Kinetics of DNA hybridization and dehybridization under weak tension

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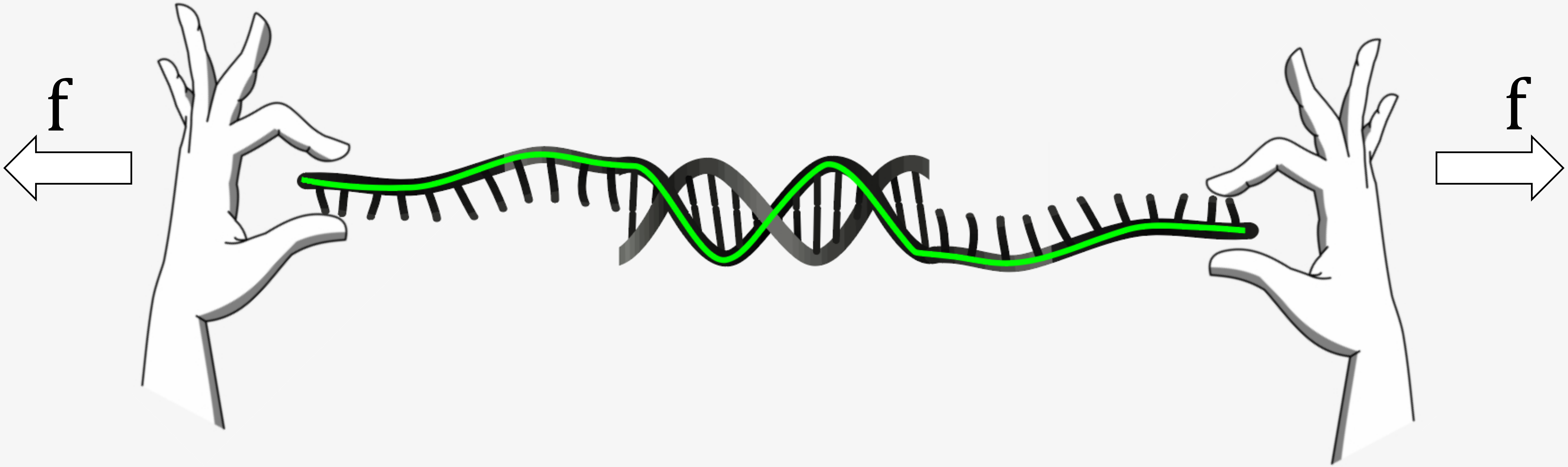
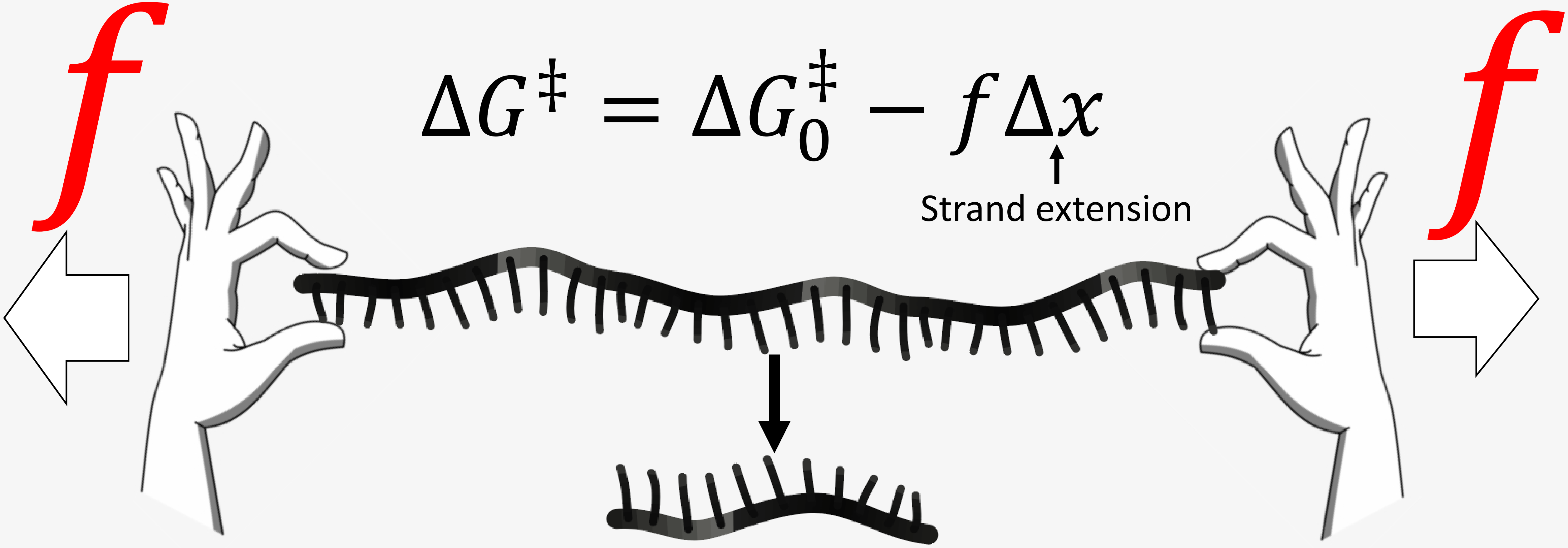
What happens when you pull on the double helix?



Large forces **destabilize** the duplex

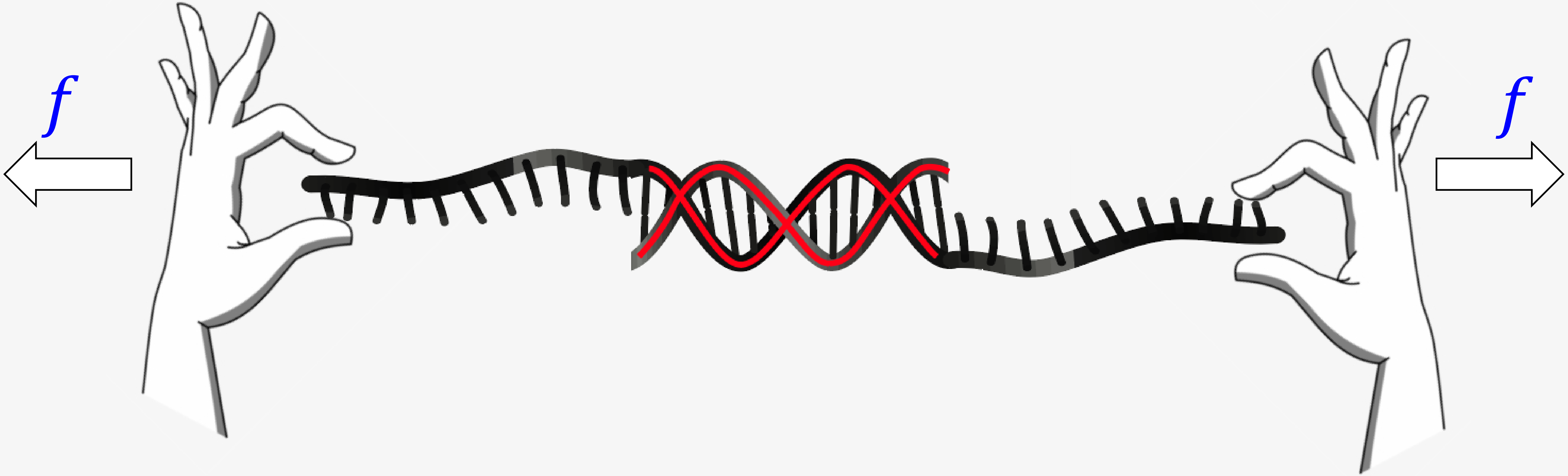
$$\Delta G^{\ddagger} = \Delta G_0^{\ddagger} - f\Delta x$$

Strand extension

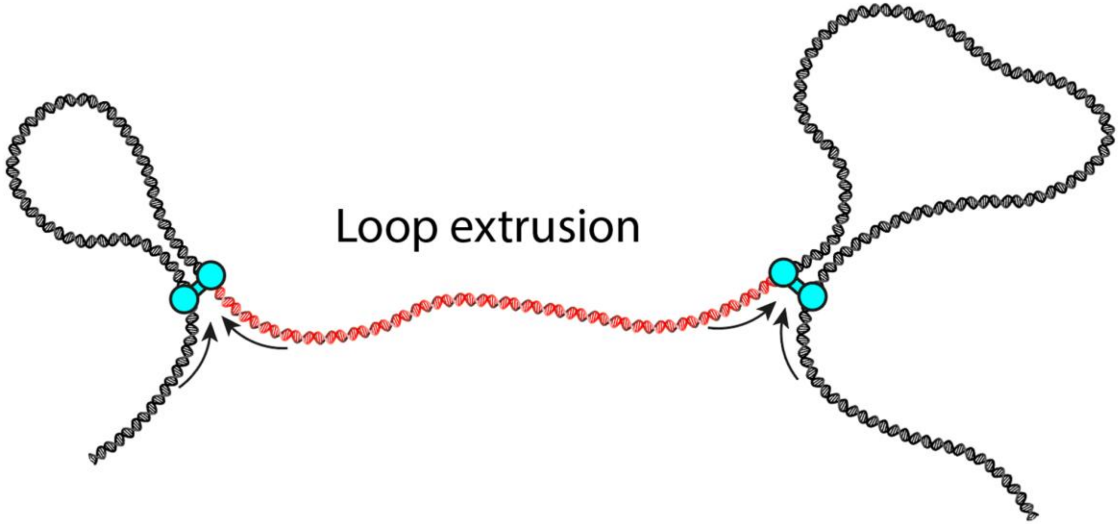


$$k_{\mathrm{off}} \sim e^{f\Delta x}$$

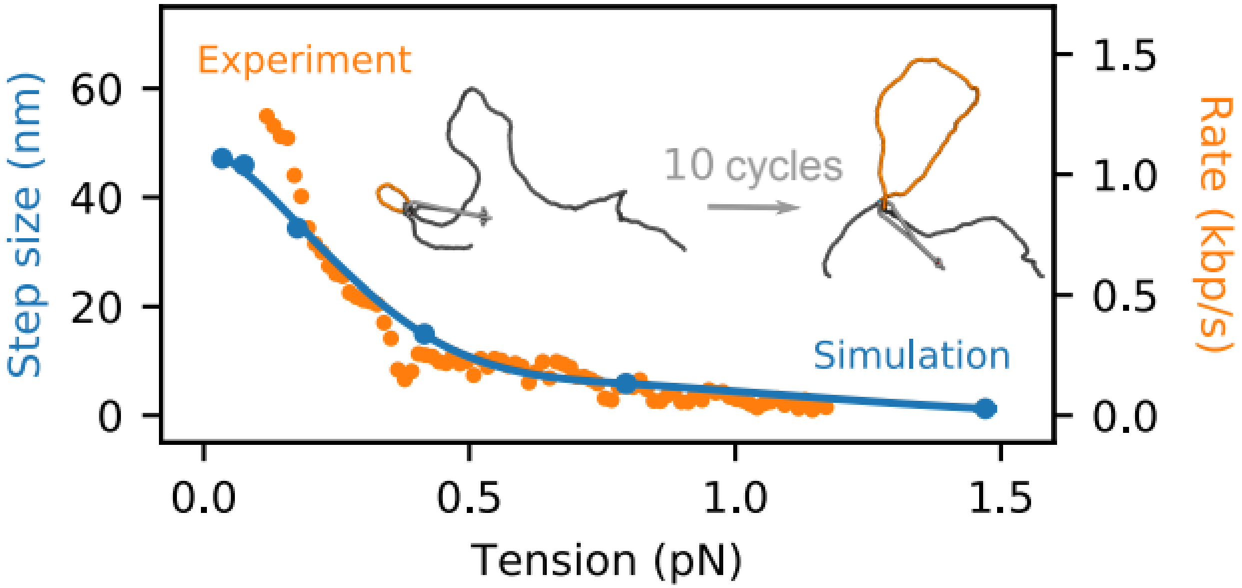
small forces (~1 pN) may stabilize the duplex?



DNA is under tension in the cell



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)

Why would weak force stabilize the duplex?

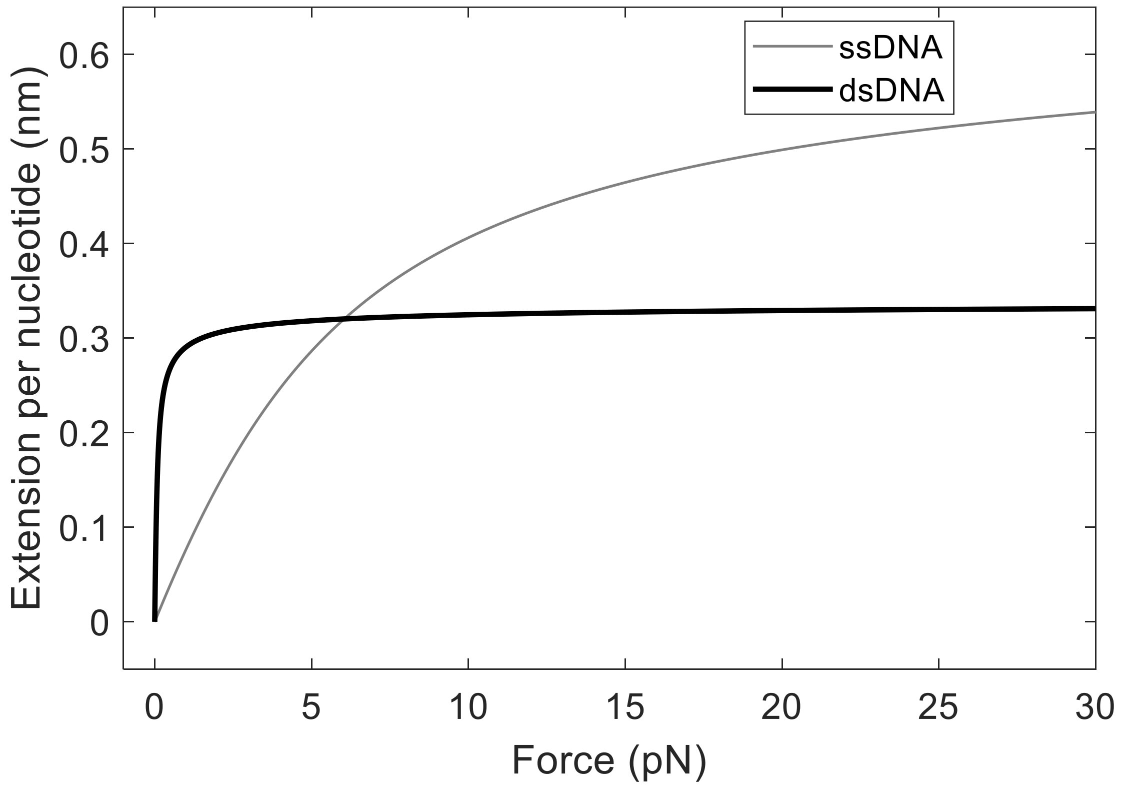
$$\Delta G^{\ddagger} = \Delta G_0^{\ddagger} - \int_0^f [x_t(f') - x_i(f')]df'$$

Transition extension

Initial extension

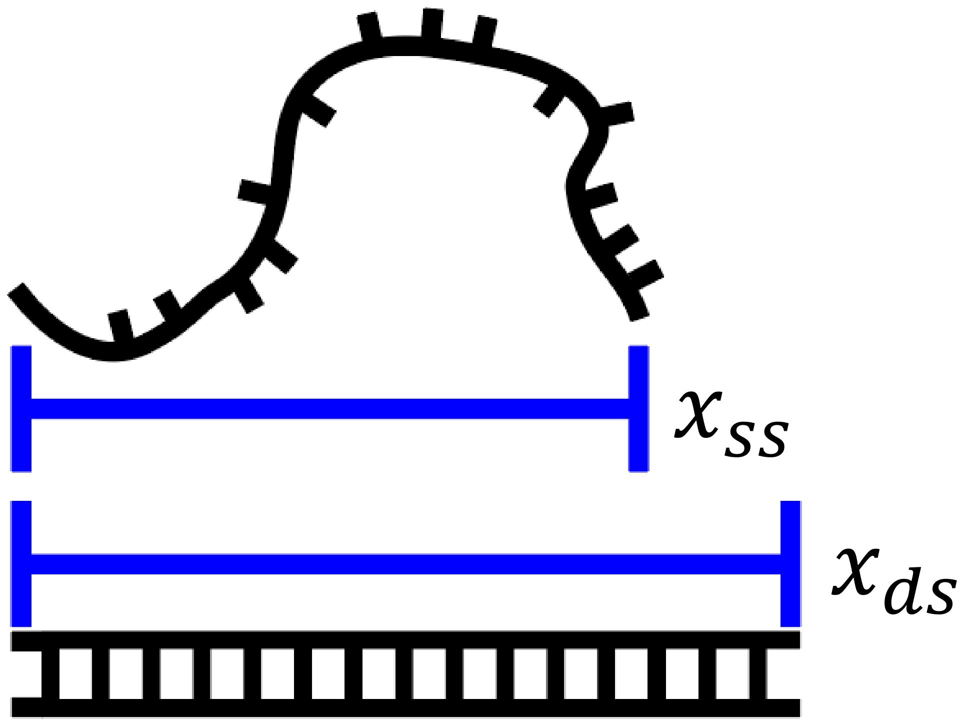
If $x_t < x_i$, then $\Delta G^{\ddagger}$ increases.

ssDNA is more flexible than dsDNA

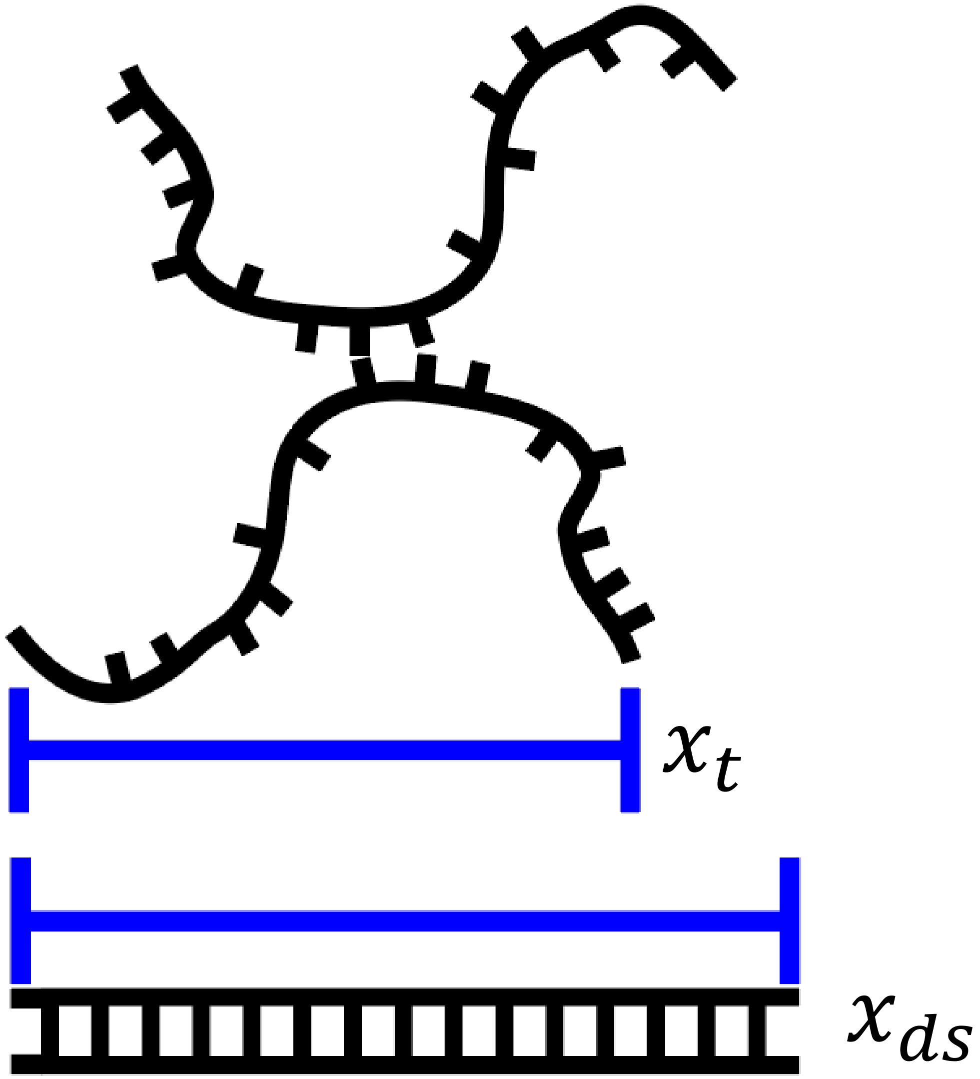


Force extension curve of DNA
(Marko-Siggia)

When $f < 5\,pN$:



Hypothesis: the transition state is also flexible (ssDNA-like)



If $x_{ss} < x_t < x_{ds}$ at a given force f,

Negative in value

$$\frac{d \log k_{\mathrm{off}}(f)}{df} = \frac{1}{k_{\mathrm{B}}T}[x_{\mathrm{t}}(f) - x_{\mathrm{ds}}(f)], \quad k_{\mathrm{off}} \text{ decreases}$$

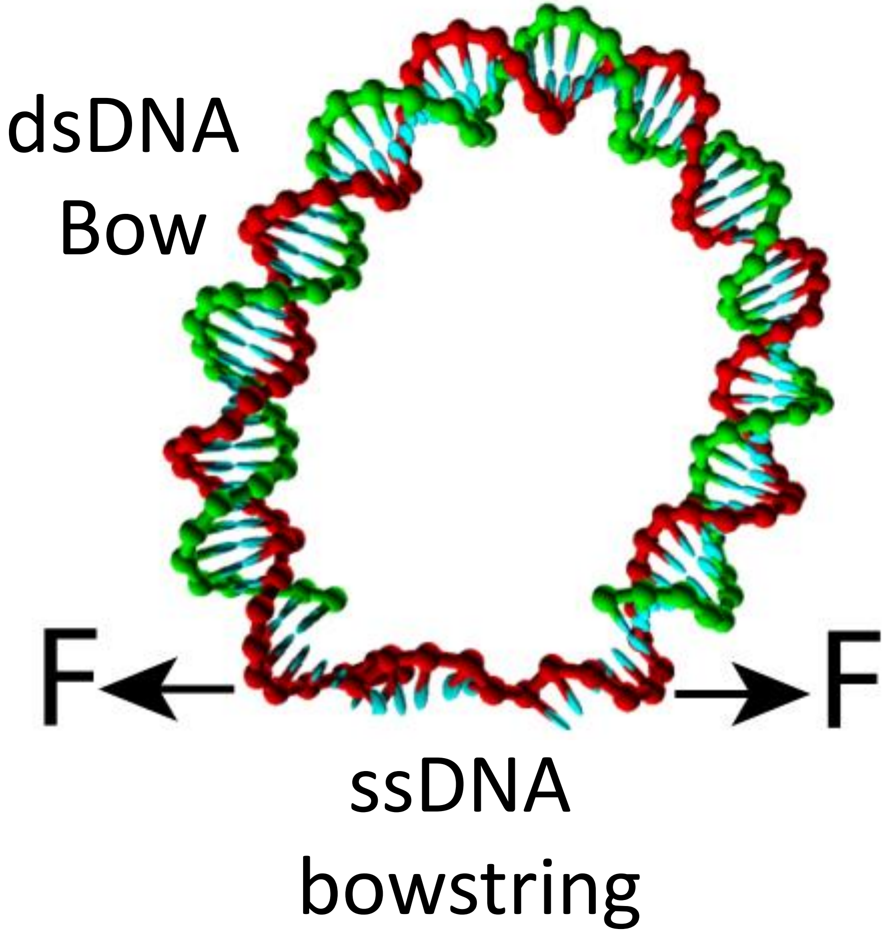
Positive in value

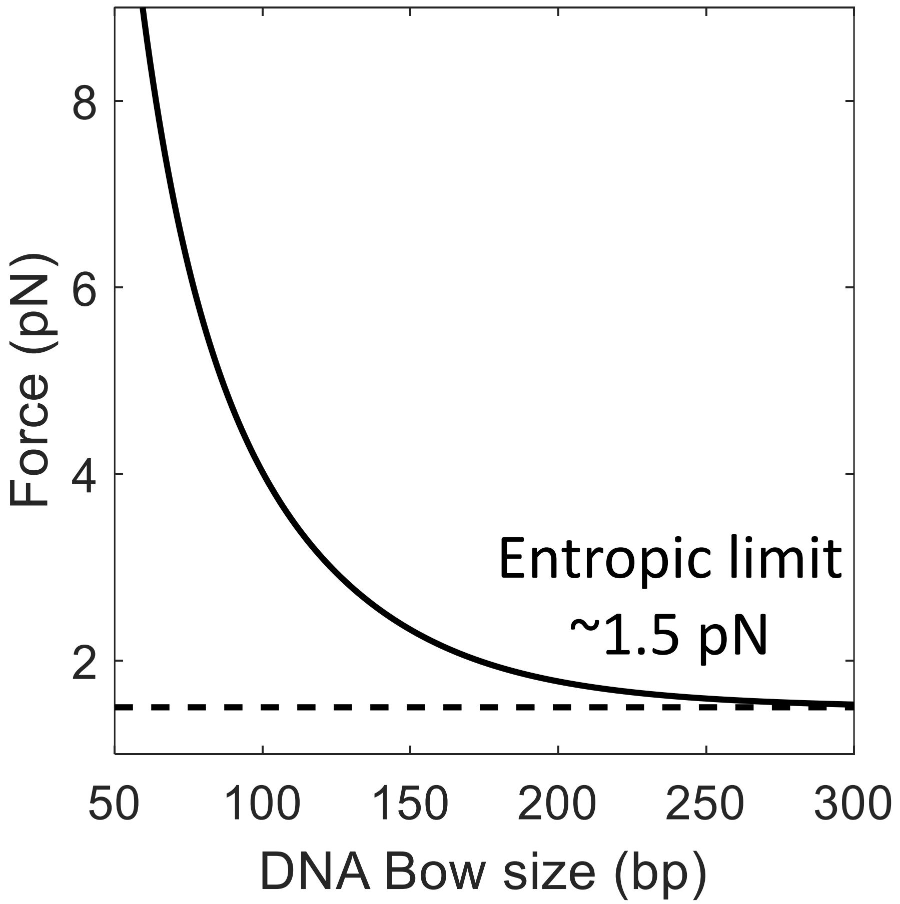
$$\frac{d \log k_{\mathrm{on}}(f)}{df} = \frac{1}{k_{\mathrm{B}}T}[x_{\mathrm{t}}(f) - x_{\mathrm{ss}}(f)], \quad k_{on} \text{ increases}$$

❑Goal

Measure binding (k_{on}) and unbinding rate (k_{off}) in the weak tension regime ($2\ pN < f < 6\ pN$)

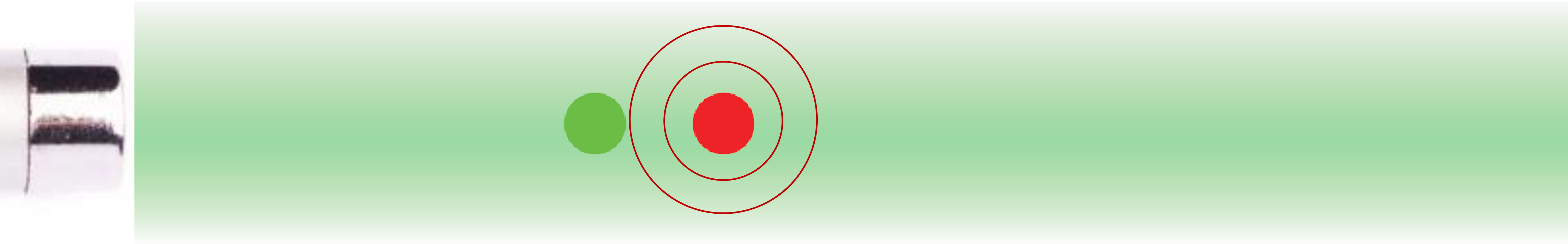
Exert weak tension with “DNA bows”



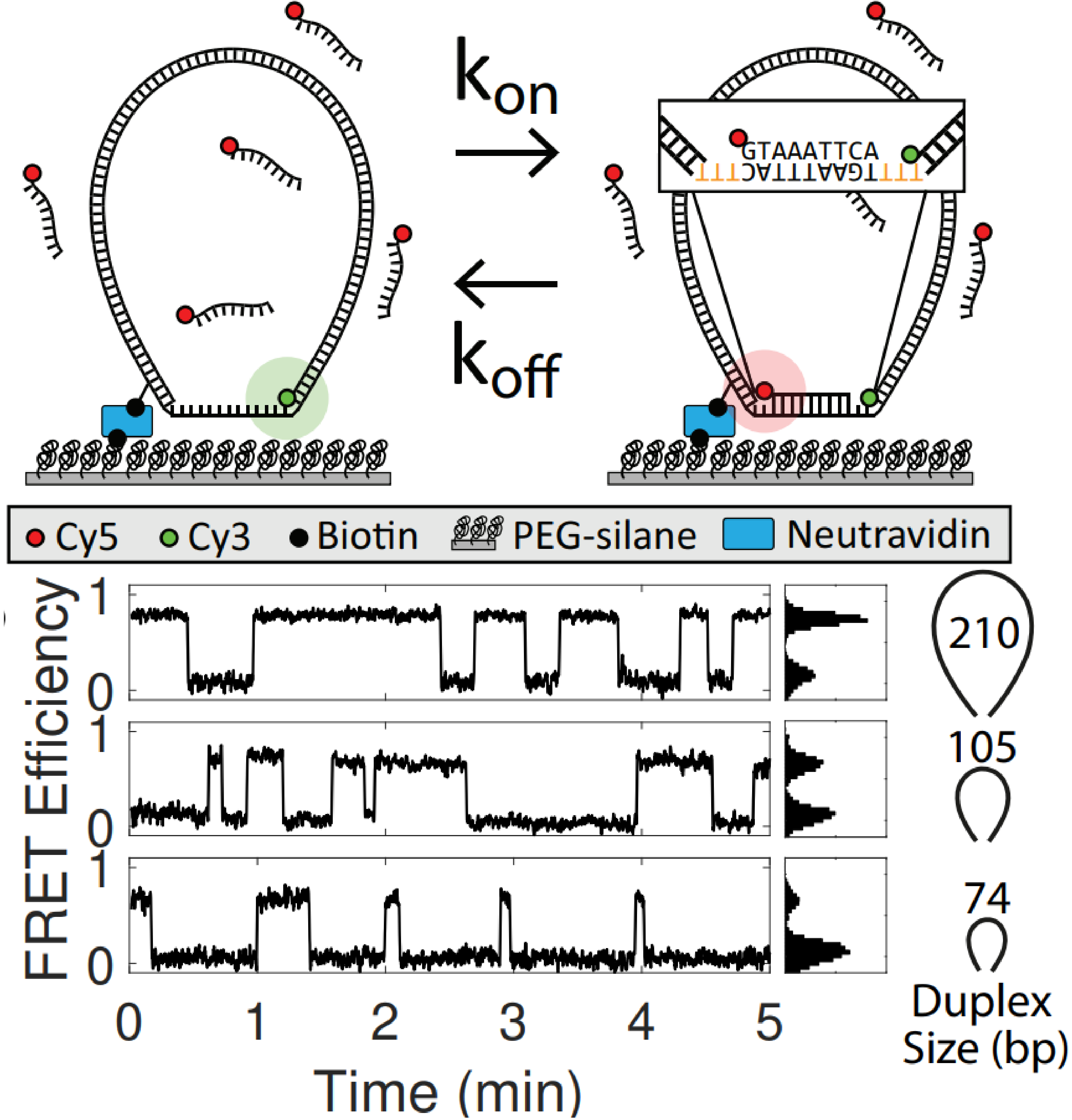


Fluorescence Resonance Energy Transfer (FRET)

Green Laser excites Donor

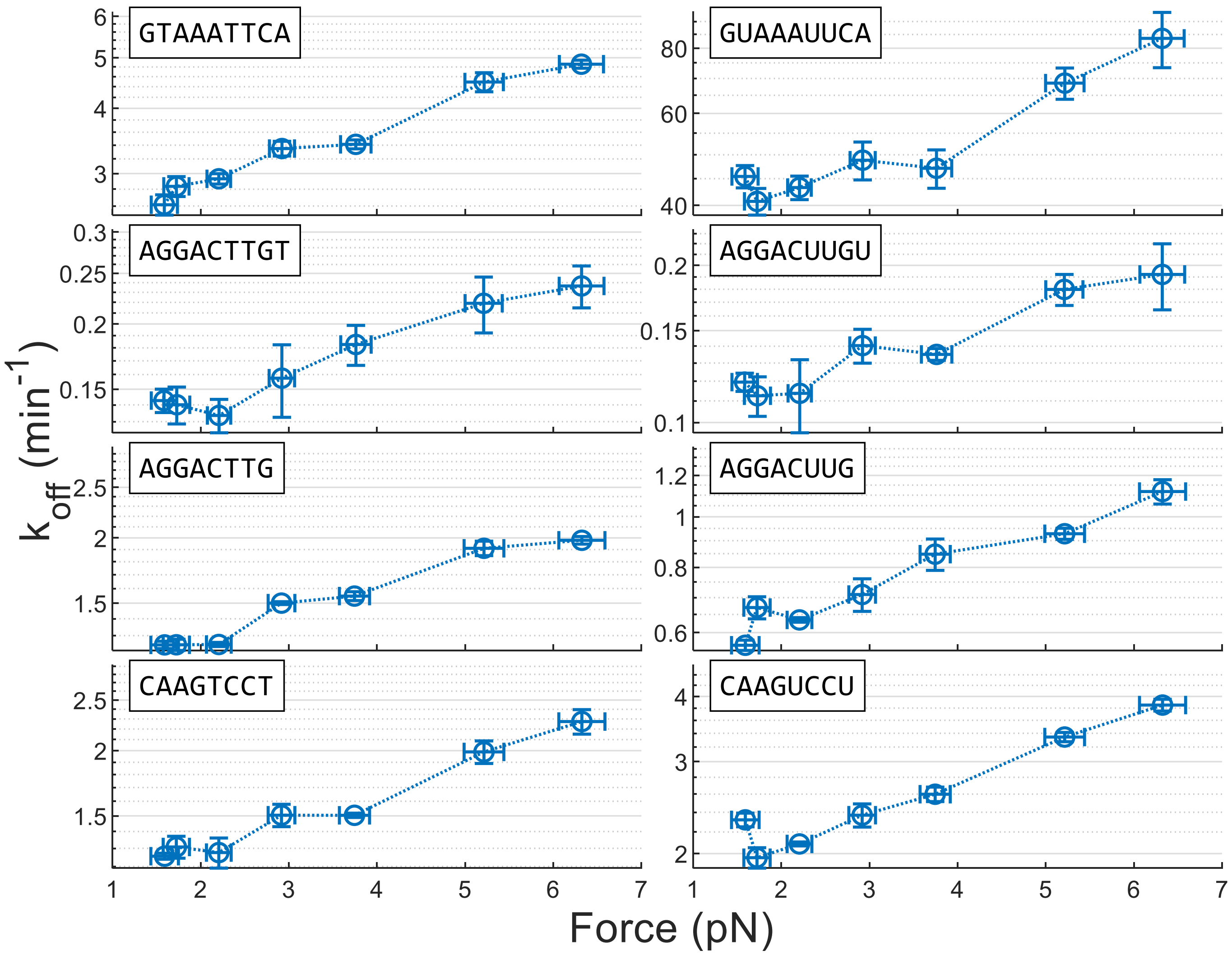


Donor gives energy to nearby Acceptor



kon
koff
GTAAATTCA
TTTCATTTAAGTTTT
Cy5
Cy3
Biotin
PEG-silane
Neutravidin
FRET Efficiency
Time (min)
210
105
74
Duplex Size (bp)

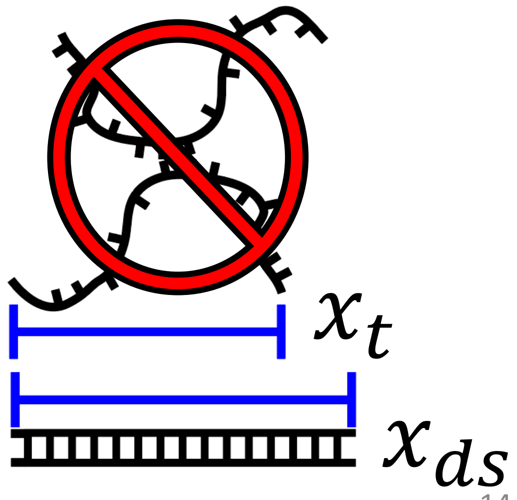
Unbinding rate vs. force



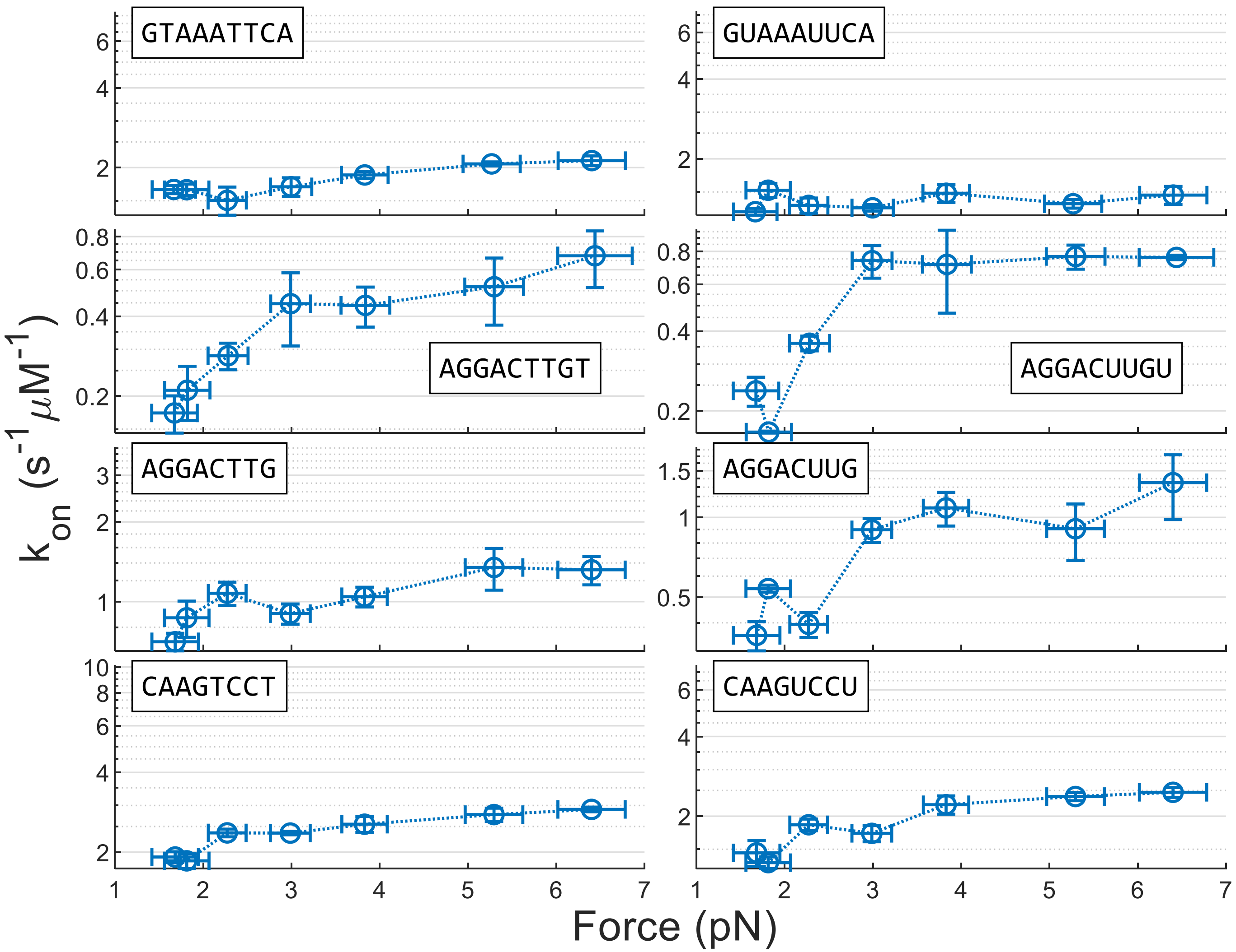
$$\frac{d \log k_{\mathrm{off}}(f)}{df} = \frac{1}{k_B T}[x_{\mathrm{t}}(f) - x_{\mathrm{ds}}(f)] > 0$$

$$\boldsymbol{x_t > x_{ds}}$$

This result contradicts the hypothesis that DNA is flexible in its transition state.



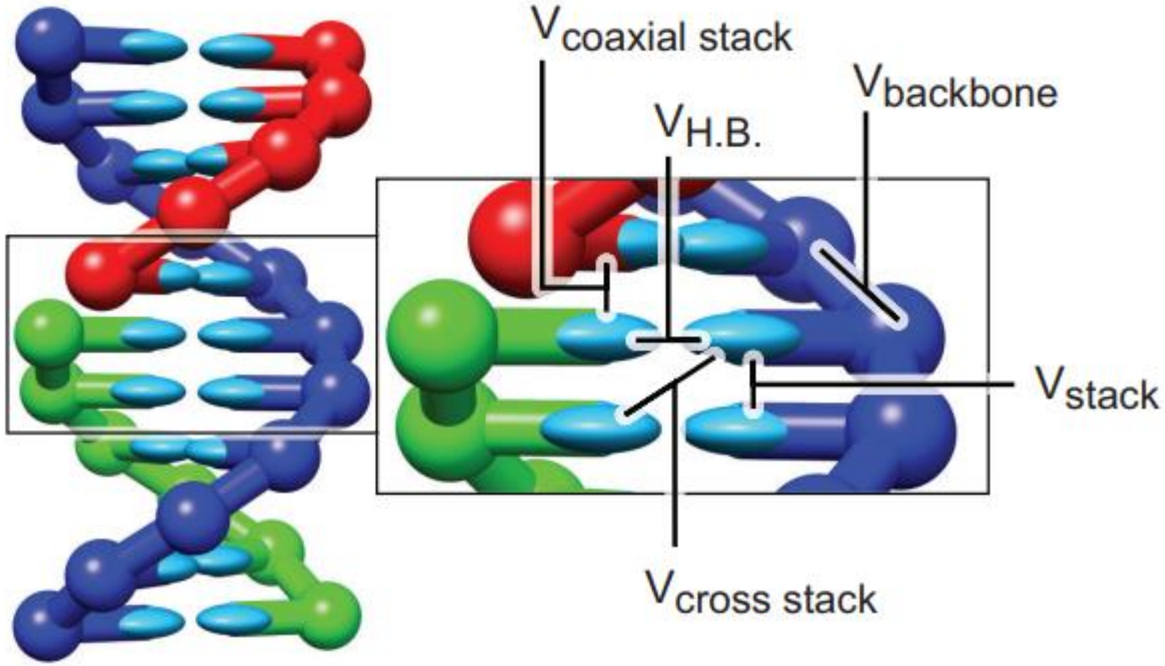
Binding rate vs. force



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

$\boldsymbol{x_t} > \boldsymbol{x_{ds}}$ as predicted

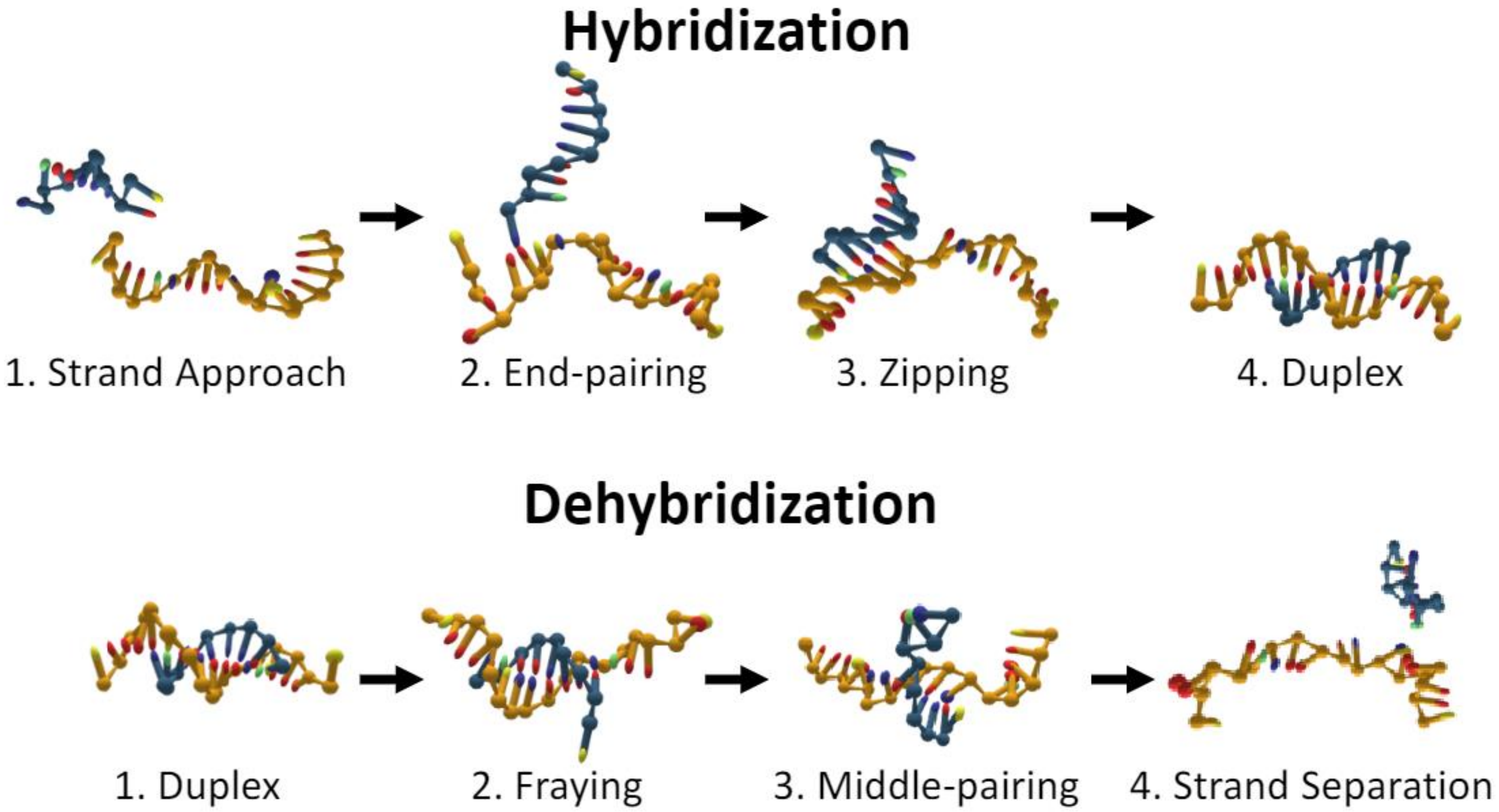
MD simulations (oxDNA)

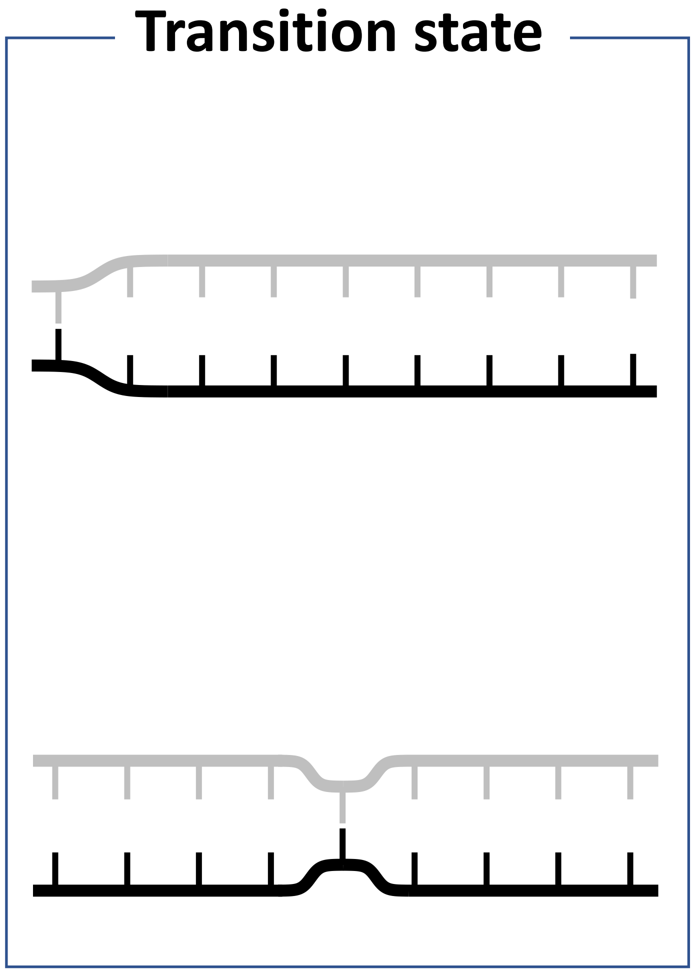


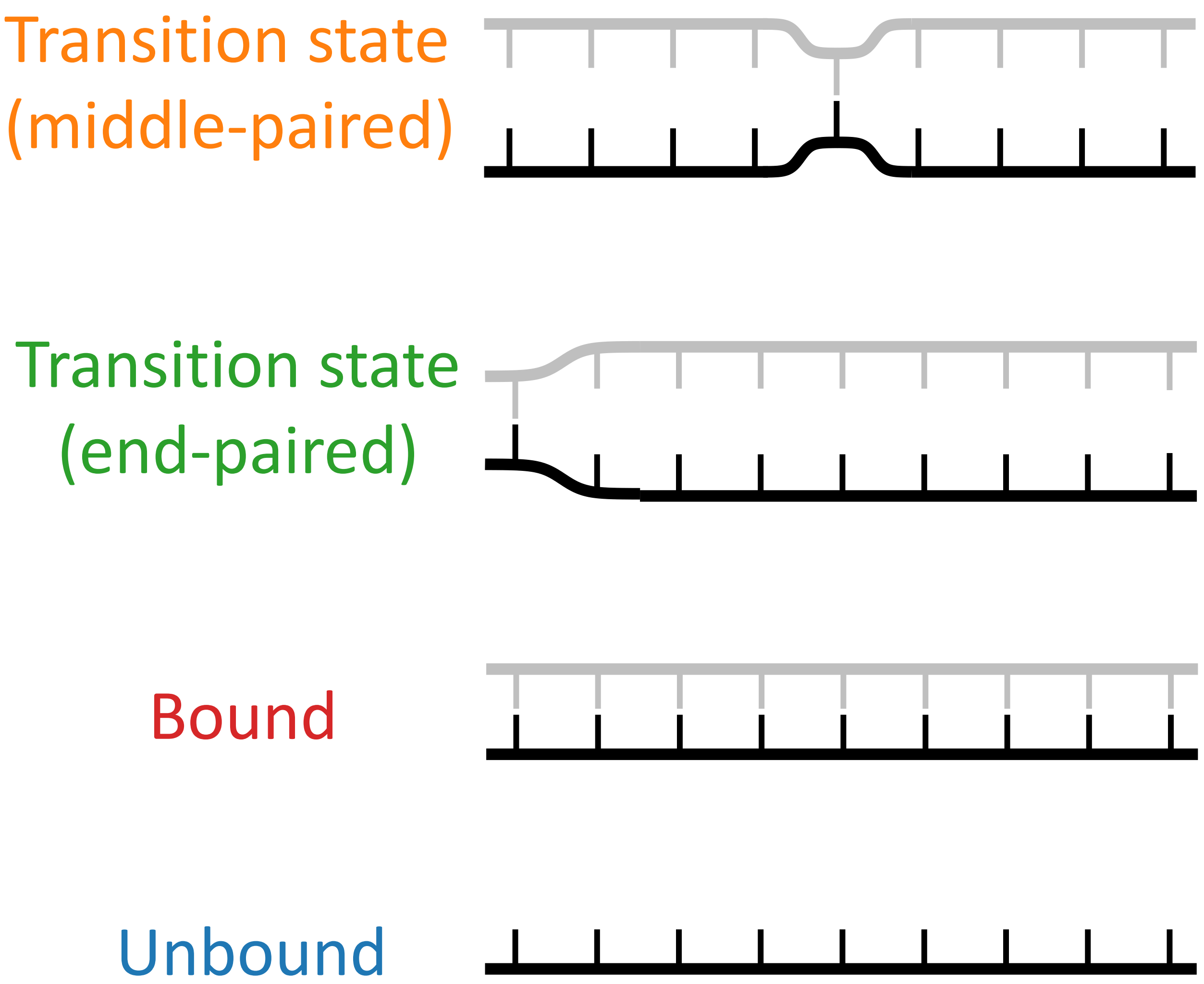
$$V = \sum_{\text{nn}} (V_{\text{backbone}} + V_{\text{stack}} + V'_{\text{exc}}) + \sum_{\text{other pairs}} \left(V_{\text{HB}} + V_{\text{c\_stack}} + V_{\text{exc}}\right)$$

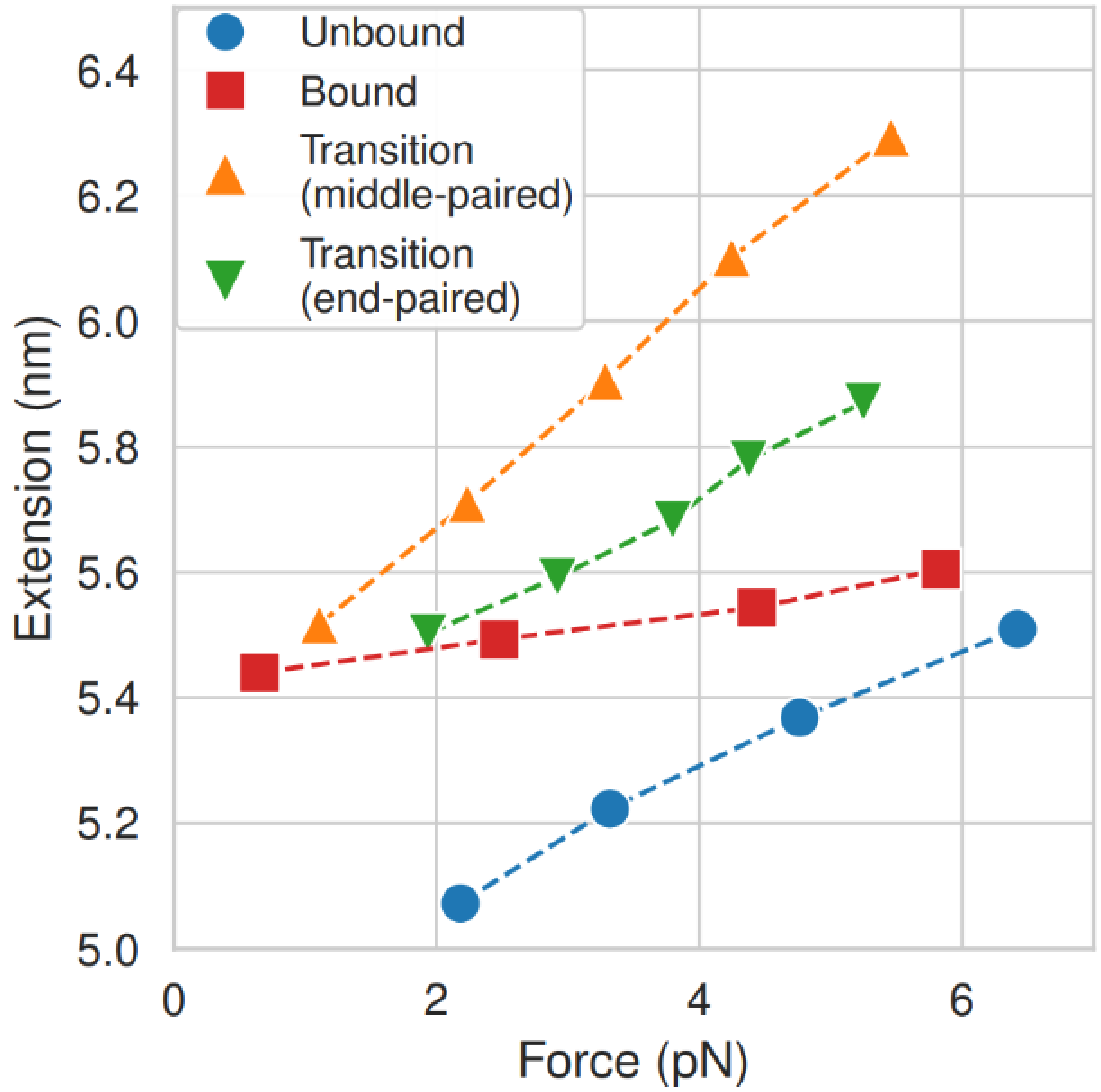
Ouldridge, T. E., Louis, A. A., & Doye, J. P. K. (2011).
The Journal of Chemical Physics

Different transition states






Transition state
(middle-paired)
Transition state
(end-paired)
Bound
Unbound


Unbound
Bound
Transition
(middle-paired)
Transition
(end-paired)
Extension (nm)
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
0
2
4
6
Force (pN)

Summary

- Using the DNA bow assay, we can measure hybridization/dehybridization in the range of 2 to 6 pN
- Both rates increase with force, with no indication of duplex stabilization at low f
- The transition state is more extended than the bound (dsDNA) and unbound (ssDNA) state, probably due to exclusion interaction

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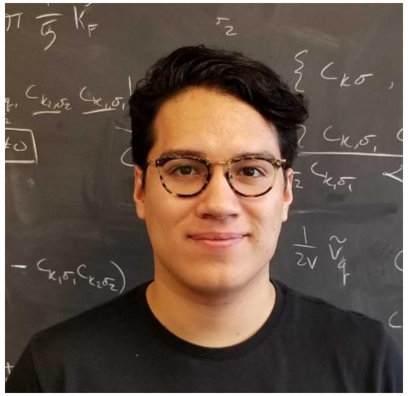

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10.1101/2022.04.19.4

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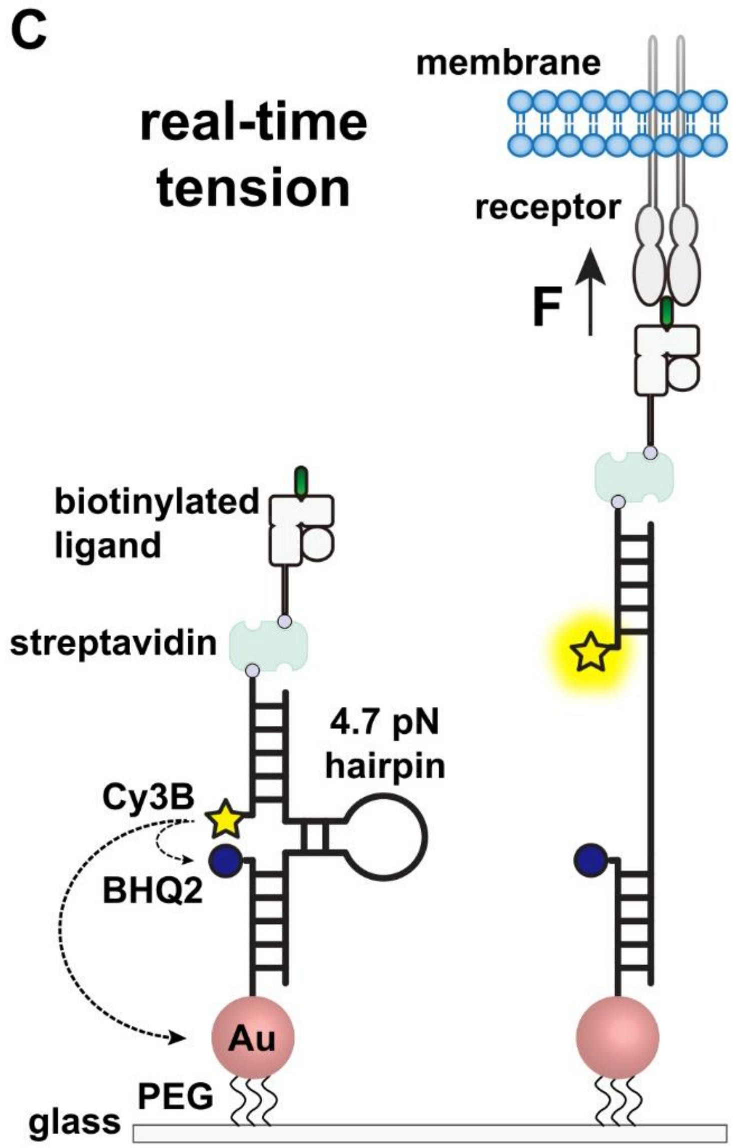
Hart, D. J., Jeong, J., Gumbart, J.C. and Kim, H.D. (2022) Weak tension accelerates hybridization and dehybridization of short oligonucleotides.



DNA Tension Probes to Map the Transient Piconewton Receptor Forces by Immune Cells.
(Ma 2021)