Improving Low Resolution Refinement with ProSMART, COOT and REFMAC5

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Introduction
Many of the structures currently in the focus of molecular biology and molecular medicine are flexible molecules and large assemblies that only yield poor quality crystals which diffracting to low resolution (2.8Å or worse). In such cases, model building and refinement become underdetermined problems with noisy data. COOT [1], Refmac5 [2] and ProSMART [3] can help to exploit the structural information contained in low resolution data, using prior knowledge to stabilize refinement and ease model building.

Spinach is a low resolution structure that has recently been determined by Warner et al. [4] in collaboration with our lab. Most of the tools presented in this poster were used. Spinach is an RNA mimic of GFP that allows for imaging of biological RNAs without large GFP fusion proteins. The structure – a quadruplex - has been crystallized with its cognate exogenous chromophore. Figures 1-3 show data from this structure solution.

Coot, a program for macromolecular modelling using X-ray and EM data, offers a number of new tools to aid model building at low resolution, among them planar peptide restraints, Ramachandran restraints and local rotation-translation searches for rigid fragments (“jiggle fit”). Model Morphing searches the best fit for each residue, while fragment possible in a selected range and then moves the chain to a robust average of these. This conserves local conformation but moves an entire region into the density (see Fig. 1). “Backrub rotamers” [5] remove several degrees of freedom in protein models; as do torsion angle restraints.

External restraints from the new software LIBG for RNA and DNA restraint generation (see Fig. 2) as well as ProSmart Restraints can be visualized, manipulated and used for changing the model in COOT. Many of these new features can also be used to fit single-particle Cryo-EM reconstructions.

Refmac5
Refmac5 is a program for refinement of macromolecular structures against crystallographic data and single-particle cryo-EM reconstructions. The program is based on Bayesian statistics, which work satisfactorily at low resolution as prior information is combined with experimental data. The most recent features for low-resolution refinement are regularizers, such as external restraints from LIBG or ProSMART, and jelly-body refinement (see box).

ProSMART
ProSMART [Nicholls et al., in press] is a tool for the comparison of macromolecular structures (DNA, RNA and proteins) and for the generation of external restraints. External restraints can be generated from homologous structures (see Fig. 3) or purely from hydrogen bonding patterns or typical conformations of secondary structure elements. Such external restraints aid refinement stability and improve geometry, and are therefore particularly useful in earlier stages of refinement. Importantly, globally rigidity is not enforced by these restraints. This is due to the nature of ProSMART restraints and the use of the Geman-McClure robust estimator function in Refmac5 [7]. ProSMART interfaces directly with COOT and Refmac5.