DARESBURY LABORATORY

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Contents

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Editorial

Many thanks to all those people who have contributed to this newsletter or to previous editions.

The film processing theme is continued here with Geoff Ford's report on recent developments at Sheffield. Several other articles describe refinement techniques and results. Future contributions on these or any other topics would be welcomed!

Arrangements for the meeting "Location and Refinement of Heavy Atom Sites" (Bristol University, 16/17 September) are well under way. Any late applicants should contact Dr. David Moss, Birkbeck College, London immediately.

Particular thanks to Pauline Harrison for the splendid photograph below!

THE PROCESSING OF OSCILLATION PHOTOGRAPHS AT SHEFFIELD

Geoff Ford (Sheffield University)

The MOSCO suite of programs for the processing of oscillation photographs has been transferred from PDP/data general FORTRAN to ICL FORTRAN on a 1906S at Sheffield. These programs were discussed recently in the CCP Quarterly News Letters dated May 1981 (The Oxford film meeting report) and September 1981 and more comprehensively in the manual of AJW. The MOSCO suite was chosen on the basis of its excellent performance in the CCP comparitive study of the processing of a set of ferritin data photographs. Coding and routines from the separate MOSCO systems at Imperial (A.J.Wonacott and others) and Bristol/Oxford (D.Stuart and others) were amalgamated and turned into batch programs. The current programs handle flat and vee-cassett films. They have been 'run-in' on ferritin data photographs which were collected on the Daresbury synchrotron source.

The MOSCO suite contains three core programs. IDXREF uses sets of partially recorded reflexions to refine the crystal unit cell dimensions and alignment parameters. The partial reflexions are often taken from two stills which have been exposed at spindle settings of, say, 0° and 90°. GENERT takes crystal and camera information and refined alignment parameters and predicts the reflexions which would be recorded on each film pack in the data set. The random-access GENERT-file holds these lists of reflexions together with their coordinates, degree of partiality and space for their measured intensities. MOSCO sums up the relevant optical densities for each spot on a photograph and places the integrated intensities in the relevant GENERT-file.

There are a number of other programs that are associated with the MOSCO suite. TAPEDISC transfers digitised optical density values from scanner mag-tapes to OD-files on a disc. FILMPIC makes a picture of an OD-file on a Tektronixs terminal. GENPLOT makes a scaled plot of the reflexions in a GENERT-file and this predicted pattern can be compared directly with the observed spots on the corresponding photographs. POSTCHEK (AJW) finds complementary partials on adjacent films and selects a sub set of those reflexions to be data for a post refinement run of IDXREF. ROTOCOR (AJW) calculates absorption, oblique incidence and Lp corrections and now transfers the reflexion data into BOSS binary data files. ABCSCAL (AJW) performs A:B:C interpack scaling and outputs binary data files for the BOSS scale-sort-merge program.

The 1906 GENERT (AJW) and IDXREF (AJW) are the standard programs with few amendments other than those needed to move from the old to the new FORTRAN dialects and I/O. The actions of IDXREF are described in the film processing report. Some additional spot indexing criteria are available and the program can now determine 'camera constants' for each cassette in the data set. The refinement of crystal unit cell dimensions and missetting angles against the discrepancy between the reflexion diffracting position and the Ewald sphere remains the same.

The MOSCO-1906 performs spot integration in the same fashion as the original MRC Cambridge programs. One line of optical densities is read and the program updates the intensities and backgrounds of all spots which cross this line. The original organisation of MOSCO (e.g. DS version) is retained but the program now uses routines from AJW to match the predicted reflexion pattern against the strong orientation spots. At present only flat or vee-cassettes can be processed but cylindrical or other geometries could be included easily.

MOSCO-1906 follows the strategy for film processing which is laid out in the Oxford film processing report. The observed film fiducial spots are approximated by a rigid-body fit of the theoretical positions in order to check the fiducial-making, developing and scanning of the film. The integration routine can now use an explicit definition of spot area and back-ground regions. The definition is held in a raster-by-raster array which can be constructed by examining the mean profile of the orientation reflexions. This addition has proved convenient and useful in the processing of ferritin data because the spots are triangular and leave irregularly shaped background regions. The integration routine also prints the optical densities for the 'bad' spots which have high or uneven backgrounds. This quality control quickly revealed dust particles, film defects, water marks on one film and an overgenerous resolution cut-off in a data set.

A batch approach to data processing is convenient and rapid. For whatever they are worth, the processing times for ferritin data on the 1906 are : IDXREF, about 4 seconds CPU time; GENERT, about 20 seconds per film of 2500 reflexions; MOSCO on a 2400 x 2400 grid of 50μ rasters, about 200 sec CPU for A + B + C films. The reliability indicators show good values : IDXREF, an RMS Δ (angle) of .021° for 2 x 20 spots which were read from stills (0° and -90° spindle) using a piece of accurate graph-paper; in MOSCO, the fiducial spots are correct

to within ± 30 - 100μ while the predicted reflexion pattern and the orientation spots agree to within 10-25μ RMS or one third of a raster. On our flat cassette data set the camera tilt and twist parameters stayed constant and close to 0.2° and 0.0° respectively.

We would like to thank A.J.Wonacott and D. Stuart for supplying copies of their MOSCO systems and the S.E.R.C. microdensitometry service for digitisation of films.

SRS X-RAY 7 PROTEIN CRYSTALLOGRAPHY WORKSTATION : POLARIZATION CORRECTION WITH MIRROR

John Helliwell

The vertically focussing mirror has now been installed and in continuous use (for small angle work) for 2 cycles. The flux through a 0.3 mm diameter standard NONIUS collimator has increased by a factor of 3 x as a result. At the focus the vertical FWHM size is 0.3 mm. Clearly, samples of size $\lesssim$ 0.3 mm will benefit the most from this geometry. The SRS is now routinely operational at 2 GeV, 200 mA; this in itself gives a factor of $\simeq$ 3 x over 1.9 GeV 100 mA operation at λ = 1.488 Å. Overall, a substantial improvement of flux (9 x) is available compared with 1.9 GeV, 100 mA, no mirror.

The value of τ' for the polarization correction is now (with the mirror added) = 0.856 for λ = 1.488 Å, machine energy 2 GeV. (Duke and Helliwell unpublished result).

Most recently, the first diffraction pattern was obtained of Kangaroo tail collagen on the beamline from the MWPC electronic area detector. At the time, the SRS was operating in one of the first single bunch mode runs 2 GeV, 0.5 mA! We expect to evaluate the MWPC system for protein crystallography during single bunch shifts in cycles 8 and 9. The single bunch shifts are very convenient for commissioning purposes whilst not interfering with the normal multibunch user shifts.

PROGRESS ON THE REFINEMENT OF HORSE SPLEEN APOFERRITIN

David Rice (Sheffield University)

The iron storage molecule ferritin consists of a protein shell comprising 24 subunits in 432 symmetry surrounding an inorganic 'core' of hydrated ferric oxide phosphate. Horse spleen apoferritin (subunit MW 19700 Daltons) crystallizes from Cadmium Sulphate solution in space group F432 with cell edge 185Å. The Xray analysis of the structure has been in progress for some years and an isomorphously phased electron density map calculated at 2.8Å clearly shows that each subunit principally consists of a four helical bundle. However, the quality of the electron density map was such that there was doubt about the connectivity between helices and the side chains were poorly resolved.

Phases for the isomorphous map were obtained from a PCMB derivative in conjunction with a Uranyl Fluoride derivative of much lower quality. There were four main reasons why this map was difficult to interpret.

1. The steep fall off in the figure of merit with resolution resulted in an electron density map with a nominal resolution of only 3.5Å
2. Severe heavy atom disturbances were visible in the map and gave rise to confusion about the direction of the polypeptide chain.
3. The tight packing of the ferritin subunits and the large number of inter- and intra-subunit interactions led to a number of strands of connecting density between adjacent stretcher of main chain. This gave difficulties in obtaining a unique assignment for the course of the polypeptide chain.
4. As ferritin is an iron storage molecule it was expected that strong metal binding sites would be found on the protein surface, and since the crystallization involves addition of Cadmium Sulphate there might be several peaks in the electron density map corresponding to bound metal ions. Two sites that are well ordered in the electron density map are clearly identifiable as non protein peaks; however, several other peaks (particularly those near heavy atom sites) could not be unambiguously differentiated from the protein.

To initiate the refinement process a tentative assignment of some side chains was made and a partial structure of the molecule was subjected to several cycles of least squares refinement using the Hendrickson-Konnert procedure. From an initial R value of .58 the refinement converged at R = .41.

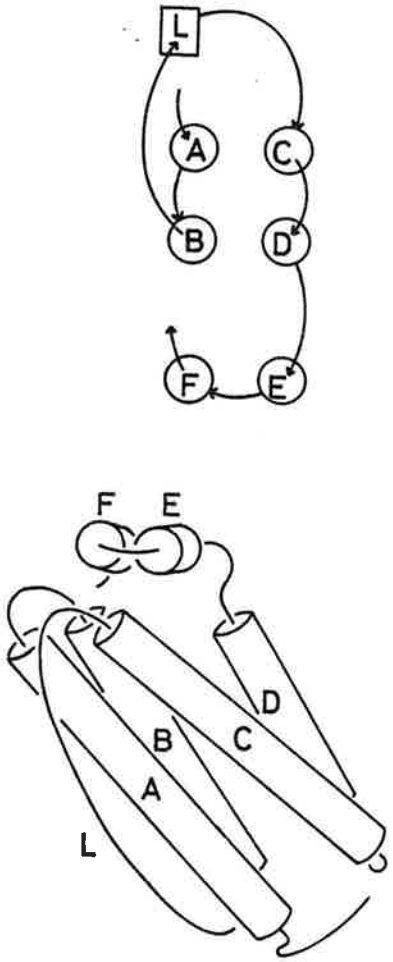

Fig. 1 Schematic representation of the tertiary structure of horse spleen apoferritin

These calculated phases were then combined with the isomorphous phase information to produce a combined electron density map in which many errors in the model were visible. A partial rebuild of the model was made using the Evans and Sutherland PS2 system at Oxford and further refinement on this model reduced the R value to .34 and more rebuilding was carried out to a new combined map.

To further improve the heavy atom derivatives the calculated phases were given Sim weighted figures of merit and then used to refine the derivatives using the procedure of phased refinement. During this process several minor sites were added to the PCMB derivative and a modest decrease in the lack of closure (10%) was achieved. The refinement of the Uranyl Fluoride derivative was more straightforward using the calculated phases and some quite large alterations were made to the heavy atom parameters although the lack of closure only decreased by 5%. The isomorphous phasing was further improved by incorporating a Silver derivative having several low occupancy sites near those of the PCMB derivative.

The isomorphous map calculated with these phases represents a considerable improvement in the electron density even though the overall figure of merit has only increased from .53 to .58. The side chain definition is much improved and some of the inter-helical connections are now unambiguous, though connectivities associated with strong inter - and intra- subunit interactions are still visible. We are currently interpreting this isomorphous map and the combined map derived from these isomorphous phases. Whilst one of the four helices is still much less well defined than the other three, the alignment of the amino acid sequence with the connectivity shown in fig.1, fits both the observed electron density and makes good chemical sense in terms of the large number of salt bridges and other interactions that can be observed.

The refinement of apoferritin is in its early stages though it should proceed quickly now. We are currently processing oscillation data photographs collected on vee-cassettes at Daresbury and this should provide us with a data set at 1.9Å resolution. We hope to include this data in our refinement and to progress to a definitive description of the molecular structure of apoferritin.

Some low resolution refinement techniques

Phil Evans, George Farrants, & Michael Lawrence
MRC Laboratory of Molecular Biology, Cambridge

In solving the structure of allosterically inhibited phosphofructokinase (PFK) by molecular replacement, two refinement methods were used which may be of general interest. The crystals of this conformation have a tetramer in the asymmetric unit, and it was possible to locate the molecular symmetry axes, and the model from the known active state, from the native Patterson, rotation functions, a one-dimensional crystal packing search, and a bit of fiddling with a poor heavy-atom derivative. This gave a model which could be improved by refinement at 7A resolution.

1. Rigid subunits

A program was written to refine the model using the subunit as a rigid body, but allowing the subunit to move relative to the molecular axes, and the tetramer relative to the crystal axes. The program is called SIMMER, because it maintains point-group symmetry, and cooks very slowly.

The orthogonal coordinates in the cell may be written

$$\underline{y} = R [Cj (Q \underline{x} + \underline{d})] + \underline{t} \qquad (1)$$

$$= R [Cj (Q (\underline{x} - \underline{x0}) + \underline{d}') - \underline{y0}] + \underline{t}' \qquad (2)$$

where

- $\underline{x}$ are the orthogonal atomic coordinates of the subunit
- R & Q are rotation matrices (expressed in Eulerian angles)
- $\underline{d}$ & $\underline{t}$ are translation vectors
- Cj are the point-group symmetry matrices (in our case 222)
- $\underline{x0}$ & $\underline{y0}$ are the centres of mass of the subunit and tetramer respectively

Thus Q and d represent the transformation of the subunit relative to the molecular axes, the operations Cj build the tetramer, and R and t give the transformation of the tetramer relative to the crystal axes. Inside the program, the rotations are done relative to the centres of mass of the subunit and tetramer, using expression (2): this helps to uncouple the rotations and translations. The usual crystallographic residual Sum w (Fobs - Fcalc)**2 is then minimized with respect to the 12 rigid body parameters which define the rotation matrices R and Q, and the translation vectors t and d, plus an overall scale and temperature factor between Fobs and Fcalc.

Because we were interested in refining a large number of atoms (~10000) with rather few reflections (~2500), the program uses the all-planes-one-atom method, that is the reflections are stored and the atoms read for each cycle, and the inner loop is over all reflections for a given atom. Doing the summation this way round is more efficient, but even so the program is slow (about 4 hours/cycle on a VAX 11/780). Many of the early trials were done using every third or fourth reflection to speed up the calculation, but this seems not to be a good idea.

2. Floppy subunits.

It became clear that it was a poor assumption that the subunit had the identical structure in the inhibited state as in the active state. This was shown mainly by the parameters refining to different values depending on the starting point, although the solution was clearly essentially correct. We decided to relax the constraint of the rigid subunit, and we considered using CORELS to keep helices and other structural elements rigid. However, CORELS would have required us to define exactly which residues to put into each rigid body, as well as being very slow. We decided to use a more simple-minded approach, smoothing the atomic shifts in the Jack-Levitt restrained

FFT least-squares procedure. The first stage of this procedure, DERIV, calculates from a difference map the diagonal least-squares matrix and the gradient vector, which define the individual atomic shifts. These were then smoothed with a moving average for each residue, by taking the shifts from the main chain atoms of neighbouring residues along the chain, using an exponential weight depending on distance with a standard deviation of 7A. This tends to preserve local conformations, particularly secondary structure elements, but for example, allows helices to move as approximately rigid units. The smoothed matrix and gradient, with the same values for all atoms in each residue, were then passed to the energy refine program, which maintains the covalent integrity of the molecule by simultaneously minimizing the calculated energy and the X-ray residual. This procedure allows the molecule to deform without any explicit statement of which parts of the molecule are to change.

The smoothed refinement was done first with four independent subunits, so that the positions of the molecular axes could be refined. We were pleased to find that the four subunits deformed in the same way, and that the position and orientation of the molecular axes was the same as that from the rigid subunit refinement to within 0.02 degrees and 0.05 A. The final refinement was done on one subunit only, taking the gradients from the difference map averaged about the fixed non-crystallographic molecular symmetry axes.

PROTEIN STRUCTURE REFINEMENT AT BIRKBECK COLLEGE USING THE CRAY-1

David Moss

Several of our research projects involving protein structure refinement are also directed towards investigation of refinement strategies. The following is a summary of some of our refinement work.

(a) Comparison of Two Refinements of Ribonuclease-A

We are collaborating with Dr Alex Wlodawer of the National Bureau of Standards, Washington D.C. in a comparison of the results of his 2.0Å joint X-ray and neutron refinement of ribonuclease-A with our 1.45Å X-ray refined structure. The mean distance between the positions of corresponding protein atoms is 0.48Å. We wish to proportion the observed differences to the following categories:

(i) Real differences between the two crystal structures (one is deuterated and has a different solvent)

(ii) Differences in map interpretation

(iii) Differences due to the random errors in the data.

The largest differences (> 2Å) involve regions of the structure where one or both structures contain disordered side chains. Comparisons of the water structure and hydrogen bond geometry have also been made.

(b) Anisotropic Temperature Factor Refinement of APP

Avian pancreatic polypeptide has been refined with anisotropic temperature factors at a resolution of 0.95Å. In general the U tensor values seem reasonable in that they can often be interpreted in terms of the concerted movement of groups of atoms.

The results of this refinement have been compared with those produced from molecular dynamics calculations.

(c) Reinvestigation of the Refinement of γ-Crystallin

The early stages of our refinement of γ-crystallin have been reinvestigated now that we have a model of the structure refined at 1.9Å resolution. In the early stages least-squares refinement had produced much smaller improvements in the model than we had anticipated when we saw the R factor drop to 30% at 2.6Å resolution. This was due to the mean error in the model being intially 1.1A. The rebuilding of the model on the graphics system after cycles of the refinement was the major cause of improvement in the early stages but in later stages the least-squares refinement played an increasingly important role. Attempts to improve the performance of least-squares in the earlier stages are in progress.

(d) The use of Realistic Energy Terms in Refinement

We are experimenting with the use of true energy terms in order to refine protein structures when little or no diffraction data is available. The idea is that it should be possible to add new energy terms to our program in a simple way. This involves firstly supplying an appropriate subroutine for function and possibly derivative calculation and secondly amending a dictionary of force constants and potential constants.

Our current dictionary allows us to minimise a function with the following terms:

$$
\begin{aligned}
2M = {} & \Sigma\, w\, (|F_o| - |F_c|)^2 \\
& + \Sigma\, w\, (\phi_o - \phi_c)^2 \\
& + \Sigma\, f_\ell\, (\ell_T - \ell_c)^2 \\
& + \Sigma\, f_\alpha\, (\alpha_T - \alpha_c)^2 \\
& + \Sigma\, f_\tau\, (1 - \cos n\tau) \\
& + \Sigma\, f_v\, |V| \\
& + \Sigma\, \left(\frac{A}{r^{12}} - \frac{B}{r^6} + \frac{q_i q_j}{r}\right) \\
& + \Sigma\, \left(\frac{C}{r^{10}} - \frac{D}{r^6} + \frac{q_i q_j}{r}\right)
\end{aligned}
$$

where the terms refer to structure amplitudes, phases, bond distances and angles, torsion angles, π-bonded systems, van der Vaals and electrostatic interactions and hydrogen bonds.

Several problems arise concerning the potentials to use in the Lennard Jones terms and the best way of allowing for the water environment. Any CCP4 members with ideas that they would like to contribute should contact us.

(e) Accelerating Structure Factor Calculations

Conventional structure factor algorithms can easily be vectorised for the Cray computer. We have produced both scalar and vector versions of structure factor subroutines and were surprised to find that 4-Gaussian approximations for scattering factors take little extra time on the Cray compared with table look-up versions. We shall be producing a Fast Fourier version of these routines soon.

Protein Crystallographers of Northern England: July 1982 Meeting

This group met again at Leeds on 13.7.82 with representatives from York, Sheffield, Leeds, Liverpool, Keele and Daresbury.

Fritjof Korber gave an overview report of the recent Erice Meeting and detailed methods and results given in some particular sessions. Since the majority of those present had not been able to attend Erice this was a most useful contribution which stimulated many questions. Guy Dodson gave an interesting description of the work in York on the structure of a bacterial ribonuclease, BARNASE. Geoff Ford both detailed the development of the Sheffield film processing programs and gave some useful hints to those collecting data on the Synchrotron Source at Daresbury. The latter aroused some lively discussion.

In addition to these formal presentations there was much useful informal discussion, exchange of magnetic tapes and molecular models.

Thanks go to Maxine McCall for organising the meetings so efficiently.

PLUTO at Daresbury

Pella Machin (Daresbury Laboratory)

Daresbury have finally bought a colour pen plotter (Benson model 1112) and it is now available for general use. The version of (Phil Evans) PLUTO implemented on the NAS AS/7000 at Daresbury is set up to utilise the colour options (black, red, green).

USE OF THE CRAY-1S BY THE OXFORD LABORATORY OF MOLECULAR BIOPHYSICS

Sheila Gover (Oxford)

Past and present members of the Laboratory have established a comprehensive suite of programs for the processing, analysis and presentation of crystallographic data. This is installed on the University ICL 2980 computer and is accessed in batch mode, complementing the on-line facilities of the Laboratory DEC PDP11/70 and the graphics of the Evans & Sutherland Picture System II. Though a powerful computer comparable in speed to an IBM 370/165, the ICL 2980 has a single processing element and lacks the flexibility of the Cray vector processor. Our access to the Daresbury Cray-1S through a workstation linked to the S.E.R.C. network has allowed us to exploit a twenty-fold reduction in the CPU time required by a Hendrickson-Konnert refinement and we hope to have similar benefit from use of the Chemistry at Harvard Molecular Mechanics programs.

Experience with the refinement of human serum prealbumin (Oatley (1976)) and human lysozyme (Artymiuk (1979)) has shown that the method of difference Fourier shifts is increasingly ineffective as the reliability index R drops below 30%. This is a consequence of the approximation used to calculate the curvature of electron density at each point of the difference map and of confusion in regions where the protein structure is highly mobile, giving rise to weak extended density. The Hendrickson-Konnert structure factor least squares refinement (Konnert (1976); Hendrickson & Konnert (1980)) calculates shifts by a conjugate gradient method and premature convergence may be avoided by use of a resolution-dependent weighting scheme (Pulford (1980)). The application of stereochemical restraints (chemical bond lengths, planarity, torsion angles, chiral volume, Van der Waals contacts .....) eliminates the need to regularise the model structure after each cycle and improves the ratio of observations to parameters so refinement at lower resolution is more successful.

Restrained structure factor least squares was first used in Oxford to further the refinement of human leukaemic lysozyme. The R-factor for the 18500 reflections to 1.5Å resolution was reduced from 27% to 19%. The validity of the method was tested by placing restraints on the covalent, but not the non-covalent structure. Non-bonded and hydrogen-bonded interactions were similar to those found in small molecules and additional features of the final electron density map could be compared with those revealed in other high-resolution protein studies (Artymiuk & Blake (1981)). At this stage the program was running on the ICL 2980 and a cycle was divided into 3 or 4 parts to deal with the complete set of structure factors and their derivatives. It was implemented on the Cray by Bill Pulford and improved to its current performance during his study of tortoise egg-white lysozyme, a refinement which succeeded despite the absence of sequence information and the lack of MIR phasing beyond 6Å (Pulford (1982)).

The versions of the program on the Cray are now extensively used by members of the Laboratory. Ten proteins have been or are in the process of being refined and details of these are given in the Table. Refinement has usually started with a few cycles of restrained least squares for a limited data set (low resolution, higher spacings omitted because they are likely to be most affected by solvent). The model is then rebuilt on the Evans & Sutherland graphics system and further cycles are interrupted by rebuilding as the data set is extended to full high resolution. Unit weighting has been found most satisfactory to date but resolution dependence is being considered for the next stage in the refinement of rabbit phosphorylase b, when the 50,000 reflections to 2.0Å will be included. This protein is the largest of those currently being studied. It has a subunit weight of 100,000 and even on the Cray the CPU time for a cycle with the 27,000 reflections from 2.5Å to 5.0Å is twenty minutes.

Progress using Hendrickson-Konnert refinement

protein	space group	resolution	number of reflections	refined atoms	current R	researcher
Lysozymes:						
Hen egg-white	$P4_32_12$	1.6Å	17000	1100	20%	P.Artymiuk/ H.Handoll
High temperature hen egg-white	$P2_12_12_1$	1.5Å	13000	1000	25%	P.Artymiuk/ J.Berthou
Tortoise egg-white	$P2_12_12_1$	1.6Å	19700	1127	19%	W.Pulford
Seal myoglobin	C2	2.5Å 2.5Å-4.5Å	6300 5140	1266	31% 28%	H.Scouloudi
Human serum prealbumin	$P2_12_12$	1.8Å	24000	1966	19%	S.Oatley
Horse muscle phosphoglycerate kinase	$P2_1$	2.5Å	13000	3200	22%	D.Rice
Triose phosphate isomerases:						
Chicken	$P2_12_12_1$	2.5Å	17000	3700	22%	P.Artymiuk/ W.Taylor
Yeast	$P2_12_12_1$	1.9Å	31000	3700	32%	P.Artymiuk/ D.Rose/ G.Petsko
Rabbit muscle phosphorylase b	$P4_32_12$	3.0Å-6.0Å 2.6Å-6.0Å	18000 25000	6625	34% 40%	D.Stuart M.Sansom
Rabbit immunoglobulin G fragment Fc	$P2_1$	2.7Å	14000	3000	33%	B.Sutton

The refinement of lysozymes from different species has suggested comparative analysis of atomic temperature factors to see if the pattern of atomic displacements can be correlated with the molecular structure. This has proved possible and observations have been made concerning the mobility of main and side-chain atoms and the significance of high mean displacements in the vicinity of the active site (Artymiuk et al. (1979)). Meaningful interpretation is limited by experimental difficulties but progress is expected through refinement of anisotropic temperature factors and the use of low temperatures to reduce anharmonic effects and distinguish static disorder from true motion (Phillips (1980)).

There has also been considerable interest in the dynamic properties of proteins following the work of the Harvard Chemistry Group (McCammon et al. (1977) & (1979); McCammon & Karplus (1980); Northrup et al. (198o)). The CHARMM suite of programs was first implemented by Bill Pulford on the ICL 2980 and he has recently installed a vectorised version on the Cray. This has been tested with a tripeptide from pancreatic trypsin inhibitor and results agreed within the limitations of differing random number generation. The method of dynamic simulation differs from others in calculating hydrogen-bond energy separately from that due to Van der Waals and electrostatic interactions. Attempts to simulate the mobility of human lysozyme over a period of O.lps have not been successful so far but it is hoped to correlate the results with the crystallographic observations of atomic displacement.

References

Artymiuk, P.J. : D. Phil. Thesis, Oxford (1979)

Artymiuk, P.J., Blake, C.C.F. : J. Mol. Biol. 152 (1981) 737

Artymiuk, P.J., Blake, C.C.F., Grace, D.E.P., Oatley, S.J., Phillips, D.C.P. & Sternberg, M.J.E. : Nature 280 (1979) 563

Hendrickson, W.A., Konnert, J.H. in Biomolecular Structure, Function, Conformation & Evolution (Srinivasan, R. ed) Pergamon Press (1980)

Konnert, J.H. : Acta Cryst. A32 (1976) 614

McCammon, J.A., Karplus, M. : Ann. Rev. Phys. Chem. 31 (1980) 29

McCammon, J.A., Gelin, B.R., Karplus, M. : Nature 267 (1977) 585

McCammon, J.A., Wolynes, P.G., Karplus, M. : Biochemistry 18 (1979) 927

Northrup, S.H., Pear, M.R., McCammon, J.A., Karplus, M. : Nature 286 (1980) 304

Oatley, S.J. : D. Phil. Thesis, Oxford (1976)

Phillips, D.C.P. : Biochem. Soc. Symp. 46 (1981) 1

Pulford, W.C.A. in Refinement of Protein Structures, Proceedings of Daresbury Study Weekend (1980) 69

Pulford, W.C.A. : D. Phil. Thesis, Oxford (1982)

Beevers-Lipson revisited (Geoff Ford)

The running time of the Hendrickson-Konnert refinement program is dominated by the time taken to calculate the structure factors and their derivatives. These quantities are evaluated by the summation of triple products of the type $\Sigma \cos(2\pi hx) * \cos(2\pi ky) * \cos(2\pi lz)$ as shown in International Tables Vol. I. C.A. Beevers and H. Lipson have pointed out (1934, Phil.Mag. 17, 855-859) that this process proceeds more rapidly if the reflexion list is ordered and if sub-sums, such as $\Sigma \cos(2\pi hx) * \cos(2\pi ky)$ are evaluated only once for each hk - line. This Beevers-Lipson summation is well suited to vector programming on the CRAY 1. In a favourable case, space group P1, 6300 atoms per asymmetric unit and 19000 reflexions, the cycle time was reduced from 500 seconds/cycle to 350 seconds/cycle. A good return for minimal effort!