Supplementary Information to "Fast, DNA-sequence Independent Translocation by FtsK in a Single Molecule Experiment"

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Off-time distributions

The distribution of times between downward translocation events (off-times) is clearly not a simple exponential function, as can be seen in Suppl. Fig. 1A, but rather is well fit by a double exponential function. The minimal chemical reaction pathway that can reproduce this kinetic behaviour, shown in Suppl. Fig. 1B, separates the free, unbound protein from the active, detectable state by a stable intermediate. In our view (see the main text discussion), the intermediate state corresponds to a single motor bound to the DNA, while the active state corresponds to two bound motors and loop formation. Both preliminary calculation and simulation of this pathway, where the rates k_i represent probabilities of transition (Keller and Bustamante, 2000), indicate the existence of two types of off-times, and thus a double exponential distribution. This can be understood as follows: An off-time begins when one motor of an active complex unbinds (through the path with rate k_4), leaving the second motor bound and the protein in the intermediate state The protein can re-enter the active state either directly (k_2) through immediate rebinding of another motor, or indirectly through complete unbinding (k_3) followed by a diffusion-limited wait for the same, or a different, complex to bind with one motor (k_1), then with a second (k_2). The direct transition will occur quite quickly, since binding of a second motor is accelerated by the complex being localized to the DNA by the bound first motor. On the other hand, the indirect transition will be relatively slow, since it involves multiple steps, including at least one passage through the diffusion-limited step k_1 (multiple passages through k_1 can occur if the protein oscillates between the free and intermediate states before entering the active state). In Suppl. Fig. 1C and 1D, we show representative data illustrating the two types of off-times: 1C shows long off-times between areas of protein activity, while 1D shows fast rebinding events corresponding to the short off-times. Further experiments are needed to quantitatively estimate the rates in this pathway; however, note that neither of the two rates extracted from fitting double exponentials to the measure distributions (as in Suppl. Fig. 1A) are directly equivalent to any of the rates in the chemical pathway, but rather are functions involving several of the pathway rates.

Event phenomenology

The majority of observed events are similar to the two plotted in Fig. 2A in the main text, i.e. a constant velocity decrease of the bead height, followed by either a fast (through protein unbinding) or slow (through direction reversal) recovery back to the initial height. Occasionally, we observe events such as those shown in Suppl Figs. 2A and 2B, wherein the recovery shows mixed behavior: either slow then fast (2A), or fast then slow (2B). We ascribe 'fast-slow' recoveries to unbinding of one of the two motors bound to the DNA, followed by a short wait (allowing the bead to quickly rise) before binding of an oppositely directed motor and a controlled increase of the bead height. 'Slow-fast' recoveries are simply explained as direction reversal (giving the slow recovery segment), followed by unbinding of one of the two motors (giving the fast segment).

We also observe an events with a pause (i.e. a zero velocity segment), as shown in Suppl. Fig. 2C. We have observed that pauses can interrupt either translocation direction, and also can occur as an intermediary between a descending and ascending (reversed) segment. However, pauses do not occur regularly, and in fact appear to occur in a given measurement only after a period of time with an excessively high protein activity, i.e. a situation where no long off-times occur, and presumably many protein complexes are active. We thus conclude that pauses are not caused solely by the protein-DNA interaction, but instead involve protein-protein interactions. Supporting this, we observe that, while pauses sometimes reoccur at the same position on the DNA within a single measurement, pause positions do not occur at the same position in different measurements that utilize the same DNA substrate, thus ruling out sequence-based pause mechanisms.

The final type of event we observe is one with twice the normal translocation velocity, as compared to other events in the same data set. Such an event is shown in Suppl. Fig. 2D. These events are consistent with our model for the activity of a protein complex outlined in Fig. 6 of the main text: a single multi-motor complex is usually localized to the extremity by a single motor before forming loops (see main text discussion). However, it is possible for a second motor to bind before the first motor stalls- this would create a loop that is extruded by two motors simultaneously, thus causing a doubled velocity of decrease of the bead height. Alternatively, one could imagine two complexes (one at each extremity) causing a double velocity event. We find this highly improbable, since both would have to start and stop simultaneously to explain an event such as the one plotted in Suppl. Fig. 2D.

Two-state model for protein-induced DNA distortion

We can model binding of a single $Ftsk_{50C}$ motor to a DNA molecule under tension as a two state system (Evans and Ritchie, 1997; Rief et al., 1998), where one state represents the unbound motor, the second represents the bound motor/DNA complex, and the two states are separated by a higher-energy transition state, as sketched in Suppl. Fig. 3. Unbinding of the motor will occur at a rate $k_{u,0} \sim \exp(-\Delta G_b/kT)$, where ΔG_b is the zero-force difference in free energy between the transition and bound states, and kT is the thermal energy. If the transition state and bound state involve different end-to-end distances of the DNA, then application of a force F will change the energy difference by an amount $\Delta\Delta G_b(F) = -Fx_b$ (Evans and Ritchie, 1997; Rief et al., 1998), where x_b is the difference in DNA extension between the transition and bound states. The unbinding rate under an applied force will then be $k_u(F) = k_{u,0} \times \exp(Fx_b/kT)$, thus predicting an exponential dependence of the mean on-time (the inverse of the unbinding rate) on the force, as we have measured. Fitting the force dependence of the on-times gives $x_b = 0.36 +/ 0.03$ nm.

The above argument can be repeated for the effect on the binding rate of a length difference x_u between the unbound and transition states; this would manifest in the measured off-times. In our experiment, however, this situation is considerably more complex than that of the unbinding rate. The binding rate needed to estimate x_u is not equivalent to the rate k_2 in the chemical pathway diagrammed in Suppl. Fig. 1B. k_2 involves both the time it takes the second motor (of a complex already tethered to the DNA by one motor) to perform a constrained diffusion until loosely contacting the DNA, and the rate of converting that loose contact into an actual binding contact; the binding rate from the two-state model corresponds only to the latter process. Even if we were currently able to estimate k_2 at various forces, the relation of that parameter to x_u would not be clear. Thus, x_u remains unknown, and $x_b = 0.36 +/ 0.03$ nm represents a lower bound of the full binding-induced change in length $x_b + x_u$.

References

Evans E, Ritchie K (1997) Dynamic strength of molecular adhesion bonds. *Biophys J* **72**: 1541-1555

Keller D, Bustamante C (2000) The mechanochemistry of molecular motors. *Biophys J* **78**: 541-556

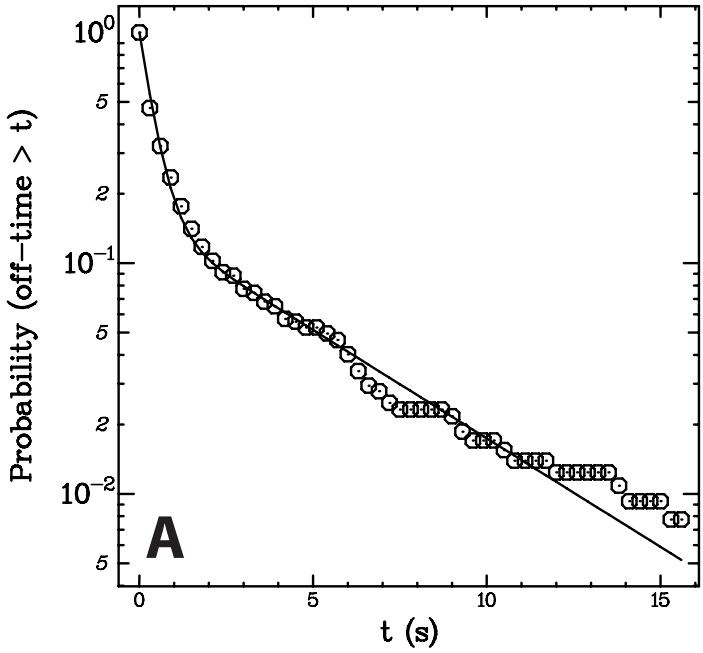
Rief M, Fernandez JM, Gaub HE (1998) Elastically coupled two-level systems as a model for biopolymer extensibility. *Physical Review Letters* **81**: 4764-4767

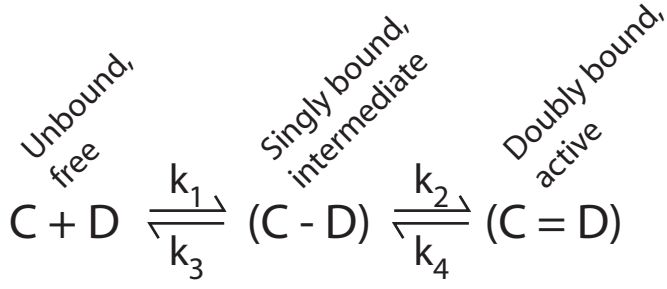
Supplementary Figures

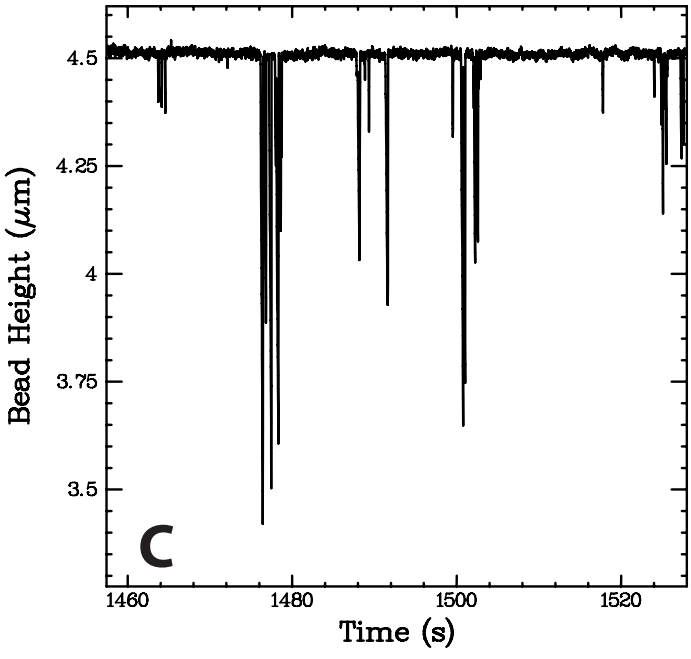
Suppl. Fig. 1 Analysis of the off-times. **A.** The probability of measuring an off-time greater than a time *t*. The circles are data points extracted from a measurement utilizing 5 mM ATP and $F =$ 18 pN. The solid line is a fit of those data points by the double exponential function $A \times \exp(-k_a t) + (1-A) \times \exp(-k_b t)$, where the best fit values were $A = 0.85$ +/- 0.01, $k_a = 2.5$ +/- 0.1 s^{-1}, and $k_b =$ 0.22 +/- 0.01 s^{-1}. **B.** The minimal chemical pathway that can reproduce a double exponential distribution of the off-times. *C* represents the $Ftsk_{50C}$ protein complex, *D* the DNA, *C-D* the complex with one motor bound to the DNA, and *C=D* the complex with two motors bound to the DNA. The state *C=D* is the only detectable state in our measurement, as only when two motors are bound will the bead height change (see main text discussion). Preliminary calculation and simulation of trajectories of single complexes among the different states, where the rates k_i represent probabilities of transition, produce double exponential distributions of off times. Note, however, that the rates k_i are not simply related to the rates k_a and k_b that can be extracted from fits such as the one in **A**. **C, D.** Measurements of bead height vs. time that illustrate either long (multisecond) off-times (**C**), or short (sub-second) off-times (**D**). Data are from the same measurement as **A**.

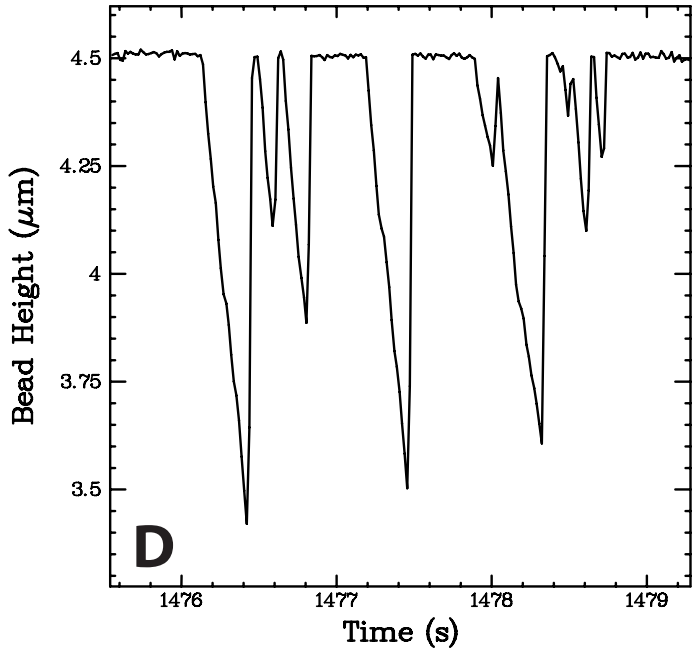
Suppl. Fig. 2 Event phenomenology. **A.** An event exhibiting a mixed-speed recovery: first slow, then fast. The velocity of the various segments is plotted. In this event, the protein brought the bead to the capillary surface; after a short delay, it reversed direction (thus the slow recovery segment) but fell off before reaching full height (thus the fast recovery segment). **B.** Similar to **A**, but with the opposite order of recovery: first fast, then slow. Here, after lowering the bead height, one motor unbound, allowing a fast recovery segment. Before full bead height was reached, a second oppositely directed motor bound to the DNA, causing a slow recovery segment. **C.** An event showing a distinct pause (marked with an arrow) in the midst of a translocation event. Such pauses are rare, and appear to occur due to protein-protein interactions, as described in the main text of the supplementary section. **D.** A double-velocity event (green line) compared to an event with the standard velocity (red line) for the given conditions (10.7 pN, 5 mM ATP). The two events occurred a few seconds apart in the same data set.

Suppl. Fig. 3 Free energy diagram of a two state system relating binding of a single $Ftsk_{50C}$ motor to a change of length of the bound DNA. See supplementary text, and references therein, for details.

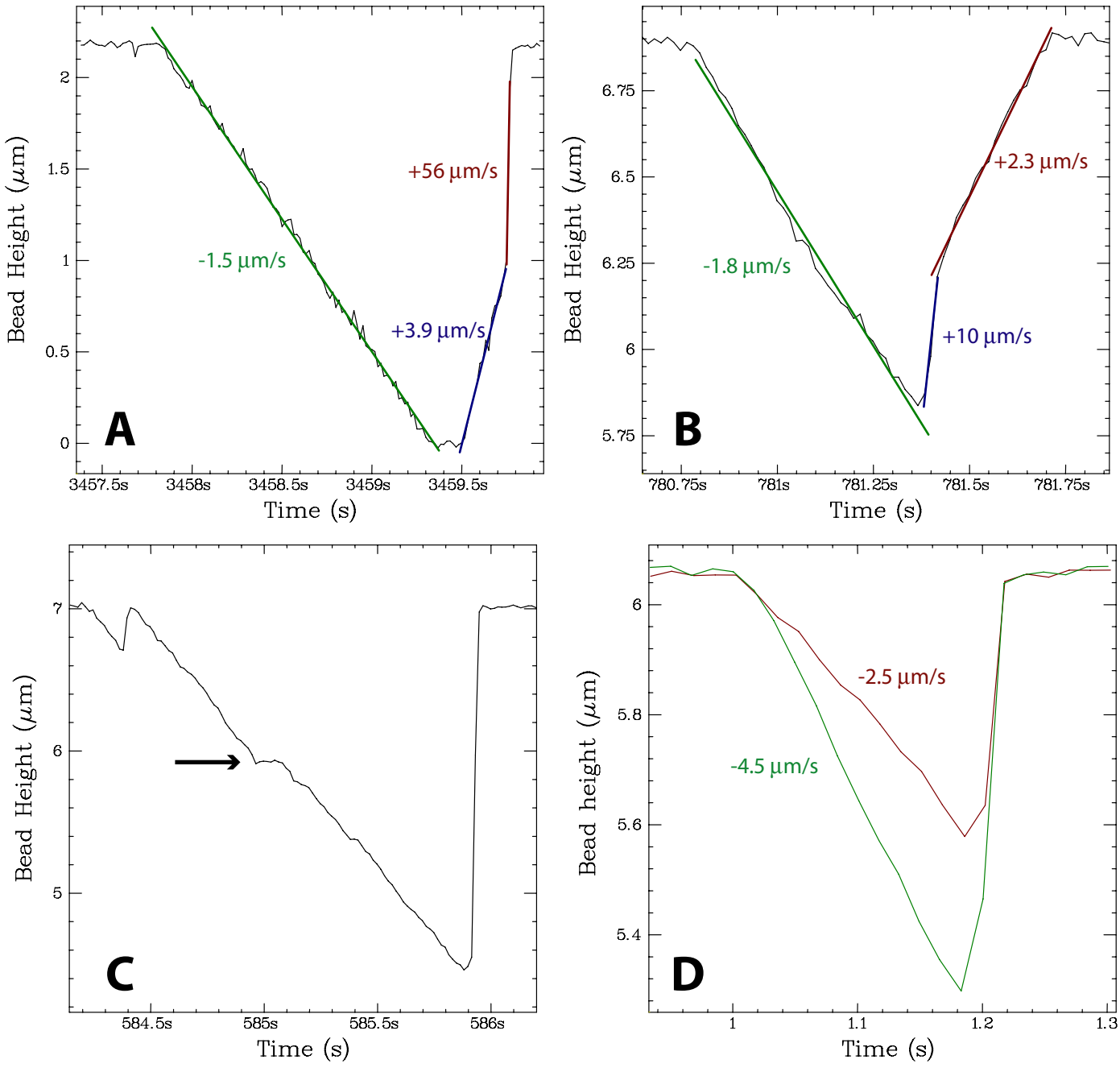




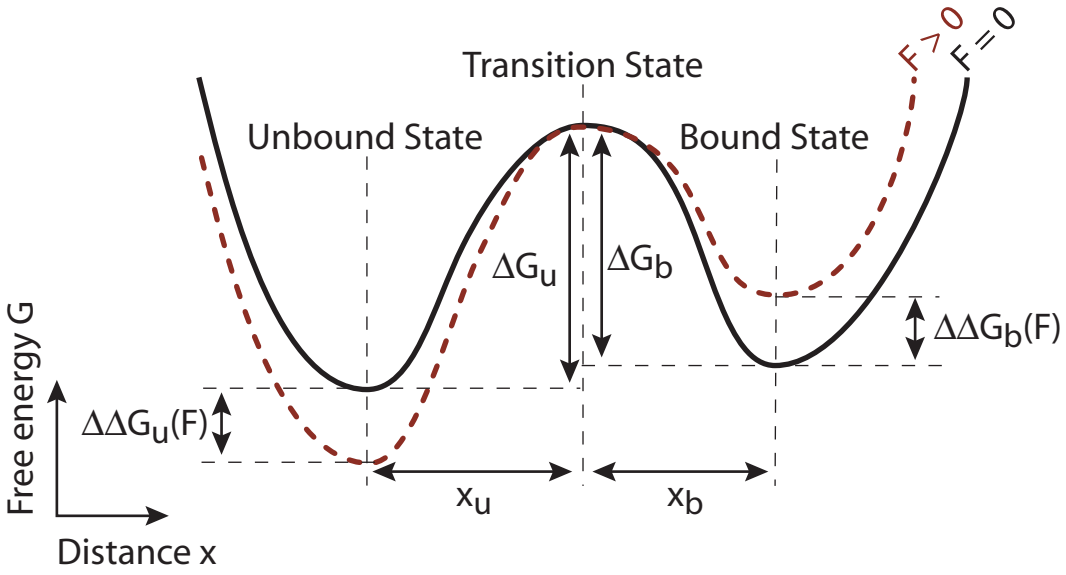




Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3