to twice at maximum.

Because of the 3.11 big earthquake in Japan, J-PARC was damaged as well as iBIX. They are being recovered and recovery is expected to take at least a year.

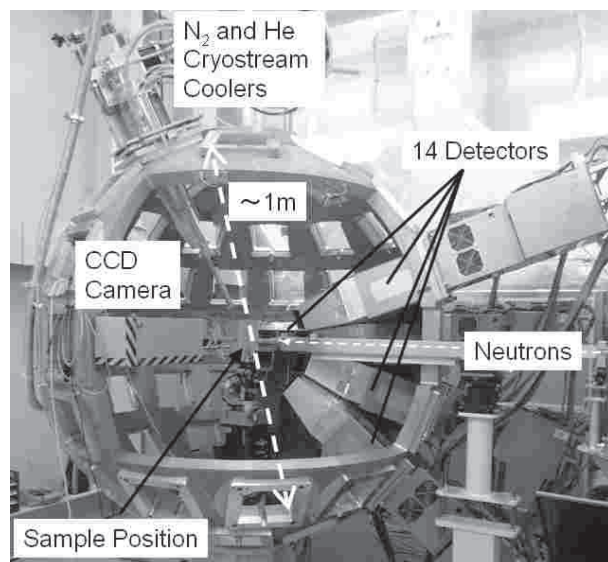

Fig. iBIX sample position and 14 detectors.

[1] I. Tanaka, K. Kusaka, T. Hosoya, N. Niimura, T. Ohhara, K. Kurihara, T. Yamada, Y. Ohnishi, K. Tomoyori and T. Yokoyama, *Acta Cryst. D* **2010**, *66*, 1194-1197.

Keywords: biology, neutron, diffractometer

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Large volume crystal growth in restricted geometery for neutron crystallography

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Neutron Macromolecular Crystallography (NMC) is the prevalent method that accurately determines the positions of hydrogen atoms in macromolecules. There are two major limiting factors in determining protein structure with neutron diffraction; one is obtaining large crystal volumes and the other is the extended experimental duration. We demonstrate that crystallizing hyperthermophilic proteins by counter-diffusion crystallization can produce suitable protein crystals for NMC. Counter-diffusion crystallization in restricted geometry, such as in capillary tubes, allows diffusion to govern mass transport processes so that a spatial-temporal gradient of supersaturation is formed along its length. Consequently, proteins can crystallize in an optimized supersaturated condition where a single crystal can fill up the diameter of the capillary. We have employed the same process in capillary vessels having diameters exceeding 1mm while minimizing the ratio between buoyant and viscous forces. As a result, protein crystals suitable for neutron crystallography can be grown in this configuration with volumes greater than $1mm^3$ while eliminating invasive crystal manipulation. Using crystals grown by this method, we have undergone neutron diffraction analysis of recombinant proteins from a hyperthermophilic archaeon. These proteins are excellent crystallization targets because of their thermal and mechanical stability and high propensity to crystallize. As an initial proof of principle, a novel inorganic pyrophosphatase (IPPase) was among the first of these proteins to be grown in large diameter capillaries resulting in a $9mm^3$ volume crystal. The next generation neutron beamlines at the Spallation Neutron Source (SNS) along with an effective counter-diffusion crystallization procedure can improve structures of a diverse range of proteins of interest to the crystallographic community. Thus the crystallization of macromolecules can grow to volumes never before achieved, potentially leading to enormous increases in the number of neutron crystallographic protein structures.

Keywords: neutron crystallography, counter-diffusion crystallization, large-volume protein crystals

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Neutron structure of retaining glycosyltransferase GTA

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With at most one electron, hydrogen atoms can be difficult to observe in protein structures by X-ray crystallography even at atomic resolution. This can pose a barrier to the critical evaluation of enzymatic mechanisms that involve proton translocation as the ionization states of active site residues cannot always be reliably determined by the chemical environment in which they reside.

One such class of enzyme is the glycosyltransferase. Glycosyltransferases catalyze the biosynthesis of oligosaccharides and glycoconjugates by the transfer of a monosaccharide unit from an activated donor molecule to an acceptor molecule with high stereospecificity. The anomeric stereochemistry of the donor sugar will either be inverted or retained upon formation of the new glycosidic linkage, depending on the enzyme. Although a consensus has been reached on a straightforward S_N2 mechanism used by inverting glycosyltransferases, the nature of the retaining mechanism is still a matter of debate.

The model retaining enzyme human ABO(H) blood group A α-1,3-N-acetylgalactosaminyltransferase (GTA) generates the blood group A antigen by the transfer of N-acetyl-galactosamine from UDP-GalNAc to the blood group H antigen. To understand better how specific active-site-residue protons and hydrogen-bonding patterns affect substrate recognition and catalysis, neutron diffraction studies were conducted at the Protein Crystallography Station (PCS) at Los Alamos Neutron Science Center (LANSCE).

This is the first study of a retaining glycosyltransferase using combined X-ray crystallographic data (to 1.9 Å resolution) and neutron Laue data (to 2.5 Å resolution) [1]. These data provide the first unambiguous assignment of protons and the causative hydrogen-bond patterns in a glycosyltransferase active site.