Keywords: neutron inelastic scattering, neutron instrumentation, pulsed neutron scattering

P01.15.96

Acta Cryst. (2008). A**64**, C200

Preparation and imaging of lipidic cubic phase based protein crystallization experiments

Peter Nollert[1], Mike Owens[1], Werner Kaminsky[2], Timothy Vincent[3], Mark Mixon[1]
[1]Emerald BioSystems, 7869 NE Day Rd W, Bainbridge Island, WA, 98110, USA, [2]Department of Chemistry, University of Washington, Seattle, WA 98195, [3]Appalachian Electronic Instruments, 100 AEI Drive, Fairlea, WV 24902, E-mail：pnollert@emeraldbiosystems.com

Protein crystals grown by the cubic phase method diffract well and have been useful in determining high-resolution structures of several membrane proteins. The lipidic cubic phase methodology employed produces crystallization experiments that pose additional challenges to inspection. A versatile microscope platform, with automated x-y stage and various illumination and imaging options, has been developed with specific application to protein crystal detection. The DETECT-X microscope is a fully automated protein crystallization imaging instrument that operates in polarization, oblique angle and trans-illumination and UV-epi illumination fluorescence mode. Protein crystallization trials in traditional crystallization trays can be imaged in a fully automatic fashion. In its unique birefringence imaging mode the DETECT-X microscope produces false-color coded images of crystalline matter depicting the orientation of the slow optical axis of protein crystals and their orientation independent birefringence. Images that demonstrate the unique protein crystal imaging capabilities of the DETECT-X microscope are presented. The combination of the different observation modes allows the imaging of protein crystals that have strong contrast with respect to their facets as well as the interior body of the crystal. Colorless, transparent protein crystals appear colored. This enhanced contrast, along with UV epifluorescence, allows researchers to (i) see protein crystals through a layer of precipitate, (ii) discern between amorphous precipitate and non-facetted sphaerulites or precrystalline matter, (iii) identify crystal twins, and (iv) distinguish between protein and salt crystals.

Keywords: membrane protein crystallization, birefringence microscopy, lipidic mesophases

P01.15.97

Acta Cryst. (2008). A**64**, C200

Protein crystallization at the laboratory of molecular biology: Robotics, procedures and developments

Fabrice P.M. Gorrec, Olga Perisic, Katharine Michie, Gebhard Schertler, Jan Lowe
MRC laboratory of Molecular Biology, Structural Studies, Hills Road, Cambridge, Cambridgeshire, CB2 0QH, UK, E-mail：fgorrec@lmb-mrc.cam.ac.uk

LMB scientists can undertake initial crystallization experiments and also crystal condition refinements for each new protein sample using automated protocols. The LMB protein crystallization facility is high-throughput and includes various robots. The protocols are straightforward and setting up plates is easy. The standard initial screening protocol is comprised of 17 MRC sitting-drop plates pre-filled with a wide variety of commercially available screen kits. Process is fast and requires only small volume of protein. The 17 plates are set up within an hour using 272 ul of sample. 10,000 plates have been set up in 2007 for initial screening alone. Refinement, custom matrices and scale-up screens are made in any type of plate. A refinement screen in MRC plate is made in three minutes on the Sciclone i1000 workstation. New methods and tools are continuously developed and integrated into our crystallization facility. For example, The MRC multi-wavelength imaging system allows assessment of crystals regardless of clarity of the drops. The LMB screen database is a Web based tool to perform basic data mining about the initial screens. To further aid users, liquid handling advances, new micro-plates and specialized gas-tight and strong-binding plate seal developments have enabled the adoption of automated procedures for seeding, for experiments under oil and for crystallization at high temperature. A novel crystallization screen called Morpheus (commercialized by Molecular Dimensions) as also been developed recently. Each crystallization condition incorporates a broad range of small ligands that were found highly successful in co-crystallizations reported in the PDB.

Keywords: robotics, protein crystallization, Morpheus screen

P02.01.01

Acta Cryst. (2008). A**64**, C200

Structural studies of urate oxidase via powder diffraction

Sotonye Dagogo[1], Marion Giffard[2], Irene Margiolaki[1], Jon Wright[1], Francoise Bonnete[2], Yves Watier[1], Bob Von Dreele[3], Andy Fitch[1]
[1]European Synchrotron Radiation Facility, 11 Avenue Alsace Lorraine, Grenoble, Isere, 38000, France, [2]Centre Interdisciplinaire de Nanoscience de Marseille, Marseille, France, [3]Advanced Photon Source, Argonne, IL 60439, USA, E-mail：dagogo@esrf.fr

Modern developments of the powder diffraction technique have allowed the investigation of systems with large unit cells like proteins [1]. Polycrystalline protein precipitates are frequently obtained under a variety of crystallisation conditions and thus powder methods can be employed for structural characterisation of small proteins when single crystals are unavailable. Urate Oxidase from Aspergillus flavus (Uox) is a protein used to reduce toxic uric acid accumulation and also in the treatment of hyperuricaemia which occurs during chemotherapy . In this study, we investigate the effects of pH, salt and polyethylene glycol (PEG) concentration on the structural characteristics of Uox uncomplexed and complexed with 8-azaxanthin (AZA). Powder diffraction data were collected at both room temperature and cryocooled conditions at the ESRF (Grenoble, France). A previously unknown orthorhombic phase of uncomplexed Uox was observed and novel crystallization methods were established. In the case of Uox complexed with AZA a different orthorhombic phase is identified. Correlations between the crystallisation conditions with the structural and micro structural characteristics of Uox will be presented.
[1] Margiolaki, I., Wright, J. P. (2008). Acta Cryst. A64, 169-180.

Keywords: proteins, powder diffraction, synchrotron radiation

P02.01.02

Acta Cryst. (2008). A**64**, C200–201

A new performing space group determination algorithm

Arie Van Der Lee[1], Lukas Palatinus[2]
[1]Institut Europeen des Membranes, cc047 Universite de Montpellier II,