DARESBURY LABORATORY

JOINT CCP4 and ESF-EACBM NEWSLETTER on PROTEIN CRYSTALLOGRAPHY

An Informal Newsletter associated with the SERC Collaborative Computational Project No.4 on Protein Crystallography and the ESF Network of the European Association of the Crystallography of Biological Macromolecules

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Editors: Wojciech M. Wolf

Keith S. Wilson

Science and Engineering Research Council
Daresbury Laboratory, Daresbury
Warrington, WA4 4AD, England

EMBL c/o DESY
Notkestrasse 85, D-2000 Hamburg 52
Federal Republic of Germany

ESF Network on the Crystallography of Biological Macromolecules

J. Drenth and K.S. Wilson

Organisation

The Network started in 1987 under the chairmanship of Wim Hol with the aim of bringing established and young biomolecular crystallographers in Europe together. It was supported by seed money from the European Science Foundation in Strasbourg for the period 1987 - 1989. Different names for the Network were used which caused some confusion; its official name is in the title of this report. "Network" is the short name we commonly use.
The funds allocated by the ESF were spent with a minimum of burocracy and a maximum of scientific output. The chairman and the secretary take care of the daily business. A Coordination Committee (see Appendix 1) meets a couple of times per year to discuss the planning of the activities and the implementation of new initiatives. At the request of the ESF the Coordination Committee has a limited number of members and as a consequence not all biomolecular crystallography groups in Europe are represented. Therefore in order to obtain an as wide as possible input we have also formed an Advisory Committee, thus having nearly all research groups in the field represented (see Appendix 2). For reasons of economy the meetings of the Advisory Committee take place during major scientific events, such as the Como meeting.

Activities in the Phase 1 period (1987 - 1989)

Three major activities were begun in the Phase 1 period.

1. WORKSHOPS and MEETINGS

The Coordination Committee coordinated and initiated meetings and workshops in the field of biomacromolecular crystallography. It appeared possible to coordinate workshops and training courses supported by organisations such as EMBO, EEC and FEBS, and to have a significant influence on the timing and subjects of the courses given. In addition the Network has taken initiatives on its own, e.g. the organisation of the First European Workshop on Crystallography of Biomacromolecules held in May 1989 in Como (It.). The workshop was organised by two members of the Coordination Committee, Dr. M. Bolognesi and Dr. J.N. Jansonius. It was specifically aimed at providing opportunities for young crystallographers to present results, to

describe methods and report about problems encountered. More than 150 scientists participated from over 20 different countries. The success of the workshop was such that it was felt that it should be held every two years. Dr. G.E. Schulz and Dr. H.L. Monaco are now organizing the second meeting which will again be held in Como, on May 13 - 16, 1991.

2. WORKING GROUPS

Another initiative of the Coordination Committee has been the creation of Working Groups.

Working Group 2.1 has been devoted to the development of structure determination software. It has been successful in bringing the software specialists from the major biomolecular crystallography laboratories in Europe together - for the first time - and discuss how the expertise in Europe should be combined. The major activities of Working Group 2.1 during the Phase 1 stage have been as follows:

(a) Creation of a general forum where those activily involved in programming in the various European protein crystallography groups can meet on a regular basis, 3 - 4 times per year.

(b) The selection of a program system on which the Working Group should base its efforts and activities. Writing a *de novo* system was rejected as impractical. The final choice was to build onto the system developed in the U.K. by the CCP4 group. This is flexible and modular, and allows for forward development by many participating groups.

(c) The work in Daresbury for the CCP4 has concentrated on the standardisation of code in FORTRAN 77 and the standardisation of keywording in a subroutine library.

(d) The Working Group has concentrated its efforts on defining a file format to handle reflection data, maps and coordinates. The specifications have been made and the second version of the subroutine code to read and write the files is nearly ready for limited release. Future plans for the Group are

1) The full exploitation of new software technology such as X-windows, and

2) The continued maintenance and updating of the package. It is most important that the European Community as a whole continues to contribute to these developments. Working Group 2.1 is an ideal Group to ensure this is realized.

Working Group 2.1 has clearly identified the need for at least one postdoctoral collaborator to be paid by European funds, and to fully devoted to the goal of obtaining a modular, uniform and comprehensive package. Due to the existence of Working Group 2.1, an optimal coordination of the work of this new postdoctoral fellow with that of the counterpart in the U.K. based CCP4 program is ensured. Dr. Wojtek Rypniewski has been appointed to this post.

A second Working Group (2.2) was started and aimed at coordination of Computer Graphics and Molecular Modelling Software. After a most stimulating first meeting it proved to be difficult to find common ground for collaborative software development. Undoubtly the dominant role of commercial packages is the underlying cause of these difficulties. Some progress was made towards a definition of uniform file formats for molecular modelling purposes, but it has finally been decided to end the independent activities of Working Group 2.2 and to incorporate it into Working Group 2.1, which will take on the development of graphics (as opposed to molecular graphics) software and restrict its activities to problems related to structure determination. This will include the use of new window systems for workstations, the display and manipulation of crystallographic models and maps on workstations, and the development of a more user friendly environment for the crystallographic package.

A third Working Group (2.3) has been set up in the spring of 1989 and includes experts in synchrotron radiation. Representatives from the three "biomolecular" synchrotrons in Europe: Daresbury, Hamburg and Orsay as well as a representative of the ESRF came together to discuss joint projects and an optimal development of the new beamlines of the ESRF to be built in Grenoble. The meetings of this Working Group proved to be extremely successful.

3. NEWSLETTER

A Newsletter of the Network was created under the guidance of the Secretary, Dr. K.S. Wilson. This Newsletter has had a great impact on the awareness of researchers in the field so that they much more than before considers themselves members of a European scientific community. It has increased mobility of scientists because of advertising vacancies. It has also been used as an instrument in obtaining views from the crystallographic community on themes such as the optimal design of beamlines on the forthcoming European Synchrotron Research Facility. The Newsletter is now published as a cooperative effort between the Network and Daresbury.

Phase 2 (1990 - 1993)

Phase 2 proposals are funded by the ESF following an *a la carte* system of financing: individual members of the ESF are invited to support the proposed activities for the Phase 2 period. Fortunately our proposals were very favourably received and the budget we asked for was granted completely. It amounts to 740 000 FrFr. per year, less 120 000 FrFr. overheads for internal ESF support. Phase 2 covers a period of three years. The countries who have committed funds to the support of Phase 2 of the network as of 21.March.1991 are Belgium, Denmark, Finland, France, Germany, Italy, Netherlands, Norway, Sweden and United Kingdom.
We are most grateful to the colleagues who have spent or will spend part of their time in organising meetings or workshops in the interest of European Biomolecular Crystallography. Each of the larger structural groups in Europe is expected to organise at least one intensive workshop in order to spread the workload evenly.

We are most grateful to the European Science Foundation for their financial support which - we believe - is extremely well spent and has given a firm basis for European cooperation which will last much longer than the Phase 1 + 2 periods.

Appendix 1: Coordination Committee

Appendix 2: Advisory Committee

APPENDIX I

Coordination Committee

Prof. J. Drenth (Chairman)	Chem. Laboratoria der Rijksuniversiteit, Groningen/Netherlands
Prof. M. Bolognesi	Universita di Pavia, Italy
Prof. C.I.Branden	Sveriges Lantbruksuniversitet, Uppsala/Sweden
Prof. B. Clark	Aarhus University, Denmark
Dr. P.R. Evans	University of Cambridge, UK
Prof. T. Richmond	Inst. f. Molekularbiologie und Biophysik, Zürich/Switzerland
Prof. W. Saenger	Freie Universität Berlin, Germany
Prof. J. Sussmann	Weizmann Institute, Rehovot/Israel
Dr. J.C. Thierry	Institute de Biologie Moléculaire et Cellulaire, Strasbourg/France
Dr. K.S. Wilson	EMBL, Hamburg/Germany
Dr. S. Wodak	Université Libre de Bruxelles, Belgium

APPENDIX 2

Advisory Committee

Prof. T. Blundell	University of London, UK
Dr. W. Bode	Max-Planck-Institut, Martinsried/Germany
Dr. G. Bricogne	Université Paris-Sud, Lure/France
Dr. T. Deisenhofer	Howard Hughes Institute, Dallas/Texas
Prof. O. Dideberg	Université de Liège, Belgium
Dr. R. Haser	Laboratoire de Cristallographie et Cristallisation de Macromolécules, Marseilles/France
Prof. W. Hol	University of Groningen, Netherlands
Prof. J. Jansonius	Universität Basel, Switzerland
Prof. A.T. Jones	University of Uppsala, Sweden
Prof. M. Kokkinidis	Institute of Molecular Biology & Biotechnolgy Heraklion/Greece
Prof. G. Schulz	Albert-Ludwigs-Universität, Freiburg/Germany
Dr. D. Stuart	University of Oxford, UK
Dr. N. Walker (Industry observer)	BASF AG, Ludwigshafen/Germany

ESF WORKSHOP

14th-15th December 1990

"Synchrotron Radiation facilities for Macromolecular Crystallography : present and future"

This workshop was held at Courcelle in South West Paris. Three of the original speakers were unable to attend. Prof. Hans -Peter Weber (Lausanne), and Prof. Andrew Miller (ESRF, Grenoble) had problems with flights due to bad weather, however Prof. Weber submitted a short manuscript. Prof. Harutyunyan (Moscow) was unable to attend due to visa restrictions from the French consulate service in Germany.

Short summaries of the presentations were provided by most of the other speakers and are included in the following pages of the newsletter. Guy Dodson provided a brief summary of the discussion session, which was lively and useful. Lindsay Sawyer's report on the meeting for the SERC is also included.

Roger Fourme
John Helliwell
Keith Wilson

ESF WORKSHOP

14th-15th December 1990

"Synchrotron Radiation facilities for Macromolecular Crystallography : present and future"

Friday, 14th December — **S.R. facilities : present**

14:00	R. Fourme	"LURE"
14:45	C. Nave	"Daresbury Laboratory SRS"
15:30	K. Wilson	"EMBL Hamburg"
16:15	*Tea*	
16:45	K. Moffat	"USA SR"
17:30	I. Andersson	"Photon Factory"
17:45	J.-L. Staudenmann	"Brookhaven"
18:45	Discussion	
19:30	*Dinner*	

Applications

21:00	D. Stuart	"Phosphorylase b"
21:20	R. Goody	"Ras P21"
21:40	A. Yonath	"Ribosomes"
22:00	J.-C. Thierry	"t-RNA synthetase complex"

Saturday, 15th December — S.R. facilities : future

09:00	J. Helliwell	"Overview"
09:45	S. Bernstorff/M.Colapietro	"Trieste"
10:15	A. Kvick	"ESF : beam-lines"
10:45	*coffee*	

General Discussion

11:00	G. Dodson	Introduction
11:15	General discussion	
12:30	*Lunch*	
14:00	H.-D. Bartunik	"MPG line in Hamburg"
14:30	M. Roth	"The French CRG at ESRF"

Applications

15:00	I. Andersson	"Rubisco"
15:30	D. Stuart	"Viruses"
16:00	A. Ducruix	"Accuracy and resolution"

Final discussion

16:30	-	Discussion
	Dinner and Evening	free for participants to organise independently

** Prof. Harutyunyan was unable to obtain a visa.
* Prof. Weber and Miller had problems with flights due to weather.

ESF Workshop
14-15th December 1990

The meeting was convened first in order to review the present organisation of synchrotron radiation in relation to PX in Europe (and also USA, Japan and the USSR), secondly to identify the PX community's needs for the future and thirdly to decide how to project these needs usefully to the bodies responsible for synchrotron radiation and its planning.

There were representatives from all the European synchrotron radiation centres and two representatives from the USA. Individuals with knowledge of the state and resources of the Japanese and Russian centres were present and able to give the meeting the necessary information. Altogether these 40+ people present represented almost all the major European PX laboratories.

There were presentations designed to inform the meeting of existing and future facilities at each of the synchrotron radiation centres. There were also two scientific sessions where application of synchrotron radiation in structure determination and time resolved experiments, and in high resolution refinement were presented.

The discussion session was designed to identify priorities, to raise concerns and to define what steps were needed to ensure the PX interests were recognised. The major issues were considered to be:

1. The size of the PX community

It was realised that the PX community does not know its size. In Europe it was estimated that there are over 100 significant PX groups in over 50 labratories. Worldwide the figure could be triple this.

Recommend action to be taken to determine the size and structure of the European PX community. This will be carried out by the SRS (Colin Nave) and the EMBL (Keith Wilson) who have circulation lists and records of principal investigators. An equivalent exercise is being undertaken by the Americans.

This information to be collected for the EACBM.

The ESF Network of the EACBM to represent PX interests to ESRF, EMBO, etc.

2. The principal needs

i. Detector development for advanced, rapid, accurate, data collection facilities (often referred to as routine). The meeting identified image plate systems (with improvements in scanning technology) as the highest priority at present. High resolution data (i.e.2 → 1 Å spacing) has enabled PX to define crystal structures accurately (i.e. ca 0.2Å error in coordinates of well defined atoms). This resolution also ensures that errors can be detected and actually simplifies refinement. It is essentiall to have this data, even though its acquisition is not glamorous. Without it the chemical and physical interactions responsible for protein function cannot be reliably determined.

ii. Improved ability to deal with small crystals. The scope of PX is limited by crystal size and by the ability to measure diffraction from poor crystals. The more SR enables small and poor crystals to be used, the larger the scope of PX will become.

iii. Improved ability to measure data from crystals with very large cells. (These are often small crystals.) Large proteins, protein assemblies and viruses are increasingly becoming available for study. The importance of structural research in these systems adds urgency to getting the experimental needs recognised.

iv. Development of thechniques. Laue (especially time resolved, but not exclusively); MAD (multiple wavelenght anomalous dispersion) - this approach has been shown to work and there will always be a proportion of studies needing this technique. As the number of X -ray analyses increases, this population will become significant.

v. Very short wavelenght. The possible benefits of very short wavelenght (ca. .3Å) include longer crystal life, more efficient usage of available apertures. Assessment studies are needed.

vi. Year round availability and improved access for urgent experiments (see 3(v)).

<u>Recommend</u>

i. Investigations into and assessments of the development of large area IP sytems, including the Weissenberg system. Note that the scanning technology associated with the IP is crucial.

ii. Continued study of CCD systems and other area detector technologies.

iii. That an appropriate proportion of resources is made available for these exercises (see (3) below).

3. The principal concerns

i. The interests and experimental needs of PX be recognised.
ESF Network of the EACBM act to represent PX interests to EMBO, ESRF, etc.

ii. Detector development. This is undersupported and its importance for PX not fully recognised. Propose that the ESRF Detector Resources funding be considered to support 'PX relevant' research into detectors.

iii. Beam line priorities at the ESRF. Reiterate that the priorities are for:

a. Wiggler
b. Undulator
c. MAD

The importance of MAD has increased considerably owing to the growing population of structures which would benefit from this approach. The PX community would like to bring forward the commissioning of the MAD station by six months to one year.

The high priority of the wiggler and undulator status remains.

iv. Responsibility for beam line development. The urgency and need of the community for appropriate beam lines at the ESRF should be recognised. The proposal that the EMBL should be responsible for their development, rather than the ESRF was very strongly supported.

v. Communications with ESRF. The PX Advisory Committee would like to see improvements in the communications between the machine physicists/engineer responsible for PX beam ines. There is a particular concern over the defined performance parameters of line 4; line 3 is more satisfactory.

vi. The coordination between SR centres.

a. There would be great advantage in the SR centres coordinating their down-time and major engineering where possible. This request would benefit all users and would be best made through user groups.

b. There is a separate questions as to whether selectivity in the expertise and technique investment should be considered. The idea that some SR centres (in Europe and/or USA) would specialise in data collection, low temperature methods, MAD etc. is attractive if it meant that resources at those centres were maintained at the state of the art.

Recommend: That the ESF Network of the EACBM advise the ESRF (SC) and EMBL of these concerns and priorities.

Conclusion. It was agreed that another workshop be held to review the SR situation in about one year.

Report on the ESF Workshop "Synchrotron Radiation Facilities for Macromolecular Crystallography: Present and Future", Gif-sur-Yvette, 14-15th December, 1990

L.Sawyer

Introduction

The workshop was sponsored by the European Science Foundation and organised in a trades union training centre just outside Paris by Roger Fourme, Keith Wilson and John Helliwell. Some 40 participants attended, the majority from Europe but with a few from the US. The programme was organised in a fairly flexible manner and was designed to cover the present facilities world-wide, those which are being built or are at a fairly advanced stage of planning and the various macromolecular crystallography (PX, though note that not all of this crystallography involves proteins) applications. There was ample time for discussion and this occurred throughout, although one full session was devoted to a more general coverage of the size, needs and inter- as well as intra-community cooperation of the protein crystallographers. Throughout, it was most evident that there are two major approaches to the application of synchrotron radiation to the study of PX: the main thrust is science lead, accounting for perhaps 90 - 95% of the users, the remaining 5 - 10% is no less important but is technology lead and it appears likely that this will play an essential role in much of the exciting biology which will be at the scientific forefront into the next century.

The European facilities

The workshop was opened by Roger Fourme (LURE) who described the history and present facilities at the Paris synchrotron. 2 stations on bending magnets (D41, D43) plus one on a wiggler (W32) are equipped with oscillation cameras. A 4th station on a bending magnet (D23), shared with materials science, has an area detector. In February 1991, D43 will close and W32 will be equipped with an image plate system (MAR). In April, the Mark 3 area detector will be introduced and by next year, a station fully equipped for multiwavlength anomalous diffraction (MAD) will be in routine operation. A case, coming mainly from materials scientists, is being prepared for a replacement 2.15GeV machine with λ_c = 2.5Å and a wiggler with λ_c = 1Å. The detector is overwhelmed with applications and LURE is moving away from film as rapidly as possible. They are not planning to perform Laue experiments.

Colin Nave (Daresbury) reviewed the SRS provision on lines 7.2, 9.5, 9.6 and in part, 9.7. Whilst the FAST had many advantages of high count rates and wide dynamic range, its limited resolution, complex data processing and spatial distortion/non-uniformity were seen as a major drawback to its popularity and in future image plate systems were likely to be the preferred means for data collection. The Hamburg facilities were reviewed by Keith Wilson (EMBL outstation) who stressed that the success of the Hamburg operation was an active, in-house PX group. Whilst the present (until the ?June shutdown) facilities were either parasitic or rather low-powered by say, DL, standards, the great stability and rapid refilling coupled with advanced data collection and processing capabilities, meant that the data which could be obtained were nearly as good as could be collected anywhere in the world. The upgrade to station X31 was scheduled for completion in June with the wiggler station becoming available at the end of the year, but these were seen as somewhat optimistic. A second point which was stressed was that access time to a facility should not be limiting - ample time (and facilities) to collect, process (at least some) and re-collect if necessary was seen as the most cost effective way to use the facility and this in turn meant groups bringing sufficient people to do this.

The Italian facilties were described by S.Bernstorff and M.Colapietro. The proposed Trieste facility is a 2.6GeV machine to run at 400mA. For diffraction experiments a 23 pole wiggler will form the basis producing some

10^{13} photons/sec/mm^2 with a $d\lambda/\lambda$ of 0.00015 and a λ_c of 1.13Å. A liquid Ga-cooled double crystal monochromator is planned and the detector, like that at Frascati, will be a Huber 4-circle with automatic alignment via window-based software.

Unfortunately, visa problems prevented E.Harutyunyan from telling us about the USSR facilities, and snow prevented Andrew Miller from talking on ESRF timescales. However, A.Kvick covered both the timescales and the beam lines proposed on the ESRF at Grenoble. The ring is due to have 7 insertion devices in routine operation by the summer of 1994, and is funded until 1999 when there should be 30 beam lines operating. Descriptions were given of wiggler line 3 and undulator line 4 which are suitable for PX but which will be shared facilities to start with. Whilst some of the problem of beamline geometry and heat dissipation were discussed, little was said about detectors at this stage.

As far as specific beamline proposals from CRG's are concerned, Hans Bartunik outlined the proposal for a German biotechnology beamline at Hamburg. A survey of the German users (actual and potential) showed that the line should be able to provided both tunable monochromatic and white radiation in a fairly routine manner. The plan is to have a 28-pole wiggler with a double mirror and a double crystal monochromator either or both of which can be removed from the beam in order to achieve focussed and unfocussed white, and focussed monochromatic radiation. In addition to FAST and image plate detectors, a cooler, laser, microspectrophotometer and beam chopper are planned as ancillary equipment for kinetic experiments. The French CRG at t he ESRF is to be a national facility satisfying the needs of both materials scientists and PX. Such a shared facility may not be optimal for either community. The need is seen to be for high resolution data and this requires high intensities between 0.5 and 2.5Å (MAD experiments). A station on a 0.8T bending magnet with a double crystal monochromator and vertical focussing by parabolic mirrors is planned with some 38MFFr already committed to build and operate the facility for ? years.

The American and Japanese scene

The US synchrotron provision was covered by Keith Moffatt (Chicago/Argonne). At CHESS a wiggler station specifically for viruses with proper biohazard containment facilities was available together with stations on an undulator becoming available from July. The Stanford facility was only available for a short time each year. Of the 2 stations at Brookhaven, X12 with a FAST detector was flux limited to 10^{10} photons/s and X25 was a wiggler station being used with white radiation. At Berkley the ALS has a wiggler insertion device team in life science which is involved in X-ray imaging and in PX, though the latter does not involve short wavelngth radiation. The APS at Argonne, which was what was discussed mostly, is a 7 GeV machine with 34 sectors, each comprising a bending magnet and an insertion device. Whilst the source is being provided by materials science money, that for the beamlines must be provided from elsewhere. (At this point we were introduced to the main output so far from the APS: a multibunch beam of acronyms!) There is an SBC (Structural Biology Consortium) funded by IBM, Exxon, DuPont and the State of Illinois. There are CATs which are the US equivalents of CR- or IR-Gs and each Collaborative Access Team can apply for 1 sector, specifying its choice of insertion device. There is CARS (Consortium of Advanced Radiations Sources) and Biosync, which is an organisation from within the structural biology community with some 400 PI's of whom 1/3 are users and of those 1/3 are major users. A small group of Biosync is involved in looking at the CAT proposals of which there are perhaps 4 involved with PX, to ensure the optimum provision of experimental facilities and to present a united front to the APS management. (The high energy physicists have a successfully working model of this idea.) Such a powerful group was thought to be singularly lacking in Europe. Still in the US, J.-L. Staudenmann, outlined the proposals for the 3 stations planned in the Howard Hughes Medical Institute building at Brookhaven. These are for MAD, Laue and high resolution data collection and will certainly include a biological containment laboratory associated with at least one of the stations. Comment was made about the need for developments in both monochromator and detector (particularly image plate scanner) design, and the need to ensure that new

facilities were tailored to the needs of biology, rather than biologists' having to make do with machines developed for other purposes.

Some details were given by I.Andersson about data collecting at the Photon Factory using the Weissenberg camera (radius 570mm, Image plate, wavelength = 1.04Å) A 4-5 deg. oscillation photograph on Rubisco took about a 10s exposure (a film exposed for a comparable time was blank!) gave a data set in about 20 min from 1 crystal. The readout per 20 x 40 cm plate was about 2 min. and the Rmerge = 6.9%. Some criticism of the uniqueness of the system and of the physical layout of the station was made.

General Discussion

Guy Dodson (York) chaired a general discussion session to review the current state of the provision of SR for protein crystallography in Europe. It was seen as important that the size of the user community in Europe be accurately determined: at present somewhere in excess of 50 laboratories were easily identified as having members who used the various facilities. Some means of coordinating the world resources was also seen as essential in the present financial climate. Significantly, much of the technological developments were being generated by the materials science community, who because of the obvious commercial applicability of their work, were generally well funded. This had tended to dictate the basic features of the various beamlines being constructed and had led to non-optimal, often shared, stations for PX. The experience of the US (and other) workers with hybrid stations (and beam lines) was one of frustration, delay and inefficient usage. It was noted that although the ESRF was starting off with effectively nothing but such shared facilities, they were aiming at achieving single-use stations as soon as possible (though this appeared to be around 1999!).

Much of the discussion centred around what were the perceived needs now and in the future. Crystals were being obtained from ever larger and more fragile biological systems, but the crystals were often small, had large unit cells and in many cases were of poor quality (one might have to screen 100 crystals to find one which gave only fair diffraction). Since high resolution and high accuracy were most important if sense was to be made of the biological questions being asked, improved means of handling such crystals and sufficient access for screening and for collecting and processing the data were seen as unavoidable.

Three types of data collection were seen as important in addition to accurate, high resolution measurements. These were multi-wavelength anomalous dispersion, Laue and time resolved measurements. Coupled to this was clearly the type of detector which would be ideal: one with a large area, a large dynamic range, a rapid read-out and small pixel size. Whilst a purely electronic device has the great advantage of allowing integration of small slices (0.1° typically), it was obvious that not enough work was being done at the fundamental physics level, particularly if the next step to shorter wavelength was to around 0.3Å.

The need for additional, peripheral support was also regarded as important: crystal handling, low temperature and flow cells are generally available anyway but there is a need for spectral measurements on crystals concomitant with data collection. There is also a need for data processing. The feeling of the meeting was that it should be possible to process data on site, if not on-line, more or less immediately after the data have been collected. It is absolutely essential that some processing be done during the access time to the beam so that data missed can be collected or duff data recollected.

As to availability, year round aceess at reasonable short notice is seen as essential. What appeared to be singularly lacking was any form of coordination of major shutdowns. It was seen as inevitable that downtime would overlap and to some extent was not always predictable, improvement should be possible if the various centres tried.

The remaining seven talks ere about the science which is being done around Europe, and covered such topics as viruses, RuBISCo, ribosomes, ras P21, phosphorylase b and tRNA-synthetase complexes.

Conclusions

The meeting was a worthwhile forum for the coordination of SR research in PX and it was felt anothr meeting should be arranged in about a year.

The size of the PX community in Europe needs to be ascertained accurately.In view of the perceived needs for better detectors, improved handling of small crystals and the data collected therefrom, especially with large unit cells, the technique development in Laue and MAD and the move to even shorter wavelengths, it was recommended that continued study of CCD systems and of large area IPs (including Weissenberg devices) be made and that approaches be made to ESRF to ensure that detector resource funding went towards PX-relevant research into detectors. Such concerns would be best transmitted to EMBO, ESRF etc. via EACMB. At ESRF it was seen as vital that the priorities should be for a station on the Wiggler, then the Undulator and facilities provided for MAD, the usefulness of this latter being likely to grow as the technique becomes more routine. It was also felt that responsibility for PX beamline development might be better taken by EMBL than ESRF, since it was also stated that the communications between the PX community and the ESRF machine physics/engineering was less than satisfactory.

An Overview of Facilities for Macromolecular Crystallography at SR Sources

John R. Helliwell, Department of Chemistry, University of Manchester M13 9PL England.

Instrumentation dedicated to macromolecular crystallography is available at all synchrotron X-radiation sources around the world.

The first published X-ray diffraction experiments performed at an SR source were those of Holmes and coworkers (1971) on muscle at DESY, Hamburg. The first protein crystallography work published was that of Hodgson and coworkers (1976) on a variety of crystals at SPEAR, Stanford. These experiments provided a platform for an expansion in this field that is still continuing apace.

Several types of facility or instrument can now be distinguished. Firstly, there are those for routine data collection i.e. measurement of high resolution data (the largest category of projects), virus and and other large unit cell projects (e.g. ribosome), and weakly scattering examples (high "B factor", small crystal volume or combinations of these). Such instruments are available in Paris (DCI), Hamburg (DORIS), Daresbury (SRS), Cornell (CHESS), Stanford (SSRL), Brookhaven (NSLS) and Tsukuba (Photon Factory). The detectors commonly used here are, depending on unit cell size, photographic film, imaging plate or television area detector. The user is, rightly, interested in a high degree of automation, ideally to the point of full data reduction. One type of data collection which is still labour intensive is that from virus crystals whereby use of 500 crystals for the first data set is common and photographic film is obligatory because of the dense diffraction patterns. The use of ultra short radiation ($\lambda \approx 0.33$Å) from undulator sources on the next generation of machines (ESRF, Grenoble; APS, Chicago; SPRING-8, Nishi Harima) may help solve this problem.

A second category of experiment is that of multiple wavelength data collection using tunable instruments ideally coupled to the most accurate means of data collection (four circle diffractometer, MWPC or, more recently, image plates). Obviously, the first set of instruments above can, to some extent, be used for such experiments. However their paramount design requirement of a very high intensity and the constraint of presently available source sizes (emittance), which have to be focussed down considerably to the typical crystal cross-section (~0.3

$\times$ 0.3 mm^2), means that they are not rapidly tunable. Although, SIROAS (single isomorphous replacement with optimised anomalous scattering) is feasible - and not without a good deal of technical ingenuity! - MAD (multi-wavelength anomalous dispersion) is not feasible on those workstations. The currently available instruments for rapidly tunable work at Hamburg (X-31), Paris (DCI), Daresbury (SRS-9.5), Stanford (Line I-5) and Brookhaven achieve the freedom to change λ at the expense of flux. This is not a severe limitation since the size of the effects that can be stimulated with (atomic) anomalous scattering restricts the projects of interest to relatively small unit cells and therefore well scattering crystals. There is a small, but significant, number of projects which seek these methods as a way to avoid an impasse in phase determination. Ultimately, the methods developed can speed up structure solving in general.

A third category of work, which is completely within the realm of SR sources, are those projects which require the speed of SR data collection. These projects particularly require use of the white beam stationary crystal (Laue) methods. This geometry completely harnesses the full polychromatic emission of the SR source. Exposure times have been realised, for a large fraction of a data set, in times certainly as short as tens of milliseconds and with feasibility tests performed in the sub-nanosecond region. To the instrumentation requirements here it is necessary to add, ideally, fast reaction initiation (e.g. by lasers or light flash), reaction monitoring and rapid data evaluation (i.e. sufficient to guide experiments on the beam line). Potentially the methods developed here could interest a wide community of users, in the longer term. In Europe multipole wiggler developments at DORIS and ESRF will be very important for pushing this work forward, especially the time resolution into the microsecond region.

The future may (!) take the following form. Undulator X-ray sources can provide λ's $\approx$ 0.33Å at ESRF, Grenoble (or Petra, Hamburg) for virus data collection using a single crystal; some feasibility tests are needed on the intrinsic perfection of virus crystals akin to those done on protein crystals at Frascati. Multipole wiggler sources at ESRF, Grenoble and on the DORIS bypass Hamburg will be used for time resolved work on enzyme (or other photo stimulable protein) crystals; the slower enzymes can be studied on existing wigglers (e.g. at SRS or DCI). Routine data collection, MAD and SIROAS is exceptionally well suited to the existing national facilities such as SRS or DCI. By the year 2020 SRS and DCI will be overtaken by the provision of compact (X-ray) sources - see for example SR News Nov/Dec 1990 for details of a 1.2 GeV machine with a 6T magnet - in the bigger universities. Synchrotron radiation usage will then fall into two categories - the central facility

"special" experiments (using 200m long wigglers and undulators) and experiments done on the home SR machine.

The main competing technology that is developing for structure solving is NMR. Structure determination is currently limited to ≈ 15000 molecular weight. Enthusiasts claim progress will be made upto 30000 molecular weight. This is still a relatively low limit in terms of needs. The year 2020, then, promises a strong activity in X-ray analysis of proteins and viruses supported with an increasingly large number of SR machines, from the small, home university sources upto the "mega"-central facility such as ESRF (and its successor.)

The overall aim of this research is to achieve a huge database of such structures, a detailed knowledge of the catalytic properties of these molecules and a full understanding of biology at the molecular level such that it can be properly harnessed for medical applications including drug design. The challenge to physics is strong in terms of instrumentation and methods and to chemistry in terms of the knowledge and prediction of reactivity and interactions of one structure with another.

PROTEIN CRYSTALLOGRAPHY AT THE SRS

Colin Nave
SERC Daresbury Laboratory, Warrington WA44AD, UK

The SRS is a 2GeV electron storage ring with a λ_c = 3.9Å and circulating currents of 200-300mA. A 5 Tesla superconducting wiggler is used as a wavelength shifter to give λ_c = 0.9Å. The machine was upgraded in 1987 to give a reduced source size of 2.4mm fwhm horizontal and 0.3mm vertical. The SRS runs at high currents for approximately 5000 hours each year. During the past 10 years four facilities for protein crystallography have been developed. Each facility is optimised for a particular type of data collection. However, particularly in the earlier beamlines, some flexibility was built in to gain experience of different types of experiment. The facilities are used for routine data collection where the demand exceeds the time available. In addition, less routine experiments, such as anomalous scattering and Laue diffraction are being undertaken. All the protein crystallography groups in the UK, as well as many from abroad, use the facility. Agreements with the Medical Research Council, Sweden and the EEC provide access for users not supported by the SERC. These agreements are used to fund some of the developments of the protein crystallography facilities.

Each station is briefly described below. The intensity for each station is quoted for 200mA current taking into account losses in the beamline. Measured values are in reasonable agreement with these figures.

Station 7.2 - Monochromatic
Bent triangular Ge(111) monochromator with 4mrad horizontal acceptance giving 9:1 horizontal focusing at 1.488Å.
$\Delta\lambda/\lambda = 0.0004$
Fused quartz mirror 1:1 vertical focusing
Focal spot size 0.5mm x 0.3mm HxV
Intensity $6X10^{11}$ photons/sec/mm^2
Arndt Wonacott oscillation camera and film data collection.

This was constructed in 1980 to use radiation from a bending magnet. It is available for approximately 75% of the time for protein crystallography.
Some wavelength tunability was incorporated into this station. However, it is now operated at a fixed wavelength of 1.488Å for routine data collection from protein crystals. There are no plans at present to develop this facility any further.

Station 9.6 - Monochromatic
Bent triangular Si(111) monochromator with 3mrad horizontal acceptance giving 8:1 horizontal focusing at 0.895Å.
$\Delta\lambda/\lambda = 0.0004$
Platinum coated fused quartz mirror 1:1 vertical focusing
Focal spot size 0.5mm x 0.3mm HxV
Intensity $1.3X10^{12}$ photons/sec/mm^2
Arndt Wonacott oscillation camera and film data collection.
Enraf Nonius FAST TV detector

This station on the wiggler has also been used for developing multiwavelength techniques and also focused Laue. It is now operated routinely as a fixed wavelength station at 0.895Å. The reduced wavelength on this station (compared with station 7.2) gives reduced absorption errors, a reduction in radiation damage, and enables higher resolution data to be collected. After many problems, the FAST TV detector is now operating routinely. It is also hoped to install an image plate based detector system on the station.

Station 9.7 - Laue
Wavelength range 0.2-2.6Å.
10^{10} photons/sec/mm^2 in $\Delta\lambda/\lambda = 0.00015$ at 1Å
Modified oscillation camera with film data collection
Shutter opening times down to 20 millisec

This station is available approximately 25% of the time for protein crystallography. It is the preferred station for those Laue experiments which require wavelengths of less than 0.5Å.

Station 9.5 - Focused Laue and Tunable Monochromatic
Wavelength range 0.45-2.6Å.
Water cooled channel cut Si(111) monochromator.
$\Delta\lambda/\lambda = 0.00015$
Platinum coated fused quartz toroidal mirror.
Aperture 1.4 mrad horizontal, 0.1mrad vertical
Focal spot size 1.5mm x 0.4mm HxV
Calculated intensity $3.6X10^{11}$ photons/sec/mm^2 at 1Å within $\Delta\lambda/\lambda = 0.00015$
Modified Arndt Wonacott oscillation camera and film data collection.
Shutter opening time down to 50 microseconds

This station is constructed via a collaboration with the Swedish research council. It is planned to install an image plate based detector system on the station. The station has been commissioned for the Laue experiments and gives the expected reduction in exposure time compared with station 9.7. Some test data for anomalous dispersion studies has been collected and is now being evaluated.

Other Facilities
Cooling to -20C and down to near liquid nitrogen temperature
Microdensitometer for film scanning
Microscopes
Biochemistry Laboratory
Computing facilities
Software Development - CCP4, Laue

Future Developments
The facilities described above are a result of many years effort in the development of X-ray beamlines optimised for protein crystallography. The intention is now to put more effort into improving the detector and computing facilities. This will include the purchase of image plate detector systems and possibly upgrading the FAST detector to improve its resolution. The computing facilities presently available will also need upgrading to enable people to process data during or as soon as possible after collection. The development of multiwavelength and time resolved experiments will proceed, with the involvement of experienced users with challenging problems to solve.

The stations on the SRS will continue to be useful, for the majority of protein crystallography projects, for the forseeable future. A facility on an ESRF bending magnet will have characteristics which are only slightly better than those of the SRS Wiggler stations. The multipole wiggler and undulator beamlines of the ESRF will have a major advantage, particularly for small crystals, large unit cells and time resolved experiments.

BEAM LINES FOR PROTEIN CRYSTALLOGRAPHY AT THE EMBL OUTSTATION IN HAMBURG.

Keith S.Wilson,

European Molecular Biology Laboratory, Hamburg Outstation, c/o DESY,

Notkestrasse 85, 2000 Hamburg 52, FRG.

PAST AND PRESENT

The EMBL outstation was set up in 1974 to apply synchrotron radiation (SR) to problems in molecular biology. The beam lines use radiation generated by the DORIS storage ring at the DESY (Deutsches Electronen Synchrotron) site. DORIS is operated in two different modes. In parasitic mode the high energy physicists are the main users, the ring is run at 5.3 GeV, with electrons circulating in one direction and positrons in the other. The needs of the high energy physicists dominate the ring parameters. The ring current after injection is about 40mA for each set of particles, and the time between injections is 1 to 1 1/2 hours. The ring is run in single bunch mode. The synchrotron radiation generated is nevertheless well suited to many of our experiments.

In the second mode of operation (main-user time) the DORIS ring is dedicated to the production of SR. The beam parameters are dictated by the SR users and various parameters such as beam energy and lifetime are significantly different from parasitic time. Only electrons circulate in the ring during main user time. The ring energy is 3.7GeV, the beam current is 80-100 mA after injection, and the time between injections about 3 hours. The ring is run in multi-bunch mode The beam-position is usually sufficiently stable under parasitic and main user modes such there is no need for realignment of the equipment after injection. DORIS is run roughly 2/3 of the time in parasitic mode and 1/3 in dedicated synchrotron mode.

The situation as of 1990 was as follows. EMBL had five SR beam-lines in Hamburg : two for protein crystallography, two for small angle scattering and one for EXAFS. The two beam lines for protein crystallography were X11 and X31.

X11 was situated in the EMBL building and took X-rays from the circulating positrons. It was thus only available during parasitic user time. X11 is the high intensity line for protein crystallography. The X11 line consists of a bent Fankuchen triangular Ge(111) crystal, segmented flat quartz mirrors, slits, and a moveable bench with a mount for either rotation camera or an imaging plate scanner. All movable elements are remotely controlled from a computer. The distance from the source to the central miror is 23.5 m and from central

mirror to focus 4 m, so that the demagnification is roughly 5:1. The monochromator is bent to achieve focussing in the horizontal plane. The available wavelength range is 0.95-2.3 Å. The wavelength is tunable in theory but changing the wavelength requires some time as it necessitates changing the take-off angle by rotating the whole bench. Vertical focussing is carried out by bending the bench on which the mirror segments have been prealigned. The set-up is shown in Figure 1. The dimensions of the focussed beam are about 1.2 x 0.7 mm^2. The crystallographic cradle on the line can be optimally oriented into the beam by a fully automatic procedure taking 1-2 minutes. This optimisation is achieved through the use of four motors which move the cradle about the first collimator slits as pivot point. The intensity is monitored after the first collimator slits for the translation and after the second slits for the rotations.

The intensity of the beam-line allows data collection at roughly 200 x the speed on a conventional source. This makes X11 one of the more intense beam-lines currently available for protein crystallography.

The X31 beam line, Figure 2, is in the Hamburg Synchrotron Laboratory (HASYLAB). The line takes radiation generated by the electrons and is available in both main-user and parasitic mode. X31 consists of a channel cut Si(111) crystal monochromator, double focussing segmented toroidal mirrors and slits. The distance from source to mirror centre is 16 m and is equal to the distance between mirrors and focus. Thus the demagnification is 1:1. The line has relatively large focal dimensions. The same type of automatically aligned crystallographic cradle as X11 is used, also allowing use of an imaging plate scanner or an oscillation camera.

The overall intensity on the crystal is some 30 x weaker on X31 in comparison to X11. One advantage of the line is its high positional stability resulting from the large focus. In addition the wavelength can be very easily tuned, involving only a rotation of the monochromator crystal with the outgoing beam remaining parallel and merely undergoing a small vertical shift. This property, coupled with the narrow wavelength band-pass of the Si(111) channel cut crystal, makes the line work well for optimised anomalous scattering studies.

Until 1988 data collection was restricted to photographic methods. At the end of that year a scanner for storage phosphor imaging plates, developed in-house by the instrumentation group, provided an automatic method of data collection with direct transfer of the two-dimensional images to the computer disc. The software currently in use for data reduction is a modified version of the MOSFLM system developed at Imperial College and Cambridge. The images are treated essentially as film. It is usually possible to reduce data to a merged set of amplitudes and sigmas within 1-2 days of finishing data collection.

FUTURE DEVELOPMENTS

The construction of a 'by-pass' to one of the high energy physics stations on DORIS is currently underway. In actual fact this no longer a by-pass as the physics experiment originally to be by-passed has now been removed from the ring. Thus the construction in practice entails the building of an asymmetric ring, DORIS III, the first ring to be intentionally asymmetric. This will provide seven straight sections in the ring where insertion devices, wigglers or undulators, can be installed. These lines will provide a gain of between one and two orders of magnitude in intensity at the sample compared to the present beam lines, given appropriate optical elements. EMBL is designing a beam-line for insertion device 7, a multipole wiggler. This will hopefully be completed early in 1992. The initial plan is for crystallographic data collection on this line.

A second part of the reconstruction allows the repositioning of line X11 in the new HASYLAB building to take radiation from the electron beam. This will allow the use of X11 during both parasitic and main-user modes of DORIS operation and will considerably extend the beam-time available for users in future years. Essentially complete years (about 40 weeks) of beam operation are foreseen for 1992-1994, with no major shutdowns planned.

ALLOCATION OF BEAM TIME.

The priorities committee of the EMBL is made up of external scientists who meet to consider the proposals received after roughly every 1 year of beam-time. The EMBL staff scientists do not sit on the committee but do play an advisory role regarding feasibility. The day to day allocation of beam-time is carried by the experimental groups in the outstation on the basis of the recommendations of the committee. Some beam-time is also reserved for new proposals received after the committee has met and judged by the staff to warrant urgent investigation. Beam proposal forms can be obtained from the author or any of the staff scientists at the above address. Further information can be obtained by :

1) Tel. 040-8908010

2) FAX 040-89080149

3) BITNET KEITH@DHHEMBL5

THE GBF/MPG WIGGLER BEAMLINE FOR PROTEIN CRYSTALLOGRAPHY AT DESY
Design principles and instrumentation

Hans D. Bartunik

Max-Planck Society, Research Unit for Structural Molecular Biology,
c/o DESY, Notkestraße 85, 2000 Hamburg 52, F.R.G.

The Society for Biotechnological Research (GBF) and the Max-Planck Society (MPG) together with HASYLAB are setting up a wiggler station (BW6) on DORIS III for X-ray diffraction studies of biological macromolecules. The facility is planned to be operational by the end of 1991. Potential user groups from research institutes and universities in Germany were invited to the first users' meeting which was held recently. The total beamtime requested by the potential users for structural biology applications in 1992 amounts to more than 300 days. For possible additional use of the beamline for small structure research, further 70 days of beamtime were requested. One may therefore expect that the new national facility will be extensively used, and that it will help to satisfy the rapidly increasing demand for synchrotron beamtime for protein crystallography in Europe.

The beamline is being designed as a general purpose instrument for all types of applications in macromolecular crystallography which require the high spectral brilliancy of wiggler radiation from DORIS. These include in particular studies of high molecular weight structures, experimental phasing by anomalous techniques, diffraction data collection to very high resolution, and investigations of protein dynamics and enzyme kinetics. The beamline will function in four different modes providing

- focused narrow-bandwidth monochromatic radiation ($\Delta\lambda/\lambda \approx 0.1\%$),
- focused medium-bandwidth monochromatic radiation ($\Delta\lambda/\lambda \approx 5\%$),
- focused polychromatic radiation ($\lambda \geq 0.6$ Å),
- unfocused polychromatic radiation ($\lambda \geq 0.3$ Å).

BW6 will be equipped with a 28-pole wiggler (k=13.2, field 1.0 T, power density 119 W/mA·mrad²). The X-ray optics includes a plane mirror (SiC on graphite, Pt coating) followed by a toroidal mirror (consisting of the same material) for 3:1 demagnifying geometry. A double-crystal monochromator (Si(111) for 0.6 <λ< 2.3 Å; Si(220) for 0.3 <λ< 1.5 Å) is located between the mirrors. Alternatively, a graphite double-monochromator positioned at short distance (0.5 m) from the sample may be used in medium-bandwidth applications. Such a bandwidth is of particular interest for Laue diffraction studies of structures with high molecular weight or broadened crystal mosaicity, and for diffuse scattering measurements. Considering narrow-bandwidth monochromatic conditions, a gain in intensity by one order of magnitude over a bending magnet beamline at DORIS like X11 may be expected.

For crystal orientation and data acquisition, rapid interchange between different instruments and detection systems will be possible. A 4-circle diffractometer is available which may be used with a FAST area detector (ENRAF-NONIUS) or with image plates. The FAST support permits rotation and inclination of the detector along a spherical surface in order to follow an efficient strategy in high-resolution data collection. Alternatively, a 3-axis goniometer may be used in combination with the FAST, image plates or photographic film. Computing facilities and software are available for OnLine data reduction and evaluation, both for monochromatic and Laue methods.

Auxiliary equipment may be used for low-temperature (> 100°K) and flow-cell experiments. Powerful instrumentation is further available for time-resolved protein crystallography and external stimulation of reactions in the sample crystal. This includes a chopper for μs time resolution, an excimer (308 nm) and a dye laser (350 - 950 nm) for laser pulse excitation, and a diode-array microspectrometer for time-resolved optical monitoring during synchrotron diffraction studies. Laboratory facilities will be provided for crystallization, preparation of heavy-atom derivatives etc. to be carried out by the users.

Beam-lines for Macromolecular Crystallography at the ESRF

Ake KVICK

ESRF

Abstract

A 6 GeV synchrotron storage ring is presently being built in Grenoble by 12 European Countries. The experimental start-up for general users is expected by 1 September 1994, when the initial 7 beam-lines are scheduled to be operational. One year later 18 facility beam-lines will be in use.

The scientific basis for these beam-lines was obtained at a General User's Meeting in March 1989.

The first seven beam-lines are in some cases shared by different scientific disciplines to allow broad access to the facility at an early stage. It is foreseen that the complications caused by hybrid beam-lines will be corrected when the full experimental capability of 30 facility stations is realized.

Macromolecular crystallography will primarily be concentrated to beam-line 3; a Lave wiggler with a monochromatic option and to beam-line 4; a high-flux undulator.

BL 3

The wiggler has been optimized to give a maximum integrated flux in the energy range 5 - 60 keV. It will be a 1.6 m long device with a 70 mm period and a K = 4.7. The total power produced is 1.8 kW and the critical energy is 17.2 keV. The maximum flux is 6.10^{14} ph/sec. mradh. 0.1 % bandwidth. Calculations show that the device will produce ~ 1.10^{10} ph/bunch in single bunch mode (10 mA) with a focussing toroidal mirror into an area of 0.2 x 0.2 mm^2.

BL 4

This is a tunable undulator with a period of 46 mm and a K = 2.08. The ID is 1.6 m. The total generated power is 737 W. The fluxes through a slit of 0.7 (V) x 1.5 (H) mm^2 will vary from 10^{14} to 5 x 10^{13} ph/sec. 0.1 % bandwidth in the energy range 5 - 30 keV with considerable flux even at higher energies.

Heat-load on optical elements

The heat-loads on the optical elements will be discussed. The present prototype studies under way to cope with the thermal distortions include adaptive mirror technology and cryogenic cooling.

In addition to BL3 and 4, beam-lines 1 and 2 have characteristics suitable to some macromolecular problems.

Efforts will also be made to instrument an early bending magnet station for MAD research.

The design of the beam-lines is being made on advice from a Crystallography Advisory Group including Drs. J. Helliwell, R. Fourme and K. Wilson. The macromolecular community is represented in the ESRF Scientific Advisory Committee by Dr. Liljas.

THE SWISS-NORWEGIAN BEAM LINE AT ESRF

A) THE PARTICIPANTS AND THEIR RESEARCH INTERESTS

ACCURATE INTENSITIES (CHARGE DENSITY, THERMAL MOTION, HIGH PRESSURE, DIFFUSE SCATTERING)

Hans-Beat Bürgi
Laboratorium für chem. und mineral. Kristallographie, Univ Bern, Bern
Dieter Schwarzenbach,
Institut de Cristallographie, Université de Lausanne, Dorigny
Hans-Peter Weber, Principal Investigator for Swiss-Norwegian Beam Line Project,
Institut de Cristallographie, Université de Lausanne, CH-1015- Lausanne-Dorigny
Emil Samuelsen,
Dept of Physics, University of Trondheim-NTH

DIFFRACTION PHYSICS: MULTIPLE DIFFRACTION PHENOMENA

Frode Mo, Bjørn Hauback
Dept of Physics, University of Trondheim-NTH

ANOMALOUS SCATTERING (INCOMMENSURATE STRUCTURES)

Gervais Chapuis
Institut de Cristallographie, Université de Lausanne, Dorigny

ANOMALOUS SCATTERING (MACROMOLECULES)

University:

Johan N. Jansonius,*)
Biozentrum, Universität Basel, Basel
Timothy J. Richmond
Institut für Molekülarbiologie
und Biophysik, ETH, Zürich
Jan Derk Smit
Biochemisches Institut, ETH, Zürich
A. Hordvik, Edward Hough, Lars Hansen
Dept of Chemistry, IMR, Tromsø

Industry:

Max Grütter,*)
Pharma, CIBA-Geigy, Basel
Malcolm Walkinshaw,*)
Preclinical Research, Sandoz, Basel
Fritz Winkler,*)
ZFE, Hoffmann-LaRoche, Basel

*) Will join at a later stage

ANOMALOUS SCATTERING (EXAFS)

Roel Prins
Institut für Technische Chemie, ETH-Zentrum, Zürich
David Nicholson,
Dept of Chemistry, University of Trondheim

TOPOGRAPHY

Melchior Fehlmann
Institut für Kristallographie und Petrographie, ETH-Zentrum, Zürich

POWDER (MONOCHROMATIC AND LAUE AT AMBIENT AND HIGH PRESSURES)

Christian Bärlocher & Lynne McCusker
Institut für Kristallographie und Petrographie, ETH-Zentrum, Zürich
Klaus Yvon
Laboratoire de Cristallographie aux Rayons X, Université de Genève, Genève
H. Fjellvåq,
Dept of Chemistry, University of Oslo

FIBER DIFFRACTION

Stuart Edelstein,
Département de Biochimie, Université de Genève, Genève

B) INSTRUMENTATION

Multi-purpose Diffractometer

Except for having 2 additional degrees of freedom, a single-crystal diffractometer does not differ fundamentally from a powder diffractometer. A single-crystal diffractometer can easily be converted into a powder diffractometer by idling the two additional axes of rotation. We have opted for a design with 6 circles.

Environmental Chambers

Access to temperatures between ~10K and at least 1400K is a prerequisite for the solution of many structural problems. We plan to purchase a gas-flow cooling device (with open access) for protein crystallography work and a closed-chamber cryostat for materials science work at lower temperatures. A thermal-imaging furnace for high temperature work (up to 2300K) is being designed at the University of Lausanne, where it will also be constructed.

Detectors

The use of area detectors is mandatory, if optimal use is to be made of the facility. In contrast to film -until recently the favored 2-D recording medium- electronic detectors give on-line control of the experiment. We foresee extensive use of this counter type in:

1) determining the orientation of a sample crystal (particularly a microcrystal)
2) charting of phase transitions
3) searching for very weak and/or diffuse intensities
4) time-resolved observations

For protein work, image plate detectors have come to the fore. Their main advantage is their high sensitivity, particularly at short wavelengths where absorption by protein and capillary become negligible (thereby increasing the lifetime of the sample). The sensitivity is at least 20 times higher than for film, and as a result exposure times can be substantially reduced while preserving the same signal-to-noise ratio.

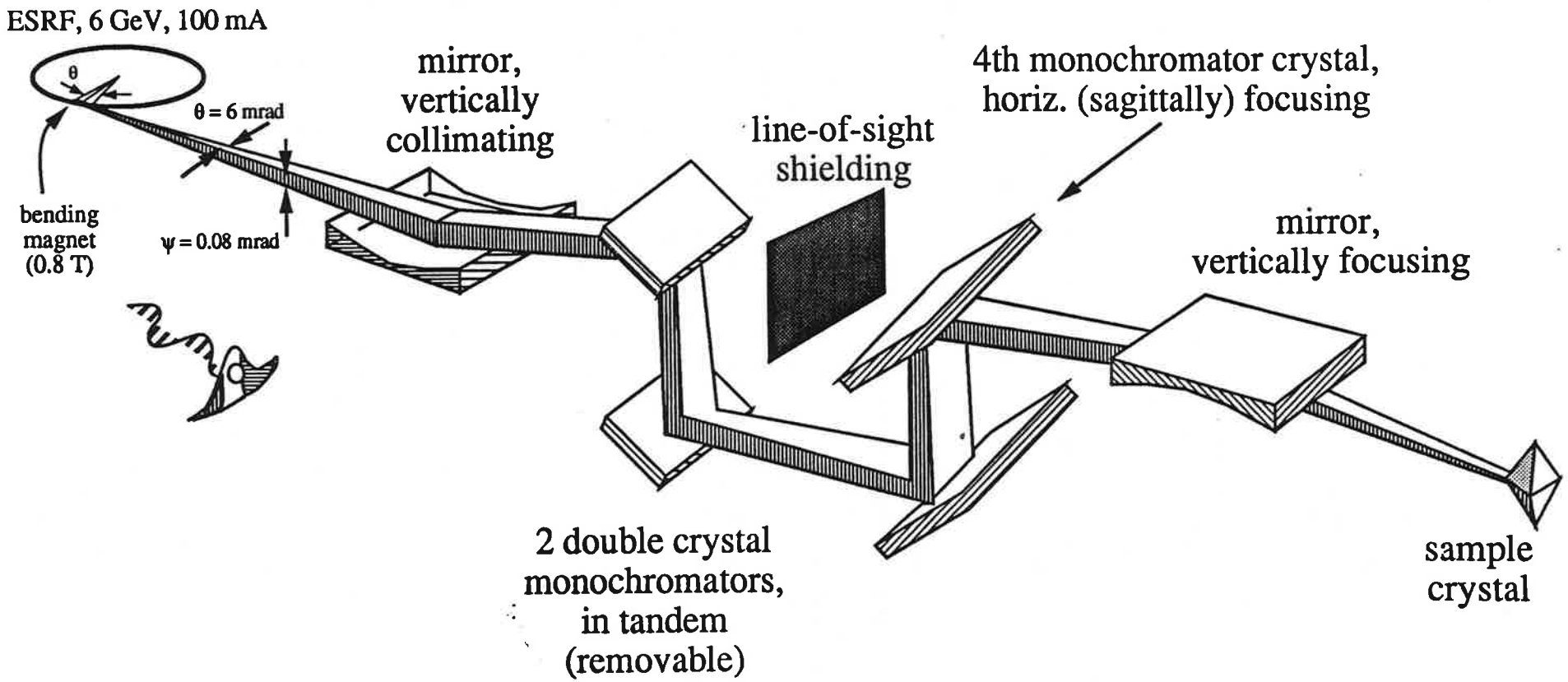
Image plates are re-usable, which matters if many exposures are to be made. The image plate, however, does not provide its user with an immediate read-out, the latter being an essential condition for most other operations other than intensity recording.

We will purchase an image plate and budget an equivalent amount for an electronic area detector while defering its purchase till the latest possible date.

Hutch

We propose to make it evacuatable down to $<10^{-3}$ mbar to reduce air scattering. Boysen et al. (1989) as well as Frey et al. (1988) have recently shown that background noise is reduced by a factor of 400 times when the air pressure within the beam path is lowered down to 10^{-3} mbar. The background is then considerably lower (~20 times) than if helium had replaced the air in the beam path. This tremendous improvement in the signal-to-noise ratio has permitted these investigators to detect in a urea inclusion compound weak peaks and diffuse layers which had previously not been observed. In the case of the mineral hollandite ($K_{1.54}Mg_{0.77}Ti_{7.23}O_{16}$), the same authors demonstrated unambiguously the absence of a peak even though the model predicted its presence.

To be able to evacuate the hutch on request is therefore indispensable if one is looking for faint reflections, collecting intensities on microcrystals or scanning close to the beam exit point. The prospect of having permanently a higher signal-to-noise ratio conforms with our general belief whereby it is better to eliminate errors at their sources than trying to correct for them afterwards. Evacuatable hutches are also planned for some of ESRF's beam lines (Riekel, pers. commun.).



Swiss-Norwegian Beam Line X-ray optics configuration as of 1990

BIOLOGICAL CRYSTALLOGRAPHY EQUIPMENT AT LURE

R. Fourme
LURE B209D, Université Paris-Sud, 91405 Orsay Cedex, France

At LURE, the french national synchrotron radiation facility, 3 stations (two full time and one half time) on beam lines of the positron storage ring DCI are dedicated to biological crystallography.

One instrument (W32) takes advantage of the radiation emitted by a five pole superconducting wiggler (critical wavelength 1.1Å) and is normally operated in the wavelength range 1-1.5Å (it has also been designed to be readily used for Laue studies with a non focused X-ray beam). The double focusing optics features two elements: a curved crystal monochromator filled with helium and equipped with Ge(111) or Si(111) crystals; and a curved Si/W multilayer (period 30 Å, length 150mm, efficiency about 60% at 1.5Å) for harmonic rejection and focusing in the vertical plane. Goniometer alignment on the X-ray beam is automatic, using VME electronics. Currently, a carrousel of photographic films is available. An imaging plate detector supplied by the EMBL Outstation in Hamburg (J. Hendrix, A. Lentfer) will be installed by april 1991; with this detector, data acquisition and data reduction will be performed by a VAX Station 3100. Two low temperature systems are available, including equipment for shock cooling.

Another instrument (D23) on a bending magnet line is used part time (50%) for protein crystallography (the rest is used by chemists), and especially MAD studies in the wavelength range from 0.98 A to 2Å. It includes a two crystal monochromator with sagittal focusing, a 6 axis goniometer and a spherical drift multiwire proportional chamber with a fast digital position encoder. This instrument provides accurate data, with typical Rsyms in the range 2.7-4% As an example, we compare diffraction data sets obtained from collagenase crystals with three laboratory instruments and the D23 setup; the last set is more accurate and extend to higher resolution (in collaboration with I. Broutin, B. Arnoux, A. Ducruix and P. Tucker).

The last instrument (D41) is also on a bending magnet. It has a curved crystal monochromator and a rotation camera. This camera will be replaced mid-1991 by a four circle diffractometer and a spherical drift multiwire proportional chamber. This detector has a fast encoder which performs a fast determination of the centroid of cathodic clusters, thus providing a smaller point spread function, a better spatial resolution and a reduced differential non-linearity. D41 is controlled by a VAXStation 3200 and electronic images are stored on 8mm video tapes. This instrument will operate between about 1.1 and 2Å and will be dedicated to high resolution data collection.

We have the project to build a new MAD instrument on a new beam line (magnet H6). This instrument will be installed at about 7-

8m from the source, in order to use a simple and very stable non focusing two crystal monochromator. D23 should then be used by chemists only.

Our laboratory is actively preparing the technical and scientific case for a new 2.15 GeV positron storage ring which would replace both DCI and SuperACO by the end of the century. Protein crystallography is planned on this machine which will be well suited for a variety of projects which do not require the ultimate performance of ESRF.

acknowledgements: I acknowledge the collaboration of R. Kahn, J.P. Benoit, A. Bentley, R. Bosshard, A. Bahri, P. Dhez and the technical staff at LURE

Programme d'Accompagnement pour l'Utilisation de l'E.S.R.F. par la Communauté Scientifique Française

Projet Ligne de lumière D 2 A M

Diffraction et diffusion anomales multi-longueur d'onde

CRG D2AM

A COLLABORATIVE RESEARCH GROUP FOR MULTIWAVELENGTH ANOMALOUS DIFFRACTION AND SCATTERING EXPERIMENTS AT ESRF IN BIOLOGICAL MACROMOLECULE CRYSTALLOGRAPHY and MATERIAL SCIENCE.

Instrument proposal on a synchrotron beam line with 0.8T bending magnet at E.S.R.F.

*****

PROPOSERS OF THE PROJECT AND WORKING TEAM for BIOLOGICAL MACROMOLECULE CRYSTALLOGRAPHY:
Michel ROTH, Laboratoire d'Ingénierie des Protéines, CEA, C.E.N.- Grenoble.
TRAN QUI Duc, Eric FANCHON, Jean VICAT, Laboratoire de Cristallographie, C.N.R.S., Grenoble, Claudine COHEN-ADDAD, Laboratoire de Biologie Structurale,CNRS-CEA, URA 1333,Grenoble, Jean-Claude THIERRY, Institut de Biologie Moléculaire et Cellulaire, Strasbourg.

*****

DESIGN OF THE BEAM LINE:

The instrument will match the following requirements:

1. **Point focusing** of the beam on the crystal.
2. **Fixed sample position** i.e. fixed beam height and fixed focusing distance
3. **High energy resolution:** 2 - 5 eV
4. **High Q resolution:** $2\ 10^{-3}$ Å^{-1}
5. **Accessible wavelength range:** 0.5 - 2.5 Å 25 - 5 keV
   with possibility of very fast wavelength changes
6. **High rate of harmonic rejection:** 10^{-4}
7. **General instrument characteristics:** robustness
   reliability
   simplicity in use (including alignment)
   i.e. "user friendliness"

The optics of the beam-line is constituted by a symmetrical two crystal monochromator between two quasi-symmetrical cylindrical parabolic mirrors in a compact arrangement. The symmetry is a (quasi) two-fold axis symmetry.
The **vertical** focusing results from the reflection on the 2 curved mirrors.
The **horizontal** focusing is produced by sagittal bending of the 2nd crystal of the monochromator.

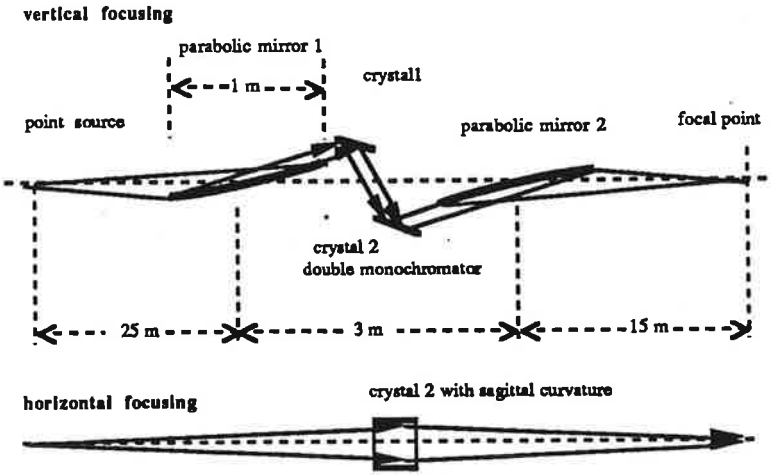
Advantages:

The beam between the mirrors has vanishing vertical divergence. Therefore:
1) one can use **flat crystals:** good cooling conditions for the 1st crystal
possibility of sagittal bending of the 2nd
2) the beam position at sample position is independent, in the vertical direction, from the beam position on the second mirror after the monochromator.

Problems:

1) Difficulty to control the vertical divergence of the beam between the mirrors, which determines the energy resolution.
2) Cost of the mirrors

3)The full vertical divergence of the incoming beam cannot be used at short wavelengths ($\lambda < 1$ Å) because of the limited length ot the mirrors (maximum loss in intensity ~ 25 %)



Schematic view of beam focusing

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Multi-wavelength anomalous diffraction (MAD) is a technique for solving the phase problem in **Biological Macromolecule Crystallography.**
There are two main experimental phasing methods:
* multiple isomorphous replacement technique MIR
* multi-wavelength anomalous diffraction MAD

Because MAD technique does not require many isomorphous heavy atom derivatives, this technique will allow to solve structures in all cases where the preparation of these derivatives is difficult.

Application to biological macromolecular crystallography:
* natural metalloprotein example: Fe, Cu, Mn, Zn
* replacement of natural metals example: Tb or Yb substituted to Ca
* conventional heavy atom derivatives example: Pt, Hg, U
* proteins with Se substituted to S in methionin
* brominated bases (uridin) in nucleic acids
* proteins complexed with ligands containing anomalous scatterers

Experimental conditions required for MAD technique.
* high energy resolution (better than 5 eV)
* fine tuning of λ to maximise the anomalous differences
* good reproducibility of λ adjustements
* high intensity, because the anomalous difference effects are small

Accessible absorption edges. The accessible wavelength range will be **0.5 Å - 2.5 Å**. The corresponding accessible absorption edges are:

K edges:
from Z = 22 (Ti) to Z = 46 (Pd) i.e. for instance:
Ti, V, Cr, Mn, **Fe, Co, Ni, Cu, Zn**,..., Ge, As, **Se, Br**,...,Rb, Sr, Y, Zr, Nb, Mo,..., Pd

L_{III} edges:
from Z = 55 (Cs) to Z = 92 (U) i.e. for instance:
Cs, Ba, ..., W,...,Pt,...,**Hg**, ..., Pb, Bi and rare earthes like: Sm, Eu, Gd, **Tb, Ho, Yb**

*****

The instrument is due on July 1994.
The instrument will be supported by the C.E.A. and the C.N.R.S. and operated by a french technical staff. For biological macromolecule crystallography, there will be 1000 hours beam time for ESRF users and 2000 hours for CRG D2AM users
More information by: M.ROTH, LIP/PSY, C.E.N.G., B.P.85X, F 38041 GRENOBLE-CEDEX, France.tel.: (33) 76 88 59 19 fax: (33) 76 88 51 22

Synchrotron Radiation facilities for macromolecular crystallography planned for Trieste

S. Bernstorff, M. Colapietro[+] and A. Savoia

SINCROTRONE TRIESTE, Trieste, Italy
[+]Dipartimento di Chimica, Univ. "La Sapienza", Roma, Italy

The storage ring ELETTRA at the SINCROTRONE TRIESTE in Italy will be a new third generation light source for synchrotron radiation. The ring will contain up to 11 insertion devices, the electron energy will be 1.5-2 GeV and electron currents of up to 400mA will be stored. ELETTRA is scheduled to start operation at the end of 1993.

One of the insertion devices will be a permanent magnet wiggler consisting of three segments with 23 poles each. With a magnetic field of 1.5 T it will have a critical energy of 4 keV (at 2 GeV) and thus produce a usable photon flux up to photon energies of 25 keV (0.5Å)(see figure). The total power emitted by this wiggler will be about 10 kW into a solid angle of about .5 (vertically) x 9 (horizontally) $mrad^2$. In some circumstances this power can be reduced by "switching off" one or two wiggler sections. The wiggler radiation will be used simultaneously for three beamlines: The central 4 mrad of the radiation cone will pass straight into a beamline for single-crystal diffraction, while two small-angle scattering experiments will use about 1.5 mrad each.

In the diffraction beamline the SR will be monochromatized in the photon energy range 4-25 keV (0.5-3 Å) by a fixed exit double-crystal monochromator with the crystals in a nondispersive antiparallel setting. Using Si(111) or Si(220) crystals, an energy resolution of about $\Delta E/E = 1.3*10^{-4}$ or about $5.3*10^{-5}$ (at λ=1.542 Å), respectively, will be obtained. The light will be focussed ~1:1 at the sample position about 40m after the source point. The focal spot size is to be 1-2mm^2 in diameter to ensure that the sample (usually much smaller) is homogeneously lit.

The first monochromator crystal absorbs almost all of the incoming radiation power, i.e. up to 5 kW (for 2 GeV electron energy, 400 mA beam current and 4 mrad horizontal acceptance) with a peak power density of up to 7 W/mm^2. That means, that this first crystal would

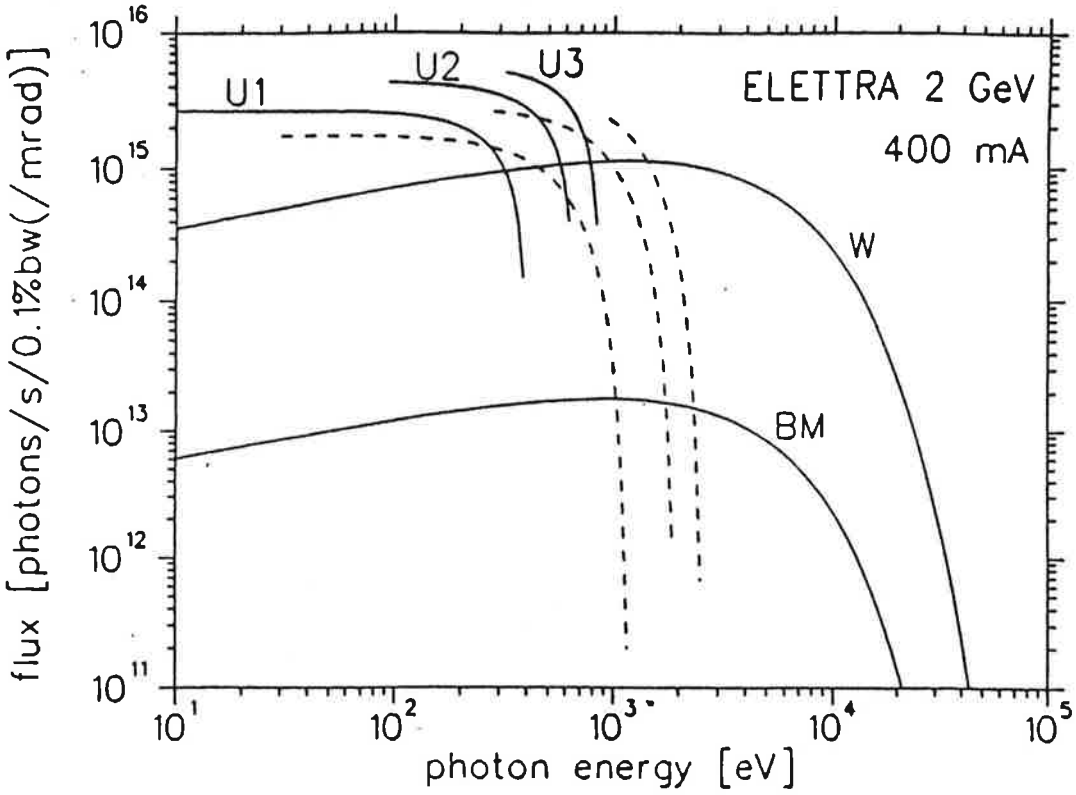
reach a temperature well above its melting temperature of ≈1410°K (!), unless it is cooled very efficiently. Therefore to ensure an efficient and homogeneous crystal cooling, the first crystal will have rectangular cooling fins, and liquid Gallium will be used as cooling fluid. To avoid temperature induced changes in focus position at the experiment 20m away, the second crystal will be heated roughly to the temperature of the first crystal. Then, with a piezo element acting on one of the crystal turning axes, the angular position of its crystal will be fine tuned in order to further minimize the thermal detuning effects and also to suppress the higher harmonics. For this the output X-ray intensity will be monitored with a ionisation chamber and its output will be used as a signal for a feedback loop.

The first monochromator crystal can be removed completely from the photon beam and reinstalled in its position of use without breaking the vacuum. This opens the possibility to also use the direct white SR beam for e.g. Laue diffraction experiments.

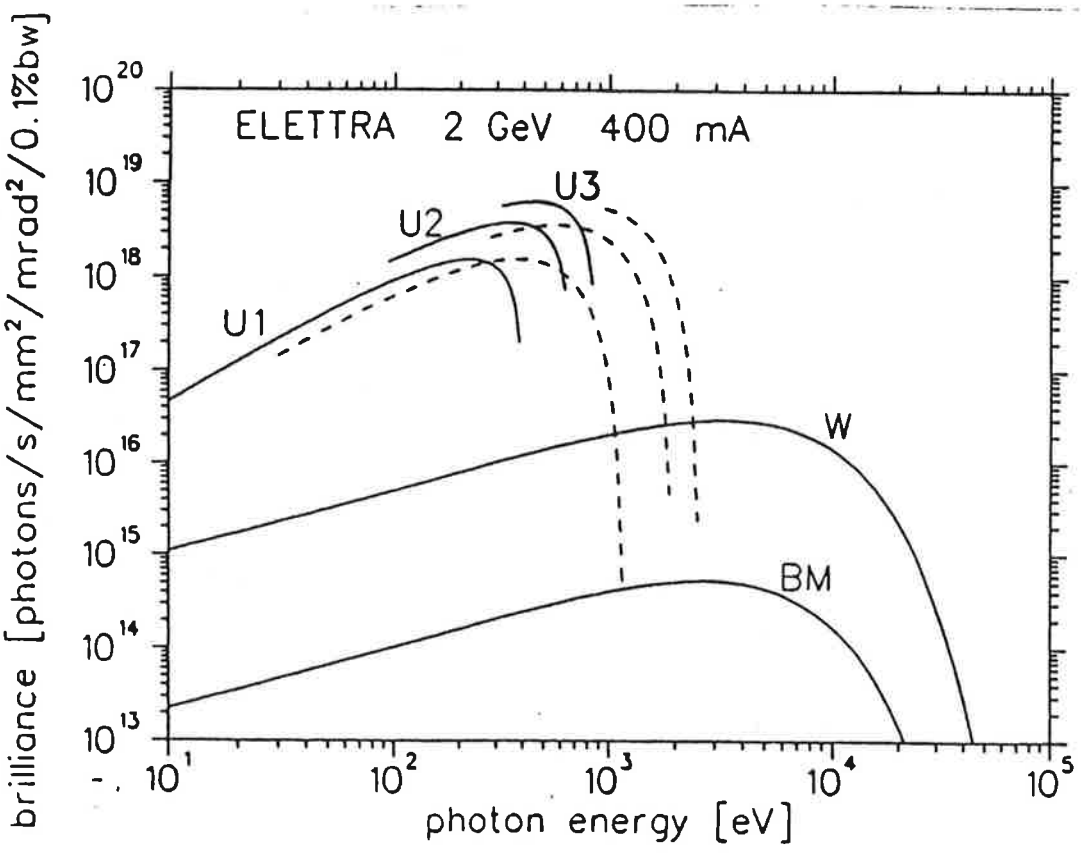
The experimental station will be build similar to the station at the wiggler beamline of ADONE in Frascati, which is currently used by us as a prototype. However for the ELETTRA diffraction station a Huber 6 circle diffractometer will be chosen with the χ-circle having a diameter of 40 cm. The diffractometer will again be mounted vertically in order to avoid intensity losses due to the horizontal polarization of the SR. The motors of the diffractometer will be controlled with highly reliable switching actuator cards which allow to achieve a resolution of 0.001° in the angular position of each circle. The diffractometer can be controlled either via an operator keybord or automatically by a computer using the CS-program system developed for and currently tested at the ADONE station.

Photomultipliers with NaI(Tl)-scintillator, an image-plate detector and an area-detector based on a CCD in combination with fluorescent optical fibers (pixel size down to $20*20\mu m^2$) will be used for the recording of the signal.

For the data analysis and the presentation of the results the SIR-CAOS software will be used.



Spectral flux of radiation sources in the storage ring at 2 GeV.



Spectral brilliance of radiation sources in the storage ring at 2 GeV.

Existing and Planned Facilities for Macromolecular Crystallography at Synchrotron Sources in the USA

Keith Moffat
Department of Biochemistry and Molecular Biology
The University of Chicago

Existing facilities (SSRL, CHESS and NSLS) are planning a series of upgrades. SSRL has installed its dedicated injector which allows the laboratory to run independent of SLAC and will permit much more extended running, 6 - 9 months per year, if the operations budget can be increased substantially. CHESS has just completed the "CHESS East" extension which includes four new experimental stations, two served by a permanent magnet wiggler, and one of which is optimized for virus crystallography and embedded in a biohazards facility at the BL3 level. At NSLS, the Howard Hughes Beam Lines will shortly be operational. The FAST detector is now running routinely and effectively on the Brookhaven Biology Beam Line, X12C.

Planned facilities, with the exception of a modest wiggler facility at the lower energy Advanced Light Source at Lawrence Berkeley Laboratory, largely center on the Advanced Photon Source (APS) at Argonne National Laboratory. The APS plans to develop 16 sectors in Phase I and Letters of Intend requesting access to a total of 24 sectors were received from 22 groups ("CATs") in 1990. Full proposals are due in March 1991, and sectors will be allocated in the summer of 1991 on the basis of review of these proposals. However, since the Department of Energy (DOE) is funding the APS itself but only a few of the beamlines, the key will the ability of the CATs to raise money independently from federal, state, local and foundation sources to design, build and operate the sectors. Initiatives are operating at various national levels to ensure that money will be available, but the funding climate is undeniably tight across all scientific disciplines.

In structural biology, 4 main CATs are developing proposals: the Argonne Structural Biology Center (Westbrook; wiggler source); the Consortium for Advanced Radiation Sources (Smith/Moffat; undulator); the Industrial Macromolecular Crystallography Association (Jones/Watenpaugh; wiggler); and BioCAT (Bunker; wiggler). Other CATs contain smaller elements of structural biology and some members may elect to join with one or more of these four CATs. The national organization of synchrotron users in structural biology, BioSync, has nearly completed a national survey of opportunities and needs, in conjunction with funding agencies, and a report will be available in Spring 1991. Two other reports with a somewhat broader scope are also at an advanced stage: the proposed Structural Biology Initiative sponsored by DOE; and a report on "Technology for the Future: Opportunities and Needs im Structural Biology and

Molecular Medicine" sponsored by NIH. All three reports are intended to convey to funding agencies and to the US Congess a strong sense of the excitement in the field, and a realistic estimate of the resources needed to develop existing and future facilities effectively.

A distinction may be drawn between "today's state-of-the-art" experiments and "frontier" experiments, both of which stand to benefit enormously from the new sources such as the APS and ESRF. Such experiments may attract different communities of users. How can these new facilities be planned, both to build on the large and vigorous communities that conduct "today's state-of-the-art" experiments and to push the limits through "frontier" experiments? At a more practical level, there is considerable scope for collaboration between European and American scientists and ways to enhance this should be explored.

The Howard Hughes Medical Institute (HHMI) SYNCHROTRON RESOURCE

J.-L. Staudenmann

HHMI-SR, NSLS-X4, Bldg 725

BNL, Upton, NY 11973-5000

Remarks about the Biology effort at NSLS

In general, there are few fully dedicated biology stations in the US synchrotron laboratories, at NSLS in particular. The representation of biology stations seems not to be in proportion of the overall funding for biology, as compared, for instance, to solid state physics. For example, the HHMI effort, when completed, on the X-ray ring of NSLS represents about 10% of the beam line constructions, but approximately 40% of its biological applications.

It is fair to state that biologists in the US have been slow to form groups for financing and exploiting synchrotron facilities. Moreover, the scope of the X-ray techniques used by the biology community is not as diverse as, for example, that in solid state physics. This is not due to lack of interest but rather that, in general, biologists have not yet pass the stage of the structure determination and, consequently, that there are not enough biologists thinking of follow-up experiments beyond the structure determination. In a world dominated by materials physicists, this is a serious handicap because the structure determination --and the many complexities it includes-- of a biological sample is not appreciated to its fullest but, rather, taken as "one more structure."

The diffraction method consists of using a crystal --the difficulties of making such a crystal will only be appreciated by biologists, not by synchrotron managements--, a diffraction tool such as

rotation, Laue, MAD, Weissenberg, precession, ...

instrument to collect integrated intensities. The enormous amount of reflections to be measured creates problems which are specific to biology and, therefore, this scientific community has to solve them alone. In my mind, no biology group has satisfactorily optimized the data collection rate for present synchrotron laboratories. With the coming of new synchrotron sources, such as APS and ESRF, the rate of data collection, solely based upon the X-ray flux ratios for bending magnet lines, will increase by at least one order of magnitude. Thus, the optimization problem will become even more serious. What kind of solutions will be found to handle such data collection rates? When compounding the

enormous quantity of reflections per frame to be processed, the optimization dilemma is even more difficult to consider. Is the biology community ready to finance such optimization research? Frankly, I did not get any indication that this problem will be addressed beforehand: I see the same methodology, as that applied in existing laboratories, being scaled up with a performance increase for each of the main components of the beam line.

Since biology samples are markedly different --they are progressively destroyed when exposed to the ionizing nature of the X-ray beam-- from those of other research fields using synchrotron radiation, the instrumentation must be specifically optimized for biology.

The HHMI Synchrotron Resource (HHMI–SR)

The overall objective of the HHMI-SR is to develop a facility that will enhance the capabilities for determining the three-dimensional structures of biological macromolecules by a substantial margin. Bottlenecks in structural biology include crystallization, data collection, phase determination, and structure interpretation and refinement. Synchrotron radiation can have an impact on all of these aspects in that very small crystals can be used, data measurement rates can be very fast, anomalous dispersion permits essentially direct-phase determination, and improved resolution and accuracy from accurate high-resolution data can markedly facilitate fitting and refinement. We have planned a facility with the versatility to be effective in relation to all of the above aspects.

The anticipated needs of the HHMI-SR in biological diffraction will mainly serve three kinds of diffraction experiments:

(1) MAD determination for direct structural analysis of macromolecule reflection phases,

(2) routine, rapid diffraction data collections for crystals and fibers through the rotation method for high-resolution refinements of preliminary models, for determining structures of complexes with inhibitors, activity effectors and other ligands, and for the analysis of site-directed mutant proteins, and

(3) Laue diffraction measurements as a backup of the rotation method, and also for time-resolved analysis of enzyme reactions and other macromolecular interactions.

The plan for developing the HHMI-SR research facility necessarily involves an intimate matching of experimental requirements for the beam lines with the constraints of the synchrotron source. Moreover, the various applications that

the HHMI organization wish to accommodate require rather different x-ray optics and detection apparatus. Efficiency and reliability dictate the construction of three specialized beam lines rather than one multipurpose line.

The X4 port is the second exit (10°) on a bending magnet of the NSLS x-ray storage ring. Out of the 48-mrad maximum-emitted radiation swath, three beam lines will be constructed. Due to the 25-m limit (from the source) in beam line length and that the beam lines are spread apart only by about 20-mrad, floor space is therefore very limited: it is imperative to optimize the component locations with great care. Since NSLS is a low emittance storage ring, the vertical FWHM of a bending magnet is dominated by the SR natural angle: about 0.2 mrad at the critical wavelength. This yields a beam FWHM of about 5 mm at the MAD line sample position. Thus, it has been decided not to install a focusing mirror for the vertical divergence in the initial setup. However, the other two lines will have horizontal and vertical focusing optics.

The HHMI-SR will be dedicated to structural analyses of biological macromolecules, primarily through crystallography. The facility is under construction at the X4 port of the National Synchrotron Light Source at Brookhaven National Laboratory, and it will comprise three beam lines. The first line, X4A, is almost completed and it is expected to be operational by April 1991. X4A has been devised to apply the multiwavelength anomalous diffraction method which provides direct estimates for the phases of reflections. The second line, X4C, will be devoted to rapid and essentially routine diffraction measurements, mainly through the rotation method. X4C construction is well on the way and it is expected to be operational by the Autumn of 1992. These two experimental stations are contained within refrigerated radiation enclosures that will maintain clean environments, narrowly defined constant temperatures (as low as 10°C), and protection against biohazard at biosafety level BL-2. The swath center is planned, in a collaborative effort with accelerator physicists of the National synchrotron Light Source, to first serve as a monitor of the white x-ray beam vertical stability. Later, this third line, X4B, will be equipped for Laue experiments to study, for instance, dynamical processes in proteins.

All three beam lines of the HHMI-SR will be equipped with storage phosphor detector plates (standard size: 200mm x 250mm or 8"x10".) The darkroom, containing the scanner, is conveniently located within the X4 area.

In a first stage of development for the HHMI-SR detector systems, we have chosen the "Digirad" scanner on the basis of comparative tests conducted in the first half of 1989. This scanner has a 12 bit digitizer, a 50+ mW He-Ne laser, and the photostimulated light is collected through bundles of fiber optics. It can automatically handle stacks of 25 plates at the time and process them with a duty cycle of 40 sec; 10 sec of handling and 30 sec of scanning. The plates remain flat during the entire procedure. As a corollary of the scanner capabilities, the HHMI-SR is also thinking of adapting or developing an automatic plate exchanger containing magazines of 25 plates. Subsequent developments will concentrate on improving the characteristics of the scanner to increase its efficiency and its dynamic range.

SYNCHROTRON RADIATION IN CRYSTALLOGRAPHIC STUDIES ON RIBOSOMES

A. Yonath

Weizmann Inst. of Science, Rehovot, Israel & MPG/ASMB, Hamburg, FRG

Ribosomes are the universal cell organelles which facilitate the biosynthesis of proteins. They are built of two independent subunits, containing together some 56 proteins and 3 RNA chains. The molecular weights of a typical bacterial ribosome and of its subunits are: **2.3, 1.45 and 0.85 million daltons**, respectively.

Besides being complex and of enormous size, the ribosomes are notoriously unstable and flexible, hence very difficult to crystallize. Nevertheless, we have obtained at least one crystal type from each ribosomal particle (Table I) and are currently collecting crystallographic data from six different crystal types (Table II).

1. Guidelines for choosing Crystal Types for Crystallographic Studies.

Two of the crystal types, of the large ribosomal subunits (50S) from Halobacterium marismortui and of the small ones (30S) from Thermus thermophilus diffract almost to molecular resolution, 4.5 and 7.3 Å respectively.

The third and the fourth types are of whole (70S) ribosomes from T. thermophilus and of their complex, mimicking a defined functional state, diffracts well and sharply at low resolution. Preliminary results led us to anticipate that similar, however more sophisticated complexes, should yield crystalls which diffract to much higher resolution can be constructed.

The fifth and the sixth types are of 50S subunits from two thermophilic bacteria. These diffract to medium resolution but are expected to provide critical information: One of these bacteria, B. stearothermophilus, was mutated by us and we used this mutant for specific derivatization with super-dense heavy-atom cluster (see below). Despite considerable effort, we are still not able to develop a similar procedure for the halophilic ribosomes, the source of our best diffracting crystals. Since we assume that the structures of ribosomes from different sources are rather similar at medium resolution, and since we plan to solve the structure iteratively, we are meanwhile using the crystals of 50S subunits from B. stearothermophilus to obtain intermediate information about locations of specific sites.

The other type is of 50S subunits from T. thermophilus, which completes a series of crystals from all ribosomal particles from the same source. This series should enable the investigation of the conformational changes which take place upon the association of ribosomes from their subunits as well as upon binding of components which participate in protein biosynthesis.

Our current collection of crystals is also adequate for comparative studies of the structures of the same particles from radically different sources, including moderate and extreme thermophiles, belonging to eubacteria, alongside with extreme halophiles, which are classified as archaebacteria.

2. Data collection: problems and improvements

Due to the large unit cells and weak diffracting power of our crystals, we are using intense synchrotron radiation for all our crystallographic studies, namely characterization, preliminary screening and data collection. To avoid the fast decay in the X-ray beam, we are experimenting at cryo-temperature. Under these conditions complete diffraction data sets can be obtained from individual crystals. A summary of the data collected since November 1987, is given in Table III. **The reasons for repetition in data collection, and for the frequent occasions of collecting data to lower than the potential resolution are due mainly to time constraints, to the quality of crystals, to the specifications of the beamline and to the mode of its operation.**

Fig. 1 shows the **heterogeneity** of the resolution of two crystal types, at ambient and at cryo temperature. Consequently, large fractions of our beam periods are dedicated to the search for suitable crystals, and frequently forces compromises regarding the resolution of measured crystals (a typical "still" or small-rotation exposure requires 5-10 minutes, even when using Polaroid or Imaging plate).

Table I

Classification of the different crystals of ribosomal particles

	30S	50S	70S	Complexes of: 70S+poly(U) (1)	70S+poly(U) +tRNA (2)	50S+tRNA+ nascent chain (3)	mutant (-L11) 50S	mutant (-L11) 70S
E.c.			+					
B.st.	+	+ +	+			+	+	+
T.t.	+	+	+ +	+	+			
H.m.	+	+		+		+		

1. Complex of 70S ribosomes together with an oligomer of 35 ± 5 uridines, serving as mRNA.

2. Complex of 70S ribosomes together with an average of 1.5-1.8 equivalents of $PhetRNA^{Phe}$ and an oligomer of 35 ± 5 uridines, serving as mRNA.

3. Complex of 50S subunits from H. marismortui or B. stearothermophilus together with a segment of 18-20 mers of poly(Phe) or poly(Lys), respectively, and the corresponding tRNA.

B.st = Bacillus stearothermophilus, T.t. = Thermus thermophilus,
H.m. = Halobacterium marismortui

Table II

CHARACTERIZED THREE-DIMENSIONAL CRYSTALS OF RIBOSOMAL PARTICLES

Source	Grown Form	Cell Dimensions (Å)	Resolution(Å)
70S T.t	MPD *	524x524x306; $P4_12_12$	app. 20
70S T.t + m-RNA & t-RNA #	MPD	524x524x306; $P4_12_12$	app. 15
30S T.t.	MPD	407x407x170; $P42_12$	7.3
50S H.m.	PEG *	210x300x581; $C222_1$	4.5
50S T.t.	AS *	495x495x196; $P4_12_12$	8.7
50S B.st. ~	A *	360x680x920; $P2_12_12$	app. 18
50S B.st. ~<	PEG	308x562x395; 114°; C2	app. 11

* MPD, PEG, A, AS = crystals were grown by vapor diffusion in hanging drops from solutions containing methyl-pentane-diol (MPD), polyethyleneglycol (PEG), ammonium sulphate (AS) or low molecular weight alcohols (A).

A complex including 70S ribosomes, 1.5-2 equivalents of $PhetRNA^{Phe}$ and an oligomer of 35 ± 5 uridines, serving as mRNA.

~ Same form and parameters for crystals of large ribosomal subunits of a mutant (missing protein BL11) of the same source and for modified particles with an undecagold-cluster.

< Same form and parameters for crystals of a complex of 50S subunits, one tRNA molecule and a segment (18-20 mers) of a nascent polypeptide chain.

B.st = Bacillus stearothermophilus, T.t. = Thermus thermophilus,
H.m. = Halobacterium marismortui

Table III. Data colleted in the period November 1987- May 1990

number	Crystal type	Resolution (Å)	Rotation in degrees	
November 1987, beamline A1 at CHESS (Cornell U.):				
1	H 50S nat (in oil)	15.0	140	
2	T 30S nat	25.0	108	
3	T 30S nat	12.0	105	
4	H 50S GC (soak),(in oil)	20.0	64	
December 1987, beamline VII at SSRL (Stanford U.):				
5	B 50S mutant (TST)	15.0	103	
6	B 50S nat	15.0	92	
7	B 50S nat	16.0	90	(split beam)
8	H 50S with Poly Phe	16.0	100	
April 1988, beamline 9.6 st SRS (Daresbury, UK):				
9	B 50S nat	12.0	92	
July 1988, beamline X11 at EMBL (DESY/Hamburg):				
10	T 30S nat	25.0	99	
11	T 30S GC (soak)	18.0	56	
12	B 50S GC	14.0	91	
13	H 50S IC	13.0	94	
14	H 50S GC	18.0	65	
September 1988, beamline X11 at EMBL (DESY/Hamburg):				
15	T 30S nat	10.0	50	
16	T 30S TAMM (soak)	11.5	60	
17	T 30S GC	12.8	49	
18	T 30S W12P(soak)	13.0	48	
20	T 30S Pt4 (soak)	10.6	50	
21	H 50S nat	4.7	30	
21	T 30S IC (saok)	10.0	92	
22	H 50S nat	6.5	47	
23	B 50S GC	10.0	70	
24	H 50S poly phe	11.0	91	
25	H 50S W12P(soak)	8.1	91	
November 1988, beamline X31 at EMBL (DESY/Hamburg):				
26	H 50S nat	7.5	92	(IP test)
27	H 50S nat	7.5	91	(IP test)
March 1989, beamline X11 at EMBL (DESY/Hamburg):				
28	H 50S nat	6.5	99	(stored 5months #22)
29	T 70S nat	22.0	111	
30	T 30S nat (in 30%MPD)	7.9	45	(stored 8h)
31	B 50S GC	17.0	97	(IP,stored 5m #23)
32	T 30S GC (soak)	11.0	49	(IP)
July 1989, beamline X11 at EMBL (DESY/Hamburg):				
33	T 30S GC (soak)	11.0	11	(IP)
34	H 50S W12S (soak)	9.6	36	(IP)
35	H 50S W12P (soak)	10.0	93	(IP)
36	H 50S W12S (soak)	9.6	92	(IP,stored 3d #34)
37	B 50S mutant (TST)	16.0	185	(IP)
38	H 50S nat	10.0	95	(IP)
39	T 70S nat	22.5	271	
40	T 70S GC (soak)	22.5	108	
October 1989, beamline X11 at EMBL (DESY/Hamburg):				
41	T 30S GC	16.0	90	(IP)
42	H 50S nat	7.5	185	(IP)
43	H 50S nat	11.0	30	(IP)
44	H 50S nat	6.0	105	(IP)
December 1989, beamline L6A1 at KEK (Japan):				
45	H 50S nat	16.0	110	(Weissenberg, IP)
46	T 30S nat	10.0	50	(Weissenberg, IP)
February 1990, beamline A1 at CHESS (Cornell U.):				
47	T 50S	12.0	22	
48	T 70S complex	22.5	110	
49	B 50S nat	16.0	184	
50	T 30S GC	11.0	60	
51	T 50S	18.0	50	
May 1990, beamline X11 at EMBL (DESY/Hamburg):				
52	T 50S	33.0	90	(IP)
53	B 50S GC	18.0	180	(IP, stored, 14m)

H = Halobacterium marismortui
B = Bacillus stearothermophilus
T = Thermus thermophilus

TST a mutant of B. stearothermophilus missing protein BL11, obtained by thiosterpton

T 70S complex = T 70S ribosomes together with a chain of 35 uridines and two molecuis of phe-tRNA(Phe)

GC, IC = undeca-gold and tetra-iridium clusters, respectively, covalently attached, unless marked: (saok)

TAMM = tetrakis(acetoxymercuri)methane.

W12P = H3PO4.12WO3.6H2O, W12S = SiO2.12WO3.6H2O

IP = imaging plate, Weissenberg = Weissenberg camera;

oil = crystals immersed in an innert hydrocarbon as in Hope et al., 1989.

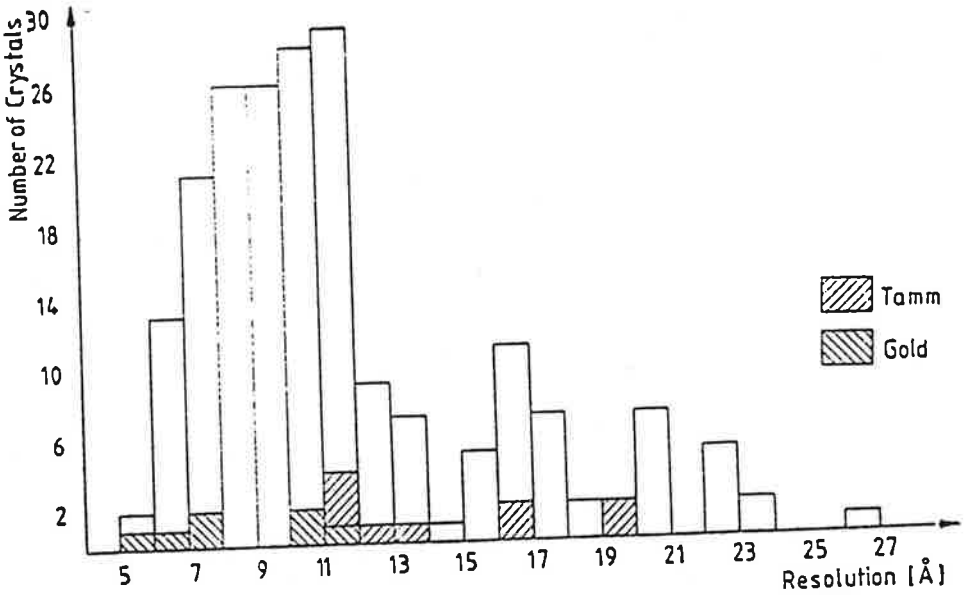


Fig. 1. The heterogeneity of crystals: Approximate resolution deteted in the first exposure of each of about 200 crystals of 50S subunits from H. marismortui, investigated at 4°-9°C (X11/EMBL/DESY); (b) Approximate resolution of about 80 crystal of 30S subunits from T. thermophilus, investigated at cryo temperature at four synchrotron radiation sources.

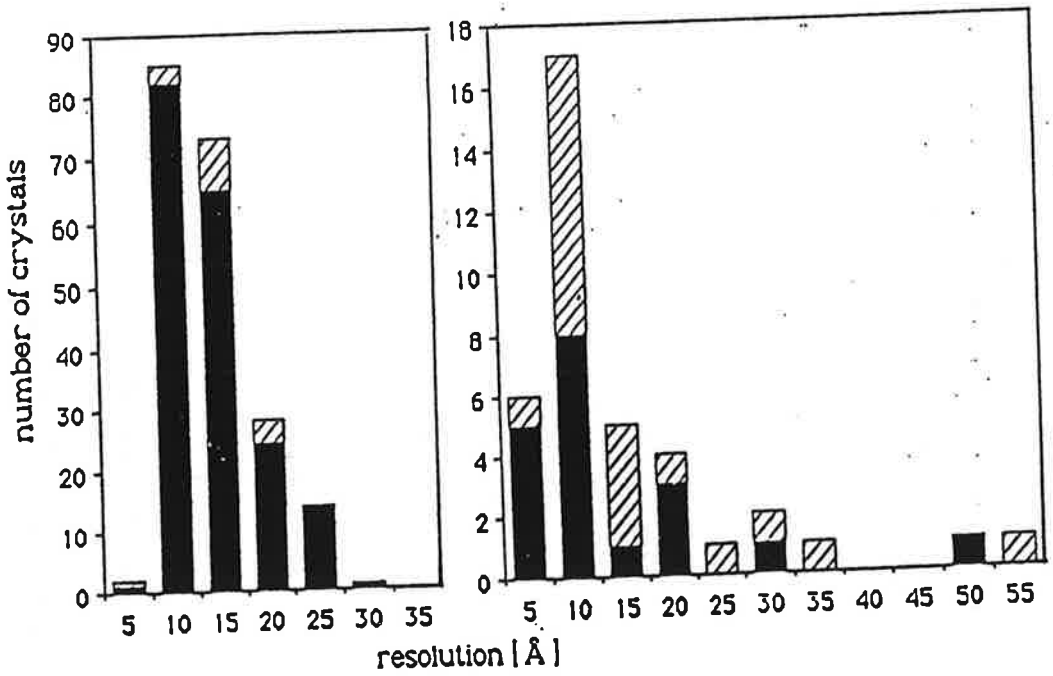


Fig. 2. The improvement in the distribution of the potential resolution of crystals of 50S subunits from H. marismortui with freezing: distribution of the approximate resolution (a) at 4-9° C, as in Fig. 1a; (b) at cryo-temperature, immersed in 18% ethylene glycol for 15-90 minutes prior to freezing in liquid propane at 90-93 K. Note the higher fraction of crystals diffracting to 5-10 Å.

Table IV: Evaluated data from 2 crystals of 50S subunit of B. stearothermophilus (at A 2σ level):

Crystal form:	Monoclinic	
Spacegroup:	C2	
Cell dimensions:	a=304, b=549, c=384 Å, beta=115°	
Crystal (# as in Table III):	Native(#9)	GC(#23)
Evaluated resolution:	14 Å	12.5 Å
Mosaicity:	0.4°	0.3°
Total observations:	13063	27987
Fully recorded reflections:	4036	4788
Measurements:	3047	3215
Unique significant:	26%	20%
Completeness:	5.8%	6.4%
R-merge (I):		
Added partials:	7883	7981
Measurements:	5322	5829
Unique significant:	18 Å	20 Å
Last shell with 50% completeness:	46%	37%
Overall completeness:	7.9%	6.8%
R-merge (I):		

Table V.: Evaluated data from two crystals of 70S ribosomes of T.thermophilus (at a 2 σ level):

Crystal form:	Tetragonal	
Spacegroup:	$P4_12_12$	
Cell dimensions:	a=524, b=524, c=315 Å	
Crystals:	Native (#39)	GC soak (#40)
Evaluated resolution:	22 Å	22.5 Å
Mosaicity:	1.0°	1.0°
Total observations:	34610	24210
Fully recorded reflections:	1804	1362
Measurements:	1053	827
Unique significant:	25%	20%
Completeness:	6.9%	8.3%
R-merge (I):		
Added partials:	8632	6798
Measurements:		

Most of our crystals grow as thin plates or needles. Some are so thin (0.01-0.05 mm), **that when irradiated by X-ray beam which is focused by several mirror segments, they cause interference of it** ("split beam" see #7 in Table III). In addition, being multi-layered thin plates, these crystals tend to crack while being handled, and their diffraction patterns frequently contain reflections of the main crystal as well as of its smaller fragment(s). The existence of the "second" crystal is not always obvious in the initial orientation and data collection steps, and in many unfortunate cases it was discovered only after a large part of the data has been collected. In fact, except for one case (Table IV), all data sets collected so far from crystals of 50S subunit of H. marismortui suffer from apparent "twinning". This, together with the very high **mosaicity** of these crystals (0.6-2°), introduced extreme difficulties in data evaluation.

The high mosaicity dictates collecting data at large rotation angles. Therefore, long exposure times are required, resulting in a high background level. Hence, significant portions of our beam periods are lost by screening for crystals not only of the highest resolution but also with the lowest mosaicity. Consequently, less than 15% of the mounted crystals on the X-ray cameras are actually being used for data collection.

Although the current percentage of useful crystals (15%) is still rather disappointing, it is an order of magnitude higher than that of two years ago, and far better than the ratio obtained three years ago. As example, is the last experiment carried out at 4° C: 232 irradiated crystals (Fig. 1) yielded only 5° of rotation at 12 Å resolution. Even at the initial stages of measurements at cryo-temperature (1986-1987), when we immersed the crystals in an inert hydrocarbon, only 8% of the crystals found their way to the X-ray beam, and between them only 8% were measured, namely total of 0.64% of the crystals yielded diffraction results. This was the main reason for us to introduce the new concept of **shock freezing at close to the natural environment** of the crystals. A thorough search led to the establishment of individual freezing conditions for each crystal form. In several instances, an improvement in the distribution of the potential resolution was observed (Fig. 2).

We have also designed a system for storing irradiated crystals for long periods: "**the solid propan liquid nitrogen procedure**". A careful statistical analysis of crystallographic data sets collected at cryo temperature from freshly cooled crystals as well as after a long storage of irradiated crystals, have been a major concern for us, as only very little experience has so far been accumulated world-wide.

In typical experiments we examined exposures, taken from a crystal at the beginning of data collection and after being irradiated for 48 hours with an intense synchrotron beam. Likewise, data have been remeasured after 153 days of storage. In all cases there was no loss of maximum resolution or required exposure time (Fig. 3).

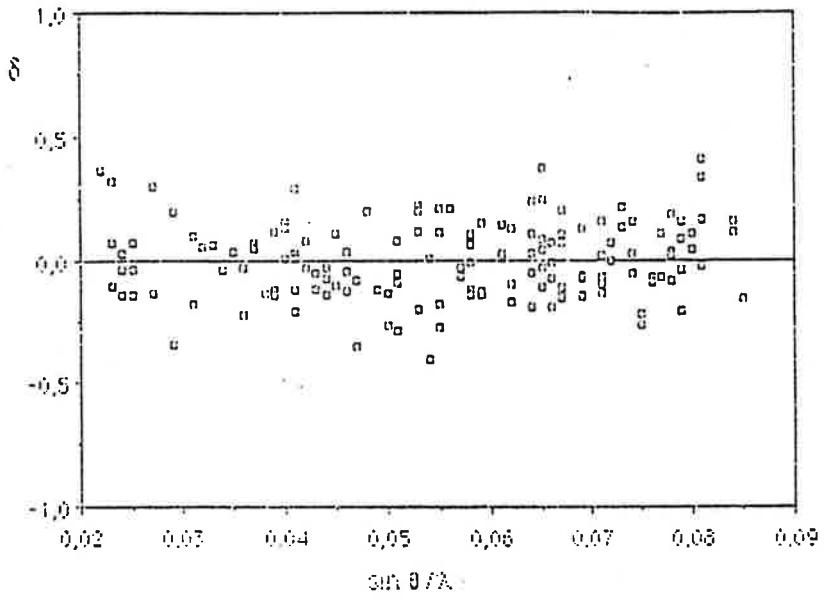


Fig. 3: A graphic representation of the stability of data after storage of 153 days in liquid propane of an irradiated crystal of 50S subunits from H. marismortui

3. Data Evaluation

The large cell dimensions, the exceptionally high mosaicity and the dramatic drop of intensities at around 15 Å resolution, forced us to modify and further develop most of the available packages for data evaluation. This is an ongoing process, since several routine application (e.g. autoindexing) are still not suitable for our data (we are using FILM and FILME, both originated at MPI/Martinsried but modified for interactive use on workstations with DEC-windows and X-windows; MOSFLM, the version developed by EMBL/Hamburg; OSC from Purdue U. and WEIS, for data collected at KEK). Despite the above mentioned difficulties, evaluation of these data yielded reasonable intensities (e.g Tables IV and V).

4. Attempt on phasing

Our strategy is to elucidate the structure of ribosomes in a progressive fashion, starting at low resolution and advancing iteratively to higher.

(a) MIR - the Multiple Isomorphous Replacement Method

Clusters which contain a core of several metal atoms linked directly to one another, comprise a system of especially high electron density. We have designed a **monofunctional undecagold cluster** (GC) for covalent binding either to isolated ribosomal components which could subsequently be incorporated into the core particles, or to exposed chemically reactive groups on the surface of the ribosomes, or to natural or tailor-made compounds which bind specifically to ribosomes.

(i) We have developed a mutant of B. stearothermophilus, lacking one ribosomal protein, (BL11). The monofunctional undecagold cluster was bound qualitatively to the isolated protein BL11, and the modified protein was subsequently incorporated into the mutated core to form labeled 50S subunits. Since we obtained isomorphous crystals from the wild-type, mutated and modified subunits, this system has a high potential for phasing. A difference Patterson map, based on data described in Table IV, was constructed. Despite the severe lack of common centric reflections, and the poor resolution (the maps were constructed at 20 Å, a resolution range where the disordered solvent is expected to contribute noise to the map), the resultant difference Patterson map appears to contain four rather correlated peaks, in accord with expected four 50S subunits per asymmetric unit.

(ii) Direct binding of the gold cluster to the surfac2e of the ribosome was possible after locating exposed -SH groups, using a radioactive model reagent. This way we could obtain quantitative binding to one exposed sulfhydryl on the 30S subunits from T. thermophilus. The crystals of the modified subunits are smaller than, but isomorphous with, the native ones and diffract to the same resolution.

(b) Rotation-translation searches

For initial phasing at low resolution, we use the models of the ribosome and of its 50S subunit which have been reconstructed by us at low resolution from electron micrographs of tilt series of two-dimensional sheets. Represented as pseudomolecules, these models were employed in rotation and translation searches (ULTIMA, MERLOT, XPLORE) with X-ray crystallographic data. For 70S ribosomes, these efforts yielded a few distinct packing arrangements with R factors of around 40-53%. After rigid-body refinement and solvent flattening the R factor of one arrangement dropped to 29.8%.

(d) Neutron Diffraction

Single crystal neutron diffraction is a technique which allows to carry out selective structural studies by contrast variation. Hence, it should be appropriate for ribosomes, which are composed of RNA and proteins. A few sets of neutron-diffraction data from crystals of 50S subunits from H. marismortui have been collected at ILL (in collaboration with Dr. M. Roth, E. Pebay-Peyroula, A. Benteley and P. Metcalf). One of these sets, collected to 30 Å resolution, could be evaluation to completion. It was then phased by direct methods (an updated version of MITRILL, J.Gilmore). The resulting maps are relatively clean, and contain features with the shape and size of this subunit.

Yeast $tRNA^{Asp}$-Aspartyl-tRNA synthetase Complex: data collection using synchrotron sources for the determination of the structure of two crystal forms of the complex

M. Ruff, S. Krishnaswamy, M. Boeglin, J. Cavarelli, A. Poterszman, A. Mitschler, J.C. Thierry and D. Moras

Laboratoire de Cristallographie biologique
Institut de Biologie Moléculaire et Cellulaire du CNRS
15 rue René Descartes, 67084 Strasbourg cedex, France

Introduction

The fidelity of the translation of the genetic information during protein synthesis relies upon the sharp recognition of the aminoacyl-tRNA synthetases and their nucleic-acid substrates, the tRNA. This leads to the specific attachment of the correct amino acid to its cognate tRNA.

Yeast aspartyl-tRNA synthetase, a dimer of molecular weight 125000, and two molecules of its cognate tRNA (Mr = 24160) cocrystallize in the cubic space group I432 (a = 354 Å) and in the orthorhombic space group $P2_12_12$ (a = 210.25 Å, b = 146.17 Å, c = 86.13 Å).

The crystal structure of the cubic form was solved to low resolution (7.5 Å) using neutron data collected at the Institute Laue Langevin and X-ray diffraction data collected on synchrotron sources in Lure and in Chess.

The structure of the orthorhombic crystal form diffracting to high resolution (2.7 Å) was solved recently using data collected at the EMBL outstation in Hamburg for the high resolution data and on a Siemens 2D area detector for low resolution heavy atom search.

The structure of the complex to high resolution was solved using MIR techniques. This new structure, a member of class II aminoacyl-tRNA synthetases, exhibits the characteristic signature motifs shared by these enzymes. This structure is different from the structure of the complex $tRNA^{Gln}$ Glutaminyl-tRNA synthetase from E. coli, recently solved in Yale which belong to class I.

New crystals of the same complex, but from E. Coli, have recently been obtained. Their diffraction power is stronger than in the case of the complex from yeast. Nevertheless data collection without the help of a synchrotron source remains an almost impossible task if data to high resolution are to be collected and even this requires an efficient 2D detector (space group $C222_1$, a = 102.5 Å, b = 128.109 Å, c = 232.11 Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$).

Data collection of the cubic crystal form (complexes from yeast)

X-ray data could only be collected on synchrotron sources due to the extremely weak diffracting power of the crystals. This was done both in Lure and in Chess. Advantage was taken of the high intensity of the beam at Chess to get more data to the diffracting limits of the crystals. This more intense beam allowed also to get more data out of one given crystal. 5271 reflexions were obtained to 7.5 Å of resolution with an R factor of 10.7 %.

Data collection of the orthorhombic crystal form (complex from yeast)

Diffraction data were collected in the laboratory using a Siemens 2D area detector for resolution data sets between 40 Å and 6 Å (essentially native and gold substituted crystals) and at the EMBL synchrotron outstation by DESY in Hamburg, using the imaging plate system (locally developed by J. Hendrix and A. Lentfer) for high resolution data (2.7 Å) of the native crystals and heavy atom derivatives. High resolution data could only be obtained on a synchrotron source due to the size of the crystals and of their diffraction power. Crystals appear as plates of 0.4 mm by 0.8 mm in their largest dimensions and a thickness varying between 0.06 mm for the earlier crystals and 0.2 mm now. Systematic errors due to absorption effects were minimized by collecting data at a wavelength set around 1.0 Å. Typically native data were derived from a collection of 500 oscillations (data from over 30 crystals were collected) out of which 310 oscillations from 22 crystals were kept for the final merge. Oscillation range could vary according of crystal setting from 0.7 to 1.5°. Advantage was taken of the brilliance of the synchrotron X11 beam line to get diffraction information to 2.7 Å of resolution with an overall R factor of 9.0% obtained from 327516 observations of 70895 independent reflexions.

	Resolution min.	max.	Rsym (%)	Number of observations observed/independent	delta F (%)
Native	40.0	2.7	9.0	327516/70895	
Hg	40.0	3.7	6.5	72399/20256	19.1
Au	40.0	5.7	7.2	16068/4896	21.1
Sm	40.0	3.5	7.0	77909/22560	15.2

delta F = $\Sigma(|FPH-FP|)/\Sigma|FP)$

Class II aminoacyl tRNA synthetases: Crystal structure of yeast aspartyl-tRNA synthetase complexed with $tRNA^{Asp}$

M. Ruff, S. Krishnaswamy, M. Boeglin, A. Poterszman, A. Mitschler, A. Podjarny, B. Rees, J.C. Thierry, D. Moras

The crystal structure of the binary complex $tRNA^{Asp}$-aspartyl tRNA synthetase from yeast was solved using MIR techniques to 3 Å resolution. Crystals belong to space group $P2_12_12$ with unit cell parameters of a = 211 Å, b = 140 Å, c = 86 Å and one dimetric molecule per asymmetric unit. Diffraction data were collected in the laboratory using a Siemens 2S area detector for the low resolution data set, and at the EMBl outstation of DESY (Hamburg) using an imaging plate system for the high resolution data sets. Three derivatives were obtained : gold chloride was used to low resolution, mercury (PCMB) and samarium (SmCl2) were used to 3.5 Å resolution. However, the poor phasing power of these derivatives clearly indicates a lack of significant contribution below 4 Å. The 6 Å resolution map enabled the tracing of the boundary of the molecular dimer and the location of the tRNAs. The phases were then improved using a combination of MIR contribution, solvent flatening techniques and averaging around the molecular two fold axis. Modeling of the tRNA molecules and the chain tracing of the synthetases was possible using a 3.5 Å resolution map. Extension to 3 Å resolution using solvent flattening techniques and two fold averaging enabled the location of a number of significant side chains.

The dimeric synthetase (AspRS), a member of class II aminoacyl tRNA synthetases, exhibits the characteristic signature motifs shared by these enzymes. Each catalytic site is built around a antiparallel β-sheet flanked by three a helices which form the pocket in which ATP and the CCA end of tRNA bind. This domain contains the three sequence motifs formed to be conserved in eight aaRS. Motif 1 is a key component of the dimer interface, motifs 2 and 3 are involved in the binding of the acceptor end. Another structural domain, barrel-like, binds to the anticodon loop of tRNA. The $tRNA^{Asp}$ molecule approaches the synthetase from the variable loop side. Two major contact areas are involved : the acceptor end and the anticodon stem end and loop. In both positions the protein interacts with the tRNA from the major groove side. The molecular association leads to the following characteristic features : i) the backbone of the GCCA single-stranded portion of the acceptor end exhibits a regular helical conformation, ii) the loop of sequence motif 2 interacts with the acceptor stem in the major groove and is in contact with the discriminator base G and the first base pair UA, iii) the anticodon loop undergoes a large conformational change in order to bind to the protein.

ACCURACY AND HIGH RESOLUTION

I. BROUTIN, B. ARNOUX AND A. DUCRUIX
ICSN, CNRS, 91198 Gif sur Yvette Cedex, France
AND P.A. TUCKER
EMBL Meyerhofstrasse 1, 6900 Heidelberg, Germany
AND R. KAHN, R. BOSSHARD AND R. FOURME
LURE, Bt 209D, University of Paris Sud, 91405 Orsay Cedex, France

Collagenase from the fly larva *Hypoderma lineatum* (MW 25,223 daltons for 230 amino acid residues) was crystallised at neutral pH in the absence of inhibitor. Crystals belong to the tetragonal space group I422, a=111.7Å and c=165.8Å, with two molecules in the asymmetric unit. Delaunay reduction gives a reduced cell with dimensions a=111.7Å b=111.7Å c=114.5Å α=119.2° β=119.2° γ=90.0° with 32 molecules per unit cell. The solvent content is about 55%. All crystals used in the following work had the same morphology (trigonal prisms) and roughly the same dimensions (0.8x0.8x0.4mm^3).

The structure was solved to 3Å resolution (to be published) using data recorded with a Philips (PW1100) diffractometer. In order to refine the structure to higher resolution, new data were measured at EMBL Heidelberg on two commercially available area detector based instruments, a FAST system (Enraf-Nonius), named data set (F) and an X100A system (Siemens), named data set (X) using a rotating anode X-ray source in each case. When the LURE Mark II detector became available new diffraction data were recorded using that instrument with synchrotron radiation, in order to obtain the highest possible resolution.

For (X), data processing was performed with the XENGEN package (Howard et al, 1987). The background was estimated from an updated global background and is a standard XENGEN procedure. Shape fitted, rather than simple summed intensities, were used. The programs in the XENGEN package subsequent to integration were not used. Instead, (X) data set was scaled internally by the method of Fox and Holmes (1966) and outliers from the distribution of symmetry related observations rejected.

For (F), the crystal to detector distance was 80mm with the detector swung from the central position by 28°. Data collection was made at 21°C. Data were processed using the program MADNES (1987) and integrated using a background plane fitting algorithm followed by the profile analysis of Kabsch (1988).

The LURE system detector consists of an Eulerian cradle with a two dimensional detector (Mark II), the whole instrument being rotated such that the ω axis was in the horizontal plane because of the horizontal polarisation of the synchrotron beam (Kahn et al, 1986). The crystal to detector distance was 580mm with the detector swung to 24° from the symmetric position. The detector entrance window (effectively 512x512mm) and shorter wavelength resulted in a maximum resolution of 1.7Å. Two data sets (L1-L2) were recorded at 18°C in two different parts of the Ewald sphere and on two different crystals. The data were processed with the MADNES program (1989). As for (F) the ellipsoidal masking procedure was used but a global background array was initialised in the first 2° of data and updated as

data collection proceeded. In addition the profile analysis of Kabsch (1989) was applied to the data. Some 15° of the (L1) data set were treated in space group P422 to check the absence of reflections H+K+L = 2n+1 corresponding to systematic extinctions in space group I422.

The CCP4 program package was used for the comparison of the various data sets. The final symmetry averaged data sets were used. All data sets were put into the (same) unique region of reciprocal space, sorted and combined to give a single file. The data sets were then scaled pairwise on F^2. The values of Rmerge are acceptable given that the data sets were measured on different crystals, different instruments, using different beam properties and at slighthly different temperatures. Furthermore the area detector data sets were not corrected for absorption effects, other than that part of the absorption correction removed by internal scaling and averaging of symmetry equivalents.

Linearity of Wilson plots is respected until the mean intensity no longer changes. Since the scattering factors continue to fall as a function of resolution the implication of a constant $\langle I \rangle$ is that the measurements are at noise level.

This comparisons show all data sets exhibit the same gross behaviour but, in order to judge the data quality, consideration of intensity alone is insufficient. The standard deviations of the intensities are also important since, at least, they give a guide to the precision if not accuracy of the intensities.

For $\langle I/\sigma(I) \rangle$ as a function of resolution, the larger this quantity the more precise the data, provided always that random errors are dominant. For (X) and (F) this was not the case and for the (X) and (F) data sets the standard deviations were multiplied by 1.38 (X) and 1.29 (F) for the subsequent analysis. The segment of the curve where the value of $I/\sigma(I)$ starts to level off to a constant value can be considered the useful resolution limit. Clearly (X) and (L) are superior in this respect but it would appear that for (F) the useful resolution limit has not been obtained.

If the percentage of reflections with $I/\sigma(I) > 3$ is plotted as a function of resolution, discontinuities in the slope of the curve suggest the point at which there is useful data which agree very closely with the effective resolution limits from the Wilson Plot. This type of plot is also useful in preparation for refinement. Information such as the cut-off value required by X-Plor at the start of refinement can be determined.

Conclusion

It is clear that the primary difference between data sets is not one of detector but rather one of the combination of detector and source. The combination of a synchrotron beam with the LURE Mark II detector allows collection of high resolution data of a quality not normally attainable even with area detectors and a rotating anode source.

HARD X-RAYS AND A FOCUSSED WHITE BEAM.

Janos Hajdu

Laboratory of Molecular Biophysics, Oxford University, South Parks Road, Oxford, OX1 3QU, U.K.

A) On the wavelength range of radiation for Laue diffraction experiments:

In the Laue geometry, a wider wavelength range brings more lattice planes into diffracting position. There are certain practical limits which need to be looked at when chosing a spectral range for the experiments (see e.g. Buras & Gerward, 1975; Clifton et al, 1990). In the long wavelength region, absorption becomes a dominant factor producing increased radiation damage. This is a serious problem. At wavelengths beyond 3A, several common atoms have absorption edges (e.g. Ca K edge at 3.07A). As wavelength lengthens, the solid angle subtended by a set of diffracted beams increases and many longer wavelength reflections escape the film. In addition, the d_min=lambda/2 boundary sets the upper limit for the highest resolution at any particular wavelength: soft X-rays don't carry high resolution data. There seems to be little advantage in opening up the spectrum towards long wavelength radiation. An upper limit of about 2.0 A has been used with proteins and viruses but this value needs to be reduced with strongly absorbing organic or inorganic crystals to avoid heating.

At the short wavelength limit, there is a tail-off in both the sensitivity of the detector (X-ray film) and of the magnitude of atomic scattering factors. The use of short wavelength radiation may still be favourable for two reasons: i) Reduced absorption leads to increased sample life times. We have found that a spectral bandwith of 0.2-0.6 A gave about 1000 times longer sample life time in the beam with protein crystals than the full spectrum of 0.2A - 2.2A (Pattison, Hajdu, Clark, Andersson and Clifton, unpublished results). ii) The narrow cone of diffraction with short wavelengths allows for longer crystal to detector distances, and gives a better peak to background ratio for hard radiation reflections. The drawback lies with the reduced scattering power of the crystal and the less than perfect detective quantum efficiency of photographic film at shorter wavelengths. Hard radiation requires a more sensitive detection system. A short wavelength limit of about 0.2 A was used in most of the Laue diffraction studies completed so far. The 0.2 A data were week on film but came up strong when image intensifier screens were used.

B) The choice of focussing optics:

The spectrum of the incident beam is modulated by filters and various optical elements between the sample and the source. Filters cut out soft radiation and hard X-rays are absorbed on focussing mirrors (see e.g. Bilderback and Hubbard, 1982). It will be necessary to use focussing optics for attempts with single bunch exposures at the ESRF. Cutting out hard X-rays decreases the number of reflections accessible in the Laue geometry. A way of preserving hard radiation is possible if tube or capillary optics are used for focussing. Capillary optics have been around for quite some time (Jentzsch & Nahring, 1931, Hirsch, 1960) and have been used in a number of applications recently (e.g. Nakazawa, 1983; Rindby, 1986).

Using tube or capillary optics with white radiation gives a focussed beam of medium to soft X-rays on top of an unfocussed core containing the full spectrum including hard radiation. Note that the divergence of hard X-rays in synchrotron radiation is usually smaller than the divergence of soft radiation. We suggest to use tube or capillary optics for those experiments where high

intensity and a broad spectral bandwidth is required. Optical elements of this size are small, cheap, spendable and could be placed anywhere in the beam pipe. With their inner surface tapered outwards or toroidal, and the tube positioned close to the source, many orders of magnitude more intensity may be gained at the sample (Clark, Hajdu, Nave, Pattison and Rindby in preparation).

Heat load: In the storage ring, the small cylindrical elements will be subjected to an even and symmetrical heat load. It is therefore hoped that they will be less sensitive to distortions during operation than large flat or toroidal mirrors where uneven heating causes problems.

Manufacturing: Microfocus X-ray capillaries used on rotating anodes or sealed X-ray tubes are drawn by hand from ordinary glass tubes and have given gains in intensity more than 1000 fold (e.g. Rindby, 1986). It is likely that ESRF will need a more sturdy material and better directional accuracy but the surface smoothness inside a drawn out glass/quartz tube may well be sufficient for these applications.

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HIGH SPEED, HIGH RESOLUTION DATA COLLECTION ON ORTHOROMBIC CRYSTALS OF SPINACH RUBISCO AT THE PHOTON FACTORY.

Inger Andersson (1), Ian J. Clifton (2), Vilmos Fulop (2), Janos Hajdu (2)

(1) Department of Molecular Biology, Swedish University of Agricultural Sciences, Box 590, S-751 24 Uppsala, Sweden.
(2) Laboratory of Molecular Biophysics, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

Ribulose bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the initial reaction of two competing metabolic pathways in plants, algae and photsynthetic bacteria; the carboxylation of ribulose-bisphosphate (RuBP) which initiates photosynthetic carbon dioxide fixation and the oxygenation of RuBP which is the starting point for photo-respiratory carbon oxidation (1). Both reactions occur at the same catalytic site of the enzyme. The latter process is wasteful since it removes RuBP from the Calvin cycle and also because recovery of the products of oxygenation is an energy consuming process which results in further loss of carbon dioxide. The relative rates of the two reactions affect the efficiency of net carbon assimilation. As a consequence there is considerable interest in experiments aiming to modify the structure of Rubisco from crop plants in order to increase the proportion of carboxylation relative to oxygenation. Knowledge of the 3-dimensional structure of the enzyme and in particular its active site is a prerequisite for a better understanding of the catalytic mechanism and the role of individual residues in the process.

The enzyme from spinach is a hexadecamer (L8S8, 550 kDa) consisting of eight large (L, 55 kDa) and eight small subunits (S, 13 kDa). The structure of the activated enzyme with a bound transition state analogue (carboxyarabinitol-bisphosphate) has previously been determined to a resolution of 2.4 Å (2,3). The present crystallographic R-factor is 0.19 for all data to 2.4 Å resolution. At this resolution, there are certain ambiguities in the orientation of the sugar analogue and, consequently, in the possible roles of certain catalytic residues around it.

Crystals of the enzyme are large and diffract to better than 1.6 Å resolution. Diffraction data to such a high resolution are extremely difficult to record from large unit cell crystals like Rubisco (space group: $C222_1$, a=157.2 Å, b=157.2 Å, c=201.3 Å). Many reflections would be overlapped on a flat film using the rotation method. To overcome this problem, we have explored the use of a giant Weissenberg camera developed at the Photon Factory (4).

EXPERIMENTAL

Diffraction data to about 1.5 Å resolution were collected at BL6A2 (wavelength = 1.04 Å; I = 350-270 mA; E = 2.5 GeV) using the Weissenberg camera for macromolecular crystallography with an IP radius of 573 mm and a 100 um collimator. The total detector area was 40 cm x 40 cm consisting of two 20 cm x 40 cm Fuji BASIII imaging plates. 24 such double images were recorded for the a* axis mounting using an oscillation range of 5.5 deg/exposure and 0.5 degree overlap between frames. Total exposure time was 20 minutes/data set (120 degrees) and the total data collection time was 2 hours. A single crystal was used and the data were collected from a single position on the crystal. The crystal diffracted to better than 1.5 Å throughout. From a second crystal, some 30 images were collected under similar conditions for the a*/c* diagonal setting with an oscillation range of 4.5 deg. The imaging plates were digitized on a BA100 IP scanner (Fuji Film Co., Japan).

RESULTS

All data were processed at the Photon Factory with the WEIS program (5) using a FACOM 360 computer. About one million measurements were made in the 2 data sets. The final combined and merged data set (R-merge = 0.069) contained 277,448 unique reflections. Fig. 1A shows the distribution of reflections in resolution bins. Refinement of the model against the data is now under way. Fig. 1B shows the first high resolution electron density map after 120 cycles of conventional Jack-Levitt type refinement using the Xplor package on a Convex C2.

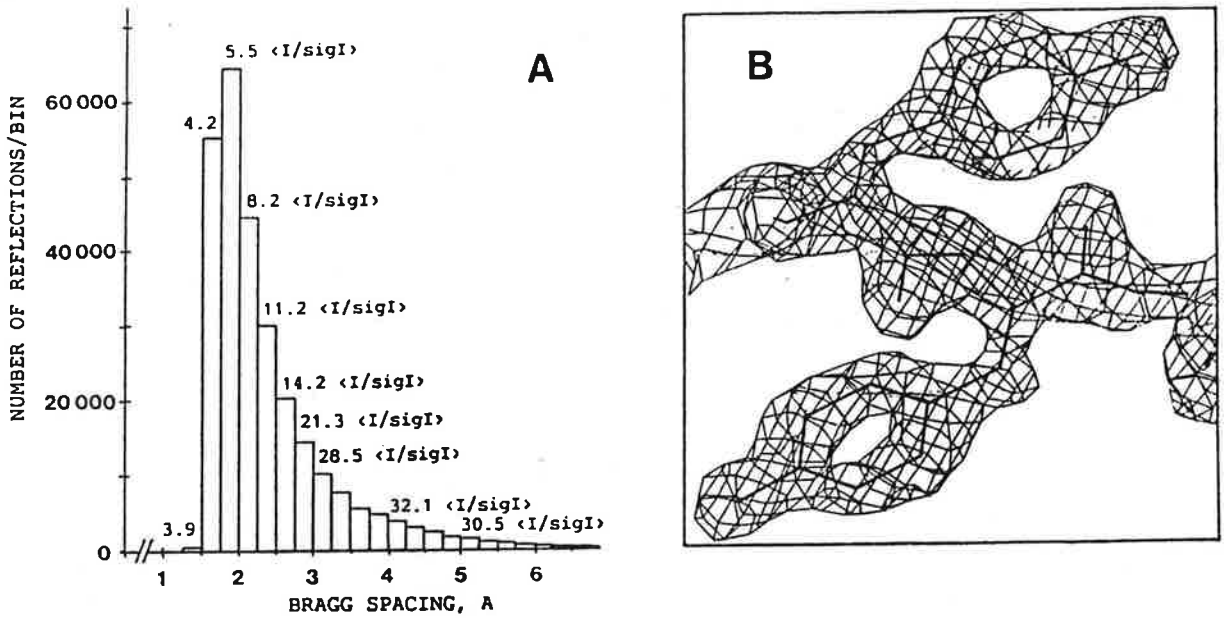

Fig. 1 (A) Distribution of the unique reflection set for spinach Rubisco. 277,448 unique reflections were obtained from about one million observations. Space group: $C222_1$, a=157.2 Å, b=157.2 Å, c=201.3 Å. The average ‹I/sigI› values for the resolution bins are given above each columns. (B) Tyrosine side chains (61I-62I, see ref. 3 for numbering) from the initial 2Fo-Fc electron density map with data to 1.7 Å resolution only. The map was calculated after 120 cycles of conventional Jack-Levitt type refinement with the Xplor package against the previously obtained 2.4 Å model on a Convex C2. Full refinement of the structure with data to 1.5 Å resolution is now under way.

ACKNOWLEDGEMENTS:

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Reaction initiation for time resolved structural studies

R. S. Goody

Abteilung Biophysik, Max-Planck-Institut für medizinische Forschung, Jahnstr. 29, D-6900, F.R.G.

and G. Rapp

EMBL, c/o DESY, Notkest. 85, D-2000 Hamburg 52, F.R.G.

Developments in the use of synchrotron radiation have reduced the time of data collection for X-ray diffraction investigations of biological systems to a time scale which is similar to that of single steps in enzymatic reactions. However, full expoloitation of the resulting potentential is heavily dependent on the ability to start the reaction of interest uniformly throughout the sample on the appropriate time scale. Except for the case of x-ray scattering in solution, classical rapid mixing methods will not be applicable due to diffusional delay in the ordered systems (crystals or fibres) used. Another technique which has been used for classical perturbation studies in solution, the temperature jump method, has been applied to fibres and partially ordered systems (Rapp et al., 1989; Kriechbaum et al., 1990) using an erbium laser at a synchrotron source, but it is not likely that this will be generally applicable to crystals. Special properties of the system under investigation can be exploited, such as electrical stimulation of muscle contraction or initiation of the reaction cycle in systems converting light into other forms of energy. However, such properties will not be available in the majority of biological systems of interest, so that other methods must be sought.

One of the most promising approaches involves the use of photosensitive derivatives of substrates or other ligands which are inert to the action of the system under investigation but which can be converted to the desired reactant by rapid photolysis. The best known derivative of this type, caged ATP, has been used extensively in investigations on the mechanism of muscle contraction. In this application, caged ATP interacts weakly with the active site of myosin, and most of the ATP generated by the light flash (xenon flash lamp or frequency doubled ruby laser) is initially free in solution. The resulting transitions (rigor to relaxation in the absence of Ca^{2+} ions, rigor to activation in its presence) can be studied by a variety of techniques, including low angle x-ray diffraction using a synchrotron source (Rapp et al., 1986; Poole et al., 1988). Caged calcium (a photosensitive calcium chelator) has also been used to study the transition from the relaxed to the active state after rapidly increasing the Ca^{2+} ion concentration by flash photolysis (Rapp et al., 1989).

The required properties of a caged compound for use in crystallographic experiments are different from those of caged ATP in the actomyosin system. The caged precursor should be present at the active site of the protein in the crystal, so that after release of the substrate or other ligand there is no diffusional delay involved in reaching their site of action. This has been realized in the case of the protein product of the H-ras protooncogene, which is a small GTPase known as p21. Caged GTP binds with high affinity to its active site, and the complex can be crystallized (Schlichting et al., 1989). After photolysis at the active site, the relatively long half life of the p21.GTP state allowed its structure to be determined by the Laue method at the EMBL Oustation, Hamburg. This work has led to the identification of the important conformational change in the protein which occurs as a result of GTP hydrolysis and which constitutes a molecular switch in this system

(Schlichting et al., 1990).

The temporal resolution in the work on p21 was of in the range of a few minutes. Several technical improvements can lead to improvements in this respect. Firstly, a more sensitive detector than x-ray fim (probably image plates) would reduce the exposure time, resulting in the dual benefit of increased temporal resolution and the ability to collect more successive data sets before beam damage to the specimen becomes limiting. Secondly, modifications in the chemistry of the photsensitive protecting group together with improvements in the light source for photolysis could lead to a higher degree of release of substrate per flash. As these improvements begin to lead to potentially better temporal resolution, several other problems begin to become significant, including rapid changing of detector cassettes and of crystal orientation, since in the work on p21, 3 orientations were needed for a reasonably complete data set. Thus, with the x-ray intensity available at synchrotron sources, it is not likely to be the time needed for individual exposures which limits the temporal resolution in the near future, but problems of detection and of the chemistry of the photolysis reactions.

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New Strategies for Microgravity Protein Crystallisation.

By Patrick D. Shaw Stewart
Douglas Instruments Ltd, 25 J Thames House, 140 Battersea Park Road, London SW11 4NB, England.

and David M. Blow
Blackett Laboratory, Imperial College, London SW7 2BZ, England.

The greatest barrier to protein structure analysis is the difficulty associated with obtaining large, well-ordered crystals for X-ray diffraction. Recent results apparently show that crystals grown in microgravity are of better quality than equivalent crystals grown on Earth (L. J. DeLucas and C. Bugg, Methods, a Companion to Methods in Enzymology, vol.1, no.1, 1990, pp105-109). This has been explained by the lack of precipitation of small crystals which are formed in the bulk of the solution to the bottom of the droplet, and by the lack of convection currents around the crystals.

Results in microgravity crystallisation have been inconsistent, however, and we believe that this may be explained in part by the failure to optimize conditions for microgravity crystallisation. If microgravity is having a significant effect on crystallisation, in particular by removing convection currents, it is probable that the ideal conditions for crystallisation in microgravity are different from those for crystallisation on Earth.

There are other reasons why current methods of crystallisation in a space laboratory are likely to prove problematic. In the first place, very minor heat flows through a vapour diffusion apparatus cause water to be transferred from the hotter to the cooler areas, which may reduce the concentration of crystallisation trials, sometimes causing any crystals to redissolve.

Another relevant phenomenon is that the conditions and time period of storage of protein prior to crystallisation may effect the nucleation and growth of crystals. At Imperial College, we have found that the incubation of a commonly-used protein for a few hours in water at 4 C causes an increase in the number of crystals by a factor of up to 1000.

For all these reasons, we have identified two approaches to ensure that the best possible conditions for crystallisation in space are found. The first is to set up a large number of different conditions, covering an area which experiments on earth indicate is likely to yield positive results. It is intended to provide about 48 sample chambers for each of 20 or more proteins. The second approach is to use a "Telescience" or remotely controlled apparatus, enabling the experimenter to control a series of crystallisation trials over and extended period where the conditions in each trial can be adjusted in the light of previous trials.

These two strategies form the basis of a recent proposal for a microgravity project submitted to the European Space Agency. If any European experimenter would be interested in providing protein samples for microgravity crystallisation using either of these approaches, please contact the authors.

Making the most of your Phases

A case study with 5-carboxymethyl,2-hydroxymuconate isomerase

D.B. Wigley and E.J. Dodson

University of York, Heslington, York, YO1 5DD

Abstract

The problem of obtaining an interpretable electron density map using data from poor derivatives is hardly a new one! The recent determination of the structure of 5-carboxymethyl, 2-hydroxymuconate isomerase (CHMI) in York was just such a case, where we had low resolution, non-isomorphous data from one derivative, and high resolution data from another isomorphous, but very poorly substituted, derivative. The following is a description of how we finally obtained an interpretable map, and how quickly the structure could be built and refined, once this map was obtained, as a result of improvements in computing power.

Introduction

The degradation of homoprotocatechuic acid is one example of the rich and complex chemistry referred to as secondary metabolism. This particular pathway is found in certain strains of *E.coli* and involves several steps. Examination of two of these steps reveals a close similarity between them; the substrates for these reactions differing by a single carboxyl group, which is removed in a separate reaction between these two isomerisations. The two isomerases show a strong specificity and each will only act upon only one of the two substrates. Consequently we want to discover how this specificity is achieved and how the enzymes are able to discriminate against the wrong substrate.

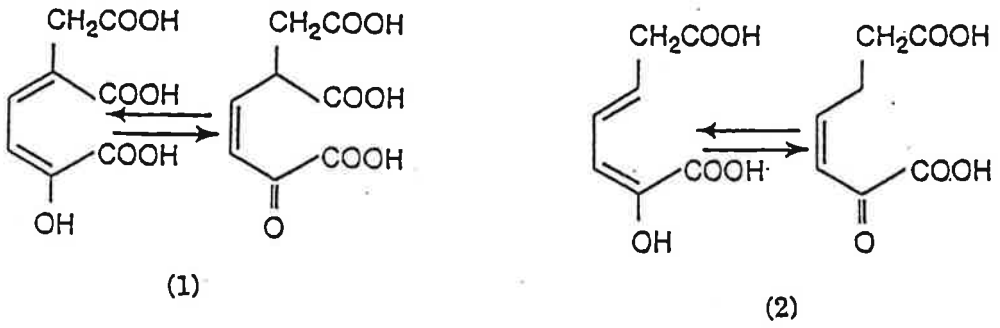


Figure 1: Degradative pathway of homoprotocatechate in *E.coli* Structure determination. Reaction (1) is catalysed by 5-carboxymethyl-2-hydroxymuconate (CHM) isomerase, and reaction (2) by 2-hydroxyhepta-2,4-diene-1,7-dioate (HHDD) isomerase.

Background

The crystals of CHM isomerase are tetragonal, P41212, with unit cell dimensions a = b = 90.3, c = 129.8, and contain one enzyme trimer in the asymmetric unit. The solvent content is around 60%. Native data have been collected to 2.1Å resolution in collaboration with Drs. Z. Dauter and K.S. Wilson at the EMBL outstation in Hamburg. Data from two heavy atom derivatives (including anomalous scattering measurements) were also collected. The K2PtCl4 derivative had three fully occupied sites and proved to be a good derivative at medium resolution, but analysis by ANISOSC revealed a lack of isomorphism beyond about 4Å resolution. Data to 3.7Å , however, were used in the phasing. The NaAuCl4 derivative proved to have two sites, both of low occupancy. Attempts to increase the occupancy of these sites by increasing the NaAuCl4 concentration resulted in a loss of diffraction by the crystals. However, 2.6Å data were collected for this derivative at a low concentration of NaAuCl4, and 3.7Å data at a higher concentration of the gold reagent. Phases were obtained by combining the information from all three derivative datasets. This was done by refining each of the gold datasets independently with the platinum data in PHARE. The refined parameters were noted, and a final run with PHARE, using the 'phase' option, produced an LCF file with phases to 2.6Å (Table 1).

Table 1

1. a) Data collection

Data	Resolution	Rmerge (%)	Riso (%)	No. refs	Completeness (%)
Native	2.1	4.9	—	28,776	89.4
K2PtCl4	3.7	4.3	28.2	6,063	98.6
NaAuCl4	3.7	3.6	9.2	6,045	98.3
NaAuCl4	2.6	***	7.0	***	****

2. b) Phasing statistics

Derivative	Resolution	No. sites	Cullis R(%)	x y z (absolute scale)	occupancy	Phasing power
K2PtCl4	3.7	3	54.9	0.73 0.24 0.35	1.16†	3.40
				0.35 0.29 0.77	1.05	
				0.38 0.03 0.71	0.98	
NaAuCl4	3.7	2	83.9	0.12 0.63 0.94	0.31	1.05
				0.32 0.11 0.88	0.21	
NaAuCl4	2.6	2	88.0	0.12 0.63 0.94	0.11	0.52
				0.32 0.11 0.88	0.07	

†Clearly an occupancy greater than 1 is an overestimate, and is probably a consequence of applying too low a B value and/or the presence of chloride ions around the Pt ion. For all heavy atoms, B factors were fixed at 25.

It was clear that the phasing power of the gold derivative was not good, and previous experience suggested that the phasing, and consequently the calculated electron density maps, would be rather poor. Despite this, electron density maps were calculated at 3.7Å and 2.6Å resolution. Although some secondary structural elements could be picked out, both maps was uninterpretable, though it is important to note that the 2.6Å map did show more detail than the 3.7Å map, thus some useful phase information could be obtained from the higher resolution data.

The maps were used for solvent flattening in an attempt to improve the phases. This did improve the situation, with breaks in β-strands healing up for example, but the maps were still uninterpretable. In an alternative procedure, solvent flattening was used to extend the phasing to 2.6Å resolution by gradually increasing the resolution of the data from 3.7Å , in steps of 0.1, and combining with the SIR (with anomalous) gold phases at each stage. This resulted in a map which was interpretable and allowed a partial model (comprising ~40% of the protein) to be built. It is worth noting that although the non crystallographic symmetry was known by this stage, for reasons discussed below, this was not utilised. Pieces of the model were built by using a combination of all three maps for one subunit, before the local symmetry was applied to generate the other subunits of the trimer. Initial cycles of PROLSQ refinement applied non crystallographic restraints (mode 2 - strict NCS for main chain but less strict for side chains). This model (R = 48%) was then used for phase combination at 2.6Å resolution to improve the phases further. Several subsequent models were built, each containing a bit more of the protein. Once the entire chain had been built (at least as polyalanine) and chain connectivity was certain, a round of refinement was carried out using XPLOR, with the resolution of the data increased to 2.1Å . The R-factor before XPLOR was 39%, and after heating, cooling and energy minimising the structure, the R-factor had only fallen to 34%. However, both the geometry of the structure and the quality of the density maps were greatly improved. This allowed correction of a couple of mistakes (e.g. where six residues were misplaced by one position along an α-helix), and allowed the assignment of side chains to those residues which had been built as polyalanine. Refinement was completed by cycles of PROLSQ and model building to fit solvent and correct some side chain conformations. The final model contained all 375 residues of the CHMI trimer, 201 water molecules and 7 sulphate ions. This model has an R-factor of 16.6% using all data between 10Å and 2.1Å resolution, with deviations from ideality of 0.018 and 0.017 for bond lengths and planes respectively.

Discussion

There were several key points in the structure determination which should be emphasised.

1. The derivatives were, of course, far from ideal. The non-isomorphism of the platinum derivative was evident from an increase in the unit cell dimension along the c-axis of 1. Analysis of this derivative data by ANISOSC confirmed this lack of isomorphism with respect to l, but analysis with respect to h and k showed that the data were still good at high h and k index. Consequently, the platinum was still a very powerful derivative at low resolution (as indicated by its phasing power).

2. The structure determination was dependent upon very accurately recorded, high resolution, but low occupancy, gold data. The Riso for this derivative was only 7% - not dissimilar to the expected Rmerge for two native datasets - and in fact was only 4% at 3.5Å resolution! The accuracy of data collected on the Hamburg image plate was absolutely essential in this case, particularly for the anomalous measurements which become more useful at high resolution because unlike Fh, the anomalous contribution remains constant with respect to resolution. Despite the very low substitution in the gold derivative, the signal was sufficient to see very clear peaks in both difference and anomalous pattersons. This has important implications for the future of data collection as it is clear that it is possible to get useful phase information from a derivative with a very low level of substitution, if one can measure the differences accurately enough. With image plate data, clearly one can still get such information with levels of gold substitution as low as 7% (i.e. equivalent to about 6 electrons). It is probably also important that because of the high sensitivity of the image plate, all of the derivative datasets could be collected from single crystals.

3. The reasonably high level of solvent in these crystals was obviously an important factor in the structure solution, and solvent flattening (using the CCP4 programs) played a major role in improvement of the protein phases. However, we found that it was important how the solvent flattening was carried out. Calculation of the mask is very important. We found that the best results were obtained if the mask were calculated at low resolution, rather than the resolution of the map you are trying to solvent flatten. Not surprisingly, heavy atom phasing is inevitably better at lower resolution, resulting in a clearer molecular boundary. Consequently the mask that you calculate is better when extracted from an MIR map at low

resolution than at higher resolution. The boundary will be less detailed, of course (which is actually often a good thing!), and the molecular volume will tend to be overestimated, but this is easily compensated for by assuming a slightly lower solvent content than you actually have, to fill in the little crevices which are lost in the lower resolution map. It would seem sensible to calculate a mask at as high a resolution as you think that the phases are good to, and probably to actually use a slightly lower resolution than this to compensate for over confidence! In our case the phases were good to 3.7Å but we used a mask calculated from a 5Å map.

4. Another important procedure in the structure was the use of several skewed maps, rather than an average of these. Although this goes against general belief, it was clear that, in this case at least, the averaging did not work as well as inspection of individual maps. Rather, it was found to be more informative to skew the maps such that the density for each monomer was in the same orientation, and then inspecting each of the maps individually rather than averaging them. The reason for this was that often the "local phasing" was very much better for one or other of the subunits, and this information was lost by averaging with poorly phased regions, so by skipping from one map to another it was easier to build up a picture of the structure. Whether this is a result of phasing with one poor derivative, such that only a small proportion of reflections are phased well, is not clear. However it does seem plausible that if, for example, one has three-fold non-crystallographic redundancy to utilise, then unless at least one third of the phases are good, then the quality of the maps will actually deteriorate after averaging. In this case, the good phases become submerged beneath the poor ones. In other words, the detail of good areas in the map can be lost when combined with the poor areas. As refinement progresses, the proportion of the map which is good will increase and at a certain stage, averaging will produce a better map. For whatever reason, it was clear that in the case of CHMI that the skewing approach was much better than averaging.

5. Model building and refinement of this structure went extremely quickly, and in fact the entire procedure was completed in just 10 days after calculating the wanged, phase-extended 2.6Å map. This was a consequence of a number of factors. The first model contained just 40% of the protein atoms, which proved sufficient, after phase combination, to see a major improvement in the map. Model bias was avoided by always combining phases from the partial model back with the initial wanged, phase-extended MIR phases after each cycle of model building. It is obviously helpful to be able to fit real sequence into the map, but in this case the initial maps were so poor

that this was only possible for two small sections. The rest of the model comprised polyalanine placed in density which was clearly either β-sheet or α-helix. After phase combination (using SIGMAA), the side chains on the polyalanine regions could be assigned and in this way we were able to bootstrap our way up to fitting the complete sequence. Model building was aided, as described above, by the use of several maps at a time. FRODO, running on an ESV workstation allowed large areas of several maps to be displayed at the same time (more than was possible with the old PS390), which was a considerable aid to model building.

6. The refinement process was aided, of course, by the cautious use of molecular dynamics, but we were careful that we were sure of chain connectivities before launching into this procedure. Perhaps the major contribution to the speed of the refinement procedure resulted from the use of our new SGI computer, which allowed the entire XPLOR job, described above, to run in just 12 hours real time, and allowed almost interactive Hendrickson-Konnert refinement, taking just a couple of minutes per cycle.

Conclusion

The speed with which structure solutions can proceed clearly has implications for the future of crystallography. Experiences both in York and elsewhere have shown that, given a reasonable degree of luck, it is now possible to solve and refine small and medium size structures in just a few months, making crystallography start to become an analytical technique accessible to biochemists as well as die-hard crystallographers.

Life under Unix at York

Eleanor Dodson

Department of Chemistry, University of York, York YO1 5DD

In December, the York protein structure group replaced the VAX cluster of a VAX 11/750, 2 MicroVax II's, a 3400 and 3600 with an SGI Power series 380SX and a 340S with 8 Gbyte of disk space. This means that:

1. There is a great deal more computing power available
2. the operating environment is now completely Unix.

The change over has been much less painful than most of the local Luddites expected. We have been greatly helped by the work done by Kim Henrick, John Campbell and Peter Daly at Daresbury when they transferred all the programs to the Convex. They designed a working procedure which we have adopted wholesale.

There are about 65 registered users on the VAX cluster, so the transfer took some organisation. The system manager has set up batch queues, printer queues and so on although the SGI boffins suggested that this was not necessary — *everything* could be done interactively. The SGI does not provide facilities for implementing disk quotas, so at present we are all having a field day.

Work was transferred in two stages. For the first period, two of us ran test runs on all the commonly used programs and compared the results. This served several purposes:

- the operating system was tested on a small subset of users
- we uncovered many of the problems with transferring VAX Fortran to another system
- we set up example command files tailored to the local environment which could be used as templates as more users moved over
- we became quite slick at moving the binary data (mostly LCF files) and command files from one machine to the other.

After two weeks the rest of the group was let loose, and the working environment became fairly profane! A communal terminal area is almost essential for such an exercise — the nearly blind guided the totally blind. Luckily there were some members of the group who were familiar with Unix, and they have run an almost continuous tutorial session as we bleated pathetically, "Is it '`grep ~/../me.com agr`' or the other way round ..."

The commonest errors have been those due to case sensitivity; a file *must* be assigned to HKLIN, not hklin, and never HklIn!

The worst feature of Unix must be its ludicrously unfriendly mnenomics, '`ls -lt`' is less easy to commit to memory than the VAX equivalent dir/date/size.

The best feature is the wealth of tools available to do almost anything — providing you can find the appropriate "man" page on the machine. I have completely failed to find a method to access most the information wihout asking a more experienced user. For example, "man dd" will tell you many useful things about reading binary data from magnetic tapes. But why dd? No mnemonic springs to mind. You can cut columns of data from your files in a more flexible way even than edt/tpu allows, if you follow the instructions in "man cut"[1]

In conclusion, three months on and virtually all the group is Unix enabled; the SGI is performing well; the computer managers have not strangled anyone; and the tremendous increase in speed and the expanded disk space available have given everyone a great incentive to make the change over.

It is perhaps worth noting some of the problems in transferring old bad Fortran code to the SGI. The compiler is the MIPS Fortran, now running on many Unix workstations (ESV, DEC3100). It is truly Fortran 77, if you think your extension is legal , forget it! Particular problems I recall have been:

- Arrays passed to subroutines in calls may not be dimensioned Array(1) — they must be array(*).
- Character and numeric data must not be included in the same COMMON block.
- **polarrfn** equivalences an integer * 2 array to a real array beginning at the integer element (4). This is an illegal line up of elements and has had to be replaced; the scratch file is marginally larger, but this is no problem.

[1]Editors Note: the command *apropos* can be used to look up manual page infomation and some manufacturers do not support cut - use that wonderful program *awk* instead

- **almn** includes some antique but quite legal code. The indices are packed in one word using this statement:

```
word = 2**16( ih+128)  + 2**8 ( ik+ 128)  + il + 128
```

We were not able to get the same results on the VAX and the SGI, and the problem has been traced back to this line. The MIPS Fortran compiler fails to do correct arithmetic with the integer 2**16. In fact the same error occurred when we ran a test program on the ESV. It seems to be a "feature" of all MIPS compilers. We have been promised a fix in the next release, but at present we run **almn** with this modification!

```
word = 40000( ih+100)  + 200 ( ik+ 100)  + il + 100
```

A further warning, after a few days the wealth of disk space was all gone, as each program had failed in one way or another it had dumped a core image ready for the debugger. These files are enormous and are not automatically deleted[2]

[2]Editors Note: BSD Unix contains the command *limit coredumpsize 0* to overcome this problem

XDL_VIEW - X-WINDOWS ROUTINES

John W. Campbell, Daresbury Laboratory

INTRODUCTION

This article introduces a set of 'view-object' XDL_VIEW routines, developed originally for use by the SERC Daresbury Laboratory Protein Crystallography project team for the development of the Laue software and for potential use with a much wider range of applications. These routines are designed to be used by applications to provide a user interface within an X-windows environment.

In developing the software, the basic requirements were considered to be as follows:

1) To use X-windows.

2) To make use of a colour display.

3) To provide 'high level' view-objects for easy use in building application software.

4) To be able to set up and manipulate such objects via Fortran callable routines (as well as providing a C interface).

5) To provide code which would be portable across a range of workstations with a certain minimum specification.

6) To enable view-objects to appear in individual windows or to be laid out in a tiled fashion on a larger window.

7) To provide a means for specifying the view-objects from which the application is prepared to accept input at any particular time.

8) To provide documentation to cover user and programmer requirements.

VIEW-OBJECTS CURRENTLY AVALAIBLE

The view-objects basically fall into two categories. These are the general purpose view-objects which are written without any particular application in mind (e.g the menu area view-object or the I/O window view-object) and those which are written with a particular application or group of applications in mind (e.g. the film image view-object).

The following view-objects are currently available:

Base Frame View-object	(general purpose)
Menu Area View-object	(general purpose)
Parameter Table View-object	(general purpose)

I/O Window View-object (general purpose)

Pop-up Notice View-object (general purpose)

Progress Bar View-object (general purpose)

Blank Object View-object (general purpose)

Film Image View-object (Protein Crystallography)

Some standard Panel Items for use within the coding of view-objects are also available. The are currently as follows:

Panel Choice Item (a drop down menu)

Panel Slider Item

Panel Button Item

Panel Value Item

Panel Label Item

A number of the view-objects contain an 'active strip' area which indicates whether or not the application program is currently ready to receive input from the view-object in question.

View-objects may either be output to individual windows or may be layed out on one or more base-frame windows as required. An example is given in Fig. 1 of the output from the program 'LAUESPOTS' in which a number of the view-objects currently available are output on a single base frame window. The example shows a menu area, a parameter table, an I/O window and a film image view-object. The film image view-object uses Panel Slider, Choice, Value and Label items.

THE FILM IMAGE VIEW OBJECT

This view-object is designed specifically for displaying film or image-plate images used in Protein Crystallography. It may be used for measuring spot positions and has facilities for overlaying symbols on top of the displayed film image. A magnifying window within the view-object shows a magnified version of a portion of the image from the main image display area. A control panel allows for a series of adjustments to the display. The film image view-object has an 'active strip' at the top to indicate when program is waiting for the input of spot or symbol positions. When the program is waiting for such input, a message is displayed in the active strip indicating the nature of the input required e.g. the default message 'Input spot positions'.

The main image display area at the bottom of the view-object displays the film or image-plate image using a gray or colour scale pallette. It may also be overlayed by symbols when the program requires it e.g. to mark the film centre position or to display a predicted spot pattern.

The magnifying window area (top left below the active strip) shows an

enlarged section from a small part of the image in the main display area. The portion of the image magnified normally follows the cursor position on the main image display area though its position may be fixed when required.

The control panel area is situated at the top right of the view-object below the active strip. The exact layout of the control panel depends on whether a film or image plate image is being displayed. For a film image, it contains the following panel items:-

A slider to control the contrast in the displayed image

A label indicating the cursor position (or pixel intensity)

A drop down menu to select overlaying options

A drop down menu to select the image display colour options

A drop down menu to select the magnification for the magnifying window

For an image-plate image the control panel also contains two value items for the minimum and maximum threshold values used in scaling the display levels.

SOME DESIGN CONSIDERATIONS

The XDL_VIEW software falls into two main categories:

1) A set of routines for managing a set of view-objects.

2) The software for the individual view-objects, some of which are of general use (menu area, parameter table etc.) and some of which are more application specific (e.g. the film image view-object).

The management software is required partly for convenience and partly as a consequence of requiring a Fortran interface to the view-object routines. The XLIB library routines interface provided is C based and makes extensive use of structures, and a variety of data types which do not map naturally on to Fortran 77. Because of the Fortran requirement, it was decided that the view-objects should be identified indirectly through a 'view-object handle' which is a unique integer, selected by the application programmer, for each view-object created within an application. (cf Fortran file logical unit numbers). One of the main functions of the management routines is to maintain a table relating the view-object handles to the actual view-objects and their associated data. The management routines also provide an initialisation routine and a routine for polling for input to the application from selected view-objects.

USING THE ROUTINES IN A FORTRAN PROGRAM

The basic structure for a program using XDL_VIEW view-objects is as follows:

1) Call the XDL_VIEW initialisation routine. This **must** be called and must be the first call to an XDL_VIEW routine.

(xdlf_open_view for Fortran)

2) Create view-objects using calls to the appropriate routines

   (e.g. xdlf_menu_area for a menu area etc.)

3) Set up a list of the view-object handles for the view-objects from which the program is prepared to accept input.

4) Poll for input from the listed view-objects

   (call xdlf_get_events in Fortran)

5) Service the returned input.

   The polling routine returns the view-object handle of the view-object for which the input was made. Specific routines are then called to access the input data (e.g. xdlf_menu_area_getitem to determine the item selected from a menu) and the approriate action is taken by the program.

The procedure then followed will depend on the nature of the program. For example the program may return to step 4 to repeat the polling from the same view-objects or go back to step 3 to change the list of view-objects from which input can be accepted before next polling for input. Alternatively, when required, the user may create new view-objects (and/or delete unwanted ones) and then have a new polling loop. A program will normally have at least one polling loop and more complex programs will often have several.

DOCUMENTATION

The documentation available covers the XDL_VIEW routines from a number of different stand points. These are:-

1) The person using an application program which makes use of the view-objects.

2) The application programmer who wants to use the available view-objects from within his/her Fortran or C program.

3) The programmer who wishes to develop new view-objects for general or specific requirements.

It is divided into three volumes:

Volume 1: User Documentation

Volume 2: Programmer's Guide

Volume 3: XDL_VIEW Routine Calls

Figure 1: Layout of view-objects on a base frame (Lauespots program)

Developing an X-windows based user interface for protein crystallographic software

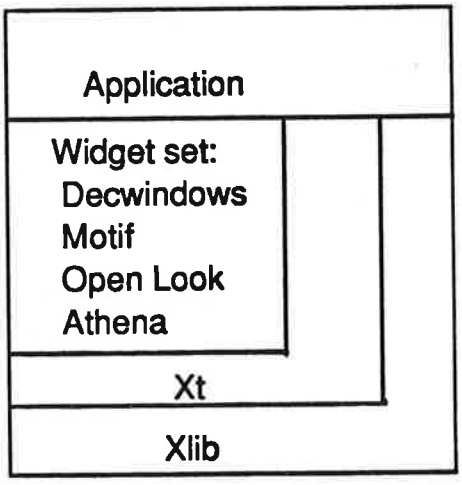
David Wild
EMBL
Postfach 10.2209,
D-6900 Heidelberg,
Germany.

This article is a progress report on work being done at EMBL to develop an X-windows based user interface to the CCP4 suite, and also a brief report on the recent EACBM/CCP4 workshop held in Heidelberg to discuss the related issue of the keywording of CCP4 programs.

Although protein crystallographers have been quick to adopt newer and faster hardware platforms, the user interface to protein crystallographic software has tended to be neglected in the past. Most protein crystallographic software still uses the traditional 80 column "card image" format adopted in the early 1970's for input. However, modern workstations, coupling powerful processors with graphical user interfaces, offer the protein crystallographer the possibility of a more interactive method of performing crystallographic calculations.

In designing a graphical user interface to the CCP4 program suite, a number of considerations need to be made. Thje interface should be capable of being used on different operating systems (VMS, Unix) and should have the possibility of being used to execute programs on a remote machine over a network. It should be easy to use for new users and yet provide experienced users with the ability to run the programs in the traditional manner, if so desired. It would also be avantageous if the underlying CCP4 programs could be modified as little as possible. It was these last considerations which led to the interface being designed as a "wrapper" for the CCP4 programs which outputs a standard CCP4 command file. The CCP4 program can then be spawned as a subprocess (VMS), run in the background (Unix) or submitted to a batch queue using this file as input.

Once the decision to use X as the windowing system was taken, the problem arose of which higher level programming toolkit to use. The figure below shows the various layers of libraries involved in writing an application which uses a set of "widgets".



"Widgets" are reuseable pieces of code which provide the "gadgets" of the user interface (buttons, menus, dialog boxes etc). The Xlib and Xt libraries, along with the Athena widget set are publically available from MIT on the X distribution tape. The Athena widgets, however, do

not provide a complete development enviroment, and it is easier to use one of the commercially available widget sets. The two main contenders here are Motif (from the Open Software Foundation) and Open Look (from AT&T/Sun). Sun also provides an alternative C language toolkit; XView, which does not use widgets or the Xt intrinsics, but which produces "Open Look" style interfaces. A public domain version of XView is available on the MIT X distribution tape.

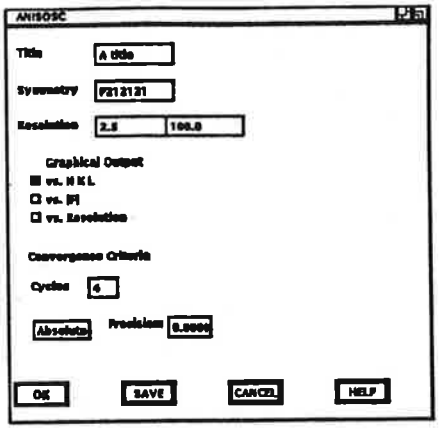
Because of the preponderance of DEC hardware at EMBL, the first version of our interface was written using the Decwindows toolkit. Both Decwindows and Motif allow the interface description to be written in an object-oriented User Interface Language (UIL) and compiled separately from the application, which is written in C. Each widget in the interface (push buttons, text widgets etc) is associated with a "callback routine" which is activated when something happens (eg: a button is pressed or something is typed). To make the interface more portable, the code was then ported to Motif, which is available for both VMS and Unix platforms from several manufacturers (and is due to replace Decwindows on DEC hardware in the near future).

On startup, the user is presented with a pull-down menu bar comprising (at the moment) 4 pull-down menus: *File, Calculation, Utilities and Help*. On selecting the *Calculation* menu, the user is presented with a pull-down menu comprising the types of calculations involved in a structure determination: *data reduction, scaling, phasing, molecular replacement, refinement* etc. For each calculation type there is a "side" pull-down menu listing the CCP4 programs relevant to that type of calculation. The user selects a program by releasing the left mouse button whilst that program's push button is highlighted.

When a program has been selected two things happen: buttons in the *File* pull-down menu containing the names of input and output files appropriate to the program (eg: *input MTZ file, output MAP file* etc) become active. At the same time, the user is presented with one or more "input" dialog boxes. These contain all the input data required by the program, and are designed to use simple input devices: on/off toggle buttons , "radio" toggle buttons, "option menus" and text fields. These input devices correspond to different types of keyword in the CCP4 input data:

- on/off toggle buttons for "switch" type keywords
- "radio" toggle buttons or "option menus" for exclusive "multiple choice" type keywords
- text fields for numeric or character data
  (any of the above may also be followed by a text field)

The figure below shows the input dialog box designed for ANISOSC.



At any time during the "input phase" the user can click on the *Help* button in the dialog box, and receive on-line help on the input keywords in a separate help window.

When all the input data is completed the user must select the appropriate input and output files from the *File* menu. On selecting an appropriate item (say *input MTZ file*) a file browser window is presented. The user either clicks on the required file name or types it into a text field. Another item in the *File* menu will allow the user to assign column labels in the MTZ files. When all files have been selected the file browser is dismissed and the *OK* button in the input dialog box is clicked. The user is then presented with a "run box" which allows the user to select the method of program execution (spawn/batch/background).

Since the input keyword types map to a relatively small number of input gadgets, it would obviously be helpful if the CCP4 input keywords conformed to the above general types. It was to try to address this question that a small workshop was recently held in Heidelberg, with participants funded by the EACBM, CCP4, NSF and IBM. Starting with an analysis of the current CCP4 keywording carried out by Kim Henrick, we were able to draw up a list of about 20 keywords which were common to many programs. We were also able to write down a "keyword grammar" which could be used as a guide in future keywording efforts . An example for a new version of ANISOSC from Eleanor Dodson, corresponding to the interface shown above, is given below.

```
TITLE string;
SYMMETRY string;
RESOLUTION real[2] default 2.5 100.0;
GRAPH    list {
            HKL  toggle default "ON";
            FMOD toggle default "OFF";
            RESO toggle default "OFF";
          };
CONVERGE  list {
              CYCLES integer default 4;
              one_of {
                    ABS toggle default "ON";
                    REL toggle default "OFF";
```

```
        };
    TOLERANCE real default 0.00001;
};
```

At the workshop, the input for this example program was changed to conform to this keyword grammar, and input dialog boxes were designed for both Motif and XView, based on the new keywords, using commercially available interactive "interface builder tools" (ICS's the builder Xcessory for Motif and Sun's GUIDE for XView). Work was also begun on a lex/yacc based parser, which will provide some preliminary error checking for input coming from the user interface. Starting from the keyword grammar, the lex/yacc parser will also enable "skeleton" code for the various interface objects (UIL or GIL) to be generated, which can then be edited by the interactive builder tools. This will be further developed by Lynn Ten Eyck at the San Diego Supercomputer Center.

Apart from the parser, and the extension of our prototype to cover the most popular CCP4 programs, future planned developments also include:

- o a port to the XView toolkit
- o graphical and summary text output in an X-window
- o provision for the execution of CCP4 programs on a remote processor over a network.

User Survey of CCP4 Program Suite

Peter J. Daly

Daresbury Laboratory, Daresbury, Warrington WA4 4AD

1 Introduction

In December 1990, I sent out a user survey on the CCP4 Bulletin Board in an attempt to determine the usage of the CCP4 Program Suite and perhaps trawl for new programs. Over thirty laboratories sent back replies, which enabled a quick analysis to take place. The results are not rigorous but provide useful indicators of the usage of the suite and where programming effort may be most suitably used.[1]

The list was not comprehensive, but contained the main body of CCP4 programs, perhaps the most serious deficiency of the survey was that there was no room to indicate whether the suite was used under VMS or Unix (or both).

2 The Questionnaire

Users were asked to indicate on a scale of 1 to 5 their usage of each program, the user documentation, the programmer documentation and the general documentation. A mark of 1 (or left blank) meant that they had not used it, whereas a score of 5 indicated a program that was vital in their work.

A quick glance through the replies seems to indicate that virtually *nobody* reads the documentation although in the

[1]The survey has already shown its worth in this way by allowing Francesca Young (at Glasgow) to concentrate on those programs that are the most important in the porting of CCP4 to an an IBM 3090 under CMS

comments section it was not recorded whether or not this was the users fault or the quality of the documentation.

3 The Index Formula used

Each program was first processed to remove all responses of 1 (not used). Then a simple sum and average was used to give each program a weight, however, since some programs are vital to a few people the Index needs to reflect the number of responses (clearly, its not useful to work on programs that are important to 1 or 2 labs, where they may do the work anyway) — purely arbitrary, I admit, but we can get some global information from it. The formula used is thus:

$$Index = \frac{N_i * 5}{N_r} + \frac{S_i}{N_i}$$

where:

N_r is the number of replies received (30)
N_i is the number of responses for that program
S_i is the sum of the responses for that program

4 Results

The appendix contains the list of programs sorted in order of popularity, the Index according to the formula used above is indicated after each program. The values in brackets indicate the sum and number of responses respectively.

5 Conclusion

The survey has provided a useful indication of the usage of CCP4 programs, and will enable maintainence to be directed at the more used and important programs. Even though the survey did not ask if the suite was under VMS or Unix it is possible to get some feel for this since the programs using graphics are not entirely implemented under

Unix and certain programs (eg laser) are more likely to be used under Unix. It may be worthwhile making the survey an annual event.

A League Table of CCP4 Programs

AGROVATA	9.3 (129 28)	HKSHIFT	4.8 (9 2)	BRKSORT	3.4 (13 5)
ROTAVATA	9.3 (130 28)	PREPARE	4.8 (23 6)	ONES	3.4 (11 4)
FFTKW	9.2 (126 26)	PROFIT	4.8 (20 5)	PLTDEV	3.4 (11 4)
LCFDUMP	9.0 (120 26)	PRTMAP	4.8 (33 11)	ADDRADII	3.3 (15 7)
EXTEND	8.9 (118 25)	MADLCF	4.7 (25 7)	CDSFIT	3.3 (6 2)
LCFUTILS	8.9 (119 28)	SHRINK	4.7 (30 10)	GENNEW	3.3 (6 2)
SORTLCF	8.9 (120 27)	TMPNAM	4.7 (16 4)	ICOEFL	3.3 (6 2)
GENSFC	8.3 (104 25)	GENERATE	4.6 (24 7)	LCCPDEBUG	3.3 (6 2)
TRUNCATE	7.9 (91 20)	MAPSIG	4.6 (24 7)	TAPELCF	3.3 (6 2)
PHASE	7.7 (88 20)	PRFLCF	4.6 (19 5)	ADDORIENT	3.2 (13 6)
MAPBRICK	7.6 (82 18)	RECONST	4.6 (24 7)	ADORIENT	3.2 (13 6)
ANSC	7.4 (82 23)	EXPAND	4.5 (28 10)	CUTOFF	3.2 (10 4)
REFINE	7.4 (82 20)	RESECTION	4.5 (12 3)	ENVRED	3.2 (8 3)
ANISOSC	7.2 (77 24)	TIDYTEXT	4.5 (21 6)	OPTSHIFT	3.2 (8 3)
PLUTO	7.2 (76 18)	BAVERAGE	4.4 (26 9)	PATVEC	3.2 (13 6)
ALMN	7.1 (75 20)	HKPROLSQ	4.4 (15 4)	RECOMPILE	3.2 (12 5)
EDITLCF	7.1 (76 22)	HKPROTIN	4.4 (15 4)	ROTATE	3.2 (8 3)
PROLSQ	6.9 (68 16)	INTERPOL	4.4 (18 5)	TURN	3.2 (3 1)
RSTATS	6.9 (70 18)	LCCP	4.4 (18 5)	BEEPEEP	3.0 (11 5)
SFC	6.8 (68 18)	ANGLES	4.3 (25 9)	HANA	3.0 (11 5)
CADLCF	6.7 (68 20)	SELECT	4.3 (25 9)	RESSEL	3.0 (11 5)
PEAKMAX	6.7 (65 17)	ABSORB	4.2 (24 9)	MPLANE	2.9 (9 4)
PROTIN	6.7 (63 15)	BSORT	4.2 (23 8)	SYMFIT	2.9 (9 4)
COMBINE	6.6 (65 19)	HEADSKEW	4.2 (14 4)	ASC_2_P84	2.8 (5 2)
UNIQUE	6.5 (63 17)	LCFDUMPB	4.2 (4 1)	CADPRO	2.8 (10 5)
MKLCF	6.3 (59 19)	SKEWPRO	4.2 (17 5)	CHKPLT	2.8 (5 2)
DERIV	6.2 (45 10)	TORSION	4.2 (21 7)	CPK7	2.8 (5 2)
DE_ASS	6.2 (42 9)	MATRICES	3.9 (13 4)	CPKMAP	2.8 (5 2)
RFACTOR	6.2 (57 18)	TRNSFMABG	3.9 (19 7)	FIX	2.8 (5 2)
ABSCALE	6.1 (54 15)	DIFFOP	3.8 (20 8)	LINKCCP	2.8 (10 5)
COMPLETE	6.0 (52 15)	DLPLUTO	3.8 (10 3)	PAIROT	2.8 (10 5)
PHARE	6.0 (51 14)	P84_2_ASC	3.8 (10 3)	PROFIT2	2.8 (5 2)
POLARRFN	6.0 (53 15)	RFPLOT	3.8 (17 6)	RDOPT	2.8 (5 2)
POSTREF	6.0 (53 15)	SHIFTS	3.8 (17 6)	SIMMAP	2.8 (7 3)
ULYSSES	5.9 (47 12)	DABS	3.7 (16 6)	TFORTH	2.8 (5 2)
LCFPREC	5.8 (36 8)	DEG	3.7 (16 6)	SIMMER	2.5 (6 3)
SKWPLANES	5.6 (43 12)	HGEN	3.7 (18 7)	LASER	2.3 (4 2)
TFSGEN	5.6 (39 10)	PRMAP	3.7 (20 9)	TED	2.3 (4 2)
SCALENEW	5.5 (42 12)	RECNV6	3.7 (12 4)	TEMP	2.3 (4 2)
TSEARCH	5.4 (41 12)	SUPERPOSE	3.7 (12 4)	TEMPD	2.3 (4 2)
LCFMERGE	5.3 (40 12)	SYMBUILD	3.7 (12 4)	MAPCONV	2.2 (2 1)
LOCAL	5.3 (42 15)	ACCESS	3.6 (18 8)	MAPMIX	2.2 (2 1)
LCFDMP	5.2 (22 5)	CDSLIM	3.6 (17 7)	MIDASLCF	2.2 (2 1)
VECSUM	5.1 (30 8)	PLOT	3.6 (14 5)	TE	2.2 (2 1)
COLLATE	5.0 (33 10)	PRD	3.6 (18 8)	CON3D	0.0 (0 0)
ECALC	4.9 (34 11)	RECENV	3.6 (14 5)	MRGSORT	0.0 (0 0)
EXCHANGE	4.9 (34 11)	WATERSORT	3.6 (18 8)		
CONTACTS	4.8 (33 12)	SUMACCESS	3.5 (16 7)		
HKREF	4.8 (9 2)	TRANSFN	3.5 (9 3)		
HKSETUP	4.8 (9 2)	VIRVE	3.5 (16 7)		

Porting CCP4 onto IBM Platforms

Francesca Young

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ

At Glasgow a project was undertaken to implement the CCP4 program suite on the IBM 3090 mainframe. This has been achieved, although there have been problems in making it available from Daresbury. Ports are currently taking place to the IBM RS/6000 running under AIX. Initial tests show this to be an extremley fast computer.

0.1 THE CMS VERSION

One of the major aims of this project was to produce a full working version running under CMS, that only differed from the Daresbury version in the implementation of some of the low level library routines. This would make it easy to maintain compatability with the Daresbury version, which is important as CCP4 is continually being updated and debugged and new programs are regularly included.
Apart from replacing the system calls in uxsupport.c and the sort routines, there were several other major problems, all of which have now been solved. These problems were caused in the main by fundamental differences between the UNIX and CMS operating systems.
There is no directory structure on CMS. Instead, the user has a virtual disk and is allowed to allocate temporary disks.
On UNIX it is easy for the program to interact with the Operating system through C routines. There is no such interaction under CMS, although it can be achieved using assembler REXX and temporary files.
Initially we were unable to access C routines from FORTRAN programs (although several C compilers are available under CMS), whereas C is an integral part of UNIX.

Problems included:

1. STANDARD FORTRAN Most of the programs in CCP4 were either written or developed on VAX/VMS systems. The compiler on the VAX will compile anything from FORTRAN to MONGOLIAN and appear to execute the programs correctly. Unfortunately, not all compilers are intelligent enough to decipher what the programmer is really trying to achieve. The VS compiler under CMS is particularly

strict. Although this caused the initial, time consuming problem of removing all the non-standard FORTRAN, hopefully we are left with portable code.

2. DIRECT ACCESS FILES When a direct access file (LCF or MAP file) is opened under CMS it defaults to a length of 50 records. This has been solved by allowing the user to define the number of records needed. When the program has completed all unused records are removed from the end of the file.

3. BINARY SORT On UNIX the interface to the standard quick sort has been written in C. Initially we were unable to access C routines from FORTRAN programs and an interface to the operating sort had to be written in FORTRAN. The calls to this look the same as the UNIX version. However this solution is limited by the amount of memory available under CMS (approx. 16 Mbytes) and is therefore unsuitable for large data sets.

4. SCRATCH AREA Files can be assigned to logical files before starting the program, but this is restricted to the users disk which is usually fairly small. This causes problems with scratch files but by storing the file assignments in a file, temporary disks can be accessed.

1 IMPROVEMENT AND AVAILABILITY

Although the CMS specific routines are now stored at Daresbury no test installation has been completed. Therefore, although the programs will compile, the installation will be need to be tailored to the facilities at the local site.
Now that we have access to a C compiler the sort can be improved. It will also allow the use of the new diskio routines to improve the diskio speed which is very slow under the old FORTRAN library routines.

2 THE AIX VERSION

Recently we have been testing CCP4 on the RS/6000. This port was very easy, showing that the IBM unix, AIX, conforms to the usual UNIX standards. From tests carried out so far it is extremely fast, taking about 60s for GENSFC to calculate Fc's from a data set of 6621 atoms and 43615 reflections. (The data used was Xylose Isomerase provided by Kim Henrick for the test suite). Details of time tests can be provided on request.
It is intended that the testing will be completed by mid-April and that the IBM version will then be available from Daresbury.

Notes from Glasgow

Neil Isaacs

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ

1 The crystallisation and characterisation of integral membrane proteins

The light harvesting antenna complexes of photosynthetic bacteria are oligomeric integral membrane proteins whose function is to absorb radiant solar energy and to transfer it to photochemical reaction centres. Crystals of the B800-850 complex from Rps. acidophila strain 10050 have been grown previously and diffraction data to a resolution of 3.2A have been collected by M. Papiz(1). The variability in the quality of the crystals has severely hindered the search for isomorphous derivatives. Current collaborative work in Glasgow between the departments of Botany, Chemistry and Biochemistry is aimed at improving the quality and reliability of crystal growth of the B800-850 complex and at crystallising other complexes for structure analysis. Crystals of the B800-820 complex from Rps. acidophila strain 7750 have been grown. Data collected on the Siemens area detector shows that these crystals are isomorphous with those of the B800-850 complex previously reported. The crystals diffract to 4.5A. Crystals of the same complex from Rps. cryptolactis have also been grown. Experiments with the first small crystals showed diffraction to 6.5A and which could be indexed in a cubic cell. To date the crystallisation has been reproducible and all of the crystals examined have diffracted suggesting that larger crystals will give higher resolution data.

2 Protein crystallography in Virology

Dr. Ben Luisi has taken up a Research Fellowship position in the MRC Institute of Virology. Ben worked with Max Perutz for his Ph.D. then was with Paul Sigler in Chicago and Yale working on DNA binding proteins. In Virology, he and his colleagues will be studying transcriptional regulatory proteins from herpes and related human viruses. They are presently trying to prepare crystals of these proteins in complexes with DNA or in higher-order complexes involving modulating factors. A related research

topic under development is high resolution crystallographic studies of nucleic acids. In conjunction with electrostatic calculations, these structures will be analyzed for charge density distribution and polarisabilities of the atoms in the bases. The objective is to understand base-stacking preferences, and to relate the rules to the problem of recognition of DNA targets by regulatory proteins.

Frog - High-Speed Restraint-Constraint Refinement Program for Macromolecular Structure

By A.G. Urzhumtsev, V. Yu. Lunin & E. A. Vernoslova

from Research Computer Center, USSR Academy of Sciences, Pushchino, Moscow Region, 142292, USSR

The new program complex FROG can be used for:

1) atomic model refinement against X-ray and other data,
2) energy refinement or conformational analysis,
3) protein engineering computing simulations.

FROG provides for the user all the basic possibilities of modern refinement programs. Some advantages of the FROG complex over, for example, the Hendrickson-Konnert (Finzel) program are

1) special composition of the model: it may consist of arbitratry rigid groups of atoms and of individual atoms at a time;
2) models of various types of macromolecules, such as proteins, DNA's etc., may be refined with their own stereochemical restraints at any one time;
3) adaptability to the available memory: the program is easily adapted to any memory of more than 512Kb; the more memory is used, the less computer time is taken;
4) criteria for refinement: original method of the FROG construction allows one to utilize any criterion which can be written in terms of rigid group or atomic parameters, density disitribution or structure factors; for example, Diamond-type criterion, account of a non-crystallographic symmetry, intermolecular interactions and others may be included into the summary criterion.

Other main features of the FROG complex are:

1) very low computer time: a refinement cycle of a middle-size protein (3000 atoms) at a resolution of 2.2Å (16000 reflections) takes about 5 min at a computer EC-1061 with about 1,2 millions oper/sec; computer time is linearly dependent on molecular size;
2) single language; only FORTRAN-IV has been used;
3) Optional service programs: service programs are added to the refinement program which facilitates the work in basic regimes;
4) program blocks may be used in other programs, e.g. those for phase refinement.

REF.: 1. Lunin & Urzhumtsev, 1985, Acta Cryst., v.A41, 327-333.
2. Urzhumtsev, Lunin & Vernoslova, 1989, J. Appl. Cryst., in print

Refinement Criteria

The following is a draft of the criteria suggested by the coordination committee for the publication of refined protein structures. Please send any comments on the draft to KSW at EMBL Hamburg.

Criteria for Refined Macromolecular Structures

Publication of refined structures should require that the following data are submitted, at the very least to the referees, even if not always for full publication.

QUALITY OF DATA

1. The quality of data should be indicated by internal (or external) consistency, based on ALL measured data. No intensity cutoff should be applied to data (small I's have the smallest absolute error (on I)). It is valid to exclude a SMALL number of outliers, but some programs (people) reject large numbers of reflections because of poor agreement : a wonderful but not allowable way of improving Rmerge.

The quality and effective diffraction resolution should be described clearly by giving the following information as a function of resolution ranges:

1) The number of unique reflections.

2) The number of measurements (or mean redundancy).

3) The internal consistency (Rmerge).

4) The completeness (fraction of theoretical data).

5) The number (or fraction) of measurements rejected from the statistics.

6) The mean(I) / r.m.s.(scatter).

ALL data should be included in the merging and statistics : data less than 1 σ or 0 must not be rejected.

PROCEDURE

2. Adequate details should be provided regarding the steps followed in constructing the model and refining the structure. The number of solvent atoms should be given, along with

information regarding solvent B-values, and the approach that was used to identify solvent sites. The report should include the history and salient details of the refinement methods employed, including the restraints used; a description of how the thermal parameters have been treated; and how solvent sites were treated. It should be clear if van der Waals distances were restrained, ether explicitly or by energy minimisation methods.

FIT OF MODEL TO DATA IN RECIPROCAL SPACE

3. The crystallographic R-index (Σ / F_{OBS} - F_{CALC} / /Σ / F_{OBS} /) should be tabulated as a function of resolution. The value for all reflections should be given along with any other values based on selected data.

FIT OF MODEL TO DATA IN REAL SPACE

4. The Real space CORRELATION COEFFICIENT Σ (/ρ_{OBS}-ρ_{CALC}/) /$\Sigma\rho_{OBS}$ should be provided for main chain and side chain atoms independently as a function of residue number. Alternatively the correlation coefficient of ρ_{OBS} against ρ_{CALC} could be calculated as a function of the same parameters. It is not yet completely clear that this is a good measure of quality : it will depend on resolution and will need experience of what is good and what is bad. However it probably is a good indicator of where there are problems along the chain. It is probably highly correlated with the mean B-factor.

REASONABLENESS

5. A final Ramachandran plot should be given, with the favourable energy regions indicated. Any non-glycine residues with angles outside the allowed regions should be discussed in detail.

6. The average B-values should be given for individual residues, including the average values for main chain atoms and the average values for the side chain atoms. In addition, it may be worthwhile to include the rms deviations in B-values within each residue's main chain and side chain atoms.

7. Any buried and unpaired charged residues should be identified and discussed.

8. Any other structural features that are considered somewhat unusual should be described. Examples include unoccupied volume inside the protein; or any unusual distributions of polar and hydrophobic groups within the molecule.

DESCRIPTIVE POINTS : NOT PRESCRIBED

The above points are essential in evaluating the 'quality' or 'correctness' of a crystal analysis. In addition the following points were felt to be useful in many cases, but not essential for inclusion in the publication.

9. Hydrogen bonding patterns within the protein should be described, especially for refined structures. In particular, the number of hydrogen bond donors that are not involved in hydrogen bonding should be given, with particular attention to unsatisfied buried main chain H-bonds.

10. Close intramolecular and intermolecular van der Waals contacts should be described. The character and stereochemistry of major crystal contacts should be discussed.

11. Conformations of side chains can be a sensitive indicator of problem areas, especially for structures refined at high resolutions. Distributions of x1, x2 values for side chains should be presented, along with an analysis of deviations from expected minimum energy ranges.

12. There should be an electron density stereo picture to illustrate the most important (or problem) part of the structure.

NUCLEIC ACIDS

13. For nucleic acids a full analysis with Dickerson's program NEWHEL should be provided.

NEW POSITION

Lecturer (Assoc. professor) in Molecular Biophysics, Lund University, Sweden

The department has excisted for more than two years and is now well equipped for macromolecular crystallography. For data collection we use a rotating anode with a Xentronics area detector equipped for liquid nitrogen cooling of the crystal. We share five VAX computers and work stations with inorganic chemists. For graphics work we use an ESV3+ and a PS390. A 1.5 GeV syncchrotron is being built at Lund university. A 7.5 T wiggler will produce X-ray radiation and gives a challenging opportunity.

About ten persons are currently employed at the department. The main fields of interest are enzyme structure and function and structure and interactions of components of the ribosome and protein synthesis machinery. The new position is best filled by someone with a complementary research program. This would most likely be in macromolecular crystallography, particularly concerning computational aspects or synchrotrone usage. Scientists with experience from molecular genetics (cloning, site directed mutagenesis) or from the translation system are also of interest.

The starting salary will be about 20 000 SEK/month or more depending on experience.

Persons interested in this position, which will be announced shortly, should for further information contact

Prof. Anders Liljas
Molecular Biophysics
Chemical Center
Lund University
Box 124
S-221 00 Lund
Sweden

Telephone: 46-46-104681 FAX: 46-46-104543 Telex: 33533

Binet: ANDERS.LILJAS MBFYS.LU.SE

In the Groningen Protein Crystallography Group a

POSTDOCTORAL POSITION

is available for crystallographic and molecular modelling studies aimed at

RATIONAL DESIGN OF TRYPANOCIDAL DRUGS.

African trypanosomiasis, or sleeping sickness, is caused by the *Trypanosoma brucei*, a unicellular organism. This parasite is entirely dependent on glycolysis and therefore we are focussing, in a European collaborative effort, on selective interference with the proper functioning of a variety of glycolytic enzymes. For one of these, several high resolution complexes of the enzyme with inhibitors have been elucidated. The gene has been overexpressed recently so numerous co-crystallizations with newly designed inhibitors are being initiated. Also for a second glycolytic enzyme from the parasite the structure of two crystal forms is available and also here the gene has been overexpressed. Unique opportunities exist therefore to participate in a multi-disciplinary approach towards designing new sleeping sickness drugs.

This project is a collaboration with several other European laboratories in particular Dr. F.R. Opperdoes in Brussels, Dr. R.K. Wierenga in Heidelberg and Prof. J. Périé in Toulouse. Regular international microsymposia serve to discuss the latest results and to set new research goals.

The Groningen Protein Crystallography Group is well equipped with protein purification instruments, conventional and rotating anode generators, two FAST television area detectors, a computer network, and several computer graphics systems.

For further information write or contact Prof. W.G.J. Hol, BIOSON Research Institute, Department of Chemistry, University of Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands. Telephone **-31-50-634383/634378, Telefax **-31-50-634200.

In the Groningen Protein Crystallography Group a

POSTDOCTORAL POSITION

is available for

MOLECULAR MODELLING FOR PROTEIN ENGINEERING.

We investigate a large number of enzymes by X-ray crystallographic techniques in close collaboration with biochemical groups which perform site-directed mutagenesis experiments on proteins with a well-characterized three-dimensional structure. A crucial role is to be performed by the successful applicant in analyzing structures of wild type and mutant enzymes in order to suggest new mutations to be made. If time permits there might be possibilities to include X-ray studies as well, although the main emphasis will be to apply existing software tools - as well as develop new tools - for protein engineering studies of a wide variety of proteins including dehalogenases, sugar transferases, lipases and phospholipases, hydroxylases and dehydrogenases, as well as isomerases. The successful candidate will have tremendous opportunities to see modelling studies being created into actual new proteins with known three-dimensional structure.

The Groningen Protein Crystallography Group is well equipped with protein purification instruments, conventional and rotating anode generators, two FAST television area detectors, a computer network, and several powerful computer graphics systems.

For further information write to or contact Dr. B.W. Dijkstra at telephone **-31-50-634381/634378 or Prof. W.G.J. Hol at telephone **-31-50-634383/634378, Department of Chemistry, University of Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands, Telefax **-31-50-634200.

In the Groningen Protein Crystallography Group a

POSTDOCTORAL POSITION

is available for crystallographic and molecular modelling studies aimed at

PROTEIN ENGINEERING OF TRIOSE PHOSPHATE ISOMERASE

Triose phosphate isomerase, or TIM, is a beautiful dimeric enzyme, each subunit having a 8-fold pseudo-symmetry. Starting from known three-dimensional structures and genes of prokaryotic as well as eukaryotic TIM's it is our objective to use this enzyme for a large number of different engineering purposes:

* to achieve greater thermostability
* to obtain functional monomeric enzymes
* to obtain complexes with peptides
* to create new enzymatic activities

In addition there is scope for other ideas within the framework of this BRIDGE project which includes besides the Groningen Protein Crystallography Group, five groups in Germany and Belgium involved in site directed mutagenesis, enzymology, peptide synthesis, protein crystallography and molecular modelling.

The Groningen Protein Crystallography Group is well equipped with protein purification instruments, conventional and rotating anode generators, two FAST television area detectors, a computer network, and several computer graphics systems.

For further information write to or contact Prof. W.G.J. Hol, BIOSON Research Institute, Department of Chemistry, University of Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands. Telephone **-31-50-634383/634378, Telefax **-31-50-634200.

In the Groningen Protein Crystallography Group a

POSTDOCTORAL POSITION

is available for crystallographic studies on

LIPASES.

In collaboration with research groups in Bochum (Germany), Brussels and Delft research will be carried out towards the elucidation of the three-dimensional structure and catalytic mechanism of lipases from *Pseudomonas aeruginosa* and *Bacillus subtilis*. The project is sponsored within the framework of the BRIDGE T-project "Lipase" of the commission of the European communities. The position is available for 3 years.

The Groningen Protein Crystallography Group is well equipped with protein purification instruments, conventional and rotating anode generators, two FAST television area detectors, computer power organised in a network, and several powerful computer graphics systems. Projects are regularly discussed in a collaborative manner in various research and group meetings.

We invite candidates from all nationalities to apply. For further information write or contact Dr. B.W. Dijkstra at telephone **-31-50-634381/634378 or Prof. W.G.J. Hol at telephone **-31-50-634383/634378, Department of Chemistry, University of Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands, Telefax **-31-50-634200.

In the Groningen Protein Crystallography Group a

GRADUATE STUDENT POSITION

is available for crystallographic studies on

HUMAN PROTECTIVE PROTEIN.

Human Protective Protein (HPP) resides in the lysosome and has a crucial function in ensuring the proper assembly of lysosomal galactosidase into large functional aggregates. The genetic disorder 'galactosialidosis' appears to be due to a defect of Human Protective Protein. HPP is a heterodimer of two proteins of 32 and 20 kD linked by a disulphide bridge. A structure determination of HPP is essential for understanding its functioning. Since its gene has been cloned and overexpressed, the structure provides immediately a framework for well-designed site-directed mutagenesis studies enabling in-depth exploration of protein-structure and function relationships.

The Groningen Protein Crystallography Group is well equipped with protein purification instruments, conventional and rotating anode generators, two FAST television area detectors, computing power organised in a network, and several powerful computer graphics systems. Projects are regularly discussed in a collaborative manner in various research and group meetings.

This project is a collaboration between Dr. S. d'Azzo and Prof. H. Galjaard, Department of Cell Biology and Genetics of the Erasmus University in Rotterdam and the protein crystallography group of the BIOSON Research Institute in Groningen. Interest in or experience with molecular biology techniques is an advantage for this interdisciplinary crystallographic investigation.

For further information write or contact Prof. W.G.J. Hol, BIOSON Research Institute, Department of Chemistry, University of Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands. Telephone **-31-50-634383/634378, Telefax **-31-50-634200.