MS04.12.03 CRYSTAL STRUCTURES OF HIV-1 RT AND NON-NUCLEOSIDE INHIBITOR COMPLEXES: IMPLICATIONS FOR DRUG DESIGN. Jingshan Ren[1], Robert Esnouf[1], Andrew Hopkins[1], Carl Ross[2], E.Yvonne Jones[1], Dave Stammers[2] and D.Stuart[1]., [1]Laboratory of Molecular Biophysics, Rex Richards Building, South Parks Road, Oxford, OX1 3QU, UK., [2]The Glaxo-Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, UK.

Crystal structures of a number of complexes of HIV-1 RT with non-nucleoside inhibitors (NNIs), including nevirapine, α-APA, 1051U91, HEPT, 9-Cl-TIBO, MKC-442 and TNK-651, have been determined at high resolution (Ren *et al.* 1995 *Nature Struct. Biol.* **2**, 293-302; Esnouf *et al.* 1995 *Nature Struct. Biol.* **2**, 303-308; Ren *et al.* 1995 *Structure* **3**, 915-926; Hopkins *et al. J. Med. Chem.* in the press). All the inhibitors we have studied bind at same pocket formed between two β-sheets of the p66 palm, some 10 Å from the polymerase active site. The internal surface of the pocket is predominantly hydrophobic being constructed mainly from leucine, valine, tryptophan and tyrosine residues. The structures reveal a common mode of binding for these chemically diverse compounds. Each compound has its own particular structural characteristics and there is sufficient plasticity in certain regions of the surrounding protein to allow some unfavourable contacts to be relieved without changing the overall binding mode. The volume of the pocket varies with the inhibitors, ranging between some 600 and 700 Å^3, of which the inhibitors occupy in the order of 250-350 Å^3. However, much of the pocket lining remains very similar in all cases so that there is a very marked matching of shape in those compounds that occupy this volume. In some cases this is achieved by conformational rearrangement of the compound from its lowest energy structure in solution. These results allow us to understand the structural basis of the potency of the inhibitors and suggest possible modifications which should improve interactions with the enzyme.

MS04.12.04 AN INFLUENZA VIRUS NEURAMINIDASE VARIANT WITH DECREASED SENSITIVITY TO THE NEURAMINIDASE INHIBITOR 4-GUANIDINO-NEU5AC2EN. J.N.Varghese, P.M.Colman, T.J. Blick, T.Tiong, S.Sahasrabudhe, & J.L. McKimm-Breschkin, Biomolecular Research Institute, Parkville, Victoria 3052.

A variant of influenza virus with 1000-fold less sensitivity in vitro to the anti-influenza drug 4-guanidino-Neu5Ac2en has been isolated by multiple passage of influenza virus NWS/G70C with the N9 subtype neuraminidase in MDCK cells in the presence of 4-guanidino-Neu5Ac2en. The crystal structure of this variant of influenza virus NWS/G70C neuraminidase, has been determined to 2 Angstrom resolution for the native enzyme and a complex with 4-guanidino-Neu5Ac2en. This study has confirmed a single site mutation of a conserved active site residue in the floor of the active site pocket, Glu 119 to Gly, and suggests that the reduced affinity for the 4-guanidino derives partly from loss of stabilising interaction between the guanidino moiety and the carboxylate at residue 119, and partly from alterations in the solvent structure of the active site. A water molecule was found close to the position formerly occupied by one of the glutamic acid carboxylate oxygens in the wild type structure, and this water molecule Hydrogen-bonds with the secondary amino nitrogens of an active site arginine (156).

MS04.12.05 STRUCTURE BASED DESIGN OF THROMBIN ACTIVE-SITE INHIBITORS. John S. Sack[1], Shari L. Ohringer[1], ChiehYing Y. Chang[1], Mary F. Malley[2], Lydia Tabernero[1,3] and Howard M. Einspahr[1], Departments of Macromolecular Crystallography[1] and Solid State Chemistry[2], Bristol-Myers Squibb Pharmaceutical Research Institute, P.O Box 4000, Princeton, NJ 08543-4000 U.S.A. [3]Current address: Department of Biological Sciences, Lilly Hall of Life Sciences, Purdue University , West Lafayette, IN 47907 USA.

Human thrombin, an important enzyme in the blood coagulation response, has been crystallized in the presence of a variety of high affinity, synthetic active-site inhibitors. X-ray crystallography has been used to determine the orientations of the inhibitors in the thrombin active site and the modes of inhibitor binding. Knowledge of the specifics of inhibitor binding proved useful in the design of successively more potent inhibitors.

Our laboratory used high molecular weight polyethylene glycol precipitations at acidic pH to produce crystalline ternary complexes of thrombin with active site inhibitors and an exosite blocking agent. This inhibitor design effort will be described in the context of the overall thrombin effort in the pharmaceutical community. In our case, the most important contributions were to examine those structures that did not fit the expected structure-activity relationships. It was by analyzing these structures that we were able to provided the chemists with new and useful design information.

MS04.12.06 SPELUNKING STROMELYSIN: A CASE HISTORY OF STRUCTURE BASED DRUG DESIGN Jens J. Birktoft, R. Crowther, U. Kammlott, B. Graves, D. Waugh, W. Levin, N. Fotouhi, K. Hull, A. Hanglow, S. Pietranico, C. Michoud and M. Visnick. Roche Research Center. Hoffmann La-Roche Inc., Nutley, NJ,. USA.

Stromelysin-1 (MMP-3) is a member of a group of zinc containing proteolytic enzymes named the metalloproteinases (MMPs). Stromelysin has been implicated in rheumatoid arthritis and osteoarthritis as well as in the metastatic phase of cancer. A characteristic feature of these diseases is the irreversible erosion of connective tissue. The established roles of MMPs in these demographically important diseases make these enzymes ideal targets for pharmacological intervention. Two routes have been taken in the development of MMP inhibitors, one starting from the structure of putative substrate (and product) - enzyme complexes, while the other approach, to be discussed here, is rooted in the perceived organization of the pro-form of the MMPs. Specifically, the interactions of the so-called switch region (#73-PRCGVP-#78) in the pro-segment with the protease domain featuring ligation of zinc by cysteine formed the starting point for the design of inhibitors targeted toward stromelysin (Fotouhi et al, JBC, 269, 30227, 1994). Comparison of high resolution crystal structures of a truncated form of prostromelysin and of a complex formed between active stromelysin and a tight binding cyclic peptide demonstrated that the switch region peptide binds to stromelysin in nearly identical manner in prostromelysin and the cyclic peptide. In both prostromelysin and the inhibitor complex a mixed beta-sheet structure is formed between the RCGV peptide and the protein. Furthermore, the orientation of this peptide is opposite to that observed with peptides bound in a substrate-like mode. Additionally, analysis of these two crystal structures revealed that the zinc-bound cysteine side chain only partially fills the S1' substrate binding site. The remainder of this large cavity, which extends through the protein core, is occupied by solvent molecules. Additional, but smaller binding cavities form the S1-S3 and S2'-S4' subsites. The binding properties of the large S1' binding site have been exploited in the development of inhibitors derived from a cysteine or iso-cysteine building block and incorporating aromatic moieties. The analysis of several crystal structures will be described, and will include descriptions of enzyme conformational changes induced by different ligands and as well as changes in ligand interactions resulting from ligand modifications.