

Combined MR-SAD using CCP4i

From Phaserwiki

All files for this tutorial are distributed from the Phaser web page

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Reflection data: lyso2001_scalal.mtz
Lactalbumin model: 1fkq_prot.pdb
Sequence file: hewl.pir
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This tutorial illustrates a common molecular replacement/experimental phasing scenario, when refinement is hindered by very strong model bias, but there is some experimental phasing signal available.

Goat α -lactalbumin is 45% identical to hen egg-white lysozyme. Although it is possible to solve lysozyme using α -lactalbumin as a model, it is very difficult to refine the structure, partly because of model bias. Unfortunately, low solvent content of this crystal form limits the ability of density modification to remove the bias. However, one can use anomalous scattering from intrinsic sulfur atoms to improve phases dramatically. It is noteworthy that the anomalous signal from the sulfur atoms is not sufficient for *ab initio* phasing (it is not possible to locate the anomalous scatterers from the data alone).

1. Solve the structure with the α -lactalbumin model. Follow the "Molecular replacement tutorial" if necessary.
2. For a fairer comparison of phase quality, we will treat the molecular replacement solution as a source of experimental phase information. (If you use the "automated model building starting from PDB file" mode, the current version of ARP/wARP will be able to build the structure, but older versions coupled with older versions of Refmac5 failed.) Do a quick solvent flattening with Parrot (choose "Use PHI/FOM instead of HL coefficients" because the MTZ file produced after MR doesn't include Hendrickson-Lattman coefficients; set PHI=PHIC and FOM=FOM; select "Use map coefficients", then set F=FWT and PHI=PHWT).
3. Start up ARP/wARP Classic in "automated model building starting from experimental phases" mode. Select the MTZ file from Parrot. To start from the Parrot map, select "Use a different (pre-weighted) Fobs for initial map calculation" under the ARP/wARP flow parameters folder, then set FBEST=parrot.F_phi.F, PHIB=parrot.F_phi.phi and FOM=Unassigned. Select the sequence file, and note there are 129 residues in lysozyme. To save time, do 3 cycles of autobuilding instead of 10.
4. Now add the S-SAD phase information. Bring up the GUI for Phaser SAD pipeline in the Automated Search & Phasing section of the Experimental Phasing module
5. All the yellow boxes need to be filled in.
 - Set "Mode for experimental phasing" to SAD with molecular replacement partial structure.
 - Uncheck the Parrot and Buccaneer steps of the pipeline (to allow control for better comparison with the MR model alone).
 - Set LLG-map calculation atom type to S.
 - Under the "Define atoms" heading, set "Partial structure" to the molecular replacement solution (output PDB-file) you have obtained in step 1.
6. Run Phaser after you entered all the information.
7. Solvent flatten with Parrot using a similar protocol to step 2. However, you should **not** choose "Use PHI/FOM instead of HL coefficients". Choose the HlanomA/B/C/D coefficients because these describe the phase information obtained only from the anomalous scattering information and not from

- the molecular replacement model; using HLA/B/C/D would include the model phase information, which would bias maps from subsequent cycles of phase improvement to look like the model.
8. Run ARP/wARP using a similar protocol as in step 3, except you should open the Refmac parameters folder and choose the option to include HL phase restraints. In the MTZ data section, choose the HlanomA/B/C/D coefficients.
 9. How many anomalous scatterers has Phaser found? Check them against the model and guess what they may be! Why is it not important to specify the exact element type in this case?
 10. If you did not know the correct space group (from the MR step), would you have to run Phaser SAD-phasing twice?
 11. Compare the two ARP/wARP runs! Which one has built more residues?

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