

Grabbing the Cat by the Tail

By Andrew E. Pelling and Robin L. Hayes

Biological processes are governed not only by chemical reactions but also by the nanomechanical properties of the biomolecules themselves. Professor Carlos Bustamante of the University of California, Berkeley, has been using optical tweezers to examine the nanomechanics of a wide spectrum of single biomolecules, including DNA, RNA, and proteins.

Viruses provide an excellent example of how mechanical properties become important at nanoscopic scales. A virus must pack a genome into its capsid in order to reproduce and infect other cells. The capsid is a hollow protein shell with an opening surrounded by a portal complex that hydrolyzes ATP to package DNA (Box 1). The bacteriophage $\phi 29$ (a virus that infects bacteria) will typically pack a 20 kilobase pair genome ($\sim 6.5 \mu\text{m}$ long) into a capsid having a volume of $5.6 \times 10^{-3} \mu\text{m}^3$. This situation

means that the DNA must be compacted by a factor of at least 6000. Bustamante's group attempts to determine how the virus overcomes the entropic, electrostatic, and mechanical forces opposing such a large compression.

Bustamante and co-workers have recently examined the dynamics and mechanics of the packaging process of the $\phi 29$ bacteriophage. They tethered a DNA-capsid complex between two microspheres, one held in an optical trap and the other held with a micropipette (Box 2).

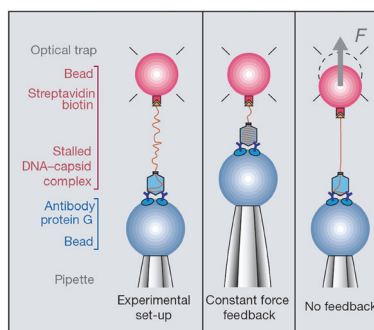
Two sets of experiments were performed. The first experiment determined the DNA packaging dynamics by maintaining a constant tension on the DNA tether. They found that it takes ca. 5.5 min. to package an entire genome at an average rate of ca. 20 base pairs per second. The motor is not perfect because pauses in packaging occur about three times per micron of DNA and last an average of 4 s. The motor can also lose its grip on the DNA, but can quickly recapture it after only 44 base pairs, on average, have slipped out.

The second experiment allowed the tension in the tether to increase, enabling Bustamante to determine the work and maximum pulling force of the motor. This

motor is the most powerful motor known to date, with an average pulling force of 57 pN, ten times greater than myosin, the motor protein in skeletal muscle. A rough estimate yields an internal pressure of about 60 atm inside the capsid. In comparison, the internal pressure within a bottle of champagne is only 5–6 atm. Bustamante speculated that the high pressures and packing density are used to drive the injection of the viral genome into the next host cell.

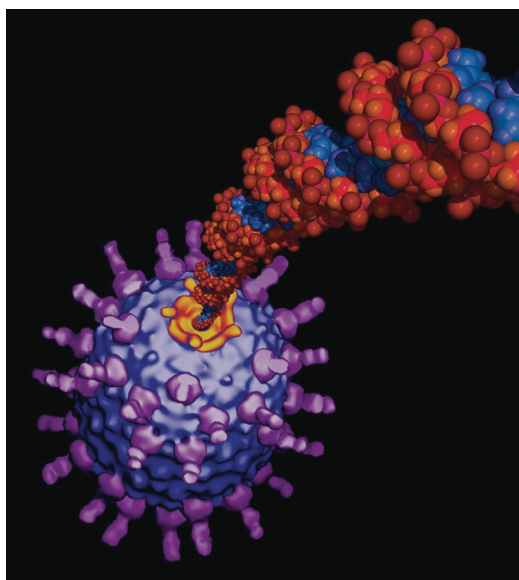
While the molecular mechanism is not known, recent results suggest that the motor is similar to that of a six-cylinder engine. The complex comprises six ATPase proteins, fueled by the hydrolysis of ATP. Preliminary results suggest that two base pairs of DNA are translated for each ATP hydrolyzed. Translation is achieved only if each “cylinder” fires in sequence.

This type of single molecule experiment has yielded the highest-resolution dynamics and mechanics of viral DNA packaging to date.

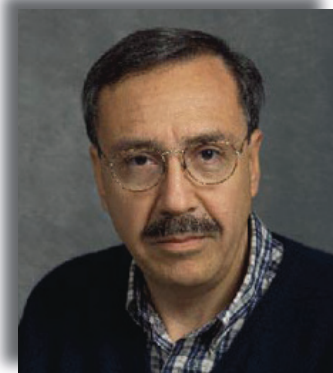


Box 2. Experimental setup used by Bustamante and co-workers. Two different experiments were performed with a DNA-capsid complex tethered between two microspheres.

Bustamante hopes to combine this work with further experiments to finally determine the exact mechanism of the molecular motor.



Box 1. Computer-generated image of a $\phi 29$ bacteriophage in the process of packaging its viral genome.



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