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_0: 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_0. 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-