The cryo-EM structure of African swine fever virus unravels a unique architecture comprising two icosahedral protein capsids and two lipoprotein membranes

German Andrés¹,*,#; Diego Charro²#, Tania Matamoros¹, Rebecca S. Dillard³, Nicola G A Abrescia²,4,*

From the ¹Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, Madrid, Spain; ²Molecular Recognition and Host-pathogen Interactions Programme, CIC bioGUNE, CIBERehd, Bizkaia Technology Park, 48160 Derio, Spain; ³NeCEN, Institute of Biology Leiden, Leiden University, 2333 CC, Leiden, Netherlands; ⁴IKERBASQUE, Basque Foundation for Science, 48013 Bilbao, Spain.

Running title: Cryo-EM of African swine fever virus

# Contributed equally

To whom correspondence should be addressed:
German Andrés: Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, Madrid, Spain; gandres@cbm.csic.es; Tel. (+34) 911964401/4548.
Nicola G. A. Abrescia: CIC bioGUNE, CIBERehd, Bizkaia Technology Park, 48160 Derio, Spain; nabrescia@cicbiogune.es; Tel. (+34) 946572523; Fax. (+34)946572502.

Keywords: African swine fever virus, virus assembly, cryo-electron microscopy, nucleocytoplasmic large DNA viruses (NCLDV)
ABSTRACT

African swine fever virus (ASFV) is a complex nucleocytoplasmic large DNA virus (NCLDV) that causes a devastating swine disease currently present in many countries of Africa, Europe and Asia. Despite intense research efforts, relevant gaps on the architecture of the infectious virus particle remain. Here, we used single-particle cryo-electron microscopy (cryo-EM) to analyze the 3D structure of the mature ASFV particle. Our results show that the ASFV virion, with a radial diameter of ~2,080 Å, encloses a genome-containing nucleoid surrounded by two distinct icosahedral protein capsids and two lipoprotein membranes. The outer capsid forms a hexagonal lattice (triangulation number $T=277$) composed of 8,280 copies of the double jelly-roll major capsid protein (MCP) p72, arranged in trimers having a pseudo-hexameric morphology, and of 60 copies of a penton protein at the vertices. The inner protein layer, organized as a $T=19$ capsid, confines the core shell and it is composed of the mature products derived from the ASFV polyproteins pp220 and pp62. Also, an icosahedral membrane lies between the two protein layers, while a pleomorphic envelope wraps the outer capsid. This high-level organization confers to ASFV a unique architecture among the NCLDVs that likely reflects the complexity of its infection process and may help explain current challenges in controlling it.

African swine fever virus (ASFV) is the causative agent of a highly lethal hemorrhagic disease of domestic pigs, for which there is no vaccine or antiviral strategy available. The disease is currently present in many countries of sub-Saharan Africa, Eastern Europe and East Asia, threatening the pig industry and food security worldwide (1-3). ASFV is the sole member of the Asfarviridae family of the proposed order “Megavirales”, a monophyletic, albeit heterogeneous, clade of nucleocytoplasmic large DNA viruses (NCLDV) that form the most complex group of DNA viruses known so far (4,5). At present, the NCLDV group contains eight taxonomic families (Ascoviridae, Asfarviridae, Iridoviridae, Marseilleviridae, Mimiviridae, Phycodnaviridae, Pithoviridae, and Poxviridae), a number that will probably increase in the near future due to the continuous discovery of new, not yet classified, giant viruses like pandoraviruses (6), faustoviruses (7), mollivirus (8), kaumoebavirus (9), cedratviruses (10), and pacmanvirus (11).

NCLDVs display a broad host tropism that includes phagotrophic protists like amoebas, plants like green algae, and animals like insects, reptiles, and mammals. ASFV mainly infects wild and domestic pigs, as well as tick vectors of the genus Ornithodoros, being the only known DNA arbovirus. The viral genome is a double-stranded DNA molecule of 170-190 kbp that contains 151-167 open reading frames (ORFs), depending on the virus strain (12,13). The ASFV particle contains about 70 different polypeptides, including multiple structural components, as well as a full set of enzymes and factors involved in viral transcription (14). Earlier structural studies based on
alddehyde-fixed samples anticipated that
the virion consists of an external lipid
membrane, an icosahedral protein
capsid, an internal lipid membrane, a
thick protein core shell and a genome-
containing nucleoid (15,16,17).
ASFV infects porcine monocytes and
macrophages, and replicates in
specialized cytoplasmic areas close to
the nucleus, known as viral factories
(16). In these assembly sites, intracellular particles acquire their inner
membrane from endoplasmic reticulum
membrane fragments (18,19). Viral
membranes become icosahedral particles
by the progressive building of the outer
capsid, which is essentially composed by
the major capsid protein (MCP) p72 and
the minor protein p49 (20,21). Concomitantly, the core shell, which is
made of the mature products derived
from polyproteins pp220 and pp62, and
the genome-containing nucleoid are
enclosed (16,22-24). Once formed,
intracellular icosahedral mature particles
move to the cell surface by a
microtubule-mediated transport system
and exit the cell by budding at the plasma
membrane, while acquiring their outer
envelope (Fig. 1A). Both intracellular
and extracellular ASFV forms are
infectious, which indicates that the
external membrane is not strictly
necessary for infectivity (24).
Many of the NCLDV, including the
phycoenavirus Paramecium bursaria
Chlorella virus (PBCV-1) (25), the
iridovirus Chilo iridescent virus (CIV)
(26), the mimivirus Cafeteria
roenbergensis virus (CroV) (27), or the
giant amoeba-infecting Faustovirus (28),
Pacmanvirus (11) and Medusavirus (29),
present large icosahedral capsids
composed of trimers of a MCP that
displays a double jelly-roll fold. Each
capsid structure consists of eight anti-
parallel β-strands arranged in two four-
stranded sheets (30); the trimeric
capsomer thus displays a pseudo-
hexameric morphology. Similar capsid
structures are found in other dsDNA
viruses such as adenoviruses and the
tailless membrane-containing
bacteriophage PRD1 (31,32).
Other NCLDV with non-icosahedral
architectures include the amphora-
shaped pandoraviruses and pithoviruses,
the spherical molliviruses (33) or the
brick-shaped poxviruses, which,
interestingly, use a double jelly-roll
scaffolding protein, D13, during their
assembly (34).
Most of the NCLDV, including ASFV
and its distant relative Pacmanvirus (11),
have an internal membrane surrounding
the genome-containing core. A
prominent exception is represented by
the ASFV’s closest relative Faustovirus,
which lacks a lipid membrane but
contains two concentric icosahedral
capsids (28).
Here, we have used single-particle cryo-
EM to characterize the three-
dimensional (3D) structure of the
extracellular mature ASFV particle and
of the virion-derived homotrimeric p72
capsomers. Our cryo-EM study shows
that the ASFV virion contains two
distinct icosahedral proteinaceous
capsids and two lipoprotein membranes.
The outer capsid resolved at ~23 Å
resolution, which is wrapped by a
pleomorphic external envelope, is
organized in trisymmetrons and
pentasymmetrons with pseudo-
hexameric capsomers composed of
homotrimers of double jelly-roll p72
protein (visualized at ~5 Å resolution)
arranged on an hexagonal lattice with a triangulation number \( T = 277 \). Juxtaposed to the base of the outer capsid, there is an icosahedrally ordered membrane which encloses an inner capsid whose pseudo-hexameric capsomers arrange on a \( T = 19 \) hexagonal lattice. This internal capsid, which is made of some mature products derived from ASFV polyproteins pp220 and pp62, confines the core-shell and the DNA-containing nucleoid. This architecture represents, to our knowledge, a unique structural organization among known NCLDVs.

Results

**ASFV structure**

Purified extracellular ASVF virions were analyzed using cryo-EM. Recorded images show a very large icosahedral virus with two differently located membranes (Fig. 1A). The outer envelope, acquired by budding during virus egress, does not follow an icosahedral symmetry. The resolution for the ASFV outer capsid, located beneath the outer envelope, is 23 Å (and ~32.0 Å for the whole virion) as judged by the Fourier shell correlation at 0.143 criteria (Fig. 1B, Table 1 and Experimental Procedures). It measures about ~2,050 Å facet-to-facet (outer capsid) and ~2,400 Å vertex-to-vertex (and radially averaged ~2,080 Å diameter; Fig 1C) and it is similar in size to only two other NCLDVs, Faustovirus and Medusavirus, determined by cryo-EM at 15 Å and 31 Å resolution, respectively (28,29). The outer capsid surrounds an icosahedral membrane whose outer and inner bilayer leaflets are discernible indicating a resolution for this less-ordered region in the range of 35 Å (Fig. 1C) (35). In turn, the inner membrane contains an icosahedral inner capsid that encloses the core shell and the nucleoid (Fig 1C-D).

The cryo-EM map of the outer capsid clearly shows the organization of the pseudo-hexagonal capsomers (Fig. 2A-B). These are composed of homotrimers of MCP p72 [determined in this study at 4.6 Å resolution as possessing a double jelly-roll fold, see Fig. 2C-D, Table 1 and Experimental Procedures; and very recently by others at 2.67 Å (36)] arranging on a hexagonal lattice with \( h = 7 \) and \( k = 12 \), giving a triangulation number of \( T = 277 \) (Fig. 1B), the same as that found in Faustovirus and Medusavirus. The p72 shell, with a thickness of ~90 Å (Fig. 1C), can thus be described in geometrical terms, as done for other NCLDVs, by trisymmetrons and pentasymmetrons, which are well-ordered collections of capsomers forming the virion facets and vertex regions, respectively (Fig. 2B) (37). The ASFV trisymmetron is made of 120 pseudo-hexameric capsomers (360 copies of MCP p72) while the pentasymmetron contains 30 pseudo-hexagonal capsomers (90 copies of MCP p72) plus of 1 penton complex with plausibly five copies of the minor penton protein p49 (B438L) (Fig. 2A-B), as deduced from the ultrastructural phenotype of a recombinant inducible virus expressing p49 and immunogold labeling (14,20). The icosahedral asymmetric unit (IAU) is therefore composed of 138 copies of p72 plus 1 copy of the penton protein (as p72 displays two jelly-rolls, 138 x 2 = 276 plus 1 copy of penton protein give 277 which recapitulates the \( T \) number) (Fig. 2C). This organization replicates the use of the pseudo-hexameric capsomer
footprint in capsid assembly also seen in smaller virus members of the PRD1-adenovirus lineage (Fig. 3A) (31,32). However, this is one of the solutions for viral capsid assembly as other smaller dsDNA viruses for example use coat proteins with the so-called HK97 fold characterized basically by two domains, the P-domain with a long α-helix and a three stranded β-sheet, and the A-domain with two α-helices and a β-sheet (31).

Juxtaposed to the p72 proteinaceous shell the icosahedrally ordered membrane is apparent with an overall thickness of ~34 Å (peak-to-peak distance in Fig. 1C) that is in line with other membrane-containing viruses (32,38-39). Weak density across the p72 shell and the outer leaflet of the membrane support the contact of these two architectural constituents (Fig. 1C inset). At about ~110 Å (peak-to-peak distance) inside from the inner leaflet of the membrane a further icosahedral structure, the inner capsid, contains the entire core (Fig. 1C-D). The cryo-EM map shows that this capsid, with a thickness of ~50 Å and with a radial diameter of ~1,580 Å, is composed of capsomers displaying a pseudo-hexameric morphology sitting on an hexagonal array with the triangulation number $T = 19$ (Fig. 4). The centre-to-centre distance between the capsomers is approximately 75 Å although further density seem to occupy the interspace between capsomers, possibly cementing them together (Fig. 4, insets). This value matches the conserved centre-to-centre distance observed in virus capsid based on vertical double jelly-roll MCPs as those of the PRD1-adenovirus lineage (Fig. 4). At the apices the density becomes turreted indicating the presence of a specific penton protein. The protein components of the inner capsid shell are mature products derived from viral polyproteins pp62 (p35, p15, p8) and pp220 (p5, p14, p34, p37, p150) (14,40,41). When the sequences of the medium size proteins (17 kDa < MW < 42 kDa) were submitted to Phyre2 protein fold recognition server for secondary structure prediction, p35, p14, p34 and p37 - returned predicted as being mainly (> 60%) α-helical whereas p15 was predicted to be a mixture of α-helices (36%) and β-strands (26%(42). The determination of whether p15 is the MCP forming the capsomers of this inner capsid, due to its β-strand content, will require a higher resolution map. The corresponding IAU thus contains three pseudo-hexameric capsomers and one protein at the vertex (Fig. 4). Further density within the inner capsid corresponds to the presence of the core shell and the nucleoid (Fig. 1C-D).

**Discussion**

**Overall architecture of ASFV**

Most of NCLDVs possess an icosahedral protein capsid, which is based on a MCP with a double jelly-roll fold, and an underlying lipoprotein membrane that encloses the genome-containing core (25,27,38,43). An exception to this overall structure is represented by Faustovirus, the closest relative of ASFV (7). The Faustovirus particle, with a diameter of 2,600 Å, lacks an internal membrane but possess a double-layered protein capsid enclosing the viral genome (28). Its outer layer is composed of a double jelly-roll MCP that is 40% identical to the ASFV MCP p72 (Fig. 3B). The contiguous inner protein layer is assembled by one or more proteins
with a likely distinct fold from the jelly-roll (28).

The structure of ASFV appears to combine both architectural components of Faustovirus and those of membrane-containing viruses such as PBCV-1, PRD1, STIV and Pacmanvirus (11,32,38,44). ASFV possesses two icosahedral concentric capsids interspaced by an icosahedral membrane along with an additional pleomorphic envelope over the outer capsid (Fig. 1C-D).

Structure and assembly of the outer capsid

The outer capsid, composed of 8,280 MCP p72 copies is organized in 20 trisymmetrons, and 12 pentasymmetrons, composed of 120 and 30 homotrimeric p72 capsomers, respectively. Further, five copies of the penton protein, putatively the minor capsid protein p49 protein, plug each of the 12 icosahedral vertices. The use of ordered arrays (trisymmetrons and pentasymmetrons) of pseudo-hexameric capsomers allows to form large icosahedral capsids on a curved two-dimensional lattice (26,37). These viruses use the pseudo-hexameric capsomers generated by trimers of vertical double jelly-roll MCPs as building blocks, containing a rotational symmetry that facilitates equivalent interactions to be formed. The orientation of these capsomers is equivalent in each trisymmetron but their orientation across adjacent trisymmetrons is related by a 60° rotation (27). Smaller viruses of the PRD1-adenovirus lineage use the same pseudo-hexameric double jelly-roll capsomer morphology to build the whole triangular facet of the icosahedral particle leaving space at the vertices only for the five copies of the penton protein, usually with a jelly-roll fold (31,32,39). Also vertical single β-barrel viruses have been found to generate heterodimeric vertical double jelly-roll with a pseudo-hexagonal shape that recapitulate the close packing see in vertical double jelly-roll viruses (39). Thus, the use of vertical jelly-roll structures forming capsomers with pseudo-hexagonal footprint offers a means to scale-up the size of capsid exploiting the internal symmetry of these basic building blocks.

The presence of α-helices at the N-terminus of ASFV p72 [Fig. 3C and (36)] located at the base of the homotrimer and the high hydrophobicity score of the first 10 residues [relative to the first 40 assessed using ProtScale (45)] supports a model in which these helices contact the underneath membrane as also seen for PRD1 MCP P3 (32). Further contacts might also occur at the five-fold vertices between penton proteins and the apex of the inner membrane as observed in membrane-containing viruses such as STIV and HCIV-1, which interact with the above penton (39,44). It has been proposed that the capsid assembly of CroV and related giant viruses may initiate at a five-fold vertex to continuously proceed outwards in a spiralling fashion (27) and the same pathway might be used by ASFV. This progressive spiral assembly might serve, in turn, to organize the icosahedral structures of the underlying membrane and the inner capsid. It is not known, however, what ASFV inner membrane protein(s) may be involved in the interaction with the five-fold capsid vertex. At present, seven known
transmembrane viral proteins (p17 [pD117L], pE183L, p12 [pO61R], p22 [pKP177L], pH108R, pE199L, and pE248R) have been identified on the inner membrane, while only one viral protein (pEP402R), a homologue of the T-lymphocyte surface antigen CD2, has been located on the external envelope so far (14). However, about half of the 15 putative transmembrane virion proteins remains uncharacterized (14).

Core organization
In contrast to other known lipid-containing viruses, the inner icosahedral membrane surrounds an icosahedral capsid that encloses the core shell and the central DNA-containing nucleoid (16,22). This inner capsid, assembled by pseudo-hexameric capsomers, displays a different triangulation number (\(T=19\)) than the outer capsid (\(T=277\)). The density at the apices, which present a turreted morphology (Fig. 1C) distinct of regions close to the two- and three-fold axis (Fig. 4), might indicate specific interaction sites with the above membrane. Such interaction would be consistent with the hierarchical and tightly coordinated assembly of ASFV (16). It has been reported that the correct assembly of the inner capsid and core shell is dependent on the assembly of the outer capsid and the inner membrane – but not vice versa. Thus, inducible ASFV recombinants in which the expression of the core shell polyproteins pp220 and pp62 is inhibited, produce icosahedral core-less particles containing a normal outer capsid over the inner membrane (23,46). At variance, recombinant viruses in which the expression of MCP p72 is inhibited, produce aberrant capsid-less structures formed by unprocessed ASFV polyproteins (19). This structural dependency would explain the fact that both capsids are overall aligned along their relative icosahedral axis despite the lack of direct interactions. Higher resolution reconstruction will better underpin the spatial relationship between the two capsids.

It is known that after ASFV endocytosis, the incoming particles undergo an acidic pH-driven disassembly in late endosomes that involves the loss of the outer membrane and the outer capsid, and the subsequent fusion of the inner membrane (47). As a result, the viral cores are delivered into the cytosol to initiate the transcription of the early viral genes. It is tempting to speculate that the structured inner protein capsid may serve to confine and protect the viral genome from host nucleases as well as from host dsDNA sensors that may activate the innate immune response during the first infection stages (48).

On the other hand, it is interesting to note that Faustovirus and Pacmanvirus encode not yet characterized homologs of the ASFV core shell polyproteins pp220 and pp62 as well as the viral protease (7,11). This suggests that both viruses may use a similar solution to ASFV for their core organization and genome packaging.

Apart from the structural and sequence homologies with dsDNA Faustovirus, double protein-layered viruses with different capsid arrangements (and \(T\) numbers) have been found primarily in RNA viruses of the Reoviridae family such as BTV and Reovirus and in bacteriophage \(\Phi 6\) where the outer-protein shell serves to introduce the inner shell into the cytoplasm (49-51).
The multi-layered architecture of the virion, with an outer envelope and an inner membrane in between two icosahedral capsids, makes the ASFV structure unique among the NCLDVs.

Conclusions and prospects
Overall, the structure described herein reflects the complexity of ASFV assembly and disassembly and showcases the challenges associated with the identification of strategies to both prevent and control ongoing infections. For instance, the non-essential role of the outer envelope in the ASFV infectivity (24) suggests that the outer capsid may play relevant roles during the virus entry. Consistent with this, neutralizing antibodies to MCP p72 have been reported to inhibit in vitro the virus attachment to the host cell (52). The identification of neutralization epitopes as well as of putative binding sites to cell receptors in the capsid proteins can benefit from this and future structural studies on ASFV.

Experimental Procedures
Cells, viruses and antibodies
Vero cells (Chlorocebus sabaeus kidney fibroblasts; ATCC number CCL-81) were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS), which was reduced to 2% during viral infection. The Vero cell-adapted ASFV strain BA71V has already been described (53). The mouse monoclonal antibody (MAb) 17LD3 against the major capsid protein p72 has been already described (19).

ASFV purification
Extracellular ASFV particles were purified according to the Percoll gradient sedimentation method (54) from supernatants of infected Vero cells. In brief, extracellular virions were collected at 72 h postinfection (hpi) from clarified infection supernatants and concentrated by high-speed centrifugation. Then, the virus sample was subjected to two successive Percoll (GE Healthcare) density gradient centrifugations, and the obtained virus preparation was further purified by Sephacryl S-1000 (GE Healthcare) gel filtration chromatography. The resulting virus-enriched fractions were collected and concentrated by centrifugation. Purified virus particles were resuspended in phosphate-buffered saline and kept at 4°C until vitrification (Fig. 1A).

Purification of MCP p72 from virion
To purify ASFV MCP p72, Percoll-purified extracellular virions were sequentially dissociated as follows. Typically, 200-300 µg of purified viruses were first dissociated with 1% Octyl β-D-glucopyranoside (OG) in Tris 10 mM pH 8.0 for 30 min at 4 °C and then centrifuged at 100,000g for 15 min in a A-100/30 Airfuge rotor (Beckman). The sediment was then dissociated for 30 min at 4 °C Tris 10 mM pH 8.0, 1% OG and 500 mM NaCl. Subsequently, the sample was ultracentrifuged as above and the resulting sediment was incubated for 30 min at 4 °C in 1% OG, 0.5M NaCl and 0.1 M dithiothreitol (DTT) in Tris 10 mM pH 8.0. Finally, the dissociated particles were ultracentrifuged once more at 100,000g for 15 min and the resulting supernatant, containing purified major capsid protein p72, was analyzed either by negative-stain...
electron microscopy (EM), SDS-PAGE and Coomassie staining or density gradient sedimentation (Fig. 5).

Sample preparation and cryo-EM data collection of MCP p72 and whole virion

MCP p72 sample (4 µl at 0.9-1.2 mg/ml) was pipetted onto 200-mesh Quantifoil R 2/2 graphene oxide coated grids (to improve particle spread on grid) which were then vitrified using a Vitrobot (Mark III - FEI). Prior to shipping the grids for HR imaging to large EM facilities in northern Europe such as NeCEN (Netherlands) and Electron Bio-Imaging Centre (eBIC-Diamond Light Source; UK) (our gateway to access high-end Titan Krios microscopes) (55), a few of them were tested for ‘quality control’ on the in-house JEM-2200FS/CR (JEOL, Ltd.) electron microscope operating at 200 kV and equipped with a 4K x 4K CCD camera. Forty frame movies (1,541 total movies) were recorded with a Titan Krios equipped with a Cs-corrector and a Falcon-3EC direct electron detector (energy filter slit width of 20 eV) using SerialEM with virtual maps to target each image to individual virions on the grid (56,57). Movies (60 frames) were recorded in the same defocus range, at a nominal magnification of 130,000x and a total dose of 47.5 e/Å², producing a pixel size of 1.09 Å at specimen (Table 1).

Image processing, 3D reconstruction and analysis

Beam-induced sample motion on recorded frames was corrected using MotionCor2 (58) and the CTF estimated with CTFFIND4 (59).

For the preparation of the virion grids purified ASFV sample at 1.0-1.2 mg/ml was pipetted onto 200-mesh Quantifoil R 2/2 grids which were vitrified using a Vitrobot (Mark III - FEI). Because of the particle’s large size different blot-pad positions and blotting times were tested with offset number -2 and 3 seconds blotting giving satisfactory results in terms of ice thickness relative to particle’s size and rupture. Two datasets were acquired for the virion sample. The first one was collected on a Titan Krios equipped with a Cs-corrector and a Falcon 3EC direct electron detector in linear mode using the EPU software (Thermo Fisher Scientific). Movies (40 frames) were recorded with a defocus range from -0.8 to -2.2 µm with a dose of 47.7 e/Å² at a nominal magnification of 59,000x producing a pixel size at the specimen of 1.13 Å (Table 1). Because of the low chance of getting one or two full particles per view using the above set-up (~1 whole virion per 14 movies), the second ASFV dataset was collected more efficiently on a Titan Krios equipped with a Gatan K2 Summit direct electron detector (56) (energy filter slit width of 20 eV) using SerialEM with virtual maps to target each image to individual virions on the grid (56,57). Movies (60 frames) were recorded in the same defocus range, at a nominal magnification of 130,000x and a total dose of 47.5 e/Å², producing a pixel size of 1.09 Å at specimen (Table 1).
pseudo-hexameric footprint typical of vertical double jelly-roll MCP (Figs. 2C and 3A). This set of particles was 3D classified in 3 classes using as a starting reference a map generated from the adenovirus hexon trimer (PDB ID 1P2Z) filtered at 60 Å resolution and imposing C3 symmetry (Faustovirus MCP PDB ID 5J7O was not used to avoid potential bias). This process was then repeated but using as a reference a derived 3D map from a previous class which clearly showed the pseudo-hexameric footprint and overall 3D shapes typical of β-barrels. Out of the three resulting classes (class-1: 46.9%, class-2: 39.2% and class-3: 13.9%), class-3 with 327,803 particles was selected for refinement on the basis of better map interpretability, angular distribution and estimated resolution. Selected particles were re-extracted with a box size of 192x192 pixels and 3D refined with a threshold derived mask.

Post-processing with a soft mask led to a map with a nominal resolution of 4.6 Å at the 0.143 criterion (Fig. 6A), however local resolution estimation shows that the resolution is anisotropic and it ranges from 4.3 to 6.5 Å according to the region of the capsomer (Fig. 2C-D). This resolution anisotropy and possibly elongated structure (Fig. 2C-D and 3C) might be consequence not only of the presence of top/bottom preferential views (Fig 6B) but also of the structural flexibility and possibly heterogeneity of a sample which has been dissociated from the virion. All together the above factors oblige for a conservative interpretation of the 3D cryo-EM map of MCP p72 outside the core of the double jelly-roll domain.

For the virion image processing, the following approach was used. From the first data collection 583 ASFV particles were visually selected and extracted in RELION (60), and 3D classified in two classes (as a reference an initial symmetrized model was generated in RELION). The largest class with 450 particles was refined and a preliminary icosahedral model was produced; this model served as reference for the 3D classification of the second dataset (684 starting particles) which led to a largest class with 650 particles. Then both set of particles were interpolated to the same pixel size and merged for 3D reconstruction. The merged 1,110 virions were 3D refined in RELION (with ‘skip-padding’ for GPU memory contraints). A threshold mask derived from the outer capsid shell was then used in the latest cycles of refinement until convergence. The corresponding unfiltered half-maps were high-pass filtered to 2,700 Å and used for postprocessing in RELION using a threshold mask with soft edges for the outer capsid (automatic B-factor $-1,236 \, \AA^2$) which led to the final resolution of the ASFV outer capsid of $\sim$23 Å as judged by the gold-standard FSC at 0.143 (31.6 Å at 0.5 criterion, see Fig. 6C). The discrimination of the outer and inner membrane leaflets of the inner lipid bilayer (a region known to be less ordered than the proteinaceous capsids), usually occurs when the resolution is equal or higher than 35 Å (see double peak in Fig. 1C) (35), thus supporting our resolution estimates. The handedness of the whole ASFV map (dextro) was assumed equivalent to that of Faustovirus (28). The resolution for the inner capsid of $\sim$24.3 Å (FSC at 0.143)
was estimated as above using a threshold mask with soft edges delimiting the corresponding protein shell. For visualization, the inner capsid was obtained by subtracting from the refined density of the whole virion the signal corresponding to the outer capsid and inner membrane, and then the box resized to 600x600x600 pixels. Map analysis and interpretation was performed in UCSF- Chimera (61) that was also used to prepare the figures.

**Author Contributions** - G.A. and N.G.A.A. designed research; G.A. D.C., TM, R.S.D. and N.G.A.A. performed research; G.A. D.C., T.M. and N.G.A.A. analyzed data; and G.A. and N.G.A.A. wrote the paper with contributions from the rest of the authors.

**Acknowledgments** - We thank Milagros Guerra at the Electron Microscopy core facility of CBMSO for technical assistance in monitoring sample preparation by ns-TEM and Isaac Santos-Pérez at the CIC bioGUNE for support in cryo-grid sample preparation and in-house cryo-EM imaging. We thank Ludovico Renault at NeCEN (Leiden-Netherlands) and Yun Song and Daniel Clare at eBIC Diamond (Harwell-UK) for valuable assistance in cryo-EM data collection. We are grateful to Juan Anguita (CIC bioGUNE) for careful reading of the manuscript and helpful comments. G.A. and N.G.A.A are supported by grants (PGC2018-098701-B-I00 and RTI2018-095700-B-I00, respectively) from the Spanish Ministerio de Ciencia, Innovacion y Universidades. G.A. is supported by the Amarouto Program for senior scientists from the Comunidad de Madrid. N.G.A.A. is supported by the Basque Departamento de Educación, Política Lingüística y Cultura (Refs: PRE_2016_2_0151, PRE_2018_1_0102). MICINN is also thanked for the Severo Ochoa Excellence Accreditation to the CIC bioGUNE (SEV-2016-0644).

This work benefited from access to the NeCEN, an Instruct-ERIC centre. Financial support was provided by Instruct-ERIC PID 3548. We also acknowledge Diamond Light Source for access and support of the Cryo-EM facilities at the UK national electron Bio-imaging Centre (eBIC) (proposal EM17171), funded by the Wellcome Trust, MRC, and BBSRC.

**Competing interests:**
The authors declare that they have no conflict of interests with the contents of this article.

**Data Deposition**
All cryo-EM maps have been deposited in the Electron Microscopy Data Bank (EMDB Deposition EMD-10346 for whole virion and inner capsid; EMD-10325 for p72 MCP).
REFERENCES

1. Dixon, L. K., Sun, H., and Roberts, H. (2019) African swine fever. *Antiviral Res* **165**, 34-41

2. Revilla, Y., Perez-Nunez, D., and Richt, J. A. (2018) African Swine Fever Virus Biology and Vaccine Approaches. *Adv Virus Res* **100**, 41-74

3. http://www.fao.org/ag/againfo/programmes/en/empres/ASF/index.html

4. Iyer, L. M., Aravind, L., and Koonin, E. V. (2001) Common origin of four diverse families of large eukaryotic DNA viruses. *J Virol* **75**, 11720-11734

5. Koonin, E. V., and Yutin, N. (2019) Evolution of the Large Nucleocytoplasmic DNA Viruses of Eukaryotes and Convergent Origins of Viral Gigantism. *Adv Virus Res* **103**, 167-202

6. Philipp, N., Legendre, M., Doutre, G., Couté, Y., Poirot, O., Lescot, M., Arslan, D., Seltzer, V., Bertaux, L., Bruley, C., Garin, J., Claverie, J. M., and Abergel, C. (2013) Pandoraviruses: amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes. *Science* **341**, 281-286

7. Reteno, D. G., Benamar, S., Khalil, J. B., Andreani, J., Armstrong, N., Klose, T., Rossmann, M., Colson, P., Raoult, D., and La Scola, B. (2015) Faustovirus, an asfarvirus-related new lineage of giant viruses infecting amoebae. *J Virol* **89**, 6585-6594

8. Legendre, M., Lartigue, A., Bertaux, L., Jeudy, S., Bartoli, J., Lescot, M., Alempic, J. M., Ramus, C., Bruley, C., Labadie, K., Shmakova, L., Rivkina, E., Couté, Y., Abergel, C., and Claverie, J. M. (2015) In-depth study of Mollivirus sibericum, a new 30,000-y-old giant virus infecting Acanthamoeba. *Proc Natl Acad Sci U S A* **112**, E5327-5335

9. Bajrai, L. H., Benamar, S., Azhar, E. I., Robert, C., Levasseur, A., Raoult, D., and La Scola, B. (2016) Kaumoeaviruses, a New Virus ThatClusters with Faustoviruses and Asfarviridae. *Viruses* **8**, pii: E278.

10. Andreani, J., Aherfi, S., Bou Khalil, J. Y., Di Pinto, F., Bitam, I., Raoult, D., Colson, P., and La Scola, B. (2016) Cedratvirus, a Double-Cork Structured Giant Virus, is a Distant Relative of Pithoviruses. *Viruses* **8**, pii: E300.

11. Andreani, J., Khalil, J. Y. B., Sevvana, M., Benamar, S., Di Pinto, F., Bitam, I., Colson, P., Klose, T., Rossmann, M. G., Raoult, D., and La Scola, B. (2017) Pacmanvirus, a New Giant Icosahedral Virus at the Crossroads between Asfarviridae and Faustoviruses. *J Virol* **91**, pii: e00212-17

12. Yanez, R. J., Rodriguez, J. M., Nogal, M. L., Yuste, L., Enriquez, C., Rodriguez, J. F., and Vinuela, E. (1995) Analysis of the complete nucleotide sequence of African swine fever virus. *Virology* **208**, 249-278

13. Dixon, L. K., Chapman, D. A., Netherton, C. L., and Upton, C. (2013) African swine fever virus replication and genomics. *Virus Res* **173**, 3-14

14. Alejo, A., Matamoros, T., Guerra, M., and Andres, G.
15. Carrascosa, J. L., Carazo, J. M.,
Carrascosa, A. L., Garcia, N.,
Santisteban, A., and Vinuela, E.
(1984) General morphology and
capsid fine structure of African
swine fever virus particles.
*Virology* **132**, 160-172

16. Salas, M. L., and Andres, G.
(2013) African swine fever virus
morphogenesis. *Virus Res* **173**, 
29-41

17. Andres, G., Simon-Mateo, C.,
and Vinuela, E. (1997)
Assembly of African swine
fever virus: role of polyprotein
pp220. *J Virol* **71**, 2331-2341

18. Andres, G., Garcia-Escudero,
R., Simon-Mateo, C., and
Vinuela, E. (1998) African
swine fever virus is enveloped
by a two-membraned collapsed
cisterna derived from the
endoplasmic reticulum. *J Virol* **72**, 
8988-9001

19. Suarez, C., Andres, G.,
Kolovou, A., Hoppe, S., Salas,
M. L., Walther, P., and Krijnse
Locker, J. (2015) African swine
fever virus assembles a single
membrane derived from rupture
of the endoplasmic reticulum.
*Cell Microbiol* **17**, 1683-1698

20. Garcia-Escudero, R., Andres,
G., Almazan, F., and Vinuela, E.
(1998) Inducible gene
expression from African swine
fever virus recombinants:
analysis of the major capsid
protein p72. *J Virol* **72**, 3185-
3195

21. Epifano, C., Krijnse-Locker, J.,
Salas, M. L., Salas, J., and
Rodriguez, J. M. (2006)
Generation of filamentous
instead of icosahedral particles
by repression of African swine
fever virus structural protein
pB438L. *J Virol* **80**, 11456-
11466

22. Andres, G., Alejo, A., Salas, J.,
and Salas, M. L. (2002) African
swine fever virus polyproteins
pp220 and pp62 assemble into
the core shell. *J Virol* **76**, 12473-
12482

23. Andres, G., Garcia-Escudero,
R., Salas, M. L., and Rodriguez,
J. M. (2002) Repression of
African swine fever virus
polyprotein pp220-encoding
gene leads to the assembly of
icosahedral core-less particles. *J Virol* **76**, 2654-2666

24. Andres, G., Garcia-Escudero,
R., Vinuela, E., Salas, M. L.,
and Rodriguez, J. M. (2001)
African swine fever virus
structural protein pE120R is
essential for virus transport from
assembly sites to plasma
membrane but not for
infectivity. *J Virol* **75**, 6758-
6768

25. Fang, Q., Zhu, D., Agarkova, I.,
Adhikari, J., Klose, T., Liu, Y.,
Chen, Z., Sun, Y., Gross, M. L.,
Van Etten, J. L., Zhang, X., and
Rossmann, M. G. (2019) Near-
atomic structure of a giant virus.
*Nat Commun* **10**, 388

26. Yan, X., Yu, Z., Zhang, P.,
Battisti, A. J., Holdaway, H. A.,
Chipman, P. R., Bajaj, C.,
Bergoin, M., Rossmann, M. G.,
and Baker, T. S. (2009) The
capsid proteins of a large,
icosahedral dsDNA virus. *J Mol
Biol* **385**, 1287-1299

27. Xiao, C., Fischer, M. G.,
Bolotaulo, D. M., Ulloa-
Rondeau, N., Avila, G. A., and
Suttle, C. A. (2017) Cryo-EM
reconstruction of the Cafeteria
roenbergenensis virus capsid
suggests novel assembly
pathway for giant viruses. *Sci Rep* **7**, 5484

28. Klose, T., Reteno, D. G., Benamar, S., Hollerbach, A., Colson, P., La Scola, B., and Rossmann, M. G. (2016) Structure of faustovirus, a large dsDNA virus. *Proc Natl Acad Sci U S A* **113**, 6206-6211

29. Yoshikawa, G., Blanc-Mathieu, R., Song, C., Kayama, Y., Mochizuki, T., Murata, K., Ogata, H., and Takemura, M. (2019) Medusavirus, a Novel Large DNA Virus Discovered from Hot Spring Water. *J Virol* **93**, e02130-18

30. Krupovic, M., and Koonin, E. V. (2017) Multiple origins of viral capsid proteins from cellular ancestors. *Proc Natl Acad Sci U S A* **114**, E2401-E2410

31. Abrescia, N. G., Bamford, D. H., Grimes, J. M., and Stuart, D. I. (2012) Structure unifies the viral universe. *Annu Rev Biochem* **81**, 795-822

32. Abrescia, N. G., Cockburn, J. J., Grimes, J. M., Sutton, G. C., Diprose, J. M., Butcher, S. J., Fuller, S. D., San Martin, C., Burnett, R. M., Stuart, D. I., Bamford, D. H., and Bamford, J. K. (2004) Insights into assembly from structural analysis of bacteriophage PRD1. *Nature* **432**, 68-74

33. Abergel C., Legendre M., Claverie JM. (2015) The rapidly expanding universe of giant viruses: Mimivirus, Pandoravirus, Pithovirus and Mollivirus. *FEMS Microbiol Rev.* **39**,779-96

34. Bahar, M. W., Graham, S. C., Stuart, D. I., and Grimes, J. M. (2011) Insights into the evolution of a complex virus from the crystal structure of vaccinia virus D13. *Structure* **19**, 1011-1020

35. Cockburn, J. J., Abrescia, N. G., Grimes, J. M., Sutton, G. C., Diprose, J. M., Benevides, J. M., Thomas, G. J., Jr., Bamford, J. K., Bamford, D. H., and Stuart, D. I. (2004) Membrane structure and interactions with protein and DNA in bacteriophage PRD1. *Nature* **432**, 120-125

36. Liu, Q., Ma, B., Qian, N., Zhang, F., Tan, X., Lei, J., and Xiang, Y. (2019) Structure of the African swine fever virus major capsid protein p72. *Cell Res* **0**, 1–3

37. Sinkovits, R. S., and Baker, T. S. (2010) A tale of two symmetrons: rules for construction of icosahedral capsids from trisymmetrons and pentasymmetrons. *J Struct Biol* **170**, 109-116

38. Yan, X., Olson, N. H., Van Etten, J. L., Bergoin, M., Rossmann, M. G., and Baker, T. S. (2000) Structure and assembly of large lipid-containing dsDNA viruses. *Nat Struct Biol* **7**, 101-103

39. Santos-Perez, I., Charro, D., Gilmarton, D., Azkargorta, M., Elortza, F., Bamford, D. H., Oksanen, H. M., and Abrescia, N. G. A. (2019) Structural basis for assembly of vertical single β-barrel viruses. *Nat Commun* **10**, 1184

40. Simon-Mateo, C., Andres, G., and Vinuela, E. (1993) Polypeptide processing in African swine fever virus: a novel gene expression strategy for a DNA virus. *EMBO J* **12**, 2977-2987

41. Simon-Mateo, C., Andres, G., Almazan, F., and Vinuela, E. (1997) Proteolytic processing in African swine fever virus:
evidence for a new structural polyprotein, pp62. *J Virol* **71**, 5799-5804

42. Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., and Sternberg, M. J. (2015) The Phyre2 web portal for protein modeling, prediction and analysis. *Nat Protoc* **10**, 845-858

43. Xiao, C., Kuznetsov, Y. G., Sun, S., Hafenstein, S. L., Kostyuchenko, V. A., Chipman, P. R., Suzan-Monti, M., Raoul, D., McPherson, A., and Rossman, M. G. (2009) Structural studies of the giant mimivirus. *PLoS Biol* **7**, e92

44. Veesler, D., Ng, T. S., Sendamarai, A. K., Eilers, B. J., Lawrence, C. M., Lok, S. M., Young, M. J., Johnson, J. E., and Fu, C. Y. (2013) Atomic structure of the 75 MDa extremophile Sulfolobus turreted icosahedral virus determined by CryoEM and X-ray crystallography. *Proc Natl Acad Sci U S A* **110**, 5504-5509

45. Gasteiger E., Hoogland C., Gattiker A., Duvaud S., Wilkins M.R., Appel R.D., Bairoch A.; *Protein Identification and Analysis Tools on the ExPASy Server*; (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press (2005). pp. 571-607

46. Suarez, C., Salas, M. L., and Rodriguez, J. M. (2010) African swine fever virus polyprotein pp62 is essential for viral core development. *J Virol* **84**, 176-187

47. Hernaez, B., Guerra, M., Salas, M. L., and Andres, G. (2016) African Swine Fever Virus Undergoes Outer Envelope Disruption, Capsid Disassembly and Inner Envelope Fusion before Core Release from Multivesicular Endosomes. *PLoS Pathog* **12**, e1005595

48. Wu, J., and Chen, Z. J. (2014) Innate immune sensing and signaling of cytosolic nucleic acids. *Annu Rev Immunol* **32**, 461-488

49. Grimes, J., Basak, A. K., Roy, P., and Stuart, D. (1995) The crystal structure of bluetongue virus VP7. *Nature* **373**, 167-170

50. Reinisch, K. M., Nibert, M. L., and Harrison, S. C. (2000) Structure of the reovirus core at 3.6 A resolution. *Nature* **404**, 960-967

51. Huiskonen, J. T., de Haas, F., Bubeck, D., Bamford, D. H., Fuller, S. D., and Butcher, S. J. (2006) Structure of the bacteriophage phi6 nucleocapsid suggests a mechanism for sequential RNA packaging. *Structure* **14**, 1039-1048

52. Gomez-Puertas, P., Rodriguez, F., Oviedo, J. M., Ramiro-Ibanez, F., Ruiz-Gonzalvo, F., Alonso, C., and Escriabano, J. M. (1996) Neutralizing antibodies to different proteins of African swine fever virus inhibit both virus attachment and internalization. *J Virol* **70**, 5689-5694

53. Enjuanes, L., Carrascosa, A. L., Moreno, M. A., and Vinuela, E. (1976) Titration of African swine fever (ASF) virus. *J Gen Virol* **32**, 471-477

54. Carrascosa, A. L., del Val, M., Santaren, J. F., and Vinuela, E. (1985) Purification and properties of African swine fever virus. *J Virol* **54**, 337-344

55. Stuart, D. I., Subramaniam, S., and Abrescia, N. G. (2016) The democratization of cryo-EM. *Nat Methods* **13**, 607-608

56. Mastronarde, D. N. (2005) Automated electron microscope
tomography using robust prediction of specimen movements. *J Struct Biol* **152**, 36-51

57. Schorb, M., Haberbosch, I., Hagen, W. J. H., Schwab, Y., and Mastronarde, D. N. (2019) Software tools for automated transmission electron microscopy. *Nat Methods* **16**, 471-477

58. Zheng, S. Q., Paloveck, E., Armache, J. P., Verba, K. A., Cheng, Y., and Agard, D. A. (2017) MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nat Methods* **14**, 331-332

59. Rohou, A., and Grigorieff, N. (2015) CTFIND4: Fast and accurate defocus estimation from electron micrographs. *J Struct Biol* **192**, 216-221

60. Zivanov, J., Nakane, T., Forsberg, B. O., Kimanius, D., Hagen, W. J., Lindahl, E., and Scheres, S. H. (2018) New tools for automated high-resolution cryo-EM structure determination in RELION-3. *Elife* **7**, e42166

61. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **25**, 1605-1612

62. de la Rosa-Trevin, J. M., Oton, J., Marabini, R., Zaldivar, A., Vargas, J., Carazo, J. M., and Sorzano, C. O. (2013) Xmipp 3.0: an improved software suite for image processing in electron microscopy. *J Struct Biol* **184**, 321-328

63. Abrescia NG, Grimes JM, Kivelä HM, Assenberg R, Sutton GC, Butcher SJ, Bamford JK, Bamford DH, Stuart DI. (2008) Insights into virus evolution and membrane biogenesis from the structure of the marine lipid-containing bacteriophage PM2. *Mol Cell* **31**, 749-761

64. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947-2948

65. Robert X, Gouet P. (2014) Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res.* **42**(Web Server issue):W320-4
Table 1. Cryo-EM data collection and 3D image processing of whole virion and virion-purified MCP p72

| ASFV virion data collection | 1st dataset | 2nd dataset |
|-----------------------------|-------------|-------------|
| Nominal magnification       | 59,000      | 130,000     |
| Voltage (kV)                | 300         | 300         |
| Camera                      | Falcon-3EC  | K2 counting |
| Electron exposure (e⁻/Å²)   | 47.7        | 47.5        |
| Defocus range (μm)          | -0.8 – 2.2  | -0.8 – 2.2  |
| Sampling interval (Å/pixel) | 1.13        | 1.09        |
| Movies                      | 8,519       | 955         |
| Frames                      | 40          | 60          |
| Selected Particles          | 583         | 684         |

| ASFV MCP p72 Data collection | 3D Reconstruction |
|-----------------------------|--------------------|
| Contributing particles      | 1,110              |
| Box Size (pixel)            | 1,000              |
| Pixel size (Å)              | 2.938              |
| Symmetry                    | I2                 |
| Map resolution (Å)          | 23.0               |
| FSC threshold               | 0.143              |
| Map sharpening B factor (Å²)| -1235.8            |
| EMDB code                   | EMDB-10346         |

| ASFV MCP p72 Data collection | 3D Reconstruction |
|-----------------------------|--------------------|
| Nominal magnification       | 59,000              |
| Voltage (kV)                | 300                 |
| Camera                      | Falcon-3EC counting |
| Electron exposure (e⁻/Å²)   | 46.6                |
| Defocus range (μm)          | -0.5 – 2.5          |
| Sampling interval (Å/pixel) | 1.13                |
| Movies                      | 1,541               |
| Frames                      | 40                  |
| Selected Particles          | 2,710,425           |

| ASFV MCP p72 Data collection | 3D Reconstruction |
|-----------------------------|--------------------|
| Contributing particles      | 337,803             |
| Box Size (pixel)            | 192                 |
| Pixel size (Å)              | 1.13                |
| Symmetry                    | C3                  |
| Map resolution (Å)          | 4.6                 |
| FSC threshold               | 0.143               |
| Map sharpening B factor (Å²)| -320                |
| EMDB code                   | EMD-10325           |
Figure 1. ASFV overall layout and outer capsid organization and structure

(A) Cryo-images of ASFV virions with some Percoll particles derived from the purification process (see Experimental Procedures); white arrowheads mark the outer envelope of the virion (in some cases broken); magenta arrowheads mark the inner membrane. Scale-bar 1000 Å. (B) Surface rendering of the ASFV density displayed using Chimera (61) and colored by radial distance (radial color scale bar in Å) with the white arrows marking the $h$ and $k$ vectors of the hexagonal lattice with triangulation number $T = 277$; the white pentagons mark the icosahedral five-folds of a triangular facet. (C) Left bottom half, surface rendered slice of virion density color-coded as Fig. 1B with outer...
capsid and inner membrane at threshold 0.018 (inner capsid and core shell at lower threshold for clarity). Bottom right, central section of the 3D reconstructed map of ASFV showing the architectural organization of the different structural components of the virion; green rectangle indicates the region where weak density contacts both capsid and inner membrane with green arrowheads in the enlarged inset marking these densities; green circle marks the turreted density at the vertices of the inner capsid. On top, the radial density profile of the virion density (from center virus to the edge box) with light-blue OC outer capsid, dark-yellow IM inner membrane, red IC inner capsid and pink N nucleoid (horizontal red and cyan lines show the width used to estimates thickness of both capsids). (D) Schematic layout of the ASFV organization as derived from this study with location of some of the viral proteins as from (14).
Figure 2. ASFV constituents and inner capsid organization
(A) Surface rendering of a ASFV virion facet (grey) at 0.024 threshold in Chimera with
at the top right the inset showing the penton protein (five copies of putative p49) and
peripentonal capsomers (homotrimers of MCP p72) viewed along the icosahedral five-
fold axis. The below insets show in blue the density corresponding to a capsomer viewed
from the top along its three-fold axis (left) and viewed from the side orthogonal to its
three-fold axis (right). The black arrows point at the same density (40% transparency) in
which the atomic model corresponding to a capsomer of Faustovirus composed of three copies of the double jelly-roll MCP (represented as cartoon tube in red; PDB 5J7O) has been docked (for clarity only the double jelly-roll core domains have been depicted). (B) Schematic representation of the organization of the trisymmetrons and pentasymmetrons in ASFV; five trisymmetrons are colored in red, blue, yellow, green and magenta; the pentasymmetron is outlined by a black pentagon with at its centre the penton (grey density). (C) Layout of one virion facet (the black outlined triangle marks those capsomers composing a trisymmetron) with grey hexagons describing the pseudo-hexameric morphology displayed by MCP p72 trimers depicted as magenta triangles. Black ovals and triangles mark the two and three-fold icosahedral symmetry axes, respectively and the black zig-zag line defines the capsomers composing the IAU. The black semi-transparent zig-zag line stemming from the icosahedral three-fold identifies the remaining two IAUs of the facet. The inset at the right-bottom is a slice of the cryo-EM map of homotrimers of MCP p72 viewed along the molecular three-fold axis and it shows the double jelly-roll fold adopted by the p72 and with the pseudo-hexameric footprint (Fig. 3A); this is displayed using the local resolution calculated in RELION (60) and mapped (color-coded resolution bar in Å) onto the capsomer density at a 0.03 threshold level in Chimera (this work; for details on map interpretability see Experimental Procedures and Figs. 3B-C and 6A-B). (D) Rendering of the homotrimeric p72 capsomer as in C inset but viewed perpendicularly to the three-fold axis (the horizontal black line and arrowheads show the height at which the density has been sliced in (C inset) while the black rectangle marks the region of the inset corresponding to a wall of a β-barrel (grey density at 0.032 threshold) where the separation of the β-strands is incipient with fitted in a jelly-roll of MCP Faustovirus (red cartoon tube).
Figure 3. Cryo-EM map of homotrimers of MCP p72 purified from the virion at 4.6 Å resolution
(A) Left, top-view of a 2D class-average obtained during 2D classification of ASFV capsomers (composed of MCP p72) unequivocally showing the presence of a pseudo-
hexameric symmetry with a true 3-fold axis perpendicular to the viewing plane (no symmetry can be imposed during 2D classification in RELION). Right, similar top view as left but density generated by using the known minimalist vertical double β-barrel PDB model with ID 2VVF (represented with cartoon tube and color coded according to chain identity) and corresponding to the MCP P2 of lipid-containing marine bacteriophage PM2 (63). The similarity between the ‘lobes’ corresponding to the individual β-barrels of the two molecules is striking as seen from the top views. (B) The alignment was performed using ClustalW (64) (top Faustovirus sequence and bottom ASFV sequence) and the figure was prepared with ESPript (65) (similarity color scheme based on % of equivalent residues; red box, strict identity, red character with blue frame, similarity). The secondary structural elements derived from the Faustovirus PDB model (ID 5J7O) are shown as helices (α-helix) and black arrows (β-strands) at the top of the alignment. (C) Fitting in Chimera of the whole trimeric Faustovirus capsomer PDB model (each molecule differently colored) into the p72 homotrimeric map (grey semitransparent; threshold 0.032). While the overall match in the region corresponding to the double β-barrel core is acceptable, the upper density deviates from the atomic model (see Experimental Procedures); the black rectangle marks the density corresponding to one of the β-barrel walls. The map also shows some density at the base and top that might not belong to the p72, as a consequence of its purification from the virion. The left and the right density are not scale.
**Figure 4. ASFV inner capsid organization**
Surface representation of the icosahedral inner capsid colored by radial distance (radial color scale bar in Å) with the five-, three and two- fold icosahedral symmetry axes labeled with cardinal numbers, and with white triangle marking a facet [the volume was re-symmetrized in XMIPP (62), Gaussian filtered with a width of 2.94 Å and displayed in Chimera at 0.083 threshold]. Inset below, enlarged view of a facet with pseudo-hexameric capsomers marked by black hexagons and with curved white arrows marking the $h$ and $k$ vectors of the hexagonal lattice with triangulation number $T = 19$; white hexagons mark other capsomers within the facet. Insets left, enlarged views of (i) a capsomer with the appearance of six petals of a daisy and of (ii) the penton and peripentonal capsomers viewed along the five-fold axis; additional density in between capsomers marked by white arrowheads and dotted lines.
Figure 5. Purification of homotrimers of MCP p72 from ASFV virions

(A) Percoll-purified MCP p72 from virions analyzed by SDS-PAGE and Coomassie blue staining. ASFV particles were dissociated successively with 1% Octyl β-D-glucopyranoside (OG), 0.5M NaCl and 0.1M DTT. The proteins solubilized in each dissociation step were analyzed by SDS-PAGE together with total virus (V). (B) Cryo-EM image of virion derived MCP p72 with some Percoll particles present in the sample; white circles mark some the capsomers formed by MCP p72 present in the image and extracted for image processing. Bar, 50 nm. (C) Sucrose gradient sedimentation of MCP p72. Purified p72 was analyzed through a 10-40% sucrose density gradient in the presence of the indicated molecular weight markers. Gradient fractions were analyzed by western immunoblotting with an anti-p72 antibody. Note that p72 sedimentation peak is around 200-kDa marker.
Figure 6. Fourier-Shell-Correlation and euler angular coverage

(A) FSC curve of homotrimers of p72 with reported resolution of 4.6 Å at the 0.143 criterion (black curve: corrected map; other colored curves represent FSC for: blue line, masked maps; red line, phase randomized masked maps; green line, unmasked maps) as derived by postprocessing in RELION; for the corresponding local resolution estimation see Fig. 2C-D. (B) Different views of the Euler angular coverage of the particles contributing to the 3D reconstruction for the ASFV homotrimeric p72 capsomer from RELION and visualized in Chimera; the height of each cylinder relates with the number...
of particles at that Euler angle and shows that the top and bottom views are overrepresented and caution should be used in interpreting the map outside the double jelly-roll core domain (see Fig. 3C). (C) FSC curve of ASFV with reported resolution of 23 Å at the 0.143 criterion (black line: corrected map; other colored curves as in A) as derived by postprocessing in RELION. The ‘spiky’ appearance of the FSC curve is due to the limited number of virions (1,110 particles contributing to the final 3D reconstruction) and defocus spread, and thus indicates that not all frequencies are equally covered – the map is nevertheless informative. The horizontal black lines mark the FSC at 0.5 and 0.143, the latter not hitting the spikes present beyond the 20 Å resolution.
The cryo-EM structure of African swine fever virus unravels a unique architecture comprising two icosahedral protein capsids and two lipoprotein membranes
German Andres, Diego Charro, Tania Matamoros, Rebecca S Dillard and Nicola G.A. Abrescia

*J. Biol. Chem.* published online October 24, 2019

Access the most updated version of this article at doi: [10.1074/jbc.AC119.011196](http://10.1074/jbc.AC119.011196)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts