MS04.14.07 AFFINITY MATURATION OF ANTIBODIES *in vivo* AND *in vitro*. Cara Marks[1], Kim Henric[2], Greg Winter[3], Ray Stevens[4], Thomas Simon[5], and James D. Marks[6]. Struct. Biol. Div., Lawrence Berkeley Laboratory, Univ. of Calif., Berkeley[1], M.R.C.-Mill Hill, U.K[2], L.M.B., Cambridge, U.K[3]. Dept. of Chem., Univ. of Calif.,Berkeley[4]. E.L.I.A.S.,Freiburg,Germany[5], Dept. of Pharm. Chem., Univ. of Calif., San Francisco[6].

During the secondary and tertiary immune response, the humoral immune system produces high affinity antibodies by mutating the variable region genes (V) of lower affinity antibodies (Ab). Structural analysis of low and high affinity antibodies indicates these mutations predominantly yield changes in residues that do not contact antigen. Indeed, *in vitro* mutagenesis of contact residues to increase antibody affinity has been largely unsuccessful. To analyze how changes in non- contact residues could result in increased affinity, we determined the atomic structures of an antibody that binds a large protein antigen and a second antibody that recognizes a small hapten. In both Abs, the somatic mutations were located in residues that do not tend antigen but rather contribute indirectly to the rigidity of the antigen binding site. Examination of the 2.0Å structure of 48G7(Fab) -para-nitrophenyl phosphonate complex indicates that none of the nine somatic mutations that contribute to a 10^4 increase in affinity contact this transition state analog. Similarly, the 2.0Å structure of FvRS1, a D1.3 variant which binds hen egg lysozyme with 8 fold higher affinity indicates that none of the somatic mutations or the mutations generated *in vitro* contact the protein antigen. Comparison of additional high affinity antibodies to models of their germline antibodies also reveal an antibody combining site composed of a well packed, easily perturbed network of residues that is stabilized by distal somatic mutations. Low frequency random mutagenesis of residues in this combining site is likely to perturb the synergy, and accounts for the largely unsuccesful *in vitro* results. In contrast, an increase in affinity could be obtained by drastically altering the binding site by simultaneous mutation of multiple residues coupled to a powerful selection scheme.

MS04.14.08 X-RAY STRUCTURE OF INSULIN MUTANTS GLYB20GLN AND GLYB8SER-GLUB13GLN: ROLES OF GLYCINE IN THE STRUCTURAL STABILITY Zhiping Yao, Yonglin Hu, Dacheng Wang, Institute of Biophysics, Chinese Academy of Sciences

Two crystal structures of insulin mutants whose wild type glycine were substitute by with side-chain residues have been determined at high resolution. With the characteristic of not having sidechain, glycine plays an important role in protein 3D structure: it usually appears at the subtle position of peptide folding. and it is often highly conservative in evolution. Thus the engineered protein of glycine substitution becomes a helpful tool to investigate the function of glycine to protein conformation. Two kinds of insulin mutant, GB20Q and GB8S-EB13Q, were obtained, whose biological activity is badly decreased. It is always glycine that appears at B8 and B20 sites of wild insulin in all mammals found so far, hence great importance comes naturally for determining the structure of glycine mutant substituted by long sidechain residues. B20Q and B8S-B13Q Human Insulin mutant are both in orthorhombic crystals with the space group $P2_12_12_1$. Reflection data was collected on synchrotron radiation with Sakabe's Weissenburg Camera System in Photon Factory, Japan. The structure were solved by Molecular Replacement method, and were well refined by X-PLOR at 1.8Å and 1.6Å resolution, respectively. The final R-factor is 0.190 and 0.183, while the bond and angle RMS deviations are 0.017Å, 2.535° and 0.016Å, 2.281°, respectively. Substituted residues have clear density in Fo-Fc map. No big conformation changes were found, while the dimmer similar to that in 2Zn insulin could still be formed. The main chain angles (Phi and Psi) of B8 and B20 are located in the unfavorable area of Ramachandran plots, in an unstable state with high local energy. The possible relation between the structure change caused by substitution of sidechained residue for glycine and the decrease of its biological activity will be discussed with the comparison to different kinds of wild type insulin.

PS04.14.09 A COMPARISON OF CRYSTAL STRUCTURES OF ENGINEERED ANTIBODY FRAGMENTS. M J Banfield, D J King* & R L Brady. Dept of Biochemistry, University of Bristol BRISTOL BS8 1TD. UK; *Celltech Therapeutics Ltd., 216 Bath Road, Slough SL1 4EN.

The 3-dimensional structure of the Fab-fragment from the murine monoclonal antibody A5B7 which binds Carinoembryonic antigen (CEA), a protein expressed on carcinoma cell surfaces has been determined to a resolution of 2.1 Å. Forms of this antibody have potential application in the treatment of colerectal cancer (Lane et al., 1995). A5B7 has been the subject of extensive protein engineering studies to produce engineered human constructs that retain high antigen binding. However, although less immunogenic than the murine form, these constructs often have reduced antigen binding, and hence reduced efficacy.

In addition to the murine form of A5B7 we have determined the crystal structure of a engineered human construct that retains ~50% binding affinity. A comparison of the binding site of the two forms shows small changes, some of which might be explained by packing in a different crystal lattice, although others partially explain the observed alterations in antigen affinity. A further engineered human construct of A5B7 that retains 100% antigen binding affinity is also under study.

A second engineered human antibody, derived from the mouse antibody CTM01, which binds to Polymorphic Epithelial Mucin (Muc1), is also being investigated. For this antibody we have a peptide mimic of the antigen and hope to analyse the structure of this Fab-fragment in both native, and peptide-bound forms.

This series of Fab crystal structures provides a unique opportunity to examine the molecular consequences of antibody humanisation, and further our understanding of protein engineering in general.

Lane, D.M., Eagle, K.F., Begent, R.H.J., Hope-Stone, L.D., Green A.J., Casey J.L., Keep, P.A., Kelly, A.M.B., Lederman, J.A., Glaser, M.G. & Hilson A.J.W. (1994) British Journal of Cancer 70, p.521-525.

PS04.14.10 X-RAY STRUCTURES OF A DESIGNED BINDING SITE IN TRYPSIN-ECOTIN COMPLEX SHOW METAL-DEPENDENT GEOMETRY Linda S. Brinen‡, W. Scott Willett§, Charles S. Craik§ and Robert J. Fletterick‡. ‡Department of Biochemistry and Biophysics, §Department of Pharmaceutical Chemistry, University of California at San Francisco, San Francisco, CA 94143

The three-dimensional structures of complexes of trypsin N143H, E151H bound to ecotin A86H are determined at 2.0 Å resolution *via* X-ray crystallography in the absence and presence of the transition metals Zn^{2+}, Ni^{2+}, and Cu^{2+}. The binding site for these transition metals was constructed by substitution of key amino acids with histidine at the trypsin-ecotin interface in the S2'/P2' pocket. Three histidine side chains, two on trypsin at positions 143 and 151, and one on ecotin at position 86, anchor the metals and provide extended catalytic recognition for substrates with His in the P2' pocket. Comparisons of the three-dimensional structures show the different geometries that result upon the binding of metal in the engineered tridentate site and suggest a structural basis for the kinetics of the metal-regulated catalysis. The structural results indicate that the geometry of the engineered metal binding