An Undulator Resource for Structural Biology

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Appendix A  Resource Advisory Committee Report
Appendix B: Sector Review Report and NE-CAT Response
Appendix C: NE-CAT Memorandum of Understanding
Appendix D: NE-CAT Safety Plan
Appendix E: P41 RR 015301-2006 Progress Report
Introduction to the revised application

The general assessment of the review panel that met in March 2006 was that while the proposal was backed by outstanding science, the application failed to show creativity, innovation and uniqueness in the proposed technological research and development program. The review panel also noted the apparent absence of true collaborative science that would drive Resource development. We have taken these criticisms to heart, and on the basis of the reviewer comments as outlined in the summary statement and on the basis of recommendations from the Resource Advisory Committee (Drs. John Chrasz, Ashley Deacon, Keith Hodgson and Janet Smith; Appendix A) we have redefined the core technologies to focus on (1) microdiffraction, (2) hardware for challenging samples and (3) computing for challenging samples, and we have explicitly defined the way which collaborative science will drive technology development.

Technological Cores:

The review panel expressed concerns that the Undulator Resource lacked the core competency to develop a large offset monochromator (previously Core Project 1). We accept this criticism, and we have deferred these plans until such time as the scientific need is fully justified, the Resource has acquired staff expertise in optics development, and the technical design details are sufficiently advanced to justify the investment.

The review panel was generally supportive of Core 2 (experiments with challenging samples). Resource strengths in beamline engineering approaches were noted along with the excellent properties of the APS. The major criticism was the lack of an innovative internal development program resulting from the involvement of the collaborative scientists. In the present application, we have recast this core to include all initiatives related to hardware development and adaptation (section D.1.2, p. 356). We have chosen collaborative scientists who will interact with Resource beam line staff to derive the development of new technologies. New and innovative approaches in the research plan include the use of a compound refractive lens to reduce horizontal emittance, automation of beam focus and automations of beam steering. Finally, we have initiated collaborations with Dr. Gerd Rosenbaum for white beam position monitoring and with Dr. Robert Thorne for development and implementation of new crystal freezing and mounting technologies.

The review panel expressed the greatest enthusiasm for microdiffraction (previously Core 3). We have retained microdiffraction as core technology (section D.1.1, p. 349), added new core collaborators, and included research plans to fully exploit the capabilities of microdiffraction.

The review panel was moderately enthusiastic about Core 4 (robotics for sample mounting and crystal screening), but it noted that our plans consisted largely of implementing off-the-shelf technology and as such lacked innovation. The panel also expressed concern that Resource collaborative scientists appeared uncommitted to robotic technology. In response, we have made a commitment to provide robotics capabilities for all Resource beam lines. We have identified core collaborators who will demand and drive this technology, and we have initiated a collaboration with Dr. Thomas Earnest through which we will implement the next generation of sample automounters. This initiative is now included as part of the new Core 2 (section D.1.2, p. 356).

The review panel was mildly enthusiastic about Core 5 (completion of station 24-BM). The panel felt that this core lacked innovation but that it would provide a valuable, cost effective resource. In response, we now view this core as infrastructure in support of various aspects of core technology, collaborative research and service. The relocation, refurbishment and build-out of beam line 24-BM will be completed using institutional funds, and it will be available for all aspects of Resource operations.
The review panel was enthusiastic about Core 6 (Crystallographic Computing) but, while noting the importance this core for the overall success of Resource, also expressed need for innovation. This core has been recast as the new Core 3 (section D.1.3, p. 384). It includes all aspects of beamline and crystallographic computing. The collaborative science projects have been redefined to provide a clear driving force for development. We have added to this core several new and innovative initiatives, and we have begun a collaboration with Drs. Wladek Minor and Zbyszek Otwinowski through which HKL3000 will serve as a platform for implementation.

Revision and consolidation of the previously proposed core technologies results in three new technological cores: Microdiffraction (Core 1), Hardware for Challenging Samples (Core 2) and Computing for Challenging Samples (Core 3). Stephen Harrison is the Core 1 Scientific Director and Kanagalaghatta Rajashankar is the Core 1 Technical Director. Wayne Hendrickson is the Core 2 Scientific Director and Malcolm Capel is the Core 2 Technical Director. Steven Ealick is the Core 3 Scientific Director and Igor Kourinov is the Core 3 Technical Director. The NE-CAT staff as, outlined in section E.4. p.478, will assist the core lead scientists in developing the technologies described in section D.1, p.349.

**Collaborative science:**

The review panel expressed substantial concerns about the apparent lack of connection between the Resource and the core collaborators, and how the needs of the core collaborators would drive the development of core technology. In the revised application, we have made an effort to define in detail the relationship between core technologies and collaborative science. Several general points are relevant to how we have redefined our collaborative science component.

(1) The NCRR-funded Undulator Resource was conceived by a group of scientists with a shared interest in developing synchrotron beam lines for challenging samples. The goals of the founding scientists provide the driving force for technology development. Their history of accomplishment in the implementation of new techniques and their record of innovative approaches to problem solving are an enabling force, which together with a talented staff of beamline developers and scientists creates a unique opportunity to advance the frontiers of structural biology. The founding scientists persuaded their home institutions to make a collaborative access team agreement and to commit matching funds, which together with NCRR funding allowed the initial build out of the resource beamlines.

(2) The core technologies presented in the revised applications were chosen to emphasize the ultimate mission of the undulator resource, namely to determine structures of important biological macromolecules, such as macromolecular assemblies and membrane proteins. Solving these problems often involves working with small crystals, large unit cells, and poor resolution of diffraction. Towards this goal we are determined to develop the best microdiffraction beamline in the country, to optimize all beamlines for difficult samples, and to develop software, scripts and computing environments to optimize both data collection and data analysis. We believe that the collaborative science we outline, which comprises a wide range of biologically important problems, will indeed drive the three core technologies, and we have explicitly cross referenced in tabular form the three cores and the collaborative projects that will push each core forward.

(3) In response to reviewer comments and in recognition of national mission of NCRR-funded research resources, the collaborative science component has expanded to include representatives from institutions outside of the founding group. These collaborative scientists were chosen using the same criteria that drove the conception of the research resource. Their projects and their relationships to the Undulator Resource are described in section D.2, p. 424.
Service:

The review panel raised concerns that the resource allocated a disproportionate amount of beamtime for scientists from the founding institutions, and as a result enthusiasm for the Resource as a national facility was diminished. We have therefore outlined a scheduling plan so that user beamtime will be allocated at 50% to scientists from NE-CAT institutions and 50% to the general user community. Procedures are now in place to ensure that beamtime within the NE-CAT institutions is allocated fairly. The general user beamtime will be allocated through the APS general user program (section D.3.1, p. 462).

Training:

The review panel was concerned about our commitment to training. In response, we have described in more detail the existing training activities and added new training initiatives. We note that all NE-CAT institutions have major graduate programs, and thus a substantial fraction of beamtime allocated to NE-CAT scientists will involve graduate-student training. At the level of user training more broadly defined, we will organize an annual workshop related to the Resource mission, modeled on the successful and well-attended workshop of microdiffraction we held in May 2006. Also in response to the reviewer comments, we have included a plan to address the special problems associated with inexperienced and non-specialist users, and a plan for user follow up to obtain a clearer understanding of the users’ experiences at the beamline.

Dissemination:

The review panel expressed concerns about how the Resource plans to disseminate technical expertise and innovation developed in the cores and in the collaborative research program. In response, we immediately added statistics gathering to our web site, and in the proposal we have outlined more aggressive strategies for dissemination. These include an electronic newsletter, publication of technical notes and a series of mini-symposia. The newsletter is intended to inform the scientific community of Resource developments and opportunities, and it will provide the focal point for establishing a Resource community of users.

Administration:

The review panel raised a number of concerns regarding Resource administration and management. To address these concerns we have implemented several organizational changes, and we have more clearly defined staff responsibilities, committee responsibilities and the relationship between NE-CAT and the Resource. To avoid potential conflicts of interest, Dr. Ealick has stepped down as the Chair of the NE-CAT Executive Committee and as the Cornell representative to the Executive Committee. Dr. Harrison is the new Executive Committee Chair, and Dr. Hao Wu is the new Cornell representative. Dr. Ealick remains NE-CAT Director, Resource Director, and Principal Investigator of the NCRR grant. Dr. Capel has been appointed Resource Deputy Director and NE-CAT Deputy Director. Drs. Capel, Harrison, and Hendrickson are named as co-Principal Investigators of the NCRR grant. Dr. Ealick is an ex officio member of both the NE-CAT Executive Committee and its Steering Committee (Drs. Harrison, Dr. Hendrickson and Dr. Darst). NCRR participation will be invited for all Resource and NE-CAT meetings.

The main advisory group to the Resource Director is the Resource Advisory Committee, whose members are listed in the first paragraph of this introduction. Dr. Ealick’s primary obligation is to the NCRR program, while Dr. Harrison and the Executive Committee are responsible to the NE-CAT institutions and scientists. To ensure continued cooperation between NE-CAT and the Resource, the chains of command, the responsibilities and the obligations have been clearly defined for each organization.
Rewriting:

The revised proposal has been extensively rewritten. The Specific Aims in Section A have been rewritten to reflect the new core technology organization. Section B (Significance) has been edited and contains new material in Section B.1 (p. 115). Sections B.2 - B.4 (pp. 117-122) contain background material from the previous application; section B.5 (p. 122) is new and describes the Sector 24 facilities. Section C (Progress Report) contains all of the material from the Progress Report of the previous application, and progress in technological R&D since October 2005 can be found in sections (C.3, p. 149; C.5, p. 259; C.7, p. 284; and C.9, p. 315). Progress on scientific accomplishments since October 2005 has been updated in section C.11, p. 335, and training and dissemination progress for that period is in section C.13, p. 343. Section D.1, p. 349, of Experimental Design and Methods has been completely rewritten to reflect the reorganization of the three core technologies. Section D.2, p. 424, has been completely rewritten to emphasize the relationship between core technology development and collaborative science. Sections D.3 - D.5, pp. 460-471, have been edited to reflect new commitments to service, training and dissemination. Section E (Resource Organizational Structure, p. 472) has been largely rewritten and expanded to reflect recent organizational changes and to define more clearly staff responsibilities and Resource operating procedures. The nomenclature for beamlines has been changed such that Phases I, II and III are now called beamlines 24-ID-C (undulator 1 end station), 24-ID-E (undulator 2 side station) and 24-BM (bending magnet end station), respectively.
A. Specific Aims

The overall goal of this proposal is to design, construct and operate synchrotron beamlines for technically challenging problems in structural biology. This will be accomplished using the unique undulator X-ray sources of the Advanced Photon Source (APS), Argonne National Laboratory. We have constructed a fully tunable undulator end station (24-ID-C) and a monochromatic undulator side station (24-ID-E) at Sector 24 of the APS. The design utilizes a canted undulator configuration in which two undulators in a single straight section are operated independently. In early 2007 we will relocate station 8-BM to Sector 24 (24-BM), to complete the initial build out of our experimental facilities. The completion of 24-BM will include several upgrades and will be fully tunable. The goal for the next grant period is to implement microdiffraction capabilities and to optimize all three stations for challenging samples through the development of beamline hardware and computing. We have identified collaborative research projects to drive the technological development and created strong links between the collaborators and the beamline staff. The user program will be expanded and all beamline technology will be made available to the general user community. We have defined new training initiatives to address the wide range of skill levels with the user community. Finally we have defined new proactive plans for dissemination of technology to the structural biology community. The specific aims for the next five years are as follows.

A.1. Microdiffraction (Technological Core 1)

Microdiffraction is an important capability currently lacking in the United States. We have defined a plan to develop the best microdiffraction beamline in the country. The instrumentation is based on the commercially available MD-2 microdiffractometer, which will be commissioned early in 2007. The MD-2 microdiffractometer will be implemented using Console, the Resource's command language environment for controlling beamline components. The plan includes a research program to study the basic properties of microcrystals and for optimizing data collection and processing. Four collaborative projects have been identified to drive the development of microdiffraction technology.

A.2. Hardware for Challenging Samples (Technological Core 2)

The goal of this core is to optimize beamlines for technically challenging samples, including data collection for small crystals, large unit cells and weakly diffracting samples. Investigators are often faced with important biological problems for which extending the resolution of diffraction by only a few tenths of an Angstrom may be critical. This requires the best possible beam focus, beam stability and signal-to-noise ratio. The technological R&D program addresses new approaches for improving beam stability and beam focus, and for lowering background. These include improvements in monochromator stability, beam position monitoring at the sample, upstream white beam position monitoring, undulator upgrades, implementation of bimorph mirrors, beam autofocusing and special beam stop and collimation needs. The core will also address how to improve data quality by attending to sample stability and efficient sample handling using automounters. Studies will be carried out to determine the optimal implementation of this technology. Three collaborative science projects have been identified to drive the development of new hardware technologies. In addition, we have initiated technical collaborations with Dr. Thomas Earnest to develop the next generation of sample automounters and with Dr. Robert Thorne to develop new methods for sample mounting and freezing.

A.3. Computing for Challenging Samples (Technological Core 3)

The goal of this core is to develop software, scripts and a computing environment to obtain the best possible data for challenging samples. The core includes the development of data collection strategies for difficult, radiation sensitive crystals and software for automatic crystal
recognition and centering. Software development includes beamline control software, programs for optimizing multiple crystal data collection strategies and analysis of non-merohedrally twinned crystals. The core will continue the development of Console, the integrated control system development system that allows rapid reconfiguration and automation of beamline environments. The core will also develop procedures and scripts to optimize and automate data processing for challenging samples and for implementing post data collection corrections such as radiation damage and absorption corrections. The core will develop automated procedures for identifying non-merohedral twinning and for obtaining the best possible integrated intensities from twinned crystals. To improve data collection and efficiency the core will implement structure determination packages, efficient computing hardware for data transfer, storage and backup and data base management for sample automounting. Four collaborative projects have been identified to drive the development of computing and software technology. In addition, we have established a technical collaboration with Drs. Wladek Minor and Zbyszek Otwinowski through which we will co-develop and test new data processing software for HKL3000. We have also established collaborations with Dr. Nicholas Sauter to develop optical and efficient data collection software and and Dr. Zheng-Qing Fu to implement automatic structure determination packages.

A.4. Collaborative Research

We have defined a collaborative science program (entirely new since the previous submission), in which the collaborating scientists require development of new technologies and in which these scientists are committed to working with the staff of the Resource to drive the relevant technical developments forward. Each collaborating scientist has a primary association with one of the three cores, but experiments on almost all technically challenging samples will benefit from some aspect of each of the core technologies. Collaborating scientists will be drawn from institutions both within the NE-CAT consortium and outside it. A table of showing the relationship between the core technologies and the collaborative research is given in section D.2. In addition to the core research collaborations we have initiated several key technical collaborations to assist us in core technology development.

A.5 Service

Beamtime for the service component of the Resource is divided evenly between general APS users and scientists from the Northeast Collaborative Access Team (NE-CAT). All technologies will be made available to both categories of users. Beamtime for the APS general users will be administered through the APS user program. Beamtime for the NE-CAT users is divided evenly among the seven NE-CAT institutions and each NE-CAT institution has developed a procedure for allocating beamtime. Future plans call for implementation of remote data collection using open source network collaboration tools.

A.6 Training

The resource will provide training in several contexts. (a) A major activity is on-site training of users, including general crystallographic instruction in addition to the necessary introduction to site-specific instrumentation and procedures. While some user groups are very experienced, both in data collection and in structure analysis, others require training in sample, handling, data collection, data processing, and structure determination. An hour or two of staff time is required in the former case, 6-8 hours or more, in the latter. This training mode is supplemented by instruction manuals, available on line, and web-based tutorials on both basic (from others) and advanced topics in crystallography (prepared by the NE-CAT staff). (b) We will design a web-based training program, which we will offer at four to six-month intervals. In this program, students will be able to log on and watch, or even control, experiments remotely. During a two-to four-hour session, data will be collected remotely and the structure determined. (c) We will offer an annual on-site workshop, covering some aspect of structural biology relevant to our mission.
A.7. Dissemination

Resource technology will be disseminated both proactively through the publication of technical notes and presentations at meetings, and passively through the Resource web site. The Resource typically presents a poster describing our facilities and developments 2-3 times per year at national meetings such as the annual meeting of the American Crystallographic Society, the Protein Society and the Biophysical Society. Collaborative scientists will also describe developments in research publications. We propose an electronic newsletter to keep the structural biology community abreast of our technological advances and research opportunities. The large number of graduate students and postdoctoral fellows involved in the research at all seven NE-CAT institutions will automatically enhance our training and dissemination programs, as these personnel move to establish their own laboratories at other institutions.

A.8. Time Table for the Research Plan

In this section, time lines are presented for many of the major tasks which are described throughout this proposal. A Gantt chart (Figure A.8.1) is presented at the end of this section summarizing graphically a number of the important time lines, particularly those relating to major acquisitions and installations of instrumentation. The time lines shown in this chart include the times allocated for procurements, deliveries, installations, and commissioning before the instrumentation is available for use. It should be emphasized that the actual times for all major installations will be dependent upon the opportune times for installations, e.g., periods of time needed that will not negatively impact on-going user programs, APS permitting access to the accelerator ring (installation of the undulator), etc.

A.8.1. Microdiffraction (Technological Core 1)

The MD2 diffractometer, very recently delivered to us in December of 2006 by ACCEL/Maatel, will be installed on the 24-ID-E beamline during late spring 2007 after bench testing and integration of the software delivered with the unit into the Console operating system. After a two month crystallographic commissioning period with beam, the microdiffractometer will be placed into full operation for core collaborators during the summer of 2007, before the new grant period actually begins.

With the availability of a fully operational microdiffractometer on 24-ID-E, the Core 1 technology research activities can begin immediately at the start of the research grant. As the demand for microdiffraction capabilities quickly grows during the first year, additional demand throughout the remainder of the grant period will be met with the introduction of microdiffraction capabilities on the second beamline, 24-ID-C. Upgrade of the 24-ID-C beamline to microdiffraction capability with installation of a higher precision goniometer and an on-axis visualizer will start mid-year of the first year of the grant and be completed by start of the second year.

A.8.2. Hardware for Challenging Samples (Technological Core 2)

The 24-ID-E beamline commissioning will be completed by late spring 2007 and the beamline immediately placed into full user operation before this grant period begins.

The procurement order for acquisition of the 3.0 cm undulator will be issued to APS at the beginning of the second year of the grant cycle. We anticipate that APS can deliver the undulator within six months and install it during the earliest available APS scheduled month long down period for accelerator maintenance, best estimated as September 2009.

The contract for purchase of the compound refractive lens will be issued at the beginning of the second year of the grant. Delivery, installation and commissioning will be completed before the end of the second year of the grant.
The contract for acquisition of the bi-morph mirror will be issued at the beginning of the third year of the grant. Delivery is expected within six months of award and installation will be completed within two months.

The time frame for implementation of the white-beam beam position monitor (BPM) depends strongly on APS’s schedule for design, fabrication, and testing of the first prototype. It is estimated that the white-beam BPM for NE-CAT will be available during the mid-point of the third year of the grant.

In regard to the sample placement robotic systems, the ALS robotic system currently being commissioned will be installed on the 24-ID-C beamline during late summer 2007, before the current grant begins. The second generation ALS robot will be developed in collaboration with Thomas Earnest and is anticipated to be available for installation and commissioning on 24-ID-E mid-point in the second year of the grant.

The move of the 8-BM beamline to 24-BM will begin during the late spring of 2007, before this grant period begins. We anticipate that the bending magnet beamline will be ready for full user operation during the summer of 2008, mid-way through year 1.

A.8.3. Computing for Challenging Samples (Technological Core 3)

Procurement of the computing cluster will be made early during the first year of the grant. Software development and implementation will continue throughout the term of the grant.

During the first and second year of the grant, effort will be directed to analysis of non-merohedrally twinned crystals. Starting at the beginning of the grant and continuing intensively for the first three years will be the development of crystal recognition software, automated data collection strategies, and optimum data collection strategies for multiple crystals.

The initial stand-alone version of WebIce will be installed and tested during the first year. During the second and third year, the software will be fully integrated with the Console beamline and detector control software. During the third and fourth years the software will be integrated with the sample mounting robots. During the mid-point of the grant period, implementation of remote operations will begin.

A.8.4. Collaborative Research

The collaborative research activities are expected to begin at the start of the grant period and continue to grow throughout the entire grant period.

A.8.5. Service

The Service activity, providing high-quality beamtime to the users, will begin at the start of the grant period with both insertion-device beamlines, 24-ID-C and E, in full user operation. The available service capacity will be further increased at the mid-point of the first year of the grant with the 24-BM beamline coming into full operation.

A.8.6. Training

During the first few years of the grant, the web-based instructional manuals will be further updated in detail. Development of the web-based tutorials and “screencasts” training sessions will take place over a period of the first few years of the grant.
A.8.7. Dissemination

Dissemination of information from NE-CAT to the broad-based crystallographic community is an important ongoing activity throughout the entire grant period. Dissemination of information through publications and presentations at meetings will increase dramatically as we progress from building to operating beamlines and as more and more of the trainees (postdoctoral fellows and graduate students) who have used the Resource move on to other positions. Early in the first year of the grant, NE-CAT will begin issuing electronic news letters to the crystallographic community.

Figure A.8.1: Gantt chart showing the time lines for major acquisitions and installations of instrumentation.
B. Significance

B.1. Background and Introduction

As an introduction to this application for renewal of funding for the NE-CAT Research Resource, we pose three questions. Where does structural biology stand at the end of 2006? In what direction is it headed? How will NE-CAT and the Resource it operates contribute to fundamental discovery in structural biology during the coming five years?

Where does structural biology stand? We understand the basic principles of protein, DNA, and RNA structure. Atomic models are available for members of most of the important classes of proteins, several kinds of RNA, and a modest set of larger assemblies. A few of these assemblies -- the ribosome is a particularly striking example, simple viruses, another -- are relatively well-defined “molecular machines”, with more or less fixed composition and a set of structural and functional states. Picturing those multiple states is nonetheless a major challenge. Even in protein synthesis, initiation and elongation factors that enter and leave at specific steps are a large part of the story: a full molecular movie of translation will probably take at least another decade even to outline, much less to complete with kinetic as well as structural parameters. But in most cases, the situation is even more complicated, because the relevant molecular machine is neither so abundant nor so well defined. One must turn to recombinant expression and biochemical dissection, “groping in the dark” to some extent when tackling a new problem, because genetic analyses and interaction networks rarely give more than a hint of the underlying structural framework.

The modularity of proteins -- mixing and matching of domains, both in evolutionary time and in the laboratory -- turns out to be one of their essential characteristics. Modularity simplifies analysis to the extent that we can dissect proteins into component domains and study the latter independently, but a number of examples illustrate that how the domains interact within a multi-domain protein or between one protein and another cannot readily be modeled from domain structures alone. Indeed, the folded structures of interacting domains often depend on their association with each other. Moreover, the essence of intracellular regulation turns out to be the timed assembly and disassembly of transient complexes. Thus, knowing the folded structures of the individual domains of a protein -- a task that may be further simplified by results of projects in structural genomics -- is usually little more than a beginning. The job of a structural biologist eager to solve problems such as the transduction of signals when a cytokine binds its receptor or the mechanism of the mitotic-spindle assembly checkpoint or the sequence of events during DNA recombination is to figure out how to put together relevant pieces of the various participating proteins into complexes that represent the stages of a multi-step process, snapshots from which dynamics can be deduced or (from suitable experiments) interpolated.

With this perspective, where are we headed? First, it is clear that structure is more, rather than less, important for sorting out problems such as the three just mentioned. The new “systems biology” may contribute powerful advances by developing computational methods to sort out relevant interactions in signaling and regulation, but mechanistic understanding and rational molecular intervention will still require three-dimensional pictures. Second, no problem is solved with a single structure. Formation of complex protein assemblies is invariably a multi-stage process. If our goal is to acquire mechanistic insight, then a large series of structures will be needed. As an illustration, consider the large number of structures required simply to work out the way hydrolysis of GTP by a small GTPase is coupled to a GAP, a GEF, and various effectors (1). Third, various structural techniques must be integrated. Our relatively advanced understanding of how myosin moves on actin has required a combination of structural approaches, including crystal structures of myosin and actin in various states, cryoEM reconstructions of actin filaments decorated with myosin under various conditions, single-molecule analysis of actin filaments moving under the impetus of single myosin motors, and small-angle diffraction from intact muscle (2). Detailed analysis of polypeptide-chain initiation
and elongation on ribosomes will likewise continue to need crystallography and electron microscopy, augmented by single-ribosome biophysical measurements and classical mechanistic enzymology (3-5). For less regular structures, cryoelectron tomography will be needed as well.

The emphasis in the preceding paragraphs has been on assemblies with multiple states and potentially variable composition, but we should not neglect the importance of very high resolution studies of single proteins (or smaller complexes) nor should we overlook the continuing challenge of individual membrane proteins. A superb example of the value of very high resolution is the discovery of a light atom at the center of the MoFe complex in nitrogenase, through analysis of the 200 kDa FeMo protein heterotetramer at 1.16 Å resolution (6). Similarly, a full understanding of potassium selectivity and transport rate in K-channels required a 2.0 Å resolution structure, ultimately made possible by co-crystallization of the bacterial KcsA channel with monoclonal Fabs (7). Moreover, the difficulty and significance of a problem is not always proportional to the size of the unit cell. Membrane proteins may crystallize with “ordinary” unit-cell dimensions, but if the crystals remain quite tiny and if the diffraction extends only a bit beyond 4 Å, the experimental setup needed to achieve adequate signal-to-noise in the 3.5 to 4 Å “water ring” region will be very similar to the one required for recording diffraction from crystals of large assemblies.

How will NE-CAT and the NE-CAT Research Resource contribute to solving the kinds of problems just posed? Our mission is indeed to provide a facility adapted, both in design and operation, to the most demanding and most complex diffraction problems and organized to allow operation to be driven by the requirements of the users. The research programs of many of the principal users at the seven member institutions and of the named outside collaborators illustrate the need for a facility with these characteristics. The crystallography of viruses and ribosomes are obvious examples, but the demands of the structural biology of transient signaling complexes and of membrane proteins may well be greater, because it will almost always be necessary to work with limiting amounts of protein and potentially unstable assemblies. For example, Hao Wu (Cornell) studies signaling downstream from TNF and its relatives (8). The pathway, which bifurcates into a survival signal under some circumstances and a death signal under others, involves assembly of various intermediate complexes that activate a multi-subunit kinase in the survival branch and a caspase cascade in the death branch. Wu's goals include understanding assemblies of increasing complexity and size, for which the combination of high collimation and high energy resolution (for accurate anomalous signal measurments) at NE-CAT beamlines will be important. Similarly, Nikola Pavletich (Sloan-Kettering) studies the regulation of the cell cycle, particularly as it becomes altered in cancer cells. The major underlying processes of this regulation are protein phosphorylation, ubiquitin-dependent proteolysis, and transcription. The cell-cycle transcription program is controlled by the E25-DP family of transcription factors and the retinoplastoma (Rb) family of proteins. Pavletich's work on a variety of complexes containing proteins that participate in this regulatory network has helped to unravel a complex control mechanism, a paradigm for many other cellular control circuits. As these circuits become more elaborate, so do the protein complexes that embody them, and the hardware developments at NE-CAT beamlines will clearly be necessary for the next stages of research on these questions.

The research programs of principal users also illustrate the link between the applications of NE-CAT and other techniques in contemporary structural biology. Studies of virus structure were among the most noteworthy early successes in applying synchrotron radiation to structural biology (9, 10), and they also led the way (together with studies of muscle proteins) in illustrating how best to combine moderate-resolution information from cryoEM with high-resolution structures from X-ray crystallography (11-14). Now that single-particle analysis in cryoEM can produce images at subnanometer resolution, it is possible to recognize protein secondary structure and hence dock high-resolution models with considerable accuracy (15-17). There is, in effect, a continuum of information available from the two approaches, and the next five years will surely see dramatic synergy. For example, high-resolution diffraction has been obtained
from crystals of ribosomal subunits derived from only a few, special sources (T. thermophilus; H. marismortui) and in only a restricted number of states (4). Electron microscopy, at last approaching truly useful resolutions, is starting to fill in some of the gaps, and the crystallographic work from the Yale group will certainly provide one important high-resolution anchor for these efforts.

The link between structures of molecular machines in defined states and their underlying dynamics, both in vitro and in the context of a living cell, will be provided in the coming years by increasingly powerful optical methods. Two examples from NE-CAT investigators and their collaborators illustrate this assertion. The first example is a study of how the base-excision DNA repair enzyme, human oxoguanine DNA glycosylase 1 (hOgg1) locates the rare 8-oxoguanine lesions that it will excise (18). A collaboration between the laboratories of Verdine and Xie (Harvard) has shown that this enzyme, for which elegant structural studies have outlined the excision mechanism (19, 20), diffuses along DNA by an essentially barrierless Brownian sliding. Thus, hOgg1 locates lesion bases by a massively redundant search, in which it ultimately binds 8-oxoguanine (as shown by the crystallography) under kinetic control. While this is a relatively simple example, the experiments clearly presage application of similar methods to problems in replication and transcription and represent the advent of single-molecule enzymology more generally. The second example is an analysis of clathrin coated-vesicle dynamics in living cells. The trajectory of nucleation, growth, budding, and rapid disassembly of a clathrin coat has been followed for individual coated pits in living cells (21) and can be integrated with structural information about the molecular organization of clathrin and associated proteins in the assembly (17). Some of the next stages of this integration, combining crystallographic studies of proteins that direct or modulate coated-pit assembly, cryoEM analysis of their locations and interactions, and live-cell imaging to work out mechanisms, are described in Harrison’s research program (Section D.3.5).

Molecular animations, such as those hinted at here, of all the principal activities of a cell will ultimately derive from resources like NE-CAT. We believe that developments in the areas represented by the three cores are among the most important technical advances needed to realize the X-ray crystallographic component of our vision for structural biology. The requirements of work on structures as diverse as ribosomes, RNA polymerases, transient subcellular assemblies, and membrane proteins will ultimately determine how best to develop: (1) microdiffraction; (2) beamline hardware necessary for optimal recording of data from very small crystals, poorly diffracting crystals, and crystals with large unit cells, as well as for obtaining the highest possible resolution from well-ordered crystals; (3) computing adapted to the same needs. The proposal that follows outlines a Research Resource suitable for solving the kinds of problems described in this introduction and for disseminating the technology for those solutions to the broader structural biology community.

B.2. Description of the APS

The Advanced Photon Source (APS) is a 3rd generation synchrotron source designed to optimize X-ray brilliance. Planning for the APS began in 1984 and the project was fully funded by the Department of Energy in 1990. Five years later the APS produced its first X-rays. Since 1995, 26 CATs have signed memorandums of understanding with the APS to develop one or more of the 34 available sectors. Currently, two sectors remain unassigned.

The Advanced Photon Source (APS) at Argonne National Laboratory is a synchrotron-radiation light source funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences (Figure B.2.1). The APS provides insertion device- (ID) and bending magnet- (BM) based synchrotron X-ray radiation for use in forefront scientific and technological research. Synchrotron radiation is produced by the precise, controlled motion of a low-emittance, high-energy particle beam that is generated and stored by the accelerator system. X-ray beams produced by IDs and BMs are transported via beamlines to research stations, where materials under study are illuminated. The users of the APS, as members of Collaborative Access Teams
(CATs), finance, manage, and operate their beamlines. Other users not affiliated with CATs gain access to beamtime as independent investigators.

The beam acceleration and storage process begins at the electron gun, a cathode-ray tube much like one in a television, which emits electrons that exit the gun at 100 keV. Under typical operations, 30 nS long pulses of electrons are raised to an energy of 200 MeV at 48 pulses per second by a series of accelerating structures in the linear accelerator, or linac.

Ten to 12 pulses of 450-MeV electrons are injected as one group into the booster synchrotron, a 368-m-long, racetrack-shaped ring of electromagnets that raise electron energies at a rate of 32 keV per turn. The accelerating force is supplied by electrical fields within radio-frequency (rf) cavities operating at 352 MHz, the same frequency used by the storage ring (SR) rf cavities. In 0.25 sec, electrons orbit the booster 200,000 times as their energy climbs to 7 GeV — approximately the speed of light.

The electrons are then injected into the 1,104-m-circumference SR. This circular chain of 1,097 electromagnets contained within a concrete shielding enclosure lies on the inner perimeter of the experiment hall, an annular structure with an exterior radius of 191.4 m, an inner radius of 164.6 m, and a height of 9.8 m. The particle beam is steered and focused by the electromagnets as it circulates within a closed system of 240 aluminum-alloy vacuum chambers running through the magnet centers. Design vacuum inside these chambers is $10^{-9}$ Torr with beam present. The beam decelerates at a rate of about 6 MeV per turn as it emits synchrotron radiation. This energy loss is replaced by the 352-MHz rf cavities in the storage ring.

Electrons were used for early accelerator commissioning activities. On July 7, 1996, operations with positrons began and carried through the final day of the accelerator run period that ended.

**Figure B.2.1:** Overview of the Advanced Photon Source. (Credit: Argonne National Laboratory).
on September 28, 1998. At that time, the APS switched back to electrons, partly in support of a new operating mode known as “top-up.”

In normal operations, the storage ring is filled to a current of 125 mA and then the orbiting beam is allowed to decay to approximately 80 mA in a 24-hour period, at which point the beamline shutters are closed (interrupting delivery of beam to the users) while the storage ring is re-filled to 100 mA. In top-up, the beam current is not allowed to decay, but instead is maintained at 100 mA through frequent (every 190 s) injections while the shutters stay open. Top-up maintains a constant level of current in the storage ring, constant heat load on X-ray optics and storage ring components, constant power demands on rf generators, and sends a constant signal strength to beam-position monitors.

A typical APS storage ring sector comprises two dipole magnets, ten quadrupole magnets, seven sextupole magnets, eight corrector magnets, nine rf beam-position monitors, and six extruded aluminum vacuum chambers and associated vacuum pumping equipment, all mounted on girders, five per sector (Figure B.2.2). The girders are aligned in the SR tunnel to a tolerance of ± 0.1 mm. The SR magnets are configured to provide 40 periods, with 40 corresponding sectors. Five of the sectors are taken up with beam-injection and rf equipment. The remaining 35 are equipped to provide ID and BM radiation. This arrangement, the Chasman-Green lattice, is named for its designers, the late Renate Chasman and G. Kenneth Green of Brookhaven National Laboratory. Their ideas on the periodic arrangement of the bending and focusing magnets in a storage ring produced a particle beam of very small size and low angular divergence, beam qualities that are highly prized by users of synchrotron light sources.

The APS magnet lattice and high energy combine to provide a small X-ray source size with low angular divergence (emittance). These characteristics allow for the operation of undulators, periodic magnetic devices that cause the positrons to undergo a series of transverse oscillations. Because of the low emittance and the periodic spacing of magnets, X-ray beams from the individual undulator poles combine to produce a harmonic spectrum. The harmonic peaks of the undulator spectrum are the source of the high X-ray brilliance. By changing the undulator gap, the harmonics can be tuned to cover the entire X-ray spectrum of interest to crystallographers.
Because the APS undulator radiation is both more intense and more highly collimated than the X-ray beams of 1st or 2nd generation sources, it is possible to consider new and challenging crystallography experiments. The high brilliance beams are advantageous for small, weakly diffracting samples, very large unit cells and other experiments limited by signal to noise.

B.3. Structural Biology at the APS

Altogether, APS has 34 sectors available for beamline development (Figure B.3.1). The main focus for eight of these sectors is macromolecular crystallography. BioCARS-CAT, IMCA-CAT, SBC-CAT, SER-CAT, SGX-CAT and NE-CAT have all declared at least one beamline operational and most are developing additional beamlines for structural biology. GMCA-CAT has finished commissioning phase and LS-CAT has one operational beamline and one under construction. In addition, a small portion of DND-CAT is devoted to biological crystallography and BIO-CAT focuses on non-single crystal biological studies. Taken together, these CATs will support about 20 beamlines for crystallography. It is unlikely that any additional biological CATs will be approved in the foreseeable future.

As the APS approaches full occupancy, it appears that only about 25-30% of the facility will be available for structural biology. Although the APS is the only high energy 3rd generation synchrotron source in the United States -- and the only such machine likely to be built in the foreseeable future -- surprisingly little effort has been invested in technically challenging experiments. Several CATs, such as SBC, IMCA, SER-CAT and the GMCA-CAT, will focus primarily on high throughput structure determination. These CATs will provide data collection facilities for a wide variety of structural studies including MAD phasing, high resolution refinements, small, weakly diffracting crystals and large unit cells. BioCARS-CAT is unique in that Laue protein crystallography for time resolved studies is a major research thrust. The goal of NE-CAT is to develop facilities that will address the problems associated with the most technically challenging experiments. These facilities will be used for a wide variety of structural studies that cannot be studied at second generation sources or, in many cases, other beamlines at the APS. We have developed a plan to optimize undulator radiation for...
crystallographic studies that will be complementary to other existing and proposed sector developments. We will develop new techniques and instrumentation driven by the need to solve technically challenging crystal structures. Thus, the NE-CAT sector will not only expand capacity for macromolecular crystallography but will also extend capabilities.

B.4. NE-CAT

The Northeastern Collaborative Access Team (NE-CAT) is a consortium of seven institutions: Columbia University, Cornell University, Harvard University, Massachusetts Institute of Technology, Memorial Sloan-Kettering Cancer Center, Rockefeller University and Yale University. The active participants comprise about 75 principal investigators at those seven institutions, including a group of established investigators who have been primarily responsible for organizing this proposal plus a group of younger investigators, some of whom have already made major contributions. The 75 research groups extend to include several hundred graduate students and postdoctoral research fellows who are training for careers in the biological sciences. The participants in NE-CAT share a common interest in research at the frontiers of structural biology and share a common need for access to 3rd generation synchrotron sources.

Authorization to develop sectors at the APS is granted to Collaborative Access Teams (CATs). Beginning almost ten years ago, a group of crystallographers from universities in the northeast set out to plan the development of an APS sector for structural biology. This core group included Stephen K. Burley, Rockefeller University; Jon Clardy, Cornell University; Steven E. Ealick, Cornell University, Stephen C. Harrison; Harvard University; Wayne A. Hendrickson, Columbia University; James M. Hogle, Harvard University; John Kuriyan, Rockefeller University; Nikola P. Pavletich, Memorial Sloan-Kettering Cancer Center; Paul B. Sigler, Yale University; Tom Steitz, Yale University and Don C. Wiley, Harvard University. This group, which also makes up the 11 core collaborators of the original NIH/NCRR proposal, submitted a proposal to the APS in July of 1998. After a presentation to the Proposal Evaluation Board in September, 1998, NE-CAT was given approval by the APS to develop a sector for structural biology.

In the summer of 2001, Cornell University was awarded an NIH grant to develop an APS sector and on May 3, 2002, NE-CAT, APS and NIH signed a memorandum of understanding, allowing construction to begin at Sector 24. The experimental facilities of NE-CAT are operated under an agreement approved by the APS, the NIH and the home institutions of the core collaborators. The management plan is described in section E of this proposal and includes an Executive Committee consisting of one member from each participating institution. The current Executive Committee members are Seth Darst, Rockefeller University; Stephen C. Harrison, Harvard University (Chair); Wayne A. Hendrickson, Columbia University; Nikola P. Pavletich, Memorial Sloan-Kettering Cancer Center; Robert Sauer, Massachusetts Institute of Technology; Thomas A. Steitz, Yale University; and Hao Wu, Cornell University. The NE-CAT Director, Stephen E. Ealick is an ex officio member.

A number of changes have taken place since NE-CAT was first conceived. Sadly, two core members, Paul Sigler and Don Wiley, died unexpectedly. In addition several senior investigators have taken new positions outside of the seven NE-CAT institutions. But as the result of new hires, the original 24 research groups have grown to 75, and NE-CAT is loaded with young, talented scientists pursuing exciting new research projects, to complement the core of senior investigators.

In addition to the Executive Committee Members, NE-CAT participants include Qing Fan, John Hunt, Lawrence Shapiro, Liang Tong and Ming Zhou at Columbia University; Olga Boudker, Richard Cerione, Brian Crane, Along Ke, Min Lu, and Holger Sondermann at Cornell University; Stephen Blacklow, Lewis C. Cantley, Bing Chen, Jon Clardy, Philip Dormitzer, Michael Eck, Barbara Furie, Bruce Furie, Rachelle Gaudet, James Hogle, David Jeruzalmi, Daniel Kahne, Robert Kingston, Keith Miller, Anjana Rao, Tom Rapoport, Steven Shoelson, Piotr Sliz, Timothy Springer, Greg Verdine, Suzanne Walker, and Jia-huai Wang at Harvard University; Tania Baker,
David Bartel, Catherine Drennan, Barbara Imperiali, Amy Keating, Stephen Lippard, Paul Matsudaira, Alexander Rich, Thomas Schwartz, JoAnne Stubbe and Michael Yaffe at Massachusetts Institute of Technology; Jonathan Goldberg, Christopher Lima, Dimitar Nikolov and Dinshaw Patel at Memorial-Sloan-Kettering Cancer Center; Günter Blobel, Roderick MacKinnon, Charles Rice, Thomas Sakmar and Erec Stebbins at Rockefeller University; and João Cabral, Pietro de Camilli, Ya Ha, Elias Lolis, Yorgo Modis, Peter B. Moore, Thomas Pollard, Anne Marie Pyle, Karen Reinisch, Scott Strobel, Yufeng Zhou and Yong Xiong at Yale University.

The scientific program of the NE-CAT research community focuses on applications of X-ray crystallography to a variety of biological systems, including a large number that relate to the causes and treatments of human diseases. Particular emphasis is placed on signal transduction, DNA transcription initiation and regulation, cell cycle regulation, virus structure and function, membrane proteins, protein folding and enzyme structure and function. Much of the research focuses on how biological macromolecules interact to form large macromolecular complexes. NE-CAT research will affect a variety of biomedical areas including cancer biology, immunology and virology as well as the basic disciplines of biochemistry, cell biology, molecular biology and biophysics. The results produced using NE-CAT facilities will be relevant to human health care, pharmaceutical development, biotechnology and other areas of economic as well as scientific importance.

NE-CAT investigators have a strong history of technological innovation, even though none of them has methodology development as the major focus of activity. It is the demands of challenging biological crystallography that drives technology here, and investigators at NE-CAT schools have led by example. Methods and protocols introduced by them have been adopted widely. Examples abound: structure refinement as now practiced was introduced by Hendrickson (Columbia) and Brünger (Harvard, Yale); molecular averaging methods were first put into practice by Harrison (Harvard) and Wiley (Harvard); cryopreservation methods took over like wild fire based on the example of applications in laboratories at Harvard and Yale following the introduction of cryoloops (Teng, Cornell); the Yale system of home-source X-ray optics was broadly emulated; MAD phasing and selenomethionyl proteins were developed by Hendrickson (Columbia); diffraction methods for very large unit cells came from the demands of viruses (Harrison) and ribosomes (Steitz, Yale); a model for the interplay of synchrotrons with membrane-protein crystallization came from MacKinnon (Rockefeller); and world-leading synchrotron beamlines were developed with leadership from Moffat (Cornell) and Ealick (Cornell) for CHESS A1 and F1, Hendrickson for NSLS X4A, and Sigler (Yale) for APS 19-ID.

The scientists of the NE-CAT are well funded through government agencies such as the National Institutes of Health and private organizations such as the Howard Hughes Medical Institute.

B.5. Available Facilities

NE-CAT’s beamline optical plan exploits the APS Tandem-Offset Undulator (TOU) located at Sector 24. The TOU provides two white X-ray beams offset from each other by 1.0 mrad. Sector 24, like all other APS sectors, also has a dipole (bending) magnet beamline port. NE-CAT will construct and operate at least two undulator and one bending magnet crystallography beamlines. The 3 NE-CAT beamlines have very different optical trains, but use a common control system, end station and detector system. Figure B.5.1 provides a floor plan for the sector and elevation views of each beamline.

The first phase of construction (24-ID-C) uses the outboard facing undulator beam (originating from the upstream undulator). The 24-ID-C beamline is tunable using a Kohzu cryo-cooled double crystal silicon monochromator, from 5 – 23 KeV with spectral bandpass of approximately 2 x 10-4, and a focus spot size of 20 µm x 60 µm (vertical x horizontal) full-width.
The second phase of construction (24-ID-E) uses the inboard-facing (downstream) undulator beam. 24-ID-E’s monochromator is a single-crystal horizontally-deflecting system. Two silicon crystals (220 and 311 cuts) are incorporated in the monochromator, selected by a vertical translation of the crystals, to provide monochromatic beams of 12.662 or 14.785 KeV at a common takeoff angle. The focus spot size of the 24-ID-E beamline is very similar to that of 24-ID-C.

The Sector 24 bending magnet beamline (24-BM-B) will be constructed from optical and end station components presently installed at sector 8’s dipole beamline. 24-BM-B will provide monochromatic beam over a spectral range of 6 to 16 KeV, with a focus spot of dimension roughly 100 x 100 µm².

NE-CAT owns 3 Area Detector Systems Corp. Q315 detectors, supported by LR Design A-frame detector supports. The standard NE-CAT end station consists of a configurable collimator assembly incorporating 2 sets of x-y slit arrays a quadrant diode beam position monitor, multiple attenuator assemblies and a fast shutter. All beamlines currently use Huber goniometers with x,y,z sample alignment head and one or more high magnification video microscopes. NE-CAT has an inventory of 4 Oxford Cryosystems liquid nitrogen boil-off cryojet sample coolers and a Joule-Thompson chilled air sample cooler for data collection between -30 and +30 °C. Each end station has an X-ray fluorescence and flux counting trains. NE-CAT has recently acquired a Mattel MD2 microdiffraction station, with an extremely high precision goniometer and an inline-concentric sample visualizer capable of imaging micron-size samples.

Each beamline end station has at least four workstations for display and analysis of diffraction data. All workstations can access a Hewlet Packard EVA 5000 fiber channel raid system, using a proprietary shared-concurrent file system. The EVA 5000 has 30 Terabytes of disc space that can be extended to 75 Terabytes. A 32-node linux-based compute cluster will be installed to provide bulk and parallel computational capacity for the NE-CAT beamlines.

The Sector 24 Laboratory Office Module (LOM) provides six enclosed offices, a conference room and commons area, further partitioned into office modules used by staff and outside users. The office space is equipped with copiers, a variety of large and small format ink-jet and laserjet printers and APS-supplied wireless and copper Ethernet systems.

Two laboratories are present in the LOM. One laboratory is used by NE-CAT staff for instrumentation construction, commissioning and maintenance activities. The other laboratory space is configured as a wet lab and is used by NE-CAT staff and outside users. The wet lab has a large, walk-in cold room, two large incubators, an ultra-pure, filtered water deionizer, a liquid chromatograph for protein purification, centrifuge, UV-visible spectrophotometer, a light scattering photometer, and liquid nitrogen sample storage systems.
Figure B.5.1: Plan view of Sector 24 (top) and elevation views of individual beamlines. 24-ID-D is a possible fourth beamline sharing upstream undulator beam with 24-ID-E.
C. Progress Report

C.1. Introduction (from previous application)

The main goal for the first five-year funding period was to design and construct three stations for macromolecular crystallography. This goal will be accomplished before the end of year 05 with two undulator stations at Sector 24 (24-ID-C and 24-ID-E) and a bending magnet station at Sector 8 (8-BM). The construction schedule slipped by about a year, mostly because of APS delays in developing the tandem undulator front end. We also experienced problems with the 8-BM optics, which were designed and installed by Oxford Instruments. Other minor delays occurred in the design and procurement of major optical components for Sector 24 and in the installation of shielding and utilities at Sector 24. In year 02 we began commissioning 8-BM and began accepting users in year 03. The user program has grown each year and we anticipate the highest productivity in year 05. Nearly 50 papers have been published from data collected at 8-BM with many more submitted or in preparation. This is an impressive record for an APS bending magnet station. We have now formally declared 8-BM to be operational and have begun taking general users. The 24-ID-C end station was designed, constructed and commissioned, and is now in early stage operations. Problems identified during commissioning have been resolved and a number of structures have already been solved using 24-ID-C data. The 24-ID-E side station has been designed and all major beamline components procured. Construction will be completed in the next six months and commissioning will be completed by the end of year 05. The following sections provide a detailed progress report. Sections C.2 and C.3 describe progress at Sector 8, sections C.4, C.5, C.6 and C.7 describe progress at Sector 24 (24-ID-C and 24-ID-E), sections C.8 and C.9 describe support facilities including robotics (section C.8.3) and sections C.10 and 11 describe core and collaborative science based on data measured at 8-BM and 24-ID, and sections C.12 and C.13 describe progress in training and dissemination.

C.2. Technological Core Developments: 8-BM/24-BM

The NE-CAT bending magnet beamline, 8-BM, at the Advanced Photon Source (APS) was the first of four (three in this funding period) NE-CAT beamlines to be built. 8-BM was originally authorized by the Whitehead Institute and contracted to Oxford Instruments for design and construction. NE-CAT assumed full responsibility for the beamline when MIT joined NE-CAT in 2002. At this time most of the beamline components had been installed. However, Oxford Instruments failed to meet the contractual specifications for flux delivery and was declared in default. Consequently, NE-CAT had to identify the existing deficiencies and correct them, which lead to a lengthy commissioning period. Initial beamline commissioning took place in the last half of 2002 and in 2003 and 2004 NE-CAT initiated early user operations. In October of 2004, 8-BM was declared operational and opened to general users of the APS. During 8-BM commissioning a large number of technical problems were identified. These problems have either been solved or solutions have been proposed and scheduled for implementation. In the long term, we plan to relocate 8-BM to 24-BM. The 8-BM commissioning process and user program are described in detail below.

C.2.1. Description of 8-BM

C.2.1.1. Optical Layout of 8-BM

Figure C.2.1.1 shows the basic optical layout of 8-BM. M1 is a vertically oriented, collimating mirror, used to maximize the flux per unit bandwidth of the double crystal monochromator (DCM). The double crystal monochromator is tunable over an energy range of 6.5 keV to 14 keV and an energy resolution of 2-3 eV. The second crystal of the DCM is sagittally bent to focus the beam in the horizontal direction. M2 is a mechanically bent vertical focusing mirror. The first mirror is located at 24.9 m from the source, the monochromator at 27.3 m, the vertical focusing mirror at 29.5 m and the sample is at 49.7 m. The primary objective in the design of the beamline was to
minimize the divergence of the beam. Thus the optics are positioned to provide a demagnification ratio close to 1:1.

Figure C.2.1.1: Basic 8-BM optical layout.

The overall technical specifications of the beamline are summarized in Table C.2.1.1.

Figure C.2.1.2 is a photograph of the experimental end station of 8-BM showing: 1) Huber Kappa goniostat, 2) ADSC 315 CCD detector supported by an 3) LR-Design (Mesa, Az). A-frame detector manipulator and support. The sample to detector distance can be varied from 160 – 1100 mm. The detector support incorporates a vertical lift that allows placement of the point of impact with the direct beam anywhere within the vertical span of the detector.

Table C.2.1: Technical Specifications of the 8-BM Beamline as of 9/1/2005.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beam Source</td>
<td>Bending magnet</td>
</tr>
<tr>
<td>Energy Range:</td>
<td>6.5 – 14 keV</td>
</tr>
<tr>
<td>Accessible Absorption Edges</td>
<td>Mn K (6.539 keV) to Br K (13.474 keV)</td>
</tr>
<tr>
<td>Energy Resolution</td>
<td>2 – 3 eV</td>
</tr>
<tr>
<td>Collimator Aperture</td>
<td>0.2 x 0.2 mm</td>
</tr>
<tr>
<td>Detector</td>
<td>Q315</td>
</tr>
<tr>
<td>Sample Detector Distance Range</td>
<td>160 – 1100 mm</td>
</tr>
</tbody>
</table>
Figure C.2.1.2. A view of the inside of the experimental end station of 8-BM. The Kappa goniostat, Quantum 315 detector and the A-frame detector support (yellow), can be seen.

C.2.1.2. Operations Staff

Beamline 8-BM and the Sector 24 beamlines are staffed by a group of five crystallographers. Dr. Craig Ogata is NE-CAT Associate Director for Operations and is assisted by staff scientists Dr. Igor Kourinov, Dr. Narayanasami Sukumar, Dr. Jun Wang and Dr. K. (Raj) Rajashankar. Biosketches for the operations group are provided at the beginning of this grant application.

C.2.1.3. User Area and Computing Oag

Two user areas are provided at 8-BM. One is adjacent to the experimental hutch. It encompasses all of the computing facilities that are available to the users during the data collection. A second area is located in the associated Sector 8 Laboratory Office Module (LOM) and can be used by users that need additional time for data archiving or data processing, or as a rest area for the current experimenters.

Experimenters have access to four dual processor AMD Athlon workstations (1.5 – 1.7 GHz) each with 2-4 GB of memory. All workstations are equipped with dual screen monitors. One workstation is equipped with a fast, high-resolution CRT monitor to support stereo graphics visualization. Shared, NFS-distributed storage is accessible by all workstations. In the summer of 2005, an existing 2 TB Raidtec fiber channel storage system that was configured for Global File System (GFS) was replaced by a 4 TB NFS mounted RAID array. GFS promised fast, concurrent access to the Raidtec system, however consistent performance of the GFS system was never achieved.

A common suite of crystallographic data reduction and analytical software is available on all workstations in the Sector 8 and Sector 24 user areas. The programs cover all aspects from data reduction to structure solution. The suite of programs includes HKL2000, LABELIT, CCP4/CCP4i, SOLVE/RESOLVE, ARP/WARP, SHELX-97, PHENIX, RAVE, O and COOT. We are
reviewing the various available “data to structure” software packages (discussed in more detail in Section D.2.6.).

User workstations are attached to a duplex 1 Gb network. Data backup and transport is effected by use of inexpensive, high density portable discs, DVD+/-RW recorders, and network file transfer. USB2 (480 Mbps) drives formatted with either ext2 or ext3 file systems are our preferred transport/backup medium. DHCP access for laptops on up to 4 ports are available at the beamline. A view of the user control area is shown in Figure C.2.1.3.

![Figure C.2.1.3](image)

**Figure C.2.1.3:** Left: the view looking toward the experimental enclosure. Right: the experimenter’s view of the computers.

C.2.1.4. 8-BM Control Software

The data collection system at 8-BM uses a combination of the Area Detector Systems Corporation Q315 control system called ADSC and Console, a scriptable, distributed beamline control system developed by M. Capel. A description of Console is presented in Section C.3.9. The ADSC control system has been modified to interact with Console in a manner such that the complexity of beamline operations (e.g. energy changes, monochromator optimization, etc.) is shielded from the user. Direct user interaction with the Console Graphical User Interface (GUI) is limited to EXAF scans, manual energy changes, and manual beam flux optimizations.

Users interact with the ADSC Graphical User Interface to program data collection. The ADSC program then interacts transparently with Console through client-server transactions to sequence detector, goniometer and shutter motions that occur during a data collection sequence. Screen captures of the interfaces of the ADSC and Console GUIs are shown in Figure C.2.1.4. The Double Crystal Monochromator Console script (right side of C.2.1.4) runs continuously during data collection. In single wavelength mode, the experimenter interacts predominantly with the ADSC window, simplifying the interaction of the programs with the user. The left side of Figure C.2.1.4 shows five component windows of the ADSC control system. From left to right the windows are “Manual Mode”, “Snapshot” for taking single pictures for evaluation, “Runs” for data collection, ADX for selecting the windows and “Status”.

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C.2.2. 8-BM Commissioning

Upon completion of installation of the beamline and development of basic control system scripting, Oxford Instruments and NE-CAT staff carried out studies to assess its optical performance (in April of 2002). The first macromolecular crystallographic data was collected in early April of the same year. Steven Ealick’s group was able to collect data on three MAD projects: arginine decarboxylase, a glutaminase domain, and an AMP nucleosidase. Two of the structures, the arginine decarboxylase and the glutaminase domain, were solved form data collected on this trip, which gave proof that all major optical and end station components of the beamline were more or less functional.

Over the next eight months, five other visits were scheduled. Two were from the Ealick group, one from Catherine Drennan’s group at MIT, and two were commercial companies (RibX and Exxon). In all cases, useful data were collected.

In this section the focus will be on the main changes that have been implemented from December of 2002 to September of 2005 as part of the crystallographic commissioning of the beamline. This overlaps slightly with the opening of the beamline to APS general users in October of 2004. Steady improvement has been made with further upgrades and commissioning scheduled for the near future.

Oxford Instruments completed 8-BM installation with an initial characterization of the beamline. The emphasis was primarily on flux measurements at the sample position and possible problems in the design of the monochromator crystal. Clearly absent in their report was any attempt to demonstrate the stability of the optics. As repeated throughout the optics characterization, there were high demands placed on stability due to the 1:1 demagnification of the optical plan. The vertically focusing mirror and the sagitally focusing second crystal are placed over 20 meters from the sample position. Any vibrations causing angular displacements of the mirror of five microradians conceivably produce a 100 μm displacement of the vertical beam position at the goniometer. Two factors compensated for some of the stability problems. One was the relatively large beam size, 500 μm in the horizontal by 400 in the vertical. The second was that the experimenters were encouraged to use a large collimator size (300 μm x 300 μm).

In 2003 a larger user load was imposed on the 8-BM beamline. Users tended to prefer collimator apertures of order 200 μm x 200 μm. However, under conditions of reduced aperture,
beam stability problems were exacerbated, complicating automatic energy changes. Requests for even smaller slit apertures (100 µm x 100 µm) or placement of the beam focal point at the detector position resulted in significant loss of flux density at the spindle.

One problem contributing to beam stability was an undersized engagement spring that secured the second monochromator crystal to its pitch and roll platform drives. The 8-BM DCM (interior shown in Figure C.2.2.1) was one of the first Oxford monochromators to incorporate a sagittal crystal based on the ESRF design. The ESRF bender assembly is significantly heavier than prior Oxford monochromator second crystal assemblies. Beyond a certain Bragg angle, the engagement spring was not strong enough to insure contact with the piezoelectric actuators that drive the second crystal’s pitch and roll platforms (Figure C.2.2.2). In the early summer of 2003, the spring was replaced and beam stability was demonstrably improved. Numerous small mechanical and software problems of a similar magnitude were encountered and solved during this first intensive round of beamline use.

**Figure C.2.2.1:** A view toward the source showing the second crystal assembly and the water cooled first crystal.

**Figure C.2.2.2:** The arrow and finger point to the undersized spring that contributed to the instability of the monochromator.
In spite of our ability to mitigate hardware and software problems encountered during commissioning, the flux density and total flux delivered at the goniometer spindle were lower than levels predicted by ray tracing calculations performed by Oxford Instruments as part of their design effort (data not shown). A systematic survey of the alignment of the beam from the monochromator to the sample position revealed a small but significant misalignment of the monochromatic beam. Once corrected, the reliability of fully automated energy setpoint changes improved.

Successful rectification of commissioning problems encouraged us to officially open the beamline to APS general users in the fall of 2004. The problems with the alignment of the beam and the dissatisfaction with the larger than expected beam size have led to a re-examination of the optical components in the beamline (see section C.2.5.1). Recently we have realigned the beam from the source to sample and resolved a reliability problem with the yaw motion of the second crystal of the monochromator. The results have resulted in at least a 25% increase in flux through the 200 µm aperture and an increase in the reliability of the energy changes. The aperture size is now routinely set 200 µm x 200 µm during all data collection at beamline 8-BM.

C.2.3. **Crystallographic Commissioning**

Beamline operations for NE-CAT started officially in Oct. 2004. At this time we declared the bend magnet beamline, 8-BM, open to APS general users. The crystallographic commissioning mode preceded the official opening. Commissioning started in April of 2002 with the visit from Professor Steven Ealick’s group. Ealick’s group was able to collect several MAD data sets. The first structure solved from that visit was pyruvoyl-dependent arginine decarboxylase from *Methanococcus jannaschii*. For convenience we will define the start of beamline operations as the beginning of the calendar year 2003. A few months prior to this date, the first of the local staff crystallographers were hired. At the current time there are five full time staff crystallographers and a programmer that constitute the operations group.

An on-line database program developed and used at BIO-CARS (authored by K. Brister) has been adopted for use at NE-CAT. The program, CARPS, handles all of the mechanics of beamtime request submission and tracking, as well as safety approval documentation on 8-BM. The CARPS database system will be expanded for use on all of the NE-CAT beamlines. All users of the 8-BM facility have been using this system since the fall of 2004.

Operational time assigned to users has slowly but steadily increased from approximately 28 percent in 2003 to 50 percent of available beamtime in 2005 (1/1 – 8/25/2005). The number of publications has also increased from 8 publications in 2003 to 15 publications to date in the first 8 months of 2005. Operations will soon expand to Sector 24 as the first of the NE-CAT insertion device beamlines becomes available.

C.2.4. **User Program**

By definition, the start of the NE-CAT 8-BM User Program at 8-BM is early 2003, a compromise between commissioning and the official declaration of operationality of 8-BM to the APS General Users Program. It can be assumed that the early experiments were more commissioning than data collection. In 2002 there were six visits to the beamline from three independent investigators. Expectedly, Steven Ealick’s group bore the brunt of early commissioning efforts. The other three investigators in the first year were Cathy Drennan, MIT, RibX (a startup pharmaceutical company) and Raynor Kolb from Exxon. Structures were successfully solved by both the Ealick and Drennan groups. RibX has been a regular visitor to the beamline through commissioning, to the present. RibX uses structure determination of 50S ribosome-complex studies as a basis for drug design and discovery with a view towards commercialization. Thus, RibX is a proprietary user and therefore reimburses the DOE for beamtime.
C.2.4.1. User Visits in 2003

The early success of the beamline meant that all major components were working and that data of good quality could be obtained. In early 2003, we planned to balance an increasing load of users with local efforts to continue development of the beamline. The beamline was not completely optimized and refined. The defining slits were typically set to 0.3 x 0.3 mm to offset beam stability problems.

After halting user operations to deal with a variety of control software issues and the monochromator problems discussed in section C.2.2, the user program resumed in mid summer and continued to the end of the year. In August, preparations were made for the use of the staff crystallographers (Jun Wang, Igor Kourinov and Narayanasami Sukumar) to host users. Both beam and beamline support were now in quasi commissioning mode. By the end of the year 2003, the beamline had hosted 31 separate data collection trips by 22 independent investigators. Approximately 58.5 days out of the total of 211 days or 28% of the total time was used for experiments. There were eight publications on structures solved with data collected on the NE-CAT beamline 8-BM (Table 2.4.1)

Table 2.4.1: Structures That Were Solved at 8-BM During Commissioning.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Author</th>
<th>Journal</th>
</tr>
</thead>
<tbody>
<tr>
<td>tRNA-70S ribosome complex</td>
<td>Schmeing … Steitz</td>
<td>RNA</td>
</tr>
<tr>
<td>Semaphorin-3A Recept. Bind. Mod</td>
<td>Antipenko…Nikolov</td>
<td>Neuron</td>
</tr>
<tr>
<td>NgR</td>
<td>Barton …Nikolov</td>
<td>EMBO J.</td>
</tr>
<tr>
<td>PPC Synthetase</td>
<td>Manoj…Ealick</td>
<td>Structure</td>
</tr>
<tr>
<td>ThiO</td>
<td>Settembre … Ealick</td>
<td>Biochemistry</td>
</tr>
<tr>
<td>PPC Decarboxylase</td>
<td>Manoj…Ealick</td>
<td>Acta Cryst. D</td>
</tr>
<tr>
<td>Pyruvyl-dep. Arg Decarboxylase</td>
<td>Tolbert…Ealick</td>
<td>Structure</td>
</tr>
</tbody>
</table>

Table 2.4.2 shows a cross section of structures solved in the Ealick lab during their trips to the beamline in the time period from April 2002 to December 2003. As can be seen in the table, the number of amino acids in the asymmetric unit (aa in asu) goes from 51 for insulin to 1,452 for AMN. In the case of insulin, the sulfur atoms were used for phasing. The signal size of 1.4 % is similar to that of crambin. In the case of AMN, the resolution of the data is marginal at 3.94 Å and the predicted signal size is relatively small. The facts that these problems could be resolved were positive indicators of the quality of the data.
Table 2.4.2: Additional Structures That Were Solved at 8-BM During Commissioning.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Res. (Å)</th>
<th>Progress</th>
<th>aa in asu</th>
<th>Method</th>
<th># of scat</th>
<th>Signal Size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ThiO -SeMet</td>
<td>2.3</td>
<td>Published in Biochemistry</td>
<td>780</td>
<td>SAD</td>
<td>24 Se</td>
<td>7.3</td>
</tr>
<tr>
<td>ArgDC -SeMet</td>
<td>2.11</td>
<td>Published in Structure</td>
<td>957</td>
<td>SAD</td>
<td>30 Se</td>
<td>7.1</td>
</tr>
<tr>
<td>CoaB -SeMet</td>
<td>2.9</td>
<td>Published in Structure</td>
<td>620</td>
<td>SAD</td>
<td>10 Se</td>
<td>5.1</td>
</tr>
<tr>
<td>YaaE -SeMet</td>
<td>2.5</td>
<td>Published in J. Biol. Chem.</td>
<td>392</td>
<td>MAD</td>
<td>10 Se</td>
<td>6.4</td>
</tr>
<tr>
<td>Insulin -native</td>
<td>1.7</td>
<td>Published in Acta Cryst. D.</td>
<td>51</td>
<td>SAD</td>
<td>6 S</td>
<td>1.4</td>
</tr>
<tr>
<td>PTD -mercury</td>
<td>3.0</td>
<td>for phasing only</td>
<td>501</td>
<td>MIR+SAD</td>
<td>3 Hg</td>
<td>6.3</td>
</tr>
<tr>
<td>TenI -SeMet</td>
<td>2.1</td>
<td>Published in Biochemistry</td>
<td>820</td>
<td>SAD</td>
<td>20 Se</td>
<td>6.3</td>
</tr>
<tr>
<td>TMSAMDC -S63A</td>
<td>2.9</td>
<td>Published in J. Biol. Chem.</td>
<td>260</td>
<td>MAD</td>
<td>2 Se</td>
<td>3.5</td>
</tr>
<tr>
<td>ThiSG -SeMet</td>
<td>3.4</td>
<td>Published in Biochemistry</td>
<td>624</td>
<td>SAD/MR</td>
<td>14 Se</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>native</td>
<td></td>
<td>624</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMN -formycin</td>
<td>3.94</td>
<td>Published in Structure</td>
<td>1452</td>
<td>MR/SAD</td>
<td>9 Se</td>
<td>3.2</td>
</tr>
<tr>
<td>kinase -SeMet</td>
<td>3.2</td>
<td>Published in Structure</td>
<td>638</td>
<td>SAD</td>
<td>10 Se</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>-SeMet/AI</td>
<td></td>
<td>638</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The year 2003 ended with a number of users. Progress had been made toward the regular use of a 0.2 x 0.2 mm collimator aperture size. The second major improvement effected in this period was the implementation of fully automatic MAD data collection. Prior to this time, communication problems between the ADSC program and Console had prevented the option from being used. After fully automated energy transitions became possible the probability of success of an unattended energy change was directly proportional to the size of the energy shift.

Due to improvements in beamline reliability and function, the decision was made to open up 8-BM to general users in the fall of 2004. The declaration of participation in the APS General User Program (GUP) meant that up to 25% of available beamtime was given over to the APS GUP for scheduling of outside users.

We launched our online NE-CAT User Database (www.NECAT.org) to handle requisitions for beamtime. All users to the beamlines have entered the system. Early in 2004, there were thirteen member groups and eight non-NE-CAT members that were registered in the database.

C.2.4.2. User Visits in 2004

In the calendar year 2004, 8-BM hosted 35 visits from 21 independent investigators accounting for 32% of the total beamtime. Staff members served as instructors in two crystallographic schools: the ACA 2004 Workshop on “MAD/SAD data collection, processing, phasing and structure solution” and the ACA Summer School in “Macromolecular Crystallography”. Three structural genomics initiatives, the New York Structural Genomics Initiative (NYSGXRC), the Northeast Structural Genomics consortium, and the Joint Center for Structural Genomics
(JCSG), and RibX Pharmaceuticals, a structure based drug design company concentrating on the 50S ribosome, were part of the user pool.

The beamtime use from this and the previous year resulted in 17 publications, Table 2.4.3. The publications ranged from radiation damage studies (Wang, J. and Ealick, S.E. (22) to the biologically significant protein-conducting channel, SecY (van den Berg, B. et al. (23)) that appeared on the January 1 cover issue of Nature. There were four PDB deposits from the three genomics initiatives.

**Table 2.4.3:** Structures Solved at 8-BM in 2004.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Author</th>
<th>Journal</th>
</tr>
</thead>
<tbody>
<tr>
<td>SecY</td>
<td>Van den Berg…Rapoport</td>
<td>Nature, 427, 36</td>
</tr>
<tr>
<td>Bacterial Fatty Acid Transporter Fadl</td>
<td>Van den Berg…Rapoport</td>
<td>Science, 304, 1506</td>
</tr>
<tr>
<td>Bnlp FH2 domain</td>
<td>Xu…Eck</td>
<td>Cell, 116, 711</td>
</tr>
<tr>
<td>YaaE</td>
<td>Bauer…Ealick</td>
<td>JBC, 279, 2704</td>
</tr>
<tr>
<td>AMP nucleosidase</td>
<td>Zhang…Ealick</td>
<td>Structure, 12, 1383</td>
</tr>
<tr>
<td>Purine 2’-deoxyribosyltransferase</td>
<td>Anand …Ealick</td>
<td>Biochem., 43, 2384</td>
</tr>
<tr>
<td>Formylglycinamide ribonucleotide amidotransferase</td>
<td>Anand…Ealick</td>
<td>Biochem., 43, 10343</td>
</tr>
<tr>
<td>Thiazole Synthase</td>
<td>Settembre…Ealick</td>
<td>Biochem., 43, 11647</td>
</tr>
<tr>
<td>S-adenosylmet. Decarboxylase</td>
<td>Toms…Ealick</td>
<td>JBC, 279, 33837</td>
</tr>
<tr>
<td>Aminomidazole Riboside Kinase</td>
<td>Zhang…Ealick</td>
<td>Structure, 12, 1809</td>
</tr>
<tr>
<td>Lysine 5,6-Aminomutase</td>
<td>Berkovitch…Drennan</td>
<td>PNAS, 101, 15870</td>
</tr>
<tr>
<td>Cytochrome C &amp; Cytochrome C Peroxidase</td>
<td>Kang…Crane</td>
<td>JACS, 126, 10836</td>
</tr>
<tr>
<td>tRNA nucleotidyltransferase</td>
<td>Xiong…Steitz</td>
<td>Nature, 430, 640</td>
</tr>
<tr>
<td>Bacterial nucleoside transporter</td>
<td>Ye &amp; Van Den Berg</td>
<td>EMBO J., 23, 3187</td>
</tr>
<tr>
<td>Protein primed DNA polymerase</td>
<td>Kamtekar …Steitz</td>
<td>Mol. Cell, 16, 609</td>
</tr>
<tr>
<td>SspB Adapter/AAA+ ClpXP Protease</td>
<td>Bolon … Sauer</td>
<td>Mol. Cell, 16, 343</td>
</tr>
</tbody>
</table>

C.2 4.3. User Visits in 2005

For the first eight months of the year, there have been 36 visits to the beamline from 24 independent investigators and one crystallographic school. The beamline has been used for 68.5 days or 50% of the total available time. An additional 25.5 days (19% of the time) has been used for repairs and upgrades. Five of the groups have come from the APS General User Program.

User groups from this and previous years have published 15 papers to date. Table C.2.4.4 contains the publications for this year. One of the more interesting structures was that of the Group I ribozyme-product complex published early in the year. Golden and co-workers solved the structure of the ribozyme in complex with a four-nucleotide product RNA. The structure, solved at 3.6 Å resolution, allowed the authors to identify the potential binding sites for the catalytic metals. Figure C.2.4.1 is a ribbon model of the structure.
Table C.2.4.4: Structures Solved at 8-BM from 1/1 – 9/15/05.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Author</th>
<th>Journal</th>
</tr>
</thead>
<tbody>
<tr>
<td>α/β serine hydrolase</td>
<td>Arnt…Wilson</td>
<td>Proteins</td>
</tr>
<tr>
<td>Uridine Phosphorylase</td>
<td>Bu…Ealick</td>
<td>Acta Cryst. D</td>
</tr>
<tr>
<td>Group I ribozyme (crystallization)</td>
<td>Chase…Golden</td>
<td>Acta Cryst. F</td>
</tr>
<tr>
<td>Ubiquitin Hydrolase</td>
<td>Misagh…Gaudet</td>
<td>JBC</td>
</tr>
<tr>
<td>Amicyanin</td>
<td>Sukumar…Davidson</td>
<td>Acta Cryst. D</td>
</tr>
<tr>
<td>Permutated Arc repressor</td>
<td>Tabtiang…Sauer</td>
<td>PNAS (2005)</td>
</tr>
<tr>
<td>TenA and Tenl</td>
<td>Toms…Ealick</td>
<td>Biochemistry</td>
</tr>
<tr>
<td>Adenosine Kinase (crystallization)</td>
<td>Wang…Li</td>
<td>Acta Cryst. F</td>
</tr>
<tr>
<td>14-3-3</td>
<td>Wilker…Yaffe</td>
<td>JBC</td>
</tr>
<tr>
<td>3-hydroxyanthranilate-3,4-dioxygenase</td>
<td>Zhang…Wang</td>
<td>JBC</td>
</tr>
<tr>
<td>Purine Nucleoside Phosphorylase Complex</td>
<td>Zhang…Wang</td>
<td>JBC</td>
</tr>
<tr>
<td>HppE</td>
<td>Higgins…Drennan</td>
<td>Nature</td>
</tr>
</tbody>
</table>

Figure C.2.4.1: Ribbon diagram of the 3.6 Å structure of the group I ribozyme-product complex (24).

C.2.5. 8-BM Optics Upgrades

Although 8-BM is a relatively new beamline, we are investigating ways to improve the performance and reliability of the beamline optics. We desire to improve beam positional stability, energy tunability, obtain a smaller beam size, and improve the flux. In order to achieve these goals, we have instituted an exhaustive investigation of the causes of the present instabilities and inefficiencies in the optics of the 8-BM beamline.
C.2.5.1. Planned improvements for 8-BM Optics

At 8-BM the source to sample distance is approximately 50 meters. This large optical leg between the monochromator and the goniometer amplifies positional instabilities or manufacturing deficiencies of the focusing optics. The optical plan of 8-BM cannot be radically altered but the demagnification problem will be remedied when the optics are reinstalled at Sector 24, where the horizontal demagnification ratio will be a more optimal 2.6 : 1

Characterization of the optics started at the entrance of the beam into our first optical enclosure (FOE) and followed through to the end station. We systematically determined the alignments of all optical elements, including the slits, collimating and focusing mirrors and the monochromator. Small but significant mis-settings for the collimating pitch angle were discovered. An apparent problem with the second crystal yaw rotation stage was discovered to result from mechanical binding of the driving lead screw for the axis and was readily fixed. Likewise, mis-settings of the water-cooled slits upstream of the monochromator were uncovered and corrected. The most important impact of these efforts was that the white beam was completely centered on the first crystal and that the monochromatic beam was projected down to the end station on a trajectory much closer to the optical plan than prior to these studies.

Figure C.2.5.1 shows a beam image at the sample position at 12.658 keV after centering of the white beam on the first crystal and optimizing with the yaw motion (left) as well as the beam image before centering (right). A comparison of the two images clearly shows a qualitative improvement in the focus of the beam. For a quantitative comparison, a new beam profile measurement was carried out using the one dimension slit scan along horizontal and vertical directions. Figure C.2.5.2 shows the horizontal and vertical measured beam profile, respectively, at 12.658 keV. The FWHM of the current horizontal focus is narrowed down to 0.33 mm from 0.5 mm. The FWHM of the vertical focusing has not changed. In preliminary tests, we found that the vertically focusing mirror needs to be optimized. Further studies on the vertical focusing will be a top priority. Photon flux measurements through both 0.3 x 0.3 mm$^2$ slits and 0.2 x 0.2 mm$^2$ slits during top-up mode with a ring current of 102 mA and a 1 mrad horizontal beam acceptance, are shown in Figure C.2.5.3. The 0.3 mm or 0.2 mm beam size at the sample position is estimated by visualizing the beam image on a phosphor paddle making it difficult to compare the absolute flux values measured at different times. Despite this difficulty, it is apparent that 0.2 mm is much less than the beam focus size. The difference of photon flux through a 0.2 mm x 0.2 mm aperture before and after the improvements is much larger than the error of the estimated beam image size defined by slits. Unfortunately we did not measure the photon flux through the 0.2 mm x 0.2 mm slits prior to the improvements. In fact, one of the problems that prevented a systematic comparison of this measurement was that the beam was not stable through the 0.2 mm aperture during energy changes. A direct comparison over the entire energy range is not available. Comparisons based on the flux values recorded in the 8-BM beamline notebook, indicate an increase by at least 20%-25%. Figure C.2.5.4 is the beam image at ~30m

**Figure C.2.5.1:** Beam image at sample position at 12.658 keV before (left) and after (right) beam was centered and yaw motion was fixed.
downstream after the beam has been centered and the yaw motion repaired. It clearly shows that 1 mrad of radiation is accepted (by grids on the fluorescence panel) and that the beam is centered to the beam path.

**Figure C.2.5.2:** Beam profile after the beam was centered in the horizontal and yaw motion fixed.

**Figure C.2.5.3:** Measured flux after being focused in the horizontal and yaw motion fixed.

C.2.5.2. Monochromator Redesign

The NE-CAT beamline 8-BM consists of a collimating mirror and a double crystal monochromator, followed by a vertically focusing mirror. The heart of the optical train is the monochromator. It consists of an internal (direct) water-cooled first crystal and a second sagittally bent second crystal. The second crystal is based on a flexure hinge bender design that was originated from Andreas Freund’s group at the ESRF.

Initial commissioning of the beamline revealed problems with the design of the cooling system on the first crystal. Studies with a transparent top revealed that bubbles or vapor could prevent an evenly distributed cooling of the crystal. A Finite Element Analysis (FEA) of the first crystal predicted that improper cooling and a pressure differential could produce deformities. Despite these possible problems, the monochromator has been used for experiments since the spring of 2002. Recent quantitative characterizations of the focusing of the beam revealed that the
optimum focal size has never been achieved. Given the source parameters, the measured specs of the optical components, and the placement of the optics, the expected focal sizes are 0.2 mm in the horizontal and 0.28 mm in the vertical. After improvements in the alignment of the beam and fixing the range of motion of yaw, the current focal size is 0.33 mm horizontally and 0.4 mm in the vertical direction. This discrepancy between calculated and measured focus size has forced us to take a closer look at possible improvements to the crystals in the monochromator.

C.2.5.2.1. Monochromator First Crystal

The design of the standard Oxford Danfysik directly cooled first crystal is referred to as a “top hat” design. The diffracting surface of the first crystal is a box that is the “hat” that fits over the internal water jets. Figure C.2.5.5 shows the bottom half of the design that includes the jets that guide the water to the bottom surface of the diffracting silicon crystal.

Studies with a transparent top hat simulating the silicon crystal revealed that a hydrostatic pressure gradient across the cooling jets, caused by the Bragg inclination, degraded the uniformity of cooling of first silicon crystal. In effect at useful Bragg angle settings only the lowermost row of jets actually contributed to cooling flow, resulting in the likelihood of non-uniform crystal cooling.

A second and more significant problem with the existing first crystal design is that there is at least 1 atm of pressure differential between the interior of the first crystal and the vacuum chamber of the monochromator. The first crystal is a fairly thin box-like structure with insufficient internal structural bracing to completely offset bowing of the diffracting surface of the first crystal due to the pressure differential. In fact, finite element simulations of the crystal performed by Oxford Instruments, in an attempt to understand the under performance of the beamline, indicated that an appreciable pressure-induced deformation of the first crystal was a likely explanation for the reduced flux density measured at the beam focus. In following issues of the DCM by Oxford Danfysik, the top-hat first crystal design has been abandoned in favor of a simpler, more effective design.

Oxford Danfysik’s new design for an internal (direct) cooled first crystal is based on a thin channeled-plate. In this design, a series of parallel water channels run along the direction of the beam just under the diffracting surface. The close proximity of the channels to the surface provides efficient cooling while the fins provide strength and rigidity to counter the pressure differential induced bowing of the crystal surface. Oxford has decided to adopt a two piece design. The channels are machined into the top piece of silicon which is then bonded to the lower piece which functions as a silicon water manifold that attaches to the water inlet and outlet. Figure C.2.5.6. shows views of the proposed upgrade for the NE-CAT monochromator. This design has already been installed at beamline BM20 (Figure C.2.5.7) at the ESRF and at MAX-Lab on a wiggler beamline. The crystals are being manufactured by Polovodice Silicon Division in Prague under the supervision of Dr. Joromir Hrdy of the Institute of Physics of the Czech Academy of Sciences in Prague. An important aspect of the manufacture of the crystal is the brazing of the top piece and water tubes to the lower silicon manifold. Our upgrade was due to arrive at the end of the summer run, in August of 2005. The crystals were not delivered due to leaks in the brazed joints and are now expected in October, 2005.
Figure C.2.5.6: Oxford design for the upgrade to the 8-BM monochromator.
Figure C.2.5.7: The microfinned water-cooled first crystal that was delivered to BM-20 at the ESRF.

C.2.5.2.2. Sagittal Focusing 2\textsuperscript{nd} crystal

The sagittal focusing second crystal design is licensed to Oxford from the ESRF. The second crystal assembly can be seen in Figure C.2.5.8. There are three motors that are integrated into the assembly. Two that are buried in the interior are used for bending the crystal and the third is the cylinder seen in the lower portion of the Figure C.2.5.4. This is the yaw motor that has been discussed extensively in the 8-BM Optics Commissioning and Characterization section.

We have chosen to upgrade the bending capability and reproducibility by replacing the current bend motors with motors that are supplemented with a 25:1 gear reducer and the addition of positional readout LVDT’s. The gear reduction will increase the accuracy of the motion by a factor of 25 and enhance the torque. One of the problems encountered in operation of the focuser at 8-BM is that the bender cannot always return to the same position. The smaller step size will reduce the error and the addition of the LVDT’s will act as an encoder and allow us to move to positional values indicated by the LVDT readouts. This will eliminate or significantly reduce any backlash problems. Figure C.2.5.9 points out the location of the motors and the LVDT’s. We anticipate that the energy range of the
beamline will increase when it is moved to Sector 24. Given the planned location of the optics in Sector 24-BM, the sagittal radius for an energy of 14 keV will decrease to 2.07 m from 3.5 at 8-BM. The current motors would be able to reach this radius. If however, the energy range is moved upwards to cover the uranium LIII edge at approximately 17 keV, the bend radius will decrease to 1.7 m. This is close to the 1.6 m radius limit that is quoted by Oxford in the description of the bender. At this radius, the torque of the original motors is no longer sufficient to bend the crystal. This value was established by the group at X4 at the NSLS. There the gear reducers were necessary to reach a minimum limit of 1.0 meter.

C.2.5.2.3. Increase Travel Range of the 2\textsuperscript{nd} Crystal

The final modification to the monochromator, in the near future, will be to change the limits of travel of the second crystal. The pitch angle of the collimator is presently close to 4.7 mrad. This value will be reduced in order to center the beam vertically on the first crystal. The reduction of the pitch angle will increase the available energy range. This is a constant exit height monochromator and its range of motion was customized to the request of the Whitehead scientists. In order to accommodate an increase in the higher energy limit, the second crystal will have to travel farther along the downstream direction parallel to the beam and higher in the vertical direction. This adjustment will be done during the installation of the new crystals in order to check for any collision possibilities that may be introduced.

C.2.6. Summary

Characterization of the focusing of the X-rays in 8-BM has led to the upgrade in the design of the cooling of the first crystal, addition of gear reduced motors and LVDT’s that measure displacement on the second crystal and a change in the travel limits of the second crystal. The installation was planned for the end of August 2005. Due to technical difficulties in the manufacture of the microfinned first crystal, the installation has been delayed. Once a new first crystal is delivered and installed, we will be able to decouple problems due to thermal or pressure induced distortions on the first crystal and any imperfections in the manufacture or mounting of the second. There is still a possibility that the second sagittal focusing crystal may have to be replaced. We have discussed the possibility of decreasing rib widths, decreasing the number of ribs or remounting the second crystal to relieve any strain or twist that was introduced by a poorly mounted crystal. This awaits characterization of the focusing after the installation of the first crystal.

C.2.7. Future Plans for 8-BM

Based on our characterization and analysis, we plan to continue to improve the performance of 8-BM in the following manner. In the near future, we will concentrate on the vertical focusing. As in the horizontal direction, we will first begin by centering the beam in the vertical direction. This will be accomplished by lowering the pitch angle of the first mirror. Further studies will be made by adjusting the bender of the focusing second mirror.
Our current characterization of the focusing in the horizontal direction has led to a 20-25% improvement of flux and improved consistency in energy changes. Users and staff are able to consistently change energies over a large energy range (7 – 12.6 keV).

Overall, our conclusion is that the 8-BM optical components are fundamentally sound. With planned improvements on individual components and the eventual relocation of the whole system to 24-BM, it is expected that the overall system performance will improve for protein crystallography at the bending magnet of Sector 24. Perhaps the most significant improvement will be in the change in the demagnification ratio. At 24-BM the vertical focal ratio will increase to 3:1 instead of the current ratio of 1:2 used at 8-BM. The distance between sample and vertical mirror will also be reduced from 20.5 m to 8.05 m, which will significantly diminish the slope error effect on the focal size. Similarly, the increase in demagnification ratio will improve the beam in the horizontal direction.

C.2.8. Implementation of Blu-Ice

At the present time, researchers at NE-CAT beamlines are using the Area Detector System Corp. (ADSC) interface in conjunction with the Console motion control system to conduct their experiments. Figure C.2.1.4 shows the user interfaces currently exposed to experimenters at the NE-CAT beamlines. The interface on the left side of the figure is a Console program interface that monitors and controls beamline functions (mostly the monochromator) during data collection. Users interact with this program to perform energy scans, manually change energies, optimize the beam, and to change incident slit (beam) sizes. The right side shows a few of the Q315 detector control interfaces. The interfaces are those for manual control of detector distance and the phi angle, a snapshot interface for single exposures and the main data collection interface. This system has been in use for many years at several beamlines and has proved to be a satisfactory interface for the beamline developers and experimenters. However, this system has the disadvantages of a split computer operation and a steep learning curve.

An ever increasing number of structural biology researchers are dependent upon use of synchrotron light sources for their data collection purposes. Researchers typically acquire data from one or more beamlines at different synchrotron facilities and are forced to become conversant with the user interface operating at each of these beamlines. Obviously, the overall efficiency of use of these beamlines would be improved if users only had to learn to use a single user interface and control philosophy. Additionally, adoption of a common user interface between beamlines would promote beamline protection from user errors and reduced manpower expenditure in user training; however, a single universal standard GUI is neither practical nor desirable. Existing beamlines have their own mature, functioning control systems. Crystallography beamlines have varying experimental mandates and operating protocol. Much time and effort has been spent in achieving existing levels of functionality and reliability at these beamlines. Crystallography beamlines are built using a very disparate set of hardware and low-level software resources. No single beamline control or user interface system can support this diverse hardware/driver base. None the less, the synchrotron crystallography community and their funding agencies demand some form of unification of beamline controls form and function, at least at the user interface level.

To facilitate usage of the NE-CAT beamlines in the future, we have chosen to replace our existing Console/ADSC user interfaces with Blu-Ice, a user interface system developed at SSRL. Blu-Ice has been developed at a large investment of effort over a substantial period of time with considerable user based input and testing. Blu-Ice is logically organized, highly graphic and intuitive in nature and is becoming widely accepted as a defacto standard at structural biology beamlines. Blu-Ice is installed and operating at crystallography beamlines at SSRL, ALS and the APS (GM/CA). Additionally, some beamlines, while not adopting Blu-Ice, per se have modified their local user interfaces to mimic the organization and appearance of Blu-Ice (e.g. SER-CAT,
APS). Consequently, we believe our users will be better served with the implementation of Blu-Ice on the NE-CAT beamlines.

C.2.8.1. Blu-Ice Distributed Control System

The Blu-Ice Software package consists of three major components. The Blu-Ice GUI is the graphical display of commands and status of an experimental control process. Its what the users see on the computer screen and uses to control their experiment. The organization of the GUI is the reason for the popularity of the program amongst experimenters. The second major component is the Distributed Hardware Server (DHS). DHS is the program that is directly responsible for controlling the hardware devices such as the motor, detector etc. It is also responsible for updating and displaying hardware device status. Finally, the Distributed Control System Server (DCSS) acts as a middle man to link the GUI and DHS together. The macros or scripts contained in the DCSS are believed to hold the years of experience in beamline controls.

All three components GUI, DHS and DCSS speak one language, the DCS Protocol. DCS is a simple text messaging system with well defined transactional rules. The DCS Protocol messages are sent and received over TCP/IP sockets. DCSS acts as a message router to transfer the messages between the users (GUI) and hardware devices (DHS). The following diagram (Figure C.2.8.1) illustrates the working relation between the three.

Figure C.2.8.1: Flow diagram of the Blu-Ice system.

Blu-Ice GUI
The Blu-Ice program is a client of the Distributed Control System Server. It provides the user with a graphical interface to the system.

DCSS
The Distributed Control System Server (DCSS) is a key component of the system. It functions primarily as a message router, enabling communication between multiple GUI clients and the various hardware components.

DHS
The Distributed Hardware Servers (DHS) act as translators, converting the DCS message protocol to the language of a third party hardware controller.

Hardware: Huber and Detector

C.2.8.2. Blu-Ice GUI

Most crystallographers like the visual display and organization of the Blu-Ice GUI. Its strength is that its developers have tried to maintain the experimenter’s perspective. It simplifies the sophisticated experimental process and hides the complexities of the control and computer infrastructure. The organization and display are intuitive so training of users is minimized.

The collaborative and remote access capabilities of the Blu-Ice GUI allow multiple users to run their own screens of the Blu-Ice GUI simultaneously. This allows them to view their experiment from either local or remote locations and staff members can run it from home or office to provide
assistance to users and troubleshoot problems remotely. The following figures are illustrations of a sample of the screens of the GUI.

**Hutch --- Experimental set up window.** The hutch menu (Figure C.2.8.2) provides the user with views of most relevant beamline hardware. The devices are arranged in the same manner as the real devices are located in the hutch. Users can easily associate the displayed devices with the actual instruments. A user can control the huber motion, detector motion, beam size, shutter, attenuation energy etc. by going to the appropriate figure. They are also able to align their sample manually with the embedded sample alignment feature (lower left in window) that is connected to a video stream. The resolution predictor (lower right in window) graphically indicates the position of the direct beam and the d-spacing of diffraction spots expected on the different parts of the detector face. It updates dynamically as the detector distance and energy changes.

![Hutch Window as implemented on the 8-BM beamline.](image)

**Figure C.2.8.2:** Hutch Window as implemented on the 8-BM beamline.

**Collect --- Data Collection Control.** The collect menu is used to specify, control and monitor the data collection (Figure C.2.8.3). Users can set up their data collection sequence with the run definitions by clicking on the small tabs on the right hand side. Up to 16 individual run definitions can be set. The run will continue to execute in the order of the run number. Users can start, pause and abort their data collection run by simply clicking on the corresponding button. The image will be automatically loaded and displayed on the left side of the screen. The user can also adjust the zoom level and centering of the image.
Figure C.2.8.3: Collect window as installed on NE-CAT beamline 8-BM.

Scan --- Energy Scan Window. The scan menu allows the experimenter to perform X-ray fluorescence energy scans. The periodic table (Figure C.2.8.4) allows the user to select the X-ray absorption edges. By clicking to the selected edge, it automatically generates optimal experimental parameters for the scan. By pressing the SCAN button the scan will be executed. It also automatically runs the program Chooch on the newly collected data and displays the results of f’ and f” curves. Based on these results, it automatically selects three energies for a multi-wavelength anomalous diffraction experiment. These three energies can be adjusted. They can also be loaded into a run definition on the Collect tab.
C.2.8.3. Distributed Hardware Server (DHS)

Each DHS is a dedicated server for control and status polling of one or more particular hardware devices. All hardware specific code is encapsulated within each DHS. DHS acts as a translator between DCSS and the hardware devices. It translates DCS protocol messages to the control protocol specific to the particular device. DHS is also responsible for updating DCSS with the current status of the device. A typical beamline control system would have several DHS modules (motor DHS, detector DHS, video server DHS, ion chamber DHS etc.) executing on one or more computers. A new DHS must be developed for each new device.

C.2.8.4. Distributed Control System Server (DCSS)

DCSS is the operating core of Blu-Ice. The major service that DCSS provides is a message router between the DHS and the GUI. It controls the communication between GUI and DHS. DCSS examines the incoming messages from the GUI and forwards them to a particular DHS to control a particular device. Conversely it receives the response from the DHS and forwards them to all the GUIs to update them. DCSS supports multiple GUI processes and continuously synchronizes all GUIs with updated information from the control system. This allows a user to run or monitor their experiments locally or remotely while viewing the correct updated information.

A scripting engine, based upon the TCL interpreter is embedded within DCSS. This simple declarative language definition is used to define all the complex functions and interdependencies of a beamline control system. TCL is a commonly used embedded scripting system used in many contexts by many programmers.
C.2.8.5. Beamline Control Program Implementation

Adoption of Blu-Ice has many apparent benefits: 1) large, mature script programming base, 2) many users already have experience with Blu-Ice’s GUI and behavior and 3) built-in collaborative and remote access functionality. However, a major difficulty for Blu-Ice adoption at NE-CAT beamlines is that the majority of the beamline hardware devices are not supported by Blu-Ice. Many new Distributed Hardware Servers (DHS) would have to be created in order to control the beamline exclusively through Blu-Ice. At this time writing a DHS is not a trivial task and requires a deep understanding of the Blu-Ice code base. Additionally, low-level Console scripting for control of beamline optics (e.g. beam tracking, energy changes, beam focus control) is stable and fully validated. Writing the necessary DHS services and translating CSL scripting to DCSS would be very time consuming and require complete re-validation of the translated scripting. Our solution to this problem is to combine the Blu-Ice GUI and its DCSS and DHS control architecture with the Console control system, placing Console in the role of a distributed hardware server (DHS).

The Console distributed control system consists of a central graphical client (the Console super client) that interacts with a variety of single-purpose servers, each responsible for status polling and control of a specific, well defined class of hardware (e.g. motion controllers, sensors, etc.), analogous to a Blu-Ice DHS. The Console super client contains the Console scripting engine (called CSL) and can communicate with Console hardware servers through different types of communications channels including: TCP/IP sockets, RPC, RS-232, IEEE-488, etc.

Multiple instances of Console can simultaneously operate within a single network, sharing hardware resources via multiple, concurrent client server connections to the Console hardware servers. Different instances of Console can pass messages to each other using a separate TCP/IP communications channel, called the Console Server Stack (CSS), in order to coordinate their actions upon shared hardware assets.

The CSS implements a simple message passing system based upon the SUN ONC-RPC communications protocol. CSS RPC transactions between different instances of the super client involve transmission of a header containing information about the IP identity of the source of the message, the IP target of the message and a string payload message. CSS transactions come in two main forms: Puts and Gets. In a CSS Put transaction, one member of a pair of transacting console super clients simply sends a message string to another super client without expecting a response from the target of the message beyond confirmation of completion of the transaction. A CSS Get transaction is identical to a Put, except that a return payload message is expected from the target as part of the transaction. The message payload string can contain specific substrings that are parsed by the target of the transaction to modify its response to the message. Messages received by the target superclient are queued in a FIFO (stack). The message stack is then acted upon asynchronously by the local Console scripting engine.

The CSS communication core and stack can be imbedded in any program, on any platform that supports ONC-RPC TCP/IP transactions (e.g. Win32, Linux, Solaris). Embedding of the CSS stack allows said program to communicate with a target Console super client on the local network and thereby marshal any resource to which the target super client has access. Resources include: CSL scripts, client-server connections, data local to the target super client, etc. This is the mechanism used to give the ADSC Q315 control system control over 24-ID-C optics and end station hardware resources. Under this integration scheme Console effectively functions as a DHSS.
Figure C.2.8.5: Flow chart of GUI, DCSS DHS and integration with Console. The Console Server Stack client-side communication core is integrated with Blu-Ice as a DCSS available client. The embedded Console server client transacts with the Console super client whose scripting engine processes the message stack and takes appropriate action on hardware resources under its control (Console Hardware Servers).

C.2.8.6. Progress to Date

(1) A Blu-Ice simulation module has been built. It has the Blu-Ice GUI look and feel. It has the basic functionality of DCSS. It includes a simulated detector DHS and motor DHS. It can be used to simulate the motion control and data collection processes (the data collection part is not working properly due to a bug in the simulated detector DHS). It has proven to be a good and effective testing tool for exploring the Blu-Ice program without any hardware devices being attached.

(2) The collaborative and remote access capabilities of Blu-Ice have been implemented. Multiple Blu-Ice GUIs have been configured and set up on multiple computers at 8-BM and in the office area. The multiple instances of the Blu-Ice GUI can be operated independently at both local and remote locations. It allows users to work together over the internet to control the experiment.

(3) Blu-Ice system configurations and ADSC detector's logical files have been modified so that it allows the Blu-Ice detector DHS to directly talk to the ADSC program "det_api_workstation" which is a critical interface program to control the detector communication via an external program.
The modified configuration file of the Q315 detector at 8 BM has been tested successfully with the Blu-Ice control. Blu-ce is not only able to send a command to and receive status from the Q315 but also is able to grab the images from all nine CCD image computers and gather this image to be displayed on the GUI. A recently installed version of the Q315 detector, located in the laboratory at Sector 24, can also successfully communicate with Blu-Ice.

(4) The Galil motor controller which controls the Huber diffractometer motions, attenuator and shutters is an essential element for a data collection process at 8-BM. A series of dcm2180 programs and scripts have been modified to work with the 8-BM control system. Preliminary testing has shown that the modified Galil DHS has worked well with the Galil motor controller at 8-BM.

(5) A data collection Script has been written to test motion control and explore the possibility for future robotic control. This Script has been tested. At this point, we are now able to start commissioning of single wavelength data collection at 8-BM. Commissioning will start at the beginning of the next run (Oct 2005).

C.3. 8-BM/24-BM Progress Since October 2005

C.3.1. 8-BM Optical Upgrades

In December 2005 the original “top hat” first monochromator crystal was replaced with the Oxford-Danfysik-supplied internal channel design (see Section C.2.5.2). At the same time the second crystal assembly was removed from the monochromator and the two stepper motors used to bend the second crystal were fitted with 25:1 gear reducers and reinstalled in the bending mechanism. Two Linear Voltage Differential Transformers (LVDT’s) were installed in the bender to provide a direct measurement of the bend-actuator deflection, and thereby the second crystal bend radius. Installation of the gear reducers in the second crystal bend actuators has increased the resolution of the step-displacement function for these motors and increased the maximum achievable deflection of the bend mechanism. This will be required by the increased crystal bend radius required when the monochromator is transferred to Sector 24. Beamline 8-BM operates with a 1:1 beam demagnification, while the demagnification of 2.6:1 is planned for the Sector 24 bending magnet beamline.

The monochromator was recommissioned in Spring 2006. Optical performance of the new monochromator crystals was performed and all crystal alignment parameters optimized. Figure C.3.1.1 shows the measured rocking curve of the new crystal — measured offline by the vendor — which has a rocking width virtually identical to the canonical, strain-free value for Si 111. Figure C.3.1.2 shows a photograph of the reassembled first and second crystal mounts, showing the second crystal deflection LVDT encoders (two tilted cylinders).

After optimization the new horizontal focal size is 0.33 mm (reduced from

![Figure C.3.1.1: Rocking curve of the new channel-cooled Si 111 first crystal provided by the vendor. FWHH = 6.12 arcsec.](image)
0.5 mm) and the vertical focus spot size is 0.4 mm (reduced from 0.5 mm). Following upgrades to the monochromator, the beamline still exceeds design targets for focus spot size (0.2 x 0.2 mm horizontal x vertical).

![Photograph of new 8-BM monochromator first and second crystals.](image)

**Figure C.3.1.2:** Photograph of new 8-BM monochromator first and second crystals.

### C.3.2. **ALS Sample Robot Commissioning**

Delivery of an Advanced Light Source sample loader robot took place in November 2005, following exhaustive acceptance testing at LBL (Figure C.3.2.1). Commissioning of the robot was conducted without X-rays during January 2006. At that time over 1,500 mount/dismount cycles were performed with less than 1% failure rate. All testing was done at liquid nitrogen temperature with sample pins loaded in all of the robot’s 64 magazine positions. In early March 2006, the robot was successfully used to mount lysozyme protein crystals for actual data collection. The robot was used to successfully mount user samples in March 2006, and after completion of optics upgrades and beamline recommissioning in November 2006.

### C.3.3. **24-BM Radiation Enclosures**

The 24-BM Preliminary Design Report was submitted to APS for review and approval in September 2005. Concurrent with submission, NE-CAT received approval from APS to begin construction of the radiation enclosures at Sector 24. Award for the construction and installation of associated utilities was made to Tecknit Shielding, Inc. On-site construction began in November 2005, and was completed within two weeks of schedule on March 30, 2006 when NE-CAT received APS approval for beneficial occupancy.
In parallel with construction of the enclosures, APS completed installation of the front end X-ray collimation and shutter systems as well as the Personnel Safety System. In May 2006 X-rays were introduced into the enclosures for the first time in order to validate the radiation integrity of the enclosures.

Figure C.3.3.1 shows the plan layout of the enclosures as well as the 8-BM optical components installed within the enclosures. The enclosures consist of a white-beam compatible wall and ceiling extending from the outboard wall of the existing ID-24-A enclosure to the out-board face of the Sector 24 accelerator system ratchet wall. The accelerator ratchet wall (red hatched outline) comprises the inboard wall of the BM enclosures. An interior lead-shielded partition wall separates the 24-BM-A First Optics Enclosure (FOE) from the 24-BM-B experimental enclosure. Both of the enclosures feature 3-panel wide doors with a 118-inch free span to simplify movement of large optical components into the enclosures from the very limited aisle space between the 24-BM and adjacent Sector 23 enclosures. Design of the component layout in the experimental enclosure, 24-BM-B, required provision for ready access to the Sector plug door (shown as solid green) as required by APS.

8-BM operated through the end of 2006 and was formally taken off-line, in preparation for decommissioning and relocation to Sector 24-BM. Relocation of optical components to Sector 24 will commence in late Spring 2007.

C.3.4. User Program

As described above 8-BM was scheduled for several upgrades to improve performance prior to its relocation at Sector 24. Debugging and commissioning continued during much of 2006 and the beamline experience some technical problems. However, 24 individual scientists from 15 research groups performed X-ray diffraction experiments. There have been 39 publications since October 2005 based on experiments performed at 8-BM.
C.4. Technological Core Developments: 24-ID-C

The main focus of NE-CAT is the development of Sector 24 and the past few years have been an exciting and productive period for the staff. During the first five year grant period from 2001-2006, NE-CAT proposed to build two undulator beamlines referred to as 24-ID-C (undulator end station) and 24-ID-E (undulator side station). In May 2002, NE-CAT received authorization to proceed with construction. Over the next three years, designs were completed, contracts were issued, and the experimental facilities began to take shape. At the time of this writing, 24-ID-C beamline is in early user operations and 24-ID-E construction will be completed before the end of the current funding period. Together with the bending magnet station at Sector 8 described above we will have our full complement of three beamlines proposed.

During design, construction and commissioning at Sector 24, a number of problems were encountered and overcome. Not the least of these was the delay by APS in delivering the tandem undulator front end. At the beginning of this project, the tandem undulator configuration was viewed as risky. However, APS took on the challenge and successfully designed a front end that was capable of handling the heat load from two X-ray undulators. APS delivered the first front end to GMCA-CAT and second to NE-CAT. Initial problems with the APS-designed front end resulted in about a six-month slip in the NE-CAT construction schedule. Various problems were identified in the NE-CAT beamline components and were solved during assembly or commissioning. Details of the design, construction and commissioning projects at Sector 24 are described below.

At present, 24-ID-C is producing small, stable X-ray beams and meets or exceeds the specifications of the original design. A number of trial user groups have visited 24-ID-C and collected high quality data; in some cases on technically challenging samples. Even at this early stage, several new structures have been solved. For the next year we plan to operate ID-24-C in

Figure C.3.3.1: Plan and elevation views of 24-BM beamline radiation enclosures and optical components.
user mode, and will only interrupt this schedule to correct deficiencies that interfere with data collection. Construction efforts will focus on 24-ID-E and performance upgrades to 24-ID-C will be limited until 24-ID-E commissioning has been completed.

C.4.1. **Preliminary Design Report: 24-ID-C and 24-ID-E Construction**

C.4.1.1. Introduction

The central design consideration of NE-CAT’s Sector 24 optical plan is the use of a Tandem Offset Undulator (TOU) to source three undulator crystallography beamlines while reserving space for one bending magnet beamline. The TOU provides two independent undulator white beams with an angular offset of 1 milliradian using two offset APS A undulators with a smaller period number (compared to a conventional undulator A, see C.2.3). Obviously, this plan requires optimal use of available floor space to situate four beamlines in an area which in typical APS sector would be used to build one undulator and one bending magnet beamline.

NE-CAT submitted the Sector-24 Conceptual Design Report (CDR) to APS in February 2002. The CDR outlined optical design and performance constraints for all 4 NE-CAT beamlines and detailed the radiation hutch layouts for the sector. The Preliminary Design Report (PDR) for NE-CAT construction 24-ID-C and 24-ID-E was submitted in August 2003 and eventually approved by APS in November 2003. The PDR specified detailed optical and end station designs as well as construction schedule for construction 24-ID-C and 24-ID-E. This section summarizes the 24-ID-C and 24-ID-E PDR and the overall Sector 24 optical design. Section C.2.14 presents corresponding material for 24-BM (bending magnet) beamline.

Beamline nomenclature and optical performance specifications for the planned NE-CAT beamlines are tabulated in Table C.4.1.1. Figure C.4.1.1 (at the end of this section) presents schema for the optical trains for the NE-CAT beamlines. Figure C.4.1.2 is a plan layout for Sector 24. The following list details the overall performance goals for all 4 Sector 24 beamlines:

24-ID-C: “Pass-Through” Undulator Beamline. The first beamline developed (using the upstream, outboard-projecting undulator) consists of a single optical train, supplying a single experimental end station with doubly focused monochromatic light from 5 to 25 keV, with a nominal bandpass of order 1x10^{-4} (ΔE/E). Monochromatization is accomplished via a cryogenically-cooled double crystal Silicon Bragg monochromator. A Kirkpatrick-Baez (K-B) mirror pair focuses the monochromatic beam.

24-ID-E: “Single-Crystal Side-Bounce” Undulator Beamline. A single, side-bounce cryogenically-cooled 220 silicon monochromator accepts the residual beam from a diamond-transmission monochromator (24-ID-D) using beam from the downstream, inwardly projecting undulator. The side-bounce mono delivers a monochromatic beam near 12.66 keV, with a nominal take-off angle (2θ) of 29.5° from the centerline. The 220 Si crystal is mounted on a cooling stage that incorporates a vertical translation. A second silicon crystal (311-cut) is mounted on this stage, with independent roll and pitch fine adjustments such that a second energy (14.84 keV) will be selectable by a vertical translation of the cooling stage (following the same 29.54° take off angle provided by the 220 crystal). This beamline also uses K-B focusing.

24-BM: Bending Magnet Beamline. The third beamline developed by NE-CAT uses the bending magnet port of the sector. The 24-BM beamline incorporates a water-cooled Si-111 monochromator with sagittal focusing, providing a spectral range from 5 to 17 keV (2 eV bandpass). Vertical focusing is accomplished with a mechanically figured mirror. Components of this beamline are currently installed and operating at Sector 8 bending magnet.
24-ID-D: Tunable Large-Offset, Side-Bounce Undulator Beamline. A pair of beamlines is sourced by the downstream, inboard-projecting undulator. Monochromatization and physical separation of the 24-ID-C and 24-ID-D beamlines accomplished with a large horizontal-offset (1.5m) diamond transmission monochromator, with a spectral range between ~8.5 and 17 keV. The undiffracted radiation passes on to the 24-ID-E Fixed-Energy Side-Bounce monochromator. As with 24-ID-C and 24-ID-E, 24-ID-D uses K-B focusing.

Table C.4.1.1: Optical Performance Specifications for NE-CAT Build-Phases.

<table>
<thead>
<tr>
<th>Build Phase Designation</th>
<th>Source</th>
<th>Energy Range (keV)</th>
<th>Energy Resolution (E/E @ 12.7 keV)</th>
<th>Focus Spot Size (HWHH (vxh))</th>
<th>End Station Identifier</th>
<th>Flux (focused) @ 12 keV Phot/mm²/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>I I</td>
<td>U.S. ID</td>
<td>5 – 25</td>
<td>2 x 10⁻⁴</td>
<td>100 x100</td>
<td>ID-C</td>
<td>10¹⁴</td>
</tr>
<tr>
<td>II III</td>
<td>D.S. ID</td>
<td>12.66 or 14.85</td>
<td>2 x 10⁻⁴</td>
<td>100 x 100</td>
<td>ID-E</td>
<td>10¹⁴</td>
</tr>
<tr>
<td>III IV</td>
<td>BM</td>
<td>5 – 17</td>
<td>2 x 10⁻⁴</td>
<td>200 x 300</td>
<td>BM-B</td>
<td>10¹²</td>
</tr>
<tr>
<td>IV II</td>
<td>D.S. ID</td>
<td>8.5 – 17</td>
<td>2 x 10⁻⁴</td>
<td>100 x100</td>
<td>ID-D</td>
<td>10¹⁴</td>
</tr>
</tbody>
</table>

Key:
- U.S. ID: Upstream (outboard-projecting) undulator
- D.S. ID: Downstream (inboard-projecting) undulator
- BM: Bending magnet.

C.4.1.2. Beamline Technical Specifications

All four Sector 24 NE-CAT beamlines are single-purpose, fixed configuration macromolecular crystallography beamlines. The following functional elements are provided by the various beamline subsystems:

- Shielding and safety systems include enclosures for the optics and the experimental hardware and are capable of protecting personnel from radiation and equipment from faults such as loss of power, cooling fluid, etc.

- Optics systems are capable of delivering a focused monochromatic beam with long-term positional stability to a sample 40-60 meters from the radiation source. The required positional stability region (horizontal and vertical) is a square of edge length 10 µm (or less) with a characteristic time measured in hours.

- Beam transport systems convey the beam from the sector front end to the sample position via ultra high vacuum paths to minimize intensity loss (due to absorption) and scattering background.

- Utility systems provide conventional infrastructure such as plumbing of water and compressed gases; electrical power; HVAC capability; liquid nitrogen; and mechanical support.
Control systems must provide interactive (and secure) local and remote control of all optics, sample handling and detection subsystems.

Front ends, insertion devices and dipole magnet must provide an X-ray beam with an energy range of at least ~5 keV to ~30 keV.

The lab and office module must provide for mechanical equipment setup and maintenance; wet laboratory capability for sample preparation; and office space for operations staff and data reduction.

C.4.1.3. Beamline Performance Characteristics / Design Constraints

24-ID-C “pass through” beamline, using the outboard-projecting undulator:
• An energy range of 5-25 keV.
• An unfocused beam of approximately $10^{12}$ X-rays/mm$^2$/sec to a sample about 65 meters from the source.
• A focused beam of approximately $10^{14}$ X-rays/mm$^2$/sec to a sample about 65 meters from the source.
• A focal spot size using mirrors of ≤100 μm horizontal by ≤100 μm vertical.
• A beam with an energy bandwidth of ΔE/E ≈ 1 x 10$^{-4}$ at an energy of ~12.66 keV.
• A beam that is stable to <10% of its size (e.g., a 100 μm beam will have a positional stability of 10 μm).

24-ID-E single-crystal side-bounce beamline using the inboard-projecting undulator:
• A fixed energy at ~12.66keV or 14.84 keV, with a fixed take-off angle (2θ) of 29.5°.
• An unfocused beam of approximately $10^{12}$ X-rays/mm$^2$/sec to a sample at 58 meters from the source.
• A focused beam of approximately $10^{14}$ X-rays/mm$^2$/sec to a sample at 58 meters from the source.
• A focal spot size using mirrors of ≤100 μm horizontal by ≤100 μm vertical.
• A beam with an energy bandwidth of ΔE/E < 1 x 10$^{-4}$ at an energy of ~12.66 keV.
• A beam that is stable to <10% of its size (e.g., a 100 μm beam will have a positional stability of 10 μm).

24-BM bending magnet beamline:
• An energy range of ~5-17 keV.
• An unfocused beam of approximately $10^{10}$ X-rays/mm$^2$/sec to a sample about 37 meters from the source.
• A focused beam of approximately $10^{11}$ X-rays/mm$^2$/sec to a sample about 37 meters from the source.
• A focal spot size (using either zone plates or mirrors) of <500 μm horizontal by <300 μm vertical.
• A beam with an energy bandwidth of ΔE/E < 4 x 10$^{-4}$ at an energy of ~12 keV.
• A beam that is stable to <10% of its size (e.g., a 500 μm beam will have a positional stability of 50 μm).

24-ID-D diamond-transmission beamline, using the inboard-projecting undulator:
• An energy range of ~8.5-17keV.
• Horizontal offset monochromatic beam by 1.5 m.
• An unfocused beam of approximately $10^{12}$ X-rays/mm$^2$/sec to a sample about 42 meters from the source.
• A focused beam of approximately $10^{14}$ X-rays/mm$^2$/sec to a sample about 42 meters from the source.
• A focal spot size using mirrors of ≤100 μm horizontal by ≤100 μm vertical.
• A beam with an energy bandwidth of ΔE/E < 2 x 10$^{-4}$ at an energy of ~12.66 keV.
• A beam that is stable to <10% of its size (e.g., a 100 µm beam will have a positional stability of 10 µm).

C.4.1.4. End Station General Design Parameters.

The Sector 24 end stations incorporate similar designs and subsystems used in the NE-CAT 8-BM end station. The principal components of all four end stations are:

• Collimation system, consisting of two independent arrays of pairs of vertical and horizontal slit blade pairs, separated by ~ 1 m. Individual slit blade positions have a reproducibility of ~ 5 µm and operate in roughing vacuum to minimize air scatter and beam intensity degradation via absorption.
• Fast monochromatic beam shifter with opening and closing times less than 5 ms, synchronized, precisely with the motion state of the crystallographic spindle.
• Precision crystallographic goniometer, with remotely controlled X,Y and Z spindle adjustments. The radius of the sphere of confusion of the crystallographic axis will be less than 10 µm. The minimum stepping unit of the crystallographic scanning axis less than 1.75 x 10⁻³ rad. Maximum stepping rate must be greater than 10°/sec to efficiently support “Friedel Flipping” (inverse beam) data collection.
• Crystal cryocooler, pneumatically-actuated beam attenuators and split diode beam position monitors (two sets in order to measure beam position and angle.
• Ionization and sample fluorescence monitors.
• Precision, remotely steered miniature beam stop.
• Video microscope capable of visualizing crystals with edge lengths <10 µm.
• Multi-element fast CCD and detector positioner and data flow systems with the following characteristics.

1. Active area consisting of at least 6000 x 6000 pixel elements with an effective pixel size less than 60 µm x 60 µm and resolution of order 90 µm x 90 µm. The Q315 was selected as the detector of choice for all NE-CAT beamlines (see section C.2.8).

2. Aggregate CCD array readout times less than or equal 1 s with high sensitivity and low noise.

3. Maximum spindle to detector distance (SDD)> 1 m, minimum SDD < 0.15 m, with ability to pitch detector about the 2θ axis (LR Design A-frame).

4. High performance data flow and computational cluster. Data flow (and storage systems) must have sufficient performance to avoid becoming a logistical bottle neck. Computational systems for data reduction and analysis must have sufficient capacity to enable users to assay data quality and evaluate the efficacy of data collection strategies on the same time scale as data collection itself.

C.4.1.5. Principal Component Descriptions

C.4.1.5.1. Common Optical Components

*Instrumentation Design Technology LDT (IDT) 24-ID-D Power Limiting Aperture Mask Assembly (non-standard).* The purpose of this component is to limit the power deposited on the first crystal of the 24-ID-D large horizontal offset diamond monochromator, that will be installed in the first renewal phase of our NIH funding approximately 3-4 years hence. The design is conceptually similar to that of the APS L5-92 mask, modified to accommodate two undulator beams with an angular separation of 1 mrad. Until the build 24-ID-D monochromator is installed this aperture will be locked in its most permissive geometry, equivalent to a square aperture 4.5 x 4.5 mm.
The design rationale behind all IDT apertures (Power-Liming (three instances) and Fixed aperture (one instance) are discussed in detail under Section 6.4.2 of this document.

IDT 24-ID-C and 24-ID-E Fixed Aperture Mask Assembly (non-standard). The principal purpose of this element is to protect a tungsten bremsstrahlung collimator located immediately downstream from this mask from overheating by a mis-steered white undulator beam. The design is functionally similar to an APS L5-92 mask, modified for use in the context of the tandem undulator.

**Tungsten Bremsstrahlung Collimator 4 (modified-standard).** This collimator is the first non-front-end associated bremsstrahlung collimator. The design is a slight modification of a standard APS tungsten collimator (APS K5-20). Instead of a single wide bore, this collimator has two bores 9.5 x 9 (h x v) mm, centered on the two undulator beams. Bore size was determined by the spread of the extremal synchrotron rays with sufficient leeway to provide a clearance of at least 2 mm horizontal and vertical. This mask is protected from illumination by the white beams by the fixed aperture mask.

**Lead Bremsstrahlung Collimator 5 (modified-standard).** This is a standard lead collimator sized in the horizontal direction to comply with bremsstrahlung shielding requirements. This collimator is connected to the first tungsten collimator via a rotatable flange. The tungsten collimator has an intrinsic formed bellows to provide compliance at this flange join. The internal channel width and height of this collimator is such that only the tungsten collimator is considered during survey and alignment of this pair of collimators (via the kinematic table surface adjustments). The fixed mask has its own independent motorized translators to facilitate alignment.

C.4.1.5.2. 24-ID-C – Specific Components

**IDT 24-ID-C Power Limiting Mask Assembly (non-standard).** This mask pair is used to limit the power load on the 24-ID-C monochromator. Both mask elements have one precision L-shaped tungsten insert in its respective corner of the conjoint aperture formed by the mask pair to minimize scatter from the aperture.

**Kohzu HLD-8-24 Cryocooled Silicon Double Crystal Monochromator (standard).** The 24-ID-C monochromator is a modification of the standard Kohzu HLD-4 monochromator in operation at COM-CAT and SGX-CAT. This is a vertically offset monochromator with a built in video-based beam position monitor (HKD-3.B), originally designed to operate with a pair of water-cooled diamond crystals. The main modification from the HLD-4 design is the inclusion of a liquid nitrogen bayonet-type rotational feed-through in addition to the normally available water feed-throughs so that we can operate with a pair of liquid-nitrogen cooled silicon crystals. The complete design specification of the HLD-8-24 and assembly drawings is presented in section C.4.6.

We used the crystal and crystal mount design used by APS Sector 4 in its Kohzu APM-5 monochromator. The first crystal is a monolithic block with a simple LN$_2$ cooling channels and no undercut. The second crystal passively cooled through a LN$_2$-cooled OFHC block and is long enough that no parallel translation of the crystal is required to track the beam across its entire spectral range.

**Oxford-Danfysik UHV Split-diode Beam Position Monitor (non-standard).** This device allows determination of the monochromatic beam position to an accuracy of 2-5 μm using the SBC-developed split diode BPM installed in a UHV cross.

**P4-50 Integrated White Beam Collimator Beam Stop (standard).** The 24-ID-C photon shutter is an APS standard P4-50 integrated collimator white beam stop, situated on an APS standard support table.
Oxford Danfysik Integrated Kirkpatrick-Baez Focusing System (non-standard). The 24-ID-C beamline uses Kirkpatrick-Baez focusing. To conserve longitudinal space in the experimental enclosure we have worked with Oxford-Danfysik to develop an integrated support system for enclosing both the Horizontal Focusing Mirror (HFM) and the Vertical Focusing Mirror (VFM) in a single vacuum vessel, supported from a single vibration isolation platform. This design, in addition to conserving linear space also provides increased stability since both the HFM and VFM are exposed to similar mechanical/vibrational environment. Both mirrors are fabricated from ULE, have rhodium, platinum and uncoated strips, and are clamped in 4-point mirror benders (SESO). The HFM mirror is situated upstream of the VFM and is 1.2 meters in length. The VFM is 1 m in length. Mirrors have the expected kinematic positioning systems that also provide pitch and roll adjustments. All axes are associated with precision incremental encoders. Additionally, a fast-response piezoelectric actuator is associated with the singular leg of the kinematic mount to provide rapid, fine adjustment of the pitch of both mirrors (see C.4.7)

C.4.1.5.3. 24-ID-E – Specific Components

IDT 24-ID-E Power-Limiting Mask Assembly (non-standard). This mask pair is used to limit the power load on the 24-ID-E monochromator. Both mask elements have one precision L-shaped tungsten insert in its respective corner of the joint aperture formed by the mask pair to minimize scatter from the aperture.

Oxford-Danfysik CryoCooled Single Crystal Side-Bounce Monochromator (non-standard). The 24-ID-E monochromator consists of a liquid nitrogen-cooled Si crystal (220) aligned to provide a fixed-energy beam (12.66 keV) with a take-off angle of 29.5°. The Si crystal is mounted on a cooling plate along with another Si crystal (311), capable of providing a monochromatic beam at 14.85 keV along the same traverse as the first crystal. Selection between the two crystals is effected by a simple vertical translation of the entire cooling block. The second crystal incorporates independent fine pitch and roll adjustments. We plan to use this configuration to perform MAD data collection in the 2-energy mode, or monochromatic data collection at the high or low energy. Critical issues of the design include thermal stability of the crystal stack and the practicality of fine steering of the low and high energy beams over the same narrowly-defined traverse. We are collaborating with Oxford-Danfysik to effect the design and construction of this monochromator using subassemblies Oxford-Danfysik has used in prior designs.

24-ID-E Permanent and Temporary Water-Cooled Beam Stop (non-standard). A water-cooled white beam stop is situated downstream of the main support stage of the 24-ID-E monochromator to permanently stop residual pink beam. The beam stop consists of an inclined block (< 10°) of glidcop with internal joint-free copper water-cooling channels. Until the 24-ID-E monochromator is installed, the inboard-projecting undulator will be administratively locked at maximum permissible gap. An additional safety margin will be provided by installing a temporary water-cooled glidcop white beam stop in a spool-piece attached downstream to the lead collimator (7) (without interference to the outboard undulator beam). The temporary stop will also block residual radiation originating from the Sector 24-dipole that enters the canted tandem front end. The temporary 24-ID-E stop will be designed by S. Sharma’s group.

P8 Monochromatic Shutter (standard). The monochromatic 24-ID-E beam will be stopped using a standard APS P8 photon shutter, situated on a shortened standard support table.

Oxford-Danfysik Horizontal Focusing Mirror System (non-standard). As with 24-ID-C, 24-ID-E focusing uses the Kirkpatrick-Baez geometry. However, due to space limitations in the SOE and 24-ID-E, we cannot use the integrated solution used in 24-ID-C without reverting to an undesirably high demagnification geometry for the VFM. We will instead use a conventional design that physically separates the VFM and HFM. The HFM will consists of a 1.2 m long ULE pre-figured meridional cylinder with rh, pt and uncoated strips, selectable by translation of the kinematic mount. The mirror will be mechanically fine-figured with a SESO 4 point bender.
Oxford-Danfysik Vertical Focusing Mirror System (non-standard). The VFM will be located immediately next to the SOE – 24-ID-E partition and will use a 1 m long ULE mirror in a 2-point SESO bender. As with the 24-ID-C K-B system, the 24-ID-E K-B system will have piezo-electric pitch adjusters for rapid-fine tune of both pitch axes.

C.4.1.6. Common End station Components

All elements of the Sector 24 end station design will recycle designs successfully used in the construction and operation of the NE-CAT beamline 8-BM (see C.4.9).

C.4.1.7. Detailed Optical Designs and Component Listings

Details of the 24-ID-C hutch and optical train layout are provided by Figures C.4.1.3 and C.4.1.4. A tabulation of components indicated in these figures is given in Tables C.4.1.2 and C.4.1.3. These tables also reference the sections of this document providing design and commissioning details for each optical element.

24-ID-E and IV hutch and optics layouts are detailed in Figures C.4.1.5 and C.4.1.6, respectively. Except for 24-BM (bending magnet beamline) a common end station design is used in all build phases. Thus Figure C.4.1.4 and Table C.4.1.3 provide details for the end stations of 24-ID-C, 24-ID-E and IV.

C.4.1.8. Ray Tracing

Bremsstrahlung and synchrotron ray traces are provided for build 24-ID-C, 24-ID-E and 24-BM Figures C.4.1.7 to C.4.1.12.

C.4.1.8.1. Bremsstrahlung Ray Tracing

The principal purpose of the second tungsten bremsstrahlung collimator, present in the secondary optics enclosure (24-ID-B, see Figure C.4.1.7) is to limit the vertical extremal bremsstrahlung rays permitted by the vertical bore of tungsten collimator 4, which would have passed through the vertical bore of lead collimator 7.

Note that a two channel water-cooled high-heat-load fixed copper mask (bore dimensions: 4.5 x 4.5 mm) is placed immediately upstream of the first bremsstrahlung (FOE) collimator to protect the interior bores from possible beam mis-steering. Additional protection for the two tungsten collimators is provided by the 2 sets of power-limiting apertures on the in-board optical line. The bore of the fixed copper mask and the geometry of the tungsten collimator bores provide at least 2 mm clearance between the extremal synchrotron rays and the interior bores of both tungsten collimators. All collimators are mounted on high load support tables with a kinematic surface plate. The surface plate incorporates precision jacks and dove-tail slides for precise alignment of the tungsten mask bores relative to the two undulator beams. The large internal bores of the lead collimators guarantee no interference with the alignment of the tungsten collimators. All synchrotron masks are mounted on the same tables through motor-driven translator stages with independent X,Y, pitch and yaw adjustments. Thus, only the alignment of the tungsten collimators is involved in setting the position of the surface plate of the support tables.

There is an asymmetry in the horizontal bore dimension of the second tungsten collimator, mandated by the minimum clearance between bore interior and the extremal synchrotron rays. Dimensions listed for the lead collimators are those for the interior of the lead bore not the spool piece bore (shielding aperture not optical aperture). The optical aperture of the lead collimators is used in all ray trace diagrams.
C.4.1.8.2. Synchrotron Radiation Ray Tracing – Thermal Protection Design

The vertical synchrotron ray traces for 24-ID-C and 24-ID-E are shown separately in Figures C.4.1.11 and C.4.1.12, respectively. The internal bores of all collimating elements (lead and tungsten) are separated from the nearest extremal synchrotron ray by at least 2 mm. All synchrotron masking is accomplished using a similar dual channel mask design to be supplied by Instrument Design Technology IDT (Manchester U.K.). Two classes of mask are employed: 1) fixed aperture and 2) power-limiting.

A fixed aperture mask consists of a pair of high-heat load collimating elements installed in tandem in the optical train. Each collimating element of the pair is fabricated from 200 mm long oxygen-free copper (OFHC) blocks with internal water cooling channels and two bores: 1) a working bore 4.5 x 4.5 mm in size, centered on one of the two undulator beams; 2) a large 20 x 20 mm “compliance” bore, centered on the opposing undulator beam. The opposite element of the pair has the same configuration, but rotated about the central axis of the beamline by 180°. The working bore follows a 150 mm long lead-in taper with a 3° opening angle in order to keep maximum power density deposited on the OFHC block below 235 W/mm² (maximum beam mis-steering). Both mask elements are mounted on independent Y-Z precision translators. Mask pairs are interconnected via bellows with an internal radius providing 13 mm of clearance relative to the beam. The compliance bore permits unconstrained alignment of the working bore relative to the beam it is intended to mask. The fixed aperture bores are aligned so that both undulator beams pass down the central channel of its respective working bore.

A power limiting mask has a similar configuration to the fixed aperture mask pair, except that both working bores of the mask pair are aligned on the same undulator beam. An L-shaped tungsten blade is brazed into opposing corners of the mask pair’s working bores. The two tungsten blades are then translated along a diagonal connecting the two blades to independently limit the Y and Z extent of the passed beam and thereby the power deposited on downstream monochromatizing elements. Each planned ID monochromator will have a power-limiting mask pair installed immediately upstream of its position.

We contracted with IDT for a complete thermal analysis of the fixed-aperture mask, prior to mask fabrication. This analysis, using the performance characteristics of a full-length undulator A, operating at 200 mA ring current predicted a maximum temperature rise of the OFHC mask block of less than 120 °C under conditions of maximal permitted beam mis-steering. Simulated peak inner coolant wall temperature did not exceed 40 °C, so local coolant boiling was not considered a likely concern. The 24-ID-D power limiter masks will be administratively locked in its most permissive configuration until installation of the 24-ID-D large offset transmission monochromator. The 24-ID-C and 24-ID-E power limiters are placed at positions where the maximum power density is a fraction of that the fixed aperture is exposed to.

Since the canted tandem undulator produces approximately 80% of the power output of a conventional undulator A and since the APS-sanctioned thermal rise for OFHC is 150 °C we should have sufficient operational “head-room” to accommodate a planned 130-150 mA APS operational modes.

The 24-ID-C optical train ends in the SOE with a standard P4-50 integrated collimator- white-beam stop, centered on the outboard undulator beam at approximately 54 m from the center of the straight section. Prior to the installation of the 24-ID-E single-side-bounce monochromator a water-cooled inclined glidcop block will be installed in a spool attached to the downstream flange of lead collimator 7 (48.2 m) to specifically stop the inboard undulator white-beam. This stop was designed by the APS and fabricated locally and is capable of tolerating the unattenuated output of the inboard undulator with a temperature rise < 150 °C. The inboard undulator (sourcing the 24-ID-E optical train) will be administratively “locked” to open gap until the 24-ID-E monochromator and beamline are installed. An APS P-8 monochromatic shutter will be placed immediately after the 24-ID-E monochromator on the monochromatic optical leg that
diverges from the undulator ray with a fixed take off angle of 29.5° (Si 220 tuned to the peak of the selenium K-edge).

Figure C.4.1.2: Sector 24 plan layout.
Figure C.4.1.3: 24-ID-C plan (top) and elevation (bottom) views. See Table C.4.1.2 for component descriptions (24-ID-C components indicated by red lettering).
Table C.4.1.2: Listing of 24-ID-C Optical Components.

<table>
<thead>
<tr>
<th>Component / Function</th>
<th>Figure C.4.13 Indicator</th>
<th>Distance from Sector Straight (m)</th>
<th>Details in Section</th>
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</thead>
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<tr>
<td>Front End Terminal Components</td>
<td>A</td>
<td>25.5</td>
<td>NA</td>
</tr>
<tr>
<td>Mask Exit collimator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dual Fixed Synchrotron Mask Thermal protection</td>
<td>B</td>
<td>32.4</td>
<td>C.4.5</td>
</tr>
<tr>
<td>Tungsten Bremsstrahlung Collimator Lead Bremsstrahlung shield</td>
<td>C</td>
<td>33.0</td>
<td>C.4.5</td>
</tr>
<tr>
<td>Gladcop Power Limit Mask Limits thermal load of monochromator first crystal</td>
<td>D</td>
<td>51.4</td>
<td>C.4.5</td>
</tr>
<tr>
<td>Kohzu HLD8-24 LN$_2$-cooled, double crystal monochromator Energy range: 4.5 – 25 keV 1-2 eV energy resolution Crystal cut: Si 111</td>
<td>E</td>
<td>52.5</td>
<td>C.4.6</td>
</tr>
<tr>
<td>Oxford-Danfysik Cryopump Monochromator cooling</td>
<td>F</td>
<td>NA</td>
<td>C.4.6</td>
</tr>
<tr>
<td>P4-50 Integrated monochromatic shutter, white mask and stop</td>
<td>G</td>
<td>54.0</td>
<td>C.4.5</td>
</tr>
<tr>
<td>Oxford Danfysik UHV quadrant diode beam position monitor</td>
<td>H</td>
<td>55.5</td>
<td>C.4.9</td>
</tr>
<tr>
<td>Oxford-Danfysik integrated Kirk-Patrick Baez focusing system 4-point, elliptical benders 1.2 1.0 mirrors (h,v) Rh, Pt, bare strips Demagnification (h,v): 7.5:1 9.5:1</td>
<td>I</td>
<td>57.0</td>
<td>C.4.7</td>
</tr>
<tr>
<td>Larry-Rock Associates auxillary support stand End station collimator Slits Attenuators shutters Quadrant-diode Beam Position Monitor Ionization, Fluorescence monitors</td>
<td>J</td>
<td>62.5</td>
<td>C.4.9</td>
</tr>
<tr>
<td>Larry-Rock Associates goniometer Support, collimator translators Huber 515m Goniometer Video Microscopes Oxford Instruments CryoJet</td>
<td>K</td>
<td>64.0</td>
<td>C.4.9</td>
</tr>
<tr>
<td>Larry-Rock Associates Detector Support ADSC Q315 9-element CCD detector</td>
<td>L</td>
<td>65.0</td>
<td>C.4.9</td>
</tr>
</tbody>
</table>
Figure C.4.1.4: 24-ID-C, 24-ID-E & IV end station. See Table C.4.1.3 for component descriptions (components indicated by red lettering).

Table C.4.1.3: Listing of 24-ID-C, 24-ID-E, and IV End Station Components.

<table>
<thead>
<tr>
<th>Component / Function</th>
<th>Figure C.4.14 Indicator</th>
<th>Distance from Sector Straight (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 cm x 6 cm x 0.2 mm Beryllium Window Terminates ultra-high vacuum</td>
<td>A</td>
<td>62</td>
</tr>
<tr>
<td>Larry-Rock Associates Auxiliary Support Stand Support for upstream gimbal of collimator Assembly. Transverse and lift translations of upstream end of collimator assembly</td>
<td>B</td>
<td>62.5</td>
</tr>
<tr>
<td>JJ-xray X-Y vacuum slit screen array Monochromatic beam defining slits.</td>
<td>C</td>
<td>62.2</td>
</tr>
<tr>
<td>Oxford Danfysik quadrant diode beam position Monitor. Principal beam position monitor</td>
<td>D</td>
<td>62.7</td>
</tr>
<tr>
<td>XIA attenuator assemblies. Pneumatic safety shutter Beam attenuation, interlocking slow shutter</td>
<td>E</td>
<td>62.9</td>
</tr>
<tr>
<td>Component / Function</td>
<td>Figure C.4.14 Indicator</td>
<td>Distance from Sector Straight (m)</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------------------</td>
<td>-------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Telescoping collimator assembly. Vertical counter mass, pneumatic compensator</td>
<td>F</td>
<td>63</td>
</tr>
<tr>
<td>ADC blue slits (guard slits)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miniature ionization monitor</td>
<td>G</td>
<td>64</td>
</tr>
<tr>
<td>Scatter guard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast rotary shutter (data shutter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huber 515 m phi goniometer with X,Y,Z sample servos.</td>
<td>H</td>
<td>64</td>
</tr>
<tr>
<td>Larry-Rock Beam Stop assembly, X,Y motorized beam stop translation. Pneumatic beam stop withdrawal system.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertical and Horizontal video microscopes.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxford Instruments Cryojet and mount.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTS cooling system.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larry-Rock Associates Detector lift mechanism. Differential lift (pitch), transverse motion of detector. Newall magnetic hall effect lift position encoders.</td>
<td>J</td>
<td>64.2 – 65.4</td>
</tr>
<tr>
<td>ADSC Q315 9-element CCD area detector</td>
<td>K</td>
<td>64.2 – 65.4</td>
</tr>
<tr>
<td>Newall magnetic hall effect position encoder for sample to detector distance</td>
<td>L</td>
<td>66</td>
</tr>
<tr>
<td>Larry-Rock Associates A-Frame Detector Support Longitudinal detector position.</td>
<td>M</td>
<td>66</td>
</tr>
</tbody>
</table>

**Table C.4.1.3** (continued): Listing of 24-ID-C, 24-ID-E, and IV End Station Components
**Table C.4.1.4:** Listing of Phase 24-ID-E Optical Components.

<table>
<thead>
<tr>
<th>Component / Function</th>
<th>Figure C.4.15 Indicator</th>
<th>Distance from Sector Straight (m)</th>
<th>Details in Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-ID-E glidcop monochromator power-limiting apertures</td>
<td>A</td>
<td>47</td>
<td>C.4.5</td>
</tr>
<tr>
<td>Tungsten Bremsstrahlung Collimator Lead Bremsstrahlung shield</td>
<td>B</td>
<td>47.7</td>
<td>C.4.5</td>
</tr>
<tr>
<td>Oxford Danfysik 2-energy, single-crystal side bounce monochromator LN$_2$ / H$_2$O crystal cooling 29.65° takeoff angle Crystal cuts: Si 220, 311 Energy set points: 12.66 14.78 keV</td>
<td>C</td>
<td>48.5</td>
<td>C.4.7</td>
</tr>
<tr>
<td>Oxford-Danfysik Cryopump Monochromator cooling</td>
<td>D</td>
<td>NA</td>
<td>C.4.6</td>
</tr>
<tr>
<td>Water-cooled inboard white beam stop</td>
<td>E</td>
<td>49</td>
<td>C.4.5</td>
</tr>
<tr>
<td>Modified P8 monochromatic shutter</td>
<td>F</td>
<td>49.5</td>
<td>C.4.5</td>
</tr>
<tr>
<td>Oxford Danfysik horizontal focusing mirror. 4-point elliptical bender 1.2 m ULE mirror. Rh, Pt, bare strips Demagnification: 5.96 : 1</td>
<td>G</td>
<td>50.8</td>
<td>C.4.8</td>
</tr>
<tr>
<td>Oxford Danfysik vertical focusing mirror. 2-point bender 0.8 m ULE mirror. Rh, Pt, bare strips Demagnification: 11.1 : 1</td>
<td>H</td>
<td>54.4</td>
<td>C.4.8</td>
</tr>
</tbody>
</table>
Figure C.4.5: Plan (top) and elevation (bottom) views of 24-ID-E beamline. Components (labeled in red) are listed in Table C.3.1.4.
Figure C.4.1.6: Plan view of Phase IV beamline. Components (labeled in red) are listed in Table C.4.1.6.
**Table C.4.1.5**: Listing of 24-ID-D Optical Components.

<table>
<thead>
<tr>
<th>Component / Function</th>
<th>Figure C.4.15 Indicator</th>
<th>Distance from Sector Straight (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-ID-D monochromator power-limiting apertures (exists, in place)</td>
<td>A</td>
<td>27.3</td>
</tr>
<tr>
<td>Bragg-transmission diamond monochromator Pure-rotational component</td>
<td>B</td>
<td>28</td>
</tr>
<tr>
<td>Water-cooled diamond (111)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target energy range: 8.5-17 keV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>To be designed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bragg-transmission diamond monochromator Rotation+translation component</td>
<td>C</td>
<td>30.5</td>
</tr>
<tr>
<td>1.5 m horizontal offset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>To be designed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P8 shutter (on hand)</td>
<td>D</td>
<td>33.3</td>
</tr>
<tr>
<td>Kirk-patrick Baez focusing system</td>
<td>E</td>
<td>36.3</td>
</tr>
<tr>
<td>To be designed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End Station</td>
<td>F</td>
<td>42</td>
</tr>
<tr>
<td>Similar to 24-ID-C and 24-ID-E</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure C.4.1.7: Horizontal bremsstrahlung ray trace for 24-ID-C and 24-ID-E.
Figure C.4.1.8: Vertical bremsstrahlung ray trace for 24-ID-C.
Figure C.4.1.9: Vertical bremsstrahlung ray trace for 24-ID-E.
Figure C.4.1.11: Vertical synchrotron ray trace for 24-ID-C.
Figure C.4.1.12: Vertical synchrotron ray trace for 24-ID-E.
C.4.2. Hutch Construction

C.4.2.1. Layout and Timeline

Detailed design of the radiation enclosures (hutches) and utilities (electrical, water, compressed air and distribution) layouts for Sector 24 24-ID-C, 24-ID-E and 24-ID-D were completed, reviewed and approved by APS and let for contract in September of 2002. NE-CAT followed the general hutch layout of SGX-CAT with some revisions to all hutch floor plans, intended to increase floor space for the 24-ID-E monochromator in the secondary optics enclosure (SOE) and a longer 24-ID-D end station. An additional door on the outboard face of the SOE was added to improve access to 24-ID-E optical components. Table C.4.2.1 lists the lead shield thickness required for each hutch wall. Table C.4.2.2 lists all hutch entryways. Figure C.4.2.1 shows the general Sector 24 hutch plan.

Designs for construction of the 24-BM (bending magnet beamline) hutches were completed in late summer of 2005. Construction of 24-BM hutches and utilities is expected to begin in the fourth quarter, 2005.

NE-CAT (24-ID-C, 24-ID-E and 24-ID-D) and GMCA-CAT hutch contracts were sent out for bid as a combined package in order to exploit the economies of scale. The two contracts were also consolidated in an attempt to derive a rational and non-competitive construction schedule for both projects. The U.S. firm Tecknit won the bid and contracts for both sectors were finalized on February 17, 2003.

APS scheduled installation of Sector-24’s front end and upstream undulator for the fall 2003 long shutdown (October). Both undulators were installed during the spring shutdown of 2004. Radiation leak checking of 24-ID-C hutches occurred immediately after the Personal Safety System was installed by the APS in December of 2003. All utility distribution systems were installed immediately following hutch assembly and finishing. All utility distributions were run along the inboard side of the hand rail system running around the hutch roof margins.

All hutches were equipped with bridge cranes of 2 ton capacity to facilitate installation and handling of optical and end station components. All cranes run parallel to the white and/or monochromatic beam trajectories in each hutch. An extensive, two-tier cable tray system was installed to support power, control and signal cable distributions.

In February 2004 air conditioning ducting was installed in all the insertion device end-station hutches (24-ID-C, 24-ID-D and 24-ID-E) to offset thermal loads generated from electronics racks to be installed in them. NE-CAT contracted with Quality Cryogenics (Memphis TN) to design a liquid nitrogen distribution system to supply the 24-ID-C and 24-ID-E monochromator cryopumps and the end station sample cryo coolers. APS provides each sector with a liquid nitrogen distribution point, located on the inner mezzanine wall of the accelerator. Delivery and installation of the liquid nitrogen distribution system by NE-CAT staff occurred in April 2004. All Sector 24-ID hutches were ready for beneficial occupancy in April 2004.

C.4.2.2. Radiation Enclosure Specification

The lead shielding wall thickness for each hutch, was designated in accordance with the Guide to Beamline Shielding Design at the Advanced Photon Source (May 2005). Table C.4.2.1 summarizes required lead shielding thickness on a per-wall, per-hutch basis. Table C.4.2.2 summarizes all hutch door specifications.
Table C.4.2.1: Lead Thickness Requirements per Hutch.

<table>
<thead>
<tr>
<th>Hutch</th>
<th>Upstream Wall Panel (mm)</th>
<th>Lateral Panel (mm)</th>
<th>Roof Panel (mm)</th>
<th>Downstream Wall Panel (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-ID-A White Beam</td>
<td>NA</td>
<td>19</td>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td>24-ID-B White Beam</td>
<td>19</td>
<td>19</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>24-ID-C Mono Beam</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>24-ID-D Mono Beam</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>24-ID-E Mono Beam</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>

Table C.4.2.2: Hutch Door Inventory.

<table>
<thead>
<tr>
<th>Enclosure ID</th>
<th>Type</th>
<th>Placement</th>
<th>Figure C.4.2.1 Key</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-ID-A</td>
<td>Recessed, Triple Panel: 1) upstream door opens pneumatically, upstream. 2) Middle door opens manually up or downstream. 3) Downstream door opens manually, downstream.</td>
<td>Outboard Wall</td>
<td>A</td>
</tr>
<tr>
<td>24-ID-B</td>
<td>Recessed, Double Panel: 1) Upstream door opens manually, upstream. 2) Downstream door opens pneumatically downstream</td>
<td>Upstream, Outboard Wall</td>
<td>C</td>
</tr>
<tr>
<td>24-ID-B</td>
<td>Recessed, Double Panel: 1) Upstream door opens pneumatically, upstream. 2) Downstream door opens manually, downstream.</td>
<td>Downstream, Inboard Wall</td>
<td>D</td>
</tr>
<tr>
<td>Enclosure ID</td>
<td>Type</td>
<td>Placement</td>
<td>Figure 4.2.1 Key</td>
</tr>
<tr>
<td>--------------</td>
<td>------</td>
<td>-----------</td>
<td>------------------</td>
</tr>
</tbody>
</table>
| 24-ID-C      | Non-recessed, Double Panel:  
1) Upstream door opens manually, upstream.  
2) Downstream door opens pneumatically downstream. | Downstream, Outboard Wall | G |
| 24-ID-D      | Non-recessed, Double Panel:  
1) Upstream door opens manually, upstream.  
2) Downstream door open pneumatically, downstream. | Outboard Wall | B |
| 24-ID-E      | Non-recessed, Double Panel:  
1) Inboard door opens pneumatically, inboard.  
2) Outboard door opens manually, outboard. | Downstream Wall | F |
| 24-ID-E      | Non-recessed, Double Panel:  
1) Upstream door opens manually, upstream.  
2) Downstream door opens pneumatically, downstream. | Wall Parallel to Mono Beam | E |
| 24-BM-A      | Recessed, Double Panel: Down Stream Panel double width  
1) Upstream door (single width) opens pneumatically, upstream.  
2) downstream door (double width) opens manually downstream. | Outboard Wall | H |
| 24-BM-B      | Recessed, Double Panel: Upstream Panel Double width  
1) Upstream door (double width) opens manually, upstream.  
2) Downstream door (single width) opens pneumatically downstream | Outboard Wall | I |

*Table C.4.2.2 (continued): Hutch Door Inventory.*
**Figure C.4.2.1:** General Sector 24 hutch layout. Black: bridge cranes. Red-hatched: accelerator saw-tooth wall. Green: egress ways. Red lettering: door placements, see Table C.4.2.2.
C.4.3.  **Tandem undulator / front end installation**

NE-CAT’s plans to build multiple undulator beamlines within a single APS sector depend upon a recent APS technical development: the tandem offset undulator (TOU). Figure C.4.3.1 shows a conceptual diagram of the APS TOU.

![Figure C.4.3.1: Top: rendering of TOU. Bottom: TOU functional schematic. Center of accelerator is towards the vertical direction. Angular offsets are greatly exaggerated.](image)

The TOU generates two, essentially independent white X-ray beams within a single APS straight by inducing an electron trajectory offset between two shortened undulator A’s, via a “dogleg” dipole, situated between them. A small dipole placed upstream of the TOU induces a 0.5 mrad outboard excursion (relative to accelerator center) of the electron beam before it passes through the first undulator. The dogleg induces a 1.0 mrad inboard excursion in the electron beam trajectory before it enters the downstream undulator gap. Finally, a second dipole, downstream of the second undulator forces the electron beam back onto the nominal trajectory, through a 0.5 mrad outboard trajectory offset. The two white X-ray beams exit the TOU with a 1.0 mrad angular separation, on a center line that is slightly offset, outboard from the nominal X-ray exit trajectory.

The gaps of the two undulators are set independently. Two complete sets of particle and X-ray beam position monitors are installed in the TOU straight so that the accelerator control system
can monitor and regulate the electron beam trajectory through both undulators, to minimize potential “cross-talk” between the spatial positions and trajectories of the two X-ray beams induced by independent undulator gaps changes.

The magnetic structures used in the TOU are identical to a standard undulator A (UA) except for a reduced length (number of magnetic periods reduced from 72 to 60). Thus, each TOU undulator produces an X-ray beam of reduced power compared to an UA, otherwise the spectral properties of the TOU beams are identical to those of an undulator A (Figure C4.3.2) (25).

![Figure C.4.3.2: Calculated on-axis brilliance tuning curves for the first, third and fifth harmonics (left to right) for standard (2.4 m) and shortened undulator A’s. The curve family related to L=2.1 m corresponds to the undulators used in the TOU. Beam energy 7.0 GeV, current 100 mA (25).](image)

At the maximum planned accelerator operating current (200 ma), the APS TOU will generate nearly 20.4 kW with both undulators set at minimum gap (10.5 mm). This output corresponds to a power density of approximately 280 kW/mrad² (normal incidence) for front end components. These issues necessitated a complete re-design of the APS undulator front end system (masks, photon shutters, bremsstrahlung shielding, collimators) in support of safe TOU operation.

APS Experimental Facilities Division (XFD) completed initial design of the tandem undulator and front end by early 2003 and immediately let contracts for fabrication of front end components for Sectors 21, 23 and 24 (LS-CAT, GMCA and NE-CAT, respectively). The undulators and front-ends for Sector 23 and 24 were installed during the fall of 2003 APS shutdown.

APS XFD released the front end design for inspection by GMCA and NE-CAT in late summer of 2003. The designs for the exit collimators and safety shutters were found wanting on a number of safety-related and operational issues. An independent committee from APS-Accelerator Systems Division, including members of GMCA and NE-CAT met to review the front end designs in August of 2003. The review committee found that the designs for the exit collimator, some of the front-end bremsstrahlung shielding and the safety shutters did not adhere to APS safety engineering policy and required revision. XFD staff eventually agreed to a redesign of effected front end components. However, both GMCA and NE-CAT asked that in order to maintain their
commissioning schedules, that the APS proceed with the installation of the initial, faulty front end components and be permitted limited operation in order to commission upstream radiation enclosures. APS agreed to this request and on December 3, 2003 both GMCA and NE-CAT commissioned their FOE’s, demonstrating stable operation of both tandem undulator installations.

APS XFD completed the redesign of the tandem undulator front end components and submitted these designs for fabrication in January of 2004. Mitigation of the exit collimators and front end bremsstrahlung shield faults were complete by March of 2004. The revised safety shutters were installed in the spring 2004 APS shutdown, permitting routine operation of the NE-CAT tandem undulator and front end.

On March 23, 2004, following delivery and installation of the Sector 24 fixed aperture masks the Second Optics Enclosure (24-ID-B) was radiation commissioned. This commissioning milestone required the installation of the entire Sector 24 (temporary) front end and all 24-ID-C and 24-ID-E masks and bremsstrahlung collimators, the white beam transport system, as well as the 24-ID-C integrated shutter (in the SOE). In 24-ID-B we installed a temporary beryllium window, protected by a He purge, bounded by dual diamond exit windows. After surveying operations, the white beam was successfully transported to the SOE with minimal beam steering required. Both undulator white beams were of roughly the same power density and were steered successfully to target positions with in the SOE (Figure C.4.3.3). On March 25, 2004, the FOE, white beam transport and the SOE were validated via APS radiation safety protocols.

![Image of X-ray sensitive "burn-paper" showing positions of in-board (right) and out-board (left) undulator beams (accelerator operating at minimum current).](image)

Figure C.4.3.3: Both undulator beams projected to SOE. Photo of X-ray sensitive “burn-paper” showing positions of in-board (right) and out-board (left) undulator beams (accelerator operating at minimum current).

C.4.4. **Personnel Safety and Equipment Protection Systems**

C.4.4.1. Personnel Safety System

The Personnel Safety System (PSS) and the Equipment Protection Systems (EPS) are two critical interlock systems intended to protect users and beamline components (respectively)
from radiation-induced damage or degradation. The principal function of the PSS system is control of the state of front-end and beamline shutters such that it is impossible to expose beamline personnel to radiation. The EPS is actually an ancillary sensing subsystem of the PSS and forces closing (or inhibits opening) of the front end shutters when some operational condition of the beamline necessary for safe operation of the beamline is not satisfied (e.g. unacceptably high vacuum or inadequate cooling fluid flow to some critical optical component).

The PSS system consists of a set of distributed Programmable Logic Controllers (PLC) whose ultimate output is a go/nogo signal to pneumatic actuators for the front end safety and photon shutters (which control the white beam access state of all beamlines in a sector). All hutch doors, front end and beamline shutters, present redundant state signals to the PSS logic that guarantee accurate representation of the physical state of said doors and shutters to the PSS. Part of the PSS system is a sequence of switches in each hutch (call PSS search switches) that must be activated in a proscribed sequence in order to condition the PSS to a state in which it (the PSS) will permit opening of the front end shutters. The locations of the PSS search switches within a hutch are intended to force a physical inspection of the hutch by the operator prior to shutter open grant condition. A set of accessible emergency override switches is also present in hutch that allow an operator to override the search state of the PSS and block shutter opening.

The APS is responsible for design and implementation of all elements of the PSS, but interact with the beamline designers in order to produce a PSS system that satisfies DOE and APS safety standards while presenting beamline users with a minimally intrusive operational protocol. NE-CAT is responsible for all costs associated with PSS design and implementation. APS is responsible for all maintenance of the PSS, in perpetuity. The APS has completed installation of the PSS systems for the First Optics Enclosure (24-ID-A), the Secondary Optics Enclosure (24-ID-B) and two end stations ID-24-C and ID-24-E. PSS systems for the end station ID-24-D and both bending magnet beamline hutch (24-BM-A, 24-BM-B) will be installed in the near future.

APS has publicly discussed the possibility of a mandatory global revision of the PSS standard for all APS sectors. The new APS PSS would be constructed from currently available, advanced PLC modules, and maximize standardization of the PSS implementations across all APS sectors. This plan, if adopted, will incur unanticipated costs to all existing APS beamlines, but will minimize recurrent costs to the APS for maintenance of the PSS infrastructure. Current, per hutch cost of a PSS system is of order $75K. Thus, this policy could significantly impact NE-CAT financial planning.

C.4.4.2. Equipment Protection System

The EPS system is designed and implemented by beamline staff. The function of the EPS is to provide a go/nogo signal (grant) to the PSS that forces closure (or inhibits opening) of front end shutters when some state of the beamline falls outside of normal operational range. The NE-CAT EPS, like the PSS is constructed from distributed PLC elements. Each beamline has its own dedicated EPS, but these beamline-specific EPS's may share sensor systems (e.g. flow sensors for masks and collimators having apertures for both inboard and outboard white beams). All EPS PLC nodes can be queried by beamline control systems via ethernet for state information. A master PLC node aggregates the go/nogo state of all subsidiary nodes to provide a master grant to the PSS.

EPS PLC nodes monitor both analog and two-state logic elements, including:

1) Cooling flow rates to masks, collimators, monochromator crystals, mirrors and compton shields (analog / binary)
2) Ionization vacuum gauges (analog / binary).
3) Gate valve open/close state (binary).
4) Thermocouple sensors monitoring monochromator crystal temperature (analog / binary).

Figure C.4.4.2 is a connection diagram for the 24-ID-C EPS. The rectangular blocks represent individual EPS PLC nodes (master node at top). Each node consists of a DirectLogic DL-250 process/bus module with multiple A/D or binary I/O PLC modules installed. The logic state and the state of individual input or output lines, and analog levels are accessible to the Console super client (see C.4.9) via Ethernet through the HEI remote procedure call server. EPS PLC modules can be programmed remotely via their ethernet connections.
Figure C.4.4.1: Sector PSS layout.
Figure C.4.4.2: Connection diagram of 24-ID-C EPS system. Master PLC node at top. The EPS interfaces to the PSS through the "Master Grant" line exiting the diagram at the top.

C.4.5. 24-ID-C and 24-ID-E Mask, Beam Stop and Shutter

An important component of the NE-CAT optical plan was design of thermal masks, bremsstrahlung collimators and shields, white beam stops and shutters. In most instances we could not make direct use of APS reference designs due to the fact that NE-CAT implementation of these optical elements had to provide masking and shielding for two, closed spaced undulator white beams and consequent increased power loadings. Figure C.4.5.1 is a Sector 24 layout drawing showing the placement and nature of masks, collimators, beam stops and shutters.
Figure C.4.5.1: Sector 24 mask, collimator, beam stop and shutter plan.
C.4.5.1. Fixed Apertures and Power-Limiting Masks

All masks and power limiting structures (thermal shielding) were designed and fabricated by Instrument Design Technology Ltd. (Manchester, U.K.) to NE-CAT specification for construction of 24-ID-C, 24-ID-E and 24-ID-D. Thermal shielding designs fall in to two categories:

1) Fixed aperture masks: a pair of fixed apertures located near the downstream wall of the First Optics Enclosure (FOE). The purpose of the fixed aperture masks (Figure C.4.5.2.) is to delimit the effective white beam steering cones. This action protects all optical elements downstream of the masks from heat-induced damage resulting form large mis-steering events of the two undulator white beams.

2) Power Limiting Masks: a pair of power-limiting masks situated just upstream of each of the three undulator monochromators. The power-limiting masks (Figure C.4.5.3) constitute a variable aperture (in both directions orthogonal to the beam), that can be used to limit the power load deposited on the first crystal of all undulator monochromators.

NE-CAT’s mask designs are derived from proven APS standard designs, but modified to accommodate two white undulator beams with an angular separation of 1 mrad. Both the fixed aperture and the power-limiting masks consist of two long, complementary glidcop mask elements, installed in series (Figure C.4.5.4). The first element of a mask pair has a narrow rectangular-conical defining bore that determines the cross-section of one of the two white beams. The same mask element also has a large compliance bore to permit unimpeded passage of the opposing undulator beam. The following element of the pair is identical to the first, but rotated about the longitudinal axis by 180°, to operate on the complementary undulator beam. The conical bores have an opening angle of 3°, with an input aperture significantly larger than the beam mis-steering footprint permitted by the front end exit aperture. Both elements of each mask pair are mounted on separate X-Y translation stages. The fixed apertures are situated 32.3 m from the center of the sector straight and have defining apertures 3 x 2 mm (H x V).

The power limiting mask elements have a 5 x 5 mm bore with a tungsten chamfered L-shaped slit blade brazed into opposing corners (between mask pairs) to serve as the actual active surface of the mask.

Prior to construction, all mask designs were subjected to exhaustive finite-element simulations of thermal and mechanical stress response. These studies mandated a significant modification of the design of the fixed aperture geometry (chamfering of the actual bore) was necessary to extend the functional life-time of the apertures to 20-years. Figure C.4.5.5 shows examples of the graphical output from one of the finite element thermal-stress simulations of a fixed aperture mask.
Figure C.4.5.2: Conceptual Design of a Fixed Aperture Mask. The two undulator beams are indicated as “downstream” (=inboard, red) and “upstream” (=outboard, green).

Figure C.4.5.3: Conceptual Design of a Power-Limiting Mask.
Figure C.4.5.4: **Left:** View of downstream sides of fixed aperture masks. The out-board beam mask is shown on the right (in-board beam mask on left). The small oval-shaped bores are the working bores (2x3 mm, HxV). The large square bores are the compliance (bypass) bores, permitting un-constrained alignment of a mask relative to the white undulator beam it is intended to mask, without interference with the complimentary beam. The main body of the mask is fabricated from a single block of glidcop, using electron beam machining. Explosion bonding was used to attach 8” flanges to the mask bodies. The masks are attached to a motorized X-Y translator via a kinmatic mount that permits un-impeded thermal expansion or contraction of the mask body. **Right:** View toward downstream wall of 24-ID-A (FOE), showing (in downstream order): 1) outboard beam fixed aperture mask; 2) inboard beam fixed aperture mask; 3) first tungsten bremsstrahlung collimator; 4) first lead bremsstrahlung collimator; 5) ion pump cross; 6) gate valve delimiting the white beam transport tube.

Figure C.4.5.5: **Left:** Simulated equilibrium temperature of a glidcop fixed mask aperture under conditions of maximal permitted mis-steering of the white beam. **Ring current:** 200mA Thermal model 3859 Watts Maximum temp. 173 °C. **Right:** Simulated thermal stress of a glidcop fixed mask aperture. **Ring current:** 200m A Thermal model 3859 Watts. Maximum Thermal Stress 261 MPa.
C.4.5.2. Bremsstrahlung-shielding

Like the synchrotron thermal masks, all bremsstrahlung shielding and mask designs used in the NE-CAT optical layout were derived, by NE-CAT from standard APS designs, modified to accommodate two tandem white beams designs (Figures C.4.5.6, C.4.5.7). The final bremsstrahlung shielding plan consists of two groupings of a tungsten aperture, followed by a lead collimator assembly, located near the downstream wall of the FOE and the upstream wall of the SOE. The shielding designs were reviewed and accepted by APS safety authorities and a contract let with Johnson-Ultravac (Ontario, CA) for fabrication. Delivery of finished components occurred in fall of 2003.

Figure C.4.5.6: Schematic of dual-channel tungsten bremsstrahlung collimator.
Figure C.4.5.7: Schematic of lead bremsstrahlung collimator.

C.4.5.3. Beam Stops

All monochromatic beams stops used in the NE-CAT optical plan were based upon standard APS designs. Table C.4.5.1 lists these shutters and the APS design provenance for them. All shutters used in the ID line constructed were contracted for fabrication by Johnson Ultravac (Ontario CA), using designs provided by NE-CAT.

Table C.4.5.1: Tabulation of Beam Stops Used in Construction.

<table>
<thead>
<tr>
<th>Effected End Station</th>
<th>Location</th>
<th>Function</th>
<th>APS Reference Designation</th>
<th>Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-ID-C</td>
<td>D.S. 24-ID-B</td>
<td>Photon Shutter</td>
<td>P4-50</td>
<td>No</td>
</tr>
<tr>
<td>24-ID-E</td>
<td>O.B. 24-ID-B</td>
<td>Photon Shutter</td>
<td>P8</td>
<td>Yes</td>
</tr>
<tr>
<td>24-ID-D</td>
<td>D.S. 24-ID-A</td>
<td>Photon Shutter</td>
<td>P8</td>
<td>Yes</td>
</tr>
<tr>
<td>NA</td>
<td>24-ID-B</td>
<td>Inboard Undulator White Stop</td>
<td>New design</td>
<td>NA</td>
</tr>
<tr>
<td>24-BM-B</td>
<td>24-BM-A</td>
<td>Photon Shutter</td>
<td>P7-20</td>
<td>Yes</td>
</tr>
</tbody>
</table>

We modified an APS monochromatic shutter design used at Sector 2 back scatter-beamline that has a very compact design, compatible with the limited space available in the SOE for the 24-ID-E photon shutter (P8 APS reference design; see Figure C.4.5.8). The same shutter design will be used as the photon shutter for NE-CAT’s 24-ID-D construction (large offset diamond transmission monochromator); adjustments hold the spool and beam stop in a sufficiently well...
defined position to avoid administrative controls on the unused inboard undulator. Two steel struts were welded between the two flanges of the beam stop to provide additional rigidity for the unsupported downstream (leftward) flange and bellows assembly.

A custom, non-moveable white beam stop to block the inboard undulator white beam was designed, fabricated and installed to permit safe operation of 24-ID-C (which uses the outboard undulator beam), without administrative controls on the inboard undulator gap controls. This device was designed by Sushil Sharma’s group (APS ASD-ME) and consists of a 50 cm long water-cooled glidcop cylinder with an internal conical bore (2 degree opening angle) brazed onto an 8” conflat flange. Also attached to the flange is a 1” tube comprising a vacuum transport for the outboard undulator white beam.

After installation of the 24-ID-E single-crystal side-bounce monochromator, this structure will be mounted on a support stand downstream of the 24-ID-E monochromator, and used as the permanent white beam stop for the inboard projecting undulator beam (Figures C.4.5.9, C.4.5.10).

Figure C.4.5.8: Compact monochromatic shutter for 24-ID-C and 24-ID-D beamlines. This design is a modification of the APS P8 monochromatic shutter. Our design incorporates a spool for connecting an ion pump to the shutter vacuum vessel and strengthening of the supporting gussets.

Figure C.4.5.9: Cut-away rendering of 24-ID-E white beam stop (view from below, relative to operating configuration. White beam from inboard undulator enter tapered channel (yellow) and spreads out over a large area of the glidcop beam stop surface (tan). Beam stop has an internal bore (blue) for water circulation). The tube (light grey) below the beam stop is the transport spool for the outboard undulator (24-ID-C).
C.4.6. **24-ID-C Monochromator**

In this section we summarize development and commissioning of the 24-ID-C monochromator. The 24-ID-C monochromator is the first release of a new monochromator from Kohzu Precision (model HLD8-24). NE-CAT adopted a liquid nitrogen-cooled first and second crystal design developed by APS (Sector 4 ID) for use in the monochromator. The invar crystal mounting frame and cooling system was fabricated locally. First and second crystals were fabricated by Crystal Scientific Ltd, Warwickshire, UK.

The Kohzu HLD8-24 is a double crystal, fixed exit geometry device. Figure C.4.6.1 is a schematic of the 24-ID-C monochromator. The pivot point of the Bragg axis is a point co-linear with the second crystal diffraction face. Therefore a small adjustment of the vertical axis of the first crystal is required to maintain fixed exit geometry. Table C.4.6.1 calls out the mechanical and optical characteristics of the system.

The monochromator was commissioned, offline, prior to installation in the SOE. After repair of damage to the monochromator incurred during shipment from Japan, vacuum conditioning of the system was attempted. Following a month of continuous pumping we failed to achieve minimum acceptable vacuum performance. Kohzu was called in to mitigate a number of vacuum-related design and fabrication deficiencies thought to affect vacuum performance (eg. lack of vacuum relief holes for major fasteners, lack of heat conditioning of mechanical components, other sources of trapped gas volume). The monochromator was disassembled and the vacuum vessel was chemically cleaned and subjected to a low temperature heat soak. We incrementally reassembled the monochromator and assayed for improvement in vacuum. Finally, after achieving reasonably high vacuum stability ($< 10^{-8}$ torr) we installed the crystal assemblies and surveyed the monochromator into position within hutch 24-ID-B (SOE).

The first (Figure C.4.6.2) and second (Figure C.4.6.3) crystal assemblies were assembled and leak-checked. We completed installation and testing of the monochromator’s liquid nitrogen cooling system and first light was brought into the 24-ID-C experimental enclosure on June 24
2004. With monochromatic light available, testing of the monochromator’s motion control system revealed additional design and manufacturing problems associated with the second crystal motion axes, which were remedied by Kozhu technicians in July 2004.

Since installation, we have worked to characterize and improve all principal optical performance parameters of the monochromator, including spectral bandpass and range, rocking curve widths, along with spectral and positional stability.

**Figure C.4.6.1**: Schematic of Kohzu HLD8-24. **Cyan**: vacuum vessel. **Blue**: 1st and 2nd crystals. **Orange**, **Grey**, **Red**: translation and rotation mechanism. **Green**: cooling lines.
Table C.4.6.1: Characteristics of the Kohzu HLD8-24 Monochromator.

<table>
<thead>
<tr>
<th>Item</th>
<th>Characteristic / Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bragg Axis $Q$</td>
<td>Range $-0.5 - 40^\circ$</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.1 arcsec / servo step</td>
</tr>
<tr>
<td>Repeatability</td>
<td>1 arcsec</td>
</tr>
<tr>
<td>Max Angular Velocity</td>
<td>$0.5^\circ$ / sec</td>
</tr>
<tr>
<td>Crystal Cooling</td>
<td>Liquid Nitrogen / Water</td>
</tr>
<tr>
<td>Nominal Vertical Offset</td>
<td></td>
</tr>
<tr>
<td>Between white and monochromatic beam</td>
<td>20 -25 mm</td>
</tr>
<tr>
<td>Ultimate Vacuum</td>
<td></td>
</tr>
<tr>
<td>Ion Pump</td>
<td>$1 \times 10^{-7}$ torr</td>
</tr>
<tr>
<td>Cryopump</td>
<td>$3 \times 10^{-8}$ torr</td>
</tr>
<tr>
<td>First Crystal:</td>
<td></td>
</tr>
<tr>
<td>Vertical Translation</td>
<td>Range $25$ mm</td>
</tr>
<tr>
<td>Resolution</td>
<td>$0.2 \mu$</td>
</tr>
<tr>
<td>Repeatability</td>
<td>$1 \mu$</td>
</tr>
<tr>
<td>Roll, Pitch error</td>
<td>3 arcsec</td>
</tr>
<tr>
<td>Second Crystal:</td>
<td></td>
</tr>
<tr>
<td>Transverse Translation</td>
<td>Range $12.5$ mm</td>
</tr>
<tr>
<td>Resolution</td>
<td>$0.2 \mu$</td>
</tr>
<tr>
<td>Repeatability</td>
<td>$1 \mu$</td>
</tr>
<tr>
<td>Rotation $Q_2$</td>
<td>Range $\pm 1^\circ$</td>
</tr>
<tr>
<td>Stepper-driven Arc</td>
<td>Resolution $0.05$ arcsec</td>
</tr>
<tr>
<td>Repeatability</td>
<td>$2$ arcsec</td>
</tr>
<tr>
<td>DPT Trim</td>
<td>Range $\pm 60$ arcsec</td>
</tr>
<tr>
<td>Repeatability</td>
<td>$0.5$ arcsec</td>
</tr>
<tr>
<td>Longitudinal Translation</td>
<td>Range $120$ mm</td>
</tr>
<tr>
<td>Resolution</td>
<td>$2\mu$</td>
</tr>
<tr>
<td>Repeatability</td>
<td>$5\mu$</td>
</tr>
<tr>
<td>Yaw, Pitch error</td>
<td>10 arcsec</td>
</tr>
<tr>
<td>Support Table:</td>
<td></td>
</tr>
<tr>
<td>Transverse Translation</td>
<td>Range $\pm 25$ mm</td>
</tr>
<tr>
<td>Resolution</td>
<td>$0.05 \mu$</td>
</tr>
<tr>
<td>Yaw Pitch error</td>
<td>20 arcsec</td>
</tr>
<tr>
<td>Vertical Translation</td>
<td>Range $\pm 25$ mm</td>
</tr>
<tr>
<td>Resolution</td>
<td>$0.056 \mu$</td>
</tr>
<tr>
<td>Yaw Pitch error</td>
<td>20 arcsec</td>
</tr>
</tbody>
</table>
Figure C.4.6.2: First crystal and mount. **Top:** First crystal and mount. Top: view of disassembled components. **Bottom:** Final assembly. Entire construction uses invar. Crystal is shown with protective membrane in place, following successful leak check, and just prior to installation. Liquid nitrogen is pumped into the first VCR connection, circulates through channels cut into the crystal, and shunted back into opposing crystal channels via a return channel bored into the leftward element of the support frame. The first crystal assembly is secured to the monochromator’s first crystal vertical lift stage via four G-11 composite posts that serve as the thermal break between the first crystal kinematic platform and the rest of the monochromator.

Figure C.4.6.3: Second crystal assembly. **Top:** Unassembled components. **Bottom:** finished assembly (inverted relative to installation). The crystal has a serpentine cross-section (strain break). The crystal is lightly clamped to a nickel-plated copper cooling block. A gallium-indium eutectic coating mediates the contact between the crystal and cooling block. G-11 composite posts beneath the cooling block constitute a thermal break between the cooling block and the upper stage of the monochromator serve as the thermal break between the first crystal kinematic platform and the rest of the monochromator.
Figure C.4.6.4: Crystal mount assemblies installed in HLD8-24. Beam enters from the left. Liquid nitrogen feed bellows originate in the photo at lower left, connects to the first crystal mount, and then to the second crystal cooling block. The large copper plate is a water-cooled Compton shield. The smaller copper plate attached to the second crystal cooling block is a Compton shield for the second crystal motion system. The Bragg rotation axis passes through a point above the center of the first crystal, collinear with the surface of the second crystal. The first crystal is mounted on a vertical translation stage. The second crystal has stepper-motor driven roll, parallel, transverse and pitch axes. The second crystal also has a fine pitch trim implemented as a digital piezoelectric transducer (DPT).

We have developed control scripts that permit facile measurement of crystal rocking curves (Figure C.4.6.5), XAFS spectra (Figure C.4.6.6) and feedback systems for maintaining peak tune between first and second crystals under varying thermal loads and programmed energy changes. Use of active position feedback permits un-interrupted use of the beam following undulator gap changes or re-establishment of beam following un-programmed loss of beam. Oxford-Danfysik quadrant diode beam position monitors (BPMs) have been installed immediately upstream of the Kirkpatrick-Baez focusing system in the 24-ID-C experimental end station and in the collimator assembly about 0.75 m upstream of the goniometer. The BPMs are
used to obtain accurate simultaneous measurements of the x and y position of the monochromatic beam (precision of order 1 \( \mu \)m) with measurement times on the order of 1 second. After establishing a “reference” position within the coordinate frame of the BPMs, the beam can be steered to minimize the difference between the observed and reference positions by imposing fine trimming corrections on the yaw angle of the horizontal focusing mirror (horizontal steering) or the pitch angle of the vertical focusing mirror (vertical steering). (See section C.4.7 covering the 24-ID-C focusing system).

\[\text{Figure C.4.6.5: Rocking curve, using second crystal pitch DPT, at an energy setting of 12.658 keV (main Bragg axis fixed. Flux was measured by BPM located near sample goniometer). The full-width at half-maximum of this curve is 7.8 arcsec (expectation value for an ideal Si 111 crystal pair is \(\sim\)7.4 arcsec at 1.54 Å). The symmetry and rocking width, and quasi-Gaussian form of the curve argues for a relatively strain free mount of both crystals. Initially, measured rocking curve widths for the same crystal setup and installation was \(\sim\) 22 arcsec, due to over-tightening of the first crystal frame (required to achieve minimum acceptable vacuum performance). After several cycles between room temperature and 90ºK the frame-induced strain on the first crystal decayed to the current measured value presumably due to inelastic decompression of the indium seals between the first crystal and the invar frame.}\]

Incident power levels on the monochromator vary due to the operational mode of the APS accelerator (e.g. non-topoff modes), undulator gap changes or from other factors requiring feedback control on the fine trim angle of the second crystal (relative to the first crystal). The BPM’s provide accurate, non-invasive flux measurements (summation of all 4 diode response channels). We optimize the pitch angle tune by varying the pitch angle of the second monochromator crystal over a few microradians above and below the current pitch angle setting, using the second crystal DPT. We continuously monitor total flux through this operation and set the DPT to the angular trim position providing maximum flux after correcting the scan results for hysteresis effects. In effect we acquire limited range rocking curves. Both BPMs are upstream of the data shutter so that we can perform position and flux trim corrections, independent of the data collection state and without subjecting the sample to additional radiation exposure. DPT-driven flux trim corrections induce small vertical beam position shifts (\(\sim\) 7 \(\mu\)m) at the spindle and a \(\sim\)2% variation in delivered flux so this correction is not actually performed during crystallographic data frame exposures.
Cryogenic cooling of the monochromator is effected with an Oxford-Danfysik cryopump, revision D. We were the recipient of serial number 1 of this device. Nonetheless operational experience with this device has been generally positive. The revision D pump incorporates a large body of experience with the O.D. cryopump at the APS. Its PLC-based control system is simpler than prior releases and is controlled by a networked, computer. The pump’s SCADA control system includes a TCPIP-based server that permits other computers or controllers to query and set the pump’s state and set points. Figure C.4.6.7 shows a trace of pump performance parameters over 20 hours of operation.

Figure C.4.6.6: Selenium foil XAFS scan (scan step resolution 1 eV). **Red trace (blue data points):** relative fluorescence (YAP/Cyberstar); **Light Blue trace (red data points):** discrete energy derivative of fluorescence; **Green trace:** relative incident flux. For this scan, the monochromator was operated as a quasi-channel cut crystal, i.e. only the Bragg axis was rotated, all other crystal motions and trims were defeated. Step time (Bragg axis motion + data acquisition) per point was < 0.35 sec. Energy resolution is of order 2.0 – 2.5 eV, based on the width of the white line.
Figure C.4.6.7: Monochromator cryopump performance logs. **Cyan:** Main Dewar fill level (%max); **Orange:** High pressure buffer fill level (%max); **Magenta:** HPB output pressure (psi); **Dark Blue:** HPB return pressure (psi); **Violet:** LN$_2$ flow rate (liters/min); **Olive:** pump current (amps). The perturbation of the high pressure buffer internal pressure, associated with main Dewar refill, drives the beam position instability associated with Dewar fill. The inset figure (upper right) shows a magnified view of the fill pressure artifact from the fill cycle in the center of the timeline on the main plot. Note that the form of the pressure perturbation in the HP buffer pressure is nearly identical (although inverted about the abscissa) to that of the associated vertical beam position perturbation shown in Figure C.4.6.8, left.

An important source of instability in the monochromator is the cyclic filling of the main liquid nitrogen Dewar of the pump. The cryopump consists of a closed loop liquid nitrogen pump loop (called the “high pressure buffer” or HPB), immersed in a large liquid nitrogen dewer. Once purged and stabilized, the HPB operates at constant volume, as long as it is fully immersed with LN$_2$. Heat extracted from the monochromator crystals by the HPB pump loop is transferred to the LN$_2$ bath of the main Dewar causing evaporative loss from this volume, thereby requiring cyclic refilling of the main Dewar. When main Dewar refilling is initiated, the plumbing of the HPB is exposed to a mix of warm N$_2$ gas and LN$_2$ until the transfer lines are at thermal equilibrium with the LN$_2$ feed. Thus Dewar filling is associated with pressure transients in both the HPB and the main Dewar. O.D. attempts to minimize this effect in its internal plumbing design, but is not entirely successful. We have installed a “keep cold” circuit in the Sector 24 LN$_2$ distribution circuit that attempts to keep most of the local distribution circuit near LN$_2$ temperatures. However, the final section of transfer line serving the cryopump cycles between room and LN$_2$ temperatures due to a design defect in O.D. LN$_2$ plumbing scheme.

The magenta-colored time line in Figure C.4.6.7 shows that a ~6 psi pressure transient is associated with the onset of every main Dewar refilling. The LN$_2$ transfer lines between the pump and the monochromator crystals are flexible bellows-like conduits. Pressure transients in this plumbing cause small length changes in the transfer lines that induce small torques on the second crystal mount (i.e. The system acts as a very sensitive barometer). Figure C.4.6.8 shows...
time traces of flux and the X and Y positions of the monochromatic beam near the crystallographic spindle during refilling of the cryopump’s main Dewar, without (left) and with (right) positional feedback systems enabled. When positional feedbacks are not operating we observe a 20 µm (1 beam height) vertical beam position perturbation, whose time series exactly matches the form of the pressure perturbation seen in the HPB time series of Figure C.4.6.8 (except for inversion about the abscissa). The time series on the right side Figure C.4.6.8 shows that the position feedback systems effectively nulls the vertical refill perturbation. The large spikes in both vertical time series are manifestations of a position transient of the white beam associated with accelerator top-off events.

In spite of the effectiveness of both positional feedback systems, we desired to minimize Dewar refilling pressure artifacts. The cryopump’s Dewar transfer lines were modified such that so a three-way purge valve is situated just upstream of the “bayonet” connection that serves as the interface between the house LN₂ distribution system and the cryopump’s main Dewar. The modification allows a purge of the entire transfer line up to the cryopump Dewar, minimizes the temperature transient seen by the HPB and thereby nulls the induced positional instability of the beam. Figure C.4.6.9 demonstrates the effectiveness of the purge modification upon eliminating the pressure transient of the HPB, associated with Dewar refill.

![Figure C.4.6.8](image-url)

**Figure C.4.6.8:** Beam X (blue), Y (red) position and flux (yellow) stability traces through cryopump Dewar refill, with (right) and without (left) positional feedback systems enabled.
Figure C.4.6.9: HPB (blue) and Dewar (red) fill levels (percent) are traced in the upper two curves through three fill cycles. The lower two curves trace the HPB output (blue) and return (red) pressures. In the first and last fill cycles, the transfer line purge system was active. During the second (middle) fill cycle the purge was manually defeated.

Cycling of front end shutters and substantial change of undulator gap subjects the monochromator first crystal to large changes in thermal power loading. The rest of the monochromator is affected, indirectly by associated changes in Compton scatter from the first crystal. The monochromator achieves stable positional output within 5 minutes of onset of illumination using the first undulator harmonic. Following transition to closed undulator gap (3rd undulator harmonic) thermal equilibration occurs within 15 minutes of shutter opening, from a cold-equilibrated state. When transitioning between undulator harmonics, a 5 minute equilibration period is required (in the absence of active feedback-driven steering). A simple second crystal DPT pitch trim operation eliminates almost all of the vertical deflection associated with power loading changes seen by the first crystal. All of the vertical or horizontal position stabilities induced by gap change or front end shutter cycling are nulled by the positional feedback controls of the K-B focusing system.

We have established that most monochromator drive systems deliver the range and precision called for in the technical specification generated during the procurement phase. We have observed substantial backlash in the second crystal’s roll and stepper motor-driven pitch axes. The roll axis irreproducibility is caused by inadequate thermal isolation of the second crystal cooling system from the pitch and roll axis mechanisms, which causes partial binding of the roll axis arc stage. The theta-2 pitch problem is due to a mechanical fault of the gearing system of said axis. Neither of these issues present insurmountable difficulties in present use of the monochromator. We understand the source of these backlash effects and will correct them as time permits.
To first order, the 24-ID-C monochromator normally operates as a quasi-channel-cut crystal monochromator (only Bragg axis rotation is required to change output energy). However, a small vertical trim of the first crystal position is necessary to maintain fixed-exit geometry. Following a Bragg displacement, a DPT-driven pitch trim is also executed to maximize output flux, and help maintain fixed exit geometry. Figure C.4.6.10 is a calibration of the first crystal vertical displacement vs. energy set point, required for fixed exit. DPT pitch trimming is accomplished by executing a short-range rocking curve measurement, using the summed outputs of the downstream BPM as a flux monitor (execution time ~ 5 seconds).

**Figure C.4.6.10:** Calibration of first crystal offset (Y1) vs. monochromator energy set point (required to maintain fixed exit geometry). Y1 position manually set at each energy by observation of fiducialized YAG fluorescence beam image. A quadratic polynomial was fit to the data and used as the calibrator of Y1 as a function of energy. 1mm Y1 travel = 5040 motor steps (calibration span ~0.45 mm for energy range of 6 to 14 keV).

We have achieved fully automatic and stable monochromatic energy transitions over an energy range from 4.5 to 22 keV. The Console script (see section C.4.9) responsible for controlling the 24-ID-C monochromator automatically transacts with the sector channel access server to set the upstream undulator gap, tune the second crystal pitch DPT and vertical crystal position. The same Console script adjusts the position of the monochromatic beam at the goniometer axis by trimming the yaw and pitch of the horizontal and vertical focusing mirrors (HFM,VFM), respectively using feedback information from the downstream quadrant diode beam position monitor. Current Console scripting assures that following significant energy changes (multiple keV) the final optimized beam position will deviate from the canonical beam position coordinate by less than 5 µ in the horizontal and vertical directions. Energy transitions are accomplished in a maximum of 1 minute.
The 24-ID-C monochromator scripting can effect a change in the mirror reflective strip selection via vertical translation of the HFM and transverse displacement of the VFM in order to minimize third harmonic contamination. Third harmonic transmission is a problem at monochromator energy set points below 7.5 keV (cutoff energy of the rhodium strip is ~ 22 keV). Use of the uncoated ULE strips (cutoff energy ~ 10.5 keV) of the focusing mirrors greatly diminishes the third harmonic component of the monochromatized beam (see Figure C.4.6.11). Third harmonic contamination can be further suppressed by forcing a small mis-tune between the Bragg angles of the first and second crystals.

![Figure C.4.6.11: Elimination of third harmonic contamination, using the bare reflective strips of the focusing system. Red: Overall transmission using Rh reflective strip. Blue: Transmission using bare ULE reflective strip. Transmission depression near 6.5 KeV is due to absorption of Cr foil of quadrant BPM. Calculated using XOP.](image)

Automatic beam tracking associated with energy changes, effected through yaw and pitch trims of the HFM and VFM (respectively), relies on accurate and reproducible measurements of the beam position via the downstream quadrant diode BPM. The performance of the quadrant diode BPM has a strong dependence on incident energy since the BPM relies on fluorescence from a metal foil upstream of the diodes. Secondly, the dynamic range of the current amplifiers of the BPM has to be adjusted over the operational range of the 24-ID-C monochromator to avoid saturation (due to changes in undulator gap and undulator harmonic transitions). Thus, the energy-dependency of the calibration of HFM yaw and VFM pitch trim vs. BPM position are quite complex and subject to perturbations from many factors. We have attempted to simplify this calibration problem using a phenomenological approach.

We derived a series of calibration curves relating the observed optimal BPM beam position coordinates at a series of energy points after manually adjusting the beam position (observed with the goniometer-mounted YAG fluorescence visualizer) using the VFM pitch DPT and the HFM’s downstream nanomotor positioner. Calibration curves were constructed for each non-saturating current-amplifier integration time (see Figures C.4.6.12, C.4.6.13). The result of these studies is a set of look-up tables relating the monochromator energy set point with a pair of target BPM coordinates (for each BPM integration time range).
Figure C.4.6.12: Target BPM coordinate vs energy calibration for BPM integration range 4.

Figure C.4.6.13: Target BPM coordinate vs energy calibration for BPM integration range 5.
C.4.7.  24-ID-C K-B Mirror

The Kirkpatrick-Baez focusing system for the 24-ID-C beamline was designed in collaboration with and built by Oxford-Danfysik (Oxford-Danfysik Beamlines Ltd, Oxford UK). The Horizontal Focusing mirror (HFM) and Vertical Focusing Mirror (VFM) and their benders were supplied by Seso (Aix-en-Provence, Fr). The system consists of independent, elliptically-bent focusing mirrors, mounted on a single support system and contained within a single vacuum chamber. The resulting, integrated design is intended to minimize the longitudinal footprint of the focusing system and result in superior inertial isolation of the mirrors (due to the large mass of the integrated system: 7.4 metric ton). Figure C.4.7.1 shows a rendering of the 24-ID-C system, and Table C.4.7.1 summarizes various physical and optical characteristics of the focusing mirrors.

![Rendering of the entire 24-ID-C K-B focusing system. HFM left, VFM right.](image)

**Key:**
- Light green: vacuum vessel lid
- Dark grey: mirror bending mechanisms and supports
- Light grey: vacuum vessel base
- Blue: support girders
- White: 600 l/s ion pumps
- Black: Synthetic granite kinematic driver supports
- Tan: Pre-stressed ferroconcrete inertial base
- Brass: Base support pads

The focusing system consists of a large ferroconcrete support plinth supporting two synthetic granite plinths each supporting the through-vacuum kinematic support structures for the HFM and VFM. The system’s vacuum vessel is supported by a series of girders, mounted to the
ferroconcrete plinth, and mechanically isolated from the mirror support structures by layered-composite damping pads.

Both mirrors are positioned by gear-reduced stepper-motor-drive kinematic mounts that incorporate high precision optical encoders. This set of drives controls the vertical position, pitch and roll of the HFM and VFM. A pair (each) of piezoelectric vibratory servo-motors (nanomotors) control the transverse position and yaw of the HFM and VFM. The step resolution of the transverse servos is 0.01 µm, providing approximately 0.2 microradian steps in yaw. Contact between the upstream kinematic leg of the VFM and its support structure is mediated by a high precision piezoelectric actuator (Jena DPT) for fine control of the VFM’s pitch (0.05 microradian repeatability).

The mirrors are prefigured as meridional cylinders and bent to the desired figure by 4-pole mechanical benders, in order to approximate and ellipsoidal surface figure. The focal point of both mirrors can be readily set to any position between the goniometer’s spindle axis and the detector face. The detector to spindle distance varies between 160 and 1100 mm.

**Table C.4.7.1: Mirror Properties and Specifications**

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>Horizontal Focusing Mirror (Specification)</th>
<th>Vertical Focusing Mirror (Specification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demagnification (spindle)</td>
<td>7.5 (1200)</td>
<td>9.5 (900)</td>
</tr>
<tr>
<td>Slope Error (microrad)</td>
<td>1.88 (2.5)</td>
<td>1.21 (2.0)</td>
</tr>
<tr>
<td>Roughness (angstrom RMS)</td>
<td>2.34 (&lt;3.0)</td>
<td>2.57 (&lt;3.0)</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>1200 (1200)</td>
<td>900 (900)</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>85 (85)</td>
<td>85 (85)</td>
</tr>
<tr>
<td>Strips</td>
<td>Rh, bare, Pt</td>
<td>Rh, bare, Pt</td>
</tr>
<tr>
<td>Status Radius (kM)</td>
<td>6.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Upon receipt, the optical properties of both the VFM and HFM were characterized at the APS metrology facility. In addition, the bend vs. displacement functions of the bender mechanisms of both mirrors were measured (e.g., see Figure C.4.7.2). All results from APS metrology were consistent with information provided by the mirror manufacturer, Seso.

In July 2004, the 24-ID-C Kirkpatrick-Baez focusing mirror system was installed in the experimental hutch, using a high-capacity air lifter to manipulate the vacuum vessel and support structure. With the assistance of Oxford-Danfysik engineers and the APS survey crew, the mirror vacuum vessel was precisely aligned to the nominal beam trajectory. Following metrological survey and bender calibration in the APS metrology lab, the vertical (VFM) and horizontal (HFM) mirrors and their bending mechanisms were installed (e.g. see Figure C.4.7.3).

**Figure C.4.7.2: Calibration of 24-ID-C HFM bend radius function at APS metrology, using the long-based line profiler. The HFM, like the VFM, was prefigured with a meridional status curvature (6 kM for HFM).**
Locally acquired metrological data agreed well with data provided by the vendor (Seso) and were in compliance with the optical performance criteria established for the system, prior to acquisition. Reproducible bend-radius calibrations for both the HFM and VFM were established.

Figure C.4.7.4 is a schematic of the motion system of the 24-ID-C focusing system, and provides nomenclature for the following discussion.
Figure C.4.7.4: Motion axes of the 24-ID-C focusing system. Involvement of each axis in compound motions (e.g. pitch, roll & yaw) of each mirror is indicated. All axes, except the mirror benders, are equipped with high precision incremental encoders. Motion axes labeled FABx are piezoelectric nanomotors, used for mirror strip selection and yaw control. All others are gear-reduced stepper-motor driven axes. Axis Dev0 AU also incorporates a piezoelectric DPT (Jena DPT) for fine pitch trim of the VFM. Fine yaw control of the HFM is provided via small trims of the HFM transverse axis FAB2.

We have developed distributed control systems for the 24-ID-C mirror, whose software and hardware components will be applied to all subsequent NE-CAT’s focusing systems, without modification, aside from calibration issues. Figure C.4.7.5 is a schematic of the transverse and yaw 24-ID-C mirror control system. Figure C.4.7.6 is a similar diagram of the vertical, roll and pitch control system. All motion axes have been calibrated (determination of step displacement functions, encoder response, backlash etc) using a linear position transducer (Heidenhain) with a sensitivity of 0.25 µm. Figure C.4.7.7 shows the measured displacement function for the Jena pitch trim DPT.
Figure C.4.7.5: Transverse and yaw motion control of 24-ID-C mirror system.

Figure C.4.7.6: Vertical, roll and pitch control system of 24-ID-C mirror system.
Initial testing of the transverse and controls revealed that the installed motion-limiting systems were inadequate. Furthermore, the digital piezoelectric transducer (DPT) for the vertical focusing pitch trim was inoperative and a number of lift jack position encoders were either defective or mis-installed. These defects were remedied in August 2004 by Oxford-Danfysik.

Both mirrors were surveyed into their nominal positions (elevation, transverse position, pitch roll, and yaw) using laser rotator fiduciation to reference survey monuments installed by the APS survey crew. The vacuum vessel lid was installed and vacuum established (vacuum < 10^{-6} torr was obtained in ~1 day of pumping). We verified the yaw and pitch angles of the HFM and VFM by orienting both mirrors such that a small portion of the unfocused beam was able to transit both mirrors to strike fluorescent paddles placed at downstream reference positions. Using fluorescence spotting and an optical level and transit we adjusted the angle of attack of both mirrors and placed reference lines on the 24-ID hutch floor to guide installation of end station components.

Following installation of all major end station components we focused the mirrors and verified the bend radius calibrations obtained during metrological validation of the mirrors and their benders. A reasonably high quality focus was obtained immediately by setting both mirrors to the bend radius parameters provided by Seso.

A ytrium-aluminum garnet converter, affixed to a small prism and aligned to the crystallographic spindle was used to assess the focus spatial profiles in real-time and fine-tune mirror bending mechanism calibration procedures (Figure C.4.7.8). The beam image was visualized using the crystal video microscope and provided ~5 
\mu m spatial resolution. Focus spatial profiles were quantitatively assayed with calibrated slit scans (Figure C.4.7.9). Measured profiles were essentially Gaussian in both dimensions ($\sigma_x = 20$, $\sigma_y = 7 \mu m$) and manifested no significant coma-distortion, due to use of a 4-moment bending mechanism to better approximate an elliptical working surface for both mirrors.
Figure C.4.7.8: **Left:** Fluorescent image of attenuated monochromatic beam (color inverted), at crystallographic spindle position. Horizontal graduations are separated by 40 µm. **Right:** Fluorescent imager mounted on a goniometer head. The imager consists of a YAG-coated glass disc glued to a small prism. The back (diagonal) face of the prism is aligned on the spindle axis. The fluorescent image was captured from the video stream of the vertical sample microscope (95% beam attenuation). Reduction of attenuation reveals no additional profile features (in the vertical or horizontal directions).
Figure C.4.7.9: Spatial profiling of monochromatic beam by horizontal (left) and vertical (right) slit scans, through the beam, near its focus position. Blue points (left figure) and red line (right figure) represent raw transmission vs. slit position trace, measured by an ionization monitor downstream of the spindle position. Red points (left figure) and blue points (right figure) are the calculated discrete positional derivative of the transmission traces. The lines (red left, blue right) are non-linear least squares Gaussian fits to the derivative traces. The slits used in the scan are 5.5 cm upstream of the spindle position. The beam was focused using a YAG-fluorescence screen placed on the crystallographic spindle, using the sample video microscope for visualization.
Beam steering in the vertical direction is accomplished using the Jena digital piezoelectric transducer (DPT) incorporated in the upstream (single) leg of the VFM's kinematic mount platform (see Figure C.4.7.10). Horizontal steering uses the downstream transverse piezoelectric motor of the HFM for control (FAB 2).

**Figure C.4.7.10:** Rendering of VFM DPT used for pitch angle trim (cylindrical structure behind conflat flange (dark grey disk, with 6 bolts)). Also seen is the nanomotor drive for the upstream VFM transverse motion (light grey L-shaped box, below DPT). The nanomotor contacts a dark-grey ceramic thrust pad, bonded to the transverse precision slide (orange). The L-shaped, dark grey brackets to the right of the nanomotor are the hard stops and limit switch mount brackets for this axis.

Our vertical beam steering protocols exhibit control resolution better than 70 nanoradians (corresponding to ~0.5 µm spatial resolution at the crystallographic spindle position). Steering resolution in the horizontal direction is ~200 nanoradians. The beam’s spatial position is monitored with an Oxford-Danfysik quad-photodiode beam position monitor (BPM) situated ~1 meter upstream of the spindle, providing a resolution of ~ 0.5 µm, x and y axes (0.5 µm thick chromium converter foil). Figure C.4.7.11 shows the raw calibration data for the HFM yaw trim axis.

The linearity of the HFM yaw and VFM pitch trim displacement functions permit us to periodically correct the beam position by simply measuring the X and Y displacements of the beam from a reference BPM position, calculating the appropriate response for the HFM yaw and VFM pitch trim axes and then issuing the calculated corrective displacements to the appropriate trim, at appropriate times (e.g. frame exposure not in effect).

Closed-loop, continuous feedback is not feasible due mechanical shocks induced by the nanomotors and VFM DPT on the mirror supports. The BPM and attenuators are situated upstream of the sample shutter and their operation does not impose a radiation exposure burden on the sample.
We have extensively studied monochromatic, focused beam position stability over a variety of accelerator operating modes, changes in undulator gap and monochromator energy set point. Sources of instability intrinsic to the beamline optics (e.g. monochromator’s cryopump) have also been studied. From these efforts, we have developed rapid steering protocols that offset all important sources of beam instability, without impacting data collection throughput. Figure C.4.7.12 shows data logs of the Y coordinate of beam position and delivered flux during non-top-off accelerator operation, where the power density, incident on the first monochromator varies by ~ 20, over an 8 hour period. During logging interval shown in Figure C.4.7.12 the DPT VFM and nanomotor HFM trim corrections were invoked at every 30 seconds, while beam position and flux data was recorded every 60 seconds. The large discontinuity in the flux trace (top) at time point 221 is due to an accelerator fill-on-fill event, corresponding to a barely perceptible fluctuation in the measured y-beam position. The two small spikes in the deflection trace at time points 113 and 278 represent the residual impact of periodic monochromator main Dewar refills on beam position. On average we can steer the beam within a cone of diameter (at spindle) +/- 1 µm RMS in both directions over the long term (seconds to days). We cannot correct for short term (< 1 sec) beam displacements originating with the accelerator itself or from APS experimental floor vibrations.

Total monochromatic flux (maximally permissive slit aperture) at 12.5 keV is better than 2 x 10^{13} photons/sec. In commissioning studies to date, crystallographic data quality is optimized with 50-90% attenuation and exposure times of order 1 second. Although our spindle-shutter synchronization is accurate to ~ 1 ms, and data collection with exposure times significantly less than 100 ms. are feasible from an instrumentation standpoint, data collected with with exposures < 100 ms show relatively poor quality due to a number of factors:

1) When working with small beams (24-ID-C beam V x H dimension: 28 µm x 80 µm, 4σ) sample spatial stability is an issue. Crystal samples are suspended in a low temperature cryocoolant gas flow during beam exposure. Any aerodynamically induced movement of the sample can easily perturb the fraction of beam intercepted by the sample (especially...
small samples). With long exposure times these problems tend to “average-out”, hence the observed improved data quality with longer exposure times.

2) We have observed beam induced flexure of conventional Dacron sample mounts. This effect may be caused by thermal impact of the beam on the twisted (and therefore internally stressed) sample loop filament. The effect is very pronounced when the LN$_2$ cooling stream is curtailed or reduced.

3) Floor vibrations and short-term instability of the X-ray source.

4) Mechanical instability in beamline optical components due to variations in thermal loading or vibrations induced by cooling flows.

Procedures have been developed to over- or under-focus the monochromatic beam in order to obtain a vertical beam spot size, at spindle of approximately 50 μ, for use with samples with characteristic dimensions > 50 μm. Our efforts to date, have produced a beam with moderate spatial modulation of flux density in the vertical direction and a pure Gaussian horizontal profile. The peak-to-peak spatial frequency of vertical flux density is of order 20 μm and consists of 3 superimposed Gaussians with a σ of 7 μm. Given that the effective resolution of the Q315 detector is 90 μm, deviations from a simple Gaussian profile in the vertical dimension of the spot profile are expected to have insignificant impact on diffraction data quality. We observe no evidence of vertical modulation in measured diffraction spot profiles. Figure C.4.7.13 shows intensity profiles of the under-focused direct beam, imaged at the spindle (lower left) and its image projected to the detector positioned downstream from the spindle by 170 mm (approximate location of beam focus). Figure C.4.7.14 is a similar study of diffraction spot intensity profiles from an actual protein crystal, with a sample to detector distance of 300 mm.
**Figure C.4.7.12:** Y-beam position stability, during non-topoff operations. **Top:** Flux delivered to spindle (arb. Intensity). **Bottom:** Y-beam position deviation from reference position (µm). Spikes in deflection trace are due to cryopump Dewar fill.
We are working with APS AOD to characterize residual positional instability of the source and to improve the decoupling between the two Sector 24 undulators. Finally, APS provides access to a facility for directly measuring the inertial stability of optical components (mobile precision 3D micro accelerometer). We will use this system to insure residual vibrations from stepping motors, shutters, etc. are not somehow manifesting themselves as fluctuation in beam position at the spindle.

Overall, we are quite satisfied with the performance and stability of the 24-ID-C KB focusing system. The measured focus spot profile is very similar to that predicted by shadow ray tracing modeling of the system. We have a greater flux density at the spindle than can effectively be used (without attenuation). Stability of the fully-focused beam (at spindle) is more than adequate for crystals 40 μ or greater in size.
Figure C.4.7.14: Attempt to observe effects of under focusing in diffraction spot profiles from a protein crystal. **Left:** small section of diffraction bitmap. **Right:** spot profiles through center of second diffraction spot from bottom of image bitmap (Top: vertical, Bottom: horizontal). Detector distance 300 mm.

C.4.8. **24-ID-C and 24-ID-E Detector and Computing Systems**

C.4.8.1. CCD Detector System

All NE-CAT beamlines use Area Detector System Corp. (ADSC) Q315 multi-cell CCD area detectors. Table C.4.8.1 summarizes the physical and performance characteristics of the Q315. The Q315 detector head consists of a 3 x 3 array of detector cells. Each detector cell consists of a Thompson 7899 2K x 2K pixel CCD, epoxy bonded to an Incom fiber optic taper with a 3.7:1 demagnification ratio. The Thompson 7899 CCD chip has a full well depth of ~270,000 e⁻, 16-bit readout and operates at a temperature of -45 °C, maintained by thermoelectric coolers. The active area of the detector head is 315 x 315 mm (6144 x 6144 pixels), with an effective pixel size of 50 x 50 µ. Spatial resolution (accounting for point spread) is of order 90 x 90 µ. Contiguous tapers are separated by polyimide spacers, yielding dead strips between cells about 200 µ wide.

All four readout channels of the Thompson 7899 are utilized yielding an unbinned aggregate readout time of approximately 1 sec (< 250 ms, 2 x 2 binned). Dark current is ~ 0.015 e⁻/pixel/sec. Front end gains are typically set to 8.5 (or 2.5 ADU).

Each detector cell connects (using a proprietary optical fiber medium named “Hotlink”) to 1 member of a 9-element “frame grabber cluster” consisting of 3 GHz Pentium 1 U servers, each with 2 GB RAM and 1 CCD chip controller (including Peltier cooling controls). One element of the...
cluster provides read-synchronization signals for the other CCD controllers. The backbone of the frame grabber cluster is a 1 GB duplex Cu Ethernet switch. Each frame grabber host is responsible for caching the raw data stream from its associated CCD into a FIFO (First In First Out) implemented in local memory. About 1/2 of available local RAM is reserved as data cache. A background process (relative to data caching) performs inline correction of the raw data sub-frames for detector response non-uniformities and geometric distortions of diffraction images caused by optical imperfections of the tapers.

A dual Xeon 4 GHz host termed the compositor aggregates the corrected sub-frames from the frame grabber cluster and stores the composited diffraction images either to a local 1.5 TB SATA-RAID (internal to the compositor) or an external high volume, high performance disc storage system.

Because of the parallelism of the Q315 readout and the fact that raw data streams are cached in RAM (rather than disc) the sustained data frame throughput (measured in collected data frames per minute) exceeds any other multi-cell detector system. The 24-ID-C and 24-ID-E detector systems, when run without constraints of goniometer synchronization can accumulate more than 44 2 x 2 binned frames per minute (100 sec effective exposure). The same system can collect more than 30 1 second exposure frames per minute when data collection is constrained by performance limits of the goniometer (backlash, shutter dead time, spindle acceleration and deceleration corrections imposed). Unbinned images can be acquired at a maximum rate of approximately 20 frames / min (1 sec exposure).
Table C.4.8.1: Physical / Operating Characteristics of the ADSC Q315 Detector.

<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometry</td>
<td>3 x 3 array</td>
</tr>
<tr>
<td>Active Area (mm, pixels)</td>
<td>315 x 315 6144 x 6144</td>
</tr>
<tr>
<td>Inter-segment dead zone (pixels, µ)</td>
<td>8 400</td>
</tr>
<tr>
<td>Phosphor optimized for operation at (keV)</td>
<td>12.4</td>
</tr>
<tr>
<td>Front End Pixel Size (µ)</td>
<td>51 x 51</td>
</tr>
<tr>
<td>Effective Spatial Resolution (µ)</td>
<td>90 x 90</td>
</tr>
<tr>
<td>CCD</td>
<td>Thompson TXH 7899</td>
</tr>
<tr>
<td>Taper-CCD bonding</td>
<td>Direct Epoxy bond</td>
</tr>
<tr>
<td>CCD Pixel Size (µ)</td>
<td>14 x 14</td>
</tr>
<tr>
<td>Taper Demagnification Ratio</td>
<td>3.7 : 1</td>
</tr>
<tr>
<td>Readout dynamic range (bits)</td>
<td>16</td>
</tr>
<tr>
<td>Front End Gain at 12.4 keV (e⁻/photon)</td>
<td>8.5 (2.5 ADU)</td>
</tr>
<tr>
<td>Front End Gain at 8.9 keV (e⁻/photon)</td>
<td>6.5 (1.9 ADU)</td>
</tr>
<tr>
<td>Full Well Depth (e⁻)</td>
<td>~270000</td>
</tr>
<tr>
<td>Dark Current (e⁻/pixel/sec)</td>
<td>0.015</td>
</tr>
<tr>
<td>Electron / ADU at 12.4 keV</td>
<td>3.36</td>
</tr>
<tr>
<td>Read Noise (1 MHz rate, e⁻)</td>
<td>~18</td>
</tr>
<tr>
<td>Readout time (unbinned, 2 x 2, sec)</td>
<td>1 0.3</td>
</tr>
<tr>
<td>CCD Operating Temperature (°C)</td>
<td>-45</td>
</tr>
<tr>
<td>Weight of Head (kg)</td>
<td>141</td>
</tr>
</tbody>
</table>
C.4.8.2 Data Flow System

We have developed a computational and storage data flow system (Figure C.4.8.1) to support sustained, rapid crystallographic data storage, reduction and analysis at Sector 24. The principal design goals for this system are:

1) Minimize the time required for acquisition and storage of corrected image data frames from a Q315 multi-cell CCD detector, which itself is a component of the overall data flow system.

2) Provide rapid, parallel access to archived data to multiple workstations;

3) Provide sufficient mass storage so that available disc storage is never a significant logistical factor in the data collection process.

![Figure C.4.8.1: Sector 24 data flow system schematic, showing the main system components and their interconnections. Left: Q315 detector; Middle: management, control and computational nodes; Right: HP EVA 5000 SAN. Sufficient capacity exits in the fiber channel fabric to connect two additional Q315 systems to the SAN.](image)

The time required to transfer data from the detector to disc storage is potentially a serious impediment to sustained, rapid data collection at NE-CAT beamlines. Each CCD element of the Q315 has four independent readout sections operating simultaneously. Additionally, all 9 cells readout in parallel, so that the aggregate readout time for the entire 6120x6120 pixel array over 36 readout channels is of order 1 second in unbinned mode (~250 ms in 2x2 binned mode). The size of a Q315 data frame is large, even within the context of currently available gigabit Ethernets (72 MBytes unbinned, 18.6 MByte 2x2 binned). Since typical exposure times per frame are 1 second, or less, we must minimize the overhead of associated with all data flow downstream of the CCD readout if we are to make optimal use of the beamlines.

The Sector 24 data flow system consists of the following components:

1) Q315 Detector (two at present, future expansion to three):
   a) detector head (9 cells, 4 readout channels / cell)
   b) frame grabber cluster, 2 GB RAM per host
c) frame compositor: dual Xeon processor, 2GB RAM, 1.5 TB RAID

2) Gigabit duplex Cu Ethernet network

3) Multiple dual Opteron and dual Xeon workstations (at least 3 / beamline)
   a) 2 GB RAM.
   b) Gigabit Ethernet.
   c) at least 1 duplex gigabit fiber channel HBA.
   d) dual graphics head.

4) Hewlett Packard EVA 5000 SAN.
   a) 15 TB disc space, expandable to 75 TB.
   b) Completely redundant internally with automatic failover for all major components.

5) Multiple dual Opteron file servers for control of data flow between the
   Workstations and the SAN. File servers, connected to the SAN via redundant fiber
   channel interconnects.

6) Duplex, gigabit fiber channel fabric and brocade switches.

Two networking fabrics are used in the NE-CAT data flow system: copper-based duplex gigabit
Ethernet and duplex gigabit fiber channel. All elements of the data flow systems have one or
more Ethernet connections. Ethernet also forms the interconnect fabric for the ADSC Q315
frame grabber cluster. All compute and management node are connected to a fiber-channel
fabric based on two Brocaide gigabit fiber channel (FC) switches. The Hewlett Packard EVA
5000 SAN also uses a dual FC internal fabric for all of its interconnections.

The core of the NE-CAT data flow system is a Hewlett Packard EVA 5000 SAN (Storage Area
Network). This system is a fully redundant dual loop FC based virtual RAID storage system. All
data paths within the EVA5000 have two-fold redundancy (controllers and data interconnects).
The EVA 5000 connects to the outside world via a FC fabric, based on Brocaide FC switches.
The NE-CAT EVA 5000 uses dual-ported 300 GB FC disc drives, providing a total of 15 Terabyte
of virtual storage. Our system is expandable to 75 TB by populating currently unused disc slots.
Maximum sustained write rate of the EVA 5000 is approximately 75 MB/sec. We expect that the
existing EVA 5000 has sufficient performance and storage capacity to concurrently support two or
three Q315 detectors.

Management of the data partitions (termed virtual drives) and their exposure to hosts connected
to the fabric is effected through a web-based system resident the management appliance host
(center of Figure C.4.8.1). The EVA 5000 supports most platforms, including win32 and Linux.
The EVA 5000 is capable of supporting sustained 75 MB/sec write speeds.

Currently we expose the EVA 5000 SAN to the Q315 and Sector 24 work stations via NFS
mounts through 1 of 2 file Linux servers (middle, Figure C.4.8.1). The two file servers connect
directly to the EVA via FC host-bus adapters. The file servers then accept or distribute SAN data
to the rest of the storage network via gigabit Ethernet.

In the near future we will reconfigure the storage network to support the IBM Global Parallel File
System (GPFS). This revision will allow all member nodes of the data network to directly access
the EVA SAN via FC, in a parallel fashion. GPFS mediates concurrent read and write access to
virtual drives and their file systems to any node running GPFS, possessing appropriate system
privilege. GPFS is available for Linux as a binary distribution (not open source) from IBM.

Data flow within the NE-CAT data flow network follows the scheme shown in Figure C.4.8.2, and
involves the following discrete stages:


1) Following an exposure, a synchronization signal is sent to all 9 cells of the Q315 detector head, initiating readout. Each CCD has 4 independent readouts sections whose outputs are multiplexed for transmission over proprietary optical fiber communications system (named “HOTLINK”) and sent to one member of the ADSC frame grabber cluster. Raw sub-frames are cached in a RAM-based FIFO (1.5 GByte per grabber node).

2) The ADSC frame grabber node corrects cached raw sub-frames for a variety of systematic errors (geometric distortions, non-uniformity of response, etc).

3) Corrected sub-frames are transferred by gigabit ethernet to the compositor node of the Q315 frame grabber cluster, where sub frames are composited into a single, corrected data frame.

4) The compositor node transmits composited data frames to the EVA 5000 SAN via gigabit Ethernet or fiber channel networks.

5) All work stations can access stored frames from the SAN via duplex GB ethernet or fiber channel. Hosts external to the Linux cluster can obtain stored composite frames via NFS (network file system) or SCP (secure shell copy) over the local GB ethernet, mediated by the file server nodes.

The compositor frame grabber cluster node executes the ADSC detector control software, including the graphical user interface used to setup and run data collection sequences. In general, users interact only with the ADSC GUI in order to setup and execute data collection sequences. The ADSC control software has been modified so that it can control all necessary functions of the beamline during crystallographic data collection, including: 1) change of the energy set point of the monochromator; 2) tuning of the beam trajectory to maximize flux delivered to the sample position 3) repositioning the Q315 detector head; 4) all relevant axes of the sample goniometer.

Typical data volumes collected by users in a single visit to the beamline are too large to make tape, CD or DVD storage a practical means of data transport and storage (given that users are usually collecting data up to the point in time were they must release control of the beamline to the succeeding user team). Users are required to provide large volume, inexpensive firewire or USB-2 disc drives for this purpose. These drives can be attached to a firewire hub on workstations without a workstation power cycle. The high relative speed of these drives compared to tape or optical disc media permit users to make effective use of all of the time allotted them.

Three or more workstations per beamline are available for running tasks associated with crystallographic data reduction and analysis. HKL 2000 and Mosfilm is installed on all workstations along with CCP4, EXPLORER, CNS, SOLVE, etc. All workstations are either dual head (two monitors per workstation) or have 30" diagonal LCD monitors attached to provide large, unconstrained visual workspaces. A DHCP server permits users to attach laptops or their own workstations to the sector network.

![Figure C.4.8.2: Data flow scheme. Color code: Nodes: black; Interconnect type: blue; Node activity: green.](Image 328x100 to 564x390)
The Sector 24 data flow system succeeds in its primary task of minimizing the dead-time of the beamline associated with data transfer within the system. That component of the overall inter-frame dead time (1.2 sec) attributable image correction process and to data flow within this system is small compared to the irreducible dead time imposed by the read-out time of the CCD chips themselves (1 sec).

The HP EVA 5000 is capable of concurrent support of at least one and possibly two more Q315 detectors and the anticipated number of workstations. Disc space will be expanded to a maximum of 75 TB using currently available 300 GB FC disc drives. Use of the IBM GPFS will provide a more scalable expansion path than NFS and provide enhanced manageability of the NE-CAT data flow system.

C.4.9. 24-ID-C and 24-ID-C Experimental Systems

C.4.9.1. Introduction

NE-CAT construction 24-ID-C, 24-ID-E, and 24-ID-D utilize identical end station designs. The end station is here considered to consist of the following subsystems:

- Beam collimation and associated positioning systems.
- Beam stop and beam attenuation systems.
- Fast sample shutters, synchronized with goniometer motion.
- Beam position monitors to provide the driving signal for steering of the beam via tuning of the yaw and pitch angles of the horizontal and vertical focusing mirrors (respectively).
- Goniometer, goniometer support stand, associated motion systems.
- Sample visualization and cooling systems.
- Multi-cell CCD detector and associated control system.
- Detector positioning systems for control detector sample distance, elevation, transverse position and pitch control.
- X-ray fluorescence and flux counting trains and flux feed back controls.
- Data flow network, consisting of control computers, a TB SANS raid system and fiber channel network.
- Real-time, distributed control system for programming and monitoring of all critical low-level beamline functions.
- High-level, intuitive graphical interface for data collection that hides much of the optical and mechanical complexity of the beamline from the user.

Detailed descriptions of the CCD detector and data flow systems are presented in section C.4.8. Progress in development and commissioning of all other experimental end station components are presented in the sequel.

C.4.9.2. Collimation System

The NE-CAT collimator consists of two sets of in-vacuum X,Y slits arrays separated by a variable distance ranging from 1.25 to 1.5 meters, driven via translation of the moveable collimator component, carrying the downstream slit array. The upstream slit acts as the defining slits, downstream as guard slits. The beam attenuators, shutters, quadrant diode beam position monitors and an ionization chamber are integrated into the collimation system. Both ends of the collimator are suspended from gimbaled bearings that are themselves supported by vertical and transverse translation stages. The downstream collimator gimbal also has a 25 cm longitudinal translation stage for setting the distance between the guard slits and the goniometer spindle axis.

Figure C.4.9.1 shows a schematic of the collimator and indicates the dispositions of each end station component supported by the collimation system. The translation of the downstream
element of the collimator is intended to simplify operation with kappa goniometers (i.e. minimize risk of collision between the goniometer kappa arm and the downstream components of the collimator).

A set of four pneumatic actuators offsets the compression force induced by the evacuated bellows connecting the mobile and stationary collimator components. Additionally, a counterweight attached to the stationary components through a linear bearing offsets the gravity loading of the vertical lift and transverse slides of the downstream gimbal support. This enables virtual force-free movement of the downstream slit array in both X,Y and Z directions, improving the mechanical precision and reproducibility of these motions. This property of the system is crucial for high resolution slit scans, used to measure beam intensity profiles (see Figure C.4.7.9) and calibration of the quadrant diode beam position monitor.

C.4.9.3. Attenuators and Beam Stop

Third generation synchrotron crystallography beamlines typically operate with the monochromatic beam heavily attenuated (50 – 90 %), in order to control the rate of X-ray damage to the sample and to optimize synchronization between the goniometer and data shutter. Two sets of pneumatically actuated XIA PF4 (4 aluminum attenuator elements per PF4) are installed in the collimator component stack, downstream of the quadrant diode beam position monitor. A mix of attenuator thicknesses are available to control the attenuation at both high and low beam energies.

The beam stop is a tungsten 1 mm thick (vertical) tapered slat, terminating in 1x1 mm plug with a conical bore. The conical bore in the beam stop proper acts as a scatter and fluorescence trap. This design was developed at APS Sector 19 (Randy Akire, Structural Biology Center). The beam stop is supported by a LR-Design (Mesa, AZ) pivot arm, providing remotely-controlled X and Y direction translation of the beam stop, driven by miniature stepper motors. The beam stop can be positioned along the length of the pivot arm to optimize the low resolution limit of a data frame or to minimize air scatter from the direct beam.

The beam stop translation stage is mounted on a pneumatically-driven transverse translation stage that is used to withdraw the beam stop from the vicinity of the spindle, during sample mounting (or recovery). The beam stop can be remotely installed, but not remotely withdrawn. The pneumatic slide has position sensors used as interlocks to prevent shutter opening when the beam stop is in a withdrawn state.

C.4.9.4. Fast Data Shutters

The beamline was initially commissioned using a commonly used commercial pneumatic shutter (PF2S2 from Xray Instrumentation Associates). The commercial shutter was found to induce perceptible vibration in the sample, in spite of the fact that the shutter was not directly mounted to the goniometer support structure. Additionally, poor reliability and slow response of this system forced us to develop our own shutters. We developed (in parallel) two shutter mechanisms to rectify these problems: 1) a low shock, high speed pneumatically actuated system based in part on components salvaged from the XIA shutter; and 2) a very high speed stepper-motor based rotary shutter.
**Figure C.4.9.1:** Schematic of extensible collimator assembly. Structure is supported by two gimbals (mount points indicated) that are supported by transverse/vertical compound translation stages. Downstream gimbal rides on a longitudinal translator stage (providing compression and extension). Pneumatic compensators (red) offset compressive force of the bellows connecting the moving and static sections of the collimator assembly.
Figure C.4.9.2: Details of collimation system. **Top:** Upstream components (right-to-left): Be window, terminating UHV, defining X,Y slits, upstream support and gimbal, O.D. quad-diode BPM, pneumatic attenuator selectors, variable stroke pneumatic shutter, linear bearings of collimator assembly. **Bottom:** bellows, downstream support and gimbal, guard X,Y slits, ionization monitor, fast-rotary shutter and scatter guard. Beam stop manipulator in foreground.

The pneumatic shutter incorporates two counter-poised, variable stroke pneumatic actuators to rapidly emplace and remove two tungsten blades from the beam (Figure C.4.9.3). This system uses dual-acting pneumatic actuators to displace the shutter elements salvaged from an XIA PF2S2, and is built on the PF2S2 vacuum chamber. We designed a system for limiting the
stroke length of the pneumatic actuators, such that the vertical aperture formed by the two shutter blades when open, was centered on the beam. We hoped that by minimizing the stroke length we could minimize the latency period between issuance of a command alter the shutter state and actual traverse of the shutter blades through the X-ray beam. Furthermore, we hoped that the limited shutter stroke would reduce mechanical shock induced in the collimator system by shutter actuation. The variable-stroke shutter achieved its first design goal with reproducible opening and closing times of order 4 ms. Unfortunately, we were still able to detect residual shutter-induced vibration of sample loops with the sample visualizer. High resolution diffraction spots from data frames acquired with this shutter also manifested shape pathologies, indicative of sample vibration (albeit to a lesser degree compared to data acquired with a conventional PF2S2).

Using an entirely different approach to shutter design, we developed a shutter based on partial rotation of a slotted, X-ray absorbing plug. Figure C.4.9.4 shows components of the final rotary shutter design. The heart of the system is a miniature, high-performance stepping motor – motor driver combination from Vexta (PMC35B3). This device is a 500 step/rotation 24 VDC stepper that will accelerate at rates up to $8 \times 10^3$ steps/sec$^2$ with no measurable loss of steps. In our design, the motor rocks 1 cm diameter aluminum plug with a 1mm slot milled in one end, to block or unblock the beam. When centered on the beam, a 26 motor step displacement is required to block the beam. Beam transit times measured with an ionization gauge placed downstream of the shutter and readout with a digital oscilloscope are 1.2 – 2 ms (Figure C.4.9.5). Variability of the midpoint of the time course for shutter opening or closing is too small to be measured reliably with our diagnostics (< 0.2 ms).

The rotary shutter is mounted on the downstream end of the collimator assembly after the guard slit array and the compact inline ionization chamber. The vacuum space of the collimator assembly is terminated by a 0.0005" kapton window, bounding the upstream side of the ionization chamber. The gas space of the ionization chamber directly communicates with the internal space of the rotary shutter and terminal scatter guard. This space is actively purged with He to minimize gas scatter in the optical path.

Synchronization of the rotary shutter with goniometer rotation during data collection is optimized by using the same motion controller (Galil 2180) to drive both the goniometer and shutter stepping motors. The Galil motion controller stores "macro" programs in its local memory that execute with very small program step latencies. A single macro is loaded into the Galil controller's working memory that handles all details of spindle motion (e.g. backlash correction, pre-exposure acceleration compensation) and the rotary shutter for each data exposure.
**Figure C.4.9.3:** Right: Variable stroke pneumatic shutter. Base of structure is an XIA shutter base, along with its shutter blade carriers and slides. Steppers drive stopping plates on linear rails to define stroke of dual acting pneumatic actuators.

**Inset:** Components of shutter: Foreground: dual-acting, double-ended pneumatic actuators.

**Background:** Linear slides, lead screws and stopping plates.

**Below:** Schematic of stopping plates and drive mechanism of plates, that control stroke length of pneumatic actuators.
Figure C.4.9.4: **Top:** Components of rotary shutter. Inset shows detail of shutter cylinder. The slot has a width of 1 mm. **Bottom Left:** Rotary shutter mounted on collimator. Note optical limit switch and alignment flag affixed to knob on the shaft of the shutter’s stepping motor. **Bottom Right:** View of rotary shutter, facing upstream, with scatter guard removed.
Figure C.4.9.5: Time course of 200 ms exposure using rotary shutter, recorded with multichannel digital oscilloscope. **Left:** Opening (1\textsuperscript{st} 17 ms of trace). **Right:** Closing (last 20 ms of trace).

**Key:** Red: Flux measured by ionization monitor downstream of shutter. Yellow: Data Flag indicates onset of opening and closing motion sequence. Cyan: Spindle motor steps. Green: Shutter motor steps.

C.4.9.5. Beam Position, Flux and Fluorescence Monitors

Two quadrant diode beam position monitors (QDBPM) are installed in the 24-ID-C hutch, both supplied by Oxford-Danfysik. The first QDBPM is installed immediately upstream of the 24-ID-C focusing system, and operates in UHV. This device is built into a 3 cm thick 6\textquotedbl{} conflat flange and has a conventional diode array that rides on a small X,Y translator for alignment of the bore of the diodes to the beam trajectory. The UHV-QDPBM (Figure C.4.9.6, left) has a small stepper-motor driven foil carrier that permits selection of 1 of 2 installed fluorescence foils or complete removal of the fluorescence source from the beam (in support of low energy data collection).

A second Oxford-Danfysik QDBPM (model DQM-100) is incorporated in the collimator, upstream of the variable-stroke shutter and attenuators (Figure C.4.9.6, right). A very thin aluminum foil (most upstream) is always left in place to minimize perturbation of the readout of the downstream QDBPM by back scatter and fluorescence from the pneumatic shutter and the aluminum attenuators. Both QDBPMs are read out by an Oxford Danfysik YMM-0012 4-channel current amplifier module.
Spatial response calibration of the downstream QDBPM is accomplished by affixing a high precision Heidenhain linear position transducer (sensitivity of 0.00001") to the top or lateral surfaces of the QDBPM vacuum housing, at positions collinear with the PIN diode array, and then following the response of the QDBPM as a function of the linear transducer output as the collimator is jogged in the X and Y directions, using the translation stages of the moveable collimator element. Figure C.4.9.7 shows typical calibration traces. These calibrations provide the response functions used in calculations for beam steering using yaw and pitch tunes of the horizontal and vertical focusing mirrors.

Since the downstream QDBPM is upstream of all attenuators and shutters, beam steering can be constantly maintained without illuminating the sample. The distance separating the downstream QDBPM from the spindle position (normally 1.4 m) complicates beam steering at the goniometer spindle due to effects of parallax. This problem is dealt with using a secondary calibration relating the raw QDBPM coordinates with the beam position at the spindle measured with a YAG fluorescence converter, fudicialized by the crystal visualizers, as a function of monochromator energy set point (see Figures C.4.6.12 and C.4.6.13). Secondly, after selecting and steering to a given set of target BPM coordinates the goniometer spindle axis is aligned to the beam position using a scanning method described in the next section.

The sum of all four output channels of the downstream QDBPM provides a relative measure of beam intensity, downstream of the defining slits, independent of attenuation and shutter states. This signal drives “microrocking” curve tuning of the pitch of the second monochromator crystal used to peak flux. Finally, after suitable cross calibration it is possible to use the ratio of the 4-channel sum output of the downstream QDBPM to the UHV BPM to measure the aggregate optical transmission of all optical elements positioned between them.

A low aspect ionization chamber is built into collimator assembly, immediately downstream of the guard slit assembly. The ion chamber is used for measuring flux downstream of all
attenuators and slits. The chamber has a single polyimide window on its upstream surface that terminates the roughing vacuum space of the collimator.

Figure C.4.9.7: Spatial calibration functions for downstream QDPBM X coordinate (left) and Y coordinate (right). Data from forward and reverse scans are included in both plots. Some degree of hysteresis is evident in both calibration traces, likely due to backlash in the vertical and horizontal motions of the downstream collimator translator stages.

Fluorescence from samples mounted on the goniometer spindle is measured with an Oxford-Danfysik Cyberstar system using a detector head fitted with a YAP (cerium-activated yttrium aluminum perovskite) fluorescence converter. The detector head is mounted on a linear translator that permits the user to remotely position the detector head to avoid detector saturation. The detector head lies in the horizontal plane of the X-ray beam (see Figure C.4.9.2).

C.4.9.6. Goniometer and Crystal Visualization and Sample Cooling

The 24-ID-C and 24-ID-E goniometer consists of a Huber 515m goniometer, consisting of a servo-driven X,Y,Z sample alignment head mounted on a Huber 510 rotation stage. The goniometer is mounted on an LR-Design goniometer support (Figure C.4.9.8).

The goniometer support has precision vertical and horizontal translation stages for the goniometer and a number of translation stages for the moveable portion of the collimator assembly. Figure C.4.9.9 consists of photographs of the assembled system, including crystal visualizers, Oxford Instruments Cryojet cooling system and its mount.

Crystal visualizers are two Infinity K2 video lenses fitted with high resolution CCD cameras. One visualizer is mounted directly under the spindle (17 cm object distance) in a vertical orientation, the other is mounted at approximately 35° to horizontal, approximately 12 cm from the spindle. Both visualizers are suspended from the stage supporting the goniometer via independent X,Z precision translation stages, fitted with micrometer translators. The effective magnification of the image displayed on the associated LCD screens is approximately 300x (field of view 600 x 450 µ). The CCD cameras of the visualizers are connected to Axis camera servers for network distribution of the image stream from both cameras. Crystal illumination is provided by fiber
optic ring and spot sources. Source intensity is remotely controllable from the beamline control area.

The Huber 515m XYZ alignment head provides about 1.5 cm travel in the Z direction (parallel to spindle axis) and 1 cm of travel in the X and Y directions, with approximately 5 µ step resolution.

**Figure C.4.9.8:** LR-Design goniometer stand, showing translation stages for moveable component of the collimator. The mounting gimbal for the collimator is the fork-like structure to the left of the beam stop mount (dark blue upright structure is the beam stop mounting block). The collimator gimbal stage translates along the linear rail bearing located far left and center of support structure. The goniometer mounts on the upright gusset (top center). Goniometer can be translated vertically and transversely. Both motions use 50:1 gear reducers for improved motion resolution.

**Figure C.4.9.9:** Left: View of Huber 515 m goniometer, crystal viewing microscopes. Right: cryosystem mount, beam stop inserter stage.
C.4.9.7. Detector Support

The detector (Q315) is mounted from an LR-Design A-frame detector positioner (Figure C.4.9.10). This system provides a very stable mechanical platform for the detector with translations controlling the sample to detector distance, vertical and transverse positions of the detector, as well as detector pitch. The greatest virtue of this detector platform is that it provides unhindered access to the goniometer spindle. All translation axes (except for the transverse motion) are fitted with magnetic Hall-effect absolute encoders with micron resolution.

Accessible sample to detector distance is presently 160 to 1400 mm (limited by mechanical details of the goniometer support). The detector lift consists of two independent vertical translators than can be differentially run out to provide changes in detector pitch. Accessible pitch is a function of detector distance and ranges from a maximum of 10° at 1000 mm detector distance to 35° at 160 mm detector distance.

Figure C.4.9.10: LR-Design A-frame detector support.
Figure C.4.9.11: 3 views of the LR-Design A-frame and detectors support. **Top-left:** A-frame and collimator system (foreground). **Bottom-left:** View of Q315 detector and strain-relief systems for its cabling. **Right:** Detail of detector manipulator. The manipulator can lift and/or pitch the detector about an axis centered on the detector face. The manipulator also has a transverse motion. The sample to detector distance and manipulator differential lift axes all use hall-effect absolute position encoders.
C.4.9.8. Beamline Controls

The control software developed for NE-CAT, named Console, has been developed specifically for controlling X-ray beamlines. Console is not a control system per se, but rather an integrated programming environment used for designing, debugging, and deploying distributed beamline control systems. The Console development environment is described in extensive detail in section D.1.3.2 and is a central effort of our third technological core: “Computing for Challenging Samples.”

Console control systems are implemented as compiled scripts written in a scripting language called Console Scripting Language (CSL), and executed by the Console Execution Unit (CEU, also called the Console Super Client) that runs under Microsoft win32 or win64 operating systems. The CEU communicates via Ethernet, serial ports or other communication modes with a set of dedicated hardware servers called Console Server Drivers (CSD’s) to move motors, acquire analog and digital data and to integrate and monitor all beamline functions.

CSD’s are implemented under linux (predominantly) or win32/64 platforms. CSD’s are C or C++ programs that run as persistent demons to control a specific hardware asset (e.g. motion controller, analog-digital converter, etc). A table of available CSD’s and the hardware that they control are listed in Table D.1.3.1.

CSD’s receive and parse command strings from the CEU’s using the unix Remote Procedure Call (RPC), and then change and/or report (to the CEU) the state of the hardware asset they control. CSD’s also maintain a small data base reflecting the real-time state of the hardware they drive.

Console has facilities for seamless interaction with other control systems including EPICS, DCS/DSH and the Area detector Systems Corporation (ADSC) detector control system.

NE-CAT’s beamline control system consists of multiple CEU’s interacting with each other, sharing a host CSD’s. Figure D.1.3.2.A and B are connection diagrams of the 24-ID-C’s Console-based control system. Numerous scripts have been written for control of the monochromator, focusing, collimation and detector systems. User’s interact with a single CEU to set the optical configuration for a data collection experiment and align samples.

Crystal diffraction data collection is programmed using the ADSC Q315 control system. We have embedded the Console RPC communication core into the Q315 control system so that it can directly program those beamline assets used during data collection: goniometer spindle axis, shutter state, detector position, slits, etc. Additionally, the Q315 control system can pass RPC messages to various CEU’s in order to change the energy set point of the monochromator, adjust the focusing mirror positions, maximize flux and tune the position of the X-ray beam relative to the spindle (via mirror-driven beam steering). The ADSC control program is used at many beamlines and most users have some familiarity with it. The modified ADSC control system can program multiple energy MAD data collection sequences.

C.4.10. 24-ID-C Commissioning

C.4.10.1. Introduction

Monochromatic light was first projected into the 24-ID-C end station on June 23 2004. The 24-ID-C Kirk-Patrick Baez mirror system was installed and commissioned and a reasonably stable, focused monochromatic beam was achieved by late August. The goniometer and detector systems were assembled and commissioned between September and October. First data trials using standard crystals (tetragonal lysozyme, ribonuclease) were performed in mid November 2004.
Initial data trials using frozen tetragonal lysozyme, a minimal beam focus spot size ($\mu_x=20\ \mu$, $\mu_y=7\ \mu$) and short exposure times ($<2$ sec) indicated sample positional instabilities induced by mechanical shock from the XIA pneumatic shutter. Shock-induced movement of the sample caused a characteristic cusped perturbation in the shapes of high resolution diffraction spots. At times, shutter-related sample vibration was directly detectable in the video stream from the alignment microscope. Aerodynamic interactions between conventional loop-mounted samples and the LN$_2$ cooling stream are also a potential source of significant sample positional instability. With large sample loops (> 200 c in length), we could directly observe cold-stream induced sample movement by inspection of the alignment camera video feeds, under conditions that eliminated Schlieren effects from the cold stream, as the cause of apparent sample motion.

We initiated a program to develop shock-free fast data shutters (see C.4.9.3) and implemented multiple phi-sweeps in the goniometer macro code in an attempt to help “average-out” shock-effects and other sources of sample and beam movement from diffraction data. First attempts at data acquisition from small (<100 $\mu$), non-standard crystals from the Ealick lab took place in late December, 2004. This run was largely unsuccessful due to spatial instability of the monochromatic beam caused by problems with the monochromator liquid nitrogen cooling system (see C.4.6.7).

By March of 2005 we had completed development of the fast rotary shutter and associated software. We had also rectified the most significant beam stability issues through improvement of the stability of the monochromator cryopump loop and development of mirror-based beam steering routines (see sections C.4.7.11-13).

C.4.10.2. Commissioning Studies with Standard Crystals

Data trials with frozen lysozyme, using the rotary shutter, immediately yielded reasonably high quality data sets, even with sub-second exposure times. Figure C.4.10.1 shows a bitmap of the first frame from a lysozyme data set using 200 ms exposure time. Table C.4.10.1 lists the scaling statistics summary for this data set. Figure C.4.10.2A,B consists of an HKL2000 graphical data summary for the 200 ms exposure time data set ($\lambda=1$ degree(singel sweep), 100 frames, $\varphi=0.97946$ Å, distance=167 mm, 2x2 binned, $R_{\text{min}}=1.42$ Å). The test crystal was a $\sim120$ x 120 x 60 $\mu$ plate. Data from this particular run were acquired with intentionally low completeness, as we used the same crystal to test data acquisition under many different conditions.
Figure C.4.10.1: Bitmap of first data frame from 200 sec lysozyme data set. Inset in upper right corner shows a magnified area on the meridian of the diffraction pattern from frame 25, centered on R=1.4 Å.

Table C.4.10.1: Scaling Statistics Summary for 200 ms Exposure Lysozyme Data Set.

<table>
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<tr>
<th>Lower Shell Limit</th>
<th>Upper Shell Limit</th>
<th>Average I</th>
<th>Average error</th>
<th>Average Stat.</th>
<th>Norm. Chi**2</th>
<th>Linear R-fac</th>
<th>Square R-fac</th>
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<td>1.784</td>
<td>0.042</td>
<td>0.047</td>
</tr>
</tbody>
</table>
Data sets from the same crystal, with 100 ms exposure time showed significant degradation of statistical quality, in the absence of data culling (Figure C.4.10.2A,B). Culled data (12%, cut determined by HKL2000) showed no apparent correlation with respect to reflection Miller indices, and were uniformly distributed over the data frame space. After culling (data not shown) statistical properties were similar to the 200 ms data set. We interpret this finding to indicate that with short exposure times (< 200 ms), the effects of residual vertical beam motion negatively impacts data statistical quality. Short exposure data acquired with vertically under-focused beam ($\alpha_y \sim 20 \mu$) or multiple sweeps demonstrate significantly improved statistics (data not shown).

Figure C.4.10.2A: HKL2000 data reduction summary for 100 frame, 200 ms exposure lysozyme data set.
Figure C.4.10.2B: HKL2000 data reduction summary, continued (200 ms exposure).

For comparison, Table C.4.10.2 and Figures C.4.10.3A,B provide the scaling statistics for another data set from the same crystal, with 1 second exposures (all other data collection parameters unchanged). Beam attenuation was adjusted to provide approximately the same number of pixel overflows that were observed with the 200 ms data set. Note that overall, the two data sets have very similar statistical properties, in spite of the 5-fold exposure time difference.

Table C.4.10.2: Scaling Statistics Summary for 1.0 Sec Exposure Lysozyme Data Set.

<table>
<thead>
<tr>
<th>Lower Shell Limit</th>
<th>Upper Shell Limit</th>
<th>Average I</th>
<th>Average Error</th>
<th>Average Stat.</th>
<th>Norm. Chi**2</th>
<th>Linear R-fac</th>
<th>Square R-fac</th>
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<td>30.00</td>
<td>3.02</td>
<td>17700.2</td>
<td>343.2</td>
<td>212.1</td>
<td>0.988</td>
<td>0.021</td>
<td>0.025</td>
</tr>
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<td>3.02</td>
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<td>7697.4</td>
<td>152.0</td>
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<td>2.116</td>
<td>0.036</td>
<td>0.044</td>
</tr>
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<td>2.39</td>
<td>2.09</td>
<td>5271.1</td>
<td>109.2</td>
<td>72.4</td>
<td>2.206</td>
<td>0.040</td>
<td>0.048</td>
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<tr>
<td>2.09</td>
<td>1.90</td>
<td>3272.7</td>
<td>72.5</td>
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<td>1.546</td>
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<td>0.043</td>
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<tr>
<td>1.90</td>
<td>1.76</td>
<td>1897.2</td>
<td>49.2</td>
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<td>1.76</td>
<td>1.66</td>
<td>1275.0</td>
<td>37.7</td>
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<td>0.044</td>
<td>0.047</td>
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<td>1.66</td>
<td>1.58</td>
<td>1046.8</td>
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<td>29.7</td>
<td>1.061</td>
<td>0.048</td>
<td>0.050</td>
</tr>
<tr>
<td>1.58</td>
<td>1.51</td>
<td>762.9</td>
<td>30.7</td>
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<td>1.145</td>
<td>0.061</td>
<td>0.064</td>
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<td>1.51</td>
<td>1.45</td>
<td>605.2</td>
<td>29.2</td>
<td>27.1</td>
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<td>0.077</td>
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<td>1.45</td>
<td>1.40</td>
<td>466.9</td>
<td>28.5</td>
<td>27.1</td>
<td>1.306</td>
<td>0.094</td>
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<td>All reflections</td>
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<td>3844.8</td>
<td>85.9</td>
<td>59.7</td>
<td>1.383</td>
<td>0.033</td>
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</table>
Figure C.4.10.3A: HKL2000 data reduction summary for 100 frame, 1.0 second exposure lysozyme data set.
Figure C.4.10.3B: HKL2000 data reduction summary continued (1second exposure).

Table C.4.10.3 provides a listing of data reduction parameters for commissioning data collection using recombinant bovine pancreatic ribonuclease A. A number of data sets were obtained from a large 200 µ crystal. The 1.0 second exposure, 3 phi-sweep set was the last acquired in this set and manifests substantial sample damage.
Table C.4.10.3: Data Reduction Summaries for Ribonuclease Commissioning Study.

<table>
<thead>
<tr>
<th>Exposure Time (sec)</th>
<th>Phi Sweeps</th>
<th>No. Frames</th>
<th>Overall $R_m$ (%)</th>
<th>$R_m$ LS 40-3.66 Å (%)</th>
<th>$R_m$ HS 1.76-1.70 Å (%)</th>
<th>Redun</th>
<th>$I/\sigma$</th>
<th>%Rej</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1</td>
<td>100</td>
<td>4.6</td>
<td>2.5</td>
<td>25.4</td>
<td>3.2</td>
<td>11.9</td>
<td>0.08</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>100</td>
<td>4.6</td>
<td>2.1</td>
<td>37.1</td>
<td>3.1</td>
<td>10.9</td>
<td>0.05</td>
</tr>
<tr>
<td>0.2</td>
<td>1</td>
<td>100</td>
<td>4.3</td>
<td>3.0</td>
<td>16.6</td>
<td>3.2</td>
<td>16.1</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Key: $R_m$ LS = R merge for low resolution shell
$R_m$ HS = R merge for high resolution shell
Redun = Reduncency
%Rej = % rejected reflections

C.4.10.3. Core User Commissioning Studies

During the final month of the fall 2005 APS run 70% of available beamtime was committed to core user studies. During this period the 24-ID-C beamline operated continuously without any significant beamline hardware or software problems.

C.4.10.3.1. Steven E. Ealick Group (Cornell University)

The Ealick laboratory has been involved in the bulk of commissioning activities. There have been four trips, March 24-25, April 15-17, June 28-30 and August 3-5 of 2005. The following section summarizes their experience with the 24-ID-C beamline.

March 24-25, 2005

AdoP. *Bacillus cereus* adenosine phosphorylase (AdoP) belongs to the high-molecular-mass purine nucleoside phosphorylase class on the basis of amino acid sequence homology and molecular mass determination, but it differs from the other members of this subfamily because it exhibits a high preference for adenosine over inosine. Using this and subsequent data sets, we determined the structures of the wild type enzyme and an active site mutant, both complexed with substrates, to determine the structural basis of the unusual substrate specificity shown by *B. cereus* AdoP. A poster describing this work was presented at the IUCr meeting in Florence, Italy and a manuscript is being prepared.

A mutant of AdoP and AdoP complexed with various ligands (ino = inosine) constituted seven useful data sets on the first trip. In addition, the crystals were used for testing loops from various manufactures and for characterization of the beamline. In the April trip, five additional data sets were collected on AdoP complexes with inosine and adenosine to high resolution, 1.17 – 1.6 Å, with the CCD detector vertically offset. Four additional data sets of AdoP-inosine and AdoP-deazainosine were collected in the visit at the end of June.

PF1337. Many of the reactions catalyzed by the enzymes in thiamin biosynthesis involve unprecedented chemistry and elucidation of catalytic mechanism is a major goal. By studying these enzymes, we have also discovered interesting evolutionary links to other pathways. This year we published the structures of TenA and TenI from this pathway. The structures of these enzymes with additional substrate analogs will provide additional insights into the active site and the pathways. PF1337 is a TenA homologue.

Two useful data sets were collected in the March visit: one of native PF1337 (2.4 Å) and one of PF1337 with hydroxymethyl pyrimidine (HMP) at 2.8 Å. Two additional data sets of PF1337
complexes one with thiamin (3.0 Å) and the other with hydroxymethyl pyrimidine (2.5 Å) were collected in the April trip. Analysis is underway.

Deoxycytidine kinase. Because deoxycytidine kinase (dCK) is the critical enzyme in the activation of several compounds that exhibit in vivo antitumor activity, we are interested in learning more about the characteristics of the active site. To that end, we are determining the crystal structure of dCK in complex with a number of substrate analogs, inhibitors, and cofactors. Previous studies indicated that replacement of the 4'-oxygen with sulfur significantly reduced the substrate activity of nucleoside analogs with dCK. Additional insights will aid in our drug discovery program.

The data was used to determine the structure of the complex of dCK with ADP and clofarabine using molecular replacement. A manuscript that has been submitted to *Acta Crystallographica Section D*. The coordinates (2A7Q) and structure factors have been deposited in the Protein Data Bank. In August two useful data sets of dCK complexed with deoxycytidine and uridine diphosphate were collected (3.47 Å and 3.50 Å). This data will help to further elucidate active site features.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Space Group</th>
<th>Res. (Å)</th>
<th>Rsym (Hi res.)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdoP-D204N</td>
<td>P6₃22</td>
<td>1.9</td>
<td>6.7(19.2)</td>
<td></td>
</tr>
<tr>
<td>AdoP</td>
<td>P6₃22</td>
<td>1.7</td>
<td>10.1(25.5)</td>
<td></td>
</tr>
<tr>
<td>AdoP</td>
<td>P6₃22</td>
<td>1.4</td>
<td>7.7(48.0)</td>
<td>Detector Offset</td>
</tr>
<tr>
<td>AdoP</td>
<td>P6₃22</td>
<td>1.4</td>
<td>5.8(45.7)</td>
<td>Litho loop test</td>
</tr>
<tr>
<td>AdoP+Ino</td>
<td>P6₃22</td>
<td>2.25</td>
<td>8.7(34.0)</td>
<td></td>
</tr>
<tr>
<td>AdoP+Ino</td>
<td>P6₃22</td>
<td>1.7</td>
<td>8.2(28.0)</td>
<td></td>
</tr>
<tr>
<td>AdoP+Ino</td>
<td>P6₃22</td>
<td>1.5</td>
<td>7.9(43.0)</td>
<td></td>
</tr>
<tr>
<td>PF1337</td>
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<td>2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF1337+HMP</td>
<td></td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dCK+ADP+clofarabine</td>
<td>P4₃212</td>
<td>2.3</td>
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<td>MR, submitted</td>
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</tbody>
</table>

April 15-17 2005

Toxoplasma gondii Adenosine Kinase (TGAK). Morbidity and mortality due to infections with *Toxoplasma gondii* are an increasing problem in the HIV-infected population. Management of toxoplasmosis in these patients is complicated by a high incidence of side effects with the most commonly used compounds and the inactivity of these compounds against the latent stage of the parasite. In conjunction with our collaborator, we will characterize the TgAK and to use the information gained to develop potent and selective subversive substrates as potential anti-toxoplasmic agents. For the crystals used in these data sets 6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (NBMPR) was used in the crystallization conditions. The structure was solved by molecular replacement. Although NBMPR could not be modeled into the active site, these data helped clarify active site residues. A manuscript has been submitted to *Acta Crystallographica Section D*.

Bovine uridine phosphorylase. Uridine phosphorylase (UP) catalyzes the reversible phosphorylorysis of uridine to uracil and ribose 1-phosphate, and is a key enzyme in the pyrimidine salvage pathway. UP is a member of the large nucleoside phosphorylase I family of proteins. As such, it shares both a common fold and common mechanism. Interest in this enzyme has stemmed from its involvement in activating a key chemotherapeutic molecule, 5-fluorouracil (5FU). Recently, experiments have indicated that the tolerance of non-cancerous cells to 5FU toxicity can be increased by co-administration of 5FU based drugs with UP inhibitors. Our studies have focused on further characterization of the enzyme with the ultimate goal of improved mechanistic understanding and the design of new inhibitors. We collected two
potentially usable data sets, to 2.5 Å and 2.8 Å resolution; however, we were unable to solve the structure by molecular replacement. Se-met crystals have been prepared, but so far are too fragile to use for an entire SAD or MAD data set.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Res. (Å)</th>
<th>Rsym(Hi res.)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
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<td>8.7(44.9)</td>
<td></td>
</tr>
<tr>
<td>AdoP-D204N +inosine+SO₄</td>
<td>1.4</td>
<td>7.5(47.3)</td>
<td></td>
</tr>
<tr>
<td>AdoP+ inosine+SO₄</td>
<td>1.25</td>
<td>10.6(30.9)</td>
<td>Detector offset</td>
</tr>
<tr>
<td>AdoP+ inosine+SO₄</td>
<td>1.17</td>
<td>4.5(44.8)</td>
<td>Detector offset</td>
</tr>
<tr>
<td>AdoP+ inosine+SO₄</td>
<td>1.2</td>
<td>5.1(41.9)</td>
<td>Detector offset</td>
</tr>
<tr>
<td>TgAK+nbmpr+ACP</td>
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<td>11(28)</td>
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</tr>
<tr>
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<td>1.3</td>
<td>7.7(21)</td>
<td></td>
</tr>
<tr>
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<td>1.5</td>
<td>4.2(30.7)</td>
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<tr>
<td>PF1337+hydroxymethyl pyrimidine</td>
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<td></td>
<td></td>
</tr>
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<td>Uridine Phosphorylase</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uridine Phosphorylase</td>
<td>2.8</td>
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<td></td>
</tr>
</tbody>
</table>

June 28-30, 2005

*Thermitoga maritima* YaaDE. In *Bacillus subtilis*, YaaE is a member of the triad glutamine amidotransferase family and functions in a recently identified alternate pathway for the biosynthesis of vitamin B6. YaaD appears to be responsible for incorporating the ammonia released by YaaE into the pyridine ring of vitamin B6. Through their role in the production of B6, YaaD homologues in Fungi have been shown to play an important role in the resistance of these organisms to oxidative stress and understanding the mechanisms of these enzymes may eventually allow the development of new antibiotics. We are looking at the fused gene in *Thermitoga maritima* to provide additional insights into this pathway. Two usable data sets to 3.15 Å and 2.90 Å were collected. The structure has been solved and refinement is continuing.

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</tr>
<tr>
<td>AdoP+inosine</td>
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<td>1.25</td>
</tr>
<tr>
<td>AdoP+deazainosine</td>
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<td>2.00</td>
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<tr>
<td>AdoP+deazainosine</td>
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<td>1.30</td>
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<tr>
<td>YaaDE</td>
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<td>YaaDE</td>
<td>I222</td>
<td>2.90</td>
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</tbody>
</table>

August 3-5 2005

*Caulobacter crescentus* ThiC. This enzyme is part of our continuing explorations into the reactions involved in thiamin biosynthesis. We published a structure of ThiC from *Bacillus subtilis*. By studying the enzyme from this organism, we hope to further elucidate the mechanism and evolutionary links among species. We obtained three useful data sets, two native (3.0 Å and 2.85 Å) and one with ThiC complexed with HMP. Analysis of the data is progressing.

*Purine nucleoside phosphorylase (PNP)*. Because of the role of purine and pyrimidine nucleotide metabolism in diseases such as cancer and viral infection, many of the enzymes
involved are targets for drug design. We also study bacterial purine nucleoside phosphorylase because of its application in prodrug activation via gene therapy. One usable data set of the E. coli PNP mutant dod7.13 to 3.2 Å was collected. We expect to be able to solve the structure by molecular replacement.

<table>
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<th>Protein</th>
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<td>ThiC</td>
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</tr>
<tr>
<td>ThiC+HMP</td>
<td>P1</td>
<td></td>
</tr>
<tr>
<td>dCK</td>
<td></td>
<td>3.47</td>
</tr>
<tr>
<td>dCK</td>
<td></td>
<td>3.50</td>
</tr>
<tr>
<td>Dod7.13 PNP</td>
<td></td>
<td>3.20</td>
</tr>
</tbody>
</table>

C.4.10.3.2. Stephen C. Harrison Group (Harvard University)

August 17-20, 2005

Rotavirus. Rotavirus is a non-enveloped, dsRNA, icosahedral virus that is the major cause of gastroenteritis in children, resulting in 440,000 child deaths worldwide each year. Development of a safe and effective vaccine is an important global health priority. Like other non-enveloped dsRNA viruses, rotavirus must traverse the cell membrane to deliver an intact internal core particle (ICP) into the cytoplasm of the infected cell. The proposed experiments involve the use of X-ray crystallography to determine the atomic resolution structure of the transcriptionally active rotavirus ICP, the ICP bound by the C-terminal region of a virally encoded membrane spanning ER protein, NSP4. Results of these experiments will illuminate the stages of viral entry into the cell and ICP budding into the ER lumen. Increasing the basic understanding of ER functioning will shed light on the processes of protein maturation and transport. Furthermore, these structures will give insight into the potential liquid crystalline packaging of the dsRNA genome. Overall these structures will serve the longer-term plans of designing subunit vaccines.

To date, only survey studies have been completed at the NE-CAT 24 beamline to investigate the suitability of the existing optical performance for study of unfrozen rotavirus crystals (see Figures C.4.10.4 and C.4.10.5). Quartz capillary mounted crystals were approximately 300 μ per edge and typically provided 2 to 3 separate exposures via crystal translation. Crystals were cooled to approximately 4°C using an FTS compressed air cooler. The images were used to define a P2_1 2_1 2_1 space group with unit cell parameters of a = 740.75 Å, b = 1198.07Å, c = 1345.41 Å, enough for one full ICP/asymmetric unit.

Herpes Simplex Virus Fusion Protein. Herpes simplex viruses (HSV-1 and -2), infect their hosts for life, causing cold sores, eye and genital infections, and encephalitis. To enter host cells, HSV requires four viral envelope glycoproteins: gB, gD, and gH/gL complex. The subject of this project, HSV-1 envelope glycoprotein gB is the required component of the viral fusion machinery, but the mechanism by which fusion occurs remains elusive and no structural information is available on gB. To understand the function of gB in virus entry, we are pursuing the structure of the soluble ectodomain of gB. Knowing the structural mechanism of HSV entry into host cells will allow us to design inhibitors of viral entry for preventative and therapeutic purposes.
HSV-1 gB Data Collection Statistics:

<table>
<thead>
<tr>
<th></th>
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<th>HSV-1 gB</th>
<th>HSV-1 gB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Se-xtal 1Peak</td>
<td>Se-xtal 2Peak</td>
<td>Se-xtal 2Remote</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>50 (2.6)</td>
<td>50 (2.8)</td>
<td>50 (2.9)</td>
</tr>
<tr>
<td>Space group</td>
<td>P1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unit cell (Å)</td>
<td></td>
<td>a = 83.6</td>
<td>α = 67.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b = 100.1</td>
<td>β = 78.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c = 100.0</td>
<td>γ = 70.6</td>
</tr>
<tr>
<td>Redundancy</td>
<td>3.8(2.6)</td>
<td>5.2(3.0)</td>
<td>4.0(2.5)</td>
</tr>
<tr>
<td>Completeness</td>
<td>77(28)</td>
<td>75(28)</td>
<td>79(53)</td>
</tr>
<tr>
<td>R_{sym} (%)</td>
<td>10(37.3)</td>
<td>11.8(47.5)</td>
<td>10.3(52.5)</td>
</tr>
<tr>
<td>I/σ</td>
<td>15.6(2.16)</td>
<td>17.5(2.02)</td>
<td>24.5(1.63)</td>
</tr>
</tbody>
</table>

Values for the highest resolution shell are given in parentheses. 

\[ R_{sym} = \frac{\sum |I_i| - \langle I \rangle}{\sum \langle I \rangle}, \] where \( \langle I \rangle \) is the mean intensity of the N reflections.

**Figure C.4.10.4:**
Diffraction image from an unfrozen rotavirus crystal. Inset: magnified view of lune to right of beam stop. Detector distance: 750 mm. Peak separations within layer lines of the lunes of the inset represent a lattice spacing of 1345 Å.
Figure C.4.10.5: 3D mesh image of the top, left, innermost lune in the inset of Figure C.4.10.4. The lattice spacing between peaks, within layer lines is 1345 Å.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Space Group</th>
<th>Res. (Å)</th>
<th>Rsym (Hi res.)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1 gB</td>
<td>P2₁2₁2₁</td>
<td></td>
<td></td>
<td>a=741</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b=1198</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c=1345</td>
</tr>
<tr>
<td>HSV-1 gB (xtal 2)</td>
<td>P1</td>
<td>2.6</td>
<td>10(37.3)</td>
<td>Peak SAD</td>
</tr>
<tr>
<td>HSV-1 gB (xtal 2)</td>
<td>P1</td>
<td>2.8</td>
<td>11.8(47.5)</td>
<td>Peak MAD</td>
</tr>
<tr>
<td>HSV-1 gB (xtal 2)</td>
<td>P1</td>
<td>2.9</td>
<td>10.3(52.5)</td>
<td>remote MAD</td>
</tr>
</tbody>
</table>
C.4.10.3.3. Nikola Pavletich Group (Sloan-Kettering Memorial Cancer Center)

Data described in this section are the result of two trips to the 24-ID-C beamline, April 18-20 and August 8-11, 2005.

Skp1-Skp2-Cks1-p27 ubiquitin ligase-substrate complex. The ubiquitin-mediated proteolysis of the p27<sup>Kip1</sup> Cdk2 inhibitor plays a central role in cell cycle progression, and enhanced degradation of p27<sup>Kip1</sup> is associated with many common cancers. Proteolysis of p27<sup>Kip1</sup> is triggered by Thr187 phosphorylation, which leads to the binding of the SCF<sub>Skp2</sub> (Skp1-Cul1-Rbx1-Skp2) ubiquitin ligase complex. Unlike other known SCF substrates, p27<sup>Kip1</sup> ubiquitination also requires the accessory protein Cks1. The crystal structure of the Skp1-Skp2-Cks1 complex bound to a p27<sup>Kip1</sup> phosphopeptide shows that p27 binds to both Cks1 and Skp2. The phosphorylated Thr187 side chain of p27<sup>Kip1</sup> is recognized by a Cks1 phosphate-binding site, while the side chain of an invariant Glu185 inserts into the interface between Skp2 and Cks1, interacting with both.

The structure was initially determined at 8-BM to 3.0 Å resolution. 24-ID-C allowed extension to 2.3 Å with a commensurate increase in the interpretable details of the interface (26). High resolution data is particularly important in this case, as the interfaces between p27 and Cks1 and Skp2 are a target for anti-cancer drug design.

Retinoblastoma C-terminus bound to an E2F-DP heterodimer. The Rb pathway links growth-regulatory signals to a transcription program required for DNA synthesis, cell cycle progression and cell division. This transcription program is activated by the E2F transcription factors and repressed by E2F-Rb complexes. Building on earlier studies of the Rb pocket domain, we have recently demonstrated that Rb C-terminal domain (RbC) contains a hitherto unknown binding site for E2F-DP heterodimers and have determined the crystal structure of an RbC-E2F-DP complex. The structure, in conjunction with biochemical data, revealed that this interaction is regulated by Cdk-mediated phosphorylation of RbC, answering, at least in part, the longstanding question of how phosphorylation dissociates Rb-E2F complexes.

The structure was initially solved with data sets from APS 8-BM and BNL-X4A to ~3.0 Å resolution. 24-ID-C allowed us to extend the resolution to 2.55 Å (27).

ATR-ATRIP sub-complex active in DNA binding. The ATR phosphoinositide 3-kinase-like protein kinase has a key role in the cell’s response to DNA damage. ATR and its accessory factor ATRIP sense DNA double strand breaks and other related lesions that arise during replication, and initiate a signaling cascade that induces cell cycle arrest and increases the cell’s repair capacity. The structure of a ~ 100 kDa ATR-ATRIP subcomplex that retains the DNA-binding activity of the full-length proteins has been determined, and attempts are being made to obtain the structure of this complex bound to DNA.

The structure was initially determined with Se-MAD data sets obtained at 8-BM to ~3.8 Å resolution. Due to crystal variability, we had to screen multiple crystals to obtain the highest possible resolution data set (currently ~3.1 Å). In addition, due to a rapid loss of diffraction intensity beyond ~6 Å, measuring the high resolution reflections was critically dependent on the small beam size and low divergence of the 24-ID-C beamline.

Publication: We anticipate that we will submit the structure for publication within the next year. Nabil Chehab and Nikola Pavletich (unpublished).

DDB1-DDB2-DNA complex involved in nucleotide excision repair. Xeroderma pigmentosum (XP) is a genetic disease characterized by hypersensitivity to UV irradiation and high incidence of skin cancer caused by inherited defects in DNA repair. Mutational malfunction of damaged-DNA binding protein 2 (DDB2) causes the XP complementation group E (XP-E). DDB2 together with DDB1 comprises a heterodimer called DDB complex, which is involved in damaged-DNA
binding and nucleotide excision repair.
The structure of the DDB1-DDB2 complex was determined using Se-Met MAD (24-ID) and Au/HG MAD (8-BM) methods and refined to 3.3 Å resolution with native data collected at 8-BM (with 3x translated crystal). The initial structure of the DDB1-DDB2-DNA complex was determined by molecular replacement using the DDB1-DDB2 structure as a search model. While the resolution of the DDB1-DDB2-DNA data set is only ~4.0, it is allowing us to refine our choice of DNA fragments to obtain better ordered crystals, and is also providing initial insights to how DDB1-DDB2 recognizes diverse DNA lesions.

We anticipate that we will submit the structure for publication within the next year: Nico Thoma and Nikola Pavletich (unpublished).

**RAD4-RAD23-DNA complex involved in nucleotide excision repair.** The RAD4 (Xeroderma Pigmentosum C)-RAD23 complex acts early on in the NER pathway, in parallel to DDB1-DDB2, to recognize DNA lesions such as cyclobutadiene dimers and bulky base adducts. How a diverse set of lesions is recognized among the many more lesions present in DNA, and how they get handed to the NER repair machinery are the major questions we are attempting to address. Towards this goal, we have recently determined the 2.6 Å structure of a RAD4-RAD23 complex, and have obtained a preliminary, low-resolution structure of the complex bound to damaged-DNA.

The structure was initially determined using Hg-MAD at 8-BM to 3.3 Å resolution, and refined against a 3.0 Å native data set (8-BM). 24-ID-C allowed extension to 2.6 Å. Several different RAD4-RAD23-DNA crystals were screened at 24-ID-C, and the best data (~4.2 Å) has allowed us to build a low-resolution model of the DNA. We are currently screening a large number of damaged-DNA substrates, designed based on the low-resolution structure, to obtained better ordered crystals.

We anticipate that we will submit the structure for publication within the next year. Jung-Hyun Min and Nikola Pavletich (unpublished).

<table>
<thead>
<tr>
<th>Protein</th>
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<th>Space Group</th>
<th>Res. (Å)</th>
<th>Rsym (Hi res.)</th>
<th>Comments</th>
</tr>
</thead>
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<td>P321</td>
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<td>5.6(42.2)</td>
<td></td>
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<tr>
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<td>C2221</td>
<td>2.55</td>
<td>5.6(50.2)</td>
<td></td>
</tr>
<tr>
<td>ATR-ATRIP</td>
<td>8</td>
<td>P4222</td>
<td>3.1</td>
<td>5.2(39.4)</td>
<td></td>
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<tr>
<td>DDB1-DDB2</td>
<td>6</td>
<td>H32</td>
<td>4.2</td>
<td>23.7(48.4)</td>
<td>a=b=267, c=471 MAD (Se)</td>
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<td>DDB1-DDB2-DNA</td>
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<td>C2</td>
<td>3.94</td>
<td>(52.3)</td>
<td>Screening for DNA seq.</td>
</tr>
<tr>
<td>RAD4-RAD23</td>
<td>12</td>
<td>C2</td>
<td>2.6</td>
<td>6.7(40.2)</td>
<td></td>
</tr>
<tr>
<td>RAD4-RAD23-DNA</td>
<td></td>
<td>C2221</td>
<td>4.1</td>
<td>10.9(52.3)</td>
<td>Screening for DNA seq.</td>
</tr>
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</table>
Post-translational protein modification by SUMO. The small ubiquitin-like modifier SUMO is known to regulate nuclear transport, stress response, and signal transduction in eukaryotes, a process that is essential for cell cycle progression in eukaryotes including yeast. Analogous to ubiquitin modification, SUMO conjugation occurs on lysine residues and is catalyzed by E1, the SUMO activating enzyme, E2, the SUMO conjugation enzyme, E3-like conjugation cofactors, and proteases that catalyze SUMO processing and deconjugation. SUMO modification does not appear to target proteins for degradation, but rather alters the target protein function through changes in cellular localization, biochemical activation, or through protection from ubiquitin-dependent degradation. Our research has focused on several SUMO processes that include its activation, conjugation, and deconjugation to and from cellular target proteins. We make extensive use of structure-based mutagenesis, genetics, and in vitro biochemical reconstitution of the sumoylation process to reveal the mechanisms used for substrate binding and SUMO modification of various substrates and are using insights uncovered by our studies to elucidate mechanisms common to all post-translational modification pathways. Several papers in this area have been published in journals such as Cell, Nature, and Molecular Cell. This work is currently funded by NIH RO1 GM065872, PI-C.D. Lima and by the Rita Allen Foundation.

There are at least 4 human isoforms of SUMO and little is known about how enzymes in the SUMO pathway discriminate between them. We successfully collected two useful data sets: (1) a protease-SUMO-2 complex and (2) SUMO-2 in a four-protein complex that includes substrate, E2, and E3. Based on our previous structures of related proteins, we have been successful in obtaining molecular replacement solutions with the data collected at 24-ID-C and are in the process of model building and refinement. Both data sets were collected in binned mode and both have exceptional statistics at low resolution.

Messenger RNA maturation and decay. Co-transcriptional and post-transcriptional processing of eukaryotic messenger RNA (mRNA) plays a central role in regulating the activity and lifetime of a particular message. The 5' m'GppN cap is the first co-transcriptional modification of eukaryotic mRNA and is required for efficient pre-mRNA splicing, export, stability, and translation. Cap formation entails three activities that are catalyzed by RNA triphosphatase, RNA guanylyltransferase, and RNA methyltransferase. Capping requires recruitment of these enzymes directly to RNA polymerase II (RNAPII) during initiation and early elongation and recruitment is mediated by interactions between the capping apparatus and the Ser5 phosphorylated C-terminal domain (CTD) of the largest RNA polymerase II subunit. Transcriptional regulation and mRNA decay work in conjunction to control the abundance and lifetime of cellular mRNA, and both serve to regulate the time that messages interact with the translation apparatus. Removal and degradation of the RNA cap structure occurs in both major RNA decay pathways. After deadenylation of polyadenylated mRNA, enzymes in the 5'-3' decay pathway hydrolyze the mRNA cap to expose the 5' RNA end to 5'-3' exoribonuclease activities. In the 3'-5' decay pathway, exosome-mediated degradation of RNA occurs from the 3' end after deadenylation, ultimately generating a cap structure that is hydrolyzed by enzymes in this pathway. We are continuing our studies in this area through structural and mechanistic characterization of enzymes involved in cap formation, CTD interaction and metabolism, cap and RNA degradation, and associated complexes with RNA polymerase II. We have published several papers in this area in journals such as Cell and Molecular Cell. This work is currently funded by NIH RO1 GM61906, PI-C.D. Lima and by the Rita Allen Foundation.

We have co-crystallized the intact capping apparatus (heterotetramer of ~180 kDa) in the presence of phosphorylated CTD and nucleotide. Although the data is weak, we were able to collect one potentially useful data set at 24-ID on this trip.
Structural studies of the anti-oxidant defense pathways of Mycobacterium tuberculosis. We have been investigating the mechanisms by which Mycobacterium tuberculosis (Mtb) evades the substantive response of the host immune system that includes both oxidative and nitrosative stress. This is accomplished in part by adopting metabolic enzymes to catabolize these toxic compounds. Lipoamide dehydrogenase (Lpd), dihydrolipoamide acyltransferase (DlaT; formerly termed succinyl transferase), an alkylhydroperoxidase termed AhpC, and the protein (AhpD) encoded by an adjacent gene, have been shown to take part in this defense pathway. All of these enzymes are oxidoreductases and each contains redox centers that reduce or oxidize adjacent partners in the pathway. We are actively engaged in the structural and mechanistic characterization of these enzymes, alone, in complex with one another, and in complex with various putative inhibitors. We have published work in this area in Science and JBC. This work is currently funded by NIH RO1 HL072718, PI-C. Nathan, Co-PI-C.D. Lima.

No structure currently exists for any intact DlaT from any organism. We successfully collected native and derivative data sets (TaBr) for this system on our trip to 24-ID-C. While we are still in the process of determining the number and quality of TaBr sites, initial results appear promising. Although the native data set collected at 24-ID is not the highest resolution, we have observed, it will be useful for SIRAS phase determination (isomorphous wavelengths and conditions).

<table>
<thead>
<tr>
<th>Protein</th>
<th># of data sets</th>
<th>Type</th>
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<td>Swvl</td>
<td>2.37</td>
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<tr>
<td>SUMO2:E2:Substrate:E3</td>
<td>1</td>
<td>Swvl</td>
<td>2.8</td>
<td>3.9(29.2)</td>
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<tr>
<td>Capping Apparatus-CTD-nucleotide</td>
<td>1</td>
<td>Swvl</td>
<td>3.2</td>
<td>10.2(68.4)</td>
<td></td>
</tr>
<tr>
<td>Dla T</td>
<td>1</td>
<td>Swvl</td>
<td>3.8</td>
<td>10.3(51.3)</td>
<td></td>
</tr>
<tr>
<td>Dla T:TaBr</td>
<td>1</td>
<td>SAD</td>
<td>4.0</td>
<td>6.7(14.0)</td>
<td>$E=10$keV</td>
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</table>

C.4.10.3.5. Michael Eck (Harvard University)

In a trip at the end of the run, August 20-22,2005, the Eck group collected 27 data sets on a variety of projects in his lab as well as one from Jia-huai Wang’s group. The table below is a summary of the data that was collected in that trip.
C.4.10.3.6. Gregory Verdine (Harvard University)

August 22-24, 2005

Structural Studies of Bacterial Nucleotide Excision Repair Enzymes. Damage to DNA by endogenous and exogenous agents are the major cause of genetic mutations that give rise to different forms of cancer. Nucleotide Excision Repair (NER) is one of the major DNA repair pathways functioning in both eukaryotes and prokaryotes. What NER stands out from other repair processes is its ability to recognize and repair a broad spectrum of DNA lesions, such as cis-syn thymine dimers caused by UV light, bulky benzo[a]pyrene diol epoxide(BPDE) guanine adducts, and N-acetyl-2-aminofluorene(AAF) guanine adducts.

The NER system in bacteria consists of three major proteins, UvrA, UvrB, and UvrC, all of which are essential for successful damage correction. UvrA, which has strong affinity to damaged sites in DNA, has a key role in the recognition process. It also has a domain that interacts with UvrB. UvrA recognizes the site of damage, facilitates the interaction between UvrB and DNA, and then leaves the damaged site. UvrB forms a stable complex with DNA, followed by the recruitment of catalytic UvrC, which cleaves the 4th phosphodiester bond at 3' end of the lesion, and then the 8th phosphodiester bond at 5' end of the lesion. Interestingly, without UvrA, UvrB or UvrC do not have any affinity to damaged DNA.

Although this repair pathway is known for decades, only UvrB and domain structures of UvrC are available to date. In this project, our goal is to obtain the crystal structure of full-length UvrA. We believe that this structure will be useful for our understanding of how NER can recognize many different types of damages and how UvrA can hand the damaged DNA to UvrB in order to complete its repair process.

About 80 bacterial UvrA crystals were surveyed and 8 full data sets were collected. Data was collected to a resolution of ~3.1 Å with I/σ less than 2 and linear R-factor less than 0.5. The space group was determined to be P2_1 corresponding to 2 molecules per asymmetric unit. This is the highest resolution and best quality data obtained to date. The beam at 24-ID was essential to obtain this data from the small, poorly diffracting crystals. Currently,

<table>
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<th>Protein</th>
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<th>Res. (Å)</th>
<th>Rsym(Hi res.)</th>
<th>Comments</th>
</tr>
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<tr>
<td>Focal adhesion kinase</td>
<td>10</td>
<td>C222_1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal adhesion kinase</td>
<td>1</td>
<td>P2_12_12_2</td>
<td></td>
<td></td>
<td>Discovered new space group</td>
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<tr>
<td>β-catenin/inhibitor complexes</td>
<td>3</td>
<td>P2_12_12_2</td>
<td></td>
<td></td>
<td>No inhibitor bound</td>
</tr>
<tr>
<td>f-spondin reelin domain</td>
<td>2</td>
<td>P4_32_2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammalian diaphanous protein 1</td>
<td>4</td>
<td>P2_1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGF-R kinase mutants</td>
<td>8</td>
<td>I23</td>
<td>2.7</td>
<td>6.6(56.3)</td>
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</tr>
</tbody>
</table>
selenomethionine incorporated crystals are being optimized to obtain phase information of this protein structure.

<table>
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<th>Res. (Å)</th>
<th>Rsym (Hi res.)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>UvrA</td>
<td>8</td>
<td>P2₁</td>
<td>3.1</td>
<td></td>
<td>Screened 80 crystals</td>
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C.4.10.3.7. Thomas Steitz (Yale University)

August 24-26, 2005

DNA Polymerase III. DNA polymerase III (PolIII) is the main replicative polymerase of prokaryotes. No structural information is available for PolIII and the PolIII family displays no sequence similarity to any other polymerase family. As Eukaryotes utilize a different family of polymerases (Family B) for their genomic replication, bacterial PolIII is also a potential drug target. A structure of this class of polymerase would give valuable information on understanding its function at the replication fork and show how PolIII relates to other polymerase families.

DnaB Helicase. DnaB helicase is a ring hexamer that functions to unwind duplex DNA at the replication fork of prokaryotes. Unwinding is in the 5’ to 3’ direction and requires the hydrolysis of ATP. DnaB is a two domain protein having a C-terminal RecA like motor domain and an N-terminal domain whose function in unwinding is not clear. To date structures have been solved for the E. coli DnaB N-terminal domain and of a homologue of the C-terminal domain from T7 gp4 helicase. There are no structures of the helicase with its DNA substrate.

A total of 35 usable datasets were collected. In general the data was of high quality and displayed a lower mosaic spread than was normal for other beamlines. Very useful data was collected from crystals of apo PolIII which have allowed the determination of a 4.0 Å electron density map. To our knowledge the first electron density calculated for this important enzyme and potential drug target.

Mosaic spread of all polymerase data was < 0.5 degrees

15 datasets were collected for crystals of nucleotide bound DnaB helicase, the majority of the data being heavy atom soaks. The data was of comparable quality to data collected at other beamlines (diffraction limit between 5 and 6 Å), although the mosaicty of some crystals was lower than seen before.

<table>
<thead>
<tr>
<th>Protein</th>
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<tr>
<td>Pol III</td>
<td></td>
<td>3.0</td>
<td>7.1</td>
<td></td>
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<tr>
<td>Pol III – Hg derivative</td>
<td></td>
<td>3.1</td>
<td>8.5</td>
<td></td>
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<tr>
<td>Pol III – W derivative</td>
<td></td>
<td>3.7</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>DnaB helicase</td>
<td>15</td>
<td>5-6</td>
<td></td>
<td>Screening Heavy atoms</td>
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</table>
C.5.  24-ID-C Progress Since October 2005

C.5.1.  Beam Position Stabilization

Liquid nitrogen (LN$_2$) cooling flow-induced vibration of the first and/or second crystals of the 24-ID-C monochromator is the principal source of beam position instability of this beamline. 24-ID-C’s beam position stability is quite good compared to most APS crystallography beamlines (see Figure C.5.1.1), but must be improved for microdiffraction crystallography. We have developed a long-term plan to eliminate this source of instability (see D.1.2.3.1), by a redesign of the monochromator’s coolant distribution system. The new design attempts to reduce non-laminarity of coolant flow and the susceptibility of the coolant distribution lines to vibration.

![Beam position traces of 24-ID-C monochromatic beam, after elimination of cooling line clamps.](image)

**Figure C.5.1.1:** Beam position traces of 24-ID-C monochromatic beam, after elimination of cooling line clamps. Vertical grid intervals represent 5 $\mu$m beam displacement. Green: horizontal beam position, Red: vertical beam position, Blue: intensity (arbitrary units). Vertical pips on horizontal and vertical traces represent times at which script-driven horizontal (cyan) or vertical (pink) beam position adjustments were made by yawing or pitching the horizontal and vertical focusing mirrors (respectively). Data were logged every 2 seconds. Vertical beam position adjustments occurred every 30 seconds, while horizontal adjustments were applied every 55 seconds. The interruption in data logging in center of figure was due to a hutch entry for a crystal change. The saw tooth pattern in the intensity trace (blue) was due to top-off ring fills. The monochromatic beam profile at the sample position was 30 $\mu$m x 60 $\mu$m (full width).

However, we have made significant gains in stability by simply removing clamps on the monochromator’s internal liquid nitrogen distribution lines (see Figure C.4.6.4). These clamps were intended to limit bulk transverse vibrations of the LN$_2$ distribution lines caused by density fluctuations and non-laminar flow of the coolant. In practice, however these clamps apparently increased the mechanical coupling between the crystal mounts and length changes (longitudinal vibration) in the bellows-like cooling lines caused by pressure fluctuations in the cryopump loop. These fluctuations seem to occur mainly in response to non-optimal settings of the pressure control system of the cryopump (see D.1.2.3.1) and intermittent phase changes within the coolant (e.g. boiling). Removal of the coolant line clamps resulted in a roughly 30-40% reduction in the magnitude of vertical beam excursions on time scales of 0.1 to 5 seconds.

C.5.2.  Build 24-ID Monochromator Second Crystal Mount Replacement

The second crystal mount-C of the 24-ID-C build monochromator (see C.4.6) is a cooling block fabricated from oxygen free copper, incorporating an internal liquid nitrogen circulation channel. The second monochromator silicon crystal is end-clamped onto the downward facing surface of the cooling block, which is thermally isolated from the second crystal motion stages by four 1.5 cm diameter cylinders of G11 epoxy composite. A thin film of liquid gallium-indium eutectic (80%:20%) forms the interface between the silicon crystal and the cooling block. The copper
cooling block is electroplated with nickel to prevent amalgamation between the copper surface and the eutectic film.

The first implementation of the second crystal mount should have had an effective surface roughness and flatness of approximately 0.001” (set by manufacturing tolerances). The cooling block manifested numerous scratches and other surface defects of uncharacterized depth. A photograph of the original cooling block (following removal of the second crystal and 1.5 years use) is shown in the upper panel of Figure C.5.2.1. 1.5 years operational experience with the original cooling block indicated that the block was, in fact, not manufactured to specified tolerance and that significant voids between the second crystal contact surface and the cooling block developed because of thermal cycling involved with development and routine maintenance of the monochromator. Immediately after initial installation, the temperature difference between the block and crystal was approximately 20 °K, with a similar differential between the first and second crystals. Following each warming and cooling cycle, associated with monochromator maintenance, the temperature difference between the second crystal cooling block increased. Indeed, near the end of the first operational year, this temperature difference had grown to nearly 100 °K.

A large temperature differential between the first and second monochromator crystals results in a mismatch in the D-spacings of the first and second crystals, which in turn forces a mis-steering of the monochromatic beam if the second crystal’s pitch is adjusted for maximum flux transmission, and/or small uncertainties in the effective energy set point if the temperature differential between the two crystals is not constant in time. In the past, we corrected for vertical mis-steering caused by first and second crystal D-spacing mismatch by adjustment of the pitch of vertical focusing mirror. Thermally induced energy set point perturbations were compensated for by in situ EXAFS measurements from calibration standards and by imposition of thermal equilibration operational hiatuses (15-30 min) following lengthy (> 1 hr) accelerator downs.

In late summer of 2006 we manufactured a new cooling block and contracted with the APS Optics Fabrication and Metrology group to polish the new cooling block to 5 µm RMS surface flatness, prior to nickel electroplating. A photograph of the new second crystal cooling block is shown in the lower panel of figure C.5.2.1. Comparison of the upper and lower panels of Figure C.5.2.1 demonstrates the improved smoothness of the second block, over the first. During the Fall 2006 APS shutdown we replaced the second crystal cooling block. Since installation, the new assembly has experienced 2 thermal cycles and the temperature difference between the cooling block and the second crystal has not exceeded 1 °K. Similarly, the temperature differential between the first and second crystals has never exceeded 1 °K.
Figure C.5.2.1: Photographs of original (upper panel) and new (lower panel) second crystal cooling blocks. In the upper panel, the second monochromator crystal has been removed, along with the gallium-indium eutectic film. Inspection shows that, some degradation of the surface has occurred, presumably due to amalgamation between the eutectic and some component of the cooling block surface. Regions of the original block outside of the degraded area show high surface roughness compared to the new cooling block.

C.5.3. Control System and Console Scripting

In order to simplify the working environment for users, we have radically revised the Sector 24 Console scripting systems that sequence and monitor beamline optics functions and sample handling. Previously, users had to interact with two separate Console installations (labeled Master and Auxiliary nodes) in order to select the monochromator's energy set point, tune the beam position relative to the goniometer and install and align a sample to the beam. The Master Console installation was responsible for all optics systems control, while the Auxiliary installation managed the goniometer position, and sample alignment. Under this setup users were required to continually move their attention between the Master and Auxiliary nodes and to remember the partitioning of functions between the two nodes.

The new scripting configuration isolates all low-level optics motion control and monitoring from direct user access and exposes a simple one-button approach to energy selection, XAFS scan programming, flux tuning and beam position optimization. This was accomplished by revision of both the monochromator control system executing on the Master and goniometer user interfaces running on the Auxiliary nodes. The goniometer user interface (see Figure C.5.3.1) now possesses controls for energy selection, XAFS scan sequencing, beam position tuning. The monochromator Console script executing on the Master console node is still responsible for the sequencing of events that actually control the monochromator and beam steering systems during XAFS scans and crystallographic data collection, but the goniometer user interface executing on the Auxiliary node initiates these events on the Master node using the Console client-to-client remote procedure call messaging channel.
Under the revised scripting system the beamline user interacts only with the Auxiliary node during routine XAFS scanning and data collection. The monochromator control script graphical user interface (executing on the Master node) was modified to reflect its user-passive role: low-level controls were removed and replaced with real-time displays (strip-charts) of beam position and flux (Figure C.5.3.2). Default monochromator scan parameters for all useful chemical elements are stored in a set of configuration files that the user selects from to elicit an EXAFS scan. Likewise, wire scanning to align the spindle axis to the vertical position of the monochromatic X-ray beam has been greatly simplified to a single button strike. Users can now call up the CHOOCH (28) with a single button strike to calculate critical points of EXAFS spectra and determine the energy set points for anomalous data collection.

Additional controls were added to the goniometer user interface for storing and recovering the state of actuators and controllers of the monochromator and beam steering systems. Users can recover a tuned, working state by simply striking the RECOVER TUNE STATE button of the interface. Loss of working state occurs when the automatic, periodic tuning and steering systems are interrupted by loss of source beam or when the user unexpectedly (very rarely) closes the beamline photon shutter.

A right mouse clicks within the confines of the video window initiates sample alignment relative to the beam center fiducials (white vertical and horizontal lines in Figure C.5.3.2). Typically one mouse click on the sample center for each of two orthogonal spindle angles provides reasonable sample alignment. Fine tuning of sample alignment is effected via strikes on the VER buttons (again, from two orthogonal phi settings).

The VIDEO OPS button calls a menu that permits the users to acquire or display previously acquired video snap shots or mpeg movies of the sample.
Figure C.5.3.1: Revised goniometer Console script GUI. The panel of buttons located in the upper left of the interface enable energy selection, maximization of flux, trimming of beam position, and XAFS scan programming from the Auxiliary control node. Below them are indicators for energy, flux and beam position and a status indicator for the busy-state of the monochromator script executing on the Master node. Right mouse clicks within the video window cause the sample translators to move the selected point to align with the center of the white beam position fiducials. Two such selections from orthogonal phi settings serve to align the sample to the beam fiducial.
Figure C.5.3.2: Revised monochromator Console script GUI. All low level motion controls have been replaced with a continuous strip-recording of the X- and Y-position beam coordinates and relative flux. Low level motion controls are available to executive users after striking the OPERATIONS button labeled MOTION CONTROLS. The button panel at upper right are controls for setting the parameters of flux maximization and beam position feedback loops.

A new Console server implementing text-to-voice synthesis was implemented and incorporated into the Console scripting language. This system enables audible prompting and warning to be incorporated into Console scripts. Audible prompting is used to direct the user’s attention to emergency or error conditions that might be otherwise overlooked in the fast-paced, stimulus rich environment present during data collection. The audible prompting system is also used to indicate completion of operations that have durations longer than a few seconds such as XAFS and goniometer alignment scans.

C.5.4. Improvements in Sample Visualization, Shutter and Attenuator Systems

A second sample visualization system identical to the first was installed at roughly 35 degrees from horizontal, upstream of the goniometer. Ideally we would install the second visualizer in an orthogonal orientation relative to the first (vertical) system, but its angular declination is constrained by collision with the collimation system.

The second visualizer provides additional visual queuing for crystal alignment. Additionally, the second visualizer is set to 2X magnification relative to the vertical visualizer. Figure C.5.4.1 is a photograph showing the two visualizers, relative to the 24-ID-C goniometer.
We have developed a simple mechanism for remote optimization the focus of the sample visualizers (Figure C.5.4.2). This mechanism consists of a gear fitted to the focusing section of the visualizer microscope, driven by a gear-reduced stepper motor through a timing belt. The remote focusing system allows the user to fine-tune the visualizer focus without entering the hutch and without manual interaction with the microscope body and the risk of perturbing its orientation. The remote focusing drive also simplifies use of the spindle-mounted yttrium-aluminum-garnet (YAG) beam visualizer, which requires a change of focus due to the fact that the YAG converter is situated ~ 0.5 cm upstream of the goniometer spindle axis.

Figure C.5.4.1: Photograph of 24-ID-C goniometer (left) and vertical and “horizontal” sample visualizers.

All sample illuminator output levels are now set by a control panel next to the main control console, so that users can adjust light levels without entering the hutch. The illuminator control system also accepts analog drive signals so that illumination levels can be set via Console script controls.
In order to improve discrimination of small crystals against optical background we installed a pneumatically-activated sample trans-illuminator. The trans-illuminator consists of two orthogonally situated white reflective surfaces (one for each sample visualizer), driven to a position downstream of the sample by a pneumatic actuator. These reflective surfaces induce a light-field background for observing the sample when inserted. Withdrawal of the trans-illuminator yields a dark-field illumination of the sample. The positioning of the trans-illuminator is such that it does not alter the flow pattern of the cryo-stream from the delivery nozzle of the crystal cryogenic cooling system. Scripting insures that the trans-illuminator is always in a stowed position prior to onset of data collection. Figure C.5.4.3 shows a photograph of the trans-illuminator in the inserted configuration.

The effectiveness of the rotary shutter has made use of the adjustable stroke pneumatic shutter unnecessary. We have replaced the adjustable shutter with an additional 4-channel attenuator assembly. The new attenuator is configured to support operation at low energy when very fine gradations of attenuator thickness are required.

A common problem reported by users of quadrant diode beam position monitors (BPMs) is perturbation of BPM performance by attenuator or shutter elements downstream of the BPM. This interaction is caused by backscatter and fluorescence from downstream beamline components exciting fluorescence and scatter from the fluorescent screen of the BPM, which then acts as a strong noise source for the BPMs position sensing scheme.
The principal 24-ID-C quadrant diode beam position monitor (BPM) is located just upstream of the attenuator arrays, within the collimator assembly. This placement of the BPM is necessary to avoid large perturbations of flux (and concomitant effects on the energy calibration of the BPM) received by the BPM as a result of changes in attenuator selection. Thus the 24-ID-C BPM is subject to the same downstream interactions seen at other beamlines. In the past we have dealt with this issue by always maintaining insertion of one very thin attenuator (the guard attenuator) just downstream of the BPM, thereby providing a constant perturbation upon the BPM. This work around becomes problematic at energies below 8 KeV where a guard attenuator of sufficient thickness to block back scatter from other downstream attenuators reduces the flux to unacceptably low levels.

We attempted to reduce the impact of attenuator back scatter and fluorescence on the BPM by greatly reducing the solid angle subtended at the BPM fluorescence screen relative to the most upstream attenuator element. To accomplish this, we designed a cylindrical aluminum plug, 4 cm long, with a central 2 mm circular channel that fits precisely within the bore of the BPM housing, downstream of the quadrant-diode assembly and the fluorescent screen (see Figure C.5.4.4). The plug has semicircular slots milled into its perimeter at 60 degree intervals so that the plug does not interfere with the vacuum clearance rate of the BPM housing. After insertion of the backscatter-blocking plug we were unable to detect significant perturbation of the BPM response as a result of alteration of the attenuator settings. Thus we no longer need to impose a minimum attenuation in order to stabilize the BMP against back scatter and fluorescence originating from the attenuators.
C.5.5.  **User Program.**

Since Oct 2005, user operations for 24-ID-C have been ramped up. During the previous calendar year 171 individual scientists, both from member institutes and from general user community, visited 24-ID-C in 292 separate trips to carry out X-ray diffraction experiments. Beamline usage statistics are provided in Figure C.5.5.1. As can be noted from this figure, the beamtime consumed for development work has gradually reduced and user beamtime has correspondingly increased. Three of the scientific highlights described in section C.11.1 are from experiments carried out at 24-ID-C. Since Oct 2005 there were 13 published or in press manuscripts based on experiments performed at 24-ID-C.

During the 2005-3 run cycle (09/2005-12/2005) research groups from six of the seven member institutes visited 24-ID-C to collect high quality data. This run involved 17 separate user visits and provided ~45% of available time to users. This provided us an opportunity to obtain feedback from users to implement improvements to the beamline.

The 2006-1 run cycle (01/2006-04/2006) provided ~60% of the available beamtime to institutional members. This run cycle involved participation of research groups from all member institutes in data collection efforts at 24-ID-C. A total of 24 separate visits were made by 57 scientists from these groups during this run cycle.

Commissioning of the 24-ID-E beamline began during the 2006-2 run cycle (05/2006-08/2006). Since the 24-ID-C and 24-ID-E beamlines share a common SOE, commissioning of 24-ID-E affected user operations at 24-ID-C. However, we made significant efforts to minimize this effect.
In spite of 24-ID-E commissioning, ~55% of the available beamtime at 24-ID-C was used for user experiments in over 17 individual visits from 62 experimenters from member institute groups.

The 2006-3 run cycle (09/2006-12/2006) was one of the busiest run cycles for 24-ID-C so far. During this run cycle 24-ID-C started accepting APS general users. Indeed 24-ID-C exceeded the APS requirements in terms of delivered general user beamtime. Ten general user groups used 24-ID-C in 11 individual visits, including visit from an industrial user performing proprietary research. 82% of the total available time was used for user operations, including 26% of beamtime provided to APS general users. More than 34 individual trips were made by researchers from member institutions and general users. A total of 112 scientists visited 24-ID-C during this run cycle. Despite such a busy schedule, beamline enhancements, such as “user slit control”, were implemented during this run cycle.

![Pie charts showing distribution of available 24-ID-C beamtime among member institutions, general users and beamline development.](image)

**Figure C.5.5.1:** Pie charts showing distribution of available 24-ID-C beamtime among member institutions, general users and beamline development.

### C.6. Technological Core Developments: 24-ID-E

#### C.6.1. 24-ID-E Monochromator

NE-CAT has collaborated with Oxford-Danfysik to design and build a multiple single-crystal branch (side-bounce) monochromator for the 24-ID-E beamline. This monochromator will provide monochromatic undulator radiation at two fixed energies (12.660 and 14.845 keV) at a
single take-off angle of 29.5° via single diffraction from one of two liquid nitrogen-cooled silicon crystals with different face cuts (311 and 220, respectively), secured to a single rotation stage. Selection between the two energy settings will be effected by a vertical translation of a linear translation stage on which the two crystals are mounted, while maintaining a fixed exit angle. This design will enable two-energy MAD data collection in addition to monochromatic studies at the 24-ID-E beamline.

Use of indirect cooling for both Si crystals is due to space limitations imposed on the monochromator vacuum vessel and attempts to minimize complexity and cost. Both the 311 and 220 crystal mounts will incorporate separate coarse and fine pitch and roll adjustments through piezoelectric actuators to facilitate conjoint beam steering and energy tuning. Figure C.6.1.1 provides details of the 24-ID-E monochromator crystal geometry, while Table C.6.1.1 lists their design parameters.

![Figure C.6.1.1. 24-ID-E monochromator silicon crystal design. The side of the crystal labeled “Contact Surface” and its apposing surface are in direct contact with the liquid nitrogen cooling plates. A channel milled between the upper section of the crystal and the main body retards propagation of mechanical strain induced by the thermal clamp into the upper section, containing the diffracting surface of the crystal.](image)

The monochromator design effort included extensive finite-element thermal transport and mechanical stress simulations to validate candidate crystal mounting and cooling strategies. These simulations indicated that operation at 12.660 keV, using the 220 Si crystal involves no technological risk, since the first harmonic of the undulator will be used (with a relatively large gap setting and modest on-crystal power densities). The second operational mode of the monochromator, using the 311 cut crystal at 14.845 keV, will require use of the third undulator harmonic with a commensurate 3-5 fold increase in power density. Thermal transport simulations indicated that avoiding heat-induced distortion of the 311 crystal surface (causing loss of monochromator acceptance) requires very effective cooling and may force significant reduction of the incident power load by use of the power-limiting apertures installed immediately upstream of the 24-ID-E monochromator.

The 24-ID-E monochromator has an option for mounting a water-cooled diamond crystal in place of the Si (311) crystal to improve the stability of operation of the system following a crystal change. With the current 3.3 cm undulator periodicity, a change of undulator harmonic is forced by the transition between 12.660 and 14.845 keV (assuming that the first undulator harmonic is...
used at 12.660, and the third harmonic at 14.845 keV). This transition involves a 3 fold increase in thermal load on the first crystal and possible stability problems driven by this thermal load change.

Table C.6.1.1: 24-ID-E monochromator crystal specification

<table>
<thead>
<tr>
<th>Operating position</th>
<th>Horizontally deflecting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Si (220), Si (311)</td>
</tr>
<tr>
<td>Diffracting face size</td>
<td>18 x 16 mm</td>
</tr>
<tr>
<td>Orientation Accuracy</td>
<td>± 0.02°</td>
</tr>
<tr>
<td>Diffracting Surface Flatness *</td>
<td>&lt; 2 µm</td>
</tr>
<tr>
<td>Roughness</td>
<td>&lt; 5 nm</td>
</tr>
<tr>
<td>Orientation between crystals</td>
<td>± 0.005°</td>
</tr>
<tr>
<td>Cooling</td>
<td>Cryogenically side-cooled</td>
</tr>
</tbody>
</table>

*Diffraecting face to be lapped, polished and etched to give an FWHM rocking curve to within 0.2" of theoretical value.

In January 2005, we completed the design process for the 24-ID-E single-crystal side-bounce monochromator and finalized contract negotiations with Oxford-Danfysik for its construction and delivery. Although the design phase was protracted (> 1 year) and exceeded our schedule time line for this procurement item, the final product should show improved performance and reliability, due to the increased effort placed in the design phase (compared to the design as it existed last year).

The main points at issue in the extended design process were:

1) Improvement of the stability and reliability of the crystal mount and motion mechanisms (via simplification).
2) Modularization of the crystal mount mechanism, facilitating “off-line” pre-alignment of the crystals.
3) Elimination of potential sources of mechanical strain and vibration on the crystal mounts originating from the liquid nitrogen circulation system.
4) Improvement of the thermal break and Compton scatter guard between the crystal mounts and the monochromator’s swing cage.
5) Reduction of the overall “foot print” of the vacuum vessel to enable insertion in the confined space allowed in the SOE.

Delivery of the 24-ID-E monochromator is scheduled for October 2005. This will delay the installation of the 24-ID-E beamline by approximately 6 months compared to our original schedule, due to the fact that installation is forced to coincide with one of the two long annual APS shutdowns, in order to avoid interruption of commissioning and user activities at the 24-ID-C undulator beamline. Obviously, this delay will have the serendipitous benefit of reducing the competition between the time demands for maturation of the 24-ID-C beam and the installation of the 24-ID-E beamline.

Figure C.6.1.2 shows plan and elevation views of the 24-ID-E optical layout in the SOE and hutch 24-ID-E and points out the location of the 24-ID-E monochromator. Figure C.6.1.3 is a rendering of the monochromator vacuum vessel and support structure. Details of swing cage and crystal supports are given in Figure C.6.1.4. The modular crystal mounts and their pitch, roll and yaw drive mechanisms are rendered in Figures C.6.1.5 and C.6.1.6.

The crystals will also be mounted on individual mounting plates which can be adjusted for height, pitch and roll using fine motorized adjustments. The crystals are mounted in a 3 point kinematic mount with height and pitch are driven using Phytron in-vacuum stepper motors.
through planetary gearboxes (without feedback) and roll using an in-vacuum Picomotor with internal feedback control.

A different crystal design, with an undercut crystal will increase the height by up to 5 mm. This can be accommodated if required at a later stage.

**Table C.6.1.2: Swing and Translation Frame Motions.**

<table>
<thead>
<tr>
<th>Motion</th>
<th>Parameter</th>
<th>Design Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pitch (Bragg Rotation about Z Axis)</td>
<td>Drive</td>
<td>UHV Nanomotion HR8 driven translation stages. 0.01µm Encoder and interpolator</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>12.5°-15.8°</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td>&lt; 0.25 µrad</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>&lt; 0.4 µrad</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td>Nanomotion HR8</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td>Renishaw RGH25 (UHV) with 200x interpolator</td>
</tr>
<tr>
<td>Crystal Selection (Translation along Z Direction)</td>
<td>Drive</td>
<td>UHV AML stepper motor driven translation stage.</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>130 mm</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td>2.5 µm</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>5 µm</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>Parallelism to Bragg Axis</td>
<td>100 µrad</td>
</tr>
</tbody>
</table>

**Table C.6.1.3: Crystal Pitch, Roll and Yaw Adjustments.**

<table>
<thead>
<tr>
<th>Motion</th>
<th>Parameter</th>
<th>Design Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (Translation along X Axis)</td>
<td>Actuator</td>
<td>Stepper motor acting on fine pitch screw</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>5 mm (±2.5 mm)</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td>&lt;0.01 µm</td>
</tr>
<tr>
<td>Pitch (Rotation about Z Axis)</td>
<td>Actuator</td>
<td>Height actuators acting in opposition</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2° (±1°)</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td>&lt;2 µrad</td>
</tr>
<tr>
<td>Roll (Rotation about Y Axis)</td>
<td>Actuator</td>
<td>New Focus Picomotor (closed loop)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2° (±1°)</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td>&lt;1 µrad</td>
</tr>
</tbody>
</table>
Figure C.6.1.2: Plan and Elevation views of 24-ID-E optics.
Figure C.6.1.3: Rendering of 24-ID-E monochromator vacuum vessel and support structure. The grey U-shaped structure is a synthetic granite inertial dampening support. A 300 l/sec ion pump fits inside the support plinth (rendered in orange). Immediately above the ion pump is the input/output manifold for liquid nitrogen cooling. Both the inboard and outboard white beams enter the vacuum vessel via the port on the right side of the vacuum tank. The 24-ID-E monochromatic beam exits via the canted spool piece on the front face of the vacuum vessel. The brown colored, paddle-like structures located near the white beam entrance and exit ports are tungsten scatter guards.
Figure C.6.1.4: Swing cage construction. Attached to the center of the base plate of the monochromator’s vacuum vessel (orange) is a kinematic support (grey-color, open-faced box-like structure). Two bearings support the swing cage (located in center of the bottom and top plates of the swing cage (blue-colored and transparent plates, respectively)) within the kinematic support. Rotation of the swing cage is driven by a nanomotor (piezoelectric drive, not shown in this view). The swing cage supports the vertical translation slide (green) that supports the two modular crystal mounts (copper, green colored). The paddle-shaped structure in the foreground is the upstream tungsten scatter guard.
**Figure C.6.1.5:** Expanded view of a single crystal mount module.

**Color key:**

<table>
<thead>
<tr>
<th>Color</th>
<th>Object</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tan</td>
<td>Silicon crystal</td>
</tr>
<tr>
<td>Copper</td>
<td>Cooling blocks</td>
</tr>
<tr>
<td>Dark Green</td>
<td>Composite Thermal break.</td>
</tr>
<tr>
<td>Dark Brown-</td>
<td>Tungsten scatter guard.</td>
</tr>
<tr>
<td>Orange</td>
<td>Module back plate.</td>
</tr>
<tr>
<td>Green</td>
<td>LN$_2$ transfer line strain relief.</td>
</tr>
<tr>
<td>Dark Grey</td>
<td>Tungsten Compton scatter guard.</td>
</tr>
</tbody>
</table>

**Figure C.6.1.6:** Front (left) and back (right) views of the crystal modular mounts and the yaw, pitch and roll drives for each crystal mount. The crystals (tan) are sandwiched between copper cooling blocks (copper-colored). The diffracting face of the crystals are obscured by H-shaped Compton scatter guards (grey). Input and output LN$_2$ transfer lines are strain-relieved by dark green-colored clamps. G-11 thermal breaks (light green) secure the crystal clamp the each crystal’s modular support (light grey). A tungsten scatter guard (brown) is sandwiched between the thermal breaks and modular mount plates. The modular mounts are supported via spring-loaded quasi-kinematic mounts, each driven by a linear motor. Two legs of the kinematic drives are gear-reduced, stepper motor-driven (Phytron) linear translators (grey cylinders with stripe). The kinematic legs implementing the roll axis are Jena digital piezoelectric transducers (DPT’s) (red-colored boxes).
C.6.2.  24-ID-E Focusing System

NE-CAT, in conjunction with Oxford-Danfysik, has designed a Kirk-Patrick Baez focusing system for the 24-ID-E NE-CAT beamline. This system is functionally very similar to that used in the 24-ID-C beamline design, consisting of two large ULE mirrors, mechanically deformed to approximate elliptical surfaces of rotation with practically-minimal focusing aberrations. Space limitations of the 24-ID-B hutch preclude the use of a single vacuum vessel as in the 24-ID-C design. The horizontal focusing mirror (HFM) will be situated in 24-ID-B, while the vertical focusing mirror will be located in a separate vacuum vessel in 24-ID-E. Otherwise, the 24-ID-E and 24-ID-C focusing systems will be virtually identical in terms of the mechanical axes available for positioning and configuring the reflective surfaces. Figure C.6.1.2 shows plan and elevation views of the SOE and hutch 24-ID-E and placements of the 24-ID-E focusing system components. Figures C.6.2.1 and C.6.2.2 consist of renderings of the vacuum vessels and mirror mounts of the 24-ID-E HFM and VFM, respectively.

The 24-ID-E VFM system uses a previously purchased mirror originally configured as an HFM, with a simple U-bender. The VFM, due to its forced proximity to the goniometer axis will have a relatively high demagnification. We have worked with Oxford-Danfysik and its subcontractor for mirrors (Seso) to develop a scheme for minimizing the focusing aberrations resulting from the high demagnification ratio. The on-hand VFM has been returned to SESO for refitting with a new asymmetric two-pole bending mechanism that will form an approximate elliptical bend in the VFM for a single-fixed focal point. Figure C.6.2.3 is a photograph of the VFM prior to shipment to Seso for the quasi-elliptical bend modification. The 24-ID-E HFM is virtually identical to the HFM of the 24-ID-C K-B system, but with a meridional surface figure appropriate for the demagnification and placement of the 24-ID-E HFM.

Both mirrors, their benders and vacuum vessels were delivered to the APS in July 2005. The 24-ID-E K-B focusing system incorporates precisely the same motion controls and position sensing systems installed in the 24-ID-C focusing system. Therefore, the Console control scripting generated for the 24-ID-C focusing system will be used with only minor modification with the 24-ID-E focusing system.

C.6.2.1. HFM Mirror

Table C.6.2.1: HFM Mirror Specifications.

<table>
<thead>
<tr>
<th>Operating position</th>
<th>Horizontally deflecting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate material</td>
<td>ULE</td>
</tr>
<tr>
<td>Active area</td>
<td>1100 mm x 70 mm</td>
</tr>
<tr>
<td>Blank dimensions</td>
<td>1200 mm x 85 mm</td>
</tr>
<tr>
<td>Shape</td>
<td>Flat (R &gt; 6 km)</td>
</tr>
<tr>
<td>Source distance</td>
<td>50.768 m</td>
</tr>
<tr>
<td>Focus distance</td>
<td>8.511 m</td>
</tr>
<tr>
<td>Tangential slope error</td>
<td>2.5 µrad</td>
</tr>
<tr>
<td></td>
<td>on 1000 mm</td>
</tr>
<tr>
<td></td>
<td>2 µrad on 800 mm</td>
</tr>
<tr>
<td>Sagittal slope error</td>
<td>25 µrad</td>
</tr>
<tr>
<td></td>
<td>on 600 mm</td>
</tr>
<tr>
<td>Coating</td>
<td>Rhodium of maximum practical width</td>
</tr>
<tr>
<td>Thickness of Coating</td>
<td>&gt; 200 µ</td>
</tr>
<tr>
<td>Surface Roughness</td>
<td>3 Å RMS</td>
</tr>
<tr>
<td></td>
<td>Best Effort 2 Å RMS for lengthwise lines</td>
</tr>
</tbody>
</table>
Table C.6.2.2: HFM Mirror Specifications.

<table>
<thead>
<tr>
<th>Operating position</th>
<th>Vertically deflecting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate material</td>
<td>ULE</td>
</tr>
<tr>
<td>Active area</td>
<td>800 mm x 60 mm</td>
</tr>
<tr>
<td>Blank dimensions</td>
<td>900 mm x 80 mm</td>
</tr>
<tr>
<td>Shape</td>
<td>Flat (R &gt; 6 km)</td>
</tr>
<tr>
<td>Source distance</td>
<td>54.380 m</td>
</tr>
<tr>
<td>Focus distance</td>
<td>4.899 m</td>
</tr>
<tr>
<td>Tangential slope error</td>
<td>5 µrad RMS</td>
</tr>
<tr>
<td></td>
<td>(&lt;3 microradians RMS measured over 800 mm)</td>
</tr>
<tr>
<td>Sagittal slope error</td>
<td>25 µrad</td>
</tr>
<tr>
<td>Coating</td>
<td>3 stripes</td>
</tr>
<tr>
<td></td>
<td>20 mm without coating</td>
</tr>
<tr>
<td></td>
<td>&gt;18 mm Rh</td>
</tr>
<tr>
<td></td>
<td>&gt;18 mm Pt</td>
</tr>
<tr>
<td>Thickness of Coating</td>
<td>&gt; 200 µ</td>
</tr>
<tr>
<td>Surface Roughness</td>
<td>5 Å RMS (2.65 Å RMS measured).</td>
</tr>
</tbody>
</table>

C.6.2.2. VFM Mirror

The VFM is reconfigured from the mirror previously supplied to NE-CAT as a cylindrically bending HFM. The bending range is similar to that before modification (1.5 km – 30 km), however the bending moments was modified to give an elliptical term to the shape in the bent condition. As there is only a single actuator it is impossible to vary the elliptical term and the bend radius independently; however this is acceptable as the mirror is to be used for a limited range of focus distances. The focusing distance was optimized for a distance of 4.899 m from center of the mirror to the focus position.

The mirror was re-tested in the reconfigured bender, at the working radius, in the SESO factory to ensure compliance with the following specification. Look up tables of motor steps to bend radius and elliptical term were provided.
### Table C.6.2.3: VFM Mirror Mechanical Specifications.

<table>
<thead>
<tr>
<th>Motion</th>
<th>Parameter</th>
<th>Req'd Specification</th>
<th>Design Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pitch</strong> (Rotation about Z Axis)</td>
<td>Drive</td>
<td>UHV Nanomotion HR8 driven translation stages. 0.1µm Encoder</td>
<td>UHV Nanomotion HR8 driven translation stages. 0.1µm Encoder</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>± 1ϒ</td>
<td>± 1ϒ</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td>&lt; 0.2 µrad</td>
<td>&lt; 0.2 µrad</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>1.3 µrad</td>
<td>&lt; 1 µrad (0.2µrad)</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td>Nanomotion HR8</td>
<td>Nanomotion HR8</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td>Renishaw RGH25 (UHV)</td>
<td>Renishaw RGH25 (UHV)</td>
</tr>
<tr>
<td><strong>Roll</strong> (Rotation about Y Axis)</td>
<td>Drive</td>
<td>Vertical jacks. 0.1µm Encoder</td>
<td>Vertical jacks. 0.1µm Encoder</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>± 1ϒ</td>
<td>± 1ϒ</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td>0.8 µrad</td>
<td>0.8 µrad</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>+/- 1 µrad</td>
<td>+/- 1 µrad</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td>Mclennan 5 phase</td>
<td>Mclennan 5 phase</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td>Renishaw RGH 24</td>
<td>Renishaw RGH 24</td>
</tr>
<tr>
<td><strong>Yaw</strong> (Rotation about X Axis)</td>
<td>Drive</td>
<td>Vertical jacks. 0.1µm Encoder</td>
<td>Vertical jacks. 0.1µm Encoder</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>± 1ϒ</td>
<td>± 1ϒ</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td>&lt; 0.2 µrad</td>
<td>&lt; 0.2 µrad</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>+/- 1 µrad</td>
<td>+/- 1 µrad</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td>Mclennan 5 phase</td>
<td>Mclennan 5 phase</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td>Renishaw RGH 24</td>
<td>Renishaw RGH 24</td>
</tr>
<tr>
<td><strong>Vertical (Z Direction)</strong></td>
<td>Drive</td>
<td>Vertical jacks. 0.1µm Encoder</td>
<td>Vertical jacks. 0.1µm Encoder</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>+/- 40.0 mm</td>
<td>+/- 40.0 mm</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td>&lt; 0.2 µm / motor step</td>
<td>&lt; 0.2 µm / motor step</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>+/- 1 µm</td>
<td>+/- 1 µm</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td>Mclennan 5 phase</td>
<td>Mclennan 5 phase</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td>Renishaw RGH 24</td>
<td>Renishaw RGH 24</td>
</tr>
<tr>
<td><strong>Lateral (X Direction)</strong></td>
<td>Drive</td>
<td>UHV Nanomotion HR8 driven translation stages. 0.1µm Encoder</td>
<td>UHV Nanomotion HR8 driven translation stages. 0.1µm Encoder</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>+/- 35.0 mm</td>
<td>+/- 35.0 mm</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td>&lt; 0.2 µm</td>
<td>&lt; 0.2 µm</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>+/- 1 µm</td>
<td>+/- 1 µm</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td>Nanomotion HR8</td>
<td>Nanomotion HR8</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td>Renishaw RGH25 (UHV)</td>
<td>Renishaw RGH25 (UHV)</td>
</tr>
</tbody>
</table>
### Table C.6.2.4: VFM Mechanical Specifications.

<table>
<thead>
<tr>
<th>Motion (VFM)</th>
<th>Parameter</th>
<th>Performance</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pitch (Rotn about X Axis)</td>
<td>Drive</td>
<td></td>
<td>Vertical jacks. 0.1µm Encoder</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td></td>
<td>± 1°</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td></td>
<td>&lt; 0.2 µrad</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td></td>
<td>+/- 1 µrad (&lt; 1 µrad (0.2 µrad))</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td></td>
<td>Mclellan 5 phase</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fine Pitch (VFM) (refer Note)</td>
<td>Drive</td>
<td></td>
<td>Piezosystem Jena PAHL40 piezo stack type actuator.</td>
</tr>
<tr>
<td>(Rotn about X Axis)</td>
<td>Range</td>
<td></td>
<td>± 20 µrad (40µm travel)</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td></td>
<td>&lt; 0.5 nrad</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td></td>
<td>0.3 µrad (&lt; 0.05 µrad)</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td></td>
<td>Piezosystem Jena PAHL40</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td></td>
<td>Integral strain gauge feedback</td>
</tr>
<tr>
<td>Roll (Rotn about Y Axis)</td>
<td>Drive</td>
<td></td>
<td>Vertical jacks. 0.1µm Encoder</td>
</tr>
<tr>
<td>(Rotn about Y Axis)</td>
<td>Range</td>
<td></td>
<td>± 1°</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td></td>
<td>0.8 µrad</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td></td>
<td>+/- 1 µrad (2 µrad)</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td></td>
<td>5 phase stepper</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td></td>
<td>Renishaw RGH 24</td>
</tr>
<tr>
<td>Yaw (VFM) (Rotn about Z Axis)</td>
<td>Drive</td>
<td></td>
<td>UHV Nanomotion HR8 driven translation stages. 0.1µ</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td></td>
<td>± 1°</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td></td>
<td>&lt; 0.2 µrad</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td></td>
<td>+/- 1 µrad (&lt; 1 µrad)</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td></td>
<td>Nanomotion HR8</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td></td>
<td>Renshaw RGH25 (UHV)</td>
</tr>
<tr>
<td>Vertical (Z Direction)</td>
<td>Drive</td>
<td></td>
<td>Vertical jacks. 0.1µm Encoder</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td></td>
<td>± 40.0 mm</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td></td>
<td>&lt; 0.2 µm / motor step</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td></td>
<td>+/- 1 µm (&lt; 0.5 µm)</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td></td>
<td>Mclellan 5 phase</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td></td>
<td>Renishaw RGH 24</td>
</tr>
<tr>
<td>Lateral (X Direction)</td>
<td>Drive</td>
<td></td>
<td>UHV Nanomotion HR8 driven translation stages. 0.1µ</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td></td>
<td>± 35 mm</td>
</tr>
<tr>
<td></td>
<td>Resolution</td>
<td></td>
<td>&lt; 0.2 µm</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td></td>
<td>+/- 1 µm (&lt; 0.2 µm)</td>
</tr>
<tr>
<td></td>
<td>Motor type</td>
<td></td>
<td>Nanomotion HR8</td>
</tr>
<tr>
<td></td>
<td>Encoder</td>
<td></td>
<td>Renshaw RGH25 (UHV)</td>
</tr>
</tbody>
</table>
Figure C.6.2.1: Side and perspective renderings of 24-ID-E horizontal focusing mirror. The U-shaped structure rendered in grey or black is a synthetic inertial damping structure (plinth). An ion pump (light grey) is mounted on the underside of the vacuum vessel. The mirror and bending mechanism is very similar to that used in the 24-ID-C HFM. The reflective surface of the mirror is on the opposite side of the rendering. The kinematic vertical lifts are mounted on both ends of the plinth.
Figure C.6.2.2: Side and perspective renderings of 24-ID-E vertical focusing mirror. The U-shaped structure rendered in grey or black is a synthetic inertial damping structure (plinth).
Figure C.6.2.3: Left: 24-ID-E vertical focusing mirror separated from bending mechanism. The mirror, itself is situated on the right; the bend mechanism is on the right. The mirror’s reflective surface is facing downwards in this photograph. Right: Assembled VFM mounted on support mechanism.
C.7. 24-ID-E Progress since October 2005

The 24-ID-E focusing system components were delivered to NE-CAT in July 2005. The dual-energy, side bounce monochromator passed factory acceptance testing in December of 2005 and delivery at APS occurred in January 2006. Upon deliver, the monochromator was subjected to extensive off-line testing and reworking to correct fabrication errors and some shipping damage (see following section). The monochromator and horizontal focusing mirror were installed in the secondary optics enclosure (24-ID-B), and the vertical focusing mirror in hutch 24-ID-E during the Spring 2006 APS shutdown. 24-ID-E end station installation took place in the summer and fall of 2006.

At the time of submission of this proposal, installation and commissioning of the hardware elements of 24-ID-E beamline are essentially complete. Experimental commissioning of the beamline will commence, upon resumption of acceleration operations in February 2007.

C.7.1. 24-ID-E Dual energy, side bounce monochromator

The 24-ID-E monochromator design and operational characteristics are detailed in section C.6.1. In short, the 24-ID-E monochromator consists of two cryogenically-cooled crystals of differing cuts (220 and 311), selectable by a simple vertical translation. Both crystals are mounted on separate kinematic platforms that permit independent, fine adjustment of pitch, roll and yaw relative to the white beam. The monochromator is designed to provide a monochromatic beam at either 12.6 or 14.8 KeV with a fixed exit angle of 29.65 degrees relative to the incident beam. This design will support two-energy Se MAD, Se or Br SAD data collection, in addition to monochromatic studies at either wavelength.

The 24-ID-E beamline uses the inboard undulator white beam, while the 24-ID-C white beam passes through the 24-ID-E monochromator vacuum vessel (outboard to the 24-ID-C monochromator crystals). With the current 3.3 cm undulator periodicity, a change of undulator harmonic is forced by the transition between 12.568 and 14.784 KeV (assuming that the first undulator harmonic is used at 12.568, and the third harmonic at 14.784 KeV). This transition involves at least a 5 fold increase in thermal load on the first crystal, raising the possibility for beam position and energy set point stabilization problems. This problem can be remedied in two ways: 1) by using the third harmonic for both crystal selections; or 2) by transitioning to a short period undulator (3.0 cm period device) with a useful first harmonic mode extending past 15 KeV. Option 1) imposes power densities on the Si 220 crystal beyond the tolerable power dissipation limits predicted by finite element simulations of thermally driven stress-strain calculations supplied by the Oxford Danfysik design study for this monochromator, but this issue can be dealt with by using the power limiting aperture, located upstream of the 24-ID-E monochromator (see C.4.5.1) to limit power deposition on the Si 220 crystal (at the expense of flux at 12.568 KeV) Option 2) is discussed in detail in section D.1.2.3.4. and involves substantial capital expense.

C.7.1.1. Factory acceptance stability testing

Factory acceptance testing involved validation of the monochromator cooling and motion systems and an attempt to determine the severity of cooling flow-induced crystal surface vibration. Figure C.7.1.1 shows a schematic of the test rig used for these measurements. Oxford Danfysik leased a Vicon Motion Systems MX-40 (Vicon Motion, Redwood CA) motion capture system to directly assay the mechanical stability of the crystal mounts in the presence and absence of liquid nitrogen cooling flow. The MX-40 is a high-speed interline CMOS CCD camera capable of region-of-interest (ROI) read-out speeds up to 1000 Hz. The pixel size of the MX-40 is 6.5 μm x 6.5 μm which establishes the spatial resolution of the stability measurements. Beam from a stabilized Zygo laser was reflected from the surface of one of the two monochromator crystals (at the design angle of incidence of the X-ray beam), heavily attenuated with a color filter and focused with a plano-convex lens onto the read head of the MX-40 motion
capture system. A constant ROI of the MX-40 image frame approximately five times larger than the diameter of the focused laser spot was read out and stored at 800 Hz while the flow rate of the liquid nitrogen cooling loop was varied. The MX-40’s data acquisition system was programmed to perform an on-the-fly calculation of the intensity centroid of the laser spot, in the reference frame of the ROI.

Figure C.7.1.1: Schematic of crystal mechanical stability test rig. Both legs of optical path of the laser beam are ~1 m in length.

Figure C.7.1.2 shows time trajectories of the laser spot centroid over a span of 5 seconds at 0 (left) and 20 Hz (right) cryo-pump rate, with a time step of 1.25 milliseconds between each discrete step in the trajectory (4000 MX-40 data frames). A pump rate of 20 Hz corresponds to approximately 2 liters/min flow through the overall liquid nitrogen circulation loop. Figure C.7.1.3 shows histograms of the x or y distance component between each trajectory time point and the overall average position of the beam. Trajectory displacement coordinate data were binned into 0.65 micron intervals for construction of the histograms. The histograms corresponding to 20 Hz liquid nitrogen flow (blue) were arbitrarily translated to center them on their baseline (0 Hz) counterparts. The mean positions of the X and Y direction histograms were both significantly shifted relative to baseline histograms due to shifting of the reflective crystal surface by thermal perturbation of the overall structure during cool down, and by the effects of pressurization of the liquid nitrogen distribution lines under conditions of non-zero liquid nitrogen flow.

Displacement of the laser spot centroid in the trajectory data is due to translational and/or rotational motion of the silicon crystal surface relative to the laser source and the MX-40 detector. A simple position measurement is not capable of discriminating between these two types of measured displacements. Also, the lengths of the incoming and outgoing optical legs of the test configuration are very different than those of the final monochromator installation at the APS. Thus no attempt was made to quantitatively analyze the trajectory data in terms of angular displacement of the incident beam. Rather these measurements were considered effective only for qualitative comparisons of stability of the crystal surfaces under various liquid nitrogen flow rates.
Inspection of the raw trajectory data provided insight into the magnitude and frequency of large excursions from the average position of the laser spot because of total overlap in the cores of the trajectory plots. Large excursions in the trajectories were correlated to mechanical disturbances originating in the floor of the Oxford Danfysik factory and were completely irreproducible and independent of cooling flow rate.

The histograms of the trajectory data give a more useful comparison of the relative stability of the crystal surface under different cooling flow regimes. The full width at half height (FWHH) of the X (vertical) centroid coordinate histogram for 20 Hz liquid nitrogen flow is significantly smaller than the FWHH of the 0 flow histogram, implying statistically smaller vibration range of the cooled crystal surface in this direction. This observation can be explained by asserting a damping of vibrations in the Y-plane due to pressurization (stiffening) effects from the liquid nitrogen distribution bellows. Conversely, comparison of the baseline and 20 Hz Y (horizontal) histograms demonstrates a slight increase of the FWHH for the 20 Hz histogram relative to baseline.

We concluded from these studies that the liquid nitrogen cooling system-induced vibration was less likely to be a source of output beam instability than floor-induced vibrations and factory acceptance of the monochromator was granted by NE-CAT.

C.7.1.2. Monochromator installation and commissioning

Upon delivery of the 24-ID-E monochromator we attempted to achieve target working vacuum under conditions of liquid nitrogen flow. We discovered significant leaks in the vacuum vessel due to microscopic factures in the corners of the vessel lid. In situ touch-up welding resolved this problem and we achieved reasonable vacuum levels without heat treatment of the monochromator vacuum vessel.
Installation of the 24-ID-E monochromator was scheduled not to interfere with 24-ID-C beamline operations (May 2006 APS shutdown). Just before installation we attempted to locally validate and calibrate all motions of the 24-ID-E monochromator. We immediately discovered damage to the Bragg axis nano-motors and one of the closed-loop pico-motors caused by mishandling during packing and shipping. We were able to replace the nano-motors in time for installation, but the replacement timeline for the pico-motor was too long to be considered prior to installation of the monochromator. This problem will be corrected during the 2007 winter APS shutdown. For this reason, initial commissioning of the 24-ID-E monochromator only involved the Si (220) crystal axes.

Aside from the Si (311) roll axis, all other rotation and translation axes were found to operate at or beyond functional specification. Figure C.7.1.4 shows an example step-displacement analysis for the Si (220) roll axis. This scan demonstrates surprisingly low hysteresis, considering that the picomotor is a peizoelectrically-driven device.

The Si (220) and (311) crystals were mounted in their respective cooling blocks (see Figure C.7.1.5) and APS Metrology was asked to topographically assay them for induced strain from the clamping action of the cooling blocks (see Figure C.7.1.6). The strain field measurements indicated that no significant strain was present and measured rocking curve widths (data not shown) were close to their expected canonical values.
**Figure C.7.1.4:** Example axis step-displacement analysis for Si (220) roll axis. The roll axis, which is driven by an encoded picomotor, was scanned from 0 to -20000 steps, to +20000 steps and finally back to 0. All data from this scan is shown superimposed in the plot. Inset shows a 7-fold zoom of central region of scan. Horizontal axis: steps vertical: mrad.

**Figure C.7.1.5:** Photograph of Si 220 crystal mount module. The diffracting face of the Si crystal is the dark square structure near the center of the figure. Two copper cooling blocks are clamped above and below the crystal body. Two thin sheets of Indium foil mediate contact between the crystal and cooling blocks. A copper Compton scattering shield bolts to the cooling blocks, leaving a channel for the incoming and outgoing beam. The cooling blocks are secured to a delrin block, which in turn is secured to the crystal’s kinematic stage (implementing the thermal break between the cooling blocks and the crystal mount module).
The 24-ID-E monochromator was installed in the SOE in early May 2006, along with the water-cooled white beam stop for the inboard undulator (Figure C.7.1.7). Figure C.7.1.8 shows a photograph of the monochromator installation, prior to installation of the horizontal focusing mirror for 24-ID-E. In order to align the Bragg axis of the monochromator to vertical, we designed a small pendulum that inserted coaxially into the precise center of the upper bearing of the Bragg axis. The lower Bragg axis bearing was fitted with a fixture with a centered reference needle.

**Figure C.7.1.6:** Topograph of Si 220 crystal diffracting surface. The x- and y-direction scales of the topograph are not commensurate due to asymmetry in the vertical and horizontal stepping of the crystal under topographic scanning. Bottom: crystal topograph. Top: strain scale (arbitrary units) providing a color-strain mapping. Note the narrow range of variation and overall uniformity of the topographic strain field (except for crystal edges and a small vertical strip in the left of the topographic field).

**Figure C.7.1.7:** Photographic detail of 24-ID-E monochromator. **Upper Right:** Si 220 and 311 Crystals. Diffracting surface is the square upper most face. **Lower Right:** View of rear of swing cage and tandem nanomotor Bragg axis drive. **Left:** Roll cage and crystal mount modules. Upper module houses the 220, while lower houses the 311 cut crystal. The drives for the crystal kinematic stages and crystal selector stage are behind the facing wall of the swing cage (not seen in this view). The Bragg axis bearing housing can be seen at the top and bottom center of the swing cage. The vertical tube at the back and right of the swing cage is a water-circulation line for temperature stabilization of the crystal kinematic plates (coupled via copper braids) and the swing cage. Thermal stabilization is required to offset imperfect thermal isolation between the liquid nitrogen circulation and the kinematic crystal mount plates.
The Bragg axis was considered aligned to vertical when the pendulum bob needle aligned to the needle in the lower bearing (see Figure C.7.1.9). The 24-ID-E monochromator inertial plinth is supported by 3 “z-wedge” lifts, which are slotted into a swash plate. The swash plate is driven in the x- and y-direction my mechanical screws. Differential displacement of the z-wedge lifts effected overall vertical positioning of the monochromator and vertical alignment of the Bragg axis (see Figure C.7.1.9).

Critical elements of the 24-ID-E monochromator have been fitted with thermocouples to monitor crystal cooling effectiveness and to monitor thermal leaks between the cooling system and the crystal module kinematic plates. Following evacuation of the vacuum vessel and onset of liquid nitrogen flow, the “head” region of both crystals attained a temperature of approximately -142.0 °C, about 10-15 °C above the cooling block temperature. The crystal module kinematic mount plates dropped to an equilibrium temperature of 3 °C and the crystal kinematic motor drives stabilized at 17 °C in their deactivated state.

The best vacuum achieved, to date is 1.4 x 10^-7 torr. Leak checking studies indicate that the VCR joins of the internal liquid nitrogen distribution are the likely cause of poor vacuum performance. During the Spring 2007 monochromator intervention we will replace all extant seals with indium-coated seals to correct for surface imperfections of the VCR glan surfaces.

Figure C.7.1.8: Photograph of 24-ID-E monochromator (center) and white beam stop (left of center) installed in secondary optics enclosure. The 300 l/sec ion pump (below monochromator vacuum base plate) has been replaced with a 600 l/sec ion pump, mounted from the top of the vacuum vessel chamber. The turbomolecular pump, shown above, was moved to the location previously occupied by the 300 l/sec ion pump.
C.7.1.3. Monochromator energy set point calibration

The planned diameter of the wall penetration between the secondary optics enclosure and the 24-ID-E end station is only 50 mm in diameter so it was imperative that the correct monochromatic beam take-off angle was determined prior to finalizing the layout of the beamline downstream of the monochromator.

Prior to cutting the penetration between 24-ID-B and 24-ID-E and installation of the 24-ID-E focusing system, the exit port of the monochromator was terminated in a He-scrubbed beryllium window and monochromatic beam was brought out to the hutch wall separating 24-ID-B and 24-ID-E. We taped a fluorescent screen on the partition wall at the likely point of intersection for a 12660 eV beam. We observed the fluorescent screen with a video camera while sweeping the monochromatic beam (Bragg rotation) through a fairly thick Se metal foil mounted on a linear translation stage, in front of the fluorescent screen. We noted the point in the Bragg scan that resulted in a sudden diminution of screen fluorescence. Aluminum absorbers were placed upstream of the Se filter to avoid saturating the fluorescent screen. The Bragg axis was rotated to the point representing the absorption edge of Se. We removed the Se foil and Al attenuators and permitted the beam to “burn-in” the fluorescent screen to generate a fiducial mark for the wall penetration. A vertical laser rotator was aligned to the fiducial burn and a fiducial mark on the beryllium window plugging the exit port of the monochromator. After drilling a 3” diameter hole in the partition wall we extended the take-off angle survey line to the outboard wall of 24-ID-
E using the laser rotator beam as reference. This procedure yielded a high-confidence survey line for layout of the focusing system and the rest of the beamline’s end station equipment.

Following installation and initial commissioning of the 24-ID-E focusing system (see section C.7.2), we precisely calibrated the desired take-off Bragg angle using a calibration tool built by K. D’Amico (SGX-CAT). This tool is a simple reflectometer, consisting of two concentric rotation tables, one of which (very high mechanical precision) supports a strain relieved silicon crystal with a 111 cut. The other rotation table (low precision) supports a visible light-shielded PIN diode. This device (design and procedure attributed to B. Batterman) is used to obtain very accurate measurements of the angular offset between the peaks in the rocking curves for the 111 and 333 reflections from the 111 Si crystal cut. Once the angular offset between the two rocking curves is obtained, the energy set point (incident wavelength) in effect for the measurement is given by the following relation:

\[ \lambda = 2 \ d_{111} \ [1 + (d_{111}/d_{333} - \cos(\Theta_{333} - \Theta_{111}))^2 / \sin(\Theta_{333} - \Theta_{111})^2]^{1/2} \]

\[ d_{111} = \text{Si 111 D-spacing} \quad d_{333} = \text{Si 333 D-spacing} \]

\[ \Theta_{111} = \text{angular position of the peak of the 111 rocking curve} \]

\[ \Theta_{333} = \text{angular position of the peak of the 333 rocking curve} \]

This calibration method has a precision of order 1 eV and is reasonably insensitive to small imprecisions in the experimental configuration, as long as the rotation axis of the Si 111 crystal face intersects the center of the incident beam and the diffractive face of the crystal. Figure C.7.1.10 is a photograph of the reflectometer setup in 24-ID-E.

We plan (see section D.1.2.4.3) to build a simplified version of this device for permanent installation on the 24-ID-E goniometer to facilitate rapid energy calibration of the 24-ID-E monochromator Bragg axis setting.

The horizontal and vertical focusing mirrors can be yawed to offset small angular displacements of the 24-ID-E monochromatic beam associated with energy calibration of the monochromator. The bore diameter of the transport spool penetrating the 24-ID-B and 24-ID-E wall limits the angular throw of the monochromatic beam to about +/- 50 eV from the nominal Bragg angle set point. It may be possible to perform limited XAFS scanning in the vicinity of the Se edge by yawing the HFM to compensate for scan-associated beam movement and maintain intersection between beam and sample.
Figure C.7.1.10. Photograph of the beam energy calibration reflectometer, installed at the approximate position of 24-ID-E gonionstat. Note the serpentine cross-section of the Si crystal, intended to minimize strain propagation from the point where the crystal is secured to the mount and the diffracting surface (pointing vertical in this view). The device above and right of the crystal is the PIN diode housing. The entire device is mounted on a transverse translation stage.

C.7.2. Focusing System Installation and Commissioning

The design and construction of the 24-ID-E Kirkpatrick-Baez focusing system is detailed in section C.6.2. Unlike the 24-ID-C focusing system the vertical (VFM) and horizontal (HFM) focusing mirrors are installed in two individual vacuum vessels, separated by the partition wall between 24-ID-B and 24-ID-E. The VFM of 24-ID-E incorporates a modified U-bender for setting the curvature of its mirror and an elliptical bender for the HFM. The 24-ID-C KB focusing system uses two-pole (elliptical) bending mechanisms for both mirrors. Otherwise, the 24-ID-E focusing system is very similar to that of 24-ID-C in terms of its mirror positioning and control systems. Like the 24-ID-C KB mirror, the transverse position and yaw of both 24-ID-E mirrors are controlled by pairs of nanomotor piezoelectric servomotors. The upstream kinematic mount of the VFM bender mechanism also uses a Jenna Digital Piezoelectric Transducer (DPT) for fine control of the mirror pitch. Console scripting for control of the 24-ID-C KB focusing system was applied to the 24-ID-E KB focusing control system with only minor modifications.

The modified VFM U-bender incorporates a spring loaded screw to induce a secondary bend moment on the mirror, near the downstream engagement of the bender actuator. This secondary moment enables the surface figure of the VFM to closely approximate an elliptical curvature (see Figure C.7.2.3) over a limited range of focal length.

Oxford-Danfysik delivered both mirrors to NE-CAT in July 2005. Upon delivery both vacuum vessels were evacuated and leak checked. Appropriate working vacuum was immediately obtained. At the same time we validated the operability of all motion systems. One nanomotor transverse motion, associated with the HFM was found to be defective. Just before installation of the 24-ID-C HFM and VFM (May 2006) Oxford-Danfysik technicians replaced the defective HFM nanomotor and we proceeded to re-validate and calibrate all of the 24-ID-E focusing system translation axes. The performance of all translation axes was found to meet or exceed design
specifications. For example, Figure C.7.2.1 shows the step-displacement function measured for the downstream transverse axis of the HFM which controls the yaw angle and transverse position of the horizontal focusing mirror in relation to the beam. This axis is used for horizontal steering of the beam. Figure C.7.2.2 presents a calibration study of the VFM DPT used for fine tuning its pitch angle and vertical steering of the monochromatic beam.

Due to delay in the delivery of the 24-ID-E monochromator we decided to postpone installation of the 24-ID-E beamline until the spring 2006 APS shutdown, so as to not interfere with user operations at 24-ID-C. The 24-ID-E monochromator was installed in the secondary optics enclosure (SOE) during the first week of the spring 2006 shutdown (see C.7.1). Immediately thereafter we installed the 24-ID-E HFM in the SOE. Figures C.7.2.3 and C.7.2.4 show photomontages of the HFM and VFM installations, respectively.

Following mirror installation, a commissioning beryllium window was installed on the downstream exit port of the VFM vacuum vessel and the entire 24-ID-E optical train was evacuated. The relatively poor working vacuum of the 24-ID-E monochromator currently imposes a less than optimal pumping limit for downstream elements of the 24-ID-E optical train. At present, the 24-ID-E shutter tank and both mirror vessels work at approximately $1 \times 10^{-3}$ torr. We have confidence that improvements to the monochromator’s liquid nitrogen distribution system (section C.7.1.3) will result in improved vacuum performance of all 24-ID-E optical components.

Both the 24-ID-E HFM and VFM were pre-aligned to the optical reference line (section C.7.1.4) and the vertical level of the white beam prior to installation of the vacuum tanks, using laser rotators. Therefore, only small adjustments to the vertical level, yaw and pitch of both mirrors were required to align their reflective surfaces to the monochromatic beam. The yaw angle of the HFM and the pitch angle of the VFM were calibrated by survey techniques applied to an image of the direct and reflected monochromatic beams displayed on a fluorescent screen located at a known distance downstream from the monochromator. The bend radii of both mirrors were driven to vendor-supplied presets to obtain an approximate focus for a point coincident with the planned position for the 24-ID-E goniometer. Further discussion of the 24-ID-E KB focusing system’s performance is deferred to section C.7.3.
Figure C.7.2.1. Example HFM step-displacement calibration: HFM downstream transverse translation (driven by a nanomotor, used for horizontal beam steering). Linear displacement measured using a high precision Heiden Hain linear transducer. Plot shows superimposed data from forward and reverse runs of 12 mm. Inset: Residuals of linear step-displacement function from linear fit to step displacement data (vertical lines = 1 µm). Step resolution (set by the axis’s encoder interpolator) = 0.05 µm, corresponding to an yaw angle step of 0.04 microradians and a theoretical steering step displacement of 0.35 µm at the goniometer spindle. The average residual step displacement over the entire measurement range (see inset) is far larger than 0.05 µm due to imperfections in the nanomotor servoloop.
Figure C.7.2.2. 24-ID-E VFM DPT step-displacement calibration. This axis is used for vertical beam steering. Vertical displacement measured by the DPT’s internal strain gauge is plotted against the measured vertical physical displacement (using a Heiden Hain linear transducer). 1 µm vertical DPT displacement corresponds to 1.14 microradians angular displacement of the VFM mirror surface and a 5.7 µm vertical displacement of the beam at the position of the goniometer spindle axis. The DPT displacement is controlled by an internal digital to analog (DAC) driver with a resolution of 0.0005 µm per digitization unit (step-displacement unit). Differential nonlinearities in the DPT response (see plot) are much larger than the minimum step-displacement. Neglecting this issue, the theoretical accuracy of vertical beam steering at the position of the goniometer is of order 0.003 µm per DPT step.
Figure C.7.2.3: Photomontage of installation of HFM. **Upper left:** HFM vacuum vessel placed in SOE, prior to installation of the shutter. 24-ID-E monochromator is located to right of the mirror, in this view. **Lower left:** Horizontal mirror bender mechanism placed on kinematic lift. The horizontally aligned cylindrical objects are the bend actuators. **Right:** Mirror installed in bender mechanism. The three reflective strips of the mirror can be seen (top to bottom: Rh, bare ULE, Pt). The upstream transverse nanomotor drive can be seen in the extreme lower left of the panel.
Figure C.7.2.4: Photomontage of installation of VFM. **Upper left:** Validation of range of motion of Jenna closed-loop digital piezoelectric actuator (DPT) used for fine pitch control of the VFM. A Heiden Hain linear transducer is positioned over the body of the DPT. The digital display on the right shows the Jenna delivering its specified 40 µm vertical displacement. **Lower left:** Mirror bend actuator undergoing range of motion validation and calibration (foreground and vertical focusing mirror, prior to assembly with the mirror (shown upside down, towards background). **Right:** VFM assembled and mounted on its kinematic positioner. The two-point bend actuator (U-bender) is suspended beneath mirror. The spring-loaded screw situated above the left (downstream) bender-mirror engagement applies an asymmetric bend moment to the mirror to force an approximate elliptical curvature to the mirror.
C.7.3.  End Station Installation and Commissioning

The 24-ID-E end station layout and componentry are identical to those of 24-ID-C except for two elements: 1) the 24-ID-E goniometer is mirror inverted relative to the beam axis, compared to 24-ID-C; 2) the lengths of the optical legs between the goniometer spindle axis and the focusing mirrors differ from those of 24-ID-C.

Installation of the 24-ID-E end station commenced in July of 2006, starting with installation of the goniometer and auxiliary support stands. This installation permitted completion of the beam transports from the 24-ID-E VFM and installation of the collimation system, including: defining slit arrays, principal quadrant beam position monitor, attenuators guard slits and data shutter. The 24-ID-E detector support was then assembled and the 24-ID-E Q315 detector installed. End station construction was essentially complete by November 2007. Figure C.7.3.1 is a photograph showing the current state of the 24-ID-E end station construction.

The distributed control system for 24-ID-E is virtually identical with regard to overall architecture and hardware to that of 24-ID-C (see C.4.9.8.3), except for monochromator related components and function. Console scripting for 24-ID-E operations was developed simultaneously with end station component installation. Scripting for the HFM, VFM auxiliary stand, slits, shutter and goniometer motion controls are nearly identical to those developed for 24-ID-C. There is negligible overlap between form and function of the 24-ID-C and 24-ID-E monochromators, so development of ab initio Console scripting for the 24-ID-E monochromator was required.

At the time of proposal submission, the ADSC Q315 control system is undergoing final integration with the 24-ID-E optics control system and data commissioning studies are scheduled to coincide with resumption of APS accelerator operations in February of 2007.

C.7.3.1. Beam focus and stability

Once the end station collimator, goniometer, flux counting trains and sample visualizer were installed we optimized the beam focus using a YAG beam imager (see Figure C.4.7.8). The optical field of the sample visualizer was spatially calibrated using a graduated optical graticule.

The monochromatic beam was attenuated and steered to the center of the sample visualizer’s fiducial cross, using the yaw adjustments of the HFM and DPT pitch axis of the VFM. The Bragg angle setting of the monochromator was left unperturbed once adjusted to 12662 eV using the method described in section C.7.1.4. Only minor changes to HFM yaw and VFM pitch were required to center the beam in the visualizer field, so both mirrors were operating very near the design angle of incidence of 3 mrad.

The transverse position of the HFM and the vertical position of the VFM were adjusted to maximize flux measured by the principal quadrant BPM (average sum of all channels) and an ionization chamber downstream from the goniometer. The principal BPM is the most upstream component housed in the collimator assembly, close to the upstream collimator gimbal (see Figure C.4.9.2). The vertical and horizontal translations of the auxiliary support stand were used to null the x- and y-channels of the BPM. The beam position, relative to the center of the bore of the BMP vacuum housing (and therefore the rest of the collimator assembly) was checked with burn paper. We observed that the null-signal position of the BPM coincided with the center of the BPM bore to less than 1 mm in both directions.
Figure C.7.3.1: Photographs of 24-ID-E end station. **Top:** View downstream from VFM. **Left Bottom:** Goniometer, cold stream and visualizer. **Right Bottom:** Q315 and detector carriage.
At the time of submission, we have not yet implemented beam slit scan scripts for 24-ID-E so our characterization of the beam profile is presently rather qualitative. Using the spatially calibrated beam visualizer we observed an initial quasi-gaussian focus with spot size of approximately 70 µm x 150 µm (vertical x horizontal, FWHH), obtained by simply applying manufacturer-supplied parameters for the mirror bend actuators. Adjustments to the two HFM bender moments and the single VFM moment eventually resulted in a gaussian beam of dimensions ~15 µm x ~50 µm, FWHH.

Direct observation of the beam with the YAG visualizer allows us to make qualitative judgments concerning beam stability at the crystallographic spindle, down to a time interval of 31 ms (frame rate of the sample visualizer’s CCD camera). The principal BPM can be polled with sub-micron position accuracy at approximately 3 Hz. We calibrated the output of our quadrant BPM’s by scanning the BPM diode array relative to the beam, using the vertical and horizontal translators auxiliary support stand to manipulate the BPM, while simultaneously monitoring the output of the BPM and the physical step displacement using a Heiden Hane linear transducer (see Figure C.7.3.2).

Figure C.7.3.3 shows a time trace of the output of the principal 24-ID-E BPM under conditions of 2.5 liter/min liquid nitrogen coolant flow, with data polling at 0.5 second intervals. Noticeably superior stability is observed in the vertical, relative to the horizontal direction. A similar stability asymmetry is seen with the 24-ID-C beam. Both beamlines manifest superior beam stability in the direction perpendicular to the direction of Bragg rotation of its monochromator crystal (horizontal in the case of 24-ID-C and vertical in the case of 24-ID-E). This finding alone, confirms that the principal source of beam instability for both beamlines is not the APS electron beam itself, but is beamline related, and is most likely cooling flow induced vibration of the monochromator crystals.

Day-to-day beam stability is very good (order ±1 µm vertical and ±5 µm horizontal), even through prolonged accelerator downs.
**Figure C.7.3.2:** Physical calibration of the 24-ID-E principal quadrant diode beam position monitor, used for beam steering. BPM was scanned through beam while monitoring position coordinate output the BPM and from a Heiden Hane linear transducer (in contact with the BPM, over or to the side of the BPM's fluorescent screen).
Figure C.7.3.3: 24-ID-E y- (magenta) and x-positional (blue) stability measured with calibrated quadrant diode beam position monitor, situated approximately 1 m upstream of goniometer spindle. Vertical gradations represent 5 μ displacement.

At this time, the main outstanding Console scripting and commissioning tasks remaining before turning the beamline over to the NE-CAT operational staff are the following:

1) Repair of the monochromator Si 311 crystal module translators and replacement of liquid nitrogen cooling distribution seals (completed in January 2007).

2) Completion of optical characterization of the beam:
   a) focus spot profiling (slit scans),
   b) study of energy set point stability of both monochromator crystals,
   c) characterization of thermal-stability issues associated with crystal selection,
   d) study of impact of upstream gap changes on 24-ID-E optical performance.

3) Completion of Q315 integration at 24-ID-E (completed in January 2007).

4) Console scripting for monochromator crystal selection, including automatic pitch and roll adjustments for the two crystal modules (to be completed by February 2007).
5) Completion of scripting for beam position steering via HFM yawing and VFM pitching, driven by data from the principal quadrant BPM. The beam position stability, even without active feedback, is sufficient to support routine data collection.

Most of the optical characterizations listed under task 2 above, can be interleaved with data commissioning. Trial data commissioning will start in February 2007. We expect that 24-ID-E will enter routine use in mid to late spring 2007.

C.8. Technological Core Developments: Infrastructure and Support Facilities

C.8.1. 24-ID-C and 24-ID-E User Area

C.8.1.1. Design of Experimental User Space

The design of the experimental user space was based on the need to ultimately accommodate user groups at four beamlines in Sector 24. For our first two insertion device beamlines, the goal was to define distinct user areas. The largest available space is in the area adjacent to the 24-ID-C hutch. In this area, we chose to physically separate two user groups working in the 24-ID-C hutch and eventually the 24-ID-E hutch, and to provide an additional shared area for any pre- and post-run user activities.

The most challenging problem was to accommodate the three separate areas in a rather small triangular space bounded by the Advanced Photon Source (APS) safety egress (top of Figure C.8.1.1), the experimental hall walkway (bottom left of Figure C.8.1.1) and the back wall of the ID-C hutch (to the right of Figure C.8.1.1). A further consideration was to avoid cross traffic in common areas. After repeated discussions between the operations group and the furniture manufacturer (Wrightline), the plan presented in Figure C.8.1.1 below was implemented.

![Conceptual design for the user space for the 24-ID-C and 24-ID-E beamlines.](image)

Figure C.8.1.1: Conceptual design for the user space for the 24-ID-C and 24-ID-E beamlines.
The 24-ID-C user area consists of 24 linear feet of sitting/working space and enough room to accommodate a user group of up to six people. In this area six computers are currently installed: two computers for the beamline operation (controls, motors, etc), another computer for the ADSC detector data collection, and three dual processor computers for data integration, scaling and structure determination. In this area there are also three standing cabinets for vacuum controllers, motor controllers, and the ADSC detector computer cluster. All work benches have full network and phone access integrated into the office furniture.

The 24-ID-E endstation hutch consists of 20 linear feet of sitting/working bench space. Here we will be installing five computers and two standing cabinets for computer/network equipment. The computers will be purchased as the 24-ID-E beamline nears completion.

Both user groups will have access to the shared chemical lab bench for sample manipulations and storage. The Chemical Bench area in Figure C.8.1.1 has a stereo zoom microscope. Seven overhead locked cabinets for individual storage are installed. Across the experimental hall walkway all beamline users will have 24 hrs/7 days a week access to our state-of-the-art biochemical laboratory. The laboratory is fully equipped to cover a wide range of experimental procedures from growing cells to protein crystallization (see LOM/lab description, Section C.8.2.).

Figure C.8.1.2: Top view of 24-ID user’s area: the 24-ID-C user area is in front and the common area is in the back; the shared chemical bench is on the left side.

The common area is for any pre- and post-run user activities, including data backups, additional data processing, rest and socializing. This is provided for convenience to users of all the
anticipated beamlines in Sector 24. Five dual processor computers will be installed in this area, in addition to the user amenities of a small refrigerator, microwave, coffee maker and sofa.

Figure C.8.1.3: General view of the user's area for 24-ID-C.

C.8.1.2. User Computers, Crystallographic Software and Network Environment

24-ID is fully equipped with several powerful computers for processing and backup of all data, and solving structures. Currently, all computers are NFS mounted to storage space on a 14Tb HP EVA5000. It is planned to convert NFS mounting to a faster parallel accessing GPFS mounting. All of the Sector 24-ID beamline users have access all data users via three computers: two dual processor Xeon (3.4 GHz, 1MbL2 cache) computers with 2 GB DDR2 memory and one high-performance 64bit double processor Opteron AMD (2.4GHz) computer with 4 GB DDR2 memory. On the beamline there is at least one 21 inch ultra-high resolution CRT monitor for stereo graphics visualization. Several other computers are used mainly for writing DVDs, mounting firewire drives and hard disk drives with non-linux partitions, and to manage network switches.

All user computers have the same set of crystallographic software to integrate and scale experimental data and to solve and refine structures. The following packages are installed and maintained: HKL2000, LABELIT, CCP4/CCP4i, SOLVE/RESOLVE, ARP/WARP, SHELX-97, CNS, PHENIX, RAVE, O and COOT. Every effort is made to install the newest version of the software as soon as it is available. A high-throughput GUI for fast automated structure solution is planned for testing and installation.

Data backup and archival storage will be the responsibility of the users. We will provide a USB2 interface from each computer for users to backup their data to their own hard disk storage. The recommended interface is USB2 (480 Mbps). Additional support will be provided for DVD+/-RW
creation and fast network data transfer to home institutions. We fully support only linux (ext3 journaling, ext2) file systems at this time.

The networking environment consists of storage, networks, and computers. Data collected by the CCD detector will be transported using Ethernet and Fiber Channel networks to storage. The data are then read from storage, and available for processing, backups, and archiving.

The network architecture, shown in Figure C.8.1.4, consists of a fiber core network for transport of ethernet and Fiber Channel traffic. All of our currently installed fiber is capable of transporting 10 Gigabit traffic. NE-CAT ethernet switches are ready (module installation) to adopt 10 Gigabit transport over selected core links. In the near future, we will evaluate the 10 Gigabit ethernet solutions for faster transport of data. Currently the 24-ID-C core ethernet fiber network is configured to run at 4 Gigabit. Each workstation has both a gigabit ethernet connection over copper Network Interface Card, and a 2 Gigabit Fiber Channel Host Adapter Card.

**Figure C.8.1.4:** Ethernet network architecture.

The physical layout of the network (wiring diagram) is shown in Figure C.8.1.5. below. The installation of wiring for 24-ID-C, 24-ID-E, and the Common Area was completed in November 2004. The fiber-core network and all switches were installed in February 2005. The orange lines represent multi-gigabit fiber core network (between switches), obtained using LACP protocol. The individual computers are connected using copper 1 Gigabit Category 6 wiring. The green, red, and blue colors represent the separation of the network as divided by the individual switches. This is to allow for fast access within the different areas but to reduce interference with the data collection in the 24-ID-C and 24-ID-E areas.
C.8.2. Laboratory Office Module (LOM)

Two years ago the laboratory portion of the Lab Office Module (LOM) was a generic synchrotron lab consisting of a single bench, fume hood, and a sink with non-potable water. Today, it has been transformed to our vision of a state-of-the-art working biochemistry lab for both users of the facility and the staff scientists. With the lengthening of the existing lab bench and the addition of another lab bench with overhead cabinets and shelves, assigned spaces for all of the resident scientists plus open bench space for users of the Sector 24 facilities. Clean power and high quality water from a Millipore system flow through the lab. It is now routinely used for protein purification and crystallization.

In addition to the remodeling of the lab space, we are now well equipped for handling proteins for crystallization. Our philosophy is that a lab that is constantly used by the staff is the best way to provide for user needs. This means that most of the supplies and tools that are often forgotten or overlooked can be found in the lab. Our users will be able to conveniently perform time-sensitive protein crystallization and crystal soaking experiments. The lab also contains many chemicals for making solutions, analytical balances, a pH meter, an ultrasound dismembrator, table-top refrigerated shaker, a high-speed refrigerated Eppendorf centrifuges, a UV/Vis spectrophotometer and twopowerful stereo zoom microscopes. Finally, the touches that separate this lab from other beamline labs are the availability of an Agilent 2100 Bioanalyzer for fast and precise characterization of molecular weights and concentrations of proteins and DNA, a dynamic light scattering system, which provides unique insights into the behavior of biomolecules in solution by measuring the hydrodynamic radius and polydispersity of macromolecules, and an AKTA/FPLC system for high throughput automated analytical and preparative protein purification. These machines will primarily be used by the staff in their research projects but can be used by the experimenters that visit as well. Figure C.8.2.1 provides several views of our biochemical lab.

Figure C.8.1.5: Ethernet wiring diagram.
We have also addressed the problems of stable temperature control for both storing biochemical materials and for growing and storing crystals. Our 4 °C box houses the AKTA/FPLC purification system. There are two incubators for crystallization setups, one at 4 °C the other at 18 °C. A small -35 °C refrigerator is used for storing proteins and chemicals, a liquid nitrogen Dewars are available for long term cold storage. Finally, our 9'x10' walk-in 4 °C cold room is in full operation and equipped with basic stainless steel and plastic furniture, and has a dedicated microscope for crystal manipulation.

C.8.3. Automated Sample Mounting System

C.8.3.1. Introduction:

In recent years, automation of synchrotron beamlines has become an important area of development. Due to limited availability of synchrotron beamtime and the increasing difficulty of projects that are being pursued, a sample automounter that can efficiently help screen a large number of crystals has become a priority. Screening of large numbers of crystals is particularly time consuming at the APS due to the delays introduced by door pneumatics and beamline safety system timing constraints. “Time and motion studies” that have been validated by actual experiments at APS have demonstrated that the time to survey a crystal can be reduced by factors of 2-5 by employing a robotic sample mounter system. Many crystals are preloaded in a liquid nitrogen containing Dewar located next to the goniometer, the shielding door is closed and the researchers can then survey the entire suite of crystals without entering the radiation enclosure again.
To meet the needs of NE-CAT users, we conducted an assessment of sample placement robotic systems that are currently available. Ultimately, we chose the ALS robot (described in section C.8.3.2). Our conclusions of the other contenders are summarized below:

**ACTOR:** Abbott Laboratories was the original inventor of this robot which was then subsequently licensed to Rigaku/MSC for commercial production. The major advantage of this robot is that it can be adapted to almost any synchrotron beamline. An increasing number of synchrotron beamlines and academic laboratories are using or are planning to install this automounter. Here at the APS, the Industrial Macromolecular Crystallographic Association (IMCA) CAT insertion beamline has had one in operation for over two years. The features of this system include automatic crystal mounting, automatic crystal orientation, automatic crystal retrieval and storage. The Dewar of the robot can accommodate five magazines and each magazine can hold up to 12 samples mounted on standard 18mm Hampton Research pins. These magazines can be transported to the synchrotron site by a standard Taylor-Wharton CP100 dry shipper. Due to all these attractive features, we seriously considered this robot. The major drawback is a very high cost compared to the automounters developed by academic institutions and the dependence upon the supplier to customize its operation based on future changing needs.

**SSRL’s SAM:** Stanford Auto-mounting (SAM) system is one of the most reliable robots available today. It is being used extensively at the protein crystallography beamlines of SSRL. Software support for this robot is integral to the Blu-Ice control system and GUI. We were impressed with the performance of this robot during our visit to SSRL in June, 2004. The Dewar of this robot can hold three cylindrical cassettes, each of which can hold up to 96 samples mounted on the Hampton Research Pins. The cassettes can be transported to the beamline using a conventional CP-100 dry shipper, MVE SC 4/2V dry-shipping Dewar or international cryogenics IC-7VS. The cost of the robot is comparable to that of the ALS robot. The drawbacks of the SSRL robot are its complicated arm movements and availability. It can only be obtained from an outside workshop with the supplied design diagrams. SSRL does not have a dedicated group to supply the robot construction to outside customers.

**Marcsc-Cryogenic Sample Changer:** This is a product of MarResearch Company and currently being used at three APS protein crystallography beamlines, Structural GenomiX CAT (SGX), COM CAT, and Dupont-Northwestern-Dow (DND) CAT. The Mar sample mounter is in use extensively at SGX. A limitation of this robot is that only 19 samples can be mounted in its compact Dewar. The major disadvantage is that the robot is attached to the MarResearch DTB detector base and being is sold only as an integrated system.

After comparing these robotic systems, we made the decision to procure an ALS robot based on the following four important factors: proven performance, simplicity of design, cost, and a user community committed to the design and continuing improvement of the robot. Currently, three protein crystallography beamlines at the Advanced Light Source (ALS) are equipped with this robot. Other robots based on the ALS design are either being installed or are in the commissioning phase at four beamlines at Brookhaven National Laboratory (BNL), Cornell University’s MacCHESS facility, and two other beamlines at the APS.

**C.8.3.2. Description of the ALS Robot Sample Changer**

The details of ALS automated sample mounting system have been published (29). Figure C.8.3.1 shows the robot integrated into the experimental setup of crystallography beamline BL 5.0.3 at the ALS.
The ALS automated sample mounting system consists of three important parts: 1) the gripper that holds the sample; 2) the X-Y-θ stage that performs the transport of the sample to the goniostat, and 3) the Dewar that stores the samples in liquid nitrogen. Figure C.8.3.2 shows a sample gripper currently in use at the ALS. The gripper has a conically shaped, brass split collet, which can be opened and closed by translation of a fixed tube via a small pneumatic actuator. There is a sensor inside the collet to monitor its temperature. The gripper has a small, low-force, linear stage (“Small move” in Figure C.8.3.2) that is used for the final positioning of the gripper on the sample. This permits gentle handling during mounting and dismounting actions.

The gripper is mounted on a pneumatic X-Y-θ stage which is used to transport the samples between the goniometer and the storage Dewar. A vertical Y stage moves the gripper in and out of the Dewar, a 90° rotational θ stage orients the gripper either horizontally or vertically, and a long horizontal stage moves the gripper between the Dewar and the goniometer head. The pneumatic stages are equipped with magnetic sensors, which indicate whether the stage is in an extended or retracted position. These sensors are also used to prevent collisions as a part of an interlock system.

Figure C.8.3.1: ALS’s BL5.0.3 end-station setup with sample mounting system (29).
Depending upon the state of the automounter, certain motions will be forbidden. For example, the horizontal stage cannot be extended if the beam stop is in place or the gripper is in the Dewar. The motorized stages of the Dewar are also part of the interlock system. The sample Dewar and cassettes are shown in Figure C.8.2.3. It is a cylindrical Dewar that can accommodate seven cassettes, on which 112 samples can be mounted. The Dewar is mounted on a R-θ motorized stage, which is used to position the selected sample for gripper access. The Dewar can be positioned such that the gripper can be pre-cooled in an unoccupied space. This precaution is necessary to protect the samples from boiling liquid nitrogen. The Dewar can be automatically filled from the liquid nitrogen supply system. The gripper can reach the samples inside the Dewar through a small access port in the cover.

Figure C.8.3.3: Sample Dewar with Cassettes (29).

Figure C.8.3.4 shows a close-up view of cassettes with magnetic bases and a sample cassette holder that are in use at the ALS. The cassettes were designed for safe transport and storage of frozen samples using conventional shipping Dewars. For safety during transportation and
storage, the cassettes and bases are locked together by two springs on the side of the assembly. A magnetic sheet on the bottom of the base holds the samples within the base and also holds the base within the automounter. The cassettes are labeled with cryocompatible barcodes for tracking. Seven such cassettes can be packed in a Taylor-Wharton CP100 dry shipper. A set of special tools were also designed and developed by the staff of the ALS to handle the cassettes and bases.

Figure C.8.3.4: Sample transport and storage system (29).

C.8.3.3. Implementation in the Experimental Station at NE-CAT’s 8-BM

The current end-station setup at 8-BM is shown in Figure C.8.3.5. During the integration of the sample mouser with the 8-BM end station, consideration will be given to ensure that manual mounting will not be complicated by the presence of the robot. It is expected that a significant number of users would still prefer manual mounting, especially in the early stages of the commissioning of the robot and on challenging projects.
C.8.3.4. Progress

NE-CAT has contracted with the Engineering Division at LBNL to construct a stand alone-robot with software to control it. The current status of the NE-CAT robot being assembled at LBNL is shown in Figure C.8.3.6.

The Dewar is covered with polyethylene foam to reduce the heat conduction into the Dewar. The length of the gripper is increased by one inch with respect to the original design, so that the level of liquid nitrogen over the samples at the Dewar can be increased considerably. This modification will help to reduce frost buildup at the top of the Dewar and reduce the turbulence introduced by the autofill. For the sake of reliability and compactness, the dimensions of the Dewar were reduced to accommodate four cassettes instead of seven. Currently, we are in the process of procuring a supporting table for the robot. The supporting table will have a fine kinematic height adjustment. Lateral adjustment that is parallel to the beam and a small angular adjustment will be built into the three point mount as well as pushers on the table top. At present, the plan is to fix it to the floor and have it act as a standalone unit relative to the phi axis.

Figure C.8.3.5: Present end station setup at NE-CAT’s 8-BM (picture taken looking toward the source).

Figure C.8.3.6: Current status of assembly of the NE-CAT automounter.
C.8.4. NE-CAT User Database

In preparation for the growth of the user load, we have adopted the BIO-CARS online database system to handle beamline proposals. This program, written by Dr. Keith Brister while at BIO-CARS, is used to handle all beamtime requests. Users are required to enter a brief description of the projects and the experimenters’ names. The database will handle submission of the APS Experimental Safety Approval Forms (ESAF) as well as provide an online calendar for scheduling and viewing scheduled users. It is capable of handling multiple beamlines. Since Oct. 2004, all experimenters that have used the NE-CAT beamlines have used this system. This includes all users that are using the Sector 24-ID beamline during commissioning mode. It also includes all APS general users. In the case of the APS general user program this is a redundant procedure; however, we have been able to get 100% compliance from these users as well. Due to its extensive usage at BIO-CARS, the system has worked well up to this date.

C.9. Infrastructure and Support Facilities Progress Since October 2005

C.9.1. Computational

The General Parallel File System (GPFS), developed by IBM has been fully implemented and tested in the Sector 24 data flow system. Section D.1.3.5.1 provides a detailed description of the data flow system of Sector 24. GPFS enables all computer nodes interfaced to the SAN to directly and concurrently access stored data without mediation by a file server. GPFS co-exists with NFS, so that EVA-5000 SAN data store can be accessed via NFS, if need be. Conversion from NFS-distributed file systems to GPFS has resulted in very substantial gain in the speed of data reduction and analysis and modest improvement in data acquisition throughput. During August 2006 GPFS system was upgraded to version 2.3.0.12. This resolved the sporadic network latency issues we had on specific workstations.

It should be noted that NE-CAT’s data storage is heavily used because of high data flow rate. In fact during the 2006-1 user run, the entire 15 TB of then available storage on the HP EVA 5000 disk storage system was used. The current storage capacity allows us to store user data for approximately 3 months.

After EVA 5000 SAN storage capacity was increased to a physical size of 30TB, an additional GPFS file system was created (called 24-ID-E) to serve the upcoming 24-ID-E beamline. It currently has a capacity of 7TB and will be increased as needed since GPFS has tools that enable us to change this size on-the-fly without interference with current data storage.

To help users analyze and archive their data, two powerful workstations were installed in the common user area during early 2006. These computers have access to all the file systems used at the beamline workstations. This has been very useful as we are making the transition to a full user operation mode, since users are usually scheduled back-to-back.

NE-CAT has an up-to-date distribution of crystallographic software suites and we are continuously adding additional software as users request. Every few months software suites are updated (if updates are available). The beamline manual is constantly updated as new improvements are made at the beamline or new feedback is obtained from users.

C.9.2. Hardware

Running a user program is the best way to learn about the strengths and weaknesses of any beamline. As we are progressing towards a full user operation mode, we have learned and implemented several upgrades to the beamline hardware.
To better serve users with samples that cannot be frozen in liquid nitrogen, such as virus samples, we have installed a FTS compressed air cooler, operating from -40 °C to 100 °C. This co-exists with the Oxford Cryojet nitrogen cold-stream crystal cooling system. Interchange between the two crystal cooling systems can be made within a few minutes.

To improve crystal visualization, several upgrades to the imaging long-range microscopes were made. A remotely operated trans-illuminator was installed, which increases the sample contrast for better visualization. Higher-resolution monitors were added at the control console. Also, users can now perform fine adjustments to the focus of the sample visualizer remotely from the control area.

In response to user requests, we have installed a finer range of attenuators to provide for better control of beam intensity onto the crystals at all energies. The current assembly of attenuators includes aluminum foils of thickness 12, 25, 50, 125, 250, 500 and 1000 μm. Any combination of these attenuators can be selected by remote operation in the control area.

Software upgrades have been completed to facilitate automated energy changes. MAD experiments can now be carried out without user intervention for energy changes. These upgrades have also enabled inverse beam MAD experiments.

Slit control was implemented in Console, which now allow users to choose pre-defined beam sizes (20x20, 20x30, 20x40, 20x60 and 20x80 μm²). Also the wire scan based beam alignment routine is now automated.

C.9.3. **Wet lab facilities**

The refrigeration compression system for the cold room was upgraded, resulting in improved temperature stability. Individual chart recorders were discarded and a centralized data logger was installed. This logs temperature data from the cold room as well as all crystallization incubators and the cold box. This data can be made available on the web in the future.

Staff crystallographers routinely use the wet lab facilities. In addition, users with samples that could not be frozen as well as users who need freshly grown crystals made use of the NE-CAT wet lab facilities to set up crystallization trials. At least one non-NE-CAT member used the wet lab facility to grow cells and to purify protein on site.

C.9.4. **User operations**

During 2006 we have ramped up the user operations at 24-ID-C. 2006-1 and 2006-2 run cycles involved ~60% user beamtime which was increased to over 80% during the 2006-3 run cycle. During 2006, over 30 research groups made one or more visits to NE-CAT beamlines. There were a total of 292 individual visits (some scientists visiting multiple times) from 171 scientists from seven member institutes and ten non-member institutes.

During the 2006-3 run cycle (beginning October 3, 2006) NE-CAT’s 24-ID-C beamline was opened for the APS General Users Program and NE-CAT provided the committed 25% of time to APS general users. As the user program was gradually growing at Sector 24, it was imperative to add additional support staff for operations. In October 2006, Narayanasami Sukumar was transferred from the 8-BM operation to provide support to 24-ID users, supplementing the support already provided by Kanagalaghatta Rajashankar and Igor Kourinov.

The common user area has now been outfitted with two powerful workstations, a combination printer-FAX, and creature comforts such as a sofa and a refrigerator. This area allows users to analyze and archive their data as well as relax, before, during, or after their experiments.
At the beamline steel and foam dewars are now provided for sample handling (instead of glass dewars). This reduces the risk of breaking glass dewars. A simple tool for washing iced-up crystals is also provided (it is just baby food spoon with a hole at the bottom, but works very well, to drip liquid nitrogen on samples).

C.10. Scientific Accomplishments

C.10.1. Core Research (NE-CAT staff)

C.10.1.1. Atrial Natriuretic Peptide (ANP) Receptor Binding Domain

The cardiac hormone, Atrial Natriuretic Peptide (ANP), stimulates salt excretion and dilates blood vessels. ANP plays a major role in blood pressure and salt-fluid volume regulation. Anomalies in its activities may cause heart failure, hypertension, and other cardiovascular diseases. ANP activities are mediated by a specific cell membrane receptor coupled to its intrinsic guanylate cyclase (GCase) activity. The receptor functions as a dimer of a single-span transmembrane protein, each consisting of an extracellular ANP-binding domain and an intracellular GCase catalytic domain. ANP binding to the extracellular domain activates GCase catalysis by an unknown mechanism (30-32).

Our aims are to determine the structure of the ANP receptor and to elucidate the mechanisms of receptor-hormone binding and transmembrane signaling. During the past year, we determined the crystal structure of the extracellular domain of the receptor (ANPR) in complex with the hormone ANP. Through comparisons with the apo-receptor structure that we determined previously, we have identified a possible structural basis for ANP receptor signaling. We have found a unique hormone-induced rotation motion of the two juxtamembrane domains in the receptor dimer that may initiate the transmembrane signal transduction. Additionally, we have determined the crystal structure of the ANPR containing a non-covalently associated bromide ion (instead of chloride ion in the native receptor) (manuscript in preparation). We also have obtained preliminary structures of two constitutively active ANPR mutants. These studies will provide better understanding of the mechanism of ANP and ANP receptor activities and will facilitate development of drugs targeted at the ANP receptor, which may lead to effective treatment of heart failure, hypertension, and possibly other cardiovascular diseases.

C.10.1.2. Complex of PKR and HIV Tar RNA

The human dsRNA activated protein kinase (PKR) is a product of an interferon-induced gene and a key component of the human innate defense system. In response to viral infection, PKR inhibits translation factor eIF-2α via phosphorylation of its serine 51 residue. PKR is involved in transcription by regulating some important transcription factors. A critical step in the activation of PKR is the interaction of dsRNA in a sequence-independent manner. Upon binding, PKR undergoes conformational rearrangement and autophosphorylation. The long-term objective of this study is to understand the molecular nature of the dsRNA:PKR interaction and protein kinase activation. HIV-1 TAR dsRNA activates PKR both in vivo and in vitro, exhibiting a concentration-dependent, activation-inhibition curve typical of dsRNA. Neutron scattering studies of the PKR and HIV-1 TAR 57 RNA complex indicate that the addition of TAR RNA to PKR results in a major conformational change in the protein, forming an activation complex with one TAR to two PKR molecules.

This is a long standing collaboration with the ultimate goal of determining the structure of the native and complex structures of dsRNA with PKR. Although many attempts to crystallize this protein have been attempted, but no reproducible crystals have been obtained. Last year, while screening crystals, a 5.0 Å resolution data set was collected on the HIV TAR RNA. Attempts to obtain better crystals of the RNA alone are being pursued in parallel with that of the PKR.
C.10.1.3. Continuous Phi Mode Data Collection in Protein Crystallography

In the traditional start/stop rotation method of data collection, the overhead for proper implementation of the shutter timing and rotation is high and this overhead increases as exposure time or oscillation width decrease. A more efficient alternative approach is a continuous rotation mode. In this mode the spindle axis is rotated at constant speed with the shutter opening and closing at specific motor positions to select which rotation ranges are recorded. The objective is to minimize overall system overhead as exposure time per image becomes small (less than one second); this overhead should be closely related to the detector readout speed and diminishes with a fast readout detector. As detector readout speeds decrease, as in the case of pixel array detectors, this method may become a viable alternative to conventional, start/stop, data collections.

As a prototype test case of this strategy, sample data sets were collected on lysozyme crystals on 8-BM. The data sets were collected in multiple passes using the Q315 detector. Multiple passes were made to test the hardware timing, overall strategy and readout times for the Q315 detector. Due to the readout times of the detector, it was necessary to collecting every second or third image while performing two or three passes to complete a data set. Preliminary results indicate that this mode of collection may be possible. Methodology and characterizations necessary for implementing continuous mode data collection in general is also described. This work was presented at NOBUGS 2004, Swiss Light Source, October, 2004 (33).

C.10.1.4. Use of Powder Diffraction for Detection of Early Leads in Crystallization

The use of powder diffraction applied to biological macromolecules has been gaining interest in recent years. This is in part due to the successful demonstration by Dr. von Dreele of the use of powder diffraction data and molecular replacement to solve a protein structure. Another more basic application of the method is in the identification of crystalline properties of precipitates in crystallization trials. These applications could provide early leads in the crystallization of complex macromolecules (Figure C.10.1.1).

In the early experiments on 8-BM, we have built a simple prototype system that holds a 1536 well plate. The results of this experiment show that it was feasible to scan through a sample number of the wells and easily see diffraction from single crystals of salt and protein as well as powder patterns of both. Currently, we are automating the procedure in conjunction with the introduction of the Blu-Ice software onto 8-BM. The use of the Blu-ce system has allowed us to quickly advance in the control of motors and the Q315 detector.

C.10.1.5. Simultaneous MAD Data Collection

Multiwavelength Anomalous Diffraction (MAD) has grown to a conventional technique used in the solution of novel protein structures. The use of this technique has been limited by the need to collect multiple data sets from the same or a few crystals. Radiation damage from stronger sources, 3rd generation bend magnets and insertion device beamlines on 2nd and 3rd generation, have contributed to the limitation. In many cases the beam is attenuated in order to collect single as well as multiple wavelengths of data.

Figure C.10.1.1: A view of the X-ray beam (white spot) on one of the wells of the crystallization plate. The dark semi-circle coming from the top of the figure is the scatter guard.
One method that would decrease the effects of radiation damage, increase the accuracy of the anomalous differences and provide a number of data sets at different energies is to use a variable bandpass curved crystal monochromator (polychromator).

We have shown that this type of polychromator could be appropriately tuned to efficiently collect MAD data. A set-up has been put together to take advantage of a MAR345 commercial set-up on beamline 1-BM at the APS. The high demagnification ratio used to focus the beam was used to collect swaths of data on a test copper containing protein. EXAF scans of the crystal were used to locate the edge and a three wavelength filter was used to collect images (Figure C.10.1.2). Due to the length of time necessary for the set-up, a full data set was not collected.

C.10.1.6. Phasing Methods Development

Direct Methods in SAD/MAD. Phasing methods based on anomalous diffraction signals such as multiple (MAD) or single wavelength anomalous diffraction (SAD) are widely used in novel macromolecular structure determinations. An essential and often most difficult step in these methods is to solve the anomalous substructure that provides the reference phases. While the Patterson methods are quite successful in this step for small to medium substructures, the direct methods based procedures, such as SnB, are often required for locating a relatively large number of anomalous scatterers in SAD or MAD phasing (34).

Since SnB was first successfully applied to determine anomalous scattering substructures in 1998, this procedure has been widely used especially for large substructures (35). For further improvement of this program, extensive experimental tests with a number of data sets are necessary. We have used the 16 existing data sets available from our group to investigate the various parameters in SnB in order to find the optimum values for solutions. The 16 data sets provide a broader range of examples for testing in terms of space group, the residues in the asymmetric unit, the number of anomalous scatterers of the asymmetric unit, the resolution of data and the size of the unit cell. The results indicate that, in general, default values are successful, however, difficult cases with low data quality require inputs which deviate considerably from the default ones. Despite the investigation of a variety of different input parameters, calculated SAD phasing electron density maps were comparable with the maps produced by MAD phasing. Our results show SAD phasing coupled with density modification can produce interpretable electron density maps to determine protein structures for most of the testing structures.

Sulfur Phasing. The presence of naturally occurring sulfur atoms offers an alternative approach to Se-Met or other heavy atom incorporating techniques for SAD/MAD phasing. Since the pioneering work of Hendrickson and Teeter in 1981, increasing efforts have been made by several groups worldwide to solve protein structures using only anomalous scattering of sulfur. Since sulfur exists in almost all proteins, these successes demonstrate the possibility of using sulfur anomalous scattering as a "universal phasing" method to determine protein structures.

Since the sulfur absorption edge of 2.5 keV is too low for multiple-wavelength near-edge data collection, sulfur phasing has to be performed at a single wavelength far from its absorption edge. Thus the anomalous signal for sulfur is generally very weak, and data collection requires
minimization of all experimental errors, optimization of data processing and reduction, and high redundancy for multiple intensity measurements. We have started to investigate all these effects in sulfur SAD phasing at NE-CAT.

A 1.5 Å native data set on cubic insulin has been collected at NE-CAT beamline 8-BM at the X-ray wavelength of 1.5 Å. Weak anomalous scattering in this dataset has been improved by radiation damage correction, which is one of the essential steps for weak anomalous signal phasing. The improved data set resulted in the solution of the cubic insulin structure based on the sulfur anomalous scattering signal. Our experience with the sulfur phasing of cubic insulin demonstrates that a redundancy of 5 is sufficient to locate the positions of the sulfur atoms and obtain a mean phase error and to solve the structure. Relying on lower redundancy data sets could save valuable measurement time and minimize crystal decay, leading to a greater usefulness of sulfur anomalous scattering in the protein structure determination. This work illustrates the importance of data redundancy and radiation damage correction in retrieving weak signals. Results were shown in the poster at the ACA meeting in Cincinnati, Ohio, 2003. A region of the density map is shown in Figure C.10.1.3.

We have also developed a more accurate, first-principle theoretical approach to estimate anomalous scattering signals in protein crystals. The approach includes the possible nearest-neighbor correlation effects that may enhance the anomalous signal in the case of disulfide bonds or other anomalous scatterer clusters (36).

**Evaluation of Dose Effect on Direct Methods.** We have tested the dose effect on direct methods using a single insulin data set collected on the NE-CAT 8-BM beamline. Different data sets at different dosages were obtained based on a single conventional data set by performing our new strategy stated in the following section. Weak sulfur anomalous signals were used to locate sulfur atoms by SnB from the series of data sets with different dosages. Six sulfur atoms’ positions could be obtained by SnB, however, success rate decreased as dose increased for the same SnB input parameters. Our test showed the SnB success rate to be 2% at zero dose where the data set was corrected to accumulated exposure time t=0. The success rate decreased to 0.6% at the highest dose to which a single crystal was exposed. This was for the data set obtained by the strategy at accumulated exposure time t=7200s. The success rate decreased because the sulfur atoms were disordered or shifted gradually as the dose increased. Our test indicates that it is important to correct for the dose effect on a data set to get a successful SnB solution (22).

C.10.1.7. Time-dependent Structures and Radiation Damage

**New Strategy for Time-Dependent Structure Observations.** We have developed a new strategy for standard crystallography experiments to allow direct observations of time-resolved structural changes on the atomic scale in a protein crystal. The basic principle of our method is that any structural change will be reflected in an intensity change of Bragg reflections. Tracking the intensity changes leads to descriptive curve fits as a function of accumulated exposure time. These fits give intensities of all Bragg reflections at any given time of interest, even though they are measured at different exposure times. This strategy leads to a time-resolved structure at a given time of interest in a chemical reaction or other process that may result in a structural change. At present, the fastest time scale we can study with this method is limited by exposure time, which is mainly limited by the shutter speed in our conventional data collection methods and can be substantially shortened with future improvements.
The new strategy represents a universal time-dependent method for studying: (a) Chemical reaction processes; (b) X-ray driven catalysis; and, (c) X-ray induced structural changes. Besides structural studies, there are two other important applications: (d) determining a correct way to make use of true redundancy in single-crystal data collection; and, (e) high resolution crystallography.

This method has been applied to a single insulin data set and a single data set of a uridine phosphorylase collected on the NE-CAT 8-BM beamline. For insulin, time-resolved changes in disulfide bonds induced by X-ray irradiation were observed (22).

Further studies and applications of the new strategy are underway, and three examples are briefly described below.

Protein-folding pathways: The observation of the sequence of the breakage of disulfide bonds using the strategy stated above may have implications for protein-folding pathways. We have employed this method to study the disulfide bond breaking sequence due to X-ray radiation as a function of time in RNase A protein. Datasets were collected from native and several mutant crystals. Different sequences of disulfide bonds breaking for the native and mutant crystals are observed. Further analyses and explanations are underway to correlate our results with other biochemical measurements related to protein folding.

High resolution structures by minimizing radiation effects: Ultra high resolution structures are most sensitive to radiation damage. This new method can be used to remove or minimize radiation effects and thus yields the real high resolution structure from a standard dataset. In collaboration with Dr. Cynthia Fuhrmann, from Dr. David Agard’s laboratory in the Department of Biochemistry & Biophysics, University of California, San Francisco, we applied the strategy on an ultrahigh resolution crystal structures of alpha-lytic protease. Figure C.10.1.4 displays the difference map of the local electron density around one of the disulfide bonds before and after the radiation damage correction. The disulfide atoms positions shifted to the red positions due to radiation damage.

Catalytic pathways: Liberated electrons by X-rays during data collection could alter active sites of an enzyme or be a trigger to initiate a chemical reaction. By using this method one could extract structures at any given time during the data collection, to trace the structural changes due to the reaction. In collaboration with Steve Ealick’s group at Cornell University, we have applied the approach on a manganese-dependent enzyme oxalate decarboxylase crystal and a uridine phosphorylase intermediate structure. Preliminary results are currently being summarized and analyzed.

Time-normalized data reduction and improved map presentation. We have developed software for time-normalized data reduction and are working on improving difference-map presentations when structural changes are relatively large.

Time-normalized data reduction: Program FitScale has been developed for data reduction. Experimental data are integrated and scaled globally using HKL2000. The scaling is done without merging symmetry equivalent observations in order to investigate the intensity variations of a unique reflection as a function of increasing accumulated exposure time. The FitScale program fits all individual intensity changes within one unique reflection sub-dataset. A linear least-squares procedure is used. A slope which we call time-normalized factor is obtained for each unique reflection by the fits. This procedure can produce intensities of all Bragg reflections...
at any given time (t). These intensities are then scaled by SCALA as a function of batches which are ordered according to accumulated exposure time. The final merged data at any given time, t, are then used for phasing the structure.

**Improved presentation of Fourier difference maps:** In connection with our research on time-resolved structural changes, we are working on more precise representations of electron density map changes using a Fourier difference technique. Standard methods in the literature make use of measured structure-factor magnitude differences \(|F_o| - |F_c|\), but assume no changes in the structure-factor phases: \(\Phi_o = \Phi_c\). While this may be valid when electron density changes are small, it may introduce significant errors when the structure around an anomalous scatterer or active site is modified substantially as we have observed in time or dose dependent measurements. A more precise description of the density changes is to use experimentally determined structure factor phases \(\Phi_1\) and \(\Phi_2\) to evaluate the difference map. This requires a different data analysis because now it is necessary to refine the phases at different times, \(t_1\) and \(t_2\). Preliminary results show that our new strategy produces more reasonable electron density maps as a function of time or dose in the case of the insulin experiment. Further studies are in progress to make a general routine for this type of difference map structural analysis.

C.10.1.8. Modulating the Reductive Unfolding Pathway of RNase A

The reductive unfolding pathways of the four disulfide-containing protein, RNase A, proceed through the reduction of its (40-95) and (65-72) disulfide bonds (independent processes) to form des [40-95] and des [65-72] (in parallel reductive unfolding pathways). These des species are, in turn, reduced to form two-disulfide-containing intermediates that are rapidly reduced to form the fully-reduced protein (R). It was found that mutating Y92 of RNase A to Leu, Ala, or Gly results in the rapid reduction of the (40-95) disulfide bond to form a sole intermediate, (mutant) des [40-95].

The structures of RNase A mutants Y92A, Y92L and Y92G were solved at 1.4-1.5 Å resolution and compared with the native structure. Structurally, the mutation of Y92 to any of the aforementioned residues results in a loss of the interactions between the side chains of Y92 and P93. Structural studies of the loops (residues 33-41 and 87-96) containing the (40-95) disulfide bond in the mutants and the WT protein, coupled with molecular dynamics simulations of disulfide-bond exposure in Y92G and WT RNase’s, suggest that the \(\pi\)-non-aromatic interaction is essential for maintaining the rigidity of the loop. The resulting destabilization of the environment around Y92 in the mutants appears to result in a lower free energy cost for exposing the (40-95) disulfide bond [relative to wild-type (WT) and to the (65-72) bond] and hence they are rapidly reduced in the mutants. Furthermore, the kinetics of reduction of the mutants suggests that the solvent exposure of the (40-95) disulfide cysteine is an important factor in their reductive unfolding processes. Our results demonstrate that a single aromatic/non-aromatic interaction can be the cause of heterogeneity in the unfolding pathways of RNase (37).

C.10.1.9. New Structure of Neurophysin Type I

The protein neurophysin plays a central role in the targeting of the hormones oxytocin and vasopressin to neurosecretory vesicles and to the storage of the hormones within these vesicles. The questions about the structure-function relationship, the mechanisms underlying allosteric changes and the thermodynamics of ligand binding cannot be answered without the knowledge of the detailed crystal structure.

Neurophysin I is a small protein of 86 amino acids having 15 sulfur atoms, thus making it the ideal candidate for structure solution using the sulfur phasing. All previous attempts to solve the structure of the protein by either molecular replacement or by heavy atom derivatives failed. This is a challenging project since the neurophysin crystals currently don’t diffract perfectly to collect
significant anomalous sulfur signal and the protein itself seems to possess high structural and
dynamical disorder, so an additional search for the strong protein-ligand complexes may be
necessary.

C.10.1.10. Membrane-Associated (S)-Mandelate Dehydrogenase

(S)-Mandelate Dehydrogenase (MDH) from Pseudomonas Putida is a membrane associated,
flavin mononucleotide (FMN)-dependent α-hydroxy acid oxidizing enzyme. MDH catalyses the
oxidation of (S)-mandelate to give a flavin hydroquinone intermediate which is subsequently
reoxidized by an organic oxidant residing in the membrane.

It belongs to the monotopic class of integral membrane proteins, which are inserted into only
one side of the phospholipids bilayer. Because of their amphipathic nature and peculiar
solubilization properties, membrane proteins are notoriously difficult to crystallize. To make it
amenable for crystallization, the 39 residue internal binding segment of MDH was replaced with a
20-residue segment derived from one of its soluble homologues, glycolate oxidase (GOX).
This resulted in a soluble chimera of MDH, called MDH-GOX2, which retained full catalytic activity
of wild type MDH. The X-ray structure of MDH-GOX2 at 2.15 Å and subsequently at 1.35 Å was
reported previously along with its structure after reduction by (S)-mandelate, also at 1.35 Å
resolution. The overall fold of the molecule is that of a TIM barrel and forms a tight tetramer
within the crystal lattice (38-40).

The structures of the G81A mutant of MDH-GOX2 and of the mutant in complex with the slow
substrates 3-indolelactate and 2-hydroxyoctanoate have been determined at 1.8 Å, 2.2 Å, and 2.5
Å, respectively (3). The G81A mutant has a 60-fold lower activity with (S)-mandelate than MDH-
GOX2, but similar reactivity towards the slow substrates with the artificial electron acceptor
DCPIP (2,6-dichlorophenoliodophenole). However, it exhibits 10-fold greater reactivity toward
oxygen in the oxidative half reaction than MDH-GOX2 with (S)-mandelate as substrate.

In the reduced G81A mutant, the sulfate anion bound to the active site of native MDH-GOX2 in the
oxidized and reduced states was replaced by water molecules. Both substrates, 3-indolelactate
and 2-hydroxyoctanoate, bound to the enzyme by displacing these and additional water
molecules. 2-Hydroxyoctanoate binds to the enzyme in a productive mode for subsequent
reaction; its orientation is such that the α-hydrogen can either be abstracted as a proton by a
histidine residue in one possible catalytic reaction mechanism, or it can be transferred to the N5
of FMN as a hydride anion, or as a hydrogen atom followed by a single electron transfer, in
alternate mechanisms. However, 3-indolelactate is bound to the enzyme in an abortive mode.
An analysis of the differences in the mode of binding of different enzyme-ligand structures in this
enzyme family leads to speculation that the G81A-3-indolelactate complex might be a transient
binding intermediate of the reductive half reaction (40).

C.10.2. Collaborative Research

C.10.2.1. Thomas A. Steitz, Yale University

Structural Studies of the Prokaryotic Primosome. The primosome is a fundamental component
of the prokaryotic replication fork, composed of 2 proteins helicase and primase. The helicase
functions to unwind duplex DNA in the 5’ to 3’ direction with concomitant ATP hydrolysis,
providing a single-stranded template for the primase and polymerase. The primase primes DNA
synthesis by producing short RNA primers, from which the polymerase can elongate the
daughter strands. While previous structures have provided valuable insights into the replicative
helicase and primase, a lack of structural information on their complexes with DNA substrate
leaves several fundamental questions about their mechanism unanswered. The primary
questions remain of how the helicase unwinds duplex DNA, and how the primase initiates
primer synthesis,. In addition, as the helicase and primase only function optimally in complex
with each other, a detailed understanding of the mechanism of primosome function can only
come with structural information on the entire primosome complex: helicase, primase, and forked-DNA substrate.

The aim of the project is to gain insight into these questions through a crystallographic analysis of the primosome and its components. Screening for helicase co-crystals using a variety of pseudo replication fork substrates has given rise to 3 diffraction quality crystal forms: 2 of the apo enzyme (diffracting to 4.0 and 3.0Å, respectively) and a 3rd (diffracting to 2.2Å), which grows in the presence of DNA substrate. Phase information for the 3.0 Å resolution apo crystal form has been obtained, and the structure of the monomeric form of the DnaB helicase has been determined. Work on the primase has resulted in the determination of its helicase-binding domain to 1.7 Å, revealing this previously unknown structure to be composed of a novel all helical-fold. In addition, initial maps (3.2Å) describing full-length primase have recently been generated and a model is being built.

**Mechanism of Transfer RNA 3'-End Maturation.** Transfer RNA nucleotidyltransferases (CCA-adding enzymes) are responsible for the maturation or repair of the functional 3' end of tRNAs by means of the addition of the essential nucleotides CCA. However, it is unclear how tRNA nucleotidyltransferases polymerize CCA onto the 3' terminus of immature tRNAs without using a nucleic acid template.

We have determined the crystal structure of the *Archaeoglobus fulgidus* tRNA nucleotidyltransferase in complex with tRNA, as well as the structures of ternary complexes of this enzyme with both RNA duplex mimics of the tRNA acceptor stem that terminate with the nucleotides C74 or C75, as well as the appropriate incoming nucleoside 5'-triphosphates. A single nucleotide-binding pocket exists whose specificity for both CTP and ATP is determined by the protein side chain of Arg 224 and backbone phosphates of the tRNA, which are non-complementary to and thus exclude UTP and GTP. Discrimination between CTP and ATP at a given addition step and at termination arises from changes in the size and shape of the nucleotide binding site that is progressively altered by the elongating 3' end of the tRNA (41). Data were collected at NE-CAT beamline 8-BM on crystals of the class I CCA-adding enzyme with substrates.

**DNA polymerases Require primers to Initiate Synthesis.** Replication at the ends of linear genomes thus presents a problem. One solution is to use a protein as a primer and attach the 5' terminal nucleotide to a hydroxyl provided by a "terminal" protein. Bacteriophage φ29 is the system in which such protein-priming mechanisms have been best studied. φ29 polymerase is also of interest because it contains an intrinsic strand displacement activity and is highly processive.

We have determined the structure of φ29 polymerase to 2.2 Å resolution. This is the first structure of a protein-primed DNA polymerase. It contains a novel domain involved in the recognition of φ29 terminal protein. Homology modeling of DNA from the structure of RB69 DNA polymerase shows that the downstream template DNA bound by φ29 DNA polymerase passes through a tunnel before entering the polymerase active site. This tunnel is only large enough to allow the passage of a single strand of DNA and thus provides a structural basis for both the intrinsic strand displacement and processivity of the polymerase. The polymerase has an unusual thumb, which also appears to be positioned so that it might encircle upstream duplex DNA, acting as a clamp to further enhance processivity (42). We are also in the final stages of determining the structure of φ29 polymerase bound to terminal protein at 3.1 Å resolution (R_free 32%). The mechanism of the initiation phase is suggested by the structure of this complex which shows a four helix bundle domain containing the primary serine in the product duplex DNA binding site.

**Deacylated tRNA Mimics Bound to the E Site of the Large Ribosomal Sumunit.** During translation, tRNAs cycle through three binding sites on the ribosome: the A, the P and the E site. We have determined the structures of the complexes between the *Haloarcula marismortui* large
riboosomal subunit and two different E site substrates, a deacylated tRNA acceptor stem minihelix and a CCA-acceptor end. Both of these tRNA mimics contain analogues of adenosine 76, the component responsible for a large proportion of E-site binding affinity. They bind in the center of the loop-extension of protein L44e and make specific contacts with both L44e and 23S rRNA including bases that are conserved in all three kingdoms of life. These contacts are consistent with the footprinting, protection and cross-linking data that have identified the E site biochemically. These structures explain the specificity of the E site for deacylated tRNAs, as it is too small to accommodate any relevant aminoacyl-tRNA. The orientation of the minihelix suggests that it may mimic the P/E hybrid state. It appears that the E site on the 50S subunit was formed by only RNA in the last common ancestor of the three kingdoms of life, since the proteins at the E sites of H. marismortui and Deinucoccus radiodurans large subunits are not homologous (43).

Data for the complex of the 50S subunit and the CCA RNA nucleotide were collected to a resolution of 2.9 Å at the NE-CAT beamline at the APS. The other complex of the minihelix and the large ribosomal complex was collected to 3.1 Å at the Structural Biology Center beamline (19-ID) at the APS.

C.10.2.2. Nikola Pavletich, Memorial Sloan-Kettering Cancer Center

The Multiple Endocrine Neoplasia1 (menin) Tumor Suppressor. Multiple Endocrine Neoplasia Type (MEN) is an autosomal dominant malignancy that is characterized by tumors of endocrine tissues. The genetic mechanism responsible for this malignancy has been linked to mutations of the Menin tumor suppressor. Despite the wealth of genetic data that support Menin’s role in MEN1 disease, the biochemical function of Menin is still unknown. However, recent studies have demonstrated that the tumor suppressor function of Menin resides in its ability to repress the transcriptional activity of a number of growth-promoting transcription factors, including JunD, Smad3 and NF-kB.

In order to better understand the biochemical functions of Menin, we have determined the three-dimensional structure of Menin using multi-wavelength anomalous diffraction (MAD) at the APS NE-CAT 8-BM beamline. We are currently addressing a structure-based model of menin function using in vitro biochemistry.

Structural Studies of the Rb-E2F interaction and its regulation by phosphorylation. The transition between the G1 and S stages of the mammalian cell cycle requires the transcriptional activation of a number of genes involved in growth and DNA synthesis. This process, primarily carried out by E2F proteins, is tightly regulated in G0 and early G1 by Rb, which inhibits E2F activity and recruits other repressors to E2F promoters. Phosphorylation of Rb by cyclin-dependent kinases leads to its inactivation and subsequent progression through the cell cycle towards division via E2F activation. Accordingly, genetic alterations, either in Rb itself or in the proteins that regulate it, that render Rb constitutively inactive are present in a number of human cancers. Rb inhibits the transcription of E2F genes in part by binding the E2F transactivation domain at the A/B pocket; however, the C-terminal domain (RbC) is also necessary for full Rb activity in growth suppression, E2F transcription and binding assays.

Previous biochemical studies in our laboratory suggested that RbC interacts with the leucine rich (LR), and marked-box (MB) domains of E2F, as well as its dimerization partner, DP. A primary objective of this project is to characterize the interaction structurally and to determine how phosphorylation of RbC may regulate it. To this end, we have crystallized the ternary complex and have determined the structure using multi-wavelength anomalous diffraction (MAD) at the APS NE-CAT 8-BM beamline.

Structure Determination of the ATR-ATRIP Complex. The ATM- and Rad3- related kinase (ATR) plays a critical role in DNA damage recognition and repair pathways. Deletion of the ATR gene is embryonic lethal, and ATR cell lines generated by siRNA exhibit sever sensitivity to DNA
damage. ATR play an essential role in the recognition and repair of cross-linked DNA, DNA double stranded breaks, as well as transcription-coupled repair. It phosphorylates a number of tumor suppressor proteins such as chk1, chk2 and p53. Recently ATR was reported to bind DNA immediately after DNA damage, and this binding was facilitated by the newly identified protein ATRIP (ATR interacting protein). ATRIP recruits ATR to sites of DNA damage where ATR phosphorylates ATRIP as well as other substrates, including the Rad17, Brca1 and the NMR (Nbs1 Mre11 and Rad50) complex.

We have crystallized a ~100 kDa complex that contains parts of ATR and ATRIP and which is active in DNA-binding assays. We have collected several Se-Met MAD data sets, the best ones giving us ~4.5 Å phases, and native data sets to ~3.3 Å at the 8-BM beamline. While we have been able to build and refine >85% of the structure, several regions remain unassigned, and we plan to use the new NE-CAT undulator beamline to obtain higher resolution native data to complete the refinement.

**Structure of the SWI2/SNF2 chromatin-remodeling domain of eukaryotic Rad54.** SWI2/SNF2 chromatin remodeling proteins mediate the mobilization of nucleosomes and of other DNA-associated proteins. SWI2/SNF2 proteins contain the seven sequence motifs characteristic of SF2 helicases, but they do not have helicase activity. Rather, they appear to use ATP hydrolysis to generate superhelical torsion in DNA.

The structure of zebrafish Rad54, involved in Rad51-mediated homologous recombination, reveals that SWI2/SNF2 enzymes are comprised of two α/β lobes similar to SF2 helicases. The Rad54 helicase lobes contain insertions that form two helical domains (HD1 and HD2), one within each lobe. HD1 and HD2 contain SWI2/SNF2-specific sequence motifs likely to be central to SIW2/SN2 function. Rad54 further contains an N-terminal domain and a zinc-stabilized C-terminal domain that are unique to the Rad54 family. The structure has a broad cleft, formed by the two lobes and flanked by HD1 and HD2, that contains residues conserved in SIW2/SNF2 helicases and motifs implicated in DNA-binding by SF2 helicases. The Rad54 structure suggests that SWI2/SNF2 proteins use a mechanism analogous to helicases to translocate on dsDNA (44). Native and derivative data sets were collected both at the NE-CAT beamline 8-BM and at the IMCA CAT beamline ID17. The structure was solved using MAD data around the gold absorption edge.

**Structural Analysis of Fanconia Anemia Proteins.** Fanconia Anemia (FA) is an autosomal recessive disorder causing a decrease in production of all types of blood cells. Patients with FA are also at a high risk for development of malignancies. There are at least seven FA genes (A, C, D2, E, F, G and BRCA2) that are associated with repair of DNA cross-links. Native and mercury MAD diffraction data were collected at the NE-CAT beamline 8-BM. This data was used to solve the structures of the FA proteins.

**C.10.2.3.** Catherine Drennen, Massachusetts Institute of Technology

**Intramolecular Communication in Enzymes: Protection from Radical Damage in Lysine-5,6-Aminomutase.** Radical enzymes have the challenge of harnessing the reactive power of a radical generating cofactor to form a radical-based intermediate specifically where needed, and without damaging the protein. For most adenosylcobalamin (AdoCbl)-dependent enzymes, substrate binding results in a ~10^{12} fold rate acceleration of Co-C bond homolysis, raising the questions of how substrate binding is sensed, and how an enzyme protects itself from radical damage in the absence of substrate. Lysine-5,6-aminomutase (5,6-LAM), an AdoCbl and pyridoxal-5'-phosphate-dependent (PLP) enzyme, is an excellent model system to address these questions.

Using data collected at the NE-CAT beamline 8-BM, we have determined the structure of 5,6-LAM to 2.8 Å resolution (45). This enzyme catalyzes the reversible 5,6 shift of the amino group in DL-lysine and βL-lysine, and has been implicated in the biosynthesis of antibiotics such as
viomycin, and nourseothricin. The structural analysis reveals that 5,6-LAM uses the same structural motifs as several other AdoCbl-dependent enzymes: a Rossmann-like domain for binding the AdoCbl, and an (α/β)$_9$ TIM domain for housing the active site. However, the orientation of the domains with respect to each other is not the same. A linkage between PLP and Lys$_{444}$ of the Rossmann domain enforces an alternate conformation that positions the AdoCbl cofactor 25 Å away from the active site. The covalent linkage between the Rossmann domain and PLP effectively locks 5,6-LAM into a non-catalytic configuration until substrate binds and releases Lys$_{444}$ via a transaldimination reaction. An untethered Rossmann domain could rotate, moving the AdoCbl into the active site at the top of the barrel. This mechanism would represent a novel means of coupling substrate binding to conformational change in an AdoCbl dependent enzyme. Our future plans include determining the structure of a substrate-bound form of 5,6-LAM and investigating the rate at which this conformational change occurs.

**Crystal Structures of the Nickel-Responsive Transcription Factor NikR.** Nickel uptake in *Escherichia coli* occurs via an ABC transporter that is transcriptionally controlled by the NikR repressor. To understand how nickel activates NikR for transcriptional repression, we have determined the first two structures of NikR: the full-length apo-repressor at 2.3Å resolution and the regulatory nickel-binding domain at 1.4Å resolution with nickel ions bound to the high-affinity site (46).

NikR is the only known metal-responsive member of the ribbon-helix-helix (β-β-α)$_2$ family of transcription factors, and its structure displays an interesting quaternary arrangement: two dimeric (β-β-α)$_2$ DNA binding domains separated by a regulatory domain responsible for nickel binding and tetramerization. The presence of this regulatory domain between the DNA binding domains explains the observation that the NikR operator has a relatively large binding site spacing compared to other (β-β-α)$_2$-responsive operators. The regulatory domain of NikR contains a nickel-binding site at the tetramer interface with a novel square-planar coordination by three histidines and one cysteine. Differences between the apo-NikR and nickel-binding domain structures reported here suggest mechanisms of DNA-binding activation upon nickel binding, and contribute to our understanding of intracellular metalloregulation.

The apo-NikR structure was solved by Se-SAD using a selenium peak dataset collected to 2.5 Å resolution at the NE-CAT beamline (8-BM). This data was collected very early on in the commissioning stage of the beamline. In addition to the scientific importance of the work, it contributed to the early development of the facility.

C.10.2.4. Seth Darst, The Rockefeller University

**Structural Studies of the Basis for Bacterial Transcription Coupled Repair.** Transcription Repair Coupling Factor (TRCF) is a widely conserved bacterial protein that couples DNA repair with transcription. TRCF recognizes RNA polymerase (RNAP) stalled at a non-coding template site of DNA damage, disrupts the transcription complex, and recruits the DNA excision repair machinery to the site. The mechanism of RNA release has been illuminated by the discovery that TRCF causes forward translocation of RNAP, using an ATP-dependent motor that is homologous to that of the Holliday branch migration protein RecG. TRCF is a 130 kD protein with a complex structure/function relationship that is not understood. We have undertaken structural studies to elucidate the structure/function relationship of TRCF, to reveal conformational changes involved in the ATP-hydrolysis cycle and its coupling to the DNA translocase activity, and to reveal the interactions of TRCF with the transcription complex.

In the last year, we crystallized the full-length *Escherichia coli* TRCF, and improved crystal growth, quality, and cryoprotection protocols to obtain 3.2 Å diffraction. Diffraction data from crystals of SeMet-TRCF has been collected at the NE-CAT beamline (8-BM) at the APS. Using this data together with ab initio and difference Fourier techniques, 48 of the possible 60 Se sites in the asymmetric unit were located. SAD phases, combined with density modification yielded a 4 Å resolution map. Since the NE-CAT undulator beamline (24-ID) was not available at the time,
beamtime was obtained on the Structural Biology Consortium (SBC) insertion device beamline (19-ID). On this trip the resolution of the diffraction data was extended to 3.2 Å resolution. SAD phases, combined with density modification and phase extension against the 3.2 Å amplitudes, yielded an excellent map. The refinement is nearly complete and we are pursuing biochemical experiments that have been suggested by the structure in order to confirm our interpretation.

C.10.2.5. Hao Wu, Cornell University

**Structural Studies of the Tumor Necrosis Factor Receptor (TNFR) Signaling Machinery.** Tumor necrosis factor receptor (TNFR) signaling is important in many aspects of mammalian biology such as embryonic development, immune regulation and maintenance of cellular homeostasis. A remarkable dichotomy of the TNFR superfamily is the ability of the receptors to induce two opposing effects of gene transcription. In one direction, the transcription is for cell survival, proliferation and differentiation, while the other direction leads to apoptotic cell death.

Two projects in TNFR signaling have been carried out during the past year. The first project is a structural study on the death-inducing signaling complex. Using data collected on the NE-CAT beamline 8-BM, we determined the crystal structure of one of the components in this signaling complex known as FLIP. FLIP is an NF-κB-inducible gene product that is involved in the TNFR-induced apoptosis. The second project is a structural study of a deubiquitinase known as A20, which is important for down regulating TNF signaling. We collected data of A20 on NE-CAT, but its structure has not been determined.

C.10.3. **Service/User Program**

NE-CAT’s bending magnet beamline 8-BM has provided beamtime to users outside of the central pool of collaborators from the very beginning of its commissioning period. The startup company RibX, a structure based drug design company concentrating on ligand complexes of the large ribosomal subunit, has been a regular user of the beamline since June of 2002. Three other companies have taken advantage of access to the beamline, Shamrock Structures, Exxon and most recently Anadys Pharmaceuticals, another startup company focusing on small RNA molecules. The locally based company, Shamrock, has come through the APS rapid access queue and while Anadys was assigned by the normal General User Program (GUP).

In addition to industrial users, three different structural genomics initiatives have used the beamline. All three have been renewed for the second round of funding from the genomics initiative. Two of the experimenters are from member institutions, Professor Lawrence Shapiro and Professor Liang Tong, both from Columbia University in New York City. Prof. Tong is a member of the North East Structural Genomics (NESG) consortium while Prof. Lawrence Shapiro belongs to the New York Structural Genomics group. The Joint Center for Structural Genomics (JCSG) took data on 8-BM during the time of the SSRL ring upgrade. All three groups have deposited coordinates of structures into the Protein Data Bank based on data from 8-BM.

The largest group of users in the service category came from the member institutions. Eighteen independent investigators have made at least one trip to the beamline. Thirteen publications have resulted from these visits. The representative abstracts that follow are primarily publications from this group of researchers. Abstracts are from the work of Dr. Robert Grant, head of the crystallographic facility at MIT, and his collaborators, Dr. Richard Cerione (Cornell University), Dr. Michael Eck (Harvard University), and Dr. Tom Rapoport (Harvard University).

More recently, Oct. of 2004, we declared 8-BM open to the APS general users. We are obligated to provide 23% of the total operation days of a run to the GUP. In the summer of 2005, six general user groups and the ACA crystallography school, came through the GUP and were scheduled on 8-BM. Outside users can also obtain beamtime directly by submitting a request through the NE-CAT database (www.NECAT.org). One of the abstracts that follow is from...
Professor Barbara Golden, Purdue University, exemplifying an outside user that applied directly to the beamline.

The following are summaries for selected projects from 8-BM users. For complete list see publications in section C.10.4 and C.11.2.

C.10.3.1. Robert Grant, Massachusetts Institute of Technology

The study of 14-3-3 family of proteins which includes seven isotypes in mammalian cells that play numerous diverse roles in intracellular signaling. One mammalian isoform, 14-3-3σ, expressed primarily in epithelial cells, appears to play a unique role in the cellular response to DNA damage and in human oncogenesis. The biological and structural basis for these 14-3-3σ-specific functions is unknown. The group have solved the X-ray crystal structure of 14-3-3σ bound to an optimal phosphopeptide ligand at 2.4 Å resolution. It was shown that endogenous 14-3-3 preferentially forms homodimers in cells, a finding that can be rationalized at the structural level by sequence differences and selective interactions at the dimer interface. The structure reveals the presence of stabilizing ring-ring and salt bridge interactions unique to the 14-3-3σ homodimer structure and potentially destabilizing electrostatic interactions between subunits in 14-3-3σ containing heterodimers, rationalizing preferential homodimerization of 14-3-3σ in vivo. The interaction of the phosphopeptide with 14-3-3 reveals a conserved mechanism for phospho-dependent ligand binding, implying that the phosphopeptide binding cleft is not the critical determinant of the unique biological properties of 14-3-3σ. Instead, the structure suggests a second ligand binding site involved in 14-3-3σ specific ligand discrimination. These findings may assist in understanding the molecular basis for 14-3-3σ-specific function in cell cycle control and cancer (47).

Another study was on versatile modes of peptide recognition by the AAA+ adaptor protein SspB (48). Energy-dependent proteases often rely on adaptor proteins to modulate substrate recognition. The SspB adaptor binds peptide sequences in the stress-response regulator RseA and in ssrA-tagged proteins and delivers these molecules to the AAA+ ClpXP protease for degradation. The structure of SspB bound to an ssrA peptide is known. The crystal structure of a complex between SspB and its recognition peptide in RseA is reported in Nature. Notably, the RseA sequence is positioned in the peptide-binding groove of SspB in a direction opposite to the ssrA peptide, the two peptides share only one common interaction with the adaptor, and the RseA interaction site is substantially larger than the overlapping ssrA site. Competition between two peptides occurs because they bind to overlapping regions of the peptide-binding cleft of SspB. This marked diversity in SspB recognition of different target proteins indicates that it is capable of highly flexible and dynamic substrate delivery. It is possible that SspB may prove to be a versatile and adaptable scaffold for peptide recognition.

C.10.3.2. Michael Eck, Harvard University

Formin proteins are direct nucleators of actin filaments and they participate in a wide range of cytoskeletal processes in all eukaryotes. The defining feature of formins is a highly conserved approximately 400 residue region, the Formin Homology-2 (FH2) domain, which has recently been found to nucleate actin filaments. The "Diaphanous-related" formins (DRFs) are effectors for Rho-family GTPases, and contain an N-terminal GTPase-binding domain (GBD), an FH3 region that may be important for localization, a proline-rich FH1 domain, and a characteristic C-terminal FH2 domain, which alone can nucleate actin filaments. The binding of Rho-GTP is thought to activate DRFs by relieving an autoinhibitory interaction between the GBD and short segment at the C-terminal end of the FH2 domain, the diaphanous autoinhibitory domain (DAD). The crystal structures of the S. cerevisiae Bni1p FH2 domain have been determined using beamline 8-BM, and helped to understand its regulation by Rho. The mostly alpha-helical FH2 domain forms a unique "tethered dimer" in which two elongated actin binding heads are tied together at either end by an unusual lasso and linker structure. Biochemical and crystallographic observations indicate that the dimer is stable but flexible, with flexibility between the two halves of
the dimer conferred by the linker segments. Although each half of the dimer is competent to interact with filament ends, the intact dimer is required for actin nucleation and processive capping. The tethered dimer architecture may allow formins to stair-step on the barbed end of an elongating nascent filament (49).

C.10.3.3. Tom Rapoport, Harvard University

Tom Rapoport published several papers based on the results of data obtained on 8-BM. Two of the more interesting projects are published in *Science* (50) and *Nature* (23).

The mechanisms by which hydrophobic molecules, such as long-chain fatty acids, enter cells are poorly understood. In Gram-negative bacteria, the lipopolysaccharide layer in the outer membrane is an efficient barrier for fatty acids and aromatic hydrocarbons destined for biodegradation. The Rapoport group reported crystal structures of the long-chain fatty acid transporter FadL from *Escherichia coli* at 2.6 and 2.8 Å resolution. FadL forms a 14-stranded beta barrel that is occluded by a central hatch domain. The structures suggest that hydrophobic compounds bind to multiple sites in FadL and use a transport mechanism that involves spontaneous conformational changes in the hatch. The formation of a channel that is shielded from the surrounding lipid may be a general mechanism by which hydrophobic molecules efficiently cross a membrane without being partitioned into the hydrophobic phase.

A conserved heterotrimeric membrane protein complex, the Sec61 or SecY complex, forms a protein-conducting channel, allowing polypeptides to be transferred across or integrated into membranes. Drs. Tom Rapoport and Steve Harrison determined the crystal structure of the complex from *Methanococcus jannaschii* at a resolution of 3.2 Å using data collected at NE-CAT and BioCARS. The structure suggests that one copy of the heterotrimer serves as a functional translocation channel. The α-subunit has two linked halves, transmembrane segments 1-5 and 6-10, clamped together by the γ-subunit. A cytoplasmic funnel leading into the channel is plugged by a short helix. Plug displacement can open the channel into an “hourglass” with a ring of hydrophobic residues at its constriction. This ring may form a seal around the translocating polypeptide, hindering the permeation of other molecules. The structure also suggests mechanisms for signal-sequence recognition and for the lateral exit of transmembrane segments of nascent membrane proteins into lipid, and indicates binding sites for partners that provide the driving force for translocation.

C.10.3.4. Barbara Golden, Purdue University

Barbara Golden reported a crystal structure of a phage Twort group I ribozyme-product complex (24). Group I introns are catalytic RNAs capable of orchestrating two sequential phosphotransesterification reactions that result in self-splicing. To understand how the group I intron active site facilitates catalysis, the structure of an active ribozyme derived from theorf142-I2 intron from phage Twort bound to a four-nucleotide product RNA has been solved at a resolution of 3.6 Å. In addition to the three conserved domains characteristic of all group I introns, the Twort ribozyme has peripheral insertions characteristic of phage introns. These elements form a ring that completely envelopes the active site, where a snug pocket for guanosine is formed by a series of stacked base triples. The structure of the active site reveals three potential binding sites for catalytic metals, and invokes a role for the 2' hydroxyl of the guanosine substrate in organization of the active site for catalysis.

C.10.4. Publications from 8-BM and 24-ID

C.10.4.1. Core Research (NE-CAT Staff)


Recently submitted:


Solved structures - manuscript in preparation:

Ogawa, H., Qiu, Y., Ogata, C. M., and Misono, K.S. Crystallographic evidence for a reversibly bound chloride ion in the atrial natriuretic peptide receptor hormone-binding domain: A conserved structural motif for the chloride binding sites.

C.10.4.2. Collaborative Research


Recently submitted:

Deaconescu, A. M., Darst, S. A. Crystallization and preliminary structure determination of *Escherichia coli* Mfd, the transcription-repair coupling factor.


Lehmann, C. Begley, T. P., and Ealick, S. E. Structure of the *Escherichia coli* ThiS-ThiF complex, a key component of the sulfur transfer system in thiamin biosynthesis.

Zhang, Y., el Kouni, M. H., Ealick, S. E. Crystal structure of *Toxoplasma gondii* adenosine kinase in complex with an ATP analog at 1.1 Å resolution.
Zhang, Y., Secrist, J. A. III, and Ealick, S. E. The crystal structure of human deoxycytidine kinase in complex with clofarabine reveals key interactions for prodrug activation.

Solved structures - manuscript in preparation:

Nikola Pavletich (Sloan-Kettering):

Menin tumor suppressor for Multiple Endocrine Neoplasia (MEN) (1.8 Å)

RAD4-RAD23 complex (acts early in the NER pathway to recognize DNA lesions) and RAD4-RAD23-DNA complex

ATR phosphoinositide 3-kinase-like protein kinase which plays a key role in the cell's response to DNA damage.

Skp1-Fbw7 complex bound to doubly phosphorylated cyclin E peptide. Cyclin E binds and activates Cdk2 and catalyzes the G1-S phase transition.

DDB1-DDB2 complex, involved in damaged DNA-binding and nucleotide excision repair associated with Xeroderma pigmentosum.

C.10.4.3. User Service


Recently submitted:

Banerjee, A., Santos, W., Verdine, G. L. Structural elucidation of the DNA interrogation strategy employed by a DNA glycosylase searching for lesions.

Clardy, J. (Harvard University) - The first structure of an N-acyl synthase for a bacterial communicating molecule.

Losey, H. C., Ruthenburg, A. J., Verdine, G. L. Structure of *Staphylococcus aureus* tRNA adenosine deaminase, TadA, in complex with RNA.


Xue, S., Calvin, K., and Li, H. Structural Mechanism of RNA Recognition and Catalysis by the Splicing Endonuclease. (in preparation).

Recently solved:

Jon Clardy (Harvard University) - The structure of PaaA, the enzyme that catalyzes the first committed step in the synthesis of pantocin A, a potent antibiotic.

C.11. Scientific Accomplishments Progress Since October 2005

C.11.1. Scientific Highlights

C.11.1.1. The Structure of *T. aquaticus* DNA Polymerase III

As a part of NE-CAT’s collaborative research program, Thomas A. Steitz’s group from Yale University, determined the crystal structure of the *Thermus aquaticus* DNA polymerase III α subunit (Figure C.11.1; (51)). The crystals of this DNA polymerase subunit belong to orthorhombic space group C2221 with cell dimensions 175.1, 186.9 and 125.8 Å with one molecule of 140 kDa per asymmetric unit. These crystals diffracted X-rays to 3.0 Å resolution. The results reveal that the structure of the catalytic domain of the eubacterial replicative polymerase is unrelated to that of the eukaryotic replicative polymerase but rather belongs to the Polβ-like nucleotidyltransferase superfamily. A model of the polymerase complexed with both DNA and β-sliding clamp interacting with a reoriented binding domain and internal β binding site was constructed that is consistent with existing biochemical data. Within the crystal, two C-terminal domains are interacting through a surface that is larger than many dimer interfaces. Since replicative polymerases of eubacteria and eukaryotes/archaea are not homologous, the nature of the replicative polymerase in the last common ancestor is unknown. Although other possibilities have been proposed, it is suggested that the plausibility of a ribozyme DNA polymerase should be considered.

![Image of Taq DNA Polymerase III α Subunit](image.png)

Figure C.11.1. Taq DNA Polymerase III α Subunit. (Top middle) A schematic diagram of the domain positions in the PolIIIα sequence. (Center) Two orthogonal views of the surface of PolIIIα colored as in the schematic above. Ribbon diagrams of the individual domains are shown around the outside.
C.11.1.2. The Structure of Glycoprotein B from Herpes Simplex Virus 1

Stephen C. Harrison’s group from Harvard University determined the crystal structure of glycoprotein B from herpes simplex virus 1 (Figure C.11.2; (52)) as a part of the NE-CAT collaborative research program. Glycoprotein B (gB) is the most conserved component of the complex cell-entry machinery of herpes viruses. The crystal structure of the gB ectodomain from herpes simplex virus type 1 reveals a multidomain trimer with unexpected homology to glycoprotein G from vesicular stomatitis virus (VSV G). An α-helical coiled-coil core relates gB to class I viral membrane fusion glycoproteins; two extended β-hairpins with hydrophobic tips, homologous to fusion peptides in VSV G, relate gB to class II fusion proteins. Members of both classes accomplish fusion through a large-scale conformational change, triggered by a signal from a receptor-binding component. The domain connectivity within a gB monomer would permit such a rearrangement, including long-range translocations linked to viral and cellular membranes.

![Figure C.11.2:](image)

**Figure C.11.2:** (A) Domain architecture of gB. Domains observed in the crystal structure are highlighted in different colors, and their corresponding first residue positions are shown. (B) Ribbon diagram of a single gB protomer, in same colors as in (A). Disordered segments are shown as dots of appropriate color. Disulfides are shown in ball-and-stick representation. Cysteines are numbered according to (A) (C) gB trimer. Protomer A is the same as in (B). Protomer B is shown in white and protomer C in gray. (D) Accessible surface area representation of gB trimer. The coloring scheme is the same as in the rest of this figure.

C.11.1.3. Structural Basis for Processivity and Single-Strand Specificity of RNase II

As a part of the user service program of NE-CAT, Arun Malhotra's group from University of Miami Miller School of Medicine visited NE-CAT through APS general user program to carry out X-ray diffraction experiments on Rnase II. They collected data for Rnase II in P1 space group with cell dimensions 55.8, 118.4, 122.4 Å; α=107.8, β=98.4, γ=91.4° and determined the structure at 2.35 Å. (Figure 11.3; (53)). RNase II is a member of the widely distributed RNR family of exoribonucleases, which are highly processive 3’→5’ hydrolytic enzymes that play an important
role in mRNA decay. The crystal structure of *E. coli* RNase II, reveals an architecture reminiscent of the RNA exosome. Three RNA-binding domains come together to form a clamp-like assembly, which can only accommodate single-stranded RNA. This leads into a narrow, basic channel that ends at the putative catalytic center that is completely enclosed within the body of the protein. The putative path for RNA agrees well with biochemical data indicating that a 3’ single strand overhang of 7–10 nt is necessary for binding and hydrolysis by RNase II. The presence of the clamp and the narrow channel provides an explanation for the processivity of RNase II and for why its action is limited to single-stranded RNA.

![Figure C.11.3: The Structure of RNase II](image)

C.11.1.4. Reconstitution, Activities, and Structure of the Eukaryotic RNA Exosome

Christopher Lima’s group from Sloan-Kettering Memorial Cancer Center made use of NE-CAT’s user service program to collect data and determine the structure of the eukaryotic RNA exosome (Figure C.11.4; (54)). It is a 286 kDa complex of nine proteins crystallized in cubic space group with cell dimension 308 Å with one complex in the asymmetric unit. The RNA exosome is a multisubunit 3’→5’ exoribonuclease complex that participates in degradation and processing of cellular RNA. To determine the activities and structure of the eukaryotic exosome, Lima’s group did a reconstitution of 9-subunit exosomes from yeast and human and reconstitution of 10- and 11-subunit exosomes from yeast. Comparative biochemical analysis between purified subunits and reconstituted exosomes using AU-rich, polyadenylated (poly[A]), generic, and structured RNA substrates reveals processive phosphorolytic activities for human Rrp41/Rrp45 and the 9-subunit human exosome, processive hydrolytic activities for yeast Rrp44 and the yeast 10-subunit exosome, distributive hydrolytic activities for Rrp6, and processive and distributive hydrolytic activities for the yeast 11-subunit exosome. To elucidate the architecture of a
eukaryotic exosome, its conserved surfaces, and the structural basis for RNA decay, Lima’s group carried out the X-ray structure determination for the 286 kDa nine-subunit human exosome at 3.35 Å.

**Figure C.11.4.** Surface representation of hExo9 depicting opposing views from top (left), and bottom (right).

### C.11.1.5. Electron Transfer Complex between Aromatic Amine Dehydrogenase and Azurin

This part of the science highlights comes from core research of NE-CAT. NE-CAT staff Narayanasami Sukumar in collaboration with F. Scott Mathews' group of the Washington University School of Medicine, St. Louis, determined the crystal structure of an electron transfer complex of aromatic amine dehydrogenase (AADH) and azurin (Figure C.11.5; (55)). In this complex electrons are transferred from the tryptophan tryptophylquinone (TTQ) cofactor of AADH to the type I copper of the cupredoxin azurin. This structure is compared with the complex of the TTQ-containing methylamine dehydrogenase (MADH) and the cupredoxin amicyanin. Despite significant similarities between the two quinoproteins and the two cupredoxins, each is specific for its respective partner and the ionic strength dependence and magnitude of the binding constant for each complex are quite different. When the MADH-amicyanin complex is aligned with the AADH-azurin complex, the amicyanin lies on top of the azurin but is oriented quite differently. Although the copper atoms differ in position by ~4.7 Å, the amicyanin bound to MADH appears to be rotated ~90° from its aligned position with azurin. Comparison of the structures of the two complexes identifies features of the interface that dictate the specificity of the protein-protein interaction and determine the rate of inter-protein electron transfer.
C.11.2. Publications Since October 2005

C.11.2.1. Core Research (NE-CAT Staff)


C.11.2.2. Collaborative Research


C.11.2.3. User Service


Ma, J. K., Carrell, C. J., Mathews, F. S., and Davidson, V. L. (2006) Site-directed mutagenesis of proline 52 to glycine in amicyanin converts a true electron transfer reaction into one that is conformationally gated, Biochemistry 45, 8284-93.


### C.12. Training and Dissemination

NE-CAT staff members have participated in a number of training activities. The most important training activity is direct interaction with users at the beamline. All users receive specific training in safety procedures, the use of NE-CAT data collection hardware and the use if NE-CAT computational facilities for data analysis and data archiving. The training takes the form of personal instruction by an NE-CAT staff member, instruction manuals and procedures, and web based technical information. During the most recent annual report, NE-CAT trained 21 independent investigators and their group members, totalling 134 senior scientists, postdoctoral fellows and graduate students. In some cases, when a small number of inexperienced students and/or postdoctoral fellows report for data collection, the staff also provides instruction in basic principles of data collection and crystallographic analysis.

In a more formal type of training, Dr. Sukumar served as an instructor for the American Crystallographic Association summer school in Crystallography held at the Illinois Institute of Technology, Chicago, Illinois, July 18-30, 2005. Dr. Wang served as a beamline instructor for the same summer school. NE-CAT beamline 8-BM was made available for the summer school so that students could get hands-on experience with data collection. In July, 2004 Dr. Kourinov served as an instructor for the American Crystallographic Association workshop on "MAD/SAD Data Collection, Processing, Phasing and Structure Solution".

NE-CAT disseminates information in a variety of ways. Most important is the NE-CAT web site http://necat.chem.cornell.edu. The website provides basic information about NE-CAT and the APS, and contains many useful links. The web site contains information about the user program, including safety, shipping information, user accommodations, etc. The web site is also a source for documentation for the NE-CAT facilities. All technical reports are available from the NE-CAT web site including descriptions and drawings for NE-CAT beamline designs. The web site contains information about the NE-CAT staff, including contact information. The site also lists structures resulting from NE-CAT data and provides a list of upcoming meetings and workshops.

NE-CAT staff present a beamline poster one or twice a year, usually including the American Crystallographic Association annual meeting. The title of the poster is fixed as "Northeastern Collaborative Access Team Beamlines at the Advanced Photon Source"; however, it is updated for each meeting to reflect recent facilities developments, technological research and structural results. The poster was presented by Dr. Ogata at the 2005 ACA meeting in Orlando, by Dr. Kourinov at the 2004 Protein Society Meeting in San Diego, by Dr. Ealick at the 2004 ACA meeting in Chicago and 2003 ACA meeting in Cincinnati. The poster has also been presented at the APS Users Meetings.

Staff scientists regularly publish papers in scientific journals and present research papers at scientific meetings. In the period since 8-BM commissioning was first completed, Drs. Ealick, Ogata, Wang, Kourinov, and Sukumar have published 24 papers based on experiments at 8-BM.
or ID-24 with four submitted papers and several others in preparation. NE-CAT staff or group members have presented approximately 25 papers at scientific meetings, usually the annual meeting of the American Crystallographic Association. Abstracts have also been contributed to the 2005 International Union of Crystallography meeting in Florence, the 2005 meeting on Technologies for Structural Analysis of Metallo-Membrane Proteins, and the 2004 Keystone Symposium on Frontiers in Structural Biology.

In November, 2003, NE-CAT organized a workshop on structural biology. The one day workshop featured both NE-CAT and outside speakers and covered a range of topics related to the goals of NE-CAT. The morning session featured talks by Robert E. Thorne, Cornell University (Cryocystallography), Earl W. Cornell, Lawrence Berkeley National Lab (Robotics at the Advanced Light Source), Carey S. Rodgers, General Electric Company (Large Aspect X-ray Detectors Using Amorphous Silicon, Hirotsugu Tsuruta, Stanford Linear Accelerator Center on Molecular Envelopes for Phasing Using Small Angle Scattering), Raimond B. Ravelli, ESRF/EMBL, Grenoble, (Using Radiation Damage for Phasing) and Frank von Delft, The Scripps Research Institute (Structure of HPHMT; a Protein with 160 SeMet Residues/Asymmetric Unit). The afternoon session consisted of technical reports from the NE-CAT staff. Although NE-CAT staff have focused heavily on the construction effort the last two years, we plan to make the workshop an annual event covering a variety of topics related to the mission of NE-CAT.

Finally, Dr. Capel has been heavily involved with technical activities at the APS. He co-organized an APS User's Organization "Special Technical Workshop for Protein Crystallography CATs" in January 2004 and presented a talk entitled "Middleware and Software Standardization". In May 2004, Dr. Capel co-organized a workshop on "The Protein Crystallography Technology and Logistics Collaboration" at the annual APS User's Meeting Workshop and presented a talk entitled "Update from the Protein Crystallography Technical Working Group". Dr. Capel is co-chair of an ongoing APS Technical Working Group (TWG) for unification of crystallography GUI specifications. The TWG has met eight times and is addressing all levels of GUI logistics.

C.13. Training and Dissemination Progress Since October 2005

C.13.1. Training

NE-CAT provides a number of training opportunities for its members, the APS General Users, and the general crystallographic community. In designing the training to be provided to its beamline users, NE-CAT has taken into consideration the broad spectrum of users which NE-CAT supports, ranging from very experienced crystallographers who want to conduct each facet of their experiments needing minimal support from the support staff, inexperienced students and postdocs eager to learn crystallographic techniques at a synchrotron, to biologists and chemists with little crystallographic experience who simply "want to get a structure" and expect a great deal of support. To address this broad spectrum of needs NE-CAT has attempted to provide a multi-tiered "graded" approach to training for its users.

C.13.1.1. NE-CAT User Training Procedures

Training at NE-CAT beamlines can be divided in to three parts. (1) NE-CAT sector orientation training (2) Beamline software and hardware training (3) Hands-on training with user samples. Each of these is described below. This procedure of training has been proven to be effective from our experience at NE-CAT.

C.13.1.1.1. NE-CAT Sector Orientation Training

This is APS required training (valid for two years) and the target of this training is NE-CAT’s safety plan and user’s general safety. Format of this training is face-to-face presentation by NE-CAT staff and an escorted tour of the facility. The following items are covered under this training.
• NE-CAT’s environmental, safety and health plan.
• Introduction of the NE-CAT’s safety coordinator and safety captains
• NE-CAT’s policies pertaining to management of chemicals and control of hazards
• ANL “stop work” and “work alone” policies.
• Location of safety equipment such as fire extinguisher, emergency shower, eye wash station, tornado shelter etc.
• Location of safety documentation, including MSDS
• Location of nearest exits and telephones
• Methods of contacting emergency medical and security personnel
• Means of contacting the floor coordinator
• Responding to alarms and other warnings
• Procedures for using liquid nitrogen and storing samples on NE-CAT site
• Procedures for disposing waste
• Wet laboratory training, if appropriate
• Paging the floor coordinator to post the APS Experimental Safety Approval Form.
• Interlocking the experimental station to enable the X-ray beam
• Escorted tour of NE-CAT facility
• User restricted activities

Time duration for this part of the training is approximately 30-45 minutes.

C.13.1.1.2. Beamline software and hardware training

This training covering beamline-specific software and hardware is required by NE-CAT. Format of this training is real time demonstration at the beamline, often using user’s sample. Some of the items that covered are:

• Description, location and peculiarities of all user- and beamline-computers
• Optimizing and monitoring the X-ray beam
• Handling and Mounting the sample
• Aligning the sample
• Collecting and analyzing the fluorescence spectrum from samples
• Selecting and setting appropriate X-ray energy for the experiment
• Selecting appropriate beam-size
• Selecting appropriate attenuation factor
• Selecting appropriate exposure duration
• Selecting appropriate mode of CCD camera (binned or un-binned)
• Collecting test diffraction images
• Discussing the optimal strategy for data collection
• Collecting complete data sets
• Using NE-CAT data storage system and appropriate backup of user data
• Description of crystallographic software that are available at NE-CAT for analysis of the data
• Connecting user’s data transport hard disk to NE-CAT workstations and creating data backups

Time duration for this part of the training is approximately 60-120 minutes.

C.13.1.1.3. Hands-on Training with Users' Samples

This is a NE-CAT required training and the goal of this training is to make the user comfortable using NE-CAT beamline’s software and hardware system. Format of this training is real time experiment at the beamline with user’s sample.

In this training user will mount their sample and drive the hardware and software. NE-CAT staff will interact with the user to collect optimized data from the sample. Sometimes several samples will be used in sequence to make a judgment on the best strategy to collect optimized diffraction
data. Based on the goals of the user, the staff will make recommendation of specific software and help the user, if needed, to run the software.

Time duration for this part of the training is approximately 60-120 minutes.

First time users go through all three parts of the training. However, users who have used the specific beamline recently do not go usually need to go through elaborate training, but instead will be provided with an update of all sections. This update will include new procedures that have been implemented and upgrades to beamline hardware or software that have been made, if any. In such cases overall training duration will be significantly less.

C.13.1.2. Some Statistics and Further Comments on NE-CAT User Training

In the past calendar year over 30 research groups made one or more visits to NE-CAT beamlines. A total of 195 individual scientists were trained at the beamline (171 at 24-ID-C and 24 at 8-BM). There were a total of 318 individual visits (292 at 24-ID-C and 26 at 8-BM, some scientists visiting multiple times). Some of these individuals were trained multiple times.

A measure of the effectiveness of the training and support provided to beamline users is gauged through informal discussions with the users during their runs as well as through user responses to the “End of Run” summary, which users are required to fill out to provide feedback on how successful they were in obtaining data and solving structures, and to provide recommendations they have for further enhancements to the beamlines and the support provided. NE-CAT beamlines have received positive feedback on the “End of Run” summary forms, reflecting the effectiveness of training.

We recognize that in the real world many of the users coming to the beamlines are junior staff, students, and postdocs who may be reluctant to provide negative information or are just too exhausted after a 24 hour run or in a hurry to catch a flight after the run to fill out a form in detail. A very important element of the entire feedback process is learning what the users convey to their senior investigators when they return to their home institutions. To gather this additional important information, our support staff periodically interact directly with the principal investigators who were not on site during their allocated beamtime, either by direct telephone or email contact, discussions at Executive Committee Meetings, etc.

C.13.2. Dissemination

During the previous year NE-CAT used several effective mechanisms to disseminate information to its users as well as to the general crystallographic community. These are (1) Journal publications (2) Presentations at meetings/conferences (3) NE-CAT website (4) Informal discussions with academic peers at APS floor (5) Organizing workshops on current topics.

The science resulting from use of the NE-CAT beamlines is clearly our most important product to be disseminated. These results are primarily disseminated by users of our beamlines and our staff through journal publications, presentations at professional meetings, APS reports, and through our website. Since Oct 2005, 57 structures have been deposited in the protein data bank and at least 51 papers have been published or in print.

The most general and widely used tool for dissemination of NE-CAT operational information is its website (necat.chem.cornell.edu), which has been receiving ~8000 hits per month. The website contains up to date information on the NE-CAT facility itself, the status and capabilities of the beamlines, how to apply for beamtime, safety information, highlights of the activities underway, information on upcoming events, structures solved, links to other important resources, etc. All the beamline design documentation, beamline operating manuals, a detailed description of the Console operating system, chemical laboratory capabilities, software available, proposal submissions and reviews, a chronological photo gallery etc. are also
available through the website. Few if any, of the APS CAT’s disseminate details as openly as NE-CAT.

All NE-CAT developed designs and software are made freely available to the scientific community. The availability of the designs and software are announced to the community at large through the Technical Working Group Meetings within APS, through presentations at crystallographic meeting and workshops, the NE-CAT website, and through publications.

Another effective mechanism for dissemination of design information and experience gained is through one-on-one interactions among peers. Several times a week the NE-CAT staff members are visited by staff from other CAT members at APS or from other synchrotron light sources, internationally. Some examples of recent dissemination of information from NE-CAT to members of other APS CAT’s are the design and implementation of a fast rotary photon shutter (several other CAT’s have already implemented such shutters based on NE-CAT’s design), procedures for successfully mounting silicon monochromator crystals on liquid-nitrogen cooling blocks, operational experiences with the Oxford Instruments cryo-coolers. Beamline developers from the National Synchrotron Light Source (NSLS), the Advanced Light Source (ALS), the Canadian Light Source (CLS), the Swiss Light Source (SLS), and the Australian Light Source (AS) have all visited NE-CAT during the past year to learn of the optics and control system designs we are currently implementing. Also, after NE-CAT successfully implemented GPFS for high-performance storage, an informal seminar was arranged at NE-CAT for the benefit of high storage demand facilities at APS. This seminar was attended by approximately 15 people from APS and ANL.

NE-CAT scientists participated at several scientific meeting with presentations both about overall beamline technological developments and individual research projects. Moreover, NE-CAT organized several workshops at scientific meetings in order to disseminate emerging new information of interest to the crystallographic community. Recently NE-CAT organized a one day workshop titled “Microdiffraction in Structural Biology” during 2006 APS User Meeting (described below in section C.13.2.1).

NE-CAT staff participated in the 2006 recent ACA meeting and American Chemical Society meeting and presented a poster about NE-CAT itself as well as core research carried out at NE-CAT.

C.13.2.1. Microdiffraction workshop summary

A workshop organized by Steven Ealick and Stephen Harrison was devoted to microdiffraction in macromolecular crystallography. Technology for recording accurate diffraction data from protein crystals 20 µm or less in typical dimension is likely to change dramatically the course of protein crystallographic studies. Qun Shen (APS) began the workshop with an overview of current APS capabilities in microdiffraction, most of them with applications in areas other than macromolecular structure. Ehmke Pohl (Swiss Light Source) followed, with a description of the way the MD-2 diffractometer has been installed and used at SLS. Michael Sawaya (UCLA) then described a specific application -- determining the structure of an amyloidogenic peptide, yeast Sup35, at ESRF beamline ID-13. The very small crystals (50 µm x 2 µm x 2 µm) yielded an excellent, high-resolution structure, with SAD phasing from a Zn ion. David Flot (ESRF) concluded the morning session with a description of the new, dedicated microdiffraction beamline for protein crystallography at at ESRF. It takes advantage of innovations adopted at ID-13. In the afternoon, Florent Cipriani (ESRF), who designed the microdiffraction instruments at ID-13, outlined the design principles and described the differences between the initial instruments and the current MD2x generation of microdiffractometers. K. Rajashankar (APS and Cornell) described plans at NE-CAT for microdiffraction, and Gwyndaf Evans (Diamond, UK), plans for its implementation at Diamond. Gerd Rosenbaum (APS and Univ. of Georgia) concluded with a talk on tradeoffs between damage and signal in the context of plans for a new microdiffraction facility at the APS Structural Biology Center.
C.13.2.2. Twinning Mini-symposium

NE-CAT staff scientists interact with students, postdocs and principal investigators who visit NE-CAT beamlines with their projects. This interaction ranges from routine beamline user training to a close interaction involving deciphering the problems with their data sets to solve the structure. However, given the frequency with which such problems are faced by practicing structural biologist, we realized that the practical knowledge could be disseminated to a broader audience through mini-symposia.

With this goal in mind, a half-day mini-symposium was organized at Cornell University during September 2006. The title of the mini-symposium was “Solving Structures from Difficult Data Sets”. The workshop was run in two 2-hour sessions in an informal setup. Approximately 30 participants, including faculty, post-docs and students, from various groups across campus attended the workshop. A Linux workstation connected to a projector was used as the media of presentation. In the morning session, Dr. Rajashankar walked the audience through solving non-merohedrally twinned thiamin binding protein using SAD data. Ways of identifying non-merohedral twinning, indexing the two twin domains independently, locating selenium sites, phasing, density modification with and without use of non-crystallographic symmetry, etc. were discussed. Informal setup helped very useful discussions during the workshop.

Weak anomalous signal from micro-crystals of bovine uridine phosphorylase was the topic for the afternoon session. The critical step in this case was obtaining the selenium sub-structure. Dr. Rajashankar showed that figure-of-merit may not clearly discriminate sub-structure “solutions” and “non-solutions”. However, often one can decide the right “solution”, based on a comparison of co-ordinates from so-far best trials. A set of repeating peaks, indeed proved to be the correct selenium sites, though they did not stand out in terms of figure-of-merit from rest of the trials. The power of non-crystallographic symmetry averaging technique was also proved to be an essential part for the success of this structure determination.

Dr. Rajashankar was able to index two additional non-merohedral twinned data sets during post workshop interactions with faculty, students and post-docs. Success of this event influenced us to propose a broader program of mini-symposia described later (sections D.4.3. and D.5.2)

C.13.3. Web site statistics

At the recommendation of the proposal review panel we have begun keeping statistics on web site hits. Hits for the NE-CAT main page are shown in Figure 13.3.1.
Figure C.13.3.1: NE-CAT main page hits for the period May 2003 to December 2006.
D. Experimental Design and Methods

D.1. Technological Core Research and Development

D.1.1. Core 1: Microdiffraction

Stephen C. Harrison, Harvard Medical School and HHMI, Core 1 Scientific Director

Primary Collaborative Projects:

Crystallization of Protein Complexes (D.2.1.)
James Berger, University of California, Berkeley and Stephen Quake, Stanford University

Structural Basis of Amyloid Formation (D.2.4)
David Eisenberg, UCLA and HHMI

Molecular Architecture of Complex Protein Assemblies and Small Organelles (D.2.5.)
Stephen C. Harrison, Harvard Medical School and HHMI

Structural Biology of Ion Channels (D.2.7.)
Roderick MacKinnon, Rockefeller University and HHMI

Additional Collaborative Projects:

Structural Biology of the Cell Cycle and the DNA-Damage Response (D.2.8.)
Nikola P. Pavletich, Memorial Sloan Kettering Cancer Center and HHMI

Post-translational Modification by Ubiquitin-like Proteins (D.2.9.)
Brenda A. Schulman, St. Jude Children’s Research Hospital and HHMI

Core 1 NE-CAT Staff (see section E.4.):

Kanagalaghatta Rajashankar (40%), NE-CAT, Core 1 Technical Director

TBA (30%), Operations Lead, Core 1 support
TBA (20%), Beamline Physicist, Core 1 support
Malcolm Capel (10%), Deputy Director, Core 1 support
Anthony Lynch (10%), Technician, Core 1 support
James Withrow (10%), Technician, Core 1 support
TBA (20%), NE-CAT Technician, Core 1 support

D.1.1.1. Background and Rationale

The principal barrier to determining structures of large macromolecular complexes and membrane proteins is expression and preparation of biochemically suitable material and growth of adequate crystals. It is frequently the case that very small crystals appear at some stage in a project but long delays ensue in obtaining larger ones. Moreover, miniaturization of crystal-growth devices (crystallization robots, microfluidics, and the like), which has enabled work on much smaller quantities of sample, and hence vastly accelerated the time between initial expression and crystallization screening, generally limits the size of initial crystals obtained. Thus, apparatus for recording accurate data from very small crystals is an essential part of any biological synchrotron radiation facility aimed at frontier applications.
How small is “very small”? Most routine structure-determination projects currently aim for crystals of at least 50 μm in typical dimension, and most standard beamline geometries provide beam diameters (at the crystal) of 100 μm or larger. But crystals of 10-20 μm in the largest dimension are the rule, rather than the exception, at many stages of work on challenging problems. Moreover, calculations from radiation-damage experiments indicate that complete data sets can probably be obtained even from (frozen) crystals as small as 10-20 μm, unless the unit-cell is unusually large or the crystal unusually sensitive (56). It is thus appropriate to customize a beamline for data collection from crystals of this size. Key issues are shaping the beam to match the size of the crystal, introducing modification to the beamline to reduce background, enhancing the accuracy of the phi axis to limit the sphere of confusion (SOC) to 1-2 μm, and improving the sample and beam visualization components of the beamline so that small crystals can be positioned at the center of a tiny beam (57, 58). Microdiffraction facilities at ESRF (beamline ID13: (59)), the Swiss Light Source (beamline X06SA P1X), and ALS (bending magnet 7.3.3: (60)) have demonstrated what can be done with suitable apparatus. Several important structures have been determined at the ESRF facility (e.g., Pebay-Peroula, et al. (61); Weichenrieder et al., (62); Fotinou et al. (63)).

We have shown at 24-ID-C that our existing optical configuration can produce a beam of about 20 μm in the vertical direction and 60 μm in the horizontal direction (at 3σ), with a total flux of order 10^{13} photons/sec (at 12 KeV) and with a focus either at the crystal or at the detector. The beam divergence in the vertical and horizontal directions is ~4 μrad and ~23 μrad, respectively (the latter depending somewhat on the focus point). Beam stability is also good (10% of beam size at 20 μm), even at these dimensions. Thus, the beam shape produced by our optics is already extremely well suited to microdiffraction. Likewise, at the other end, detector readout is extremely rapid. Addition of a proper microdiffraction capability to a line with these beam and recording characteristics therefore requires only the installation of a suitable microdiffractometer, with the low-background, low-SOC, and improved visualization characteristics mentioned above. We note that our current diffractometer at 24-ID-C has a SOC of about 5 μm (larger than optimal for very small crystals) and that we do not currently have on-axis visualization.

D.1.1.2. Core instrumentation

We propose to implement microdiffraction in two stages. Stage 1: We have purchased from Accel, Inc., the microdiffractometer model MD2, built by Maatel, Inc. (for whom Accel is the distributor). We will install this diffractometer as described below. This will be the first installation of an MD2 in the US. Stage 2: We will design an on-beam-axis visualization system for our other undulator station and outfit it with a suitable, low-SOC goniometer. Decisions concerning the specific instrumentation will depend on our experience with the MD2 and on our experience with its advantages and limitations.

(a) Stage 1.

(i) MD2 microdiffractometer.

The MD2 is based on experiments carried out at ESRF in the late 1990's (59) Figure D.1.1.1 shows the recent installation at the Swiss Light Source.

The built-in beam shaping consists of a beam defining aperture made of platinum (available at various sizes from 5 to 200 μm), followed by a molybdenum capillary with I.D. 340 μm and O.D. 510 μm. Collimated beam from this capillary enters a cleaning aperture with I.D. 100 μm. This beam conditioning system can deliver X-ray beams of variable size in the range 5 – 100 μm. This feature allows the experimenter to match the beam size to the sample. Figure D.1.1.1 shows the beam at various stages of shaping. In addition to the beam shaping and capillary shielding, the air scatter is minimized by keeping the path length of the microbeam as small as possible (5 mm from defining aperture to crystal). There is a tungsten beam stop, either 5 or 10 mm
downstream of the crystal. The capillary and beam stop retract downward for sample mounting and dismounting.

The PHI axis of the MD2 has an SOC of less than 2 µm. There is a built-in, on-beam-axis video microscope, so that the experimenter can visualize the sample precisely as seen by the X-ray beam, without parallax error. A high-resolution objective lens gathers light from the sample, and a mirror, tilted at 45°, directs the image onto a high-resolution CCD camera. Each has a molybdenum-coated hole to allow passage of the X-ray beam. A scintillator can be moved into position to view the beam size and shape; the sample, if mounted, is shifted so that the scintillator is in the plane of the crystal. A cryocooler mounting table is provided, and a motorized sample transfer arc facilitates mounting of pre-frozen samples. Control electronics are mounted in a rack, which communicates with the control computer through a TCP/IP link. The unit comes with operating software, as well as with a CORBA device server to allow control with other software if desired.

(ii) MD2-beamline integration

*Installation.* The MD2 will initially be installed and commissioned on the 24-ID-E beamline (dual-energy, side bounce station). This choice is mandated by the high user demand on the 24-ID-C beamline, as well as for the following technical reasons. At present, the 24-ID-E beamline has higher vertical positional stability than the 24-ID-C beamline. Table D.1.1.1 compares the long term positional stability of the two beamlines, with beam steering feedback in effect. The greater vertical stability of the 24-ID-E beamline directly translates to greater intensity stability of the collimated beam delivered to the MD2 spindle axis.

![Image](image.png)

*Figure D.1.1.1:* The SLS installation of the MD2. (a) General view of the setup. (b) Beam at various stages of shaping. The size of the spot on the right is 30 microns.

<table>
<thead>
<tr>
<th>Beamline</th>
<th>Vertical Stability* (µm RMS)</th>
<th>Horizontal Stability* (µm RMS)</th>
<th>Vertical Demagnification Ratio**</th>
<th>Horizontal Demagnification Ratio**</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-ID-C</td>
<td>± 5</td>
<td>± 3</td>
<td>9.5 : 1</td>
<td>7.5 : 1</td>
</tr>
<tr>
<td>24-ID-E</td>
<td>± 1</td>
<td>± 4</td>
<td>10.8 : 1</td>
<td>5.9 : 1</td>
</tr>
</tbody>
</table>

*Root Mean Square positional fluctuation of beam position. Measured with a quadrant diode beam position monitor, sampled at 1 Hz for period of ~1 hr.

**Beam focus plane situated at goniometer spindle axis.

The average of the vertical and horizontal demagnification ratios of the 24-ID-C and 24-ID-E beamlines are very similar (24-ID-C 8.5:1, 24-ID-E 8.4:1). So flux density delivered at the
entrance aperture of the MD2 beam shaper is similar for installation of the MD2 on either line. The 24-ID-E beamline has a lower horizontal demagnification, however, so the effective horizontal divergence of the beam will be somewhat smaller on at 24-ID-E station and spot resolution correspondingly superior. The difference in the vertical demagnification ratio for the two beamlines is insignificant given the large asymmetry of x and y components of the source divergences ($\Sigma y' = 3.9, \Sigma x' = 23.0 \mu\text{rad}$).

One limiting feature of 24-ID-E is the lack of continuous energy tunability. Two energy set points will be available by crystal switching: 12.66 and 14.78 KeV, corresponding to the Se and Br K edges. Thus, it will be possible to collect SAD data for Se or Br substituted crystals. In practice, the lifetime of very small crystals will generally make SAD a preferable (or necessary) approach, and we do not anticipate that lack of continuous tunability will be a severe limitation. Moreover, as described in the next section, our MD2 support structure will allow facile transfer to the 24-ID-C beamline when appropriate.

**Mechanical Support and Integration.** NE-CAT’s MD2 support structure has been designed to permit rapid, facile installation of the MD2 on either the 24-ID-C or 24-ID-E beamlines, immediately downstream of the existing Huber goniometer stands. Figure D.1.1.2 shows multiple views of the MD2 support table, its construction and planned installation, relative to existing 24-ID-E goniometry.

The MD2 mount consists of a table supporting a large inertial damping mass and a static kinematic upper surface, supported by three jacks for leveling and rough setting of the pitch, yaw and roll of the kinematic surface. The static kinematic surface supports two additional stages, one for alignment of the MD2 along an axis transverse to the X-ray beam. The transverse stage supports a yaw plate that pivots about a point lying directly beneath the center of the MD2’s beam shaper. Finally, the yaw plate supports three very high precision vertical translation stages (z-blocks), one for each leg of the MD2. The z-blocks have nominal positioning reproducibility of approximately 1 µm and will be used to fine tune the elevation, pitch and roll axes of the MD2 beam shaper to the X-ray beam. The MD2 mounts to the Z-blocks via ball-cone-slot kinematic fixtures. Care has been taken in the support design to decouple (orthogonalize) all of the alignment axes.

The footings of the support stand will have hardened steel fiducial balls attached to their undersides. The fiducial balls will engage steel cones inset into the concrete floor of the end station in order to effect a highly reproducible installation of the support.

The MD2 does not contain its own shutter mechanism. NE-CAT’s MD2 microdiffraction installation will use the existing high speed rotary shutter installed as the terminal component of our collimator system. An evacuated tube (roughing vacuum) will complete the flight path between the 24-ID-E collimator assembly and the MD2. The existing Huber goniometer is supported by a transverse stage and can easily be translated out of the way of the flight path. Our Kirkpatrick Baez focusing system can place the beamline’s focal plane anywhere between the existing goniometer and the Q315 detector.

The MD2 has a significant internal air-filled flight path. We will examine the feasibility of internally purging the MD2's casing with He to minimize the impact of gas scatter on data quality.
Software Integration. The MD2 is delivered with a complete set of motion controls and a self-contained control system, based on ESRF’s Tango distributed control system. Maatel also provides a win-32 based graphical user interface program that communicates with the Tango server embedded in the MD2 motion control system and handles display of the in-line crystal visualizer. Our native control system (Console) cannot in its current form communicate via Tango’s transaction layer (CORBA). Programmers at the Swiss Light Source (SLS) have developed an EPICS channel access gateway IOC (EPICS IO controller) hosted under win-32 for controlling the MD2 through their EPICS-based control network. We have made arrangements to port the SLS MD2-EPICS gateway to our system. Console already contains a complete internal channel access implementation, so interfacing the MD2 to our control network should be straightforward.

The channel access interface to the MD2 will permit sequencing of the MD2 via invocation of validated, high level methods built into the MD2’s Tango server software. Little or no low-level programming of the MD2 should be required, apart from issues related to data shutter-spindle rotation synchronization.

We will develop Console scripts that will align the MD2 to the beam, periodically check the beam-spindle alignment and automatically steer the beam to maintain optimal alignment with the beam-shaper input and the MD2’s spindle axis.

Sample-Loading Robotics. NE-CAT is completing the integration of an ALS-derived sample loading robot with our bending magnet beamline. An early priority of the microdiffraction program will be to investigate the compatibility of the ALS sample loader with the MD2 microdiffactometer. All motions of the ALS sample loader are pneumatically driven, so there is a reasonable concern that delicate sample handling mechanisms of the MD2 might be incompatible with the current generation ALS robot. Nonetheless, we recognize importance of minimizing the logistical cost of sample mounting (time wasted in hutch interlock cycles and manual sample mounting). Our new collaboration with T. Earnest with the next generation ALS robot should resolve any compatibility problems with the MD2 (see section D.1.2.4.5.4.

(b) Stage 2.

We will adapt microdiffraction capability to our existing 24-ID-C beamline, probably in year 2 of this grant. Even during stage 1, we will be able to move the MD2 readily from 24-ID-E to the 24-ID-C hutch. The support table will be designed for easy transport and positioning, using fiducial detents on the floor. Our specific plan for Stage 2 will depend on the demand for microdiffraction experiments using the MD2 in the 24-ID-C station, on our experience with that instrument, and on budget available from institutional funds for capital equipment purchase. The biggest expenditures would be for the low SOC goniometer, a better focusing system and an in-line visualizer.

(i) Goniometer. APS has designed and constructed a high precision goniometer that may serve as a cost-effective alternative to the MD2. The APS goniometer consists of a gas-spring-loaded x-y-z stage, mounted to a high precision air-bearing rotation table. The stage system includes three DC-motor driven linear stages and a gas-spring-based heavy preloading structure, which offsets variable gravitational loading to ensure that the stage system keeps high positioning performance independent of goniometer orientation. These stages have a motion range of several mm with sub-micron resolution and repeatability. The goniometer x, y, z stage has a load capacity of 0.4 Kg and costs around $25K. Figure D.1.1.3 shows a rendering of the x-y-z stage, which is supported by an air-bearing-based omega stage.
(ii) Focusing. Bimorph mirrors are probably the best option for getting a better control over focusing. See D.1.2.3.1 for a description of our plans.

(iii) In-line visualizer. We have designed an in-line visualizer that could be installed downstream of the sample, as opposed to upstream in MD2. This design is based on a parallax correction depending upon the beam position visualized on two YAG crystals – one upstream of the sample and another down stream of the sample. A preliminary drawing is shown in Figure D.1.1.4. We will also consider purchasing an on-axis visualizer from Maatel (~$80K), if detailed analysis of the downstream visualizer design suggests that it will not be as effective.

D.1.1.3. Implementation

(a) Microfocus vs. microbeam. We anticipate two optical regimes. When a somewhat divergent beam can be tolerated (modest unit-cell size), it may be advantageous for very tiny samples to focus the beam on the crystal. This is the mode employed at ESRF beamline ID13, and we anticipate its use for applications such as those of Eisenberg and co-workers (see D.2.4). For larger unit cells and weakly diffracting crystals, it is in principle optimal to focus on the detector. The intensity of the Bragg diffraction decreases proportionally with crystal-to-detector distance (D), while the intensity of diffuse scatter from interstitial water in the crystal and from most other unavoidable sources of background falls off as D². The pixel size of current CCD detectors (about 50 µm) will limit the advantages gained in this way, as most “microcrystals” will be smaller than 50 µm in typical dimension and hence the diameter of the diffracted beams will be smaller than 50 µm. We will test various beam configurations (see sections D.2.1, D.2.2, D.2.3, D.2.4, and D.2.5.5), to optimize signal-to-noise for different kinds of problems. In practice, the divergence of the beam at ID-24-C when focused either on the crystal or on the detector is about 4 µrad vertical and 23 µrad horizontal. Except for an utterly perfect crystal, this divergence will contribute less than the divergence from crystal mosaic spread and other imperfections. The inherent beam divergence will in any case lead to little more than doubling of the spot width from a 25 µm crystal at a detector distance of 1 m. These calculations illustrate that we have reached a regime in which decreasing the mosaic spread of a frozen crystal could bring substantial advantages. As the cryopreservation process itself is often the source of much of the mosaic structure, in situ freezing techniques such as those described by
Quake and Berger in their collaborative section (D.2.1) and experiments of the kind described in D.1.1.4.d, below, will be important for discovering optimal data collection procedures.

(b) Non-circular apertures. We will explore the application of non-circular apertures (e.g., slits or ovals), for exposing needle-like crystals over a length greater than their width, without simultaneously illuminating surrounding ice. The MD2 has an aperture holder, which is magnetically attached to its support table. We will design additional aperture holders with, for example, oval-shaped apertures to replace circular ones. The MD2 has aperture scan routines for easy centering of the beam, so that aperture change and centering is easy and quick.

(c) Helium. As delivered, the MD2 does not come with a helium purge along the X-ray path (~130 mm) within it. To reduce the background from scattering by air in the path, we will implement a purge for as much of its length as possible. The design of the MD2 makes this modification straightforward. The entrance aperture of the on-line visualizer will be about 10 mm downstream of the thin, kapton window that seals the evacuated beamline flight path.

D.1.1.4. Applications

(a) Choice of aperture to match crystal. With our existing slit assembly on the 24-ID-C beamline, we have experimented with slitting down the beam to 20 µm x 20 µm. We will simplify user-driven beam slitting by installing pre-set routines. This will make application to relatively small crystals (e.g., 20 µm) straightforward, even with the existing goniometer.

(b) Needle-like crystals. Many macromolecular complexes crystallize as needles, often substantially longer than 100 µm but less than 20 µm in width. This morphology results from crystal packings for which addition of molecules in one direction is much more rapid than in the other two. When illuminated by a suitably small beam (approximately equal in diameter to the crystal width), needles can be translated along their length, to minimize radiation damage. We will implement control software to optimize scans of this kind, with automatic phi-axis translation.

(c) Crystal scanning. Protein crystals are mosaic. Within a small region, the mosaic spread may be less than for the entire crystals, and there will then be advantage in exposing a smaller volume with a microbeam, as demonstrated by Cusack et al. (64). Different small volumes may each have lower mosaic spread, even if they differ in mean orientation from each other, so when one volume begins to suffer from radiation damage, the crystal can be moved to illuminate another.

(d) Freezing crystals without cryoprotectant. Very tiny or very thin crystals, suitable for electron diffraction, can sometimes be frozen without specific cryoprotectant (see Taylor and Glaeser, 1974 (65)). We will experiment with direct freezing in liquid nitrogen, working with crystals in the 1-5 µm range. This will be very interesting in conjunction with Robert Thorne’s “hyperquenching” crystal freezing method (66).

(e) Nanoscale crystallization. We will collaborate with Quake and Berger (see D.2.1) to adapt our microdiffraction configuration to the 10-25 nanoliter scale crystallization methods, with direct mounting, being developed by their groups.

D.1.2. Core 2. Hardware for Challenging Samples

Wayne A. Hendrickson, Columbia University and HHMI, Core 2 Scientific Director

Primary Collaborative Projects:

Protein Biosynthesis (D.2.2.)

Jamie H. Doudna Cate, University of California, Berkeley
Structural Biology of Protein:Protein Interactions (D.2.6.)
Wayne Hendrickson, Columbia University and HHMI

Structural Biology of Apoptosis and NF-κB Signaling (D.2.11.)
Hao Wu, Weill Medical College

Additional Collaborative Projects:

Crystallization of Protein Complexes (D.2.1.)
James Berger, University of California, Berkeley and Stephen Quake, Stanford University

Chemical Biology of Metabolic Enzymes (D.2.3.)
Steven E. Ealick, Cornell University

Molecular Architecture of Complex Protein Assemblies and Small Organelles (D.2.5.)
Stephen C. Harrison, Harvard Medical School and HHMI

Structural Biology of the Cell Cycle and the DNA-Damage Response (D.2.8.)
Nikola P. Pavletich, Memorial Sloan Kettering Cancer Center and HHMI

Post-translational Modification by Ubiquitin-like Proteins (D.2.9.)
Brenda A. Schulman, St. Jude Children’s Research Hospital and HHMI

Recombination, Replication, Transcription and Translation (D.2.10.)
Thomas A. Steitz, Yale University and HHMI

Core 2 NE-CAT Staff (see section E.4.):

Malcolm Capel (40%), Deputy Director, Core 2 Technical Director

TBA (40%), Beamline Physicist, Core 2 support
Narayanasami Sukumar (20%), Staff Scientist, Core 2 support
Jun Wang (10%), Staff Scientist, Core 2 support
Anthony Lynch (20%), Technician, Core 2 support
TBA (20%), Technician, Core 2 support

D.1.2.1. Background and Rationale

X-ray optics and control are at the heart of a synchrotron beamline, and beam characteristics connect intimately to effectiveness in handling challenging crystallographic problems. While the X-ray beams that we now produce at ID-24-C and at ID-24-E have excellent properties, we nevertheless recognize substantial opportunities for improvement. Indeed, their very excellence leads to problems. When very bright beams are focused as is already achievable at ID-24-C (~20 μ vertical by ~60 μ horizontal) there is great sensitivity to any vibration in the system and there are challenges in assuring uniform intensity through the beam profile. The fineness of focus and high flux density in these beams also places demands on the capacity and precision of end-station instrumentation. Our beam diagnostics make us aware of existing limitations and we propose improvements to realize opportunities that we see to meet prospective challenges and also to address current user needs.

Technical challenges in biological crystallography arise both at the level of fundamental properties of the molecules themselves and in terms of practical consequences of the particular crystal parameters. Fundamental challenges arise from radiation damage, which afflicts all samples since X-rays are a form of ionizing radiation, and the limited diffraction order that commonly arises from intrinsic flexibility in our molecules and assemblages. Although beamline instrumentation can do little to address these fundamental properties, these properties certainly do affect the experiment and effective instrumentation can mitigate the
consequences. In particular, the conservation of scattering means that the loss of diffraction whether from intrinsic disorder or from radiation damage results in inelastic scattering contributions to the background. This exacerbates what is typically already a poor signal-to-noise situation. Beamlines can, and to large extent the NE-CAT beamlines already do, address the practical consequences of difficult crystals such as large unit-cell dimensions, very small crystal sizes, asymmetric diffraction, sharp resonances from anomalous scatterers, and the demand for a wide range of X-ray energies to meet anomalous scattering edges. These place diverse and often conflicting demands on the instrumentation, however, and we recognize the need for versatility and for intimate collaboration between experimenters and beamline scientists.

One major thrust in our hardware development effort concerns beam stability. Through the control of vibration sources, through feedback monitoring of beam positions, and through heat-load reduction by exchange to a shorter period undulator, we expect to achieve appreciable improvement in beam stability. This will have multifarious and sometimes unexpected implications for experiments. It is obvious, for example, that if 10 μ beams are to be useful they must be very stable, but it may not occur at first that beam stability is also crucial for achieving small beam stops for the measurement of low-order reflections.

A second major thrust concerns beam focusing. Here we anticipate exciting developments and important practical consequences from new opportunities in X-ray optics. By replacing the existing vertical mirror with a piezo-controlled bimorph mirror, we will be able to remove beam structure, independent of focal position. Moreover, the added control provided from the bimorph design coupled with improved focus diagnostics will also better permit us to defocus readily, when appropriate such as in circumstances of larger crystals. Finally, we are excited by the prospect of implementing low-Z refractive optics elements to reduce beam divergence, which is especially needed in the horizontal dimension. This has immediate practical consequences that will be felt most especially in the sideways-deflected 24-ID-E beamline. The refractive optics correction will permit a full match to monochromator crystals for the optimization of peak Se and Br anomalous scattering signals. It will also be an important element in delivering a highly brilliant, nearly round beam to the MD2 microdiffractometer.

Finally, we propose diverse elements of instrumentation to meet the challenges of specific problems. The resulting versatility is, in fact, the very essence of the NE-CAT concept. Many of the specific proposals described in this section (D.1.2.4) below aim to address generic or specific issues already encountered, such as the general need for background reduction which becomes more acute for long crystal-to-detector settings, or special collimating and beam stop configurations. These suggestions are really only illustrations of a general philosophy, however. We expect our collaborative scientists to drive these developments by working with the beamline scientists to adapt the beamlines for optimal measurements on the most challenging of samples. We expect innovation from both sides, and we have designed beamline adaptability and versatility into the program.

D.1.2.2. X-Ray Beam Stability

Crystallographic data collection using small X-ray beams and even smaller samples demands the maximum possible positional stability of the incoming X-ray beam. Additionally, the sample itself must exhibit high off-axis positional stability. This is a condition difficult to guarantee, given that the sample is typically mounted in a more or less flexible nylon loop, exposed to a cold nitrogen stream and potentially subject to vibrations induced by rapid motions of the goniometer spindle axis drives or mechanical shocks from shutters, etc.

Table D.1.2.1 attempts to summarize various sources of beam and sample positional instability with the potential to effect crystallographic data quality in effect at microdiffracton beamlines. Many of the sources of X-ray source and sample instability, listed below, have been present at conventional (non-microfocus) synchrotron crystallography beamlines but their impact on data.
quality was masked or muted by the relatively large focus spot sizes of their beams. Furthermore, the lower flux density of older beamlines forced longer exposure times which mitigate the effect of high temporal frequency positional instabilities through time averaging imposed by longer sample exposure times.

Averaged over all sectors, APS’s stated long term stability goal is of order 0.2 μrad (vertical), but will not be achievable with currently operating beam diagnostics or position feedback methods.

We have studied the beam stability of both NE-CAT undulator beamlines using quadrant diode beam position monitors and a high-resolution Yttrium Aluminum Garnet (YAG) beam visualizer. These measurements show that the overall positional stability, on short times scale is approximately +1-5 μ with transient displacements (< 0.2 sec) of higher magnitude, in the direction perpendicular to the Bragg rotation axis. Both the 24-ID-C and 24-ID-E beamlines manifest significantly greater stability along the axis parallel to the Bragg rotation axis of the crystal. Figure D.1.2.1 shows a 10 minute x- and y-position trace for the 24-ID-E beamline, sampled at approximately 5Hz. During acquisition of this data all steering feedback controls were defeated to show intrinsic stability of the monochromatic beam.
Table D.1.2.1: Sources of sample and beam positional instability.

<table>
<thead>
<tr>
<th>Source of Instability</th>
<th>Induces Sample Movement</th>
<th>Induces Beam Movement</th>
<th>Solution(s)</th>
</tr>
</thead>
</table>
| Accelerator Beam Instabilities |                         | +                     | 1) Source feedback improvements.  
2) Accelerator beam steering based on beamline X-ray beam position monitor data.                                               |
| Experimental Floor Mechanical Vibrations |                         | +                     | 1) Inertial isolation of mirrors & monochromator  
2) Elimination of sources of vibration (e.g. pumps).  
3) Use of > 1 demagnification beamline optics                                           |
| End-station mechanical vibrations (e.g. shutters) | +                       | +                     | 1) Inertial isolation of mirrors & monochromator  
2) Elimination of sources of vibration (e.g. pumps).  
3) Use of > 1 demagnification beamline optics                                           |
| Optics Cooling flow-induced vibration. |                         | +                     | 1) Stabilized cooling flow  
2) Maximize laminarity of cooling flow.  
3) Minimize vibrational coupling.  
4) Minimize pressure transients in coolant flow loop.                                   |
| Undulator Harmonic Shift – 10 fold increase in power density on monochromator |                         | +                     | Optimize undulator periodicity to eliminate need for harmonic transition.                                                               |
| Hutch Temperature Instabilities | +                       | +                     | 1) Thermostat hutches  
2) Isolate optics from air flow perturbations (e.g. door openings).                                                                   |
| Goniometer Imprecision / goniometer vibration | +                       |                       | 1) Improved goniometer precision  
2) Hutch Temperature stability                                                            |
| Sample Cooling Flow-induced vibration | +                       |                       | 1) Minimize cooling flow velocity and optimize angle of attack of cooling flow.  
2) Use more rigid, shorter sample mounts                                                  |
Figure D.1.2.1: 24-ID-E y- (magenta) and x-positional (blue) stability measured with calibrated quadrant diode beam position monitor, situated approximately 1 m upstream of goniometer spindle. Vertical gradations represent 5 µ displacement.

The 24-ID-C beamline’s vertical demagnification (9.5:1) greatly reduces the impact of some intrinsic (beamline induced) and all extrinsic (accelerator induced) beam position instabilities at the spindle. Nonetheless, the large size of optical leg separating the last effective accelerator steering element and the goniometer spindle (62 m) results in a vertical steering envelope of order ±5-6 µ (including focusing system demagnification) due to extrinsic position instability sources only.

NE-CAT has developed plans to improve sample and beam stability to levels more in line with microdiffraction experimental requirements. These plans will address both sample and intrinsic (beamline-related) and extrinsic (source-related) beam stability issues. These development plans are summarized in the following sections.

D.1.2.2.1. Elimination of Monochromator-Associated Beam Position Instability

As previously mentioned, the current short-term (< 2 sec) positional stability limits of the 24-ID-C and 24-ID-E beamlines are governed by monochromator crystal vibration induced by LN₂ coolant flow. Both monochromators show increased beam instability in the direction perpendicular to their Bragg rotation axes (vertical for 24-ID-C and horizontal for 24-ID-E).

Both the 24-ID-C and 24-ID-E monochromator’s liquid-nitrogen (LN₂) coolant flows induce disturbances in their crystals’ orientation by two mechanisms:

1) Coolant flow turbulence in the internal LN₂ plumbing or phase inhomogeneities within the LN₂ coolant itself cause the plumbing to vibrate. Plumbing vibrations induce small torques on the crystal mounts.
2) Presently installed LN₂ plumbing consist of flexible bellows-like conduits. Hydrostatic pressure changes in the monochromator’s cooling loop (e.g. from cryopump dewar filling) cause length changes in the cooling lines which in turn produce small torques on the crystal mounts.

The first source of monochromator instability can be remedied by redesign of the in-vacuum liquid nitrogen distribution lines. We will replace the flexible conduit implementing the current distribution system with straight wall stainless steel tubing with short welded bellows segments to permit crystal motion at critical points in the LN₂ circulation system. Flow in the revised LN₂ plumbing should display a much greater degree of uniform, laminar flow and thus produce significantly less flow-induced vibration of the monochromator crystals.

The second cause of cooling flow-induced monochromator instability – pressure changes in the cooling loop – will be partially corrected by the conversion to smooth-wall tubing. Only small sections of the tubing will be flexible so the overall longitudinal length change of the coolant lines, due to pressure perturbations will likely be reduced. Further stability gains will be made by eliminating the pressure fluctuations at their source. We have already eliminated the major pressure instability in the LN₂ coolant loop by installation of a purge-valve system in the LN₂ input transfer line to the main LN₂ dewar. The remaining pressure pulses in the coolant loop, arise from a feature of the Oxford-Danfysik cryopump that was intended to stabilize the hydrostatic pressure of the loop. The Oxford-Danfysik LN₂ cryopumps incorporate a packet-heater in the “high-pressure” cooling loop to regulate long term pressure stability of the cooling loop. This heater is activated when pressure falls below a pre-set value and throttles down as pressure approaches the preset. The packet-heater system itself causes small pressure perturbations presumably due to non-optimal PID settings in the process controller that controls the heater. These heater-related pressure bumps cause small changes in the monochromatic beam position that we offset via beam steering with the focusing mirrors. Since mirror steering occurs intermittently we experience temporary fluctuations in sample to beam alignment that noticeably degrade data quality.

We will attempt to optimize the PID settings of the process controller driving the heater pressure regulator. If the heater PID programming cannot be modified to completely eliminate short-term pressure instabilities we will install an external pressure regulator in the cryopump’s external circulation loop. The pressure regulator can be implemented through a fast variable flow restrictor or a gas-phase pressure capacitor.

D.1.2.2.2. Improved White-Beam Position Monitoring.

The steering envelope of the APS global feedback system could made more restrictive if the APS feedback system had access to beam position information provided by sensors closer to the end station than the existing front end BPM’s. NE-CAT, along with a number of other APS crystallography groups (GMCA, SBC, SER) are collaborating with APS Accelerator Operations Division (AOD) staff to develop methods and technologies for improved steering and stability of the X-ray source. Planned efforts along these lines are listed below. These research projects are an adjunct to APS’s long term efforts to continually improve the effectiveness and reliability of the APS global feedback steering system.

1) Primary Aperture thermal beam position monitor. G. Rosenbaum (SBC, SER) pioneered the use of thermal sensors, embedded within the body of the fixed aperture or white slits as white-beam position sensors. Figure D.1.2.2 shows a conceptual scheme of the thermal BPM built into the NE-CAT first aperture assemblies. Miniature thermocouples or resistive temperature probes are inserted in fine bores within the body of the aperture block, that terminate near the restrictive area of the working surface of the aperture. (rectangular aperture cross-section, one for each aperture face). The outputs of the four thermal sensors, after reference corrections for variation in coolant temperature are ratioed by the following scheme:
The ratios $R_x$ and $R_y$ are to first order, linearly related to $x$ and $y$ displacements of the white beam from a position of perfect alignment relative to the mask bore centerline. This device has a position sensitivity of order $10-20 \ \mu\text{m}$ and a relatively long response time (order seconds).

Figure D.1.2.2: Schematic of thermal BPM, embedded in both elements of the 24-ID-C fixed aperture. Left: transverse cross-section through body of mask at point indicated by red arrows. Right: Longitudinal cross-section through mask body, viewed from above the white beam. Larger channel, of square cross-section is the compliance bore, that permits un-restrained movement of mask to center it on the inboard undulator beam. The smaller bore of rectangular cross-section is the working bore for the inboard undulator beam. Thermocouple bores are indicated in transverse cross-section. The outboard undulator white beam aperture block is complementary and situated downstream relative to one shown in the drawing.

2) White-beam Quadrant Diode Beam Position Monitors (QDBPM). G. Rosenbaum (SER-CAT, SBC-CAT) has designed a white-beam compatible form of the quadrant diode beam position monitor and has received funding from the APS to build and test a prototype device. The white-beam quadrant diode BPM operates in ultra-high vacuum, upstream of the beamline’s monochromator. It consists of quadrant diode array that is differentially illuminated by fluorescence from water-cooled copper beam scrapers, situated downstream of the diode array. The scrapers are each emplaced on translator slides for alignment, and calibration. Scrapers are moved to intercept fringes for the white beam and the resulting copper fluorescence differentially illuminates 4 diodes (2 horizontal, 2 vertical). Effective beam position is determined from the diode responses by the same -/+ ratio method mentioned above (using fluorescence instead of temperature). The white-beam QDBPM is expected to have a positional resolution of order $1 \ \mu\text{m}$ in both directions. Figure D.1.2.3 is a diagram of a prototype white beam QDBPM that has only horizontal position sensing capability.
NE-CAT has not yet commissioned our fixed-aperture thermal BPM, but the output of a similar device has been used at SER-CAT to provide steering information to the APS global feedback system, during studies operations. The SER-CAT thermal BPM is located 52 m from source and has bore dimensions smaller than the NE-CAT mask. This system was shown to have an effective position resolution of 20 \( \mu \) in the vertical direction, 40 \( \mu \) horizontal, with a time response of order 10’s of seconds. The geometric constraints associated with use of a tandem undulator forced placement of the NE-CAT aperture much closer to source (32.5 m) than the SER-CAT device. Additionally, the NE-CAT apertures are larger than those of the SER-CAT device. Thus, it is expected that the NE-CAT aperture thermal BPM will have poorer position resolution than the SER-CAT thermal BPM. Nonetheless, when commissioned, the NE-CAT thermal BPM will be useful for detecting large scale errors in beam steering that occasionally accompany mode changes in APS accelerator, and when resuming accelerator operations following long maintenance shutdowns. Recovering from large scale disturbances of white beam position can cause significant operational lapses. Even a coarse objective measure of white beam position will speed recovery from such occurrences.

The prototype white beam QDBPM underwent testing in the fall 2005 APS run at sector 19 (SBC). This technology will be deployed at Sector 24 and a program initiated with APS AOD to incorporate the white-beam QDBPM in the Sector 24 feedback system. A letter of intent, formalizing this collaborative arrangement is included in L.1.

**Figure D.1.2.3:** Schematic of a prototype white-beam quadrant diode beam position monitor, horizontal channel only. **Upper right:** plan view, beam direction right to left. **Lower right:** view along beam propagation direction. **Left:** isometric projection, beam direction: right to left.

**Key:** Gold: diode housing. Orange: copper beam scrapers. Green: scraper cooling block.
D.1.2.2.3. Improved Monochromatic Beam Position Monitoring

At present, 24-ID-C and 24-ID-E’s monochromatic BPMs are installed upstream of each beamline’s attenuator array and data shutters, in order to avoid crosstalk induced by backscatter and fluorescence from attenuators and shutters and to simplify the wavelength-dependent BPM steering calibration. A distance of ~1 meter separates the BPM and the goniometer axis. In this configuration, mirror mediated beam steering insures that the monochromatic beam transits a fixed point in coordinate space of the BPM, and in the absence of electron beam angle shifts, this condition is sufficient to guarantee that it also intersects the aligned spindle axis. If the electron beam suffers an angle perturbation the beam can miss the spindle while still transiting the fixed steering point of the monochromatic BPM, due to its large separation from the spindle axis.

In order to correct for source angle perturbations we will develop a feedback system incorporating a second monochromatic BPM placed as close to the spindle as possible, but upstream of the data shutter. The second BPM will experience radical variation in incident flux due to attenuator selection and thus be extremely difficult to calibrate. Nonetheless, the downstream BPM can still augment beam steering by serving in a “sample-and-hold” mode, during data collection. In this scheme, the upstream (existing) quadrant diode BPM serves as the driver of the position feedback system for maintaining constant beam position through monochromator energy changes, monochromator second crystal pitch tuning, mirror focus changes, etc. The new, downstream BPM will be used to measure a “relative” or arbitrary beam position just prior to the onset of data collection. The feedback system will then be configured to use the downstream BPM as the driver throughout the duration of a data collection sequence. The downstream BPM is upstream of the data shutter and therefore can provide a continuous driver signal for the position feedback during data collection. At the end of data collection, the feedback system will be reconfigured to use the upstream BPM as the driver. The dual feedback system should provide a much greater degree of insensitivity to source angle shifts than we currently enjoy. It is predominantly a software development effort.

We are developing a second method for monitoring beam position using a miniature in-line CCD-based beam imager situated immediately downstream of the goniometer spindle, but outside of the cooling stream. The CCD camera will image a 100 μm thick YAG fluorescence converter that will provide a image of the beam with minimal bloom, since the YAG converter is an optical (non granular) continuum. Output from the CCD will be digitized and geometric properties of beam, including position determined. This device is described in more detail in section D.1.2.3. The YAG imager will be inserted into the attenuated X-ray beam with a very high precision, vertically oriented linear slide. The YAG imager will be used mainly for objective assay of the geometry of the beam focus spot (see section Core 2.2.2), but if suitably fiduciated, will also serve as a highly accurate beam position sensor, with minimal beam-parallax errors. We expect to be able to insert the YAG-imager, measure the beam position and effect beam steering in a matter of a few seconds, so this system could be used to cyclically correct the beam-spindle alignment without significant degradation of data frame rate.

D.1.2.2.4. Short-Period Downstream Undulator Upgrade

The magnetic period of both undulators of the Sector 24 Tandem Offset Undulator (TOU) is 3.3 cm, the standard periodicity for the APS undulator A. The brilliance-vs. gap curve of the first harmonic of the APS standard 3.3 cm period undulator falls precipitously between the K absorption lines of Se and Br, forcing a transition to the third harmonic of the undulator for experiments in this spectral region. The 3.3 cm undulator harmonic transition is also bracketed by the two operating modes of the 24-ID-E monochromator (12.658 and 14.784 KeV). Both 24-ID-C and 24-ID-E monochromators experience large thermal load increases during the transition between 18th-36th harmonic. Consequently, energy set point changes spanning the harmonic transition impose significant dwell period to allow for thermal equilibration of the monochromator and stabilization of monochromatic beam position.
The operational impact of undulator mode switching could be greatly reduced if the useful range of the first undulator harmonic could be extended approximately 1 KeV higher than the current limit. In this section we discuss the benefits of replacement of the existing inboard undulator with a short period device, in order to reduce the necessity of mode switching.

Figure D.1.2.4 shows a plot of total power and on-axis power density for a conventional 72 period undulator A, as a function of undulator gap (67). Total delivered power, under the standard accelerator operating mode, at minimum gap is about 6 KW, with power densities approaching 170 KW/mm2 at the exit collimator (front end). The undulators used in the TOU have 62 periods so corresponding peak performance parameters for the TOU undulators are reduced by about 12%, compared to a conventional undulator A.

![Figure D.1.2.4: Plot of total power and on-axis power density vs. undulator gap for an Undulator A with 3.3 cm magnetic period, 72 periods. Ring current 100 mA, beam energy 7.0 GeV.](image)

Figure D.1.2.5 is a plot of total power and on-axis power density as a function of undulator energy setting, using the first undulator harmonic (undulator periodicity 3.3 cm) (67). Note that the total power emitted by the undulator for a first harmonic energy setting of 13.5 KeV (gap ~ 35 mm) is 50 W and on-axis (peak) power density is 10 KW/mm².

Figure D.1.2.6 shows a comparison of on-axis brilliance vs energy (tuning curves) for the 1st, 3rd and 5th harmonics of 3.3 cm (red traces) and 3.0 cm (blue traces) periodicity undulator A. The 1st harmonic of the 3.3 cm period undulator useful for energy set points between 3 and 13.5 KeV. Beyond 13.5 KeV the 3rd undulator harmonic is used. The undulator gap corresponding to an energy set point of 13.5 KeV using the third undulator harmonic is ~12.4 mm which corresponds to a total power of 3.9 KW and an on-axis power density of 135 KW/mm². Total power and peak power density on the first monochromator crystal jump by more than an order of magnitude at the first to third harmonic transition point.
This logistical issue is remediable now that APS has successfully deployed a modified undulator A incorporating jaws with a magnetic period of 3.0 cm. Figures D.1.2.6 and D.1.2.7 compare the spectral properties of 3.3 and 3.0 period undulators. Use of a 3.0 period undulator extends the usefulness of the range of the first undulator harmonic to 15 KeV, enabling both the 24-ID-C and 24-ID-E monochromators to remain in a low thermal loading regime throughout the most commonly used region of the undulator spectrum. Stability of monochromator first crystal power loading greatly simplifies and improves the reliability of automated monochromator energy transitions.

APS is willing to convert NE-CAT’s 3.3 cm period TOU to a 3.0 cm period device if NE-CAT assumes responsibility for procurement of the 3.0 cm period magnetic jaws. NE-CAT can optionally exchange only one set of jaws to minimize costs. In this case only the inboard-projecting undulator (servicing 24-ID-E and 24-ID-D) would be transitioned to the longer period device. Costs associated with installation and recalibration of the revised TOU would be borne by APS. As of fall 2007 stated cost for the period conversion is approximately $220 K per undulator.

**Figure D.1.2.5:** Total power and on-axis power density vs. energy set point using first undulator harmonic.
Figure D.1.2.6: Comparison of calculated brilliance vs energy for 3.3 (red) and 3.0 (blue) period undulators (62 periods). Brilliance curves for 1st, 2nd and 3rd harmonics are plotted (ordering of harmonics left to right). The two vertical dashed lines show the positions of the inflection points of Se and Br K fluorescence emission spectra.

Figure D.1.2.7: Comparison of calculated peak power density vs. energy for 3.3 (red) and 3.0 (blue) period undulators (62 periods). Power density curves for 1st, 2nd and 3rd harmonics are plotted (ordering of harmonics left to right). The two vertical dashed lines show the positions of the inflection points of Se and Br K fluorescence emission spectra.
D.1.2.3. Improved Beam Focus

The focusing system of crystallography beamlines controls two (typically opposing) elements of an experiment’s optical configuration: 1) the longitudinal position of best focus and 2) focus spot size (and flux density) on sample. Users may prefer to place the beam focus at the detection plane (or at some intermediate position between sample and detector), in order to minimize diffraction spot overlap with large unit cell samples. In this case the sample is exposed to a larger beam (and lowered flux density) compared to the case where the focus is adjusted to coincide with the sample plane. Alternatively, the desired optical configuration is set by signal to noise concerns and users may prefer a small beam, on sample, in order to minimize the illuminated volume of frozen solvent.

Presently both of NE-CAT’s undulator beamlines can achieve very small focus spot sizes and the position of best focus can be adjusted to any position between the sample goniometer and the detector plane. This flexibility is achieved through use of large horizontal demagnification and modest vertical demagnification Kirkpatrick-Baez focusing optics. In this section we discuss plans to improve the versatility, ease of use and performance of our focusing systems to better accommodate the large variety of data collection configurations required by NE-CAT users.

D.1.2.3.1. Improved Vertical Beam Focus Using a Bi-morph Mirror

The focusing systems of both 24-ID-C and 24-ID-E beamlines produce uniform Gaussian focus spots, with parameters very close to design specifications. However, the 24-ID-C beam vertical focus manifests pronounced vertical spatial inhomogeneities (structure) when the focal plane is moved to positions upstream and downstream of the point of observation (see Figure D.1.2.8).

The optical configuration in effect during this study placed the beam focus on the detector, 500 mm downstream from the goniometer spindle. The attenuated beam was imaged at the focal plane with the Q315 detector (upper left panel) and at the goniometer spindle (lower left panel) using a YAG converter (perpendicular to the beam) mounted on a small prism, centered on the goniometer axis, and imaged with the crystal alignment camera. The effective resolution of the YAG imager is 2-5 μm, while the effective resolution of Q315 (accounting for point spread) is approximately 80 μm x 80 μm. The lower left panel shows the vertical structure in the under-focused beam, observed at the goniometer. If the beam were perfectly stable, this intensity structure would have little relevance. However, under the vertical stability regimen currently in effect, the vertical intensity density variation results in non-uniform sampling of the Bragg spots during phi-rotation of crystals of size less than or equal to the vertical beam at the spindle.

The off-focus beam structure observed at the 24-ID-C beamline is typical for conventional reflective optics and is caused by imperfections of the mirror surface figure. Recent developments in adaptive X-ray optics provide a solution to this problem. Bimorph mirrors, developed at the ESRF and marketed by SESO consist of a relatively thin, pre-figured optical substrate, epoxy bonded to a piezo-electrically perturbed (actuated) substructure. This substructure is capable of undergoing local distortions (in response to activation of embedded piezoelectric actuators) resulting in changes in local curvature of the overlying optical substrate. Between 8 and 32 independent piezoelectric actuators (each connected to its own high-voltage op amp) can be embedded in a bimorph mirror to enable highly accurate and reproducible manipulation of the surface curvature of the mirror surface. This technology has successfully been deployed at a number of third generation synchrotron crystallography beamlines, including several at the APS (e.g. GMCA).

We plan to replace the existing conventionally bent vertical focusing mirror with an appropriately configured bimorph mirror to provide a more uniform beam at the spindle axis, when the focus plane is located upstream or downstream of the spindle axis. Funding for this procurement will be derived from NE-CAT’s institutional funds.
D.1.2.3.2 Implementation of Beam “Autofocusing”

24-ID-C and 24-ID-E beamlines use stepper motor-driven 4 and 2 pole bending mechanisms to independently adjust the vertical and horizontal focus of the monochromatic beam. Currently, we rely on fixed calibration look-up tables to bend the mirrors of both beamlines to provide the desired focus properties.

We find that significant irreproducibility exists in the system currently used to set the mirror bend conditions, and that the main cause of this irreproducibility is end station hutch temperature fluctuations. Hutch temperature instabilities indirectly result in un-programmed changes in mirror bend radius due to slippage of the bender-mirror engagements resulting from thermally excited expansions and contractions of the bender mechanisms. As a result, beam focus change operations are usually carried out by NE-CAT staff, not beamline users.

In order to give NE-CAT users the ability to change the focus characteristics of both beamlines at will, we will develop automated beam shape diagnostics and controls that will ensure that user-commanded focus properties are actually in effect, at all times.

We have developed an in-line beam imager consisting of a thin crystalline YAG (Yttrium Aluminum Garnet) fluorescence X-ray converter, imaged by a short focal length video microscope. The spatial resolution of the camera is of order 1 µm x 1 µm per CCD pixel (V x H, neglecting converter point spread). The YAG converter consists of a thin, extremely uniform
optical continuum that provides a minimally-dispersive image of the beam, under appropriate attenuation conditions. Figure D.1.2.9 shows a photograph of a prototype of the YAG imager camera and magnified view of an unmounted YAG crystal.

Two YAG imagers will be installed on both 24-ID-C and 24-ID-E beamlines. One imager will be situated so that the YAG converter is 1 cm downstream from the goniometer spindle, perpendicular to the X-ray beam, riding on a precision vertical slide. The beam stop is carried by a pneumatic transverse slide so that the beam stop can be automatically moved out of collision with the YAG imager before it is inserted in the beam. The second YAG beam imager will be carried on a vertical slide attached to the top of Q315 detector support. The slide will insert the beam visualizer just upstream of the detector face. This imager will be able to observer the focus spot anywhere between the spindle and the maximum detector distance. Output from both cameras will be digitized by video frame grabbers and the position and dispersion of the attenuated beam measured. These parameters will be fed back to Console scripting to iteratively optimize the beam focus for the spindle or the detector plane. As previously discussed, the output from the YAG imager proximal to the goniometer spindle, may also be used as the driving signal for rapid alignment of the spindle axis to the beam.

![Photograph of prototype miniature beam imager](image)

**Figure D.1.2.9:** Photograph of prototype miniature beam imager. In this version the YAG converter is affixed to a mount that threads into the end of the extension tube of the video microscope. Alternatively, the camera can be positioned vertically to image a prism-mounted YAG converter. The CCD camera has 800 x 470 effective pixels and with the lens shown images an area 300 x 400 microns. Inset: unmounted YAG converter (scale cm).

D.1.2.3.3. Reducing Beam Divergence

The effective bandpass of the 24-ID-E monochromator is quite large due to its single crystal, side bounce design. Both the energy bandpass of this system and the size of the focus spot in the horizontal direction could be significantly reduced if the inboard undulator’s white beam were collimated, far upstream of the monochromator. Reduction of the bandpass of the 24-ID-E monochromatic beam will increase the effectiveness of the beamline for SAD data collection at the Se and Br k-edges. A decrease in divergence of the beam, originating at a point close to
source will increase the brilliance at the entrance of the MD2 microdiffraactometer's beam shaper or reduced focus spot size (compared to the uncollimated state) when the 24-ID-E conventional goniometer is in use.

Until recently, synchrotron beam focusing and collimation relied exclusively on reflective (mirrors) or diffractive (bent crystals, zone plates) optical technologies. Recent work at the ESRF (68) and SPRING-8 (69) has introduced refractive elements as a practical alternative to reflective and diffractive optical elements. Refractive X-ray optic elements are comprised of arrays of precisely figured holes with diameters of order 1 mm, in a low Z metal (e.g. Li, Be or Al) or a radiation-resistant carbon-based polymer (e.g. polyimide, pyrocarbon). Compound Refractive Lenses (CRL) fabricated from metal have holes with 2-dimensional curvature and focus the beam only in the plane of hole curvature. Two orthogonally situated CRL’s can be used for 2D focusing. Polymer-based CRL’s have been constructed by forming precisely-spaced air bubbles in a polymerizable substrate. The resulting CRL holes have 3-dimentional curvature and can focus in both planes. Metal and polymeric CRL’s have been successfully used for monochromatic beam focusing. Radiation damage susceptibility of carbon polymers force the use of cooled-metal CRL’s in white beam applications.

CRL’s are relatively simple, inexpensive and have a much smaller footprint (along the longitudinal optical axis) than reflective optics. CRL’s operate at normal incidence to the beam and are less sensitive than reflective optics to slope and roughness errors of the optical surface. However, the focus profile of a CRL is determined by aberration of the lens and small angle scattering from shape errors in the holes or density fluctuations (grain structure, etc) in the material used to fabricate the lens.

Application of CRL’s to synchrotron focusing and collimation are limited by absorption (at energies less than 10 KeV), their relatively small numeric aperture (due to off-axis absorptive effects), and chromatic aberration. In spite of these limitations, CRL’s offer an attractive alternative to conventional beam shaping techniques under certain circumstances.

A CRL’s optical behavior is determined by material from which it is fabricated and the geometric figure of the holes (circular, parabolic elliptical), their size and spacing. Use of parabolically figured holes in the CRL array reduces chromatic aberration. CRL’s can be configured for both high demagnification focusing or beam collimation applications.

In collimation applications the focal point of the CRL is adjusted to coincide with the origin of the source (10’s of meters). Water-cooled Be CRL’s have successfully been used to reduce the vertical or horizontal white beam divergence at the ESRF (ID-28), SPRING-8 (beamline 47XU) and APS (beamline ID-3, (70).

At beam 47XU, SPRING-8, a Be CRL was used the reduce the vertical divergence of the monochromatic beam (14.4 KeV) from 14.0 µrad to 2.8 µrad with only 10% absorptive loss (71). Very similar results were obtatined at ID28, ESRF where a series of Be CRL’s with 1 mm diameter holes, a fixed inter-hole web thickness of 0.1 mm and a varying number of holes was used to collimate the white beam, situated 28.5 m from the undulator source point. The vertical divergence at 14.4 KeV was reduced from 14 µrad native to 1.7 µrad with less than 8 % loss due to absorption (see Figure D.1.2.10).
Figure D.1.2.11 shows photographs of the white beam CRL collimator installed at the Inelastic Scattering Beamline (ID-28) at the ESRF. The entire CRL, its cooling system and a water cooled aperture used to limit off-axis power absorption (due to CRL misalignment) are suspended from a single 8° vacuum flange.

We will investigate the practicality of horizontally collimating the white beam of the inboard Sector 24 undulator (serving 24-ID-E) with a water-cooled Be CRL, very similar to the one shown in Figure 2.4.2. This device will be contained in a large vacuum-T located 28 m downstream from the undulator center and 20.5 m upstream of the 24-ID-E monochromator. The CRL will be configured to minimize the chromatic aberration for a point between somewhere between the K edges of Se and Br (the two energies available from the 24-ID-E monochromator, via crystal selection are 12.66 and 14.78 KeV).

Using the “CRL" tool of XOP 2.11 we have simulated the performance of a Be CRL comprised of 8, 1.0 mm diameter circular holes with a 0.1 mm inter-hole web thickness (see Figure D.1.2.12). CRL ray tracing calculations used the current values for horizontal emittance and beta function for the APS accelerator and used modified undulator A parameters (to match those of the tandem offset undulator). Figure D.1.2.13 compares the x-direction intensity profiles for a monochromatic beam observed at the mid-point of the 24-ID-E horizontal focusing mirror, with (bold) and without (fine) the CRL located 28 m downstream from the undulator center. The simulated CRL reduces the horizontal foot print of the beam at the horizontal focusing mirror by 65% with 16% absorptive loss. With the focal plane set to coincide with the 24-ID-E goniometer, the pre-collimated beam gives a spot profile (3σ V x H) of approximately 20 x 22 µ compared 20 x 65 µ (V x H) for an uncollimated beam. Horizontal divergence is reduced from 23 µrad to 8 µrad.

The relatively large radius of the CRL’s hole in collimation applications means that the device will be relatively easy to align, because if its comparatively large numeric aperture. It happens that the proposed placement for our CRL is identical to that used at ESRF ID28. The vertical and horizontal emittances of the ESRF are approximately twice that of the APS, thus we expect to readily be able to achieve or exceed the performance of the ESRF ID28 device at Sector 24.
The small size of the CRL (in the transverse direction) enables the device to be mounted in the outboard undulator beam very far upstream, and not interfere with the inboard undulator white beam. The horizontal separation between the inboard and outboard white beams at the planned insertion point of the CRL is ~15 mm, while the width of the Be CRL will be approximately 10 mm (resulting in a 10 mm clearance between the CRL and the inboard white beam). Like the ESRF ID28 device, our CRL will be suspended from a compound x-y translation stages for removing (vertical) and alignment the CRL. Pitch and yaw adjustments will be effected with peizo-electric screws (picomotors). Thermal protection for the CRL will be provided by the 24-ID-D power-limiting aperture, located at 27.3 meters downstream of the undulator center. Funds for the CRL will derive from NE-CAT’s institutional funds, not from NCRR funding. Several synchrotron optical components vendors source Be CRL’s including Accel.

If successful, we will consider installation of a horizontally aligned CRL on the 24-ID-C line, to reduce that beamline’s horizontal divergence and focus spot size. Construction of the 24-ID-D beamline would force us to move the CRL into the sector front end, and add a thermal-protective aperture. Again, the small transverse extent of the device makes this a viable proposition.
D.1.2.4. Supporting Special Data Collection Requirements

Along with efforts to improve the stability and beam focusing capabilities of NE-CAT beamline optical systems we plan to develop operational methods and techniques intended to facilitate sample handling and improve quality of diffraction data. Methods directly associated with the microdiffraction program are discussed in section D.1.1 and will not be repeated here.

D.1.2.4.1. Minimization of background scatter

The 24-ID-C beamline displays a low background noise field (measured at the Q315 detector). Typically, noise counts per pixel per second exposure are less than 100 out to the “water ring” (under attenuation levels typical for data collection). Figure D.1.2.13 is a 1 second exposure from a 50 µm lysozyme crystal. The lower panel of the figure is a “cut” through the diffraction image showing the magnitude of the back ground scatter field. The low noise intensity demonstrates the high optical quality of the 24-ID-C focusing system mirror surfaces and the effectiveness of the variable length collimation system.

Another reason for low noise levels in 24-ID-C diffraction patterns is stringent elimination of all sources of gas scatter in the optical train, down to the scatter guard (1 cm upstream of the

Figure D.1.2.12: Simulation of CRL performance for reduction of horizontal divergence of inboard undulator white beam, using the ray-tracing code “CRL” of XOP 2.11. Bold trace: simulated horizontal intensity distribution imaged at center of 24-ID-E horizontal focusing mirror (50.8 m from source) with CRL situated at 28 m from source. Fine trace: same as bold trace without CRL installed. Energy: 12662 eV. Hole radius: 1.0 m, N=8 with 0.1 mm inter-hole web, using current APS source and undulator A parameters.
goniometer axis). The entire length of the variable length collimator operates at micron scale roughing vacuum. There is one window in the system, bounding the upstream ionization chamber gas space, fabricated from 0.0005" thick polyimide. The ionization chamber operates with He gas as the working medium, with no downstream window. Instead, the He flow originating in the ionization chamber serves to purge the entire gas space downstream of the ionization chamber, down to the scatter guard tube (including the rotary shutter). The helium gas flow is sufficient to maintain 100% He partial pressure in the downstream components of the collimator assembly and shutter, but insufficient to perturb the cryostream flow.

D.1.2.4.2. Helium Flight Paths and Smaller Beam Stops

Further improvement of the background scatter for our conventional crystallography installations might be achieved through improved beam stops and development of ad hoc noise limiting facilities such as collapsible helium flight paths installed between the beam stop and the detector.

In some circumstances users desire to acquire diffraction data with very long sample to detector distances at low energy, in order to obtain extreme-low order diffraction data from large unit cells. Under these circumstances air absorption can noticeably degrade data quality of weak reflections. We will design and fabricate a number of gas-tight, large cross-section bellows, of varying lengths, to form helium-purged flight path between the beam stop and the detector. Bellow sections will consist of square-cross section folded-paper constructions, coated with a thick elastomeric film to make them gas tight. Bellow section ends will be held in thin aluminum frames that can be bolted together to form a flight path of the required length, supported by spring suspensions from the detector rails of the LR-Design detector A-frame.

Our standard beam stop is a 1x1 mm tungsten stop supported by a 1 mm thick horizontal arm. The beam stop is mounted from a transverse translation stage which removes the beam stop assembly from the spindle area during sample installation and automatically reinserts before the commencement of data collection. The repositioning error following beam stop reinsertion is less than 20 µm. A triangular cup is Electron Discharge Machined (EDM) into the upstream face of the beam stop and acts to minimize the scatter and fluorescence from the beam stop surface projecting towards the YAP fluorescence detector, used for XAFS measurements. In fact, we fine tune the alignment of the beam stop by simply translating the beam stop to minimize the output of the YAP detector, in the absence of a sample.

Figure D.1.2.13: One second lysozyme diffraction image (upper). Lower: cut through diffraction image at position indicated by dotted line, intentionally avoiding all diffraction spots. The base line background (central beam stop shadow) is approximately 40 counts.
We have experimented with 0.5 x 0.5 mm tungsten beams stop of the same design but current levels of beam stability discourage their routine use, without severely cutting down the beam size with the collimator slit arrays. Use of smaller beam stops allows us to reduce the direct beam air scatter path between the sample and the stop, without severely increasing the number of low order reflections lost to the shadow cone of the beam stop.

We expect that previously discussed plans to improve beam position sensing and steering will eventually result in a stability regime permitting use of small beam stops, placed closer to the sample. Several of our collaborators have urged that we make this repositioning of the beam stop possible.

D.1.2.4.3. Facile Energy Calibration of 24-ID-E Beamline

The 24-ID-E monochromator is a horizontally deflecting single crystal system. As such, calibration of the precise energy set point of the monochromator is complicated by horizontal angle shifts of the source beam and by the distorting effects of the thermal loading of the crystal surface.

By precise measurement of the angular offset between the 111 and 333 reflections from a strain-relieved 111-cut silicon crystal face, aligned on the goniometer axis, one can determine the effective wavelength to a precision of order $10^{-4}$ (method attributed to B. Batterman) using the following relationship:

$$\lambda = 2 \ d_{111} \ [1 + (d_{111}/d_{333} - \cos(\Theta_{333} - \Theta_{111}))^2 \ / \ \sin(\Theta_{333} - \Theta_{111})]^{1/2}$$

- $d_{111}$ = Si 111 D-spacing
- $d_{333}$ = Si 333 D-spacing
- $\Theta_{111}$ = angular position of peak 111 rocking curve
- $\Theta_{333}$ = angular position of peak 333 rocking curve

The existing 24-ID-E goniometer has sufficient angular resolution to support this measurement. We will install a low precision half-arc stage, concentric with the omega rotation stage for orienting a PIN diode used for measurement of the reflection rocking curves. With this infrastructural improvement, the user will simply mount and align the Si crystal and run a Console script to acquire the necessary rocking curves to determine the precise wavelength of the beam.

D.1.2.4.4. Special Collimation Conditions

GMCA CAT is developing a beam shaper that is conceptually very similar to that used in the MD2 microdiffractometer, to reduce the size of the beam and limit background scatter for microdiffraction experiments. The GMCA beam shaper mounts on a small kinematic plate and magnetically couples to the end of their main collimator assembly, replacing the scatter guard. The GMCA beam shaper consists of a 10 $\mu$m circular EDM aperture in a thin platinum plate, bonded to a 20 $\mu$m diameter molybdenum scatter guard tube, ~2 cm long. A small 300 $\mu$m x 300 $\mu$m beam stop is carried by a separate arm. Using this device, near microdiffractometer performance might be achievable with our conventional goniometry. Our existing collimator cannot achieve the same beam shaping performance without generating large slit flares from the down stream slit array.

Obviously, use of this device imposes a severe tradeoff between flux and the overall background scatter level. We will fabricate one or more of these inexpensive devices with various aperture sizes and test them at the 24-ID-C beamline. The main goal of this effort will be to lower background and increase the largest accessible d-spacing at the detector for experiments attempting to obtain phase information from low order diffraction data.
D.1.2.4.5. Special Sample Handling

D.1.2.4.5.1. Crystal Annealing

Users frequently attempt to improve (or salvage) data quality with crystal annealing techniques. Usually, this effort involves manual, essentially uncontrolled blockage of the cryostream with an ad hoc blocker (e.g. paper, foil). We will design and install a small pneumatically-actuated shutter to block the cryostream for a pre-determined duration and speed. This small device will mount from the cryostream head itself, underneath the existing transilluminator pneumatic actuator. The pneumatic actuator will be controlled by a small programmable logic device that enables the user to specify a precise, reproducible duration of stream blockage and eliminate accidental disturbance of the sample by the blocking device.

D.1.2.4.5.2. Kappa Goniostats

During initial beamline commissioning at Sector 24 we have intentionally avoided the issue of providing and supporting kappa goniometry. Use of kappa goniometers involve a number of complications avoided by simple phi goniostats: 1) maintenance of alignment and eucentricity of the kappa goniostat’s various axes and 2) collisions with surrounding beamline components (e.g. collimator and detector). In addition, only a minority proportion of users insist upon access to kappa goniometry.

The potential benefits of kappa goniostats are obvious and unassailable. NE-CAT presently owns two kappa goniometers: 1) a Huber 515 W, currently installed at the sector 8 bending magnet and 2) a LR-Design mini kappa system based upon a mechanical design by G. Rosenbaum and similar to the instrument installed at the Structural Biology Center beamline. The Huber 515 has the benefit of stability that accrues from its relatively large size and heavy construction. It is fitted with the same sample x,y,z sample alignment stage present on the 24-ID-C and 24-ID-E phi goniometers. However, the 515 has large, and quite slow stepper motor-driven omega stage and has until now been used exclusively as a phi goniometer. The LR-Design mini kappa lacks the x,y,z sample-spindle alignment axis and has proven to be very difficult to maintain in a fully functional state under the day to day battering users subject it to (personal communication J. Chrzas, SER-CAT).

Given the present state of maturity and stability of the 24-ID-C end station and its controls, NE-CAT support of kappa goniostats can now be considered. We will revise the 24-ID-C goniometer support stand to interchangeably mount the Huber 515M phi goniometer, the Huber 515M kappa goniometer or the LR-Design mini kappa. This effort involves construction of offset supports for the two kappa goniometers to adjust for differences in the diameters of the two omega stages and their height offsets relative to the existing Huber 515M phi goniometer.

A moderately large software effort will be required to integrate the two new goniometers into the 24-ID-C control network and the data collection system. The 24-ID-C beamline’s extensible collimator permits retraction of the downstream end of the collimator upstream, for collision avoidance. Users will be permitted to specify the goniometer of choice but reconfiguration of the goniometer will be effected by beamline staff, so advance notice of goniometer preference will be required.

D.1.2.4.5.3. Robotic Sample Loaders

As a matter of long term logistical planning NE-CAT postponed installation of robotic sample loaders on the 24-ID-C beamline until all other critical beamline functions were mature and vetted. Robotic sample loaders will greatly increase the rate and facility with which NE-CAT users can survey large numbers of samples, mainly by eliminating time lost through lengthy safety interlock cycles, associated with hutch entries.
In the initial phase of the project NE-CAT purchased one robotic sample loader from the group at Lawrence Berkeley National Laboratory (LBNL) that developed and implemented the first synchrotron-based crystal automounter at the Advanced Light Source (29). The LBNL robotic loader is presently installed and operating at NE-CAT’s sector 8 bending magnet beamline. During the last calendar year the robot and its software have undergone extensive testing. In its current state of development, the robot has an overall successful loading rate of between 96 and 98%.

The automounter will be integrated into the 24-ID-C control network and made available as an optional adjunct to the 24-ID-C beamline, operating in conjunction initially with the Huber 515 M phi goniometer and eventually with the Huber 515 W kappa goniometer. Plans for automation of sample loading with the MD2 at 24-ID-E are currently undecided due to uncertainties in the mechanical compatibility between the MD2 and the LBNL automounter. Maatel / Accel are planning to market a robotic sample loader (the SC3) for use with the MD2. In the long run, NE-CAT plans to support robotic sample loaders on all of its beamlines. In this regard we are collaborating with the group at LBNL that developed our current system to co-develop and implement the next-generation system under development at LBNL (72) through an NIH/NIGMS R01 with site-specific optimizations, high-level integration into the beamline control system, and capabilities of facile interfacing with downstream data analysis and structure determination programs. A sensor to determine whether crystals have been successfully mounted or unmounted will be developed and installed on the robot to inform the control system of the success/failure state following each load attempt. Currently a proximity sensor is used on the prototype automounter as described in the collaborative section.

Following decommissioning of the sector 8 beamline, in early 2007, we will install footings for mounting the robot support stand outboard of the 24-ID-C goniometer. The robot and its support stand will be modified to reflect differences between the 24-ID-C and sector 8-BM beamline layouts. The presence of the robot installation will not seriously impede manual access to the 24-ID-C goniometer spindle.

The ability to rapidly and reliably screen numerous crystals to determine which ones have optimal quality in terms of resolution, mosaicity, and anomalous signal, for example, is particularly critical for crystals of membrane proteins and large biomolecular assemblies since they frequently are small, mosaic, and suffer radiation damage rapidly, and in general demonstrate more technical challenges. Intelligently-controlled automation for sample mounting and alignment, well-integrated into the beamline control system at the high-brightness beamlines of NE-CAT will provide a powerful tool for scientists to collect data with higher throughput and accuracy, and thus more successfully proceed in using structure to answer important biological questions.

D.1.2.4.5.4. Collaboration with LBNL to Develop a Second Generation Automatic Sample Loading Robot.

A collaboration between NE-CAT and LBNL will develop, implement, and improve the automation of crystal mounting under cryogenic conditions at the biological crystallography beamlines in APS Sector 24. Three next-generation automounters will be developed based on the system described in Cork, et al., (72) that will be optimized for the beamlines at NE-CAT and the demands of their scientific user base, particularly in regard to increasing the accuracy and throughput of diffraction data from challenging projects such as biomolecular complexes and integral membrane proteins. In addition, this robot will be compatible with the Mattel MD2 microdiffraction station.

Automation of the structure determination process, from target selection to structure interpretation, significantly benefits the biology community by providing tools for improved and more rapid structure determination (73), thus increasing the overall speed and accuracy of data collection, and by providing the capability for the rapid screening of crystals to select those with
the best merit for data collection. There are several benefits to using an automounter system for biological crystallography, especially on high-brightness beamlines: i) the use of synchrotron beamtime is more efficiently used; ii) it facilitates advanced data collection techniques; iii) higher-quality data can be collected from pre-screened crystals; iv) the risk to crystals during cryo-mounting and unmounting is significantly reduced; v) systematic studies of alternate and optimal data collection strategies are more easily undertaken.

Over the past six years, an automated crystal mounting and alignment system (automounter) has been developed at Lawrence Berkeley National Laboratory and installed on three of the protein crystallography experimental stations at the Advanced Light Source (ALS) synchrotron (29). The Berkeley automounter, in its first-generation design, has subsequently been implemented at a number of other facilities - NSLS, CHESS, and three CATs at the APS, including NE-CAT. Systems of similar purposes for rotating anode sources have been developed at Abbott Laboratories (74), as well as for experimental stations at the Stanford Synchrotron Radiation Laboratory (75).

Based on the experience gained in developing and using the first-generation system, and in consideration of further developments in technology, a second-generation system was developed (72) that retained many of the components and strong points of the first-generation system while making improvements in flexibility, reliability, and serviceability. The first-generation automounter features a transport stage based on a set of very compact pneumatic stages. This has the advantage of being very low cost, has excellent speed performance, and is relatively safe to operate (low force actuators). However, the pneumatic stages are somewhat difficult to align relative to the sample positioner and the storage Dewar. The design also requires a motorized Rθ stage under the storage Dewar in order to position the sample beneath the gripper. In the development of the next-generation system, the pneumatic and the Rθ stages are replaced with a motorized XYZ Cartesian stage consisting of three SmartModules (Adept Technology) as shown in Figure D.1.2.14. The stage is equipped with absolute encoders for error-free initialization. Realignment is accomplished by adjustment of software parameters, not mechanical shims and clamps. New capabilities will be added to the NE-CAT system beyond the current design (e.g. new docking procedures and a special storage crib for alignment tools).

The first-generation storage Dewar design incorporates a cylindrical vacuum-insulated vessel with an internal platform for supporting the sample cassettes. The subassembly is mounted on an Rθ stage that positions the sample directly beneath the fixed transport stage pickup location. The samples are kept a few centimeters below the LN₂ surface and the liquid level is controlled within 4-5 mm. The storage Dewar subsystem has been the most problematic component of the first-generation design. Condensation from the Dewar tends to accumulate on the Rθ stage and leads to maintenance problems with the stage and motors. The LN₂ level controls are also difficult to make reliable with a moving Dewar design. Finally, system alignment is much more complicated with the somewhat sloppy tolerances of the vessel design. For this project the Rθ stage will be replaced with the overhead-mounted motorized Cartesian XYZ stage (see Figure D.1.2.14a,b). This will remove all moving parts from the area susceptible to condensation buildup. The cylindrical Dewar will be replaced with a rectangular design with a high-degree of flexibility in its footprint for better space utilization and sample handling capacity. A stationary Dewar design will also permit us to use a secondary LN₂ supply Dewar for better level control and buffer capacity (Figure D.1.2.14c). A gravity-leveling design will be used where the storage and supply Dewars are coupled directly by a flexible transfer line and the supply Dewar is equipped with a reliable float valve level control.
An improved gripper design (see Figure D.1.2.15) was made consisting of modifications of the previous design to correct some of the more significant issues while maintaining interchangeability. Our goal for this was to make improvements in alignment, accuracy, and ease of assembly, including redesigning the support tubes to improve accuracy, servicing, and reliability, a three-jaw collet to improve concentricity and gripping made from aluminum bronze rather than brass giving a stiffer spring material. This allows for a more precise and consistent grip contact surface. Teeth were also added to enable gripping under very icy conditions. Slots were added to the outer tubing to improve warm-up and cool-down time. This version has been extensively tested using LBNL pucks and unipucks, and shows near-perfect reliability when coupled with a well-designed Dewar baseplate, occasional warmup and drying cycles, and optimized mounting protocols. A number of these grippers are under production for ALS, APS, NSLS, and CHESS that will go into use by March 2007.

**Figure D.1.2.14:** Prototype and CAD for second-generation automounter. a) prototype robot in development lab at LBNL, b) CAD of full system with goniometer, c) CAD cut-away showing Dewar, puck baseplate independently supported from three stands with fiducial balls. Berkeley and Universal Pucks can be accommodated as well as the Rigaku magazine.
The NE-CAT and LBNL collaboration will develop and install automated sample handler systems with the following features: 1) they will be optimized for NE-CAT research goals; 2) they will have significantly improved performance, reliability, availability, and serviceability compared with current systems; and 3) they will have extensive flexibility to permit easy incorporation of new requirements and instrumentation into a complex biological crystallography end station environment. The new design will be based on the second-generation Berkeley automounter (72) which already has the following enhanced features: 1) a motorized XYZ stage with above-Dewar mounting. This protects the stages from exposure to LN$_2$ and moisture, and permits alignment and calibration to be done purely in software; 2) absolute encoders for error-free initialization; 3) a stationary Dewar for improved LN$_2$ handling and top-up control and with a rectangular shape for optimized space utilization. The Dewar also features a low-cost foam-insulated construction and uses a gravity-fill compartment for reduced turbulence in the sample compartment (Figure D.1.2.14C). Interchangeable mounting plates can be made to accommodate LBNL, Rigaku, or UNIPUCK style cassettes. This plate is mounted off the support table, not the Dewar, for stability and improved alignment. The prototype system at LBNL has been extensively tested and demonstrates extremely high reliability.

At NE-CAT the Adept (http://www.adept.com/products/details.asp?pid=65) stages can be elevated and of reduced size for better user access. This also increases system safety as all large moving parts are now above the operator's head. The current pneumatic FLIP stage can be replaced with motorized stage for improved reliability and reproducibility (e.g. http://www.intelligentactuator.com/pdf/manuals/RCP2_Actuator_Rotary.pdf).

For fully automated calibration and alignment of the robot and goniometer, smart cameras (e.g. http://www.cognex.com/products/DVT/default.asp) will be used. The fiducial balls, as seen in Figure D.1.2.14c, are mounted outside of liquid nitrogen on the support stand that holds the puck mounting plate inside the Dewar, and are used to provide a reference. The known geometry between these landmarks and the positions of the pucks and the crystals can be precisely calculated. These cameras can also be used to detect cassette occupancy and missing pins. Proximity detectors are currently used on the prototype system to detect whether the crystal and its pinbase have been picked up or removed. If the state is not what is expected based on the action taken, then retrying, for example, the pickup of a crystal can be done and heat/dry cycles can be performed.

The LBNL group will provide support in the hardware and software integration of the automounter and work with NE-CAT scientists and engineers to write the Distributed Hardware Server (DHS) for BLU-ICE data acquisition system. Software development for interfacing to the robot controller and for optimizing the gripper transport protocols will be collaboratively performed between NE-CAT and LBNL. A standalone JAVA server for diagnostic testing and incorporation into other control systems will be written with the assistance of LBNL collaborators. Improved sample tracking is also planned including the use 1D or 2D barcodes on cassettes and 2D barcode on base of pins with a local sample database containing information on the sample and its location.
The high-brightness NE-CAT beamlines equipped with crystal mounting hardware integrated into the beamline control system can be coupled to downstream data analysis and structure determination programs, as well as automated data archiving and laboratory information management databases and software to maintain and track the information from the experiments. This can be used for the screening and selection of the highest-quality crystals for further data collection, as well as for optimal multi-crystal data collection.

D.1.2.4.5.5. Improving Sample Stability

Improved sample positional stability during data collection is an essential complement to attempts to maximize X-ray beam position stability. Sample stability can be decomposed into two components: 1) aerodynamic stability of the flexible loop containing the sample (embedded in a cryo-coolant gas flow); 2) mechanical stability of the magnetic coupling between the goniometer head and the sample mount. Another concern (albeit not to the stability issue) is that of sample format standardization. Mount format, stem length, etc are critical to the successful implementation of the robotic sample loader.

Aerodynamic interaction between sample loops and the cryostream can result in uneven excitation of Bragg reflections as they cross the Ewald sphere and as a consequence increased errors in their integrated intensities. The errors manifest as high R-factors in single crystal diffraction data. Figure D.1.2.16 demonstrates the effect of aerodynamically-driven loop vibration on the rotx parameter of crystal orientation matrix (experiments carried out at sector 31ID in APS using a lysozyme crystal, Rajashankar, unpublished). Figure D.1.2.16A shows the value of rotx as a function of image number (1 degree rotation per image). For an unstable, vibrating sample preparation, the rotx value fluctuates strongly across the data set. However, under identical experimental conditions (cryostream velocity and temperature), the same crystal used in the first data set acquisition, removed from the nylon loop and remounted in a home made polyimide loop, the rotx values remain far more constant (Figure D.1.2.16B). A similar trend was noticed for the crysy and crysz parameters, though with a smaller magnitude difference, consistent with the orientation of the cryostream flow relative to the spindle axes. The overall merging R-factor for the data in Figure D.1.2.16A was 24% as compared to 4% for the data in Figure D.1.2.16B.

Figure D.1.2.16: Rotation matrix crysx parameter for a lysozyme crystal mounted in A) a 500 µ nylon loop and B) a 500 µ home made kapton loop.
NE-CAT, SER-CAT and SBC-CAT have compared the aerodynamics of new mylar and polyimide-based mounting technologies with conventional twisted-loop nylon mounts. Both of these new mounting systems are commercially available and are photolithographically or laser cut from polymer sheets: “Litholoop™” mounts from Molecular Dimensions Limited (Cambridgeshire, U.K.) are photolithographically cut from mylar sheets. “Micromounts™” from MiTeGen (Ithaca, NY) are photolithographically cut from polyimide sheets. Figure D.1.2.17 shows renderings of both crystal mounting systems. Litholoops and MicroMounts are sold in variety of loop sizes, and both incorporate slots and cuts to promote wicking of excess mother liquor away from the sample crystal, prior to flash freezing.

In our experience both Litholoops and Micromounts demonstrate superior aerodynamical stability in the cryostream compared to conventional mounts (assayed by data quality, positional chi trends and visual inspection when subjected to intentionally excessive cryo-stream flow rates). Micromounts seem be more fragile than cryoloops (higher rates of breakage). Both mounting technologies produce acceptably low levels of background scatter.

All APS crystallography CATs recommend that their users convert to one of the polymer sheet-based mounting systems. NE-CAT will work with other APS CATs in promote further standardization in mounting technologies (e.g. loop length, magnetic base, etc).

**Figure D.1.2.17:** Renderings of “engineered” crystal mounts. Left: Litholoop, a planar mount photolithographically cut from mylar sheets. Right: MicroMounts are laser cut from polyimide sheets and sandwiched between two curved surfaces to induce curvature of the polymide “loop”. Stability of the loop is increased in the direction orthogonal to the mount curvature.

**D.1.3. Core 3. Computing for Challenging Samples**

Steven E. Ealick, Cornell University, Core 3 Scientific Director

Primary Collaborative Projects:

Chemical Biology of Metabolic Enzymes (D.2.3.)
Steven E. Ealick, Cornell University

Structural Biology of the Cell Cycle and the DNA-Damage Response (D.2.8.)
Nikola P. Pavletich, Memorial Sloan Kettering Cancer Center and HHMI

Post-translational Modification by Ubiquitin-like Proteins (D.2.9.)
Brenda A. Schulman, St. Jude Children’s Research Hospital and HHMI

Recombination, Replication, Transcription and Translation (D.2.10.)
Thomas A. Steitz, Yale University and HHMI

Additional Collaborative Projects:

Protein Biosynthesis (D.2.2.)
Jamie H. Doudna Cate, University of California, Berkeley

Molecular Architecture of Complex Protein Assemblies and Small Organelles (D.2.5.)
Stephen C. Harrison, Harvard Medical School and HHMI
Structural Biology of Protein:Protein Interactions (D.2.6.)
Wayne Hendrickson, Columbia University and HHMI

Structural Biology of Apoptosis and NF-κB Signaling (D.2.11.)
Hao Wu, Weill Medical College

Core 3 NE-CAT Staff (see section E.4.):

Igor Kourinov (40%), Staff Scientist, Core 3 Technical Director
TBA (40%), Staff Scientist, Core 3 support
Jun Wang (30%), Staff Scientist, Core 3 support
Malcolm Capel (20%), Deputy Director, Core 3 support
Xiaochun Yang (60%), Programmer, Core 3 support
James Withrow (30%), Technician, Core 3 support

D.1.3.1. Background and Rationale

Challenging crystals present special problems for both data collection and data processing. This core focuses on the computational needs associated with small crystals, weakly diffracting crystals, radiation sensitive crystals and large unit cells. The development of computational technologies is driven by collaborative projects involving frequently problematic systems such as macromolecular complexes and membrane proteins. These crystals often yield data in the 3.5 - 4 Å range and extending the resolution by even a few tenths of an Angstrom may be critical.

Problematic crystals often require special data collection strategies and beamlines with readily reconfigurable optical characteristics. While most modern synchrotron beamlines are designed for routine data collection, challenging samples require easily configurable beamlines in which the experimenter is able to control parameters such as beam size, beam focus position, data collection procedures, data collection strategy, and others. A key component of this core is the ongoing development of Console. Console is a control system development environment that allows the beamline staff and advanced experimenters to control all aspects of the beamline environment via graphically driven scripts. Scripts are written in simple declarative language, embedded in the Console development environment. In contrast to the intense, low level programming required to make changes at a typical beamline, Console scripts can be quickly constructed and implemented using the scripting automation capabilities of the Console development environment.

Difficult crystals are often radiation sensitive and consequently data collection may require multiple samples. Therefore optimal data collection strategy may include accounting for multiple crystals in different orientations. Data collection strategies may also benefit from monitoring or even anticipating radiation damage, and data quality may benefit from improved corrections for radiation damage.

Computational methods also include efficient screening using crystal automounters, and cataloging of results using data base management software. This capability allows the experimenter to select the best crystals and devise strategies to obtain the desired resolution, data set completeness and redundancy. At the same time an efficient data transfer, storage and backup environment is required to handle the large quantities of data generated by simultaneous experiments at multiple beamlines. Rapid data analysis akin to the methods used in structural genomics are needed to determine as quickly as possible whether or not the experiment was successful.

Finally, any macromolecular crystal is potentially affected by problems such as high mosaicity, split or otherwise deformed diffraction spots or twinning. In most cases these data sets are...
discarded without further analysis, yet with some effort these data may easily provide an experimental result with the desired level of accuracy. Non-merohedral twinning is a common problem in macromolecular crystallography that often can be overcome by detection and appropriate data processing procedures.

The computational core must take into account the wide range of experience levels of the visiting scientists. Our overall strategy will be to provide a Blu-Ice like GUI for inexperienced users performing routine experiments, and a Console GUI for intermediate and advanced users with special needs. Resource staff will collaborate with scientists to design experimental strategies, carry out data collection and process data. Novel strategies and experimental design that become frequently used will be added as options to the GUI's.

The technologies of the computational core will be developed in an environment considerably different than of a decade or two ago, in which nearly every practicing crystallographer listed computer programmer as a secondary occupation. Today software packages are largely the product of professional developers and users interact through efficient but rigid GUI's. Underlying the GUI is often a scripting language that allows the user greater flexibility and control. Our efforts will include both writing software and developing scripts for the control of existing software. In the former case we may collaborate with software developers to implement algorithms or the computer programs may be standalone. In the latter case the scripts will be cataloged and readily available and in some cases added to the GUI as a user option.

The organization of the computation core is as follows: (1) beamline control using Console, (2) development of experimental strategies for difficult crystals, including automated screening, (3) development of optimal data processing procedures for challenging crystals, including decay and absorption corrections, and handling of pathological cases such as non-merohedral twinning and (4) development of network and storage systems for efficient data management, and efficient high throughput capabilities for efficient structure analysis.

D.1.3.2. Development of beamline control software

D.1.3.2.1. Introduction to Console

A brief description of Console is presented in section C.4.9.8. of the Progress Report. A more in depth treatment is given here.

D.1.3.2.1.1. Abbreviations

AD/DA: Analog to Digital / Digital to Analog
ADSC: Area Detector Systems Corp.
CORBA: Common Object Resource Broker Architecture
CSL: Console Scripting Language
CEU: Console Execution Unit
CSD: Console Server Driver
CDHS: Console Distributed Hardware Server – interfaced between Console and Blu-Ice
DCS/DHS: Distributed Control System/Distributed Hardware System (SSRL Control system)
EPICS: Experimental Physics & Industrial Control System
GUI: Graphical User Interface
IOC: EPICS I/O Controller node
RPC: Remote Procedure Call
SDK: System Developer’s Kit
VPN: Virtual Private Network
XAFS: X-Ray Absorption Fine Structure
D.1.3.2.1.2. Overview

The control software implemented at NE-CAT, named Console, has been developed specifically for controlling X-ray beamlines. Console is not a control system per se, but rather an integrated development environment for designing and deploying distributed control systems.

A “distributed control system” refers here to a control network in which any member of the network can program both local hardware resources (attached to local host bus or communications port) and remote hardware resources (standalone or connected to other member nodes of the network). Controllers are interconnected to control resources (e.g. motion controllers, A/D or D/A systems, detectors, etc) via ethernet, RS-232 serial connections, IEEE-488 (GPIB) buses, or any communications mode supported under Win32 or Linux.

The Console development environment consists of the following components:

1) A simple, readily extensible procedural scripting language, named CSL (Console Scripting Language).

2) Tools for composing and editing interactive graphical user interfaces for Console scripts (the GUI Generator). These interfaces are implemented using CSL primitives. CSL interface code is automatically generated by the GUI-Generator and inserted into operational CSL scripts via an include mechanism. A rich set of low and high level interface elements and widgets are available to construct CSL GUI’s.

3) A fast, standalone compiler (the "Interpreter") for the scripting language that outputs a parsed executable byte code file, read and executed by the Console Execution Unit (not system binaries).

4) An environment for debugging and executing compiled byte code files (CSL scripts). The Console Execution Unit (CEU) (also called the Console super-client) is responsible for loading compiled CSL scripts, managing their GUI’s and executing compiled CSL codes, with or without debugging. The Console super-client is a multi-threaded win-32 application. Its principal threads of execution are:

   • Scripting Engine: loads and executes compiled CSL byte codes.
   • Script Debugger: real-time symbolic debugger.
   • GUI Polling Thread: creates and manages script GUI’s. GUI elements are registered with this thread. User selection events (button strikes, cursor movements) are associated with GUI elements and the Scripting engine is continuously informed of these associations.
   • Console Server: handles communication between different instances of Console and external control systems containing an embedded console client. Implements a virtual shared memory space for use by Console clients.

5) A set of light-weight Linux-based and win32 servers (called Console Server-Drivers or CSD’s) that parse and pass command streams from a CEU to hardware resources under their control (e.g. motion controllers, A/D systems, etc). CSD’s also maintain a real-time local record of the state of the devices under their control. This control strategy permits multiple clients on the network to program or query a shared set of hardware assets. Server-drivers communicate with the Console superclient (CEU) via one or more of the following transaction protocols:
Figure D.1.3.1 (at the end of this section) shows a block diagram of the overall organization of Console and names the currently implemented driver-servers used to communicate with and control hardware resources within the Sector 24 network.

Console Server-Drivers (CSD’s) for a large number of motion controllers, AD/DA systems have been developed for both Win32 and Linux operating environments. Generic CSD’s have been implemented to program devices connected to control nodes via RS-232 serial ports or IEEE-488 (GPIB) interfaces. A CSD exists for communicating with IO devices connected to Linux-based controllers using the COMEDI application interface. The COMEDI API supports nearly all analog-to-digital and digital-to-analog (A/D D/A) interface boards currently available. Finally, a CSD has been developed for communicating with Programmable Logic Controllers (PLC) connected to the control network via Ethernet. Table D.1.3.1 lists currently available CSD’s, their scope and function.

Client-server connections are secured by a simple password exchange transaction at the time of creation of each client-server connection. Once secured, Console RPC and socket channels remain open and active until closed by the Console super client (except for transient socket connections (e.g. HTTP puts and gets). RPC-based transactions typically occur within a time frame of approximately 10 milliseconds (time between sending of a message and posting of a response from the target host, apart from the time target host takes to actually complete the action requested by the message).

CSD implementations use the same RPC and socket transaction cores and all RPC or socket-related CSL operators take a common syntactic form. It is therefore a simple, if somewhat repetitive, matter to write server-drivers for new instrumentation and control devices.

Each instance of the CEU (Console super client) maintains a thread of execution dedicated to receiving, queuing and parsing RPC messages originating from other instances of the CEU within the control network. This thread implements the Console message-passing server (called the Console Server). Through this mechanism a Console script running on a given host can receive, process, and respond to RPC requests from another CEU within the network. The Console Server enables multiple instances of the Console super client to access and program a shared set of hardware resources in a coherent and synchronized fashion.

The latter type of embedding has been used to integrate the ADSC Q315 detector control system within the Sector 24 control network (see Figure D.1.3.1 at the end of this section). The entire source tree of the ADSC detector control system is provided with the Q315 detector package, enabling the embedding of the Console client-side within the Q315 control system. We have embedded the Console client-side RPC routines for the Console message passing server and a variety of motion controllers and A/D D/A systems in the CCD_BL API of the Q315 control system. The ADSC control system thereby acquires direct, low latency access to those components of the Sector 24 control network it requires for precision synchronization and maximum data throughput (e.g. goniometer and shutter controls). Furthermore, the ADSC control system can pass requests to high-level Console scripts running on a CEU for a variety of control functions not directly accessible to it, e.g. monochromator energy changes and flux optimization, beam steering, focusing mirror adjustments.
Table D.1.3.1: Currently Implemented Console Server-Drivers.

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Transaction Protocol</th>
<th>Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Console Server</td>
<td>Console-console transactions</td>
<td>RPC</td>
<td>Win32</td>
</tr>
<tr>
<td></td>
<td>Console – embedded client transactions</td>
<td></td>
<td>Linux</td>
</tr>
<tr>
<td>Epics Channel</td>
<td>Communication with CA Servers</td>
<td>Epics CA</td>
<td>Win32</td>
</tr>
<tr>
<td>Access</td>
<td></td>
<td></td>
<td>Linux</td>
</tr>
<tr>
<td>GEN SOCKET</td>
<td>Generalized Socket Servers</td>
<td>Socket</td>
<td>Win32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transient Persistent</td>
<td>Linux</td>
</tr>
<tr>
<td>OMS</td>
<td>Oregon Microsystems PC68 motion</td>
<td>RPC</td>
<td>Win32</td>
</tr>
<tr>
<td></td>
<td>controllers.</td>
<td></td>
<td>Linux</td>
</tr>
<tr>
<td>MCL</td>
<td>McClennan motion controllers.</td>
<td>RPC</td>
<td>Win32</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Linux</td>
</tr>
<tr>
<td>GAL</td>
<td>Galil DMC motion controllers.</td>
<td>RPC</td>
<td>Linux</td>
</tr>
<tr>
<td>D TAU</td>
<td>Delta Tau PMAC2 motion controllers</td>
<td>RPC</td>
<td>Win32</td>
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<td></td>
<td></td>
<td></td>
<td>Linux</td>
</tr>
<tr>
<td>AXIS</td>
<td>Axis Communications streaming</td>
<td>Active X</td>
<td>Win32</td>
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<td></td>
<td>network video servers</td>
<td>(COMM)</td>
<td></td>
</tr>
<tr>
<td>SERIAL</td>
<td>Generic IEEE 488 interface server</td>
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<td></td>
<td></td>
<td></td>
<td>Linux</td>
</tr>
<tr>
<td>HEI</td>
<td>Direct 32 PLC systems interface</td>
<td>RPC</td>
<td>Linux</td>
</tr>
<tr>
<td></td>
<td>Control of EPS and other PLC’s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COMEDI</td>
<td>COMEDI A/D D/A general IO server</td>
<td>RPC</td>
<td>Linux</td>
</tr>
<tr>
<td>D Tran</td>
<td>Data Translation A/D D/A card server</td>
<td>RPC</td>
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<td>Diamond Systems general PC104 IO</td>
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<td>devices server.</td>
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<td></td>
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<tr>
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<td></td>
<td>devices server.</td>
<td></td>
<td></td>
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<tr>
<td>TTS</td>
<td>Text to speech synthesizer server.</td>
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<tr>
<td></td>
<td>Audible alarms and user prompts.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTILITY</td>
<td>Monochromator cryopump, Thermocouples,</td>
<td>RPC</td>
<td>Win32</td>
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<td></td>
<td>Thermocontrollers</td>
<td>HTTP put/get</td>
<td>Linux</td>
</tr>
<tr>
<td>NTGRAPH</td>
<td>Real-time Intelligent Plotting and</td>
<td>Active X</td>
<td>Win32</td>
</tr>
<tr>
<td></td>
<td>printing.</td>
<td>(COMM)</td>
<td></td>
</tr>
</tbody>
</table>

Multiple instances of the Console system can execute on a single control node and interoperate with instances of Console running on other nodes within the same network. Different instances of Console may interoperate as peers or in a hierarchical mode incorporating master and slave relationships.

An immediate benefit of this sort of control distribution is that one can remotely control a beamline using exactly the same set of scripts on a remote CEU (e.g. a home environment) that normally execute on a local CEU, given a reasonably high bandwidth ethernet connection and the ability to bypass facility firewall restrictions. NECAT’s staff operators can remotely assist users or correct problems using a CEU running on their home PC’s, thereby reducing response times to beamline problems and reducing wear and tear on the beamline operators.
D.1.3.2.1.3. Why use and develop Console?

Use of Console for low level and high level beamline control, as opposed to one of the commonly used control systems (e.g. Epics, MX, Blu-Ice), has substantial advantages. The principal benefits that accrue to NE-CAT through the use of Console are:

1) Console is an integrated control system development environment with support for all aspects of control system development, including:
   a) Rapid source code development and compilation.
   b) Automated Graphical User Interface (GUI) generation.
   c) Script debugging and validation.
   d) Network distribution and remote operation.
   e) Sharing of control resources (motor controllers, data collection systems, etc) among a group of controllers.

2) Total control of all source code and ease of extension. We can rapidly develop new communications, control and GUI capabilities to answer unanticipated or changing needs of NE-CAT users in an “on-the-fly” mode.

3) The process of developing the purely operational components of a Console script is completely decoupled from the process that generates the GUI of the script. Thus the form and function of a Console GUI may be modified without requiring wholesale revision of the operational script source code.

4) Console possesses a unique real-time tracing and debugging system to aid script development and validation.

5) Console is designed to interoperate facilely with foreign or self-contained control systems (e.g. EPICS, detector, robot control packages) to exploit their capabilities and resources.

To take full advantage of these benefits, the NE-CAT research resource will pay attention to the following requirements:

6) We will continue to maintain, develop and validate both the Console program and the beamline Console script code bases.

7) NE-CAT programmers will be trained to use the Console scripting system (CSL).

8) A staff member will be recruited with Console development and management as a primary responsibility, under the direction of Capel (who has been to date the sole developer and manager).

D.1.3.2.1.4. Console scripting language

Console’s embedded scripting language is named “Console Scripting Language (CSL)” . CSL is a simple procedural, strongly typed language using an entirely flat memory model (all variables are of global scope). CSL supports the following general data types: short (16 bit) and long (32 bit) integers, double precision reals, C-strings, 16-bit Booleans and arrays of all data types up to rank 10.

All CSL command lines (except for some program flow control statements) take the syntactical form of:

Operator [operand1],[ operand2],..
The first syntactical element is the operator string, corresponding to one of the approximately 950 operators defined under CSL. Subsequent syntactical elements are operands of the operator. All operators and their calling conventions are defined in a single syntax declaration file named OPERATOR.DAT. The syntax declaration file also defines the types and lexical ordering of all operands required by each operator, using a simple grammatical notation. Each operand string must correspond to a unique variable name, defined under a valid type declaration statement. All unary operators return the result in the calling operand. In all other cases, the operator returns its result in the first (result) operand.

In order to add a new function definition to the Console operator repertory, the programmer simply adds a new operator definition record to the OPERATOR.DAT file. An operator definition record consists of:

1) Unique operator index.
2) Unique operator name.
3) One or more operand type and order declaration statements written in the Console type grammar notation.

The Console compiler automatically generates appropriate hash table entries for its internal parsing routines in response to the addition of a new operator definition record to the OPERATOR.DAT configuration file. Finally, a corresponding function or subroutine that actually implements the action of the new operator must be added to an appropriate dynamic link (dll) library loaded by the CEU at run time, referenced by a subroutine entry point that is a unique function of the operator index of the new operand. Aside from low level program flow control and variable memory allocation operands, lexical extension of CSL is quite simple.

Console defines operators for all the commonly used calculation and program flow control constructs expected of a procedural language, including: DO ENDDO and DO WHILE loops; IF THEN ELSE ENDF block; conditional and unconditional GOTO's; SUBROUTINE calls; etc. A CSL script can permanently or temporarily pass execution control to another pre-compiled CSL script (an operation called CHAINing).

D.1.3.2.1.5. Generating GUIs

A central intent of CSL is to simplify the process of generating Graphical User Interfaces (GUIs) within the context of Console scripts. A very rich set of CSL drawing operators are defined that map to the Win32 Direct-Draw API, providing high graphics performance. All functions of the PGLOT scientific plotting package for generating publication-quality graphs are exposed as CSL operators. CSL provides a series of client calls to an open source Active-X control called NTGraph for real-time, self-scaled function and data plotting. In addition, Console defines many high level graphical widgets to facilitate the programming of real-time data displays or control GUI's.

The GUI Generator is used to create and modify GUI's for CSL scripts. The output of the Console GUI Generator is a set of text files that consist of CSL source code statements encoding each element of a CSL GUI. This “auto-generated” source code is inserted in the body of the target Console script using the “INCLUDE <filename>” operator construct, where <filename> refers to the auto-generated source code file. During compilation of the target script, the auto-generated source code is loaded by the Console Interpreter at the point where the INCLUDE operator occurs and the GUI source code is compiled within the context of the target script.

The programmer inserts appropriate operators within the target script that cause “registration” or event queuing of included GUI control elements with the GUI polling thread of the CEU. The GUI polling thread of the CEU sends event information back to the executing CSL script when a registered GUI element is selected by a mouse click or other input selection mode. The
executing script then calls an appropriate subroutine or registered callback function (called a dispatch) in response to the GUI polling event, completing the GUI-response cycle.

This method of creating GUI’s decouples the operational components of a script from its purely interfacial elements. Thus, the GUI elements of a Console script may be relocated, rescaled or undergo changes in appearance without requiring modification of the operational parts of the script. Addition of new GUI elements (buttons, readouts, etc) to an existing GUI requires modification of the associated CSL script to enable polling of and to install callback mechanisms for the new GUI elements.

D.1.3.2.1.6. Script debugging

CSL scripts of moderate or higher complexity require formal mechanisms for debugging and validating the correctness of their operation. The Console Interpreter provides the first level of script debugging. All syntax and program logic errors encountered during interpretation are logged and annotated to describe the error and suggest corrective action. The compiled byte codes for a script are not recorded until all recognized syntactical and logical errors are eliminated from it’s source file.

The CEU provides a rich suite of debugging tools for analysis of the operation of scripts that successfully compile. The CEU can be configured to generate time-stamped trace-displays of all CEU-CSD transactions. A transaction trace display is available for all CSD’s. The transaction trace output can be saved to a file for off-line analysis. Additionally, the CEU can generate a time-stamped trace display (and save file) for detailed analysis script execution. For each program step the execution tracer logs the program counter, operator identity and a listing of all associated operands, including the value of the pointer to each operand and its value following the execution step. Script execution may be temporarily stopped and resumed to examine and analyze the trace information.

Recently, a complete real-time symbolic debugging system has been developed for the Console Execution Unit (see below). The symbolic debugger lets the programmer browse script source and the state of all named variables (operands) during script execution. Browsing of source and variable states can be accomplished using symbolic or string searches or by interaction with graphical controls of the debug browser windows. The Console Debugger implements standard debugging constructs such as break points, conditional breaks, watch points and operator-controlled program stepping.

D.1.3.2.1.7. Sector 24 control networks

Figure D.1.3.2A, B is a block diagram of the 24-ID-C Console-based control network currently in place at Sector 24, and is segmented to demarcate the main optical systems (dashed lines). A parallel network of very similar constitution and complexity supports the 24-ID-E beamline (not shown). The motion controllers (blue boxes labeled GALIL, DELTA TAU, PC68) are intelligent stepping and servomotor controllers used to control the positions of the collimating and focusing mirrors, monochromator crystal positioning drives, a variety of white and monochromatic slits as well as the sample goniometer axes and detector positioners. The second crystal of the monochromator has an elaborate set of piezoelectric effectors for coarse and fine tuning its roll and pitch axes. Steering of the monochromatic beam to a fixed sample point is effected by these controls (in conjunction with the vertical focusing mirror’s pitch axis), during energy shifts or to compensate for residual synchrotron source positional instability.

The Sector 24 equipment protection system is a PLC (programmable logic controller) – based system that polls a variety of hardware interlocks and sensors, including cooling flow rates for all white-beam exposed optical systems, vacuum readouts and gate valve status to continuously monitor the operating state of beamline infrastructure systems. Significant departures from normal operating conditions by any monitored component of the beamline automatically causes
closure of the beamline front end shutter and gate valve to protect optical components and vacuum equipment from damage due to overheating or inappropriate exposure to APS white radiation or vacuum breech. The EPS systems can be both programmed and monitored via the 8-BM control system.

Nodes in the network that support user interfaces are indicated by the black boxes labeled “cortex1 and cortex2”. Cortex 1 is the master CEU control node of the network (i.e. CSL scripts running on this node have control access to all hardware resources of the network).

The master node, Cortex 1, is responsible for periodic adjustments of beam position (via mirror steering, driven by quadrant diode beam position monitors), optimization of flux (pitch tuning of the monochromator’s second crystal) and controlling the energy set point of the monochromator in response to requests from Cortex 2 or the ADSC Q315 detector control system. Cortex 1 scripting also logs all beamline set points, motion control states, performs XAFS scans and is responsible for monitoring the health of the beamline as a whole.

The Cortex 2 CEU is the node that beamline users interact with for aligning a sample and configuring the beamline optics during data collection. Scripts running on Cortex 2 are designed to expose only those controls that users require for crystallographic data collection. Typically, multiple instances of the CEU run on Cortex 2, executing CSL scripts for 1) sample alignment via video streams from two quasi-orthogonal video microscopes; 2) aligning the X-ray beam to the goniometer spindle; 3) monitoring the sector front end status which provides information on the stability of the white X-ray beam.

The user interface node labeled “VPN Console CLIENT” represents a Virtual Private Network (Cisco Systems)-based tunnel through the APS firewall that permits Console CEU’s running on NE-CAT staff’s home computers to issue Console RPC messages to part or all of the Sector 24B control network.

The Console RPC message passing core has been embedded in the ADSC detector control software to permit the ADSC detector control software to directly pass control messages to the CSD’s in control of the monochromator, sample shutter, detector positioner and all axes of the Goniometer. Thus, the ADSC control system can program the position of the Q315 detector, goniometer settings and initiate energy changes and fine tuning of the monochromator to maximize delivered flux and correct the beam trajectory after an energy shift. Direct interaction between the ADSC control system and Console CSD’s helps reduce the inter-data frame dead time by shortening communications paths within the control network. The ADSC control system can also access the master CEU via the Console client-server RPC messaging system to post service requests for those hardware resources for which the ADSC control system lacks direct access or functions involving collective configuration changes in the beamline optics (e.g. monochromator energy changes). This mechanism is used to periodically post RPC messages to the master CEU to initiate tuning of pitch and roll for the monochromator’s second crystal, in order to maintain peak flux on the sample crystal during long data collection sequences.

The embedding of critical beamline control functions within the ADSC control system means that users need only be trained in the use of the ADSC control interface itself in order to set up data collection protocols that involve energy changes, without being trained in the direct use of beamline controls via the master CEU. This property minimizes the training burden with new users and also increases system security in that user access to beamline controls may be restricted to those exposed only in the ADSC interface.

**D.1.3.2.18. Example CSL scripts**

Figure D.1.3.3 is a screen grab of the Console script responsible for handling all critical optical systems during crystallographic data collection. This CSL script is an “executive” script used by NE-CAT staff to manually configure the 24-ID-C monochromator and beam steering and flux.
optimization feed back systems. This script also parses the Console message-passing stream from the Sector 24 Auxiliary CEU and the ADSC detector control system in order to maintain beam position lock, peak flux tuning and handle energy set point changes.

The 15 control structures in the upper portion of screen are readouts for the monochromator’s servo and stepper motor drives. The readouts can be configured to display physical units or motor steps. The buttons on the left of each readout (labeled with arrows) are configurable jog actuators. By left clicking on the display, itself the user calls up a dialog for specifying an absolute motion of the relevant axis. The displays to the right of each motor control indicates the state of limit switches and indicates the power state of the associated motor drive (rectangular indicator below limits). The readout boxes below the motor controls provide information on the energy set point, current flux (from downstream quadrant diode BPM), the undulator gap current BPM coordinates of the monochromatic beam (leftmost lower level displays). The rightmost displays provide status information for the monochromator cryopump.

The blue beveled buttons to the right and below the readouts are triggers for a wide variety of functions, some of which are:

<table>
<thead>
<tr>
<th>Button Label</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>SET ENERGY</td>
<td>Initiate dialog for energy set point.</td>
</tr>
<tr>
<td>EDGE SELECTOR</td>
<td>Chain to CSL script for discrete edge selection using a periodic</td>
</tr>
<tr>
<td></td>
<td>table-based GUI.</td>
</tr>
<tr>
<td>QG ROCKING CURVE</td>
<td>Dialog for rocking curve acquisition (see Figure C.2.6.5)</td>
</tr>
<tr>
<td>XAFS SCAN</td>
<td>Dialog for XAFS curve acquisition (see Figure C.2.6.6)</td>
</tr>
<tr>
<td>TUNE X POSITION</td>
<td>Optimize beam X position by trimming HFM yaw.</td>
</tr>
<tr>
<td>TUNE Y POSITION</td>
<td>Optimize beam Y position by trimming VFM pitch.</td>
</tr>
<tr>
<td>TUNE PERIOD</td>
<td>Dialog for periodic position trim and flux optimization.</td>
</tr>
<tr>
<td>SET PITCH QUEENSGATE</td>
<td>Dialog to manually control second crystal DPT.</td>
</tr>
<tr>
<td>PITCH DPT TUNE</td>
<td>Coarse &amp; fine step optimization of flux (second crystal rock) followed</td>
</tr>
<tr>
<td></td>
<td>by Y position optimization.</td>
</tr>
<tr>
<td>FINE PITH DPT TUNE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>CHAIN PHI_HFM</td>
<td>Chain to CSL scripts for HFM and VFM control.</td>
</tr>
<tr>
<td>CHAIN PHI_VFM</td>
<td></td>
</tr>
<tr>
<td>DOCUMENTATION</td>
<td>Dialog to display documentary information.</td>
</tr>
<tr>
<td>NOTEBOOK</td>
<td>Dialog for run logs and user notebook.</td>
</tr>
</tbody>
</table>

The lower, left section of the display provides real-time information on state the monochromator script (topmost), Console server transactions (middle), and beam steering parameters (bottom). The ADSC control system interacts with the monochromator CSL script via the Console server message passing mechanism. The Console Server client side functions have been embedded within the ADSC control system. When appropriate the ADSC control systems passes a simple message string to the monochromator script (e.g. VFM pitch tune for beam position trim, second crystal pitch tune to optimize flux, change monochromator energy set point, etc). The monochromator script parses the message and activates corresponding control subroutine. The ADSC control system dwells until receiving a message from the monochromator script indicating completion and completion status.

Figure D.1.3.4 is a screen dump of the CSL script that beamline users interact with to configure the Sector 24-C optics for data collection, including: monochromator energy set point, beam position, flux and beam size. This script is also used to collect and analyze XAFS data from samples and to align samples to the crystallographic spindle. This script manages critical optical settings by communicating with the Sector 24-C Master CEU, using the Console server communication channel.
The large sub-window in the upper right portion of the display shows a 16-frame/sec video stream from and Axis camera server connected to the vertical visualizer’s CCD camera. The Axis camera server is capable of supporting four simultaneous mpeg3 client connections. Left clicking on the sample image causes the goniometer sample alignment axes to move the selected sample position to the center of the white cross hairs, corresponding to the center of the current beam position steering target. The digital readouts to the left of the video window are real-time readouts of the energy set point of the monochromator, flux and current beam position. Buttons above the digital readouts perform the following actions:

<table>
<thead>
<tr>
<th>Button Label</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>MONO ENERGY</td>
<td>Request change the energy set point (via Master CEU)</td>
</tr>
<tr>
<td>MAXIMIZE FLUX</td>
<td>Maximize flux (optimize second crystal pitch tune)</td>
</tr>
<tr>
<td>TRIM X POSITION</td>
<td>Optimize x-position of the X-ray beam</td>
</tr>
<tr>
<td>TRIM Y POSITION</td>
<td>Optimize y-position of beam</td>
</tr>
<tr>
<td>XAFS SCAN</td>
<td>Configure and initiate XAFS scan (via Master CEU)</td>
</tr>
<tr>
<td>MASTER OPS</td>
<td>Miscellaneous Utility operations on Master CEU</td>
</tr>
<tr>
<td>VIDEO OPS</td>
<td>Configuration options for video stream server</td>
</tr>
<tr>
<td></td>
<td>Save still image</td>
</tr>
<tr>
<td></td>
<td>Display saved still image</td>
</tr>
<tr>
<td></td>
<td>Record mpeg stream</td>
</tr>
<tr>
<td></td>
<td>Playback mpeg stream</td>
</tr>
<tr>
<td>SAVE TUNE STATE</td>
<td>Manually save all parameters of current optical config.</td>
</tr>
<tr>
<td>READ TUNE STATE</td>
<td>Assert saved optical configuration</td>
</tr>
<tr>
<td>SLIT PRESETS</td>
<td>Manual selection of slitted beam size</td>
</tr>
<tr>
<td>CHOOCH</td>
<td>Run CHOOCH analysis on acquired XAFS data</td>
</tr>
</tbody>
</table>

The large buttons left and below the video window set the goniometer spindle angle. The arrow buttons in the screen area labeled “VER” cause a jog of varying magnitude to the appropriate X or Y servo of the Huber 515M to align a sample relative to the screen graticules, which show the position of the beam in the coordinate frame of the visualizer camera. The horizontally-aligned arrow buttons at the bottom of the screen trigger jogs of the Z-axis servo.

The buttons immediately below the video window have the following functional assignments:

<table>
<thead>
<tr>
<th>Button Label</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEAM STOP</td>
<td>Forced insertion of beam stop</td>
</tr>
<tr>
<td>HELP</td>
<td>Launch HTML help for script</td>
</tr>
<tr>
<td>EXIT</td>
<td>Exit script</td>
</tr>
<tr>
<td>TOGGLE SHUTTER</td>
<td>Open/Close data shutter</td>
</tr>
<tr>
<td>YAP POSITION</td>
<td>Set position of YAP fluorescence detector</td>
</tr>
<tr>
<td>NULL X,Y,Z</td>
<td>Dialog for setting and moving to X,Y,Z reference position</td>
</tr>
<tr>
<td>GONIOMETER SCAN</td>
<td>Dialogs for vertical and horizontal wire scans to align goniometer spindle axis to beam</td>
</tr>
<tr>
<td>GONIOMETER JOG</td>
<td>Dialogs for vertical and horizontal jogs of the goniometer. Used in conjunction with fluorescent probe mounted on spindle axis</td>
</tr>
<tr>
<td>ATTENUATORS</td>
<td>Dialog for attenuator control</td>
</tr>
<tr>
<td>TRANSILLUM</td>
<td>Insert / Remove sample transilluminator</td>
</tr>
<tr>
<td>FOCUS JOG</td>
<td>Dialog for adjusting camera focus position and trimming focus</td>
</tr>
</tbody>
</table>
Figure D.1.3.1: Console architecture, emphasizing transaction modes. Programs: GUI Generator (upper right), CSL compiler upper middle. Console execution unit (CEU) magenta boxes. Console server drivers (CSD’s): blue boxes. Client-server connections: red lines. Local/Remote hardware resources: black boxes. Orange box on far left represents the ADSC Q315 control system and indicates that foreign control systems with embedded console CSD and Console-Server Clients can share hardware resources and data with the Console script engine.
**Figure D.1.3.2A:** Schematic of Sector 24 beamline control system. Connection types: ethernet-cyan, bus connections-magenta, serial rs2323-red, data/signal-dark blue. Computational nodes: black rectangles. Motor controllers: blue rectangles. A/D D/A: magenta rectangles. Encoders/sensors: green.
Figure D.1.3.2B: Schematic of Sector 24 beamline control system (continued). See Figure D.1.3.2.A for description of color coding.
Figure D.1.3.3: 24-ID-C monochromator CSL script.

Figure D.1.3.4: Main user interface CSL script. This script used to align samples to the goniometer spindle, set the monochromator energy, acquire XAFS spectra, maximize flux and adjust the beam position relative to the spindle and align the spindle to the xray beam.
D.1.3.2.2. Console development plan

D.1.3.2.2.1. Channel access and Epics

CSL includes operators that map to all of the major EPICS Channel Access client functions (e.g. EPICS data base puts, gets, monitor creation, etc.). This feature of CSL permits the CEU to communicate directly with the Sector 24 EPICS Channel Access Server to set running parameters of the undulators, acquire status information from the sector beam position monitors or communicate with any EPICS IOC (I/O controller) with appropriate permission status. This feature of CSL will enable NECAT to migrate low level device handling from CSD’s to EPICS IOC’s if deemed necessary. At present the majority of devices used in our control network are not effectively supported by existing EPICS driver records. Migration from Console’s CSD’s to EPICS IOC’s will have the benefit of lowering development and maintenance costs associated with the CSD’s.

D.1.3.2.2.2. CSL training

To enable NE-CAT programmers and beamline scientists to learn CSL more readily, we are developing a number of facilities and programming tools. The Console Interpreter, GUI generator and the Super-client (CEU) all have online help systems (mainly HTML or windows SDK help-based) that document each tool’s capabilities and methods. There is also an extensive on-line help for CSL specification and the Console scripting engine. The help systems are updated in parallel with the scripting engine and with CSL.

An important new training adjunct will be the Console Operator Templator, currently under development. This tool will be used in conjunction with the editor used to manipulate CSL source files. The Templator will contain a hierarchical browsing tree representing all CSL operators and their various invocations. Selection of a given operator name (an invocation) will cause a corresponding functional help summary of the operator and its calling conventions to appear in a scroll window.

Simultaneously, an invocation template for the selected operator will appear in a clipboard window, immediately below the help scroll window. This operator template will consist of the operator name and a sequence of self-descriptive dummy operands (arguments) for each required operand of the operator. The user will edit the operator template, replacing each dummy operand with an actual operand string. The Templator will have access to the script source file being edited and determine if each actual operand corresponds to a variable name already type-declared in the source file. In the case where the actual operand is not defined, then an appropriate type declaration will be generated in the clipboard window for the actual operand. In the case where the actual operand replacement corresponds with a previously declared variable name, the Templator will check the replacement operand for correctness of type and warn of any inconsistencies between the inputted replacement argument and the invocation pattern for the selected operator. After all of the dummy operands have been replaced with actual arguments the code template will be pasted into source code file at the appropriate location, along with any auto-generated type declaration. Figure D.1.3.5 shows a prototype of the Templator tool interface.

The Console Templator will spare the novice Console programmer from memorizing hundreds of operator calling conventions, and should make the task of learning CSL much less burdensome.
D.1.3.2.2.3. Console real-time symbolic debugger

As previously described, a symbolic debugger is under development for interactive, on-line debugging of CSL scripts. The symbolic debugger, an optional execution thread of the super-client enables the programmer to examine, in depth, the execution of a compiled CSL script and the content of any of its named variables throughout the course of script execution.

The debugger is implemented as a series of independent browser windows:

• **Source Browser**: (Figure D.1.3.6A consisting of a scroll window containing an annotated representation of the compiled CSL source. The source browser is used to set and clear execution break points by left-clicking on a displayed line of source. Right-clicking in this scroll causes a search dialog to display that can be used to position the scroll to a specific line of source. The scroll window is automatically adjusted to show a source view port centered on the current program counter, during script execution and when a break point interrupts script execution. The source browser has a set of buttons for manual control of program stepping and for stepping over or clearing established program break points. The Source browser also continuously displays the current operator and all of its operand types and values.

• **Variable browsers**: (Figure D.1.3.6B) each variable or operand type (short and long integers, real, Boolean, string) has its own state browser. The variable browsers have a scroll window listing each named variable in the order in which it was declared in the scripts source, along with its pointer into the memory space allocated for its variable type. A left click on the variable name in the selector scroller causes a listing of the variable’s state (content) to be displayed in a scroll window below the variable selector scroller. If the selected variable is an array the entire array content is displayed. A right click anywhere in the selector scroller elicits a search dialog whereby the user can enter a search key for easy location of a desired variable in
long lists. The variable browsers are active regardless of the execution state of the script engine.

The debugger operates without significant degradation of the execution rate of the CEU’s scripting engine. The debugger currently supports most elements expected of a symbolic debugger, including in-line break points, a variety of manual program stepping modes and real-time, searchable access to all variable data and source code.

Under development are a number of new debugger capabilities:

- **Watch-points**: Program execution continues until user-specified variables experience a change of state. When a change of selected variable state occurs program execution is halted until the user commands a resumption of execution. Watch-points will set by mouse selection in a variable browser window or keyed search on a variable name.

- **In-line variable modification**: This facility will allow users to alter the state of any selected variable at break- or watch-point interruptions.

- **In-line code modification**: This facility will allow the user to modify source code from within the debugger and immediately apply changes to executing code.

- **Profiler**: Will calculate and display histograms of program line number call frequency with and without execution latency weighting. This tool will aid the Console programmer in locating “hot spots” (frequently executed) or high latency regions of a script, where effort would best be expended in improving script speed or efficiency.
**Figure D.1.3.6A**: Debugger Source Browser. Upper scroll window shows annotated source and is used to set break point positions (left click on selected line). Thin structure on far left shows current execution point (blue bar), a break point at line 3084 (red circle) and a successful search point (green circle). Buttons control execution mode and break point activity and activate keyed source searches. Window near bottom shows current operator and operand states at the time of encounter with the break point.
Sector 24’s CSL scripting repertoire is being extended to improve systems reliability and to enhance the user’s ability to configure the beamline optics and end station for their particular needs, without NE-CAT staff intervention. A sampling of planned scripting efforts in these directions follows:

- **Detector Orienter.** NE-CAT’s Q315 detectors are suspended form the A-frame support via a differential lift mechanism that permits the detector to be elevated, with or without a change in detector pitch (relative to the beam vector). Maximum detector pitch is a function of Sample to Detector Distance (SDD), ranging from 6.5° at 1000mm SDD to 37° at 110 mm SDD. The detector can be translated to place the point of intersection with the beam anywhere in the detectors vertical extent and +1 detector element, in the horizontal. The Detector Orienter script will permit users to assert pitch and elevation set points for the detector anywhere in the physically allowed orientation space. The script will provide graphical feedback indicating the diffraction limits of any proposed change in elevation and pitch. Additionally, the script will perform collision checks prior to any move commitment.
• **Beam Shaper.** Presently all changes in the placement of the focal point of the X-ray beam by modification of the bend radii of the vertical and horizontal focusing mirrors (HFM,VFM) are performed by NE-CAT staff, only. The placement of the focus can be anywhere between the spindle and detector axes. The size of the beam on sample is controlled by the degree of over or under-focus of both mirrors and can be further adjusted by slitting. Presently the only unsupervised user modification of beam size is via selection from a number of preset collimator settings provided by the Camera alignment script, running on the Auxiliary CEU (see above). The Beam Shaper script will utilize the miniature beam imager described in section D.1.2.4.4. Two beam imagers will provide a high resolution image of the beam at two points: 1) 1 cm downstream from the sample spindle and 2) 1 cm upstream of the Q315 detector face. Both beam imagers will be temporarily inserted in the beam using high precision vertical slides. Digitized output from the beam imager's CCD camera will be used to determine the position and geometric properties of the beam. This feedback information will be used to finely control the mirror benders to set the desired beam size and shape. We have found that mirror adjustments cannot be usefully driven by simple lookup tables or a priori calibration functions due to hysteresis of the mirror benders and temperature effects on bender performance.

• **Rapid Spindle Aligner.** Vertical alignment of the goniometer spindle to the beam is currently effected using a scanning process. A fine tungsten wire is aligned to the spindle axis and the entire goniometer is scanned vertically so that the tungsten wire passes through the X-ray beam. Backscatter and fluorescence from the wire is measured by a YAP fluorescence detector situated upstream of the goniometer. A CSL script calculates the second moments of the wire scan profiles for 2 or 4 orthogonal phi settings. Finally, the goniometer is translated to the vertical position corresponding to the average of the scan profile second moments. This procedure accurately aligns the spindle axis to the beam but is time consuming and requires installation of the scanning wire. Given the current angular stability of the APS source, intermittent realignments are required to maintain perfect alignment of small X-ray beams (< 50 μm) and even smaller samples. The Rapid Spindle Aligner will use digitized image information from the miniature beam imager just downstream of the spindle to accurately measure the position of the beam relative to the crystallographic spindle and optimize its alignment relative to the spindle. This process will be accomplished without removal of installed samples and be so rapid that intermittent beam-spindle position optimizations can become part of the normal data collection cycle. We also describe in D.1.3.3.4 a procedure for "on-the-fly" spindle alignment that involves diffracted intensities. The two methods may have complementary advantages, either for precision or convenience.

D.1.3.2.3. Integration of a Blu-Ice-like GUI

SSRL’s Blu-Ice is a popular crystallography GUI for configuration of beamlines and programming data collection. Blu-Ice is architecturally decoupled from its native control system DCS/DHS so that it is possible to overlay it upon “foreign” control systems such as EPICS. NE-CAT is currently exploring the possibility of using Blu-Ice as the user interface for sequencing data collection and user-initiated beamline configuration, for situations not requiring the flexibility and re-configurability of Console or where the beamline user has a preference for the Blu-Ice interface.

NE-CAT has developed a DHS (Distributed Hardware Server, called Console-DHS or CDHS) that provides a transaction gateway between Blu-Ice and Console. The CDHS contains an embedded Console-Server client that permits CDHS to pass command strings to the sector Master CEU. Thus we can marshal all existing CSL functionalities (e.g. monochromator and focusing system controls) through a Blu-Ice interface by relative simple modifications to Blu-Ice. The downside of this strategy is added indirection in communication pathways between the GUI and the control resources being used, and increased response latencies and difficulties in system debugging and validation.
The CDHS is currently undergoing development and validation at the 8-BM beamline. Following relocation and re-commissioning of the bending magnet beamline at Sector 24, NE-CAT will devise a configuration system whereby the operative GUI can be readily switched between native Console interfaces and CDHS-Blu-Ice. This system will be extended to the insertion device beamlines following validation of the Console-Blu-Ice hybrid and the outcome of user preference testing.

D.1.3.2.4. Integration of the MD2 microdiffractometer

The MD2 micro-diffractometer is supplied with its own control system built on the ESRF’s Tango distributed control system. Tango uses the Common Object Broker Architecture (CORBA) as its transaction layer. Console presently has no internal CORBA transaction interface. Instead we will use the Swiss Light Source’s EPICS interface to the MD2 Tango server to synchronize and coordinate the MD2 with NE-CAT’s beamline controls. New CSL scripting will be developed to integrate the MD2 with the beamline, using Channel Access transactions to monitor and control the microdiffactometer. Alternatively, Maatel/Accel provides a standalone win32 control interface for crystal visualization, alignment and MD2 setup.

In either case, a second version of the ADSC Q315 control system will be developed for use with the MD2 instead of the Huber 515m. Revision of the ADSC system will be a modest development effort, involving of order a few hundred codes lines.

D.1.3.2.5. Integration of the ALS robot

The ALS robot, undergoing development at testing at 8-BM, is provided with a standalone control interface, as well as a complete SDK for developing novel control interfaces from external control systems. Additionally, a Blu-Ice/DCS/DHS interface has been developed for this sample loading robot (see following section).

Users tend to strongly disfavor having to access multiple program interfaces on multiple control nodes in order to configure and monitor data collection. Thus, there is a strong imperative to develop some form of integration of the ALS robot into the beamline control system.

Console integration of the ALS robot will use the vendor-supplied java-based SDK. The SDK uses simple string transactions with the robot’s hardware server mediated by persistent socket connections, to invoke high-level functions of the robot (e.g. load sample, recover sample, index magazine, etc). CSL contains a complete winsock32 implementation so it possesses the necessary communication modes to interact with the ALS robot. A CSL script will be developed to provide direct user control over all user-pertinent high level functions, and the ADSC control system will be modified to call this script via its embedded console-server client.

D.1.3.2.6. Remote data collection

Integration of the ALS sample mounting robot into the Sector 24 control network provides the possibility of offering our users remote access to beamline resources for data collection. The possible modes of “remote data collection” range over two extremes: from 1) mail-in sample handling to 2) collaborative use of the beamline.

Under mode 1) remote data collection, users mail in frozen samples in a format acceptable to the sample magazine of the ALS robot; beamline staff load the sample mounter magazine and users remotely control all aspects of sample loading and data collection with minimal intervention from beamline staff. Under mode 2 remote use, staff present and/or users at the beamline collaborate with remote users (e.g. the user group’s PI) to operate the beamline, acquire diffraction data and carry out data analysis.
Mode 1 remote use is contingent upon the availability of a highly reliable, high volume sample loading robot and data management software for tracking samples and associated data, as well as high performance network connections to support the large volume of network traffic attending crystallographic data collection and analysis.

Mode 2 remote use does not necessarily require sample loading automation but depends upon collaboratorium or telepresence software and high performance network infrastructures. These are software tools that enable low latency communication (visual and/or aural) between local and remote users (or beamline staff) and sharing of the control system and data analysis computational environments.

To some extent, NE-CAT already relies upon the Mode 2 collaborative use pattern. All NE-CAT user support staff have working home installations of Console and relevant beamline Console scripts. Because all sanctioned instances of Console have peer-to-peer level access to beamline control system assets, NE-CAT staff have the ability to remotely take control of NE-CAT beamlines from their home computers. Presently, this facility is used mainly to assist users in diagnosis and recovery from fault conditions reported by users via phone conversations.

We could extend Console’s "circle of trust" to remote users by simply providing them with working Console installations and scripting. Remote users would circumvent the APS firewall restrictions by requesting an APS Virtual Private Network (VPN) client account. Users would presumably already have been trained in the use of Console scripting through prior, local use of the beamline.

Desktop sharing tools, such as those based upon Virtual Network Computing (VNC) offer easy to implement collaboration modes for NE-CAT’s beamlines (76). The VNC client-server protocol enables real-time sharing of the beamline control computer's desktop environment with one or more remote client workstations. Local and remote users of the shared environment experience the same graphical display and have peer-to-peer level control of the beamline through the shared desktop (possibly with some network latency). The remote collaborator need only have a VNC client installed on his/her workstation and possess appropriate APS network privileges. VNC is based upon open source communication protocols and many freeware implementations are available. VNC is platform and hardware neutral (supports intel PC’s, Mac’s, win32, linux OSX).

Both of these simple models of remote beamline use demand an unrealistically high degree of discipline, clarity of communication and cooperation amongst the participants. Desktop sharing and Console distribution are easy to set up but are difficult to manage and completely vulnerable to unintended or rogue misuse, without continuous oversight of remote user network privileges by beamline staff.

A variety of collaborative tools exist to support managed mode 1 and 2 remote beamline use. SER-CAT has pioneered the use of the collaboration suite Access-grid for mode 1 and 2 remote use of their beamlines. Access-grid (77) is architecture and platform neutral, and open source. It provides multiple, synchronous video and sound communication channels and a mechanism for browser-based proxy-control of any software package that is source available. Access-grid’s hardware requirements and installation are quite demanding. In addition, Access-Grid requires a complex network fire-wall penetration agreement between the network security authorities of both the APS and the remote user’s home institution. Nonetheless, Access-Grid provides an extremely rich telepresence and the ability to expose virtually any program through a network browser interface.

NE-CAT will survey commercially-available and open source remote collaboration software packages and adopt a system that enables complete, but secure remote access to those beamline assets required for sample mounting (in the case of availability of automounter), beamline configuration and data collection. The selected package will not require heroic efforts
to install and special hardware procurements on the remote user’s part. NE-CAT cannot
 guarantee that mode 1) remote use will be extended to a first time CAT user or to APS general
 users, because of issues surrounding user beamline and safety training and software
 installation and validation.

D.1.3.2.7. Console server-driver state monitoring

Console requires a number of different control nodes to be fully functional and executing their
assigned Console Server Driver programs (CSD's). CSD failure results in loss of specific
functions or stalling of Console scripts depending on continuous access to a failed service.
CSD failure is a rare event, but overall system reliability requires a method for assuring continuity
of CSD service in the form of frequent, periodic monitoring of the health of all CSD's in a given
control network and a mechanism for automatically restarting and recovering the internal state of
a failed CSD.

NE-CAT has established a prototype CSD monitoring system based on a proprietary java tool
base intended for intermittent integrity probing of large server networks (PortSensor, Web Group
Media, Inc). The current prototype monitoring system informs beamline staff, via email and an
audible alarms system, when one or more CSD’s fails to answer a probe interaction from the
CSD monitoring system. The CSD monitor maintains a running log of all fault indications and
can be accessed by HTML-based clients from any browser to remotely check the health of the
Sector 24 CSD network. We will extend this system to automatically restart and recover faulty
CSD’s.

D.1.3.3. Optimal and efficient data collection

D.1.3.3.1. Integrated beamline control and data analysis

In collaboration with SLAC and LBNL, we will implement, install and maintain a new package,
WebIce, designed to integrate beamline control and data analysis tools, in order to facilitate
collection of high quality data. Optimal strategies for data collection are influenced by many
factors: some are general, and others depend on the particular characteristics of both crystal
and detector. Selection of proper data collection parameters is often a compromise between
several competing requirements. WebIce will help in this decision-making step.

Web-Ice is a browser originally developed to increase the level of automation of experiments
carried out at the SSRL Macromolecular Crystallography beamlines. WebIce is currently under
redesign to optimize data collection strategy and to facilitate remote monitoring and control of the
experiments. The current SSRL version of Web-Ice provides tools to 1) monitor the beamline
instrumentation and control panel via the beamline video system; 2) view diffraction images; 3)
perform a preliminary automated analysis of the diffraction pattern; 4) monitor automated
data collection on a single GUI. We will need to devote substantial programming effort to the complete integration of this package with ADSC detector software and our beamline control systems, but we believe that the outcome of this effort will make it much easier for relatively inexperienced users to solve

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difficult problems and to recognize when there are unanticipated crystallographic complexities that can otherwise lead to failed data collection sessions and unused data sets.

The implementation of WebIce on our beamlines is planned as a three step process: First, a standalone version of the software necessary only for determination of optimal data collection strategy will be tested and installed, in collaboration with Berkley and Stanford. This software is also being considered for implementation by GM/CA-CAT (Ward Smith), so it will be easier for us to test and troubleshoot together the initial software installation and testing. At the first stage, the beamline users will collect two snapshots (at 0 and 90 degrees) and the program will then immediately analyze the two images and estimate an optimal data collection strategy. The default optimality decision is based on several default parameters, such as I/sigma at highest resolution, desired resolution, or minimum data collection time. If users choose to provide any additional information about the size of the crystal, the solvent content and specific parameters of the beamline (such as slit size, flux, energy), a calculation of radiation dose and a rough estimation of radiation damage may be done BEFORE the real experiment starts. Radiation dose calculation prior to data collection will provide a way for users to obtain the maximum amount of useful information from a given crystal. Users will be presented with the calculated data optimal data collection strategy, which they can either accept or, if they choose, edit by changing the initial input model parameters. The WebIce software may also be “trained” for different data collection protocols, such as those optimal for molecular replacement, native data collection, or a SAD/MAD experiment. Our studies of strategy optimization will be carried out in collaboration with users during collection of data for actual structure determinations, and they will be followed by analysis of the data sets collected and of the final quality of the model resulting from the project.

In a second stage, after thorough testing, the working version of the WebIce will be fully integrated with the ADSC detector software and our CONSOLE beamline control software. This integration will permit the direct transfer of parameters, either estimated or edited by experienced users (such as new detector distance, exposure time or thickness of attenuators, starting phi-angle, oscillation range, number of frames, possibility of doing several runs with different flux, etc) to the ADSC detector and the corresponding beamline motors. Thus, after collecting the first two snapshots of the crystal, the user can start data collection just by pressing a “start optimal data collection” button, provided the optimality score of the estimated strategy is high enough.

In a third stage, the software will be fully integrated with automatic robotic applications, to provide tools for simple database management during multiple crystal screening and for use in remote data collection. These applications of WebIce are currently under development and testing at SSRL and are not yet generally available.

Users often wish to collect a large number of data sets from many different crystals. Fast computers now allow the user to index, integrate and scale data in real time during data collection, but the importance of monitoring the quality of the diffracted intensities and scaled data is often ignored. In all subsequent computational stages of structure determination, especially in the automatic ones, the quality of the primary data plays a very decisive role and determines the precision of the final model. Very often, a quick look at a “good” R-merge after scaling encourages a user to move on to another project or crystal. Because data collection is the final experimental stage in a multi-step project, and one that is often difficult to repeat, we believe it is important to spend time interpreting properly the initial diffraction images, in order to avoid subsequent mistakes.

We therefore propose to implement an additional, fully automated and rapid step, to assess the quality and characteristics of the processed dataset and to adjust the strategy of data collection while the crystal is still mounted on the goniometer. Obviously, the identification of hidden problems in the freshly scaled dataset cannot guarantee that the problem might be immediately solved, but any red flags can trigger a change in the data collection strategy or alert the user to apply special procedures for subsequent structure determination. Our approach is based on a
small set of subroutines created by the group of Paul Adams (83); the beta-version of these programs is available through the open source CCTBX libraries and is currently being tested at the NE-CAT beamline. The software, called XTRIAGE, performs many quick, basic tests of the quality of the experimental data: unit-cell content analyses, anisotropic Wilson scaling, Wilson plot analysis, exploration of the higher metric symmetry, outlier rejections, ice rings detection, anomalous signal analyses, detection of pseudo translational symmetry, and several twinning tests and statistics. We will create a simple program, which will monitoring the user’s process directory, so that once the scaled data file is created (by HKL2000 or MOSFLM), it will start an automatic check, using additional parameters gathered from the same directory. A pop-up window will present a short summary of the output of XTRIAGE, with an explanation and several decision-making suggestions for how to overcome problems or for possible strategy optimization. For example, detection of anisotropic diffraction or twinning, while the crystal is still mounted on the goniostat, may help users to change collection strategy to collect better or more complete data from the same crystal; an incorrectly indexed and scaled dataset may be fixed by pointing the user to the most likely point group (based on the calculations of R-values scoring function similarly implemented in LABELIT (79)).

Manual running of XTRIAGE on an average, scaled dataset now takes less than 5 seconds, so the background daemon to run this software will not slow down our computers. The software can read both scapack data and .mtz files. A similar approach is also now implemented in the newest version of CCP4 (i.e. SCALA and TRUNCATE), but the data statistics are well hidden in the long output log-files and they are difficult to retrieve by inexperienced users. Moreover, HKL2000 is now de facto the software of choice for data processing at synchrotron beamlines, and a simple script to quickly calculate statistics on scaled data (scalepack .sca) will be very useful. The proposed development of the data analysis script is based on a modular approach, so if we wish to include (or a user requests) any additional test/statistics, it will be very easy to incorporate the relevant routines into existing source code. For example, we will include in this script the ability to calculate redundancy-independent and precision-indicating R-merge factors, as suggested by Diederichs and Karplus (84) and Weiss (85). These statistically correct R-merge factors are believed to be more objective and meaningful global indicators of X-ray data quality, since there are too many ways to decrease the value of the “usual” R-merge.

D.1.3.3.2. Data collection using multiple crystals or translated crystals

There are many cases in which the complete data set cannot be collected from a single crystal, mainly because of the high radiation damage (examples include microcrystals, virus crystal data collection at 0 °C, etc). The easiest scenario to handle is illustrated by diffraction data collection from a small, needle-like crystal (e.g., 10 μm x 10 μm x 200 μm). In this case it is possible to cut the beam down to 20 μm x 20 μm, or even smaller, and to collect ~ 10 consecutive data sets, each from a fresh portion of the same crystal (assuming the crystal needle is oriented nearly perpendicular to the beam). The naive data collection strategy, starting the oscillation range for a fresh portion of the crystal at the angular position at which the last range terminated, is not always the optimal one. Indeed, examination of a so-called “leapfrog” strategy in most symmetry groups, especially low-symmetry ones, suggests data collection to be more optimal when using separated oscillation regions (better completeness in a shorter total oscillation range). The software STRATEGY (86), which is now a part of MOSFLM, reads the starting crystal orientation and cell parameters from the ”.x” denzo files, simulates all recordable reflections during full 360° crystal rotation and calculates the optimal strategy for the desired data completeness. We will make small changes in the code, to take into consideration the estimated number of separated region for data collection and to make the script more user-friendly. This strategy was recently checked and proven to produce good data with higher than expected completeness from extremely radiation sensitive crystals of E. coli ribosomes (J. Cate’s group).

Another more difficult case involves data collection from different crystals, mounted in different orientations (these problems will be extremely important for microcrystals, with or without the microdiffractometer). First, STRATEGY is run to get the optimal starting oscillation angle for the
first crystal. Data are then collected, integrated, and properly scaled. The next crystal is mounted and its orientation determined; the software, taking into consideration the available scaled data from first crystal, calculates the new optimal starting angle for data collection from the second crystal. All data are properly scaled and used as input for the next round of strategy calculations. These steps are interactively repeated for all subsequent crystals until a full data set has been collected with the desired completeness and redundancy. At the end, it is also necessary to check also that the redundancy is uniformly distributed.

D.1.3.3.3. Automatic crystal recognition and centering

Crystal centering, generally by manual adjustment using visual recognition, has been a source of difficulty at synchrotron beamlines since their inception. Contemporary technologies allow implementation of automated centering procedures, including location of the crystal in the loop and alignment of the crystal with the X-ray beam. There are many software packages for image recognition, and several of them have already been applied specifically for crystal recognition, especially in connection with crystallization robots. These approaches allow detection of sub-micron crystals in a clear crystallization drop. Cryocooled protein crystals mounted in different loops pose a much greater challenge, however. There are a large number of difficult situations, including highly transparent and plate-like crystals, bad freezing with a lot of ice on the surface, invisible crystals because of non-vitreous drop, inclusions of non-crystal artifacts, and lens effect due to the geometry of the drop.

There are currently two main crystallographic software suites for automated crystal centering: XREC (87) and C3D (88). XREC uses a combination of four different texture-based algorithms, which are targeted towards direct identification of the crystal rather than the loop or surrounding liquid. To increase the accuracy of the estimated coordinates of the crystal center the result from four different image recognition algorithms are weighted dynamically. The XREC software was tested on 104 sets (containing eight or nine images with 40° increments) and showed ~78% success rate. It is a standalone software, which is available from EMBL.

C3D software implements the idea of recognizing what the human eye uses to detect the crystal: crystal edges and its geometrical shape. The software implementation uses the Canny edge-detection algorithm, and the scoring system is based on the Radon transform. C3D has a longer (as compared to XREC) history of use at different synchrotron beamlines and smooth integration with microdiffactometer MD2. C3D is also a standalone program. It carries out the processing of the images, and it operates with client programs associated with the visualization cameras, the goniometer, and the lighting devices. The software is continuously being evaluated and optimized using the growing annotated database of crystal images. The software is freely available from EMBL for non-commercial use.

We will implement one or both of these programs in two steps. First, we will carry out broad testing of both software packages, using an available library of crystal images from the BIOXHIT image database and from our own collected set of frozen crystals. Based on this research, the software will be “trained” and optimized for current beamline hardware (cameras properties, illuminations etc). The second step will involve integration of the software to the current beamline control software (CONSOLE). We expect that implementation of one or both of these software packages will increase user productivity, both by automatic centering of the crystal while the hutch is being searched, and by fully automating robotic screening of crystals.

D.1.3.3.4. Sample/Spindle auto-alignment software

Maintaining alignment between micrometer-scale X-ray beams and the goniometer axis can be problematic, even at a synchrotron source as reliable and stable as the APS. The positional stability of the white beam varies with the fill mode of the accelerator. Furthermore, the Sector 24 white beam can undergo significant excursions from its normal steering point when a sector
upstream from Sector 24 requests local steering changes, or when the APS accelerator global and local steering systems suffer some temporary degradation in performance.

The current method used to align the phi axis to the beam is a wire scanning procedure. A fine tungsten wire is aligned to the spindle and fluorescence from it is monitored by a scintillation detector, situated upstream of the spindle axis, as goniometer is translated such that the tungsten wire transits the entire vertical extent of the beam. The resulting fluorescence scan profiles are typically quasi-gaussian and are proportional to the convolution product of the wire surface emission and vertical beam intensity profiles. The scan is repeated for 2 or 4 orthogonal phi orientations. The second moments of the scan profiles are averaged and the goniometer translated to the corresponding vertical position. This procedure is insensitive to misalignment of the wire to the spindle axis, takes a few minutes to execute and is accurate to order 1 μm. However, installation of the wire involves a hutch entry and removal of mounted samples from the spindle.

In section D.1.2.2, we discussed plans to improve the accuracy and efficiency of our beam position measurement technology. Here we describe software developments for 1) automatic alignment of the vertical position of the goniometer spindle axis to the beam or 2) optimization of the sample alignment to the spindle axis, using sample diffraction data as the source of steering information. Both alignment procedures will adjust the relevant axis position(s) by “symmetrizing” integrated intensities of corresponding low order reflections from pairs of diffraction images separated by a phi rotation of 180°. The proposed method is not completely original, and similar methods have been proposed previously (e.g. W. I. Karain et al. (89)).

Our alignment optimization scheme involves two cases:

**Case 1): Optimize vertical alignment of goniometer phi axis to X-ray beam.**

Sample presumed to be aligned to the goniometer spindle.

1) Beam is heavily attenuated (~90%).
2) Two short exposures are acquired, separated by a phi rotation of 180° (hereafter called image pairs).
3) Low order diffraction spots (inside water ring) are discriminated against scattering background.
4) The radial dependence of scattering background is calculated, for image pairs, filtering out all pixel intensities within a given radius of discriminated diffraction spots.
5) Discriminated diffraction spots are box integrated and background corrected using the radial background intensity function to estimate local background for diffraction spots.
6) The average of intensity ratios between symmetric spot integrals in image pairs (symmetry ratios) is calculated.
7) The vertical position of the goniometer is stepped by a small increment and steps 2-6 of the scheme repeated.
8) If the goniometer steps in the direction that improves beam alignment then the average symmetry ratio converges to 1, else step direction is reversed.
9) Optionally, after locating the vertical goniometer position that minimizes the difference between unity and the average symmetry ratio, the starting phi axis position is incremented 90° and the entire process repeated.

**Case 2): Optimize alignment of sample to phi axis.**

Spindle presumed to be aligned to the X-ray beam and the sample is close to perfect alignment.

The Case 2) alignment algorithm is identical to that of Case 1), except that the spindle sample x-y- axes are stepped rather than the position of the goniometer (see Figure D.1.3.7).
The principal benefit of the proposed methods (beyond automation) is that no alteration to mounted samples is required for their application. The down side is potential radiation damage to the sample resulting from the potentially numerous exposures required by the method.

Radiation damage is minimized by high attenuation and short exposures used in the scans and by optimizing the overall logistics of the scanning procedure.

There are many alternatives to the optimization objective function (symmetrization of intensity ratios) and simple grid stepping system discussed above. For example, correlation analysis could replace the ratio optimization. Azimuthal blocking of data and segmentation of background profile may be required to account for azimuthal inhomogeneities in the scattering background field. More efficient search techniques like gradient techniques (linear-descent methods) require far fewer steps than simple grid stepping.
The method described above is not intended to replace optical sample alignment methods but to serve as a means of automatic re-alignment of misaligned samples or the goniometer rotation axis (to the beam). Sample diffraction-based alignment optimization could also be incorporated in a multi-tier sample alignment protocol that first uses optical methods (e.g. the C3D program, B. Lavault et al) to obtain an approximate sample-beam alignment and then diffracton based techniques to optimize it. Under this constraint, the “size” of the space that must be searched to realign (or optimally align) the sample or spindle is much reduced, and concomitantly, the radiation dose imposed by the scanning process.

D.1.3.4. Data processing and scaling in non-standard cases

D.1.3.4.1. Data processing and analysis for microcrystals

a. Obtaining integrated intensities: profile fitting

In the description of the microdiffraction core, we distinguish between a microfocus geometry, in which the beam is sharply focused on the crystal and then diverges, and a microbeam geometry, in which the low divergence of the current optical configuration is preserved, reducing the spot size with a suitable aperture to match the crystal dimensions. In the former case, the spot shape at the detector will generally be determined by the beam divergence convoluted with the mosaic characteristics of the crystal, and the profile fitting procedures currently implemented in HKL2000 or in MOSFLM can probably be applied directly (90). In the latter case, however, it may be possible to obtain more accurate measurements of weak reflections with alternative integration methods. Preliminary collaborative work by the Harrison group with a 20-25 µm beam and different types of crystals leads to the following conclusions. (a) If the crystal is significantly mosaic (as is often the case with “frozen” crystals), then the spot shape is determined by the mosaic profile of the crystal, the size of the block (often large enough not to contribute) and the angular spread. That is, the beam is so narrowly collimated that we can detect the contributions of the crystal properties to the shape of the spot. One can parameterize these properties, refine the parameters for all spots, and thus obtain a good model for the (substantial) variation of spot shape with position in reciprocal space (Figure D.1.3.8). The point-spread function of the detector (about 80 µm FWHM for the Q315) must also be determined, as it convolutes with the actual spot profile. (b) If the crystal has little or no detectable mosaic spread, as is frequently the case with “unfrozen” virus crystals (Figure D.1.3.9) then the spot shape is determined by the beam profile (and the detector point spread). In this case, the “profile” may depend on the position of the spot centroid with respect to the pixel lattice, and profile fitting may be less accurate than summing values within a certain mask and subtracting a suitably scaled background. We will carry out a systematic exploration of these issues, once the MD2 is fully commissioned, and we will modify data-processing software to reflect what we find are optimal strategies.
**Figure D.1.3.8:** Diffraction pattern taken at 24-ID-C with a 25x25 micron beam (1 degree oscillation; crystal-to-detector distance = 350 mm) from a cryopreserved dengue-virus envelope protein crystal, space group P3\(_1\)2\(_1\)1, a=b= 81 Å; c= 287 Å. The crystals are needles, about 25 microns in the short direction and 100-150 microns in the long direction. The diffraction is correspondingly anisotropic. In the upper right is a magnified view of reflections from the row designated by arrow a. Note the elongation of the spots in a direction roughly perpendicular to the c* axis. In the region designated by the arrow b, the spots are enlarged, but more uniformly (see magnification on the left). The spot shapes can be predicted from the anisotropic mosaic spread, the mosaic block size, and the way the spot passes through the Ewald sphere. Collaboration with E. Settembre, A. Schmidt, and S. Harrison.

**Figure D.1.3.9:** Oscillation frame (0.1 degree) taken at 24-ID-C from a crystal of capillary mounted rotavirus inner capsid particles (ICPs), recorded at 0°C. Space group P2\(_1\)2\(_1\)2\(_1\), a = 740 Å , b = 1198 Å, c = 1345 Å. Crystal-to-detector distance = 800 mm. On the left is a magnification of the region in the box on the full figure, and a further magnification of one spot is also shown. The full width at half maximum of the spot is slightly less than 4 pixels, or about 180-200 microns. It varies little across the entire pattern. Collaboration with E. Settembre and S. Harrison.
b. Collecting and scaling data from multiple crystals

For very small crystals (e.g., 10 µm), of the size we expect to study routinely with the MD2 on 24-ID-E, it will generally be necessary to assemble a complete data set from several crystals. Assuming that all are soaked in cryoprotectant at the same time and frozen in the same loop or grid, then we can anticipate that they will be closely isomorphous. The simplest procedure will then be to move from crystal to crystal, calculating orientation from two orthogonal frames from a new crystal and selecting a rotation range by running the data-collection strategy program to be developed as described in section D.1.3.3.2. We will implement control scripts for the MD2 to incorporate these procedures and to make them as straightforward for the user as possible.

For scaling data from multiple crystals, it is important that parameters such as mosaic spread be refined for the entire set of frames corresponding to one crystal and that the scale factors and “thermal” parameters (B’s) be consistent from frame to frame within a set from one crystal. We will modify the scaling programs if needed, in order to be sure that the parameters obtained during scaling and postrefinement are constrained correctly.

D.1.3.4.2. Non-merohedral twinning

There are many tools available to detect and handle, merohedral and pseudo-merohedral twinning. Todd Yeates’ twin server, the detwin program of CCP4, and the twin refinement methodologies of SHELEX and CNS are a few of the many that could be mentioned. However, there are not many tools to handle non-merohedral twins. TWINABS (91), CELL_NOW (91), GEMINI (92), SAINT (93) and TWINLAW (94) are among some of the recent developments dealing with non-merohedry, but most of these programs are written for small-molecule crystallography. Though some of these tools could be useful for macromolecules, detailed visual inspection of diffraction pattern is still the best way to identify and resolve non-merohedral twinning in macromolecules.

In a non-merohedral twin, the reciprocal lattices of different twin domains do not overlap exactly. Auto indexing routines often fail to report the real cell. A pseudo cell with at least one of the axes being a multiple of real axis or a wrong indication of higher symmetry (e.g. a real monoclinic cell being reported as a orthorhombic cell) are some of the results of non-merohedral twinning. Data sets with such symptoms are often discarded.

We will implement tools to identify non-merohedral twinning and process the intensity data in such cases. We demonstrate some of our plans using one example, a structure determined using data collected at beamline 24-ID-C. The example comes from thiamin binding protein (TbpA), with a tetramer of ~140 kDa (35 kDa per protomer) in the asymmetric unit. Crystals of seleno-methionine substituted TbpA diffracted to 2.5 Å, but all crystals were found to be non-merohedrally twinned (see below).

Figure D.1.3.10A shows part of a diffraction pattern from a non-merohedrally twinned TbpA crystal. Auto indexing routines had difficulty in indexing these data sets. Choice of right image and right resolution cut-off would arrive at an indexing with the c axis either two or three times the real c value, depending upon how peaks were chosen (Figure D.1.3.10B). Close inspection of the predictions showed that many had no intensities and that many spots were not well predicted. Based on visual observation of the peaks and predictions, the real c axis could be derived and input by hand into the program to force that value. Even with the correct cell, there were many spots that could not be accounted for from a single lattice, but once non-merohedral twinning was taken into account and two twin domains indexed independently, all spots could be accounted for. Figure D.1.3.7C and D show reciprocal lattices of the two non-merohedral twin domains indexed independently. The structure of TbpA could be determined from data with only one of the twin domains. Figure D.1.3.8 illustrates a selected region of the experimental electron density for TbpA.
It is well documented that such non-merohedral twins are not uncommon (95-98). Fortuitous cell lengths and angles associated with unexplainable spots and systematic absences are some indications of non-merohedral twins. For e.g. a primitive monoclinic unit cell with fortuitous values of $a$, $c$ and $\beta$, so that $4.a.\cos\beta+c=0$ could be non-merohdraly twinned with a two-fold twin relationship, leading to an overlap of reflections with $l=2n$, which means half of the reflections will include contributions from both twin domains and rest of the half will have contribution from only twin domain. TbpA has similar fortuitous cell, which satisfies $11.3.c.\cos\beta+a=0$. This relationship results in reflections on every $6^{th}$ (approximately) layers along $a^*$ to overlap (See Figure D.1.3.10C and D as well as Figure D.1.3.11). It was approximately $6^{th}$ layer, because the twin was not that perfect (note the 11.3 factor in the relation which is not quite 12). This resulted in approximately $1/6^{th}$ of the reflections to be perfectly overlapped. A twin model for TbpA is provided in Figure D.1.3.12.

(1) What can we learn from TbpA? As seen from Figure D.1.3.10, for a cell with three times real $c$ axis, if we count intensities of spots, reciprocal spots that are due to artifacts of twinning will have no or extremely small intensity counts. Indeed there will be some kind of systematicity in the strong/weak distribution. One can systematically analyze the strong/weak distribution to derive the real cell. However, care has to be exercised not to confuse non-crystallographic translational symmetry to be non-merohedral twin, because such symmetry also would give rise to a distribution of alternating strong and weak spots.
(2) We will develop routines to determine the twin law. Two twin domains when indexed independently will provide two orientation matrices, say $U_1$ and $U_2$. Twin operation $T$ will transform $U_1$ to $U_2$, i.e. $U_2 = T U_1$. Twin law $T$ can be derived from the product $U_1^{-1} U_2$.

(3) From this twin law we will predict spot overlaps and de-convolute the overlapping reflections.

(4) If data from two twin domains are scaled together, their relative scales will provide an estimation of the twin fraction of each domain.

(5) We will also consider the method employed by Liang et al (1996) to visualize the peaks graphically using a graphical visualizer such as Geomview (www.geomview.org).

D.1.3.4.3. Radiation Decay and Absorption Corrections

a. Radiation decay.

Radiation damage is a major source of systematic errors in the data collected at high brilliance synchrotron sources. We will therefore develop optimized data scaling procedures to reduce effects of radiation damage during routine oscillation data collection. Originally the method was developed to partially correct the diffraction intensities during data reduction of highly redundant data (99). The computational method consisted of redundancy-based zero-dose extrapolation of an exponential decay function, as currently implemented in XSCALE (part of the XDS package). We will integrate this software on our computers and provide easy scripts to run it. We will modify the source code, as needed, to take into consideration any non-linear behavior of the effects of radiation damage (especially at early stages of data collection: we have observed in some
cases a non-linear effect of radiation during first 40-90 seconds of data collection, after which the radiation decay is slower and linear with time).

There are several limitations of the method just described: the most important is that for appropriate analysis of the time dependence of the intensities and sigmas, a high redundancy of measured reflections is necessary. This requirement limits the application of either to crystals belonging to high-symmetry space groups or to properly optimized SAD experiments that need highly redundant data to extract very low anomalous signals (e.g., from sulfur or phosphorus). We will therefore perform systematic studies to search for the best theoretical model to realistically represent the intensity changes at different stages of radiation decay at different energies. Such a model will be useful for correcting the experimental intensities for radiation damage, when a low redundancy data set precludes independent fitting of a model. We will also consider specific modeling of radiation damage in low-energy data sets as an approach to enhancing the weak anomalous signal for sulfur/phosphorus phasing.

b. Absorption corrections

Structure determination using weak anomalous scatterers like sulfur usually requires data collected at longer wavelengths, typically higher than 1.5Å. Such experiments provide rather weak anomalous signal, hence very accurate data are essential to derive phase information. Metals such as Zn, Cu, Co and Fe, functional or structural elements in some proteins, also have absorption edges at longer wavelengths. To make use of such atoms for phasing purposes, data collection at longer wavelengths is necessary. At these wavelengths, anisotropic absorption effects introduce significant errors in the measurements. Correction for absorption anisotropy may be applied through empirical approach as proposed by R. Blessing (100) and implemented in several data processing software packages. We have shown (ACA meeting 2006, P016) that significant improvement in data quality can be achieved by applying empirical absorption corrections. This approach needs to have enough redundancy of measurements, however, and should be used with care. We will continue our research on practical applications of automated analysis of absorption correction as implemented in different software packages, such as HKL2000, SCALA, XABS2 etc. and we will provide user-friendly scripts for proper absorption correction calculations with intelligent decision-making protocols. Our goal is to facilitate practical use of different absorption correction calculations for improving data quality.

D.1.3.5. Efficient Data Management

D.1.3.5.1. Beamline Computer Network and Infrastructure

Figure D.1.3.13 is a diagram of the current Sector 24 data collection and data flow system. The system now includes two Area Detector Systems Q315 detectors, servicing 24-ID-C and 24-ID-E, a high performance Storage Area Network (SAN, Hewlett Packard EVA 5000) with 30 Terabyte of disk space and a number of file servers and workstations.

Control and data flow within the system is mediated by both gigabit Ethernet and fiber channel networks. Each compute node and the Q315 detector controllers contain both Gigabit Ethernet and Fiber Channel Host Bus Adapters (HBA). The EVA 5000 SAN can communicate with attached workstations and detector systems via either network in the event of failure of the external fiber channel fabric. The default configuration of the system uses fiber channel as the data flow medium. The EVA 5000 itself possesses two-fold redundancy in all of its internal fiber channel fabric elements as well as its disc controllers.
Figure D.1.3.13: Sector 24 Data collection and data-flow system. Red-outlined components represent the 24-ID-C and 24-IC-E ADSC Q315 detector systems and “online” user workstations associated with each detector installation. The magenta-outlined system is the Hewlett-Packard EVA5000 fiber channel SAN and fiber channel switching fabric. The upper green block indicates the existing “offline” workstation area. The lower green block represents the planned Beowulf compute cluster. 100 Gigabit copper Ethernet connectivity lines are rendered in violet, while fiber channel optical connections are shown in blue. Ethernet switching elements are not shown.

Both Q315 detectors are controlled by a dual xenon processor node (controller node) with a built-in 1.5 Terabyte RAID disc array, used as emergency disc store in the event of failure of the EVA 5000 SAN or data flow network. Each of the nine CCD elements of the Q315 is interfaced to a winXP-based frame buffer via a proprietary optical link (Q-link). The nine frame-buffer nodes communicate with the Q315 controller node via a dedicated Gigabit Ethernet network. Each frame buffer node contains two gigabytes of local memory, 1 gigabyte of which is allocated as a data cache for “sub-frames” downloaded from the CCD attached to the frame buffer node. Upon download to the frame buffer, subframes are corrected for response and geometry artifacts and sequentially accumulated in each frame buffer’s cache. One of the threads of execution of the Q315 controller node asynchronously composites corrected, cached subframes from the nine frame buffer nodes and forwards composited frames to the EVA 5000 SAN or the local disc store. Local correction processing and caching of data subframes decouples the overall data flow rate from the write-speed limitations of the working disc store, at least until saturation of the 1 gigabyte cache of the frame buffer nodes occurs. The maximum data frame rate of the Q315 detectors under NE-CAT’s data flow architecture is 28 frames per minute with 2x2 binning (18 Megabyte frames) and 18 frames per minute, unbinned (72 Megabyte frames), with a 0.1 second data exposure.

The EVA 5000 SAN (see Figure D.1.3.14) has been upgraded from its original 15 Terabyte disc space to 30 Terabyte using high performance 300 Gigabyte fiber channel disc drives. The EVA 5000 disc store is configured as a level-5 RAID so that individual disc failures have negligible impact upon data flow within the network, as long as no more than two simultaneous two disc
failures within a single bank of discs occurs. Hewlett-Packard provides a volume management system with the EVA 5000 that enables beamline staff to easily reconfigure disc partitions (called volumes) and control the exposure of disc volumes to work stations associated with either undulator beamline and external users.

**Figure D.1.3.14:** Photograph of the EVA 5000 SAN system. There are 12 banks of disk, currently populated to yield 30 Terabyte of useful disc space. The dual disc controllers are situated in the middle of the rack. The main external fiber channel fabric switch fills the uppermost rack slot. We can expand useful disc space to ~ 50 Terabytes, without adding additional disc banks and power supplies. The entire system will be transferred to one of the LOM laboratories in early 2007 to reduce particulate inhalation by the SAN. The rack to the left of the SAN enclosure houses the data flow system's NFS servers and management systems for the EVA 5000. The rack to the right of the SAN will house the compute cluster.

All elements of the Sector 24 Data Flow Network are commissioned with the exception of the "compute cluster" which is currently under design. The compute cluster will consist of a commercial. Linux-based 32-node Beowulf cluster (Penguin Computing) that will support bulk data reduction and analysis operations of the sector. Each node of the cluster will consist of a dual-core 64 bit processor with a minimum of 2 Gigabits of local memory, with dual Gigabit Ethernet and fiber channel HBAs.

The General Parallel File System, developed by IBM has been fully implemented and tested in the Sector 24 data flow system. GPFS enables all compute nodes interfaced to the SAN to directly and concurrently access stored data without mediation by a file server. Offsite users with appropriate APS fire wall penetration privileges can still access the EVA-5000 SAN data store via NFS, which gracefully co-exists with GPFS.

Conversion from NFS-distributed file systems to GPFS has resulted in modest improvement in data acquisition throughput and very substantial gain in the speed of data reduction and analysis. Figure D.1.3.15 documents this gain by comparing the scaling behavior of GPFS to conventional NFS.
D.1.3.5.2. On-site Structure Analysis

On-site structure analysis is a valuable adjunct to all our beamlines. During a single visit, users collect data for projects of varying complexity, including some “routine” structure determinations even when the main purpose of the visit is a very difficult crystallographic challenge. Moreover, in many cases of very complex problems, such as those with tiny crystals, the structure determination itself can be relatively straightforward, once good data have been obtained. We aim to increase the productivity of the beamlines by facilitating and accelerating on-site phasing and preliminary model building, so that an accurate assessment of results can provide feedback for selecting the next crystals to study during a single visit. Even a partially built structure will minimize the number of collected data sets and decrease the overall synchrotron time required for structure determination.

We are currently exploring different software packages for fully automated structure determination pipelines that deal with all steps from diffraction image data processing to structure determination without customized scripts or format conversion. Several fully automated suites are under consideration, including X-solve (Ashley Deacon), ACrS (Wayne Anderson) and HKL3000 and HKL2000-PH (Wladek Minor). These automatic or semi-automatic pipelines combine different sets of existing crystallographic programs and differ mostly in their GUIs and in the decision-making protocols and structure solution pathways. It appears that the most advanced software is HKL2000-PH with a very streamlined approach to image integration, phasing and model building. Intuitive user interface and navigation, limited choice of input parameters, real-time data analysis and use of very powerful underlying crystallographic packages will increase user productivity, especially with less experienced users (even from very experienced laboratories!). Until we have committed to implementation of HKL2000-PH, we will focus on providing full support for several other powerful semi-automated software packages (such as PHENIX, AutoSHARP, SGXPRO, BnP, etc.), which start automated structure determination at the stage of scaled hkl data. Several dozen structures have been determined within hours of data collection at beamline 24-ID, using available common crystallographic software. In most cases that was done with the guidance of experienced beamline support.

Figure D.1.3.15: Comparison of GPFS to NFS scaling performance. The same 100 frame unbinned data set (~76 MByte/frame) was stored on GPFS and ext3 file systems. Upper trace shows execution time for HKL2000 to integrate and scale the data set stored on GPFS as the number of workstations concurrently performing the data reduction on the same data set increases from 1 to 6. Lower trace shows performance of NFS under the same scaling regime.
personnel. To help in this aspect of user training, we will create sets of well-documented, user-friendly scripts for the right choice of input parameters for the existing crystallographic software. In addition, we will include a detailed separate crystallographic computing section in the beamline user’s manual.

We will also continue a different level of collaboration with software engineering groups developing new automated pipelines (e.g., SER-CAT, SLAC), and we will beta-test future “black box” structure solution software, as it becomes available.

D.1.3.5.3. Parallel Computing Applications

Parallel computation will be essential for efficient on-site data reduction and structure analysis. We will therefore acquire a 32-node (minimum) multiprocessor LINUX cluster (see D.1.3.5.1) for CPU-intense crystallographic calculations, to make structure determination a nearly real-time process. Communication within the cluster will be mediated by a gigabit dedicated Ethernet fabric, and duplex-1 gigabit fiber channel will connect the cluster to our existing EVA 5000 SAN. The cluster will free the main beamline computers for the most important tasks of a real-time strategy estimation and data integration and scaling.

We will use open-source clustering tools to manage the cluster and share its computational resources over all NE-CAT beamlines. One attractive management solution is OpenMosix (101). OpenMosix is a linux kernel module that provides fully automatic or managed process assignment and load balancing over an ethernet-connected cluster. From any workstation, a user initiates processes on an executive (master) Mosix cluster node. The Mosix executive then migrates the new process to some node within the cluster such that overall memory and processor cycle utilization are load-balanced across the entire cluster. Mosix nodes continuously communicate with each other and with the executive to provide resource utilization information required by the load balancing algorithm running on the executive. The process migration and load balancing system have extremely low overheads and are entirely transparent to the user. No modification of our code base is required to run it on a Mosix-managed cluster. OpenMosix provides a rich set of tools for monitoring cluster node health and loading. Mosix gracefully co-exists on clusters with the common message-passing parallel computing systems such as MPI and PVI (although adaptive load-balancing process migration does not improve execution performance of MPI or PVI codes).

Parallel computational tasks will include calculations with wide input parameter space searches (such as multiple resolution cutoffs or different seeds for SHELXD), intense parallel calculation of the composite omit maps, and various possibilities for combining programs in different pathways as implemented in the SGXPRO suite (collaboration with SER-CAT). The current version of SGXPRO was designed and developed to automate structure determination using multiprocessor LINUX clusters. SGXPRO has a built-in parallel algorithm and workflow engine to automate and manage communication between different processes. It can build systematic searches of different computational algorithms and a range of input parameters to generate the best possible result for a given data set. The initial testing of SGXPRO on the SER-CAT beamline computational cluster showed a substantial increase in productivity. The multiprocessor LINUX cluster will also be useful for running in parallel several CPU intensive instances of model refinement and rebuilding, as implemented in ARP/wARP and in the PHENIX project.

D.1.3.5.4. Database Management for Robotic Applications

NE-CAT is frequently visited by groups with projects that require screening of hundreds of crystals in order to find just one with acceptable diffraction. An easy-to-use database and associated software to automatically mount and align the crystals, collect a few snapshots, analyze them, and produce a score will greatly reduce the burden on these users. We will therefore automate as much as possible the collection and processing of data and streamline
the process of automatic screening and ranking of the crystals based on their diffraction properties. We will also augment robotic usage with appropriate database management tools to organize and analyze the large quantities of data obtained with the use of the robot.

Current crystallographic database management ranges from a rather simple, EXCEL-like sheet to huge multi-functional Protein Information Management Systems (PIMS) (www.pims-lims.org). We do not expect to need the huge data bases that are required for structural genomics projects (the maximum is likely to be several hundred crystals per user-group visit), so we will start with a simple, EXCEL-based database, which should be fully integrated with the control robot software and the beamline data collection software. With the “Screening” interface of Blu-Ice, the user may select several samples of interest and define the data collection action to be performed on each sample. Once started, the script should allow unsupervised data collection of selected crystals. We have successfully interrogated our crystal mounting robot with the Blu-Ice package installed at 8-BM (see C.8.3.4.). The next step will be to associate the Blu-Ice interface with the EXCEL spreadsheet, containing information about the different crystals, and with the crystal centering module. This approach is similar to the SSRL robotic application.

Another, more advanced approach will be to populate the spreadsheet database not only with the names of the collected snapshots, but also with additional crystallographic information after rapid processing of the first few snapshots, thereby allowing the system to provide a relative score for each crystal. Most of the analysis of the collected snapshots will be done using the integrated WebIce software (see D.1.3.3.1), and the following information will be entered into the database for every successfully mounted crystal: maximum resolution, I/sigma, unit cell parameters, mosaicity, quality of the spots, ice rings, Bravais Lattice, detector parameters, expected radiation damage, possible strategy for data collection, and a final score. After screening of all crystals in a particular batch by the automated robot, the user (or, in fully automated cases, the system) can use the score to enhance rational decision making. After selection of the good crystals using data in the spreadsheet, the robot will mount again the selected crystals, the software will propagate the optimal data collection strategy to the beamline control software, and full data sets will be collected for all selected crystals without further users intervention. In the implementation of this approach, the most challenging part will be the integration of several different software packages into one fully functional suite. Similar integration has been accomplished at several beamlines (mainly at ALS beamlines 5.0.1 and 5.0.3), so we are confident that this can be done successfully. The database management feature of the WebIce is under intense development at SSRL and LBNL, and our collaboration with Nicholas Sauter will be important for full implementation of this valuable tool.

D.2. Collaborative Research

Section D.1 describes three core technologies that will be developed by the Resource. A total of 11 collaborative projects described in section D.2 will drive the development of the core technologies. The collaborative projects represent a wide variety of technically challenging crystallographic problems and as well as a wide variety of biological applications. Each core collaborator was chosen for a particular core; however, it is often the case that a collaborator will interact with additional cores. Table D.2.1 shows the relationship between the core collaborators and the three core technologies. The table also shows the Resource staff member with whom the core collaborator will directly interact.
D.3.1.2. Rights of the Beamline

As stated earlier, the beamline representative can reject a proposal on technical grounds or conflict of interest. The beamline is required to offer no more than the normal capabilities of the beamline. In regard to scheduling, as long as the lead time is reasonable, any CAT is allowed to schedule CAT members prior to contacting and scheduling the general users. The beamline is also allowed to choose their distribution of the type of beamtime that is given out. In the case of macromolecular crystallography, the percentage of rapid access beamtime can be chosen.

D.3.1.3. Plan of Action

Approximately six weeks prior to the start of the next cycle, representatives from NE-CAT institutions will be asked to submit beamtime requests. Requests from collaborative scientists will be given first priority in scheduling. The next priority will be given to NE-CAT and general users with technically challenging projects. The remaining slots will be filled by NE-CAT and general users with routine crystals.

D.3.2. NE-CAT

D.3.2.1. Time Available

The NE-CAT institutions will control the allocation of 90.5 days per station per year, or 30 days per trimester. The beamtime is divided evenly giving each of the seven institutions 4.5 days per beamline per trimester. Scientists associated with NE-CAT are also eligible to apply for general user beamtime through the APS. This beamtime is allocated independently of NE-CAT.

D.3.2.2. NE-CAT Participants

NE-CAT is comprised of seven member institutions: Columbia University, Cornell University, Harvard University, Massachusetts Institute of Technology; Memorial Sloan-Kettering Cancer Center, Rockefeller University and Yale University. The number of principal investigators per institution varies; however, the beamtime is allocated equally to each institution.

The amount of time available for the upcoming run will be announced to the membership approximately two months prior to the run. It will be the responsibility of each of the member institutions to govern its own members. Approximately six weeks prior to the start of the run, requests for NE-CAT time will only be recognized from an representative appointed by each of the institutions. A list of the names of the contact person, the amount of time, and assigned beamline will be submitted to the NE-CAT Administrator, Cyndi Salbego. Institutions are encouraged to combine two or more investigators in one visit. This would give the institutions the flexibility to break a one time day time period into any size shift. The local staff at the synchrotron will not be involved in the distribution of time within a member institution. The NE-CAT staff will control scheduling.

D.3.3. User Tracking Database

NE-CAT worked with BIO-CARS CAT to adapt their user program database. This web based database was constructed to make requesting beamtime easier for the users, and reporting on that usage much more accurate.

The original database was modified slightly to reflect NE-CAT as its owner. The database was given a more user friendly, dynamic drop down menu, some of the policies of the program changed, and the color palette was made more neutral.

This database can keep track of all the groups that come to our beamline. It contains group members and all their personal information, samples, chemicals, and equipment that they have
brought or anticipate bringing on future visits. This information never has to be re-entered by the user and can be modified/updated as needed. With the database, we can also send out mass mailings asking people if they needed some of our available time. Messages can also be sent to groups letting them know what publications we have on record for them and if there are any new ones that they would like to report.

Communication between staff about beamtime schedules can be easily made through the database. “Straw” schedules (Figure D.3.3.1) can be completed to make sure we are using all the available beamtime efficiently, with an official schedule of that time taking place when confirmation has been made with user groups. Groups requesting time can be given an accurate description of available time on the first contact with them. Rapid access time can also be left on our schedule for crucial experiments in a short time frame.

![Figure D.3.3.1: A sample "straw" schedule.](image)

For our reporting purposes, we can keep track of their requests for time, along with time that was scheduled and used. We have an area for publications to be entered. This allows us to generate a report more easily.

Step by Step through the Database

A new group would register at www.NECAT.org with a group name, password, primary email address, group PI, and institution affiliation. This will generate an automatic email to the email address entered with a secure URL that includes their access key. This URL and access key allow them to securely login to the database (Figure D.3.3.2). This URL and password are meant to be shared within the group, for all the people who would need to be able to have access.
Once the group is established, personnel can begin filling in group members. Each person enters his or her name, position, highest degree obtained, citizenship, and gender. There is a link associated with citizenship directed to the APS website with instructions on what needs to be completed based on the country of citizenship. It also gives the user a timeframe in which the paperwork is likely to be approved. We then collect an email address, office phone and fax number. The email is used to contact users regarding their beamtime, publications, and to let them know if we have available time that we are looking to fill. We ask for institution information, including departments, and we keep track of safety training information. There is an option for making a person active in the group or not. This is so that we do not remove information we may need for reports of who used the beamline during a certain time period, but those people will not be included in updated group information. We suggest that groups change the password when people move on from the group.

![Image](https://example.com/image.jpg)

**Figure D.3.3.2:** A screen for registering lab personnel in the data base.

Groups can then proceed to add samples and chemicals and all the safety/biohazard information we need. When a group is ready to get beamtime, they fill out the beamtime request page (Figure D.3.3.3). This page asks for information that we will need to submit the Experimental Safety Approval Forms (ESAF). Information such as title of the experiment, PI, the team coming to our facility, the samples being used, and an abstract are required. We also allow a spot for any notes; for example, the requested date for data collection.

Once the group has submitted all the information, we review it and schedule a trip. We send an email letting them know their schedule and request that they update the ‘trip’ for any changes, such as a new person coming or someone dropping out. After they give us that information, we have our safety officer check the information and submit the ESAF form to APS for approval.
NE-CAT has been using this system with 8-BM since October of 2004, and currently has added Sector 24-ID-C users to the system. All users coming to our beamlines are now routed through www.NECAT.org.

**Figure D.3.3.3:** The experiment description and beamtime request screen.

### D.3.4. **Infrastructure**

All facilities of NE-CAT will be made available to both general users and CAT members. In addition to the standard beamline componetry, users will have access to the MD-2 microdiffractometer and the crystal automounters. Users will have access to a cold room and standard biochemical equipment within the NE-CAT biochemistry laboratory. Users will have access to station computing and to additional computing set aside for post data collection analysis and backup. The Resource will install a 24-node computer cluster for fast data processing and on site structure determination. All standard crystallographic software will be available to all users.

The Structural Biology Grid (SBGrid; http://www.sbgrid.org), sponsored by the Harvard Medical School Center for Molecular and Cellular Dynamics (http://cmcd.med.harvard.edu), provides software distributions and computational support to its participating members. It pools the expertise of several X-ray crystallography, NMR and electron microscopy laboratories to define the various programs and program suites that are important for contemporary structural biology, and it maintains a constantly updated library of that software, appropriately licensed for use by all member laboratories. Member laboratories support the licensing and updating efforts through an annual subscription. Participating laboratories include groups at Harvard Medical School, Harvard University and its affiliated research institutions such as Massachusetts General Hospital, Dana-Faber Cancer Institute, and the Brigham and Women’s Hospital, as well as
many laboratories from other institutions, including Yale University, Rockefeller University, Cornell University, Stanford University, California Institute of Technology, and UCSF. NE-CAT is a member of SBGrid (see letter from Piotr Sliz in section L).

SBGrid also provides some access to parallel computing grid for participating laboratories. The grid currently includes three clusters: a 28-CPU 64-bit OS X cluster, a 50-CPU 64-bit Opteron cluster and a 30-CPU 32-bit Athlon cluster. Clusters are fully integrated into a unified grid using United Devices GridMP software. The grid is supplemented by 10+TB of RAID storage and full distribution of the SBGrid software.

D.4. Training

As described in section C.12 and C.13, NE-CAT provides a number of training opportunities for its participating scientists, the APS General Users, and the general crystallographic community.

D.4.1. On Site User Training

Multi-tiered “graded” on-site training and pre-arrival training opportunities via NE-CAT website are the current mode of NE-CAT user training.

Before arriving at the NE-CAT beamline facility, users have an opportunity to prepare themselves to conduct their experiments by reviewing the information contained on the NE-CAT website. The website currently contains detailed information on the beamlines themselves, operational manuals on how to use the beamlines, information on the available data reduction and analysis software, etc. This web-based information will be constantly updated to reflect new developments and enhancements made to the beamlines.

On arrival at the facility, new users at the APS facility undergo general facility orientation and mandatory safety training conducted by APS. Having met the APS requirements, users then proceed to the NE-CAT beamlines where they receive beamline orientation and safety training specific to the NE-CAT beamlines as well as extensive training on how to conduct experiments at the beamline as described in section C.13.1. Beamline training provided by the highly experienced PhD level crystallographic support staff generally includes the mounting and alignment of a users’ crystal sample, use of the beamline control system, detector data acquisition controls, use of the various software packages available, archival of data, etc. Usually, the first round of data collection is performed by user support personnel who will explain every step and procedure involved to the user. Support staff will discuss with the user an optimal data collection strategy involving optimal exposure, optimal attenuation, optimal energy, etc. After the first round of data collection, users start independent beamline operation with the support personnel closely watching and giving additional instructions as necessary. The beamline specific training for new users generally lasts for 2-5 hours. Training for returning users is generally shorter and consists of a “refresher training”, which emphasizes changes in the beamline operation and operating policies. This section of training changes frequently as APS introduces new policies or NE-CAT implements new capabilities. Hence it is under permanent evolution and greatly tailored to the specifics of different groups and it is based on our previous training experience.

During the period when users are performing their experiments, the support staff periodically interact with the users, with the frequency dependent upon the proficiency of the users. The purpose of these interactions is to identify as quickly as possible if a user is having problems operating the beamline and in obtaining best quality data. Based on their extensive experience operating the beamline and solving structures with the software packages available at the beamline, the support staff provides the users with recommendations to solve their problems as well as obtain the quality of data desired. This type of interaction has been highly successful in the past and we are planning to spend more time in this area. It has helped graduate students gain more crystallographic experience to solve structures that they previously could not solve,
anneal crystals to obtain higher quality data, index and derive useful data from split or non-
merohedrally twinned crystals, and establish better optimized data collection strategies. By
providing close interactions between the users and the support staff, the users’ productivity and
success will be maximized for each visit to the beamline.

Users can and usually do contact support personnel during the evening and early morning
hours by phone. During these off hour periods the users also rely on the written beamline
operational manuals as a first attempt to resolve operational problems that they encounter.

D.4.2. Workshops

As an “out reach” activity to provide training to a broader spectrum of crystallographers, we have
organized selected workshops, made presentations at professional meetings, and made
presentations at APS meetings. These activities will continue in the future. As past examples,
NE-CAT has organized successful workshops on addressing problems working with
challenging samples and microdiffraction at annual APS Users Meetings, participated every year
in hands-on crystallography training courses hosted by the ACA, described various new
technical advancements at the APS Technical Working Group Meetings, and conducted a
training workshop at one of its member universities-Cornell. As NE-CAT transitions more and
more from building to operating beamlines, it will dramatically increase its activities in the
training area through these various media as well as pursue new opportunities.

During the first funding period we focused primarily on construction; however, as we shift our
effort to operations, we plan an annual workshop in an area related to the NE-CAT mission. NE-
CAT organized one such workshop in November, 2003 and last year NE-CAT organized a
workshop on microdiffraction.

The one day workshops will have both invited speakers from outside NE-CAT and presentations
by NE-CAT staff and NE-CAT scientists. These workshops will serve our training and
dissemination mission, but will also allow us to explore new research directions. NE-CAT has
been active in organizing workshops for the annual APS user meetings and other local
workshops focusing on various aspects of macromolecular crystallography.

D.4.3. Off-Site Minisymposia

One new opportunity we will initiate shortly is the organization of annual symposia to be
presented at the member locations, described in more detail under “Dissemination”. These half
day mini-symposia will feature presentations on the beamline operations with information on
enhancements made to the beamlines and changes in operations and procedures, invited
speakers presenting new techniques for obtaining higher quality data and better structure
refinements, highlights of research from NE-CAT beamlines, sharing of experiences, etc.

D.4.4. Web tutorials

Currently under development are website sections that will contain recommendations and “Tips
and Tricks” as to how to improve data quality and structural determinations that are based on
beamline user experiences as well as new developments in the field. We intend to include
sections such as, but not limited to, data collection optimization, high resolution data collections
(low pass and a high pass), phasing on weak anomalous signal, absorption correction, dealing
with a poor MR solution, Non-merohedral twinning etc. Other topics will be included as we
progress. These sections will include detailed step-by-step instructions on how to deal with
specific cases. In addition to these advanced topics in crystallography the website will contain
pointers to tutorials on the basics of X-ray crystallography.
D.4.5. **Web-based Screencasts**

We are now beginning to prepare a web-based interactive electronic media (“screencasts”) help resource to better prepare users before arriving at the beamline as well as while they are using the beamline. We will prepare a set of “screencasts” providing clear instructions to carry out any particular operation at the beamline. For example, instructions on changing energy will be in one clip, which will show step-by-step mouse movements and key clicks in video with synchronized audio instructions to execute the process.

D.4.6. **NE-CAT Network of Training**

All the institutions associated with NE-CAT have substantial graduate programs, and graduate-student training is a central part of the research activities of nearly all the laboratories that will use the Resource. The training and research missions of the Resource are thus intimately linked. Approximately 200 graduate students and 130 postdoctoral fellows are currently working in laboratories of NE-CAT member institutions on projects that are likely to make use of at least one of the beamlines, and essentially every user visit will bring to the beamlines more graduate and postdoctoral trainees than long-term technical staff or senior research associates. The same will be true of the majority of general users, particularly in view of our emphasis on frontier projects rather than high-throughput structure determination.

D.5. **Dissemination**

NE-CAT is committed to the dissemination of technological and scientific information to its users as well as to the general crystallographic community (see section C.12 and C.13 for ongoing activities in this area). To reach a larger community in the future, NE-CAT will maintain and continually increase its presence and participation at scientific meetings. As NE-CAT transitions from focussing on constructing beamlines to facilitating and performing research on them, our pofile in the literature will increase as well. The success of the workshop titled “Microdiffraction in Structural Biology” organized by NECAT during 2006 APS User Meeting calls for the future planning of similar effective ways of scientific dissemination. NE-CAT is planning to organize or host at least one such workshop a year. To improve our dissemination and to become more proactive, we plan to implement (1) a semi-annual electronic newsletter, and (2) mini-symposia at the participating institutions.

D.5.1. **Electronic Newsletter**

In order to reach a broader range of crystallographers, NE-CAT will issue semi-annual electronic newsletters providing up to date information on its facilities, newly implemented technologies, and highlights of its research activities. This newsletter will be transmitted to NE-CAT beamline users via e-mail as well as the general crystallographic community. The newsletter will also feature recent research highlights resulting from users and collaborators. The newsletter will feature an easy “unsubscribe” function to minimize annoyance; however, we hope that it will be beneficial to the structural biology community. A major function of the newsletter will be to aid NE-CAT in developing a user base who will benefit from our technological R&D.

D.5.2. **Mini-symposia**

As a new initiative designed to better disseminate new developments and information among our institutional members, we will begin holding annual mini-symposia at the participating institutions. (See section C.13.2.2 for a description of a test run.) These mini-symposia will consist of presentations from the facility staff on newly implemented capabilities, upcoming improvements to the beamlines, recommendations on how to improve the quality of data taken and determination of structures, etc. There will also be presentations of the highlights of recent research from each of the participating institutions. Other speakers will be invited to present information on topical issues such as new capabilities and new scientific challenges. The mini-
symposia will be open to all that are interested in attending. But particular attention will be
devoted to promoting interactions and possible collaborations between researchers at the
participating institutions as well as disseminating timely information on the developments taking
place at the NE-CAT facility. As appropriate material presented in these mini-symposia will be
edited and adapted for subsequent timely publication on our web-site and the electronic
newsletter. The mini-symposia will be rotated through various member institutions and will be
extended to other institutions, such as those associated with collaborative scientists or frequent
users of the NE-CAT facilities.

D.5.3. Contributions to the APS Annual Report

NE-CAT will contribute regularly to the APS Annual Report. The current article on microdiffraction
is under preparation for the section on "Novel X-ray Techniques & Instrumentation" in the
upcoming APS annual report. Since APS bears the costs of publication this is a particularly
efficient mechanism by which NE-CAT can inform the entire APS user community and beyond of
our new capabilities.

D.5.4. NE-CAT Web Site

One of NE-CAT’s most effective dissemination tools is its web site, NECAT.chem.cornell.edu,
which has averaged about 10,000 hits per month over the last two years. The web site contains
information on the user program, technical facilities, NE-CAT background, NE-CAT organization,
results from NE-CAT and listings of upcoming meetings and workshops. The web site also
contains links to the APS, ANL, NCRR and other important web sites. All technical reports
submitted to the APS and NIH are available on the web site, as are all sector design details. The
web site also provides updates on construction progress. The web site contains a link to our
user database, which is used to track all NE-CAT collaborators, NE-CAT users, and independent
investigators. The web site is a valuable tool for users and potential users, and also contains
design information of use to beamline scientists and results of potential interests to the entire
biological community. We will continue to update and improve the web site as new needs are
identified.

D.5.5. APS Design and Software Sharing

All NE-CAT generated sector and instrument designs are freely available to the scientific
community. Proprietary designs obtained from outside sources are protected; however, the NE-
CAT staff will freely provide any information on implementation and adaptation to the NE-CAT
sector. NE-CAT designs are available from the NE-CAT web site or by contacting the Director or
Deputy Director.

Software developed by NE-CAT is also freely available to the scientific community.

D.5.6. Publications and Presentations at Meetings

The NE-CAT staff is expected to publish scientific and technical results in a timely manner. Each
staff member is involved in ongoing research projects and regularly generates publishable
results. The projects include both structure determination and analysis, as well as the
development and testing of methods and techniques. As attention to construction is now
easing, we will publish more technical notes. For example, Malcolm Capel is preparing a
description of the vibration free rotary shutter developed for beamline ID-24-C. The scientific staff
also regularly attends conferences and meetings related to structural biology and synchrotron
radiation research. NE-CAT is present at the annual meeting of the American Crystallographic
Association and regularly at meetings such as the Protein Society and the Biophysical Society.
D.5.7. **NE-CAT Network of Dissemination**

In addition to the methods just outlined for disseminating the technological developments of the Resource, we also note that a particularly high proportion of the postdoctoral fellows working in NE-CAT member laboratories move on to directing their own research laboratories, often within a year or less of completing their NE-CAT-related project. While not a substitute for more formal mechanisms for communicating and spreading information about new experimental methods, new hardware, and new computational approaches, this dispersal of talent within the international research community guarantees a further level of dissemination and enhances the broader effectiveness of the Resource.
E. Resource Organizational Structure

The primary goal of this research is the use of synchrotron radiation in structural biology, specifically the use of single crystal diffraction to provide high quality three-dimensional structures of important biological materials. Three groups will be play important roles. First, the NE-CAT staff is responsible for design, constructing and operating the beamlines. Second, the core collaborators, who are established structural biologists with demanding research programs, will provide the driving force for beamline innovation and for ongoing evolution of the facilities. Third, the beamline users will translate the beamline development into scientific results with impact on a wide variety of biomedical areas.

Most of the core collaborators have worked together for many years through various collaborations at MacCHESS, NSLS or APS. However, coordination of the staff, collaborators and users requires a well defined management plan. The plan proposed here is an evolution of the plan proposed five years ago based on the experience we have gained during the current five year funding period. Project management involves many interactions between the APS and NE-CAT. The lines of management must be clear and responsive to the policies of the Department of Energy, the APS management and the NIH. The organization has the following key components: an Executive Committee, a Director to provide overall scientific guidance, one Associate Director for Technological R&D and one Associate Director for User Operations. The management team will be assisted by a technical advisory committee, a resource advisory committee, a proposal review committee and various APS review panels.

E.1. Overall Organizational Structure

The Resource is a national facility that must operate in compliance with NCRR/NIH regulations, APS/ANL regulations and the NE-CAT Consortium. The majority of funding comes from NIH; however, the NE-CAT institutions raised a significant amount of matching funds ($7.2M plus in kind contributions from MIT including 8-BM componentry and a Q315 detector). NE-CAT and the Research Resource have compatible but occasionally competing needs. The NE-CAT Executive Committee represents the interests of the NE-CAT scientists and the Resource Director represents the interest of NCRR with oversight from the Resource Advisory Committee and the NCRR Program Officer. The Executive Committee and the Resource Director must cooperate and work closely together is the venture is to succeed. Finally, the APS structure requires that collaborative access teams develop sectors, thus imposing yet another level of organizational structure. Cornell University is the lead institution for NE-CAT and the applicant for NCRR funding, thus providing practical constraints to the organizational structure.

In this section we describe the Resource and NE-CAT organizational structure (Figure E.1.1). Section E.2 describes the relationship between NE-CAT and the Research Resource, highlighting overlap and potential areas of conflict. Resource operating procedures are described in Section E.3, Resource Staff responsibilities are described in section E.4 and the relevant oversight committees are described in section E.5.

The Resource and NE-CAT Director is Steve Ealick. The Director receives input from the NE-CAT Executive Committee (and its Steering Committee), the Resource Advisory Committee and the Technical Advisory Committee. The Director conveys to the Deputy Director, Malcolm Capel, who the day-to-day supervision of the Resource and NE-CAT staff. The key staff functions are (1) core technological R&D, (2) collaborations with scientists using the core technologies, (3) user program, (4) technical support and maintenance, (5) training, (6) dissemination and (7) environmental safety and health. The individuals performing these functions and the percentage of effort devoted to each function is described in detail in section E.4.

Both the NE-CAT Executive Committee and the Resource Advisory Committee provide advice on priorities and direction. The Director is responsible for developing strategies and the Deputy
Director is responsible for implementing those strategies. The Director is responsible for communicating plans and progress to the NCRR and to the Executive Committee. The NE-CAT/Resource staff is responsible for compliance with APS/ANL policies and the Deputy Director and Director are responsible for reporting to the APS/ANL. The Director is responsible for implementing recommendations of the APS Scientific Advisory Committee. The Director is responsible for ensuring effective interactions between the core technologies and the collaborative science while the technical staff is responsible for training and oversight of the users program.

E.2. Relationship of the Research Resource to NE-CAT

APS assigns sectors to Collaborative Access Teams (CATs). A prospective CAT presents a scientific case and a fully outlined technical design, both of which must be approved before a final sector assignment is made. NE-CAT was set up by the six (later, seven) founding institutions to propose a plan for development and operation of a sector at APS and to carry out that plan following its approval. Sector 24 was assigned to NE-CAT in 2002. A more complete history of NE-CAT appears in section B.4.
Each institution has designated the current roster of scientists likely to use the facility, and each is represented on an Executive Committee by one of those scientists. To launch the effort, each founding institution provided seed funding ($1.2M). The Executive Committee made the early decision to operate the sector as a national facility and in that spirit chose to apply for funding as an NCRR Research Resource. That application was successful, and this proposal is a request for a five-year renewal. The guidelines of the NCRR are fully compatible with those of APS and with the goals of NE-CAT. We describe below the NE-CAT organizational structure, which has been designed both to ensure that we realize our scientific and technical goals and to reconcile any potential conflicts between our mandate as an NCRR resource and our obligations to the member institutions. We also emphasize how the Research Resource and the NCRR procedures fit into the overall operation of the CAT, as established by the original NE-CAT agreements and the requirements of APS.

**E.2.1. Management plans**

Both NCRR and APS require formal management plans. The two plans are essentially the same, although the oversight and reporting responsibilities are somewhat different. The NE-CAT management plan must be reviewed and approved by APS; the Resource management plan was reviewed by the initial NIH review panel and implemented with input from NCRR. The NE-CAT management plan specifically addresses work breakdown structure, quality assurance, construction scheduling, and safety. The Resource management plan addresses technological R&D and user operations.
E.2.2. **Organization and oversight**

Steven Ealick, the Principal Investigator of this grant, serves as both Resource Director and NE-CAT Director, and Malcolm Capel serves as Resource and NE-CAT Deputy Director. Stephen Harrison serves as Chair of the NE-CAT Executive Committee. Funding for the NCRR Resource is administered by Cornell University, the home institution of the P.I. Figure E.2.1 is an organization diagram.

The Biomedical Technology division of NCRR oversees the Resource, with the help of the Resource Advisory Committee. The Resource must operate in accordance with NIH guidelines and the established agreements between NIH and Cornell. The Executive Committee oversees NE-CAT staff and operations, as managed by the Director and Deputy Director of the Resource, on behalf of the member institutions. NE-CAT must operate in accordance with the memorandum of understanding signed by the member institutions of NE-CAT and APS. To assure successful operation, any potentially conflicts between the goals of the Resource and those of the CAT institutions will be resolved through discussion and agreement among the representative of the NCRR Program Office, the NE-CAT Executive Committee Chair, and the Resource/CAT Director.

The APS provides additional oversight through sector reviews, which take place every three years. In 2007, the APS is also conducting a cross-cut review of all structural biology at APS. NCRR of course requires annual non-competing renewal applications and five-year funding applications.

NE-CAT is expected to participate in the APS Annual Users Meeting and in the Partner User Council, which consists of all CAT Directors (or their designated representatives). The Council addresses issues of common interest to all CATs or to broad disciplines. The NCRR organizes an annual meeting of all Resource Directors. The purpose of this meeting is to foster interactions among resources and to cover administrative issues relating to all resources.

E.2.3. **Reporting**

The NCRR requires each resource to submit a detailed annual report citing technological progress, scientific progress, and statistics relating to user programs, training, and dissemination. The APS requires all CATs to submit citations to the APS publication database. Contributions to the APS annual report are expected.

E.2.4. **Scientific Direction**

The NE-CAT Executive Committee establishes the general scientific direction for the CAT and for the Resource. Founding members from the NE-CAT institutions prepared the scientific case used to establish the CAT, and this case was the basis for the NCRR grant. The NE-CAT Executive Committee, acting in consultation with the core collaborators from non-NE-CAT institutions, the Resource Advisory Committee, and the APS Sector Review Panel, continues to set the scientific directions.

E.2.5. **Allocation of Resources.**

The APS requires that a CAT make available 25% of the user time to the general user community. NCRR does not set a specific number, but allocation to users must be consistent with the operation of a national user facility funded largely by the NIH. Consistent with this guideline, NE-CAT will allocate 50% of its beamtime to the general user community. The APS does not require a CAT to schedule general user time until one year after the beamline has formally been declared operational, and under this policy it is in principle possible for a CAT to have extended periods of exclusive usage. To conform to the character of a national user facility,
we will declare our beamlines operational as soon as possible and waive the one-year grace period. This was our practice for both 8-BM and 24-ID-C, and we will follow the same policy for 24-ID-E and 24-BM. We will also follow the same policy for beamline enhancements and special instrumentation such as the MD-2 microdiffractometer. During user commissioning period, early access will be given to all core collaborators, irrespective of their NE-CAT affiliation, and once commissioning has been completed, we will make facilities available to all users.

E.2.6. Technology Development

NCRR depends on the grant review process to evaluate the feasibility of technology development. The APS requires approval of multiple design levels, designated conceptual design, preliminary design, and final design. APS committees must approve each level. The NE-CAT management plan also requires internal review by the Technical Advisory Committee prior to submission to the APS. Thus, the NCRR and APS procedures complement each other, while overlapping in some respects.

E.2.7. Priority Setting

Setting of priorities is ultimately the responsibility of the Resource/NE-CAT Director. The process is informed by the NE-CAT Executive Committee and the Resource Advisory Committee, and priorities must be consistent with the Specific Aims of the NCRR grant proposal and with the goals of the APS sector proposal. Both of these proposals focused on developing beamlines for challenging problems at the frontier of structural biology.

E.2.8. Planning

Five-year planning is built into the NCRR application cycle. In addition, new opportunities will occur on a shorter time frame. Planning for such opportunities will be a joint effort of the NE-CAT Executive Committee, the core collaborators, the Resource Advisory Committee, and the NCRR Program Office. Agreement on how to proceed with new directions, even those not directly dependent on NCRR funding, will come primarily from discussions among the Resource/CAT Director, the NE-CAT Executive Committee Chair, and the NCRR Program Officer.

E.2.9. Operations

Resource operating procedures are described in section E.3. The Resource/CAT Deputy Director is responsible for day-to-day operations, and the Resource/CAT Director establishes priorities, based on input from NCRR and the Executive Committee. Potential conflicts or uncertainties in operational issues (e.g., allocation of funds or personnel decisions) will be resolved through consultation with the NCRR Program Officer and the NE-CAT Executive Committee Chair.

E 2.10. Health and Safety

The terms of the NIH grant require compliance with a variety of personal and biological safety issues. The APS requires that a detailed safety plan be prepared and approved before a sector is allowed to operate. The NE-CAT safety plan (Appendix D) specifies training and operating procedures for sector 24. Each NE-CAT/Resource users must undergo both APS general safety training and NE-CAT specific safety training. The NE-CAT safety officer is Dr. Sukumar. The APS assumes responsibility for installation and maintenance of radiation safety interlocks. It also requires equipment safety systems to assure that APS and sector instrumentation is protected from damage. The NIH and APS/ANL safety requirements are fully compatible.
E.3. Resource Operating Procedures and User Scheduling

This section summarizes briefly the processes which NE-CAT uses to manage the resources and conduct the “business” of operating the facility. Commensurate with operating a national resource, these processes are designed to insure good planning with broad based input from the user community, be highly transparent to the user community and its sponsors through reporting and reviews, and follow well established standards and good business practices.

E.3.1. Decision Making

Decision making within NE-CAT flows from the vision and direction set for NE-CAT by its Executive Committee for the grant period, consistent with the NIH/NCRR P41 Grant Guidelines and the specific objectives presented in the approved grant award. The Executive Committee, currently chaired by Steven Harrison, is formally convened to meet as a body at least twice each year, with additional meetings or teleconferences called on a as need basis. The NE-CAT Resource Director is then charged with operating the facility and managing the resources within the general guidance and directives provided by the Executive Committee. Before each grant year, the Director meets with the Deputy Director to discuss and agree upon the staffing, expenditures, specific milestones to be met for the coming year- consistent with the longer term guidance provided by the Executive Committee. Based upon this meeting the Director then prepares and presents his annual staffing and spending plan recommendations to the Executive Committee for review and approval. Following the Executive Committee's approval, the Director and his Deputy then proceed to execute the plan for the coming year.

During the year unanticipated events or new opportunities may arise which require changes to the plan. If the Director believes that the changes proposed represent a minor change, not negatively impacting the goals proposed or requiring a substantial change in costing, he will seek approval from the Executive Committee to make such changes. If the changes represent a substantial change in direction (a highly unlikely occurrence), the Director will seek approval from both the Executive Committee and NCRR.

To aid in the decision making process and to solicit independent expert recommendations, the Director will in a timely manner seek input from specially appointed committees. A Technical Advisory Committee will be convened to address technical issues. The Research Advisory Committee will be convened to provide recommendations related more to management and resource distribution issues.

E.3.2. Reporting

Reporting of issues, highlights, events, potential problems, etc. occur at a number of different levels- beginning at the staff level. In addition to the day-to-day open communications, NE-CAT holds bi-monthly Group Meetings attended by all APS resident members with the NE-CAT Director in attendance- either in person or via audio-video teleconferencing. The Director begins each of these meetings with “News from the Director” – providing specific instructions of what he wants done, important upcoming events, and milestones. This is followed by status reports from each group member, discussion and resolution of problems encountered, near term priorities of tasks to be completed, etc.

Each week a report is submitted to the Director summarizing the events of the week such as experiences of users during the week, technical progress, problems with recommendations for solution, action items originating from the Group Meeting, etc.

In meetings with the Executive Committee, the Technical Advisory Committee, and the Resource Advisory Committee the Director generally provides the committees with timely status reports summarizing the user resource program, highlights of research, and technical developments.
Reporting to the broader NE-CAT membership and crystallographic community is primarily made through the NE-CAT website, the newly proposed Newsletter, presentations at meetings, specially convened workshops, etc.

NE-CAT makes a number of reports to APS, including progress reports for the APS Annual Report, NE-CAT contributions to the APS Publications Database, cross-cut activities, APS convened meeting, etc.

NE-CAT submits annual progress reports to NIH/NCRR documenting how the resources have been applied to meet the objectives described in the grant proposal as well as scientific highlights of the research conducted, statistics on resource usage, etc.

**E.3.3. Oversight**

There are a number of different levels of independent oversight and review conducted at the NCRR level, by APS, through the NE-CAT Technical Advisory and Resource Advisory Committees, internally within NE-CAT itself by the Executive Committee, and the users of the facility.

The NCRR reviews the annual progress reports and periodically reviews the progress through site reviews. APS conducts intensive reviews of each CAT’s performance (through its Scientific Advisory Committee) every three years as part of the Memorandum of Understanding Agreement renewal process. Also on about the same schedule APS conducts “cross-cut reviews”, reviewing all the CATs conducting research in the same discipline area, e.g., structural biology, inelastic scattering, high-pressure, etc. In addition, APS conducts special “design reviews” of NE-CAT’s construction and installation activities as well as numerous special safety reviews of operations, safety documentation and practices. At each convened Executive Committee, Technical Advisory, Resource Advisory Committee meeting, reviews are conducted on specific aspects of NE-CAT’s activities.

Each visiting user group provides a review of NE-CAT’s operations. We require each user group to submit a “User Summary Form” at the end of their runs asking specifically to comment upon their experience using the beamline and support provided as well as recommendations as to how the operation can be improved.

**E.3.3. User Scheduling.**

User scheduling begins with APS issuing the “Long- Range Operations Schedule” which defines the time periods APS will provide beam for the user program during the next two run cycles. One to two months before a run cycle is scheduled to begin, the Operations Leader, designated as responsible for scheduling of the users, issues a call for requests for beamtime to NE-CAT’s institutional users requesting that each applicant provide preferred dates as well as alternate dates for their requested beamtimes. In parallel APS, several months before each run, solicits proposals from General Users. An APS committee prioritizes these requests and distributes the approved proposals to NE-CAT and the other CAT’s for scheduling. With all the information in hand, the Operations Leader then prepares the detailed day-by-day schedule taking in account as best as possible the time preferences of each approved requester and allocates the total time available, commensurate with the earlier agreed upon distribution of time assigned to beamline maintenance and development, the 50% commitment of available time as a NCRR National resource (APS General Users) and 50% of available time for NE-CAT institutional members. Once the schedule is finalized, the NE-CAT Administrator then contacts each requester and provides the support needed by the users-e.g., access into the Laboratory, scheduling of required training, shipment of sample containers, etc. Once on site, the users must have their required APS orientation and safety training completed and receive the NE-CAT beamline orientation training, before being allowed to use the beamlines.
The NE-CAT Administrator is responsible for maintaining a database on beamline utilization. Summary information is routinely transmitted to the NE-CAT Director and available to the Executive Committee, NCRR, APS and others to assure everyone that the time is being allocated equitably and as per the agreements in place.

**E.3.4. Administrative Practices**

All procurement requests originating from the staff must be reviewed and approved by the Deputy Director. Once approved, the Administrator prepares the appropriate procurement materials and justifications. For procurements > $500 the procurements packages are submitted to the office of the NE-CAT Director. Once Director approval is granted, the procurement is processed by the Cornell Purchasing Department in conformance with Cornell’s procurement policies. Small approved procurements <$500 are procured by the Administrator using an authorized credit card. Cornell maintains pre-funded accounts with Argonne to be used to pay for APS and Argonne services and construction activities, as required by the Laboratory and APS.

The Deputy Director is responsible for initiating personnel actions, e.g., hires. All hiring activities are required to be reviewed and processed by the Cornell Human Resources Department. Open positions must be posted by Cornell and degreed positions are widely advertised in appropriate publications. Potential candidates are interviewed by the Deputy Director and selected members of his staff. Recommendations are then forwarded to The Director for approval and processing by Cornell University.

The Director is responsible for budgeting and control of expenditures. At Cornell he is assisted by an Administrator in the Department of Chemistry and Chemical Biology, who is expert in finance and the Cornell procurement system, who maintains the official financial records for NE-CAT. Informal financial and procurement data bases are maintained at the NE-CAT site and used to respond rapidly to urgent local inquiries and needs for information.

**E.4. Resource Staff and Responsibilities**

The Resource staff responsibilities are shown in Table E.4.1. In addition to the P.I., there are twelve scientific or technical staff plus one administrator at APS and two scientific personnel plus half time of an administrator at Cornell. Four of the APS positions will be filled when the grant is renewed. We describe here the staffing of each core and then the responsibilities of each person. We refer to the four two-be-appointed positions here as operations leader, beamline physicist, computational protein crystallographer, and beamline technician. The first three of these positions will be filled by PhD scientists. Each core has two or three staff scientists associated with it, who are the NE-CAT participants in the collaborative research program, as outlined below and in section D.1. Each collaborating group will have a principal “go-to” person, although of course we anticipate that additional collaborative interactions will develop as work progresses.
Table E.4.1: NE-CAT Staff Responsibilities (percent effort)

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E.4.1. Responsibilities for cores and for collaborative research

Core 1, microdiffraction. Rajashankar and the to-be-appointed operations leader are the scientific staff of Core 1. Development of that core will also require contributions from Capel, the beamline physicist, and the technical staff, as outlined in the Table. Harrison and Eisenberg are already collaborating with Rajashankar; the operations manager will be the collaborative “go-to” person for Mackinnon and Berger. We expect that many service users, from both NE-CAT and non-NE-CAT institutions, we become interested in the microdiffraction beamline, and we therefore believe that it makes sense to assign some responsibility for this core to the operations leader.

Kanagalaghatta Rajashankar of the NE-CAT staff will have overall responsibility for the activities within this core. Rajashankar (40%) and the Operations Lead (20%) will spend the majority of their efforts initially preparing the MD2 for operation and continue to augment its capabilities throughout the early years of the grant period, e.g., adding a robotic sample placement system, automatic sample alignment, etc. to the MD2. They will also work closely with Malcolm Capel implementing micro-diffraction capabilities on the 24-ID-C beamline. Throughout the entire period of the MD2 operation and the upgraded micro-diffraction capabilities on 24-ID-C they will be responsible for developing new capabilities in support of users requiring micro-diffraction capabilities on both ID beamlines. Malcolm Capel (10%) will be responsible for initially integrating the MD2 software into the Console beamline operating system as well as upgrading the 24-ID-C beamline with micro-diffraction capabilities by installing a higher precision goniometer, an on-axis sample alignment visualizer system, as well as other planned upgrades.
throughout the grant period. To support these ongoing activities will require approximately a half-man year per year of technician effort, planned to be contributed by Anthony Lynch (10%), James Withrow (10%) and the TBA technician (20%).

**Core 2, beamline hardware.** Capel, the to-be-appointed beamline physicist, and Sukumar are the principal scientific staff of Core 2. The technical staff and Wang will contribute as shown. Capel and Cate have an established collaborative interaction that will continue; Hendrickson and the beamline physicist will work together; Sukumar, whose responsibilities include robotics, will collaborate with Wu.

Malcolm Capel will have overall responsibility of the Core 2 activities. Capel (40%) working together with the TBA Beamline Physicist (40%), will bring into fruition new capabilities on both ID beamlines for enhanced beam stability, decreased beam divergence, and tighter focus. These activities will include setting the specifications, procurement, installation, and commissioning of a new 3.0cm period undulator, bi-morph focusing mirror, white-beam position monitor, and a refractive lens system. Sukumar (20%) assisted by Wang (10%) and under the supervision of Capel will have the responsibility of working with Thomas Earnest of LBNL in developing, installing, and commissioning the new generation of the ALS sample placement robotic system. Successful pursuit of these major installation activities is expected to require the support of 40% of a man year each year of technician effort, provided by Anthony Lynch (20%) and the TBA technician (20%).

**Core 3, computing.** Kourinov, Wang, and the to-be-appointed computational protein crystallographer are the scientific staff of Core 3. A programmer (Yang) and an electronics technician (Withrow) also contribute, as does Capel, the developer of Console. The crystallographer will work closely with Capel on Console. Kourinov will be the “go-to” staff person for Steitz and Schulman; Wang, for Ealick; the protein crystallographer, for Pavletich.

Igor Kourinov will have overall responsibility for all Core 3 development activities. Kourinov will be responsible for bringing into operation new software to aid in data collection and structural determinations (30%). A primary focus will be on the development of an integrated beamline control and data analysis system through the integration of WebIce with Console (10%). He will be assisted by the Lead Scientist TBA who will contribute to development of new data collection and structural determination software (20%) and parallel processing applications (20%). Capel (20%) will be responsible for continuing development of Console as well as working with others to integrate new software systems into Console. He will also be responsible for design, procurement, and installation of all major new computer and networking hardware, e.g., the computing cluster. Wang (30%) will be working on non-merohedral twinning and radiation decay and absorption corrections as well as other tasks. Yang (60%) will be responsible for the implementation of the BLU-ICE Graphics User Interface, sample placement robotics software implementations, and sample auto-centering. Approximately 30% of Jim Withrow’s technician effort will be needed to perform general computer installations, maintenance and electronics support.

**E.4.2. Individual staff responsibilities**

All scientific and technical staff participate in training, through regular interactions with users; all scientific staff participate in dissemination, through organizing workshops, developing web-site text and through helping general users to apply methods developed in connection with one of the cores. All scientific and technical staff (except the Deputy Director) provide service, through direct user support during running times. The remaining responsibilities of each staff person are specified below. All staff except Kinsland spend 100% of their time on this project; percentages not included explicitly below are for Service, Training, and Dissemination, as outlined in Table E.4.1.
Malcolm Capel, Deputy Director, NE-CAT. Core 1 (10%). Capel will design the mechanical support and positioner for the microdiffactometer and oversee its commissioning; he will design the retro-fits for integration with NE-CAT beamlines; he will design and implement the software interface between the MD2 and NE-CAT beamline controls. In subsequent stages, he will design software and hardware for microdiffraction using existing NE-CAT beamline hardware, with improved positional stability, sample visualization, etc. Core 2 (40%). Capel designs, implements, and commissions beamline optical and end-station components: monochromators, mirrors, detectors, and sample-handling hardware. He will work closely with the beamline physicist on these activities. He will design improvements and modifications to end-station components such as shutters, attenuator controls, etc., particularly in response to requirements of the collaborative scientists. Core 3 (20%). Capel will continue to develop and implement Console, including instructing and supervising the computational protein crystallographer (in Console development) and the programmer. He will also participate in software development and maintenance for detection systems. Administration (10%). The responsibilities of Deputy Director include various administrative tasks, especially in connection with the interface between NE-CAT and APS.

Kanagalaghatta Rajashankar, Senior Scientist. Rajashankar has been our principal liaison with users. Some of these responsibilities will be assumed by the to-be-appointed operations leader. He continues to have an important role in user support and dissemination, especially of the procedures we develop for microdiffraction at APS. Core 1 (40%). Rajashankar will oversee and help carry out installation, commissioning, and modification of the MD2. He will collaborate with Harrison and Eisenberg in developing procedures for optimal use of the microdiffactometer for different kinds of samples. He will work with the operations leader as needed in the collaborations with Mackinnon and Berger.

Operations leader, Senior Scientist or Staff Scientist (to be appointed). We will recruit for this position an experienced protein crystallographer with strong administrative and interpersonal skills. Core 1 (30%). The operations leader will work with Rajashankar in developing, implementing, and maintaining the microdiffraction facilities described in section D.1.1. He/she will also be the NE-CAT collaborative interface for the Mackinnon and Berger projects. Administration (10%). The operations leader will be in charge of scheduling users and matching staff support to user visits. This position is a replacement for Craig Ogata whose NE-CAT appointment ended February 7, 2007.

Igor Kourinov, Staff Scientist. Kourinov has installed and maintained most of our computing systems and software. He will continue to oversee all NE-CAT computing. Core 3 (40%). He will take particular responsibility for collaborations with Steitz and Schulman. He will implement the various data-processing software projects we outline in the body of the proposal.

Narayanasami Sukumar, Staff Scientist. Sukumar is in charge of installing, commissioning and testing the ALS sample-mounting robot at 24-ID-C and 24-BM. He will also be involved in whatever robotics we work out for the MD2. He has a substantial service commitment, for direct user support, including supervising and maintaining the chemical laboratory. Core 2 (20%). Sukumar will provide the collaborative interface for Wu.

Jun Wang, Staff Scientist. Wang works on specialized data processing software. Core 3 (30%). Wang will mediate the Ealick collaboration and take responsibility for implementing and testing user-friendly versions of the approaches developed in that and other Core 3 collaborative efforts.

Beamline physicist, Staff Scientist, to be appointed. The various hardware developments proposed in Core 2, including bimorph mirrors and other optical improvements, will require appointment of a staff scientist with specialized experience in beamline optical design. Core 1 (20%). The beamline physicist will be responsible (in collaboration with Capel, Rajashankar, and the operations leader) for adapting beamline optics for microdiffraction applications, including the MD2 at 24-ID-E and changes in the optics at 24-ID-C, as we implement...
microdiffraction on the latter beamline. Core 2 (40%). The beamline physicist’s principal responsibility will be to the beamline hardware in Core 2. He/she will collaborate with Hendrickson.

Computational protein crystallographer, Staff Scientist, to be appointed. We will recruit a protein crystallographer with experience in programming and software implementation, to work with Capel on Console development and to help less experienced users with structure determinations. Core 3 (40%). The crystallographer will collaborate with Pavletich. By combining this responsibility with that of assisting users with data collection and structure determination, we will accelerate dissemination of new procedures.

Xiaochun Yang, Programmer. Yang has responsibility for all the programming efforts that involve integrating Blu-Ice and related user applications. He also devotes some time to direct user support.

Anthony Lynch, Research Support Specialist. Lynch has participated in the construction and installation of essentially all NE-CAT hardware. He coordinates with all APS machine shops and other support facilities. He will continue in these responsibilities. He has begun to devote some time to user support during running time, and this will continue to be a component of his activities.

James Withrow, Research Support Specialist. Withrow has been involved in fabrication, testing, and installation of beamline electronic systems, such as motion control devices, equipment protection systems, and interface hardware. The design and construction of electronics to support research efforts will continue to be his principal activity. He will contribute to our user support program, including user emergency contact 24/7.

Technician, Research Support Specialist. The technician will have general computing and networking skills to complement the mechanical, electronic and programming skills already available. The technician will also have general mechanical and electronics skills. The technician will provide support for beamline and laboratory computing and will assist in maintaining web manuals and creating screencast. The technician will contribute to our user support program, including user emergency contact 24/7.

Cynthia Salbego, Administrator. Salbego is the on-site administrator for all aspects of NE-CAT activity, from user communications to procurement. She also maintains and updates the web site.

Yang Zhang, Research Associate (Cornell). Zhang is the scientific liaison between the Resource Director and the operation of the Resource. She coordinates synchrotron experiments for the PI’s laboratory, makes frequent trips to APS, and provides a direct collaborative interface with the entire NE-CAT operation.

Rachel Koralewski, Graduate student (Cornell). Koralewski is a graduate student in the Ealick laboratory. She is responsible for various aspects of Cores 2 and 3. She will make frequent trips to the APS and interact with Malcolm Capel, Igor Kourinov and other NE-CAT staff members to develop data collection and data processing technologies.

Leslie Kinsland, Research Administrator (Cornell; 50% effort). Kinsland is the administrative liaison between the PI and the Resource, including the Executive Committee, grant management, grant and report preparation, and related activities.
E.5. Committees

The Resource receives oversight from several committees.

E.5.1. NCRR Committees

NCRR Advisory Committee: A Resource Advisory Committee will advise NE-CAT on matters related to resource Technological R&D, resource core and collaborative research, the user program, training and dissemination. An NCRR representative and Dr. Stephen Harrison (NE-CAT Executive Committee Chair) are invited to attend meetings. The committee meets at least once a year. The most recent report of the Advisory Committee (August 21, 2006) is located in Appendix A.

Resource Advisory Committee Members

Dr. Ashley Deacon (Chair)
Dr. Keith Hodgson
Dr. Janet Smith
Dr. John Chrzas

Technical Advisory Committee: A Technical Advisory Committee will advise the Resource Director on technical design issues. The committee members are experts in beamline design and considerable experience in design and building beamlines. The committee members have reviewed all aspects of our sector design and last met about two years ago to review the design for beamline ID-24-E, which is now in commissioning. We will call upon this committee (or a modified version of the committee) as needed when new beamline design initiatives or modifications are undertaken.

Technical Advisory Committee Members

Dr. Peter Siddons (Chair)
Dr. John Chrzas
Dr. John Quintana
Dr. Albert Thompson

E.5.2. NE-CAT Committees

NE-CAT Executive Committee: Collaborative Access Teams provide the organizational structure required to develop APS sectors. An Executive Committee oversees the operation NE-CAT. The NE-CAT member institutions are Columbia, Cornell, Harvard, MIT, Rockefeller, Sloan Kettering and Yale and each institution designates one member of the Executive Committee. The Executive Committee meets 2-3 times per year and an NCRR representative is invited to all meetings. The main responsibilities of the Executive Committee are:

- Define and develop the scientific mission of the NE-CAT with appropriate consultation with all members.
- Appoint the Director and Deputy Director, define their responsibilities, and guide the staffing plan.
- Add or remove members of NE-CAT and approve institutional representatives to the Executive Committee.
- Monitor the technical and administrative efforts of the NE-CAT staff and their performance with respect to cost, schedule and technical scope.
Approve the use of construction and contingency funds when the authority of the Director is exceeded and to approve major changes in beamline design or scope.

Assure that the beamlines are designed, built and operated so as to pose no hazard to the staff, independent investigators, APS personnel, the community, or the environment.

Assure that APS-developed policies relating to users are followed.

Oversee NE-CAT’s reporting activities and institute effective communications between NE-CAT management, members, independent investigators, and the APS User Support and Administration Office.

Develop plans and procedures to allocate beamtime to NE-CAT members and independent investigators consistent with APS user policy, the independent investigator policy, and the Memorandum of Understanding.

**NE-CAT Executive Committee Members**

- Dr. Stephen Harrison (Chair)
- Dr. Seth Darst
- Dr. Wayne Hendrickson
- Dr. Nikola Pavletich
- Dr. Robert Sauer
- Dr. Tomas Steitz
- Dr. Hao Wu
- Dr. Steve Ealick (ex officio)

**NE-CAT Steering Committee: The NE-CAT Steering Committee is a subcommittee of the NE-CAT Executive Committee. This committee is authorized to make decisions and recommendations not requiring a meeting of the full Executive Committee, as judged by the Executive Committee Chair. The Steering Committee operates largely through e-mail and conference calls. An NCRR representative will be invited to participate in meetings when issues relevant to NCRR are discussed.**

**NE-CAT Steering Committee Members**

- Dr. Stephen Harrison (Chair)
- Dr. Seth Darst
- Dr. Wayne Hendrickson
- Dr. Steve Ealick (ex officio)

**E.5.3. APS Committees**

Sector Review Panel: The operation of NE-CAT is under the oversight of the APS and is reviewed every three years by a Sector Review Panel and the APS Scientific Advisory Committee. NE-CAT was last reviewed by on October 18, 2005. The report is given in Appendix B.

**Sector Review Panel Members:**

- Dr. Jennifer Doudna (Chair)
- Dr. Bruce Bunker
- Dr. Frank Collart
- Dr. John Helliwell
- Dr. Alfonso Mondragon
- Dr. Mark Rivers
- Dr. Janet Smith
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