Mechanics of DNA packaging and ejection from elastic phage capsid

Long Li, Jizeng Wang,a) and Youhe Zhou

Key Laboratory of Mechanics on Environment and Disaster in Western China, the Ministry of Education of China, School of Civil Engineering and Mechanics, Lanzhou University, Lanzhou 730000, China

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Abstract This study intends to investigate how the elasticity of a bacterial phage can affect the process of DNA packaging and ejection. For this purpose, we propose a unified continuum and statistical mechanics model by taking into account the effects of DNA bending deformation, electrostatic repulsion between DNA–DNA strands and elastic deformation of the phage capsid. Based on such a model, we derive the quantitative relations between packaging force, elasticity of capsid, DNA length remaining in the capsid, osmotic pressure and ejection time. The theoretically predicted results are found to agree very well with in vitro experimental observations in the literature. © 2013 The Chinese Society of Theoretical and Applied Mechanics. [doi:10.1063/2.1305403]

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As a typical Escherichia coli, lambda phage can package chromosome genome into its capsid through DNA packaging motor by consuming adenosine triphosphate (ATP) molecules. The capsid needs to withstand a high inner pressure for confining the DNA, which can be subsequently injected into the cell cytoplasm for infection by releasing the confinement free energy as long as the phage binds to the target Escherichia coli cell, and opens its tail. Such remarkable capability of genome delivery makes the lambda phage to be proposed as a potential drug delivery container by specific modification on phage coat. Therefore, understanding the molecular biomechanics of lambda phage is of great interest to advancing our fundamental biological knowledge as well as many practical applications in fields such as drug delivery, global ecology and bacterial pathogenicity.

Theoretical models of DNA packaging have been developed to determine the forces involves in packaging DNA into a rigid capsid. It has been pointed out that the packaging motors supply driving energy to package DNA into the phage capsid as concentric circular loops by overcoming the energy barrier of DNA bending and electrostatic repulsion between DNA strands. In general, experiments have confirmed that lambda phage can sustain DNA with lengths in the range of 78%–106% of a standard value, 16.5 μm. In the case of packaged genome length being less than 78%, the confining effect becomes weak, which will not be able to provide large enough ejection force to overcome the osmotic pressure of cytoplasm, making the phage loses its effective infection ability. For the case of genome length being larger than 106%, the phage capsid will be broken by the high confining pressure. Experimental measurements have shown that the lambda phage capsid can only sustain an internal pressure below a critical value corresponding to a DNA packaging force of 50 pN.

The confinement free energy can be released to drive the DNA ejection overcoming the viscous friction between DNA–DNA, DNA–capsid, and DNA–tail and the resistance from the osmotic pressure in external buffer solution or cytoplasm. For such dynamic processes, developments in experimental techniques have enabled real-time monitoring, resolving a longstanding puzzle about the ejection speed. Wang et al. have theoretically considered the DNA ejection from phage as a compressed wormlike chain moving out of confined space. The theoretically predicted relations between the ejection speed, ejection time, ejection length, and other physical parameters, such as the phage type, total genome length, and ionic state of external buffer solutions, show reasonable agreement with in vitro experimental observations.

Despite of the above progresses, in most existing theoretical models on DNA packaging and ejection, energetic contribution from the elastic deformation of the capsid has been unfortunately neglected, though, which might be critical in understanding bacterial pathogenicity, and designing of release-controllable drug delivery systems. Hence, in order to quantitatively understand the role that elastic deformation of phage capsid has played in the dynamics of DNA ejection, in this letter, we develop a coupled continuum and statistical mechanics model by taking into account the elastic deformation of capsid, the bending of confined DNA chain, the electrostatic interaction between DNA strands, and the viscous friction during the ejection process. Such a study might be of interest to broad research areas including toxicologists, nanotechnologists, and virologists.

To illustrate the model problem considered in this study, Fig. 1 shows a DNA chain confined in an elastic spherical capsid with Young’s modulus $Y$, and Poisson’s ratio $\mu=0.3$. The configuration of the confined viral DNA is assumed to be a continuous spool consisting of nearly concentric loops. We assume that the capsid in empty state has the inner and outer radiuses, $R_i$ and $R_{out}$, respectively. For the filled capsid, we denote $R_{eq}$ as its equilibrium inner radius, and $R$ as the inner radius of the DNA spool (as shown in Fig. 1). According to Refs. 5 and 6, we have the following expression for the

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a)Corresponding author: Email: jzwang@lzu.edu.cn.
confinement free energy of the DNA chain

\[ E_{\text{DNA}} = \sqrt{3} F_0 L_{\text{in}} (c_0^2 + c_0 d_s) \exp \left( -\frac{d_s}{c_0} \right) - \]

\[ \frac{4\pi \xi k_B T}{\sqrt{3} d_s^2} \left( \sqrt{R_{\text{eq}}^2 - R^2} + R_{\text{eq}} \ln \left( \frac{R_{\text{eq}} - \sqrt{R_{\text{eq}}^2 - R^2}}{R} \right) \right), \]

where \( k_B \) is the Boltzmann constant, \( T \) is the temperature, \( \xi = 50 \) nm and \( d_s \) are constants that characterize the strength and decay length of electrostatic interaction between DNA strands, and \( L_{\text{in}} \) is the length of packaged DNA chain, which can be related to \( R_{\text{eq}} \), \( d_s \), and \( R \) as

\[ L_{\text{in}} = \frac{8\pi}{3\sqrt{3} d_s^2} \left( R_{\text{eq}}^2 - R^2 \right)^{3/2}. \]

Once the DNA chain is packaged, the phage capsid will be deformed due to a confining pressure, \( p_{\text{in}} \). According to elastic theory, the normal stresses in radial and tangential directions in the elastic capsid can be given by \( \sigma_r = a/r^3 + b \) and \( \sigma_\theta = -a/(2r^3) + b \), respectively, where \( a = p_{\text{in}} R_{\text{in}}^3 R_{\text{out}}^3/(R_{\text{in}}^3 - R_{\text{out}}^3) \) and \( b = -p_{\text{in}} R_{\text{in}}^3/(R_{\text{in}}^3 - R_{\text{out}}^3) \). The radial displacement in the elastic capsid should satisfy the boundary condition, \( u_r(R_{\text{in}}) = R_{\text{eq}} - R_{\text{in}} \). Substituting \( u_r(R_{\text{in}}) \) into the geometric relations and then the constitutive relations yields

\[ p_{\text{in}} - \frac{Y (R_{\text{eq}} - R_{\text{in}})}{GR_{\text{in}}} = 0, \]

where \( G = [(4\mu - 2)R_{\text{in}}^3 - (1 + \mu)R_{\text{out}}^3]/[2(R_{\text{in}}^3 - R_{\text{out}}^3)] \). Then, the strain energy stored by the capsid can be obtained as

\[ E_{\text{cap}} = \frac{2\pi Y R_{\text{in}}}{G} (R_{\text{eq}} - R_{\text{in}})^2. \]

The total energy of the DNA loaded phage head is the sum of the confinement free energy in Eq. (1) and the elastic deformation energy of the capsid in Eq. (4), \( E_{\text{total}} = E_{\text{DNA}} + E_{\text{cap}} \). For a given DNA genome length \( L_{\text{in}} \), the geometrical configuration of the DNA inside the elastic capsid can be characterized by three parameters \( R_{\text{eq}} \), \( d_s \), and \( R \). Inserting Eq. (2) into the expression of the total energy and eliminating the unknown parameter \( R \), we obtain \( E_{\text{total}}(R_{\text{eq}}, d_s) = E_{\text{DNA}}(R_{\text{eq}}, d_s) + E_{\text{cap}}(R_{\text{eq}}) \). In order to minimize the system energy, we consider the variation of the total energy functional, which gives

\[ \frac{Y R_{\text{in}}}{G} (R_{\text{eq}} - R_{\text{in}}) = \frac{\xi k_B T}{\sqrt{3} d_s^2} \left( R_{\text{eq}} \sqrt{R_{\text{eq}}^2 - R^2} + R^2 \ln \left( \frac{R_{\text{eq}} - \sqrt{R_{\text{eq}}^2 - R^2}}{R} \right) \right), \]

\[ \sqrt{3} F_0 \exp \left( -\frac{d_s}{c_0} \right) = \frac{\xi k_B T}{R_{\text{eq}}^2 d_s} + \frac{3\xi k_B T}{d_s} \left[ \frac{1}{R_{\text{eq}}^2 - R^2} + \frac{R_{\text{eq}}}{R_{\text{eq}}^2 - R^2} \right] \exp \left( -\frac{d_s}{c_0} \right) + \frac{\xi k_B T}{2 R^2}. \]

In the limit of very large Young’s modulus \( (R_{\text{eq}} = R_{\text{in}}) \), Eq. (5) is reduced to the same equation as has been derived by Purohit et al.\(^5,6\) for the rigid capsid. Then, an energetic packaging force can be obtained by differentiating the total energy \( E_{\text{total}} \) with respect to \( L_{\text{in}} \), as

\[ F = \frac{\partial E_{\text{DNA}}}{\partial L_{\text{in}}} = \sqrt{3} F_0 (c_0^2 + c_0 d_s) \cdot \]

\[ \exp \left( -\frac{d_s}{c_0} \right) + \frac{\xi k_B T}{2 R^2}. \]

It is interesting that the packaging force in Eq. (6) has the same form as that given by Purohit et al.\(^5,6\) However, different from Purohit et al.,\(^5,6\) the parameters for characterizing the packaging state, \( R_{\text{eq}}, d_s, \), and \( R \), become dependent on the elastic deformation of the capsid.

In our previous study,\(^12\) we have analyzed the viscous motion of a semiflexible polymer chain coming out of a strongly confined space as a model to investigate the effects of various structure confinements and frictional resistances encountered during the DNA ejection process. By assuming the overdamped motion of the DNA chain, then the ejection force will be balanced by the resistance forces consisting of total viscous friction\(^12\) and osmotic pressure,\(^12\) and the time interval from \( t_0 \) to \( t_1 \) (corresponding to the packed DNA length decreasing from \( L_0 \) to \( L_1 \)) for describing the relation between the ejection time and the DNA length remaining in the capsid can be expressed as

\[ t_1 - t_0 = \int_{L_0}^{L_1} \frac{\eta(L_{\text{in}})}{F(L_{\text{in}}) + F_{\text{osm}}} dL_{\text{in}}, \]

where \( \eta(L_{\text{in}}) \) is the total friction coefficient of the DNA chain with sections inside the phage head, tail tube and
the external solution, whose expression can be found in Ref. 12, and \( F_{\text{osm}} = -\pi R_{d}^{2} \Omega \) is the osmotic resistance force from external buffer solution, \( R_{d} \) and \( \Omega \) are the effective DNA radius and osmotic pressure, respectively.

In the following, we take typical values \( R_{\text{out}} = 31.5 \text{nm} \), \( R_{\text{in}} = 29 \text{nm} \), \( R_{d} = 1 \text{nm} \), tail tube length 150 nm and total genome length 16.5 \( \mu \text{m} \). Young’s modulus of mature and immature lambda phage capsids are 1 GPa and 0.3 GPa, respectively. The viscosity and osmotic pressure for dilute ionic solution are taken to be equal to 0.001 Pa·s and 1 kPa.

Roos et al. have demonstrated that the maturation process of bacteriophage HK97 accompanies increase of Young’s modulus of the phage capsid, implying that Young’s modulus of phage capsids can be very different. Figure 2 shows the packaging force as a function of the percent of DNA packed into the capsid. Figures 2(a) and 2(b) represent the cases that capsids are filled with 10 mM MgCl\(_2\) solution with low electrostatic repulsion and 5 mM MgCl\(_2\) and 50 mM NaCl solution with high electrostatic repulsion, respectively. It can be seen from Fig. 2 that the packaging force is strongly dependent on the DNA length remaining inside the particle. Comparing the packaging force in Fig. 2, we can find that Young’s modulus of the phage capsid has weak effect on the DNA packaging force for 10 mM MgCl\(_2\) buffer, however, for the buffer with high ionic strength, as shown in Fig. 1(b), obvious difference on the effective packaging forces can be found for the capsids with different Young’s modulus. Usually, a softer nanoparticle sustains weaker internal confining pressure and implying an easier DNA package process.

In a previous optical tweezers experiment on DNA packaging in lambda phage, the authors have used different buffer solutions respectively containing 12.5 mM Tris-HCl, 2.5 mM MgCl\(_2\), and 25 mM NaCl for cell centrifugation, 25 mM Tris-HCl, 5 mM MgCl\(_2\), and 50 mM NaCl with \( F_{0} = 2.3 \times 10^{4} \text{ pN/nm}^{2} \) and \( c_{0} = 0.27 \text{ nm} \) for procapsid purification, and 20 mM Tris-HCl, 10 mM MgCl\(_2\) with \( F_{0} = 1.2 \times 10^{4} \text{ pN/nm}^{2} \) and \( c_{0} = 0.30 \text{ nm} \) for microsphere washing. We thus hypothesize that the actual buffer solution that influences the electrostatic repulsion between DNA strands during the packaging process should be a mix of all the above solutions, for which we choose the parameters \( F_{0} = 1.6 \times 10^{5} \text{ pN/nm}^{2} \) and \( c_{0} = 0.27 \text{ nm} \) to characterize its ionic property. Figure 3 shows the packaging force as a function of the percentage of DNA packed into capsule. It can be seen from Fig. 3 on DNA packaging into the immature phage, predictions based on the present elastic model matches experiments very well. For the DNA packaging into the mature phage, Fig. 3 shows that the packaging force is about 50 pN at the 100 percent packaging, which agrees well with the experiments.\(^9\)

Osmotic pressure can resist the DNA ejection. The resistance force felt by the DNA chain is proportional to an effective area around the area of cross-section of a DNA molecule. For the osmotic pressure of PEG buffer, Grayson et al. have adopted an effective radius as 1 nm (bare DNA) plus 0.2 nm, which is the half PEG monomer length found experimentally. Figure 4 shows the relation between the length of DNA ejected from the capsid filled with 10 mM Mg\(^{2+}\) solution (corresponding to \( F_{0} = 1.2 \times 10^{4} \text{ pN/nm}^{2} \) and \( c_{0} = 0.30 \text{ nm} \)) and the osmotic pressure in various external buffer solutions. It can be seen from Fig. 4 that the result based on the present elastic model agrees better with the experimental results than that based on the rigid model by Purohit et al.\(^5\,6\) In addition, the ejection process of DNA from
the elastic capsid can be inhibited by different values of osmotic pressure. And softer capsid corresponds to a lower value of the inhibition osmotic pressure. The underlying mechanism is that the softer capsid can be easily deformed, making the DNA spool inside the capsid become looser. Then the confining effect due to the electrostatic repulsive interaction and DNA bending is reduced, and eventually leading to a smaller ejection force.

For phage capsids of various stiffness filled with 10 mM NaCl solution (corresponding to \( F_0 = 660 \text{ pN/nm}^2 \) and \( c_0 = 0.52 \text{ nm} \)).\(^5\) Fig. 5 shows the comparison between theoretical predictions (dashed line) and experimental results (hollow circles) by Grayson et al.\(^11\) on the ejected DNA length as a function of the ejection time in 10 mM NaCl external buffer solution. Good agreement between theory and experiment can be observed from Fig. 5.

In addition, Fig. 5 also shows the DNA ejected into the cytoplasm of *Escherichia coli* (dotted and dash-dotted lines) with an osmotic pressure of 3.3 atm (1 atm = 101 325 Pa).\(^17\) From Fig. 5, it can observe that the ejection process strongly depends on the capsid stiffness. This finding may be of interest to researches on release-controllable drug delivery systems.

In summary, we have developed a unified continuum and statistical mechanics model for describing dynamics processes of DNA packaging and ejection from elastic phage capsids. Based on this model, we have quantitatively derived the relations between Young’s modulus of the capsid, ejection time, ejected DNA length, osmotic pressure, ejection force, and various physical parameters. In comparison with the rigid-capsid model by Purohit et al.,\(^3\) the present model can give more precise predictions than the rigid one according to in vitro experimental observations in the literature. This study deepens our understanding on the molecular biomechanics of DNA packaging and ejection from bacteriophage, which is crucially important to not only fundamental biological understandings but also practical applications such as the design of advanced site-specific and controllable drug delivery systems.

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