DARESBURY LABORATORY

INFORMATION QUARTERLY for PROTEIN CRYSTALLOGRAPHY

An Informal Newsletter associated with Collaborative Computational Project No. 4 on Protein Crystallography

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Contents

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EDITORIAL

Thanks are due to the contributers to this edition of the newsletter and to Peter Brick for organising the collection of contributions. A copy of the papers in this newsletter have been sent to Keith Wilson for inclusion in the EACBM (European Association for Crystallography of Biological Macromolecules) newsletter.

I would like to take this opportunity to give advance publicity for a meeting to be organised by the CCP4 and Daresbury Laboratory. The meeting will take place on the 5-6 of Februaury 1988 and will be entitled 'Improving Protein Phases'. Practical applications of solvent flattening, density averaging, direct methods etc. will be covered. A notice announcing the meeting is to be circulated in the near future.

Sue Bailey
8th October 1987

β-LACTOGLOBULIN: a transport protein.

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β-Lactoglobulin (BLG), the major protein component of bovine milk whey has recently been established to be a transport protein. Its distribution is widespread, occurring in the milk of many other species including dolphin, dog, echidna and marsupials.

BLG is now known to be just one example of a family of similar transport proteins (L. Sawyer, Nature 627, 359; 1987). The topology of the polypeptide chain fold of BLG appears to be common to a group of ten or more proteins which include insecticyanin, human plasma retinol binding protein (RBP) and a number of odorant-binding proteins. RBP is one example with remarkable overall similarity, it shares the striking "cross-hatching" of strands of anti-parallel β-sheet, although there are distinct differences in detail (RBP has 182 residues) the extra length is accommodated in the loop regions. Retinol can be bound by BLG apparently in the analogous pocket to that of RBP with a dissociation constant $K_d = 2 \times 10^{-8}$M, stronger by a factor of ten than RBP. This basic framework appears to be suitable for binding small hydrophobic and labile molecules and functioning as a transporter within the aqueous environment.

The bovine molecule is dimeric under physiological conditions, each subunit having a MW of about 18400, corresponding to 162 residues. It is very resistant to proteolysis, passing through the stomach at pH2. As the pH is raised BLG undergoes several pH-dependent conformational changes; that occurring between pH 6 and 8, the Tanford (N ⇌ R) transition, results in a carboxyl group being exposed, and increased reactivity of cysteine. The 3-D structures of two crystal forms grown on either side of the Tanford transition are under investigation.

X-ray studies on the low pH form of bovine BLG variant A have been carried out in Leeds. Crystals of the triclinic lattice X form, space group P1, unit cell dimensions a = 38.1, b = 49.7, c = 56.6A, α = 122.2, β = 97.5, γ = 104.0° are grown from 4M $(NH_4)_2SO_4$, pH 6.5. Reflection data were collected from 33 native crystals to 2.0A resolution on an ENRAF-Nonius CAD-4 diffractometer.

Data were processed using the CCP4 program suite. Background/peak analysis was performed using the Bayesian statistics and each peak profile-fitted by the 3-stage regression model of Stuart Oatley and Simon French, Acta. Cryst. A38, 537 (1982) using Tim Richmond's PROFIT program. Corrections for radiation damage, absorption and Lorentz-polarisation effects were applied and the

programs ROTAVATA and AGROVATA used to scale batches. TRUNCATE was used to eliminate negative intensity values. The merged data contains 21462 reflections, 98% of the unique reflections to 2.0A spacing, with R_{merge} on intensity = 0.058.

Studies on the higher pH form, BLG lattice Y, were perfomed by L. Sawyer, S Hambling and colleagues in Edinburgh. Orthorhombic crystals of lattice Y, space group $B22_12$, unit cell dimensions a = 55.7, b = 67.2, c = 81.7 are grown from 2M $(NH_4)_2SO_4$ pH 7.8. Data were collected from 5 native crystals to 1.8A resolution at the SRS Daresbury.

The data were processed using the MOSFILM suite of programs implemented at Daresbury and data from the 5 crystals merged to give 89% of the unique reflections and R_{merge} on intensity = 0.097.

For lattice Y, the low resolution data collected earlier (Papiz et al., Nature 324, 383; 1986) were scaled and combined to produce a data set comprising 91% of the unique data set to 1.8A. The phases produced by combining MIR, solvent flattening and partial structure information have been used to produce an electron density map of superior quality to its predecessors. Work is in progress refitting the model to this map in order to extend the phases and continue refinement.

The lattice X structure was initially determined by aligning the lattice Y model with a 6A MIR map (Green et al., J Mol Biol. 131, 375; 1979) and by a single mercurial derivative between 6A and 3A. Taking this initial model (136 residues of poly-Alanine) and the new 2.0A amplitudes, phase extension was carried out in stages by means of the Hendrickson-Konnert refinement program. This gave an R-factor of 38% to 2.3A. Currently about 80% of one subunit has been fitted to a $3F_o-2F_c$ map.

The initial comparison of the main chains of the low and high pH forms show that they are broadly similar: the disulphide (106-119) is maintained in both forms and Trp-19 has a similar disposition at the foot of the binding pocket. However, small variations can be seen which need to be examined more fully to distinguish between those caused by different crystal packing and those resulting from the Tanford transition. Further refinement is required before this detailed comparison can be made.

ELECTRON DENSITY MAPS FROM LAUE PHOTOGRAPHS OF PROTEIN CRYSTALS.

Janos Hajdu (1) in collaboration with Pella A. Machin (2), John W. Campbell (2), Ian J. Clifton (2), Trevor J. Greenhough (2;3), Sebastian Zurek (2) Sheila Gover (1), Mike Elder (2) Gregory K. Farber (4), Steve Almo (4,5), Gregory A. Petsko (4) and Louise N. Johnson (1)

1: Laboratory of Molecular Biophysics, Oxford University, U.K.; 2: SERC Daresbury Laboratory, Daresbury, U.K.; 3: Department of Physics, University of Keele, U.K.; 4: Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA; 5: Department of Biophysical Chemistry, Harvard University, Cambridge, MA 02139, USA

It is with great sadness that we note the deaths of Pella A. Machin and Mike Elder who were killed in a mountaineering accident in Scotland on the 7th of March 1987. Most of this work was made possible through their help and enthusiasm and through their inspiration. We all miss them so much.

We report here on the first difference Fourier maps obtained from Laue diffraction photographs of a protein crystal, glycogen phosphorylase b and give an account of results with xylose isomerase where Laue data were used successfully to find heavy atom positions. These are the first two cases where structural information could be extracted from Laue photographs of protein crystals. Both data sets were collected at station 9.7 of the Synchrotron Radiation Source (SRS) at Daresbury and software written at Daresbury was used.

The extremely high data rates available with the Laue technique at synchrotron radiation sources (more than 100,000 reflections per second can be recorded) open up new areas in crystallography, eg. the time resolved analysis of various phenomena in crystals. Crystallographic studies of structural CHANGES face the problem that a structure determined by diffraction methods is averaged over the diffracting volume of the crystal and over the time needed to record the diffraction data. Attempts to use X-ray crystallography to extract three dimensional information on transient phenomena in crystals have been hampered primarily by long data collection times. The high intensity and the broad effective spectrum (0.2-2.1 A) of the white X-radiation generated on the SRS wiggler magnet allow the recording of Laue diffraction photographs on the millisecond time scale. Here we show that good quality electron density maps can be calculated from Laue data taken in a very short time. The combination of this approach with techniques resembling cine photography allows the recording of 3-dimensional movies at atomic resolution and opens up new areas in structural biology and chemistry. Transient phenomena like structural changes, phase transitions or catalysis may now be investigated by X-ray crystallography provided the volume averaging is favourable.

GLYCOGEN PHOSPHORYLASE:

The structure of glycogen phosphorylase b (EC 2.4.1.1, subunit M.W.=97,434) has been solved to 1.9 A resolution using monochromatic data collected at the SRS (K. R. Acharya et al., in preparation). Maltoheptaose is a substrate of the enzyme which binds to the glycogen storage site of the protein. Under the conditions of the experiment, the time needed to reach half saturation binding with maltoheptaose was about 8 minutes. The structure of the bound oligosaccharide is known to 2.5 A resolution (P.J.McLaughlin et al. in preparation). In order to test a new difference method in the treatment of Laue data, the binding of maltoheptaose to phosphorylase b was monitored. The crystal was kept in a thermostated flow cell and Laue data sets of three exposures (with 6 films in a pack) were taken at different angular settings before, during and after the addition of the ligand (50 mM maltoheptaose) at station 9.7, SRS Daresbury.

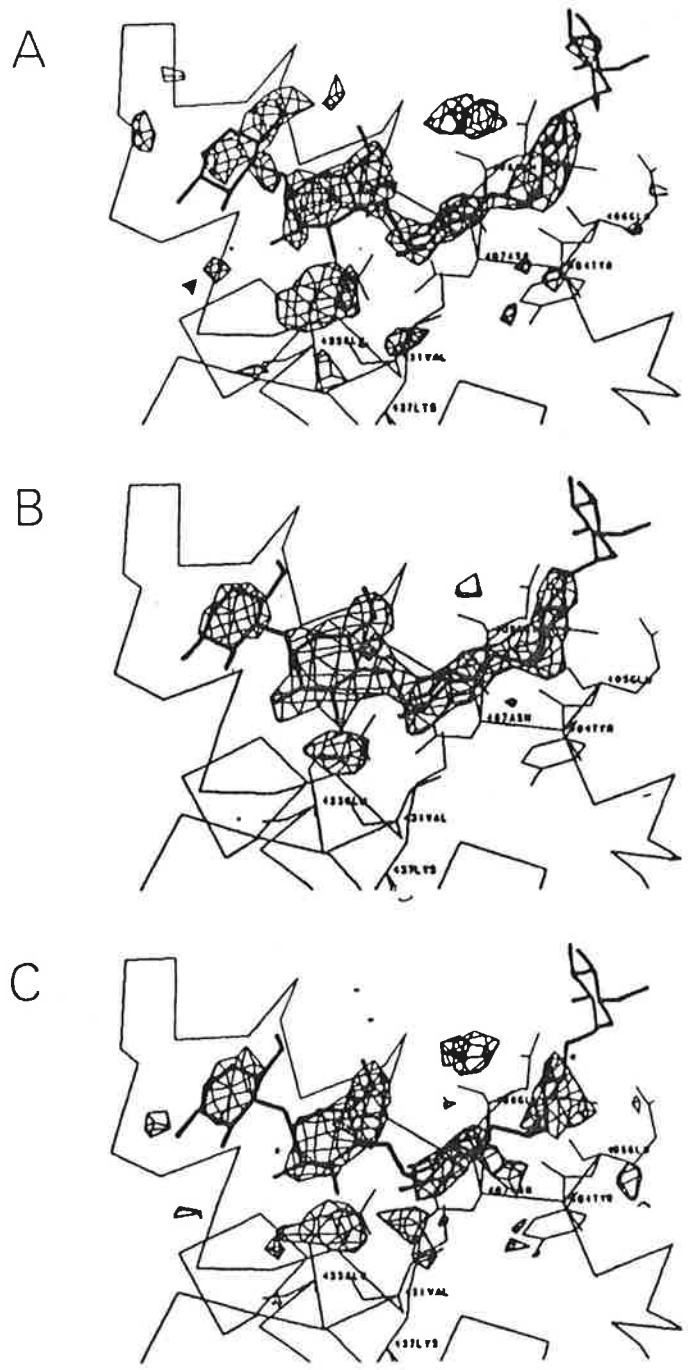

FIGURE 1:
DIFFERENCE FOURIER MAPS SHOWING BOUND MALTOHEPTAOSE IN PHOSPHORYLASE B CRYSTALS.

A: Laue map calculated with a unique set of 9,029 reflections. B: Monochromatic map to 2.5 A resolution calculated with a unique set of 29,689 reflections. C: Monochromatic map calculated with 9,029 reflections common to both the monochromatic and the Laue data sets. A positive contour is displayed at half maximal peak height in all 3 maps. Side chains of the protein are displayed as they appear in the unliganded native structure. Only 4 of the 7 sugar units can be localised. This is due to the increased mobility of the sugar units at each end of the maltoheptaose molecule. Movements of Glu 433, Lys 437 and Gln 408 produce the two extra density lobes on either side of the oligosaccharide in B and can clearly be seen in A (and C).

Data sets in the sequence were scaled to the starting (native) Laue data set using an anisotropic temperature factor to compensate for variations in exposure time, beam decay, radiation damage, etc. Each film in a film pack was individually scaled to its counterpart in the native Laue data set and the measurements were kept unmerged. Since the initial photographs in the sequence represent the native protein and the spectral properties of the X-ray beam were not altered during the experiment, fractional intensity changes on subsequent photographs with the crystal in the same orientation were used to calculate difference Fourier maps with coefficients

$$\frac{(F\_laue\_deriv - F\_laue\_native)}{F\_laue\_native} \times F\_monochrom\_native$$

and phases from the monochromatic data. F_laue_native is a structure factor amplitude measured on a starting, native photograph; F_laue_derivative is the corresponding measurement after the addition of the ligand; and F_monochrom_native is the measurement from the reference monochromatic data set. The advantage of this DIFFERENCE TECHNIQUE is that wavelength and position dependent corrections are not needed.

In this experiment, a unique set of 15,529 reflections remained with positive intensity measurements after 46.3% of all predicted reflections had been rejected due to energy and/or spatial overlaps. Energy overlap ('multiplets') occurs when diffraction maxima for different wavelengths but the same diffraction angle are superimposed on the film. Although multiplets may be deconvoluted from multiple-film exposures, they represent a small portion of the data (never more than 27.2%) and were not included in the present processing. Spatial overlap occurs due to the very dense nature of protein crystal Laue patterns. The unique set was further reduced to 9,029 by selecting reliable measurement pairs only. The difference Fourier map showing maltoheptaose bound at the glycogen storage site of phosphorylase b is shown in figure 1A. Figure 1B depicts a similar map calculated with a full monochromatic data set to 2.5 A resolution (29,689 unique reflections). Figure 1C, which represents the real control for the quality of the Laue data, shows a monochromatic map calculated using ONLY those 9,029 reflections common to the monochromatic and Laue data sets. The quality of the Laue map (Fig. 1A) is better than the quality of the equivalent monochromatic map (Fig. 1C). This could be attributed to 3 major factors: i) reduced radiation damage, ii) identical conditions for obtaining the native and derivative data sets, iii) no need for various corrections in the treatment of the data. To achieve the quality of the full monochromatic map (Fig. 1B), the number of reflections contributing to the Laue map (Fig. 1A) has to be increased. Deconvoluting multiplet spots into component reflections is an obvious step and so is attempting to reduce the number of reflections rejected due to spatial overlap.

XYLOSE ISOMERASE:

It is, in principle, possible to extract structural information from Laue data without the knowledge of a starting, native structure. The success of this approach does, however, depend on a satisfactory treatment of wavelength dependent effects such as incident intensity, absorption, Lorentz and polarization factors, obliquity and detector response. The determination of the so called "wavelength normalization curve" to compensate for these effects poses the greatest challenge in trying to extract independent structural information from Laue photographs. None of these factors had to be considered with the difference method outlined above. We report here the first successful use of Laue data to characterize a heavy-atom derivative of a protein crystal using a wavelength normalization technique developed in Daresbury.

Crystals of xylose isomerase were chosen for the Laue experiments for several reasons. The structure of this enzyme has just been solved at 3 A resolution using the multiple isomorphous replacement method with monochromatic data collected by conventional diffractometry. Large crystals which are not

FIGURE 2:
DIFFERENCE FOURIER MAP BETWEEN LAUE_NATIVE AND LAUE_Eu OF XYLOSE ISOMERASE.

The Eu density is the only significant feature of the map. The lowest contour level is 4 standard deviations

especially susceptible to radiation damage are easy to grow. These crystals belong to space group P22121 but have strong pseudo-body centering. To 3 A resolution, the systematic absences corresponding to the pseudo space group I222 are almost perfectly obeyed. The unit cell dimensions are a=98.7, b=93.9, c=87.7, and the asymmetric unit of the pseudo space group contains one subunit of molecular weight 40,000. Using the Laue method at the SRS, 70% of a complete data set to 2.5 A resolution can be collected in 1 sec on these crystals.

We decided to use europium as a probe for the divalent cation binding site. In addition to being a test of the ability to the Laue diffraction technique to produce interpretable electron density maps of the binding of small molecules to the active site of a protein, the europium experiment also provides a way to test whether Laue data can beused to locate heavy atoms in a protein crystal.

All data were collected at station 9.7 at Daresbury Each film pack had 6 or 10 films (A-F or A-J); no metal foils were interleaved between the films. Crystals were mounted with c* along the capillary tube and perpendicular to the x-ray beam. Three sets of photographs with crystal to film distances of 110 to 120 mm were taken on each crystal. The x-ray beam was aligned along a* or b* or was 45 o between the two. The crystal was translated between each photograph. Exposure times were 1 or 2 seconds. Data reduction has four phases: finding and refining the crystal orientation, indexing spots and measuring their intensity, scaling the intensities of spots measured on films A-F, and normalizing these intensities based on the wavelength causing diffraction of a particular reflection.

Patterson maps calculated from both Laue and diffractometer data using only this small set of reflections (1205 out of a possible 7300 between 6 and 3 A resolution) were uninterpretable. However, a difference Fourier between Laue native and Laue Eu data using monochromatic phases produced a map practically identical to the Difference Fourier calculated with a complete set of diffractometer data. The map clearly showed two europium atoms binding in the central cavity that had previously been identified as the active site (Fig. 2). No other significant features were present in either difference Fourier.

The crystals used in these experiments provided a difficult test case for the Laue method. There were problems with pseudo symmetry and with non-isomorphism. In addition, the low symmetry of the crystals made it impossible to collect a complete data set on a single photograph. Despite these problems, the data from the Laue experiments were good enough to find the heavy atoms. The Laue difference Fourier was, in fact, as clean and interpretable as the corresponding monochromatic difference Fourier.

FUTURE POSSIBILITIES

Time resolved X-ray studies at the SRS may be carried out on systems with intermediate half-lives ranging from hours, through a few seconds, down to picoseconds (stroboscopic experiments using the time structure of the storage ring). These methods have great potential in the three dimensional study of a variety of phenomena, not only in protein crystallography but in other areas of solid state physics too.

In the study of catalytic reactions in crystals, the initial priority is the determination of the rate-limiting steps of the reaction in the crystal since this determines which of the following experimental techniques is applicable for the 3-dimensional study of intermediates: i) The flow-cell technique is possible where the rate-limiting step is not the diffusion or binding of the substrates. ii) Relaxation techniques including T-jump, pH jump, pressure jump and photo- -dissociation. iii) Photo-activation of 'caged' substrates by flash photolysis.

Of particular importance is the time scale of the reaction which will determine which of the following data collection techniques, with additional time-scale manipulation by crystal cooling, may be possible: i) Collection of a sequence

of complete monochromatic data sets if the half life of the intermediates is of the order of a few hours. ii) Collection of a sequence of Laue photographs if the half life of the intermediates is longer than about 30 seconds. iii) Collection of streaked Laue photographs to study phenomena in the 0.1-30 second interval. iv) The use of electronic area detectors such as the FAST system at the Daresbury SRS to collect frames of diffraction patterns as a function of time. The current minimum time per frame is 17 secs but this will be reduced to 5.3 secs in the near future. Use of the FAST clearly depends on establishing whether the detector can provide the necessary resolution. v) Stroboscopic experiments using the time structure of the storage ring for events down to the picosecond time scale.

Having established the conditions and the experimental techniques to be applied in a particular case, collection of a sequence of3-D data sets over the time course of the reaction should be carried out. The analysis of the data will require software development. With unfavourable volume averaging, i.e. with mixtures of structural states, it might still be possible to extract structural information on some of the species. This, however, needs further work particularly in structure refinement.

The development of the theories, software and experimental techniques outlined above offers a route to the dissection and detailed understanding of molecular events associated with catalysis, ligand binding and structural transitions in proteins. This area has only recently become accessible to the crystallographer and developments in millisecond and sub-millisecond data collection methods are of vital importance.

CRYSTAL STRUCTURE DETERMINATION USING INTENSITY DATA FROM LAUE PHOTOGRAPHS

by Jennifer A Clucas, Marjorie M Harding and Stephen J Maginn, Department of Inorganic, Physical and Industrial Chemistry, Liverpool University.

A preliminary account of the use of Laue diffraction patterns to measure reflection intensities has been given (1) and an extensive account is in preparation (2). This structure determination was undertaken as a pilot study to confirm the potential of the Laue method for intensity measurement. Eventually we would expect the method to be of value for very small crystals, and for time dependent studies. Its potential for showing changes in electron density within a short time scale in a protein crystal has already been beautifully demonstrated (3). The crystal studied here was of 'normal' size and the complete determination of a previously unknown structure has been achieved; there are no problems of disorder or poor resolution like those which we reported recently in a similar study of another organometallic compound (4). At present the R factor for 2800 reflections with $F > 6\sigma(F)$ is 0.16. Failure to achieve a better refinement suggests the presence of a systematic error in the F's; we suspect that this is, at least in part, sample absorption, but are invesitgating further.

Experimental

SRS workstation 9.7 was used (July 1985), with the SRS running at 2GeV(?) and 15mA (single bunch mode); 9 film packs, each consisting of F,F,Al,F,Al,F,Al,F,Cu,F (where F is film, and Al and Cu are metal foils), were recorded at 20° intervals in ϕ, for a crystal of dimensions 0.3 x0.3 0.3 mm^3 in an arbitrary orientation, with crystal-film distance 59mm, exposure time 10 sec, and a collimator of diameter 0.2mm.

Crystal Data

$Rh_6(CO)_{14}$dppm where dppm is $Ph_2P.CH_2.PPh_2$, M.W. 1394
Monoclinic, a = 16.26, b = 18.58, c = 13.49 A, β = 87.19°, (e.s.d. of absolute values 1-2%, e.s.d. of ratio 0.3%), space group $P2_1/n$, Z = 4, Dcalc = 2.27 g cm^{-3}. (An approximate unit cell was derived from Weissenberg photographs taken with Cu$K\alpha$ radiation; the axial ratios were refined from the Laue photographs within the program GENLAUE.)

Film Processing

The crystal orientation was found using the 'index' facility in the program LGEN on the ICL PERQcomputer (5). The orientation and unit cell were refined with GENLAUE on the VAX computer; r.m.s. displacements of observed from predicted spot positions were 0.05 to 0.075 mm for 100 spots selected as nodals. Careful comparison of the original film with printed versions of the LGEN predictions then allowed the soft limits to

be estimated:

λmin 0.28 A (from the innermost observable reflections)

λmax 2.5 A

dmin 0.88–0.92 A (slight increase through data set)

Integration by INTLAUE (6) used box parameters 11 11 3 3 1 for the film packs ϕ = 0 to 120°, and 13 13 5 1 1 for ϕ = 140 and 160°; the spot is integrated over an area 0.5 x 0.5 mm or 0.6 x 0.6 mm and the background estimated from a border 0.05 mm all round. The spots increased slightly in size in successive film packs, suggesting a small increase in mosaic spread (7) with Xray exposure, and the box size was adjusted to accomodate this. Scaling and merging with AFSCALE yielded 2000–2400 reflections for each of seven film packs with merging R's of 0.09 to 0.13; two other film packs for which the integration or merging seemed much less satisfactory were rejected at this stage, and no multiplet reflections were retained. LAUENORM (8) derived the wavelength normalisation curve f(λ) and the film pack scale factors. The most satisfactory data set was obtained using only the data for λ = 0.54–0.91 A, with I > 2σ(I); this consisted of 3958 reflections with a merging R (on intensity) of 0.12.

Structure Solution and Refinement

SHELX86 (9) with its automatic Patterson search routine PATSEE located an octahedron of rhodium atoms; the remainder of the structure was found from a series of electron density maps and then refined. Throughout the solution and refinement all Fobs were modified by the factor $\exp(-4\sin^2\theta/\lambda^2)$; without this factor the data appeared very 'sharp' and zero or negative vibration parameters, U, were obtained in the refinement; with the factor, the vibration parameters are very like those in similar compounds. The need for this modification factor indicates that some systematic error is present. Sample absorption is serious; the absorption coefficient of Rh increases from μ = 14.2 cm-1 at λ = 0.6A to μ = 45 cm-1 at λ = 0.9A; we have avoided using wavelengths very close to the rhodium absorption edge at 0.53A. An absorption correction should be calculated for the crystal and diffraction geometry and applied before wavelength normalisation and merging; We are not confident that this would account for all of the present error.

The structure

The structure is illustrated in Fig 2. An octahedron of rhodium atoms has four face-capping carbonyl groups, ten terminal carbonyl groups and the diphosphine ligand bridging two rhodium atoms. Bond lengths and stereochemistry are normal for this type of compound (e.g. see reference (10)), and the structure is consistent with n.m.r. evidence and expectations from its chemistry (11).

Discussion

The work shows that meaningful intensities can be measured from Laue diffraction patterns for the determination of an

be estimated:

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Discussion

The work shows that meaningful intensities can be measured from Laue diffraction patterns for the determination of an

unknown and fairly complex structure. There are several areas that require further work, the absorption correction and investigation of other possible causes of systematic error in the data. If the method were applied to a very small crystal it would be necessary to determine cell dimensions by a means other than conventional Weissenberg photography; the axial ratios can be well determined from Laue photographs, and it should be possible to combine this information with accurate powder diffraction data to get good absolute values.

Acknowledgement

We are grateful to John Campbell, Ian Clifton and John Helliwell for help at various stages, but the project would not have been possible at all without the software development work of Pella Machin and Mike Elder, so tragically killed in the Cairngorms in March; we mourn their loss.

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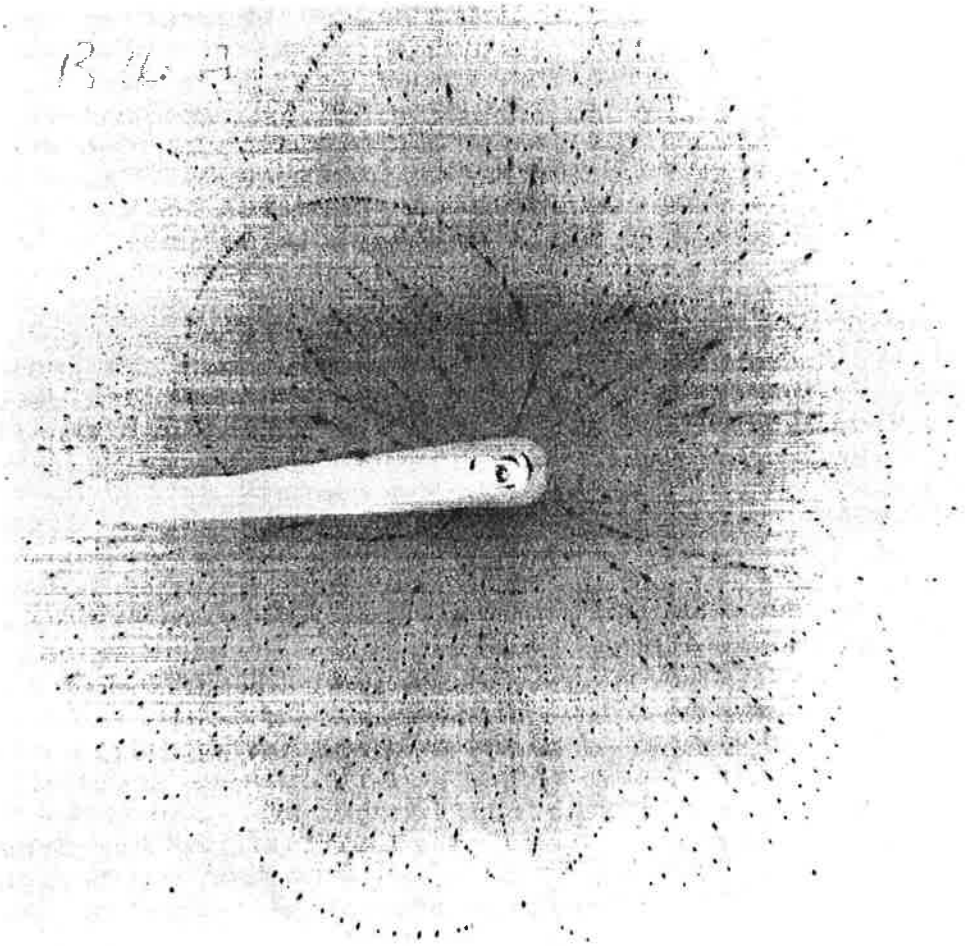

Fig 1 One of the Laue diffraction photographs used in this structure determination.

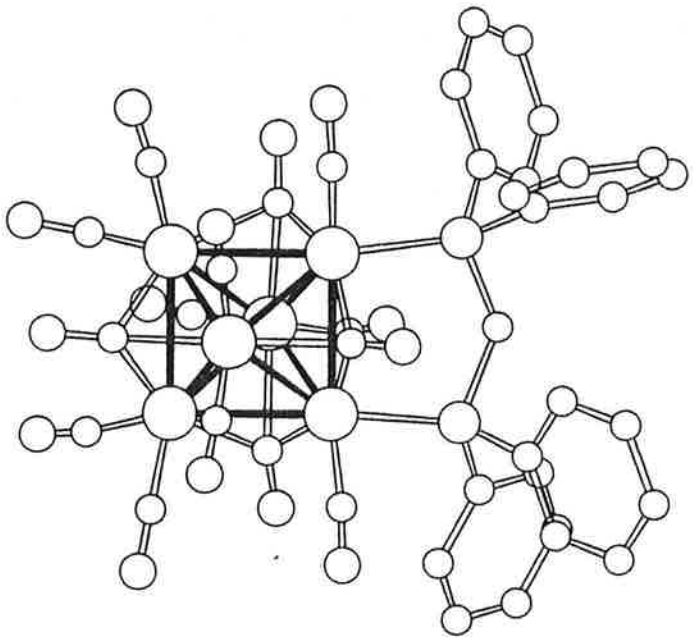

Fig 2 The structure found for $Rh_6(CO)_{14}$ dppm. The largest circles represent (six) Rh atoms, the next largest (two) P atoms, then oxygen; the smallest represent carbon atoms.

Measurement of Oscillation Photographs collected on the SRS: Recent Practical Experience

Peter Brick, Charles Collier, Kim Henrick, Andrew G.W. Leslie & Alan J. Wonacott.
Imperial College, London.

The initial step in the measurement of oscillation films is the determination of the precise crystal orientation and reciprocal cell parameters. This is usually achieved by indexing reflections appearing on a pair of "still" photographs taken at two widely separated spindle settings and then refining the cell parameters by minimizing a function involving the distance of the reciprocal lattice points from the Ewald sphere. Thus satisfactory refinement of the parameters is critically dependent upon the accuracy of the initial estimates of the parameters used in the indexing procedure.

The wavelength must be known accurately: Typically the reciprocal cell dimensions must be known to better than 1 %. In practice, we have found that the wavelength on station PX9.6 can be sufficiently different from the nominal value to cause difficulty in indexing "still" photographs. For example, with recent data collected on PX9.6, the nominal wavelength was 0.88 Å while our measured value was 0.873 Å (obtained from comparing the diffraction of a number of crystals with known cell dimensions). On an earlier occasion the measured wavelength was 0.90 Å. These differences can be outside the rather limited range of convergence of the indexing procedure. In contrast, we have generally found the wavelength on station PX7.2 to be close to the nominal value of 1.488 Å.

The camera constants must be known accurately: In order to index the reflections it is important to know the position on the film of the direct beam relative to the fiducial marks. The beam position can be recorded directly on the film by momentarily moving the beam-stop away using a "flying beam-stop". In practice we have sometimes found difficulty in obtaining the correct exposure — either recording a large splodge or nothing at all. On station PX9.6 the camera constants (position of the direct-beam compared with the mid-point of the fiducial marks) can be significantly different from zero. For data collected recently on this station the horizontal offsets for different cassettes ranged between 0.73 and 1.03 mm with vertical offsets between -0.59 and -0.91 mm.

If the camera constants are unknown and the cell dimensions are reasonably large, we have found it possible to mis-index all the reflections by one and still end up with a reasonably small residual in the refinement procedure.

The direct-beam would simplify oscillation photograph measurement: When using the SRS we currently do not record the straight through beam on the oscillation films themselves as it is tedious to do and the equipment can be unreliable. However, when we come to measure data collected from crystals with large cell dimensions we have to manually align the calculated and observed diffraction patterns using an interactive graphics terminal. For example, the trigonal crystals of tyrosyl-tRNA synthetase have a **c** cell spacing of 240 Å. For 2.5 Å data this corresponds to a spot spacing on the film of 0.5 mm which means that the camera constants have to be known to

better than about 0.2 mm. In practice, we have found that it is possible to measure synthetase films with all the spots misindexed by one in the l index without any very obvious indications from the film measurement program. Not until we obtain a value for the agreement between supposedly symmetry related reflections on the film is it obvious that something is wrong.

Accurate misorientation angles from oscillation photographs: If the cell parameters are known but no "stills" are available, it is possible to obtain accurate misorientation angles directly from an oscillation photograph by simply treating the photograph as if it were a "still" taken at the mid-point of the oscillation range.

Although in the refinement procedure it is assumed that the reflections should lie exactly on the Ewald sphere, we find that the technique gives values for the missetting angles in very good agreement with those obtained from the usual refinement method. The differences are typically less than 0.03°. The main limit to the accuracy appears to be set by the misalignment of the cassette on the camera. For an accurate measure of the rotation of the crystal around the X-ray beam ($\Delta\psi_x$ in our notation), the angle made by a line joining two fiducial mark positions and the rotation axis must be known. This angle (the ω camera constant in our notation) is normally less than 0.1°.

We currently digitise our "still" photographs and use a computer program (STILLS) to find the coordinates of the reflections and fiducial marks. The same program (with increased array sizes) can be used to find the reflection coordinates on an oscillation photograph and then the program IDXREF (also with increased array sizes) used in the normal way to index the reflections and refine the crystal orientation. The success of the indexing procedure can be judged by the agreement between the observed and calculated spot positions on the film.

As an example, using recent data collected on PX9.6 from a trigonal crystal of xylose isomerase ($\mathbf{a}$ = 105.67 Å $\mathbf{c}$ = 154.3 Å), the program STILLS found 885 reflections out to a radius of 40 mm on a film collected in the rotation range 7.30–8.95°. Using the middle value of 8.125°, IDXREF indexed all the reflections and gave missetting angles within 0.02° of the values obtained using the normal procedure. The rms difference between observed and calculated spot positions was 0.06 mm and the rms value of the quantity minimised $(r_{obs} - r_{calc})/d^*$ is 0.375°. By starting with the reflections near the center of the film and increasing the radius for included spots during the course of the refinement, the radius of convergence can be extended to 2°.

For this spacegroup the two cell dimensions can be refined from a single "still" photograph. However, with an oscillation photograph, the refinement results in poorly determined parameters although they are within 3 standard deviations of the known values. In the present case, refinement gives $\mathbf{a}$ = 106.46(.36) Å and $\mathbf{c}$ = 154.59(.35) Å.

A COMPUTER-CONTROLLED SYRINGE SYSTEM FOR CRYSTALLIZATION SCREENING

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Macromolecular crystallization screening trials typically require fairly large volumes (at least a few microliters) of material at high concentration and involve a great deal of tedious pipetting. As the available quantities of proteins of interest to the crystallographer may be very small, screening methods using smaller volumes are required. The repetitive nature of the screening procedure, and the occasional requirement for setting up crystals under non-ambient conditions (eg. in a reducing atmosphere or under microgravity conditions) makes it an ideal candidate for automation. We will describe an inexpensive system using computer-controlled motorized syringes which can produce screens using sub-microliter volumes of material. The system consists of a set of syringe modules (up to 64) which will hold any standard size of Hamilton (or similar) syringe, interface boxes (1 per 4 syringes) and the controlling software. This may be run off any of a number of microcomputers, preferably of the PC type. In preliminary tests we used 3 syringes containing, respectively, protein solution, precipitating agent and barrier fluid (silicon oil). The syringes are arranged to feed into a tube such that program-specified volumes of the 2 reactants mix forming drops that are separated within the tube by the barrier fluid, which isolates each drop from its neighbors. There are various ways to store the output, in our tests we used siliconized melting-point tubes or petrie-dishes.

Preliminary tests crystallizing the known proteins lysozyme (1) and ferritin (2) were very encouraging. Crystals were produced at the appropriate conditions with perfectly standard morphology following the usual time course. They were marginally smaller than are generally produced by the standard methods (1), but most of the trials were done using smaller drops than can usually be used in batch-type procedures. (The apparatus should reliably produce drops anywhere in the range from a few tens of nanoliters to a few ml depending on the size of syringe fitted.) We expect to be able to devise much more complicated screens using more syringes, hopefully including vapor diffusion, and trials of other proteins are in progress.

Using larger (60 ml) syringes, the setup can also be used as an adjunct to manual procedures. We use 2 syringes with different concentrations of precipitating agent (and one with air as a separator) to fill the wells of Linbro tissue culter plates used in hanging-drop vapor diffusion experiments. At top speed the wells can be filled essentially as fast as one can move the output tube from one well to the next. The system is produced by The DBSS Partnership.

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ACA PROJECT - PROTEIN CRYSTALLIZATION PLATE
(A Status Report)

Recognition for bringing this project to the ACA should be given to Noel Jones of the Eli Lilly Company. Noel worked with a number of colleagues at other laboratories in the crystallographic community to optimize the design of a protein crystallization plate that would be compatible with robotics. Crystallographers contributing to the final design were Keith Ward and Mary Ann Perozzo of the Naval Research Laboratory. Because of this collaboration, Noel Jones did not consider the final design to be proprietary to his employer. He conceived the idea of giving the ACA ownership of the design for the mold to produce the crystallization plate. In this instance, the ACA is a "neutral third party," i.e., the ACA will own the design, as well as the mold itself, to ensure continued availability of the crystallization plate to users.

In addition, Noel Jones worked to assure funding to the ACA to cover the cost of the fabrication of the injection mold to produce the protein crystallization plate. He did this by obtaining pledges of funds to be donated to the ACA for this purpose.

As of March 31, 1987, the ACA has received $21,000 in donations. The ACA has already contracted the machining of the mold; the delivery date is estimated to be in April. Fabrication of the mold is expected to cost $24,000. Negotiation and completion of a license agreement with the proposed manufacturer of the plate, Flow Laboratories, will incur additional legal fees. Other costs would include copyrighting and/or patenting the mold design or the plate and possibly introductory promotions and advertisements.

The ACA is in the process of requesting fulfillment of the pledges made earlier. However, because of costs in addition to the basic cost of fabrication of the mold, the ACA is still seeking donations to the protein crystallization plate project. If you think your organization would be able to contribute to this project, please contact Catharine Foris, ACA Treasurer, at (302) 772-3687 [(302) 695-3687 after May 1, 1987].

Organizations that have contributed or pledged are listed below along with the crystallographic contact.

Abbott Laboratories	C. Abad-Zapatero
E. I. du Pont de Nemours & Co.	P. C. Weber
Genentech, Inc.	A. Kossiakoff
Hoffman-LaRoche, Inc.	J. F. Blount
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Naval Research Laboratory	K. B. Ward, M. A. Perozzo
Nicolet Instruments Corp.	M. J. Janiak
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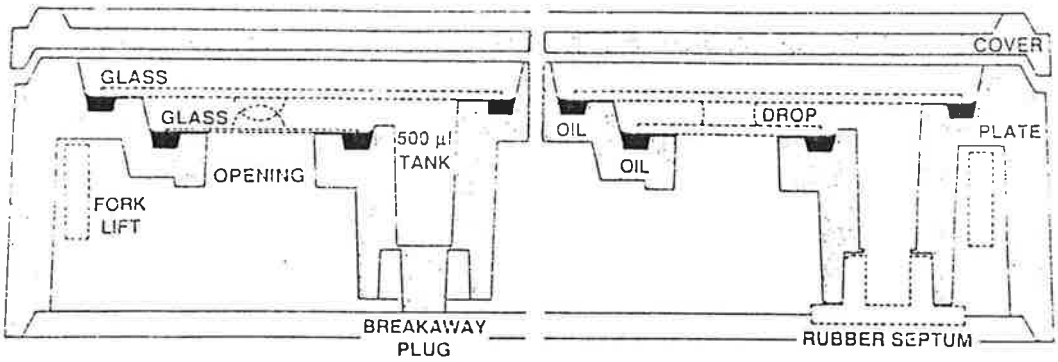

Instructions for Use

In this envelope are enough glass slips to set up fifteen sandwiched drop crystallizations. The slips should be cleaned and silylated before use so that your protein solution will not wet the glass. If desired, the breakaway plug in the bottom of a reservoir may be removed and a rubber septum inserted so that the equilibrating solution may be changed with a hypodermic syringe. Prepare the plate by filling the upper and lower troughs of each well with ordinary hydrocarbon vacuum pump oil. A monomolecular fluorocarbon polymer coating on the plate prevents the oil from creeping on the polystyrene surface. Into each reservoir put 500 µl of the desired equilibrating solution. Position one of the 14 x 14 x 1.5 mm glass slips over the hole in each well. If the troughs are sufficiently full of oil, the slips should seal quickly. Put a 5 to 25 µl drop of the protein solution (mixed with equilibrating solution, if desired) in the middle of the lower glass slip and then set one of the 24 x 30 x 1.0 mm glass slips in position on the upper trough. If the glass slips have been properly silylated, a sandwiched drop should form.

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