Tutorial

I - MOLECULAR REPLACEMENT PHASING

Resolution of the structure of hen lysozyme from diffraction of Hen lysozyme crystal and a coordinates file of Bovine lysozyme :

Hen lysozyme sequence :

```
>sp|P00698|LYSC_CHICK Lysozyme C OS=Gallus gallus GN=LYZ PE=1 SV=1
MRSLLILVLCFLPLAALGKVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQA
TNRNTDGSTDYGILQINSRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCAKKIVSDG
NGMNAWVAWRNRCKGTDVQAWIRGCRL
```

Bovine lysozyme sequence:

```
>sp|P04421|LYSC_BOVIN Lysozyme C OS=Bos taurus GN=LYZ1 PE=1 SV=2
MKALVILGFLFLSVAVQGKVFERCELARTLKKLGLDGYKGVSLANWLCLTKWESSYNTKA
TNYNPSSESTDYGIFQINSKWWCNDGKTPNAVDGCHVSCRELMENDIAKAVACAKHIVSE
QGITAWVAWKSHCRDHDVSSYVEGCTL
```

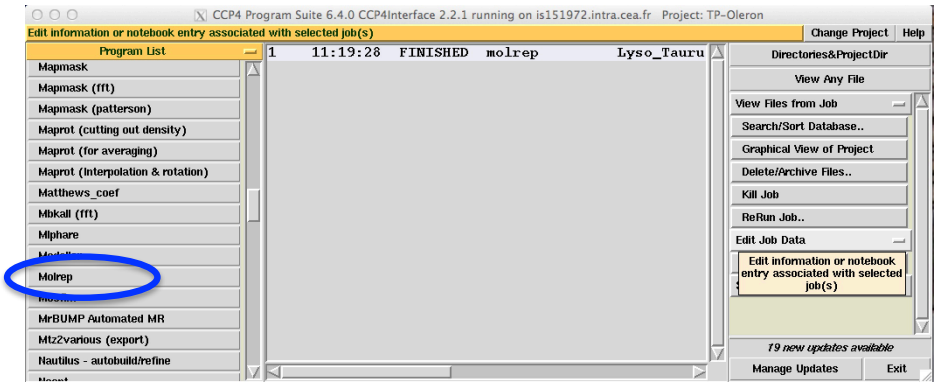
Sequence associated to PDB coordinates file of Bovine lysozyme:

```
>2Z2F:A|PDBID|CHAIN|SEQUENCE
KVFERCELARTLKKLGLDGYKGVSLANWLCLTKWESSYNTKA
TNYNPSSESTDYGIFQINSKWWCNDGKTPNAVDGCHVSCSELMENDIAKAVACAKHIVSE
QGITAWVAWKSHCRDHDVSSYVEGCTL
```

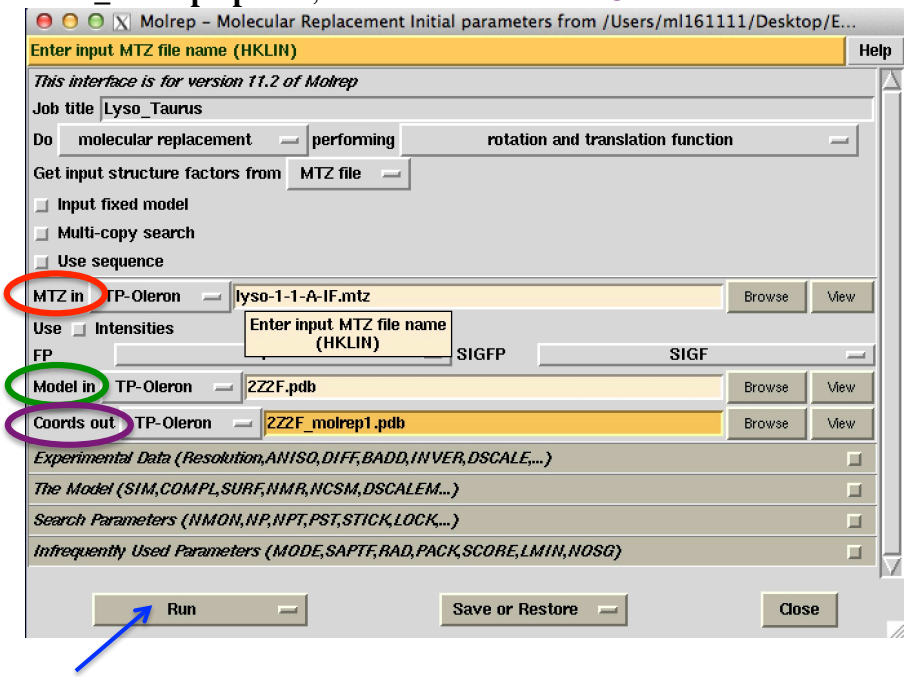
Hen and bovine lysozymes sequence alignment :

```
>2z2f_A Lysozyme C-2; stomach lysozyme, 1,4-beta-N-acetylmuramidase C, bacteriolytic
enzyme, hydrolase; 1.50A {Bos taurus} SCOP: d.2.1.2
Length = 129    Score =  154 bits (389), Expect = 1e-38,    Method: Composition-based
stats.  Identities = 71/130 (54%), Positives = 96/130 (73%), Gaps = 2/130 (1%)

Query: 19  KVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDG-STDYGILQIN 77
           KVF RCELA  +K+ GLD Y+G SL NW+C  K+ES++NT+ATN N    STDYGI QIN
Sbjct: 1   KVFERCELARTLKKLGLDGYKGVSLANWLCLTKWESSYNTKATNYNPSSESTDYGIFQIN 60

Query: 78  SRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCAKKIVSDGNGMNAWVAWRNRCKGTD 137
           S+WWCNDG+TP + + C++ CS L+ +DI  +V CAK IVS+  G+ AWVAW++ C+  D
Sbjct: 61  SKWWCNDGKTPNAVDGCHVSCSELMENDIAKAVACAKHIVSE-QGITAWVAWKSHCRDHD 119

Query: 138 VQAWIRGCRL 147
           V +++ GC L
Sbjct: 120 VSSYVEGCTL 129
```

You will now calculate the phase of the observed amplitudes Fobs(hkl), by the molecular replacement method using the software **MOLREP** from CCP4i package.
The interface of the software opens by clicking on **Molrep** :

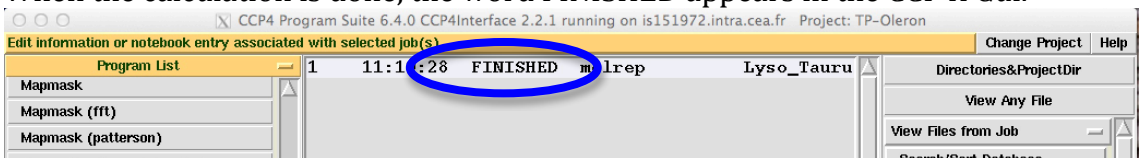


The amplitudes and their associated errors will be extracted from the file « lyso-1-1-A-IF.mtz » that you must enter on line « MTZ in ». The phase information will be calculated from the PDB file "**2Z2F.pdb** ", line « Model In ». The « solution » PDB file will be named **« 2Z2F_molrep1.pdb »**, as indicated on line « Coords out ».

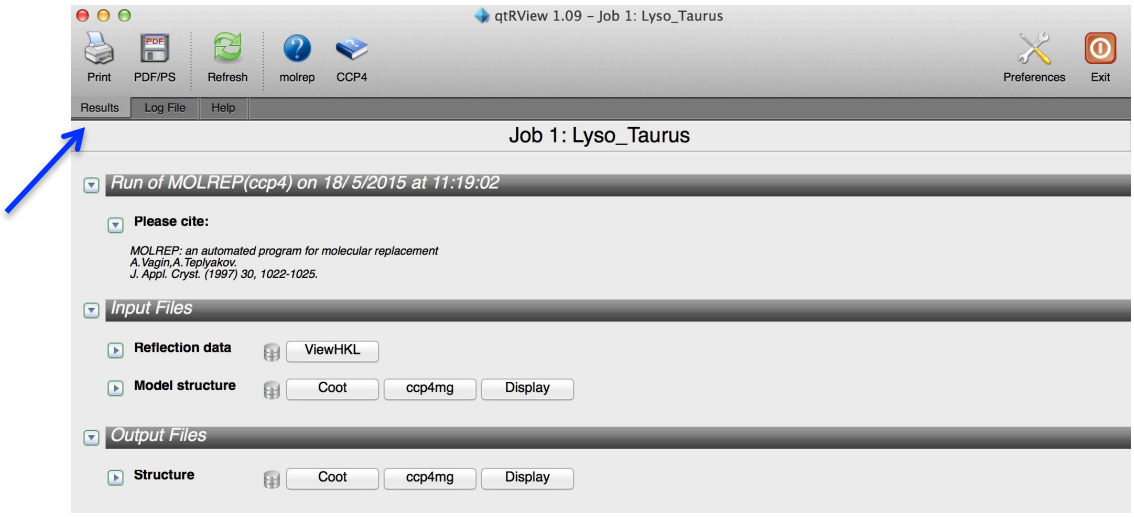


Press Run to start the calculation.

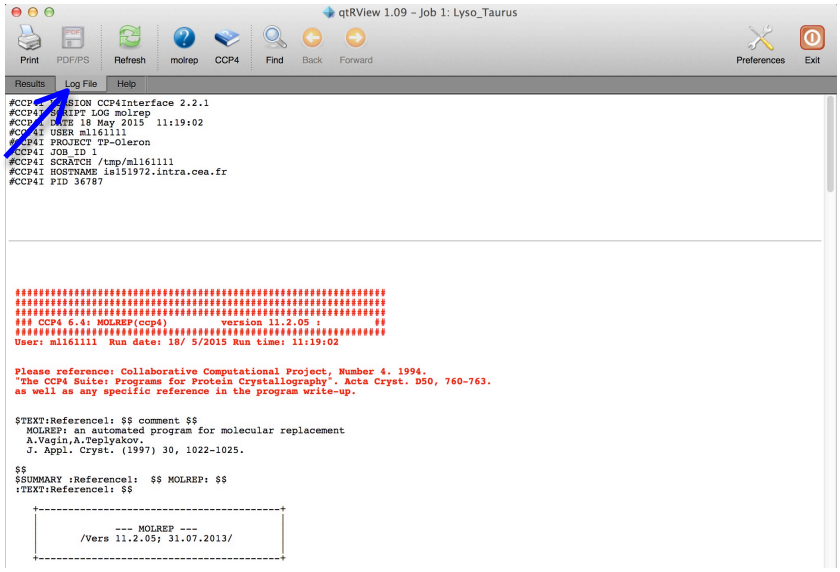
When the calculation is done, the word FINISHED appears in the CCP4i Gui.



You can open the output file by double click on the job line
Two tabs are going to allow you to analyze the results of your calculation :



The Results section allows you to open the input and output with COOT, ccp4mg or Display.



The Log file contains the information regarding the calculation itself.

Questions

1. Which parameters will allow to determine the correctness of **MOLREP** solution ? / *Quels paramètres vont permettre de déterminer si la solution trouvée par **MOLREP** est correcte ?*
2. What is the reliability factor Rfac of the solution found by MOLREP ? / *Quel est le facteur de confiance Rfact de la solution trouvée par **MOLREP** ?*
3. What contrast has the solution found by MOLREP ? / *Quel contraste a la solution trouvée par **MOLREP** ?*
4. Open the output file structure with **COOT**, and visualize the solution. What can tell you that the solution is probably correct (or wrong) ? *Ouvrez le fichier de sortie avec **COOT** et visualisez la solution. Qu'est-ce qui peut vous indiquer que la solution est coorecte (ou fausse) ?*

II - REFINEMENT OF MOLECULAR REPLACEMENT SOLUTION WITH REFMAC

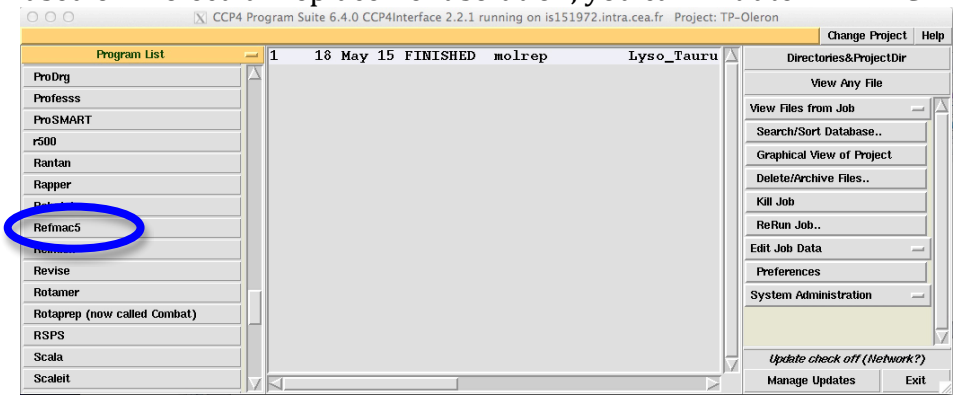
1) Before running the refinement with Refmac ...
Check that a **free Rfactor flag** (FreeRflag) has been set in your mtz data file.
If you have followed the XDS data processing tutorial, the FreeRflag has already been added to the mtz file (loot at the uniquemtz.out log file).
If you have used imosflm, you should add the FreeRflag as follow:
- Check whether the FreeRflag is present in the mtz file:
type in the terminal window:
mtzdump hklin lyso-1-1-A-IF.mtz
go

if FreeRflag is absent, type:
freerflag hklin lyso-1-1-A-IF.mtz hklout lyso-1-1-A-IFfree.mtz << eof
freerfrac 0.05
end
eof

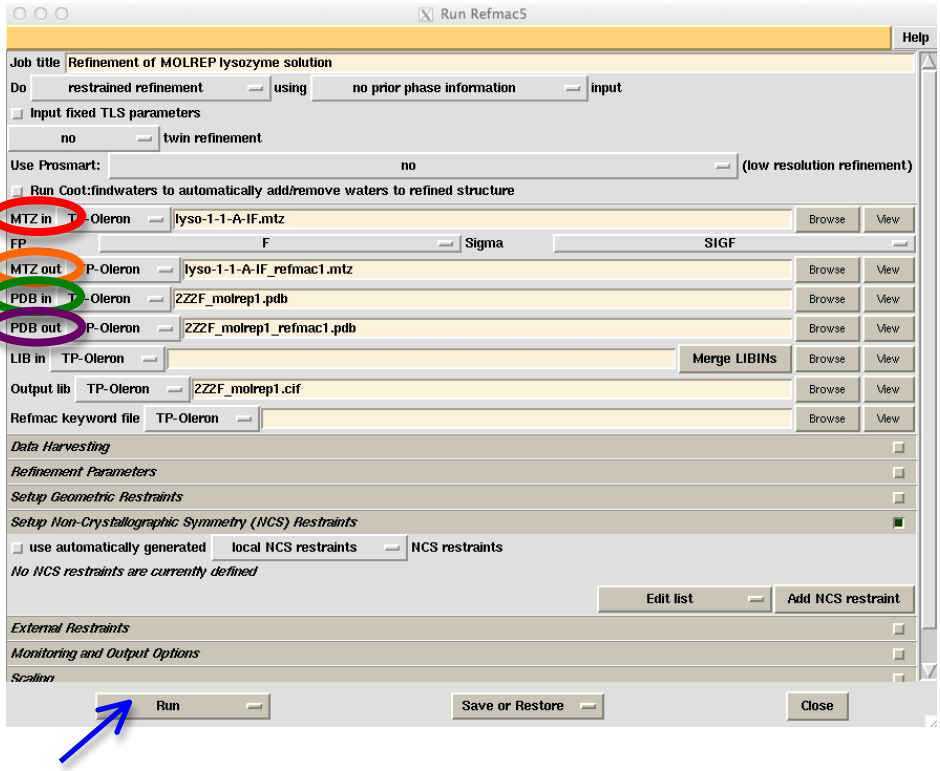
and check again:
mtzdump hklin lyso-1-1-A-IFfree.mtz
go

if the FreeRflag is there now, lyso-1-1-A-IFfree.mtz is the file to use for the next steps.

2) Refinement with Refmac
Based on molecular replacement solution, you can initiate REFMAC :

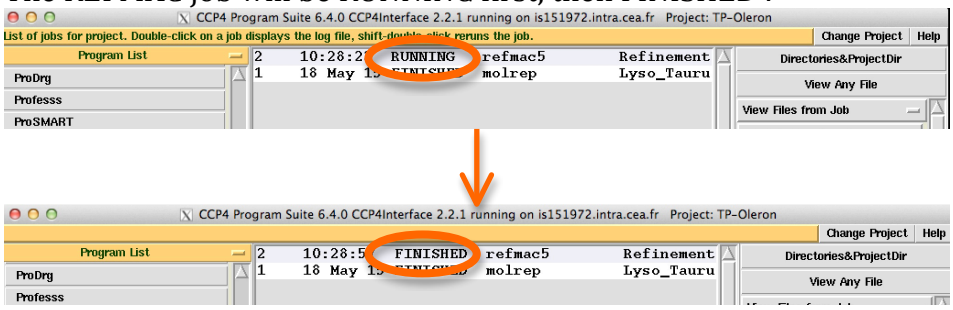


As previously, the amplitudes and their associated errors will be extracted from the file « lyso-1-1-A-IF.mtz » that you must enter on line « MTZ in ». The phase information will be directly calculated from the MOLREP solution PDB file "**2Z2F_molrep1.pdb** ", line « Model In ». After refinement, the refined model PDB file will be **« 2Z2F_molrep1_refmac1.pdb »**, as indicated on line « PDB out ». The amplitudes, and electron density map coefficient will be in the binary file **« lyso-1-1-A-IF_refmac1.mtz »**, as indicated on line MTZ out.

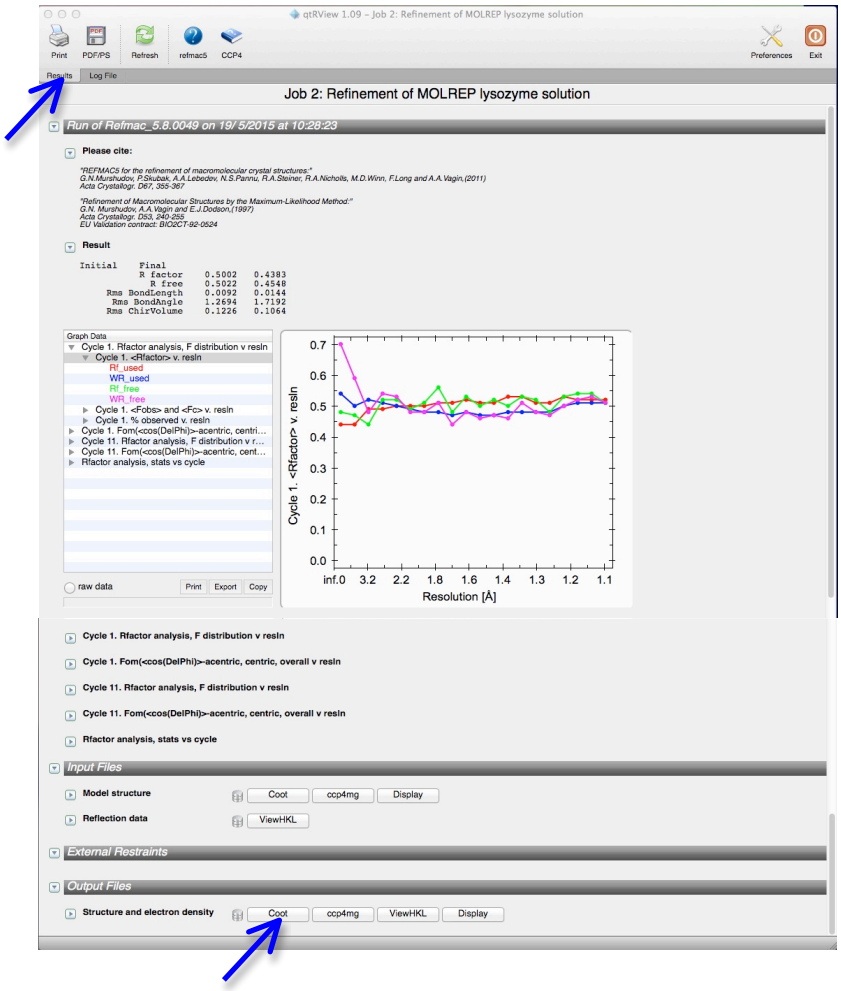


Press Run to start the calculation.

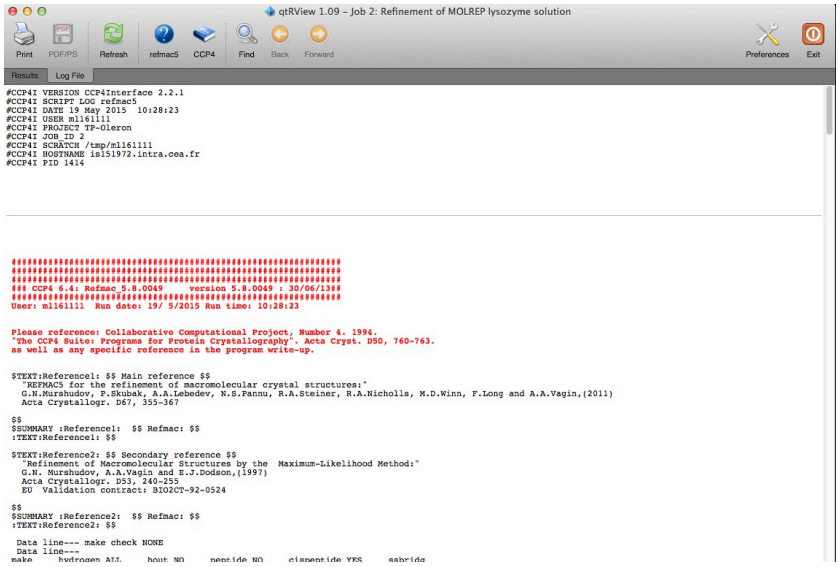
The REFMAC job will be RUNNING first, then FINISHED :



As previously, you can open the output file by double click on the job line. There is also one Results section and one Log File section in the output file.



Press Coot to open the output files (pdb and mtz files) in coot.



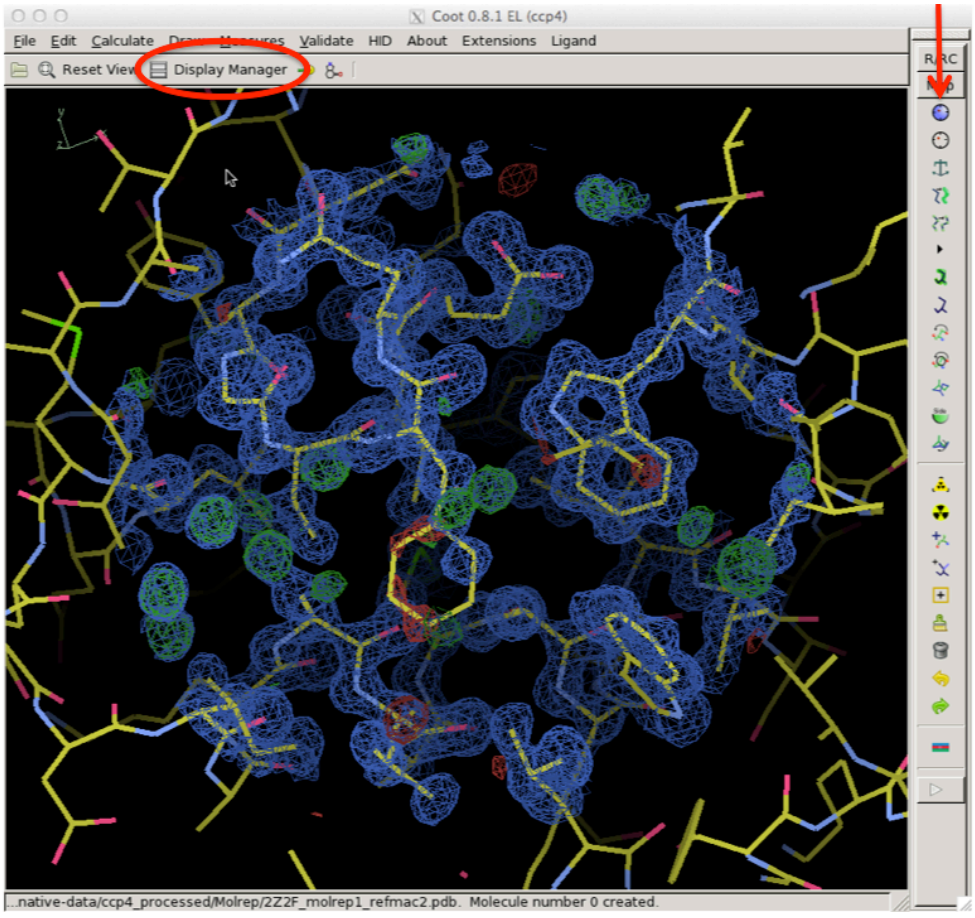
Questions

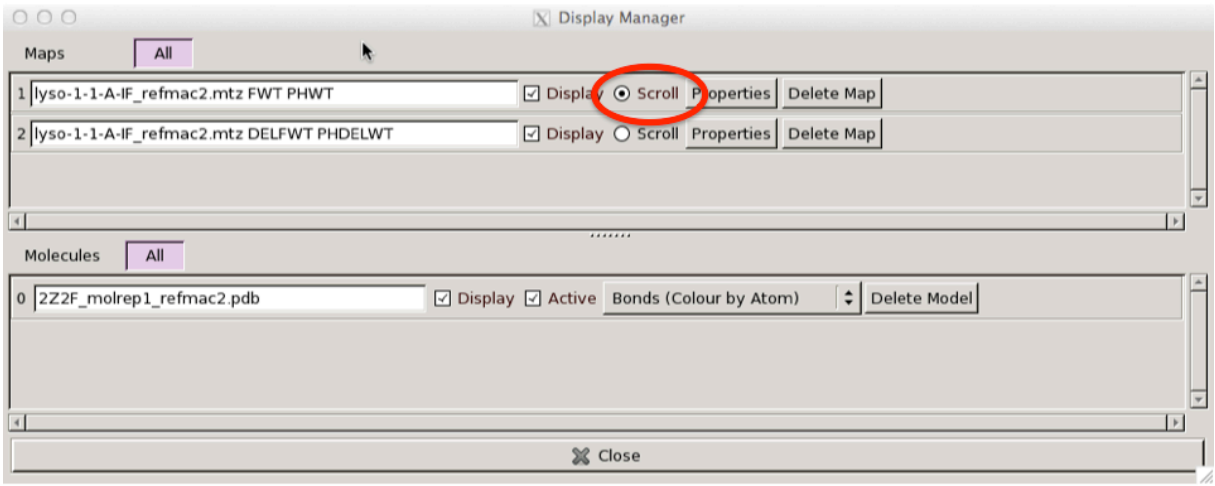
1. What parameters inform on the refinement quality?
2. What appears in the Coot window?
3. Based on the lysozyme sequences alignment, can you correct the refined structure in Coot?

3) Model building with Coot

The minimum to know about Coot:
Coot is fairly "intuitive": most of the necessary action can be selected from the icons on the right icon bar of the Coot window: just put the mouse arrow on the icon to know what it does.

Before to start model building
Check out the contouring level of electron density maps:
{2Fobs – Fcalc} maps (FWT, PHWT) should be contoured at 1 rms
{Fobs – Fcalc} residual maps (DELFWT, PHDELWT) should be contoured at +3 or -3 rms
Clic on "Display Manager" and select the scroll button corresponding to the desired map; the use the middle wheel of your mouse to increase or decrease the level.

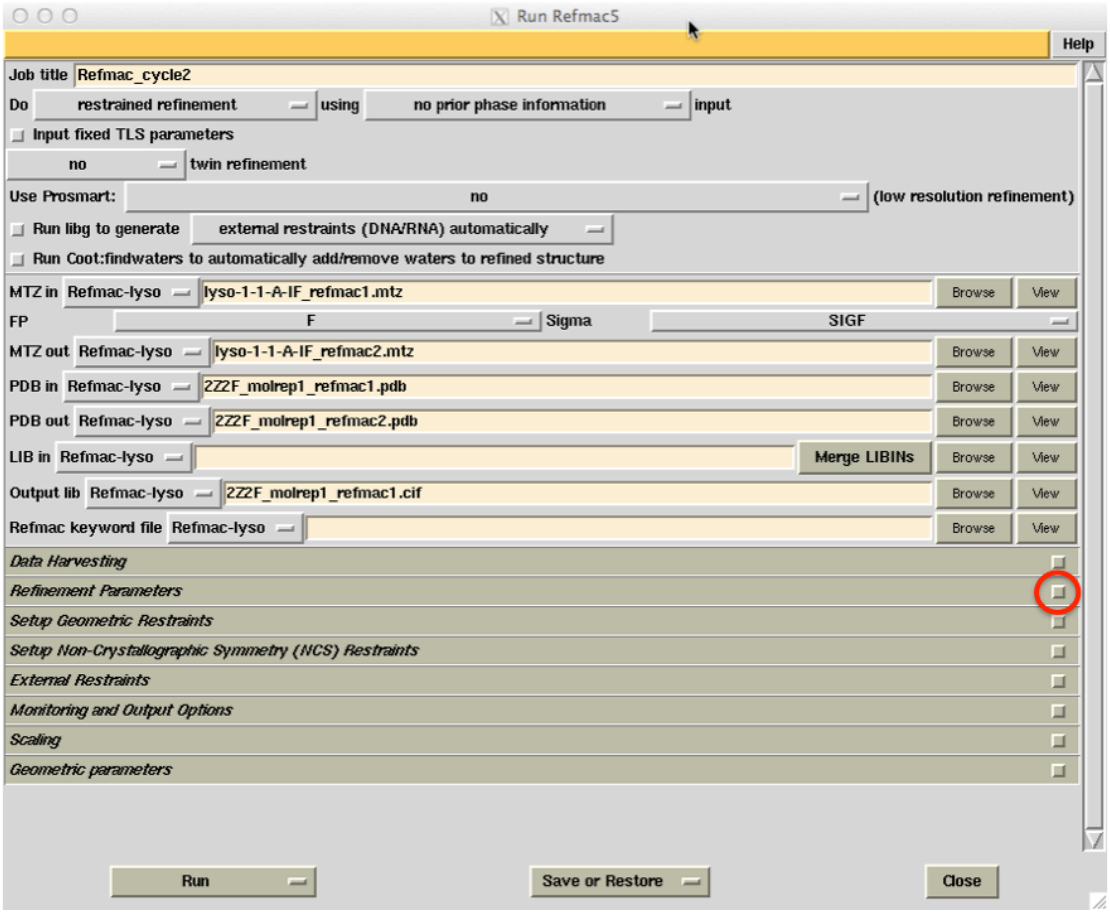


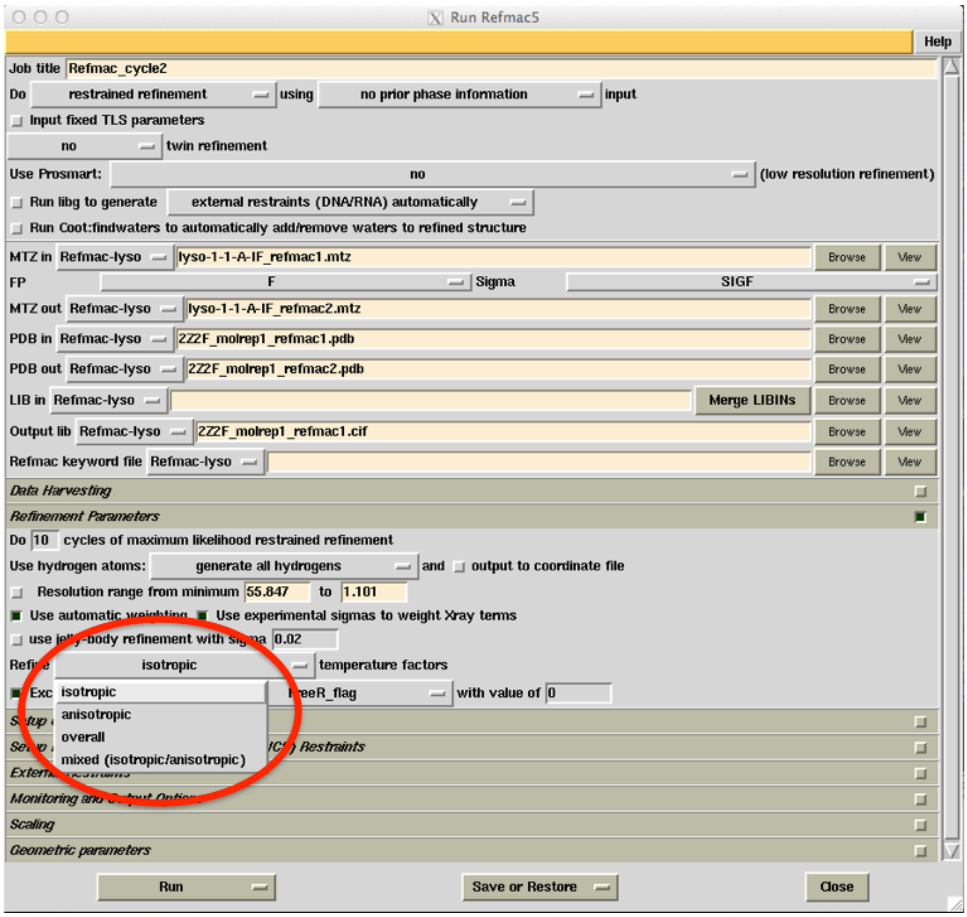


- Once you have made the desired structural change in coot, you can save the new coordinates of the model ("file" menu) and run refmac again.

- You do cycle coot and refmac steps until you have interpreted all the significant electron density, with a good fit between your model and the electron density.

At some point (but you might not reach it during the practical), you may need to look at the refinement parameters submenu in Refmac:





You may have to modify:

- the number of cycles: put more than 10 if your Rfactor is not stable after 10 cycles
- the B factor (temperature factor) : isotropic (default, one B factor per atom), overall (one B factor for the entire model, recommended for low resolution data, i.e. d_{max} > 3.2 Å), anisotropic B factor (6 parameters per atom, model the anisotropy of atom motion, useful for high resolution data, i.e. d_{max} < 1.3 Å)