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Structural characterization of genomic RNA-coat protein contacts in single-stranded RNA viruses by high-resolution cryo-EM

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Recent developments in cryo-electron microscopy (cryo-EM) hardware along with continuously evolving software tools have led to the discovery of many novel structures that it was not possible to solve until now, resulting in what is termed “the resolution revolution”. In structural virology, it has also led to a re-evaluation of known structures. Most virion structures solved by X-ray crystallography or cryo-EM are focused on the capsid protein (CP) as a result of the application of icosahedral symmetry averaging to “improve” the electron density maps. However, this has the consequence that the intrinsic asymmetry of important components of virions, such as the viral genome and structural proteins lacking such symmetry, are masked. Single-stranded (ss), positive-sense RNA viruses are major pathogens in all kingdoms of life. Asymmetric cryo-EM structure determination of a major model virus in this class, bacteriophage MS2, reveals the limitations of a symmetrized view. As well as the presence and interactions made by the unique Maturation Protein, it also reveals multiple gRNA-CP dimer contacts corresponding to our previous prediction that dispersed, sequence-degenerate RNA motifs (Packaging Signals, PSs) play important roles during the virion assembly. Here, we describe how relaxing symmetry during structure determination can image such gRNA-PS contacts in a range of ssRNA viruses including the picornavirus Bovine Enterovirus-1, the alphaviruses Sindbis and Semliki Forest Viruses, as well as the plant virus Turnip Crinkle Virus. The revelation of these functionally important gRNA-CP contacts changes our fundamental understanding of assembly in these pathogens and may have further translational importance.

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