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Conference: From DNA-Inspired Physics to Physics-Inspired Biology

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Kinetics of Force-Induced Melting of Single DNA Molecules

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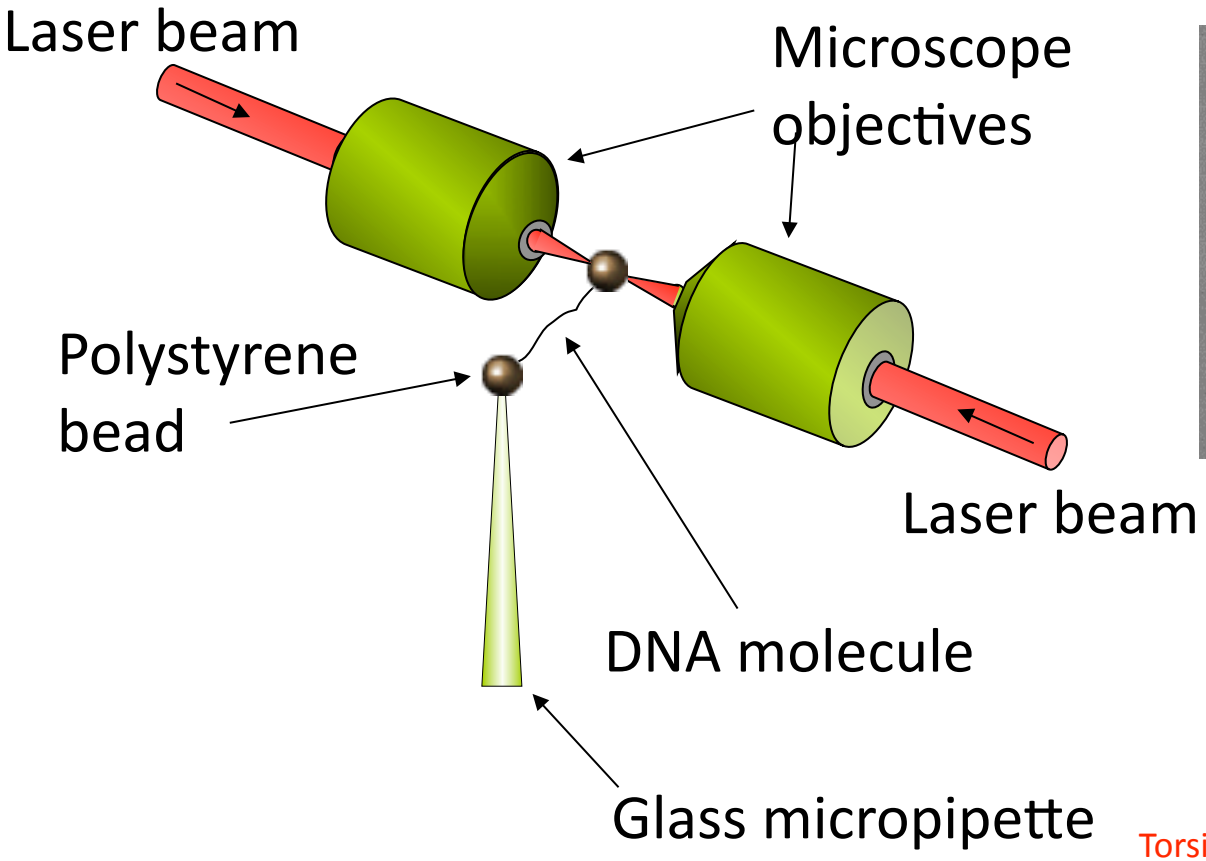
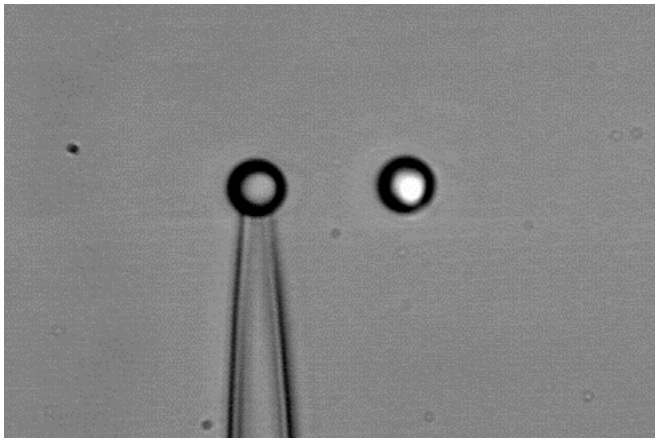
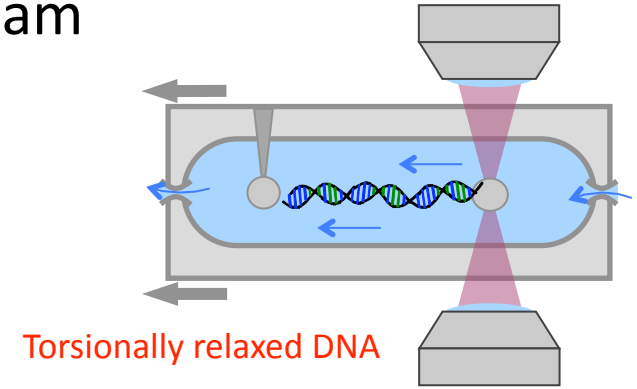
Kinetics of Force-Induced melting of single DNA molecules

Ioulia Rouzina, June 2009, Trieste

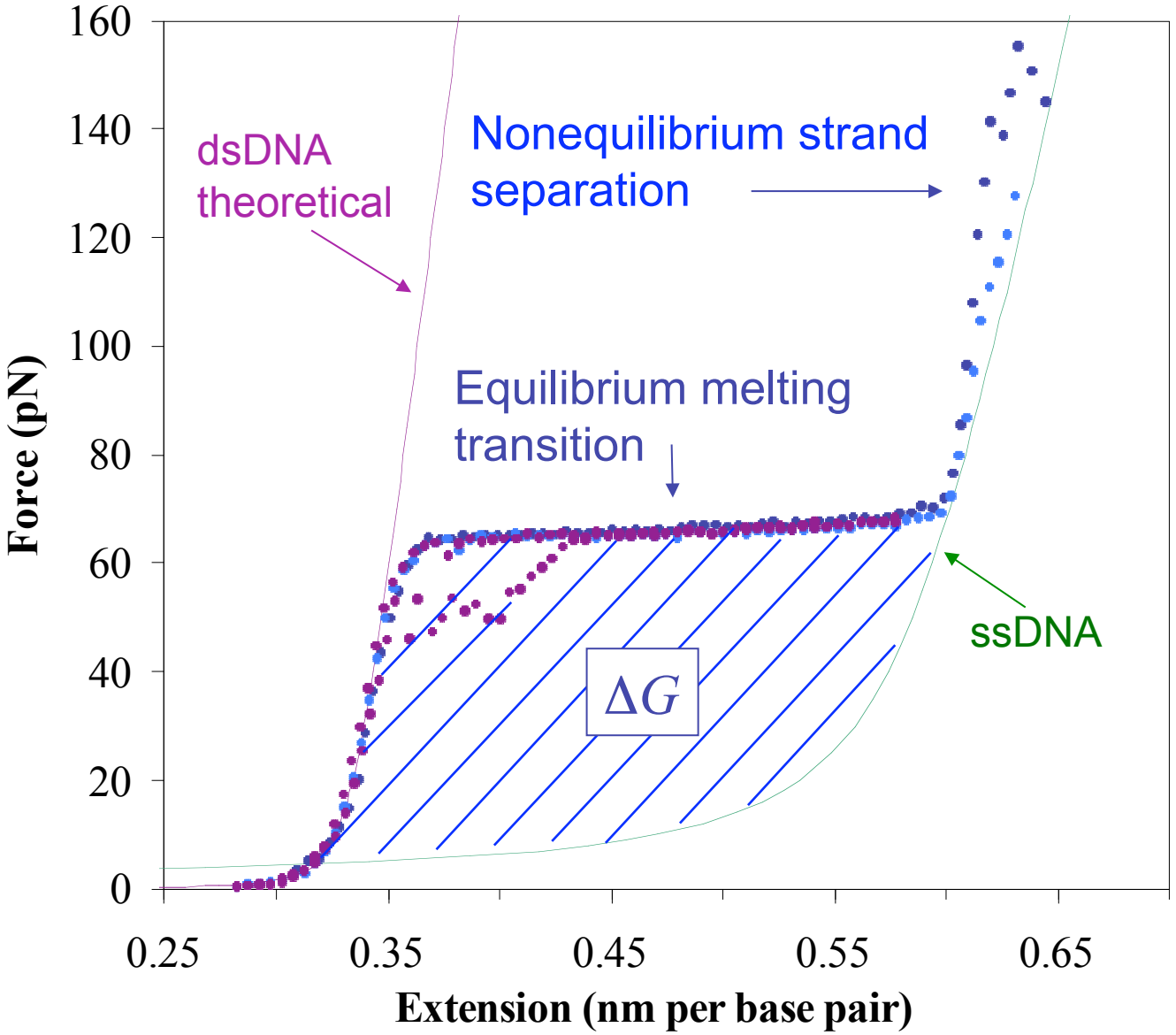






Experiment: Mark Williams lab, Boston

DNA force-induced overstretching



ΔG = area between dsDNA and ssDNA

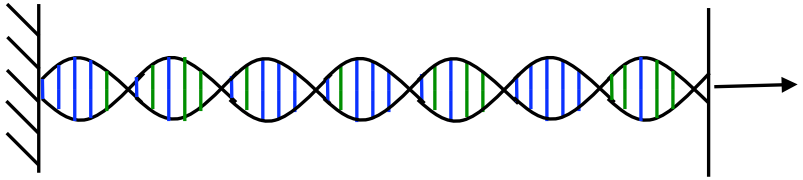
ΔG = DNA melting free energy when reversible

Hysteresis observed when DNA reannealing is inhibited

DNA force-induced melting

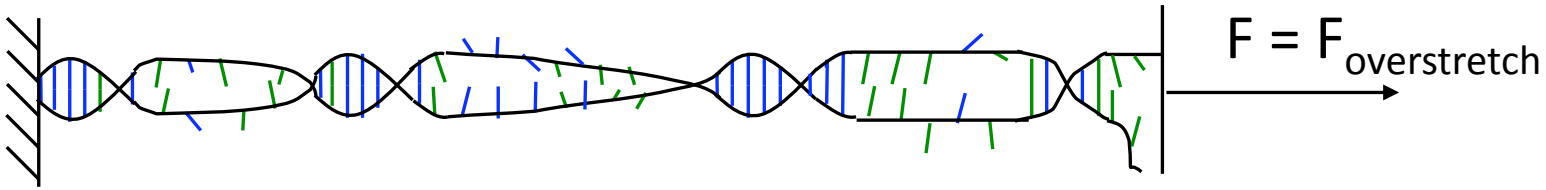
Helical base-paired DNA

Length per base pair 0.34 nm



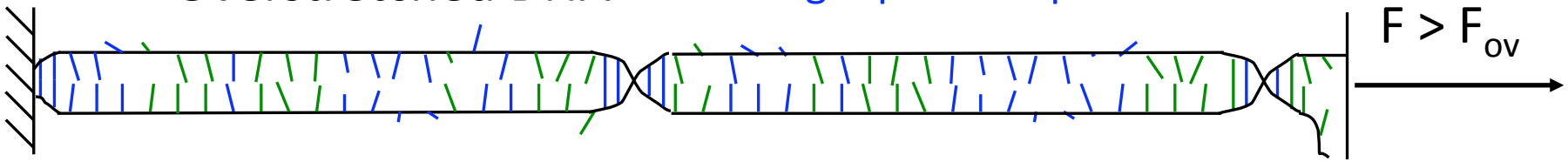
Partially overstretched DNA

Stacking and pairing interactions broken

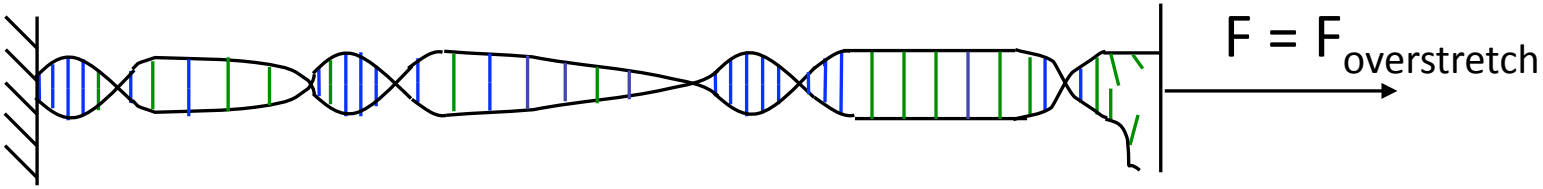


Overstretched DNA

Length per base pair 0.58 nm



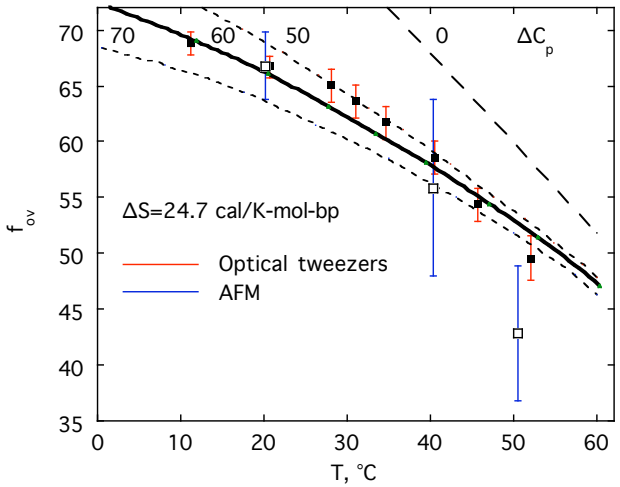
"S-DNA"

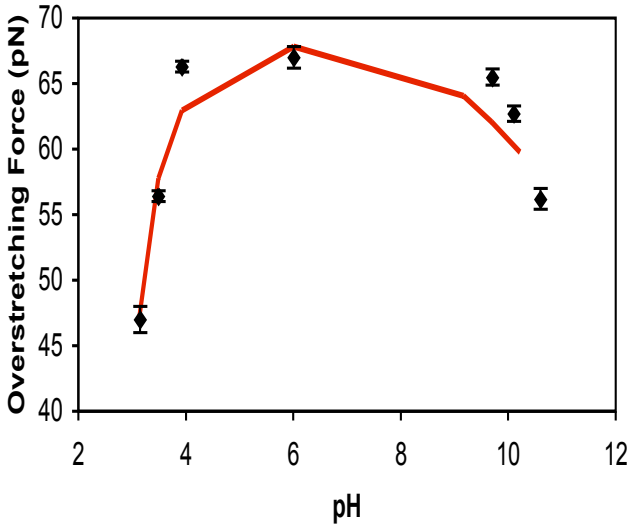


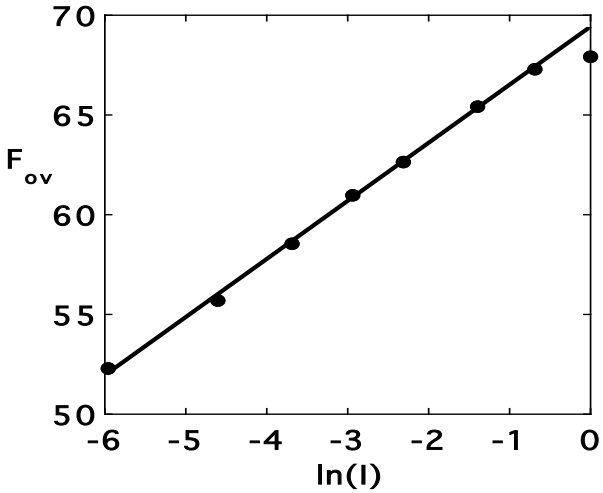
Only stacking interactions broken

Pro- arguments for DNA FIM

- All solution conditions, (i.e. temperature, salt, pH) affect the transition force just as they affect T_m;
- Ligand covalently modifying only ss DNA (Gyoxal) does so on DNA length that is overstretched;
- All single stranded binding proteins bind ds DNA with rates proportional to the force-facilitated probability of DNA melting to create ss DNA binding site;
- Intercalators that stabilize and elongat ds DNA predictably increase transition force and decrease elongation upon transition...

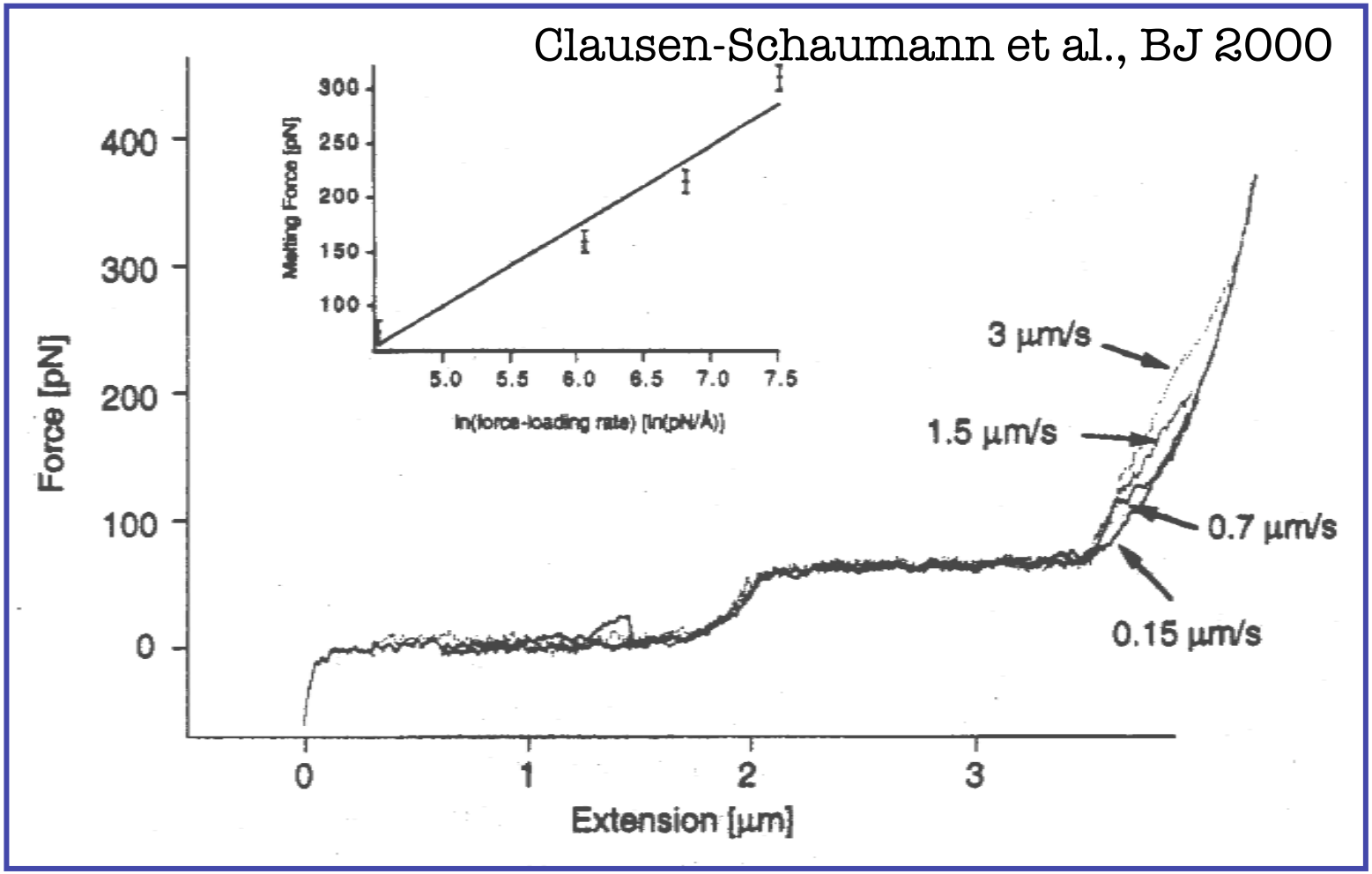




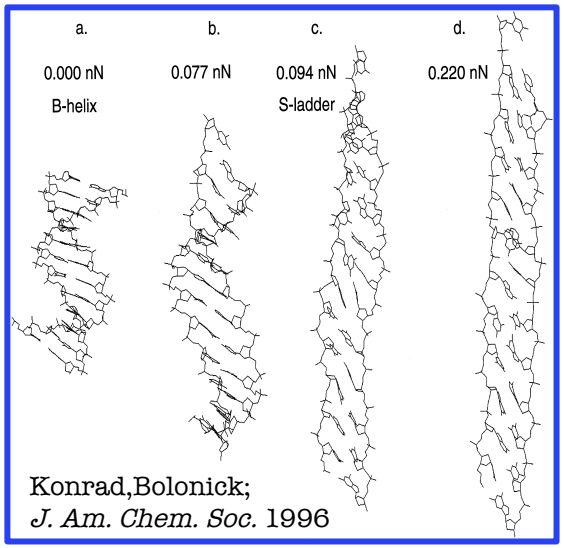


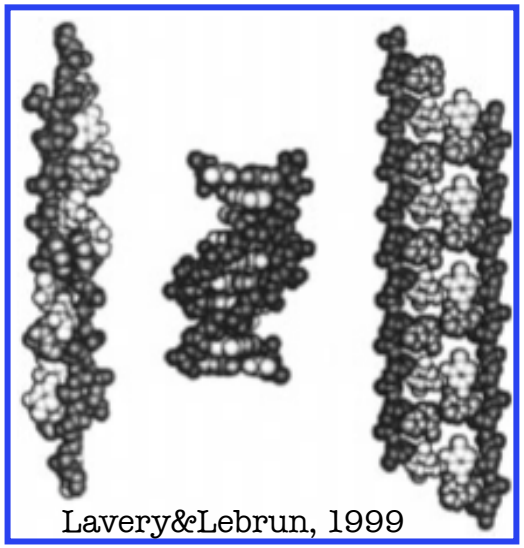
Counter- arguments for DNA FIM

- If transition is DNA melting, how much higher forces can be supported by few bp at the end of transition?
- Force-extension curve above plateau is different from 1 or 2 ss DNA.
- Rate-independence of the 1st transition and rate-dependence of the 2nd



Modeling of S DNA structure

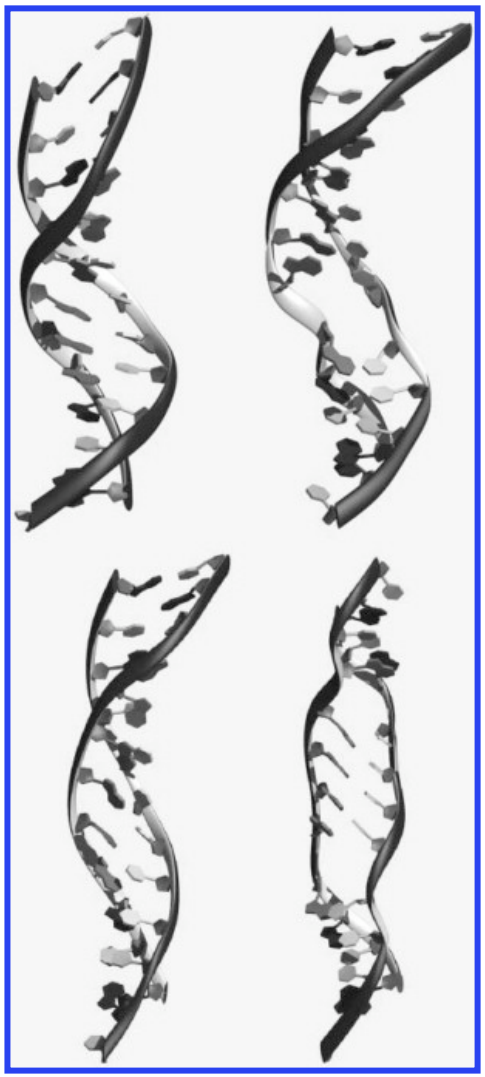




- All modeled B/S transitions have forces much higher then observed 65 pN. Energies of B/S are ~15-20 kcal/mol bp, compared to ~2-3 kcal/mol bp of observed transition and of thermal DNA melting.
- No reason for the observed high B/S cooperativity.
- B/S transition is expected to depend on attachment (5'5', 3'3', 5'3', 3'5'), but experimentally it does not;

S DNA is unstable with respect to melting

S. Harris et.al. BJ, 2005



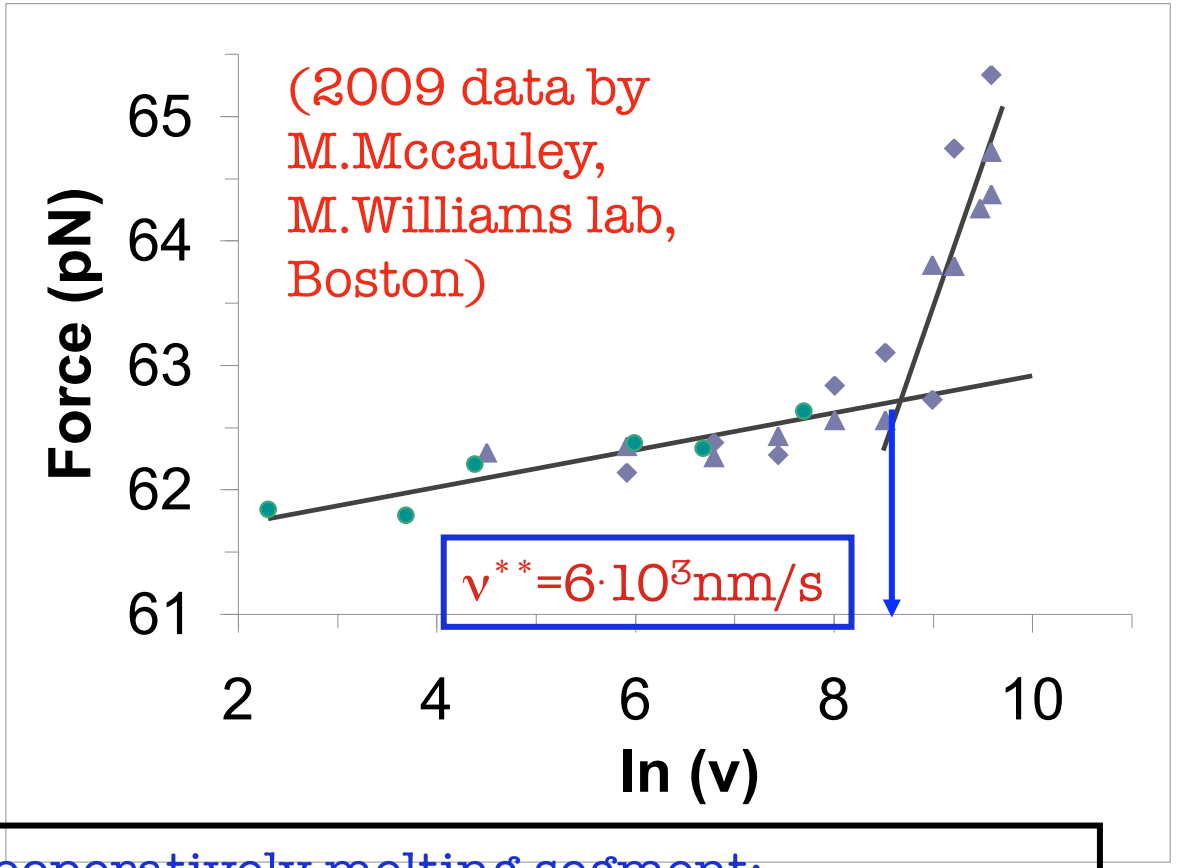
- Modeling of DNA melting is complicated due to large entropy of ss DNA;
- For the same reason ss DNA is energetically more favorable then any ds stretched DNA;
- Most of ss DNA entropy is retained in the force-melted state. Only backbone degrees of freedom are pulled out;
- When entropy of ss DNA is properly sampled ss is always more stable then S DNA

DNA oversretching force does depend on pulling rate

$F_{1/2}=F^{*}+k_BT/(n\cdot\Delta x)\cdot\ln(v)$

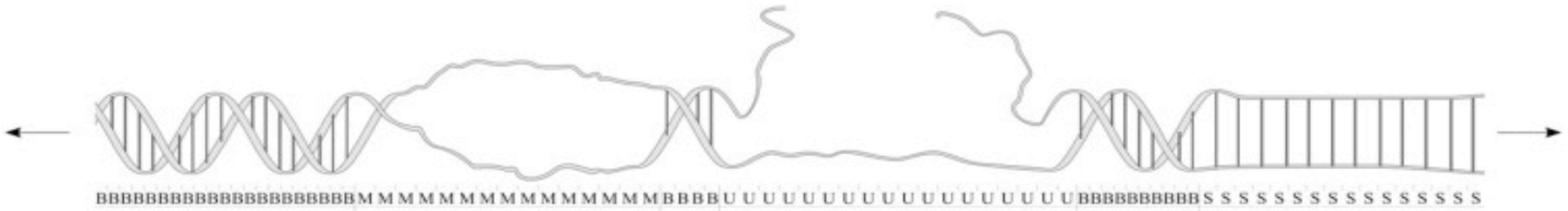
$v < v^{**}$, n~100 bp;
$v > v^{**}$, n~8 bp;
$v^{**}=6\cdot10^{3}$nm/s,

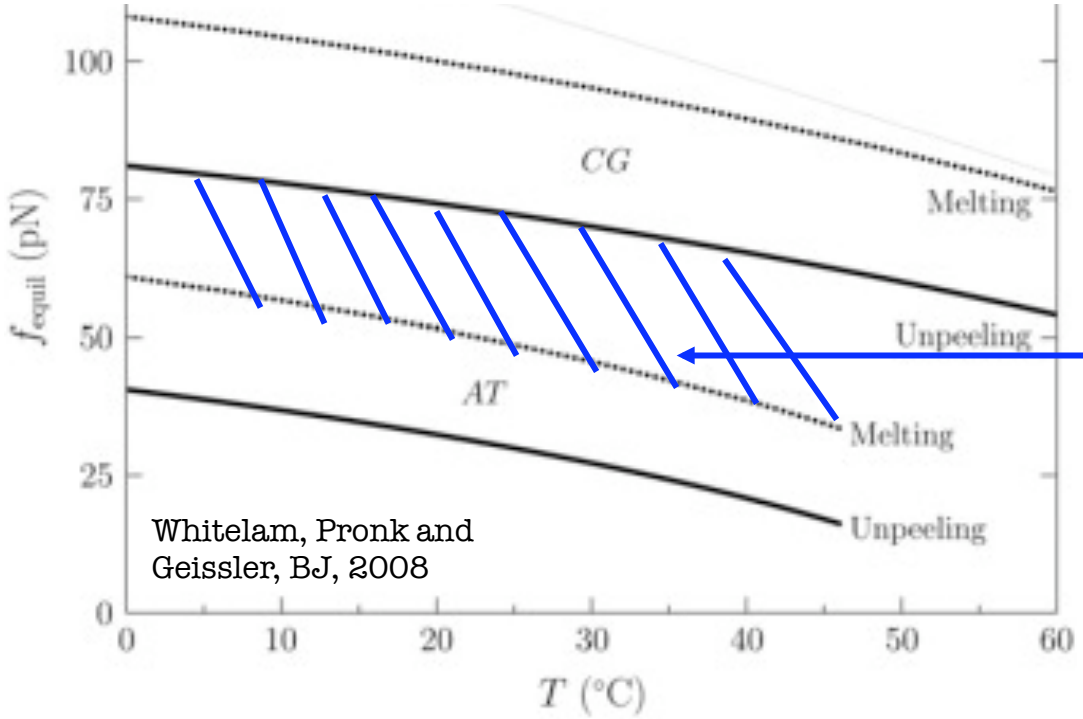
This $F_{1/2}(v)$ is inconsistent with B/S model. Is it consistent with melting?



- n is typical # of bp in cooperatively melting segment;
- n is large ~100 for slow pulling, but decreases with rate to less then 1 bp, i.e.melting becomes non cooperative at $F>F_m$
- At still higher rates slope would become as high as for strand separation force.

Inside melting is entropically unfavorable compared to unpeeling, but can still happen due to heterogeniety of DNA bp stability





Force range where inside melting of heteropolymeric DNA in high salt is possible

$\Delta F_H = F_{mGC} - F_{mAT}$ ~ 40pN -melting force range due to DNA heterogeniety

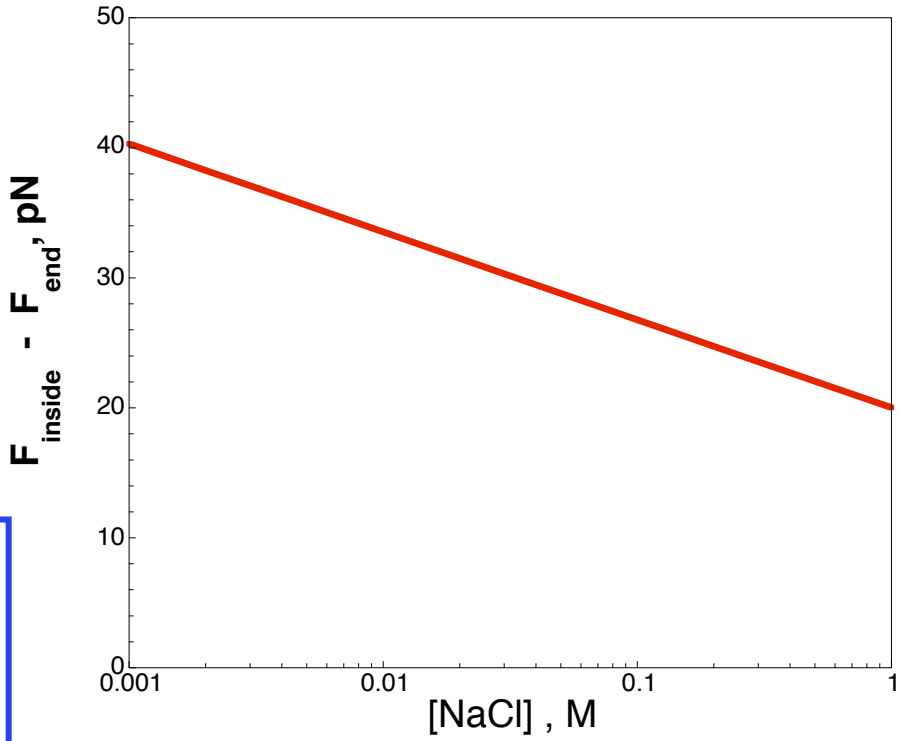
In high salt [Na]>~150 mM the average melting force difference between inside and end melting is ~20 pN. It comes from additional penalty of ~1 k_BT/bp melting due to both strands stretching inside.

Low salt makes inside melting more unfavorable due to electrostatic strand repulsion

Electrostatic free energy of melted strand repulsion per bp

$$\Delta G_{el} = k_B T \cdot \left(\frac{h_2}{l_B} - \frac{h_1}{l_B} \right) \cdot \ln\left(\frac{I}{I_0} \right)$$

h_1, h_2, are length per unit charge in inside- vs end- melted state, $l_B = e^2/\kappa kT$ -Bijerum length, I_0=1 M



Conclusion:

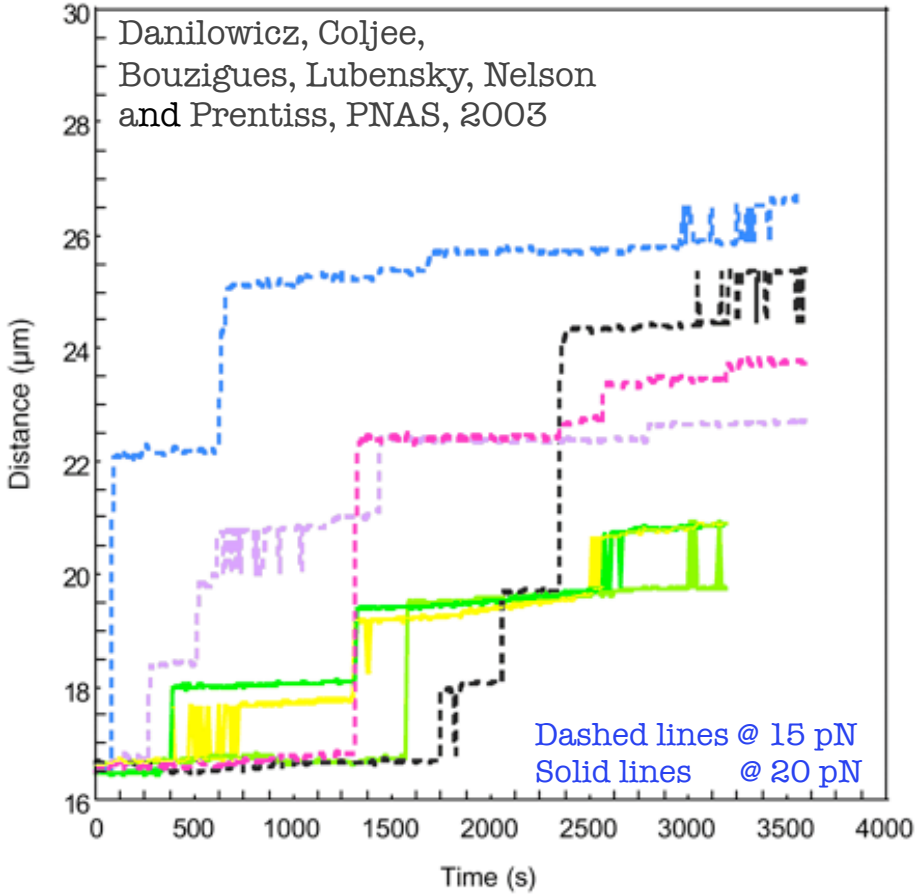
As F_{inside}-F_{end} approaches ΔF_H ~ 40pN at [Na]<~50 mM inside melting becomes impossible.

$$F_{inside} - F_{end} = \left(F_{inside} - F_{end}\right)_{highsalt} + \frac{k_B T}{l_B} \cdot \ln\left(\frac{I_0}{I}\right) \cdot \left(\left(\frac{h_{ss} - h_{ds}}{x_{ss} - x_{ds}} \right)_{inside} - \left(\frac{h_{ss} - h_{ds}}{x_{ss} - x_{ds}} \right)_{end} \right) =$$

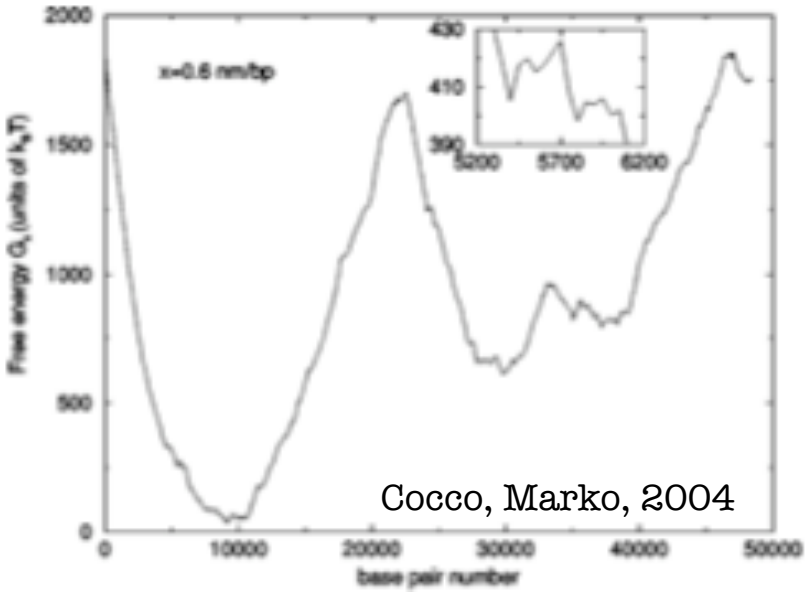
$$20\,pN + 4.2\,pN \cdot \ln\left(\frac{I_0}{I}\right) \cdot 0.7$$

DNA force-melting from the end via unzipping or unpeeling are both strongly kinetically inhibited

DNA unzipping @const.force



Free energy of DNA unpeeling



- Heteropolymeric DNA melting from the end at $F<F_{m\,GC}$ is slow due arbitrary large energy barriers for melting long stable sequences.
- Statistics of such unpeeling is similar to that of DNA unzipping.

- DNA unpeeling from 1 boundary at const.pulling rate should show large (~40 pN) or more force fluctuations. Unpeeling from >=2 boundaries should show smooth F(x) with $F_{1/2}(v)$ as observed in OT experiment (cooperative melting)

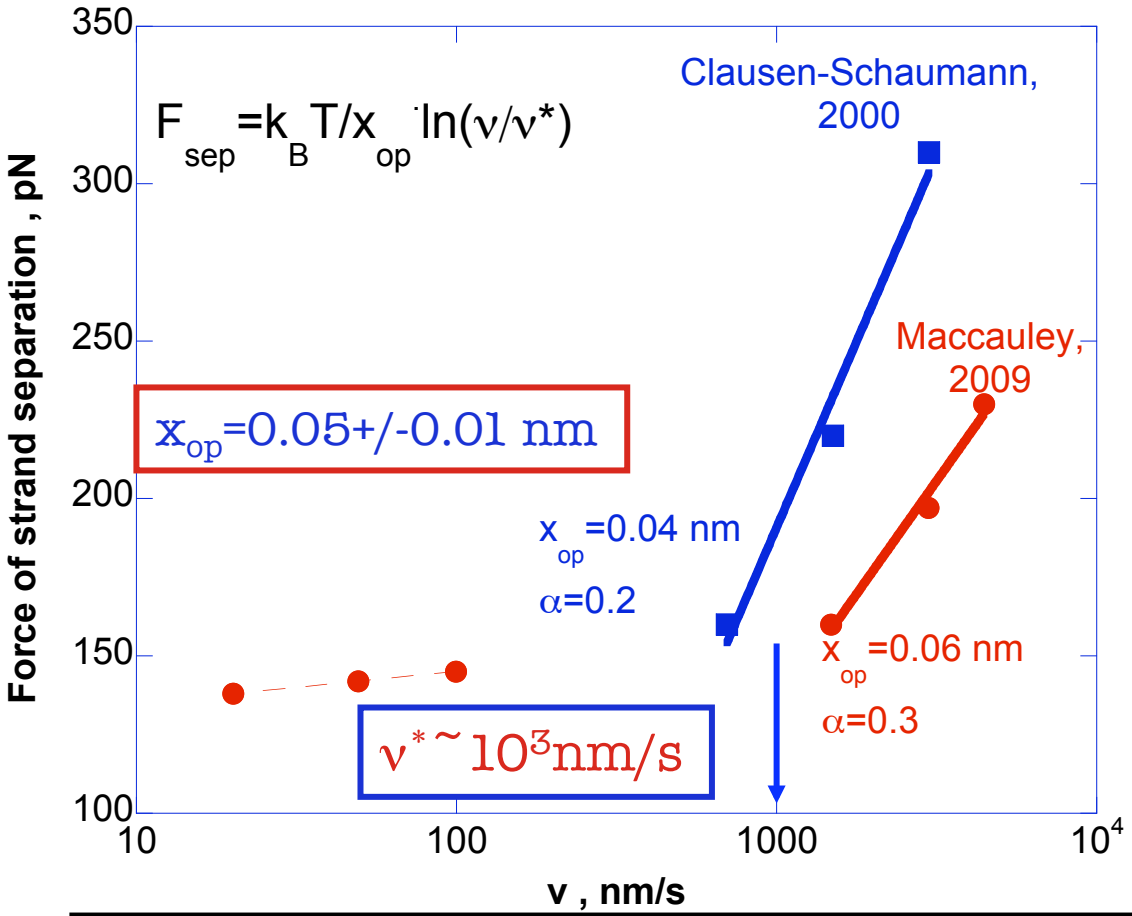
Common features of DNA unzipping and unpeeling

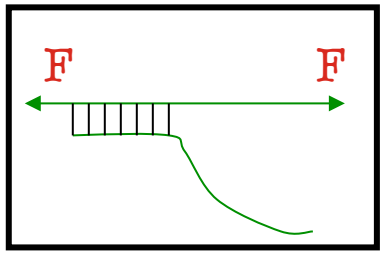
- Opening free energy is DNA sequence determined.
- Long DNA have arbitrary high opening energy barriers at average DNA melting force, F_m. May have infinitely long opening times.
- Opening force at a constant pulling rate may have large fluctuations between $F_{m\,AT}$ and $F_{m\,GC}$ (i.e. vary within ~10pN range for unzipping and within ~44 pN range for unpeeling).
- Very weak rate dependence of opening force due to large length associated with cooperative melting of a large DNA segment.

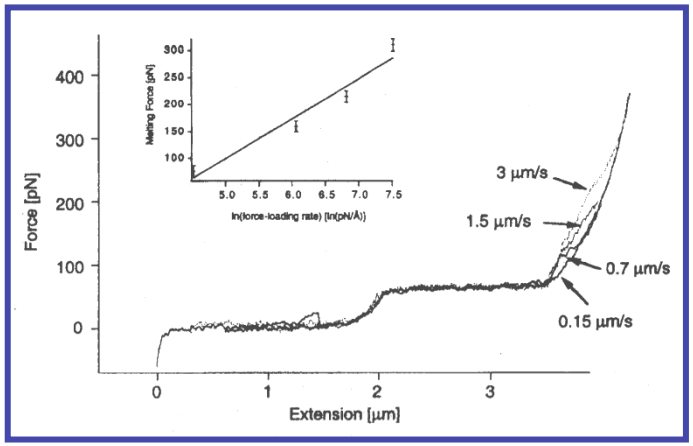
Different features of DNA unzipping and unpeeling

- In case of DNA unpeeling force is applied not to the DNA end, but along the whole molecule. Therefore, inside melting of the low stability regions is possible if their equilibrium melting force is lower then the unpeeling force.
- Melting of inside regions is also highly cooperative due to large boundary free energy.
- Unpeeling and inside melting coexist leading to the smooth DNA melting force close to F_m that is weakly rate-dependent with typical length of cooperatively melting segment ~100 bp

Strand separation transition





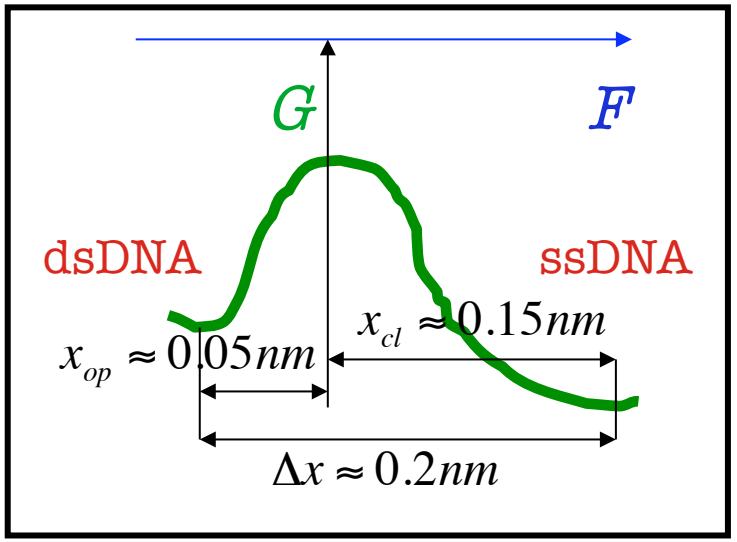


- Force of final strand separation grows sharply at $v > v^* \sim 10^3$ nm/s.
- For $v >> v^*$: $F_{sep}=k_B T/x_{op} \cdot \ln(v / v^*)$; $x_{op}=0.05\text{nm} << \Delta x=0.2$ nm
- Critical rate for strand separation $v^*=10^3$ nm/s is smaller then critical rate for transition midpoint, $v^{**}=6 \cdot 10^3$ nm/s because few, (compared to several at transition midpoint) boundaries remain.

Effect of force on bp opening and closing rates

$$k_{op}(F) = \frac{k_0}{s_0} \cdot e^{Fx_{op}/k_BT} \qquad \Delta x = x_{op} + x_{cl}$$

$$k_{cl}(F) = k_0 \cdot e^{-Fx_{cl}/k_BT} \qquad x_{op} = \alpha \cdot \Delta x$$



$k_0 \sim 10^6\, s^{-1}$ Rate of end bp closing

Porschke, 1976;Russu NMR

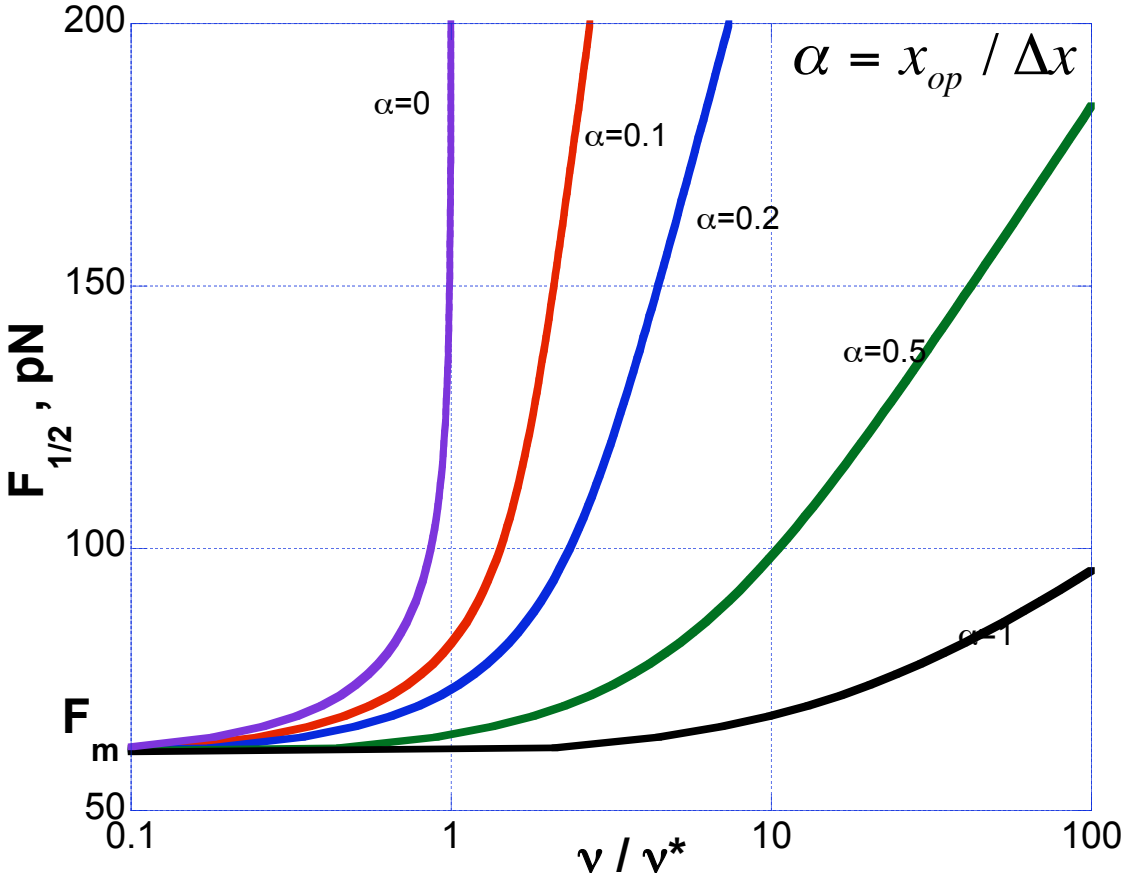
$k_0 / s_0 \sim 10^6 / 30 \sim 3 \cdot 10^4\, s^{-1}$ average rate of bp opening at room temp

$s_0 \approx 30$ average bp stability at room temp

$x_{op} << x_{cl}$, i.e. force has much weaker effect on opening then on closing;

Force-facilitated opening rate is slow: $k_{op}(F) \geq 3 \cdot 10^4\, s^{-1}$

Small bp elongation upon opening leads to high and very rate-dependent opening force at pulling rates >~ 10^3 nm/s.



$$\nu^* = \Delta x \cdot \frac{k_0}{s_{GC}} \approx$$

$$0.2nm \cdot 1.5 \cdot 10^4 s^{-1}$$

$$= 3 \cdot 10^3 nm / s$$

Last bp are the strongest GC

The fitted value is:

$$\nu^* \sim 1000 nm / s$$

$$\nu(F) = \Delta x \cdot \left(k_{op}(F) - k_{cl}(F)\right) = \Delta x \cdot \left(\frac{k_0}{s_0} \cdot e^{Fx_{op}/k_BT} - k_0 \cdot e^{-Fx_{cl}/k_BT}\right) =$$

$$\Delta x \cdot \frac{k_0}{s_0} \cdot e^{Fx_{op}/k_BT}\left(1 - e^{-(F_m - F)\Delta x/k_BT}\right) = \nu^* \cdot e^{F\alpha\Delta x/k_BT}\left(1 - e^{-(F_m - F)\Delta x/k_BT}\right)$$

Conclusions

- Weak rate dependence of overstretching force is consistent with cooperative pseudo-equilibrium melting of ~100 bp segments of DNA. This cooperativity is due to DNA heterogeniety. At $v > v^{**} \sim 10^4$ nm/s melting segments get shorter, force gets higher, and finally melting becomes non-equilibrium (ripping);

- At low salt (<~50 mM NaCl) force-induced DNA melting can only proceed from DNA ends. At higher salt it can also happen inside weakly stable DNA regions (at $v < v^{**}$);

- DNA unpeeling from a single end will happen via sequence-determined force-jumps, that weakly depend on rate.

- Melting from more then one boundary, or inside the duplex will happen at weakly rate-dependent force slightly higher then the average equilibrium melting force.

Conclusions
(continued)

- Strand separation force becomes high and very rate dependent at $v > v^* \sim 10^3$ nm/s. v^* is the natural end bp opening rate. It is slow, becaue it happens at room temp, and the force facilitated opening only weakly.

- Melting force destabilizes DNA duplex by slowing down bp closing, while having little effect on bp opening. Natural bp opening/closing rates at F_m are $\sim 10^4$ s^{-1}, i.e. much slower then at T_m $\sim 10^6$ s^{-1}.

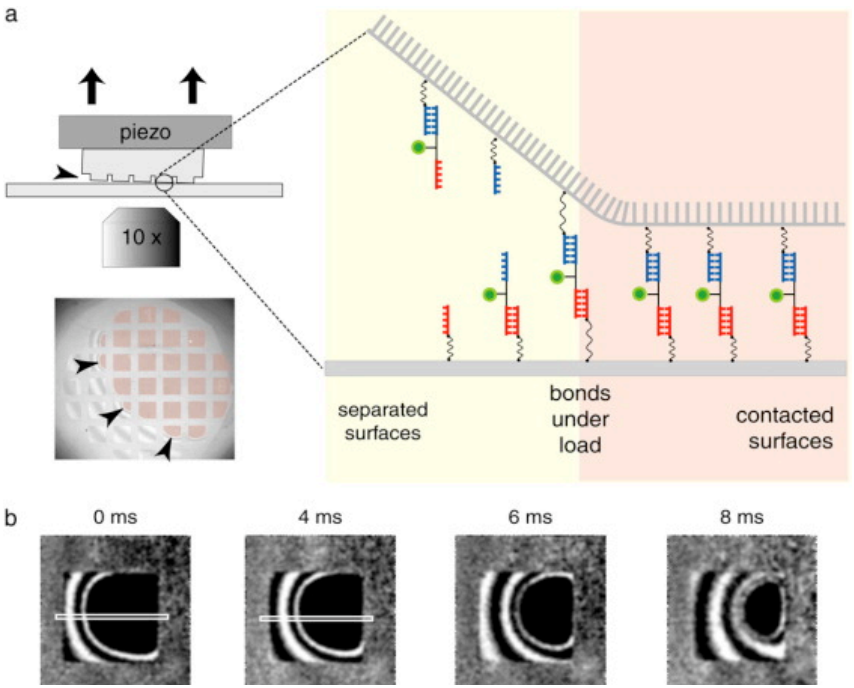
- Non-equilibrium ripping upon strand separation at $v > v^*$ should depend on DNA strand attachment. Pseudo-equilibrium melting is not sensitive to strand attachment.

- $v^{**} \sim 10\, v^*$ because melting at the transition midpoint happens from ~ 10 boundaries (depends on DNA length);

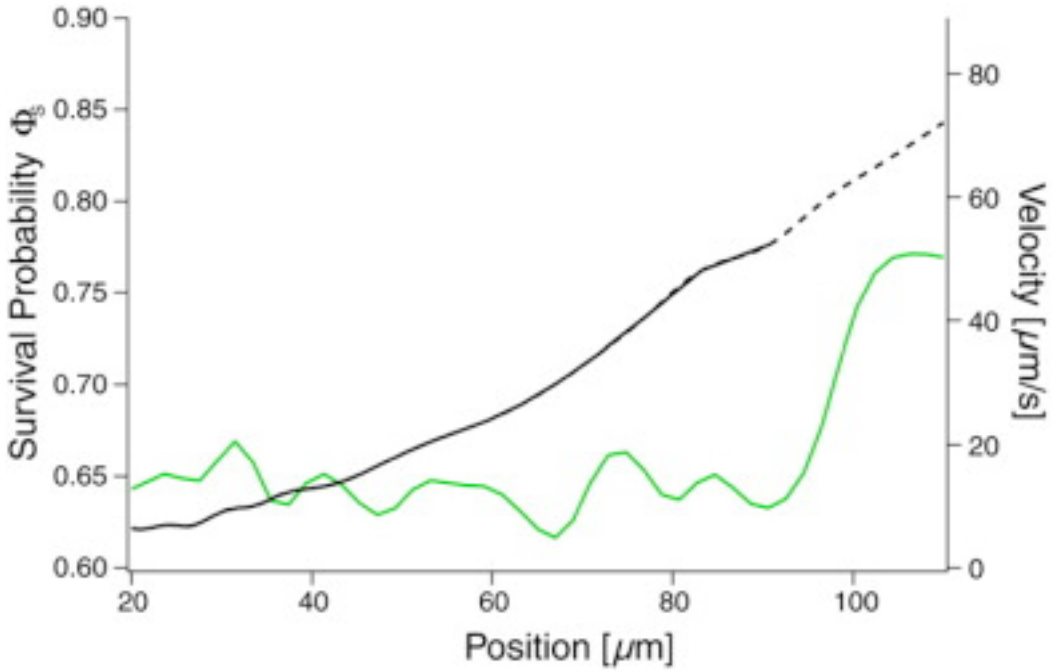
Molecular Force Balance Measurements Reveal that dsDNA Unbinds Under Force in Rate- and attachement- Dependent Pathways

Albrecht, Neuert, Lugmaier and Gaub, Biophys. Journal, 2008

Experimental design



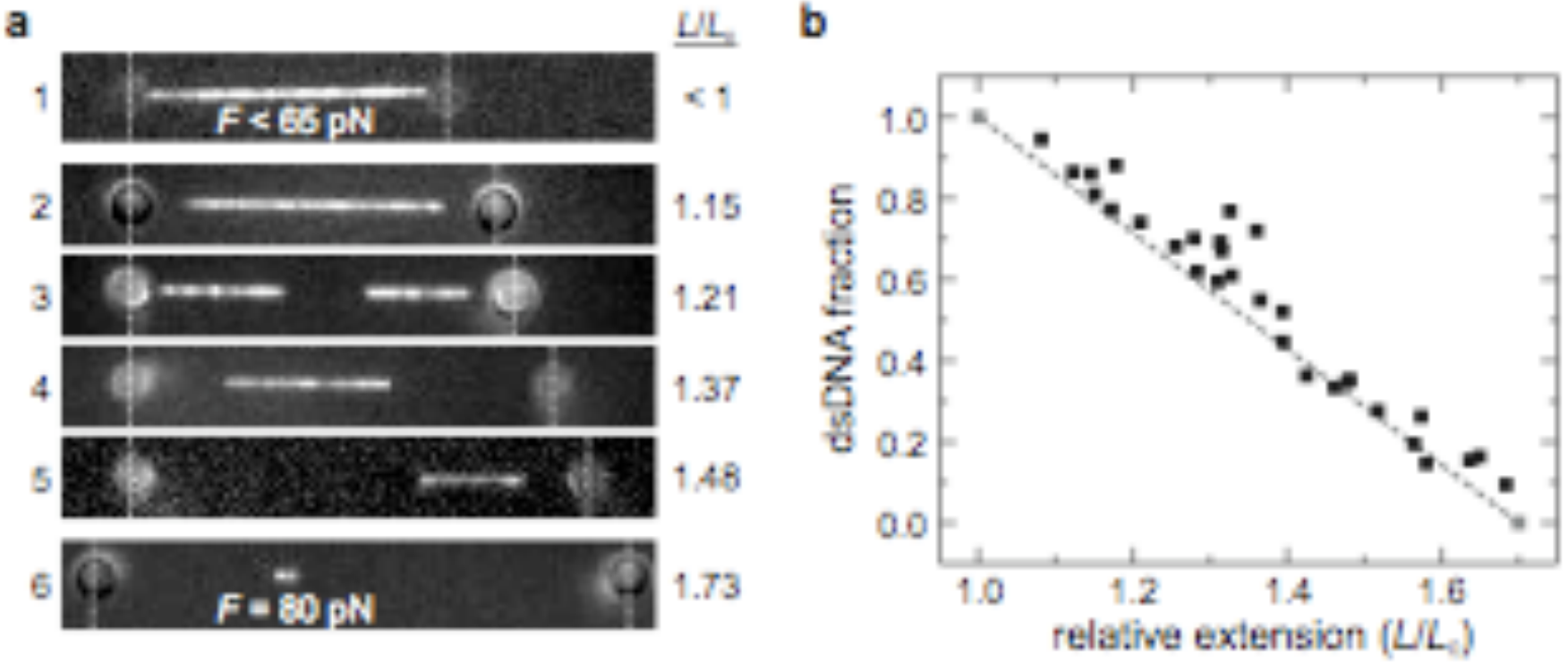
Duplex survival probability (green line) and pulling velocity (black line) vs position on the slide.



Survival probability increases sharply above loading rate ~40 μm/s and becomes dependent on DNA oligo attachment geometry. This is how non-equilibrium ripping is expected to behave.

All overstretched DNA is melted

(unpublished data from Gijs Wuite's lab. Joost van Mameren PhD Thesis)



dsDNA is fluorescently labeled by YOYO. Melted regions are dark. Fraction of the bright (i.e. ds) length accounts for all DNA elongation during transition

Conclusions:

- All overstretched DNA is melted.
- All melting is from the DNA ends (Because of 50 mM NaCl).