MS09 O1

MTases and helicases: a medium-throughput approach to viral protein structures M. Milani, E. Mastrangelo, M. Bollati, G. Sorrentino and M. Bolognesi.
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In the context of the European VIZIER project, our lab is involved in the study of flaviviral methyltransferases (MTase) and helicases (Hel). Flaviviruses are enveloped positive-strand RNA viruses, which code for 3 structural and 7 non-structural (NS) proteins. Among the NS, particularly important are the multifunctional proteins NS3 (protease/helicase) and NS5 (Mtase/RNA-polymerase).
Flaviviral Hel participate in RNA replication separating the RNA template and daughter strands. We report here the three-dimensional structure (at 3.1 Å resolution) of the NS3 helicase domain (residues 186-619; NS3:186-619) from Kunjin virus, an Australian variant of the West Nile virus. As for homologous helicases, NS3:186-619 is composed of three domains, two of which are structurally related and held to host the NTPase and RTPase active sites. The third domain (C-terminal) is involved in RNA binding/recognition. In addition, we analyzed the activity of the full-length protein and its structure in solution using small angle X-ray scattering (SAXS). Our results show a strong influence of the NS3 protease domain on the helicase activity that can scarcely be explained in term of domains organization and requires further investigations.
MTases are involved in the mRNA capping process, resulting in the transfer of methyl groups from the cofactor S-adenosyl-L-methionine (AdoMet) to a capped RNA substrate. We solved the crystal structures of the Wesselsbron virus methyltransferase (^{Wv}MTase) in complex with AdoMet and with both the cofactor and the capped substrate GpppG, at 2.0 Å and 1.9 Å resolution, respectively. Wesselsbron is an African mosquito-borne *Flavivirus* belonging to the Yellow Fever virus group that affects animals and human beings. Comparison of the two structures shows that the presence of GpppG stabilizes the N-terminal subdomain, as indicated by the higher B-factor values relative to the other MTases. In order to further characterize the function and catalytic activity of ^{Wv}MTase, assays with different substrates are in progress.

MS09 O2

Crystal structure of the postfusion form of the baculovirus GP64 protein Jan Kadlec[a], Silvia Loureiro[b], Ian M. Jones[b] and David I. Stuart[a], [a] *Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, UK.* [b] *School of Biological Sciences, University of Reading, UK.*
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Keywords: viral entry, fusion protein, baculovirus

Viral entry of the enveloped viruses to the host cells requires recognition and attachment to cell receptor or receptors followed by a fusion of the viral and host membranes that results in the delivery of the viral genetic material into the cytoplasm of the host cell. The fusion reaction is driven by viral fusion proteins that undergo a major conformational change triggered by the interactions with target cell. We determined the crystal structure of baculovirus surface glycoprotein GP64 in its postfusion conformation at 2.95 Å resolution. The structure reveals a multidomain trimer with unexpected homology to recently reported structures of VSV G and herpesvirus gB proteins. GP64 is thus the third example of a fusion protein that does not belong in either of the previously established classes of type I and type II fusion proteins. Structural similarities within this new group as well as across all three types of fusion proteins will be discussed. Identification of the fusion loops in domain IV based on the homology to VSV G protein allows for genetic manipulation of GP64 that might improve its ability to promote membrane fusion and consequently facilitate the use of baculovirus as a vector in gene therapy, protein display and protein expression in insect cells.

MS09 O3

Structural and mutagenic analysis of foot-and-mouth disease virus 3C protease Trevor R. Sweeney[a], Patricia A. Zunszain[a], Núria Roqué-Rosell[b], Robin J. Leatherbarrow[b] Stephen Curry[a]. [a]*Biophysics Section, Division of Cell and Molecular Biology and* [b]*Biological and Biophysical Chemistry Section, Department of Chemistry, Imperial College London.*
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Keywords: foot-and-mouth disease virus, 3C protease, enzyme activity

The 3C protease from foot-and-mouth disease virus (FMDV) is a potential target for anti-viral drug design. We have determined the structure of a new crystal form of FMDV 3C^{pro}, which reveals features that are important for catalytic activity [1]. In particular we show that a surface loop, which was disordered in previous structures [2], adopts a β-ribbon structure that is similar in conformation to equivalent regions on other picornaviral 3C proteases and some serine proteases. This β-ribbon folds over the peptide binding cleft and clearly contributes to substrate recognition. Substitution of Cys142 at the tip of the β-ribbon with different amino acids has a significant impact

on enzyme activity and shows that higher activity is obtained with more hydrophobic sidechains. Comparison of the structure of FMDV 3C^{pro} with homologous enzyme-peptide complexes suggests a cogent explanation for the effects of these mutations. We are now working on recently-obtained crystals of a 3C-peptide complex and will present our new insights into the molecular basis of the specificity and mechanism of this important enzyme.

[1] Sweeney *et al.* (2007) *J. Virology* 81, 115-124.
[2] Birtley *et al.* (2005) *J. Biol. Chem.* 280, 11520-11527.