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Biological XAFS at the BioCAT Undulator Beamline 18ID at the APS

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Abstract

The Biophysics Collaborative Access Team (BioCAT) undulator beam line at the Advanced Photon Source, Argonne, IL is a user facility devoted to the study of partially ordered and disordered biological materials by X-ray scattering, diffraction and absorption spectroscopy. Two sagital focussing, double-crystal (Si(111) and Si(400)) monochromators and a 1 m mirror provide monochromatic, horizontally and vertically focussed X-ray beams in the range of 4–35 keV. The small focal spots produced by this optics are well matched to novel XAFS detectors developed by BioCAT: a multilayer analyzer array and Bent Laue crystal based analyzers. Fast on-the-fly scans (∼5 s/scan) have been implemented to take full advantage of the high X-ray flux at the Advanced Photon Source. Such fast scans not only allow high throughput but also reduces radiation damage to labile biological samples. A closed–cycle displex cryostat is routinely used for low temperature XAS measurements. For continuous flow and time resolved XAS experiments, a dual syringe stopped-flow system has been implemented.

1. Introduction

The Biophysics Collaborative Access Team (BioCAT) undulator beam line, 18ID, at the Advanced Photon Source, Argonne, IL, is a high performance instrument designed for and dedicated to the study of partially ordered and disordered biological materials. X-ray Absorption Spectroscopy experiments include studies of dilute metalloprotein solutions (with a special emphasis on small volumes and time resolved XAS studies), oriented films, and oriented single crystals. High flux delivered into a small spot size is required for many practical time-resolved XAFS experiments and high throughput modes that employ flow systems. To this end, the beam line was designed to deliver doubly focused undulator beam, scannable over a wide energy range while maintaining a fixed position on the sample. The beamline has been operating since 1997 as an open facility to all researchers on the basis of peer reviewed research proposals. Here we describe the beamline optics, EXAFS specific instrumentation and performance. Representative experimental results of low temperature, room temperature flow experiments and time-resolved experiments are presented.

2. Beamline Optics

APS undulator "A" [1] provides a source of very intense monochromatic radiation in the 3.2–14 keV range (first harmonic) and 9.6–42 keV (third harmonic). The undulator gap may be scanned at rates of ca. 1 mm/sec under beamline control, permitting very fast scans over a typical XAS range of ca. 1 keV in 10 seconds. The undulator gap can also be tapered to deliver a smooth energy range on the order of 1 keV but with a significant loss in peak intensity. A differential pump separates the beamline vacuum structure from the storage ring permitting windowless operation so that experiments near the calcium edge at 4 keV are practical. Two independent double-crystal monochromator assemblies [2] cover the energy range of BioCAT interest. The two monochromators have identical mechanisms, but monochromator #1 has Si (111) crystals to cover an energy range from 3.4 keV to 14.6 keV, while those in monochromator #2 have a (400) orientation for energies from 7.9 keV up to 33.8 keV. Both monochromators have a cryo-cooled first crystal [3] and sagittal focusing second crystal assemblies for horizontal focusing of the beam. Horizontal focal spot sizes with a FWHM of 120–150 μm are typical for the energy range of 4.0–10.0 keV. An ULE glass-ceramic mirror (Rocketdyne Corporation) is used for harmonic rejection and it can be either used flat or eliptically bent to allow vertical focusing independently of any horizontal focusing. The mirror surface is divided into three lanes: bare ULE, Pd coated, and Pt coated to cover the full energy range of the beamline. When focused at the center of the experimental table the beam profile has been observed to be as small as 40 μm (FWHM). Additional details of the beamline optics can be found elsewhere [4].

3. Control software

The beamline control software is based on the Experimental Physics and Industrial Control System (EPICS) which is a distributed system using VME-based electronics with crate controllers running the proprietary real-time UNIX-like operating system VxWorks (Wind River Systems). To take full advantage of the high X-ray flux at the Advanced Photon Source and to reduce radiation damage to labile biological samples, two types of fast on-the-fly scans have been implemented: a "generic" fast scan and "energy" fast scan. With the generic scan we can scan any servo or stepper motor at the beamline while recording the output into the Joerger VCS16 scaler, with 16 inputs. The scan may use one of three different algorithms but all of them have the same lower limit to the time resolution of ∼150 ms/point. As a result, the typical generic scan time is ∼15–60 seconds. These scanning protocols have been very useful in beamline diagnosis and alignment and have found use in various experimental protocols. The fast energy scan is implemented for both the beamline monochromators. This scan makes use of a Struck 7201 multichannel scaler, with 32 inputs and 4k memory arrays per each input, which simultaneously record the monochromator encoder outputs and

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the X-ray intensities. The minimum time per point for this scan is ∼1 ms and the total scan time is typically 1–10 s. We have also included the synchronous motion of monochromator and the beamline undulator into the energy scans so that the energy of the two devices can be changed simultaneously. Finally, all the energy and generic scans provide for two or three dimensional scans with stepwise motion of the second and the third motors respectively retaining continuous scanning in the first dimension.

For positional feedback, we have implemented an in-vacuum beam position monitor (BPM) developed by SBC-CAT [5]. This BPM is linear over a range of a few millimeters with a resolution of less than 2 μm. Using this BPM and a feed-forward approach to adjust the beam angle, we have been able to maintain constant exit height to ±25 μm over an XAS scan. In addition, a variable offset in the equations of motion of the monochromator conditions has been used to minimize energy-dependent beam position changes.

To maintain peak X-ray beam intensity during energy scans we have installed an analog feedback system consisting of: 1) a piezo-electric actuator, mounted to adjust the tilt angle of the second crystal relative to the first crystal 2) a lock-in amplifier (Stanford Research Systems model SR830 DSP), and 3) a custom-designed analog electronic feedback control unit. A small amplitude sine wave of frequency, f, is fed to the piezo actuator to impose a periodic variation on the beam intensity. When the two crystals are misaligned, the lock-in amplifier detects this intensity signal with signal strength proportional to the slope of the rocking curve. With the proper choice of phase angle, the sign of this correction signal will indicate the direction to drive the piezo in order to recover peak intensity. The correction signal is fed into an integrator, the output of which is summed with the dither sine wave and a manually adjustable offset, and then transmitted to the piezo amplifier. Amplitude of about 20% of the FWHM of the rocking curve is sufficient to drive the second crystal to the peak. The feedback system is capable maintaining a lock on the peak intensity for energy changes at a rate of 200 eV/s, allowing monochromator scans of 1000 eV in 5 seconds without losing intensity.

4. Experimental Equipment

Transmission ionization chambers modified from the CHESS design (Advanced Design Consultants, Lansing NY) are available for incident flux and transmission measurement. There are 4 current amplifiers (Keithley model 428), which are interfaced through the RS232 ports on the beamline workstations. For fluorescence detection there are fluorescence ionization chambers and large area plastic scintillator/photomultiplier tubes that can operate in either pulse counting or integrating modes in conjunction with a two dimensionally focused Soller slit assembly designed for the point-focused beam. Highly dilute systems require the capabilities of the multilayer analyzer [6, 7] and a bent Laue crystal based analyzer [8] for experiments at the Cd K edge, Mo K edge and Zn K edge.

XAS measurements on biological samples are conventionally performed at low temperature to reduce the radiation damage caused by the production of free radicals, and also to reduce the damping effect of temperature in the EXAFS oscillations. A low vibration, closed–cycle displex cryostat permits such experiments at temperatures down to 10 K. A Lakeshore Model 330 controller allows regulation of the Displex temperature under EPICS control.

In some cases it is desirable to measure the samples in solution at room temperature to characterize the dynamic changes in structure that bridge the static endpoints provided by crystallography and to avoid the artifacts that can be introduced by freezing. Furthermore, flow systems can reduce the duty cycle for a given measurement providing faster throughput of samples that will permit more elaborate types of experiments to be designed and executed than is possible with conventional approaches. For continuous and stopped-flow XAS experiments, a dual syringe stopped-flow system [9] has been implemented at BioCAT. The stop flow chamber can be cooled to temperatures as low as 233 K to lengthen the lifetime of a reaction. A portable UV-Vis spectrometer (Ocean Optics model 2000) is integrated into the stopped flow device through optical fiber and lenses allowing optical spectra to be collected simultaneously with an XAS measurement.

5. Results and discussion

The combination of the doubly focused beam and the efficient detectors available at BioCAT allow the study of dilute samples (ca. 1 mM). A typical scan time is 20–60 seconds with ca. 50 scans typically required to obtain good statistics, thus, a total time of 20–60 minutes suffices to complete an XAFS spectrum for each sample with either frozen samples or using the continuous flow system. Low temperature XAS experiments use the Displex

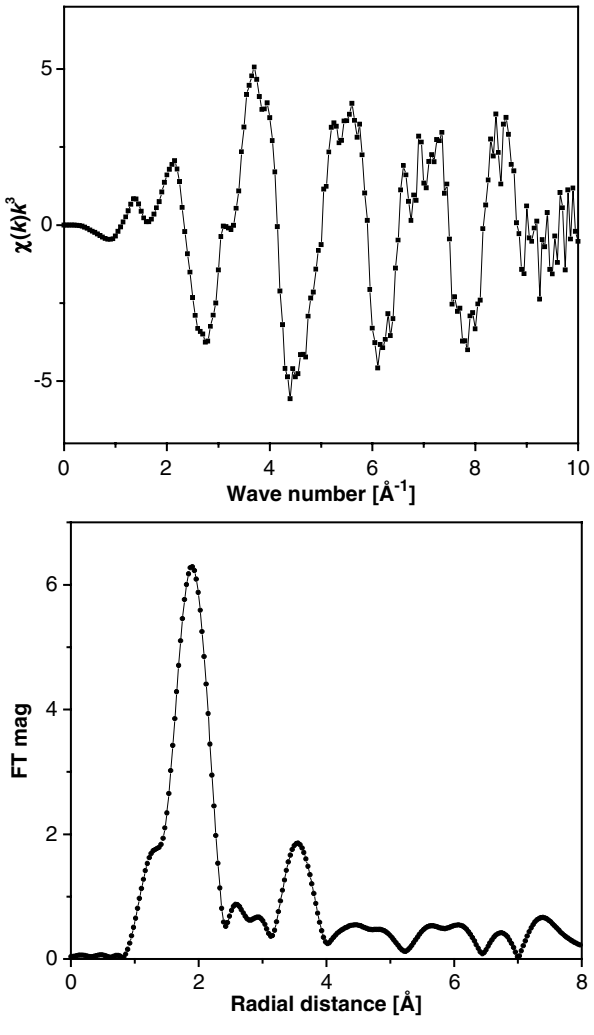

Fig. 1. EXAFS signal of a ∼1 mM Ca sample measured at 30 K with the multilayer analyzer (courtesy of Prof. C. Carmeli- Tel Aviv University).

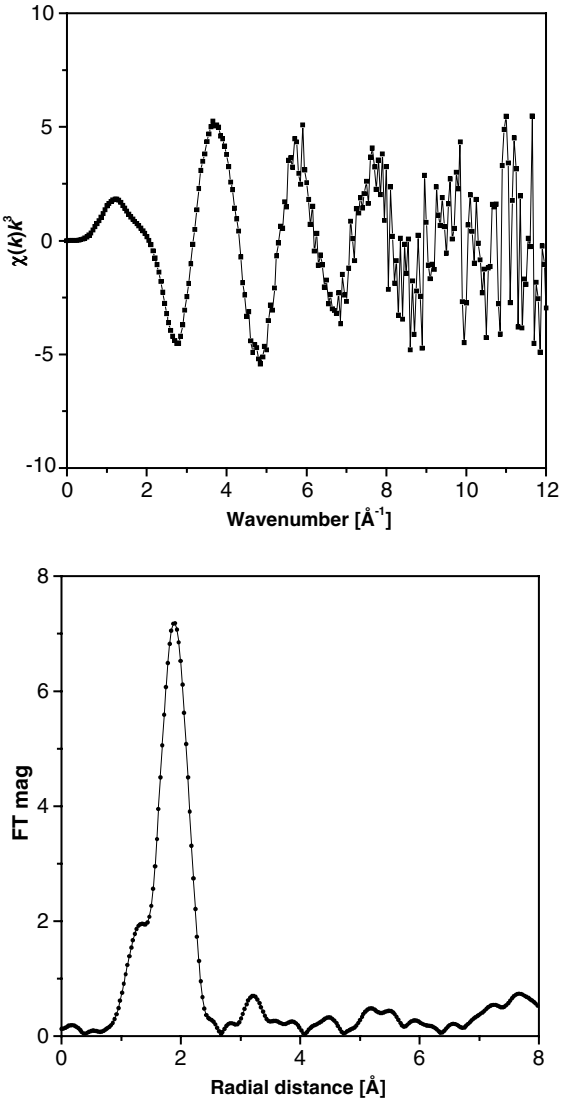

Fig. 2. EXAFS signal of a ∼1 mM Npl4 Zinc Finger protein measured at 40 K with the Zn Bent Laue analyzer (courtesy of Prof. T. Stemmler – Wayne State University).

cryostat and an aluminum sample holder, which can contain up to 5 sample cells. The multilayer analyzer or bent Laue analyzer, as appropriate, is mounted on one side of the cryostat exit windows, while the other window is used for sample exchange. The cryostat itself is mounted on an x-y stage that allows the user to raster scan the sample in two dimensions during the experiment. Depending on the particular case, each sample spot is exposed to the beam from 20 s to 120 s maximum and then a new spot is selected for subsequent scans. More than 200 spots may be measured in a single cell in our standard sample holders. This feature has been demonstrated to be very useful when working with samples that are sensitive to radiation damage. Figures 1 and 2 show EXAFS signal of a ∼1 mM Ca sample measured at 30 K measured with the multilayer analyzer (courtesy Prof. C. Carmeli-Tel Aviv University), and Zn protein samples at low temperature (35 K) measured with the Laue analyzer (courtesy Prof. T. Stemmler – Wayne State University).

The stop-flow system has also proved to be a versatile instrument for both time-resolved XANES studies [10] and continuous flow experiments. The Multilayer analyzer is currently being upgraded from 2 to 18 detection channels to increase maximum count rate. The Laue analyzer detector is also being upgraded to increase solid angle acceptance. Enhancements continue to improve overall beamline performance.

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