stronger chemical constraints. One such constraint is the connnectivity of the macromolecule. We have developed a rapid algorithm for measuring the connectivity of a map which shows promise in reducing the multiplicity of solutions to the phase problem. We have also developed a refinement method (PRISM) which exploits the connectivity constraint to iteratively improve phases. An initial electron density map is generated with inaccurate phases derived from a partial structure or from isomorphous replacement. A linear connected skeleton is then constructed from the map using a modified version of Greer's algorithm and a new map is created from the skeleton. This "skeletonized" map is Fourier transformed to obtain new phases, which are combined with any starting phase information and the experimental structure factor amplitudes to produce a new map. The procedure is iterated until convergence is reached. The method has been applied to problems with starting phase information from either molecular replacement or isomorphous replacement and appears to be a significant improvement over solvent flattening in both cases.

DS-02.07.04 DIRECT METHODS AND MACROMOLECULAR CRYSTALLOGRAPHY: LIGHTS AND LIMITS. By C. Giacovazzo*, A. Guagliardi, Dipartimento Geomineralogico, Universita' di Bari, 70124 Bari, Italy; D. Siliqi, Department of Inorganic Chemistry , University of Tirana, Tirana, Albania.

Several papers can be found in literature which describe the application of Direct Methods to macromolecules. Their efficiency is tested both for *ab initio* phasing and for phase refinement and extension. In this paper the role of direct methods in the field of macromolecular crystallography is analyzed. A criterion is formulated which suggests the necessary conditions for the success or the failure of the *ab initio* direct procedure. Most of the experimental protein data do not satisfy such a criterion, therefore their ab initio solution is a quite improbable event.

DS-02.07.05 JOINT X-RAY AND NMR REFINEMENT. By B Shaanan, Department of Biological Chemistry, The Institute of Life Sciences, The Hebrew University of Jerusalem, Israel

PS-02.07.06

AMoRe, An Integrated Molecular Replacement Program in Protein Crystallography : Some Applications to Multibody Systems. By J.Navaza[1], Y.Mauguen[1], P.Saludjian[2], T. Prangé[2], P.Alzari[3] and G.A.Bentley[3]. [1]Laboratoire de Physique, Faculté de Pharmacie, 92290 CHATENAY-MALABRY, France. [2]Chimie Biomoléculaire, URA 1430 CNRS, UFR Biomédicale, 93012-BOBIGNY CEDEX, France. [3]Unité d'Immunologie Structurale, URA 359 CNRS, Institut Pasteur. 25 rue du Docteur Roux, 75724 PARIS CEDEX, France.

A new strategy for Molecular Replacement calculations in protein crystallography has been implemented in the AMoRe package of programs. The algorithms have now been extensively tested in several crystal structures containing multiple copies of the proteins in the asymmetric unit. The examples discussed in the present communication include :

-The complex between Fab F9.13.7 and Guinea-fowl lysozyme with two molecules in the asymmetric unit, using three different search probes (lysozyme, variable and constant regions of the Fab), five out of the six subunits could be sequentially positioned in a single run of AMoRe.

-The trigonal form of Tumor Necrosis Factor, with six copies in the asymmetric unit (a dimer of trimers), using a trimer as the search model.

-A new orthorhombic form of Erabutoxin-b cristallized in presence of KSCN (two copies in the asymmetric unit).

-The complex of a bacterial ribonuclease, barnase, with its specific proteic inhibitor, bastar. There are three copies of the complex in the asymmetric unit. The barnase structure, representing approximately 1/6th of the a.u., was used as the search model.

-An hexagonal form of bastar with four molecules in the a.u., using as the search model the inhibitor subunit, taken out from the above refined complex.

PS-02.07.07 **PHASE PERTURBATION AS A MEANS OF REDUCING MODEL BIAS IN MACROMOLECULAR CRYSTALLOGRAPHY.** By M.V.Hosur and K.K.Kannan Solid State Physics Division, Bhabha Atomic Research Centre,Trombay, Bombay-400085,INDIA.

The **Molecular Replacement Method** is being increasingly used to solve protein structures by X-ray crystallography. It has also been recognized that the search model used in the above method, introduces a phase bias that complicates interpretation of the calculated electron density maps. A number of attempts have been made to reduce this model bias either by calculating OMIT maps or by using modified amplitudes in Fourier calculations. However, the features of a Fourier map are determined more by the phase rather than the amplitude of the coefficient. We have therefore explored the possibility of altering the phases of the coefficients as a means of reducing model bias in Fourier calculations. A variety of schemes of phase perturbation have been tried, with interesting results. These results and their implications to solving protein structures by the Molecular Replacement Method will be discussed.

PS-02.07.08

ASSESSMENT OF BULK SOLVENT MODELS BY CROSS-VALIDATION. By A.T. Brünger and J.-S. Jiang*, Department of Molecular Biophysics and Biochemistry, Yale University, U.S.A.

Bulk solvent models can play an important part in the modeling of macromolecular diffraction data. Bulk solvent models are aimed at reducing the residual for the low-resolution reflections. A low R value is not necessarily an indicator for the quality of the model. For example, we have shown earlier (Brünger, A.T., *Nature* **355**,472-474, 1992) that a bulk solvent model consisting of a disordered liquid of point atoms actually worsens the information content of the crystal structure. Cross-validation showed considerable promise to avoid this type of overfitting.

50 02-Methods for Structure Determination and Analysis, Computing and Graphics

We have assessed the relative information content of a number of bulk solvent models using cross-validation. The free R approach was generalized to study the quality of the fit for all reflections as opposed to a single test set. We compared the simple homogeneous solvent mask model (Wang, B.-C., *Methods Enzymol.* **115**,90-112, 1985), the multiple-shell model (Schoenborn, B.P., *J.Mol.Biol.* **201**,741-749, 1988; Cheng, X.D. and Schoenborn, B.P., *Acta Cryst.* **B46**,195-208, 1991), and the difference-map model (Badger, J. and Caspar, D.L.D., *Proc. Natl. Acad. Sci.*, 622-626, 1991). The relative merit of these models will be discussed.

PS-02.07.09 PHASE DETERMINATION OF MACROMOLECULAR CRYSTALS FROM MULTIPLE X-RAY DIFFRACTION USING A CONVENTIONAL LABORATORY SOURCE. By Mau-Tzai Huang, Chien-Mei Wang, and Shih-Lin Chang*, Department of Physics, National Tsing Hua University, Hsinchu, Taiwan

Coherent interaction among diffracted waves in x-ray multiple diffraction has been successfully utilized in determining phases of structure-factor multiplets for organic crystals. For macromolecular crystals, synchrotron radiation is generally believed to be an indispensable source for phasing the structure factors in multi-beam experiments, because the signal-to-noise ratio of the multiply diffracted intensities are usually very small. However, the limited synchrotron beam time and the fast deterioration of crystals under high photon flux are the disadvantages of using synchrotron radiation.
To overcome this difficulty, we have used a rotating-anode x-ray generator together with a z-axis four-circle diffractometer to carry out multiple diffraction experiments. The z-axis is a vertical arm for the detector mounted perpendicular to the diffracting plane. Secondary reflections can therefore be easily detected. We have applied this geometry to observe the intensities of many 3-beam and 4-beam diffractions for lysosome crystals. The associated phases are correctly determined. The condition of observing the weak multi-beam intensities is derived and the difficulties encountered in the experiments are discussed.

PS-02.07.10
COMPARISON OF REAL-SPACE AND RECIPROCAL SPACE CRITERIA TO ASSESS THE ACCURACY OF MACROMOLECULAR CRYSTAL STRUCTURES. By J.-S. Jiang*, R.E. Hubbard and A.T. Brünger, Department of Molecular Biophysics and Biochemistry, Yale University, U.S.A.

A number of real-space and reciprocal space criteria to assess the quality of atomic models has been compared. Among the criteria are the free R value (Brünger, A.T., *Nature* **355**,472-474, 1992), the real space R value (Bränden, C.I. and Jones, T.A. *Nature* **343**,687-689, 1990), continuity of density maps (Engh, R.A. and Huber, R., *Acta Cryst.* **A47**,392-400, 1991) and satisfaction of geometric and stereochemical restraints. We have also generalized the real space R value to measure the agreement between atomic shapes of observed and computed density maps.
Two examples are presented: TAKA alpha-amylase (Swift, H., et al., *Acta Cryst.* **B47**,535-544, 1991) and the B72.3 Fab chimeric antibody fragment (Brady, R.L., et al., *J. Mol. Biol.* **227**,253-264, 1992). We compared partially incorrect models with the correct model. Results show that the various criteria are correlated to the mean phase error of the models.

PS-02.07.11 CONNECTIVITY AND THE PHASE PROBLEM IN MACROMOLECULAR CRYSTALLOGRAPHY.

D Baker*, C Bystroff, A Krukowski, C Wilson and D Agard, Department of Biochemistry and Biophysics, UCSF

The crystallographic phase problem is indeterminate in the absence of additional chemical information. The commonly employed chemical constraints—positivity, atomicity, and a solvent boundary—leave the phase problem greatly underdetermined for Fourier data sets of moderate (2.5–3.0Å) resolution. A successful *ab initio* approach must make use of high resolution Fourier data and/or stronger chemical constraints. One such constraint is the connectivity of the macromolecule. We have developed a rapid algorithm for measuring the connectivity of a map which shows promise in reducing the multiplicity of solutions to the phase problem. We have also developed a refinement method (PRISM) which exploits the connectivity constraint to iteratively improve phases. An initial electron density map is generated with inaccurate phases derived from a partial structure or from isomorphous replacement. A linear connected skeleton is then constructed from the map using a modified version of Greer's algorithm and a new map is created from the skeleton. This "skeletonized" map is Fourier transformed to obtain new phases, which are combined with any starting phase information and the experimental structure factor amplitudes to produce a new map. The procedure is iterated until convergence is reached. The method has been applied to problems with starting phase information from either molecular replacement or isomorphous replacement and appears to be a significant improvement over solvent flattening in both cases.

PS-02.07.12 COLLAGEN FIBRIL STRUCTURE BY X-RAY SYNCHROTRON RADIATION STUDIES OF MULTIPLE ISOMORPHOUS DERIVATIVES

A. Miller*, T.J. Wess, L. Wess, Biochemistry Department, Edinburgh University, U.K.
M. Saad and A. Hammersley, ESRF, Grenoble, France.

Multiple isomorphous derivatives tested against the native X-ray diffraction pattern, were used to phase the (h, k, l) reflections and produce an electron density map of the collagen fibril. This allows conclusions to be drawn about the molecular packing in native fibrils and the molecular conformation of the telopeptides which contain the inter-molecular crosslinks.