Biological Applications of Synchrotron Radiation:

An Evaluation of the State of the Field in 2002

A BioSync Report.

Issued by the Structural Biology Synchrotron users Organization, October, 2002.

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Introduction

Maxwell's equations show that electromagnetic radiation is generated when charged particles are accelerated. For the builders of the first circular accelerators of subatomic particles, the radiation their devices *inevitably* produced, which they called "synchrotron radiation", was a nuisance because it dissipated the energy of the particles being accelerated. By the 1960s, however, it was realized that synchrotron radiation has properties that make it ideal for experiments that are difficult or impossible to do using the electromagnetic radiation produced by conventional sources, and consequently there are synchrotrons operating all around the world today dedicated not to the production of high-energy beams of subatomic particles, but rather to the production of synchrotron radiation. Most of them produce X-rays beams of remarkable brightness, and the availability of those beams has had a major impact on many areas of science, including biology.

The Structural Biology Synchrotron Users Organization (BioSync) was formed in 1990 to promote the access to synchrotrons of *North American scientists interested in using synchrotron radiation to study biological systems. These scientists, who regardless of disciplinary affiliation are referred to below as "biologists"* , do four different kinds of experiments with synchrotron radiation: (1) macromolecular crystallography, (2) spectroscopy, (3) X-ray scattering (from non-crystalline samples), and (4) imaging. BioSync includes biological scientists active in all four areas.

BioSync has produced reports on the status of biological research at synchrotrons periodically. The first, which was issued in 1991, included the results of surveys of both the managers of synchrotron radiation facilities and biological users. The 1991 report argued that the demand for synchrotron access in the biological community would ultimately prove to be far larger than 1991 user statistics indicated because of the existence of a large number of biologists anxious to have structures determined, who were neither crystallographers nor users. It urged that facilities be built to meet that latent demand.

BioSync published its second report in 1997. It included statistics documenting an accelerating growth in the number of structures determined using synchrotron radiation, consistent with the predictions of the 1991 report, and it demonstrated that synchrotron radiation was becoming ever more important to the biological community. It urged the construction of additional beam lines for biological research, especially macromolecular crystallography, and the upgrading of existing beam lines.

This report is the third produced by BioSync. It deals with the application of synchrotron radiation to macromolecular crystallography, X-ray spectroscopy, and X-ray scattering, but it does not cover X-ray imaging, the smallest by far of the four fields. (This obvious gap in coverage will need to be addressed in the next BioSync report.) Like its predecessors, this report provides information about the current status of the biological research being done at synchrotron facilities around the country, and includes the results of surveys sent to biological users and to facility managers.

The members of the committee responsible for this report are:

Martin Caffrey, Ohio State University
Charles Carter, University of North Carolina
Jon Clardy, Cornell University
Christopher Hill, University of Utah
Michael Maroney, University of Massachusetts
Peter Moore, Yale University (chairman)
Ronald Stenkamp, University of Washington
Raymond Stevens, The Scripps Research Institute.

The Committee thanks Dr. Robert Sweet (Brookhaven National Laboratory) for making available the results of his recent survey of synchrotron users. A copy of his report is appended to this text. The Committee made extensive use of the 2001 Hodgson-Lattman report on biological synchrotron facilities, and thanks its authors for allowing it to access this document. *The first version of this report, which was completed in early June, 2002, was reviewed by a panel of experts: Michael Chapman, Johann Deisenhofer, David Eisenberg, Thomas Earnest, Wim Hol, Keith Moffat, James Penner-Hahn, and Ivan Rayment. Their comments were taken into account during the preparation of the final version, and the Committee thanks them for their input.*

Peter B. Moore
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Abbreviations and Definitions:

APS: Advanced Photon Source. The third generation synchrotron light source at Argonne National Laboratory (Chicago).

ALS: Advanced Light Source. The third generation synchrotron light source at Lawrence Berkeley National Laboratory (Berkeley).

BioSync: The Structural Biology Synchrotron Users Organization.

brightness*: the number of photons delivered to a sample at a given wavelength each second per unit energy bandwidth, per unit area illuminated, per unit solid angle of beam divergence.*

CAMD: Center for Advanced Microstructures and Devices. For users interested in X-rays, the CAMD synchrotron is the least useful of the synchrotron light sources now available. It is operated by Louisiana State University (Baton Rouge).

CCD: Charge-coupled device. A descriptor applied to a class of solid-state detectors of X-rays and other electromagnetic radiation.

CHESS: Cornell High Energy Synchrotron Source. This light source operates parasitically as an adjunct to an accelerator used for high energy physics. It is located on the campus of Cornell University (Ithaca, NY).

FedEx: A system for crystallographic data collection that obviates the need for crystallographers to travel to synchrotrons in order to collect data. Investigators are to ship their crystals to synchrotrons already mounted and frozen (often by Federal Express), as many already do. Crystals are placed in robotic crystal mounting devices by beam line staff, and the data collection process managed either locally by beam line staff, or remotely by the group that produced the crystals.

MAD: Multi-wavelength anomalous diffraction. A technique for obtaining phases for crystal diffraction patterns based on anomalous diffraction.

NSLS: National Synchrotron Light Source. The second generation synchrotron light source located at Brookhaven National Laboratory (Upton, NY).

SSRL: Stanford Synchrotron Radiation Laboratory. SSRL operates a synchrotron called SPEAR, which is being converted into a third generation light source. This facility is part of the Stanford Linear Accelerator Center (SLAC) (Palo Alto).

Executive Summary

The growth in the biological use of synchrotron radiation predicted by the 1991 BioSync Report, and documented in the 1997 BioSync Report, continues unabated. In order that the promise of this important area of biophysics be fully realized, a number of issues should be addressed now. They are summarized below, starting with those the Committee considers most urgent.

! Funding for synchrotron operations must be increased. The Department of Energy and, to a lesser extent, the National Science Foundation funded the construction of most of the synchrotron light sources in the USA, and pay directly for their on-going operating expenses. In recent years, the Department of Energy and the National Science Foundation have not had budget increases that match those received by the National Institutes of Health, the chief sponsor of the biological research that consumes an ever increasing fraction of the available synchrotron beam time. The Department of Energy and the National Science Foundation need help with the financial burden of synchrotron operations, which is growing faster than the corresponding parts of their budgets, at least in part because of the growth in biological demand. Either Congress must be persuaded to increase the relevant lines in the budgets of the Department of Energy and the National Science Foundation, or a mechanism must be found that enables the National Institutes of Health to pay for the radiation used by the scientists it sponsors.

! CHESS and NSLS should be upgraded, and resources provided so that existing beam lines at ALL synchrotrons can be upgraded also. Every effort should be made to upgrade NSLS. NSLS has been remarkably productive, but it is a second generation light source, and its value to users will decline unless it is upgraded. Furthermore, if CHESS were operated as a dedicated light source, its beam lines could be competitive with the best in the world, and thus a major national asset. In considering these recommendations, it should not be forgotten that CHESS and NSLS are the facilities closest to most east coast synchrotron users. If they are not upgraded, members of that large community will increasingly find themselves forced to travel long distances to gain *hands-on* access to state of the art beam lines.

It equally is vital that resources be made available to those responsible for the maintenance and operation of all synchrotron beam lines of all kinds so that they can purchase the optical elements, sample handling devices, detectors, computers, etc. necessary to keep their beam lines up to date. The amounts of money required for such activities are much smaller than those required to upgrade entire facilities *or build new beam lines*, but benefits can be very large.

! The level of staffing of beam lines needs to be increased. Synchrotron beam lines can generate prodigious amounts of data, but will not do so unless users get round the clock staff support. It takes approximately 5 staff members per crystallographic beam line to get 24 hour a day, 7 days a week coverage, which is significantly more staff than the average beam line has today. Staffs this large may not be required for non-

crystallographic beam lines, but they too will not produce the output they should unless adequately staffed, which none of them are today.

Given the huge increase in the number of beam lines that will have occurred by the end of 2002, the fact that staff levels at the beam lines now operating are lower than desirable and that salary money is in short supply, staff shortages are likely to persist for years to come. While additional funds will be required, money alone will not solve this problem. Beam line staff jobs must be configured to make them more attractive than they are today. BioSync should organize a committee to study this problem.

! The number of crystallographic beam lines available is sufficient to meet demand for the immediate future, but additional beam lines may be required for other kinds of biological experiments. In November, 2001, there were 27 beam lines in the U.S. dedicated to biological crystallography. By the end of 2002, the number will be close to 48. Provided these beam lines are adequately staffed and well-instrumented, they should suffice to meet demand for the next several years. It should be recognized, however, that the structural genomics initiative, which is still in its infancy, could generate demand beyond what this Committee anticipates, and that beam line construction takes years. For these reasons, this issue should be revisited by 2005.

Those responsible for the 2005 assessment should pay careful attention to the tendency of facility operators to convert beam lines instrumented for techniques like X-ray absorption spectroscopy and X-ray small angle scattering into crystallographic beam lines. It is not obvious that the number of beam lines instrumented for non-crystallographic experiments (~ 10) is sufficient; beam lines for X-ray absorption spectroscopy, for example, are currently oversubscribed. Furthermore, the number of non-crystallographic beam lines is already so small that a local decision to convert even one of them into a crystallographic beam line could have serious implications for an entire field of research nationwide.

! Efforts to automate crystallographic beam lines should be vigorously supported. The Committee's forecast about the sufficiency of crystallographic beam line resources assumes that substantial increases in productivity will be realized from the automation initiatives now underway. Not only is automation essential for increasing throughput, it is essential if "FedEx" crystallography is to work as its proponents hope.

! Beam line designers and operators should be encouraged to agree on both equipment standards and software standards. There would be substantial advantages for users if all crystallographic beam lines accepted the same crystal mounting hardware. If this were the case, mounted crystals could be shipped from their laboratory of origin to any facility that had beam time available. Similarly, there would be big benefits if the interfaces that control beam line operations were the same at all beam lines. One advantage would be a reduction in the time wasted on user *training*.

! It would be helpful if the beam line application process were standardized and streamlined. According to the latest user survey, the greatest impediment to access is the length of time that passes between the submission of applications for time and the date

that time awarded actually gets used. ALS and SSRL have recently implemented access review processes that result in data collection within about a month of application submission. Other facilities should adopt similar procedures. In addition, the application themselves should have a short, highly defined format, and be standardized so that a single proposal is acceptable at all synchrotrons.

General Concerns.

Synchrotron radiation is used by biologists for several different purposes: X-ray crystallography, X-ray absorption spectroscopy, X-ray small angle scattering and diffraction, and X-ray imaging. While the needs and interests of scientists engaged in these different kinds of research are not identical, on four important issues they coincide, and it is to these issues that we turn first.

Synchrotron operations and maintenance . One of the most important problems confronting synchrotron users today is infrastructure support. In the Hodgson-Lattman survey, support for basic facility operations was the top concern of those responsible for synchrotron operations. As it now stands, facility operations are supported primarily by the Department of Energy and the National Science Foundation, the two agencies that financed the construction of most of the synchrotrons now operating. The huge increase in funding for the National Institutes of Health that occurred recently is driving a strong increase in demand on the part of biological users. At the same time, the budgets of the Department of Energy and the National Science Foundation, which are effectively declining, are seriously constraining the ability of synchrotrons to maintain base operations, let alone respond to increased demand.

SSRL provides a useful example of the kind of difficulties now being experienced. Following the upgrade of SPEAR3 now in progress, SSRL will require significant new funding to provide the 5000 user hours/year it has set as its goal, and to provide the staff support appropriate for a third generation synchrotron. Where will those funds come from? Limited funding for operational costs also restricts the ability of facility staff everywhere to do preventive maintenance, thereby reducing overall efficiency.

One could argue that the appropriate way to solve this problem is to make users pay for the photons they consume, at least in part, rather than relying entirely on block allocations of funds to synchrotron operators. It would be inappropriate to rehearse here the arguments for and against user fees. Suffice it to say, they have been discussed many times in the past, and the conclusion always reached has been that facilities like synchrotrons are unlikely to thrive if required to support themselves largely, or entirely on user fees. That being the case, the only alternative *for the moment* is to seek increased funding for these activities from the National Science Foundation and the Department of Energy, and/or to develop a mechanism that enables the other large, interested agency, National Institutes of Health, to contribute.

In the future, the case for extracting at least some of the funds required to support synchrotron operations from users is likely to strengthen. Organizations that solve macromolecular crystal structures for biological scientists on a service basis will probably develop at synchrotrons (see below). The biologists who would be the "customers" of these organizations routinely pay for the other kinds of technical support they receive on a fee-for-service basis. In this case it would be reasonable to include in the fee charged for the determination of a crystal structure an allowance for the cost of facility operations.

NSLS, CHESS and the geographical distribution of beam lines . Much of the growth in beam line number, quality and capability in recent years has occurred at APS, ALS and SSRL, i.e. in the mid-west and the Bay area. While these developments are welcomed by all because of their positive impact on the Nation's scientific capabilities, they pose a significant logistical problem for investigators based on the east coast, who increasingly find themselves having to travel long distances to collect data hands-on at state-of-the-art beam lines.

As Table 1 shows, crystallographic synchrotron users prefer to collect data close to home. By so doing they reduce the time and money spent on travel, and those costs are not trivial. Synchrotrons operate 24 hours a day, and thus user groups must send at least two people to the synchrotron every time they have a run. Table 1 also shows that in 2000-2001, ~38% of the days of crystallographic data collection used were consumed by groups based in the northeast, and their needs were met largely by NSLS and CHESS, the two increasingly obsolete synchrotrons located in their area.

Table 1. Synchrotron usage and home institution location, 2000-2001.

Days of beam use	SSRL 2001	Chess 2001	NSLS 2000	ALS 2001	APS 2001	Row sums
Canada	3	6	31	0	26	66
Mexico	3	0	0	0	0	3
Midwest	79	0	67	4	366	516
Northeast	39	80	672	5	160	956
Northwest	13	0	124	24	4	165
Southeast	44	2	48	0	33	127
Southwest	34	0	16	1	8	59
West	284	13	32	282	14	625
Totals	499	101	990	316	611	2517

Synchrotron usage is measured in days of beam time. Table compiled by Robert Sweet (Brookhaven National Laboratory).

Remote crystallographic data collection (see below) should alleviate the impact of this geographical distribution problem. Nevertheless, those crystallographers who are involved in methods development. and other non-routine projects, like *all* non-crystallographic users, must travel to synchrotrons to do their work, and thus there is no technological fix for their problem.

The proposals from the operators of NSLS and CHESS for facility upgrades should be considered in this context, at least in part. Both NSLS and CHESS are outclassed by their more recently constructed (or upgraded) competitors elsewhere, especially for the execution of cutting-edge experiments. Upgraded, they could both deliver excellent service to *the national scientific community for many years to come, as well as meeting the needs of east coast users who require hand-on access.*

CHESS operates today as a parasitic user of radiation generated by an accelerator operated for other purposes. If CHESS were converted into a dedicated source, beam lines could be constructed on it that compete on even terms with those at third generation light sources, like ALS or APS. In the case of NSLS, nothing so dramatic is possible because of the way it was designed. Most of the upgrades being discussed for it today are improvements of specific beam lines. However, schemes for replacing NSLS with a new, Brookhaven-based light source are being actively considered, and it is important that they be pursued. An NSLS replacement could be the most attractive option for meeting national needs later in this decade.

It should be noted that a similar geographical problem confronts scientists base in the southeast. CAMD, the synchrotron operated by the State of Louisiana, is being modified for crystallographic data collection, which will certainly help this group, but CAMD is unlikely to meet more than a modest fraction of their needs.

Beam line staff, and beam line upgrades . All the constituencies that use synchrotron radiation for biological purposes agree that the beam lines they use are less effective than they should be both because staff levels are sub-optimal, and because beam lines are being upgraded less aggressively than they should be. The way these issues play out varies from discipline to discipline, and for that reason they are addressed again below. However, the bottom-line message is the same. Synchrotrons are enormously costly to build and operate, and even the individual beam lines that use the radiation they produce are expensive. The construction cost of a single crystallographic beam line is $5,000,000 to $15,000,000. A few hundred thousand additional dollars for a new piece of hardware or an additional staff member is money well spent if it increases the output of such instruments by a factor of two, and it often can.

Macromolecular Crystallography.

X-ray crystallography has been the primary method for obtaining atomic resolution information about the structures of biological macromolecules since the late 1950s, and is, if anything, more dominant today than it was even as recently as 10 years ago. This is true in large measure because macromolecular crystallographers can collect data using synchrotron X-ray sources, rather than the home equipment most relied on until the early 1990s.

Synchrotrons can provide beams of X-rays of wavelengths suitable for macromolecular crystallography (~ 1 Å) that are orders of magnitude brighter than those produced by the best laboratory equipment. Consequently, synchrotron beam lines built for macromolecular crystallography are vastly superior to laboratory equipment. Complete data sets can be obtained from ordinary sized crystals of proteins of ordinary molecular weight in minutes to hours, instead of days to weeks, and crystals much too small for home-source data collection can often be dealt with successfully. The extraordinary brilliance of synchrotron sources also makes it practical to collect data from weakly diffracting crystals with large unit cell dimensions that would otherwise be impossible to study. In addition, synchrotron beam lines can be used to collect data over

a range of wavelengths because they are tunable, which home sources are not. The tunability of synchrotrons sources has sparked a rapid growth in the use of anomalous diffraction techniques for solving the phase problem, which in turn has greatly accelerated the speed with which macromolecular structures can be determined. It is unlikely that anyone would be thinking about high-throughput protein structure determination today if synchrotrons did not exist.

Current Status . Macromolecular crystallography is an expanding activity, and the impact of synchrotron radiation on crystallography is growing even more rapidly. In 1991, the year the first BioSync report appeared, 18% of the 127 of the 428 crystal structures deposited in the Protein Data Bank that required experimental solution of the phase problem *de novo* were solved using synchrotron radiation. In 1999, the last year for which such data have been compiled, 62% of the 778 structures deposited in the Protein Data Bank that required solution of the phase problem were synchrotron-connected. The total number of structures deposited in the Protein Data Bank last year (2001) is so large (3298) that it is impractical to compile statistics like those just cited, but there is every reason to believe that the growth in the importance of synchrotron radiation has not stopped.

The scientific rewards for using synchrotron radiation are enormous. For example, everything else being equal, the resolution of data sets obtained using synchrotron X-ray sources is almost always superior to that of data sets collected using home laboratory equipment, and high resolution structures are better than low resolution structures. In addition, powerful phasing methods like MAD *and SAD* absolutely require synchrotron radiation.

Trends . Macromolecular crystallography is gradually being transformed from an experimental technique available only to specialists into a methodology available to every biological scientist. Determining the crystal structure of a protein may soon be no more remarkable than determining the sequence of a DNA oligonucleotide. Most biologists, it should be pointed out, have the DNAs they care about sequenced at centralized facilities using technologies many of them are barely able to describe. Crystal structure determination is headed in the same direction.

The development of user-friendly crystallographic beam lines at synchrotrons has streamlined data collection, an aspect of crystallography that used to tie up expensive (by ordinary laboratory standards) instruments for prolonged periods of time. In addition to making data collection faster, the development of crystallographic beam lines has converted it from an activity done at home on instrumentation available to only a few into an activity that occurs at centralized facilities accessible to all qualified scientists. From the point of view of most members of the biochemical community, synchrotrons are crystallographic data factories, and developments now underway will make them even more effective and more dominant than they are today.

At the same time that data collection has become centralized, increasingly sophisticated computer codes run on increasingly powerful computers have made the downstream processing of crystallographic data much faster and more efficient. In the near future, macromolecular crystal structures are likely to be solved automatically, the

way small molecule crystal structures are solved today. However, this does NOT mean that the need for well-trained macromolecular crystallographers is about to vanish. Even today small molecule crystals are encountered that require human intervention to solve. The same will be true for macromolecular crystals for decades to come.

There is general agreement that macromolecular crystallography has a vital role in the biological sciences going forward. The structural genomics initiative, which is being sponsored by the National Institute of General Medical Sciences, is eloquent testimony to that belief. Beyond taking care of the general problems discussed above, what steps should be taken now to ensure that this enterprise continues to thrive?

Staffing . The average staffing levels for crystallographic beam lines has increased from 3.1 to 3.7 full time equivalents per beam line in recent years, due largely to a welcome and much appreciated increase in support from the National Institute of General Medical Sciences and the National Center for Research Resources. Nevertheless, additional staff is still needed at existing beam lines. Users of a beam line that has a staff of 3 or 4 cannot be supported properly on nights and weekends. On average, it would take another 1.5 full time equivalents per beam line to obtain 24/7 coverage (Hodgson-Lattman). If this level of staffing could be achieved, the efficiency of beam line utilization would increase.

The staffing of crystallographic beam lines is likely to become a crisis in 2002 because of the pressures that develop as the huge number of new beam lines now under construction come on-line. It is further aggravated by the trend towards providing users with shorter, but more frequent periods of access, and by the increasing demand from inexperienced users for X-ray crystallographic data. Staff shortages will hamper all areas of structural biology that use synchrotron radiation, not just macromolecular crystallography, and at present, both the funding required to support new staff and the mechanisms to attract talented young scientists to such positions are lacking.

In addition to needing more staff, changes in the user community are going to require staff with skills different from those of existing staff. The first wave of crystallographic users moved ongoing experimental programs from their laboratories to the synchrotron. Their runs were lengthy by today's standards, and during each run, their laboratory members actively participated in all phases of data collection and analysis. Today the user population includes users who are new to protein crystallography, users who want to make only brief visits for limited data collection or crystal screening, users who don't want to visit the synchrotron in person, but would prefer to send their crystals in so that data can be collected either by others or be collected in a mode that enables them to control the process remotely, i.e. engage in “FedEx” crystallography. These constituencies are effectively transferring to beam line staff functions that in earlier times would have had to be performed by members of their own groups.

In the not too distant future, a new group of users will be added to the mix: users who expect facilities to provide them with solved structures instead of raw data. The history of small-molecule crystallography shows that facilities of this kind can have a positive effect. A large number of publications in the chemical literature today include structures that were not solved by their authors. Macromolecular crystallography is headed in the same direction; some universities already have facilities where an

investigator can drop off a protein crystal and, at least in principle, get a solved structure back. Facilities of this kind require a staff that is well trained in all aspects of crystallography, not just data collection, and because of the centralizing role already played by synchrotrons, it seems likely that synchrotrons will become the homes for the most important of these full-service, macromolecular crystallography operations.

The recruiting of scientists to run full-service crystallographic facilities will not only require increased salary funding, it will also require the creation of an environment and a career path that encourages talented young scientists to work at synchrotrons, and to devote a large fraction of their energies to advancing the science of others. One reason this Committee recommends that the average number of staff per beam line be raised to 5 is its belief that only when staff levels reach that level will it become possible for staff members both to assist users and to pursue their own research. Past experience indicates that scientists who work at user facilities are the people most likely to understand how the equipment everyone is using could be improved, and to have both the resources and the inclination to pursue the technologies that will make those improvements possible. Their motivation to do such work is highest when it is driven by their own research interests. Thus there are two reasons for enabling beam line staff to do their own research. It will help facilities recruit quality staff, and it will ensure the advance of beam line technology. *Two points further points should be made, both of quite obvious. First, it is not essential that the staff of every beam line be composed entirely of scientists with Ph.D.s in crystallography. Technicians with B.S. or M.S. degrees in physics, chemistry or biology, for whom career path issues are much less important, should be able to do at lot of the routine work. Second, there is strength in numbers. Problems like the support of users late at night are easier (and cheaper) to solve if the staff of all of the biological beamlines at a synchrotron cooperate, as they already do at some facilities.*

Beam line upgrades . In many instances beam line upgrades can be the most cost-effective way to increase the output of a synchrotron facility. Although the installation of CCD detectors has greatly increased the efficiency of many beam lines, there are still several beam lines that could benefit, including some under development, that require funds for the purchase of better detectors. From the point of view of beam line efficiency, the single most important advantage of CCD detectors is that data can be downloaded from them in seconds, compared to the minutes it takes to read an image plate, which is the next best option. The reason this difference is critical is that on high brilliance beam lines a single frame of data can often be collected in a seconds, and time spent downloading data is time that could more profitable be spent collecting data. Significant advances in detector technology are likely in the next few years. It would make sense to budget for the replacement/upgrade of detectors at every beam line every five years or so.

It is also important that funds be provided for the upgrading of the optical and other hardware components of existing beam lines. Although a great deal has been learned about synchrotron beam line design and construction, it is by no means a mature art form. Improvements in materials, manufacturing methods, and optical and hardware design continue to be made, and it is important that existing beam lines take advantage of

them. Often modest changes in a beam line can result in increases in data collection rate of factors of 2 or greater.

The increasing rate of data acquisition and need for real-time optimization of data collection already taxes, and often exceeds, the computer resources available at most beam lines. Significant investment must be made to increase computer power and data storage capability.

Beam lines Resources . As of November, 2001, there were 27 synchrotron beam lines operating in the United States dedicated to X-ray crystallographic studies of biological macromolecules, and 21 beam lines were under design or construction (see Appendix, Table 1). This phenomenal growth has been financed by the agencies that sponsor biological research, and reflects their belief in the importance of structural biology in the post-genomic era. Their investment has already engendered a huge increase in the productivity of US crystallographers.

A key question this committee has been asked to address is whether or not the 48 crystallographic beam lines that will shortly be available are going to be enough. While it is certain that demand for beam access will continue to grow, there is no reason to authorize the construction of additional crystallographic beam lines now beyond that which may be required to make best use of an upgraded CHESS or NSLS. First, by the time this report is published, the number of beam lines will have almost doubled with no corresponding increase in the size of the user community. Second, as will be described below, initiatives already in progress should dramatically increase the productivity of existing (and future) beam lines, further expanding the resources available.

There are two sources of uncertainty in this assessment.

(1) The impact of the structural genomics initiative on demand for beam time cannot be predicted accurately at this point. On the one hand, several of the beam lines now under construction are being built for structural genomics, and technologies for high-throughput crystal and data handling, which are being developed at least in part in response to the structural genomics initiative, should also reduce the impact of the initiative on beam line availability. Also, it seems that with few exceptions, the wildly optimistic predictions of the level of crystallographic activity that would result from structural genomics, made when the enterprise began, have been scaled back. On the other hand, in at least one case, beam time has been guaranteed for a structural genomics project without the construction of a new beam line to provide it.

(2) Implicit in the estimate that beam line resources will be adequate is the assumption that all the beam lines now in existence or under construction will be operated, staffed and maintained to best advantage, which may or may not turn out to be the case.

New synchrotron sources . Astonishing increases in the brilliance of synchrotron radiation sources have been achieved by those who design and build synchrotrons since the field began, and strategies are being pursued today, e.g. the free electron laser, that may result in huge future improvements. Consequently, it is likely that any new synchrotron built in the USA in the future will be considerably more powerful than those

that now exist. The benefits of such instruments to non-biological synchrotron radiation may justify their construction. The biological case is less obvious.

Biological users have benefitted enormously from machine improvements over the years; the brighter the source, the faster data are collected. Nevertheless, further increases in synchrotron brilliance are unlikely to result in proportional increases in biological throughput. The reason is that at today's brightest beam lines, for most biological crystals, source brilliance does not limit the rate at which data are collected, let alone the rate at which structures are solved. Almost all of the data collected from biological crystals today are obtained from crystals that have been frozen to reduce the chemical damage that otherwise is caused by the diffusion of the reactive species generated by exposure to X-rays. In addition to damaging crystals chemically, X-rays heat them, and the brighter a beam, the shorter the exposure required to melt a crystal along the path traversed by the beam. Those who collect data at the brightest beam lines today commonly control crystal heating by attenuating the beam, and thus effectively throw away most of the (expensive) photons those beam lines deliver. Thus, as things stand, the crystallographic community is not taking full advantage of today's brightest sources, and unless data collection strategies can be elaborated that obviate the problems just described, the only biologists likely to benefit from even brighter sources are those interested in solving the structures of crystals that diffract very weakly, either because the crystals want to study are very small, or because their unit cell dimensions are very large.

Automation . Much of the anticipated growth in demand for crystallographic beam time will probably be met by the increases in the efficiency of beam line utilization that result from automation. This will require considerable investment, but much less than would be required to satisfy the same demand by building more beam lines. Thus it is vital that efforts to automate beam lines continue.

The objective of the automation effort is to reduce to a minimum the need for user intervention during the collection and analysis of crystallographic data. This can save beam time several ways. For example, beam lines at third generation synchrotrons can collect the data required to solve a protein structure from a single, frozen crystal in less than one hour. But before data collection can begin, someone has to turn off the X-ray beam, enter the chamber where crystals are exposed to X-rays (the hutch), mount a crystal on the goniometer, exit the hutch, turn on the beam, and align the crystal in the beam. This takes time, ~ 15 minutes per crystal, and during that time no data are collected. The fraction of the available beam time so wasted becomes particularly significant when crystals are being screened for their suitability for data collection.

Major improvements in beam time utilization would result if crystal mounting and alignment was done robotically. Additional improvements would be obtained if programs were developed to assess the data a crystal is providing in real time so that data collection can be stopped if the data are poor or the data collection strategy optimized if the data are good. Beam line operators estimate that automation should improve beam line throughput by not less than a factor of two.

Automation projects are underway at most of the synchrotron facilities in the U.S.A.. The goal is the development of a flexible, but robust and user-friendly system

that can be installed on many beam lines. It is to include a crystal mounting robot, a program for automated crystal alignment, programs to control and monitor data collection, and a system for reducing data in real time. Almost all the components of such a system exist at one synchrotron or another, at least in prototype form, but nowhere has a complete package become operational.

At present the Berkeley Center for Structural Biology and the Bio-instrumentation group at ALS have progressed as far as anyone. They installed a robotic crystal mounting and alignment system at beam line 5.0.3 in April 2001 that has already been used to collect many data sets. It relies on locally designed, task-specific hardware instead of commercially available robots, and it is physically compact and made of low-cost components. The sample storage and transport system uses puck-shaped cassettes that can hold sixteen crystals each. They can be loaded with mounted, frozen crystals anywhere in the world and shipped frozen to ALS in standard containers. The dewar in the hutch can hold either sixty-four (four pucks) or one hundred twelve crystals (seven pucks). This system was replicated at beam line 5.0.2 in early 2002. A second copy is being made for beam line X12-B at NSLS, and the system is being considered at other crystallographic beam lines.

The group at ALS is now trying to make their system "smart". Standard crystal screening and data collection protocols have been implemented, and integration of data collection, data processing, and analysis is underway. The goal is a system that will make it possible to collect data efficiently in a mode that requires no user input. The benefits this system offers "high-throughput" experimental programs are obvious, but others will also benefit. Ultimately, this system will be interfaced with analysis and refinement programs such as PHENIX, which is being developed by LBNL's Computational Crystallography Initiative, to provide an automated pipeline from crystal to refined structure.

Progress in solving the computational aspects of automation problem is being made at several other locations. The Argonne Structural Biology Center Collaborative Access Team is currently co-developing the data reduction program HKL2000 so that beam line data collection equipment at APS can be connected with data processing software. The goal is a system that will enable users to determine optimal data collection strategies in real time, and to process data on-line. The Brookhaven Biology Department group, which operates four beam lines at the NSLS, has pioneered the use of graphical user interfaces for beam line operation, integration of beam line operations and data collection, pipelining of data to data-reduction software, and remote observation and remote operation of beam lines. This system is the heart of their successful, courier-based (FedEx) data-collection program. In addition to adapting the LBNL/ALS robotic sample changer for use at the NSLS, the BNL group is constructing a database to harvest and integrate the information obtained during every synchrotron experiment. This database will include everything from the original beam time application through data reduction, and will produce suitable for deposition in the Protein Data Bank. Like the Argonne Structural Biology Center Collaborative Access Team, the NSLS group has an integrated system operating on beam line X9-B that uses HKL2000 to enable efficient data collection and processing without user intervention.

The automation program at SSRL has produced a system that enables both local and remote operation. The goal is to make it possible for users to send cassettes loaded with mounted crystals that can be used for data collection on any of the SSRL SMB-crystallography beam lines. A complete prototype of the sample management system has been installed at BL11-1, and most of the rest of the package is expected to reach prototyping stage in the second quarter of 2002. SSRL has also developed Blu-Ice/DCS, which is a unified diffraction data collection environment that integrates with existing and anticipated control systems in a distributed fashion. It is currently operational at all SSRL crystallographic beam lines, and copies of the development version of the software have been provided to most US and many international synchrotron light sources for evaluation and implementation. Several ALS beam lines are now operated using derivatives of the Blu-Ice system.

Standardization . System standardization should be a priority at beam lines around the country. In addition to reducing user burdens, it would almost certainly reduce the time support staff spends training new users. That said, the drive for standardization should not be allowed to stifle innovation, but it should loom large in consideration of all issues that directly affect users

Sample mounting procedures should become standardized for the various robotic systems currently under development. Specifically, standards need to be agreed upon so that all robotic systems will accept the same types of mounting pins and storage cassettes. This is not the case now. Different robotic systems uses different types of pins and cassettes, and thus once crystals have been prepared for use with one system, the number of beam lines on which data can be collected from them is reduced to a handful, which is clearly inefficient. Why should a crystallographic laboratory have to provide itself with two or three different sets of hardware to accomplish the same objective? This is akin to the situation confronted 5-10 years ago where mounting pins suitable for one beam line did not fit the goniometer head of another.

One reason users are reluctant to shop around for beam time as much as they ought to is the efficiency that derives from familiarity with the procedures peculiar to the beam lines they already know. To the extent possible, interfaces for beam line operation, data collection, and data processing should be standardized. This will become increasingly important as remote data collection becomes common place, and there is increasing reason to encourage users to collect data at whatever suitable beam line is available, without regard to geographical location.

Users have long identified the length of the interval between submission of proposals for beam time and the day the time awarded is actually used as a serious impediment to progress. In some cases the gap can be as much as a year, which is a very serious problem for junior investigators, delays beam line access for projects of high urgency, and often results in data collection time awarded for one project being used for another that was never reviewed. Another deficiency is that guidelines for proposals are not always clearly defined, with the result that applicants often write several pages when a few paragraphs would do. Furthermore, because of uncertainty and the long review process, applicants often submit the same proposal to two more different

synchrotrons/beam lines, each of which will usually has distinctly different proposal requirements, which makes extra work for everyone.

Many of the inefficiencies in the current system could be fixed by fairly simple organizational changes. Recently, ALS and SSRL have instigated a system of rapid review that uses a short, standardized application form, and makes beam time available within about one month of proposal receipt. This practice should be adopted nationally.

X-ray Absorption Spectroscopy

X-ray absorption spectroscopy plays an important role in the structural biology of metal-containing biomolecules (e.g., metalloproteins and metalloenzymes), and also has a significant role in the study of biological sulfur and selenium centers. Proteins of this kind constitute a large fraction of the proteome, perhaps 30%, and the heavy atoms found in proteins are frequently components of enzyme active sites, and are therefore of great functional importance. The technique provides information regarding the coordination number and geometry of metal centers, the identity of ligands, and provides accurate metal-ligand bond lengths. Because it does not require crystalline samples, X-ray absorption spectroscopy information can be obtained on any biological molecule that contains a heavy atom, and it is well-adapted to mechanistic studies because any reaction intermediate that forms in high abundance can be examined directly. X-ray absorption spectroscopy is complementary to macromolecular crystallography in that the distance information it provides can be used to refine the structure of metal centers. X-ray absorption spectroscopy also provides a means for verifying the oxidation state metals and assessing integrity of the metal site in crystals being used for crystallographic studies.

Current status. At present, there are five beam lines at SSRL dedicated to X-ray absorption spectroscopy, with three of them being effectively biology beam lines, and consequently SSRL is the best facility for performing most biological X-ray absorption spectroscopy experiments in the U.S. In addition, ALS has one soft X-ray absorption spectroscopy beam line (e.g., S K-edge spectroscopy), APS has a single beam line best utilized for brightness-limited X-ray absorption spectroscopy, and NSLS has 0.5 beam lines dedicated to biological X-ray absorption spectroscopy. There are in addition another three beam lines at NSLS where biological X-ray absorption spectroscopy experiments could be done, but because users would have to provide the detectors, cryostats, etc. required, they are not attractive to biological users.

Trends . While the demand for X-ray absorption spectroscopy beam time is not growing as fast as demand for crystallographic beam time, existing facilities are oversubscribed. This has two important consequences. First, it is difficult for new researchers to compete with established researchers for beam time. Second, it is difficult to get beam time for the development of new techniques. Both of these problems need to be addressed if X-ray absorption spectroscopy is going to reach its full potential as a tool

in structural biology. There is a clear need for new X-ray absorption spectroscopy beamlines.

Automation and remote data collection are not likely to have as great an impact on X-ray absorption spectroscopy as it will on crystallography. Since X-ray absorption spectroscopy experiments are done in many different ways, each requiring its own equipment and experimental protocol, it is unlikely that researchers will ever be able to submit samples in a standard format for standard data collection. Further, since the size of the X-ray absorption spectroscopy community is much smaller than the crystallographic community, the cost/benefit ratio for automation is not as favorable.

An important corollary of these facts is that regional facilities will continue to be far more important to the X-ray absorption spectroscopy community than they are to the crystallographic community. Someone interested in doing an X-ray absorption spectroscopy experiment is almost certainly going to have to do it in person, and it will be impossible to avoid the associated cost of travel. In this regard, it is noteworthy that 3 of 5.5 existing beam lines for biological X-ray absorption spectroscopy work are located in California. It is thus very important that existing facilities at NSLS be maintained for the benefit of researchers on the East Coast, and the tendency of X-ray absorption beam lines to be converted into crystallographic beam lines must be resisted. It has already led to less than optimum experimental conditions for X-ray absorption spectroscopy on beam line X9 at NSLS, for example.

New opportunities. Third generation synchrotron sources make it possible to do new experiments with X-ray absorption spectroscopy. These include spatially-resolved X-ray absorption spectroscopy measurements using microprobe techniques, and time-resolved measurements using stopped-flow and rapid-scan methods. It is anticipated that stopped-flow techniques will do for mechanistic metallobiochemistry what they did earlier for mechanistic inorganic chemistry; they will provide detailed insights into kinetics and reaction mechanisms. If this approach proves to be as powerful as it seems likely, there could be a large increase in the demand for X-ray absorption spectroscopy beam time. In addition, the minimum sample concentrations required for experimentation on third generation beam lines are much lower than on less brilliant sources, enabling experiments to be performed at close to biological concentrations. This both circumvents the aggregation that often accompanies high sample concentrations, and makes it possible to investigate the structural effects of aggregation.

Along with the brighter sources have come technical challenges. Despite advances in detector technology that have provided 30-element Ge detectors that saturate at count rates about 4 times greater than older detectors, X-ray absorption spectroscopy data collection is still detector-limited due to saturation. Even faster detectors are needed. A wavelength-resolving detector that may solve this problem is under development at BioCAT at APS. Additional development work on this and other possible solutions to the detector problem is urgently needed. In addition, it should be recognized that the higher the X-ray flux, the greater the problem posed by radiation damage. Research is needed into techniques that either prevent radiation damage or provide rapid data collection in order to minimize exposure time.

Small Angle Scattering.

Synchrotron radiation is used to investigate the X-ray scattering properties of biological samples that are less than fully crystalline. Although the number of scientists who do such experiments is much smaller than the number engaged in macromolecular crystallography, they do a much wider variety of experiments. Nevertheless, for the purposes of this report, their activities are categorized as small angle X-ray scattering/diffraction because most of the data sets they collect do not extend to scattering angles outside the range where $\sin\theta \approx \theta$, and there is usually a premium on observing scatter at very small scattering angles.

The samples studied by scattering techniques range from macromolecular solutions, which are totally disordered, to near-crystalline preparations of biological fibers. X-ray small angle scattering/diffraction experiments can produce useful, low resolution information about molecular size and shape, state of folding and aggregation, mesophase identification and characterization, membrane structure, membrane fusion, membrane protein crystallization, muscle structure and function, etc. X-ray small angle scattering/diffraction experiments can identify situations where macromolecular crystal structures are misleading because lattice interactions have stabilized non-native conformations, and to explore the response of biological systems to changes in temperature and ionic conditions under near- *in vivo* conditions, which often cannot be done crystallographically. They can also be used to characterize macromolecular aggregates and assemblies, even those that form transiently, and thus in combination with crystallography and spectroscopies of different kinds will contribute to our understanding of organisms as integrated molecular devices. X-ray scattering also has applications in the area of disease diagnosis and the characterization of disease conditions. For example, it is currently being used to study collagen in breast cancer, crystallins in cataract disease, and the plaques found in patients suffering from Alzheimer's, Parkinson's, and Creutzfeldt-Jakob diseases.

X-ray small angle scattering/diffraction does not compete with X-ray crystallography because it does not yield atomic resolution structures, but it does compete with X-ray crystallography for synchrotron access. X-ray small angle scattering/diffraction beam lines deliver highly collimated X-ray beams, and have low background radiation, especially at small scattering angles, a combination of properties that is very attractive to crystallographers interested in crystals with large unit cells. The temptation to turn X-ray small angle scattering/diffraction lines over to crystallographic users has been succumbed to in the past (e.g. at NSLS). It should be resisted.

Prior to the advent of synchrotrons, X-ray small angle scattering/diffraction experiments were done using laboratory equipment. Unfortunately, X-ray small angle scattering/diffraction laboratory equipment is expensive, temperamental, and often must be home-built. Furthermore, the fluxes that reach samples in such equipment are so low that data collection times are often discouragingly long. Thus, prior to the advent of synchrotrons, the cost of obtaining X-ray small angle scattering/diffraction information, measured either as time or dollars per bit of information obtained, was so high that it

could be justified in only a limited number of contexts. Not surprisingly, the number of scientists doing X-ray small angle scattering/diffraction experiments was very small.

Synchrotron X-ray sources are ideal for X-ray small angle scattering/diffraction studies both because they are orders of magnitude brighter than home sources, and because it is easy to extract from them the highly collimated beams of X-rays essential for X-ray small angle scattering/diffraction work. Routine X-ray small angle scattering/diffraction experiments can be executed on properly equipped synchrotron beam lines in a tiny fraction of the time it would take to do them on home equipment, and thus the time-cost of such data has fallen dramatically. It is almost certainly true today that the number of biologists whose programs could benefit from X-ray small angle scattering/diffraction data is much larger than the number who are collecting such data. Rather than provide each of these potential users with his/her own in-house X-ray small angle scattering/diffraction set up, it makes sense to service their needs centrally at synchrotron facilities, where high brilliance beams, state-of-the-art instrumentation, real-time data reduction, and expert assistance can all be laid on.

Current Status . X-ray small angle scattering/diffraction beam lines operate currently at APS (18ID), CHESS (D1), and SSRL (BL4-2). The beam lines at APS, CHESS, and SSRL devote >50 % of the time available to biological applications. ALS identifies beam line 12.3.1 as being a bio-X-ray small angle scattering/diffraction beam line, but we have no detailed information about it. The CAMD web site also refers to a SAX capability, but again details are lacking. Finally, the G1-line at CHESS is being developed as a D1 clone to serve the Cornell community, and it will be used for SAXS studies of biomaterials.

The literature shows that a significant amount of bio-X-ray small angle scattering/diffraction data has been collected using small angle scattering/diffraction beam lines that were not specifically designed for biological purposes. The A1 and F1 beam lines at CHESS and the 8-ID beam line at APS are examples. It would be interesting to know how extensive this activity is, and whether those beam lines are being used in preference to dedicated bio-X-ray small angle scattering/diffraction lines, or just because they are available.

Trends. X-ray small angle scattering/diffraction samples are as sensitive to radiation damage as macromolecular crystals, and from a practical point of view may be even more so because they often cannot be studied in the frozen state. So far radiation damage has been mitigated by flowing sample solutions continuously past the X-ray beam, and by continuous translation for fibrous and liquid crystalline samples. SSRL is developing a system for low temperature solution experiments, which may help. In addition the mechanisms of radiation damage are being investigated in hopes of finding ways of defeating them. Deoxygenation of samples and the use of additives are both being studied. Clearly it makes sense to support the development of smart/fast systems for controlling shutters/attenuators and sample translation devices so that sample exposure can be minimized during alignment and between exposures.

Eventually it may become possible to obtain significant amounts of structural information by analyzing the scattering produced when a sample is exposed to a single,

ultra-short (femtosecond), ultra-high intensity pulse of X-rays of the sort that free-electron lasers are expected to generate. Radiation damage should not be an issue for data collected this way because the data will have been generated before radiation damage has had time to become manifest. (Samples, of course, will be destroyed in the process.) It may even be possible to solve structures at high resolution using data of this kind obtained from single macromolecules (Neutze et al., Nature 406:752, 2000). (For a less optimistic view see R. Henderson, Nature 415:833, 2002.) Free electron laser sources are under development at SSRL and DESY (Germany), and so these concepts are likely to be tested before long. If those who question the validity such approaches are wrong, major new opportunities for X-ray small angle scattering/diffraction will open up.

A much less ambitious *ab initio* method for determining macromolecular structure is under development that combines crystallography and small angle X-ray scattering. Molecular replacement will be done using small angle X-ray scattering-derived, low-resolution models for macromolecules, which specify their size and shape. The low-resolution phases that emerge will then be the starting point for a process that extends the phases out to the limit of the diffraction data. The attraction of this approach, if it can be made to work, is that would eliminate the need to phase diffraction patterns experimentally.

Many of the most important applications of X-ray small angle scattering/diffraction involve the collection of data as a function of time from samples undergoing a change that has been initiated by the experimenter. Even at synchrotrons, flux can be limiting for such applications because flux determines how fine the time-slicing can be. The spread of X-ray wavelengths ($\Delta\lambda/\lambda$) in the synchrotron beams available is almost never limiting for X-ray small angle scattering/diffraction experiments under any circumstances, and therein lies an important opportunity. Accurate small angle data can be collected using beams with far wider wavelength spreads than can be tolerated crystallographically, and the broader the wavelength spread accepted by the optics of a synchrotron beam line, the higher its flux. A study done at SSRL, for example, showed a flux gain of about 10x when the bandpass at 10 keV was increased to 150 eV, with no significant loss in point-to-point resolution. It is important that support be provided for the development of wide-bandpass, multilayer monochromators for synchrotron beam lines.

Kinetics and mechanistic studies require that the process of interest be triggered rapidly. This can be done by abruptly changing pH, ionic conditions, ligand concentrations, temperature, pressure, light, magnetic/electric field strength, stress/strain, etc. Traditionally, users have supplied the specialized instrumentation needed for such studies. The X-ray small angle scattering/diffraction beam line of the future will come equipped with much of this instrumentation and with user-friendly software interfaces.

Interesting opportunities would be created if microfocus X-ray beams suitable for scattering experiments could be produced, possibly using zone plate technology. A microfocus SAXS beam line could be used to study of the nucleation phase of macromolecular crystal growth.

Operational Issues . Like their crystallographic colleagues the members of the X-ray small angle scattering/diffraction community confronts a number of problems that are primarily organizational in nature.

Standardization. The X-ray small angle scattering/diffraction community would benefit from standardization so that all SAXS beam lines operate the same way. It should not be forgotten that standardization and streamlining of the beam time application process would also have a beneficial impact.

Staffing. X-ray small angle scattering/diffraction beam lines, like crystallographic beam lines, are understaffed. Improvements in this area would certainly improve productivity. Again the reader is referred to the discussion of this topic in the crystallography section.

Automation. X-ray small angle scattering/diffraction does not lend itself to automation the way X-ray crystallography does. The processes, the time scales, scattering power/angle, data collection modes, etc., vary so much from one experimental system to the next that it is a rare when two consecutive user groups on a X-ray small angle scattering/diffraction line perform the same type of experiment using the same experimental setup. Not surprisingly, therefore, Fedex X-ray small angle scattering/diffraction experimentation is not being contemplated anywhere today. That said, it might make sense to organize beam lines so that X-ray small angle scattering/diffraction data collection could be remotely monitored and controlled. This would not eliminate the need to send people to synchrotrons, but it would reduce the number of people that have to be sent, and it would make it easier to make decisions about ongoing experiments.

With time, however, certain methods will probably emerge as being generally useful and a community of users will grow that wants to do such experiments on a routine basis. If that should begin to happen, opportunities for robotization may arise, and beam line scientist need to anticipate such eventualities.

The role of beam line scientists. Until X-ray small angle scattering/diffraction experiments become far more standardized than they are today, it will make sense for users to engage beam line scientist as a collaborator rather than service providers. (The difference between X-ray small angle scattering/diffraction users and crystallographic users in this regard is large.) Collaborative arrangements of this sort will ensure that the instrumentation, etc., brought to facilities is properly configured so that beam time is used with maximum efficiency.

Sustaining the field. As pointed out earlier, biological X-ray small angle scattering/diffraction is a small field, and as such, has always been in danger of going extinct as a result of accidental fluctuations in the number of experts willing to remain engaged in it. This problem will become acute as X-ray small angle scattering/diffraction experimentation becomes increasingly concentrated in a small number of facilities around the country. The staff of those facilities may ultimately become the only people in the country who really understand the nuts and bolts of such experiments. It is important, therefore, that qualified personnel be recruited to staff these facilities, and that career paths be developed for them where service earns rewards comparable to those normally associated with research and development.

Finally, there are many scientists around the country who could make good use of small angle X-ray scattering/diffraction data, and it is critical that efforts be made to reach out to them. They will not use the X-ray small angle scattering/diffraction beam lines at synchrotrons unless they are supported in every area, starting with instrumentation, sample manipulation and environment control systems, data collection, and finally data analysis.

Appendix

Table 1. Synchrotron Beam lines for Macromolecular Crystallography (from information assembled by Keith Hodgson and Eaton Lattman, November 2001)

Station	PRT	Use	Status*	% General User	Current Staff	Current Detector
ALS						
BL 5.0.2	Ind/Acad	Mad/Mono		40	4.33	2x2 CCD
BL 5.0.3	GNF.Syrrx	Mono		25	4.33	2x2 CCD
BL 5.0.1	Ind/Acad	Mono		40	4.33	2x2 CCD
BL 8.3.1	UCB/UCSF	MAD	D / C 10/01	25	Tbd	2x2 CCD or BP300
BL 8.2.1	HHMI	MAD	D / C 10/01	25	Tbd	2x2 CCD
BL 8.2.2	HHMI	MAD	D / C 11/01	25	Tbd	2x2 CCD
BL 4.2.2	MBC	MAD	D/C 1/02	25	Tbd	BP300
BL 12.2.2	SIBYLS	MAD	D/C 1/03	25	Tbd	3x3 CCD
APS						
5-ID-B	DND	MAD		25&	2.3	MAR IP
5-BM-B	DND	MAD	C	25	Tbd	Tbd
14-BM-C	BioCARS	Mono		100	5	2x2 CCD/IP
14-BM-D	BioCARS	MAD/Laue		100	5	2x2 CCD/IP
14-ID-B	BioCARS	Mono		100	5	2x2 CCD/IP
17-BM	IMCA	MAD/Mono		25	4.7	2x2 CCD
17-ID	IMCA	Mono		25	4.7	MAR CCD
19-BM	SBC	MAD		75	5.7	3x3 CCD
19-ID	SBC	MAD		75	5.7	3x3 CCD
22-BM	SER	MAD/SG	C	25	6.5	Tbd
22-ID	SER	MAD/SG	C	25	6.5	BP300
31-BM	SGX	MAD/SG	D	25	Tbd	Tbd
31-ID	SGX	MAD/SG	D	25	Tbd	Tbd
31-ID	SGX	MAD/SG	D	25	Tbd	Tbd
32-ID-B	ComCAT	MAD/Mono	C		Tbd	MAR CCD
Tbd-ID	NE	MAD	D	40	4 est	CCD tbd
Tbd-ID-side	NE	MAD/Mono	D	40	4 est	CCD tbd
Tbd-BM	NE	MAD/Mono	D	40	4 est	CCD tbd
Tbd-ID	NIGMS/CA	MAD/SG	D	50	4 est	Tbd
Tbd-ID	NIGMS/CA	MAD/SG	D	50	4 est	Tbd
Tbm-BM	NIGMS/CA	MAD/SG	D	50	4 est	Tbd
CAMD						
GCPCC	Acad. Consort.	MAD	C	25	2	CCD
CHESS						

A1		Mono		100	4	2x2 CCD/IP
F1		Mono		100	4	2x2 CCD/IP
F2		MAD/Mono		100	4	2x2 CCD/IP
NSLS						
X12B	BNL Bio	MAD		75	2.5	2x2 CCD
X12C		MAD		75	2.6	2x2 CCD
X25		MAD/Mono		37.5	2.2	3x3 CCD
X26C		Mono		25	1.8	2x2 CCD
XBC	LANL	MAD/SG		25	1.6	2x2 CCD
X4A	HHMI	MAD		25	n/a	2x2 CCD
X6		MAD	C	70	4	2x2 CCD
X9A	AE	MAD		60	2.5	CCD
X9B	AE	MAD (50%)		25	3.5	2x2 CCD
SSRL						
BL 1-5		MAD		100	3.6	2x2 CCD
BL 7-1		Mono		100	3.6	2x2 CCD
BL 9-1		Mono		100	3.6	MAR345 IP
BL 9-2		MAD/Mono		100	3.6	3x3 CCD
BL 11-1	TSR/SU	Mono		33	3.6	3x3 CCD
BL 11-3	Ind. Consort.	Mono/SG	C	25	Tbd	2x2 CCD

* C: under construction; D: under design; PRT: participating research team; Tbd: to be determined.
& Not all devoted to macromolecular crystallography