

mth685, high resolution data

1. Intro

There are two essential things helping with molecular replacement in this case.

Firstly, a large number (up to 100) of rotation peaks are to be tested by translation function.

Secondly, starting from the point, where two domains are found, the partial structure should be refined (rigid body refinement in MOLREP and then restrained refinement in REFMAC) before using it as a fixed model in molecular replacement search of the next domain. (Restrained refinement of the first found domain alone is not stable and thus is not reliable; one can try it to see fluctuations of R and R-free.)

Also, the second copy of a given domain can be easier found using this domain from the latest refined partial model.

In this way both low and high resolution data contribute to the structure solution: low resolution is used in MR, high resolution contributes to the restrained refinement and helps to improve search model.

In the protocol known to be successful, the domains were found in the following order: 1, 2, 2, 1, 3, 3.

2. Alignment

In the successful protocol domains were 3d-aligned against target sequence (Alignment option in MOLREP).

Possibly, this is not important, but the protocol without alignment have not been tested.

3. First copy of the domain 1

At this stage one copy of domain 1 is to be found. (In tested protocol domain 1 includes residues up to 89 of the aligned model.)

The search is straightforward, the standard run of MOLREP will give a solution. It is good idea to refine the solution as rigid body (appropriate option of MOLREP). The restrained refinement (REFMAC) at this stage is unlikely useful.

4. First copy of the domain 2

The domain 1 found in the previous step is the fixed part of model; the original aligned domain 2 (residues 90 to 162) is the search model.

This search may require up to 50 peaks of the rotation function to be tested (maybe even more). The contrast in the translation function is going to be quite convincing.

The resultant model is to be refined as two rigid bodies using MOLREP (rigid body refinement, domain yes).

This is to be followed by restrained refinement with REFMAC, where a stable decrease of R and R free confirms correct solution.

Two models are to be stored after this refinement: the final one after REFMAC and part of it corresponding to the domain 2. This two models will be used in the search of the second copy of the domain 2.

5. Second copy of the domain 2

The procedure is similar to the previous step. The search model is the refined domain 2 from previous step. The fixed model is refined domains 1 and 2 from previous step.

The solution is expected among first 50 peaks of rotation function (it is good idea to test 100).

The output from MOLREP will contain: first copy of domain 1 (chain A in the pdb-file), first copy of domain 2 (chain B) and the second copy of domain 2 (chain C).

This model is to be refined as 3 rigid bodies (MOLREP) and then with REFMAC.

To be stored from this step are: the whole model after REFMAC and its fraction corresponding to domain 1 (chain A).

6. Second copy of the domain 1

The search model is the refined domain 1 from previous step. The fixed model is refined domains 1, 2 and 2 from previous step.

The procedure is as before.

To be stored from this step is the whole model after REFMAC.

At this stage the ARP/wARP can be tried with target sequence. (As I can remember the double sequence has to be in the input)

However, it may be worth of trying to find at least one copy of the third domain.

7. First copy of the domain 3

The search model is aligned original domain 3 (163-232). The fixed model is refined domains 1, 1, 2 and 2 from previous step (output from REFMAC).

At this step the top of the scoring table may be shared by 2-3 peaks that are very close to each other in terms of both translations/rotations and correlation/R-factors. This indicates significant conformational changes in the domain compared to the search model.

The output model corresponding to the top solution can be refined (rigid body refinement in MOLREP followed by restrained refinement in REFMAC). It contains now domains 1, 1, 2, 2 and 3.

To be stored from this step are: the whole model after REFMAC and its fraction corresponding to domain 3 (chain E).

8. Second copy of the domain 3

The search model is the refined domain 3 from the previous step. The fixed model is refined domains 1, 1, 2, 2 and 3 from the previous step.

The procedure is as before.

The top solution may be wrong one in this step. It should be examined using COOT. The terminal residues of all domains are to be close enough to appropriate terminus of neighbouring domain or its symmetry equivalent.

If the solution is wrong, then it is better to go one step back, and use the final model from the previous step to start with model rebuilding and refinement. The electron density at the previous step is already good enough for this.