Molecular Replacement Practical

Introduction

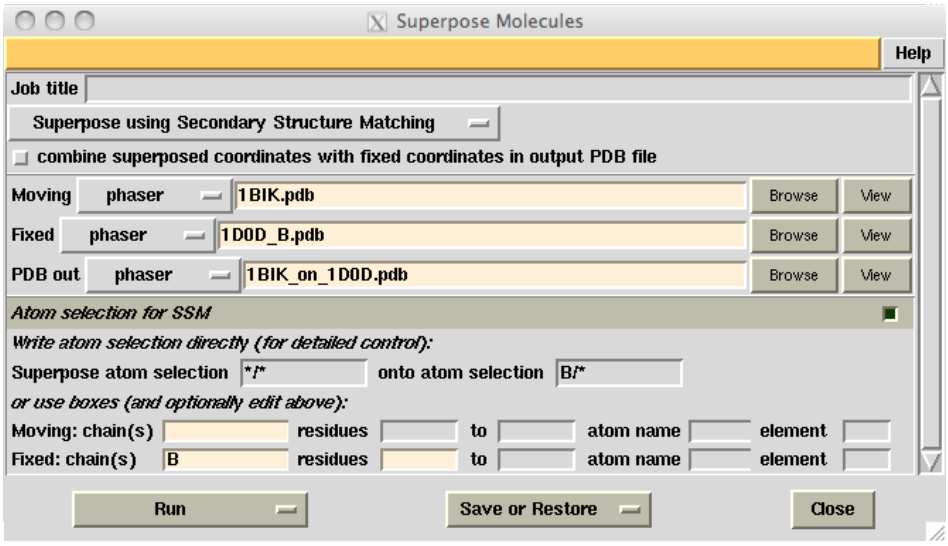
During the practical you will learn 1) how to use ensembling to construct a search model and 2) how to solve a heterodimeric complex 3) how to solve a homo-oligomer from a monomer

1. MR using ensemble search models: TOXD

α-Dendrotoxin (TOXD, 7139Da) is a small neurotoxin from green mamba venom. You have two models for the structure. One is in the file 1BIK.pdb, which contains the protein chain from PDB entry 1BIK, and the other is in the file 1D0D_B.pdb, which contains chain B from PDB entry 1D0D. 1BIK is the structure of Bikunin, a serine protease inhibitor from the human inter-α-inhibitor complex, with sequence identity 37.7% to TOXD. 1DOD is the complex between tick anticoagulant protein (chain A) and bovine pancreatic trypsin inhibitor (BPTI, chain B). BPTI has a sequence identity of 36.4% to TOXD. Note that models making up an ensemble must be superimposed on each other, which has not yet been done with these two structures.

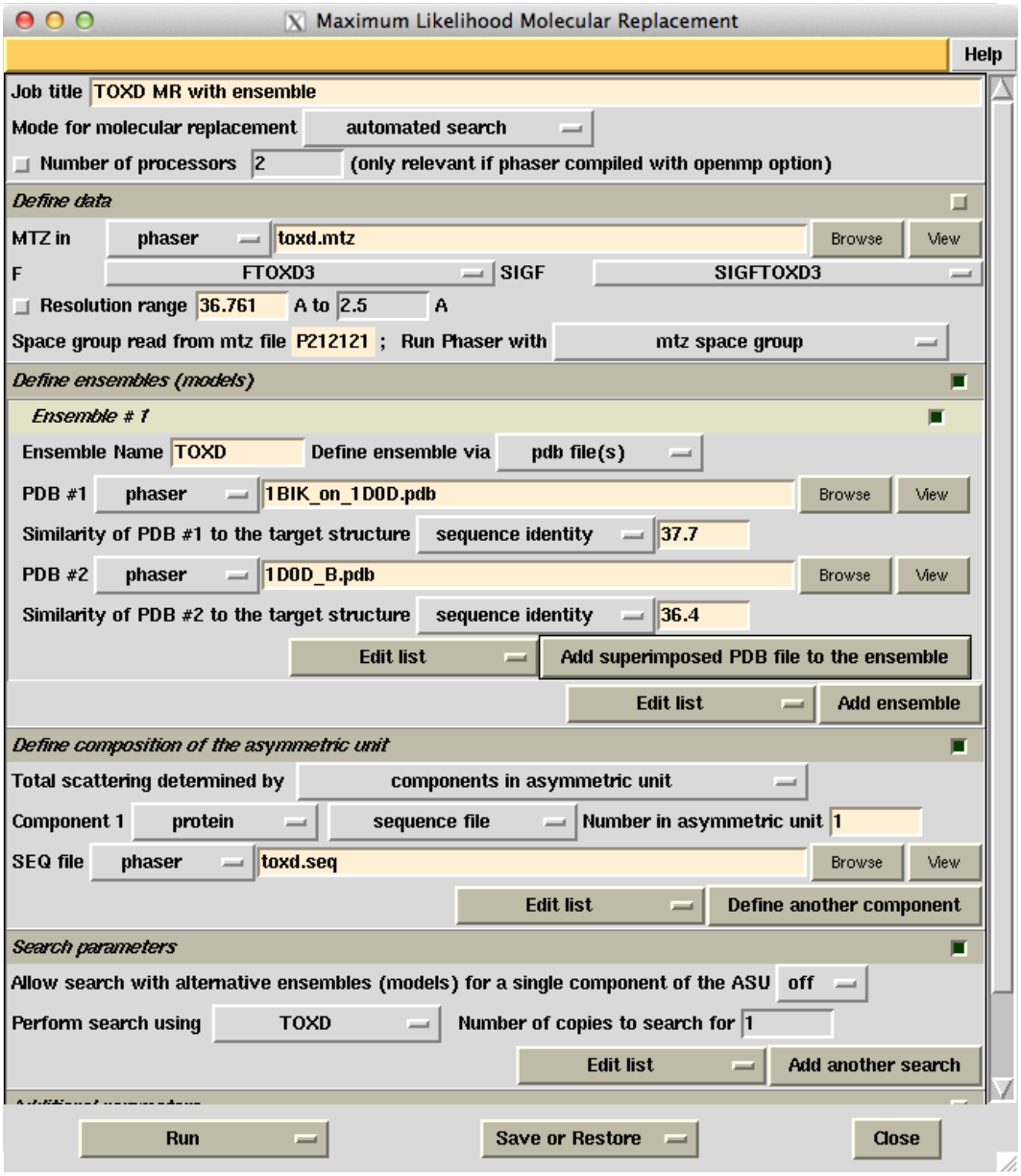
1.1 Superimpose the two pdb files that will make the ensemble

- View 1BIK.pdb and 1D0D_B.pdb in Coot. Are the two structures superimposed?
- Launch ccp4i by typing ccp4i on the command line.
- Click on “Change Project” and select “phaser” from the list of available projects.
- From the drop-down module list, select “Co-ordinate Utilities”
- Select “Superpose Molecules”
- From the drop-down menu, select “Superpose using Secondary Structure Matching”
- In the “Moving” field, select 1BIK.pdb
- In the “Fixed” field, select 1D0D_B.pdb
- In the “PDB out” field, enter 1BIK_on_1D0D.pdb
- In the atom selection section, enter chain “B” in the “Fixed: chain(s)” field.
    - *Normally you would also select a chain for the moving molecule, but there is no chain identifier in the 1BIK.pdb.*
- Run this job
- View the structures in Coot. Are they superimposed?



1.2 Run Phaser for Molecular Replacement

- From the drop-down module list, select “Molecular Replacement” and launch “Run Phaser”
- Enter the input as shown in the figure below
    - *Make sure you enter the superimposed pdb files for the ensemble*
    - *Make sure the pdb files are entered as the one ensemble and not as two ensembles*
- Run this job



1.3. *Inspect the log file.*

- Write down the steps in the structure solution in the order in which they were taken.
- Find the pieces of information listed in Table 1 in the log file
- Look at the .sol file. Copy the annotation at the top of the solution. To what do the values in the annotation refer? Find the same values where they are first reported in the log file.
- Has Phaser solved the structure?

2. Solving a heterodimeric complex using MR: BETA/BLIP

β-Lactamase (BETA, 29kDa) is an enzyme produced by various bacteria, and is of interest because it is responsible for penicillin resistance, cleaving penicillin at the β-lactam ring. There are many small molecule inhibitors of BETA in clinical use, but bacteria can become resistant to these as well. Streptomyces clavuligerus produces beta-lactamase inhibitory protein (BLIP, 17.5kDa), which has been investigated as an alternative to small molecule inhibitors, as it appears more difficult for bacteria to become resistant to this form of BETA inhibition. The structures of BETA and BLIP were originally solved separately by experimental phasing methods. The crystal structure of the complex between BETA and BLIP has been a test case for molecular replacement because of the difficulty encountered in the original structure solution. BETA, which models 62% of the unit cell, is trivial to locate, but BLIP is more difficult to find. The BLIP component was originally found by testing a large number of potential orientations with a translation function search, until one solution stood out from the noise.

2.1 *Consider the MR problem*

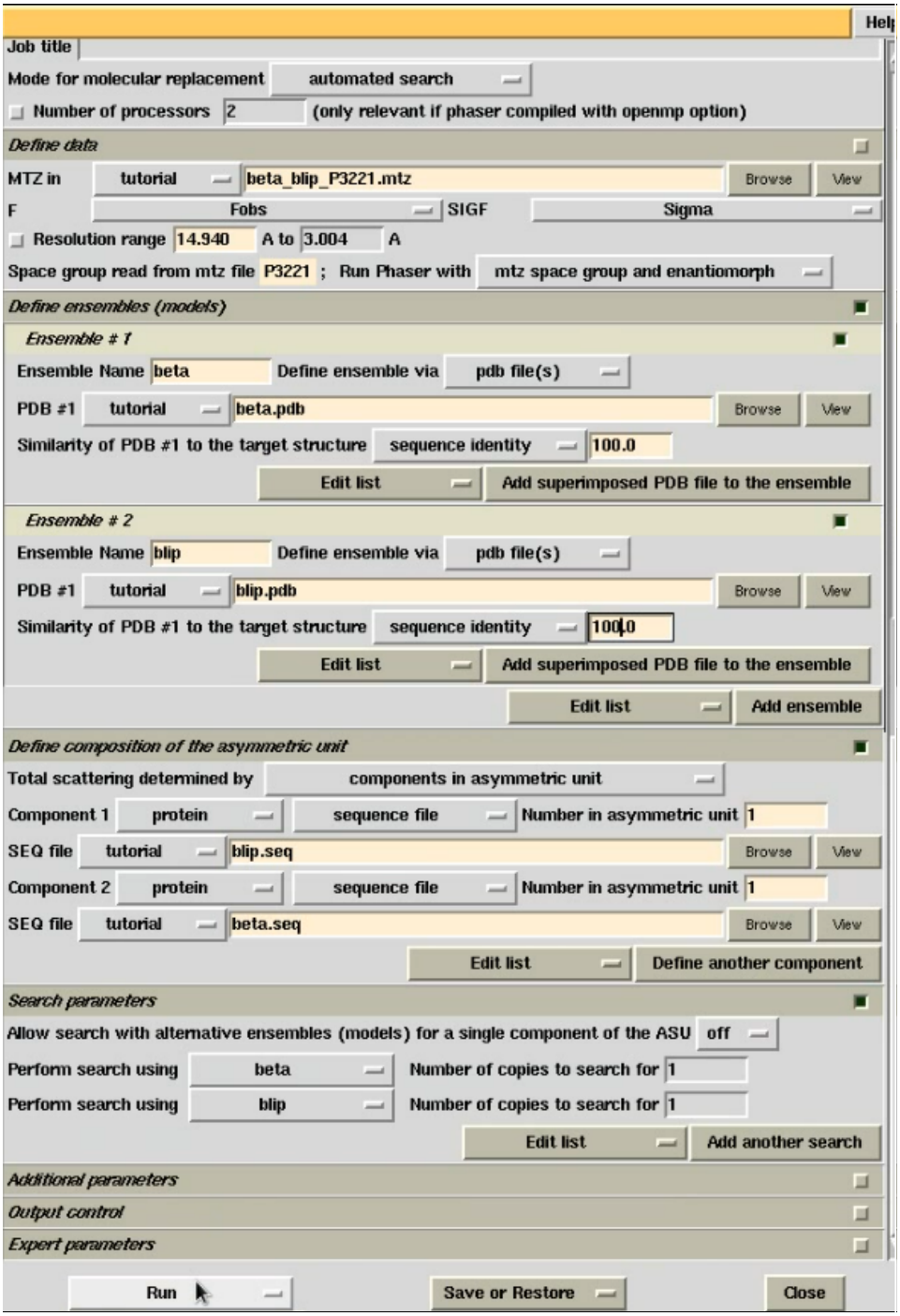
- What is the space-group recorded on the mtz file? If you had not solved this structure, would you know that this was the space-group? If not, what other space-group(s) must you consider?
    - *Consider handedness and possible enantiomorphs*

2.2. *Run Phaser for Molecular Replacement*

- From the drop-down module list, select “Molecular Replacement” and launch “Run Phaser”
- Enter the input as shown in the figure below.
    - *Make sure the pdb files are entered as two ensembles*
- Run the job.

2.3. *Inspect the log file*

- Write down the steps in the structure solution in the order in which they were taken.
- How are the steps different from the TOXD example
- Find the pieces of information listed in Table 1 in the log file
- Which space group is the solution in?
- Why doesn’t Phaser perform the rotation function in the two enantiomorphic space groups?
- Which reflections in the data are particularly important for deciding the translational symmetry of the space-groups to search? Under what data collection conditions might you not have recorded these important reflections? Are there any other space-groups that you might want to consider when solving BETA/BLIP?
- How big is the anisotropic correction for the data?
    - *How does this compare to TOXD?*
- Has Phaser solved the structure?

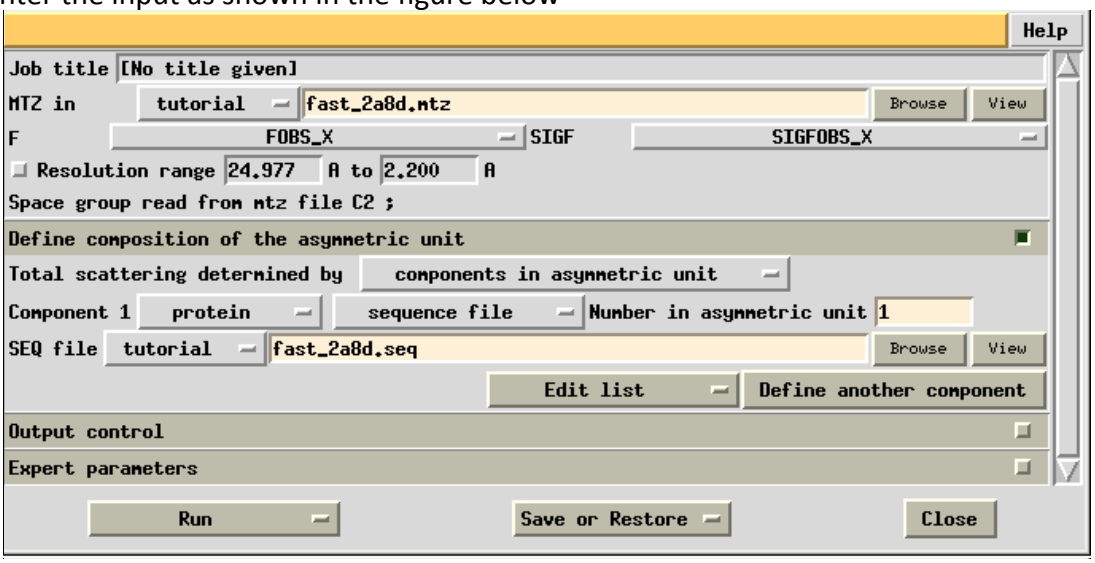


3. Solving a homo-oligomeric complex: HICA

Carbonic anhydrase is an enzyme that assists rapid inter-conversion of carbon dioxide and water into carbonic acid, protons and bicarbonate ions to aid removal of carbon dioxide from the blood in respiration. This ancient enzyme has three distinct classes; alpha, beta and gamma. Carbonic anhydrase from mammals belong to the alpha class, the plant enzymes belong to the beta class, while the enzyme from methane-producing thermophillic bacteria forms the gamma class. Members of these different classes share very little sequence or structural similarity. The alpha enzyme is a monomer and the gamma enzyme is trimeric. The beta enzyme can be a dimer, tetramer, hexamer or octamer. *Haemophilus influenzae* β-carbonic anhydrase (HICA,2a8d) is an allosteric protein. The model you have for this structure is *E. col*i β-carbonic anhydrase, which has 61% sequence identity to HICA.

3.1 *Consider the MR problem*

- From the drop-down module list, select “Molecular Replacement” and select the “Phaser Cell Content Analysis” tab
- Enter the input as shown in the figure below



- Run the job
- Inspect the log file
- How many monomers of β-carbonic anhydrase can fit in the asymmetric unit? Which of these possibilities is most probable? Which of these are possible? What are the oligomeric associations that could correspond with the possible asymmetric unit contents?
    - *Consider the application of crystal symmetry*

3.2 Run Phaser for Molecular Replacement

- From the drop-down module list, select “Molecular Replacement” and launch “Run Phaser”
- Enter the input with the number of copies you think should be in the asymmetric unit
    - *Make sure you enter both the number of copies of the sequence in the composition AND the number of copies of the ensemble to search for, and that this number is the same*
- Run this job

3.3. Inspect the log file

- Write down the steps in the structure solution in the order in which they were taken.
- Find the pieces of information listed in Table 1 in the log file
- Has Phaser solved the structure?
    - *How many molecules are there in the asymmetric unit?*
    - *View the structure in Coot. What is the biological oligomeric association?*

Table 1

cell content analysis	probability of input composition
anisotropy correction	anisotropic B-factor
translational ncs	translational ncs vector if any
ensembling	input VRMS of members of the ensemble
rotation function	selection criteria for rescoring fast RF orientations with full RF number selected for rescoring with full RF highest RFZ in full RF final (purging) selection criteria number selected for TF
translation function	selection criteria for rescoring fast TF positions with full TF number selected for rescoring with full TF highest TFZ in full TF number of TFZ > 8 final (purging) selection criteria number selected for packing
packing function	number of clashes allowed number of solutions accepted
refinement	increase in LLG for top solution refined VRMS TFZ equivalent
automated MR decision making	resolution for searches expected difficulty of search search order for ensembles (if more than one type) cutoff selection changes (if any) amalgamation (if any)

4. Advanced

If you have time revisit the TOXD and BETA-BLIP examples and look at the following.

4.1 TOXD

- Run Phaser again without using ensembling, instead just using 1BIK or 1D0D as a search model. What are the LLGs of the final solutions? What are the Z-scores of the translation functions? Was ensembling a good idea?
- Run Phaser again using the two pdb files before superposition. What does Phaser report?

4.2 BETA-BLIP

- Run Phaser again with the anisotropy correction turned off. What effect does this have on the structure solution?