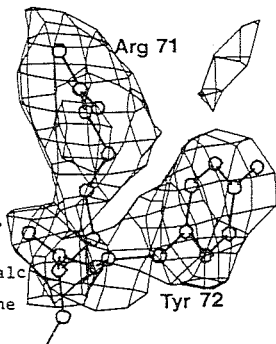


lines than it does in crystals.

It has been necessary to develop for fiber diffraction many methods already established in crystallography. Restrainted least-squares refinement has proved very useful, despite the limited number of data available. Information from partial structures will be increasingly important as crystal structures become available for the monomers of important biological assemblies. The difference Fourier method has been developed for fiber diffraction; the peak heights, noise levels and optimal Fourier coefficients are quite different from those found in crystallography, for example, in TMV at 3Å resolution, coefficients  $6F_{obs} - 5F_{calc}$  have been shown in both theory and practice to be most effective. These methods have been used in this laboratory to solve TMV at 2.9Å by MDIR and layer-line splitting, and to refine the structure by restrained least-squares in conjunction with difference Fourier-Bessel maps. Other helical viruses are under study; work on cucumber green mottle mosaic virus is at an advanced stage. The structure of microtubules has been solved at low-resolution (18Å) in collaboration with Drs. Cohen at Brandeis and Beese at Yale, using a model from electron microscopy and density modification refinement. The structure of Pfl is being determined by Dr. Makowski's group at Columbia using model-building and restrained least-squares refinement. Supported by NIH grant GM33265.

Figure: Part of a  $6F_{obs} - 5F_{calc}$  map of TMV with Arg 71 omitted from the model clearly shows the position of the side-chain.



#### 01.2-2 X-RAY CRYSTALLOGRAPHIC STUDIES OF HUMAN SERUM AMYLOID P-COMPONENT (SAP)

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Serum Amyloid P-Component (SAP) is a normal plasma glycoprotein belonging to the pentraxin family of plasma proteins, of which the other member in man is the homologous protein C-Reactive Protein (CRP), the classical acute phase reactant (Mantzouranis et al., JBC, 280, 7752-7756, 1985; Pepys & Baltz, Adv. Immunol. 34, 141-212, 1983). SAP is composed of ten glycosylated subunits, each of Mr 23,500, which are non-covalently associated in two pentameric discs interacting face-to-face, though under non-physiological conditions the pentamers can dissociate.

Pentameric SAP crystallizes in space group  $P2_1$ , cell constants  $a=69.0$ ,  $b=99.3$ ,  $c=96.8\text{\AA}$ ,  $\beta=95.1^\circ$ , with one pentamer per asymmetric unit. The crystals diffract well to at least 2Å resolution. Data from native crystals and two isomorphous heavy atom derivatives have been collected on a FAST TV-detector diffractometer.

Analysis of the self-rotation function clearly indicates the orientation of the non-crystallographic 5-fold symmetry axis. Progress on the analysis of the structure both by molecular replacement, using as search-model an unrelated pentameric molecule, and by isomorphous replacement will be presented.

01.2-3 MULTIPLE-WAVELENGTH PHASE DETERMINATION IN PROTEIN CRYSTALLOGRAPHY. Janet L. Smith\*, Arno Pähler, H. M. Krishna Murthy and Wayne A. Hendrickson, Dept. of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, USA.

The tunability of synchrotron radiation provides an excellent means to exploit the dispersive anomalous-scattering properties of appropriate atoms in order to solve the phase problem. We report on methods developed to extract phase information from multiple-wavelength data.

Data were measured to 3.0Å spacings from crystals of selenobiotinyl streptavidin, lamprey hemoglobin (with W.E. Love) and Urechis hemoglobin (with R.L. Stanfield and M.L. Hackert) and to 2.5Å spacings from crystals of a bacterial ferredoxin (with W.H. Orme-Johnson) on the area-detector facility at the Stanford Synchrotron Radiation Laboratory with R.P. Phizackerly and E.A. Merritt. Absorption edges were measured from the data crystals in order to select wavelengths for data collection where anomalous-scattering effects are maximal. Bijvoet pairs were measured at each of four or five wavelengths.

Careful scaling of the integrated intensities from area-detector images was a crucial part of phase determination. Uncorrected systematic errors were minimized by local scaling first of Bijvoet pairs and then of data from multiple wavelengths. Dispersive differences in total scattering were considered only for ferredoxin, where iron anomalous scattering is a significant part of the total. The data were placed on an approximately absolute scale by calculation of unit cell contents.

The phase equation for a single type of anomalous scatterer (adapted from J. Karle, Int. J. Quantum Chem. (1980) 7, 357-367) is

$$|F_{\lambda}^{\dagger}|^2 = |F_{\lambda}|^2 + (f_{\lambda}^{\dagger}/f_0)^2 |F_{\lambda}|^2 + 2(f_{\lambda}^{\dagger}/f_0) |F_{\lambda}| |F_{\lambda}| \cos(\phi_{\lambda} - \phi_a) \pm 2(f_{\lambda}^{\dagger}/f_0) |F_{\lambda}| |F_{\lambda}| \sin(\phi_{\lambda} - \phi_a)$$

where  $|F_{\lambda}^{\dagger}|$  is the observed structure-factor amplitude for the + or - mate of a reflection at wavelength  $\lambda$ ,  $|F_{\lambda}|$  and  $\phi_{\lambda}$  are the amplitude and phase for the total normal structure factor, and  $|F_{\lambda}|$  and  $\phi_a$  are for the normal scattering of the anomalous scatterer. The scattering factors  $f_{\lambda}$  and  $f_{\lambda}^{\dagger}$  for the anomalous scatterer at wavelength  $\lambda$  were derived from absorption spectra measured from the data crystal;  $f_{\lambda}^{\dagger}$  is the magnitude of anomalous scattering and  $f_0$  is the normal scattering factor. A refinement procedure was developed to determine  $|F_{\lambda}|$ ,  $|F_{\lambda}|$  and  $(\phi_{\lambda} - \phi_a)$  from the multiple measurements for each reflection.

Patterson maps with coefficients  $|F_{\lambda}|^2$  yielded the anomalous-scatterer positions for lamprey hemoglobin (1 Fe) and streptavidin (2 Se). Atomic positions of anomalous scatterers were refined against the  $|F_{\lambda}|^2$ 's. The ferredoxin and Urechis hemoglobin data are being analyzed. Values of  $\phi_{\lambda}$  were obtained by adding the calculated  $\phi_a$  values to the refined phase differences. Electron-density maps were calculated from weighted  $|F_{\lambda}|$  coefficients and  $\phi_{\lambda}$  phases. Weights were based on lack-of-closure errors calculated for each reflection. Analysis of the streptavidin electron-density map is in progress.

The lamprey hemoglobin crystal structure is known and a model has been refined at 2.0Å resolution (Honzatko, Hendrickson & Love, J. Mol. Biol. (1985) 184, 147-164). Phases calculated from the refined model were used to judge the accuracy of the multiple-wavelength phases and to evaluate various error models. The multiple-wavelength analysis produced phases of equal quality to the isomorphous-replacement phases from which the structure was solved. Both sets of experimental phases have a mean phase error of 51° when compared with the model phases.

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