Microcrystallography With In Meso Grown Membrane Protein Crystals

Vadim Cherezov, Michael Hanson, Peter Kuhn and Raymond C. Stevens

Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA

This work was supported by NIH Roadmap grant P50 GM073197.

Abstract

Membrane protein crystallization in lipidic cubic phases (or in meso) often produces small but very well-ordered crystals. They appear to be a perfect match for the recently commissioned minibeam capability at the GM/CA CAT at Advanced Photon Source, Argonne, IL. We describe challenges and solutions to the problems related to growing, manipulating and collecting data from in meso grown microcrystals. Primary attention is focused on automated setting up nanofluidic cubic phase (LCP) crystallization drop, visual detection of initial crystals hits of colorless proteins in LCP, harvesting microcrystals from LCP, alignment of in situ grown microcrystal embedded in opaque frozen lipid mesophase with 10 µm in diameter minibeam, and strategies for data collection. Utilizing technologies developed under this project we recently determined a structure of a modified β2-adrenergic receptor at 2.4 Å resolution (Cherezov et al., 2007; Rosenbaum et al., 2007). Directions for extending utility of microcrystallography with frozen as well as in situ crystals are proposed.

In meso crystallization.

Robotics for in meso crystallization.

Minibeam at 23-ID beamline

GM/CA CAT, APS, Argonne

Microcrystal alignment

Microdiffraction

Diffraction resolution from in meso grown crystals scales roughly with crystal size.

References


Acknowledgments

The authors acknowledge the support of Janet Smith, Robert Fischetti and Nukri Sanishvili at the GM/CA CAT beamline at the Advanced Photon Source. For assistance in developing both of the alignment and detecking. The GM/CA CAT beamline (23-ID) is supported by the National Cancer Institute (Y1-C0-1006) and the National Institute of General Medical Sciences (Y1-M0-11). This work was supported in part by the NIH Roadmap Initiative grant P50GM073197.

The authors acknowledge Tom Kuhn and Brian Kalbick from Stanford University, Huipei Cho and Joseph Fine from the University of California, San Diego for all the excellent collaborations used in the work. Justin Whitefield and Yumei Zheng from The Ohio State University for the generous loan of the in situ robot.