Investigation of Mechanical Strength of the Nanoshell of Bacteriophage Phi-29

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ABSTRACT

The shell of a virus is composed by protein and intends to protect the DNA in the virus. The DNA will release from viral capsid by internal pressure when virus attaches the cytoplasm. The structure of capsid is well known from scanning-force microscopy (SFM) or cryo-EM studies. Bacteriophage Phi-29 can package double-stranded DNA with the length of 6.6 um into a capsid. When packaged, the DNA is kept under high pressure (about 6MPa) inside the viral shell. Therefore, the bacteriophage capsid serves as a high-pressure container. Ivanovska placed the nano tip of an atomic force microscope (AFM) onto the shell of a bacteriophage, increased the force applied by the tip slowly and recorded the deformation of the shell. The result shows that empty shells withstand nanonewton forces while being indented up to 30% of their height. By combining with simulation and theoretical method, the Young's modulus of bacteriophage shell is 1.8GPa in empty.

In this research, by using the analytical solution (method 1, method 2) and finite element method that include three approaches were proposed to find the stress in the nanoshell when phi-29 packages the dsDNA. Method 1 is assumed that the capsid is simplified as a hollow sphere loaded by an inner pressure of 6 MPa and its outer pressure is 0 MPa. From analytical solution the stress in the capsid is 47.6 MPa. When it comes to method 2, the capsid is assumed to be a cylindrical vessel with ellipsoidal heads and loaded by an inner pressure, 6MPa. The stress in the capsid calculated from method 2 is 83.4 MPa. To method 3, the analysis software is applied to simulate the true-structure-like phi-29 capsid mode. From the simulation result, the stress in the capsid shell is 116.6 MPa. The results of the numerical simulation show the best agreement to the experimental data among these three methods. The method proposed here would be used to study the mechanical behavior in DNA packaging and release from viral capsids in the future.

Keywords: Atomistic-Continuum mechanics method (ACM), DNA, Finite element method, Morse functions.

1 INTRODUCTION

Before considering the architecture of viruses, it is worth noting that, although proteins may have regular secondary structure elements in the form of an α helix and β structure, the tertiary structure of the protein is not symmetrical. This, of course, is a consequence of hydrogen bonding, disulphide bridges and the intrusion of praline in the secondary structure. Although one might consider that the nucleic acid could be enveloped by a single, large protein molecule, however, the actual situation is that the proteins are irregular in shape, whereas most virus particles have a regular morphology. Bacteriophage phi-29 has a dsDNA genome and infects Bacillus subtillis, and Phi-29 is a relatively small virus built by only seven different structural polypeptides, including a scaffolding protein directing the assembly. Novel methods based on reconstruction of phi-29 particles were devised to determined the profiles of the 42*54nm prohead (as shown in Fig1) [1-2] that consists of gp8, gp8.5, gp10 and gpRNA, but most of the mechanically coherent shell is made of gp8 (the presence of one additional component (the fiber protein) being dispensable for structural integrity). The organization of these proteins within a prohead is obtained from cryo-electron microscopy (EM).

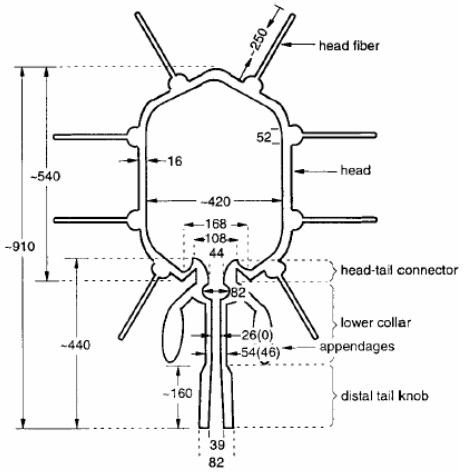


Fig. 1 Illustration of phi-29 structure [1-2]. Unit:Å

A prolate shell is constructed from 235 gp8 subunits formed in a T= 3 lattice, which is composed of two parts:

one is 20 hexameric joining with 11 pentameric units to form the icosahedral end caps and another is 10 hexameric units forming the cylindrical equatorial region. Prohead, the first particles assembled in the phi-29 morphogenetic pathway, consist of a head-tail connector protein gp10, scaffolding protein gp7, a major capsid protein gp8, head fiber gp8.5, and a 174 base RNA. The viral genome is packaged into the prohead. Note that the DNA gp3 packaging requires an addition of a virus-encoded gp16 and ATP. The scaffolding protein gp7 leaves during this stage. Finally, gp16 and pRNA are released, and the lower collar, tail knob as well as the appendage proteins are added to form the tail of the mature virion. The dsDNA can be ejected by the incubation with $NaClO_4$. There are symmetric mismatches between the five-fold-symmetric capsid and the 12-fold symmetric connector or the six-fold-symmetric tail.

2 THEORY

Finite element method

The finite element method considers the minimization of the total potential energy, which includes internal energy, bending energy, twisting energy, the contact energy and the external energy. Moreover, the geometry of the single-stranded DNA can be described by discrete finite element with few geometrical limitations.

Using the principle of minimum potential energy, one can generate the equations for a constant-strain finite element. For each specific time ($t = t_i$), the total potential energy is a function of the nodal displacements $X(x, y, z)$ such that $\pi_p = \pi_p(X)$. Here the total potential energy is given by

$$\pi_p\Big|_{t=t_i} = U + \Omega_p\Big|_{t=t_i} \quad (1)$$

where U and Ω_p represent strain energy and energy of external loading, respectively. The above equation can be rewritten as a finite element integrated form [5]

$$\pi_p\Big|_{t=t_i} = \frac{1}{2}\iiint_V \left[\rho\{d\}^T[N]^T[N]\{\ddot{d}\}dV + \{d\}^T[B]^T[D][B]\{d\}\right]_{t=t_i} dV - \left[\{d\}^T\{P\}\Big|_{t=t_i} + \iint_S \{d\}^T[N_S]^T[T_S]\Big|_{t=t_i} dS\right] \quad (2)$$

where the $\{d\}$ represents the nodal vector, $\{\ddot{d}\}$ represents the nodal acceleration, ρ represents the density, $[B]$ is the strain-displacement matrix, $[D]$ is modulus of elasticity matrix, $[N]$ is the shape function matrix, $\{P\}$ is the external load vector and $[T_S]$ is the traction force matrix. The minimization of total potential energy with respect to each nodal displacement requires that

$$\frac{\partial \pi_p}{\partial \{d\}}\Big|_{t=t_i} = \left(\iiint_V [\rho][N]^T[N]\{\ddot{d}\} + [B]^T[D][B]dV\{d\}\right)_{t=t_i} - \left[\{P\} + \iint_S [N_S]^T[T_S]dS\right]_{t=t_i} = 0 \quad (3)$$

namely,

$$at\ t = t_i,\ \left(\iiint_V [B]^T[D][B]dV\right)\{d\} + \left(\iiint_V \rho[N]^T[N]dV\right)\{\ddot{d}\} = \{P\} + \iint_S [N_S]^T[T_S]dS \quad (4)$$

Finally, solving the linear system shown in Eq.(3) at each specific time, one could obtain the {d} and the global nodal vector could be revealed.

3 RESULTS

3.1 Hollow cylinder

Assuming the phi-29 appearance is a hollow structure. Let a and b denote the inner and outer radii of the cylinder, and p_i and p_o the uniform internal and external pressure, as shown in Fig 2.

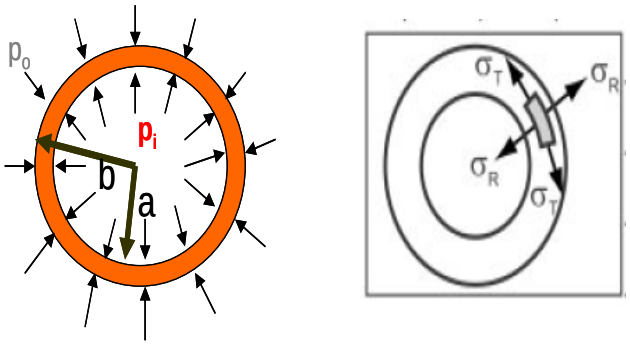


Fig. 2 The phi-29 shape is assumed to be similar to a hollow cylinder structure

By using the stress equation [6]

$$\left(\frac{\partial^2}{\partial r^2} + \frac{1}{r}\frac{\partial}{\partial r} + \frac{1}{r^2}\frac{\partial^2}{\partial \theta^2}\right)\left(\frac{\partial^2 \phi}{\partial r^2} + \frac{1}{r}\frac{\partial \phi}{\partial r} + \frac{1}{r^2}\frac{\partial^2 \phi}{\partial \theta^2}\right) = 0 \quad (5)$$

When the stress function depends on r only, the equation of compatibility becomes

$$\left(\frac{\partial^2}{\partial r^2} + \frac{1}{r}\frac{\partial}{\partial r}\right)\left(\frac{\partial^2 \phi}{\partial r^2} + \frac{1}{r}\frac{\partial \phi}{\partial r}\right) = 0 \quad (6)$$

Expand the equation (6) and rewrite it

$$\frac{1}{r}\frac{d}{dr}\left\{r\frac{d}{dr}\left[\frac{1}{r}\frac{1}{dr}\left(r\frac{d\phi}{dr}\right)\right]\right\} = 0 \quad (7)$$

The solution has four constants of integration, which must be determined from the boundary condition. By substitution, it can be checked that

$$\phi = A\log r + Br^2\log r + Cr^2 + D \quad (8)$$

is the general solution.

And from the boundary condition, we can obtain

NSTI-Nanotech 2007, www.nsti.org, ISBN 1420061828 Vol. 1, 2007

$$A = \frac{a^2 b^2 (p_0 - p_i)}{b^2 - a^2}$$

$$2C = \frac{p_i a^2 - p_0 b^2}{b^3 - a^2}$$

$$B = 0$$

$$D = 0$$

And the stress components are obtained:

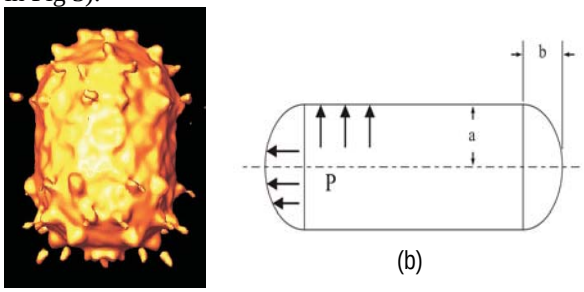
$$\sigma_r = \frac{a^2 b^2 (p_0 - p_i)}{(b^2 - a^2) r^2} + \frac{p_i a^2 - p_0 b^2}{b^2 - a^2}$$

$$\sigma_\theta = -\frac{a^2 b^2 (p_0 - p_i)}{b^2 - a^2} \frac{1}{r^2} + \frac{p_i a^2 - p_0 b^2}{b^2 - a^2} \quad (9)$$

Then, let a=24nm, b=25.6nm, p_o is 0MPa and p_i is 6MPa. The maximum tangential stress is 47.6MPa.

3.2 Cylinder vessel with ellipsoidal heads

In this approach, the shape of phi-29 is assume to be similar to a cylinder vessel with ellipsoidal heads (as shown in Fig 3).



(a) (b)

Fig. 3 (a) A 3D cryo-EM reconstruction of a head of phi-29[4] (b) the phi-29 structure is assumed to be similar to the cylinder vessel with ellipsoidal heads (based on the profile obtained from (a))

The symbol b in Fig 3b represents the height of ellipsoidal head of 10.8nm; the symbol a in Fig3b is a radius of cylinder vessel and equals to 21nm. The thickness of phi-29 surface is 1.6nm. The outer pressure is 0 MPa and inner pressure is 6 Mpa. From the stress equation based on thick-plate assumption [7]

$$\sigma_\theta = \frac{ap}{t}\left[1 - \frac{1}{4}\left(\frac{a^2}{b^2}\right) * (-0.042) + \frac{3\upsilon}{4\sqrt{3(1-\upsilon^2)}} * 0.15\right] \quad (10)$$

Where p represents inner pressure, and ν represents the passion ratio, and t is the thickness of the surface. After the calculation, the maximum tangential stress from the equation (10) is 83.4Mpa

3.3 Numerical method

The profile of phi-29 is measure from scanning-force microscopy (SFM) or cryo-EM studies [8] as shown in Figure4. In the numerical method, the analysis software is applied to simulate the true-structure-like (according to the profile indicated in figure 4) phi-29 capsid model (as shown in Figure 5). The experimental data proposed by Bustamante et al revealed that when the phi-29 package DNA into the capsid, the inner pressure may estimate to be 6 MPa and the stress of phi-29 shell may up to 100~300MPa [3-4]. Therefore, assume that there is a 6MPa inner pressure in the model. From the simulation result, the stress in the capsid shell is 116.6 MPa, as shown in Fig 6. Comparing with the experimental results, good agreements in tendency were achieved.

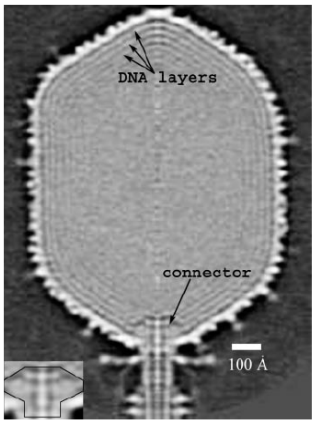


Figure 4. The central section of the reconstructed cryo-EM density map [8]

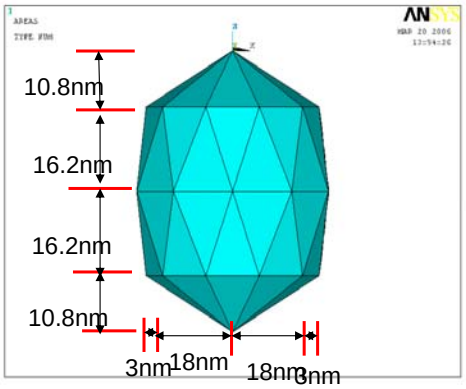


Figure 5. Finite element model of the capsid of Phi-29

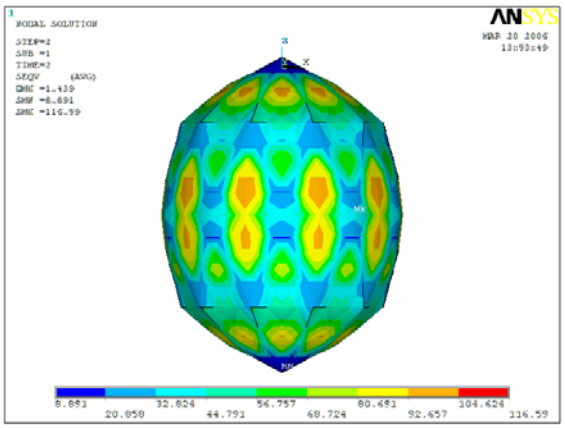


Figure 6. The von Mises stress in the capsid when Phi-29 packages the dsDNA.

4 CONCLUSION

According to the results, the stresses of method 1 and 2 are below the experiment data. The reason is that in these two methods, the surface of the phi-29 are all smooth, hence the roughness of the phage surface cannot be taken into consideration. On the contrary, in method 3, a true-structure-like capsid model was built, therefore it can obtain a more accurate stress in the phi-29 surface. The method proposed here would be used to study the mechanical behavior in DNA packaging and release from viral capsids in the future.

ACKNOWLEDGEMENT

The authors like to thank the National Science Council of Taiwan, R.O.C for financially supporting this research under Contract No. NSC 93-2120-M-007-005.We are grateful to Dr. S. B. Smith and Dr. C. Bustamante. The authors thank Dr. C. Yuan for discussions on numerical simulation technique as well. Moreover, we thank Dr. Y. Cui for the valuable suggestions.

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