

Keywords: phenazine biosynthesis, domain swapping, substrate channelling

FA1-MS12-P06

The secretome of parasitic nematodes: Analysis of host-parasite cross-talk. Markus Perbandt^{a,c}, Raphael Eberle^a, Kai Lüersen^b, Eva Liebau^b, Christian Betzel^a.

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We analyse functional macromolecular systems in filarial nematode worms causing major infectious diseases like river blindness and lymphatic filariasis. These helminths parasitize for long periods their immunocompetent hosts, provoking only restricted pathology in their tissue habitats although the pathogens can accumulate to large assemblies. Because of their size, helminths cannot sequester in niches like many bacteria, fungi and protozoa. By co-evolution, these “king-sized” pathogens have developed other strategies to secure survival. Recent research on the interaction between helminths and vertebrate hosts has provided information and insights about evasion, immunomodulation and protection mechanisms [1]. Here, molecules are most important that are secreted into the host tissue or are associated with the surface of the parasite. They represent the first molecules exposed to and affecting the host immune apparatus and are thus likely to be involved in the establishment and maintenance of the parasite within the host and in the avoidance, modulation and skewing of the host immune response. Immune modulation is suggested to be beneficial to both, the human host and the parasite, as it protects the worm from being eradicated, and at the same time protects the host from excessive inflammatory responses that may lead to tissue damage.

We analyse the composition of excretory-secretory products (ESPs) and selected surface proteins of parasitic nematodes, since these are likely to present the principle players in parasite-host cross-talk and have the capacity to actively shape the immunological environment [2,3].

We presently analyse the structures of all important and identified key proteins and here we present the structural insights of the first targets like the OvGST1 from *Onchocerca volvulus*, the causative agent of river blindness.

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Keywords: river blindness, immunomodulation, host-parasite cross-talk

FA1-MS12-P07

Crystal structure of *Enterococcus faecalis* Thymidylate synthase. C. Pozzi^a, M. Benvenuti^a, S. Ferrari^b, R. Luciani^b, A. Catalano^c, R.M. Stroud^d, M.P. Costi^b, S. Mangani^{a,m} ^aDepartment of

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Thymidylate synthase (TS) is an enzyme that catalyzes the reductive methylation of 2'-deoxyuridine 5'-monophosphate (dUMP) to thymidine 5'-monophosphate (dTMP), using the cofactor 5,10-methylene-5,6,7,8-tetrahydrofolate (mTHF) as a one-carbon donor and reductant [1]. This reaction is the only the *novus* source of thymidylate for the cells [2]. The inhibition of TS leads to pronounced changes in cellular protein and RNA, cessation of DNA replication and eventually cell death [3]. Because of its critical function, considerable effort has been focused on the design of TS inhibitors for the treatment of cancer [4]. Less attention has been directed towards the design of species-specific TS inhibitors aimed at treating diseases caused by bacterial, fungal or opportunistic pathogens. However, taking into account the rise in antibiotic resistant bacteria, the relative toxicity of treatments for fungal infections and the poor therapies available for several opportunistic infections in immunocompromised patients, the successful development of pathogen-specific TS inhibitors may offer an important alternative to current antibiotic, antiparasitic and antifungal drugs [5,6]. TS enzymes show an highly conserved structure, but some differences of the active site may be exploited for the design of inhibitors able to discriminate pathogen TSs vs. human TS [7].

In the effort to increase our knowledge about bacterial TS, we have determined the 2.18 Å crystal structure of *Enterococcus faecalis* TS (EfTS), which is the first structure determination of this enzyme, as prepared and in complex with a specific inhibitor.

EfTS is a homodimer showing subunit heterogeneity as only one of the subunits brings bound a derivative of the tetrahydrofolate intermediate of the catalyzed reaction. The binding of the inhibitor is accompanied by large rearrangement of the protein, determining the ordering of a mobile loop that appears disordered in the inhibitor free subunit. The designed inhibitor replaces the naturally occurring inhibitor while leaving the other subunit free.

The determination of the two crystal structures provides interesting clues about the catalytic mechanism of EfTS and its inhibition that might be relevant for the rational design of more powerful and selective inhibitors towards bacterial targets.

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Keywords: macromolecular crystallography, drug design, thymidylate synthase, drug discovery and design

FA1-MS12-P08

Binary complex of 14-3-3 σ /p53 pT387-peptide and implications for stabilization. Benjamin Schumacher, Justine Mondry, Philipp Thiel, Michael Weyand^a, Christian Ottmann^{a,*}. *Chemical Genomics Centre of the*

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In its active form p53 exists as a tetramer and is known to be a tumor suppressor protein with cell cycle checkpoint control function [1]. The adapter protein 14-3-3 σ binds to p53 and stabilizes the functional tetramer and thereby enhances anti-tumor activity. Several binding sites for 14-3-3 proteins have been identified in the C-terminus of p53 [2]. We could solve the crystal structure of the C-terminus of p53 (residues 385-393) in complex with 14-3-3 σ at a resolution of 1.2Å. The accommodation of the peptide in the 14-3-3 binding pocket implies a starting point for discussion of binding of 14-3-3 σ to the active p53 tetramer and its stabilization. Furthermore the structure reveals the existence of a pocket for small molecules which could be used to stabilize the 14-3-3/p53 interaction and which could be used as a possible starting point for therapeutic intervention.

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Keywords: 14-3-3 protein, Stabilizing molecule, protein-protein-interaction

FA1-MS12-P09

Recognition of the CD1d-Alpha-GalactosylCeramide analogues by the NKT T Cell Receptor. Kwok S. Wun^a, Siew S Pang^a, Garth Cameron^b, Onisha Patel^a, Daniel G Pellicci^b, James McCluskey^b, Dale I Godfrey^b, Steven A Porcelli^c, Jamie Rossjohn^a. ^aDepartment of Biochemistry and Molecular Biology, Monash University, Clayton, Australia. ^bDepartment of Microbiology and Immunology, University of Melbourne, Parkville, Australia. ^cDepartment of Microbiology and Immunology, Department of Medicine, Albert Einstein College of Medicine, Bronx, USA.

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Unlike the Major Histocompatibility molecules (MHC), CD1d molecule is suited to capture and present lipid-based antigen for T cell recognition [1]. One of the diverse range of lipids that CD1d can present includes the potent immune stimulator glycolipid: α -galactosylceramide (α -GalCer) consisting of a galactose sugar head group connected by two lipid tails. This CD1d- α -GalCer molecule can be recognised by a unique class of T cells, termed Type I NKT cells that expresses T cell receptor (TCR) encoding an invariant α chain and a restricted β chain repertoire. Through the crystal structure of the NKT TCR-CD1d- α -GalCer complex, it can be observed that the NKT TCR recognises CD1d- α -GalCer in a very distinct manner when compared to any other TCR-peptide-MHC (TCR-pMHC) complexes [2]. More specifically, the NKT TCR docks the CD1d- α -GalCer molecule in a parallel conformation with its V α domain contacting both the α 1 and α 2 helices of CD1d, a phenomenon that is not observed in any TCR-pMHC complexes. Using the crystal structure as a guide, an alanine scanning mutagenesis of the residues on the NKT TCR and CD1d molecule as well as the use of different α -GalCer analogues enabled the minimal binding requirement of CD1d- α -GalCer restriction to be defined [3]. Collectively,

these results highlight the fundamental differences of the way the immune system recognises peptide and lipid-based antigens. The current focus of the project involves the use of different α -GalCer analogues that the NKT cells can recognise. Importantly, these analogues have been tested *in vivo* to be shown to induce bias cytokine responses, thus, illustrating the potential of using these analogues for future immuno-drug therapy. Here I shall present recent findings pertaining to the recognition of these α -GalCer analogues.

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Keywords: structural immunology, T cell receptors, lipids