DNA ligase

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Dynamics of phosphodiester synthesis by DNA ligase

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Ligases are essential actors in DNA replication, recombination, and repair by virtue of their ability to seal breaks in the phosphodiester backbone. Ligation proceeds through a nicked DNA-adenylate intermediate (AppDNA), which must be sealed quickly to avoid creating a potentially toxic lesion. Here, we take advantage of ligase-catalyzed AMP-dependent incision of a single supercoiled DNA molecule to observe the step of phosphodiester synthesis in real time. An exponentially distributed number of supercoils was relaxed per successful incision-resealing event, from which we deduce the torque-dependent ligation probability per DNA swivel. Premature dissociation of ligase from nicked DNA-adenylate accounted for ~10% of the observed events. The ability of ligase to form a C-shaped protein clamp around DNA is a key determinant of ligation probability per turn and the stability of the ligase-AppDNA intermediate. The estimated rate of phosphodiester synthesis by DNA ligase (400 s^{-1}) is similar to the high rates of phosphodiester synthesis by replicative DNA polymerases.

DNA ligation | DNA relaxation | magnetic tweezers

The DNA ligases are essential guardians of genome integrity. They seal 3′-OH/5′-PO_4 DNA nicks via three chemical steps (Fig. 1*a*): (*i*) ligase reacts with ATP (or NAD^+) to form a covalent ligase–adenylate intermediate, in which AMP is linked via a phosphoamide (P–N) bond to Nζ of a lysine on the enzyme; (*ii*) AMP is transferred from the ligase to the 5′-PO_4 strand at a nick to form a DNA–adenylate intermediate (AppDNA); and (*iii*) ligase catalyzes attack by the 3′-OH of the nick on AppDNA to form a phosphodiester bond and release AMP (1). Recent biochemical and crystallographic studies have illuminated the mechanism of nucleotidyl transfer, how ligases recognize nicks as "damaged," and how protein domain movements and active-site remodeling are used to choreograph the sequential steps of the ligation pathway (2, 3). In particular, the crystal structures of nick-bound ligases have revealed a conserved theme whereby ligases envelope the DNA duplex in a C-shaped protein clamp and elicit changes in DNA conformation, including bending at the nick and the adoption of A-form helical structure on the 3′-OH side of the nick (4–6).

Chlorella virus ligase (CVLig) is a minimized (298 aa) pluripotent exemplar of the ATP-dependent DNA ligase clade. It consists of an N-terminal nucleotidyltransferase domain and a C-terminal OB-fold domain. Although lacking the accessory domains found in cellular ligases, it has an intrinsic nick-sensing function and can sustain mitotic growth, excision repair, and nonhomologous end joining in budding yeast when it is the only ligase present in the cell (7–10). Accordingly, CVLig has proven to be an instructive model system for mechanistic and structural studies (11–15). For example, the atomic structure of the CVLig-AMP intermediate bound to duplex DNA with a 3′-OH/5′-PO_4 nick highlighted the key role of a β-hairpin "latch" module emanating from the OB domain in forming the C-shaped protein-DNA clamp (6) (Fig. 1*b*).

The least understood phase of nick sealing is phosphodiester bond synthesis (step 3 in Fig. 1*a*). Here, we use CVLig in the context of single-molecule nanomanipulation to directly analyze the kinetics and DNA dynamics of phosphodiester bond formation by a ligase–AppDNA complex formed *in situ* on a linear DNA. Our single-molecule experiments take advantage of the microscopic reversibility of step 3 of the ligation reaction, whereby ligase can catalyze attack of AMP on the DNA phosphodiester backbone to form a nicked DNA-adenylate. This nicked DNA-adenylate is then resealed by forward catalysis of step 3 (16). If the starting DNA substrate is a covalently closed supercoiled DNA, and if ligase releases the 3′-OH end of the AppDNA nick before executing forward step 3, the net result is incremental supercoil relaxation. AMP-dependent DNA supercoil release is a feature of many DNA ligases, including *Escherichia coli* LigA (16), T4 DNA ligase (17), vaccinia virus DNA ligase (18), and (as shown presently) *Chlorella* virus DNA ligase. This process is roughly analogous to the reactions catalyzed by type I DNA topoisomerases (TopI), except that TopI enzymes do not require AMP but instead use a tyrosine nucleophile on the enzyme to attack the phosphodiester backbone and form a covalent protein-linked DNA nick (19). The present single-molecule studies of DNA ligase provide key insights into nick sealing, especially the probability of sealing when torque is applied to a nick, the influence of protein structural elements on the stability of the ligase–AppDNA intermediate, and the rate of the chemical step of phosphodiester formation.

Results and Discussion

Ensemble and Single-Molecule Assays of Supercoil Relaxation by DNA Ligase. Purified recombinant CVLig relaxed negatively supercoiled plasmid DNA in the presence of 10 mM AMP to generate a mixture of partially relaxed topoisomers, fully relaxed circles, and nicked circular products (Fig. 1*c*). No supercoil relaxation by CVLig was detected when AMP was omitted (data not shown), indicating that the observed activity was not attributable to a contaminating topoisomerase.

In the single-molecule experiments, ~100 plectonemic superhelical turns were introduced into a 22-kb linear duplex DNA held under constant tension by a magnetic tweezer [see *Materials and Methods*, Fig. 1*d*, and supporting information (SI) Fig. S1*a*]. Infusion of 6 nM CVLig, 5 mM $MgCl_2$, and 10 mM AMP into the reaction chamber elicited a stepwise increase in DNA extension (i.e., the distance from the surface to the magnetic bead) observable in real time (Fig. 1*d*), where each step is the result of a single cleavage-religation cycle. The simultaneous action of two enzymes has negligible probability because the delay between successive steps (typically ~1 min; Fig. 1*d*) largely exceeds their duration (typically ~0.1 s; see Fig. 4). The occurrence of successive cleavage/religation cycles by the same enzyme separated by a short enough pause that they appear as a single step cannot strictly be excluded, but is unlikely in view of the low specific activity of the reverse step 3 reaction. Control experiments showed that CVLig required AMP

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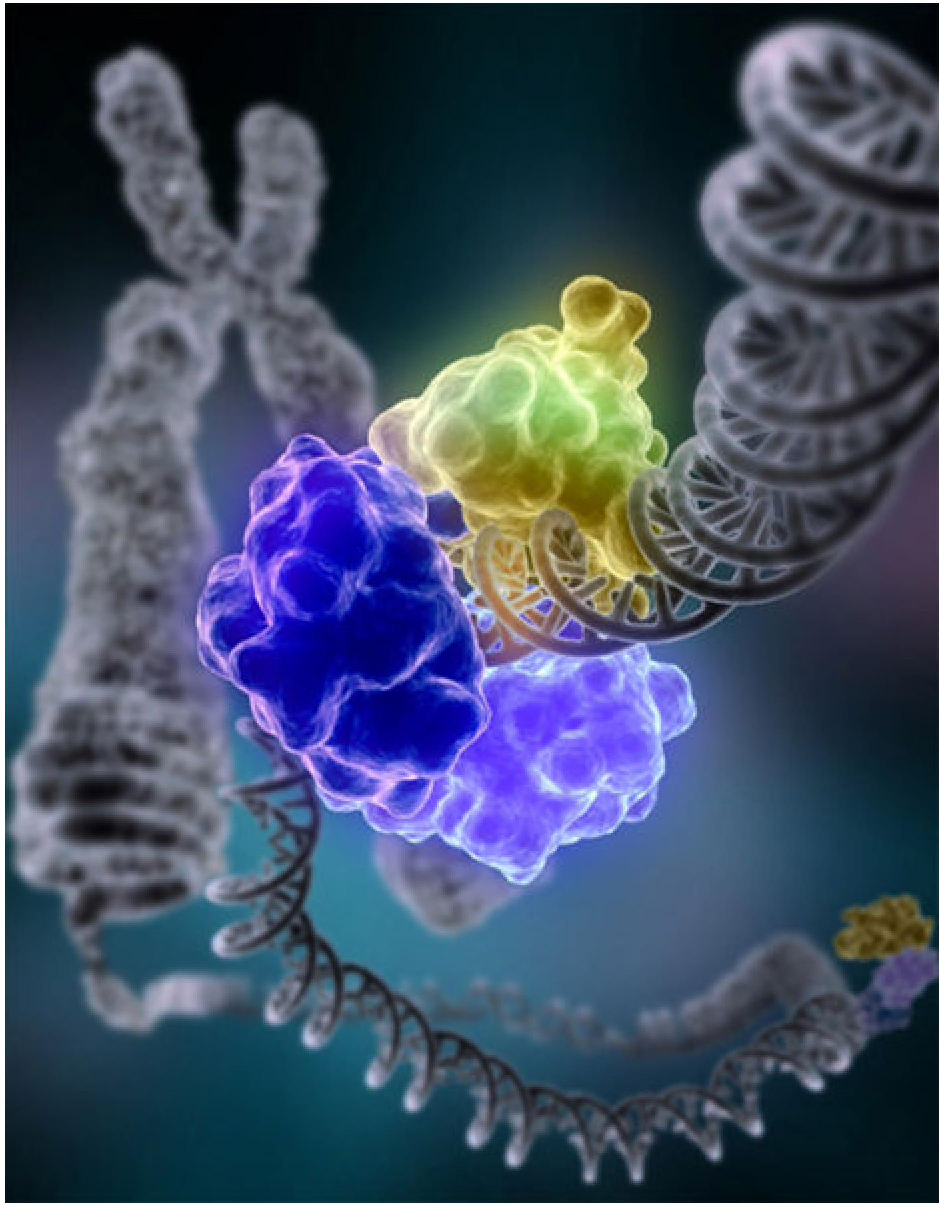
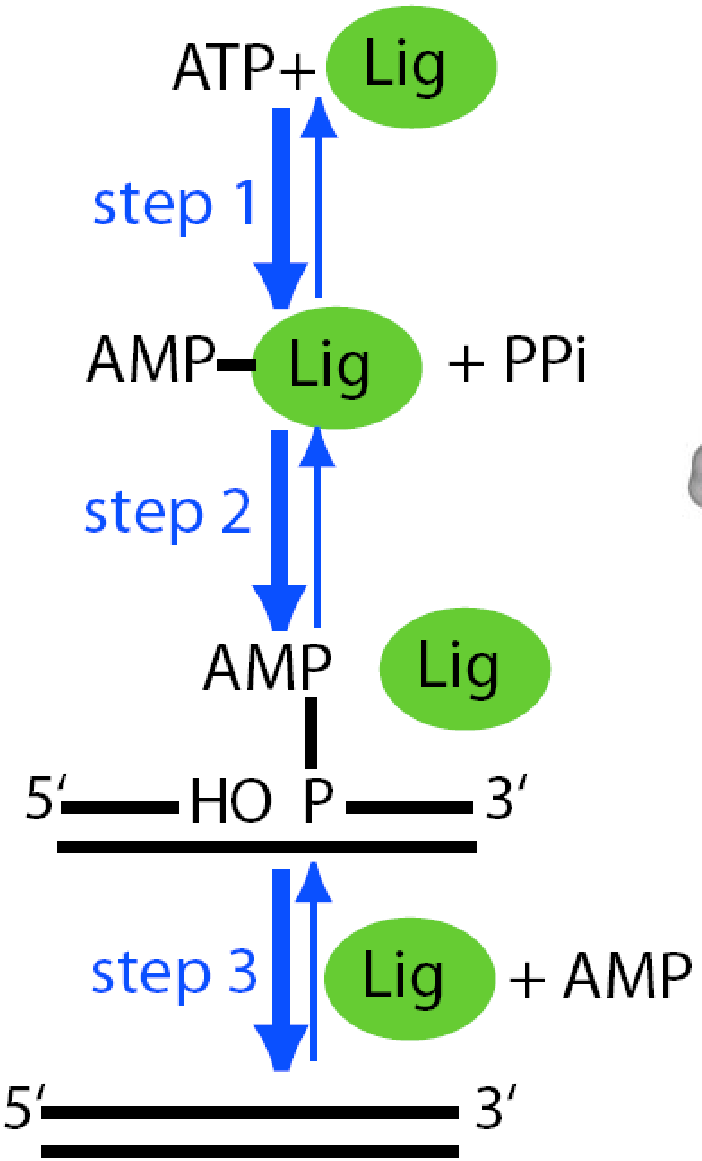
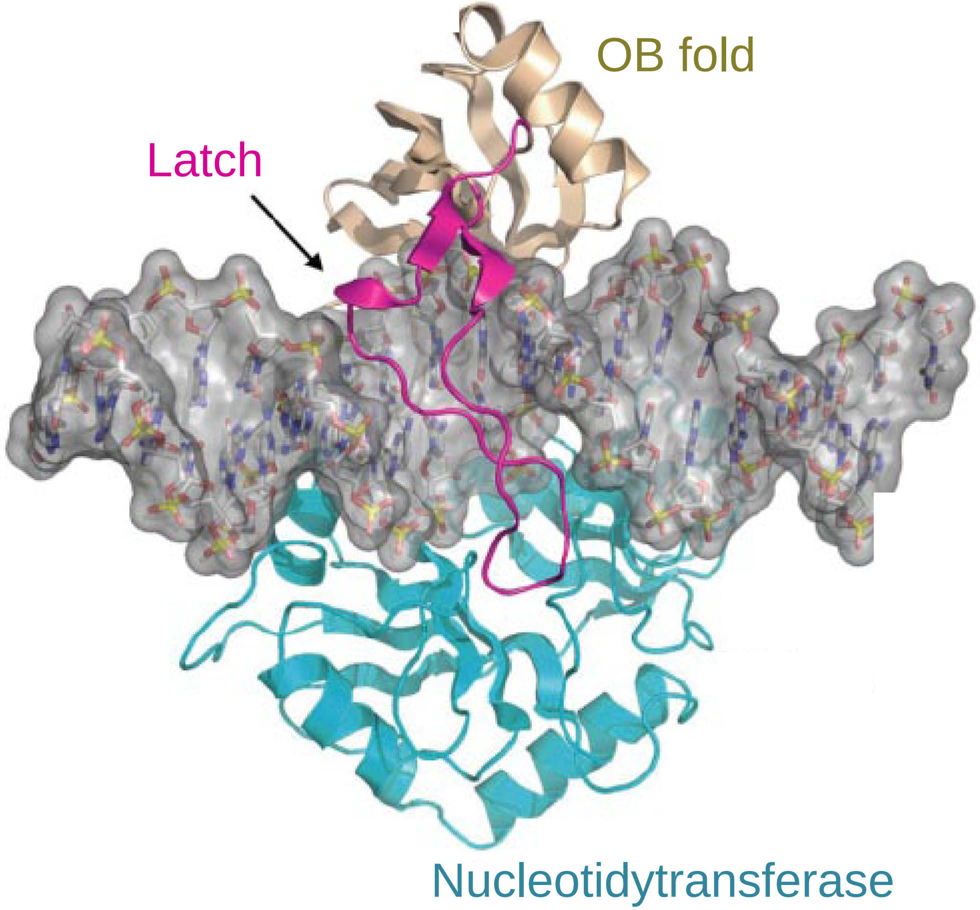


July 19, 2008

DNA ligase





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DNA ligase

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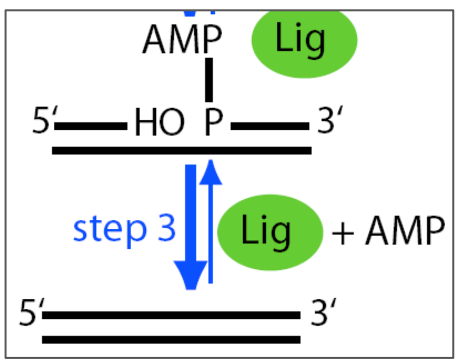
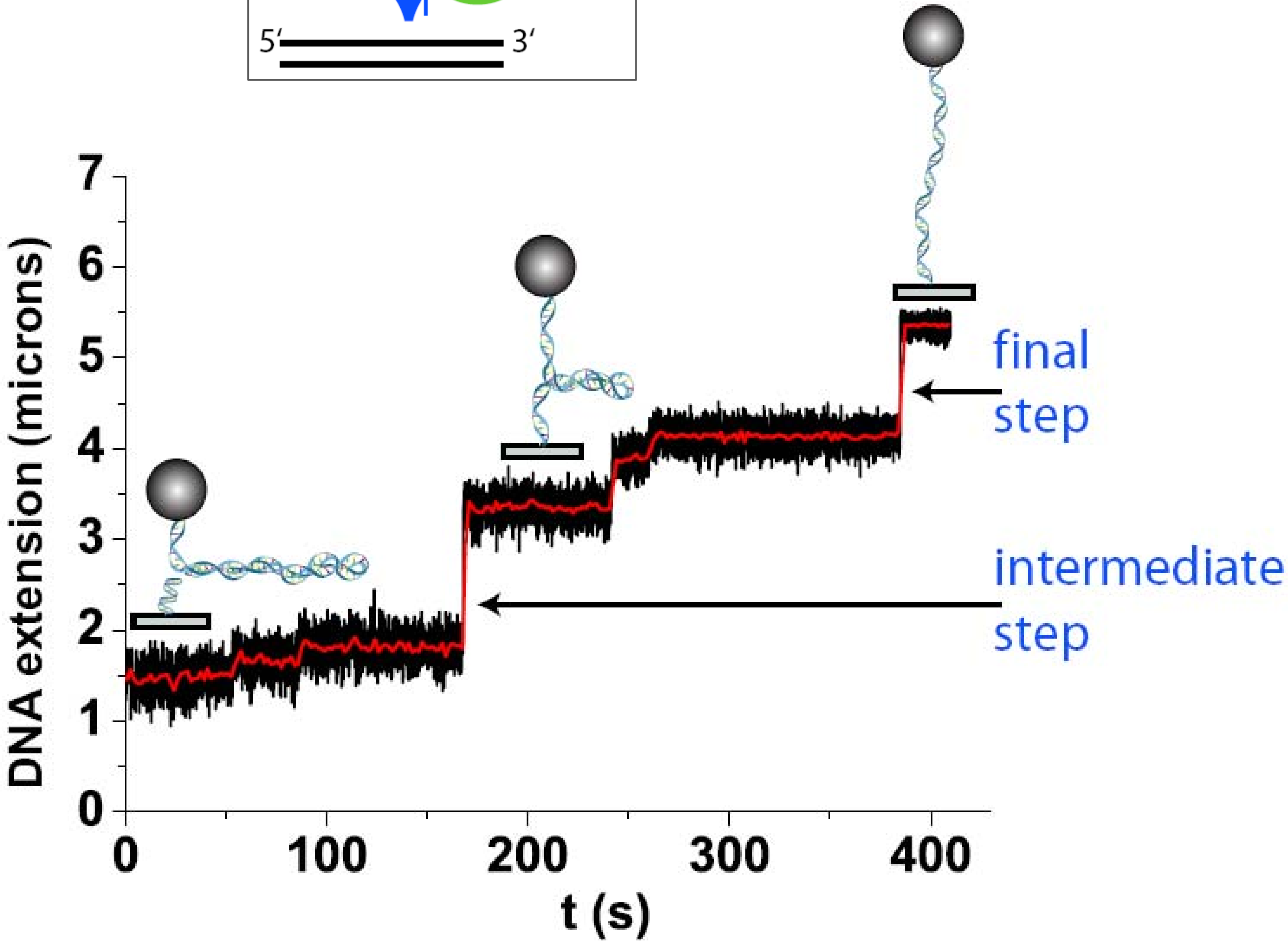
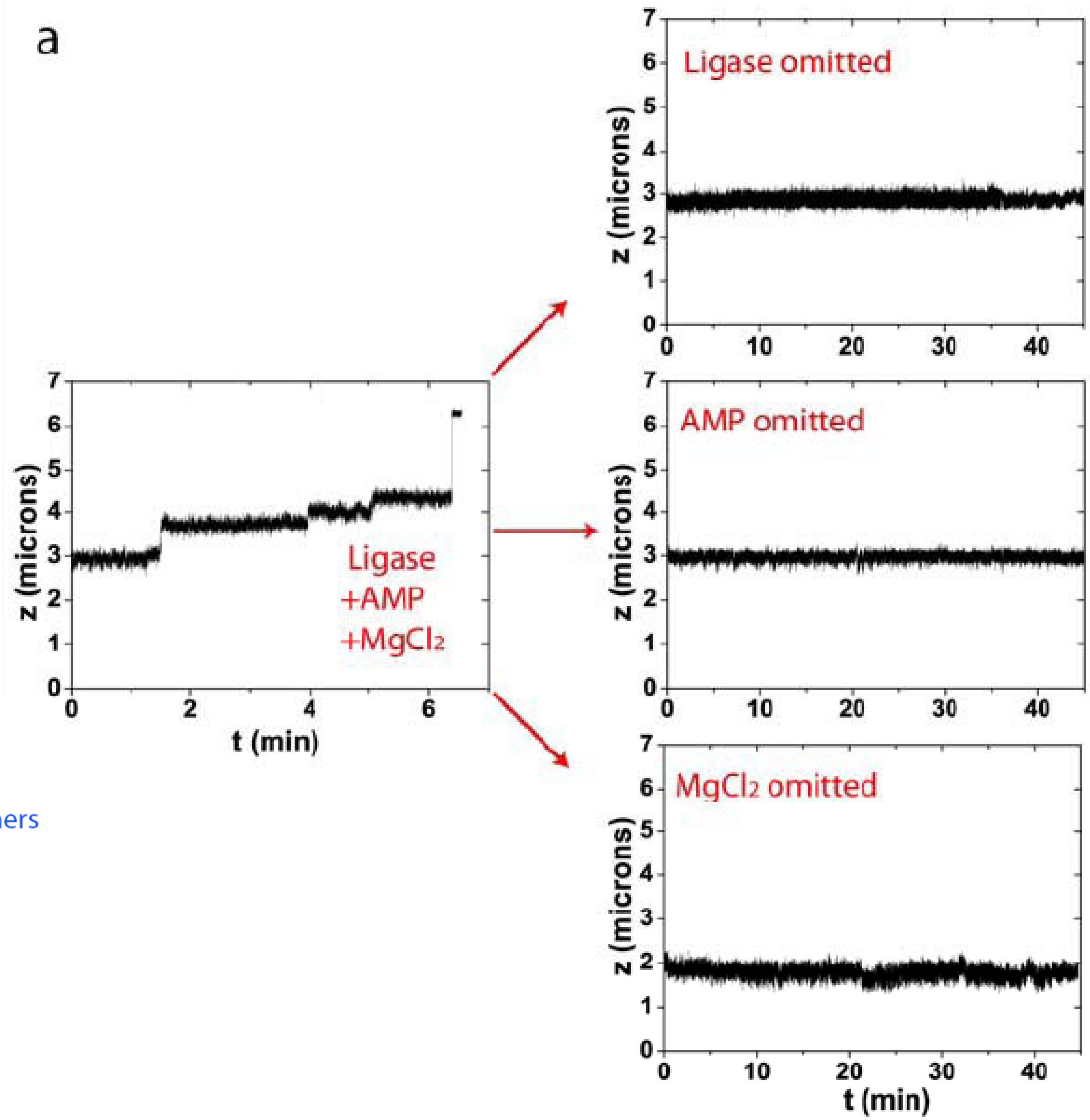
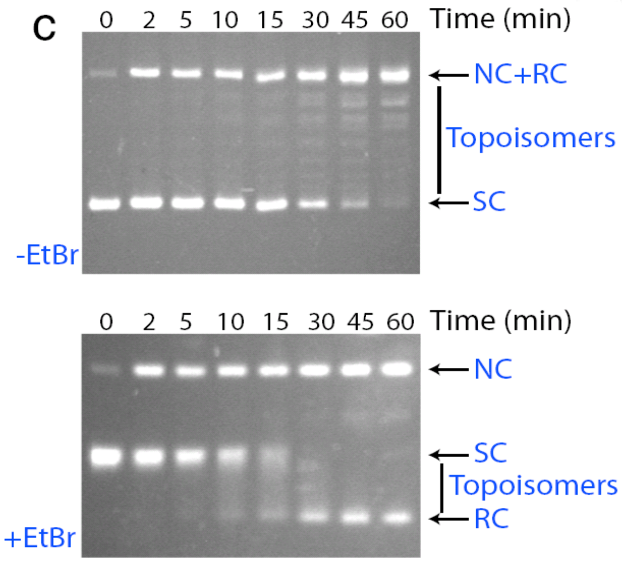




DNA ligase - control





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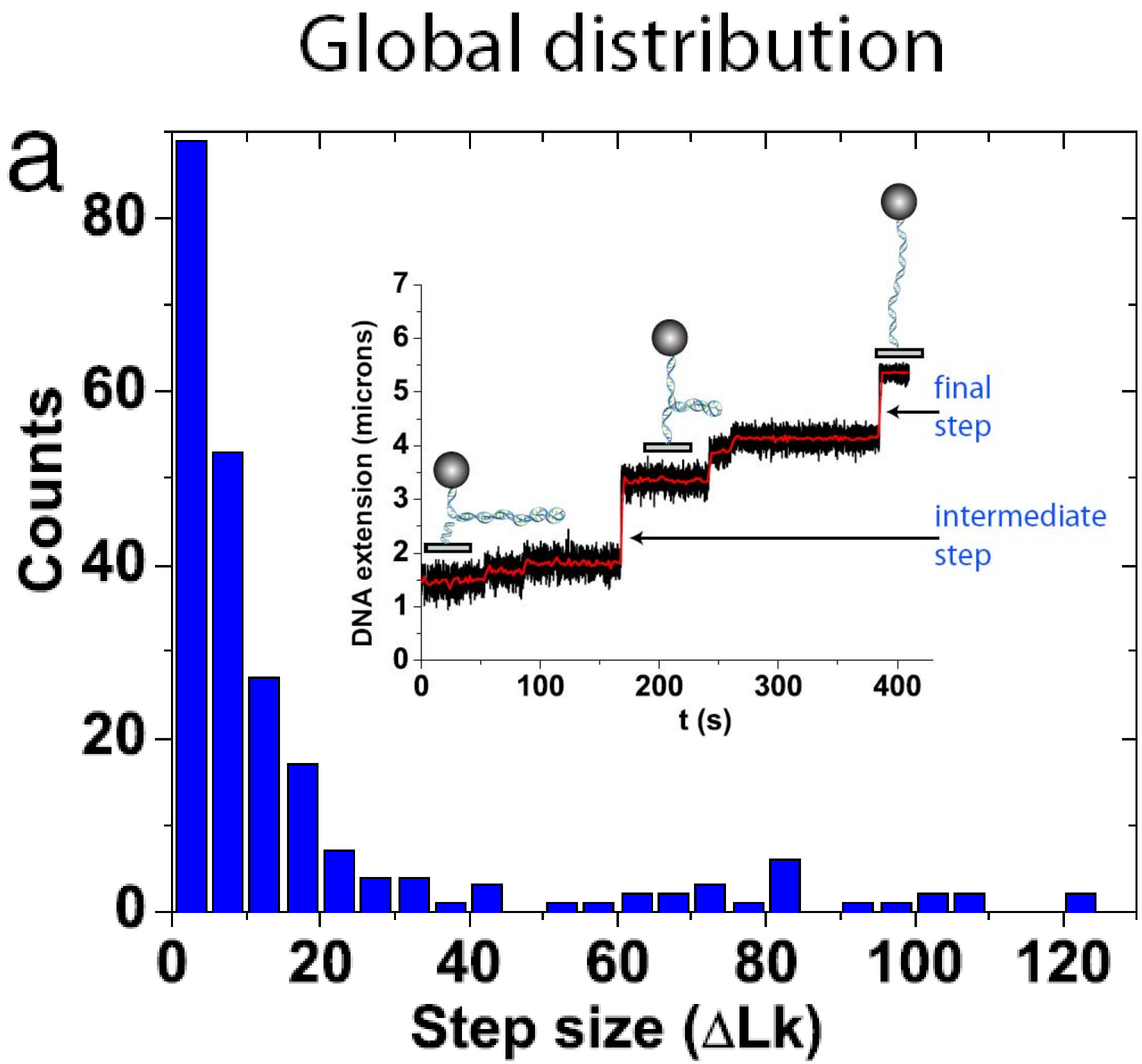
Step size distribution



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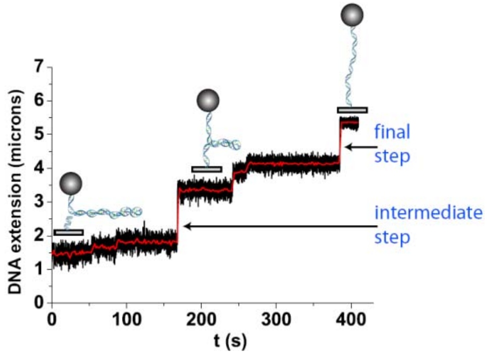
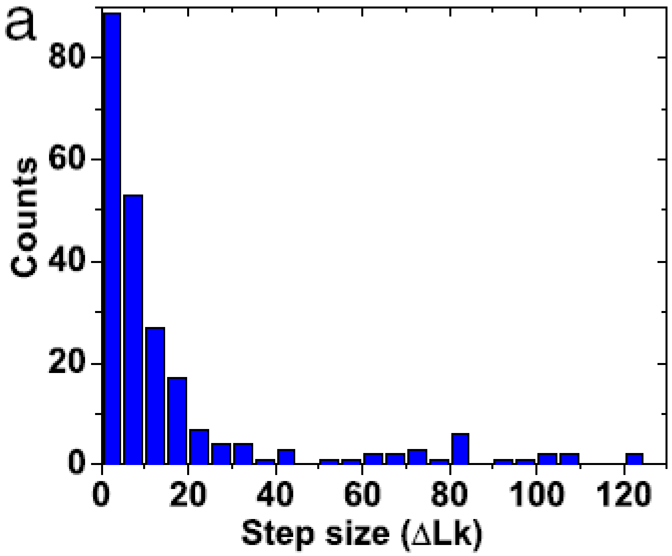
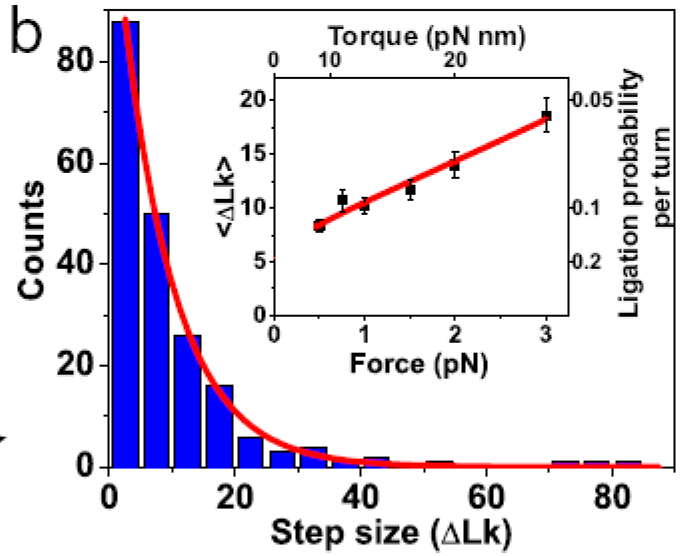


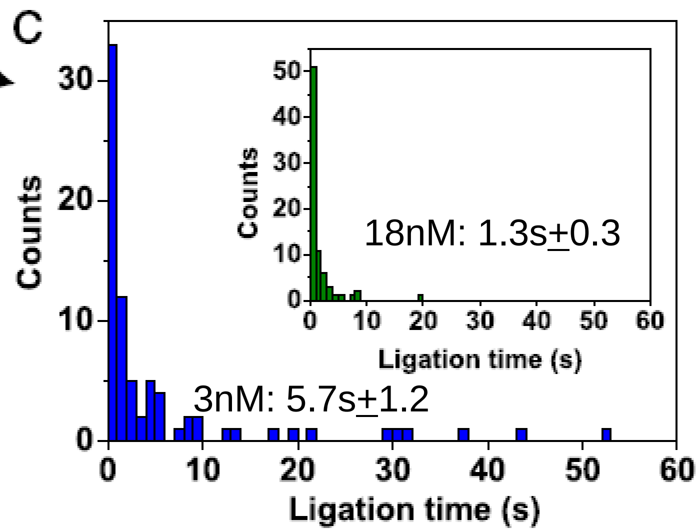
Global distribution



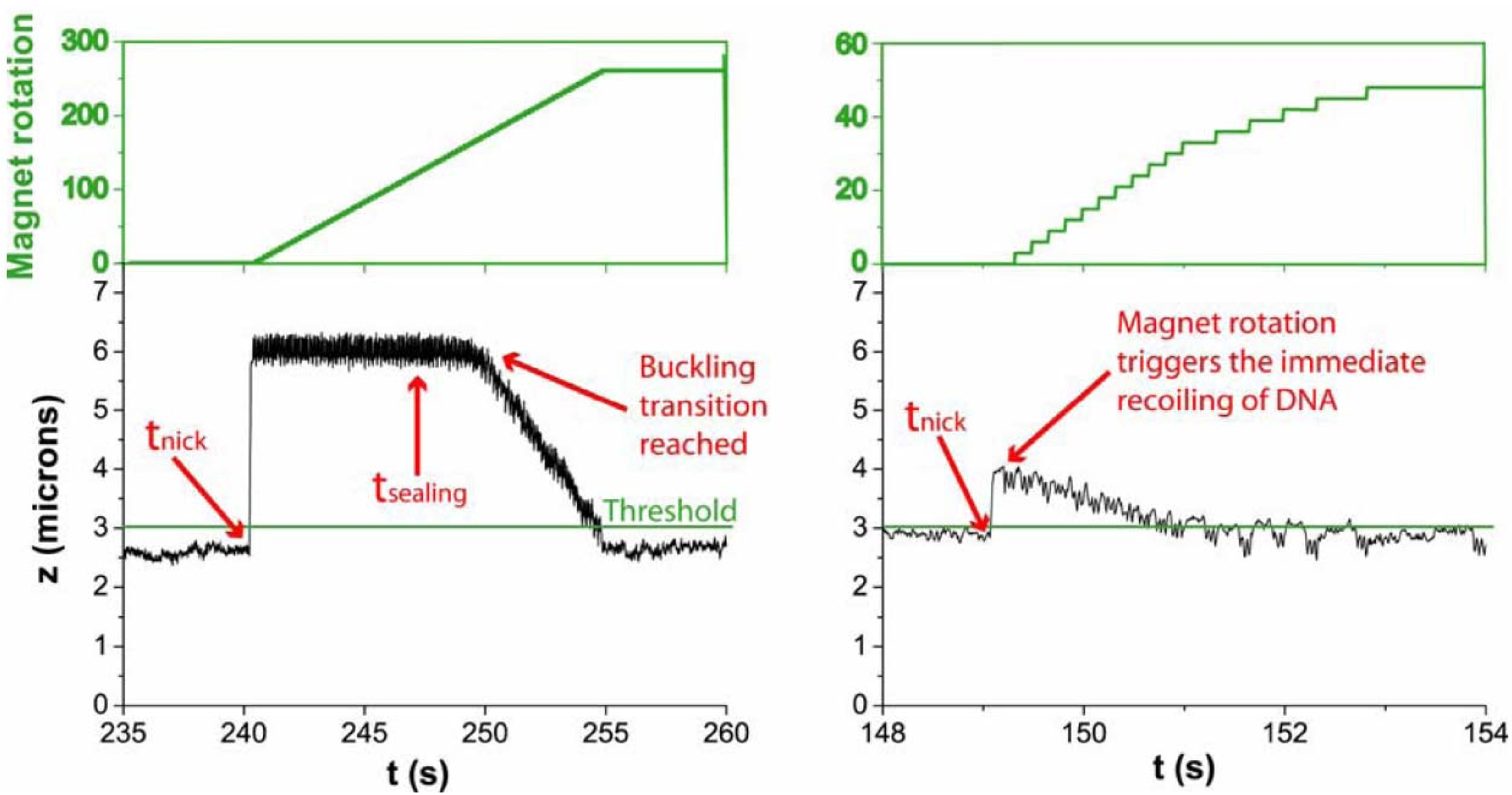
Analysis restricted to intermediate steps



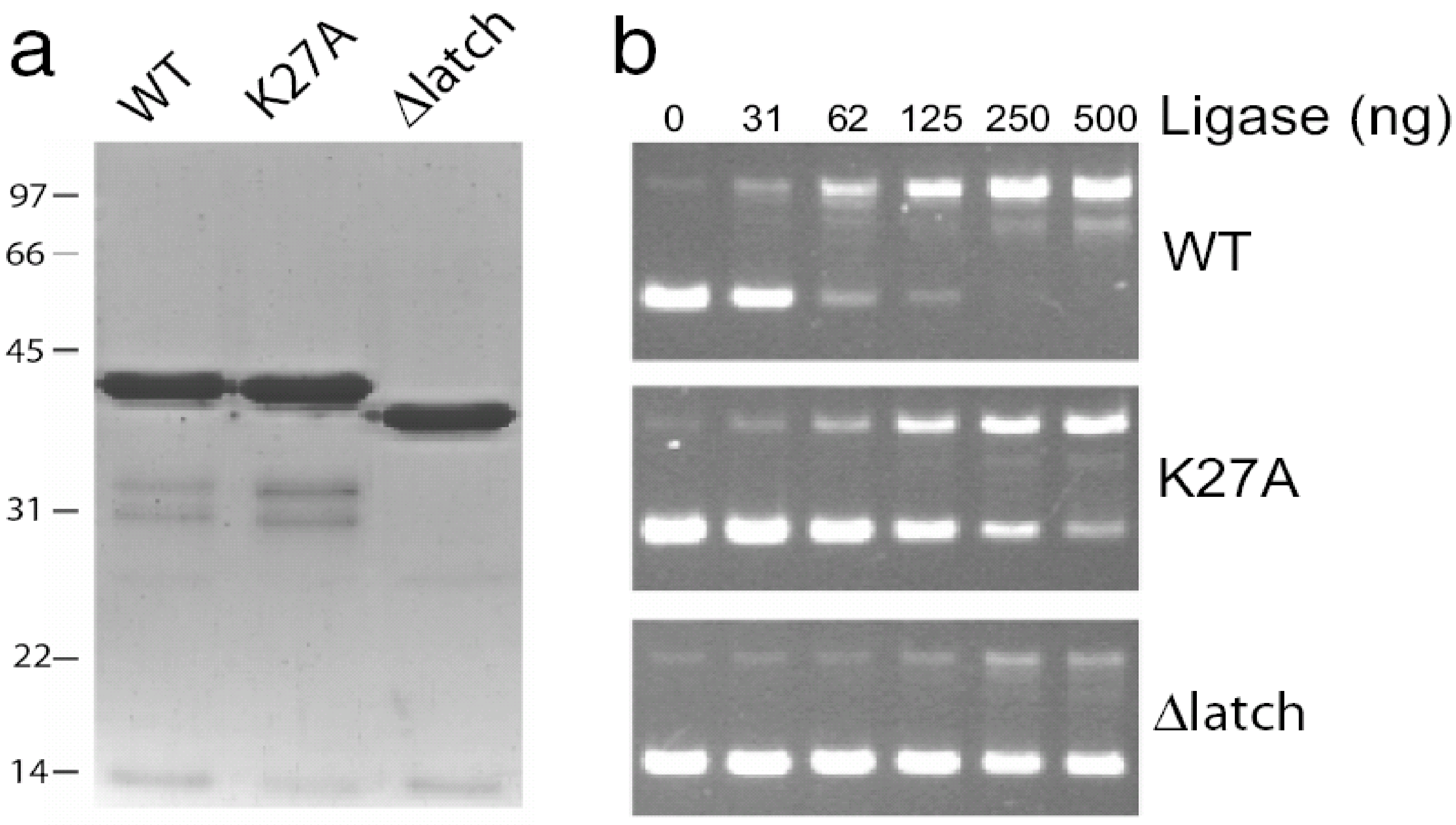
Analysis restricted to large final steps



DNA ligase


Magnet rotation
300
200
100
0
7
6
5
4
3
2
1
0
z (microns)
235
240
245
250
255
260
t (s)
tnick
tsealing
Buckling transition reached
Threshold
60
40
20
148
150
152
154
Magnet rotation triggers the immediate recoiling of DNA

Mutant K27A


a
WT
K27A
Δlatch
97
66
45
31
22
14
b
0
31
62
125
250
500
Ligase (ng)
WT
K27A
Δlatch

DNA ligase

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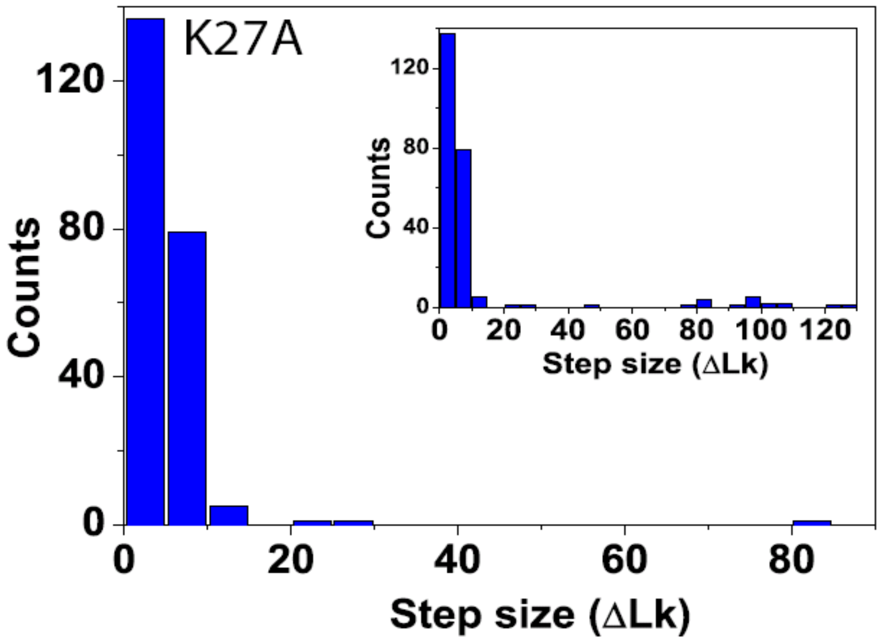
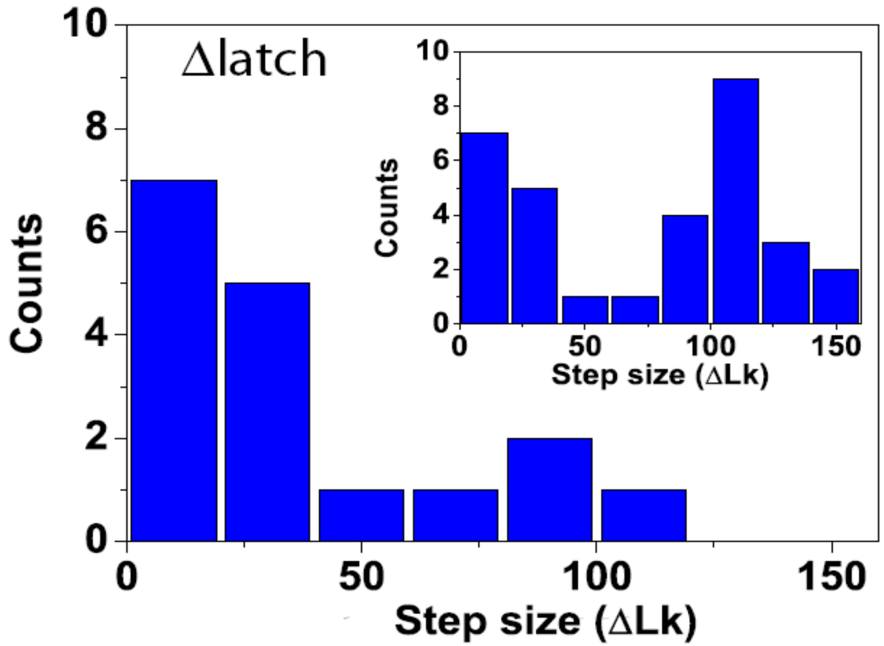
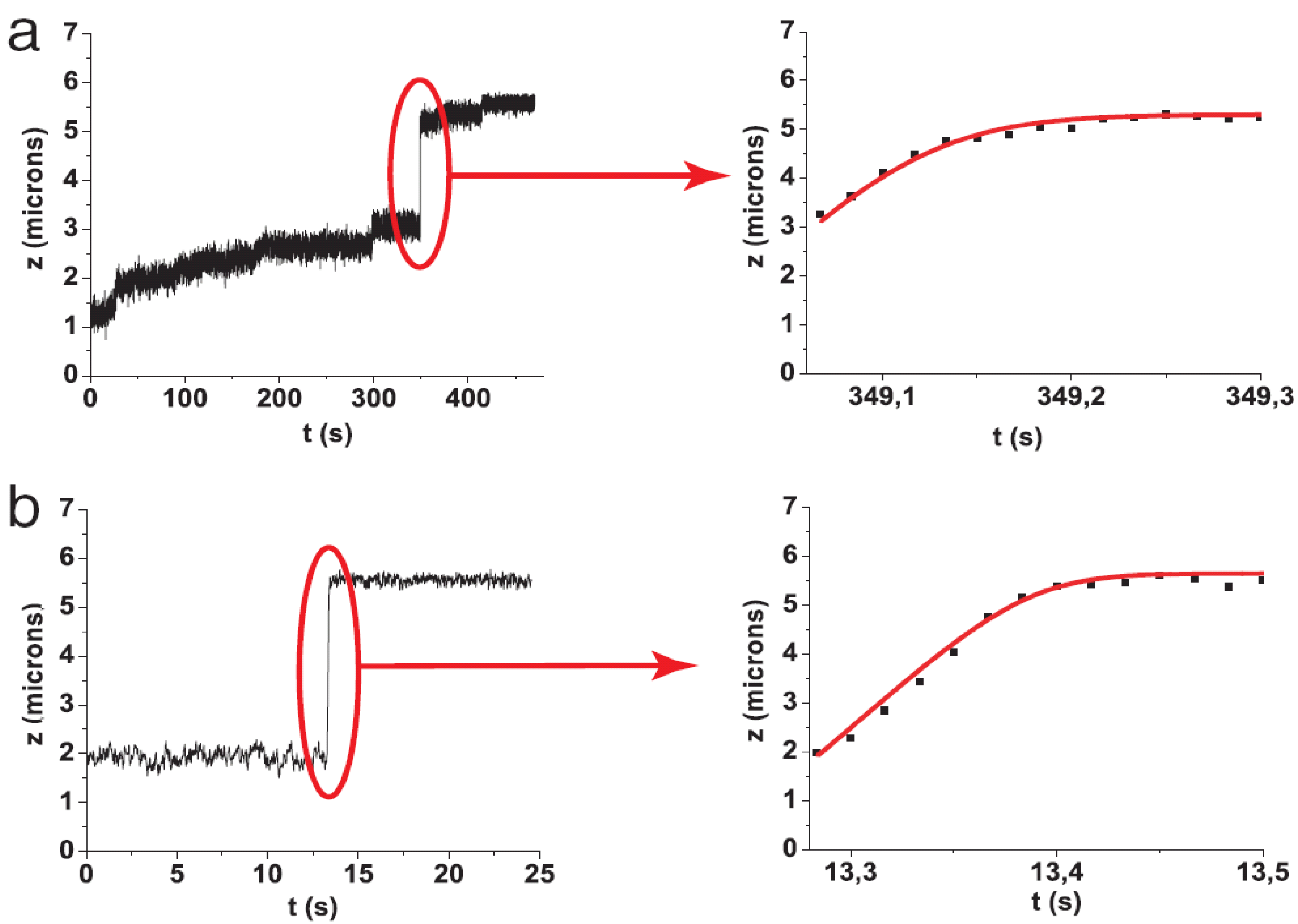




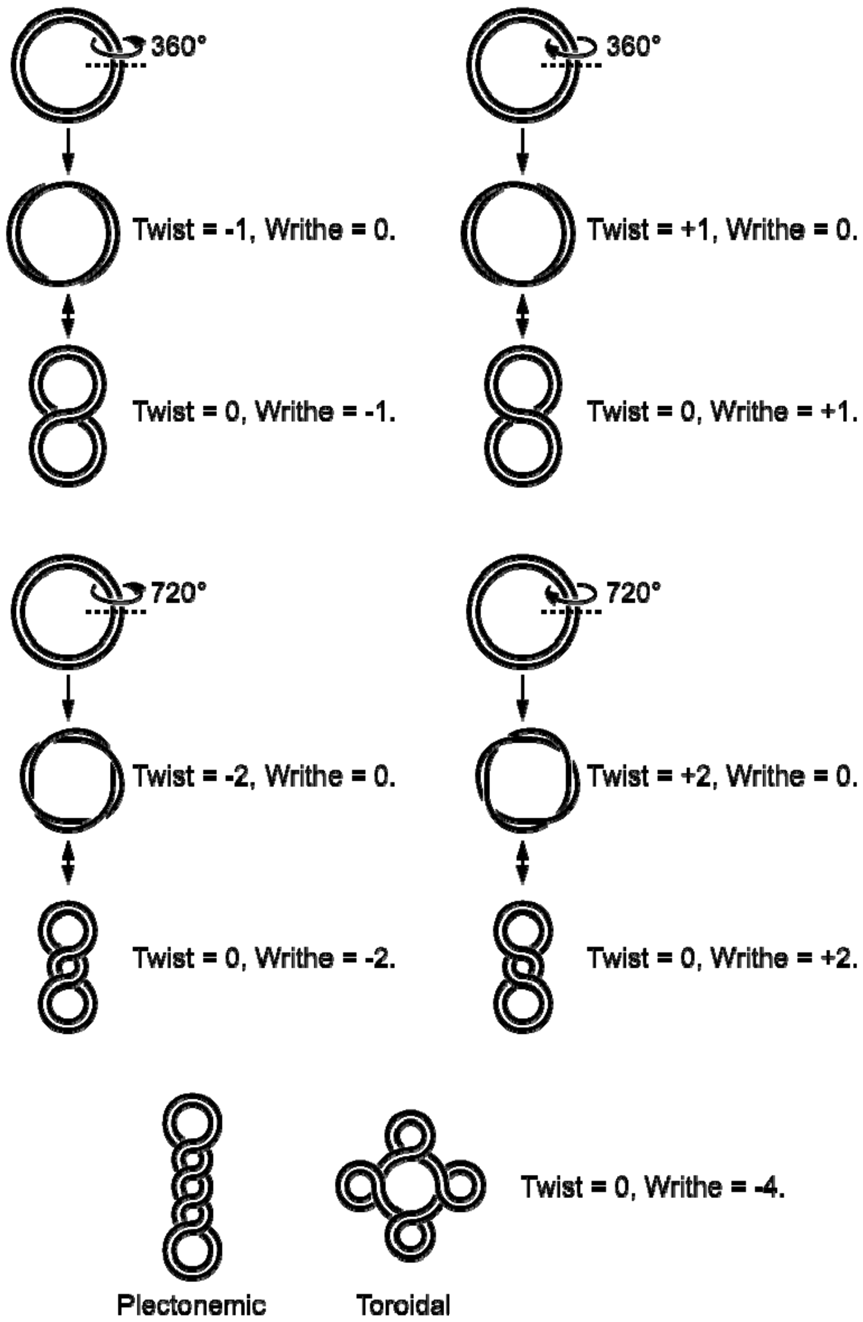
DNA ligase


a
b
z (microns)
t (s)
0
1
2
3
4
5
6
7
0
100
200
300
400
349,1
349,2
349,3
0
5
10
15
20
25
13,3
13,4
13,5

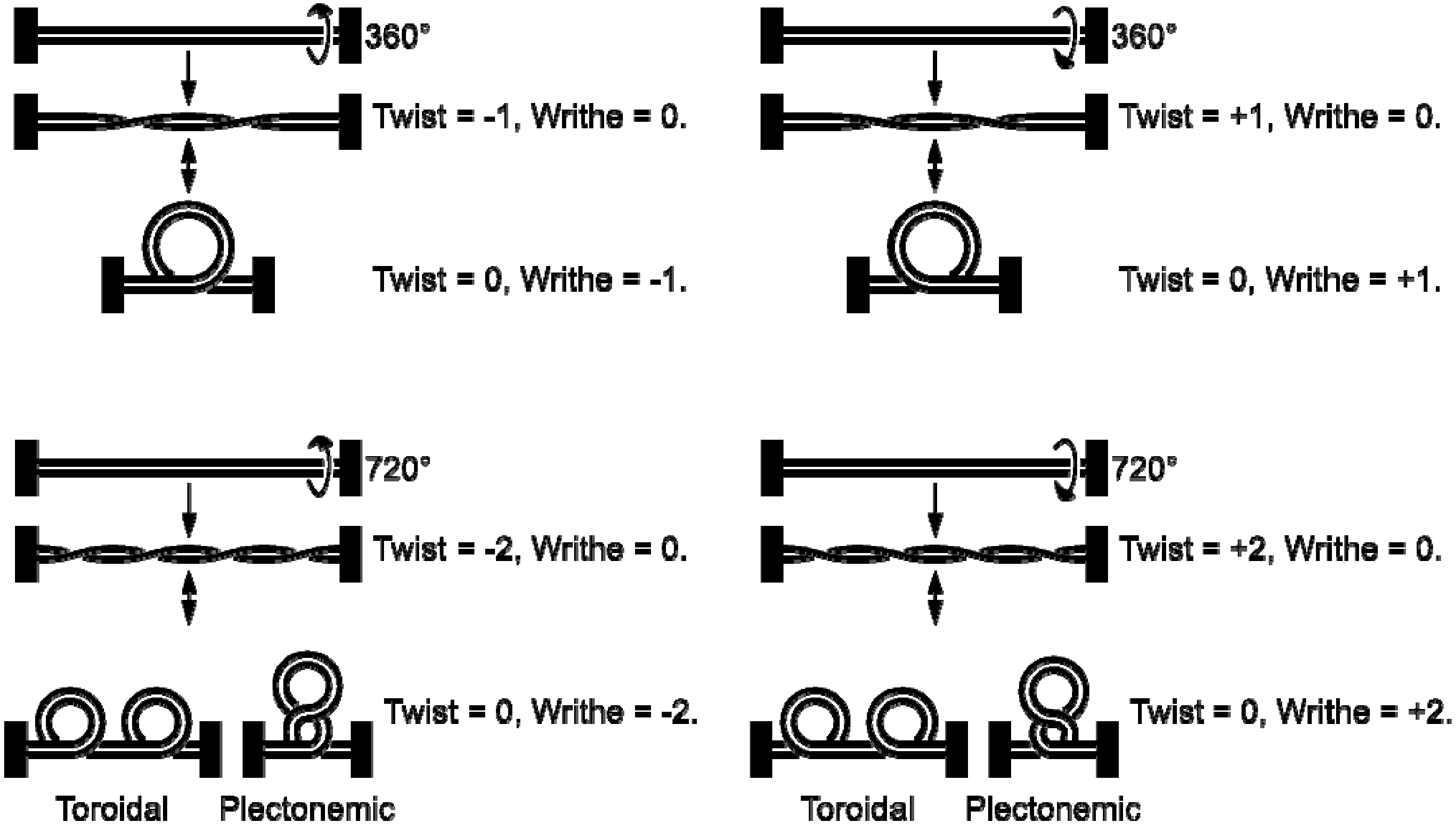
DNA ligase

SUPPLEMENT

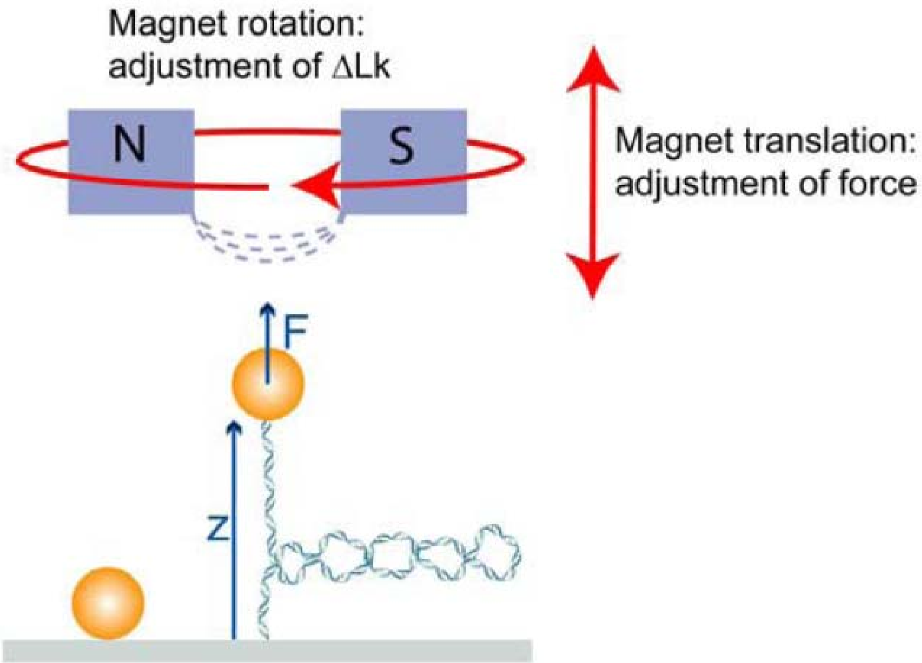
DNA Spercoiling

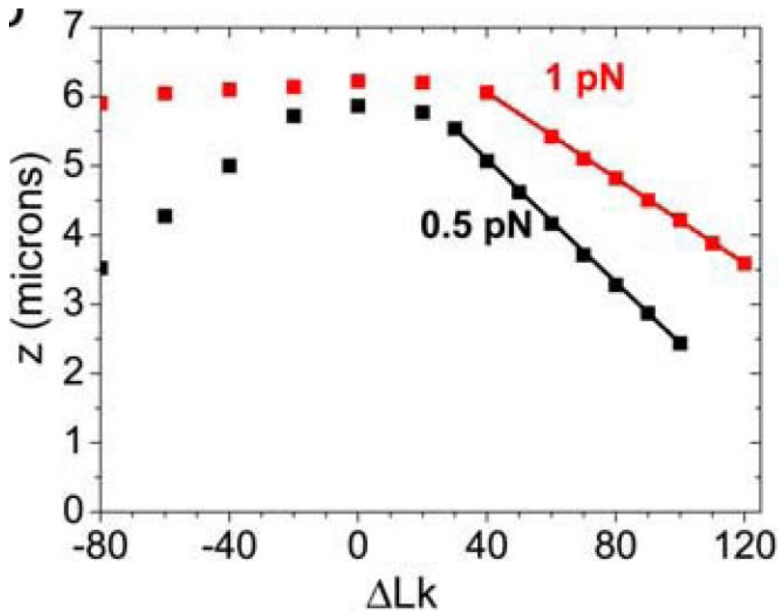


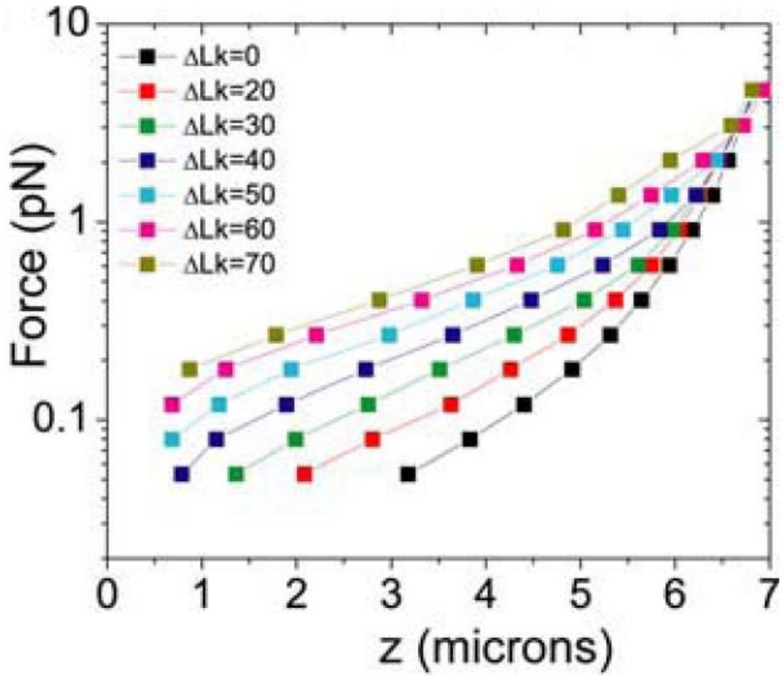
DNA Spercoiling



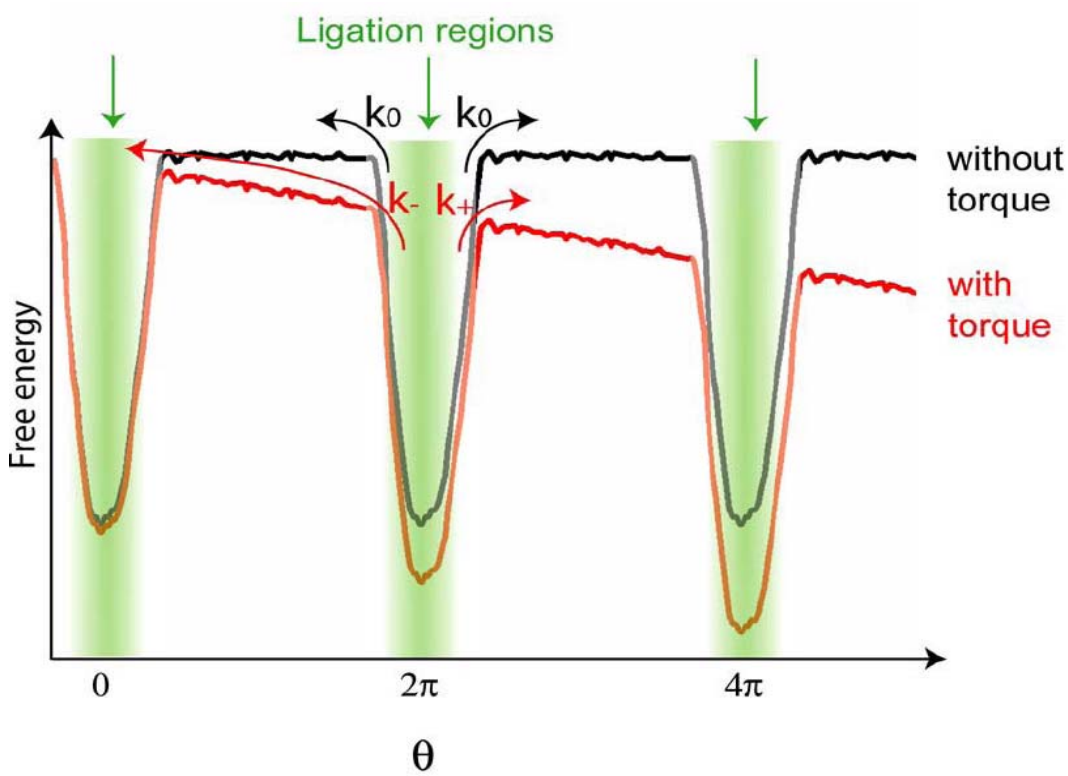
Magnetic tweezer

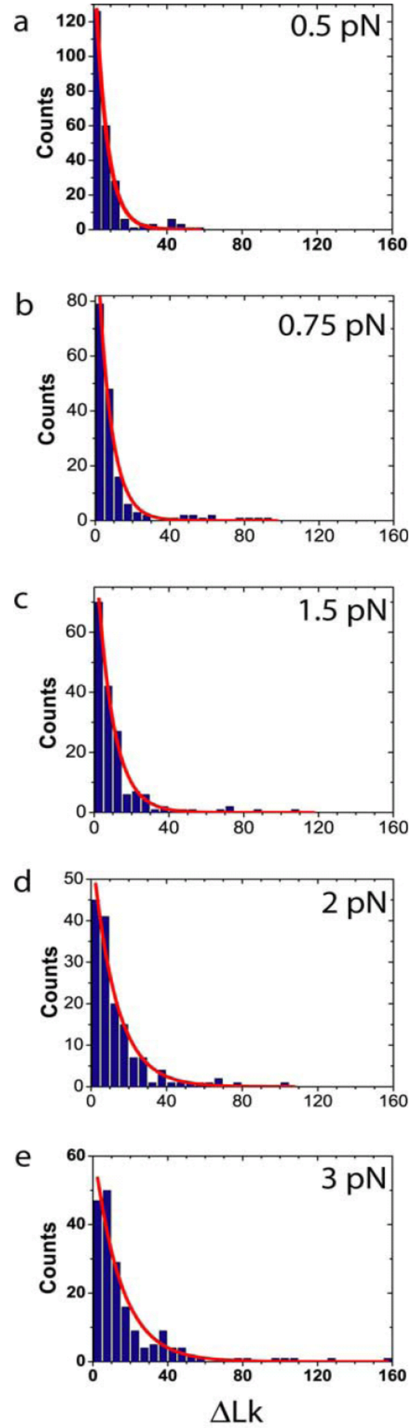




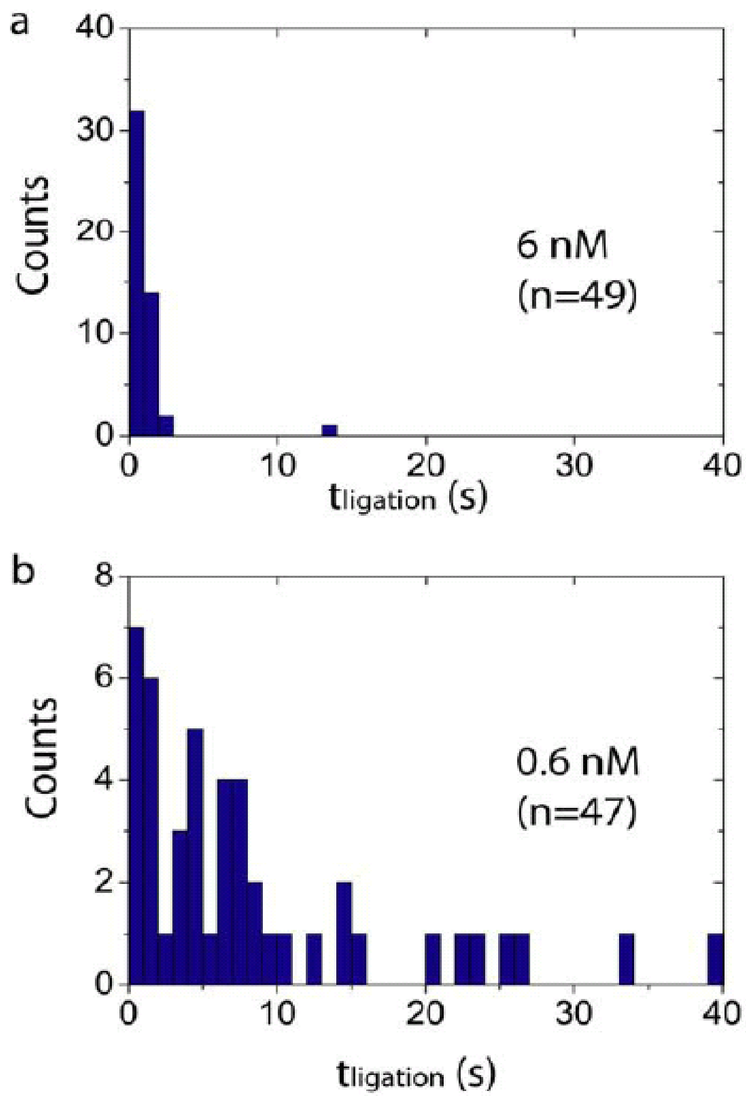


Distribution of intermediate step sizes

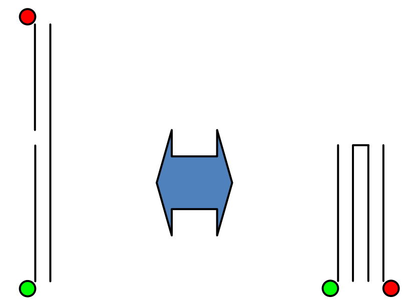




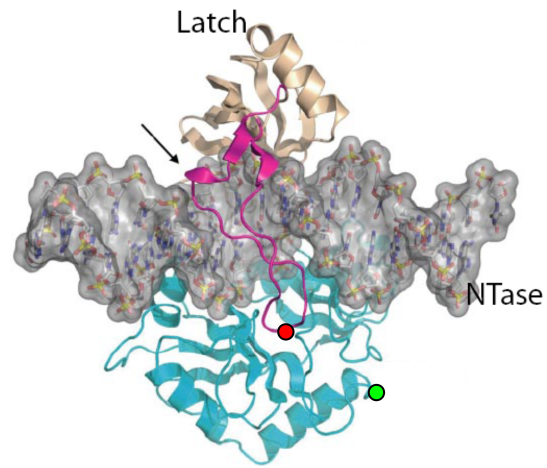
K29A mutant

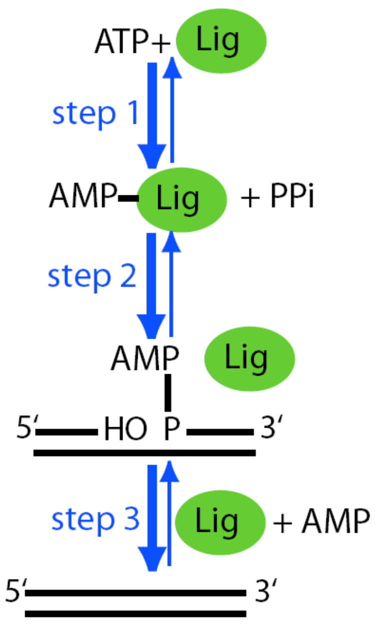

a
40
30
20
10
0
Counts
6 nM
(n=49)
0
10
20
30
40
tligation (s)
b
8
6
4
2
0
Counts
0.6 nM
(n=47)
0
10
20
30
40
tligation (s)

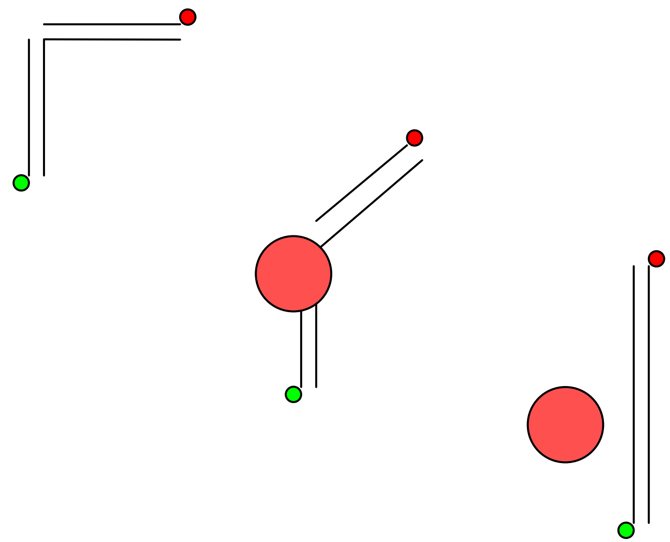
Enzyme	Force, pN	Average step size ± SEM	Ligation probability per turn, %	Fraction of dissociation events
WT	0.5	8.3 ± 0.6	12	18/174 (10%)
	0.75	10.7 ± 1.0	9	13/123 (11%)
	1	10.2 ± 0.8	10	12/128 (9%)
	1.5	11.7 ± 1.0	9	10/113 (9%)
	2	14.0 ± 1.2	7	16/94 (17%)
	3	18.6 ± 1.5	5	5/60 (8%)
K27A	1	5.4 ± 0.4	19	17/204 (8%)
ΔLatch	1	43 ± 16	2	13/25 (52%)



Salt dependent







Kd, ligation time etc..