Chemical mapping of DNA and counter-ion content inside phage by energy-filtered TEM

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Abstract Double-stranded DNA in many bacterial viruses (phage) is strongly confined, which results in internal genome pressures of tens of atmospheres. This pressure is strongly dependent on local ion concentration and distribution within the viral capsid. Here, we have used electron energy loss spectroscopy (EELS), energy-filtered TEM (EFTEM) and X-ray energy dispersive spectroscopy to provide such chemical information from the capsid and the phage tail through which DNA is injected into the cell. To achieve this, we have developed a method to prepare thin monolayers of self-supporting virus/buffer films, suitable for EELS and EFTEM analysis. The method is based on entrapment of virus particles at air–liquid interfaces; thus, the commonly used method of staining by heavy metal salts can be avoided, eliminating the risk for chemical artifacts. We found that Mg^{2+} concentration was approximately 2–4 times higher in the DNA-filled capsid than in the surrounding TM buffer (containing 10 mM Mg^{2+}). Furthermore, we also analyzed the DNA content inside the phage tail by mapping phosphorus and magnesium.

Keywords Energy-Filtered Transmission Electron Microscopy (EFTEM) · Electron Energy Loss Spectroscopy (EELS) · Magnesium · Phage lambda · Spermine · Chemical mapping

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1 Introduction

Viruses are among the simplest reproducing biological objects; yet, there are many unknown mechanisms involved in the viral replication cycle during cell infection. Detailed knowledge of the chemical composition within viruses might give clues about how the release of the viral genome is powered and triggered. Viruses consist of a protein shell (viral capsid) that protects the viral genome (DNA or RNA). In many viruses, the negatively charged genome is significantly larger in its contour length compared with the inner diameter of the capsid. This implies that the genome is highly compacted inside the capsid and might exert outward forces on the capsid walls. This is particularly the case for motor packaged double-stranded DNA (dsDNA) viruses. The resulting genome pressure on the capsid was estimated for several bacterial viruses (phage) and was shown to be as high as tens of atmospheres [1–3]. Specifically, in the case of phage λ , which has a 17,000 nm long dsDNA molecule packed in an icosahedral capsid [4] with external diameter of 63 nm, the internal genome pressure was estimated to be as high as 40 atm [2]. The internal pressure and force presumably play a crucial role in the DNA release into the cell from many tailed bacteriophages during the first step of infection. Therefore, having a deeper understanding of the physics of DNA confined in viruses has importance for the general understanding of mechanisms controlling viral DNA ejection and packaging. Theoretical [5–7] and experimental [1, 2, 8] analysis have shown that the internal capsid pressure is mainly due to bending and repulsive forces between negatively charged DNA strands. Furthermore, the effective interactions between neighboring strands of the strongly confined viral genome are determined by the concentrations of mono- and multi-valent salts in the host solution [8–10] since viral capsids are permeable to water and many ions (including Mg^{2+} and the tetravalent polyamine spermine $^{4+}$). In the theoretical work of Tzlil et al. [7], it was shown that it is precisely these chain–chain repulsions that dominate the internal pressure and are likely the forces ejecting the DNA from phage. It has been shown experimentally that the osmotic pressure required to completely inhibit DNA ejection from phage λ is dramatically decreased in the presence of the polyamine cation spermine (Sp^{4+}) or increased magnesium (Mg^{2+}) ion concentration [8]. This is due to the screening of negative charges on the DNA and also to the introduction of an attractive interaction in the case of added spermine. The attractive interaction is introduced by bridging and interhelical binding between bases of the DNA and spermine ions. Thus, DNA condenses in the presence of many multivalent ions [11]. However, it should be noted that even with addition of Sp^{4+} ions, dsDNA inside the capsid still remains under pressure. The average interstrand DNA–DNA distance in a toroidal bulk DNA condensed by Sp^{4+} is approximately 28 Å. The interstrand distance in a fully packaged wild-type (wt) DNA-length phage λ capsid is ≈ 25 Å, implying a strong repulsive force even in the presence of screening and attractive interactions [8].

Determining the spatial distribution of the viral genome and ions in the capsid allows better correlation between theoretical values of internal DNA energy stored in phage and experimental observations. Chemical element analyses with a sub-particle resolution level can provide this information. Energy-filtered transmission electron microscopy (EFTEM) has the capability to map out the elemental distribution with the required spatial resolution [12, 13] (see Section 2). A common obstacle when using EFTEM is the very large background associated with the electron energy loss spectroscopy (EELS) signal. This background signal, which is due to inelastic multiple electron scattering, is individual for each edge energy, i.e., each element. It increases very rapidly with sample thickness or addition of heavy elements, i.e., stains [12, 14–16]. For EFTEM, it is thus most important to make the samples as thin as possible and omit additions of any heavy elements during the preparation.

EFTEM has previously been used to study viral morphogenesis in infected cells [13, 17–19] but has to our knowledge never been applied in the study of viral structures without extensive preparations for imaging including embedding, staining, and sectioning.

Here, we have used a simple preparation technique to study viruses by EFTEM and X-ray energy dispersive spectroscopy (XEDS) and to map the distribution of DNA and local ion distribution in phage λ . Thin, self-assembled films of only one layer of phage λ particles and buffer are formed, without the addition of any heavy metal staining chemicals, thus eliminating interference both from the supporting film and from negative stains. We show here that even a monolayer virus particle film of this kind is sufficiently resistant to electron radiation to allow mapping of several elements in sequence within a single virus particle using EFTEM. Images corresponding either to traditional negative staining or positive staining can be recorded by selective imaging of the chemical constituents of the solvent surrounding the virus particles or the chemical constituents in the particles themselves.

We used phosphorous EFTEM mapping to visualize DNA and magnesium mapping to visualize the distribution of Mg^{2+} ions that affect the internal capsid pressure. To verify our findings, we used an independent analysis method, XEDS, based on X-ray emission, for chemical composition and quantification and high-angle annular dark-field imaging for comparison of image features. To determine whether magnesium ions are tightly bound to the phage interior or if they can easily be replaced by other ions, we also exchanged the magnesium-supplemented buffer with the buffer supplemented with spermine through a membrane wash.

2 Experimental methods

2.1 Bacteriophage strain and preparation of phage stock

Wt λ cI857 bacteriophage with a genome length of 48.5 kbp was produced by thermal induction of the lysogenic *Escherichia coli* strain [20] AE1. The strain was modified to grow without Lamb protein expressed on the cell surface in order to increase the yield of phage induced in the cell. The culture was then lysed by temperature induction. Phage purification details are described elsewhere [2]. Phage samples were purified by CsCl equilibrium centrifugation and thereafter dialyzed from CsCl against TM (Tris–Magnesium) buffer (10 mM $MgSO_4$ and 50 mM Tris–HCl at pH 7.4) for 3 h. The final phage titer was 5×10^{12} virions/mL, as determined by plaque assay [21]. To investigate the chemical composition of phages without magnesium present in the buffer, TM was replaced with 50 mM Tris–HCl and 1 mM spermine HCl by using Millipore 10 K filter devices.

2.2 Transmission electron microscopy

2.2.1 TEM imaging

Overviews to determine the quality of the buffer-virus films were recorded with a Philips CM 120 Biotwin instrument operating at 120 kV. All other imaging was performed on a JEOL 3000 F field emission gun instrument at 300 kV. Most images are recorded with a conventional, stationary electron beam, but all HAADF images and XEDS line traces were recorded using a focused probe in scanning mode (STEM). The XEDS is an Oxford Instruments INCA system, with full quantification by top-hat filtering, least square refinement to standards.

2.2.2 Direct deposition on TEM grids

Two microliters of droplets of highly concentrated virus suspensions (5×10^{12} virions/mL) were spread onto copper grids. The copper grids were coated with a lacy carbon supporting film that had been treated with a glow discharge shortly before application. Excess liquid was removed by touching the edge of the grid with a filter paper and the grid was left to dry.

2.2.3 Surface enrichment method

Forty microliters of droplets of diluted virus suspension (1×10^{12} virions/mL) were put on parafilm in a humid environment. After 2 h, lacy carbon filmed grids were put on top of separate droplets. Two types of grids were used, either exposed to a glow discharge or non-treated. After 1 h of exposure, the grids were carefully lifted from the droplets and excess liquid was removed by touching the edge of the grid with a filter paper before letting the grid dry.

2.2.4 Virus monolayer film formation

The method employed to enrich virus particles at the surface of large droplets of diluted virus suspensions gave thin self-supporting virus-buffered films for all three buffers (Fig. 1a–c). However, the standard method of glow-discharging the grids (to increase the hydrophilicity of the carbon film) should not be used for this type of sample. When glow-

Fig. 1 Virus films, prepared by the surface enrichment method. In (d–f), the lacy carbon film has been glow discharged before pick-up of the viruses. The buffers used are Tris–HCl supplemented with (a, d) magnesium sulfate, (b, e) magnesium chloride, and (c, f) spermine. Only in (a–c) monolayer films, usable for imaging and EFTEM, are spanning the holes. The viruses (phage λ particles) have been enriched in the surface layer of droplets applied on parafilm, and kept in humid conditions for 3 h

discharged grids are used, the virus particles are preferentially found in agglomerates on the supporting carbon film, as shown in Fig. 1d–f. These agglomerates have viruses in several disordered layers, thus preventing sub-particle chemical mapping and single virus imaging. Also, as these agglomerates add thickness to the supporting carbon film, more liquid is held within the perforated holes, and thick virus-buffered films, suitable for neither imaging, nor analysis, are formed.

Using the direct application method for highly concentrated virus suspension in buffer supplemented with magnesium sulphate, some very small suitable regions for TEM imaging and analysis were present on grids (not shown). However, when the direct application method was used for other buffers, the coverage of the grid was uneven. We found both areas with and without virus-buffered films, and when present, the virus-buffered film was far too thick for imaging. The results were similar to that when glow-discharged grids were used for the entrapping method, as shown in Fig. 1e and f.

2.2.5 Electron energy loss spectroscopy

Electron energy loss spectra were collected on a 2×2 k CCD with a Gatan Imaging Filter (GIF 2000) on a JEOL 3000 F microscope in diffraction mode. Camera length was 25 cm, the size of the entrance aperture of the spectrometer 3 mm and the collection angle 1.90 mrad.

In EELS, the elements of the sample are detected as protruding edges at specific energies over a locally varying background, usually many times greater in magnitude than the element specific edges. For quantifying EEL spectra, the most crucial part is to model the background locally, and subtract it from the edge. Edges were identified by using the ID function in the program suite Digital Micrograph 3.7.0.

To verify that the single scattering approximation is fulfilled, the effective sample thickness (t) can be estimated from integrated intensities in the acquired spectra according to

$$\frac{t}{\lambda} = \ln \left(\frac{I_0}{I_{I_0}} \right).$$

λ in this expression is the mean free path of the inelastic scattering of primary electrons, I_0 is the integrated intensity under the elastic zero peak and I_{I_0} under the whole spectra [12, 16], generally approximated by the integrated intensity up to about 200 eV energy loss, including the zero loss peak. The value of $\ln(I_0/I_{I_0})$ was mapped out across the specimen (“thickness maps”), and areas with values <0.45 were selected for further analyses.

Detectable edges were the $L_{2,3}$ edge for magnesium (Mg), phosphorous (P), sulfur (S) and chlorine (Cl), whereas the K edge proved better for carbon (C), nitrogen (N) and oxygen (O). The edges were in general most clearly distinguishable for the films with spermine supplemented buffer.

2.2.6 Energy-filtered transmission electron microscopy

Energy-filtered images were recorded on a 2×2 k CCD camera with a post column filter at a JEOL 3000 F microscope. The experimental conditions were as follows; the condenser aperture used had a diameter of 150 μm , the size of the entrance aperture of the spectrometer was 3 mm and the setting of the spot size was 2. Convergence angle was <1 mrad and collection angle about 5 mrad. Acquisition time was 1 s for unfiltered and zero-loss images and up to 20 s for energy filtered images used for maps.

Energy-filtered images were recorded and elemental maps constructed, either by the three energy window method [17, 22] (with two images recorded below and one image recorded above the ionization edge energy), or with the jump ratio method [19, 23] (just using one image recorded at energies below, and one image recorded above the edge energy of interest).

Electrons with the acquired energy losses were selected by raising the accelerating voltage of the microscope by an amount corresponding to the edge energy loss, to maintain perfect alignment. The lowest-energy edge for the target element in the spectra was usually selected for energy-filtered imaging, since K ionization edges have the best signal-to-background ratio (SBR). However, at higher energy losses, the K edges suffers from low intensity, thus giving preference for $L_{2,3}$ edges. Thickness maps were recorded to assure a homogeneous thickness across the field of view.

2.3 EFTEM energy windows

Images to calculate Mg maps were recorded in the energy loss interval ranging from 41 eV to 61 eV with a $\delta E = 10$ eV energy window. Energy-selective images for P maps were recorded in an interval ranging from 90 to 162 eV with a $\delta E = 20$ eV energy window. C maps were calculated from images recorded in the energy loss interval ranging from 242 to 304 eV with a $\delta E = 20$ eV energy window. The acquisition times were 1 s for Mg, 12 s for P, 20 s for C.

2.3.1 Visibility of edges

For phosphorous, carbon, nitrogen, and oxygen, it was concluded that the closest edge at lower energy was situated sufficiently far away to use the three energy window method to create trustworthy maps. In this method, the first two energy windows are used to model the background by the power law $B = A \times E^{-R}$ [22].

Preceding edges make background fitting more complicated for magnesium, chlorine, and sulfur. For Mg maps, it is the proximity of the $L_{2,3}$ edge to the plasmon that makes background subtraction delicate, forcing the use of a smaller energy interval for background modeling. Due to the higher intensity in this low loss region, it is possible to obtain suitable signal with smaller energy windows, but with a smaller energy window the sensibility to energy drift becomes more pronounced. An alternative to using smaller energy windows is to use the two window (jump ratio) method. The jump ratio method is known only to be valid for specimens that have a thickness, t , less than about half of the total inelastic mean free path. This criterion was fulfilled by the self-supporting virus buffer films, having a thickness of $<0.45\lambda$. The jump ratio method generally gives higher signal-to-noise ratios than the three window method, as the latter uses two parameters instead of one to fit the background shape to the shape of the energy loss spectrum. For each parameter there is an added statistical uncertainty [14].

The P and C maps are created with the three window method, whereas the Mg maps are calculated by the jump ratio method (Fig. 2). Note that while absolute quantification between elements is cumbersome, the relative occurrence of an element in a map is reliable due to the fact that the same energy edge and background are used for all data points.

Fig. 2 Maps, created from EFTEM of a thin dry film of virus particles in 50 mM Tris-HCl buffer supplemented with 10 mM MgCl_2 , showing details of the capsid wall and the DNA content by selective imaging: **a** carbon map, **b** phosphorous map, and **c** magnesium map. It is clear that the phosphorous signals originate from the core of the capsid, i.e., the DNA content, whereas the carbon signal is the highest immediately outside the core, where the capsid wall is projected along the beam. Note also the different appearance of the phage tails, marked with a *T*. In the carbon map, they are bright, in the phosphorous map, they are not distinguishable, and in the magnesium map, they appear as if negatively stained (dark) by the buffer. This suggests that there is no DNA in the tail. In the magnesium map, the signal is high from the capsid interior (marked *C*), rather intense from the buffer film, but approaching zero for the capsid head protein boundary and the tail. This is due to the absence of magnesium in the protein but a high level of magnesium in the surrounding matrix of dried buffer. Carbon and phosphorous maps were achieved by the two-window background subtraction method, while the jump ratio method was used for the magnesium map due to interference from the preceding plasmon peak. Note that the level of the mapped elements is very similar for all particles in the maps, ensuring reproducibility

3 Results and discussion

3.1 Self-supporting virus buffer films

By creating a virus-buffered film that spans over holes in the supporting carbon film, without any addition of heavy metal stains, we have achieved minimal background for TEM and EFTEM. We have also been able to verify the validity of our maps by XEDS together with HAADF-STEM.

A high concentration of the virus particles in the film is needed to make the film sufficiently stable. As grids with supporting carbon film are hydrophobic, droplets with such a volume do not spread well on the film. Treatments such as glow discharge may be used to increase the hydrophilicity of the grid, and increase the spreadability of water-based suspensions, but this may also increase the adsorptive property at the carbon film. The virus particles in this study serve as an example as they are preferentially bound to the support carbon film that has been treated with glow discharge. On glow-discharged grids, it is therefore not possible to create thin, self-supporting virus-buffered films spanning over the holes in the carbon film. A way to circumvent this is by dilution of the suspension, which makes it possible to use droplets of larger volume. A droplet of bigger volume has a more suitable surface curvature for spreading on the grid and no pre-treatment of the grid to increase spreading properties is necessary. The virus particles, reaching the surface by Brownian motion, are trapped in the air-liquid interface and accumulated with time [24, 25]. This property has previously been used for immunogold labeling [26] and negative staining

of virus particles for cryo-TEM [27]. From Fig. 1, it is clear that ordered arrays of phage λ are formed in the self-supporting films without any additions.

The thickness of the self-supporting monolayer films created of virus and buffer is dependent on the diameter of the virus particles. The average effective thickness is $<0.45 \lambda$ (inelastic mean free paths) for the virus particles and even lower for the surrounding film, and does not vary more than 15% over good quality films spanning holes in the carbon film (see Fig. 1).

3.2 Elemental distribution

Based on the information in the spectra, energy windows for image acquisition (see Section 2) were set up to calculate maps for magnesium (Mg), phosphorous (P), and carbon (C) (Fig. 2).

3.2.1 Carbon mapping

Carbon (Fig. 2a) is present both in the capsid and in the DNA. Still, the phage particles often appear as hollow hexagonal particles with a tail in the carbon maps. This is due to the projected carbon mass along the incident electron path. The diameter of the particle in the carbon map is about 8 nm larger in diameter than the particle in the phosphorous map. This corresponds well with the thickness of the λ capsid [4, 28].

3.2.2 Phosphorous mapping

The phosphorous map (Fig. 2b), may be used as a marker for DNA and points out the position of DNA packed within the capsid head. Background subtraction for phosphorous is complicated as the phosphorous $L_{2,3}$ edge is situated at about five times the energy of the first plasmon peak, which is fairly close. Only the single scattering spectrum obeys the power law in this range. At $t = 0.5\lambda$, which is close to the thickness of the self-supporting films of buffer and phage λ , both the second and triple scattering contribution exceed the single scattering contribution to the background. However, the phosphorous signal measured by XEDS is zero in the tails of the capsids, which supports the EFTEM results that no DNA is visible in the tail of phage λ (Fig. 2b).

3.2.3 Magnesium mapping

For the buffers containing magnesium, the magnesium maps have a very low contrast. Probably this is because magnesium ions are both bound within the virus particles and situated in the surrounding film of the buffer salts. Sufficient signal is achieved from the interior of the capsid and from the buffer film. This suggests that magnesium is situated in the capsid interior. Verifying with STEM and line traces by XEDS (Fig. 3a), we show that concentration of Mg^{2+} ions inside phage heads is approximately 2–4 times higher than in the surrounding buffer solution. Figure 3c shows the XEDS line trace composition for a filled capsid next to an empty capsid along the A–B line (Fig. 3a). A clear Mg signal (blue) is visible both outside and inside the capsid, with the maximum approximately four times higher on the inside, whereas P (red) is only present inside the filled capsid. Darker areas (almost black) correspond to the overlap between blue and red. Complementary point analyses by XEDS of adjacent virus particles and film area, allows quantification in relation to other elements present (Table 1 and Fig. 4). As the projected mass and composition differ between the film and the particle, it is, however, difficult to find a fixed reference

Fig. 3 HAADF-STEM images of monolayers of phage λ viruses. In (a), the TM buffer has been replaced by 50 mM Tris-HCl and 10 mM MgCl_2 , and in (b), the TM buffer has been replaced by Tris-HCl and 1 mM spermine. **c** XEDS line trace composition for a filled capsid next to an empty capsid along the A-B line in (a). A clear Mg signal (blue) is visible both outside and inside the capsid, with the maximum approximately four times higher on the inside for this particle, whereas P (red) is only present inside the filled capsid. Darker areas (almost black) show overlap of blue on red. **d** Line trace composition along the A-B line in (b). The P signal is strong inside the capsid, but the Mg signal is only at the noise level. The dark horizontal line in (a) shows the location of a previous line scan. The line traces comprise about 150 independent analyses across the particle. Typically, results are binned over adjacent points in order to reduce statistical noise, but this is not done here, in order to show the noise level (which is ± 1 count)

element. By referring the Mg signal to the Cu signal, which is a system peak caused by scattering from the sample to the specimen grid, or the carbon signal, which is dominated by a (constant) carbon film on the holder, we do, however, see a significant increase of Mg inside the particle with a factor 2 or higher. This shows, for the first time, that ion distribution within the viral capsid can be analyzed and quantified with EELS, EFTEM and XEDS. Identification of the 2–4 fold difference in Mg-ion concentration between the interior and exterior of the capsid is an important finding for modeling of DNA packaging in capsids and internal pressure calculations, since this should significantly affect the internal genome pressure within the capsid [8].

To pack a full length genome of 48.5 kbp into the phage λ capsid, the expanded procapsid head, consisting of the phage head protein gpE (gp stands for gene product),

Table 1 Quantification of XEDS point spectra 1 (center of capsid), and 2 (buffer film next to particle), using top-hat filter and least square refinement towards library spectra in the Oxford instruments INCA program suite

Element peak	Atomic% spectrum 1	Atomic% spectrum 2
C K	76.23	69.35
N K	13.27	16.40
O K	9.33	11.38
Mg K	0.23	0.47
Si K	0.62	0.09
P K	0.24	2.13
S K	0.07	0.17
Totals	100	100

An accuracy of better than 0.1 at-% is generally achieved from minor components in this type of matrix

has to be stabilized by gpD (the head stabilization protein) [29]. The binding site for gpD is hidden internally within the capsid [4, 30]. It has been reported that magnesium is necessary to stabilize the procapsid during gpD binding [4] and it also stabilizes the viral genome [8]. Phages with genomic deletions (with 80% DNA length compared to the wild type genome) can be grown without the addition of gpD, in the presence of high magnesium concentrations [29]. If magnesium ions are removed by EDTA from a solution of fully packed phage λ , the phage particles will rupture, releasing their genomes [31]. Phages, however, remain stable if magnesium is replaced with tetravalent spermine ions (Sp^{4+}). It has also been shown experimentally that the osmotic pressure required to completely suppress DNA ejection from phage λ can be decreased dramatically in the presence of magnesium (Mg^{2+}) or spermine (Sp^{4+}) ions [8]. In order to verify whether spermine ions would easily replace magnesium within the capsid, we performed EELS analysis and EFTEM on phage in Tris-buffer containing spermine (instead of Mg^{2+}), where magnesium has been replaced through single membrane filtering. In the spectra for phage λ in buffer complemented with spermine (50 mM Tris-HCl and 1 mM SpHCl) a small

Fig. 4 XEDS spectrum from virus capsid showing all elements present in the sample, including the copper signal, and an additional carbon signal, which are both from the specimen holder. The Mg signal is clearly above the noise level. Quantification of the elements is listed in Table 1

L_{2,3} edge for magnesium is however still distinguishable. This indicates that magnesium is relatively strongly bound to the viral DNA inside the capsid. Mapping magnesium by EFTEM in phage λ embedded in spermine-supplemented Tris-HCl buffer confirms this (see Fig. 2c as well as XEDS analysis in Fig. 3b and d). Note that magnesium is absent in the tail (Fig. 2c), suggesting that there is no DNA throughout the tail in a fully-packaged phage λ .

4 Conclusions

This work shows the potential of using self-supporting buffer virus films for analysis of distributions of elements within the virus particles by the use of EFTEM. A self-sustained monolayer film has the lowest possible thickness but still allows imaging of phages without using any embedding or sectioning techniques. It is durable enough to withstand a fully focused electron beam, which is used for XEDS analysis, which verifies the chemical identification by EFTEM. This works with a spread out, low-intensity beam, thus making it superior for less sturdy samples.

The buffer, forming part of the film, should be chosen depending on the question to be addressed, minimizing the overlap of elements used for mapping. We have shown by two independent methods, EFTEM and XEDS, that, for bacteriophage λ , the distribution of DNA counterions, such as Mg^{2+} , can be investigated in the viral solution. Specifically, we found that Mg^{2+} ions exhibit 2–4 times higher local concentrations inside the phage capsid compared to the background concentration in the buffer. Our results also show that magnesium is not completely removed from phage DNA even after buffer replacement with spermine-containing buffer (instead of magnesium buffer) through a single membrane filtering. Furthermore, through phosphorous and magnesium mapping of phage particles, none of these elements were observed in the tails of the fully-packaged phage λ . This observation suggests the absence of DNA in the phage tail, something that has been up for debate on many occasions.

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References

1. Castelnovo, M., Evilevitch, A.: DNA ejection from bacteriophage: towards a general behavior for osmotic-suppression experiments. *Eur. Phys. J., E. Soft Matter* **24**, 9–18 (2007)
2. Evilevitch, A., Lavelle, L., Knobler, C.M., Raspaud, E., Gelbart, W.M.: Osmotic pressure inhibition of DNA ejection from phage. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 9292–9295 (2003)
3. Smith, R.A., Jiang, Z.G.: Neuronal modulation and plasticity in-vitro. *Intl. Rev. Cytol.* **153**, 233–296 (1994)
4. Dokland, T., Murialdo, H.: Structural transitions during maturation of bacteriophage lambda capsids. *J. Mol. Biol.* **233**, 682–694 (1993)
5. Kindt, J., Tzliil, S., Ben-Shaul, A., Gelbart, W.M.: DNA packaging and ejection forces in bacteriophage. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 13671–13674 (2001)
6. Purohit, P.K., Kodnev, J., Phillips, R.: Force steps during viral DNA packaging. *J. Mech. Phys. Solids* **51**, 2239–2257 (2003)

7. Tzlil, S., Kindt, J.T., Gelbart, W.M., Ben-Shaul, A.: Forces and pressures in DNA packaging and release from viral capsids. *Biophys. J.* **84**, 1616–1627 (2003)
8. Evilevitch, A., Fang, L.T., Yoffe, A.M., Castelnovo, M., Rau, D.C., Parsegian, V.A., Gelbart, W.M., Knobler, C.M.: Effects of salt concentrations and bending energy on the extent of ejection of phage genomes. *Biophys. J.* **94**, 1110–1120 (2008)
9. Bode, V.C., Harrison, D.P.: Distinct effects of diamines, polyamines, and magnesium ions on stability of lambda-phage heads. *Biochemistry* **12**, 3193–3196 (1973)
10. Evilevitch, A., Castelnovo, M., Knobler, C.M., Gelbart, W.M.: Measuring the force ejecting DNA from phage. *J. Phys. Chem. B* **108**, 6838–6843 (2004)
11. Bloomfield, V.A.: DNA condensation. *Curr. Opin. Struct. Biol.* **6**, 334–341 (1996)
12. Diociaiuti, M.: Electron energy loss spectroscopy microanalysis and imaging in the transmission electron microscope: example of biological applications. *J. Electron. Spec. Relat. Phenom.* **143**, 191–205 (2005)
13. Ottensmeyer, F.P., Andrew, J.W.: High-resolution microanalysis of biological specimens by electron energy loss spectroscopy and by electron spectroscopic imaging. *J. Ultrastruct. Res.* **72**, 336–348 (1980)
14. Leapman, R.D., Jarnik, M., Steven, A.C.: Spatial distributions of sulfur-rich proteins in cornifying Epithelia. *J. Struct. Biol.* **120**, 168–179 (1997)
15. Leapman, R.D., Ornberg, R.L.: Quantitative electron energy loss spectroscopy in biology. *Ultramicroscopy* **24**, 251–268 (1988)
16. Wang, Y.Y., Ho, R., Shao, Z., Somlyo, A.P.: Optimization of quantitative electron energy loss spectroscopy in the low loss region: phosphorus L-edge. *Ultramicroscopy* **41**, 11–31 (1992)
17. Harauz, G., Evans, D.H., Beniac, D.R., Arsenault, A.L., Rutherford, B., Ottensmeyer, F.P.: Electron spectroscopic imaging of encapsidated DNA in vaccinia virus. *Can. J. Microbiol.* **41**, 889–894 (1995)
18. Ozel, M., Pauli, G., Gelderblom, H.R.: Electron spectroscopic imaging (ESI) of viruses using thin-section and immunolabelling preparations. *Ultramicroscopy* **32**, 35–41 (1990)
19. Quintana, C., Marco, S., Bonnet, N., Risco, C., Gutierrez, M.L., Guerrero, A., Carrascosa, J.L.: Optimization of phosphorus localization by EFTEM of nucleic acid containing structures. *Micron* **29**, 297–307 (1998)
20. Evilevitch, A.: Effects of condensing agent and nuclease on the extent of ejection from phage lambda. *J. Phys. Chem. B* **110**, 22261–22265 (2006)
21. Silhavy, T.J.: Experiments with gene fusions. Cold Spring Harbor: Cold Spring Harbor Laboratory, New York (1984)
22. Egerton, R.F.: Electron energy-loss spectroscopy in the electron microscope. Plenum Press, New York (1986)
23. Hofer, F., Warbichler, P., Grogger, W.: Imaging of nanometer-sized precipitates in solids by electron spectroscopic imaging. *Ultramicroscopy* **59**, 15–31 (1995)
24. Cyrklaff, M., Roos, N., Gross, H., Dubochet, J.: Particle-surface interaction in thin vitrified films for cryoelectron microscopy. *J. Microsc.* **175**, 135–142 (1994)
25. Johnson, R.P.C., Gregory, D.W.: Viruses accumulate spontaneously near droplet surfaces—a method to concentrate viruses for electron microscopy. *J. Microsc.* **171**, 125–136 (1993)
26. Roos, N., Cyrklaff, M., Cudmore, S., Blasco, R., Krijnse-Locker, J., Griffiths, G.: A novel immunogold cryoelectron microscopic approach to investigate the structure of the intracellular and extracellular forms of vaccinia virus. *EMBO J.* **15**, 2343–2355 (1996)
27. Adrian, M., Dubochet, J., Fuller, S.D., Harris, J.R.: Cryo-negative staining. *Micron* **29**, 145–160 (1998)
28. Yang, F., Forrer, P., Dauter, Z., Conway, J.F., Cheng, N., Cerritelli, M.E., Steven, A.C., Plückthun, A., Wlodawer, A.: Novel fold and capsid-binding properties of the λ -phage display platform protein gpD. *Nat. Struct. Mol. Biol.* **7**, 230–237 (2000)
29. Sternberg, N., Weisberg, R.: Packaging of coliphage lambda DNA—II. The role of the gene D protein. *J. Mol. Biol.* **117**, 733–759 (1977)
30. Wurtz, M., Kistler, J., Hohn, T.: Surface structure of in vitro assembled bacteriophage lambda polyheads. *J. Mol. Biol.* **101**, 39–56 (1976)
31. Parkinson, J.S., Huskey, R.J.: Deletion mutants of bacteriophage lambda. I. Isolation and initial characterization. *J. Mol. Biol.* **56**, 369–384 (1971)