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

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Structural studies of GH31 and GH32 glycoside hydrolases

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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.

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

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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.

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Structural and functional characterization of transactivation Domain of BRCA1

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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