01.4–1 EXPERIENCES IN PHASE EXTENSION BY SYMMETRY AVERAGING APPLIED TO HEMOCYANIN. By C. E. Nordman, Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA.

A symmetry-averaging procedure in which electron density values to be averaged are retrieved, and averages inserted, by means of a superposed symmetry grid (Nordman (1980), Acta Cryst. A36, 747-754) was used in the low-resolution structure analysis of satellite tobacco necrosis virus (STNV), where 10 Å phases from double isomorphous replacement (DIR) were used as the starting point in a stepwise phase extension to 4.0 Å (Unge et al. (1980), Nature 285, 373-377). To evaluate the procedure in a case less extreme (i.e., less favorable) than the 60-fold noncrystallographically symmetric STNV, we have applied an improved version to hemocyanin, with 6-fold noncrystallographic symmetry (Gaykema et al. (1983), Chemica Scripta 21, 19-23) using $|F_{obs}|$ values to 3.2 Å resolution and DIR phases to 5.0 Å kindly provided by W. G. J. Hol, University of Groningen.

Phase extension from 10 to 6.7 Å resolution gives good, and steadily improving, agreement with refined 6.7 Å DIR phases. The latter phase set, extended to 3.7 Å shows agreement in the 6.7-5.0 Å range with the refined 5.0 Å DIR phases to av. $\Delta\phi$=14°, and apparent convergence, beyond 5.0 Å, to phases generated by extending the 5.0 Å phases, in small steps, to 3.2 Å (150 cycles, 2.4 min CPU/cycle, Amdahl 5860). 1.5% of the measured reflections beyond 5.0 Å resolution were treated as "unknown" and their true magnitudes were not used in the phase extension. Good agreement between Fc and Fo for these reflections (R = 0.25-0.35) provides unbiased support for the essential correctness of the phases, as does emergence of interpretable detail matching that found by Hol and co-workers in maps based on 4.0 Å DIR phases. Observations on rate of convergence, treatment of missing or unobserved data, further progress, and possible pitfalls will be discussed.

01.4–2 "COMPARISON OF DIFFERENT DENSITY MODIFICATION METHODS FOR IMPROVING THE IMAGE OF A PROTEIN MAP AT HIGH RESOLUTION". By A.D. Podjarny,*@#,J.L. Sussman*+ , T.N. Bhat*,E.M. Westbrook @, , M. Harel+ , A. Yonath+ and M. Shoham+ .* NIH,NIADDK,Building 2. , Bethesda, Md., USA;+ Dept. of Structural Chemistry, Weizmann Institute of Science, Israel. @ Dept. of Biophysics and Theoretical Biology , University of Chicago, USA.

X-Ray data extending up to 2.2 A resolution from a 2Fe-2S ferredoxin from Halobacterium from the dead sea [Sussman et al.,(1979), J.Mol.Biol. 134,375-377] were used for the comparison of different methods of electron density modification. A set of phases was available from a Pt derivative and anomalous scattering from the Fe atoms. A partial tentative model was available based on molecular replacement methods [Sussman et al., in preparation]. However, the quality of the SIR+ANOMALOUS electron density map did not permit further improvement of the model. Density modification methods were applied to this work and compared. These methods were:
1) Attenuation of negative density and truncation of positive values.
2a)Automatic boundary definition based on an electron density mask calculated from the entire data after multiplying it in reciprocal space with the transform of a smearing function. This has been shown (E. Westbrook, personal communication) to be the reciprocal space analog of defining the molecular envelope on the basis of a real space average calculated over a large radius, e.g., 9 A (B.C. Wang, submitted 1984).
2b)Solvent levelling to impose non negativity outside the mask determined in 2a.
3a)Automatic boundary definition using the principle that the local average of electron density in the neighbourhood of the protein region should be high and either form a cluster of electron density points of large volume or if a tentative model exists, it should be connected with this model density. [Bhat and Blow, Acta Cryst.,(1982), A38, 21-29]

3b)Merging the model (if model exists) with the observed electron density, along with solvent flattening outside the mask determined by step 3a)[Bhat and Blow, Acta Cryst., 1982), A38, 21-29].

Applying step 1 without phase combination with SIR phases was shown to be ineffective. Therefore, steps 1, 2, and 3 were applied separately including at each step phase combination with SIR phases, and the resultant three maps were compared. The results are the following:
1)The map shows increased detail overall.
2)The map clearly shows the protein region relative to the solvent.Increased detail in the protein region, rendering some zones interpretable.
3)The map is improved over the previous step. The interpretability is sufficient to be used to arrive at the present model for the entire structure.

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01.4–3 CRYSTAL STRUCTURE OF BENCE JONES PROTEIN PAV. By J. Rose, D. Yang, Z.B. Xu, W. Furey, C.S. Yoo*, B.C. Wang and M. Sax, Biocrystallography Laboratory, P.O. Box 12055, VA Medical Center, Pittsburgh, PA 15240, U.S.A. and the Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260, U.S.A.

Bence Jones protein Pav (M.W. 45,000) is an immunoglobulin light chain dimer (λ type) isolated from the urine of a myeloma patient. Crystals have been grown in our laboratory (Wang, et.al., J.Mol.Biol. 116, 1977). The crystals are orthorhombic with space group $P2_12_12_1$. The cell parameters are: a = 94.5 Å, b = 93.4 Å and c = 72.7 Å. Complete sets of 4.5 Å data for both native and a derivative containing $K_2Pt(CNS)_4$ were collected at low temperature using a Picker 4 circle diffractometer. Phase information for Pav has been obtained to this resolution by the ISIR method (Wang, ACA Winter Meeting, March, 1982).

A preliminary protein structure at 4.5 Å resolution has been determined by fitting the coordinates of Bence-Jones protein Mcg to the electron density map produced by the ISIR method. A rigid body refinement of this model converged to an R factor of 0.42.

A native data set to 2.8 Å has been obtained using oscillation photography and a 10 KW rotating anode x-ray source. The film data has now been processed and we are currently extending the phasing by a number of different techniques. The results of this study will be presented. This work is supported by NIH grant Am Ca 18827 and the Medical Research Service of the VA Medical Center.

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