

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.

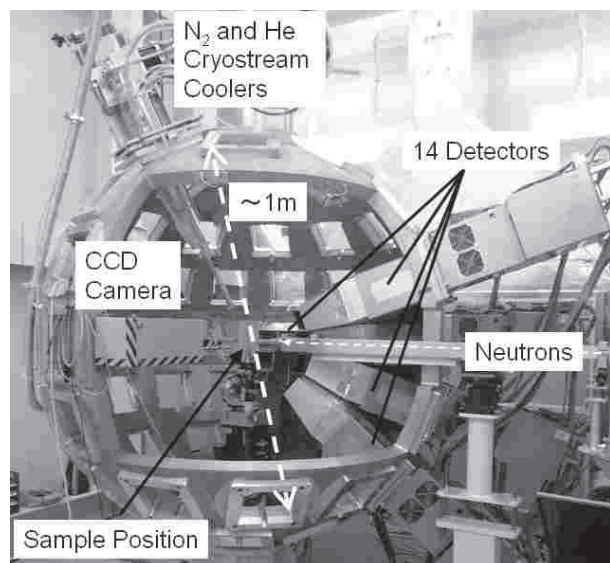


Fig. iBIX sample position and 14 detectors.

[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

MS82.P04

Acta Cryst. (2011) **A67**, C733

Large volume crystal growth in restricted geometry for neutron crystallography

Joseph D. Ng,^a Ronny C. Hughes,^a Leighton Coates,^b Stephen J. Tomanicek,^b Juan M. Garcia-Ruiz,^c Matthew P. Blakeley,^d Paul Langen,^b ^aLaboratory for Structural Biology and Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, AL 35899, (USA). ^bOak Ridge National Laboratory, Neutron Scattering Science Division and Environmental Sciences Division, 1 Bethel Valley Road, Oak Ridge, TN 37831, (USA). ^cLaboratorio de Estudios Cristalográficos (IACT), CSIC-Universidad de Granada, Av. de la Innovación s/n, Armilla, Granada, (Spain). ^dInstitut Laue Langevin, 6 rue Jules Horowitz, BP 156, 38042 Grenoble, (France); E-mail: ngj@uah.edu

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 1mm³ 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 9mm³ 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

MS82.P05

Acta Cryst. (2011) **A67**, C733-C734

Neutron structure of retaining glycosyltransferase GTA

Brock Schuman,^a Suzanne Z. Fisher,^b Svetlana Borisova,^a Monica M. Palcic,^c Leighton Coates,^d Paul A. Langan,^{b,d} Stephen V. Evans,^a ^aDepartment of Biochemistry and Microbiology, University of Victoria, Victoria, BC, (Australia). ^bBioscience Division, Los Alamos National Laboratory, Los Alamos, NM (USA). ^cCarlsberg Laboratory, Gamle Carlsberg Vej 10, (Denmark). ^dOak Ridge National Laboratory, Neutron Scattering Science Division, Oak Ridge, TN (USA). E-mail: brock.schuman@gmail.com

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.

Poster Sessions

[1] B. Schuman, S.Z. Fisher, A. Kovalevsky, S.N. Borisova, M.M. Palcic, L. Coates, P. Langan, S.V. Evans, *Acta crystallographica. Section F, Structural biology and crystallization communications* **2011**, *67*, 258-262.

Keywords: neutron, glycosyltransferase, mechanism

MS82.P06

Acta Cryst. (2011) A67, C734

Joint Neutron/X-ray crystallographic study on the mechanism of pectate lyase

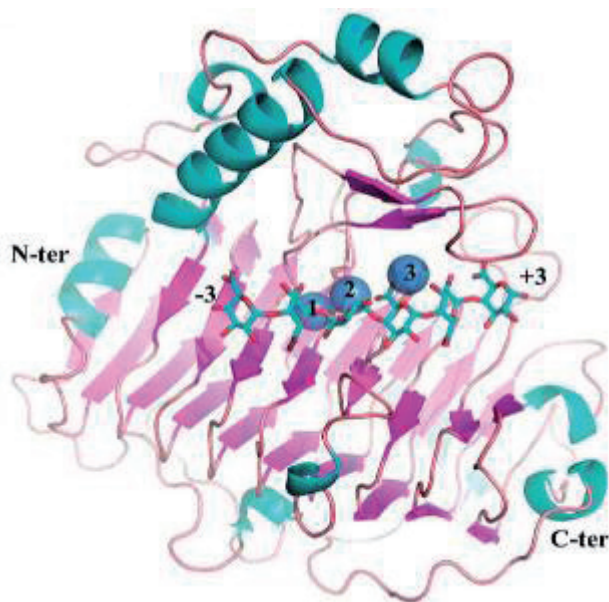
S. Ali,^{a,b} S. Teixeira,^{a,c} R.W. Pickersgill,^b ^aDeuteration Laboratory, ILL, 6 Rue Jules Horowitz, BP 156, F-38042, Grenoble, Cedex 9, (France). ^bSchool of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London, (UK), E1 4NS. ^cEPSAM, School of Physical and Geographical Sciences, Keele University, Keele, Staffordshire, ST5 5BG. E-mail ali@ill.fr

Bacterial soft-rot disease is a major problem for plants in the field and in storage. It is caused by the invasion of pathogens after cell wall damage by pectate lyases secreted by bacteria such as *Bacillus subtilis* (BsPel).

BsPel belongs to a family of lyases that cleave α -1,4-linked galacturonic acid units of the pectate component of plant cell walls via an *anti*- β -elimination reaction. A proposed catalytic mechanism [1] features a conserved arginine acting as a base as low as pH4.5. Where calcium ions are required for activity; a primary Ca^{+2} ion binds substrate and an additional 2 stabilise the intermediate.

At present the major mechanistic question is the protonation state of this active site arginine, which at physiological pH (7.0) is expected to be protonated. Therefore, proton abstraction initiating the reaction is likely to result from a local shift of pKa that has yet to be proven.

A joint Neutron and X-ray study has been carried out to study the structure of BsPel and the active site residues in particular. We have produced perdeuterated BsPel, crystallised it, and collected both Neutron and X-ray data on the same crystal sample.



Above: Cartoon representation of the parallel β -helix architecture (arrows) of BsPel bound with hexasaccharide (stick representation) and 3 calcium ions (spheres).

[1] A. Seyedarabi, T.T. To, S. Ali, S. Hussain, M. Fries, R. Madsen, M. Clausen,

S. Teixeira, K. Brocklehurst, R. Pickersgill *Biochemistry* **2010**, *49*, 539–546.

Keywords: neutron, X-ray, pectate-lyase

MS82.P07

Acta Cryst. (2011) A67, C734

Transthyretin amyloidosis – insights from neutron crystallography

Melina Haupt,^{a,b} Matthew P. Blakeley,^b Susana C.M. Teixeira,^{a,b} Edward P. Mitchell,^c Mark B. Pepys,^d V. Trevor Forsyth,^{a,b} Johnathan B. Cooper,^d ^aEPSAM/ISTM, Keele University, Staffordshire, ST5 5BG (UK). ^bInstitut Laue-Langevin, 6 rue Jules Horowitz, 38042 Grenoble (France). ^cEuropean Synchrotron Radiation Facility, 6 rue Jules Horowitz, BP220, 38043 Grenoble (France). ^dTTR University College London, Laboratory for Protein Crystallography and Acute Phase Proteins, Royal Free Ca, Rowland Hill Street, London, NW3 2PF (UK). E-mail: haupt@ill.fr

Human Transthyretin (TTR) is a homotetramer protein that transports thyroxine in the blood and cerebrospinal fluid. TTR is intrinsically amyloidogenic and associated with three major amyloid diseases. Familial amyloidotic polyneuropathy (FAP) manifests itself by deposition of fibrils and amorphous aggregates in the liver, whereas familial amyloidotic cardiomyopathy (FAC) afflicts the heart. Both diseases are hereditary and due to point mutations in the genome, rendering the protein more labile and thus prone to dissociation and aggregation. The third, senile systemic amyloidosis (SSA) is linked to native TTR and is the most widespread; it affects about 25% of the population over 80 years old. Early diagnosis and new therapies, including small molecule compounds stabilizing the native fold, offer the possibility of a prolonged remission of this otherwise inexorable disease. The analysis of the protonation states of the subunit interface and changes occurring upon ligand binding is therefore of great interest for an understanding of the factors that stabilise the homotetramer, prevent dissociation and ultimately amyloidosis. Perdeuterated human TTR has been overexpressed in *E. coli* in fermenters of the ILL Deuteration Laboratory and large crystals have been grown. Neutron quasi-Laue data to 2.1 Å resolution and room temperature X-ray data to 1.9 Å resolution were collected on perdeuterated TTR crystals and used for a joint X-ray/neutron structural refinement with phenix.refine. The results are being used to study protonation and hydration in native TTR with a view to follow-up studies of TTR in complex with a number of promising ligands.

Keywords: ttr, amyloidosis, neutron crystallography

MS82.P08

Acta Cryst. (2011) A67, C734-C735

The first neutron structure analysis of protein with ibix in j-parc

Katsuhiro Kusaka,^a Taro Yamada,^a Takaaki Hosoya,^a Takashi Ohhara,^{b,c} Kazuo Kurihara,^d Katsuaki Tomoyori,^a Takeshi Yokoyama,^a Ichiro Tanaka,^a and Nobuo Niimura,^a ^aFrontier Research Center for Applied Atomic Sciences, Ibaraki University, Tokai Ibaraki (Japan). ^bJ-PARC Center, Japan Atomic Energy Agency (JAEA), Tokai Ibaraki (Japan). ^cResearch Center for Neutron Science & Technology, Comprehensive Research Organization for Science and Society (CROSS) Tokai Ibaraki (Japan). ^dQuantum Beam Science Directorate, (Japan) Atomic Energy Agency (JAEA), Tokai Ibaraki (Japan). E-mail: kusakats@mx.ibaraki.ac.jp

Since 2004, Ibaraki prefecture has constructed the TOF neutron