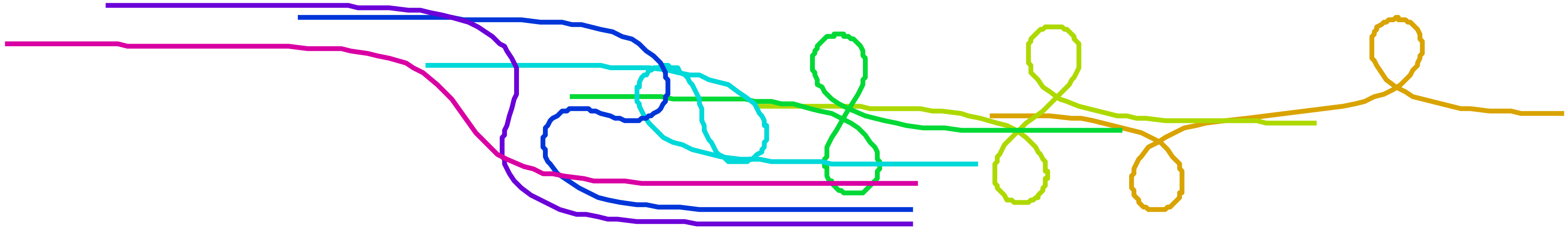
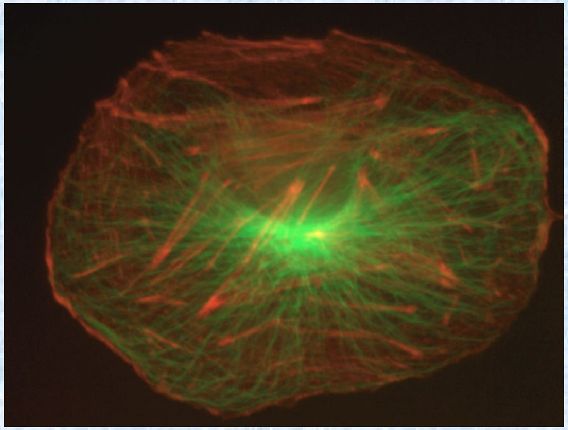
Chem 163

10 Nov. 2022
Adam E. Cohen

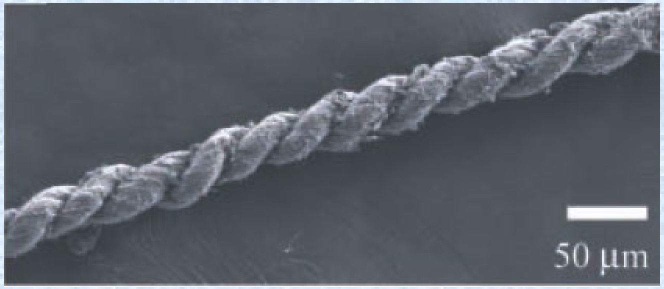


Mechanics of rods

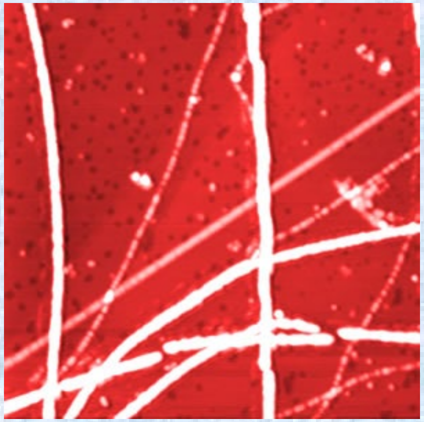
Fibrils and Us



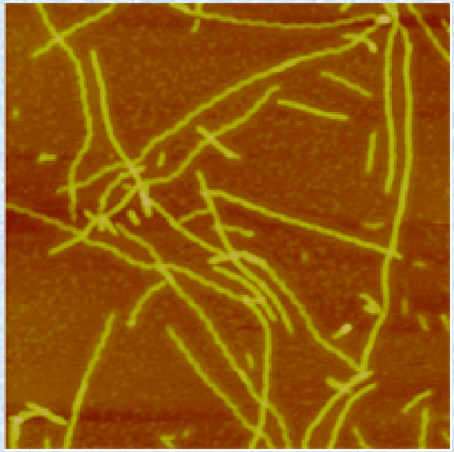
actin & tubulin



rope of carbon nanotubes

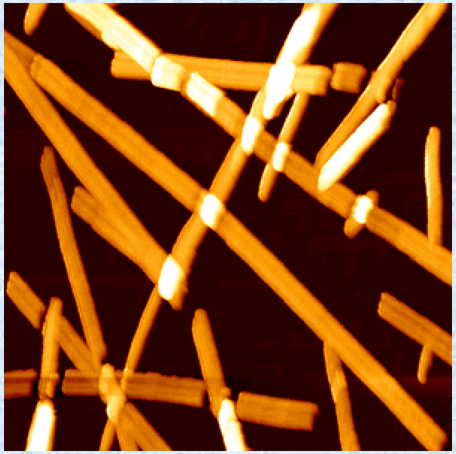


j-aggregates

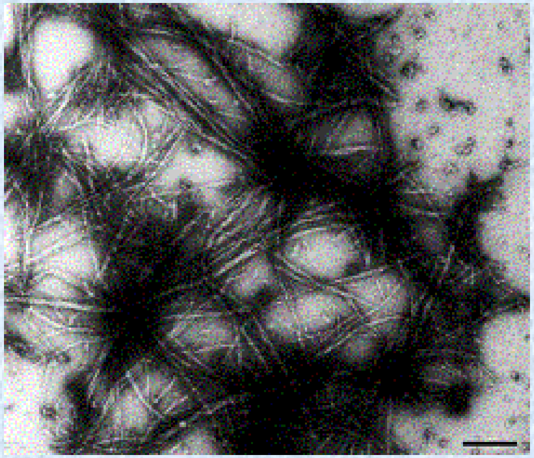


prions

sickle-cells

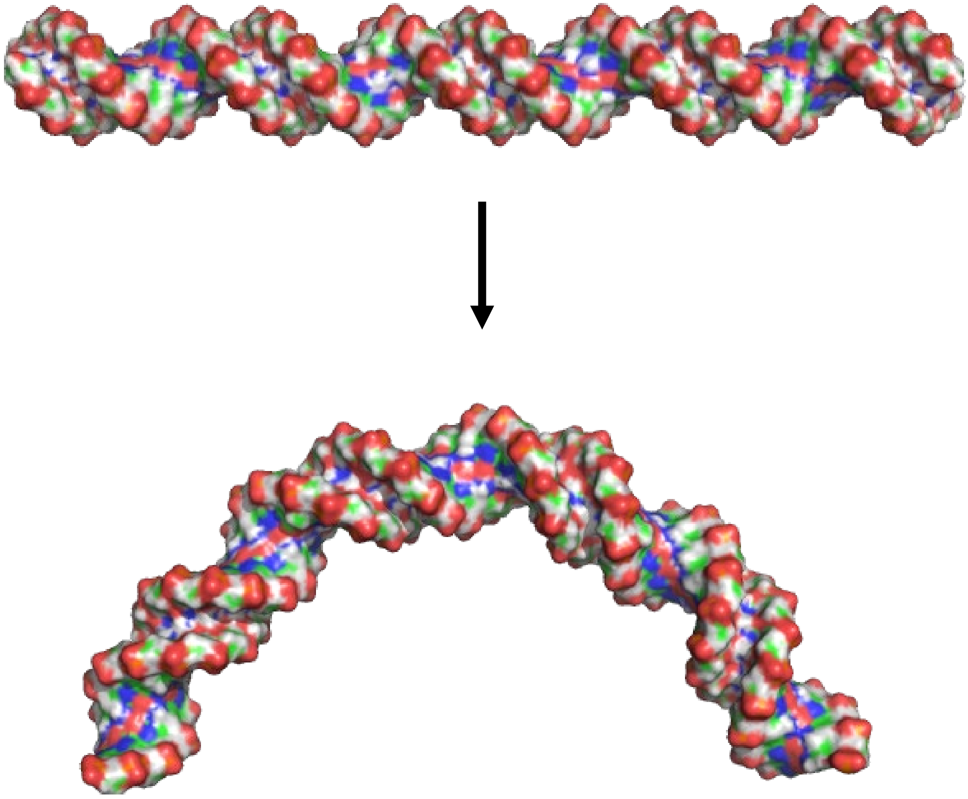


amyloids



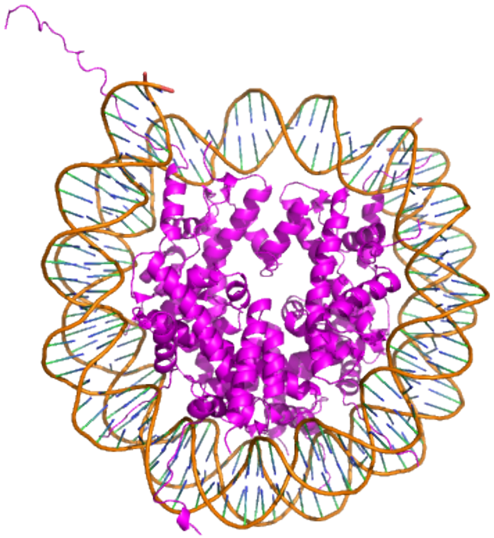
cataracts

DNA Bending

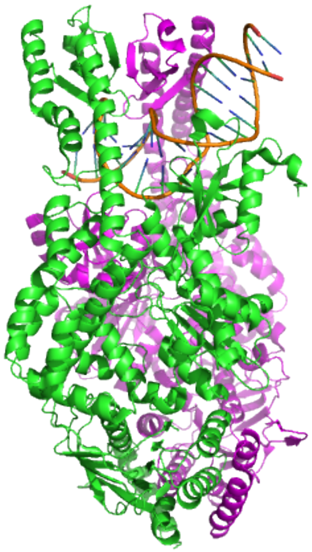


Nucleic Acid Builder (NAB)
Macke and Case, *Mol. Model. Nucleic Acids* **682**, 379 (1998).

DNA bending in nature



Nucleosome (1AOI)
25°/bp

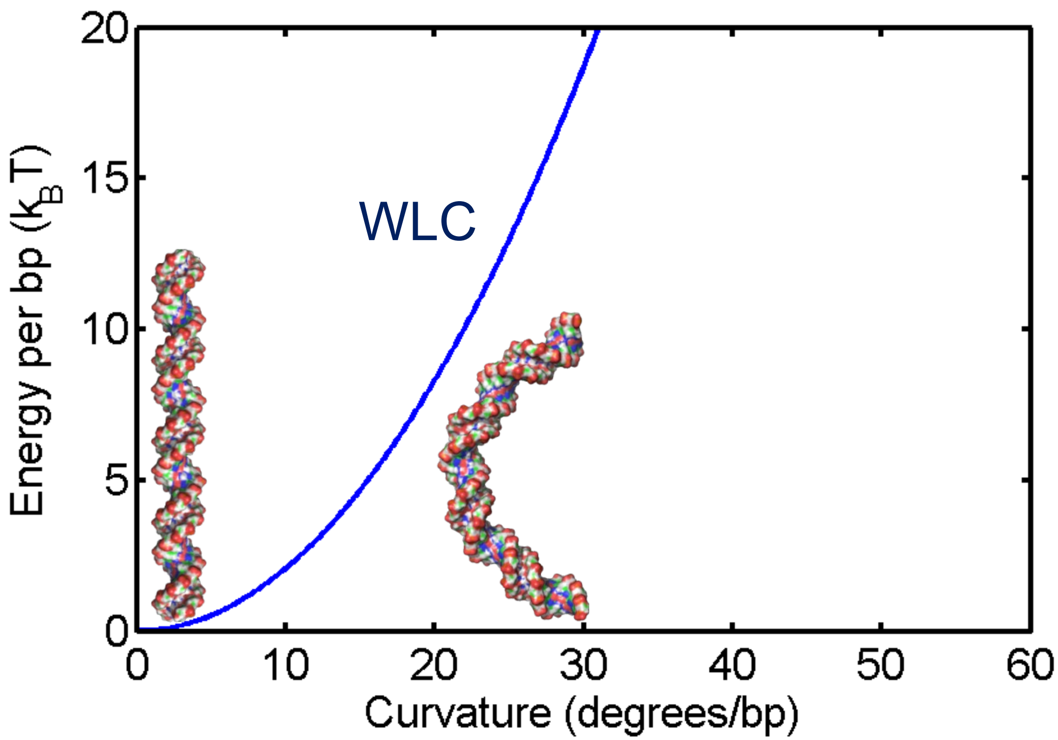


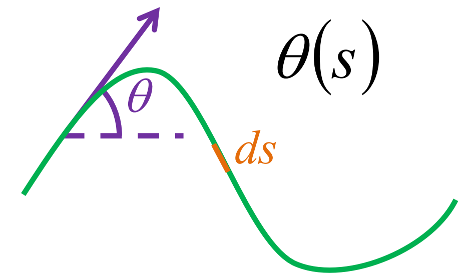
MutS + G-T mismatch (1E3M)
60°/bp at mismatch

Catabolite activator protein (1CGP)
40°/bp

Redundancy of codons allows for an underlying mechanical code

The wormlike chain model of DNA bending





Wormlike chain (WLC):

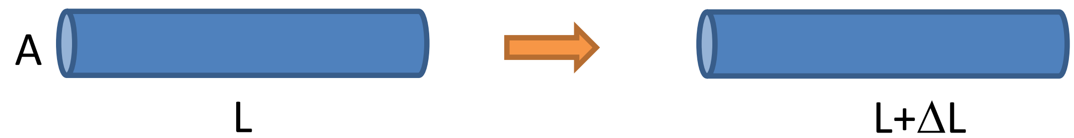
$$H = \int_0^L \frac{1}{2} \alpha \left(\frac{\partial \theta}{\partial s} \right)^2 ds$$

α → bending modulus

- Problems with using the WLC to describe DNA bending in the context of protein binding
  - WLC treats DNA as mechanically homogeneous, but interactions reflect local variation (sequence, damage, modifications)
  - Proteins induce higher curvature than is experimentally accessible
- Idea: induce curvature in short pieces of DNA

Bending of a rod

Appetizer: **stretching**



Young's modulus

Spring constant $k = Y\frac{A}{L}$

Elastic energy U $= \frac{1}{2} Y \frac{A}{L} \Delta L^2 = \frac{1}{2} Y A \int_0^L \left(\frac{\Delta L}{L}\right)^2 dL$

Main course: **bending**

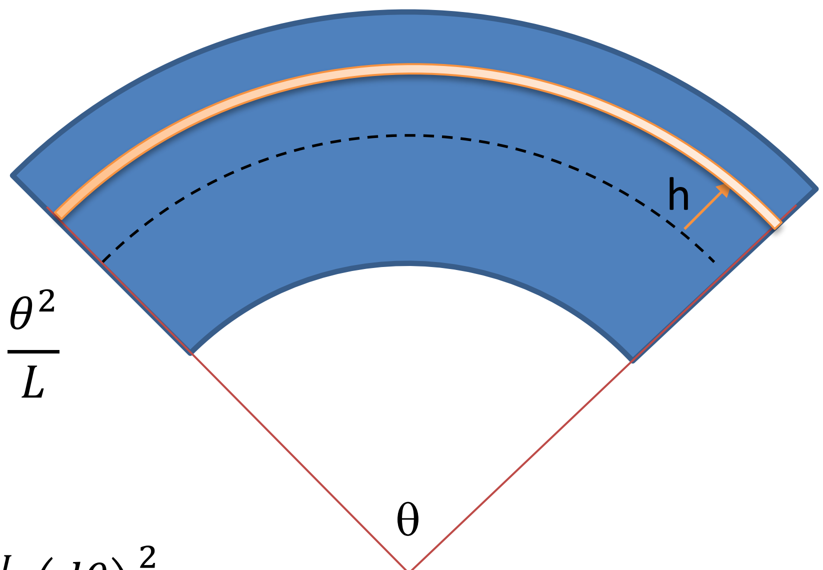
$\Delta L = h\theta$

$$dU = \frac{1}{2} Y \frac{dA}{L} h^2 \theta^2$$

$$U = \frac{1}{2} Y \left(\int_{surf} h^2 dA \right) \frac{\theta^2}{L}$$

I

$$U = \frac{1}{2} Y\, I \frac{\theta^2}{L} = \frac{1}{2} Y I \int_0^L \left(\frac{d\theta}{dL}\right)^2 ds$$



Relation of bending modulus to material properties

$$\alpha = Y\, I$$

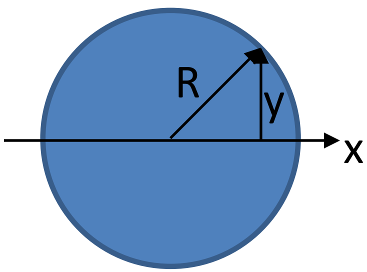
Y: Young's Modulus (N/m^2)

I: Area moment of inertia (m^4)

Why are microtubules hollow?

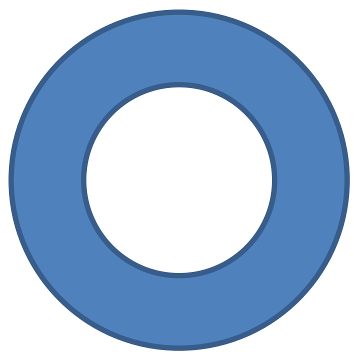
Coherent bending ($I \sim R^4$) vs. incoherent bending ($I \sim R^2$)

$$I = \int_A y^2 dA$$



$$I_x = \frac{\pi R^4}{4}$$

(easiest to see by calculating I_z and then applying perpendicular axis theorem)



$$I = \frac{\pi(R_2^4 - R_1^4)}{4}$$

Mechanics of rods

$$H = \int_0^L \frac{1}{2}\alpha\left(\frac{\partial\theta}{\partial s}\right)^2 ds$$

Loop energy: $\frac{\partial\theta}{\partial s} = \frac{1}{R}$

$$U_{loop} = 2\pi R \frac{1}{2}\alpha\left(\frac{1}{R}\right)^2$$

$$= \frac{\pi\alpha}{R}$$

Consider $U_{loop} = \frac{1}{2}k_BT$

→ $R = 2\pi\frac{\alpha}{k_BT}$

Persistence length $l_p = \frac{\alpha}{k_BT}$

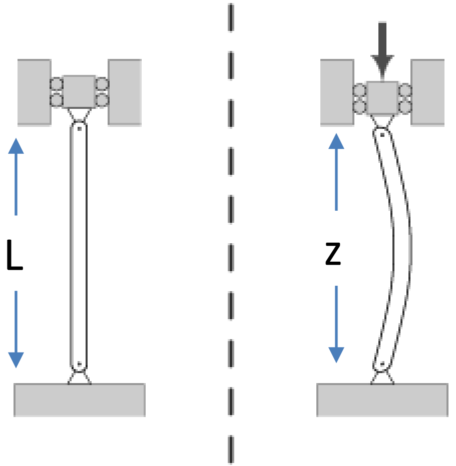
e.g. for dsDNA, l_p ~ 53 nm

Autocorrelation of unit tangent $\langle \mathbf{t}(s_a)\cdot\mathbf{t}(s_b)\rangle = e^{-|s_a-s_b|/l_p}$

Example: DNA bending around a histone

Histone: R ~ 4 nm, 2 revolutions
U_{bend} ~ 70 k_BT

Rods with forces: Euler buckling



$$U_{tot} = \frac{1}{2}\alpha \int_0^L \left(\frac{d\theta}{ds}\right)^2 ds - F(L - z)$$

Guess: $\theta(s) = A\cos\frac{\pi s}{L}$

$$\left(\frac{d\theta}{ds}\right)^2 = \left(\frac{A\pi}{L}\sin\frac{\pi s}{L}\right)^2$$

$$U_{bend} = \frac{1}{2}\alpha\left(A\frac{\pi}{L}\right)^2\frac{L}{2} = \frac{\alpha A^2\pi^2}{4L}$$

$$z = \int_0^L \cos\theta\, ds = \int_0^L \cos\left(A\cos\frac{\pi s}{L}\right) ds \sim \int_0^L \left[1 - \frac{1}{2}\left(A\cos\frac{\pi s}{L}\right)^2\right] ds = L - \frac{A^2 L}{4}$$

$$U_{mech} = -\frac{FA^2L}{4}$$

$$U_{tot} = A^2\left(\frac{\alpha\pi^2}{4L} - \frac{FL}{4}\right)$$

$$F_{buckle} \geq \frac{\pi^2\alpha}{L^2} \qquad L_{buckle} \geq \sqrt{\frac{\pi^2\alpha}{F}}$$

The elasticae

For a rod under compression $U(s) = \frac{\alpha}{2}\left(\frac{\partial\theta}{\partial s}\right)^2 + F\,cos\theta$

Lagrangian of a physical pendulum

$$\mathcal{L} = KE - PE$$
$$= \frac{1}{2}mL^2\left(\frac{d\theta}{dt}\right)^2 + mgL\cos\theta$$

Lagrangian is minimized via Calculus of Variations

$$\frac{\mathrm{d}}{\mathrm{dt}}\left(\frac{\partial\mathcal{L}}{\partial\dot{q}}\right) = \frac{\partial\mathcal{L}}{\partial q}$$

→ Newton's eqns of motion

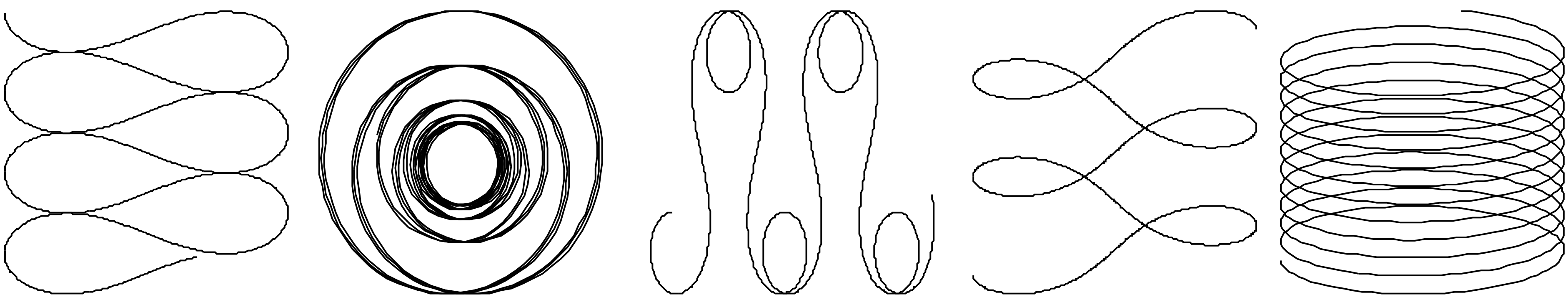
For a rod:

$$\frac{\mathrm{d}}{\mathrm{ds}}\alpha\left(\frac{\partial\theta}{\partial \mathrm{s}}\right) = -F\sin\theta$$

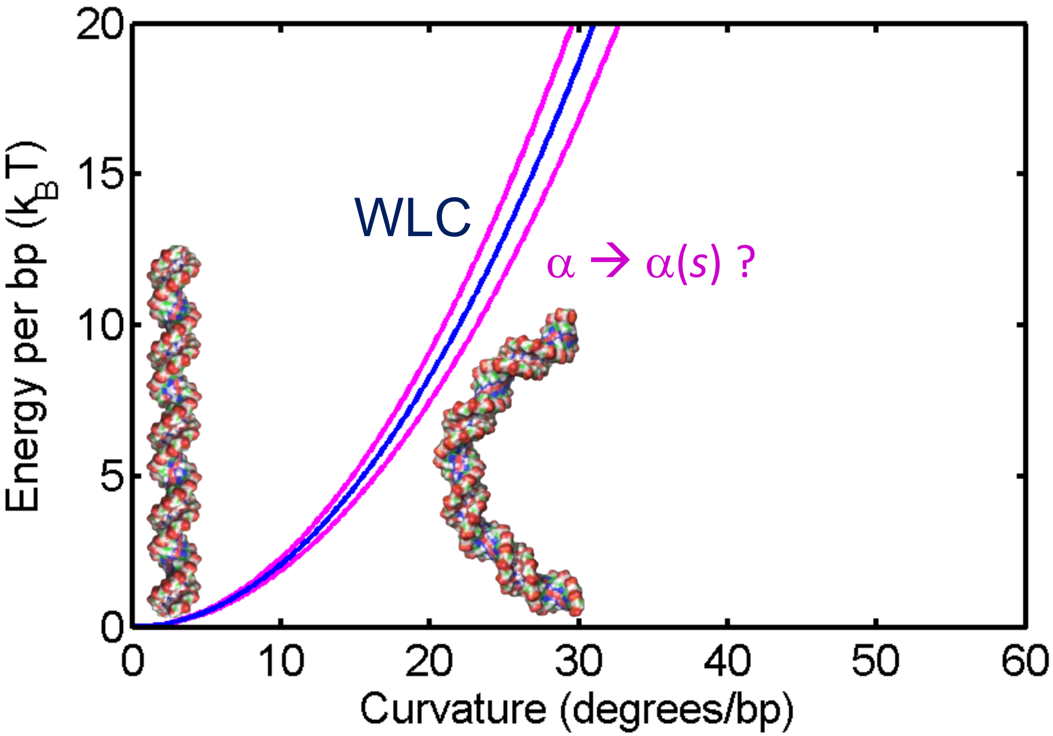
Small-angle limit:

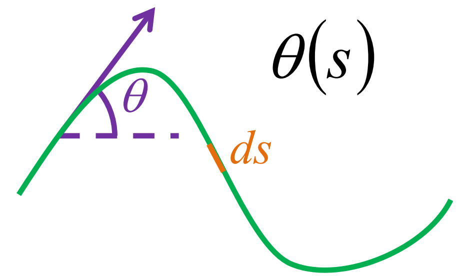
$$\alpha\frac{d^2\theta}{ds^2} = -F\theta$$

$$\theta(s) = A\sin\sqrt{\frac{F}{\alpha}}\,s + B\cos\sqrt{\frac{F}{\alpha}}\,s$$



The wormlike chain model of DNA bending





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bending modulus

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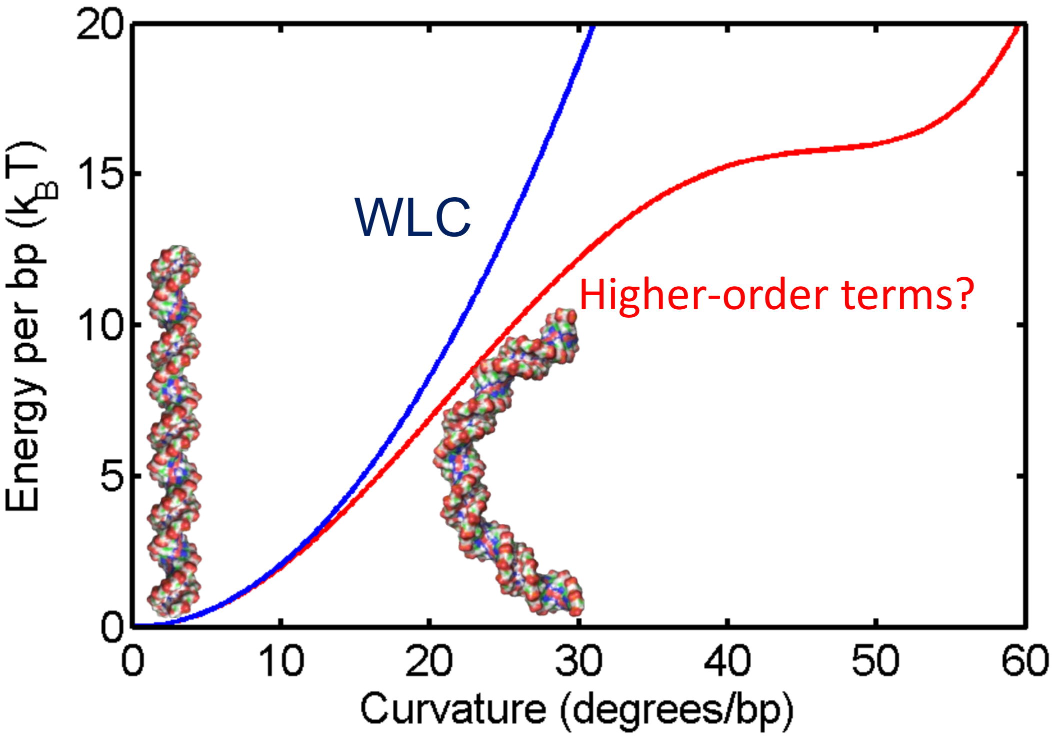
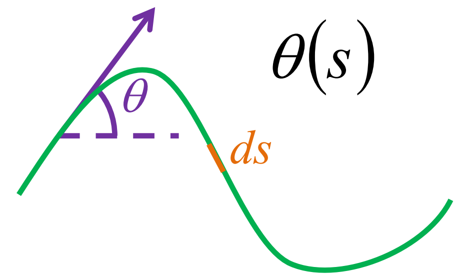
The wormlike chain model of DNA bending





Wormlike chain (WLC):

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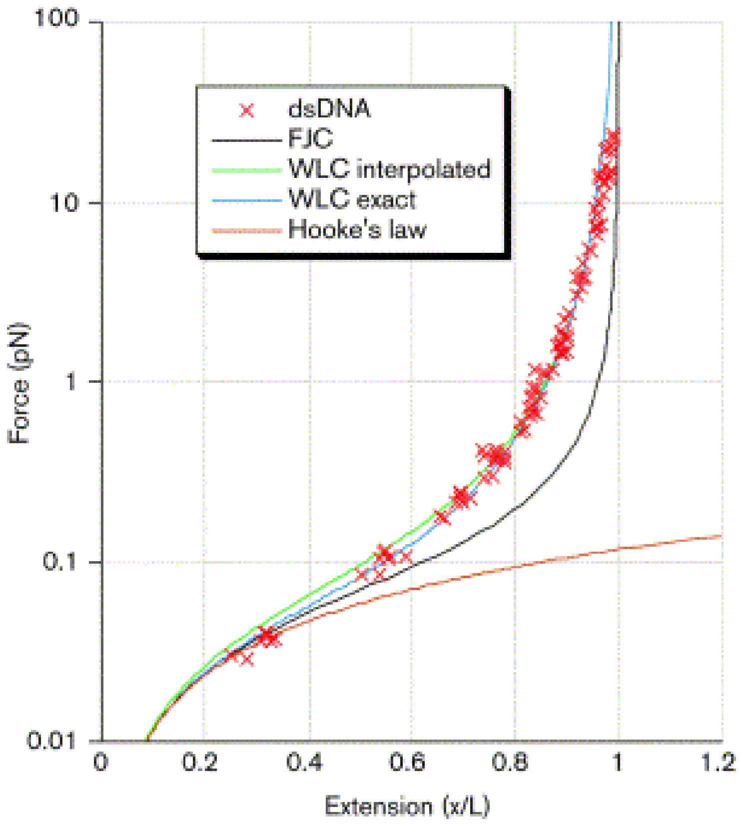
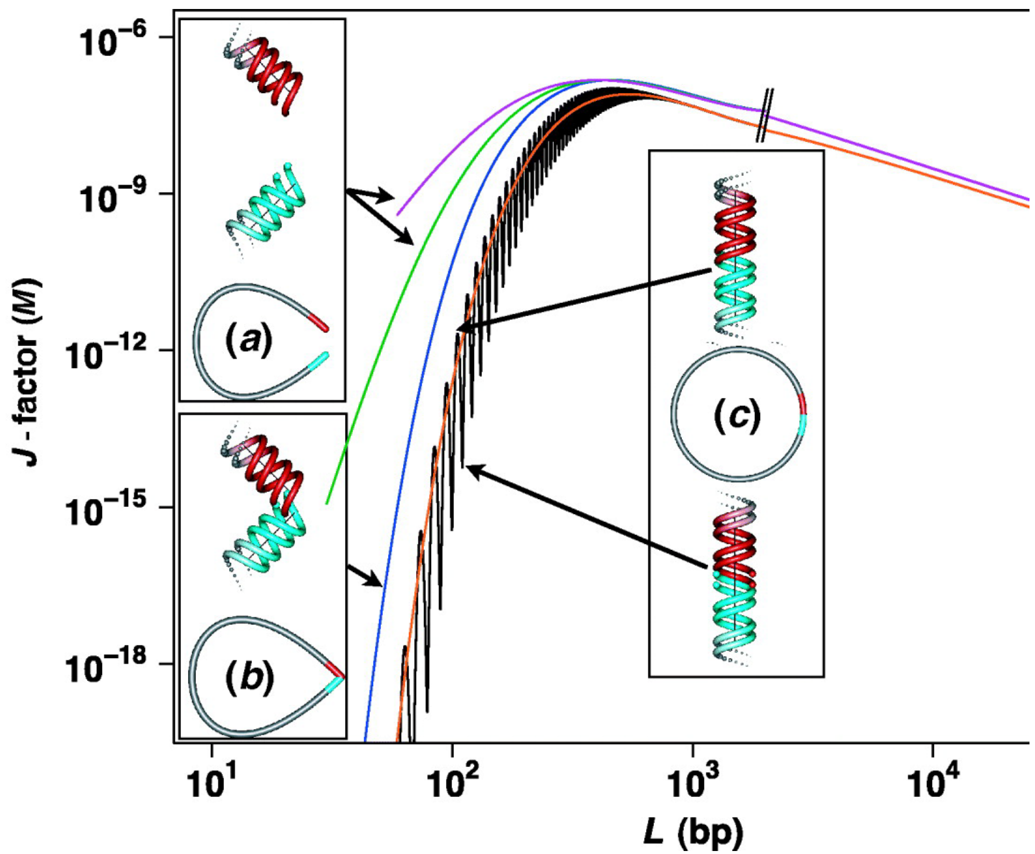
Experiments consistent with elastic rod model



Bustamante *et al.* (2000), *Curr. Opin. Struct. Biol*

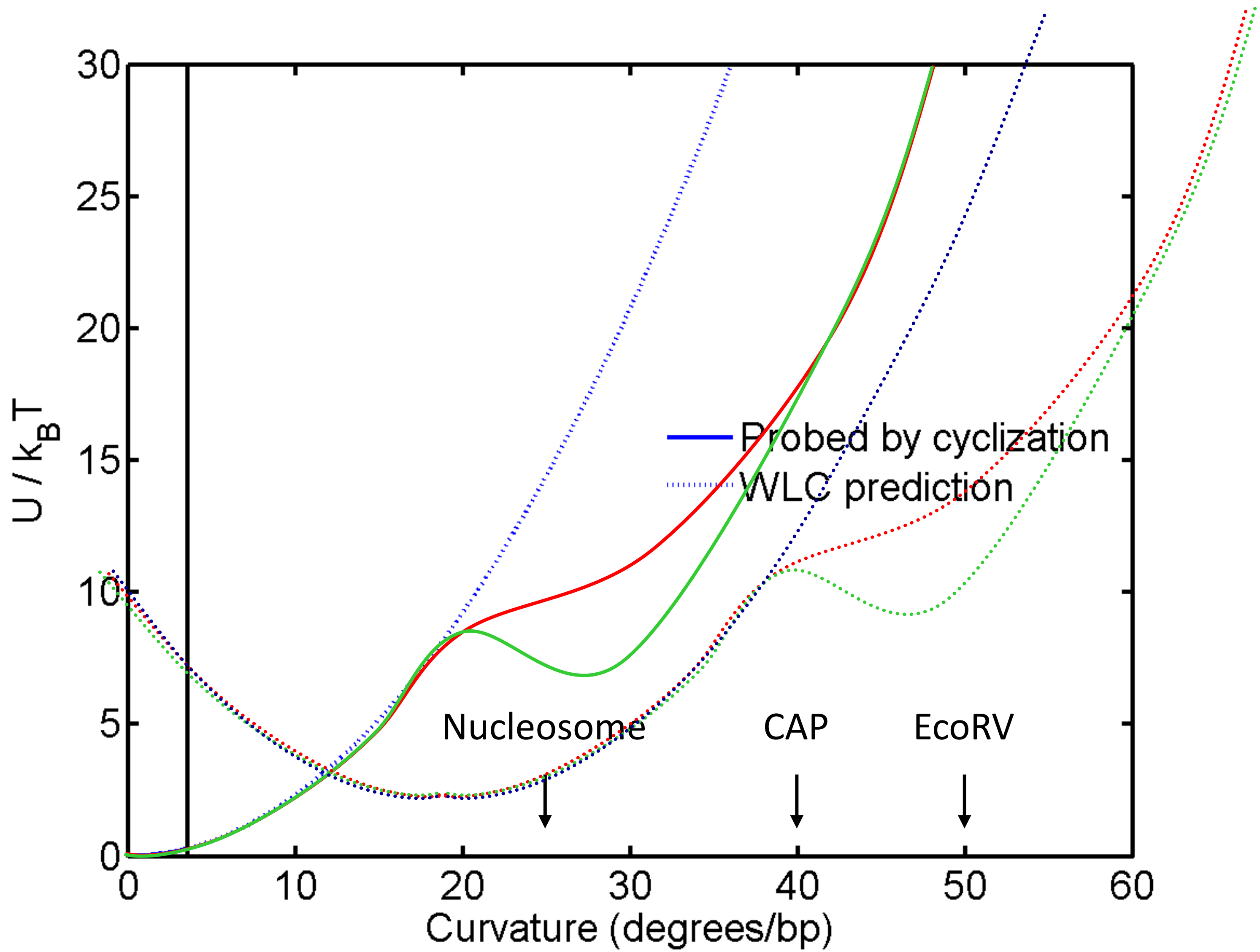


Peters and Maher (2010), *Quarterly Rev. of Biophys.*

WLC model is powerful because it is insensitive to molecular details

but sometimes details matter…

Current experiments are limited to thermally accessible bending energy



Where might the homogeneous elastic rod model fail?

- Particular sequences

  Nucleosome positioning sequences; protein binding sites

- Regions of high imposed curvature

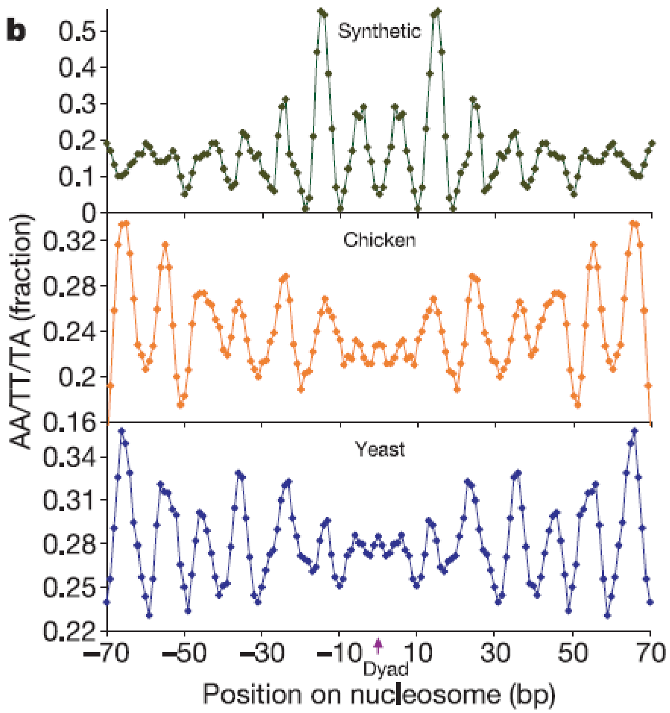
  Under bound proteins; in a virus capsid; regulatory loops

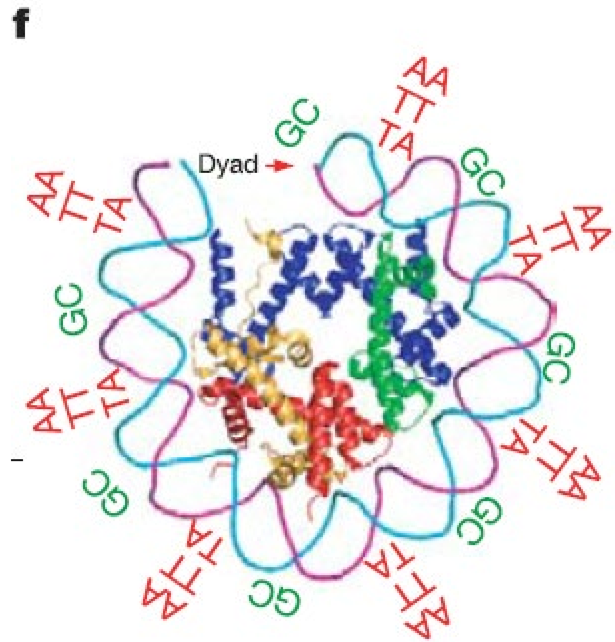
- Damage sites

  Thymine dimers, oxidation, alkylation, hydrolysis, deamination

- Base pair mismatches
- Nicks
- Gaps
- Epigenetic modifications

Nucleosome positioning sequences

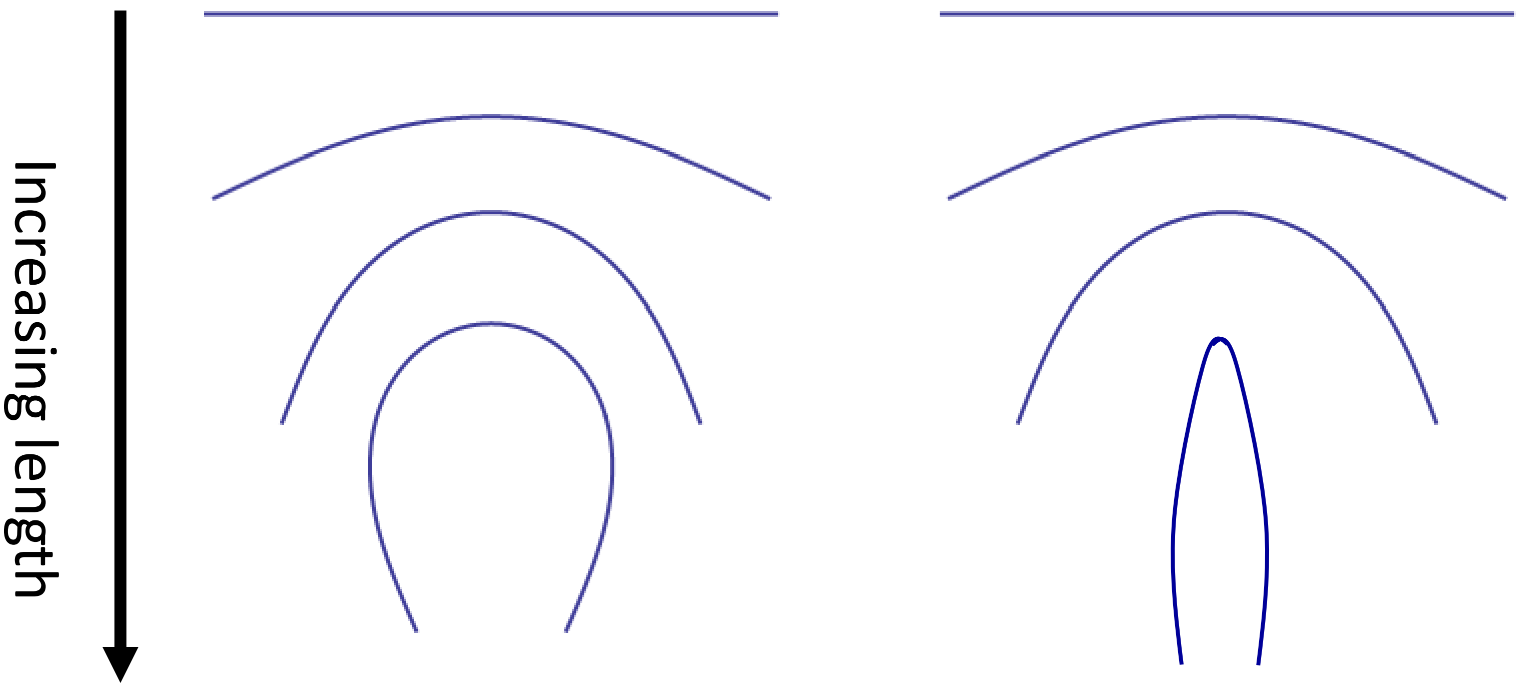




Segal, Eran, et al. "A genomic code for nucleosome positioning." *Nature* 442.7104 (2006): 772-778.

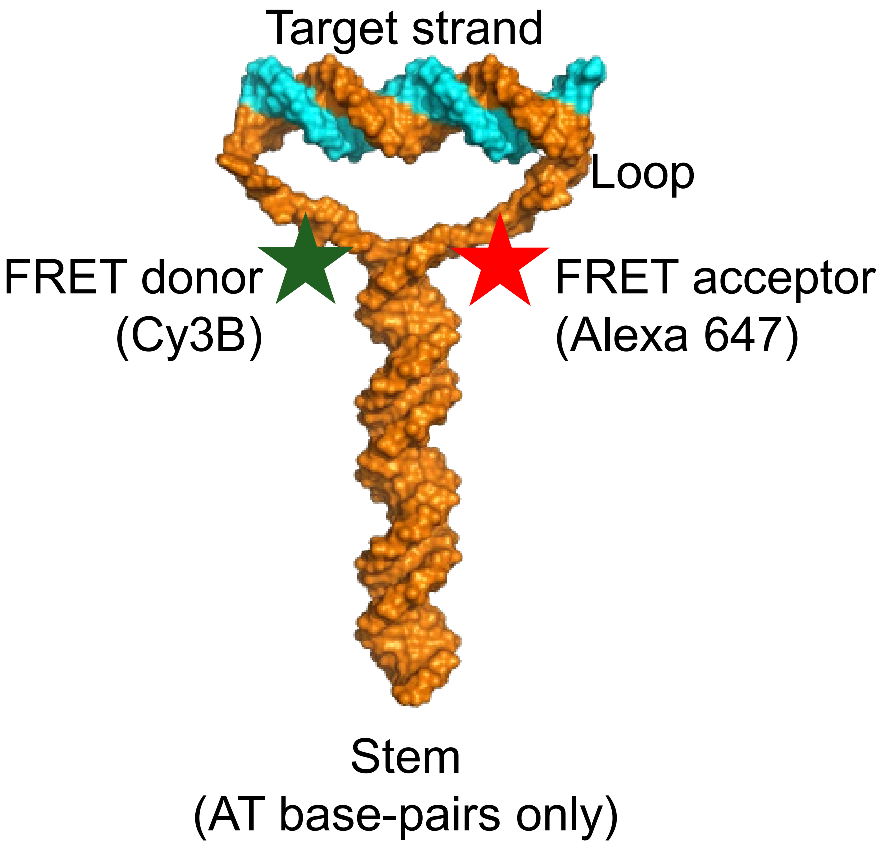
Behavior beyond buckling

Buckling (linear elasticity) | Kinking (nonlinear elasticity)

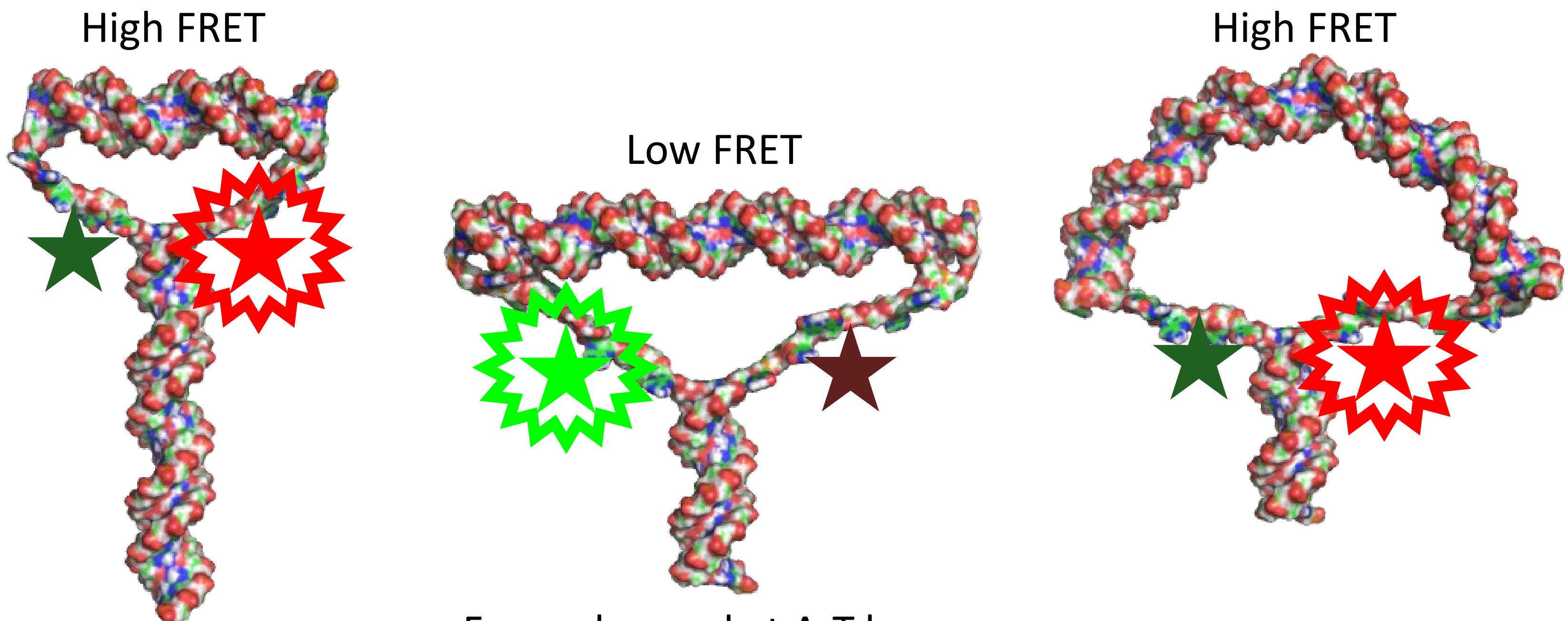


Constant compressive force

Molecular vise



Increasing complement length →



Force clamped at A-T base-pairing force (9 pN [1])

$$F_{AT} \approx 9 \text{ pN} \, [1]$$

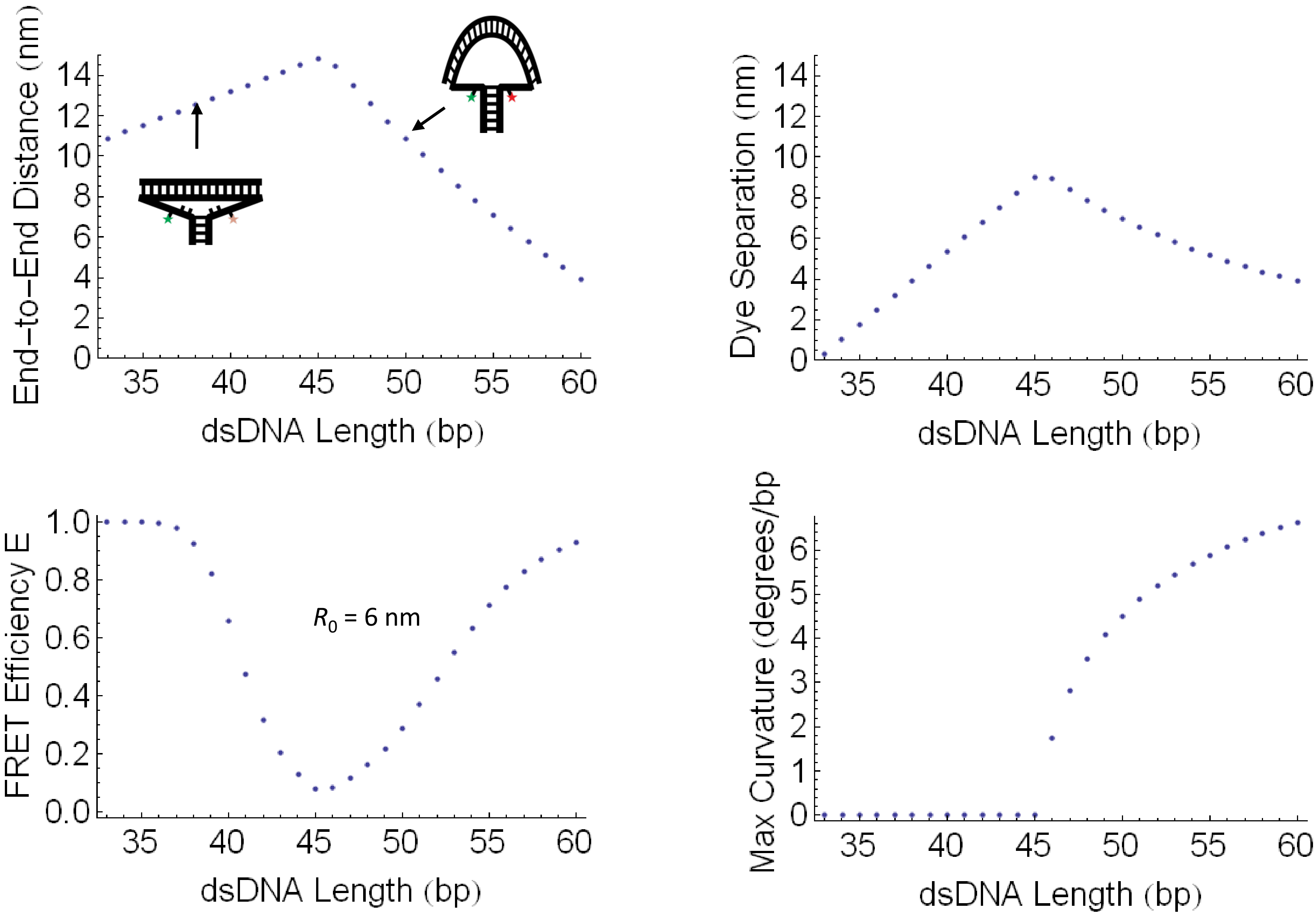
$$\text{persistence length} \equiv \frac{\alpha}{k_B T} \approx 44 - 55 \text{ nm} \, [2]$$

$$L_{buckle} = \sqrt{\frac{\pi^2 \alpha}{F}} \approx 14.1 - 15.7 \text{ nm} = 41 - 46 \text{ bp}$$

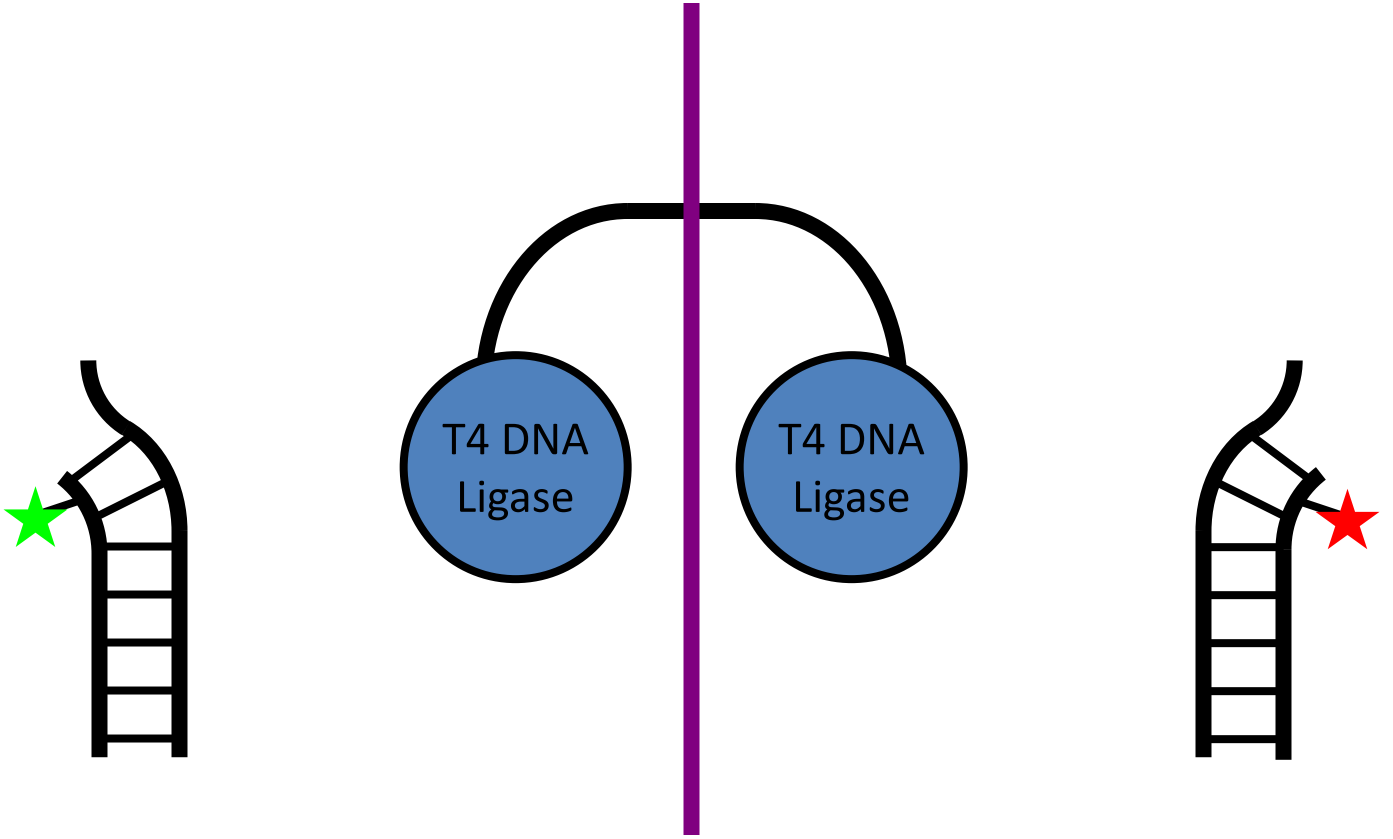
Stem composition	Buckling length	
A-T (9 pN)	45 bp	15 nm
G-C (21 pN)	29 bp	10 nm

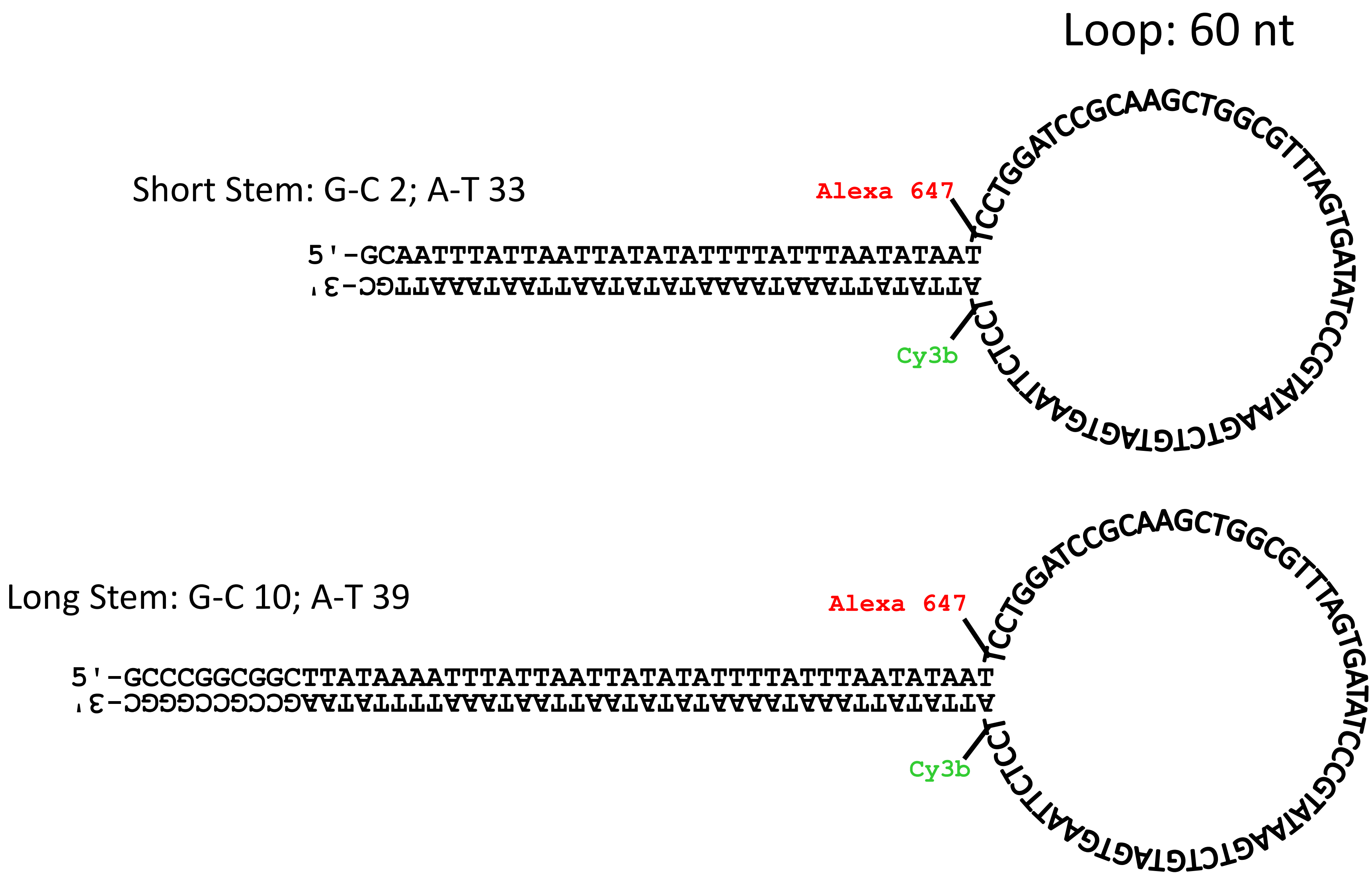
1. Woodside *et al.*, *PNAS* **103**, 6190 (2006).
2. Lu, Weers, Stellwagen, *Biopolymers* **61**, 261 (2002).

Predictions of linear elasticity

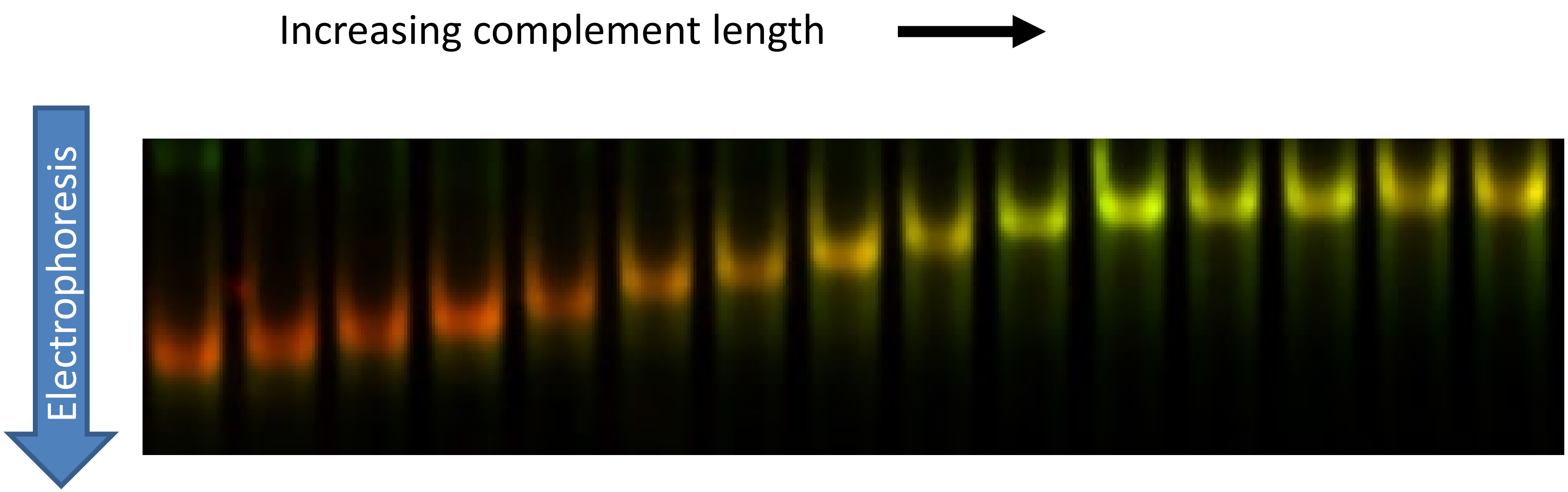


Hairpin Synthesis

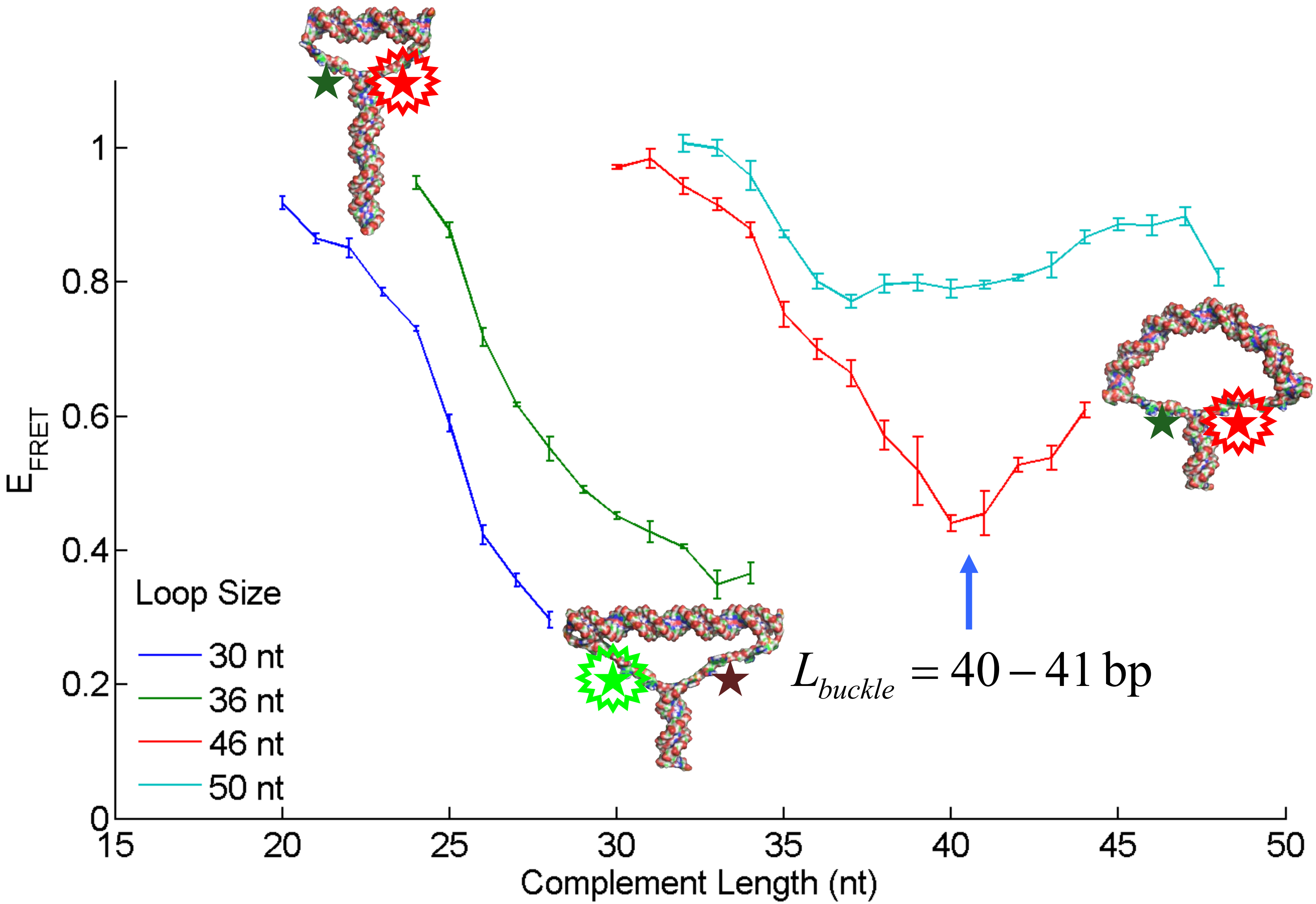



Loop: 60 nt
Short Stem: G-C 2; A-T 33
Alexa 647
5'-GCAATTTATTAATTATATATTTTATTTAATATAAT
Cy3b
Long Stem: G-C 10; A-T 39
Alexa 647
5'-GCCCGGCGGCTTATAAAATTTATTAATTATATATTTTATTTAATATAAT
Cy3b

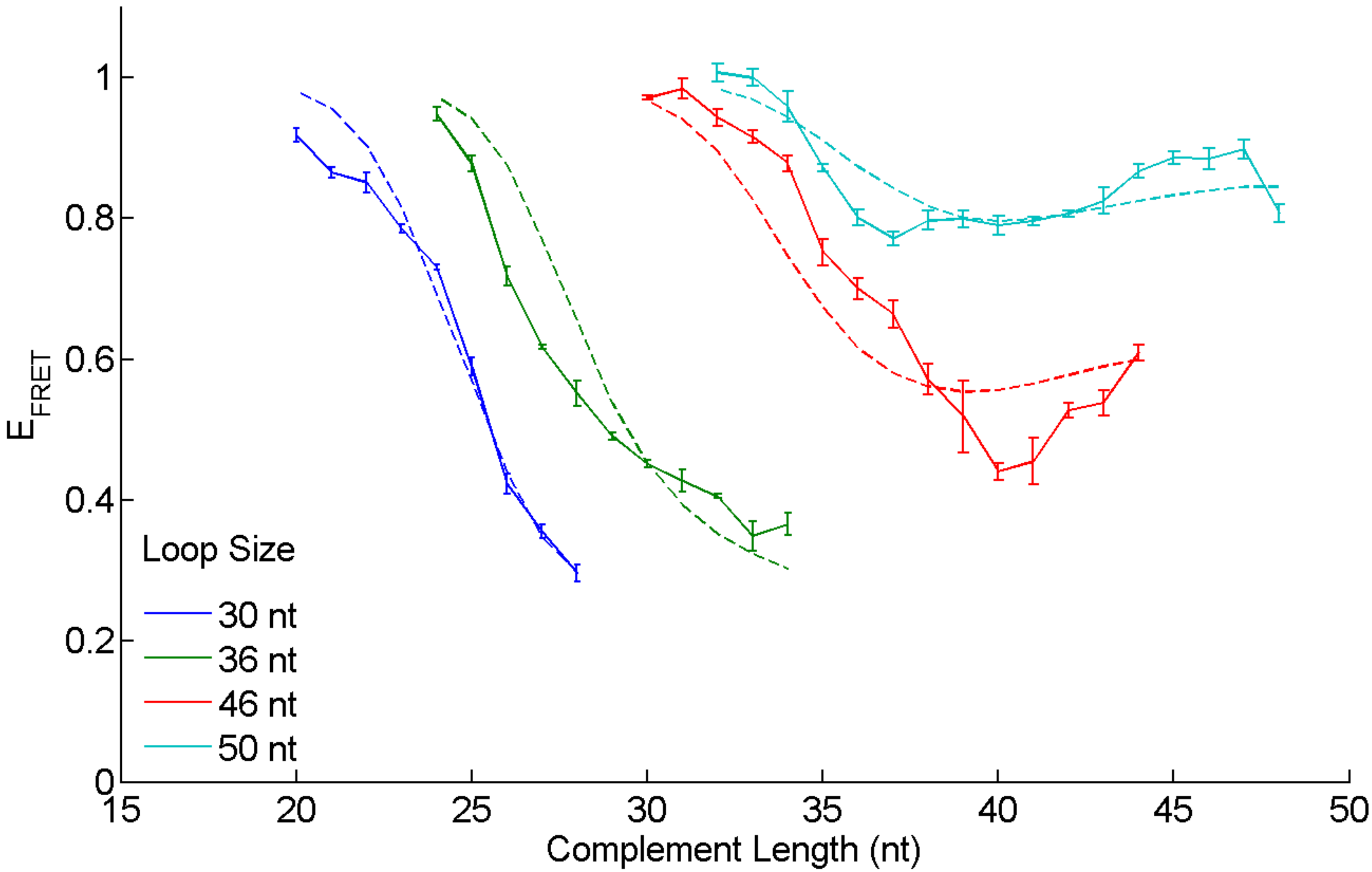
Data collection: native PAGE



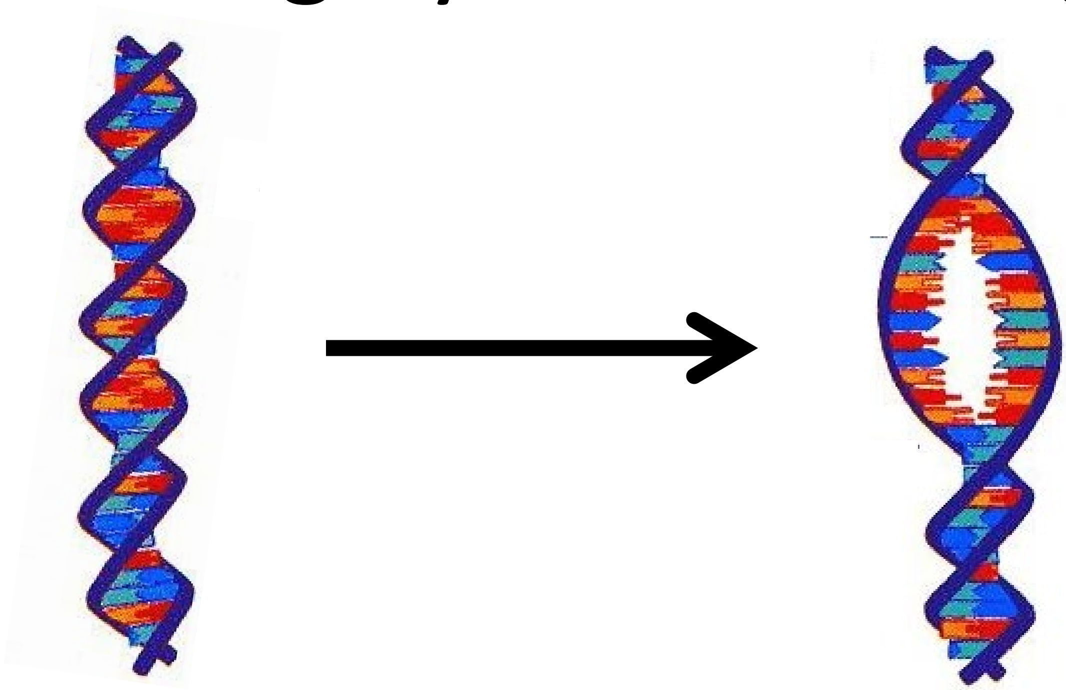
Euler buckling in dsDNA



A simple statistical mechanical model

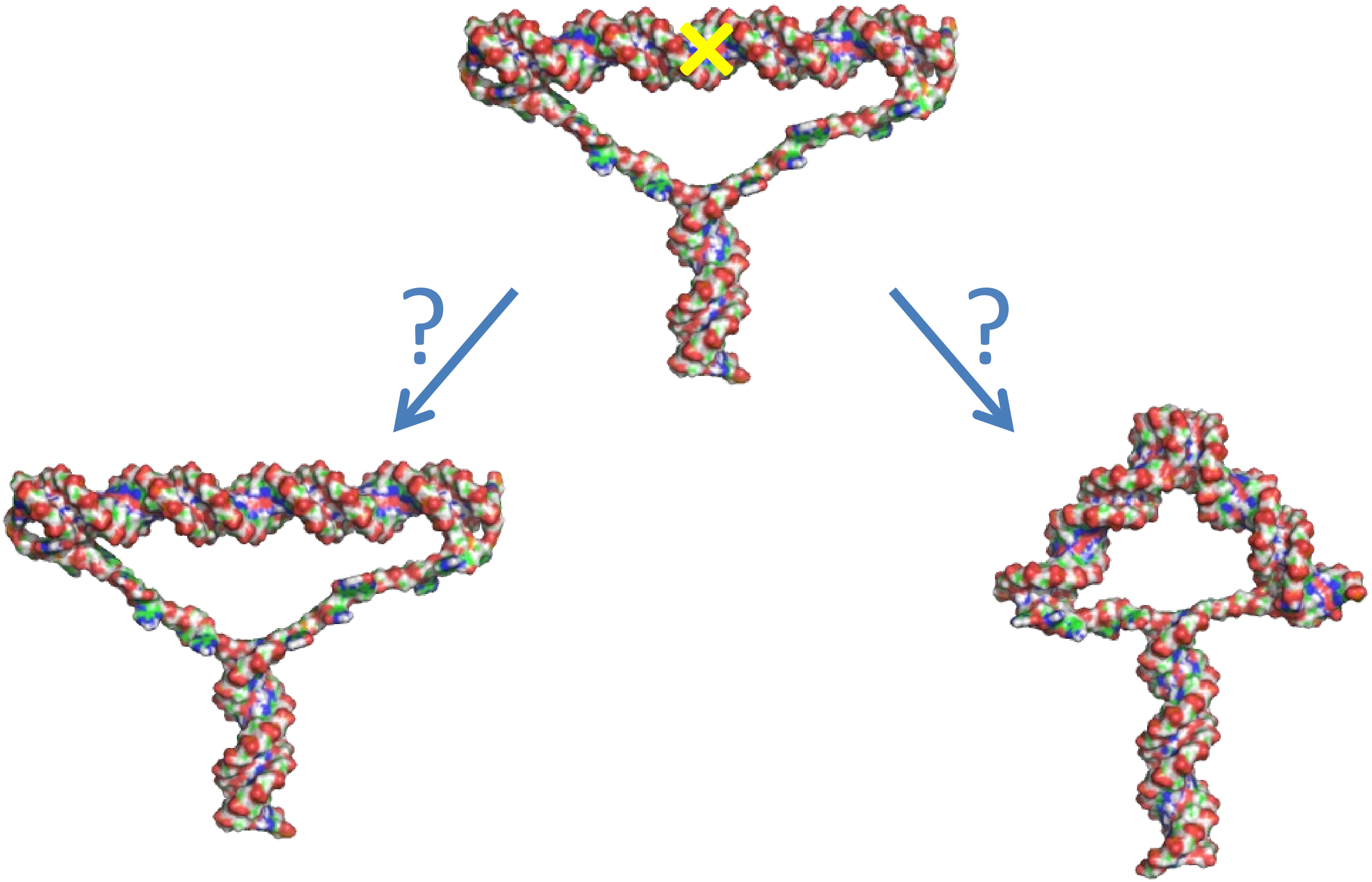


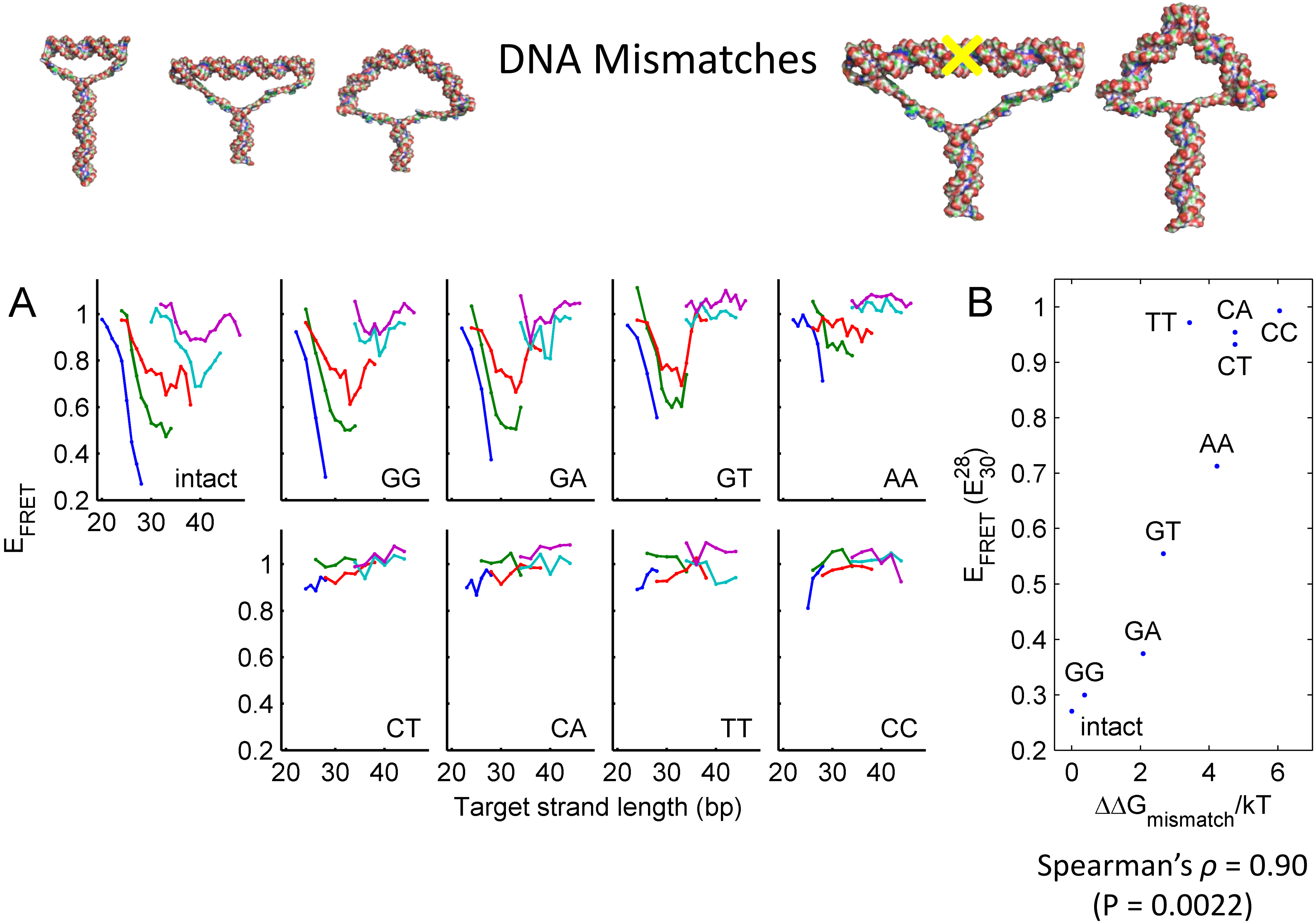
Kinking by local melting



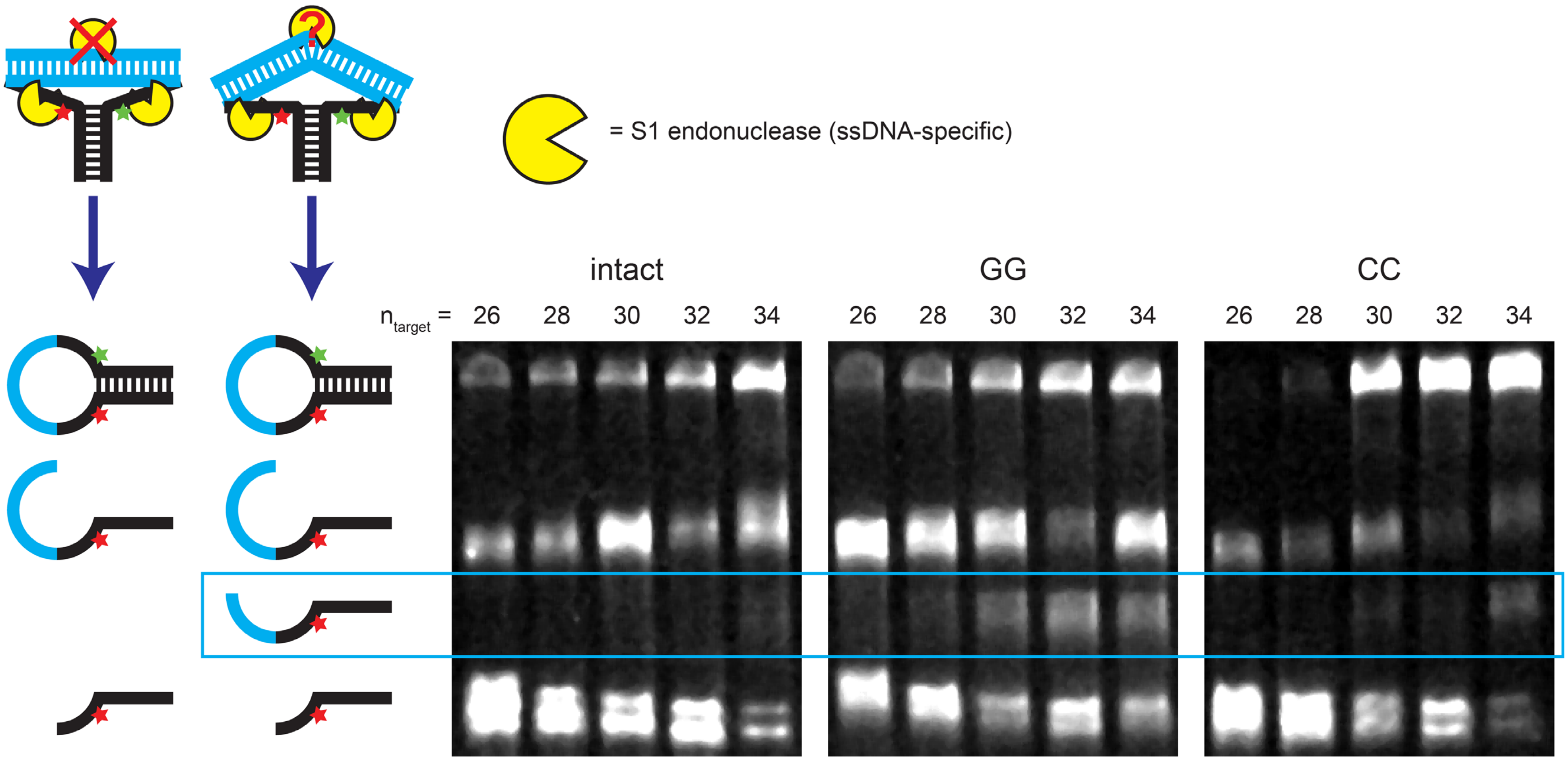
- Hypotheses:
  - Kinking should correlate with “meltability”
  - Kinked structures should be recognizable as single-stranded DNA

DNA Mismatches

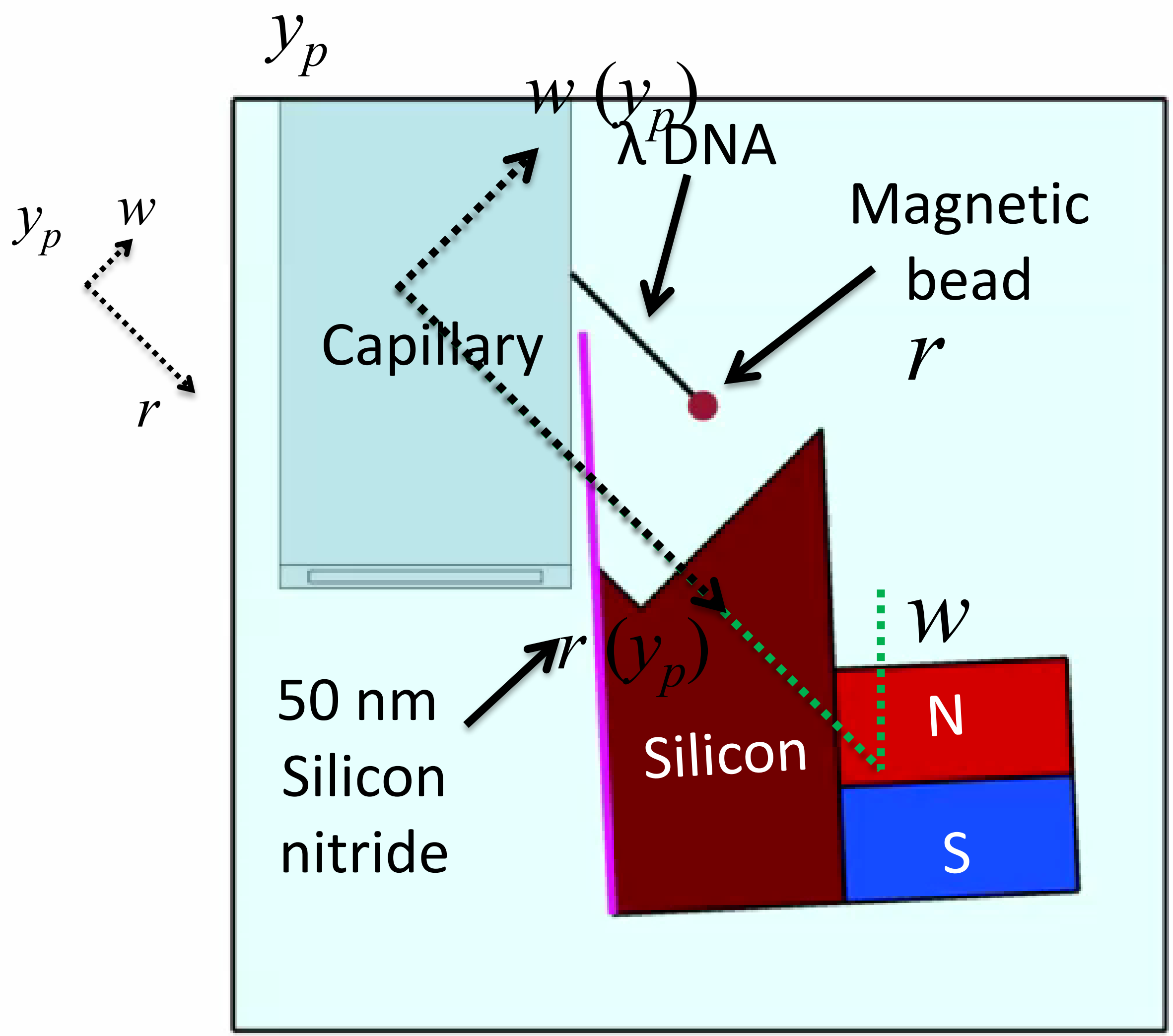



DNA Mismatches
A
1
0.8
0.6
0.4
0.2
20
30
40
E_FRET
intact
GG
GA
GT
AA
CT
CA
TT
CC
Target strand length (bp)
B
E_FRET (E^28_30)
ΔΔG_mismatch/kT
0
2
4
6
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Spearman's ρ = 0.90
(P = 0.0022)

S1 nuclease cleavage

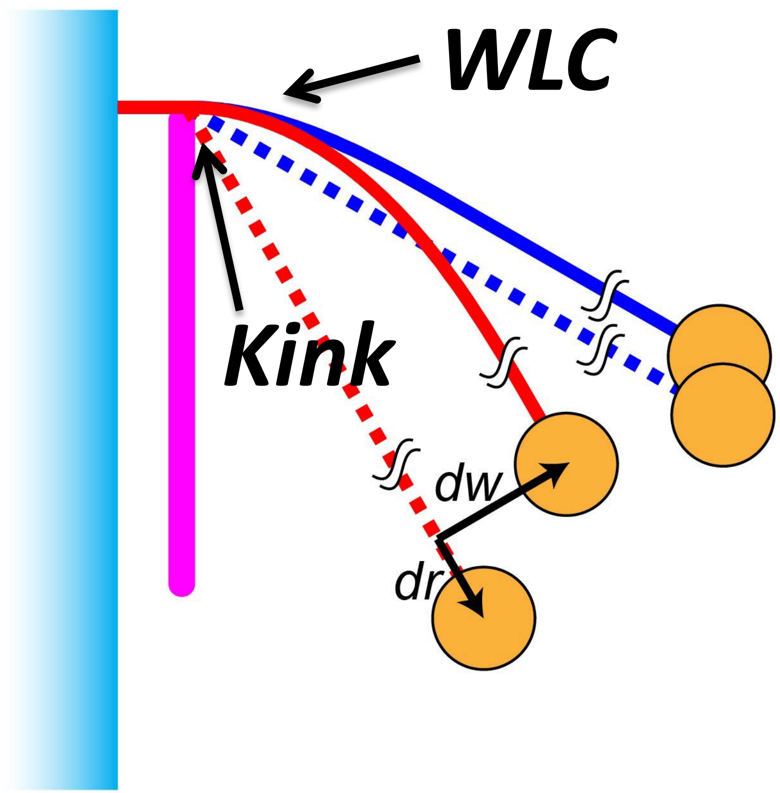


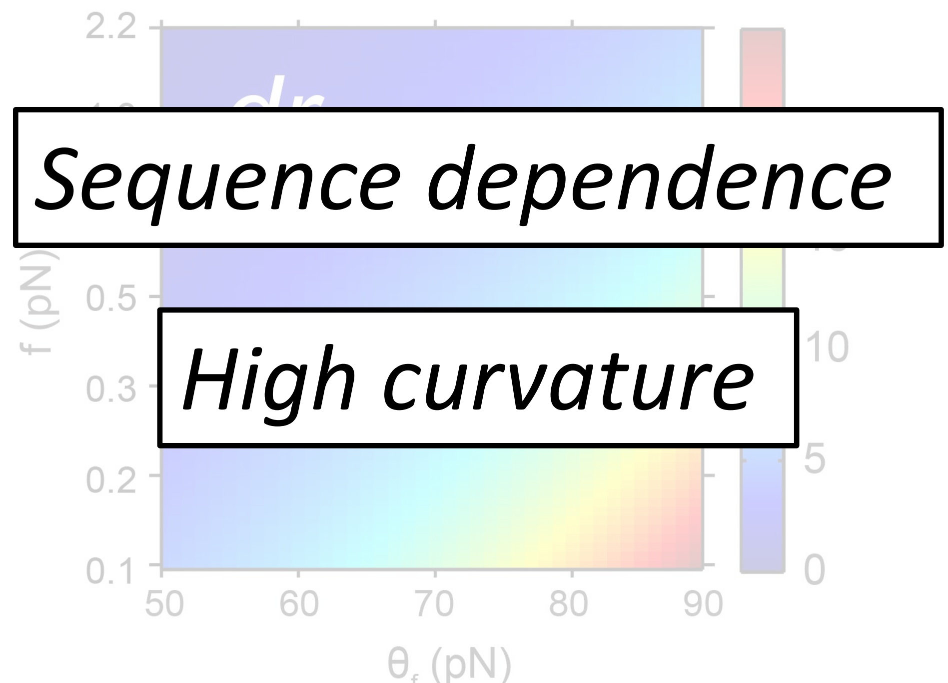
DNA Pulley



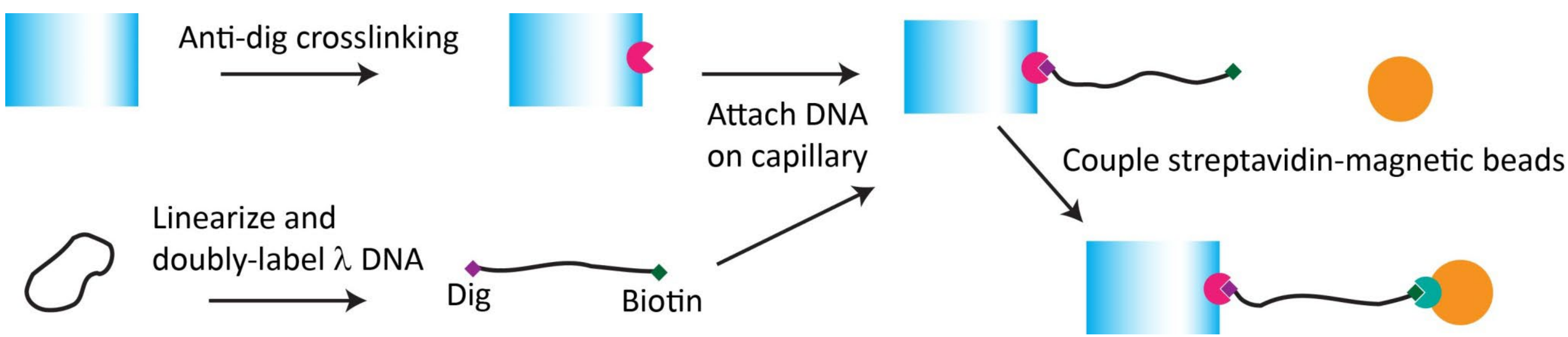
y_p
$w\,(y_p)$
λ DNA
Magnetic bead
r
y_p
w
r
Capillary
w
$r\,(y_p)$
50 nm Silicon nitride
Silicon
N
S

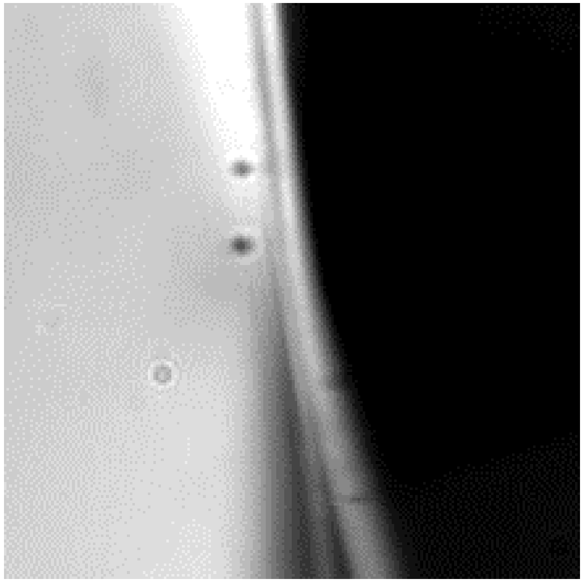
Nano-mechanics of DNA



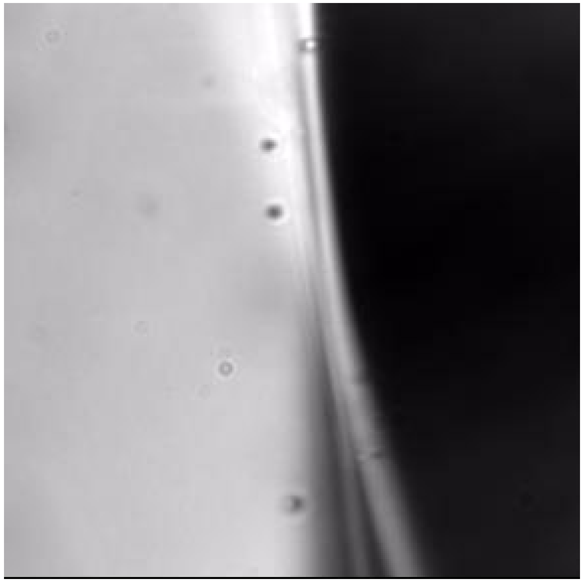


λ DNA construct



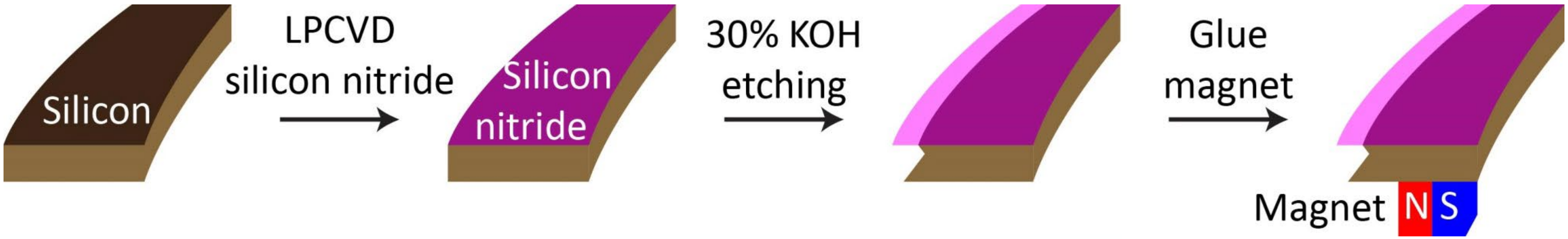


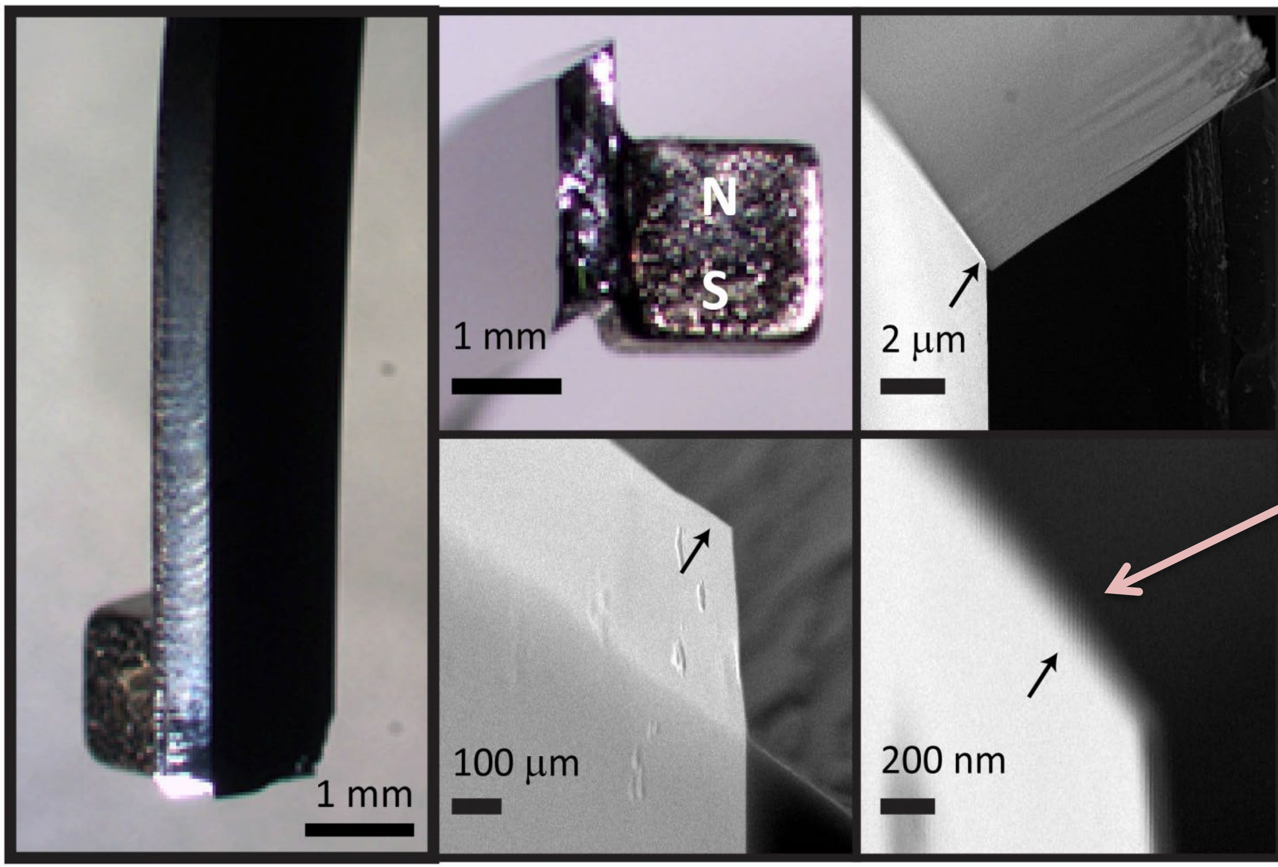
Rotating force



Varying force

Silicon nitride knife

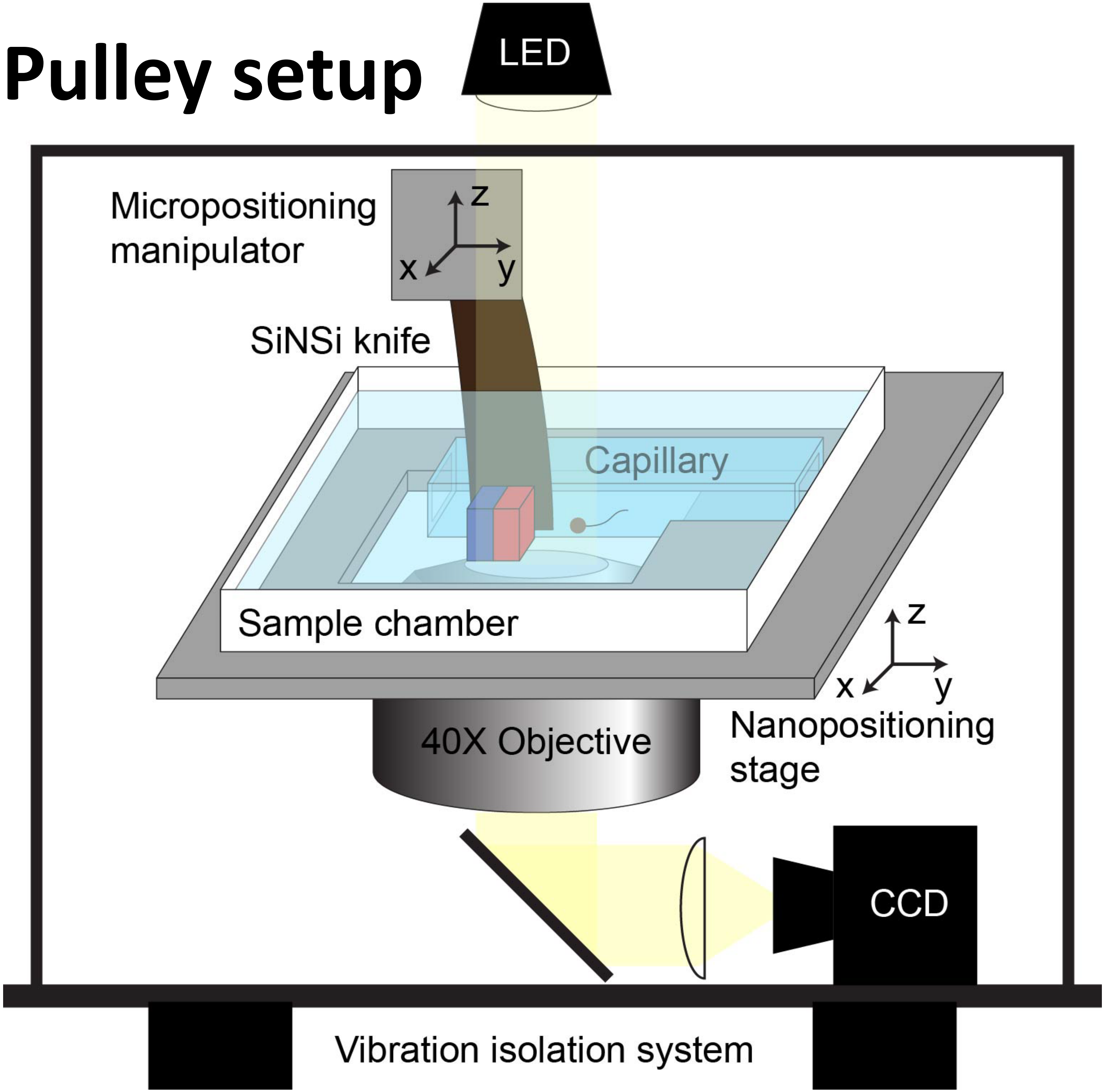


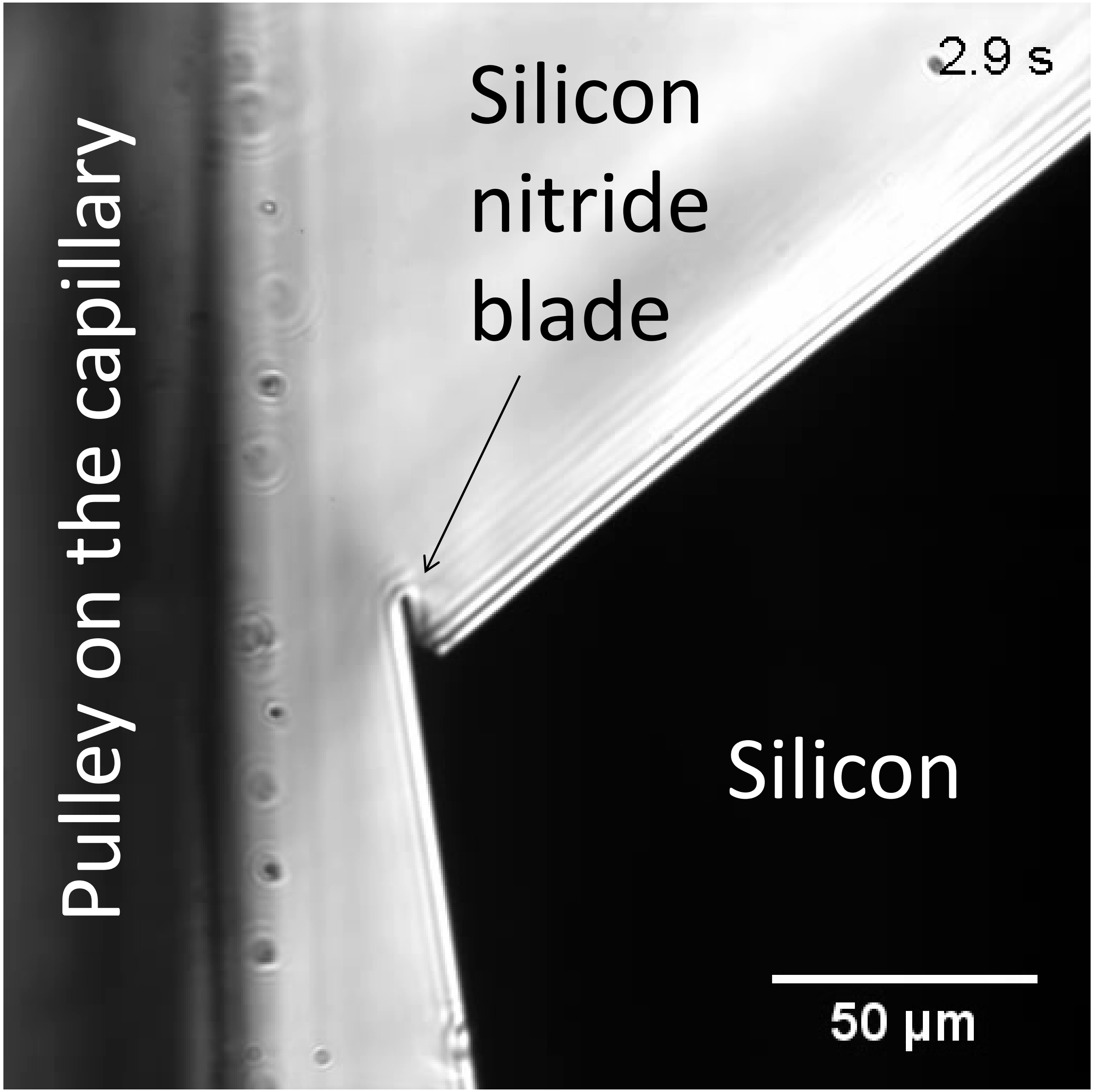


Stereomicroscope and SEM Images

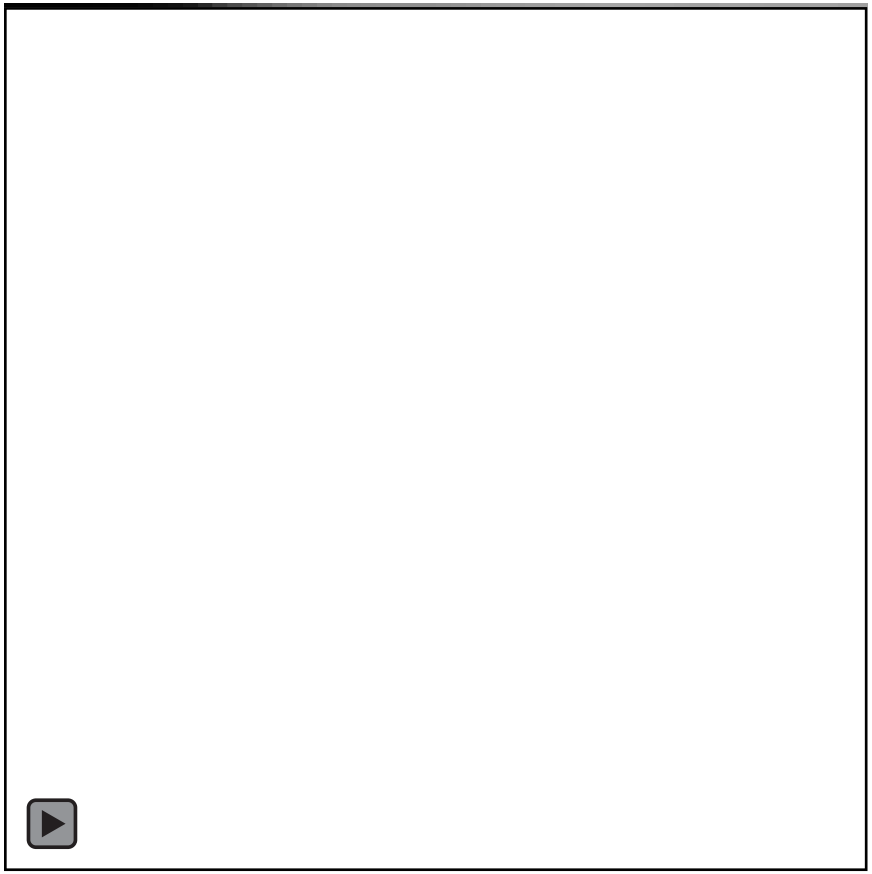
Nanoscopically smooth edge

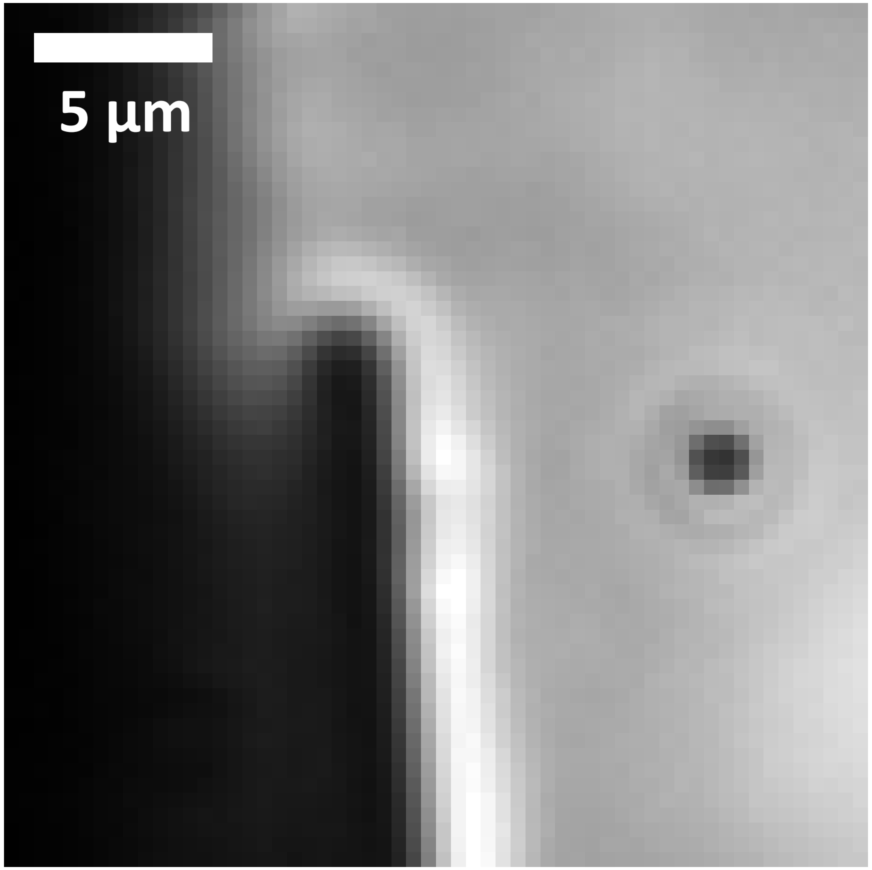
DNA Pulley setup




2.9 s
Silicon nitride blade
Pulley on the capillary
Silicon
50 µm

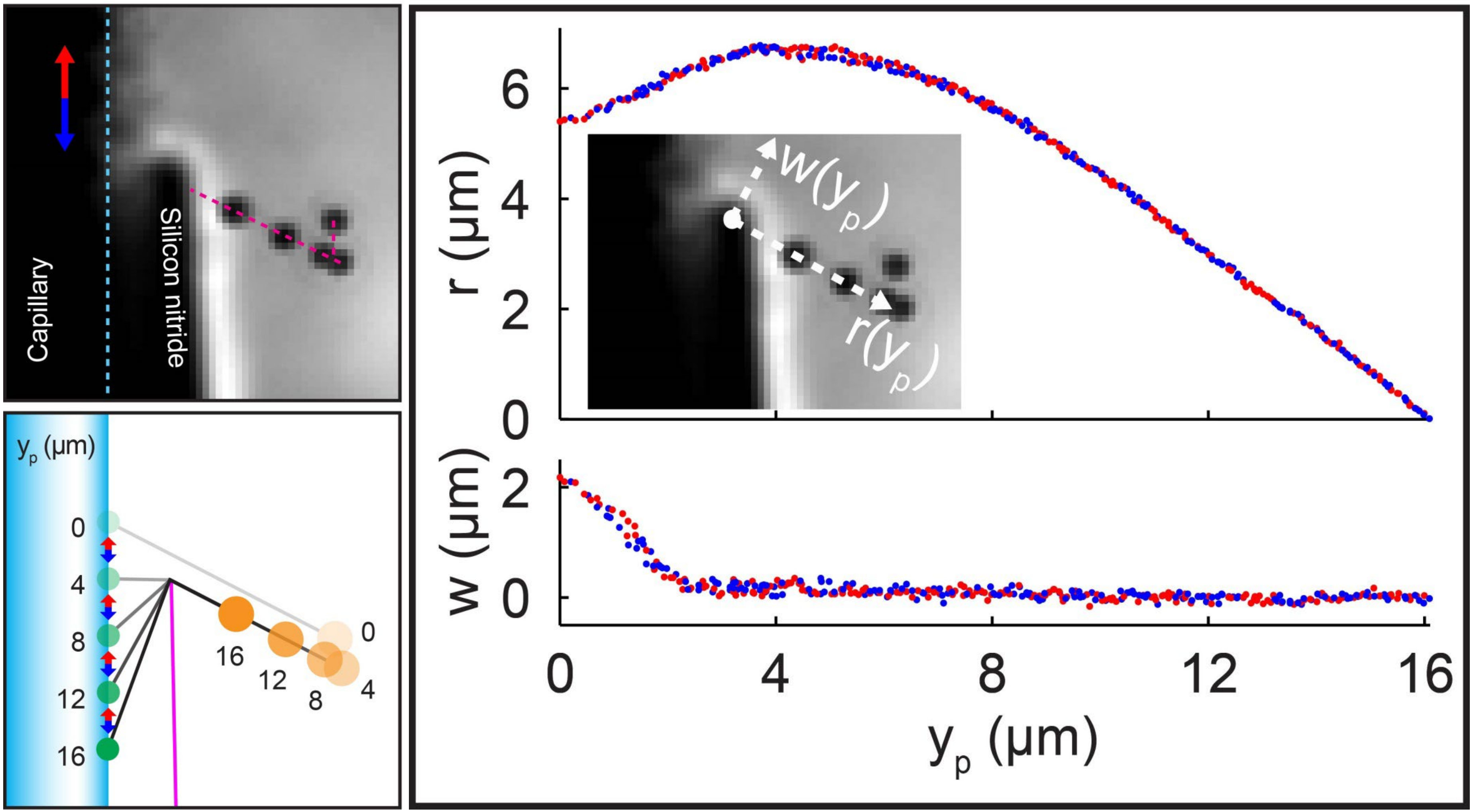
Simple scanning



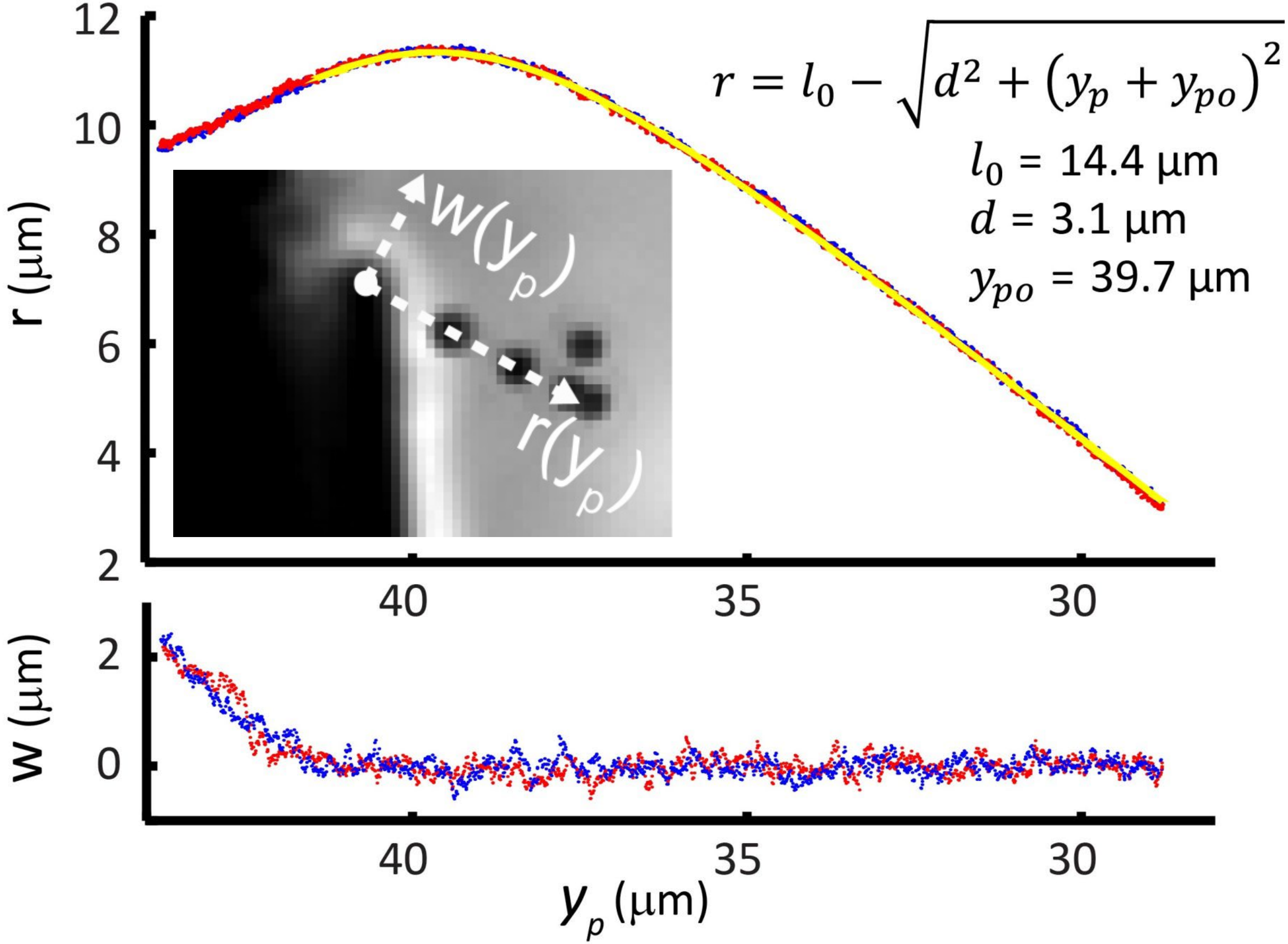


$$r = l_o - \sqrt{d^2 + \left(y_p + y_o\right)^2}$$

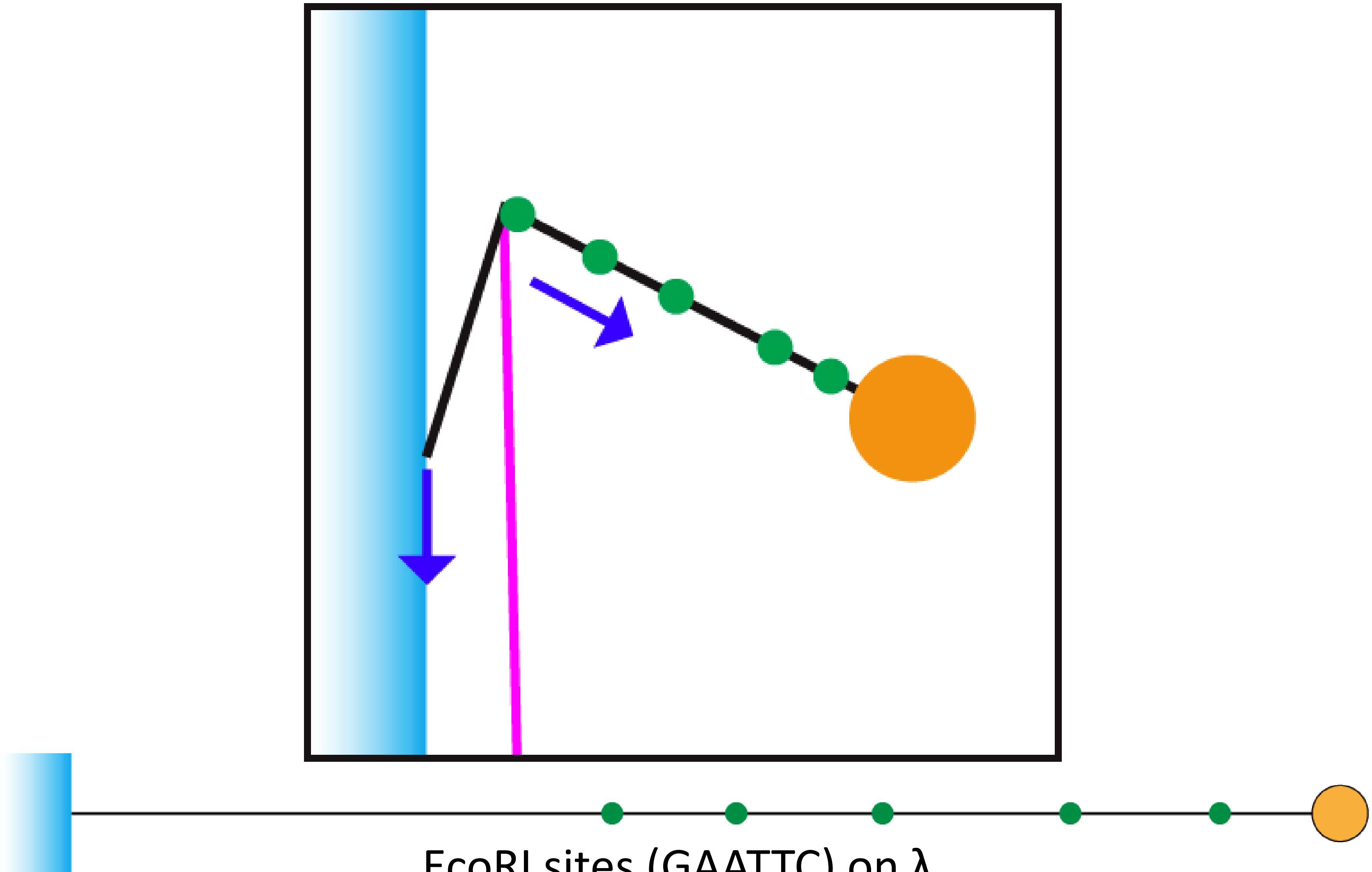
l_o: total extension; d: capillary distance; y_o: offset in y



Bead trajectory

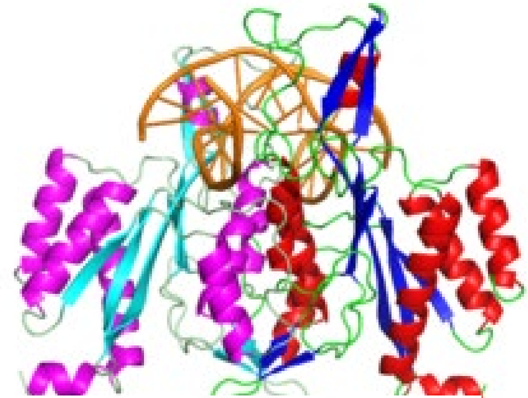


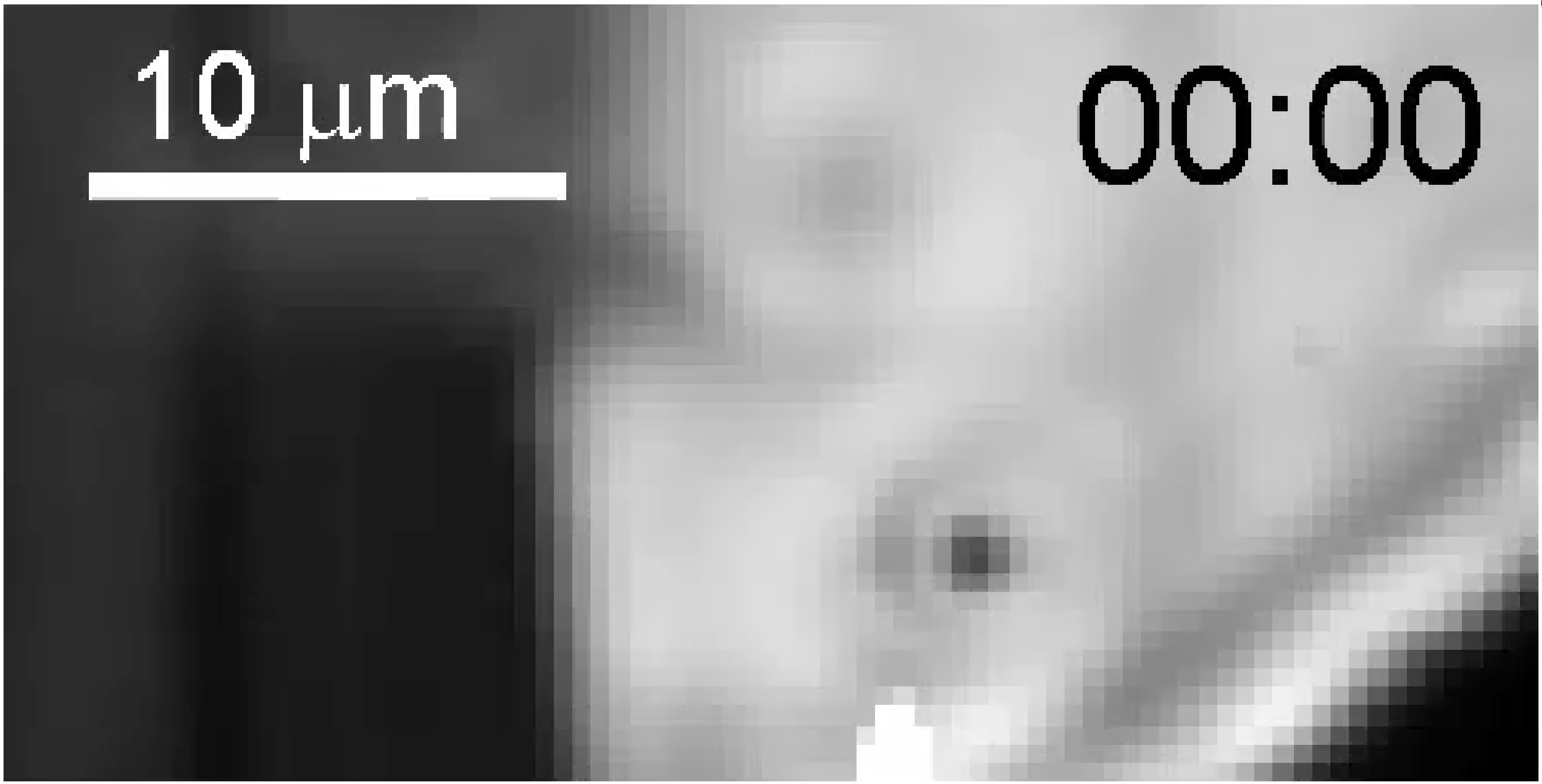
Fiducial marker: EcoRI



EcoRI sites (GAATTC) on λ

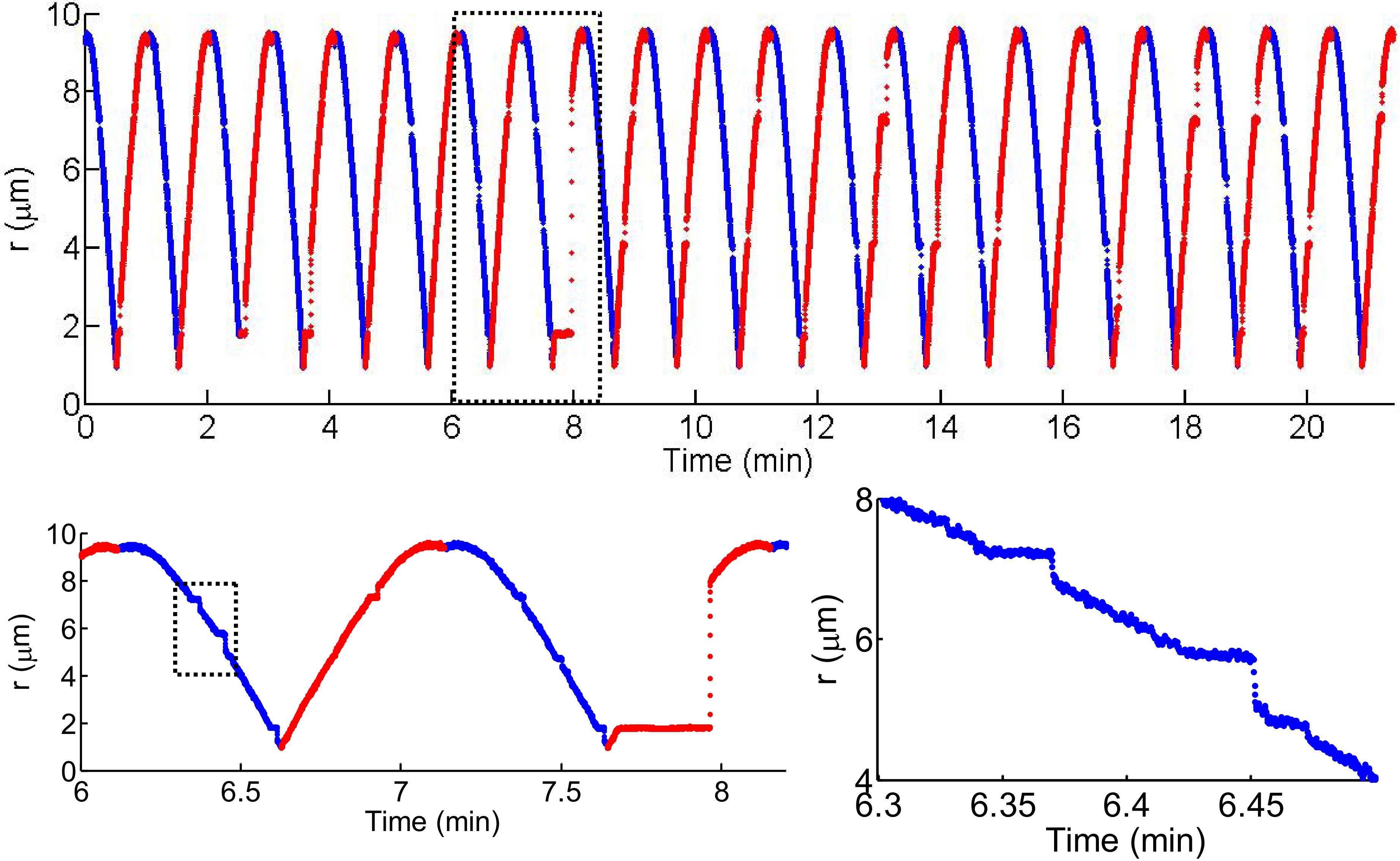
EcoRI-incubated λ DNA



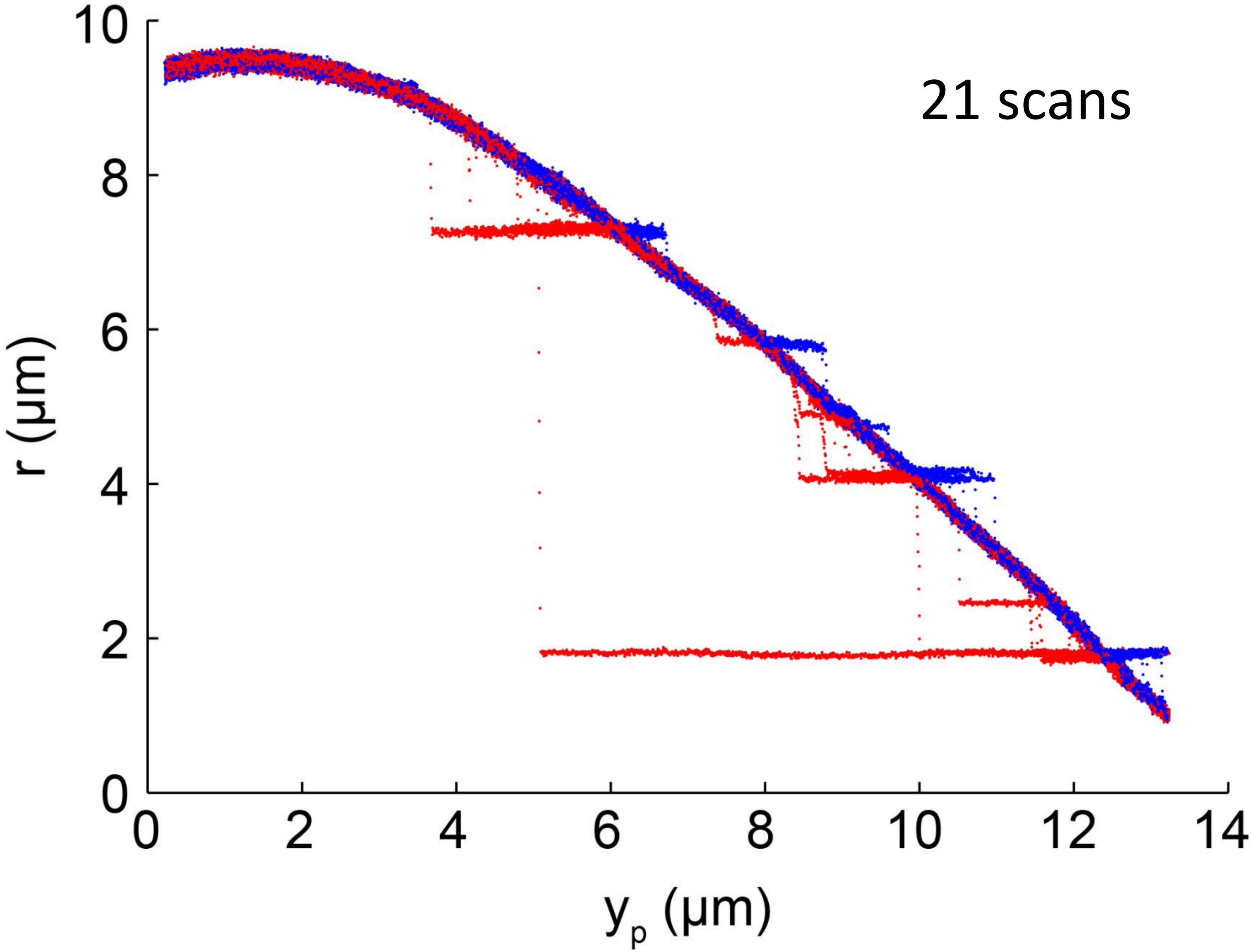


λ DNA with 10 nM EcoRI, 1 mM $CaCl_2$

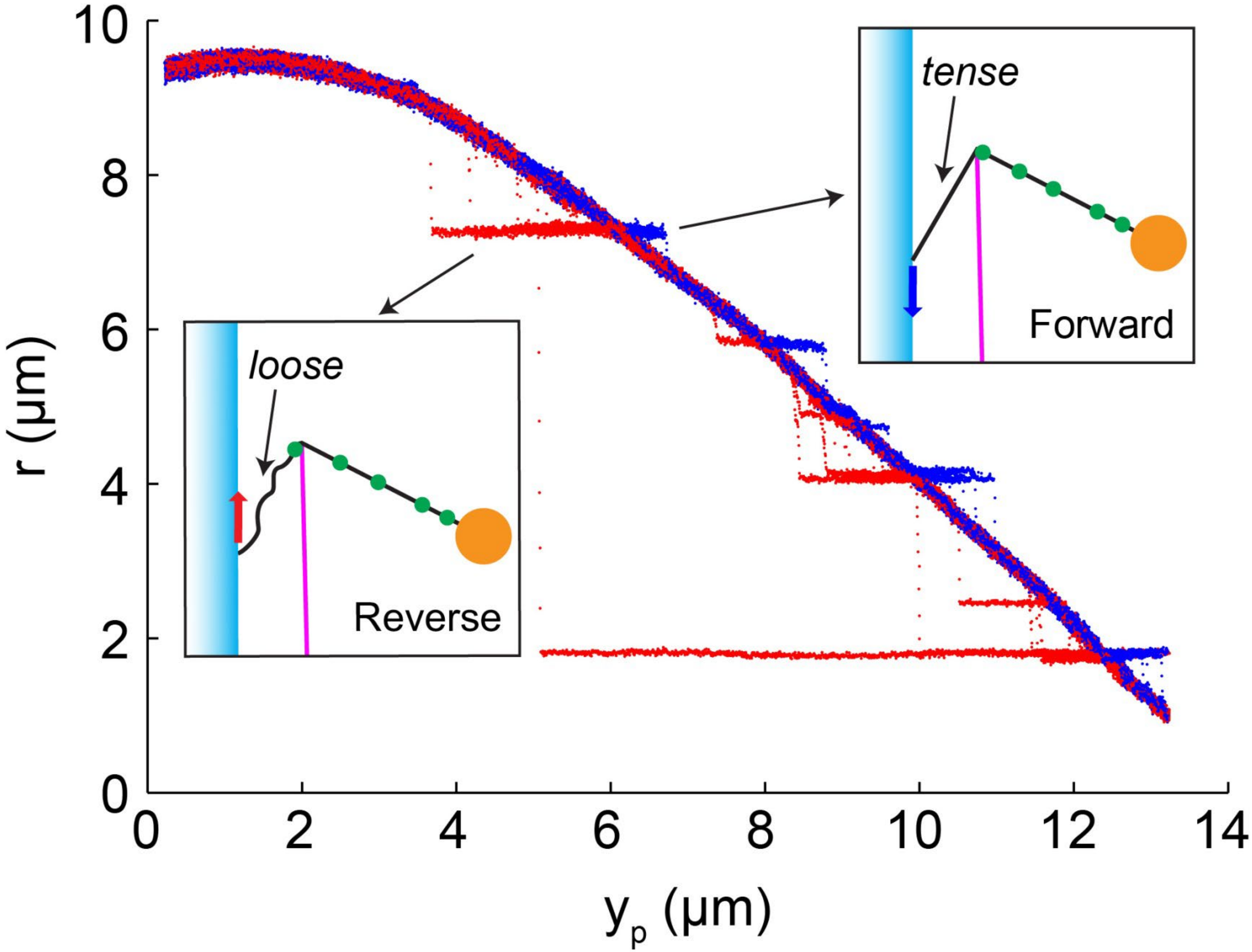
EcoRI-incubated λ DNA



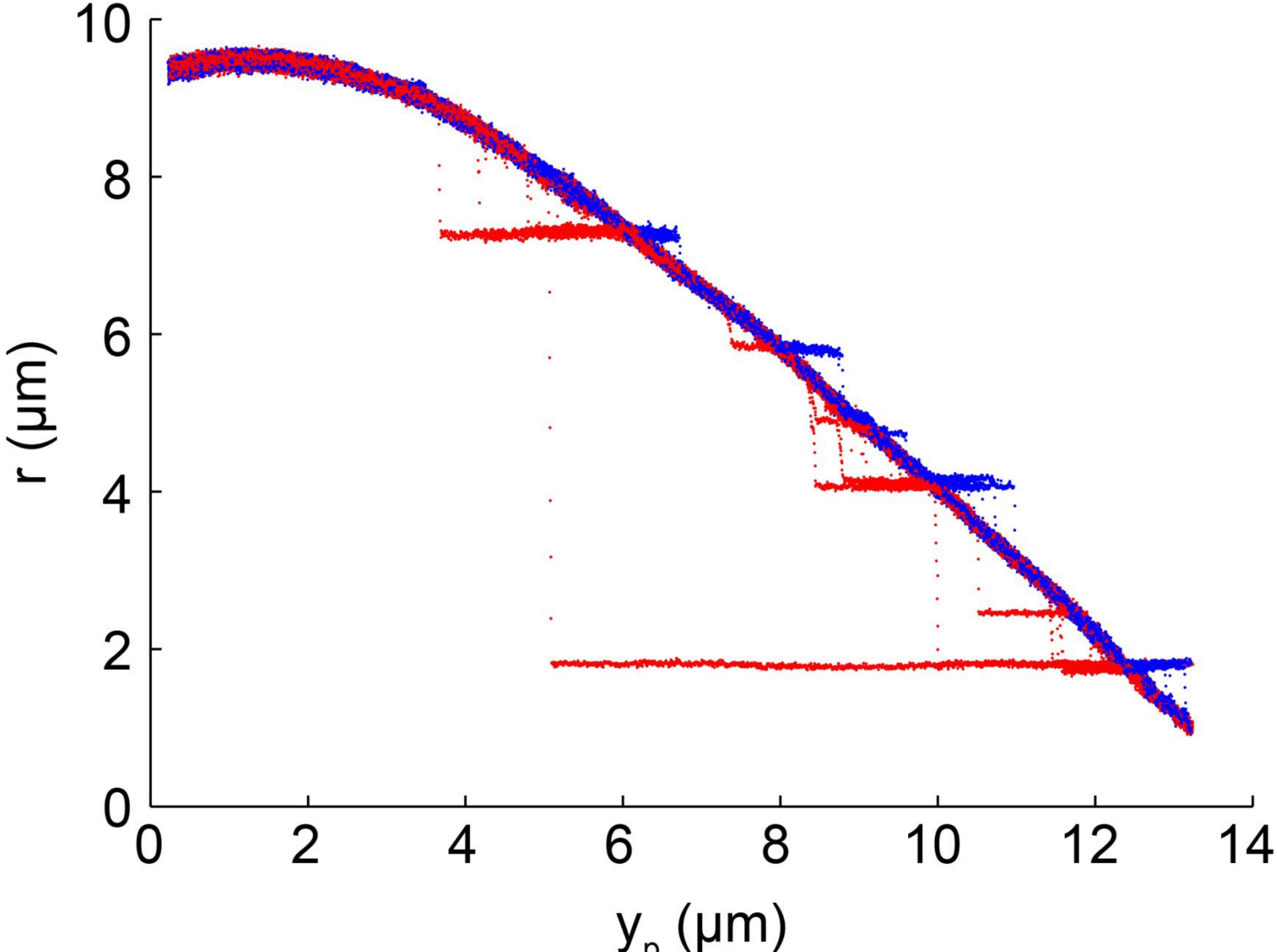
EcoRI on DNA



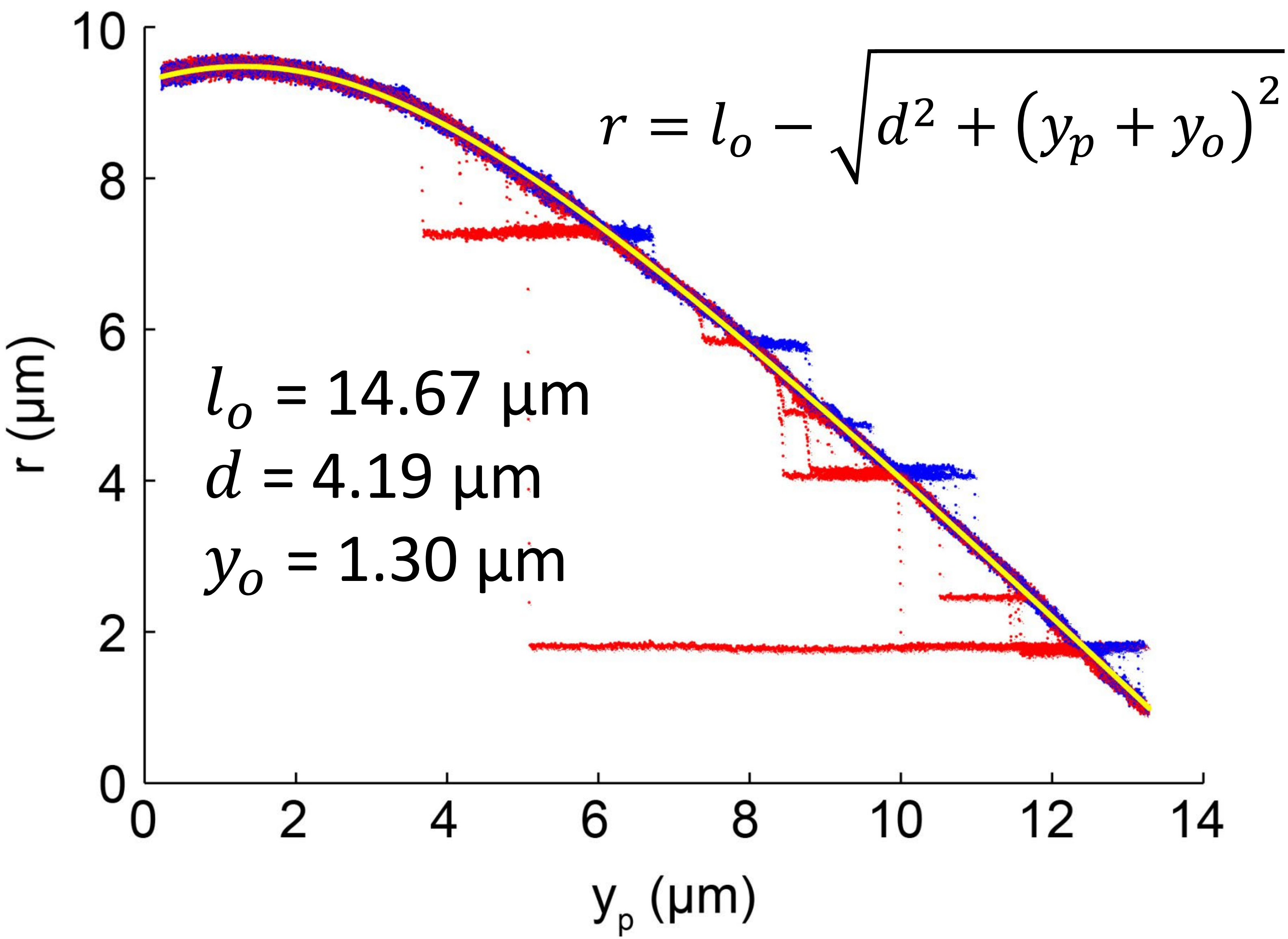
Asymmetry in the direction



EcoRI on DNA

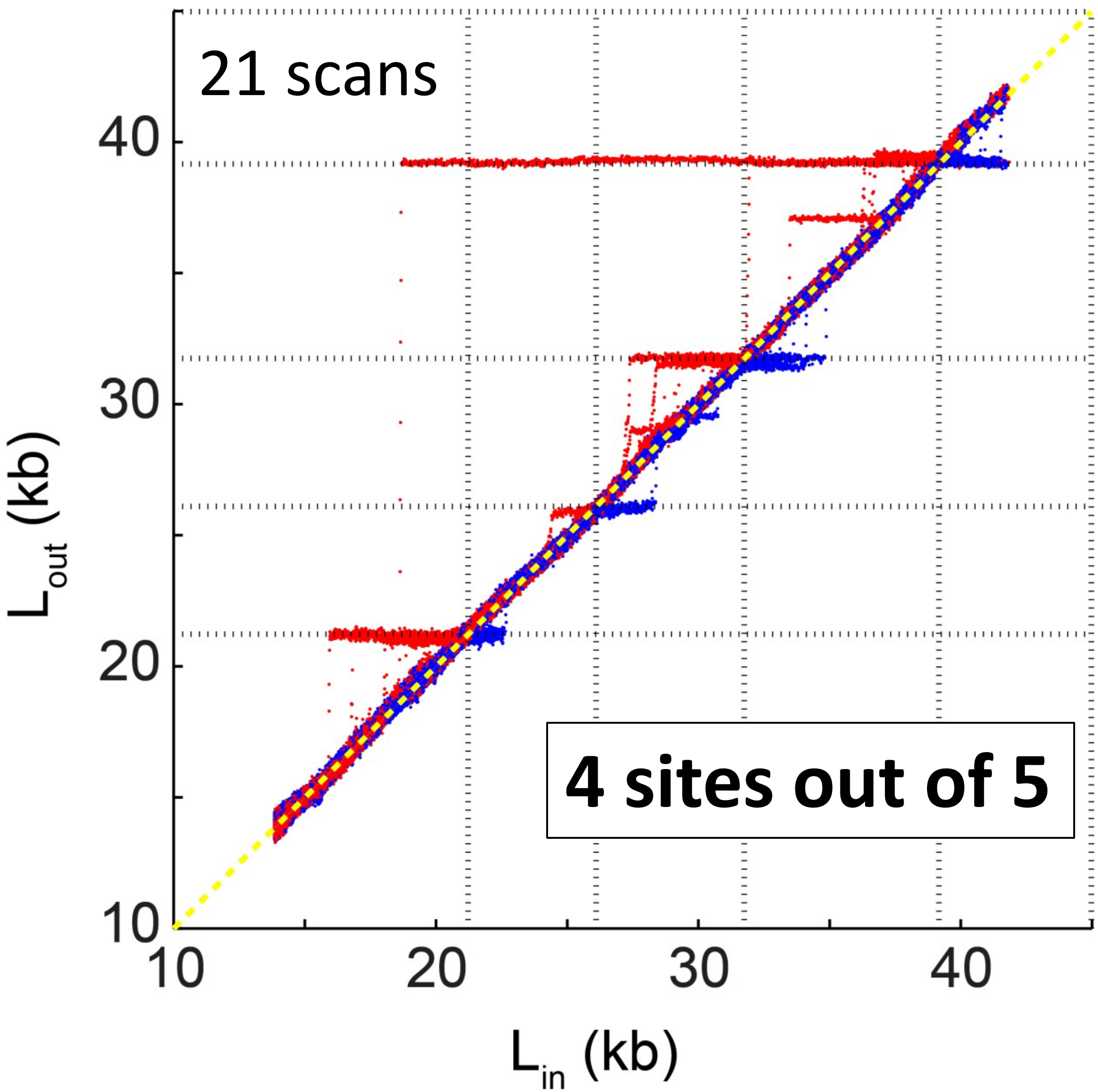


EcoRI on DNA



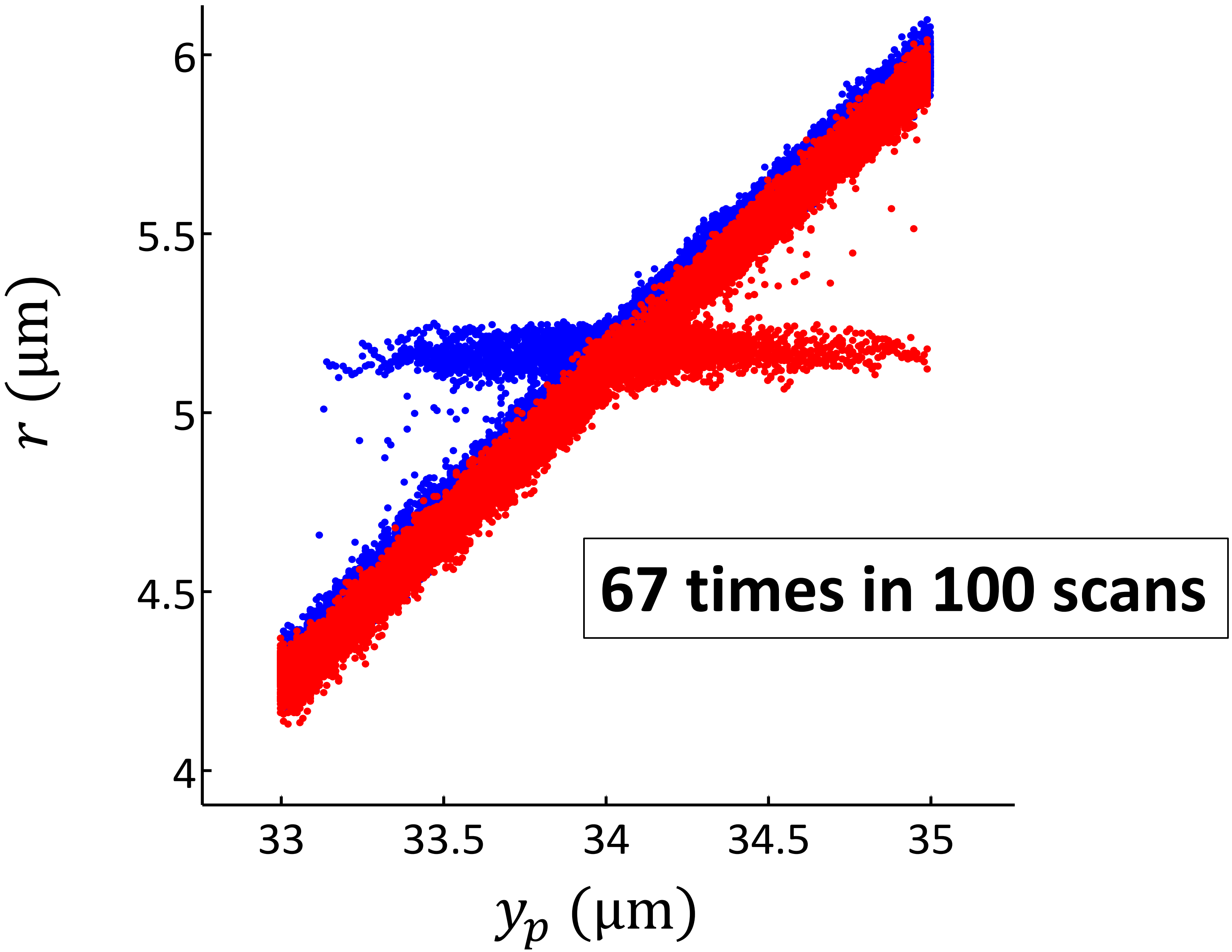
10
8
6
4
2
0
0
2
4
6
8
10
12
14
r (µm)
y_p (µm)
$r = l_o - \sqrt{d^2 + (y_p + y_o)^2}$
l_o = 14.67 µm
d = 4.19 µm
y_o = 1.30 µm

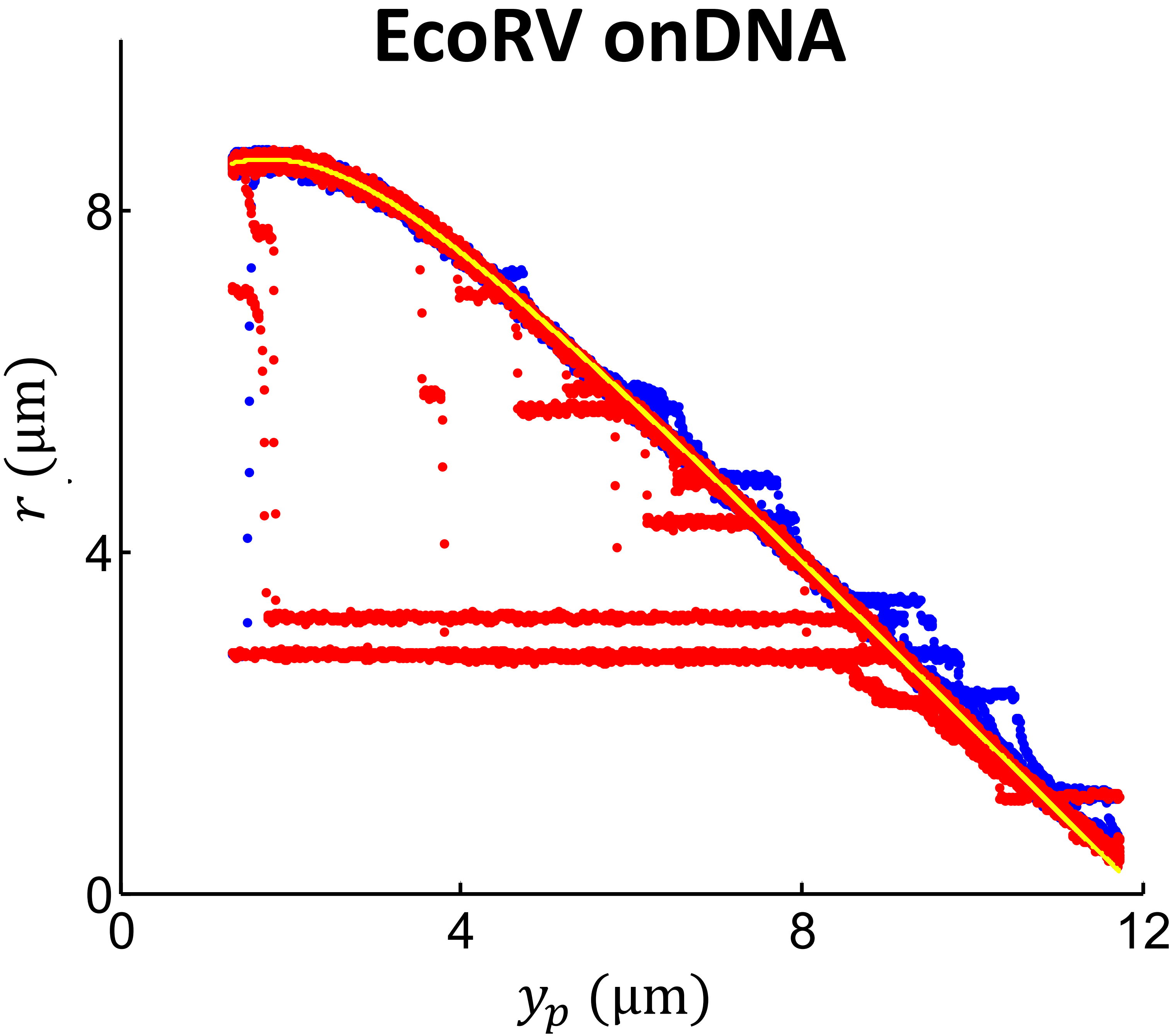
Mapping recognition sites



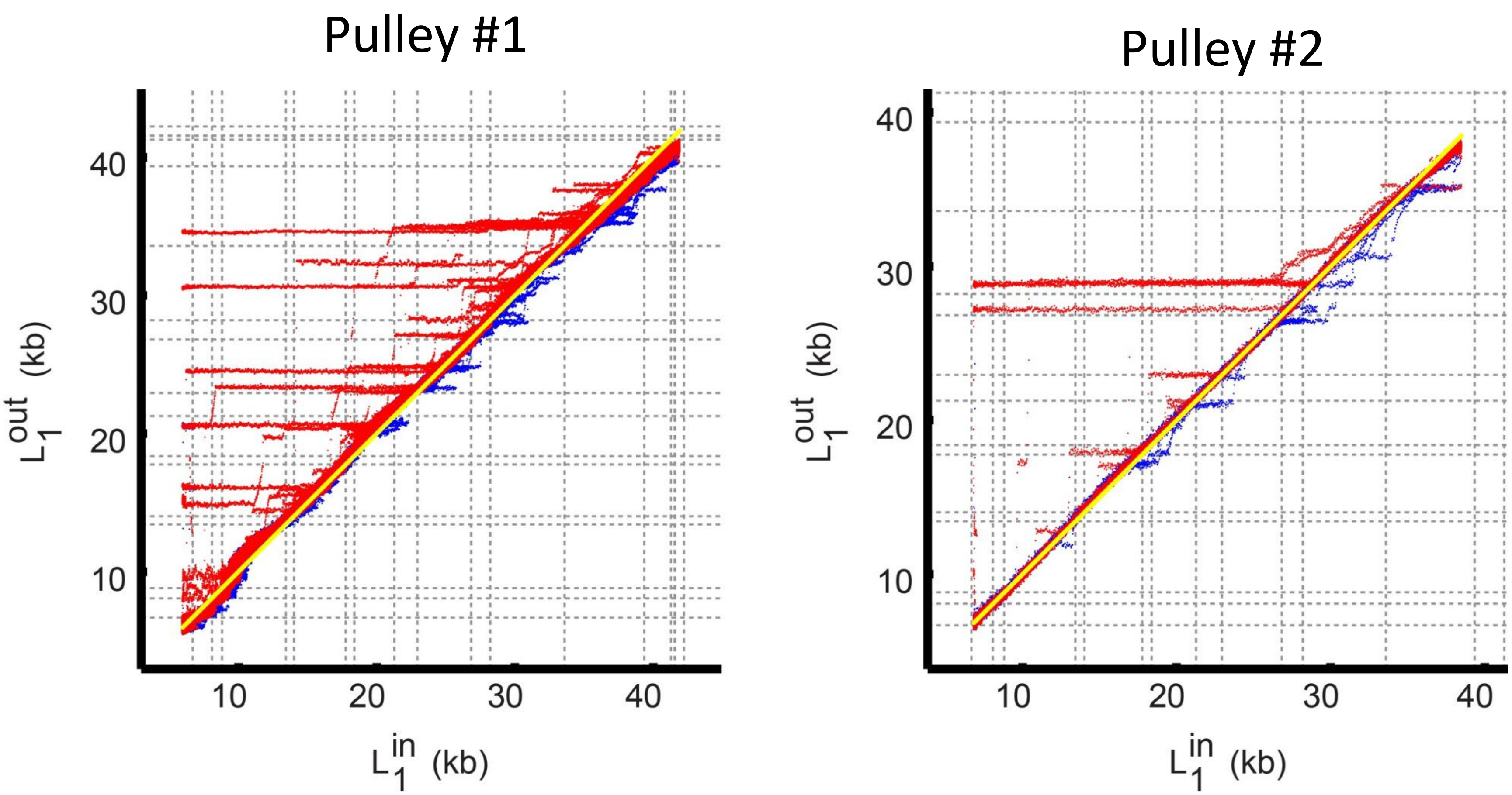
← **EcoRI sites**

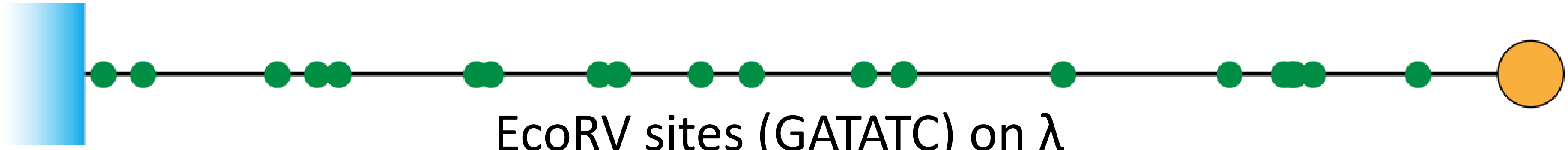
Detection efficiency




EcoRV onDNA
r (μm)
y_p (μm)
0
4
8
0
4
8
12

EcoRV bound to DNA





EcoRV sites (GATATC) on λ