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Refinement of macromolecular structures at low resolution

Oleg Kovalevskiy[1], Robert A. Nicholls[1], Garib N. Murshudov[1]
[1]*Structural Studies Division, MRC Laboratory Of Molecular Biology, Cambridge, United Kingdom*
E-mail: okovalev@mrc-lmb.cam.ac.uk

Poor diffraction quality of macromolecular crystals is a common problem: various types of disorder result in the weakening of high-resolution observations, anisotropic diffraction and other problems. Such datasets have low information content; hence the ratio of the number of observations to adjustable model parameters is small. This ratio could be improved by using complementary information - our prior knowledge about macromolecular structures. Restraints produced based on known related structures could be used as such an additional source of information; ProSMART is one of the programs that can generate restraints using reference protein/RNA/DNA structures as well as for secondary structure elements. These restraints are used by REFMAC5 to stabilise refinement of an atomic model against low-resolution diffraction data.

We have tested various refinement strategies and different REFMAC5 [1] and ProSMART [2] parameters on a test set of more than a hundred structures with resolution below 3.0 Å, for which structures of high-resolution homologues are available. We found that refinement with external restraints is sensitive to the selection of homologous structures for restraint generation; some homologues of the same target structure may improve refinement much better than others.

The best-performing refinement protocols have been implemented in LORESTR: an automated pipeline for structure refinement at low resolution [3], distributed as part of the CCP4 suite. The pipeline facilitates the fully-automated selection of optimal external restraints from ProSMART for structure refinement by REFMAC5. It can automatically run a BLAST search to identify homologues, and download the corresponding models from the PDB. It automatically detects twinning, and finds the optimal scaling method and parameters for solvent modeling. The pipeline runs a number of refinement protocols in order to find the best protocol for each particular case. In our tests, LORESTR was able to produce substantially better quality models in the vast majority of cases, improving both R-factors and model geometry for 94% of test cases. The dramatic improvement in R-factors and the geometric quality of low-resolution models observed when using the fully-automated mode of the pipeline demonstrates its potential use for researchers working with low-resolution cases, especially during the initial stages of refinement, or when unable to further progress with refinement.

Currently we are working on multi-crystal refinement: treatment of the special case where several low-resolution X-ray diffraction datasets and models are available for a particular protein. We have designed a procedure to co-refine all structures simultaneously, executing multiple concurrent REFMAC5 refinements, generating external restraints for each model using all others, and iterating until convergence. This technique allows information transfer between the structures, which could potentially improve refinement and thus the quality of resulting models.

[1] Murshudov, G.N. et al. (2011) Acta Cryst D67, 355-367
[2] Nicholls R.A. et al. (2012) Acta Cryst D68, 404-417
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