augmented with time-averaged x-ray restraints [1] to produce a series of Boltzmann-weighted structures that represents the conformational space sampled during a simulation. The resulting ensemble typically contains 100-250 structures and is shown to significantly improve the model error (as judged by Rfree), in comparison with traditional methods. This new method is suitable for diffraction data with upper resolution limits in the range of 1-3Å d-spacing. This method does not require excessive computation time and can be run on a standard desktop machine.

Ensemble refinement was developed, and is available, within the PHENIX software suite [2]. It utilises a maximum-likelihood target function in conjunction with a dual explicit- and bulk-solvent model and can be used with any heterogeneous atom or group.

In addition to the improved global statistics, ensemble refinement reveals highly-resolved local disorder features which are demonstrated to reflect important functional details for a number of test cases.

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Keywords: macromolecular, refinement, disorder

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Low resolution refinement in the program – REFMAC

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Despite rapid advances in Macromolecular X-ray Crystallographic (MX) methods, derivation of reliable atomic models from low resolution diffraction data still poses many challenges. The main reason for this is that the number of observations relative to the number of adjustable parameters is small and furthermore signal to noise ratio in the experimental data is very low. As a consequence derivation of biologically meaningful information from such data is challenging. Intrinsic mobility of macromolecules means that in many cases growing crystals diffracting to higher resolution is not possible and low resolution data must be used to derive some useful structural information.

Statistically sensible analysis of low resolution diffraction data requires tackling of two related but distinct problems i) stabilisation of ill-posedness of refinement procedures - reduction the effective number of parameters without sacrificing completeness of atomic models ii) calculation of maximal signal/minimal noise electron density that would not suffer from bias towards model errors. Solving the first problem is necessary to derive reliable atomic model and the second problem to calculate interpretable electron density that is used in model (re)building.

1) The first problem is usually tackled using additional restraints based on structural information. Available structural information are a) known similar three-dimensional structures b) secondary structures; c) NCS if present; d) in addition it is also possible to exploit the fact that during refinement inter-atomic distances should not change dramatically. It has already been shown that using these restraints improves reliability of the derived models. As a result of model improvement errors in the derived atomic models are reduced, and it means that calculated phases have less error hence reducing noise in the electron density related to the model errors.

2) Sharpening of an electron density while increasing signal amplifies noise masking out “true” signal. There are several approaches to such problems. These include: a) regularisation using Tikhonov-Sobolev method; b) Wiener filters and c) Bayesian filters. These techniques attempt to answer to one common question: how to enhance signal without noise amplification? Another problem in map sharpening is that it assumes that all atoms have the same B values. It is in general not true and there is a distribution of B values – inverse gamma distribution. Moreover individual atoms’ oscillation depends on its position in the asymmetric unit. These facts need to be accounted for if accurate map sharpening tools to be designed. In this presentation some approaches to these problems will also be discussed.

Keywords: refinement, macromolecule, restrained

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Better ligand representation in BUSTER protein-complex structure determination

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The generation of reliable restraints for novel small molecule ligands in protein complexes is of great importance for both model placement into density and subsequent refinement. We have recently released GRADE [1], a procedure whose main source of restraint information is the Cambridge Small Molecule Database (CSD), queried using the MOGUL program [2], developed by the CCDC. Where small-molecule information is lacking, grade uses quantum chemical procedures to set restraint values. GRADE automatically produces restraints that are compatible with the Engh and Huber EH99 restraints used for the protein during building and refinement. Particular care has to be taken when interpreting CSD data in order to produce restraints for torsion angles. This is likely to be because small molecule crystal structures are often less strained than those found in protein complexes.

An alternative to conventional stereochemical restraint functions is provided by the direct use of quantum mechanics to compute the potential energy of the ligand. This involves invoking a quantum chemical program to provide the potential energy and its gradients for the ligand conformation in each cycle of BUSTER refinement. It will be shown how the results of the direct use of QM for ligands in refinement complement the use of CSD data.

[1] BUSTER package http://www.globalphasing.com/buster/. [2] I.J. Bruno et al, *J. Chem. Inf. Comput. Sci.* **2004***, 44,* 2133-2144.

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Structure solution by molecular replacement using *ab initio* protein models

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Molecular Replacement uses a known search model to solve the unknown crystal structure of a related protein, but is dependent on the availability of a model having sufficient structural similarity. *Ab initio* modelling has developed to the extent that its results can sometimes be used to successfully phase diffraction data. Thus, *ab initio* models can be tried as search models where structural homologues are not available and experimental phasing is difficult [1].