Supporting Information for:

Dynamics of the Translocation Step Measured in Individual DNA Polymerase Complexes

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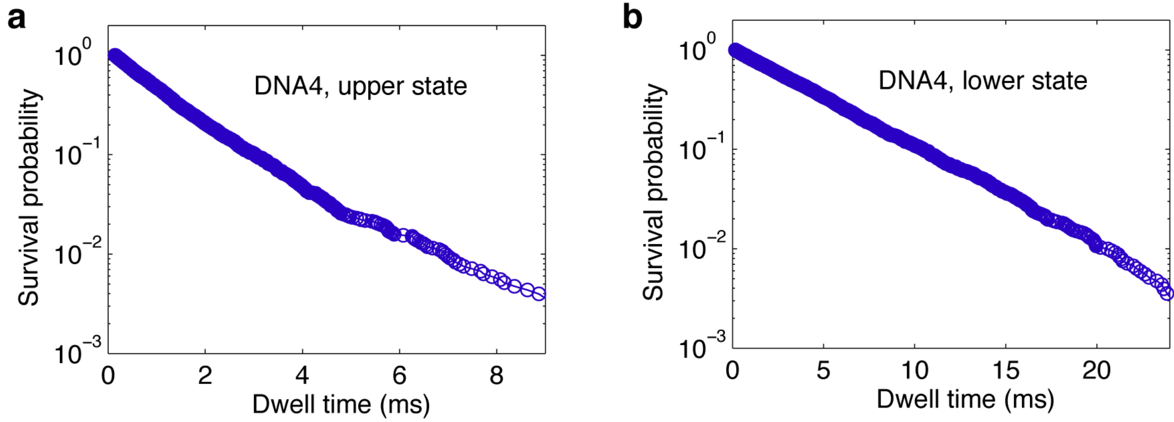

Figure S1. **Two discrete states along the direction of translocation in phi29 DNAP-DNA complexes**. Survival probability plots for the dwell times of phi29 DNAP-DNA4 complexes in (a) the upper amplitude state, and (b) the lower amplitude state. Complexes were captured at 180 mV. The sequence of the DNA4 substrate is shown in Figure 3a, i.

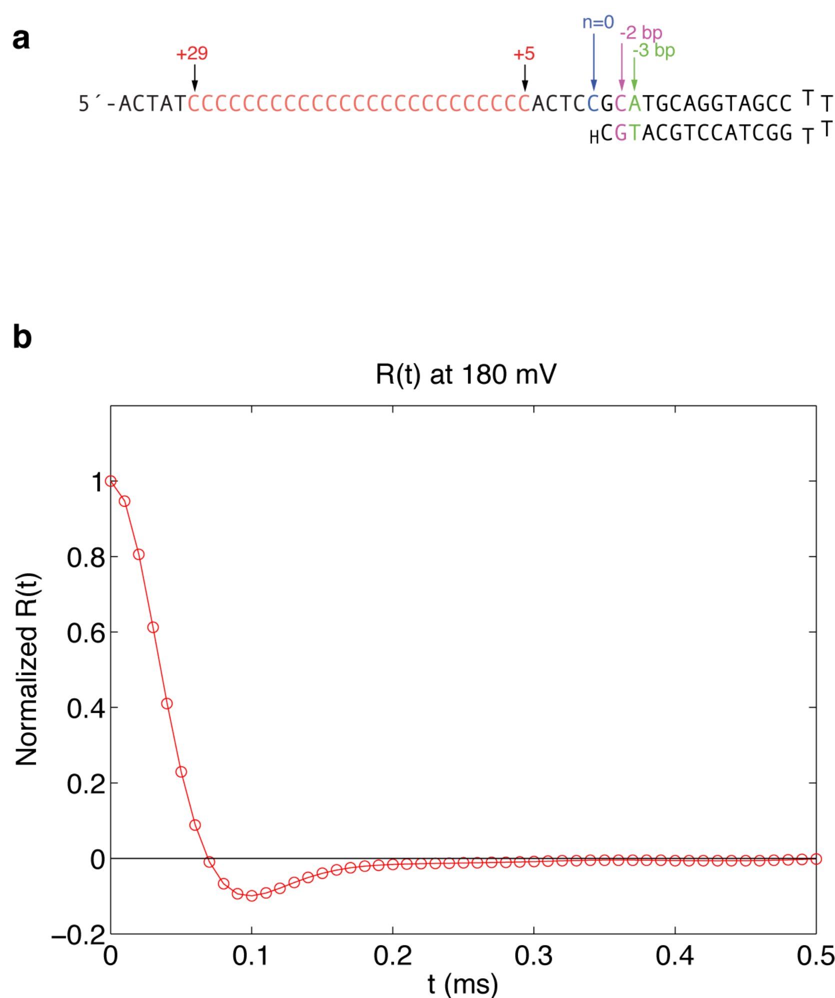

Figure S2. **Effect of colored noise and filtering at 5 kHz on the auto-correlation function.** (a) DNA substrate bearing a homopolymeric template strand. This substrate lacks the abasic reporter group, and features poly(dCMP) spanning positions +5 to +29 of the template strand (the entire length of the segment suspended through the nanopore). The active-site proximal DNA sequences (the entire duplex region and the template strand from positions n=0 to +4) are identical to those in the DNA1 substrate shown in Figure 1c. (b) Normalized auto-correlation function $R(t)$ measured at 180 mV for a complex formed with the DNA substrate shown in panel (a). Normalized auto-correlation function is defined as $R(t) = E[(Y(t_0)\text{-}E[Y])(Y(t_0+t)\text{-}E[Y])]/\text{var}[Y]$. Since the segment of template strand that is suspended through the nanopore consists of poly(dCMP), the measured amplitude fluctuates around a single level. If there is no filtering and the noise is white, the normalized auto-correlation function is $R(t) = 0$ for $t > 0$. Thus, the deviation from $R(t) = 0$ is the effect of colored noise and filtering. Panel (b) demonstrates that the effect of colored noise and filtering on $R(t)$ is limited to $t < 0.2$ ms (most of the effect is concentrated in the region $t < 0.1$ ms).

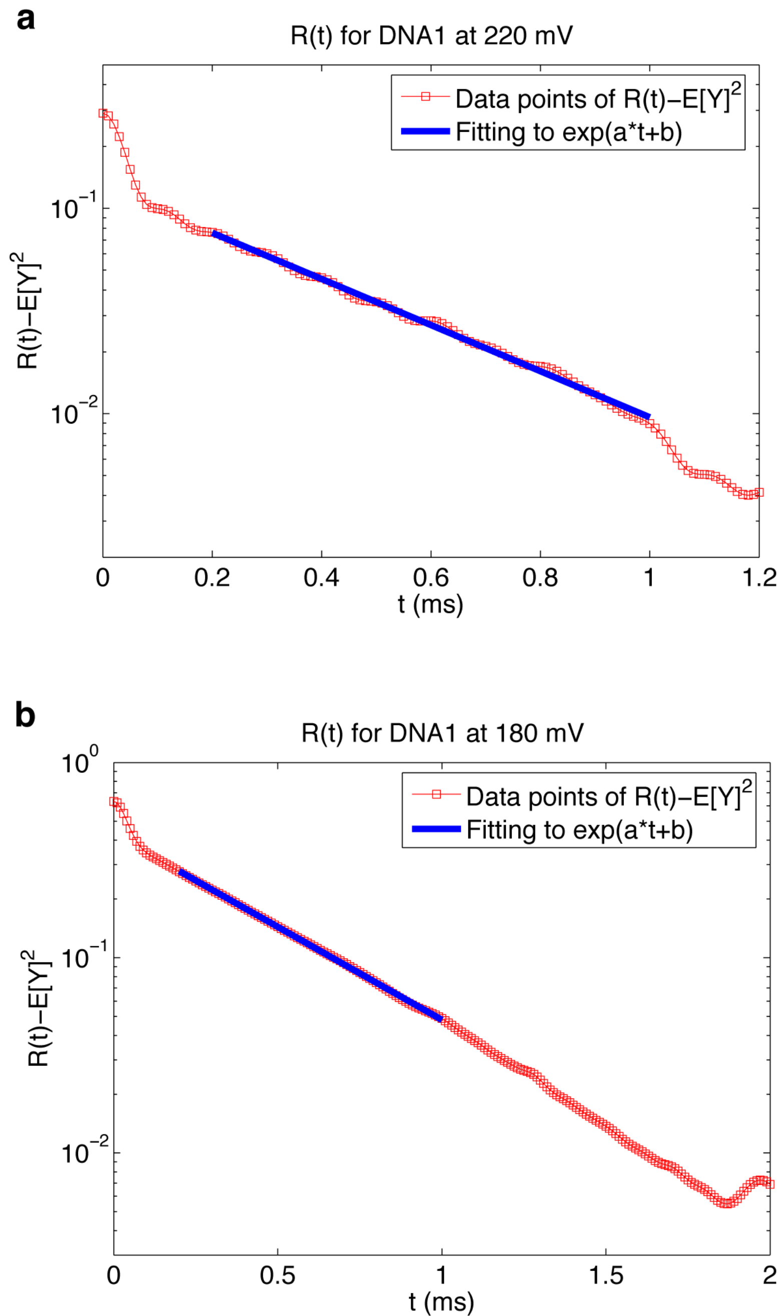

Figure S3. **Fitting an exponential function to the measured auto-correlation.** To exclude the effect of colored noise and filtering on the auto-correlation function, we only use $R(t)$ for $t >$ 0.2ms. Since the auto-correlation decays exponentially, the relative error in the measured auto-correlation increases with t. Eventually, for large t, the measured auto-correlation will have no accuracy. (a) The auto-correlation measured at 220mV. This is the case with the largest transition rate ($r_1 + r_2 = 2722 \pm 71\ s^{-1}$). The measured auto-correlation shows an accurate exponential

decay for 0.2ms < t < 1 ms before losing accuracy. The exponential fitting is based on the measured auto-correlation for 0.2ms < t < 1 ms. (b) The auto-correlation measured at 180mV. At 180mV the transition rate is smaller and, as expected, the measured auto-correlation resolves the slower exponential decay accurately over a longer time interval than [0.2ms, 1.0ms]. For consistency, all exponential fittings to measured auto-correlations are performed in the interval [0.2ms, 1.0ms].

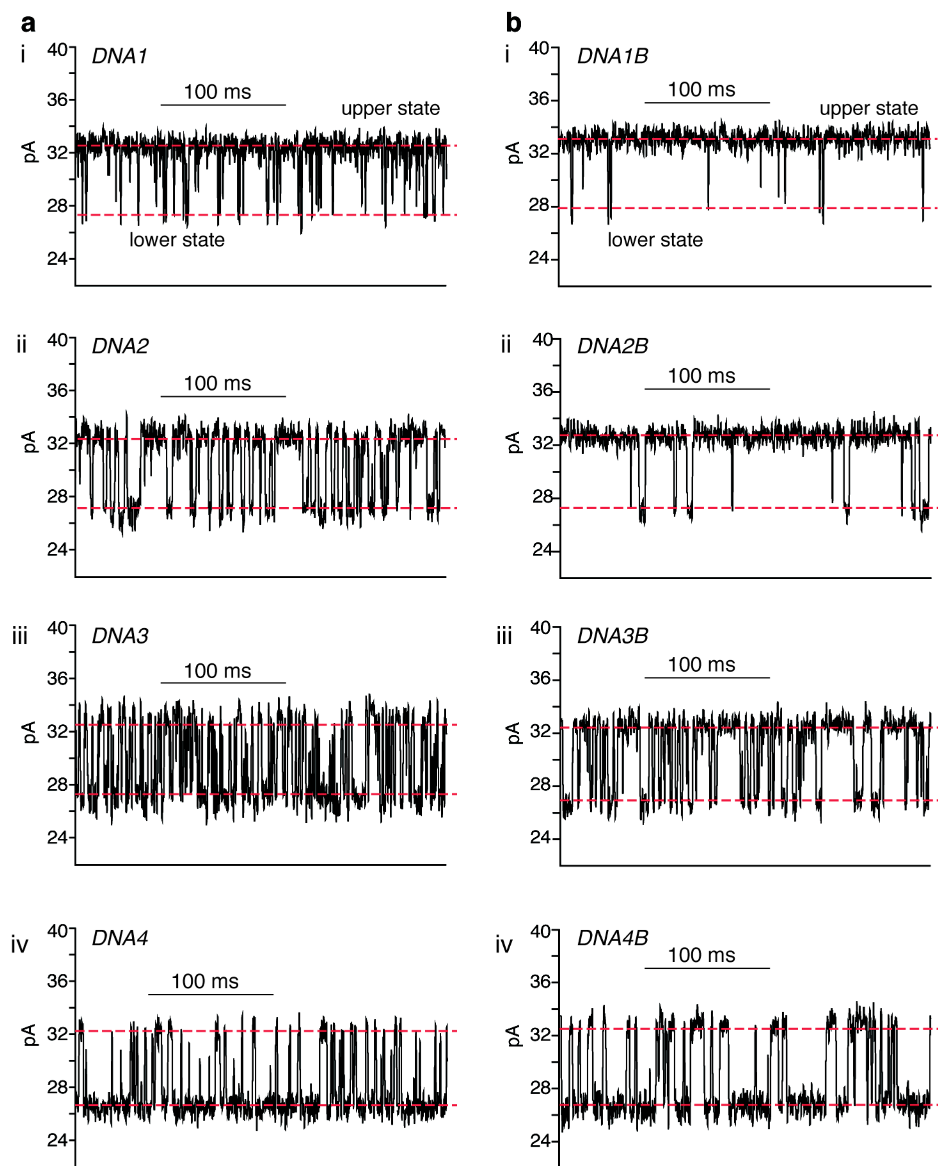

Figure S4. **Ionic current traces for complexes formed between phi29 DNAP and DNAs 1-4 and 1B-4B**. Representative ionic current amplitude traces for complexes formed between phi29 DNAP and (a, i) DNA1; (a, ii) DNA2; (a, iii) DNA3; (a, iv) DNA4; (b, i) DNA1B; (b, ii) DNA2B; (b, iii) DNA3; and (b, iv) DNA4. Complexes were captured at 180 mV applied potential.

Table S1. Number of phi29 DNAP-DNA captured complexes used to determine the translocation rates.

DNA substrate[a]:	DNA1	DNA2	DNA3	DNA4
Voltage (mV)	Number of captured complexes [b]			
140	22	46	21	16
150	19	50	12	13
160	24	49	21	17
170	15	42	35	14
180	90	41	14	63
190	36	44	28	18
200	20	54	26	22
210	19	44	23	44
220	20	49	27	28
DNA substrate[a]:	DNA1B	DNA2B	DNA3B	DNA4B
Voltage (mV)	Number of captured complexes [b]			
130	39	24	25	24
140	54	21	29	23
150	74	17	20	23
160	42	20	24	25
170	32	20	14	25
180	42	22	31	20
190	28	24	26	21
200	22	23	27	14

[a] DNA sequences are shown in Figure 3.
[b] The number of independent capture events used to extract the rates, at each voltage, for complexes between phi29 DNAP-DNA and each of the eight DNA substrates.