Abstract

The ejection of DNA from a bacterial virus ("phage") into its host cell is a biologically important example of the translocation of a macromolecular chain along its length through a membrane. The simplest mechanism for this motion is diffusion, but in the case of phage ejection a significant driving force derives from the high degree of stress to which the DNA is subjected in the viral capsid. The translocation is further sped up by the ratcheting and entropic forces associated with proteins that bind to the viral DNA in the host cell cytoplasm. We formulate a generalized diffusion equation that includes these various pushing and pulling effects and make estimates of the corresponding speed-ups in the overall translocation process. Stress in the capsid is the dominant factor throughout early ejection, with the pull due to binding particles taking over at later stages. Confinement effects are also investigated, in the case where the phage injects its DNA into a volume comparable to the capsid size. Our results suggest a series of \textit{in vitro} experiments involving the ejection of DNA into vesicles filled with varying amounts of binding proteins from phage whose state of stress is controlled by ambient salt conditions or by tuning genome length.

1 Introduction

A crucial first step in the life cycle of most bacterial viruses involves binding of the virion to a receptor protein in the host cell membrane followed by injection of viral DNA. The viral genome is typically about 10 microns long, and its translocation from outside to inside the host cell is accomplished over times that vary from seconds to minutes. The wide range of mechanisms responsible for injection of phage genomes has recently been systematically reviewed [1,2,3], including many references to the last few decades of relevant literature. In the present paper we formulate a general theory of chain translocation that takes...
into account many of the physical processes involved in actual phage life cycles. These processes include: diffusion of the DNA chain along its length; driving forces due to stress on the DNA inside the viral capsid; resisting forces associated with osmotic pressure in the host cell; cell confinement effects that constrain the injected chain; and ratcheting and pulling forces associated with DNA-binding proteins in the host cell cytoplasm.

Considerable effort has been focused on the energetics of packaging and ejecting DNA in phage. In particular, theoretical work has shown that the dominant source of stress on the DNA in the capsid results from strong repulsive interactions between neighboring portions of double helix that are confined at average interaxial spacings as small as 2.5 nm. Another major contribution comes from the bending stress that arises from the capsid radius being smaller than the DNA persistence length. The force needed to package the genome against this resistance is provided by a virally encoded motor protein that pushes in the DNA along its length. Recent laser tweezer measurements have confirmed that this force increases progressively as packaging proceeds, i.e., as the chain becomes more crowded and bent, reaching values as large as 50 pN upon completion. Conversely, the force ejecting the DNA upon binding of the phage to its membrane receptor has been shown to decrease monotonically from tens of piconewtons to zero as crowding and bending stress are progressively relieved. In the current paper we consider the dynamics of phage ejection and attempt to distinguish the relative importance of these large, varying, “internal” forces and the binding particles in the external solution (bacterial cytoplasm).

It is useful at the outset to consider the simple diffusion limit of the translocation process. More explicitly, consider the case in which a chain is threaded through a hole in a membrane dividing one solution from another. If the chain is free, i.e., in the absence of pushing or pulling forces and of binding particles, it will simply diffuse along its length, experiencing a friction associated with its passage through the membrane and the viscosity of the solution. The time required for its translocation from, say, the left to the right will be

$$\frac{L^2}{2D} = \tau_d,$$

where $L$ is the length of the chain and $D$ is its effective translational diffusion coefficient.

Suppose now that particles are added to the right-hand solution which bind irreversibly to the chain at regularly spaced sites as soon as they diffuse into the solution. Then, if $s$ is the spacing between these binding sites, the diffusion of the chain will be ratcheted each time another length $s$ has entered the solution, corresponding to the fact that the chain cannot move backwards through the hole at a site where a particle is bound. Accordingly, the time it takes for the entire chain to appear on the right is simply given by

$$\frac{s^2}{2D} - \tau_d,$$

the time required for diffusion between a pair of neighboring binding sites – times the total number of sites, $L/s$. It follows that the overall translocation time in the presence of perfect ratcheting is reduced by a factor of $s/L$ over that for free diffusion. When the binding of particles is reversible – they do not remain bound indefinitely, thereby allowing some sites to diffuse backwards through the hole – the translocation time is increased by a factor of $(1 + 2K)$ compared to perfect ratcheting, where $K$ is the ratio of “off” and “on” rates for particle binding. Finally, note that the ideal ratcheting time of $Ls/2D$ corresponds to a velocity of $2D/s$ and hence, by the Stokes-Einstein relation, to a force of $2k_BT/s$ pulling the chain into the particle-containing solution.

When the particle binding is reversible, however, it turns out that there can be an
additional correction to the ratcheting dynamics, one that can significantly shorten the translocation time below $L_s/2D = \tau_{\text{idealratchet}}$. This effect requires that the diffusive motion of the chain is slow enough and is due to the fact that the entropy of reversibly bound particles increases when there is more chain for them to explore. As a result, the entropy is an increasing function of chain length available in the right-hand solution. Indeed, in the limit of fully equilibrated binding, the system is equivalent to a one-dimensional Langmuir adsorption problem [17, 18] (P. G. de Gennes, personal communication). More explicitly, the 1D Langmuir pressure can be written in the form $P_{1D} = (k_BT/s) \ln \{1 + \exp[(\epsilon + \mu)/k_BT]\}$, where $\epsilon > 0$ is the energy lowering of the adsorbing particles upon binding and $\mu$ is their chemical potential in solution. Note that in the limit of large binding energy ($(\epsilon + \mu)/k_BT \gg 1$) this pressure reduces simply to $(\epsilon + \mu)/s$, which – because pressure is force in a 1D system – can be directly interpreted as the force pulling on the chain due to the reversible binding of particles. Note further, in the large binding energy limit, that this force is necessarily large compared to the ideal ratcheting force, $2k_BT/s$ [17].

Ambjornsson and Metzler [18] have recently clarified the various timescales that determine the different regimes of chain translocation in the presence of “chaperones”, i.e., binding particles. The first, $\tau_0$, is the time needed for the chain to diffuse a distance of order $s$, the separation between binding sites. The second and third are $\tau_{\text{occ}}$ and $\tau_{\text{unocc}}$, the characteristic times that a binding site remains occupied and unoccupied, respectively. $\tau_{\text{occ}}$ and $\tau_{\text{unocc}}$ are related by the equilibrium relation,

$$\frac{\tau_{\text{occ}}}{\tau_{\text{unocc}}} = \exp \left( \frac{\epsilon + \mu}{k_B T} \right),$$

Finally, $\tau_{\text{unocc}}$ can be approximated by the typical time it takes for a particle to diffuse a distance of order $R \simeq c_0^{-1/3}$ between binding free particles:

$$\tau_{\text{unocc}} = \frac{R^2}{2D_0} \simeq \frac{1}{D_0 c_0^{2/3}};$$

where $D_0$ is the diffusion coefficient of the particles. One can then distinguish between three different regimes:

1. Diffusive regime: $\tau_0 \ll \tau_{\text{unocc}}, \tau_{\text{occ}}$. Here the binding particles are irrelevant to the chain translocation because the chain diffuses its full length in a time too short for the particles to bind.

2. Irreversible binding regime: $\tau_{\text{unocc}} \ll \tau_0 \ll \tau_{\text{occ}}$. Here particles bind essentially irreversibly on a time scale short compared to the time it takes for the chain to diffuse a distance between binding sites. We shall refer to this as the “ratcheting” regime.

3. Reversible binding regime: $\tau_{\text{unocc}}, \tau_{\text{occ}} \ll \tau_0$. Here diffusion of the chain along its length is slow compared to the time required for an “on”/“off” equilibrium of the binding particles to be achieved. We shall refer to this as the “Langmuir” regime.

It is also important to clarify some relevant length scales involved in the problem.
Specifically, we distinguish between two extremes of how the separation, s, between binding sites compares with the range, δ, of the attractive interaction between binding particle and chain. Pure and "perfect" ratcheting will arise when τ_{unocc} ≪ τ₀ ≪ τ_{occ}, independent of the relative values of δ and s. "Imperfect" ratcheting will arise when τ_{unocc}, τ_{occ} ≪ τ₀, but δ ≪ s. The translocation time for the "imperfect" ratchet is higher than the "perfect" ratchet by a factor of (1 + 2K). Finally, when τ_{unocc}, τ_{occ} ≪ τ₀ and δ ≈ s, in addition to the "imperfect" ratchet we also have a Langmuir force on the chain. Note that if the binding free energy between DNA and the binding proteins is very large then K ≪ 1, and the imperfect ratchet is no different than the perfect one. In this paper we will always take this limit.

Before proceeding further it is instructive to make some numerical estimates. Within this simple translocation model all time scales are naturally referenced to that for pure translational diffusion of a chain along its length, and hence to the diffusion coefficient D introduced earlier. In reality, however, the DNA ejection process is enormously more complicated, since the chain moving through the tail of the phage is feeling not only the friction associated with the few hydration layers surrounding it but also the viscous effects arising from interaction with the inner surface of the tail just nanometers away. Furthermore, this chain portion is connected to the lengths of chain inside the capsid and outside in the cell cytoplasm. The chain remaining inside the capsid moves by reptating through neighboring portions of still-packaged chain and/or by overall rotation of the packaged chain. All of these latter motions involve viscous dissipation that is insufficiently well-characterized to enable realistic estimates of diffusion time scales, even though one can distinguish between different dependence on chain length for each of these dynamical processes [19, 9]. As a result, in pursuing the simple translocation picture as a model for overall phage ejection kinetics, we resort to using an effective diffusion coefficient D to define the unit of time, τ_d = L^2/2D.

A strong upper bound for D can be obtained by considering the part of the dissipation arising as the chain moves through the tail portion of the virus. Taking into account only the friction between the DNA and the fluid in the tail we have, for example [20, 19], ζ = 2πlη/ln(∆/d). Here ζ is the friction coefficient, l is the length of the tail, η is the viscosity of water, ∆ is the inner diameter of the tail, and d is the diameter of the double-stranded DNA. Taking l = 100nm, η = 10^{-9}pN-s/nm², ∆ = 4nm [21] and d = 2nm, we find ζ = 9 × 10^{-7}pN-s/nm and hence a diffusion coefficient (D = k_BT/ζ) of 5 × 10^6nm²/s. For a typical phage genome length (L) of 10µm, this in turn leads to a diffusional translocation time (τ_d = L^2/2D) of about 10 seconds, not unlike ejection times measured for phage λ [22]. Recall, however, that this estimate is based on a value for D which is a strong upper bound, because of all the viscous dissipation contributions that were neglected, suggesting that the actual unassisted diffusional time is likely several orders of magnitude larger than this 10 seconds estimate. Indeed, the outcome of the work presented below is that the translocation time is shortened beyond τ_d by several orders of magnitude by a combination of effects dominated by pressure in the capsid and binding particles in the external solution. A schematic of the role of these various effects is shown in Fig. 1.

The outline of our paper is as follows. In the next section we include the effect of capsid pressure by formulating a Fokker-Planck description of translocation driven by a
Figure 1: Schematic showing the various physical effects which assist bare diffusion in the process of phage DNA ejection. The DNA cross-section is not shown to scale: its diameter is $2 - 4 \text{ nm}$, as compared with a capsid interior diameter that is ten times larger. The spring denotes schematically the stored energy density resulting in a force $F$ acting along the length $L - x$ of chain remaining in the capsid. The small spheres denote particles giving rise to an “external” (cytoplasmic) osmotic pressure $\Pi_{\text{osmotic}}$, while the green particles labeled $i$ and $i + 1$ are successive binding particles.

The combination of diffusion and spatially varying force, i.e., a force pushing the chain from one side that depends on the length of chain remaining on that side (corresponding to the portion still in the capsid and hence experiencing stress due to crowding and bending). We evaluate the mean-first-passage-time (MFPT) for translocation of an arbitrary length and thereby calculate the length ejected as a function of time, using estimates of the spatially-varying ejection force from recent theories of phage packaging energetics. We find that the translocation times are 2 to 3 orders of magnitude faster than the diffusional time. We also treat the case of ejection into a volume comparable to the capsid size (mimicking, say, studies in which phage are made to eject into small vesicles that have been reconstituted with receptor protein [23, 24]) and find that the ejection time decreases from its maximum value when the confinement scale is made larger or smaller than the capsid size. In Sec. 3 we treat the further speed-up in translocation due to ideal ratcheting and to reversible particle binding, respectively. We find that the simple ratcheting effect is small compared to that arising from the entropic force of reversible particle binding. The effect of reversible particle binding decreases the translocation time by another order of magnitude beyond that due to capsid pressure effects. We conclude in Sec. 4 with a discussion of related work by others, of additional contributions to ejection dynamics that will be studied in future theoretical work (in particular, the effect of RNA polymerase acting on the ejected DNA), and of experiments planned to test the various predictions made in the present work.

## 2 Kinetics of ejection driven by packaging force

As discussed in Sec. 1 we focus here on a chain which has been confined in a viral capsid and which is ejected from it through a hollow tail just big enough to accommodate its diameter. To elucidate the essentials of this ejection process we describe the translocation
of the chain as a “diffusion-in-a-field” problem \[25, 26, 27\]. In the present case, involving the translocation of a linear polymer along its length, the diffusion coordinate is a scalar, i.e., the length of chain \(x\) that has been ejected from the tail of the virus. The external field is described by the potential energy \(U(x)\) that gives rise to the force \(F(x) = -dU(x)/dx\), pushing on the chain when a length \(x\) of it has been ejected. This force is due to the remaining chain length \(L - x\) being confined inside the capsid and thereby subjected to strong self-repulsion \((U_{\text{rep}})\) and bending \((U_{\text{bend}})\). The corresponding potential \(U(x)\) is the free energy calculated in recent theories of DNA packaging in viral capsids \[8, 7, 11\]. This energy is seen to decrease dramatically as ejection proceeds (i.e., as \(x\) increases), and so does the magnitude of its slope that constitutes the driving force for ejection.

The one-dimensional dynamics of a diffusing particle in the presence of an external field is a classic problem in stochastic processes \[28\], and, as argued above, can be tailored to treat the translocation of phage DNA under the action of an ejection force \(F(x) = -dU(x)/dx\). Accordingly, the probability \(p(x,t)\) of finding a length \(x\) ejected at time \(t\) is given by the Fokker-Planck equation

\[
\frac{\partial p(x,t)}{\partial t} = \frac{\partial}{\partial x} \left( D \frac{\partial p(x,t)}{\partial x} + \frac{D}{k_B T} \frac{\partial U(x)}{\partial x} p(x,t) \right). \tag{3}
\]

As part of this stochastic description of the translocation-under-a-force process, it is natural to define a mean-first-passage-time (MFPT), \(t(x)\), that gives the average time it takes for a length \(x\) to be ejected in the presence of the external field \(U(x)\), namely \[29\],

\[
t(x) = \frac{1}{D} \int_0^x dx_1 \exp \left( -\frac{U(x_1)}{k_B T} \right) \int_{x_1}^x dx_2 \exp \left( \frac{U(x_2)}{k_B T} \right). \tag{4}
\]

It is useful to consider several limits of this general equation, the first corresponding to the familiar case of no external field. From \(U \equiv 0\) the integrals in MFPT reduce trivially to \(x^2/2D\), giving the expected diffusion time, \(t(x) = x^2/2D\).

For the case of constant force, i.e., \(U = -Fx + \text{constant}\), the integrals in MFPT can also be evaluated analytically, giving \[16\]

\[
t_{\text{Constant Force}}(x) = \frac{x^2 \exp[-\beta F x]}{D} \frac{(\beta F x) - 1}{(\beta F x)^2}. \tag{5}
\]

Here we have written \(\beta\) for \(1/k_B T\), and taken \(F = -dU(x)/dx > 0\) to denote the constant force driving translocation of the chain to the right. In Sec. 3 we will apply Eq. 5 locally, over each segment of length \(s\) associated with a binding site, to calculate the ideal ratcheting corrections to force-driven translocation. Note that simple and ratcheted diffusion are overwhelmed by force-driven translocation when \(\beta F L \gg 1\) and \(\beta F s \gg 1\), respectively.

In the most general instance of spatially varying “external” field \(U(x)\), as in the case of capsid-pressure-driven translocation, the integrals in Eq. 4 must be evaluated numerically. In this way we calculate \(t(x)\) from Eq. 4 for the \(U(x)\) determined from a recent treatment \[7, 11\] of the packaging energetics in phage capsids. This provides a one-to-one correspondence between each successive time \(t(x)\) and the fraction of chain
ejected $x(t)/L$ at that instant. 

In Purohit et al. \cite{7, 11} the DNA inside the phage capsid is assumed to be organized in a hexagonally packed inverse-spool. The potential $U(x)$ is expressed as a combination of the bending energy and the repulsive interaction between the DNA strands, and is given by,

$$U(x) = U_{\text{rep}}(L - x) + U_{\text{bend}}(L - x)$$

$$= \sqrt{3}F_0(L - x)(c^2 + cd)\exp(-d/c)$$

$$+ \frac{2\pi k_B T \xi}{\sqrt{3d}} \int_{R_{\text{in}}}^{R_{\text{out}}} \frac{N(r)}{r} dr.$$  \hspace{1cm} (6)

$F_0$ and $c$ are experimentally determined constants \cite{30} describing the interaction between neighboring DNA strands, $\xi$ is the persistence length of DNA, $d$ is the inter-strand spacing, $R_{\text{out}}$ and $R_{\text{in}}$ are the radius of the capsid and the inner radius of the DNA spool, respectively, and $N(r)$ is the number of hoops of DNA at a distance $r$ from the spool axis. We are interested in finding the internal force on the phage genome as a function of genome length inside the capsid. We do so using Eq. 6 and simple geometrical constraints on the phage genome inside the capsid. The number of loops $N(r)$ in Eq. 6 is given by $z(r)/d$, where $z(r) = (R_{\text{out}}^2 - r^2)^{1/2}$ is the height of the capsid at distance $r$ from the central axis of the DNA spool. The actual volume available for the DNA $- V(R_{\text{in}}, R_{\text{out}})$ can be related to the genome length $L - x$ in the capsid, and the inter-strand spacing $d$, giving an expression for $R_{\text{in}}$ in terms of $d$, $R_{\text{out}}$ and $L - x$. This relation can be substituted for $R_{\text{in}}$ in Eq. 6 which then can be minimized with respect to $d$ to give the equilibrium inter-strand spacing as a function of the genome length $L - x$ inside the capsid. In this way we determine the total packing energy as a function of genome length inside the capsid $(L - x)$ or as a function of the DNA length ejected $x$, i.e., $U(x)$. Using this result and Eq. 4 we can evaluate the MFPT, $t(x)$, for the DNA ejection in $\lambda$ as a function of the length ejected. The corresponding fraction ejected, $x(t)/L$, is shown as a function of time in Fig. 2, with the label “no confinement”.

The value of $D$ can be estimated on the basis of this simple model by the following procedure. The experiment by Novick and Baldeschwieler \cite{22} showed that in a buffer containing 10 mM of Mg$^{2+}$ it took roughly 50 seconds for phage $\lambda$ to completely eject its genome. The values for $F_0$ and $c$ in buffers containing Mg$^{2+}$ have been measured \cite{30}. Since the values measured for 5mM and 25mM Mg$^{2+}$ were not significantly different, we assume that the forces at 10mM will be identical, i.e., $F_0 = 12000$ pN/nm$^2$ and $c = 0.3 \text{ nm}$. Using these values in Eq. 4 and numerically evaluating it for $x = L = 48500 \times 0.34 \text{ nm}$ we find the total time for $\lambda$ to eject its genome of 48.5 kbp is $t \approx (10^5 \text{nm}^2/D)\text{seconds}$. Then, since this value is experimentally estimated to be around 50 seconds \cite{22}, we infer that $D \approx 10^3 \text{nm}^2/s$. This is about 3 orders of magnitude smaller than the $D$ estimated in Sec. 1, consistent with all the sources of dissipation that were left out of that estimate.

An interesting application of our estimates is to the experiments in which viruses eject their DNA into lipid vesicles \cite{2, 22, 24, 31}. Here lipid vesicles are reconstituted with the receptors recognized by the phage of interest, and then mixed with a solution of the phage. The phage binds to the receptor and ejects its DNA into the vesicle. We argue
that the amount of DNA ejected into the vesicle and the corresponding time depend on the radius of the vesicle. In particular if the vesicle has a radius comparable to that of the viral capsid there will be a build-up of pressure inside the vesicle due to the ejected DNA. Ultimately, the ejection process will come to a halt when the force on the DNA from the capsid equals the force from the vesicle side – this can be thought of similarly from the free energy perspective as a free-energy minimizing configuration. Hence, the ejection will not, in general, be complete.

We can work out the ejection rate for this process as follows. If $x$ is the length of genome ejected into the vesicle, we denote the free energies of the DNA inside the viral capsid and the vesicle by $U_{\text{capsid}}(L-x)$ and $U_{\text{vesicle}}(x)$, respectively. The total free energy will be given by,

$$U(x) = U_{\text{capsid}}(L-x) + U_{\text{vesicle}}(x).$$  \hfill (7)

As explained before, we already know $U_{\text{capsid}}(L-x)$ – see Eq. 4 the expression for $U_{\text{vesicle}}(x)$ can be obtained similarly by assuming that the vesicle is like a spherical capsid and the DNA configuration inside is similar to that inside the viral capsid. Our assumed structure for the DNA in the vesicle is a highly idealized model, though we note that electron microscopy on such vesicles demonstrates that DNA within them can adapt highly ordered configurations [2]. In the limit where the vesicle radius is large compared to that of the phage capsid we will recover the free injection result (DNA ejecting from phage into the surrounding solution).

The injection process will stop when the total free energy reaches a minimum, i.e., the
total force on the DNA is zero. The time for DNA injection is given by Eq. 4. We have worked out the kinetics of the ejection for the bacteriophage λ (radius ≈ 29 nm) ejecting its genome into vesicles of radius 29, 50, 100 nm. The phage is taken to be suspended in a solution of Mg$^{+2}$ ions, and similarly the vesicle, with concentration that approximately gives the same values for $F_0$ and $c$ as discussed earlier. This yields a prediction for the kinetics of injection for different vesicle radius. It can be seen from Fig. 2 that when the size of the vesicle is comparable to the capsid size there is only a partial ejection of the DNA. When the vesicle size is almost twice the size of the capsid nearly the entire genome is ejected, except for the last part of the DNA, which takes “extra” time because of the resistance offered to it from the DNA inside the vesicle. Finally, when the vesicle is more than three times the size of the capsid, DNA gets completely ejected from the phage capsid as if there were no vesicle. It is interesting to note that in the initial stages of ejection all the curves for various vesicle sizes fall on one another because there is no resistance to the injection, but as the ejection proceeds each curve reflects a different resistance.

It is also possible that the arguments given above for in vitro ejection into vesicles could be relevant to thinking about ejection into the crowded environment of a bacterial cell [32, 33]. As a result of the crowding within the host bacterium, the viral DNA may be subject to confinement effects like those induced by vesicles.

3 DNA ejection in the presence of DNA binding proteins

The *E. coli* cell has as many as 250 types of DNA binding proteins [34]. Some fraction of these proteins likely bind either specifically or non-specifically to the phage genome as it enters the host bacterium. Accordingly, we consider what happens if the phage DNA is swarmed with binding proteins upon its entry into the host cell. Depending on the binding on/off rates, binding site density, and the strength of binding, we have a corresponding speed-up of the DNA injection into the bacterial cell. In this section we explore this effect and see how, in addition to the speed-up, it helps the phage inject its DNA against the osmotic pressure in the host cell.

Throughout the following analysis of particle binding effects, we assume that the chain is stiff on length scales (e.g. 10’s of nanometers for double-stranded DNA genomes) large compared to the size of the relevant binding particles (typically a few nanometers). We also assume that the binding particles are comparable in size to the distance between sites; for an estimate of Langmuir forces in the more general case of larger binding particles, see [18].

3.1 DNA ejection due to the ratchet action.

Consider a scenario in which host cell binding proteins irreversibly bind on to the DNA at a rate much faster than the translocation rate. In such a case, once a binding site is inside the cell, it is immediately occupied by a binding protein. If the protein stays bound long enough, compared to the translocation time, it will prevent thermal fluctuations from
retracting the DNA back into the capsid. As a result, the DNA will diffuse only between consecutive binding sites, instead of along its complete length. Depending on the spacing between the consecutive sites, it will bring about a speed-up in the translocation compared to when it is only force-driven [16].

For simplicity we assume that the protein binding sites are uniformly distributed along the length of the genome. If the distance between the consecutive binding sites, \( s \), is small compared to genome length, i.e., \( L \gg s \), we can assume that the internal force on the genome due to the packaged DNA is effectively constant while the DNA chain is diffusing between sites. In that case the MFPT, \( t_i \), for the DNA to translocate the distance \( s \) between the binding sites \( i-1 \) and \( i \) is simply given by Eq. 5 with \( x \) replaced by \( s \), and \( F \) replaced by \( F_i \). The internal force \( F \) is of course a varying function of ejected length \( x \), but to a good approximation is constant over each interval of length \( s \). The subscript \( i \) on the force \( F \) denotes this approximately constant force on the DNA chain when the translocation is taking place between the \( i-1 \) and \( i \)th binding sites, i.e., when length \((i-1)s\) has been ejected.

The total translocation time for ejecting length \( x \) of the DNA is given by a sum over the MFPTs for all the sections of length \( s \), along the length \( x \) ejected. The MFPT as a function of \( x \) is given by,

\[
t(x)_{\text{Ratchet}+U(x)} = \sum_{i=1}^{x/s} t_i(F_i) \bigg| \text{Eq. 5} \tag{8}
\]

The corresponding plot for the fraction ejected, \( x(t)/L \), as a function of time is shown in Fig. 3 for \( s = 20 \) nm: the ratcheting reduces the injection time by half as compared to when the ejection results exclusively from the internal force. From Eq. 5 it can be seen that the time will decrease exponentially as the spacing \( s \) decreases. But, since \( s \) can not be smaller than the size of the binding proteins, which is of the order of 10 nm, we have a lower bound on \( s \). The important qualitative consequence of the ratchet will be seen (see Sec. 3.3) to be its helping with internalization of the complete phage genome against osmotic pressure, when internal force alone is insufficient to carry it out.

### 3.2 Reversible force from the binding proteins

Consider another extreme scenario where DNA injects into a reservoir of binding particles and the rate of translocation is slow compared to the time required for the particles to bind and unbind from the DNA. In this case, the binding proteins will come to equilibrium with the DNA. As a result there will be an adsorption force pulling on the DNA, given by [17] (P. G. de Gennes, personal communication),

\[
F = \frac{k_B T}{s} \ln \left\{ 1 + \exp \left( \frac{\varepsilon + \mu}{k_B T} \right) \right\}.
\]

\[
\approx \frac{\Delta G}{s}, \quad \Delta G \gg k_B T\tag{9}
\]
Figure 3: The fraction of DNA injected in phage $\lambda$ as a function of time (in units of $L^2/D$) in the presence of binding particles that form a ratchet. The DNA injection purely due to the internal force is used as a benchmark, and the spacing between the binding sites $s = 20$ nm. It can be seen that the ratchet reduces the translocation time. The time required to internalize the genome solely by the ratcheting mechanism (see lower, straight line) is around twice the time taken for the purely internal force-driven mechanism.

Here $\mu$ is the chemical potential maintained by the reservoir of binding proteins, $\epsilon$ is the binding energy of the proteins with the DNA, and $\epsilon + \mu = \Delta G (> 0)$ is the binding free energy for the proteins. This adsorption force is the 1D Langmuir pressure discussed in Sec. 1. Now, since we have assumed that $\Delta G \gg k_B T$, the binding proteins are mostly bound “on” the DNA and very rarely “off”. So in addition to the force there will also be a Brownian ratchet as discussed earlier. In order to evaluate the MFPT we follow exactly the same process as in the previous section with the addition of this Langmuir force $\Delta G/s$ to $F_i$. The total MFPT is then given by Eq. 9 with $F_i$ replaced by $F_i + \Delta G/s$. We take a typical value of non-specific DNA-protein binding free-energy of $\Delta G = 8k_B T$. The plot corresponding to $s = 20$ nm is shown in Fig. 4. It can be seen that the Langmuir force speeds up the genome translocation by almost an order of magnitude. Not only that, but even if we do not have an internal force, this mechanism (see Pure Langmuir) will inject the complete genome faster than the internal force-driven mechanism. This is because after about 50% ejection, the internal force begins to drop below the constant value of the Langmuir force. Indeed, from Fig. 4 we see that it is at an ejected fraction of about 0.5 that the slope of the “Internal Force” curve drops below the constant slope (rate) of the “Pure Langmuir” plot.

The two cases we described are really two opposite extreme cases for the DNA binding proteins. In reality the rate of binding and the equilibration times may not be very fast (compared to translocation times) and the translocation rates would lie somewhere in between the rates evaluated in this section; for these cases it is necessary to treat the dynamical coupling between particle binding and chain diffusion.
Figure 4: The fraction of DNA injected in phage λ in the presence of binding proteins that bind reversibly, as a function of time (in units of $L^2/D$). The presence of reversible binding proteins result in a pulling Langmuir force (see text). This pulling force significantly enhances the DNA ejection rate over that of the purely force-driven mechanism, by almost a factor of ten.

3.3 Binding proteins enable DNA ejection against osmotic pressure

Due to macromolecular crowding, the *E. coli* bacterium has internal osmotic pressures of about 3 atm. The work of Evilevitch *et al.* showed for phage λ that the ejection process can be partially/completely inhibited by an application of osmotic pressure. Hence, it appears that if the phage were to rely entirely on the driving force due to the packaged DNA to eject its genome, the time scale for full ejection would be prohibitively long. On the other hand, since we know that the genome is completely internalized it seems likely that the particle-binding mechanisms described above may play a key role in *in vivo* DNA translocation. In this section we will see that the task can be accomplished by the Brownian ratchet and the 1D Langmuir force mechanism discussed in the preceding Secs. 3.1 & 3.2.

To see how the Brownian ratchet can internalize the genome against the osmotic pressure, we use the following procedure. If the osmotic pressure in the host cell is $\Pi_{\text{osmotic}}$, the resisting force acting on the DNA can be approximated by $F_{\text{osmotic}} = \Pi_{\text{osmotic}} \pi R_{\text{DNA}}^2$, where $R_{\text{DNA}}$ is the radius of the DNA (about 1 nm). For an osmotic pressure of 3 atm the osmotic force is then estimated to be around 1 pN. We can now replace the term $F$ in Eq. 4 with $F_i - F_{\text{osmotic}}$ to evaluate the MFPT, $t_i$ for the injection of the DNA segment between binding sites $i-1$ and $i$. This time $t_i$ is then summed over all $i$, as in Eq. 8 to give the time $t(x)$ and hence $x(t)/L$. This fraction is plotted in Fig. 5 for the case of spacing $s = 20$ nm between binding sites, and for an osmotic pressure of 3 atm.

It can be seen from the figure (bottom curve) that the time required for internalizing the genome is comparable to the time it takes for phage to inject its genome purely by the
internal force, when there is no osmotic pressure. The internal force for λ (data not shown) at around 50% DNA ejection is approximately 1 pN, i.e., of the order of $F_{\text{osmotic}}$. It can be seen from Fig. 5 that the slope of the curve showing ejection in the presence of ratcheting and osmotic pressure starts decreasing at that percentage of ejection. The average force produced by a Brownian ratchet is $2k_BT/s \approx 0.4\text{pN}$ for $s = 20\text{nm}$ [17, 18]. At 60 – 70% ejection the internal force is around 0.5 pN; the total driving force is then approximately $0.5 + 0.4 = 0.9\text{ pN}$, which is almost the same as $F_{\text{osmotic}}$. This force hence works to eject the genome against the external osmotic force. When around 15% of the genome is left in the phage capsid, the internal force is almost zero. At this point there is only a small amount of the genome still to be ejected and a small differential of $F_{\text{osmotic}} - F_{\text{ratchet}} \approx 0.5\text{ pN}$ to be worked against. This is accomplished by the Brownian motion of the DNA.

Now take the second case, when in addition to forming a ratchet we have a 1D Langmuir pressure, as discussed in Sec. 3.2. To include the effect of the osmotic pressure we have to subtract the osmotic force $F_{\text{osmotic}} = \Pi_{\text{osmotic}}\pi R_{\text{DNA}}^2$ from the driving force $F_i + \Delta G/s$ and use the result in Eq. 5. This means that, so long as $(\Delta G/s - \Pi_{\text{osmotic}}\pi R_{\text{DNA}}^2) \geq 0$, we will always have DNA ejection faster than or the same as that for the purely force driven non-osmotic pressure case. For the numbers we took in the preceding sections, $\Delta G/s = 8k_BT/s \approx 1.6\text{pN}$, which is greater than $F_{\text{osmotic}} \approx 1\text{pN}$. This implies that the phage would inject its genome faster than in the purely pressure-driven mechanism.

Figure 5: The fraction of DNA injected in phage λ as a function of time (in units of $L^2/D$) for the case in which there is a resistive force due to osmotic pressure. We compare the roles of the Langmuir force and the ratchet effect in ejecting the phage DNA against osmotic pressure. The spacing $s$ is taken to be 20nm and the osmotic pressure in the cell is around 3atm. It can be seen that the Langmuir force easily pulls the DNA against this pressure. The DNA translocation by the Brownian ratchet requires a much longer time, but it still succeeds in pulling out the genome at time-scales not much longer than the ejection by internal force alone with zero osmotic pressure.
4 Discussion and Conclusion

This paper addresses the problem of the kinetics of phage injection and the various mechanisms responsible for it. We make use of the available experimental data, existing models for phage packaging, and classical Fokker-Planck theory, to make predictions about translocation rates for phage DNA ejection. The key quantitative predictions described in this paper are:

- **Dependence of ejection rates on driving pressure.** As shown in Fig. 2, the driving force due to the packaged DNA speeds up the ejection process by $2 - 3$ orders of magnitude over free diffusion, and thus is a major contributor to the process of injection. Also, in the *in vitro* setting, the smaller the vesicle into which ejection occurs, the smaller the amount of DNA injected. In addition, for genomes of the same size, the time required for the ejection of the DNA is larger than when into a bigger vesicle.

- **Dependence of ejection rates on the presence of irreversible DNA-binding proteins.** Ratcheting enhances the DNA ejection rate from the viral capsid. The speed-up is minor when compared to internal force-driven ejection (see Fig. 3), but as seen from Fig. 5, it is sufficient to pull out the genome against osmotic pressures of up to 3 atm found inside the bacterial cell.

- **Dependence of ejection rates on the presence of reversible binding proteins.** The reversible binding of proteins exerts a 1D Langmuir force on the DNA. It can be seen from Fig. 4 that the presence of this phenomenon significantly enhances the DNA ejection rate beyond that due to pressure in the viral capsid. From Fig. 5, it is clear that this force is sufficient to efficiently internalize the phage genome against osmotic pressures of up to 3 atm in the bacterium.

We have several biological examples in mind when we treat these ejection mechanisms. In bacteriophage T5 the DNA injection occurs in two steps. The first step transfer, which involves ejection of around 10% of the phage genome, is driven by the internal force. There is then a brief pause, when a protein is synthesized that is implicated in the degradation of the host chromosome, thereby freeing the large number of proteins that had been bound to it. These latter proteins are now available for binding to the injected portion of the phage genome and for pulling the remaining DNA into the cell, via the ratcheting and Langmuir mechanisms.

Similar ideas to those proposed here might also prove useful in those cases where the viral genome is translocated as a result of the binding of motor proteins which themselves translocate along the DNA. One such example is the pulling force by the NTP-driven RNA polymerase (RNAP). RNAP is a very strong motor and can exert forces of up to 14 pN. As described by Molineux and coauthors, transcription by RNAP is the major mechanism for DNA injection from wild-type T7 into *E. Coli* and is an intriguing additional active mechanism that is of great interest to treat theoretically as well. The calculations presented here call for a more systematic experimental analysis of the extent to which proteins bind onto phage DNA as it enters the infected cell.
In this work we have analyzed various effects of DNA translocation of internal capsid pressure and “exterior” (cytoplasmic) binding proteins that can be tested by a variety of in vitro experiments involving phage ejection kinetics into synthetic vesicles and through membranes formed over holes in planar partitions. In these ways one can separately control the capsid pressures (by varying salt concentrations or genome length, for example) and the nature and concentration of DNA-binding proteins inside the capsid or on the other side of the membrane. In addition, it will be important to examine the role of these various mechanisms in determining the kinetics of genome delivery in vivo.

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