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.

Research supported by NIH grants GM21589, AR19626 and a Molecular Biophysics Training Grant.

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-

ment which includes a cis-tran proline isomerization and an asparagine swapping the side-chain oxygen for a main-chain carbonyl. In contrast, several structures of other bound ligands shows the gate to the cavity in a closed conformation and one ligand disorders the gate so that it is no longer visible in the electron denisty. Solution-state kinetics studies indicate that this conformational change is not an artifact of the crystallization conditions. The trapping of the open conformation provides a unique view of the extent to which protein dynamics can allow small molecule penetration into "inaccesible" protein cavities.

Fitzgerald, M.M., Churchill, M.J., McRee, D.E., and Goodin, D.B. (1995). "Small Molecule Binding to an Artificially Created Cavity at the Active Site of Cytochrome c Peroxidase." Biochemistry 33, 3807-3818.

PS04.14.15 TOXIN INTO VACCINE: STUDIES OF AB5 BACTERIAL TOXINS. Ethan A Merritt, Ingeborg Feil, Wim G J Hol: Dept of Biological Structure and HHMI, University of Washington, Seattle WA 98195-7742 Randall K Holmes: Dept of Microbiology, University of Colorado Health Sciences Center, Denver CO 80262, Rino Rappuoli: IRIS, Via Florentina 1, 53100 Siena, Italy

Cholera toxin and *E. coli* heat-labile enterotoxin are closely related AB5 hexameric assemblies secreted into the intestine during bacterial infection. Together they are responsible for over a million deaths annually. As with many other bacterial toxins the catalytic activity resides in a separate 'A' subunit, while receptor binding and delivery of the toxin to the target cell is mediated by a separate' B' fragment, in this case a pentamer which binds to the oligosaccharide of ganglioside GM1.

In addition to their deleterious biological effect as toxins, however, these molecular assemblies exhibit a remarkable ability to stimulate the immune system. In particular they are capable of evoking a strong mucosal immune response when administered orally or intranasally, and have been reported to confer the same sort of evoked response to co-administered antigens. It is therefore of great interest to determine whether these toxins can be engineered to lose toxicity while retaining their immunological properties.

We report here our recent crystallographic studies aimed at understanding both carbohydrate recognition by the receptor binding site and substrate recognition by the catalytic site. In particular we report the structures of oligosaccharide complexes with mutant toxins exhibiting altered receptor binding specificity, and also the structure of an engineered mutant at the active site with altered toxicity.

PS04.14.16 THE STRUCTURE OF A DESIGNED PEPTIDE REFINED TO 2.1 Å RESOLUTION. Nancy L. Ogihara, Manfred S. Weiss, William F. DeGrado, and David Eisenberg, UCLA-DOE Laboratory of Structural Biology and Molecular Medicine, Box 951570, University of California, Los Angeles, Los Angeles, CA 90095-1570

The three-dimensional structure of the designed peptide Acetyl-E VEALEKK VAALESK VQALEKK VEALEHG- amide has been determined and refined to a crystallographic R-factor of 21.4% for all data from 10 to 2.1 Å, resolution. In the trigonal crystal, three molecules, related by a crystallographic 3-fold axis form a parallel three helix bundle. The bundles are stacked head-to- tail to form a continuous coiled coil along the z direction of the crystal. The contacts between neighboring helices within the coiled coiled are mainly hydrophobic; four layers of valine residues alternating with four layers of leucine residues form the core of the bundle. Mostly hydrophilic contacts mediate the interaction between trimers. Here, a total of 2 solvent mediated hydrogen bonds and 2 direct protein- protein hydrogen bonds are found. Based on the structure, we propose a rule for designing crystals of peptides containing continuous 2-, 3-, and 4-helix bundles.

PS04.14.17 BINDING OF SMALL ELECTRON-DENSE LIGANDS IN LARGE PROTEIN CAVITIES. Michael L. Quillin, Walter A. Baase, and Brian W. Matthews, Howard Hughes Medical Institute, Institute of Molecular Biology University of Oregon, Eugene, Oregon 97403.

The extent to which disordered water molecules occupy hydrophobic cavities in proteins has been the subject of considerable debate. In some cases, the techniques of NMR spectroscopy and X-ray crystallography have provided seemingly contradictory estimates of the solvent content of apolar cavities (Ernst et al. (1995), Science 267, 1813-1817; Matthews et al. (1995), Science 270, 1847-1848). In an effort to resolve whether it is possible to detect fully occupied yet disordered molecules in a protein cavity using crystallographic methods, we have determined the crystal structures of several complexes of small, electron-dense molecules bound within cavity-containing mutants of T4 lysozyme. Two classes of probes have been studied in this manner: noble gases, such as krypton and xenon; and alkyl halides, including alkyl bromides and iodides. Although these ligands bind within the cavity, they do not do so in a disordered fashion. Rather, it appears that there are preferential binding sites which are highly conserved among the different ligands. Factors which influence the location of these sites will be discussed.

PS04.14.18 CRYSTAL STRUCTURE OF A STABLE ALPHA-1-ANTITRYPSIN VARIANT REVEALS THE STABILIZATION MECHANISM. SeongEon Ryu, Hee-Jung Choi, Kee Nyung Lee, Ki-Sun Kwon and Myeong-Hee Yu, Protein Engineering Division Korea Research Institute of Bioscience and Biotechnology, KIST P.O. Box 115, Yusong, Taejon 305-600, South Korea

α-1-antitrypsin, which is a member of serpin family, controls the level of neutrophil elastase in plasma by inhibiting its activity. The conformation of the mobile reactive loop and the relative instability of the native α-1-antitrypsin are implicated in the inhibitory mechanism. The crystal structure of a mutant form of the uncleaved α-1-antitrypsin with stabilization mutations at seven different positions (hepta α-1-antitrypsin) has been determined at 2.7 Å resolution. The structure was compared with the structures of other serpins to understand the stabilizing effect of the mutations. We found that hepta α-1-antitrypsin is stabilized by various mechanisms. i) relaxation of a conformational strain by removing unfavorable overlaps of Van der Waals radii. ii) stabilization of hydrophobic cores by addition of better hydrophobic interactions. iii) destabilization of the loop inserted structures. Among these, the stabilization by the relaxation of the conformational strain by the F51L mutation underscores the importance of the central hydrophobic core region in the sheet A opening of the serpins.

PS04.14.19 X-RAY ANALYSIS OF HEAT-RESISTANT MUTANTS OF HU PROTEIN Takahiro Tominaga*, Shunsuke Kawamura, Makoto Kimura, Atsushi Nakagawa*, Isao Tanaka*, *Division of Biological Science, Graduate School of Science, Hokkaido University, Sapporo, 060, Japan, Laboratory of Biochemistry, Faculty of Agriculture, Kyushu University, Fukuoka, 812, Japan

HU protein is ubiquitous in eubacterial kingdom. This small basic protein of molecular weight 9,500 is also known as DNA bending protein. Its amino acid sequence is highly conserved, e.g., *B.stearothermophilus* and *B.subtilis* have only 12 different residues. However the HU proteins of the two species have quite different thermal denaturation temperature (*B.st* 65°C, *B.su* 48°C). To elucidate the relationship between structure and thermostability of *B.st* and *B.su* HUs, we constructed T13A, T33L, E34D, and K38N mutants where the amino acids in BstHU were changed to the corresponding ones in BsuHU. Mutant proteins were expressed,