PS04.15.11 CRYSTAL STRUCTURE OF MFE-23, A CLINICALLY IMPORTANT ANTI-CEA SCFV ANTIBODY FRAGMENT, AT 0.28 NM RESOLUTION. M. K. Boehm[1], A. L. Corper[2], T. Wan[2], M. Sohi[2], B. J. Sutton[2], J. D. Thornton[1], P. A. Keep[1], R. H. J. Begent[1], S. J. Perkins[1], [1]Depts. Biochemistry Molecular Biology, and Clinical Oncology, Royal Free Hospital Sch. Med., Rowland Hill St., London NW3 2PF, UK, [2]Randall Institute, King's College London, 26-29 Drury Lane, London WC2B 5RL, UK

MFE-23 is a single-chain Fv (scFv) antibody fragment that was selected from a phage-display library for high affinity binding to carcinoembryonic antigen (CEA), and has important clinical uses in the detection, monitoring and targetting of colon cancer. CEA is a seven-domain cell adhesion molecule which is expressed at high levels on colon carcinoma cells. MFE-23 (M_r 27,150; 256 residues) consists of an N-terminal V_H domain, a 15-residue linker, a V_L domain and a 12-residue tag. MFE-23 was expressed in E. coli and purified on CEA coupled to Sepharose. Crystals were produced by the hanging-drop method. MFE-23 at 2 mg/ml was mixed 1:1 with precipitant (100 mM Tris-HCl at pH 6.5 containing 45% saturated ammonium sulfate), and a 10 µl drop of this mixture was equilibrated against 0.5 ml of precipitant at 18°C. Well-formed crystals formed within several days, and diffracted to 0.2 nm resolution. Diffraction data were collected using an R-AXIS-IIC mounted on an RU200 rotating anode X-ray source. The crystal belongs to the trigonal space group $P3_221$ and has unit cell dimensions of a = b = 6.170 nm and c = 12.794 nm. A single crystal was used for structure determination. Data to a resolution of 0.28 nm were processed using DENZO and the CCP4 program package. The structure was solved by molecular replacement using the Fv coordinates from a murine IgA Fab fragment (Brookhaven code 2FBJ). The antibody complementarity determining regions, the linker and the tag were omitted for the calculation of initial 2Fo-Fc electron density maps. Refinement of the MFE-23 structure is currently in progress, utilizing the rigid-body refinement and the positional refinement algorithms of X-PLOR and manual rebuilding of the model in O.

PS04.15.12 ARE "HOT SPOTS" [SUPER-FLEXIBLE REGIONS] ON THE SURFACE OF AN ANTIGEN ESSENTIAL FOR ANTIGEN-ANTIBODY BINDING? Gerson H Cohen, David R Davies, National Institutes of Health, NIDDK/LMB, Bethesda, MD 20892 USA

The lysozyme molecule has been studied crystallographically in complex with a number of Fv and Fab antibody fragments. In these complexes the structure of the lysozyme molecule shows significant changes at one or more of three points in its sequence. The Ca positions at residues 47, 70-71 and 101-103 of lysozyme in one complex differ from the corresponding position in a second by 1-2 Å on the average and by as much as 7 Å. The average general difference in Ca positions is 0.5-0.75 A. This main chain flexibility demonstrated by bound lysozyme is found in the published structures of native unbound lysozyme and is not merely a distortion resulting from the association with antibody fragments. In all of the complexes reported at least one of these three regions is partially buried as a result of complex formation. In an attempt to determine whether this flexibility is required for antibody binding or is merely coincidental, we examined neuraminidase and its complexes with NC41 and NC10. The neuraminidase structures do not exhibit the flexibility shown by lysozyme; the maximum difference noted in the comparison of the neuraminidases is only 1Å. Neuraminidase does not support the requirement of hot spots in the antigen-antibody interface.

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PS04.15.13 THE HIGH RESOLUTION STRUCTURE OF AN ANTI-hCG FAB. Constantina Fotinou[1], Jeremy Beauchamp[1], Annemarie deHaan[2], Ebo Bos[2] & Neil W. Isaacs[1]. [1]Dept. of Chemistry, University of Glasgow, Glasgow. G12 8QQ U.K.; [2]Dept. of Biotechnology & Biochemistry, N.V. Organon, Molenstraat 110, PO Box 20, 5340 BH, Oss, The Netherlands.

The structure of Fab3A2 - an anti-human chorionic gonadotropin (hCG) antibody - has been solved to 2.0Å resolution, with data collected to 1.4Å resolution.

hCG is a hormone essential for the maintenance of the early stages of pregnancy. It is a member of the glycoprotein hormone family which includes follicle stimulating hormone (FSH), luteinising hormone(LH) and thyroid stimulating hormone (TSH), which have a common α-subunit. The biological specificity being determined by the β-subunit. A high degree of sequence similarity exists, with hCG and LH binding to a common receptor. hCG is unique in having a C-terminal extension on the β-subunit. This C-terminal peptide of hCG has been used in WHO sponsored research to produce an anti-fertility vaccine. As some forms of cancer secrete hCG, specific antibodies against the hormone can be used as immunodiagnostics.

Antibody 3A2 is an hCG C-terminal specific antibody. The structure of the Fab fragment (Fab3A2) has been solved to 2.0Å resolution. Data were collected at Daresbury SRS under cryocooled conditions (100K). The structure was solved by molecular replacement using AMoRe(Navaza, (1994) *Acta Cryst. A*, **50**, 157-163). After some refinement, the crystallographic R-factor is 22.3%. A high resolution dataset has recently been collected to 1.4Å which is currently being used to refine the structure further. Comparison of the complementarity determining regions (CDRs) of Fab3A2 with those from the Macromolecular Structures Database reveals a mainchain conformation consistent with the canonical structure hypothesis (Chothia *et al.*, (1994) *Nature*, **342**, 877-883).

PS04.15.14 CHARACTERIZATION OF CHEMICALLY SYNTHESIZED HYBRID CXC CHEMOKINES. Tai Y. Fu, Yu Wai Chen, Yaoguang Luo, Ian Clark-Lewis and Gary D. Brayer, Department of Biochemistry and Molecular Biology, and the Protein Engineering Network of Centres of Excellence, 2146 Health Sciences Mall, University of British Columbia, Vancouver, BC, Canada V6T 1Z3

Interleukin-8 (IL-8) is a chemoattractant cytokine (chemokine) that stimulates the accumulation and activation of neutrophil leukocytes at sites of inflammation. This small protein (72 residues) has been linked to a wide variety of inflammatory events and is thought to be the causitive agent in their associated immuno-pathology. IL-8 is one of a family of related proteins termed the CXC chemokines that have four conserved cysteines, with the first two separated by one amino acid. Interferon-γ inducible peptide-10 (IP-10; 74 residues) is also a member of the CXC chemokine family and is expressed by a variety of cell types on induction with interferon-γ and lipopolysaccharide. Although IL-8 and IP-10 share 24% primary sequence identity, they appear to have very different physiological roles. IP-10 does not compete with IL-8 for binding to neutrophil receptors and has unique properties associated with T-cell mediated inflammatory reponses and host-mediated anti-tumor effects. To characterize the components of IL-8 that are responsible for its specific functions, four IL-8/IP-10 molecular hybrids were chemically synthesized in which portions of the sequence of IL-8 were replaced with the corresponding IP-10 sequence to determine the resultant functional and structural changes induced. Two of these hybrid CXC chemokines have activities comparable to wild-type IL-8, while the other two