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