ESF Studies on Large Research Facilities in Europe

Review of the needs for European synchrotron and related beam-lines for biological and biomedical research

ESF Study Report
November 1998

To:

The European Science Foundation

At the request of the European Science Foundation (ESF), we have examined the needs for European synchrotron and related beam-lines for biological and biomedical research.

Our findings and conclusions are presented in this Review, for which text we assume full responsibility. We endorse the report of the Reference Group.

September 1998

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Review of the needs for European synchrotron and related beam-lines for biological and biomedical research

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Foreword

More than 20 years ago the European Science Foundation predicted a bright scientific future for synchrotron radiation research in Europe. The ability of hard X-rays to cast new light on the intrinsic properties of matter promised to open up new areas of research and analysis in fields ranging from chemistry to geophysics and from engineering to biology. In fact, we were so convinced of its value to European science that we went on to argue the scientific case and develop the blueprint for what became the European Synchrotron Radiation Facility, built in Grenoble, France and now used by several thousand European scientists each year.

In the autumn of 1997, we were asked to look once again at the European prospects for synchrotron radiation. The initiative for the review came originally from the UK's Medical Research Council (MRC). However, other national funding agencies in Europe shared the MRC's feeling that the demand for synchrotron radiation from biomolecular researchers is evolving rapidly and that an overview of projected needs and resources was required.

The MRC and a number of other national research funding agencies were in the position that they would have to take policy decisions which would have long-term consequences for such research and which would benefit from an authoritative and independent assessment of future scientific needs for synchrotrons for biological and biomedical research.

The timetable was tight. But the task was clearly within the ESF's range of competencies. Moreover, it was also in line with our new strategic plan 1998-2001, which places emphasis on the ESF's role as a provider of high quality and independent scientific advice on a range of science policy issues, particularly on those related to large research facilities.

Having accepted the project, the Foundation followed its well-established processes for carrying out this type of assessment. For the review's results and recommendations to be credible, it was important that the assessment be conducted independently of the users and providers of synchrotron facilities, while, at the same time, drawing on their unique expertise and advice. Twin requirements that were met by the creation of two separate but linked groups.

Under the chairmanship of ESF Board member, Professor Gunnar Öquist (a plant phsyiologist and Secretary General of the Swedish Natural Science Research

Council) a six member Review Panel interacted with a much larger Reference Group made up of representatives from both the user and supplier communities. Their two reports, together with the Review Panel's recommendations, all of which were subject to wide-ranging external consultation, are presented in this document.

The ESF thanks Professor Öquist and the members of both the Review Panel and the Reference Group for the very substantial work that they have put into the preparation of their reports. They have produced an authoritative document within the original timeframe set by the MRC which can serve as a factual basis for decision-makers. Since the completion of the report, a number of funding decisions have been or are being taken with regard to new synchrotron facilities. In addition, the advent of the EC Fifth Framework Programme provides another opportunity to take forward the clear recommendations of the report. ESF will now, over the coming months, consider the ways in which it can help further in the implementation of the report.

Professor Enric Banda
ESF Secretary General

November 1998

Part 1

Review Panel

Executive Summary

We are already in the beginning of a new scientific era, the pursuit of a detailed understanding of life processes at the molecular level. It has been brought about by the confluence of several major scientific advances: the recombinant DNA revolution; the ability to analyse genes (DNA); and the accompanying revolution in our ability to determine the three-dimensional structures of biological macromolecules at high resolution. These developments will open totally new perspectives and possibilities of enormous importance for mankind, e.g. for public health and the environment.

In this context, synchrotron radiation has transformed the prospects for structural analysis of biological macromolecules. The present review of the needs for European synchrotron radiation and related beam-lines for biological and biomedical research is therefore both timely and desirable.

Synchrotron radiation has many different applications in the Life Sciences but quantitatively the dominating use is in X-ray protein crystallography. In this report we emphasise the rapidly growing need for three-dimensional structure analysis of proteins in the Life Sciences. Internationally, this field of research not only develops rapidly, it is also extremely competitive and Europe must secure a proper supply of synchrotron radiation in order to maintain a front-line position in both research and application of new knowledge on molecular structure and function. Other important techniques for structure determination are largely complementary to those based on synchrotron radiation.

The needs in other applications of synchrotron radiation are also growing, e.g. in fibre diffraction, small angle scattering, time-resolved studies, spectroscopy, microscopy and medical applications. It is important that these uses of synchrotron radiation are allowed to expand and novel techniques to emerge.

The Review Panel emphasises three key issues:

- The European community needs greater access to more, properly equipped beam-lines.

- The present system of beam-time allocation does not fit the needs of the crystallographic community. Protein crystallography requires frequent but short access to beam-lines.

- The protein crystallography beam-lines are inadequately staffed. The large number of

projects and the heterogeneity of the rapidly growing user community places a particularly heavy burden on the staff.

The Review Panel offers the following recommendations:

Immediate actions

(a) The efficiency of currently available beam-lines for protein crystallography can be increased immediately and at relatively low cost by installing commercially available, large-size CCD detectors (with short read-out times and high quantum efficiency).

(b) The application procedures for beam-time have to be adjusted to the specific needs of the biological community. The field is extremely competitive, fast moving and characterised by relatively short experiments. We recommend a twin-track system: a block booking for long-term projects and a fast track that would allow access to synchrotron radiation within a short time period.

(c) The staffing of beam-lines has to be improved to ensure efficient operation around the clock. Clearly, the operation of a beam-line dedicated to protein crystallography requires more staff than other synchrotron radiation lines, owing to the large turn-over of projects and the heterogeneous composition and rapid growth of the user community.

(d) The provision of services for data collection and quality assessment of crystals that have been pre-checked and shipped in a frozen state to the site should be explored.

(e) There is a need for a committee of European providers and users of synchrotron radiation to monitor and give advice on the biological use of synchrotron facilities. This would uncover bottlenecks that prevent efficient use, identify ways of solving the problems, and provide a forum for discussing future expansion, such as the creation of new beam-lines, upgrading of existing ones, and the possible provision of new facilities. The appropriate framework for such a provider/user organisation is the ESF, which could give administrative support.

Medium-term needs

(a) The Review Panel strongly endorses the current plans for the replacement of national synchrotron radiation sources (SRS in UK and Lure in France) and for the construction of new sources such as the Swiss Light Source (SLS), the German sources ANKA (Karlsruhe) and BESSY II (Berlin), and the proposed Spanish Light Source in Barcelona (LLS). These developments appear

absolutely essential for satisfying the future needs of the biological community in Europe and for ensuring a geographical distribution of synchrotron facilities. At present, it is very difficult and probably impossible to estimate the precise number of new beam-lines needed during the next five years. Based on the rapid increase foreseen in the need for synchrotron radiation in the Life Sciences, we fully endorse the building and upgrading of existing and planned beam-lines and recommend a close monitoring of the development of demand and supply so that corrective steps can be taken in time.

(b) The demand for high optical quality beam-lines, including those with micro-focusing (a focal spot size of 10 - 20 µm) is bound to increase, because it alleviates the requirements on crystal size and quality.

(c) Investments in the development of area detectors (e.g. solid state) and in data acquisition and handling are needed. The panel urges the Life Sciences community to take an active part in these developments.

(d) The needs in other areas using synchrotron radiation (e.g. non-crystalline diffraction, spectroscopy, microscopy, medical applications) are in principle comparable with those of X-ray crystallography. The relative priorities of the various applications are likely to vary over time. It must be ensured that there is an adequate research base with appropriate beam-lines and detectors to allow these applications to expand and to allow novel techniques to emerge.

European dimension

At present, EMBL has two outstations, one at DESY, where 7 beam-lines have been built and are now operated and maintained by the outstation, and one at Grenoble next to ESRF. In view of the growing demand for synchrotron radiation for biology, there is an acute need to upgrade further the EMBL facility at DESY. The Review Panel is furthermore persuaded by the evidence of the users that there is insufficient support for full biological use of the protein crystallography beam-lines at ESRF. EMBL and ESRF are urged to find the means to put this right, perhaps by setting up a joint working group.

In view of the very strategic role of synchrotron radiation in the Life Sciences and its applications in Europe over the next few decades, there may also be a need (besides the proposed committee of providers and users; see above under Immediate actions) for a European organisation to develop

and operate beam-lines at the national synchrotron facilities. The organisation would support beam-lines dedicated to individual applications rather than be divided between different areas of science. This would improve the access to synchrotrons for laboratories whose home countries do not run national facilities and it would avoid unnecessary redundancy across Europe.

Background to the Review

The British Medical Research Council (MRC) approached the ESF in the autumn of 1997 concerning a Europe-wide assessment of the present and future scientific needs for synchrotron radiation (SR) for biological and biomedical research. The request reflects the substantial commitment of MRC and other Life Sciences funding agencies to the support of SR activities. The background to the request is a perceived growing need for SR in the European Life Sciences research community, particularly with respect to determining macromolecular structures, mainly proteins. This need is reflected by discussions and plans already underway in many European countries. Evidence of current interest in SR is also shown for example by the recent article in Nature Structural Biology (August 1998, 5, 657-658 + supplement).

The ESF Board, at their meeting on 15 October 1997, approved the setting up of an ESF review on the above-mentioned topic. Following this decision the Core Group of the Standing Committee for Life and Environmental Sciences (LESC), and the Core Group/Executive Group of the Standing Committee for Physical and Engineering Sciences (PESC) and of the European Medical Research Councils (EMRC), gave advice on the Review and suggested experts that could carry out the task. The Board gave final approval to set up the Review on 23 January 1998.

This assessment of the needs for SR for biological and biomedical research is well in line with the ESF plan 1998 - 2001 emphasising the important role of ESF for science policy issues at the European level, by providing high quality and independent scientific advice on various matters such as, for example, the need for the development of Large Research Facilities.

The Terms of Reference are given in Annex 1. They emphasise that the review should concentrate on current and future needs in beam-line provision, current and projected demand for access, how that demand is and should be met, and the impact of future technical developments. The review is intended to form an authoritative "platform" of scientifically oriented advice, on the basis of which concerned organisations can take policy decisions.

The Terms of Reference specify that the review should be conducted independently of the users and providers of synchrotron facilities, whilst

drawing on their expertise and advice. Along these lines an independent Review Panel was established. Furthermore, a Reference Group consisting of representatives from the user and supplier communities was created. The task of the latter group of experts was to assist the Review Panel by assembling and editing pertinent background information. In addition, the Review Panel asked for advice from a number of distinguished scientists working with SR and with techniques complementary to SR.

The members of the Review Panel were Arnold Hoff, Leiden University (NL), Bruno Lengeler, RWTH, Aachen (DE), Richard N. Perham, University of Cambridge (UK), Tilman Schirmer, Biozentrum der Universität Basel (CH) and Michel van der Rest, CEA (F). Professor Gunnar Öquist, NFR (SE), member of the ESF Board, was appointed chairman of the Review Panel. The members of the Reference Group were Kenneth C. Holmes, Max Planck Institut für Medizinische Forschung (DE), chairman, Keith Wilson, University of York (UK), vice chairman, Carl I. Bränden, Karolinska Institute (SE), José Carrascosa, CSIC Centro Nacional de Biotecnología (ES), Peter Day, The Royal Institute (UK), Bauke Dijkstra, University of Gröningen (NL), Guy Dodson, National Institute for Medical Research (UK), Wayne A. Hendrickson, Columbia University (US), Anita Lewit-Bentley, LURE (FR), Peter Lindley, ESRF (FR), Dino Moras, IGBMC, CNRS (FR), Wolfram Saenger, Freie Universität Berlin (DE), Jochen Schneider, HASYLAB (DE) and Jean-Claude Thierry, IGBMC, CNRS (FR). Advice on the review as a whole was given by Dennis Bamford, University of Helsinki (FI), Martino Bolognesi, University of Genova (IT), Hartmut Michel, Max-Planck-Institut für Biophysik (DE), Catherine Moody/Diane McLaren, MRC (UK) and Francisco José Rubia Vila, Consejería Educación y Cultura (ES). Advice on complementary methods was given by Marius Clore, NIH (US), Werner Kuhlbrandt, Max-Planck-Institut für Biophysik (DE), Peter Roepstorff, University of Odense (DK), Benno P Schoenborn, Los Alamos National Laboratory (US), Alasdair Steven, NIH (US) and Kurt Wüthrich, Eidgenössische Technische Hochschule Zürich (CH). Information related to industry was supplied by Fritz Winkler, F. Hoffmann-LaRoche Ets (CH) and Malcolm Weir, Glaxo Wellcome (UK). Further details on the members of the Review Panel, the Reference Group and the additional advisers are given in Annex 4.

Initial information on the review was given in a letter to the members of the Review Panel/ Reference Group respectively (Annex 3). The Reference Group met for the first time on the 2 February 1998 in Paris. Subsequent meetings were held on the 2-3 and 27 March 1998. The material produced by the Reference Group is found in part 2 of this book. It includes a survey of existing and planned synchrotron facilities in Europe and a survey of the opinions of users of SR. The findings and views of the Reference Group are summarised in a report. The Review Panel met in Strasbourg on 24-26 April 1998. During this meeting it had the opportunity of meeting and discussing with members of the Reference Group. A review report was produced and, after extensive consultations, approved by the members of the Review Panel following a telephone meeting on the 12 August 1998.

The Review Panel wishes to emphasise that structural biology is a rapidly evolving and expanding field. The Panel therefore stresses that although applying a long-term perspective to identify the need for SR, the recommendations given in this report are based on a five-year time span. The Panel emphasises the importance of carefully monitoring the development in order to make any necessary revisions to the projected needs, and suggests that a follow-up review should be conducted after five years.

In discussing the Terms of Reference, the Panel was of the opinion that at present it is most appropriate to concentrate on the current and projected needs of the academic community, since it will most probably continue to lead the development of structural biology. The pharmaceutical industry will certainly also increase their usage (cf: the Reference Group report) but most of the increase is likely to occur through cooperation with the academic community. This view is based on the observation that only 1-2% of the beam time at ESRF is currently used for proprietary research. Analysis of the accepted proposals from the academic community however indicates that approximately 20% of the beam time is used for non-proprietary collaborative research with industry and this may well be an underestimate. As the convenience and availability of suitable beam-lines increases, and as the availability of numerous genome sequences grows, it is likely that the pharmaceutical industry will become an increasingly important user of SR. This development must be watched carefully so that any steps necessary to maintain access to SR for industry are taken in good time.

The Scientific Case

The growing need for three-dimensional structure analysis

The latter part of the 20th century has seen the dawn of a new scientific era, the pursuit of a detailed understanding of life processes at the molecular level. It has often been said, but that does not make it any the less true, that we are witnessing in biology an amazing transformation comparable with that which changed the face of physics earlier this century and is familiar to everyone in the achievements of the past 20 years in computing and information technology. This transformation has been brought about by the confluence of several major scientific advances: the recombinant DNA revolution; the ability to analyse gene (DNA) sequences which in turn has led to the current genome projects; and the accompanying revolution in our ability to determine the three-dimensional (3D) structures of biological macromolecules at high resolution.

Knowledge of molecular structure in 3D has underpinned many of the advances in chemistry in the 20th century. Likewise, if we are to understand the functions of biological macromolecules and their mechanisms of action, we must have a detailed knowledge of the structures of the molecules themselves and of the way in which they interact with each other and with a myriad of small molecules *in vivo*. This covers a vast range of undertakings: from analysing the way in which enzymes catalyse the reactions on which life itself depends, to understanding the way in which a metre of DNA in each human cell is condensed into a manageable nucleoprotein structure about 1 µm in diameter and replicated at each cell cycle; from establishing the way in which complex proteins in plant cells harvest the light energy of the sun and use it in the production of organic matters and oxygen, to analysing the signalling processes that underlie nerve transmission, hormonal control of metabolism, tissue differentiation, and so on.

The importance of this activity worldwide can readily be assessed by analysing the number of new protein and nucleic acid structures

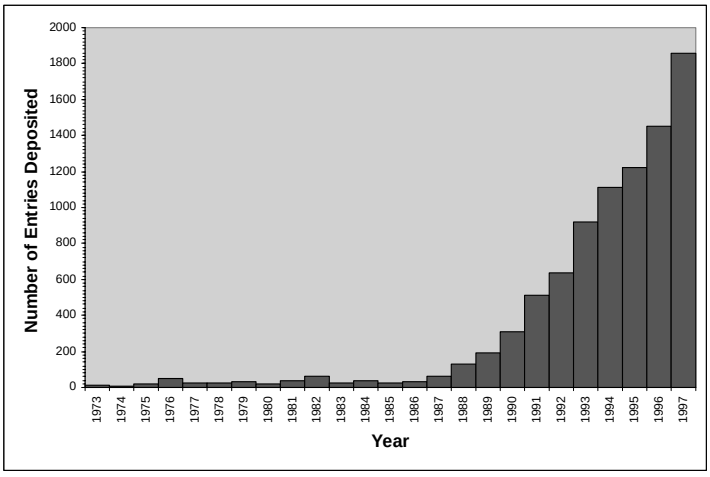


Figure 1: Depositions in the Protein Data Bank. All entries deposited in the Brookhaven Portein Data Bank are included. The vast majority of these are coordinate sets from crystallographic experiments.
Source: Protein Data Bank web site (http: / / www.pdb.bnl.gov)

deposited in the protein data bank (PDB), shown in Figure 1. The dramatic rise in the past decade has been driven by the technological advances already referred to, coupled with the increasing recognition of its value to the pharmaceutical and biotechnology industries.

Genome projects

The blueprint for all life processes lies in the DNA sequence of the genome of the organism in question. The quantum jumps in technology adumbrated above have led to a concerted world-wide effort to determine the complete DNA (genome) sequences of a widespread selection of key organisms, including man. Sequences of about a dozen such genomes, chiefly bacterial (both benign and pathogens) but also including yeast, have now been reported. The human genome sequence is well under way (about 4% of the total of $3x10^9$ base pairs have been completed and up to 15% is available for screening; more if one includes expressed sequence tags) and it is confidently predicted that it will be finished well before 2005. The flood of new information, including the inferred amino acid sequences of all the potential proteins that could function in these organisms, is awesome. It is chastening to note that in yeast, for example, one of the best known and best understood of organisms (it is after all the basis of the world's oldest biotechnology industry, brewing), we can recognise at present fewer than one third of the potential proteins encoded in the genome! The stage is set for yet another quantum leap in knowledge.

Importance to public health and environment

There is a pressing need for the development of more effective pharmaceutical agents as well as of active agents for agriculture more compatible with the drive for a clean environment. Structure determination is rapidly becoming a critical step in the development of new active agents in both the pharmaceutical (drugs) and agro-chemical (pesticide, herbicide) industries. In the development of an active new agent, structure determination intervenes as soon as a potential target is identified. High-throughput screening of libraries, chemical or biological, is usually undertaken to identify lead compounds. These compounds are then improved by cycles of structure determination of agent-target complexes, of biological assays and of modelling of the interaction of modified agents with their target to improve specificity and efficiency. An example of this process is the development of inhibitors of HIV protease based on an estimated

200 structure determinations. The design of these drugs would not have been feasible without detailed knowledge of such a large number of structures. A growing number of critical health-related problems (e.g. the current emergence of pathological strains of bacteria resistant to all known antibiotics) will similarly require a massive and rapid search for new targets and active agents. The prevention of severe epidemics may well depend on the use of SR for 3D-structure determination.

The use of SR in medical imaging and diagnostics is in its early stages at the moment, exploiting the excellent characteristics of third-generation sources. The development of these methods, and possibly their applications in therapy, could be of some importance in the future.

New systems and products

In addition to actively contributing to the design of new drugs, genomic and structural research will form the basis for engineering of new systems and products such as artificial photosynthesis and modified plant fibres and polysaccharides with improved and/or novel properties. In the food industry, the results of these developments are already taking shape and will increasingly have an influence on our daily life.

Biological applications of synchrotron radiation

Crystallography

Among the many applications of SR in the Life Sciences, crystallography certainly represents the major consumer of beam-time. Crystallography is normally the technique of choice to obtain detailed structural information of macromolecules and macromolecular complexes at atomic resolution. Resolutions down to 0.8 Å have been reported. From the above considerations, it is clear that the need for high resolution structural analysis will continue to increase in the coming years.

SR has opened completely new horizons for X-ray crystallography of biological macromolecules. The leading scientific journal Science has nominated SR as the runner up for the scientific breakthrough of the year 1997, after the cloning of the sheep “Dolly” and the Mars Pathfinder. The three applications of SR quoted by Science (the structures of the nucleosome, bacteriorhodopsin and bluetongue virus) emphasize the fact that the use of SR has permitted the determination of structures of very large and complex macromolecular assemblies that could not be studied by any other means owing to the very large size of the crystal asymmetric unit

and/or because of the very small size of the crystals obtained.

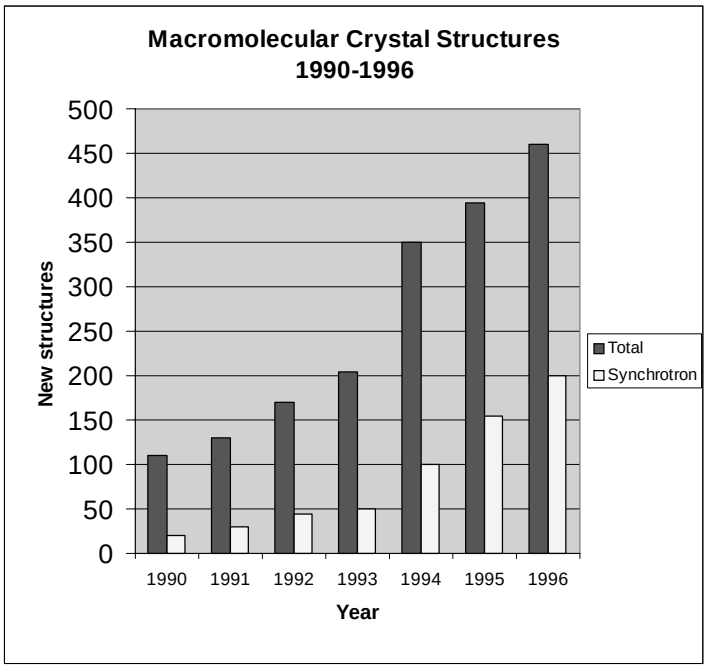
There are clear additional benefits that make the use of synchrotron light beams almost mandatory for efficient X-ray crystallography. The high brilliance of the beam makes very short exposure time feasible, thereby permitting a better control of damage to the sample and a high throughput. The small cross-section of the beam permits the study of small crystals or of selected regions of imperfect crystals, thus allowing the determination of the structures of many macromolecules that would not be possible otherwise. The quality of the optics attained on synchrotron lines also has a beneficial influence on resolution and quality of the data.

Tunability of the wavelength also provides an important benefit by permitting the use of anomalous diffraction for the elucidation of structures (MAD technique). As detailed in the Reference Group report, this technique eliminates the need for multiple isomorphous replacement and reduces the number of sets of data that have to be acquired for a given molecule and the risks inherent in the soaking of fragile crystals in several different heavy atom-containing solutions.

A further advantage of short exposure time and/or the pulse structure of the beam is that it uniquely makes possible the provision of 3D structures of kinetic intermediates in biological processes. Several examples, such as the elucidation of enzyme reaction pathways and the molecular basis of muscle contraction, are described in the report of the Reference Group. Although quantitatively this is not the foreseeable major use of SR, its importance is profound.

The evolution of the number of structures solved using synchrotron sources compared with those solved using standard rotating anode X-ray generators (see Figure 2) is a striking indication of the realisation of the above-mentioned advantages by the crystallography

Figure 2. New crystal structures published. The figure, which is based on Table 1, p. 47 in the report of the Reference Group, shows the total number of new crystal structures published and the number determined with SR respectively.



community. It is clear that if SR had been more accessible during the period considered, the ratio of structures solved on synchrotron beam lines as well as the total number of structures solved would have been significantly higher.

Other uses of SR

For the same reasons that it has become an indispensable source of X-rays for crystallography, namely its collimation, brilliance and tunability, SR has led to major advances in other applications. Fibre diffraction, which lies at the historical root of structural biology for both DNA and proteins, has been supremely important in allowing the determination of structures such as those of helical viruses and muscle fibres (most spectacularly in a time-resolved mode in elucidating the mechanism of contraction of living muscle). Its importance will continue as further higher-order fibrous or filamentous structures are tackled. Likewise, as an energy source for spectroscopy, SR is unparalleled. The use of Extended X-ray Absorption Fine Structure (EXAFS), for example, provides information about interatomic distances in metalloproteins beyond the limit of diffraction methods, even in the absence of crystallographic data. Metalloproteins catalyse many of the fundamental reactions of life: photosynthesis, electron transport, oxygen transport; and the biochemistry of bioenergetics is being transformed by the new understanding made possible by detailed structural analysis. In the UV it is now possible to attain wavelengths as low as 150 nm. This will make possible new experiments on protein folding, notably in the study of the kinetics of the folding and unfolding processes. The study of protein folding is a burgeoning field, fed in part by the advances in structural techniques and by the flood of new sequence information emerging from the genome projects.

Other potentially important applications of SR in the field of biological and medical research are described in the report of the Reference Group.

Complementary methods

Synchrotron radiation is not alone in being able to provide three-dimensional structural information about biological macromolecules. Other techniques are important, but they offer different opportunities and should not be seen as being in competition with protein crystallography.

NMR

Most notable and most successful of the complementary technologies, perhaps, is high

resolution solution NMR spectroscopy. This too has made very impressive advances in the past decade, such that it is now possible to envisage structural analysis of proteins up to 30-40 kDa in molecular mass. It has the advantage of yielding the structure in solution, and can be applied to proteins that have resisted attempts at crystallisation. Furthermore, once a first protein has been assigned and studied, NMR can quickly provide details on possible changes in the structure due, for example, to a mutation. At present, however, NMR can not handle the *large* macromolecular complexes that are now the focus of so much molecular cell biology and are increasingly under study by X-ray crystallography. In contrast, it scores over X-ray crystallography in being able to analyse the dynamical behaviour of macromolecules, the dynamics of solvent (water) interaction, and the formation of transient complexes between the target protein and a ligand, be it another protein or a small molecule.

Moreover, given suitable isotope labelling strategies, solid-state NMR spectroscopy can provide detailed information on limited domains of potentially very large structures. This technique is growing rapidly, and may soon provide information on, for example, membrane proteins, which as a general rule are particularly difficult to crystallise. Similarly it may be able to offer information about drug-receptor binding that is presently not obtainable by other means.

Furthermore, the upper molecular weight limit of applicability of NMR is constantly being extended. Novel NMR approaches are being developed, e.g. TROSY (transverse relaxation-optimised spectroscopy), which enables solution NMR studies of macromolecular assemblies much larger than those accessible with conventional solution NMR techniques.

It is the view of the Review Panel that NMR is an important complementary technique to X-ray crystallography, and certainly not in competition with X-ray crystallography.

Neutron diffraction and spectroscopy

A recent survey of the scientific applications of neutron scattering, including biology, has been published by ESF ("Scientific prospects for neutron scattering with present and future sources" ISBN 2-9031 48-90-2). Neutron diffraction plays a small but important part in structural biology, notably because of its ability to detect hydrogen and distinguish between its isotopes ^{1}H and ^{2}H. Neutron diffraction

experiments on protein crystals, fibres and other oriented systems such as membranes, provide relatively high resolution information on hydrogen locations, hydrogen bonding and other components that can be deuterium-labelled such as specific lipids or membrane components. Low resolution neutron diffraction studies are particularly important for virus studies, large complexes such as ribosomes, and membrane protein crystals; by using H_2O:D_2O contrast variation, the different components can be distinguished within these structures. Contrast variation and deuterium-labelling also make neutrons particularly useful for small angle scattering studies of biological complexes in solution. Neutron spectroscopy is a powerful method to study molecular dynamics in the nano- to pico-second time range - the range relevant to the weak forces (e.g. H-bonding) that stabilise biological molecules and contribute to thermal motions. The neutron approach is unique in providing simultaneously the energy transfers involved and the amplitudes of the motions. Neutron studies in general provide information that cannot be obtained by other methods and are strongly complementary to X-ray, electron microscopy and NMR investigations. The use of neutrons in biology, however, has been severely restricted by lack of beam time due to the shut-down of reactors and the strong demand on the few existing instruments that have the necessary instrumentation.

Mass spectrometry

Another exciting area of great technological advance in the past decade is that of mass spectrometry. It is now possible to determine molecular masses with high accuracy up to 50 kDa, and the newer FTICR (Fourier Transform Ion Cyclotron Resonance) mass spectrometers coming on the market will extend this to, say, 200 kDa. The application of H/D exchange techniques, coupled with the high precision of mass spectrometry of derived protein fragments, has already permitted the detection and mapping of conformational changes in proteins. It is conceivable that this can be extended further to mapping the interface regions of protein complexes; but it is inconceivable that it can be used to determine three-dimensional structures as such.

In addition to the studies based on H/D exchange, another promising concept for mass spectrometric studies of higher order structures, notably protein surface topology and protein interactions, is chemical surface labelling (and/or cross-linking) followed by the identification of

the labelled (cross-linked) sites by mass spectrometric peptide mapping. This approach gives substantial information on the protein structure and the interaction interface. It is also likely to be a valuable addition to structure modelling based on analogy to proteins of known structure because the information obtained can be used as restraints in the modelling process.

In fact, these new developments in mass spectrometry emphasise the growing need to have the detailed three-dimensional structures of the interacting proteins to make the mass spectrometric analysis possible.

Electron microscopy

Electron microscopy (EM) is a rapidly evolving technique whose resolution has recently been dramatically improved with the advent of field emission guns, improved cryoholders and advances in image processing. Its particular strength lies in the structure determination of macromolecular complexes that cannot be crystallised easily and are too large for NMR. The gap in resolution between structures obtained by X-ray diffraction and images obtained by EM, is almost filled. Cryo-EM of symmetrical objects such as icosahedral viruses has yielded information at close to 7Å resolution. The study of asymmetrical objects is also possible at this level although it requires the collection of a much higher number of images than for symmetrical objects to get meaningful information. However, with anticipated progress in automated data acquisition and processing, this will be less of a limitation than at present. Cryo-EM is thus a method of choice for the study of large complexes, especially when structures of sub-components determined at atomic resolution by X-ray crystallography can be fitted within the shape obtained at lower resolution by cryo-EM (e.g. ribosomes, filamentous muscle proteins and virus-antibody complexes). Recently, the complementarity of cryo-EM and crystallography has been demonstrated in elucidating electron density maps of the 50S ribosome particle.

In addition, the absence of the phase problem in electron image processing is a definite advantage that can actually be combined constructively with X-ray crystallography to phase native X-ray data sets in some circumstances, thus providing an alternative to MIR (multiple isomorphous replacement) or molecular replacement. The cryo EM-derived molecular envelope can be used for solvent flattening in combination with non-crystallographic symmetry for this purpose.

Electron microscopy and electron diffraction of regular arrays of macromolecules, including 2D-crystals, has now reached a resolution of 3-4 Å, at which reliable atomic models have been built, as in the case of bacteriorhodopsin, the plant light-harvesting chlorophyll complex and tubulin. This method is particularly promising for the study of membrane proteins for which often only 2D-crystals can be obtained. Like NMR spectroscopy, it should be considered as highly complementary to X-ray analysis.

Modelling

Modelling is a very broad field that should be considered as entirely complementary to X-ray diffraction and other techniques of structural biology. It is an integral part of the structure refinement process in X-ray crystallography and NMR spectroscopy, and can be very useful for the detailed analysis of catalytic mechanisms and conformational changes of macromolecules with known structures. When a template structure is available, the structure of homologous proteins can be reliably predicted with an overall accuracy of, say, 3 Å. It is however notoriously ineffective (and probably so for a long time) in *ab initio* structure prediction.

Other methods

Several new methods, such as atomic force microscopy (AFM), and scanning tunnelling microscopy (STM), are being developed at the moment. They should provide new ways to look at cells and macromolecules. It is, however, very unlikely that these methods will ever reach the atomic resolution obtained by X-ray crystallography; indeed with AFM it is the *surface* of the molecule that is being probed, not its interior.

Structure determination by specialised companies

The high demand for structures at atomic resolution coupled with the establishment of standard routine techniques for the analysis of relatively simple macromolecules should be a strong driving force for the creation of specialised companies that could be contracted by biological research laboratories or by bio-industries for the determination of 3D-structures. A similar trend has been observed for example with DNA sequencing. The access of such companies to SR should be encouraged since it should have a positive impact on the development of European bio-industries. A greatly facilitated access of academic biological laboratories to 3D-structures could also result from the creation of such companies.

However, even if there is potential for a rapidly growing market for structure determination by such specialised companies, the Review Panel wants to caution against unrealistic expectations in the near future. We would recommend an open attitude on this matter and suggest that the SR community should start seriously to consider issues of importance that may help to optimise such a development when the time comes.

Long-term developments

The next generation of SR facilities might well be the free-electron-lasers (FELs) being developed in different laboratories in and outside Europe. These sources have unprecedented characteristics. The average brilliance is 5 orders of magnitude higher than that of the best third-generation sources available at present, and the beam is even better collimated; the peak brilliance is even 10 orders of magnitude larger. The radiation has a time structure 1000 times shorter than that at ESRF, reaching then about 100 fs. In addition, the beam has an excellent lateral coherence. However, there are a large number of problems to be solved before users can profit from these outstanding sources: the SASE principle (Self Amplified Spontaneous Emission) has to be demonstrated for hard X-rays and the heat load may cause serious problems for the optics and for biological samples. But the outlook is very promising. Imaging in phase contrast might have a strong impact on medical applications. Fresnel holography should be possible if high-resolution detector systems are available. The dynamics of biological reactions down to the time scale of electronic rearrangements in a molecule may become observable. Structural biology has always been at the forefront in exploiting SR and therefore should associate itself with this interesting development in order to find and exploit new biological applications for these revolutionary sources.

Key issues

The Review Panel endorses the analysis of the expanding needs for SR in biological and biomedical research outlined in Part 2, the Reference Group report, pp. 43-45, and exemplified by Figures 1 and 2 in Part 1 of this report. We also refer to the recent report of BioSync (Structural Biology and Synchrotron Radiation: Evaluation of Resources and Needs. Report of BioSync – the Structural Biology Synchrotron Users Organisation, 1997), the structural biology synchrotron user organisation in USA. Clearly, there is a high demand in Europe for access to SR. The demand is increasing

rapidly, in particular for protein crystallography, but the application of SR in other areas of structural research will also grow in the foreseeable future.

The Review Panel would like to emphasise the current needs and difficulties:

- The European community needs greater access to more, properly equipped, beam-lines.

- The present system of beam-time allocation does not fit the needs of the crystallographic community. Protein crystallography requires frequent but short access to beam-lines. This is necessitated by the unpredictability of crystallisation, the delicacy of the crystals themselves, and the need for repetitive rounds of structural analysis.

- The protein crystallography beam-lines are inadequately staffed for optimal utilisation of the equipment. The large number of projects and the heterogeneity of the user community place a particularly heavy burden on the staff.

These three issues severely reduce the efficiency of the protein crystallography beam-lines available at present in Europe.

Recommendations

The Review Panel endorses strongly the recommendations put forward by the Reference Group. In the following the Panel elaborates on their major points and raises a few further issues.

Immediate actions

(a) The efficiency of currently available beam-lines for protein crystallography can be increased immediately and at relatively low cost expenditure by installing commercially available, large-size CCD detectors (with short read-out times and high quantum efficiency). This would give the potential to increase the throughput at the beam-lines by a factor of 5 to 10 in terms of data collection.

(b) The application procedures for beam-time have to be adjusted to the specific needs of the biological community. The field is extremely competitive, moving fast and is characterised by relatively short experiments. We recommend a twin-track system be developed: a block booking for long-term projects with a round of proposals every 3 months, and a fast track that would allow access to SR for quick experiments within, say, one week. The latter route would cater for the needs of users with crystals that are difficult to obtain, to reproduce or to store. For highly qualified research groups it may be appropriate to allow negotiations of even longer time periods than 3 months of block booking.

(c) The staffing of beam-lines has to be improved to ensure efficient operation around the clock. Clearly, the operation of a beam-line dedicated to protein crystallography requires more staff than other synchrotron radiation lines owing to the large turn-over of projects and the heterogeneous composition of the user community. In order to attract qualified beam-line scientists it is necessary to offer long-term perspectives to them. This is particularly important to those who are not getting tenure at the facility. One way to achieve this goal is to tighten the links between the synchrotron radiation facilities and European universities and research organisations. When post-doctoral fellows fill the role of beam-line scientists, it is important that they be given enough time for their own project work so that they can pursue their scientific career in a competitive way.

(d) The provision of services for data collection and quality assessment of crystals that have been pre-checked and shipped in a frozen state to the site should be explored. This would ensure the most efficient turn-over for data collection of routine samples. Obviously, the user would also benefit largely from such a service

by saving travel time and expense. Charging for the service could partially finance the cost of the additional beam-line personnel required.

(e) There is a need to coordinate the use of beam-lines at the various synchrotron radiation facilities, the ESRF and the national machines in Europe. In addition, there is a need to monitor the biological use of synchrotron facilities. This would uncover bottlenecks that prevent efficient use, identify ways of solving the problems, and provide a forum for discussing future expansion, such as the creation of new beam-lines, upgrading of existing ones, and the possible provision of new facilities. These needs could be met by the formation of a dedicated organisation like Biosync, a committee of providers and users that operates effectively in the USA and whose recent report (1997) we have studied. The appropriate framework for such a supply/user organisation is the ESF, which could give administrative support.

Medium-term needs

(a) The Review Panel strongly endorses the current plans for the replacement of national SR sources (e.g. SRS in UK and Lure in France) (SRS, Lure), and the construction of new sources such as the Swiss Light Source (SLS), the German sources ANKA (Karlsruhe) and BESSY II (Berlin), and the proposed Spanish Light Source of Barcelona (LLS). These developments appear absolutely essential for satisfying the future needs of the biological community in Europe and for ensuring a geographical distribution of synchrotron facilities. The Panel wants to emphasise that beam-lines for crystallography at newly planned synchrotrons should be equipped with undulator sources, because of their inherently superior optical properties. To reach photon energies around 12 keV, as needed for crystallography, the storage rings have to be operated at electron energies above 2.5 GeV.

At present, it is very difficult and probably impossible to estimate the precise number of new beam-lines needed during the next five years. Based on the rapid increase foreseen in the need for SR in the Life Sciences, we fully endorse the building and upgrading of existing and planned beam-lines and recommend a close monitoring (see above) of the development of demand and supply so that corrective steps can be taken in time.

(b) The demand for high optical quality beam-lines, including those with micro-focusing facility (with a focal spot size of 10-20 μm) is bound to increase, because it alleviates the requirements on crystal size and quality. There is need for technical development in this field.

(c) Investments in the development of area detectors (e.g. solid state) and in data acquisition and handling are needed. Since this is a requirement for all diffraction experiments, protein crystallography will benefit from developments in other areas of SR research. The panel urges the Life Sciences community to take an active part in these developments.

(d) The needs in other areas using SR (e.g. fibre diffraction, small angle scattering, time resolved studies, spectroscopy, microscopy, medical applications) are in principle comparable with those of X-ray crystallography. Such experiments can only be done using SR and are yielding results of high scientific value. The relative priorities of the various methods are likely to vary over time. It must be ensured that there is an adequate research base with appropriate beam-lines and detectors to allow these applications to expand and to allow novel techniques to emerge.

European dimension

At present, EMBL has two outstations, one at DESY, where 7 beam-lines have been built and are now operated and maintained by the outstation, and one at Grenoble next to ESRF. In view of the growing demand for SR for biology, there is an acute need to upgrade the facility at DESY. The Review Panel is furthermore persuaded by the evidence of the users that there is insufficient support for full biological use of the beam-lines at ESRF. EMBL and ESRF are urged to find the means to put this right, perhaps by setting up a joint working group.

In view of the very strategic role of SR in the Life Sciences and its applications in Europe over the next 20 to 30 years, there may (in addition to the proposed committee of providers and users; see above under Immediate actions) also be a need for a European organisation to oversee the management of European facilities for biological applications of SR. Such an organisation would take responsibility for operating, maintaining and developing existing and planned beam-lines at national SR facilities in specific areas of structural biology. The organisation would support beam-lines dedicated to individual applications rather than be divided between different areas of science. This would also improve the access to synchrotrons for laboratories whose home countries do not run national facilities and it would avoid unnecessary redundancy across Europe.

If these three sets of recommendations are adopted, the Review Panel is of the opinion that the European needs for biological and biomedical use of SR should be met.

Acknowledgements

This review is the result of outstanding co-operation between experts drawn from a wide variety of fields.

I particularly wish to thank the five members of the Review Panel for the excellent work they have put into this report. Their broad knowledge has been most impressive.

I am also extremely grateful to all the members of the Reference Group for their invaluable and extensive work with assembling and processing the substantial background material for the review. In particular I would like to thank the chairman of the Reference Group, Kenneth C. Holmes and its vice-chairman, Keith S. Wilson for their unflagging dedication.

My special thanks also go to all those others that have contributed to the report and given comments on the many draft versions.

Finally I am indebted to Annette Moth-Wiklund and Philippa Rowe of the European Science Foundation (ESF) for their substantial work with the production of the report.

Gunnar Öquist

Chairman of the Review Panel

Stockholm, September 1998

Part 2

Reference Group

Part 2: Reference Group

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Report of the Reference Group

Interpreting the Remit of the report requested from the ESF, the Reference Group understood that the MRC and other sponsors of life sciences research saw current and anticipated synchrotron usage as dominated by macromolecular crystallography, but that other techniques including spectroscopy, non-crystalline diffraction, microscopy and angiography should also be supported.

The Reference Group understood that its remit covered the following issues:

- The short-term needs in terms of optimal use of present facilities, i.e. the hardware, software and staffing levels of the presently available beam-lines on existing machines.

- The medium and longer-term needs of the community in terms of the need for additional beam-lines, the utilisation of planned new sources, the need for additional sources, and the potential impact of new technologies such as free electron lasers.

- Biology was understood to have provided a substantial proportion of the scientific case for building the ESRF and other recent SR sources. Because the needs of the structural community are increasing fast, there is a need to ensure that an appropriate proportion of facilities is indeed made available for the foreseen expansion in biological applications.

The Group was to seek information from the community and assemble a science-based report to be passed to the Review Panel.

Summary Recommendations

General

ESF should express strong support for the use of SR facilities in Europe for structural biology. The needs of the biological community (which will amount to many beam-lines) must be taken into account in planning future use of SR.

- There is a considerable over-subscription of current resources. More undulator beam-lines for the life sciences are needed. Users only have access for the minimum time for experiments and would benefit from longer times.

- To alleviate the over-subscription, the installation of commercially available CCD and other state-of-the-art detectors on current beam-lines should be encouraged with the greatest urgency.

- Replacement of SR sources which are obsolescent (LURE, SRS, etc.) by machines which are complementary to ESRF with energy-range 2.5-3.5 GeV to enable the use of X-ray undulators should be supported. New sources should be positioned in Europe so as to ensure user proximity.

- We recommend the proper support (hardware, software and staff) of existing beam-lines with a life expectancy of greater than 5 years.

- The SR machines themselves must continue to be centrally funded, as at present.

- Attention should be given to mechanisms for funding beam-lines, which could follow one of a number of models. The cost of beam-lines (1-2 MECU) is out of reach of biologists and biological granting agencies but might, for example, be carried out by pan-European CRG-like consortia. The funding agencies should be encouraged to look favourably on such applications.

- The sharing of European facilities could be improved by setting up a European network of biological synchrotron users. A pan-European organisation is called for to extend or replace the role of EMBL which presently does not control adequate funds for the task.

- Beam-lines should be dedicated to individual applications, not generically shared lines as on the bending magnets at ESRF. Again some form of pan-European consortium (a) should orchestrate this.

- The potential of novel sources, in particular free electron lasers such as that planned at DESY,

must be considered. Structural Biology has always been at the forefront of exploitation of synchrotron X-ray sources and will certainly find new applications for these revolutionary sources.

Macromolecular Crystallography: present and future

The panel believed that the interests of the life sciences community in synchrotron radiation (SR) centred on protein crystallography (PX), with a rough division of needs for resources as 75% crystallography, 10% non-crystalline diffraction, 10% spectroscopy, 5% others such as microscopy and angiography. The importance of the latter were seen potentially to increase as they reached maturity. This summary therefore concentrates on PX.

- X-ray crystallography has become a standard technique in cell biology and molecular biology and protein crystallographers now use SR on a routine basis. More and more biochemists and molecular biologists enter the field of protein crystallography.

- The explosive impact of the genome projects will only increase these growth problems. Macromolecular crystallography will be a key contributor in Biomedicine and Biotechnology, both commercial and academic, well into the next millennium. The growth of macromolecular crystallography will be limited only by funding for some decades.

- Biochemical experiments need *rapid access* to provide the requisite feedback for optimal experimental design.

- For micro-crystal diffraction and for optimal MAD phasing one needs the brilliance and collimation available from undulators. These methods are expected to gain in importance. Therefore the need for undulator X-ray beam-lines is paramount.

- In the immediate future a great deal of new capacity can be generated by investment in new beam-lines at existing facilities and the upgrading of existing beam-lines.

- Particular attention should be given to detectors and detector development, which present a very serious bottleneck to data collection. There is need for a new generation of detectors with high spatial resolution such as silicon pixel detectors and appropriate funds should be made available. An urgent need is for the installation of high performance commercial CCD detectors on the majority of PX beam-lines. This will increase the efficiency of 2nd generation lines by a factor of 5-10, and at ESRF even more. The cost per line is less than 0.5 MECU.

- SR sources are designed to run for 24h per day, seven days per week. It is most important that staffing levels at the beam-lines should be adequate to allow 24h usage in an optimum manner, to maximise the return on the investment in the machines.

The needs in non PX areas are in principle comparable. The relative priorities of the methods are likely to vary as time passes. It must be ensured that there is an adequate research base with appropriate beam-lines and detectors to allow these applications to expand and to allow novel techniques to emerge.

The Central Role of Crystallography

Complementary techniques

This paper concentrates on the synchrotron radiation needs of protein crystallography in Europe. We recognise that in addition to X-ray crystallography, NMR and cryo-electron microscopy play important complementary roles. However, only in exceptional cases can they approach the precision of X-ray crystallography: high precision is essential for an understanding of protein function in chemical terms.

Cryo-electron microscopy is particularly advantageous for two-dimensional crystals. Recently the technique has produced outstanding results such as the structures of tubulin and bacteriorhodopsin. For many years only two-dimensional crystals of bacteriorhodopsin could be obtained. Extensive and innovative research by the group at the MRC, Cambridge (led by R. Henderson) using cryo-electron microscopy finally led to a structure at 3.0 Å resolution. Subsequently the development of cubic lipid gel phase crystallisation techniques produced small three-dimensional crystals which could be measured by novel developments at the ESRF. This led to the significantly improved resolution of 2.4 Å. The complementarity of the two techniques is nicely illustrated since the electron microscope structure was used to provide the initial model for the calculation of the higher resolution X-ray structure.

NMR methods are relatively time-consuming and need very large quantities of protein. Currently, NMR structure determination is restricted to molecular weights of 30 kDa or less. Nevertheless, in cases where the production of three-dimensional crystals has proved elusive, NMR has made significant contributions (e.g. growth factors) and is rightly considered as a powerful complementary technique. However, the unique power of NMR is in the mapping of the dynamics of macromolecules rather than in the production of high resolution structures.

The central role of crystallography

Small molecule crystallography underpinned the structural chemistry of the 20th century. It is the only technique which reveals full 3-D information of all atoms in a molecule. Macromomolecular crystallography will carry out a similar role for Biochemistry, Molecular Biology, Pharmacology, Molecular Medecine and Biotechology in the 21st century. Synchrotron X-ray techniques are absolutely central to these developments.

X-ray crystallography requires single crystals, which can present problems. However, although the process of crystallisation of macromolecules is far from being understood, striking progress has been made over the past decade so that the majority of problems should become accessible to X-ray crystallography.

The three-dimensional structural information that results from an X-ray analysis is at a resolution (precision) inaccessible to other methods. Moreover, the molecular weights presently accessible range up to 0.5 m da. It is very unlikely that NMR, Cryo-electron Microscopy or any other technique will compete with macromolecular X-ray crystallography in the short or medium term for a large proportion of such systems.

Given the improvements in crystallisation techniques and the opportunities opened up by microfocus beam-lines for very small crystals, the minimisation of radiation damage by cryogenic methods, the increasing speeds of data collection, and MAD phase determination, synchrotron X-ray methods will be the work horse of structural biology for the foreseeable future.

Background: Synchrotron radiation in biology

Biology is in a state of flux. It is traditionally a descriptive science with few discernible laws, but more and more biological phenomena can be analysed at the molecular level by the laws of chemistry and physics. Nearly four centuries ago René Descartes foresaw that one day such an analysis would be possible:

...si on connaissait bien quelles sont toutes les parties de la semence de quelque espèce d'animal, on pourrait déduire de cela seul, par de raisons entièrement mathématiques et certaines, toute la figure et conformation de chacun de ses membres.

Two techniques are the pillars of our burgeoning understanding of biology at a molecular level, recombinant DNA technology and X-ray crystallography. Structural studies of macromolecules provide the essential molecular anatomy, which is the basis for an understanding of cell physiology. X-ray crystallography enables us to "see" the positions of thousands of atoms that form the three-dimensional structures of proteins, nucleic acids, and their complexes. Since the local arrangements of atoms in these molecules determine their biological function and specificity, knowledge of the structures allows us to understand how these systems work. Moreover, since we are far from a theoretical prediction of protein function from DNA sequence, the empirical methods of structure research will long be essential for bridging the break in the chain of causality from genotype to phenotype.

On account of its excellent collimation and brilliance, synchrotron radiation (SR) has become an *indispensable* X-ray source for protein crystallography. For many years SR X-ray sources were considered to be reserved for difficult problems. However, the accuracy of the data obtainable coupled with the unique ability to vary wavelength continuously means that the use of SR sources for protein structure determinations has become routine. In the last year, ca. 70% of all published structures were determined using data taken at a synchrotron source, and this tendency is still rising. Moreover, protein structure determination is no longer carried out solely by esoteric specialists but rather is increasingly carried out by cell biologists themselves. There will be a rapid growth in the need for protein structure determination. On this account one can reliably predict a considerable increase in the demand for synchrotron

beam-lines dedicated to biological research. The size of the increase will be determined by the future funding of biological science rather than by scientific need, which is open-ended.

The development of synchrotron sources for biological research not only benefits fundamental research. Francis Bacon, a near contemporary of Descartes, urgently advocated new ways by which men might establish a legitimate command over nature to the glory of God and the relief of man's estate. Bacon advocated the empirical method. It is noteworthy that the application of protein structure determination underpinned by synchrotron radiation has already yielded abundant reward in the Baconian manner. The development of effective drugs against Aids and the design of immunization strategies against influenza are but two examples. It is fair to say that cellular immunology would not have been understood without the atomic structures of some of the components. As a further example, we are close to understanding the molecular basis of muscle contraction. None of this would have been possible without synchrotron X-radiation. Genome projects are already producing a large number of DNA sequences of individual genes, many of interest per se, since they allow us to locate mutations leading to genetic diseases. The three-dimensional structures of the encoded proteins are the necessary requirement for a rational drug design. Thus through its ability to yield protein structures, synchrotron X-radiation constitutes a key to the development of the health and pharmaceutical industry in the USA and Europe, and is of ever increasing importance to the development of biotechnology for the food and agriculture sector.

With the tremendous progress in genetic engineering, proteins and nucleic acids from many different sources are more and more often expressed in sufficient quantities for biophysical and crystallographic studies. There are recent initiatives in different countries to express systematically all the genes to be sequenced in the human genome project (ca. 100,000) and of other genomes (for example from archaebacteria which have potential industrial applications because of their thermostability), to purify the expressed proteins, and to determine their structures by crystallographic or NMR-spectroscopic methods. This will again multiply the need for synchrotron X-radiation, especially if we consider that thousands of clones are already available all over Europe, ready to be expressed! The number of

structures to be determined is much higher than this in practice, as an enormous number of mutant and ligand complex structures will be required if we wish to understand and exploit the function of these proteins.

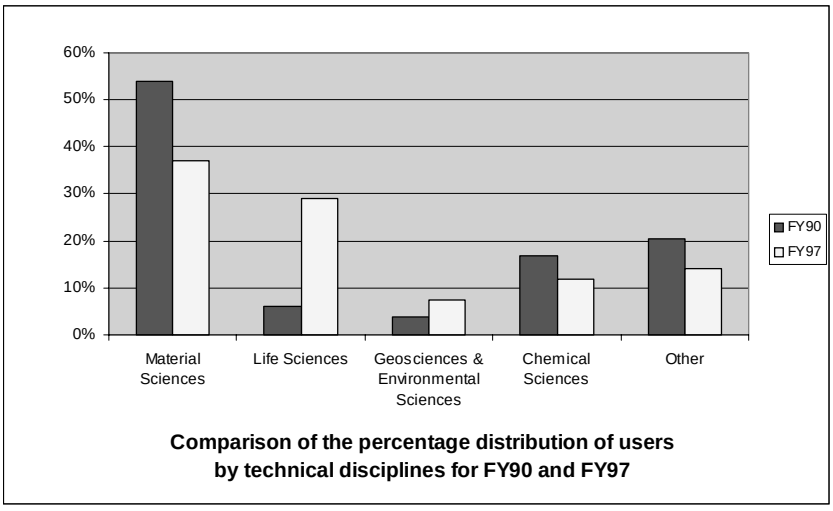
In addition to the availability of biological materials, methodologies in purification and crystallisation are rapidly advancing. This progress cannot be separated from the development of new crystallographic technologies, e.g. high-power synchrotrons fitted with undulators producing brilliant X-radiation. In combination with suitable area detectors such sources provide more accurate data much more quickly. Moreover, the superior optics allows the use of very small crystals. In addition, they allow the use of an optimised wavelength so that anomalous dispersion effects can be utilised in the phase determination that is an essential part of a structure determination.

Biological applications of synchrotron radiation in non-crystalline systems continue to gain in importance. The time-structure of synchrotron radiation is attractive for many kinds of spectroscopy. Moreover, the high brilliance combined with continuous wavelength range allows spectroscopic measurements of much higher quality than with any other source.

The growing importance of synchrotron radiation in biology is illustrated by the figure from the "Report of the Basic Energy Sciences Advisory Committee Panel *("Birgeneau Panel"*) on D.O.E. Synchrotron Radiation Sources and Sciences" in the USA (Fig. 3).

The relative number of Synchrotron radiation users from Geosciences are increasing, whereas those from Materials and Chemical Sciences are decreasing in number. In sharp contrast, the number of users from Life Sciences has been dramatically rising in recent years.

Figure 3, from the "Report of the Basic Energy Sciences Advisory Committee Panel *("Birgeneau Panel"*) on D.O.E. Synchrotron Radiation Sources and Sciences" in the USA.



Comparison of the percentage distribution of users by technical disciplines for FY90 and FY97

Macromolecular crystallography

More and more structures

The growth in the number of protein and nucleic acid structure determinations can be gauged by the growth in the yearly rate of data sets deposited in the protein data bank (PDB).

Since 1989 the rate of deposition has been increasing steadily by about 200 pa. The present total is more than 7,000. Since there is sometimes a 1-2 year lag between publication and appearance in the PDB, this number is an underestimate of the real number of structures solved. Of these, 80% were carried out by X-ray crystallography.

An analysis of "new" crystal structures published (i.e. not counting variations on known structures) shows a rising tendency (460 in 1996 compared with 394 in 1995). Of these, in 1996, 44% were determined with synchrotron radiation. Our user survey shows a marked rise in the rate of solving structures in Europe in the last years and moreover, shows that most are now solved with synchrotron radiation. Several new techniques have contributed to this growth.

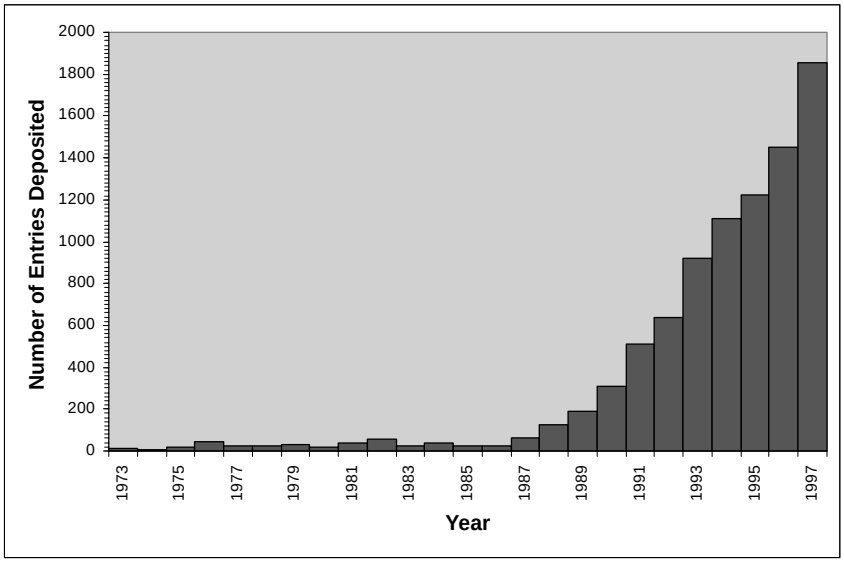


Figure 4: Depositions in the Protein Data Bank. All entries deposited in the Brookhaven Portein Data Bank are included. The vast majority of these are coordinate sets from crystallographic experiments. Source: Protein Data Bank web site (http: / / www.pdb.bnl.gov)

Table 1
Macromolecular Crystal Structures[1] 1990-1996

Year	1990	1991	1992	1993	1994	1995	1996
New crystal strcutures	109	127	165	204	352	394	460
New structures radiation with synchrotron	19	30	44	50	100	158	202
Percent	18%	24%	27%	25%	28%	40%	44%
Journals	**New structures with synchrotron radiation / Total new crystal structures**						
Structure	-	-	-	3/7	20/50	33/67	34/68
Nature	4/22	8/18	15/38	14/29	25/42	36/51	25/38
Nat Struct Biol	-	-	-	-	8/26	15/43	22/56
J Mol Biol	4/18	4/21	6/33	12/39	8/45	10/42	27/59
Biochemistry	4/15	1/7	1/8	0/18	2/34	11/40	11/46
Science	1/12	5/26	2/20	4/25	8/30	13/33	19/31
PNAS	1/6	1/14	3/14	2/25	6/27	8/26	8/25
Cell	0/2	0/1	1/4	3/9	5/12	10/25	12/23
Acta Cryst	2/5	5/8	2/4	2/10	2/17	4/18	6/22
EMBO J	0/5	2/7	4/5	2/13	9/22	9/18	17/25
J Biol Chem	1/6	0/9	1/13	1/10	2/15	4/13	5/20
Protein Sci	-	-	2/3	2/6	0/10	1/7	7/21
Other	2/18	4/16	7/23	5/13	5/22	4/10	9/26

[1] Source: Macromolecular structures, 1991-1997, eds., W.A. Hendrickson & K. Wüthrich, Current Biology Ltd., London. All published crystal structures of biological macromolecules are abstracted in Macromolecular Structures if they meet the criterion of crystallographic uniqueness, i.e., they are not isophormous with previously reported crystal structures. Approximately half of the abstracted structures were determined by molecular replacement. Not included are new ligand states, mutants, etc. that crystallize isomorphously with previously published structures.

Cryo-crystallography

The widespread adoption of cryo-techniques and the resulting minimisation of radiation damage have contributed greatly to the successful application of synchrotron radiation as a routine method. Data quality is considerably improved by measuring all the data from one crystal. Moreover, the labour-intensive changing of crystals during data collection has been much reduced.

Harder Radiation

A major source of systematic error in data collection from

macromolecular crystals is absorption in the crystal. By using radiation at 1.0 Å and harder (>12.5 KeV) absorption can be reduced to a negligible level which leads to much better data. Comparable conventional sources of sufficient brilliance do not exist.

Micro-crystallography

The high brilliance of the third generation synchrotron sources permits data collection from very small crystals (10-50m). This development holds great promise for the study of integral membrane proteins and macromolecular complexes, systems of enormous biological significance but which are very challenging crystallographic problems. Moreover, even from more mundane proteins, micro-crystals are often obtained relatively quickly. Also crystals frequently persist in growing as plates where one dimension is limited to a few microns or as needles which are only extensive in one dimension. The ability to deal with such specimens is an important factor in the design of new beam-lines.

At the ESRF a micro-focus beam line permits good quality data to be obtained on previously unusable small crystals only a few tens of micrometers in size. The current experimental arrangement is based on a 30 mm focused undulator beam, but beams down to a few microns in size are feasible. A major problem concerns sample alignment and much effort has been invested in this area. In addition radiation damage makes the use of cryogenic techniques mandatory and fast data collection is essential through the use of CCD-based detectors. A recent highlight has involved Bacteriorhodopsin. This protein is found in the purple membrane of *Halobacterium halobium* and acts as a light driven proton pump across the cell membrane to convert light into chemical energy. Present structural knowledge is based on electron diffraction studies, but these are limited in resolution to about 3.0 Å. At ESRF beamline ID13, diffraction patterns of three-dimensional Bacteriorhodopsin crystals of about 30 mm were, for the first time, measured to high resolution giving data from which a 2.5 Å structure has been determined.

Phase determination - the use of MAD

Synchrotron radiation, which is inherently polychromatic and therefore tuneable through the use of monochromators, has enabled the development of multi-wavelength anomalous diffraction (MAD) for phase determination in macromolecular

crystallography. MAD has the advantage of accurate and rapid structure determination from the diffraction measured from one single crystal, and cryo-crystallography typically makes this possible. Any of the conventional heavy atoms used in isomorphous replacement e.g. mercury, uranium, and platinum can also be used for MAD but so can a number of lighter elements. The greatest impact has come from selenium, which can be systematically incorporated into proteins as selenomethionine which will replace methionine in biological protein synthesis. Increasingly, brominated nucleic acids are also being used in MAD structure determination. With appropriate X-ray optics, a beam line suitable for MAD experiments is also optimal for single wavelength data collection (such as from ligand complexes and mutant variations). There is in fact a powerful argument for more general use of experimental phases from MAD, since the model-bias from molecular replacement can rather easily lead to wrong structures of which there are certainly already representatives in the literature.

We estimate that the percentage of experiments requiring MAD tunability is unlikely to exceed 30% of the total. Many SR experiments will continue to be: (1) extension of the resolution compared to the home source for optimum structure refinement and accuracy; (2) collection of data on series of protein-ligand complexes and mutants. Many of the latter will be with sets of isomorphous crystals; (3) molecular replacement.

Atomic resolution structure determination

Using harder radiation, in favourable cases (well ordered protein crystals) data can be collected out beyond 1.0 Å resolution. The intrinsic interest in such high resolution stems from a wish to understand chemical mechanisms. However, there is also a lively technical interest in high resolution data, as they permit to determine the missing phases by direct methods as is now routine for organic molecules.

Computing technology

Macro-molecular research makes large demands on computing. These include the storage and processing of large data sets generated by modern data collection devices. The development of very powerful networking and modern work-stations has matched the demands of synchrotron technology. There have also been important developments in crystallographic algorithms, which have greatly increased the power of phasing and refinement methods.

Beam-lines

The attractive features of SR for protein crystal data collection are its excellent optical properties (low cross-fire, small cross section) and brilliance. Data are taken with monochromatic X-rays (for an application of the Laue method, see below) usually with an energy around 12 KeV. Bending magnets, wigglers and undulators have all been pressed into service. An often-used optical system is a double crystal monochromator (silicon) followed by focusing mirrors. However, since protein crystals tend to be small and can accept only 1-2 mrad cross-fire there is increasing interest in undulator beams where the beam's optical properties match the crystal properties in an optimal way. Undulators are very advantageous for all the new techniques listed above. One undulator beam line can be used for MAD, micro crystal diffraction, or normal data collection. There is still a lot of useful work that can be done with existing bending magnets, but new designs of undulators are preferable.

A number of present and planned lines, especially those on undulators, provide for MAD experiments. There remains a case for the non-tunable single monochromator lines presently in use, and for the Quadriga-style ESRF lines, provided they are optimised for a wavelength near 0.9 Å.

Time-resolved crystallography

The problem

Biological macromolecules may undergo distinct conformational changes during their function. This is especially true of enzymes where binding of substrate can induce substantial changes in conformation and where the conformation of enzyme and bound substrate may evolve through several different intermediates and transition states in the course of reaction. A full understanding of the functioning of macromolecules must therefore include a description of the structural changes involved in their function. Time-resolved crystallography aims to provide a structural basis for such a description.

Strategies for time-resolved crystallography depend on the lifetime of the intermediates whose structure is to be determined. The fastest chemical reactions involving the breakage of covalent bonds happen on the femtosecond to picosecond time scale. Provided that a trigger is available that starts the reaction synchronously for all molecules in the crystal, and that a sufficiently fast data collection method is available (picosecond time scale), a

full movie of the conformational transitions could be obtained. However, many reactions are much slower, or can be slowed down sufficiently to allow data collection over a period of several days.

Three approaches are currently being used in “time-resolved” crystallography.

(1) “Physical trapping” stabilises intermediates by cryogenic techniques, and conventional crystallographic techniques and instruments (even in-house rotating anode generators) can be used to visualise the frozen intermediates.

(2) The use of “chemical trapping” in which the lifetime of intermediates is prolonged by chemical manipulation. Either the substrate is modified, or the enzyme is slowed down by appropriate site-specific mutations, or changes in e.g. pH.

(3) The most challenging form of time-resolved crystallography is provided by “no trapping”, in which the chemical and physical manipulation of intermediates and associated artefacts are avoided, but the necessity for ultra rapid crystallographic measurement is introduced. This approach requires the polychromatic Laue diffraction method, or ultra-fast monochromatic diffraction. In these studies synchronisation of the catalytic course of all enzymes in the crystal is required. This is most easily done by a (laser-) light trigger, and this approach has been restricted to proteins or substrates that carry a chromophore. An alternative that has been explored is the use of caged substrate-like compounds, such as caged-GTP in ras-P21.

The ultimate goal of the no trapping approach is to combine X-ray structural and fast spectroscopic studies, and to define (structurally) the kinetic intermediates in the reaction. The X-ray pulse duration emitted by a single particle bunch circulating in the ESRF is about 150 ps, and repeats every few µs. A 150 ps exposure from an undulator is sufficient for reasonable diffraction from a well ordered crystal.

Synchrotron needs for time resolved crystallography

The collection of time-resolved data for 3-D macromolecular crystallography is particularly challenging on account of the very large numbers of independent diffraction data to be recorded in a short time. This makes data collection even more challenging than for non-crystalline systems such as muscle.

Applications of type (1) and (2) need no special SR or other biophysical instrumentation. Their execution is essentially identical to conventional SR data collection on macromolecules, and their needs can be considered together with macromolecular crystallography in general.

Experiments of type (3) are presently limited in number. This is largely a problem of the associated biophysical chemistry and kinetics. Triggering mechanisms have to be identified other than just photactivation of chromophores. The approach only works when a substantial build-up of key intermediates occurs at defined time intervals. This is where Bragg and Boltzmann are in conflict; i.e. not all molecules in the lattice continue to have the same conformation at the same time. The problems of deconvoluting overlapping sets of conformations is truly awesome.

This problem is *not* related to the capability of the beam-lines now available on third generation sources such as the ESRF or APS, but rather to the chemistry. More instrumentation in terms of spectrophotometers and other triggering mechanisms will be required, but not new synchrotrons or beam-lines. The number can be expected to increase as the chemistry and kinetics evolves, albeit slowly. The existence of a beam line(s) with sufficient capability for such experiments at the ESRF should be sufficient to handle these experiments at present. Other synchrotrons should probably continue to concentrate on non-time resolved work.

Recommended actions

The different European X-ray sources are presently heavily oversubscribed. The problem could be alleviated by simplification of the application procedures. In addition the introduction of efficient commercial CCD detectors on existing beam-lines would improve their effectiveness by roughly an order of magnitude.

Access

The integration of synchrotron radiation into biochemistry and cell biology has a strong impact on the way synchrotron beam time should be allocated. For a working biochemist the need for rapid feedback is paramount. A 2 week block-booking in 8 months time is neither useful nor efficient.

Beam time is usually only allocated after applications have been approved by a committee. The procedures are different at different European SR facilities. Waiting times can be as long as 4 to 8 months, and measuring time is allocated not only depending on

the scientific quality of the application but also on the availability of time on beam-lines. It frequently happens that crystals/samples are no longer available at the time of beam allocation on account of difficulties with protein preparation or crystal stability. What is needed is faster, less bureaucratic and easier access to synchrotron X-radiation.

Support

Enough technical/engineering support should be provided to ensure 24h usage of the beam-lines. Most facilities seem to have insufficient staff to exploit the initial large investment.

Detectors

This is a consistently under-funded area where the potential improvements could be dramatic. The lack of detectors with high speed read out, high quantum efficiency, and high spatial resolution is limiting in practically all fields of SR applications and in X-ray diffraction in particular.

Even though the time required to get good statistics for one exposure is now measured in seconds or (ESRF undulator) milliseconds, imaging plates take minutes to read out so that data collection (which may consist of 100-200 exposures) requires the order of hours. CCD's can bring the read out time down to seconds but large CCD's are expensive. One modern CCD detector on every major beam line in Europe would improve data output by a factor of at least 10 if the organisational and administrative hurdles could be overcome.

Non-Crystalline Diffraction

Fibre diffraction

Fibre diffraction played an important role in the development of molecular biology since it yielded the structure of DNA. Moreover, recording time-resolved fibre diffraction from living muscles during contraction was the problem which drove the initial development of synchrotron X-ray sources and which still has an active following.

The extreme collimation of the synchrotron beam is very advantageous for fibre diffraction, which is subject to much higher intrinsic background scattering than crystal diffraction. For time-resolved studies sufficient X-ray intensity can only be provided by synchrotron sources. However, because the natural occurrence of biological fibres is limited, and since most of them have been worked on it is unlikely that fibre diffraction will be a major growth area.

Low angle scattering

For well-defined systems low angle X-ray scattering can yield structural information about (for example) the status of an allosteric enzyme in solution, information, which would be difficult to obtain by any other method. Moreover, with synchrotron radiation sources, it can be used to monitor enzyme kinetics. Low angle scattering can also be very useful for following processes of protein aggregation.

Time resolved studies

Over the years time-resolved fibre diffraction or low angle scattering studies in biology have been largely restricted in Europe to EMBL Hamburg and to the SRS. At both EMBL and SRS the needs of muscle diffraction drove beam line and detector development. Areas covered have included self-assembly of complex systems such as tubulin and collagen, the chemistry and physiology of muscle contraction and more recently organisational changes in phospholipid membranes. In all of these, synchrotron radiation has played an essential role in allowing time-resolved measurements.

Recommended actions

The only dedicated synchrotron radiation lines for these methods in biology are at EMBL Hamburg, with shared lines at Grenoble, LURE and SRS. One of the Hamburg EMBL lines is soon to be converted to protein crystallography very much against the wishes of its (international) user community. While recognising that the protein crystallography community is much larger (and growing), the

needs of the non-crystalline community must be surveyed and their needs in the future adequately addressed. Their need is for a brilliant well collimated beam, and for fast electronic area detectors with high spatial resolution. These criteria will probably be met by most of the undulator beam-lines being used for crystalline diffraction. The special requirements for low angle fibre diffraction are a long specimen-detector distance and space for the specimen cells and any associated equipment. The experiments tend to be of non-standard, complicated design and need more beam time per experiment than macromolecular crystallography.

Spectroscopy and microscopy

Present

X-Ray Absorption Spectroscopy, XAS

Extended X-ray Absorption Fine Structure (EXAFS) and X-ray Absorption Near Edge Structure (XANES) measurements can be used to probe the local environments of metal sites in biological macromolecules both in solution (often at very low concentrations of the metal, *i.e.* less than 0.5 mM) and the solid state. It is also possible to study changes in the local environment of the metal as the macromolecule undergoes chemical changes such as during a catalytic cycle. A combination of crystallographic and X-ray absorption measurements in a complementary fashion can be very useful in structural biology. In cases where crystallisation has proved problematic or only very small amounts of the protein are available, then spectroscopic methods can provide powerful tools for structural studies.

Time-resolved fluorescence studies in solution.

This technique is routinely used in several European centres, such as SRS, MAX, DESY, and most frequently at LURE, using the UV storage ring Super-ACO.

The most interesting use of this technique is in the study of dynamic properties of biological macromolecules, where the observed time-scales are complementary to those attained by NMR. Using the intrinsic protein fluorescence it is possible to study conformational changes of proteins and their interactions with other macromolecules. By using other site-targeted extrinsic fluorescent probes, similar physico-chemical information can be obtained on isolated, as well as integral biological systems, under near-physiological conditions. Compared with laser sources, synchrotrons present higher stability and, moreover, easy and reproducible tunability of wavelengths.

Recent developments include the coupling of the types of experiments mentioned above with a confocal microscope (SRS) to follow events in a cell.

UV spectroscopy

The SRS at Daresbury has developed SR circular dichroism spectroscopy for the study of proteins in solution. The secondary structures of proteins are best studied at wavelengths below 190 nm, corresponding to the absorption band of the peptide bond. This is not easy with standard spectrometers, even when all precautions are taken to reduce background, since the

signal-to-noise ratio is very unfavourable. The synchrotron source provides a much more intense and very "clean" source of light and can attain wavelengths down to 150nm, giving spectra of excellent quality. With sufficient intensity, furthermore, it should be possible to study the kinetics of events in solution, such as protein denaturation or folding, as well as the kinetics of reactions involving proteins and nucleic acids.

IR spectroscopy

The application of IR radiation to the study of biological systems is a fairly recent advance that has been developed in particular at the SRS, Daresbury. At Brookhaven a microscope has been coupled to an IR spectrometer for the study of biological samples. First results of experiments carried out by scientists from LURE (France) on intact cells show the feasibility of mapping the chemical composition of the cell contents and its evolution in time. In this case the characteristic "signature" arises from chemical bonds typical of organic and macro-molecules.

X-ray microscopy

This technique has been developed mainly at soft X-ray sources, such as BESSY, NSLS (Brookhaven), ALADIN (Wisconsin) and ISA (ASTRID). Experiments carried out so far show the capacity of this method to provide images of entire cells, both in aqueous environment and embedded in ice. The resolution is better than 1m and, with the development of phase contrast, the images are of very good quality.

Phase contrast imaging using interferometers for hard X-rays has become a powerful technique for studying samples of medical relevance such as rat cerebrum.

New developments in soft X-ray microscopy are presently taking place at the ESRF, using high energy X-rays. Aims include the exploitation of the possibility of tuning the wavelength accurately to the absorption edge of several interesting elements, thus permitting a "chemical analysis" of the samples studied.

The existing technology for soft X-rays achieves a resolution of 30nm. The development of the optical elements (zone plates) currently taking place is quite spectacular and the improvement of the resolution of the images should make 20nm realistic. The technology is spurred by the development of microlithography techniques, which use synchrotron radiation itself to form zone plates. In fact, synchrotron radiation has now become the main technology for the manufacture of the finest zone plates and other optical elements. In the field of imaging

using high-energy X-ray sources, the development of the focusing Bragg-Fresnel optical elements is a speciality of Russian scientists. Several of them have recently joined European synchrotron facilities, in particular ELETTRA and the ESRF, to take part in the development of imaging instrumentation.

The future

Spectroscopy

The synchrotron radiation beam in the VUV/UV/vis/IR may be readily focused to the size of a few microns, which is comparable to the dimensions of cells. The wavelength domain corresponds to the energy range in which physicochemical analyses may be performed, since it is the range in which electronic and vibrational molecular transitions are found. Furthermore, at wavelengths longer than 325 nm, damage to biological samples tends to be small, allowing experiments on living material. The excellent long-term stability of synchrotron sources, the perfect pulse reproducibility, the picosecond pulse duration, and the typically 5 to 10 MHz repetition frequency make synchrotron sources very attractive for time-resolved studies.

A further area of photobiology that exploits the range and ease of tunability of synchrotron radiation in the VUV/SXR is the study of specific radiation damage. Damage by photons is qualitatively different from damage by electrons: the latter cause a cascade of reactions that are difficult to control, whereas with photons, if the energy of photons corresponds to the absorption band of the chromophore studied, very little damage is caused to the sample. It is thus possible to design pump-probe experiments that address a precise chemical species within the sample. This opens new lines in photobiology, allowing for the first time insight into the primary mechanisms of radiation damage.

The combination of optical microscopes with synchrotron sources and the above-mentioned techniques would give very powerful installations, since different techniques could be brought to bear simultaneously upon the same biological sample. The goal would be to obtain time-resolved spectro-imaging of subcellular organelles.

Microscopy

Similar advances can be envisaged in the field of X-ray microscopy. The advantage of X-ray microscopy over electron microscopy is that it tolerates much thicker samples than the electron microscope, thus making the imaging of a whole cell practicable. It is not necessary to work under vacuum, nor on

dehydrated samples, allowing studies under near-physiological conditions. In the regions of intermediate resolution, corresponding to large molecular assemblies and subcellular compartments, X-ray microscopy should be able to give extremely useful information. The rapid development of optical systems will bring the spatial resolution to a value which allows detailed investigation of organelles and sub-cellular compartments. Accurate tunability of the X-ray beam is another feature of synchrotron radiation that will be developed allowing the mapping of a given element, such as calcium, within a cell by selective X-ray absorption. With very intense beams it should, furthermore, be possible to observe these phenomena evolving in time e.g. the spread of calcium within a cell after stimulus.

Recommended actions

The more recent applications of spectroscopy and microscopy have not yet had time to gather a regular clientele of biological users. At present, therefore, it is rather difficult to estimate how fast the demand might grow. Fluorescence depolarisation and UV dichroism would seem to be good candidates. Both methods are most powerful in time-resolved applications. The time structure of the beam (i.e. single bunch mode) is then of utmost importance. Single bunch mode operation is in fact unpopular at most installations. Thus it is necessary to find solutions that allow both time-resolved and high-flux applications fair access to the resources.

X-ray microscopy can best provide its full potential at a low-emittance source. However, present applications are limited and it would seem that supply and demand may be adequately matched. Nevertheless, we should be prepared for surprises if one of these techniques provides a breakthrough in some aspect of cell biology.

Medical research with synchrotron radiation

Since the early days of X-ray synchrotron radiation research medical applications have been persued intensively. At the ESRF the medical beam-line has been constructed to perform three different types of experiment, *computed tomography*, *coronary angiography*, and *micro-beam radiation therapy (MRT)*, and there is a close collaboration with the Centre Hospitalier Universitaire de Grenoble. The beam-line will be used essentially as a medical research tool. The patient positioning system, (the medical chair), has been constructed so that in addition to the normal angiography scans, it can also perform spiral scans, a technique where the X-ray source and the detectors appear to move in a helical path as seen in the reference frame of the patient; this motion can be very useful for computed tomography studies. The first micro-beam radiation therapy experiments have confirmed the very high absorbed dose levels for tissue as well as cellular necrosis that form the basis of the MRT concept.

More recently the use of diffraction and phase contrast has opened new opportunities to image soft tissue, e.g. in mammography. To date only coronary angiography has reached the state where patients are successfully investigated in a routine manner.

Minimal invasive coronary angiography

Selective coronary angiography is the established routine imaging technique for patients with coronary artery diseases. A catheter is introduced into the ostium of the coronary artery of interest via the arterial system. In 1995 in Germany 409,159 patients were subject to such surgery, 30% of those were follow-up investigations. On account of the arterial catheterization there is a certain risk inherent in this method. The morbidity is about 1.5% (0.5% severe complications) and mortality about 0.1%. Therefore, physicians are particularly interested in a non-invasive or a minimally invasive technique.

This can be achieved by application of the contrast agent via the veins rather than the arteries. This leads to imaging problems from the dilution of the contrast agent by a factor of 40 or 50, the superposition of the coronary arteries on large iodine-filled structures, and the fast motion of the heart. To overcome dilution a subtraction method using two wavelengths that straddle the absorption edge of iodine can be used to enhance the contrast. Because of the fast

motion of the heart the two images must be taken simultaneously. This can be done by using monochromatic X-rays at different energies for the two images. One energy is below to K-edge of iodine at 33.17 keV, and one above, where the absorption coefficient is 6-fold higher for an energy separation of 300 eV. After subtraction of the images the sensitivity to iodine is 10,000 higher than for soft tissue.

This technique, named dichromography, has been realised at HASYLAB at DESY in Hamburg, where a line-scan system is installed at a wiggler beam line of the storage ring DORIS. The experience gained by investigating a large number of patients (300 to date) and the cost-estimate for a special storage ring for medical applications will provide the basis for a final cost evaluation. If the price per patient is acceptable, DESY plans to build a suitable storage ring and, together with the University Hospital, set up a regional centre for minimum invasive coronary angiography in Hamburg.

Future

The needs in this area are not easy to define as it is relatively new. However the potential for medical research, biopsy of tissue samples for both diagnosis and eventually treatment of patients is high. One can only assume that increasing numbers of dedicated beam-lines, possibly dedicated storage rings and associated facilities will be required in the coming years.

Managing Beam-Lines

User needs

The average user increasingly needs and expects quick and non-bureaucratic access to beam-lines. The industrial community will put special emphasis on this aspect (see below). Moreover, for the normal protein crystallographer, the biological problems require rapid feedback rather than a wait of several months as often happens at present. The ranking of a priorities committee might be related to the larger research field of a qualified group and not so much to individual projects. This allows the scientist at the facility to optimise beam time allocation according to the availability of samples and equipment. Encouragement and beam time should be given to newcomers. Moreover, the training aspect could be taken into account in evaluating the performance of groups.

In addition, the management of the ESRF is developing a policy for long term proposals, which has proved to be very successful at the SRS and HASYLAB. Groups (ranging from individuals to collaborations between institutes) with projects of outstanding merit will be awarded beam time over an extended period (up to 2 years). Within this guaranteed time they will be able to set their own scientific priorities, ensuring reasonably fast access for key projects and changes in scientific priorities during the allocation period. Peer review will be used to assess the quality of the science being produced. It is hoped that this will increase the commitment of the long-term project groups to the facility (as opposed to the transient interest of most users) and may result in Ph.D students and post-docs being assigned to the ESRF for extended periods. A similar policy has been carried out successfully by the SRS for a number of years. (Similar flexible policies should be encouraged at other sources.)

Beam-line operation

The facility or the scientist running the beam-line has to ensure that the optics are optimal for the respective source, i.e. for bending magnet, wiggler or undulator. State of the art instrumentation including cryo-cooling, alignment facilities, and modern detectors have to be made available. Availability of good software is essential.

The SR facility should make sure that all beam-lines are equipped with adequate computer control and that they are easy to operate. Ideally, the user interface should be as standard as possible, not just where beam-lines of similar type are concerned, but for all beam-lines susceptible to interest the biology community. Furthermore,

this interface should be as similar as possible between different SR facilities. The facilities must provide sufficient computer power to allow users immediate and rapid data processing, so they can manage their experiment as efficiently as possible.

Beam-line scientists

Beam line scientists need reserved access with sufficient beam time in order to develop new methods. This need varies between methods, but it is especially important in those that are entirely dependent on synchrotron radiation (such as XAS, use of variable wavelengths etc.)

The scientist in charge of a beam-line should have the opportunity to pursue his/her own research. In many cases this will be done in collaboration with external groups. In order to be efficient the beam-line scientist should if possible work closely together with PhD students, i.e. good relations between the facility and universities is very important. Moreover the relationship with visiting scientists deserves careful evaluation. The role of the beam-line scientist as a collaborator in subsequent publications needs to be clearly and fairly defined at the conception of projects. The degree of collaboration varies strongly with the type of application, and for example, is generally rather limited in routine crystallographic studies.

General management of beam-lines

At the various synchrotron radiation facilities across Europe, beam-lines are operated in different ways. They are run by the facility, or by a Collaborating Research Group (CRG) or by a mixture of both. At Daresbury, LURE and the ESRF, the beam-lines are mainly operated by the facility although exceptions are the EPSRC sponsored CRG in surface science at Daresbury and the various national and bi-national CRG's at the ESRF. At DESY the beam-lines for structural biology are operated either by the EMBL Outstation or the Max-Planck/GBF collaboration together with the HASYLAB staff. DESY provides the SR beam free, but the instruments and the beam-lines themselves are operated by the external user groups; the same applies to the CRG's at the ESRF. The medical beam line for coronary angiography at Hamburg is operated by HASYLAB in conjunction with the University Hospital in Hamburg-Eppendorf. At the ESRF the medical research beam line is operated by the facility in conjunction with the Centre Hospitalier Universitaire de Grenoble (CHU).

In the USA at SSRL in Stanford beam-lines are mainly operated by the facility, whereas at the NSLS in Brookhaven most of the beam-lines are operated by a mixture of Participating Research Teams (PRT's) and Collaborative Access Teams (CAT's) and the central facility. (CAT's are broadly synonymous with CRG's). A report recently submitted to the Department of Energy in the USA indicates that the CAT/PRT system employed at the APS in Argonne and the users generally regard the NSLS at Brookhaven as a good model for the funding situation in the USA. In this system the X-ray photons are provided by the facility, but the beam-lines are funded as a result of peer review. There is a general consensus that at least 25% of the available beam time should be dedicated to the general users who are not involved in setting up or operating the beam. The system has the major attraction that strong links and commitments are encouraged between the facility and the sections of the user community which run the CAT's. The system is generally regarded by users as providing innovative beam-lines with strong science programmes. However there are generic concerns regarding long-term maintenance and technical support for the beam-lines, inconsistent and incompatible software and equipment between beam-lines, and duplication of effort (and mistakes). Problems can also arise if the external user groups responsible for the CATs do not find sufficient funds to modernise a beam line or to carry out repairs.

Within Europe the EMBL Outstation in Hamburg is an outstanding example of a successful CRG in structural biology, providing technical and scientific expertise and strong user support. A major contribution to this success is consistent funding and staffing levels from a pan-European organisation and dedication of resources to a specific discipline. Other perhaps less ideal examples are some of the CRG's at the ESRF where the pressure of obtaining national funding has caused the beam-lines to be multi-purpose in nature, lacking optimisation in any one particular discipline. There are firm plans at both HASYLAB and the ESRF to move towards a system of operation in which user groups are given the opportunity to be significantly involved in beam line operation. This may range from providing, maintaining and operating specialised equipment on a particular beam line, to full responsibility for individual or groups of beam-lines according to the EMBL Hamburg Outstation model. This compromise between

the complete CAT model at the APS and the complete facility models has the potential to minimise the problems indicated above, whilst encouraging participation and support from user groups practising the highest quality of science. We regard this increased external support as essential for the efficient operation and scientific development of the European facilities and an economic way of alleviating in part the problems of staffing levels arising from the intensely operator-dependent nature of synchrotron use.

An essential difference between the situation in the USA and Europe is the lack of National boundaries in the former. This means that CRG's can be based on the whole USA community and that beam-lines are dedicated to a particular application. Nationality-based funding in Europe leads to lines shared between many applications, which are slaves of all applications but masters of none. To circumvent this problem we envisage the development of further pan-European CRG's (EUROCATs?) at facilities such as ESRF and others, which could take the responsibility for operating, maintaining and developing existing beam-lines in specific areas of structural biology. These would work in close conjunction with the facility, so that the transfer of expertise and knowledge and use of facility infra-structure would be readily facilitated. Such pan-European CRG's would support lines dedicated to individual applications rather than be divided between different areas of science. A pan-European organisation appears to be called for to extend or replace the role of EMBL which presently does not control adequate funds for the task.

Novel Developments in SR Sources

Storage rings

The third generation synchrotron radiation facilities optimised for the use of undulators perform extremely well. The machines work very reliably. They have been able to increase the highest possible brilliance by about a factor of 250 in the last three years. By increasing the brilliance in the 1 Å wavelength range, one also increases the brilliance available in the spectral range of very hard X-rays, i.e. at photon energies of 30 keV and above. This new development may have some impact for structural biology, e.g. in reducing radiation damage. By going to higher electron energies in the storage rings and/or by using minigap undulators, maybe even superconducting undulators, the available intensities in the very hard part of the radiation spectrum will certainly increase in the near future.

Minigap undulators, in superconducting technology, should enable the storage rings operating at electron energies around 2 GeV to reach the spectral range between 12 and 18 keV which is very important for macromolecular crystallography.

Nevertheless we are approaching the limits of storage-ring based synchrotron radiation sources, especially with respect to brilliance, time structure and degree of coherence of the beam. ESRF is only about two orders of magnitude away from the theoretical limit of a storage ring.

Free electron laser

SR sources driven by linear accelerators (LINAC), such as Free Electron Lasers (FEL) based on the principle of Self-Amplified Spontaneous Emission (SASE), open qualitatively new possibilities for synchrotron radiation research. They are therefore the most promising candidate for fourth generation SR sources. The average brilliance which can be achieved for wavelengths down to 1 Å is about six orders of magnitude higher than the best performance of the ESRF today. Moreover, these FELs provide pulses in the 100 fs time regime, which again opens qualitatively new fields of research. Comparing the peak brilliance of a storage-ring-based undulator source with that of a free electron laser, the improvement is about 10 orders of magnitude (such peak intensities do raise problems since the electric field strengths would probably be sufficient to turn a biological sample into a plasma). In addition the beam is fully coherent and has a very high degree of polarisation either linear or circular depending on the undulator of the FEL.

At DESY an X-ray FEL is planned using a linear accelerator with superconducting technology, which provides bunch trains containing about 12,000 individual pulses of 100 fsec at an interval of 80 nsec with a 5 or 10 Hertz repetition rate. The spectral distribution of the spontaneous undulator radiation compares well with the performance of third generation storage ring based sources.

A LINAC-based FEL not only poses a challenge to the machine physicists, but is also a challenge for potential users. One needs to demonstrate the principle of a SASE FEL to find out to what extent these machines work reliably, and to learn how to do experiments at such a machine (especially how to solve the heat load problem on the optical elements and the samples). For this reason at DESY a VUV-FEL based on SASE is under construction, which has its fundamental between 6 and 2 nm and which should be in operation in the year 2002. The first step will be a proof-of-principle experiment in the summer of 1999 where the SASE principle should be demonstrated for the wavelength range 120 - 40 nm.

X-ray microscopy, is considered to profit strongly from the availability of such a VUV-FEL. Based on the experience with microscopes installed at BESSY and a pulsed plasma source one can show that it is possible to take a high resolution image of initially live, unfixed biological specimen with one pulse of the FEL. To image a 25 nm protein structure in ten µm water layer with a wavelength of 25 nm in phase contrast one needs a photon density in the object of about $2.0 \bullet 10^8$ photon/µm2 corresponding to a dose of $2.7 \bullet 10^6$ Gy. To get this photon density it is necessary to focus a beam with an energy of several µJ onto an area of about 10 x 10µm2. Because of the high power density in the irradiated part of the object this part will be destroyed but the exposure time of 400 fs is much smaller than the time scale of hydrodynamic motion which is in this case about 50 ps.

One very interesting possibility opened up by such intense pulsed sources is to use them to excite intense collimated Mössbauer scattering from an Fe-containing crystal. The nuclear scattering cross section for Fe radiation is equivalent to about 500 electrons-worth of Thompson scattering, which opens up the possibility of using iron as a super-heavy atom for phasing really large crystalline objects.

The Attitude of Industry

Preamble

Many pharmaceutical companies are very actively engaged in protein crystallography even to the level that they run their own beam-lines. Thus it is rumoured that the structure of the HIV protease which is necessary to activate the reverse transcriptase was solved more than 200 times. All major European pharmaceutical companies now have active PX groups. Synchrotron radiation is a vital resource for industrial PX, for the solution of novel and liganded structures. Indeed a consortium of US-based companies have collaborated to build a beam-line at APS. However, the situation in Europe is more fragmented, with industrial groups making mostly local arrangements with domestic synchrotrons. A more concerted approach in Europe would produce economies of scale but would require greater collaboration. Such a situation should change in the future. The intention of Hoffmann LaRoche and Novartis to become users of the crystallography beam-line at the Swiss light source has been important to raise the design energy to 2.4 GeV so as to produce an efficient source for macromolecular crystallography.

Glaxo-Wellcome

Glaxo-Wellcome is one of the major pharmaceutical companies using SR in Europe and its views are particularly valuable. The pace of research is increasing and is accompanied by increased throughput and turnaround time of protein projects. Typically there are plans for overall several-fold expansion on the 3-5 year time scale which includes a proportion of SR based-research. The availability of human and pathogenic genome sequences, which is a major element in this growth, will lead to a further focus on protein structures. Speed in characterising proteins of potential pharmaceutical interest is vital. SR has the hugely important advantage that it enables the pharmaceutical research programmes to be scheduled accurately by exploiting techniques such as seleno-methionine substitution and MAD phasing, high-resolution data, and the usage of small crystals. Moreover, an increasing proportion of research consists of surveying complexes of target proteins with a wide range of ligands. This exercise is routine, but needs to be carried out expeditiously. Facilities are required which allow the rapid collection of diffraction data sets with fast access.

Glaxo-Wellcome intends to increase their usage of SR for

Glaxo-Wellcome intends to increase their usage of SR for proprietary research, their estimate is by about a factor of four in the next three years. The factors determining actual usage are:

- The relative costs of the data sets at home and at the facility. Usage fees should be as flexible as possible; smaller time allocations more frequently would be advantageous.

- Convenience and availability (vital to the increasingly competitive pharmaceutical research programmes). Rapid access is essential to meet drug discovery time-scales.

- Ease of access, i.e. straightforward administrative arrangements.

An ideal scenario might involve shipping large numbers of frozen crystals for routine data collection of liganded structures, or for characterisation of novel crystals, as well as other spells of time when Glaxo-Wellcome employees would be present. This would cut travel costs and time, and lower activation barriers for use.

Proprietary versus collaborative research

Currently at the ESRF only some 1-2% of the beam time is used for proprietary research, *i.e.* when industry pays for beam time at commercial rates on the basis that any results will be not be released to the public domain. Far more encouraging, however, is the fact that the *non-proprietary* involvement of industry is substantially more than this and could well exceed 18%. Such involvement is difficult to quantify precisely, but involves direct and/or non-direct collaborations with academia and government sponsored research institutions.

Survey of Synchrotron Radiation Facilities in Europe

● Facilities in operation, used substantially by biologists

○ Facilities in operation, not used substantially by biologists

● Facilities under construction or planned

Facilities in operation, used substantially by biologists

1.	**ASTRID (SA)**	**Institute for Storage Ring Facilities** Ny Munkegade Bygning 520 DK-8000 Aarhus C
2.	**ELETTRA**	**Sincrotrone Trieste S.C.p.A.** Strada Statale 14 km. 163.5 Area Science Park IT-34012 Basovizza - Trieste
3.	**EMBL Outstation**	**European Molecular Biology Laboratory** c/o DESY Notkestrasse 85, Geb 25a DE-22603 Hamburg
4.	**ESRF**	**European Synchrotron Radiation Facility** B.P. 220 FR-38043 Grenoble Cedex
5.	**DORIS III**	**Hamburger Synchrotronstrahlungslabor (HASYLAB)** c/o DESY Notkestr. 85 DE-22603 Hamburg
6.	**LURE**	**Laboratoire pour l'utilisation du rayonnement électromagnétique** Batiment 209D Centre Universitaire Paris-Sud FR-91405 Orsday Cedex
7.	**MAX**	**Swedish National Electron Accelerator Laboratory** Box 118 SE-221 00 Lund
8.	**SRS**	**Synchrotron Radiation Source** CLRC - Daresbury Laboratory Daresbury UK-Warrington WA4 4AD

Facilities in operation, not used substantially by biologists

1.	**BESSY I**	**Berliner Electronenspeicherring Gesellschaft für Synchrotronstrahlung GmbH** Lentzeallee 100 DE-14195 Berlin
2.	**DELTA**	**Dortmund Electron Test Accelerator** Institute for Accelerator Physics and Synchrotron Radiation Universität Dortmund Emil-Figge Str. 74b DE-44221 Dortmund

3.	**ELSA**	**Electron Stretcher and Accelerator** Physikalisches Institute Universität Bonn Nussallee 12 DE-53115 Bonn
4.	**EUTERPE**	**Eindhoven University of Technology Cyclotron Laboratory** P.O. Box 513 5600 MB Eindhoven The Netherlands

Facilities under construction

1.	**ANKA**	**Angströmquelle Karlsruhe** Forschungszentrum Karlsruhe GmbH Postfach 3640 DE-76021 Karlsruhe
2.	**BESSY II**	**Berliner Electronenspeicherring Gesellschaft für Synchrotronstrahlung GmbH** Lentzeallee 100 DE-14195 Berlin
3.	**SLS**	**Swiss Light Source** Paul-Scherer Institute (PSI) CH-5232 Villingen

Planned facilities

1.	**LLS**	**Light Source of Barcelona** Laboratorio de Llum de Sincrotró de Barcelona - IFAE Edifici CN, Universidad Autonoma de Barcelona ES-08193 Bellaterra (Barcelona)
2.	**DIAMOND**	United Kingdom
3.	**SOLEIL**	France

Synchrotron Radiation Sources in Europe

Machine and Location	Energy GeV	Storage ring circumference m	Injected current mA	Lifetime hours	Hard X-ray range	Emittance (*) nm-rad
LURE DCI – Orsay (FR)	1.85	-	200	>50	Yes	15.0
ESRF – Grenoble (European)	6.0	844	200	>50	Yes	4.0
BESSY I – Berlin (DE)	0.8	62.4	500	5	No	50.0
DORIS III – Hamburg (DE)	4.45	289.2	120	-	Yes	404.0
SRS – Daresbury (UK)	2.0	96.0	250	>20	Yes	130.0
ELETTRA – Trieste (IT)	2.0	259.2	300	20	Yes	7.0
MAX II – Lund (SE)	1.5	90.0	200	>10	Yes	8.8
ASTRID – Aarhus (DK)	0.58	-	200	10	No	140
Under Construction						
SLS Villingen (CH)	2.1	288.0	400	-	Yes	3.0
BESSY II – Berlin (DE)	1.7	240	100	-	Yes	5.5
DELTA – Dortmund (DE)	1.3	115	200	-	Yes	5.2
ANKA – Karlsruhe (DE)	2.5	103	400	>17	Yes	40-80
Planned						
DIAMOND – ? (UK)	3.0	345	300	>20	Yes	14.0
SOLEIL - ? (FR)	2.5	336.0	500	30	Yes	3.0
LLS – Barcelona (ES)	2.5	251.8	250	-	Yes	8.0

(*) The lower the emittance, the smaller the electron beam and therefore photon beam giving rise to a higher brilliance.

Structural Biology Beam-Lines of European Facilities

- SRS – Daresbury, ESRF – Grenoble
- EMBL Outstation at DESY- Hamburg
- LURE/DCI - Orsay
- MPI-GBF - Hamburg
- MAX-II - Lund
- ELETTRA - Trieste
- ELSA - Bonn
- LURE/ACO - Orsay(#)

Laboratory	Station	%Biology	Brilliance ($x10^{12}$) (*)	λmin(Å)	λmax	λfix	Dλ/λ ($x\ 10^{-4}$)	Typical beam size as sample HxV (mm^2)	Detector	Comments(+)
1. Macromolecular Crystallography – Essentially Fixed Wavelength										
SRS	7.2	87	0.5	1.2	1.5	Fixed	3.0	0.4 x 0.4	IP	PX and Fibre Diffraction
	9.6	91	2.0	-	-	0.87	3.0	0.4 x 0.4	IP	-
ESRF	ID02	50	10.0	0.7	1.6	0.97	3.0	0.05 x 0.05	IP	Shared with SAS
	ID13	30	1×10^{-4}	0.78	2.0	Fixed	1.0	0.007 x 0.007	IP/CCD	Microfocus
	ID14-1	100	9.2	-	-	0.92	2.0	0.05 x 0.05	CCD	Available Jan 1999
	ID14-2	100	9.2	-	-	0.92	2.0	0.05 x 0.05	CCD	Available Jan1999
	ID14-3	100	9.2	0.92	1.44	Fixed	2.0	0.05 x 0.05	CCD/IP	a) λ dependent on ID14-4 b) IP 80 x 80 cm robot controlled off-line system
EMBL	X11	100	1.0	-	-	0.91	5.0	0.1 x 0.1	IP	
	BW7B	100	50.0	-	-	0.88	5.0	0.05 x 0.05	IP	
LURE DCI	W32	100	0.3	0.9	1.6	0.9	10.0	0.7 x 0.2	IP	
	D41a	50	0.016	1.2	1.8	1.38	10.0	0.6 x 0.6	IP	IP shared with DW21b

(#) All facilities claim to have adequate biological support laboratories
(+) All PX stations appear to have cryo-cooling facilities
(*) This column should be read with caution – it is not clear that all facilities have used the same definition of brilliance

Laboratory	Station	%Biology	Brilliance ($x10^{12}$) (*)	λmin(Å)	λmax	λfix	Dλ / λ ($x\ 10^{-4}$)	Typical beam size as sample HxV (mm^2)	Detector	Comments(+)
2. Macromolecular Crystallography – Tunable Wavelength										
SRS	9.5	93	2.0	0.45	2.60	-	2.0	0.5 x 0.6	IP	
ESRF	ID14-4	100	45.0	0.34	1.44	-	2.0	0.05 x 0.05	IP	
	BM14	100	1.0	0.60	1.80	-	2.0	0.15 x 0.15	CCD/IP	Bending Magnet. CCD-Image Intensifier
EMBL	X31	100	0.01	0.7	1.80	-	1.5	0.10 x 0.10	IP	
	BW7a	100	5.0	0.50	1.80	-	1.5	0.10 x 0.10	IP	
MPI/GBF	BW6	100	0.5	0.6	2.0		3.0	2.0 x 0.40	IP	
LURE/DCI	W21b	30	0.3	0.62	3.0	-	0.1	0.7 x 0.2	IP	Detector shared with D41a
MAX-II	PX	90	6.0	0.9	2.4	-	10.0	0.6 x 0.4	IP/CCD	CCD is SMART 1000
ELETTRA	b1 Diffr-action	50	10.0	0.5	3.0	-	-	1.0 x 1.0	IP	
3. Laue, and Time-resolved measurements										
SRS	9.7a	3	0.5	0.2	3.0	-	-	Unfocussed	IP	Laue station
ESRF	ID09	50	2.0 for Laue 0.8 for fixed λ	0.3	2.0	Tunable	2.0	0.2 x 0.19	CCD/IP	CCD – Image Intensifier IP 30 x 40 cm off-line MD
4. SAXS – Small-angle scattering										
SRS	16.1	49	2.0	-	-	1.41	300	5.0 x 1.0	MWPC/IP	Water bath/ LINICAM
	2.1	52	0.5	-	-	1.54	300	5.0 x 1.0	MWPC/IP	Water bath/ LINICAM
	8.2	19	0.5	-	-	1.54	40	3.0 x 3.0	MWPC/IP	Water bath/ LINICAM
	2.2	48	0.5	-	-	1.54	1.5	4.0 x 1.0	Ge Solid State (SS)	Water bath
ESRF	ID2	20	10.0	-	-	0.97	3.0	0.05 x 0.05	MWPC/ CCD/IP	a) Continually variable detector-sample distance b) Off-line IP
EMBL	X33	100	1.0	-	-	1.5	50	-	MWPC	SAXS/WAXS
	X13	100	1.0	-	-	1.5	50	-	MWPC	Muscle/Lipids
LURE DCI	D24	55	0.032	1.2	1.9	Fixed	10	0.1 x 0.1	MWPC/IP	
	D43	20	0.016	0.7	1.8	Fixed	100	0.07 x 0.07	CCD/IP	Diffuse Scattering Disordered systems IP is off-line MD
ELETTRA	b1 SAXS	50	5.0	0.77	2.3	Fixed	25	5.4 x 1.8	ID & 2D MWPC	SAXS/WAXS; microfocus 3λ = 0.7, 1.54, 2.30 ≈

Laboratory	Station	%Biology	Brilliance ($x10^{12}$) (*)	λmin(Å)	λmax	λfix	Dλ / λ ($x\ 10^{-4}$)	Typical beam size as sample HxV (mm^2)	Detector	Comments(+)
5. X-Ray Absorption Spectroscopy (only lines with significant biological usage are listed)										
SRS	8.1	21	0.5	1.1	3.5	-	3	3.0 x 1.2	SS-13 element	
	9.2	8	2.0	0.37	1.77	-	0.6	Unfocused	SS-13 element	
ESRF	ID26	New BL	5	0.5	2.5	-	1 – 10	0.2 x 0.02	Multi-channel Si drift	Ultra-dilute samples and QEXAFS
LURE DCI	D21	30	0.016	0.4	6.2	-	1	-	SS – 7 element	
ELSA	-	10	0.01	6.2	12.4	-	-	2 x 10	Ionisation chambers	
	-	10	0.01	6.2	12.4	-	-	2 X 10	Semi-conductors	
EMBL	EXAFS	100	0.01	0.4	2.0	-	0.14	-	SS – 13 element	
6. VUV and IR										
SRS	3.1	18	0.005	350	5000	-	5.0	6.0 x 1.0	Photo-multiplier	Circular Dichroism
	12.1	14	0.005	2000	8000	-	10.0	0.5 x 0.5	Photo-multiplier	Time-resolved (TR) Fluorescence & Energy-Resolved luminiscence
	13.1a	100	-	2000	7000	-	100	100 x 100 mm	Photo-multiplier	Confocal Microscopy
	13.1b	73	-	1900	10000	-	10	1.0 x 1.0	Photo-multiplier	TR fluorescence & CD
LURE/ SACO	SA1	50	-	2000	7000	-	10	0.1 x 0.1	Micro-channel plates	
	SB1	50	-	2000	10000	-	10	0.1 x 0.1	Micro-channel plates	TR fluorescence Used in 2-bunch mode only
	SA4	50	-	2100	12000	-	10	10.0 x 10.0	Photo-multipliers	
MAX-I	-	20	-	2000	24000	-	-	2.0 x 2.0	Micro-channel plates	TR fluorescence in UV and visible

Further beam lines for structural biology applications will become available in the next 5 years at ANKA (Karlsruhe) and the Swiss National Light Source. At the SRS, Daresbury, 2 additional beam lines are in the construction phase. Replacement synchrotrons are being considered in France (SOLEIL) and the UK (DIAMOND) and a new source is being planned for Spain (LLS at Bellatera). No information was made available from BESSY (Berlin).