nicotinamide ring of NADPH. The diphospho bridge and adenosine portion of NADPH was clearly bound to those complexes in typical fashion, but the density for the nicotinamide ring was not visible. That portion of the cofactor was disordered but appeared to extended into solvent, being displaced from its normal binding site by the inhibitor. The width of the binding cleft of *C. albicans* DHFR was significantly larger (1.3-2.0 Å) than the corresponding dimensions of human DHFR and may be a feature that is exploited by the 5-arylthioquinazoline inhibitors. *C. albicans* DHFR crystallized in space group $P2_1$ with two molecules in the asymmetric unit. Final structures had R-factors of 0.155-0.199 at resolution values of 1.6-1.85 Å.

PS04.12.38 STEREOCHEMICAL CONSIDERATIONS IN DRUG DESIGN: THE CRYSTAL STRUCTURES OF HUMAN α-THROMBIN COMPLEXED WITH TWO TRIPEPTIDYL ALDEHYDE INHIBITORS AT 2.1Å RESOLUTION. E. Zhang, R. K. Arni, O. E. Levy#, A. Tulinsky, Department of Chemistry, Michigan State University, East Lansing, MI48824, USA, #Corvas International, Inc. San Diego, CA 92121, USA

Thrombin is a trypsin-like serine protease that plays a central role in thrombosis and hemostasis, inhibitors of which are potentially pharmacologically important as antithrombotics. The structures of many small molecule inhibitors complexed with thrombin reveal that the active site of thrombin has one anionic subsite (S1) and two hydrophobic subsites (S2 and S3) in addition to the catalytic site (Ser-195 and an oxyanion hole) .

The binding of transition-state analog inhibitors, such as peptidyl aldehydes to trypsin-like serine proteases may be modelled by two kinetic steps, a rapid, reversible binding of enzyme and inhibitor involving the three subsites of the active site followed by a slower reversible covalent-like interaction at the catalytic site. Two isomeric peptidyl aldehyde thrombin inhibitors containing 3-guanidylpiperidyl alaninal at the P1 position were designed and synthesized to examine the contributions of steric and dynamic features to selectivity and kinetics. The two inhibitors were identical except for the absolute configuration of the chiral Cγ atom. The inhibitors were soaked into thrombin-hirugen crystals and the structures of the complexes were determined and refined at 2.1Å resolution to R values of ~ 0.15. From the structures it was possible to assign an absolute configuration to each isomer and thus to correlate the structures/configurations with the observed inhibition kinetics. The kinetically fast inhibitor(Ki=5nM) was identified as the R configuration and the more normal slow-tight binding kinetics (Ki=0.3nM) was associated with the S configuration at the P1-Cγ.

The structures of the R- and S- configurations were very similar at the S2 and S3 subsites and the catalytic site, but are different at the S1 subsite. In the R-configuration, the piperidyl ring is in a chair conformation with the guanidyl group forming energetically favorable interactions with Asp-189 of thrombin. In contrast, the piperidyl ring of the S-configuration is in an energetically less favorable boat conformation with its guanidyl interacting with Asp- 189. At the catalytic site, the oxygen atom of the aldehyde group of both isomers is not in the oxyanion hole. These observations suggest that the kinetically slow step is related to the interactions of the boat form in the S1 site.

PS04.12.39 CRYSTAL STRUCTURE OF THE NOVEL CYSTEINE PROTEASE, CATHEPSIN K, IN COMPLEX WITH THE INHIBITOR E-64. Baoguang Zhao, Cheryl A. Janson, Ward W. Smith, Mike McQueney, Christopher Jones, Sherin S. Abdel-Meguid, Departments of Macromolecular Sciences and Protein Biochemistry, SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd, King of Prussia, PA 19406 USA

Cathepsin K is a recently discovered human cysteine protease with significant sequence homology to cathepsin S and cathepsin L. Cathepsin K is abundantly and selectively expressed in osteoclasts, the cells responsible for bone degradation. This observation has led to the suggestion that this enzyme plays an important role in bone resorption. The design of potent, selective inhibitors of cathepsin K should represent a new approach to the prevention of excessive bone loss in diseases such as osteoporosis.

We have determined the three dimensional structure of human cathepsin K in complex with the cysteine protease inhibitor E-64 at 2.2 angstroms resolution. The complex crystallizes in space group $P2_12_12_1$ with unit cell dimensions a = 38.4, b = 50.7, c = 104.9Å. The structure was solved using molecular replacement with the coordinates of papain as the basis for a search model. The resulting electron density confirms that cathepsin K has the identical secondary structure and the same overall fold as papain. The position and conformation of the E-64 inhibitor are clearly evident. We will describe the structure of cathepsin K including the active site of the enzyme and the interactions with the inhibitor and compare this structure with other known cysteine proteases. Knowledge of the structure of cathepsin K will be useful in the structure-based design of inhibitors of the enzyme.

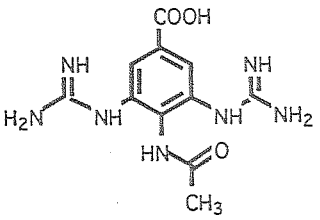
Muscle & Motor Proteins

MS04.13.01 STRUCTURAL BASIS OF MYOSIN MOTILITY. I. Rayment, A.J. Fisher, C.A. Smith, A. Gulick, R. Smith, H.M. Holden and K. Sutoh , Institute for Enzyme Research and Department of Biochemistry, University of Wisconsin, Madison WI 53705, and Department of Pure and Applied Sciences, University of Tokyo, Komba, Tokyo 153 Japan

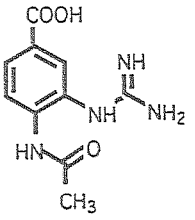
The mechanism by which chemical energy is transduced into directed movement in muscle and myosin-based motility is a fundamental question in biology. Recently considerable progress has been made towards establishing the molecular basis of the sliding filament model that was proposed over 40 years ago through the determination of the three-dimensional structures of actin (Kabsch et al., 1990, Nature, 347, 37-44) and myosin subfragment-1 (Rayment et al., 1993, Science, 261, 50-58). These have provided a structural framework for a molecular hypothesis for muscle contraction (Rayment et al., 1993, Science, 261, 58-65). Even so many questions remain concerning the structural transitions that underlie the conversion of chemical energy into directed movement. In an effort to understand how ATP hydrolysis is coupled to movement we have determined the structure of a genetically truncated myosin head in the presence of more than seven substrate analogs including MgADP, MgPPi, MgATPγS, MgAMPPNP, MgADP·BeF_x, MgADP·AlF_4, MgADP·VO_4 and several non-ATP derivatives that support tension. These complexes suggest a structural mechanism for ATP hydrolysis and new model for the conformational changes that underlie myosin-based motility.

PS04.12.34 CRYSTAL STRUCTURE OF INFLUENZA VIRUS NEURAMINIDASE WITH INHIBITOR 3,5-DIGUANIDINO-4-(*N*-ACETYLAMINO)BENZOIC ACID E. A. Sudbeck,[1] M. J. Jedrzejas,[1,2] S. Singh,[3] W. J. Brouillette,[3] G. M. Air,[2] W. G. Laver,[4] Y. S. Babu,[5] S. Bantia,[5] P. Chand,[5] N. Chu,[5] J. A. Montgomery,[5] D. A. Walsh,[5] M. Luo,[1,2] [1]Center for Macromolecular Crystallography, [2]Department of Microbiology, [3]Department of Chemistry, University of Alabama, Birmingham, AL, [4]John Curtin School of Medical Research, Australian National University, Canberra, Australia, [5]BioCryst Pharmaceuticals, Inc., Birmingham, AL.

We have designed a series of benzoic acid derivatives to fit into the active site of influenza virus neuraminidase and inhibit its enzymatic activity. Because the residues in the active site of neuraminidase are conserved for all known strains of influenza virus, these types of inhibitors have a potential to be highly effective drugs against the virus. One such compound, 3,5-diguanidino-4-(*N*-acetylamino)benzoic acid (**1**), shows measurable inhibition of neuraminidase in activity assays. We have solved the X-ray crystal structure of B/Lee/40 neuraminidase complexed with **1** at -180°C and 2.2Å resolution. The inhibitor interacts with amino acid residues in the active site of neuraminidase through specific hydrogen bonds and hydrophobic interactions. The structure is compared to the complex of neuraminidase with a related inhibitor, 3-guanidino-4-(*N*-acetyl-amino)benzoic acid (**2**). Compounds **1** and **2** interact similarly in the active site of neuraminidase except for a displacement of two structural water molecules by the 5-guanidino group of **1**.



1 (IC50 = 0.07mM)



2 (IC50 = 0.01mM)

PS04.12.35 SIALIDASE INHIBITORS TO PREVENT BACTERIAL VAGINOSIS RELATED PREMATURITY. J. Tsao, C. White, M. Luo, Center for Macromolecular Crystallography, University of Alabama in Birmingham, Birmingham, AL.35294

Bacterial vaginosis (BV) is one of the common vaginal infectious disorder affecting women of reproductive ages, including 15-20% of pregnant women. Many studies have indicated the link between BV and prematurity, but the pathophysiological mechanism are still poorly understood. However, elevated sialidase activity is directly correlated with the prematurity outcomes of pregnant BV patients. In many bacteria infections, bacteria sialidases play an important roles in host cell adhesion and have been implicated in pathogenicity. We have designed three new classes of bacteria sialidase specific inhibitors based in the active site structure of *Salmonella typhimurium* LT2 sialidase. The inhibitors were constructed using two benzoic acid-based lead compounds and targeting towards a hydrophobic pocket located near Trp 128 and a strong anionic pocket located between Asp 100 and Glu 231 in the bacterial sialidase active site. Several synthesized inhibitor compounds show measurable inhibition of sialidase activity. We soaked two of the compounds into the *Salmonella typhimurium* crystals and determined the structure of the complexes. The inhibitors interact with the active site residues through hydrogen binding and hydrophobic interactions. Those complex structures will help to design more effective inhibitors that are specific to the bacterial vaginosis related bacteria sialidase.

PS04.12.36 HIGH RESOLUTION X-RAY STRUCTURE OF BOVINE PURINE NUCLEOSIDE PHOSPHORYLASE WITH INHIBITORS. Weiru Wang and Steven E. Ealick, Section of Biochemistry, Molecular and Cell Biology, 209 Biotechnology Building, Cornell University, Ithaca, NY 14853

Purine nucleoside phosphorylase (PNP, EC 2.4.2.1) is a salvage enzyme important to the T-cell-mediated part of the immune system and as such is an important therapeutic target. This paper describes a series of high resolution x-ray structures of PNP inhibitors-9(((phosphono)-alkyl)-benzyl)-guanines, (((guaninyl)alkyl)-phosphinico)-methyl)-phosphonic acids and 9-deazaguanine derivatives, complexed with bovine PNP. These inhibitors are multi-substrate analogues and are able to bind the purine bind site, the ribose binding site and the phosphate bind site simultaneously in the PNP active site. These competitive inhibitors have high binding affinity with Ki ~10nM and lower. X-ray diffraction data were obtained at low temperature with the Macromolecular Diffraction Facility at the Cornell High Energy Synchrotron Source (MacCHESS). Enzyme-inhibitor structures were refined to resolutions ranging from 1.7 to 2.0 Å with XPLOR. Detailed inhibitor-enzyme interactions were investigated based on these high resolution structures. Residue His64 which is close to the phosphate binding site moves in and out of phosphate binding site surface when different inhibitors are bound. This movement changes the number of positive charge in the phosphate binding site and suggests that electrostatic interactions in the phosphate binding site are different for different inhibitors. A phosphonyl group attached to a 9-deazaguanine derivative interacts with two positively charged residues on the pocket surface without His64 while a free phosphate, or di-phosphate analogue group interacts with three positively charged residues including His64. The difference in electrostatic interactions might contribute to difference in binding energy. Interactions between the inhibitor and enzyme in the purine binding site and the ribose binding site are similar to the results from previous study. Small conformational and positional deviation will be described. Conformational changes of the residues in the PNP active site were also observed. These changes are complementary to the inhibitor conformation and may also contribute to binding energy.

PS04.12.37 STRUCTURES OF *CANDIDA ALBICANS* DIHYDROFOLATE REDUCTASE: HOLOENZYME AND TERNARY INHIBITOR COMPLEXES Marc Whitlow, Andrew J. Howard, David Stewart, Karl D. Hardman (Genex Corp., Gaithersburg, MD 20877); Lee F. Kuyper, David P. Baccanari, Mary Fling, Joseph H. Chan, Robert L. Tansik (Glaxo Wellcome Inc., Research Triangle Park, NC, 27709, USA).

Crystal structures of *Candida albicans* dihydrofolate reductase (DHFR) were determined as part of an effort to develop selective inhibitors for the treatment of systemic fungal infections. The incidence of systemic fungal infections has risen considerably in recent years: *C. albicans* is now the fifth leading cause of microbial infection in the hospital setting, and opportunistic fungal infections present a major problem to AIDS patients. The success of selective DHFR inhibitors as antibacterials and antimalarials prompted us to explore the development of compounds for the selective inhibition of *C. albicans* DHFR (versus human DHFR). The enzyme was cloned, expressed, and crystallized, and its structure was determined as the holoenzyme and in ternary complex with a number of inhibitors. A potent but nonselective inhibitor (a 2,4-diaminopyrrolo[3,2-d]quinazoline) bound to the enzyme as expected. However, a selective class of inhibitor (2,4-diamino-5-arylthioquinazolines) showed unusual binding properties. Two of the most selective inhibitors bound to the enzyme with the 5-arylthio moiety positioned in the site that is normally occupied by the