MX Tutorial 2

Determination of the crystal structure of Hen Egg White Lysozyme by the Molecular Replacement method

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In this tutorial, the crystal structure of Hen Egg White Lysozyme will be determined by the Molecular Replacement method, using as phasing model the 3D structure of the related protein from *Bos taurus* (cow).

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Getting started

- Login to your account and start a shell terminal;

- Type the command:

  %cd ECS7/Lysozyme ↵

- Check that the tutorial files are present in this directory:

  % ls ↵

  2z2f-model.pdb – coordinates of the Lysozyme from cow

  alignment.aln – alignment between the sequence of both proteins (cow lysozyme on top)

  cu_Lyso_test1_1_0m.prp – logfile with the data processing statistics

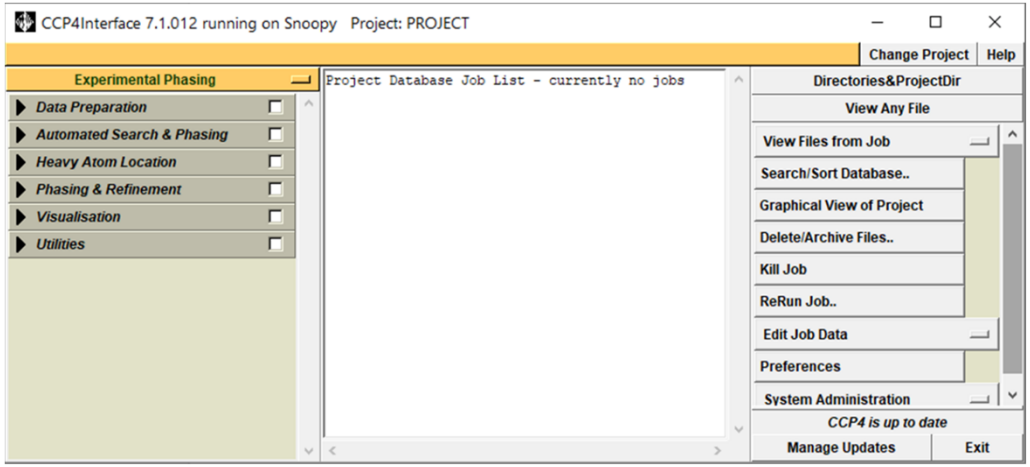
  cu_Lyso_test1_1_0m.sca – diffraction data

  Lysozyme.pir - aminoacid sequence in 1-letter format

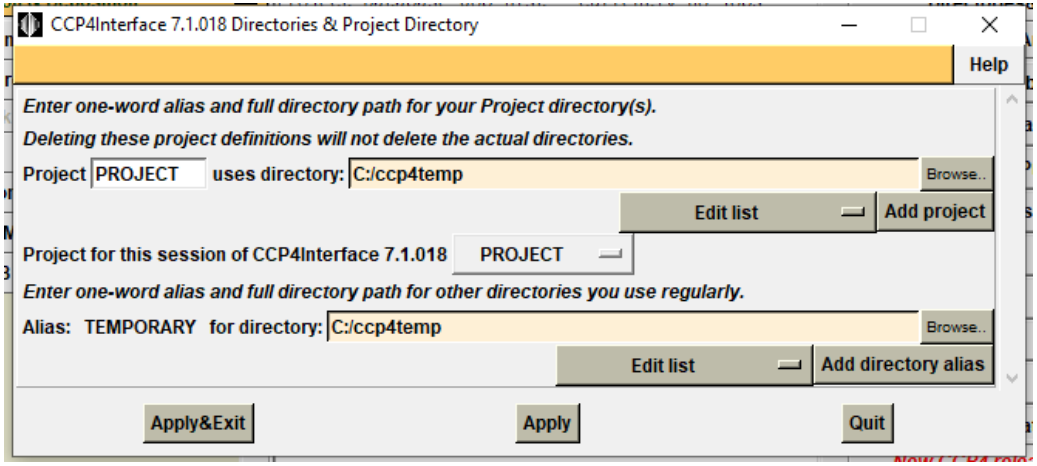
Start CCP4i and create a new project

- Start CCP4i

```
% ccp4i & ↵
```

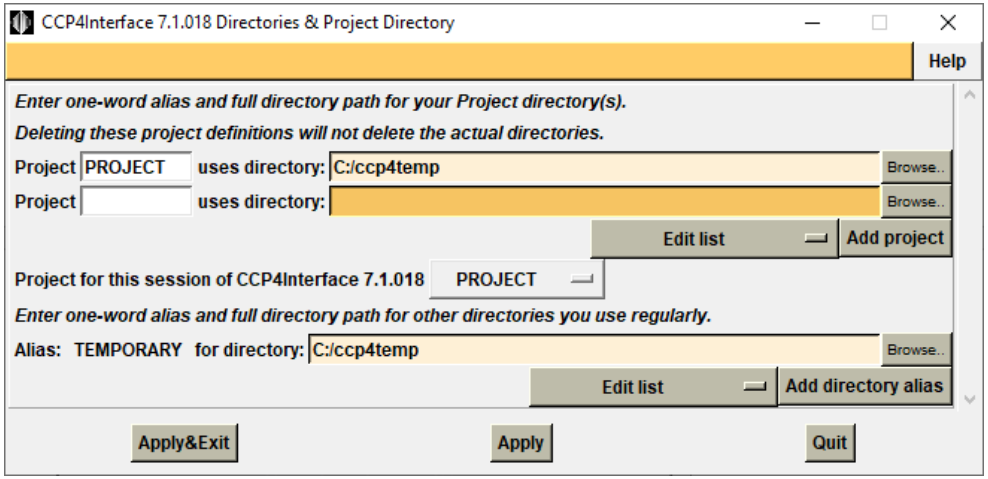


- Click on the **Directories&ProjectDir** button

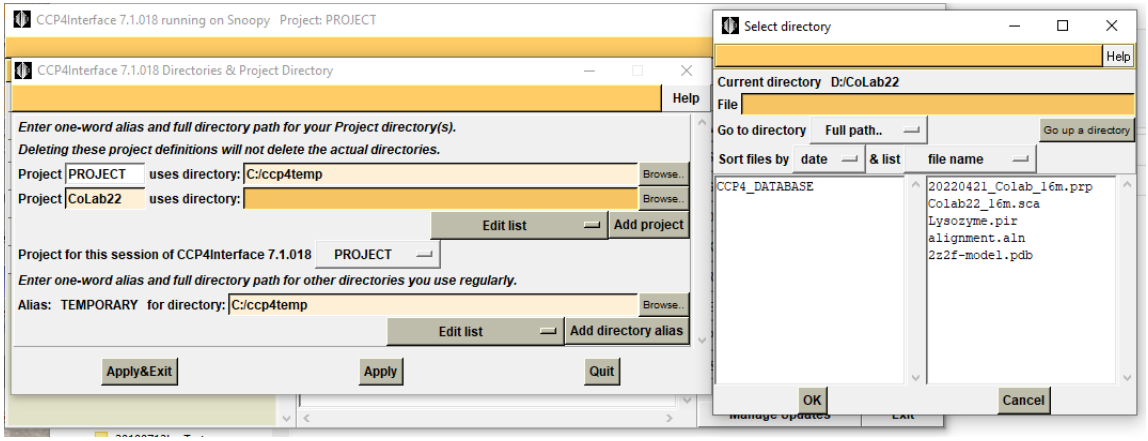


Start CCP4i and create a new project

- Click on the Add Project Button



- In the Project box you can type a word to name the project (e.g., ECS7). Click on the **Browse** button near the empty box and select **Full Path** as shown. You should see the files listed. Click **OK** to close the directory selection window. Then, click on the **Project for this session** button, select the name you gave to this project and click **Apply&Exit**



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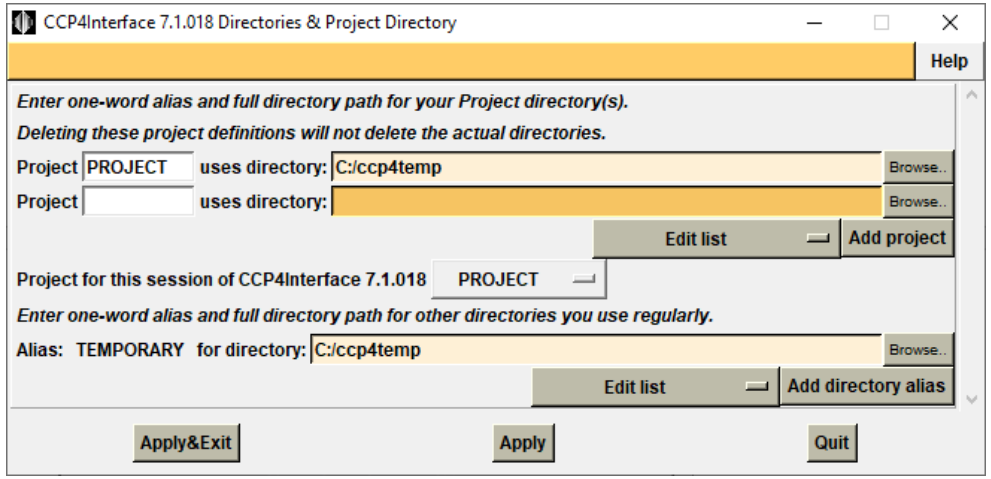

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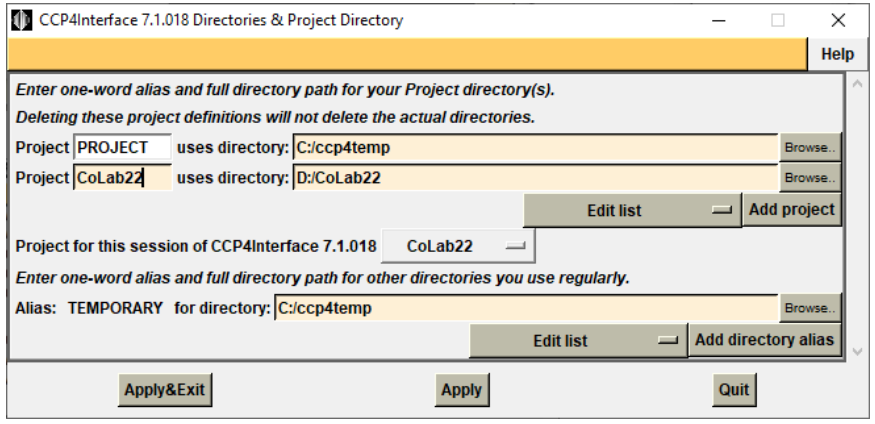
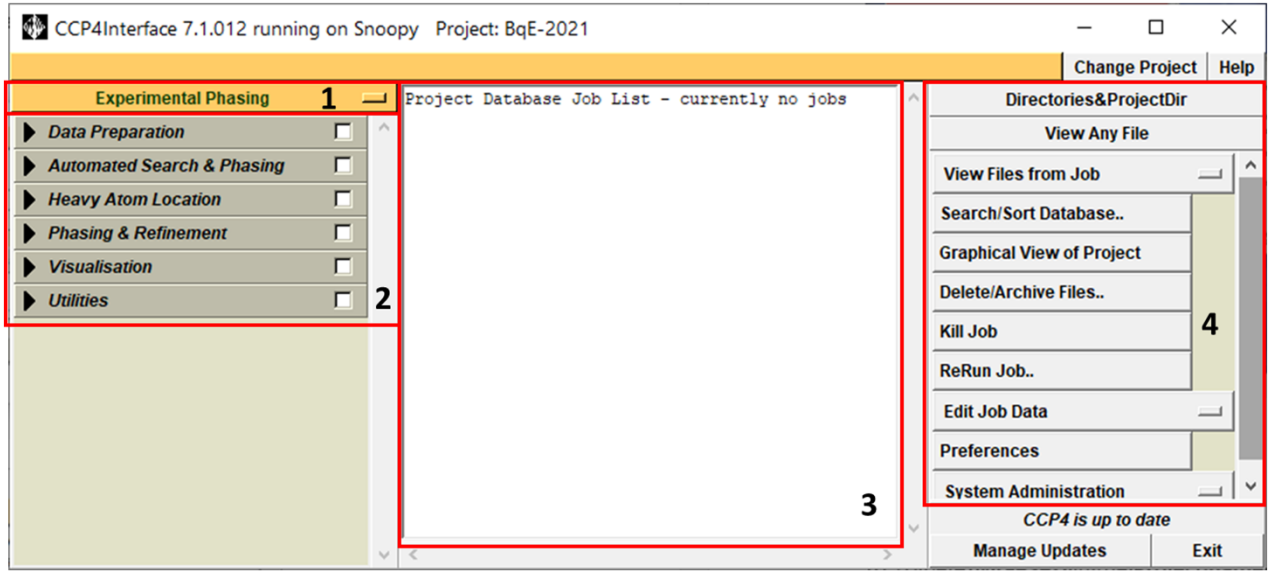


Overview of the CCP4i interface

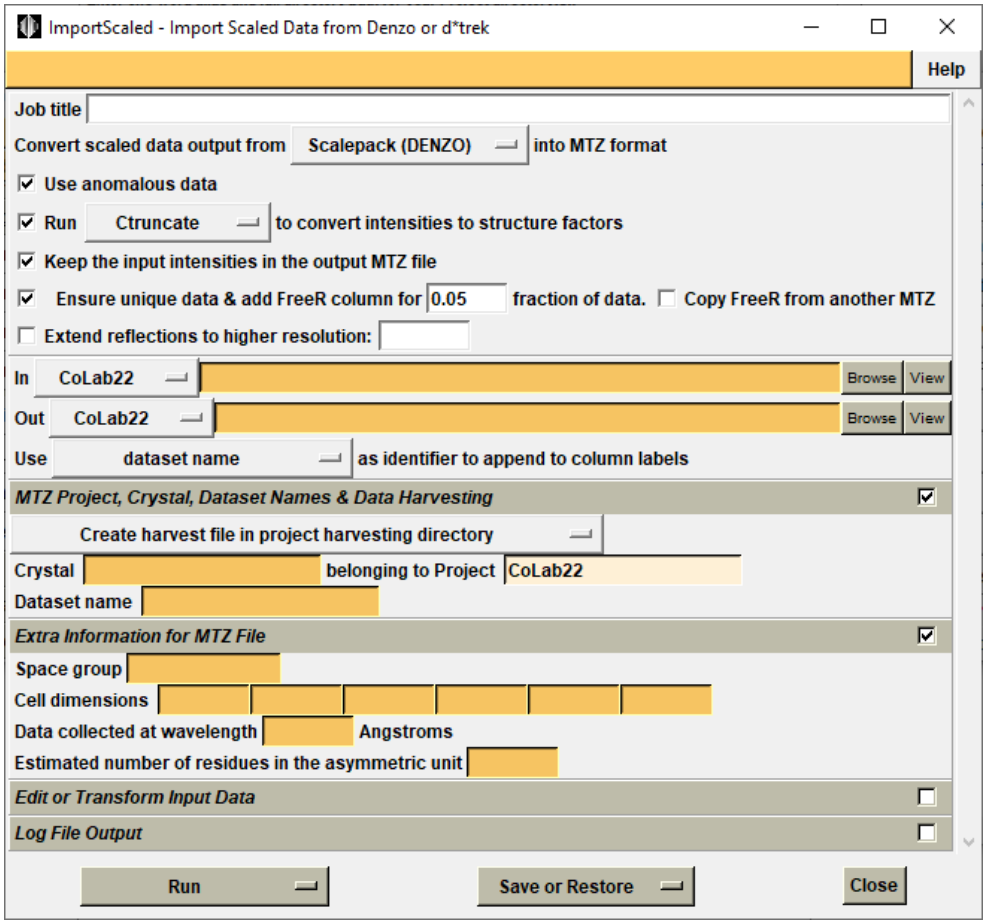
This is what the main window of the CCP4 GUI looks like:



1. *This button selects groups of programs;*
2. *Buttons in this window open a program list or select a program in the group;*
3. *This window lists all the run and running jobs in the current project;*
4. *This window contains buttons for acting on jobs and interface management;*

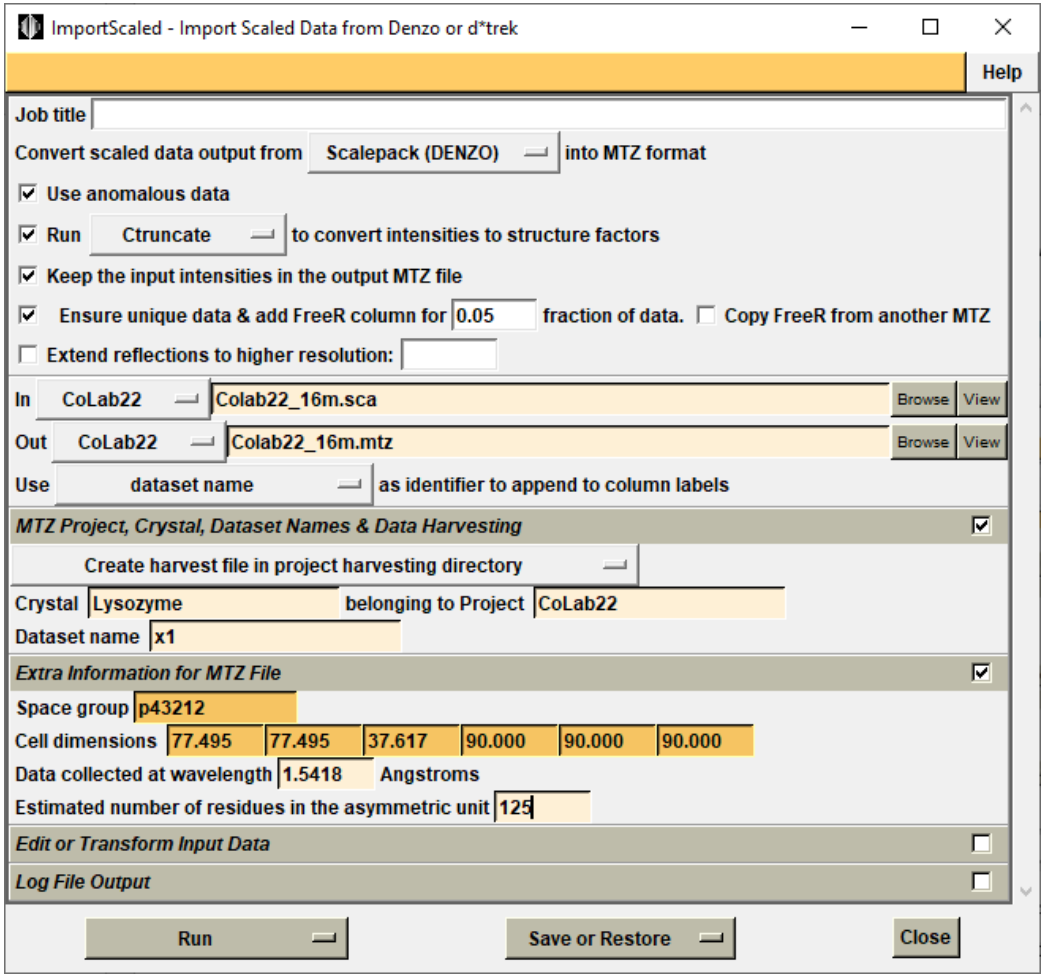
Importing the diffraction data into CCP4

Select the **Data Reduction and Analysis** group of programs in **1**, click on the **Import Integrated Data** subgroup and then select the **Import Merged Data** task:



Importing the diffraction data into CCP4

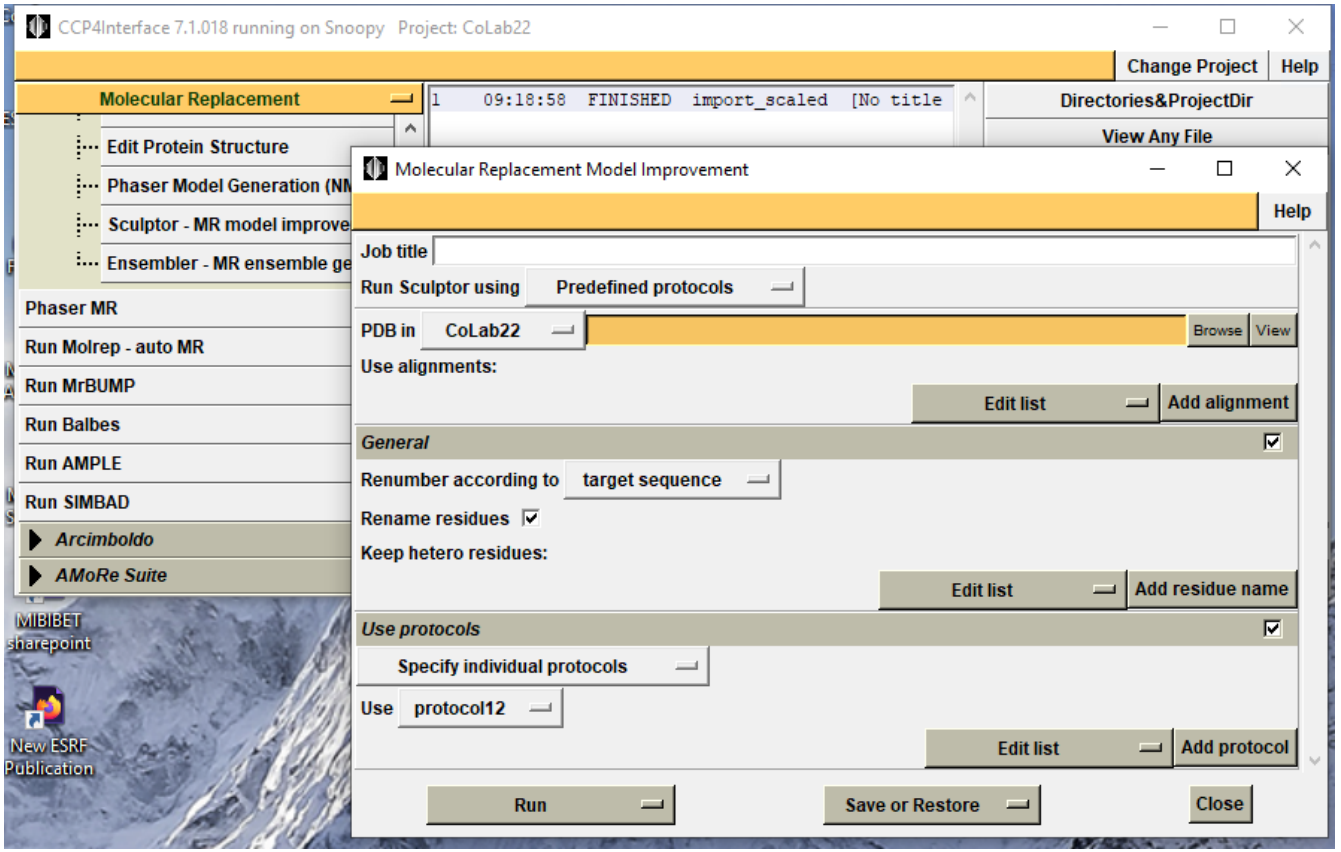
Load the **.sca** file and complete the remaining empty boxes as shown :



Click on the **Run** button, select **Run Now** from the drop-down menu and then click the **Close** button. When the program is **FINISHED** in the central GUI window you have now converted the .**sca** (text) file to the **.mtz** (binary) format used in CCP4 and most crystallographic programs.

Preparing the bos taurus model for MR

Select the **Molecular Replacement** program group, click on the ***Model Generation*** line in **2** to expand its program list and then click on the **Sculptor** button:



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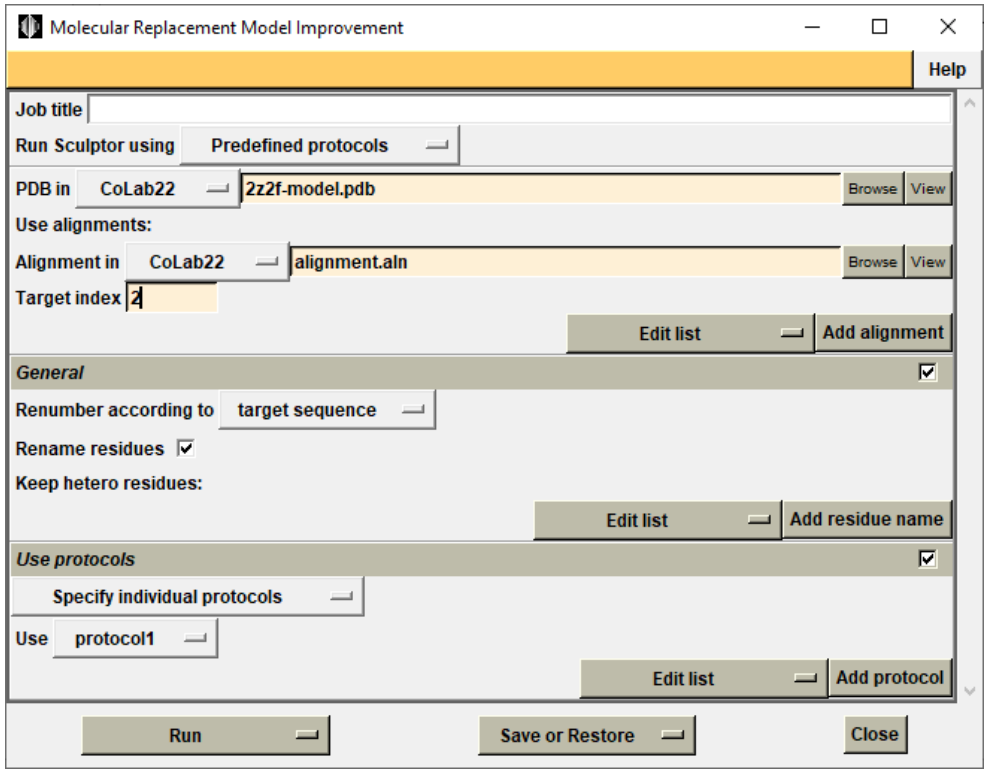
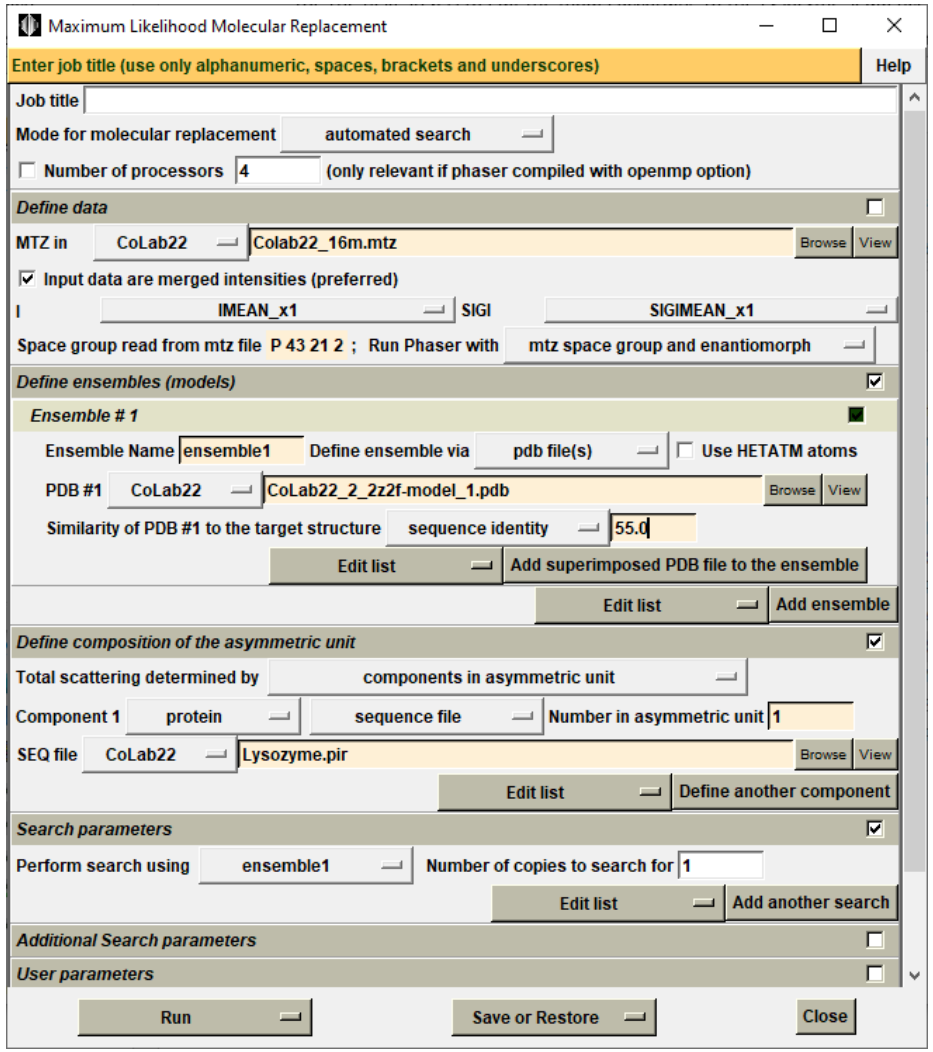


Load the **2z2f-model.pdb** file in the **PDB in** window, click on the **Add alignment** button and load the **alignment.aln** file in the **Alignment in** window. Use **Target Index 2** and select **Specify individual protocols** and **protocol1** to be used in the calculation. Click on the **Run** button and select **Run Now** from the drop-down menu and then click the **Close** button.

You should now have a **new PDB file** in your project directory which has been edited to remove the largest expected differences with your unknown 3D structure, based on the sequence alignment.

Running the MR calculations



Close the ***Model Generation*** program group and click on **Phaser MR** to open a new window. Load the **Colab22_16m.mtz** file into the **MTZ in** window; notice the program automatically selects the measured structure factor intensities and their estimated errors.

Load the PDB file from Sculptor into the **PDB#1** window and specify a **sequence identity** of **55.0**% (from the PDB file header). Load the sequence file **Lysozyme.pir** into the **SEQ file** window. Scroll down the job window and select **ensemble1** in the Search parameters pane.

Click on the **Run** button and select **Run Now** from the drop-down menu and then click the **Close** button.

Once the program is FINISHED, click on **View Files from Job** and then on **View Result of MR in Coot** to Load the output PDB and MTZ files in Coot and inspect the results. *Can you see any problems with the model and/or electron density?*

How do I know MR worked?

```
Top Peaks Without Clustering
Select peaks over 67.5% of top (i.e. 0.675*(top-mean)+mean)
   Also store peaks over 52.5% of top
There were 116 sites over 52.5% of top
116 peaks selected
The sites over 52.5% are:
#      Euler1 Euler2 Euler3    FSS    Z-score
1        64.4   73.0  168.4    100.000  6.05
2        64.2   72.5  167.0     98.670  5.97
3        64.0   72.0  165.6     97.471  5.90
#Sites = 116: output truncated to 3 sites
```

A Z-score **higher than 4** for the rotation function calculations.

```
Top Peaks Without Clustering
Select peaks over 67.5% of top (i.e. 0.675*(top-mean)+mean)
There were 8 sites over 67.5% of top
8 peaks selected
The sites over 67.5% are:
#      Frac X Frac Y Frac Z    FSS    Z-score
1       0.237  0.754  0.458     465.14 16.13
2       0.238  0.754  0.215     259.27  8.91
3       0.238  0.754  0.367     258.07  8.87
#Sites = 8: output truncated to 3 sites
```

A Z-score **higher than 8** for the translation function calculations.

```
Packing Table
-------------
Solutions accepted if pairwise clashes less than 10 % of trace atoms
#in   #out Clash-% Symm TF-SET  ROT TFpk#        TF     TFZ    SpaceGroup
1     Top1  0.428  --       1     1    1        222.42 16.13  P 43 21 2
2     2     9.519  --       1     1    2         10.43  8.69  P 43 21 2
3     3     1.604  --       1     1    3         -8.82  8.87  P 43 21 2

3 accepted of 3 solutions
   3 pack of 3 accepted solutions
```

Very few or no **packing clashes**.

```
Refinement Table (Sorted)
-------------------------
Refinement to full resolution
#out =#out #in =T (Start LLG Rval TFZ) (Refined LLG Rval TFZ==) SpaceGroup  Cntrst
Top1 ---   1           494.7 50.8 n/a         494.8 50.8 24.8   P 43 21 2    n/a
```

The **R-value** is not very informative because there is no scaling between F_o and F_c

How do I know MR worked?

```
   $TEXT:MR Result: $$ Baubles Markup $$

** SINGLE solution

** Solution written to SOL file:  /home/users/matias/ECS7/Lysozyme/ECS7_Lyso_3.sol

** Solution written to PDB file:  /home/users/matias/ECS7/Lysozyme/ECS7_Lyso_3.1.pdb
** Solution written to MTZ file:  /home/users/matias/ECS7/Lysozyme/ECS7_Lyso_3.1.mtz
   Solution annotation (history):
   SOLU SET  RFZ=6.1 TFZ=16.1 PAK=0 LLG=275 TFZ==16.5 LLG=495 TFZ==24.8 PAK=1 LLG=495 TFZ==24.8
   SOLU SPAC P 43 21 2
   SOLU 6DIM ENSE ensemble1 EULER  151.4   71.6  168.4 FRAC -0.25 -0.25  0.22 BFAC -0.26 #TFZ==24.8
   SOLU ENSEMBLE ensemble1 VRMS DELTA +0.0143 #RMSD  0.76 #VRMS  0.77
```

This is also a good sign.

```
Number LLG Z-Score
$$ loggraph $$
1      20.00  6.13
2       5.18  4.24
3       4.02  4.10
$$
```

Rotation function: 20.00

```
$$
Number LLG Z-Score
$$ loggraph $$
1     222.42 16.13
2      10.43  8.69
3      -8.82  8.87
$$
```

Translation function: 222.42

```
$$
Number final-LLG initial-LLG final-R initial-R
$$ loggraph $$
1     274.60     222.42 39.21 41.49
2      83.50      10.43 47.60 49.12
3      76.13      -8.82 48.34 50.23
$$
```

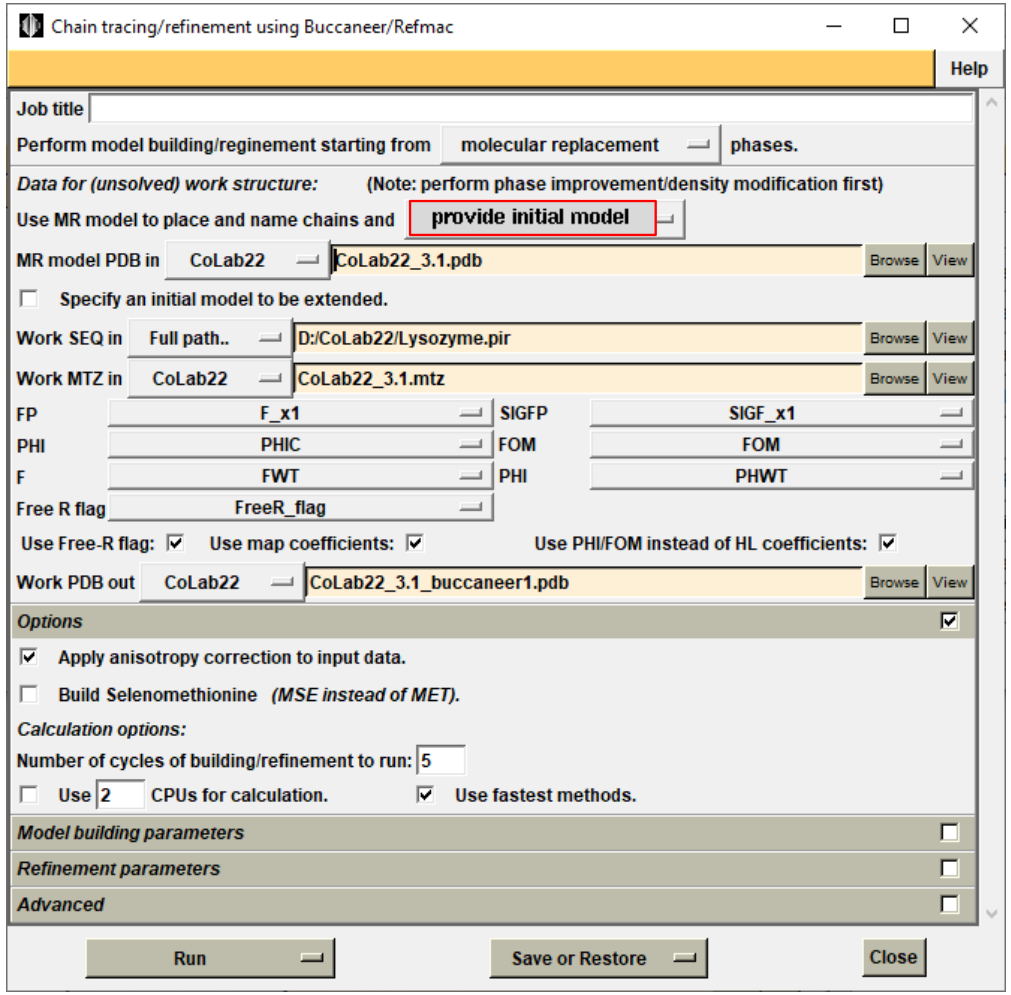
Refinement to 3.8 Å resolution: 276.40

```
$$
Number final-LLG initial-LLG final-R initial-R
$$ loggraph $$
1     494.81     494.72 50.82 50.82
$$
```

Refinement to 1.68 Å resolution: 494.81

The LLG (Log-likelihood gain) **must increase** during the calculations

Automated model editing and rebuilding with buccaneer

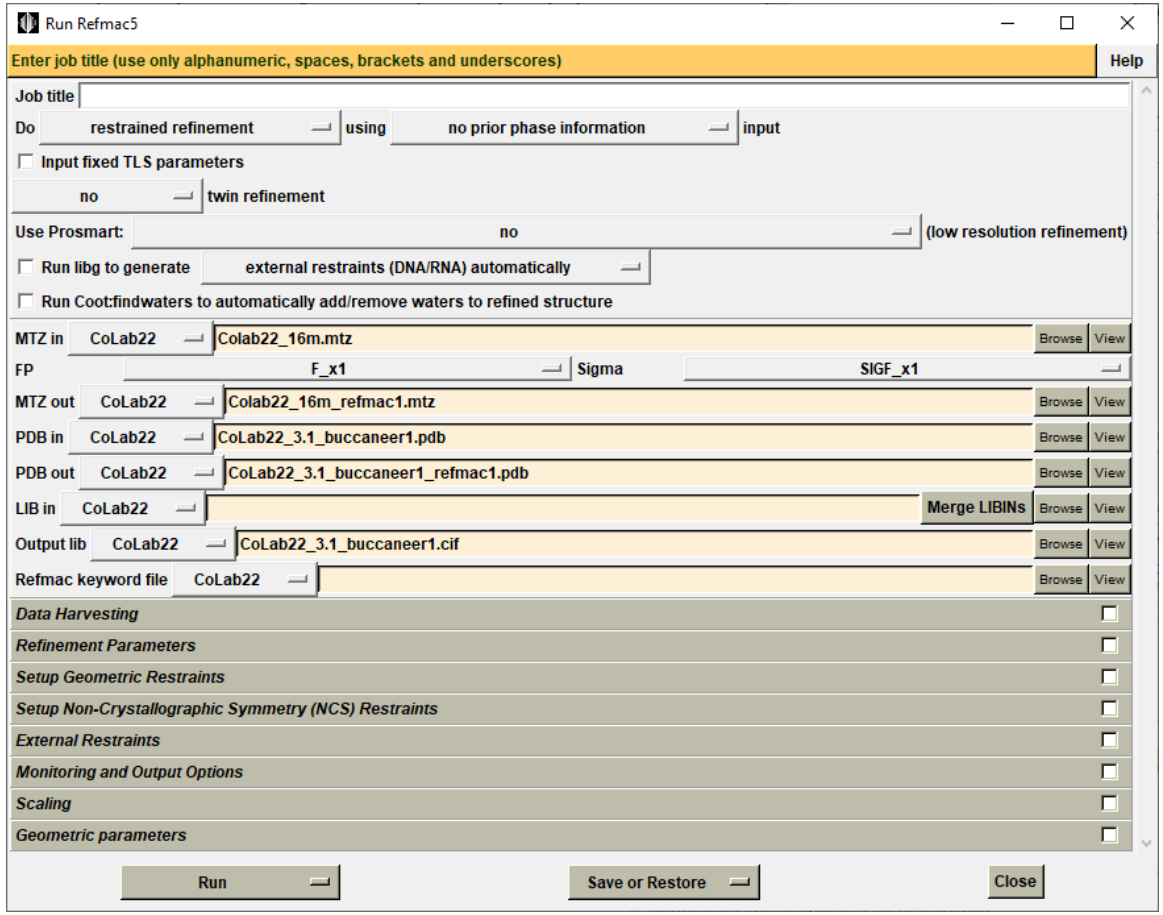


The next step is to edit the model according to the Lysozyme sequence. Remember, only **55%** of the aminoacids are identical between the two structures. This can be done manually in Coot or in a more automated way using **Buccaneer**.

Select the **Model Building** program group, click on the **Buccaneer** button and fill out the data as shown (be sure to select the **Molecular Replacement** mode in the first line):

Click on the **Run** button and select **Run Now** from the drop-down menu and then click the **Close** button. When the program is FINISHED you can inspect the results in Coot.

Automated model editing and rebuilding with buccaneer



To make life easier, let us first do a round of refinement using **Refmac5**. Select the **Refinement** program group, click on the **Run Refmac5** button and fill out the data as shown (you only need to supply the datafiles for the “MTZ in” and “PDB in” boxes):

Click on the **Run** button and select **Run Now** from the drop-down menu and then click the **Close** button. When the program is FINISHED you can inspect the results in Coot. Click on **View Files from Job** and then on **View result of refinement in Coot**.

NEXT STEPS – correct the model in Coot (today) and continue the refinement in Refmac5 (tomorrow)