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BioCAT NEWS

CSRRI Center for Synchrotron Radiation Research and Instrumentation
Illinois Institute of Technology

A Message From the Director

Grant Bunker
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I would like to extend greetings to our old friends, and to potential new ones. It is a pleasure to provide this update on the Biophysics Collaborative Access Team (BioCAT). After five long years in gestation the project is now funded and on the road .

BioCAT's purpose is to construct and operate facilities at Argonne National Laboratory's Advanced Photon Source (APS) for the study of biological structure and dynamics at the molecular level, using x-ray scattering/diffraction and x-ray absorption spectroscopy (XAFS/EXAFS/XANES). The main focus of BioCAT is non-crystalline systems, with an emphasis on time resolved experiments and studies of small ordered domains (down to ~50-100 micrometer). The specific intent is to complement the several APS CATs oriented primarily toward macromolecular crystallography. BioCAT's facilities are available to the entire biological/biomedical research community.

We believe that, despite the importance and spectacular successes of macromolecular crystallography, there is a broad range of important and interesting biophysical and biochemical phenomena that can only be explained by studying them under conditions similar to those *in vivo* - in many cases, in solution, or in membranes; in some cases (e.g. muscle fibers) actually in living cells. Often, important biological interactions entail subtle conformational changes involving only small changes in free energy, which may be masked or altered in crystals. In addition, many biological structures are inherently non-crystalline. When crystallographic structures are available, they provide excellent starting points for modeling experimental data obtained in non-crystalline form. High performance computing adds another important tool for these problems, which we intend to exploit.

As of Sept 30, 1995, the National Institutes of Health (NIH) has committed funds ($8.6 M total) to construct and operate one undulator beamline at the APS for the first 5 years, which takes us to the turn of the millenium; funds beyond that point are expected for future R&D and operations. NIH staff in the Biomedical Research Technology Program and up through the Director's office went to extraordinary efforts to fund the project, which we greatly appreciate. Financial support from Kraft General Foods (Allen Blaurock), the State of Illinois, and the Illinois Institute of Technology was essential; productive interaction with the other CATs (IMCA-CAT an MR-CAT) within IIT's Center for Synchrotron Radiation Research and Instrumentation (CSRRI), and the interest and enthusiasm of the research community all have been essential for the successful launch of BioCAT.

We have assembled a talented and motivated staff, most with experience running facilities similar to BioCAT. Recognizing the prominent role of x-ray scattering to BioCAT, and because my own background is in XAFS spectroscopy, I have asked Dr. Tom Irving, formerly of MacCHESS, to join BioCAT as Associate Director. Our project manager, Edgar Black, formerly of the APS, was in charge of engineering and installing most of the magnets in the ring; Ed now coordinates the various projects and manages the overall timeline and budget.

We are fortunate that Dr. Robert Fischetti, formerly of the Biostructures PRT (X9 at the NSLS), has recently joined us as Senior Beamline Scientist, bringing his experience in diffraction and XAFS, as well as a novel detector project. Dr. Shengke Wang brings his background in polarized XAFS of metalloproteins to his new position as Beamline Scientist. Both Bob and Shengke are working closely with Gerd Rosenbaum and Ed Black on beamline design and construction.

INSIDE

Dr. Ke Zhang, Assistant Director for XAFS Instrumentation, also formerly of the Biostructures PRT, is in charge of several important core projects, including a novel detector for fluorescence XAFS, which appears very promising, and is a high priority to develop. Our CAT Coordinator, who will be the principal user contact during routine operations, is Ms. Homer Harwood.

Unfortunately for us, but fortunately for him, Dr. Pathikrit ("Pat") Bandyopadhyay has returned to work in India so that he can be reunited with his wife (a high energy physicist) and son. I would like to express my thanks to him for his dedicated work to help launch BioCAT. We all wish him the best.

We are especially fortunate to have the participation and access to designs of Dr. Gerd Rosenbaum, who built one of the first synchrotron x-ray beamlines about 25 years ago at DESY in Hamburg for fiber diffraction studies of muscle, and who subsequently built high quality beamlines (X9) at the NSLS. Personally I find it satisfying that those pioneering studies have come full circle, with the BioCAT facilities finally providing the capabilities needed for the muscle problem. Although a full time employee of the Structural Biology Center at Argonne, with which BioCAT has a close relationship, Gerd is a collaborator on several of our detector projects and is the principal designer of our beamline optics. In addition, we will have the participation of Jonathan Schug, programmer, whom many will know as the author of XDC, the data acquisition software on beamline X9.

In this newsletter, we describe the primary beamline facilities that will be available when initial experiments commence in late 1997. One of the strengths of our project is detector development; several of our projects originated in our work at the Biostructures PRT in Philadelphia and continue in Chicago. Beyond the first two years, subject to additional funding, we intend to add a wide bandpass (~1.0% bandwidth) multilayer optic to increase the flux approximately two orders of magnitude for certain types of scattering experiments, to add a high resolution monochromator, and implement numerous and continuous improvements to the capabilities of the beamline. Long range goals include extending the energy range to the phosphorus and sulfur K-edges, and to construct a bend magnet line. I believe that in the long term there is an intriguing potential to devise novel insertion devices which could be used as an adjunct to the existing APS undulator A. Microfocussing capillary optics is another intriguing possibility
among many.

Many exciting scientific opportunities will be available upon commissioning of the undulator beamline late next year. We welcome and solicit your comments, suggestions, and participation in BioCAT. For more information please contact me at bunker@biocat1.iit.edu or Tom Irving at irving@biocat1.iit.edu.

BioCAT Undulator Beamline as a Premier Instrument for Biological X-ray Scattering and Diffraction

Tom Irving
Associate Director of BioCAT

One of the major roles of BioCAT will be to provide outstanding facilities for performing X-ray diffraction experiments on non-crystalline biological specimens. This class of experiments is often collectively referred to (not always accurately) as SAS (small angle X-ray scattering). This includes solution scattering of protein containing solutions, diffraction from model membrane systems and L-B films as well as diffraction from fibrous protein systems (including muscle).

Synchrotron radiation although almost always helpful for SAXS studies is essential for the following broad classes of experiments:

kinetics
anomalous scattering
small scattering volumes
measurements of particularly weak signals

Many if not most biological small angle scattering problems fall into one or more of these categories. Scattering from such systems is usually relatively weak, other factors such as the short duration of the physiologically interesting state (often ms or less) plus small sample sizes conspire to reduce the available signal.

The BioCAT undulator beamline should be just about ideal for these studies. The BioCAT beam line will deliver at least 2.4×10^{12} ph/s/mm^2 without focusing of the incident beam (this will provide extremely low beam divergence) and about 5×10^{14} ph/s/ mm^2 with the beam focused at the sample position (this providing comparable or less beam divergence to existing bending magnet beam lines) both with an energy resolution, $\Delta E/E$ or 10^{-4}. Most existing bending magnet beam lines deliver about 3×10^{11} ph/s/mm^2. This factor of 10 to 1000 X increase in flux density will allow, at least in principle, dramatic increases in time resolution in time resolved experiments and allowing to extend such experiments to much smaller specimens than ever before. The predicted beam spot sizes and divergence characteristics are shown in Table I. First order resolution will be at least 5000 Å in one direction. Because the beam focusing is accomplished using horizontal sagittal focusing with an independent vertical focusing mirror, the beam size, shape, and focus can be tailored to match the sample dimensions and desired direction of best resolution. This will be especially appreciated by the muscle community. A further advantage of independent focusing elements is being able to control the divergence of the focused beam.

A range of evacuated lengths of flight tubes from a few cm to 10 m will be available cover a wide range of reciprocal space so as to be optimal for a given experiment. The accessible

energy range will be 4-15 keV (in the first harmonic) allowing selection of wavelength for anomalous dispersion studies.

Table I
Insertion Device Beam Line Design Parameters

Insertion Device	APS undulator A
Monochromator Type	Cryogenically Cooled Si[111] Crystal
Energy Range(Fundamental)	4 - 13 keV
Energy Resolution (DE/E)	2×10^{-4}
Spot size	< 50 μm (FWHM vertical)
(focused at sample)	< 200 μm (FWHM horizontal)
Angular Resolution	.16 mrad (FWHM vertical
(focused at sample)	.19 mrad (FWHM horizontal)
Spot size	< 100 μm (FWHM vertical)
(focused at detector 10 m from sample)	< 600 μm (FWHM horizontal)
Spot Size	1200 μm (FWHM vertical)
(no focusing)	2500 μm (FWHM horizontal)
Angular Resolution	.025 mrad (FWHM vertical)
(no focusing)	.056 mrad (FWHM horizontal)

SAS Detectors at BioCAT

The characteristic times for conceivable small angle x-ray diffraction experiments may lie anywhere between picoseconds (e.g. primary photochemical events in photosynthesis or sensory transduction) to years (e.g. organismal growth processes, racemization of certain amino acids). For the diffraction experiments that one might imagine doing on the BioCAT undulator beamline, one would like a position-sensitive x-ray area detection system capable of responding and reading out on time scales between microseconds to seconds (e.g. protein folding/unfolding, dynamics of muscle contraction, macromolecular conformational changes in solution)

The high count rates at the APS limit the range of detector options. At present, image plates remain a detecting medium of choice due to their combination of relatively good spatial resolution, large active area (typically 20 x 25 cm), high dynamic range (~10^4), sensitivity and lack of instantaneous count rate limitations. We plan to have an image plate system operational when the beam line is commissioned. We also plan to acquire a 1000 x1000 pixel (active area of about 50 mm^2) CCD based area detectors developed by E. Westbrook and colleagues at Argonne National Labs. These detectors will be used to acquire high-quality area diffraction patterns. By using a fast shutter and strobing you can get a single frame to arbitrary time resolution. They will not be useful for multiframe time resolved experiments.

Bob Fischetti in his article describes his Multielement Array detector which will be able to acquire one dimensional time framed data to 16 μs resolution at APS count rates We expect this to be very useful for solution scattering, model membrane and some muscle studies. We have also acquired a television-based area detector (80 mm active area, ~500 μm spatial resolution) from Brandeis University which allow limited two dimensional continuous 'movies' to ms scale time resolution.

These detectors, although very useful, will not be deeply satisfying to those who desire full frame, image-plate type spatial resolution and e.g. 10 μs time resolution which has been achieved with two dimensional proportional counter type detectors at Daresbury Laboratory. The present generation of their detectors would saturate at the flux rates expected at the APS. Next generation detectors of this type from Daresbury laboratories coming on line over the next year or so should be capable of handling the diffraction from small specimens such as single muscle fibers at the APS. There are also a number of proposals to build silicon strip detectors (well established in the high energy physics field) which would be expected to yield similar performance. All these detectors, however, are expensive, about $500,000 - $600,000, to reproduce. At the present stage of development, they also require a small team of experts to maintain them, which along with the acquisition cost will be beyond the present means of BioCAT. We feel that development of these technologies will be essential in the long term for optimum use of the BioCAT beamline. We would like to spearhead any efforts to acquire such detectors but these will require input from the community to back the proposals with solid science to raise the necessary funds.

BioCAT Advanced Capabilities for XAFS

Grant Bunker

The principal benefit of undulator radiation over wigglers and bend magnet radiation is high brilliance: high flux in small angular divergence, and a small source size. Undulators also have desirable properties for polarized XAFS studies: at the peak of the fundamental and odd harmonics the beam is essentially perfectly polarized in the plane of oscillation (horizontal).

With the focusing optics, which demagnify the source size approximately 10 to 1, spot sizes less than 50 micron in the vertical direction, and twice that in the horizontal direction, will be achievable (in theory, much better than that). In unfocussed mode, the beam size will be approximately 2mm vertical by 4mm horizontal. With independent horizontal and vertical focusing a wide range of beam aspect ratios and sizes will be achievable. Fluxes are expected to approach 10^14 photon/sec for 2 eV bandwidth. Various tradeoffs are possible between resolution, flux, and focal size. Initially the monochromator will be cryogenically cooled Si(111), with a low end cutoff of about 4 KeV and a high energy cutoff of 15 KeV in the fundamental, and 45 KeV in the third harmonic. Cryogenic cooling is used to keep the silicon near its temperature of vanishing thermal expansion coefficient, so the high power undulator density doesn't distort the first crystal and broaden the rocking curve.

The high flux into a small spot size will be important for time resolved XAFS studies and experiments on small samples, or small ordered domains in samples, such as single crystals. BioCAT plans to develop a dedicated stopped flow system, optical monitoring system, and polarized XAFS instrumenta-

tion to take advantage of these possibilities. We are fortunate to have Dr. Ke Zhang directing these core projects, as well as the multilayer analyzer/detector, which will offer important advantages over existing multielement detectors (see article by Ke Zhang in this newsletter).

The intrinsic energy width of the undulator peaks is on the order of 100 eV. The energy position of the peaks can be controlled by adjusting the undulator gap, which is the separation between the magnet pole faces. The closer they are together, the stronger the magnetic field felt by the electron beam, the larger the deflection, and the lower the energy of the undulator peak.

To scan over an XAFS scan range of 1 KeV or so, there are two approaches. The APS has assured us they will permit scanning of the undulator gap in concert with the monochromator. This requires attention to detail, but tests to date suggest there is no good reason why it shouldn't work. Full scans should be achievable on the scale of a few seconds.

An alternative approach is to taper the undulator gap so there is a linear gradient in gap along the length of the undulator. This broadens the undulator energy spectrum, but at the cost of peak flux. This approach probably will be most useful for fast edge scans on a time scale shorter than seconds. We plan to make the control system very flexible so that it can accommodate unusual experiments, while trying to preserve ease of use for typical experiments.

Our initial priorities are to get the basic beamline up and running without compromising optical quality. Once the beamline is running, additional features and amenities will be added. Among these will be enhanced data analysis capabilities. The BioCAT facilities as a total package will offer exciting possibilities for biological XAFS experimenters.

Multilayer analyzer XAFS detector

Ke Zhang, Assistant Director for XAFS Instrumentation

It is expected that the photon flux will increase by a factor of 100 to 1000 at the ID line of BioCAT over the spectroscopic beamlines at NSLS. The development provides real opportunities for biological XAFS applications in determining the structure and time course of a time-resolved reaction, and on even more dilute systems. However, these dramatic photon flux increases will have little effect on XAFS data collection speed of dilute systems using currently available detector systems. The commonly used energy-resolving fluorescence detector, the multi-element Ge detector, will have to be operated very inefficiently to avoid detector saturation. The same is true, to a greater or lesser extent, for any detector with energy discrimination through processing of detector signals. Only a detector system that removes the unwanted background *before* it reaches the detector electronics can, in our opinion, solve the dilemma of detectors always lagging behind increasing available fluxes.

BioCAT plans to develop an energy-resolving detector system using synthetic multilayers. The lack of dequate detectors have been a significant impediment to efficient XAFS data collection at existing synchrotron sources and would become very much so at the APS. The proposed multilayer-based detector will essentially removes that bottleneck by combining high data collection efficiency with very high count rate capability, and a reasonable solid angle of acceptance. Simulation and comparison of the multilayer array detector with the conventional detectors, such as the ionization chamber and 13-element Ge detectors, shows that the detector will be superior to them, in particular with its ability to handle the increased photon fluxes available from insertion devices, and to allow decreased sample concentration. The design specifications for this detector are shown in Table II. This type of detector will provide tremendous opportunities for XAFS measurements on dilute systems, such as biological systems, at third generation synchrotron sources.

Table II

Design Specifications for Energy Resolving Fluorescence Detector with 40 Multilayer Array.

Energy Range	5 to 9.5 keV (Mn - Zn Kα)
Solid Angle Acceptance	0.5 to 0.6 Sterradian
Energy Resolution	200-300 eV @ 7 KeV
Background Rejection Factor	> 80-100
Count Rate Limitation	None (current integration mode)
Time Resolution	20 ns in Photon Counting Mode
Sample Vertical Dimension	< 0.4 mm

A prototype multilayer detector will be designed and fabricated at the beginning of 1997. A full scale array detector will be constructed at the end of 1997. Test and operation of the detector will take place in early 1998 when the ID line is fully operational.

Multi-Element Detector for Sub-Millisecond Time-Resolved X-ray Diffraction

Bob Fischetti
Senior Beam line scientist

One of the main missions of the BioCAT undulator beamline is to perform time resolved experiments on dynamic systems. The high flux available at the Advanced Photon Source, however, would saturate most existing time slicing single or multiwire proportional counter detectors. While two-dimensional CCD detectors scan handle the high in-

stantaneous flux available at synchrotron sources, their time-resolution is typically limited to on the order of seconds because of the long readout times. Time frames of 1 msec or greater have been achieved when the CCD detector is used as a one-dimensional streak camera. A Multi-Element Detector (MED) was designed and developed by Gerd Rosenbaum, Bob Fischetti and Kent Blasie to address these detector limitations. The MED provides the capability for time resolved X-ray diffraction experiments on microsecond and longer time scales at the flux levels anticipated at the APS.

The current design of the (MED) incorporates 128 independent channels of fast plastic scintillator fibers which are read out in parallel via photon counting electronics. The detector is constructed of scintillator fibers of 1 x 1 mm cross section which are arranged to form a linear position sensitive detector with an active area of 1 mm by 128 mm. An advantage of this detector design is that the X-ray sensitive front end is constructed of relatively inexpensive materials, and thus one can exchange the front end to match the detection geometry of a particular experiment. Many experiments (e.g. solution scattering, model membrane systems and many muscle experiments) can make good use of a linear (one dimensional) detector. Other experiments require two dimensional information. The individual elements can be arranged into a grid for peak profiling experiments or they may be separated to monitor individual reflections in kinetic experiments. We will seek funding to upgrade the detector to 256 channels, in which case non-linear arrangements will be even more useful.

The maximum useful count rate in photon counting mode for a stochastic source is limited to ~10 MHz per channel for a 10% dead time loss due to pulse pile up. The timing structure of the electron storage ring at the APS may further limit the count rate per channel. Thus we plan to add a current integration mode which will extend the maximum count rate to 10^9 cps per channel resulting in a global count rate of ~10^{11} cps over the entire detector. The 1/2 inch diameter photomultiplier tubes are cooled in order to reduce the dark count rate to ~1 cps per channel. Thus an individual channel will have an extremely large dynamic range and the detectors dynamic range should exceed 10^4. (Currently the dynamic range of the detector is limited by nearest neighbor channel-to-channel cross talk which we are working to reduce.)

The fast time-framing capabilities will allow diffraction patterns to be recorded in up to 1024 contiguous time frames with a minimum duration of 16 µsec and a blind time between frames of only 200 nsec. Noncontiguous time frames of 1 µsec minimum duration can be spaced as closely as 16 µsec.

Meridional X-ray diffraction data have been successfully recorded with a 16-channel prototype of the detector. The 1 mm x 16 mm detector was oriented such that the 16 channels simultaneously recorded diffraction along the meridian. The figure shows the diffraction recorded from a 10-bilayer Langmuir-Blodgett multilayer film which was elastically bent to simultaneously provide diffraction of high intensity over an extended range of reciprocal space. The diffraction pattern was recorded in a 100 msec snap shot using the high incidence flux provided by the sagittally focusing monochromator on beamline X9B at the National Synchrotron Light Source at Brookhaven National Laboratory. The maximum count rate recorded in this experiment was $5x10^6$ cps from the low angle specularly reflected beam.

The inset shows the diffraction recorded from a similarly bent 5-bilayer L-B film. A comparison is shown between the data recorded simultaneously with all 16-channels and that recorded in a step scan (The detector was used a single channel scintillator by placing a very narrow slit over one channel and recording the output of that channel as the detector was scanned through reciprocal space.) The pattern demonstrates the ability of the detector to accurately record a diffraction pattern over an extended range of reciprocal space simultaneously. The small differences between the two measurements are within the uniformity of response correction of the detector.

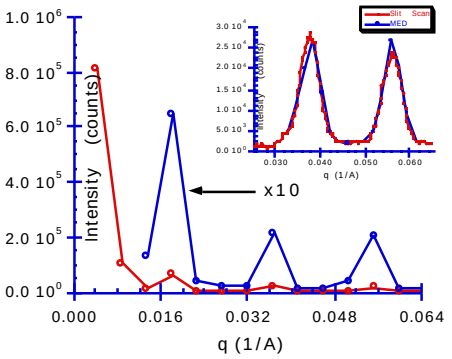

Figure 1.

X-ray diffraction pattern recorded in 100 msec with the 16-channel prototype of the Multi-Element Detector from a curved 10-bilayer Langmuir-Blodgett multilayer film. The inset compares the diffraction pattern recorded with the 16-channel prototype verses a "slit-scan" from a 5-bilayer L-B film.

Lab/Office Module

Ed Black
Project Manager/Engineer

The layout of the Lab Office Module (LOM) is shown in Fig. 2. It consists of approximately 3600 square feet of space of which 2100 square feet are office space including user access area, and 1500 square feet of laboratories. The office space has three distinct sections, the left wing will have permanent BioCAT staff offices, the right wing will contain a large partitioned area for user office space which can be rearranged as needs evolve. The central part contains a workstation room and an additional office. The workstation room will contain a graphics workstation and Xterminals for preliminary data analysis, while the office will be overflow for BioCAT staff or visitors. All offices will have ethernet hookups and telephones. The central space will contain a fax machine and photocopier.

There are two laboratories each of 750 square feet, one set up as a chemical laboratory, the other as electrical/mechanical. Beginning in fiscal year three (Oct., 1997) we have funds to install basic laboratory equipment (balances, spectrophotometer, ultrapure water). Suggestions of what sort of equipment will be needed are welcome. Part of the mechanical lab will be dedicated for detector and other equipment storage and maintenance, the other part will serve as a staging area for experimenters equipment. It will be possible to use some of the biochemical laboratory space for this purpose as well.

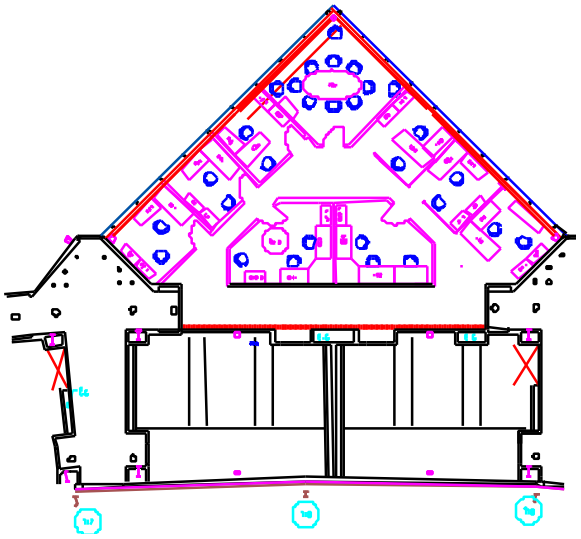

Fig. 2

Construction Time Lines

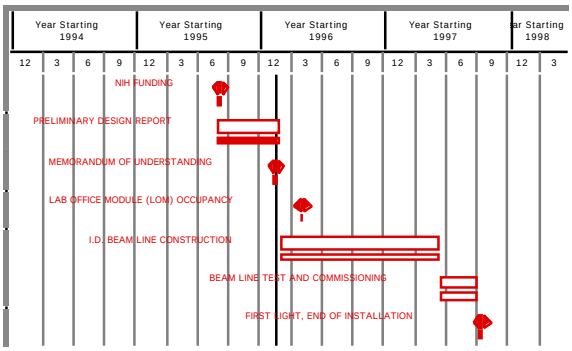

Fig 3

First Light Fall 1997!

Postscript:

The BioCAT beamline will be a superb research instrument that you can count for delivering the beam you need for cutting edge experiments. For it to reach its full potential **we need your input**. We can only guess at the full range experiments that will be possible. For BioCAT to continue to be funded at a level commensurate with the need, the facility needs to produce excellent science in order to justify the continuing interest of our funding agencies. If you have an potential application please talk to us. We have many design decisions to make and although we have assembled a committed team of experts to make these decisions we cannot forsee every eventuality and we need to know what experiments you would like to do. Budgets are tight and to provide additional capabilities, we need to seek additional funding. We can coordinate such efforts but we need your ambitions and vision to help make it happen.
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