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The effect of genome length on ejection forces in bacteriophage lambda

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Abstract

A variety of viruses tightly pack their genetic material into protein capsids that are barely large enough to enclose the genome. In particular, in bacteriophages, forces as high as 60 pN are encountered during packaging and ejection, produced by DNA bending elasticity and self-interactions. The high forces are believed to be important for the ejection process, though the extent of their involvement is not yet clear. As a result, there is a need for quantitative models and experiments that reveal the nature of the forces relevant to DNA ejection. Here, we report measurements of the ejection forces for two different mutants of bacteriophage λ, λb221cI26 and λcI60, which differ in genome length by ~30%. As expected for a force-driven ejection mechanism, the osmotic pressure at which DNA release is completely inhibited varies with the genome length: we find inhibition pressures of 15 atm and 25 atm, for the short and long genomes, respectively, values that are in agreement with our theoretical calculations.

Keywords: Bacteriophage; Lambda; LamB; Maltoporin; Genome delivery; DNA ejection; Pressure

Introduction

Over the past 30 years, a series of experiments and theoretical work have produced many insights about the importance of internal forces in the bacteriophage life cycle: Early measurements on λ capsids showed that they contained tightly packed DNA (Earnshaw and Harrison, 1977), and subsequent experiments established that DNA packed at these densities exerts a pressure of tens of atmospheres that is dependent on the DNA density and salt conditions (Rau et al., 1984). It was expected that similar pressures would be found within λ capsids, resulting in forces that push apart the crowded strands of DNA, stress the capsid, and power the ejection of DNA. Any effect of the λ genome length on its life cycle (independent of any particular genes) suggests that these internal forces are important, and there are several such effects known: there are upper and lower bounds on the amount of DNA that can be packaged into a λ capsid (Feiss et al., 1977); and mutants of λ with long genomes are killed if magnesium ions are removed by chelation (Parkinson and Huskey, 1971). While magnesium ions reduce the forces between DNA, stabilizing the phage particles, DNA-condensing ions such as putrescine prevent DNA ejection (Parkinson and Huskey, 1971; Mackay and Bode, 1976; de Frutos et al., 2005a), and external osmotic stress can stabilize the genome within phages (Serwer et al., 1983). Another point of evidence that the compression of DNA is important in phage delivery comes from the *ptsM* gene, which codes for a cytoplasmic membrane protein. Mutations in *ptsM* prevent DNA ejection from λ phages with short genomes, while phages with longer genomes are not significantly affected (Scandella and Arber, 1974; Emmons et al., 1975; Elliott and Arber, 1978). The product of *ptsM* may reduce the amount of force required to penetrate the cytoplasmic membrane.

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The evidence above indicates that internal forces have an important role in the function of λ: phages with low ejection forces are incapable of transferring their DNA across the cell wall, while phages with high forces are unable to package their genome or are unstable when fully packaged. A variety of theoretical models of DNA packaging in bacteriophage have been proposed (Riemer and Bloomfield, 1978; Serwer, 1988; Black, 1989; Odijk, 1998, 2004; Kindt et al., 2001; Tzlil et al., 2003; Purohit et al., 2003, 2005). The models are consistent with each other, and all treat the DNA packaging problem in very similar ways. In our view, a significant advance is that the predictions of recent models are more quantitative: physical constants that were treated as unknown parameters in earlier work have been given numerical values based on related experimental work. These developments were inspired by recent experiments quantifying the forces required to tightly pack DNA into capsids (Smith et al., 2001) and the forces driving DNA ejection (Evilevitch et al., 2003, 2005); the nature of these experiments demands models that can make specific, quantitative predictions about virus packaging. Most recently, experimentally measured genome lengths and capsid geometries, with realistic ionic conditions, have been used in order to compute the measured forces (Purohit et al., 2005).

The aim of this paper is to examine the effect of genome length on ejection in bacteriophage λ and to use this information to test the theory more stringently than had been possible before. To vary the genome length in λ, we use λcI60, a clear-plaque mutant with a 48.5-kbp genome (the same length as wild type λ), and λb221cI26 (λb221), which has a much shorter genome of 37.7 kbp (Parkinson and Huskey, 1971). To measure the ejection force, we use the experimental technique of Evilevitch et al. (2003), with improved precision, applying an external osmotic stress to the capsids during ejection and halting the ejection at the point where the internal and external forces balance.

Why is a comparison to this kind of experiment a stringent test? In general, it is expected that phage ejection forces will increase as genome length increases. The correlation between forces and genome length has not previously been tested, but it is essential to our interpretation of the experimental evidence—that phage survival can be affected by genome length differences, due to the resulting changes in pressure. Even more, the theoretical model contains no unknown parameters that need to be fit to data, and it has a precise dependence on genome length; without consulting the experimental results, we are able to make specific, quantitative predictions for exactly how the forces will increase. The experiment therefore is a demanding test of the model. In fact, we demonstrate here that theory and experiment are in good numerical agreement even as we vary the genome length, giving strong support to the correctness of the model.

Results and discussion

Fig. 1 shows our experimental results. Partial ejection in both λb221 (37.7 kbp) and λcI60 (48.5 kbp) is similar to that measured earlier in EMBL3 (41.5 kbp): the ejected fraction decreases quickly to 50% at several atmospheres of osmotic pressure, then descends more slowly to 0%. As shown by Evilevitch et al. (2005), this "ejected fraction" corresponds to all phages ejecting the same percentage of their DNA, rather than a percentage of the phages ejecting all of their DNA. Most importantly, at every pressure where a measurable amount of DNA is ejected, λb221 ejects less DNA than λcI60. Conversely, for any point in the ejection process, the osmotic force required to stop ejection is always lower in λb221 than in λcI60. For example, the highest forces occur at the point where osmotic pressure completely inhibits the ejection of DNA. This

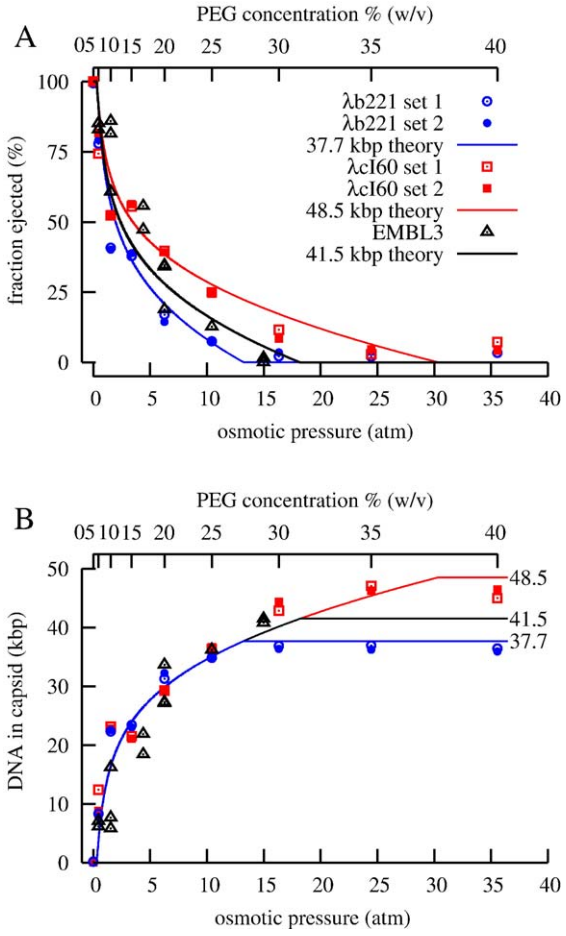

Fig. 1. Measured DNA ejection from λcI60 and λb221 at various external PEG concentrations (points), compared to theoretical predictions for 37.7 kbp and 48.5 kbp genomes (curves). Also plotted: data reported earlier on EMBL3 (Evilevitch et al., 2003), with corrections for the density of PEG made to the earlier analysis, and the corresponding 41.5 kbp theory. The datasets consistently show a dip between 1.5 and 3.5 atm, whose reason is unknown. Otherwise, the precise trends predicted by theory are seen in the data. Panel A shows the percentage of total DNA ejected. At all pressures below 20 atm, λcI60 ejects more DNA than λb221. There is a larger scattering error in the EMBL3 data than in the λcI60 and λb221 data; taking this into consideration, the datasets for all three phages are completely consistent with the expectation that phages with longer genomes eject more DNA at all pressures. Panel B shows an alternative view: the inferred amount of DNA remaining in the capsid after ejection, calculated as $N(1-p)$, where N is the number of base pairs in the genome, and p is the ejected fraction plotted in panel A. As expected, the data points fall on a single curve below 10 atm, then diverge as the maximum pressure is reached for each phage, stopping on the horizontal lines that correspond to the full phage genomes.

inhibition pressure is in the range 10–15 atm for λb221 and 20–25 atm for λcI60—a ~100% increase in pressure results from a 30% increase in genome length.

In Fig. 1, we have included a comparison of our results with earlier work on phage EMBL3 (Evilevitch et al., 2003), for which the genome length is 41.5 kbp. Within the experimental uncertainty, the ejection curve for the intermediate length genome lies between those of the 48.5 kbp and 37.7 kbp phages reported here.

The predictions of our parameter-free model are also shown in Fig. 1. Except for the dip, the theory predicts the data quite well. Both the absolute magnitude and the general shape of the curves are predicted correctly. This is a significant result because no fitting was done to match the theory to the data. A straightforward inverse-spool model of the DNA arrangement, taking into account only the measured bending elasticity and interaxial forces, correctly predicts the dependence of ejection force on genome length.

An alternative view of the ejection data is shown in Fig. 1B. From the ejected fraction p, we can compute the amount of DNA remaining in the capsid as $N(1 - p)$, where N is the number of base pairs in the genome. Since ejected DNA is digested by DNase I, the amount remaining in the capsid should depend only on the external osmotic pressure, and it should be independent of the genome size of the phage. Indeed, Fig. 1 shows that all of the data below 15 atm falls roughly on a single curve. Above 25 atm, the pressure is sufficient to hold the entire genome within the capsid, so the amount of encapsidated DNA is just equal to the genome length of the phage.

For λcI60, which is almost identical to the wild-type λ, our model and measurements indicate that DNA is ejected with a force of around 10 pN that drops steadily as the genome enters the host cell. (See Table 1 for a summary of our quantitative predictions for the phage ejection process.) What advantage does λ gain from having such a high ejection force? The answer may lie in the internal osmotic pressure of the *Escherichia coli* cell, which must be overcome for the phage DNA to penetrate the cytoplasm. The cellular contents of *E. coli* were analyzed in great detail by Stock et al. (1977), who paid particular attention to changes in the cytoplasmic and periplasmic composition under varying osmotic conditions. By assuming that the measured solutes can move freely within the cell, these authors compute a theoretical pressure of 3.3 atm for *E. coli* growing rapidly but approaching saturation. The internal osmotic pressure may also be directly measured by several methods, but it is very difficult to characterize precisely. Hypertonic solutions induce plasmolysis, a shrinkage of the cytoplasm away from the outer membrane, which was used by Knaysi (1951) to estimate the internal osmotic pressure throughout the growth of a culture of *E. coli*. Apparently, the relative osmotic pressure between the growth medium and the cytoplasm increases to as much as 15 atm during an initial phase of rapid growth, before it levels off to 2 atm. In another study, the pressure within *S. aureus* cells was measured by vapor equilibration at 20–25 atm and compared to the fraction of the cells' dry weight made up by small osmolytes; the dry weight analysis of *E. coli* indicates that it will have a pressure several times lower, though the relevant experiments on *E. coli* were apparently not completed (Mitchell and Moyle, 1956). More references and discussion about the osmotic pressure within *E. coli* can be found in Neidhardt (1996), p. 1211.

Table 1
Predictions from our parameter-free theoretical model for various mutants of λ

Phage	Genome (kbp)	L (nm)	ρ (%)	d_s (nm)	F (pN)	E (k_BT)
λcI60	48.5	16.5	50.7	2.65	13.8	13.5×10^3
EMBL3	41.5	14.1	43.4	2.85	8.24	7.2×10^3
λb221	37.7	12.8	39.4	2.98	5.85	4.8×10^3

For each phage, we tabulate the genome length L, the fraction ρ of volume taken up by the genome assuming that DNA has a radius of 1 nm, the initial ejection force F, and the total energy E stored in a packaged capsid. For all three phages, we compute $V_{capsid} = 1.02 \times 10^5$ nm^3. The energy is given here in units of the thermal energy scale k_BT; Boltzmann's constant times the experimental temperature of 310 K.

Based on the preceding discussion, it seems reasonable to assume that *E. coli* has an osmotic pressure of at least 2 atm under most conditions. Fig. 1 shows that λcI60 can eject roughly 60% of its genome against this osmotic pressure, using internal force alone. However, the rest of the genome must be actively transported into the cell. Reversible diffusion, in particular, is incapable of transporting the rest of the genome into cell, since the energy barrier that must be overcome is several times $10^3 k_BT$ (Smith et al., 2001; Purohit et al., 2005). A two-step process is implied: first a quick pressure-driven injection of half of the DNA, then a slower protein-driven importation of the remainder (this slower importation has been measured in detail for RNA polymerase in phage T7—see Kemp et al., 2004). The two-step process has been observed in vivo for the phages T5 (Letellier et al., 2004) and ϕ29 (González-Huici et al., 2004), though similar experiments have not been done on phage λ. A experiment that seems to contradict our model was done by Maltouf and Labedan (1985), who showed that λ DNA can enter the cell even if ATP and the proton motive force are absent in the cell due to energy-poisoning agents. One possible resolution is that the energy could be derived from another source; for example, the binding of proteins to DNA would release energy even in the absence of ATP. Additionally their measurements indicate that only about 40% of the DNA enters the cell under normal circumstances, so it is unclear what the results imply for a two-step ejection process, and the osmotic pressure of energy-poisoned cells has not, in any case, been quantified. Other force-generating mechanisms have been hypothesized, including the influx of water into the DNA-transporting pore (Molineux, 2006).

If we again assume a 2 atm osmotic pressure in the cytoplasm, Fig. 1 shows that only 40% of the genome, 15 kbp, is injected in the first step for λb221. Since phages with genomes much shorter than λb221 are not infectious, we speculate that 15 kbp is close to the minimum amount of DNA required to initiate the second (non-pressure driven) stage of importation. In fact, the osmotic pressure measurements of Knaysi (1951) suggest that λb221 genome ejection should be completely inhibited by the osmotic pressure (15 atm) of rapidly growing *E. coli*. However, in our laboratory and elsewhere, this

phage is routinely used to infect cultures growing rapidly at a density of 10^8 cells/ml, with no apparent problems. It may be that the cellular osmolytes are somewhat smaller particles than PEG monomers, decreasing the effective radius of the DNA and allowing a greater ejection than would be expected by taking the *x*-axis in Fig. 1 literally.

In the case of bacteriophage T7, the entire genome is actively transported at a slow, constant speed (Molineux, 2001; Kemp et al., 2004) even though calculations indicate that its internal pressure is similar to that in λ (Purohit et al., 2005). Something apparently slows down the process in T7 (perhaps the observed expulsion of internal proteins from the T7 capsid), whereas some other dsDNA bacteriophages experience a first stage of fast, pressure-driven ejection. This is the case for λ, for which the ejection time is less than 2 min (García and Molineux, 1995), and for bacteriophage T5 in which recent experiments (de Frutos et al., 2005b; Mangenot et al., 2005) show that DNA ejection proceeds very rapidly between discrete stopping points along the genome. Furthermore, it has been shown in unpublished measurements by Evilevitch et al. that T5 ejection can be inhibited by external osmotic pressure, suggesting a pressure-driven ejection process. A detailed discussion about possible mechanisms for genome delivery and the role of pressure can be found in Molineux (2006). It would be useful to carry out further in vivo ejection experiments with λ and T5 and in vitro experiments with T7 to clarify the differences in the ejection mechanisms of these phages.

Materials and methods

Phages λb221cI26 (λb221) and λcI60 were extracted from single plaques, grown in 3 L cultures of *E. coli* C600 cells and purified by PEG precipitation, differential sedimentation, and equilibrium CsCl gradients, resulting in $\sim 10^{13}$ infectious particles. After purification, phages were dialyzed twice against a 500-fold greater volume of TM buffer (50 mM Tris, 10 mM $MgSO_4$, pH 7.4).

To check the genome lengths of the phages used in this experiment, we used phenol and chloroform to extract the DNA from approximately 5×10^9 phages of each type into 500 μL of 0.5× TBE buffer. A quantity of 1 μl (10^7 genomes, or 0.5 ng) was removed from each extraction, mixed with loading dye, heated briefly to 65 °C to separate cohesive ends, and pipetted into a 0.5× TBE, 1% agarose gel. A 10 ng quantity of a standard ladder (λ-mix, Fermentas) was included for size comparison. We ran the gel using the method of Birren et al. (1990), with a simple electrophoresis box (Owl Separation Systems B1A) and a homebuilt voltage inverter, pulsed at 100 V forward for 0.8 s, 60 V backward for 0.8 s, for a total time of 19 h. The gel was stained with SYBR Gold (Molecular Probes) and photographed with an Alphaimager HP (Alpha Innotech). Results were consistent with the expected 37.7 kbp genome for λb221, obtained by averaging the values reported in Davidson and Szybalski (1971), and a 48.5 kbp genome for λcI60.

The λ receptor LamB (maltoporin), required to trigger ejection, was extracted from the membranes of *E. coli* pop154 cells: these cells express a lamB gene from *S. sonnei* known to be compatible with a variety of λ strains, allowing ejection in the absence of chloroform (Roa and Scandella, 1976; Graff et al., 2002). An overnight culture was sonicated, then the membranes were pelleted, homogenized, and washed in 0.3% *n*-octyl-oligo-oxyethylene (oPOE; Alexis Biochemicals #500-002-L005) at 40 °C for 50 min. A second wash was performed in 0.5% oPOE, followed by extraction in 3% oPOE at 37 °C. LamB was affinity purified in amylose resin and spin filtered to replace the buffer with TM buffer containing 1% oPOE. Based on the sequence of LamB, it follows that a 1 cm absorbance of 1.0 at 280 nm corresponds to 0.34 mg/mL of protein, which we use for computing LamB concentrations in the experiment. Accordingly, from 2 L of cells, we were able to obtain at least 1 mg of protein, enough for many ejection experiments.

Our method for measuring ejection forces is substantially the same as that described earlier (Evilevitch et al., 2003), with minor refinements that have improved precision. We paid particular attention to the difficulty of pipetting the viscous PEG solutions, trying to minimize systematic and statistical errors that occur when the solution adheres to the pipette tips. A solution of 50% (w/w) polyethylene glycol (PEG) 8000 (Fluka PEG Ultra) was prepared in TM buffer with 0.5% oPOE, and its density was measured at 1.09 g/mL (see also González-Tello et al., 1994). This solution was used to prepare solutions of PEG/TM 0.5–1% oPOE at various specified %(w/v) values (see Fig. 2) on an analytical balance. The mass measurements allowed us to set the quantity of PEG in each sample within 0.2 mg, which corresponds to an error in concentration of approximately 0.1% (w/v). Phage solution was added to a final concentration of $\sim 10^{11}$/mL, and DNase I was added at 10 μg/mL. The sample tubes were turned slowly for several minutes to mix the viscous PEG solutions. Purified LamB was added with a wide-mouth tip, and the resulting 200 μl solution was mixed quickly by pipetting. A wide-mouth tip is an inaccurate pipetting device, but it is necessary for quickly mixing viscous solutions. To minimize the effect of inaccurate pipetting on the measurements, we used a final concentration of LamB that was sufficient for maximal ejection, ~2 μg/ml. After the addition of LamB, the samples were incubated for 1 h at 37 °C, which was sufficient for the reaction to reach its endpoint—complete digestion of the ejected genome fraction by DNase I. Finally, the capsids were separated from the ejected DNA fragments by a centrifugation for 20 h at 18,000 × *g*.

After centrifugation, 120 μL of supernatant from each tube was removed to a UV-transparent plastic cuvette (Ocean Optics UVettes), and DNA concentrations were measured with a UV–visible spectrophotometer (LKB Biochrom Ultrospec II). The absorbance curves were aligned at 300 nm (immediately after the DNA absorbance peak) to compensate for absorbance not due to DNA. The resulting curves are shown in Fig. 2. The absorbance values at 260 nm, A_{260}, are linearly related to the amount of DNA ejected from the phage capsids. In contrast with earlier experiments, there was no measurable background DNA absorbance due to ruptured phage capsids: samples at high PEG concentrations or without LamB had similar values of A_{260} to samples prepared without phages. This is probably because phages were used within 1 month of dialysis; in contrast,

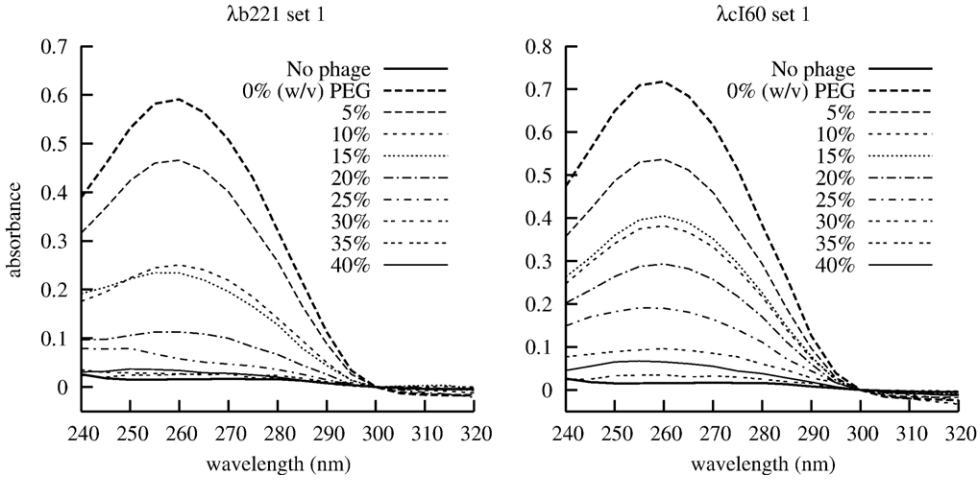

Fig. 2. UV absorbance of the supernatant containing fragments of the DNA ejected from λb221 (dataset "λb221 set 1", left) and λcI60 (dataset "λcI60 set 1", right), aligned at 300 nm. Absorbance curves are shown for various concentrations of PEG, corresponding to different osmotic pressures, and for tests done in the absence of phages. A second dataset taken for each phage is not shown.

samples of λb221 measured after 5 months of storage in TM buffer at 4 °C had a background $A_{260} \approx 0.1$.

When there is no inhibiting osmotic pressure, ejection reaches completion (Evilevitch et al., 2005), and when no phages are added, no DNA is ejected. We use these two limiting cases as calibrations for 100% and 0% ejection, respectively, and find intermediate values with a linear interpolation:

$$\text{ejected fraction} = 100\% \cdot \frac{A_{260,\ \text{with PEG}} - A_{260,\ \text{no phages}}}{A_{260,\ \text{no PEG}} - A_{260,\ \text{no PEG}}}. \qquad (1)$$

An alternative procedure is to use no LamB as a calibration for 0%; this can account for background absorbance due to DNA that escaped from phage capsids during extended storage (Evilevitch et al., 2003). In our experiments, there was no measurable background absorbance, so both methods gave similar results.

The weight measurements set m_{PEG}, the mass of PEG, and m_{tot}, the total mass, for each sample. The %(w/w) weight–weight fraction was computed as $w = m_{\text{PEG}}/m_{\text{tot}}$. The osmotic pressure at each PEG concentration was then determined with the empirical formula (Michel, 1983)

$$\Pi(\text{atm}) = -1.29G^2T + 140G^2 + 4G, \qquad (2)$$

where T is the temperature (°C) and $G \equiv w/(100 - w)$. Note that the osmotic pressure is an increasing function of the PEG concentration and a decreasing function of temperature. For this experiment, $T = 37$.

Two complete sets of samples were prepared for each of λcI60 and λb221. Each sample has a statistical error due to weight measurements, pipetting, and spectrophotometry. To minimize systematic effects on the ejected fraction, the two "no phages" and "no PEG" tubes were averaged for each phage. Statistical errors were propagated to yield x and y errors, which were consistent with the observed scatter in the data.

Earlier work on EMBL3, a 41.5 kbp λ mutant (Evilevitch et al., 2003), can compared with ours, if a simple correction is applied to the data. In this earlier work, the osmotic pressures of the solutions were calculated from the %(w/v) concentrations, without taking into account the density of the PEG solution. Here, we convert them to %(w/w) taking into account the density of the PEG solution. The density differences can be corrected using the relation between weight fraction and density of PEG solutions (González-Tello et al., 1994). After this correction, we find that the largest measured pressure, for example, is 15 atm instead of the 19.6 atm reported earlier. The correction has a smaller effect at lower PEG concentrations; the difference at 10% (w/v) is only 0.2 atm.

Our theoretical model is based on earlier work that describes the packaging energy as a function of the length of DNA in the capsid (Riemer and Bloomfield, 1978; Tzlil et al., 2003; Purohit et al., 2003, 2005). We model the λ capsid as a sphere and its genome as a long semiflexible rod. We assume that the rod is wound into a cylindrically symmetric spool (Cerritelli et al., 1997) with local hexagonal packing. The total energy of the packaged DNA can then be approximated by a sum of interaxial repulsion energy and the DNA bending energy:

$$E = E_{\text{interaction}} + E_{\text{bend}} = \sqrt{3}F_0L\left(c^2 + cd_s\right)\exp(-d_s/c) + \frac{2\pi k_B T\xi}{\sqrt{3}\cdot d_s}\int_{R_{\text{in}}}^{R_{\text{out}}}\frac{N(r)}{r}dr, \qquad (3)$$

where F_0 and c are empirically determined constants describing the interaction between neighboring DNA double-helices, ξ is the persistence length of DNA, L is the length of the DNA within the capsid, d_s is the interaxial spacing, R_{out} and R_{in} are the radius of the capsid and the inner radius of the DNA spool, respectively, and the number of loops of DNA at a distance r from the spool axis is given by $N(r)$. Boltzmann's constant is denoted by k_B; at 37 °C the Boltzmann factor k_BT is approximately equal to 4.28 pN nm. For the persistence length ξ, we use 50 nm, though its value in Mg^{2+} buffer may be ~10% smaller (Hagerman, 1988). The spacing between sequential bases of DNA varies, depending on the base types, from 0.33 to

0.34 nm (Olson et al., 1998). To compute L, we disregard this variation and use 0.34 nm times the number of base pairs within the capsid. The interaxial forces in buffers containing Mg^{2+} ions have been measured by Rau et al. (1984). In their paper, the forces are plotted as a function of the interaxial spacing for various concentration of Mg^{2+}; 5 mM, 25 mM, 100 mM, etc. The authors did not measure the forces at 10 mM, the concentration used in our experiments. However, the plotted curves for 5 mM and 25 mM Mg^{2+} are not significantly different from each other, so we assume that the curve for the unmeasured intermediate value of 10 mM is also similar to these. A least squares fit to the combined 5 mM and 25 mM data in (Rau et al., 1984) gives $F_0 = 12{,}000$ pN/nm 2 and $c = 0.30$ nm. The radius of the phage capsid R_{out} is around 29 nm (Earnshaw and Harrison, 1977). Once we know d_s, R_{in}, and $N(r)$, we can use Eq. (3) to calculate the internal force on the phage genome as a function of genome length inside the capsid, providing an interpretation of the experimental results.

We calculate the remaining variables as a function of L according to the following recipe, which involves only simple geometrical considerations and elementary calculus. The number of loops $N(r)$ in Eq. (3) is given by $N(r) = z(r)/d_s$, where $z(r) = 2 \cdot \sqrt{R_{out}^2 - r^2}$ is the height of the capsid at distance r from the central axis of the DNA spool. The actual volume $V(R_{in}, R_{out})$ occupied by the DNA spool can be related to the genome length L and the interaxial spacing d_s to get an expression for R_{in} in terms of d_s, R_{out} and L (Purohit et al., 2003). This expression for R_{in} is substituted into Eq. (3), which can then be minimized with respect to d_s to give the equilibrium interaxial spacing as a function of the genome length L inside the capsid. From d_s, R_{in}, and $N(r)$, Eq. (3) now gives us the total packing energy as a function of genome length inside the capsid. The internal force $F(L)$ acting on the genome is obtained by taking the derivative of Eq. (3) with respect to L (Purohit et al., 2003).

The preceding construct is a parameter-free model that predicts the ejection force from a λ capsid. Table 1 shows exactly what this model predicts for the phages used in this experiment. There are experimental uncertainties in the quantities quoted above, which should in particular should lead to errors of 10–50% in the magnitude of the predicted forces, but general trends and relative forces will not be strongly affected by these errors (these tests of the parameters are not shown).

DNA ejection in our experiment is halted at the point where the internal force balances the osmotic force. We have described above how to obtain the internal force (and hence, the external osmotic force because of the equilibrium) acting on the genome. But the experimental variable is an osmotic pressure (Tzlil et al., 2003). Thus, we need to translate this force into a pressure. The force is given approximately by

$$F(L) = \Pi \cdot \pi R_{DNA}^2, \tag{4}$$

where Π is the osmotic pressure, and R_{DNA} is the effective radius of the DNA. The effective radius is the smallest possible distance between the center of a PEG monomer and the center of DNA, so that $L \cdot R_{DNA}^2$ gives us the total volume excluded to PEG because of the DNA. We take R_{DNA} as 1.0 nm (bare DNA) plus 0.2 nm, half the PEG monomer length found experimentally (Abbot et al., 1992; Marsh, 2004), and use Eq. (4) to compute Π.

Eq. (4) is a formula for the osmotic force on a very large area, and it should provide an approximately correct description of the DNA-PEG interaction. It is worth pointing out, however, that the tip of the ejected DNA has an area that is only several times larger than the size of a PEG monomer. In fact, the relevant scale is the correlation length (mesh size) of PEG, ~1–3 nm (de Gennes, 1979, pp.78–80), which is approximately the same as the diameter of DNA, implying that Eq. (4) is not exact. Eq. (4) can be corrected using a scaling expression (de Vries, 2001; Castelnovo et al., 2003; Evilevitch et al., 2004), but since this result is good only up to a multiplicative constant, its importance is unclear. Hence, to maintain clarity in our analysis, we do not use the correction. On the other hand, including this effect could result in better agreement between theory and experiment.

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