Medium-term upgrades enabling new science at BioCARS

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Introduction

In this document, we propose a set of beamline upgrades that will enable new and enhance existing core research and development activities in both static and time-resolved crystallography at Sector 14. These upgrades include a large-area pixel-array detector suitable for time-resolved/Laue diffraction and replacement of the 14-ID-B experimental station with a larger enclosure to house the new detector and its positioning aparatus. An automated crystal handling system with an associated liquid nitrogen autofill system is being proposed for 14-ID-B and 14-BM-C to carry out rapid sample placement and screening. Additionally, new optics for the sector 14 bending magnet beamline will fully utilize the properties of the source for both 14-BM-C and 14-BM-D experimental stations and provide extra capacity for BSL2-3 experiments (BioCARS is the only faclility in the USA capable of performing these studies). This is especially important since it is forseen that 14-ID-B will be used primarly for time-resolved studies.

Science

BioCARS' chief scientific emphasis lies on time-resolved crystallography on time scales from 100 ps to s, in which a substantial collaborative user community is developing. Our recently completed upgraded insertion device beamline 14-ID is served by two dissimilar, collinear undulator sources that deliver tightly-focused, intense, tunable, monochromatic or pink X-ray beams to the 14-ID-B station, suitable for both time-resolved and standard monochromatic experiments. The bending magnet beamline 14-BM is particularly suited to study crystals with large unit cells, or at ultra-high resolution. A laser laboratory houses ps and ns pulsed lasers and other optical instrumentation essential to the time-resolved experiments. Three experimental stations, one ID station and two bending magnet stations, are embedded in a BSL-3 facility to allow safe study of biohazards. Both the time-resolved and biohazards capabilities are unique in the USA. In all cases, we assist users in solving transient and static, atomic-level structures of direct relevance to significant biomedical problems. Some of these structures will be of biohazards classified at the BSL-3 level; examples include human pathogenic viruses such as West Nile virus and prions. Others such as anthrax-related toxins or enzymes are key to the metabolism of pathogenic microorganisms. They are both of substantial public health interest in their own right and may yield information directed at responses to bioterrorism. Yet others, particularly the transient structures, provide a novel view of mechanisms of action, and thus underlie the development of more effective therapeutic agents.

Based on the proposed medium-term upgrades, we will implement new methods for exploring irreversible reactions based on pixel array detectors; develop a new capability to study small to microcrystals with both monochromatic and Laue techniques, and examine the ultrafast time course of radiation damage at room and cryo temperatures. Service emphasizes study of biohazards, both as single crystals (often with large unit cells) and where feasible, as fibers or less-ordered samples; and of small to microcrystals. The intellectual merit of the proposed project are two-fold: to expand the knowledge of the three-dimensional structure of macromolecules important to the life sciences, *e.g.* the structure of edema factor, one of three toxins that make anthrax a deadly biohazard (Drum et al, 2002); and to improve the understanding of their functions and reaction mechanisms, *e.g.* viruses and their mechanisms for infection of the human cells (Rossmann et al., 2005). The robotic system will significantly improve the efficiency and experimental capabilities of both the ID and bending magnet beamlines at BioCARS by increasing x-ray data quantity and quality, and thus improve the output of high-impact science by the users of the facility.

Added value of a medium-term upgrade

Pixel Array detector for time-resolved/Laue crystallography

An ideal X-ray detector for time-resolved macromolecular crystallography would include the following characteristics (among others). First, it must have a large area, on the order of 40 x 40 cm^2, to enable high-resolution data to be collected at a large crystal-to-detector distance and thus minimize the background and maximize the mean spot separation. Second, good spatial resolution is crucial if crystals with large unit cells are to be examined, leading to the requirement that individual pixels be ~100 μm. Third, it should be possible to take multiple consecutive frames with an inter-frame rate as high as possible. Ideally, this would be matched to the X-ray pulse frequency of the storage ring, 6.5 MHz in the standard 24-bunch fill pattern of the APS. Each X-ray pulse has a duration of ~100 ps FWHM and repeats every 153.4 ns in this mode. This would allow one laser pump pulse (or other reaction initiation pulse) to be followed by many probe X-ray pulses, each of which generates a diffraction pattern that could be separately recorded and stored. Although this detector would be capable of isolating data from a single X-ray pulse electronically, a mechanical chopper is still necessary to protect the sample from radiation damage due to prolonged X-ray exposure. The ability to take many consecutive frames quickly is an essential requirement for the efficient study of irreversible systems. At first reading these characteristics are daunting. However each has been realized technologically, though only in separate detectors; they have not yet been combined in a single detector. We propose to collaborate in the development of a prototype detector with the APS and others by providing feedback regarding ways in which they can be optimized for high-count-rate, high-frame-rate experiments such as ours.

Pixel array detectors (PAD), in which each pixel has its own signal processing and readout electronics to enable the pixels to be read out in parallel, are being developed as an alternative to CCD detectors. As a result of their quite different design, these PAD detectors are capable of much higher time resolution and faster readout compared to currently available CCD detectors. Recently the promise of PAD has led to the commercialization of this exciting technology by a group at the Paul Scherrer Institute that developed the PILATUS detector (Broennimann et al., 2006). In groundbreaking work, Gruner's group at Cornell University is developing an analog-integrating PAD or APAD (Ercan et al., 2006). Although the PILATUS and APAD detectors are conceptually similar and both have analog and digital circuitry, there is a crucial difference between them. The PILATUS is essentially a photon-counting detector, in which absorption of each individual photon produces an electrical pulse that is discriminated by a comparator and counted. The maximum instantaneous count rate of this detector is limited by the temporal width of the electrical pulse to 1 MHz for the PILATUS II. In other words, two photons arriving at a single pixel within 1 μs of each other will be counted - erroneously - as one. This detector is therefore unsuited to our ultrafast, time-resolved, Laue measurements where instantaneous count rates can be as high as 50 THz. In contrast, an APAD stores the charge produced by each photon in a capacitor and thus effectively integrates the X-ray flux. An APAD is limited by the well depth (well capacity), i.e. the total amount of charge that can be stored per pixel (proportional to the number of photons absorbed, and to the photon energy), and not by the rate at which photons arrive. This version of the PAD is in principle ideal for our time-resolved measurements where several thousand photons can arrive at a single pixel within a single X-ray pulse.

Unfortunately, of the various prototypes of the APAD produced to date, none has exactly the specifications required for the ultrafast time-resolved measurements described in this proposal. However, the key elements of the technology are in place and with sufficient funding, could be further adapted for time-resolved crystallography experiments. We propose entering into a collaboration with the APS to develop and manufacture such a detector.

Automated crystal handling system

This proposal requests funds to acquire, install and commission an automated crystal handling system – also called an Automount Robot or robotic sample handling system – and associated liquid nitrogen (LN2) autofill system that will permit uninterrupted operation of the device. This new instrumentation will be installed on both the insertion device endstation 14-ID-B and the bending magnet endstation 14-BM-C of sector 14 at BioCARS and will enhance their throughput. It will offer scientists at all levels of expertise an improved environment to pursue their research by reducing the routine, repetitive, error-prone and time-consuming manual activities related to crystal mounting and screening; by assuring reliability and reproducibility of all critical manipulations carried out by the robotic system; and by enhancing use of the facility through remote access.

Enlargement of the 14-ID-B endstation

The 14-ID-B endstation is rather small at present and cannot accommodate a large-area detector and motion control system. Additionally, infrastructure associated with the laser delivery system consumes much of the space required for a crystal mounting robot. Therefore, we propose to remove the existing enclosure and replace with Tecknit-built station large enough to accommodate the additional infrastructure necessary to house a large-area detector system, laser delivery system, second-stage focusing to afford focused pink and mono beams ~10 μm in diameter, and an automated sample changing robot.

Replacement of 14-BM optics

Sector 14's bending magnet beamline splits the BM radiation into 1.2 mrad and 2.0 mrad slices and delivers it to 14-BM-C and 14-BM-D respectively. The 14-BM-C side-bounce station is, in principle, tunable in energy from 8-16.5 keV however, scanning is not possible. The 14-BM-D station is tunable in energy from 6.8-18 keV moreover; the energy can be scanned for Multiple-wavelength Anomalous Diffraction (MAD) experiments. Both stations suffer from highly degraded mirrors and an outdated monochromator. Replacing the mirror system and upgrading the monochromatic capabilities will enhance throughput and improve the quality of the data. It should be stressed that these stations are BSL2-3 compatible and are unique in the country in that regard. We envision the bending magnet beamline becoming a resource for the entire CARS CAT as both ChemMatCARS and GSECARS plan to utilize the 14-BM-D station for small-molecule crystallography studies.

Table 1: Enhancements expected from the medium-term upgrade.

Upgrade	Time–Resolved Crystallography	BM Service Crystallography
Analog pixel array detector	Fast large-area detector with on-board frame storage will allow for multiple consecutive frames. Large unit-cell crystallography.	N/A
Detector motion control system	Provides accurate placement of detector.	N/A
Replace 14-ID-B station	A larger hutch is needed to house a large-area detector.	N/A
Sample robot	Increase throughput and efficiency.	Increase throughput and efficiency.
Bending magnet beamline optics	N/A	Current optics are over 10-years-old and do not efficiently condition the beam. New state-of-the-art optics would improve the beam quality and flux.

Expected user communities

We successfully conducted the first ns time-resolved diffraction experiments at the BioCARS ID beamline in the summer of 2000 and hosted the first outside user group in March 2001. We have focused a considerable effort in fostering the development of a time-resolved user community since that time. The community has been growing steadily. Following the recent complete upgrade of the 14-ID beamline and the implementation of the 100ps time-resolved capability, we have just resumed time-resolved user operation during the 2008-1 APS run cycle. We expect substantial growth of the time-resolved user community as a result of the BioCARS laser, X-ray chopper and optics upgrade. This X-ray upgrade greatly expands beamtime availability since the isolation of a single X-ray pulse is now possible in the 24-bunch mode. Both hybrid and 24 bunch modes can therefore be used for time-resolved experiments.

The past time-resolved user community involved ~20 user groups. In addition to users interested in studies of fast ns-μs processes in inherently photosensitive systems (W. Royer, University of Massachusetts Medical School; M. Schmidt, Technische Universität München, Germany; H. Ihee, KAIST, South Korea; J. Norris, University of Chicago; W. Montfort, University of Arizona; K. Moffat, University of Chicago), as well as an increasing number of users that seek to use caged compounds for initiation of irreversible reactions. Several of them were developing an efficient cage-based reaction initiation and have conducted initial, feasibility studies (E. Pai, University of Toronto; C. Stauffacher, Purdue University; D. Ohlendorf, University of Minnesota; M. Hrmova, University of Adelaide, Australia). Other user groups explored the use of a flow cell for steady-state studies of reaction intermediates (A. Mesecar, University of Illinois; G. Petsko and D. Ringe, Brandeis University; M. Hasson, Purdue University). Yet other groups conducted preliminary Laue measurements to test their crystals for suitability for time-resolved experiments, while developing a method for reaction triggering in their systems (B. Edwards, Wayne State University; T. Hurley, Indiana University Medical School; A. Rosenzweig, Northwestern University). Finally, a novel temperature jump experiment aimed to explore the initial steps of partial protein unfolding upon crystal heating by ns laser pulses at 1064nm (D. Agard, University of California). With the latest upgrade and the new 100ps time-resolution capability, about 10 new user groups have already joined the BioCARS user community.

Enabling technology and infrastructure

The central technological effort foreseen in this proposal is the development of a large-area analog pixel-array detector. Prototypes have been built by Gruner's group at Cornell University and tested with fuel-spray experiments at the APS however; these detectors have a relatively small active area and are not suited for measuring diffraction patterns from macromolecular crystals which typically have large unit cells. Moreover, building a large-area version of this detector in collaboration with the APS will benefit not only time-resolved crystallography, it will also significantly enhance fast x-ray imaging techniques, where one would like to take in rapid succession a set of images of a mechanical process such as the time-evolution of a fuel spray as described earlier.

Partnerships and user interest

Philip Anfinrud and Friedrich Schotte	Laboratory of Chemical Physics, NIDDK/NIH
Alfred Batschauer	Philipps-University Marburg
Roberto Bogomolni	University of California Santa Cruz
Katrina T. Forest	University of Wisconsin-Madison
Maria Hrmova	The University of Adelaide
Thomas D. Hurley	Indiana University School of Medicine
Hyotcherl Ihee	KAIST
John E. Johnson	The Scripps Research Institute
Andrew Mesecar	University of Illinois at Chicago
Christopher Min	Columbia University
Douglas H. Ohlendorf	University of Minnesota
Joseph Orgel	Illinois Institute of Technology
Emil F. Pai	University of Toronto
Stanley Prusiner	University of California San Francisco
Vijay S. Reddy	The Scripps Research Institute
Michael Reedy	Duke University
Dagmar Ringe and Gregory Petsko	Brandeis University
Michael Rossmann	Purdue University
William E. Royer	University of Massachusetts Medical School
Gebhard Schertler	MRC Laboratory of Molecular Biology
Marius Schmidt	University of Wisconsin-Milwaukee
Cynthia V. Stauffacher	Purdue University
Gerald Stubbs	Vanderbilt University
Wei-Jen Tang	The University of Chicago:
Jasper van Thor	Imperial College London
Bidadi Venkataram Prasad	Baylor College of Medicine
Gregory L. Verdine	Harvard University
Stanley J. Watowich	University of Texas Medical Branch
Carrie M. Wilmot	University of Minnesota

Industry and technology transfer

The pixel array detector technology can be transferred easily to industry both as a diagnostic tool and as product for manufacture.

Budget

	Year 1	Year 2	Year 3	Year 4	Year 5	Total
M&S	50K	50K	50K	50K	50K	250K
Pixel Array Detector/ Motion Control	250K	2000K	-	-	-	2250K
Automated crystal handling system	-	250K	-	-	250K	500K
14-ID Secondary Focusing		300k				300k
14-ID-B Construction	350K	-	-	-	-	350K
14-BM Optics Upgrade	-	-	-	1000K	-	1000k
Sum	650K	2300K	50K	1050K	300K	**4900K**

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