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The Vault particle, with an astounding molecular weight of 13 MDa, is the largest Ribonucleoprotein described. Although its function is still unclear, it has been related to cell signalling, proliferation and immune responses. The Vault complex shows an overall barrel-shaped structure organized in two identical moieties, each consisting of thirty-nine copies of the major vault protein MVP. Earlier data indicated that Vault particles can dissociate at acidic pH.

Recently, we solved the crystal structure of the Vault particle at 8 Å resolution, together with the 2.1 Å structure of the seven N-terminal domains (R1-R7) of MVP. The structure reveals the interactions that govern Vault association and provides an explanation for a reversible dissociation induced by low pH. [1]. This information complemented the 3.5Å X-ray structure of the vault particle, published at the beginning of 2009 by other authors [2], showing the MVP monomers folded into twelve distinct domains: nine repeat domains, a shoulder domain, a cap-helix and a cap ring. The comparison between the structures of R1-R7 and the equivalent region in the 3.5Å structure of the entire particle showed important discrepancies in the tracing of domains R1 and R2. Furthermore, in the structure reported by Tanaka et al., all the atomic positions and temperature factors of the 39 copies of MVP found in the crystal asymmetric unit were included explicitly in the refinement (PDB ids. 2ZU0, 2ZV4 and 2ZV5, with an averaged temperature factor of 121.4 Å2). In this report, refinement of the vault structure was performed using the DEN protocols as included in the Refine low-resolution package of CNS [3]. During refinement the 39-fold non-crystallographic symmetry was always strictly maintained with constrains applied to a single MVP monomer, which was generated as a hybrid model containing the R1R7 structure at the N-terminus (residues form Glu4 to Val380) and the coordinates corresponding to monomer A (PDB id: 2ZV4) at the C-terminus (residues from P381 to G814). The deposited structure factors of the 3.5 Å vault structure (PDB id: 2ZU0) were used as experimental data. Refinement was combined with averaging and solvent flattening with DM and the quality of the resulting maps was also enhanced by applying a negative Bsharp value in a resolution dependent weighting scheme (map sharpening).

This refinement suggests important movements in the N-terminus of the MVP structure during the closing of the two Vault halves and provides an improved view of the C-terminal region.

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Preparation and Characterization of the complex [Co^{III}(TpivPP)(4,4'-bipy)(Cl)]

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Cobalt porphyrin species are specially used as models for hemoproteines and actually investigations concerning these species includes many areas such as organic reaction catalysis [1], [2] biosensors [3] and materials presenting non-linear optical activities.The (4,4'-bib yridine)(chlorido)(α,α,α,α-tetrakis(*o*-pivalamidophenyl)porphyrinato) cobalt(III) has been synthesized and characterized by UV-vis, IR and proton NMR spectroscopy which indicate, inter alia, that the cobalt ion presents the oxidation state III. The X-ray molecular structure shows that the Co(III) ion is hexa-coordinated by the four nitrogen atoms of the pyrrole rings of the TpivPP anion, the chlorido axial ligand on the pocket side of the porphyrin and the nitrogen atom of 4,4'-bipyridine ligand. The average cobalt-pyrrole nitrogen atoms is Co-N_p = 1.983 (2) Å, the Co—Cl and Co—N(bpy) bond lengths are respectively 2.230 (1)Å and 2.030 (3)Å. The compound [Co^{III}(TpivPP)(Cl)(4,4'-bipy)] crystallizes in the monoclinic space group C2/c with unit cell dimensions *a*=18,906(5) Å, *b*=19,112(6) Å, *c*=18,264(8) Å, *β*=92,112°. The structure was refined to *R*=4,61%, WR_2=13,78% and S=1,079 .

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Keywords: porphyrins, single-crystal X-ray

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Interaction of Alzheimer β-amyloid and metal complexes with lipid bilayers

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Alzheimer's disease is an age-related disorder characterized by progressive cognitive decline and neurodegeneration. One of the key features of this disease is the presence of amyloid plaques associated with neuritic degeneration [1]. The amyloid plaques are composed predominantly of 40-42-residue peptides, the amyloid β-peptide (βA), being the βA(1-42) the most toxic. Soluble βA peptides interact with cell membranes and have been proposed to affect membrane integrity leading to apoptosis. The molecular mechanisms of these interactions are still unclear. In the context of these observations we investigated the molecular mechanisms that might explain the abnormal accumulation of aluminum in the brains of patients with Alzheimer's disease.

There are reports indicating that Al(III) interacts with cell membranes inducing structural and functional perturbations. They might be due to: a) direct interaction of Al(III) with proteins forming ion channels, receptors and enzymes, b) induction of structural alterations in the membrane lipid bilayer, or c) action on the lipid-protein interfaces. To elucidate among these alternatives, and given the structural complexity of native cell membranes, we utilized molecular models consisting of bilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoyl-phosphatidylethanolamine (DMPE), representative of phospholipid classes located in the outer and inner monolayers of many cell membranes, respectively.

The capacity of βA and of its complexes with Al, Zn, Cu and Fe to perturb the multibilayer structures of DMPC and DMPE was evaluated by X-ray diffraction. In the context of these studies we report that βA, in our experimental conditions, did not alter any of the bilayer structures while the addition of the βA-Al complex deeply disordered that of DMPC membrane [2]. Our findings imply that Al, compared to the other βA-metal complexes tested could have a specifically relevant effect in enhancing βA toxicity. It is important to stress that, in our experimental conditions, it is not Al alone which causes such alterations but the

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