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APPROACHES TO MODELING PROTEIN FLEXIBILITY DURING DRUG DESIGN

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Using high-resolution crystal structures in structure-based drug design affords the ability to determine detailed interactions but does not properly account for ligand-induced enzyme conformational changes. The assumption of a single protein conformation imparts bias into the discovery and design of novel inhibitors. We have implemented a new algorithm to predict a minimal manifold of enzyme conformations and applied this algorithm to structures of the enzyme, thymidylate synthase. Experimental structural evidence of thymidylate synthase from *Pneumocystis carinii* bound to two potent inhibitors reveals a large conformational change in the enzyme. Energy scores calculated from docking each inhibitor in the structure of the opposite enzyme are poor, reflecting the bias in rational drug discovery with the use of a single structure of the active site. We relegated conformational changes in thymidylate synthase to a few specific residues and projected their trajectories in order to compute a minimal ensemble of possible structures of the enzyme. The minimal ensemble is a more exactly defined docking target and resulted in better scores for the known inhibitors, confirming the necessity of modeling protein flexibility during drug design. In order to overcome difficulties in defining residues most likely to undergo conformational change, we have recently expanded the algorithm to more completely screen the conformational space of rotamers within the active site using computational biophysical filters. Evaluating the energies of protein and ligand complexes with an ensemble-based scoring scheme resulted in good correlations between known inhibitors and docking scores.

Keywords: DRUG DESIGN, PROTEIN FLEXIBILITY, DOCKING

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THE STRUCTURE OF A NOVEL RNA-BINDING AND TRANSLOCATING PROTEIN – TBRBP

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Testis/brain-RNA-binding protein (TB-RBP) suppresses the translation of stored mRNAs which contain 15 base RNA sequences called H and Y elements. TB-RBP is a 228 residue, 26 kDa protein with no obvious homology to any known protein. In addition to influencing temporal expression of mRNA TB-RBP also influences the spatial expression of mRNA.
TB-RBP crosses intercellular bridges between developing haploid spermatids and is abundant in brain cells where it binds to brain mRNAs for movement along neuronal processes. TB-RBP is the analog of the human protein translin, which mediateschromosomal translocations causing lymphoblastic leukemia. We report the 2.65 Å crystal structure of mouse TB-RBP. The structure is predominately alpha-helical and exhibits a novel protein fold. A Dali search confirms that the topology of TB-RNB is unique.
The protein has a complex mode of assembly. Although it may function as a cyclic octamer in solution, the crystal structure shows an ellipsoidal octamer with a central cavity large enough to accommodate a Y or H RNA element. Basic amino acid residues, known from mutagenic studies to affect RNA and DNA binding, are located on the interior surface of the assembled particle.
GTP is known to release RNA binding by TB-RBP; addition of GTP to our crystals causes them to crack, suggesting a substantial conformational change. The putative guanine nucleotide binding domain that controls RNA binding is located at a dimer interface.

Keywords: **RNA BINDING, TEMPORAL CONTROL, NOVEL STRUCTURE**

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STRUCTURES OF MOLECULAR CHAPERONE ClpB REVEALS ITS MECHANISMS FOR REGULATED PEPTIDE BINDING

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E. coli Heat shock protein 100 (Hsp100) ClpB has recently been identified to function as molecular chaperones by dis-aggregating denatured proteins. The ClpB N-terminal domain (ClpBN) may interact directly with non-native polypeptides and play essential roles in ClpB chaperone activity. To investigate the molecular chaperone mechanisms of Hsp100 ClpB, we have determined the crystal structures of ClpBN to 1.95 Å by MAD methods. The crystal structure revealed a putative peptide-binding groove on the molecular surface. The groove is primarily composed of hydrophobic residues. Modeling a peptide into the putative peptide-binding groove of ClpBN indicated that ClpB prefers to bind an extended peptide substrate with the minimum length of five residues through hydrophobic interactions. Interestingly, the crystal structure suggested that the putative peptide-binding groove was gated by a salt bridge formed by R101 from the upper side of the groove and E141 from the lower side of the groove. Four ClpBN molecules are present in one asymmetric unit. In two of those four molecules, the salt bridges are tightly formed and salt bridges seal the putative peptide-binding groove. However, in the other two molecules, the salt bridges are not formed. The putative peptide-binding grooves stay in open conformations that can readily accommodate peptide substrates. ClpB may regulate its peptide substrate binding through the formation of the salt bridge between R101 and E141.The phage display screening of ClpBN revealed a peptide substrate of ClpB with high affinity. The structure determination of ClpBN-peptide complex is under way.

Keywords: **MOLECULAR CHAPERONE, PROTEIN FOLDING, HSP100**

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STUDIES ON THE DESOSAMINE PATHWAY MAY AID IN THE DESIGN OF NOVEL ANTIBIOTICS

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Nature produces an impressive array of compounds that have useful biological activities, including antibiotics, insecticides and antiparasitic agents to name but a few. One important group of these bioactive compounds produced by microorganisms are the polyketides, which show great diversity in both their chemical structures and biological activities.
The deoxysugar glycosides found on many polyketides frequently prove to be the necessary and often essential components needed for their biological activity. This has led to the idea of using combinatorial biosynthesis, a process of manipulating antibiotic genes clusters in certain microorganisms in order to incorporate different sugars or to glycosylate different positions on the polyketide concerned, to produce novel natural products for drug development. This requires an understanding of how these sugars are synthesized and then transferred to the polyketide. In bacteria, the most abundant group of sugar moieties found in bioactive compounds are the 6-deoxyhexoses. *Streptomyces venezuelae* produces the polyketides methymycin, neomethymycin, pikromycin and narbomycin. All of these compounds contain the single 3-(dimethylamino)-3,4,6-trideoxy sugar desosamine, which also exists in erythromycin and several other macrolide antibiotics. Seven genes in the des cluster of *S. venezuelae* are presumed to be responsible for the biosynthesis of TDP-D-desosamine (desI to desVI and desVIII); also found is desVII, which encodes a glycosyltranferase. We have purified a number of the desosamine enzymes from *S. venezuelae* and are currently proceeding with structural studies on these proteins.

Keywords: **DEOXYSUGAR DESOSAMINE ANTIBIOTIC**