MR using CCP4i:BETA/BLIP

From Phaserwiki

Data for this tutorial are found here (http://www.phaser.cimr.cam.ac.uk/index.php/Tutorials)

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Reflection data: beta_blip_P3221.mtz
Structure files: beta.pdb, blip.pdb
Sequence file: beta.seq, blip.seq
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This tutorial demonstrates a difficult molecular replacement problem.

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.

1. What do you think is the best order in which to search for BETA and BLIP? Under what circumstances could the lower molecular weight search model be the easiest to find by molecular replacement?
2. 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?
    - Think about handedness (enantiomorphs)
3. Run Phaser for solving BETA/BLIP
    - Bring up the GUI for Phaser
    - All the yellow boxes need to be filled in.
    - Search for BETA and BLIP in the one job.
4. Has Phaser solved the structure?
    - Look at the Z-scores for the rotation and translation functions
5. What search order was used?
    - If you wanted, you could force the other search order and see what difference this makes.
6. Which space group was the solution in?
7. Look though the job.sum file and identify the anisotropy correction, rotation function, translation function, packing, and refinement modes, for the two search molecules, and all the space groups. Draw a flow diagram of the search strategy.
8. Why doesn't Phaser perform the rotation function in the two enantiomorphic space groups?
9. 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?

10. How big is the anisotropic correction for the data? How does this compare to TOXD?
11. Run Phaser again with the anisotropy correction turned off. What effect does this have on the structure solution?