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

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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.

[1] Ng K.K.S., Kolaktar A.R., Park-Snyder S., Feinberg H., Clark D.A., Drickamer K., Weis W.I., *J. Biol. Chem*., 2002, **277**, 16088-16095. [2] Shrive A.K., Tharia H.A., Strong P., Kishore U., Burns I., Rizkallah P.J., Reid K.B.M., Greenhough T.J., *J. Mol. Biol.*, 2003, **331(2)**, 509-523.
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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