

STRUCTURAL BIOLOGY
AND
SYNCHROTRON RADIATION:
ASSESSMENT OF RESOURCES AND NEEDS

Edited by

Janet L. Smith and Keith D. Watenpaugh

Report Sponsored by

BioSync,

The Structural Biology Synchrotron Users Organization

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July 1991

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TABLE OF CONTENTS

Preface.....	i-ii
Members of the Study Group and Authors of the Report.....	iii
Other Workshop Participants.....	iv
Reviewers.....	iv
Executive Summary.....	1-4
I. Introduction.....	5-6
II. Crystallography.....	7-22
III. X-ray Microscopy.....	23-28
IV. Small-angle Scattering.....	29-32
V. X-ray Spectroscopy.....	33-42
VI. Other Disciplines.....	43-44
VII. Other Concerns.....	45-48
VIII. Costs.....	49-56
Appendix A. Data from User Questionnaires.....	57-62
Appendix B. Data from Questionnaires to Synchrotron Radiation Facilities.....	63-78
Appendix C. Written Comments by Questionnaire Respondents.....	79-84
Appendix D. User Questionnaire.....	85-88
Appendix E. Questionnaires to Synchrotron Radiation Facilities.....	89-96
Appendix F. Glossary of Technical Terms and Acronyms.....	97-99

PREFACE

The Structural Biology Synchrotron Users Organization (BioSync) was formed in 1989 to facilitate access to synchrotron radiation sources for structural biologists working in North America. One of the first tasks that the membership set before the newly elected Steering Committee in 1990 was to document both the future needs of the structural biology community for synchrotron radiation and the strengths and weaknesses of the current status of synchrotron research in structural biology. This report is the result of a study conducted by BioSync to address these issues.

The bulk of the quantitative data are derived from responses to two questionnaires. A user questionnaire was sent in September, 1990, to all independent investigators working in fields of structural biology where synchrotron radiation can be used as a research tool. These 364 people are the membership of BioSync, and are all such individuals known to BioSync. The basic research fields that are major users of synchrotron radiation are crystallography, X-ray microscopy, scattering from noncrystalline materials and X-ray spectroscopy. A questionnaire was sent in Fall, 1990, to scientists at all synchrotron radiation facilities worldwide where structural biologists from institutions in North America can go to do experiments.

BioSync convened a study group to analyze and interpret the data and to write this report. The study group membership, which includes all members of the BioSync Steering Committee, is listed on page iii. The study group met on January, 13-14, 1991, in Washington, D.C., and members of the group wrote and edited the report subsequently. The four major research areas of structural biology for which synchrotron radiation is a research tool were each represented by at least two members of the study group.

The achievements and promise of structural biology, and the biological questions that structure research can answer, have been dealt with very effectively by recent publications, especially reports entitled "Form and Function: Perspectives on Structural Biology and Resources for the Future" (available as PUB-682/12-90 from the Office of Health and Environmental Research, U.S. Department of Energy, Washington, DC 20545), "Technologies for the Future: Opportunities and Needs in Structural Biology and Molecular Medicine" (available from the Biomedical Research Technology Program, National Center for Research Resources, NIH, Bethesda, MD 20892), and "Finding the Critical Shapes" (available from the Howard Hughes Medical Institute, 6701 Rockledge Drive, Bethesda, MD 20817).

This report is more specialized; within the broad framework of structural biology defined by these other reports, it documents and quantifies the nature and needs of that segment of the structural biology community that uses the intense X-ray light produced by synchrotron sources. The main conclusions from the deliberations of the study group are presented in the Executive Summary (pages 1-4). Section I of the report describes the development of synchrotron sources in the U.S. and their use for structural biology research. Sections II through V describe the particular situation for each of the four major disciplines of structural biology with regard to use of synchrotron radiation. Other uses of synchrotron radiation in structural biology are described in Section VI, and additional technical points not limited to a particular discipline are discussed in Section VII. In Section VIII is a detailed discussion of the costs of building and operating a synchrotron radiation beamline for structural biology research. Quantitative data from the user and facility questionnaires are given in Appendices A and B, unedited user comments in Appendix C, and sample questionnaires in Appendices D and E. Appendix F is a glossary of technical terms and acronyms.

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I. INTRODUCTION

Synchrotron radiation has been used as a research tool in structural biology for over fifteen years and has grown in prominence as its value has been realized.

“First generation” synchrotron sources were constructed as machines for experiments in high-energy physics. In the U.S. these machines are CHESS* at Cornell University and SSRL (as originally operated) at Stanford University. The synchrotron radiation produced by these machines is a by-product of the primary high-energy physics experiment. Experiments that exploit the special properties of synchrotron radiation are parasitic to the primary physics experiment and offer synchrotron radiation to users only with frequent interruptions or for limited periods of time. Through the years, the use of synchrotron radiation has become a more prominent activity at both of these facilities, as it has proven to be a successful research tool in many scientific disciplines. In particular, insertion devices* have been designed to optimize certain properties of synchrotron radiation and are now used on several beamlines at both facilities.

“Second generation” sources are fully dedicated to the production of synchrotron radiation, and do not run in a parasitic mode with respect to other experiments. In the U.S. these facilities are NSLS at Brookhaven National Laboratory, SSRL (starting in 1991), and Aladdin at the University of Wisconsin. Users are offered long experimental times of steady “beam” at these facilities.

A “third generation” of synchrotron radiation sources has been designed to maximize the brilliance of the radiation produced. The two third-generation facilities under construction in the U.S. are ALS at Lawrence Berkeley Laboratory and APS at Argonne National Laboratory. These synchrotron facilities will push the technology to its limits through the low-emittance design of the sources, which will produce an extremely high photon flux from an extremely small area in an extremely small solid angle. That is, they are very brilliant sources. Several orders of magnitude more photon flux to small samples will be possible compared to current synchrotron sources. Several technical problems having to do with the high heat load and radiation levels must be solved to take full advantage of these sources. The photon brilliance and flux will be further increased by the use of insertion devices.

Of the seven synchrotron sources operating or under construction in the U.S., four produce primarily hard X-rays (CHESS, SSRL, the X-ray ring at NSLS and APS) and three produce primarily soft X-rays and ultraviolet radiation (Aladdin, the UV ring at NSLS and ALS). All sources but Aladdin are being or will be used extensively for structural biology research. Several synchrotron radiation sources outside the U.S. have excellent facilities for structural biology research, and third-generation sources are under construction in both Europe and Japan. A growing number of researchers in the U.S. have used facilities abroad, as sources in the U.S. have become oversubscribed, are temporarily shut down, or have not been equipped to meet their needs.

Structural biologists have used synchrotron radiation since it has been available for research. The first experimental facilities for structural biology at CHESS and at SSRL were developed under the direction of structural biologists at Cornell and at Stanford who were associated with the synchrotron laboratories because of a desire to use synchrotron radiation in their own research. Notable successes by these scientists and others interested in developing the technology for structural biology led to greater demand for synchrotron radiation resources and to an increase in the number of users. Financial support and staff were sought specifically to support the structural biology effort of outside users at these facilities. Peer-review procedures were established at both laboratories to assign experimental time equitably as the demand grew. Much of the cost of operating the structural biology facilities at CHESS and at SSRL has been borne by the respective synchrotron radiation laboratories, and the funding for structural biology at both laboratories has been quite limited relative to the need.

Beginning with NSLS, and continuing with the laboratories at ALS and APS, the design, construction and operation of beamlines and the funding for these activities have been the responsibilities of teams of users who then have exclusive use of their facilities for most of the available beamtime. The balance of the experimental time must be available for general users who are not members of the team. At these facilities, no structural biologists with ties to the central synchrotron laboratory direct the development of beamlines for the structural biology community. Structural biologists

who wish to use the facilities either belong to teams who develop beamlines or apply for general-user time. A few of the newer beamlines at SSRL have also been operated in this user-team mode.

Existing facilities for structural biology at synchrotron sources have been quite successful despite limited resources, and there is a large demand for them. The support needed to operate existing facilities optimally and to construct and operate new facilities is beyond that traditionally available for structural biology research. The needs of the structural biology community for synchrotron radiation must be carefully evaluated in order that limited financial and scientific resources be used most wisely. This report describes the current status of synchrotron radiation facilities for structural biology and projects what needs will exist for these resources through the year 2000.

II. CRYSTALLOGRAPHY

A. Description of Current and Future Research Using Synchrotron Radiation

Experiments now being performed in biological crystallography with synchrotron radiation fall into four major categories:

- fixed wavelength (monochromatic) crystallography,
- MAD (multiwavelength anomalous diffraction) experiments,
- time-resolved crystallography (Laue investigations), and
- diffuse scattering studies.

Most of the monochromatic experiments have exploited the flux and tunability characteristics of synchrotron radiation while employing basically the same methods for data measurement and analysis as experiments done in the home laboratory. MAD, Laue methods and diffuse scattering use the unique properties of synchrotron radiation (tunability, energy dispersion and high flux, respectively) for experiments that would be extremely difficult or impossible with conventional X-ray sources and crystals of biological macromolecules.

1. Monochromatic Crystallography

Over the past decade, the major demand for synchrotron radiation beamtime for biological crystallography has been for fixed wavelength studies directed at structure determinations. The properties of extremely high brilliance and tunability give rise to six distinct advantages of synchrotron radiation over radiation from standard laboratory sources for monochromatic experiments.

Rapid Data Collection Rates: Crystals of biological macromolecules diffract X-rays weakly, and data collection using conventional laboratory sources can take several weeks or even months to complete. Due to the high brilliance of synchrotron radiation, data can generally be collected faster by orders of magnitude than is possible with laboratory sources. This can greatly decrease the time required to determine the structure of a complex macromolecule or assembly and can increase the throughput of structural information, factors which are particularly important in an industrial setting.

Data Collection from Crystals with Large Unit Cells: Large biological macromolecules or assemblies produce crystals with proportionately large unit cells. Individual spots in diffraction patterns occur at smaller separations in proportion to larger unit-cell sizes. The highly collimated beams from synchrotron sources permit diffraction spots from large unit cells to be resolved. In principle, it is possible to resolve reflections from unit cells that are many hundreds or even thousands of angstroms on an edge. Crystals with large unit cells also diffract X-rays less strongly than crystals of comparable size and quality that have small unit cells, and the brilliance of synchrotron radiation is critical to many such experiments. Crystallographic data from viruses and ribosomes are being measured routinely using synchrotron radiation. Most problems of this type cannot practically be handled with standard laboratory X-ray sources.

Data Collection with Minimized Radiation Damage to Crystals: The extent of crystallographic data that can be measured from a crystal of a biological macromolecule is severely limited by radiation damage to the crystal. Much of the damage to crystals is due to diffusion of free radicals that are generated by X-ray photons. The high brilliance of synchrotron radiation permits large quantities of data to be obtained before the crystals are destroyed by these time-dependent degradation processes. The tunability of synchrotron radiation allows for the selection of wavelengths where the effects of radiation damage are minimized.

Higher Resolution Data: Structural results from crystals of biological macromolecules are nearly always resolution limited. In general, macromolecular crystals yield data to higher effective resolutions with synchrotron radiation. This is due to rapid data collection with reduced radiation damage and to the improved signal-to-noise ratios with the "clean" monochromatic, brilliant radiation available from synchrotron sources. Even when data have been obtained from standard laboratory sources for an initial structural analysis, high-resolution data from synchrotron sources are

essential to obtain the most accurate crystal structure and to draw chemical and biochemical conclusions from that structure with the most certainty.

Data Collection from Small Crystals: It is not unusual to obtain microcrystals of biological macromolecules quickly and easily, whereas years may be required to produce crystals large enough for analysis on conventional X-ray sources. Some macromolecules never form large crystals despite major effort. The high brilliance of synchrotron radiation permits structural studies using extremely small crystals of macromolecules and thus greatly widens the range of possible projects. Data have been collected from crystals with dimensions as small as 40 microns using synchrotron radiation, and it should be possible to go to even smaller crystal sizes with third-generation synchrotron sources.

Selection of Wavelengths for Optimizing Diffraction Experiments: Absorption of X-rays by the sample limits the accuracy of diffraction data. Absorption effects can be minimized by using higher-energy radiation than is available from conventional laboratory sources. In addition, wavelengths can be selected to seek or to avoid absorption edges of specific elements present in the biological sample.

2. Multiwavelength Anomalous Diffraction (MAD)

Phase determination is the fundamental problem in crystallography and stands between data measurement and calculation of an image of the crystallized molecule. Synchrotron sources enable the phase problem to be overcome, since MAD experiments permit direct determination of phases from X-ray diffraction amplitudes. By selecting wavelengths that optimize anomalous-dispersion effects from selenium, phosphorous, halides or metal ions that are bound to the crystallized macromolecules, it is possible to directly determine the phase angles from crystals of these materials. Since the alternative methods of multiple isomorphous replacement or molecular replacement are very time-consuming and often unsuccessful, MAD techniques are becoming a major application of synchrotron radiation.

3. Use of Polychromatic Radiation: Time-resolved Crystallography

Laue methods use polychromatic, "white" radiation in diffraction experiments, and, therefore, cannot use conventional laboratory X-ray sources for experiments with crystals of biological macromolecules. Large amounts of data can be collected extremely rapidly (in fractions of a second) from a single crystal using Laue methods with intense synchrotron radiation. The power of Laue methods lies in time-resolved kinetic studies from macromolecular crystals. These kinetic studies are being used to understand reaction mechanisms, conformational changes, and other important structural changes that occur in biological macromolecules. Such experiments are inaccessible with laboratory X-ray sources.

4. Diffuse Scattering

Information about molecular motions in crystals of proteins and other biological materials is contained in the diffuse scattering that occurs around Bragg reflections in the diffraction patterns. Diffuse scattering data can be used to understand both intra- and intermolecular motions in these crystals. Useful diffuse scattering data on crystalline macromolecules can be obtained only with difficulty from conventional radiation sources, but much more readily from synchrotron sources. The availability of synchrotron radiation has opened up this new field.

5. Future Prospects for Crystallographic Studies with Synchrotron Radiation

The future of macromolecular crystallography holds the potential for explosive growth in the demand for three-dimensional structures. The scientific payoff from a three-dimensional structure is large and the investment in time and materials is rapidly shrinking. Several factors contribute to this trend.

- The scientific impact of the large number of three-dimensional structures now known has increased the importance of structure in all fields of biological sciences at the molecular level. Revolutions have occurred in certain fields of biology following determination of the structure of a key macromolecule. Examples include molecular immunology and the structure of human histocompatibility antigen, photosynthesis and the reaction center structure, and molecular virology and the first spherical virus structures.
- Much of the effort of the biotechnology industry is directed toward engineering new or altered functions into proteins. Structural information about the proteins being investigated and also about other, unrelated proteins is critical to these efforts.
- The rapidly growing ability of structural biologists to produce macromolecules of interest in sufficient quantity and purity will continue to expand the array of problems amenable to crystallographic analysis.
- Crystallographic structure determination no longer requires the Herculean effort it once did. The advent of more streamlined macromolecular crystallography will mean that nonspecialists will be able to carry out many routine structure determinations, as is currently possible for modifications to previously determined structures.
- The ease of structure determination and of macromolecular preparation will result in more detailed structure/function studies. Many of these will require batteries of crystallographic studies of mutant or liganded proteins.
- The explosion of protein sequence data, particularly deriving from the Human Genome Project, will reveal many interesting proteins for which crystals and structures will be sought.
- Clinical success of any pharmaceutical agent developed by structure-based design methods will fuel a large increase in demand for structures of target proteins and of protein:drug complexes.

Analogous to the case of the critical structure revolutionizing a field of molecular biology, demonstration of success in a crystallographic pursuit brings with it increased use of the method. This is already happening with multiwavelength anomalous diffraction, where several new structures have been determined recently. Of the "new"

crystallographic technologies, MAD brought the strongest positive response from crystallographer respondents to our user questionnaire. As soon as the Laue method achieves a major new structural success, the same increase in demand for appropriate facilities is expected. In addition, as Laue techniques for time-resolved crystallography mature, it is likely that they will be more widely incorporated in studies of structure/function relationships. The same can be said for demonstrated success at producing a major structural result from diffuse scattering studies. All three fields are developing rapidly and the future for each is judged to be bright.

An important factor is the success of the synchrotron radiation facilities themselves. Existing facilities such as SSRL, CHESS and NSLS are moving from the "heroic" mode of experimentation to the "user-friendly" mode. Facilities now being planned at the ALS and APS have a strong user-friendly component in their design. The success of these efforts is more dependent on the level of support that each facility receives than on any other single factor. The extent to which the trend toward user friendliness is successful will determine the level of demand for these facilities in the future. "Helpful, readily accessible support staff" and "user friendliness" are two of the most important features of synchrotron radiation facilities, according to questionnaire respondents (Appendix A, Table A-10). As more user-friendly facilities for pursuing crystallographic analyses are developed, there will be a rapid growth in use of them by biologists and other nonspecialists as well as by crystallographers.

The capabilities of synchrotron radiation will influence the nature of future crystallographic experiments. There will be a trend toward studies of larger and more complex macromolecules and macromolecular aggregates, using smaller crystals, and to a higher accuracy. These factors will all increase the data-collection time required for structural analyses relative to today's problems.

A new standard for structural accuracy in macromolecular crystallography is likely to be defined when highly accurate data can be obtained rapidly and routinely, and as refinement techniques increase in sophistication. Very accurate data sets from synchrotron sources will be used to refine most crystallographic models. It is likely that data from synchrotron sources will also be used to redetermine, with much higher accuracy, many of the protein structures that have been determined using laboratory X-ray sources during the past two decades.

The advent of new, highly brilliant synchrotron sources and advances in data collection techniques will further enhance the usefulness of extremely small crystals. Since only microcrystals of many proteins and macromolecular assemblies can be obtained readily, improved methods for working with small crystals will greatly increase the number of problems amenable to crystallographic analysis. It should be possible to obtain accurate diffraction data from microcrystals with dimensions as small as 10-20 microns. There are also several potential advantages of working with microcrystals, including decreased absorption of X-rays, improved success of flash-freezing techniques, and sharper diffraction profiles.

Techniques for resolving reflections from crystals with extremely large unit cells will be developed further in the future. Better optics and detectors must be developed and made available to permit analysis of larger macromolecular aggregates. Future structural studies are also likely to include weakly diffracting two-dimensional crystals of proteins and other biological macromolecules. Future research may be directed at new types of studies in diffraction physics performed using biological crystals. Biological materials have unique physical properties that will be of interest in various fields of materials science.

B. Description of Current and Planned Facilities

There are six stations at synchrotron sources in the U.S. where experiments can be done today in macromolecular crystallography. Six more stations at existing synchrotron radiation facilities are being commissioned. The types of crystallographic experiments described in the previous section (fixed wavelength, multiwavelength, Laue and diffuse scattering) have different experimental requirements and usually cannot be done at the same experimental station. The fundamental differences in instrumental requirements for these types of experiments are described here.

Laue Diffraction: The sample is irradiated with white radiation, so no monochromator is needed. It is usually desirable to have an optical device to determine the upper and lower bounds of the energy range being used. The fastest time-resolved experiments require very high brilliance and an insertion device is recommended. The Laue experiment,

because it uses the white synchrotron radiation beam, involves the highest levels of radiation, and extra shielding in the experimental hutch is needed together with special air handling to remove any ozone. Because time-resolved experiments require specialized equipment for starting, stopping and detecting a chemical reaction, the set-up and testing time usually far exceeds the data collection time and experimental stations cannot have a high throughput.

Fixed-Wavelength Crystallography and Diffuse Scattering Experiments: The main requirement is for a fixed, single, user-selected wavelength of X-rays. This wavelength is generally used for an entire experiment, so the time required to change wavelength and optimize optics is not critical to any one experiment. The experimental station must be equipped with a monochromator that can select wavelengths over the range of about 0.5Å to about 3.0Å (~4-25 keV). Brilliance is important in nearly all experiments, so an insertion device and focusing optics on the beamline are desirable.

Multiwavelength Anomalous Diffraction: It is important in MAD experiments to change the wavelength frequently, reproducibly and rapidly. This is best done with a fixed-exit monochromator in which the position of the monochromatic exit beam does not change and optimization of optics can be automatic as the wavelength is changed. In addition, energy resolution is critical for many experiments and a high-quality narrow-bandpass monochromator is essential. It is important to have high brilliance to MAD experimental stations because a very narrow energy range is being selected; without it, the flux incident on the crystal can be low.

A summary of the experimental stations for crystallography that are in operation or being commissioned is given in Table II-1. Detailed information on beamline optics and station instrumentation is given in Appendix B, Tables B-4 and B-5.

Table II-1. Experimental Stations for Crystallography

Experimental Station	Experiment Type	Date of First Operation	Operating Time* (days/year)
In Operation:			
SSRL 1-5AD	MAD	1982	100
SSRL 7-1	Monochromatic	1984	210
CHESS A1	Monochromatic (1.56Å)	1980	143
CHESS B2	Laue	1990	44
CHESS F1	Monochromatic, MAD	1990	187
NLSL X12-C	Monochromatic, MAD	1986	250
Being Commissioned:			
NLSL X4-A	MAD	1991	250
NLSL X4-C	Monochromatic	1991	250
NLSL X8-C	Monochromatic, MAD	1991	125
NLSL X25	Laue	1991	25
NLSL X26-C	Laue	1991	125
SSRL 10-2	Laue	1992	20

* Maximum time that beam is scheduled to be available to each station for macromolecular crystallography.

Table II-2 contains an estimate of the number of data sets that could be measured at each experimental station if the station, as currently configured, operated without interruption for as many days per year as synchrotron radiation should be available to that station for crystallographic experiments. Also listed is the maximum number of days of usable beam that have ever been achieved for crystallography from each station. It is important to note that only the CHESS A1 station has thus far achieved its stated limit. Other existing stations have not achieved their stated limits for a variety of reasons. CHESS F1 and B2 began operations in late 1990; SSRL begins dedicated operation, for which potential operating time is calculated, in mid-1991; and NLSL X12-C has had considerable development time and experienced significant staff shortages since its operations began.

Table II-2. Potential Throughput of Stations for Crystallography

Experimental Station	Operating time (days/year)		Maximum Data Rate	
	Achieved	Potential	Data Sets/Day	Data Sets/Year
Monochromatic				
SSRL 7-1	56	210	4	840
CHESS A1	124	143	4	572
CHESS F1 ¹	25	up to 187	6	up to 1122
NSLS X4-C	-	250	4	1000
NSLS X8-C ¹	-	up to 125	4	up to 500
NSLS X12-C ¹	125	up to 250	4	up to 1000
MAD²				
SSRL 1-5AD	30	100	0.25	25
CHESS F1 ¹	25	up to 187	1.5	up to 280
NSLS X4-A	-	250	2	500
NSLS X8-C ¹	-	up to 125	1	up to 125
NSLS X12-C ¹	125	up to 250	1	up to 250
Laue				
CHESS B2	14	44	0.25	11
NSLS X25	-	25	0.50	12
NSLS X26-C	-	125	0.25	31
SSRL 10-2	-	20	0.25	5

¹ CHESS F1, NSLS X8-C and NSLS X12-C are available for both monochromatic and MAD experiments; the data rates in the table are for 100% operating time being used for each type of experiment. In practice, time spent for one type of experiment cannot be spent for the other.

² Each MAD data set includes Bijvoet data at 3-5 wavelengths and is therefore 6-10 times larger than a monochromatic data set from the same crystals.

C. Evaluation of Current Situation and Projection of Future Demands

Currently there are a total of six stations (soon to be twelve) at synchrotron sources in the U.S. that are devoted between 10% and 100% to biological crystallography. Six of the twelve experimental stations can be used for routine, rapid data collection. The queues are long for use of all the current facilities, and the speed and system friendliness with which data can be collected leave much to be desired at certain facilities. Perhaps a total of 1,000 data sets will be collected at all the facilities operational in 1991 and 2,000 data sets in 1992. Great improvements are being made at a number of these facilities, which will improve their performance in succeeding years. With proper funding and development, they could reach their full potential of around 5,000 data sets per year by 1995.

The early success of crystallographic facilities at CHESS and at SSRL, as well as at synchrotron sources abroad, has proven the outstanding capabilities of synchrotron radiation for macromolecular crystallography, as the facilities have provided exceptional data for large numbers of investigators. They continue their evolution from being largely experimental to being fully developed user-friendly facilities. Crystallographic experiments at all existing synchrotron radiation facilities still maintain something of a "heroic" flavor. This is a serious concern if the facilities are to realize the enormous potential they have for speeding and improving the quality of crystallography. There is strong support for synchrotron radiation facilities among crystallographers, but often this is not turned into use of synchrotron sources because of the real or perceived difficulties of carrying out the experiments. Nearly all these difficulties could be addressed by addition of appropriate support staff to the stations used for crystallography. Existing stations are woefully understaffed (see also Section VIII).

A major part of the "heroism" necessary for data collection at most existing facilities is the need for a lengthy data-

processing effort in the home laboratory subsequent to the measurement of raw data. At three of the four existing stations for monochromatic data collection (SSRL 7-1, CHESS A1 and CHESS F1), most data are measured on film and then processed away from the synchrotron source. Few crystallographers have ready access to film-scanning instruments because few use film any longer for their home data collection. In addition, the effort needed to process film data is both labor- and compute-intensive. All three of these stations are, or will soon be, equipped with imaging-plate, or storage-phosphor, detectors, which are far superior to film, but which still require a comparable data-processing effort subsequent to the experiment. Much of the speed that is gained by data collection with synchrotron radiation is lost through manipulation of films and imaging plates on the collection instrument, through retrieval of digital data from the images later, and particularly through labor- and compute-intensive data processing. This consideration often tips the balance in cases where some, albeit inferior, data can be obtained with conventional X-ray sources and electronic area detectors. The development effort at synchrotron radiation facilities, until now, has been directed appropriately towards optics, diffractometry and detectors. In the years since most existing experimental stations for crystallography were designed, the standard for data collection and processing in the home laboratory has evolved such that relatively rapid, semi-automated data processing is the norm. This evolution is now being transferred to the synchrotron sources.

There is no question that the "PRT" mode of operation has slowed access to NSLS by crystallographers. At NSLS, only station X12-C was operating for crystallographic experiments at the end of 1990, and it is a relative newcomer to the facility. In fact, the Department of Biology at Brookhaven (PRT for X12) operates X12-C as a community resource and not primarily for its own researchers. Three of the stations being commissioned at NSLS (X8-C, X25 and X26-C) will also be operated more in the "facility" than in the "PRT" mode when they are used for crystallographic experiments. The Howard Hughes Medical Institute beamline at NSLS (stations X4-A and X4-C) will be the first for crystallography operated in the true "PRT" mode, although a large proportion of beamtime is expected to be available for non-PRT experimenters. The NSLS experience is in striking contrast to the experience of crystallographers at SSRL and at CHESS, where scientific staff at the facilities have developed and run effectively the stations that are used for crystallographic experiments.

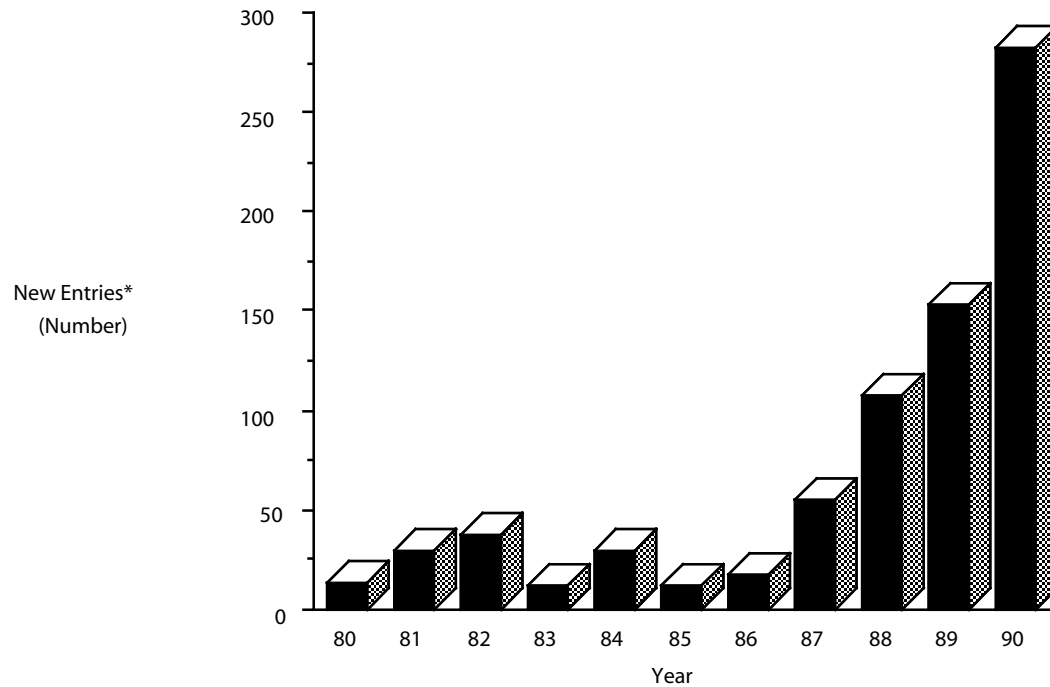
The crystallographic community is unaccustomed to the design, construction, operation, fund-raising and organizational tasks required of a successful PRT, has been slow to start at NSLS, and has not used its traditional sources of support for "PRT"-mode operations there. Rather, individual crystallographer investigators raise their individual funds to carry out individual research projects in their individual laboratories. This cottage-industry approach is well suited to supporting individual investigator-initiated research but is untenable for developing and maintaining synchrotron radiation facilities. A radical departure from traditional behavior is required at synchrotron radiation sources where the development and operation of beamlines is the responsibility of users, as is the case at NSLS, at ALS and at APS.

The number of biological crystallography laboratories in universities is expanding rapidly, and several hundred new crystallographers are now in training in the U.S. (see Appendix A, Table A-4). The biotechnology and pharmaceutical industries continue a recent expansion into crystallography. There will be a steady and continuous expansion in the demand for beamtime at synchrotron sources by professional crystallographers during the coming decade. However, it is expected that the major expansion of biological crystallography will involve not full-time crystallographers, but biophysicists, biochemists, molecular biologists, and cell biologists. Crystallography will become an important tool that accompanies many other types of biological and biochemical studies, and there will be a great increase in demand for crystallographic facilities that are suitable for use by nonspecialists. The expected demographics closely parallels the growth that occurred in small-molecule crystallography beginning in the early 1970's. Prior to 1970, essentially all crystallographic studies were performed by specialists in crystallography. At present, nearly all research chemistry departments include crystallography as a standard analytical technique. While many structure determinations demand the skills of a trained crystallographer, much of the small-molecule crystallography now is done by chemists, who use crystallography as a routine tool.

Expansion in macromolecular crystallography will be accompanied by a corresponding increase in demand for access to synchrotron radiation facilities, especially if these facilities are operated in a user-friendly mode that includes a data processing capability. The growth that is likely to occur can be appreciated by examining the growth in the Brookhaven Protein Data Bank, as shown in Figure II-1. While many of the atomic coordinate entries are new ligand states or mutants of previously known macromolecular structures, each entry required new crystallographic data collection. Based on the second-order growth rate of the coordinate data sets deposited over the last five years, there should be 15 times as many deposited per year in ten years. Another indicator of the growth potential in biological crystallography is the expansion of the protein sequence database of the National Biomedical Research Foundation, which now contains

18,000 entries with a current growth rate of several thousand new sequences per year. This number will accelerate rapidly as the Human Genome Project progresses, and many of these problems will be subjected to structural analysis by crystallography.

Figure II-1 Brookhaven Protein Data Bank
New Atomic Coordinate Entries



* Data from the January edition of the Protein Data Bank Newsletter for each year. (Available from the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973 USA.) Total entries = number of atomic coordinate entries available + number in preparation - number of model structures available and in preparation. Number displayed = total entries in year (i+1) - total entries in year i.

The very bright promise that is offered to the biological community by the potential for rapid increase in the production of new crystal structures of important macromolecules and assemblies depends in part on the availability of readily accessible, user-friendly, adequately supported synchrotron radiation facilities. The tremendous biological payoff to be gained by making crystallography a usable tool to the biophysicist/biochemist/molecular biologist will not be realized if there are not bright, accessible synchrotron sources, or if the sources are not adequately instrumented, or if the instruments are extremely difficult to use, or if there are not staff to help nonspecialist users, or if the entire crystallographic experiment is not streamlined.

We have documented the growth in the crystallographic community and have described the growing demand for crystallographic results. Below are two projections of the need for synchrotron radiation facilities in the year 2000, estimated by number of data sets to be measured.

Projection 1: Based on numbers of researchers reported in responses to the user questionnaire.

The following assumptions are made.

- Only the 138 crystallographer investigators (57%) who responded to the user questionnaire will be identified as synchrotron radiation users. All others will be considered uninterested in synchrotron radiation for the purposes of this projection.
- There will be no accelerated growth due to the Human Genome Project or to biotechnology breakthroughs.
- 5% of the 387 postdoctoral associates of the 138 investigators will become independent investigators in the U.S. each year. No graduate students will become independent investigators directly. 5% of investigators with more than 20 years of independent experience will retire each year. The resulting growth rate is 13% per year, or 468 independent investigators by the year 2000 from current crystallography groups alone.
- By the year 2000, one-half of the research groups determining macromolecular structures will be headed by biochemists, molecular biologists, and other biological scientists with or without the supervision of crystallographers. This adds another 468 groups doing crystallography by the year 2000.
- Each of the 468 crystallographic groups will collect an average of 50 data sets per year. With the increased ease of data collection, there will be more data sets collected (for example, more heavy-atom feasibility data sets, more substrate binding studies, more site-directed mutagenesis experiments, more high-resolution data, more small crystals examined, etc.). Each of the 468 biological groups will collect 20 data sets per year.

By these assumptions, the following is projected.

$$\begin{aligned} &50 \text{ data sets/group/year} \times 468 \text{ crystallographic groups} + \\ &20 \text{ data sets/group/year} \times 468 \text{ biological groups} = \\ &32,800 \text{ total data sets/year} \end{aligned}$$

Projection 2: Based on numbers of institutions and investigators.

The following assumptions are made.

- By the year 2000 at least 50% of macromolecular structures will be determined by biochemists, molecular biologists and other biological scientists under the advice of crystallographers; this is particularly true for mutant proteins and altered ligand states for which an initial crystal structure is known. At most 50% of macromolecular structures will be determined by professional crystallographers.
- There are about 100 active research universities in the U.S. This is based on the fact that, when ranked in order of funds received, the top 100 universities receive about 85% of all Federal expenditures to universities. (Science and Engineering Indicators - 1989, National Science Board, Washington, DC (1989) U.S. Government Printing Office, NSB89-1.)
- In each university there will be two crystallographer investigators with five structural projects each, or a total of 10 projects/research university. The current BioSync mailing list includes 178 crystallographer investigators at 80 universities. This group of investigators is young and growing; one-third were working in someone else's laboratory five years ago.

$$10 \text{ total projects/university} \times 100 \text{ universities} = 1,000 \text{ projects}$$

- In each university there will be 15 other biological scientists with two structural projects each, or a total

of 30 projects/university.

40 total projects/university x 100 universities = 4,000 projects

- Each university project needs 1-10 data sets/year with an average of 10 data sets/project/year in a crystallography lab and 6 data sets/project/year in a biological lab. Determination of a homologous structure (new species or crystal form for a macromolecule of known structure) or extension of an existing structure to high resolution will require one data set, while 10 data sets will be required for isomorphous replacement or MAD structure determination and for series of mutants or ligand states.

1,000 projects in crystallography labs x 10 data sets/project/year +
3,000 projects in biological labs x 6 data sets/project/year =
28,000 data sets/year

- Industrial needs are different. For each drug receptor or target protein, there will be many structures of drug:macromolecule complexes to be determined; each project will require 50 data sets/year. There will be ~60 companies involved in structural research (20 pharmaceutical and 40 biotechnology) and each company will have an average of two structural projects. Currently there are 50 crystallographer investigators at 20 companies on the BioSync mailing list; this group is also young and growing.

60 companies x 2 projects/company x 50 data sets/project/year =
6,000 data sets/year

By these assumptions the following is projected.

28,000 data sets/year at universities + 6,000 data sets/year at companies = 34,000 total data sets/year

Note that Projections 1 and 2, though founded on different assumptions and approached in rather different ways, result in a very similar assessment of about 33,000 data sets per year in the year 2000.

The fraction of all data sets that will be collected at synchrotron sources is expected to increase for a variety of reasons. For example, more accurate, more rapid, more user-friendly data collection methods quickly replace older methods. In small-molecule crystallography laboratories, film methods quickly became unacceptable with the development of automated diffractometers. In macromolecular crystallography, area detectors are rapidly replacing other data collection systems. As user-friendly synchrotron radiation facilities are developed where experimenters can collect data rapidly and can also quickly reduce raw data to integrated intensities, the facilities will be in very high demand. A significant demand for synchrotron radiation resources is also expected from biochemists who will be doing crystallography and who will rely on outside facilities for data collection, rather than maintaining their own equipment, which is both labor and cost intensive.

We conservatively estimate that 50% of the estimated number of data sets, or ~16,000 data sets/year will be collected at synchrotron sources, if the sources are available.

D. Facilities Needed

Need for a total of 18 experimental stations for rapid collection of monochromatic data is projected for the year 2000. There are currently, or will be in the near future, six stations where data can be collected in an efficient, purely production-line mode (see the "Monochromatic" section of Table II-2). It should be possible to collect up to 5000 data sets/year from these stations in toto. Only a fraction of the potential maximum number of data sets has been collected from any of the existing stations to date. To collect the projected 16,000 data sets, three times the existing six, or 18, stations will be required. Additionally, a means could be devised for vastly improving the efficiency of single stations. (See the next section, II. E., for ideas to improve throughput.)

Rapid data-processing is an essential component of an efficient crystallographic data collection facility at a synchrotron radiation laboratory. The availability of on-line data processing at the synchrotron source would have an

enormous impact on the projected demand for beamtime at synchrotron sources in macromolecular crystallography. If appropriate user-friendly facilities are developed, then demand will be significantly greater than the 16,000 data sets/year that we have projected. If such facilities are not developed, then demand will be lower and the important increase in speed that synchrotron radiation offers to macromolecular crystallography will not be realized. It is particularly important that computational means be developed to handle and process the very rapidly accumulated data that are anticipated at third-generation synchrotron sources equipped with fast detectors.

Many experiments, including time-resolved crystallography, diffuse scattering and some multiwavelength anomalous diffraction, cannot be treated as production-line jobs. These generally require specialized experimental stations, and in some cases, specialized beamline optics. The interest of respondents to the user questionnaire in MAD and time-resolved experiments is particularly high. At least a doubling in number of facilities dedicated to MAD and time-resolved experiments is needed. This represents a minimum of two more fully dedicated MAD stations and the equivalent of one more fully dedicated time-resolved station.

E. Special Consideration: Enhancing the Speed of Crystallographic Data Collection

It is projected that several new experimental stations of the style currently in operation may be needed for macromolecular crystallography by the year 2000. Another approach to the large anticipated demand is to use more efficiently the radiation that is now available or that will soon appear in the facilities under construction. Creative ideas are needed to rethink the whole process about how to collect thousands of data sets on thousands of crystals. For instance, one can envision many hutches in tandem on one beamline with X-ray optics switching the beam from one hutch to the other as new crystals are mounted, particularly when the mounting and centering time for small crystals substantially exceeds the X-ray exposure time, as is generally the case. Alternatively, with some developments in X-ray optics, it may be possible to make monochromators with high transmission as well as reflectivity that can split the beam (with minimal loss of intensity) in, for example, five ways at five different X-ray energies to five independent X-ray stations. Another idea is to mount crystals in precision capillaries in a manner conducive to handling with robotic arms or sample manipulators. Innovative sample handling to mount and expose a new crystal every minute or so in a fully automated manner may be possible in analogy to integrated circuits in a wafer manufacturing plant that can be aligned with micron precision and processed through complex fabrication steps without human intervention. If each crystal requires five minutes of beamtime, then of order 300 crystals per day or 70,000 per year per experimental station might be studied if the right "mass production" hardware and software were available. Rapid and automatic processing of data from the X-ray detector is essential to successful implementation of this idea.

Along with advanced sample handling, more efficient training techniques are needed such as better instruction manuals for the use of complex apparatus and/or personnel instruction in a video tape format. Thus the productivity on experimental stations emphasizing routine data collection can be greatly enhanced when compared to the present operations on existing stations.

F. Special Consideration: Detectors for Macromolecular Crystallography

Most experimental results today in protein crystallography with synchrotron radiation are not limited by the X-ray flux, but by the efficiency and speed of the detectors being used to record these data. Whereas commercial area detectors for protein crystallography are now available that can use rotating-anode X-ray sources efficiently, this match between source and detector has not yet been achieved for synchrotron sources. It is very important that X-ray experimental stations employed for protein crystallography be rapidly fitted with detectors having high absolute efficiency and high speed data readout to computer storage. In addition, these area detectors must cover a large solid angle of the diffraction geometry, while at the same time they must be designed with many pixels (high spatial resolution), to record the dense information content of the diffraction patterns. Also important are the properties of linear response to X-ray dose, spatial homogeneity in sensitivity, minimal geometrical distortion of the image, and stability over time.

Physical Size and Spatial Resolution: Diffraction patterns from macromolecular crystals exhibit high information density: many closely-spaced Bragg reflections must be accurately measured, and the amplitude of the diffraction pattern changes rapidly over short distances. Each Bragg spot must be recorded on more than one pixel to minimize

systematic errors and to permit accurate modelling of spot profiles. Furthermore, spots must be clearly separated on the recorded image, to avoid contamination by neighbors. Thus, the beam must be collimated tightly, the detector must be positioned sufficiently far away to separate the peaks by at least five pixels, and the point-spread function of the detector must be less than, or commensurate with, the pixel size.

Coherent Bragg diffraction data are contaminated by Compton and other diffuse background X-ray scattering, which decrease with distance as the reciprocal square. However, the Bragg spots spread more slowly, so there is virtue in placing a detector far back from the sample crystal. At the same time, protein crystals diffract X-rays into a large solid angle, and failure to record these data amount to, in effect, a reduction in the efficiency of the experimental detection apparatus. These combined criteria argue for large detectors, or many detectors operating in grouped arrays, having dense sampling by many pixels.

How large do detectors need to be? If a square detector with 512 pixels on each side were positioned normal to and centered on the direct X-ray beam, reciprocal lattice points could be recorded approximately 50 orders from the center. This is 3.0-Å resolution for a crystal with maximum cell dimension of 150Å, but 6.0-Å resolution if the cell dimension were 300Å. This is not adequate. If the detector were a square of 1,024 pixels on a side, this detector would record 3-Å data from the crystal with the larger unit cell, without swinging the detector center from the origin of reciprocal space. Physically large pixels would permit this detector to be moved far away from the crystal sample.

Higher resolution data could be recorded if the detector center were swung away from the reciprocal space origin: the 1,024-pixel detector could collect 1.5-Å data from the 300-Å cell crystal while continuing to record very low-resolution data near the origin. For much larger unit cells, the 1,024-pixel detector is still inadequate: virus crystals with 600-Å cells or greater need a detector with 2,048 pixels on an edge, or larger.

Detector Speed: Protein crystals are highly labile in strong X-ray beams; at modern synchrotron sources, crystals may deteriorate markedly in less than ten minutes. Therefore, speed is an important design imperative. To achieve significant statistical quality of data, we require large integrated total counts for each Bragg spot: for 1% accuracy (all else being corrected properly), 10,000 counts per spot above background are needed, on average. For typical crystals, about 200 spots might be imaged on a detector screen simultaneously, and to approach this 1% statistical error, a data frame of 0.1° typically records several million counts over its surface before it is read out. Whereas this X-ray dose requires two or three minutes to record on a rotating anode X-ray source, such integrated doses are summed in a few seconds on modern synchrotron sources. On some experimental stations today, and on stations at future sources, data frames will require substantially less than one second of integration time, if the station is fitted with efficient electronic area detectors. Therefore development of fast computer readout systems, and fast data processing, are essential for the efficient use of synchrotron sources.

Detector Efficiency: Detectors must record X-ray flux efficiently. Data of equal quality require twice the collection time with a 50% efficient detector as with a 100% efficient detector. Since the sample is labile, time lost cannot be regained by any means. Detectors should also be spatially efficient. In this context, efficient detectors must record data over the largest possible solid angle, since data missed during one pass must be measured on another pass. Both counting efficiency and spatial efficiency are very important when the sample crystal is deteriorating.

Other Considerations: Uniformity of detector sensitivity leads to simplified algorithms to correct recorded images. Large differences in sensitivity over the detector face are difficult to correct properly, and can reduce overall data quality. However, smaller deviations in sensitivity are entirely correctable and need not be harmful. Similarly, geometrical distortion of images should be minimized, but smooth, differentiable distortions can be corrected. Likewise, nonlinear response to X-ray dose is correctable but should be minimized.

Detectors meeting many of these criteria are now being developed in a few laboratories, but no current design satisfies every design goal. It is critical for proper application of synchrotron radiation to macromolecular crystallography that detector development proceed and accelerate, and that new ideas about hardware components be introduced into the field.

G. Summary

Synchrotron radiation has already made an enormous impact on the field of macromolecular crystallography. It has the potential to be a critical component in vastly improving the rate and quality of structural results in crystallography. For this potential to be realized several things are needed.

- Approximately 15 new experimental stations dedicated to macromolecular crystallography will be needed by the year 2000 to handle the anticipated demand both from professional crystallographers and from other biological scientists who will be doing structural work.
- The efficiency and user-friendliness of existing experimental stations need to be improved. These efforts must include facilities for rapid data processing at the synchrotron radiation laboratory.
- User support will be needed for biological researchers who will use synchrotron radiation facilities but who are not trained crystallographers.
- New technologies to increase data throughput need to be developed. Ideas include crystal-handling robotics and optical arrangements allowing a single beamline to serve multiple experimental stations.
- New and efficient software is needed to process rapidly collected data, both monochromatic and polychromatic. This is particularly true for the third-generation, high-brilliance synchrotron sources.
- There is a need to develop new, fast, efficient X-ray detectors that can handle the high count rates of third-generation synchrotron sources.
- Geographically distributed synchrotron radiation facilities are needed to minimize travel costs, crystal handling, etc.

III. X-RAY MICROSCOPY

A. Description of Current and Future Research Using Synchrotron Radiation

It has been appreciated for most of this century that imaging with short-wavelength X-rays offers the potential of a microscope with many times greater resolution than optical microscopy. However, X-ray imaging of quality even equal to that of the light microscope could not be achieved. These limitations were largely the result of an inability to focus X-rays effectively. As a consequence, the utility of X-rays for microscopic imaging in biology has been minimal, and the development of an effective high-resolution electron microscope in the 1940's appeared to eliminate any substantial reason to pursue development of an X-ray microscope for biological research. The recent construction of synchrotron sources of bright, partially coherent soft X-ray radiation, and the ability to fabricate diffractive X-ray lenses, particularly Fresnel zone plates, capable of focusing such radiation to a small spot size, have now made it possible to develop new X-ray microscopes that are able to overcome many of the earlier limitations of the method. This has led to a new interest in the development of such devices.

These microscopes are now capable of high resolution, around 40-50 nm today and approaching 20 nm in the near future. The resolution of X-ray microscopy, as of electron microscopy, is ultimately limited by radiation damage to unprotected specimens, although further technical advances may permit a closer approach to wavelength-limited resolution. As recent advances in visible light microscopy have demonstrated, it is possible to obtain useful, sometimes model-dependent, information beyond the resolution limit. Similarly, with modest improvements in resolution and the application of contrast enhancement techniques, X-ray microscopy may allow the direct visual examination of objects of extremely small size, of supramolecular or even macromolecular dimensions.

Thus, X-ray microscopy has now reached a state of development where it can be exploited for biological research. The potential of the X-ray microscope lies not so much in the ability to provide high resolution information (the electron microscope is superior in this regard), but rather in allowing biologists to view samples at such resolution under more normal physiological conditions. This is due to the penetrating power of X-rays, which eliminates the need for vacuum; and the soft X-ray water window, a wavelength region in which water is transparent to X-rays relative to bioorganic molecules. These features make it possible to view many cells and all subcellular structure without having to place them in high vacuum, or to prepare thin sections. Additionally, satisfactory imaging contrast can be provided by the natural constituents of the sample due to the differential absorption of X-rays by different elements in it. One can obtain quantitative information about the spatial distribution of specific elemental and chemical constituents. These are extremely valuable properties for biological microscopy and are unavailable at such high resolution by other methods.

Because of the potential for high resolution, X-ray microscopy provides the first clearly complementary means to assess current concepts of cell ultrastructure derived from electron microscopy. X-ray microscopy should be a valuable tool for evaluating the effects on samples of the stringent preparation methods required for electron microscopy because X-ray images can be made of unaltered objects in physiological environments. Finally, X-ray images permits time-resolved studies in a physiological environment at high resolution. This capability cannot presently be achieved in other ways, and has permitted dynamic structural studies on isolated cellular organelles at high resolution.

Since 1988, synchrotron radiation-based X-ray microscopy has been in transition from a predominantly developmental mode, to an operational one. As a result, important biological studies are being undertaken for the first time. Three important demonstrations of the method have been provided recently: 1) samples can be imaged at high resolution, whole and unaltered in any way other than extraction from their source tissue or cell, suspended in and containing water as they normally do, at ambient or body temperature; 2) high resolution holograms of biological samples can be formed and reconstructed using the coherence property of synchrotron radiation; and 3) structural changes can be followed, and quantitative measures of changes in protein content made, as they evolve in real time in single structures.

The study of cellular secretion is becoming a major focus of study. Research on higher-order chromosomal structure and folding, as well as muscle function and pathology, are other subjects presently being pursued. The use of elemental X-ray absorption edges to provide chemical maps has begun; for example, calcium measurements are made in muscle and cartilage by tuning the X-rays across the calcium L-edge.

Although substantial progress has been made, such studies are only in their infancy. They need to be carried beyond the pilot study stage, and many other samples and experimental questions await examination. Limitations imposed by radiation damage must be determined. In addition, technical development is far from complete. Under way are attempts to explore elemental analysis for a broad range of elements using L and K absorption edges. Various approaches to three-dimensional imaging have begun, from the ambitious holography to simple stereo images. Phase contrast imaging shows promise, and is being developed around the world. A substantial challenge remains in the design and microfabrication of sample holders and chambers.

Important strides have also been made in X-ray fluorescent emission microscopy, or microprobe analysis. Although this is a low-resolution method compared to absorption microscopy (the current state-of-the-art is $1\mu\text{m} \times 1\mu\text{m}$ resolution), it offers the potential for a complete quantitative analysis of elements of mid to high atomic number in samples in their native state, with a remarkable sensitivity in the femtogram range. Further detector development will allow for improvements in this sensitivity by orders of magnitude. The X-ray microprobe has not yet been exploited in a major way for research in structural biology, although it is of substantial potential value.

B. Description of Current and Planned Facilities

The only dedicated facility in the U.S. at present is the NSLS X1-A experimental station. It makes use of the soft X-ray undulator that it time-shares with X1-B. The beamline is equipped with feedback stabilization, a spherical grating monochromator and two end stations operating simultaneously. One of these end stations is in routine use for scanning soft X-ray microscopy, based on zone plate focusing. The resolution is presently 500\AA . Images are recorded in digital form, with dwell time presently being $1\text{msec}/\text{pixel}$. The other end station is used for the development of X-ray holography and other new ideas. Support facilities include optical microscopy (phase contrast and interference contrast), incubator, dark room, numerical image analysis and display. These facilities are being improved with a new mirror and grating in 1991.

Biological fluorescence microscopy is approximately 25% of the program at NSLS station X26-A. Resolution at present is about $3\mu\text{m}$, with optics under development to reach $1\mu\text{m}$.

C. Evaluation of Current Situation and Projection of Future Demands

High resolution soft X-ray imaging is a brightness-limited technique. The only currently available high brightness synchrotron source is the X1-A station at NSLS. It was available in 1989-90 100% of the time, but was reduced to ~50% starting in 1991 due to sharing with X1-B. The 50% time constraint leads to a painful shortage of available time for this new and growing activity. X-ray imaging (as electron microscopy) requires much "on-line" time, unlike other forms of structural biology, where most of the effort involves the off-line analysis of collected data. The X1-A station is used by a consortium of five institutions (SUNY-Stony Brook, NSLS, Lawrence Berkeley Laboratory, University of California-San Francisco and IBM), as well as general users from other institutions.

Since 1988 the capabilities at X1-A have moved from developmental to operational, the resolution has improved substantially, and the rate of image collection has increased by a factor of three or more. Much work has been done on the design of appropriate specimen environments (microfabricated wet cells with thin windows, helium enclosures, etc.) and on the software. It is a major weakness that there is no technical support available - the scientists, postdocs and students are also called upon to do the maintenance, development and user support.

X-ray microscopy offers the hope of unique capabilities in the study of wet, unaltered biological specimens and in the mapping of elemental constituents. Much development work remains to be done to determine the utility of X-ray microscopy in structural biology. Rapidly expanding demand for the technique could follow if its utility improves with the developments outlined below. By the year 2000 there could be as many as 20 groups involved in major X-ray imaging projects, with a somewhat larger number doing smaller experiments or feasibility studies. One fully supported station, operating full time at top efficiency, could accommodate about 5 groups or 25% of these users.

D. Facilities Needed

Two fully operational X-ray microscopy facilities dedicated primarily to life sciences are planned in the U.S. for the year 2000. These will be at NSLS and at ALS, which are able to provide soft X-ray beams of the necessary coherence and brightness. Each facility will consist of two microscopes; one that will be in a predominantly operational mode, and the other used partly for development purposes. If more facilities become available, the demand will surely grow to saturate them. Each facility requires a complement of ancillary biological and optical microscopy laboratories. Several specific facility needs exist.

Upgrade of current stations: The critical need at NSLS X1-A is for support personnel. The addition of technical staff is required to increase efficiency and scientific productivity. To support efficient utilization of the station, a second microscopy station is required, while the current microscope should be further upgraded to keep pace with continuing developments in X-ray optics. In addition, ancillary biological and microscopy facilities are needed.

For fluorescence microscopy, part-time support at NSLS X26-A should be provided.

New stations: New facilities for soft X-ray imaging are planned for ALS. They are based on an undulator beamline with several branches. In addition, the structural biology community needs to participate in the construction of new facilities for X-ray fluorescence imaging. Rather than building entire beamlines, it would be cost effective to share with the geological and materials science communities, and arrange for about a one-third share of new facilities at the APS and CHESS.

Instrument development: The upgrade of the X1-A station, and especially the twenty-fold increase in brightness expected at ALS, dictates that instrumentation must be developed to match the increased beamline capabilities. For example:

- Continuing development of high resolution zone plates by the Center for X-ray Optics at Lawrence Berkeley Laboratory in collaboration with IBM is essential.
- Detectors with higher count-rate capability must be developed.
- Electronics, data handling and image analysis must be upgraded to keep up with the data rate, and to present the less sophisticated user with a friendly, easy-to-learn environment.

The higher brightness expected at the upgraded facilities also opens up qualitatively superior imaging capabilities based on:

- very rapid imaging microscopy ($\ll 1$ msec/picture),
- phase contrast microscopy, and
- diffraction tomography and holography for three-dimensional imaging.

These and other new approaches will require a significant amount of instrument development to make them useful and accessible for the biological user.

E. Special Consideration: Fluorescent Microprobe for X-ray Imaging

The two-dimensional distributions of chemical elements within a tissue section or a cell can be determined with an X-ray fluorescence microprobe with high spatial resolution. Although sensitivity varies from element to element, data have been collected for such elements as chlorine, potassium, calcium, iron, manganese, copper, and zinc present at levels of 10-100 femtograms with resolution elements of sizes from $1\mu\text{m} \times 1\mu\text{m}$ to $10\mu\text{m} \times 10\mu\text{m}$. Current microprobes obtain this spatial resolution by the use of optical components such as total reflection mirrors, multilayers, zone plates, and pinholes coupled to intense sources of synchrotron radiation. The demand for these types of experiments

would increase if much better spatial resolution were available, e.g. 0.05-0.10 μm . Pushing to higher spatial resolution necessitates the use of low-emittance, high-brilliance storage rings coupled with developments in optics to produce a beam with enough intensity in a small enough area.

One possible nemesis for experiments of this kind is the damage inflicted to the sample by the incident radiation. Both beam heating and the loss of sample structure by radiation damage can be minimized by cooling samples to cryogenic temperatures, but it is not known exactly how much this effect will ultimately limit the spatial resolution obtainable.

The opportunity to examine an individual cell with spatial resolution sufficient to directly image its contents is a tantalizing prospect.

F. Summary

As a result of the construction of synchrotron light sources, particularly at NSLS and at ALS, soft X-ray microscopy is becoming a new imaging modality for biologists. There is a growing realization worldwide that X-ray imaging provides unique capabilities and will be in growing demand.

- In Germany, an institute for biological microscopy with five scientific staff, four support staff and ten students has been created under the direction of Günter Schmahl. The institute operates three stations at the BESSY synchrotron source in Berlin, will be building additional facilities at ESRF and at BESSY II, and is in close contact with Zeiss to explore opportunities for commercial development.
- In England, a major effort involving seven faculty, three postdocs, one technician and five students has been mounted under the direction of Ronald Burge at King's College, London. Experiments are carried out at the Daresbury synchrotron source and in the future also will be done at the ELETTRA synchrotron source in Trieste and at ESRF. The group hosted the International Symposium on X-ray Microscopy, attended by 140 scientists from a dozen countries in September, 1990.
- In Denmark, a new synchrotron radiation laboratory is being built at Aarhus. A major part of its scientific program is biological microscopy.
- In Japan at least five groups at Tsukuba, Osaka, Nagoya, Okazaki and Tokyo are active in the development of biological X-ray imaging with synchrotron radiation. They make use of the Photon Factory and UVSOR, and work in close contact with Nikon, Canon and Hamamatsu in anticipation of commercial developments.
- There are additional projects under development in Italy, China, and elsewhere.

In the U.S., there is only one synchrotron-based X-ray microscope at present. It is undercapitalized and is not available all of the time because of time-sharing commitments to another research team. This greatly limits the potential for satisfying the needs of biological research groups. Microscopic investigation is a time-intensive method, requiring the investigator to spend much time at the machine looking at many samples. At present, it is impossible to carry out more than a handful of projects.

We project a substantial increase in demand if the special features of the method are developed productively for biology and become more generally known. Even with the addition of a new facility at ALS, expansion of the capabilities at NSLS, and improvements in facility utilization, X-ray microscopy will, for the foreseeable future, be a facilities-limited method.

Given two well equipped and adequately funded facilities for the nation, each with two imaging stations, we project that as many as 20 serious projects could be accommodated per year, as well as a somewhat larger number of smaller experiments and pilot studies. Such a capability would produce important new biomedical research, and represents a relatively modest national investment in this unique form of advanced microscopy.

With a relatively modest additional investment, the structural biology community could obtain a share of fluorescence microprobe facilities at higher energy sources (APS, CHESS, NSLS). Developments in optics, detectors and other instrumentation will almost certainly lead to new capabilities that combine imaging with other techniques in structural biology. We foresee soft X-ray absorption spectroscopy and small-angle scattering with spatial resolution from micron- or even submicron-size regions, as well as image formation using spectral features of interest. To implement routine use of such combined approaches, a second straight section would be needed at the ALS, with associated experimental stations and instrumentation by the year 2000.

IV. SMALL-ANGLE SCATTERING

A. Description of Current and Future Research Using Synchrotron Radiation

Experiments using small-angle X-ray scattering to study noncrystalline biological systems provide a broad range of structural and functional information available by no other technique. In many cases, the systems can be studied in a near-native state and sometimes even within living tissue. Since scattering from such systems is usually relatively weak, other factors such as the short duration of a particular state and small intrinsic sample size further reduce the available signal. High brilliance and total flux from synchrotron sources are crucial to carrying out these experiments, which fall into the following four main classes:

- kinetics,
- anomalous scattering,
- intrinsically small scattering volumes, and
- measurement of particularly weak signals.

In addition, use of synchrotron radiation is found, effectively, to decrease radiation damage in many biological specimens, thus providing data superior to that available using conventional X-ray sources.

Current experiments using synchrotron radiation to study noncrystalline specimens involve a large number of experimental groups who study many macromolecular systems and supramolecular assemblies. Kinetic experiments are currently being performed on muscle systems under a variety of dynamic conditions; on mesophase transitions in lipids and other biological liquid crystal systems; on structural transitions in membranes; on assemblies of macromolecular systems such as microtubules and actin; and on enzymes undergoing conformational changes in solution. Anomalous scattering is being used in structural studies of acetylcholine receptors, Ca^{++} ATPase and the filamentous bacteriophages. Intrinsically small objects that can be studied include single muscle fibers, chromosomes, preparations of multi- and unilamellar lipid vesicles, thin layers of rapidly frozen specimens preserved in vitreous ice, and particularly well-ordered domains of fibers oriented magnetically or by other means. Weakly scattering materials that are measurable on a useful time-scale only with synchrotron radiation include dilute solutions of enzymes, super lattices in certain specimens, and reflections to the highest possible resolution from a range of specimens such as lipid mesophases and virus fibers.

Synchrotron sources with higher brilliance will make new classes of experiments possible in the future that are impossible with current sources and instrumentation. Higher brilliance will not only provide better time resolution for studies of the dynamics of systems such as muscle and lipid phase transitions, but it will make possible detailed dynamic measurements on many parts of the diffraction diagram from the single muscle fibers that are used in coordinated mechanical and biochemical manipulations of the contractile system. These are essential for understanding the contractile mechanism. Higher flux will allow studies of rapid structural transitions in enzymes at physiologically relevant concentrations. The ability to obtain diffraction patterns from volumes as small as a cubic micron will make feasible the development of a molecular histology. The structural organization of extracellular matrix or pathological deposits may be determined by scanning a specimen to determine the spatial distribution of molecular orientations and order, which can then be correlated with standard histological methods. Use of micromanipulation and ordering of small specimens with laser beams may create an entirely new technology of specimen preparations for structural analysis.

B. Description of Current Facilities

Station X12-B at NSLS, whose commissioning has recently been completed, is dedicated primarily to small-angle X-ray scattering. Station X9 at NSLS has about 25% of its time allocated to small-angle scattering. The remainder of the experiments are carried out on crystallography stations at SSRL, CHESS and NSLS, which generally are not optimized for small-angle scattering and are in heavy demand by the crystallographic community.

C. Evaluation of Current Situation and Projection of Future Demand

Current use of synchrotron sources for small-angle X-ray scattering is limited in the U.S. by availability of instrumentation. The single station dedicated primarily to small-angle X-ray scattering is both underfunded and understaffed. Most small-angle scattering experiments are carried out on instruments designed for single-crystal diffraction and are not optimized for small-angle scattering data collection or for time-resolved studies. Only one experimental station in the U.S. (X12-B at NSLS) is optimized for small-angle scattering with regard to time-resolved studies and none is under construction. Experimental stations capable of rapid wavelength tuning for anomalous scattering studies are limited. In addition, they are incapable of covering the range of wavelengths needed for many critical experiments. The deficiencies in instrumentation demonstrate the fact that the U.S. lags far behind Europe and Japan in development of an advanced capability for small-angle X-ray scattering.

There is a strong community of researchers involved in small-angle scattering in North America. There are 45 independent investigators in this field who are known to BioSync. Two-thirds of the 27 responding to the user questionnaire consider synchrotron sources very important or essential for their research. Current demand by the 45 research groups is estimated to be about 500 beam-days per year. Assuming that 20% of the current trainees will eventually become independent investigators and that growth is limited to current types of experimentation, we estimate a conservative growth rate of 50 beam-days (10%) per year. Assuming efficient utilization of new synchrotron sources capable of extending the variety of specimens that can be studied and of increasing time resolution, we would project a growth rate of 125 beam-days (25%) per year. These growth rates result in the upper and lower projections of needed beamtime in Table IV-1.

Table IV-1. Estimates of Beamtime Needed for Small-angle X-ray Scattering

	Beam Days/Year			
	1991	1992	1995	2000
Lower estimate	500	550	730	1180
Upper estimate	500	625	1220	3725

Current estimated demand could be met if existing stations operated at peak performance. However, the mismatch of instrumentation to experiment and the need to move equipment in and out of experimental stations used primarily for crystallography effectively reduce usable time by at least a factor of two.

D. Facilities Needed

Three new stations will be needed by the year 2000 to meet anticipated demand. We conclude that meeting current demand requires two stations fully developed and optimized for small-angle scattering with capabilities for time-resolved studies and for anomalous scattering (rapid wavelength tunability); one dedicated station is currently in operation. This estimate is based on the assumption that each station provides about 250 days of usable time per year. Since the feasibility of many experiments depends on the highest intensity attainable, any station built for biological small-angle X-ray scattering at an existing synchrotron radiation facility should be on a beamline equipped with an insertion device. The experimental station at NSLS dedicated to small-angle scattering is not on an insertion-device beamline. Meeting future demand will require development of at least two more stations by the year 2000. A small proportion of this demand may be met by parasitic use of crystallography stations that are not optimized for small-angle scattering experiments.

The following conditions should be met to optimize the results from high-resolution and time-resolved small-angle scattering experiments:

- brilliant beam with stable position for small samples,
- continuous and rapid tunability over a wide energy range with a variable band width,
- computer-controlled fast shutters for the incident beam during stroboscopic experiments,
- controlled environment (temperature, pressure and electric and magnetic field strength) and chemical composition for static/equilibrium and time-resolved studies,
- provision for temperature- and pressure-jump and rapid-mixing experiments for time-resolved studies,
- fine control over sample position for small samples, for minimizing radiation damage and for samples with large crystallite size,
- ability to monitor the applied experimental variable in the sample adjacent to the interrogating X-ray beam during the course of the experiment, and
- biochemical laboratory outfitted with standard chemicals and solvents, centrifuges, walk-in refrigerators, ice-making machines, etc.

It is expected that future developments will be made in combining time-resolved X-ray scattering with simultaneous use of differential scanning calorimetry, polarized light microscopy, Raman scattering, FTIR or electron spin resonance, but specific requirements for these are not included in the above list of needs.

E. Special Consideration: Detectors for Scattering from Noncrystalline Samples

Fast, two-dimensional detectors with large active areas of the multiwire or solid-state type will be needed for dynamic measurements on fibers and oriented samples and for quantitative powder diffraction. The possibility of using storage-phosphor or imaging-plate detectors should also be considered in this application. The ability to rapidly evaluate and to process, store and network data on-site is deemed critical to the successful execution of small-angle X-ray scattering studies.

F. Summary

Small-angle scattering from noncrystalline biological specimens has the capability of answering many questions about the structure and function of biological systems that cannot be addressed by any other technique. Use of high-intensity synchrotron radiation greatly enhances the capabilities of this technique making possible many experiments that cannot be done using conventional sources. With the inception of the very high-intensity sources planned for the coming decade, an entirely new generation of innovative experiments will be possible using small-angle scattering. However, current facilities lag far behind their counterparts in Europe and Japan. Only about half the current demand for synchrotron radiation for small-angle scattering can be met with current facilities, and many experiments that are feasible at facilities abroad cannot be carried out here. To meet the anticipated demand for small-angle scattering experiments, one new, well equipped experimental station should be developed in the near future, and two more by the end of the decade.

V. X-RAY SPECTROSCOPY

A. Description of Current and Future Research Using Synchrotron Radiation

X-ray absorption spectroscopy (XAS) is the premier technique for determining to high resolution the local structural environment of metal ions in metalloproteins. Functionally important changes in the structures of metal centers are often very subtle, and XAS is particularly powerful in characterizing small changes in metal-site structure for proteins whose structures have been determined previously by crystallography or NMR. XAS is conventionally divided into extended X-ray absorption fine structure (EXAFS) for spectral structure well above the absorption edge, and X-ray absorption near-edge structure (XANES) for spectral structure within ~50 eV of the absorption edge.

Most of the current biological use of XAS involves EXAFS studies of metalloproteins. These have typically been static studies of stable intermediates. In many cases, little or nothing is known about the structure of the site, and the goal of the EXAFS studies is to determine the ligation of the metal atom. Notable successes include structure elucidation of the Fe-Mo cofactor in nitrogenase and the Mn cluster in the photosynthetic oxygen-evolving complex, where important details of completely unknown structures were developed. In addition, other examples include the metal sites in cytochrome oxidase, hemerythrin, and hemocyanin, where structural features were refined using EXAFS. Although XANES is often utilized as a "fingerprint" for a particular structural feature, little quantitative use is made of XANES at present. Most current XAS studies utilize hard X-rays (5-25 keV) with sample concentrations greater than 1 mM metal. Most of the current biological XAS work in the U.S. is performed by approximately 15 experimental groups, with occasional use by approximately 15 other groups.

Several new experimental methodologies are being developed that will increase the utilization of XAS in structural biology. These are being driven by past successes of XAS, by the anticipation of new high-brilliance synchrotron sources of the third generation, and by numerous technical developments applicable to both new and existing synchrotron sources. The following are among the new experimental methodologies.

Low energy XAS: Recent work has demonstrated the utility of studies at low energies (e.g. the sulfur K-edge at 2500 eV) for determining electronic structure. Future experiments are likely to utilize even lower energies (e.g. the manganese L-edge at 650 eV) for determining metal-ion electronic structure. While these experiments are possible using current synchrotron sources, perhaps with new detectors, they are very well suited for the soft X-ray source at ALS.

Polarization Dependent Studies: Most biological applications of XAS have taken advantage of the ability of XAS to characterize samples that cannot be crystallized. There is, however, increasing recognition that polarized XAS offers the possibility to enhance significantly the information that can be obtained from an oriented sample (crystalline in two or three dimensions or membranes ordered in one dimension). These experiments are possible using the current synchrotron sources.

Magnetic Circular Dichroism: X-ray magnetic circular dichroism (MCD) has been demonstrated at room temperature for ferromagnetic samples. No experiments have yet been reported for biological materials in the X-ray region. However, the use of low temperatures should permit application of X-ray MCD to paramagnetic samples. Efficient utilization of this method will require new experimental stations to optimize the production of circularly polarized X-rays.

X-ray Raman Scattering: X-ray Raman scattering has been used to measure low-energy absorption (e.g. carbon K-edge) using hard X-rays. This may be a useful approach for measuring soft X-ray spectra of proteins. Application to dilute systems will require the third generation of X-ray sources.

Time-resolved Studies: Most present XAS studies are static investigations of stable intermediates, the primary exception being observation of photo-dissociated hemoprotein carbonyl complexes. The two promising approaches to time-resolved XAS that have been put forward are dispersive XAS and stopped flow experiments. Both will require high-brilliance, third-generation synchrotron sources to be feasible for biological systems. Controlled freeze quenching after rapid mixing can be used to trap and study intermediates if fast energy-resolved detectors are developed. For example, rapid mixing of an enzyme and its substrates followed by freeze quenching after as little as 5 msec may allow structural studies of reaction and enzyme intermediates. With new and efficient detectors, even present stations would

be able to handle a large variety of kinetics problems involving metalloenzymes reacting with substrates. This would broach a whole new research area where structural changes in catalytic sites of metalloenzymes could for the first time be followed during the catalytic cycle.

High Resolution Fluorescence: There is increasing interest in utilizing XANES for investigation of metal-ion electronic structure. A useful complement to XANES is high-resolution fluorescence. To be successful on dilute biological samples, these experiments will require high-brilliance synchrotron sources.

Micro-spot XAS: New high-brilliance synchrotron sources will permit XAS to be performed on increasingly small sample volumes. Although this may pose formidable problems for sample integrity, micro XAS will have two important implications for structural biology. It will permit studies of proteins that can only be prepared in small amounts, and it may permit studies to be performed on intact cells, including localization of metal atoms in organelles.

Site Dynamics: Accurate measurement of XAS as a function of temperature has the potential for revealing details of the vibrational coupling of scattering centers. This may be very important in understanding the chemical properties of catalytic and structural metal centers. Intrinsic to developments in this area is the need for good cryogenic equipment specifically married to needed developments in new detectors.

B. Description of Current and Planned Facilities

Experimental stations for XAS are described in Appendix B, Tables B-1, B-4 and B-5. In Table V-1 we estimate the maximum number of days per year that each station is available for biological XAS experiments. Station U9-B at NSLS, used for UV spectroscopy, is not included here.

Based on past experience, we estimate that approximately 20% of the XAS beamtime at NSLS and CHESS will be allocated to "biological" studies. Historically SSRL has had a greater emphasis on biological experiments, and thus we estimate that approximately one-third of the SSRL XAS time will go to "biological" experiments. We estimate 210 days/year of experimental time at SSRL (based on SSRL projections for time after the facility is converted to a fully dedicated synchrotron source), 220 days/year at CHESS and 250 days/year at NSLS. For NSLS we assume 25% of the time on each station goes to general users (~60 days/year) with the remainder assigned to PRTs. The total experimental time available for biological X-ray spectroscopy on all experimental stations, now existing or being commissioned, is approximately 850 days/year if all stations operate at full capacity.

Table V-1. Beamtime Estimates for Current and Planned XAS Experimental Stations

Synchrotron Station	Total Days/Year For Biology	Comments
SSRL		
4-1	70	
4-2	70	
7-3	70	
10-2	20	Expected to be available for users in 1992.
2-3	70	Weak beam.
6-2	70	PRT beamline; one-third of time for general users.
1-5	35	Weak beam; suitable for feasibility studies in SSRL's "rapid turnaround" program
CHESS		
C2	65	No biological equipment.
NSLS		
PRT and General Users		
X9	190	
X10-C	60	
X19-A	100	
General Users Only		
X11-A	12	
X18-B	12	
X23-A	12	
Total	856	

C. Evaluation of Current Situation and Projection of Future Demands

Successes of XAS experiments, recent advances in XAS theory, and a growing consensus regarding the proper methods for XAS data analysis have brought the field to a point of maturity where many potential experimenters are contemplating how to add XAS to their array of biophysical methods. However, the practicalities of carrying out XAS experiments are still hindering many investigators from becoming involved. Documentable demand for XAS facilities today is seriously affected by two factors, one technical and one logistical.

On the technical side, two opposing effects, both arising from sample concentration, limit the use of XAS. The 1 mM concentration typical of biological samples is quite dilute with respect to detection of an XAS signal from a metal (weak signal), but is very concentrated with respect to the biological macromolecule (strong noise of background scatter). The signal is difficult to detect. In addition, the very large amounts of biological macromolecule that are needed severely limit the problems for which XAS can be used as an experimental method. Enormous benefit could be gained by the availability of appropriate energy-resolving detectors, which could discriminate the large background of Compton (scattered) photons from the slightly less energetic fluorescence signal. Such detectors would immediately improve the throughput of current samples, and, more importantly, would allow XAS investigation of new molecules that cannot be prepared in concentrations high enough for analysis with current detectors. Even with the existing synchrotron radiation sources, most biological XAS experiments are detector limited. The mismatch between detector and source capabilities will become even more severe with the synchrotron sources of the third generation, such as APS. New detectors will be absolutely required to take advantage of the unique capabilities offered by APS. The combination of new synchrotron sources and better detectors has the potential to transform this field.

On the logistical side, biological XAS is seriously limited by the unnecessarily "heroic" nature of the experiments themselves. A lack of support facilities at the current laboratories (biological laboratory space, anaerobic sample handling, inexpensive housing) is largely responsible for this and limits the effectiveness of even experienced users. There is virtually no effective user support for the occasional user of XAS methods. This is particularly detrimental in a field where most of the investigators who would be natural XAS experimenters have rather broad-ranging research programs in biophysics or bioinorganic chemistry. These investigators simply cannot afford the effort necessary to do XAS when it would be only one of several experimental methods they use. Biological XAS is also limited by the scarcity of beamtime. At present, beamtime is sufficiently scarce that XAS experiments are limited to the most pressing, but abundant, samples (i.e. samples where little or no other structural information is available).

We have estimated current demand for the case where all groups known to be using XAS do a modest but significant amount of data collection - 15 days/year. Such a group would employ a half-dozen workers making samples, collecting and analyzing data, and doing all the other measurements that go into a typical biophysical research program on macromolecules. Thirty biological XAS groups have been identified from the BioSync mailing list, clearly a lower limit. We estimate the current total demand by these groups to be approximately 450 days a year. This minimum is in excess of the number of days reported by the synchrotron radiation facilities to have been available in 1990, and underscores the critical limitation in resources. Currently XAS work is so difficult, because of beamtime and detector limitations, that, if the experiments became truly reliable and routine, we estimate that a minimum of twice as much beamtime could be used by each active group, without attracting any new workers, i.e.

$$30 \text{ XAS groups} \times 30 \text{ days/year/group} = 900 \text{ days/year.}$$

This number misses an important consideration, namely that at the 100 or so vigorous research universities in the U.S. there are two or more bioinorganic groups who would use XAS routinely, were it not so difficult to get a program launched. Using this conservative estimate for number of groups gives

$$200 \text{ bioinorganic groups} \times 15 \text{ days/year/group} = 3000 \text{ days/year.}$$

There are presently ~400 research groups identified as "bioinorganic" from the Bioinorganic Chemistry Electronic Mail List; most of these would have at least occasional need for XAS. This upper estimate for number of groups gives

$$400 \text{ bioinorganic groups} \times 15 \text{ days/year/group} = 6000 \text{ days/year.}$$

The pool of user groups will grow considerably beyond those identified as “bioinorganic” when facilities for soft X-ray spectroscopy at the ALS make absorption edges of lighter elements available for study.

In the previous section, we estimated the upper limit of beamtime availability to be approximately 850 days/year, less than the upper estimate of 900 days of potential demand from current XAS groups, and less than 30% of our conservative estimate of potential demand, at 15 days/year (a lower limit), by the potential 200 or so groups that would come on-line provided the technique were reliably available.

D. Facilities Needed

Photons: Assuming only an evolutionary increase in number of experiments, there will be a shortfall of approximately 9 station-years. (3000 days demand – 850 days supply = 2150 days shortfall = 9 station-years @ 250 days/year/station)

A limited list of additional special-purpose stations, for which a need can readily be identified, includes:

- circularly polarized XAS,
- soft X-ray biological XAS,
- time-resolved, flow experiments, and
- high resolution fluorescence.

Equipment: The most pressing need (needed immediately to take advantage of the capabilities of existing stations) is the development of new detectors. Cryostats, ready availability of equipment for anaerobic sample handling, some general laboratory equipment, and suitable space to house it are also needed. The next most pressing need is for user-friendly support facilities. The extreme difficulty of gaining access to synchrotron radiation and to performing XAS experiments at existing synchrotron radiation facilities blocks the entry of new investigators. User-friendly facilities are necessary in order to adequately exploit the scientific opportunities possible in biological XAS. (This would also improve the efficiency of existing users.) Housing/travel costs (particularly at SSRL) block the full use of synchrotron radiation, particularly by new research groups.

E. Special Consideration: The Role of New Detectors in Biological XAS

The single most important issue limiting progress in biological XAS is the need for development of new X-ray detectors. Most biological XAS experiments are limited not by the available X-ray flux, but by the characteristics of available X-ray detectors.

The XAS signal from biological samples is usually detected as fluorescence from the absorbing atom(s). The signal is very weak and is embedded in a much larger background of Compton scatter. Detectors are required that can handle extremely high count rates (up to 10^8 counts/sec from current synchrotron sources) in order to detect the weak XAS signal. The detector proposed here would address the count rate problem by using multiple electronic channels.

One electronic channel of a solid-state detector has a maximum count rate of $\sim 10^5$ sec⁻¹. Higher count rates give significant nonlinearities (saturation) due to detector deadtimes. The experimental strategy for handling count rates of $\sim 10^8$ sec⁻¹ with single detectors on current synchrotron sources is to reduce the count rate by moving the detector away from the sample and to increase the measurement time. The result is typically 8-12 hours to measure data with marginal signal-to-noise from a very concentrated biological sample. The detector is clearly limiting.

How many detector channels are needed?

The fluorescence signal from a typical sample (1 mM Fe) is

$$\begin{aligned} & 10^{10} \text{ photons/sec incident on the sample} \\ & \times 0.001 \text{ fraction of photons absorbed by 1 mM Fe} \\ & \times 0.8 \text{ fluorescent yield} \\ & \times 10\% \text{ of } 4\pi \text{ solid angle intercepted by the detector} \\ & = 8 \times 10^5 \text{ photons/sec of fluorescence.} \end{aligned}$$

This signal is embedded in a scattering background that is typically 100 times larger, to give $\sim 10^8$ total photons/sec on the detector. One detector channel can record 10^5 counts/sec at most. This limit is fundamental; even if detector deadtime could be overcome, count rates significantly higher than 10^5 sec^{-1} could mean > 1 photon per synchrotron pulse - thus leading to detector pileup. ~ 1000 channels per detector are needed! (The largest detector array available at present has 13 channels.) This is based on current synchrotron sources; the problem would be even worse at the third-generation, high-brilliance sources. To permit separation of the fluorescence signal from the scattering noise, the ideal detector would also have energy resolution of 200-300 eV.

How many counts are needed for a "good" EXAFS spectrum?

$$\text{EXAFS} = \frac{\mu}{\mu_0} \quad (\mu = \text{observed absorption coefficient,} \\ \mu_0 = \text{theoretical free-atom absorption coefficient})$$

The typical EXAFS spectrum for a biological sample has values oscillating between +5 and -5 from $k \sim 4 \text{ \AA}^{-1}$ to $k \sim 15 \text{ \AA}^{-1}$ above the absorption edge of the absorbing atom. Because the spectrum is taken as χk^3 , the desired signal within the X-ray fluorescence from the absorber decreases with increasing resolution. "Good" data is defined as having a signal-to-noise ratio of 10 or better. Since the uncertainty in N counts is \sqrt{N} , for any resolution both the minimum counts needed to achieve the desired signal-to-noise and the minimum counting time given a rate of 8×10^5 fluorescence counts/sec/mM Fe can be determined. A summary for pertinent resolutions is given in Table V-2.

Table V-2. Factors in Measurement of EXAFS at Various Resolutions

	Resolution (k)		
	4 \AA^{-1}	6.5 \AA^{-1}	15 \AA^{-1}
modulation of	15%	3%	0.3%
For signal-to-noise > 10 :			
minimum counts	1/70	1/300	1/3300
minimum counting time:			
1 mM Fe	6 msec	0.1 sec	12 sec
0.1 mM Fe	60 msec	1 sec	120 sec

How fast could a scan be done with a 1000-element detector?

If there are 1000 detector channels, each capable of a count rate of 10^5 sec^{-1} , it would be possible to count 10^8 counts/sec. If as little as 0.1% of the radiation is fluorescence, it would still be possible to count 10^5 useful counts/sec. (This corresponds roughly to 0.1 mM Fe.) The desired statistics could then be obtained in 120 sec per point at the end of the scan (10^7 counts at $k = 15 \text{ \AA}^{-1}$) and $\ll 1$ sec per point at the beginning of the scan (10^4 counts at $k = 4 \text{ \AA}^{-1}$). In fact, 10^5 counts/sec will give acceptable statistics in 1 sec out to $k \sim 6.5 \text{ \AA}^{-1}$. The ideal counting times would then be

~1 sec for energies below 7\AA^{-1} (shorter integration times will not give any improvement in data collection speed due to the time required to move the monochromator). Above $k \sim 7\text{\AA}^{-1}$, the integration times should increase steeply to 120 sec at $k = 15\text{\AA}^{-1}$. If the average measurement time is 20 sec in the $7\text{-}15\text{\AA}^{-1}$ range (this assumes a more rapid than necessary increase in measurement time), measurement of the $k = 7\text{-}15\text{\AA}^{-1}$ region with a step size of 0.05\AA^{-1} would require 3200 sec. The initial region would require ~400 sec (1 sec to move the monochromator and 1 sec to measure for each of 200 points) for a total of ~1 hour per sample. Table V-3 compares the proposed detector with the current situation and shows the dramatic improvement that could be achieved in experimental conditions.

Table V-3. Experimental Parameters for Two Detector Types

	Current Detectors	1000-element Detector
Fe concentration	1.0 mM	0.1 mM
k range	4-13 \AA^{-1}	4-15 \AA^{-1}
signal-to-noise	2-3	10
measurement time	8-12 hours	1 hour

Conclusions

- Current stations would provide sufficient flux to give data with significantly enhanced k range in dramatically reduced time if adequate detectors were available.
- XAS data collection time could be decreased to be comparable to the time required for a UV-visible or epr spectrum, or sample concentration could be decreased by two orders of magnitude with the current data collection time. This would completely change the use of XAS in structural biology.

F. Summary

Instrumentation: A concerted effort to develop optimized detectors would yield enormous improvements in XAS with relatively modest costs (see previous section). A limited number of 13-element Ge detectors are now available; however demand exceeds availability, and the detectors are far from ideal. There has been a large imbalance in resource allocation with respect to XAS. Much effort has gone to experimental station development and operation, while detector development has been neglected. As a result, virtually none of the present biological XAS experiments are run under ideal (i.e. flux limited) conditions. It is clear that low-temperature measurements are required in order to obtain the best signal-to-noise ratio and to avoid sample decomposition. Good temperature control ($\leq \pm 5\%$) in the 4-300K domain is essential for taking advantage of the available and potential data quality. In addition, improvements are needed in monochromator stability and design, particularly in addressing the problem of high heat-load stability.

Scientific Developments: In the future, it should be possible and desirable to apply XAS to a much wider range of samples. One of the forces driving this increase will be the increase in the number of proteins for which three-dimensional structures are determined using NMR and crystallography. In this environment, XAS is an important part of the general strategy in biology, of using experimental variational methods to probe function in macromolecules. Use of EXAFS on derivatives and rapidly frozen intermediate states of systems for which an initial crystallographic analysis is already available has the potential to be the best current approach to a detailed analysis of structure changes occurring in a catalyst during turnover. As an increasing number of proteins are selectively modified using the techniques of molecular biology, there will be an increasing number of samples of "mutant proteins" for which XAS analysis will be the most efficient approach for initial structure characterization. In addition, recent advances in XAS theory and the growing consensus regarding the proper methods for XAS data analysis will open the field to an increasing number of occasional users. This again will dramatically increase the demand for XAS time. Finally, knowledge of the metal-ligand distances (as determined from EXAFS) can provide an important constraint in refining three-dimensional models from crystallography and NMR.

Synchrotron Radiation Sources: The most pressing demand for biological XAS will be the availability of beamtime for “conventional” experiments at existing synchrotron radiation sources. Because of the limitations of current detectors, relatively few experiments will be able to take advantage immediately of the high brilliance of third-generation synchrotron radiation sources. However, increasing demand for beamtime at the (already oversubscribed) existing sources will surely be followed by an increase in demand for the high-brilliance lines as their utility becomes manifest. The unique capability of the ALS to produce intense radiation in the soft X-ray region will allow study of additional elemental absorption edges that are of biological significance.

Support: Only limited support is available for XAS. There are at least 30 groups of structural biologists that could currently utilize XAS, and without doubt there will be increasing demand for support for the “occasional” XAS user. The present lack of such support is a significant barrier to the entry of new users and limits the effectiveness of experienced users. In addition to financial support directed to investigators, user support at synchrotron radiation facilities is absolutely essential for the occasional user of XAS methods. A useful analogy can be made with the evolution of NMR facilities from a rare resource (“the East Coast 220 MHz machine”) to a situation where almost any qualified scientist can gain appropriate access to the technique. In the case of XAS, the current stage of development in “service” is much more primitive and may never become a “local” utility, but the full development even of presently available facilities demands close attention to user-friendliness on the part of proprietors of the technology.

While the net effect of advances in detector technology could be to decrease the demand for beamtime (less time per experiment), it is far more likely to increase the net demand. Rather than less time per experiment, there will be more numerous cases of less sample per experiment with no change in experiment time. This will be the means for making more projects accessible by advancing to more realistic sample concentrations (<1 mM). Lower concentrations, or the ability to use small (<0.1 ml) sample volumes, will also make feasible new kinds of experiments, i.e. flow kinetics, photochemical activation and freeze-quenching. Thus, a substantial increase in pressure on the facilities is projected as they become better at doing experiments under conditions closer to those in cells, and more amenable to kinetic and photochemical methods. A strong historical precedent exists in biophysics: EPR at low temperatures, using homodyne detection, and pulse and FT NMR methods, caused explosions in utilization, even though the initial effect was to make rather simple improvements in required sample concentrations. The eventual effect of these improvements was underestimated by all concerned. Thus the projections here should be regarded as conservative.

VI. OTHER DISCIPLINES

A. Internal and Interfacial Structure of Membranes Using X-ray Standing Waves

The X-ray standing wave (XSW) method developed in the mid-sixties was used originally to determine to atomic resolution the positions of heavy atoms in and on perfect single crystals of silicon and germanium. The advent of layered synthetic microstructures, used primarily as wide-bandpass X-ray monochromators, heralded a new era in the use of XSW to study biologically relevant structures of order 10-100Å in size. The original measurements were performed on model membrane Langmuir-Blodgett films and served to establish the utility of the XSW approach in determining heavy-atom locations in such systems to sub-angstrom resolution and in tracking the heavy-atom layer as it moves during a thermotropic transition. Recent results show that the XSW is well defined at a distance of nearly 1000Å from the XSW-generating surface. Thus, XSW is useful in probing objects of this size without a compromise in resolution. The stage is now set to extend these measurements to detailed structural studies of cellular and reconstituted membranes and to thin protein and lipid films.

In addition to the above measurements on well ordered systems, the XSW method has been used successfully to "directly" profile ion distribution at the membrane/aqueous interface. With this method, the theories of membrane electrostatics developed over the past 70 years finally are experimentally tractable.

Only one group is working in this area. Up to ten groups, each using about five weeks of experimental time per year, are expected by the year 2000.

B. Photon Activation Therapy (PAT)

PAT is a radiological technique wherein the radiation dose delivered to a tumor is enhanced by the introduction of a heavy atom (typically stable ^{127}I as iodinated deoxyuridine, IdU) into the DNA of proliferating tumor cells. Irreversible cytotoxic effects of IdU are expressed upon activation by external and implanted photon sources. Synchrotron radiation is used in enhancement studies to determine the radiobiological parameters for the calculation of dose enhancement and for PAT optimization. Typically, measurements involve the irradiation of cells with and without the heavy atom, above and below the K-absorption edge of the heavy atom and over a range of dose rates. The thrust of this research is to understand the mechanism of radiation enhancement and to exploit it in selective tumor inactivation.

Only one group is working in this area to date. Up to three groups, each using about two weeks of experimental time per year, are expected by the year 2000.

C. X-Radiation Damage

While synchrotron radiation offers many advantages for X-ray studies, radiation damage can limit its usefulness. Considering the importance of X-rays as a structural probe and the anticipated use of new, highly brilliant synchrotron radiation sources in structural biology, the problem of radiation damage must be carefully examined. The thrust of this effort is to understand the mechanism of radiation damage and the environmental factors influencing radiation effects. Such information will be used for devising prudent experimental strategies for making measurements at synchrotron sources while minimizing the effects of radiation damage and should provide insights into the biochemical and biological consequences of radiation damage.

This represents a minor component of several groups' efforts. It is expected to be the principal concern of up to five groups, each using about three weeks of experimental time per year, by the year 2000.

VII. OTHER CONCERNS

A. Impact of Improvement in Synchrotron Sources and Hardware

The design of storage rings for synchrotron radiation research is moving into a mature phase. Over the last 15 years there has been a general movement from parasitic operations on existing storage rings built for high energy physics to dedicated sources fully optimized for experiments with synchrotron radiation. The third generation of synchrotron sources in machines such as the ALS and the APS is now under construction. These radiation sources equipped with insertion devices such as wigglers and undulators will bring unsurpassed opportunities to many fields of study including structural biology. It is difficult to envision designing synchrotron sources that exceed the design characteristics of this current generation. A very few speciality insertion devices may be made for producing special effects such as circularly polarized light, for instance, but the likelihood of revolutionary developments in this area is small. It appears that storage rings with a significantly lower design emittance than for the ALS or the APS could be designed. Although there is a community that would like a diffraction-limited, hard X-ray source, such rings would benefit only a handful of biological experiments, for example soft X-ray microscopy. Thus other directions should be pursued in making improvements to the overall optimization of the experimental design.

Improvement of beamline hardware and operating techniques may yield rich dividends. Paying attention to beam position monitoring at individual experimental stations and maximizing beam stability are key to consistently obtaining high quality data. Of particular concern is how the running of many undulator devices will influence the beam quality and stability on all beamlines. New designs for heat-resistant X-ray optical elements must be developed and tested in order not to diminish the brilliance of the new sources from heat-induced distortions in the optics.

B. Impact of Molecular Biology on the Need for Synchrotron Radiation

Current molecular biological techniques are increasing dramatically the need for structural information at the molecular, molecular complex, suborganelle, organelle, and cellular levels. This, in turn, impacts the demand for synchrotron radiation in explosive proportion.

The techniques of gene identification, cloning and gene expression will allow an unparalleled number of protein, RNA and DNA molecules to be produced in qualities and quantities amenable to three-dimensional structural study, which will be fundamental for understanding the biological function of these molecules. Once the structure of a molecule is known, molecular biological techniques such as site-directed mutagenesis and protein engineering will generate an explosion of molecular variants or molecular complexes whose structures have to be determined. The explosive increment in the need for structural information on variants and complexes is even greater in the field of drug-design and improvement. Variant molecules can be studied by crystallographic, small-angle scattering and absorption methods using synchrotron radiation.

On the cellular level, the modern molecular biological techniques of gene transfer and mutagenesis in eukaryotic cells will dramatically increase the production of cells with altered phenotypes, which can be studied at the cellular, organelle and suborganelle levels in the "natural" state by imaging and scattering. Molecular biological techniques can also allow preferential, localized observation of suborganelles and organelles distinguished by their elemental composition by scattering and imaging experiments.

The impact described above is being greatly amplified by the results of the Human Genome Project.

C. Unanticipated Research Opportunities from New Facilities

Construction of new facilities for synchrotron radiation will provide the potential for carrying out new science not possible with current sources. These facilities will not merely allow us to do more of the same types of experiments currently being done, but will provide whole new classes of experiments. These new sources will provide both incremental and dramatic improvements over current capabilities. The availability of more intense sources of radiation will indeed provide the potential for greater throughput: the ability to solve more structures and more variants of each structure.

Proteins that form smaller crystals will be studied. Larger unit cells will be characterized. Higher resolution data will be obtainable. Kinetic studies will have higher time resolution. Each of these, taken alone, is an incremental improvement in design of experiments currently being performed. Taken together, however, they represent a dramatic increase in our abilities to characterize macromolecular structure and correlate it to function. Evolutionary change in experimental capabilities will also lead to revolutionary change in our understanding of macromolecular function.

New sources will also provide the potential to work in entirely new experimental regimes. Time resolution completely inaccessible currently will become possible. Structural characterization of 100 mutants of a single protein will be realizable. Point-by-point scanning of histological sections could provide characterization of molecular order as a function of position, which could be the basis for a "scanning molecular biology".

New science will derive from evolutionary improvements in experimental design. The result will be revolutionary changes in our understanding of macromolecular function. New sources will further provide the potential for entirely new experimental approaches. These should similarly provide whole new views of macromolecular structure and insight into biomolecular function.

D. Computational Resources to Match Synchrotron Radiation Resources

The development of computational resources in parallel with development of synchrotron sources will be critical for the storage, visualization and processing of the vast amount of structural data to be generated. Computational abilities can, in and of themselves, provide a driving force for technical innovations. For instance, the wide variety of scanning microscopes now being applied to biological systems can only be implemented with computational support. Three-dimensional imaging with visible light based on scanning confocal microscopy, reconstruction from through-focus series or tomographic methods require substantial computation. Correlating, comparing, contrasting or simply visualizing three-dimensional data sets or three-dimensional images demand substantial computer effort. It is critical to the success of structural biology in the 1990's that the initiative in experimental resources is mirrored by provision of the computational resources needed to manipulate the data being collected. The complexity of biomolecular structures can only be visualized with substantial graphics capabilities. The remarkable advances in computer hardware provide the potential for manipulation of this vast amount of structural data. Provision of the necessary computational resources, both hardware and software, will be critical for efficient use of synchrotron sources for structural biology.

VIII. COSTS

A. Costs of Current Facilities

A reasonably accurate estimate of the cost of operating existing experimental stations for structural biology can be obtained from the responses by facility staff at NSLS, CHESS and SSRL to their questionnaire, as in Table VIII-1. Annual support for structural biology, including direct and indirect costs, over all three facilities has increased from around \$2.5M in 1985 to \$4.3M in 1990.

We emphasize that this is an incremental support cost; not included are the much larger base costs of running the entire synchrotron radiation facility, and the accelerator itself. These base costs should strictly be distributed over all users, in proportion to their usage of the facility. However, since no facility that we know of levies charges on structural biology funding agencies or users in this way, we do not include base costs here.

In 1990, roughly ten experimental stations were supported (all or part of U9-B, X1-A, X9, X11-A, X12-B, X12-C and X19-A at NSLS; the equivalent of two full stations at CHESS; the equivalent of three full stations at SSRL) for an average cost per station of \$430K. These stations were staffed by a total of 34 personnel, at an average of 3.4 staff per station and an average cost of \$126.5K per staff member per year. We note that, as an internal check, this number seems reasonable. If we assume an indirect cost rate of 65%, then indirect costs are \$49.8K per staff member per year. The balance of \$76.7K is direct costs and may be divided as \$25K for supplies and operations, \$40K salary and \$12K fringe benefits if we assume a 30% fringe benefit rate. The \$40K average salary is realistic; a range from \$20K to \$65K is likely.

However, do these staffing levels truly represent the staff support for structural biology? Existing facilities for structural biology such as CHESS or SSRL are effective only because there is, in effect, a hidden staff subsidy that supports structural biology, but is not directly accountable to structural biology and hence not included in the above staffing levels. Examples include the CHESS operators, machinist and electronics specialist, paid by the overall NSF grant or by Cornell University, and their counterparts at SSRL, paid by the DOE grant or by Stanford University. Further, the administrative load is shared over all users without regard to scientific discipline; for example, there is a single Proposal Evaluation Panel, a single administrative manager, and a single beamtime scheduler at both facilities. The economies of scale are substantial, and allow these facilities to function effectively.

We believe that all existing facilities are seriously understaffed. This leads to less effective user support, inadequate development of new technologies and techniques, staff burnout, and fiscal diseconomies that arise from underutilization of expensive capital equipment. It is preposterous to invest tens (or hundreds) of millions of dollars on construction of a synchrotron radiation facility, and then to slowly strangle it by seriously inadequate operating budgets.

In cases where this hidden staff subsidy from the facility is reduced or absent, as in many of the NSLS beamlines, it is sometimes supplied by the host institutions of the PRT members. For example, a host institution may supply accounting and purchasing functions, or designate a scientist as the administrative manager. This is an ineffective mode of operation; when the hidden staff subsidy is minimal or remote, user support and the effectiveness of the facility suffer.

An additional capital cost is associated with construction of new apparatus and experimental stations. Capital cost was almost certainly not included in the above figures by the questionnaire respondents; they therefore represent incremental operating costs only. Capital cost can vary widely depending on the extent of the construction; it is sometimes difficult to distinguish a true capital cost (hardware only) from a total construction cost (hardware + staff costs + supplies to execute the construction). We supply one reasonably current figure, that for construction in 1988-90 of the CHESS East Laboratory and the new B2 station. This laboratory includes a wiggler source, a bending magnet source, three front ends, the X-ray optics and beam transport to serve five experimental hutches, a biohazards facility and five experimental stations ranging from diffraction physics through materials science to structural biology. The total capital cost is estimated at \$3.0M, an average of \$600K per experimental station. However, the bending magnet line required little optics as it is substantially used as a white beamline; and other hardware was relatively modest.

B. Costs of Future Facilities

Capital costs: On the national scale, the total anticipated costs clearly depend on the total number of new beamlines and experimental stations to be constructed, on a level that is appropriate to meet the user demand. They also depend on the mix of new stations: for example, the costs of an XAS station are somewhat lower than for a diffraction or soft X-ray microscopy station. However, the bulk of the hardware costs lie in the beamlines, X-ray optics and hutches, rather than in the X-ray experimental apparatus itself. The beamlines are becoming more expensive as third generation synchrotron sources such as the ALS and APS are to be equipped. They are of necessity much more sophisticated. The brilliant, high-power sources require better optics with much more elaborate cooling schemes to deliver X-rays to the sample; the greatly increased X-ray intensities demand superior X-ray detectors that can accept the much higher local and global counting rates, and computing systems that can process, partly reduce and store the data in real time; greater radiation shielding is needed for the higher-energy, high-intensity sources that may also be used for white-beam experiments rather than monochromatic experiments; and the experiments themselves are becoming more complicated and hence require additional equipment such as pulsed and CW lasers and single-crystal microspectrophotometers. Finally, the beamlines are larger; for example, they can be up to 80 m long at the APS, compared to a maximum of 25 m at CHESS. All these factors point to substantially increased capital costs at third-generation sources, by comparison with existing, first- and second-generation sources.

As examples of capital costs of new beamlines and experimental stations, we offer careful estimates of the total capital cost of one sector (two beamlines) at APS, that CARS/BioCARS (Consortium for Advanced Radiation Sources) proposes to devote almost entirely to diffraction experiments in structural biology. This capital cost, in 1990 dollars but excluding any contingency, is estimated at \$4.26M (Table VIII-2). This figure does not include the capital cost of an insertion device or the front end (to be provided by the APS) but does include the complete beamlines, all optics, hutches, the experimental apparatus in all hutches, a BL3 facility, and all control electronics. This sector will share four hutches, three of which can be used simultaneously, and the apparatus for six different styles of experiments that can be introduced into these hutches: virus crystallography, time-resolved diffraction, microcrystal diffraction, surface scattering, MAD phasing/chemical crystallography, and small-angle scattering. In this way, the capital costs of the beamline itself, the hutches, the associated biohazards facility and the optics are distributed over several experimental stations, in a cost-effective manner. If we assume that this sector is equivalent to four experimental stations, then the capital cost per station is \$1.07M; if it is equivalent to three experimental stations, then the capital cost per station is \$1.42M.

Another single sector (two beamlines and two experimental stations) to be developed at the APS for single-crystal diffraction experiments is estimated at a capital cost of \$3.8M, or \$1.9M capital cost per station.

Another careful study of the capital cost of construction of X-ray microscopy facilities at the ALS yielded a figure of \$6.6M including the undulator source, front end, beamline, all optics and two end stations. If we estimate the undulator and front end to cost \$2.5M, then the balance is \$4.1M; and the capital cost per station is one-half of this, or \$2.05M. These figures include engineering, design and inspection costs.

Finally, a proposal to develop one new beamline with four experimental stations at SSRL (for X-ray absorption spectroscopy, small-angle scattering, Laue crystallography, and MAD), of which three can be used simultaneously, is estimated at a capital cost of \$6.3M, which includes some instrumentation development. If we estimate the wiggler and front end to be \$1.3M, then the balance is \$5.0M. If this beamline is equivalent to four stations, then the capital cost per station is \$1.25M; if it is equivalent to three stations, then the capital cost per station is \$1.67M.

The range from \$1.07M to \$2.05M per experimental station in part reflects differences among disciplines (and uncertainties in the estimates), but more the economies of scale that arise when several stations can be served by a single beamline. For planning purposes, a capital cost per station of $\$1.6M \pm \$0.3M$ in 1990 dollars is indicated by the above figures, with the lower number appropriate only to multi-station facilities or to those with minimally equipped experimental stations. In a realistic budget, a 25% contingency should be added to all these figures.

There are further capital costs not included in the above, for ancillary but essential facilities such as a small, modestly equipped biochemistry laboratory, stockroom, machine shop and electronics shop for user access. We generously estimate these at a total of \$1.0M. Here too, large economies of scale can be achieved if these facilities can be constructed and operated in common, to serve several groups of users. Common operation is in place at laboratories

such as CHESS, and is under active discussion by, for example, the structural biology groups planning to use the APS.

How large a staff is needed to design, construct, install and test a new facility? The answer is clearly very variable, depending on the scale of the facility and on how much of the work is contracted out to others. As one example, we use the CHESS East construction referred to above. This occupied the entire 20-person staff of CHESS for approximately 24 months, plus additional design effort prior to construction and further effort after the main construction, estimated at 40 person-months. Thus the total staff effort was 520 person-months for five experimental stations; say, 104 person-months per experimental station. As a second example, the complete CARS/BioCARS staffing plan for two sectors at the APS (of which only one is devoted to structural biology) calls for about 23 staff during the bulk of the construction phase from 1990 through 1995, in which a total of 103.5 person-years or 1240 person-months are to be associated with design, construction, installation and testing. The two sectors will house a total of roughly ten experimental stations; thus the construction staff is 124 person-months per experimental station. For construction of the single APS sector referred to above, the construction staff is estimated at 267 person-months, or 134 person-months per experimental station. For smaller-scale facilities, the concept of critical staff size comes into play. What is the minimum staff necessary to design and construct a small-scale facility, such as a single beamline and single experimental station at NSLS? We estimate it at 6 persons and likewise estimate a 30 to 36 month construction period, or 180-216 person-months per station.

Although the exact numbers of person-months per station may be open to argument, it is clear that the general levels are correct, and that economies of scale can be substantial; larger facilities such as CHESS and CARS permit more efficient staffing.

For a larger facility, a construction effort of 140 ± 20 person-months per station is indicated, and for a smaller facility, perhaps 190 ± 30 person-months per station. Since the personnel costs were estimated above at \$126.5K per staff member per year or \$10.55K per person-month, the total personnel costs of construction per station lie around \$1.5M for a larger facility and \$1.9M for a smaller, in 1990 dollars.

Operating costs: As seen in the questionnaire responses above, the bulk of operating costs arise from staff salaries, fringe benefits, their associated supplies and other costs. It is therefore reasonable to ask what are appropriate staffing levels for an effective user facility and first-rate research facility; and how these levels might vary with the size of the facility.

Staff may be broadly classed into three levels: operators, research staff, and administrative or other support staff. The first typically hold B.S. or M.S. degrees in science or technology and serve as instrumentation and user support specialists, engineers or technicians. The second typically hold Ph.D. degrees and conduct research to advance some aspect of synchrotron radiation science and its application to structural biology, provide user support often via scientific collaboration, and offer high level scientific management of the facility. The third include machinists and stockroom support as well as administrative, fiscal, personnel and user scheduling functions.

Since synchrotron radiation facilities operate 20-24 hours per day, effective user support demands a shift system for the operators. The high-stress nature of such facilities cannot be over-emphasized and indeed, staff burnout is a recurring management problem. This can largely be alleviated by a staff that is large enough to allow rotation of individuals in and out of high-stress positions, on a 3- or 6-month basis.

At existing facilities, operators and research staff train users and act as troubleshooters, but generally do not participate actively in data collection by the users unless they are scientific collaborators. We denote this the "current support mode". As noted above, these are generally supplemented by the "hidden staff subsidy". We denote the "optimum support mode" the same operator and staff functions, but at increased, optimum staffing levels. That is, the "hidden staff subsidy" is made explicit and included (as it should be) in the true staff. The "optimum support mode" generally has staffing levels 50% higher than the "current support mode". There is a further, novel operating mode which we denote the "complete mode". In it, the data for established experiments (for example, monochromatic, high resolution crystallographic data sets or straightforward XAS data) are collected by the operators and facility staff, not by the users. The user role becomes that of sample preparation and consultant to the staff. This, at first sight, radical proposal is based on the idea that the greatest throughput of high-accuracy data sets for established experiments will be achieved, not by newly trained users, but by facility staff whose chief responsibility this is. In effect, the facility is offering a comprehensive data collection service. To do this requires a substantially larger staff, as each station at which this service is in place must

be staffed 24 hours per day. However, users now do not need to bring a large group to the facility; two will suffice, adept at sample preparation. Of course, this style works only for established experiments, and not those where the user has to conduct a great deal of on-site data evaluation or apparatus refinement or reconstruction.

With these three modes in mind, Table VIII-3 presents staffing levels. These may be readily translated into operating costs by assuming an average of \$130K per staff member per year for direct and indirect costs. For example, the cost of operating a two station facility in the "current support mode" is estimated at $6 \times \$130K = \$780K/\text{year}$, or \$390K/year/station. Supplies and operations expenses are included in the \$130K per staff member estimate.

There is one further important operating cost not included in the above. If effective progress is to be made in developing the synchrotron X-ray technology and techniques, then the research staff must indeed conduct their own research as well as fulfill their user support role. (In the terminology of the NIH National Center for Research Resources, the staff must participate in "Core" and "Collaborative" research.) We estimate the direct + indirect cost of this staff research as \$100K/research staff member/year.

By combining the staffing levels (Table VIII-3) with the estimated average staff cost of \$130K/year, and including the staff research, we arrive at estimates of the total operating cost for facilities of various sizes, and the total operating cost per station, as in Table VIII-3.

Table VIII-1. Personnel and Fiscal Support of Experimental Stations for Structural Biology

		Number of		Total Annual
Synchrotron		Personnel to	Funding	Support (\$)
Beamline	Year	Operate/Manage	Source	(direct + indirect)
NSLS				
U9-B	1985-1990	1.5	DOE	205,000
X1-A ¹	1985-1990	3	NSF/DOE	200,000
X9	1988	NR	NIH	836,392
	1989	8.5	NIH	1,225,646
	1990	8.5	NIH	987,636
X11-A	1985-1990	3	DOE/Industry	350,000
X12-B	1985-1990	2	DOE	215,000
X12-C	1985	2	DOE	150,000
	1986	2	DOE	150,000
	1987	2.1	DOE	180,000
	1988	2.2	DOE	220,000
	1989	1.7	DOE	230,000
X19-A	1990	2.2	DOE	250,000
	1988	1	U. of Michigan	50,000
	1989	1	U. of Kentucky	50,000
	1990	2	U. of Kentucky, NSLS	100,000
CHESS ²				
A1, B2, F1	1985	5-MacCHESS	NIH	375,000
		14-CHESS	NSF	1,000,000
	1986	5-MacCHESS	NIH	534,000
		14-CHESS	NSF	1,000,000
	1987	5-MacCHESS	NIH	403,000
		14.5-CHESS	NSF	2,000,000
	1988	5-MacCHESS	NIH	1,000,000
		20.5-CHESS	NSF	2,000,000
	1989	5-MacCHESS	NIH	700,000
		23.5-CHESS	NSF	2,000,000
	1990	5-MacCHESS	NIH	700,000
		25-CHESS	NSF	2,500,000
SSRL				
structural biology	1990	10	NIH/DOE	1,580,000

¹ Funding sources are for SUNY-Stony Brook, which provides ~80% of operations. Additional support comes from the other three PRT members. Major hardware (undulator) is provided by NSLS.

² MacCHESS provides the operation and user support for the three beamlines that are used for biological diffraction. The overall facility budget from NSF also supports these beamlines.

Table VIII-2. Capital Costs for APS Hardware

Item	Total	1991	1992	1993	1994	1995	1996
A1-Monochromator	\$50,000	\$10,000	\$25,000			\$10,000	\$5,000
A1-Mirror	45,000			\$30,000	\$10,000	5,000	
Monochromator tank	25,000		10,000	15,000			
Mirror tank	35,000		10,000	20,000		5,000	
Mono Crystals	40,000	15,000	15,000				10,000
Crystal Cooler	10,000					10,000	
Beryllium windows	40,000		10,000	20,000	10,000		
A1-station equipment	130,000			40,000	40,000	40,000	10,000
A1-experimental station	115,000			20,000	75,000	20,000	
BL3 facility	400,000			40,000	360,000		
SP scanner	400,000				400,000		
Benchcamera plus	65,000			25,000	20,000	20,000	
SAS equipment	200,000			20,000	30,000	130,000	20,000
Surface scattering	120,000		30,000	30,000	30,000	30,000	
POE	160,000			30,000	100,000	30,000	
A2-Monochromator	50,000		20,000	20,000		10,000	
A2-Mirror	45,000		30,000			10,000	5,000
Monochromator tank	45,000		15,000	15,000		15,000	
Mirror tank	45,000		20,000	10,000		15,000	
Mono Crystals	35,000	10,000	15,000				10,000
Electronic Feedback	25,000	5,000	5,000	5,000		5,000	5,000
Beryllium windows	25,000		10,000	15,000			
Experimental station	80,000			20,000	40,000	20,000	
A2-station equipment	130,000			40,000	40,000	40,000	10,000
Benchcamera simple	35,000			20,000	15,000		
6-axes diffractometer	120,000				30,000	80,000	10,000
B-line Monochromator	50,000		20,000	20,000		5,000	5,000
Monochromator tank	65,000	10,000	30,000	20,000		5,000	
Propane cooler	130,000	20,000	80,000	25,000			5,000
Mono crystals	45,000	10,000	20,000			5,000	10,000
Electronic feedback	20,000	5,000	5,000	5,000			5,000
Beryllium windows	15,000		5,000	8,000		2,000	
POE	200,000			45,000	100,000	55,000	
B1 station	75,000			20,000	45,000	10,000	
B1-station equipment	130,000			40,000	40,000	50,000	
Surface Scattering Optics	80,000			30,000	30,000	20,000	
Micro Xtal Diffraction	140,000			30,000	30,000	70,000	10,000
B2-station	75,000				50,000	25,000	
B2-station equipment	165,000			55,000	55,000	55,000	
Storage Phosphor Scanner	400,000					400,000	
Time-resolved equipment	200,000		20,000	50,000	55,000	60,000	15,000
Subtotal	\$4,260,000	\$85,000	\$395,000	\$783,000	\$1,605,000	\$1,257,000	\$135,000

Table VIII-3. Staffing Levels and Operating Costs

	# of Stations	Mode		
		"Current"	"Optimum"	"Complete"
Operators (number, full time)	1	1	3	5
	2	2.5	4	9
	3	3	5	12
	4	4	6	18
Research Staff (number, full time)	1	1	2	2
	2	2	3	3
	3	3	4	4
	4	3	5	5
Administrative/Support Staff (number, full time)	1	1.5	2	3
	2	1.5	3	4
	3	2	4	5
	4	3	4	5
Total (number, full time)	1	3.5	7	10
	2	6	10	16
	3	8	13	21
	4	10	15	28
Total per Station (number, full time)	1	3.5	7	10
	2	3	5	8
	3	2.7	4.3	7
	4	2.5	3.75	7
Personnel Costs (\$K)	1	455	910	1300
	2	780	1300	2080
	3	1040	1690	2730
	4	1300	1950	3640
Staff Research Costs (\$K)	1	100	200	200
	2	200	300	300
	3	300	400	400
	4	300	500	500
Total Operating Costs (\$K)	1	555	1110	1500
	2	980	1600	2380
	3	1340	2090	3130
	4	1600	2450	4140
Total Operating Costs/Station (\$K)	1	555	1110	1500
	2	490	800	1190
	3	447	697	1043
	4	400	613	1035

EXECUTIVE SUMMARY

Structural biology in its many different forms plays a predominant role in our understanding of biological systems. It is impossible to understand how molecules or cellular components function without knowledge of their organization and structure. For many experiments concerning the structure of biological matter, intense X-ray light produced by synchrotron radiation sources is a crucial research tool. This report describes the status of current resources and quantitates the future needs for synchrotron radiation in structural biology. Four major scientific disciplines of structural biology currently require synchrotron radiation: crystallography, X-ray microscopy, scattering from noncrystalline materials and X-ray spectroscopy. Much of the data used in the report is derived from questionnaires widely distributed to structural biologists in these disciplines in North America and to staff at synchrotron sources worldwide. While the current need for synchrotron radiation by these disciplines varies widely, there is a common potential for rapid growth. A threefold increase in the need by U.S. scientists for synchrotron radiation is projected through the year 2000, based on current growth rates.

The BioSync study group has examined how the recognized growth and importance of structural biology research are changing the needs of structural biologists for synchrotron radiation, how the status of current facilities influences the research, and what specific needs exist to improve access to existing synchrotron radiation sources by structural biologists and to exploit opportunities at new sources. The study group reached the following major conclusions.

1. Structural research is providing ever more significant insights into biological systems, and the demand for structural information is increasing in all fields of biology.

A larger percentage of all biological research relies on structural information than at any time in the past. Several factors account for this trend: the critical impact of key structural results on many fields of biology, the availability of materials for structural research in quantity and quality heretofore unseen, and the revelation of many new structural projects by the Human Genome Project. The demand for structural information is so strong that non-specialists are willing to use structural methods, particularly crystallography, if these are made readily accessible to them. The reliance on structure is expected to increase further as more biologists identify structural information as a key to solving their biological problems and realize that structural information can be obtained from available materials with accessible methods.

2. Synchrotron radiation can make significant contributions to the speed, quality and nature of structural results in biology.

Several spectacular results have been achieved in structural biology as a direct result of the use of synchrotron radiation. Over the past fifteen years, the role of synchrotron radiation in structural biology has been growing steadily. The unique properties of synchrotron radiation, particularly photon brilliance and flux and wavelength tunability, provide an additional promise for greater productivity and improved accuracy of structural biology. Research on both more complex biological samples and samples of smaller size greatly benefits from these and other unique properties of synchrotron radiation.

3. Strong resources in structural biology, including synchrotron radiation, are critical to maintaining and enhancing the U.S. competitive advantage in the pharmaceutical and biotechnology industries.

A very strong basic research enterprise in both industrial and academic settings is responsible in part for the lead now enjoyed by the U.S. in the pharmaceutical and biotechnology industries. Increasing reliance of basic biological research on structural information and of structural biology on synchrotron radiation means that synchrotron radiation facilities for basic structural biology are important to maintaining this international competitive advantage. In addition, the opportunities for commercial development in biotechnology and in modern pharmaceutical design have attracted a strong and growing industrial effort in structural biology, especially in the field of crystallography and (to a more restricted extent) in X-ray microscopy. Without timely access to state-of-the-art synchrotron radiation facilities, the U.S. will not remain at the forefront in these industries.

4. The rapidly growing structural biology community and the large number of non-specialists willing to undertake structural studies result in a very large "latent" community of users of synchrotron radiation for structural biology.

The excitement and promise of structural biology are clearly perceived by younger scientists. The four disciplines of structural biology that need synchrotron radiation are currently training approximately 700 Ph.D. students and 900 postdoctoral fellows. The scientists are young; one-third of the independent investigators themselves have headed independent laboratories for five or fewer years. Less formal evidence suggests that there is a growing number of researchers trained in other fields of biology who are now attempting to learn and apply the methods of structural biology.

The structural biology community recognizes synchrotron radiation as a vital research tool; questionnaire responses show that 64% view synchrotron radiation as critical or very important to their own research, and 90% agree that synchrotron radiation is important to the research of individual scientists. This strong consensus by practitioners in the field, their growing numbers, the strong demand by the biological community for structural results, and the new willingness by non-specialists to do structural research adds up to a very large potential demand for synchrotron radiation resources by the existing biology community. By the year 2000, the number of independent investigators using synchrotron radiation in structural biology could increase sevenfold, based on current growth rates.

5. The achievements of synchrotron radiation in structural biology far outpace the support for existing facilities, and lagging support limits the use of synchrotron radiation by structural biologists today.

Beamtime at synchrotron sources is a precious commodity for structural biologists. At most experimental stations, the wait for beamtime is long and the assigned time is short. Users often express frustration at their attempts to use synchrotron radiation, and rate a helpful, accessible staff and user friendliness as essential to their research with synchrotron radiation. The equipment is too often unique to the individual experimental station and may be difficult to operate effectively; support staff are too few and seriously overworked; there is inadequate documentation for instrument use and computer programs; and the facility administration and support may be minimal. The enormous benefits that synchrotron radiation offers to structural biology cannot fully be realized in such circumstances. It is further a waste of the nation's resources to invest in the major construction costs required to build synchrotron radiation sources and then to strangle their use by inadequate operating support for instrumentation and staff.

Scientific communities including structural biologists have driven the development of technology for exploitation of synchrotron radiation. The need for more rapidly measured, more extensive and more accurate data continues to exert pressure for improved technology at synchrotron radiation sources. Among the most pressing current needs are new, ultra-rapid detectors of scattered radiation; X-ray optics to handle high heat loads; robotic sample handling; and computer software and hardware to analyze and store data at greatly increased rates. The bright promise of structural biology to produce major results rapidly will be realized only if synchrotron radiation sources are accessible to large numbers of researchers. This requires all of the following.

- reliable sources of synchrotron radiation
- instruments and computer programs at synchrotron radiation facilities that are relatively easy to use
- adequate user support at synchrotron radiation facilities, especially for personnel
- a user-friendly atmosphere and attitude on the part of support staff and facility administrators
- research support for individual investigators that is sufficient for development of synchrotron radiation projects

6. Substantial new support for new experimental facilities and for improvement to existing facilities is needed to match the substantial growth of the structural biology research community.

The need for new experimental stations is projected through the year 2000 based on the increased number of investigators, their increased productivity and the improved efficiency of new experimental stations. The following numbers of new stations are the best estimates of the study group.

Crystallography	15
X-ray spectroscopy	9
Scattering from noncrystalline materials	3
X-ray microscopy	2
Total	29

Construction costs are estimated to be \$3-4M/station in 1990 dollars with no contingency, and operations costs are estimated to be \$0.5-1.0M/station/year in 1990 dollars. Total support for new stations is approximately \$100M for construction, in 1990 dollars spent over a three- to five-year period, and approximately \$20M/year for operations in 1990 dollars.

The projected need for new stations is based on the assumption that all existing stations will be fully supported and will operate at full capacity, which has never been the case for any of them. Full support of existing experimental stations for structural biology will require a total increment to their current support of approximately \$3.2M/year in 1990 dollars.

7. Improved mechanisms for funding, design, construction and operation are needed for the rapid, efficient development of synchrotron radiation facilities for structural biology.

All new U.S. synchrotron radiation facilities (Brookhaven, Berkeley and Argonne) provide only photons and not instrumentation for individual beamlines. Individual beamlines and experimental stations are provided by users who must compete for access to the synchrotron sources, construct and operate their own beamlines, and raise funds to support these activities. Compared to scientists in most other disciplines, structural biologists are at a competitive disadvantage in the areas of design and construction of beamlines at synchrotron sources. There is little precedent in the biological community for building instrumentation of the required sophistication, and the traditional funding sources for structural biology have seldom provided support on the required scale. In addition, there is little precedent for widespread collaboration of researchers to share in the development and management of such instrumentation or of interagency collaboration to fund such projects for structural biology. The synchrotron radiation facilities, funding organizations and BioSync must aid in bringing about these collaborations. Collaboration must extend to the design and development of novel instrumentation and must include the best expertise in the community whether residing in university, synchrotron radiation, industrial or government laboratories.

In less than two decades, synchrotron radiation has evolved from a possibly useful concept to an extremely valuable, but oversubscribed, resource. Development of new synchrotron radiation sources, allocation of beamlines and building of experimental stations and ancillary facilities requires considerable scientific talent, planning, time and money. The importance of structural biology to our fundamental understanding of biological systems mandates long-term support. Collaboration of the biological community, synchrotron radiation facilities and funding organizations is essential to the success of the endeavor.

APPENDIX A. DATA FROM USER QUESTIONNAIRES

In September, 1990 BioSync distributed a user questionnaire to structural biologists in order to learn about past and anticipated future use of synchrotron radiation, factors affecting use of synchrotron radiation, and attitudes about synchrotron radiation. The user questionnaire is contained in Appendix D. All written comments, unedited, from the user questionnaire are in Appendix C. Responses to the user questionnaire are tabulated here in Appendix A. Comments preceding the tables are intended to clarify and, in some cases, to prevent misinterpretation of the quantitative data.

We attempted to distribute the user questionnaire to all independent investigators working in North America in fields of structural biology where synchrotron radiation can be used as a research tool. With only a handful of exceptions, these investigators work in four scientific disciplines, which we have used for much of our data analysis. The disciplines and our abbreviations for them are listed below.

Crystallography	XTAL
Scattering from noncrystalline materials	SAS
X-ray spectroscopy	XAS
X-ray microscopy	IMG

We believe that our list of investigators is more complete in the discipline of crystallography than in any other area. Ten investigators who we contacted work in Canada. All others are in the United States.

Number of questionnaires distributed	364
Number of questionnaires returned	209 (57%)
Returned questionnaires from nonindependent investigators	16
Number of questionnaire responses used in this analysis	193

It is important to note that the numbers below represent only ~57% of the independent investigators known to us.

Table A-1. Work Environment of Structural Biologists

Current Employer	Number of Investigators
University	135 (70%)
Industry	26 (13%)
Government Laboratory	20 (10%)
Other Institutions	12 (6%)
Retired	2 (1%)

Table A-2. Years of Independent Experience and Size of Research Group

Years as an Independent Investigator	Number of Investigators	Average Size of Research Group (σ)	Total Number of Researchers	Average Anticipated 5-Year Change in Group Size (σ)
0-5	59 (31%)	4.0 (2.9)	236	+3.2 (2.2)
6-10	45 (23%)	6.4 (4.7)	288	+2.6 (3.3)
11-15	21 (11%)	7.8 (5.8)	164	+1.3 (3.6)
16-20	26 (13%)	8.7 (5.6)	226	+1.3 (2.2)
>20	41 (21%)	7.3 (4.7)	299	+0.8 (2.7)
unspecified	1			
Total	193	1213		

Table A-3. Years of Independent Experience by Scientific Discipline

Years as an Independent Investigator	Number of Investigators					Total
	XTAL	SAS	XAS	IMG	Other	
0-5	49	5	5	0		59
6-10	34	8	3	0		45
11-15	14	4	3	0		21
16-20	19	2	3	2		26
>20	22	7	7	4	1	41
unspecified		1				1
Total	138	27	21	6	1	193

Table A-4. Number of Structural Biologists in Training by Scientific Discipline

	XTAL	SAS	XAS	IMG	Total
Independent Investigators (trainers)	138	27	21	6	192
Graduate Students	258	50	87	18	413
Postdoctoral Associates	387	47	68	9	511
Trainees/Trainer	4.7	3.6	7.4	4.5	4.8

Table A-5. Level and Sources of Research Support

Annual Research Support	Number of Investigators	Source of Research Support	Number of Investigators
0-\$25,000	10	NIH	125
\$25,000-\$100,000	39	NSF	54
\$100,000-\$200,000	48	DOE	20
>\$200,000	92	Industry	49
		Foundation	34
		Other	40

Tables A-6 and A-7 show the users' reported past and anticipated future use of synchrotron radiation at facilities worldwide.

Synchrotron radiation sources used by structural biologists:

SSRL	Stanford Synchrotron Radiation Laboratory, Stanford, California
CHESS	Cornell High Energy Synchrotron Source, Ithaca, New York
NSLS	National Synchrotron Light Source, Brookhaven, Long Island, New York
SRS	Synchrotron Radiation Source, SERC Daresbury Laboratory, Warrington, England
LURE	Laboratoire pour l'Utilisation du Rayonnement Electromagnétique, Orsay, France
DESY	Deutsches Elektronen-Synchrotron, Hamburg, Germany
PF	Photon Factory, National Laboratory for High Energy Physics, Tsukuba, Japan

Synchrotron radiation facilities now under construction (estimated date for operations to begin):

ALS	Advanced Light Source, Berkeley, California (1993)
ESRF	European Synchrotron Radiation Facility, Grenoble, France (1994)
APS	Advanced Photon Source, Argonne, Illinois (1995)
SPring-8	Super Photon Ring, Harima, Hyogo, Japan (1998)

We show in Table A-6 the use of each of the synchrotron radiation facilities as reported by the 57% of independent investigators in North America who responded to the user questionnaire. A significant fraction of those not responding are known to be users of synchrotron radiation.

Table A-6. Past Use of Synchrotron Radiation

Year	Total Days Used Each Year							Total
	SSRL	CHESS	NSLS	SRS	LURE	DESY	PF	
1985	426	175	249	73	24	60	0	1007
1986	346	163	420	52	24	47	2	1054
1987*	302	176	249	22	0	21	14	784
1988*	146	121	294	60	0	36	43	700
1989*	121	137	528	60	0	60	52	958
1990	77203	591	47	15	19	35	987	

* The existing U.S. synchrotron sources all experienced significant downtime in the 1987-1989 period, and it appears that questionnaire responses are substantially in error for those years. See Appendix B, Table B-10, below.

Starting with 1991, the users anticipate a need for more than three times as many days of beamtime per year as they were able to obtain in each of the past six years (average = 915 days/year). The numbers of days needed at each facility as estimated by respondents have been scaled to take into account those investigators who were unable to estimate their needs in future years. The fraction not making estimates for each year are 30% for 1991, 35% for 1992, 40% for 1993, 53% for 1994, 57% for 1995, 61% for 1996, 63% for 1997 and 64% for both 1998 and 1999. Scaled estimates are reported in Table A-7.

Table A-7. Anticipated Future Need for Synchrotron Radiation

Year	Estimated Number of Days Needed										
	SSRL	CHESS	NSLS	SRS	LURE	DESY	PF	ALS	ESRF	APS	Total
1991	539	433	1837	69	3	86	79	-	-	-	3046
1992	669	517	2034	66	3	146	54	-	-	-	3489
1993	612	543	2018	72	0	120	45	413	-	-	3823
1994	581	577	2226	64	0	132	15	532	32	-	4159
1995	542	316	2026	58	0	93	0	581	47	12814944	
1996	562	208	2008	64	0	79	0	641	13	16975272	
1997	578	176	2024	14	0	46	0	676	14	18515379	
1998	553	181	2078	14	0	47	0	694	14	19085489	
1999	553	181	2217	14	0	47	0	694	14	19315651	

Inability to obtain sufficient experimental time is the single most important factor limiting the questionnaire respondents' use of synchrotron radiation (question 5). About one-third of the responding crystallographers indicated that they can do their current research without synchrotron radiation. About one-quarter of the respondents have never used synchrotron radiation, based on their answers to question 2.

Table A-8. Factors Limiting Use of Synchrotron Radiation, by Scientific Discipline

	Number of Respondents				Total
	XTAL	SAS	XAS	IMG	
Don't need it for current research	46	2	0	0	48
Can't readily get beamtime	61	10	7	1	79
Can't get support at synchrotron facility	15	6	3	1	25
Insufficient support for travel	28	5	5	0	38
Didn't respond to question 5	33	11	10	4	58
Never used synchrotron radiation	46	4	1	0	51

Synchrotron radiation will be important for the future research effort of nearly all respondents.

Table A-9. Importance of Synchrotron Radiation to the Investigator's Future Research Plans

	Number of Respondents				
	XTAL	SAS	XAS	IMG	Total
Critical	35	8	12	5	60
Very important	47	10	7	0	64
Moderately important	45	5	2	1	53
Unimportant	8	4	0	0	12
No opinion	3	0	0	0	3

Questionnaire respondents clearly indicated that four features of synchrotron radiation facilities are most important to them: state-of-the-art X-ray detectors, high photon flux to the experiment, user-friendly experimental facilities with helpful staff, and accessibility.

Table A-10. Relative Importance of Various Features of Synchrotron Radiation Facilities

Feature	Number of Respondents					
	Essential	Very Important	Important	Useful	Unimportant	No Answer
Fast, efficient X-ray detectors	138	18	12	2	3	19
Detectors with high spatial resolution	92	42	16	4	9	29
High intensity*	110	35	11	2	4	30
High brilliance*	107	28	17	3	4	33
High flux*	96	31	15	4	6	40
Helpful, readily accessible support staff	110	52	9	2	3	16
User friendliness	98	47	17	4	4	22
Ready accessibility for short-term, feasibility studies	100	47	10	4	5	26
Ready accessibility for long-term projects	97	41	16	4	5	29
High energy resolution	66	35	28	15	9	39
Good auxiliary facilities	61	63	29	10	5	24
On-site data processing	55	49	28	13	18	29
Good housing and convenient services	34	63	47	13	9	26
Good communication	31	40	63	21	7	30
Intellectually stimulating environment	27	41	55	18	22	29

* Many respondents indicated confusion among these categories.

There is overwhelming agreement by respondents to the user questionnaire that synchrotron radiation is important to the research of individual scientists.

Table A-11. General Importance of Synchrotron Radiation

	Number of Respondents			
	Agree	Disagree	Neutral	No Answer
Money spent on synchrotrons could be better directed to individual research grants.	12	113	53	14
Synchrotrons are:				
essential to my future research	132	18	36	6
essential to industrial growth in drug design/biotechnology	107	17	61	7
not cost effective	10	117	54	11
“big science”	70	47	62	13
important for the research of individual scientists	173	1	8	10

No consensus exists among questionnaire respondents about how to proceed if all synchrotron radiation facilities cannot be supported. Many respondents could not fit their opinions into the options offered in this question (see Appendix C for additional comments).

Table A-12. Fiscal Advice If Funds Are Limiting

Option	Number of Respondents
Close them all; don't need them; waste of money	0
Don't build new facilities; continue to support existing facilities	87
Build new facilities, but	54
Close SSRL	32
Close CHESS	27
Close NSLS	4
No answer	45

APPENDIX B. DATA FROM QUESTIONNAIRES TO SYNCHROTRON RADIATION FACILITIES

There are seven synchrotron radiation sources where structural biologists currently do research. They are:

SSRL	Stanford Synchrotron Radiation Laboratory, Stanford, California
CHESS	Cornell High Energy Synchrotron Source, Ithaca, New York
NSLS	National Synchrotron Light Source, Brookhaven, Long Island, New York
SRS	Synchrotron Radiation Source, SERC Daresbury Laboratory, Warrington, England
LURE	Laboratoire pour l'Utilisation du Rayonnement Electromagnétique, Orsay, France
DESY	Deutsches Elektronen-Synchrotron, Hamburg, Germany
PF	Photon Factory, National Laboratory for High Energy Physics, Tsukuba, Japan

Each of the three synchrotron radiation facilities now in operation in the U.S. and used by structural biologists is run somewhat differently from the others. Below we describe each facility.

CHESS: The Cornell High Energy Synchrotron Source is operated parasitically on a high-energy storage ring, CESR. The 11 beamlines at CHESS are available for experiments approximately 220 days per year through peer-reviewed competitive proposals. Beginning in 1991, CHESS will have a one-month "dedicated" run each year for the production of undulator radiation and biological experimentation. The CHESS facility is supported by NSF; a grant from NIH (NCRR) supports operation and development of facilities for structural biology, which accounts for approximately 30% of all research at CHESS.

SSRL: The Stanford Synchrotron Radiation Laboratory has in recent years operated in both the "dedicated" (50%) and "parasitic" (50%) modes using the high-energy ring SPEAR at the Stanford Linear Accelerator Center (SLAC). Virtually no structural biology experiments have been done in the "parasitic" mode of operation. All experimental time is available through peer-reviewed competitive proposals. SSRL has not operated in a normal mode (two "dedicated" runs per year of 6-8 weeks each) since 1987, largely due to funding constraints. SSRL begins a new phase of operation in 1991 when it converts SPEAR into a fully dedicated synchrotron radiation source. After an initial shakedown, SSRL expects 6-8 months of dedicated operation each year. Most beamlines at SSRL are operated by SSRL staff as general user facilities. A few beamlines are operated by Participating Research Teams for their own experiments, but are available for general users 33% of the operating time through the SSRL peer-review process. Structural biology accounts for about one-third of all experiments done at SSRL, and a grant from NIH (NCRR) supports the biological facilities. The U.S. Department of Energy supports the overall SSRL facility.

NSLS: The National Synchrotron Light Source at Brookhaven National Laboratory has the first X-ray storage ring in the U.S. that was built as a dedicated synchrotron radiation source. The Light Source, which includes both X-ray and ultraviolet storage rings, was built and is operated by the U.S. Department of Energy. Individual beamlines were built and are operated by user groups called Participating Research Teams (PRTs). Unlike the situation at CHESS and SSRL, which are operated as integrated facilities, each beamline at NSLS is run independently of the others and has independent facilities and independent operating plans. NSLS has a general user program, and each PRT is required to provide at least 25% of its beamtime to general users. Since NSLS is dedicated to production of synchrotron radiation, X-rays are available for experiments approximately 250 days per year. Approximately 7% of the experiments on the X-ray ring at NSLS are biological, as are less than 5% of the experiments on the UV ring.

CHESS, SSRL and NSLS all experienced recent long periods of down time for facility upgrades. There were virtually no photons available in the U.S. during 1988. NSLS started up again in early 1989, CHESS in late 1989 and SSRL slowly in 1990.

Two synchrotron radiation facilities are under construction in the U.S.

ALS: The Advanced Light Source at Lawrence Berkeley Laboratory is being built by the U.S. Department of Energy and will be run in the PRT mode. There will be a total of 34 beamlines using radiation in the ultraviolet and near X-ray regions. Two among the initial group of PRTs specialize in structural biology. ALS will have a general user program for researchers not affiliated with PRTs to gain access to the ALS. After commissioning, ALS is expected to operate about 250 days per year. The start of operations is planned for 1993.

APS: The Advanced Photon Source at Argonne National Laboratory will be a dedicated synchrotron radiation source providing X-rays that are orders of magnitude more brilliant than at any existing facility. The ring is being built and will be operated by the U.S. Department of Energy. Collaborative Access Teams (CATs) will build and operate pairs of beamlines, known as sectors. The ring will have a total capacity of 68 beamlines. Four CATs submitting proposals to APS have a substantial component of structural biology in their research plans. APS operations are planned to begin in 1995.

In late 1990 BioSync sent questionnaires to scientists at all appropriate synchrotron sources worldwide. Of facilities in the U.S., we asked detailed questions about configuration of beamlines, instrumentation for structural biology research, use of time on experimental stations for structural biology, demand for beamtime on these stations and financial support for structural biology stations. Of synchrotron radiation facilities abroad, we asked about numbers of research groups from the U.S. doing structural biology experiments and numbers of days used by these groups. Sample questionnaires are contained in Appendix E.

The data from synchrotron radiation facilities is of variable reliability. It was apparent as we gathered these data that most facilities are grossly understaffed. In most cases, data were not available in the form we requested, and staff were not available to extract the information we wanted from existing data. In all cases scientific or administrative staff were cooperative. They simply did not have the time or support to answer our questions fully. Of the U.S. facilities, data from CHESS (interestingly, the smallest facility) are nearly complete. The user data from SSRL are incomplete but accurate; those from NSLS are clearly estimates for nearly all experimental stations. All data from NSLS come from individual beamline staff. We were unable to obtain data for some stations that are used a small fraction of the time for structural biology and are mentioned in other sections of the report. The central NSLS administration does not record user data in a form that would be helpful to us (i.e. structural biology as distinct from other scientific disciplines). Data from the Photon Factory are accurate and nearly complete. Data from the other synchrotron radiation facilities abroad are clearly estimates and, we believe, underreport actual use by investigators from the U.S.

Table B-1. Beamlines Currently Available for Structural Biology

Synchrotron Beamline*	Experiments
NSLS	
U9-B	UV circular dichroism and time-resolved fluorescence. PRT: Department of Biology - Brookhaven National Laboratory.
X1-A	IMG: Scanning microscopy, holography and diffraction from cultured cells and organelles; imaging of unstained specimens and elemental mapping. PRT: SUNY-Stony Brook, NSLS, Lawrence Berkeley Laboratory, IBM.
X9	XAS, SAS: X-ray absorption, spectroscopy, diffraction and small-angle scattering of biological metal clusters and proteins; time-resolved studies of multilayers and monolayers of lipids, proteins, and muscle fibers. PRT: University City Science Center - University of Pennsylvania.
X11-A	XAS: Biological EXAFS. PRT: North Carolina State University, NSLS.
X12-B	SAS: Diffraction from fluid samples, muscle filaments, etc., including both amorphous and organized systems; time-resolved studies. PRT: Department of Biology - Brookhaven National Laboratory.
X12-C	XAS: Fixed-wavelength crystallography; multiwavelength anomalous diffraction. PRT: Department of Biology - Brookhaven National Laboratory.
X19-A	XTAL: Biological EXAFS and XANES. PRT: EXAFS consortium.
CHESS	
A1	XTAL, SAS: Fixed-wavelength crystallography (unit cells up to 1000Å); small-angle scattering.
A3	XAS, SAS: General spectroscopy; small-angle scattering.
B2	XTAL: Laue diffraction.
C2	XAS, SAS: General spectroscopy; small-angle scattering.
F1	XTAL, SAS: Fixed-wavelength crystallography (unit cells up to 1000Å); multiwavelength anomalous diffraction; small-angle scattering.
SSRL	
1-4	SAS: General small-angle scattering.
1-5	XAS: EXAFS.
1-5AD	XTAL: Multiwavelength anomalous diffraction.
2-1	SAS: Small-angle scattering.
2-3	XAS: General spectroscopy.
4-1	XAS: General spectroscopy.
4-2	SAS, XAS: Small-angle scattering; general spectroscopy.
6-2	XAS: General spectroscopy. PRT: Lawrence Berkeley Laboratory, Exxon.
7-1	XTAL: Fixed-wavelength crystallography
7-2	SAS: Small-angle scattering.
7-3	XAS: General spectroscopy.

* For appropriate beamlines, the Participating Research Team (PRT) or Insertion Device Team (IDT) that operates the beamline is also listed.

Table B-2. Beamlines Being Commissioned for Structural Biology

Synchrotron	
Beamline	Experiments
NSLS	
X4	XTAL: Multiwavelength anomalous diffraction; fixed-wavelength crystallography. PRT: Howard Hughes Medical Institute.
X8-C	XTAL: Fixed-wavelength crystallography; multiwavelength anomalous diffraction. PRT: Physics and Materials Science programs at Los Alamos National Laboratory and Sandia Laboratory, Biology Division of Argonne National Laboratory.
X25	XTAL: Laue diffraction. IDT: R. Sweet, Department of Biology, Brookhaven National Laboratory (one of eight members of the IDT).
X26-C	XTAL: Laue diffraction. PRT: Applied Sciences Department of Brookhaven National Laboratory, Consortium for Advanced Radiation Sources, Biology Division - Argonne National Laboratory.
SSRL	
10-2	XTAL, XAS: Laue diffraction, spectroscopy. PRT: Lawrence Livermore Laboratory, University of California system.

Table B-3. Laboratory-wide Ancillary and Computing Facilities at U.S. Synchrotron Sources

	NSLS	CHES	SSRL
# Stations for structural biology	7	4	11
Total # stations	79 X-ray 29 ultraviolet	11	24
Ancillary Facilities:			
(availability)			
Stockroom	24 hrs.	24 hrs. (Mech. & Elect.)	24 hrs.
Machine shop	24 hrs.	24 hrs.	24 hrs.
(user shop)			
Support staff	40 hrs/week	24 hrs.	24 hrs.
Biochemistry lab	24 hrs.	24 hrs. (by prior arrangement)	24 hrs.*
Cold room (4°)	24 hrs.	24 hrs. (by prior arrangement)	none
Inert atmosphere chamber	no	no	24 hrs.
Biohazard containment	no	BL3	BL2
Other	dark room	2 darkrooms (24 hrs.)	darkroom (24 hrs.); liquid N ₂ and liquid He cryostats.
Central computing	connection to international networks	microVAX 3800 host networked to 12 VAXstations and two DECstation 5000's. Connection to internet, bitnet, and phone lines.	VAX8810 (VMS). Networked to beamline computers, to data transfer system, to terminals and printers throughout the lab, and to the world via HEPnet, bitnet and 8 phone lines. PDP-11/34 data transfer system with tape drive, and disk drives and dual floppy drive.

* Stereomicroscope, optical analyzer, ultracentrifuge, microcentrifuge, UV-VIS spectrophotometer, ultrafiltration cells, convection oven, pH meter, analytical balance, water purifier, refrigerated circulator, refrigerator/freezer, ice machine, dishwasher, vortex mixer, ultrasonic cleaner.

Table B-4. Characteristics of U.S. Synchrotron Radiation Beamlines Used for Structural Biology

Synchrotron Beamline	Insertion Device	Energy* Selection	Optics
Beamlines currently in operation:			
NLSL			
U9-B	none	up to 9 eV	Off-axis ellipsoidal mirror.
X1-A	undulator	0.25-0.73 keV	Zone plate.
X9	none	3.5-18.3 keV	Vertical mirror focusing, horizontal monochromator focusing.
X11-A	none	4.0-35.5 keV	No focusing.
X12-B	none	7.7-20.7 keV	Two mirrors, doubly focused.
X12-C	none	7.5-13 keV	Bent cylindrical, Rh-coated mirror.
X19-A	none	2-20 keV	Post-monochromator mirror.
CHESS			
A1	6-pole wiggler	7.9 keV	Horizontally focused Ge (111) monochromator followed by vertically focusing mirror.
A3	123-pole undulator	4-25 keV	None (undulator during dedicated runs only).
B2	none	4-50 keV	Flat mirror for high energy cutoff - no focusing.
C2	none	4-20 keV (focused)	Double-crystal monochromator with sagittal focusing.
		4-33 keV (unfocused)	
F1	25-pole wiggler	6-14 keV	Horizontally focused Si (111) monochromator followed by vertically focusing mirror.
SSRL			
1-4	none	6.7-10.8 keV	Fused-silica, Pt-coated mirror. 2.0 mrad into curved-crystal monochromator.
1-5	none	2.8-30 keV	No focusing. 1.0 mrad into double-crystal monochromator.
2-1	none	2.8-8.9 keV	Bent cylinder, fused quartz, Pt-coated mirror, 0-4.8 mrad into double-crystal monochromator.
2-3	none	2.8-30 keV	No focusing. 1.0 mrad into double-crystal monochromator.
4-1	8-pole wiggler	2.8-50 keV	No focusing. 1.0 mrad into double-crystal monochromator.

Table B-4. Characteristics of U.S. Synchrotron Radiation Beamlines Used for Structural Biology
(continued)

Synchrotron Beamline	Insertion Device	Energy* Selection	Optics
Beamlines currently in operation:			
SSRL			
4-2	8-pole wiggler	2.8-10.2 keV (focused)	Bent cylinder, fused quartz, Pt-coated mirror. 0-4.6 mrad into
		2.8-50 keV (unfocused)	double-crystal monochromator.
6-2	54-pole wiggler	2.2-22 keV (focused)	Bent cylinder, fused quartz, Pt-coated mirror. 0-2.3 mrad
		2.2-50 keV (unfocused)	(focused), 0-1.0 mrad (unfocused) into double-crystal monochromator.
7-1	8-pole wiggler	6-13 keV	Fused-silica, Pt-coated mirror (bendable for vertical focusing). 1.0 mrad into curved single-crystal monochromator.
7-2	8-pole wiggler	2.8-10.2 keV (focused)	Bent cylinder, fused quartz, Pt-coated mirror. 0-4.6 mrad
		2.8-50 keV (unfocused)	(focused), 0-1.0 mrad (unfocused) into double-crystal monochromator.
7-3	8-pole wiggler	2.8-50 keV	No focusing. 1.0 mrad into double-crystal monochromator.
Beamlines being commissioned:			
NSLS			
X4	none	3-40 keV	8 mrad into monochromator with sagittal focusing.
X8-C	none	5-25 keV	Toroidal focusing mirror. 4 mrad into double-crystal monochromator.
X25	31-pole wiggler	white radiation	Double-focusing, Pt-coated mirror. 3 mrad.
X26-C	none	white radiation	Focusing mirror. 4 mrad.
SSRL			
10-2	31-pole wiggler	2.8-22 keV or into	Mirror. 0-2.3 mrad (focused), 0-1.0 mrad (unfocused)
		white radiation	double-crystal monochromator.

* Wavelengths, in angstroms, can be obtained from the relation $\lambda = 12.39854/E$, where E is in keV.

Table B-5. Equipment Associated with Beamlines for Structural Biology

Synchrotron	
Beamline	Equipment
Beamlines currently in operation:	
NSLS	
U9-B	2.2T electromagnet for magnetic CD; emission monochromator for fluorescence experiments; temperature control of samples (10-373K); 80386 computer for control of spectrometer; microVAX II.
X1-A	Scanning X-ray microscope; image acquisition and analysis computer and graphics equipment; clean room with Nomarski, inverted phase microscopes, fume hood, shaking incubators, refrigerated centrifuge. PRT members only: Gabor holography chamber; CCD camera for Fourier transformation holography.
X9	Absorption and fluorescence ion chambers; amplifiers; V-F convertors; X-Z translation table; lab equipment (UV-VIS spectrometer, EPR, glove box, pH-meter, balance, microscope); VAX-730, Packard Bell, MacII computers. PRT members only: 13-element Ge detector and electronics (also available to general users upon request), position detector; SIT detector.
X11-A	Specimen dewars. PRT members only: Displex cryostat; special detector.
X12-B	Time-slicing data collection in one and two dimensions; temperature control, slow temperature jump and stopped-flow equipment; auto sample-changer; 3-circle goniometer; two microcomputers for experimental control and data analysis.
X12-C	FAST (Enraf-Nonius) video-based area diffractometer; specimen cooling to -40°C; two microVAX III's, ~2GB disk storage, 8mm tape drive.
X19-A	13-element Ge detector array; fluorescence ion chamber; transmission ion chambers; liquid He cryostat; microVAX II.
CHESS	
all stations	Flexible oscillation camera with many degrees of freedom; FTS crystal cooler (-60 to +80°C); liquid N ₂ based crystal cooler (80 to 230 K); DECstation 5000 for analysis of diffraction patterns; biohazard containment level BL2 for stations A1 and B2, level BL3 for station F1; six-circle diffractometers; scintillation counters; Si and Ge solid-state detectors; Kodak storage-phosphor scanner system.
SSRL	
1-4	IBM PS/2; 1024-element array detector (25-μ pixel).
1-5	microVAX; 4-circle diffractometer (CAD-4).

Table B-5. Equipment Associated with Beamlines for Structural Biology (continued)

Synchrotron

Beamline	Equipment
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Beamlines currently in operation:

SSRL

1-5AD	One San Diego Multiwire System 2-D multiwire proportional counter; 5-circle Huber diffractometer; microVAX II/GPX.
2-1, 2-3, 4-1, 4-2, 7-3	microVAX II.
6-2	PDP 11/34.
7-1	Rotation camera; PDP 11/23.
7-2	Huber 6-circle diffractometer; microVAX II/GPX.
all stations	XTAL: Dark room; cryocooling for crystals; conventional generator for fiducial marking; cooling to 4K; considerable software for data processing. XAS: 3-SSRL 6" ion chambers on optical bench with Huber X-Y slits; 3-Stern/Heald/Lytle ion chambers with soller slits and filters; 2 adjustable arrays of 4 NaI(Tl) scintillation detectors; 13-element Ge solid-state detector (~200 eV resolution at liquid N ₂ temp.); liquid He cryostats. SAS: Strobe for sample excitation, optics for simultaneous absorption; temperature-controlled sample enclosure.

Beamlines being commissioned:

NSLS

X4	4-circle diffractometer; storage phosphors and scanner; instrument control and data-reduction computers; crystal-cooling apparatus; cold room hutch (10°C); biocontainment BL-2.
X8-C	CCD electronic area detector (1024 x 1024 pixel); 6-circle diffractometer; microVAX 3200; VAX station 4000; FTS crystal cooler; liquid N ₂ cryostat.
X25, X26-C	Rotation camera with film or imaging plate detector; CCD camera; temperature control of experiment; flash lamps and lasers for reaction initiation and photolysis; flow cells.

SSRL

10-2	Home-built goniometer and spectrometer.
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Data on use of synchrotron radiation facilities for structural biology, as reported by facility staff, are given in Tables B-6 and B-7. Data from CHESS are nearly complete. It was not possible to obtain data from SSRL for each beamline; SSRL is run as a fully integrated laboratory. Data from NSLS came from the PRT for each beamline, and some of the questionnaire responses are clearly rough estimates.

Table B-6. Use of Beamtime on Experimental Stations Used for Structural Biology

Synchrotron Beamline	Year	Number of Days					No Beam
		Structural Biology	Other Experiments	Development	Repair	Unused	
NSLS ¹							
U9-B ²	1985	179	0	75	22	89	141
	1986	193	0	72	25	75	132
	1987	195	0	65	16	89	130
	1988	216	0	90	11	48	135
	1989	227	0	101	15	22	120
	1990	198	0	123	10	34	75
X1-A	1985	120	0	50	50	5	80
	1986	90	0	50	50	5	80
	1989	180	0	50	50	5	80
	1990	240	0	20	10	5	80
X9	1989	149	19	16	1	0	168
	1990	179	32	22	0	0	126
X11-A	1985	12	NR	NR	NR	NR	NR
	1986	12	NR	NR	NR	NR	NR
	1987	12	NR	NR	NR	NR	NR
	1989	12	NR	NR	NR	NR	NR
	1990	12	NR	NR	NR	NR	NR
X12-B	1989	128	110	18	18	0	91
	1990	128	110	18	18	0	91
X12-C	1986	73	0	0	98	66	128
	1987	70	2	0	0	36	257
	1989	95	3	60	0	30	176
	1990	125	0	20	40	80	100
X19-A	1989	90	90	30	30	< 5	120
	1990	100	100	20	10	< 5	120

¹ NSLS was down during 1988.

² Total days/year is greater than 365 because development and repair were done in part on days with no beam to the station.

Table B-6. Use of Beamtime on Experimental Stations Used for Structural Biology (continued)

Synchrotron Beamline	Year	Number of Days					No Beam	
		Structural Biology	Other Experiments	Development	Repair	Unused		
CHESS ³								
A1	1985	81	99	6	4	5	170	
	1986	54	52	4	3	6	246	
	1987	124	60	6	4	6	165	
	1988	17	17	7	2	2	320	
	1989	17	10	3	2	2	331	
	1990	105	22	8	3	3	224	
A3	1985	10	160	10	10	50	125	
	1986	10	75	10	10	30	230	
	1987	10	180	10	10	50	125	
	1988	5	115	10	10	50	175	
B2 ⁴	1990	14	22	5	2	10	224	
C2	1985	20	180	10	10	0	125	
	1986	15	80	10	10	0	230	
	1987	25	175	10	10	0	125	
	1988	20	130	10	10	0	175	
	1989	25	175	10	10	0	125	
F1 ⁴	1990	25	0	20	1	5	40	
SSRL								
all stations	1985	XAS	181	821 ⁵	not readily available			
		XTAL	85					
		SAS	55					
	1986	XAS	121		not readily available			
		XTAL	77					
		SAS	74					
	1987	XAS	204		not readily available			
		XTAL	100					
		SAS	38					
	1988	SAS	5		52	not readily available		
	1989	XAS	39		277	not readily available		
		XTAL	28					
		SAS	14					
	1990	XAS	49		322	not readily available		
		XTAL	34					
SAS		17						

³ CHESS was down during most of 1988-89 to upgrade High Energy Physics detector.

⁴ CHESS stations B2 and F1 began operations during 1990.

⁵ Reported number is the total for all nonbiological experiments on all SSRL beamlines.

Table B-7. Number of Structural Biology User Groups and Number and Duration of Experimental Runs

Synchrotron Beamline	Year	Number of User Groups	Number of Runs/Group (Range or Average)	Number of Days/Run (Range or Average)
NSLS				
U9-B	1985	13	3	4
	1986	11	3	4
	1987	12	3	4
	1988	14	3	4
	1989	15	3	4
	1990	13	3	4
X1-A	1985	3	3	16
	1986	3	3	16
	1989	4	3	16
	1990	5	3	16
X9	1989	34	1-3	2-3 XAS, 5-7 SAS
	1990	37	1-3	2-3 XAS, 5-7 SAS
X11-A	1985	2	NR	NR
	1986	2	NR	NR
	1987	2	NR	NR
	1989	2	NR	NR
	1990	2	3	2
X12-B	1989	3	2	6
	1990	5	2	6
X-12C	1986	15	1	4
	1987	16	1	5
	1989	36	1	7
	1990	16	2	5
X19-A	1989	9	3	4
	1990	10	3	4
	CHESS			
A1	1985	15	1-8	3-9
	1986	11	1-3	1-7
	1987	33	1-6	1-11
	1988	12	1-2	2-5
	1989	6	1-2	2-5
	1990	22	1-5	1-9
A3	1985	1	2	5
	1986	1	2	5
	1987	1	2	5
	1988	1	1	5
B2	1990	8	1-3	2-6
C2	1985	3	2	3
	1986	3	2	3
	1987	4	2	3
	1988	3	2	3
	1989	4	2	3
F1	1990	4	1-2	2-5
SSRL				
all stations	1985	31	1-9	1-9
	1986	35	1-9	1-10
	1987	37	1-10	1-11
	1988	1	1	5
	1989	23	1-3	1-6
	1990	18	1-3	1-11

Use of synchrotron radiation facilities abroad by U.S. researchers, as reported by scientific staff at the synchrotron facilities, is given in Table B-8. Data are complete from the Photon Factory, except for one beamline where we made estimates based on user responses. Data from SRS, LURE and DESY appear to be very rough estimates.

Table B-8. Use of Synchrotron Sources Abroad by Structural Biologists in the U.S.

Year	Number of Days					Number of User Groups			
	SRS	LURE	DESY	PF	Total	SRS	LURE	DESY	PF
1985	2	~20	10	0	32	1	~2	1	0
1986	4	~20	10	0	34	2	~2	1	0
1987	0	0	7	14	21	0	0	1	1
1988	3	0	19	33	55	1	0	4	2
1989	13	0	21	45	79	2	0	3	5
1990	9	~20	7	31	67	1	~2	1	5

Use of U.S. synchrotron sources for structural biology by researchers based outside the U.S. was reported only for CHESS and SSRL. Use of CHESS was reported to be 11 days in 1990, 7 days in 1987, 5 days in 1986 and 2 days in 1985, all on station A1. This amounts to 4% of the total beamtime used for structural biology. SSRL staff estimate that 4% of all beamtime at SSRL is used by research groups from outside the U.S. for all experiments. They could make no separate estimate for structural biology experiments, but told us that a total of three non-U.S. research groups have done structural biology experiments at SSRL that were not scientific collaborations with SSRL staff.

The fraction of their synchrotron radiation beamtime that U.S. structural biologists have spent at facilities outside the U.S. increased sharply in 1988 when no U.S. facilities were operating.

Table B-9. Proportion of Structural Biology Beamtime Used Outside the U.S.

	1985	1986	1987	1988	1989	1990
Total beamtime for structural biology (# days)	596	560	604	102	856	1095
Time spent at facilities abroad						
# days	32	34	21	55	79	67
percent	5	6	3	54	9	6

There is a large discrepancy between the amount of beamtime reported by synchrotron radiation users and the amount reported by staff of the synchrotron facilities. In nearly all cases, the 57% of structural biologists who responded to the user questionnaire reported more beamtime than was reported by the facility staff. This is not limited to particular scientific disciplines or to particular synchrotron facilities. We can think of several reasons for the discrepancy.

- Users were asked how many days they spent at synchrotron sources while data from synchrotron facilities were, in nearly all cases, days of usable beam. There is often a large difference between time assigned and useful experimental time. In addition, we should have asked users about experimental time rather than “time spent at synchrotrons”.
- Users reported very little reduction in beamtime for 1988 (Appendix A, Table A-6), when CHESS, SSRL and NSLS were all down for major facility work. This leads us to suspect that user-reported synchrotron radiation beamtime may be overestimated in general, particularly in earlier years. The discrepancy is smallest in later years.
- Facility-reported data must be considered a lower estimate of true experimental time. “Unofficial” structural biology experiments would not appear on facility records. Where we have only rough estimates of beamtime use, facility staff seemed to be reporting time used by only those research groups they could remember.
- Many users reported synchrotron radiation use for years when they were not independent investigators, although we asked them not to do so. Our user data can be corrected for this.

In Table B-10 we show the beamtime reported by users (corrected to remove time when the investigator worked in another’s lab) in comparison with beamtime reported by facility staff.

Table B-10. Comparison of Beamtime Use Reported by Users and by Facility Staff

Year	Number of Days Reported									
	XTAL		XAS		SAS		IMG		Total	
	Users	Sync	Users	Sync	Users	Sync	Users	Sync	Users	Sync
1985	300	194	139	207	221	75	140	120	800	596
1986	245	234	183	142	328	94	150	90	906	560
1987	221	304	232	235	137	65	140	0	730	604
1988	216	64	202	14	100	24	150	0	668	102
1989	323	181	288	272	74	223	193	180	878	856
1990	379	331	296	297	139	227	172	240	986	1095

We asked facility staff to estimate the ratio of demand to supply for each beamline. The data in Table B-11 are rough estimates. Several respondents told us that users do not apply for beamtime if they believe that the wait for beamtime is long or that they will not get time or if the available facilities are difficult to use.

Table B-11. Estimated Ratio of Demand to Supply of Beamtime

Synchrotron Beamline	Demand for Beamtime/Beamtime Available					
	<0.5	0.5-0.8	0.9-1.1	1.2-1.5	1.6-2.0	>2.0
NSLS						
U9-B ¹						
X1-A ²						
X9 ²						
X11-A						
X12-B ³						
X12-C ⁴						
X19-A ⁵						
CHESS						
A1 ⁶						
B2 ⁷						
C2 ⁸						
F1 ⁹						
SSRL						
all stations ¹⁰						
1-5AD						
7-1						

- 1 NSLS U9-B: "Increasing! Limiting factor is personnel to assist users. Demand would increase greatly if we had the ability to support more users."
- 2 NSLS X1-A, X9: "Increasing."
- 3 NSLS X12-B: "Increasing, have not advertised availability."
- 4 NSLS X12-C: "Increasing/static - it's self-limiting. People don't really state an intention to come if the wait will be longer than two months."
- 5 NSLS X19-A: "Ratio is increasing as beamline performance improves and people realize beamline strengths."
- 6 CHESS A1: "This ratio is static at the moment at about 1.1."
- 7 CHESS B2: "Static for the few months that the station has been in operation."
- 8 CHESS C2: "Static."
- 9 CHESS F1: "Many crystallographers are just realizing how good this station is for their projects and that it is available for users."
- 10 "SSRL has not operated in a normal manner since 1987. The paper user community is shrinking. However, there is every indication that the large demand is still out there."

APPENDIX C. WRITTEN COMMENTS BY QUESTIONNAIRE RESPONDENTS

Below are all comments, unedited, that were written by respondents to the user questionnaire.

"Synchrotrons are a terrific example of "big science" benefitting a wide variety of scientists by making unique resources accessible to a community. There has to be a continuing interactive development that occurs between on-site scientists and users who travel periodically to the source. Structural biologists should be united in recognizing the current and future value of these resources even if their current experiments don't require synchrotron radiation."

"Some of the questions seem quixotic, especially under #6. For example, why not word the second statement "Synchrotrons are essential to industrial growth", i.e. in general, what, precisely, is "big science"? Who is not an "individual scientist"? Also, the questionnaire is top-heavy with crystallography. What about EXAFS and small-angle diffraction? Less attention is given to these areas."

"This kind of survey is extremely useful if BioSync is willing to make hard choices and come out for certain things strongly. As stated in my questionnaire, my limitation is research funds to employ postdocs, students to analyze data. We can collect data extremely fast and efficiently. I can't afford the number of people in my lab that I could give good X-ray projects to. I think both APS and ALS may be too expensive - focus on ALS and BNL (and perhaps a SSRL rebuild). We really need more funding for individual investigator grants probably not to new synchrotrons."

"Prefer image plates with an option of on-site reduction or take home reduction."

"I believe development of time-resolved crystallography/small-angle/XAS ... is probably the most unique advance in biophysical capabilities for synchrotron radiation."

"My answer to question 9 is a disquieting one to me personally. If a presently working facility must be abandoned at some point, my choice for that facility would involve many considerations, none of which I am asked to provide descriptions of on this form. There is also a temporal factor—my answer today could change dramatically over time."

"Coming from an industrial group that is a member of the consortium planning to build a facility at APS, funding per se for beamtime access is not a direct issue. However, funding from federal sources for nonproprietary beamlines is essential to foster the environment in which an industrial beamline can function efficiently."

"Awkward questionnaire. Should standardize answer types."

"I think the use of synchrotrons for RO1 type science is extremely important. It's "big science" for sure, but there's so much "little science" that benefits. It's in a different class from other "big science projects" where relatively few benefit."

"My group's synchrotron radiation activities, in structural chemistry, consist of crystallographic studies on extremely small single crystals of systems important in catalysis, or of advanced materials such as high T_C metal oxides. My biological work is restricted to the Protein Data Bank."

"Biophysics, molecular biology, X-ray crystallography, all, will profit from better synchrotron sources. Many new wonderful things are becoming available and will continue to do so because of the fantastic flux and tunability. Time-resolved experiments on ligand-binding and enzyme kinetics can now be planned that were impossible 5-10 years ago. The future is bright and we don't even know for sure what the limitations are."

"I'm a minor consumer of synchrotron techniques, thus my opinions could be cheerfully downweighted. I submit them only as a trained, but largely neutral, observer."

"The most severe limitation is access to operational beamlines. I would put priority #1 as funding all existing synchrotron radiation labs to run continually."

"There are research problems not well suited for conventional (i.e. rotating anode) X-ray sources; a well equipped and staffed center is our essential national resource - it is shocking how slow the U.S. has been in developing and providing this resource."

"I might use NSLS to do a problem now getting underway. Synchrotrons are not essential for my work. Any sensible person has to ask why more synchrotrons must be build now when the ones we have are not fully supported or made efficient use of. Given the current budget problems, starting new sources may mean closing the ones we have now."

"For question #10 I would be very surprised if film-based data collection isn't totally replaced by image plate technology within three years at all synchrotrons!"

"Our group is developing electronic area detectors, not doing structural biology research per se, so my answers to these questions are unorthodox, since the questions seem to be designed for other potential synchrotron users."

"EXAFS is an important component of my research on metalloproteins, but it is only one of many techniques we use."

"It's damned hard to get beamtime unless you are one of the big users. I have waited 3 years for time at Cornell.

SSRL was a total bust."

"Synchrotron sources are centrally important to structural biology. They should be supported as well as finances permit."

"Some of the questions cannot be answered as multiple choice questions, but need more explanation for a well balanced answer."

"Using conventional X-ray generators we are able to collect X-ray diffraction data to $\sim 2.7 \text{ \AA}$ resolution on crystals of gamma interferon. We hope to extend this to $\sim 2.0 \text{ \AA}$ resolution using synchrotron radiation."

"For me, the biggest consideration would be to have fully reduced data by the time we left. Moreover, expense is a major factor - not only transportation but also accommodation. Thus, remaining long after the data collection to process data becomes very costly: e.g. on-site densitometry, etc. As we would use synchrotron data collection as an alternative to area detectors or even diffractometry, our data handling programs at home start with reduced, corrected data. So we really need fast data reduction as well as collection. Time = money!

Generally, I think that the greatest number of users might be achieved by having good support staff and documentation and access to data reduction. Perhaps it would be possible to go home and reduce the data over the network (if good documentation were available)."

"I am a very limited user for now - weight this form accordingly."

"Go for it."

"Prior to becoming an independent investigator, I have had experience at SSRL and Daresbury. The one flaw I see at the facilities where I have worked, (add NSLS) is the attitude among many scientists that the synchrotron beamlines is a resource they are entitled to. Individuals should contribute time and/or financial resources to the beamlines, scaled to their total research dollars."

"Depending upon conditions, etc., I would like to have available all of the various options listed under (10)."

"My synchrotron requirements are still developing - it is too early for me to answer all questions accurately."

"Will be less active researcher in the near future."

Support for these facilities is important for reasons in addition to "production data collection" - new methods and techniques are frequently developed in such facilities."

"Macromolecular crystallography should move forward and not stagnate. For this to be successful new technologies must be tried and developed and made available in a user friendly way to the researchers."

"We are in the early stages of a long term investigation of the human factor XIII blood coagulation factor. We will need synchrotron radiation to go to atomic resolution (or even 2.8 \AA). This protein is medically important so I think we will be successful in getting its structural investigation funded."

"Synchrotron radiation is obviously essential for some structural biology. In other cases it may be difficult to anticipate whether use of a synchrotron will be essential. More frequent use of feasibility studies would be good."

"I would only use synchrotron radiation for protein crystallography when:

- 1) small crystals - require high brilliance,
- 2) large unit cells - require high collimation,
- 3) only one or two heavy atom derivatives which benefit from MAD,
- 4) impossible to get derivatives and need to attempt selenomethione MAD method.

Otherwise, in-house area detectors are sufficient.

I have no plans to do Laue and/or time-resolved experiments, but appreciate that these methods require synchrotron radiation. I regard on-site data processing as essential!"

"I plan to do general crystallography on protein mutants and may or may not actually use synchrotron radiation."

"I expect my use to evolve from mostly EXAFS \varnothing diffraction work."

"I have just started as a P.I. so projections are difficult. Projects I have worked on did not require a synchrotron."

"Some of these questions are hard to answer, i.e. #10. I would use whatever is the best method available at the time for the particular project in hand.

What is important is that synchrotrons still stay on top of new developments so users do not have to worry about the details."

"I have used SSRL once for small-angle scattering of muscle in the mid-1970's, but have no plans to re-enter this field at present."

"Small-angle scattering is not possible unless suitable cameras are available. What cameras are planned? Why no mention?"

"Must improve availability and ease of use. Perhaps less hands-on experimentation, more service facility stressing production and throughput. Obviously, there should always be time and support (and priority) for pioneering efforts."

"Build broadly towards the future for synchrotron-based biology! X-ray diffraction is both important and a proven method, but it should not define what is meant by "biosynch"."

"I have not used synchrotron sources very much because of the lack of convenient, easy availability in the U.S."

"The costs associated with using a synchrotron source are very high (travel, preparation, film, etc.). We have spent about \$10,000 this year to complete a Laue diffraction experiment and have been unsuccessful due to repeated 'ring' failures. A very important consideration to us is whether existing rings can be made to operate more reliably with less down time for injection and also will the new rings (Argonne & Berkeley) be more or less reliable than existing rings?"

"An oversight committee must help in the budget process to ensure that limited money is concentrated in a few sites.

The facilities should pick an optical disk standard for frame transfer to labs."

"I've come from the SASX/SAXD group at Stanford working with Seb Doniach and Keith Hodgson. Even at Stanford where SAXD is practiced, it represents a very small group of investigators (<4 with biological applications). I think the anomalous SAXD that we've done is very promising, but it needs a larger commitment of beamtime to sort out the artifacts. I am also using E.M. and fluorescence spectroscopy to study the acetylcholine receptor, because SSRL's operation was compromised in the race to produce Z0's. This is a strong argument for a separate state of the art facility for producing X-rays. Furthermore the very large SSRL downtime/uptime ratio of the past few years proved to be very discouraging to a new assistant professor trying to use anomalous scattering to study the acetylcholine receptor.

I am quite ambivalent about the cost effectiveness of synchrotrons. They provide the ability to "see" things not seen by other techniques. However, they are incredibly expensive and if the choice were between my grants @\$70,000/grant and a synchrotron @\$500,000,000, I would probably opt for my grants. Fortunately until now, both have been funded.

I've never seen a synchrotron really hum! While I was there, SSRL was always limping along on a shoestring. Subsequently funds have been spent to upgrade administrative facilities: looks nice, but does it make the machine work better? I've never been to Brookhaven or any of the foreign synchrotrons, so my view is quite provincial. I very much like the medium scale operation represented by SSRL and CHESS, but to get a synchrotron that really hums, maybe the APS and the NLSL state of the art facility is necessary.

I look forward to further communications from the BioSync group."

"Ideal beamlines for my science are not available in the U.S."

"In question 10, image plates might be used with dispersive EXAFS. Hence, electronic area detectors could be used for time resolved dispersive EXAFS."

"For the projects in my lab, the most important use of the synchrotron would be to collect native data to a higher resolution than possible on the rotating anode. Also tuning the wavelength to be close to the absorption edge of the heavy-atom would be of interest.

My plans for the next few years depend on the applications that will be done within a year or so, how useful will they be for the progress of the project."

"My group is still mostly at the rotating anode stage, where there are lots of good, cheap experiments, provided we can afford to have graduate students. However, this could change rapidly. The issue really comes down to one of cost-effectiveness in supporting research."

APPENDIX D. USER QUESTIONNAIRE

APPENDIX E. QUESTIONNAIRES TO SYNCHROTRON RADIATION

FACILITIES APPENDIX F. GLOSSARY OF TECHNICAL TERMS AND ACRONYMS

A source of general information about synchrotron radiation and hardware is "Introduction to Synchrotron Radiation" by G. Margaritondo (Oxford University Press, 1988).

ALS - acronym for Advanced Light Source, synchrotron radiation source under construction at Lawrence Berkeley Laboratory.

APS - acronym for Advanced Photon Source, synchrotron radiation source under construction at Argonne National Laboratory.

beamline - path of radiation emitted by a bending magnet or insertion device through an aperture in the storage ring. The beamline is tangential to the particle orbit in a bending magnet and collinear with the mean orbit in an insertion device. The term as generally used includes all optical and experimental components "downstream" of the aperture, and may include several experimental stations.

brightness - flux per unit solid angle, usually expressed in units of photons/sec/mrad²/mA stored current, emitted over 0.1% fractional bandwidth. (Brightness is defined identically to brilliance by some authors.)

brilliance - flux per unit source area and unit solid angle, usually expressed in units of photons/sec/mm²/mrad²/mA stored current, emitted over 0.1% fractional bandwidth. Brilliance is a measure of beam quality that takes into account the divergence of the synchrotron beam, and is of importance for those experiments that require excellent angular collimation.

CAT - acronym for Collaborative Access Team, used by APS to denote groups of researchers who develop and operate beamlines.

CHESS - acronym for Cornell High Energy Synchrotron Source, synchrotron radiation source at Cornell University.

DESY - acronym for Deutsches Elektronen-Synchrotron, synchrotron radiation source in Hamburg, Germany.

emittance - a measure of the size and angular extent of the particle beam in the storage ring, usually expressed in units of nm-radians. Particle beams of lower emittance produce synchrotron radiation of greater brilliance.

ESRF - acronym for European Synchrotron Radiation Facility, synchrotron radiation source being constructed by the European Community at Grenoble, France.

EXAFS - acronym for Extended X-ray Absorption Fine Structure. EXAFS analysis is the study of the modulation of X-ray absorption that occurs just above an absorption edge and is due to backscatter by atoms that are near neighbors (<~5Å) of the absorbing atom. Structural information is obtained about the number and type of backscatterers and their distances from the absorbing atom.

experimental station - instrumentation for performing an experiment with synchrotron radiation. Usually includes a hutch and the experimental instrumentation it contains, together with electronic and computer hardware to control the station.

flux - beam brightness integrated over the entire source area and over all vertical angles, usually expressed in units of photons/sec/mrad/mA stored current, emitted over 0.1% fractional bandwidth.

hutch - enclosure for the experimental apparatus on a beamline. This protects personnel from the high radiation levels around the experiment.

insertion device - periodic array of magnets inserted in one of the straight sections of the storage ring. These are wigglers or undulators and serve to boost the intensity of the synchrotron radiation or to shift or drastically modify its energy spectrum for the particular beamline they precede in the ring.

LURE - acronym for Laboratoire pour l'Utilisation du Rayonnement Electromagnétique, synchrotron radiation source at the Université Paris Sud, Orsay, France.

MAD - acronym for Multiwavelength Anomalous Diffraction. MAD is used to solve the crystallographic phase problem directly from intensities measured at different wavelengths where a component of the crystal scatters anomalously due to the proximity of the wavelengths to an absorption edge.

NSLS - acronym for National Synchrotron Light Source, synchrotron radiation source at Brookhaven National Laboratory.

Photon Factory - synchrotron radiation source at the National Laboratory for High Energy Physics, Tsukuba, Japan.

PRT - acronym for Participating Research Team, used by NSLS and ALS to denote groups of researchers who develop and

