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