[MS4-02] Macromolecular Refinement At Low Resolution With REFMAC5 And ProSMART.

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Regularisers are often used to stabilise macromolecular crystallographic refinement, and ensure consistency between the derived models and available prior knowledge. Using the Bayesian framework, such regularisers are often implemented in the form of restraints, which are often referred to as geometry terms. Regularisers may be used at all resolutions, although more may be required at lower resolutions in order to achieve an acceptable effective parameter-to-observation ratio Restraints representing chemical information are commonly used at all resolutions (e.g. bond and angle restraints), which help local structure adopt chemically reasonable conformations.At medium resolution, TLS, local NCS, and B-value restraints may be used. In some cases, effects such as crystal mosaicity and molecular disorder lead to poor diffraction quality, weak intensities, and result in only low-resolution data being available. Other factors such as twinning and other crystal peculiarities also reduce information content and thus effective resolution. In such cases, the weak signal, noisy data, and low observation-to-parameter ratio often cause unstable refinement, a higher risk of over-fitting, and ultimately result in an unreliable/suboptimal model. Such complications in crystallographic refinement may be lessened to some degree by introducing further regularisers such as jelly-body and external restraints. These techniques have been recently developed with the intention of allowing high-quality models to be routinely achieved even in cases where only low resolution data are available (e.g. >3Å). Jelly-body restraints stabilise refinement without imposing any externally-derived information. These effectively restrain the structure to its existing conformation, ensuring smoother parameter changes during refinement. This strategy has been found to be extremely effective at medium to low resolution. External restraints (available for protein and DNA/RNA models) are designed to utilise structural information as a source of prior knowledge, helping local interatomic distances to agree with previous observation without inappropriately enforcing global rigidity. Such structural information may be derived from homologous models where available, even if in a different global conformational state or from a different crystal form. Otherwise, more generic types of information can be utilised, such as knowledge of hydrogen bonding patterns, or typical conformations of secondary structural elements and other structural fragments. External restraints may be generated by ProSMART [1, 2], and are typically short (2.5-4.2Å) stabilising local structure whilst allowing global conformational flexibility between target and reference structures. Challenges include determining suitability of reference structures, and ensuring robustness to destructive external information. ProSMART's structural analysis features are intended to aid such assessment, allowing quantitative and visual analysis of localised differences between related structures. In this context, these features are useful for comparing target and reference structures, and for identifying/investigating any structural changes that the model may undergo during the model building and refinement process. Other recent developments in refinement, map calculation, analysis and validation tools will also be discussed. These tools have been implemented to aid refinement by REFMAC5 [1, 2, 3], although it should be noted that similar techniques have also been implemented in other modern crystallographic refinement software packages, e.g. BUSTER-TNT [4], phenix.refine [5], SHELX [6] and CNS [7].

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