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Keywords: antibody structure, photophysics, protein dynamics

P.04.11.3
Acta Cryst. (2005). A**61**, C230
Engineering Immune System Glycoproteins to form Uniform Crystalline Lattices
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Interactions of antibodies and Fc receptors (FcR) play a fundamental role in immunity. However, antibodies and FcR are heavily glycosylated, making it difficult to obtain strongly diffracting crystals. Consequently, most structures for these proteins have been determined at low to medium resolutions (<3.0-2.6 Å). Without modifying the carbohydrates, we are developing procedures for generating crystals of immune system glycoproteins that diffract to high resolutions.

A 2.0 Å structure was previously determined for FcγRIIa with a point mutation of Ser to Phe at position 88[1]. The Phe88 side chain is involved in a key lattice contact by completing a hydrophobic pocket that traps a proline "guest ligand" from a symmetry related receptor monomer. As a result the crystals are robust, allowing a variety of receptor glycoforms to be resolved and the structural analysis extended to 1.5 Å. The role of Phe88 in promoting uniform crystalline lattices has been shown by determining the 2.3 Å resolution structure of the "wild-type" (Ser88) FcγRIIa glycoprotein. The Ser88 receptor crystals were fragile with receptors arranged in a different and more loosely packed crystalline lattice compared to Phe88 receptor crystals.

The improved properties of FcγRIIa crystals containing the lattice forming Phe88 mutation may have profound implications for the field of macromolecular crystal engineering.

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Keywords: immune system proteins, cell surface receptors, crystal engineering

P.04.11.4
Acta Cryst. (2005). A**61**, C230
Crystal Structure of Leukocyte Ig-like Receptor 9 (LIR9/ILT11 /CD85f)
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Leukocyte Immunoglobulin(Ig)-like receptors (LIRs) are human Ig-like receptors that have activating and inhibitory function in leukocytes. LIRs can be subdivided into three groups: inhibitory, activating and secreted molecules. LIR9 is mainly expressed on monocytes and neutrophils as both membrane bound and secreted forms. The membrane bound LIR9 is an activating receptor with arginine residue in its transmembrane region, and the cross-linking of LIR9 induces activation of monocytes. Whereas LIR1 and LIR2, the inhibitory receptors of LIR family, are known to bind to a broad range of human MHC class I molecules (MHCIs), the binding property of LIR9 is unknown. LIR9 shows less homology with LIR1/2/6 recognizing MHCIs (less than 60% amino acid identity with LIR1/2/6). Here we demonstrated that LIR9 had no or very weak affinities to MHCIs by biosensor analysis. Furthermore, we determined the crystal structure of extracellular domain of LIR9 at 1.9 Å resolution by MAD method. The structure showed large structural differences in the region corresponding to the MHCI binding site of LIR1, resulting in the disability of LIR9 to bind to MHCIs. These results raised the possibility that LIR9 recognizes a non-MHCI ligand.
Keywords: immune regulation, immunoglobulin-like receptor, structural immunology

P.04.11.5
Acta Cryst. (2005). A**61**, C230
The Crystal Structure of Human CD1d with and without α-Galactosylceramide Bound
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The glycolipid α-galactosylceramide binds with high affinity to CD1d and stimulates NKT cells. Here we report the crystal structure of human CD1d in complex with synthetic α-galactosylceramide at 3.2 Å resolution. The structure reveals a tightly fit lipid in the CD1d hydrophobic binding groove, with the sphingosine chain bound in the C' pocket and the longer acyl chain anchored in the A' pocket. Pocket volumes and hydrogen bonds to the glycolipid head group optimize α-galactosylceramide binding to CD1d. The structure of CD1d without lipid is also presented which shows a more open conformation of the binding groove than is seen in lipid-bound CD1d, suggesting a dual conformation of CD1d in which the "open" conformation is more able to load lipids than the lipid-bound "closed" conformation.
Keywords: humanCD1d, empty MHC class I-like protein, α-galactosylceramide

P.04.11.6
Acta Cryst. (2005). A**61**, C230
Structure, Function and Evolution of the Serum Pentraxins
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Wide-ranging studies on the serum pentraxins C-reactive protein and serum amyloid P component are aimed at the investigation of the structural, functional and evolutionary relationships, and of the humoral and cellular recognition properties, of the pentraxins from species ranging from invertebrate to man. Recognition properties of the pentraxins, homologues of which have been found in mammals, fish, amphibians, and invertebrates, include cell wall phospholipids and fungal and bacterial polysaccharides. In the absence of highly developed adaptive immunity, a diverse array of humoral components, such as the pentraxins, provides an essential and effective strategy for recognising and destroying disease-causing pathogens.

The structures of pentraxins from man [1], rat, Mustelis canis and L. polyphemus [2,3,4] reveal variable aggregation of the conserved protomer fold, details of novel binding properties and insights in to the relationships between structural, functional and sequence homology.

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Keywords: pentraxin, innate immunity, evolution

P.04.11.7
Acta Cryst. (2005). A**61**, C230-C231
Structural Studies of Human CD81 Extracellular Domain
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CD81 is a four transmembrane protein of 236 amino acids, belonging to the tetraspanin protein family, involved in various

immune responses. Besides the four hydrophobic transmembrane regions, CD81 hosts two extracellular domains, known as large and small extracellular loops (LEL and SEL, respectively). Human CD81 is held to act as (co)receptor for hepatitis C virus (HCV), thus a key participant in the infection.

To widen our knowledge on the roles played by CD81 LEL in binding the HCV E2 glycoprotein, the LEL crystal structure was approached. Three different crystal forms have so far been obtained. We report here on the most recently grown form (R32). Marked conformational fluctuations in the molecular regions held to be involved in binding to the viral protein, suggest rules for recognition and assembly within the tetraspan web.

Keywords: cell surface receptor, structural analysis of molecular crystals, virus receptors

P.04.12.1

Acta Cryst. (2005). A**61**, C231

Structure of MRG15 reveals a Novel Fold and provides Insights into its Biological Functions

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MRG15(MORF4 related gene on chromosome 15) and MORF4 (mortality related gene on chromosome 4) belong to a newly identified protein family which share high sequence homology. Recently, the MRG proteins have been shown to function in transcription regulation (histone modification). We determined the crystal structures of the C terminal domains of MRG15 (MRG15C) and MORF4 (MORF4C) to 2.2 Å and 3.0 Å resolution, respectively.. A structure homology search with the DALI algorithm indicates that MRG15C and MORF4C have a novel protein fold with no obvious similarity to those of other proteins of known structures. The proteins are mainly consisted of α-helices and form homodimer in both solution and structures. Structure analysis and multiple sequence alignment indicate that there are a negatively charged hydrophilic patch at the C-terminal and a hydrophobic pocket at the dimeric interface both of which are composed of several highly conserved residues. These structural locations could be putative binding sites for other proteins or substrates. Biochemical assays indicate that the hydrophilic patch might be involved in binding with PAM14. Additionally, we have also obtained a 2.3 Å resolution diffraction data for the N-terminal domain (1-90 amino acids) of MRG15(MRG15N) which shows limited similarity to Chromo domain. Structure determination of MRG15N is ongoing. Analysis of these structures will shed new light on the biological functions of MRG15 protein and other members of the MRG protein family.

Keywords: MRG15, novel fold, chromo domain

P.04.12.2

Acta Cryst. (2005). A**61**, C231

Crystal Structure of the Small G Protein M-Ras and its Implications

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Ras proteins are involved in a wide variety of cellular responses. They undergo conformational changes in two conserved regions, named “switch I” and “switch II” by cycling between GTP-bound active and GDP-bound inactive states, and thereby function as ‘on/off’ biological switches. In the active state, they bind to their specific effectors to initiate various signaling events.

We have determined the crystal structure of a Ras-family protein, M-Ras (residues 1-178), in complex with GDP and GTP. The overall structure of M-Ras resembles those of other Ras-family proteins excluding its characteristic conformations in switch regions. In the GTP-bound form, Ras proteins, including H-Ras and Rap2A, are known to conserve several intra-molecular interactions essential for conformational stabilization of switch I. In particular, hydrogen bonds between Thr-35 and the ã-phosphate of GTP play important roles to stabilize this effector loop, yielding a preferable conformation for effector recognition. In the case of M-Ras-GTP, the corresponding interaction through Thr-45 is completely lost, and this lack causes a distinctive switch I conformation, where switch I loop is pulled away from the guanine nucleotide and shows an open unstable conformation. In addition, the orientation of the á2-helix in switch II shows a remarkable difference from those of H-Ras and Rap2A. These structural features may provide new information to investigate effector recognition mechanisms by Ras proteins.

Keywords: Ras, switch region, crystal structure

P.04.12.3

Acta Cryst. (2005). A**61**, C231

Structural Studies of Plant RKIP/PEBP Family Members

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Intracellular signalling is a critical cellular function, and one that is often perturbed in a variety of diseases. The RKIP/PEBP protein family is highly conserved across a wide range of organisms from humans to bacteria and plants. This family is involved in the regulation of kinase signalling pathways. Two members of this family, TFL1 and FT, from *Arabidopsis thaliana* act antagonistically to control meristem fate. However the exact mechanism of their action remains unknown.

To help elucidate the means by which these proteins act, crystallographic studies are being undertaken. While previous work has determined the structures of TFL1 and FT alone, current efforts are focused on extending the structural information to include protein:ligand complexes. This includes complexes of these proteins with phosphorylated amino acid, and attempts to co-crystallise these proteins with their respective protein ligands.

Because of the high level of conservation within the RKIP/PEBP protein family it is hoped that studying the functional mechanism of plant members will help elucidate the action of these proteins in humans, for which crystallisation of their complexes has proven intractable.

Keywords: signal transduction, kinase regulation, RKIP/PEBP

P.04.12.4

Acta Cryst. (2005). A**61**, C231-C232

Inwardly rectifying Potassium Channels

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Inwardly rectifying potassium (Kir) channels are integral membrane proteins selective to conducting K^+ ions into cells. These channels are found in many organisms and are involved in a wide range of physiological processes such as propagation of action potential and hormone regulation. The basic topology of Kir channels consist of a tetramer of two transmembrane helices, and a loop containing the selectivity filter. Both the N and C termini of each monomer in the tetramer are intracellular, and these interact to form a large globular domain, which is involved in gating. One of the difficulties in solving the structure of membrane proteins such as Kir channels is obtaining significant quantities of highly purified protein. Rather than relying on the isolation of soluble proteins from natural sources, structural biologists are focused on producing large amounts of target proteins heterologously. Therefore, a number of strategies in this project have been employed to overcome this problem. Murine Kir2.1 has been successfully cloned and overexpressed in the membrane of the methylotrophic yeast *Pichia pastoris*. This yeast was chosen as an expression host as it has many advantages of higher eukaryotic expression systems but it is as easy to manipulate as *E.coli* or *Saccharomyces cerevisiae.* Further investigations into purification and characterisation of the expressed Kir2.1 are currently being undertaken. The intracellular extramembranous domains of the murine Kir2.1 channel have been overexpressed in *E.coli.* This protein forms a stable tetramer and can be purified to a very high level. Crystallisation trials with this pure protein are in progress, as are