2015 Summer School on Polymers in Biology

DNA mechanics and structural diversity of DNA

@ KIAS, 22 Jun – 3 July

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Korea University

Lecture 1

- Hierarchy of biological organization
- Biomolecules: 1D polymers
- Examples of Polymers in Biology: DNA, RNA, Proteins, and Polysaccharides
- DNA: genetic material; double helix
- Central Dogma
- DNA thermodynamics

Lecture 2

- Watson-Crick base pair
- Effects of chemical factors on DNA stability
- DNA sequence vs. charged polymer
- Mechanical models: Freely Jointed Chain model
- Persistence length, end-to-end extension, radius of gyration, force response

Lecture 3

- Mechanical models: Worm Like Chain model
- DNA supercoils: definition (sign, magnitude)
- Linking number, twist, writhe
- Călugăreanu-White-Fuller theorem
- Energy associated with DNA supercoiling
- Non-canonical DNA structures (induced by SC)

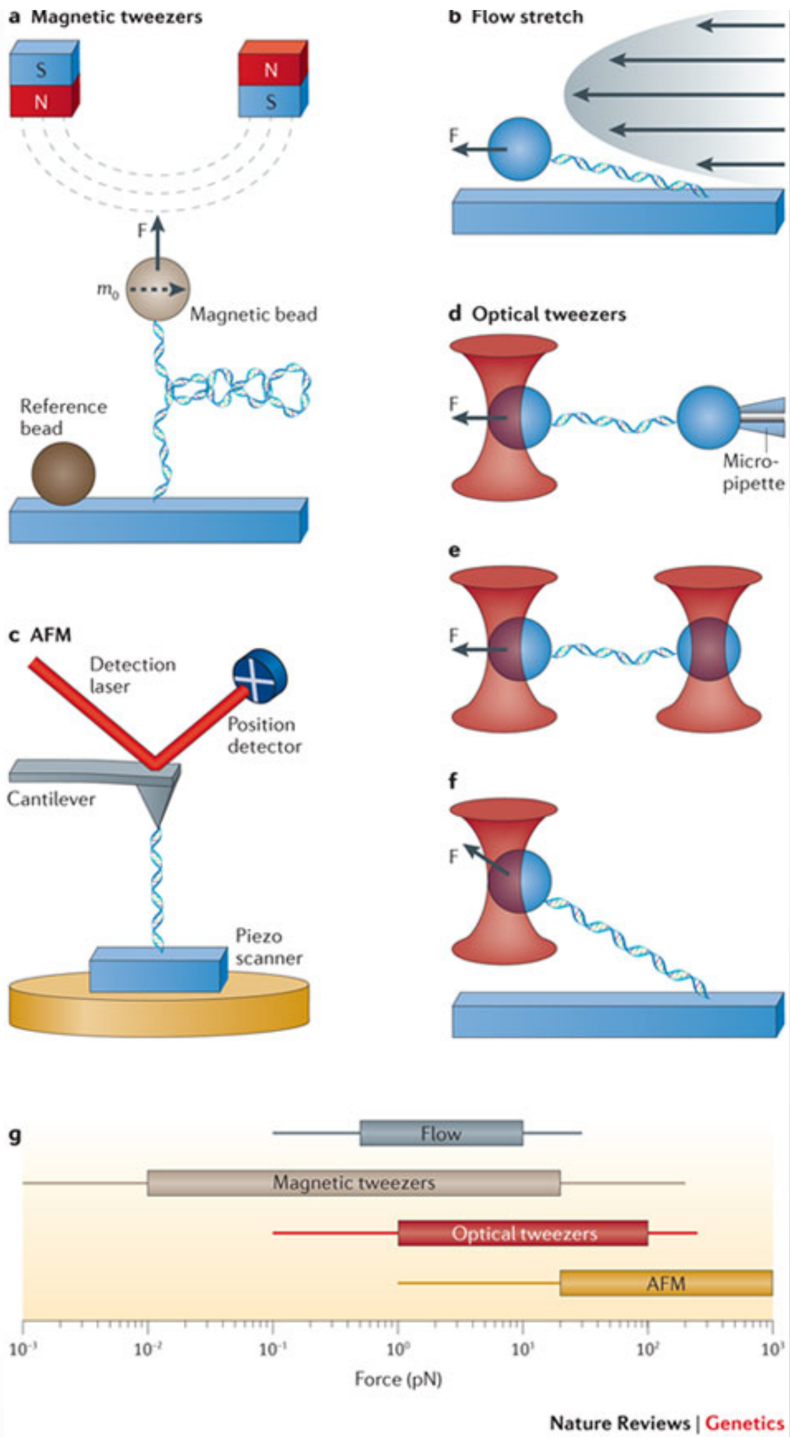
Lecture 4

- Single molecule methods (revisit)
- Hybrid single molecule technique of smFRET & MT
- Case studies: DNA mechanics via single-molecule methods
- Case studies: Non-canonical DNA and its dynamics via single-molecule methods

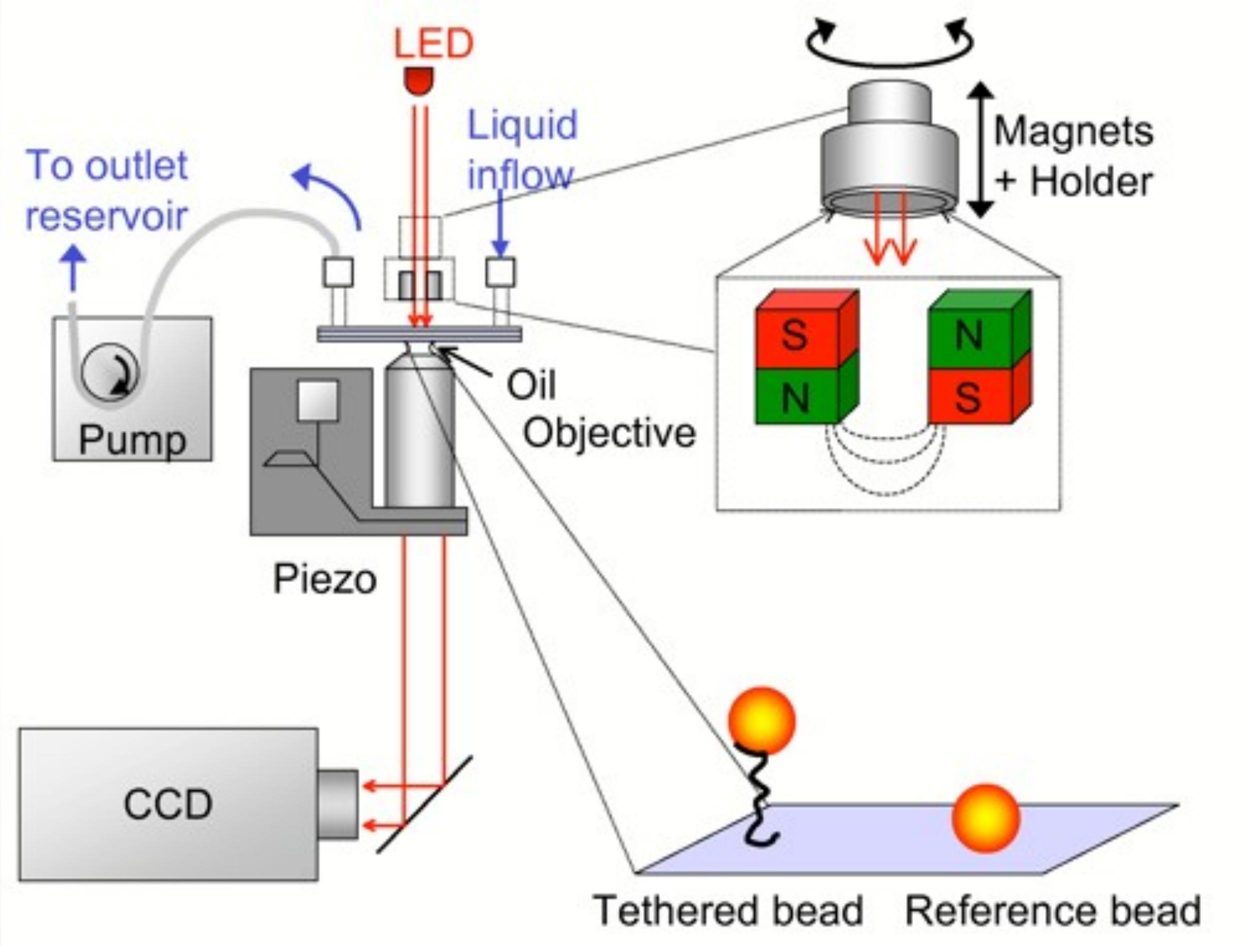
Single molecule manipulation

See Omar Saleh's lectures!

- Optical tweezers
- Magnetic tweezers
- AFM
-



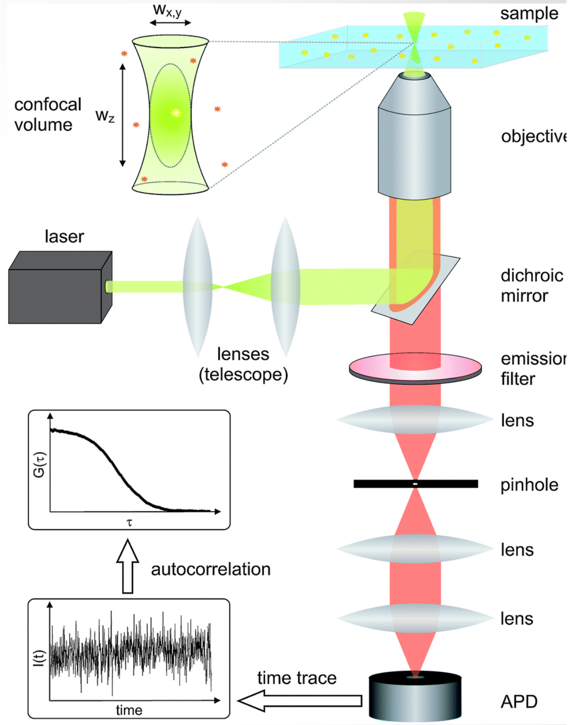
Magnetic tweezers



Single molecule fluorescence detection

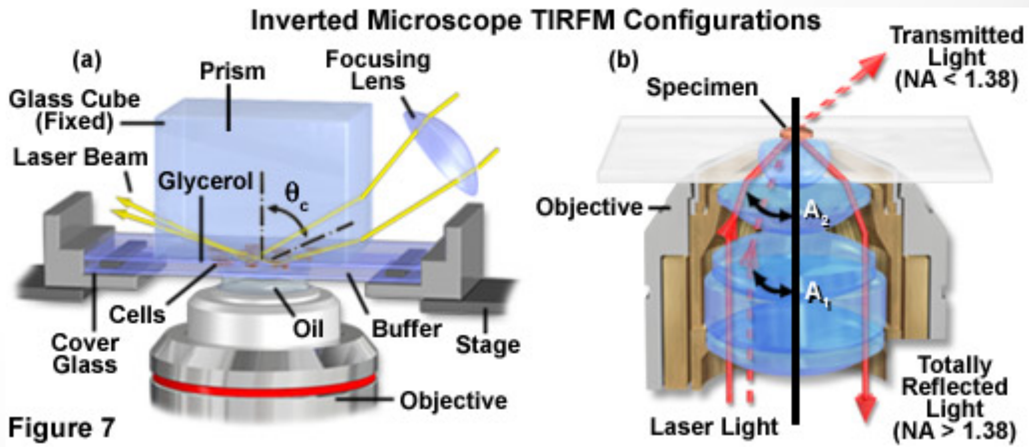
See Ben Schuler's lectures!

- Confocal based detection



- Total internal reflection based detection

- immobilize target molecules → long-term observation
  - Prism type
  - Objective type



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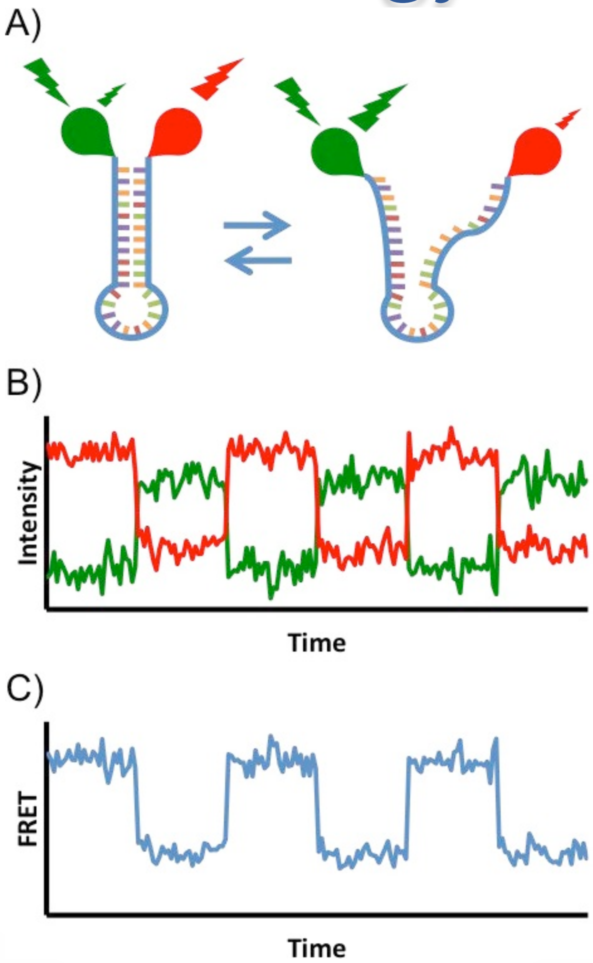
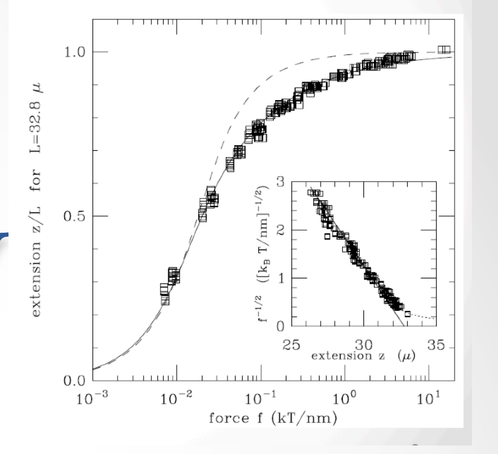
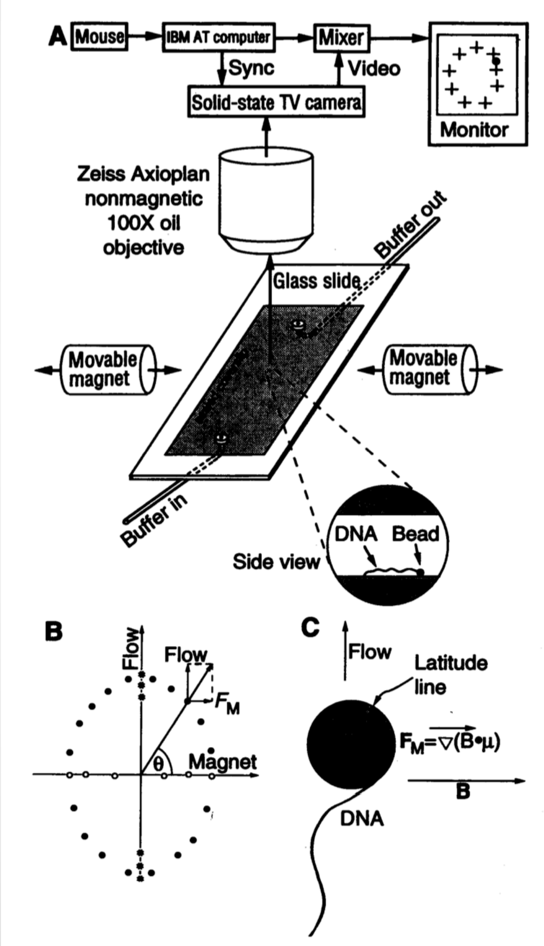
smFRET (single-molecule Fluorescence Resonance Energy Transfer)



DNA elasticity





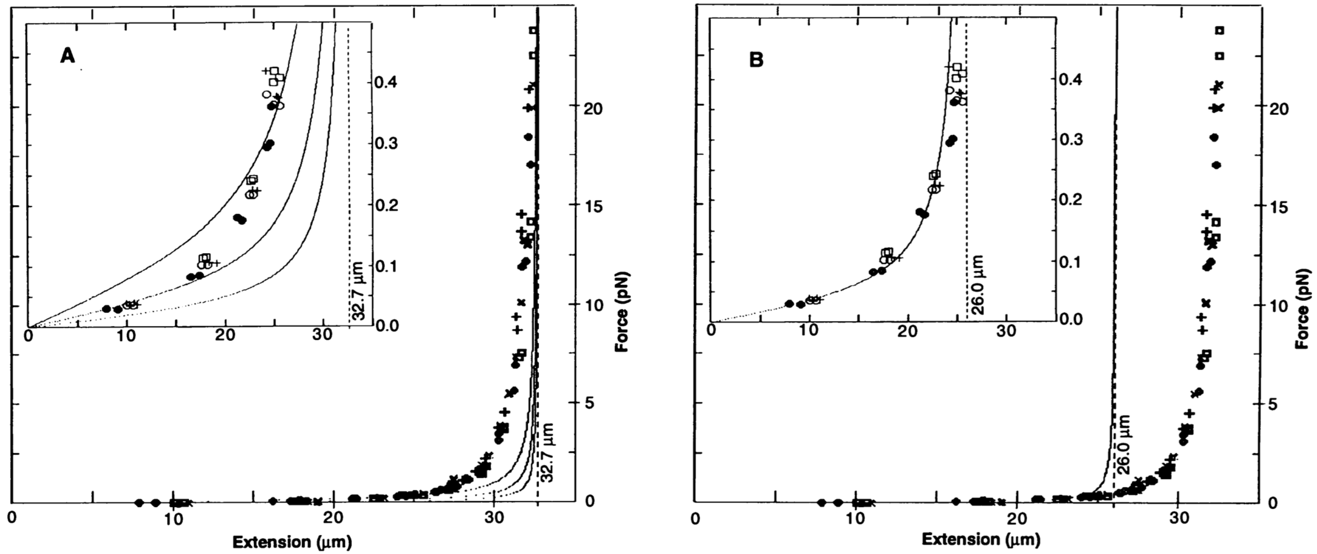
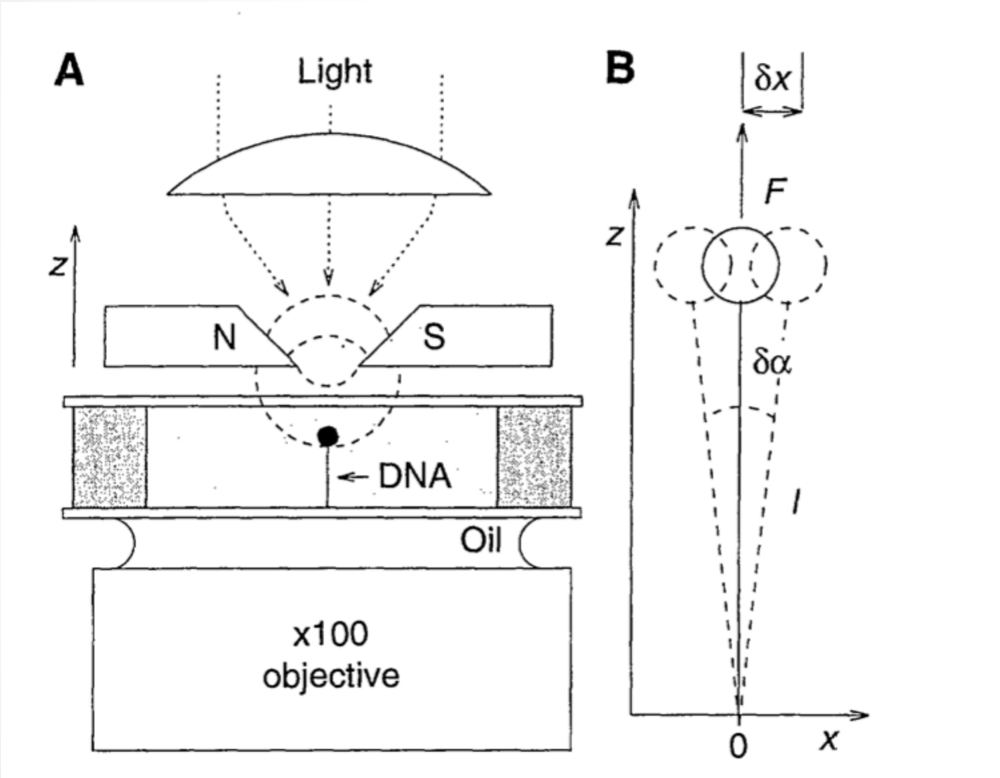


Fig. 3. (**A**) Force versus extension data for four different λ-dimer molecules (●, □, +, and ○) in 5 mM Na_2HPO_4 buffer (10 mM Na^+, pH 8.3). Inset: expanded vertical scale (0 to 0.5 pN). Continuous curves are from Eq. 2 assuming *L* = 32.7 μm and *b* = 500 Å (top), 1000 Å (middle), and 2000 Å (lower). *L* = 32.7 μm was chosen to agree with the accepted value of 3.37 Å rise per base pair (*30*), not to fit the data. (**B**) The same data compared with a Langevin curve *L* = 26 μm and *b* = 1400 Å. These values were chosen to match the low-force slope.

S. Smith, L Finzi, C. Bustamante, Science 1992

DNA supercoiling



T. Strick, V. Croquette, et al, Science (1996)

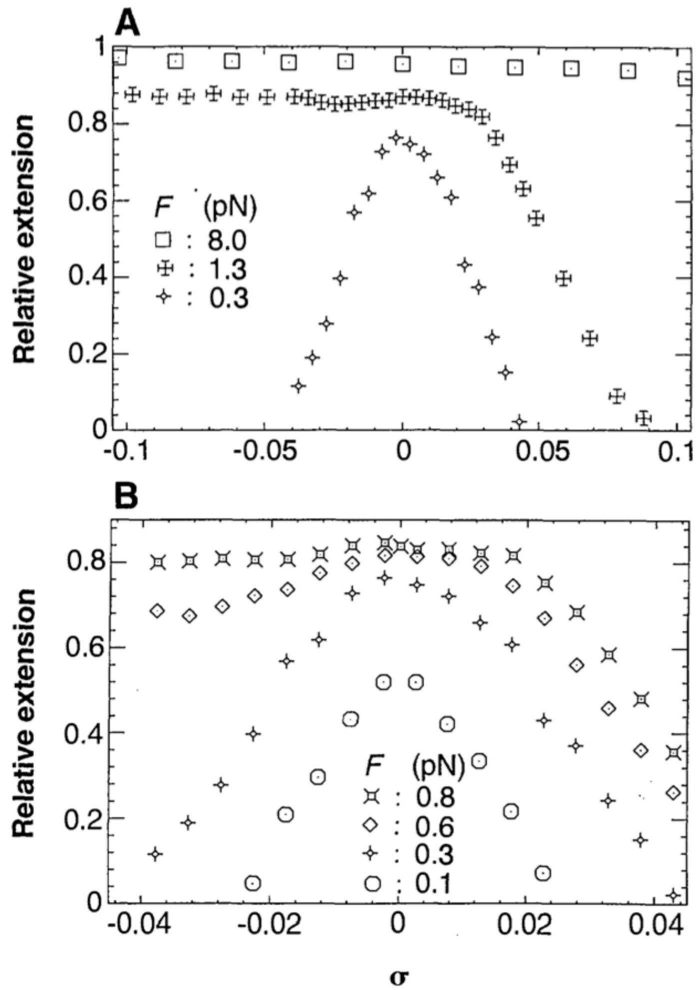


Fig. 3. Relative extension versus degree of supercoiling σ at various forces. (**A**) $F = 8$ pN, 1.3 pN, and 0.3 pN. (**B**) $F = 0.8$ pN, 0.6 pN, and 0.3 pN [as in (A)] and $F = 0.1$ pN. As in Fig. 2, one notices the symmetric behavior under $\sigma \rightarrow -\sigma$ at smaller forces and the transition to an extended state at greater forces, first at negative supercoilings (above 0.45 pN) and then at positive supercoilings (above 3 pN).

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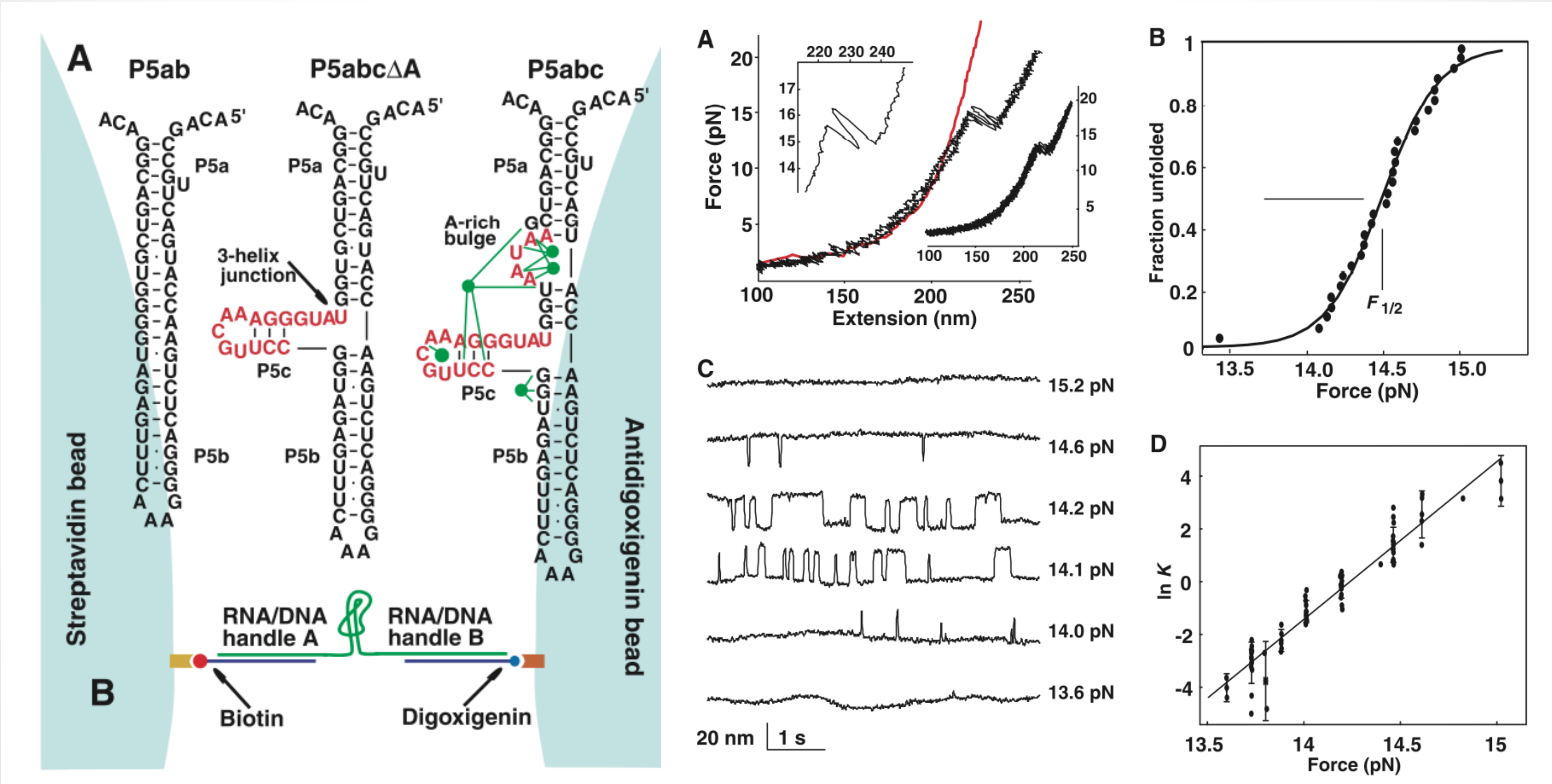
(RNA) hairpin unzipping



Liphardt, Tinoco, Bustamante, Science (2001)

DNA overstretching

S. Smith, Bustamante, et al, Science 1996

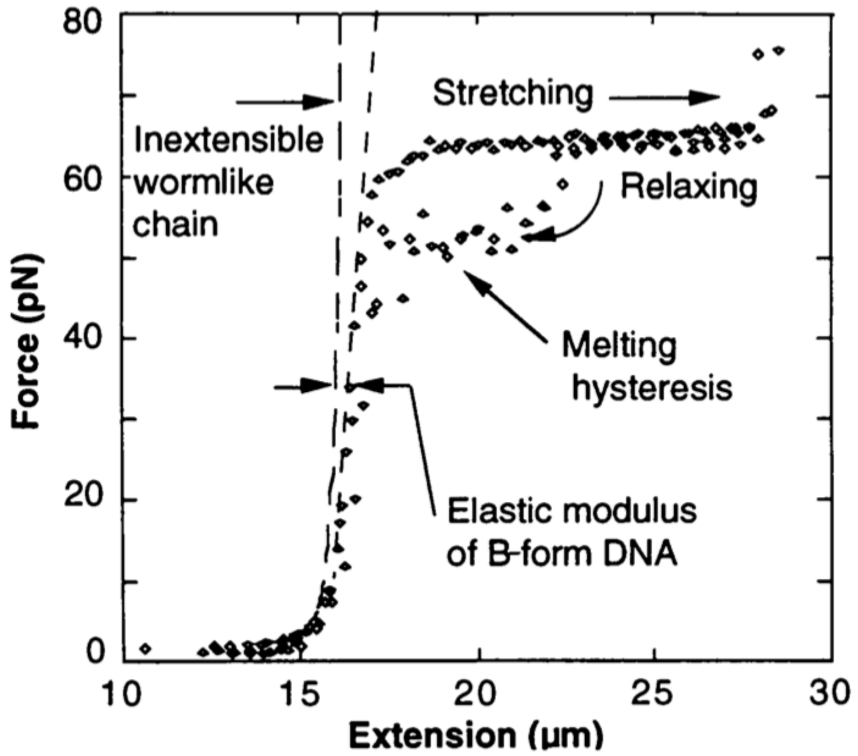
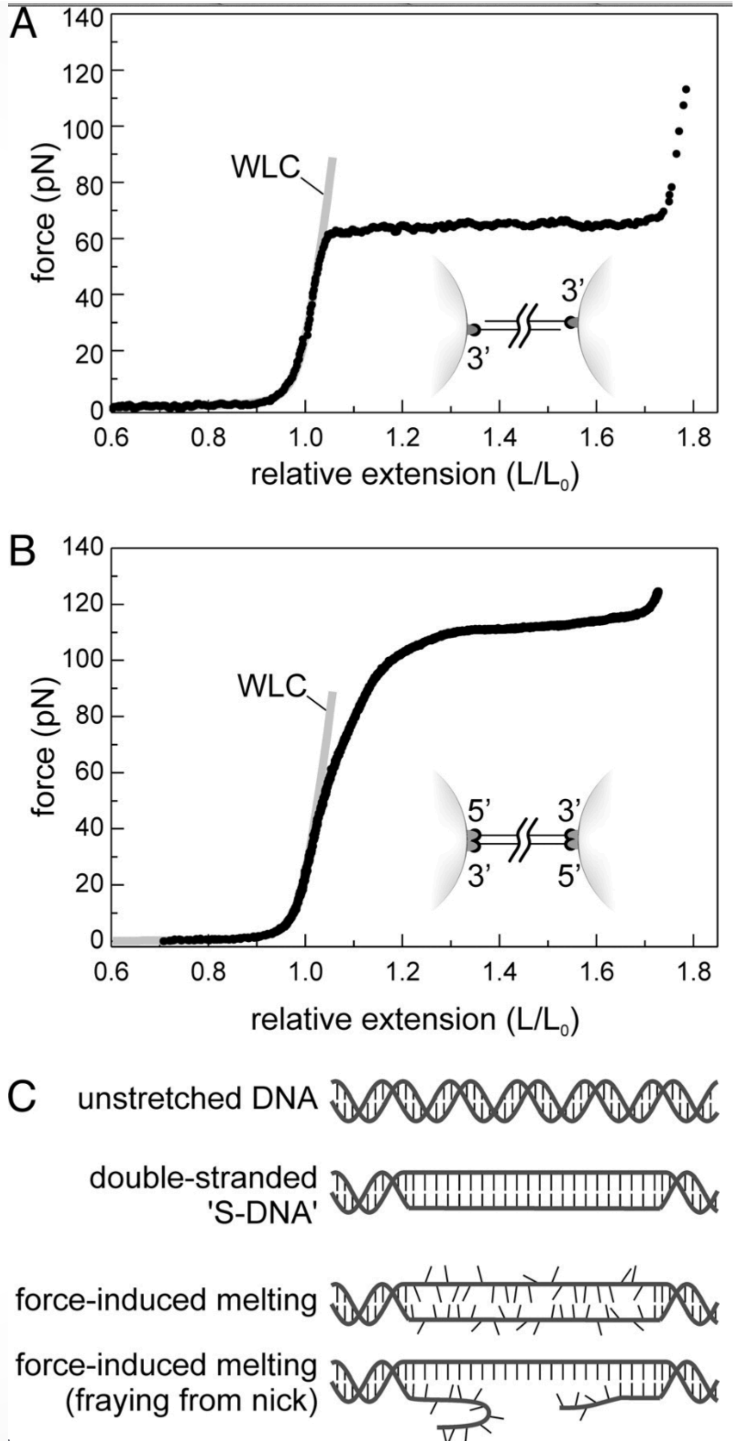
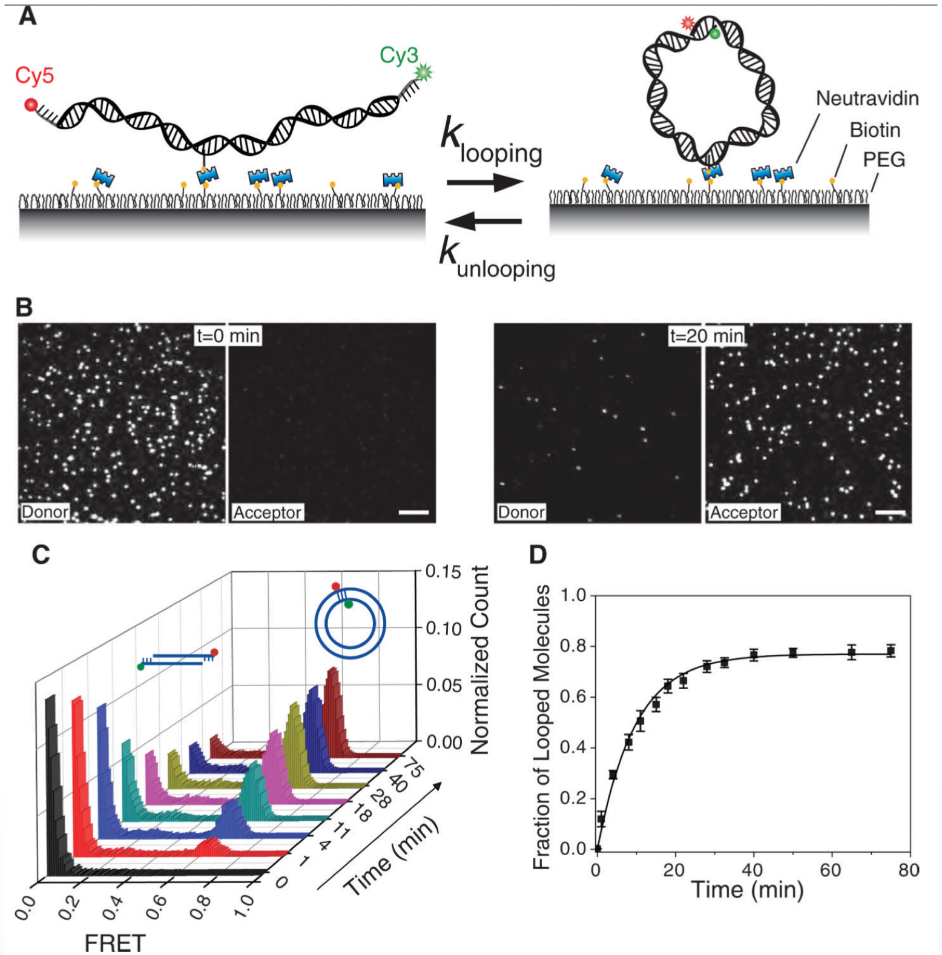


Fig. 2. Stretching of λ-phage DNA (NEB) in 150 mM NaCl, 10 mM tris, 1 mM EDTA, pH 8.0. The "inextensible wormlike chain" curve is from Bustamante *et al*. (2) for a *P* value of 53 nm and contour length of 16.4 µm.



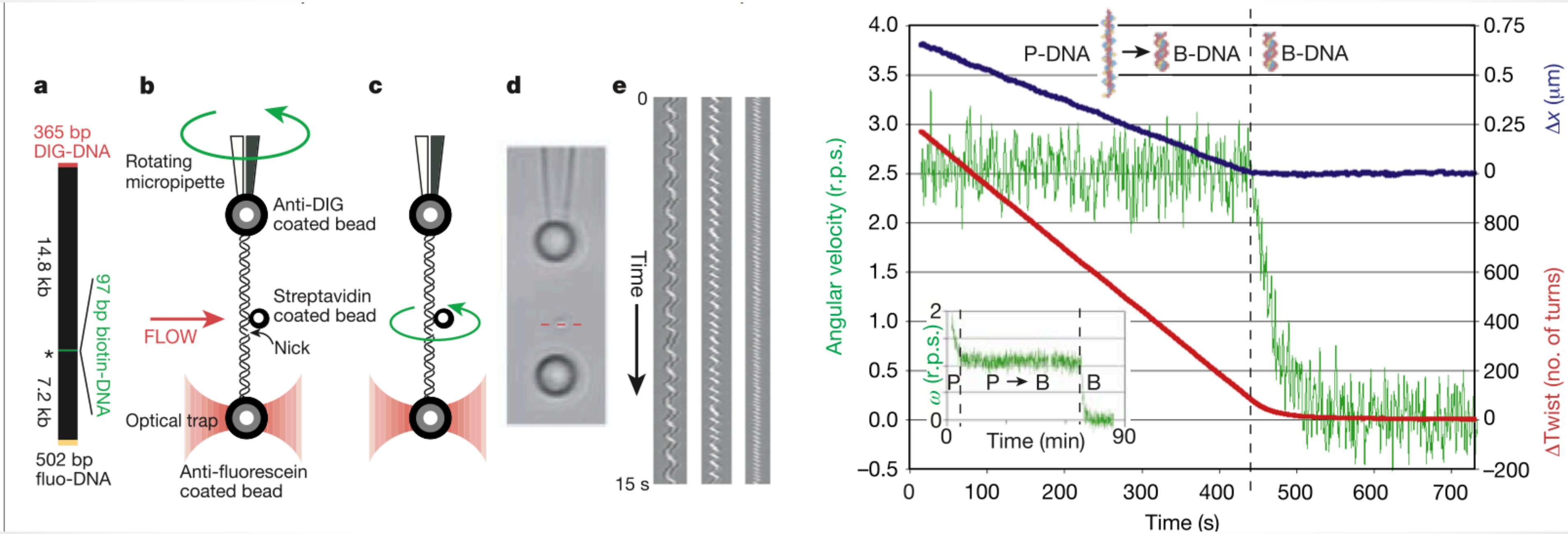
DNA looping & ligation



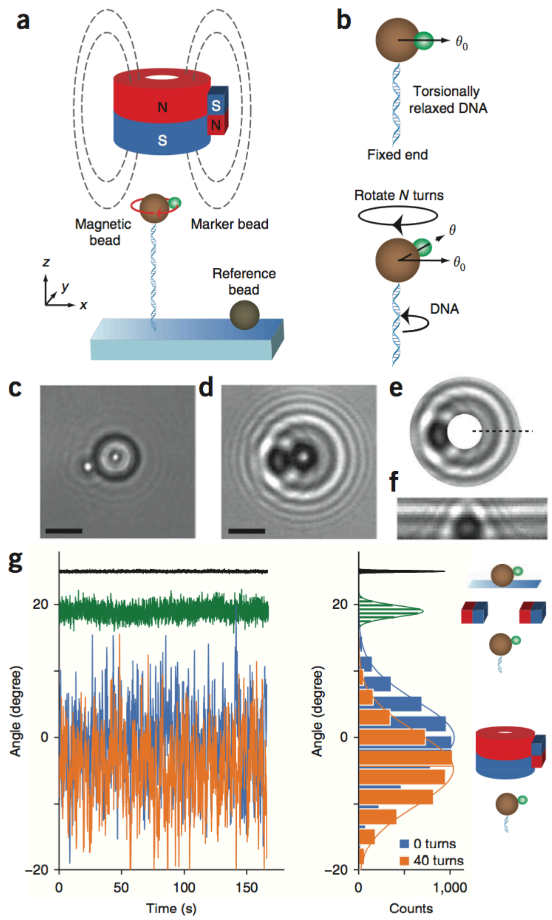
Vafabakhsh, Ha, Science 2012

DNA torsional stiffness C

- Bouchiat, Mezard PRL (1998): 86 nm
- Bryant, Bustamante, et al, Nature (2001) ~ 120 nm



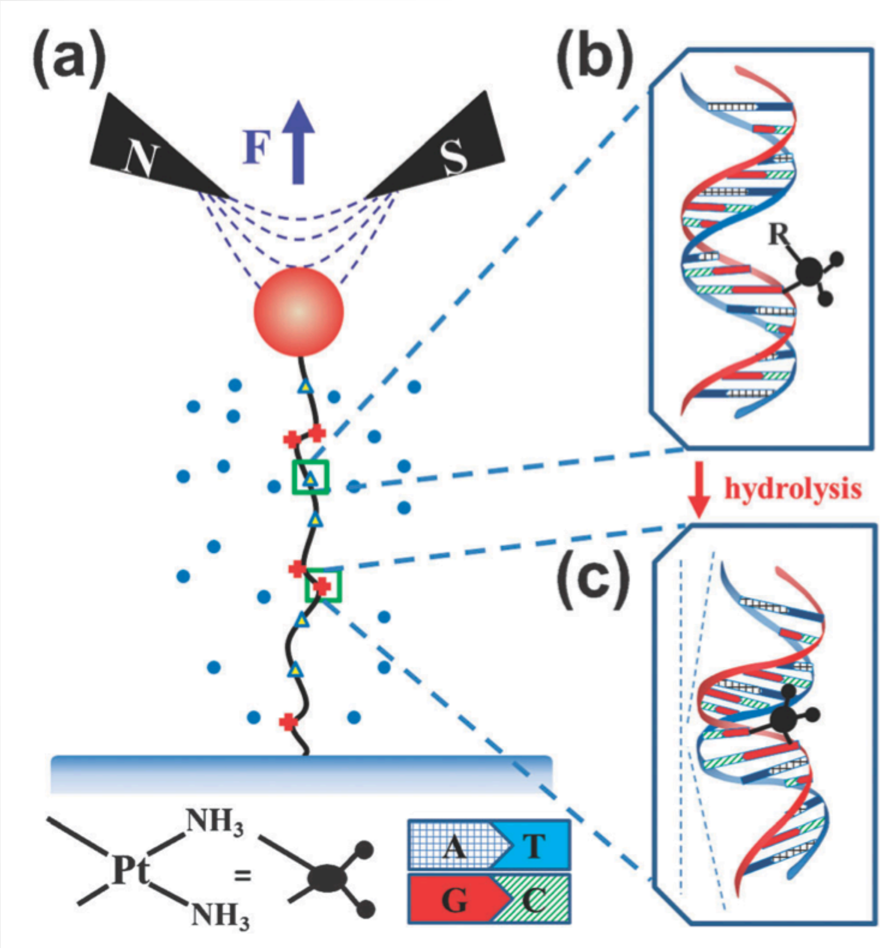
Magnetic torque tweezers

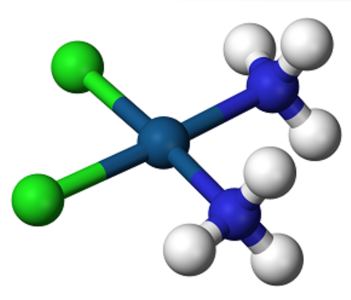


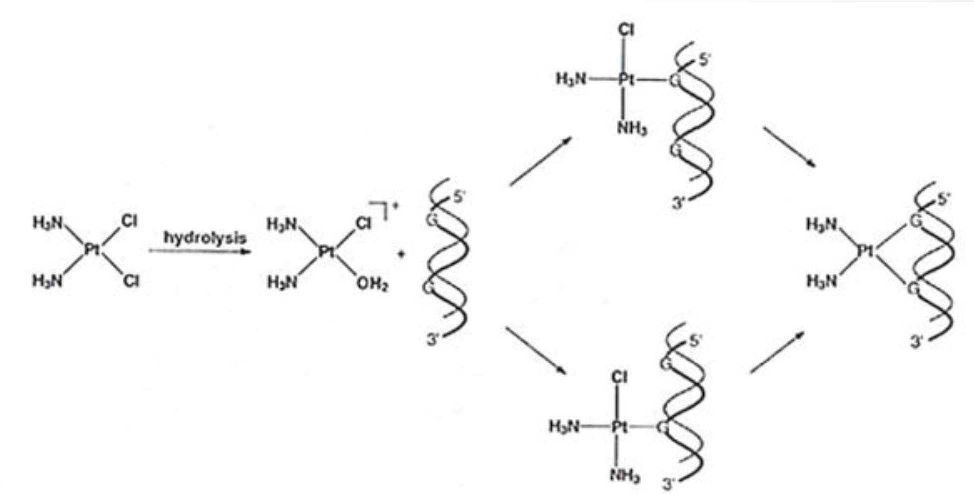
Lipfert, Dekker, Nat. Meth. 2011

The effect of kinks on DNA mechanics

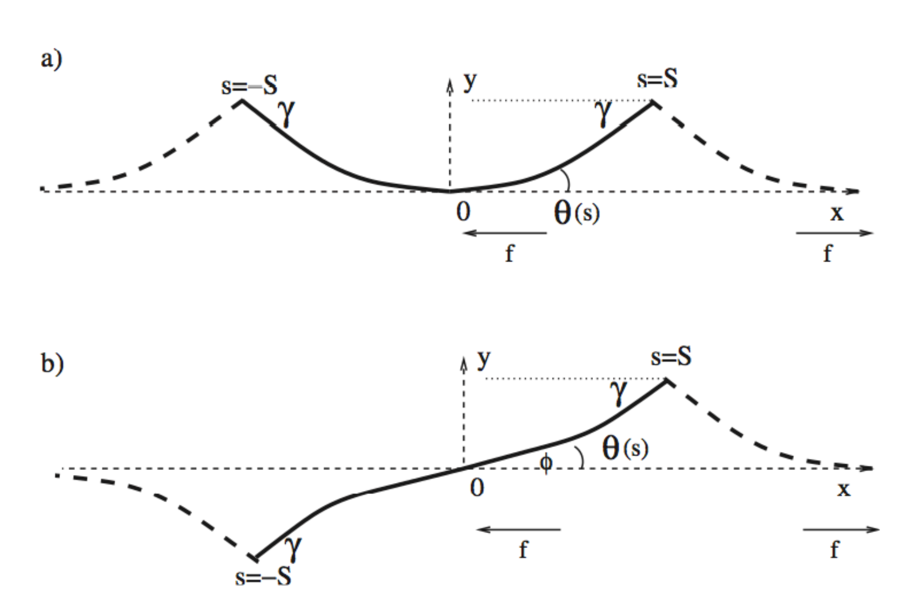
Cisplatin: anti-cancer drug







Euler elastica; Euler-Lagrange method



Large force limit

$$\frac{E}{k_BT} = \frac{l_p}{2}\int_{-S}^{S} ds\left(\frac{d\mathbf{u}(s)}{ds}\right)^2 - \frac{\mathbf{f}}{k_BT}\cdot[\mathbf{r}(L)-\mathbf{r}(0)],$$

$$L = \int ds\left[\frac{l_pk_BT}{2}\left(\frac{d\theta(s)}{ds}\right)^2 - f\cos\theta(s)\right]$$

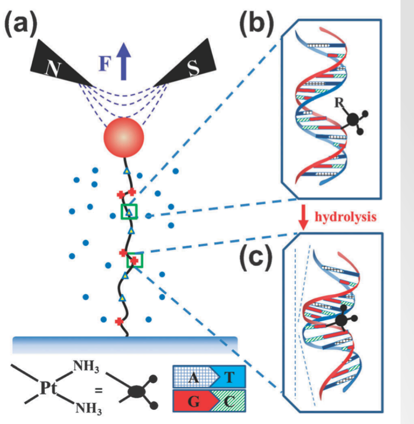
$$l_pk_BT\frac{d^2\theta}{ds^2} - f\sin\theta(s) = 0.$$

$$\tan[\theta(s)/4] = \tan(\gamma/4)\exp(-s/\Lambda), \quad \Lambda = \sqrt{k_BTl_p/f},$$

$$x = L - \frac{L\Lambda}{2l_p} - \frac{L}{S}\frac{\gamma^2\Lambda}{4} \equiv L - \frac{L}{2}\sqrt{\frac{k_BT}{f}}\frac{1}{\sqrt{\Gamma^K}}$$

$$\frac{1}{\sqrt{\Gamma^K}} = \frac{1}{\sqrt{l_p}}\left(1+\gamma^2\frac{l_p}{2S}\right)$$

Elasticity of DNA reveals the degree of cisplatin binding.



Small force limit

$$\langle \cos\ \theta(s)\rangle = e^{-s/l_p},$$

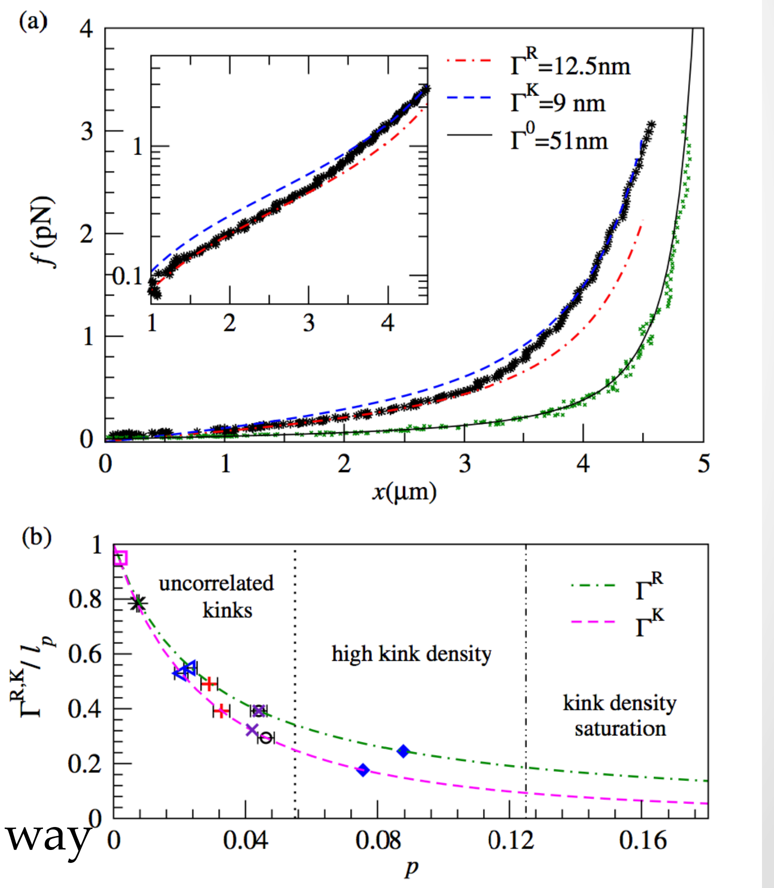
$$\cos\ \theta_k = e^{-s_k/l_p}$$

$$K \equiv \langle \cos\ \theta_1\rangle = (1-p)e^{-a/l_p} + pe^{-a/l_p - s_k/l_p}$$
$$= e^{-a/l_p}[1 - p(1 - e^{-s_k/l_p})].$$

$$\langle \cos\ \theta_m\rangle = K^m$$

$$\Gamma^R = -a/\ln(K)$$

$$\frac{1}{\Gamma^R} = \frac{1}{l_p} + \frac{p}{a}(1 - e^{-s_k/l_p})$$



Determine cisplatin binding in a self-consistent way

N.-K. Lee, J.-S. Park, …, S.-C. Hong, PRL 2008

σ-extension measurement permits the degree of cisplatin binding.

were comparable under the reaction conditions. From the total peak shift, the degree of total cisplatin binding, initially either mono- or bi-functional, can be estimated by the relation introduced above. From Fig. 4, total $\delta\sigma$ is $\sim$1.1% and therefore p is $\sim$2.9%. From the elasticity measurements described in the previous section, p was $\sim$2.3%. The degrees of cisplatin binding evaluated from two separate methods are in reasonable agreement.

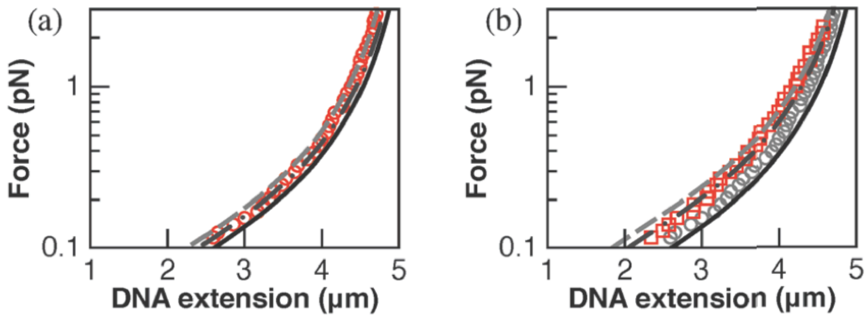


Fig. 2 Monofunctional cisplatin adducts in the presence of 24 mM $NaHCO_3$ and their conversion to bifunctional adducts. (a) The force–extension curves of a dsDNA molecule measured at t = $\sim$30 min after the DNA was incubated with cisplatin in [$NaHCO_3$] = 24 mM (red circles). (b) After washing with [NaCl] = 10 mM, ξ dramatically decreased resulting in a large shift in the force–extension curve

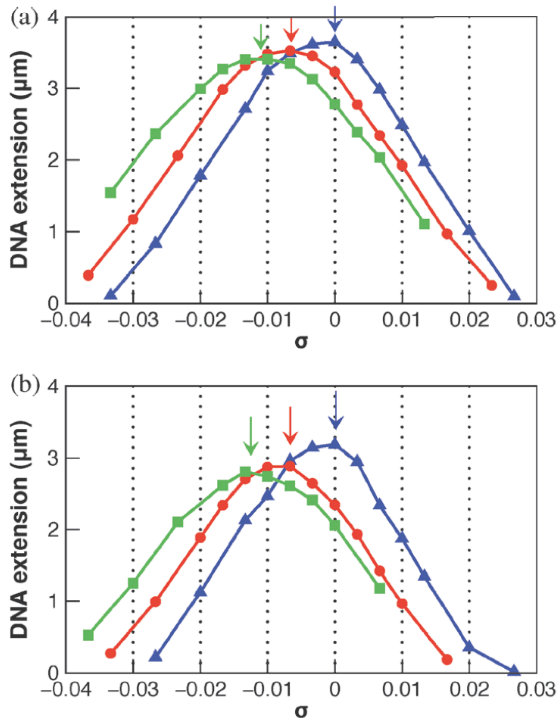
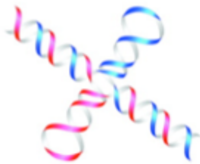
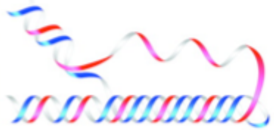
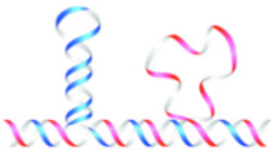
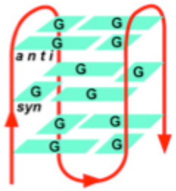
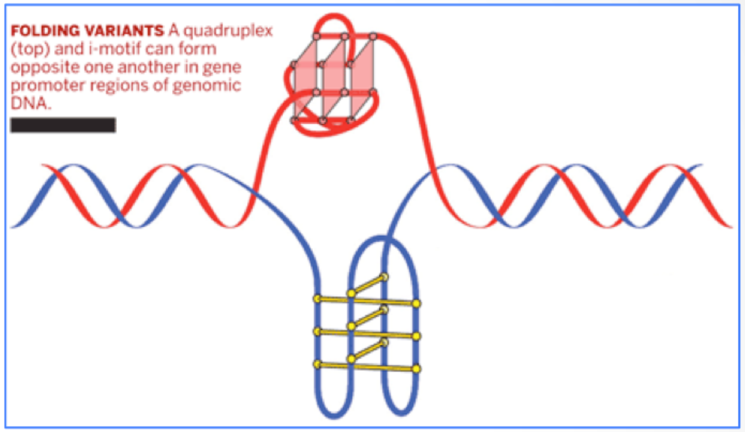


Fig. 4 DNA-twisting measurements show the existence of a considerable amount of monofunctional cisplatin adducts in carbonate buffer. A set of three σ-*vs.*-extension curves was obtained sequentially for each DNA molecule at (a) 0.28 pN and (b) 0.15 pN. Red circles and green squares show the σ-*vs.*-extension data of a DNA molecule incubated with cisplatin (1.65 mM) in 24 mM carbonate buffer before and after washing with 10 mM NaCl, respectively. The blue curve with triangles was obtained for the same DNA in 24 mM carbonate prior to any treatment with cisplatin. The arrows indicate the peak positions in the σ-*vs.*-extension curves.

J.-S. Park, S. H. Kim, N.-K. Lee, K. J. Lee, S.-C. Hong, PCCP 2012

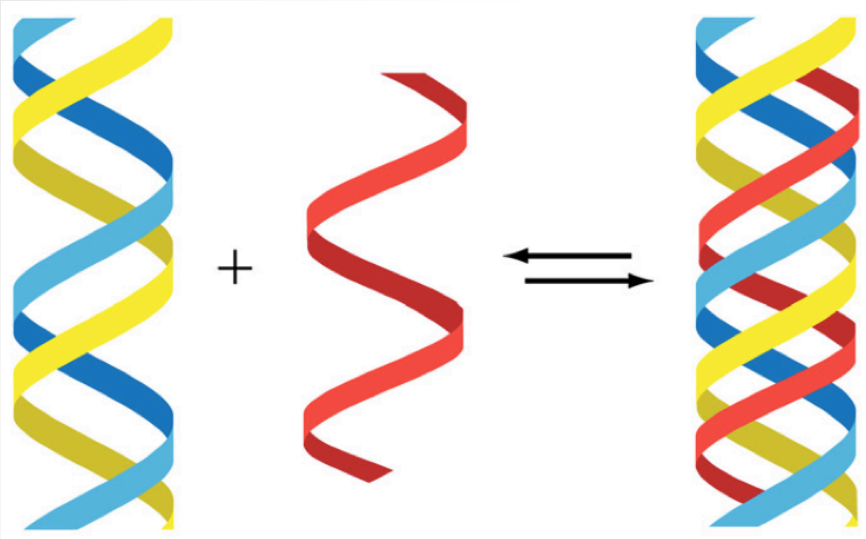
Non-canonical DNA conformations

Name	Conformation	General Seq. Requirements	Sequence
Cruciform		Inverted Repeats	TCGGTACCGA AGCCATGGCT
Triplex		$(R \bullet Y)_n$ Mirror Repeats	AAGAGGGGAGAA TTCTCCCCTCTT
Slipped (Hairpin) Structure		Direct Repeats	TCGGTTCGGT AGCCAAGCCA
Tetraplex		Oligo $(G)_n$ Tracts	$AG_3(T_2AG_3)_3$ single strand
Left-handed Z - DNA		$(YR \bullet YR)_n$	CGCGTGCGTGTG GCGCACGCACAC



- They may serve as structural motif to induce downstream reactions.
- They are believed to play important roles in DNA metabolism.
- They are implicated in genetic disorders.

H-DNA

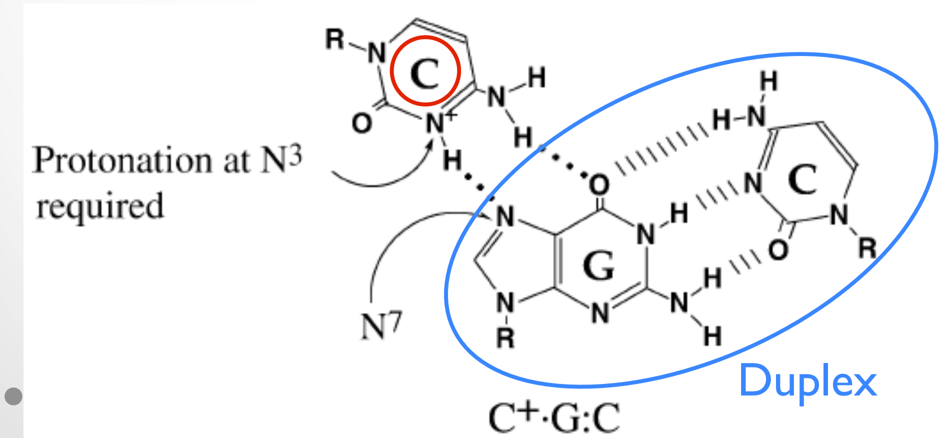


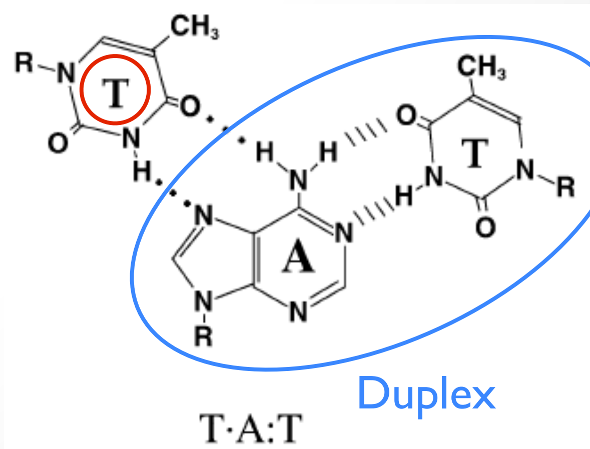
dsDNA ssDNA Triplex DNA

○ Double strand DNA(dsDNA)
+ Single strand DNA(ssDNA)

= Triple Helical DNA
= Triplex DNA

◎ Hoogsteen Base Pairing





Prediction & discovery of triple helix

L. Pauling, PNAS 1953

A. Rich, JACS 1957

the five-membered ring 0.5 Å from the plane of the other four, as report
by Furberg[6] for cytidine) is 4.95 Å. It is found that it is very difficult
assign atomic positions in such a way that the residues can form a brid
between an outer oxygen atom of one phosphate group and an outer oxyg
atom of a phosphate group in the layer above, without bringing some ator
into closer contact than is normal. The atomic parameters given in Tab

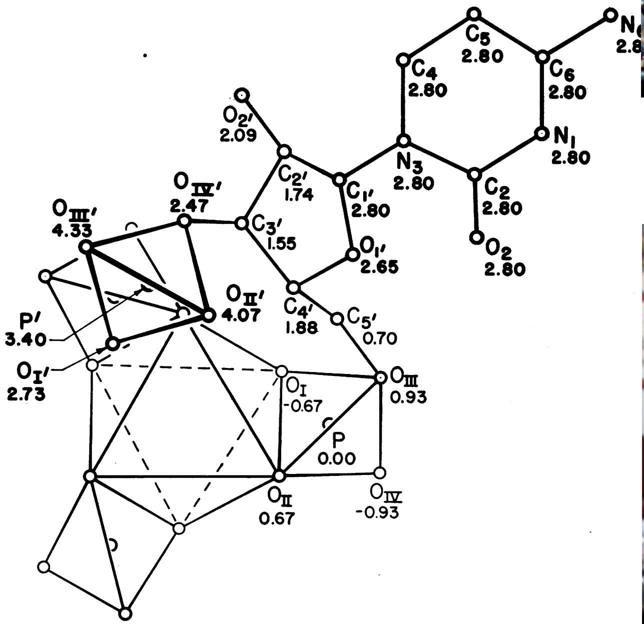
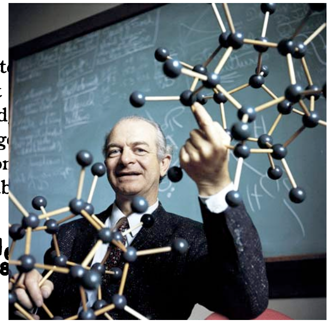


FIGURE 5

A plan of the nucleic acid structure, showing four of the phosphate groups, one rib
furanose group, and one pyrimidine group.



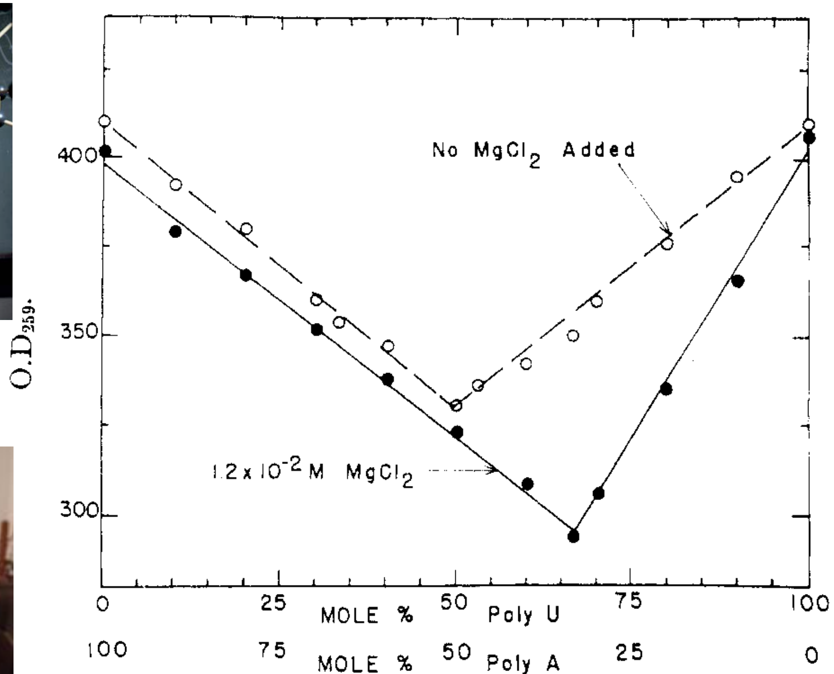
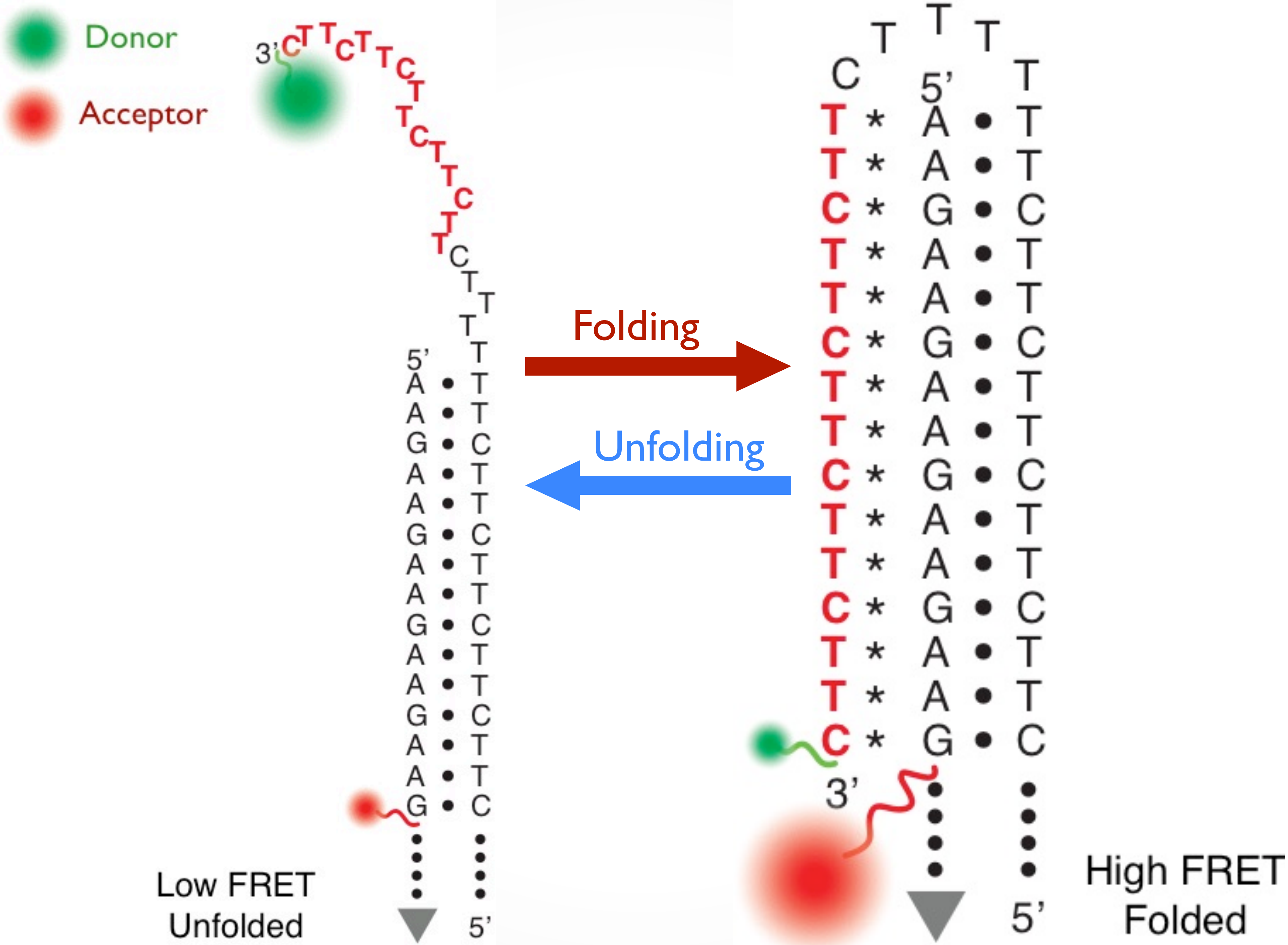
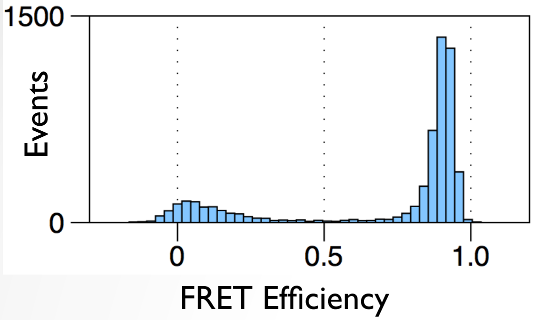


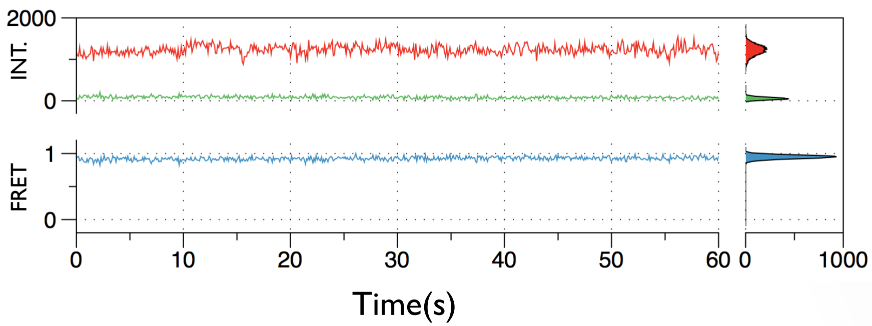
Fig. 1.—The optical density of various mixtures of poly-
denylic acid and polyuridylic acid. Optical densities were
neasured two hours after mixing. All solutions are in 0.1 *M*
NaCl, 0.01 *M* glycylglycine, *p*H 7.4, T = 25°.

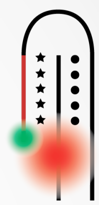

Donor
Acceptor
Folding
Unfolding
Low FRET
Unfolded
High FRET
Folded

◎ Low pH (= 6.5) → High FRET

Folded

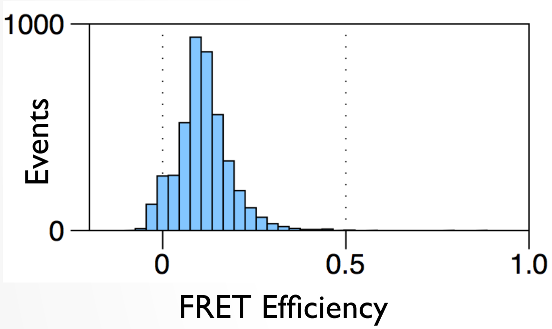


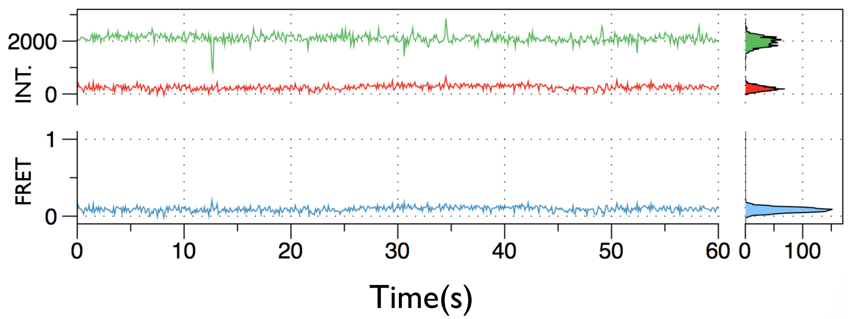


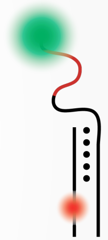


◎ High pH (= 8.5) → Low FRET

Unfolded

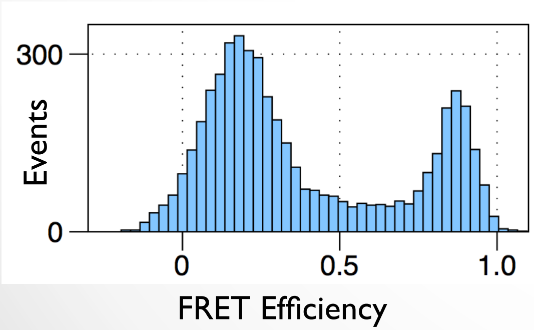


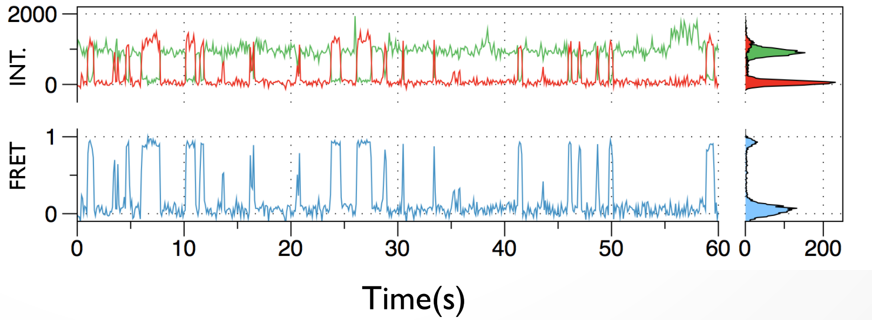


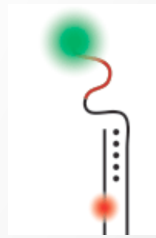


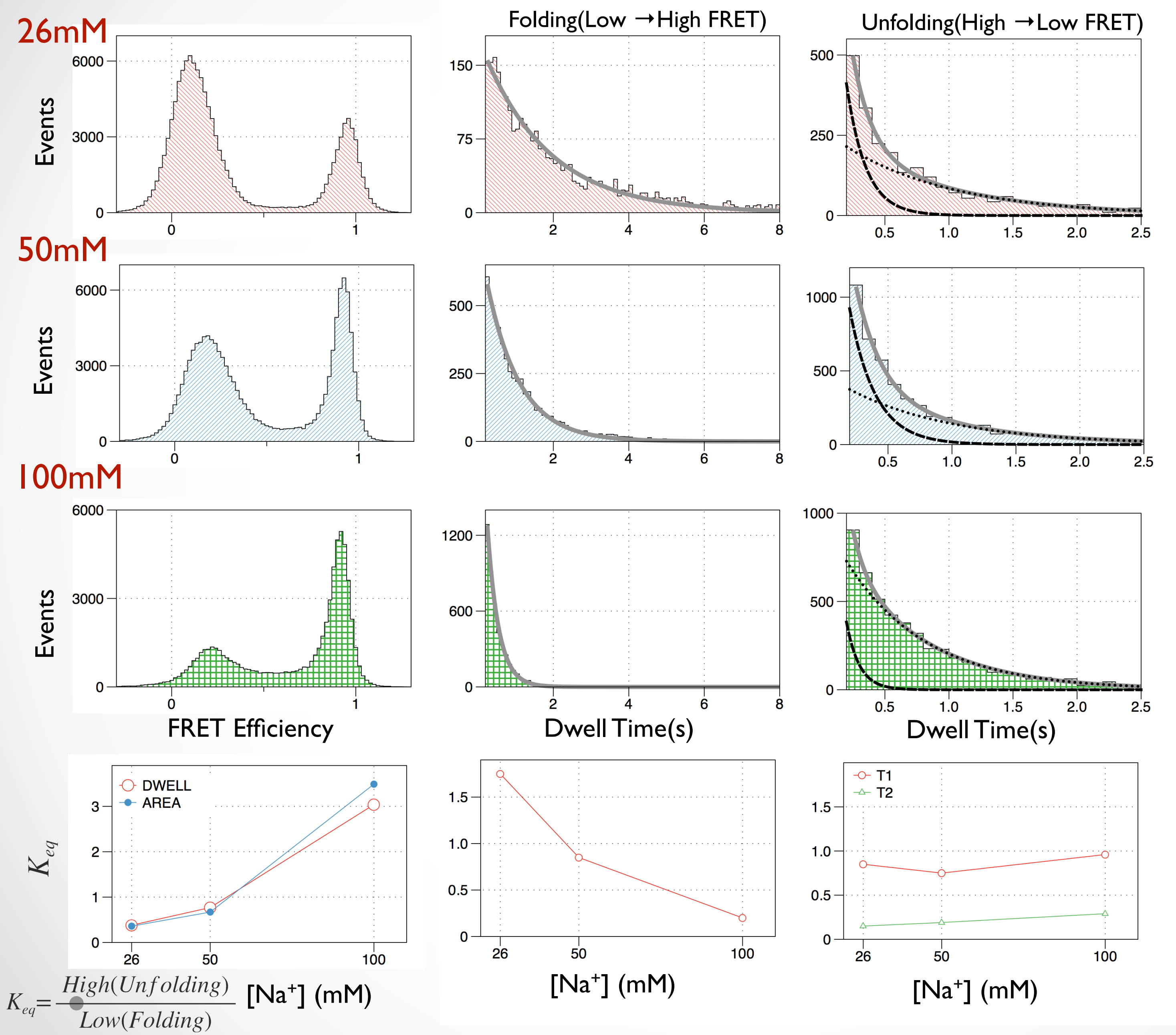
◎ Neutral pH (= 7.5) → Low & High FRET

Folded & Unfolded









◎ Folding is expedited by high salt concentration (more screening).

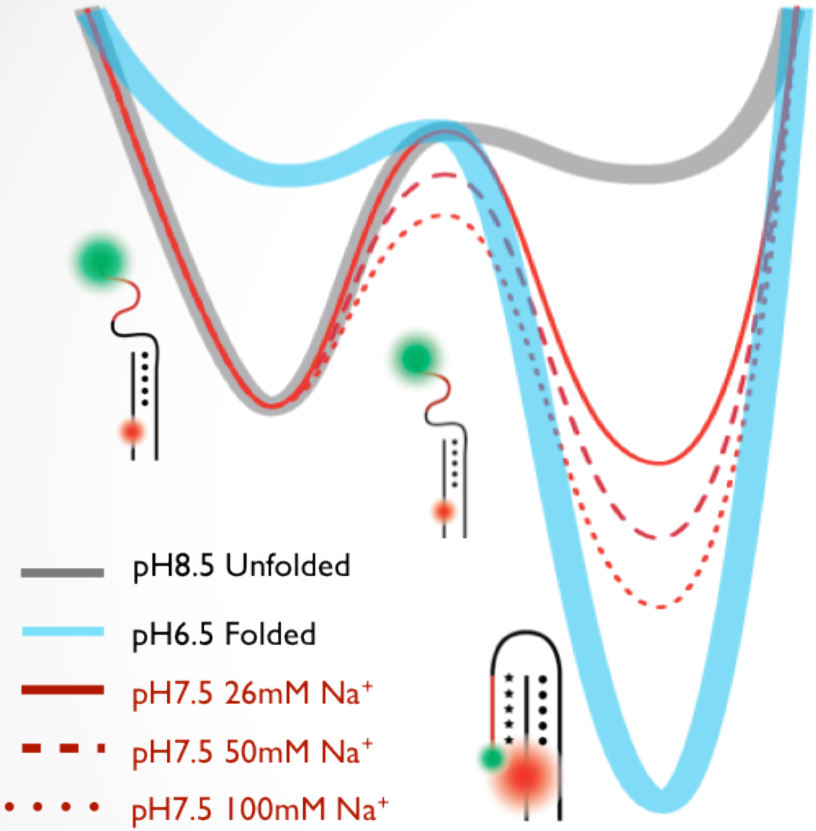
◎ Unfolding kinetics is heterogeneous .

◎ The switching time is macroscopic (order of seconds).

◎ RNA polymerase stalls more easily (rate : 10~20 nt/s).

* 생리식염수 : 150mM

◎ Schematics of energy landscape and model

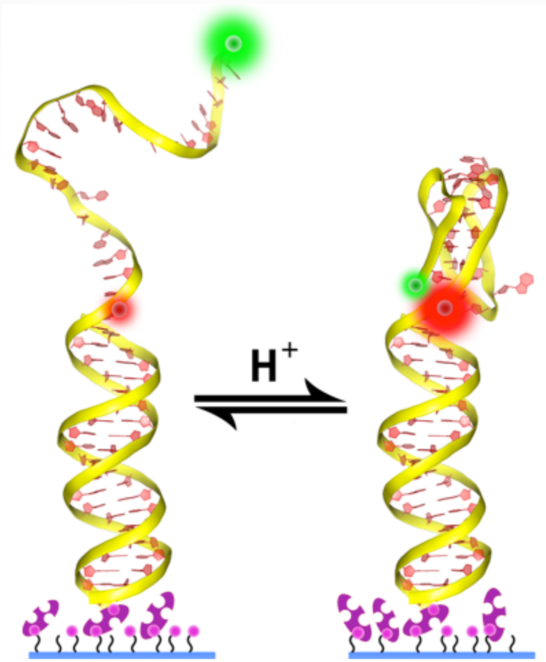


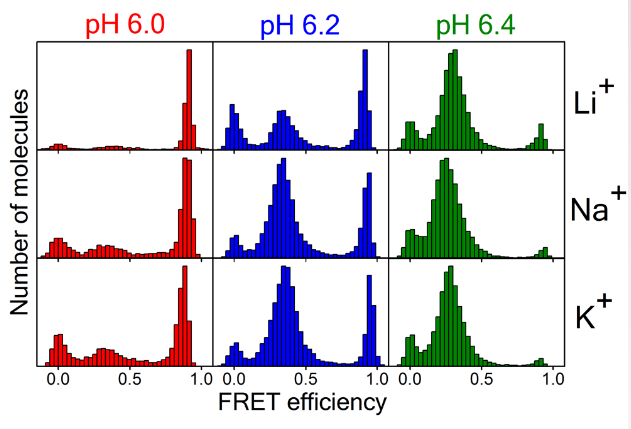
◎ The energy barrier of the transition varies according to the salt concentration.

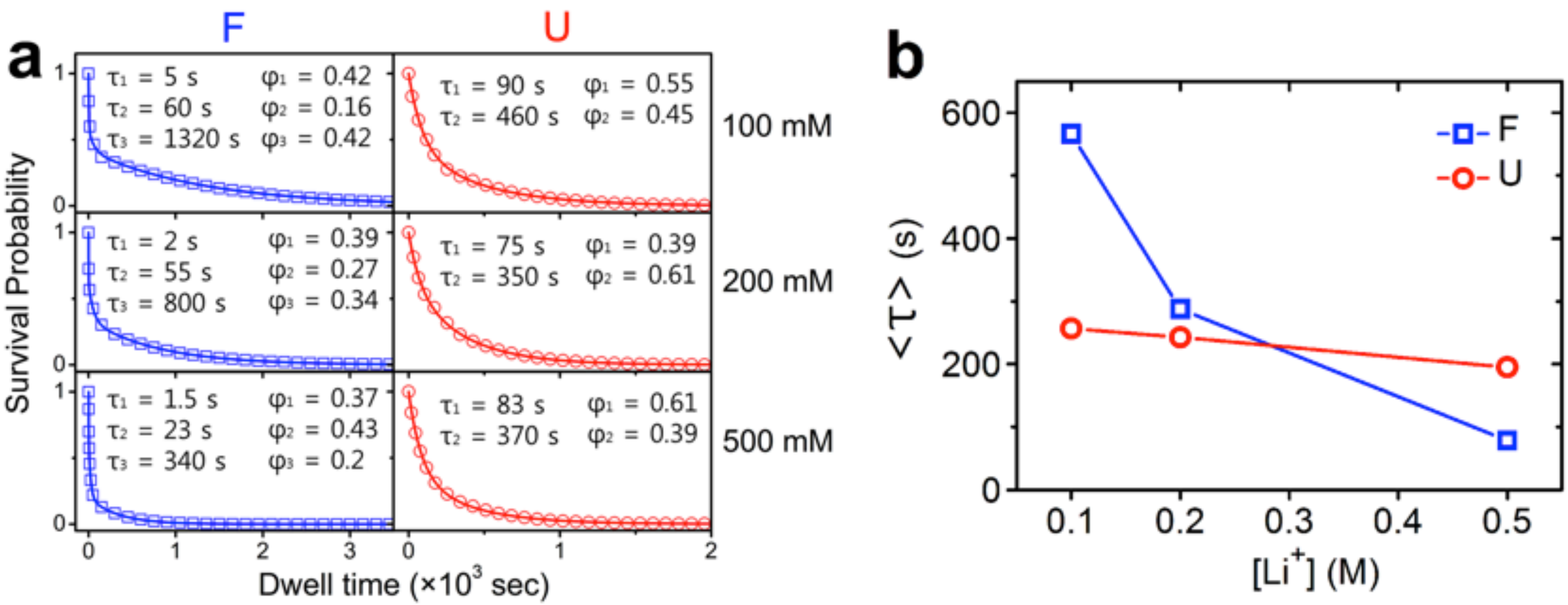
◎ Depending on the folded states that were meta-stable or folded, the kinetics of unfolding appears differently.

I. B. Lee, N.-K. Lee, S.-C. Hong, BJ 2012

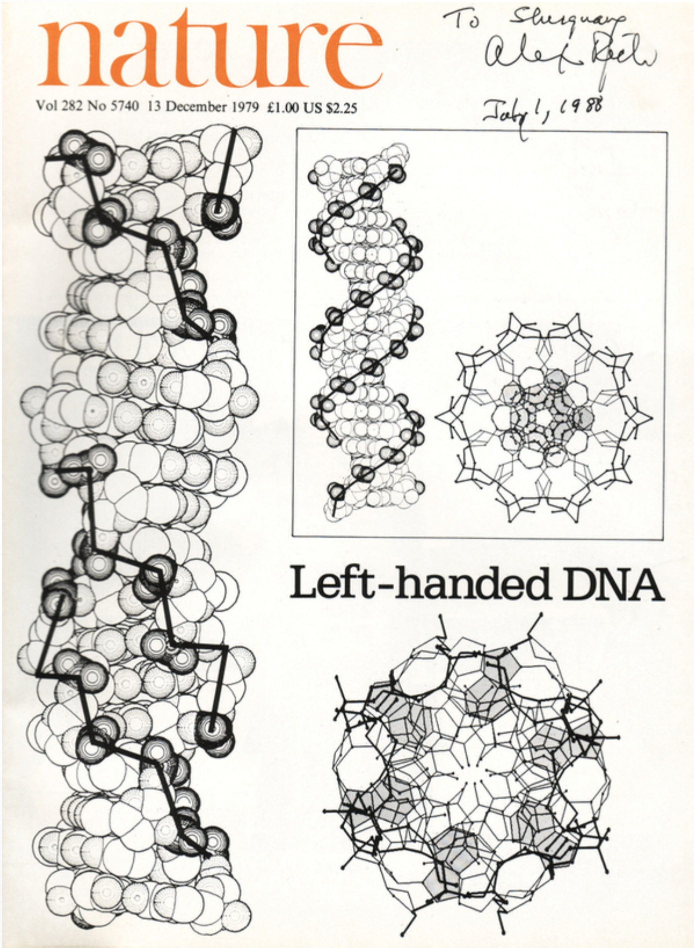
i-motif

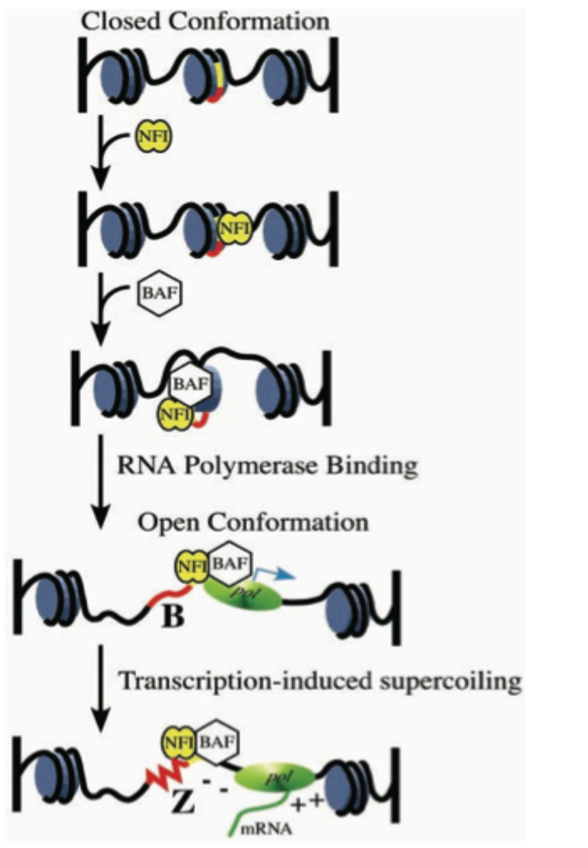

H+


pH 6.0
pH 6.2
pH 6.4
Li+
Na+
K+
Number of molecules
0.0
0.5
1.0
FRET efficiency


a
F
U
Survival Probability
τ1 = 5 s φ1 = 0.42
τ2 = 60 s φ2 = 0.16
τ3 = 1320 s φ3 = 0.42
τ1 = 90 s φ1 = 0.55
τ2 = 460 s φ2 = 0.45
100 mM
τ1 = 2 s φ1 = 0.39
τ2 = 55 s φ2 = 0.27
τ3 = 800 s φ3 = 0.34
τ1 = 75 s φ1 = 0.39
τ2 = 350 s φ2 = 0.61
200 mM
τ1 = 1.5 s φ1 = 0.37
τ2 = 23 s φ2 = 0.43
τ3 = 340 s φ3 = 0.2
τ1 = 83 s φ1 = 0.61
τ2 = 370 s φ2 = 0.39
500 mM
Dwell time (×10³ sec)
b
<τ> (s)
F
U
[Li+] (M)
0
200
400
600
0.1
0.2
0.3
0.4
0.5

Z-DNA

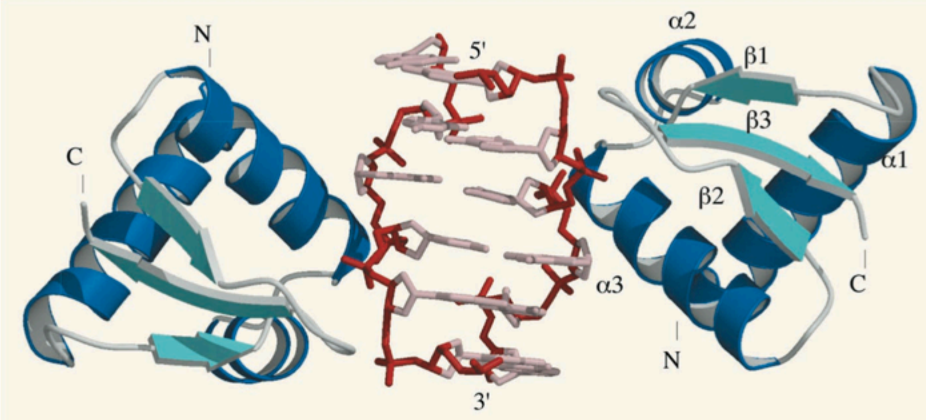




Z-DNA coupled transcriptional regulation in BAF regulated genes.

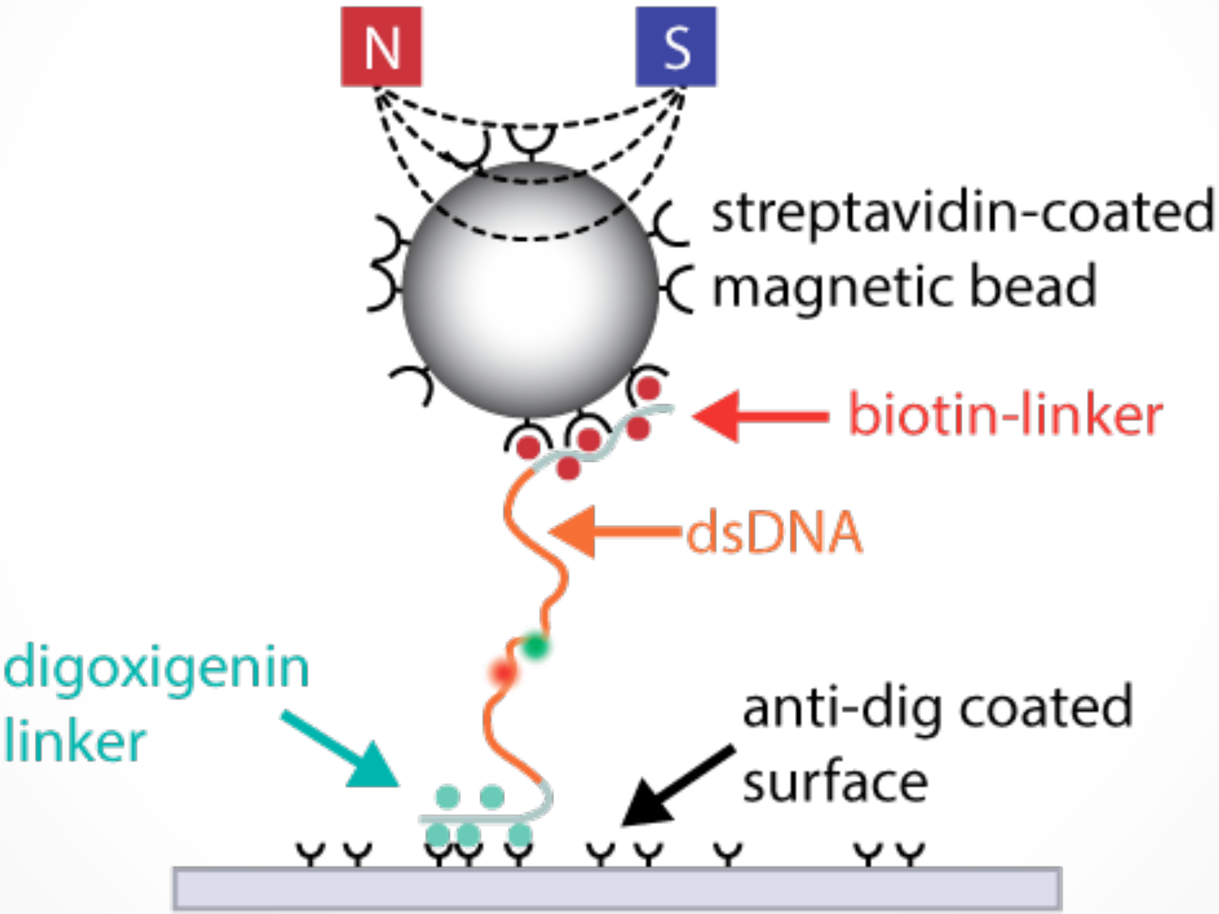
Factors that affect Z-DNA

- Sequence (alternating **purine** and **pyrimidine**)
  - $(GC/GC)_n > (TG/CA)_n > (AT/AT)_n$
- High salt conditions
  - $[Na^+]$ ~ 2.5 M for $(GC/GC)_n$
- Z-DNA binding proteins
  - ADAR1 (hZ_{aADAR1}), DAI, E3L

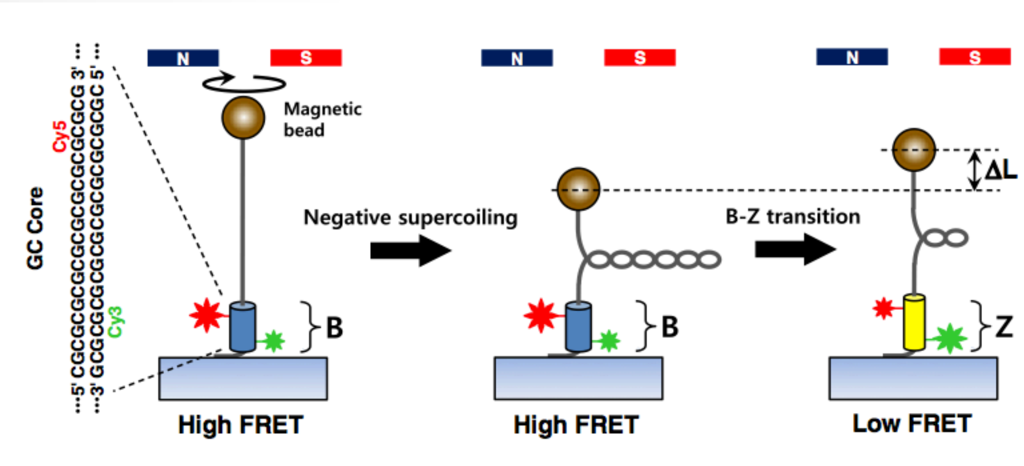


- Negative supercoiling
  - σ ~ -3 % ~ -9 % is sufficient for the BZ transition in physiological salt conditions (~100 mM).
    e.g. -3 % ~ 97 (=100-3) turns in 1000 bp DNA (Lk_0 ~ 100 for 1000 bp DNA; $\Delta Lk = Lk - Lk_0$; $\sigma = \Delta Lk / Lk_0$)

We first developed hybrid technique of smFRET and magnetic tweezers!

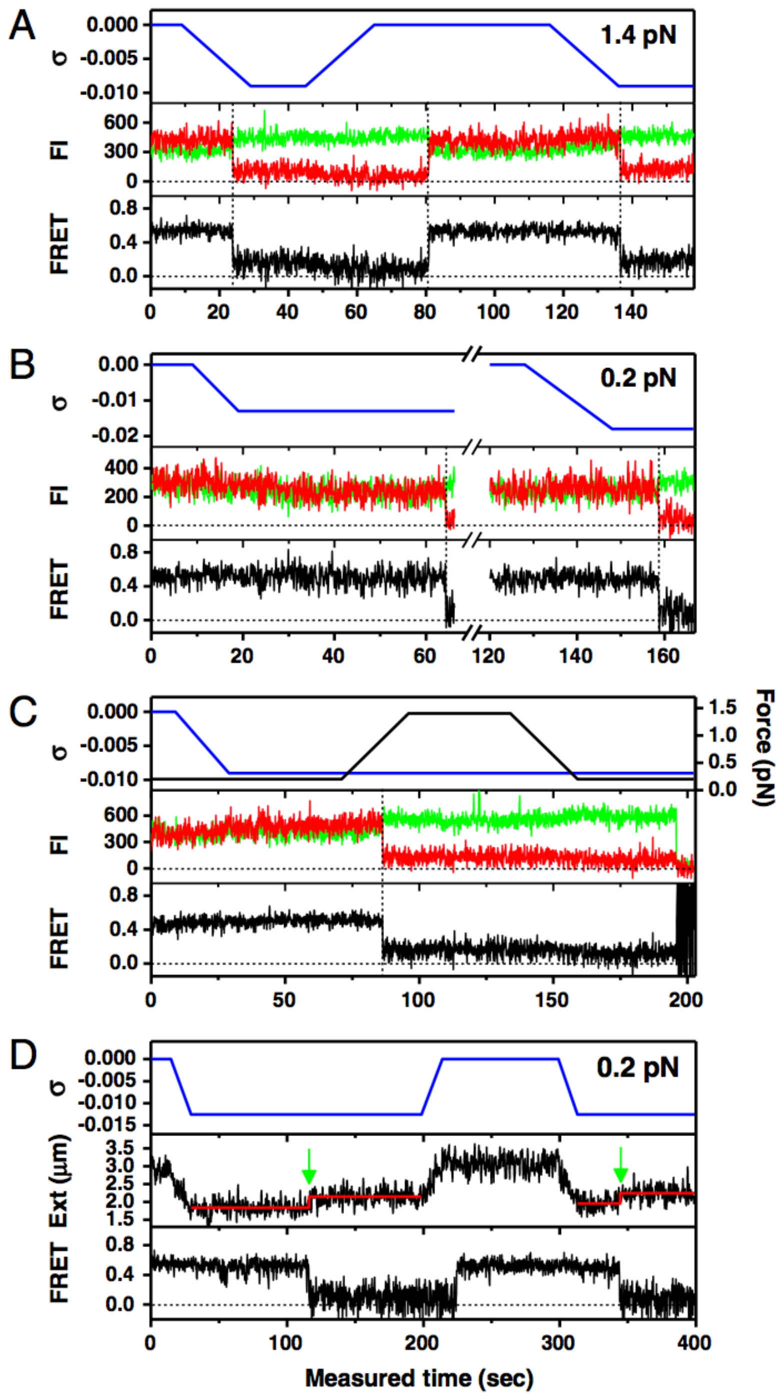


Synchronized measurements of smFRET and z-extension



$$\Delta Lk = \Delta Tw + Wr$$

M. Lee, S. H. Kim, S.-C. Hong, PNAS 2010



Summary

- Thermodynamic properties of DNA can be elucidated by empirical approach.
- DNA exhibits intriguing mechanical properties, which can be well understood by polymer models.
- DNA can adopt a variety of interesting non-canonical conformations, which have biological significance.
- Single-molecule methods are powerful techniques to characterize (bio)physical properties of DNA and explore dynamics and conformations (their heterogeneity) of (non-canonical) DNAs.

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