MS03.03.04 JOINT MAXIMUM ENTROPY AND NON-CRYSTALLOGRAPHIC SYMMETRY CONSTRAINTS IN MODEL INDEPENDENT MAP REFINEMENT. Charles W. Carter, Jr., Valentin Ilyin, Xin Huang, and GenPei Li*. Department of Biochemistry and Biophysics, CB 7260 University of North Carolina at Chapel Hill, Chapel Hill, NC 27514 USA and *Department of Physical Chemistry, Beijing University, Beijing, Peoples Republic of China.

We have extended previous studies using entropy maximization under the constraint of solvent flatness (MESF) to refine electron density maps having non-crystallographic symmetry. *Bacillus stearothermophilus* Tryptophanyl-tRNA synthetase (TrpRS) crystallizes in a family of different polymorphs, either in the absence of biochemically relevant ligands or when bound to such ligands in the absence of high (10mM) ATP concentrations. Ligand-free enzyme grows either as triclinic (P1) or monoclinic (C2) crystals, and liganded complexes grow as monoclinic ($P2_1$) crystals. Each case presents strong non-crystallographic 32 symmetry in reciprocal space. Placement of the known structure using AMORE has now shown that each asymmetric unit contains either one or two units of three enzyme dimers characterized by 3_1 screw symmetry; all three crystal forms are closely related to space group $P3_121$, previously observed at 18 Å resolution in the $P2_1$ crystals, and thought to be space group P321 (Carter, et al., 1990, *Acta Cryst.* **A46:**57-68). This high redundancy provides important phase constraints.

To preserve subtle differences in TrpRS conformation in each crystal, we are pursuing model-independent refinement of each structure, in order to minimize possible model-induced phase biases. From the initial model phases, cycles of phase improvement consist of the following operations: (1) maximum entropy solvent flattening constrained by subsets of roughly the best third of the current phases (Xiang, et al., *Acta Cryst* **D50:**193-212); (2) phase permutation, if necessary, involving small numbers of reflections with large renormalized structure factors (Doublié, et al., 1994, *Acta Cryst* **A50:**164-182); (3) Non-crystallographic symmetry averaging of centroid $q^{ME}(x)$ maps obtained by MESF. This process is iterated to convergence. Examples will be described, and compared with examples from studies at low resolution, involving Likelihood ranking of phase sets generated by conventional direct methods and subsequent phase extension. (Supported by NIH GM48519-02 and NSF MCB 9304674)

MS03.03.05 HOLOGRAPHIC METHODS IN CRYSTALLOGRAPHY. Abraham Szoke, Hanna Szoke Lawrence Livermore National Laboratory, Livermore, CA 94551 and John R. Somoza Dept. of Biochemistry and Biophysics, UC San Francisco, CA 94143

The holographic method is a novel method for generating electron density maps from crystallographic data. The advantage of this method over conventional Fourier maps is that it can easily take into account additional information and, in this process, it is capable of changing the phases of calculated reflections. Additional information includes: positivity of the electron density everywhere; near constancy of the electron density in the solvent region (similar to solvent flattening); a known part of the molecule (similar to molecular replacement); MIR; MAD and noncrystallographic symmetry. The program that implements the holographic method, EDEN, uses any and all these kinds of information simultaneously and consistently. We will present results of tests both on artificial examples and on real protein data. For example a model protein of 207 residues (Thaumatin) could be perfectly recovered from its last 47 residues (23% of the total) using a low resolution solvent mask that covers 1/2 of the volume of the unit cell. Results will be presented on studies of solvent structures and on MIR.

References: 1. Szoke, A. (1993) Acta Cryst. A49, 853-866. 2. Somoza, J. R., Szoke, H., Goodman, D. M., Beran, P., Truckses, D.,Kim, S-H. & Szoke, A. (1995) Acta Cryst. A51, 691-708. 145

MS03.03.06 HOW DOES SOLVENT FLATTENING REMOVE THE PHASE AMBIGUITY. Bi-Cheng Wang, Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, U.S.A. and Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260, U.S.A.

Although solvent flattening is now widely used for improving electron density maps, the theoretical basis of how this approach can remove the phase ambiguity has not been fully discussed. This presentation will address the basic phasing power of solvent flattening. It will show how this approach can, in principle, systematically remove the phase ambiguity by removing the noise associated with it. The lecture will also address the precautions in the use of single isomorphous replacement and single-wavelength anomalous scattering data as well as data accuracy which is essential to this technique. Calculated and experimental data will be used in the illustration. An extension of solvent flattening techniques to multi-wavelength anomalous scattering data will also be presented.

MS03.03.07 SEMI-AUTOMATED DENSITY FITTING WITH XTALVIEW. Duncan McRee, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037, dem@scripps.edu

The newest release of XtalView from CCMS and MSI includes new features for semi-automated density fitting in XFIT. The program will search the density to find a likely position for the next C-alpha. Five alternatives are also found. The user can also use the mouse to adjust the C-alpha manually using a "baton". After a fragment is built it can be changed to poly-ala with by automated building of overlaaping penatamers. At this point the user can specify position in the amino acid sequence and the program mutates the side chains and find the rotamer with the highest overlap with the density. A number of features have been added to allow naming residues, reversing chains, sorting fragments, and adding chain ID's. The new version can be obtained from CCMS by sending email to ccms-request@sdsc.edu with a blank subject and the message 'get xtalview'.

MS03.03.08 TOWARDS THE FULL AUTOMATION OF MAP INTERPRETATION Thomas Oldfield, Department of Chemistry, University of York, Heslington, York, Y01 5DD UK

Recent developments in recombinant DNA techniques, crystallisation protocols, X-ray data collection techniques and devices, and computing have led to a substantial increase in the speed and number of protein structure determinations in modern crystallographic laboratories. However, there still remains a number of key stages in the crystallographic process which limit the rate of structure determination. One of these is fitting electron density maps, either in the initial stages of tracing a chain to a new map, or in the manual rebuilding during refinement. This is a particularly onerous task, requiring many days and often weeks of working at a graphics terminal with maps and model.

The electron density applications available within the new version of QUANTA (QUANTA 96), represent novel and effective tools for speeding up this process. The various modules (X-AutoFit, X-Ligand, X-Solvate and X-Build) have been developed over the past year in close collaboration with the large number of crystallographers working on projects in the Protein Group at York. Crucially, these new tools are easy to learn and natural to use, providing up to a 10 fold reduction in the time spent at the graphics terminal.

During my presentation, I will show how the semi-automated tools within this application can be used for the interpretation of maps calculated with experimental phase information. I will also present methods which go some way towards the automatic interpretation of electron density, and the limitations of these methods as a function of resolution and quality of maps.