

# Stuffing a virus with DNA: Dissecting viral genome packaging

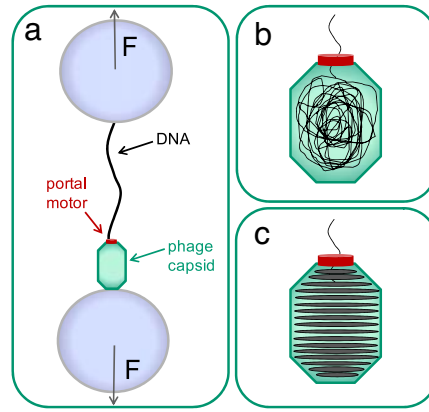
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It is rare that a fully functional biological system presents a simple biophysical problem. The problem of viral DNA packaging comes tantalizingly close: What forces are required to stuff double-stranded DNA of fixed length into a container of fixed volume? Now that biophysicists have developed the capability to measure such forces, theoretical solutions to this problem can be directly tested, as shown in the article by Fuller *et al.* (1) in this issue of PNAS. Because the theories describing these experiments involve relatively simple thermodynamics, the experiments present an excellent model system to test our understanding of DNA biophysics. Surprisingly, the authors find that recent models to describe DNA packaging in bacteriophages do not quantitatively predict the results of experiments in which solution conditions are changed.

Over the past several years, new techniques have been developed to measure the pressure required to hold packaged DNA inside a bacteriophage capsid (2–4) and the forces required to achieve such packaging (1, 5). These two complementary experiments have yielded several theoretical attempts to describe the biophysical mechanisms governing the DNA packaging and ejection processes. Because the biophysical properties of double-stranded DNA under a variety of solution conditions have been studied for many years, it should be possible to make detailed quantitative predictions for DNA packaging as solution conditions are varied.

Theoretical treatments of DNA packaging have typically separated the energetics of the final packaged structure into two components: the energy required to bend the DNA in order for it to fit into the small viral capsid and the interaction energy between portions of the DNA as it becomes tightly packed (6–10). Because DNA is a highly negatively charged polymer, it is expected that one of the primary contributions to the energetic cost of packaging DNA is due to the electrostatic repulsion between different parts of the DNA molecule, and the magnitude of this repulsion will depend strongly on the presence of cations in solution, which condense on DNA, effectively screening the electrostatic repulsion (11) and al-



**Fig. 1.** Measurements of DNA packaging forces. (a) Schematic diagram of the optical tweezers experiment by Fuller *et al.* (1), in which the rate of DNA packaging by bacteriophage  $\phi 29$  is measured as a function of force for high and low filling fractions. (b) Disagreement between theory and experiment may be due to the inability of the DNA to achieve an equilibrium structure during initial packaging. (c) Theoretical calculations of the internal packaging force assume an idealized DNA configuration.

tering DNA hydration (12). Therefore, the reformulated problem becomes: What is the energetic cost to package a negatively charged polymer with known elasticity into a small container when a fixed concentration of cations is available to screen the electrostatic repulsion?

Of course, there are complications to this problem. First, the elasticity of the DNA itself may depend on cation concentration. Second, the interaction energy between portions of the DNA molecule is not purely electrostatic. Additional energetic costs to condensing DNA into a small space arise from hydration changes, in which water is excluded from space occupied by the DNA, and entropic effects due to the change in the number of available molecular configurations for a given condensed structure. Although Odijk (13) recently discussed the contributions of these effects to viral DNA packaging, quantitative first principles calculations of DNA interaction energies are not yet available. Fortunately, the elasticity of DNA (14–16) and the forces required to compact DNA strands close together (12, 17) have both been independently measured at various cation concentra-

tions. Therefore, in principle one can use these measurements to calculate the elastic and interaction energies under given solution conditions and then quantitatively predict the forces required to package DNA in a specific configuration into a given viral capsid. Such a calculation was recently done by Purohit *et al.* (7), for which they used an empirical function for the interaction between compacted DNA segments from osmotic pressure measurements and assumed a standard wormlike chain elasticity for DNA. Those results provide a quantitative prediction for the packaging forces in bacteriophage  $\phi 29$ , which are directly tested in the work by Fuller *et al.* (1).

To investigate the effects of simple changes in counterion valence and concentration on DNA packaging and portal motor function, Fuller *et al.* (1) measured the rate of DNA packaging by single bacteriophage  $\phi 29$  portal motors under a fixed applied force by using an optical tweezers instrument (Fig. 1a). Surprisingly, they found that the packaging rate did not directly correlate with the fractional DNA charge screened under specific ionic conditions. Therefore, a simple model in which packing is inhibited by electrostatic repulsion does not hold, because additional screening of these interactions does not necessarily result in faster packaging, but changes in ionic conditions do strongly affect the packaging rate. They also found that packaging was inhibited in the absence of magnesium, suggesting that the portal motor requires magnesium as a cofactor.

The fact that both the amount of DNA packaged and the packaging velocity did not vary consistently with cationic screening suggested that the cations alter motor function. By obtaining constant force DNA packaging rates at both low and high capsid filling and factoring out the changes in motor function, the authors separated the effects of solution conditions on the properties of the motor from the effects due to DNA confinement. They then calculated the relative packaging rate for a given

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fraction of genome packaged, and, by determining the force at which that packaging rate occurred, obtained the internal force as a function of the percentage of genome packaged. This internal force can be compared directly with theoretical calculations.

The calculated internal force as a function of genome packaged showed the expected trend that the internal force decreased at higher ionic strength and for counterions of higher valence (1). However, the model of Purohit *et al.* (7) provides specific quantitative predictions for the expected internal force, and, surprisingly, the observed internal forces are much higher than those predicted by the model. The model by Odijk (13) also predicts a lower force than that observed in the packaging experiments. There are several possible reasons for this significant disagreement between experiment and theory.

Although the model under consideration involves relatively straightforward thermodynamics, several assumptions are made that may be reconsidered. First, it is assumed that all of the work done to package the DNA is stored as potential energy in the packaged DNA configuration. However, if friction played a significant role in the packaging process itself, this would explain a higher observed packaging force, although several authors have concluded that friction is likely negligible during packaging (1, 7, 13). To fully resolve this question, experiments are needed to independently test the role of friction in viral packaging. The assumption of thermodynamic equilibrium also may not hold, because the molecular configura-

tion achieved during packaging might not be the same as that achieved in the final equilibrium state (Fig. 1*b*). Finally, the equilibrium coaxial spool structure assumed inside the virus may not be correct, although x-ray diffraction studies suggest an ordered structure (Fig. 1*c*) (18). In addition, errors in measurements of capsid volume will significantly affect the calculated internal force (19).

## The elasticity of DNA may depend on cation concentration.

Higher-resolution structures of DNA packaged inside the bacteriophage are needed to answer these questions.

Along with questions regarding the assumptions that go into a thermodynamic model for DNA packaging, there also may be questions about the validity of the parameters chosen to describe the packaging energy. For example, the calculations of Purohit *et al.* (7, 8) assume that the DNA persistence length, which is roughly the length scale over which thermal fluctuations cause the molecule to bend, is 50 nm, which holds for high monovalent salt concentrations (14–16). In contrast, the persistence length is reduced by more than a factor of two (indicating a much more flexible DNA molecule) (14, 15) in the presence of divalent and trivalent counterions, and eventually the DNA will collapse in the presence of high concentrations of multivalent cations (20). Because the diame-

ter of a virus capsid is typically of the order of 50 nm, small changes in DNA flexibility may have a significant effect on the energetics of DNA packaging. However, this correction should lower the predicted internal force, so it cannot explain the current disagreement between theory and experiment. Another primary input to the model is the interaction energy measured in osmotic pressure experiments. Although these data are available under a variety of solution conditions, the exact same solution conditions are not used in Fuller *et al.* (ref. 1; see also refs. 12 and 17).

The results from Fuller *et al.* (1) present significant new information on a biologically important model system that will allow further testing of biophysical theories that describe DNA bending and DNA interaction. Although the results clearly call for the reevaluation of theories that describe DNA compaction and DNA interaction forces, new experiments are needed to provide more complete quantitative input to the biophysical models being developed. Additional work should include independent measurements of DNA interaction energies and DNA flexibility under different solution conditions, high-resolution structures of DNA inside the viral capsid, and experiments to test the possibility of frictional forces or nonequilibrium dynamics during the packaging process. Finally, further experiments measuring DNA ejection forces as a function of solution conditions as well as DNA packaging forces will shed significant new light on the important biophysical problem of DNA packaging.

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