refmac_tutorial.tar.gz
cd refmac_tutorial

There are several files to play around. These files can only give you some idea about refinement in general and refmac5 in particular.
Start ccp4i. For each case you should create a project in ccp4i using "Directories&ProjectDir" on the top right of the interface. It will help you to trace back what you have done and find files you need. There are four subdirectories. Each subdirectory is for one tutorial. They are: **rnase** – a simple refinement, **1n5b** - tls refinement, **twin** – twin refinement, **sad** – sad refinement.

I) Simple refinement.

On the top of the ccp4i select "Refinement". Click "Run Refmac5". It will bring up refmac5 interface. Define input mtz and coordinate files. They are rnase115_unique1.mtz and rnase.pdb. Press Run/Run now on the left bottom side.

Current version of the program uses automatic weighting. For many cases it works sufficiently well. Sometimes (especially at high resolution) default weighting may give a relaxed geometry. In this case you can play with the weighting of X-ray and geometry terms. If you want to change weights then click "Refinement Parameters", under this unclick "Use automatic weighting" and add an appropriate number into the field "Use weighting term". For low resolution you may want to use very small values - 0.01 or even smaller. For higher resolution this number may need to be as high as 10. You may need to run refinement with different values to get it right. If after refinement run rms bond distances are more than 0.02 then you may want to reduce weighting, if rms bond value is less than 0.01 then you may need to increase. Smaller weighting value means tighter geometry.
Now click on the "run" button. Program should run.
While it is running you can have a look at the log file. When refmac finishes this job you should use coot (or another proram) to view coordinates, maps and make corrections if you need to.
coot –pdb rnase_refmac1.pdb –data rnase115_refmac1.mtz

and analyse the electron density.

II) TLS refinement

This example of TLS refinement in Refmac5 uses 1n5b, a molecular chaperone.
The asymmetric unit contains 2 dimers, chains A + B and chains C + D.
Data is in P212121 to 2.0A

To run Refmac5, we need to specify what TLS groups we wish to use. CCP4 wiki site has a page about different ways of creating TLS groups.
To create TLS group using ccp4i:

1. Go to ccp4i -> Refinement -> Model preparation -> Create/Edit TLS file.
3. Give "TLS out" a name such as 1n5b_in.tls
4. Define 4 groups (use "Add another TLS group" to create additional entries):
   Chain A, residues 3 to 130
   Chain B, residues 3 to 130
   Chain C, residues 3 to 129
   Chain D, residues 3 to 130
5. Click "Run now" to create the file.

We are now ready to do TLS refinement

6. Select the task Run Refmac5
7. Select mode "TLS & restrained refinement" from the protocol folder (top left button after the “Job title”.
8. Set the files:
   HKLIN 1n5b.mtz
   XYZIN 1n5b.pdb
   TLSIN 1n5b_in.tls (from above)

Note 1: If you do not give input tls file then each chain will be taken as a tls group.
Note 2: Waters close to tls groups will be added to those groups (it is a new feature and available from ccp4 6.0.99c onwards)
Note 2: If you do not give tls file then refmac will choose each chain as single tls group. So you can start from step 6 and in step 8 do may not define TLSIN (Select mode “TLS & restrained refinement”). I.e. you do not have define file with tls groups.

9. In the folder "TLS parameters" select "Set initial Bfactors"
10. For a quick run, use 5 TLS cycles and 5 restrained cycles

When Refmac5 has finished, check the logfile for the usual things: Rfree, geometry, warning messages. TLS section shows the raw TLS parameters at each cycle. These don't mean much on their own, but you can see if the TLS refinement is converging. Now we need to analyse the TLS parameters. It is available from ccp4 6.1.1 onwards.

11. In the “Model Completion & Analysis” section select "Analyse TLS parameters" task.
12. Set the files:

TLSIN is TLSOUT from previous Refmac job
XYZIN is XYZOUT from previous Refmac job

13. Click “Output file containing the axis ..” and give the AXES file a name 1n5b_tls.vector
14. In the Axes folder, select mmCIF format
15. In Other Options, select "Analyse derived atomic ...."

Check the log file for "NON-POSITIVE DEFINITE WARNINGS" (I think there will be none!)
Output coordinates contain ANISOU records derived from the TLS plus residual B factors. Use coot to view them. Axis can be viewed using ccp4mg (I think?)

III) Twin

Run refmac5 as usual. Click Twin refinement button on the interface. It will activate twin refinement.

In some versions of ccp4i there was a bug and twin refinement was not activated. To check if it is activated check the log file. If it has TWIN keyword then everything is fine. If twin does not run automatically then use “Include keyword file”. Click on “Browse” and select file keyw.dat. This file contains a single keyword - TWIN. Adding this should activate twin refinement.

Keyword file could be used to add options that are not available on the current interface.

Note that in this case Rfactor and Rfree are diverging. It is because 1) This data set is from pdb and there was no twin flag there 2) Twin relates two (or more) set of reflections. Usual free R selection does not take this fact into account. Refmac5 puts twin related reflections to the same set (i.e. either all related reflections belong to “free” or to “working” set). In general free reflection selection should be done before starting refinement accounting for potential twin operators. One way of achieving this is to select free reflections at higher group and expand them. It will ensure that throughout refinement and all other treatment of data all related reflections will belong to the same set.

Look at the maps.

IV) SAD

Refinement against F+ and F- directly (SAD refinement).

Start refmac5 as usual.On the top right side of the interface choose “SAD data directly” instead of “no prior phase information”. Type in the “Anomalous atom” field SE, f’ field -8.0 and f’’field 4.0. You can also use wavelength. Then all atoms that have significant f’’ will be used in anomalous refinement.

After refinement finished you should look at the maps. There are coefficients for anomolous difference map also (corresponding mtz labels are FAN and PHAN for amplitudes and phases of anomalous difference map).

I) Dictionary

If you have new ligand then find out about its chemistry (mainly valence bond properties). It is very important to understand ligand you are dealing with. First time it may take some time. But once you have done many times it becomes easier.

Select from ccp4i → Refinement → Restraint preparation → Monomer library sketcher

There are helpful hints on the top of the sketcher. On the right hand there are templates. Once you start sketching your molecule on the left hand of sketcher atom names and their charge will appear. You can change atom names, element names or atomic charges.

There are several ways of creating dictionary file:

1) From scratch. You can draw your ligand using sketcher and then: file→ Create library description. New window will appear. Give a reasonable name for your ligand and run.
2) Editing the existing dictionary file from distributed area: File→ Read File → Load monomer library. Choose one of them that is similar to your ligand and edit it. Then click run button.
3) Dictionary from existing coordinates: You should be very careful with this. Make sure that file you are working with is very reliable. Possible sources are: CSD, CORINA, PRODRG or your favourite energy minimiser (It is better to use quantum chemical minimisers but make sure that your minimisation was performed in a water or in an environment that is closer to the conditions where your ligand leaves).
   File → Read file → Read pdb
4) From a smile string. You can use either coot or sketcher to create a library file from a smile string. In sketcher under pull down menu "File -→ Read file → Read Smiles file" give smile file. It should create a dictionary file for you.

Other very good options to create dictionary files:
1) MSDchem: from the server http://www.ebi.ac.uk/msd/ choose MSDchem
2) PRODRG server:
http://davapc1.bioch.dundee.ac.uk/programs/prodrg/

Other useful websites for "ideal" coordinate generation:
3) DrugBank: http://www.drugbank.ca/ - This site has MarvinSketch as plug in
4) ZINC: http://zinc.docking.org/index.shtml

5) CACTVS: http://www2.chemie.uni-erlangen.de/software/cactvs/

Using JLigand. JLigand is an interface to create description of ligands and covalent links that can be used in refinement. The program can be downloaded from:
http://www.ysbl.york.ac.uk/~pyoung/JLigand/JLigand.html
There is some documentation there also.

How to deal with covalently linked ligands:

1) Create dictionary for ligand if there is not one. It can be done either using monomer sketcher or prodrg server or MSDchem
2) Create library description for links. At the moment it is not automatic. See example: dict/test4.cif
3) Add link record into your pdb before CRYST card. See example: dict/1.pdb
4) Run refinement and provide dictionary with monomer and link descriptions (in this case test4.cif)
5) Analyse pdb and map using coot
6) Or just use JLigand. It will help you to create link description.