Correction

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Correction for “Solid-to-fluid–like DNA transition in viruses facilitates infection,” by Ting Liu, Udom Sae-Ueng, Dong Li, Gabriel C. Lander, Xiaobing Zuo, Bengt Jönsson, Donald Rau, Ivetta Shefer, and Alex Evilevitch, which appeared in issue 41, October 14, 2014, of Proc Natl Acad Sci USA (111:14675–14680; first published September 30, 2014; 10.1073/pnas.1321637111).

The authors note that Fig. 2 appeared incorrectly. The corrected figure and its legend appear below.

Fig. 2. Histograms of AFM-measured spring constants of empty and WT DNA phage λ capsids at different temperatures in 10 mM MgCl2–Tris buffer (A) and 10 mM MgSO4–Tris buffer (B). Above the DNA phase transition temperature, the spring constant of WT DNA-filled λ capsids is equal to that of an empty capsid. (C) Spring constants of empty and DNA-filled phage λ capsids as a function of indentation rates at 32 °C and 37 °C in MgCl2–Tris buffer. The error bars show the SE. (D) Spring constants of encapsidated DNA, \( k_{\text{DNA}} \), measured at 32 °C and 37 °C in MgCl2–Tris buffer. The error bars show SE. The black dashed line shows the indentation rate of 60 nm/s, at which the DNA relaxation rate during the indentation is faster than the AFM tip indentation rate (i.e., indentation occurs at equilibrium). The gray dashed line shows the indentation rate corresponding to the DNA sliding/ejection rate of 60,000 bps determined in ref. 9.

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Solid-to-fluid–like DNA transition in viruses facilitates infection

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Releasing the packaged viral DNA into the host cell is an essential process to initiate viral infection. In many double-stranded DNA bacterial viruses and herpesviruses, the tightly packaged genome is hexagonally ordered and stressed in the protein shell, called the capsid. DNA condensed in this state inside viral capsids has been shown to be trapped in a glassy state, with restricted molecular motion in vitro. This limited intracapsid DNA mobility is caused by the sliding friction between closely packed DNA strands, as a result of the repulsive interactions between the negative charges on the DNA helices. It had been unclear how this rigid crystalline structure of the viral genome rapidly ejects from the capsid, reaching rates of 60,000 bp/s. Through a combination of single-molecule and bulk techniques, we determined how the structure and energy of the encapsidated DNA in phage λ regulates the mobility required for its ejection. Our data show that packaged λ-DNA undergoes a solid-to-fluid–like disordering transition as a function of temperature, resulting locally in less densely packed DNA, reducing DNA–DNA repulsions. This process leads to a significant increase in genome mobility or fluidity, which facilitates genome release at temperatures close to that of viral infection (37 °C), suggesting a remarkable physical adaptation of bacterial viruses to the environment of Escherichia coli cells in a human host.

Nucleic acids constitute one of the main components of many viruses by weight. The viral genome is packed tightly into a small volume within a protein shell called the capsid. This is true for most prokaryotic viruses, such as double-stranded DNA (dsDNA) viruses (1–5), as well as many eukaryotic viruses [e.g., herpesviruses (6) and reoviruses (7)]. The length of the ds-genome in these viruses is several hundred times longer than the diameter of the capsid. This tight packaging leads to genome bending stress and strong repulsive interactions, resulting in internal capsid pressures reaching tens of atmospheres. The extreme efficiency of viral replication is associated with a rapid transfer of the genome from the capsid to the host cell. This pressure-driven genome ejection occurs through a single portal opening in the capsid with a cross-section of a few nanometers (8), allowing the passage of one dsDNA chain at a time. The energy and structure of the confined viral genome are closely related and determine the rate of major viral replication steps, such as genome ejection and packaging (9–13). We have shown in vivo that the internal DNA pressure will affect the probability of infecting the cell (11), whereas the rate of packaging is the limiting step for viral assembly (14). Although DNA is always condensed inside the cell (15), it is not condensed to the same extent as inside a viral capsid. Other than in sperm nuclei, in vivo packaging densities range from 5–10% by volume (16, 17). DNA confined in viral capsids, on the other hand, is at the extreme end of the packaging scale, where it is confined to 55% by volume, forming a hexagonally ordered structure (2, 3, 10). At only a few angstroms of DNA–DNA surface separation [e.g., 7 Å surface separation in the wild-type (WT) DNA length of 48,500 bp packaged in phage λ (10), hexagonally ordered DNA has been shown to have very restricted mobility (16, 18). It has been proposed that so-called Coulomb sliding friction between neighboring DNA helices plays a significant role in DNA mobility at high packing densities in the viral capsids (19, 20). Indeed, recently it was shown that interhelical sliding friction leads to a kinetically trapped, glassy DNA state inside the capsid. This high-friction genome state was found to significantly affect the rates of DNA packaging in vitro (21). This occurs from dragging closely packed, negatively charged DNA helices past other helices. Despite decades of investigations of the encapsidated genome structure and its energetics (3, 22), it is not known what provides the required mobility to the hexagonally ordered viral DNA during the initiation of its ultrafast ejection, reaching 60,000 bp/s (9). In this work, we provide an answer to this fundamentally important question.

The well-known concept of viral metastability often refers to the viral capsid that must be sufficiently stable to protect the viral genome, and unstable enough to release its genome into the cell (23). In this work, using bacteriophage λ as a model system, we discovered a novel concept of viral metastability attributed to the viral genome. The energetics, structure, and mobility of the encapsidated DNA are studied as a function of temperature, a parameter that rarely is varied in biophysical measurements on viruses but is pertinent to viral replication and survival. This study revealed a remarkable structural transition of dsDNA in phage λ capsids, close to the ideal temperature for infection, i.e., 37 °C. Because phage λ infects Escherichia coli that originate in the human gut, the human body temperature makes phage DNA fluid-like and thus optimized for rapid release into bacterial cells.

Significance

The efficiency of viral replication is limited by the ability of the virus to eject its genome into a cell. We discovered a fundamentally important mechanism for translocation of viral genomes into cells. For the first time, to our knowledge, we show that tightly packaged DNA in the viral capsid of a bacterial virus (phage λ) undergoes a solid-to-fluid–like structural transition that facilitates infection close to 37 °C. Our finding shows a remarkable physical adaptation of bacterial viruses to the environment of Escherichia coli cells in a human host.


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At the same time, at lower temperatures outside the host, DNA inside the capsid is more restricted or more solid-like when the conditions are less favorable for infection, which helps prevent spontaneous genome release.

**Results and Discussion**

We used a unique experimental approach by combining bulk and single-molecule techniques to verify the solid-to-fluid-like transition of DNA in phage λ capsids. Isothermal titration calorimetry (ITC) revealed an abrupt transition in the internal energy of the encapsidated DNA as a function of temperature (Energetics of Intracapsid DNA Structural Transition). Solution small-angle X-ray scattering (SAXS) shows that this transition is associated with the sudden decrease in the amount of ordered DNA inside the capsid (Ordering of Encapsidated DNA). This abrupt structural change leads to a dramatic increase in genome mobility, verified by atomic force microscopy (AFM) nanoindentation (Solid-to-Fluid-Like DNA Transition). SAXS data and cryo-electron microscopy (cryo-EM) reconstructions of DNA-filled λ-capsids suggest that the disordering transition of DNA transfer occurs in the center of the capsid volume. This results from the temperature-induced interplay between the DNA bending stress and the interstrand repulsive interactions (Structural Changes of Encapsidated DNA). DNA in the capsid becomes more disordered and locally less densely packed with increased interstand separations, which reduces DNA–DNA repulsions and increases genome mobility. Finally, we show how intracapsid DNA mobility influences the kinetics of initiation of DNA ejection from the capsid in vitro by using single-molecule fluorescence measurements (Effect of Intracapsid DNA Mobility on the Kinetics of Initiation of DNA Ejection). These results reveal favorable as well as inhibitory in vivo conditions for phage infectivity and replication.

**Energetics of Intracapsid DNA Structural Transition.** As mentioned above, the viral genome organization resulting from intracapsid confinement is closely associated with its energetic state (12, 24). The effect of temperature on the energy state of the pressurized genome in the viral capsid was not investigated previously. We designed a new microliterometric assay that provides the most direct method to measure the internal energy of the confined viral genome. Using ITC, the enthalpy change (ΔH) associated with DNA ejection from phage λ is measured as heat released when concentrated phage particles are titrated into a LamB (maltoporin) receptor solution, which triggers DNA ejection in vitro (25) (see details in SI Materials and Methods). Because the total volume of the system does not change during the DNA ejection, and the pressure is constant, the change in internal energy and in enthalpy is approximately equal (25). The temperature in the reference cell is continuously equilibrated to that of the sample cell after each titration of phage in LamB solution. The differential power between the reference cell and the sample cell after each titration of phage in LamB solution. The discontinuity demonstrates an abrupt transition, which may be attributed to the DNA inside the capsid or the DNA that has been ejected. However, differential scanning calorimetry analysis of free λ DNA in solution confirmed that there is no structural transition in this temperature range, with double-to-single-stranded DNA melting occurring at significantly higher temperatures (27). Therefore, it is the encapsidated DNA that undergoes the structural transition not observed previously.

Before the transition, the absolute value of the ejection enthalpy change |ΔHej(T)| shows a strong linear increase with increasing temperature. |ΔHej(T)| increases nearly four times when the temperature is raised from 22 °C to 32 °C. This increase in the internal energy indicates an increase in the stress of the confined genome as temperature is being raised. At the transition temperature, T*, the internal energy is reduced by almost half, suggesting partial relief of the stressed state. After the transition, |ΔHej(T)| shows only weak temperature dependence when the temperature is increased further to 42 °C (Fig. 1A). This observation demonstrates that the DNA inside λ capsid may exist in two energy states.

The critical genome stress is reached at temperature T* and is required for the structural transition to occur. This suggests that varying the DNA stress inside the capsid should affect the temperature of transition, T*. We test this hypothesis by repeating the ITC measurement of phage DNA ejection enthalpy under different ionic conditions, using Tris buffer with 10 mM MgSO4 instead of 10 mM MgCl2. Because all viral capsids are permeable to smaller ions, ionic conditions of the host solution have a direct influence on the internal DNA stress by affecting the DNA–DNA repulsive interactions (28). Our previous studies showed that DNA pressure inside λ capsids and DNA–DNA spacing are larger in Tris solution with 10 mM MgSO4 than with 10 mM MgCl2 (28). This is related to the difference in ion pairing energies of Mg2+ with Cl− and SO42−, leading to considerably fewer Mg2+ ions bound to DNA when the cation is SO42−. This results in a weaker screening of the electrostatic repulsive forces between the DNA strands and therefore stronger repulsion (28). As a result, we found that the intracapsid DNA transition in 10 mM MgSO4–Tris buffer
occurs at a lower temperature of $T^* \sim 28$ °C, instead of 33 °C in 10 mM MgCl$_2$ (Fig. 1B). This finding confirms that there is a strong interdependence between the DNA structural transition temperature in phage $\lambda$ and the internal genome stress. In Ordering of Encapsidated DNA, we investigate which structural changes of DNA in the capsid lead to the observed transition.

Ordering of Encapsidated DNA. Solution SAXS is a powerful technique that provides structural information about the encapsidated genome (3, 29). Protein capsids and DNA have different scattering profiles and are well-resolved (29) (Fig. S2). We collected SAXS scattering data for WT DNA length phage $\lambda$ in 10 mM MgCl$_2$–Tris buffer in the temperature range between 22 and 40 °C (Fig. 1C and D). The short-range DNA interaxial spacings determine the position of the DNA diffraction peak, whereas the area of this peak provides information on the total number of ordered DNA base pairs of the encapsidated genome (3). When DNA inside the capsid becomes less ordered, the DNA peak area decreases as a result of less coherent diffraction. If the genome is completely disordered, the DNA diffraction peak disappears. Within the measured temperature range, the short-range DNA ordering appears to be unaffected with the diffraction peak position at scattering vector $q \sim 0.26$ Å$^{-1}$. This corresponds to an average interlayer spacing of 23.8 ± 0.1 Å, which in turn is converted to a DNA interaxial spacing of 27.5 ± 0.1 Å, assuming hexagonal packing (Fig. 1C). The area of the DNA scattering peak shows only a small decrease with the increasing temperature up to the DNA structural transition (Fig. 1D). However, at ~33 °C, the DNA diffraction peak area undergoes a sudden drop. This area drop signifies a loss of the amount of ordered DNA inside the capsid. This observation supports the above ITC measured abrupt transition of the internal energy occurring at the same temperature $T^*$. As mentioned above, the decrease in genome ordering should provide a more fluid-like DNA state inside the capsid because of the locally reduced DNA packing density, resulting in weakened interaxial repulsion. AFM indentation of DNA-filled capsids, we verify this mechanical solid-to-fluid intra-capsid DNA transition as a function of temperature.

Solid-to-Fluid-Like DNA Transition. The mechanical properties of the DNA inside phage capsids are measured by recording the force resisting the indentation when the AFM tip is brought into contact with the DNA-filled capsid in solution (Fig. 2 and Fig. S3) (30, 31). Because viral capsids are permeable to water, indentation leads to displacement of water molecules hydrating the DNA (30), occurring with compression of the packaged DNA strands. The resisting force attributed to the deformation of the empty capsid shell may be determined separately by AFM indentation of empty $\lambda$-capsids. Hence, the force resisting DNA-filled $\lambda$-capsid AFM indentation (in addition to the stiffness of the empty capsid) is associated with the free energy required to remove water molecules hydrating the closely packed DNA strands and the change in the interhelical phosphate–phosphate correlations (30). As mentioned above, this energy is described by DNA–DNA repulsive interactions and DNA bending stress (12, 24). Furthermore, if the AFM tip rate of indentation is faster than the relaxation dynamics of DNA during deformation, there may be an additional contribution to the measured DNA stiffness from the interstrand sliding friction. Unless specified otherwise, the rate of indentation in all measurements was 60 nm/s, which we have verified is slower than the relaxation rates for AFM-induced capsid and DNA deformations. Thus, measured stiffness reflects the free energy of the capsid and the encapsidated genome. A decrease in capsid stiffness attributable to DNA indicates an increase in genome fluidity or, equivalently, mobility. The observed variation in the stiffness and relative compressibility of the encapsidated $\lambda$-genome is explained by the structural DNA transitions determined with SAXS and cryo-EM measurements below (3, 29).

The force–distance curve is linear, suggesting elastic deformation of the capsid (Fig. S3) (30). The slope of the force–distance curve is equal to the spring constant $k$ (newtons per meter), describing the capsid stiffness. Fig. 2 shows histograms of the measured spring constants for WT DNA length phage $\lambda$ in 10 mM MgCl$_2$–Tris buffer (Fig. 2A) and in 10 mM MgSO$_4$–Tris buffer (Fig. 2B), as well as empty $\lambda$-capsids as a function of temperature between 19 °C and 37 °C. The spring constant for the empty capsids shows no temperature or coion dependence with an average value of $k = 0.089 \pm 0.005$ N/m. On the contrary, DNA-filled capsids display an increase in stiffness with increasing temperature, indicating growing internal genome stress. This observation corresponds to the increase in DNA internal energy measured above with ITC. At temperatures above the transition temperatures seen with ITC, i.e., $T^* \sim 33$ °C in 10 mM MgCl$_2$ and $T^* \sim 28$ °C in 10 mM MgSO$_4$, there is an abrupt drop in the capsid stiffness, and the spring constant for the DNA-filled capsid becomes equal to that of the empty $\lambda$-capsid. This implies that DNA in the capsid no longer is resisting the AFM indentation and does not contribute to the overall capsid stiffness. This observation suggests that closely packed DNA strands can slide past each other without restriction when the capsid is deformed with the AFM tip. Thus, the encapsidated DNA is more fluid-like or compressible after the structural transition, rather than solid-like with restricted mobility before the transition. Furthermore, Fig. 2 demonstrates that despite the initial differences in intracapsid DNA stress between 10 mM MgCl$_2$ and 10 mM MgSO$_4$–Tris buffers, the critical spring constant at which the DNA transition occurs (at two different temperatures) is approximately the same: $k^* \sim 0.2$ N/m ($k$-values are summarized in Table S2). This observation shows that both a structural and a mechanical solid-to-fluid-like DNA transition occurs when the genome stress inside the capsid has reached a unique critical state induced by an increase in temperature.

It has been shown for several bacteriophages (9, 32), including phage $\lambda$, that DNA ejection rates in vitro might be as high as 60,000–75,000 bp/s. Assuming that DNA strands are sliding past each other during the ejection process (19, 20), these ejection
velocities correspond to an interhelical sliding rate of $10^{-10} - 10^5$ nm/s (using 0.34 nm/bp). As mentioned above, when a λ-capsid is compressed with an AFM tip, the DNA strands inside the capsid slide past each other as well. By choosing the AFM indentation rates corresponding to the DNA sliding rates during ejection, we attempt to simulate the friction effects occurring during the genome ejection process. Therefore, in addition to the above measured DNA stiffness associated with the free energy of genome confined inside the capsid, we also investigated whether the DNA sliding friction is contributing to the restricted genome mobility at the higher rates of indentation. The intracapsid DNA stiffness, $k_{DNA}$, was measured as a function of AFM tip indentation rate (Fig. 2 C and D). $k_{DNA}$ was derived from $k(DNA$-filled capsid) − $k$(empty capsid), where the spring constants for DNA-filled and empty λ-capsids were measured separately for each rate of indentation. (We assume that capsid and DNA deformations are independent of each other.) Fig. 2C shows the spring constant, $k$, versus the indentation rate for empty and DNA-filled capsids in 10 mM MgCl$_2$–Tris buffer before the DNA transition (at $T = 32 \, ^\circ C$) and after the DNA transition (at $T = 37 \, ^\circ C$). Fig. 2D showing $k_{DNA}$ only confirms that at the 60-nm/s indentation rate used for all spring constant measurements above, the measured DNA stiffness does not depend on the rate of indentation within the measured temperature range ($T$), because the spring constants measured at 60-nm/s and 7.8 $\times 10^{-3}$-nm/s indentation rates are the same. However, as the indentation rates approach $10^{-10} - 10^5$ nm/s (corresponding to the DNA sliding rates during ejection), the DNA stiffness rises by nearly 95% at $32 \, ^\circ C$ (before the transition). At the same time, at $37\, ^\circ C$, after the intracapsid DNA transition has occurred, the DNA stiffness remains essentially unchanged and is close to zero within the entire indentation rate interval ($10^{-5} - 10^3$ nm/s). These observations suggest that the increase in DNA stiffness at lower temperatures is accompanied by an interhelical sliding friction caused by the strong repulsive interactions between the DNA strands (9). When DNA–DNA repulsions are reduced at the temperature of infection because of the structural transition, the genome remains fluid-like, with little frictional contribution even at very high indentation rates. Our AFM data suggest that both repulsive interstrand interactions and interhelical sliding friction restrict the mobility of the intracapsid genome at temperatures below that of the host environment of humans at $37 \, ^\circ C$, likely inhibiting the initiation of DNA ejection from the capsid. At the same time, at $37 \, ^\circ C$, the dramatic increase in mobility of the encapsidated viral DNA helps initiate genome ejection into the cell, facilitating the infection process. The energetic, structural, and mechanical analyses above provide evidence for a temperature-dependent metastable behavior of the encapsidated viral DNA. For a complete description of this important phenomenon for viral replication, we investigated where in the capsid volume this DNA-disordering transition takes place.

**Structural Changes of Encapsidated DNA.** The asymmetric cryo-EM single-particle reconstruction of WT DNA phage λ in Fig. S4 reveals that the entire capsid volume is filled with DNA, extending all the way to the center of the capsid. Starting from the capsid walls, there are well-ordered, multiple concentric DNA layers. The layers are spaced evenly, indicating that DNA has adopted an ordered repetitive structure characteristic of a liquid crystalline state. However, toward the center of the capsid, the ordered layers disappear, suggesting a less-ordered DNA structure with lower packing density than in the periphery of the capsid. Similar dsDNA distributions within the capsids also were observed for other viruses (2, 3, 29, 32–36).

The DNA structure in viral capsids is determined by DNA–DNA interactions, bending stress, and packing defects (10, 29). With the help of osmotic stress measurements on bulk DNA arrays condensed in solution by an osmotic stress polymer [polyethylene glycol (PEG); see details in SI Materials and Methods], we analyzed the effect of temperature on the DNA–DNA interaction energy alone without capsid-induced bending (10, 28, 29, 37). We found that the DNA–DNA repulsive interactions for linearly packaged DNA, at the same interaxial distances as in the capsid, are not significantly affected by the temperature increase from 5 to 50 °C, and no structural transition occurs (Fig. S4).

At the same time, intracapsid confinement requires DNA to bend along radii that are energetically unfavorable given the internal λ-capsid radius of $\sim 30$ nm (38) and 50-nm dsDNA persistence length (12), which creates bending stress on the packaged genome. (Persistence length defines the stiffness of a polymer, describing the minimum radius of curvature it can adopt by the available thermal energy. Bending it to a smaller radius requires additional work.) To relieve the bending stress, helices are packed closer to the capsid wall, decreasing the bending radius and also decreasing the spacing, therefore increasing the interaction energy. At the same time, the repulsive DNA–DNA interactions will push DNA strands as far from one another as possible, filling the entire capsid volume and maximizing the interstrand separations (10). There is a tradeoff between bending and interaction energies. Furthermore, when DNA is bending inside the capsid, the initial correlation between two helices that have slightly different radii of curvature is lost, and the mutual orientation between helices must be re-established. This leads to the packing defects that are absent for linear packaging of DNA in solution. Packing defects are required to re-establish a favorable phosphate–phosphate “phasing” of helices, reducing the repulsive interactions due to bending (10, 39, 40). The higher temperature likely will hinder the determination of this optimum correlation between the helices. The spacing remains the same because DNA simply fills a volume (as shown by SAXS in Fig. 1C), but the interhelical repulsion will increase. This is confirmed by an increase in the internal energy (measured by ITC; Fig. 1 A and B) and an increase in the stiffness of DNA in the capsid (measured by AFM; Fig. 2) when the temperature is increased before the transition. In parallel with the increasing interstrand repulsions, the increase in temperature will decrease the DNA persistence length (41), leading to less bending stress. If the bending stress decreases and if there is room to expand in the capsid, spacings would increase and interaction energy decrease. However, the spacing remains the same (DNA–DNA repulsions are reduced at the temperature of infection because of the structural transition, the genome remains fluid-like, with little frictional contribution even at very high indentation rates.) Cutaway view of the asymmetric cryo-EM reconstruction of WT DNA phage λ, showing more ordered DNA (green) in the periphery of the capsid and less ordered DNA in the center. Density (green) in the center of the channel formed by the portal complex (red) likely is the end of the λ DNA that is being packaged last, and ejected first. (B) Central slices through the 3D cryo-EM isosahedral reconstructions of the WT DNA phage λ in the absence (Upper) and presence (Lower) of 1.3 mM spermine (4+). Radial averages of the 2D slices are shown to the right. The central slice of each reconstruction was extracted along the fivefold symmetric axis, providing cross-sections of density in which the capsid and packaged genome appear most circular. (C) One-dimensional plots of the radially averaged central slices of the phage symmetric reconstructions. DNA layers are marked with red vertical lines.

Fig. 3. (A) Cutaway view of the asymmetric cryo-EM reconstruction of WT DNA phage λ, showing more ordered DNA (green) in the periphery of the capsid and less ordered DNA in the center. Density (green) in the center of the channel formed by the portal complex (red) likely is the end of the λ DNA that is being packaged last, and ejected first. (B) Central slices through the 3D cryo-EM isosahedral reconstructions of the WT DNA phage λ in the absence (Upper) and presence (Lower) of 1.3 mM spermine (4+). Radial averages of the 2D slices are shown to the right. The central slice of each reconstruction was extracted along the fivefold symmetric axis, providing cross-sections of density in which the capsid and packaged genome appear most circular. (C) One-dimensional plots of the radially averaged central slices of the phage symmetric reconstructions. DNA layers are marked with red vertical lines.
where DNA bending stress is stronger and packing defects are larger than for DNA closer to the capsid wall, making the DNA in the center more destabilized and therefore more sensitive to increasing temperature. At the transition temperature, the DNA bending stress becomes sufficiently small, allowing a fraction of the ordered DNA layers closest to the capsid’s center to undergo a disordering transition. The disordered DNA will have a lower packing density than the ordered DNA, which maximizes DNA–DNA spacings and simultaneously reduces the repulsive interactions. This yields an overall lower energy state of the encapsidated genome and increases DNA mobility in the center of the capsid.

We test this assumption and reconcile SAXS and cryo-EM observations by adding spermine (4+) ions to the WT λ-DNA phage. Spermine introduces attractive interactions between the DNA strands and strongly reduces the interstrand repulsions (10–42). This allows the interstrand packing defects (which were hindered by the increased temperature) to be re-established and therefore increases ordering of the DNA in the capsid. Cryo-EM reconstruction cross-sections in Fig. 3 demonstrate that addition of 1 mM spermine induces an increased ordering of the DNA in the center of the capsid, whereas it does not affect the ordered DNA in the periphery of the capsid. We observed that the concentric DNA layers now extend further toward the center of the capsid and that their number is increased from seven to nine layers. However, the DNA ordering and the interaxial distance for the first seven DNA layers in the periphery of the capsid remain unaffected, which is explained by the dominant short-range DNA–DNA hydration repulsion over the electrostatic repulsion interaction at interaxial spacings of \( a_0 = 27.5 \text{ Å} \) in WT DNA phage λ (10). SAXS measurements on WT DNA phage λ in 1 mM spermine confirm the increased amount of ordered encapsidated DNA with an increased DNA diffraction peak area compared with the case without spermine (shown in Fig. 1D). Furthermore, with spermine, the DNA diffraction peak area varies only slightly in the entire temperature range (20–40 °C) and no abrupt transition is observed. This is because the increase in DNA repulsive interactions at increasing temperatures (resulting from hindrance of the packing defects) are offset by the spermine-induced attractive interactions. As a result, the interstrand repulsive interactions in the center of the capsid are not large enough to induce the transition. These observations support the validity of our assumption that the structural DNA transition occurs closer to the capsid core, as shown schematically in Fig. S5.

Effect of Intracapsid DNA Mobility on the Kinetics of Initiation of DNA Ejection. AFM data above suggest that repulsive interstrand interactions restrict the mobility of the intracapsid genome, which may lead to an energy barrier for the initiation of genome ejection from the capsid at temperatures below that of the structural DNA transition. To test this hypothesis, we triggered DNA ejection from phage λ in vitro by adding purified LamB receptor. Using single-molecule fluorescence, we measured the average ensemble kinetics of the number of phages that ejected their DNA versus time once LamB was added (Fig. 4). It is important to emphasize that the ejection time for individual phage particles is significantly shorter than the time frame for our measurements, which we confirmed with single-molecule fluorescence measurements and which also was verified in refs. 9 and 43. The LamB was used in excess (1:10,000 phage-to-LamB ratio) so that the initiation of DNA ejection was not limited by the LamB diffusion (which was confirmed experimentally).

We performed fluorescence measurements of DNA ejection using a spinning-disk confocal microscope. Phage λ particles were adsorbed to a hydrophobically modified glass surface in a flow cell chamber. Fluorescent dye (YOYO-1) was flown in together with LamB at time 0. The diffusion of YOYO into the capsid interior was strongly kinetically limited. Once the DNA was ejected, YOYO instantly bound to it and indicated the number of phage particles that ejected genome, appearing as fluorescent spots over time (Fig. 4). The ejected DNA remained attached to the capsid, which also was observed in ref. 9. Furthermore, the ejected DNA immediately adhered to the modified glass surface and appeared partially stretched in the flow, helping us visualize phages that had ejected their genomes. Fig. 4 demonstrates that ejection from all phage particles does not start simultaneously.

We measured population ejection kinetics in MgSO₄–Tris buffer below and above the DNA transition temperature \( T^* \) (28 °C), at 22 °C and 31 °C. The data show that before the transition at \( T = 22 °C \), all phages have ejected their DNA after \( ∼8 \text{ min} \). At the same time, at \( T = 31 °C \) after the DNA transition has occurred, all phage particles have ejected their DNA after only \( ∼3 \text{ min} \). To confirm that this significant rate increase in the observed ensemble kinetics is associated with the structural transition and increased mobility of the encapsidated DNA, rather than the temperature effect on the portal-complex opening kinetics, we repeated those measurements at the same temperatures (22 °C and 31 °C) but in MgCl₂–Tris buffer (data shown in Fig. S6). Both these temperatures were below the DNA transition temperature of \( T^* \) (33 °C). Fig. S6 shows that this time, temperature had essentially no effect on the ensemble ejection kinetics. At both 22 °C and 31 °C, it took \( ∼8 \text{ min} \) for all phage particles to eject their genomes. These kinetics data suggest that the intracapsid DNA mobility, regulated by the temperature and ionic conditions, strongly affects the ability of the virus to initiate its genome release, which likely affects the rate of viral replication in vivo.

These observations provide new insight into the physical conditions in vivo required for successful delivery of the phage genome into the cell. Our data suggest that variations in temperature and ionic conditions in the cellular cytoplasm might affect viral infectivity and the rate of infection spread. Interestingly, using plaque assays, we found that the average area of phage λ plaques formed on a fixed layer of E. coli cells during the same incubation time of 12 h has strong temperature dependence at temperatures above the intracapsid DNA transition temperature \( T^* \) (33 °C in MgCl₂–Tris buffer; Fig. S7). Both phages and cells were resuspended in MgCl₂ Tris buffer (also used for the in vitro measurements above; see SI Materials and Methods for a detailed description of the method). Remarkably, the plaque area essentially was unchanged between 30 °C and 35 °C; however, it increased rapidly once the favorable temperature of infection was reached at \( T \geq 37 °C \) and almost doubled when the temperature increased from 37 °C to 42 °C. Likewise, many factors might contribute to this behavior (44), although the temperature-sensitive variation in intracapsid DNA mobility also might play a role.

Conclusions

We discovered that dsDNA in phage λ-capsids undergoes a solid-to-fluid-like transition as a result of decreased genome ordering occurring close to the optimum temperature for infection in the environment of the human host (i.e., 37 °C). This finding explains how a tightly packed, kinetically trapped
encapsidated viral genome (21) can be ejected readily into the cell. To our knowledge, this is the first demonstration of viral metabolatability attributed to the genome rather than the capsid. Because phage infectivity in vivo is significantly affected by the efficiency of viral DNA translocation into the cell (11, 14), the metastable state of the tightly packaged DNA likely is rate limiting for the viral replication cycle. Thus, at lower temperatures outside the host, the DNA in the capsid is solid-like with restricted mobility, which helps prevent its spontaneous release. Once inside the host, the increased temperature induces the necessary mobility of the viral genome, facilitating its infection of bacterial cells. This demonstrates an evolutionary physical adaptation of viruses to their host environment. We recently found for human herpes simplex virus that its intracapsid stressed DNA state leads to pressure-driven DNA ejection analogous to that of phage ϕ (6). This observation suggests that this unique metastable state of DNA in viral capsids may be universal for many pressurized viruses and may serve as a new target for drugs interfering with viral replication.

Materials and Methods
A detailed description is provided in SI Materials and Methods.

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