THE ADVANCED PHOTON SOURCE

Capturing Protein Motions from Thousands of Fragile Protein Crystals

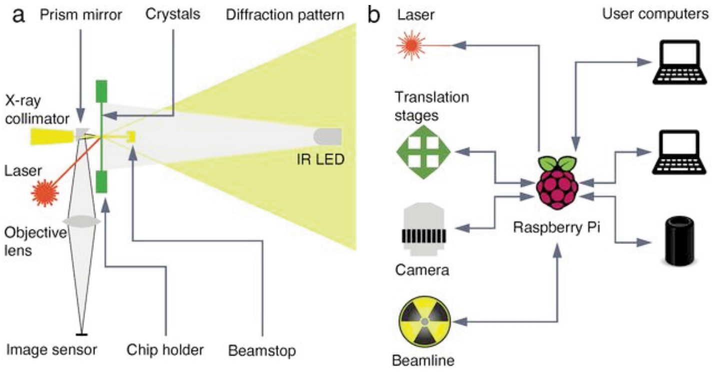

Fig. 1. Compact inSituX diffractometer. (a) Schematic layout of the diffractometer at the beamline. Each subsystem is colored differently: the crystallization device and motion stages are green, the beamline components for x-ray diffraction are yellow, the optical imaging components are gray and the excitation light is red. (b) Schematic flowchart of control system. At the center of the control system is a Raspberry Pi microcomputer, which coordinates the translation stages that carry samples with the imaging, x-ray, and excitation subsystems, and communicates with multiple user computers for data analyses.

Proteins can be fragile and data collection conditions for investigating them with x-rays (commonly performed at below-freezing temperatures to reduce radiation damage) don't correspond to the protein's physiological temperature. A team of collaborators made changes to the basic diffractometer design to address these issues and using the U.S. Department of Energy's Advanced Photon Source (APS) demonstrated how their new diffractometer platform automatically conducts large-scale, serial Laue diffraction at room temperature directly from the devices where the crystals were grown.

The two major hurdles to successful collection of structural information about proteins using x-rays are (1) difficulty in collecting data in physiologically relevant conditions and (2) successful navigation of the complicated procedures to transfer, mount, load, and collect data on protein crystals without exposing them to physical stress or drying them out. To overcome these hurdles, the team of researchers from the University of Illinois at Chicago, Renz Research, Inc., and the University of Maryland developed an automated diffractometer platform with additional capabilities which collects monochromatic and Laue diffraction data of large arrays of protein crystals. The platform minimizes the radiation damage to the crystals by requiring only a single x-ray exposure for each crystal. The platform collects data on large arrays of in situ protein crystals—crystals which are assessed in the same chamber where they were grown, allowing fragile proteins to be studied more easily without requiring sample manipulation. Figure 1 shows the diffractometer.

To demonstrate the efficacy of their platform, the team worked with two photosensitive bilin-based photoreceptors, which are representative of difficult types of proteins because they typically diffract weakly, their diffraction degrades quickly, and they are highly sensitive to visible light. The team collected Laue diffraction data at room temperature using the diffractometer platform with the BioCARS x-ray beamline 14-ID-B and the Life Sciences Collaborative Access Team x-ray beamlines at the APS, an Office of Science user facility at Argonne National Laboratory.

The well-resolved structures of these fragile example proteins collected by the team at room temperature demonstrate the potential of this prototype diffractometer platform to reveal protein activities in physiologically-relevant environments, bringing us closer to better understanding of the cellular processes in which proteins participate. — Mary Alexandra Agner.

See: Zhong Ren* Cong Wang, Heewhan Shin, Sepalika Bandara, Indika Kumarapperuma, Michael Y. Ren, Weijia Kang, and Xiaojing Yang**, IUCrJ **7**, 1009 (2020). DOI: 10.1107/S2052252520011288

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CALL FOR APS GENERAL-USER PROPOSALS

The Advanced Photon Source is open to experimenters who can benefit from the facility's high-brightness hard x-ray beams.

General-user proposals for beam time during Run 2021-3 are due by Friday, July 2, 2021.

Information on access to beam time at the APS is at http://www.aps.anl.gov/Users/apply_for_beamtime.html or contact Dr. Dennis Mills, DMM@aps.anl.gov, 630/252-5680.

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