01.X-01 REFINEMENT OF BIOLOGICAL MACROMOLECULAR STRUCTURES - AN OVERVIEW. By J.L. Sussman Department of Structural Chemistry, Weizmann Institute of Science, Rehovot, Israel

During the past five years there has been a virtual revolution in the crystallographic refinement of biological macromolecules. This is primarily due to:

1) Refinement in reciprocal space, i.e. against structure factors, rather than in real space.
2) The explicit introduction of stereochemical information into refinement programs to overcome the severe limitation of the low data/parameter ratio.
3) The development of very fast algorithms for the rate limiting steps of the refinement process.

The advantage of working in reciprocal space is that the observed X-ray data are used directly in the refinement process, yielding much faster convergence than generally found in real-space methods.

The ways in which stereochemical information has been introduced can be looked at in terms of three different approaches to the manipulation of the structural parameters. At one extreme are the programs where the cartesian coordinates of each atomic position can be varied independently, while the stereochemistry is restrained to standard values by spring-like energy terms between atoms (Hermans & McQueen, Acta Cryst. (1974) A30, 730; Konnert, Acta Cryst. (1976) A32 614; Hendrickson & Konnert in "Biomolecular Structure, Conformation, Function and Evolution" Vol. 1, (ed. Srinivasan), pp. 43-57 (Pergamon, Oxford, 1979); Jack & Levitt, Acta Cryst. (1978) A34, 931; Dodson, Issacs & Rollett, Acta Cryst. (1976) A32, 311). At the other extreme are programs where the bond lengths and bond angles are strictly constrained and the only variable parameters are the dihedral angles of the backbone and side chains (Diamond, Acta Cryst. (1971) A27, 436; Fitzwater & Scheraga, Acta Cryst. (1980) A36, 211; Arnott, Dover & Wonacott, Acta Cryst. (1969) B25, 2192). The third approach, between these two, consists of a constrained group refinement procedure (Sussman, Holbrook, Church & Kim, Acta Cryst. (1977) A33, 800; Hoard & Nordman, Acta Cryst. (1979) A35, 1010). Here the groups need not be completely rigid but may contain any number of flexible dihedral angles and the stereochemistry between groups is maintained by restraints as described in the first method.

Fast algorithms have been introduced in the solution of the least-squares normal equation matrix by means of the conjugate-gradient procedure as well as in the calculation of the structure factors and derivatives via the fast-fourier transform, FFT, (Agarwal, Acta Cryst. (1978) A34, 791).

01.X-02 EXPERIENCES IN REFINEMENT WITH CHAIN CONSTRAINTS IN REAL SPACE AND ENERGY RESTRAINTS IN RECIPROCAL SPACE. By W. Steigemann, Max-Planck-Institut fuer Biochemie, 8033 Martinsried, F.R.G.

The problem of low overdetermination of the least-squares system in the refinement of protein structures may be overcome by mainly two approaches: 1) reduction of the number of parameters or 2) increase of the number of observations.

A refinement method of the first kind is the "real space refinement procedure" (Diamond, Acta Cryst. (1971) A27, 435). It has been used extensively in our laboratory for the refinement of several protein structures. This procedure has the general advantage of being a volume fitting method which allows the use of any kind of electron density map (calculated either with m.i.r. phases or model phases or combination of the two). Major disadvantages are the absolute rigidity of stereochemical parameters in the model and the ignorance of non-bonded interactions, two facts which are prohibitive to good convergence, especially in the case of manual intervention and in the case of resolution lower than 3A. For these reasons the main application of this method is nowadays initial refinement in a m.i.r. map when no reliable calculated phases are available.

For refinement with phases calculated from the atomic model the procedure of Jack & Levitt (Acta Cryst. (1978) A34, 931) is used (implemented by J. Deisenhofer (Biochem. (1981), in press) in our laboratory). This method falls into the second class and is a combination of crystallographic least squares refinement in reciprocal space with simultaneous minimization of potential energy. The stereochemical parameters, bond lengths and angles, known from small molecule crystallography, and their associated force constants, roughly known from spectroscopy, have to be regarded as additional observations introduced into the least-squares problem. The discrepancies to the values calculated from the model are minimized on the basis of energy values. One may here also talk of restraints (or flexibility) in stereochemical parameters in contrast to constraints (or absolute rigidity) in the real space procedure. The Jack-Levitt method has its major advantage in the inclusion of non-bonded interactions which is of particular importance in low to medium resolution refinement. In addition, repair of stereochemical distortions is easily possible. This makes this procedure most valuable in a progressed state of refinement.

Another refinement method, which makes use of constraints within groups of atoms and restraints between them, is established in the program CORELS (Sussman, Holbrook, Church and Kim, Acta Cryst. (1977) A33, 800). We found this procedure particularly useful for orientational and translational refinement of domains (Marquart, Deisenhofer, Huber & Palm, J. Mol. Biol. (1980) 141, 369).

Examples of best application of each of the methods will be given.