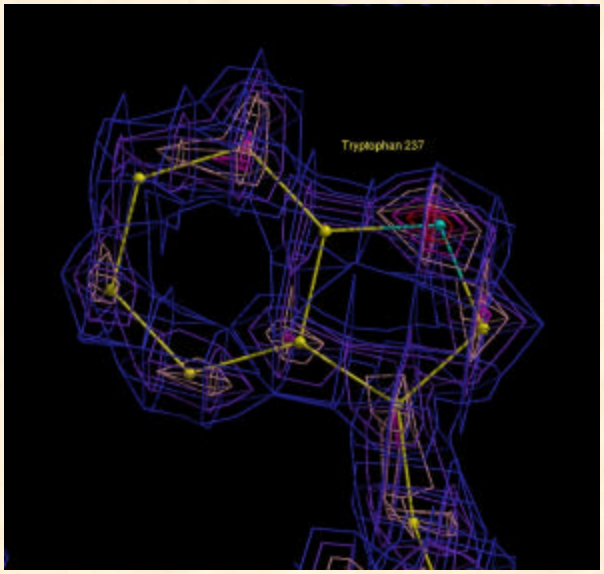
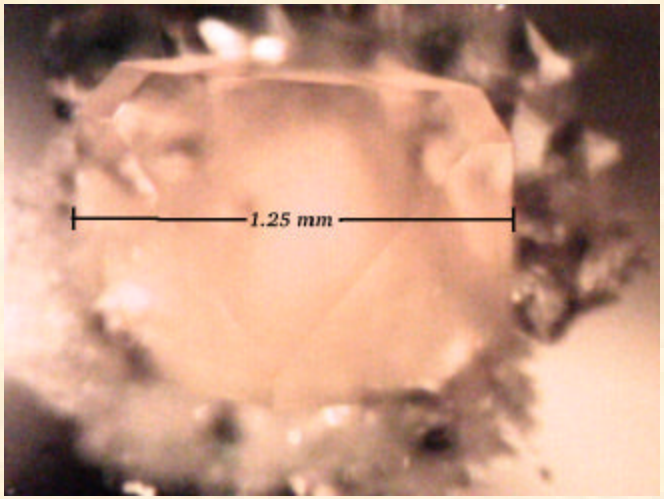
Comparison of X-ray and Neutron Crystallographic Structures of Glucose Isomerase

Studies are underway to compare structural information obtained from ultra-high resolution x-ray and neutron diffraction data to help define the future role of macromolecular neutron diffraction at the Spallation Neutron Source. The potential advantages of neutrons over X rays include much easier visualization of hydrogen atoms and water structure. Additionally, the absence of radiation damage from thermal neutrons allows for data collection at room temperature. Therefore, neutron structures reveal biological macromolecules in their physiological state.



Tryptophan 237 from 0.94 Å resolution X-ray data prior to final refinement. Electron density resolves individual carbon and nitrogen atoms, but hydrogens are not yet clearly resolved.



Large crystal of glucose isomerase prior to starting deuterium exchange in preparation for neutron data collection.

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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) beamline, 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 compare structural information obtained from ultra-high resolution X-ray data and neutron diffraction data to help define the future role of macromolecular neutron diffraction at the Spallation Neutron Source. The potential advantages of neutrons over X rays include much easier visualization of hydrogen atoms and water structure. Additionally, the absence of radiation damage from thermal neutrons allows for data collection at room temperatures. Therefore, neutron structures reveal biological macromolecules in their physiological state.

Ultra-high resolution X-ray data (~0.94 Å) for glucose isomerase were collected at the IMCA-CAT beam line. The crystals were flash cooled and maintained in cryogenic nitrogen or helium during data collection. The nitrogen data have refined to a merging statistic value of 3.06%. The molecular refinement is underway using CNS and SHELX software. Prior to model fitting the R value for working data is 19% and the test set data 20%. Both values indicate the overall high quality of these data. Position assignment of waters and hydrogens should be finished shortly and the completed ultra-high resolution X-ray structure will be available for comparison to a neutron diffraction structure of glucose isomerase.

Within macromolecules, biochemical mechanisms can be elucidated and modeling studies facilitated from knowledge of hydrogen positions. Thus, neutron macromolecular crystallography, with its greater ease and accuracy of visualizing hydrogen atoms and water structure than ultra-high resolution X-ray crystallography, is intrinsic to understanding fine-scale biochemical interactions within and between proteins. In other areas such as *in silico* structure studies, knowledge of protein solvent structure and hydrogen position will improve understanding of protein folding, structure based drug design and protein-protein interactions, and will improve the empirical basis of protein folding algorithms and *de novo* structure determination.

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