Supporting Information

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SI Materials and Methods

DNA Packaging Components. The empty expanded proheads and gp17 were purified according to the procedures described (1). Different batches of proheads or empty phage heads (2) or gp17 (full-length gp17 with a hexa-histidine tag at the N terminus) showed the same behavior in response to varying the ATP concentration and other variables tested.

Defined Bulk DNA Packaging Assays. Bulk packaging assays were conducted as described (1). Empty purified heads (10^{10}), 1.5 μM purified gp17 monomers, and 600 ng of λ DNA were incubated at room temperature (RT) for 30 min in the presence of 1 mM ATP, in a 20-μL reaction volume containing 30 mM Tris·HCl at pH 7.6, 5 mM $MgCl_2$, and 80 mM NaCl. After this incubation, 2 μL of 7 mg/mL DNase I was added to terminate the reaction and digest the unpackaged DNA by incubating this mixture for 30 min at 37 °C. The packaged, DNase I protected DNA was released from the capsid by the addition of 3 μL of Proteinase K mixture (1:1:1 mixture of Proteinase K, 10% SDS, and 500 mM EDTA at pH 8) for 30 min at 67 °C. The sample was then loaded on a 0.8% agarose gel and electrophoresed for 1–2 h at 100 V, and the amount of DNA packaged was quantified in comparison with a known amount of DNA included in the same gel.

Single-Molecule DNA Packaging Assays by Using High-Resolution Dual Optical Tweezers. Stalled head–gp17 packaging complexes were formed by incubating 10^{10} purified proheads, 1.5 μM purified gp17 monomers, 500 ng of 120-bp dsDNA, and 1 mM of the nonhydrolyzable analog ATP-γS in a buffer containing 30 mM Tris·HCl at pH 7.6, 5 mM $MgCl_2$, and 80 mM NaCl, as described (2). Proheads were purified from the 17*am*18*am*rII mutant and contain the dodecameric portal at the special fivefold vertex of the icosahedral head. The reaction mixture lacked the small terminase, gp16, similarly to previously reported single-molecule experiments (3). The complexes were immobilized on 0.79-μm T4-antibody coated polystyrene microspheres by incubating the reaction mixture with the beads for 30 min at RT. The PCR-amplified 10-kb λ DNA molecules biotinylated at one end were attached to 0.86-μm streptavidin-coated polystyrene microspheres.

The high resolution dual-trap optical tweezers were set up and calibrated as described (4). The microspheres were captured in separate traps and brought into near contact for ∼1 s and then separated—a procedure referred to as "fishing"—in the presence of packaging buffer (30 mM Tris·HCl at pH 7.6, 5 mM $MgCl_2$, and 80 mM NaCl) containing 1 mM ATP, and an oxygen scavenging system (100 mg/mL glucose oxidase, 20 mg/mL catalase, and 4 mg/mL glucose) to prevent damage by the reactive singlet oxygen species (5). This fishing procedure was repeated a few times or until packaging was initiated (Fig. 1*C*), as evident by a rise in force when the motor captured the DNA.

Data Analysis. Data were collected at 100 Hz in force-feedback mode, where the distance between the traps was adjusted to maintain a constant external load of 5 pN. Packaging was measured as a decrease in tether length as a function of time. Data analysis was done by using custom programs written in Matlab (Mathworks). The contour length of DNA was calculated from the measured force and extension between the microspheres by using the worm-like chain model assuming a persistence length of 53 nm, a stretch modulus of 1,200 pN, and a distance per base pair of 0.34 nm as described in ref. 6.

The instantaneous velocity of DNA packaging was determined from a linear fit of the packaged DNA contour length vs. time over a sliding window of 0.1 s (10 data points, sliding the window one data point at a time). For each packaging trace, a histogram of the velocity from all 0.1-s windows was plotted and fitted to a double Gaussian distribution, with the two peaks corresponding to packaging and pause velocities. The mean packaging (unpackaging) velocity of a trace was calculated from the Gaussian fitting of the packaging (pause) peak. Pauses were scored as any region of the trace having a velocity either 3 SD less than the packaging velocity peak, or <50 bp/s (whichever was less), for a duration longer than 0.1 s.

Kinetic Simulations. To test the validity of our four-state model, we performed stochastic simulations by using custom Matlab programs. The simulations followed closely the master equation formalism used in ref. 7. Using as input the number of states, transition rates connecting these states (as depicted in Fig. 6), and step sizes corresponding to packaging and unpackaging, the simulations generated time courses of the states occupied by the motor and the DNA length packaged as a function of time. We assumed a fixed step size of +2 bp for packaging, and –2 bp for unpackaging. To model ATP or force-dependent steps, the relevant kinetic rate constants were multiplied by the concentration [ATP] or by an Arrhenius-like factor $\exp(-F\Delta x/k_BT)$, respectively. (Here T is the temperature, k_B is Boltzmann constant, and Δx represents the distance to the transition state along the mechanical reaction coordinate). We ran simulations at each ATP concentration used in the experiments, generating a population of packaging traces (Fig. S4). From these simulations, we determined the packaging and pause characteristics as a function of ATP and force: mean packaging velocity including and excluding pauses (Fig. 1*E*), mean DNA length packaged between pauses (Fig. 2*A*), mean pause duration (Fig. 2*B*), mean unpackaged DNA length (Fig. 2*C*), and mean unpackaging velocity (Fig. 3*B*). These quantities were compared with the experimental results, and the transition rates were adjusted until the best global match was achieved.

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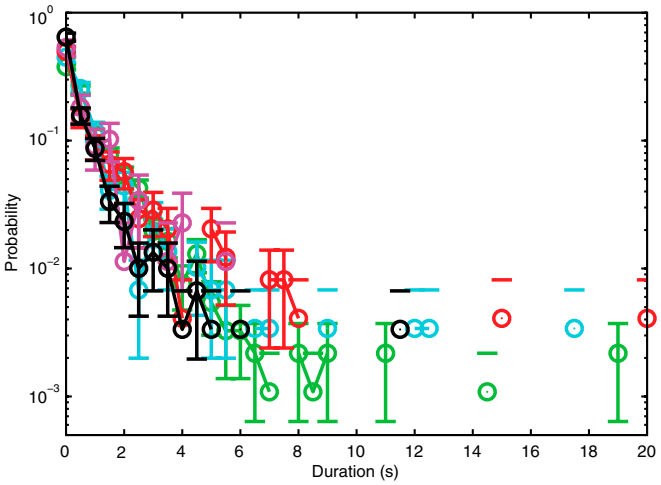

Fig. S1. Pause durations are uniform and exponentially distributed. Distributions of pause durations over various ATP concentrations—25 μM (green), 50 μM (cyan), 100 μM (red), 250 μM and 500 μM (averaged together, magenta), and 1,000 μM and 2,000 μM (averaged together, black)—indicate that pauses span similar durations across ATP concentrations and are exponentially distributed.

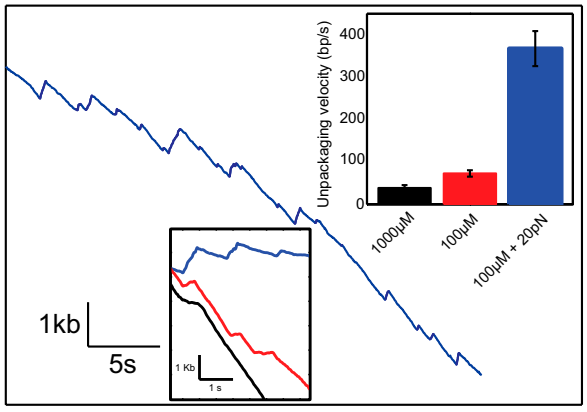

Fig. S2. Higher external loads cause faster unpackaging. Representative packaging trace at a tension of 20 pN and 100 μM ATP. *Lower Inset* compares representative pauses at 20 pN and 100 μM ATP (blue), 5 pN and 100 μM ATP (red), and 5 pN and 1,000 μM ATP (black). *Upper Inset* compares unpackaging velocities under the above set of conditions.

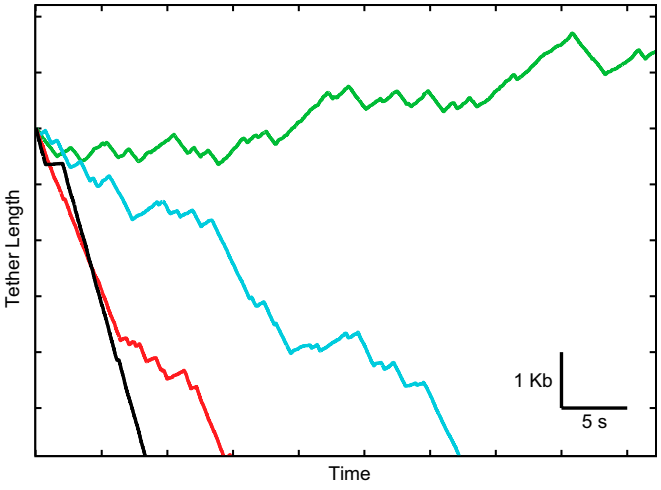

Fig. S3. Simulated traces at different ATP concentrations. Representative simulated traces at different ATP concentrations: 25 μM (green), 50 μM (cyan), 100 μM (red), and 1,000 μM (black) obtained from stochastic simulations (*SI Materials and Methods*). Simulated traces qualitatively resemble experimentally derived traces (Fig. 1*D*).