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

target for the first anti-HIV drugs, it still has potential for development of new drugs including the targeting of as yet unexploited regions such as the RNaseH active site and tRNA primer binding.

Keywords: HIV reverse transcriptases, inhibitor binding, drug resistance

MS36.26.5

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Structure-based Vaccine Design of Human Rhinovirus: HIV Chimeras as Candidate AIDS Vaccines

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Our laboratory team has developed a system for generating combinatorial libraries of cold-causing human rhinoviruses (HRVs) that effectively display immunogenic peptide segments from a variety of pathogens. We have used this system to generate chimeric HRV-HIV-1 viruses displaying regions of the HIV-1 membrane-spanning protein gp41 that are part of the conserved and critical viral fusion machinery. We have generated chimeric HRVs displaying the so-called ELDKWA epitope of this region of gp41 that elicit immune responses able to broadly and potently cross-neutralize HIV-1 primary isolates, the first neutralizing responses reported for any ELDKWA-based immunogens. Ultimately, such immunogens might serve as valuable constituents in an AIDS vaccine.

Structural considerations for this vaccine engineering system will be discussed. We have obtained diffraction data at CHESS and BNL for several HRV:HIV-1 chimeras; structure determination is in progress. We are also investigating structures of chimeric virus complexed with anti-HIV neutralizing antibodies or Fab fragments. An important long-term goal is to identify three-dimensional correlates of immunogenicity and apply the knowledge to facilitate vaccine design and development using a structure-based approach.

Keywords: virus structure, virus engineering, immunology

MS37 INTRACELLULAR TRAFFICKING OF BIOMOLECULES

Chairpersons: Yoshiro Yoneda, David Owen

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Transport out of the Nucleus and Beyond: Molecular Mechanisms

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The movement of proteins and RNAs between the nucleus and cytoplasm of eukaryotic cells is mediated by nucleo-cytoplasmic transport receptors. Most receptors belong to the karyopherin β family of protein, which are also known as importins or exportins according to whether they import or export cargo into/from the nucleus. The directionality of import and export processes depends on the small GTPase, Ran. In contrast to most proteins/RNAs, mRNAs are transported out of the nucleus by a transport factor unrelated to the karyopherin family. mRNA export is linked to quality control mechanisms that make sure that only correctly transcribed and processed mRNAs are exported and translated. A ubiquitous quality control mechanism is nonsense-mediated mRNA decay (NMD). NMD is a surveillance pathway that detects mRNAs containing premature translation termination codons (PTCs) and degrades them before they give rise to truncated protein products. In humans, detection and degradation of PTC-containing mRNAs is dependent on splicing. The splicing-dependence is correlated to the exon junction complex (EJC), a multiprotein assembly that is deposited on mRNAs at the end of splicing upstream of exon junctions. EJC components mark aberrant mRNAs for detection by the NMD machinery and deliver the targeted mRNA to degrading enzymes such as the exosome.

X-ray structures of components of the mRNA export/surveillance machinery give insights on the molecular mechanisms with which they function.

Keywords: protein-RNA interactions, macromolecular assemblies, intracellular trafficking

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Cse1: the Structure of an Exportin in its Closed, Cytosolic State

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Cse1 mediates nuclear export of importin-alpha, the nuclear localization signal (NLS) import adaptor. We report the 3.1Å resolution structure of cargo-free Cse1, representing this HEAT-repeat protein in its cytosolic state. Cse1 is compact, consisting of N- and C-terminal arches that interact to form a ring. Comparison with the structure of cargo-bound Cse1 shows a major conformational change leading to opening of the structure upon cargo binding.

The largest structural changes occur within a hinge region centered at HEAT repeat 8. This repeat contains a conserved insertion that connects the RanGTP and importin-alpha contact sites and that is essential for binding. In the cargo-free state, the RanGTP binding sites are occluded and the importin-alpha sites are distorted. Mutations that destabilize the N- to C-terminal interaction uncouple importin-alpha and Ran binding, suggesting that the closed conformation prevents association with importin-alpha.

Keywords: Cse1, exportin, nuclear transport

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Cracking of the Targeting Signal Embedded in Mitochondrial Presequences

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Most mitochondrial proteins are synthesized in the cytosol as precursor proteins with a cleavable N-terminal presequences and are imported into mitochondria. Protein import into mitochondria is mediated by protein assemblies in the mitochondrial membranes. A subunit, Tom20, functions as a general protein import receptor by recognizing presequences of preproteins. Although no consensus sequence is found, Tom20 recognizes a wide variety of presequences.

To understand the structural basis of the presequence recognition, we determined the NMR and crystal structures of Tom20 in a complex with a presequence peptide. Note that the presequence was fixed to Tom20 via a designed intermolecular disulfide bond to obtain crystals. The bound presequence forms an amphiphilic a-helix. NMR titration experiments indicated the presence of a unique presequence binding site in Tom20, and defined a common five-residue pattern in different presequences. To refine this pattern, we introduced a new peptide library approach using the formation of an intermolecular disulfide bond. We propose that a presequence is regarded as a collective entity of short amino acid sequences that are recognized by several proteins including Tom20. The organization (position, order, and overlapping) of these binding segments is unique for each presequence. This view explains why no consensus sequences are found by simple sequence comparisons.

Keywords: protein transport, molecular recognition, crystallographic and NMR solution state structures

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Vps29: a Phosphoesterase Fold that acts as an Interaction Scaffold in the Assembly of Retromer

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