Virion Structure and In Vitro Genome Release Mechanism of Dicistrovirus Kashmir Bee Virus

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ABSTRACT  Infections with Kashmir bee virus (KBV) are lethal for honeybees and have been associated with colony collapse disorder. KBV and closely related viruses contribute to the ongoing decline in the number of honeybee colonies in North America, Europe, Australia, and other parts of the world. Despite the economic and ecological impact of KBV, its structure and infection process remain unknown. Here, we present the structure of the virion of KBV determined to a resolution of 2.8 Å. We show that the exposure of KBV to acidic pH induces a reduction in interpentamer contacts within capsids and the reorganization of its RNA genome from a uniform distribution to regions of high and low density. Capsids of KBV crack into pieces at acidic pH, resulting in the formation of open particles lacking pentamers of capsid proteins. The large openings of capsids enable the rapid release of genomes and thus limit the probability of their degradation by RNases. The opening of capsids may be a shared mechanism for the genome release of viruses from the family Dicistroviridae.

IMPORTANCE  The western honeybee (Apis mellifera) is indispensable for maintaining agricultural productivity as well as the abundance and diversity of wild flowering plants. However, bees suffer from environmental pollution, parasites, and pathogens, including viruses. Outbreaks of virus infections cause the deaths of individual honeybees as well as collapses of whole colonies. Kashmir bee virus has been associated with colony collapse disorder in the United States, and no cure for the disease is currently available. Here, we report the structure of an infectious particle of Kashmir bee virus and show how its protein capsid opens to release the genome. Our structural characterization of the infection process determined that therapeutic compounds stabilizing contacts between pentamers of capsid proteins could prevent the genome release of the virus.

KEYWORDS  RNA, capsid opening, genome release, ilflavirus

Viruses that have the greatest impact on honeybees and other pollinators from the order Hymenoptera belong to the families Dicistroviridae and Iflaviridae (1–3). Kashmir bee virus (KBV) is an Aparavirus from the family Dicistroviridae. KBV, together with Israeli acute paralysis virus, and acute bee paralysis virus constitute a complex of closely related viruses that are distributed worldwide, with different members predominating in distinct geographical regions (4). The viruses ordinarily cause subclinical infections that are interspersed with sudden outbreaks marked by high virus titers and virulent pathology. KBV and the related viruses cause honeybee colony collapses, especially when the hives are infested with the parasitic mite Varroa destructor (4).

The structures of several viruses from the family Dicistroviridae have been determined: Israeli acute bee paralysis virus from the genus Aparavirus, triatoma virus and...
black queen cell virus from the genus *Triatovirus*, and cricket paralysis virus from the genus *Cripavirus* (5–8). Viruses from the family *Dicistroviridae* have nonenveloped capsids with icosahedral symmetry and diameters of about 30 nm. Capsids of dicistroviruses protect linear single-stranded positive-sense RNA genomes that are 9,000 to 10,500 nucleotides long (9). The genomes contain two open reading frames. The first one encodes nonstructural proteins that ensure the replication of dicistroviruses in infected cells. The second open reading frame contains structural (capsid-forming) proteins. The polyproteins are cotranslationally and posttranslationally cleaved by virus proteases to produce functional subunits. The capsid proteins originating from a single polyprotein form a protomer—the basic building block of the dicistrovirus capsid. The major capsid proteins VP1, VP2, and VP3 have β-sandwich jelly roll folds and form capsids with pseudo-$T=3$ icosahedral symmetry (5–8, 10). VP4s are peptides that are 70 to 90 residues long and attach to the inner faces of capsids of aparaviruses and cripaviruses. In contrast, VP4s are present in the virions of triatoviruses but are not attached to the shells formed by the other capsid proteins (5, 6, 8). Capsids protect dicistrovirus genomes from degradation by RNases present in the extracellular environment. To initiate infection, dicistroviruses bind to as-yet-unknown receptors at the cell surface. After endocytosis, the capsids release genomes and ensure their delivery into the cytoplasm. It has been shown that acidic pH induces the genome release of Israeli acute bee paralysis virus *in vitro* (11). Virions of Israeli acute bee paralysis virus convert to activated particles, which are characterized by the externalization of VP4 and the N termini of VP1 from a capsid and redistribution of their genomic RNA (11). Holes in the capsids of activated particles positioned on fivefold and twofold axes of picorna(-like) viruses were speculated to serve as channels for genome release (12–17). However, the dimensions of these holes are too small to enable the passage of single-stranded RNA. The dissociation of the capsid into pentamers was described as the mechanism of genome release of foot-and-mouth disease virus (18). It has been recently indicated that enterovirus particles expel pentamers of capsid protein protomers to release their genomes. The resulting openings, which are more than 120 Å in diameter, are sufficient to release the RNA genomes even if they contain double-stranded segments (19). It has been speculated that triatoma virus may release its genome by particle opening (20, 21).

Here, we present the virion structure of KBV. In addition, we show that the exposure of KBV to acidic pH induces the reorganization of its RNA genome and a reduction in interpentamer contacts within capsids. Capsids of KBV crack into pieces, resulting in the formation of open particles lacking pentamers of capsid proteins. The large opening of a capsid enables the rapid release of the genome and thus limits the probability of its degradation by RNases during the process.

**RESULTS AND DISCUSSION**

**Virion structure of KBV.** The structure of the KBV virion was determined to a resolution of 2.8 Å (Fig. 1; see also Fig. S1, S2, and S3 and Table S1 in the supplemental material). The capsid of KBV is built from VP1 subunits, which form pentamers around fivefold symmetry axes of the capsid, and VP2 and VP3 subunits that assemble into heterohexamers around the threefold symmetry axes (Fig. 1A and B). Sixty copies of the minor capsid protein VP4 are attached to the inner face of the capsid (Fig. 1B). The cryo-electron microscopy (cryo-EM) density map enabled building of the complete structure of 208 residues of VP1, residues 10 to 259 out of 259 of VP2, and the complete structure of 300 residues of VP3, and residues 12 to 69 out of 69 of VP4 (Fig. 1B and C). The cores of major capsid proteins VP1 to VP3 have jelly roll β-sandwich folds with β-strands named B to I according to a convention (Fig. 1B) (10, 22, 23). VP4 of KBV forms two short α-helices at the C terminus (Fig. 1B and C). The surface of the KBV virion is decorated with 60 spiky protrusions located between fivefold and threefold axes of icosahedral symmetry of the capsid (Fig. 1A and B). The spikes, which protrude 23 Å above the capsid surface, are formed by two antiparallel β-strands from the CD loop of VP3 and the C-terminal β-strand of VP1 (Fig. 1B). Similar spikes decorate the
particles of Israeli acute bee paralysis virus, black queen cell virus, and triatoma virus (5, 6, 8). The spikes of Israeli acute bee paralysis virus and black queen cell virus were speculated to play a role in receptor recognition (5, 6). Being the most prominent surface features, the spikes of KBV may have a similar function.

The capsid of KBV is built from 12 pentamers of capsid protein protomers VP1 to VP4. Interfaces between neighboring pentamers are predominantly formed by VP2 and VP3 subunits (Fig. 2A). Two β-strands from the N terminus of VP2 extend the β-sheet CHEF of the VP3 subunit from a neighboring pentamer, which is an example of domain swapping that is common in virus capsids (Fig. 2A) (24). The contacts between two pentamers mediated by the N termini of VP2 subunits account for 54% of the interface, which has a buried surface area of 4,900 Å².

Maturation cleavage of VP0. Immature particles of picorna-like viruses assemble from protomers containing a precursor protein, VP0, which in dicistroviruses is cleaved...
into N-terminal VP4 and C-terminal VP3 (Fig. 1C) (25). The cleavage occurs after the particles are filled with RNA genomes (26–28). It has been speculated that the Asp-Asp-Phe motif from the VP1 subunit cleaves the VP0 subunit of cricket paralysis virus and the capsid protein of black beetle virus from the family Nodaviridae (7, 27–29). Homologous residues Asp186, Asp187, and Phe188 from VP1 of KBV are positioned in the vicinity of the site of cleavage of VP0 into VP4 and VP3 (Fig. 3A).

When viewed from inside the capsid, the VP4 of KBV resembles the Greek letter lambda (Fig. 3B). In contrast, VP4 of cricket paralysis virus has the shape of the letter sigma (Fig. 3C). VP4s of KBV, Israeli acute bee paralysis virus, and cricket paralysis virus interact with the other capsid proteins through interfaces with buried surface areas of 1,350, 1,600, and 1,400 Å², respectively. The buried surface area of the interface between two neighboring copies of VP4 is 800 Å² in KBV and Israeli acute bee paralysis virus and 1,000 Å² in cricket paralysis virus. These extensive contacts between adjacent copies of VP4 may enable their synchronized release from particles together with the genome, as discussed below.

**Intersubunit contacts in capsids of dicistroviruses.** The availability of atomic structures enabled the comparison of intersubunit contacts in capsids of KBV, Israeli acute bee paralysis virus, cricket paralysis virus, black queen cell virus, and triatoma virus (5–8). Although the overall sequence conservation among the viruses is less than 41%, they share five interactions between capsid proteins that are formed by conserved residues. The interface between subunits VP1 and VP3 from one protomer contains two hydrogen bonds, which connect Arg93 and Lys95 from β-strand D of VP1 to Asp27 from the N terminus of VP3 (KBV residue numbering) (Fig. 4A and B). Another common interaction is that of Tyr148 from the GH-loop of VP1, which is linked by a hydrogen bond to Glu34 from the N terminus of the neighboring VP1 subunit (Fig. 4A and C). These interprotomer interactions may play a role in establishing the proper positioning of protomers during capsid assembly. In addition, Ser1 and Lys2 from the N terminus of VP3 form hydrogen bonds with Arg93 from β-strand D and Asp186 from the C terminus of the VP1 subunit from another protomer within the same pentamer (Fig. 4A and D). These contacts position the N terminus of the precursor subunit VP0 for cleavage by the putative proteolytic site formed by Asp186, Asp187, and Phe188 from VP1, as discussed above.

**Exposure of KBV to acidic buffer generates various kinds of particles.** At neutral pH, the RNA genomes uniformly fill the capsid cavities of KBV virions (Fig. 5A and C). Empty or broken particles constituted less than 1% of the purified virus (Fig. 5A). In contrast, after exposure to acidic pH, the population of particles became structurally heterogeneous (Fig. 5B). Three-dimensional classification differentiated five types of particles: (i) particles that have a uniform distribution of RNA genomes and the same...
capsid structure as a native virus at neutral pH, except for missing resolved electron density for nine C-terminal residues of VP3 (Fig. 5B and D; Fig. S1, S2, and S3; Table S1); the residues which became disordered are located at the particle surface and probably became mobile at the acidic pH; (ii) activated particles with reduced interpentamer contacts and RNA reorganized into regions of high and low density (Fig. 2B and Fig. 5B and E; Fig. S1, S2, and S3; Table S1); VP4 remained attached to the shells formed by the other capsid proteins of these particles (Fig. 2B); (iii) open particles missing genomes and pentamers of capsid protein protomers (Fig. 5B and F; Fig. 6A and B; Fig. S1, S2, and S3; Table S1); (iv) empty particles in which one pentamer bulges out from the capsid (Fig. 5B and G; Fig. 6C; Fig. S1, S2, and S3; Table S1); (v) empty particles with intact capsids, which lack VP4 (Fig. 2C; Fig. 5B and H; Fig. S1, S2, and S3; Table S1). The formation of the different particles, after exposure to acidic pH, may be related to differences in the folds of the genomic RNA. The structures of packaged RNA genomes probably differ from particle to particle (30), and their reactions to exposure to acidic pH may be different. Furthermore, genomes of single-stranded RNA viruses are packaged in capsids together with positively charged polyamines, which shield the negative charge of the RNA (31–33). Individual particles might also differ in their polyamine content, which may cause differences in the reactions of KBV particles to acidic pH.

**Capsids of KBV open to release genomes at acidic pH.** Capsids protect virus genomes from degradation in the extracellular environment, but viruses have to be able to release their RNAs to initiate infection. The genome release of KBV was induced by incubation at pH 6 at 34°C for 30 min, mimicking the conditions in endosomes (Fig. 5B). The acidic pH or the changes in the structure of the capsid induced by the acidic
pH trigger the reorganization of RNA genomes in activated particles of KBV (Fig. 5E), which is a characteristic feature of activated particles of the well-studied enteroviruses from the family *Picornaviridae* (11, 19, 34–37). Changes in the capsid-genome interactions were also observed in particles of triatoma virus which were induced to release their genomes by exposure to alkaline pH (21). Furthermore, particles of KBV with non-uniform genome distribution lack the density resolved in the native virion for 53 residues from the N terminus of VP2, which causes a reduction in interpentamer contacts from 4,600 to 1,450 Å² (Fig. 2B). This reduction in interpentamer contacts, which was also observed in Israeli acute bee paralysis virus and triatoma virus (11, 20), is another characteristic feature of activated particles. In contrast to the activated particles of enteroviruses, those of KBV are not expanded relative to virions at neutral pH (Fig. 5C and E) and do not contain pores along twofold axes of icosahedral symmetry of their capsids (Fig. 5E), and their VP4s remain attached to the shells formed by the other capsid proteins (Fig. 2B).

The asymmetric reconstruction of an open particle of KBV missing one pentamer of capsid protein protomers was determined to a resolution of 18 Å (Fig. S1 and S2; Table S1). The application of fivefold symmetry averaging enabled the determination of the structure of the open particle to a resolution of 11 Å (Fig. 6B; Fig. S1 and S2; Table S1). Furthermore, the micrographs of KBV exposed to acidic pH contain fragments of capsids (Fig. 5B). The observation of capsid fragments and open particles lacking a pentamer indicates that, at least in vitro, the genome release of KBV is enabled by capsid opening. It is likely that the reduction in interpentamer contacts, which is caused by the loss of structure of the N terminus of VP2 subunits, destabilizes the KBV capsid. At the same time, the genome reorganization may induce an increase in the pressure
exerted by the genome on the inner face of the capsid. The combination of capsid weakening and/or increased internal pressure enables capsid opening—either the cracking of the whole capsid into several pieces or expulsion of a pentamer of capsid proteins (19).

The genome release mechanism of dicistroviruses. Activated and empty particles of dicistroviruses are not expanded relative to native virions and do not contain holes which may serve as channels for genome release (20, 38). It has been proposed that genomes of dicistroviruses are released from particles as single-stranded RNAs through transiently formed pores along twofold or fivefold axes of the icosahedral symmetry of their capsids (11). The observation of numerous empty particles in the sample of KBV exposed to acidic pH indicates that this mechanism of genome release is a possibility. However, extrusion of the 10,000-nucleotide-long RNA through a narrow pore would be a slow process, which would expose the genome to degradation by RNases (39, 40). The speculations about single-stranded RNA (ssRNA) release through a narrow pore in a capsid were based on the comparisons of icosahedrally symmetrized structures of capsids of native virions and those of empty particles resulting from the genome release (11). The reliance on icosahedrally symmetrized structures in the previous studies prevented the identification of asymmetric features that are essential for the genome release. In contrast, it has been indicated that the genome release of triatoma virus, which can be induced by alkaline pH, depends on capsid disruption or the lid-like opening of pentamers of capsid proteins (20, 21).

Here, we show that acidic pH induces the formation of activated particles of KBV, which are characterized by a reduction in interpentamer contacts and a genome organized into regions with low and high density (Fig. 7). The activated particles crack open or lose pentamers to release their genomes (Fig. 7). VP4s are probably released from KBV particles together with genomes. It has been shown that VP4 of triatoma virus forms toroidal pores in membranes and thus enables the delivery of the virus RNA from endosomes into the cytoplasm (41). It is likely that VP4 of KBV has the same function. Fragments of KBV capsids may reassemble into empty particles (Fig. 7). A subpopulation of the empty particles contains a pentamer of capsid proteins bulging out
from their capsid (Fig. 7). Since these particles lack genomes, it is possible that they represent incompletely reassembled capsids, which have released their RNAs.

We analyzed disassembly intermediates of triatoma virus exposed to denaturing conditions. Particles of triatoma virus fragment into pentamers when exposed to urea at a concentration higher than 2.5 M (Fig. S4A). In contrast, holes with various shapes open in capsids of triatoma virus when treated with chaotropic salts (Fig. S4B). It has been shown previously that capsid proteins of triatoma virus reassemble into empty particles after the genome release (21).

The observation of open particles of both KBV and triatoma virus indicates that capsid opening may be a shared genome release mechanism of dicistroviruses. Previous in silico simulations of particles with properties similar to those of KBV demonstrated that disruption of a capsid enables genome release in less than a microsecond, which limits the probability of its degradation by RNases during the process (19). The dependence of dicistrovirus genome release on capsid opening indicates that compounds overstabilizing interpentamer interactions may block the infection process.

**MATERIALS AND METHODS**

**KBV propagation in honeybee pupae.** The propagation of KBV was carried out as described previously (5, 6). In brief, brood areas with white-eyed pupae of *Apis mellifera* were identified by the color and structural features of the cell caps. White-eyed pupae were carefully extracted from the brood combs to prevent their injury. The pupae were placed on paper furrows with the ventral side up. The virus inoculum (1 μl) was injected into each pupa using a Hamilton micropipette with a 30-gauge 22-mm-long needle through the intersegmental cuticle between the 4th and 5th sternite. Pupae that leaked hemolymph after the injection were discarded. The optimal concentration of the virus in the inoculum for virus production was determined experimentally, by comparing virus yields when using different virus concentrations in the injection inoculum. Inoculated pupae were placed into petri dishes and incubated at 30°C and 75% humidity for 5 days. After incubation, the pupae were frozen at −20°C. For long-term storage, the pupae were kept at −80°C.

**KBV purification.** Fifty experimentally infected honeybee pupae were homogenized with a Dounce homogenizer in 30 ml of phosphate-buffered saline (PBS), pH 7.5 (Sigma-Aldrich). Nonionic detergent NP-40 was added to a final concentration of 0.5%, and the homogenate was incubated for 1 h at room temperature. The homogenate was centrifuged at 8,000 × g for 30 min. The pellet was discarded, and the supernatant was centrifuged at 150,000 × g for 3 h in a Ti50.2 fixed-angle rotor (Beckman-Coulter).
The resulting pellet was resuspended in PBS to a final volume of 5 mL. MgCl₂ was added to a final concentration of 5 mM as well as 20 μg/mL DNase I and 20 μg/mL RNase. The solution was incubated at room temperature for 30 min and centrifuged at 4,000 × g for 15 min. The resulting supernatant was loaded onto a CsCl (0.6 g/mL) solution prepared in PBS. The ultracentrifugation proceeded for 16 h at 30,000 rpm in a Beckman SW-41 Ti rotor (Beckman Coulter Optima XPN-80 ultracentrifuge) at 10°C to establish the CsCl gradient. Virus bands were collected by gently piercing the ultracentrifuge tubes with an 18-gauge needle. The viruses were transferred to PBS by several rounds of concentration and dilution through centrifuge filter units with a 100-kDa-molecular-weight cutoff. This procedure yielded about 700 μg of the virus with sufficient purity for cryo-EM analysis.

Preparation of capsid protein pentamers and open particles of triatoma virus. Native virions of triatoma virus were prepared as described previously (26). Purified virions at a concentration of 0.2 mg/mL were dialyzed against 8 M urea or 1 M guanidinium thiocyanate in NMM buffer (10 mM NaCl, 1 mM MgCl₂, 50 mM Tris-HCl, pH 7.5) for 24 h at room temperature. The treated particles were applied to the nitrocellulose-covered copper grids and stained with 2.5% aqueous uranyl acetate for observation under an electron microscope.

Induction of genome release of KBV. Purified KBV was diluted in 50 mM morpholineethanesulfonic acid (MES), pH 6.0, to a final virus concentration of 2.5 mg/mL and incubated at 34°C in a heat block for 30 min. The temperature was selected to mimic conditions in a beehive. After incubation, samples were immediately vitrified for cryo-EM analysis.

Preparation of samples for cryo-EM. A solution of freshly purified KBV at a concentration of 2.5 mg/mL was applied onto holey carbon-coated copper grids (Quantifoil R2/1, mesh 300; Quantifoil Micro Tools), blotted, and plunge-frozen in liquid ethane using an FEI Vitrobot Mark IV, set to a 2-s blotting time and 0 s blot force. The Vitrobot sample application chamber was held at 5°C and 100% humidity throughout the whole vitrification process. The samples were stored under liquid nitrogen until use.

Cryo-EM data collection. Grids with the vitrified samples of KBV and KBV incubated at acidic pH were transferred to an FEI Titan Krios electron microscope operated at 300 kV and aligned for parallel illumination in nanoprobe mode. Images were recorded with an FEI Falcon II (native KBV) and FEI Falcon III (KBV incubated at acidic pH) direct electron detection camera under low-dose conditions (20 e⁻/Å²). Images were recorded using defocus values ranging from 1.0 to 2.5 μm at a nominal magnification of ×75,000, resulting in a pixel size of 1.063 Å/px for native KBV. For the KBV sample treated with acidic pH, the underfocus ranged from 0.2 to 2.0 μm at a magnification of ×75,000, resulting in a pixel size of 1.07 Å/px. Each image of native KBV and KBV at acidic pH was recorded in movie mode with 0.5 s (7 fractions) and 1 s (39 fractions) of total acquisition time, respectively, and saved as separate movie frames. MgCl₂ 50 mM Tris-HCl, pH 7.5, for 15 min. The resulting supernatant was filtered (50-Å) electron density map of the native virion of Israeli acute bee paralysis virus (EMD-4114) was used as an initial model for three-dimensional (3D) classification and for subsequent refinements of all the KBV reconstructions with imposedicosahedral symmetry, which was performed using the RELION 3dautorefine procedure (45). To further homogenize the data set, after initial refinement the particles were subjected to another round of 3D classification omitting the alignment step and using the particle shifts and orientations estimated in the previous refinement step. Particles belonging to the best class were reextracted and recentered. Reextracted particles were subjected to another round of autorefinement in RELION 3.1. The electrostatic potential map of the KBV particle featuring a missing pentamer, obtained from the initial asymmetric 3D classification, was subsequently used as an initial model for further 3D classification and subsequent refinement of the open particle. Fivefold symmetry was applied in all classifications and refinements of the open particle. Final reconstructions, according to the gold standard (46), were calculated using RELION 3dautorefine. The resulting maps were masked with a threshold mask and B-factor sharpened using the RELION postprocess procedure.

The final resolution was estimated using the 0.143 Fourier shell correlation (FSC) cutoff.

Building of molecular structures. The PDB structure of Israeli acute bee paralysis virus (PDB: 5CDC) was rigid-body fitted into the B-factor-sharpened electrostatic potential map of the KBV virion, using the program Phenix and manually rebuilt using the program Coot (5, 47, 48). The structure was refined by alternating rounds of real-space refinement in Phenix (47) and reciprocal space refinement in Refmac5 (49). After each refinement step, the structure was carefully inspected in Coot and Coot refinements were fixed manually. The structure quality was monitored throughout the model building and refinement using the MolProbity tool (50). The structure of the native KBV virion was used as the initial model for building the structures of particles treated with acidic pH.

Analysis of intersubunit contacts. For the analysis of the intersubunit contacts, the following PDB models and their corresponding sequences were used: Israeli acute bee paralysis virus (PDB: 5CDC),
cricket paralysis virus (PDB: 1B35), black queen cell virus (PDB: 5MQC), and triatoma virus (PDB: 3NAP) (5–8). Pairwise MUSCLE alignments of biological protomers were performed using the software Geneious Prime 2019. Structures of icosahedral asymmetric units of the five viruses were symmetry expanded to obtain complete capsids. Contacts representing all possible protein-protein interactions within the capsid were analyzed using the EMBL-EBI PDBePISA tool (51).

Preparation of figures. The figures presented in this article were generated using the programs PyMOL (The PyMOL Molecular Graphics System, version 1.2r3pre; Schrödinger, LLC), Chimera (52), and Gimp (The GIMP Development Team, 2019, GIMP, https://www.gimp.org). Tables and graphs were generated using Microsoft Excel and Gnuplot (http://gnuplot.info), respectively.

Data deposition. Cryo-EM maps and coordinates of the following structures were deposited in the Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB; https://www.ebi.ac.uk/pdbe/emdb) as follows: KBV virion at neutral pH, EMD-12182 and PDB 7BG8; particle with uniform genome distribution at acidic pH, EMD-12137 and PDB 7BG3; activated particle at acidic pH, EMD-12173 and PDB 7BG8; empty particle at acidic pH, EMD-12154 and PDB 7BE9; asymmetric reconstruction of open particle at acidic pH, EMD-12155; fivefold-symmetrized reconstruction of open particle at acidic pH, EMD-12167; and asymmetric reconstruction of particle with bulged pentamer of capsid protein protomers at acidic pH, EMD-12168.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 2.3 MB.

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