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Neutron Protein Crystallography Station (PCS)

(Funded by U.S. Department of Energy)

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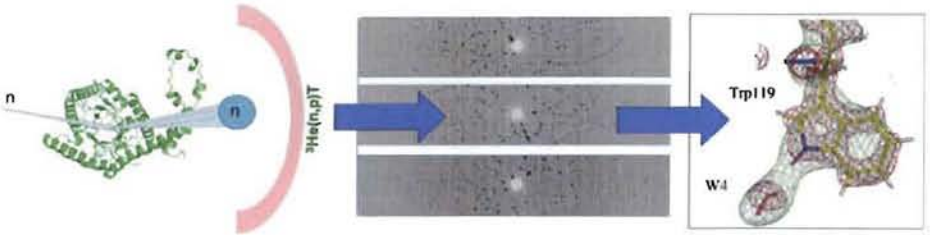
The PCS (Protein Crystallography Station) at Los Alamos Neutron Science Center (LANSCE) is a unique facility in the USA that is designed and optimized for detecting and collecting neutron diffraction data from macromolecular crystals. PCS utilizes the 20 Hz spallation neutron source at LANSCE to enable time-of-flight measurements using 0.6-7.0 Å neutrons. This increases the neutron flux on the sample by using a wavelength range that is optimal for studying macromolecular crystal structures. The diagram below show a schematic of PCS and photos of the detector and instrument cave.

Methodology and Services Provided by PCS

PCS can help with sample production, purification, all the way to structure determination. Expression of perdeuterated proteins involve algae-derived media made in 100% D_2O. Bacteria (eg. BL21 strains) are adapted to deuterated conditions and expression continues as usual. We have 2 GE Life Science chromatography systems and various column media for protein purification. Once sufficiently pure sample is produced, we have a liquid-handling system, Oryx-8 from Douglas Instruments, to assist with screening and crystallization optimization.



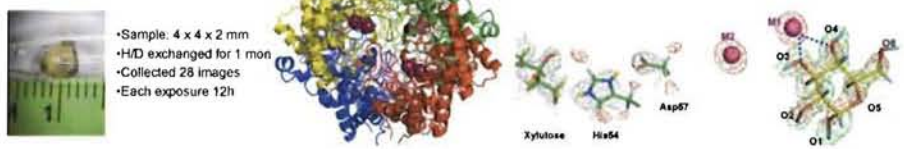
If crystals are of suitable size (> 0.4mm^3), data can be collected at PCS at LANSCE. As we use time-of-flight Laue techniques, less data is needed for a complete structure, thus overcoming the long (~20h) exposure time. Data is processed and refined with in-house modified software based on D*trek and CNS.

Recent Success from PCS

Protein	Space Group	Crystal Volume (mm^3)	No. settings	Resolution (Å)
PYP	$P6_3$	0.79	15	2.5
DFPase	$P2_12_12_1$	0.43	37	2.2
DHFR	$P6_1$	0.40	15	2.2
Hb deoxy	$P2_1$	20	36	1.8
XI	I222	4	37	2.2
HCA II	$P2_1$	1.2	41	2.0

Towards 3rd Generation biofuels

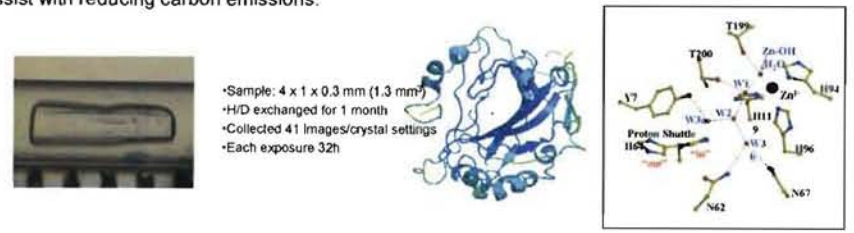
D-xylose isomerase (XI) catalyzes the conversion of D-xylose and D-glucose to the keto sugars xylulose and fructose. XI is an fundamentally important enzyme in sugar metabolism as well as the commercial applications, notably for biofuels. The XI-catalyzed reaction involves a formal transfer of two H atoms, facilitated by the presence of two divalent metal cations, M1 and M2, in the active site. We have determined 5 neutron structures of XI representing snapshots along the reaction pathway. A revised mechanism has been proposed which will allow engineering of XI for better performance and stability.



USERS: Leif Hanson, Jenny Glusker, Andrey Kovalevsky, Horace Carrell - Fox Chase Cancer Center, LANL, University of Toleo

Environment and carbon management

Carbonic anhydrase (**CA**) catalyzes the reversible interconversion of CO_2 to HCO_3^- and is rate-limited by a proton transfer (PT) event between a Zn-bound water and internal proton shuttle, His64. Despite the availability of atomic resolution X-ray structures, it is still unknown whether the Zn-bound solvent is water/hydroxide, if H64 is charged or neutral, and how the solvent is oriented with respect to each other and active site residues. Understanding how the enzyme facilitates CO_2 hydration and proton transfer will help in devising methods for using biological systems for carbon sequestration. This could ultimately assist with reducing carbon emissions.



USERS: John Domsic, Robert McKenna, David Silverman, Zoe Fisher - University of Florida, LANL

Publications

Fisher, S.Z., Kovalevsky, A., Domsic, J., Mustyakimov, M., Silverman, D., McKenna, R., Langan, P. (2009) *Acta* **F65**, 495-498.

Kovalevsky, A., Katz, A., Carrell, H., Hanson, L., Mustyakimov, M., Fisher, S.Z., Coates, L., Schoenborn, B.P., Bunick, G., Glusker, J., Langan, P. (2009) *Biochemistry* **47**, 7595-7597.

Blum, M.M., Mustyakimov, M., Rutenjans, H., Kehe, K., Schoenborn, B.P., Langan, P., Chen, J.C. (2009) *Proc. Natl. Acad. Sci* USA **106**, 713-718.

Langan, P., Fisher, S.Z., Kovalevsky, A., Mustyakimov, M., Valone-Sutcliffe, A., Unkefer, C., Waltman, M.J., Coates, L., Adams, P.D., Afonine, P., Bennett, B., Dealwis, C., Schoenborn, B.P. (2008) *J. Synchrotron Rad.* **15**, 215-218

Kovalevsky, A., Chatake, T., Shibayama, N., Park, S.Y., Ishikawa, T., Mustyakimov, M., Fisher, S.Z., Langan, P., Morimoto, Y. (2008) *Acta Cryst* **D64**, 270-273.

Fisher, S.Z., Anderson, S., Henning, R., Moffat, K., Langan, P., Thiyagarajan, P., Schultz, A. (2007) *Acta Cryst* **D63**, 1178-1184.