05-Molecular Modelling and Design for Proteins and Drugs

PS-05.01.10 Tampering with Success: Using Structure to Develop Second Generation Engineered Subtilisins" By. R. Bott, J. Dauberman, L. Wilson, B. Schmidt, G. Ganshaw, J. Sanford, D. Estell, H. Sagar, and T. Graycar, Genencor International, So. San Francisco CA 94080 USA

We have determined the three-dimensional structure of a variant of *Bacillus lentus* having four site-specific substitutions: K27R/V104Y/N123S/T274A which has the benefit of providing twice the commercially important performance as the native enzyme. This structure has been used to identify additional sites which can confer increased chelant and thermal stability, as well as, sites which can dramatically alter the kinetic parameters of this variant enzyme. Substituting S128G results in an enzyme with substantially increased activity on a synthetic substrate and reduced performance of proteinaceous substrates while substitutions at other sites produce the reverse effects. We are in the process of determining how the structure function relationships deduced from these structures can be incorporated to produce a second generation engineered enzyme.

PS-05.01.11 MODELS FOR CATALYTIC ANTIBODIES: A TOOL FOR UNDERSTANDING THEIR MECHANISM OF ACTION. By M. Eisenstein[1], D.S. Schindler[2], R. Zemel[2] B.S. Green[3] and Z. Eshhar[2], Departments of Structural Biology[1] and Chemical Immunology[2], The Weizmann Institute of Science, Rehovot, Israel, and Pharmaceutical Chemistry[3], The Hebrew University, Jerusalem, Israel.

Immunization with transition state analogs of a chemical reaction can give antibodies which catalyze the reaction. Such catalytic antibodies have the potential to provide us with proteins that have enzymatic activities with novel specificities. In order to engineer these antibodies and understand their mechanism of action we are studying catalytic antibodies with esterolytic activity on p-nitrophenyl esters.
Antibodies were raised in mice by immunization with p-nitrophenyl phosphonate as the hapten and six monoclonal antibodies with catalytic activity were found by extensive screening. Biochemical experiments divide these antibodies into two groups according to their substrate specificity and inactivation, sensitivity to chemical modification and affinity to transition state analogs.
Sequencing of these antibodies showed that the two groups have different light chains. Models of the antibodies were built on the basis of their sequence and the known three-dimensional structures of antibodies. A deep L-shaped groove is present in one group of the antibodies and it is proposed that this is where the transition state analog binds. This groove is wider and shallower in the other group of antibodies explaining the difference in binding affinity. The phosphonate moiety of the transition state analog binds to a Tyr residue which is conserved in all six antibodies and is located at the bend of the groove. It also binds to a Gln / Tyr from the heavy chain. Additional interactions between the antibodies and the p-nitrophenyl and the aliphatic chain of the transition state analog stabilize the binding.
An evident structural difference in the binding grooves of the two groups of antibodies is that a Tyr residue in one group, which is bound to the phosphonate-binding Tyr, is replaced by an Arg in the other group. This change can explain the differences in substrate inactivation and chemical modification. It also hints at possible dissimilarities in the ester hydrolysis mechanism between the two groups of antibodies.

PS-05.01.12 MODELLING STUDY OF A NEUTRAL PHOSPHOLIPASE A_2 FROM THE VENOM OF AGKISTRODON HALYS PALLAS. By X.Q. Wang*, Z.J. Lin, National Laboratory of Biomacromolecules, Institute of Biophysics, Academia Sinica, Beijing, China.

The neutral phospholipase A_2 (PLA_2), isolated from *Agkistrodon halys* pallus venom, has strong presynaptic neurotoxin activity and designated as agkistrodotoxin (ATX). The sequence of ATX (Kondo,K. et al.,(1989),J.Biochem.105,196-203) is highly homologous to that of the toxic basic subunit of crotoxin. ATX has a tendency to associate with identical molecules to form dimer or higher aggregates from crystallization and M.W. determination (Jin,L.,et al.,(1991),Chinese J. Biochem.Biophys.23,269-276). Sequences alignment between ATX and non-toxic *Crotalus atrox* venom PLA_2 shows the identity of 50% amino acids and presence of almost same residues involving subunit interaction. In order to study the possibility of dimerization from a structure point of view,three dimensional models of both monomeric and dimeric ATX have been graphically built using the X-ray structure of *C. atrox* venom PLA_2 and optimized by energy minimization technique with programs QUANTA and CHARMm. The result shows that the structure seems essentially similar to that of *C. atrox* venom PLA_2(the r.m.s. deviation of corresponding C_α atoms is 1.46Å except the fragment 85-89 in a loop region. The dimer-stabilizations of ATX dimeric model are very similar to those of *C. atrox* venom PLA_2 except that the interaction between His34 and Glu6 is replaced by that between Glu34 and Asn6. The energy calculation shows that the dimer's total energy and hydrogen bond energy are 241 and 41 kcal/mol respectively lower than twice of monomer's energies. These suggest that the C.atrox-like dimer is a more stable form than monomer. We plan to complete the modeling using molecular dynamics simulation method in next step. The definitive determination of the structures will be done by X-ray crystallography, which is currently underway.

PS-05.01.13 DISTANCE GEOMETRY AND MOLECULAR DYNAMICS CONFORMATIONAL SEARCH OF A CYCLIC PEPTIDE FOR PROTEIN DE NOVO DESIGN. Zhaowen Luo*, Luhua Lai, Xiaojie Xu, Department of Chemistry, Peking University, Beijing 100871, CHINA

A cyclic peptide, designed to act as topological template of the four–helixes bundle, was investigated by distance geometry and molecular dynamics conformational search. The sequence of the cyclic peptide is (Phe–Lys–Pro–Gly–Lys–Gly)2. Firstly, 100 conformations were generated by distance geometry with constraints for ring closure. Seven conformation clusters were classified according to their mutual rms. A representative conformation from the cluster with highest density was selected to

perform a 100 ps molecular dynamics simulation at 900K. 100 conformations of the peptide were taken from the dynamics trajectory for every 1 ps. Rms graphs were used to classify the 100 minimized conformations into five conformations families.
The five conformation families were compared with the conformation from 2D nmr studies. RMS values obtained are between 2.0A and 4.0A. By studying the representative conformations of each family on graphics, the conformation group with the least rms value was found similar to the nmr structure. Conformational search by combining distance geometry and molecular dynamics has not been seen in literature. The primary purpose of this study was to obtain the model of a de novo peptide or protein.In future, more constraints from theoretical design and motif alignment will be added in distance geometry. Moreover, criteria will be developed to indicate the preferable conformation.

PS-05.01.14 ELECTROSTATIC EFFECTS IN PROTEIN: COMPARISON OF TK METHOD AND FDPB METHOD. **By Yanli Wang*, Luhua Lai & Xiaojie Xu, Department of Chemistry , Peking University, Beijing, China**

Two approaches for calculating electrostatic effects in proteins are compared. Both TK methods which is based on Tanford-Kirkwood theory and FDPB method which is based on finite-difference Poisson-Boltzmann equation are applied to calculate the pKa values of the charged residues. All of the results are compared with the experimental data. It is shown that TK method gives better results than FDPB method, especially for the residues near the surface of the molecule, and RMS between calculated data and experimental data are 2.55 and 0.99 respectively. It is found that the results from TK method are closely related to the accessibility of a residue. For the residues with accessibility higher than 0.67, the absolute values of difference between experiment and calculation are below 0.50, on the other hand , for most of the rest residues , the difference values are above 0.90. It is interesting that the two approaches compensate each other, and this phenomenon is also related to the accessibilities of the charged residues. For deeply buried residues, the calculated results from FDPB method are more accurate than those from TK method, whereas for residues near the surface of the molecule, TK method can give more accurate results than FDPB method. This can been seen from the calculated results for the residues GLU_35, ASP_8, ASP_52, LYS_97. The possible reasons are discussed, as well as the virtues , limitations of the two models.

PS-05.01.15 THE DESIGN OF TEMPLATE ASSEMBLED PARALLEL FOUR-HELIX BUNDLE PROTEIN. by Yu Luo*, Zhenwei Miao, Luhua Lai, Xiaojie Xu, Department of Chemistry, Peking University, Beijing 100871, CHINA

The packing of the hydrophobic surfaces of the four helices forming the parallel four-helix bundle were investigated using crude energy function: $E=(R/R0)^9 - 1.5*(R/R0)^6$. Based on the crystal structure of a leucine zipper protein-GCN4, 4-fold symmetry was proposed for this bundle protein. Monte Carlo technique was used to obtain the optimal orientations and displacements of the four helices with respect to the main axis of the bundle.
Single Helices of standard alpha-helix geometry were built using QUANTA/CHARMm. Up to 10 heptad repeat sequences were devised with hydrophobic side chains at position 1,4 and 5. The most frequently occurring rotamers of side chains were used. The hydrophilic surfaces were approximated by alanines. Inspection of the optimal geometry of the bundles showed that isoleucine and valine at position 1 and 5 were less favorable than leucine. Taken also into account the electrostatic interactions, we designed three 16-residue helix sequences and one contrast sequence. One of the three helix sequences were synthesized and high helicity observed.

PS-05.01.16 CONSTRAINT PEPTIDE CONFORMATIONAL ANALYSIS BY MONTE CARLO SIMULATED ANNEALING. by Leyu Wang*, Qiaolin Deng, Luhua Lai, Yuzhen Han, Xiaojie Xu, Department of Chemistry, Peking University, Beijing 100871, CHINA

Distance geometry and molecular dynamics method are currently employed in determining molecular structures with inter-atomic distances from NMR NOESY experiment. Because of the flexibility of peptide, distances obtained from NMR are usually not sufficient to confine its structure. Both distance geometry and molecular dynamics method will bias in the conformational space at this circumstance. Constraint Monte Carlo simulated annealing was established to solve this problem.
Distance constraints were included into the ECEPP force field by introducing an energy term of $E'=K(R-R0)^2$. Monte Carlo simulated annealing was performed in dihedral angle space. Conformational analysis on a pentapeptide with eight inter-atomic distances was carried out as a test. The resulting conformations agree well with experimental data.