structural modification of βA determined by the binding with the metal.

Acknowledgements. To FONDECYT (Project 1090041).

[1] G.P. Eckert, W.G. Wood, W.E. Müller, *Journal of Neural Transmission* **2001**, *108,* 1051-1064. [2] M. Suwalsky, S. Bolognin, P. Zatta, *Journal of Alzheimers Disease* **2009**, *17,* 81-90.

Keywords: X-ray, lipid bilayer, alzheimer's

MS01.P17

Acta Cryst. (2011) A**67,** C221

Structural studies of GH31 and GH32 glycoside hydrolases

Stephanie Oerum,[a] Heidi A. Ernst,[a] Sine Larsen,[a] Hiroyuki Nakai,[b] Natsuko Nakai,[b] Maher Abou Hachem,[b] Birte Svensson,[b] Jens-Christian N. Poulsen,[a] Leila Lo Leggio,[a] [a]*Department of Chemistry, University of Copenhagen, (Denmark).* [b]*Department of Systems Biology, DTU, (Denmark).* E-mail: Oerum@kemi.ku.dk

The α-glucosidase MalA from *Sulfolobus solfataricus* forms part of the carbohydrate-metabolising machinery that allows the archaebacterium to utilize carbohydrates, such as maltose, as the sole energy source. MalA belongs to the GH31 (www.cazy.org/GH31) family of α-glucosidases (EC 3.2.1.20), which hydrolyze a terminal (1→4) linked α-D-glucose moiety, resulting in release of an α-D-glucose moiety from a variety of substrates. The reported structure of MalA is in complex with β-octyl glucopyranoside (BOG) [1]. Enzymes from GH31 are not as well characterized as other α-glucosidases, making them desirable targets for crystallographers. This project aims at refining our understanding of the MalA structure, in particular of the active site, thus facilitating a deeper understanding of the mechanism of this carbohydrate-degrading enzyme. To achieve this, crystals of new MalA complexes together with inhibitors are produced, and datasets have been obtained of complexes with deoxynojirimycin and acarbose at 3.3 Å and 2.8 Å resolution, respectively. The structures have been determined and are in the last stages of refinement.

ScrB and BfrA from *Lactobacillus acidophilus* are involved in the intracellular metabolism of kesto-oligosaccharides and sucrose. Both enzymes belong to the GH32 (www.cazy.org/GH32) family but while BfrA is a beta-fructosidase, ScrB is a sucrose 6-phosphate hydrolase. Recombinant ScrB and BfrA have been produced in *Escherichia coli* along with several active site mutants of ScrB to determine the structures and establish the structural determinants of specificity. Preliminary crystallization conditions are found for BfrA, ScrB and the ScrB mutant D47A, but no usable dataset has yet been obtained.

This project is supported by the Carlsberg Foundation and the Danish Research Council for Natural Sciences. We thank MAXLAB, the ESRF and the DANSCATT program for provision of beamtime and travel support.

[1] H.A. Ernst, L.Lo Leggio, M, Willemoes, G. Leonard, P. Blum, S. Larsen, *J.Mol.Biol.* **2006**, *358*, 1106-1124.

Keywords: glycoside hydrolases; crystallization; crystal structure.

MS01.P18

Acta Cryst. (2011) A**67,** C221

Structure of the chloride dependent E290S-LeuT mutant from Aquifex aeolicus

Adriana K. Kantcheva,[a] Anne-Marie Lund Winther,[a] Matthias Quick,[b] Jonathan A. Javitch, [b] and Poul Nissen,[a] [a]*Center for Structural Biology, Dept. Molecular Biology, University of Aarhus (Denmark).* [b]*Center for Molecular Recognition, Dept. Pharmacology, Columbia University, New York (USA).* E-mail: akk@mb.au.dk

Members of the Neurotransmitter Sodium Symporter (NSS) family are essential for the proper functioning of the Central Nervous System. NSS members transport molecules such as osmolytes, amino acids and biogenic amines across the neuronal membrane and include widely studied members such as the dopamine transporter, the serotonin transporter, the GABA transporter, and others. Improper functioning of these proteins causes serious conditions such as depression, autism, epilepsy and Parkinson's, [1]. It is thus of central medical significance to understand in detail the mode of function of these transporters. The framework for structural studies of the NSS family was laid in 2005 when the structure of the Leucine transporter from Aquifex aeolicus (aaLeuT), a bacterial NSS transporter homologue, was published [1]. The structure shows aaLeuT in the open-to-out, occluded form with bound substrates. Several structures of aaLeuT with different inhibitors bound were reported in the following years, [2-4]. Many questions however remain unanswered, such as the mode of chloride dependence in the eukaryotic members of the family. Unlike the eukaryotic transporters, aaLeuT is chloride independent, [5]. We have crystallised and determined the structure of a mutated form of LeuT, where the glutamic acid residue at position 290 is substituted by a serine residue, inducing chloride dependence in aaLeuT and rendering this transporter more eukaryotic-like. The structure was determined with bound bromide to facilitate the crystallographic identification of the site. The bromide is bound near residue 290 and elucidates a possible role of chloride in the sodium gradient-driven transport mechanism.

[1] A. Yamashita, S.K. Singh, T. Kawate, Y. Jin, E. Gouaux, *Nature* **2005**, *437*, 215-23. [2] S.K. Singh, A. Yamashita, E. Gouaux, *Nature* **2007**, *448,* 952-6. [3] S.K. Singh, C.L. Piscitelli, A. Yamashita, E. Gouaux, *Science* **2008**, *322,* 1655-61. [4] Z. Zhou, et al. *Science* **2007**, *317,* 1390-3. [5] L. R. Forrest, S. Tavoulari, Y.W. Zhang, G. Rudnick, B. Honig, *PNAS* **2007**, *104/31,* 12761-6.

Keywords: structure, LeuT, chloride

MS01.P19

Acta Cryst. (2011) A**67,** C221-C222

Structural and functional characterization of transactivation Domain of BRCA1

Lumbini R. Yadav, Ashok K. Varma, *Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer, Kharghar, Navi-Mumbai-410 210, (India).* Email: lumbini.y@gmail.com

Breast Cancer susceptibility gene-1, BRCA1 comprises several functional domains that includes RING finger at N-terminus, DNA binding domain at the middle, and C-terminal transactivation domain. The crystal structure of BRCT domain of BRCA1 has been characterized as phosphospecific binding domain. However structure based transcription activation function of BRCT is still elusive. Mutation discovered at the interface between BRCTs and phosphospecific binding partners disrupt the binding and further impair its function. Most importantly, few unreported structurally uncharacterized pathogenic mutations are discovered at the N-terminal extended region of BRCT. Therefore, C- terminus of BRCA1, region comprising (1560-1859) were cloned in GST fusion vector, further FPLC purified, and crystallized using sitting drop vapor diffusion method. Good quality of crystals hexagonal shaped were obtained against the buffer 0.1M MES pH 6.5,1.4 M Ammonium Sulphate ,0.01M Cobalt chloride hexahydrate. Circular Dichroism and Fluorimetric analysis of highly purified domain has revealed that extended form of BRCT domains has correctly folded secondary & tertiary structures, and all the amino acid are in three dimensional proper folded environment. ITC analysis with Abraxas, single phosphorylated and doubly phosphorylated peptide

has been performed and will be highlighted during presentation. The crystals just have been shipped to BM-14 beam line at France and results obtained will be elaborated during presentation. The structure studies of BRCA1 (1560-1859) will help in understanding the pathogenicity of mutations which may provide very important information for predisposing someone at the high risk of cancer.

Keywords: BRCA1, transactivation domain.

MS01.P20

Acta Cryst. (2011) A**67,** C222

The crystal structure of TBC domain of human GapCenA

Robert Janowski, Maïlys Boutin, Miquel Coll, *Institute for Research in Biomedicine, Instituto de Biologia Molecular de Barcelona, CSIC, Parc Cientific de Barcelona, Barcelona, (Spain).* E-mail: robert.janowski@irbbarcelona.org

In eukaryotic cells, the centrosome serves as a main microtubule organizing centre and plays a key role in one of the most important process in biology, the cell division. It is composed of two orthogonally arranged centrioles surrounded by an amorphous mass of proteins termed the pericentriolar material. Despite its importance, little is known about its precise molecular structure. The ultimate goal of the Centrosome 3D project is to gain a better structural understanding of the centrosome function and its relation to a series of human pathologies including Parkinson and Huntington, Bardet–Biedl syndrome, development of cystic kidneys, and most notably, cancer.

GapCenA (RabGap1) is one of the centrosome associated protein, it activates the small GTPases, regulators of membrane trafficking and receptor localization in eucariotic cells. The human genome encodes at least 70 Rab GTPases and more than 50 putative Rab GTPase-activating proteins (GAPs). GapCenA is composed of 1069 amino-acid residues, contains PID (phosphotyrosine interaction domain) and TBC (Tre-2/Bub2/Cdc16) domains, as well as long coiled-coli C-terminal tail. After testing a number of different constructs of GapCenA we have managed to crystallize and determine the structure of the TBC domain to the resolution of 1.85 Å.

Keywords: biocrystallography, protein, X-ray_structure

MS01.P21

Acta Cryst. (2011) A**67,** C222

Expression, Purification, Crystallization and preliminar X-ray crystallographic analysis of Human Malonyl Coa Decarboxylase

D. Aparicio,[a] R. Pérez,[a] X. Carpena,[a] P. Loewen,[b] I. Fita,[a] [a]*Institut de Biologia Molecular de Barcelona (CSIC) and Institut de Recerca Biomèdica (IRB), Parc Cientific de Barcelona, 08028 Barcelona, (Spain).* [b]*Department of Microbiology, University of Manitoba, Winnipeg, Manitoba R3T2N2, (Canada).* Email:daacri@ibmb.csic.es

Malonyl Coa Decarboxylase (MCD), which catalyses the conversion of malonyl coa to acetyl coa, plays an important role in the pathways of the novo fatty acid biosynthesis and fatty acid oxidation. Malonyl coa is an allosteric inhibitor of Carnitine Palmitoyltransferase 1, the enzyme that normally control the flux throw the mitochondrial β-oxidation. In order to provide structural and function evidences of the molecular mechanism of malonyl coa decarboxylation, we have overexpressed and crystallized human MCD. The crystals belongs to the triclinic P1 space group with a unit cell a = 79.55 b = 103.58 c = 134.23 α = 95.40 β = 90.11 γ = 94.82. The self rotation function shows binary axes at 90º from each others which would agree with a tetramer in the crystal asymmetric unit, having D2 symmetry and a volume solvent content of about 75%. Structure determination (with phases obtained from selenomethionine derivative collected at the ESRF Grenoble) are in progress.

Keywords: malonyl coa decarboxylase, malonyl coa, fatty acids

MS01.P22

Acta Cryst. (2011) A**67,** C222

The crystal structure of the p27 component of human dynactin

Anna K, Kowalska, Meiying Zheng, Urszula Derewenda, Zygmunt S. Derewenda, *Department of Molecular Physiology and Biological Physics, University of Virginia, (USA).* E-mail: akk7s@virginia.edu

Dynactin, (or dynein activator), is a 1.2 MDa complex essential for the most aspects of cellular function of dynein in eukaryotic cells [1]. Dynein, a retrograde microtubule-based molecular motor, is not only essential for intracellular transport, but is also required for mitosis and for cell viability. Inhibition or depletion of dynactin, its partner complex, leads to loss of dynein function. Dynactin contains eleven different polypeptide chains, and the structure of most is either known or can be inferred from homologous proteins. However, the structure of the so-called pointed-end complex remains an enigma, although it is thought to participate in interactions with membranous cargo. Two components of this complex, the p25 and p27 proteins, show unique amino acid sequence features that suggest an unusual left-handed β-helix fold, so far seen in several prokaryotic enzymes [2]

We here report the crystal structure of human p27 protein determined to 2.15 Å resolution. The structure was successfully solved in the automated Molecular Replacement pipeline BALBES [3] even there is very limited amino acid sequence similarity of p27 to any of the structures in the PDB. As expected, the main domain displays a tertiary fold of the left-handed parallel β-helix (LβH) structural motif, encoded by the 'hexapeptide repeat' amino acid sequence motif.

The parallel β-helix is a highly repetitive fold, made up of parallel β-strands connected by turns or loops. Those structures coil up to form helical 'rungs'. The structure is stabilized mainly by interstrand hydrogen bonds. Each rung of the β-helix has 2 to 3 untwisted parallel β-strands interrupted by a loop region or one to two turns. The helical rungs are aligned and form a cross-β structure. In this form β-strands are linked by hydrogen bonds and are parallel to the axis of helix. This repetitive motif creates a hydrophobic core of a cylindrical shape. In the β-helix, the strands are almost planar, the surfaces of a helix are nearly flat, all of which results in forming a triangular prism shape.

The p27 protein represents the only one in the human proteome with the β-helix fold, although p25 is expected to be structurally related. The only other similar protein in any eukaryotic proteome is an antifreeze protein from spruce budworm, although it represents type I of LβH, and consists of five residues per strand (15 per rung), rather than six, as found in the p27 structure. There is a homodimer in the asymmetric unit of the p27 crystal, and its quaternary structure strongly suggests that the mutual disposition of the two molecules is representative of the structure of the p25/p27 heterodimer found in dynactin.

We hope that the three-dimensional model will assist in studies intended to unravel the details of interaction of dynactin with cargo particles.

[1] T.A. Schroer,. *Annu Rev Cell Dev Biol* **2004,** *20*, 759-779. [2] J.H. Choi, C. Govaerts, B.C. May, F.E. Cohen, *Proteins*. **2008,** *73(1)*, 150-60. [3] F. Long, A. Vagin, P. Young, G.N. Murshudov. *Acta Cryst.* **2007,** *D63*.

Keywords: dynactin, beta-helix fold, automated molecular replacement