Three-dimensional architecture of the bacteriophage \( \phi 29 \) packaged genome and elucidation of its packaging process

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Abstract

The goal of the work reported here is to understand the precise molecular mechanism of the process of DNA packaging in dsDNA bacteriophages. Cryo-EM was used to directly visualize the architecture of the DNA inside the capsid and thus to measure fundamental physical parameters such as inter-strand distances, local curvatures, and the degree of order. We obtained cryo-EM images of bacteriophage that had packaged defined fragments of the genome as well as particles that had partially completed the packaging process. The resulting comparison of structures observed at intermediate and final stages shows that there is no unique, deterministic DNA packaging pathway. Monte Carlo simulations of the packaging process provide insights on the forces involved and the resultant structures.

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Introduction

Double-stranded DNA viruses, including many bacteriophages and clinically relevant herpesviruses and adenoviruses, compact their DNA to near crystalline density during the DNA packaging stage of viral assembly (Jardine and Anderson, 2006). Although the problem of how DNA becomes organized within the head during packaging has been of interest for many decades, little experimental work directly addressing details of this process has been reported. We have used cryo-electron microscopy (cryo-EM) imaging to directly visualize the architecture of the DNA inside the capsid and thus to measure fundamental physical parameters of DNA organization. We also follow the temporal course of packaging with cryo-EM images obtained at different, defined stages of the process.

Early work inferred several possible models for the architecture of packaged DNA in bacteriophages, based on measurements of DNA inter-strand distances and DNA strand orientations obtained from various biophysical techniques. X-ray diffraction work provided evidence consistent with hexagonal packing in T2 and T7 (North and Rich, 1961). Richards et al. (1973), using EM of negatively stained samples, found that the DNA close to the capsid of several bacteriophages has a circumferential orientation. Earnshaw and Harrison and others (Earnshaw and Casjens, 1980; Earnshaw and Harrison, 1977) studied isometric P22 and wild-type \( \lambda \) particles by X-ray diffraction and determined that the DNA forms concentric layers, almost completely filling the capsid volume. The concentric spool model they formulated was considered to
better account for the experimental data than the “ball of string” model or a folded chain model. Hall and Schellman (1982a, 1982b) used flow dichroism to carry out measurements on several DNA bacteriophages. They reported an average preferential orientation of the DNA in all cases studied, orthogonal to the main axis of the particle in phage λ and parallel in T4B, but ruled out the simplest possible packing models. Instead, their results are only consistent with a combination of four possibilities: the DNA wound, at least in part, in inclined paths relative to the phage axis; orientational disorder; composite structures; and partial organization. Structural features such as reported in the work reviewed above and many others, inspired efforts in modeling the arrangement of the internalized DNA and in measuring properties of condensed DNA in solution. We highlight experimental work proposing a liquid crystal (Lepault et al., 1987), a folded toroid (Hud and Downing, 2001; Hud et al., 1995), a folded coaxial spool (Serwer, 1986), the spiral fold (Black et al., 1985), and experimental determinations of DNA self-interaction potential (Rau et al., 1984; Strey et al., 1998 and references therein). In addition, Monte Carlo simulations have provided insights on the evolution of the structure as the DNA is packed, as well as on the final structure (Petrov and Harvey, 2007). A recent review (Garcia et al., 2007) analyzed the forces associated with tightly bent DNA and the consequences for a range of fundamental biological processes including packaging in viruses.

In 1997 the first high quality cryo-EM image analysis of intact phage T7 (Cerritelli et al., 1997) allowed a direct visualization of the patterns formed by DNA strands. The cryo-EM reconstruction of isometric bacteriophage T4 (Olson et al., 2001), and later the prolate head of T4 (Fokine et al., 2004) showed several layers of DNA spaced at ~2.4–2.5 nm, clearly visible in central sections of the density map. Recently a series of cryo-EM reconstructions have allowed the visualization of DNA strands within the outer layers of packaged DNA in bacteriophage T7, P22, ε15, and φ29 (Agirrezabala et al., 2005; Chang et al., 2006; Jiang et al., 2006; Lander et al., 2006; Xiang et al., 2006). All these reconstructions had the common goal of describing the architecture of the whole phage at high resolution in order to better understand the protein components of the head, portal and tail, and the capsid subunits, along with their relative positions and interactions. In all cases, the DNA appears ordered in concentric layers, although only three layers are clearly resolved in the two P22 reconstructions (Chang et al., 2006; Lander et al., 2006) and the ε15 reconstruction (Jiang et al., 2006), while at least four layers are clearly visible in the T7 and φ29 reconstruction (Agirrezabala et al., 2005; Xiang et al., 2006). Clear density for individual DNA strands in the final degree of order for the whole genome of bacteriophage φ29, we have tackled the problem with a different approach. Our work has focused exclusively on imaging the packaged DNA in φ29 and intermediate states throughout the packaging process (Grimes et al., 2002). We have purposely designed our data acquisition parameters to minimize the protein contributions to the reconstruction process. We observe well-resolved concentric DNA layers, or shells, in the three-dimensional reconstruction of fully packaged particles, although there is very little detail in the protein capsid. In addition, image analysis of particles with partial genome content has helped us understand the degree of order present at different stages of the packaging process. We conclude that there is no unique, deterministic DNA packaging pathway. We have also performed Monte Carlo simulations that provide models that help us understand basic aspects of the packaging process.

**Results**

**Cryo-EM single particle reconstruction of the full genome in mature virus particles**

Individual projections of mature φ29 particles obtained by cryo-electron microscopy of frozen hydrated samples already show striking patterns within fully packaged particles. Fig. 1 shows typical particles, in several orientations; see Methods for details. The prolate bacteriophage φ29 head has an elongated, almost cylindrical shape, with caps that display clear five-fold (c5) symmetry. Images were acquired using defocus values chosen to optimize the contrast for the DNA spacings. Projections along the axis of the cylinder reveal concentric circles, and projections perpendicular to that axis often show a punctate pattern consistent with hexagonal close packing of the DNA (Hud and Downing, 2001). Single particle reconstructions from 8200 particles were computed, either without imposing any symmetry throughout the process, or imposing c5 symmetry after the first iterations without any symmetry. The resulting reconstructions were virtually identical, apart from the higher signal-to-noise ratio and the presence of noise symmetrization artifacts in the symmetric reconstruction. A comparison between these two reconstructions is given in Fig. S1.
Fig. 1. Typical images of mature φ29 particles. A total of 8200 mature particles were boxed from film images acquired at a magnification 48,500×. A good distribution of orientations was assured by choosing the appropriate ice thickness, and no preferential orientations were observed. Top views typically show a strong pattern of concentric circles, while side views show a punctate pattern. Imaging parameters were chosen to maximize information transfer and contrast at the resolution relevant for typical DNA spacing in viruses, i.e. ~2.1 to 2.5 nm; see Methods for details. Protein components have a secondary relevance in these images, and do not drive the refinement process. Box sizes are 384×384 pixels, with a pixel size of 0.262 nm (the box sides measure 101 nm).

Figs. 2 and 3 show the symmetric reconstruction density map. The DNA patterns discussed above, shown in Fig. 1, were dominant in aligning the particles during the reconstruction process. The protein components have thus been averaged out, and the three-dimensional reconstruction of the DNA is the main feature. At a resolution of ~2.2 to 2.5 nm, the Fourier shell correlation coefficient is 0.6 in the symmetric reconstruction and 0.5 in the asymmetric reconstruction, allowing accurate measurement of relevant spacings in the density maps; see Fig. S2 for more details. Axial or central sections of the density maps (Fig. 3) show five shells inside the capsid, as closed surfaces with irregular but quite continuous walls. At the isosurface values chosen here, the fifth layer is connected with the more disordered density at the center, but its outer boundary is well delineated (innermost density in blue in Fig. 3C).

Fig. 3 shows orthogonal (A) and axial (B) cross-sections through the center of mass of the reconstructed density, together with density profiles for each section. The red arrows indicate the maximum density peaks from the capsid, and the blue arrows the maximum density peaks from the outermost DNA layer. The fall-off of the integrated density corresponds to an outer diameter of approximately 45 nm for the orthogonal section. The peak-to-peak diameter for the capsid is ~43.5 nm, and the peak-to-peak diameter of the outermost DNA shell is ~33 nm. The first six integrated density peaks, from the outside, correspond to clearly discernible shells in the 3D map. While the radial plot shows two more peaks, there is no continuous shell inside the sixth. The radius of the innermost DNA shell is approximately 7.2 nm, the smallest radius of curvature we experimentally observe.

Spacing and organization of the fully packaged DNA

Our three-dimensional reconstruction allows us to carry out measurements from which we can examine the current theory of DNA packing in viral particles. The average distance between the six layers is 2.4 nm, and it is approximately the same value when the averages are computed for any section orthogonal to the shells. These distances were computed after resampling the density maps to a final pixel size 1/4 of the original in order to define the center of mass of density elements more accurately. Assuming that the strands are mainly hexagonally close-packed, the distance between neighboring strands would on average be ~2.8 nm, in excellent agreement...
with the value of 2.8 nm determined by X-ray diffraction (Earnshaw and Casjens, 1980).

As pointed out earlier (Petrov and Harvey, 2007), the density map from this type of reconstruction provides a probability density for the DNA strands. The facts that only a few individual strands are visible and that the contrast of the shells decreases away from the capsid indicate that there is some degree of disorder in the packing. The capsid gives a strong constraint on the radius of the outer layer, although there is enough variability in positioning strands with respect to the capsid that aligning the DNA images causes a loss of contrast for the capsid. From the falloff of contrast from one shell to the next, we can estimate that the order of 80% of each layer is in alignment with the adjacent layer. Although the DNA is clearly structured in layers, these layers are not as regularly spaced as expected; there is variability in the measured distance between them. The distances between shells seem to decrease slightly, as a trend, for shells “sandwiched” between the outermost and innermost ones. The distance variability is consistent with a lack of unique, long range order.

The radius of the inner shell, approximately $\sim 7.2$ nm, coincides with the estimate of $\sim 6.0$ nm by Earnshaw and Harrison (1977), and puts an upper limit to the curvature the DNA is constrained to adopt toward the end of the packing process. It is notable that a radius of $\sim 7.2$ nm results in a circumference of $\sim 44$ nm, much less than the persistence length of DNA ($\sim 53$ nm). Theoretical work has also predicted a void volume at the center (Odijk, 1998), but we see no indication of such a void.

Cryo-EM analysis of partially packaged genomes

Our conclusion that the fully packaged DNA does not adopt a unique structure, but rather fits an organized architecture that is filled by different DNA trajectories in different particles, implies that there is no unique packaging pathway. On the other hand, if there is a unique DNA packaging pathway, snapshots of packaging particles at intermediate states should reveal the underlying structural features common to all viral particles. To see if there are any detectable structures that develop as the packaging proceeds, we imaged particles with defined lengths of 32%, 51%, and 78% of the total genome packaged. The substrate DNA was cut by specific restriction enzymes, and proheads preferentially package the left end DNA-gp3 fragment of the restriction enzyme digest (Bjornsti et al., 1983) prior to microscopic examination; for more details see Methods. A gel showing the DNA packaged in each reaction is shown in Fig. 4.

Fig. 5 shows views of $\varphi$29 particles from three approximately equivalent orientations, for different contents of DNA. The top row shows empty capsids. Note the characteristic angles made by their projected boundaries. The second, third, fourth, and fifth rows correspond to particles which packaged 32%, 51%, 78%, and 100% of the genome, respectively. As empty capsids are filled, the shapes are gradually rounded off. There is an increase in diameter of about 3% to 4% from completely empty capsids to fully packaged ones, which results in an increase in total volume of about 9% to 12%.

The most striking feature of these two-dimensional projections is that the DNA always occupies the full volume of the capsid, for any percentage of the total genome packaged. This is observed for all orientations, throughout hundreds of images. There never is a center with lower density, which would be the case if the DNA were adopting a spool or concentric solenoidal structure.

In order to confirm these results using an independent experimental procedure, we also imaged particles obtained by
time-resolved, rapid quenching of the packaging of the full-length genome after initiating the process. \textit{In vitro} packaging reactions were started and cryo-plunged after 2 min (±4 s) of initiation, which resulted in a packaged DNA content of between 40% and 60% of the total genome. Fig. 6 shows examples of the images obtained from these samples. These results are consistent with the images of the partially packaged genomes; the DNA occupies the entire volume of the capsid quite evenly, and there are no regular patterns.

Multivariate statistical analysis applied to these data sets confirmed that there are no patterns in phage particles with as much as half of the genome packaged. No in-register average of the mass density throughout the volumes could be obtained from 2D projections of these particles. In several particles with 78% of the total genome, the two outermost DNA layers start to be present, but this pattern was not consistent.

\textit{Simulated packaging of the \varphi29 genome}

Although a distinct layer ordering is manifested in our cryo-EM reconstruction of \varphi29 upon averaging over many phage projections, the three-dimensional DNA arrangement within a single particle cannot be ascertained. As such, connecting the average DNA density with the single-phage genome arrange-
ment remains an important step in understanding the mechanism of viral packaging. Our aim in this section is to determine whether our understanding of the packaging process is sufficient to construct a model of the process that can account for the various aspects of our cryo-EM analysis.

Our simulation is composed of a DNA strand modeled as a worm-like chain (Kratky, 1949) with an experimentally measured self-interaction potential (Rau et al., 1984), inserted into a confined space with dimensions extracted from our cryo-EM reconstruction; details of the theoretical model and a comparison with that used in similar simulations (Petrov and Harvey, 2007) appear in the Supplementary data section. Our model provides a method of predicting individual packaging trajectories as well as average internal density. Fig. 7 shows four snapshots (30%, 50%, 70%, and 100% packaged) from a single packaging simulation from top and side views.

As in previous work (Arsuaga et al., 2002; Forrey and Muthukumar, 2006; Klug et al., 2005; Klug and Ortiz, 2003; LaMarque et al., 2004; Petrov and Harvey, 2007; Spakowitz and Wang, 2005), our packaging simulations do not result in spool-like structures. Rather, at all stages of the process, the DNA fills the capsid with rather uniform density, with chain segments weaving throughout the volume; this is visible in the 100% packaged conformation by the multi-colored chain segments present in the outer layer. Experimental cross-linking between DNA and capsid proteins in phage lambda (Widom and Baldwin, 1983) shows contact between the capsid protein and the DNA chain at all positions along the chain, consistent with the chain path in our simulated structures. Chain segments in Fig. 6 are locally aligned with neighboring segments, but this ordering does not persist over large distances. In particles with all of the genome packaged, this ordering leads to the appearance of one or two outer rings in top views.

A comparison of 2D projections of simulated density, class averages of such projections, actual phage particles projections, and projections of spontaneous DNA toroidal condensates provides some useful insights on structures within the particles. The punctate pattern typical in side views of several filled particles, associated with close packing of DNA, is also present in individual projections and class averages of the simulated densities, as shown in Fig. S3. Fig. S4 shows orthogonal and axial sections of the 3-D reconstruction obtained using projections of simulated, fully packaged DNA densities. The two outermost DNA shells are recovered.

Discussion

We have obtained a 3-D reconstruction that indicates that packaging of the bacteriophage φ29 genome follows local hexagonal close packing. While there is sufficient variability among phage particles that very few individual strands are resolved, most of the packaged genome is localized in concentric solenoids with a small, highly disordered central core. In the region around the portal, we can clearly see rings in the reconstructions of mature bacteriophage particles, but these account for a negligible fraction of the total DNA. Otherwise, we see no evidence of a unique, hexagonally closed-packed concentric solenoid. This conclusion is also supported by a careful analysis of the distances that separate DNA shells, obtained from orthogonal and axial cross-sections, which shows a degree of variability. These results are consistent with previous results (Agirrezabala et al., 2005; Chang et al., 2006; Fokine et al., 2004; Jiang et al., 2006; Lander et al., 2006; Xiang et al., 2006). The simplest interpretation consistent with this and previous structures is that the DNA adopts an overall architecture that is dominated by short-range hexagonal close packing, but does not adopt a single, unique structure. The fact that the DNA shells are continuous surfaces toward the poles of the elongated volume can only be explained by a distribution of DNA trajectories that are not confined to planes perpendicular to the axis.

Image analysis of particles with different percentages of the total genome packaged inside the capsid indicates that there is no unique DNA packaging pathway. The capsid volume of viral particles with 32%, 51%, and 78% of the total DNA content appears fully occupied, with an isotropic density distribution throughout. Although we clearly see concentric DNA strands in top views of mature phages, we have not been able to observe similar density profiles for phages with partial genomes packaged, except for a few top views of particles which have packaged 78% of the genome, in which one or two outermost rings are visible. The highly condensed final state reflects a high degree of order not present in the intermediate states, and this ordering transition occurs going from ∼70% to 100% of the total genome packaged. What we observe is consistent with a transition between a disordered state in which DNA is able to dynamically search for the lowest curvatures and maximum inter-strand distance, to a final compacted state in which confinement has constrained the DNA into a highly regular architecture. The three outer layers account for a negligible fraction of the total genome, and we would only observe them in particles with partially packaged genomes if the pathway consisted in the step-wise ordering of a concentric spool. Instead, these layers are formed only as the DNA is spatially
constrained to adopt a close-packed local order, most probably with some degree of local hexagonal packing.

In a previous work aimed at elucidating the structure of the φ29 motor in actively packaging particles, Simpson et al. (2000) used ATP-γS to block active DNA packaging motors. Cryo-electron microscopy was then used to obtain three-dimensional reconstructions of the particles with a partial genome contained in the capsids. The interior of the capsids had

Fig. 5. Images of partially packaged genomes. Shown here are representative images of particles projected at different orientations, with (top to bottom) 0%, 32%, 51%, 78%, and 100% of the genome packaged. For all partially packaged particles, we see an isotropic grey density distribution filling the interior of the capsids, indicating that the DNA occupies all the available volume. One quite faint, outermost layer starts to appear in top views of some of the particles with 78% of the genome packaged (leftmost image in the 78% row).
no empty spaces, and no order, and there was no layered structure such as we see in the fully packaged particles. Although their work was based on a low number of particles, and the focus was the protein components of the phage, the results are in full agreement with our findings.

Simulations of the packaging of genome-length DNA performed with substantially different models reproduce fundamental aspects of the intermediate stages of the packaging process. Monte Carlo simulations have been reported that use a more accurate energetic model of the process and provide quantitatively accurate values for the work of genome packaging. Both these simulations and ours presented here predict fully disordered intermediate states along the packaging pathway that are consistent with single-molecule force spectroscopy measurements showing that at least 50% of the genome is packaged with negligible work (Smith et al., 2001); little work is necessary to deform the DNA or bring the strands into close proximity until the DNA content approaches 70%. This could not be case if the DNA was ordered in a pre-determined and close-packed, solenoidal architecture.

Both sets of simulations reproduce some aspects of the layer structure determined by cryo-EM, and those of Petrov and Harvey (2007) produce a 3D reconstruction in close agreement with our experimental one. In both, the increase in force toward the end of the process is associated with a “phase transition” from a highly disordered state to a degree of close packing, although neither reproduces the abrupt change in slope of the experimental force curve (Smith et al., 2001). Three-dimensional reconstructions of these simulated DNA densities obtained using standard EM image analysis tools can be directly compared with corresponding reconstructions from cryo-EM data. It is encouraging that it has become possible to compute informative packaging simulations that can test these physical parameters against cryo-EM data.

Finally, we would like to point out that larger bacteriophages, such as T4 and epsilon15, have diameters and lengths that exceed the commonly accepted free DNA persistence length of ∼50–53 nm. In these cases, it cannot be assumed that the DNA is elastically constrained during the initial stages of packaging, spontaneously adopting circular trajectories close to the capsid. Yet, the fully packaged viruses show a typical layered architecture as shown here for φ29. It seems possible that confined DNA, under relatively high salt concentrations, may be significantly more flexible and exhibit a substantially smaller persistence length than currently assumed under the worm-like chain model, as suggested by

Fig. 6. Images of partially packaged genomes obtained by quenching the DNA packaging reactions. Typical images from the CCD image data set of viral particles which were cryo-plunged 2 min (± 4 s) after initiation of in vitro DNA packaging reactions.

Fig. 7. Monte Carlo simulation of DNA packaging in φ29. These images show typical snapshots throughout a packaging simulation at stages where 30%, 50%, 70%, and 100% of the genome has been packaged. The chain is colored blue at the end entering the confinement and red at the free end within the capsid at full packaging. The capsid is not shown.
recent experimental results (Wiggins et al., 2006). A more flexible DNA would explain the large re-ordering observed between 80% and 100% packaging, since it would be possible to better sample conformational space than under currently assumed stiffness. The implications, of course, apply to packaging in all viruses.

While experimental work aimed at obtaining better three-dimensional structural information continues, theoretical work finally has actual spatial parameters at hand to constrain the models. Aspects of the packaging process that were previously almost completely unexplored have been investigated here, establishing that at least in \( \phi \)29 there is no unique, ordered packaging pathway. The DNA, instead of filling the capsid in concentric hoops, seems to undergo a condensation or phase transition after at least 70% of the whole genome has been packaged. This condensation results in local close-packed order, and the DNA adopts a layered architecture with five concentric, closed, shells. These results constrain the derivation of phenomenological parameters that can be used with theoretical models of DNA elasticity and interactions in predicting the forces involved in the packaging and ejection processes, and the final pressure inside a fully packaged bacteriophage particle. While the current simplistic models and the predictions obtained from them are useful and provide values that fall within a realistic range, it is clear that we need to revise and improve current models.

Methods

Specimen preparation

Fiberless bacteriophage \( \phi \)29 were obtained as described previously (Wichitwechkarn et al., 1989). Mutant sus14(1241), which has a delayed lysis phenotype that gives a higher burst per cell than wild-type, was used for this work. The phages were purified by isopycnic CsCl centrifugation, dialyzed against 1×TMS buffer (50 mM Tris–HCl, pH 7.8, 10 mM MgCl\(_2\), 100 mM NaCl), and stored at 4 °C. Final phage titers were obtained from them are useful and provide values that fall within a realistic range, it is clear that we need to revise and improve current models.

Partial genomes and plunge-frozen packaging reactions

Left-end fragment packaged particles were prepared as follows. Fiberless proheads, gp16 ATPase, and DNA-gp3 were prepared as previously described (Grimes and Anderson, 1997). Briefly, proheads were purified from \( B. \) subtilis RD2 (sup-) cells infected with the mutant sus8.5(900)-sus16(300)-sus14(1241) by sucrose gradient centrifugation, pelleted and resuspended in 1×TMS. Prohead pRNA was removed by treating with RNase and EDTA. In vitro transcribed 120-base pRNA was reconstituted to particles prior to packaging. To produce left ends of various lengths, DNA-gp3 was digested with 3U endonuclease per microgram of DNA-gp3 for 1 h [ClaI (32% genome), EcoRI (51% genome), or NcoI (78% genome)]. Packaging reactions consisted of 17 nM digested DNA-gp3, 17 nM proheads, 250 nM gp16, and 500 μM ATP in 32.5 mM Tris–HCl (pH 7.6), 6.5 mM MgCl\(_2\), 55 mM NaCl. After 10 min, samples were treated with DNase I prior to agarose gel analysis (0.5 μg/ml) or cryo-TEM preparation (0.1 μg/ml). Packaging was assessed by running heat-extracted, Proteinase K-treated DNA samples on a 0.8% agarose TBE gel, and DNA was visualized using ethidium bromide staining. Negative controls contained no ATP. For imaging of the time-resolved, rapid quenching of the packaging of the full-length genome, a set of in vitro packaging reactions was initiated as described above, with full-length DNA-gp3. Aliquots of 5 μl were then taken directly from the packaging reaction, placed onto lacey films and cryo-plunged 2 minutes (±4 s) after initiation.

General imaging

Images were acquired on CCD and on KODAK SO163 films, using a JEOL-3100-FEF microscope equipped with a FEG electron source operating at 300 kV, an Omega energy filter, a Gatan 795 2kx2k CCD camera (pixel size 30 μm), and a cryo-transfer stage. The stage was cooled with liquid nitrogen to 80 K. Parameters common to all data sets were the following: Objective aperture diameter ~40 μm; energy filter slit width of 20eV; defocus between 1.4 μm and 2.0 μm; dose per image between 3000 and 4500 e⁻/nm².

The film data set was acquired at a magnification of 48,500× and digitized using a Nikon Super Cool 8000 scanner at a scan step of 6.35 μm per pixel, and subsequently binned to yield a final pixel size corresponding to 0.262 nm on the specimen scale.

Reconstruction

Image analysis, boxing, and three-dimensional reconstructions were done using the package EMAN (Ludtke et al., 1999). The final reconstructions discussed in this work were obtained from 8200 boxed particles, using an initial box size of 384 pixels which was later clipped to 320 pixels. CTF parameters were manually determined for each film using the program ctffit, part of the EMAN package, and the phases flipped for each set of boxed particles from each individual film. After an initial set of four refinement cycles using an angular step of 5° for projection matching, and imposing no symmetry, the resulting model was used for two separate sets of refinements. One reconstruction was refined for 12 iterations, using angular steps of 3°, without imposing any symmetry. Another reconstruction was refined for 10 cycles imposing c5 symmetry, also using 3° angular steps for projection matching. See the online Supplementary data for details about all the reconstructions computed for this work.

The measurements of distances between DNA layers, perimeters, and central cross-section areas of the DNA shells,
radial averages, and density profiles were done with the program ImageJ (http://rsb.info.nih.gov/ij/). The segmentation of DNA shells in different colors was done with the program AVS-Express (http://www.avs.com) and rendered with Chimera (http://www.cgl.ucsf.edu/chimera).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.virol.2007.07.035.

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