We have just determined the three-dimensional structure of proaerolysin at 2.8Å resolution by X-ray crystallography. The structure has a rather bizarre elongated shape comprising four domains rich in beta-sheet with one strand longer than 90Å. We have been able to relate various structural features to toxin function (secretion, activation, oligomerization, channel formation and gating) on the basis of the behaviour of a range of single-point mutants. The protoxin contains no hydrophobic stretches of sequence long enough to span a membrane, but the dimer interface has a number of hydrophobic patches. Activation to the mature toxin would expose an additional hydrophobic region and this may account for the protein's ability to penetrate lipid bilayers. Images of an aerolysin oligomer derived from electron microscopy has enabled us to construct a model of the aerolysin channel and suggested a pathway for how the water-soluble protoxin inserts into a membrane to form a voltage-gated channel.

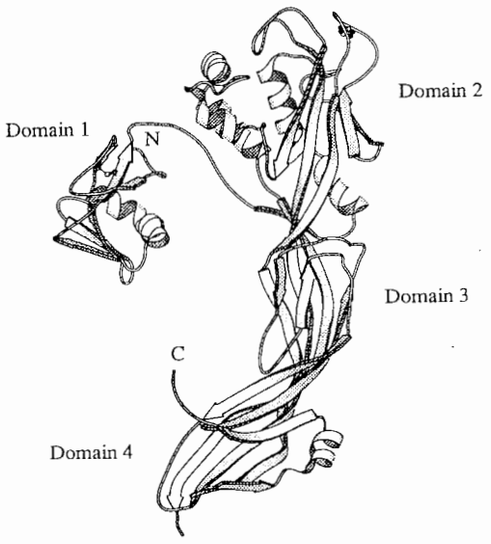

Figure: Cartoon drawing of proaerolysin as determined by X-ray crystallography. This figure was produced by the computer program MOLSCRIPT (Kraulis, J.P., J. Appl. Crystallogr. (1991) **24**, 946-950).

DS–03.08.06 CRYSTAL STRUCTURE OF THE COMPLEX BETWEEN A SINGLE CHAIN ANTIBODY AND NEURAMINIDASE: A BASIS FOR RATIONAL PROTEIN ENGINEERING. By R.L. Malby*[1], M.C. Lawrence[1], W.R. Tulip[1], V.R. Harley[2], R.G. Webster[3], P.J. Hudson[2] and P.M. Colman[1], [1]Biomolecular Research Institute and [2]CSIRO Division of Biomolecular Engineering, Parkville, Vic. 3052, Australia; [3]Department of Virology, St. Jude Children's Research Hospital, Memphis, TN 38105, U.S.A.

We have solved the structure of the complex between a recombinant single chain Fv (scFv) fragment of antibody NC10 and its antigen, neuraminidase (NA) from influenza virus. The complex crystals belong to space group $P42_12$ with cell edges a=b=141.0Å, c=217.9Å and they contain two complexes per asymmetric unit. The single chain Fv consists of the variable heavy (VH) and variable light (VL) chains of the NC10 antibody, joined by a $(GlyGlyGlyGlySer)_3$ peptide linker. Single-chain Fv molecules may be useful as therapeutic and diagnostic reagents, since they retain antigen-binding properties and are more stable under physiological conditions than double-chain Fv molecules. They are also ideal models for protein engineering studies on antibodies (Glockshuber et al., Biochemistry, 1990, 29, 1362-1367).

The scFv-NA complex structure has been solved at 3.0Å resolution using the molecular replacement routines in XPLOR, and the Fab-NA complex as a search model. Preliminary analysis of the scFv-NA structure indicates that the modes of attachment of Fab and scFv to NA are very similar. A close association between two scFv molecules related by a crystallographic twofold rotation leads to speculation that the scFvs may have crystallised in dimeric form, which has relevance to the possible utility of these molecules.

Crystallographic refinement may reveal the conformation of the peptide linker and answer the question of scFv dimerisation in this structure. Additionally, since we have recently extended the refinement of the parent NC10 Fab-NA complex structure (space group I422, a=b=171.5Å, c=160.2Å, one complex per asymmetric unit; Colman et al., Phil. Trans. R. Soc. Lond. B., 1989, 323, 511-518; Tulip, Ph.D. Thesis, University of Melbourne, 1990) to 2.2Å, refinement of the scFv-NA structure will permit comparisons to be made between the overall structures and in particular the antibody-antigen interfaces of the two NC10-NA complexes. On this basis of these two structures we intend to engineer mutations in the NC10 antibody to alter its affinity towards NA, and to design novel antibody structures.

DS–03.08.07 THE CRYSTAL STRUCTURE OF L-1 ISOZYME OF SOYBEAN LIPOXYGENASE. By Wladek Minor[1]*, Jeffrey T. Bolin[1], Janusz Steczko[2], Bernard Axelrod[2] and Zbyszek Otwinowski[3], [1]Department of Biological Sciences, [2]Department of Biochemistry, Purdue University, W. Lafayette IN 47907, [3]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510

Lipoxygenases are non-heme, non-sulfur, iron-containing dioxygenases catalyzing the hydroperoxidation of polyunsaturated fatty acids. The iron is known to participate in the catalysis. All plant and mammalian lipoxygenases that have been so far characterized contain six conserved histidine residues. Five of these occur in a fairly well-conserved region of 40 amino acid residues which contains iron ligands.

The enzyme was crystallized in space group $P2_1$ with one 839 amino acid monomer per asymmetric unit, and cell dimensions a= 95.6Å b=94.3Å c=50.3Å and b=91.3°. Diffraction data were collected using a SDMW area detector and RAXIS-II imaging plate system to a resolution of 2.6A. The structure was solved by multiple isomorphous replacement with two heavy atom derivatives. MIR phases were calculated to 3.0Å resolution. Phase improvement and extension to 2.6Å was performed by a solvent flattening technique that incorporates maximum entropy procedures (Z. Otwinowski, to be published). The position of the active site iron atom was located in an anomalous difference electron density map. The iron atom is found in a predominantly alpha helical domain and is attached to two histidines (His 499 and His 504), both of which belong to an eight turn alpha helix. The binding of the iron atom by these two histidines is in agreement with the prediction made earlier by analysis of site specific mutations (Steczko and Axelrod Biochem. Biophysics Res. Commun. 1992, 186, 668-689). Detailed interpretation of the electron density map is in progress and will be presented together with procedures used in the structure determination

DS–03.08.08 CRYSTAL STRUCTURE OF A BACTERIAL MURAMIDASE: THE SOLUBLE LYTIC TRANSGLYCOSYLASE FROM *E. COLI* by Andy-Mark Thunnissen*, Arnoud Dijkstra[1], Ilenriette Rozeboom, Kor H.Kalk, Wolfgang Keck[1] and Bauke W.Dijkstra, BIOSON Research Institute and Lab. of Biophysical Chemistry, University of Groningen, Nijenborgh 4,9747 AG Groningen, the Netherlands, [1]F.Hoffmann-La Roche Ltd., Pharma Research Department, CH-4002, Basel, Switzerland

The soluble lytic transglycosylase (SLT) is a monomeric enzyme of 618 amino acids, that is located in the periplasmic space of the *E.coli* bacterium. The enzyme is involved in the metabolism of murein, the structural component of the bacterial cell wall. In vitro, SLT is able to totally degrade intact murein to low-molecular weight muropeptides, much the same as the lysozymes do. However, in addition to cleavage of the β-1,4-glycosidic bonds, SLT carries out a unique transglycosylation reaction forming an internal 1,6-anhydro bond in the muramic acid residue.

Our interest in the structure of SLT has two main reasons. We would like to understand the mechanism by which this enzyme carries out the muramyl transferase reaction and learn more about the role of this enzyme in the metabolism of murein. Moreover, SLT is a potential target for a structure-based design of a new class of antibiotics.

The 3D structure of SLT has been determined by X-ray crystallography to a resolution of 2.7A. The crystal structure was phased to 3.3A using multiple isomorphous replacement. Solvent flattening was used to improve the MIR phases and a preliminary protein model of SLT was constructed from the electron density using the computer graphics software FRODO and O. The protein model was improved and completed stepwise by alternating rounds of partial model refinement with model building in maps calculated from combined MIR, solvent flattened and model phases. The complete model was then refined by simulated annealing using XPLOR. The current crystallographic R-factor is 22.8% for 25115 reflections from 8.0 to 2.7A with $F>2\sigma$.

The protein model of SLT shows a remarkable "doughnut-like" shape. It consists of 3 domains, all very rich in α-helices. The N-terminal domain is formed by 22 interconnecting α-helices in a super-helical arrangement. Via a loop of some 20 residues, this "U-shaped" helical arm is connected to a small linker domain, which packs against the N-terminal end of the protein, thus forming a ring structure with a large central hole of over 20A in diameter. On top of one the sides of this ring, the more globular C-terminal end of the U-domain. Interestingly, this C-terminal domain has a fold which resembles that of the lysozymes, especially that of the goose-type lysozyme. This structural similarity was not expected from sequence analysis. A structural alignment of the C-terminal domain of SLT with different types of lysozymes show that Glu-478 matches the position of the "catalytic" glutamic acid. However, SLT seems to lack the "catalytic" aspartate. The location of the active site of the transglycosylase was confirmed by an inhibitor binding study.

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DS-03.08.09 THE THREE-DIMENSIONAL STRUCTURE OF THE HISTIDINE-CONTAINING PHOSPHOCARRIER PROTEIN HPr FROM *E. coli* AT 2.0 Å RESOLUTION. By Zongchao Jia , E. Bruce Waygood, J. Wilson Quail* and Louis T. J. Delbaere. Departments of Chemistry and Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

The histidine-containing phosphocarrier protein (HPr) is a central component of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) that transports carbohydrates across the cell membrane of bacteria. The three-dimensional structure of *E. coli* HPr has been redetermined using the method of molecular replacement. The structure has been refined to 2.0 Å resolution with an *R* factor of 0.135 for all data, maintaining very good geometry. The overall folding topology of HPr is a classical open-faced beta-sandwich, consisting of four antiparallel beta-strands and three alpha-helices, and is not compatible with the original *E. coli* HPr X-ray structure (El-Kabbani *et al. J. Biol. Chem.*, 1987, **262**, 12926-12929). As the crystals were obtained from the same condition for the both determinations, the original X-ray structure of *E. coli* HPr is thus likely incorrect. While there is considerable overall structural homology between HPr from *E. coli* and from other organisms, the semi-closed conformation of the active center in this structure is unique but can be correlated to other reported HPr X-ray structures. A comparison between *E. coli* HPr and *Streptococcus faecalis* HPr (Jia *et al. Nature*, 1993, **361**, 94-97) will be presented. In addition, the relationships between some of site-directed mutagenesis results and protein structure/function have been investigated.

PS-03.08.10 REFINED STRUCTURE AND SOLVENT NETWORK OF CHICKEN GIZZARD G-ACTIN DNASE I COMPLEX AT 1.8Å RESOLUTION. By K. SASAKI, K. SAKABE*, N. SAKABE**, H. KONDO***, and M. SHIMOMURA****, College of Medical Technology, Nagoya University, Higashi, Nagoya 461,Japan, *Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan, **National Laboratory for High Energy Physics, Tsukuba, Ibaraki 305, Japan, ***Department of Biology, Faculty of Science, Nagoya Univ. Chikusa, Nagoya 464,Japan,****Faculty of Education,Tokohagakuen University, Sena 1000, Shizuoka 220, Japan.

The refined G-actin structure is essential for the the construction of F-actin and thin filament at atomic level in consideration of atomic informations obtained by other physico-chemical and biochemical techniques and for theoretical experiments.

We refined the crystal structure of chicken gizzard G-actin DNase I complex with 1.8Å resolution data collected on macromolecular data collection system with a Weissenberg camera and imaging plate(IP) at BL6A2 in the Photon Factory (N. Sakabe, Nucl. Instr. and Meth. A303(1991)448-463). The starting model of the coordinates was obtained with five heavy atom derivatives(YB, PCMB, MMA,FMA & BrATP)using MIRA method at 2.5Å resolution. The obtained coordinates were refined using "XPLOR" program. The checking of the atomic positions was carried out using omit maps with PS390 using "FRODO" program. The finding of solvent molecules was carried out on $F_o - F_c$ maps. During the refinement the resolution of the data was increased gradually. The R value is 0.188 including 350 water molecules for 50,000 reflections in 10-1.8Å resolution range.

The average B values of the main chain and side chain atoms for actin are 31.7 and 35.0 respectively. Those of main chain and side chain atoms for DNase I is 14.9 and 17.2 respectively. Thus the motion of actin is 1.5 times larger than that of DNase I. The average B value of main chain atom of DNase I is 11.0 at 2Å resolution reported by Suck et al(J.Mol.Biol.(1986)192,605-632), that is not