MS04.12.07 STRUCTURE-BASED DESIGN OF CALCINEURIN INHIBITORS. Charles R. Kissinger, Hans Parge, Daniel Knighton, Laura Pelletier, Anna Tempczyk, John Tatlock and Ernest Villafranca, Agouron Pharmaceuticals, Inc., 3565 General Atomics Court, San Diego, CA 92122

The crystal structure of human calcineurin (CaN) provides the foundation for structure-based design of novel immunosuppressive agents. CaN is a calmodulin-dependent protein serine/threonine phosphatase that plays a critical role in T-cell activation. CaN is the target of the immunosuppressive drugs, cyclosporin and tacrolimus (FK506). These macrocyclic compounds inhibit the enzyme only after forming complexes with cytoplasmic binding proteins (cyclophilin and FKBP12, respectively). We have determined the crystal structures of human CaN and of the CaN-FKBP12-FK506 complex. The structure of native CaN reveals that a calmodulin-regulated auto-inhibitory sequence binds over the di-metal active site. In the CaNFKBP12-FK506 complex, FKBP12-FK506 binds adjacent to the CaN active site but dispaces the auto-inhibitory sequence and inhibits through a non- competitive mechanism. The binding of FKBP12-FK506 appears to mimic a natural protein-protein interaction involved in CaN regulation. The structure of the complex reveals essential features of FK506 necessary to facilitate protein-protein interaction between CaN and FKBP12. The structural findings suggest strategies for design of both FK506 analogues and direct active site inhibitors of CaN.

MS04.12.08 HUMAN PLASMA ALBUMIN: THE SECOND STEP IN STRUCTURE-BASED DRUG DESIGN Daniel C. Carter, ES76 MSFC, NASA, Huntsville, AL 35812 USA

Albumin has a well known ability to alter the in vivo metabolism and distribution of a wide spectrum of pharmaceutical therapeutics. Often newly developed therapeutics are rendered less effective or ineffective by virtue of their high affinity for this abundant plasma protein. Insight into the chemistry of all major drug/ligand sites on albumin has been gained by means of crystallographic structure studies. Approaches to improving the bioavailability of potential therapeutics by structure-based design are presented.

PS04.12.09 THE MOLECULAR BASIS OF HIV-1 PROTEASE DRUG RESISTANCE. Paul Ala, E. Huston, R. DeLoskey, J. Duke, B. Korant, C.-H. Chang, The DuPont Merck Pharmaceutical Co., Wilmington, DE 19880

HIV-1 protease processes the *gag* and *gag-pol* polyproteins into mature structural and replicative proteins. Incomplete processing caused by disrupting the normal function of the protease results in the formation of immature (non-infectious) viral particles. Several potent synthetic compounds against the wild type enzyme currently exist, however, clinical studies have revealed the emergence of drug resistance during the course of treatment. Selective pressures created by treating patients with these inhibitors have caused the emergence of viruses that possess mutations in several regions of the protease sequence, most importantly in the substrate binding pocket and the flaps. We believe that all protease inhibitors will, to some degree, select for viruses which possess mutant proteases that will be able to process viral polyprotein precursors but exhibit reduced binding affinities for inhibitors. Therefore, we have attempted to identify the structural features of HIV-1 protease mutants that confer drug resistance and utilize this information to improve drug efficacy. We have crystallized wild type and several mutant HIV-1 proteases (V82I, V82F, I84V and V82F/I84V) complexed with cyclic urea inhibitors, DMP323 and DMP450. The structures indicate that a loss or gain of hydrophobic interactions between mutant proteases and inhibitors is in part responsible for altering the binding affinities for inhibitors. A detailed understanding of the structural changes caused by mutations, within the protease sequence, will be essential when designing new compounds to combat native and mutant HIV-1 proteases in future treatments.

PS04.12.10 PHOSPHOGLYCERATE KINASE FROM *TRYPANOSOMA BRUCEI:* SELECTIVE INHIBITORS, HOMOLOGY MODELING AND CRYSTALLIZATION. Bradley E. Bernstein, Christophe L. M. J. Verlinde, Wim G. J. Hol*, Biomolecular Structure Center, University of Washington, Box 357742, *Howard Hughes Medical Institute, Seattle, WA 98195-7420

Trypanosoma brucei, the causative agent of African sleeping sickness is completely reliant on glycolysis for its energy needs while infecting its human host. Trypansomal glycolytic enzymes are, therefore, excellent targets for "selective" structure-based drug design. We found that an empirically identified inhibitor of phosphoglycerate kinase, "SPADNS," has an IC_{50} of 20μM towards the trypanosomal version of this enzyme but does not inhibit a mammalian PGK in the measurable range. SPADNS has a rigid, multi-ringed structure and several functional groups which can be modified and is therefore an excellent lead compound for drug design. A topology based similarity search identified 89 commercially available derivatives of SPADNS. Two of these compounds were found to be even more potent inhibitors of trypanosomal PGK than SPADNS; with IC_{50}s of 1.2μM and 2.5μM and two orders of magnitude of selectivity. To investigate binding modes for SPADNS and its derivatives a homology model of *T.brucei* PGK has been built on the basis of the crystal structures of *B.stearothermophilus* and porcine PGK (49% and 46% identical in sequence to the *T.brucei* enzyme). Sequence conservation, as well as structure validation algorithms, suggest that this model is particularly accurate in the active site cleft region where these inhibitors are expected to bind. Based on this model a binding mode has been identified which explains: (i) the relative affinities of SPADNS and its derivatives for trypanosomal PGK and (ii) the selectivity which these compounds exhibit with respect to mammalian PGK. We are interested in obtaining crystallographic data for critical analysis of the model and binding hypothesis. Towards this end, a large scale expression and purification of *T.brucei* PGK has been carried out and conditions which yield small protein crystals are currently being optimized.

PS04.12.11 STRUCTURAL STUDIES OF INHIBITOR COMPLEXES OF HIV-1 PROTEASE AND OF ITS DRUG RESISTANCE MUTANTS. T.N. Bhat, R.S. Randad, A.Y. Lee, L. Lubkowska, S. Munshi, B. Yu, S. Gulnik, P.J. Collins, J.W. Erickson, Structural Biochemistry Program, Frederick Biomedical Supercomputing Center, SAIC, National Cancer Institute-Frederick, Cancer Research and Development Center, Frederick, Maryland, 21702-1201

HIV-1 protease is essential for the maturation of fully infectious virions and it is an important target for the design of antiviral therapeutic agents for AIDS. We have studied the crystal structures of numerous inhibitors complexed with the HIV-1 protease some of which are currently undergoing clinical trials. We have used these crystal structures to develop our understanding of the critical features of binding that contribute to potency. A major obstacle to antiviral therapy for HIV has been the emergence of drug resistance mutations in HIV-1 protease. To understand the structural basis of drug resistance, studies were undertaken to determine the crystal structures of mutants of HIV-1 protease complexed with various inhibitors. These structural studies reveal unexpected conformational rearrangements of protein and

inhibitor molecules. The changes observed in mutant complexes underscore the significance of flexibility of both the enzyme and inhibitor molecules for understanding resistance mechanisms and for designing second generation inhibitors.

PS04.12.12 COMPLEXES OF CALMODULIN WITH ANTAGONISTS. Zs. Böcskei, V. Harmat, D. Menyhárd, G. Náray-Szabó, Eötvös University of Sciences, Budapest, Hungary, B. Vértessy, J. Ovádi, Institute of Enzimology of the Hungarian Academy of Sciences, Budapest, Hungary

Calmodulin is believed to be the most important mediator of Ca^{2+}-dependent signaling in eucarytic cells and is thought to play an essetial role in processes like cell proliferation and growth. Calmodulin is therefore a target for certain drugs and consequently a target of drug design experiments.

The structures of calmodulin as well as its complexes with a number of substrates has received widespread attention recently. This is because calmodulin plays a regulatory role in a number of processes by transforming the value of the intracellular Ca^{2+} -ion concetration into a more structured information. If Ca^{2+} concentration increases than two hydrophobic binding pockets of calmodulin become exposed facilitating the binding of certain amphilphilic regulatory helices of at least 30 different proteins of high biological importance. Calmodulin mediated enzyme activation can be efficiently inhibited by a number of pharmacological agents (antipsychotics, antidepressants, muscle relaxants etc.). Crystal structure of TFP (a potent antipsychotic phenothiazine type drug, kind of a reference molecule on the area) with calmodulin is known from the literature[1]. This shows that due to the effect of the small molecular antagonist TFP, calmoduline undergoes a very similar conformational change to that it suffers when it binds regulatory oligopeptide pieces of proteins normally regulated by calmodulin.

We have recently shown that drugs fairly different form TFP result in very similar calmodulin conformational changes. Furthermore we have also demonstrated that at low, physiologically relevant TFP concentrations the binding of the second TFP occurs in the C-terminal domain of calmodulin, unlike it was proposed earlier. A quaterner complex of calmoduline with Ca^{2+} and two different type of drugs has also been investigated resulting in some new insights into the inactivation of calmodulin.

[1] Vandonselaar, M., Hickie, R.A., Quail, J.W., Delbaere, L.T.J. (1994) Nature Structural Biology 1 795-801.

PS04.12.13 STRUCTURE ANALYSIS OF KEY DRUG DESIGN TARGET ENZYMES FROM HUMAN PATHOGENS. Christopher M. Bruns[1], Andrew S. Arvai[1], Andrew J. Nowalk[2], Timothy A. Mietzner[2], Duncan E. McRee[1], and John A. Tainer[1]. [1]Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA USA 92037; [2]Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA USA 15261

Two proteins that represent promising drug design targets against human pathogens are the major ferric iron binding protein (FBP) from *Neisseria* and *Haemophilus*, and glutathione-S-transferase (GST) from *Schistosoma*. These proteins are both members of structural families for which several crystal structures are already known, permitting rational design of inhibitors specific for particular family members.

FBPs from two species of pathogenic bacteria have been crystallized. FBP crystals from *Haemophilus influenzae* (which causes many infections, including meningitis) diffract X-rays to 1.6 Å resolution. Anomalous scattering from these crystals unambiguously reveals the position of the iron atom at the active site. Crystals of FBP from *Neisseria gonorrhoeae* (which causes gonorrhea) diffract to 2.8 Å. Crystallographic determination of these protein structures is underway.

The crystal structure of GST from *Schistosoma japonicum* has previously been determined, both in native form and in complex with praziquantel, the leading drug used to treat schistosomiasis (McTigue et al 1995). We are working to evaluate other potential inhibitors by X-ray crystallography.

Difficult sequence and structure alignment problems encountered during the analysis of these two families of proteins have motivated the development of a general purpose sequence alignment program designed to incorporate tertiary structure information into traditional sequence alignment methods. The progress of these studies will be discussed.

(1) McTigue, M;Williams, D; and Tainer, J (1995) *J.Mol.Biol.* **246**:21-27

PS04.12.14 THE STRUCTURE OF A SELF-ASSOCIATED COMPLEX OF STROMELYSIN. Garold L. Bryant, Jr., Eric T. Baldwin, Laura C. Kelley & Barry C. Finzel, Structural, Analytical & Medicinal Chemistry, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49007

The matrix metalloproteinase stromelysin (MMP-3) has been the subject of intensive structural studies because of the apparent role of this class of enzymes in chronic inflammation and tumor progression. We report the 1.9 Å structure of an orthorhombic crystal form of the stromelysin catalytic domain (83-255) with two molecules in the asymmetric unit. In this form, the C-terminal residues of one molecule are bound in the P1-P3 subsites of the second molecule, with the carboxylate of Thr^{255} coordinated to the catalytic zinc. Most previously published peptidic inhibitors of both stromelysin and collagenase have bound toward the P' side of the substrate binding cleft. This self-complex reveals features of peptide binding on the P side. The substrate binding cleft is much wider on this side, and the bound peptide is found to lie along one edge of the cleft, making hydrogen bonds to the outermost strand (βIV) of the beta sheet that dominates the MMP folding topology. The S1 (Thr^{255}) and S2 (Glu^{254}) sidechains make no specific interactions with the enzyme, but the sidechain of S3 (Pro^{253}) is nestled into a strongly hydrophobic P3 specificity pocket formed by a juxtaposition of aromatic protein side chains (Tyr^{155}, His^{166}, Tyr^{168}). In the absence of any inhibitor occupying the P1'-P3' sites, a surrounding portion of the stromelysin structure possesses considerable flexibility.

PS04.12.15 RATIONAL DESIGN OF SULFONAMIDE INHIBITOR SPECIFIC FOR HUMAN CARBONIC ANHYDRASE I ISOZYME. Sugoto Chakravarty, Sunil Ghose, A. Bannerjee, K. K. Kannan, Bhabha Atomic Research Centre, Bombay - 400 085, India

Rational design of N - unsubstituted sulfonamide drugs which inhibit specifically a particular human carbonic anhydrase isozyme is of immense importance. From the refined crystal structures of human carbonic anhydrase I (HCAI) - sulfonamide complexes and subsequent molecular dynamics simulations, we have proposed a new sulfonamide inhibitor with stronger inhibition against HCAI. From the 2Å refined structures of three heterocyclic and aromatic sulfonamides complexed to HCAI the active site loop of L198, T199 and H200 was identified to be important for binding of the drug molecules (Chakravarty & Kannan, (1994). J. Mol. Biol., **243**, 298 - 309). The general features of binding of sulfonamides to HCAI were also revealed. The components of interaction energy which correlate well with the known inhibition constants for six sulfonamide complexes of both HCAI and HCAII were then