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.

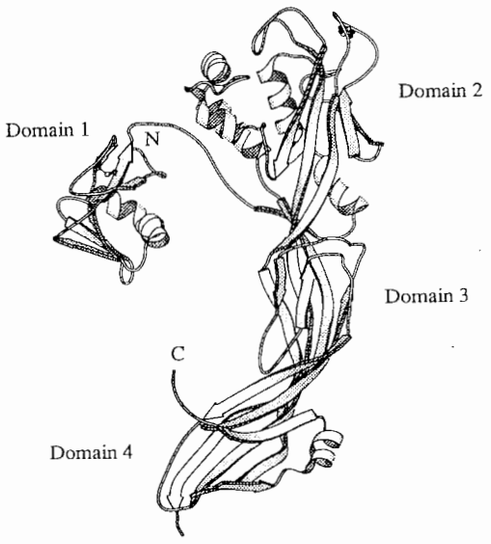

Figure: Cartoon drawing of proaerolysin as determined by X-ray crystallography. This figure was produced by the computer program MOLSCRIPT (Kraulis, J.P., J. Appl. Crystallogr. (1991) **24**, 946-950).

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

