Seeing is Believing: High-Resolution Cryo Electron Microscopy reveals Mechanism of Virus Assembly and Genome Encapsidation

Dr Emma Hesketh and Rebecca Thompson

In new work led by researchers at the University of Leeds' Astbury Centre for Structural Molecular Biology, recent advances in cryo-electron microscopy (cryo-EM) were used to investigate the structure of a small plant virus, Cowpea mosaic virus (CPMV). The work* sought to reveal the mechanism of capsid assembly and genome encapsidation of CPMV, laying the groundwork for use of this virus capsid in biotechnology applications.

CPMV can replicate to produce many billions of identical copies of itself in plants. The virus is comprised of a hollow sphere of protein, or capsid, which is in turn made up of multiple copies of the 'small' and 'large' subunits. Inside this capsid is the virus' single stranded RNA genome. The research sought to investigate how the capsid assembles and in particular how it packages its genome.

This study, which was a collaboration with colleagues at the John Innes Centre (JIC) in Norwich, builds on previous, award-winning research, in which Professor George Lomonossoff and colleagues at the JIC developed a system to create 'empty virus-like particles' (eVLPs); the CPMV capsid without the infectious RNA inside. As the eVLP has no genome, it cannot reproduce itself or mutate, making it ideal for use in biotechnology. The capsid could be engineered by changing the protein sequence to target different capsids for different purposes. However, to achieve this level of engineering, we need precise, atomic level information about the capsid structure. Eventually CPMV eVLPs could be designed as vehicles for targeted drug delivery, a source of novel diagnostic reagents, or even new vaccines.

This new research led by researchers in Leeds, shows the structure of the eVLP in unprecedented detail. CPMV is one of the smallest known viruses and so its direct visualisation requires use of an electron microscope. Cryo-EM images, such as shown in *Figure 1*, were processed to generate structures of both CPMV and the related eVLP to 3–3.5 Ångstroms (Å) shown in *Figures 2 and 3*.

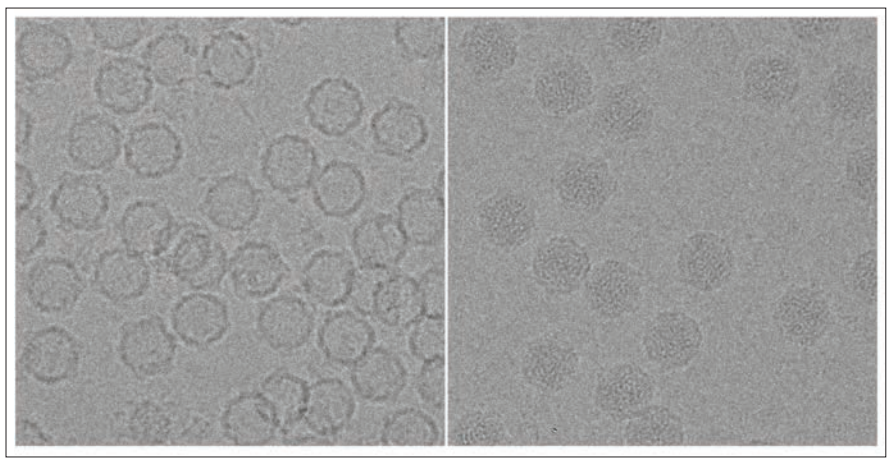

Figure 1. Cryo electron micrographs of empty CPMV particles (eVLPs, left) and 'full' CPMV particles containing its single stranded RNA genome (right).

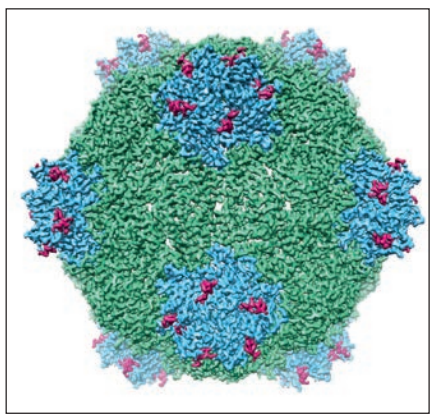

Figure 2. The structure of an empty CPMV (eVLP) produced using cryo electron microscopy.

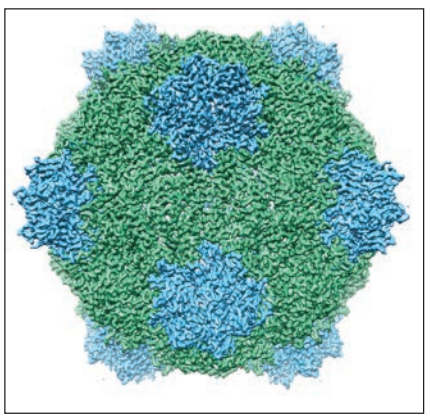

Figure 3. The structure of CPMV produced using cryo electron microscopy.

Figure 6. Researchers from the University of Leeds. Left to right Emma Hesketh, Neil Ranson, Rebecca Thompson.

Dr Neil Ranson said, "At this resolution, which rivals that achieved by X-ray crystallography, the side-chains of the individual amino acids that form the protein capsid could be built into position, providing an atomic model of the entire structure. This opens the way to manipulating those amino acids and intervening in the function of the protein capsid with unprecedented precision."

The researchers show how CPMV is a highly symmetrical protein shell formed from five-sided 'pentons' each built from five copies of an asymmetric subunit. Each asymmetric unit is made up of the large subunit (green) and the small subunit (blue). In *Figure 4* we can clearly see the single stranded RNA genome of CPMV inside the protein shell. The basic protein subunit is very simple, so the virus only needs a very small amount of genetic information to make a large protein shell. Not only is this very efficient, but CPMV capsids are highly stable, another characteristic which may be exploited in biotechnological applications.

Previously, biochemical studies by the JIC team predicted that a small, 24 residue extension at one end of the small subunits of the capsid was involved in encapsidation of the RNA genome during virus assembly. However, this feature had not been previously observed using other structural techniques such as X-ray crystallography, making it difficult to validate its role in assembly. In this work, the cryo-EM structures allowed the researchers to visualise the extension for the first time (see the pink section in *Figure 5*). It found that the 24 residue extension of the S subunit holds the pentons together as the virus' outer structure is built. These residues are also essential for the virus to package its genes, but it is cleaved from the virus when it has done its job.

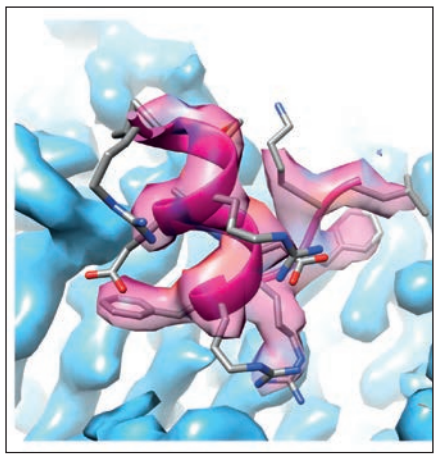

Figure 5. The structure of the 24 residue extension visualised for the first time in this study.

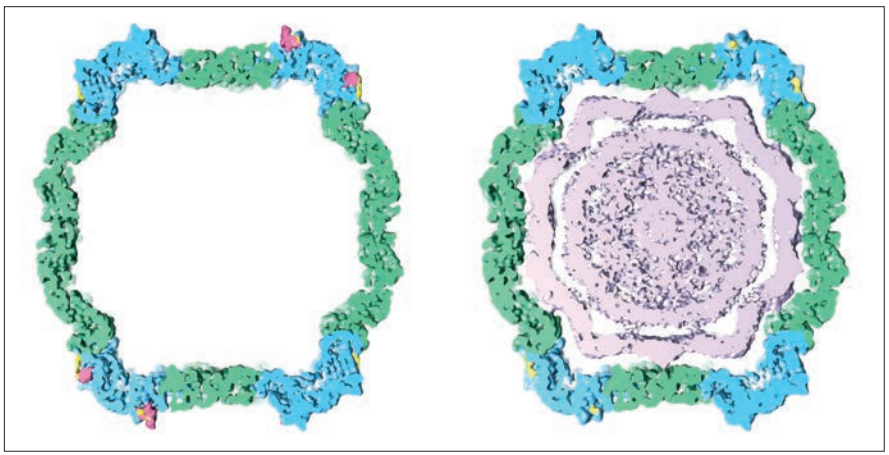

Figure 4. The empty capsid of eVLP (left) compared to the infectious particle filled with the RNA genome (lilac, right).

Professor George Lomonossoff said: "The structural studies show that the extension acts like a dab of 'molecular glue' to stabilise the capsid subunits as they are pieced together. Thanks to these wonderful new images, we've now seen with our own eyes how the extension works, both in the infectious Cowpea Mosaic Virus and in our empty virus-like particle. This is a very important finding to validate our work on eVLPs – we now know that our engineered capsids are put together in the same way as CPMV and as a result we now have a new model for virus assembly. Because CPMV is a member of a large family of viruses that includes polio, foot-and-mouth and Hepatitis A, our studies should also aid our understanding of how these important animal viruses assemble."

The research was enabled by recent advances in electron microscope hardware, in particular electron microscopes and direct electron detectors, also advances in image processing, sometimes collectively referred to as the 'resolution revolution'. These advances have transformed the level of detail that can be revealed by cryo-EM. The structures of CPMV shown here are amongst the most detailed electron microscope structures of protein complexes yet published.

Figure 7. Researchers from the John Innes Centre (JIC) in Norwich. George Lomonossoff (Left), Yulia Meshcheriakova (Right) and Pooja Saxena (Second from right).

The University of Leeds, and Wellcome Trust, have recently invested £17 million in the new Astbury Biostructure Laboratory at the University of Leeds, which will be installing two state of the art electron microscopes and direct electron detectors in spring 2016.

More information about equipment arriving at the Astbury Biostructure laboratory can be found at www.astbury.leeds.ac.uk/biostructurelaboratory.

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(Find the article at: http://rdcu.be/fIlS).