Extreme Bendability of DNA Less than 100 Base Pairs Long Revealed by Single-Molecule Cyclization

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The classical view of DNA posits that DNA must be stiff below the persistence length [<150 base pairs (bp)], but recent studies addressing this have yielded contradictory results. We developed a fluorescence-based, protein-free assay for studying the cyclization of single DNA molecules in real time. The assay samples the equilibrium population of a sharply bent, transient species that is entirely suppressed in single-molecule mechanical measurements and is biologically more relevant than the annealed species sampled in the traditional ligase-based assay. The looping rate has a weak length dependence between 67 and 106 bp that cannot be described by the worm-like chain model. Many biologically important protein-DNA interactions that involve looping and bending of DNA below 100 bp likely use this intrinsic bendability of DNA.

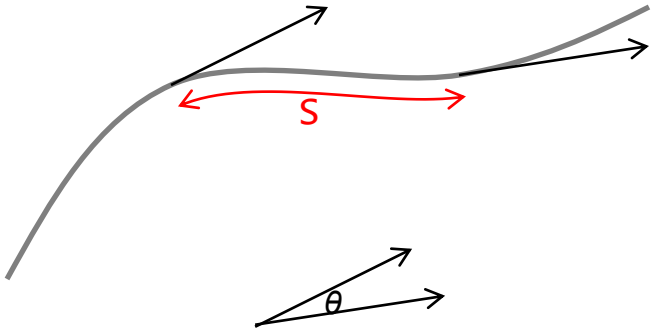
problem

Persistence length

basic mechanical property quantifying the stiffness of a polymer.

Persistence length of DNA = 50nm, 150bp

$$< \cos\theta >= e^{-s/l_p}$$



l_p : polymer's characteristic persistence length

s : tangent vector at a distance

θ : between a vector that is tangent to the polymer

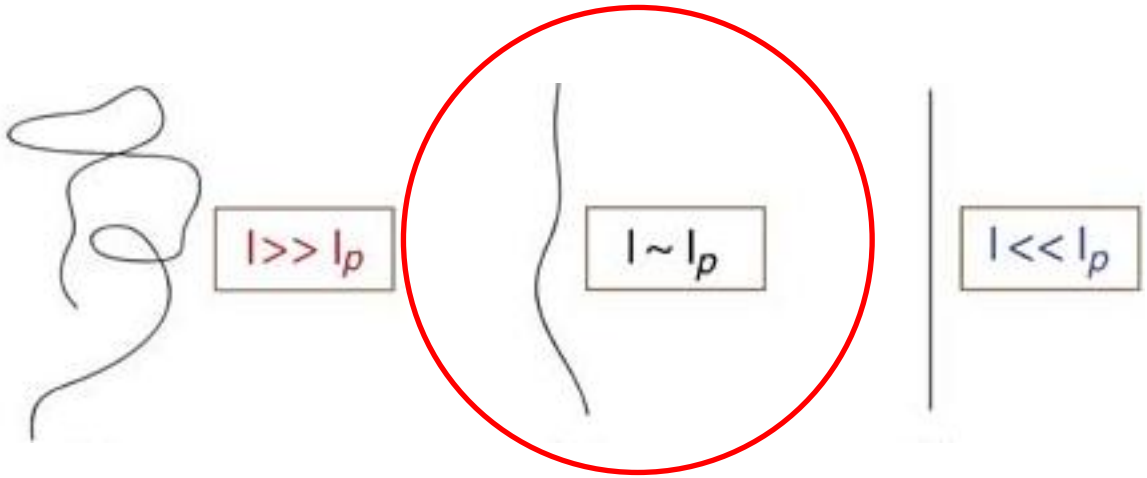
problem

Longer than the persistence length

Properties can only be described statistically like a **Three-dimensional random walk**

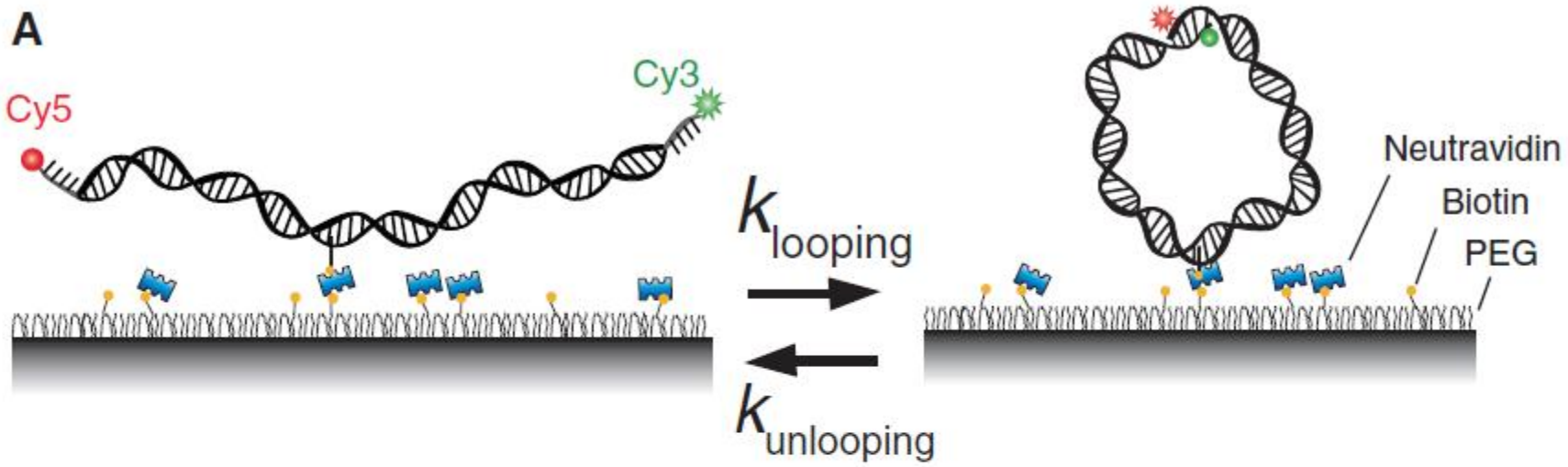
Shorter than the persistence length

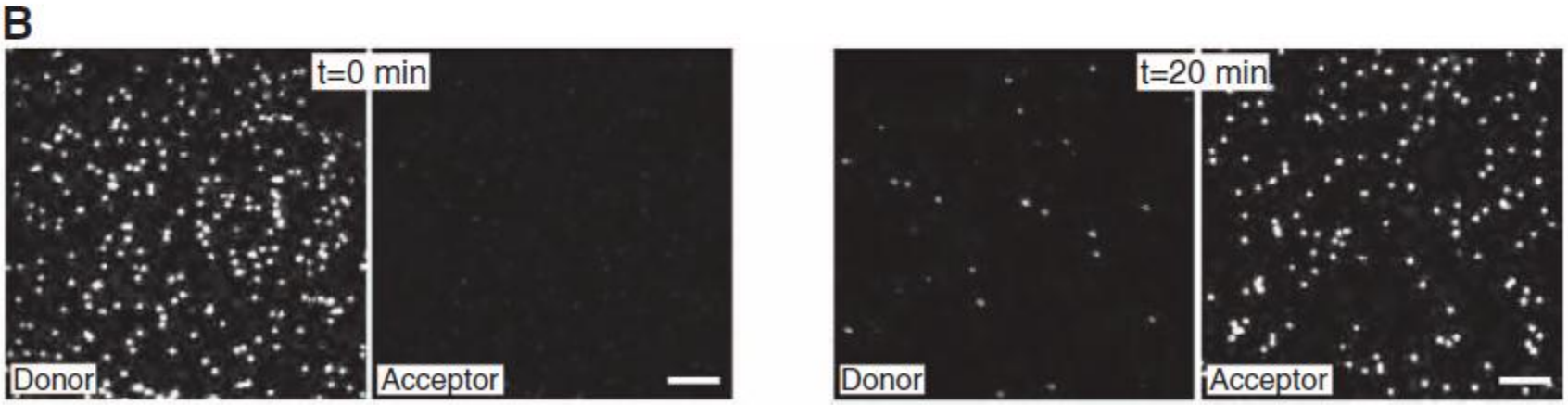
Molecule behaves rather like a **flexible elastic rod**



result

Dependence of DNA length





91-bp(base pair) DNA molecules

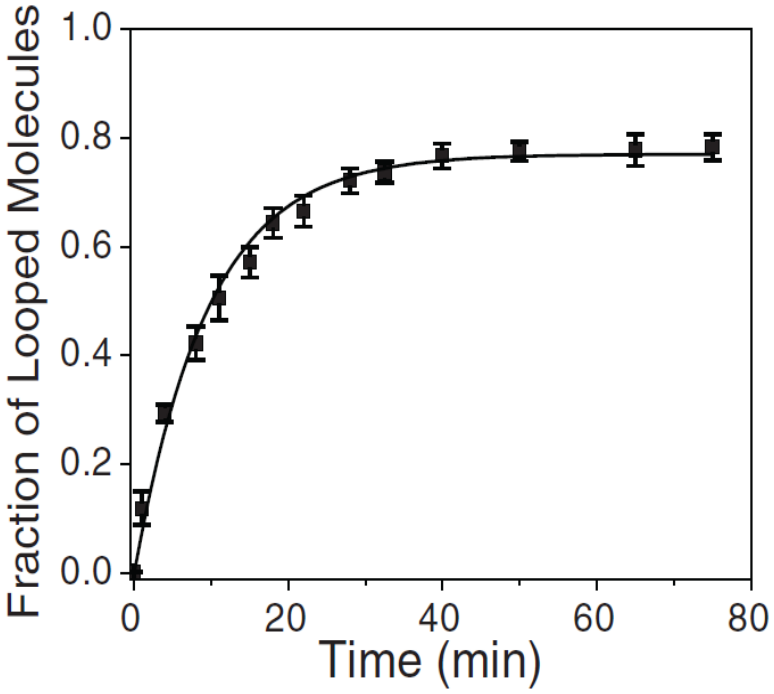
adding high salt (1 M NaCl) buffer

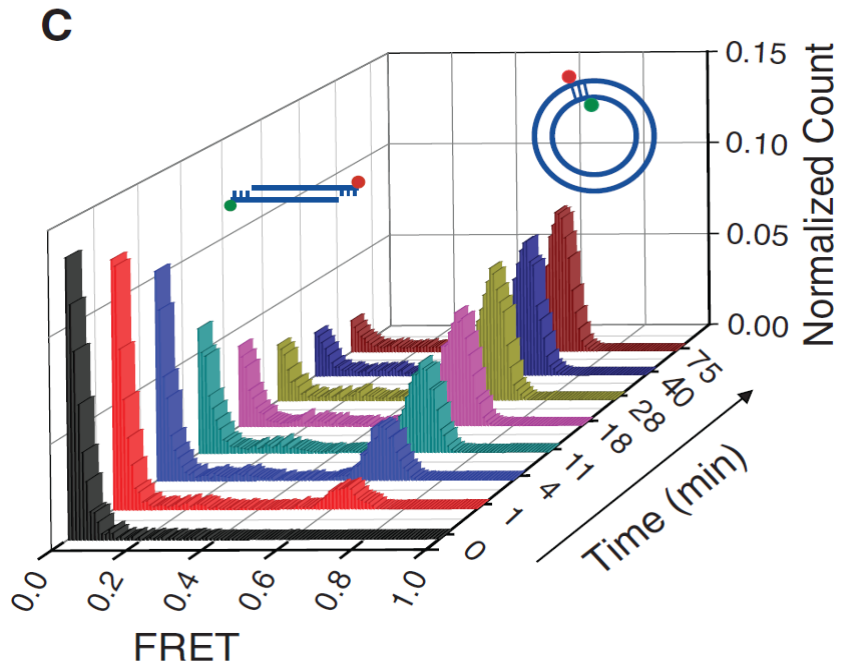
Donor -> acceptor

Looping happened

result

Dependence of DNA length





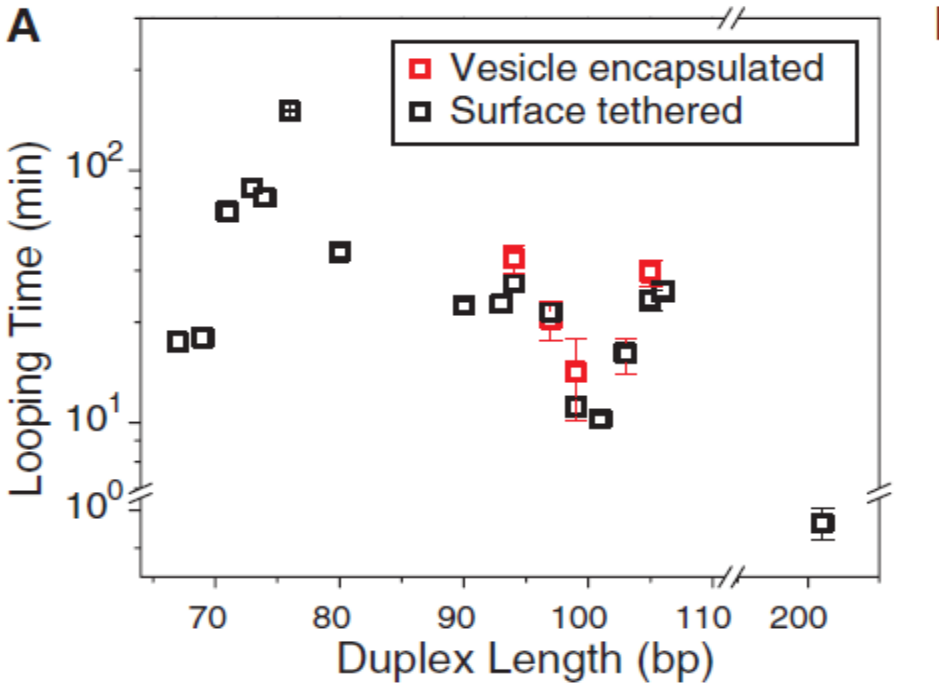
exponential fit to this curve gives R.

Using 91-bp initial dsDNA with 10-nucleotide (nt) overhangs

Obtain the looping rate R

result

Dependence of DNA length



Looping time is represented by 1/R

DNA much shorter than the persistent length : **hard to form a loop**

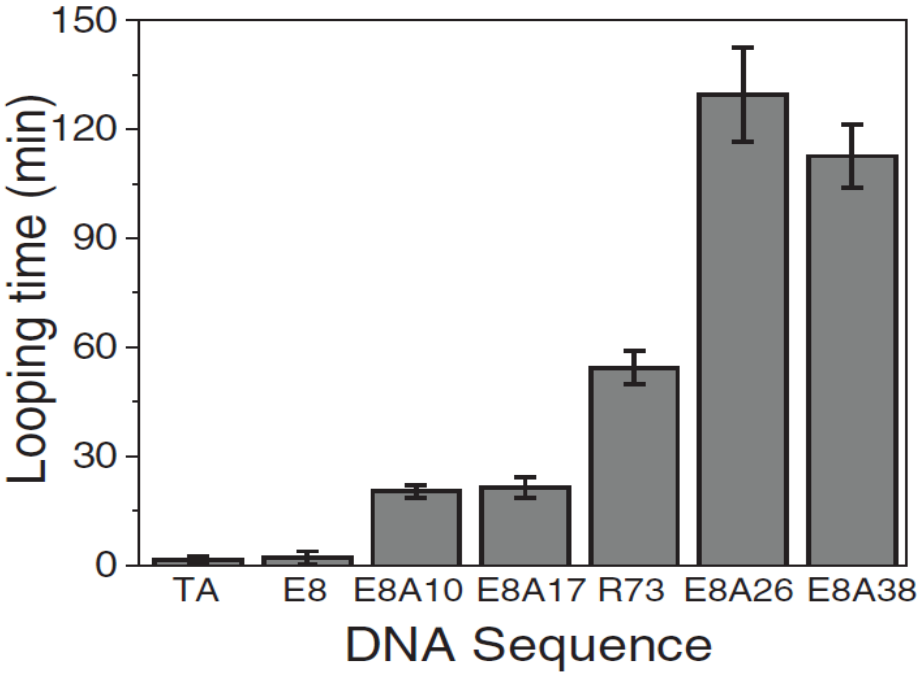
but →

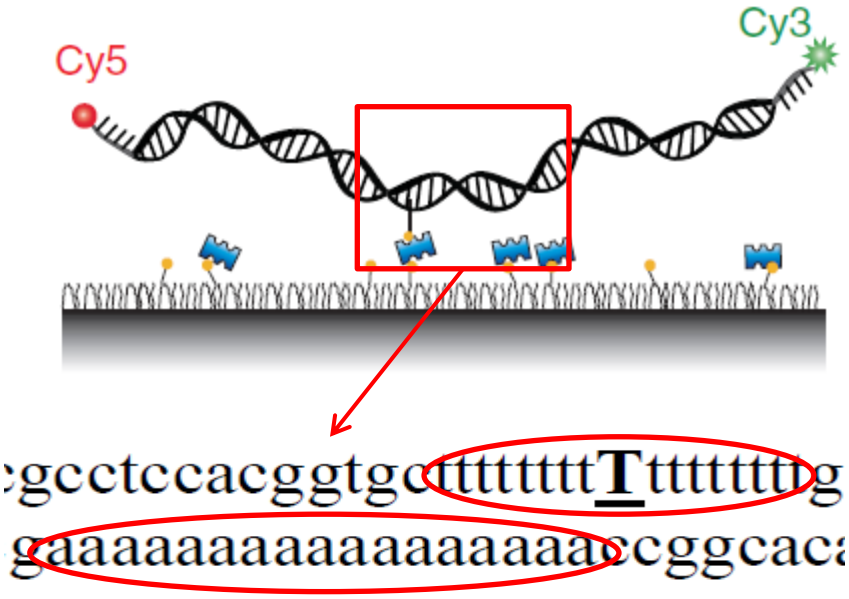
Result is different

No DNA length dependence

result

Dependence of DNA sequence





63-bp duplex length and 10-nt overhang.

TA : nucleosome positioning sequence

E8 : A bases in the middle of a random sequence

E8An : inserting A, number of n (n=0,10,17,26,38)

Change in sequence

↓

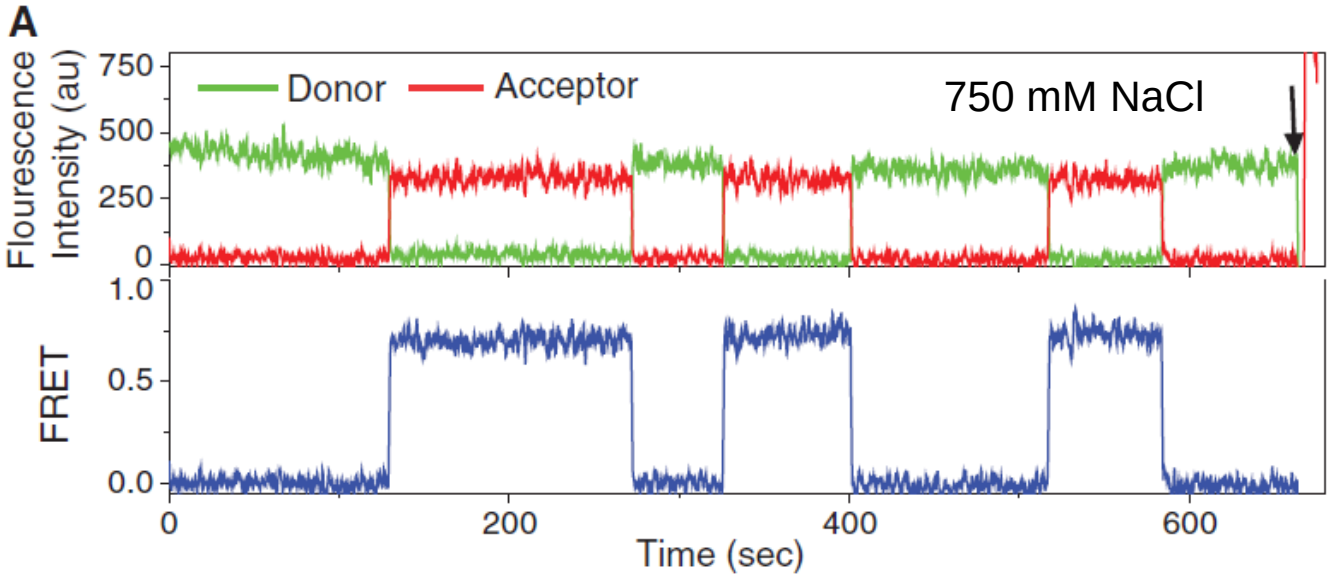
Change in looping time

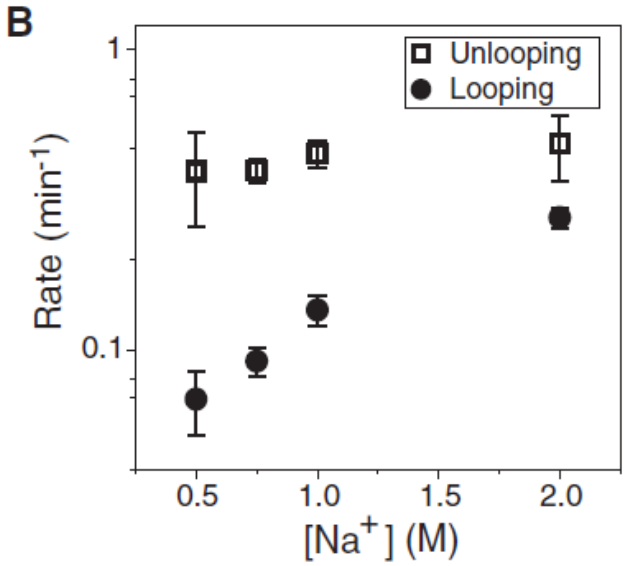
Influence of poly-A curvature-inducing

dependence of loop bendability

result

Dependence of Na+





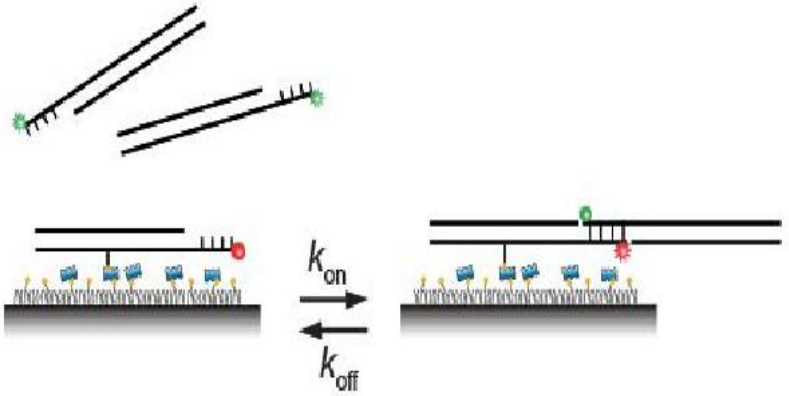
91-bp initial dsDNA with 8-nt single-stranded overhangs

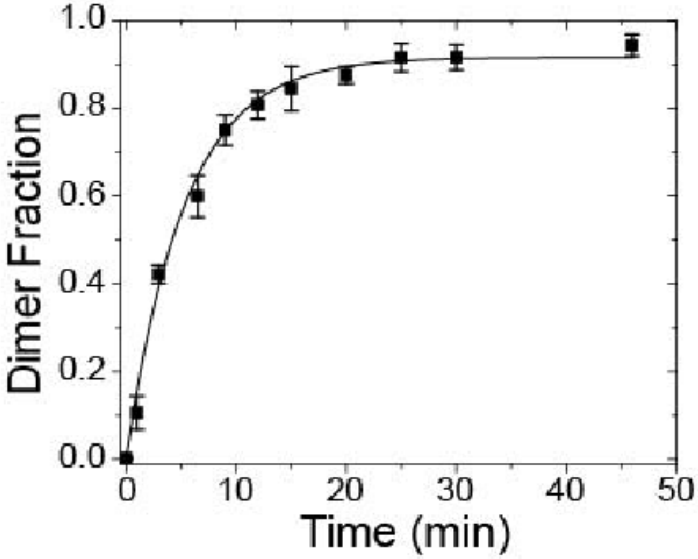
Unlooping rate **did not change** between 0.5M and 2M Na+

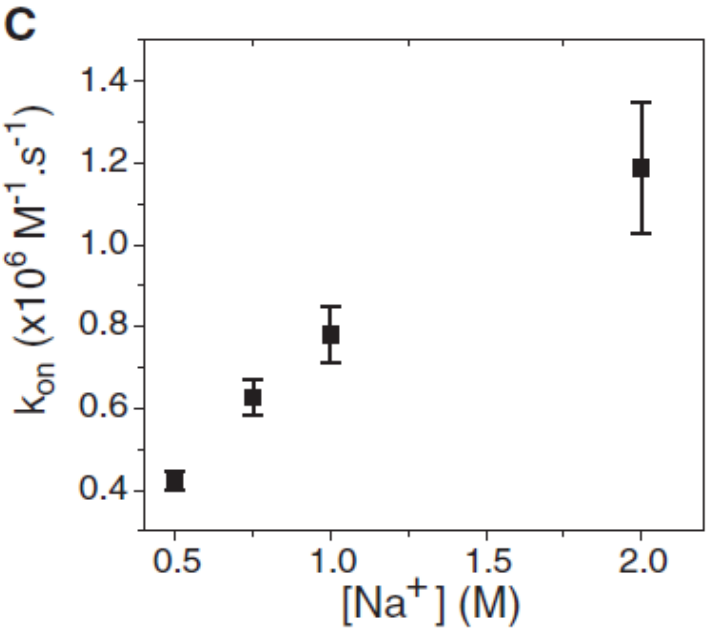
Looping rate is **increasing**

result

Dependence of Na+







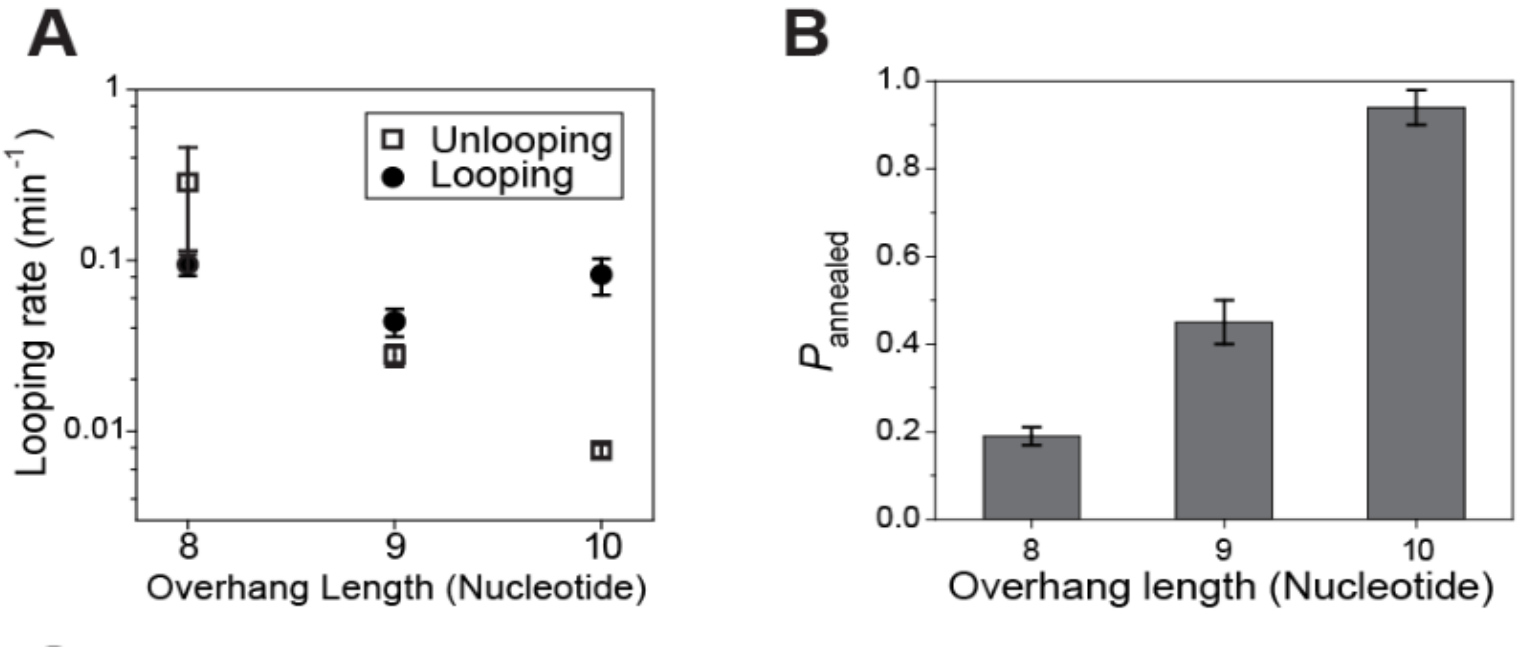
exponential fit to this curve gives R.

$$k_{on}[s^{-1}M^{-1}] = \frac{looping\ rate[s^{-1}]}{concentration[M]}$$

no dependence of loop bendability
Dependence of Kon

result

Dependence of overhang length



91-bp initial dsDNA, but with different overhang length(8,9,10 nt)

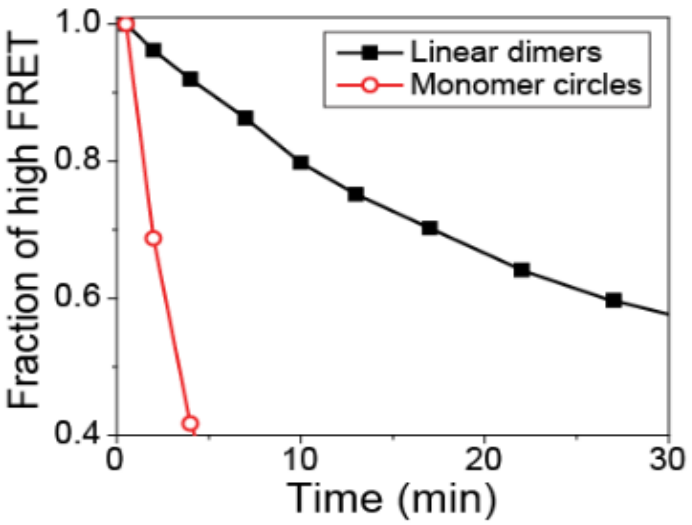
Unlooping rate is **decreasing**

Looping rate **Does not show significant difference.**

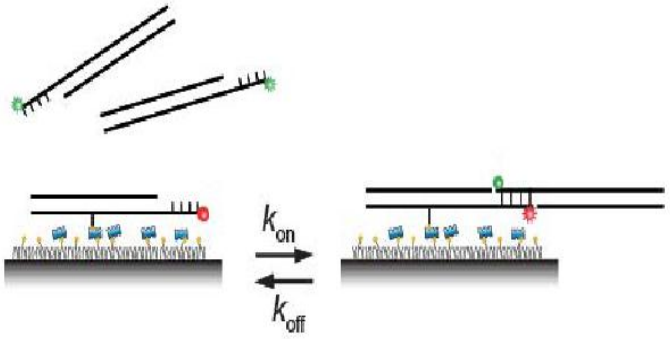
No dependence of loop bendability

result

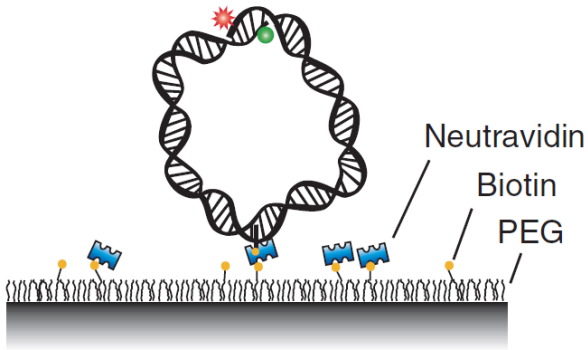
Dependence of internal force



91-bp initial dsDNA with 8-nt single-stranded overhangs



Linear dimers



Monomer circles

Monomer circles

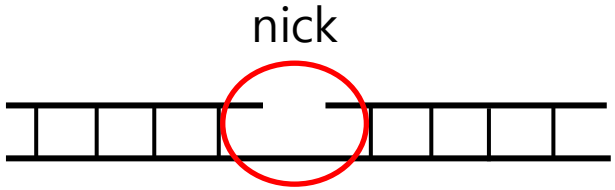
Elastic energy provide a shear force

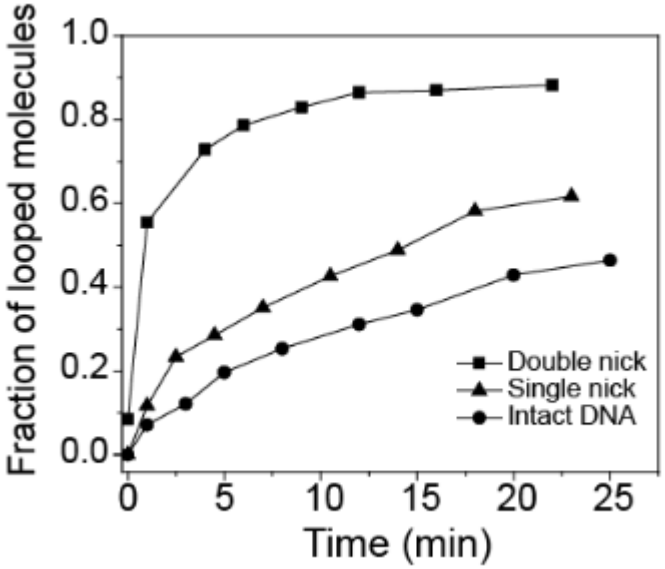
➡ **melts 20 times as fast** in a DNA circle as in a DNA dimer

dependence of loop bendability

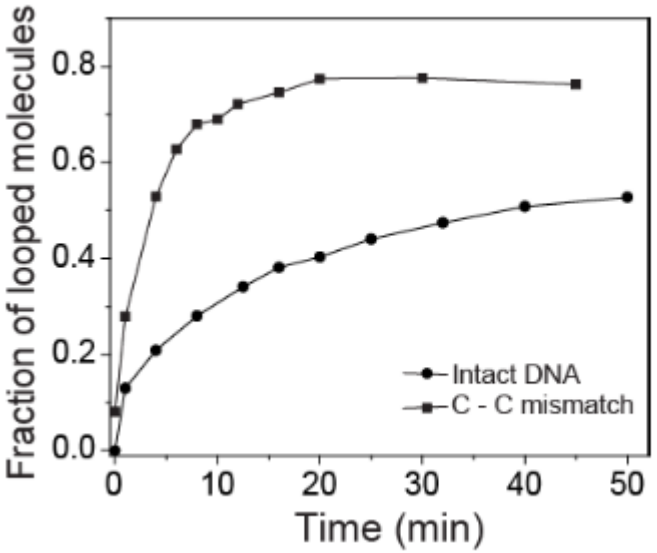
result

Dependence of nick, mismatch





87 bp initial dsDNA with 10 nt overhangs



59 bp initial dsDNA with 10 nt overhang

loop rate

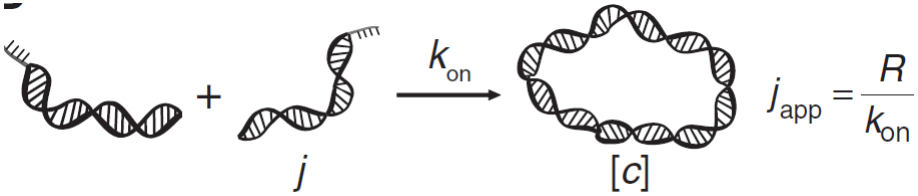
Double nick > single nick > intact DNA

loop rate

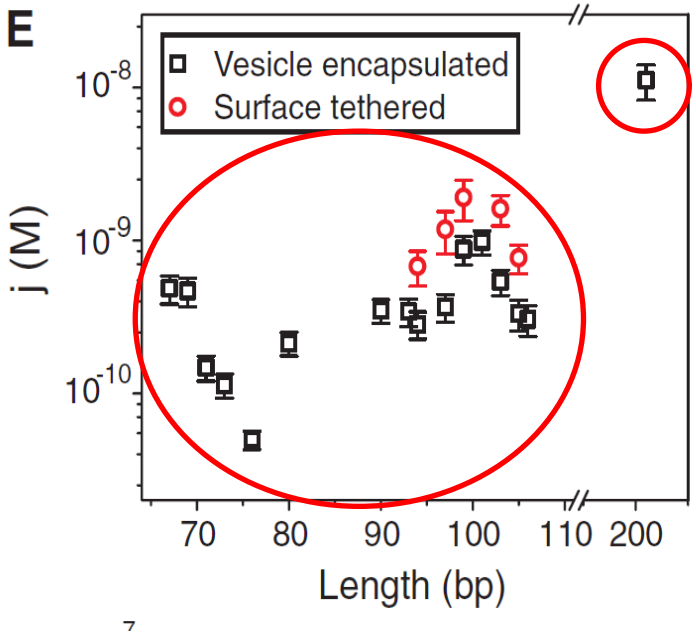
C-C mismatch DNA > intact DNA

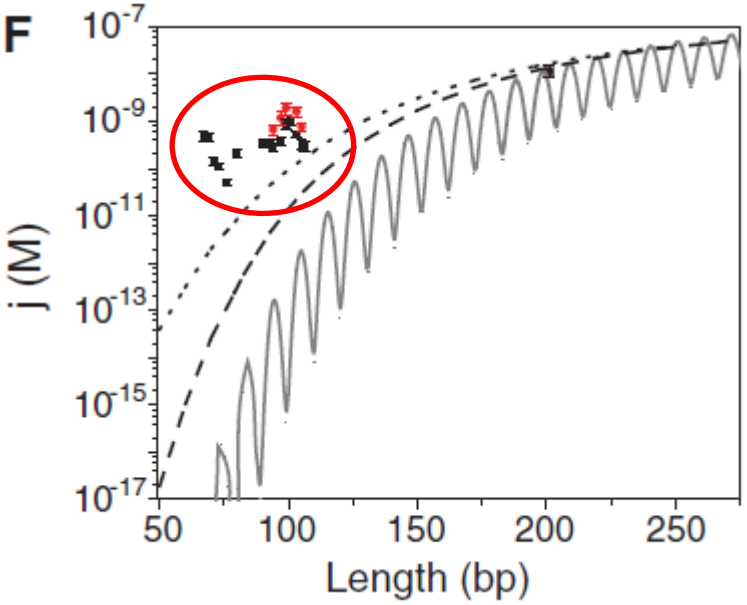
dependence of loop bendability

result



Dependence of j factor





j value means the effective concentration of the sticky ends

j values not matched the shorter length DNA

We don't get to j factor by using WLC model.

conclusion

Determination of Short length DNA loop bendability

dependence of loop bendability

DNA sequence	➡	increase poly-A, looptime ↑
internal force	➡	Unloop rate monomer circle > linear dimer
Nick and mismatch	➡	loop rate Nick, c:c mismatch > intact DNA

No dependence of loop bendability

increasing Concentration of Na+	➡	loop rate ↑ unloop rate —
increasing overhang length	➡	loop rate — unloop rate ↑