PS04.12.19 TOWARDS RATIONAL DRUG DESIGN: CRYSTAL STRUCTURES OF GLYCINAMIDE RIBONUCLEOTIDE TRANSFORMYLASE. Samantha E. Greasley[1], Vicente Reyes[1], Enrico A. Stura[1], Mark S. Warren[2], Stephen J. Benkovic[2], Nancy E. Haynes[1], D. Boger[1] and Ian A. Wilson[1]. [1]Departments of Molecular Biology and Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Rd, La Jolla, CA 92037. [2]Department of Chemistry, Pennsylvania State University, University Park, PA 16802.

Enzymes involved in purine or pyrimidine biosynthesis are potential targets for the treatment of degenerative diseases of the replication cycle. Glycinamide ribonucleotide transformylase (GAR Tfase) catalyses the first of two steps in de novo purine biosynthesis that require reduced folate cofactors. The enzyme catalyses the transfer of a formyl group from 10-formyltetrahydrofolate (10-$CHOH_4F$) to the amino group of glycinamide ribonucleotide (GAR) to form formyl glycinamide ribonucleotide (FGAR) and tetrahydrofolate (H_4F). The discovery that inhibitors of GAR-Tfase, derivatives of 5,10-dideazatetrahydrofolate, are able to stop proliferation of tumor cells in culture suggested this enzyme as a target for antineoplastic agents. Structure-based rational drug design has proven useful in the development of inhibitors for enzymes such as thymidylate synthase (TS), dehydrofolate reductase (DHFR) and ribonucleotide reductase. Therefore, the focus of our studies have been to investigate the crystal structures of both wild type [1,2] and mutant forms of GARTfase in complex with several substrate cofactors and inhibitors to delineate, design and test new drug lead compounds of this enzyme.

1. Chen, P. et al. & Wilson, I.A. (1992). J. Mol. Biol. 227, 283-292.
2. Klein, C. et al. & Wilson, I.A. (1995). J. Mol. Biol. 249, 153-175.

PS04.12.20 STRUCTURE-BASED DRUG DESIGN WITH HIV INTEGRASE. Greenwald J., Vix O., Farnet C.*, Bushman F.*, Choe S., Structural Biology and Infectious Disease* Laboratories, The Salk Institute, La Jolla, CA 92093

During the replication cycle of HIV, the insertion of the viral DNA into the host chromosome is catalyzed by HIV integrase. The crystal structure of a catalytically active fragment (residues 50-212) of HIV integrase (Dyda et al. Science 266:1981) has revealed that the structure is homologous to a number of polynucleotidyl transferases including RNAse H and RuvC. More recently, it has been discovered that HIV integrase has an intrinsic binding affinity to an HIV matrix protein (Gallay et al. Cell 83:569) only when the matrix protein is phosphorylated on a tyrosine. Gallay et al suggest that HIV integrase must form a complex with matrix protein in order to translocate to the host nucleus, where the catalytic action of HIV integrase occurs. Complex formation provides a potential target for drug design with the goal of blocking the viral replication cycle.

An array of compounds that mimic phosphorylated tyrosine has been soaked and co-crystallized with integrase. Diffraction data collected with synchrotron x-rays have not shown any significant binding sites for the compounds screened thus far; however, we have improved the resolution of the data from 2.5 Å to beyond 1.8 Å. An accurate atomic model of integrase would be essential for precise energetic evaluation of drug conformation and binding affinity. Efforts are being made to refine the protein model at 1.8 Å, which will be critical for locating possible binding sites of inhibitors.

PS04.12.21 HIGH RESOLUTION CRYSTAL STRUCTURE OF DEOXY SICKLE CELL HEMOGLOBIN. D. J. Harrington, K. Adachi, W. E. Royer, Jr., University of Massachusetts Medical Center, Worcester, MA 01605 and the University of Pennsylvania School of Medicine, Philadelphia, PA 19104

We have refined the crystal structure of deoxy sickle cell hemoglobin (B6 glu to val) at 2.0 Å resolution to an R-factor of 18 % (free R = 24%) using crystals isomorphous to those originally grown by Wishner and Love. A predominant feature of this crystal form is a double strand of hemoglobin tetramers that has been shown by a variety of techniques to be the fundamental building block of the intracellular sickle cell fiber. The double strand is stabilized by lateral contacts involving the mutant valine interacting with a pocket between the E and F helices on another tetramer. The new structure reveals some marked differences from the previously refined 3.0 Å resolution structure, including several residues in the lateral contact which have shifted by as much as 3.5Å \201. The lateral contact includes, in addition to the hydrophobic interactions involving the mutant valine, hydrophilic interactions and bridging water molecules at the periphery of the contact. This structure provides insight into hemoglobin polymerization and may be useful for the structure-based design of therapeutic agents to treat sickle cell disease.

PS04.12.22 TOWARDS UNRAVELLING THE STRUCTURE OF AN ANTIBIOTICS-INACTIVATING ENZYME - KANAMYCIN KINASE. Wai-Ching Hon, Gerard D Wright, Daniel S-C Yang & Albert M Berghuis, Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada. L8N 3Z5

The enterococcal kanamycin kinase (3', 5"-aminoglycoside phosphotransferase type IIIa (APH(3')) belongs to a class of enzymes that inactivate the aminoglycoside-aminocyclitol type of antibiotics. It has a promiscuous substrate spectrum and can regiospecifically phosphorylate the 3' and/or 5" hydroxyl group of eight aminoglycosides, some of which are clinically important. High resolution structural models of the enzyme is imperative to complement biochemical analyses in fully understanding its kinetic and chemical mechanisms. The eventual goal of this work is the development of more effective antibiotics or inhibitors to this enzyme.

We have overexpressed and purified the protein from E. coli. Crystals that are complexed with the enzyme's various cofactors, substrates and their analogs have been obtained. These crystals diffract on average to 2.7 Å. A full dataset of an ATP-cocrystal, which diffracted beyond 2.2 Å, has been collected at the X12C beamline at NSLS. Results of our work on the structure solution using the isomorphous replacement method will be presented.

PS04.12.23 CONFORMATIONAL CHANGES IN HIV-1 REVERSE TRANSCRIPTASE RELEVANT TO THE DESIGN OF POTENT INHIBITORS. Andrew Hopkins[1], Jingshan Ren[1], Robert Esnouf[1], Carl Ross[2], E.Yvonne Jones[1], David Stammers[2] and David Stuart[1]. [1]Laboratory of Molecular Biophysics, Rex Richards Building, South Parks Road, Oxford, OX1 3QU, UK; [2]Structural Biology Group, Glaxo-WellcomeResearch Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, UK.

Inhibition of HIV-1 reverse transcriptase (RT) is an important target for the development of anti-AIDS drugs. Nucleoside inhibitors such as AZT, 3TC and ddI suffer from serious toxicological effects and the development of drug resistant viral mutations. Non-nucleoside inhibitors (NNIs) consist of a variety of potent compounds such as TIBO, nevirapine and BHAP which are selective for HIV-1 RT and display low toxicity. The therapeutic efficacy of NNIs, however, is limited by the rapid developments

of drug resistant mutations. Several crystal structures of RT-NNI complexes determined at high resolution (Ren *et al*, 1995, *Nature Struct. Biol.* **2**, 303-308, Ren et al, 1995, *Structure.* **3**, 915- 926) show NNIs bind to a hydrophobic pocket in RT about 10Å from the polymerase catalytic site. Analyses of these crystal structures illustrate the mechanisms of NNI resistance to be mainly a loss of stabilizing van der Waals contacts between the protein and the inhibitor. To dissect out the structural requirements for the design of a potent NNI, we have determined the crystal structures of a series of HEPT analogues covering a wide range of potencies (Hopkins *et al, J.Med.Chem.*, in press). These complex structures reveal conformational changes in the protein some of which correlate with the potencies of the HEPT analogues. The major determinant of increased potency is the improved ring stacking interactions between the 6-benzyl ring of the inhibitors and Tyr181. The conformational switching of Tyr181 into its more exploitable position is caused by steric interactions with the 5-position substituent on the pyrimidine ring. All tight binding NNIs possess groups which throw this conformational switch.

PS04.12.24 STRUCTURE-BASED DRUG DESIGN OF A NOVEL SERIES OF HUMAN CATHEPSIN D INHIBITORS. Angela Y. Lee, Pavel Majer, Sergei V. Gulnik, Jack Collins, Abelardo M. Silva, Narayana T. Bhat and John W. Erickson, Structural Biochemistry Program, SAIC-Frederick, National Cancer InstituteFrederick Cancer Research and Development Center, Frederick, Maryland 217021201

Cathepsin D (Cat D) is a lysosomal aspartic protease implicated in many aspects of pathology such as cancer and Alzheimer disease, thus representing a novel target of therapeutic importance. Development of specific and bioavailable Cat O inhibitors would aid in delineating its role in normal and disease states. Based on our recently solved X-ray structures of human Cat D and its complex with pepstatin A, a new series of inhibitors for Cat D have been designed, synthesized and kinetically characterized. In our attempt to better understand their modes of binding and to aid in our future design, crystallographic studies of human Cat D complexed with some of these inhibitors were carried out. The crystal structure of a complex with a linear statine-based compound will be compared with that of a related cyclic analog, as well as with the model structures.

PS04.12.25 THREE DIMENSIONAL STRUCTURES OF E.COLI PNP COMPLEXES. Chenglong Li and Steven Ealick, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853

We present here several complex structures of E.Coli PNP with its substrates and substrate analogs. Purine nucleoside phosphorylase (PNP) catalyzes the reversible phosphorolysis of purine ribo or 2'-deoxyribonucleosides to the purine and ribose or 2-deoxyribose-1-phosphate. The enzyme has been isolated from both eukaryotic and prokaryotic organisms and functions in the purine salvage pathways. The human and bovine PNPs are specific for the 6-oxo-purines and many of their analogs,and both are trimers with identical subunits. E.Coli PNP represents another class of PNP identified from various sources,which is hexameric,has no sequence similarity with human and bovine PNPs and accepts both 6-amino and 6-oxopurines as substrates. E.Coli PNP has a strikingly different active site and binding features to its substrates compared with that of mammalian PNPs. The phosphate binding site consists of backbone Gly20 and three arginine residues Arg43 and Arg87 from one subunit and Arg24 from the neighboring subunit. The three arginine residue hold the phosphate to form a strong binding net and yet are flexible enough to allow phosphate to initiate the nucleophilic attack due to the long arms of arginine residues. E.Coli PNP does not undergo a large conformational change during the enzymatic reaction like in the case of mammalian PNP. The base binding site consists of residues Phe159,Phe167,Ile206,Val178 and Asp204. It is mainly made of hydrophobic residues and is more open (therefore maybe less specific) than its mammalian counterpart. Another remarkable feature is that the nucleoside binding conformations in the E.Coli and mammalian PNP are totally different. Although the ribosyl and phosphate groups bind to E.Coli PNP and mammalian PNP in similar ways,the purine base is rotated 180 degree about the glycosidic bond. E.Coli PNP has only one H-bonding residue Asp204 and mammalian PNPs have two H-bonding residues Asn243 and Glu 201 near the purine base.

PS04.12.26 THROMBIN COMPLEXES WITH THIAZOLE-BASED INHIBITORS: USEFUL PROBES OF THE S1' BINDING SITE. John H. Matthews*, Raman Krishnan*, Michael J. Costanzo#, Bruce E. Maryanoff#, A. Tulinsky*, * Department of Chemistry, Michigan State University, East Lansing, MI 48824, #The R. W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477

The serine protease thrombin plays a central role in blood clotting with the most prominent function being the conversion of fibrinogen to fibrin in the later stages of the coagulation cascade. A large number of inhibitor-thrombin complexes have been studied by X-ray crystallography. Most of these inhibitors bind to one or the other of the S1-S3 subsites of the active site or the fibrinogen recognition exosite. Less is known of the binding at the S' subsites that involve substrate residues downstream from the point of cleavage. We report here the results of S1'-binding thiazole-containing groups and the implications for the future design of inhibitors. The potent thrombin inhibitor RWJ-50353 is a tripeptide with a D-Phe-Pro-Arg motif (PPACK) with a benzothiazole group. The other inhibitor, RWJ-50215, is related to DAPA, dansylarginine N-(3-ethyl-1,5-pentanediyl) amide, an early member of a class of inhibitors based on the chemical nature of thrombin binding sites and contains a 2-ketothiazole group.

The RWJ-50353-hirugen-thrombin structure was refined to an R value of 0.168 in the (7.0-2.3) Å resolution range with 125 water molecules, while the RWJ-50215 complex converged at an R value of 0.155 in the (7.0-1.8) Å resolution range with 161 water molecules.

Binding in the S1-S3 subsites is similar to the parent compounds PPACK (RWJ50353) and DAPA (RWJ-50215). The benzothiazole in RWJ-50353 and the thiazole in RWJ-50215 bind at the S1' site of thrombin. There they are surrounded by His57, Tyr60A, Trp60D, Lys60F of thrombin, and in the case of RWJ-50215, also by the piperidine ring of the inhibitor. In RWJ-50353, the N1 atom of the benzothiazole forms a hydrogen bond with His57NE2 (2.7 Å) and the indole ring of Trp60D stacks edge onto the face of the benzothiazole ring. The sidechain of Lys60F is displaced from its normal position by the bulky benzothiazole group. Both N1 and O1 of the 2-ketothiazole of RWJ-50215 form hydrogen bonds with the sidechain of Lys60F.