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Ulrich Englich, a* Irina A. Kriksunov, a Richard A. Cerione, a,b,c Michael J. Cook, a Richard Gillilan, a Sol M. Gruner, d,e Qingqui Huang, a Chae Un Kim, a William Miller, a Soren Nielsen, a David Schuller, a Scott Smith a and Doletha M. E. Szebenyia

*MacCHESS (Macromolecular Diffraction Facility at CHESS), Cornell University, Ithaca, NY 14853, USA, bDepartment of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA, cCornell High Energy Synchrotron Source (CHESS), Cornell University, Ithaca, NY 14853, USA, dField of Biophysics, Cornell University, Ithaca, NY 14853, USA, and ePhysics Department, Cornell University, Ithaca, NY 14853, USA. E-mail: ue22@cornell.edu

The Macromolecular Diffraction Facility at the Cornell High Energy Synchrotron Source (MacCHESS) is a national research resource supported by the National Center for Research Resources of the US National Institutes of Health. MacCHESS is pursuing several research initiatives designed to benefit both CHESS users and the wider structural biology community. Three initiatives are presented in further detail: microcrystallography, which aims to improve the collection of diffraction data from crystals a few micrometers across, or small well diffracting regions of inhomogeneous crystals, so as to obtain high-resolution structures; pressure cryocooling, which can stabilize transient structures and reduce lattice damage during the cooling process; and BioSAXS (small-angle X-ray scattering on biological solutions), which can extract molecular shape and other structural information from macromolecules in solution.

Keywords: protein crystallography; microcrystallography; BioSAXS; cryo; pressure.

1. Introduction

The X-ray solution of macromolecular structures involves several steps. If the macromolecule can be crystallized, then crystals of suitable size and diffraction quality must be obtained. In general these must then be cryoprotected to mitigate radiation damage. If crystals cannot be obtained, then low-resolution structural information may still be available by BioSAXS. BioSAXS can also help phase crystalline diffraction patterns. Below, we describe technical developments at CHESS in each of the three areas microcrystallography, cryocooling and BioSAXS.

2. Microcrystallography

It is now very common for users to arrive at CHESS with crystals that are either small or have very small diffracting regions. Crystals 50 μm across are now routine and often crystals are only 10 to 20 μm in some dimension. In addition, for large inhomogeneous crystals, better diffraction is sometimes obtained by illuminating a small well ordered portion rather than the whole crystal. Small-area illumination also allows minimization of the effects of radiation damage by moving to fresh portions of the crystal when the diffraction deteriorates.

Kirkpatrick–Baez (KB) mirrors are most commonly used to produce synchrotron microbeams. KB mirrors are less practical at CHESS because of space limitations and a source size that is large and relatively divergent. In consequence, CHESS has developed single-bounce focusing monocapillary optics to generate intense microbeams (Bilderback et al., 2007; Gillilan et al., 2010). Capillaries are drawn to order on an in-house computer-controlled drawing tower. The drawing tower apparatus can be programmed to produce capillaries of a desired focal spot size and working distance to match beamline characteristics and crystal requirements. MacCHESS routinely uses 20 μm and 5 μm capillaries with modest beam divergences of 2–4 mrad, which allow collection of data on samples with lattice parameters up to about 500 Å. For example, a typical total flux of \(2.8 \times 10^{10}\) photons s\(^{-1}\) for a 20 μm capillary has been measured at the CHESS F1 station (Gillilan et al., 2010), while the weaker bending-magnet station F3 equipped with a 5 μm capillary produces \(3.2 \times 10^9\) photons s\(^{-1}\) (Englich, 2008). The capillaries are mounted in a housing that allows for easy exchange between collimator and microfocusing capillary [Figs. 1(a) and 1(b)].
X-ray beam as it rotates. The MacCHESS staff have improved the performance of the air-bearing-based rotation stage. To measure the sphere of confusion, a new Keyence laser device replaces a mechanical probe and allows for fast and accurate automated data acquisition (Fig. 2). A runout of $\pm 1\,\mu$m has been achieved by applying tight mechanical standards in conjunction with encoded stepper motors. Equally important is the implementation of a high-resolution crystal-viewing camera system with an accompanying Java-based computer interface for manual and automated crystal centering.

3. High-pressure cryocooling

Flash-cooling of biological macromolecules has been the method of choice to reduce radiation damage and prolong the lifetime of the sample in intense synchrotron beams (Hope, 1988). The method typically involves soaking the crystals in a cryoprotectant solution prior to cryocooling. Determining the right kind and amount of cryoprotectant can be a tedious task. An alternative is a novel high-pressure cryocooling procedure that requires no, or very little, cryoprotectant (Kim et al., 2005). The method involves cooling macromolecular crystals to cryogenic temperatures ($\sim 100\,^\circ$K) in high-pressure (up to 200 MPa) helium gas. Applications include successful cryocooling with little or no penetrating cryoprotectant, and native sulfur SAD (single-wavelength anomalous dispersion) phasing. Samples in capillaries can also be pressure cryocooled (Kim et al., 2007; Chen et al., 2009). The method has been extended to other gases, e.g. Kr or Xe (followed by He) for SAD phasing (Kim et al., 2006, 2007), and CO$_2$ (alone at lower pressure) to visualize an enzymatic intermediate state in carbonic anhydrase (Domsci et al., 2008). Surprising results include visualization of ligands which could not be seen using conventional flash-cooling methods (Albright et al., 2006), and unusual phase behavior of water in protein crystals (Kim et al., 2008, 2009). The method can also be used to study pressure effects on protein structures (Barstow et al., 2008, 2009). A mechanism involving high-density amorphous ice has been proposed to explain why the method works (Kim et al., 2005, 2008, 2009).

The procedure, as described earlier (Kim et al., 2005), requires samples to be coated with a thin film of hydrocarbon oil for protection against drying, pressurized in a helium atmosphere, and then flash-cooled to 77 K. Details are described in the following steps [Figs. 3(a)–3(d)]:

(i) Mount sample (usually a crystal in oil) in a cryoloop on a steel pin (Fig. 3a) with a piece of piano wire attached to the base.

(ii) Slide the pin with the mounted sample into pressure tubing (Fig. 3b), where an external magnet holds it near the top.
(iii) Place up to three tubes into a bath partially filled with liquid nitrogen (Fig. 3c).
(iv) Place the bath in a safety enclosure.
(v) Connect tubes to a manifold and pressurize the system with helium, usually to about 200 MPa (2 kbar).
(vi) Remove magnets, letting the pins fall to the bottom of the tubes (at 77 K).
(vii) Release pressure from the system.
(viii) Disconnect the tubes at top and bottom, keeping the pins with the samples under liquid nitrogen (Fig. 3d).

The pins can then be transferred under liquid nitrogen to standard bases and handled like conventional flash-cryo-cooled samples. Note that the procedure can be used to cryocool samples in capillaries within the mother liquor (Kim et al., 2007).

The original high-pressure cryocooling apparatus is housed in the Cornell Physics Department. In order to make the high-pressure cryocooling method more available to users, a second apparatus, capable of operation to 200 MPa, has been made and installed at the synchrotron, and staff members have been trained in its use. All high-pressure components are enclosed in a steel cabinet with half-inch-thick walls designed to safely contain the apparatus in case of an unexpected gas release or failure of a high-pressure part. The whole apparatus weighs in at about 1500 kg (Fig. 4).

4. BioSAXS

Small-angle X-ray scattering on biological solutions (BioSAXS) is rapidly growing in user demand. It continues to play an increasingly important role in structural biology, not only as an aid in the assembly of macromolecular complexes but also in understanding changes in oligomeric states, determining conformation in solution and assessing structural integrity. Low-resolution shapes may be obtained for large macromolecules and macromolecular complexes without growing crystals. BioSAXS also allows studies of conformational changes of proteins and other macromolecules in solution under a very wide range of conditions including those which are close to physiological. In the past, BioSAXS has been supported at MacCHESS on a limited basis through access to the CHESS G1 line. A solid-state cooled housing using specially designed disposable sample cells (Ando et al., 2008) has been used successfully in a number of cases (Gupta et al., 2010; Navarro et al., 2009; Bennett et al., 2008).

MacCHESS is currently commissioning a dedicated BioSAXS beamline at the CHESS F2 line equipped with high-flux multilayer optics, robotic sample loading and microfluidic sample handling. High-precision specially polished slits (Advanced Design Consulting USA) have been mounted.
processing (Fig. 6). To date, the robotic system has been tested on both the F2 and G1 beamlines, and is available to users. The major upgrade of the F2 beamline optics, which will significantly improve BioSAXS performance at that station, is planned for Spring 2011 with the addition of high-flux multilayer optics.

5. Summary

MacCHESS has pioneered variations on steps to obtain biomolecular structures at synchrotron sources. The use of ‘drop-in’ single-bounce monocapillary optics offers users great flexibility to choose a variety of beam sizes at a given beamline. Moreover, these optics are readily retrofitted into existing beamlines with relatively little effort. High-pressure cryocooling allows users to obtain better data in many cases where diffraction quality is compromised by conventional cryocooling procedures. Solution SAXS capabilities at CHESS complement standard crystal diffraction capabilities. It is not unusual at CHESS for users to simultaneously perform crystal diffraction and solution SAXS experiments on a given protein, on appropriately equipped beamlines, at the same time.

References