Supporting Information

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SI Text

Dependence of the Average Step Size on Torque. Here, we present a model to describe the dynamics of DNA swiveling under the application of an external torque. This model is an improvement on one that we used previously (1), as it additionally takes into account possible variations of Lk in the direction opposite to the applied torque. Thus, it is independent of initial assumptions regarding the amplitude of this torque.

We assume that the free energy of a given conformation of the DNA-enzyme complex depends *a priori* on the angle θ describing the relative rotation of the two DNA strands after a nick has been created. In the absence of torque, the free energy landscape has a 2π periodicity (Fig. S4). We assume that the free energy landscape includes a potential minimum when the 3′ and 5′ strands of the DNA are aligned to permit ligation and neglect the contribution of other free energy barriers. The application of a torque tilts the energy landscape, which results in a modification of the height of the free energy barriers opposing the escape from the ligation region. More precisely, if we denote the applied torque by Γ (where $\Gamma > 0$ corresponds to positively supercoiled DNA) and the angles from the free energy minimum to the top of the energy barrier by α, then the heights of the energy barriers are modified by $-\Gamma\alpha$ and $+\Gamma(2\pi - \alpha)$, respectively.

We now assume that the DNA is in a potential minimum corresponding to $\Delta Lk = m$. From such a configuration, three transitions are possible: (*i*) variation of ΔLk by one unit, assisted by torque: rate k_+; (*ii*) variation of ΔLk by one unit, against the torque: rate k_-; and (*iii*) ligation of DNA that terminates the reaction, with probability P_{lig}.

The ligation probability P_{lig} depends on both the rate k_{lig} of the ligation reaction and the average time T_{res} spent in the potential minimum corresponding to $\Delta Lk = m$. Using the short-time linear approximation of P_{lig} yields $P_{lig} = k_{lig}\, T_{res}$. Moreover, $T_{res} = 1/(k_+ + k_-)$, because the DNA exits from the configuration $\Delta Lk = m$ at a total rate $k_+ + k_-$. Consistent with this short-time approximation made to determine P_{lig}, we do not consider here the possibility of subsequent recleavage of DNA after its religation. Derivations accounting for this possible effect can be found in work by Marko and coworkers (2, 3).

In this framework, the average number of moves $<N>$ made before ligation, including both moves with and against the applied torque, can be shown to equal $<N> = 1/P_{lig}$, leading to:

$$\langle N\rangle = \frac{k_+ + k_-}{k_{lig}}.$$

Among the moves made before ligation, an average fraction $k_+/(k_+ + k_-)$ are made in the direction assisted by the torque, whereas a fraction $k_-/(k_+ + k_-)$ are made in the opposite direction. Therefore, the average variation of ΔLk before ligation is:

$$\langle Lk\rangle = \frac{k_+}{k_+ + k_-}\langle N\rangle - \frac{k_-}{k_+ + k_-}\langle N\rangle.$$

We can now substitute into this expression for k_+ and k_- according to the Arrhenius law:

$$k_+ = k_0 e^{\frac{\Gamma\alpha}{k_B T}} \quad \text{and} \quad k_- = k_0 e^{\frac{-\Gamma(2\pi-\alpha)}{k_B T}},$$

where k_0 represents the value of k_+ and k_- at zero torque (assumed to be equal), k_B is Boltzmann's constant, and T is the temperature in Kelvin. We note that the ratio between k_+ and k_- is given by:

$$\frac{k_+}{k_-} = e^{\frac{-2\pi\Gamma}{k_B T}}.$$

Two limits can be considered:

(*i*) Low torque regime ($2\pi\Gamma \ll k_B T$).

In this limit, $<\Delta Lk> = k_0\,(\exp(\Gamma\alpha/k_B T) - \exp(-\Gamma(2\pi - \alpha)k_B T))/k_{lig} \approx k_0\Gamma\, 2\pi/(k_B T\, k_{lig})$. In this regime, $<\Delta Lk>$ depends linearly on the applied torque, and goes to 0 as Γ goes to 0, as expected.

(*ii*) High torque regime ($2\pi\Gamma \gg k_B T$). Here, $k_+/k_- \gg 1$, so that the contribution of k_- can be neglected, and therefore $<\Delta Lk> \approx k_0\,/k_{lig}\, \exp(\Gamma\alpha/k_B T)$.

In this regime, $<\Delta Lk>$ scales exponentially with the applied torque. In magnetic tweezers experiments, the torque Γ is typically not directly measured, but is deduced according to the formula $\Gamma = (2k_B T L_p F)^{1/2}$ that converts force to torque beyond the buckling regime. However, a recent study (3) has shown that this formula may lead to a ≈25% overestimation of the torque and derived a slightly more complex relation between force and torque (formula 17 of ref. 3), which we used in this study. Using this force-torque relation, we established that the torque always exceeded $k_B T$ in the 0.5–3 pN force range of our experiments (the torque is ≈2 $k_B T$ at 0.5 pN and increases with force), allowing the use of the high-torque approximation to fit our experimental data (Fig. 2*b*).

Dynamic Analysis. A model describing the extension of a bare DNA molecule following the release of the torsional constraint has been presented (4). Briefly, three forces were considered in the equation describing bead motion: the force exerted by the magnets, the drag force opposing bead motion in liquid (in which the vicinity of the glass surface has to be taken into account), and the force of DNA on the bead. The quasi-static approach consists of assuming that internal dynamics of DNA have much faster time scales than bead upward motion (whose velocity is limited by the drag force opposing this motion). Under this assumption, the force exerted by DNA on the bead can be replaced by its equilibrium value. In this model, the influence of supercoils along DNA is therefore limited to their contribution to DNA elastic behavior (shown in Fig. S1*c*). We have shown that this assumption leads to an accurate description of the dynamics of bare DNA (4), and the present article demonstrates that the model also works for DNA relaxation induced by CVLig.

1. Koster DA, Croquette V, Dekker C, Shuman S, Dekker NH (2005) Friction and torque govern the relaxation of DNA supercoils by eukaryotic topoisomerase IB. *Nature* 434:671–674.
2. Taneja B, Schnurr B, Slesarev A, Marko JF, Mondragon A (2007) Topoisomerase V relaxes supercoiled DNA by a constrained swiveling mechanism. *Proc Natl Acad Sci USA* 104:14670–14675.
3. Marko JF (2007) (2007) Torque and dynamics of linking number relaxation in stretched supercoiled DNA. *Phys Rev E Stat Nonlin Soft Matter Phys* 76:021926.
4. Crut A, Koster DA, Seidel R, Wiggins CH, Dekker NH (2007) (2007) Fast dynamics of supercoiled DNA revealed by single-molecule experiments. *Proc Natl Acad Sci USA* 104:11957–11962.

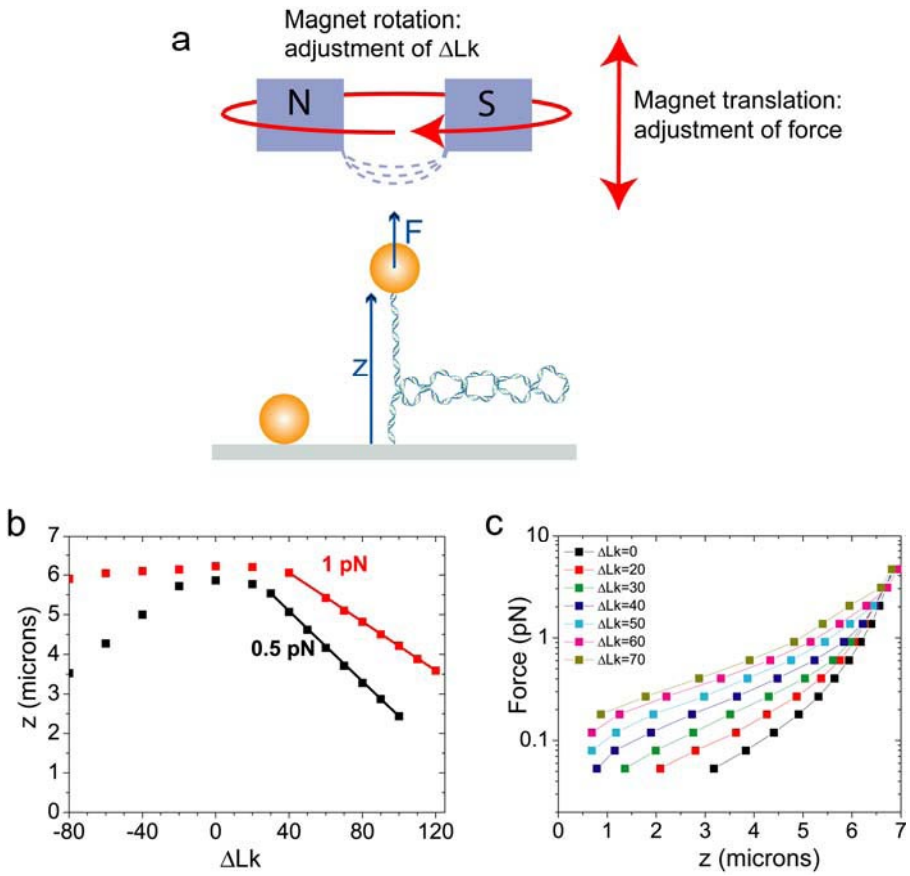

Fig. S1. Nanomanipulation and calibration of a single DNA molecule with magnetic tweezers. (*a*) Schematic representation of the magnetic tweezers setup. A pair of magnets mounted above the flow cell exerts an upward force on a magnetic bead. Rotating the magnets twists the DNA molecule, leading to the formation of plectonemes. A differential measurement of bead position involving a fixed bead is used to avoid long-term drift. (*b*) Behavior of a single DNA molecule under torsion. Under low forces such as 0.5 pN (black points), the molecule exhibits a symmetrical response to positive and negative rotations, resulting from the formation of plectonemes along the molecule. However, under larger forces (e.g., 1 pN, red points), plectonemes are formed only at positive rotations. The linear slope observed in the plectonemic regime makes possible to estimate the size of a plectoneme via a linear fit (solid lines). (*c*) Elasticity of positively supercoiled DNA. The force-extension behavior of a given molecule was measured for different values of its linking number, ranging from 0 (black) to 70 (beige). These calibrations reflect a large dependence of DNA elasticity on supercoiling and were essential for addressing the dynamics of steps that were terminated by ligation while plectonemes in DNA.

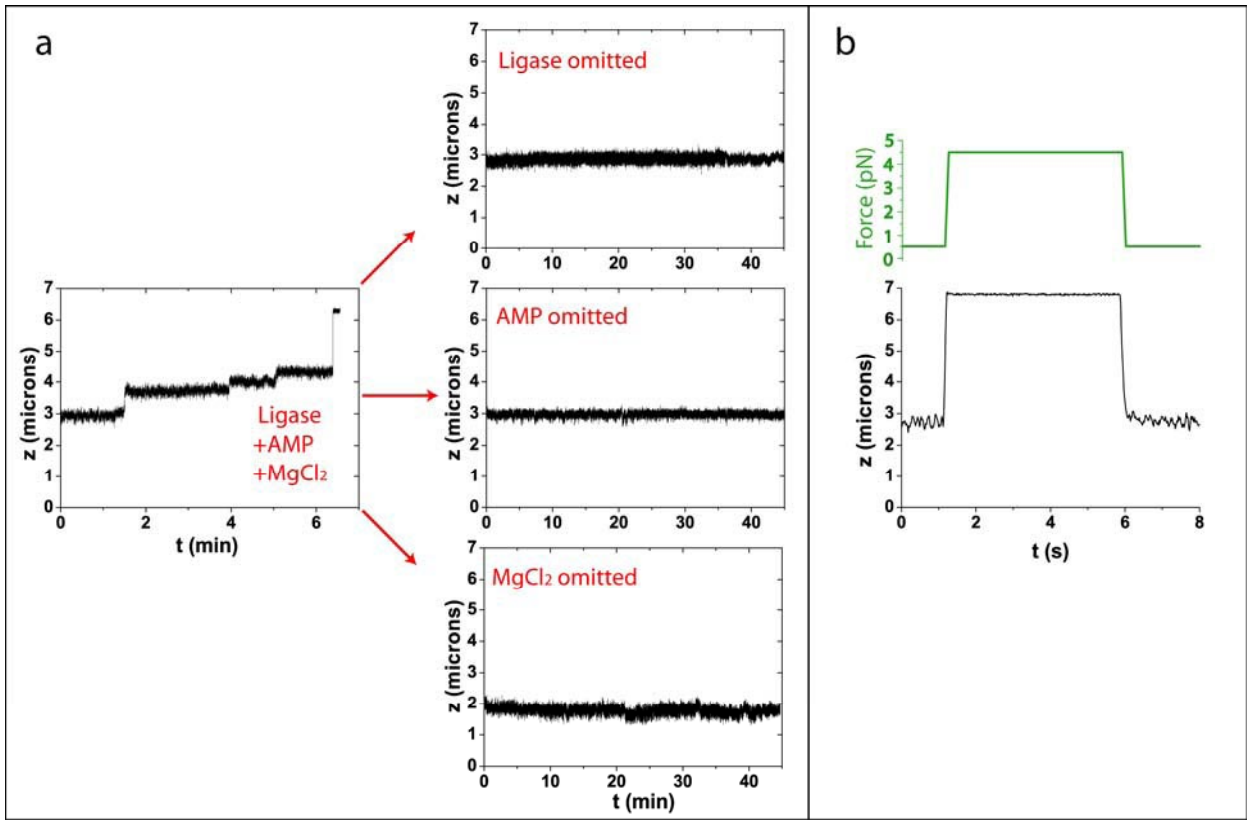

Fig. S2. Controls performed to ensure that the observed steps are the result of AMP- and magnesium-dependent action of CVLig on DNA. (*a*) AMP and magnesium requirements of the relaxation reaction. (*Left*) The combination of 6 nM CVLig, 10 mM AMP, and 5 mM $MgCl_2$ results in the stepwise relaxation of DNA in a few minutes as shown (obtained under a force of 1 pN). (*Right*) However, no relaxation was observed when any of these components was omitted. The requirement of AMP signifies that the observed activity is not caused by a contaminating topoisomerase. (*b*) Response of a DNA molecule to a force switch. A force switch can be applied on DNA molecule by the vertical translation of the magnets. In the example shown here, the force applied on a supercoiled molecule ($\Delta Lk = 100$) was temporarily increased from 0.5 to 4.5 pN within a time <0.1 s. The response of the molecule occurred on the same time scale without displaying any intermediate steps, indicating that the dynamics of the molecule were not perturbed by nonspecific interactions (e.g., interactions between the DNA and the surface). Such tests were performed regularly before experiments with CVLig.

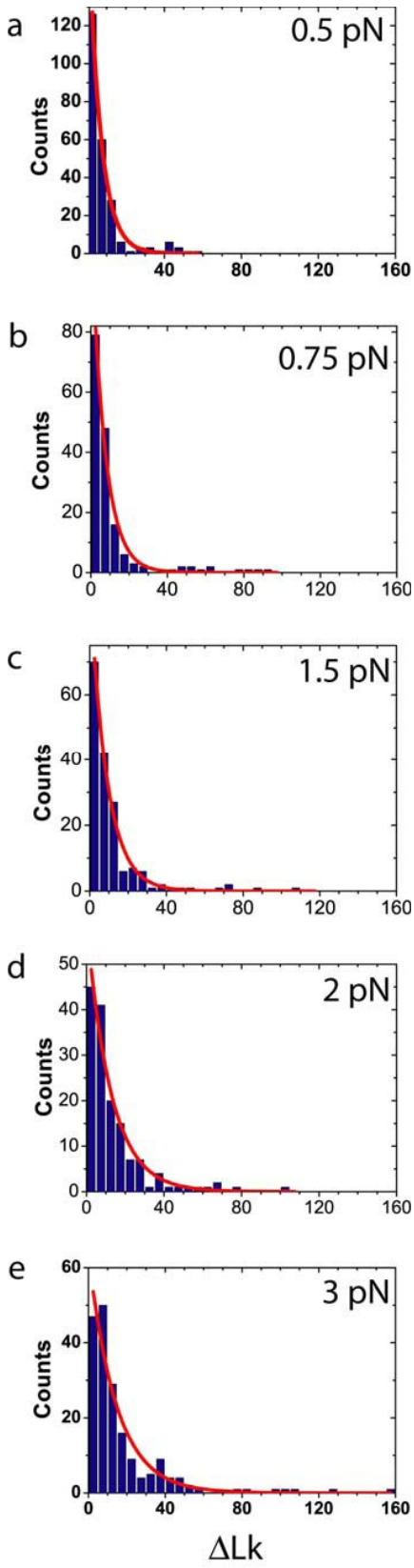

Fig. S3. Δ*Lk* distributions after AMP-triggered DNA cleavage by CVLig at various forces. (*a*) 0.5 pN. (*b*) 0.75 pN. (*c*) 1.5 pN. (*d*) 2 pN. (*e*) 3 pN. An exponential fit of the data is displayed in red in each case. These distributions illustrate the dependence of the average step size on the applied force.

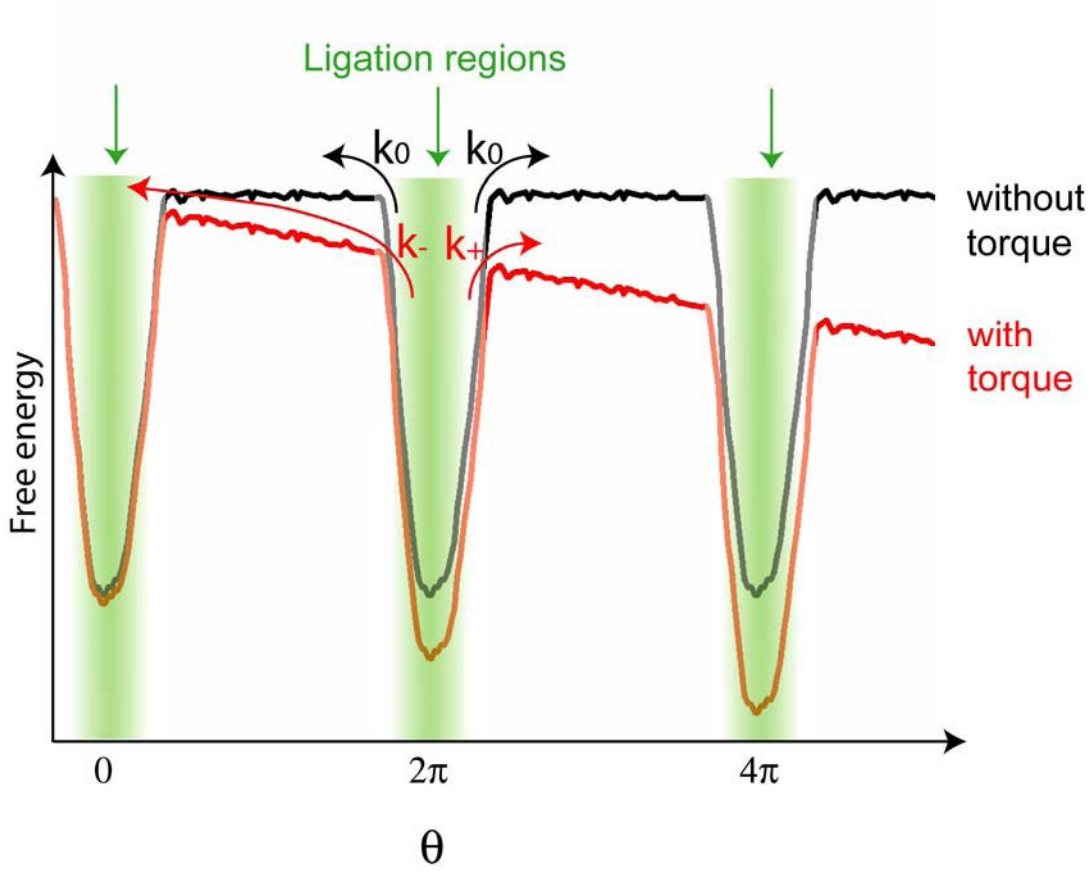

Fig. S4. Schematic of the model used to determine the torque dependence of step size. The free energy associated with the angle θ describing the relative rotation of the two DNA strands is represented. It is assumed to present wells for the orientation allowing ligation of the strands. The application of a torque modifies the height of energy barriers along the free energy landscape, and therefore the rates k_+ and k_- at which they are crossed.

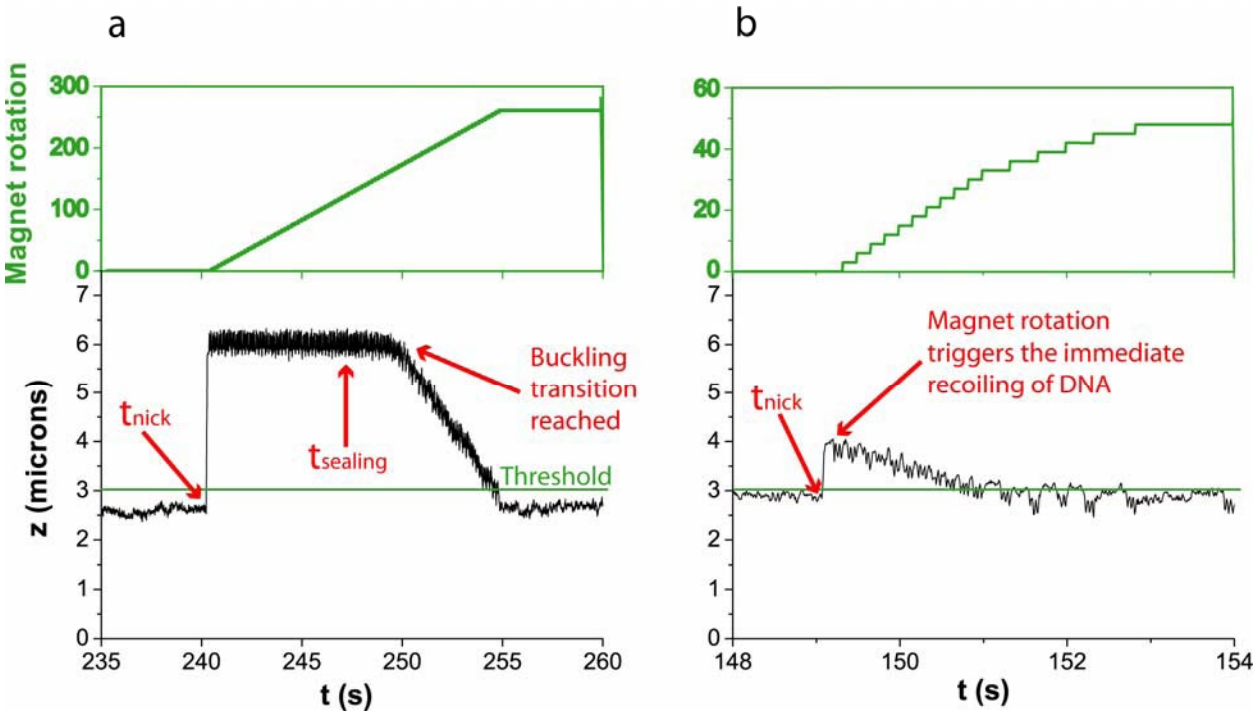

Fig. S5. Measurement of the lifetime of nicked states after large final steps. In these experiments, the increase of DNA extension above a given threshold (green line, set to 3 μm in this example) triggers the continuous rotation of magnets within a≈0.1-s delay. (*a*) Large final steps terminate with a plateau in the DNA extension, the height of which corresponds to the extension of a torsionally relaxed molecule under the magnetic force applied (here equal to 1 pN). The duration of the plateau shown here (≈10 s) largely exceeds the ''buckling time'' required to induce plectonemic supercoils in an initially relaxed molecule (≈2 s when magnets exerting a 1 pN force are rotated at 20 Hz). We infer from this observation that DNA remains in a nicked state for the additional time (≈8 s here). The times t_{nick} (at which DNA extension starts to increase) and $t_{sealing}$ (obtained by subtracting the buckling time from the time at which DNA extension starts to decrease) are indicated. The lifetime of nicked states is deduced from these times by $t_{ligation} = t_{sealing} - t_{nick}$. (*b*) The behavior of an intermediate step is shown for comparison. Here, magnet rotation immediately triggers the recoiling of the DNA molecule, indicating a much reduced religation time.

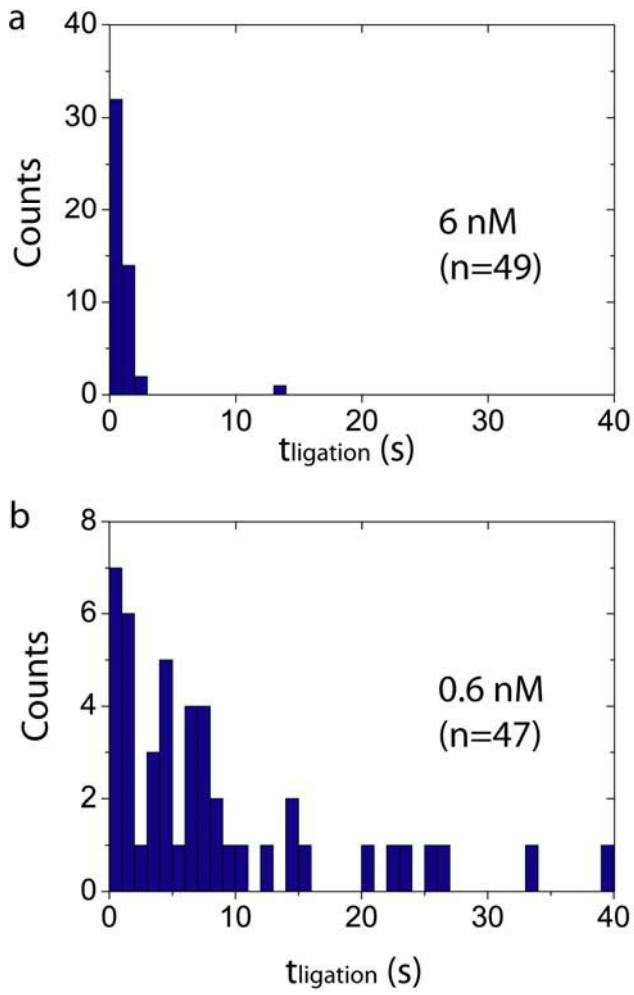

Fig. S6. Concentration dependence of ligation time after large final steps for the K27A mutant. The total number of events considered is indicated on both plots. The 10-fold decrease of ligase concentration from 6 nM (*a*) to 0.6 nM (*b*) results in a significant increase of the lifetime of nicked states after large final steps, which is very similar to the behavior of the WT enzyme. Consequently, dissociation is likely to occur for both enzymes.