

## MS6-P6 X-ray structure of *Triatoma virus* empty capsid: insights into the mechanism of uncoating and RNA release in dicistroviruses

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In viruses, uncoating and RNA release are two key steps of successfully infecting a target cell. During these steps, the capsid must undergo the necessary conformational changes to allow RNA egress. Despite their importance, these processes are poorly known in the *Dicistroviridae* family. Here we solved by X-ray crystallography the atomic structure of *Triatoma virus* empty particle (PDB code 5L7O), which is the resulting capsid after RNA release. It is observed that the overall shape of the capsid and of the three individual proteins is maintained in comparison with the mature virion. Furthermore, no channels indicative of RNA release are formed in TrV empty particle. However, the most prominent change in the empty particle in comparison with the mature virion is the loss of order in the N-terminal domain of VP2 protein. In mature virions this domain swaps with its two-fold related VP2 N-terminal domain located in an adjacent pentamer stabilizing the binding between pentamers. The loss of these interactions allows us to propose that RNA release may take place through the partial disassembly of TrV capsid into pentameric subunits. The fewer number of stabilizing interactions between pentamers and the lack of formation of new holes support this model. This model differs from the currently accepted model for rhinoviruses and enteroviruses in which genome externalization occurs by the extrusion of the RNA through capsid channels.

**Keywords:** Dicistroviridae, RNA release, uncoating, capsid disassembly

## MS7 Protein & glycobiology structure determination

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## MS7-P1 Crystal structure and biophysical characterization of ligand-free and -bound RNases 4 and 6, members of the human RNase A superfamily

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In addition to strictly conserved ribonucleolytic activities, ribonuclease (RNase) A superfamily members have been associated to a variety of biological functions such as anti-bactericidal, cytotoxic, angiogenic, immunosuppressive, anti-tumoral and/or anti-viral activities. Human RNase A members comprise eight rapidly evolving homologous enzymes with varying degrees of structural similarity and enzymatic activities. While the crystallographic and/or NMR structures of RNases 1-5 and 7 have already been elucidated, the structural characterization of RNases 6 (or RNase k6) and RNase 8 are still unresolved.

In this study, we have successfully crystallized human RNase 6 in presence of phosphate anions. We compare its crystalline structure with RNase A (bovine) and RNase 4. Despite many structural similarities with RNase A, we emphasized on a number of differences that may account for the different functions among the human RNases. Moreover, we have identified the phosphate binding residues and ionic binding architecture within the catalytic pocket of RNase 6. A new phosphate-binding site located within loop 4 and involving His67 was also uncovered.

The biophysical properties of RNases 4 and 6 were analyzed via NMR titration and isothermal titration calorimetry with two different ligands: 3'-UMP and 5'-AMP. The results were compared to RNase A and correlated to their unliganded crystal structures and their structures in presence of the ligand 5'-AMP.

Resolving the crystal structure of human RNases provides valuable insights into understanding its biological function in human, which may find applications in various fields such as drug design.

**Keywords:** Crystal structure, human ribonucleases, RNase 4, RNase 6, enzymes, ligand