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Supporting Material

-dependent dynamics of DNA ejections for bacteriophage lambda

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Supplemental Information

Spurious ejections without LamB

The rate of ejections without LamB is ~ 0.008 ejections/sec; with LamB added the rate is ~.06 ejections/sec (at 1:500,000 SYBR gold dilution). The rate of ejections is substantially increased upon addition of LamB. We also performed phage ejections in the presence of large quantities of surface blocking agents (BSA, casein, heparin, as detailed in the Materials and Methods) and saw the same rate of spontaneous ejections. We therefore conclude that the spontaneous ejections are likely the result of SYBR gold. It is possible that spontaneous ejections could be the result of interactions with the glass, although the mechanism is difficult to discern, due to the presence of spontaneous ejections both on monolayers of protein and bare glass.

Calibration of DNA length under flow

Fig. S1 is the calibration of measured length of DNA in the microscope to actual length of DNA in kbp as determined by restriction enzymes (Grayson *et al*., 2007). The result is that length measured in the microscope is roughly quadratic with actual length in base pairs. We use this calibration to determine the actual length of ejected DNA in Fig. 3, C and E, and Fig. S2.

Trajectory data

Herein we plot all trajectories (Fig. S2) used to calculate velocities in Fig. 3, E and F. In Fig. S3 and S4 we describe how the two measures of DNA ejected, length and intensity, are related to each other. In brief, fluorescence intensity consistently overestimates DNA at small lengths, and underestimates at long lengths (see Fig. S3 legend for details). Fig. S2 demonstrates characteristic "continuous" and "looped" trajectories for different salt conditions. Looped trajectories clearly show a discontinuous change in length as a function of time, while the intensity measurements suggest a continuous exit of DNA from the capsid as a function of time and that all of the DNA has exited the capsid even though the length is much shorter than the fully stretched DNA. This suggests that for the "looped" trajectories the DNA is pinned to the capsid (Fig. 2, B) and ultimately unfolds due to the presence of the flow, resulting in a discontinuous change in length as shown in the figure.

The discrepancy between measured length and intensity during the onset of phage ejection is because of our use of highly diluted SYBR gold dye (1:100000 fold), which substantially reduces the signal/noise ratio. Our segmentation algorithm finds ejections by checking for fluorescence intensity. However, due to the low S/N, one strand of DNA, which to our eyes obviously appears

as a single connected strand, looks like multiple disconnected strands to the computer. Thus, a median filter is used to smooth images so that those disconnected strands can become connected. At early times in a phage ejection, a small spot of DNA which is very dim but nonetheless detected has close to zero intensity count. However, after this small spot of DNA is smoothed by the median filter, the spot becomes elongated, resulting in the high length reported for near zero DNA intensity.

Osmotic suppression assay

Evilevitch *et al*., 2003, developed a method to measure the amount of DNA remaining in phage capsids after incubation with LamB in the presence of osmolytes using spectrophotometry (absorbance at 260 nm) and by field inversion gel electrophoresis (Evilevitch *et al*., 2005); in the former measurement, the fraction of phages that did not eject was assumed to be constant at different concentrations of PEG. On the basis of this assumption, the A_{260} measurement can be directly converted from mass to length; however, if the fraction of ejected phages changes from preparation to preparation, spectrophotometry will not produce an accurate measure of length. Moreover, sample to sample differences in preparation (DNA precipitation yield, especially) make it difficult to conclude that there is a constant unejected fraction – this is shown in Figure 5 of Evilevitch *et al*., 2005, where there is up to a 30% variability in A_{260} measurements. To circumvent this issue, we chose to visualize the DNA length directly by performing agarose gel electrophoresis (Figure S5) only. We see clearly that increasing the external pressure inhibits DNA ejection monotonically (data not shown for 10 mM Tris, pH 7.5, 10 mM $MgSO_4$), in contrast to Figure 1, A in Grayson *et al*., 2006. By measuring the migration distance in each lane, we can relate the pressure exerted on the phage to the amount of DNA that was not remaining in the capsid, and in this fashion derive a pressure corresponding to the DNA left in the capsid as a function of amount of DNA ejected (Fig. 4, A).

Above each lane in Fig. S5 is indicated the amount of external pressure induced by the presence of the PEG as measured in atmospheres. The relation between PEG concentration and pressure is described in Michel, 1983. Lanes labeled "L" correspond to the DNA ladder, with the marker lengths noted in (Fig. S5, A). Increasing amounts of external pressure results in increasing amounts of DNA left inside the capsid after ejection. Migration distance in each lane was measured by looking for the maximum fluorescence peak relative to the 48.5 kbp peak, and comparing that to the corresponding ladder. Since electrophoresis was performed in many different batches, we present the results in such a way that the relevant pressure experiment in the appropriate salt condition is to the right of its corresponding ladder. Electrophoresis parameters: 100 V forward, 0.8 sec; 60 V backwards, 0.8 sec; 7-10 hours.

Using the results from Figs. S3 and S4 (continuous velocities only), the end-on area of a double-stranded piece of DNA (radius = 1 nm), and assuming a linear relation between force and velocity, we arrive at the amount of intra-capsid friction that the DNA encounters at different lengths (Fig. 4B).

Movie S1. Biotinylated lambda phages ejected off of a 2 micron sphere, held in an optical trap. The schematic for the experiment is shown in Fig. 1, B. Both continuous and looped ejection events are evident. Time stamp is in the movie.

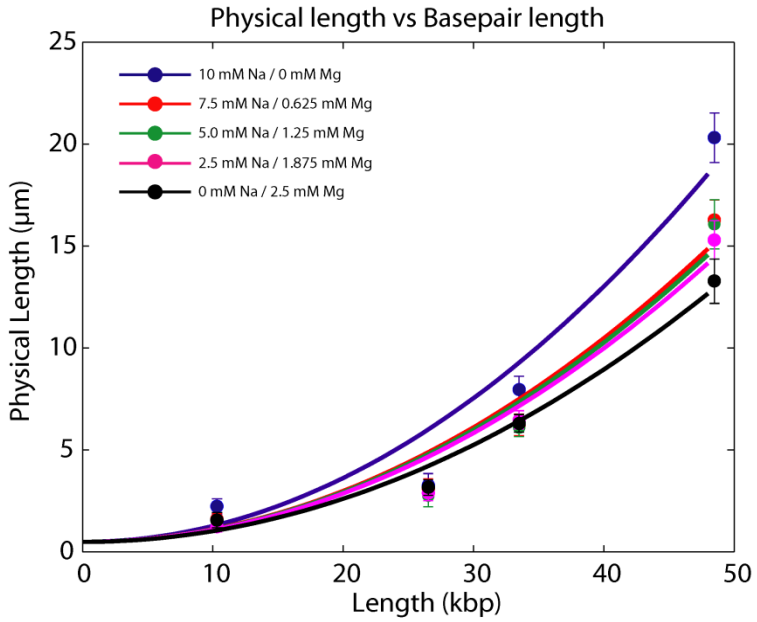

Figure S1. Calibration of tethered DNA length. To convert the physical length of DNA to the length in kbp, we performed a calibration as done by Grayson *et al.*, 2007. Briefly, biotinylated lambda genomes were either treated with restriction enzymes (XhoI, NsiI, and PmlI) or kept untreated. The DNA was attached to a glass coverslip through a biotin-streptavidin bond. The DNA was extended with a 40 µl/min flow and the lengths of different pieces of DNA were measured manually using ImageJ. The mean length was fit to a quadratic function; error bars represent the standard deviation of the measured length.

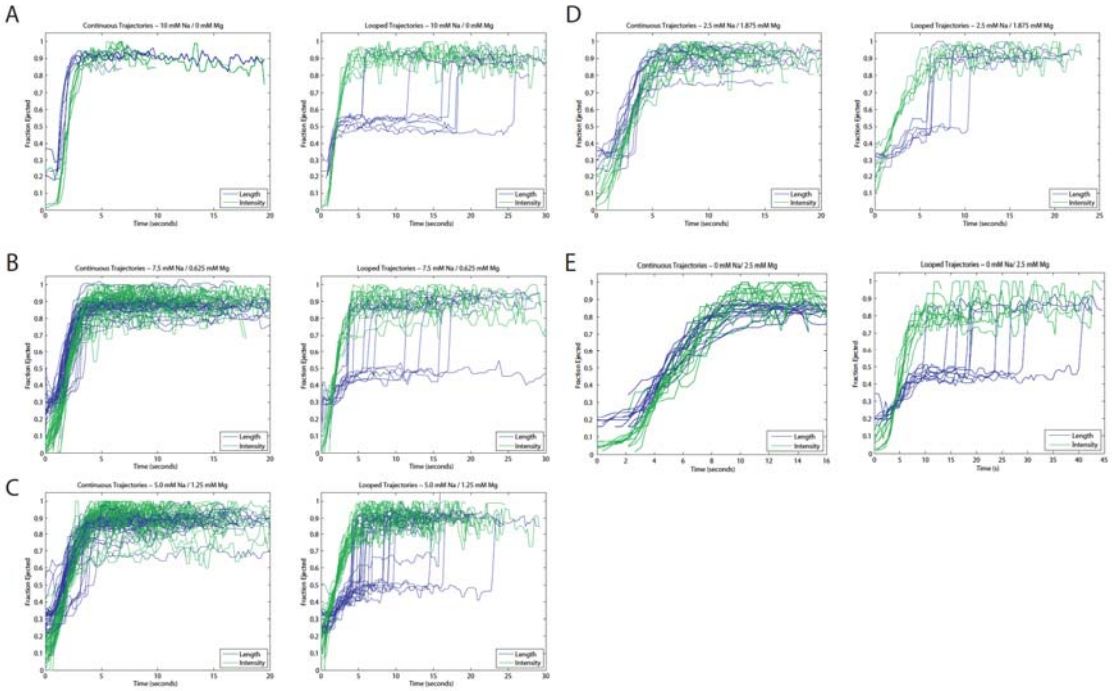

Figure S2. Trajectories used in the paper for all salt conditions. Green: intensity, blue: length. Intensity is the summed intensity of the DNA labeled by SYBR gold. The length is calibrated by measuring defined-length lambda DNA, via restriction digests, under the same flow conditions. Length is then calculated by interpolation. Looping trajectories uniformly unfold at half the maximum length. (A) Salt condition: 10 mM Tris, pH 7.4, 10 mM NaCl, 0 mM MgSO4. Number of trajectories: 5 continuous and 8 looped. (B) Salt condition: 10 mM Tris, pH 7.4, 7.5 mM NaCl, 0.625 mM MgSO4. Number of trajectories: 36 continuous and 13 looped. (C) Salt condition: 10 mM Tris, pH 7.4, 5.0 mM NaCl, 1.25 mM MgSO4. Number of trajectories: 43 continuous and 25 looped. (D) Salt condition: 10 mM Tris, pH 7.4, 2.5 mM NaCl, 1.875 mM MgSO4. Number of trajectories: 14 continuous and 6 looped. (E) Salt condition: 10 mM Tris, pH 7.4, 0 mM NaCl, 2.5 mM MgSO4. Number of trajectories: 15 continuous and 10 looped.

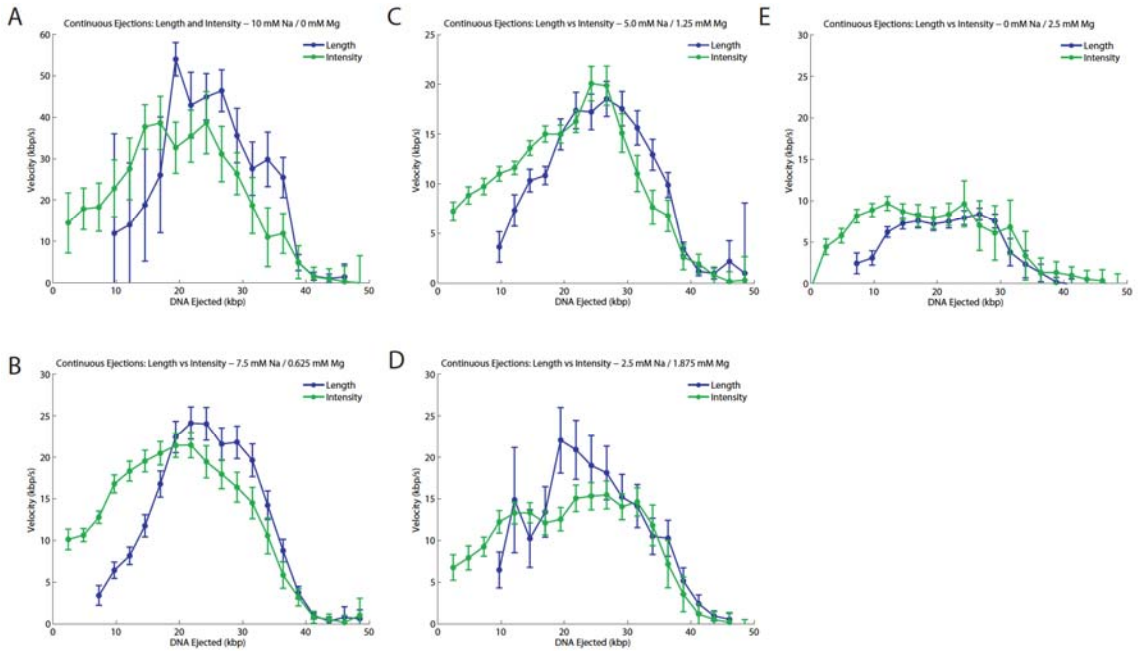

Figure S3. Velocities from the single-phage ejection experiment. Each panel has two classes – green lines denote velocities measured by intensity; blue lines denote velocities measured by length. Intensity measures give a larger estimate for the velocity of DNA ejected at early times because our segmentation algorithm misses the first bits of DNA ejection, and so changes in DNA length appear relatively large at small lengths. Since DNA lengths are hard to directly measure via restriction digest calibration at early times in ejections, we don't measure lengths less than around 5 kbp. The number of trajectories used in each velocity plot is listed in Fig. S1 caption. The errors were determined by standard error. (A) Salt condition: 10 mM Tris, pH 7.4, 10 mM NaCl, 0 mM MgSO4. (B) Salt condition: 10 mM Tris, pH 7.4, 7.5 mM NaCl, 0.625 mM MgSO4. (C) Salt condition: 10 mM Tris, pH 7.4, 5.0 mM NaCl, 1.25 mM MgSO4. (D) Salt condition: 10 mM Tris, pH 7.4, 2.5 mM NaCl, 1.875 mM MgSO4. (E) Salt condition: 10 mM Tris, pH 7.4, 0 mM NaCl, 2.5 mM MgSO4.

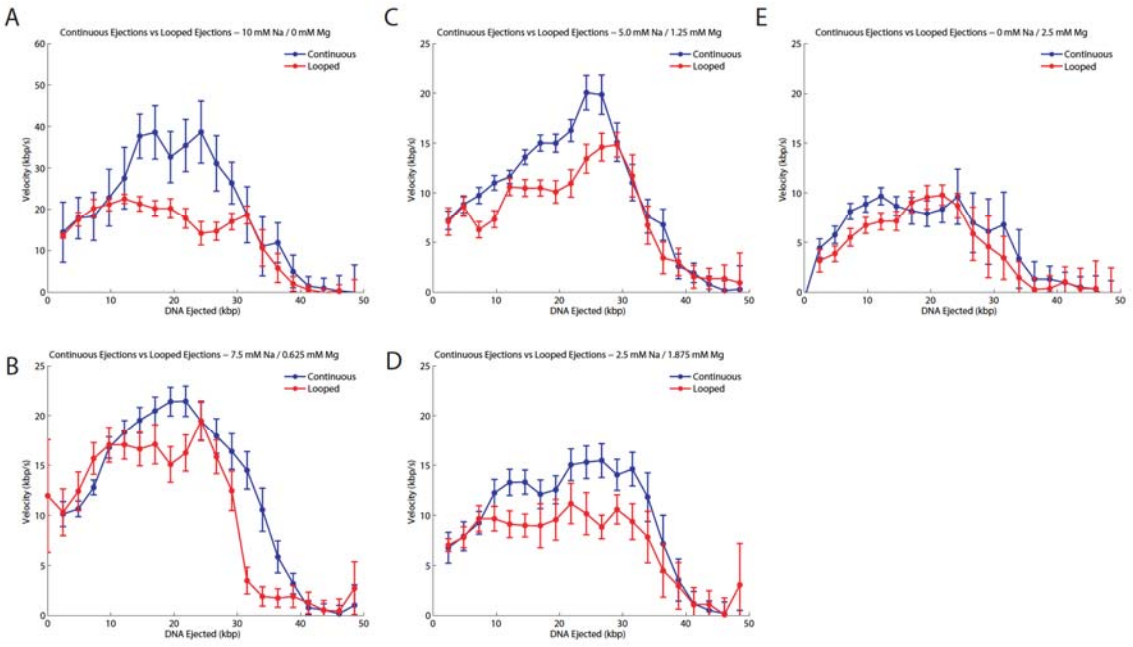

Figure S4. Velocity of DNA at different salt conditions. Red: velocity based on looped trajectories. Blue: velocities based on continuous trajectories. All velocities were determined using intensities; both classes of ejections show similar dynamics. The number of trajectories used in each velocity plot is listed in Fig. S1 caption. The errors were determined by standard error. (A) Salt condition: 10 mM Tris, pH 7.4, 10 mM NaCl, 0 mM MgSO4. (B) Salt condition: 10 mM Tris, pH 7.4, 7.5 mM NaCl, 0.625 mM MgSO4. (C) Salt condition: 10 mM Tris, pH 7.4, 5.0 mM NaCl, 1.25 mM MgSO4. (D) Salt condition: 10 mM Tris, pH 7.4, 2.5 mM NaCl, 1.875 mM MgSO4. (E) Salt condition: 10 mM Tris, pH 7.4, 0 mM NaCl, 2.5 mM MgSO4.

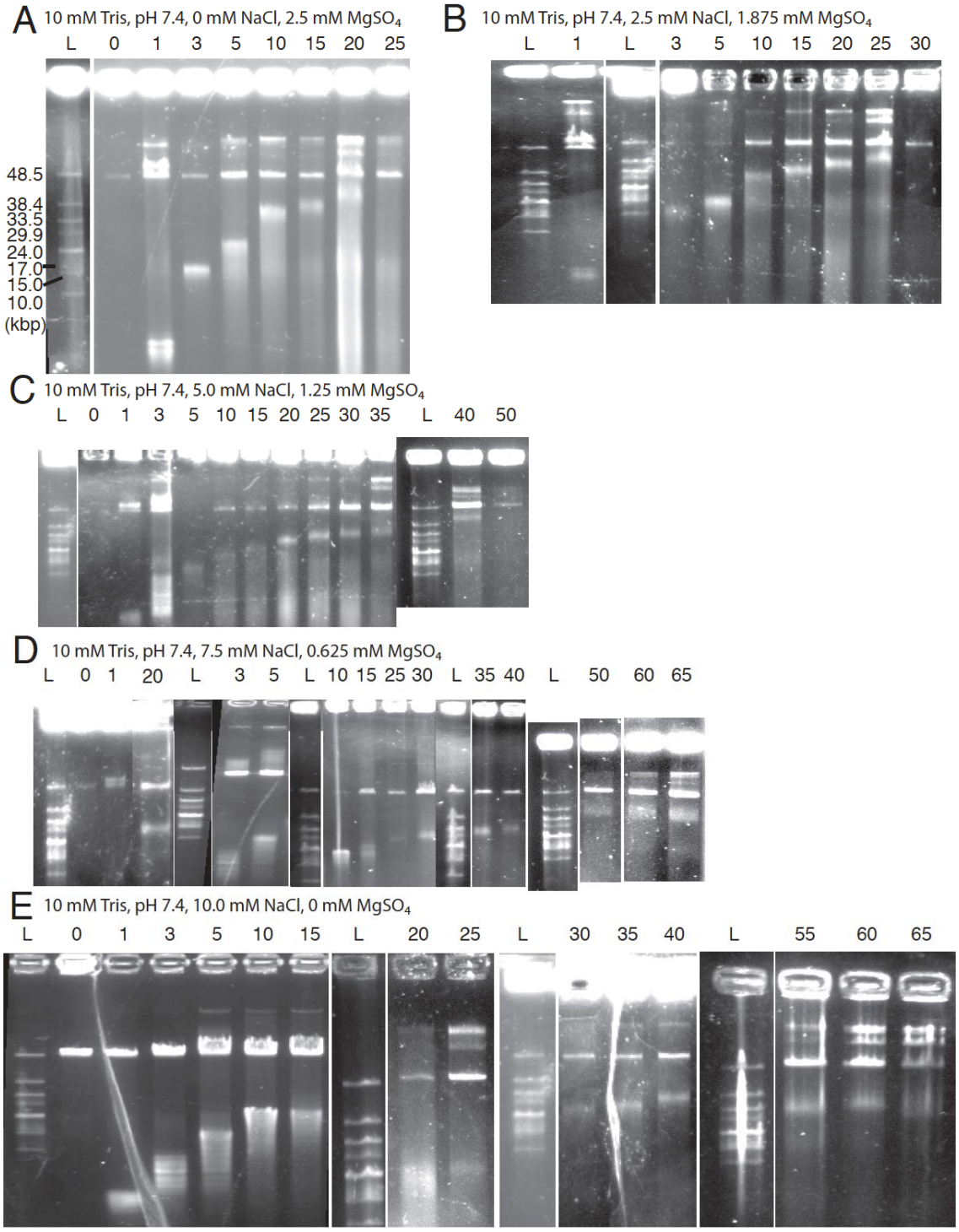

Figure S5. Field-inversion gel electrophoresis of force-dependence assay. A trend of DNA retention in the capsid with increasing external osmotic pressure is clearly visible. (A) Salt condition: 10 mM Tris, pH 7.4, 0 mM NaCl, 2.5 mM MgSO4. (B) Salt condition: 10 mM Tris, pH 7.4, 2.5mM NaCl, 1.875 mM MgSO4. (C) Salt condition: 10 mM Tris, pH 7.4, 5.0 mM NaCl, 1.25 mM MgSO4. (D) Salt condition: 10 mM Tris, pH 7.4, 7.5 mM NaCl, 0.625 mM MgSO4. (E)

Salt condition: 10 mM Tris, pH 7.4, 10 mM NaCl, 0 mM $MgSO_4$.

Additional references:

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