## MS50.P05

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**A Glue and Zipper mechanism upholds the TCR-peptide-MHC interaction**

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The immune system consists of a complex network of cells whose role is to protect the body against a large range of potential invaders or pathogens. Among these cells exists a special team of molecules: one that binds the pathogen particle (MHC) and one that recognises the pathogen particle (TCR) and eliminates the infected cells. Understanding how this team of molecules works may help us to design vaccines and drugs, such as anti-cancer agents, but also to improve the rate of transplant success.

The interaction between T-cell receptor (TCR) and the Major Histocompatibility Complex molecule (MHC) presenting the viral particle (peptide), essential as it is for the immune system to function, operates via an unclear mechanism.

Indeed, The driving force behind this weak TCR-peptide-MHC interaction is elusive so far. In this study we show by combining biophysical, structural and cellular assays, how the hot spot on the MHC molecule are spread in a manner that merely follows the TCRs gaze on the peptide. The structures of three different TCRs specific for the same peptide-MHC complex show that the peptide is the driving force of the TCRs docking, attracting the receptor like "glue", and that the MHC molecule locks the interaction like a "molecular zipper". By doing so, the hot spot on both peptide and MHC are co-localised on the peptide-MHC surface.

**Keywords: immunology, epstein-barr virus, T-cell receptor.**

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**The assembly of c4b-binding protein via its oligomerisation domain**

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C4b-binding protein (C4bp) is a soluble regulator of both the classical and lectin pathways of complement activation capable of controlling C4b-mediated reactions. Inhibition of the innate response by C4bp is achieved via three distinct mechanisms; C4bp possesses cofactor activity for proteolytic cleavage of C4b by factor I and inhibits assembly of the classical and lectin pathway C3 convertase to which it also displays decay-accelerating properties.

Human C4bp is a macromolecular assembly of alpha and beta chains each consisting of eight and three complement control protein (CCP) domains respectively. Regulation of complement is provided by the C4b binding site in CCP's 1-3 of the alpha chain. Assembly into a spider-like structure occurs via separate C-terminal oligomerisation domains with the most common stochiometry reported to be 7α1β. Recently, the C4bp oligomerisation domains from different species have shown great potential in acting as vaccine adjuvants when fused to an antigen.

Presented here are the X-ray structures of the oligomerisation domains from both human C4bp and a chicken homologue showing assembly of discrete, disulphide-bonded species. Also provided is data rationalising the stoichiometry between the alpha and beta chains and discussion of the adjuvant-like effects observed by Ogun *et al*.

**Keywords: C4bp, oligomerisation, structure**

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**Structural and Functional Investigation of the *trans*-encoded HLA-DQ8/2**

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The class II MHC molecule HLA-DQ2 (DQA1*0501-DQB1*0201) and HLA-DQ8 (DQA1*0301-DQB1*0302) confer susceptibility to type I diabetes and to celiac disease. Individuals who are heterozygous for HLA-DQ2 and HLA-DQ8 have a five-fold higher risk of developing type 1 diabetes than those who are homozygous for either HLA-DQ2 or HLA-DQ8 [1]. For celiac disease, such elevated risk for heterozygous individuals is not observed. It has been proposed that HLA-DQ2/HLA-DQ8 heterozygous individuals express disease contributing *trans*-encoded MHC molecules on the surface of antigen presenting cells. A *trans*-encoded MHC refers to a MHC heterodimer where the α-chain and β-chain are derived from HLA genes encoded on different chromosomes [2]. However, relatively little is known about the extent of *trans*-encoded HLA-DQ formation or their immunological function. Here, we report the crystal structure of HLA-DQ8/2 which is a hybrid MHC molecule made up of the HLA-DQ8 α-chain and the HLA-DQ2 β-chain. It is the first atomic structure of a *trans*-encoded HLA-DQ molecule. We also present T-cell data showing differential presentation of a celiac disease associated gliadin peptide by the *cis*-encoded HLA-DQ2 and the *trans*-encoded HLA-DQ8/2.

[1] E. Thorsby, *Hum. Immunol.* **1997**, *53*, 1-11. [2] D. J. Charron, V. Lotteau, P. Turmel, *Nature* **1984**, *312*, 157-159.

**Keywords: celiac disease, trans-encoded, MHC**

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**Crystal Structure of HLA-DQ2.5 in complex with CLIP**

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We have determined the atomic structure of HLA-DQ2.5 in complex with CLIP at 2.7 Å resolution. HLA-DQ2.5 is a class II MHC associated with type 1 diabetes and celiac disease. CLIP (Class II Invariant Chain Peptide) is a fragment of the invariant chain which is

retained in the class II MHC peptide binding pocket until it is replaced by an antigen in the endosome. CLIP serves to stabilize the MHC heterodimer and also prevents binding of endogenous peptides. CLIP-to-antigen exchange, mediated by HLA-DM, is a key determinant of subsequent immunological events. HLA-CLIP stability is thought to be an important factor for triggering autoimmunity [1,2]. We have analyzed the non-covalent interaction between HLA-DQ2.5 and CLIP in order to determine the molecular basis for the unusually long half-life of the HLA-DQ2.5-CLIP complex. This is the first HLA-DQ-CLIP structure to be reported.

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**Keywords: HLA-DQ2.5, autoimmunity, CLIP**

## MS50.P09

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**Structure, function and evolution of the serum pentraxins**

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Wide-ranging studies on the serum pentraxins C-reactive protein (CRP) and serum amyloid P component (SAP) 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. In humans CRP, when bound to a suitable physiological ligand, binds C1q and efficiently activates classical complement.

The structures of pentraxins from man [1,2] and *Limulus polyphemus* [3] reveal variable aggregation of the conserved protomer fold. Unusually LimSAP displays two distinct molecular aggregations for the same molecule, a doubly stacked octamer and a doubly stacked heptamer. Although sequence homology with human SAP is relatively low, structural homology is high. This is due in part to a "topological" equivalence of side chain position. Upon binding phosphoethanolamine, LimSAP binds a third calcium in each subunit, with all three calcium ions contributing to the binding and orientation of the ligand. New structural studies of mammalian, fish and horseshoe crab pentraxins provide further unique insights into not only the evolutionary conservation of an important functional role in immunity, but also into the diversity of molecular aggregation built from a phylogenetically conserved protomer fold.

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**Keywords: pentraxin, innate immunity, protein evolution**

## MS50.P10

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**Structural basis of recognition of pathogen-associated molecular patterns by pgrp-s**

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Peptidoglycan recognition proteins (PGRPs) are involved in the recognition of pathogen-associated molecular patterns (PAMPs). The well known PAMPs include lipopolysaccharide (LPS) from Gram-negative bacteria and lipoteichoic acid (LTA) from Gram-positive bacteria. PGRP-short (PGRP-S) has been isolated from the mammary secretions of camel (CPGRP-S). It binds LPS and LTA with affinities of $1.6\times10^{-9}$ M and $2.4\times10^{-8}$ M respectively. The crystal structures of CPGRP-S complexes with LPS and LTA revealed that both compounds were held tightly inside the CPGRP-S tetrameric complex consisting of molecules A, B, C and D. The binding cleft is formed at the interface of molecules C and D which is extendable to the interface of A and C. The interface of molecules A and B is tightly packed while that between B and D forms an open channel. The hydrophilic moieties of these compounds occupy a common region while hydrophobic chains interact with distinct regions in the binding site. The flow cytometry studies showed that both LPS- and LTA-induced expressions of pro-inflammatory cytokines, TNF-α and IL-6 were inhibited by CPGRP-S. The results of animal studies using mice models indicated that both LPS- and LTA-induced mortality rates decreased drastically when CPGRP-S was administered. The recognition of both kinds of PAMPs from Gram-negative and Gram-positive bacteria, their high binding affinities to CPGRP-S, the significant decrease in the productions of LPS- and LTA-induced TNF-α and IL-6 on introduction of CPGRP-S and the drastic reductions in mortality rate in mice models by CPGRP-S suggest that CPGRP-S may be exploited as a common antibiotic agent for the welfare of mankind. This is particularly significant as there is an alarming rise in the incidence of bacterial resistance to known antibiotics. This also brings the amino acid sequence of CPGRP-S in focus particularly the presence of residues, Pro96 and Pro151 at one of the interfaces and the absence of three N-terminal residues and Cys8 as compared to human PGRP-S. So far in the family of PGRP-S, such a homotetrameric complex has been observed only for CPGRP-S.

**Keywords: PGRP, PAMPs, LPS**

## MS50.P11

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**Structural studies of macrophage proteins using UQSG pipeline**

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Macrophages are cells differentiated from circulating blood monocytes that represent the first line of defense against pathogen invasion. Macrophages are widely distributed throughout the body and are particularly abundant at the route of pathogen entry. They play a critical role in immune defense by initiating, promoting, preventing, suppressing or terminating immune responses.

We established a high-throughput pipeline at the University of Queensland to investigate the structures and functions of novel macrophage proteins [1]. My project began with the selection of 12