02.X-7 STRUCTURAL BASIS OF THE ACTION OF THERMOLYSIN AND THE ZINC PROTEASES. Hazel M. Holden, Dale E. Tronrud, Arthur F. Monzingo, Larry H. Weaver and Brian W. Matthews, Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403 U.S.A.

High resolution X-ray crystallography has been used to determine the modes of binding to thermolysin of a series of different inhibitors including dipeptides, mercaptans, hydroxamates, N-carboxymethyl peptides and phosphonamidates. The interactions displayed by such inhibitors illustrate interactions that are presumed to occur between the enzyme and its substrates during catalysis. The crystallograhic analyses, together with model building, suggest a detailed stereochemical mechanism of action for thermolysin and, by analogy other zinc proteases such as carboxypeptidase A and the angiotensin converting enzyme. Recent analyses of a series of phosphonamidates, which are presumed to be transition-state analogues, has shown that chemically similar inhibitors can adopt dissimilar modes of binding. These different configurations provide a rationalization for large differences in the kinetics of binding that are observed for these inhibitors. Comparison of matched pairs of inhibitors allows the contribution of a single interaction (e.g. a hydrogen bond) to be determined.

02.X-8 MOLECULAR DYNAMICS AS A TOOL IN CALCULATING BINDING ENERGIES. By Paul A. Bash, Chemistry Department, Harvard University, Cambridge, MA 02138, U.S.A.

Recent access to the latest supercomputers is providing the means to develop computational methods, based on rigorous principles from quantum and statistical mechanics, that can be applied to macromolecular systems. Such methods are capable of calculating free energy and structural changes in real systems where the three-dimensional structure of the macromolecule is known in atomic detail. The development of methods that combine classical molecular dynamics, ab initio and semi-empirical quantum mechanics, and free energy perturbation techniques will be presented. Their utility will be demonstrated by calculating solvation free energies of biological molecules in solution, relative binding free energies of ligands to proteins, and the energetic pathway of an enzyme reaction.

02.X-9 PREDICTION OF THE CONFORMATION OF SHORT SEGMENTS OF POLYPEPTIDE CHAIN IN PROTEINS by J.Moult and M.N.G.James, Medical Research Council of Canada Group in Protein Structure and Function, Biochemistry Department, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

The design of segments of protein structure, the modelling of homologous proteins, and the determination of protein structure using molecular replacement methods all require an ability to predict the conformation adopted by short stretches of polypeptide chain in the presence of the rest of the structure. We report here the current state of development of a computer algorithm with such predictive power (J.Moult and M.N.G.James, PROTEINS 1 146-163 (1986)). The success of the method depends upon extracting from known protein structures a set of rules which can be used to restrict the number of conformations a chain may have, and to choose one close to the correct structure. Useful rules are the preferred values of backbone and side chain dihedral angles, the avoidance of short interatomic contacts, the optimization of electrostatic interactions, and the minimization of solvent exposed hydrophobic area. Tests of the method, simulating homologous modelling problems, show that for stretches of chain up to six residues long it is possible to identify amongst the generated conformations one with about 1Å root mean square deviation (on all non-hydrogen atoms) from the correct structure.

02.X-10 ANTIBODY-ANTIGEN RECOGNITION: THE STRUCTURE OF A FAB-LYSOZYME COMPLEX. By A.G.Amit, *R.A.Mariuzza, +S.E.V.Phillips and R.J. Poljak, Immunologie Structurale Institut Pasteur, Paris, France, *MRC Laboratory of Molecular Biology, Cambridge, UK, and +Astbury Department of Biophysics, University of Leeds, Leeds,UK.

The initial step in the activation of the immune system is the binding of foreign antigens to the surface of B and T lymphocytes, the receptor molecule on B lymphocytes being membrane bound immunoglobulin. A B lymphocyte also secretes soluble immunoglobulin molecules, or antibodies, with antigen binding domains of a single antigen specificity identical to that of its membrane bound receptors. Mild proteolysis of antibody molecules liberates these domains as antigen binding fragments (Fab) which may be purified and crystallized. A Fab is highly specific in binding its particular antigen molecule, and not others, and can even distinguish point mutations where the antigen is a protein molecule. This molecular recognition forms the basis of the specificity of the immune response, and can be studied directly in crystal structures of antibody-antigen complexes.

The crystal structure of the complex between hen egg lysozyme and the Fab fragment of a monoclonal antibody (D1.3) raised against it in Balb/c mice has been determined at 2.8Å resolution (1). Crystals of the complex grown from 15-20% PEG 6000 at pH 6.0 are monoclinic, space group $P2_1$ with a=55.6, b=143.4, c=49.1Å, β=120.5°, Z=2. X-ray intensities for native and three heavy-atom derivative crystals were collected on a 4-circle diffractometer. The MIR electron density map (<m> = 0.47 for 15592 reflextions) was difficult to interpret, but was greatly improved by the application of a simple density modification technique. The current model has been refined to R=0.27 for all reflexions in