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Supporting information for article:

Remote laboratory training for high school students: grocery store based hands-on project in protein crystallography

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Supplementary Materials:

PyMOL

Download pymol and open the Pymol graphic window.
(PyMOL: https://pymol.org/2/)

Type the following commands:

fetch 7knk (this will automatically download the pdb file and display it as “7knk”. PDB code 7knk corresponds to the structure reported in this paper.)

fetch 7knk, type=2fofc (will load the 2Fo-Fc map as “7knk_2fofc”)
fetch 7knk, type=fofc (will load the Fo-Fc map as “7knk_fofc”)

center on 7knk (Select the "center" option by clicking on the "A" next to
the "7knk" name on the right panel next to the figure display.

isomesh 2fofcmap,7knk_2fofc, 1.0, poly, carve=2.0 (will display the 2Fo-Fc map for
the whole molecule in white color).

color this map blue (select color by clicking on the “C” next to the “7knk_2fofc” name).

isomesh fofcmap,7knk_fofc, 2.5, poly, carve=2.0 (this will display difference maps in white color).

To electron density map for residues 20 to 30:

Type these commands:

select site1, resi 20-30 (this will create an object named “site1” with residues from 20 to 30.

isomesh 2fofcsite1map,7knk_2fofc, 1.0, site1, carve=2.0 (this will display the map for “site1”)

color magenta
hide the previous 2fofcmap (if not, the new map color may not be visible)

(Note: to hide the map “select the option by clicking on the “H” next to the “7knk_2fofc” name).

To Superimpose structures:

Download 7knk.pdb and 135l.pdb from the protein data bank (vide infra)

Load PDB file 1: eg. File > Open > 7knk.pdb

Load PDB file 2: eg. File > Open > 135l.pdb (PDB code “135L” corresponds to the Turkey lysozyme).

type the commad: align 135l, 7knk

This will superpose 135l structure onto 7knk

center on 7knk
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Coot

Download Coot crystallographic program.
(Coot: https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/)

Search for Protein Data Bank
type 7knk in the "3D Structures" prompt

Download PDB format. Downloaded file name: 7knk.pdb
Download Map Coefficients (MTZ format). Downloaded file name: 7knk_phases.mtz

File > Open Coordinate > 7knk.pdb (this will display the structure).
File > Open MTZ, mmCIF, fcf or phs > 7knk_phases.mtz

Amplitudes FWT
Phase PHWT

Click "OK"

(This will display the 2Fo-Fc map (blue color))

File > Open MTZ, mmCIF, fcf or phs > 7knk_phases.mtz

Amplitudes: FWT
Select Phase as: PHDELWT

Select "Is a Difference Map" option

click "OK"

(This will display Fo-Fc map in green and Fc-Fo map in red).

To superimpose structures:
Load the structures.

Go to Calculate > SSM Superpose >

Select the reference structure: One of the loaded pdb file

Select the Moving structure: Another loded pdb file

This will superpose the second structure on to the first structure.

Protein Data Bank (PDB)

Search for PDB and locate the PDB site. Eg. https://www.rcsb.org/

Go to the PDB site and search for a name of the protein or type the PDB code in the "3D Structures" section. For example: 7knk

This will display details of the crystal structure for PDB code 7knk.

The structural information is stored in various tabs (Structure Summary, 3D View, Annotations, Experiment, Sequence, etc.).

Generally, the "Structure Summary" tab will be displayed.

This shows the display of the structure on the left and text summary of the structure and data on the right. Scrolling down the page shows more details on the structure.

The top right corner of the page has pull down menus with "Display Files" and "download Files".

By clicking on the "Display Files", users can display FASTA sequence of the structure and various formats of the deposited data.

The "Download Files" section allows downloading of the FASTA sequence, PDB coordinate, structure factors, maps etc.

The PDB format display shows Title of the PDB entry, name of the protein, related publication, details of the crystal data, refinement statistics, details of the secondary structure, sequence information, unit cell dimensions and space group, and coordinate.

Each lint of the coordinate section has

ATOM 1 N LYS A 1 -9.800 2.934 -17.926 1.00 20.50 N

Numbers "-9.800 2.934 -17.926" gives the X, Y, Z position of the corresponding atom in orthogonal coordinates. Next number "1" gives the occupancy of that atom. This is followed by the b-factor (here, 20.50).

More details about the PDB data is available at https://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/introduction

Crystal mounting video links (provide by Dr. Jim Pflugrath) :

https://www.youtube.com/watch?v=45Qc3jOPaKY

https://www.youtube.com/watch?v=1y89sfsuQBw

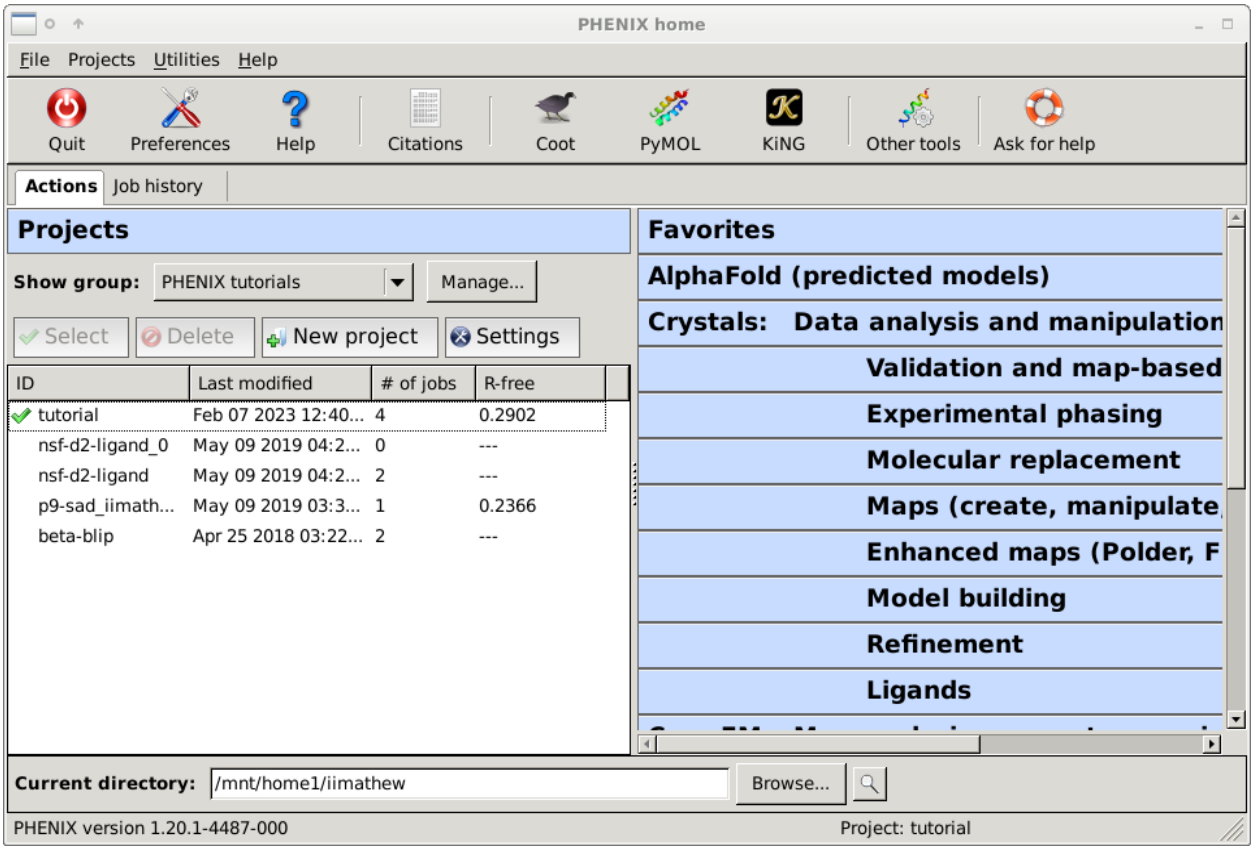
Structure solution and Refinement

Phenix (https://phenix-online.org/).

Phenix can be downloaded from this site.

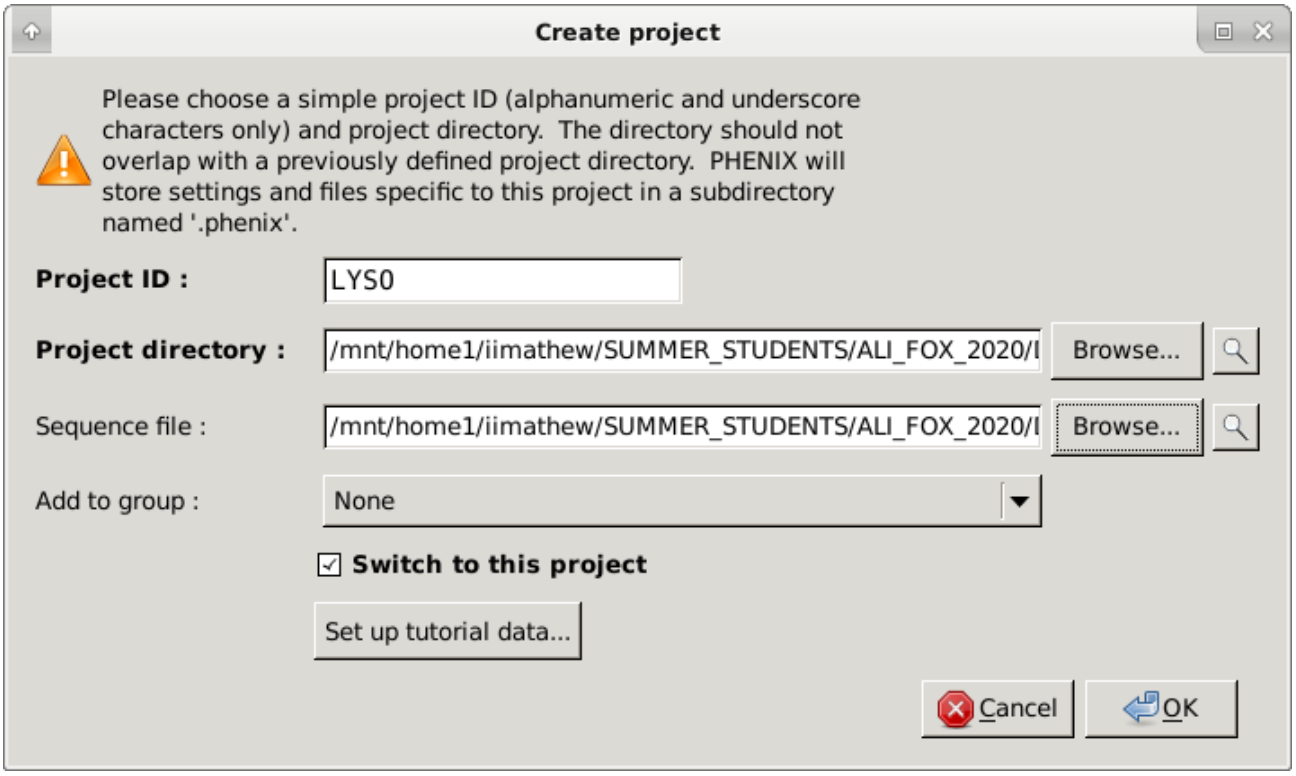
Phenix website has video tutorials and documentation for the various programs.

Start Phenix software, this will display the following window.



Click on the “New Project”

This gives the following window with an option to input the sequence file.



Give a Project ID:

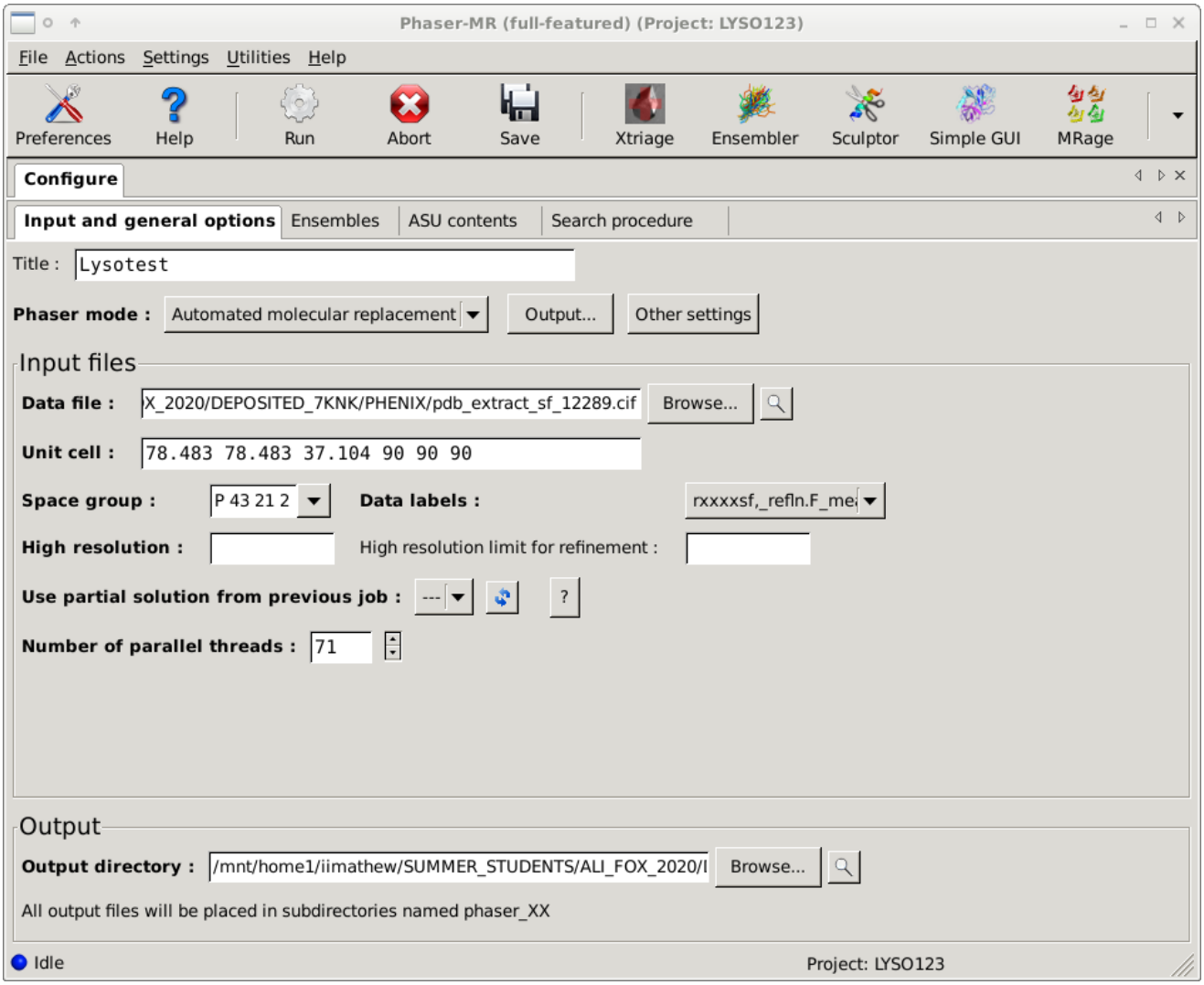
Upload the sequence file for the 193L structure in the Supplementary section or download the sequence file from the PDB ID: 193L (from the PDB site).

Click the “OK” button.

Go to the first Phenix widget and click on the “Molecular Replacement”

This will open the following widget. This widget had different Tabs to input to fill.

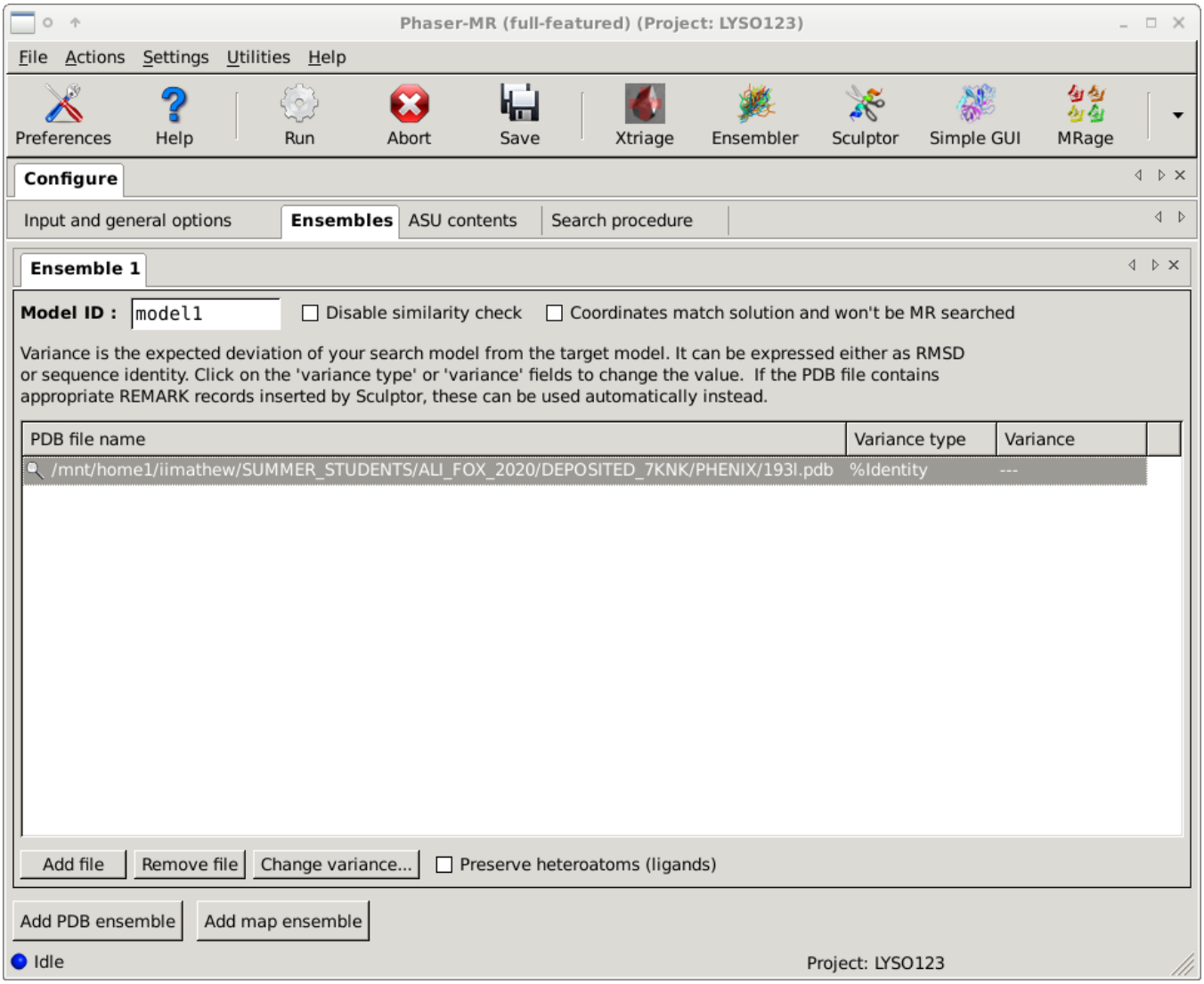
The first Tab is “Input and general options” shown below.



Put a Title and load the reflection file (pdb_extract_sf_12289.cif) in the Supplementary section.

Uploading this file will automatically fill the Unit cell and space group.

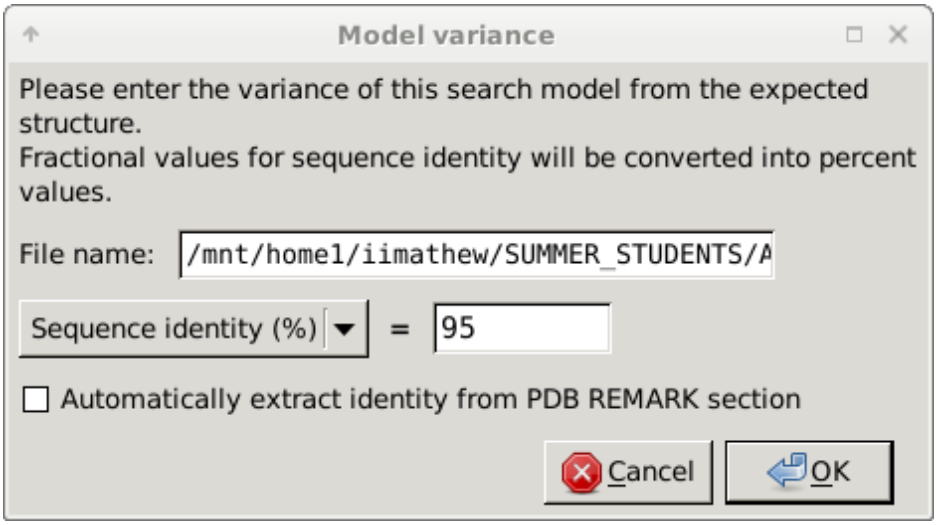
Then go to the “Ensembles” Tab (see the picture below).



Put a Model ID: (eg. model1)

Click on the “Add File” and load the pdb file: 193l.pdb (from the Supplementary section or download it from the PDB site).

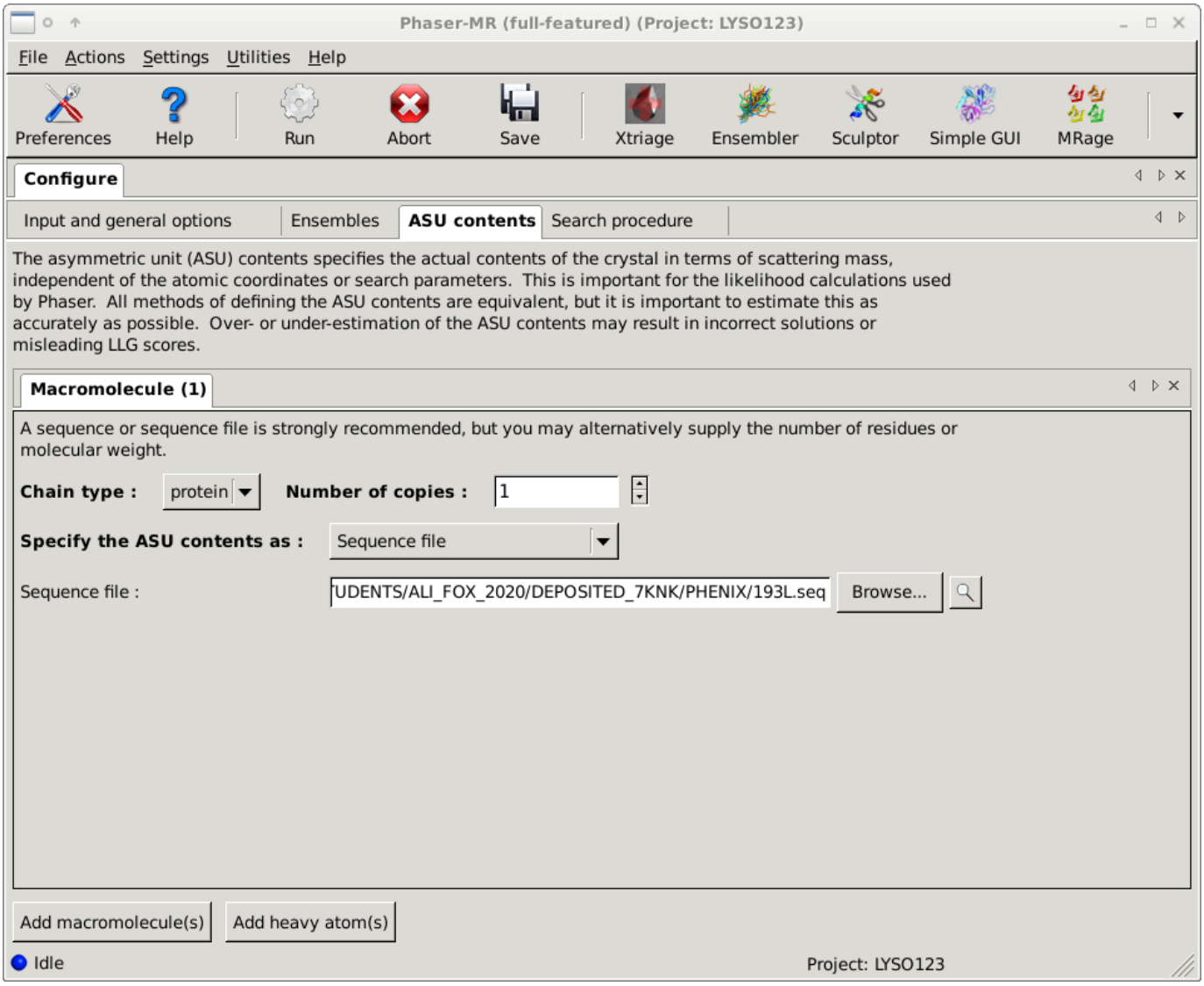
Click on the “Change variance”. This will open a new window as shown below.



Put the sequence identity: 95

Click OK to close this widget.

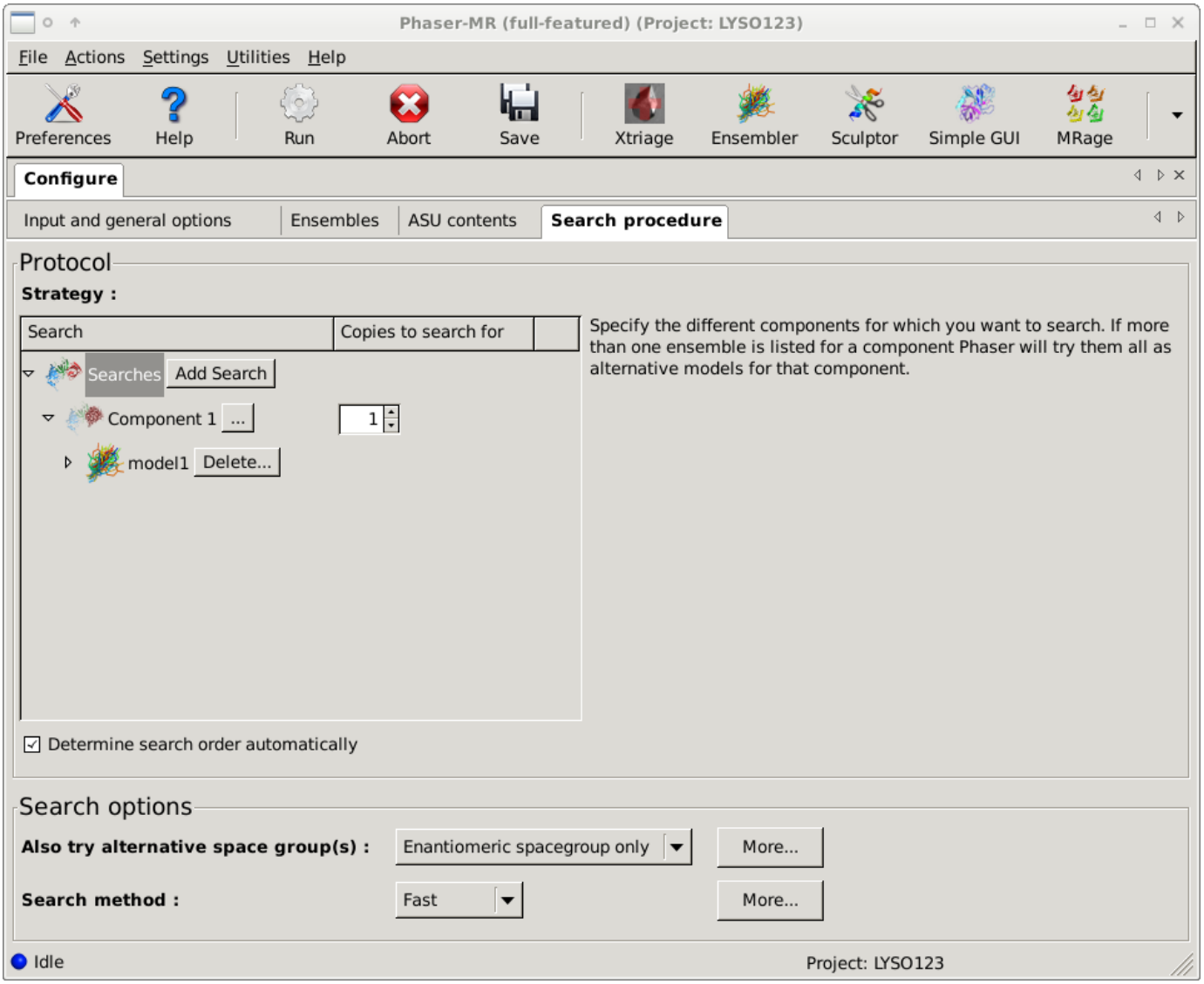
Then, go to the ASU contents Tab (see below)



Number of copies: 1

Upload the sequence file for the 193L structure provided in the Supplementary or download it from the PDB site.

Now go to the Search Procedure" Tab (see below).

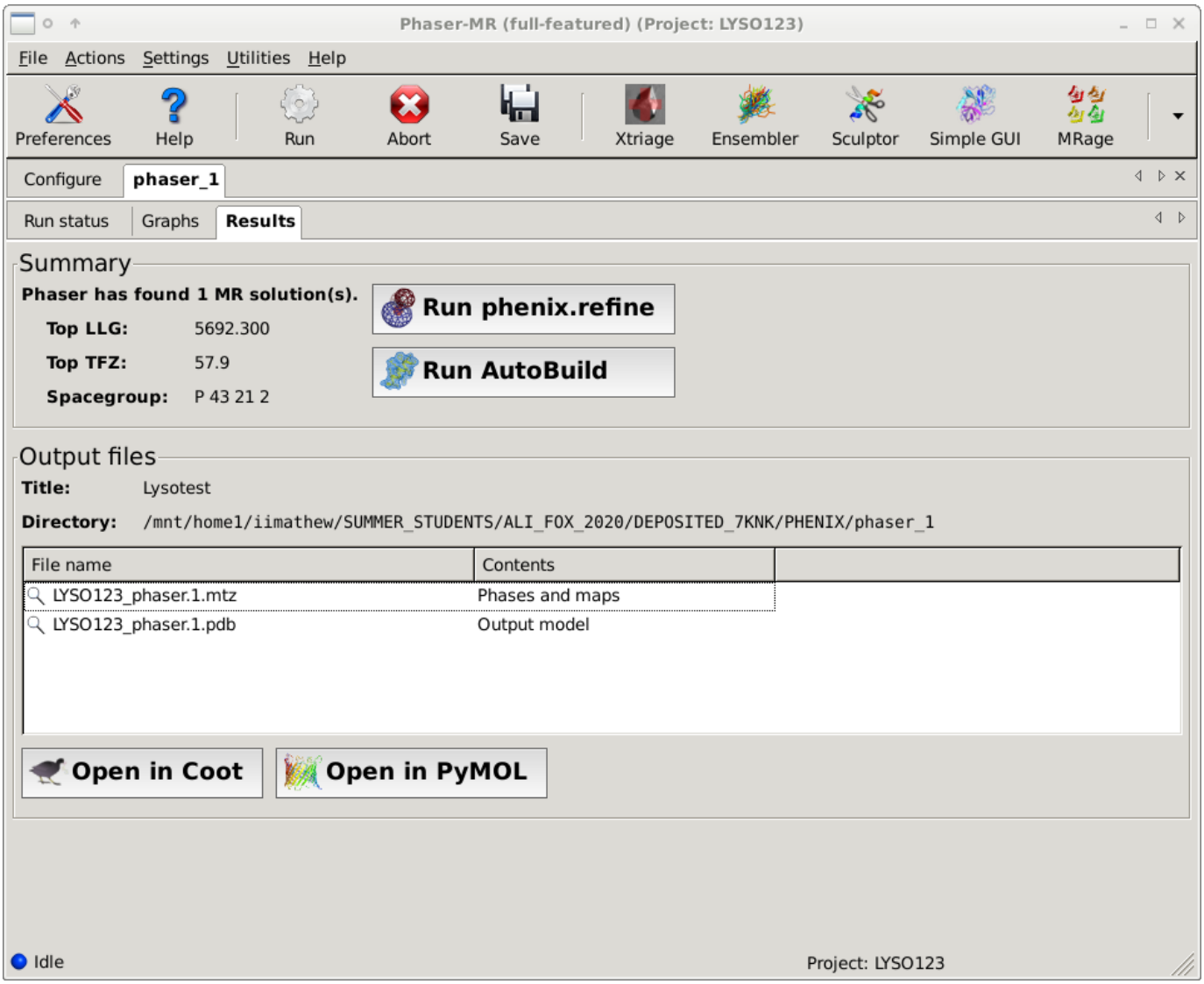


Click on the Component 1, select Add ensemble and select “model1” (this is the Model ID we provided in the Ensembles Tab.

Select “Run” from the top bar and select “Run now”. This will perform the structure solution using the Molecular Replacement method.

Program will run for some time (depending on the CPU power of the machine). There will be constant updates on the window.

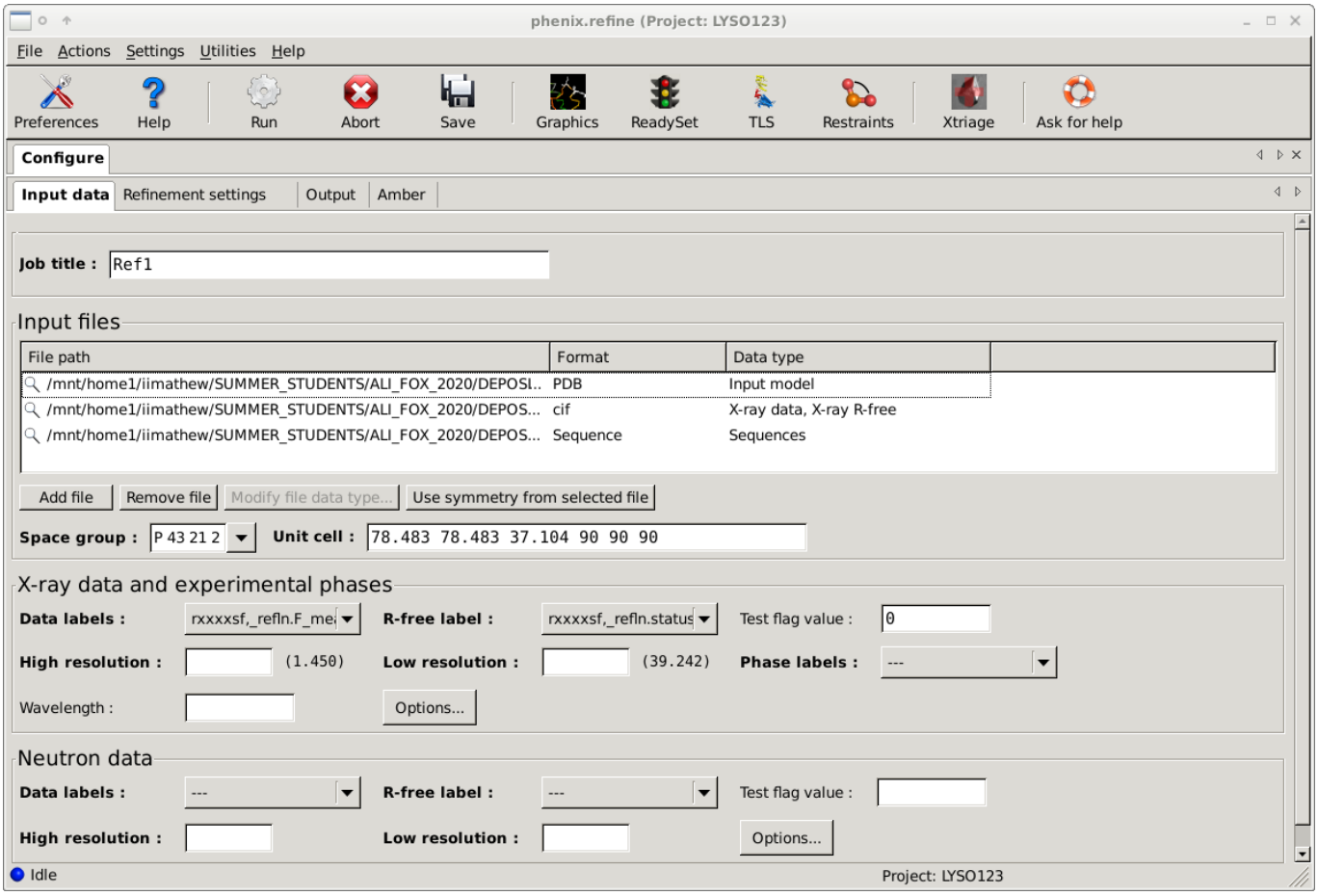
When the program completes, it will display the following window.



The high values for Top LLG (larger the better) and Top TFZ (higher than 8) show that structure is solved.

Click on the “Run phenix.refine” button.

This will open the following window. Here also information is displayed in a “Tab” format.

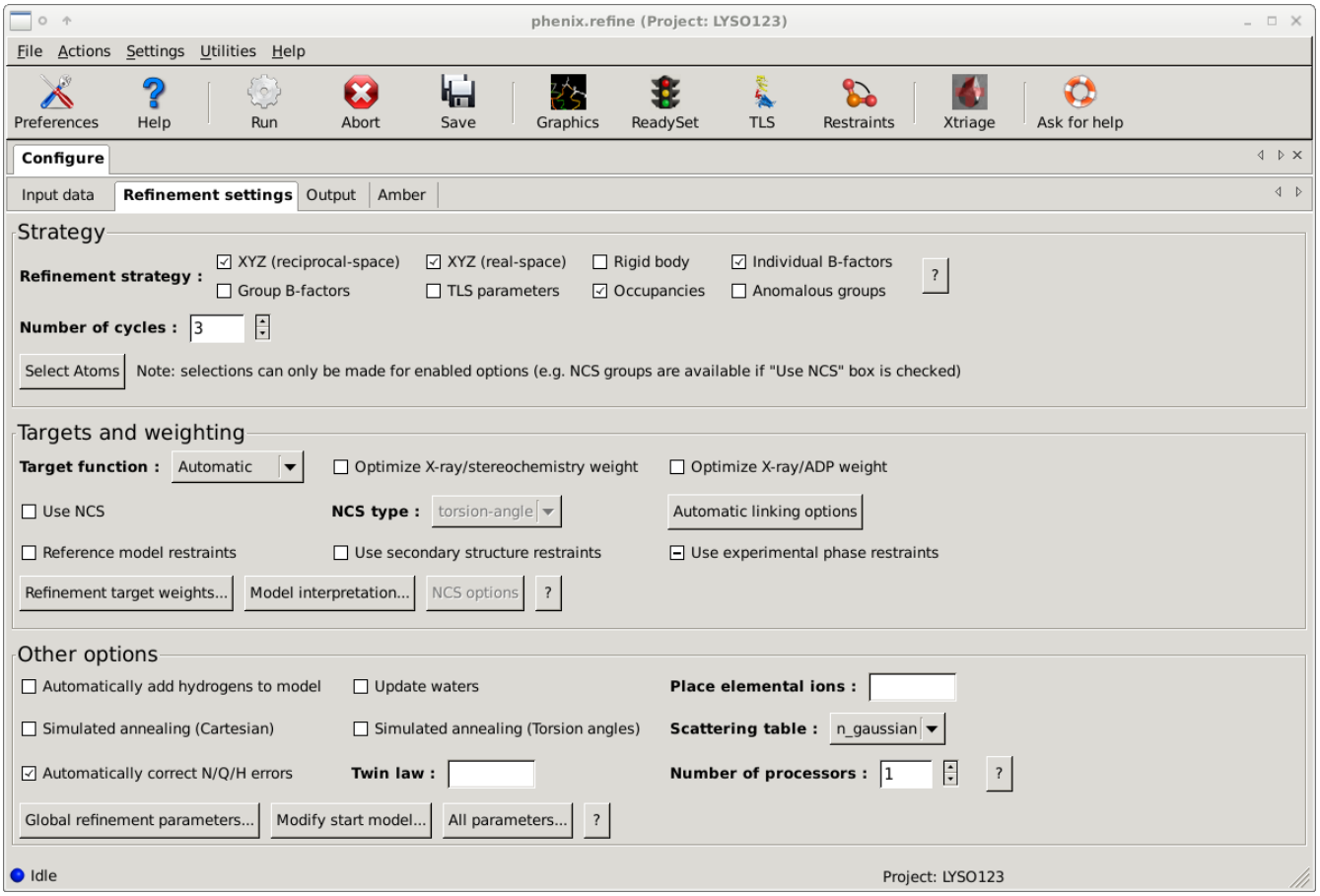


The first Tab is “Input Data”.

Put a Job title (eg: Ref1).

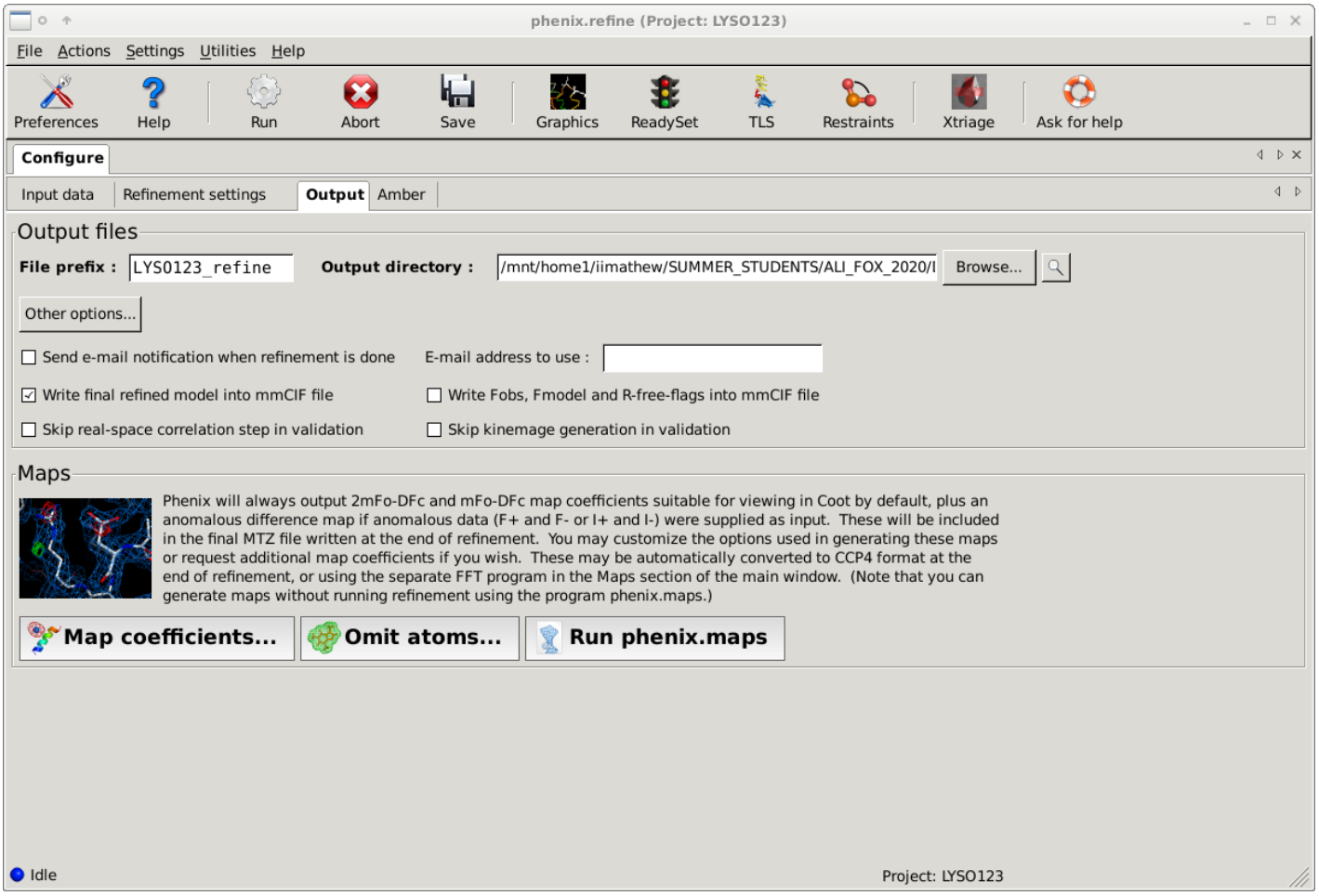
The files needed for the refinement (coordinate, reflection data and sequence) are already uploaded in the window.

Go to the “Refinement settings” Tab.



No need to change any information. However, you could increase “Number of Processors” to higher values, depending on the computing power of the computer.

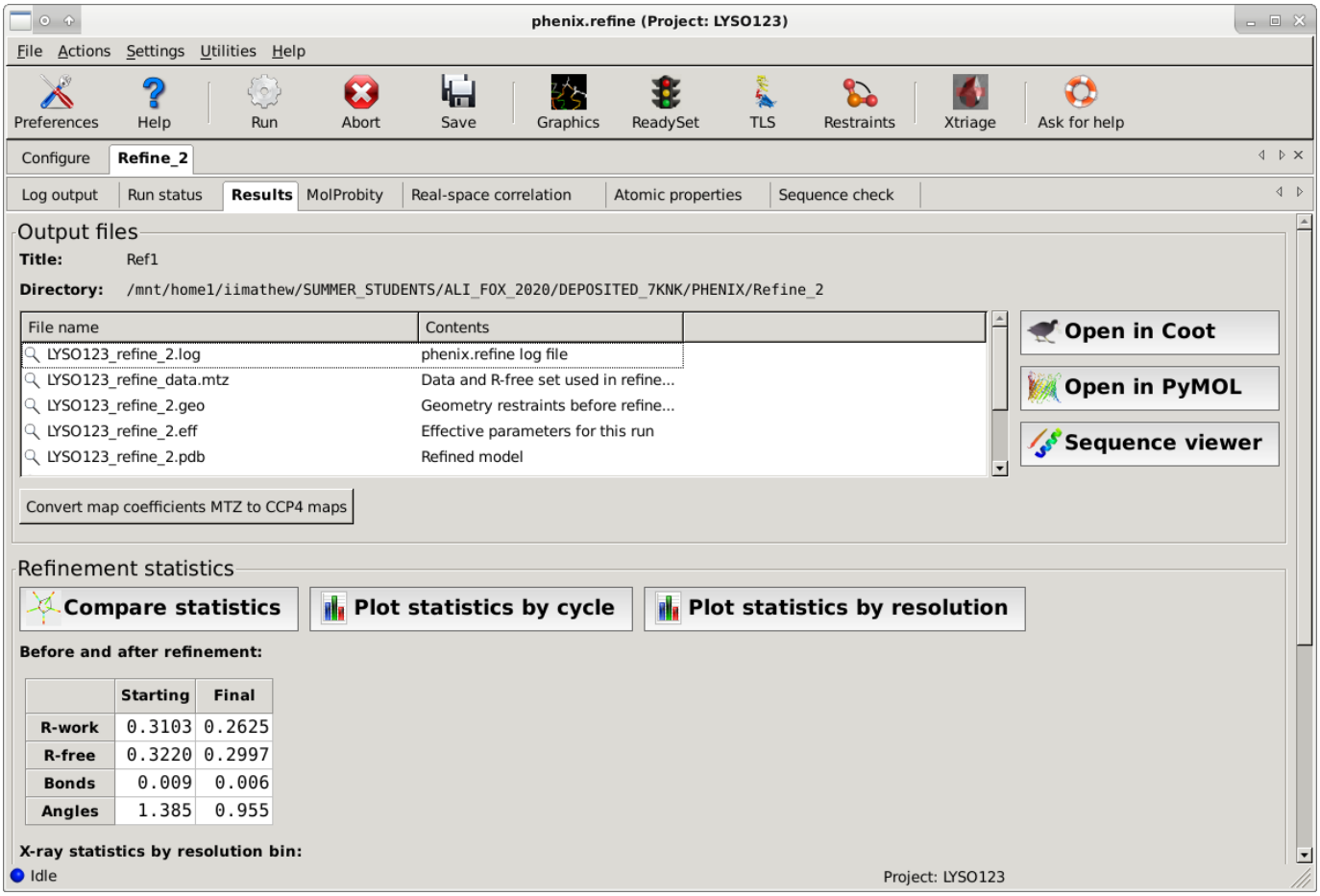
Select the “Output” Tab



Program automatically fill the “File prefix” based on the two titles we gave at the beginning of Phenix and in the “Input” section of “Refinement”.

Select “Run” from the top bar and select “Run now”. This will run the “Refinement” of the structure obtained in the MR.

When the program completes, it will display the following window.



This widget also has tabs. A short summary of the refinement results is provided in the “Results” tab (shown the above window). Other Tabs provides various information resulted from the Structure solution.

Clicking on the “Open in Coot” will display structure and electron density using the “Coot” program. (NOTE: the proper display require proper setting of the various files).