incoherent scattering. A fully deuterated sample will thus yield better diffraction data with stronger density in the hydrogen position. On this basis, a sperm whale myoglobin gene (Barry A. Springer and Stephen G. Sligar, Proc. Natl. Acad. Sci. USA, 1987, 84,8961-8965) modified to include part of the lambda cII protein gene (Kiyoshi Nagai and Hans Christian Thogersen, Nature, 1984, 309, 810-812) has been cloned into the T7 expression system. The fusion protein has been overexpressed in *E. coli* to a very high level both in protonated and deuterated media. Because of the solubility and folding problems during purification, different bacterial strains and induction conditions have been searched to work out an optimal procedure. After reconstitution with heme and cleavage with trypsin, milligram amounts of holo-myoglobin have been obtained. Crystallization trials have been successful. The crystals are large enough for both x-ray and neutron studies. The synthetic sperm whale myoglobin crystallizes in $P2_1$ space group isomorphously with the native protein crystal, which makes possible a comparison with previous studies (Xiaodong Cheng and Benno P. Schoenborn, Acta. Crystal., 1990, B46, 195-208). We are currently crystallizing deuterated myoglobin. Results of diffraction experiments on these samples will be presented.

PS–03.12.03 REFINED CRYSTAL STRUCTURE OF CHICKEN ANNEXIN V. D.A.Waller*, M.C.Bewley & J.H.Walker, Department of Biochemistry and Molecular Biology, University of Leeds, Leeds, England

The annexins are a family of widely distributed calcium dependent phopholipid binding proteins. Annexins I–XI have been sequenced. They do not contain the classical E–F hand clacium binding motif of proteins such as calmodulin or troponin C, hence they are a distinct family of calcium–binding proteins.

The structure of chicken annexin V has been solved by molecular replacement using the full coordinates of human annexin V as a search model. It has been refined by restrained least–squares methods to an R–factor of 19.0% at 2.25Å resolution. The structure includes three calcium ions and 82 water molecules.

The calcium ions are bound in three of the eight loops on the surface of the protein which is thought to bind to the membrane. Studies are underway to locate additional metal ion binding sites analogous to those found in the human protein.

PS–03.12.04 CRYSTAL STRUCTURE OF PSOPHOCARPIN B_1 : A CHYMOTRYPSIN INHIBITOR FROM WINGED BEAN SEEDS. By J.K.Dattagupta, A.Podder, C.Chakrabarti*, U.Sen, S.K.Dutta[1] and M.Singh[1], Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Calcutta 700 064, India. [1]Indian Institute of Chemical Biology, Calcutta 32, India.

Psophocarpin B_1, the winged bean (*Psophocarpus tetragonolobus*) chymotrypsin inhibitor (WCI) is a single-chain polypeptide (MW 20 KD) having 183 amino acid residues. It belongs to the Kunitz (STI) family of inhibitors and has sequence homology with other members of the family such as Soya bean trypsin inhibitor (STI), Erythrina trypsin inhibitor (ETI) etc.

The inhibitor, isolated from the seeds and purified to homogeneity, was crystallized from 25% ammonium sulphate, 0.1M-Tris·HCℓ, 0.4M NaCℓ, pH 8.0, using vapour diffusion method. The crystals are hexagonal, space group $P6_122$, cell dimensions a=b=61 Å, c=210 Å. X-ray diffraction data (2.9 Å) has been collected on an area detector and the molecular replacement method has been used to solve the structure, utilising the close homology existing between WCI and ETI. Refinement is underway using restrained least-squares and the current crystallographic R-factor is 30%.

The three dimensional structure of the inhibitor as found by us is similar to that of ETI and STI structures - there is however a difference in the reactive site loop (Leu 65 - Ser 66 is the scissile bond) which appears to be somewhat displaced. From the preliminary results of our crystal structure analysis and using the known structure of chymotrypsin, a possible mode of binding has been predicted which is consistent with other serine proteases-protein inhibitor complexes. It is observed that the rigidity of the reactive site loop in the inhibitor is not due to any `S - S' bond or salt bridge but through hydrogen bonding with the N-terminus loop, Asn 14 and P_4 Ser, the last two acting as spacers. Some structurally and functionally important residues alongwith Asn 14 and P_4 Ser are seen to be conserved in all the members of inhibitor family - this may be considered to be responsible for the same type of structural rigidity of the reactive site loop and common mode of action of the legume inhibitors of this family.

One of the authors (A.P) acknowledges the help of Prof. T.L.Blundell, Prof. D.M.Blow and their colleagues for extending the computational facilities of their laboratories at Birkbeck and Imperial Colleges during a short visit by her with a British Council fellowship. The ETI coordinates were kindly made available to us by Prof. D.M.Blow.

PS–03.12.05 PREDICTION OF WATER AROUND POLAR PROTEIN SIDE CHAINS: AN AID TO STRUCTURE REFINEMENT. By S.M. Roe, The Nitrogen Fixation Lab, The University of Sussex, Brighton, BN1 9RQ, England and M.M. Teeter*, Department of Chemistry, Boston College, Chestnut Hill, MA 02167. USA

Water is important in stabilizing the folded conformation of a protein and also is necessary for enzyme activity. Its inclusion in a crystallographic model can be beneficial during refinement or understanding the mechanism of enzyme action. We have analyzed the patterns in hydration of polar side chains around crambin and 6 other proteins which diffract to bettter than 1.4Å resolution (S.M. Roe and M.M. Teeter, J. Mol. Biol., in press (1993)).

Correspondence between the solvent positions around residue side chains can be found by superimposing identical functional groups and their accompanying hydrogen bonding spheres. Well defined hydrogen-bonding shells can be located. Solvent positions around amino side chains are more ordered than around carboxyl. This is true especially where both amino and carbonyl groups are present in the same residue, i.e. asparagine.

A template has been developed which permits prediction of water positions around polar groups. This was tested on crambin and as well as two protein not in our hydrogen bonding database. Rms deviation from water positions were less than the resolution of the structures. The algorithm was also tested in refinement of two proteins. Inclusion of water lowered the R value by 2-4% after refinement. 86-91% of the waters were judged to be well predicted and could refine within the radius of convergence.

