**PS04.15.23 CRYSTALLOGRAPHIC STUDIES OF MUTANTS OF THE CHEMOKINE MACROPHAGE INFLAMMATORY PROTEIN-1α.** J. Maclean[a], N. W. Isaacs[a], G. J. Graham[b], [a]Dept. of Chemistry, Glasgow University, Glasgow, Scotland, [b]Beatson Institute for Cancer Research, Glasgow, Scotland

Macrophage Inflammatory Protein 1, a member of the CC family of chemokines, has been isolated in two forms, namely MIP-1α and MIP-1β. Both are low mw proteins ($M_r$ 8kD) which form aggregates of mw up to 100 kD at high ionic strength and neutral pH. MIP-1α, a potent inhibitor of haematopoietic stem cell proliferation *in vitro,* is of potential use in cancer therapy.

Although the NMR structure of MIP-1β showed that protein to exist as a discrete dimer at pH values below 3.5[1], the propensity of these proteins to aggregate under physiological conditions had made crystallographic analyses impossible. On the assumption that the interactions giving rise to the aggregation were electrostatic, the self-aggregation potential of MIP- 1α was removed by systematically neutralising acidic residues in the carboxy-terminal region of the protein. Thus a single mutation (66 Glu → Gln) gave rise to a stable tetramer (PM1) of mw 32 kD. A double mutation (66 Glu → Gln; 64 Asp → Asn) gave a stable dimer (PM2) of mw 16 kD. Finally, a stable monomer (PM3) was obtained when three residues were mutated (66 Glu → Gln; 64 Asp → Asn; 60 Glu → Gln).

The structures of all three mutants have been determined by X-ray crystallography. PM3 was solved by SIRAS and the structures of PM2 and PM1 were solved by Molecular Replacement using the refined PM3 coordinates as a search model. The crystal packing is found to be identical for PM2 and PM3, and the dimer thus formed is similar to that of MIP- 1β. The structure of the tetramer shows how the predicted interactions could give rise to aggregation of the molecules.

Lodi, P. J. *et al,* Science, Vol 263, 1994, pl762-1767.

**PS04.15.24 PRELIMINARY X-RAY DIFFRACTION ANALYSIS OF AN ANTIBODY FRAGMENT AGAINST P-GLYCOPROTEIN.** Valerie Monem, Sona Vasudevan, Kathy L. Johns and David R. Rose, Ontario Cancer Institute, Department of Medical Biophysics, University of Toronto, Canada.

P-glycoprotein (P-gp) has been shown to be involved in the development of drug resistance in cancer cells, thus impeding successful chemotherapy treatment. P-gp is predicted to consist of twelve membrane-spanning domains and is thought to act as an ATP-dependent drug pump with specificity for a broad range of structurally unrelated drugs[1]. The structure of P-gp is as yet unknown. We are using monoclonal antibodies as one approach to obtaining structural information about P-gp. The Fab portion of the antibody C219 that recognizes both cytoplasmic ATP-binding domains of all known P-gp molecules[2] was crystallized with and without its peptide epitope. The unliganded Fab crystallized in the monoclinic space group $P2_1$ with 4 molecules in the asymmetric unit and data were collected to 3.2 Å resolution. Previous self-rotation analysis suggested the presence of a two-fold non-crystallographic symmetry (NCS)[3]. Subsequent analysis was done using molecular replacement methods. The super-imposed structures of several Fabs with a wide range of elbow-bends were used as starting probes. The program AMORE showed the same four strong rotation and translation peaks with two of the probes against data from 15 to 4 Å resolution. Rigid-body refinement was executed by X-PLOR, allowing for adjustment of the elbow-bend and of the heavy and light chains independently for each molecule. Only two of the four molecules in the asymmetric unit were found to be non-crystallographically related by 180°. The unrefined electron density maps support the molecular replacement solutions. Further analysis will include the exploitation of the two-fold NCS with averaging techniques. The crystals of C219 with bound peptide are orthorhombic and belong to the space group $P2_12_12_1$, most likely with 4 molecules in the asymmetric unit (42% solvent content). However, these crystals did not diffract well enough for thorough crystallographic analysis. Better crystals are being grown using seeding techniques in preparation for synchrotron data collection. Supported by NSERC and NCI (Canada).

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**PS04.15.25 THE MOLECULAR ARCHITECTURE OF TWO MICROBIAL SUPERANTIGENS** A.C. Papageorgiou[1], H.S. Tranter[2], R.D. Brehm[2] & K.R. Acharya[1]. [1]School of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK. [2]Developmental Production Department, Production Division, Centre for Applied Microbiology and Research, Porton Down, Salisbury SP4 OJG, UK.

Staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1) are a family of structurally related proteins that are produced by *Staphylococcus aureus* and have dramatic effects on the immune system. Studies with SEs and TSST-1 have revealed these to be highly polyclonal activators of T-cells in the presence of cells having class II antigens of the major histocompatibility complex (MHC class II). These toxins act at nanomolar concentrations and are not processed internally like with normal antigens. Each toxin type is capable of stimulating subpopulations of peripheral T lymplocytes possessing a specific variable β (Vβ segment of the T-cell receptor (TcR) and have been classified as superantigens. We have determined the 3D crystal structure of TSST-1 and SEC2 at 2.5Å and 2.0Å resolution, respectively (Acharya *et al.*, 1994; Papageorgiou *et al.*, 1995). The overall architecture of these molecules is similar to other known superantigen structures, namely SEB (Swaminathan *et al.*, 1992) and SEA (Schad *et al.*, 1995). A detailed comparison of the different modes of MHC class II and TcR binding adapted by this family of molecules will be presented.

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**PS04.15.26 THE MOLECULAR STRUCTURE OF THE LIPOAMIDE DEHYDROGENASE DOMAIN (DLDH) OF A SURFACE ANTIGEN FROM NEISSERIA MENINGITIDIS** Lucile Pernot[1], Inés Li de la Sierra[1,2] , Thierry Prangé[1,3], Pedro Saludjian[3], Marc Schiltz[1], Roger Fourme[1], Gabriel Padrón[2] [1]LURE , Bâtiment 209, Université Paris-Sud, 91405-ORSAY CEDEX, France ;[2]CIGB, ave 31 entre 156 y 190, Cubanacan, La Habana, Cuba, [3]Chimie Biomoléculaire (URA 1430 - CNRS), UFR-Biomédicale, 93012-BOBIGNY CEDEX, France

The domain involves the major antigenic determinant of P64k, a protein from the surface of the Nesseria Meningitidis bacteria has been localized in the X-ray structure at 2.9Å resolution. The P64k is a two domain protein comprizing a dihydrolipoamide dehydrogenase (DLDH) domain of c.a. 500 residues, and a smaller lipoic acid binding domain of 120 residues, connected together by a linker of twenty hydrophobic residues. The first domain involves a FAD prosthetic group and a NADH cofactor. The lipoic acid binding domain was easily cleaved and therefore was not observed in the X-ray structure. The structure of the DLDH domain was solved by a combination of molecular replacement (MR) and multiple isomorphous replacement (MIR) techniques using a TAMM (mercury) and a xenon derivatives.