MS36 COMBATING VIRUSES
Chairpersons: Elspeth Garman, Ming Luo

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Non-nucleoside Inhibitors of NS5B Polymerase from HCV, Genotypes 1b and 2a
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Hepatitis C virus (HCV) is an important human pathogen affecting ~ 3% of the world's population. The current antiviral therapies (a combination of pegylated interferon and ribavirin) are of limited efficacy and often have severe adverse side effects. Structures of the RNA dependent RNA polymerases from HCV, genotypes 1b [1] and 2a complexed to a variety of non-nucleoside non-competitive inhibitors reveal a common binding site that is ~ 35 Å from the polymerase active site. Two crystal forms, I and II, of the 2a genotype unbound RdRp reveal a "closed" or active form of the polymerase and an "open" or inactive form, respectively. The difference in conformation lies in the relative orientation of the fingers and thumb domains of the molecule. Inhibitors bind only to the form I (active) conformation and will not bind to the form II (inactive) crystals. The binding of the inhibitors triggers the conformational changes from the active to the inactive conformation in the crystals. (Research supported in part by CIHR, AHFMR and Virochem Pharma Inc. MNGJ gratefully acknowledges the receipt of a Canada Research Chair).

[1] Wang M., Ng K.K., et al., *J. Biol. Chem.*, 2003, **278**, 9489.
Keywords: hepatitis c virus, non competitive inhibitor, conformational change

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Crystal Structures of SARS Coronavirus Proteins
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Since the 2003 SARS outbreak, which has now subsided, our laboratory has worked to obtain a series of important results in SARS basic research. These results can be summarized as follows:

We successfully determined the structure of the SARS coronavirus main proteinase (M^{pro} or $3CL^{pro}$) and its complex with an inhibitor in 2003. This was the first structure of any protein from the SARS coronavirus to be determined in the world. The SARS-CoV M^{pro}, which is a 33.8-kDa protease (also called the 3C-like protease), plays a pivotal role in mediating viral replication and transcription and is therefore an important target for the design of anti-SARS drugs. We have used the SARS M^{pro} structure to design a series of inhibitors that are effective against four kinds of coronavirus. We have also analyzed the structures of the SARS M^{pro} and the porcine transmissible gastroenteritis virus (TGEV) M^{pro} in complex with the above inhibitors. This series of crystal structures, together with biochemical data, provide an important structural basis for rational drug design.

The second crystal structure to be determined from our laboratory is the SARS-CoV membrane fusion protein. The coronavirus spike (S) protein, an enveloped glycoprotein essential for viral entry, belongs to the class I fusion proteins and is characterized by the presence of two heptad repeat (HR) regions, HR1 and HR2. These two regions are understood to form a fusion-active conformation similar to those of other typical viral fusion proteins. The crystal structure of the SARS-CoV fusion core protein is a six-helix bundle with three HR2 helices packed against the hydrophobic grooves on the surface of a central coiled coil formed by three parallel HR1 helices in an oblique antiparallel manner. We have also determined the mouse hepatitis virus (MHV) S protein fusion core and proposed a conserved molecular mechanism by which the S protein mediates the coronavirus membrane fusion and subsequent viral entry. This work provides a new avenue for the design of anti-SARS therapeutics via strategies aimed at inhibiting viral entry by blocking hairpin formation.

Recently, a third structure has been solved in our laboratory. The complex structure between two non-structural proteins reveals exciting new functional insights into the SARS coronavirus.
Keywords: SARS, coronavirus, crystal sructure

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Structure, Mechanism and Specificity of FMDV 3C Protease
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Foot-and-mouth disease virus (FMDV) causes a widespread and economically devastating disease of domestic livestock. The viral RNA genome is translated as a single polypeptide precursor that must be cleaved into functional proteins by virally-encoded proteases. Ten of the thirteen cleavages are performed by the highly conserved 3C protease ($3C^{pro}$), making the enzyme an attractive target for anti-viral drugs. We have developed a soluble, recombinant form of FMDV $3C^{pro}$, determined the crystal structure to 1.9 Å resolution and analysed the cleavage specificity of the enzyme. The structure indicates that FMDV $3C^{pro}$ adopts a chymotrypsin-like fold and possess a Cys-His-Asp catalytic triad in a similar conformation to the Ser-His-Asp triad conserved in almost all serine proteases. This observation suggests that the dyad-based mechanisms proposed for this class of cysteine proteases need to be re-assessed. Peptide cleavage assays revealed that the recognition sequence spans at least four residues either side of the scissile bond (P4-P4´) and that FMDV $3C^{pro}$ discriminates only weakly in favour of P1-Gln over P1-Glu, in contrast to other $3C^{pro}$ enzymes that strongly favour P1-Gln. The relaxed specificity may be due to the unexpected absence in FMDV $3C^{pro}$ of an extended β–ribbon that folds over the substrate binding cleft in other picornavirus $3C^{pro}$ structures. Collectively these results establish a valuable framework for the development of FMDV $3C^{pro}$ inhibitors.
Keywords: viral protease, catalytic mechanism, antivirals

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HIV Reverse Transcriptases: Structural Basis for Inhibition and Drug Resistance
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HIV reverse transcriptase (RT) is one of the main target sites for the action of anti-AIDS drugs. Two classes of anti-RT drugs are in clinical use: nucleoside analogues (NRTIs), which bind at the dNTP site and cause DNA chain termination, whilst the non-nucleoside inhibitors (NNRTIs) bind in a pocket distal to the polymerase active site. Extensive crystallographic studies have been used to define the overall architecture of the HIV-1 RT p66/p51 heterodimer, the binding and mode of inhibition for the NNRTIs as well as the binding sites for NRTI drugs. Due to the rapid turnover of HIV and the low fidelity of transcription of RT, drug resistance rapidly emerges which presents a challenge to continued suppression of the virus. Structural studies of many mutant HIV-1 RTs resistant to NNRTIs have shed light on the structural basis for drug resistance and how 'second-generation' compounds are more resilient to the presence of mutations. For RT from the different serotype HIV-2, the crystal structure points to the mechanism of its inherent resistance to NNRTIs. The significant data base of HIV RT structures are being used in structure based design approaches. A number of successful studies have been reported and NNRTIs with greatly improved activity against common drug resistant forms of HIV are now in clinical trials. Thus, although RT was the