Structural Biology and Synchrotron Radiation:
Evaluation of Resources and Needs

Report of BioSync –

The Structural Biology Synchrotron Users Organization

1997

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Introduction

The Structural Biology Synchrotron Users Organization (BioSync) was formed in 1990 to promote access to synchrotron radiation for scientists whose primary research is in the field of structural biology. The synchrotron radiation techniques used for structural biology can be sub-divided into four principal areas: crystallography, spectroscopy, scattering from noncrystalline materials and imaging. The BioSync membership includes leaders of all such structural biology research groups in North America.

The BioSync group had two organizational meetings and convened a Study Group in 1989/90. The main result of that activity was publication of a report in 1991 on the status of structural biology research using synchrotron radiation. It included the results of surveys of both the synchrotron radiation facilities and the structural biology research community. The Study Group of experienced structural biologists and synchrotron radiation experts met to evaluate the survey data, to assess the size and needs of the community, to predict what synchrotron radiation facilities would be needed in the future, and to write the report, which was published in July, 1991.

The main conclusions of the report were that structural biology, especially crystallography, was a very rapidly growing field with a growing impact on basic and applied biology, and that the synchrotron radiation facilities available at the time were insufficient for the needs of this ever-expanding community. Both construction of additional beamlines and improved support for existing beamlines were recommended to meet the predicted need. Construction costs were estimated at $3-4M per beamline and operation costs at $0.5-1.0M per year per beamline (1990 dollars). In its most controversial conclusion, the Study Group predicted a very large "latent" demand for synchrotron beam time from biologists who were not specialists in structural methodologies. More streamlined structural experiments coupled with an intense demand for new macromolecular structures were the driving forces for the "latent" demand.

This document is intended to provide a report on the current status of the biological uses and demands of synchrotron radiation in the U.S. and is an update to the 1991 BioSync Report. The synchrotron radiation facilities and user community have once again been surveyed, and a group of experienced structural biologists has analyzed the data and written this report. The 1997 BioSync Committee met in Cambridge, MA, in July, 1997, to analyze the results of the user and facility surveys, to consult with users and with representatives of funding agencies and to reach the main conclusions presented in this report.

The 1997 BioSync Committee members, and authors of this Report, are:

Ian Wilson, D. Phil., The Scripps Research Institute, Chair
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Others attending the meeting in Cambridge, MA, in July 1997, were:

> Marvin Cassman, Ph.D., NIGMS, National Institutes of Health
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Several distinguished structural biologists reviewed this report before publication, including:

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December, 1997

Executive Summary

In the six years since the last report of the Structural Biology Synchrotron Users Organization (BioSync), the impact of structural biology in all areas of biological science has expanded greatly, as anticipated. With this continued development has come an increase in both the size and the complexity of the macromolecular structures that are being determined and in the difficulty of the experiments that are being pursued. This increase in complexity, which was not expected to occur so quickly, has meant that synchrotron-based structural biology has expanded its role and now makes a significant contribution in addressing the fundamental questions of how life processes are carried out and the practical applications of treating disease at the molecular level. Thus, it is not simply that the number of macromolecular crystal structures is growing, but more importantly, that structural biology is having an increasing impact on such diverse fields as immunology, neurobiology, cell biology, virology, physiology, molecular biology, medicine and biotechnology.

These recent advances can be attributed to three key improvements in methodology: (1) the ability to clone and express a vast array of cellular proteins in quantities sufficient for structural studies, (2) the use of cryo-crystallography to prepare extremely stable crystals, and (3) the availability of and technological innovations at synchrotron radiation facilities. Many more macromolecules are now being crystallized. With the use of frozen crystals, crystallographic studies of large multicomponent complexes have now become nearly routine. High quality data are also being obtained from poorly ordered or weakly diffracting (as opposed to merely small) crystals.   Non-crystallographic synchrotron techniques are also providing complementary information on systems with inherent conformational flexibility or with metal centers. These factors have brought many more projects of high biological significance into the realm of structural biology. Without synchrotron sources, many of these new research projects, which are often extremely challenging biophysical studies, could not yet have been undertaken. It is likely that the advances seen in the last six years represent only the beginning of an even greater explosion in the structural biology field that will accompany the on-going genome projects.

The BioSync Committee has evaluated the present synchrotron needs of the structural biology community through surveys of both the community and the synchrotron facilities themselves and through meetings with experienced users as well as representatives of government funding agencies. The object of this report is to evaluate what synchrotron facilities and support operations are currently needed, and to anticipate what will be required to sustain the exciting progress in structural biology in the coming years. The following main conclusions have been reached.

1. **Structural biology research is producing results of high biological impact that have a direct bearing on human health issues.**

   The 1991 predictions have been borne out and in many ways surpassed. Structure-based drug design, which seemed merely a trendy phrase a few years ago, has become a reality. The design of new medically important drugs, such as the HIV-protease inhibitors and the influenza neuraminidase inhibitors, is a direct consequence of research in structural biology. It is expected that this trend will continue and will become increasingly important in the fight against the plethora of emerging and re-emerging viral and microbial pathogens that are now infecting the human population. Currently, no drugs are available for protozoan diseases such as sleeping sickness, malaria and Chagas' disease. New therapeutics are also required to combat drug-resistant pathogens, such as some forms of tuberculosis, which are no longer controlled by currently available drugs. Structural biology is also becoming increasingly important in biotechnology, as for example, in the design (or re-design) of enzymes to degrade pollutants or to act as thermostable

industrial catalysts, or in the design of insecticides with increased efficacy. These biotechnology applications can have huge environmental and economic impacts. In the basic sciences, the fields of cell and developmental biology have become "molecular" through over-production of extremely interesting macromolecules in sufficient quantity for structural study. Many of these molecules are structurally challenging due to their size and complexity. On the horizon, major new insights are expected into the processes of cell biology and development that will come from the structures of key macromolecules, akin to the revolution in molecular immunology caused by the structure determination of the major histocompatibility antigen (MHC) class I molecule.

2.   **Synchrotron radiation is now a dominant contributor to new macromolecular structures.**

The role of synchrotron radiation in structural biology has been growing rapidly over the last twenty years. A recent survey of the literature shows that synchrotron radiation was used in nearly half of the new structure determinations. The benefits of synchrotron radiation include substantial increases in resolution over those available with laboratory sources and the ability to study crystals that are too small or have a unit cell too large to be studied using home X-ray sources. Time-resolved studies by Laue methods have generated snapshots of enzyme reactions, and dynamic structures in solution have been investigated by small-angle scattering.

3.   **Synchrotron radiation combined with MAD phasing has revolutionized macromolecular structure determination.**

Since 1991, the greatest technological advance in structure determination has been the full development of multiwavelength anomalous diffraction (MAD) phasing methods. This technique has the advantage of accurate and rapid structure determination using diffraction data from one crystal, once an appropriate chemical element has been incorporated in the macromolecule. Thus, MAD bypasses the rate-limiting step of finding isomorphous derivatives. The power of MAD is greatly enhanced by the widespread applicability of the selenomethionine label in proteins and the brominated uracil label in nucleic acids. A disproportionately large number of structures solved with MAD are now being reported in *Science, Nature* and *Cell*, attesting to its importance in structural problems of broad biological significance. MAD, which has an absolute requirement for tunable synchrotron radiation, will continue to have a major impact on the practice of structural biology. It is being adopted so rapidly that adequate MAD beam time is expected to be the most limiting synchrotron resource for structural biology in the next five years.

4.   **Non-crystallographic applications to structural biology continue to grow.**

X-ray absorption spectroscopy, small-angle X-ray scattering and X-ray imaging continue to provide crucial information for systems that are dynamic, are very large or include metal centers. In addition, these methods complement crystallography in providing information critical for understanding biological function. For example, X-ray crystallography and X-ray absorption spectroscopy in combination provide a complete description of metal sites in proteins that is not provided by either technique alone. Metalloproteins are involved in all biological energy-capture, conversion and transfer. Small-angle scattering can be used to determine how protein components assemble into functional units and what changes in association are relevant to function. The recent construction of dedicated small-angle scattering beamlines at SSRL and APS has made this technique accessible to a much wider array of problems. Recent advances in X-ray

microscopy and X-ray microprobe imaging offer the promise of dramatically improved images of cells and tissue.

5.   The general demand for structural information in all molecular fields of biology continues to grow very rapidly, and is paralleled by a growth in the demand for synchrotron time.

Three factors contribute to the substantially increased demand.

a.   Technological improvements in synchrotron facilities, X-ray detectors and crystal handling have brought many more biological problems into the range of structural biology and have significantly improved the success rate and quality of synchrotron experiments. The ability to freeze macromolecular crystals at cryogenic temperatures (*ca.* $-170°$ C) has effectively immortalized many crystals. No longer is radiation damage of the specimen a major concern that limits the ability to measure high-quality data. More accurate, higher resolution structures have resulted from complete data sets being obtained from a single frozen crystal. The freezing process has also allowed use of smaller crystals, which require more irradiation in order to measure their weaker diffraction. Crystals with dimensions of less than 0.1 mm now routinely furnish high quality data. The combination of smaller crystals and harder X-rays has minimized absorption errors; and the low background characteristic of good synchrotron beamlines makes it easier to record excellent data at very high resolution.   Hence, structures that were previously inaccessible have become almost routine. These advances coupled with MAD phasing have made high resolution structures attainable more rapidly from a greater proportion of crystals for a wider range of biological problems.

b.   As the complexity of the biological project increases, there is greater demand for synchrotron time to tackle more difficult problems. These projects include crystals with very large unit cells, poorly scattering crystals of macromolecular assemblies or membrane proteins, micro-crystals, and problems in dynamics where the goal is to capture snapshots of biological events along an enzyme reaction coordinate or other kinetic pathway. For such complex problems, the ability to integrate information from several types of experiments - crystallography, spectroscopy, solution scattering or imaging - is often critical.

c.   As anticipated, a significant new demand has indeed come from a "latent" community of users who are not specialists in crystallography but who have biologically significant structure determinations to carry out. These types of problems include multiple mutant structures, drug or ligand complexes of solved structures, molecular replacement structures and entirely new structures. Latent demand is difficult to quantitate, but it is already clear that many non-specialist laboratories are embarking on structure determinations. Their willingness to undertake structural work is a testament to the impact of structural results in molecular fields of biology and to the success in streamlining structural experiments. Non-specialist users are a greater challenge for synchrotron facilities because they usually need a higher level of assistance from scientific staff. In addition, the synchrotron facility may be their only available X-ray source. Non-specialist demand is also expected to rise rapidly due to structure determinations arising from the genome projects. Thus, additional, highly trained support staff are required to meet the needs of larger numbers of non-specialist users who will come to the synchrotron facilities. The designation of the specialist and non-specialist researcher is gradually blurring as structural science becomes more accessible and the biological problems to which it is applied become more challenging. Postdoctoral associates and graduate students in specialist laboratories are becoming more sophisticated biologists but are often less expert in biophysics than when structural studies were a more arduous and labor-intensive undertaking. This means that in the future even synchrotron

users from specialist laboratories will require a higher level of support at the synchrotron facility than is required now.

6.   Regional facilities will grow in importance.

Without question, there is a strong demand for regional facilities that can provide service to the regional scientific community. There was overwhelming enthusiasm and uniform support among structural biologists for keeping all of the current synchrotron facilities in the U.S. open for biological use in order to service regional needs. It was deemed of extreme importance that research groups be within driving or short flying distance of synchrotron facilities to exploit their resources fully. The ability to drive to a local facility with samples in hand was rated as extremely important by a majority of users. Graduate students and postdoctoral fellows are the majority of scientific workers who actually go to the synchrotron, and it is essential that proximity to synchrotron facilities allows them to travel in large numbers for training. As actual costs of synchrotron trips are almost never covered completely by research grants, the decreased travel costs associated with regional facilities are especially important.

7.   The most cost effective way to improve throughput at synchrotron facilities is to upgrade existing beamlines.

In the 1991 BioSync Report, it was strongly advised that multiple new beam-lines be built. Much of this increase has been realized, especially with the new APS facility at Argonne and new ALS facility at Berkeley. Additional beamlines for biological use have become available at NSLS (Brookhaven), at SSRL (Stanford) and at CHESS (Cornell). These capital investments are expensive but essential. Unfortunately, after the initial mega-investment, only limited funds are typically available to keep the beamlines current with technological advances and adequately staffed to support user research. Consequently, the X-ray detectors and computer technology on most beamlines lag behind the state-of-the-art. It is strongly encouraged that beamline instrumentation at national synchrotron facilities be upgraded approximately every three years. These upgrades could substantially improve the throughput, and hence effective beam time, with a relatively small investment of funds and without the need for construction of expensive new beamlines. Since the best hardware and software are ineffective if well-trained staff are not available to assist the users, these upgrades must be accompanied by appropriate levels of staffing for user support.

Also, the structural biology community has become more organized since 1991 in response to its substantially increased reliance on synchrotron radiation. A substantial investment has been made in new beamlines by consortia of users from both the academic and industrial communities, who have purchased exclusive rights to as much as 75% of available time on individual beamlines. This trend is likely to continue as groups wish to have greater control of synchrotron beam time allocation for more convenient and rapid scheduling. Many users noted that the wait for beam time is not well matched to either the pace of science or the lifetime of samples that cannot wait for months before being used. The ponderous peer review system currently in place for beam time allocation at most synchrotron facilities is usually not appropriate for research projects that have already been peer reviewed for their primary funding. Alternative mechanisms for quicker and easier access to beamlines should be promptly developed.

8.   Increased cooperation between organizations funding synchrotron facilities and basic research is highly recommended.

At present, the DOE, NIH/NCRR and NSF provide major funding for the national synchrotron facilities, whereas the majority of academic users are funded by NIH Institutes other than NCRR, or by NSF. The light source operations are provided mainly by DOE (ALS, APS, NSLS, SSRL) and NSF (CHESS). NIH/NCRR and DOE/OBER provide support for operation of beamlines for structural biology. Collaboration of these organizations would increase operational efficiency and planning for synchrotron source upgrades and the infrastructure required to operate the synchrotron facilities at the state-of-the-art. The periodic upgrade of beamline instrumentation and user support could be more effectively coordinated through such a collaboration. Government and other organizations funding research grants to the structural biology user community could provide up-front support in addition to the modest travel allocations in individual research budgets.

In summary, the recommendations are that all current beamlines must be effectively maintained at National centers in Brookhaven, Cornell, Argonne, Stanford and Berkeley. Allocation of funds to upgrade existing beamlines is highly desirable in order to accommodate increased demand. Collaborative funding of these resources by NIH, DOE and NSF is strongly encouraged. While it is expected that demand will always outstrip available resources, it is the strong opinion of the BioSync Committee that the increased demand is not simply a matter of an increased number of users or of more projects of the same type. Extraordinary advances have been made recently in structural biology that impact all aspects of the medical and biological sciences. Breakthroughs in complex macromolecular structures have included the proteasome, GroEl, the nucleosome, the TCR-MHC complex and muscle proteins. The fundamental difference is a move from small soluble molecules of up to 100,000 daltons to multimeric complexes of greater than 1,000,000 daltons. The consequence of this shift towards increasingly complex structures and complex biological and biophysical problems is that it is now possible to address fundamental aspects of how a cell functions and how genes and gene products control cell development and function. Understanding of the mechanisms of these fundamental life processes can be harnessed in the future for gene therapy, in the design of new drugs for treatment of a large number of human diseases and inherited conditions, and in applications to improve the environment. Structural biology has moved from the simple study of structure and function of single proteins to a molecular understanding of cellular processes that control life and death.

Macromolecular Crystallography

Current Status

The field of X-ray crystallography as applied to biological macromolecules is even more vibrant than in 1991 and continues to have a major impact on the advancement of biology at the molecular level. Knowledge of three-dimensional structures of key macromolecules fundamentally changes biological research in associated areas. However, there has been a dramatic change in the interaction of structural biology with other fields of molecular biology in the last five years. Structural results are now more eagerly sought and better understood by biologists whose primary research does not involve crystallography or NMR. The great engine of molecular biology continues to roar, producing ever more interesting and complex materials for structural study. In concert with this, crystallography has advanced to the point where structural work can now keep pace with other molecular studies and is no longer the rate-limiting step in understanding the molecular mechanism of a biological function. Thus, the predictions of growth and demand made in the 1991 BioSync report, which were controversial at the time, have been resoundingly borne out. These and other qualitative changes in the field are discussed below. The conclusions presented below derive from comparison of the user surveys of 1991 and 1997 and on the deliberations of the BioSync Committee.

More crystallographers are producing more structures. The number of research laboratories continues to expand, as evidenced by the large fraction of investigators who have been running independent laboratories for five or fewer years (about 30% of the total, Table A-4). Assuming equal penetration of the structural biology community by the 1991 and 1997 BioSync surveys, the number of independent investigators is now 25-30% larger than in 1991. Another factor that has had an increasing impact on the field of biological crystallography is the support of the Howard Hughes Medical Institute (HHMI), which has grown from an initial group of about six investigators in structural biology to about 20 today. The growth in the number of crystallographic investigators can also be obtained from the enrollment of the American Crystallographic Association (ACA). The ACA, long a bastion of small molecule crystallographers, is now strongly influenced by biological crystallographers. The membership of the Macromolecular Crystallography Special Interest Group now includes more than half the total membership of the ACA. This proportion continues to rise.

At the same time, the number of new crystal structures has grown at a substantially faster rate than in 1991, demonstrating the greatly increased productivity of the community. Four times as many new crystal structures of macromolecules were published in 1996 as in 1990 (Table 1), far outpacing the growth in number of investigators. In another measure of crystallographic output, the number of entries deposited in the Brookhaven Protein Data Bank increased similarly in the same time period (512 new entries deposited in 1991 *vs*. 1437 in 1996, Fig. 1). Of these, the considerable majority represents the result of protein crystallography and provides a benchmark for the activity in this field. Although many of these structures were determined in the users' laboratories, an increasing number have made use of synchrotron radiation and, if access to synchrotrons had been more available or quicker, these numbers could have been doubled.
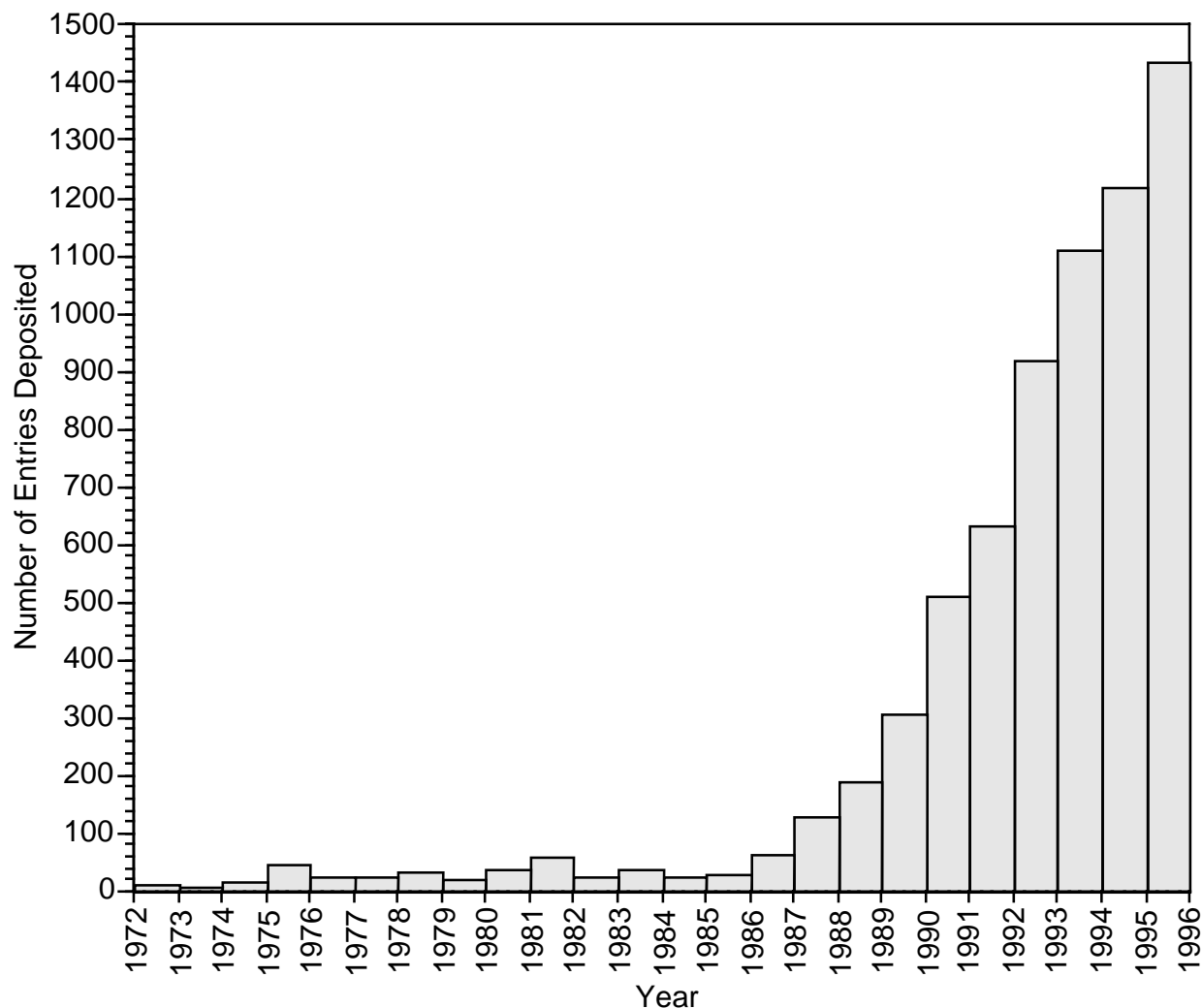
Figure 1. Depositions in the Protein Data Bank. All entries deposited in the Brookhaven Protein Data Bank are included. The vast majority of these are coordinate sets from crystallographic experiments. Source: Protein Data Bank web site (http://www.pdb.bnl.gov/statistics.html).

Synchrotron radiation has penetrated more deeply into the body of crystallographic results today than in 1991. More than 40% of the new crystal structures published in 1995 were determined using diffraction data from synchrotron sources. In 1990, this number was 18% (Table 1). This represents a major shift in the behavior of crystallographers and a major increase in the impact of synchrotron radiation in macromolecular crystallography. The reported use of synchrotron radiation per research group has doubled since 1991 (averaged over all respondents, five days per group in 1990 compared with eleven days per group in 1996, Table A-6). The attitudes of crystallographers towards synchrotron radiation have also changed. In 1991, one-third of respondents to the BioSync survey did not use synchrotron radiation because they did not need it, although virtually all respondents thought synchrotron radiation would be important for their future research. In 1997, the future has arrived. Only 7% of crystallographer respondents do not currently use synchrotron radiation because they don't feel they need it (Table A-7), but all think they will depend on synchrotron radiation in the future (Table A-8).

Table 1

Macromolecular Crystal Structures[1] 1990-1996

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

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

New technologies are responsible for the growing prominence of synchrotron radiation in macromolecular crystallography. In 1991, crystallography with synchrotron radiation was still in an "heroic" mode because extraordinary results only came from experiments that were expensive, labor intensive and more prone to failure than experiments in the home laboratory. In 1997, the "heroic" mode is largely a thing of the past, thanks to many technological advances that have dramatically improved the effective use of synchrotron radiation.

• The widespread adoption of cryogenic techniques and the near elimination of radiation damage as a major factor in synchrotron experiments is the greatest contributor to improved success of crystallography with synchrotron radiation. Data quality is substantially improved by eliminating errors due to crystal-to-crystal non-uniformity when complete datasets are measured from one crystal. The labor-intensive process of changing crystals in an experiment with intense synchrotron radiation has been largely eliminated.

• X-ray detectors have evolved from noisy photographic film with its labor-intensive development steps, to manually scanned image plates (IPs), to the automatically, but slowly, scanned IPs on most beamlines today, to the rapid, automatic charge-coupled devices (CCDs) now being introduced.

• The technology of multiwavelength anomalous diffraction (MAD) has become a routine tool, due to frozen crystals and availability of specialized beamlines.

• Construction of third-generation synchrotron sources, such as the ESRF in Europe and APS in the U.S., has provided highly brilliant, low background, stable beams that can be utilized to collect data from very small crystals, or from crystals with very large unit cells.

• Improvements to computer hardware and software allow on-line processing of raw diffraction images, permit better experiment monitoring and greatly reduce the time between synchrotron experiment and structural result.

• Improvements in protein purification and crystal preparation combined with the improvement in resolution customarily observed at synchrotron sources have resulted in an overall improvement in the quality of crystal structures in general and in the advent of very high resolution data sets for a large number of molecules.

Growing reliance of researchers on synchrotron radiation has resulted in new activism to gain access to suitable facilities. The most significant change in behavior has been self-organization of leading research groups to participate actively in the operation and construction of beamlines in order to guarantee access to synchrotron radiation. This trend was pioneered by the HHMI in providing beamline support for MAD measurements at the NSLS, which has also benefited many crystallographers outside the HHMI umbrella. More recently, five different groups of researchers have organized themselves to take over management and/or fiscal responsibility for beamlines in order to insure that they will have experimental time for their projects. These researchers are neither synchrotron experts, instrument specialists nor methodological aficionados, but are primarily structural biologists with competitive projects requiring synchrotron radiation. Such a change in behavior could not have happened during the "heroic" mode of synchrotron experimentation that existed in 1991.

Crystallography has joined the mainstream of molecular biological research tools. In the 1991 BioSync report, a strong "latent" demand for crystallographic synchrotron beam time was predicted for biologists whose primary research does not involve crystallography. The magnitude of the demand would depend on the support such researchers received at synchrotron sources. In 1997, it is clear that this prediction has been fulfilled. Many molecular biologists are adding crystallography as an experimental tool in their laboratories because of their growing dependence on structural information and the growing ease of obtaining it. The crystallographic expertise usually comes from postdoctoral associates. Following this trend, the Institute of General Medical Sciences at NIH recently began to supplement research grants of biologists with salary support for postdoctoral associates who are trained in crystallography. U.S. synchrotron facilities routinely receive applications for beam time from leading biologists who are not collaborating with established crystallographers. However, adequate support for these non-expert users remains beyond the means of nearly all synchrotron facilities. Thus, in 1997, use of synchrotron radiation for crystallographic experiments by non-specialists is limited to biologists who are able to recruit and pay for crystallographic expertise within their research programs.

Equally dramatic is the change within crystallography laboratories since 1991. The clear separation between expert and non-expert users of crystallographic synchrotron stations is markedly

blurred compared with the situation in 1991. Freed from much of the arduous work formerly required in crystal structure determinations, crystallographers are expanding their experimental repertoire beyond crystallography and today are more accurately described as structural biologists. While macromolecular crystallography is becoming easier and more streamlined, it is as a result being applied to more challenging biological problems. Thus, development and production of biologically interesting molecules for crystallization requires an ever greater effort, an effort that increasingly comes from within the structural biologist's own laboratory. Cross-training at the postdoctoral level occurs in both directions – crystallographers in molecular biology laboratories and molecular biologists in crystallography laboratories. This is a remarkable and refreshing exception to the tendency for greater specialization in scientific research.

Demand for synchrotron beam time continues to outpace supply. The U.S. national capacity for crystallographic experiments at synchrotron sources has approximately doubled since 1991 (Appendix B). However, demand continues to outpace supply by a factor of approximately two. Structural biologists report that the largest impediment to their use of synchrotron radiation today is timely access to the facilities (Table A-7).

Technological challenges remain. X-ray detectors continue to be limiting. Fast, efficient X-ray detectors with high spatial resolution and a bright X-ray beam are equally considered to be the two most important features of synchrotron radiation facilities by survey respondents (Table A-9). In the second tier of importance are adequate computer and networking services and well staffed, user-friendly experimental facilities. This reflects that fact that beamline efficiency could be improved substantially. Today's computer networks are insufficient to handle the data rate and data volume generated in typical, present-day crystallographic experiments.

Emerging Trends

More biologists will tackle more structural problems of greater complexity using the full range of technologies.

There will continue to be an increasing interest in macromolecule structures, at least in part because of the advances in ease and precision of structure determination brought on by the use of synchrotron radiation. At the 1997 Protein Society Meeting in Boston, approximately 50% of the symposium talks were based on the results of structural investigations. Even when the speaker was not a crystallographer, frequent references were made to protein structures determined through collaborations with a crystallographic group. The number of such collaborations will continue to grow. However, a more important trend is the growing number of biologists who determine structures by X-ray diffraction within their own laboratories, and the growing number of crystallographers whose research programs are more biological. This integration of crystallographic research into the framework of a modern molecular biology laboratory is a phenomenon of the last five years and is clearly growing rapidly, although the numbers are difficult to project with any accuracy. In some cases, biologists are able to purchase and maintain their own diffraction instruments, but the majority will depend on the use of outside facilities, especially synchrotron sources, for data collection. The need for synchrotron time for non-specialist and less-specialist users will continue to rise dramatically. An excellent example of this trend is the recent publication of the crystal structure of the fibrinogen core by R. F. Doolittle and coworkers (Spraggon, Everse & Doolittle (1997) *Nature 389*, 455-462).

Another trend in macromolecular crystallography is the shift from structure determinations of single protein molecules to large protein-protein and protein-nucleic acid complexes. Examples include the structures of proteasomes, chaperonins, muscle proteins, nucleosomes and integral membrane protein complexes. In addition, it is worth noting that approximately half the proteins now being sequenced in the genome projects are membrane proteins involved in cell-cell communication. Only about a dozen of these have been investigated by crystallographic methods, and it is clear that the next decade will see a surge in the structure determination of these important molecules as methods are sought for crystallizing them routinely. Membrane proteins and macromolecule assemblies will generally diffract more weakly due to their size and complexity. Thus, effective data collection will require synchrotron radiation.

The genome projects will also generate considerable new crystal structure determination. BioSync is aware of at least four projects to determine structures for all hypothetical soluble proteins encoded by open reading frames in new genome sequences for which connections to the extant structure database cannot be made. All of these projects involve production of selenomethionyl proteins for MAD structure determination with synchrotron radiation. The impact of this new area of research is unknown, just as the impact of the genome projects was unknown a few years ago, but it is likely to be substantial.

A better chemical understanding of biological processes is an emerging trend of the increasing spatial and temporal resolution of structural information. Synchrotron radiation is one of the major technological advances contributing to this trend. Ultra-high resolution crystal structures from synchrotron data will continue to have a major impact in this respect. We are only just beginning to reap the advances of these high resolution studies around 1 Å resolution.

Technological changes in several areas of macromolecular crystallography will have a major influence on the research done with synchrotron radiation.

MAD use of polychromatic radiation. Multiwavelength anomalous diffraction (MAD) offers a generally applicable and rapid method for *de novo* structure determination of biological macromolecules. This method, in conjunction with cryocrystallography, allows one to collect data and solve the structure using a single crystal, and, in favorable cases, complete the entire process in a matter of days. The high brilliance and tunability of synchrotron radiation is essential for MAD; no laboratory X-ray source can be used as a substitute. The general selenomethionine label for proteins, developed for MAD by Prof. W. A. Hendrickson, is most widely used. Statistical direct methods, developed for small-molecule crystallography, have been used very recently to locate large numbers of Se sites in proteins, demonstrating that selenomethionyl MAD is applicable to much larger protein structures than anticipated. It is certain that MAD will become a major, perhaps even the dominant, method of macromolecular structure determination in the future. Selenomethionyl MAD is a critical part of the massive projects now being planned in structural genomics. Beam time for MAD will be the most limiting synchrotron resource in the coming years.

Data collection from microcrystals. The high brilliance of the new synchrotron sources permits data collection from very small crystals. There is anecdotal evidence of 10-μm crystals having been used for data collection. This development holds great promise for study of integral membrane proteins and macromolecular complexes, systems of enormous biological significance but very challenging crystallization problems. Microcrystals are often obtained relatively quickly, whereas it may take years of effort to grow crystals to a size suitable for laboratory data collection. Also crystals frequently persist in growing as plates or thin needles with only one large dimension. Microcrystallography requires an order of magnitude greater precision in crystal and X-ray beam alignment than is implemented on most of today's crystallographic beamlines. The technology for high-precision alignment exists on microfocus beamlines used for nonbiological research. Its implementation on crystallography beamlines could have great impact.

Time-resolved crystallography. One of the most exciting recent developments in the field of experimental protein science has been the application of synchrotron radiation to obtain nanosecond-long snapshots of a protein as it changes in response to ligand dissociation. The examination of the photodissociation of carbonmonoxy myoglobin and the photochemistry of photoactive yellow protein represent a major advance in time-resolved protein crystallography. All proteins undergo some structural change when carrying out their biological function. However, the measurement and understanding of the kinetics of conformational changes in proteins has hitherto been a largely unexplored area. The use of single-pulse Laue radiation coupled to laser photoactivation permits direct observations of structural changes in the nanosecond time range. Based on the interest generated by this work, it is clear that more studies will be directed toward understanding the kinetic events associated with phenomena where photostimulated triggering is possible. With currently available sources the maximum time resolution will be about 150 psec, and this will enable direct comparison between the time dependence of atomic positions determined by X-ray crystallography and molecular dynamics calculations, and their relation to spectroscopic observations.

High resolution crystallography of macromolecules. Crystallographers are increasingly finding crystals that diffract to much higher resolution than was previously thought to be possible. The reasons for this improvement are several. Better purification procedures yield larger, more strongly diffracting crystals. Cryogenic data collection results in lower background noise in diffraction images. Great improvements in signal-to-noise are obtained with synchrotron radiation due to a cleaner, more parallel and monochromatic X-ray beam. The result is an improvement in effective resolution, in the range of 0.2 Å to 0.4 Å for typical cases, and sometimes substantially more. Crystal structures have been determined to 1.0 Å or better for at least a dozen proteins. Crambin, a small plant protein, probably still leads the pack with diffraction to about 0.67 Å, but there is now an increasing number of larger and more complex proteins for which it is possible to determine structures to what can truly be called atomic resolution. At these resolutions, the structures can be determined by the actual data without need for stereochemical assumptions. Thus, more and more structures are accessible to phasing by statistical direct methods, which are being extended to protein crystals with diffraction data beyond 1.2 Å. The immediate rewards for this improved resolution are clear: more precise atomic coordinates for structure analysis and modeling; better descriptions of active sites, including hydrogen locations for more informed models of enzyme mechanisms; better understanding of protein flexibility through individual anisotropic temperature factors. The impact is likely to be a much improved understanding of the chemistry of biological processes. In addition there will be other observations such as the distribution of bonding electrons whose utility may have to wait for future fundamental research.

Very large unit cells. A recent spectacular example of advances in large unit cells is for orbivirus with a molecular diameter of about 700 Å, and unit cell dimensions of considerably greater than 1000 Å. The highly collimated beams from third-generation synchrotron sources permit the diffraction patterns of crystals with very large unit cells to be recorded.

The growing speed of macromolecular crystallography is well matched to the rapid pace of biological science. The burst in productivity of crystallographic research groups between 1991 and today will continue. The mid-1990s burst was due in major part to the adoption of cryocooling technology, both at the synchrotron and in the home laboratory. Cryocrystallography is probably the largest single factor in increasing the use of synchrotron radiation. The adoption of two new general labels for structure determination - selenomethionine and high-pressure xenon gas - will be major contributors to increasing the speed of crystallography. The widespread use of MAD for direct structure determination will drive an even greater demand for synchrotron facilities and speed structure determination overall. The experimental electron density maps of very high quality that frequently are the result of MAD phasing will also speed structure determination. The emphasis on speed will also drive development of new models for access to synchrotron radiation, including remote data collection and more complete on-site data analysis.

Recommendations

The importance of synchrotron radiation to biotechnology, molecular medicine and all molecular fields of basic biology has come of age. The dependence of macromolecular crystallography on synchrotron radiation has impacted all fields of biology, and leads to the following specific recommendations.

- The efficiency of current beamlines should be improved. It is the strong opinion of the BioSync Committee that improving current beamlines is highly cost effective as opposed to construction of new ones at present. Improved detectors could increase throughput by at least a factor of two, and improved ergonomics could also result in a doubling of the utility of current beamlines. Another factor of two might come from improved training of users. The most important need is for increased funding support for scientific and technical staffing at the synchrotron facilities.

- The importance and growth in number of part-time or non-specialist crystallographers should be recognized. Additional support, especially in highly trained staff, is needed for this rapidly growing user group.

- More elective scheduling to ensure more rapid access to beamlines for crystallographic experiments must be developed. Beam time requests should be handled to match project needs with beamline capabilities.

- Regional synchrotron facilities are an extremely important complement to specialized facilities. These sites have lower travel costs, which allow access by junior scientists for training. We believe these facilities will grow in importance, not diminish as high-brilliance sources come on-line.

- Funding of synchrotron operation and beamlines should be coordinated across government funding agencies. The current separation of funding of beamlines, facilities, and user support is inefficient and can be improved.

- A 25-30% access of 'general users' to 'privately' managed beamlines should continue to be required, and the full range of experimental capabilities of each beamline should be available to the general user.

- Research and development on use of beamlines by remote access should be supported in order to determine whether data collection can be managed remotely. This would save travel time and expense for routine experiments and require fewer users to travel to the synchrotron facility.

- Users should exercise good judgment in the selection of experiments to be done at synchrotrons and should match needs to capabilities. Experimenters should arrive at synchrotrons prepared to make the most efficient use of the time allotted, having prepared adequately at home to ensure likely success of their synchrotron experiments. Such preparation would include finding conditions for freezing crystals, bringing frozen, pre-screened crystals and knowledge of the crystal cell parameters.

- A 'Code of Ethics' for beamline operation and use should be developed jointly by beamline managers, users, synchrotron facilities and government funding agencies. This should include concepts of integrity, training of beamline personnel on the issues of privacy and competition amongst users, and responsibilities of user consortia. Also, as the synchrotron staff becomes more heavily involved in individual research projects, these guidelines should be developed to protect both the staff and the user groups in conflict-of-interest situations that are likely to occur more frequently in the future.

Non-Crystallographic Methods in Structural Biology

Synchrotron radiation is used for a number of non-crystallographic applications. X-ray absorption spectroscopy gives detailed characterization of metal centers in biological macromolecules. Small-angle X-ray scattering is used to probe the dynamic and static size and shape of macromolecules in solution. X-ray microscopy is showing promise as an imaging tool. These biophysical methods may be applied to macromolecules in solution and do not require growth of single crystals to produce structural data. They account for a substantially smaller proportion of synchrotron beam time at present than does crystallography (Table A2). However, they are frequently applied to samples not amenable to crystallization and to properties inaccessible to the crystalline state. The complementary information they provide can be key to full understanding of biomolecular functions. This section describes the current status and future prospects of X-ray absorption spectroscopy, small-angle X-ray scattering, and X-ray microscopy.

X-ray Absorption Spectroscopy

Current Status. X-ray absorption spectroscopy (XAS) is the study of spectral details on the high-energy side of an X-ray resonant frequency or absorption edge of a metal atom. X-ray absorption spectra are frequently divided into the regions of X-ray absorption near edge structure (XANES) within ~50 eV of the edge, and extended X-ray absorption fine structure (EXAFS) from ~50 eV to ~1000 eV above the edge. The XANES region contains information about the oxidation state and local geometry of the absorbing metal atom. The EXAFS region provides structural information about the absorbing atom, and can be analyzed to give bond lengths to ± 0.02 Å, coordination numbers to ± 1, and the chemical identity of ligands to within one row of the periodic table, *e.g.* distinguishing N from Cl but not from O.

One of the principal attractions of XAS for structural biology is to provide element-specific structural information about macromolecules in solution. Many samples of interest, such as reaction intermediates, are not amenable to study as single crystals or are difficult to crystallize. A second important application of XAS is as a powerful complement to crystallography in the study of metalloproteins. Metal-ligand distances available from XAS are typically an order of magnitude more accurate than those from macromolecular crystallography. Thus XAS data can be essential for defining the details of an active site and can aid in the refinement of a crystallographic model. For example, connectivity within the unusual Mo/Fe/S cluster in nitrogenase was obtained with crystallography, from which starting point EXAFS provided the complementary high-resolution structural information required to understand the mechanism. In addition, EXAFS data for a variety of metalloproteins, *e.g.* rubredoxin, *A. vinelandii* ferredoxin and lipoxygenase, have helped to correct initial errors in metal site structures obtained from crystallography. XANES measurements can often provide a direct and unambiguous determination of metal-ion oxidation state, *e.g.* the presence of Cu(II) and not Cu(III) in galactose oxidase. This information is difficult to extract with other spectroscopic methods and impossible to obtain from crystallography.

Presently two beamlines at SSRL (7-3 and 9-3) and one at NSLS (X-9) are semi-dedicated to biological XAS. One biological XAS line (BioCAT, split with SAXS) is under construction at APS. In addition, perhaps 6 other US beamlines are used with some frequency for biological XAS studies. Finally, several special-purpose beamlines (circularly polarized X-rays, low-energy X-rays, *etc.*) are currently being designed or constructed and will be used in part for biological studies.

Approximately 20-30 US research groups make frequent use of XAS for structural biology and perhaps 20-30 other groups use XAS occasionally for studies of biological samples. A large fraction of XAS studies are collaborative ventures, involving a group with X-ray expertise interacting with a group

having biochemical expertise. Biological XAS is thus used, at least occasionally, by 100-200 research groups in bioinorganic and biophysical chemistry.

Emerging Trends. As the number of crystallized proteins has increased, so too has the number of uncrystallized proteins that have been isolated and purified. XAS will remain an important tool for characterizing the metal sites in these proteins. Realistic projections of the capabilities of the next generation of beamlines (APS, SPEAR3) and of future detectors (see below) should allow XAS to be extended both to lower concentrations and to smaller sample volumes (see section on X-ray microscopy). These will allow XAS to be applied to samples that are either too dilute or too small for present technology, and thus will keep the demand for XAS beam time at least as high as present levels of two- to three-fold oversubscription.

One particularly important growth area may be the use of polarized XAS to provide angle-resolved bond-length information to complement diffraction studies. For example, with the greatly enhanced crystal lifetimes that come with cryogenic experiments, a single crystal can be used for both crystallography and XAS. The three-dimensional structure of a metalloprotein can be determined by crystallography, the metal-ligand bond lengths to much greater precision by EXAFS, and the metal-ion oxidation state by XANES, all from one sample crystal. Improved X-ray fluorescence detectors on MAD beamlines are the only instrumental requirement for these hybrid experiments, because XANES is part of every MAD experiment. Polarized XANES data from single crystals could provide orientation-dependent anomalous scattering factors f' and f'' for use in metal-based MAD phasing. The combination of XAS with crystallography would provide a better description of the molecular structure than is possible from either technique alone. Such applications would increase significantly the demand for XAS beam time.

XAS is limited by its relatively low information content. In the 1991 BioSync report several new developments were identified, which had the potential to enhance XAS by providing more information: circularly polarized XAS, soft X-ray XAS, energy-resolved X-ray fluorescence, and time resolved XAS measurements. Over the last 6 years, all of these have been demonstrated for biological samples. Circularly polarized XAS measurements distinguish different spin-states of a sample. Soft X-ray measurements provide direct access to electronic-structure information that can only be inferred from hard X-ray XAS measurements. High-resolution energy-resolved X-ray fluorescence offers the possibility of site-selective XAS, thus obviating the limitation that XAS is a bulk measurement giving only average structural information. Temporal resolution makes possible the use of XAS to determine the oxidation state and perhaps the molecular structure of transient reaction intermediates. Stopped-flow time-resolved XAS in combination with principal component analysis has been used in studies of supported catalysts to identify the reactive intermediate in a complex mixture. This approach should be readily applicable to metalloprotein studies.

Recommendations. The 1991 BioSync report noted that the most pressing limitation to biological XAS was the lack of suitable X-ray fluorescence detectors. Although the available detector technology has improved since then, this conclusion remains true. It is routine on many beamlines for users to decrease the available flux in order to avoid detector saturation. This problem will be even greater at the third-generation synchrotron radiation sources. Piecemeal efforts to develop new detectors have been made at the different synchrotron sources and these are beginning to show results. Nevertheless, the XAS community would benefit tremendously from a concerted effort to develop optimized detectors which could fully utilize the available X-ray flux. Optimized detectors would result in an immediate increase in sample throughput by a factor of two or more. This would make a substantial impact on the current beam time shortfall, and would facilitate the projected expansion in XAS applications.

In addition to optimizing present detectors, it is important to explore other detector technologies. Promising recent developments include superconducting tunnel junctions, microcalorimeters, and

"transition edge" detectors. These offer the promise of both very high energy resolution (2-10 eV) and high count rates (20 kHz). Arrays of such detectors could revolutionize the practice of XAS. It is not clear at this point which of these detector technologies will be the most useful. It *is* clear that the XAS community needs to invest more effort in the development of the next generation of detectors.

The biological support facilities available for XAS have improved significantly since 1991, to the point that these are typically not a limiting feature in experimental progress. The availability of beam time remains a limiting factor for many studies. Based on the current over-subscription rates, a two-fold increase in the available beamlines would be completely utilized, even without the increase in demand that is anticipated. Although no increase in the number of beamlines is likely in the short term, it should be possible to increase the available XAS time through improved detectors, which will effectively increase throughput, and perhaps eventually through development of new beamlines. Any decrease in the operational time available at either SSRL or NSLS would have a serious and immediate negative impact on the field.

Many of the new XAS techniques (circular dichroism, soft X-ray, time-resolved) require unique, dedicated beamlines. These are presently under construction at ALS and APS. For the next few years, it appears that the availability of experimental time for these experiments should be sufficient to permit exploration of the range of applications of these methods in structural biology. If one of these methods proves to be widely applicable, additional beamline construction may be indicated 3-5 years from now.

In summary, the most pressing need for XAS is improved detectors. These would help alleviate the present shortfall in beam time. This is, however, a double edged sword, since improved detectors will make possible the application of XAS to smaller and more dilute samples, thus increasing the demand for beam time. In the 3-5 year time frame, some increase in XAS beamlines is likely to be necessary.

Small Angle X-ray Scattering

Current Status. X-ray scattering can be used to characterize solutes in a variety of solutions and can be performed over a range of scattering vectors. The most useful region for structural biology is small angle X-ray scattering (SAXS). SAXS is unique in providing information on macromolecular shape, *e.g.* radius of gyration, R_g, with high temporal resolution, although atomic-resolution structures cannot be retrieved. The dynamics of many important biological processes, such as protein folding, are studied by SAXS. For example, several distinct folding intermediates during renaturation of apo-myoglobin and lysozyme have been detected recently. A detailed description of protein folding is important for understanding prion virulence, *e.g.* bovine spongiform encephalopathy or "mad cow disease". Time-resolved SAXS is one of the few techniques that can provide direct information about the rate and mechanism of protein compaction during folding. Small-angle scattering is a powerful method to study molecular interactions and conformational flexibility in solution, which are key to understanding molecular communication in biological systems. Solution scattering in conjunction with high resolution structural data can provide invaluable insights into the interaction of individual components in molecular assemblies and complexes. This can be done in the absence of crystals and for the widest range of molecular weights and dimensions (~10 - 1000 Å), thus providing an important adjunct to structural data from NMR and crystallography. SAXS has been particularly useful in contributing to our understanding of biochemical regulation by providing insights into domain reorientation and protein-protein interactions important in signaling. Advances in molecular biology techniques combined with brilliant synchrotron X-ray sources have permitted studies of the interactions of regulatory proteins with active enzymes whose solubilities and stabilities are limited.

Current facilities in the US include beamline 4-2 at SSRL (semi-dedicated to SAXS), and NSLS beamlines X9B and X12B. Beam time available for SAXS at NSLS is decreasing due to increased crystallographic demand. The BioCAT sector under development at the APS will include a significant SAXS resource. These beamlines are used for structural biology by approximately 20 US groups.

Emerging Trends. Historically, SAXS has been used by a small number of laboratories because fabrication of clean small-angle cameras is a delicate art that few have mastered; and SAXS measurements on dilute solutions require lengthy exposures, and consequently have low throughput. Synchrotron radiation has allowed SAXS to be carried out much more quickly and easily. There is a great opportunity for the development of well-equipped user facilities dedicated to SAXS. In parallel with this, we anticipate several important improvements in analytical and experimental techniques, listed below. These will improve both the ease of using SAXS and the scope of problems to which SAXS can be applied. The anticipated developments suggest that the potential for growth in the use of SAXS for biological samples is large.

Analytical improvements include:

- Use of SAXS instrumentation to measure low-angle diffraction data for 3-D reconstructions of viruses.
- Use of principal component analysis to extract SAXS profiles for a number of species in a mixture, *e.g.* during protein folding or unfolding.

Experimental improvements include:

- Improved time resolution by jet-flow mixing to measure the kinetics of R_g in the sub-msec domain rather than in the ~20-msec limit of current stopped-flow time instruments.
- Circumvention of radiation damage by use of a flow cell or by addition of thiourea as a protective agent.

Recommendations. Planning for synchrotron radiation resource allocation should allow for a significant growth in the use of SAXS. The technique is now poised for expansion into a much wider range of laboratories. Investment in user support, such as software development, scattering cell fabrication, hardware for kinetics, and so on, is crucial to this expansion since these improvements are necessary for the entry of new groups into the field. At the present time, improvements in user support are more important than the construction of new beamlines for SAXS.

X-ray Microscopy

It has long been recognized that the ability to visualize an object of interest is one of the cornerstones of advancement in science. For this reason, X-ray imaging holds special promise as a technique in structural biology. Much of the promise comes from the possibility to do spectroscopy with the imaging X-ray beam. The method is under development. The primary challenge for biological X-ray microscopy is to focus an intense enough X-ray beam on a small enough spot to image interesting biological objects, yet minimize the effects of radiation damage. A variety of technologies can be used to focus X-rays, including zone plates, Kumakov lenses, waveguides, refractive optics, capillary optics, grazing incidence mirrors, and normal incidence mirrors. At present, zone plates and grazing incidence mirrors are the technologies that are most highly developed in synchrotron X-ray microscopes.

The zone-plate approach provides superb spatial resolution — as low as 30 nm in some cases. However, zone-plates have smaller apertures than are possible with mirrors and thus typically give lower flux. The lower flux, together with the fact that extremely precise alignment is required to scan energy with a zone-plate, has meant that zone-plate beamlines are most often used for fixed energy imaging, or possibly for dual wavelength differential imaging. In contrast, mirror-based beamlines have been used for a range of spectroscopic studies. Although the currently available X-ray fluxes have limited these to XANES studies, EXAFS and possibly SAXS measurements should be possible in the future. Most spectroscopic work has been done at a spatial resolution of 10-30 μm, although this has recently been extended to ~1 μm. The diffraction limit for Pt-coated Kirkpatrick-Baez mirrors is 40 nm at 7 keV (wavelength λ = 0.2 nm). Thus it should be possible eventually to extend mirror-based beamlines to a resolution limit close to that of zone-plates. However, considerable improvements in mirror technology will be required to reach this limit. Zone-plate beamlines are sometimes referred to as X-ray microscopes while mirror-based beamlines are more often described as X-ray microprobes, with the names reflecting both the differences in spatial resolution and the tendency to use the former primarily for single wavelength imaging and the latter for energy-scanning spectroscopies. In terms of the experiments for which they can be used, however, there is considerable overlap between the different types of beamlines.

Current Status. Zone-plate beamlines are presently in use both as soft and hard X-ray sources. The soft X-ray sources, NSLS beamline X1A and ALS beamlines 7.0 and 6.1, typically operate in the 0.2-1.2 keV range (λ = 7-1 nm) and produce spot sizes as small as 30 nm. This energy region is important as it includes the so-called water window, where the absorption due to oxygen is low, and thus where biological samples can be studied. The hard X-ray zone-plate beamline at APS operates at 8 keV (λ = 0.2 nm) and produces a spot size of *ca*. 0.25 μm. Mirror-based beamlines are presently operated at NSLS (X26A), APS (GeoCARS), and ALS (10.3.1 and 10.3.2). These typically operate in the hard X-ray region (5-12 keV, λ = 0.2-0.1 nm) and produce spot sizes in the range of 1 μm (APS and ALS) or 30 μm (NSLS). An additional microprobe beamline is presently being constructed at the ALS.

Most of the current applications utilize inorganic samples, addressing geological, semiconductor, and/or materials science problems. Extension to biological samples is straightforward, although some investment in sample preparation and handling will be required, since biological samples are generally less robust than inorganic samples. The technology for this is well established. Using an X-ray microprobe beamline, it should be possible to map the oxidation state and ligation of metal ions in biological samples at 1 μm resolution. This will permit studies of metal speciation in some large cells, and can be used for histochemical studies of metal ion distribution in tissues.

Currently the most prominent X-ray microscope in the US is the scanning transmission X-ray microscope (STXM) at the NSLS (beamline X1A). The operating parameters of the Brookhaven STXM and its experimental capabilities have both improved substantially in the last two years. In particular:

- The spot size is presently below 50 nm and should reach 30 nm soon.
- Quick freezing of cells in liquid ethane has been shown to provide radiation resistant but unperturbed specimens.
- A rotation stage for CAT-scan type reconstructions is now operational.

The Brookhaven STXM is usually operated in a wavelength window where oxygen has low absorption, so that water is transparent. Variation of λ within this window can be used to give elemental contrast between C and N, or between different oxidation states of a single element. Contrast based on other elements (*e.g.* Ca^{2+}) is also possible. A new version of the microscope that is much easier to operate is also ready. Existing facilities should permit a three-dimensional reconstruction of a single cell at *ca.* 50 nm resolution, with elemental contrast, so that protein-rich and nucleic acid-rich regions will show up differentially.

Emerging Trends. The existing X-ray microprobe beamline at NSLS is heavily oversubscribed. This situation is expected to ease somewhat as the new APS facility comes on line. However, with the higher flux and smaller spot size of the new beamline, new classes of experiments will become possible. At a 1 µm spot size, it is realistic to perform *in situ* XAS measurements on intact biological samples. This, together with the ability to image each of the elements in a sample at µm resolution, is likely to attract a large group of new users. It is anticipated that this expansion in user base will rapidly lead to oversubscription at least as severe as that presently experienced at NSLS.

The recent dramatic improvements in the Brookhaven STXM have expanded significantly the range of samples that can be studied. This again suggests that demand from outside users will rise rapidly in the next few years. Many of these experiments, particularly those involving 3D data sets, will place heavy demands on beam time. At a flux of 10^6 photons/second, each pixel needs about 10 ms of exposure to give good statistics. For an object of typical size 5 µm several million pixels are required, which translates into several hours for a single 3D data set.

Several beamlines at the ALS provide facilities for X-ray microscopy that can be used for biological samples. Beamline 7.0.1 houses a scanning transmission and scanning photoelectron microscope and beamline 6.1 will provide a full-field transmission X-ray microscope with zone-plate lenses. The overall impact of the new ALS facilities will be to significantly increase the available time and available modalities for X-ray microscopy. If past developments in imaging are any guide, these new facilities will generate a significant increase in the users of and applications for X-ray microscopy in structural biology. Possible future developments include new techniques for dark-field and holographic imaging and new algorithms for "super-resolution", in which the diffraction pattern rather than the transmission is recorded for each pixel.

Recommendations. It appears likely that the future demand for access to both X-ray microprobe and X-ray microscopy beamlines will grow dramatically in the next 5 years. This is likely to lead to the need for additional beamlines dedicated to these experiments. In addition, the development of improved support facilities for microscopy will be important for facilitating the entry of new users into the field.

This Brookhaven STXM represents the current state of the art in X-ray microscopy. However other technologies, such as the imaging microscopy used at ALS beamline 6.1, may end up being the methods of choice for many problems. Given the recent developments in the field, substantial increase in demand for STXM access is anticipated. When the potential impact of several very exciting technical developments is considered, one should prepare for a hitherto unanticipated demand for beamline resources on the part of the X-ray microscopy community.

APPENDIX A: DATA FROM USER SURVEY

Primary research categories of respondents:

Macromolecular crystallography	XTAL (201 investigators or 76.7% of respondents)
Scattering from noncrystalline materials	SNM (26 investigators or 9.9% of respondents)
X-ray absorption spectroscopy	XAS (24 investigators or 9.1% of respondents)
X-ray microscopy	IMG (11 investigators or 4.2% of respondents)
Other	(6 investigators or 2.3% of respondents)
No primary category	(8 investigators or 3.1% of respondents)

Total number of responses was 262. Of these, 11 indicated more than one primary category and 8 indicated no primary category. Primary category was assigned as the most frequent use indicated of synchrotrons for experiments (see Table A-2). Those that indicated equal frequency of use in more than one category were included in the analysis for each of those categories. Of those indicating *Other*, the descriptions were: interferometry; infrared radiochemistry; x-ray standing wave; and detector development.

Table A-1. Work Environment of Structural Biologists

Current Employer	Number of Investigators
University	187 (71.4%)
Government Laboratory	34 (12.9%)
Other (private and nonprofit)	18 (6.9%)
Industry	23 (8.8%)
Total	262

Table A-2. Use of Synchrotrons for Certain Experiments

Experiment Symbol		Major Use	Occasional	Once Only	Never
Macromolecular crystallography:					
XTAL	Monochromatic	109	68	16	69
XTAL	MAD Phasing	29	36	25	172
XTAL	Laue	4	10	19	229
Scattering from noncrystalline materials:					
SNM	Static	21	14	8	219
SNM	Time-resolved	12	10	2	238
X-ray absorption spectroscopy:					
XAS	Static	21	10	6	225
XAS	Time-resolved	1	7	2	252
X-ray imaging/ microscopy IMG		9	5	0	248
Other		5	1	0	256

The results for Table A-2 were obtained by adding the number of people who responded to each type of experiment for each of four possible answers.

**Table A-3. Correlation of Size of Research Group with Number of Years
as an Independent Investigator**

First Year as Independent Investigator	Number of Investigators	Number of Staff	Average Size of Research Group	Total Number of Researchers	Average Anticipated 5-Year Change in Size
1992-97	75	375	6	450	+1.9
1987-91	50	343	7.9	393	+1.2
1982-86	33	223	7.8	256	+1.5
1977-81	27	187	7.9	214	+0.8
Before 1977	77	654	9.5	731	-0.5
Total	262	1782		2044	

The results for Table A-3 were obtained by adding the number of investigators who had worked as independent investigators for periods of 5 years at a time. The total number of researchers was obtained by adding the number of postdocs, graduate students, and support staff to the number of investigators for groups of 5 years at a time. This number was divided by the number of independent investigators to find the average size of the research group for each of the periods of time. To find the average anticipated size of research groups in 5 years, that number was added to the total number of investigators, averaged, and the difference between the existing number and the anticipated number was figured.

**Table A-4. Correlation of Scientific Discipline with Number of Years
as an Independent Investigator**

Number of Investigators

First Year as an Independent Investigator	XTAL	SNM	XAS	IMG	Other	None	Total
1992-97	63	2	3	3	2	3	76
1987-91	43	1	3	3	2	0	52
1982-86	23	5	5	1	1	0	35
1977-81	18	4	4	3	1	0	30
Before 1977	54	14	9	1	0	5	83
Total	201	26	24	11	6	8	276

The results for Table A-4 were obtained by counting the number of investigators per category by groups of 5 years. The total is larger than the actual number of respondents because some investigators responded with equal usage frequency to more than one category.

Table A-5. Level and Sources of Research Support

Annual Research Support	Number of Investigators
$1K-50K	37
$50K-150K	80
$150K-250K	45
>$250K	65
unspecified	7
No external support	28

Source of Research Support	Number of Investigators
NIH	180
Foundation	66
NSF	63
Industry	57
DOE	40
Other U.S. government	24
Canada	15
Investigator's Institution	14
Other International	6
State	2
Other Unspecified	4

Table A-6. Biological Use of Synchrotron Radiation Facilities 1994-1996

Total Days Each Year Reported by Users

Year	SSRL	CHESS	NSLS	ALS	APS	SRS	LURE	DESY	PhFac	ESRF	Total
1994	439	277	883	70	0	24	45	23	75	33	1869
1995	547	275	980	280	2	15	55	37	45	48	2284
1996	671	369	1194	345	110	9	48	53	29	88	2916

These results were obtained by adding the number of days each facility was used by year.

Table A-7. Factors Limiting Use of Synchrotron Radiation by Scientific Discipline

A. Too much time between application for beam time and experiment.
B. Can't readily get synchrotron beam time.
C. Insufficient research support to enable travel to the synchrotron.
D. Burdensome requirements for beam time proposals.
E. Key instrumentation for experiment unavailable at synchrotron facility.
F. Insufficient user support at synchrotron facility.
G. Don't need it for my research.
H. Other.
I. Too much radiation damage to samples.
J. Sample problems.

Number of Respondents

Factor	XTAL	SNM	XAS	IMG	None	Other	Total (% of respondents)
A	121	8	4	2	1	1	137 (52.3)
B	112	6	3	6	1	1	129 (49.2)
C	44	5	7	0	2	2	60 (22.9)
D	41	4	4	1	2	1	52 (19.8)
E	16	4	1	2	1	2	24 (9.2)
F	16	3	0	1	1	1	22 (8.4)
G	15	1	0	0	3	2	21 (8.0)
H	9	2	4	1	0	2	18 (6.9)
I	3	4	1	1	1	2	11 (4.2)
J	6	0	0	0	0	0	6 (2.2)

The results for Table A-7 were obtained by adding the number of respondents to each factor limiting use of the synchrotron facilities by each category of investigation. Description of other factors limiting use were: too busy building beamlines; other demands on researcher's time; enough time for data analysis; we have our own line; convenience of staying home; personnel available; analysis time is rate limiting; proprietary research projects; usual experimental limitations; and small group/large time blocks.

**Table A-8. Importance of Synchrotron Radiation to the
Investigator's Future Research Plans**

A. Critical
B. Very Important
C. Moderately Important
D. Unimportant

Factors	XTAL	SNM	XAS	IMG	Other	None	Total (% of respondents)
A	127	14	16	10	3	3	173 (63)
B	56	6	5	1	2	0	70 (25)
C	18	6	2	0	1	1	28 (10)
D	0	0	1	0	0	4	5 (2)

The results for Table A-8 were found by adding the number of responses of each type of investigator by each level of importance of synchrotron radiation in the future.

Table A-9. Relative Importance of Various Features of Synchrotron Radiation Facilities

A. Fast, efficient X-ray detectors
B. High brilliance/intensity/flux
C. Detectors with high spatial resolution
D. Computing and network services
E. Repeated access for long-term projects
F. High energy resolution
G. User-friendly environment
H. Helpful, readily accessible support staff
I. Rapid access for single experiments or feasibility studies
J. User training
K. On-site data processing
L. Good communication, *e.g.*, user group, Web site, documentation
M. Good ancillary facilities, *e.g.*, cold room, biochemistry lab, stock room
N. Intellectually stimulating environment
O. Good housing and convenient services
P. Other

Feature	Essential	Important	Helpful	Occasionally helpful	Unimportant	No Answer
A	205	38	7	1	0	11
B	191	55	7	1	0	8
C	157	53	17	12	8	15
D	155	70	18	5	0	14
E	146	76	20	4	3	13
F	139	61	25	9	9	19
G	134	82	36	1	0	9
H	134	89	28	3	0	8
I	130	84	27	6	2	13
J	113	79	44	7	7	12
K	109	85	45	12	0	11
L	84	76	67	20	2	13
M	82	92	55	19	2	12
N	52	48	101	28	19	14
O	49	90	88	13	9	13
P	10	2	0	0	1	249

The results for table A-9 were obtained by adding the number of each of five possible responses (essential, important, helpful, occasionally helpful, or unimportant) or no response by each feature of the synchrotron facilities. Descriptions of *Other* were: stable beam, stable optics out; time slicing; coding system; on-site machine shop; and truly optimal MAD beamlines.

Table A-10. Future Uses of Synchrotron Radiation by Scientific Discipline

A. Crystal structure determination by monochromatic techniques
B. Crystal structure determination by multiwavelength anomalous diffraction (MAD)
C. Determination of multiple crystal structures for proteins provided by site-directed mutagenesis, or as part of drug/ligand studies
D. Time-resolved structure analysis
E. Non-crystalline diffraction
F. Solution scattering of biomolecules and their complexes
G. X-ray absorption spectroscopy
H. Direct imaging by X-ray microscopy
I. Other

Use	XTAL	SNM	XAS	IMG	Other	None	Total
A	151	6	3	2	1	0	163
B	101	3	1	2	0	0	107
C	95	2	1	2	0	0	100
D	10	11	1	1	0	0	23
E	4	10	1	1	0	0	16
F	2	10	3	1	0	0	16
G	3	4	20	3	1	0	31
H	1	3	2	9	0	0	15
I	3	2	2	1	3	0	11

The results for Table A-10 were obtained by adding the number of responses of 1 (very frequently) or 2 (regular) to each type of future use by each scientific discipline. The total use for some items is greater than the number of respondents because some investigators indicated use in more than one scientific discipline. Descriptions of *Other* were: XIFS, interferometry; resonance x-ray diffraction; infrared, radiochemistry; topography and mosaicity; development of x-ray optics; instrumentation development; and detector software development.

Table A-11 and A-12. Collaborations

A total of 151 researchers collaborated with other independent investigators who do not have expertise in synchrotron science.

Table A-11. Collaborations

	1994	1995	1996
Number of responses	95	109	125
Number of collaborations	293	332	487

Table A-12. Collaborations by Discipline (Number of respondents in parenthesis)

Discipline	1994	1995	1996
XTAL	127 (62)	158 (76)	258 (90[1])
SNM	41 (8)	38 (7)	78 (9)
XAS	106 (16)	108 (17)	118 (16)
IMG	16 (8)	25 (8)	30 (9)
Other	3 (1)	3 (1)	3 (1)

[1]One additional researcher reported 230 collaborations!

Table A-13. Publications

Number of Publications			
First Year as an Independent Investigator	1994	1995	1996
1992-97	32	52	66
1987-91	68	75	95
1982-86	43	53	64
1977-81	47	57	53
Before 1977	109	138	141
Total	299	375	419

This survey was administered by Sheryl Martin and Laura Yust of the Human Genome Management Information System at Oak Ridge National Laboratory.

APPENDIX B: DATA FROM SURVEY OF SYNCHROTRON RADIATION FACILITIES

Table B-1. Overall Summary of Synchrotron Use for Structural Biology

	Synchrotron				
	CHESS	SSRL	NSLS	APS	ALS
Macromolecular Crystallography (XTAL)	F2	BL1-5 BL9-1 BL9-2	X4A X8C X12B X12C X25 X26C	17ID 17BM 19ID 19BM 14ID 14BM	5.0.2 5.0.1
Fixed-Wavelength XTAL	A1 F1	BL7-1	X4C	5BM	None
Non Crystal Diffraction (SNM)	A1 F1 F2	BL4-2 BL10-2 BL1-4	X9B X12B X27	18ID	None
X-ray Imaging (IMG)	None	BL3-4 BL10-2	X1A	None	6.1.3
Spectroscopy (XAS)	None	BL2-3 BL4-3 BL6-2 BL7-3 BL9-3 BL5-2 BL8-2	X9 X11 X18B X19A X10C	18ID	4.0
Other	None		IR Micro. U2B, CD, Fluor. U9B	None	None
Days of beam time per year	165	220	210	200-250	210-230
% Struct. Bio.[a]	30%	35-50%	15%	25%	12%

[a]Fraction of total beam time at all beamlines devoted to the Structural Biology disciplines mentioned.

Table B-2. Beamlines for Structural Biology

Synchrotron	Beamline	Organization	Birth Date
CHESS	A1	CHESS/MacCHESS	Oct-93
	F1	CHESS/MacCHESS	Aug-89
	F2	CHESS/MacCHESS	Aug-89
SSRL	BL 1-4	SSRL (partially operated)	late 70's
	BL 1-5	SSRL	1983
	BL 2-3	SSRL	Jun-76
	BL 3-4	SSRL	Jun-79
	BL 4-2	SSRL	Feb-89
	BL 4-3	SSRL	Feb-89
	BL 6-2	SSRL (PRT: Exxon 33%)	1984
	BL 7-1	SSRL	Apr-84
	BL 7-3	SSRL	Oct-80
	BL 9-1	SSRL	Aug-96
	BL 9-2	SSRL	Dec-97
	BL 9-3	SSRL	Jul-97
	BL 10-1	SSRL (PRT: CMR 33%; IBM 33%)	Nov-87
	BL 10-2	SSRL (PRT: UC/DOE Natl. Labs 67%)	Nov-87
	BL 5-2UV	SSRL	Dec-84
	BL 8-2UV	SSRL (PRT: UC/DOE Natl. Labs 67%)	Nov-86
NSLS	X1A	SUNY Stony Brook, Physics Dept.	Aug-90
	X4A	HHMI	Jan-94
	X4C	HHMI	late 97
	X8C	LANL, UCLA, Hoffman LaRoche, Eastern Canadian Consortium	May-97
	X9A	Albert Einstein, Phys. and Biophys.	1996
	X9B	Albert Einstein, Phys. and Biophys.	1986
	X11A	X11A PRT	1984
	X12B	BNL Biology Dept.	Jan-95
	X12C	BNL Biology Dept.	1985
	X19A	NSLS & PRT	Jun-91
	X25	BNL NSLS	Feb-90
	X26C	Cold Spring Harbor, SUNY Stony Brook, U. Chicago	Jun-97
APS	5BM	DND-CAT	Jun-96
	14BM-C	BioCARS	Mar-98
	14BM-D	BioCARS	Jan-98
	14ID	BioCARS	Jun-98
	17ID	IMCA-CAT	May-97
	17BM	IMCA-CAT	Mar-97
	18ID	BioCAT	Sep-97
	19ID	SBC-CAT	Apr-96
	19BM	SBC-CAT	Dec-96
ALS	4	ALS Structural Bio.	late '98
	5.0.1	ALS Structural Bio.	Jul-99
	5.0.2	ALS Structural Bio.	Jul-97
	6.1.3	LBNL Ctr. for X-ray Optics	1997

Table B-3. Characteristics of Beamlines Used for Structural Biology

Beamline	Flux (ph/sec)	Cross-fire (mr)	Instrumentation in 1998-1999
CHESS A1	$3.0x10^{11}$	1.2	phi-axis camera with mosaic CCD
CHESS F1	$3.0x10^{11}$	1.2	phi-axis camera with mosaic CCD
CHESS F2	$1.0x10^{11}$	1.6	phi-axis camera with mosaic CCD
SSRL BL 1-4	NA	NA	SAXS Camera w/single element CCD
SSRL BL 1-5	$2x10^{9}$	4.6	300mm MAR or Image Plate w/off-line scanner
SSRL BL 2-3	NA	NA	LHe cryostat on X-Y sample positioner/ 13-element Ge Detector or Lytle Det.
SSRL BL 3-4	NA	NA	Soft X-ray microscope
SSRL BL 4-2	NA	NA	Sample translator/Jet Mixer/Stopped-flow/Single axis goniometer/CCD/Linear PSD
SSRL BL 4-3	NA	NA	LHe cryostat on X-Y sample positioner/ 13-element Ge Detector or Lytle Det.
SSRL BL 6-2	NA	NA	LHe or LN2 cryostat on X-Y sample positioner/ 13-30 element Ge or Lytle Det.
SSRL BL 7-1	$3x10^{10}$	1.0	300mm MAR
SSRL BL 7-3	NA	NA	LHe cryostat on X-Y sample positioner/13-30 element Ge Detector
SSRL BL 9-1	$1x10^{11}$	3.0	345mm MAR or 2x2 CCD on Huber Kappa
SSRL BL 9-2	$4x10^{11}$	4.7	ADSC Quantum 4 2x2 CCD on Huber Kappa
SSRL BL 9-3	NA	NA	LHe cryostat on X-Y sample positioner/single crystal goniometer w/LN2 cryo. 30-element Ge
SSRL BL 10-1	NA	NA	NA
SSRL BL 10-2	NA	NA	6-axis Huber diffractometer w/solid state det.
SSRL BL 5-2UV	NA	NA	VUV chamber and multi-element Ge Detector
SSRL BL 8-2UV	NA	NA	VUV chamber and multi-element Ge Detector
NSLS X1A	NA	NA	NA
NSLS X4A	$~10^{10}$	~2.0	Huber 3-circle/R-Axis IV
NSLS X4C	$~10^{10}$	~2.0	Huber 3-circle/ ADSC single cell CCD
NSLS X8C	$2x10^{10}$	2.0	MAR/1k CCD (eventually 2k CCD)
NSLS X9A	NA	NA	NA
NSLS X9B	$1x10^{11}$	2.0	MAR/Ge detector
NSLS X11A	NA	NA	Displex, 13-element Ge
NSLS X12B	$5x10^{10}$	2.5	MAR 300/CCD
NSLS X12C	$1x10^{10}$	2.0	CAD4/Brandeis CCD
NSLS X19A	NA	NA	He cryostat and NSLS 100+ element detector
NSLS X25	$1x10^{11}$	1.0	MAR345 or CAD4/MAR CCD
NSLS X26C	TBD	TBD	Fast goniometer/MAR image plate
APS 5BM	--	--	MAR
APS 14BM-C	$5x10^{12}$	2.0	ADSC CCD or MAR or Fuji Off-Line
APS 14BM-D	$5x10^{12}$	1.5	ADSC CCD or MAR or Fuji Off-Line
APS 14ID	$7x10^{13}$	0.3	ADSC CCD or MAR or Fuji Off-Line
APS 17ID	--	--	Siemens 2x2 CCD
APS 17BM	--	--	--
APS 18ID	NA	NA	NA
APS 19ID	$2.5x10^{15}$	0.3	Kappa stage on Omega platform/ANL 9-CCD
APS 19BM	$8.0x10^{13}$	1.0	Kappa stage on Omega platform/ANL 9-CCD
ALS 4	NA	NA	NA
ALS 5.0.1	$8.5x10^{13}$	3.0	Kappa goniometer/2x2 CCD array
ALS 5.0.2	$2.3x10^{13}$	1.5	Kappa goniometer/2x2 CCD array
ALS 6.1.3	NA	NA	NA

Table B-4. Allocation of Time and Staff

Beamline	XTAL ext/int[a]	XAS	SNM	IMG	# Personnel
CHESS A1	.90/.05[b]	--	--	--	4.0
CHESS F1	.82/.02[b]	--	.10/0	--	4.0
CHESS F2	.42/.08	--	.03/0	--	4.0
SSRL BL 1-4	--	.07	--	--	0.1
SSRL BL 1-5	.88/.12	--	--	--	2.3
SSRL BL 2-3	--	.18/.12	--	--	0.7
SSRL BL 3-4	--	--	--	.66/0	1.6
SSRL BL 4-2	--	--	.38/.10	--	1.3
SSRL BL 4-3	--	.08/.01	--	--	0.2
SSRL BL 6-2	--	.27/.02	--	--	0.7
SSRL BL 7-1	.87/.13[b]	--	--	--	2.4
SSRL BL 7-3	--	.80/.13	--	--	2.3
SSRL BL 9-1	.90/.10	--	--	--	2.4
SSRL BL 9-2	(.88/.12)	--	--	--	2.4
SSRL BL 9-3	--	(.90/.10)	--	--	2.4
SSRL BL 10-1	--	--	--	--	--
SSRL BL 10-2	--	--	.12/0	.19/0	0.9
SSRL BL 5-2UV	--	.10/0	--	--	0.2
SSRL BL 8-2UV	--	.19/0	--	--	0.5
NSLS X1A	--	--	--	.32/.03	2.0
NSLS X4A	.40/.60	--	--	--	2.0
NSLS X4C	TBD	--	--	--	2.0
NSLS X8C	(.25/.75)	--	--	--	2.0
NSLS X9A	(.25/.75)	--	--	--	1.0
NSLS X9B	(0/.4)	(.3/.15)	(0.15)	--	2.0
NSLS X11A	--	.07/.03	--	--	3.0
NSLS X12B	0.55/0.20	.25/0	--	--	2.0
NSLS X12C	.65/.35	--	--	--	3.0
NSLS X19A	--	--	--	.45/.55	0.5
NSLS X25	.32/.08	.04/.01	--	--	2.0
NSLS X26C	.40/.25	--	--	--	1.0
APS 5BM	(0/.15)	--	--	--	4.0
APS 14BM-C	--	--	--	--	4.0
APS 14BM-D	--	--	--	--	4.0
APS 14ID	--	--	--	--	3.0
APS 17ID	(.25/.75)	--	--	--	--
APS 17BM	(.25/.75)	--	--	--	--
APS 18ID	--	.25	.50	--	9.5
APS 19ID	(.75/.25)	--	--	--	6.0
APS 19BM	(.75/.25)	--	--	--	6.0
ALS 4	--	Mag. CD 0.25/0	--	--	--
ALS 5.0.1	(0.4/0.4)[b]	--	--	--	3.0
ALS 5.0.2	(0.4/0.4)	--	--	--	3.0
ALS 6.1.3	--	--	--	1.0	--

[a] Ext/Int shows the fractions of beam time used by general users and by the group operating the beamline, respectively. (#) are projections for mid-1998.
[b] fixed-wavelength.

Table B-5. User Access to Beamlines for Structural Biology

Beamline	Scheduling Period	Groups Served/Period	Left in Queue
CHESS A1	2 mos	18	50
CHESS F1	2 mos	12	50
CHESS F2	2 mos	18	5
SSRL BL 1-4	2-3 mos	0	0
SSRL BL 1-5	2-3 mos	28/yr	0
SSRL BL 2-3	2-3 mos	9/yr	0
SSRL BL 3-4	2-3 mos	--	0
SSRL BL 4-2	2-3 mos	33/yr	12/yr
SSRL BL 4-3	2-3 mos	3/yr	3
SSRL BL 6-2	2-3 mos	9/yr	4
SSRL BL 7-1	2-3 mos	90/yr	96
SSRL BL 7-3	2-3 mos	28/yr	17
SSRL BL 9-1	2-3 mos	90/yr	TBD
SSRL BL 9-2	2-3 mos	TBD	TBD
SSRL BL 9-3	2-3 mos	TBD	TBD
SSRL BL 10-1	2-3 mos	TBD	TBD
SSRL BL 10-2	2-3 mos	2-3/yr	1-2/yr
SSRL BL 5-2UV	2-3 mos	3/yr	2
SSRL BL 8-2UV	2-3 mos	6/yr	1
NSLS X1A	4 mos	10	4
NSLS X4A	1.5 mos	11	2-3
NSLS X4C	TBD	TBD	TBD
NSLS X8C	4 mos	TBD	TBD
NSLS X9A	4 mos	5	0
NSLS X9B	4 mos	16-20	5-10
NSLS X11A	4 mos	16	30
NSLS X12B	4 mos	20	40
NSLS X12C	4 mos	20	40
NSLS X19A	4 mos	15	1
NSLS X25	4 mos	17	12
NSLS X26C	4 mos	TBD	TBD
APS 5BM	TBD	TBD	TBD
APS 14BM-C	TBD	TBD	TBD
APS 14BM-D	TBD	TBD	TBD
APS 14ID	TBD	TBD	TBD
APS 17ID	TBD	TBD	TBD
APS 17BM	TBD	TBD	TBD
APS 18ID	TBD	TBD	TBD
APS 19ID	TBD	TBD	TBD
APS 19BM	TBD	TBD	TBD
ALS 4	TBD	TBD	TBD
ALS 5.0.1	TBD	TBD	TBD
ALS 5.0.2	TBD	TBD	TBD
ALS 6.1.3	TBD	TBD	TBD

TBD, to be determined.

Additional Comments about Beamlines

B. Hedman, SSRL, All beamlines:
As we have given average values for 3 years of running to get reasonably reliable numbers, trends have not been displayed. However, there continues to be a strongly increasing demand for crystallography access at SSRL. The number of active proposals for monochromatic data collection increased from below 20 in 1990 to more than 160 in FY96. When station 9-1 became available for user scheduling earlier this year, it was instantly filled and there is already a significant and growing over demand for access (with NO decrease in activity on the other monochromatic station 7-1). It is also the case that virtually every group says that they would like significantly more time than they can get and this will become increasingly true as the push toward higher (nearer atomic) resolution progresses. There are also growth trends in SAXS/D with about a 60% increase in the number of active SAXS/D proposals between 1994 and 1996. There are 8 new outside user groups using primarily BL 4-2 since 1993, in part because of new capabilities in time-resolved SAXS/D and in low angle crystallography of large unit cell assemblies. In the XAS area, there has been a 65% increase in the number of active XAS proposals from '90 to '95 with 11 new outside user groups since 1991 - all meaning we are still significantly under meeting demand in bio XAS. New capabilities in detectors to study more dilute samples (high throughput 13-element Ge systems and soon a 30-element version) and instrumentation for grazing incidence XAS studies have contributed to this growth as well. There is also a significant growth in demand and numbers of proposals for time to study problems in bio- and phyto-remediation. Overall, it is the case that bio activity at SSRL has grown to represent about 50% of the user and proposal base.

R. Sweet, NSLS, beamline X12C:
I believe the number of proposals we receive is limited by the expected waiting period. Many investigators inquire about accessibility of time, but then fail to submit a proposal when they learn that the wait is likely to be 6 months. They hope for 2-3 months. At the same time, there is significant demand for a turn-around of less than one month -- many people call (1-2 a week!) hoping that there is some time RIGHT NOW.

L. Berman, NSLS, beamline X25:
Although I can't affirm so with certainty at this point, I am sure that, when the survey responses from the other NSLS biology beamlines are submitted, you will find that the oversubscription rate at the level noted on X25 is probably typical for the other beamlines too. In fact, I believe that it is even more severe for the BNL Biology beamlines X12B/C.

G. Bunker, APS, BioCAT:
We expect demand to be very strong once we have demonstrated the capabilities of the beamline. I have no doubt it will be strongly oversubscribed.

J. Calabrese, APS, DuPont-Northwestern CAT:
As the number of facilities expand, and become more user-friendly and reliable, more users will appreciate the importance and value of SR and its demand and use will grow.

E. Westbrook, APS, Structural Biology Center:
The user program has not yet begun. We anticipate 3 day runs will be scheduled, with users advised to come early to train, and they may stay later to process. User throughput will depend on APS storage ring stability. With no advertising and explicit warnings that we are not yet ready for users, I already have over 20 unsolicited requests for beam time.

T. Earnest, ALS, All beamlines:
We have had immense interest, especially from west coast groups for beam time even as we are initially commissioning. Our partnerships with industry (Amgen, Roche) and academia (UC-Berkeley, and soon (it looks) UCSF) offer a different "hybrid" mode of funding and beam time allocation that could serve as a model for future facilities.

APPENDIX C: OTHER COMMENTS BY RESPONDENTS TO THE USER SURVEY

Other Comments from Users:

Beam time proposals should be able to be submitted electronically.

Good survey format. Should allow accounting for co-PI's and non-PI/non-postdoc research group members in demographic section (not just postdocs).

When proposals are scored, there needs to be a balance of new proposals versus proposals which have received a lot of synchrotron times, even though the scores for new proposals may be lower.

My use at present is to test detectors for mainly crystallographic use. Rapid access modes, e.g. beamline staff time, have been most useful in the past. Sample handling facilities are important. Access to local computing is not so important (but nice).

I am in the unusual situation of working in a department that runs two protein crystallography beamlines, thus my responses to the survey are not those of a typical user.

If I could always collect my data at a synchrotron, I would. There is no substitute for experimental phases; molecular replacement phasing is subtly inferior and misleading. Hence, I would hope to collect both native and MAD data for all projects.

We currently average 2 synchrotron trips per year, primarily to use MAD for protein structure determination. Each trip consumes about 3 days of beam time. I expect (hope!) our synchrotron needs will approximately double over the next 5 years.

With easier access to a reliable and efficient MAD beamline, structure determination will be greatly facilitated.

Signing proprietary users agreement (and non proprietary agreements) generates a lot of concerns inside of an industrial crystallography lab.

Thanks for working on this and good luck!

The factors you list which normally limit synchrotron use are precisely those which caused my company to appreciate the importance of having our own beamline. Access must not be impaired by governmental ignorance.

I am actually a "supplier" of synchrotron photons. My answers relate to the collaborative projects I have and those that involve my own students.

To date we have not used a synchrotron in our research. Beginning this month we will be using synchrotron radiation in our research, and hope to increase our use of synchrotrons over the next couple of years.

We plan only to use the synchrotron when we cannot successfully solve a structure at home, but I still feel good access and good quality facilities are very important.

We have one project currently that may require synchrotron use for MAD phasing. Future use will depend on specific research projects/crystal quality.

The long time between proposal and beam time makes it very difficult to organize crystal growth or biochemical preparations in general and synchronize these with the synchrotron schedule.

About 2/3 of our projects depend on the availability of synchrotron X-ray sources.

I anticipate my need for synchrotron X-ray data under cryogenic conditions to increase in coming years!

Expect to generate samples in 3-5 years that will require high brilliance for diffraction. Do not have them yet, which is why we have not used synchrotrons recently. MAD phasing likely to be used in early experiments.

Our laboratory is now starting to gear up to utilize synchrotron sources for our structure analysis. We anticipate that this project will involve several trips to radiation sources over the next 1-2 years.

A flexible approach to the experimental station is very important. I enjoy this style at the X1A and other beamlines at the NSLS.

On the limited occasions when I felt that an EXAFS measurement was desirable I have been able to persuade colleagues to collaborate with me.

Canada is presently deciding whether or not to build a synchrotron facility. It seems promising but not assured. Much of the research done in my laboratory will stop if we do not have access to a synchrotron.

All labs should have their own synchrotron [beamlines].

We are rarely able to do "experiments" at the synchrotron. Time is always filled with challenging, but routine, data collection.

Need blocks of time of at least a week in length to work with live material.

The long lag between submitting a proposal and getting beam time needs to be reduced. Notification that beam time is available needs to be increased (*i.e.* >1 month). Both problems will only be solved with increases in total beam time.

We are a team working at a SR facility. The number of publications will be available in a few weeks as the activity report 1993-1996 has been tackled (about 1.4 publications/researcher/year).

We have been reasonably satisfied with SSRL & Brookhaven trips. In the early days of our major project, we had difficulty in transporting crystals. Those problems have mainly been solved.

Synchrotron radiation absolutely essential for earthworm hemoglobin structure and oxy-lamprey, oxy-Glycera hemoglobin structures.

The research group are those dedicated to SR experiments; there are two research associates, 11 postdocs, 4 graduate students and two technicians. Total budget is $800,000.

By the time beam time is approved for a particular project, it is common for other projects to be more important for data collection with the use of synchrotron radiation.

Looking forward to using more synchrotron time in the years ahead.

Most of our work is on relatively small proteins for which heavy atom derivatives are difficult to obtain. We now routinely produce selenomethionine proteins for analysis and expect to use synchrotrons on a more regular basis.

Synchrotron radiation is becoming an essential part of protein crystallography. It permits rapid structure determination and extends the data to higher resolutions. We estimate that the requirements for beam time will expand considerably in the future.

I would rank high brilliance as being the most important for our work, as it allows to do structures, such as large macromolecular assembles, not possible otherwise.

The lag time between the time we need synchrotron radiation and the time we get it is much too long.

Federal funds must be reserved for specialized synchrotron facilities first. Ordinary bending magnet lines must derive some support directly from users – more insertion devices, especially at 2nd generation synchrotrons, are critical.

Occasional users should be supported with quick access and standard facilities. This should not require dedication of a major portion of research group. Continuous attempts to broaden the base of users are extremely important.

Of the above, good documentation/software/computing are my biggest concerns. With my small group, several days at a time are too much of a strain. However, one day at a time is not worth the travel. Catch 22.

I anticipate my future needs will be significantly greater than my past usage.

By far the biggest obstacle in the way of our making greater use of synchrotron radiation is the 9 months interval between application for time and use.

It has been a great facility and the staffs are very helpful.

The US Synchrotron inventory is a marvelous resource, and needs careful nurturing to extract its full value.

In that we are located in Houston, TX, traveling to any synchrotron source is costly and inconvenient. Every trip to a synchrotron is physically and emotionally exhausting for everybody because the crystals have to perform – or else!

There are experiments I would like to try but cannot because synchrotron time is so precious and travel is expensive.

Synchrotron availability has been critical to my research.

Many important experiments in structural biology can not be done without synchrotron radiation resources. This will only become increasingly true as scientists are able to crystallize larger and larger biologically important complexes.

The ready availability of synchrotron facilities has opened new dimensions in protein structure studies, particularly in high resolution of large molecules (e.g., bacterioferrin) and atomic resolution studies (e.g., concanavalin A).

Beautifully done survey, covers all the key points.

Better to spend available funds on improving regional facilities (e.g. SSRL, CHESS etc.) than putting all eggs into one basket that is only available to a few (APS).

I am not a crystallographer but collaborate with a crystallographer who uses the synchrotron periodically. I am most interested in rapid techniques to observe enzyme-substrate intermediates.

PRT & CAT access to NSLS and APS is ESSENTIAL for regular repeated access for long-term projects of major users best matching key instrumentation development to particular scientific projects while still allowing for general users.

Too many of the current beamlines have sub-optimal MAD facilities. There is a desperate need for MAD lines with: rapid and precise wavelength change; high energy; very small bandwidth; easy on-off cryo cooling; quick fluorescence; CCD.

I'll soon have access to the ALS as a PRT member, which will improve my own situation drastically. Rapid access for routine MAD experiments as a phasing tool cannot be overemphasized.

Location is extremely important. Access by car relative to plane reduces travel costs and facilitates transport of equipment and biological samples.

Folks at CHESS do an outstanding job of user support. Usual experimental limitations associated with x-tography – getting xtals of sufficient quality to characterize sufficiently to put in an application.

At the present time our synchrotron use is initiated by our collaborator in macromolecular crystallography. In the future, this use will be initiated by us.

Easy access to quick meals at odd hours!

My collaborators are necessarily and usually people who have more expertise in synchrotron science! Need to coordinate availability of 2-4 expert collaborators from US, UK, Germany. My main research goal is time-resolved snapshots of the motor molecule.

It is critical to reduce the time between proposed submission and beam time availability. It makes no sense anymore to plan data collection 8 months-1 year ahead of time.

Don't use synchrotron radiation now. When materials are ready (2-5 years), it will be essential.

Synchrotron data collection is of absolute necessity. We could not have done any of these without SSRL and Brookhaven.

Faster backup/archiving facilities.

Small research groups can be disadvantaged by not having a critical mass of personnel for a synchrotron trip. Development of shared time or some other mechanism would make beam time more accessible to more people who'd like to use it.

Synchrotron use will become essential for developing projects.

I expect to use the BioCAT beamline regularly from 1998 on.

We have 12 days beam time at Brookhaven with the first 3 days scheduled for 8/97. All 12 days must be completed within scheduling year.

The Stanford facility has been great. I appreciate the use and the friendly staff.

My use of synchrotron resources in the U.S. in the past has been sporadic and not terribly productive, but these were valuable learning experiences. The company for which I work is a member of IMCA - hopefully much additional beam time will be available.

My trips to CHESS were especially helpful.

Our experiences at BNL and SSRL have been very positive. The staff have been exceedingly helpful!

We expect to make more use of synchrotron sources as our IMCA beamline becomes available.

APPENDIX D: USER SURVEY QUESTIONS

Demographic Information

How many years have you been an independent investigator? ____
If you are not an independent investigator, stop here. Thank you.

What is your research environment?
___ University   ___ Industry   ___ Government   ___ Laboratory
___ Other (please specify): _____

What is your source(s) of research support? (Check all that apply)
___ NIH   ___ NSF   ___ DOE   ___ Industry   ___ Foundation
___ Other (please specify): _____

What is the total annual support (direct costs) for all projects in your laboratory that rely on synchrotron radiation? (Choose one)
___$0   ___$1K-50K   ___$50K-150K   ___$150K-250K   ___>$250K

How large is your research group today?
___ Number of postdocs, research associates or equivalent
___ Number of graduate students
___ Number of other support personnel (e.g., technician, programmer)
___ How large do you think your group will be in 5 years? (Postdocs + graduate students + technicians)

Does your use of synchrotron radiation include scientific collaboration on the projects of other independent investigators who do not have expertise in synchrotron science?
___ Yes   ___ No
     If yes, with how many such independent investigators have you done collaborative synchrotron
     experiments in the past three years?
         ___ 1994   ___ 1995   ___ 1996

Synchrotron Information

1.   **Please estimate how many days of beamtime your group used at each of the following synchrotron sources. (If none, leave blank)**

	1994	1995	1996
Stanford (SSRL)			
Cornell (CHESS)			
Brookhaven (NSLS)			
Berkeley (ALS)			
Argonne (APS)			
Daresbury (SRS)			
Orsay (LURE)			
Hamburg (DESY)			
Tsukuba (PhFac)			
Grenoble (ESRF)			

2.   **How many publications (excluding conference abstracts) from your group in the past three years reported results obtained with synchrotron radiation?**

_____ 1994   _____ 1995   _____ 1996

3.   How have you used synchrotrons in the past, for the following types of experiments?
          Choose from: 1 = Major use   2 = Occasional use
                                         3 = Once only   4 = Never

Protein crystallography: monochromatic   _____
                                            MAD phasing   _____
                                            Laue   _____
Small angle scattering: static   _____
                                          time-resolved   _____
X-ray spectroscopy: static   _____
                                       time-resolved   _____
X-ray imaging/microscopy   _____
Wide angle x-ray scattering   _____
Other: _____
          Please specify other: _____

4.   How important is synchrotron radiation to your future research plans?
___ Critical   ___ Very Important   ___ Moderately Important   ___ Unimportant

5.   What will be important future uses of synchrotron radiation for your research?
          Choose from: 1 = Very frequent   2 = Regular   3 = Occasional
                                         4 = Rare   5 = Never
___ Crystal structure determination by monochromatic techniques.
___ Crystal structure determination by multiwavelength anomalous diffraction (MAD).
___ Determination of multiple crystal structures for proteins provided by site-directed mutagenesis, or as
       part of drug/ligand studies.
___ Time-resolved structure analysis.
___ Non-crystalline diffraction.
___ Solution scattering of biomolecules and their complexes.
___ X-ray spectroscopy.
___ Direct imaging by X-ray microscopy.
___ Other (please specify): _____

6.   What factors limit your use of synchrotron radiation? (Check all that apply):
___ Don't need it for my research.
___ Can't readily get synchrotron beam time.
___ Insufficient user support at synchrotron facility.
___ Insufficient research support to enable travel to the synchrotron.
___ Too much time between application for beam time and experiment.
___ Key instrumentation for experiment unavailable at synchrotron facility.
___ Too much radiation damage to samples.
___ Burdensome requirements for beamtime proposals.
___ Other (please specify): _____

7. **Please rate the importance of each of the following synchrotron research facilities for your research.**
Choose from: 1 = Essential   2 = Important   3 = Helpful
                      4 = Occasionally helpful   5 = Unimportant

___ High brilliance/intensity/flux.
___ High energy resolution.
___ Fast, efficient X-ray detectors.
___ Detectors with high spatial resolution.
___ User-friendly environment.
___ Helpful, readily accessible support staff.
___ Intellectually stimulating environment.
___ Good ancillary facilities, e.g., cold room, biochemistry lab, stock room.
___ On-site data processing.
___ Good communication e.g., user group, Web site, documentation.
___ Good housing and convenient services.
___ Rapid access for single experiments or feasibility studies.
___ Repeated access for long-term projects.
___ User training.
___ Computing and network services.
___ Other (please specify): _____

General Comments:

APPENDIX E: ACRONYMS

ACA - American Crystallographic Association

ALS - Advanced Light Source

APS -Advanced Photon Source

CAT - Collaborative Access Team, a group of researchers who collaborate on the development of a sector (two beamlines) at the APS

CHESS - Cornell High Energy Synchrotron Source

DESY - Deutsches Elektronen-Synchrotron

DOE - U. S. Department of Energy

ESRF - European Synchrotron Radiation Facility

EXAFS - Extended x-ray absorption fine structure

HHMI - Howard Hughes Medical Institute

LURE - Laboratoire pour l'Utilisation du Rayonnement Électromagnétique

MAD - Multiwavelength anomalous diffraction

NCRR - National Center for Research Resources of NIH

NIH - National Institutes of Health of the U. S. Public Health Service

NSF - U. S. National Science Foundation

NSLS - National Synchrotron Light Source

OBER - Office of Biological and Environmental Research of DOE, formerly know as the Office of Health and Environmental Research (OHER)

PDB - Brookhaven Protein Data Bank, an international database of three-dimensional structures of biological macromolecules

PhFac - Photon Factory

SAXS - Small-angle X-ray scattering

SPEAR - Stanford Positron-Electron Accelerator Ring

SRS - Synchrotron Radiation Source

SSRL - Stanford Synchrotron Radiation Laboratory

XANES - X-ray absorption near-edge structure

XAS - X-ray absorption spectroscopy