A First Model of the Dynamics of the Bacteriophage T4 Injection Machinery

Bacteriophage T4 is one of the most common and complex of the tailed viruses that infect host bacteria using an intriguing contractile tail assembly. Despite extensive progress in resolving the structure of T4, the dynamics of the injection machinery remains largely unknown. This paper contributes a first model of the injection machinery that is driven by elastic energy stored in a structure known as the sheath. The sheath is composed of helical strands of protein that suddenly collapse from an energetic, extended conformation prior to infection to a relaxed, contracted conformation during infection. We employ Kirchhoff rod theory to simulate the nonlinear dynamics of a single protein strand coupled to a model for the remainder of the virus, including the coupled translation and rotation of the head (capsid), neck, and tail tube. Doing so provides an important building block toward the future goal of modeling the entire sheath structure which is composed of six interacting helical protein strands. The resulting numerical model exposes fundamental features of the injection machinery including the time scale and energetics of the infection process, the nonlinear conformational change experienced by the sheath, and the contribution of hydrodynamic drag on the head (capsid).

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1 Introduction

Bacteriophages, or “phages” for short, are viruses that inject their genome in a bacterial host during infection. The majority (96%) of phages possess a large protein head (icosahedral protein capsid) containing its genome and a long slender tail structure [1] that is used to deliver the genome during infection. The tailed phages subdivide into three families that are distinguished by their tail structure. They include Myoviridae which possess elaborate tails that mechanically contract during infection, Siphoviridae which have long but noncontractile tails, and Podoviridae having short, noncontractile tails [2]. One of the most complex phages from the family Myoviridae is bacteriophage T4 which infects Escherichia coli [2,3]. As illustrated in Fig. 1, bacteriophage T4 consists of a (distorted) icosahedral head and a long contractile tail assembly. The head, which contains double-stranded viral deoxyribonucleic acid (DNA), is attached to the tail assembly at the neck [4]. The tail assembly consists of a tail tube (through which the genome passes), surrounded by a springlike sheath, which powers the injection process. The lower end of the sheath attaches to a complex multiprotein baseplate with six long and six short fibers [5,6]. When the long fibers bind to the host, the short fibers anchor the cell and signal a conformational change of the baseplate from a sixfold symmetric dome shape to a star shape. Doing so triggers the sudden dynamic contraction of the (initially stretched) sheath that drives the rigid tail tube into the host through which the phage genome is then delivered [7], refer to Fig. 2.

Because contractile-tailed phages such as T4 are highly efficient genetic delivery machines, understanding their function has major implications for future nanotechnology devices. Indeed, emerging bio-nanotechnologies exploit phage machinery for the detection and control of pathogens [8], for peptide display [9,10] and for phage therapy as an alternative to antibiotics [11–13]. A large number of studies have contributed to our knowledge of bacteriophage T4 structure including the components of the injection mechanism, see, for example, Refs. [4,14–17]. Despite this wealth of data available on the structure of T4, we lack a fundamental understanding of how this intricate machinery works including the dynamics of the injection process.

In this paper, we aim to partially fill that void by contributing a first model to approximate the three-dimensional dynamics of the injection machinery of bacteriophage T4 and, by extension, to all phages possessing contractile tails (Myoviridae). We employ this model to explore the energetics and dynamics of the injection process including the large conformational changes of the energy-
storing sheath. The resulting model may also have future implications for advances in nanotechnology, which aim to harness viral machinery for nanoscale gating, sensing, and translocation, and for translocation typing, peptide display, and phage therapy as reviewed above.

To simulate the dynamics of the injection machinery, we propose a model for the helical protein chains that constitute the backbone of the elastic sheath. A single helical chain or strand is represented by an elastic rod which stores and then releases the energy to drive the injection process described above. Rod models are commonly used to represent the mechanics of biological filaments and single molecules including cilia, flagella, and DNA, see, for example, Refs. [18–24]. The large conformational change experienced by the helical strand induces large rotations and displacements of the rod elements that necessitate using the geometrically nonlinear formulation of Kirchhoff rod theory [25]. Prior numerical formulations of this theory (see, for example, Refs. [18,20–23]) successfully predict highly nonlinear looped, supercoiled, and buckled conformations of DNA.

We extend from these studies using a dynamic formulation to model the transient response of a single helical protein strand of the sheath that is coupled to a model of the capsid and tail tube at one end and to the baseplate at the opposite end. Doing so contributes an essential component model of the sheath that we aim to build upon toward our future goal of modeling the complete sheath structure coupled to the remainder of the injection machinery. Section 2 describes the structure of the T4 injection machinery. In Sec. 3, the dynamical rod model is formulated with initial and boundary conditions relevant to the bacteriophage T4 injection machinery. We first validate our numerical formulation in Sec. 4 by comparing the computed natural frequencies of a single helical strand to known results for a (nanoscale) helical spring. The resulting procedure is then employed in Sec. 5 to simulate the rapid injection process as revealed by the transient dynamics and energetics of the coupled strand–capsid–tail tube system. Section 6 provides conclusions and comments regarding the future research.

### 2 Structure of Bacteriophage T4 Injection Machinery

As mentioned above, bacteriophage T4 is one of the most complex tailed viruses [3,6] and much of its structure has been revealed by cryo-EM measurement. As shown in Fig. 3, T4 is dominated by a 1195 Å long and 860 Å wide prolate capsid (head) [26] containing the 172 kilobase (172 kb) genomic DNA [5], see Fig. 3(a). The capsid is composed of more than 3000 polypeptide chains of at least 12 types of protein [3]. The molecular weight of the head and the genomic DNA is 194 MDalton (MDa)² and 112 MDa, respectively [27]. The long and contractile tail assembly extending from the head is approximately 1200 Å long and has a 250 Å diameter [6]. During infection, the contractile tail penetrates the host cell to deliver the viral genome [3]. The capsid connects to the tail assembly through the neck which is continuous with a cylindrical tail tube that is 940 Å long and possesses a 96 Å external diameter and a 43 Å internal diameter [28]. Surrounding the tail tube is a spring-like sheath structure which stores elastic energy to power the injection process [6]. The sheath, which is 925 Å long and 240 Å diameter [17], is composed of six helical protein strands that work in unison like six helical springs, refer to Fig. 3(b). The sheath is formed from 138 subunits of gene product 18 (gp18) protein synthesized by the virus that are further coupled by 23 hexameric rings visible in Figs. 3(b) and 3(c) [29]. In the extended state, each ring is formed by six subunits 40.6 Å thick and rotated by 17.2 deg in a right-handed manner relative to the previous ring [17]. The upper end of the sheath attaches to the neck and the lower end attaches to a baseplate that is 270 Å high and 520 Å in diameter [28] and composed of at least 14 proteins [6]. Six long and six short tail fibers extend from the baseplate and are responsible for recognizing and attaching the phage to the host. Further details of the structure of T4 can be found in Refs. [3,15,17,26–28].

The infection process proceeds as follows in reference to both Figs. 2 and 3. First, the long tail fiber tips interact reversibly with cell surface receptors to recognize the host (see Fig. 2(a)). Next, the short tail fibers bind irreversibly to the host surface and the baseplate undergoes a large conformational transition from a hexagonal domelike structure to a flatter star-shaped structure which signals sheath contraction [3,6,29,30] (see Figs. 3(b) and 3(c)). The sheath undergoes a large and rapid irreversible contraction to about 37% of its extended (initial) length [29], and viral DNA is injected into the host through the tail tube (see Figs. 2(b), 2(c), 3(b), and 3(c)). Before contraction, the end of the tail tube is captured by the baseplate, but during injection, it is released from the baseplate and translates along and rotates about its long axis. In so doing, the helical parameters describing the sheath change abruptly from 40.6 Å rise and 17.2 Å twist in the extended conformation to 16.4 Å rise and 32.9 Å twist in the contracted conformation. As illustrated in Fig. 3(c), the resulting contracted sheath is 420 Å long with a 330 Å diameter [29]. The rapid collapse of the sheath induces a large change in the rotation between the first and last subunits of each strand. In particular, the 378.4 Å (1.05 turn)

\[ 1 \text{Da} = 1.66053 \times 10^{-24} \text{g}. \]
rotation in the extended sheath increases to 723.8 Å (2.01 turns) in the contracted sheath. Consequently, the head (which remains attached to the sheath by the neck) rapidly rotates by 345.4 Å about the tail tube axis while it simultaneously translates during this collapse [17]. This coupled translation and rotation of the tail tube (and the attached head) create a combination of thrust and torque that pierces the host membrane and results in about half of the tail tube penetrating into the host [29]. Simulations from a first model presented in this paper predict this rapid sheath contraction and the combined translation and rotation of the tail tube and head during the injection process.

3 First Model of Injection Machinery

The sheath is a complex assembly of six interacting helical protein strands that collectively form a shell-like structure, refer to Figs. 3(b) and 3(c). As a first step toward understanding the mechanics of the entire sheath, we focus in this paper on the behavior of a single helical strand, which forms the backbone of the sheath. Doing so lays the foundation for modeling the entire six-stranded sheath structure as part of our on-going research. As suggested in Fig. 4, a helical strand of gp18 can be modeled as an elastic rod as a coarse-grain approximation which sacrifices atomic detail in favor of describing the overall three-dimensional bending and twisting of the strand centroidal axis. Doing so extends the use of elastic rod theory that has previously been used to describe DNA loops and supercoils and other biological filaments as reviewed above.

The single helical strand connects to the capsid/neck/tail tube assembly at the upper end and to the baseplate at the lower end (see Fig. 4(a)). We represent the capsid/neck/tail tube assembly as a single rigid body in the shape of a cylinder that mimics the large capsid volume. During injection, this cylinder undergoes rapid translation along and rotation about the tail tube axis driven by the large reaction force/moment from the helical strand and subjected to hydrodynamic drag. We begin below by summarizing the dynamic rod model of the helical strand.

3.1 Dynamic Rod Model of Helical Strand. Referring to Fig. 4(b), consider a segment of a helical strand as the infinitesimal element of a Kirchhoff rod with equivalent averaged elastic properties. The shape of the rod is parameterized by the three-dimensional centerline curve $R(s,t)$ and the body-fixed frame at each cross section $\{a_i\}$, where $s$ denotes the contour-length coordinate measured from the baseplate (bottom) end and $t$ denotes time. The balance laws of linear and angular momentum for a Kirchhoff rod element resolved in the body-fixed frame are [21,31]
\[
\frac{\partial f}{\partial s} + \mathbf{\kappa} \times f = m_s \left( \frac{\partial v}{\partial t} + \omega \times v \right) - \mathbf{F}_{\text{body}} \tag{1}
\]

\[
\frac{\partial q}{\partial s} + \mathbf{\kappa} \times q = I_s \, \frac{\partial \omega}{\partial t} + \omega \times I_s \omega + f \times \mathbf{a}_3 - \mathbf{Q}_{\text{body}} \tag{2}
\]

where \(\mathbf{\kappa}(s, t)\) is the curvature/twist vector defined as the rotation of the body-fixed frame \(\{\mathbf{a}_i\}\) per unit contour length relative to the inertial frame \(\{\mathbf{e}_i\}\), \(\mathbf{\omega}(s, t)\) is the angular velocity of the cross section defined as the rotation of the body-fixed frame \(\{\mathbf{a}_i\}\) per unit time relative to the inertial frame \(\{\mathbf{e}_i\}\), \(v(s, t)\) is the velocity of the strand cross section centroid, \(m(s)\) is the mass of the strand per unit contour length, and \(I_s(s)\) denote the diagonal \(3 \times 3\) tensor of principal mass moments of inertia per unit contour length. The quantities \(f(s, t)\) and \(q(s, t)\) are the internal force vector and internal moment vector, respectively. Finally, \(\mathbf{F}_{\text{body}}(s, t)\) and \(\mathbf{Q}_{\text{body}}(s, t)\) denote the sum of all the distributed external body forces and moments per unit contour length, respectively, and \(\mathbf{a}_3\) is the unit tangent vector. (Note that \(\mathbf{F}_{\text{body}}\) and \(\mathbf{Q}_{\text{body}}\) may also be functions of the kinematic variables \(\mathbf{\kappa}(s, t)\), \(\mathbf{\omega}(s, t)\), and \(v(s, t)\).)

In Eqs. (1) and (2), the quantities \(\mathbf{\kappa}(s, t)\), \(\mathbf{\omega}(s, t)\), \(v(s, t)\), and \(f(s, t)\) define four unknown field variables which also satisfy two additional field equations

\[
\frac{\partial \mathbf{\omega}}{\partial s} + \mathbf{\kappa} \times \mathbf{\omega} = \frac{\partial \mathbf{\kappa}}{\partial t} \tag{3}
\]

\[
\frac{\partial \mathbf{\omega}}{\partial s} + \mathbf{\kappa} \times \mathbf{\omega} = \frac{\partial \mathbf{\kappa}}{\partial t} \tag{4}
\]

Equation (3) enforces assumed inextensibility and unsharability constraints for the strand, while Eq. (4) is a compatibility constraint that guarantees the smoothness of \(\mathbf{\omega}\) and \(\mathbf{\kappa}\). In Eq. (2), the internal moment \(q(s, t)\) is related to the curvature/twist vector through an assumed linear elastic constitutive law

\[
q(s, t) = B(\mathbf{\kappa}(s, t) - \mathbf{\kappa}_0(s)) \tag{5}
\]

where \(\mathbf{\kappa}_0(s)\) is the known intrinsic curvature/twist vector of the helical strand in a stress-free state, and \(\mathbf{B}\) is a diagonal \(3 \times 3\) stiffness tensor composed of the bending and torsional stiffness coefficients of the equivalent rod.

The four vector equations (1)–(4) containing the four vector unknowns \(\{v, \mathbf{\omega}, \mathbf{\kappa}, f\}\) result in a 12th-order system of nonlinear partial differential equations which are solved numerically following nondimensionalization. To this end, we introduce the nondimensional space and time variables

\[
\tilde{r} = \frac{r}{L}, \quad \tilde{t} = \frac{t}{t_0}, \quad P_0 = \sqrt{\frac{m_s L}{EJ}} \tag{6}
\]

where \(L\) is the total contour length of the strand, \(E\) is the Young’s modulus, and \(J\) is the area moment of inertia for bending of the assumed circular rod cross section. Substituting Eq. (6) into Eqs. (1)–(4) results in the following nondimensional equations:

\[
\frac{\partial \tilde{f}}{\partial \tilde{s}} + \mathbf{\tilde{\kappa}} \times \tilde{f} = \frac{\partial \tilde{v}}{\partial t} + \tilde{\mathbf{r}} \times \tilde{v} - \tilde{\mathbf{F}}_{\text{body}} \tag{7}
\]

\[
\frac{\partial \tilde{q}}{\partial \tilde{s}} + \mathbf{\tilde{\kappa}} \times \tilde{q} = I_s \frac{\partial \tilde{\omega}}{\partial t} + \tilde{\omega} \times I_s \tilde{\omega} + \tilde{f} \times \mathbf{\tilde{a}}_3 - \tilde{\mathbf{Q}}_{\text{body}} \tag{8}
\]

\[
\frac{\partial \tilde{\mathbf{\omega}}}{\partial \tilde{s}} + \mathbf{\tilde{\kappa}} \times \tilde{\mathbf{\omega}} = \frac{\partial \mathbf{\tilde{\kappa}}}{\partial \tilde{t}} \tag{9}
\]

\[
\frac{\partial \tilde{\mathbf{\omega}}}{\partial \tilde{s}} + \mathbf{\tilde{\kappa}} \times \tilde{\mathbf{\omega}} = \frac{\partial \mathbf{\tilde{\kappa}}}{\partial \tilde{t}} \tag{10}
\]

where all the quantities with an overbar are nondimensional as defined in the Appendix. Equations (7)–(10) can be written in the compact operator form

\[
\mathbf{M}(\tilde{\mathbf{y}}, \tilde{\mathbf{r}}, \tilde{t}) \frac{\partial \tilde{\mathbf{y}}}{\partial \tilde{t}} + \mathbf{K}(\tilde{\mathbf{y}}, \tilde{\mathbf{r}}, \tilde{t}) \frac{\partial \tilde{\mathbf{y}}}{\partial \tilde{s}} + \tilde{\mathbf{F}}(\tilde{\mathbf{y}}, \tilde{\mathbf{r}}, \tilde{t}) = 0 \tag{11}
\]

where \(\tilde{\mathbf{y}}(\tilde{r}, \tilde{t}) = \{\tilde{\mathbf{r}}, \tilde{\mathbf{\omega}}, \tilde{\mathbf{\kappa}}, \tilde{\mathbf{f}}\}\) and

\[
\mathbf{M} = \begin{bmatrix}
\Theta & \Theta & \Theta & \Theta \\
\Theta & \mathbf{I} & \Theta & \Theta \\
\Theta & \Theta & \mathbf{I} & \Theta \\
\mathbf{I} & \Theta & \Theta & \Theta
\end{bmatrix}, \quad \mathbf{K} = \begin{bmatrix}
\Theta & \Theta & \Theta & \Theta \\
\Theta & \mathbf{I} & \Theta & \Theta \\
\Theta & \Theta & \mathbf{I} & \Theta \\
\mathbf{I} & \Theta & \Theta & \Theta
\end{bmatrix}, \quad \mathbf{F} = \begin{bmatrix}
\tilde{\mathbf{r}} \times \tilde{\mathbf{a}}_3 - \tilde{\mathbf{\kappa}} \times \tilde{\mathbf{v}} \\
- \tilde{\mathbf{\kappa}} \times \tilde{\mathbf{v}} \\
- (\frac{\partial \tilde{\mathbf{B}}}{\partial \tilde{t}} - \frac{\partial (\tilde{\mathbf{B}} \tilde{\mathbf{K}}_0)}{\partial \tilde{t}}) + \tilde{\mathbf{r}} \times I_s \tilde{\mathbf{v}} + \tilde{f} \times \mathbf{a}_3 - \tilde{\mathbf{\kappa}} \times \tilde{\mathbf{B}}(\tilde{\mathbf{\kappa}} - \tilde{\mathbf{K}}_0) - \tilde{\mathbf{Q}}_{\text{body}}
\end{bmatrix}
\]

Here, \(\Theta\) and \(\mathbf{I}\) are the \(3 \times 3\) zero and identity matrices, respectively. For future reference, the total (nondimensional) elastic energy, \(\mathcal{U}_e\), and kinetic energy, \(\mathcal{K}_e\), for the strand are

\[
\mathcal{U}_e = \int_0^1 \left[ \frac{1}{2} (\tilde{\mathbf{K}} - \tilde{\mathbf{K}}_0)^T \tilde{\mathbf{B}} (\tilde{\mathbf{K}} - \tilde{\mathbf{K}}_0) \right] d\tilde{s} \tag{12}
\]

\[
\mathcal{K}_e = \int_0^1 \left[ \frac{1}{2} \tilde{\mathbf{r}} \times I_s \tilde{\mathbf{v}} + \frac{1}{2} \tilde{\mathbf{r}}^T \tilde{\mathbf{m}} \tilde{\mathbf{v}} \right] d\tilde{s} \tag{13}
\]

The theory is completed upon specifying the following initial and boundary conditions for the T4 injection process.

### 3.2 Initial and Boundary Conditions for T4 Injection Process

Equation (11) is a 12th-order system of partial differential equations in space and time, which is solved for the field variables \(\tilde{\mathbf{y}}(\tilde{r}, \tilde{t}) = \{\tilde{\mathbf{r}}, \tilde{\mathbf{\omega}}, \tilde{\mathbf{\kappa}}, \tilde{\mathbf{f}}\}\) under specified initial and boundary conditions (six at each boundary). For the T4 injection process, it is natural to first define the boundary conditions in the inertial
frame and subsequently transform the boundary conditions to those in the body-fixed frame consistent with Eq. (11). As shown in Fig. 5, the first model of the T4 injection process includes the (nonlinear) rod representation of a single protein strand attached at its upper end to a large rigid cylinder representing the capsid/neck/tail tube assembly at the point of attachment denoted by A. The cylinder has two degrees-of-freedom defined by the translation along and the rotation about the tail tube (e1) axis. During contraction, both the velocity \( v_{e1} \) and the angular velocity \( \omega \) about the tail tube axis are unknown while no rotation occurs about the e1 and e2 directions. Therefore, the boundary conditions at the upper end (t = 1) of the strand are

\[
\begin{align*}
\mathbf{v}_{e1}(1, t) = 0, & \quad \mathbf{v}_{e2}(1, t) = 0 \\
\mathbf{v}_{e1}(1, t) = v_{e1}(1, t), & \quad \mathbf{v}_{e2}(1, t) = \mathbf{v}_{e1} \mathbf{v}_{e3} \\
\mathbf{f}^A_{\text{drag}} = m_i \frac{\partial \mathbf{v}_{e3}}{\partial t} + \mathbf{q}_{\text{drag}} = I_i \frac{\partial \mathbf{v}_{e3}}{\partial t},
\end{align*}
\]

(14a)

\[
(14b)
\]

\[
(14c)
\]

where \( \mathbf{v}_{e1} \) and \( \mathbf{v}_{e2} \) in Eq. (14b) are the components of velocity of point A in the plane e1 – e2 induced by rotation about the cylinder axis, and similarly, \( \mathbf{v}_{e1} \) and \( \mathbf{v}_{e2} \) denote the components of the normalized position vector \( \mathbf{r} \) from the cylindrical axis to point A. Equation (14c) is the linear and angular momentum balance laws for the cylinder along e3, which are influenced by the hydrodynamic drag force and moment created by the surrounding physiological buffer defined by

\[
(\mathbf{F}_{\text{drag}} = -\mathbf{C}_i \mathbf{v}_{e3}, \quad \mathbf{Q}_{\text{drag}} = -\mathbf{C}_i \mathbf{v}_{e3})
\]

(15)

Here, \( \mathbf{C}_i \) and \( \mathbf{C}_i \) are normalized force and moment drag coefficients, respectively. \( I_i \) and \( m_i = m_i/m_i L \) denote the normalized mass moment of inertia and mass of the cylinder representing that of the capsid/neck/tail tube assembly. Finally, the vectors \( f^A_{e3} \) and \( q^A_{e3} \) are the normalized reaction force and moment of the strand, respectively, at point A along the e3 direction. At the lower end (t = 0), the strand is attached to the stationary baseplate. Thus, there is no rotation or translation at this boundary as described by

\[
\begin{align*}
\mathbf{v}_{e1}(0, t) = 0, & \quad \mathbf{v}_{e2}(0, t) = 0, \\
\mathbf{v}_{e1}(0, t) = v_{e1}(0, t), & \quad \mathbf{v}_{e2}(0, t) = \mathbf{v}_{e1} \mathbf{v}_{e3}, \\
\mathbf{f}^A_{\text{drag}} = m_i \frac{\partial \mathbf{v}_{e3}}{\partial t} + \mathbf{q}_{\text{drag}} = I_i \frac{\partial \mathbf{v}_{e3}}{\partial t},
\end{align*}
\]

(16)

As mentioned in Sec. 2, during the injection process, the sheath undergoes a large conformational change from the extended to the contracted conformation. To simulate this phenomenon, we employ a two-stage solution process that starts with the strand at rest in the contracted conformation. We first apply a constant force and torque to the cylinder sufficient to translate and rotate the cylinder to achieve the extended conformation. Second, once the extended conformation is attained, the constant force and torque are suddenly eliminated to induce the dynamic collapse of the ejection process and the return to the contracted conformation.

The nonlinear system of Eq. (11) is not integrable in closed form and this necessitates employing a numerical solution at each time step. We use a finite difference formulation based on the generalized- \( x \) method for discretizing in both space and time domains following the procedure already summarized in Refs. [31,32]. Starting with an initial condition \( \mathbf{Y}(\tau, 0) \) for the contracted state, the discretized equations are integrated over space at each successive time step. The boundary conditions are satisfied using a classical shooting method for boundary-value problems. References [31] and [32] detail this numerical procedure.

4 Validation: Limiting Case of Spring Vibration

Before employing the above formulation for evaluating the injection machinery of T4, we validate the formulation by employing the model to replicate a known result. To this end, the system of Eq. (11) is used to compute the fundamental natural frequency of a nanoscale helical spring composed of protein. The approximate fundamental natural frequency \( f_0(\text{Hz}) \) for a clamped-free spring from linear theory [33] is

\[
f_0 = \frac{d}{4\pi ND^2} \sqrt{\frac{G}{2\rho}}
\]

(17)

where \( d \) is the diameter of a thin rod forming \( N \) turns of a helical spring having diameter \( D \), shear modulus \( G \), and density \( \rho \). As an illustrative example, consider a spring composed of the protein actin that has \( G = 0.766 \text{ GPa} \) and \( \rho = 1380 \text{ kg/m}^3 \) [34]. We consider a nanoscale spring having \( d = 10 \text{ Å}, D = 300 \text{ Å} \), and \( N = 3 \). Evaluating Eq. (17), the fundamental frequency of vibration of this example spring is 15.53 MHz. Next, we examine the free vibration of the same spring using the numerical formulation outlined above. For this example, the mass and dimensions of the attached cylinder are set to zero as are the hydrodynamic drag force and moment. We select the (normalized) time and space steps of \( \Delta \tau = 0.005 \) and \( \Delta s = 0.01 \), respectively, which yield converged results for this and all other examples in our study.

We begin with stretching the spring by applying a constant vertical velocity \( v_{e1} = 0.5 \) to the free end for the first 50 time steps which constitutes phase 1 of our simulation. The free end is then suddenly released allowing the spring to freely vibrate during phase 2. Figure 6(a) illustrates the computed spring kinetic (12) and strain (13) energies calculated during phases 1 and 2. Since there is no damping in this example, the total mechanical energy shown in Fig. 6(a) remains constant (to within numerical approximation). The power spectrum of the strain energy shown in Fig. 6(b) reveals that the frequency with the largest power (ignoring aliasing error) is 15.59 MHz. This numerical result replicates that above from linear theory (17) to within 0.5%. The remaining (small) difference can be attributed to the nonlinearities present in the (nonlinear) rod formulation and by numerical approximations.

5 Simulating the T4 Injection Machinery

Having validated the above formulation, we now employ it to simulate the injection process of bacteriophage T4. While the elastic properties of the protein strand gp18 remain unknown, we can assume that they are similar in magnitude to those of other protein filaments, such as actin, whose properties are well
Fig. 6 (a) Strain, kinetic, and total mechanical energies of a nanoscale protein spring. Phase 1: Stretching phase with prescribed velocity of free end. Phase 2: Free vibration phase following the release of the free end. Energy is reported in the units of $kT$, where $k$ is the Boltzmann constant and $T$ is the temperature (kelvin), and time is reported in the units of nanoseconds. (b) Power spectrum of the strain energy of the nanoscale protein spring. Illustrated peak locates natural frequency of fundamental vibration mode.

characterized [34]. We also estimate values for the mass, mass moment of inertia, and drag coefficients for the capsid/neck/tail tube assembly in arriving at a first simulation of the dynamics of the injection machinery. In particular, we use the following material and geometrical properties for the contracted (stress-free) conformation of the helical strand

\[ E = 2.3 \text{ GPa}, \quad G = 0.766 \text{ GPa}, \quad \rho = 1380 \text{ kg/m}^3, \]
\[ d = 10 \text{ Å}, \quad D = 330 \text{ Å}, \quad N = 2.01, \quad \text{and} \quad L = 2115.5 \text{ Å} \]

and for the capsid/neck/tail tube assembly (rigid cylinder)

\[ p = 210 \text{ Å}, \quad m_s = 10^{-15} \text{ kg/m}, \quad m_t = 2.3 \times 10^{-20} \text{ kg}, \quad \text{and} \quad r_c = 325 \text{ Å} \]

where $p$ is the helical strand pitch, and $r_c$ denotes the cylinder radius.

As described in Sec. 2, the contracted helical strand, which is assumed stress-free, has about two turns ($N = 2.01$), while the extended helical strand, whose strain energy powers the injection process, has about one turn ($N = 1.05$). The simulation begins with initial conditions for the strand at rest in the contracted conformation which also defines the intrinsic curvature/twist vector $\mathbf{k}$ in Eq. (5) (a helix with 2.01 turns, diameter 330 Å, and pitch 210 Å). To create the extended conformation (a helix with 1.05 turns, diameter 240 Å, and pitch 925 Å) from the initial contracted conformation, we apply a (normalized) constant force $\mathbf{F}_c = 170$ and moment $\mathbf{m}_c = -14$ to the capsid via Eq. (14c) for 245 time steps at which time the extended conformation is achieved. This force/torque system is then suddenly eliminated which immediately initiates a rapid dynamic collapse to the contracted conformation which is partially resisted by the hydrodynamic drag force/moment system acting on the capsid.

Inferring from cryo-EM images, the tail assembly must simultaneously twist counterclockwise (about one turn) and translate downward in achieving the contracted conformation and, due to the nanoscale hydrodynamics, this injection process is expected to be overdamped [34]. The simulation yields the trajectory and associated energetics of the helical strand and the capsid/neck/tail tube assembly. In particular, to compute the system kinetic energy, we add the kinetic energies of translation and rotation of the capsid/neck/tail tube assembly to that of the helical strand given by Eq. (13). The energetics of the entire injection process is illustrated in Fig. 7 for the case of unrealistically small drag coefficients $C_v = 160$ and $C_r = 0.2$ which yield an underdamped response as evidenced by the decaying oscillations about the contracted conformation. Increasing the drag coefficients to $C_v = 285$ and $C_r = 1.8$ yields the expected overdamped response illustrated in Fig. 8(a), where the extended conformation rapidly collapses to the contracted conformation.

From Fig. 8(a), the time scale of the injection process is on the order of 700 ns. However, this is likely an upper bound estimate to the time scale since the complete sheath is stiffer as it consists of six interacting helical strands instead of the single helical strand included in this first model. For comparison, the initial collapse of a highly buckled small portion of DNA in the virus $\phi$29 is estimated to be 30–45 ns as reported in Ref. [23] using an analogous model. Importantly, the energy computations reveal that the strain energy of the extended conformation, about $7kT$, powers the rapid collapse of the sheath and the associated injection of the tail tube needed for infection. For comparison, this energy is significantly less than that (100 $kT$ required to buckle the small (90 bp) portion of DNA as reported in Ref. [23]. However, the computed energy for a single helical strand is certainly a lower bound estimate to that stored in the complete six-stranded sheath.

The time-varying kinetic energy of the helical strand and the capsid/neck/tail tube assembly is presented in Fig. 8(b), and it represents approximately 10% of the total energy (Fig 8(a)), a small but non-negligible contribution. Note that the capsid is massive in
comparison to the helical strand and therefore dominates the kinetic energy of the entire injection machinery. Figure 8(b) further exposes the relative contribution of the rotational and translational kinetic energies of the capsid/neck/tail tube assembly, and the companion translational and angular velocities are illustrated in Fig. 9. The rotational kinetic energy lags that due to translation and has a maximum about half as large as that due to translation. The source of this lag is visible in Fig. 9 where the angular velocity of the capsid/neck/tail tube assembly attains an extremum significantly later than the extremum of the translational velocity. Note that the translational velocity is negative as required during the downward collapse of the helical strand. In contrast, the angular velocity remains positive as the downward collapse also induces a positive (right-handed) rotation of the capsid. The angular velocity achieves an enormous magnitude (on the order of $10^7$ rad/s) which is anticipated since the capsid must complete one full rotation during this rapid (700 ns) dynamic collapse. Selected (snapshot) images of the system are shown in Fig. 10 during the rapid collapse from the extended conformation to the contracted conformation, for example, overdamped case.

Fig. 8 (a) Energetics of rapid collapse from extended conformation to contracted conformation. Increased drag coefficients yield the expected overdamped response. (b) Comparison of kinetic energies for helical strand and capsid/neck/tail tube assembly.

Fig. 9 Translational velocity, $v_e$, and rotational velocity, $\omega_e$, of capsid/neck/tail tube assembly during the injection process.

It is also worth mentioning that during contraction, the gp18 subunits slide (shear) relative to each other with otherwise no apparent change in their structure [16]. Therefore, the total arc length of the helical strand in the extended conformation is actually different from that in the contracted conformation, a behavior that is not captured in the present model that embeds inextensible and unshearable constraints on strand deformation. Removing these constraints will yield a softer structural model for the strand and simultaneously increase the predicted time for dynamic collapse.

6 Conclusions and Future Studies

The injection machinery of bacteriophage T4 is an intriguing nanoscale machine that includes an elastic sheath that powers the injection process through a contractile tail assembly. While the structure of T4 is reasonably well resolved, the dynamics of the injection process remain largely unknown, including the process time scale and energetics. This paper contributes a first model of the injection machinery dynamics by coupling a nonlinear model of an elastic helical protein strand to a rigid body model of the capsid/neck/tail tube assembly subject to hydrodynamic drag. The helical strand is modeled as a Kirchhoff rod that captures the large conformational change of the sheath from its extended state prior to injection to its contracted state following injection. The rod couples to the (relatively massive) capsid/neck/tail tube assembly at one boundary, and the resulting nonlinear initial-boundary value problem is solved using finite differencing (generalized-$z$ method in space and time). Numerical solutions reveal an expected rapid and overdamped collapse from the extended to the contracted state during injection. Simulations further reveal that this process occurs in approximately 700 ns, is powered by $7kT$ of elastic energy stored in the helical strand, and induces the coupled translation and rotation of the tail tube needed to penetrate a host bacterium.

This first dynamic model, that includes a single helical protein strand, represents an important step toward constructing a future model incorporating all six interacting strands that constitute the complete sheath. Recognizing the increased stiffness of the complete sheath immediately suggests that (1) the injection process is likely shorter than the 700 ns estimated above and (2) is likely powered by elastic energy greater than the $7kT$ estimated above.

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Appendix

Nondimensional parameters employed in Sec. 3 are

\[ f = \left( \frac{m_L^2}{p_0} \right) I, \quad F = \left( \frac{m_L}{p_0} \right) \mathcal{F} \]

\[ q = \left( \frac{m_L^2}{p_0} \right) q, \quad Q = \left( \frac{m_L^2}{p_0} \right) \mathcal{Q} \]

\[ v = \left( \frac{L}{p_0} \right) v, \quad \omega = \left( \frac{1}{p_0} \right) \omega \]

\[ \kappa = -\frac{L}{p_0} \kappa, \quad I_c = \left( \frac{m_L^2}{p_0} \right) I_c \]

\[ U_c = \left( \frac{m_L^2}{p_0} \right) U_c, \quad C_c = \left( \frac{m_L}{p_0} \right) \mathcal{C}_c \]

References


