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

MS.58.2

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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

MS.58.3

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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.

Keywords: refinement, quantum_chemistry, database

MS.58.4

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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].

Our method employs *ab initio* polyalanine models (or 'decoys'), produced in large numbers then clustered based on the presence of similar core structures. The largest of these clusters is likely to be closest to the native structure [2]. Such *ab initio* modelling may result in an accurate prediction of the structural core of the target, but with inaccurate loops and termini. We have been developing an automated pipeline for the processing of *ab initio* models for use in Molecular Replacement. We show that truncation and clustering of models into ensembles can give a successful result where a single search model would fail. We find that the addition of a selection of side chains can also be used to improve the success rate.

Importantly, the likely success or failure of the modelling can be predicted based on characteristics of the protein such as length and secondary structure, and by the convergence of the modelling program to produce a large cluster of models with a similar core structure. Predictions of success can be given at each stage of the pipeline as data are accumulated to give feedback to the user, and to prioritise models for use in Molecular Replacement.

The pipeline has been tested on 241 proteins between 40-120 residues long, using Rosetta to produce 1000 decoys for each target. Ensembles were produced from these decoys, and Molecular Replacement carried out using MrBUMP. For 40 proteins, at least one search model was placed within 3Å of the deposited structure by MrBUMP, and in a further 42 cases, one or more search model was positioned to within 3-6Å. In 137 cases, ARP/wARP rebuilds resulted in mapping of traced residues to sequence. Of these, 71 proteins show a 50% or greater sequence coverage ratio with 20% or more of the backbone traced.

We have thus shown that *ab initio* modelling can be a viable route to structure solution for many small proteins. Initial results with other *ab initio* programs show success where Rosetta models failed. Similarly, we are extending this work to other rebuilding programs such as buccaneer which may well improve performance further.

This pipeline will be made freely available, and may ultimately require only the input of the protein sequence along with the experimental data. Unlike other computationally intensive methods [3], this method is suitable for modest hardware, allowing for broader adoption.

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[3] B. Qian, S. Raman, R. Das, P. Bradley, A.J. McCoy, R.J. Read, D. Baker *Nature* **2007**, *450*, 259-264.

Keywords: modelling, molecular_replacement, automated_ structure_solution

MS.58.5

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Release 7.2 of ARP/wARP software suite

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ARP/wARP is a software project for automated model building and refinement in macromolecular crystallography. The software is the result of years of development in the areas of X-ray crystallography, informatics and statistical pattern recognition. ARP/wARP is an iterative procedure based on the use of hybrid macromolecular models that is integrated with model refinement and reconstruction to provide a unified approach. The software protocols are computationally efficient and provide an easy-to-use pipeline for the building of models of proteins, poly-nucleotides, bound ligands and solvent.

Version 7.2 of ARP/wARP was recently released and contains new techniques and approaches for the building models of large, low-resolution structures using non-crystallographic symmetry (NCS) and motif searching. NCS-related parts of a structure are rarely built in the same way during model building. A beneficial side effect of this is that each copy provides information that is not present in another copy. By combining this intrinsic information the model building process is improved and the overall completeness of built structures at low resolution is increased. Coupled with novel implementations for enhanced protein chain tracing, the use of NCS provides improvements of up to 18% in model completeness (from 55% to 73% at 3.2Å, for example).

Further developments include automation in modelling of bound ligands that are partially ordered. The electron density map can be automatically screened for density corresponding to any of a cocktail of potential ligands and those that fit best are automatically built. Furthermore, in instances where the density is insufficient to allow accurate identification of atomic coordinates of particular ligands, 'partial' ligands, comprised only of the fragments whose atoms are in unequivocal density, can be modelled.

The graphical front-end *Arpnavigator* allows users to view models in real time while they are automatically built and refined. With the 7.2 release the functionality of the front-end has been considerably extended, and new file formats and display styles of molecules are supported. Publication quality images in standard colours are now easier to produce.

Keywords: model_building, refinement, crystallographic_ Software

MS.59.1

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Halogen vs. Hydrogen bonding in the design of anion receptors

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Halogen bonding (XB), namely any noncovalent interactions involving the positive region of the electrostatic potential surface of halogen atoms [1], has proven its efficiency and reliability in supramolecular chemistry, crystal engineering, and materials science [2]. Its potential and use in anion coordination and anion-templated assembly has been discovered and investigated only recently [3].

In this contribution, we report some examples of anion binding driven by halogen bonding where halides anions act as halogen bonding acceptors.

We will also present how XB directs the self-assembly of oxyanions, by far the most numerous class of anions in organic chemistry, forming discrete adducts and 1D, 2D, or 3D supramolecular networks with halocarbons. Some specific examples will be discussed in order to identify new supramolecular synthons based on halogen bonding and to outline some general principles for the design of effective and selective receptors based on this interaction [4].

It will be demonstrated that the replacement of hydrogen with halogen atoms into anion receptor scaffolds may develop as a convenient strategy to improve binding and selectivity.