01.3-1 MERLOT: A PACKAGE OF COMPUTER PROGRAMS FOR THE DETERMINATION OF PHASES USING THE MOLECULAR REPLACEMENT METHOD. By P.M.D. Fitzgerald, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

The term molecular replacement, as used here, describes a technique whereby an initial phasing model can be obtained for a crystal structure by "replacing" the unknown structure with a properly oriented and positioned "molecule" of identical or related structure.
MERLOT is an integrated package of computer programs to perform all of the calculations needed for phase determination using the molecular replacement method. The package contains two versions of the rotation function, three methods for solution of the translation problem (a translation function, a packing function and an R value mapping program), a program for testing the feasibility of a potential solution, a program for optimizing a potential solution and various programs with set-up or result analysis functions. The programs are applicable to both macromolecular and small molecule phase determinations. All non-centrosymmetric space groups (excluding rhombohedral and cubic) are provided for.
The programs have been tested on a set of macromolecular test structures that were selected to represent the range of problems for which phase determination by molecular replacement has been attempted. Included are cases in which the search structure is an exact or very close match to the unknown structure and cases in which the two differ markedly; cases of low, medium and high crystallographic symmetry; and cases of one copy of the unknown structure per asymmetric unit or more than one copy per asymmetric unit.
The following general conclusions can be drawn from these test calculations:
1) In cases where the search structure is a good approximation to the structure of the unknown, obtaining a molecular replacement solution is relatively straightforward, even in cases of high crystallographic symmetry or multiple copies in the asymmetric unit. A default set of parameters for all stages of the calculation has been determined for this class of problems.
2) In cases where the correspondence between search and unknown structures is lower, achieving a correct solution is often extremely dependent on the parameters used in the calculation and no generally applicable strategy can be described. In such cases, use of a different search structure or modification of the search structure to better match the unknown can be efficacious.
The program package, which includes machine independent Fortran source code, complete documentation and three fully documented test cases is available on request from the author.

01.3-2 STRUCTURAL ANALYSIS OF THE COMPLEX OF MUNG BEAN TRYPSIN INHIBITOR WITH PORCINE TRYPSIN AT 2.8 Å RESOLUTION. By G.D.Lin, J.C. Xuan, J.H.Fu, Y.W.Yan, R.G.Zhang, Z.W.Chi, T. Q.Tsao, Shanghai Institute of Biochemistry, Academia Sinica, and Z.G.Chen, X.C.Wei, G.P. Li, G.Y.Lu, X.P.Dai, Y.Q.Tang, Institute of Physical Chemistry and Department of Biology, Peking University, Peking,China.

Mung Bean trypsin inhibitor (MBI) belongs to Bowman-Birk type inhibitor with two active domains and LYS-SER and ARG-SER as the respective active centers. We have been specially interested in the structural determination of the complex of MBI with porcine trypsin at a molar ratio 1:2. It was crystallized with unit cell of symmetry $P3_1$ and dimensions a=b=62.46,c=160.01 Å. Its rough structure has been solved with molecular replacement method. The two trypsin molecules of the complex were correctly oriented and located, and their models rebuilt. The electron density map of MBI has also been interpreted. MBI is apparently situated between the two enzymes which are related by a non-crystallographic two-fold axis perpendicular to the crystallographic three-fold screw axis. The results have shown the structural details of the complex molecule as well as the inter-molecular interactions of MBI and the two enzymes. Both the side chains of active site LYS-20 and ARG-47 respectively extend into the specific pocket of each trypsin, and approach the ASP-189 of each. The "LYS" and "ARG" active loops of MBI are found on the top and the bottom of the molecule with opposite trends of polypeptide chains. The active sites of the two loops widely interact with trypsin molecules and stabilize the complex structure. Our work is now proceeding to refine the complex structure at 2.8 Å resolution.

01.4-1 PHASE EXTENSION BY COMBINED USE OF SOLVENT FLATTENING AND ENTROPY MAXIMIZATION. E. Prince, Institute for Materials Science and Engineering, National Bureau of Standards, Gaithersburg, MD 20899, U. S. A., and L. Sjölin, R. Alenljung and A. Svensson, Institute for Inorganic Chemistry, Chalmers University of Technology and University of Göteborg, S-41296 Göteborg, Sweden.

Using an efficient algorithm for finding a maximum entropy density distribution under the constraint that $\langle \sum |F| \cos(2\pi \mathbf{h}\cdot\mathbf{r} - \phi) \rangle$, where the sum is over a set of reflections whose phases have been previously determined, is constant, a procedure has been developed for extending phases to higher resolution in macromolecular structures. A density map is calculated at low resolution using the best available set of phases. The molecular envelope is identified, and density in the solvent region is set to a constant value. Within the envelope the entropy maximization procedure, which tends to sharpen peaks and flatten valleys in the map, is applied. Structure factors calculated from this map correspond to a density distribution, which must be everywhere positive, that might be described as "least surprising, given the prior information used in the density map." Phases from this map and the observed amplitudes are used to calculate a new map at higher resolution, and the procedure is repeated until all observed reflections have been included.
Using low resolution phases from the refined structure of ribonuclease A, the procedure quickly reproduced the high resolution structure. Starting with limited sets of phases determined by single isomorphous replacement, readily interpretable maps were obtained for the previously unknown structures of calcium containing fragment 1 of bovine prothrombin and of fragment TR2C from bull testis calmodulin to 2.4Å and 3.2Å, respectively. The mathematics for the numerical solution of the maximum entropy problem and the restraint procedure will be described.