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,