MS71 STRUCTURAL BIOLOGY AND THE IMMUNE SYSTEM
Chairpersons: David Rose, Massimo Degano

MS71.29.1
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Structural Basis of HIV-1 Neutralization: Implications for Vaccine Design
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Antibodies that can potently neutralize a broad spectrum of HIV-1 primary isolates are extremely rare and invaluable for innovative HIV-1 vaccine design strategies. Crystal structures for four of the five antibodies [b12; 2G12; 447-52D; and 4E10] have been determined. Antibody b12 interacts with the recessed CD4 binding site through a long CDR H3 loop. Anti-gp120 antibody 2g12 recognizes a cluster of high-mannose sugars on the surface of gp120—an unexpected high affinity for a carbohydrate epitope. The 2g12 Fab arms dimerize via exchange of their V_H domains to form a multivalent binding surface for carbohydrates that is useful for designing a carbohydrate-based vaccine. Antibody 447-52D also uses a long CDR H3 loop, but it interacts with the V3 loop backbone of gp120, which explains its broad specificity. 4E10 is the most broadly neutralizing and is effective against all clades and subtypes of HIV-1. The 4E10 structure with a gp41 peptide shows a helical conformation for the epitope that gives insights into the membrane fusion events. Thus, these structural studies not only elucidate how each antibody interacts with its respective antigenic site in either gp120 or gp41, but also give fascinating insights into how the immune system evolves strategies to overcome challenges in accessing epitopes that are deeply-buried (b12), have low antigenicity (2g12), that vary in sequence (447-52D), and are transiently-accessible (4E10). The novel modes of antigen recognition provide a plethora of new ideas for the design of novel HIV-1 immunogens to elicit such antibody responses and are being harnessed in a retrovaccinology approach for HIV-1 vaccine design.
Keywords: antibodies, HIV-1 vaccine, antibody-antigen interactions

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Structural Basis of Ligand Recognition by the Collectins
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The biological activity of collectins is exerted through Ca-dependent binding of the terminal monosaccharide of, for example, cell surface lipopolysaccharide and phospholipids, peptidoglycans and glycosaminoglycans. The residues in the carbohydrate-binding pocket which coordinate to both the calcium ion and the ligand are highly conserved. Variability in other binding determinants in the binding pocket is, however, evident throughout the family. One of these determinants has been shown to influence bound ligand orientation in rat MBP [1], but there is, as yet, no explanation of the variability of orientation and relative affinity for the variety of ligands.

Our high resolution structures of recombinant collectin fragments, including a biologically and therapeutically active fragment of hSP-D, in both unliganded and ligand-complexed forms [2], provide preliminary data towards an understanding of the ligand specificity of the collectins. They also raise questions regarding the interaction of hSP-D with natural ligands, the regulation of its activity by calcium, and its interaction with receptors on immune effector cells.

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Keywords: lectin, immune system, ligand-protein interactions

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Do Sharks have a New Antibody Lineage?
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Sharks are the most primitive animals to have an advanced adaptive immune system. Their long evolutionary history (~400 million years) is reflected in a diverse array of shark antibodies, including the unique IgNAR (Ig new antigen receptor) isotype. IgNARs are heavy chain homodimers, there is no associated light chain and binding affinity mainly resides in two complementarity determining regions. Given that sharks also possess heavy-light chain antibodies, the question has been: did IgNARs evolve from the conventional antibody/T-cell receptor format, or do they represent an entirely separate antibody lineage?

Structural studies presented here including recent reports of the first three-dimensional crystal structures for IgNAR variable domains [1] and of the IgNAR–antigen complex structure [2] have provided significant insight into IgNAR evolutionary origin and antigen-binding strategy. Comparison of IgNAR structures to that of a range of immune molecules showed the best agreement with members of the cell adhesion family. We hypothesise that the IgNARs are an evolutionarily distinct antibody lineage, separate from heavy-light chain antibodies and T cell receptors.

[1] Streltsov V.A, Varghese J.N., Carmichael J.A., Irving R.A., Hudson P.J., Nuttall S.D., *PNAS*, 2004, **101**, 12444. [2] Stanfield R.L, Dooley H., Flajnik M.F., Wilson I.A., *Science*, 2004, **305**, 1770.
Keywords: crystal structure, Ig new antigen receptor, shark antibody

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The Structure of CD3εγ in Complex with the Therapeutic antibody, OKT3
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The T cell receptor (TCR) is responsible for the recognition of peptide antigens presented by MHC class I molecules. Upon recognition of a presented peptide, the TCR induces the cytotoxic T cell response. CD3 is a multisubunit complex that performs a fundamental role in T cell signalling, T cell development and surface expression of the αβ TCR. The CD3 complex is composed of a CD3εγ heterodimer, a CD3εδ heterodimer and a CD3ζζ homodimer and, together with the TCR, are key molecules of the T cell immunological synapse. A focus of the T cell signaling function of the CD3 complex is the interaction of the CD3ε extracellular domain with the TCR constant domains. The importance of the CD3ε extracellular domain in signal transmission is also emphasized by the binding of OKT3, a therapeutic monoclonal antibody used successfully as an immunosuppressive agent in tissue transplantation.

We solved the crystal structure of the human CD3εγ heterodimer in complex with a Fab fragment of OKT3. The mode of CD3εγ dimerization together with the OKT3 epitope provides a general structural basis for CD3 assembly and maps potential sites of interaction with TCR. Despite the important influence of OKT3 on the activity of the immunological synapse, OKT3 binds to an atypically small area and has a low affinity for the isolated CD3εγ heterodimer.
Keywords: immunobiology, therapeutic antibodies, T cell receptors

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Structural Insights into the Central Complement Ccomponent C3
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The complement system is a critical component of the mammalian immune defense against micro-organisms in plasma that links the innate and adaptive immune responses. It consists of >30 plasma proteins and cell-surface receptors. The three different pathways of activation converge in the activation of complement component C3. C3 is a 190 kDa plasma protein that, together with complement components C4 and C5, belongs to the α2-Macroglobulin family. C3 undergoes a series of proteolytic activation and degradation steps and interacts with several regulators of complement. Here we present the structure of a naturally occurring, proteolytic product of C3, called C3c, which constitutes ¾ of the total protein. This structure provides insight into C3 and its binding sites and provides the first insight into the core fold of the α2-Macroglobulin protein family.

The C3c structure shows a surprising domain composition and reveals that the two, β and α, polypeptide chains of mature C3 are heavily intertwined. The core of the protein consists of 8 homologous domains, which we refer to as macroglobulin (MG) domains. The domains display a fibronection type-3 (FN3) like fold but have no sequence homology and lack the FN3-motif.

The multi-domain structure, its potential domain-domain flexibility and the implications for complement activation and convertase formation will be discussed.

Keywords: immunology, complement, plasma proteins

MS72 HOT STRUCTURES IN PROTEIN CRYSTALLOGRAPHY
Chairpersons: Glaucius Oliva, Andrew H.-J. Wang

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Structural Studies on Carboxysomes
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Carboxysomes are microcompartments found in autotrophic bacteria; they function to sequester RuBisCO for optimal carbon fixation. Carboxysomes are essentially primitive organelles, composed entirely of protein. Genomic sequencing is revealing the surprisingly wide distribution of proteinaceous organelles that are structurally related to the carboxysome. In order to understand principles of carboxysome assembly and function, we have undertaken EM and crystallographic analyses of the carboxysome and its isolated component proteins and enzymes. We have determined the structures of two of the carboxysome shell components. Our data provide the first molecular details of carboxysome structure and assembly that show striking parallels to principles of viral architecture. Our data also provide insights into the structural basis of function, including import and export of substrate and products.

Keywords: carbon-fixation, biological macromolecules, organelle assembly

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Structure and Function of RNase E and the RNA Degradosome Assembly
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The essential enzyme RNase E is critical to RNA processing and decay regulation in *Escherichia coli*. The activity of RNase E affects the balance and composition of the transcript population, and the enzyme serves as the scaffold for a multi-component assembly known as the RNA degradosome. RNase E belongs to a widely occurring family of ribonucleases that cleave RNA internally, but whose catalytic power is determined by the 5'-terminus of the substrate, even if this lies at a distance from the cutting site. We report crystal structures of the catalytic domain of RNase E as trapped allosteric intermediates with RNA substrates. The structures explain why a tetrameric quaternary structure is required for activity, and how the recognition of the 5' terminus of the substrate triggers a conformational transition to initiate catalysis. The structure also sheds light on the question of how RNase E might selectively process, rather than destroy, specific RNA precursors. We have also solved the crystal structures of two other components of the degradosome (enolase and polynucleotide phosphorylase), and the cognate complex of enolase with a recognition site from RNase E. These structural data are used to propose a model for the organization and function of the RNA degradosome.

Keywords: gene regulation, RNA processing and decay, ribonuclease

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Crystal Structures of Proteins Involved in Membrane Traffic
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Membrane traffic plays crucial roles in cell functions such as post-translational modification of newly synthesized proteins, exocytosis and endocytosis, receptor recycling, autophagy and lipid transport. Vesicle transport mediates many of these trafficking events using an intricate network of protein-protein interactions of coat proteins, adaptor proteins (AP), cargo receptors, SNARE complexes, small GTPases, ubiquitin and various accessory proteins. I will present our most recent structures of proteins involved in membrane trafficking of proteins and lipids between different organelles: the endoplasmic reticulum, the trans-Golgi Network, endosomes and lysosomes. First, double-sided recognition of ubiquitin molecules by several adaptor proteins will be presented as a recurring structural motif, from the examples of the ubiquitin interacting motif (UIM) of Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate), and the GAT domain of GGA (Golgi-localizing, γ-adaptin ear domain homology, ARF-binding) and others. Second, structures of proteins involved in the first phase of vesicle budding from the ER; a guanine nucleotide exchange factor, small GTPases, and cargo receptors such as yeast Emp46p and Emp47p will be described using examples selected from yeast and plant proteins.

Keywords: X-ray protein crystallography, protein transport, membrane traffic

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The Structure of a Mitochondrial Peptidasome
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The pitrilysin endometalloproteases perform an essential molecular scavenger function in the cell by removing potentially harmful peptides. Especially the insulin-degrading enzyme (IDE) has obtained much attention, in part due to IDE's ability to degrade the degenerative amyloid-ß peptide associated with Alzheimer's disease. Presequence protease (PreP) is an organellar homologue to IDE and was recently identified as a protease responsible for the degradation of targeting peptides in both mitochondria and chloroplasts. The ability of PreP to degrade small, unfolded peptides in mitochondria is of particular interest in light of recent findings, which link amyloid-ß to the mitochondrial toxicity associated with Alzheimer's disease.

The 2.1Å resolution crystal structure of PreP from *Arabidopsis thaliana* represents the first structure from the pitrilysin protease family. The 995-residue polypeptide forms an enclosed chamber of