Poster Presentation

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Low Resolution Refinement with ProSMART, COOT and REFMAC5

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Membrane proteins and large assemblies are currently a major focus of molecular biology and molecular medicine. Due to their size and flexibility, these structures may only yield poor quality crystals for which diffraction intensities can be measured to merely mid-low resolution. Nevertheless, these data contain valuable structural information. Here, it will be shown how new features in COOT [1], REFMAC5 [2] and ProSMART [3] can help to exploit low resolution data for model building and refinement, as well as aid model validation. Refinement at low resolution can be stabilised with regularisers, such as jelly-body and external restraints. These allow to routinely obtain good quality models even in cases where only low-resolution data are available (e.g. >3Å). External restraints (available for protein and DNA/RNA) exploit structural prior knowledge, utilising the assertion that local interatomic distances should agree with previous observations. Sources for such prior knowledge include isomorphous and homologous structures, hydrogen bonding patterns, and typical conformations of secondary structure elements. Importantly, global rigidity is not enforced by these restraints – the approach presented allows for dramatic conformational differences between target and reference models. Consequently, restraints may be generated using homologous reference models resolved in different crystal forms. COOT facilitates model building at low resolution by removing degrees of freedom through so-called “backrub rotamers” and torsion angle restraints, as well as providing semi-automatic building options such as model morphing and jiggle fit . Map sharpening and blurring, now available in both COOT and REFMAC5, can be employed to provide further insight regarding the validity of a model, as well as aiding the model building process. General guidelines for the application of these features are provided, along with examples demonstrating their usage.

[1] *P. Emsley, B. Lohkamp, W.G. Scott, K. Cowtan, Acta Cryst D, 2010, 66, 486–501,* ***[2]*** *G. N. Murshudov, P. Skubak, A. Lebedev, et al., Acta Cryst. D, 2011, 67, 355–367,* ***[3]*** *R.A. Nicholls, F. Long, G.N. Murshudov, Acta Cryst. D, 2012, 68, 404-417*

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