MS45-O4 Low Resolution Refinement Tools in REFMAC5. Robert A. Nicholls, Fei Long, Garib N. Murshudov, *MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 0QH, UK*
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Heterogeneous organisation of molecules in the crystal, due to effects such as crystal mosaicity and molecular disorder, lead to poor diffraction quality, weak intensities, and ultimately low resolution data. Other factors such as twinning also reduce information content and thus effective resolution. Many tools have been developed to aid crystallographic refinement at medium and high resolutions in recent decades. One of the current challenges is to develop approaches allowing high-quality models to be routinely achieved using low-resolution data (e.g. >3Å). Various tools have recently been implemented to aid low-resolution refinement in REFMAC5 [1, 2] including: (i) the use of external structural information, (ii) "jelly-body" refinement, and (iii) map sharpening. Similar techniques have also been implemented in other modern crystallographic refinement software packages, e.g. BUSTER-TNT [3], phenix.refine [4], SHELX [5] and CNS [6]. The use of chemical and structural information as restraints is intended to increase consistency of the derived models with available prior knowledge. In recent years, external structural information has been utilised in various forms, such as secondary structures, homologous reference structures, and homology models. Using the Bayesian framework, such information can be incorporated as restraints during refinement, which should help the macromolecule under refinement adopt a conformation consistent with previous observations. This is similar to the use of geometry terms, which help local structure adopt chemically reasonable conformations. ProSMART [1] generates external distance restraints using reference structures, hydrogen bonding patterns (e.g. secondary structure restraints), and structural fragments (e.g. secondary structure conformations). These restraints are typically short (2.5-4.2Å) stabilising local structure whilst allowing global conformational flexibility between target and reference structures. We have also implemented DNA/RNA base pair restraints based on interatomic distances, torsion angles, and chirality. Such information can enhance the reliability of derived atomic models and stabilise refinement, particularly at lower resolutions. However, any improvement due to external restraints will be limited by reference structure quality, and the consistency between the reference structure and the (unknown) true structure of the macromolecule under refinement. Consequently, challenges include determining suitability of reference structural information, and ensuring robustness to destructive external information. "Jelly body" restraints aim to stabilise refinement by means of local distance restraints. These effectively restrain the structure to its existing conformation, ensuring smoother parameter changes during refinement. Rather than modifying the likelihood function, they influence the search direction, implicitly encouraging refinement along normal modes. Low resolution data often exhibits high isotropic/anisotropic B-values, causing smeared electron density with vanishing features, e.g. side chains. The electron density always contains noise stemming from sources such as experimental/model errors and Fourier series termination. Map sharpening recalculates the electron density in a way that reduces overall map errors, increasing clarity and possibly revealing more features. The problem is considered as an inverse deblurring problem, and solved using Tikhonov regularisers. It is demonstrated that this map sharpening can automatically produce maps with more structural features whilst maintaining connectivity. Tests show that these tools give promising results, although more work needs to be done to further exploit structural information and address the problem of reliable electron density calculation, ultimately leading towards routine refinement at low resolution.

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