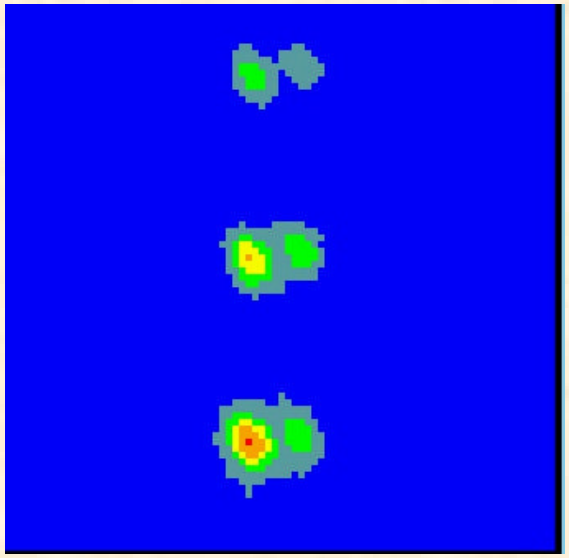
Reducing Radiation Damage to Macromolecular Crystals at Synchrotrons by using Cryogenic Helium during Data Collection



Diffraction data collected on Displex cooled crystals, in contrast to an open flow helium cryostat, verify that a variety of protein crystals manifests ‘split spot’ diffraction at decakelvin temperatures. Additionally, these experiments were conducted at the T17 LADI neutron diffraction station at ILL, proving that the ‘spot splitting’ phenomenon is temperature induced and not the result of radiation damage, as could be construed from the synchrotron X-ray studies.

The low temperature diffraction spot ‘splitting’ phenomenon, seen in the image above, suggests that previously published results using helium for macromolecular data collection did not achieve decakelvin temperatures. The earlier studies that suggested no improvement in crystal lifetime with helium cooling should be reconsidered. Further research on the ‘spot splitting’ phenomenon will lead to the use of cryogenic helium as the most reliable way of reducing the effects of radiation damage during macromolecular data collection at The Department of Energy operated synchrotrons.

Reducing Radiation Damage to Macromolecular Crystals at Synchrotrons by using Cryogenic Helium during Data Collection

Researchers in the Advanced Biomedical Science and Technology Group of the Life Sciences Division, Oak Ridge National Laboratory have teamed up with scientists at the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) beam line, Advanced Photon Source, Argonne National Laboratory, the Department of Chemistry, University of Toledo, NASA Marshall Space Flight Center, Huntsville Alabama, and the Institut Laue Langevin, Grenoble France to demonstrate the benefits of cryogenic helium for macromolecular data collection.

The extreme intensity of X-radiation sources such as 3rd generation synchrotrons and long duration data collection MAD phasing experiments necessitate the use of cryocrystallography. However, radiation damage remains a significant problem during crystallographic data collection using normal cryogenic techniques (i.e., open flow liquid N2 cryostats). Experiments have indicated that using an open high-flow-rate liquid He cryostat mitigates some of the effects of radiation damage at high flux X-ray sources. These studies with cryogenic helium were presented by invitation at the Department of Energy (DOE) co-sponsored 2nd International Workshop on X-ray Damage to Crystalline Biological Samples, held at the Advanced Photon Source.

A phenomenon encountered during cryo-helium data collection is the observation of 'split spots' in higher resolution diffraction shells. This behavior has been observed in all our cryogenic helium studies. Additional experiments were conducted to determine if this 'split spot' phenomenon is a new low-temperature phase transition, or the result of crystal splitting caused by the extreme temperature gradient that occurs when protein crystals are flash cooling in a decakelvin helium stream. Recently, our collaborator at the European Molecular Biology Laboratory- Institut Laue Langevin (EMBL-ILL) duplicated the phenomenon in crystals initially flash-cooled in liquid nitrogen (spot splitting does not occur with liquid nitrogen or cryogenic nitrogen gas), then transferred to a Displex helium dilution refrigerator for cooling to ~12K. Diffraction data collected on the Displex cooled crystals corroborate ours, that a variety of protein crystals manifests 'split spot' diffraction at decakelvin temperatures. Additionally, these experiments were conducted at the T17 LADI neutron diffraction station at ILL, proving that the 'spot splitting' phenomenon is temperature induced and not the result of radiation damage, as could be construed from the synchrotron X-ray studies.

The low temperature diffraction spot 'splitting' phenomenon suggests that previously published results using helium for macromolecular data collection did not achieve decakelvin temperatures. The earlier studies that suggested no improvement in crystal lifetime with helium cooling should be reconsidered. Further research on the 'spot splitting' phenomenon will lead to the use of cryogenic helium as the most reliable way of reducing the effects of radiation damage during macromolecular data collection at The Department of Energy operated synchrotrons.

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Technical Contact: Gerald Bunick
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