Hybridization and dehybridization of short oligonucleotides subject to weak tension

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KIM LAB

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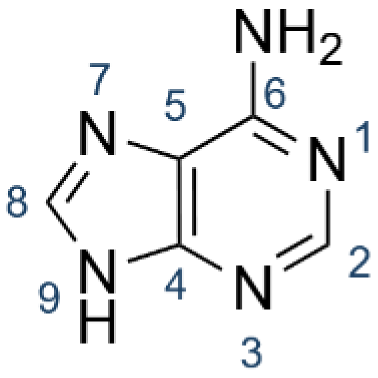
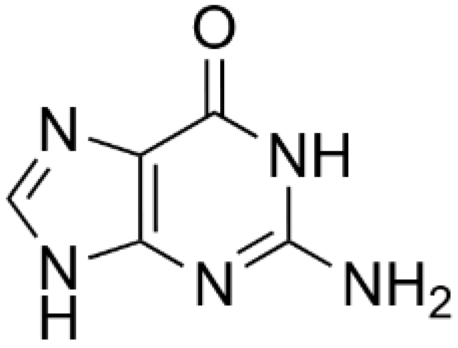
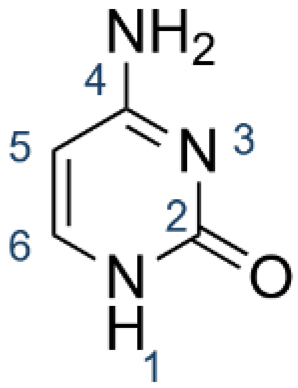
AGTCTATCGGATCGGATCGAATTCGAT



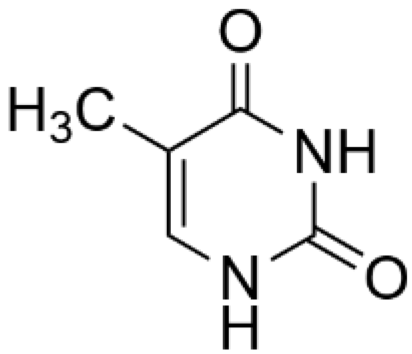
Adenine



Guanine

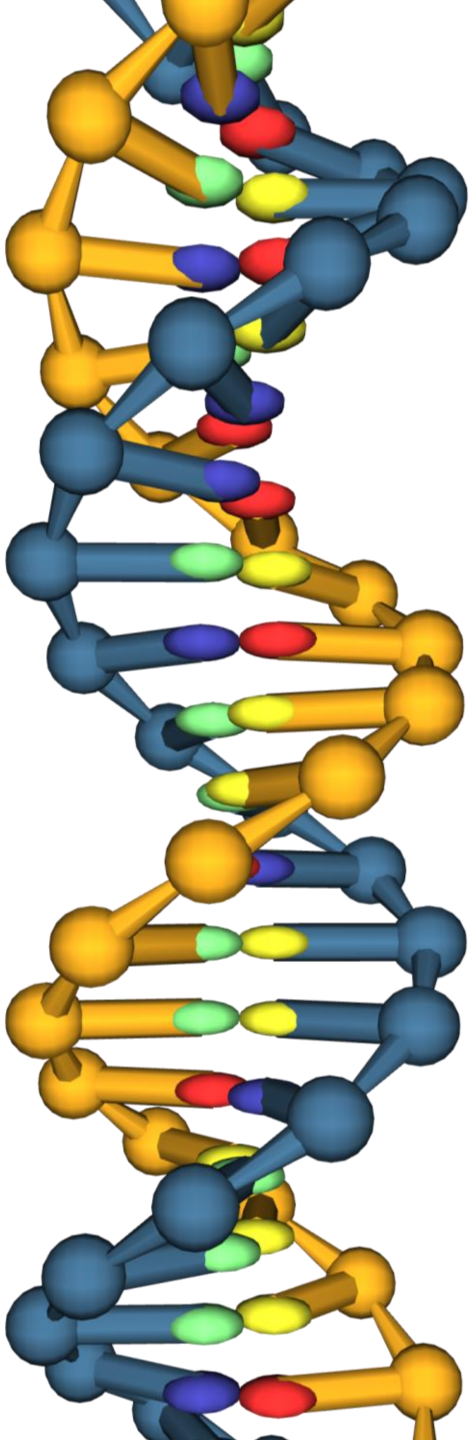


Cytosine

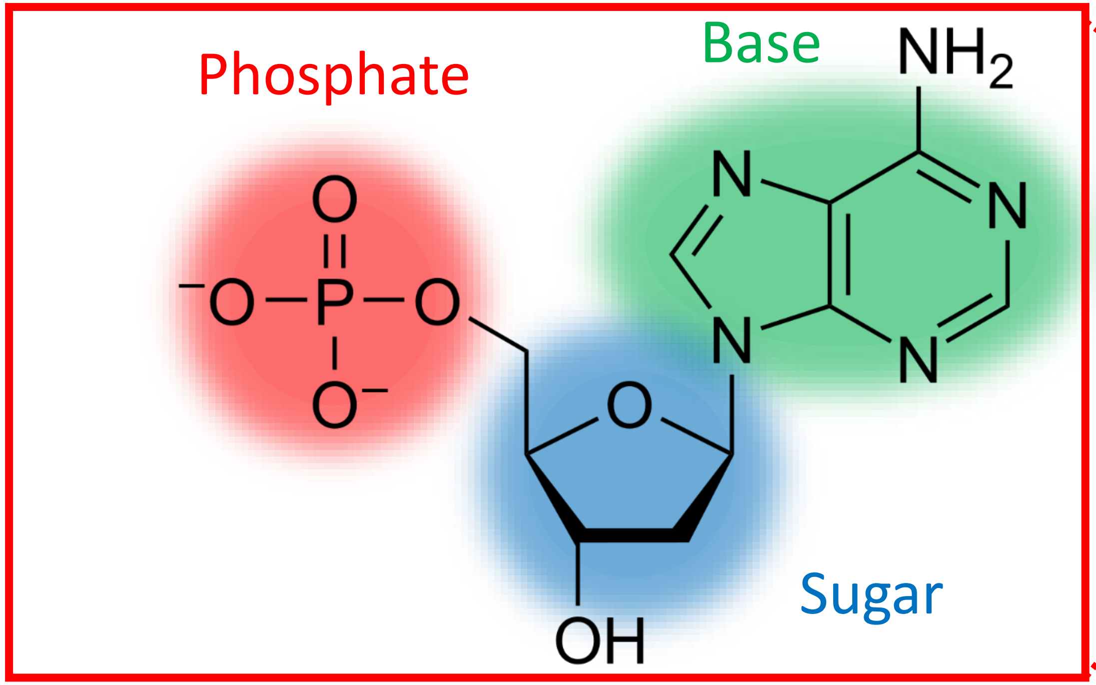


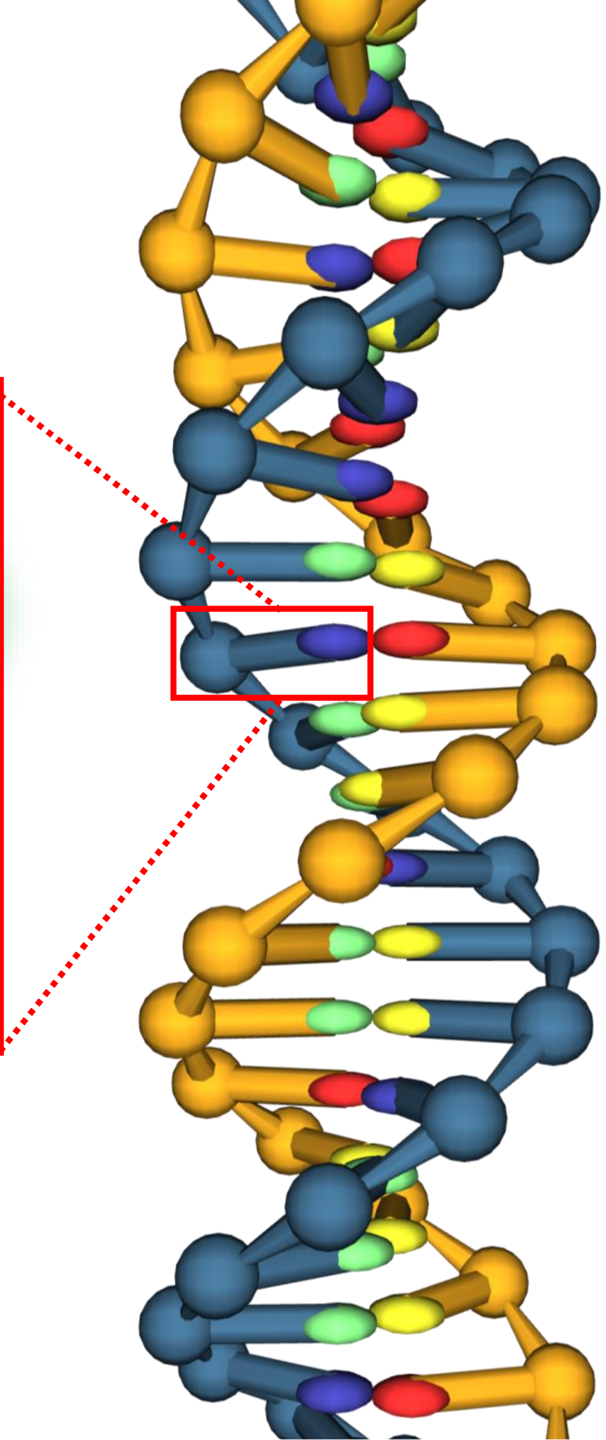
Thymine

DNA double helix
(duplex)

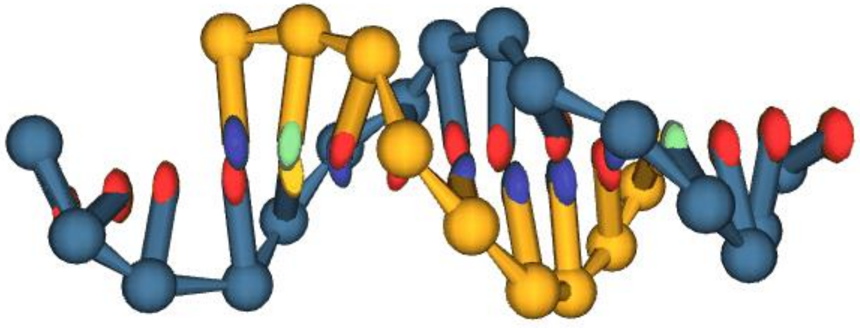


DNA Nucleotide

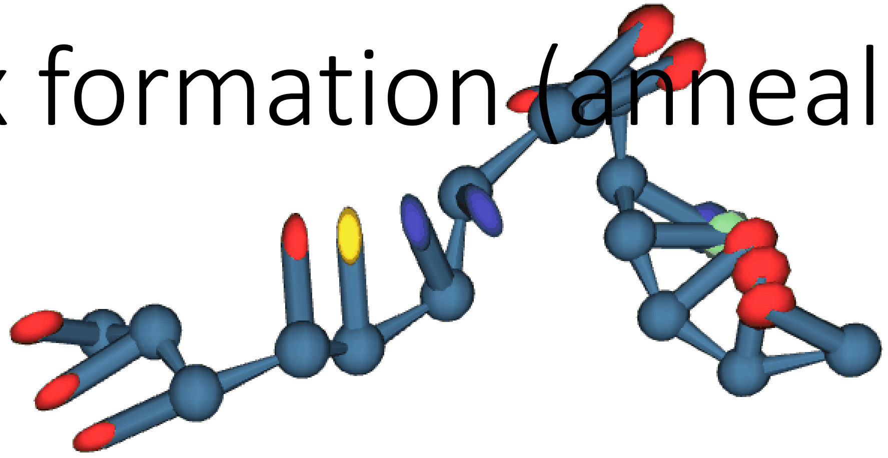


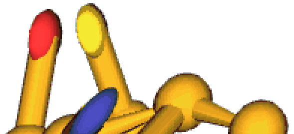


Process 1: strand separation (melting)

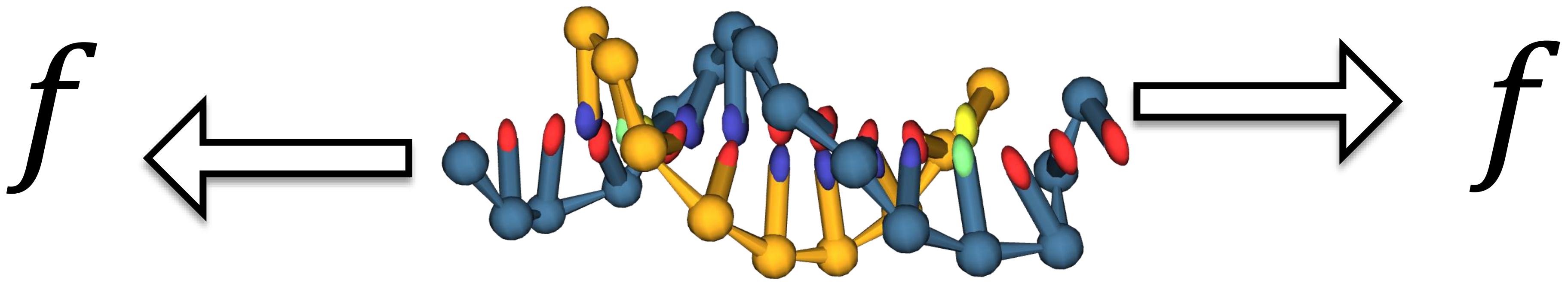


Process 2: duplex formation (annealing)

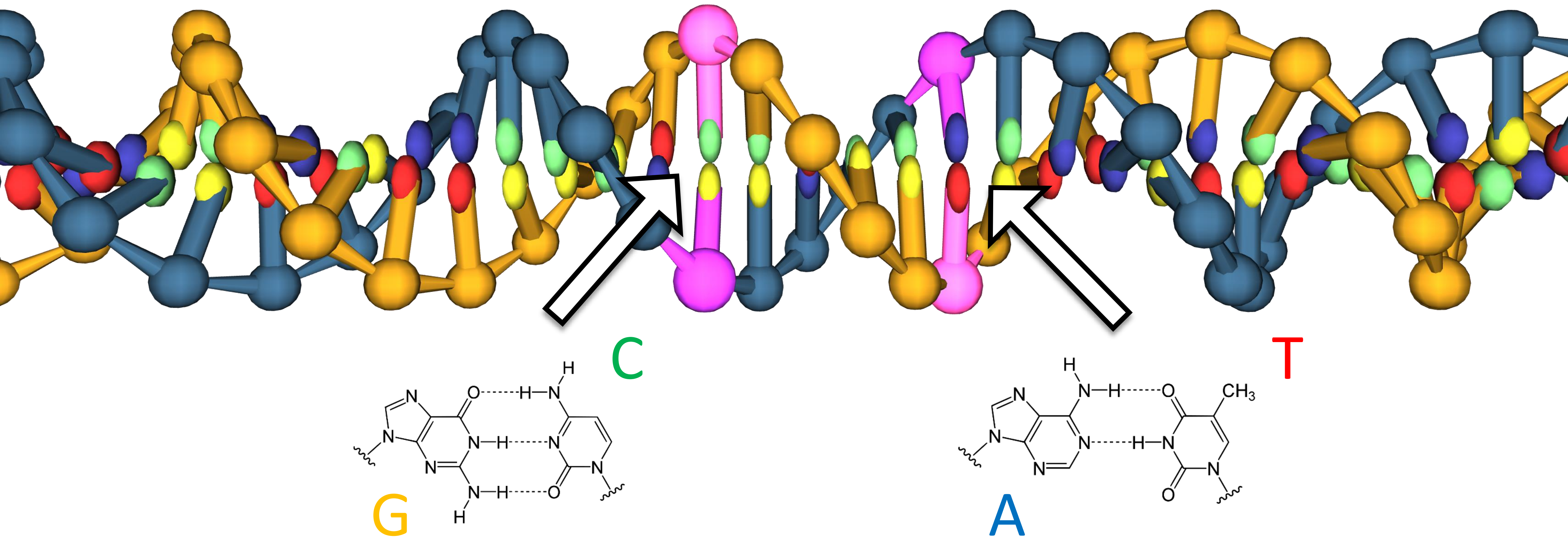




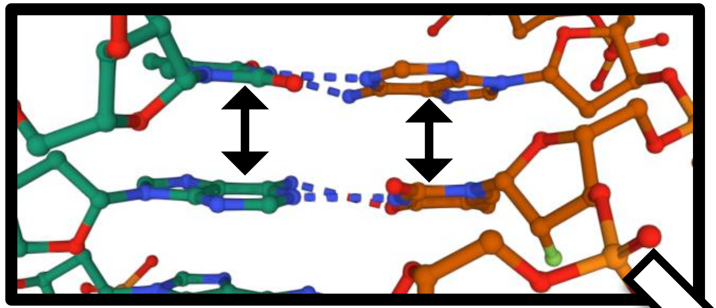
How does force affect these processes?



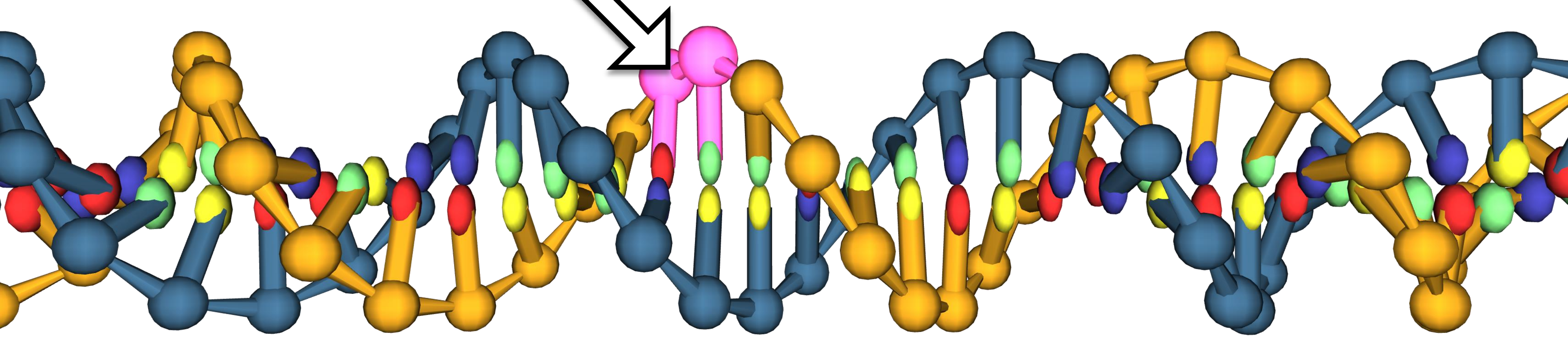
Base pairing



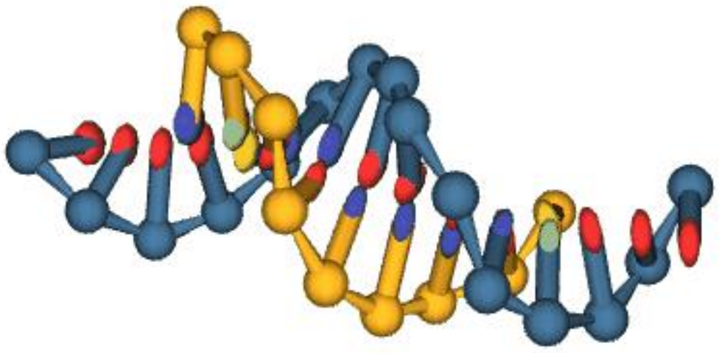
PDB ID: 436D



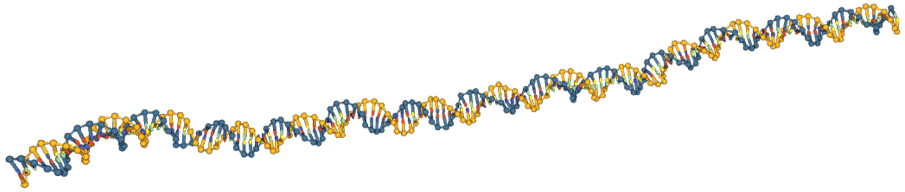
Base stacking

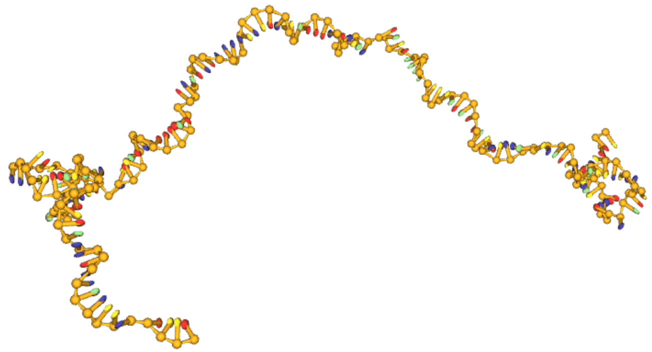


How does force affect a DNA duplex?

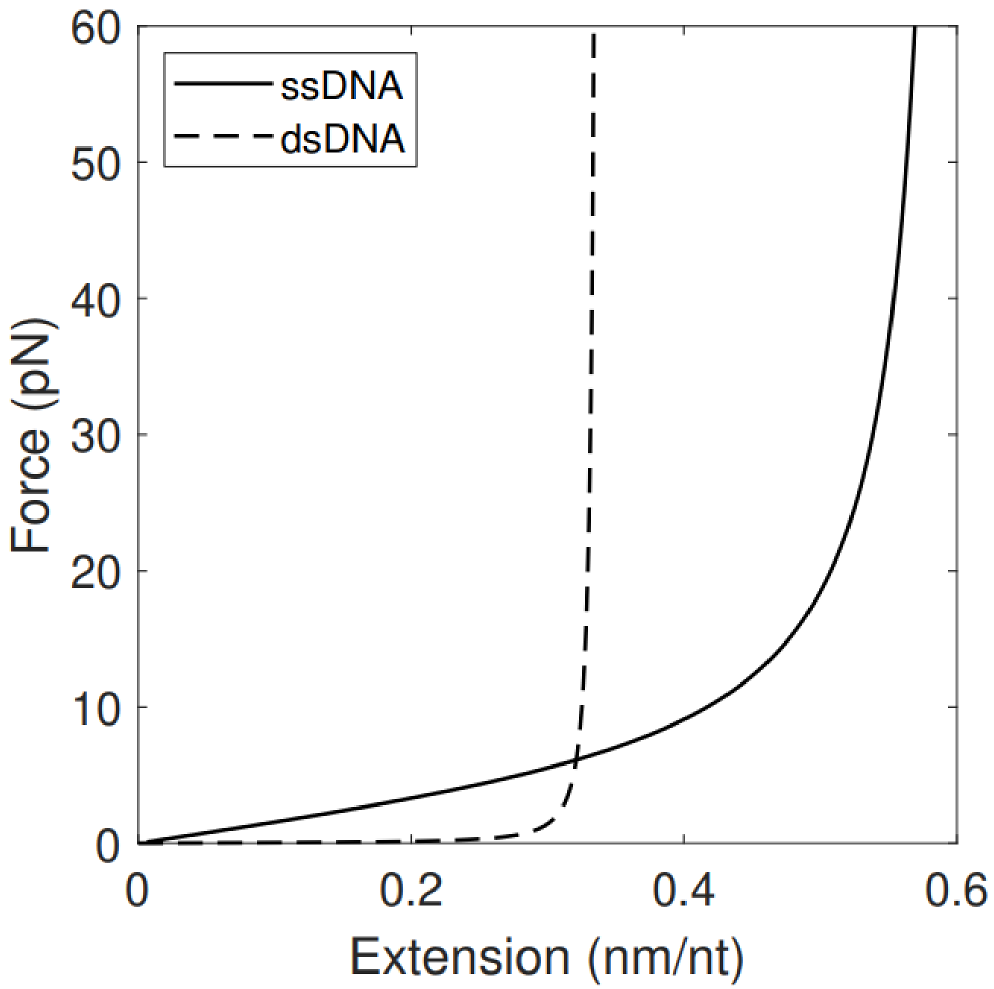


dsDNA is more rigid than **ssDNA**



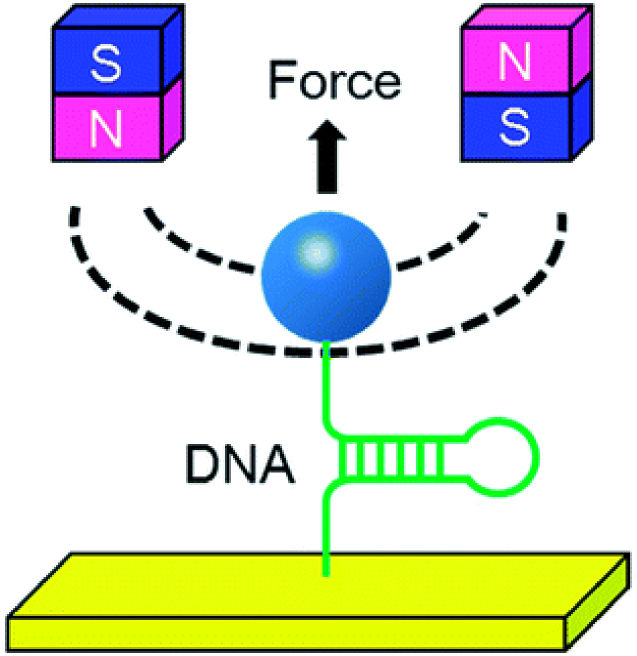


DNA force-extension



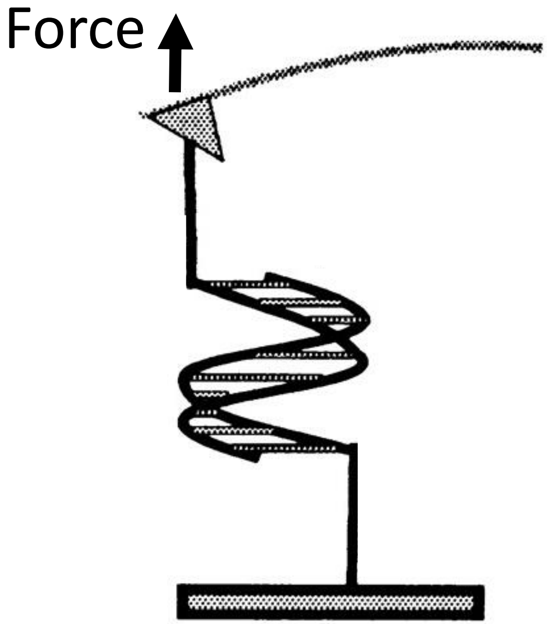
Single-molecule force spectroscopy

Magnetic tweezers



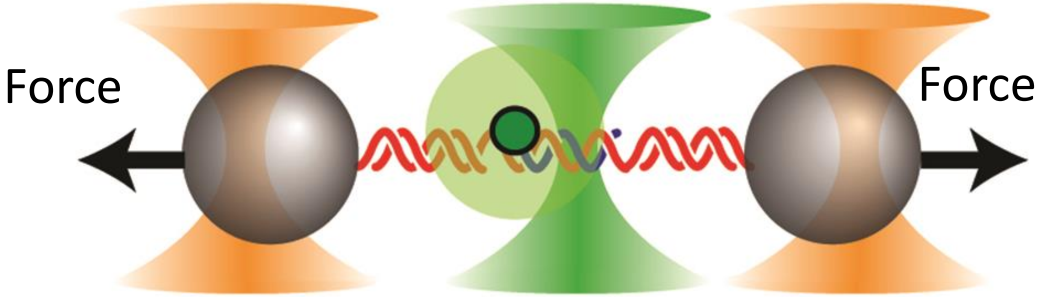
Qi Xin 2017

Atomic force microscope



Strunz 1999

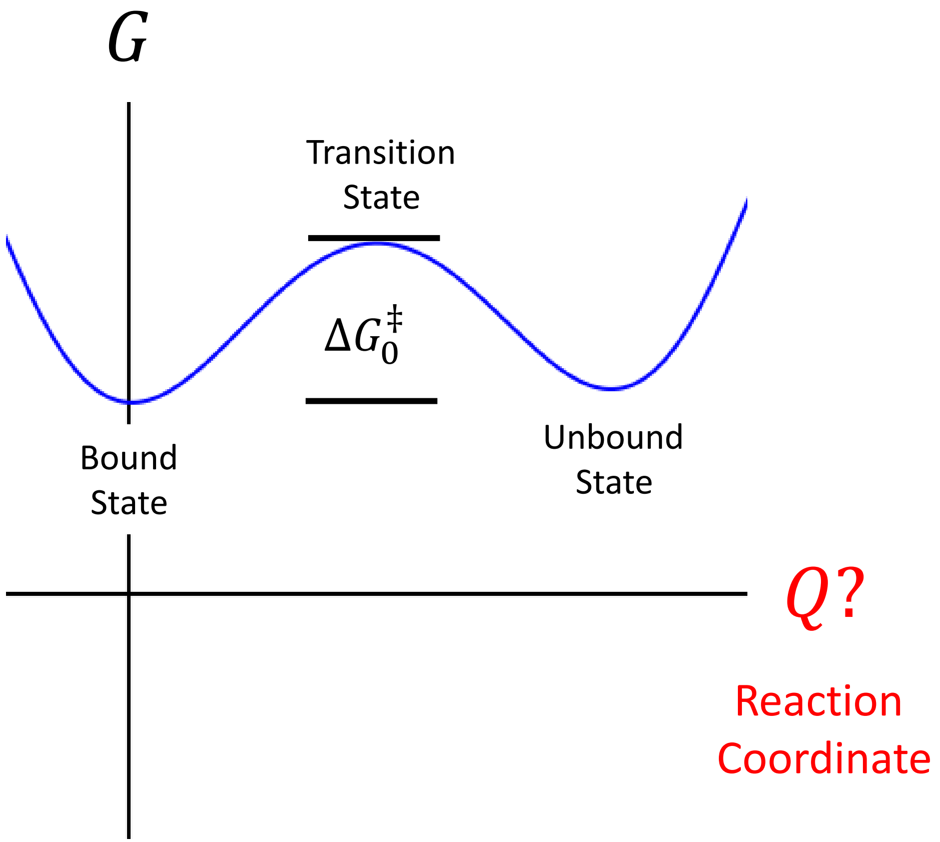
Optical (laser) tweezers



Whitley 2016

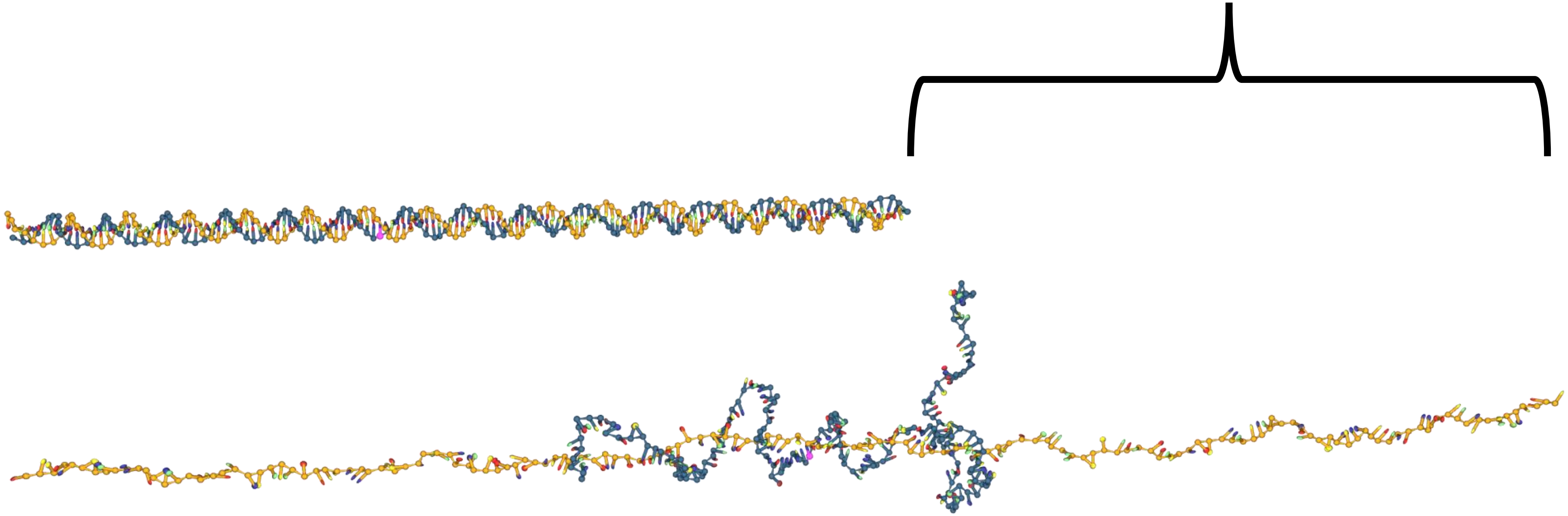
How to quantify force-dependent melting?

$$k \sim e^{-\Delta G_0^{\ddagger}/k_B T}$$



Strong force (>> 10 pN)

$\Delta x^{\ddagger}$



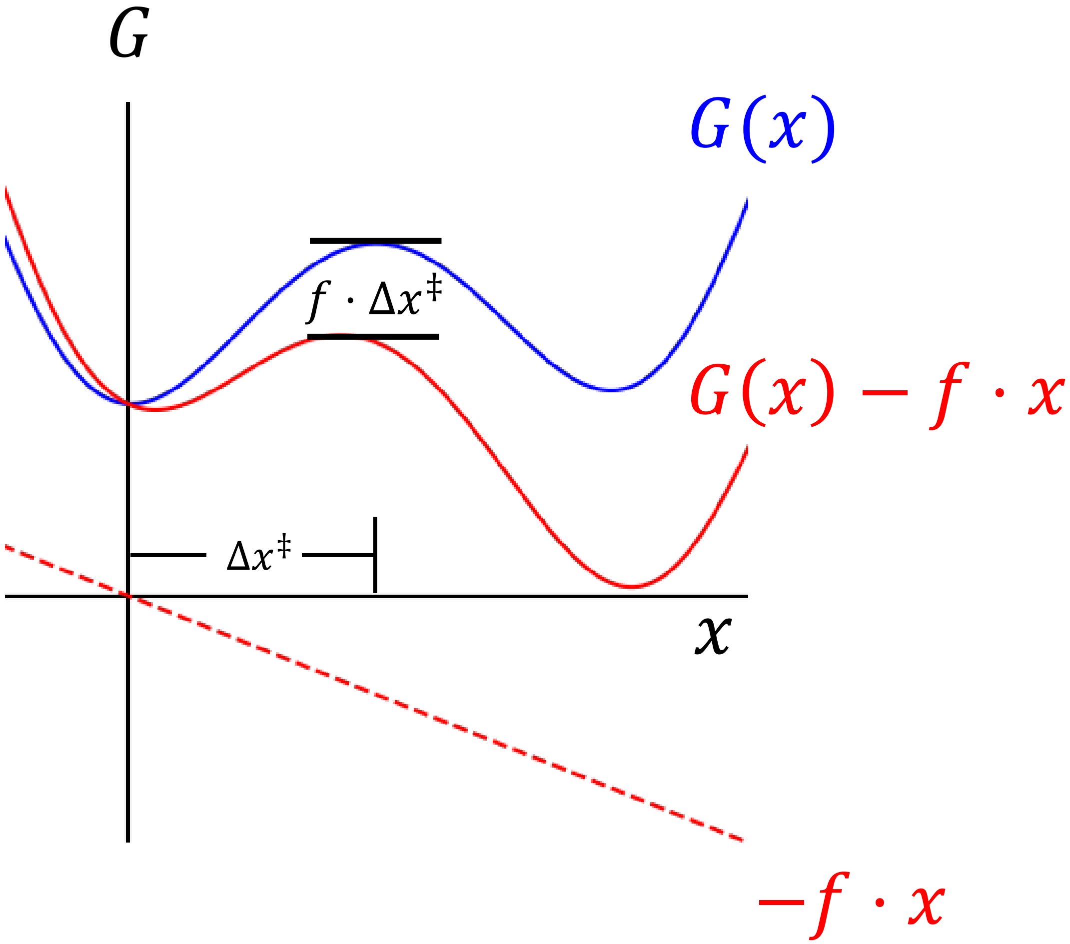
dsDNA

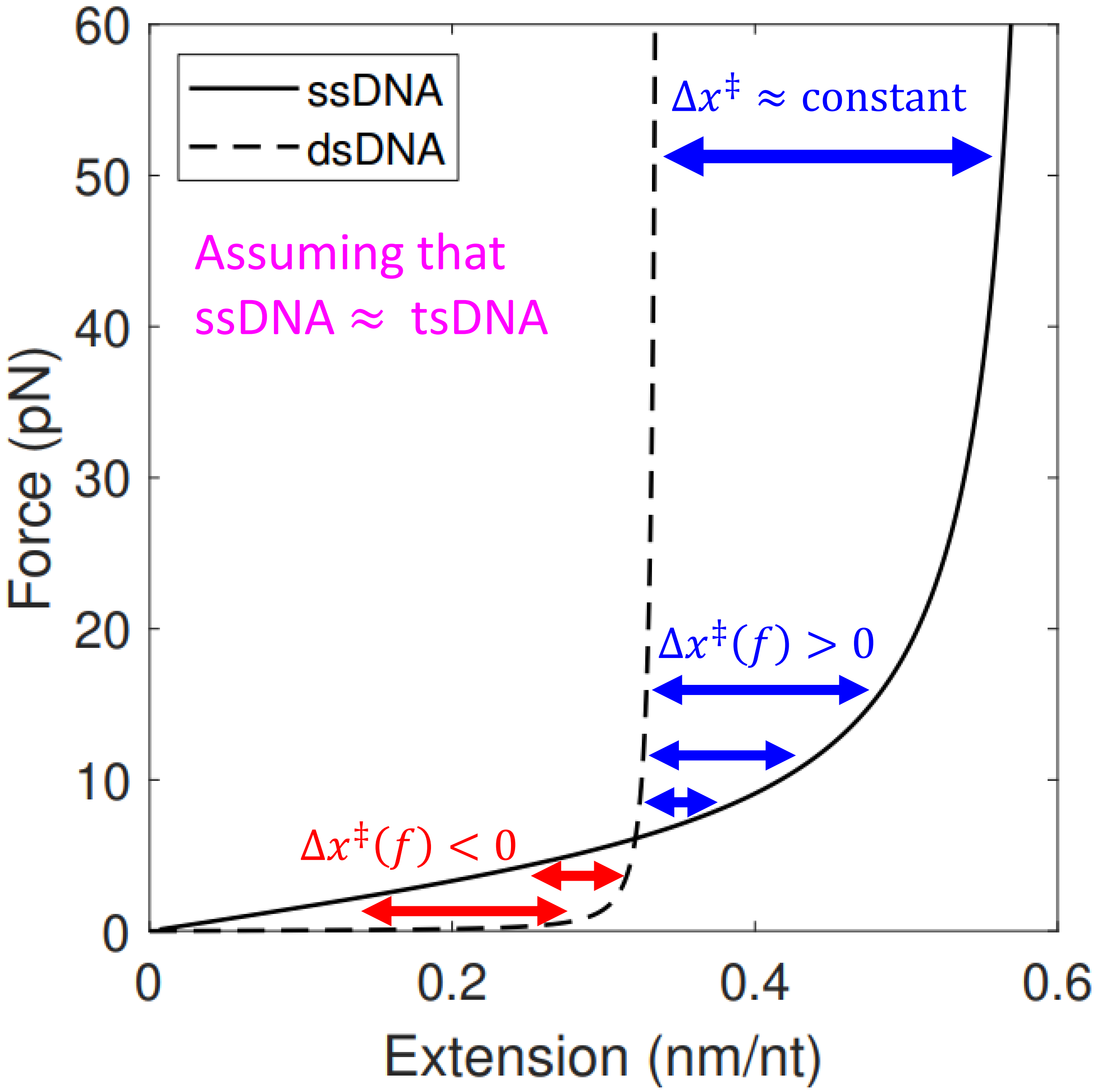
Transition state
"tsDNA"

Longer extension, like ssDNA

Constant transition distance

$$k \sim e^{-(\Delta G_0^{\ddagger} - f \cdot \Delta x^{\ddagger})/k_B T}$$

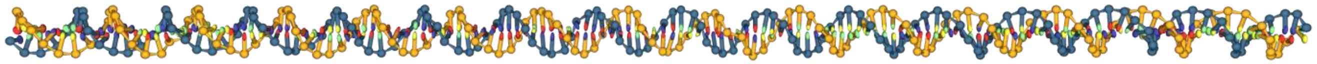




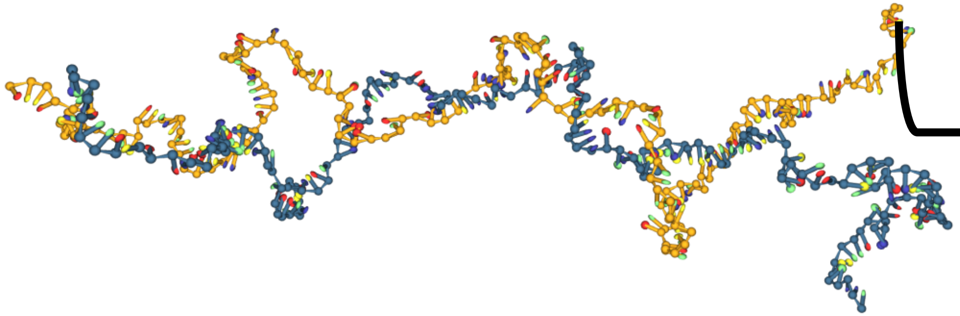
ssDNA
dsDNA
$\Delta x^{\ddagger} \approx$ constant
Assuming that
ssDNA ≈ tsDNA
$\Delta x^{\ddagger}(f) > 0$
$\Delta x^{\ddagger}(f) < 0$
Force (pN)
Extension (nm/nt)
0
10
20
30
40
50
60
0
0.2
0.4
0.6

Weak force (< 10 pN)

Bound state:



Transition state:



Flexible like ssDNA

$\Delta x^{\ddagger} < 0$

Generalized force model

$$k \sim e^{-\left[\Delta G_0^{\ddagger} - \int_0^{f} f' \cdot \Delta x^{\ddagger}(f')\, df'\right]/k_B T}$$

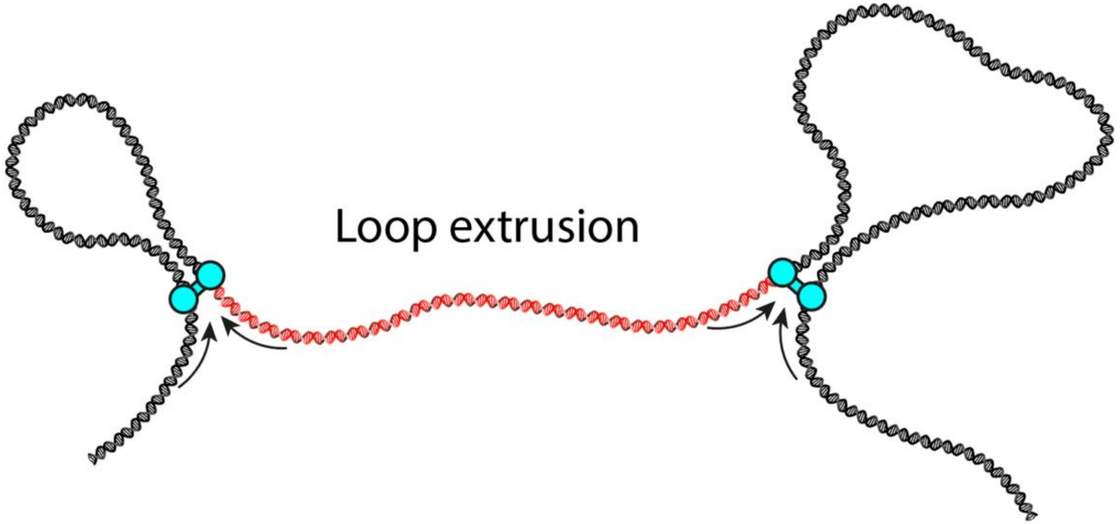
$$\frac{d \log k\,(f)}{df} = \frac{1}{k_B T}\left[\Delta x^{\ddagger}(f)\right]$$

How does the rate change with tension?

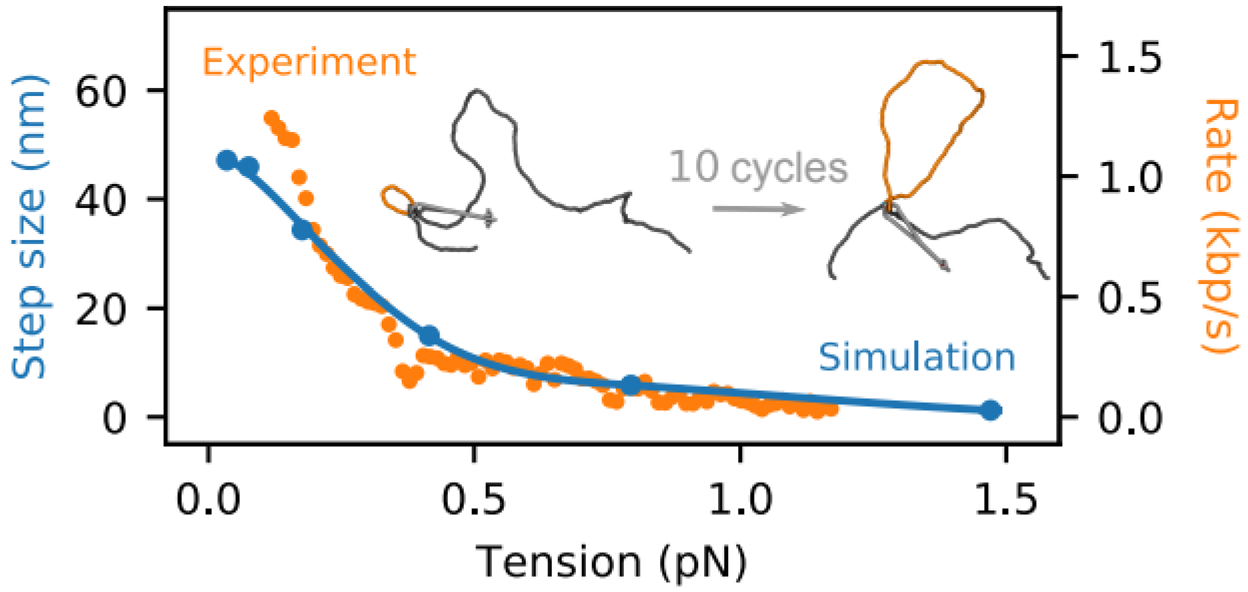
$$\frac{d\log k\,(f)}{df} = \frac{1}{k_B T}\left[\Delta x^{\ddagger}(f)\right]$$

At weak force: $\Delta x^{\ddagger} < 0$ $\quad \frac{dk(f)}{df} < 0$

Weak force (<10 pN) in the nucleus



Real-time imaging of DNA loop extrusion by condensin
(Ganji 2019)

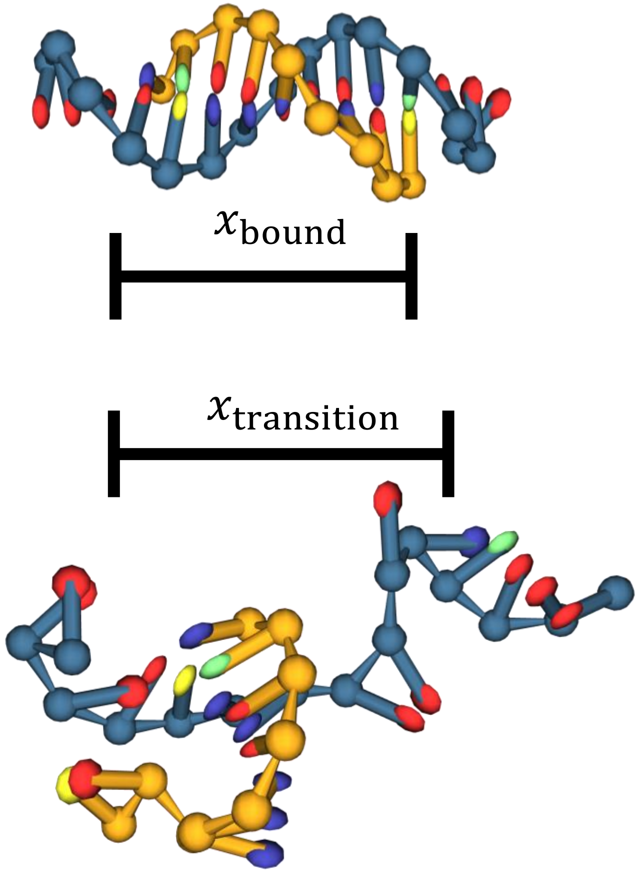


DNA tension-modulated translocation and loop extrusion by SMC complexes revealed by molecular dynamics simulations
(Nomadis 2022)

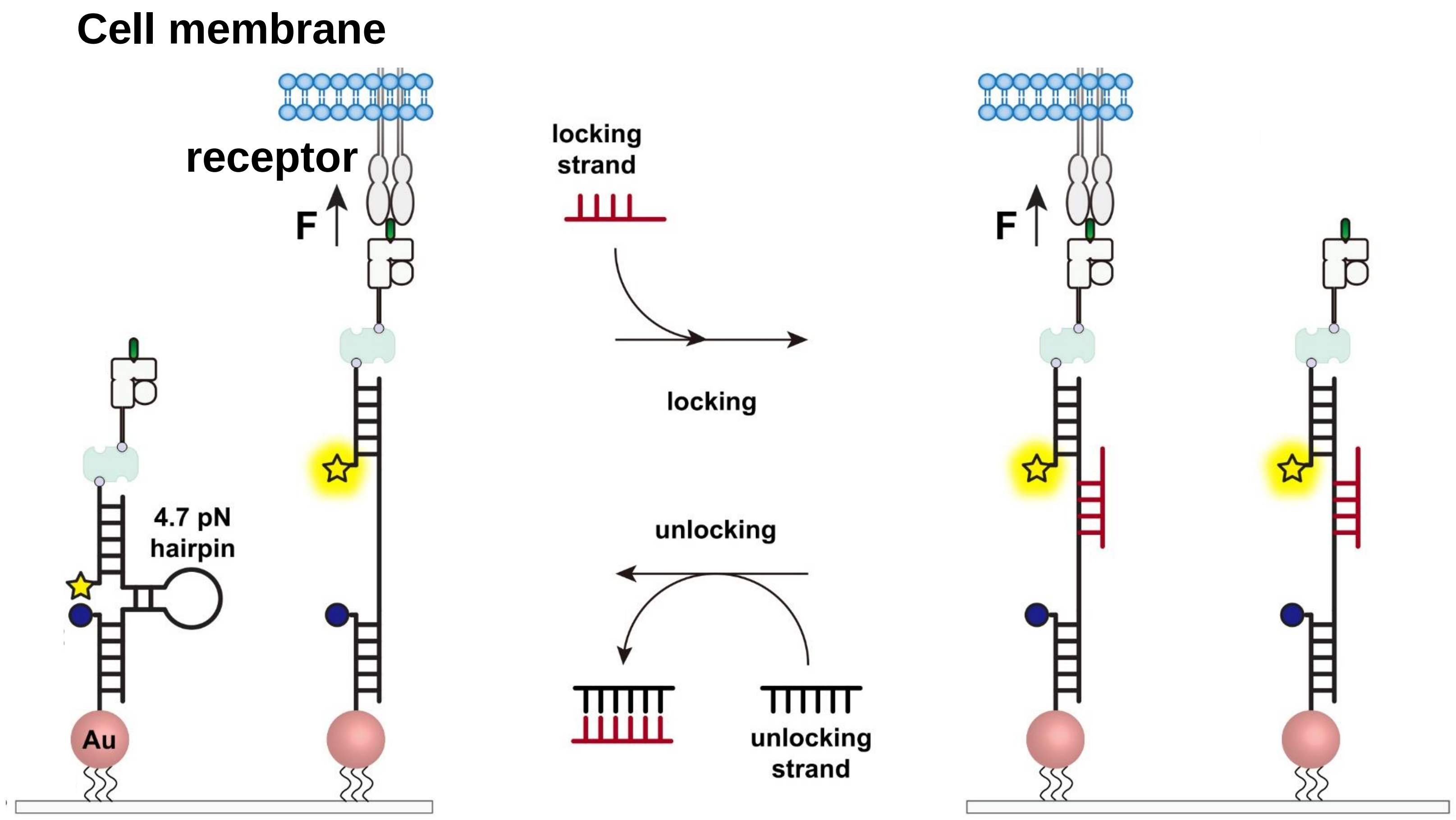
How does extension change for short duplexes at weak force?

Bound state:

Transition state:



Short DNA duplexes as a weak force (<10 pN) sensor



Ma et al. 2021, modified with permission

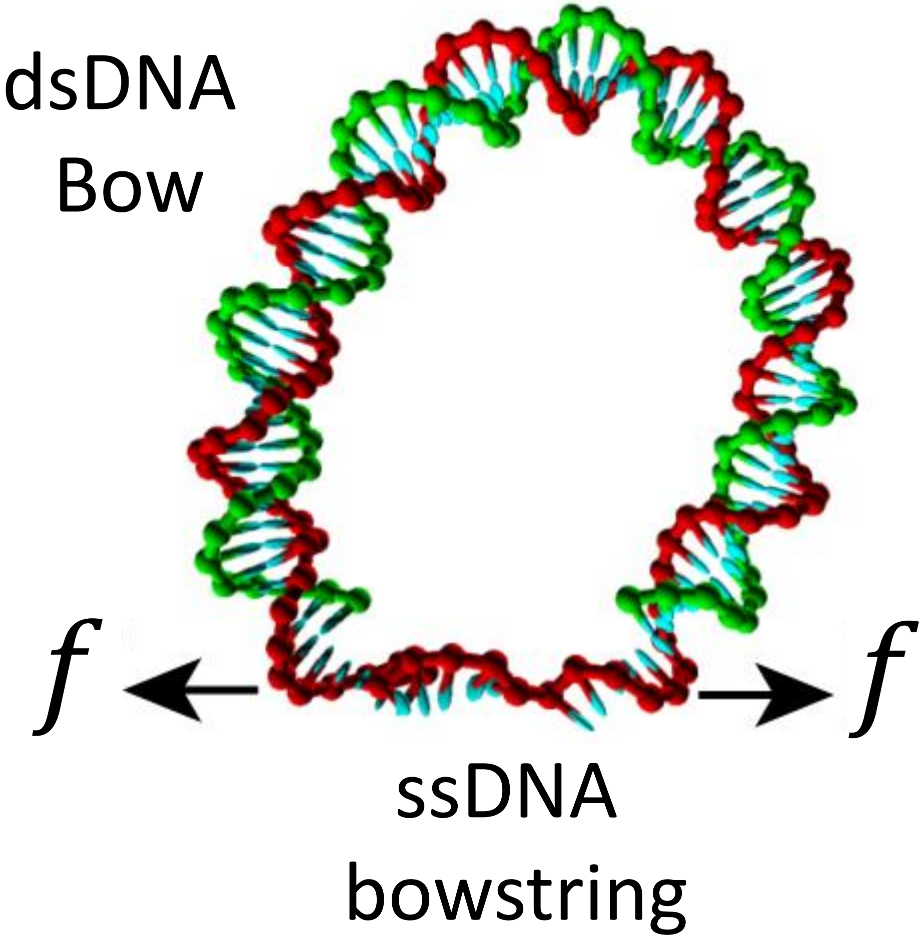
<u>Goal 1:</u>

Measure the binding and unbinding rates of a <u>**short**</u> duplex subject to <u>**weak**</u> tension

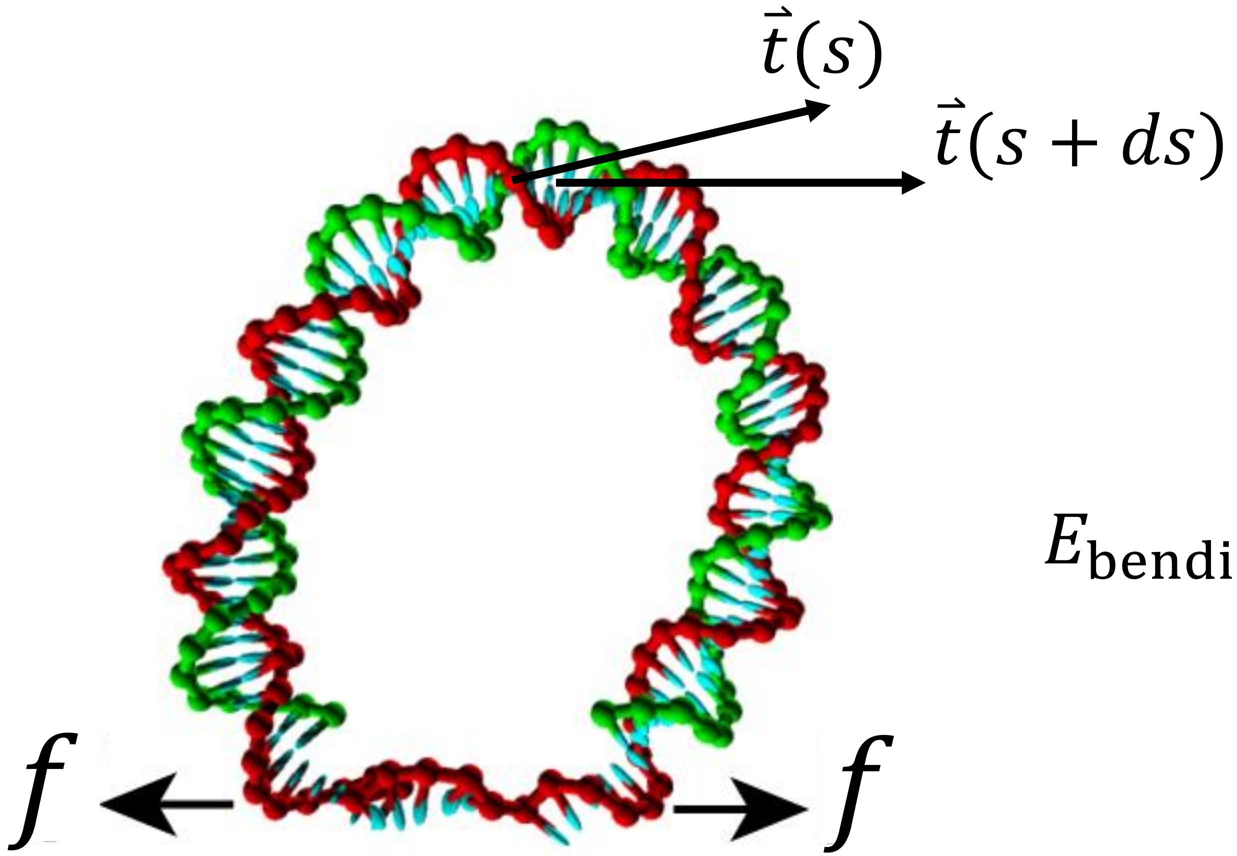
$l < 10$ base pairs

$f < 10$ piconewtons

Exerting weak tension with “DNA bows”

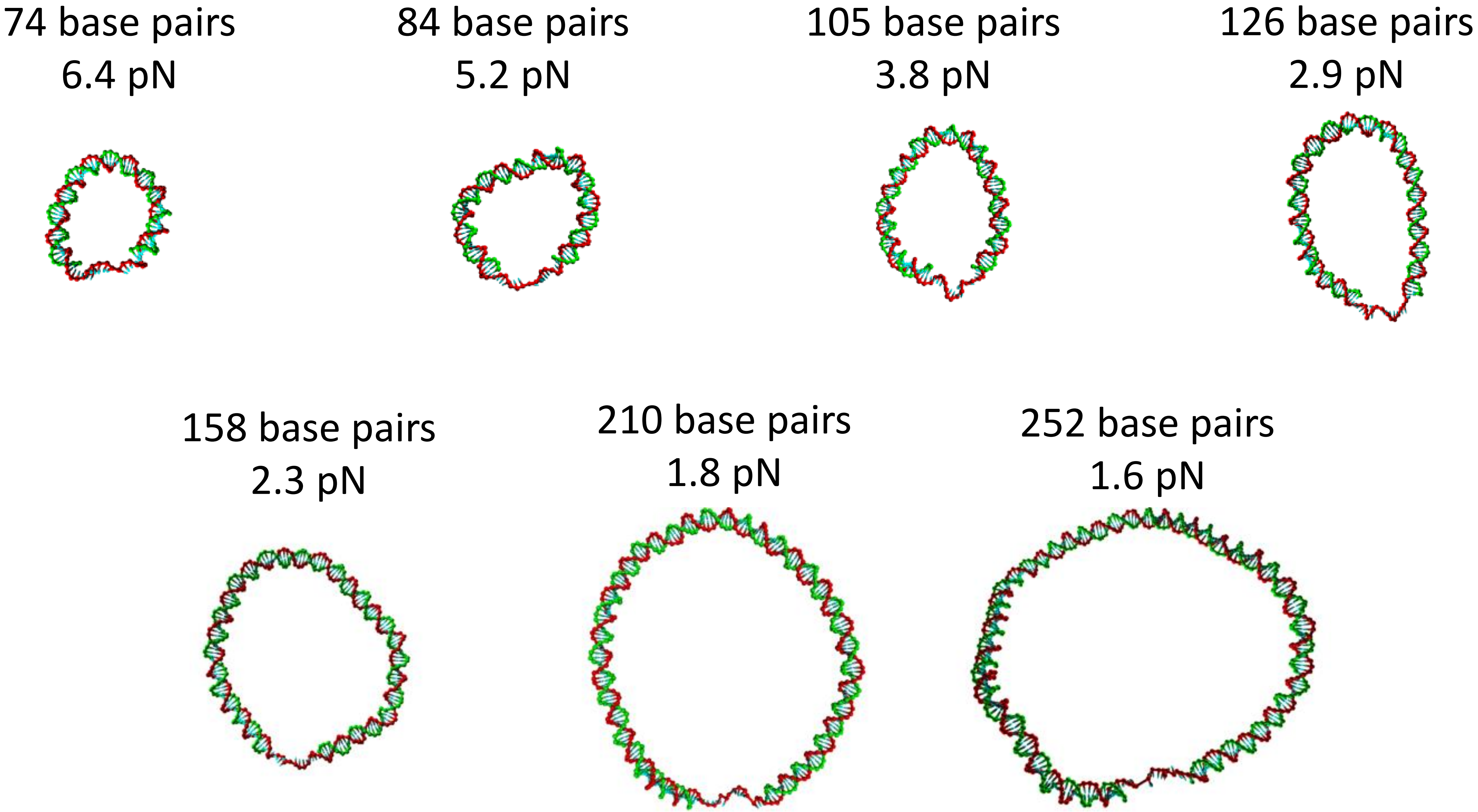


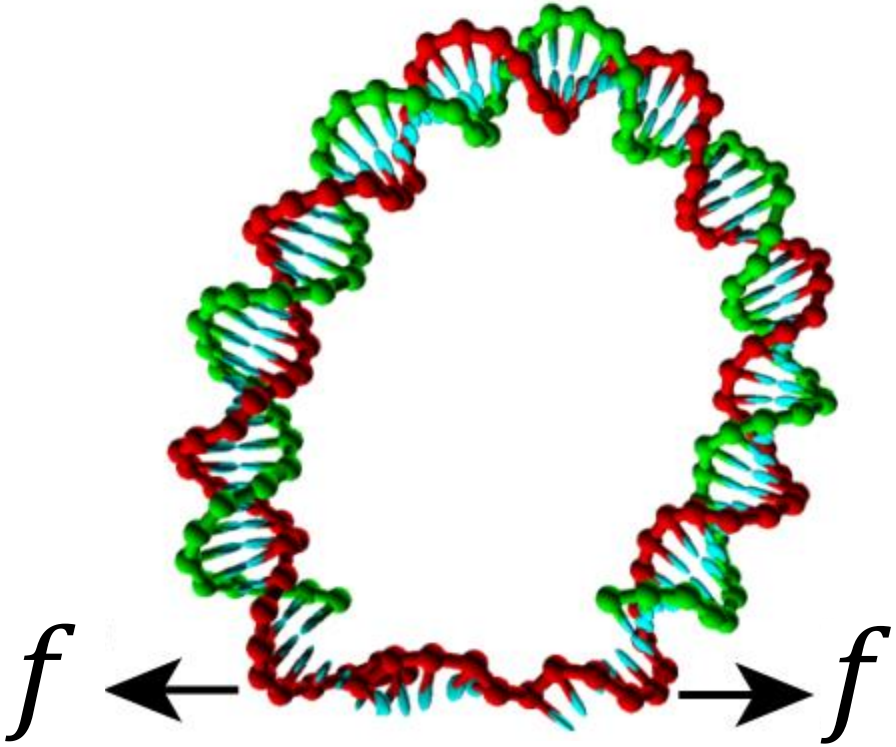
Bending force exerted by dsDNA



$$E_{\text{bending}} = \frac{k_B T}{2} \cdot P \int_0^L \left(\frac{\partial \vec{t}\,(s)}{\partial s}\right)^2 ds$$

Persistence length of dsDNA ≈ 150 bp


74 base pairs
6.4 pN
84 base pairs
5.2 pN
105 base pairs
3.8 pN
126 base pairs
2.9 pN
158 base pairs
2.3 pN
210 base pairs
1.8 pN
252 base pairs
1.6 pN

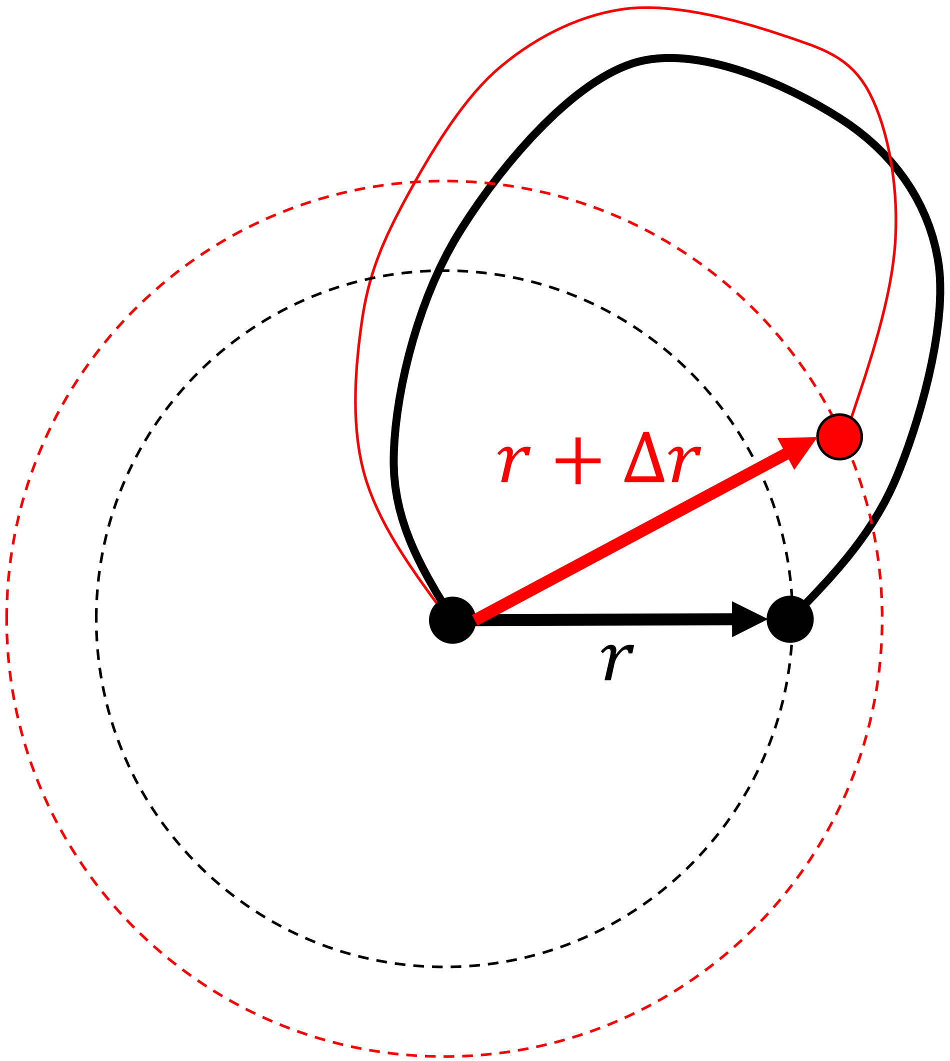


Radial partition function

Density of bent states with radius r

$$P(r) = 4\pi r^2 \cdot Q(r)$$

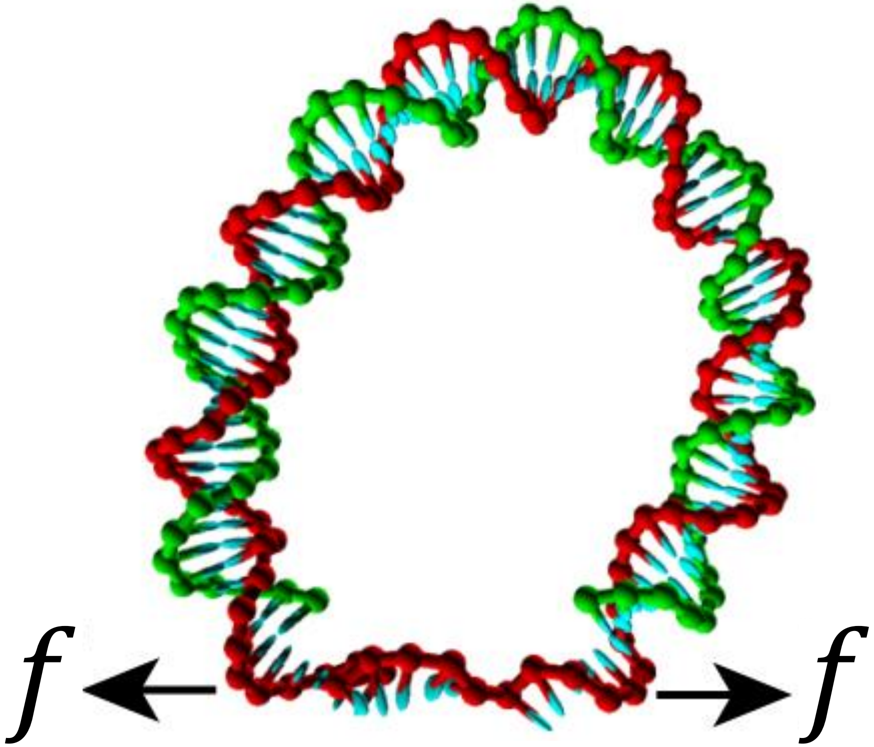
Entropic force exerted by dsDNA



Radial partition function

Density of bent states with radius r

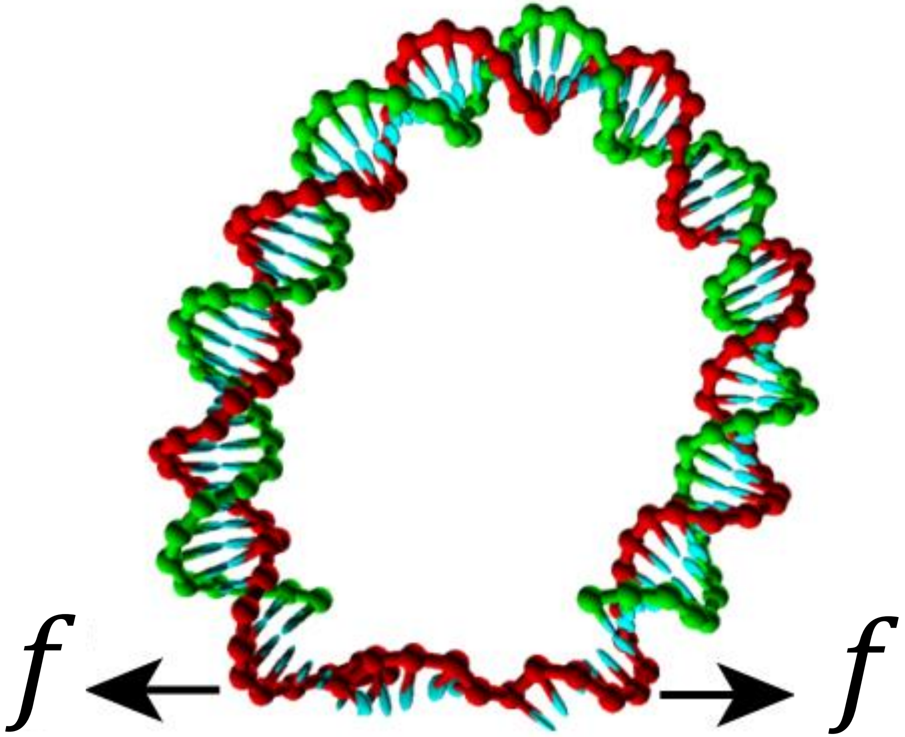
$$P(r) = 4\pi r^2 \cdot Q(r)$$

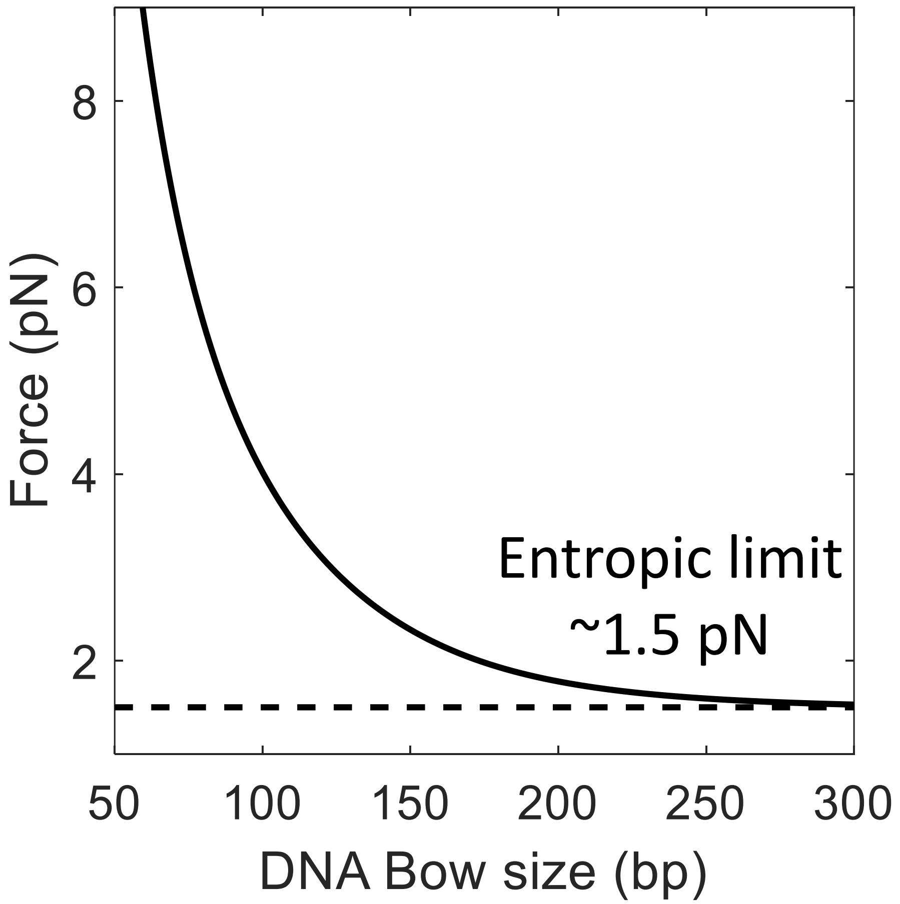


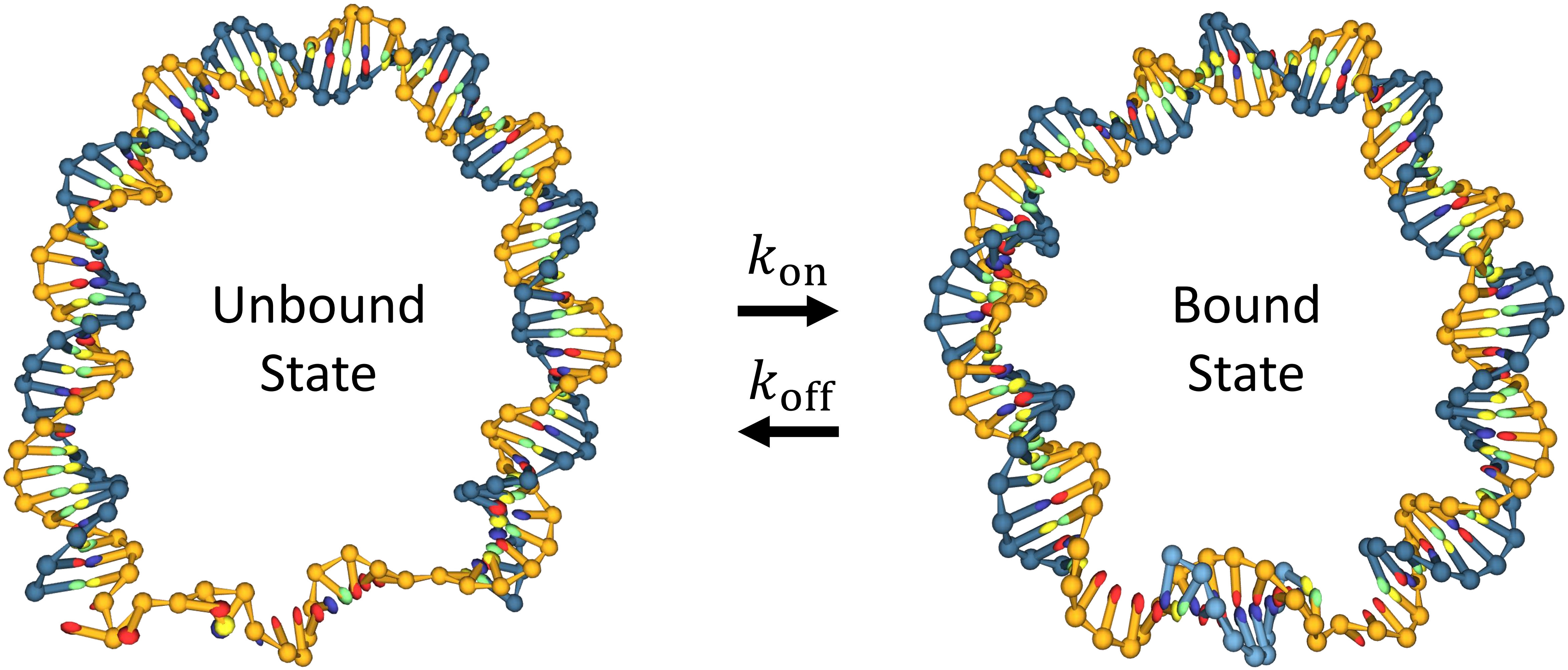
$$f_{total}(r) = k_B T \frac{\partial \ln[4\pi r^2 Q(r)]}{\partial r}$$

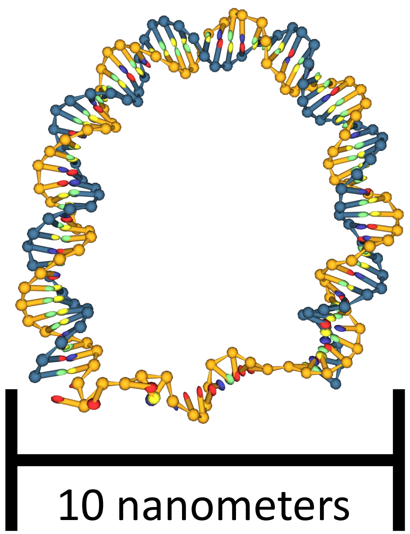
$$= k_B T \left(\underbrace{\frac{2}{r}}_{\text{Entropic force}} + \underbrace{\frac{\partial \ln Q(r)}{\partial r}}_{\text{Bending force}} \right)$$

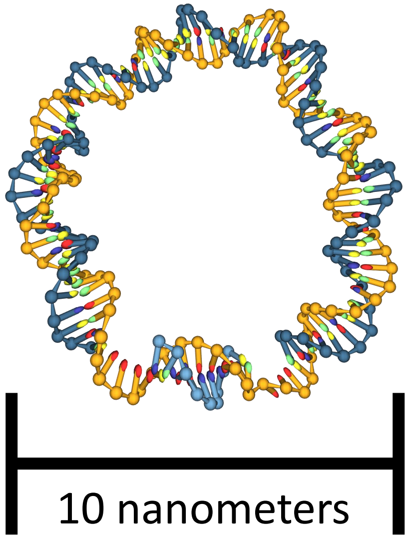
Exert weak tension with “DNA bows”





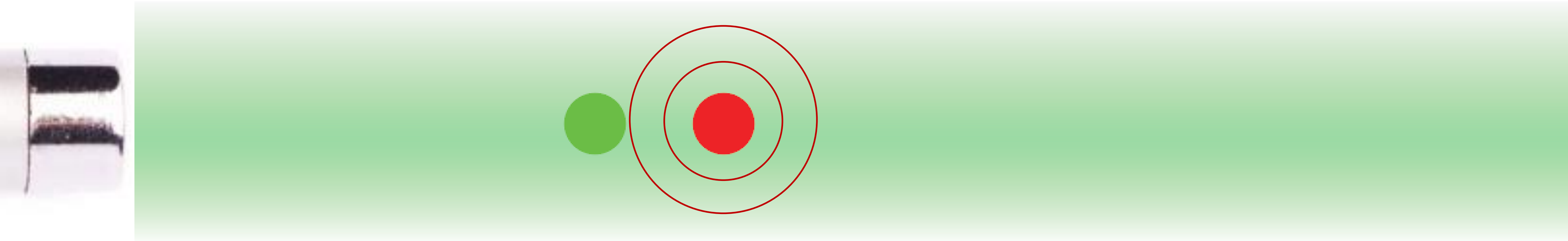

Unbound State
k_{on}
k_{off}
Bound State


10 nanometers

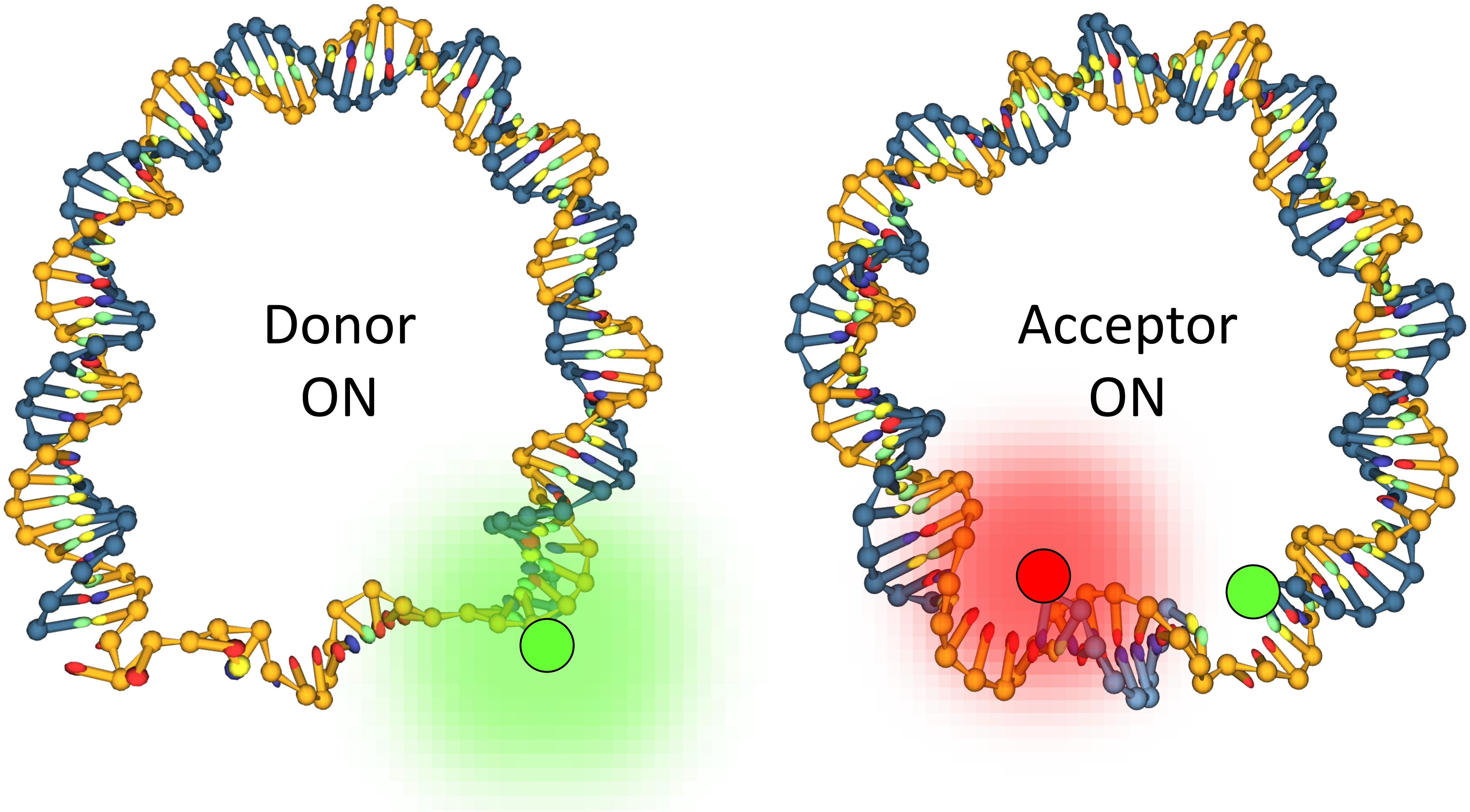

10 nanometers

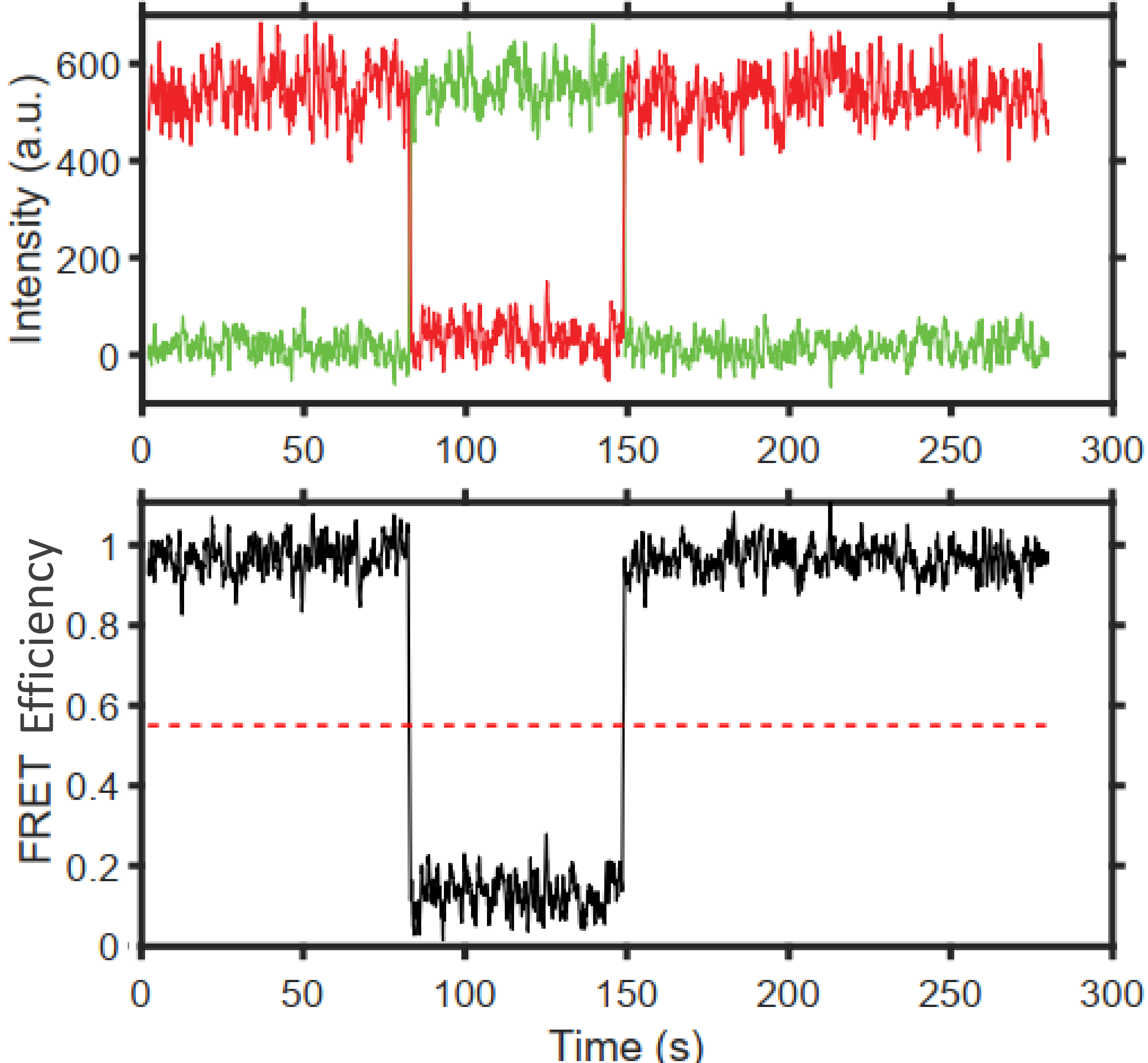
Fluorescence Resonance Energy Transfer (FRET)

Green Laser excites Donor



Donor gives energy to nearby Acceptor

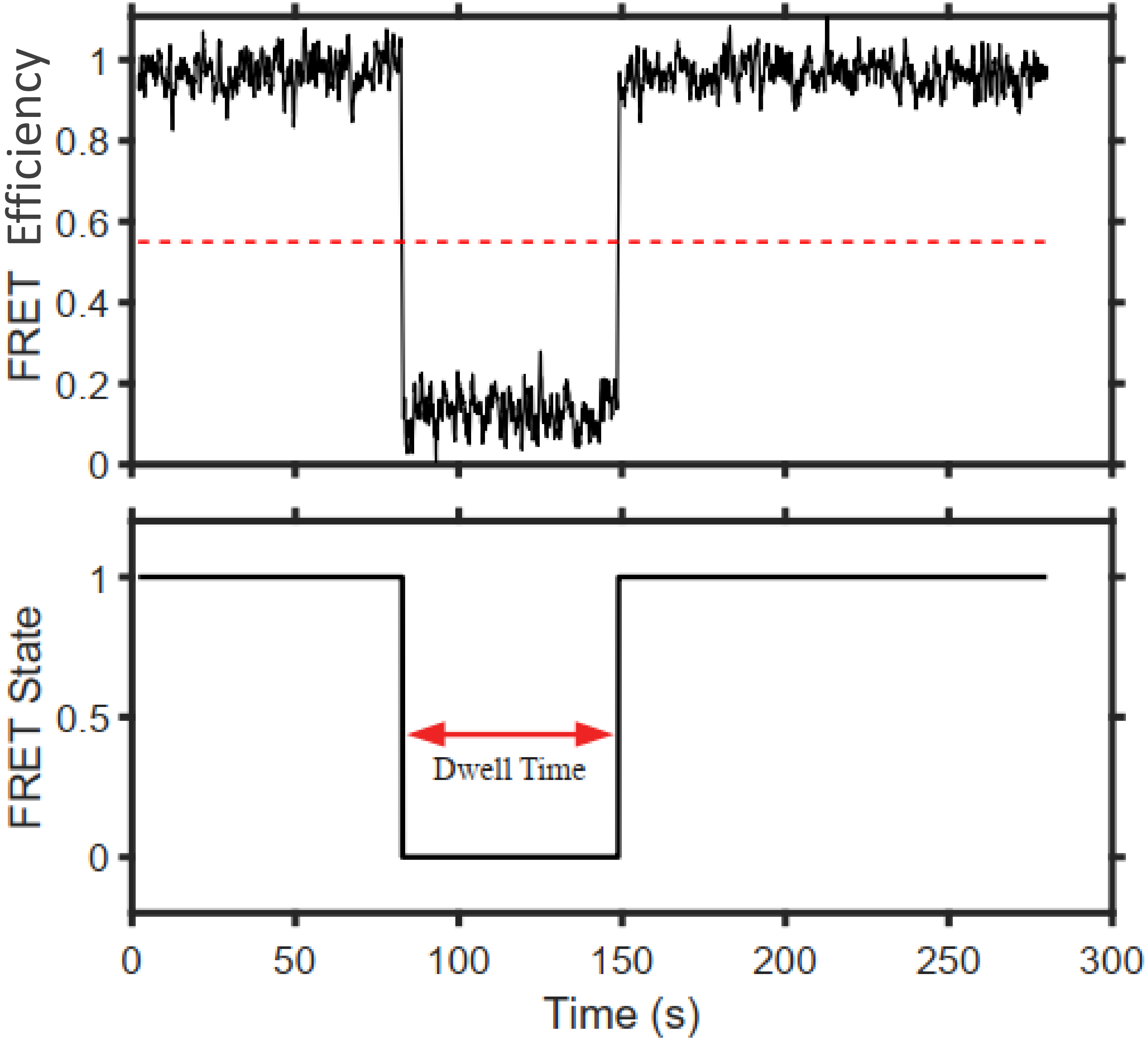

Donor
ON
Acceptor
ON



$$E_{FRET} = \frac{I_A}{I_D + I_A}$$

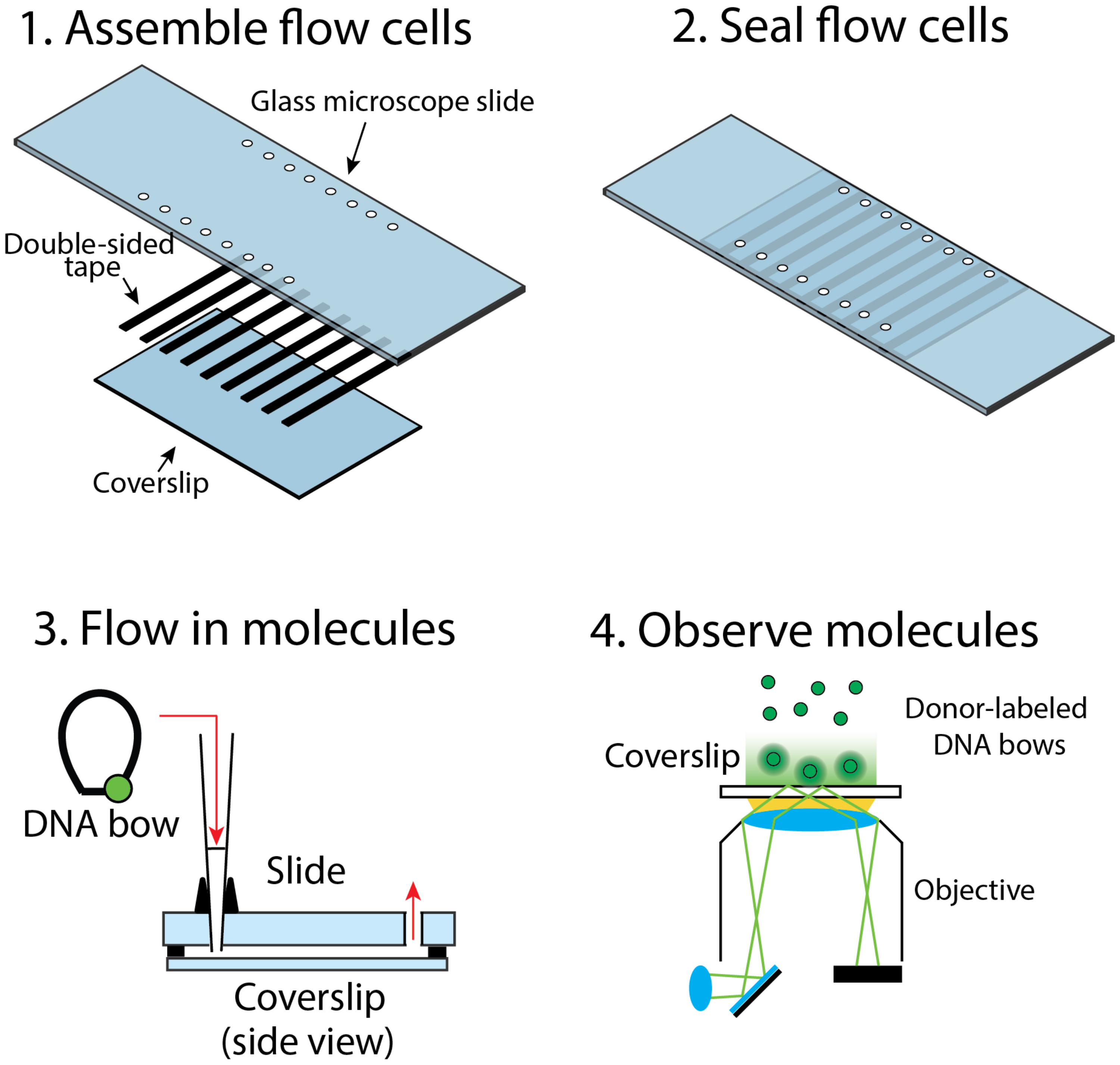
Bound

Unbound



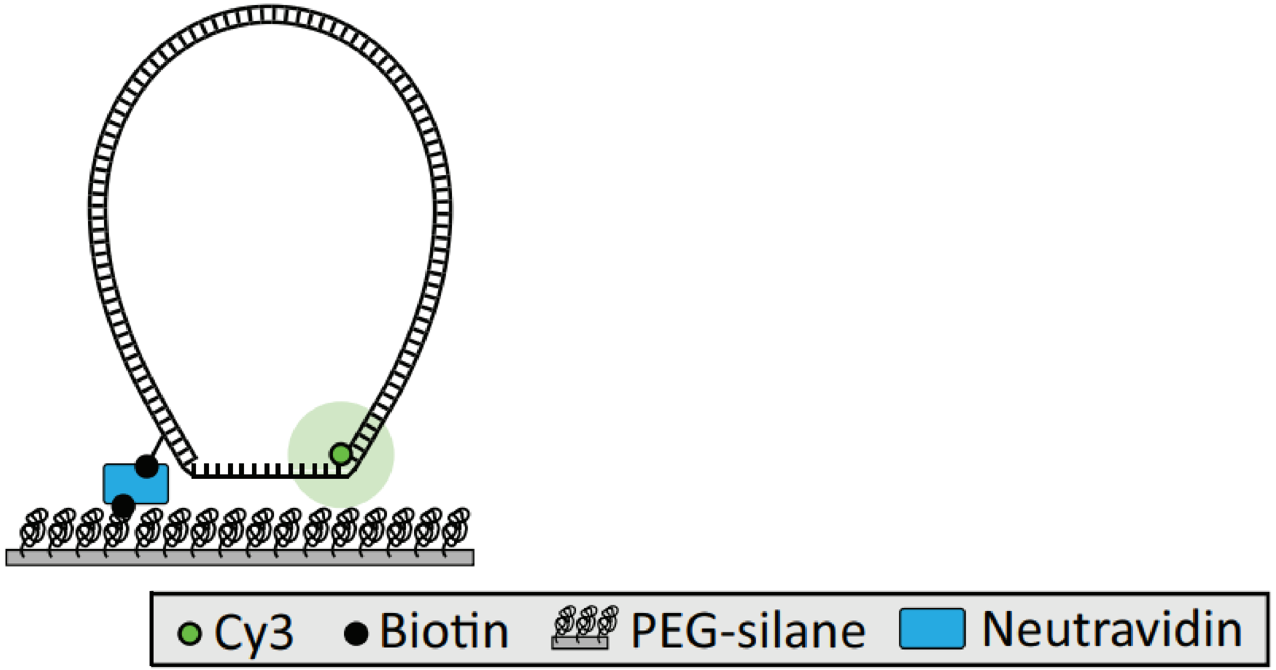
Threshold line

Binary state trace



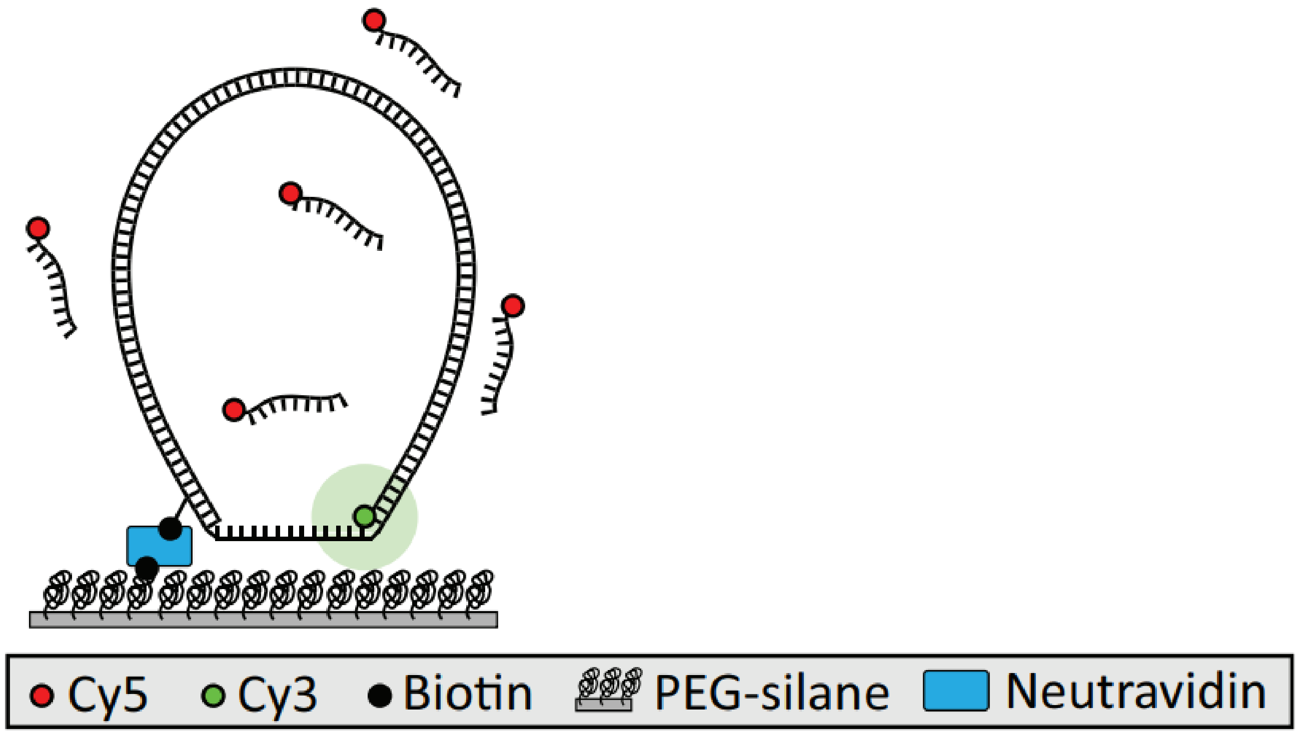
1. Assemble flow cells
Glass microscope slide
Double-sided tape
Coverslip
2. Seal flow cells
3. Flow in molecules
DNA bow
Slide
Coverslip (side view)
4. Observe molecules
Donor-labeled DNA bows
Coverslip
Objective

1. Immobilize DNA bows on the surface

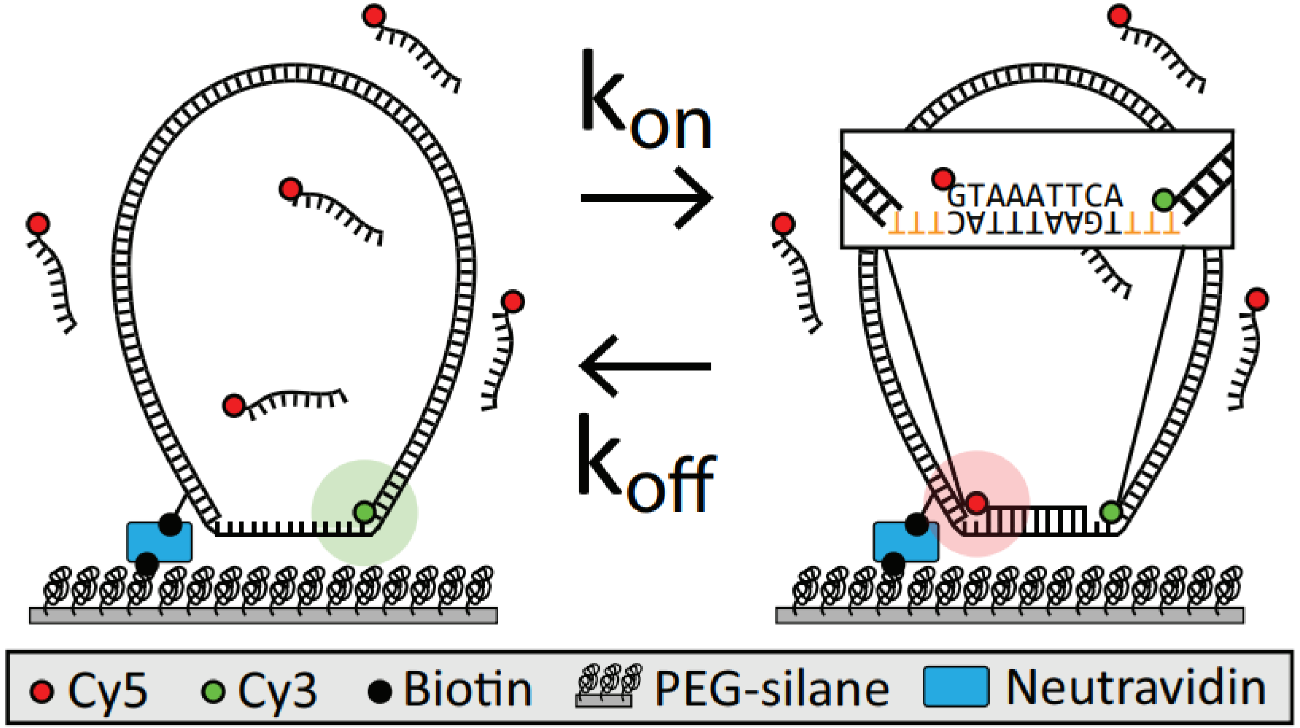


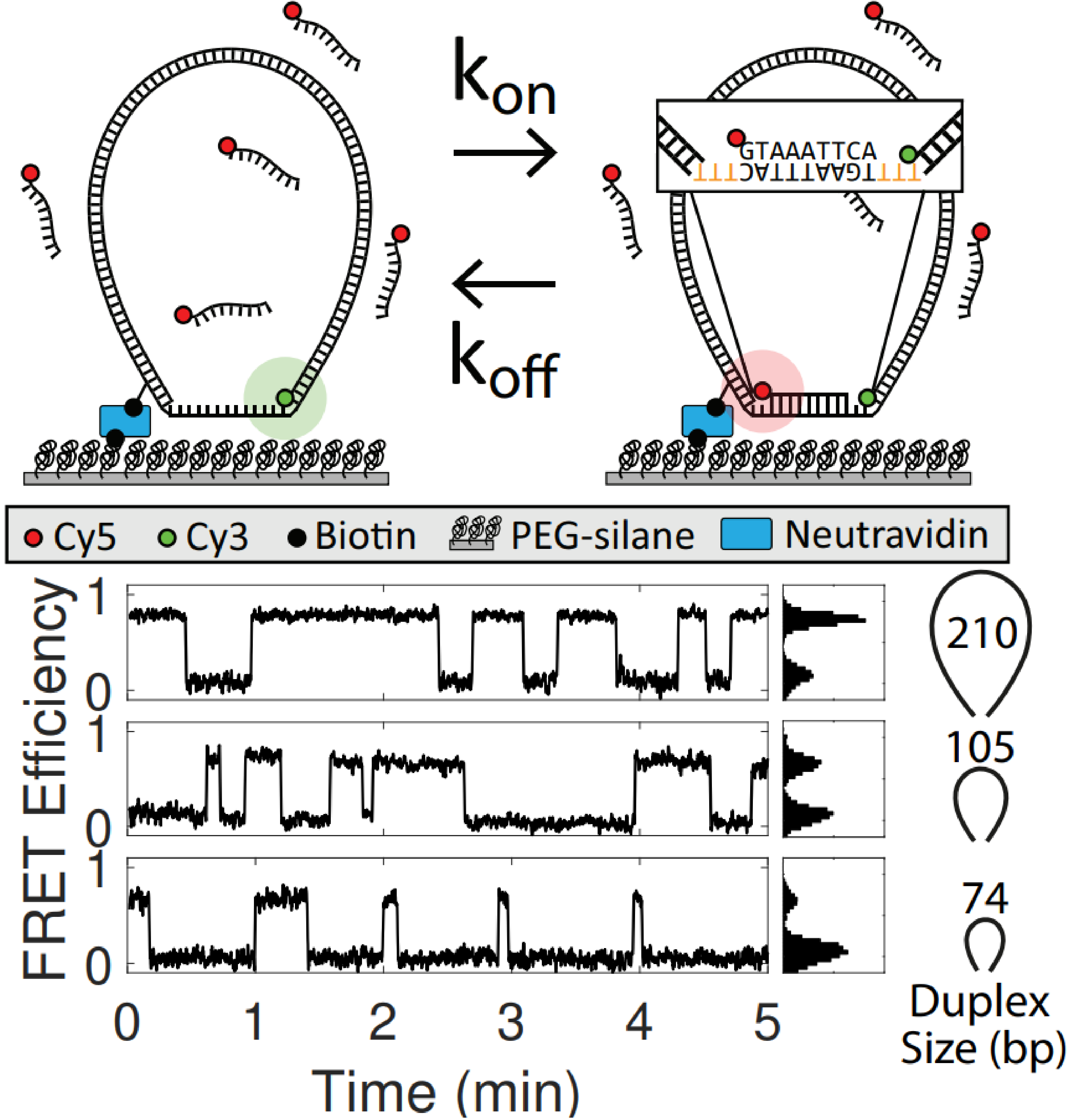
1. Immobilize DNA bows on the surface

2. Flow in buffer with dye-labeled probes



1. Immobilize DNA bows on the surface
2. Flow in buffer with dye-labeled probes
3. **Observe binding and unbinding rates**

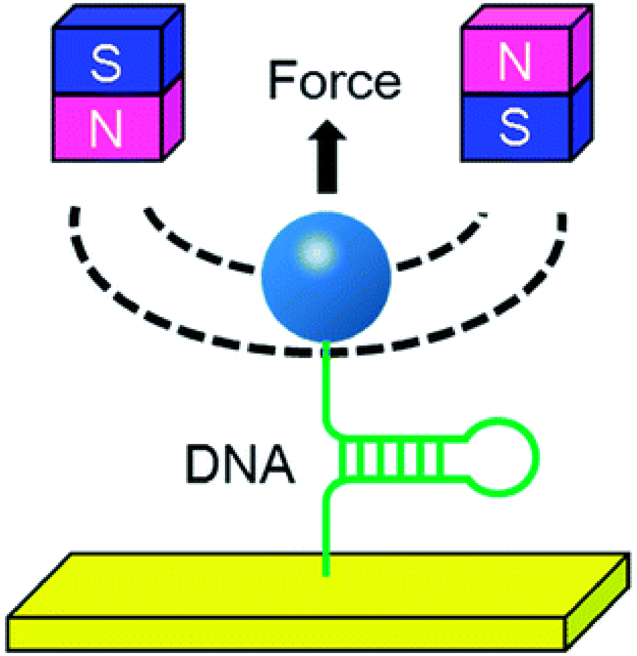




k_{on}
k_{off}
GTAAATTCA
TTTCATTTAAGTTTT
Cy5
Cy3
Biotin
PEG-silane
Neutravidin
FRET Efficiency
1
0
1
0
1
0
0
1
2
3
4
5
Time (min)
210
105
74
Duplex Size (bp)

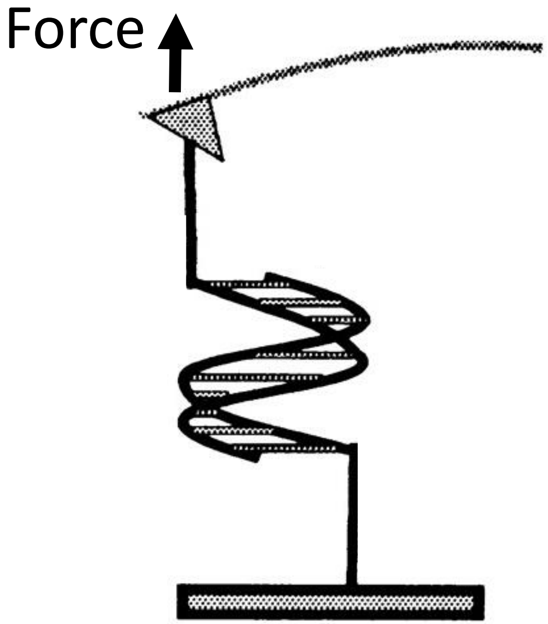
Single-molecule force spectroscopy

Magnetic tweezers



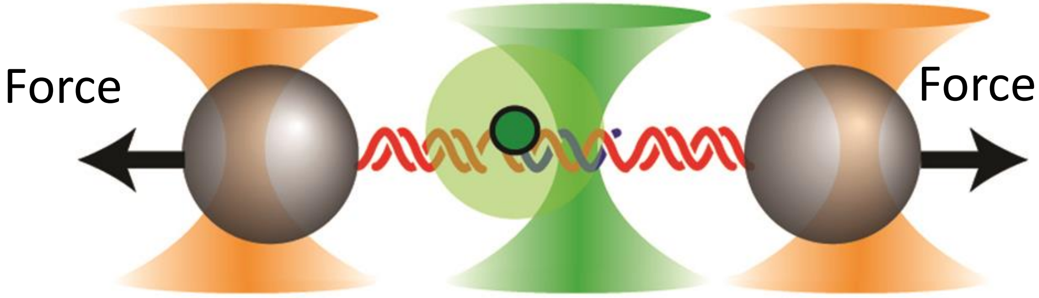
Qi Xin 2017

Atomic force microscope

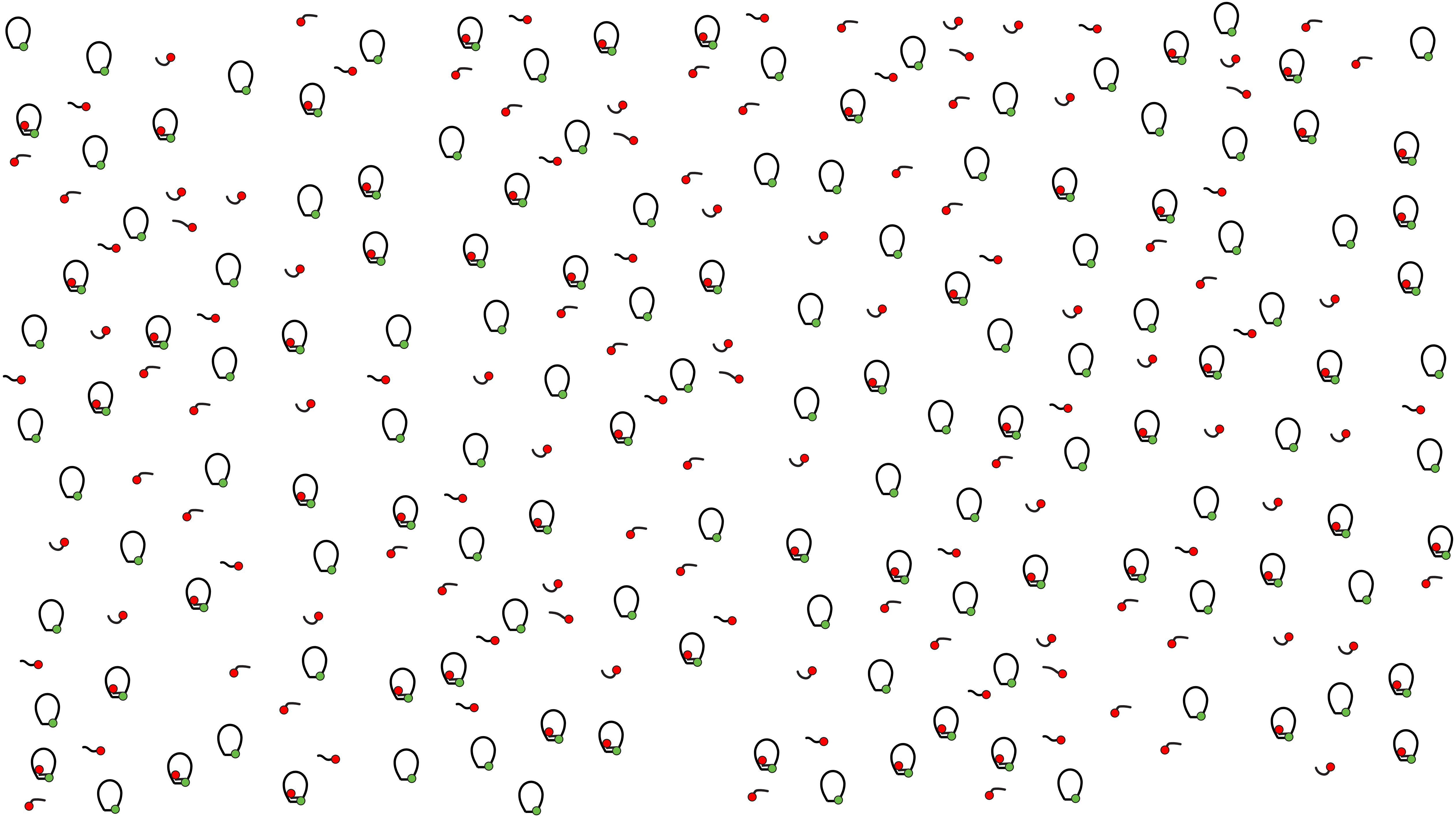


Strunz 1999

Optical (laser) tweezers



Whitley 2016



Testing force-dependence

1. Duplex sequence
2. Probe length
3. Pulling strand
4. RNA-DNA

Testing force-dependence

1. Duplex sequence
2. Probe length
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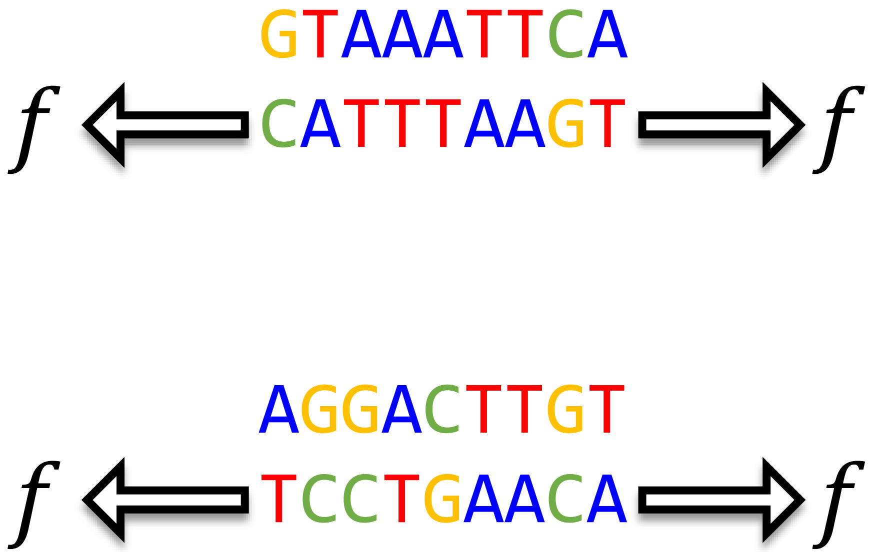


Testing force-dependence

1. Duplex sequence
2. Probe length
3. Pulling strand
4. RNA-DNA

9 bp probe

GTAAATTCA

f CATTTAAGT f

8 bp probe

GTAAATTC

f CATTTAAGT f

Testing force-dependence

1. Duplex sequence
2. Probe length
3. Pulling strand
4. RNA-DNA

Testing force-dependence

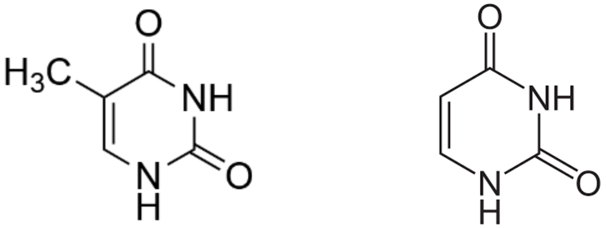
1. Duplex sequence
2. Probe length
3. Pulling strand
4. RNA-DNA

GTAAATTCA

$f \Longleftarrow$ CATTTAAGT $\Longrightarrow f$

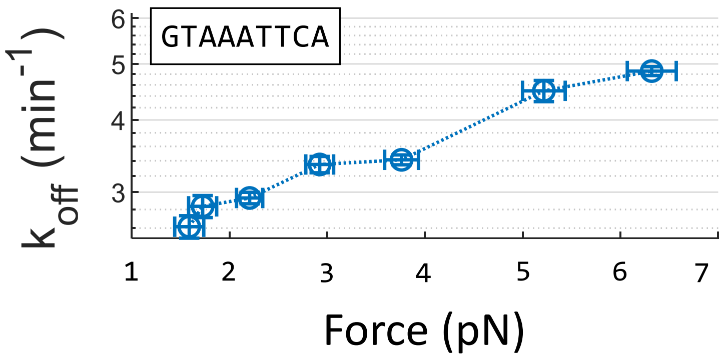
GUAAAUUCA

$f \Longleftarrow$ CATTTAAGT $\Longrightarrow f$



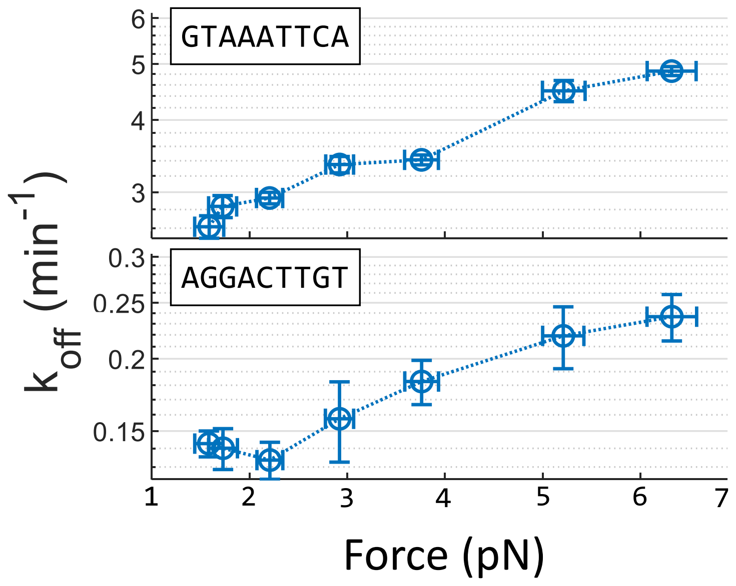
Thymine Uracil

Unbinding rate vs. force



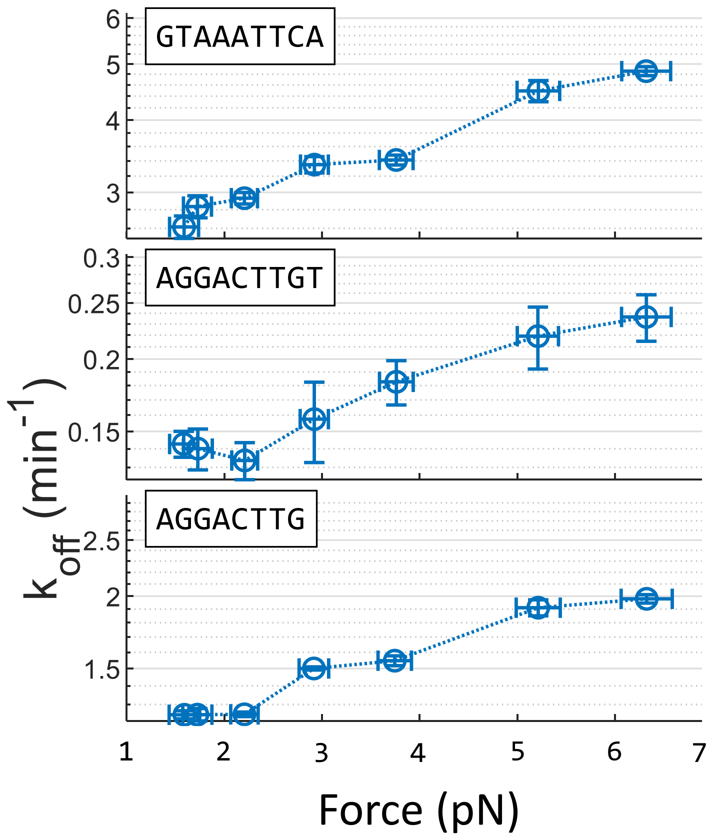
- 7 bow sizes spanning 1-7 piconewtons
- 3 trials for each bow size
- ~150 molecules measured for each trial

Unbinding rate vs. force



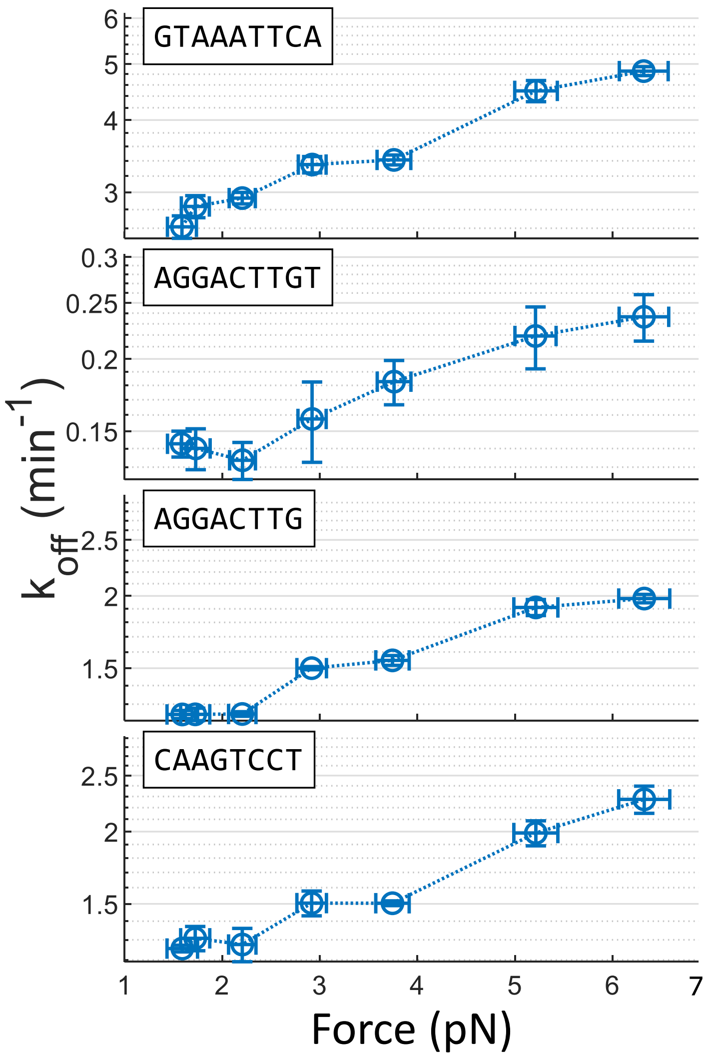
1. Duplex Sequence

Unbinding rate vs. force



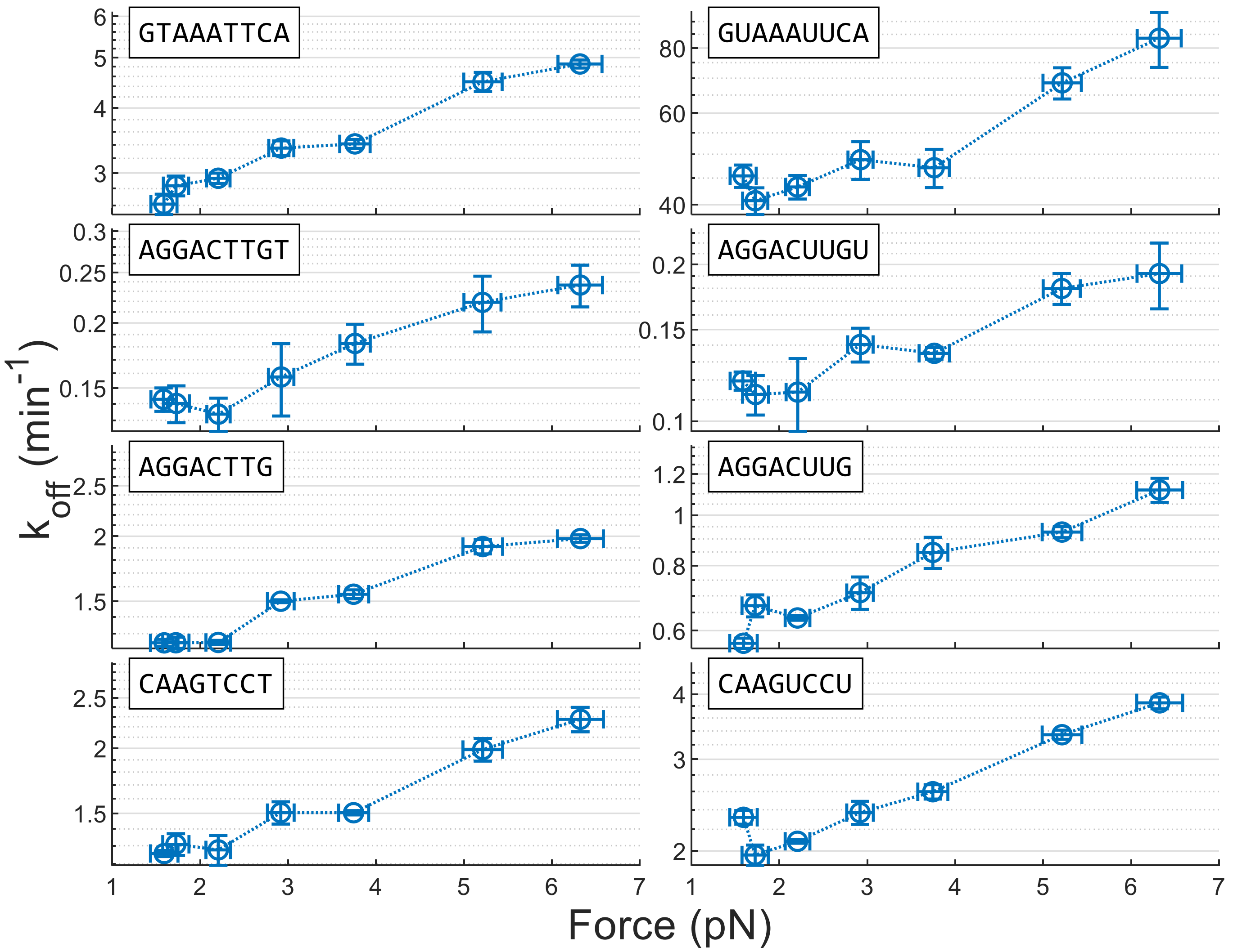
2. Probe Length

Unbinding rate vs. force



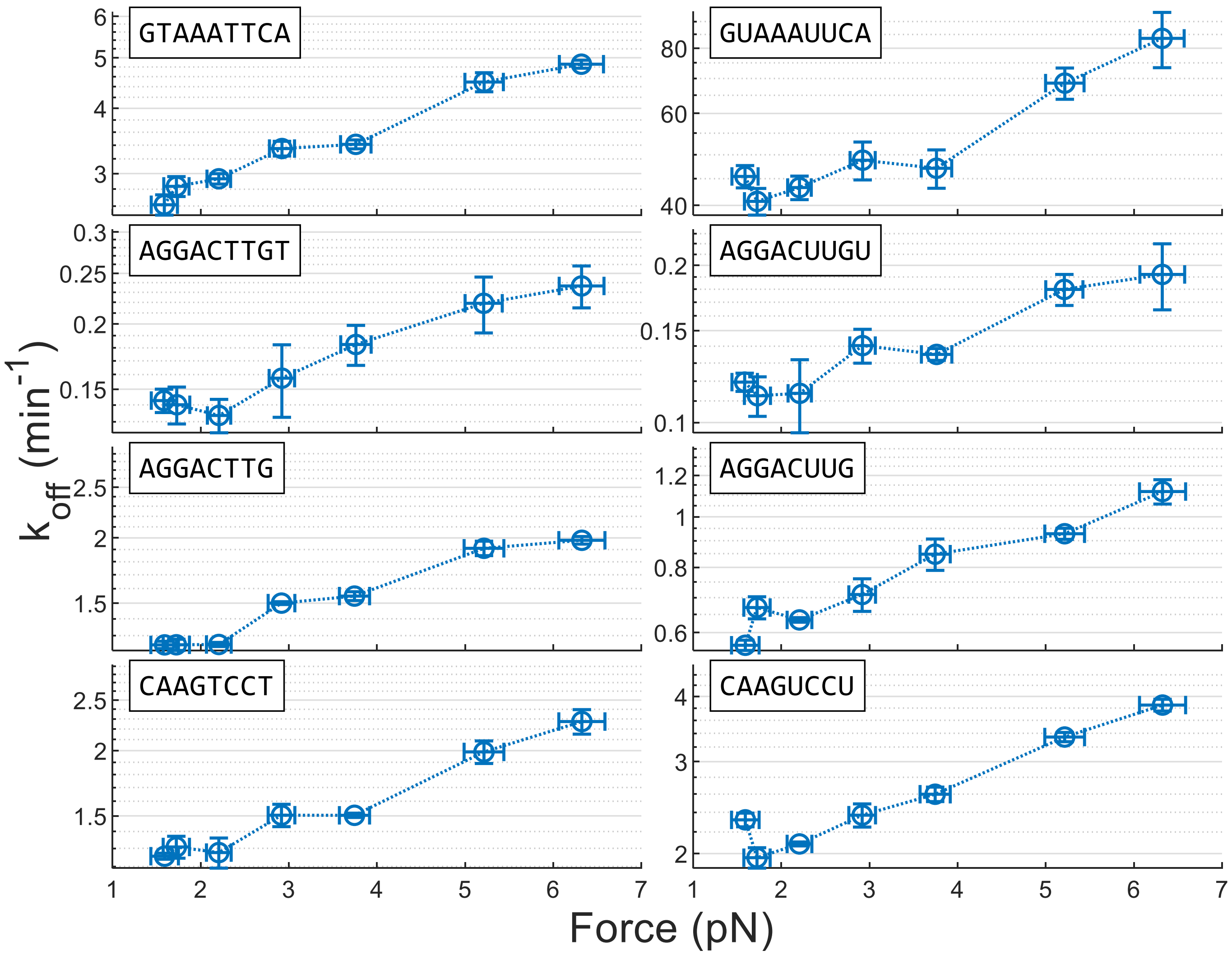
3. Pulling strand

Unbinding rate vs. force



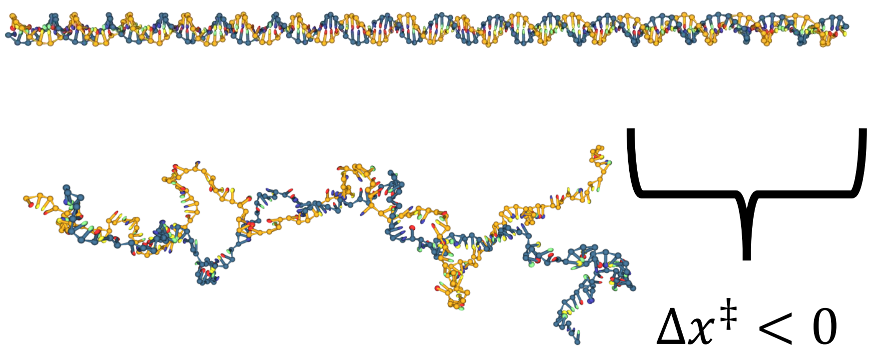
4. RNA-DNA

Unbinding rate vs. force



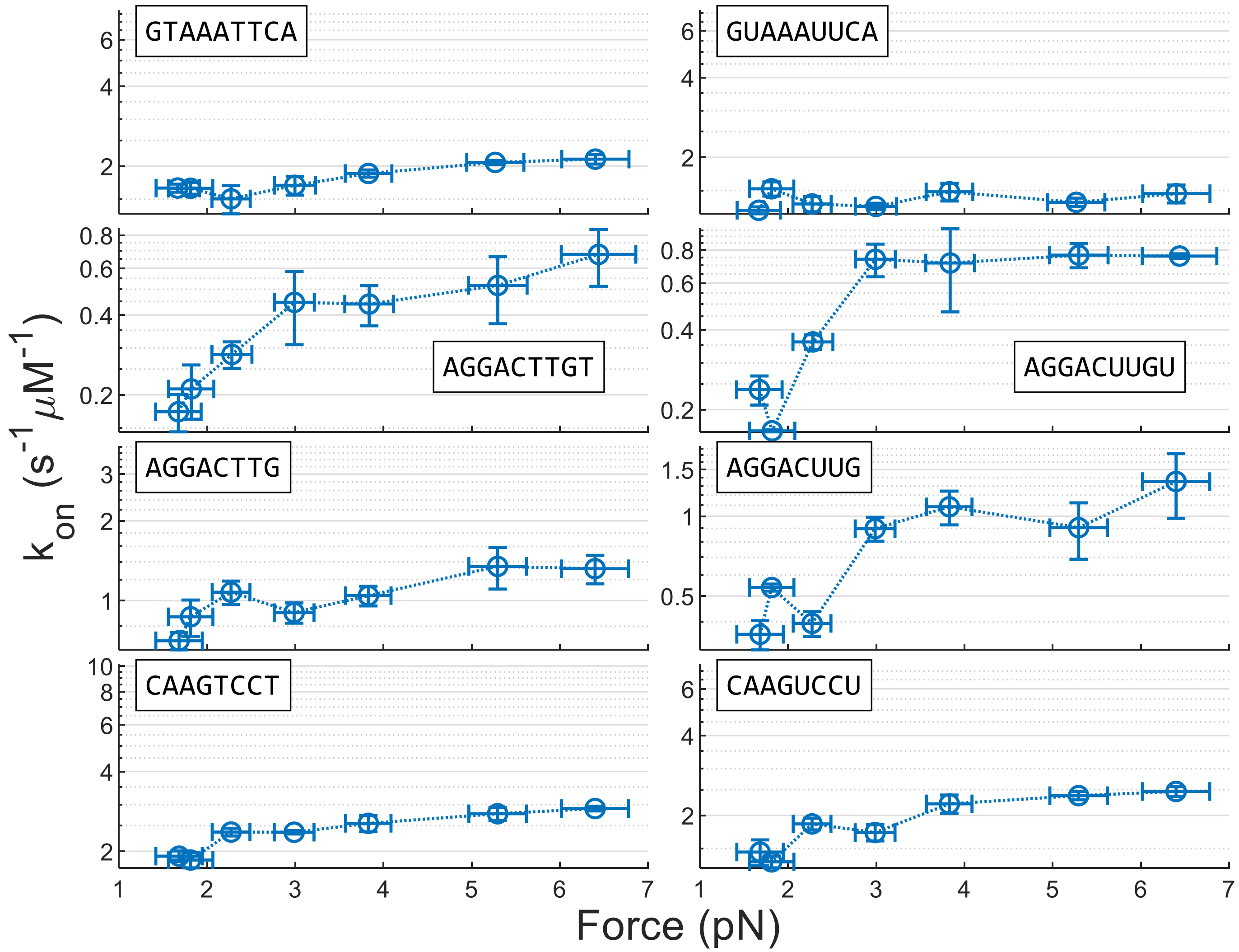
$$\frac{d\log k_{\mathrm{off}}(f)}{df} = \frac{1}{k_B T}[x_{\mathrm{t}}(f) - x_{\mathrm{ds}}(f)] > 0$$

$$\boldsymbol{x}_{\textbf{transition}} > \boldsymbol{x}_{\textbf{bound state}}$$



Short duplexes do not transition like long duplexes

Binding rate vs. force



$$\frac{d \log k_{\mathrm{on}}(f)}{df} = \frac{1}{k_B T}[x_{\mathrm{t}}(f) - x_{\mathrm{ss}}(f)] \geq 0$$

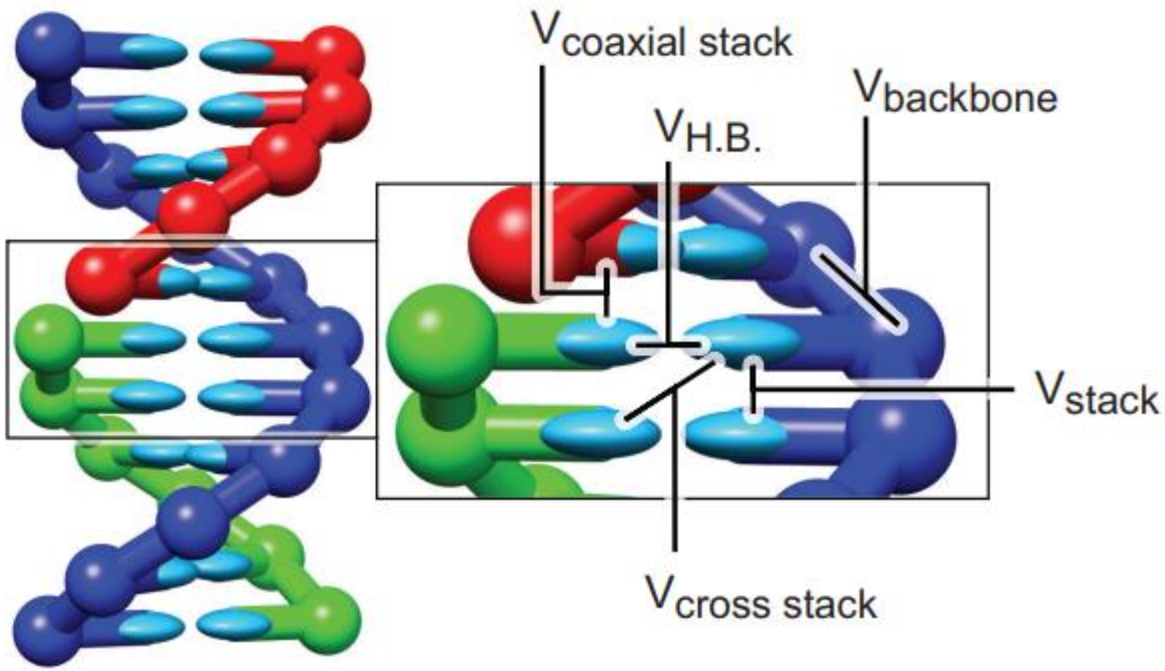
$$\boldsymbol{x}_{\mathbf{transition}} > \boldsymbol{x}_{\mathbf{unbound}}$$

Goal 2:

Simulate DNA duplex under tension

Observe extension during duplex formation/separation

“Coarse-grained” simulations (oxDNA)



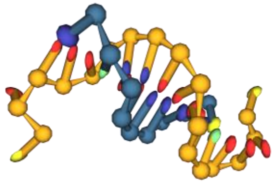
$$V = \sum_{\mathrm{nn}} (V_{\mathrm{backbone}} + V_{\mathrm{stack}} + V'_{\mathrm{exc}}) + \sum_{\mathrm{other\ pairs}} \left(V_{\mathrm{HB}} + V_{\mathrm{c\_stack}} + V_{\mathrm{exc}}\right)$$

Ouldridge, T. E., Louis, A. A., & Doye, J. P. K. (2011).
The Journal of Chemical Physics

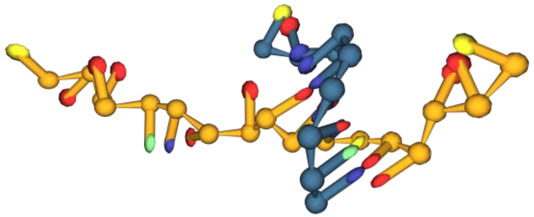
Observe duplex transitions at 7 force values

Tensile force (piconewtons)

1 2 3 4 5 6 7



Extremely weak tension



Moderate tension

How long does a ~10 bp DNA take to melt?

$$k = \underbrace{k_0}_{\text{Attempt Rate}} * \underbrace{\exp\left(-\frac{\Delta G}{k_B T}\right)}_{\text{melting probability}}$$

$$\frac{10^{-2}}{\text{sec}} \approx \frac{10^{7}\ \text{attempts}}{\text{sec}} \times \frac{10^{-9}}{\text{attempt}}$$

Experimentally, melting happens every $\sim 100\ s$

How long does it take to simulate?

Attempt Rate

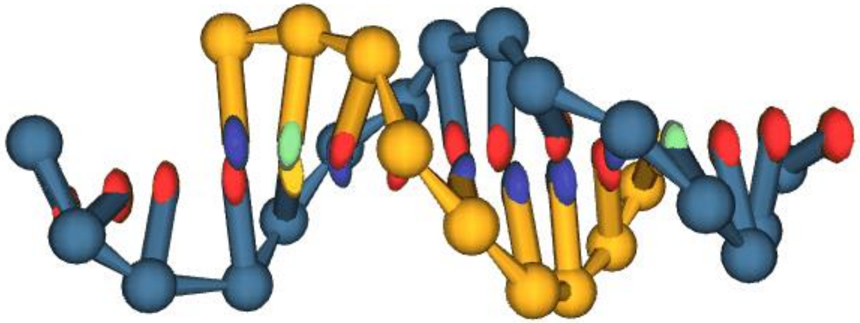
melting probability

$$k = k_0 * \exp\left(-\frac{\Delta G}{k_B T}\right)$$

$$\frac{10^{-6}}{\text{sec}} \approx \frac{10^{4}\ \text{attempts}}{\text{sec}} \times \frac{10^{-9}}{\text{attempt}}$$

Computationally, melting happens about **<u>once a day.</u>**

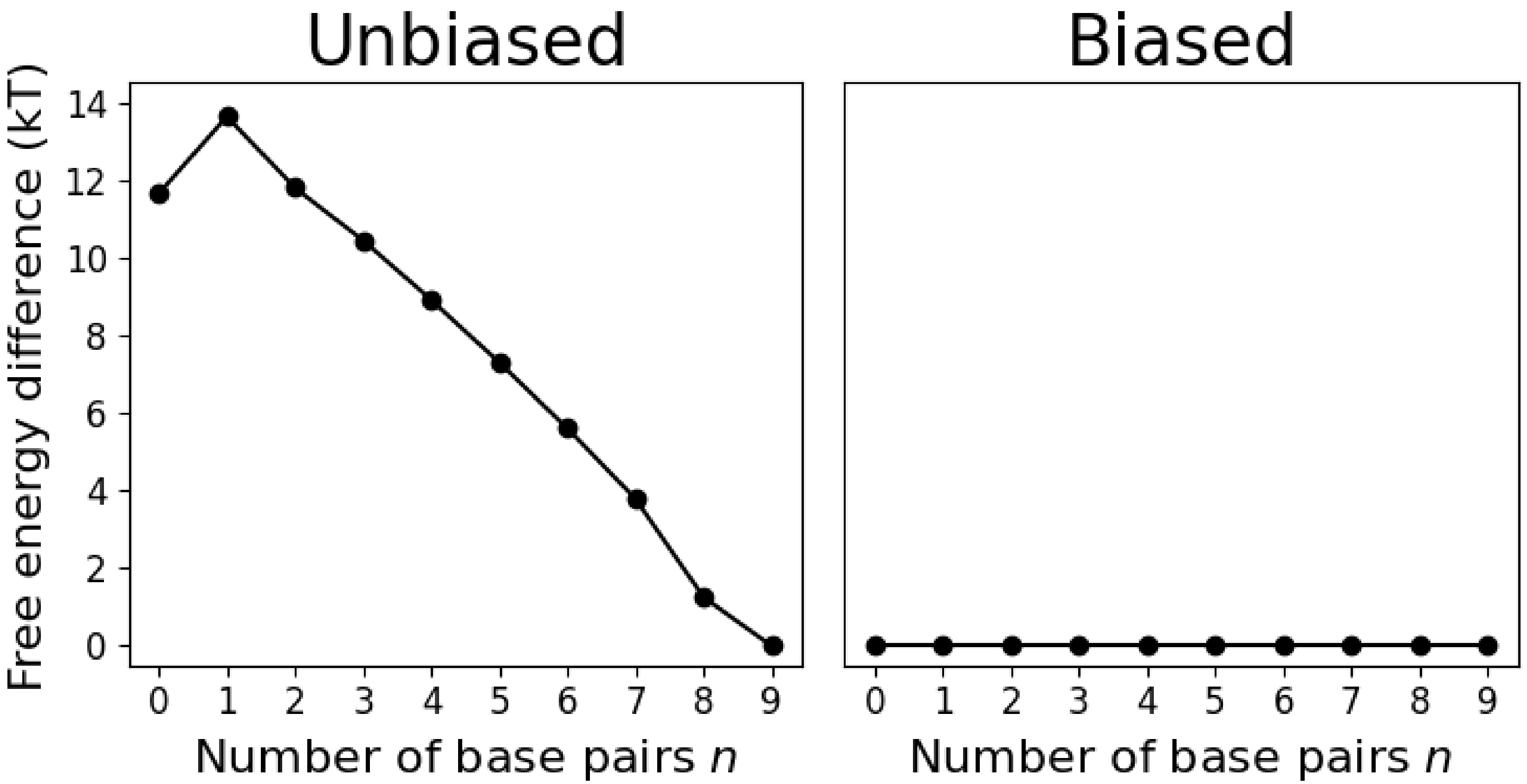
How did I simulate this?



Molecular configuration

Bias

$$\pi(\boldsymbol{r}) \propto w(\boldsymbol{r}) \exp\left(-\frac{U(\boldsymbol{r})}{k_B T}\right)$$



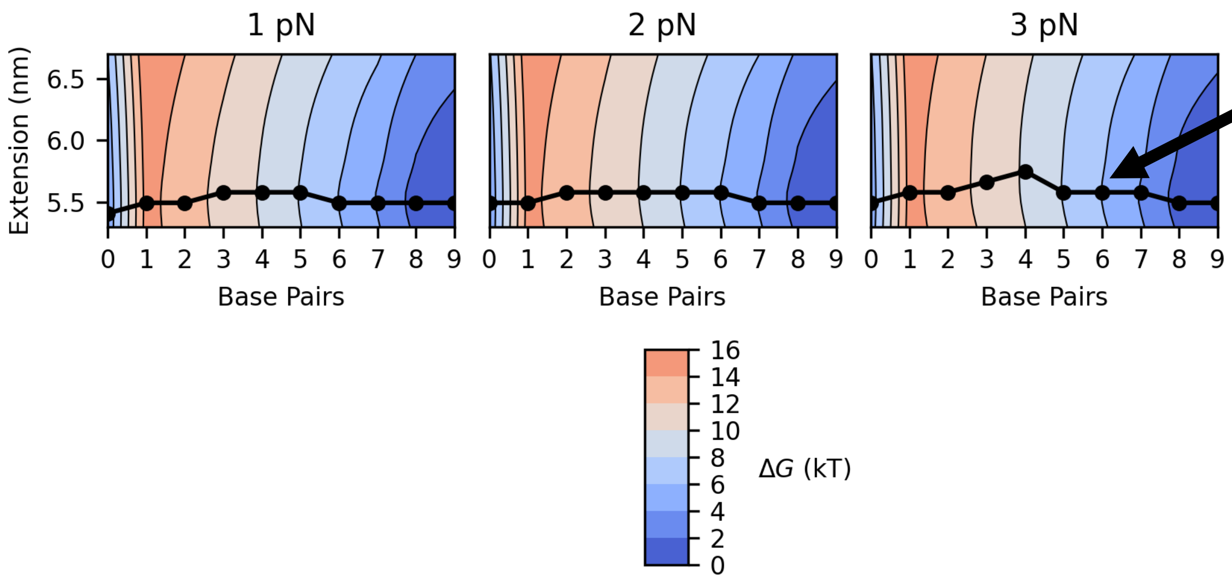
$$\Delta F_{\mathrm{unbiased}}\left(n_{\mathrm{bp}}\right) = \Delta F_{\mathrm{biased}}\left(n_{\mathrm{bp}}\right) - \mathrm{k_B T} \cdot \ln w(n_{\mathrm{bp}})$$

Sampling the melting transition

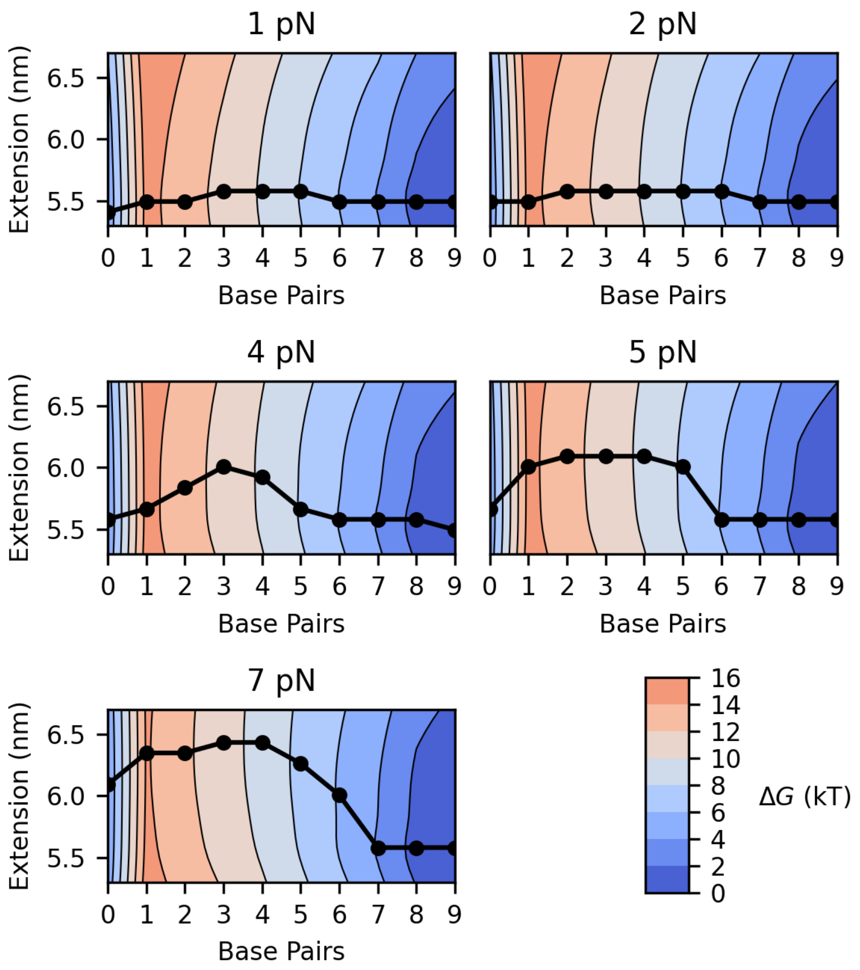
1. Simulate DNA transitions from $1 - 7\ \mathrm{pN}$

2. Calculate free energy difference: $\Delta F(x, n_{bp})$

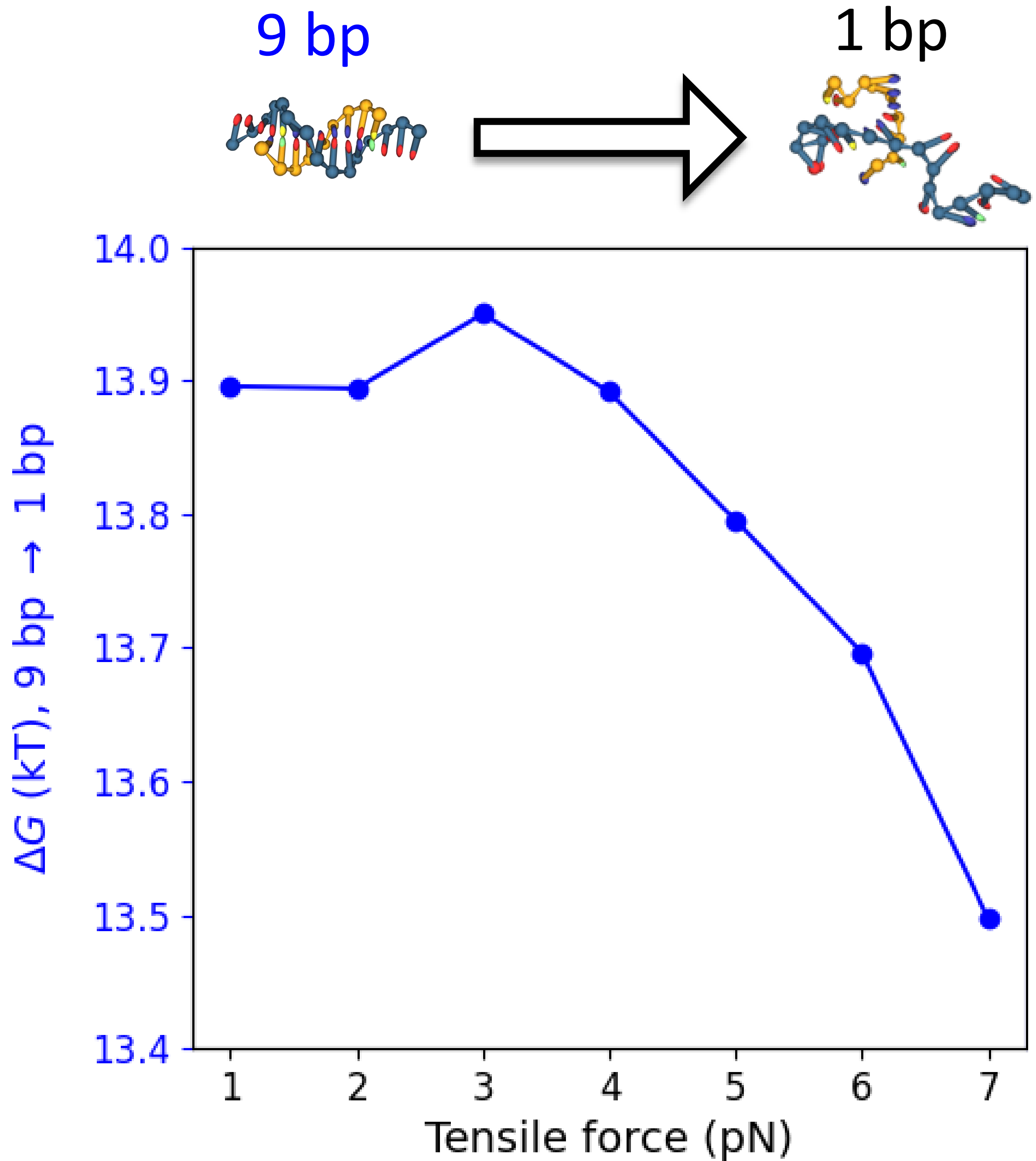
3. For each n_{bp} value, find x that minimizes ΔF

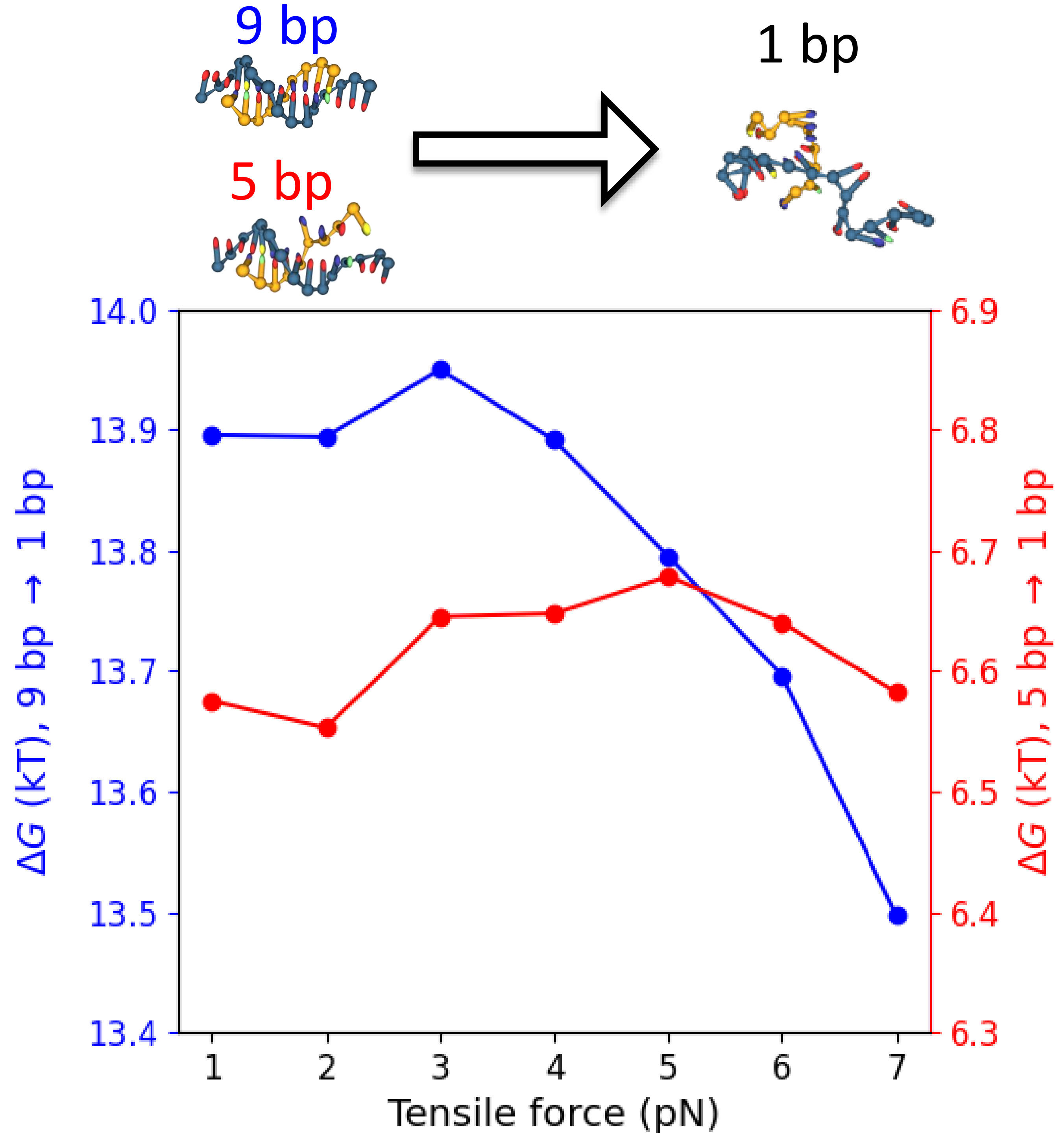


Free energy minima characterize the transition path.



1 pN
2 pN
3 pN
4 pN
5 pN
6 pN
7 pN
Extension (nm)
Base Pairs
ΔG (kT)


9 bp
1 bp
14.0
13.9
13.8
13.7
13.6
13.5
13.4
ΔG (kT), 9 bp → 1 bp
1
2
3
4
5
6
7
Tensile force (pN)



9 bp
5 bp
1 bp
14.0
13.9
13.8
13.7
13.6
13.5
13.4
6.9
6.8
6.7
6.6
6.5
6.4
6.3
1
2
3
4
5
6
7
ΔG (kT), 9 bp → 1 bp
ΔG (kT), 5 bp → 1 bp
Tensile force (pN)

Goal 3:

How do terminal dsDNA "block" attachments affect ssDNA?

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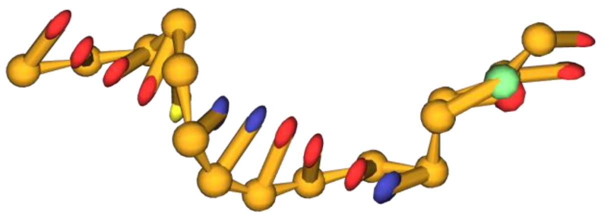

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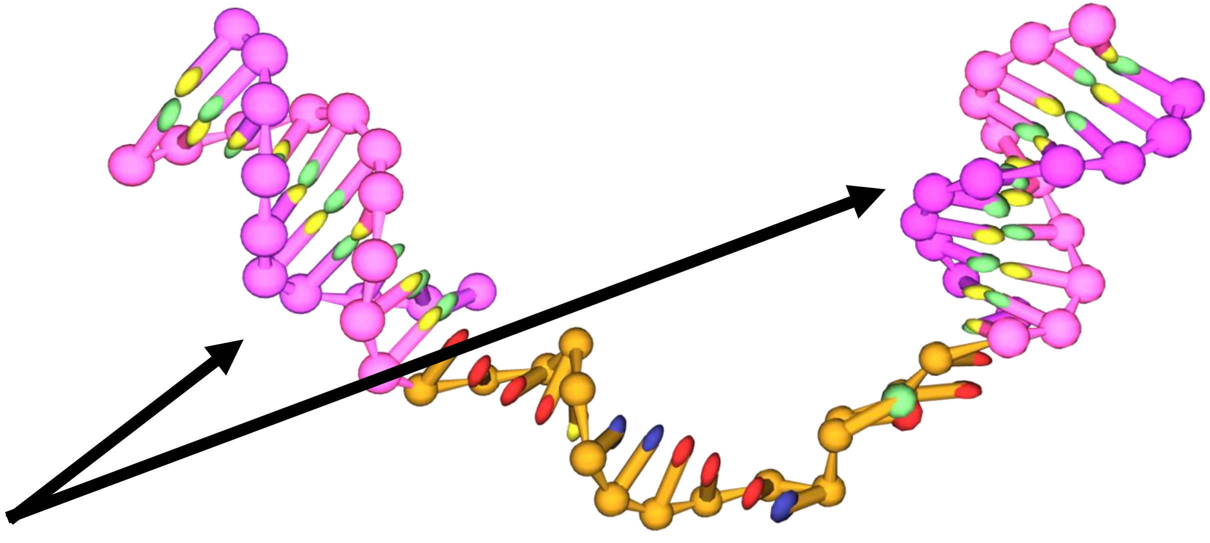
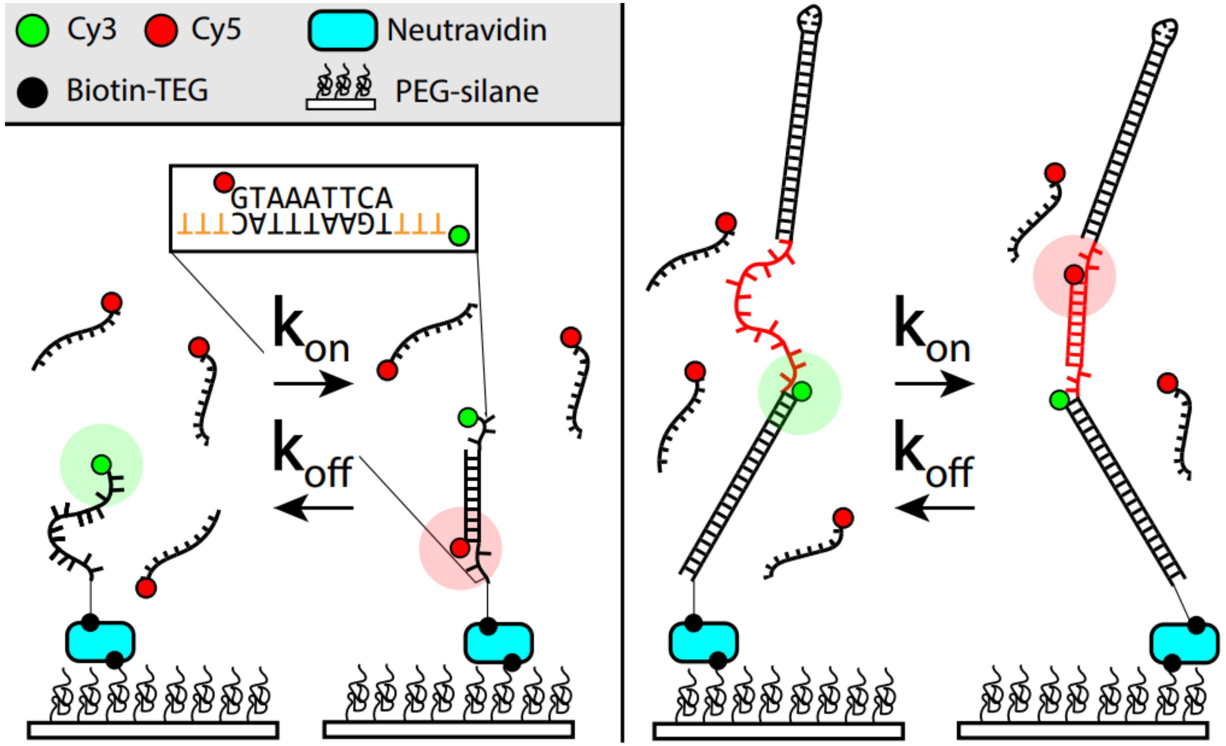
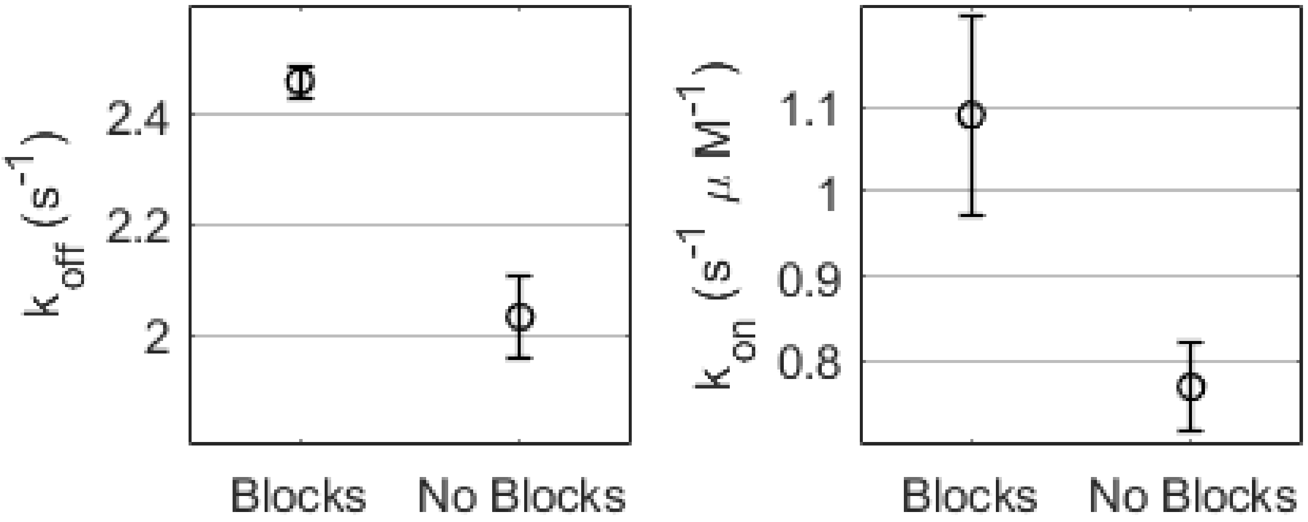


dsDNA "blocks"

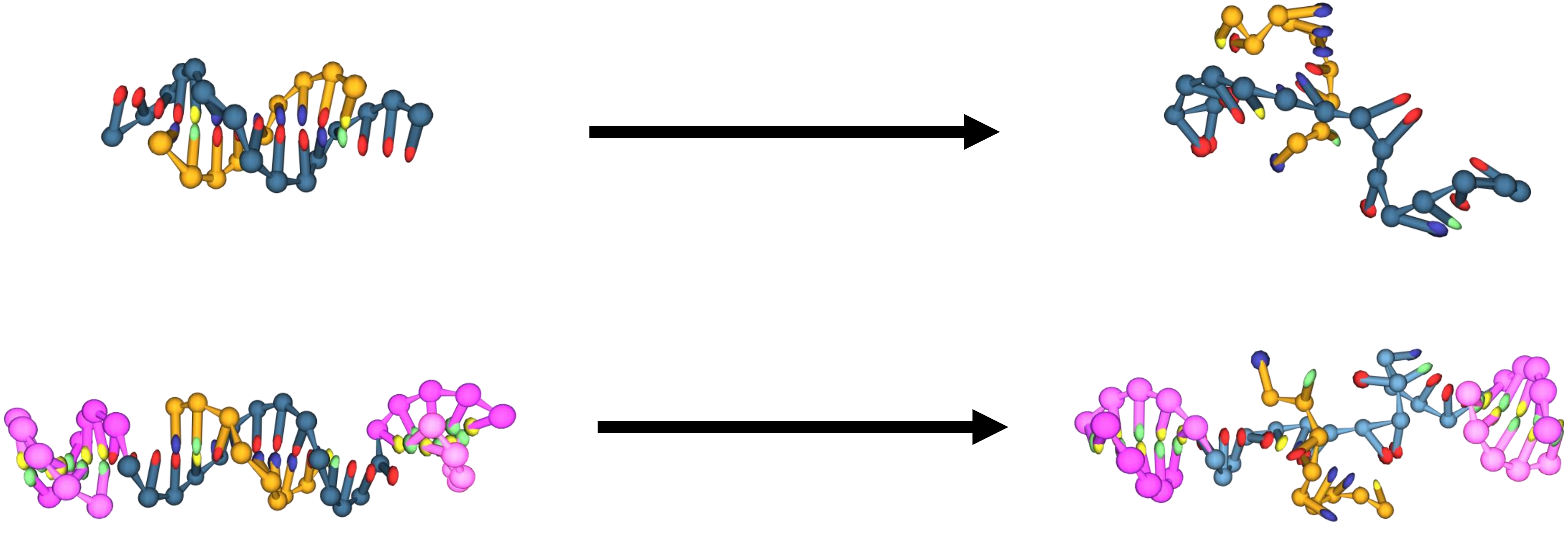


Zero dsDNA “blocks”   Two dsDNA “blocks”

Flanking dsDNA blocks accelerate binding & unbinding



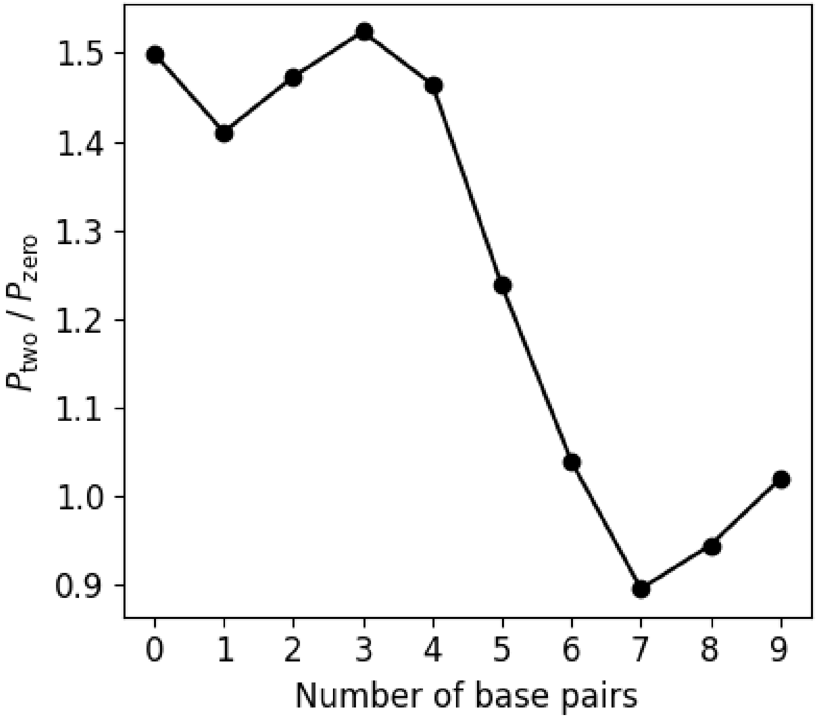
Simulate melting transition of both constructs

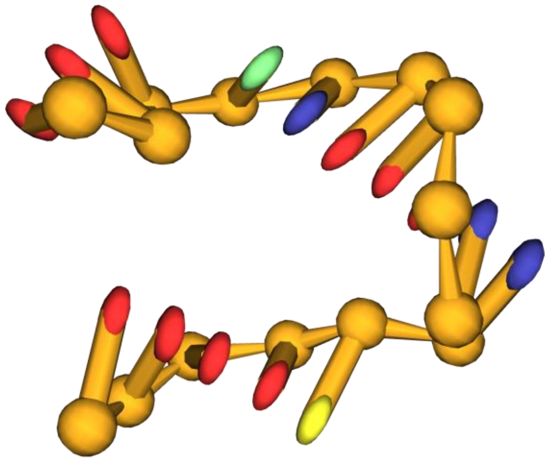


9 base pairs (bound state)

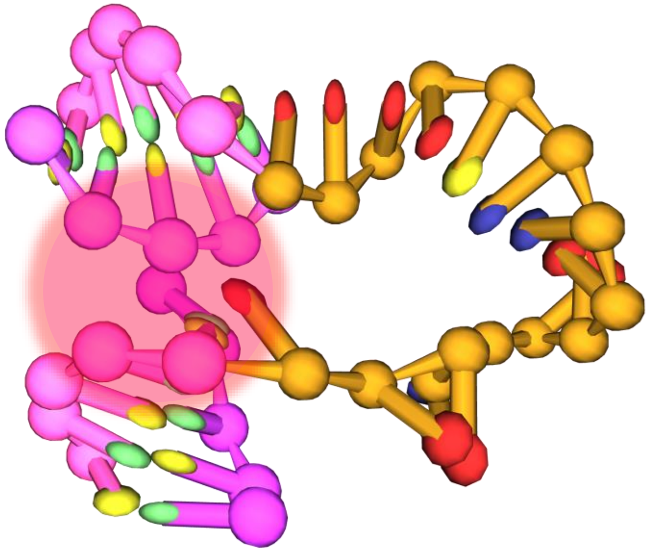
1 base pairs (near-transition)

Flanking dsDNA blocks destabilize the bound state

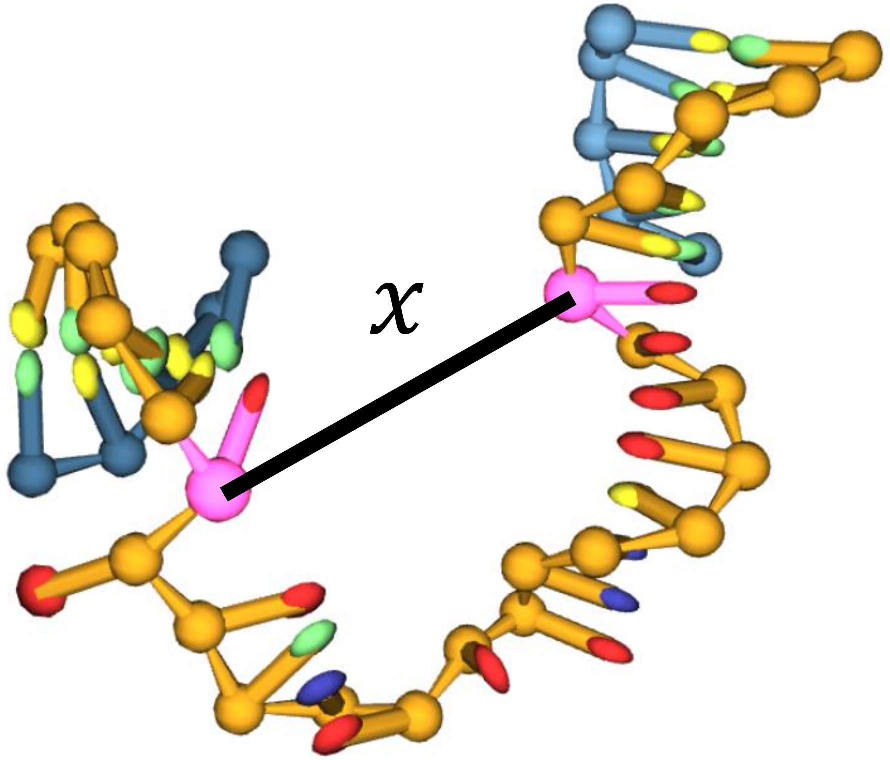


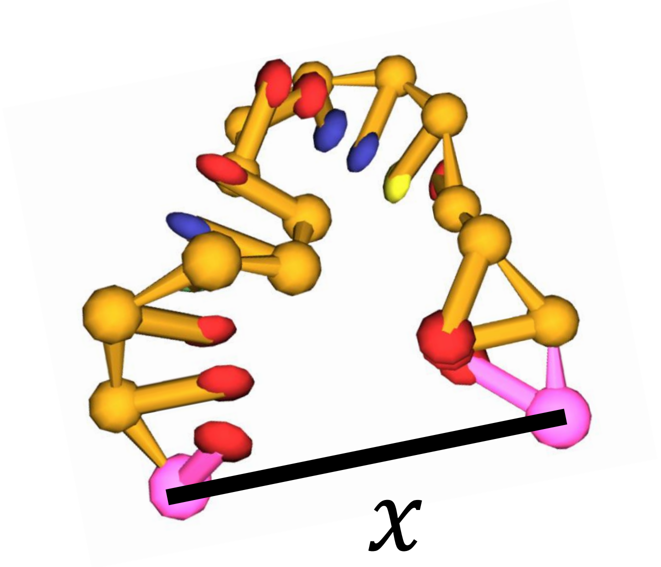


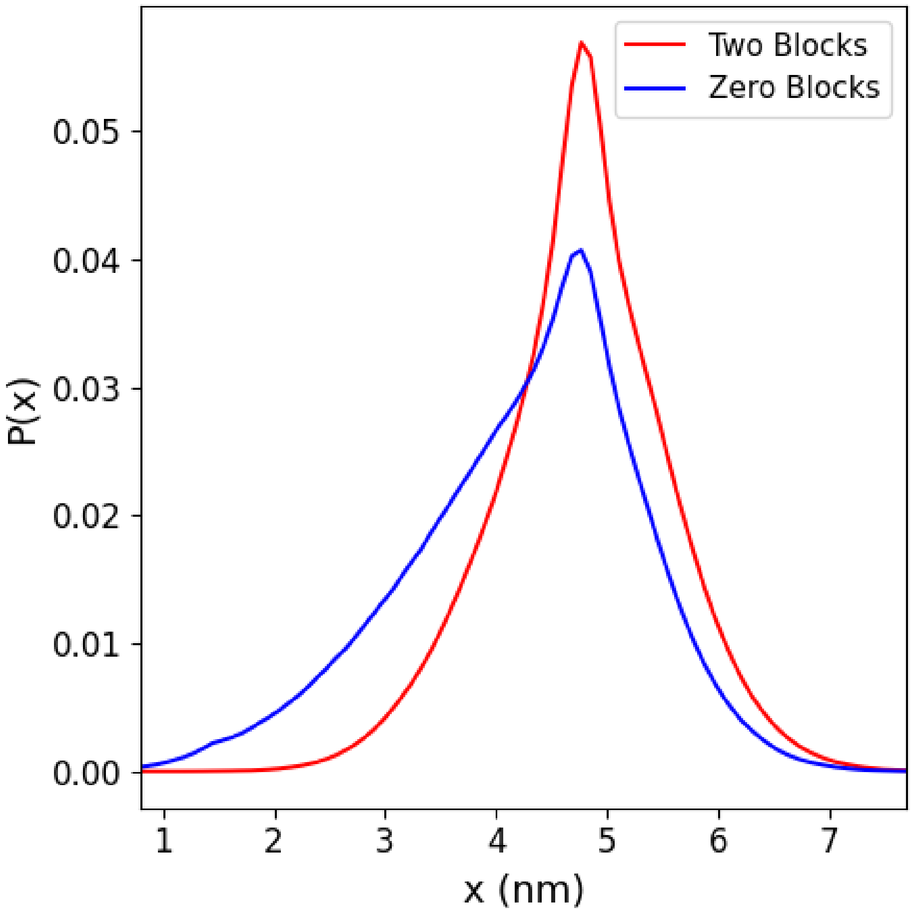
Electrostatic repulsion from dsDNA prevents ssDNA strand folding



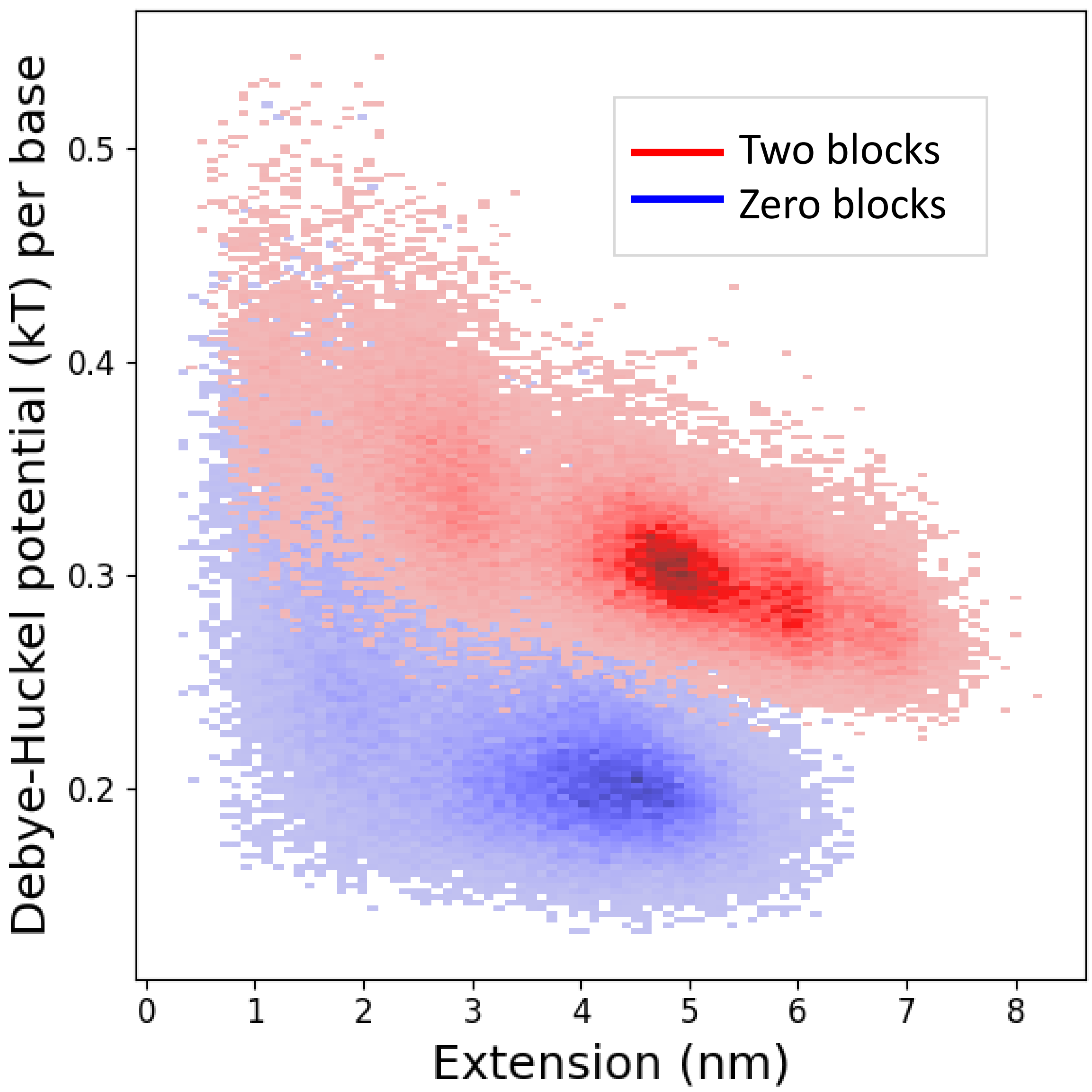
How do dsDNA blocks affect ssDNA extension?







Two Blocks
Zero Blocks
0.00
0.01
0.02
0.03
0.04
0.05
P(x)
1
2
3
4
5
6
7
x (nm)



$$V_{\mathrm{DH}}(T,I) = \sum_{ij} \frac{(q_{\mathrm{eff}}e)^2}{4\pi\epsilon_0\epsilon_{\mathrm{r}}} \frac{\exp\left\{-r_{ij}^{\text{b-b}}/\lambda_{\mathrm{DH}}(T,I)\right\}}{r_{ij}^{\text{b-b}}}$$

Goal 1: How do short duplexes subject to weak force transition?

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Weak tension accelerates binding and unbinding from 1 to 7 pN.

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Extension is a poor reaction coordinate at ~3 pN and below

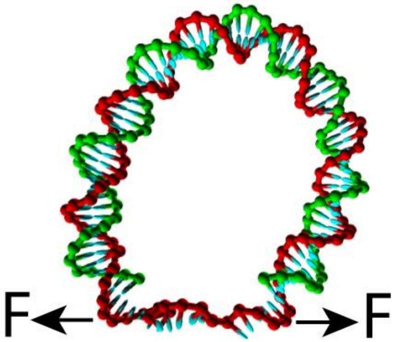
Goal 3: How do terminal dsDNA block attachments affect ssDNA?

Flanking dsDNA blocks extend DNA and accelerate duplex kinetics

Thanks!

DNA Bo

DNA Bow



JC Gumbart

Gable Wadsworth

Tony Lemos

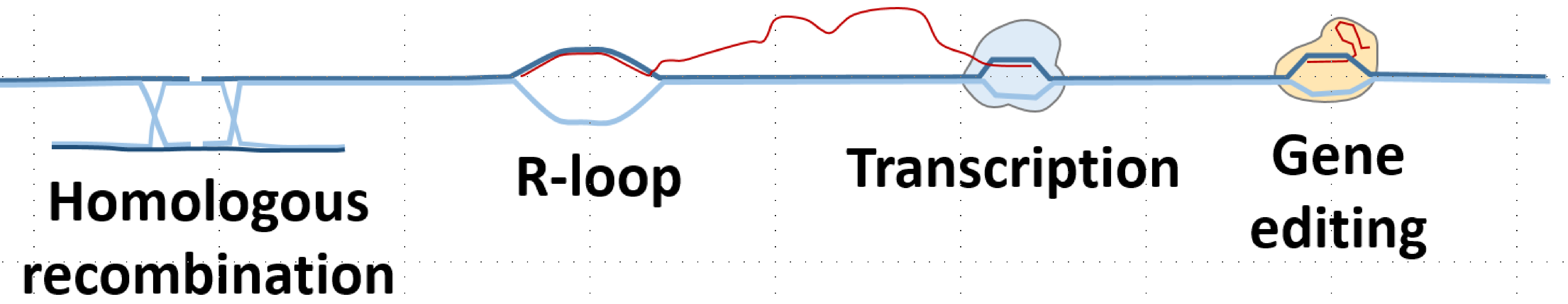
Michael Ryan

Harold Kim

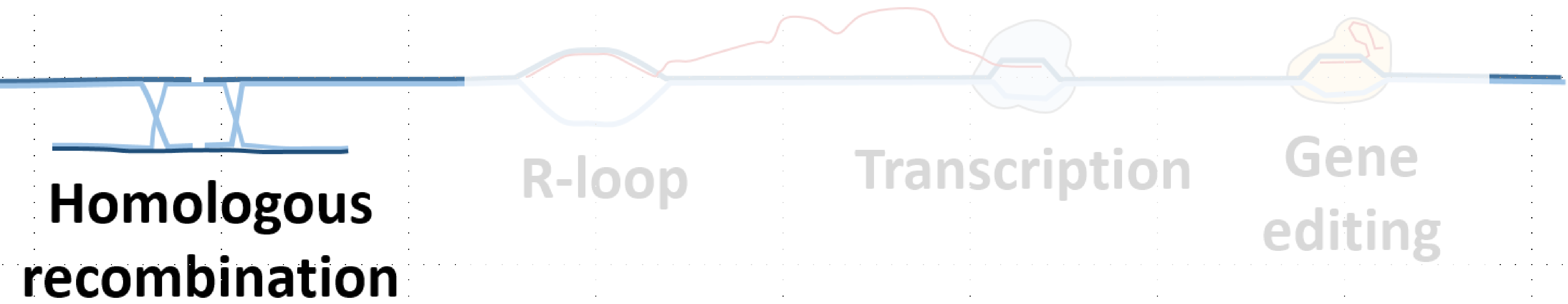
Alec Cook

Jiyoun Jeong (PhD)

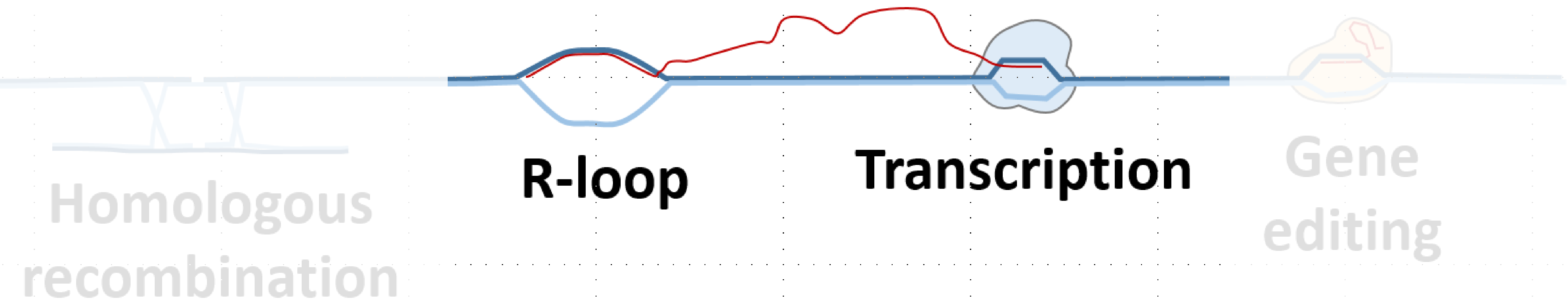
Strand separation & formation in biology



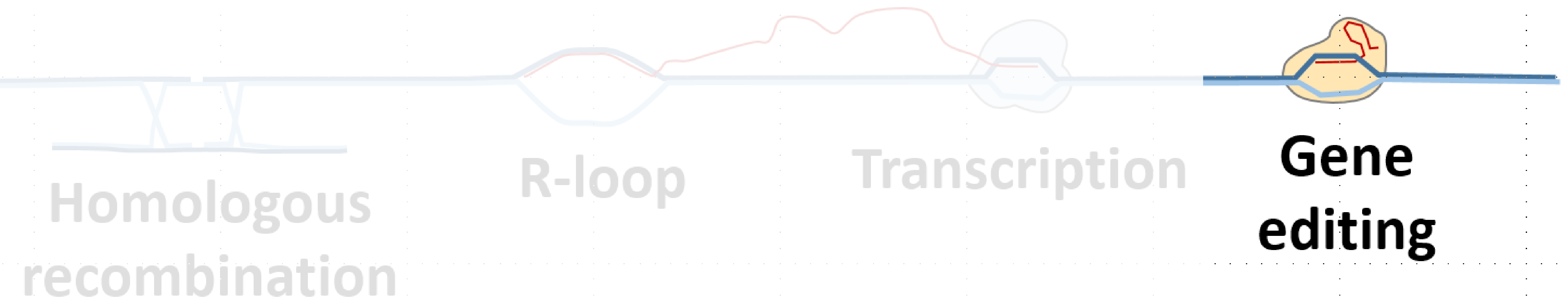
Strand separation & formation in biology

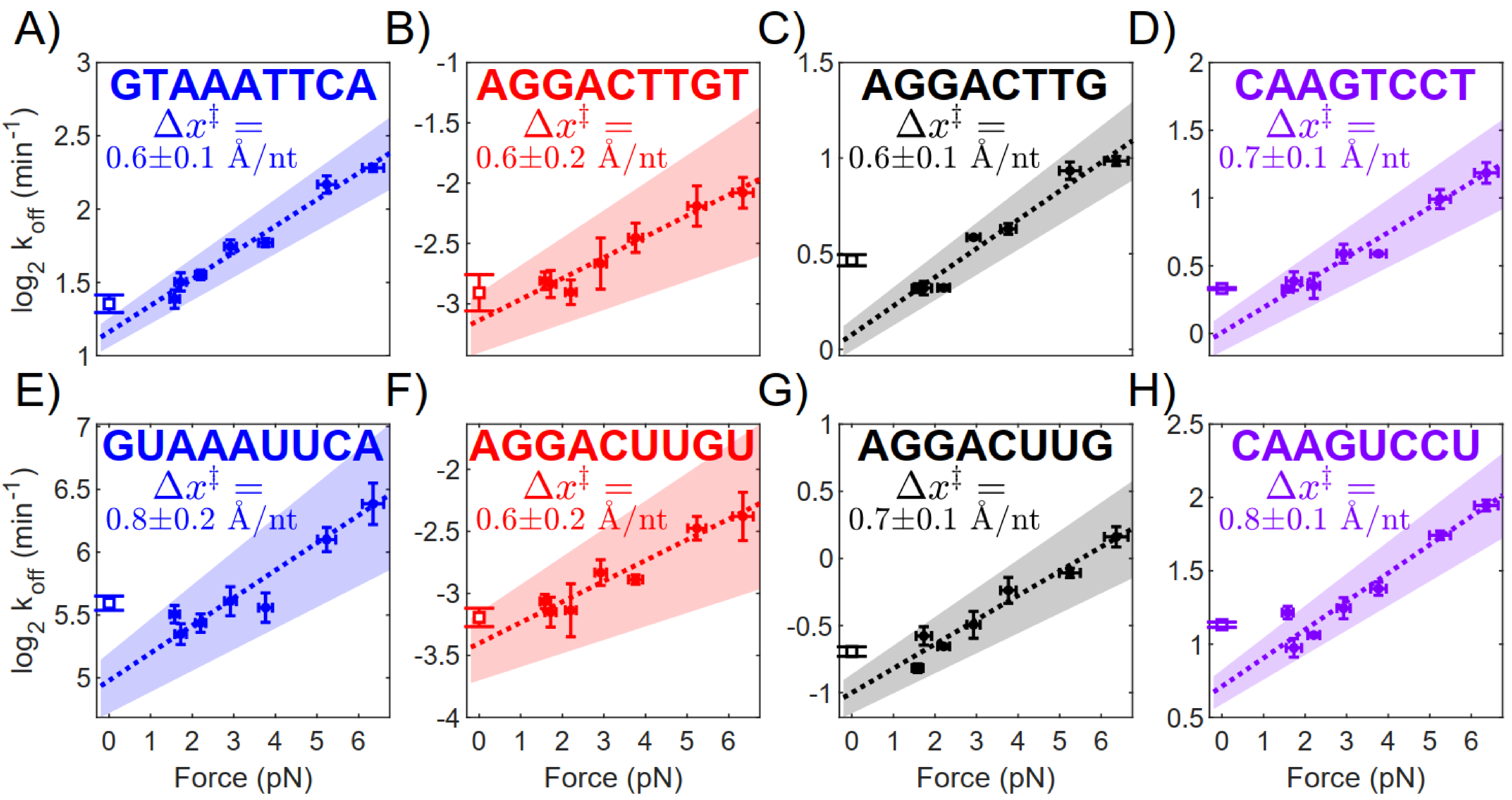


Strand separation & formation in biology

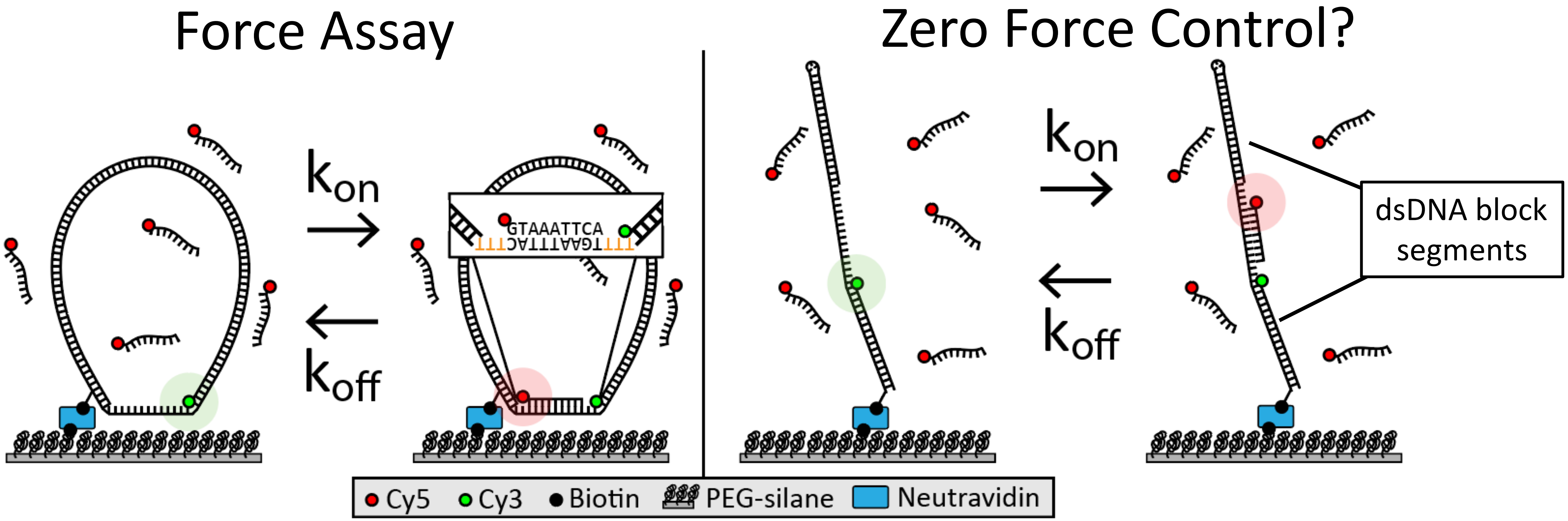


Strand separation & formation in biology





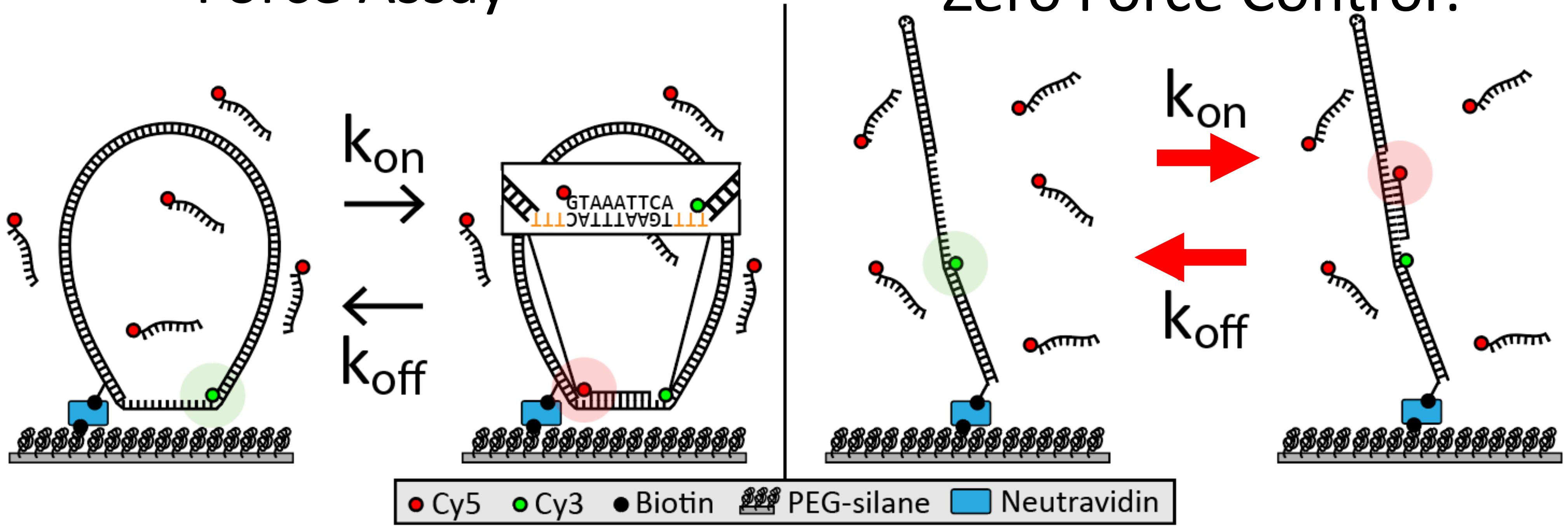
A)
GTAAATTCA
$\Delta x^\ddagger =$
0.6 ± 0.1 Å/nt
B)
AGGACTTGT
$\Delta x^\ddagger =$
0.6 ± 0.2 Å/nt
C)
AGGACTTG
$\Delta x^\ddagger =$
0.6 ± 0.1 Å/nt
D)
CAAGTCCT
$\Delta x^\ddagger =$
0.7 ± 0.1 Å/nt
E)
GUAAAUUCA
$\Delta x^\ddagger =$
0.8 ± 0.2 Å/nt
F)
AGGACUUGU
$\Delta x^\ddagger =$
0.6 ± 0.2 Å/nt
G)
AGGACUUG
$\Delta x^\ddagger =$
0.7 ± 0.1 Å/nt
H)
CAAGUCCU
$\Delta x^\ddagger =$
0.8 ± 0.1 Å/nt
$\log_2 k_{off}$ (min^{-1})
Force (pN)



Force Assay
Zero Force Control?
kon
koff
GTAAATTCA
TTTCATTTAAGTTTT
dsDNA block segments
Cy5
Cy3
Biotin
PEG-silane
Neutravidin

Force Assay

Zero Force Control?



Observed rates higher than expected for zero force

Biasing Potential

$$\pi(\boldsymbol{r}) = \frac{w(\mathbf{r})\exp\left(-\frac{U(\boldsymbol{r})}{k_B T}\right)}{\int w(\boldsymbol{r}')\exp\left(-\frac{U(\boldsymbol{r}')}{k_B T}\right) d\boldsymbol{r}'}$$