01.4-08 STRATEGIES FOR THE LEAST-SQUARES REFINEMENT OF PROTEIN STRUCTURES. By D. S. Moss, M. J. Stanford and G. J. Wistow, Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, UK.

The optimum strategy for the initial stages of least-squares refinement of a protein was investigated.

A model of the eye lens protein γ-crystallin II was built into a 2.6Å resolution electron density map using an interactive computer graphics system. Least-squares refinement of this model was undertaken using structure amplitudes and isomorphous phases to a resolution of 2.6Å. The effect of errors in the observed data was examined by comparing the results of refinements using structure factors derived either from experimental observations or calculated from a high resolution model. Various strategies were employed for weighting the structure amplitudes in several independent schemes of refinement. Each scheme was pursued until the rms atomic shift was less than 0.02Å, at which point intervention on the graphics system would have been required.

Progress was monitored by vector mapping the atomic shifts, and comparing refined coordinates with the high resolution model. The CPU time and real time required for each strategy was also considered. Schemes which gave a high weight to low resolution data for the first part of the refinement required the least CPU time and were the only ones capable of producing atomic shifts of sufficient magnitude to correct some of the larger errors in the model.

01.4-09 PHASE IMPROVEMENT BY DENSITY MODIFICATION IN THREE LARGE STRUCTURES. By R.W. Schevitz, A.D. Podjarny, M. Zwick, J. Hughes, E.M. Westbrook, D. Feldman, and P.B.Sigler, Dept. of Biophysics and Theoretical Biology, The University of Chicago, Chicago,Ill. 60637.

Density modification is a method of direct phase improvement or extension in which sensible restrictions not dependent on a detailed interpretation of the electron density map are imposed on an initial or provisional map to yield in turn more accurate phases following (fast) Fourier transformation. These phases are then merged with the initial set in subsequent iterations to give a new image of greater interpretability. Non-negativity of the electron density and constancy of the solvent regions were the restrictions exploited in three macromolecular structural studies ranging from low to high resolution. 2633 MIR phases of yeast $tRNA_f^{Met}$ which spanned from 14 to 4.5Å resolution having an average phase error of 68° were improved and extended following density modification to a 3545 reflection phase set ranging from 100 to 4Å resolution having an average phase error of 43°. Interpretability of the map was improved, and it resembled closely the map calculated from the refined molecular coordinates. A 2.5Å MIR map of phospholipase A_2 from C. atrox was improved sufficiently by density modification to substantially improve the tracing of the backbone. A 6Å MIR map of ketosteroid isomerase was improved by density modification to allow recognition of the molecular boundaries of two independent dimers in the asymmetric unit.

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01.4-10 A COMPUTER PROCEDURE FOR EXTENSION OF MODEL ELECTRON-DENSITY FOR DENSITY MODIFICATION OR FOR REFINEMENT PURPOSES. By T.N. Bhat and D.M. Blow, Blackett Laboratory, Imperial College of Science and Technology, London SW7 2BZ England.

An efficient computer procedure has been developed for the extraction of regions of continuous well-connected high density from a three-dimensional electron-density map. This may be used to produce a more realistic electron-density distribution from a poorly phased protein map at moderate resolution, by a cyclic process. The procedure may be used to generate an extended model volume from a smaller volume, based, for example, on a starting atomic model of the partial structure; or it can identify possible structural features in the uninterpreted regions of the electron-density by picking up large continuous islands of positive electron density. The procedure can also be used to trace molecular boundaries or solvent regions in an automatic way. Once the density within the extended model volume has been scaled to the starting model, they can be used together as an improved electron-density distribution of the molecule. Phases calculated from this density may be used in a phase combination procedure to improve the starting phases for the next cycle of a convergent process. Where portions of the electron-density cannot be interpreted at the atomic level, they can be incorporated in the calculation of phase angles during least-squares refinement of structural parameters, giving improved convergence to the refinement. The procedure has been applied to the structure determination and refinement of tyrosyl-tRNA synthetase.

01.4-11 ON THE PHASE EXTENSION AND REFINEMENT OF METMYOGLOBIN USING DIRECT METHODS. By G.J. Olthof and H.Schenk, Laboratory for Crystallography, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands.

The phase extension and refinement procedure of Olthof, Sint & Schenk (Acta Cryst.(1979), A35, 941-946) was applied to the protein metmyoglobin (Takano, J. Mol. Biol.,(1977), 537-568)*

For refinement the modified tangent formula was used:

$$\exp i\phi_H = \sum_K E_3 \exp\{i(\phi_K+\phi_{H-K}-s\Delta_3)\} / \left|\sum_K E_3 \exp\, i(\phi_K+\phi_{H-K}-s\Delta_3)\right|$$

The unknown signs s are chosen such as to minimize the value of $|-\phi_H+\phi_K+\phi_{H-K}-s\Delta_3|$. Where the normal tangent refinement leads to centrosymmetric phases in a few cycles, the modified procedure is both stable and enantiomorph conserving. Moreover, phases were derived for some reflections which were not reliably phased by protein methods. Inclusion of these reflections led to a density map of slightly better quality than the original one.

Two phase extension and refinement experiments were carried out, the first extending data from 2.8 Å resolution to 2.0 Å, and the second from 3.0 to 2.0 Å. Comparison of the density maps calculated with the starting phases with maps based on the final phases shows a definite improvement, although the 2.0 Å map is not as good as the map based on the Data Bank phases. For the two experiments phases deviate from the Data Bank phases by 37 and 45° respectively. The height of the Fe-atom in the maps is of the expected magnitude.

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