

A complex of atypical PKC and Par6 is a common regulator for cell-polarity related processes, which is an essential clue to evolutionary conserved cell-polarity regulation. Here, we determined the crystal structure of the aPKC and Par6 PB1 domain complex to a resolution of 1.5 Å. Both PB1 adopt a ubiquitin fold. aPKC PB1 presents an OPCA motif, 28 amino acid residues with acidic and hydrophobic residues, which interacts with the conserved lysine residue of Par6 PB1 in a front-and-back manner. Structural comparison of the aPKC and Par6 PB1 complex with the p40<sup>phox</sup> and p67<sup>phox</sup> PB1 complex, subunits of neutrophil NADPH oxidase, reveals that the specific interaction is achieved by tilting the interface so that the insertion or extension in the sequence is engaged in the specificity determinant. The PB1 domain develops the interaction surface on the ubiquitin fold to increase the versatility of molecular interaction.

**Keywords:** protein complex structure, domain structure, cell polarity

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#### The Allergenic Non-specific Lipid Transfer Protein from Peach: Structural Studies

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A large number of proteins can bind small molecules with a poor solubility in water. The function of many of these proteins is to transport small molecules which would not be soluble in the biological environment. The ligands often present a strong lipophilic characters, as, for example, retinoids and fatty acids. In addition to the well known calycins, this group of protein includes the so-called non-specific lipid transfer proteins (*ns*-LTPs). They are plant proteins of about 9 kDa molecular mass, and they bind fatty acids, steroids and other types of lipids. However, their function has not been precisely defined as yet [1].

Our study is focused on the determination of the three dimensional structure of peach *ns*-LTP. Recently, several biological and medical studies have stimulated interest in the *ns*-LTP from peach. This protein, like others *ns*-LTPs, binds fatty acids, but it has been also identified as an important food allergen [2].

Despite its strong sequence similarity with others *ns*-LTPs whose structure is already known [3], its crystal structure determination was not straightforward. It required the measurement of synchrotron anomalous data with enhanced sulphur *f'* by using a synchrotron X-ray wavelength of 2Å from SRS MPW beamline 10. In addition a stronger  $\langle I/\sigma(I) \rangle$  was then achieved using the APS undulator at SBC CAT. The molecular model is a single compact domain, made up by four  $\alpha$ -helices, which form a hydrophobic pocket where the lipid is bound. The 3-D structure is strongly stabilized by four disulphide bridges. Possible immunogenic epitopes associated with the allergenic reaction will be proposed by this study.

[1] Kader J. C., *Annu. Rev Plant Physiol. Plant Mol. Biol.*, 1996, **47**, 627. [2] Salcedo G., Sanchez – Monge R., Diaz – Casado A., Barber D., *Clin. Exp. Allergy* 2004, **34**, 1336. [3] Han G. W., Lee J. Y., Song H. K., Chang C., Min K., Moon J., Shin D. H., Kopka M. L., Sawaya M. R., Yuan H. S., Kim T. D., Choe J., Lim D., Moon H. J., Suh S. W., *J. Mol. Biol.* 2001, **308**, 263.

**Keywords:** *ns*-LTP, non specific lipid transfer protein, food allergen

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#### LRRs: A platform to build a Protein Recognition Motif

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The leucine-rich repeat domains (LRRs) of cell-surface receptors often constitute the binding regions for small protein ligands. Examples are found in many types of medically important receptors,

e.g. Insulin and growth-factor receptors, G-coupled protein receptors and Toll-like receptors. Thus they are important therapeutic targets.

The architecture of LRRs generally consists of a  $\beta$ -helix or solenoid with a prominent  $\beta$ -sheet down one face forming the ligand-binding surface. Side chains on this face are tightly packed to form a sterically well-defined surface with the chemical composition dictated by sequence. The opposite face shows considerable structural variability and here, the space occupied by the main chain appears to dictate the curvature of the ligand-binding face. Thus LRRs have a simple but elegant design, where the main chain provides a regular framework of variable size and shape and chemical nature of the site is under genetic control.

Now that a considerable number of these structures have been determined, with or without their ligands, a detailed analysis has revealed the factors which control the overall architecture for *ab initio* design of a protein-binding surface. Naturally-occurring augmentations of this standard architecture provide additional ways of creating a protein-docking site.

**Keywords:** receptor-ligand interactions, molecular recognition, protein engineering

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#### Cracking of the Targeting Signal Embedded in Mitochondrial Presequences

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Most mitochondrial proteins are synthesized in the cytosol as precursor proteins with a cleavable N-terminal presequences and are imported into mitochondria. Protein import into mitochondria is mediated by protein assemblies in the mitochondrial membranes. A subunit, Tom20, functions as a general protein import receptor by recognizing presequences of preproteins. Although no consensus sequence is found, Tom20 recognizes a wide variety of presequences.

To understand the structural basis of the presequence recognition, we determined the NMR and crystal structures of Tom20 in a complex with a presequence peptide. Note that the presequence was fixed to Tom20 via a designed intermolecular disulfide bond to obtain crystals. The bound presequence forms an amphiphilic  $\alpha$ -helix. NMR titration experiments indicated the presence of a unique presequence binding site in Tom20, and defined a common five-residue pattern in different presequences. To refine this pattern, we introduced a new peptide library approach using the formation of an intermolecular disulfide bond. We propose that a presequence is regarded as a collective entity of short amino acid sequences that are recognized by several proteins including Tom20. The organization (position, order, and overlapping) of these binding segments is unique for each presequence. This view explains why no consensus sequences are found by simple sequence comparisons.

**Keywords:** protein transport, molecular recognition, crystallographic and NMR solution structures

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#### Crystal Structure of Decameric Peroxiredoxin (AhpC) from *Amphibacillus xylanus*

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Peroxiredoxins (Prxs), also referred to as AhpCs, are a ubiquitous family of antioxidant enzymes. Bacterial AhpC is recognized as the primary scavenger of endogenously generated hydrogen peroxides. AhpC purified from *Amphibacillus xylanus* shows extremely high scavenging activity for both hydroperoxide and alkyl hydroperoxide in

cooperation with the flavoprotein, NADH oxidase. Here we report the crystal structure of *A. xylophilus* AhpC in its oxidized form. The enzyme forms a ring-like ( $\alpha$ 2)<sub>5</sub>-decamer, the structure of which is similar to those of the previously reported Prxs, and especially to that from *Salmonella typhimurium*. The dimer-dimer interface of the decamer exhibits moderate and conserved hydrophobic interactions, which have been proposed to dissociate at the physiological ionic strengths. In the crystal, electron densities of small molecules were observed between the decamers and were shown to play a unique role in the crystallization by bridging the decamers via the hydrogen bonds.

[1] Kitano K., Kita, A., Hakoshima T., Niimura Y., Miki K., *Proteins*, 2005, *in press*. [2] Kitano K., Niimura Y., Nishiyama Y., Miki K. *J. Biochem.*, 1999, **126**, 313.

**Keywords:** protein structures, antioxidants, oligomers

#### P.04.19.2

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#### Structures of CFTR NBD1 Suggest a Molecular Mechanism for Cystic Fibrosis

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Cystic fibrosis (CF) is caused by genetic defects in the cystic fibrosis transmembrane conductance regulator protein (CFTR), most commonly through omission of residue Phe-508 ( $\Delta$ F508) in the first nucleotide-binding domain (NBD1), resulting in misfolded, non-functional CFTR chloride channel. The structure of NBD1 was solved in order to better understand the effect of this deletion.

Initially, the structure of wild-type (WT) mouse NBD1 was determined [1]. This revealed a largely conventional NBD fold, compared with those of similar bacterial proteins, with the exception of additions in the N- and C-terminal regions of the protein with phosphorylatable and potentially regulatory function. Residue Phe-508 was seen to be surface exposed in a loop region and not of obvious importance to the NBD1 fold.

Recently, the structure of human NBD1 with  $\Delta$ F508 present was determined [2], which revealed that the deletion did not alter or disrupt the fold of this domain. This observation was supported by thermodynamic measurements on WT and  $\Delta$ F508 protein that showed the stability of NBD1 is unaffected as well. The effect of the deletion then appears to be the disruption of interactions of NBD1 with other domains of CFTR, most likely with the first membrane spanning domain (MSD1). This new understanding of the molecular mechanism of dysfunctional  $\Delta$ F508 CFTR will lead to improved drug discovery efforts for CF.

[1] Lewis H.A., Buchanan S.G., et al., *EMBO J.*, 2004, **23**, 282. [2] Lewis H.A., Zhao X., et al., *J. Biol. Chem.*, 2005, 1346.

**Keywords:** disease-related structures, cystic fibrosis, nucleotide-binding domain

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#### Amyloidosis: Structure of a $\lambda$ 6 Light Chain Antibody Fragment

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It has been well evidenced the preferential association of the lambda VI antibody light chain subgroup with antibody light chain amyloidosis. We have generated an engineered light chain variable region domain (rVL6aJL2) whose 99 amino terminal residues are encoded by the germline VI gene segment 6a and the complementary segment of 12 residues encoded by the gene segment JL2. *In vitro* fibril formation assays demonstrated that the rVL6aJL2 is able to slowly aggregate itself as amyloid-like fibrils under physiological conditions. The recombinant protein expressed in *E.coli*, was purified and crystallised using 1.4 to 2.0 M sodium acetate, 100mM MES pH 6.5. Crystals diffracted up to 1.9 Å resolution. The crystals grew in three different space groups and all contained fibrillar structures assembled into the crystal. These structures have helicoidal shape with a 93 Å long pitch, and a section perpendicular to the axis, 45 Å wide,

with a squared shape. The molecule that generates this structure through a 4<sub>1</sub> symmetry, is a dimer built as that formed by light and heavy chains in functional antibodies. These dimer-dimer contacts found, comprises a surface larger than 1600 Å<sup>2</sup> and the amino acids involved has been shown to participate in fibrillogenesis. As in other structures from fibrillogenic antibody light-chains, no significant conformational changes have been observed. The question we have so far is: have or not the helicoidal structures we found over 3 different space groups relation with the atomic structure of amyloidotic fibers?

This work has been supported by CONACyT. Data were collected at the L.U.E.P., U.N.A.M. and at the NSLS Brookhaven, line X6-A.

**Keywords:** amyloidosis, fibres, crystallographic structure

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#### Structural and Functional Study of the Bloom Syndrome Protein

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Bloom Syndrome (BS) is an autosomal recessive human disorder characterized by genomic instability and a predisposition to a wide variety of cancers. The gene mutated in BS encodes a three domains enzyme, the Bloom Syndrome Protein (BLM), which C-terminal extension can be divided in two subdomains: RecQ-Ct and HRDC.

We report herein that the RecQ-Ct domain, responsible for DNA unwinding, contains a zinc finger motif. In order to understand the role of this motif in BLM, we constructed a series of mutations altering its highly conserved residues. Experiments done with these mutants showed that they were severely impaired in DNA binding and for the subsequent ATPase and helicase activities, revealing the importance of the zinc finger motif for all the functions of the enzyme. We computed the three dimensional structure of the RecQ-Ct domain by homology modeling using the template structure of the RecQ helicase from *E. coli*. This model allowed us to study the consequences of mutations observed in the Bloom Syndrome Protein when associated to a cancer. The mutant enzymes have been expressed in *E. coli* and their activities have been compared to the wild type enzyme. In order to get new insight in the molecular basis of Bloom Syndrome disease, we underwent the crystallization of the RecQ-Ct and HRDC domains in presence of various DNA substrates.

**Keywords:** cancer, DNA-protein interactions, structure-function

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#### Molecular Basis for Antitumor Effect of Actin-aplyronine A Complex

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Aplyronine A is a promising candidate of anticancer agent isolated from *Aplysia kurodai*. The complex structure of actin-aplyronine A is determined via a synchrotron X-ray analysis at 1.45Å resolution. Aplyronine A binds to actin in the similar manners with the trisoxazole toxins and gelsolin segment 1 around its aliphatic tail part which should play an important role for F-actin depolymerizing activity. In contrast, the structural aspects relating to peculiar interactions between aplyronine A and actin can be found around the macrolide ring part which should be a key to the potent antitumor effect of aplyronine A. Actin-aplyronine A complex structure should suggest that each complex comes to have ability to effect other biological reactions which are essential for deaths of cancer cells. The precise charge density map was also visualized with the maximum entropy method to construct the structure model of a disordered stereoisomer of a peculiar side-chain of aplyronine A as well as to