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The structural basis for the essential PA-PB1 subunit interaction in influenza RNA polymerase

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Influenza A virus is a major human and animal pathogen with the potential to cause catastrophic loss of life. Recently, the emergence of a novel H1N1 viral strain has affected the entire world. Additionally, a highly pathogenic avian influenza caused by H5N1 strain, has a potential of a next pandemic. Most current influenza drug target is haemagglutinin(HA) or neuraminidase(NA), and these protein present at the virion surface. Sixteen different HA subtypes and nine different NA subtypes have been identified. Oseltamivir(Tamiflu) and zanamivir(Relenza) are NA inhibitors, and prevent viral particles being released from infected cells. These drugs have been stocked in world wide, but resistant influenza is already emerging. Another anti-influenza drug amantagine targets the M2 protein of the viral proton channel. However, a single residue change is sufficient to confer resistance. Both oseltamivir and amantadine target proteins with a single known function and substantial sequence variation between viral strains.Therefore, we have to prepare a new method for a new highly pathogenic and oseltamivir-resistant influenza.

The viral RNA polymerase is not yet a target of any approved pharmaceutical, but has recently become a focus for the development of new anti-influenza drugs since it is highly conserved in avian and human influenza. It carries out a number of essential processes in the viral life cycle, many of which remain poorly understood. The three subunits, PB1, PB2 and PA play different roles within the polymerase, and are all essential for viral replication but despite considerable functional analysis relatively little is known about their structure.

Here, we solved the crystal structure of PA-PB1 subunit interaction. The carboxy-terminal domain of PA forms a novel fold, and forms deep, highly hydrophobic groove into which the amino-terminal residues of PB1 can fit by forming a helix.[1] Furthermore, we have found highly conserved residues which are essential for these interactions, and demonstrated that the interruption of these interfaces dramatically reduces viral replication. These interfaces have considerable potential as a drug target sites, which are entirely independent of surface antigen type.

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Crystal structure of measles virus hemagglutinin with its human receptor CD46

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Measles virus (MV) remains as a worldwide disease even though there is an effective vaccine available since the sixties. Almost eradicated in most industrialized countries, MV still causes frequent outbreaks, although in developing countries 20 million cases and 300,000 deaths are reported every year annually mainly due to deficiencies in vaccine administration. Therefore, MV has been a priority in World Health Organization vaccination programs for its complete eradication by the end of the 20th century.

Despite all the efforts against MV, little is known about the molecular machinery used by the virus to infect host cells and the early events occurring after virus particle attachment to the host cell. Infection is initiated by attachment of the measles virus hemagglutinin (MV-H), a glycoprotein anchored to the virus envelope, to the host cell receptors CD46 or signaling lymphocyte activation molecule (SLAM).

In this work, we report the crystal structure of MV-H in complex with a CD46 protein spanning the two N-terminal domains. A unique groove at the side of the MV-H β-propeller domain, which is absent in homologous paramyxovirus attachment proteins, engages residues in both CD46 domains. Key contacts involve a protruding loop in the N-terminal CD46 domain that carries two sequential proline residues (PP motif) and penetrates deeply into a hydrophobic socket in MV-H. Viral residues within this extended groove can have certain variability whereas less accessible residues are conserved, which leads to an extended MV tropism by increasing CD46 binding affinity and preserving SLAM binding. The relatively variable and concave surface onto which the CD46 interdomain interface and the SCR2 dock represents an area well suited to accommodate diverse receptor molecules binding to an inaccessible socket in the MV-H protein. Therefore, the use of an extended surface on the side of the β-propeller domain for receptor binding by MV forms the basis for a strategy to extend the virus tissue tropism by receptor-specificity switching.

Keywords: measles, CD46, hemagglutinin.

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Studying interactions between vaccinia virus protein a46 and the cellular protein MyD88

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Vaccinia virus, closely related to smallpox virus, is a member of the poxvirus family. It is well known for its various strategies to inhibit the immune response of cells of the innate immune system, but the mechanisms of the majority of these important interactions have not been clearly studied. Vaccinia virus encodes a wide range of proteins, which target the pathogen signal transduction pathway from the plasma membrane to the nucleus, and finally prevent production of proinflammatory and antiviral cytokines. Our main goal is to define mechanisms of inhibition of NFκB (the central transcriptional factor during an antiviral response) activation by certain vaccinia proteins by solving their structure and structural mechanisms of interaction with cellular proteins. A profound knowledge of such viral interference strategies will help to understand molecular aspects of viral pathogenesis and to develop novel cell defense approaches. It will give also a deeper insight into cell defense mechanisms.

The vaccinia virus A46 protein comprises 240 a.a. and according to structure predictions has a single globular domain with a short hypothetical unstructured C-terminus. Both A46 alone and a fusion with maltose binding protein (MBP) expression vectors were constructed. Only the fused protein construct was expressed at the sufficient level for further studies. Using TEV proteolysis, we could cleave A46 protein from the MBP-A46 construct. Both MBP-A46 and cleaved A46 protein were purified to homogeneity. The MBP-A46 construct behaved as a dimer where as A46 alone behaved as a tetramer on size exclusion chromatography. A limited proteolysis experiment confirmed the presence of one domain in A46. We set up a number of crystallization screens with A46 protein but failed to obtain any reproducible crystals.

The cellular target of A46 protein is the adaptor protein MyD88, which participates in numerous signal transduction cascades during both innate and acquired immune responses. The MyD88 protein is composed of two domains, including the C-terminal TIR (Toll/IL1-like) domain. According to bioinformatic predictions, the viral A46 protein should contain a TIR fold, similar to MyD88. Thus, the TIR domain in MyD88 is believed to be the site of the interaction between A46 and its target. To test this hypothesis, the C-terminal part of the murine MyD88 protein, comprising the TIR domain (146-296 a.a.), was expressed with a GST-tag and purified. We will now study the A46 and MyD88 interactions via pull-down assays and isothermal titration calorimetry, as well as crystallization of the purified complex.

Keywords: vaccinia, A46, MyD88

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Reorganization of the shell protein during the maturation of bacteriophage T7

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Maturation of dsDNA bacteriophages involves the assembly of the virus prohead from a limited set of structural components followed by rearrangements required to acquire the stability that is necessary for infecting a host under challenging environmental conditions.

Bacteriophage T7 infects *Escherichia coli* and belongs to the Podoviridae family. It has a 40kb dsDNA, an icosahedral capsid with a triangulation number T=7, and a non-contractil tail [1]. The virus shell is made of 415 copies of protein gp10A. The prohead also contains a scaffolding protein (gp9) and a dodecameric connector (gp8) that attaches a core complex to one of the 12 5-fold vertices. The structure of of the T7 prohead and mature head have been solved by three-dimensional cryo-electron microscopy [2], and quasi-atomic models for the procapsid and the mature capsid shells have also been obtained [3, 4], revealing that the main protein gp10A has an HK97-like fold.

The comparison of both structures reveals the molecular basis of T7 shell maturation [4]. The mature capsid presents an expanded and thinner shell than the prohead, with a drastic rearrangement of the protein monomers that increases their interacting surfaces, in turn resulting in a new bonding lattice. The rearrangements include tilting, in-plane rotation, and radial expansion of the subunits, as well as a relative bending of the A- and P-domains of each subunit.

Our results suggest that T7 might represent one of the simplest primordial capsid maturation mechanisms.

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Single crystal X-ray diffuse scattering from supra-molecular assemblies

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Unlike Bragg scattering which only contains single body information, diffuse scattering contains two-body information and this enables the local structure and dynamics to be probed. In some instances static (occupational) disorder may feature in these studies but in others the disorder is of thermal origin and the resulting structured *thermal diffuse scattering* (TDS) is used to obtain details of the correlations that occur between both the inter- and intra-molecular displacements. Due to the recent advancements in computing power, methods that involve the construction of models of disordered crystals from which the diffuse scattering is calculated have become more and more effective.

Original methods for the evaluation of harmonic potentials used in Monte Carlo simulations for modeling the diffuse scattering from molecular crystals has been superseded by implementing an empirical formula that effectively simplifies this procedure [1]. The Method works so well that now not only is implementing disorder models (<50 atoms per molecule) more rapid (as applied to previous studies of problems associated with polymorphism in pharmaceuticals), we can also begin to accurately model the diffuse scattering features from larger supra-molecular systems. We have applied these novel techniques to problems involving supra-molecular host guest assemblies by performing simulations to model the diffuse scattering from single crystals of 1,11-undecadioic acid/urea and t-butylcalix-4-arene.

The urea inclusion compound (UIC) for 1,11-Undecadoic acid has a well resolved structure in which the guest is disordered over three positions with respect to the lattice repeat of the urea channels. A result of this disorder is strong layers of diffuse scattering perpendicular to the channel axis at one-third increments between the average 'Bragg' Layer lines. Our methods use Ising-type short-range order models for the site occupations of the guests to show that the direction for the correlation of the guest molecules between channels is actually trigonal, even though the average structure is orthorhombic. A good fit to the diffuse features is established from applying the correct correlation energies in the model.

t-Butylcalix-4-arene if crystallized with different mixtures of Pyridine-N-oxide (PNO) or Nitrobenzene ($PhNO_2$) can form what we know to be two isomorphous crystalline phases 'beta' or 'delta'. This is reflected by the apparent differences in cell dimensions and major guest occupation in the 'calix' cavity. Each of the crystals can have varying amounts of PNO or $PhNO_2$ depending on the growth