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Absorption Correction and Optimal Planning of Data Collection Based on a 3D Sample Model.

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A reconstruction using visual images has been used to produce a 3D model of a macromolecular crystal sample, with the crystal, supporting loop and buffer identified as separate components. The model allows path lengths through crystal, solvent and crystal mount system to be determined, as well as the calculation of the diffraction volume.

A software package, named 3DAC, was developed for building a 3D model of a macromolecular crystal, and treating X-ray diffraction data for absorption effects using the sample shape information [1]. The method offers an effective absorption correction. At lower levels of data redundancy, the algorithm provided a clear advantage, illustrating that it may be of considerable value in situations where data acquisition is limited by crystal lifetime.

The previously published work is now gradually being improved towards the automation and general use: the absorption correction work is being taken forward with a systematic study of several crystal systems and more precise measurements and descriptions of the beam profile; the software package producing the 3D model is also being enriched with a new user interface. In difficult cases (e.g. when the crystal is not fully visible), the user can now fit a polyhedron to the crystal sample.

The 3D model is also being applied to data collection strategies using the BEST software [2][3] by allowing the crystal cross-section to be included in the strategy calculations.

The methods described here are of general interest, particularly for long wavelength X-ray work and very sensitive crystal samples.

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Keywords: 3D modeling; absorption correction; data collection strategy

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Recent Advances in CRANK.

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CRANK is a software suite for automatic macromolecular structure solution. The first release of CRANK was shown to effectively detect and phase anomalous scatterers from SAD data [1]. The current release can successfully build over 200 real SAD, SIRAS, MAD and MAD+native data sets. Improvements in the latest version of CRANK have sped-up and increased the robustness of the automatic structure solution process. As of CRANK version 1.3.x the quality and completeness of an obtained substructure are validated using the Luzzati parameters refined in the program BP3. This approach allows for early termination of the substructure detection stage in a common situation where the figure of merit alone is insufficient to safely decide whether a correct solution has been reached. Furthermore, algorithmic improvements in BP3 have led to about a three-fold speed improvement in substructure refinement and phasing. Additionally, automatic model building has been improved by using SAD data directly in model refinement, made possible by the new interface for ARP/wARP and REFMAC. A modified version of REFMAC, implementing a multivariate SAD likelihood function, extends resolution and phase quality limits required for automatic model building with iterative refinement [2]. In combination with the SHELX[C/D/E] pipeline, this approach has recently been found to be very effective. The addition of Baubles markup has made CRANK log files more insightful and user friendly. These and other improvements are in the latest version of CRANK, packaged with CCP4 6.1.2 and freely available from http://www.bfsc.leidenuniv.nl/software/crank.

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[2] Pavol Skubak, Steven Ness, and Navraj S Pannu., *Acta Crystallographica. Section D, Biological crystallography* 61.Pt 12 (Dec. **2005**), pp. 1626–1635. doi: 10.1107/S0907444905032233

Keywords: crystallography macromolecular; automatic structure solution; phasing methods

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Using Molrep for Fragment-based Electron Density Interpretation at Low Resolution

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Despite the technological advances in X-ray crystallography and electron microscopy, the electron density of large macromolecular complexes is often available to low resolution and is difficult to interpret by conventional methods. Fortunately, a complex often already contains known structural components, which can be further divided into rigid fragments [1,2]. To efficiently position a fragment in an experimentally phased density map at low resolution requires density fitting in real space. Such real space molecular replacement should have a minimal signal interference from the vast "missing part" of the target structure, tolerate reasonable errors in homology models and experimental phases, and be able to find multiple copies of the similar modular domain present in the target

structure even in the absence of molecular symmetry. Here we evaluate the effectiveness of spherical averaged density matching as implemented in Molrep [3] in comparison to conventional molecular replacement methods in positioning a small domain in the electron density map of a multi-domain protein. We investigate how resolution, phase error and model error would influence the quality of density fitting.

[1] Schneider T.R. *Acta Cryst. D.* **2004**, 60, 2269. [2] Mosca R., Schneider T.R. *Nucleic Acids Res.* **2008**, 36, W42. [3] Vagin A.A., Isupov M.N. *Acta Cryst. D.* **2001**, 57, 1451.

Keywords: low resolution crystallography; electron density fitting; algorithms

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Coordinate Variations in Structural Ensembles from Different Refinement Methods. Jacopo Negroni[a], Roberto Mosca[a], Thomas R. Schneider[a].
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It is questionable whether a single model is an appropriate representation of the situation observed in a crystal of a biological macromolecule. In recent years, several authors have suggested procedures to produce ensembles of models that possibly yield a better description of the variability observed in a crystal [1,2].
In principle, the spread observed for a particular atomic position in an ensemble obtained by a particular method can provide a measure of the *precision* of the position. It may be possible to obtain a first-order approximation of the *accuracy* of an atomic position by comparing several ensembles obtained using different refinement methods. Accurate estimates of the accuracy of atomic coordinates would in fact be very useful as these could then be taken into account in structure analysis.
To compare the coordinate variability between different models produced by the same refinement method ('ensemble') and between models produced by different refinement methods, we have created a series of ensembles and single models for the tetragonal crystal form of Lysozyme.
We found that, within ensembles created by one method the coordinate variability is rather small, while between ensembles or models created by different methods, larger differences can be observed.

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Keywords: structure analysis; coordinate error estimation; precision

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Advancement of the Main Chain Tracing in ARP/wARP. Helene Dörksen[a], Victor S. Lamzin[b].
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Comprehensive structural interpretation of the crystallographic experimental X-ray data, having both observed structure factor amplitudes and phase estimates, remains one of the most challenging tasks in the protein crystallography. Our on-going development is addressed to the problem of performing accuracy and completeness of the protein structure provided by the protein chain tracing module of ARP/wARP software. The success of the model building strongly depends on the level of the informational content of the data, where a low level is indicated by noisy structure factors leading to a hardly interpretable electron density map or by the data, whose resolution is limited. We combine several methods including weighted template matching technique and optimisation of the density template alignment. We apply a number of rotation invariant characteristics defined on the density values and centre-atomic distances. This leads to the completeness of the model that is growing from iteration to iteration. We also introduce a resolution dependent parameter, which further increase the completeness of the provided structure. Overall the improvement of the built main chain by 12-25% is achieved compared to the protein chain tracing in earlier ARP/wARP version 7.0.

Keywords: software development; density modification; model building

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Intensity to Amplitude Conversion Using Ctruncate. Norman Stein[a], Charles Ballard[a]. *[a]CCP4, Daresbury Laboratory, Warrington, WA4 4AD, UK.*
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In protein crystallography, calculation of structure factor amplitudes from intensity measurements is complicated by the fact that some weak reflections will have negative intensities, due to the background subtraction process. Ctruncate is a new CCP4 program, ultimately intended to replace the original Truncate program, which uses Bayesian statistics to calculate positive structure factors from negative input intensities, using the French and Wilson algorithm. Ctruncate detects significant anisotropy in the data and performs anisotropy correction. The corrected data is used to calculate a number of data quality indicators, such as moments of intensity and cumulative intensity distributions. Potential twinning operators are calculated from first principles, thus allowing detection of cases of pseudomerohedral twinning which might otherwise be missed. A number of quantitative tests for twinning such as the H test and the L test have been introduced. Ctruncate also checks for the presence of translational Non Crystallographic Symmetry (tNCS) using a Patterson function. The optical resolution is used to give a guide to the limiting resolution of the data. The prior distribution used in Ctruncate is the Wilson distribution, which is only really appropriate in the absence of twinning and tNCS. It is hoped to extend this work to give a quantitative treatment of the modifications to the Wilson distribution induced by these effects.

Keywords: software; protein structure determination; twinning