P.04.13.8
Acta Cryst. (2005). A**61**, C237
Structural Study of Hepatitis B Virus Capsid Polymorphism and Stabilization
Christina Bourne, Adam Zlotnick, *Department of Biochemistry & Molecular Biology, OU Health Sciences Center, Oklahoma City OK USA*. E-mail: christina-bourne@ouhsc.edu

The amino acid changes between different strains of HBV affect biological properties [1] and crystallization [2]. We hypothesize that these differences manifest as capsid polymorphism within an icosohedral framework.

We have crystallized strain *adyw* HBV T=4 capsids for comparison to the previously determined *adw*-like HBV capsid [1]. To obtain this structure we engineered disulfide cross-links between the dimer building blocks, resulting in a stabalization of the capsid within the crystallographic lattice. To asses the role of this cross-link, data was also collected on a crystal without cross-linking. A critical advance for our structures was the ability to cryo-cool them, which was only possible when free capsid was included in the artificial mother liquor.

Data were collected at the APS synchrotron, beamline 14BMC. The data sets were isomorphous in *C*2 space group with one capsid per asymmetric unit. The cross-linked capsid data set is 81% complete overall to 3.95Å, while the non-cross-linked capsid is 94.9% complete to 3.95Å.

Current efforts are focused on phasing these structures with molecular replacement methods and refinement of the solution. This will allow us to visualize structural polymorphism between strains of HBV capsid and the effect of cross-linking capsid subunits.

[1] Ceres P., Stray S., Zlotnick A., *J. Virol.*, 2004, **78**, 9538. [2] Wynne S., Crowther R., Leslie A., *Mol. Cell.*, 1999, **3**, 771.
Keywords: capsids, virus crystallography, polymorphism

P.04.14.1
Acta Cryst. (2005). A**61**, C237
Structural Determination of the hTIM10 Complex
Chaille T. Webb, Michael Gorman, Jacqueline M. Gulbis, *Structural Biology Division, The Walter and Eliza Hall Institute, Melbourne, Australia.* E-mail: cwebb@wehi.edu.au

Over 99% of human mitochondrial proteins are synthesised from nuclear DNA and must be imported as immature precursors *via* a coordinated series of specific, tightly regulated events. Encoded topological signals ensure nascent proteins are ushered to their correct mitochondrial destination. Proteins destined for the inner or outer mitochondrial membranes contain internal targeting information. After transfer through the outer membrane's general import pore, preproteins encounter TIM10, a hetero-hexamer of two homologous polypeptides, Tim9 and Tim10. TIM10 mediates preprotein passage across and within the intermembrane space (IMS). Inner membrane carrier proteins (*e.g.*AAC) are transferred to the inner membrane translocase, Tim22, for insertion, whereas β-barrel proteins of the outer membrane are transferred to the sorting and assembly machinery, SAM[1]. Tim9 and Tim10 share a twin CX_3C consensus sequence, similar to a zinc finger motif. Whilst disulphide formation appears to be necessary for hexamer formation and function, there is evidence that zinc binding occurs in the cytosol prior to import, oxidation occurring later in the IMS [2].

Initial electron density maps have been calculated using SAD phasing, and I am on the way to determining the structure of the human TIM10 complex. The structure will illuminate how this key intermediate functions in the context of translocation.

[1] Koehler C.M., *Annu. Rev. Cell Dev. Biol*., 2004, **20**, 309. [2] Lu H., Allen S., Wardleworth L., Savory P., Toktlidis K., *J. Biol. Chem*., 2004, **279**, 18952.
Keywords: mitochondrial import, translocase, chaperone

P.04.14.2
Acta Cryst. (2005). A**61**, C237
Structure Determination of Proteins Involved in the Stability of Phycobilisomes during Environmental Stress
Monica Dines[1], Meira Meled Frank[1], Rakefet Schwarz[2], Noam Adir[1], [1]*Department of Chemistry and Institute of Catalysis, Science and Technology, Technion, Technion City, Haifa 32000 Israel. FAX-972-4-8233735.* [2]*Faculty of Life Sciences Bar-Ilan University.* E-mail: nadir@tx.technion.ac.il

The activities of the photosynthetic apparatus are highly controlled by the environment. Environmental parameters such as light quality, light intensity, temperature, water availability, and nutrient status play critical roles in photosynthetic complexes activities and the levels of pigments and proteins associated with those complexes.

An example of this modulation is the degradation of the light-harvesting pigment-protein complex, the phycobilisome (PBS), during starvation for sulphur and nitrogen.

The protein that appears to trigger PBS degradation during nutrient deprivation is nblA, a very small protein (6.5 kDa), whose level increases dramatically upon starvation of *cyanobactaria* for nitrogen or sulfur. This protein is apparently not a protease, and it may be a new class of proteins – a **dismantlease.** We are investigating the 3D structure of nblA from the *S. elongatus.* (a mesophilic cyanobaterium) and from the *T. vulcanus* (a thermophilic cyanobaterium). We have succeeded in cloning, expressing, purifying and crystallizing bough nblA proteins with and with out Se-methionine. Crystals of nblA from *S. elongates* were found to be amenable to be flash-frozen without addition of cryoprotectant, and X-ray diffraction was measured recently at the European Synchrotron Radiation Facility (ESRF-Grenoble) beamline ID14-I. The crystals diffracted to a resolution better than 2.5Å. Analysis of the diffraction pattern was performed using DENZO. The crystals belong to a P4 space group, with cell dimensions of a = 78Å, b = 78Å, c = 69Å, and four monomers in the asymmetric unit. We are now working on obtaining crystals that will enable collection of complete data sets with a better resolution and on structure determination.
Keywords: photosynthetic-related proteins, structural biology, stress

P.04.14.3
Acta Cryst. (2005). A**61**, C237
Cage-like Hexamers of Cold-active β-galactosidase from *Arthrobacter* sp. C2-2
Jan Dohnalek[a], Tereza Skalova[a], Jarmila Duskova[a], Hana Petrokova[a], Eva Vondrackova, Petra Lipovova[b], Vojtech Spiwok[b], Blanka Kralova[b], Jindrich Hasek[a], [a]*Institute of Macromolecular Chemistry of the Czech Academy of Science, Prague, Czech Republic.* [b]*Institute of Chemical Technology, Prague, Czech Republic.* E-mail: dohnalek@imc.cas.cz

β-galactosidase catalyzes hydrolysis of galactosyl moiety from non-reducing termini of oligosaccharides or from glycosides. Crystals of β-galactosidase from psychrotrophic bacterium *Arthrobacter* sp. C2-2 were grown by vapor diffusion technique and X-ray diffraction data were collected up to 1.9 Å [1]. Molecular replacement solution in $P2_1$ space group revealed six molecules arranged in cage-like hexameric structure with cca 60,000 non-hydrogen atoms per asymmetric unit. The compact hexamers are characterized by three types of channels, six active sites open towards the central cavity, high number of buried water molecules within the protein and Na^+ and Mg^{2+} ions bound in vicinity of the active site. Comparison to *E.coli* β-galactosidase shows both similarities and significant differences regarding the active site and oligomerization mechanism.
Acknowledgement: This work was supported by GACR (project 204/02/0843/A) and by the Grant Agency of the Academy of Sciences of the Czech Republic (project KJB500500512).

[1] Petroková H., Vondráčková E., Skálová T., Dohnálek J., Lipovová P., Spiwok V., Strnad H., Králová B., HašekJ., *Collect. Czech. Chem. C.*, 2005, **70**, 124-132.
Keywords: beta-galactosidase, X-ray structure, cold-active enzymes