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

site is dictated by preferred geometry of the metal ion and not the structural constraints of the surrounding protein. This finding, which could not be adequately predicted by modeling studies done on this system, is key to the design of a metal binding site. Of the three metals, the binding of zinc results in the most favorable binding geometry, not dissimilar to those observed in naturally occurring zinc-binding proteins. This work represents the first successful X-ray crystallographic investigation of a *de novo* engineered metal binding site in the absence and presence of metal ions.

PS04.14.11 X-RAY CRYSTALLOGRAPHIC STUDIES OF COLLAGEN-LIKE PEPTIDES. Rachel Kramer[1], Jinsong Liu[1], Jordi Bella[3], Manju Venugopal[2], Patricia Mayville[1], Barbara Brodsky[2], Helen M. Berman[1]. [1]Department of Chemistry, Rutgers University, Piscataway NJ 08855, [2]Department of Biochemistry, University of Medicine and Dentistry of New Jersey, Piscataway NJ 08855, and [3]Present address: Department of Biological Sciences, Purdue University, West Lafayette IN 47907.

Peptide models have proved extremely useful in the elucidation of the structure of collagen and the triple-helical motif. We have crystallized three new triple-helical peptides and will report the results of the structural analyses of these collagen-like peptides. One of these peptides is a homotrimer in which 12 residues from human type III collagen are embedded. This imino acid-poor region is located near the unique collagenase cleavage site and contains a glycine residue known to be the site of a lethal Gly→Ser mutation. This structure could potentially help resolve issues of interchain hydrogen bonding in imino acid-poor regions. Another of the peptides is missing a hydroxyproline at the center of the triple helix, therby interrupting the repeating Gly-X-Y pattern. This omission models a type of break that occurs frequently in nonfibrillar collagens and is found to a lesser extent in noncollagenous triple-helical proteins, such as C1q and mannose binding protein. It is possible that such interruptions may be important to molecular structure or supramolecular association. A third peptide containing lysine and glutamic acid was synthesized to examine the effect of a pair of adjacent charged residues on a triple helix and the role electrostatic interactions play in triple-helical conformation.

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PS04.14.12 STRUCTURES OF AN ENGINEERED BLOOD SUBSTITUTE AND INSIGHTS INTO HEMOGLOBIN FUNCTION. Kenneth S. Kroeger and Craig E. Kundrot. Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215, USA

The deoxy and cyanomet structures of the potential blood substitute rHb1.1 reveal a new quaternary structure for hemoglobin and demonstrate the importance of small conformational changes far from the hemes and the allosteric interface. rHb1.1 is produced by Somatogen, Inc. and contains four changes relative to human hemoglobin A_o: a glycine that covalently joins the two α-chains, the naturally occuring Hb Presbyterian mutation (Asnβ108→Lys), and Val1→Met in the α- and β-chains. The glycine bridge forces cyanomet rHb1.1 (determined at 2.6 Å resolution) to adopt a previously unobserved quaternary structure, bringing the number of observed ligated quaternary structures to three. The overall structure of the deoxy rHb1.1 at 2.0 Å resolution is very similar to deoxy human hemoglobin A_o. The Asnβ108→Lys mutation, however, produces a new hydrogen bond in the relatively rigid $\alpha_1\beta_1$ interface which does not form in the cyanomet structure. Thus, this mutation stabilizes the deoxy state relative to the ligated states and demonstrates the importance of small conformational changes in the $\alpha_1\beta_1$ interface which is often incorrectly regarded as rigid.

The plurality of high oxygen affinity forms of hemoglobin contrasts with the uniqueness of the low affinity form and suggests an important rule for allosteric proteins: one functional state is achieved only within a particular, well-defined structure (T for hemoglobin, high k_{cat} for an enzyme) while the other ("R" for hemoglobin, low k_{cat} for an enzyme) can be achieved by many structures. Mutations are more likely to affect the functional properties of the former state than the latter.

PS04.14.13 STRUCTURE DETERMINATION OF THE COMPLEX BETWEEN DIPHTHERIA TOXIN AND ITS RECEPTOR. Gordon V. Louie, Walter Yang, Marianne E. Bowman, Senyon Choe, Structural Biology Laboratory, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037

The first step in the cytotoxic action of diphtheria toxin (DT) is binding of the toxin molecule to the surface of a susceptible cell. The cellular receptor for DT binding is the membrane-anchored precursor of heparin-binding epidermal growth factor (HBEGF). We have determined the crystal structure of a 1:1 complex between DT and a soluble fragment of HBEGF. HBEGF in the complex adopts the typical EGF-like fold, with its principal ß-hairpin packed snugly against the face of a ß-sheet in the receptor-binding domain of DT. The central portion of the ~1100 Å^2 interface is predominantly hydrophobic; eleven hydrogen bonds are formed between the two molecules around the periphery of the interface.

Our structural information on the atomic interactions between DT and HBEGF is providing a basis for designing mutations that will alter the binding specificity of DT. The long-term objective is an engineered toxin that will recognize heregulin, another member of the EGF-family. Heregulin is overexpressed in some cancerous cells, and also acts as the activating ligand for the HER4 receptor, which is overexpressed in breast carcinoma cells. A heregulin-specific DT may be a useful therapeutic agent for the inhibition of growth of breast cancer cells.

Crystals of the DT-HBEGF complex belong to space group $C222_1$, with unit cell dimensions a=88.84, b=103.19, c=126.52 Å and a single copy of the complex in the asymmetric unit. The position and orientation of DT were determined by molecular replacement, with X-ray data measured on an image plate detector and a rotating anode source. Subsequently, the HBEGF portion of the complex was built into difference density. The current atomic model contains the entire DT molecule, and 40 amino-acid residues of HBEGF. It has been refined to an R-factor of 0.224 for all reflections in the resolution range 10-2.6 Å.

PS04.14.14 PROTEIN ENGINEERED HINGED GATE OPENS A CHANNEL TO AN ARTIFICIAL CAVITY. Duncan E. McRee*, Melissa M. Fitzgerald, Rabi A. Musah, and David B. Goodin, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037

Conformational changes in the structures of proteins which gate the access of substrates or ligands to an active site are important features of enzyme function. We describe an unusual example of a structural rearrangement near a buried artificial cavity in cytochrome c peroxidase (Fitzgerald et al., 1994) upon binding of a positively charged benzimidazole that opens a channel to the buried cavity and apparently represents the entry of ligands to the buried cavity. A hinged rotation at two residues, Pro-190 and Asn-195, results in a surface loop rearrangement that opens a large solvent accessible channel to an otherwise inaccesible binding cavity. High resolution crystal structures have allowed detailed characterization of this rearrange-